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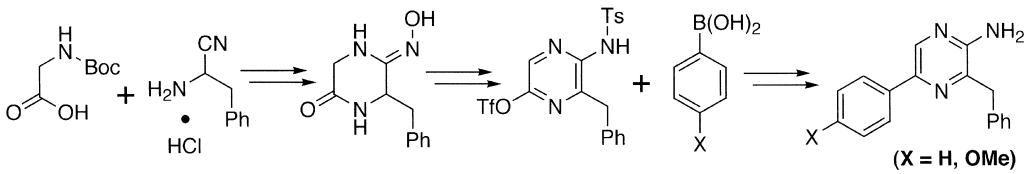
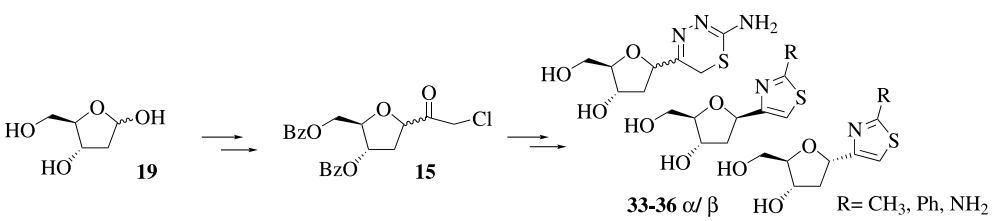
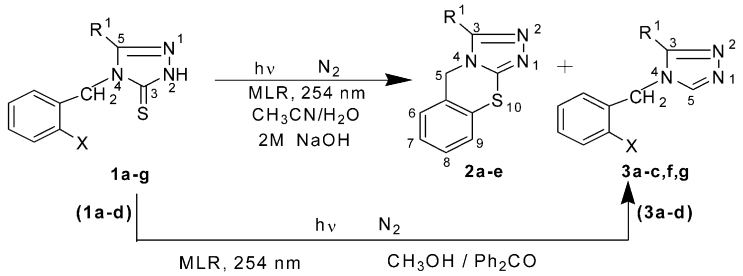
Contents

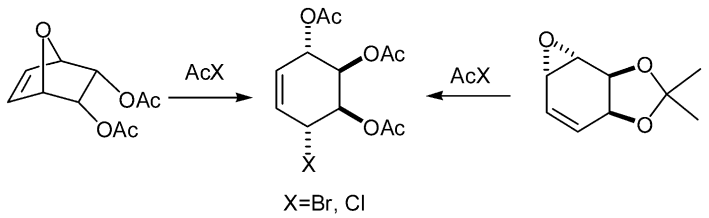
REPORT

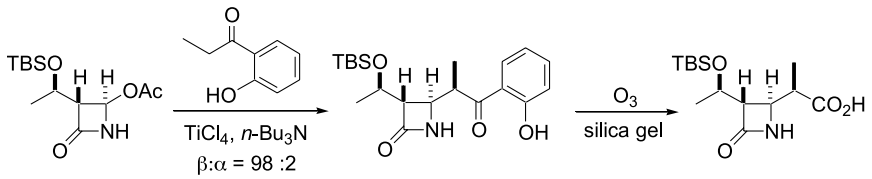
ARTICLES

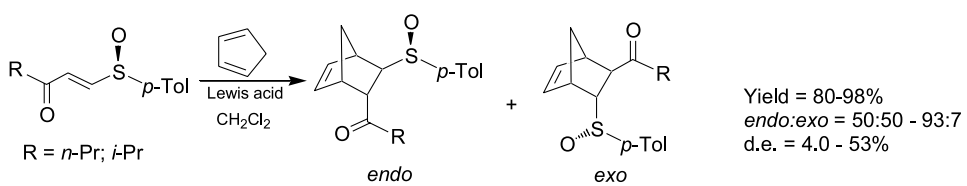


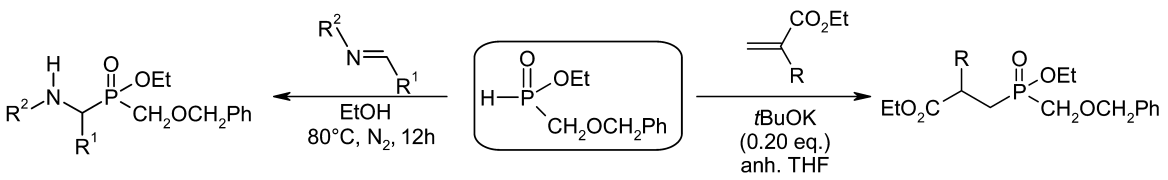


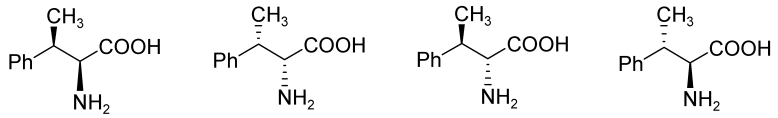


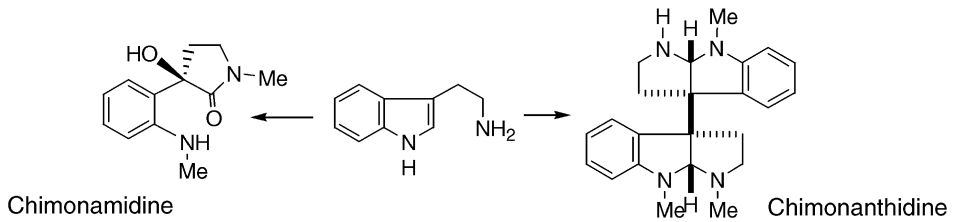


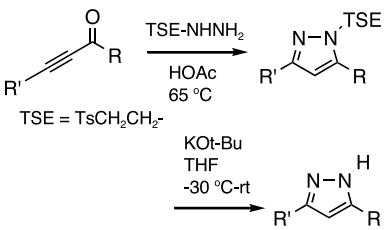




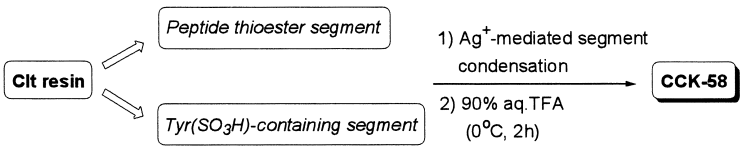


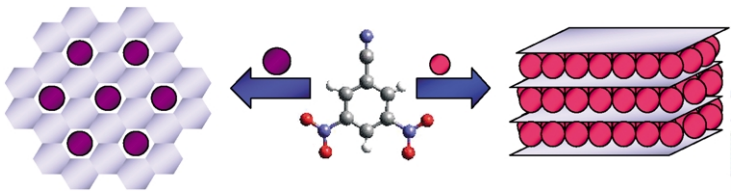


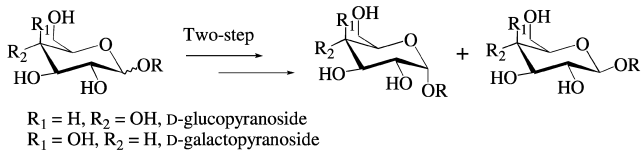


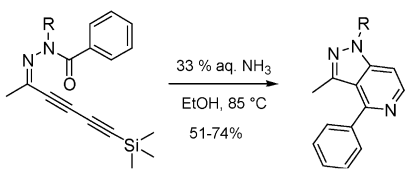


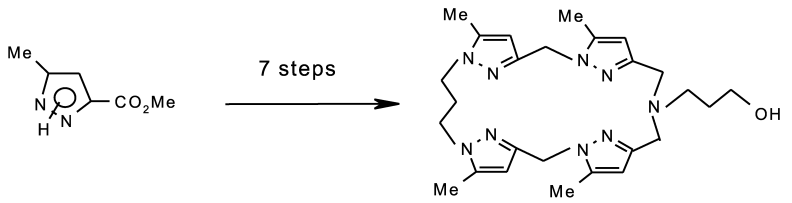
β-Tosylethylhydrazine, prepared from *p*-tolyl vinyl sulfone and hydrazine hydrate, reacts with both 1,3-diketones and conjugated ynones to provide N-tosylethyl-protected (TSE) pyrazoles in good yields. The TSE group can be removed from the pyrazoles using potassium *t*-butoxide in THF.



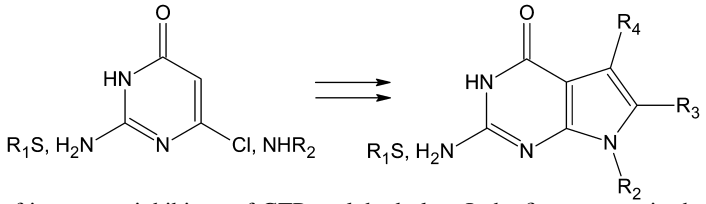




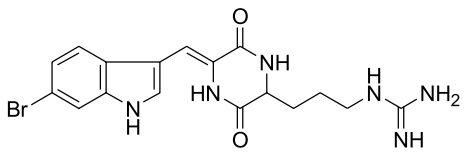


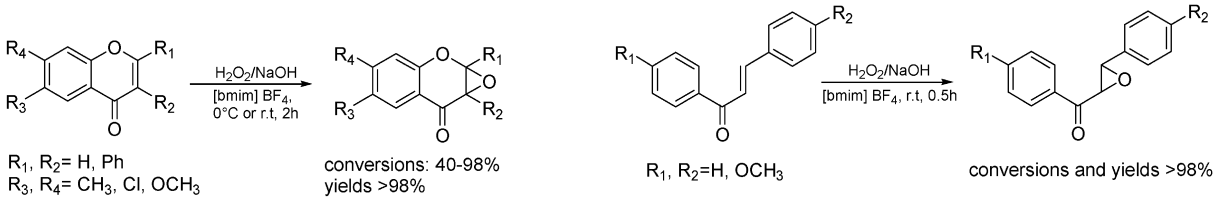


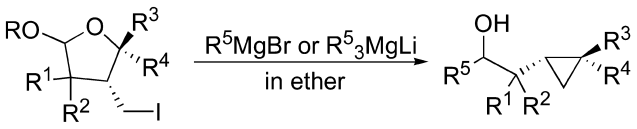
Li^+, Na^+, K^+, Cs^+, Ca^{2+}, Cd^{2+}, Pb^{2+} and Hg^{2+}

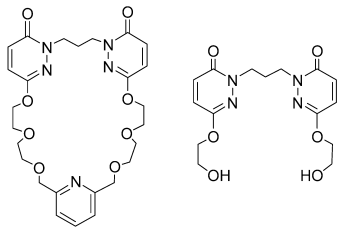


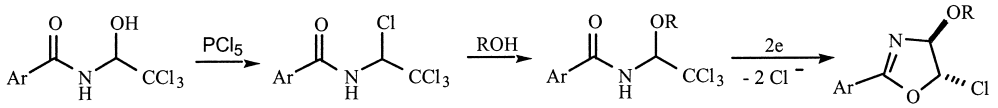
Substituted 7-deazaguanines are of interest as inhibitors of GTP cyclohydrolase I, the first enzyme in the biosynthetic pathway leading to dihydrofolate and tetrahydrobiopterin. Methods are described for the synthesis of 7-deazaguanines substituted at positions 2, 6 and 9 (purine numbering) such that a wide diversity of compounds can be prepared. Several compounds show inhibitory activity with respect to GTP cyclohydrolase I, and some show evidence for chemical transformation by that enzyme.

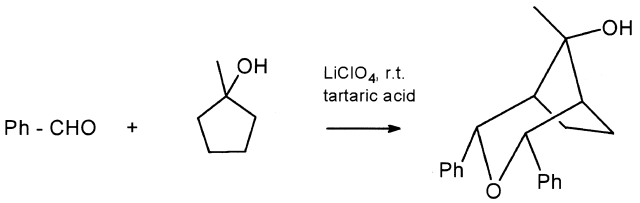




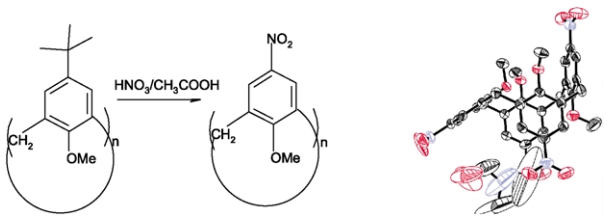








An example of unusual and mild alkylation of aldehydes by $LiClO_4$-activation in the presence of acids.



OTHER CONTENTS

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Recent advances in the application of supercritical fluids for carbon–carbon bond formation in organic synthesis

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Contents

1. Introduction

Reactions under supercritical conditions have been used for large-scale industrial production for most of the twentieth century, but the application of supercritical fluids (SCFs) in the synthesis of complex organic molecules is only just emerging. Research in this field has been particularly active in the last decade of this century, because the special properties of SCFs make them attractive solvents for modern synthetic chemistry. The idea of using supercritical fluids as reaction solvents has, however, been emerging ever since the discovery of a 'peculiar state of matter' early in the nineteenth century by Baron Charles Cagniard de LaTour, an experimental physicist[1] in France. Supercritical fluids may be alternatives to liquid solvents, but they are neither simple nor simply replacements of solvents. The experimental chemist could not modify a written synthetic method by simply crossing out the word 'benzene' and replacing it with the words 'supercritical carbon dioxide'. Many other modifications to the procedure would be necessary, because of the inferior solvent strength and need for the pressurized equipment for many SCFs.

Supercritical fluids may be defined as the state of a compound, mixture or element above its critical pressure (P_c) and critical temperature (T_c), but below the pressure required to condense it into a solid. They occupy a point where pure and applied science meet head on. This is a feature that has attracted many workers to the field. The importance of SCFs and their applications have been summarised quite effectively by Garrabos et al.,[2] who also

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Nomenclature

acac	acetylacetonate
ADMET	acyclic diene metathesis
AIBN	2,2′-azobis(isobutyryl nitrile)
BINOL	*R*(+)-1,1′-naphthalene-2,2′-diol
BINAP	2,2′-bis(diphenylphosphino)-1,1′-binaphthyl
BINAPHOS	2-(diphenylphosphino)-1,1′-binaphthen-2′-yl-1,1′-binaphthen-2,2′-diylphosphite
cod	1,5-cyclooctadiene
CNT	carbon nanotubes
DELOXAN	polysiloxane-based solid acids
DABCO	1,4-diazabicyclo[2.2.2]octane
dppb	1,4-diphenylphosphinobutane
DME	dimethyl ether
de	diastereomeric excess
DBU	diazabicyclo[5.4.0]undecene
H–H	head-to-head dimer
H–T	head-to-tail dimer
hfacac	1,1,1,5,5,5-hexafluoroacetylacetonate
LAB	linear alkylbenzenes
MWCNT	multiwalled carbon nanotube
PTC	Phase-transfer catalysis
ROMP	ring-opening metathesis polymerisation
RCM	ring-closing metathesis
SCF	supercritical fluid
SCW	supercritical water
TPPTSS	triphenylphosphine trisulphonate sodium salt
TON	turnover number
THAB	tetraheptyl ammoniumbromide
THAC	tetraheptyl ammoniumchloride

describe a series of interesting applications, as well as outlining the sometimes-overlooked effects of gravity on fluids near their critical point. The properties of SCFs are different from those of ordinary liquids and gases and are tunable simply by changing the pressure and temperature. In particular, the density and viscosity change drastically at conditions close to the critical point. It is well known that the density-dependent properties of an SCF solvent (e.g. solubility, diffusivity, viscosity and heat capacity) can be manipulated with relatively small changes in temperature and pressure. In catalysis applications, the resulting effects of these tunable solvents feature in a variety of ways, such as enhancing component[3] and catalyst[4] solubilities, influencing Kinetic rates through both temperature and pressure effects, as well as shifting equilibrium constants to favour the desired products,[5] increasing selectivity and yields (e.g. by manipulating the solvent dielectric constant[6] or viscosity,[7]) reducing mass transfer limitations in diffusion-limited reactions,[8] controlling the temperature in highly-exothermic reactions through adjustment of the solvent heat capacity[9a] and minimising heterogeneous catalyst deactivation through the prevention of coking and extraction of fouling products.[9b]

The most popular supercritical fluid, carbon dioxide, has the added benefit of being a natural, unregulated solvent, with low toxicity and high availability.[10] When carbon dioxide is used as the supercritical solvent, additional advantages can be realised. The chemical industry has become increasingly aware of environmental concerns over the use of volatile organic solvents and chlorofluorocarbons in the manufacture and processing of commercial polymer products. The use of water alleviates these problems somewhat, but it still results in large amounts of hazardous aqueous waste that require treatment. Green chemistry is much more than simply replacing hazardous materials (solvents, reagents) with less hazardous substances and can be defined as elegant chemistry on the basis of three factors,[11] environmental friendliness, chemical efficiency (selective), and economic viability. As a result of these environmental concerns, supercritical CO_2 represents a more environmentally friendly alternative to the traditional solvents. Although supercritical CO_2 has been touted as a modern remedy for many commercial problems, the use of CO_2 as a solvent is complicated by the low solubility of many reactants, even under supercritical conditions.[12] Many industrial applications are therefore hindered by this obstacle, as well as by the fact that high-pressure equipment can be quite costly. Despite these difficulties, the attraction of combining natural catalysts with natural solvents has been the driving force behind a growing body of literature concerning the stability, activity and specificity of enzymes in supercritical carbon dioxide.[13–15]

The trend towards using supercritical fluids in chemical practice[16] intensified only at the beginning of 1980s, and their use as reaction media is becoming an alternative for the reactions in which the previously described options are not suitable. The projected advantages of the reactions in supercritical fluids are the increased reaction rates and selectivities resulting from the high solubility of the reactant gases, rapid diffusion of solvents, weakening of the solvation around the reacting species and the local clustering of reactants or solvents.[17] It is also interesting to note, in a practical sense, that these fluids are easily recycled and allow the separation of dissolved compounds by a gradual release of pressure. Sequential and selective precipitations of the catalyst and product would be possible. Several recent reports have shown that $scCO_2$ can replace the conventional organic solvents in various transformations, such as radical reactions,[18] Diels–Alder reactions,[19] polymerisations,[20] homogeneous hydrocarboxylations[21] and asymmetric hydrogenations.[22] Broadly, the authors have reviewed those papers, published in recent years, which concern some aspects of carbon–carbon bond formation reactions in organic synthesis and which are considered to be of the greatest value to the synthetic organic chemist.

The most intriguing aspect of organic synthesis of paramount concern is that of the carbon–carbon bond-formation reaction.[23] The efficient generation of a carbon–carbon bond forms the backbone and is the essence of synthetic organic chemistry, organometallic reactions, metal-catalysed reactions and cycloaddition reactions leading to the formation of new carbon–carbon bonds figuring prominently in both synthetic and mechanistic organic chemistry.[24] Moreover, the development of new and more selective reagents in carbon–carbon bond-forming reactions has accelerated exponentially and still constitutes one of the most vigorous areas of organic synthesis. Two major

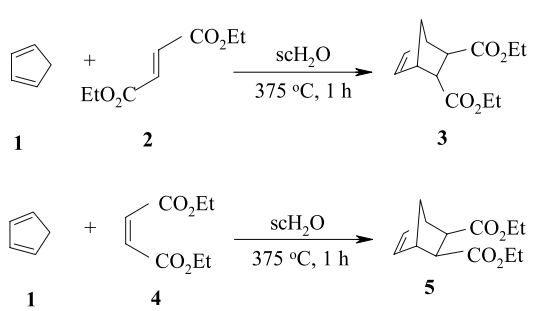
types of reagents are the carbon nucleophiles and carbon electrophiles and these are widely used to form carbon–carbon bonds in organic synthesis. The C-nucleophile group includes numerous organometallic reagents, carbanions, enolates and their precursors. The familiar alkylating, acylating and cyclopropanating reagents, together with Michael acceptors and other electron-deficient olefins, comprise a fundamental group of C-electrophiles.

The efficient formation of carbon–carbon bonds with good and, preferably, predictable stereocontrol is still a synthetic challenge in organic chemistry. On this issue during the last decade, a unique reactivity and remarkable selectivity has been exhibited on removing organic solvents in carbon–carbon bond-forming reactions, which is an important drive towards the development of environmentally benign chemical technologies. In addition, organic solvents are high on the lists of toxic or otherwise damaging compounds, because of the large volumes used in industry and the difficulties in containing volatile compounds. Replacement reaction media include ionic liquids[25,26] supercritical fluids,[27] water[28–30] and solvent-free conditions.[26,31,32] Due to the broad applicability and the vast diversity of C–C bond formation reactions in organic synthesis and, for the sake of simplicity, this review has strived to include the relevant information on the current status of the developments in the application of SCFs in C–C bond-forming reactions.

2. Carbon–carbon bond formation reactions in supercritical fluids

2.1. Diels–Alder reactions

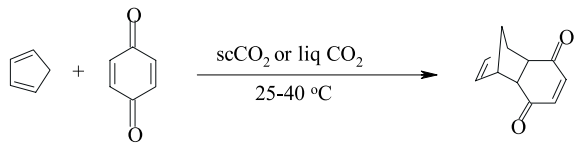
The Diels–Alder reaction is the most widely-used synthetic method for the synthesis of polycyclic ring compounds. Ikushima et al.[33] examined the Diels–Alder reaction in $scCO_2$ and found specific changes in the isomer distribution and in the rate of reaction near the critical point.[34] In the early 1980s, Breslow et al.[35] and Grieco et al.[36] reported that the rates of Diels–Alder reactions were greatly improved by using water instead of conventional organic solvents as the reaction media. Kolis et al.[37] have reported the possibility of performing Diels–Alder reactions in superheated and scH_2O due to the unique properties[38] of scH_2O. The reactions tested were the cycloadditions of cyclopentadiene **1** with diethyl furmarate **2** and diethyl maleate **4** using scH_2O as the solvent. They obtained yields of 10 and 86% for **3** and **5**, respectively, after 1 h. Although the yield of the *endo*/*exo*-2,3-diethyl ester of 5-norbornene **3** was low, equal



Scheme 1.

amounts of both isomers of **5** were formed in good yield from the *cis* diene (Scheme 1).

Renslo et al.[39] have examined the reaction selectivity in some Diels–Alder reactions in $scCO_2$ and conventional solvents. They showed that the product distribution in $scCO_2$ at pressures of 49–118 bar and at temperatures of 50 and 150 °C was very similar to that obtained in conventional solvents such as toluene. This is different from the previous observations under similar conditions[33] and Renslo et al. pointed out the importance of phase behaviour when sampling CO_2 reaction mixtures for results. Isaacs and Keating[40] carried out the Diels–Alder reaction between *p*-benzoquinone **6** and cyclopentandiene **1** in CO_2 at 25–40 °C, to form **7**. It was shown that the reaction effectively occurred throughout the liquid and supercritical ranges with no discontinuity and that the rates of the reaction were about 20% greater than those obtained in diethyl ether (Scheme 2).

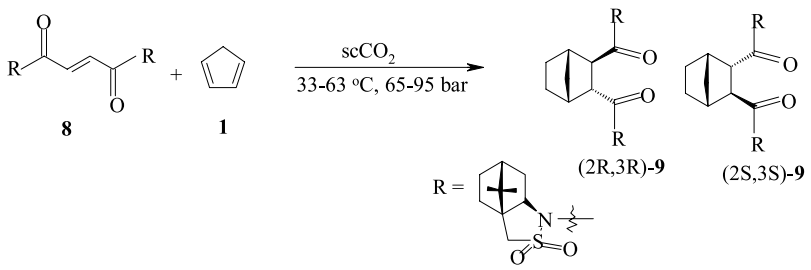


Scheme 2.

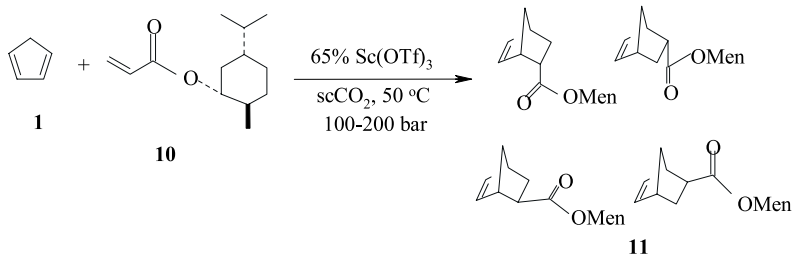
Weinstein et al.[41] studied the Diels–Alder reaction of cyclopentadiene **1** and ethyl acrylate in CO_2 from 38 to 88 °C and from 80 to 210 bar. The rate of the reaction was shown to increase with pressure (or density) for the whole range examined at a constant temperature. Paulaites and Alexander reported the earliest findings on the Diels–Alder reaction in supercritical media.[42] The first Diels–Alder reaction in $scCO_2$ controlled by a chiral auxilary[43] was reported by Chapuis et al. As part of a study into the solvent effects on stereoselectivity, the reaction between cyclopentadiene **1** and the dienophile **8** to produce **9** was performed in $scCO_2$. In conventional solvents, the *de* generally increased with polarity (58% *de* in CCl_4, 92% *de* in water), although several anomalies were observed such as in diethyl ether (87% *de*) and hexane (70% *de*). In $scCO_2$, the best selectivity was observed around the critical point (65% conversion, 93% *de* at 33 °C, 74 bar), although a similar result was obtained at a slightly higher temperature and pressure (100% converison, 92% *de* at 43 °C, 78 bar) (Scheme 3).

The Lewis acid-catalysed distereoselective Diels–Alder reaction between (−)-menthyl acrylate **10** and cyclopentadiene **1** was also investigated using scandium triflate as the catalyst.[44] The poor rate and selectivities in the uncatalysed reaction can be improved by using a Lewis acid i.e. scandium triflate, which was investigated by Oakes et al. in 1999. A moderate diastereocontrol was observed, which was once again optimised by tuning the pressure of the $scCO_2$ solvent. At a pressure of 155 bar and a temperature of 50 °C an *endo* to *exo* ratio of 9.25:1 of **11** was achieved, with a diastereomeric ratio of 3.6:1 (Scheme 4).

In 1998, Clifford et al.[45] investigated reaction controlled and

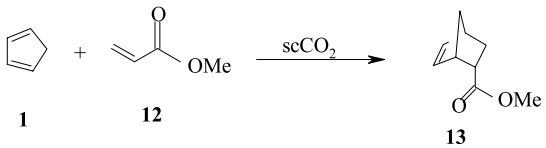


Scheme 3.



Scheme 4.

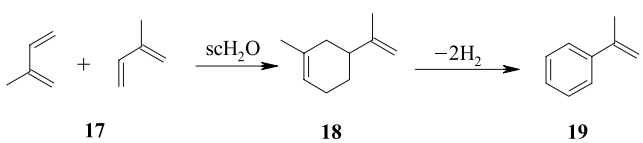
potential tuning in the Diels–Alder reaction between cyclopentadiene **1** and methyl acrylate **12** in $scCO_2$ to give **13** (Scheme 5). The theoretical explanation is based on a tuning function. The calculation using the tuning function suggested that the maximum selectivity could occur at a density of 0.540 g ml^{-1}, significantly above the critical density of 0.465 g ml^{-1} (Scheme 5).



Scheme 5.

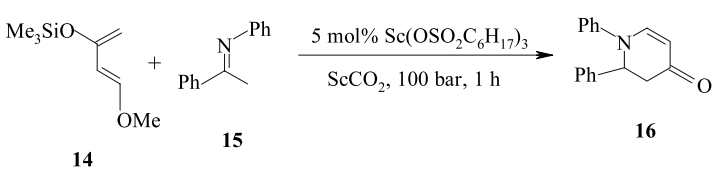
In a recent finding, Kobayashi has reported the use of scandium perfluoro-alkanesulfonates as Lewis acid catalysts for the Diels–Alder reaction in $scCO_2$.[46] It was disclosed that the catalyst activity was improved by increasing the length of the perfluoroalkyl chain and, hence, its solubility. This catalyst was also used in the aza-Diels–Alder reaction of Danishefsky's diene **14** with the imine **15** in $scCO_2$ to obtain the corresponding aza-Diels–Alder adduct **1b** in 99% yield (Scheme 6).

There are various other reports[49] on the selectivity of the Diels–Alder reaction in supercritical fluids. The reaction of isoprene **17** in supercritical H_2O was conducted in batch mode, in the temperature range of 300–410 °C at 25 MPa with an initial concentration of 8 wt%. The residence time was 1 h. The main products were Diels–Alder adducts such as dipentene **18** and some terpenes **19**. No hydration products could be detected[50] (Scheme 7).



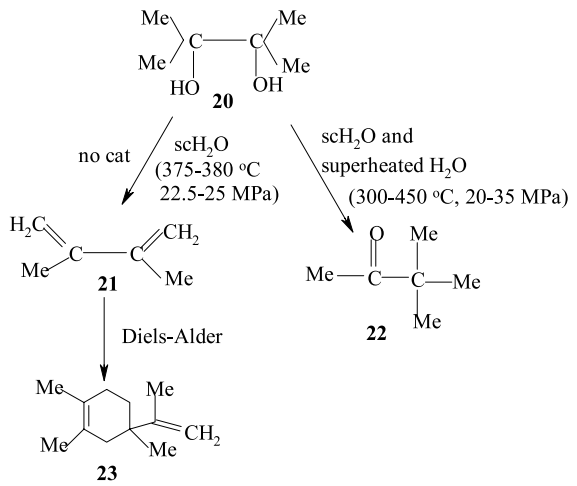
Scheme 7.

Supercritical water could be used as an acid catalyst for dehydration and other reactions if its own 'acidity' could be changed. Pinacolone **22** was the sole product under scH_2O and superheated water reaction conditions. In the very limited near-critical region of 375–380 °C at 22.5–25 MPa, however, the formation of 1,2,4-trimethyl-4-isopro-penyl-cyclohexene **23**, rather than pinacolone **22**, from pinacol **20** was observed for the first time.[51] The conversion of **20** to **23**



Scheme 6.

The silica-catalyzed Diels–Alder reaction in $scCO_2$ was carried out by Danheiser et al.[47] Here, the silica was found to significantly enhance the rate and selectivity of the reaction. Roberts investigated[48] the effect of pressure on the bimolecular rate constant of the Diels–Alder reaction between maleic anhydride and isoprene in $scCO_2$ at 35 °C. was 50–70%. Here, pinacol was completely dehydrated into 2,3-dimethyl-1,3-butadiene **21** and the intermolecular Diels–Alder reaction of **21** then results in the formation of **23**. Diels–Alder reactions have already been confirmed to occur in supercritical water in the absence of acid catalysts[52] (Scheme 8).



Scheme 8.

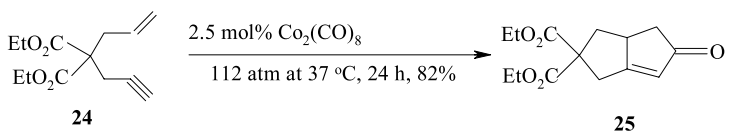
2.2. Catalytic Pauson–Khand reactions

The cocyclisation of alkynes with alkenes and carbon monoxide by cobalt, leading to cyclopentenones (known as the Pauson–Khand reaction), has been accepted as one of the most powerful tools in the synthesis of cyclopentenones. Recent developments in the Pauson–Khand reaction include the discovery of promoters, such as silica gel, tertiary amine *N*-oxides and DMSO for the stoichiometric reaction, enantioselective reactions and catalytic versions of the reaction.[53] Jeong et al.[54] have reported the first catalytic intramolecular Pauson–Khand reaction in supercritical fluids. The catalytic intramolecular Pauson–Khand reactions e.g. **24** to **25** were first performed in $scCO_2$ with dicobalt octacarbonyl as the catalyst and enynes, followed by correct pressurisation with carbon monoxide and carbon dioxide. The reaction mixture requires a higher carbon monoxide pressure (15–30 atm) to make the catalytic metal species as intact as possible (Scheme 9).

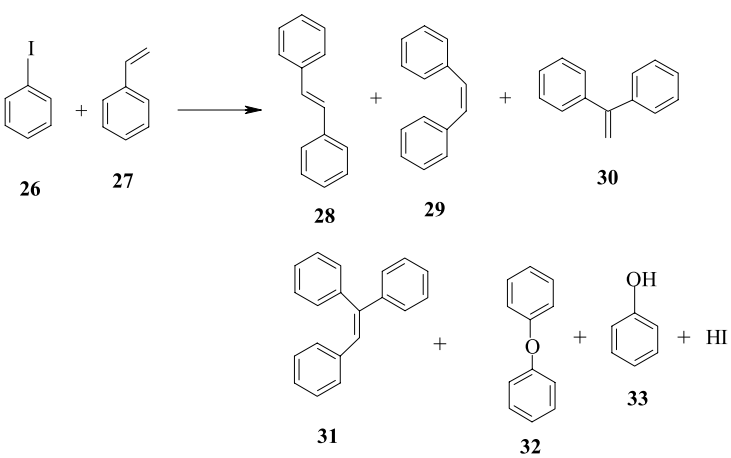
2.3. Inter- and intramolecular Heck reactions

The Heck reaction is an extremely valuable method for carbon–carbon bond formation and is now widely used in the fine chemical and pharmaceutical industries.[55] The Heck arylation of alkenes has been carried out in hot compressed water (533 K) and in scH_2O (673 K) in the presence of Pd catalysts.[56,57] Ikushima et al.[58] have recently reported the Heck coupling reaction of iodobenzene **26** with styrene **27** in supercritical water without using any catalyst, in which several alkylarenes, such as stilbene **28** and **29** and 1,1-diphenylethylene **30**, were formed, as shown in Scheme 10, besides hydrogen iodide and other products **31** to **33**. They demonstrated[59,60] a remarkable stimulation of rearrangement or disproportionation using scH_2O, which might be due to the acid and base difunctionality of scH_2O. The unusual properties of water near its critical point provide a novel method for extending the Heck reaction into water. It was shown that a high reaction rate and selectivity are possible near its critical point. The choice of base had a significant effect on the product selectivity. The best result was obtained using KOAc, which is a relatively mild base. The conversion reached 70% and the yield of stilbene was 55.6% (both *trans* and *cis* stilbene) within 10 min (Scheme 10).

Recently various approaches to the Heck reaction has been developed,[61–65] but its practical application has been restricted, due to the disadvantage of using environmentally damaging solvents and transition-metal catalysts and to problems associated with catalyst-product separation and side reactions such as decomposition. The Heck reaction using fluorinated phosphine–palladium complexes in $scCO_2$ with electron-deficient alkenes occurs in a superior yield to that reported for conventional solvents, i.e. 90% conversion and 90% selectivity for the coupling of PhI **26** with acrylic acid and styrene.[66] Similar results were obtained in the Heck coupling by Holmes[67] using isolated

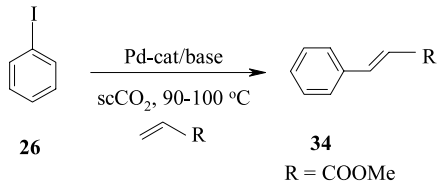


Scheme 9.



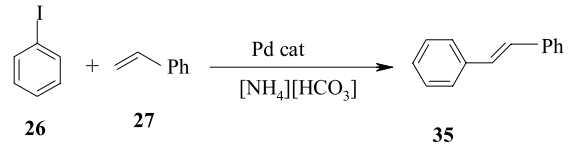
Scheme 10.

complexes of the formula $[PdL_2X_2]$, where L=$PhP[(CH_2)_2(CF_2)_6F]_2$ and X=Cl or OAc. At 100 °C, a 91% isolated yield of methyl cinnamate **34** from PhI **26** and methyl acrylate acid was achieved using 5 mol% of the acetate complex (Scheme 11).



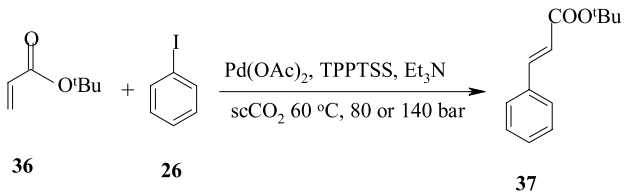
Scheme 11.

The Heck reaction has also been studied in metal-catalysed organic synthesis in SCFs other than CO_2. Supercritical and, especially, superheated water have been found to be suitable solvent media for this reaction for the synthesis of **35** using various palladium complexes without phosphine ligands[68] (Scheme 12).



Scheme 12.

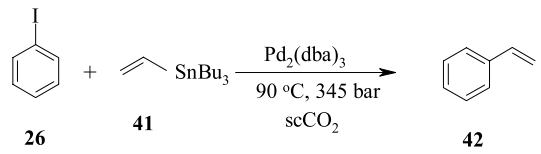
Heck reactions in $scCO_2$ using fluorinated phosphine ligands[69] or trifluoroacetate counterions,[70] or non-fluorinated phosphines[71,72] and solid-supported reactions have been reported. The application of supported reagents[73] in $scCO_2$ has received little attention.[74] Cacchi has successfully used Pd/C as a heterogeneous catalyst to facilitate the Heck reaction in $scCO_2$, although these conditions required extended reaction times to release reasonable yields[75] Arai has reported a Heck reaction using water-soluble catalysts in $scCO_2$–water biphasic systems.[76] The coupling of iodobenzene **26** with butyl acrylate **36** in $scCO_2$ to form **37** was investigated[77] using $Pd(OAc)_2$ and triphenylphosphine trisulphonate sodium salt (TPPTSS) as water-soluble ligands (Scheme 13). In the absence of a co-solvent, the catalyst remains insoluble and the yields are low (<5% at 80 bar), but the addition of a polar co-solvent such as water increases the rate. The use of a more CO_2-philic co-solvent such as ethylene glycol offered a further enhancement (Scheme 13).



Scheme 13.

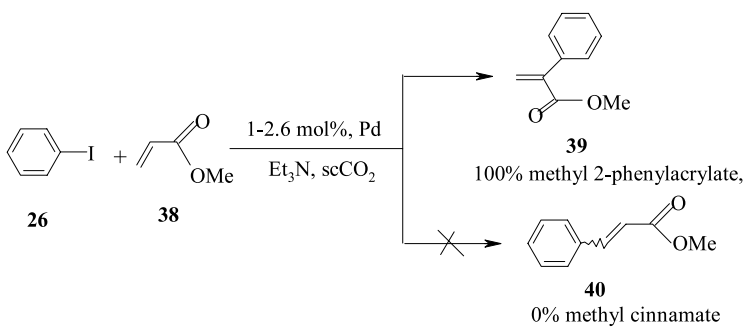
In a recent report,[78] dendrimer-encapsulated nanoparticles were shown to be versatile catalysts for both the Heck heterocoupling of iodobenzene with methyl acrylate and the hydrogenation of styrene in supercritical CO_2. Iodobenzene **26** can be coupled with methyl acrylate **38**, which is a benchmark reaction for the Heck coupling,[79] to yield exclusively methyl 2-phenylacrylate **39** (Scheme 14). The selectivity of **39** is remarkable when compared with standard palladium complexes or colloidal nanoparticles[80,81] used for Heck couplings in organic solvents, which result in only the *cis* and/or *trans* cinnamate **40**. The high selectivity is due in part to the steric environment the dendrimer template imposes on the reaction intermediates.

Tumas et al.[69] have investigated palladium-catalysed carbon–carbon bond coupling reactions, namely the Heck and Stille reactions in $scCO_2$. The reaction between iodobenzene **26** and vinyl(tributyl)tin **41** was carried out with a number of ligands using $Pd(dba)_3$ as the source of palladium. The nature of the ligand was found to significantly affect the yield of **42**. Using Ph_3P, a 49% conversion was achieved, which was only slightly better than that observed with no ligand present (38%). Tris(2-furyl)phosphine, however, showed good activity (86% conversion) (Scheme 15).

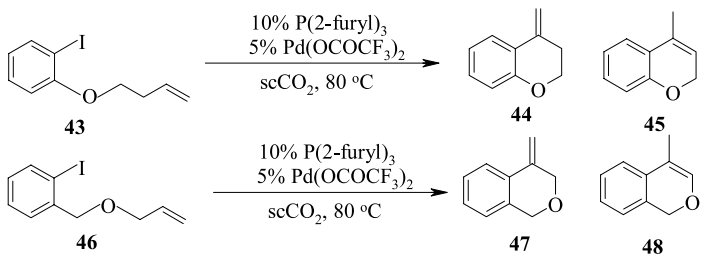


Scheme 15.

The intramolecular Heck cyclisation reaction in supercritical CO_2 has also been studied.[82] The intramolecular cyclisation of **43** and **46** in acetonitrile as the solvent gave complete conversion, but the isomerised exocyclic products **45** and **48** accounted for 76 and 80% of the yields, respectively. It has, however, been shown that, by carrying out the reaction in $scCO_2$, not only is a complete conversion



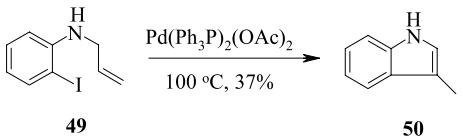
Scheme 14.



Scheme 16.

achieved, but suppression of the double bond isomerisation reaction is also observed. The desired exocyclic products **44** and **47** account for 83 and 93% of the yield, respectively (Scheme 16).

The intramolecular Heck reaction of **49** has been investigated[67] by Holmes et al. using fluorinated phosphine ligand complexes in supercritical carbon dioxide and the corresponding substituted indole **50** was obtained in superior yield to that reported for conventional solvents (Scheme 17).



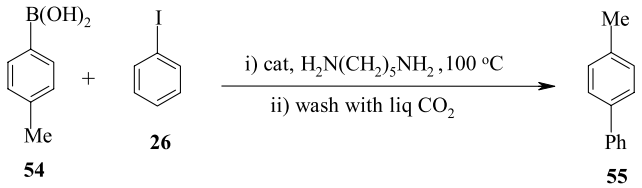
Scheme 17.

2.4. C–C bond-forming Baylis–Hillman reactions

The Baylis–Hillman reaction[83] is considered to be one of the most facile coupling protocols between activated alkenes **12** and aldehydes **51**, allowing the introduction of a hydroxyalkyl moiety at the α-position of Michael acceptors **52**. This reaction can be efficiently carried[84] out in $scCO_2$ with enhanced reaction rates relative to the comparable solution-phase reactions. At low pressure, an unprecedented dimerisation and formation of **53** is observed, which has led to the development of a novel one-pot three-component coupling reaction to form highly-functionalised ethers derived from Baylis–Hillman products (Scheme 18).

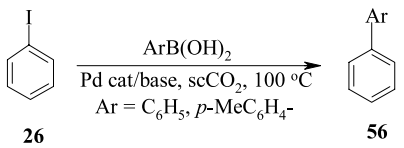
2.5. Suzuki coupling reactions

Suzuki coupling reactions[85] have been investigated in the supercritical phase in recent years.[63] Treatment of *p*-tolylboronic acid **54** with iodobenzene **26** and the base *N*,*N*,*N*,*N*-tetramethylhexanediamine in the presence of the polymer-supported phosphine–palladium catalyst in $scCO_2$ afforded the biaryl product **55**, which was isolated by venting the liquid CO_2 isolation into a beaker containing EtOAc. Washing the remaining resin and the amine salt with liquid CO_2 afforded the product in 64% yield (Scheme 19).



Scheme 19.

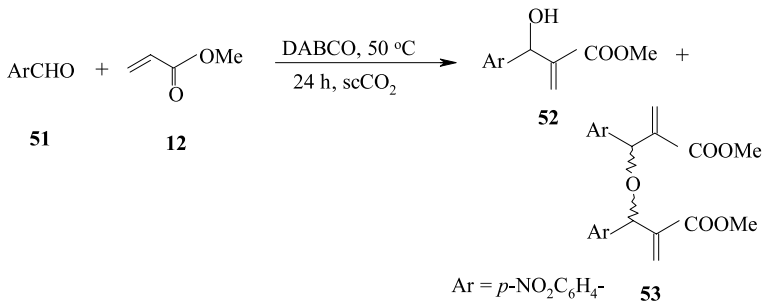
The Suzuki coupling with arylboronic acids[86] using phosphine ligands which play a crucial role in stabilising the active intermediate in $scCO_2$ has been studied. The reaction is believed to proceed via a Pd^0 intermediate, generated in situ from Pd^o or Pd^{II} precursors (Scheme 20). The fluorinated phosphine–palladium complex-mediated coupling of boronic acids with aryl or vinyl halides to yield biaryl **56**, the Suzuki reaction, has certain advantages for the coupling of two sp2 centres. This too can be conducted in $scCO_2$ in yields that are comparable to those achieved in conventional solvents.[67]



Scheme 20.

2.6. Alkylation reactions

Poliakoff and his co-workers introduced the supercritical phase to the Friedel–Crafts alkylation reactions by using $scCO_2$ or by making propene, one of the reactants, the



Scheme 18.

supercritical fluid.[87] The effect of the supercritical fluid operation on catalyst deactivation has been studied by Gao et al.[88,89] using the alkylation of benzene with ethylene on a Y-type zeolite as an example. Li Fan et al.[90] have investigated the effect of the SCF on the alkylation reaction on Y-type zeolites. Two types of alkylation reactions were studied, isopentane (T_c=188 °C, P_c=33 MPa) with isobutene and isobutane (T_c=135 °C, P_c=3.6 MPa) with isobutene. The paraffins acted as both reactant and supercritical fluid. The supercritical-phase reaction exhibited a higher catalytic activity, along with a remarkably longer lifetime, compared to the reaction in the liquid or gas phase. Recently, Clark and Subramaniam reported the 1-butene/isobutane alkylation in $scCO_2$ with USY zeolite as the catalyst. The utilisation of $scCO_2$ was considered mainly to lower the reaction temperature, as the higher reaction temperatures in other supercritical phase systems could have increased the cracking and coking reactions.[91] They showed that, using a molar excess of a low T_c diluent such as $scCO_2$, the alkylation can be performed at supercritical conditions at temperatures lower than the critical temperature of isobutane (<135 °C), resulting in a virtually steady alkylate (trimethylpentanes and dimethylhexanes) production for experimental durations of nearly 2 days.

Hitzler et al.[87] investigated the continuous Friedel–Crafts alkylation of mesitylene [$C_6H_3(Me)_3$], and anisole (C_6H_5OMe) with propene or propan-2-ol in supercritical carbon dioxide using polysiloxane-supported solid (DELOXAN) acid as catalyst in a small fixed-bed reactor (10 ml volume). Mesitylene **57** was alkylated in sc-propene (T_c=91.9 °C, P_c=46.0 bar) and the corresponding mono alkylated species **58** was obtained as the major product (25%) and the di and tri alkylated product **59** and **60** as the minor products. This work clearly demonstrates the feasibility of continuous and sustainable Friedel–Crafts alkylation in SCF solution, although no comparison was made with continuous alkylation in a conventional solvent using the same catalyst (Scheme 21). Suzuki et al.[92] have also performed Friedel–Crafts reactions, alkylations and etherifications at 350 °C, 152 bar and residence times of 120 min in the absence of any acid catalysts in scMeOH.

2.7. Photochemical reactions

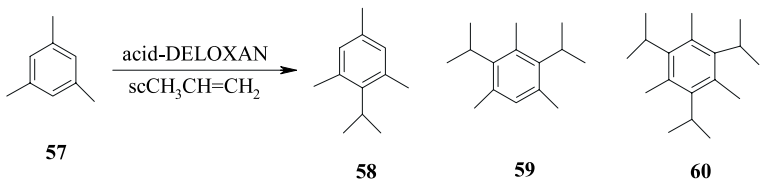
Johnston et al.[93] investigated the [2+2] photodimerisation of isophorone **61** in $scCO_2$ (38 °C) and $scCHF_3$ (34.5 °C). Three dimers were produced: a head-to-head dimer ($H-H_{anti}$), and two diasteromeric head-to-tail dimers ($H-T_{anti}$ and $H-T_{syn}$). In conventional solvents, Chapman found that more polar solvents favour the production of the more polar product.[94] Analogous results were obtained in SCF solvents, the more polar product ($H-H_{anti}$) being a major product in the more polar solvent and only a minor product[93] in CO_2 (in which the $H-H:H-T_{total}$ ratio was essentially 0.10, independent of pressure). These observations are explicable on the basis that, over the range of pressures examined, the dielectric constant varies more for CHF_3 (from 2.5 to 8.4) than for CO_2 (from 1.34 to 1.54) (Scheme 22).

Weedon et al. have examined the photo-Fries rearrangement[95] of naphthyl acetate **62** in $scCO_2$ at 35 and 46 °C. Photolysis of **62** leads to a caged pair [**63/64**] and the reaction in the cage yields the photo-Fries products, 2- or 4-acetylnaphthol (**65** or **66**). A cage escape, however, followed by hydrogen abstraction (isopropanol was present as a hydrogen-atom donor) leads to α-naphthol **67** (Scheme 23).

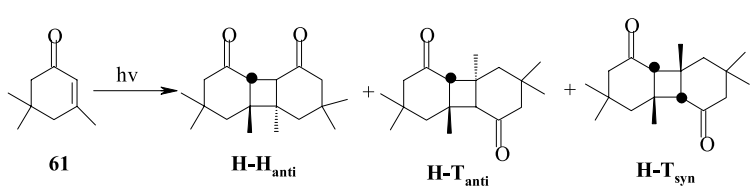
Photochemical carbonylation of the C–H bonds of liquid propane was achieved by Sakakura et al.[96] with the use of $RhCl(CO)(PMe_3)_2$ as catalyst and 3 atm of CO at 15 °C. Excellent selectivity for linear butanal (97% selectivity, 20 TON after 484 h) was obtained with only traces of 2-methylpropanal and acetaldehyde being observed.

2.8. Cyclopropanation reactions

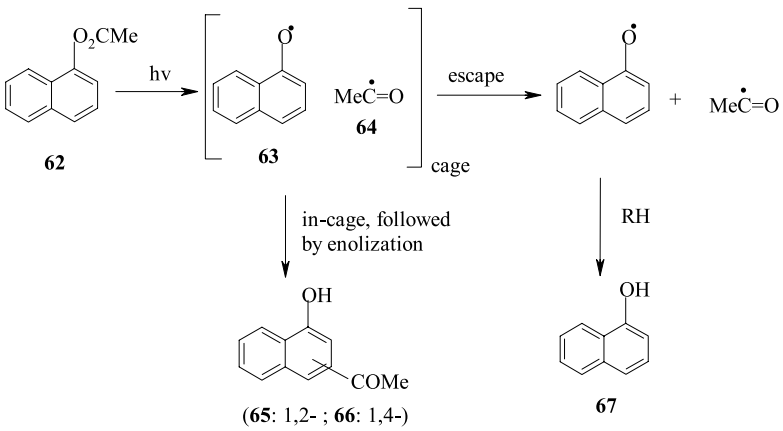
Supercritical fluoroform ($scCHF_3$) is yet another SCF that provides highly interesting opportunities for metal-catalysed C–C bond-formation reactions. Rhodium-catalysed asymmetric cyclopropanation was investigated, as it exhibits a marked selectivity dependence on solvent polarity in the liquid state[97,98] The cyclopropanation of styrene **27** with methyl phenyldiazoacetate **68** catalysed by



Scheme 21.

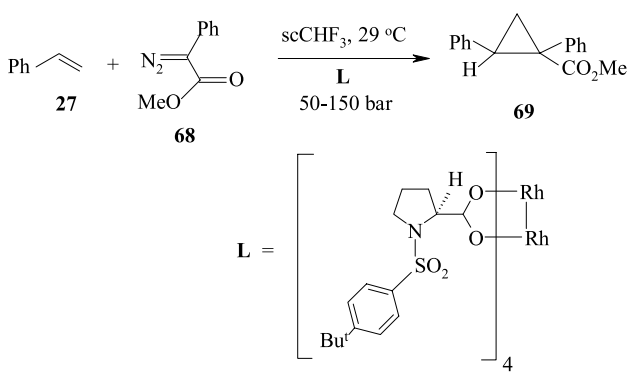


Scheme 22.



Scheme 23.

the dimeric rhodium(II) carboxylate complex L proceeds, to form **69** with a higher enantioselectivity in non-polar than in polar liquid solvents. Indeed, a strong dependence of the enantioselectivity on pressure was observed when the same reaction was performed in $scCHF_3$ at various pressures[99] (Scheme 24).



Scheme 24.

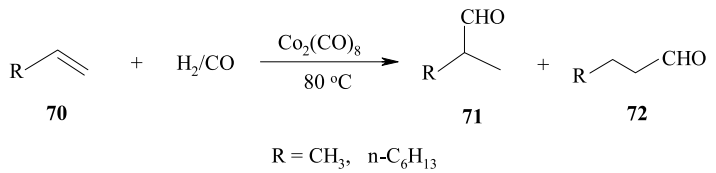
2.9. Hydroformylation reactions

The addition of CO and H_2 to a C=C double bond to yield aldehydes or, with subsequent reduction, alcohols is referred to as hydroformylation This reaction is one of the most important processes catalysed by homogenous organometallic catalysts on an industrial scale.[100] The hydroformylation catalysts are classified according to the metal used, with cobalt and rhodium-based catalysts being by far the most successful systems. The catalytic cycle proposed by Heck and Breslow[101] consists of a number of elementary steps. Depending on the catalyst and other factors, the rate-limiting step can be the reaction with H_2[102] and a rate increase could therefore be observed for some hydroformylation systems in SCFs.

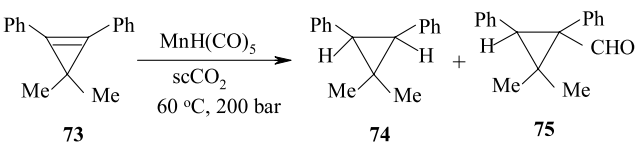
The first hydroformylation reaction of propylene in $scCO_2$ catalysed by dicobalt octacarbonyl was reported by Rathke et al.[103] The propylene **70** hydroformylation proceeded smoothly at 80 °C at $P_{H2}=P_{co}=56$ atm with $Co_2(CO)_8$ (10 mol%), hydrogen (42 bar) and carbon monoxide (42 bar), giving *n*-butyraldehyde **72** (88%). The selectivity for the desired linear aldehyde, butanal (88%), is higher than the value (83%) measured in benzene at slightly higher pressures (*Pco*=80 atm). The linear-to-branched ratio is slightly influenced by the pressure and temperature.[104] When the temperature is constant at 88 °C, the linear product selectivity increases from 73 to 81% as the pressure doubles (Scheme 25).

Noyori and his co-workers[105] reported the stoichiometric reaction of the olefin **73** with $MnH(CO)_5$ in $scCO_2$,which gave a similar selectivity for the hydroformylation product **75**, over the hydrogenation product **74**, to that found in alkane solvents. This suggested that the aldehyde **75** was primarily formed by non-radical pathways, which were independent of the solvent viscosity (Scheme 26).

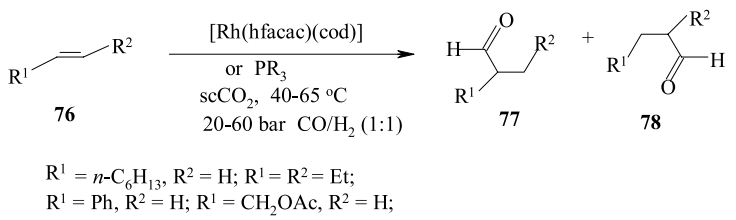
As in conventional solvents, rhodium-based systems are generally much more active than cobalt catalysts in $scCO_2$ (Scheme 27). Various alkenes **76** have been hydroformylated in $scCO_2$ to yield **77** and **78** with [Rh(hfacac)(cod)] (cod=1.5-cyclooctadiene) as the catalyst precursor without additional ligands, at substrate/Rh ratios as high as 2600:1.[106] The reaction rate was found to be considerably higher in $scCO_2$ than in liquid organic solvents, this effect



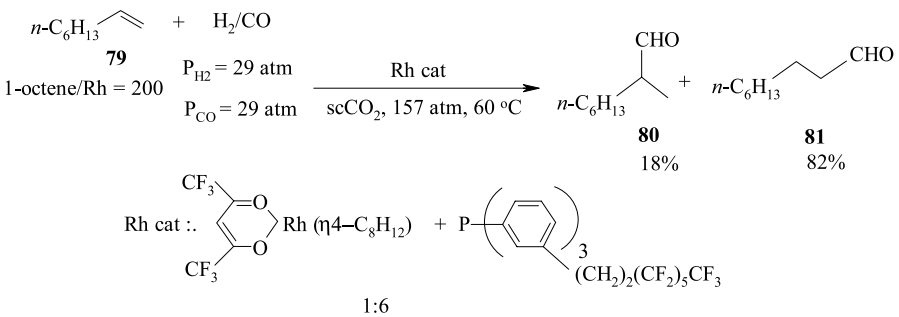
Scheme 25.



Scheme 26.



Scheme 27.



Scheme 28.

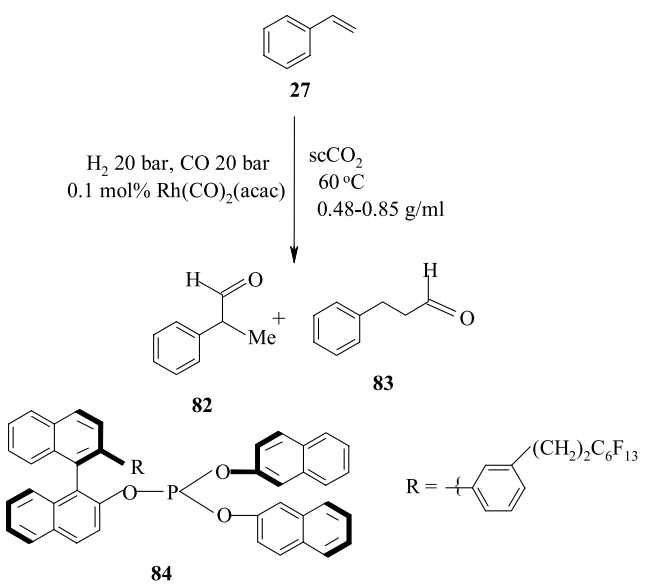
being the most pronounced for internal alkenes such as *trans*-3-hexene.

Leitner et al.[107] investigated a CO_2-soluble Rh complex with a polyfluoroalkyl substituted triarylphosphine ligand (Rh/phosphine 1:6) in $scCO_2$, which effected the hydroformylation of 1-octene **79** to give the linear aldehyde **81** in good yield and with 82% selectivity (Scheme 28). The reaction proceeds smoothly in the homogeneous supercritical phase without any side reactions such as hydrogenation or isomerisation (to **80**) of the olefin.

The introduction of fluorinated side-chains on the aromatic rings of the phosphine ligands results in an increase in the solubilities of the complexes in $scCO_2$, quantified by UV/vis spectroscopy of the Rh-hexafluoroacetylacetonate complexes with these ligands.[107] Under supercritical conditions at 45 °C and 91 atm, these complexes give bright yellow solutions with saturation concentrations of 6.3×10^{-5} and 7.5×10^{-5} mol l^{-1}, respectively.

Recently, a rhodium-catalysed hydroformylation reaction in $scCO_2$ using trialkylphosphines as simple alternatives to fluorinated arylphosphines, in order to achieve solubility, was investigated.[108] The catalyst prepared in situ from $Rh_2(OAc)_4$ (0.74 mol%) and PEt_3 (4 mol%) gave complete conversion of hex-1-ene within 2 h at 100 °C. The equivalent reaction in toluene solution showed an equivalent rate, but a poorer product ratio of 2.1:1.

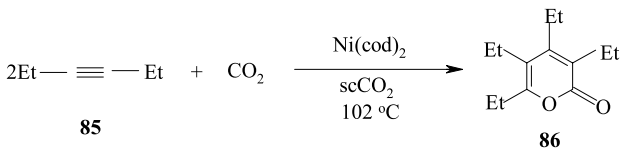
Asymmetric hydroformylation in $scCO_2$ also provides viable routes to important anti-inflammatory drugs starting from simple vinylarenes. Rhodium catalysts bearing the chiral phosphine/phosphite ligand (*R*,*S*)-BINAPHOS allow very high levels of enantiocontrol.[109] Investigations with a catalyst made up from 1,5-cod and (*R*,*S*)-BINAPHOS revealed that the ligand-bound rhodium species have insufficient solubility in the supercritical phase. Even a moderate asymmetric induction could only be obtained at low CO_2 densities, when an additional liquid phase was present at some stage of the reaction.[110] The ligand solubility problem was addressed by the addition of fluorinated chains, leading to the development of the fluorinated BINAPHOS ligand **84**. Thus the asymmetric hydroformylation of styrene **27**, using (*R*,*S*)-BINAPHOS **84** as the catalyst, favoured the asymmetric hydroformylation product **82** over its achiral regioisomer **83** with an appreciable asymmetric induction 68% ee. The use of this ligand with $Rh(CO)_2(acac)$, leads to increased levels of enantioselectivity and greater regioselectivity both in a conventional benzene solvent and in $scCO_2$ with quantitative conversions (17 h, 60 °C, 0.1 mol% cat, 0.2 mol% **84**).[111] (Scheme 29).



Scheme 29.

2.10. Coupling reactions in $scCO_2$

Reetz et al.[112] investigated the formation of tetraethyl 2-pyrone **86** from 3-hexyne **85** and CO_2 using a catalyst generated in situ from $Ni(cod)_2$ and the chelating diphosphine 1,4-diphenylphosphinobutane (dppb)[$Ph_2P(CH_2)_4PPh_2$] in $scCO_2$. The selectivity and TON of 7, although only moderate, were similar to those reported in conventional solvents. Although the phase behaviour of the low-density reaction mixture and the solubility of the metal complex were not mentioned, these results demonstrated that Ni catalysts allow catalytic C–C coupling reactions with CO_2 under conditions beyond T_c and P_c Changing the catalyst from dppb to trimethylphosphine as the ligand increased the activity, allowed lower temperatures (51 °C) to be used and gave a higher TON of 18. The catalyst did, however, have a shorter lifetime in $scCO_2$ compared with conventional solvents[113] (Scheme 30).

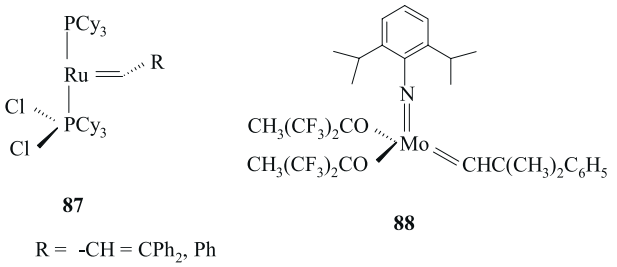


Scheme 30.

2.11. Olefin metathesis in $scCO_2$

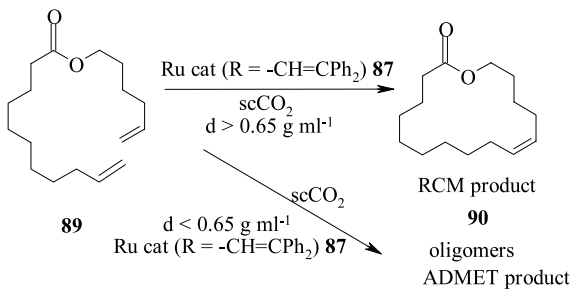
Recent literature reveals that supercritical fluids are useful reaction media for the metathesis of olefins.[114–116] DeSimone found that $[Ru(H_2O)_6](OTs)_2$ (Ts=*p*-toluenesulfonyl) catalysed the ROMP (ring-opening metathesis polymerisation) of norbornene at 65 °C in $scCO_2$ (67–296 atm). The product, an off-white spongy textured polymer, was isolated by venting the CO_2.[117] Leitner et al.[118] have reported transition metal-catalysed olefin metathesis reactions in compressed CO_2 media. Using the conventional metathesis catalysts **87** and **88** (Scheme 31) ROMP of norbornene and cyclooctene gave the corresponding polymer in excellent yields, both in liquid CO_2 and in $scCO_2$.

Ring-closing metathesis (RCM) was also investigated[118,119] using the same carbene complexes and some remarkable observations were made. It was found that the RCM of **89** was extremely sensitive to density, with the 16-membered ring **90** being formed in excellent yield at densities >0.65 g ml^{-1} whereas mainly oligomers (70%, with 10%



Scheme 31.

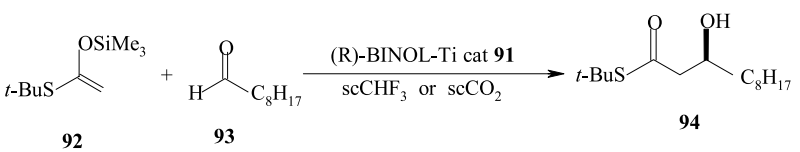
89) were produced at low densities (ADMET product) (Scheme 32). The density effect on the reaction pathway is not fully understood, but may be caused by the compressibility of the supercritical phase. Furstner et al. speculate that increasing the density at constant volume leads to a high dilution reaction condition, favouring the intramolecular reaction pathway.[119] A number of other cyclisations were performed in good yield. An interesting observation was that the catalyst **87** (R=–CH=CPh_2) (Scheme 31), which is normally deactivated in the presence of basic N–H groups, was active under such conditions in CO_2 solution.



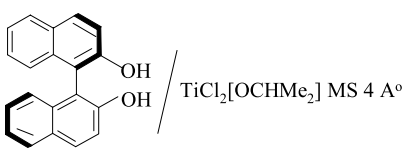
Scheme 32.

2.12. Asymmetric Mukaiyama aldol reactions in $scCHF_3$

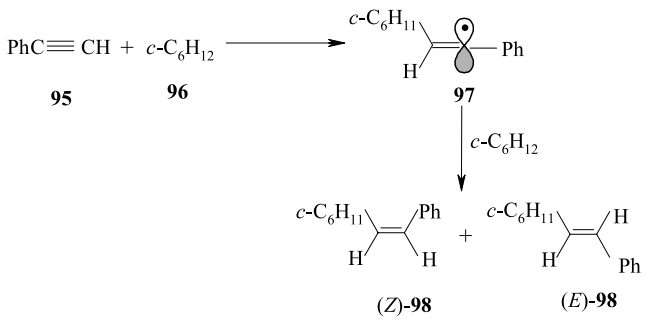
Asymmetric Lewis acid-catalysed carbon–carbon bond formation in Mukaiyama aldol reactions mediated by a β-naphthol-derived chiral titanium(IV) complex **91** proceeds smoothly in a supercritical fluid[120] such as fluoroform ($scCHF_3$). The chemical yield and enantioselectivity of the reaction in SCFs are found to be tuned by changing the supercritical fluids, ($scCHF_3$ versus $scCO_2$) and adjusting the matched polarities by varying the pressure of the CHF_3. The reaction in SCFs containing the chiral



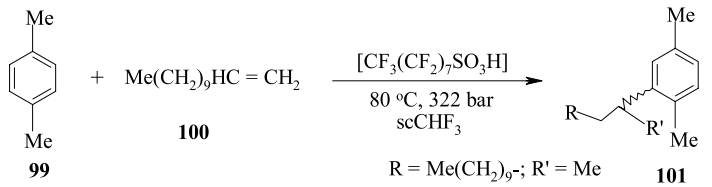
(R)-BINOL-Ti cat **91**:



Scheme 33.



Scheme 34.



Scheme 35.

Table 1. Dodecene alkylation of *p*-xylene with $CF_3(CF_2)_7SO_3H$ catalyst

Solvent	Conv. to LAB (%)	Isomerisation products[a] (%)
None[b]	0	<1
CO_2[c]	1	68
CHF_3[c]	90	7–9

[a] Value shown represents conversion of the non-alkylated α-olefin to an internal olefin mixture with 85% being close to thermodynamic equilibrium.
[b] 100 °C, 1 bar.
[c] 80 °C, 322 bar.

catalyst[121] and a ketene silyl acetal of the thioester **92** and an aldehyde **93** (1:2:3=1:20–40:20 molar ratio) proceeds smoothly to give a trimethylsilyl ether of the aldol product **94** in moderate yields (Scheme 33). The outcome of the reaction was found to be influenced by tuning the SCFs ($scCO_2$: T_c=31.0 °C, P_c=72.8 atm).

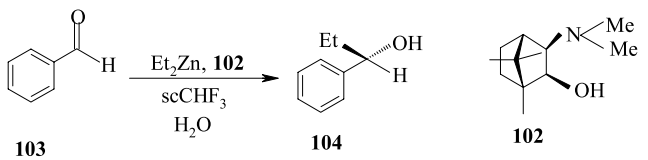
2.13. Addition of supercritical cyclohexane to phenylethyne

Metzger et al.[122] examined the addition of cyclohexane **96** to phenylethyne **95** in the temperature range from 20 to 340 °C in supercritical cyclohexane (ratio 1000:1). The addition proceeds via a 2-cyclohexyl-1-phenylethenyl radical **97** to provide 1-cyclohexyl-2-phenylethene **98**, as shown in Scheme 34. The radical chain is initiated by a bimolecular reaction of cyclohexane with phenylethylene to give a cyclohexyl radical and a 1-phenylethenyl radical. No effect on the reaction rate constant near the critical point was observed.

2.14. Formation of alkylbenzenes

Hutchenson and his co-workers[123] demonstrated the formation of linear alkylbenzenes (LAB) using a perfluorosulfonic acid catalyst in supercritical fluid reaction media. An enhanced alkylation activity was observed in fluoroforms (CHF_3) compared to carbon dioxide. The results indicates that $scCO_2$ solubilises the long-chain perfluorosulphonic acid $CF_3(CF_2)_7SO_3H$ which initiates the catalytic activity of these molecules. The poor conversion to the LAB product suggests, however, that the availability of the acid sites is still limited under these conditions despite the apparent solubility of the catalyst. The authors found that the dodec-1-ene **100** alkylation of *p*-xylene **99** using SCF fluoroform as the solvent proceeds in high yield to form **101**. The isomerisation of the α-olefin is primarily observed using $scCO_2$, with only a small yield of the alkylated product. This demonstrates tuning of the reaction pathway depending upon the solvent characteristics within the SCF media. This example illustrates the use of an SCF solvent to define the reaction chemistry in a catalysis application (Scheme 35 and Table 1).

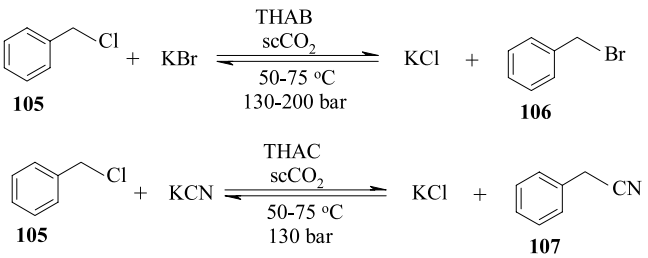
The asymmetric alkylation of benzaldehyde **103** catalysed by **102** in supercritical fluoroform (Scheme 36) to form the alcohol **104** was studied by Jessop et al.[99,124] Preliminary investigations of the reactions of benzaldehyde **103** with diethylzinc in $scCHF_3$ showed that the enantioselectivity was pressure dependent.



Scheme 36.

2.15. Phase-transfer catalysis

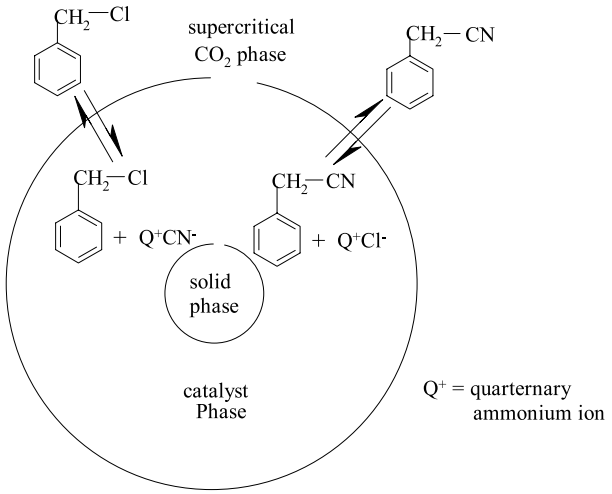
Phase-transfer catalysis (PTC) has also been used for carrying out reactions in supercritical media. The first PTC reaction in SCF was the displacement reaction of benzyl chloride **105** with potassium bromide in supercritical carbon dioxide[125,126] with 5 mol% acetone, in the presence of tetraheptylammonium bromide (THAB), to yield benzyl bromide **106** (Scheme 37). Chandler et al.[127] investigated the reaction between benzyl chloride **105** and potassium



Scheme 37.

cyanide in $scCO_2$ in the presence of tetraheptylammonium chloride (THAC) to yield benzyl cyanide **107**.

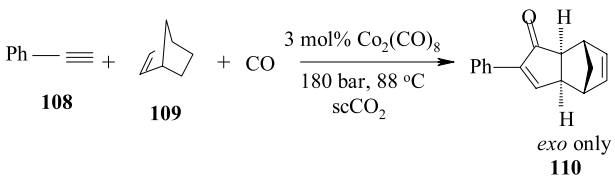
Scheme 38 depicts the three-phase system and the concentrated catalyst phase where the reaction is believed to occur. In the presence of acetone as a cosolvent, the reaction rate decreased, perhaps due to the increased solubility of the catalyst in the SCF and the detection of catalyst in the ω-phase. Although it is customary to add co-solvents to SCFs to increase the solubilities, in this case the increased solubility appears to be detrimental to the reaction rate.



Scheme 38.

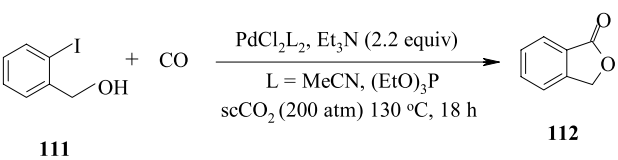
2.16. Miscellaneous reactions

An intermolecular reaction was also carried out in $scCO_2$ where[54] phenylacetylene **108** can couple with excess norbonadiene **109** catalysed by dicobaltoctacarbonyl and a CO pressure of 15 bar, to give the bicyclic compound **110** in 87% (Scheme 39).



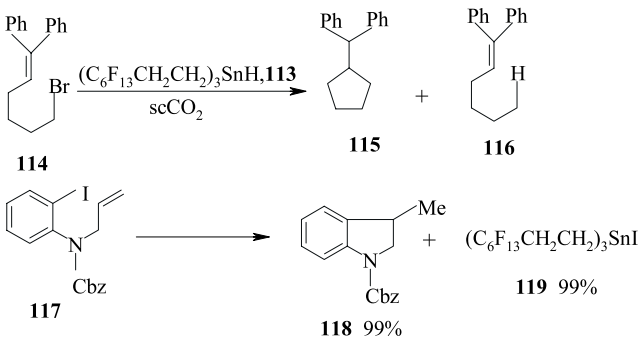
Scheme 39.

Ikaria et al. have reported[128] an efficient carbonylation of aryl halides catalysed by CO_2-soluble palladium complexes with trialkyl or triaryl phosphite ligands in $scCO_2$. The intramolecular carbonylation of 2-iodobenzyl alcohol **111** catalysed by $PdCl_2(MeCN)_2$ in $scCO_2$ proceeded efficiently to give the phthalide **112** with a TON of 1880 after 18 h (Scheme 40). Changing the ligand to the more soluble triethylphosphite gave an increase in rate, showing that the reaction is faster in $scCO_2$ than in conventional organic solvents.



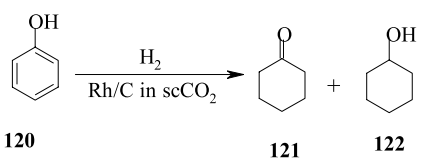
Scheme 40.

An intramolecular cyclisation[129] via the reduction of 1,1-diphenyl-6-bromo-1-hexene **114** under supercritical CO_2 conditions with the fluorous tin hydride **113** provided the 5-*exo* cyclised product **115** in 87% isolated yield, along with 7% of the reduced product **116**. Interestingly, the reduction of **114** with liquid benzotrifluoride (1 atm) provided only the cyclised product **115**, which was isolated in 75% yield. Significantly, the reduction of **114** with tributyltin hydride produced neither **115** nor **116** but recovered the starting materials, along with some tin formate. In addition, reduction of the aryl iodide **117** with the fluorous tin hydride **113** provided **118** in 99% yield, along with 99% of the tin iodide **119** (Scheme 41).

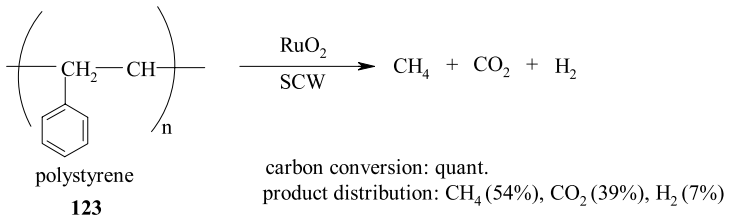


Scheme 41.

A very recent study by Shirai et al.[130] found that a charcoal-supported rhodium catalyst was highly active for the ring hydrogenation of phenol and cresols under supercritical carbon dioxide. Commercially available catalysts were used in this work viz 5 wt% carbon-supported palladium (5% Pd/C), rhodium (5% Rh/C), platinum (5% Pt/C), and ruthenium (5% Ru/C). During hydrogenation of phenol, it was found that both the hydrogenation activity and selectivity to cyclohexanol increased with increasing hydrogen pressure at 10 Mpa carbon dioxide. Phenol hydrogenation is a successive reaction in which phenol **120** is first hydrogenated to cyclohexanone **121** followed by hydrogenation of the latter to cyclohexanol **122** (Scheme 42). Cyclohexanol was, however observed at low phenol conversion under high hydrogen pressure, indicating that it would be formed not only via the cyclohexanone intermediate, but also directly from phenol. The hydrogenation activity also increased with increasing carbon dioxide pressure. Bhange et al. have observed higher conversions with increasing CO_2 pressure in the case of cinnamaldehyde under supercritical carbon dioxide.[131]



Scheme 42.



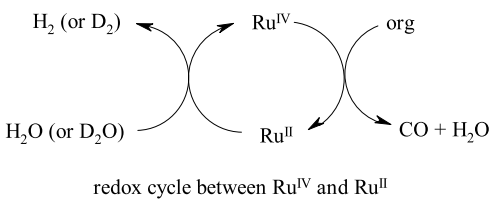
Scheme 43.

Table 2. Summary of the results on the gasification reaction of organic compounds by RuO_2 in SCW

Organic compounds (org)	Molar ratio [org]/[RuO_2]	C-conv. (%)	Product distribution (%)		
			CH_4	CO_2	H_2
Naphthalene	5.12	96.7	48.8	42.7	8.4
Carbazole	3.94	87.9	52.7	40.6	6.7
Diphenyl ether	3.87	99.9	45.8	48.8	5.4
Dibenzofuran	3.92	101.7	51.0	43.6	5.5
Polyethylene	23.5	100.6	66.6	28.0	5.3
Polypropylene	15.7	99.9	66.5	26.9	6.5
Polystyrene	6.32	100.7	53.7	39.4	6.9
Poly(ethylene terephthalate)	3.44	97.2	37.3	51.0	11.5
Cellulose	4.07	97.0	34.2	50.9	14.6

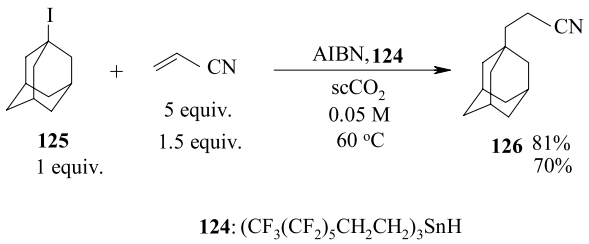
In a recent breakthrough by Tomiyasu et. al,[132] complete gasification of organic compounds by ruthenium(IV) oxide (RuO_2) in SCW has been achieved, where aromatic compounds, as well as other organic compounds including plastics, are converted into CH_4 and CO_2, accompanied by the production of H_2. The stoichiometry of the conversions strongly suggests that the hydrogen source of the fuel products is water and that the catalytic effect of RuO_2 results from a redox couple of Ru^{IV}/Ru^{II} induced by SCW. In fact, direct evidence supporting this has been obtained by a gasification experiment of polystyrene **123** in a RuO_2-supercritical deuterium oxide system. (Scheme 43 and Table 2).

Organic molecules are partially oxidised by RuO_2 to form CO and H_2O, where Ru^{IV} is reduced to the lower oxidation sate of Ru^{II}. In order to oxidise an excess number of organic molecules, Ru^{II} must be re-oxidised to Ru^{IV}, which is carried out with the reduction of SCW to H_2. The Ru^{IV} regenerated is reduced again to Ru^{II} for further partial oxidation of organic molecules. The CO produced is converted into CH_4 and CO_2 through [mCO+nH$_2$] reactions with H_2 derived from SCW. A redox cycle between Ru^{IV} and Ru^{II} is induced by SCW, in which the gasification reaction of organic compounds proceeds catalytically (Scheme 44).



Scheme 44.

Curran et al. have reported[129] radical reactions in supercritical carbon dioxide. The standard Giese reaction shown in Scheme 45 was conducted by the addition of iodoadamantane **125** to acrylonitrile (5 equiv), which provided the radical adduct **126** in 81% yield after flash chromatography when carried out in the presence of tris(2-perfluorohexyl)ethyl)tin hydride **124** in $scCO_2$. Additionally formed in this experiment was an acetone-soluble material, polyacrylonitrile.[133] When 1.5 equiv. of acrylonitrile was used, the compound **126** was isolated in 70% yield and the formation of this acetone-soluble material was not observed (Scheme 45).



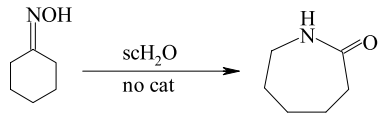
Scheme 45.

In a very recent paper, Wai et al.[134] reported a rapid, direct and green procedure to decorate multiwalled CNTs (MWCNTs) with catalytic palladium nanoparticles by a simple hydrogen reduction of a Pd(II)-β-diketone precursor using $scCO_2$ as the medium. The resulting Pd nanoparticle-MWCNT composite is an effective catalyst for the selective hydrogenation of olefins in CO_2 and for the electrochemical reduction of O_2. The catalytic capability of the Pd-MWCNT composite was tested for hydrogenation of a CO_2-soluble olefin *trans*-stilbene in liquid CO_2. The conversion of this stilbene **127** to 1,2-diphenylethane **128** was about 80 and 96% after 5 and 10 min. respectively, and indicates that the Pd-MWCNT composite exhibits a high catalytic activity for the hydrogenation of olefins in CO_2. This simple and green nanoparticle deposition technique is not limited to Pd and may be used to prepare a variety of metal nanoparticles on MWCNT surfaces for catalytic applications (Scheme 46).

127 128

Scheme 46.

A high-pressure and high-temperature FTIR method was used to study the non-catalytic Beckmann rearrangement using supercritical water.[135] A significant acceleration of the Beckmann rearrangement was achieved by using scH_2O, especially near the critical point, and even in the absence of any acid catalysts. It has been demonstrated that scH_2O acts effectively in place of the conventional acid catalysis for the rearrangement. In the case of the Beckmann rearrangement of cyclohexanone-oxime **129** into ε-caprolactam **130**, in the IR spectrum near the critical point a new CO stretching band assigned to ε-caprolactam appeared, whereas no bands for cyclohexanone-oxime were observed. The rate constant for the formation of ε-caprolactam greatly increased as the temperature approached the critical temperature of water. The accelerated rates of reaction may be attributed to a large increase in the local proton concentration around the organic reactants (Scheme 47).



129 130

Scheme 47.

3. Conclusions

The various examples illustrated in this review have demonstrated the considerable potential of supercritical fluids as alternative media for carbon–carbon bond-formation reactions in organic synthesis. Although the favourable features of using supercritical CO_2 as a reaction medium for organic synthesis have been widely cited for over a decade, it is still not very clear what types of reactions to run in supercritical CO_2 and how to run them. In order to exploit the potential of $scCO_2$, the fundamental principles behind its role must be understood and to do this requires true interdisciplinary research involving academic synthetic chemists and industrial process chemists, physical chemists and chemical engineers, all of whom have an important role to play. Further studies are therefore surely needed, and the wholesale apportioning of this reaction class to supercritical CO_2 now seems like a viable possibility. In addition, the use of highly CO_2-soluble fluorous reagents and catalysts should prove to be a valuable strategy to transport other reaction classes to CO_2. Although much work has been carried out in this area, the field of SCF reaction chemistry is far less developed than the extraction of materials. Part of the reason is undoubtedly the understandable caution of reaction chemists to embark on experiments that involve high pressures, high temperatures or even both. Nevertheless, this review has shown that new chemistry is beginning to emerge and that SCFs do provide access to new compounds. Most importantly, it is clear that SCFs offer chemists increased opportunities to control reactions. In supercritical media, chemists can manipulate the phase behaviour of a mixture and they can control the concentrations of dissolved gases, alter the morphology of the products and carry out their reaction in a cleaner, greener way. At the end of many reactions, however, the problem of separating the products from spent and unspent reagents remains. The use of environmentally friendly reaction solvents such as supercritical CO_2 makes little sense if the reactions are followed by standard extractions or chromatographies with traditional organic solvents: extractions and chromatographies invariably require more solvent volumes than the reactions that precede them. It seems probable, however, that the large differences in solubility in supercritical CO_2 between fluorous and organic compounds can be transferred into practical separation procedures. In the long run, the potential of supercritical fluid applications will continue to expand, driven by research uncovering new opportunities and substantiating their known potential for controlling surface reactions and for the synthesis of new catalytic materials.

Acknowledgements

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Biographical sketch

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Novel synthetic route of aryl-aminopyrazine

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Abstract—We report a novel synthetic route of aryl-aminopyrazine through a new cyclization reaction by using a hydroxylamine. Starting from Boc-glycine and aminonitrile, the aminopyrazine ring was prepared in several steps. After trifluoromethane sulfonylation of the aminopyrazinone, the resultant triflate was subjected to Suzuki–Miyaura coupling reaction with aryl boronic acid to afford coelenteramine.

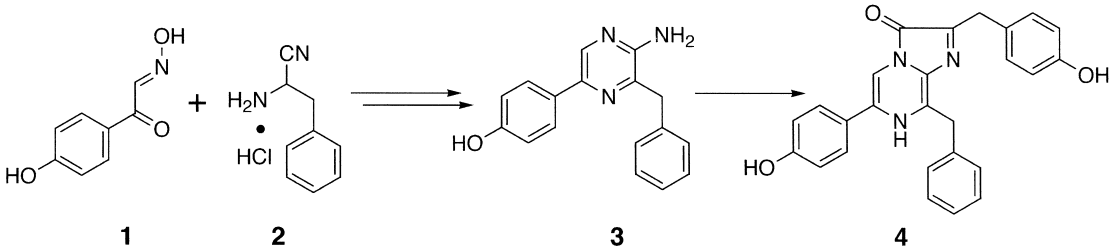
1. Introduction

Kishi and Goto established the synthetic route of coelenterazine (**4**) from coelenteramine (**3**) as a precursor, which was prepared from the condensation of keto-oxime (**1**) and aminonitrile (**2**) (Scheme 1).[1] This has been so far the only promised method for the synthesis of benzyl-aryl-aminopyrazines. Aminopyrazines are spread over many natural products, especially in luminous marine organisms like jellyfish (*Aequorea aequorea*),[2] fireflysquid (*Watasenia scintillans*),[3] and flyingsquid Tobiika (*Symplectoteuthis oualaniensis* L.),[4] etc. Their luciferin has imidazopyrazinone ring structure (**4**) that has been synthesized from aminopyrazine (Scheme 1). Since it is difficult to get many kinds of keto-oximes, it has been requested to develop alternative routes accessible to the various aryl-aminopyrazine analogs. And such methods would encourage those who study on those luminous creatures. Nakamura reported another synthetic way for coelenteramine (**3**) of great value for these reasons,[5] but their synthetic method needs 2-aminopyrazine as a starting material. They use Stille coupling reaction with tin reagents having toxicity and enforcing the tedious process in waste of the tin reagents. Therefore, we aimed at new synthetic route of aminopyrazine derivatives, starting from *N*-Boc-glycine (**8**) and aminonitrile hydrochloride (**2**), and we planned to make a 5-aminopyrazine-2-*O*-triflate (**6**) as the coupling partner for the coelenteramine synthesis. After getting 2-*O*-trifluoromethanesulfonyl-5-aminopyrazine (**6**), Suzuki–Miyaura coupling[6] reaction would enable us to synthesize various aminopyrazine analogs.

2. Results and discussion

2.1. Synthetic plan

During the research of molecular mechanism of symplectin (a photoprotein of *Symplectoteuthis oualaniensis* L.),[7] we focused on the protein structure of symplectin active site where dehydrocoelenterazine (**5**) binds.[8] Due to the limitation of the availability of variation of acetophenone



Scheme 1. Synthetic route of coelenterazine (**4**) from coelenteramine (**3**) by Kishi and Goto.[1]

Keywords: Aminopyrazine; Suzuki coupling; Hydroxylamine.
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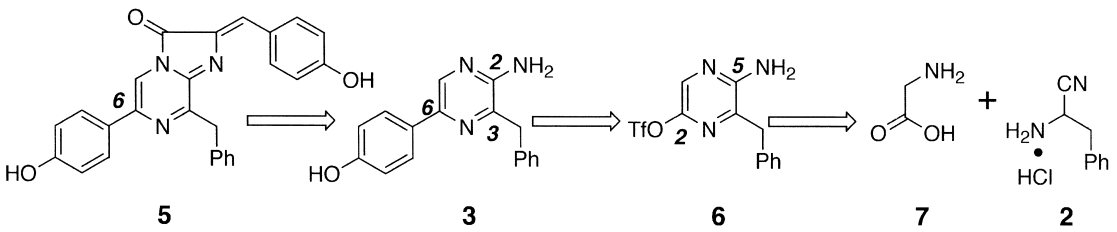
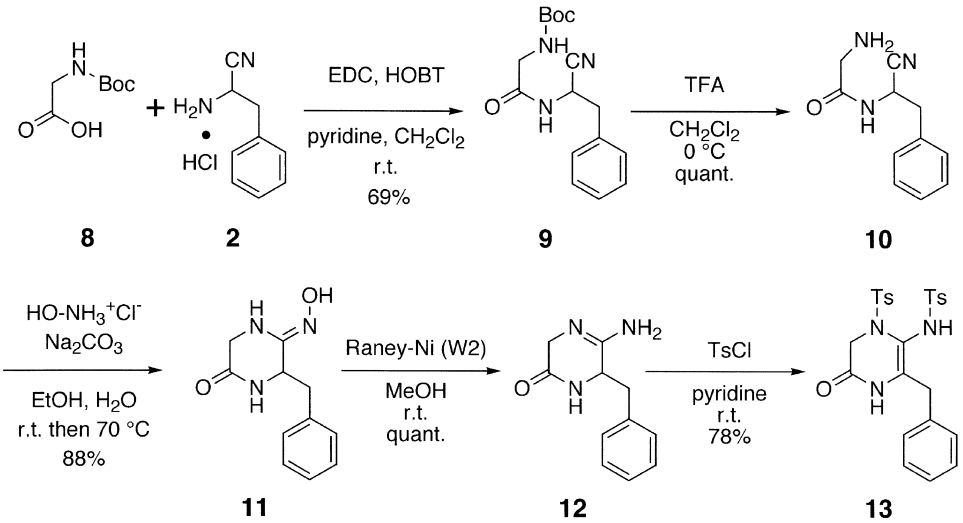
as a photoprobe, we could not modify the aromatic ring in 6-position of dehydrocoelenterazine for photoaffinity labeling experiments. Therefore, we planned to make the novel route for the synthesis of dehydrocoelenterazine having various functions at the 6-position and to devise a new synthetic route of aminopyrazine. Considering the Suzuki–Miyaura coupling as the final functionalization of aminopyrazine, we retrosynthesized dehydrocoelenterazine (**5**) as shown in Scheme 2. Since dehydrocoelenterazine (**5**) is derived from coelenteramine (**3**), we must devise a synthetic route of the triflate (**6**). We thought that the triflate (**6**) must be derived from glycine (**7**) and aminonitrile hydrochloride (**2**). Therefore, we started our synthesis from commercially available *N*-Boc-glycine (**8**) and aminonitrile hydrochloride (**2**).

2.2. Synthetic route

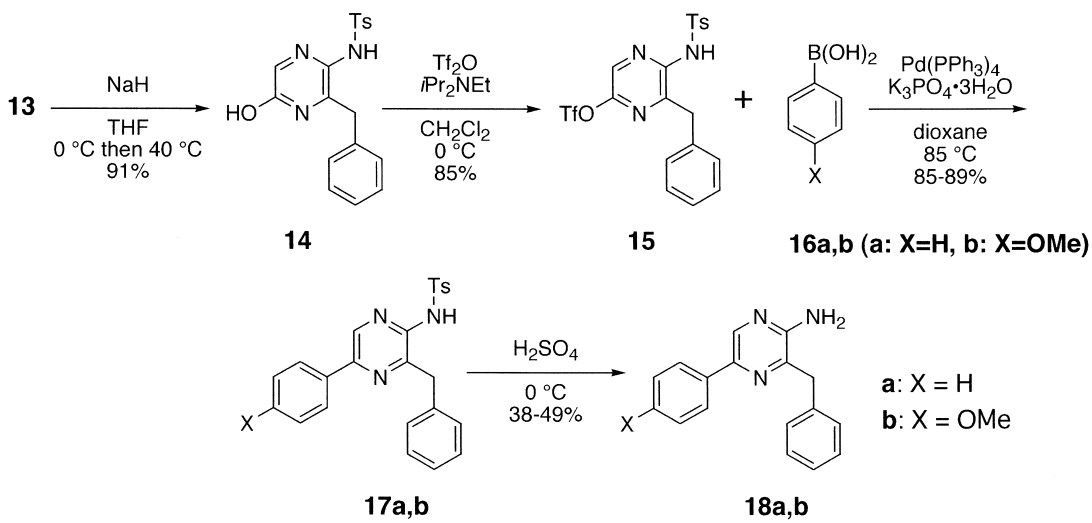
Schemes 3 and 4 show our novel synthetic route of the synthesis of triflate (**6**). Condensation of *N*-Boc-glycine (**8**) and aminonitrile hydrochloride (**2**) with EDC (1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide), HOBt



Scheme 2. Synthetic plan of dehydrocoelenterazine (**5**) from aminopyrazine triflate (**6**).



Scheme 3. Synthetic route for the formation of pyrazine core (**11**) by using the key reaction of hydroxyamine from Boc-glycine (**8**) and aminonitrile (**2**).



Scheme 4. Synthetic route for aminopyrazine (**18a**,**b**) by using triflate **15** as key intermediate.

(hydroxybenzotriazole) and pyridine in dichloromethane afforded the amide (**9**) in 69% yield.

Isolation of amide (**9**) was turned out to be much easier than the condensation by using DIC (diisopropylcarbodiimide), since the urea produced from EDC was water soluble and easily removed with acidic water. The Boc protecting group of amide (**9**) was favorably removed by treatment with trifluoroacetic acid to give amidoaminonitrile (**10**) as a white powder in quantitative yield. Unfortunately, the direct cyclization of **10** to cyclic amidine (**12**) was failed after many trials, probably due to the weak nucleophilicity of primary amine in the substrate (**10**). The subsequent cyclization of amide-aminonitrile (**10**) with hydroxylamine was the key step in this synthetic route to afford cyclic oxime (**11**). It must be noted that stirring the reaction mixture at room temperature over night should be followed at 70 °C for 3 h for obtaining cyclized product in high yield. On the other hand, stirring the reaction mixture at 70 °C for 3 h from the beginning gave the cyclic oxime (**11**) in low yield. Hydrogenolysis of the oxime (**11**) was accomplished with hydrogen under atmospheric pressure in methanol and Raney nickel (W-2) as catalyst. Due to the insolubility of the amidine (**12**) in many organic solvents, the protection of amidine (**12**) was proved to be unsuccessful. However, by using pyridine as the sole solvent, tosylation of the amidine (**12**) successfully afforded ditosylate (**13**) in 78% yield. We found the double bond migration of the ditosylate (**13**) from the amidine (**12**), and we confirmed the protected position of amine in **13** with two dimensional NMR analyses. The removal of one of the tosyl groups in **13** with sodium hydride in THF followed by recrystallization afforded aromatized hydroxypyrazine (**14**) as yellow needles in 91% yield. Hydroxypyrazine (**14**) was converted to triflate (**15**) with trifluoromethanesulfonic anhydride and *N*,*N*-diisopropylethylamine as a base in dichloromethane. This triflate (**15**) was the precursor of various aminopyrazine analogs by using Suzuki–Miyaura coupling. In this report, we selected both phenylboronic acid (**16a**) and 4-methoxyphenylboronic acid (**16b**) as coupling partners. Following the procedure established by Suzuki,[9] triflate (**15**) was coupled with boronic acids (**16a**,**b**) by using $Pd(PPh_3)_4$ and K_3PO_4 to afford *N*-tosylamidepyrazine (**17a**,**b**) in 85–89% yield. The removal of tosyl group in *N*-tosylamidepyrazine (**17a**,**b**) was accomplished by treatment with conc. H_2SO_4 at 0 °C to provide target aminopyrazine (**18a**,**b**) as a white solid in 38–49% yield. In the case of **18b**, the spectroscopic data was identical with the data of aminopyrazine that was synthesized by using Kishi and Goto route.[10] Although we purified each compound in each step for the spectroscopic analysis, we noted here that it is also possible to obtain hydroxyaminopyrazine (**14**) in 46% yield in 5 steps without any purification process starting from *N*-Boc-amide (**9**). Although 5 steps-yield was decreased probably due to the impurities of each starting material, omission of each purification process is more convenient for the preparation of 2-hydroxy-5-aminopyrazine (**14**).

3. Summary

We succeeded in developing up the novel synthetic route of aminopyrazine by using these synthetic route reported herein. This method should enable us to prepare many kinds of aminopyrazine analogs, which have potentiality many functions at the 6-position in coelenteramine through Suzuki–Miyaura coupling of triflate (**15**) with a variety of boronic acid. Furthermore, the merit of the use of Suzuki–Miyaura coupling is the convenience of boron reagents compare to the tin reagents for Stille coupling. Further progress of the current synthesis and the corresponding luminescent activity is to be published elsewhere.

4. Experimental

4.1. General

All melting points were measured on Yanaco MP-S3 and uncorrected. IR spectra were recorded on a PERKIN ELMER Paragon 1000 FT-IR spectrophotometer. Proton NMR spectra were recorded on a JEOL GSX 270 for 270 MHz, a Varian Gemini-2000 for 300 MHz, a JEOL JNML-500 for 500 MHz or a Bruker AMX-600 for 600 MHz. Chemical shift (δ) are given in parts per million relative to tetramethylsilane (δ 0.00) or CD_3OD (δ 3.30) or DMSO-d_6 (δ 2.49) as internal standard. Coupling constants (*J*) are given in Hz. Carbon NMR were recorded on a JEOL GSX 270 for 67.8 Hz, or on a Varian Gemini-2000 for 75 MHz, or on a JEOL JNML-500 for 125.7 Hz, or on a Bruker AMX-600 for 150.9 MHz. Chemical shifts are (δ) given in parts per million relative to $CDCl_3$ (δ 77.0) or CD_3OD (δ 49.0) or DMSO-d_6 (δ 45.0) as internal standard. Coupling constants (*J*) are given in Hz. Low-resolution EI mass spectra and FAB mass spectra were measured with a JEOL JMS-700 or JMS-600. High-resolution (HR) mass spectra were measured with a JEOL JMS-700. Elemental analysis and HRMS were performed by Analytical Laboratory of this school. Dichloromethane (CH_2Cl_2) was distilled from calcium hydride. Tetrahydrofuran (THF) and 1,4-dioxane were distilled from sodium metal in the presence of sodium benzophenone ketyl as indicator. Pyridine was dried over NaOH pellet and used without distillation. The other solvents were of reagent grade. Analytical thin-layer chromatography (tlc) was conducted on precoated tlc plates: silica gel 60 F-254 [E.Merck (Art 5715) Darmstadt, Germany], layer thickness 0.25 mm. Silica gel column chromatography utilized Silica Gel 60 (spherical) 40–50 μm [KANTO CHEMICAL CO., INC].

4.1.1. Boc-amidenitrile (9). To a solution of *N*-Boc-glycine (**8**) (5.00 g, 28.6 mmol) and aminonitrile hydrochloride (**2**) (5.49 g, 30.0 mmol, 1.1 equiv.) in CH_2Cl_2 (70 ml) and pyridine (20 ml) was added hydroxybenzotriazole (4.24 g, 31.4 mmol, 1.1 equiv.) and 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (6.03 g, 31.4 mmol, 1.1 equiv.) at 0 °C in an ice bath under Ar atmosphere. This mixture was stirred for 15 h at room temperature. The urea was removed by washing with 1 N HCl aq, then the reaction mixture was washed with water, saturated $NaHCO_3$ and diluted brine. Purification by column chromatography on silica gel with AcOEt/hexane (1:1) provided amide (**9**) (6.00 g, 69% yield) as a yellow curdy solid.

Compound **9**: mp 85–88 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (9H, s, *t*-Bu), 3.10–3.07 (2H, m, benzyl), 3.77 (1H, dd,

J=16.8, 5.7 Hz, C(O)CH_2NH), 3.81 (1H, dd, J=16.8, 4.8 Hz, C(O)CH_2NH), 5.11 (1H, ddd, J=15.0, 6.9, 6.9 Hz, CNCH), 5.29 (1H, brd, BocNH), 7.14 (1H, brd, amide-NH), 7.38–7.25 (5H, m, Ph) ppm. ^{13}C NMR (75 MHz) δ 28.3, 37.4, 42.6, 43.0, 78.3, 119.2, 127.4, 128.6, 129.6, 135.8, 156.0, 169.8 ppm. IR (KBr): 3335, 2957, 1679, 1533, 1208, 1127 cm^{-1}. FAB-MS (NBA) m/z 304 (MH$^+$). Anal. calcd for $C_{16}H_{21}N_3O_3$: C, 63.35; H, 6.98; N, 13.85. Found: C, 63.10; H, 7.05; N, 13.81.

4.1.2. Amidenitrile TFA salt (10). To a solution of **9** (19.5 g, 64.3 mmol) in 60 ml of CH_2Cl_2 was added trifluoroacetic acid (60 ml) at 0 °C under nitrogen. After stirring for 1.5 h at 0 °C, this mixture was quenched with ice and was evaporated to remove the solvents. Resulting white solid was TFA salt (**10**) (20.4 g, quantitative yield).

Compound **10**: mp 155 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.17–3.05 (2H, m, benzyl), 3.35 (2H, s, C(O)CH_2NH), 5.18–5.11 (1H, dd, J=6.6, 6.9 Hz, CNCH) 7.40–7.27 (5H, m, Ph) 7.85 (1H, d, J=7.8 Hz, amide-NH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 38.8, 41.0, 44.2, 118.1, 127.9, 128.9, 129.4, 134.4, 172.3 ppm. IR (KBr): 3335, 2960, 1678, 1532, 1209, 1126 cm^{-1}. EI-MS m/z 203 (M$^+$). Anal. calcd for $C_{13}H_{14}N_3O_3F_3$:C, 49.21; H, 4.45; N, 13.25. Found: C, 49.21; H, 4.63; N, 13.18.

4.1.3. Cyclic oxime (11). To a solution of HCl·NH_2OH (458 mg, 6.59 mmol, 2.0 equiv.) and Na_2CO_3 (350 mg, 3.30 mmol, 1.0 equiv.) in EtOH (5 ml) and water (5 ml) was added compound **10** (1.05 g, 3.30 mmol) at room temperature under Ar. After stirring for 16 h at room temperature, the reaction mixture was brought up to 70 °C, and then was continued to stir for 3 h. After evaporated the solvents, the obtained residue was dissolved in AcOEt–water and was extracted with AcOEt (5X). Resulted organic layer was dried over Na_2SO_4, and was evaporated to afford oxime (**11**) as a white solid (633 mg, 88% yield).

Compound **11**: mp 200–203 °C (decomposed). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.91 (2H, d, J=6.3 Hz, benzyl), 3.12 (1H, dd, J=23.4, 1.5 Hz, C (O) CH_2NH), 3.34 (1H, dd, J=23.4, 3.6 Hz, C(O)CH_2NH), 3.98 (1H, broad d, J=4.5 Hz, CNCH), 6.26 (1H, br, NH), 7.29–7.149 (5H, m, Ph), 8.07 (1H, d, J=3.6 Hz amide-NH), 9.10 (1H, d, J=1.5 Hz, OH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 41.8, 44.4, 52.2, 126.8, 128.4, 130.1, 137.1, 148.3, 169.0 ppm. IR (KBr): 3221, 1665, 1439, 1316,1087, 701 cm^{-1}. EI-MS m/z 219 (M$^+$). Anal. calcd for $C_{11}H_{13}N_3O_2$: C, 60.26; H, 5.98; N, 19.17. Found: C, 60.26; H, 5.80; N, 18.91.

4.1.4. Cyclic amidine (12). In a 300 ml round shaped flask, Raney-Ni W2 (about 2 g, ethanol wet) and MeOH (50 ml) were charged with Ar. To this suspension was added a solution of oxime (**11**) (3.50 g, 16.0 mmol) in MeOH (100 ml), then the flask was changed with hydrogen from Ar. After stirring for 3 h at room temperature, the reaction mixture was filtered through a pad of Celite washing with MeOH. Evaporation of the resultant solution afforded pale yellow solid (**12**) (3.34 g, quantitative yield).

Compound **12**: mp 220 °C (decomposed). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.59 (1H, dt, J=19.8, 1.7 Hz, C(O)CH_2N), 2.81 (1H, dd, J=13.5, 4.5 Hz, benzyl), 2.98 (1H, dd, J=13.5, 5.4 Hz, benzyl), 3.25 (1H, d, J=19.8 Hz, C(O)CH_2NH), 4.08 (1H, brd, Bn-CH), 5.83 (2H, br, NH_2), 7.13–7.25 (5H, m, Ph), 7.82 (1H, brd amide-NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 40.2, 49.6, 53.5, 126.9, 128.2, 130.3, 136.7, 157.3, 169.9 ppm. IR (KBr): 3425, 3192, 3032, 2742, 1681, 1329, 708 cm^{-1}. FAB-MS (NBA) m/z 204 (MH$^+$). Anal. calcd for $C_{11}H_{13}N_3O$: C, 65.01; H, 6.45; N, 20.68. Found: C, 65.02; H, 6.61; N, 20.61.

4.1.5. Cyclic amidine ditosylamide (13). To a solution of amidine (**12**) (256 mg, 1.26 mmol) in pyridine (5 ml) was added *p*-toluenesulfonyl chloride (1.19 g, 6.26 mmol, 5.0 equiv.) at 0 °C under Ar atmosphere. This mixture was stirred for 1 h at room temperature. Pyridine was removed by diluting with toluene and concentrating in vacuo. The resulting residue was dissolved in CH_2Cl_2–water and was extracted with CH_2Cl_2 (X3). The combined organic layer was washed with brine and was dried over Na_2SO_4. After evaporated the crude product was purified by column chromatography on silica gel with AcOEt–hexane (1:1), Concentration of the solution afforded pale yellow solid (**13**) (499 mg, 78% yield).

Compound **13**: mp 87–92 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.31 (3H, s, Ts), 2.36 (3H, s, Ts), 3.29 (2H, s, benzyl), 3.91 (2H, s, C (O) CH_2), 7.12 (2H, d, J=7.9 Hz, Ts), 7.18–7.26 (5H, m, Ph), 7.31 (2H, d, J=8.2 Hz, Ts), 7.49 (2H, d, J=7.9 Hz, Ts), 7.74 (2H, d, J=8.2 Hz, Ts), 9.26 (1H, s, amide-NH), 9.48 (1H, s, NH) ppm. ^{13}C NMR (125 MHz, DMSO-d_6) δ 20.9, 21.0, 33.3, 49.5, 110.7, 126.3, 126.9, 127.9, 128.4, 128.8, 129.2, 129.4, 133.4, 133.9, 136.4, 138.4, 142.6, 144.0, 164.9 ppm. IR (KBr): 3271, 2959, 1672, 1511, 1378, 1217 cm^{-1}. FAB-MS (NBA) m/z 287 (MH$^+$). Anal. calcd for $C_{25}H_{25}N_3O_5S_2$:C, 58.69; H, 4.93; N, 8.21. Found: C, 58.58; H, 5.05; N, 8.12.

4.1.6. 5-*N*-Tosylamide-2-hydroxypyrazine (14). To a solution of di-Ts-amidine (**13**) (102 mg, 0.20 mmol) in THF (4 ml), was added NaH (60% in mineral oil, 24.0 mg, 0.6 mmol, 3.0 equiv.) at 0 °C. After stirring for 2 h at room temperature, the reaction mixture was quenched with some drops of MeOH and was evaporated. The resulting residue was dissolved in AcOEt–water, and was extracted with AcOEt (X5). The organic layer was dried over Na_2SO_4. After evaporation, the residue was purified by column chromatography on silica gel with AcOEt–hexane (3:1) to give 5-*N*-tosylamide-2-hydroxypyrazine (**14**) as a white solid (64.5 mg, 91% yield).

Compound **14**: mp 228 °C (decomposed). FL (MeOH) Em. 435 nm (Ex. 350 nm). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.37 (3H, s, Ts), 3.30 (1H, s, OH), 4.03 (2H, s, benzyl), 7.19–7.30 (5H, m, Ph), 7.34 (2H, d, J=8.3 Hz, Ts), 7.56 (1H, s, C (OH) CH), 7.65 (2H, d, J=8.3 Hz, Ts), 9.86 (1H, s, NHTs) ppm. ^{13}C NMR (125 MHz, DMSO-d_6)[11] δ 20.9, 126.4, 126.9, 128.4, 128.7, 129.3, 138.4, 142.7 ppm. IR (KBr): 3424, 3244, 1672, 1336, 1164, 693 cm^{-1}. FAB-MS (NBA) m/z 356 (MH$^+$). HRMS (EI) calcd for $C_{18}H_{17}N_3O_3S$ 355.0991, found 355.1018 (M$^+$). Anal. calcd for $C_{18}H_{17}N_3O_3S$:C, 60.83; H, 4.82; N, 11.82. Found: C, 60.84; H, 4.79; N, 11.77.

4.1.7. 5-*N*-(*p*-Toluenesulfonyl)amide-6-benzyl-2-*O*-trifluoromethanesulfonyloxy-pyrazine (15). To a solution of 5-*N*-Ts-amide-2-hydroxypyrazine (**14**) (222 mg, 0.625 mmol) in CH_2Cl_2 (10 ml) and *i*-Pr_2NEt (300 μl, 1.74 mmol, 2.8 equiv.) was added trifluoromethansulfonic anhydride (147 μl, 0.875 mmol, 1.4 equiv.) at 0 °C under Ar atmosphere. After stirring for 2 h at 0 °C, ice water was added to the reaction mixture. This mixture was extracted with CH_2Cl_2 (X3). The organic layer was dried over Na_2SO_4, and the solvent was evaporated. The resulting residue was purified by column chromatography on silica gel with AcOEt–hexane (1:3) to give orange solid (**15**) (259 mg, 85% yield).

Compound **15**: mp 141–143 °C. FL (MeOH) Em. 419 nm (Ex. 350 nm). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.40 (3H, s, Ts), 4.15 (2H, s, benzyl), 7.39–7.17 (7H, m, Ph+Ts), 7.70 (2H, d, *J*=8.6 Hz, Ts), 8.06 (1H, s, CH-6) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.6, 39.8, 128.0, 128.4, 128.7, 129.5, 132.5, 134.4, 135.7, 142.5, 144.8, 145.9, 147.5 ppm. IR (KBr): 3424, 2926, 1604, 1431, 1219, 1163 cm^{-1}. EI-MS *m/z* 487 (M+). Anal. calcd for $C_{19}H_{16}F_3N_3O_5S_2$:C, 46.81; H, 3.31; N, 8.62. Found: C, 46.80; H, 3.26; N, 8.48.

4.1.8. 2-*N*-(*p*-Toluenesulfonyl)amide-3-benzyl-5-phenylpyrazine (17a). A mixture of triflate (**15**) (25.0 mg, 0.051 mmol) and phenylboronic acid (**16a**) (9.4 mg, 0.077 mmol, 1.5 equiv.) and $Pd(PPh_3)_4$ (5.8 mg, 0.0051 mmol, 0.1 equiv.) and $K_3PO_4{\cdot}3H_2O$ (20.5 mg, 0.077 mmol, 1.5 equiv.) in dioxane (1 ml) was heated to 80 °C for 1.5 h. The mixture was diluted with toluene (2 ml) and treated with aqueous 3 M NaOH (3 drops) and 30% H_2O_2 (3 drops) for 1 h at room temperature to oxidize the residual borane. The product was extracted with ether (X3), and dried over Na_2SO_4. After evaporated, the residue was purified by column chromatography on silica gel with AcOEt–hexane (1:3) to give **17a** as a pale yellow solid (17.9 mg, 85% yield).

Compound **17a**: mp 167–169 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.38 (3H, s, Ts), 4.27 (2H, s, benzyl), 6.92 (1H, s, TsNH), 7.48–7.20 (10H, m, 2Ph+Ts), 7.70 (2H, d, *J*=8.3 Hz, Ts), 7.93 (2H, d, *J*=7.1 Hz, Ph), 8.53 (1H, s, CH-6) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.6, 40.8, 126.3, 127.6, 128.3, 128.7, 129.0, 129.3, 129.5, 136.0, 137.3, 143.4, 144.2, 144.6, 146.8 ppm. IR (KBr): 3450, 1586, 1452, 1167, 1088, 695 cm^{-1}. FAB-MS (NBA) *m/z* 416 (MH^+). HRMS (FAB/NBA) calcd for $C_{24}H_{22}N_3O_2S$ 416.1433, found 416.1435 (MH^+). Anal. calcd for $C_{24}H_{21}N_3O_2S$:C, 69.37; H, 5.09; N, 10.11. Found: C, 69.37; H, 5.21; N, 9.99.

4.1.9. 2-Amino-3-benzyl-5-phenylpyrazine (18a). *N*-Ts-amidepyrazine (**17a**) (62.9 mg, 0.15 mmol) was dissolved in 1.0 ml of conc. H_2SO_4 at 0 °C in an ice bath. After stirring for 10 min at 0 °C, ice was poured into the reaction. It was extracted with AcOEt (X3), and the organic layer was washed with brine once. After neutralization with $NaHCO_3$, the organic layer was dried over Na_2SO_4. Purification by preparative TLC provided aminopyrazine (**18a**) (14.9 mg, 38% yield) as a yellow solid.

Compound **18a**: mp 133–136 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.20 (2H, s, benzyl), 4.46 (2H, s, amine-NH_2), 7.25–7.34 (5H, m, Ph), 7.37 (1H, dt, *J*=7.3, 1.2 Hz, Ph), 7.46 (2H, t, *J*=8.2, 7.3 Hz, Ph), 7.95 (2H, dd, *J*=8.2, 1.2 Hz, Ph), 8.39 (1H, s, Py) ppm. ^{13}C NMR (150 MHz, $CDCl_3$) δ 41.3, 125.2, 126.3, 128.2, 128.3, 128.4, 128.5, 128.6, 129.3, 129.5, 136.8, 137.2, 138.0, 151.7 ppm. FAB-MS (NBA): *m/z* 262 (MH^+). HRMS (FAB/NBA) calcd for $C_{17}H_{16}N_3$ 262.1344, found 262.1357 (MH^+). Anal. calcd for $C_{17}H_{15}N_3$: C, 78.13; H, 5.79; N, 16.08. Found: C, 78.33; H, 5.77; N, 16.01.

4.1.10. 2-*N*-(*p*-Toluenesulfonyl)amide-3-benzyl-5-(4-methoxyphenyl)pyrazine (17b). To a solution of triflate (**15**) (14.2 mg, 0.029 mmol) and boronic acid (**16b**) (10.9 mg, 0.072 mmol, 2.5 equiv.) in dioxane (0.6 ml) was added K_3PO_4 (10.2 mg, 0.048 mmol, 1.6 equiv.), PPh_3 (5.2 mg, 0.020 mmol, 0.7 equiv.) and $Pd(dba)_2$ (3.0 mg, 5.2 μmol, 0.2 equiv.) successively at room temperature. After stirring for 3 h at 80 °C in an oil bath, the reaction mixture was extracted with AcOEt (X2). The organic layer was washed with brine once and dried over Na_2SO_4. Purification by preparative TLC provided *N*-Ts-amidepyrazine (**17b**) (11.6 mg, 89% yield) as a yellow solid.

Compound **17b**: mp 169.0–169.5 °C. ^{1}H NMR (600 MHz, CDCl3) δ 2.38 (3H, s, Ts), 3.85 (3H, s, OCH_3), 4.24 (2H, s, benzyl), 6.98 (2H, d, *J*=8.4 Hz, anisole), 7.17–7.36 (8H, m, Ar), 7.70 (2H, d, *J*=8.4 Hz, anisole), 7.88 (2H, d, *J*=7.8 Hz, Ts), 8.46 (1H, s, Py) ppm. ^{13}C NMR (150 MHz, $CDCl_3$) δ 22.0, 40.6, 54.9, 114.9, 127.2, 127.6, 128.8, 129.2, 129.8, 136.0, 136.2, 136.5, 137.2, 143.6, 143.8, 146.8, 160.7 ppm. FAB-MS (NBA) *m/z* 446 (MH^+). HRMS (FAB/NBA) calcd for $C_{25}H_{24}N_3O_3S$ 446.1538, found 446.1515 (MH^+). Anal. calcd for $C_{25}H_{23}N_3O_3S$: C, 67.39; H, 5.20; N, 9.43. Found: C, 67.38; H, 5.17; N, 9.41.

4.1.11. 2-Amino-3-benzyl-5-(4-methoxyphenyl)pyrazine (18b). *N*-Ts-amidepyrazine (**17b**) (25.5 mg, 0.057 mmol) was dissolved in 1.0 ml of conc. H_2SO_4 at 0 °C in an ice bath. After stirring for 20 min at 0 °C, ice was poured into the reaction. The reaction mixture was extracted with CH_2Cl_2 (X3). The organic layer was washed with brine once and then dried over Na_2SO_4. Purification by preparative TLC provided aminopyrazine (**18b**) (8.2 mg, 49% yield) as a yellow solid and *N*-Ts-amidepyrazine (**17b**) (6.6 mg, 26% yield) as recovered starting material.

Compound **18b**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.84 (3H, s, OCH_3), 4.16 (2H, s, benzyl), 4.32 (2H, s, amine-NH_2), 6.97 (2H, d, *J*=8.8 Hz, anisole), 7.22–7.32 (5H, m, Ph), 7.86 (2H, d, *J*=8.8 Hz, anisole), 8.31 (1H, s, Py) ppm. All the other spectroscopic data were identical with reported data in Ref 10).

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11. Benzylic carbon NMR signals was not observed due the perfect overlapping with the carbon signals of DMSO-d_6.

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A parallel synthesis approach towards a family of *C*-nucleosides

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Abstract—A synthetic route was devised for a sugar based α-chloroketone, which was subsequently used to generate a family of *C*-nucleosides via parallel synthetic methodology.

1. Introduction

The discovery of new chemotherapeutic treatments for controlling microbial infections is an important topic in clinical medicine.

Many nucleoside analogues of natural origin have been found to be bioactive. Bredinine **1**[1] (Mizoribine) is an imidazole nucleoside antibiotic clinically used as an immunosuppressant;[2] Toyocamycin **2**,[3] Mycalisin A **3**,[4] and Thiosangivamycin **4**[3] are three naturally occurring nucleosides which exert potent antiviral and antineoplastic activity (Fig. 1); Pseudouridine **5**,[5] Showdomycin **6**,[6] Pyrazofurin **7**[7] and Tiazofurin **8**[8] have been shown to possess a wide range of medicinal properties, including antibiotic, antiviral, and anti-tumor activity (Fig. 2).

In recent years a large number of compounds have been prepared modelled on naturally occurring templates and subsequently tested.[9] Triciribine **9**[10a] (Fig. 3) a synthetic tricyclic analogue of Toyocamicin **2** constitutes a valid example. Triciribine monophospate (TCN-P) is now in phase II studies as a potential antineoplastic agent.[10b]

In general, nucleoside analogues can be divided into three classes: (a) nucleosides bearing modification on the sugar moiety, (b) nucleosides bearing modification on the base moiety, (c) nucleosides bearing modification on both the base and the sugar moieties. Typical modifications of class (a) analogues include the alteration of sugar stereochemistry or the removal of one or more stereogenic centre as in

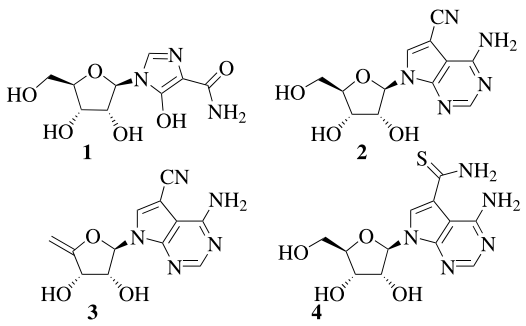

Figure 1. Naturally occurring nucleosides analogues.

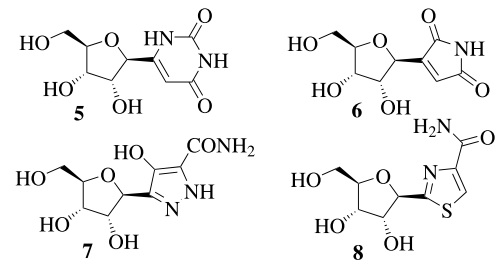

Figure 2. Naturally occurring *C*-nucleosides.

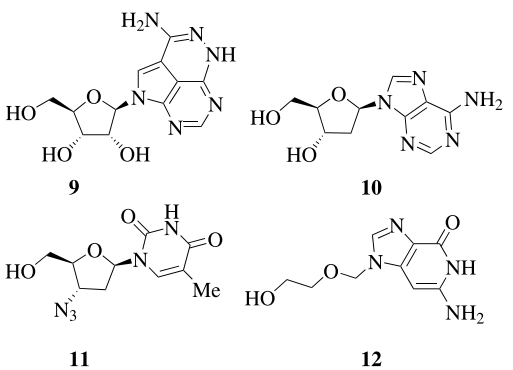

Figure 3. Synthetic nucleosides analogues.

Keywords: *C*-Nucleosides; Parallel synthesis.
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2′-deoxyadenosine **10** (Fig. 3); introduction of a different moiety such as an azido group in Zidovudine **11**, or replacement of the sugar by acyclic structures. Compounds having remarkable antiviral or antitumor activity have been obtained through sugar modification, such as acyclovir **12**.

Typical modifications of class (b) analogues include the addition or removal of structural elements, as in **9**. Modifications for class (c) analogues include all previously mentioned alterations for group (a) and (b). *C*-Nucleosides constitute a category of analogues in which the modification occurred at the linkage between the base and the sugar. In *C*-nucleosides, the sugar and the base are linked through a C–C linkage as opposed to *N*-nucleosides where a C–N bond is present. This structural alteration is believed to increase the nucleoside stability towards nucleoside hydrolase enzymes, and to inhibit in general the cascade of events leading to DNA or RNA formation.[11] Many naturally occurring *C*-nucleosides such as Pseudouridine **5**, Showdomycin **6** and Pyrazofurin **7** have been reported as strong antibiotic agents,[5–7] which has primed research on *C*-nucleoside analogues. However, despite the large amount of data collected, *C*-nucleosides and especially *C*-nucleosides belonging to the 2-deoxy-D-ribose series have scarcely been explored. Additionally, there is no data available on the biological activity of 2-deoxy-*C*-nucleosides belonging to the α-anomeric series. For these reasons, we believe that *C*-nucleosides constitute an ideal template for use in drug discovery.

2. Results and discussion

Our research focuses on the development of parallel synthetic methodologies which generate families of potentially useful drug candidates.[12–15] As part of this research we set out to develop a synthetic route to a family of *C*-nucleoside analogues of the 2-deoxy-D-series (Fig. 4). We choose the following key features to define the new family: (i) the *C*-linkage between the sugar and the heterocyclic moiety; (ii) the presence of multiple heteroatoms, as potential sites for H-bonding recognition; (iii) synthesis in both the α and β anomeric series; (iv) sugar moiety derived from the naturally occurring 2-deoxy-D-ribose **19**.

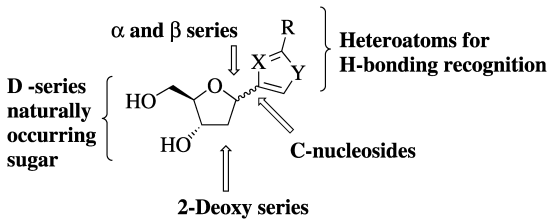
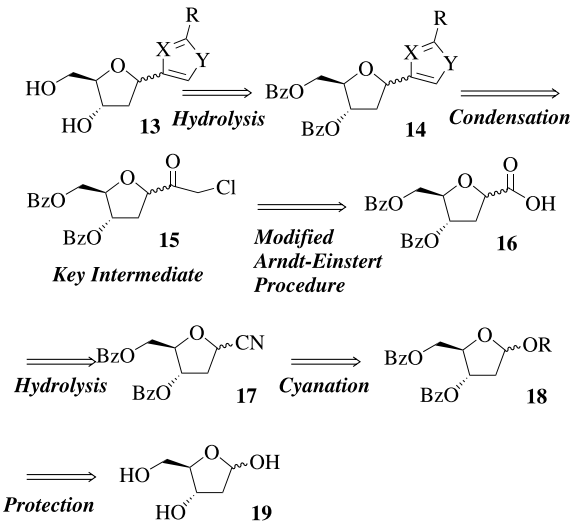


Figure 4. A family of 2-D-deoxy-*C*-nucleosides.

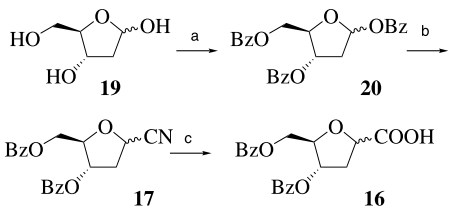
A disconnection analysis towards target **13** placed α-chloroketone **15** as the key intermediate in the synthetic plan (Scheme 1).

α-Haloketones have been shown to be versatile building blocks for the synthesis of heterocycles,[16–19] therefore we targeted compound **15** as a key intermediate in our synthetic approach. In turn, compound **15** can be accessed from the



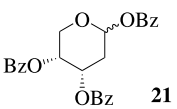
Scheme 1. Synthetic plan.

parent carboxylic acids **16**, through a modified Arndt–Einstert reaction. The synthesis of **16** has been reported from commercially available 2-deoxy-D-ribose **19** (Scheme 2).[20] As a protecting group regime was needed, attention was directed towards groups, which could be cleaved under mild conditions. The benzoyl group was selected, due to its ease of removal with aqueous base or methanolic ammonia. Thus, commercially available 2-deoxy-D-ribose **19** was firstly protected to give a tri-*O*-benzoyl deoxyribose derivative **20**, which in turn, was reacted to give nitrile **17**. Hydrolysis of **17** in mild acidic conditions furnished acid **16**. Conversion of **20** to **17** and of **17** to **16** were very effective processes, leading to high reaction yields.

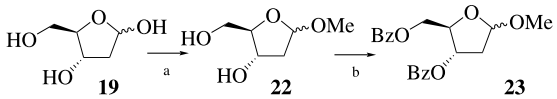


Scheme 2. *Reagents and conditions*: (a) DCM, BzCl (4 equiv.), pyridine (12 equiv.), rt, 1.5 h, 39%; (b) DCM, TMSCN (1.3 equiv.), $BF_3{\cdot}Et_2O$ (3.3 equiv.), 0 °C, 1.5 h, 73%; (c) 1,4-dioxane, HCl (1 mL, 35% HCl in water), 72%.

However, following this route, the overall yield was restricted as compound **20** could be obtained in no more than 39% yield from **19**. This was due to the concomitant formation of tribenzoylpyranose **21**, which affected the efficiency of the whole synthetic route. Furthermore, we found that the purification of **20** from **21** was laborious and time consuming.

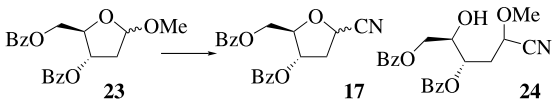


Formation of **21** could be avoided by blocking the anomeric position prior to benzoylation. Hence, selective methoxylation of the anomeric position furnished compound **22**[21] in nearly quantitative yields, which was successively benzoylated to give **23**[22] in 73% yield (Scheme 3).



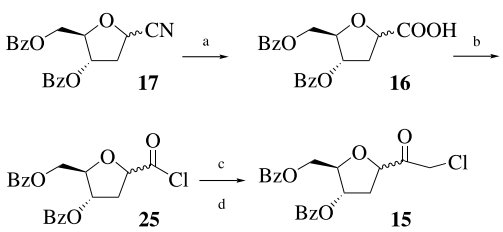
Scheme 3. *Reagents and conditions*: (a) MeOH, AcCl (6.4 mol%), rt, 25 min, 99%; (b) DCM, BzCl (4 equiv.), pyridine (12 equiv.), rt, 1.5 h, 73%.

Compound **23** was then submitted to cyanation using TMSCN in the presence of $BF_3{\cdot}OEt_2$ (Scheme 4) to give nitrile **17**. Although conversion of **23** was complete under the experimental conditions employed, the yields of **17** never exceeded 70–75%, due to the formation of acyclic derivative **24** by product. This behaviour was not observed when **20** was cyanated under similar conditions, and could be rationalised by the fact that benzoate is a better leaving group.



Scheme 4. *Reagents and conditions*: DCM, TMSCN (1.3 equiv.), $BF_3{\cdot}Et_2O$ (3.3 equiv.), 0 °C, 1.5 h, 71%.

The hydrolysis of nitrile **17** proceeded as reported and acid **16** was obtained in 72% yield (Scheme 5).[20]

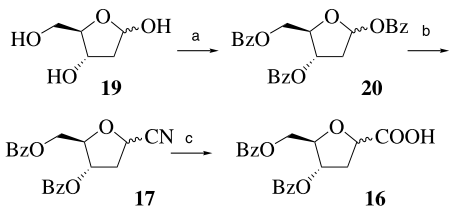


Scheme 5. *Reagents and conditions*: (a) dioxane/HCl, 70 °C, 6 h, 72%; (b) DCM, α,α-dichloromethyl methyl ether (5 equiv.), reflux, 4 h, 99%; (c) Et_2O, CH_2N_2, (3 equiv.); (d) HCl gas, 30 min, 61%.

α-Chloroketone **15** was prepared from **16** through a modified Arndt–Einstert procedure. This method involved the activation of the acid to a mixed anhydride or an acyl chloride such as **25**, followed by displacement of chloride by diazomethane and subsequent quenching with HCl (Scheme 5). With the α-chloroketone **15** in hand we explored its reactivity towards a number of polynucleophiles.

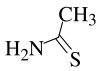
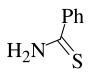
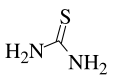
We reacted **15** with various thioamide nucleophiles and found that thiazoles **26**–**28** could be obtained in good yields (Scheme 6 and Table 1).

Importantly, the α and β anomers could be separated at this stage by simple flash chromatography.



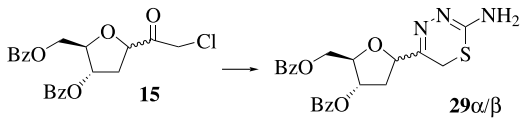
Scheme 6. *Reagents and conditions*: **15**, ethanol, thioamide (1.0 equiv.), or thiourea, reflux, 16 h.

Table 1. Formation of benzoyl nucleosides

Entry	Nucleophile	Product	Yield (%)
1		R=CH_3 **26**α	35
		R=CH_3 **26**β	53
2		R=Ph **27**α	22
		R=Ph **27**β	45
3		R=NH_2 **28**α	35
		R=NH_2 **28**β	55

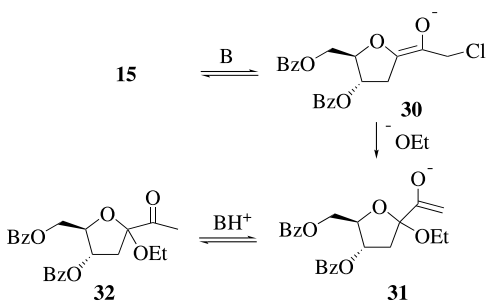
The stereochemistry at the anomeric position was assigned by NOE experiments. Positive NOE was observed between the anomeric H-1′ and H-4′ in the β anomer, and between the H-1′ and H-3′ in the α anomer.

We have also employed **15** to make heterocycles other than thiazoles. When chloroketone **15** was reacted with thiosemicarbazide hydrochloride, rapid condensation occurred and thiazidine **29**α/β was isolated in 81% yield (Scheme 7).



Scheme 7. *Reagents and conditions*: **15**, methanol, thiosemicarbazide hydrochloride (1.0 equiv.), reflux, 1 h, 81%.

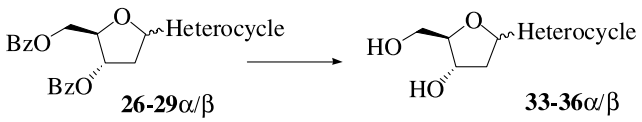
We have attempted to prepare sugar-based imidazoles by reacting α-chloroketone **15** with amidines. In these experiments no heterocycle was formed and repeatedly, compound **32**α/β was isolated in 60–70% yield (Scheme 8).



Scheme 8. *Reagents and conditions*: **15**, ethanol, benzamidine hydrochloride (1 equiv.), $NaHCO_3$ (1 equiv.), reflux, 4 h, 65% yield.

It is possible that the basic character of amidines promotes conversion of **15** to **32**. A mechanism for this reaction is proposed as follows (Scheme 8). It is noteworthy that reaction of α-chloroketones with AcOK/AcOH to give α′-propanones has been reported for substrates bearing an α′-aryloxy, or α′-phenylthio substituent.[23]

Dibenzoyl *C*-nucleosides **26**–**28**α/β, were finally deprotected using either LiOH in THF/H_2O or NH_3/MeOH. These reactions proceeded in high yields and with no epimerization at the anomeric position (Scheme 9 and Table 2).



26-29α/β **33-36**α/β

Scheme 9. *Reagents and conditions*: LiOH·H_2O (1.1 equiv.) THF–H_2O, rt, 24 h; or.MeOH,·NH_3, rt, 24 h.

Table 2. Deprotection of dibenzoyl nucleosides

Entry	Protected nucleoside	Nucleoside	Yield (%)
1[a]	**26**α	**33**α	71
2[a]	**26**β	**33**β	78
3[a]	**27**α	**34**α	82
4[a]	**27**β	**34**β	86
5[b]	**28**α	**35**α	65
6[b]	**28**β	**35**β	68
7[b]	**29**α/β	**36**α/β	75

[a] Reagents and conditions: LiOH·H_2O (1.1 equiv.) THF–H_2O, rt, 24 h.
[b] Reagents and conditions: MeOH·NH_3, rt, 24 h.

In conclusion, parallel synthetic methodology towards a family of *C*-nucleosides has been developed. This route made use of an α-chloroketone as the key intermediate derived from a naturally occurring sugar, which led to a number of synthetic nucleosides.

3. Experimental

3.1. General

Anhydrous DCM was obtained by stirring over calcium hydride for 24 h followed by distillation under nitrogen. All water used was distilled.

^1H and ^{13}C Spectra were recorded on, Brüker DPX200 (200 MHz), Varian Gemini 200 (200 MHz), Brüker DPX 250 (250 MHz), Brüker DQX 400 (400 MHz), Brüker DPX400 (400 MHz) and Brüker AMX 500 (500 MHz) spectrometers at ambient temperatures. ^1H NMR spectral assignments are supported by ^1H–^1H COSY where necessary. For ^1H NMR recorded in $CDCl_3$ chemical shifts (δ_H) are quoted in parts per million (ppm) and are referenced to the residual solvent peak. The following abbreviations are used: s, singlet, d, doublet, t, triplet, dd, doublet of doublets, ddd, doublet of doublet of doublets, dt, doublet of triplets, m, multiplet and br, broad. Coupling constants (*J*) were recorded in Hertz (Hz) to the nearest 0.5 Hz. Carbon spectra are supported by DEPT analysis where necessary.

Infrared (IR) spectra were recorded as thin films between NaCl plates on a Perkin–Elmer Paragon Fourier Transform spectrometer. Absorption maximum (ν_{max}) was reported in wave numbers (cm^{-1}) and only selected peaks are reported. The following abbreviations are used: w, weak, m, medium, s, strong and br, broad.

Low-resolution mass spectra (*m/z*) were recorded using a V.G.TRIO (GCMS) spectrometer, a Micromass Platform (APCI) spectrometer, Micromass Autospec spectrometer (CI^+) and a Micromass ZAB spectrometer (CI^+, EI). Only molecular ion (M^+) and other major peaks are reported. High-resolution mass spectra were recorded on a Micromass Autospec spectrometer and are accurate to ±5 ppm.

Melting points were obtained using a Büchi 510 Cambridge Instruments Gallen™ III hot stage melting point apparatus and are uncorrected.

Specific optical rotations were recorded using a Perkin–Elmer 241 automatic polarimeter with a cell of path length 1 dm. All concentrations are given in grams per 100 mL.

Flash chromatography was carried out using silica gel 60 0.040–0.063 mm, 230–400 mesh as the stationary phase. Thin layer chromatography was carried out on aluminium backed plates pre-coated with Merck silica gel 60 F_{254} (1.05554), which were visualized by quenching of UV fluorescence (λ_{max}=254 nm) or by staining with either 10% (w/v) ammonium molybdate in 2 M sulphuric acid or basic potassium permanganate solution (followed by heat) as appropriate. Retention factors (R_f) are reported to ±0.5.

All reactions were carried out under anhydrous conditions and an argon atmosphere unless otherwise indicated.

3.1.1. 2*R*-Hydroxymethyl-5-methoxytetrahydrofuran-3*S*-ol, 22.[21] To a stirred solution of 2-deoxy-D-ribose (5.00 g, 37.3 mmol) in methanol (60 mL) was added 1% methanolic hydrogen chloride solution (prepared by adding 170 μL acetyl chloride to 10 mL MeOH). The reaction mixture was stirred at room temperature under an Argon atmosphere (25 min) then sodium bicarbonate (2 g) added and the reaction stirring continued for further 10 min. The solids were filtered and the solvent removed in vacuo to give **22** as an orange oil (5.46 g, 99% yield). This product was found pure enough to be used without further purification; δ_H (400 MHz, $CDCl_3$) 5.11–5.08 (1H, m, CH_3O–C*H*–O), 4.50–4.01 (2H, m, $HOCH_2CHCHO$), 3.75–3.71 (2H, m, $HOCH_2CHCHOH$), 3.38 (3H, s, CH_3OCHO), 2.17–1.83 (2H, m, $MeOCHCH_2CH$).[21]

3.1.2. 3*S*-Benzoyloxy-2*R*-benzoyloxymethyl-5-methoxytetrahydrofuran, 23.[22] To a stirred solution of **22** (5.46 g, 36.9 mmol) in dichloromethane (90 mL) was added benzoyl chloride (17.2 mL, 147 mmol, 4 equiv.) and the reaction mixture cooled to 0 °C by ice bath cooling. A mixture of dichloromethane (88 mL) and pyridine (44 mL) was added dropwise and the reaction mixture was then stirred at room temperature under Ar atmosphere (1.5 h). The reaction mixture was washed with 10% sulphuric acid (4×300 mL), then with saturated potassium bicarbonate solution (2×200 mL) and finally with water (2×200 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatography on silica gel eluting with dichloromethane to give the title compound **23** as a colourless oil (9.63 g, 73% yield, α/β=40:60);[24] R_f 0.2, dichloromethane.

Compound **23**α. δ_H (500 MHz, $CDCl_3$) 8.16–8.08 (4H, m, Ar); 7.62–7.59 (2H, m, Ar), 7.50–7.45 (4H, m, Ar), 5.52–5.49 (1H, m, BzOC*H*CH_2), 5.25 (1H, d, CH_3OCHO *J*=4.5 Hz), 4.72–4.42 (3H, m, BzOCH_2CHO, BzOCH_2-C*H*O), 3.48 (3H, s, CH_3OCHO), 2.59 (1H, m, CHOHCH_2) 2.27 (1H, m, CHOHCH_2); δ_C (125.8 MHz, $CDCl_3$) 105.51

(CH_3OCHO), 81.37 ($BzOCH_2CHO$), 75.26 ($BzOCHCHO$), 64.93 ($BzOCH_2CHO$), 55.58 (CH_3OCHO), 39.54 ($OCHCH_2CHOBz$).

Compound **23**β. δ_H (500 MHz, $CDCl_3$) 8.16–8.08 (4H, m, Ar); 7.62–7.59 (2H, m, Ar), 7.50–7.45 (4H, m, Ar), 5.70–5.67 (1H, m, $BzOCHCH_2$), 5.29 (1H, dd, CH_3OCHO, J=2, 5.5 Hz), 4.72–4.42 (3H, m, $BzOCH_2CHO$, $BzOCH_2CHO$), 3.42 (3H, s, CH_3OCHO), 2.63 (1H, m, $CHOHCH_2$) 2.41 (1H, m, $CHOHCH_2$); δ_C (125.8 MHz, $CDCl_3$) 105.89 (CH_3OCHO), 82.12 ($BzOCH_2CHO$), 76.04 ($BzOCHCHO$), 65.74 ($BzOCH_2CHO$), 55.67 (CH_3OCHO), 39.85 ($OCHCH_2CHOBz$).

Compound **23**α/β. δ_C (125.8 MHz, $CDCl_3$) 166.49, 166.64, 166.69, 166.86 (PhCO); 133.76, 133.69, 133.58, 133.50, 130.37, 130.27, 130.22, 130.20, 130.13, 130.10, 130.04, 129.06, 128.99, 128.89, 128.86, 128.82 (Ar); ν_{max}/cm^{-1} (neat film) 1723s, 1602m; *m/z* (GC–CI[NH_3]) 374 (MNH_4^+, 30%), 325 (100%); HRMS found: MNH_4^+ 374.1620 ($C_{20}H_{24}NO_6$ requires 374.1604).

3.1.3. 4*S*-Benzoyloxy-5*R*-benzoyloxymethyltetrahydrofuran-2*R*/*S*-carbonitrile, 17.[20] To a stirred solution of **23** (9.62 g, 27.0 mmol) in dry dichloromethane (160 mL) was added TMSCN (5.47 mL, 38.4 mmol, 1.4 equiv.) and $BF_3{\cdot}OEt_2$ (11.9 mL, 96.0 mmol, 3.6 equiv.). The reaction mixture was stirred at 0 °C under an Argon atmosphere for 1.5 h. After this time, a saturated aqueous $NaHCO_3$ solution (300 mL) was slowly added and the reaction mixture stirred until evolution of CO_2 was finished. The product was extracted with dichloromethane (2×300 mL), the organic layer dried over Na_2SO_4 and the solvent removed in vacuo. The residue was purified by flash chromatography on silica gel eluting with chloroform/ethyl acetate (40:1) to give the title compound **17** as a yellow oil (6.72 g, 71% yield); R_f 0.85; δ_H (250 MHz, $CDCl_3$) 8.12–8.00 (4H, m, Ar), 7.51–7.47 (6H, m, Ar), 5.65 (1H, m, $BzOCHCH_2$) 5.08 (0.4H, dd, J=2, 7 Hz, OCHCN), 4.94 (0.6H, dd, J=6.5, 9 Hz, OCHCN), 4.71–4.52 (3H, m, $BzOCH_2CHO$), 2.81–2.60 (2H, m, $CHCH_2CHCN$); δ_C (62.5 MHz, $CDCl_3$) 166.56 (PhCO), 166.45 (PhCO), 166.11 (PhCO), 134.19 (Ar), 134.14 (Ar), 133.84 (Ar), 130.37 (Ar), 130.17 (Ar), 130.13 (Ar), 130.05 (Ar), 129.79 (Ar), 129.34 (Ar), 129.06 (Ar), 129.02 (Ar), 128.98 (Ar), 118.84 (NCCHO), 118.29 (NCCHO), 84.64 ($CNCHCH_2CHOBz$), 84.17 ($CNCHCH_2CHOBz$), 75.94 ($BzOCH_2CHO$), 75.42 ($BzOCH_2CHO$), 66.98 (NCCHO), 66.30 (NCCHO), 64.32 ($BzOCH_2CHO$), 64.20 ($BzOCH_2CHO$), 38.33 ($NCCHCH_2CH$), 38.22 ($NCCHCH_2CHOBz$); ν_{max}/cm^{-1} (neat film), 1722s, 1602s,; *m/z* [CI(NH_3)] 369 (MNH_4^+, 100%); HRMS found: MNH_4^+ 369.1438 ($C_{20}H_{21}N_2O_5$ requires 369.1450), and 4*S*,5*R*,6-tribenzoyloxy-2*R*/*S*-methoxy-hexanenitrile **24**: yellow oil (2.25 g, 22% yield); R_f 0.25, chloroform/ethyl acetate (40:1); δ_H (200 MHz, $CDCl_3$), 8.09–8.01 (4H, m, Ar), 7.61–7.40 (6H, m, Ar), 5.51–5.42 (1H, m, $NCCHOCH_3$), 4.61–4.17 (4H, m, $BzOCH_2CHO$, $BzOCHCH_2$), 3.45 and 3.48 (3H, 2×s, OCH_3), 2.58–2.38 (2H, m, $BzOCHCH_2CHCN$); δ_C (50.3 MHz, $CDCl_3$) 167.0 (PhCO), 166.0 (PhCO), 133.7 (Ar), 133.6 (Ar), 133.4 (Ar), 129.8 (Ar), 129.8 (Ar), 129.4 (Ar), 128.7 (Ar), 128.5 (Ar), 117.7 (NCCHOMe), 71.2 and 71.1 (NCCHOMe), 70.9 and 70.7 ($BzOCHCHOH$), 67.8 and 67.0 ($BzOCH_2CHOH$), 65.7 and 65.6 ($BzOCH_2CHOH$), 58.4 and 58.2 ($BzOCHCH_2CHCN$), 34.9 and 34.5 (CH_3OCHCN); ν_{max}/cm^{-1} (neat film) 3477m br, 1202s, 1126s; *m/z* (electrospray) 401 (MNH_4^+, 100%); HRMS found: MH^+ 384.1463 ($C_{21}H_{22}NO_6$ requires 384.1447).

3.1.4. 4*S*-Benzoyloxy-5*R*-benzoyloxymethyltetrahydrofuran-2*R*/*S*-carboxylic acid 16.[20] To a stirred solution of **17** (4.81 g, 13.7 mmol) in 1,4-dioxane (112 mL) was added conc. HCl (11 mL). The reaction mixture was refluxed at 80 °C (6 h) and then the solvent was removed in vacuo. The residue was purified by flash chromatography on silica gel eluting with ethyl acetate/petrol/acetic acid (70:30:2) to give **16** as a yellow oil (3.64 g, 72% yield); R_f 0.4, ethyl acetate/petrol/acetic acid (70:30:2); δ_H (200 MHz, $CDCl_3$) 8.90 (1H, br s, HOOCCH), 8.08–8.01 (3H, m, Ar), 7.96–7.92 (1H, m, Ar), 7.62–7.37 (6H, m, Ar), 5.58–5.54 (1H, m, $BzOCHCH_2$) 4.48–4.71 (1H, m, HO_2CCHO) 4.70–4.51 (3H, m, $BzOCH_2CHO$), 2.82–2.43 (2H, m, $BzOCHCH_2$); δ_C (100.6 MHz, $CDCl_3$) 177.03 (HOOCCH), 166.74 (PhCO), 165.90 (PhCO), 133.65 (Ar), 133.54 (Ar), 133.47 (Ar), 133.37 (Ar), 129.76 (Ar), 129.71 (Ar), 129.66 (Ar), 129.46 (Ar), 129.32 (Ar), 129.11 (Ar), 128.57 (Ar), 128.54 (Ar), 128.50 (Ar), 84.26 and 83.73 ($BzOCHCH_2$), 75.86 and 75.34 ($BzOCH_2CHO$), 67.04 (OCHCOOH), 64.43 and 64.17 ($BzOCH_2CHO$), 36.37 ($BzOCHCH_2CHO$); ν_{max}/cm^{-1} (neat film) 1721s, 1271s; *m/z* (electrospray) 393 (MNa^+, 100%); HRMS found: MNa^+ 393.0943 ($C_{20}H_{18}O_7Na$ requires 393.0950).

3.1.5. 4*S*-Benzoyloxy-5*R*-benzoyloxymethyl-tetrahydrofuran-2*R*/*S*-carbonyl chloride, 25. To a mixture of the **16** (100 mg, 0.27 mmol) in dry dichloromethane (5 mL) was added α,α-dichloromethyl methyl ether (122 μL, 1.35 mmol, 5 equiv.). The mixture was then refluxed for 4 h, allowed to cool and concentrated in vacuo to yield the desired acid chloride **25** as a light yellow oil (103 mg, 99% yield); δ_H (400 MHz, $CDCl_3$) 8.05–7.96 (4H, m, Ar), 7.62–7.55 (2H, m, Ar), 7.48–7.45 (4H, m, Ar), 5.59 (1H, app s, $BzOCHCH_2$), 5.04 (1H, app t, $ClCOCHO$, J=9 Hz), 4.61–4.51 (3H, m, $BzOCH_2CHO$), 2.79–2.64 (2H, m, $ClCOCHCH_2$); δ_C (50.3 MHz, $CDCl_3$) 175.22 ($ClCOCHO$), 173.67 ($ClCOCHO$), 166.36 (PhCO), 166.01 (PhCO), 135.25 (Ar), 133.87 (Ar), 133.50 (Ar), 129.88 (Ar), 129.73 (Ar), 129.36 (Ar), 129.25 (Ar), 128.78 (Ar), 84.36 ($BzOCHCH_2$), 83.89 ($BzOCHCH_2$), 75.38 ($ClCOCHO$), 74.95 ($ClCOCHO$), 68.02 ($BzOCH_2CHO$), 64.05 ($BzOCH_2CHO$), 53.51 ($BzOCH_2CHO$), 52.63 ($BzOCH_2CHO$), 36.62 ($BzOCHCH_2CHO$), 36.26 ($BzOCHCH_2CHO$); ν_{max}/cm^{-1} 3021 m, 2401 w, 1811 m, 1773s; this compound gave unsatisfactory mass spectral data.

3.1.6. 2-Chloro-1-(4*S*-benzoyloxy-5*R*-benzoyloxymethyltetrahydrofuran-2*R*/*S*-yl)-ethanone, 15. The crude acid chloride **25**, obtained from **16** (3.56 g, 9.62 mmol), was taken up in dry diethyl ether (100 mL) and an ethereal solution of freshly generated alcohol free diazomethane added (6.18 g Diazald, 28.9 mmol, 3 equiv.). The reaction mixture was stirred for 15 min and then bubbled through with dry HCl gas for 30 min. The solution was diluted with diethyl ether (125 mL) and the organic phase washed with water (300 mL), then with saturated aqueous $NaHCO_3$ solution (2×300 mL) and finally with water (300 mL). The

reaction mixture was dried over $CaCl_2$, filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel eluting with ethyl acetate/petrol (30:70) to give **15** as a white solid (2.38 g, 61% yield), m.p 122 °C; R_f 0.55, ethyl acetate/petrol (30:70); δ_H (400 MHz, $CDCl_3$) 8.17–7.90 (4H, m, Ar) 7.64–7.59 (2H, m, Ar), 7.55–7.44 (4H, m, Ar), 5.58–5.55 (1H, m, BzOC*H*CH_2), 4.88–4.83 (1H, m, C*H*$COCH_2Cl$), 4.64 (2H, d, *J*=5 Hz, BzOC*H*$_2$CHO), 4.48 (2H, 2×s, $COCH_2Cl$), 4.65–4.47 (1H, m, $BzOCH_2CHO$), 2.77–2.37 (1H, m, $BzOCHCH_2CH$); δ_C (100.6 MHz, $CDCl_3$) 203.31 and 201.02 ($COCH_2Cl$), 166.08, 165.85 and 165.52 (Ph*C*O); 133.65, 133.57, 133.43, 133.37, 129.66, 129.63, 129.59, 129.53, 129.44, 129.32, 129.20, 128.88, 128.62, 128.54 and 128.52 (Ar); 83.95 and 83.76 (BzO*C*HCH_2), 83.20 and 82.54 ($ClCH_2CO$*C*), 75.80 and 75.65 ($BzOCH_2$C*H*), 64.27 and 64.15 (BzO*C*H_2CH), 47.56 and 46.51 (Cl*C*H_2CO), 35.82 and 35.72 (BzOCH*C*H_2); ν_{max}/cm^{-1} (neat film) 1719s, 1269s, 1101s; *m*/*z* (electrospray) 403 (MH^+, 30%); HRMS found: 403.0944 ($C_{21}H_{20}O_6Cl$ requires 403.0948).

3.2. General procedure for the preparation of compounds 26α/β, 27α/β, 28α/β

To a solution of **15** (556 mg, 1.38 mmol) in ethanol (10.4 mL) was added the corresponding thioamide (1.38 mmol, 1 equiv.) and the reaction mixture refluxed for 20 h. Then the solvent was removed and the residue purified by flash chromatography.

3.2.1. 3*S*-Benzoyloxy-2*R*-benzoyloxymethyl-5*R*-(2-methylthiazol-4-yl)-tetrahydrofuran, 26β. Colourless oil, 310 mg, 53% yield from **15**, R_f 0.4 ethyl acetate/petrol (30:70); δ_H (400 MHz, $CDCl_3$) 8.09–8.07 (4H, m, Ar), 7.61–7.55 (2H, m, Ar), 7.48–7.40 (4H, m, Ar), 7.12 (1H, s, =C*H*S), 5.65 (1H, d, *J*=6 Hz, BzOC*H*CH_2), 5.41 (1H, dd, *J*=5.5, 10 Hz, OC*H*C=), 4.64 (2H, d, *J*=2 Hz, BzOC*H*$_2$-CH), 4.56–4.55 (1H, m, $BzOCH_2CH$), 2.68 (3H, s, CH_3C=), 2.68–2.65 (1H, m, $BzOCHCH_2$), 2.50 (1H, ddd, *J*=6, 10, 14 Hz, $BzOCHCH_2$); δ_C (100.6 MHz, $CDCl_3$) 166.51 and 166.26 (Ph*C*=O), 165.99 (CH_3C), 155.49 (OCHC=*C*H), 133.29 133.01, 129.87, 129.72, 129.69, 129.43, 128.33, 128.27 (Ar), 114.34 (OCH*C*=CH), 82.88 (BzO*C*HCH_2), 78.14 (O*C*HC=CH), 76.69 ($BzOCH_2$ *C*HO), 64.74 (BzO*C*H_2CHO), 39.71 (BzOCH*C*H_2), 19.12 (CH_3C); ν_{max}/cm^{-1} (neat film) 1722s, 1602 w, *m*/*z* (electrospray) 446 (MNa^+, 100%); HRMS found: MNa^+ 446.1042 ($C_{23}H_{21}NO_5SNa$ requires 446.1038); $[\alpha]_D^{20}$ −68.0 [*c* 1.0, $CHCl_3$].

3.2.2. 3*S*-Benzoyloxy-2*R*-benzoyloxymethyl-5*S*-(2-methylthiazol-4-yl)-tetrahydrofuran, 26α. Colourless oil, 205 mg, 35% yield from **15**, R_f 0.2 ethyl acetate/petrol (30:70); δ_H (400 MHz, $CDCl_3$) 8.10–8.05 (2H, m, Ar), 7.84–7.81 (2H, m, 2H, Ar), 7.58–7.39 (6H, m, Ar), 7.14 (1H, s, =CHS), 5.62 (1H, ddd, *J*=9, 7, 3 Hz, BzOC*H*CH_2), 5.47 (1H, dd, *J*=6, 5 Hz, OC*H*C=), 4.71 (1H, m, BzOC*H*$_2$CH), 4.61 (2H, d, *J*=5 Hz, BzOC*H*$_2$CH), 2.91 (1H, dd, *J*=9, 7 Hz, $BzOCHCH_2$), 2.69–2.60 (1H, m, $BzOCHCH_2$), 2.67 (3H, s, CH_3); δ_C (100.6 MHz, $CDCl_3$) 166.27 and 166.26 (Ph*C*O), 165.99 (CH_3C=N), 156.21 (OCHC=*C*H), 133.16, 133.11, 129.68, 129.65, 129.58, 129.21, 128.41, 128.27 (Ar), 113.72 (OCH*C*=CH), 82.13 (BzO*C*HCH_2), 77.71 (O*C*HC=CH), 76.43 ($BzOCH_2$*C*HO), 65.80 (BzO*C*H_2CHO), 38.27 (BzOCH*C*H_2), 19.22 (CH_3C=N); ν_{max}/cm^{-1} (neat film) 1722s, 1275s; *m*/*z* (electrospray) 446 (MNa^+, 100%); HRMS found: MNa^+ 446.1047 ($C_{23}H_{21}NO_5SNa$ requires 446.1038); $[\alpha]_D^{20}$ +10.0 [*c* 1.0, $CHCl_3$].

3.2.3. 3*S*-Benzoyloxy-2*R*-benzoyloxymethyl-5*R*-(2-phenylthiazol-4-yl)-tetrahydrofuran, 27β. Colourless oil, 301 mg, 45% yield from **15**, R_f 0.65 ethyl acetate/petrol (10:90); δ_H (500 MHz, $CDCl_3$) 8.17–8.13 (4H, m, *Ph*), 7.98–7.96 (2H, m, *Ph*), 7.60–7.45 (9H, m, *Ph*), 7.35 (1H, s, OCHC=C*H*S), 5.76 (1H, d, *J*=5.5 Hz, BzOC*H*CH_2), 5.56 (1H, dd, *J*=10, 5.5 Hz, OC*H*C=), 4.80–4.66 (2H, m, BzOC*H*$_2$CHO), 4.61 (1H, app d, *J*=6.5 Hz, $BzOCH_2$C*H*O), 2.78 (1H, dd, *J*=14, 5.5 Hz, BzOCH*C*H_2), 2.67 (1H, ddd, *J*=14, 10, 5.5 Hz, BzOCH*C*H_2); δ_C (125.8 MHz, $CDCl_3$) 168.65 (Ph*C*=N); 166.21 (Ph*C*O), 165.97 (Ph*C*O), 156.90 (OCHC=C*H*S), 133.45, 133.26, 132.98, 129.94, 129.73, 129.61, 128.78, 128.74, 128.38, 128.33, 128.29, 126.42 (Ar), 114.83 (OCH*C*=CHS), 82.89 (BzO*C*HCH_2), 77.90 (O*C*HC=CH), 77.64 ($BzOCH_2$*C*HO), 64.69 ($BzOCH_2$ *C*HO), 39.10 (BzOCH*C*H_2); ν_{max}/cm^{-1} 1722s, 1602m; *m*/*z* (electrospray) 486 (MH^+, 100%); HRMS found: MH^+ 486.1373 ($C_{28}H_{24}NO_5S$ requires 486.1375); $[\alpha]_D^{20}$ −30.0 [*c* 1.0, $CHCl_3$].

3.2.4. 3*S*-Benzoyloxy-2*R*-benzoyloxymethyl-5*S*-(2-phenylthiazol-4-yl)-tetrahydrofuran, 27α. Colourless oil, 147 mg, 22% yield from **15**, R_f 0.62 ethyl acetate/petrol (10:90); δ_H (500 MHz, $CDCl_3$) 8.10–8.09 (2H, m, Ar), 7.96–7.92 (2H, m, Ar), 7.80–7.78 (2H, m, Ar), 7.60–7.58 (1H, m, Ar), 7.50–7.26 (9H, m, *Ar*), 5.61 (1H, dd, *J*=6, 3 Hz, BzOC*H*CH_2), 5.58 (1H, dd, *J*=4, 8 Hz, OC*H*C=), 4.74–4.73 (1H, m, $BzOCH_2$C*H*), 4.63 (2H, d, BzOC*H*$_2$CH, *J*=4 Hz), 2.97 (1H, dt, *J*=14, 7 Hz, BzOCHC*H*$_2$),), 2.81 (1H, dt, *J*=14, 4 Hz, BzOCHC*H*$_2$); δ_C (100.6 MHz, $CDCl_3$) 168.11 (Ph*C*=N), 166.24 (Ph*C*O), 165.88 (Ph*C*O), 158.73 (OCHC=*C*HS), 133.50, 133.07, 166.06, 129.92, 129.62, 129.47, 128.79, 128.37, 128.17, 126.42 (Ar), 114.10 (OCH*C*=CHS), 82.37 (BzO*C*HCH_2), 77.92 (O*C*HC=CH), 76.44 ($BzOCH_2$*C*HO), 64.71 (BzO*C*H_2CHO), 38.17 (BzO*C*H_2CH); ν_{max}/cm^{-1} (neat film) 1722s; *m*/*z* (electrospray) 486 (MH^+, 100%); HRMS found: 486.1366 ($C_{28}H_{24}NO_5S$ requires 486.1375); $[\alpha]_D^{20}$ +6.4 [*c* 1.0, $CHCl_3$].

3.2.5. 3*S*-Benzoyloxy-2*R*-benzoyloxymethyl-5*R*-(2-aminothiazol-4-yl)-tetrahydrofuran, 28β. Colourless oil, 322 mg, 55% yield from **15**, R_f 0.55 ethyl acetate/petrol (50:50); δ_H (400 MHz, $CDCl_3$) 8.10–8.07 (4H, m, Ar), 7.67–7.56 (2H, m, Ar), 7.49–7.37 (4H, m, Ar), 6.51 (1H, s, C=C*H*), 5.61 (1H, d, *J*=5 Hz, BzOC*H*CH_2), 5.19 (1H, dd, *J*=10, 6 Hz, C*H*C=CH), 5.01 (2H, br, NH_2), 4.65–4.62 (2H, m, BzOC*H*$_2$CHO), 4.53 (1H, app s, $BzOCH_2$C*H*O), 2.56–2.45 (2H, m, BzOCHC*H*$_2$); δ_C (100.6 MHz, $CDCl_3$) 168.41 (=*C*NH_2), 166.77 (Ph*C*O), 166.48 (Ph*C*O), 151.78 (CHC=*C*H), 133.77, 133.66, 130.37, 130.24, 130.14, 128.91, 128.87, and 128.81 (Ar), 105.25 (CH*C*=CH), 83.17 (BzO*C*HCH_2), 77.93 (*C*HC=CH), 77.20 ($BzOCH_2$-*C*H), 65.23 (BzO*C*H_2CH), 39.02(BzOCH*C*H_2); ν_{max}/cm^{-1} (neat film) 3367 br m, 1721s; *m*/*z* (EI) 425 (MH^+, 100%); HRMS found: MH^+ 425.1178 ($C_{22}H_{21}N_2O_5S$ requires 425.1171); $[\alpha]_D^{20}$ −53 [*c* 1.0, $CHCl_3$].

3.2.6. 3*S*-Benzoyloxy-2*R*-benzoyloxymethyl-5*S*-(2-aminothiazol-4-yl)-tetrahydrofuran, 28α. Colourless oil, 205 mg, 35% yield from **15**, R_f 0.45 ethyl acetate/petrol (50:50) δ_H (400 MHz, $CDCl_3$) 8.08–8.05 (2H, m, Ar), 7.93–7.90 (2H, m, Ar), 7.59–7.53 (2H, m, Ar), 7.45–7.39 (4H, m, Ar), 6.49 (1H, s, C=C*H*), 5.60 (1H, ddd, *J*=10, 7, 4 Hz, BzOC*H*CH_2), 5.24 (1H, dd, *J*=8, 6 Hz, C*H*C=CH), 5.19 (2H, br, NH_2), 4.67 (1H, dd, *J*=8, 4 Hz, BzOCH_2C*H*), 4.58 (2H, d, *J*=8 Hz, BzOC*H*$_2$CH), 2.83 (1H, dd, *J*=13, 8 Hz, BzOCHC*H*$_2$), 2.53 (1H, ddd, *J*=13, 7, 6 Hz, BzOCHC*H*$_2$); δ_C (100.6 MHz, $CDCl_3$) 168.15 (=*C*NH_2), 166.32 (Ph*C*O), 166.06 (Ph*C*O), 152.64 (CHC=*C*H), 133.22, 133.14, 129.75, 129.68, 129.67, 129.62, 128.43 and 128.45 (Ar), 103.97 (CH*C*=CH), 81.87 (BzO*C*HCH$_2$), 77.46 (*C*HC=CH), 76.28 (BzOCH$_2$*C*H), 64.82 (BzO*C*H$_2$CH), 37.82(BzOCH*C*H$_2$); ν_{max}/cm^{-1} (neat film) 3367 br m, 1721s; *m*/*z* (EI) 425 (MH^+, 100%); HRMS found: MH^+ 425.1172 ($C_{22}H_{21}N_2O_5S$ requires 425.1171); $[\alpha]_D^{20}$ +4.5 [*c* 1.0, $CHCl_3$].

3.2.7. Preparation of 3*S*-benzoyloxy-2*R*-benzoyloxymethyl-5*R*/*S*-[6*H*-(1,3,4)-thiadiazin-2-amine-5-yl]-tetrahydro-furan 29α/β. To a solution of **15** (556 mg, 1.38 mmol) in methanol (20 mL) was added thiosemicarbazide hydrochloride (175 mg, 1.38 mmol, 1 equiv.) and the reaction mixture refluxed for 1 h. Then the solvent was removed and the residue purified by flash chromatography, to yield **29**α/β as a colourless oil, 490 mg, 81% yield from **15**, R_f 0.65 ethyl acetate/methanol (50:50); δ_H (400 MHz, $CDCl_3$) 8.07–7.96 (4H, m, Ar), 7.62–7.55 (2H, m, Ar), 7.49–7.42 (4H, m, Ar), 5.59 (1H, m, BzOC*H*CH_2), 5.15 (1H, m, C*H*C=N), 4.65–4.51 (3H, m, BzOCH_2C*H*), 3.41 (0.4H, d, *J*=14 Hz, N=CCH_2), 3.30 (0.4H, d, *J*=14 Hz, N=CCH_2), 3.29 (0.6H, d, *J*=14 Hz, N=CCH_2), 3.20 (0.6H, d, *J*=14 Hz, N=CCH_2), 2.92–2.84 (0.4H, m, BzOCHCH_2), 2.72–2.66 (0.4H, m, BzOCHCH_2), 2.58–2.31 (1.2H, m, BzOCHCH_2); δ_C (100.6 MHz, $CDCl_3$) 166.15(Ph*C*O), 166.05(Ph*C*O), 150.56 (=*C*NH_2), 133.46, 133.32, 133.24, 129.70, 129.66, 129.54, 129.45 and 128.59 (Ar), 95.67 (CH*C*=N), 83.36 and 82.60 (BzO*C*HCH$_2$), 80.53 and 80.08 (*C*HC=CH), 76.69 and 76.33 (BzOCH$_2$-*C*H), 64.73 and 64.54 (BzO*C*H$_2$CH), 37.37 and 36.29 (BzOCH*C*H$_2$), 20.58 and 20.36, (*C*H_2S); ν_{max}/cm^{-1} (neat film) 3320 br m, 1716s; *m*/*z* (AP^+) 439 (M^+, 100%); HRMS found: MH^+ 440.1281 ($C_{22}H_{22}N_3O_5S$ requires 440.1280).

3.2.8. 1-(2*R*/*S*-Ethoxy-4*S*-benzoyloxy-5*R*-benzoyloxymethyl-tetrahydrofuran-2-yl)-ethanone 32. To a solution of **15** (556 mg, 1.38 mmol) in ethanol (10.4 mL) was added benzamidine hydrochloride (215 mg, 1.38 mmol, 1 equiv.) and $NaHCO_3$ (115 mg, 1.38 mmol, 1 equiv.). The reaction mixture was refluxed for 4 h, then the solvent was removed and the residue purified by flash chromatography to yield **32** as a colourless oil, 370 mg (65% yield), R_f 0.45 ethyl acetate/petrol (20:80); δ_H (400 MHz, $CDCl_3$) 8.10–7.99 (4H, m, Ar), 7.62–7.56 (2H, m, Ar), 7.48–7.42 (4H, m, Ar), 5.63 (0.3H, ddd, *J*=3.5, 4.5, 7 Hz, BzOC*H*CH_2), 5.54 (0.7H, ddd, *J*=8, 5.5, 2.5 Hz, BzOC*H*CH_2), 4.71–4.53 (3H, m, BzOCH_2C*H*), 3.65 (1H, m, *J*=7 Hz, CH_3CH_2OC), 3.49 (0.7H, m, CH_3CH_2OC), 3.40 (0.3H, m, CH_3CH_2OC), 2.77 (0.3H, dd, *J*=14, 7 Hz, BzOCHCH_2), 2.63 (0.7H, dd, *J*=14, 7 Hz, BzOCHCH_2), 2.49 (0.3H, dd, *J*=15, 5 Hz, BzOCHCH_2), 2.42 (0.7H, dd, *J*=15, 2 Hz, BzOCHCH_2), 2.33 (0.9H, s, CH_3C=O), 2.24 (2.1H, s, CH_3C=O), 1.26 (2.1H, t, *J*=7 Hz, C$H_3$$CH_2$OC), 1.17 (0.9H, t, *J*=7 Hz, C$H_3$$CH_2$OC); δ_C (100.6 MHz, $CDCl_3$) 204.95 and 204.29 (CH_3*C*=O), 166.15, 166.12, 166.10, 165.82 (Ph*C*=O), 133.45, 133.38, 133.24, 133.18, 139.72, 129.68, 129.64, 129.56, 129.49, 129.22, 128.48, 128.44, 128.38, 128.26 (Ar), 109.43 and 109.15 (*C*–OCH_2CH_3), 83.30 and 83.06 (BzO*C*HCH$_2$), 74.85 and 74.72 (BzOCH$_2$*C*H), 64.34 and 64.06 (BzO*C*H$_2$CH), 59.57 and 59.23 (CO*C*$H_2$$CH_3$), 40.25 and 39.56 (BzOCH*C*H$_2$), 25.43 and 25.37 (CH_3C=O), 15.58 and 15.21 (COCH_2*C*H_3); ν_{max}/cm^{-1} (neat film) 1724s; *m*/*z* (electrospray) 430 (MNH_4^+, 100%); HRMS found: MNH_4^+ 430.1870 ($C_{23}H_{28}NO_7$ requires 430.1866).

3.3. General procedure for the preparation of compounds 33α, 33β, 34α and 34β

To solution of the benzoylated nucleoside **26**α, **26**β, **27**α and **27**β (0.4 mmol) in THF (20 mL) and water (20 mL) was added LiOH·H_2O (19 mg, 1.1 equiv.) and the solution stirred at room temperature for 24 h. The reaction mixture was concentrated in vacuo to leave a cloudy water phase, which was extracted with ethyl acetate (3×10 mL). The organic layer was dried Na_2SO_4 and concentrated in vacuo to give pure **33**α, **33**β, **34**α and **34**β.

3.3.1. 2*R*-Hydroxymethyl-5*R*-(2-methylthiazol-4-yl)-tetrahydrofuran-3*S*-ol, 33β. Colourless oil, 67 mg, 78% yield; δ_H (500 MHz, $CDCl_3$) 7.06 (1H, s, C=C*H*S), 5.24 (1H, dd, *J*=9, 1.5 Hz, HOC*H*CH_2), 4.32–4.30 (2H, m, HOCH$_2$C*H*OC*H*C=N), 3.69 (1H, dd, *J*=11.5, 4 Hz, HOCH_2CHO), 3.58 (1H, dd, *J*=11.5, 6 Hz, HOCH_2CHO), 2.70 (3H, s, CH_3C=N), 2.58 (1H, ddd, *J*=14, 9, 6.5 Hz, HOCHCH_2CHO), 2.17 (1H, m, HOCHCH_2CHO); δ_C (125.8 MHz, $CDCl_3$) 167.7 (CH_3*C*=N), 156.4 (OCHC=*C*HS), 115.8 (OCH*C*=CHS), 89.2 (HO*C*HCH$_2$), 76.4 (O*C*HCN=C), 73.7 (HOCH$_2$*C*HO), 63.6 (HO*C*H$_2$-CH), 39.7 (HOCH*C*H$_2$CHO), 19.0 (*C*H_3C=N); ν_{max}/cm^{-1} (neat film) 3366 br, *m*/*z* (electrospray) 216 (MH^+,100%); HRMS found: MH^+ 216.0684 ($C_9H_{14}NO_3S$ requires 216.0694); $[\alpha]_D^{20}$ +50.0 [*c* 1.0, $CHCl_3$].

3.3.2. 2*R*-Hydroxymethyl-5*S*-(2-methylthiazol-4-yl)tetrahydrofuran-3*S*-ol, 33α. Colourless oil, 61 mg (71% yield); δ_H (500 MHz, $CDCl_3$) 7.00 (1H, s, C=C*H*S), 5.30 (1H, dd, *J*=9.5, 6.5 Hz, HOC*H*CH_2), 4.66 (1H, d, *J*=5 Hz, OC*H*C=CH), 4.16 (1H, app s, HOCH$_2$C*H*O), 3.93 (1H, dd, *J*=12.5, 2.5 Hz, HOCH_2CHO), 3.72 (1H, d, *J*=12.5 Hz, HOCH_2CHO), 2.69 (3H, s, CH_3C=N), 2.56 (1H, ddd, *J*=13.5, 9.5, 5.5 Hz), 2.26 (1H, dd, *J*=13.5, 6.5 Hz, CH_2CHO); δ_C (125.8 MHz, $CDCl_3$) 167.7 (CH_3*C*=N), 156.7 (OCHC=*C*H), 114.9 (OCH*C*=CH), 88.9 (O*C*HCH$_2$), 76.2 (O*C*HC=CH), 75.4 (HOCH$_2$*C*HO), 64.10 (HO*C*H$_2$CH), 42.7 (HOCH*C*H$_2$O), 19.1 (*C*H_3C=N); ν_{max}/cm^{-1} (neat film) 3367 br; *m*/*z* (electrospray) 216 (MH^+, 100%); HRMS found: MH^+ 216.0694 ($C_9H_{14}NO_3S$ requires 216.0694); $[\alpha]_D^{20}$ +4.1 [*c* 1.0, $CHCl_3$].

3.3.3. 2*R*-Hydroxymethyl-5*R*-(2-phenylthiazol-4-yl)-tetrahydrofuran-3*S*-ol, 34β. Colourless oil, 95 mg, 86% yield; δ_H (400 MHz, $CDCl_3$) 7.88–7.85 (2H, m, Ph), 7.49–7.39 (3H, m, Ph), 7.37 (1H, s, OCHC=C*H*S), 5.32 (1H, dd, *J*=1.5, 9 Hz), 4.39–4.37 (2H, m HOCH$_2$

C*H*OC*H*C═CH), 3.73 (1H, dd, *J*=11, 4 Hz, HOC*H*$_2$CHO), 3.62 (1H, dd, *J*=11, 5.5 Hz, HOC*H*$_2$CHO), 2.63 (1H, ddd, *J*=14, 9, 6.5 Hz, HOCHC*H*$_2$CHO), 2.34 (1H, m, HOCHC*H*$_2$CHO); δ_C (125.8 MHz, $CDCl_3$) 169.88 (Ph*C*═N), 157.68 (OCHC═*C*H), 132.64, 130.11, 128.21, 126.34 (Ar), 116.10 (OCH*C*═CH), 89.10 (O*C*HCH$_2$), 76.22 (O*C*HC═CH), 74.10 (HOCH$_2$*C*HO), 63.71 (HO*C*H$_2$CH), 40.01(HOCH*C*H$_2$O); ν_{max}/cm^{-1} (neat film) 3030 br; *m*/*z* (electrospray) 278 (MH$^+$, 100%); HRMS found: MH$^+$ 278.0848 ($C_{14}H_{16}NO_3S$ requires 278.0851). $[\alpha]_D^{20}$ +45.0 [*c* 1.0, $CHCl_3$].

3.3.4. 2*R*-Hydroxymethyl-5*S*-(2-phenylthiazol-4-yl)tetrahydrofuran-3*S*-ol, 34α. Colourless oil, 91 mg (82% yield); δ_H (400 MHz, $CDCl_3$) 7.94–7.88 (2H, m, Ph), 7.49–7.39 (3H, m, Ph), 7.16 (1H, s, OCHC═C*H*S), 5.39 (1H, dd, *J*=9, 7 Hz, HOC*H*CH$_2$), 4.70 (1H, d, *J*=5 Hz, OC*H*C═CH), 4.21 (1H, app s, HOCH$_2$C*H*O), 3.97 (1H, dd, *J*=2.5, 12 Hz, HOC*H*$_2$CHO), 3.76 (1H, dd, *J*=9, 2 Hz, HOC*H*$_2$CHO), 2.65 (1H, ddd, *J*=14, 9, 5.5 Hz), 2.30 (1H, dd, *J*=14, 6.5 Hz, C*H*$_2$CHO); δ_C (80 MHz, $CDCl_3$) 169.88 (Ph*C*═N), 157.99 (OCHC═*C*H), 132.71, 130.34, 128.97 and 126.34 (Ar), 115.26 (OCH*C*═CH), 88.71 (O*C*HCH$_2$), 76.31 (O*C*HC═CH), 75.19 (HOCH$_2$*C*HO), 64.07 (HO*C*H$_2$CH), 42.67(HOCH*C*H$_2$O); ν_{max}/cm^{-1} (neat film) 3015 br; *m*/*z* (electrospray) 278 (MH$^+$, 100%); HRMS found: MH$^+$ 278.0854 ($C_{14}H_{16}NO_3S$ requires 278.0851). $[\alpha]_D^{20}$ +10.8 [*c* 1.0, $CHCl_3$].

3.4. General procedure for the preparation of compounds 35α, 35β, and 36α/β

A stream of NH_3 was bubbled through a solution of the benzoylated nucleoside **28**α, **28**β, and **29**α/β (0.4 mmol) in methanol (20 mL) for 5 min. The reaction mixture was stirred at room temperature for 24 h, then the solvent removed in vacuo. The residue was dissolved in water (1 mL) and washed with dichloromethane (3×2 mL). The aqueous phase was evaporated to give **35**α, **35**β, and **36**α/β.

3.4.1. 2*R*-Hydroxymethyl-5*R*-(2-aminothiazol-4-yl)tetrahydrofuran-3*S*-ol, 35β. Colourless oil, 59 mg, 68% yield; δ_H (200 MHz, D_2O) 6.33 (1H, s, OCHC═C*H*S), 4.72 (1H, t, HOC*H*CH$_2$, *J*=8 Hz), 4.09 (1H, dd, *J*=5, 2.5 Hz, OC*H*C═CH), 3.67 (1H, dd, *J*=8, 5 Hz, HOCH$_2$C*H*O), 3.45–3.31 (2H, m, HOC*H*$_2$CHO), 1.93–1.86 (2H, m, C*H*$_2$CHO); δ_C (80 MHz, $CDCl_3$) 173.82 (*C*NH$_2$), 152.88 (OCH*C*═CH), 108.67 (C═*C*–S), 89.82 (O*C*HCH$_2$), 79.16 (O*C*HC═CH), 75.60 (HOCH$_2$*C*HO), 65.10 (HO*C*H$_2$CH), 42.55 (HOCH*C*H$_2$); ν_{max}/cm^{-1} (neat film) 3030 br; *m*/*z* (electrospray) 217 (MH$^+$, 100%); HRMS found: MH$^+$ 217.0647 ($C_8H_{13}N_2O_3S$ requires 217.0647); $[\alpha]_D^{20}$ +42.0 [*c* 1.0, H_2O].

3.4.2. 2*R*-Hydroxymethyl-5*S*-(2-aminothiazol-4-yl)tetrahydrofuran-3*S*-ol, 35α. Colourless oil, 56 mg, 65% yield; δ_H (200 MHz, D_2O) 6.34 (1H, s, OCHC═C*H*S), 4.74 (1H, t, HOC*H*CH$_2$, *J*=7 Hz), 4.08 (1H, dd, *J*=7, 5 Hz, OC*H*C═N), 3.82–3.72 (1H, m, HOCH$_2$C*H*O), 3.48–3.30 (2H, m, HOC*H*$_2$CHO), 2.31 (1H, m, C*H*$_2$CHO), 1.86 (1H, m, C*H*$_2$CHO); δ_C (80 MHz, $CDCl_3$), 170.08 (*C*NH$_2$), 149.41 (OCH*C*═CH), 104.71 (C═*C*–S), 85.10 (O*C*HCH$_2$), 75.06 (O*C*HC═CH), 71.24 (HOCH$_2$*C*HO), 60.69 (HO*C*H$_2$CH), 38.06 (HOCH*C*H$_2$); ν_{max}/cm^{-1} (neat film) 3030 br; *m*/*z* (electrospray) 217 (MH$^+$, 100%); HRMS found: MH$^+$ 217.0654 ($C_8H_{13}N_2O_3S$ requires 217.0647); $[\alpha]_D^{20}$ +5.6 [*c* 1.0, H_2O].

3.4.3. 5-*R*/*S* (2-Amino-6*H*-[1,3,4]thiadiazin-5-yl)-2*R*-hydroxymethyltetrahydrofuran-3*S*-ol, 36α/β. Colourless oil, 69 mg, 75% yield; δ_H (200 MHz, D_2O) 4.86 (1H, t, HOC*H*CH$_2$, *J*=8 Hz), 4.32–4.28 (1H, m, OC*H*C═N), 3.96–3.93 (1H, m, HOCH$_2$C*H*O), 3.64–3.51 (2H, m, HOC*H*$_2$CHO), 3.32–3.19 (2H, m, C*H*$_2$S), 3.24 (2H, s, NH$_2$), 2.13–2.08 (2H, m, C*H*$_2$CHO); δ_C (80 MHz, D_2O), 171.65 (*C*NH$_2$), 154.55 (OCH*C*═N), 87.86 (O*C*HCH$_2$), 79.89 (O*C*HC═CH), 72.72 (HOCH$_2$*C*HO), 62.26 (HO*C*H$_2$CH), 38.92 (HOCH*C*H$_2$), 20.10 (*C*H$_2$S); ν_{max}/cm^{-1} (neat film) 3050 br; *m*/*z* (electrospray) 232 (MH$^+$, 35%); HRMS found: MH$^+$ 232.0764 ($C_8H_{14}N_3O_3S$ requires 232.0756).

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Photochemical synthesis of triazolo[3,4-*b*]-1,3(4*H*)-benzothiazines: a detailed mechanistic study on photocyclization/photodesulfurisation of triazole-3-thiones

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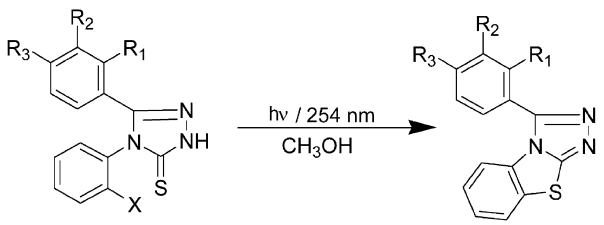
Abstract—Irradiation of 4-(2-halobenzyl)-5-substituted-1,2,4-triazole-3-thiones under base mediated (CH_3CN/2 M NaOH) condition afforded triazolo[3,4-*b*]-1,3(4*H*)-benzothiazines and desulfurized triazoles. Benzophenone sensitized photolysis of triazole-3-thiones gave desulfurized triazoles exclusively. The mechanism of the photocyclization/photodesulfurization and the involvement of singlet and triplet energy levels are discussed.

1. Introduction

The photochemistry of thiocarbonyl compounds has received much attention from both synthetic and mechanistic points of views. Earlier workers from our laboratories reported[1] the synthesis of condensed benzothiazoles from thioamides, such as the synthesis of *s*-triazolo[3,4-*b*]-benzothiazoles (Scheme 1) from 4-(*o*-halophenyl)-5-substituted 1,2,4-triazole-3-thiones by photolysis. Recently, we reported the synthesis of isoquinoline-fused benzoxazole and benzoxazine systems, under base mediated photolytic conditions.[2]



Scheme 1.

In continuation of our interest in the synthesis of biologically active nitrogen and sulfur containing heterocycles, and the importance of triazole fused benzothiazine ring systems,[3–6] we focused our work on the synthesis of condensed benzothiazines. In a preliminary communication, we reported the synthesis of triazolo[3,4-*b*]-1,3(4*H*) benzothiazines and desulfurization of 1,2,4-triazole-3-thiones.[7] In this paper, we discuss the synthesis and mechanistic aspects on the formation of triazolo benzothiazines and desulfurization of triazole-3-thiones on irradiation under basic and triplet sensitized conditions.

2. Results and discussion

Refluxing a mixture of *o*-halobenzyl isothiocyanate (1 equiv.) and the substituted acid hydrazide (1 equiv.) in ethanol[8] afforded the corresponding thiosemicarbazides which were directly converted into the respective 4-(*o*-halobenzyl)-5-substituted-1,2,4-triazole-3-thiones **1a–g** in good yield (Scheme 2) using 10% K_2CO_3 (Table 1).

The structures of triazole-3-thiones **1a–g** were confirmed by spectral and analytical data. IR spectra of thiones **1a–g** were comparable with those reported in the literature.[9,10] The presence of a strong band in the region of 1300 cm^{-1} corresponds to the thione (–C=S) form and a weak band around 2600 cm^{-1} indicates the thiol (–SH) form. In the ^1H NMR of the triazole-3-thiones, a singlet around δ 13 showed

Table 1. Synthesis of substituted 1,2,4-triazole-3-thiones **1a–g**

Compound	R^1	X
1a	Phenyl	Cl
1b	*p*-Tolyl	Cl
1c	*p*-Anisyl	Cl
1d	*o*-Tolyl	Br
1e	Benzyl	Cl
1f	*o*-Chlorophenyl	Cl
1g	*o*-Chlorophenyl	Br

Keywords: Cyclization; Benzothiazines; Desulfurization; Triazoles.
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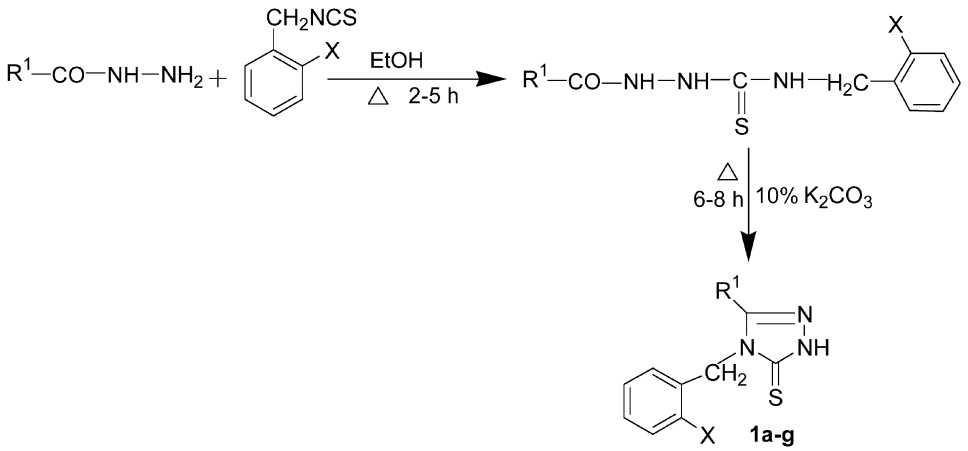


Scheme 2.

the presence of –SH proton. ^{13}C NMR also showed the presence of thiocarbonyl carbon around δ 169. In the ^{13}C NMR, the thiocarbonyl carbon appears upfield compared to the carbonyl carbon signal.[11] The signal at δ 160 for the above compounds could be represented as for the thiol form.

The ^{1}H NMR spectra of **1a** exhibited a singlet at δ 5.37 for the $-CH_2$ protons, a multiplet between δ 6.96–7.46 for aromatic protons and a singlet at δ 13.88 for the –SH proton. The ^{13}C NMR spectrum of **1a** showed a peak at δ 45.7 for $-CH_2$ carbon, which was confirmed by the DEPT-135 spectrum, which showed $-CH_2$ carbon as an inverted peak. The X-ray crystallography data confirmed the structure of **1b**.[12]

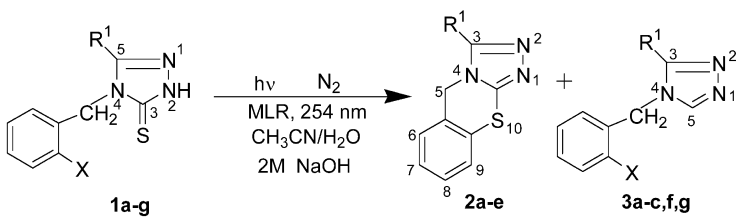
2.1. Irradiation studies

The irradiation experiments were carried out at 254 nm in an Applied Photophysics multilamp reactor (MLR). The various conditions are tabulated in Table 2. An acetonitrile solution (150 mL) of 4-(2-chlorobenzyl)-5-phenyl-1,2,4-triazole-3-thione **1a** (0.3 g, 0.9 mmol) containing 30 mL of aqueous 2 M NaOH (entry 6 in Table 2) was flushed with nitrogen for 1 h and irradiated at 254 nm for 18 h; workup and chromatographic purification furnished the photosubstituted 3-phenyl-1,2,4-triazolo [3,4-*b*]-1,3(4*H*)-benzothiazine **2a** (41%) and photodesulfurized 4-(2-chlorobenzyl)-5-phenyl-1,2,4-triazole **3a** (10%). Similarly, irradiation of triazole-3-thiones **1b**–**e** (entries 8, 9, 11 and 12 in Table 2) in CH_3CN containing 2 M NaOH in MLR for 11–21 h, furnished the corresponding photosubstituted triazolobenzothiazines **2b**–**e** and photodesulfurized triazoles **3b**,**c**. In the case of **1d**,**e**, no desulfurized product was observed (Scheme 3).

The photosubstitution reaction described here would involve the intramolecular displacement of the halogen atom present in the *N*-benzyl moiety of the triazole-3-thiones **1a**–**e** by the thiocarbonyl sulphur of the thioamide. The photodesulfurization would take place in the thiol (–SH) form of the triazole-3-thiones.

In the presence of base such as NaOH, the UV–Vis absorption behaviour of thione **1a** (λ_{max}=262 nm) in CH_3CN changed to λ_{max}=300 nm. The absorption at 300 nm in the UV spectra is believed to be the formation of anionic form of the triazole-3-thione, due to deprotonation by base. Thus, the anionic species is formed in solution, which induces the photosubstitution reaction. When the irradiation of **1a** was carried out in acetonitrile containing weak bases such as aqueous K_2CO_3 (0.5 M) or NaOH (0.5 M) (entries 4, 5 in Table 2), the product **2a** was formed in lower yield compared to using 2 M NaOH conditions (entry 6). A Similar trend was observed in the case of **1b** (entries 7, 8) also. Probably an anionic thiol of the thioamide bond was not efficiently populated in this medium for the substitution reaction.

The irradiation of **1a** in protic solvent (methanol) containing 0.5 M K_2CO_3 or 0.5 M NaOH (entries 2,3) was carried out; the formation of desulfurized product **3a** increased by more than three times compared to the substituted product **2a**. This is due to the proton donor behavior of methanol, which probably inhibits the substitution by blocking the formation of anionic sulphur of **1a** by its protonation, which invariably increases the population of the thiol (–SH) form. In order to study the reaction pathway for the photosubstitution and photodesulfurization of triazole-3-thiones, the photolysis of thione **1a** was carried out under nitrogen as



Scheme 3.

Table 2. Irradiation of triazole-3-thiones **1a-g** under various reaction condition

Entry	Compound	Reaction medium	R_1	Reaction time (h)	X	**2** Yield (%)	**3** Yield (%)
1	**1a**	CH_3OH/Ph_2CO	Ph	62	Cl	-	67
2	**1a**	CH_3OH/0.5 M K_2CO_3	Ph	45	Cl	11	50
3	**1a**	CH_3OH/0.5 M NaOH	Ph	30	Cl	15	48
4	**1a**	CH_3CN/0.5 M K_2CO_3	Ph	20	Cl	30	15
5	**1a**	CH_3CN/0.5 M NaOH	Ph	20	Cl	32	16
6	**1a**	CH_3CN/2 M NaOH	Ph	18	Cl	41	10
7	**1b**	CH_3CN/0.5 M NaOH	*p*-Tolyl	22	Cl	38	11
8	**1b**	CH_3CN/2 M NaOH	*p*-Tolyl	17	Cl	47	8
9	**1c**	CH_3CN/2 M NaOH	*p*-Anisyl	20	Cl	38	15
10	**1d**	CH_3OH/Ph_2CO	*o*-Tolyl	48	Br	-	71
11	**1d**	CH_3CN/2 M NaOH	*o*-Tolyl	11	Br	50	-
12	**1e**	CH_3CN/2 M NaOH	Benzyl	21	Cl	45	-
13	**1f**	CH_3CN/2 M NaOH	*o*-Chlorophenyl	32	Cl	-	47
14	**1g**	CH_3CN/2 M NaOH	*o*-Chlorophenyl	13	Br	-	48

well as oxygen atmosphere. Hence, an acetonitrile solution (50 mL) of **1a** (0.1 g) containing aqueous NaOH (0.5 M, 10 mL), was irradiated in a MLR (254 nm) for 20 h under nitrogen (or oxygen) atmosphere. The photoproducts of the reaction mixture were analysed by HPLC. Under a nitrogen atmosphere, the photosubstitution reaction is faster than the photodesulfurization (entry 1 in Table 3). Whereas, in oxygen atmosphere, which is a triplet quencher, the substitution reaction was not affected, but the desulfurization was reduced considerably (entry 2 in Table 3).

The irradiation of **1a** (0.3 g) in methanol (180 mL) containing benzophenone (1.2 equiv.) a triplet sensitizer, was carried out in a MLR (254 nm) for 62 h (entry 1 in Table 2) to afford exclusively the desulfurized triazole **3a** in 67% yield. In this reaction, no substitution product was formed. A Similar trend was observed in the irradiation of the bromo analogue **1d** (entry 10 in Table 2). Hence, in the base mediated irradiation reactions of triazole thiones, a singlet state is involved for substitution reaction and a triplet state in the photodesulfurization reaction.

The irradiation of 4-(2-chlorobenzyl)-5-(2-chlorophenyl)-1,2,4-triazole-3-thione **1f** in CH_3CN/2 M NaOH conditions (entry 13 in Table 2) for 32 h furnished exclusively the desulfurized triazole **3f**. In this reaction, no photosubstituted product was formed. Similarly, the irradiation of 4-(2-bromobenzyl)-5-(2-chlorophenyl)-1,2,4-triazole-3-thione **1g** in CH_3CN/2 M NaOH conditions (entry 14 in Table 2) also afforded the desulfurized product **3g** exclusively. This is due to the presence of the *o*-chlorine atom as the 5-aryl substituent of triazole thione, which increases the population of the triplet state involved for the desulfurization of triazole thiones by enhancing inter system crossing effect (heavy atom effect).[13]

Table 3. Relative rates of the formation of **2a** and **3a**

Entry	Compound	Reaction medium	Atmosphere	Relative rate	
				2a	**3a**
1	**1a**	CH_3CN/0.5 M NaOH	N_2	2.0	1.0
2	**1a**	CH_3CN/0.5 M NaOH	O_2	2.0	0.25

HPLC-Column used—Bondapak C18; Solvent—CH_3OH/H_20 (8:2); Rate—1 mL/1 min; UV_{max}—254 nm (detector).

Thus, one possible explanation for the formation of the photosubstitution product is that an anionic nucleophile of thiocarbonyl sulphur of triazole-3-thione in basic medium intramolecularly displaces the halogen of the haloarene in the singlet excited state (S_N2Ar^*).[14] Another possibility is that an anionic radical species photoinduced from anionic thiocarbonyl sulphur of the thioamide bond in basic medium intramolecularly substitutes the halogen of the haloarene ($S_N(ET)Ar^*$)[14] (Scheme 4).

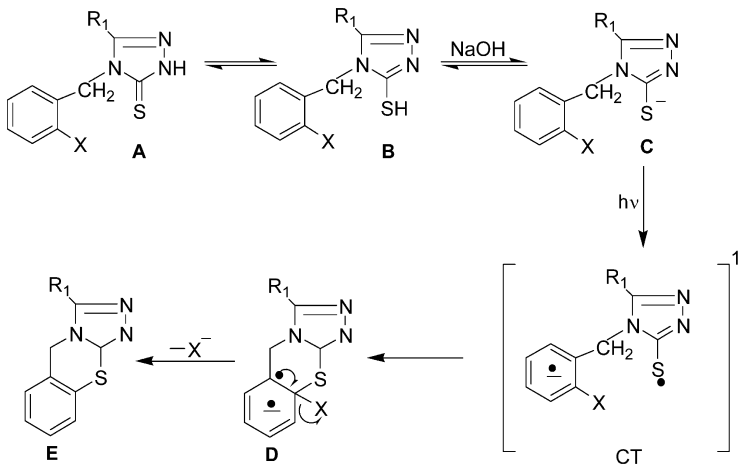
An intramolecular electron transfer mechanism has been proposed for the formation of 2-(pyridinyl)-benzoxazoles.[15] Earlier, we proposed intramolecular electron-transfer mechanism for the formation of benzothiazoles from the photoreaction of *o*-halothioacetamide,[1] and we observed the Cl_2^{-} (anion radical) at λ_{max} ~345 nm by laser flash and steady state experiments.

The structure of photoproducts **2** and **3** were consistent with the spectral and analytical data. The IR spectra of 3-(*p*-tolyl)-1,2,4-triazolo-(3,4-*b*)-1,3(4*H*)-benzothiazine **2b**, showed bands at 1593 (C=N), 748 (C–S) cm^{-1}. ^1H NMR spectrum of **2b** showed singlets at δ 2.45 and 5.20 for CH_3 and C_4-CH_2 protons, while the ^{13}C NMR showed signals at δ 21.44 (CH_3), 47.61 (CH_2). The mass spectra of **2b** showed the molecular ion M^+ (*m*/*z* 279) as a base peak. The mass fragment at *m*/*z* 161 indicates the fragmentation of Ph–CN from the M^+-1 peak. The other compounds follow the same pattern.

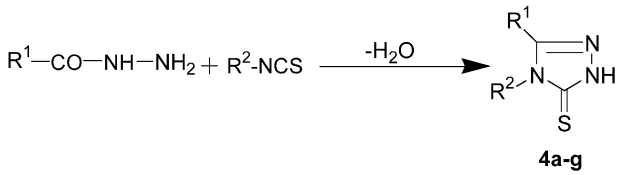
The ^1H NMR spectra of photodesulfurized triazoles **3a–d**,**f**,**g** exhibited a singlet around δ 8 for triazole C_5-CH proton and another singlet around δ 5.2 for the methylene protons. The ^{13}C NMR spectra of triazoles **3** showed a peak around δ 144 for C_5-CH carbon of the triazole ring. The mass spectrum of **3b** showed molecular ion M^+ peak (*m*/*z* 283). The fragmentation of $Cl^{\cdot}$ from molecular ion was observed (*m*/*z* 248).

2.2. Triplet sensitized photolysis of substituted 1,2,4-triazole-3-thiones 1a–d and 4a–g

The substituted 1,2,4-triazole-3-thiones **4a–g** were synthesized (Scheme 5) from the corresponding acid hydrazide (1 equiv.) and isothiocyanate (1 equiv.) in good yield (Table 4). The triazolethiones **4a–e**[16] and **4f**[1] were reported



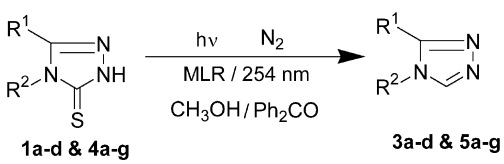
Scheme 4.



Scheme 5.

earlier. The structure of compound **4g** was confirmed by spectral and analytical data, and XRD.[17]

A methanolic solution of triazole-3-thione **4a** (0.3 g, 1 mmol), after flushing with nitrogen for 1 h, was irradiated in multilamp reactor (254 nm). Even after 100 h of irradiation, no reaction was observed. Next, thione **4a** in methanol containing 1.2 equiv. of benzophenone, was flushed with N_2 and irradiated using multilamp reactor for 62 h, to afford the desulfurized 3-phenyl-4-methylphenyl-1,2,4-triazole **5a** in good yield, (Scheme 6). A trace of sulfur was also isolated along with the desulfurized product. Likewise, irradiation of the triazole-3-thiones **4b**–**e** in methanol, also afforded the corresponding desulfurized triazoles **5b**–**e** in good yields (Table 5). The irradiation of **1a**–**d** in the presence of benzophenone yielded the respective desulfurized triazoles **3a**–**d**, instead in an experiment in the absence of benzophenone, thione **1a** did not give any product even after 100 h of irradiation. The photoproducts **5a**–**e** were confirmed by mp and mixture mp and superimposable IR with those obtained under thin film reaction (TFR) condition, which has been reported earlier.[16] Likewise the photoproducts **3a**–**c** were confirmed by mp, mixture mp and superimposable IR with those obtained under base mediated photolytic conditions. The structure of **3d** was confirmed by spectral and analytical data.



Scheme 6.

The desulfurization of 1,2,4-triazole-3-thione by (i) using Raney Ni/EtOH and (ii) using 17% HNO_3, to the corresponding 1,2,4-triazole is known.[18,19] Reaction of **4a** (0.5 g, 1.9 mmol) in the presence of Raney Ni for 23 h, afforded **5a** (47%). Likewise, the treatment of **4b** (2.1 mmol) with 17% HNO_3 (50 mL) under reflux for 5 h, afforded the desulfurized triazole **5b** in 55% yield, compared by mp, mixture mp and superimposable IR.

Benzophenone sensitized irradiation of 4-(2-chlorophenyl)-5-phenyl-1,2,4-triazole-3-thione **4f** in methanol for 50 h afforded the desulfurized 3-phenyl-4-(2-chlorophenyl)-1,2,4-triazole **5f**, instead of the respective triazolobenzothiazole, which was obtained[1] earlier by the irradiation of **4f** in methanol, in the absence of benzophenone using MLR. The structure of photoproduct **5f** was confirmed by spectral and analytical data.

Irradiation of **4g** using MLR (100 h) did not afford any product, but the starting triazole-3-thione in 82% yield. However, the irradiation of **4g** in the presence of benzophenone (1.2 equiv.) in MLR for 65 h, furnished the desulfurized product 3,4-di-(2-chlorophenyl)-1,2,4-triazole **5g** in 60% yield.

The triazole-3-thione **4a** (no *o*-halogen in the 4-aryl ring) and 4-(2-chlorobenzyl)-5-phenyl-1,2,4-triazole-3-thione **1a** in methanol, do not undergo any photochemical reaction in MLR (254 nm) in the absence of benzophenone. Whereas, the photolysis of these thiones in the presence of benzophenone, which is a well-known triplet sensitizer (E_T=69 kcal/mol) produced desulfurized triazole in MLR

Table 4. The various substituted 1,2,4-triazole-3-thiones **4a**–**g**

Compound **4a**–**g**	R^1	R^2
4a	Phenyl	*p*-Tolyl
4b	*p*-Tolyl	*p*-Tolyl
4c	*o*-Tolyl	1-Naphthyl
4d	4-Pyridyl	*p*-Tolyl
4e	Phenyl	Benzyl
4f	Phenyl	*o*-Chlorophenyl
4g	*o*-Chlorophenyl	*o*-Chlorophenyl

Table 5. Photodesulfurization of triazole-3-thiones using multilamp reactors (MLR)

Starting compound	R^1	R^2	Product	Reaction time (h)	Yield (%)
4a	Phenyl	*p*-Tolyl	**5a**	38	42
4b	*p*-Tolyl	*p*-Tolyl	**5b**	40	59
4c	*o*-Tolyl	1-Naphthyl	**5c**	30	70
4d	4-Pyridyl	*p*-Tolyl	**5d**	43	30
4e	Phenyl	Benzyl	**5e**	33	57
4f	Phenyl	2-Chloro phenyl	**5f**	50	52
4g	2-Chloro phenyl	2-Chloro phenyl	**5g**	65	60
1a	Phenyl	2-Chloro benzyl	**3a**	62	67
1b	*p*-Tolyl	2-Chloro benzyl	**3b**	53	60
1c	*o*-Anisyl	2-Chloro benzyl	**3c**	47	48
1d	*o*-Tolyl	2-Bromo benzyl	**3d**	48	71

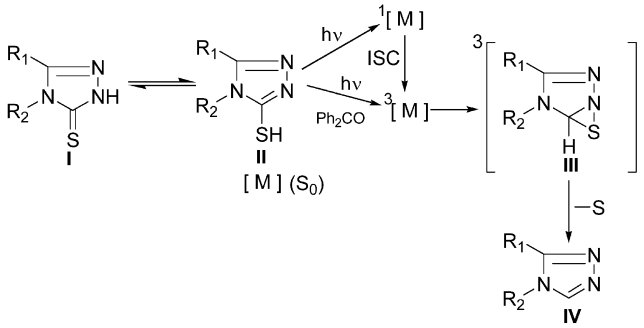
using methanol as a solvent. In this line, photolysis of **1b–d** and **4b–g** also afforded the desulfurized triazole (Table 5), in the presence of benzophenone.

Similarly, photolysis of a methanolic (180 mL) solution of **1a** containing xanthone (1.2 equiv.) (E_T=74 kcal/mol), after flushing with nitrogen, in MLR for 48 h also yielded desulfurized triazole **3a** in 75%yield.

To ensure the absorption of light exclusively by benzophenone, a longer wavelength experiment was carried out. Photolysis of **1a** in methanol (180 mL) containing benzophenone (1.2 equiv.), after flushing with nitrogen, under multilamp reactor with wave length 365 nm for 70 h, also afforded the desulfurized triazole **3a**, but in a slightly lower yield (59%), when compared with the benzophenone experiment (67% yield) at 254 nm. This indicates that some reaction proceeds from the direct excitation of the substrate [M] to S_1, followed by ISC to T_1, which will undergo the reaction in the triplet state. The slightly higher yield (75%) of the product, when xanthone with higher E_T was used, also supports the triplet state reaction for the desulfurization.

From the above observations, we believe that photodesulfurization of triazole-3-thiones using MLR, in presence of benzophenone is a triplet state reaction. This was further evidenced by quenching the triplet state with a triplet quencher. For that, the photolysis of **1a** was carried out in methanol (180 mL) containing benzophenone (1.2 equiv.) after flushing with oxygen for 1 h in MLR (254 nm). After 67 h of irradiation, chromatographic separation afforded 50% of recovered starting triazole-3-thione, in addition to a trace of desulfurized triazole **3a**. The formation of a trace amount of the desulfurized product **3a** could be due to inefficient oxygen supply, since oxygen was not bubbled throughout the reaction. From the above observations, it is concluded that the desulfurization of triazole-3-thiones must be a triplet state reaction.

The irradiation of thione in CH_3CN/NaOH medium giving cyclization product probably takes place in the singlet state by electron transfer mechanism from the thioenolate anion. On the other hand, the formation of desulfurization product could be from the thiol itself in the conventional $S_0 \rightarrow S_1 \rightarrow T_1$ pathway involving the triplet state of the thiol. A reasonable mechanism for the formation of desulfurized triazole from triazole-3-thione is represented in Scheme 7.



Scheme 7.

In this postulated mechanism, the triplet state episulfide **III** is formed initially through the thiol tautomer **II** (the thiol form was confirmed by ^{1}H NMR and IR of thione) by (i) direct population of triplet state, under benzophenone sensitized reaction (ii) population of singlet state followed by triplet state, under base mediated condition. Then the episulfide intermediate loses sulfur to give the triazole **IV**. Photoinduced desulfurization of episulfide, and desulfurization of indoline-2-thione to indole via-episulfide had been reported.[20,21] The episulfide intermediate **III** in the reaction could not be confirmed. However, irradiation in a non-protic solvent such as benzene containing **4a** and benzophenone (1.2 equiv.) using MLR for 27 h, furnished the respective desulfurized product **5a**.

3. Conclusions

The triazole-3-thiones **1a–g** and **4a–g** were synthesized from the corresponding acid hydrazides and isothiocyanates. Irradiation of 4-(2-halobenzyl)-5-substituted-1,2,4-triazole-3-thiones **1a–e** under base mediated condition afforded the respective 1,2,4-triazolo[3,4-*b*]-1,3(4*H*)-benzothiazines **2a–e** and desulfurized triazoles **3a–c** depending on the concentration of the base employed. However, photolysis of **1f**,**g** afforded only the desulfurized triazoles **3f**,**g** even under stronger basic condition (CH_3CN/2 M NaOH). Irradiation of triazole-3-thiones **1a–d** and **4a–g**, using MLR in presence of benzophenone (triplet sensitizer) furnished desulfurized triazoles **3a–d** and **5a–g**, exclusively. A reasonable mechanism for the photocyclization involving singlet state, and for the desulfurization of triazole-3-thiones, in the triplet state via an episulfide intermediate has been proposed.

4. Experimental

4.1. General

All the melting points are uncorrected. UV spectra were recorded with Shimadzu 1601 spectrophotometer. IR spectra were recorded on FTIR-8300 Shimadzu spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded with Brucker-DPX 200 (200 MHz) and Jeol-GSX 400 (400 MHz) instruments with TMS as internal standard (chemical shift in δ ppm). The mass spectra were recorded with Jeol-JMS-DX 303 HF and GCMS QP 5000 Shimadzu instruments. Chromatographic separations were done using silica gel (ACME samples). Thin layer chromatography (TLC) was performed using glass plates coated with silica gel (ACME samples) of 0.25 mm thickness. Spots were visualized using iodine vapour. The photochemical reactions were carried out in quartz vessel of different capacity in Applied Photophysics multilamp reactor (254, 365 nm, 12 lamps).

4.1.1. Synthesis of substituted 1,2,4-triazole-3-thiones 1a–g 4-(2-chlorobenzyl)-5-phenyl-1,2,4-triazole-3-thione (1a). A mixture of 2-chlorobenzyl isothiocyanate (1.34 g, 7.4 mmol) and benzoic acid hydrazide (1 g, 7.4 mmol) in ethanol (75 mL) was refluxed on a water bath for 2 h, to afford the corresponding thiosemicarbazide upon cooling. The thiosemicarbazide thus obtained was filtered, then it was refluxed in 10% K_2CO_3 for 6 h, cooled, filtered and the filtrate washed with ether. The aqueous layer was acidified with cold, dil. HCl. The separated solid was filtered and washed with water to furnish thiazine **1a**; recrystallized from ethyl acetate. Yield: 1.69 g (76%), colourless solid, mp 210–212 °C; UV (λ_{max}): 260 nm (CH_3CN); IR (KBr): 3084, 2935, 2598, 1554, 1431, 1353, 1276 cm^{-1}; ^{1}H NMR ($CDCl_3$/DMSO-d_6, 200 MHz): δ 5.37 (s, 2H, CH_2), 6.96–7.46 (m, 9H, ArH), 13.88 (s, 1H, SH). ^{13}C NMR ($CDCl_3$/DMSO-d_6, 50 MHz): δ 45.70, 125.86, 127.30, 127.40, 128.10, 129.00, 129.70, 130.90, 132.04, 132.94, 152.06 (C=N), 169.01 (C=S); ^{13}C NMR-DEPT-135 ($CDCl_3$/DMSO-d_6, 50 MHz) δ 45.60 ($CH_2\downarrow$), 127.30, 128.00, 128.90, 129.60, 130.80 (all CH). MS: *m*/*z* (%)=301 (M^+, 6), 303 (M+2, 2), 266 (100), 233 (2), 206 (2), 125 (26), 103 (14), 89 (12), 77 (8). Anal. calcd for $C_{15}H_{12}N_3SCl$ (301.794): C, 59.69; H, 4.00; N, 13.92. Found: C, 59.80; H, 4.11; N, 14.01.

4.1.2. 4-(2-Chlorobenzyl)-5-(*p*-tolyl)-1,2,4-triazole-3-thione (1b). The thiosemicarbazide obtained by the treatment of 2-chlorobenzyl isothiocyanate (1.22 g, 6.6 mmol) and *p*-toluic hydrazide (1 g, 6.6 mmol), when refluxed in 10% K_2CO_3 for 7 h, afforded **1b**.Yield: 1.81 g (87%), colourless solid, mp 166–168 °C; UV (λ_{max}): 258 nm (CH_3OH); IR (KBr): 3097, 2921, 2563, 1614, 1446, 1348, 1225 cm^{-1}; ^{1}H NMR ($CDCl_3$/DMSO-d_6, 200 MHz): δ 2.35 (s, 3H, CH_3), 5.36 (s, 2H, CH_2), 6.95–7.42 (m, 8H, ArH), 13.77 (s, 1H, SH). ^{13}C NMR ($CDCl_3$/DMSO-d_6,50 MHz): δ 21.6, 46.0, 123.1, 127.5, 128.2, 129.1, 129.9, 132.2, 141.4, 152.4 (C=N), 169.1 (C=S); ^{13}C NMR-DEPT-135 ($CDCl_3$/DMSO-d_6, 50 MHz): δ 21.6 ($CH_3\uparrow$), 45.9 ($CH_2\downarrow$), 127.4, 128.1, 129.1, 129.8 (all CH). MS: *m*/*z* (%)=315 (M^+, 5), 317 (M+2, 2), 280 (100), 247 (2), 125 (30), 117 (8), 102 (4), 89 (14), 77 (6). Anal. calcd for $C_{16}H_{14}N_3SCl$ (315.820): C, 60.84; H, 4.46; N, 13.30. Found: C, 60.97; H, 4.75; N, 13.03. The structure of the compound **1b** was further confirmed by XRD.[12]

4.1.3. 5-(*p*-Anisyl)-4-(2-chlorobenzyl)-1,2,4-triazole-3-thione (1c). Treatment of 2-chlorobenzyl isothiocyanate (1.3 g, 8.0 mmol) with *p*-anisic hydrazide (1.47 g, 8.0 mmol) in ethanol afforded the thiosemicarbazide, which on refluxing in 10% K_2CO_3 for 7 h, furnished **1c**. Yield: 2.12 g (80%), colourless solid, mp 195–197 °C; UV (λ_{max}): 260 nm (CH_3OH); IR (KBr): 3107, 2945, 2588, 1608, 1515, 1350, 1257, 1174 cm^{-1}; ^{1}H NMR ($CDCl_3$/DMSO-d_6, 400 MHz): δ 3.79 (s, 3H, OCH_3), 5.34 (s, 2H, CH_2), 6.86–7.62 (m, 8H, ArH), 13.91 (s, 1H, SH). ^{13}C NMR ($CDCl_3$/DMSO-d_6,100 MHz): δ 44.74 (CH_2), 54.49 (OCH_3), 113.53, 116.96, 126.28, 126.42, 127.69, 128.10, 128.65, 129.96, 130.91, 131.97, 150.98, 160.46 (C–OCH_3), 167.61 (C=S). MS: *m*/*z* (%)=331 (M^+, 6), 333 (M+2, 2), 296 (100), 281 (4), 265 (3), 163 (4), 133 (10), 125 (28), 89 (14), 77 (6). Anal. calcd for $C_{16}H_{14}N_3OSCl$ (331.820): C, 57.91; H, 4.25; N, 12.66. Found: C, 58.10; H, 4.43; N, 12.81.

4.1.4. 4-(2-Bromobenzyl)-5-(*o*-tolyl)-1,2,4-triazole-3-thione (1d). Refluxing a mixture of 2-bromobenzyl isothiocyanate (1.7 g, 7.5 mmol) and *o*-toluic hydrazide (1.2 g, 7.5 mmol) in ethanol yielded the thiosemicarbazide, which on treatment with 10% K_2CO_3 for 8 h, furnished **1d**. Yield: 2.23 g (83%), colourless solid, mp 188–190 °C; UV (λ_{max}): 253 nm (CH_3OH); IR (KBr): 3087, 2927, 2578, 1598, 1453, 1338, 1247 cm^{-1}; ^{1}H NMR($CDCl_3$, 400 MHz): δ 2.07 (s, 3H, CH_3), 5.25 (s, 2H, CH_2), 6.95–7.42 (m, 8H, ArH), 12.40 (s, 1H, SH). ^{13}C NMR($CDCl_3$, 100 MHz); δ 19.44 (CH_3), 47.34 (CH_2), 122.50, 124.55, 126.05, 127.63, 128.35, 129.27, 129.58, 130.76, 131.09, 132.67, 133.92, 138.27, 151.84, 168.18 (C=S). MS: *m*/*z* (%)=359 (M^+)(361-trace), 280 (100), 171 (10), 169 (10), 163, (2), 117 (6), 89 (12), 77 (4). Anal. calcd for $C_{16}H_{14}N_3SBr$ (360.271): C, 53.34; H, 3.91; N, 11.66. Found: C, 53.57; H, 3.78; N, 11.82.

4.1.5. 5-Benzyl-4-(2-chlorobenzyl)-1,2,4-triazole-3-thione (1e). The thione **1e** was prepared by refluxing the thiosemicarbazide, obtained from 2-chlorobenzyl isothiocyanate (1.83 g, 10 mmol) and benzyl hydrazide (1.5 g, 10 mmol), in 10% K_2CO_3 for 7 h. Yield: 2.29 g (73%), colourless solid, mp 150–152 °C; UV (λ_{max}): 256 nm (CH_3OH); IR (KBr): 3083, 2918, 1588, 1448, 1347, 1239 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.89 (s, 2H, CH_2), 5.29 (s, 2H, CH_2), 6.83 (d, J=7.6 Hz, 1H, H_6–ArH), 7.08–7.39 (m, 8H, ArH). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 32.03 (C–CH_2), 44.27 (N–CH_2), 127.15, 127.28, 127.57, 128.45, 128.85, 129.11, 129.63, 131.58, 132.10, 132.82, 151.66, 168.30. MS: *m*/*z* (%)=315 (M^+, 16), 317 (M+2, 6), 280 (100), 247 (8), 163 (10), 125 (80), 89 (12), 77 (4). Anal. calcd for $C_{16}H_{14}N_3SCl$ (315.820): C, 60.84; H, 4.46; N, 13.30. Found: C, 61.10; H, 4.73; N, 13.12.

4.1.6. 4-(2-Chlorobenzyl)-5-(2-chlorophenyl)-1,2,4-triazole-3-thione (1f). The thiosemicarbazide, obtained from 2-chlorobenzyl isothiocyanate (1.07 g, 5.8 mmol) and *o*-chlorobenzhydrazide (1 g, 5.8 mmol), was refluxed in 10% K_2CO_3 for 8 h to afford **1f**. Yield: 1.76 g (85%), colourless solid, mp 190–192 °C; UV (λ_{max}): 257 nm (CH_3OH); IR (KBr): 3085, 2948, 2598, 1607, 1437, 1338,

1249 cm^{-1}; ^1H NMR ($CDCl_3$/DMSO-d_6 200 MHz): δ 5.31 (s, 2H, CH_2), 6.97–7.45 (m, 8H, ArH), 13.90 (s, 1H, SH). ^{13}C NMR ($CDCl_3$/DMSO-d_6, 50 MHz): δ 44.8, 125.5, 127.1, 128.7, 129.1, 129.3, 129.9, 131.7, 132.4, 132.7, 132.8, 134.4, 149.4, 168.5 (C=S). MS: *m/z* (%)=335 (M^+, 5), 337 (M+2, 3), 300 (100)[302, 38], 280 (10), 163 (4), 137 (12)[139, 4], 125 (44), 102 (12), 89 (20), 77 (8). Anal. calcd for $C_{15}H_{11}N_3SCl_2$ (336.238): C, 53.85; H, 3.29; N, 12.49. Found: C, 53.82; H, 3.41; N, 12.66.

4.1.7. 4-(2-Bromobenzyl)-5-(2-chlorophenyl)-1,2,4-triazole-3-thione (1g). Treatment of 2-bromobenzyl isothiocyanate (1.33 g, 5.8 mmol) with *o*-chloro benzhydrazide (1 g, 5.8 mmol) in ethanol afforded the thiosemicarbazide, which on refluxing in 10% K_2CO_3 for 7 h furnished **1g**. Yield: 1.71 g (78%), colourless solid, mp 182–184 °C; UV (λ_{max}): 257 nm (CH_3OH); IR (KBr): 3097, 2948, 2578, 1612, 1436, 1332, 1238 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz); δ 5.34 (s, 2H, CH_2), 6.98–7.46 (m, 8H, ArH), 12.17 (s, 1H, SH). ^{13}C NMR ($CDCl_3$, 100 MHz); δ 47.56, 122.65, 124.84, 127.00, 127.62, 128.74, 129.38, 130.02, 131.58, 132.51, 132.66, 133.75, 134.45, 149.93, 168.54 (C=S). MS, *m/z* (%)=(379 (M^+), 381-trace), 300 (100)[302, 33], 169 (16)[171, 14], 163 (6), 137 (14)[139, 6], 102 (14), 90 (38), 77 (10). Anal. calcd for $C_{15}H_{11}N_3SClBr$ (380.690): C, 47.32; H, 2.91; N, 11.03. Found: C, 47.60; H, 3.10; N, 11.28.

4.2. Photochemical synthesis of substituted 1,2,4-triazolo [3,4-*b*]-1,3-(4*H*)-benzothiazines 2a–e and desulfurized 1,2,4-triazoles 3a–d,f,g from thiones 1a–g

4.2.1. Irradiation of (1a). (i) An acetonitrile solution (150 mL) of 4-(2-chlorobenzyl)-5-phenyl-1,2,4-triazole-3-thione **1a** (0.3 g, 0.9 mmol) containing 30 mL of aqueous 2 M NaOH was flushed with nitrogen for 1 h and irradiated at 254 nm in an Applied Photophysics multilamp reactor (MLR) for 18 h. After completion of the reaction, checked by TLC, the solvent was removed under reduced pressure from the two-phase mixture and it was extracted with ethyl acetate. The ethyl acetate layer was separated; the aqueous layer was neutralized with dil. HCl, and extracted with ethyl acetate. The ethyl acetate portions were combined together, evaporated and the residue obtained was chromatographed over a column of silica gel; elution with ethyl acetate–petroleum ether (1:1) furnished the photosubstituted 3-phenyl-1,2,4-triazolo [3,4-*b*]-1,3(4*H*)-benzothiazine **2a** (41%). In addition 4-(2-chlorobenzyl)-3-phenyl-1,2,4-triazole **3a** (10%) was obtained in the petroleum ether–ethyl acetate (3:7) elusion. The spectral and analytical data of compounds **2a** and **3a** were reported earlier.[6]

4.3. Irradiation of (1b)

Irradiation of an acetonitrile (150 mL) solution of 4-(2-chlorobenzyl)-5-(*p*-tolyl)-1,2,4-triazole-3-thione **1b** (0.3 g, 1.0 mmol) containing 2 M NaOH (30 mL), after flushing with nitrogen, in multilamp reactor for 17 h, followed by usual workup as mentioned above and chromatographic separation afforded triazolo benzothiazine **2b** and triazole **3b**.

4.3.1. 3-(*p*-Tolyl)-1,2,4-triazolo-[3,4-*b*]-1,3(4*H*)-benzothiazine (2b). Yield: 0.12 g (47%), colourless solid, mp 204–206 °C; UV (λ_{max}): 261, 243 nm (CH_3OH); IR (KBr): 1593, 1473, 748 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 2.45 (s, 3H, CH_3), 5.20 (s, 2H, CH_2), 7.22–7.60 (m, 8H, ArH). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 21.44, 47.61, 123.32, 127.30, 127.89, 127.92, 128.51, 129.07, 129.44, 129.82, 130.20, 140.64, 147.55, 154.06. MS: *m/z* (%)=279 (M^+, 100), 278 (22), 263 (3), 207 (2), 161 (14), 135 (22), 134 (44), 121 (25), 117 (12), 116 (14), 108 (12), 102 (4), 90 (20), 89 (24), 77 (12). Anal. calcd for $C_{16}H_{13}N_3S$ (279.360): C, 68.78; H, 4.69; N, 15.04. Found: C, 68.49; H, 4.93; N, 15.25.

4.3.2. 4-(2-Chlorobenzyl)-3-(*p*-tolyl)-1,2,4-triazole (3b). Yield: 0.021 g (8%), colourless solid, mp 130–132 °C; UV (λ_{max}): 236 nm (CH_3OH); IR (KBr) 1603, 1517, 1473 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 2.40 (s, 3H, CH_3), 5.29 (s, 2H, CH_2), 6.87 (d, *J*=7.32 Hz, 1H, C_6–ArH), 7.23–7.47 (m, 7H, ArH), 8.16 (s, 1H, C_5–H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 21.39, 46.53, 123.47, 127.63, 128.38, 128.57, 129.67, 130.02, 130.06, 132.76, 132.84, 140.49 144.33 (C_5–H), 154.50 (C=N). MS: *m/z* (%)=283 (M^+, 68)[285, M+2, 20], 248 (100), 166 (3), 165 (4), 138 (2), 131 (4), 125 (92)[127, 32], 117 (5), 116 (6), 104 (4), 103 (24), 90 (12), 89 (34), 77 (36). Anal. calcd for $C_{16}H_{14}N_3Cl$ (283.754): C, 67.72; H, 4.97; N, 14.80. Found: C, 67.87; H, 5.10; N, 15.02.

4.4. Irradiation of (1c)

5-(*p*-Anisyl)-4-(2-chlorobenzyl)-1,2,4-triazole-3-thione **1c** (0.35 g, 1.1 mmol) in acetonitrile (150 mL) containing 2 M NaOH (30 mL) was irradiated using a multilamp reactor (254 nm) for 20 h. Usual workup followed by chromatographic separation furnished the respective triazolo benzothiazine **2c** and triazole **3c**.

4.4.1. 3-(*p*-Anisyl)-1,2,4-triazolo-[3,4-*b*]-1,3(4*H*)-benzothiazine (2c). Yield: 0.12 g (38%), colourless solid, mp 166–168 °C; UV (λ_{max}): 260, 206 nm (CH_3OH); IR (KBr): 1614, 1467, 1245, 1031, 746 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 3.85 (s, 3H, OCH_3), 5.42 (s, 2H, CH_2), 6.95 (d, *J*=8.76 Hz, 2H, ArH), 7.26–7.40 (m, 4H, ArH), 8.00 (d, *J*=8.8 Hz, 2H, ArH). MS: *m/z* (%)=295 (M^+, 100), 294 (90), 279 (56), 223 (4), 161 (20), 135 (21), 134 (80), 133 (21), 121 (35), 108 (10), 102 (11), 90 (42), 89 (41), 77 (23). Anal. calcd for $C_{16}H_{13}N_3SO$ (295.359): C, 65.06; H, 4.43; N, 14.22. Found: C, 65.25; H, 4.68; N, 14.38.

4.4.2. 3-(*p*-Anisyl)-4-(2-chlorobenzyl)-1,2,4-triazole (3c). Yield: 0.047 g (15%), colourless solid, mp 156–158 °C; UV (λ_{max}): 248 nm (CH_3CN); IR (KBr): 1583, 1503, 1450, 1358, 1259 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 3.84 (s, 3H, OCH_3), 5.28 (s, 2H, CH_2), 6.87 (d, *J*=7.8 Hz, 1H, C_6–ArH), 6.97 (m, 2H, ArH), 7.23–7.51 (m, 5H, ArH), 8.16 (s, 1H, C_5–H). MS: *m/z* (%)=299 (M^+, 71)[301, M+2, 25], 264 (72), 236 (11), 221 (5), 131 (3), 165 (11), 125 (100), 127 (32), 104 (2), 103 (10), 90 (20), 89 (36), 77 (10). Anal. calcd for $C_{16}H_{14}N_3ClO$ (299.754): C, 64.10; H, 4.70; N, 14.01. Found: C, 64.37; H, 4.89; N, 14.20.

4.5. Irradiation of (1d)

4.5.1. 3-(*o*-Tolyl)-1,2,4-triazolo-[3,4-*b*]-1,3(4*H*)-benzothiazine (2d). (i) Irradiation of an acetonitrile (150 mL)

solution of 4-(2-bromobenzyl)-5-(*o*-tolyl)-1,2,4-triazole-3-thione **1d** (0.3 g, 1.1 mmol) containing 2 M NaOH (30 mL) in a multilamp reactor for 11 h, and chromatographic separation afforded **2d**. Yield: 0.15 g (50%), colourless solid, mp 173–175 °C; UV (λ_{max}): 259, 231 nm (CH_3OH); IR (KBr): 1585, 1470, 746 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 2.63 (s, 3H, CH_3), 5.46 (s, 2H, CH_2), 7.25–7.91 (m, 8H, ArH). ^{13}C NMR ($CDCl_3$, 100 MHz); δ 21.67, 50.68, 125.72, 127.30, 128.04, 128.42, 128.65, 128.98, 129.44, 131.11, 137.07, 147.20, 151.65, 153.15. MS: *m/z* (%)=279 (M^+, 100), 278 (10), 264 (4), 161 (3), 135 (20), 134 (16), 121 (52), 117 (14), 116 (15), 108 (5), 102, (2), 90 (14), 89 (22), 77 (14). Anal. calcd for $C_{16}H_{13}N_3S$ (279.360): C, 68.78; H, 4.69; N, 15.04. Found: C, 68.52; H, 4.97; N, 15.32.

4.6. Irradiation of (1e)

4.6.1. 3-Benzyl-1,2,4-triazolo-[3,4-*b*]-1,3(4*H*)-benzothiazine (2e). The compound **2e** was obtained on irradiation of 5-benzyl-4-(2-chlorobenzyl)-1,2,4-triazole-3-thione **1e** (0.25 g 0.8 mmol) in acetonitrile (150 mL) containing 2 M NaOH (30 mL) using multilamp reactor for 21 h, followed by chromatographic separation. Yield: 0.10 g (45%), colourless solid, mp 162–164 °C; UV (λ_{max}): 257, 208 nm (CH_3OH); IR (KBr): 1595, 1475, 748 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 4.37 (s, 2H, CH_2), 4.81 (s, 2H, CH_2), 7.09–7.42 (m, 9H, ArH). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 31.45, 46.69, 127.39, 127.52, 127.89, 128.39, 129.17, 135.10, 147.50, 153.20. MS: *m/z* (%)=279 (M^+, 100), 278 (28), 161 (12), 135 (78), 134 (20), 121 (24), 117 (15), 116 (13), 108 (6), 102 (6), 90 (25), 89 (32), 77 (24). Anal. calcd for $C_{16}H_{13}N_3S$ (279.360): C, 68.78; H, 4.69; N, 15.04. Found: C, 68.98; H, 4.90; N, 15.30.

4.7. Irradiation of 1f

4.7.1. 4-(2-Chlorobenzyl)-3-(2-chlorophenyl)-1,2,4-triazole (3f). The irradiation of 4-(2-chlorobenzyl)-5-(2-chlorophenyl)-1,2,4-triazole-3-thione **1f** (0.2 g, 0.6 mmol) in acetonitrile (150 mL) containing 2 M NaOH (30 mL) using multilamp reactor (254 nm) for 32 h, and usual workup followed by chromatographic separation using petroleum ether–ethyl acetate mixture (1:9) furnished the respective triazole **3f**. Yield: 0.085 g (47%), colourless solid, mp 100–102 °C; UV (λ_{max}): 267 nm (CH_3OH); IR (KBr): 1613, 1583, 1453 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 5.12 (s, 2H, CH_2), 6.88 (dd, *J*=7.6, 1.6 Hz, 1H, C_6–ArH), 7.14–7.50 (m, 7H, ArH), 8.25 (s, 1H, C_5–H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 47.00, 126.55, 127.42, 127.62, 130.09, 130.27, 130.32, 130.60, 132.22, 132.86, 133.91, 134.58, 144.18 (C_5–H), 152.45 (C=N). MS: *m/z* (%)=303 (M^+, 38)[305, M+2, 22] [307, M+4, 8], 268 (68)[270, 30], 242 (4), 166 (4), 165 (3), 138 (6), 131 (5), 125 (100)[127, 30], 104 (30), 103 (8), 90 (14), 89 (33), 77 (12). Anal. calcd for $C_{15}H_{11}N_3Cl_2$ (304.172): C, 59.22; H, 3.64; N, 13.81. Found: C, 59.42; H, 3.83; N, 13.63.

4.8. Irradiation of 1g

4.8.1. 4-(2-Bromobenzyl)-3-(2-chlorophenyl)-1,2,4-triazole (3g). Irradiation of an acetonitrile (150 mL) solution of 4-(2-bromobenzyl)-5-(2-chlorophenyl)-1,2,4-triazole-3-thione **1g** (0.3 g, 0.8 mmol) containing 2 M NaOH (30 mL), (after flushing with nitrogen) in multilamp reactor for 13 h, followed by chromatographic separation afforded triazole **3g**. Yield: 0.13 g (48%), colourless solid, mp 128–130 °C; UV (λ_{max}): 267 nm (CH_3OH); IR (KBr): 1601, 1591, 1447 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 5.13 (s, 2H, CH_2), 6.90 (dd, *J*=7.32, 2.44 Hz, 1H, C_6–ArH), 7.16–7.56 (m, 7H, ArH), 8.22 (s, 1H, C_5–H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 49.01, 123.55, 126.36, 127.15, 127.97, 129.83, 130.00, 130.46, 131.93, 132.63, 133.37, 133.57, 134.31, 143.89 (C_5–H), 152.13 (C=N). MS: *m/z* (%)=347 (M^+, 40), 349 (M+2, 53), 351 (M+4, 13), 268 (100) [270 (30)], 233 (4), 206 (4), 176 (12), 169 (75)[170 (74)], 137 (3), 131 (10), 104 (18), 103 (5), 90 (76), 89 (55), 77 (12). Anal. calcd for $C_{15}H_{11}N_3ClBr$ (348.624): C, 51.67; H, 3.18; N, 12.05. Found: C, 51.88; H, 3.40; N, 12.30.

4.9. General procedure for the synthesis of substituted 1,2,4-triazole-3-thiones 4a–g

A mixture of acid hydrazide (1 equiv.) and aryl isothiocyanate (1 equiv.) was refluxed in K_2CO_3 solution (100 mL, 10%) for 6–8 h, cooled, filtered and the filtrate washed with ether. The aqueous layer was neutralized with cold, dil. HCl (in the case of **4d** pH=7 was maintained). The separated solid was filtered and washed with water to get the corresponding triazole-3-thione. The product was recrystallized from ethyl acetate. The triazole-3-thione **4e** was prepared from the respective thiosemicarbazide, obtained from the corresponding acid hydrazide and isothiocyanate.

The spectral and analytical data for compounds **4a–e** were reported earlier.[16]

4.9.1. 4-(2-Chlorophenyl)-5-phenyl-1,2,4-triazole-3-thione (4f). The thione **4f** was prepared by refluxing a mixture of 2-chlorophenyl isothiocyanate (1.0 g, 5 mmol) and benzoic hydrazide 0.68 g (5 mmol) in 10% K_2CO_3 for 8 h. Yield: 1.03 g (72%), colourless solid, mp 224–226 °C (lit.[1] mp 222 °C).

4.9.2. 4,5-Di-(2-chlorophenyl)-1,2,4-triazole-3-thione (4g). A mixture of 2-chlorobenzhydrazide (1.0 g, 5 mmol) and 2-chlorophenyl isothiocyanate (1.0 g, 5 mmol) was refluxed for 8 h in K_2CO_3 solution. Yield: 1.2 g (74%), colourless solid, mp 252–254 °C; UV (λ_{max}): 257 and 219 nm (MeOH); IR (KBr): 3100, 2900, 2598, 1495, 1350, 1274, 1240 cm^{-1}; ^1H NMR ($CDCl_3$/200 MHz,): δ 7.24–7.53 (m, 8H, ArH), 12.10 (s, 1H, SH). ^{13}C NMR ($CDCl_3$/50 MHz,): δ 124.62, 125.67, 127.85, 128.40, 128.69, 129.01, 129.45, 129.88, 130.24, 130.53, 132.24, 147.48, 167.01; ^{13}C NMR-DEPT 90 ($CDCl_3$/50 MHz,): δ 124.63, 125.68, 127.86, 128.42, 128.68, 129.46, 129.88, 130.27 (all CH). MS: *m/z* (%)=321 (M^+,13), (323, M+2), (325, M+4), 288 (40), 286 (100), 149 (90), 137 (5), 125 (11), 111 (11), 90 (11), 75 (18). Anal. calcd for $C_{14}H_9Cl_2N_3S$ (322.212): C, 52.18; H, 2.81; N, 13.04. Found: C, 52.09; H, 2.79; N, 13.08. The structure of the compound **4g** was further confirmed by XRD.[17]

4.10. General procedure for the photochemical desulfurization of triazole-3-thiones 1a–d and 4a–g

A methanolic solution (180 mL) of triazole-3-thione and benzophenone (1.2 equiv.) was flushed with nitrogen for 1 h

and irradiated in a multilamp reactor (254 nm). After completion of the reaction, checked by TLC, the solvent was removed under reduced pressure. The residue obtained was chromatographed over a column of silica gel. Elution with petroleum ether–ethyl acetate afforded the desulfurized triazoles.

4.10.1. 3-Phenyl-4-(*p*-tolyl)-1,2,4-triazole (5a). Irradiation of a solution of **4a** (0.3 g, 1.1 mmol) and benzophenone (0.24 g, 1.3 mmol) in methanol (180 mL) using MLR for 38 h, followed by chromatographic separation, afforded **5a**. Yield: 42%.

An ethanolic solution of **4a** (0.5 g, 1.9 mmol) in the presence of a catalytic amount of Raney Ni (in absolute ethanol was refluxed for 23 h). After completion of the reaction, the solution was filtered, concentrated and purified over silica gel column to get **5a**.Yield: 0.12 g, 47%, mp, mixture mp and superimposable IR was found to be consistent with **5a** obtained from the photolysis of **4a**.

4.10.2. 3,4-Di-(*p*-tolyl)-1,2,4-triazole (5b). A solution of **4b** (0.4 g, 1.4 mmol) and benzophenone (0.31 g, 1.7 mmol) in methanol (180 mL) was irradiated using MLR for 40 h. After completion of the reaction, followed by chromatographic separation afforded **5b**. Yield: 59%.

The thione **4b** (0.6 g, 2.1 mmol) in 17% aq.HNO_3 (50 mL) was refluxed for 5 h. After completion of the reaction, it was cooled to room temperature and then neutralized with dil. NaOH. The solid obtained was filtered, washed with water and recrystallized from ethyl acetate to isolate the desulfurized product (0.29 g, 55%), which was compared with the desulfurized triazole **5b**, obtained under photolytic condition by mp, mixture mp and superimposable IR.

4.10.3. 4-(1-Naphthyl)-3-(*o*-tolyl)-1,2,4-triazole (5c). Photolysis of **4c** (0.3 g, 0.9 mmol) and benzophenone (0.2 g, 1.1 mmol) in methanol (180 mL) using MLR for 30 h, followed by chromatographic separation, afforded **5c**. Yield: 70%.

4.10.4. 3-(4-Pyridyl)-4-(*p*-tolyl)-1,2,4-triazole (5d). Irradiation of **4d** (0.3 g, 1.1 mmol) and benzophenone (0.24 g, 1.3 mmol) in methanol (180 mL) using MLR for 43 h, followed by chromatographic separation furnished **5d**. Yield: 30%.

4.10.5. 4-Benzyl-3-phenyl-1,2,4-triazole (5e). Irradiation of a mixture of **4e** (0.3 g, 1.1 mmol) and benzophenone (0.24 g, 1.3 mmol) in methanol (180 mL) using MLR for 33 h, furnished **5e**. Yield: 57%.

The structures of triazoles **5a–e** were confirmed by mp, mixture mp and superimposable IR with those obtained under TFR condition, which has been reported earlier.[16]

4.10.6. 4-(2-Chlorophenyl)-3-phenyl-1,2,4-triazole (5f). Photolysis of 4-(2-chlorophenyl)-5-phenyl-1,2,4-triazole-3-thione **4f** (0.25 g, 0.8 mmol) and benzophenone (0.19 g, 1.0 mmol) in methanol (180 mL) using MLR for 50 h, followed by chromatographic separation, afforded **5f**. Yield: 0.11 g (52%), colourless solid, mp 98–100 °C; UV (λ_{max}): 236, 208 nm (MeOH); IR (KBr): 1524, 1434 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 7.25–7.60 (m, 9H, ArH), 8.30 (s, 1H, C_5–H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 126.24, 127.97, 128.18, 128.62, 129.01, 130.02, 130.97, 131.28, 131.67, 132.47, 144.62, (C_5–H) 153.45 (C=N). MS: *m/z* (%)=255 (M^+, 100)[257, M+2, 34], 254 (41)[256, 33], 220 (98), 193 (8), 166 (6), 165 (5), 152 (30)[154, 11], 138 (14)[140, 6], 125 (32)[127, 10], 103 (10), 90 (51), 89 (23), 77 (26). Anal. calcd for $C_{14}H_{10}N_3Cl$ (255.701): C, 65.76; H, 3.94; N, 16.43. Found: C, 65.97; H, 4.12; N, 16.71.

4.10.7. 3,4-Di-(2-chlorophenyl)-1,2,4-triazole (5g). Irradiation of 4,5-di-(2-chlorophenyl)-1,2,4-triazole-3-thione **4g** (0.25 g, 0.8 mmol) and benzophenone (1.2 equiv., 0.17 g) in absolute methanol (180 mL), at 254 nm in MLR for 65 h, furnished **5g** after chromatographic separation. Yield: 0.12 g (60%), colourless solid, mp 138–140 °C; UV (λ_{max}): 268 and 222 nm (MeOH); IR (KBr): 1500 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6/CD_3CN/200 MHz): δ 7.30–7.50 (m, 8H, ArH), 8.75 (s, 1H). ^{13}C NMR (DMSO-d_6/CD_3CN/50 MHz): δ 126.52, 127.71, 128.67, 129.84, 130.28, 130.96, 132.01, 132.75, 133.25, 134.28, 145.49 (C_5–CH); ^{13}C NMR-DEPT-135, (DMSO-d_6/CD_3CN/50 MHz): δ 127.71, 128.67, 129.84, 130.28, 130.95, 132.01, 132.76, 133.25, 145.49 (C_5–CH) (all CH). MS: *m/z* (%)=289 (M^+, 25), (291, M+2), (293, M+4), 254 (41), 256 (23) 152 (37), 138 (13), 125 (44), 111 (35), 90 (62), 75 (100), 63 (70), 50 (45). Anal. calcd for $C_{14}H_9Cl_2N_3$ (290.146): C, 57.95; H, 3.12; N, 14.48. Found: C, 58.05; H, 3.25; N, 14.38.

A solution of **4g** (0.5 g, 1.5 mmol) and catalytic amount of Raney Ni in absolute ethanol was refluxed for 26 h. After completion of the reaction, the solution was filtered, concentrated and purified over silica gel column to get **5g**. Yield: 0.1 g, 22%, mp, mixture mp and superimposable IR was found to be consistent with **5g** obtained from the photolysis of **4g**.

4.10.8. 4-(2-Chlorobenzyl)-3-phenyl-1,2,4-triazole (3a). A methanolic solution of **1a** (0.3 g, 0.9 mmol) containing benzophenone (0.12 g, 1 mmol) after flushing with nitrogen was irradiated, in multilamp reactor for 62 h. After completion of the reaction, chromatographic separation afforded triazole **3a**.

4.10.9. 4-(2-Chlorobenzyl)-3-(*p*-tolyl)-1,2,4-triazole (3b). Irradiation of a methanolic solution of **1b** (0.4 g, 1.3 mmol) and benzophenone (0.28 g, 1.5 mmol) using MLR for 53 h, afforded **3b**.

4.10.10. 3-(*p*-Anisyl)-4-(2-chlorobenzyl)-1,2,4-triazole (3c). Irradiation of a mixture of **1c** (0.4 g, 1.2 mmol) and benzophenone (0.26 g, 1.4 mmol) in methanol (180 mL) using MLR for 47 h, furnished **3c**. Yield: 48%.

The structure of triazoles **3a–c** were confirmed by mp, mixture mp and superimpossable IR with those obtained under base mediated photolytic condition.

4.10.11. 4-(2-Bromobenzyl)-3-(*o*-tolyl)-1,2,4-triazole (3d). (ii) A methanolic solution (180 mL) of thione **1d** (0.2 g, 0.6 mmol) containing benzophenone (0.12 g,

0.7 mmol) was flushed with nitrogen and irradiated in a multilamp reactor for 48 h. After completion of the reaction monitored by TLC, the solvent was removed under reduced pressure; the residue obtained was chromatographed over a column of silica gel. Elution with ethyl acetate afforded desulfurized triazole **3d**. Yield: 0.13 g (71%), colourless solid, mp 108–110 °C; UV (λ_{max}): 266 nm (CH_3OH); IR (KBr): 1602, 1581, 1462 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 2.16 (s, 3H, CH_3), 5.06 (s, 2H, CH_2), 6.78 (d, J=7.32 Hz, 1H, C_6–ArH), 7.16–7.55 (m, 7H, ArH), 8.30 (s, 1H, C_5–H). ^{13}C NMR ($CDCl_3$, 100 MHz); δ 19.59, 48.57, 123.25, 125.83, 127.89, 129.61, 129.94, 130.27, 130.33, 130.62, 133.22, 133.69, 138.55, 143.56 (C_5–H), 153.57 (C=N). MS: *m/z* (%)=327 (M^+, 15)[329, M+2, 16], 326[328], 312 (7), 248 (100), 172 (6), 169 (20)[171, 23], 158 (59), 131 (4), 117 (7), 116 (8), 104 (8), 103 (14), 90 (30), 89 (24), 77 (27). Anal. calcd for $C_{16}H_{14}N_3Br$ (328.205): C, 58.55; H, 4.29; N, 12.80. Found: C, 58.80; H, 4.41; N, 12.93.

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Stereoselective ring-opening reactions with AcBr and AcCl. A new method for preparation of some haloconduritols

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Abstract—The actions of AcX (X=Br, Cl) on 7-oxa-bicyclo[2.2.1]hept-5-ene-2,3-diol diacetates and a *transoid*-epoxide prepared from the acetonide of cyclohexa-3,5-diene-*cis*-1,2-diol were studied. H_2SO_4-catalyzed cleavage of *exo-cis*-7-oxa-bicyclo[2.2.1]hept-5-ene-2,3-diol diacetate with AcCl gave (1α,2α,3α,6β)-6-chloro-4-cyclohexene-1,2,3-triol triacetate, from which the corresponding chloroconduritol was obtained by *trans*-esterification (MeOH/HCl). A similar reaction of the *exo*-diacetate with AcBr in the presence of H_2SO_4 resulted in bromine addition. The formation of bromine from the reaction of AcBr and H_2SO_4 was observed by independent experiments. H_2SO_4-catalyzed reaction of *endo-cis*-7-oxa-bicyclo[2.2.1]hept-5-ene-2,3-diol diacetate with AcX (X=Br, Cl) gave (1α,2α,3β,6β)-6-halo-4-cyclohexene-1,2,3-triol triacetates. The reaction of the *transoid*-epoxide with AcX (X=Br, Cl) with no catalyst gave also (1α,2α,3β,6β)-6-halo-4-cyclohexene-1,2,3-triol triacetates.

1. Introduction

Glycosidase inhibitors have become interesting as anti-obesity drugs, antidiabetics, antifungals, insecticides, and antivirals, including substances active against the human immunodeficiency virus (HIV) and metastasis.[1] Bromo-conduritol, a diastereomeric mixture of (1α,2β,3α,6β)-6-bromo-4-cyclohexene-1,2,3-triol and (1α,2β,3α,6α)-6-bromo-4-cyclohexene-1,2,3-triol, has been commonly used as a covalent, irreversible, active-site directed glucosidase inhibitor.[2]

A few synthetic procedures[2d,3] for the preparation of haloconduritols have been described. Recently, we have reported[4] a facile synthesis of haloconduritols based on Lewis acid (BBr_3 or BCl_3)-assisted ring-opening of *endo*-diacetate **1** at low temperature. Thus, our method gives haloconduritol diacetates **2a**, **3a** in the construction of conduritol-A, from which haloconduritols **2c**, **3c** were efficiently prepared (Scheme 1).

Mechanistic investigations have revealed that ring-opening of *endo*-diacetate **1** proceeds by a neighboring group participation. A neighboring group participation is not expected in *exo*-diacetate **4** due to the relative stereochemistry. Ring-opening of *exo*-diacetate **4** should afford a different haloconduritol. Thus, we planned to investigate the ring-opening of *exo*-diacetate **4**. Also, considering high reactivity of epoxides to give halohydrins, we studied the ring-opening reactions of epoxide **5** to give haloconduritols (Fig. 1).

2. Results and discussion

At first, we attempted the BX_3-assisted ring-opening of *exo*-diacetate **4**. We treated *exo*-diacetate **4** with BBr_3 or BCl_3 at −78 °C and then, the reaction mixture was stirred at room temperature for 4 h. After quenching the reaction with water and chromatographic separation, we determined that the reaction with BBr_3 gave three products (**6**, **7**, and **8**) Attempts to ring-open **4** with BCl_3 were unsuccessful (Scheme 2).

The formation of **6**–**8** may be explained via haloboranation. Haloboranation of olefins with BBr_3 has been known[5] for a long time. Formation of monobromide **6** and **7** may be easily explained by addition of BBr_3 to the double bond of **4** to give Br–C–C–BBr_2-like intermediates **9** and **10** followed by hydrolysis. The formation of **8** probably proceeds through the oxidation of C–B bond of intermediate **10** by air oxygen to give **11** (Scheme 3). Indeed, performing the reaction under N_2 atmosphere and quenching the reaction media with water gave only dibromides **6** and **7** in a ratio of 1:1 and total yield of 65%.

Cleavage of ethers by acyl halides have been known for over

Keywords: Haloconduritol; Ring-opening; 7-Oxa-bicyclo[2.2.1]hept-5-ene-2,3-diol diacetate; Cyclohexa-3,5-diene-*cis*-1,2-diol; Acetyl bromide; Acetyl chloride.
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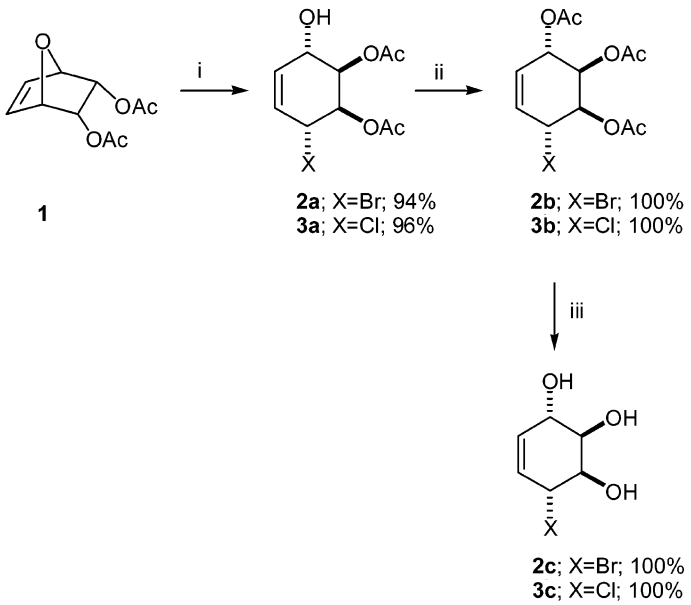


Scheme 1. (i) BX_3, CH_2Cl_2, −78 °C, then H_2O (BX_3=BBr_3 or BCl_3) (ii) CH_3COCl, CH_2Cl_2; (iii) MeOH, HCl, 0 °C.

50 years.[6] Considering the efficiency of the reaction to give alkyl halides and alcohols we applied this method to *exo*-diacetate **4**. For this, we treated *exo*-diacetate **4** with excess AcCl in the presence of H_2SO_4. As expected, the reaction gave chloroconduritol triacetate **13a** in the construction of conduritol-C, from which chloroconduritol **13b** was readily obtained by *trans*-esterification. The ^1H NMR spectra of **13a**,**b** showed an identity with similar C-type haloconduritol derivatives.[3c,7] As a surprising result, the reaction of *exo*-diacetate **4** with AcBr and H_2SO_4 gave a brominated compound **12** (Scheme 4).

We assumed that brominated compound **12** should be formed via addition of Br_2 to the double bond of **4**. As evidence, one drop of Br_2 was added to a solution of *exo*-diacetate **4** in $CDCl_3$ in an NMR tube, and the NMR spectra showed the formation **12** as a sole product. To our knowledge, there has not been any report on the formation of bromine from the reaction of AcBr and H_2SO_4. Therefore, the formation of bromine from AcBr and H_2SO_4 was provided by performing an independent experiment. To a stirred solution of 50 mmol AcBr in CH_2Cl_2 was added one drop H_2SO_4 and the reaction mixture was additionally stirred for 15 min. Meanwhile the colorless reaction mixture turned red. To determine the quantity of the bromine, 25 mmol cyclohexene was added and after purification only 0.8 mmol 1,2-dibromocyclohexane was obtained. In another experiment, after treatment of 50 mmol AcBr with 50 mmol H_2SO_4 in dichloromethane, 25 mmol cyclohexene was added to reaction mixture. This time, isolated 1,2-dibromocyclohexane was 22 mmol. These findings show that the decomposition of AcBr with H_2SO_4 is not a catalytic reaction. Most probably, H_2SO_4 seems to participate in the reaction as a reagent. At this stage we did not study the mechanism of the reaction.

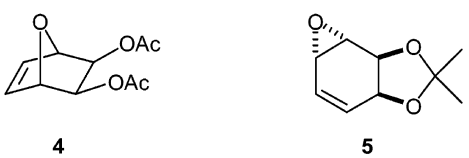
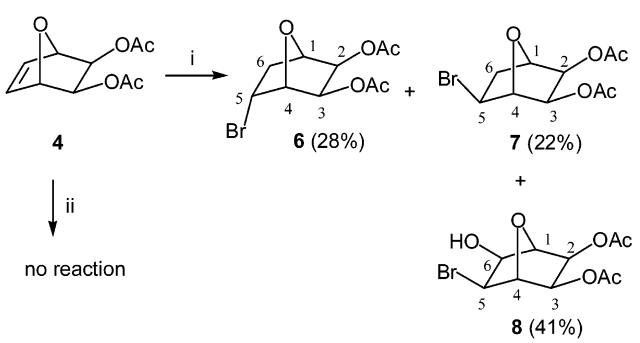


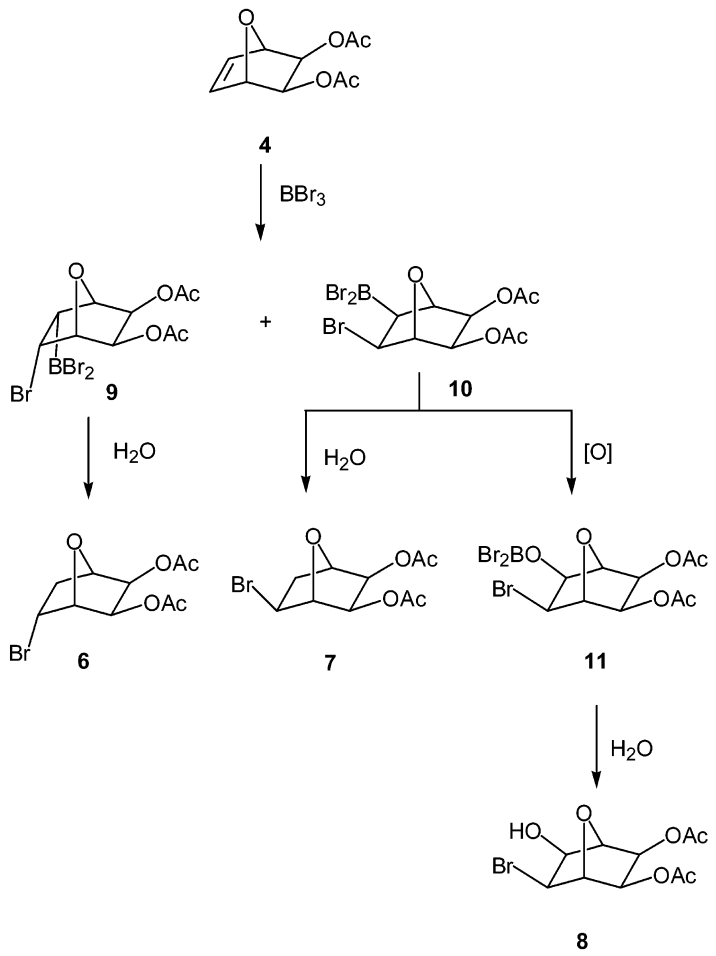
Figure 1.

Complete peak assignments of the NMR spectra of **6**, **7**, **8** and **12** were carried out. Taking into consideration the coupling constants, we easily elucidated the relative stereochemistry of H_2, H_3, H_5 and H_6. In the ^1H NMR spectra of these four compounds $J_{1,2}$ and $J_{3,4}$ were not observed probably due to dihedral angles close to 90°. Similar results were reported in benzobicylic[2.2.1]system having *exo*-substituents.[8] Again, all $J_{2,3}$ values are about 6.2 Hz. $J_{5,6}$ of **8**, similar to $J_{2,3}$, is also 6.3 Hz. While *endo*-H_5 of **7** is seen as a doublet of doublets ($J_{5,6endo}$=7.0 Hz, $J_{5,6exo}$=4.0 Hz), *exo*-H_5 of **6** is seen as doublet of triplets ($J_{5,6exo}$=10.9 Hz, $J_{5,6endo}$=5.0 Hz, $J_{4,5}$=5.0 Hz). The configurations of the groups in **7** and **8** were also confirmed by the observation of NOE effects. In **7**, irradiation of H–C_5(Br) at δ 4.03 caused signal enhancement of the resonances at H_2, H_3 and adjacent protons at C_6. In a similar way, irradiation of H–C_6(OH) of **8** at δ 3.94 caused signal enhancement of the resonances at H_1, H_2, H_3 and H_5.

In our previous studies, BBr_3 or BCl_3-assisted ring-opening of *endo*-diacetate **1** required a temperature of −78 °C.



Scheme 2. (i) BBr_3, CH_2Cl_2, −78 °C, then H_2O (ii) BCl_3, CH_2Cl_2, −78 °C, then H_2O.

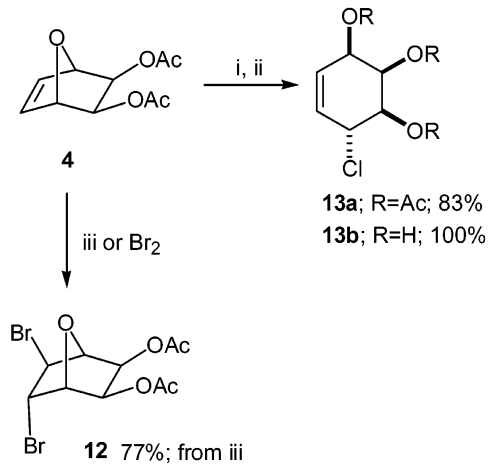


Scheme 3. Possible mechanism for the formation **6**–**8** from **4**.

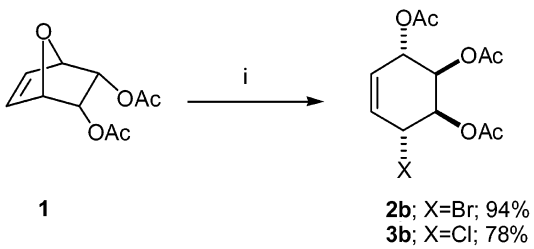
Therefore we attempted to ring-opening of **1** with AcX (X=Br, Cl) at ambient temperature. The reaction gave haloconduritol triacetates **2b** and **3b** in good yields (Scheme 5). The reaction appears to proceed by neighboring group participation as with the reaction in Scheme 1.

Epoxide **5**, readily prepared by epoxidation of acetonide **14**, is highly reactive to substitution and has been used for many syntheses in the literature.[9] Without any catalyst, the reaction of epoxide **5** with excess of acetyl halides (X=Br, Cl) gave directly **2b** and **3b** in good yields (Scheme 6).

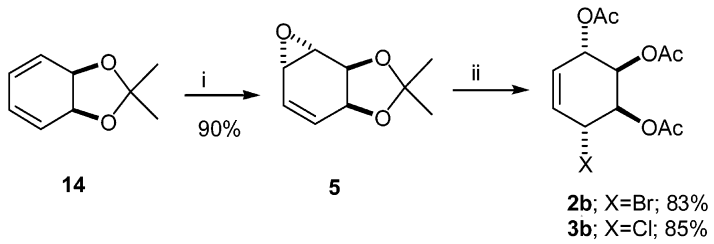
We suppose that the formation of products **2b** and **3b** proceeds by an S_N2' mechanism as outlined in Scheme 7. At first, epoxide **5** and acetyl halide should give a **15**-like tetrahedral intermediate. While halide is being transferred to the 6-position, the electron pair of the double bond could substitute carbon–oxygen bond of epoxide at C_4 to give **16**-like product. Further acetylation of ketal oxygens with acetyl halide affords haloconduritol triacetates **2b** and **3b**. Similar S_N2' substitutions were observed in the reactions of **5** or **5**-like unsaturated epoxides with some organometallic reagents.[10]



Scheme 4. (i) AcCl, CH_2Cl_2, H_2SO_4; (ii) MeOH, HCl; (iii) AcBr, CH_2Cl_2, H_2SO_4.



Scheme 5. (i) AcX (X=Br, Cl), CH_2Cl_2, H_2SO_4 (cat).



Scheme 6. (i) MCPBA, (ii) AcX (X=Br, Cl), CH_2Cl_2.

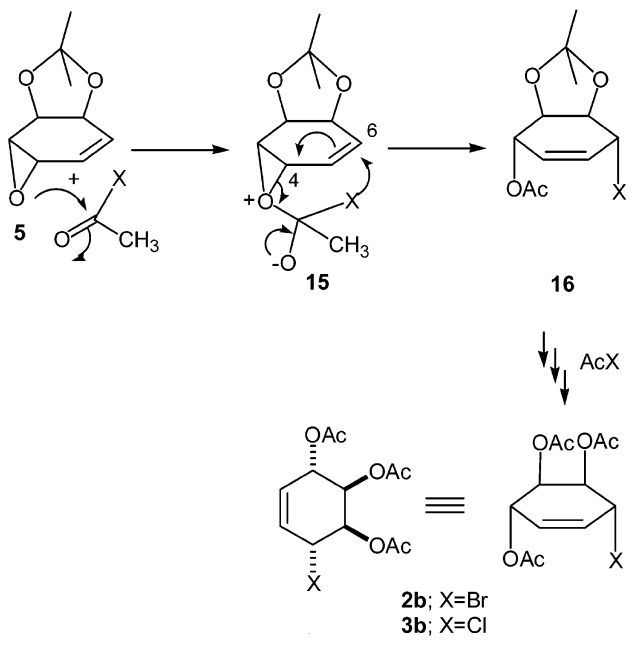
3. Conclusion

In conclusion, we describe in this paper a preparation method for a new chloroconduritol **13b** in the construction of conduritol-C. We also describe two new methods for the preparation of haloconduritol triacetates **2b** and **3b** from *endo*-diacetate **1** and epoxide **5** via cleavage with acetyl halides. Deacetylation of triacetates **2b** and **3b** by *trans*-esterification (MeOH/HCl) to give the corresponding haloconduritols was reported in our previous paper.[4]

4. Experimental

4.1. General information

Solvents were purified and dried by standard procedures before use. Melting points were determined on Büchi 539 capillary melting apparatus and uncorrected. Infrared spectra were obtained from KBr pellets or film on a Mattson 1000 FT-IR spectrophotometer. The ^1H and ^{13}C NMR spectra were recorded on 200 (50) MHz Varian spectrometers. Elemental analyses were carried out on a Carlo Erba 1108 model CHNS-O analyser. Column chromatography was performed on silica gel 60 (70–230 mesh ASTM). Thin layer chromatography was carried out on Merck 0.2 mm silica gel, 60 F_{254} analytical aluminum plates.



Scheme 7. Suggested mechanism for the formation of **2b** and **3b** from epoxide **5**.

4.1.1. *endo-cis*-7-Oxa-bicyclo[2.2.1]heptane-2,3-diol diacetate (1).[4] **1** was prepared from *endo*-cycloadduct of furan and vinylene carbonate according to our previously reported procedure.[4]

4.1.2. *exo-cis*-7-Oxa-bicyclo[2.2.1]heptane-2,3-diol diacetate (4).[4] **4** was prepared from *exo*-cycloadduct of furan and vinylene carbonate according to our previously reported procedure.[4]

4.1.3. *cis*-1,2-Isopropylidenedioxycyclohexa-3,5-diene (14). Acetonide **14** was prepared from 1,4-cyclohexadiene as described by Yang[11] et al. A direct preparation of **14** from cyclohexa-3,5-diene-*cis*-1,2-diol in a high yield is also described by Ramesh[9b] et al.

4.1.4. (3aα,5aβ,6aβ,6bα)-2,2-dimethyl-3a,5a,6a,6b-tetrahydro-oxireno[*e*]-1,3-benzo-dioxole (5). Epoxide **5** was prepared by epoxidation of acetonide **14** with *m*-chloroperbenzoic acid following the reported procedure by Banwell[9f] et al.

4.1.5. The reaction of *exo*-diacetate 4 with BBr_3. *Method A*. Under nitrogen atmosphere, to a stirred solution of *exo*-diacetate **4** (1.00 g, 4.7 mmol) in 20 mL of CH_2Cl_2 was added dropwise a solution of BBr_3 (0.5 mL, 1.30 g, 5.2 mmol) in 20 mL of CH_2Cl_2 at −78 °C over 10 min. After addition was completed, the mixture was stirred at 0 °C for 1 h, and then at room temperature for 4 h under air atmosphere. To the reaction mixture was added 5 mL of saturated $NaHCO_3$ solution. The organic phase was separated. The aqueous phase was additionally extracted with $CHCl_3$ (3×30 mL). The combined organic phases were dried over Na_2SO_4. The solvent was evaporated (30 °C, 25 mm Hg) and the product was chromatographied on a silica gel column (70 g) eluting with hexane–EtOAc (3:1).

The first fraction (2*exo*,3*exo*,5*endo*)-5-*Bromo*-7-*oxa*-*bicyclo*[2.2.1]*heptan*-2,3-*diol diacetate* (**6**). (0.40 g, %28). Colorless crystal. Mp 125–127 °C (solidified). ^1H NMR (200 MHz, $CDCl_3$) δ 5.66 (d, 1H, H_3, $J_{2,3}$=6.2 Hz), 4.97 (d, 1H, H_2, $J_{2,3}$=6.2 Hz), 4.50 (d, 1H, H_4, $J_{4,5}$=5.0 Hz), 4.42 (d, 1H, H_1, $J_{1,6exo}$=6.2 Hz), 3.98 (dt, 1H, H_5, $J_{5,6exo}$=10.9 Hz, $J_{5,6endo}$=5.0 Hz, $J_{4,5}$=5.0 Hz), 2.53 (ddd, 1H, H_6 (*exo*), $J_{6endo,6exo}$=15.9 Hz, $J_{5,6exo}$=10.9 Hz, $J_{1,6exo}$=6.2 Hz), 1.65 (dd, 1H, H_6 (*endo*), $J_{6endo,6exo}$=15.9 Hz, $J_{5,6endo}$=5.0 Hz), 2.12 (s, 3H), 2.08 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.3 (2C), 83.9, 82.7, 77.4, 75.8, 41.6, 38.4, 22.4, 22.3. IR (KBr) 2993, 2923, 2854, 1747, 1438, 1388, 1245, 1130, 1064, 1041, 906 cm^{-1}. Anal. calcd for $C_{10}H_{13}BrO_5$ (293.11): C, 40.98; H, 4.47; Found: C, 40.61, H, 4.35.

Second fraction (2*exo*,3*exo*,5*exo*)-5-*Bromo*-7-*oxa-bicyclo* [2.2.1]*heptan*-2,3-*diol diacetate* (**7**). (0.30 g, %22). Colorless crystal. Mp 134–136 °C (solidified). ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.87 (A part of AB system, d, 1H, H_2 or H_3, $J_{2,3}$=6.2 Hz), 4.80 (B part of AB system, d, 1H, H_2 or H_3, $J_{2,3}$=6.2 Hz), 4.60 (br d, 1H, H_1, $J_{1,6exo}$=5.0 Hz), 4.56 (br s, 1H, H_4), 4.03 (dd, 1H, H_5, $J_{5,6endo}$=7.0 Hz, $J_{5,6exo}$=4.0 Hz), 2.27–2.06 (AB system, m, 2H, H_6 (*endo*) and H_6 (*exo*)), 2.06 (s, 6H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.1, 171.8, 88.8, 81.8, 76.9, 75.3, 44.4, 40.5, 22.5, 22.4. IR (KBr) 3031, 3012, 1747, 1438, 1388, 1373, 1257, 1218, 1195, 1141, 1064, 1002, 944, 898 cm^{-1}. Anal. calcd for $C_{10}H_{13}BrO_5$ (293.11): C, 40.98; H, 4.47; Found: C, 40.51, H, 4.64.

Third fraction (2*exo*,3*exo*,5*exo*,6*exo*)-5-*Bromo*-7-*oxa-bicyclo*[2.2.1] *heptan*-2,3,6-*triol* 2,3-*diacetate* (**8**). (0.60 g, %41). Colorless crystal. Mp 149–151 °C (solidified). ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.94 (A part of AB system, d, 1H, H_2 or H_3, $J_{2,3}$=6.2 Hz), 4.91 (B part of AB system, d, 1H, H_2 or H_3, $J_{2,3}$=6.2 Hz), 4.56 (br d, 1H, H_4, $J_{1,4}$=2.2 Hz), 4.41 (d, 1H, H_1, $J_{1,4}$=2.2 Hz), 4.27 (d, 1H, H_5, $J_{5,6}$=6.3 Hz), 3.94 (dd, 1H, H_6, $J_{5,6}$=6.3 Hz, $J_{6,OH}$=9.5 Hz), 2.35 (d, 1H, OH, $J_{6,OH}$=9.5 Hz), 2.10 (s, 3H), 2.09 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.5, 171.4, 89.0, 88.4, 74.8, 73.5, 72.1, 55.0, 22.3 (2C). IR (KBr) 3455, 3394, 1643, 1554, 1469, 1427, 1052, 1025, 964 cm^{-1}. Anal. calcd for $C_{10}H_{13}BrO_6$ (309.11): C, 38.86; H, 4.24; Found: C, 39.10, H, 4.31.

Method B. Under nitrogen atmosphere, to a stirred solution of *exo*-diacetate **4** (0.160 g, 0.75 mmol) in 20 mL of CH_2Cl_2 was added dropwise a solution of BBr_3 (0.1 mL, 0.26 g, 1.0 mmol) in 20 mL of CH_2Cl_2 at −78 °C over 10 min. After addition was completed, the mixture was stirred at 0 °C for 1 h, and then at room temperature for 4 h under nitrogen atmosphere. After quenching of the reaction mixture with water (1 mL), organic phase was separated, dried (Na_2SO_4). Removing of the solvents gave a mixture of monobromides **6** and **7** in a ratio of 1: 1 (according to ^{1}H NMR) (0.143 g; 65%).

4.1.6. (1α,2α,3α,6β)-6-Chloro-4-cyclohexene-1,2,3-triol triacetate (13a). To a solution of *exo*-diacetate **4** (0.50 g, 2.36 mmol) in CH_2Cl_2 (5 mL) were added 1.5 mL of AcCl and one drop H_2SO_4. The reaction mixture was stirred for 24 h at room temperature. The solvent and excess of AcCl were evaporated. The residue was dissolved in $CHCl_3$ and the solution filtered over an basic Al_2O_3 (Aktiv.1; 5 g). Removal of the solvent gave **13a** (0.57 g, 83%). Colorless crystal. Mp 103–104 °C (from hexane–EtOAc). ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.90 (dt, 1H, H_5, J=10.5, 2.6 Hz), 5.69–5.58 (m, 3H, H_2, H_3 and H_4). 5.22 (dd, 1H, H_1, $J_{1,6}$=8.4 Hz, $J_{1,2}$=2.0 Hz), 4.72 (dm, 1H, H_6, $J_{1,6}$=8.4 Hz), 2.11 (s, 3H), 2.10 (s, 3H), 2.03 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.9, 171.5 (2C), 131.5, 128.4, 75.4, 71.1, 69.4, 56.7, 22.6 (3C). IR (KBr) 2969, 1754, 1430, 1373, 1226, 1157, 1075, 1033, 917 cm^{-1}. Anal. calcd for $C_{12}H_{15}ClO_6$ (290.70): C, 49.58; H, 5.20; Found: C, 49.38, H, 5.16.

4.1.7. (1α,2α,3α,6β)-6-Chloro-4-cyclohexene-1,2,3-triol (13b). A stirred solution of **13a** (0.35 g, 1.20 mmol) in 20 mL of methanol was cooled to 0 °C. At the given temperature HCl gas was passed through the solution over 20 min. The reaction flask was closed with a stopper and stirred at room temperature for 1 h. Removal of the solvent, methyl acetate and HCl under reduced pressure (30 °C, 25 mm Hg) and recrystallization from EtOAc gave chloroconduritol **13b** (0.16 g, 82%). Colorless crystal. Mp 128–130 °C (from EtOAc). ^{1}H NMR (200 MHz, DMSO-d_6) δ 5.63–5.49 (AB system, 2H, H_4 and H_5, $J_{4,5}$=10.5 Hz), 4.54 (br d, 1H, H_6, $J_{1,6}$=7.4 Hz), 4.16–3.96 (m, 4H, 3×OH and H_2), 3.82 (br s, 1H, H_3), 3.59 (br d, 1H, H_1, J=7.4 Hz). ^{13}C NMR (200 MHz, DMSO-d_6) δ 133.5, 128.5, 76.4, 74.4, 69.3, 63.6. IR (film) 3326, 2929, 2894, 1428, 1351, 1312, 1258, 1150, 1027, 927, 888, 850 cm^{-1}. Anal. calcd for $C_6H_9ClO_3$ (164.59): C, 43.78; H, 5.51; Found: C, 43.58, H, 5.53.

4.1.8. (2*exo*,3*exo*,5*endo*,6*exo*)-5,6-Dibromo-7-oxa-bicyclo [2.2.1] heptan-2,3-diol diacetate (12). To a solution of *exo*-diacetate **4** (0.25 g, 1.18 mmol) in CH_2Cl_2 (5 mL) were added 1 mL of AcBr and one drop H_2SO_4. The reaction mixture was stirred for 12 h at room temperature. The solvent and excess of AcBr were evaporated. The residue was dissolved in $CHCl_3$ and the solution filtered over an basic Al_2O_3 (Aktiv.1; 5 g). Removal of the solvent gave dibromide **12** (0.34 g, 77%). Colorless crystal. Mp 99–101 °C (from hexane–EtOAc). ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.65 (d, 1H, H_3, $J_{2,3}$=6.2 Hz), 5.06 (d, 1H, H_2, $J_{2,3}$=6.2 Hz), 4.64 (br d, 1H, H_4, $J_{4,5}$=5.4 Hz), 4.54 (br s, 1H, H_1), 4.24 (dd, 1H, H_5, $J_{4,5}$=5.4 Hz, $J_{5,6}$=3.4 Hz), 3.95 (d, 1H, H_6, $J_{5,6}$=3.4 Hz), 2.11 (s, 3H), 2.08 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.7, 171.5, 90.3, 85.2, 74.9, 74.7, 51.8, 51.5, 22.3 (2C). IR (KBr) 3018, 1757, 1433, 1379, 1248, 1086, 1067, 1020, 916. Anal. calcd for $C_{10}H_{12}Br_2O_5$ (372.01): C, 32.29; H, 3.25; Found: C, 32.16, H, 3.23.

4.1.9. Preparation of 2b from *endo*-diacetate 1. To a solution of *endo*-diacetate **1** (1.00 g, 4.72 mmol) in CH_2Cl_2 (50 mL) were added 3 mL of AcBr and one drop H_2SO_4. The reaction mixture was stirred for 5 h at room temperature. The solvent and excess of AcBr were evaporated. The residue was dissolved in $CHCl_3$ and the solution filtered over an basic Al_2O_3 (Aktiv.1; 5 g). Removal of the solvent gave oily **2b**[4] (1.49 g, 94%).

4.1.10. Preparation of 3b from *endo*-diacetate 1. To a solution of *endo*-diacetate **1** (1.00 g, 4.72 mmol) in CH_2Cl_2 (50 mL) were added 3 mL of AcCl and one drop H_2SO_4. The reaction mixture was stirred for 12 h at room temperature. The solvent and excess of AcCl were evaporated. The residue was dissolved in $CHCl_3$ and the solution filtered over a basic Al_2O_3 (Aktiv.1; 5 g). Removal of the solvent gave oily **3b**[4] (1.07 g, 78%).

4.1.11. Preparation of 2b from epoxide 5. To a solution of epoxide **5** (0.80 g, 4.76 mmol) in 50 mL of CH_2Cl_2 was added a 3 mL of acetyl bromide. The reaction mixture was magnetically stirred at room temperature for 12 h. The solvent and excess of acetyl bromide were evaporated. The residue was dissolved in $CHCl_3$ and the solution filtered over an basic Al_2O_3 (Aktiv.1; 5 g). Removal of the solvent gave oily **2b**[4] (1.33 g, 83%).

4.1.12. Preparation of 3b from epoxide 5. The procedure

described in Section 4.1.11 was applied to epoxide **5** using AcCl to give oily **3b**[4] (85%).

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A highly diastereoselective synthesis of a 1-β-methylcarbapenem intermediate using titanium enolate of 2′-hydroxypropiophenone

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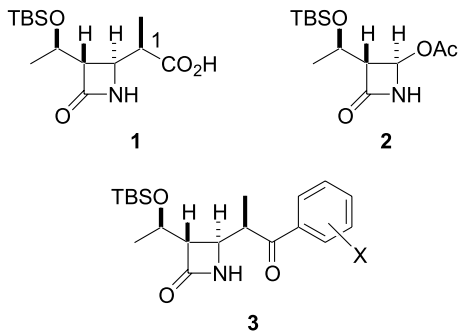
Abstract—A key 1-β-methylcarbapenem intermediate is synthesized from a highly diastereoselective condensation between the titanium enolate of 2′-hydroxypropiophenone with 4-acetoxy-β-lactam followed by ozonolysis of the resulting ketone to the carboxylic acid.

1. Introduction

In the synthesis of 1-β-methylcarbapenems with increased chemical and metabolic stability retaining the potent antibacterial activity of thienamycin, β-lactam **1** is serving as a key intermediate.[1] Most of the efforts for the synthesis of **1** have been devoted to the stereoselective introduction of 2-propionic acid moiety to the C(4) position of the commercially available 4-acetoxy-β-lactam **2**.[2] Diverse metal enolates of propionic acid derivatives having chiral and achiral auxiliaries were devised, including 2-oxazolidinones,[3a] 2-picolyl thiols,[4] and 2,3-dihydro-4*H*-1,3-benzoxazin-4-ones,[5] for the improved β-selectivity of **1**. But, still more easily accessible auxiliary and convenient reaction conditions have to be devised for more stereoselective and economic introduction of 2-propionic acid unit to **1**.



In our earlier study, we used lithium enolates of the readily available propiophenone derivatives for an aldol-type condensation with **2** followed by Baeyer–Villiger oxidation of the resulting ketone **3** with hydrogen peroxide under basic condition to generate **1**.[6] The β-selectivity in this condensation was low (<4:1), and, the transenolization between the enolate of the propiophenone and the acetate group of **2** caused about 30% of recovered **2**. Also, the oxidation of **3** with H_2O_2/NaOH provided unpredictable yield of **1** due to the β-lactam ring cleavage with a prolonged exposure of the reaction mixture to the basic media. Thus, an alternative approach has been pursued for more selective condensation between **2** and enolates of the propiophenone derivatives, together with a more reliable method for the oxidative conversion of the resulting ketone to **1**.[7]

2. Results and discussion

We used hydroxy- and methoxy-substituted propiophenones **4a–c**, which are commercially available in low cost, as a synthon for 2-propionic acid in expectation that the electron-rich aroyl group of **3** could be oxidized to the corresponding carboxylic acid in **1**. The titanium enolate of 2′-hydroxypropiophenone (**4a**), generated from **4a**, titanium tetrachloride and tri-*n*-butylamine, was condensed with **2** in high β-selectivity (Table 1). The yield of this reaction depends heavily on the amount of the enolate used. With the use of less than 2 equiv. of the enolate, the yields were sharply decreasing (entries 1 and 2). The reaction was best optimized with the use of 2.3 equiv. of the Ti(IV)-enolate to give 82% of **3a** after SiO_2 chromatography with 98:2 β/α diastereselectivity (entry 3). Conveniently, the crude mixture after the aqueous workup was directly recrystallized in ethyl acetate-hexanes to provide 78% of **3a** with

Keywords: Azetidinones; Carbapenems; Stereoselective; Condensation; Enolates; Ozonolysis.
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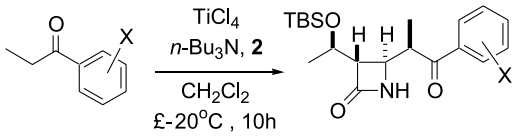
Table 1. Condensation of titanium enolate of propiophenones **4a**–**c** with **2**

Entry	Ketone (equiv.)[a]	$TiCl_4$ (equiv.)[a]	n-Bu_3N (equiv.)[a]	Product	Yield[b] (%)	De[c] (%)
1	**4a** (1.0)	1.0	3.0	**3a**	28	91
2	**4a** (1.8)	1.8	4.6	**3a**	63	93
3	**4a** (2.3)	2.3	5.6	**3a**	82 (78)[d]	96 (98)[d,e]
4	**4a** (3.0)	3.0	7.0	**3a**	32	96
5	**4b** (2.3)	2.3	3.3	**3b**	44	75
6	**4c** (2.3)	2.3	3.3	**3c**	57	85

[a] Equivalence relative to the amount of **2** used.
[b] Yields after SiO_2 chromatography.
[c] The diastereomeric excess was determined by ^1H NMR of the crude product.
[d] Yield and diastereomeric excess in the parenthesis refer to those after recrystallization of the crude product.
[e] Determined by HPLC analysis.

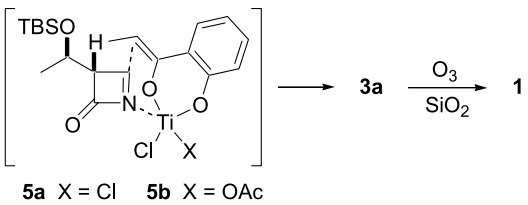
99:1 β-selectivity. The reaction did not proceed with the use of $Ti(O^iPr)_4$ or $TiCl_2(O^iPr)_2$, and, also, with the use of triethylamine instead of tri-*n*-butylamine.[8] Methoxypropiophenones, **4b** and **4c**, provided the corresponding **3b** and **3c** with much lower yields and diastereoselectivities than the use of **4a** under the same condition (entries 5 and 6). Unexpectedly, 2′-methoxy derivative **4b** showed a lower diastereoselectivity than 4′-methoxy derivative **4c**. Lack of an intramolecular chelation of the poorly basic phenyl ether oxygen in **4b** with the titanium enolate and the resulting tilt of the 2′-methoxyphenyl ring from the plane of the enolate may be the reason for the decreased selectivity in the condensation (Scheme 1).

The enhanced stereoselectivity in the formation of **3a** can be explained with the selective *Z*-enolate formation from **4a** through the bidentated Ti(IV)-enolate, and a tight coordination in the six-membered transition state of the resulting enolate with the acylimine generated from **2**, as shown in **5a**. The need for more than 2 equiv. of Ti(IV)-enolate of **4a** for an improved result may be due to the presence of less reactive acetoxy-substituted enolate **5b**.



Scheme 1.

Since the oxidation of **3a** with *m*-CPBA was too slow to be a practical method, and the use of H_2O_2/NaOH provided unpredictable yield of **1**, decomposition of 2-hydroxybenzoyl group of **3a** using ozone into a carboxyl group of **1** had been studied. Among the ozonolysis conditions studied, dry ozonation method was found to be most successful.[9] Passing a stream of ozone at −78 °C through the silica gel pre-adsorbed with **3a**, warming the mixture to ambient temperature followed by washing the silica gel with ethyl acetate provided conveniently the acid **1** in 60% yield in a reproducible manner (Scheme 2).



Scheme 2.

3. Conclusions

In conclusion, we developed a new practical method for the synthesis of a key 1-β-methylcarbapenem intermediate using a highly diastereoselective condensation of 4-acetoxy-β-lactam with a titanium enolate of 2′-hydroxypropiophenone and oxidative conversion of the resulting ketone to the carboxylic acid with a dry ozonation method. Eventually, 2-hydroxyphenyl group of the cheaply available 2′-hydroxypropiophenone served as an excellent achiral auxiliary for the highly diastereoselective introduction of 2-propionic acid needed for a 1-β-methylcarbapenem synthesis.

4. Experimental

4.1. General

IR spectra were recorded on a Bomem MB-104 spectrophotometer. Optical rotations were measured with a Rudolph Research Autopol III polarimeter. ^1H NMR spectra were recorded on a Varian Germini 300 (300 MHz) with TMS as an internal reference. ^{13}C NMR spectra were recorded on a Bruker AMX 400 (100 MHz) with TMS or $CDCl_3$ as an internal reference. Elemental analyses were obtained from Sogang Organic Chemistry Research Center, Seoul. Chiral HPLC analysis was performed on a Jasco LC-1500 Series HPLC system with a UV detector. TLC was performed on Merck silica gel 60 F_{254} precoated glass backed plates. Dichloromethane and tri-*n*-butylamine were dried by distillation over CaH_2 before use. All reactions were carried out in oven-dried glassware under an argon atmosphere.

4.1.1. (3*S*,4*R*)-3-[(*R*)-1-(*t*-Butyldimethylsiloxy)ethyl]-4-[(*R*)-1-(2-hydroxybenzoyl)ethyl]-2-azetidinone (3a). To a solution of 2′-hydroxypropiophenone **4a** (1.29 mL, 9.37 mmol) in 20 mL of dichloromethane at −78 °C was added slowly titanium tetrachloride (1.00 mL, 9.37 mmol) followed by tri-*n*-butylamine (5.50 mL, 23.0 mmol). The mixture was stirred for 30 min at the temperature and another 30 min at −40 °C. A solution of **2** (1.17 g, 4.07 mmol) in 14 mL of dichloromethane was added to the mixture dropwise, and the resulting solution was stirred at −20 °C for 10 h. The mixture was quenched with 80 mL of sat. NH_4Cl and extracted three times with 100 mL portions of ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residual solid was recrystallized in ethyl acetate and hexanes to give 1.20 g (78%, β/α=99:1) of **3a** as a white solid: 98% de by HPLC analysis (Chiralpak CAPCELL PAK C_{18}, 4:6 H_2O/CH_3CN, 1 mL/min, 254 nm UV detector), t_R=11.6 min for **3a** β-form, and t_R=13.8 min

for α-form; R_f=0.35 (1:1 ethyl acetate/hexanes); $[\alpha]_D^{25}=-79.8$ (*c* 1.4, EtOH); mp=170–171 °C; IR (KBr) 1760, 1634 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.05 (s, 3H), 0.08 (s, 3H), 0.86 (s, 9H), 1.15 (d, *J*=6 Hz, 3H), 1.34 (d, *J*=5 Hz, 3H), 2.92 (dd, *J*=5, 2 Hz, 1H), 3.75 (dq, *J*=6, 5 Hz, 1H), 4.00 (dd, *J*=5, 2 Hz, 1H), 4.12 (m, 1H), 6.00 (br s, 1H), 6.93–7.74 (m, 4H), 12.27 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.77, −4.00, 13.67, 18.17, 22.65, 25.98, 42.82, 52.01, 62.17, 65.56, 118.34, 119.27, 119.41, 130.00, 137.32, 163.60, 168.45, 209.00; Anal. Calcd for $C_{20}H_{31}NO_4Si$: C, 63.62; H, 8.28; N, 3.71. Found: C, 63.63; H, 8.42; N, 3.67.

4.1.2. (3*S*,4*R*)-3-[(*R*)-1-(*t*-Butyldimethylsiloxy)ethyl]-4-[(*R*)-1-(2-methoxybenzoyl)ethyl]-2-azetidinone (3b). To a solution of 2′-methoxypropiophenone **4b** (340 mg, 2.07 mmol) in 5 mL of dichloromethane at −78 °C was added slowly titanium tetrachloride (0.22 mL, 2.07 mmol) followed by tri-*n*-butylamine (0.71 mL, 3.0 mmol). The mixture was stirred for 30 min at the temperature and another 30 min at −40 °C. A solution of **2** (260 mg, 0.90 mmol) in 2.5 mL of dichloromethane was added to the mixture dropwise, and the resulting solution was stirred at −20 °C for 10 h. The mixture was quenched with 20 mL of sat. NH_4Cl and extracted three times with 30-mL portions of ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residual white solid was purified by silica gel flash chromatogaphy (1:2 ethyl acetate/hexanes) to give 154 mg (44%, β/α=7:1) of **3b** as a chromatographically inseparable diastereomeric mixture. The ratio of α and β diastereomers was determined by integration of the ^1H NMR signals of C(3)/H at 2.81 ppm (α-isomer) and 2.93 ppm (β-isomer). Spectral data for major β-isomer: R_f=0.25 (1:2, EtOAc/hexanes); IR (KBr) 1759, 1672 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.04 (s, 3H), 0.07 (s, 3H), 0.86 (s, 9H), 1.20 (d, *J*=6 Hz, 3H), 1.21 (d, *J*=5 Hz, 3H), 2.93 (dd, *J*=5, 2 Hz, 1H), 3.78 (dq, *J*=6, 5 Hz, 1H), 3.90 (s, 3H), 4.04 (dd, *J*=5, 2 Hz, 1H), 4.12 (quint, *J*=5 Hz, 1H), 5.89 (br s, 1H), 6.96–7.60 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.15, −4.31, 12.07, 17.83, 22.42, 25.65, 47.52, 51.71, 55.41, 61.30, 65.50, 111.38, 120.81, 127.80, 130.33, 133.60, 158.01, 168.54, 205.55; Anal. Calcd for $C_{21}H_{33}NO_4Si$: C, 64.41; H, 8.49; N, 3.58. Found: C, 64.33; H, 8.67; N, 3.36.

4.1.3. (3*S*,4*R*)-3-[(*R*)-1-(*t*-Butyldimethylsiloxy)ethyl]-4-[(*R*)-1-(4-methoxybenzoyl)ethyl]-2-azetidinone (3c). To a solution of 4′-methoxypropiophenone **4c** (0.165 mL, 0.937 mmol) in 2 mL of dichloromethane at −78 °C was added slowly titanium tetrachloride (0.10 mL, 0.94 mmol) followed by tri-*n*-butylamine (0.32 mL, 1.35 mmol). The mixture was stirred for 30 min at the temperature and another 30 min at −40 °C. A solution of **2** (117 mg, 0.407 mmol) in 1 mL of dichloromethane was added to the mixture dropwise, and the resulting solution was stirred at −20 °C for 10 h. The mixture was quenched with 20 mL of sat. NH_4Cl and extracted three times with 20-mL portions of ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residual white solid was purified by silica gel flash chromatography (1:2 ethyl acetate/hexanes) to give 92 mg (57%, β/α=12.3:1) of **3c** as a chromatographically inseparable diastereomeric mixture. The ratio of α and β diastereomers was determined by integration of the ^1H NMR signals of Ar/H doublets at 8.07 ppm (α-isomer) and 7.94 ppm (β-isomer). Spectral data for major β-Isomer: R_f=0.17 (1:2, EtOAc/hexanes); IR (KBr) 1757, 1669 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.04 (s, 3H), 0.06 (s, 3H), 0.86 (s, 9H), 1.15 (d, *J*=6 Hz, 3H), 1.27 (d, *J*=5 Hz, 3H), 2.88 (dd, *J*=5, 2 Hz, 1H), 3.66 (dq, *J*=6, 5 Hz, 1H), 3.88 (s, 3H), 3.97 (dd, *J*=5, 2 Hz, 1H), 4.16 (quint, *J*=5 Hz, 1H), 6.06 (br s, 1H), 6.95 (d, *J*=9 Hz, 2H), 7.94 (d, *J*=9 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.10, −4.33, 13.07, 17.84, 22.34, 25.66, 42.50, 51.97, 55.44, 61.65, 65.37, 113.93, 128.63, 130.62, 163.79, 168.41, 201.09; Anal. Calcd for $C_{21}H_{33}NO_4Si$: C, 64.41; H, 8.49; N, 3.58. Found: C, 64.46; H, 8.44; N, 3.32.

4.1.4. (3*S*,4*R*)-3-[(*R*)-1-(*t*-Butyldimethylsiloxy)ethyl]-4-[(*R*)-1-carboxyethyl]-2-azetidinone (1). A solution of **3a** (206 mg, 0.546 mmol) in 50 mL of dichloromethane was added 5.4 g of silica gel (70–130 mesh) followed by the removal of the solvent under reduced pressure. The silica gel pre-adsorbed with **3a** in 500 mL Erlenmeyer flask was passed with ozone at −78 °C for 10 min followed by warming the flask to room temperature. The silica gel was washed with ethyl acetate, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (3:2 hexanes/ethyl acetate) to give **1** as a white solid (98 mg, 60%): mp 140–143 °C. Spectral data (^1H NMR, IR) of **1** are identical with those reported.[1]

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Diels–Alder reaction of optically active (*E*)-γ-keto-α,β-unsaturated *p*-tolylsulfoxides with cyclopentadiene

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Abstract—The Diels–Alder reaction of enantiomerically pure (*E*)-γ-keto-α,β-unsaturated *p*-tolylsulphoxides **3** with cyclopentadiene give four easily separable diastereomers. The effect of several Lewis acids on the reaction was studied, finding a high *endo* selectivity with respect to the carbonyl group and moderate π-diastereoselectivity using $BF_3{\cdot}Et_2O$ as catalyst. The reactivity of compounds **3** as well as their *endo* selectivity are both higher than those observed for the corresponding (*E*)-3-sulfinylacrylates.

1. Introduction

The combination of Diels–Alder reaction with asymmetric induction exerted by sulphoxides represents a very powerful method for C–C bond formation in a stereocontrolled manner.[1] The sulphinyl group has equally become one of the most interesting chiral inductors in asymmetric Diels–Alder reactions due to the following facts: its ability to differentiate between diastereotopic faces of neighboring double bonds, the ease of chemical transformations into different functional groups including its clean removal under mild conditions, and the existence of several efficient methods that allow the preparation of enantiomerically pure sulphoxides. The poor results obtained in the Diels–Alder reaction using unsubstituted vinylic sulphoxides (low reactivity and only moderate stereoselectivity)[2] were substantially improved by attaching additional groups to the double bond, which increases the reactivity and simultaneously restricts the conformational mobility around the C–S bond, hence improving the stereoselectivity of the dienophile. In this sense, several electron-withdrawing groups have been incorporated to vinylic sulphoxides, such as carbonyl,[3] nitro,[4] sulphonyl,[5] sulphinyl,[6] and cyano.[7] Nevertheless, the most widely studied one is doubtlessly the ester group, the contributions by Koizumi in this field clearly being the most significant.[8]

As part of our studies involving the stereoselective preparation and synthetic application of γ-substituted-α,β-unsaturated sulphoxides, we have recently reported an efficient synthesis of enantiomerically pure (*E*)-γ-hydroxy- and (*E*)-γ-keto-α,β-unsaturated *p*-tolylsulphoxides.[9] We report herein the results obtained in the Diels–Alder reaction between enantiomerically pure (*E*)-γ-keto-α,β-unsaturated *p*-tolylsulphoxides **3** as dienophiles and cyclopentadiene. Both *endo* selectivity of alkylcarbonyl substituent and π-diastereoselectivity are strongly influenced by the nature of Lewis acid present in the reaction.

2. Results and discussion

(*E*)-γ-Hydroxy-α,β-unsaturated *p*-tolylsulphoxides **2** were obtained in excellent chemical yield by condensation of enantiomerically pure (*S*,*S*)-bis-*p*-tolylsulfinylmethane **1** with enolizable aldehydes in the presence of piperidine as base and thiophile.[9] The process involves a Knoevenagel condensation between the aldehyde and methylene active bis-sulphoxide **1**, in tandem with an allylic sulphoxide–sulphenate rearrangement and hydrolysis of the sulphenate ester promoted by the thiophilic base. The (*E*)-γ-hydroxysulphoxides **2** were oxidized with PCC and sodium acetate in dichloromethane at room temperature to afford enantiomerically pure (*E*)-γ-keto-α,β-unsaturated *p*-tolylsulphoxides **3** in high chemical yields (Scheme 1).

With the (*E*)-γ-ketosulfphoxides **3a** and **3b** in hand, their

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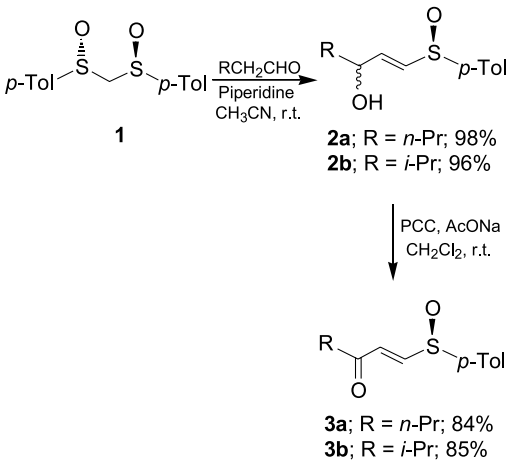


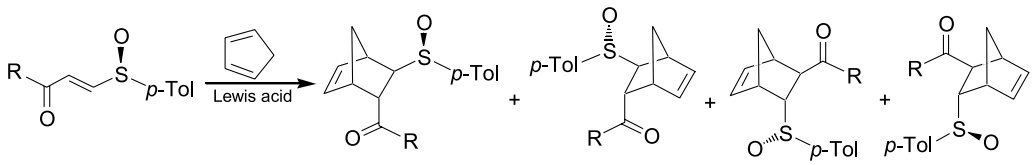
Scheme 1.

Diels–Alder reactions with cyclopentadiene under thermal conditions were studied. Initially, we carried out the cycloaddition reaction between enantiopure (*R*)-(*E*)-1-*p*-tolylsulfinyl-1-hexen-3-one **3a** and cyclopentadiene at 25 °C in toluene. After 30 min, analysis of the reaction mixture by TLC showed that the four possible diastereoisomeric norbornenes **4a**, **5a**, **6a** and **7a** were formed, which could be easily separated by flash chromatography affording the four enantiomerically pure cycloadducts (Table 1, entry 1). The *endo*/*exo* ratio (taking the carbonyl group as reference for the *endo* or *exo* designation) and the relative proportions of the four diastereoisomers, were easily determined from the relative intensities of the well-separated vinylic proton signals in the ^{1}H NMR (500 MHz) spectrum of the crude. The stereochemistry of the diastereomers **4**–**7** were assigned by irradiation of the proton signals and homonuclear shift correlation in ^{1}H and ^{13}C NMR spectroscopy.

As shown in Table 1, a similar behavior was observed for (*R*)-(*E*)-4-methyl-1-*p*-tolylsulfinyl-1-penten-3-one **3b** in its reaction with cyclopentadiene (entry 2). These results suggest that diastereoselectivity of the Diels–Alder reaction of (*E*)-γ-keto-α,β-unsaturated *p*-tolylsulphoxides **3** is almost independent of the size of the alkyl R group attached to the carbonyl. Additionally, the *endo* preference for carbonyl or sulphinyl group is found to be very similar.

There has been much interest in recent years in the effect of Lewis acids on diastereomeric ratios in the Diels–Alder cycloaddition reaction. Having established the structure and stereochemistry of the four diastereomeric adducts from the thermal Diels–Alder reaction, we turned our attention to the effect of Lewis acid promoters on the process. As expected, the use of Lewis acids increases the reactivity of the dienophile allowing the use of lower reaction temperatures. We carried out the reaction between (*R*)-(*E*)-1-(*p*-tolylsulfinyl)-1-hexen-3-ona **3a** and cyclopentadiene at −78 °C in dichloromethane as solvent in the presence of several Lewis acids, the results are collected in the Table 1. The addition of SiO_2 or $LiClO_4$ as promoters (entries 3 and 4), afforded the four adducts **4/5/6/7** in good chemical yield but it had no significant influence on both *endo*/*exo* and π-facial selectivities. The addition of $ZnBr_2$ or $SnCl_4$ (entries 5–8) gave rise to a moderate carbonyl-*endo* selectivity, affording adduct **5** as the predominant stereoisomer. On the other hand, when the Diels–Alder reaction was carried out in the presence of $TiCl_4$, Et_2AlCl or $BF_3{\cdot}Et_2O$ as Lewis acids (entries 9–13), a reversal of the π-facial selectivity was observed, adduct **4** now being the isomer obtained in higher proportion.[10,12] The best promoter for the Diels–Alder reaction of (*R*)-(*E*)-1-(*p*-tolylsulfinyl)-1-hexen-3-ona **3a** with cyclopentadiene resulted to be $BF_3{\cdot}Et_2O$, which increases both *endo*/*exo* and π-facial selectivities from 54:46 (22% d.e. *endo*) in the thermal reaction until 93:7

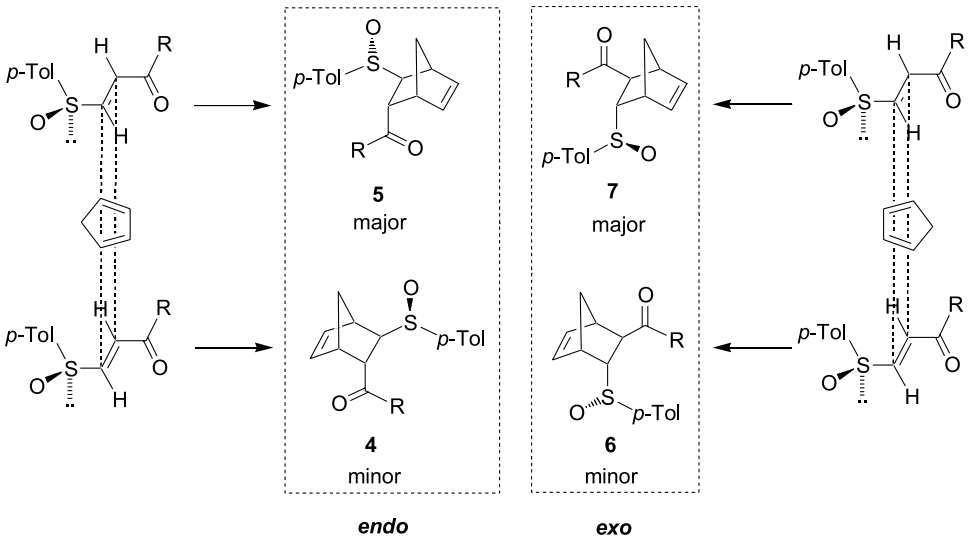
Table 1. Diels–Alder reaction of (*E*)-γ-keto-α,β-unsaturated *p*-tolylsulfoxides **3a-b** with cyclopentadiene



3 **4** **5** **6** **7**

Entry	R	Lewis acid[a]	*t* (h)	Yield (%)	**4/5/6/7**[b]	*endo*/*exo*	% d.e. *endo*	% d.e. *exo*
1	*n*-Pr	—	0.5[c]	90	21:33:17:29	54:46	22	26
2	*i*-Pr	—	0.5[c]	95	15:36:17:32	51:49	41	31
3	*n*-Pr	SiO_2	24[d]	99	19:36:16:29	55:45	31	29
4	*n*-Pr	$LiClO_4$	24[d]	95	20:35:16:29	55:45	27	29
5	*n*-Pr	$ZnBr_2$	10[d]	95	34:41:12:13	75:25	9	4
6	*n*-Pr	$ZnBr_2$[e]	10[d]	97	32:45:09:14	77:23	17	22
7	*n*-Pr	$SnCl_4$	2[d]	80	35:54:05:06	89:11	21	9
8	*i*-Pr	$SnCl_4$	2[d]	82	37:52:06:05	89:11	17	9
9	*n*-Pr	$TiCl_4$	2[d]	85	53:31:07:09	84:16	26	13
10	*n*-Pr	Et_2AlCl	2[d]	86	60:25:11:04	85:15	41	47
11	*n*-Pr	$BF_3{\cdot}Et_2O$	2[d]	98	65:26:06:03	91:9	43	33
12	*n*-Pr	$BF_3{\cdot}Et_2O$[e]	2[d]	97	71:22:05:02	93:7	53	43
13	*i*-Pr	$BF_3{\cdot}Et_2O$	2[d]	98	60:24:10:06	86:14	42	25

[a] 1.2 equiv.
[b] Determined by ^{1}H NMR integrals of vinylic protons.
[c] Reaction carried out at room temperature.
[d] Reaction carried out at −78 °C.
[e] 2 equiv. of Lewis acid were used.



Scheme 2.

(53% d.e. *endo*) in the process carried out in the presence of 2 equiv. of Lewis acid.

The observed π-facial diastereoselectivity in Diels–Alder reactions of (*E*)-γ-keto-α,β-unsaturated *p*-tolyl-sulphoxides **3a** and **3b** with cyclopentadiene may be explained assuming the transition state proposed by Koizumi for the reaction of ethyl *p*-tolylsulfinylmethylenepropionate,[11] in which the α,β-unsaturated sulphoxide adopts the *s-trans* conformation with respect to the S=O and C=C bonds, and the addition of cyclopentadiene takes place where is found the lone pair electrons at sulphur (Scheme 2).

Loss of optical purity for (*E*)-γ-keto-α,β-unsaturated *p*-tolylsulfoxides **3a**-**b** was shown to be minimal in the process by control experiments in which (*E*)-γ-keto-sulfoxides **3a**-**b** were submitted to the reaction conditions in the absence of the cyclopentadiene recovering the corresponding sulphoxide without any loss on its $[\alpha]_D$ value.

3. Conclusions

In summary, we can state that the readily obtained (*E*)-γ-keto-α,β-unsaturated *p*-tolylsulphoxides **3** are efficient chiral sulphinyldienophiles exhibiting the following advantages with respect to the corresponding sulfinyl acrilate: higher reactivity, higher degree of stereocontrol when the process is carried out in the presence of $BF_3{\cdot}Et_2O$ as promoter, good chemical yield and easy separation of diastereomeric mixtures affording the four enantiomerically pure cycloadducts.

4. Experimental

4.1. General

Melting points were determined in open capillary tubes on a Gallenkamp apparatus and are uncorrected. ^1H NMR spectra were registered on a Bruker AC-200 (200 MHz) or AMX-500 (500 MHz) and ^{13}C NMR on AC-200 (50.3 MHz) or AMX-500 (125.72 MHz). All spectra were obtained using $CDCl_3$ as solvent and TMS as internal standard. Chemical shifts are reported in ppm, and coupling constants in Hz. Optical rotations were taken on a Perkin–Elmer 241-MC polarimeter in an 1 dm tube; concentrations are given in g/100 mL. High resolutions mass measurements were performed on a Kratos MS-80-RFA spectrometer. HPLC analysis was carried out on a Waters, Millipore 600A model using a Chiral OD (Diacel) column or reverse phase column Lichrocart C-18. Routine monitoring of reactions was performed using Merck 60 F 254 silica gel, aluminium supported TLC plates. For the flash chromatography,[13] silica gel 60 (230–400 mesh ASTM, Merck) was used. Flasks, stirrings bars, and hypodermic needles used for the generation of organometallic compounds were dried for ca. 12 h at 120 °C and allowed to cool in a dessicator over anhydrous calcium sulphate. Anhydrous solvents (ethers) were obtained by distillation from benzophenone ketyl.[14]

4.1.1. (*E*)-γ-Hydroxy-α,β-unsaturated sulfoxides (2a-b). The general procedure described by Llera et al. was followed.[9]

4.2. General procedure for the oxidation of compounds 2a-b

To a solution of (*E*)-γ-hydroxy-α,β-unsaturated sulphoxides **2a**-**b** (1 mol) in dichloromethane was sequentially added sodium acetate (1 equiv.) and pyridinium chlorochromate PCC (3 equiv.). The resulting suspension was vigorously shaken at room temperature and monitored by TLC for the conversion. The reaction mixture was filtered on $SiO_2/CaSO_4$ (9:1) and washed with CH_2Cl_2/ether (1:1). After evaporating the solvent, the crude was purified by column chromatography to give (*E*)-γ-ketosulfoxide **3a**-**b**.

4.2.1. *R*-(*E*)-1-*p*-Tolylsulfinyl-1-hexen-3-one (*R*)-3a. The general procedure was followed for the oxidation of 1.19 g (4.9 mmol) of **2a**, employing 0.68 g (4.9 mmol) of sodium acetate and 3.21 g (15 mmol) of pyridinium chlorochromate for 5 h. Purification of the reaction mixture by flash

chromatography (hexane/ethyl acetate, 3:1), afforded 0.99 g (84% yield) of (*R*)-**3a** as a white solid. Mp 92.5–93.5 °C. $[\alpha]_D$=+404 (*c*=2, acetone). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.89 (t, *J*=7.4 Hz, 3H, CH_3), 1.61 (m, 2H, CH_2), 2.36 (s, 3H, CH_3Ar), 2.55 (t, *J*=7.4 Hz, 2H, CH_2–C═O), 6.94 (d, J_{trans}=15.0 Hz, 1H, CH–S═O), 7.29 (d, J_{trans}=15.0 Hz, 1H, CH–C═O), 7.28–7.47 (AA′BB′ system, 4H, aromatics). ^{13}C NMR (50.29 MHz, $CDCl_3$) δ 13.5, 17.0, 21.4, 44.3, 124.9, 129.4, 130.4, 138.2, 142.5, 148.6, 197.3. HRMS calcd *m/z* for $C_{13}H_{16}O_2S$: 236.0881. Found: 236.0842. Anal. Calcd for $C_{13}H_{16}O_2S$: C, 66.07; H, 6.83. Found: C, 65.98; H, 6.54.

4.2.2. (*R*)-(*E*)-4-Methyl-1-*p*-tolylsulfinyl-1-penten-3-one (*R*)-3b. The general procedure was followed for the oxidation of 1.18 g (4.95 mmol) of **2b**, employing 0.68 g (5.0 mmol) of sodium acetate and 3.20 g (15 mmol) of pyridinium chlorochromate for 2.5 h. Purification of the reaction mixture by flash chromatography (hexane/ethyl acetate, 4:1), afforded 1.0 g (85% yield) of (*R*)-**3b** as a white solid. Mp 76–77 °C. $[\alpha]_D$=+350 (*c*=2, acetone). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.15 (d, *J*=6.9 Hz, 6H, $(CH_3)_2$), 2.40 (s, 3H, CH_3Ar), 2.80 (m, 1H, CH), 7.10 (d, J_{trans}=15.0 Hz, 1H, CH–S═O), 7.38 (d, J_{trans}=15.0 Hz, 1H, CH–C═O), 7.32–7.50 (AA′BB′ system, 4H, aromatics). ^{13}C NMR (50.29 MHz, $CDCl_3$) δ 17.8, 21.5, 40.8, 125.1, 128.1, 130.5, 138.2, 142.6, 149.1, 200.7. HRMS calcd *m/z* for $C_{13}H_{16}O_2S$: 236.0881. Found: 236.0842. Anal. Calcd for $C_{13}H_{16}O_2S$: C, 66.07; H, 6.83. Found: C, 65.91; H, 6.53.

4.3. Diels–Alder reaction of (*R*)-(*E*)-1-*p*-tolylsulfinyl-1-hexen-3-one (*R*)-3a

A solution of (*R*)-**3a** (550 mg, 2.3 mmol) and freshly distilled cyclopentadiene (750 mg, 0.94 mL, 11.5 mmol) in toluene (10 mL) was stirred for 30 min at room temperature. The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography eluting with hexane/ether/ethyl acetate (3:2:1) to give the products **4**–**7a** (90% chemical yield).

4.3.1. Compound 4a. Yield 19.1%, white solid. Mp 89.5–91 °C. $[\alpha]_D$=0 (*c*=2, acetone). ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.64 (t, *J*=7.1 Hz, 3H, CH_3), 1.06 (m, 2H, –CH_2–), 1.47 (dd, *J*=8.7, 1.7 Hz, 1H, H_{7a}), 1.98 (dt, *J*=16.9, 7.2 Hz, 1H, $H_{1'a}$), 2.09 (m, 1H, H_{7b}), 2.18 (dt, *J*=16.9, 7.2 Hz, 1H, $H_{1'b}$), 2.36 (s, 3H, CH_3Ar), 3.00 (dd, *J*=4.7, 1.7 Hz, 1H, H_2), 3.18 (m, 1H, H_4), 3.25 (m, 1H, H_1), 3.38 (dd, *J*=4.7, 3.5 Hz, 1H, H_3), 5.90 (dd, *J*=5.6, 2.8 Hz, 1H, H_5), 6.30 (dd, *J*=5.6, 3.2 Hz, 1H, H_6), 7.22–7.37 (AA′BB′ system, 4H, aromatics). ^{13}C NMR (50.29 MHz, $CDCl_3$) δ 13.4, 16.8, 21.2, 43.0, 45.8, 46.1, 47.2, 49.0, 64.0, 123.7, 129.6, 135.5, 137.6, 140.1, 140.8, 207.3. Anal. Calcd for $C_{18}H_{22}O_2S$: C, 71.48; H, 7.33. Found: C, 71.14; H, 7.21.

4.3.2. Compound 5a. Yield 25.9%, white solid. Mp 94–95 °C. $[\alpha]_D$=0 (*c*=2, acetone). ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.72 (t, *J*=7.0 Hz, 3H, CH_3), 1.37 (m, 2H, –CH_2–), 1.55 (dd, *J*=8.9, 1.8 Hz, 1H, H_{7a}), 1.97 (m, 1H, H_{7b}), 2.11 (dt, *J*=16.9, 7.2 Hz, 1H, $H_{1'a}$), 2.22 (dt, *J*=16.9, 7.2 Hz, 1H, $H_{1'b}$), 2.36 (s, 3H, CH_3Ar), 3.99 (dd, *J*=4.7, 3.5 Hz, 1H, H_3), 3.16 (dd, *J*=4.7, 1.8 Hz, 1H, H_2), 3.24 (m, 1H, H_1), 3.28 (m, 1H, H_4), 5.90 (dd, *J*=5.6, 3.3 Hz, 1H, H_5), 6.18 (dd, *J*=5.6, 2.7 Hz, 1H, H_6), 7.27–7.53 (AA′BB′ system, 4H, aromatics). ^{13}C NMR (50.29 MHz, $CDCl_3$) δ 13.2, 16.9, 21.4, 43.2, 44.1, 46.7, 47.6, 53.4, 65.3, 125.1, 129.9, 134.9, 137.5, 139.6, 141.9, 206.8. Anal. Calcd for $C_{18}H_{22}O_2S$: C, 71.48; H, 7.33. Found: C, 71.13; H, 7.27.

4.3.3. Compound 6a. Yield 11.7%, viscous oil. $[\alpha]_D$=+24 (*c*=2, acetone). ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.81 (t, *J*=7.3 Hz, 3H, CH_3), 1.40 (m, 1H, H_{7a}), 1.45 (m, 2H, –CH_2–), 1.61 (m, 1H, H_{7b}), 2.33 (dt, *J*=17.3, 7.3 Hz, 1H, $H_{1'a}$), 2.38 (s, 3H, CH_3Ar), 2.49 (dt, *J*=17.3, 7.3 Hz, 1H, $H_{1'b}$), 2.84 (m, 1H, H_1), 2.88 (dd, *J*=4.6, 1.7 Hz, 1H, H_3), 2.97 (m, 1H, H_4), 3.65 (dd, *J*=4.6, 3.3 Hz, 1H, H_2), 6.25 (dd, *J*=5.6, 2.7 Hz, 1H, H_6), 6.35 (dd, *J*=5.6, 3.1 Hz, 1H, H_5), 7.37–7.47 (AA′BB′ system, 4H, aromatics). ^{13}C NMR (50.29 MHz, $CDCl_3$) δ 13.6, 17.0, 21.4, 44.1, 44.3, 47.0, 47.1, 51.0, 69.5, 124.2, 129.8, 134.9, 137.1, 140.8, 141.5, 209.7. Anal. Calcd for $C_{18}H_{22}O_2S$: C, 71.48; H, 7.33. Found: C, 71.23; H, 7.37.

4.3.4. Compound 7a. Yield 29.4%, white solid. Mp 109.5–110 °C. $[\alpha]_D$=+8 (*c*=2, acetone). ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.62 (t, *J*=7.2 Hz, 3H, CH_3), 1.22 (m, 2H, –CH_2–), 1.51 (dq, *J*=9.1, 1.6 Hz, 1H, H_{7a}), 1.60 (dd, *J*=9.1, 1.6 Hz, 1H, H_{7b}), 1.66 (dt, *J*=17.2, 7.2 Hz, 1H, $H_{1'a}$), 2.02 (dd, *J*=4.7, 1.4 Hz, 1H, H_3), 2.18 (dt, *J*=17.2, 7.2 Hz, 1H, $H_{1'b}$), 2.34 (s, 3H, CH_3Ar), 2.89 (m, 1H, H_4), 3.45 (m, 1H, H_1), 3.91 (dd, *J*=4.7, 3.3 Hz, 1H, H_2), 6.34 (dd, *J*=5.6, 3.1 Hz, 1H, H_5), 6.50 (dd, *J*=5.6, 2.8 Hz, 1H, H_6), 7.22–7.51 (AA′BB′ system, 4H, aromatics). ^{13}C NMR (50.29 MHz, $CDCl_3$) δ 13.4, 16.7, 21.4, 44.0, 44.3, 45.9, 47.4, 52.0, 69.5, 125.6, 129.9, 136.1, 137.4, 139.8, 142.3, 207.8. Anal. Calcd for $C_{18}H_{22}O_2S$: C, 71.48; H, 7.33. Found: C, 71.18; H, 7.31.

4.4. Diels–Alder reaction of (*R*)-(*E*)-4-methyl-1-(*p*-tolylsulfinyl)-1-penten-3-ona (*R*)-3b

A solution of (*R*)-**3a** (500 mg, 2.1 mmol) and freshly distilled ciclopentadiene (750 mg, 0.94 mL, 11.5 mmol) in toluene (10 mL) was heated under for 30 min at 25 °C. The solvent was evaporated under vacuum and the crude product was purified by column chromatography eluting with hexane/ether/ethyl acetate (2:2:1) to give the products **4b**–**7b** (95% global yield).

4.4.1. Compound 4b. Yield 18.3%, white solid. Mp 103–105 °C. $[\alpha]_D$=+68 (*c*=2, acetone). ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.59 (d, *J*=6.9 Hz, 3H, $(CH_3)_2CH$), 0.82 (d, *J*=6.9 Hz, 3H, $(CH_3)_2CH$), 1.47 (dd, *J*=8.6, 1.7 Hz, 1H, H_{7a}), 2.14 (d, *J*=8.6 Hz, 1H, H_{7b}), 2.31 (s, 3H, CH_3Ar), 2.46 (h, *J*=6.9 Hz, 1H, $(CH_3)_2CH$), 3.04 (dd, *J*=4.7, 1.8 Hz, 1H, H_2), 3.18 (m, 1H, H_4), 3.27 (m, 1H, H_1), 3.49 (dd, *J*=4.7, 3.5 Hz, 1H, H_3), 5.84 (dd, *J*=5.6, 2.7 Hz, 1H, H_5), 6.31 (dd, *J*=5.6, 3.2 Hz, 1H, H_6), 7.20–7.35 (AA′BB′ system, 4H, aromatics). ^{13}C NMR (50.29 MHz, $CDCl_3$) δ 17.8, 18.8, 21.2, 39.1, 46.0, 47.2, 47.5, 63.4, 123.8, 129.6, 135.5, 137.8, 140.0, 140.7, 211.5. Anal. Calcd for $C_{18}H_{22}O_2S$: C, 71.48; H, 7.33. Found: C, 71.20; H, 7.26.

4.4.2. Compound 5b. Yield 33.6%, white solid. Mp 133–134 °C. $[\alpha]_D$=+46 (*c*=2, acetone). ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.75 (d, *J*=7.0 Hz, 3H, $(CH_3)_2CH$), 0.88 (d,

J=7.0 Hz, 3H, $(CH_3)_2CH$), 1.56 (dq, J=8.9, 1.8 Hz, 1H, H_{7a}), 1.96 (d, J=8.9 Hz, 1H, H_{7b}), 2.34 (s, 3H, CH_3Ar), 2.52 (h, J=7.0 Hz, 1H, $(CH_3)_2CH$), 3.08 (dd, J=4.6, 3.6 Hz, 1H, H_3), 3.21 (dd, J=4.6, 1.8 Hz, 1H, H_2), 3.26 (m, 2H, H_1 and H_4), 5.83 (dd, J=5.6, 2.7 Hz, 1H, H_5), 6.18 (dd, J=5.6, 3.7 Hz, 1H, H_6), 7.24–7.52 (AA′BB′ system, 4H, aromatics). ^{13}C NMR (50.29 MHz, $CDCl_3$) δ 17.7, 18.9, 21.4, 39.1, 44.1, 46.9, 47.7, 51.6, 65.1, 125.3, 129.9, 134.7, 137.4, 139.5, 141.9, 210.6. Anal. Calcd for $C_{18}H_{22}O_2S$: C, 71.48; H, 7.33. Found: C, 71.28; H, 7.36.

4.4.3. Compound 6b. Yield 13.0%, viscous oil. $[\alpha]_D$=+79.5 (c=5.94, acetone). ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.90 (d, J=6.9 Hz, 3H, $(CH_3)_2CH$), 1.06 (d, J=6.9 Hz, 3H, $(CH_3)_2CH$), 1.41 (ddd, J=8.9, 3.4, 1.7 Hz, 1H, H_{7a}), 1.67 (d, J=8.9 Hz, 1H, H_{7b}), 2.38 (s, 3H, CH_3Ar), 2.72 (h, J=6.9 Hz, 1H, $(CH_3)_2CH$), 2.90 (m, 1H, H_1), 2.96 (m, 1H, H_4), 3.05 (dd, J=4.5, 1.7 Hz, 1H, H_3), 3.70 (dd, J=4.5, 3.3 Hz, 1H, H_3), 6.28 (dd, J=5.6, 2.7 Hz, 1H, H_6), 6.38 (dd, J=5.6, 2.7 Hz, 1H, H_5), 7.28–7.47 (AA′BB′ system, 4H, aromatics). ^{13}C NMR (50.29 MHz, $CDCl_3$) δ 18.3, 18.4, 21.3, 40.3, 45.2, 46.7, 47.4, 49.4, 69.2, 124.2, 129.8, 134.9, 136.9, 140.7, 141.4, 213.4. Anal. Calcd for $C_{18}H_{22}O_2S$: C, 71.48; H, 7.33. Found: C, 71.30; H, 7.31.

4.4.4. Compound 7b. Yield 29.7%, white solid. Mp 154.5–156 °C. $[\alpha]_D$=+16 (c=2, acetone). ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.46 (d, J=6.9 Hz, 3H, $(CH_3)_2CH$), 0.87 (d, J=6.9 Hz, 3H, $(CH_3)_2CH$), 1.49 (ddd, J=9.0, 3.2, 1.6 Hz, 1H, H_{7a}), 1.58 (d, J=9.0 Hz, 1H, H_{7b}), 2.12 (dd, J=4.6, 1.6 Hz, 1H, H_3), 2.21 (h, J=6.9 Hz, 1H, $(CH_3)_2CH$), 2.31 (s, 3H, CH_3Ar), 2.89 (m, 1H, H_4), 3.45 (m, 1H, H_1), 3.99 (dd, J=4.6, 3.4 Hz, 1H, H2), 6.34 (dd, J=5.6, 3.4 Hz, 1H, H_5), 6.50 (dd, J=5.6, 2.8 Hz, 1H, H_6), 7.20–7.50 (AA′BB′ system, 4H, aromatics). ^{13}C NMR (50.29 MHz, $CDCl_3$) δ 18.1, 21.3, 40.1, 44.4, 45.6, 47.6, 50.7, 68.7, 126.0, 129.9, 136.2, 137.3, 139.6, 142.5, 211.5. Anal. Calcd for $C_{18}H_{22}O_2S$: C, 71.48; H, 7.33. Found: C, 71.29; H, 7.36.

4.5. Diels–Alder reaction of (*R*)-(*E*)-1-(*p*-tolylsulfinyl)-1-hexen-3-ona (*R*)-2a with Lewis acids

The ketosulphoxide (50 mg, 0.2 mmol) was dissolved in anhydrous dichloromethane (5 mL) under a N_2 atmosphere and a solution of Lewis acid (0.25 mmol) was added dropwise at −78 °C. The solution was stirred for 20 min and treated at −78 °C with freshly distilled ciclopentadiene (2.3 mmol). After being stirred for (2–24 h) at −78 °C, the reaction was quenched with water (2 mL). The organic phase was separated and washed successively with 10% hydrochloric acid solution, saturated aqueous $NaHCO_3$ solution and saturated aqueous NaCl solution and dried over Na_2SO_4. The solvent was removed under reduced pressure and the proportion of cycloadducts was analyzed by ^{1}H NMR (500 MHz).

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Synthesis of new α or γ-functionalized hydroxymethylphosphinic acid derivatives

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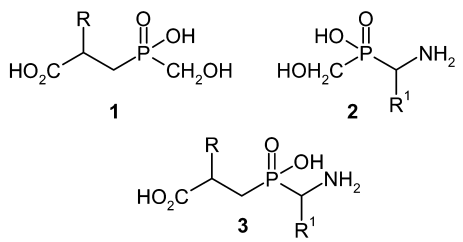
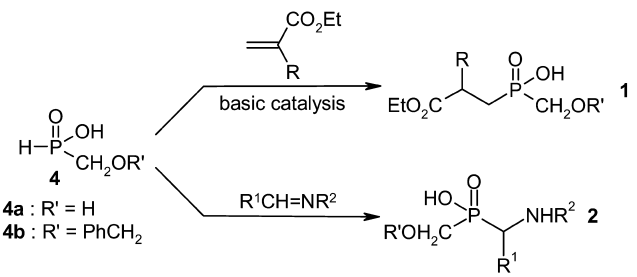
Abstract—The syntheses of new γ-ethoxycarbonyl- and α-amino-alkyl hydroxymethylphosphinic acid derivatives are described. These compounds were conveniently prepared by Michael addition or Kabachnik–Fields reaction of an original precursor, ethyl benzyloxymethyl hydrogenophosphinate, respectively to α,β-unsaturated esters using a basic activation or to imines. Selective deprotection of the alcohol function was achieved by hydrogenolysis on Pd/C, whereas lithium bromide was used to selectively cleave the phosphinate ester group. Acidic hydrolysis readily gave the free hydroxymethylphosphinic acids.

1. Introduction

The phosphonic group [$-P(O)(OH)_2$] belongs to a wide range of biologically active compounds.[1–4] But, its high ionic character limits the transmembranar transport and consequently some phosphonic acids derivatives show a very low bioavailability.[5] Searching for new biologically active phosphorus compounds, we investigated the possibility to use the hydroxymethylphosphinic group [$-P(O)(OH)(CH_2OH)$] as a substitute for the phosphonic one. This substitution should modify the physical and chemical properties of the substrates,[6] particularly their ionic character, and consequently improve their biological activity. To evaluate the potential of such replacement, we decided to synthesize new functionalized phosphinic acids of structure **1** and **2**, which can be considered as analogs of valuable inhibitors of various proteases,[7] the phosphinic pseudo-dipeptides **3**.



Currently, in the syntheses of hydroxymethylphosphinic derivatives, the hydroxymethyl group is introduced in the last step. There are only few publications dealing with the synthesis of a general precursor which could react with various functional electrophiles.[8] Herein, we describe the synthesis of compounds **1**–**2**, using a common precursor, phosphinate **4b** which is consequently added to α,β-unsaturated esters or to imines (Scheme 1).



Scheme 1.

2. Results and discussion

2.1. Synthesis of hydroxymethylphosphinic acid 4a

We first chose to prepare hydroxymethylphosphinic acid **4a**, as common precursor of the target compounds. Dihydrogenophosphinic acid, H_3PO_2 **5** and formaldehyde were selected as starting material.[9] Reactions were performed in various conditions using acidic activation (see Section 4). Unfortunately, reaction mixtures were generally composed by the unreacted H_3PO_2 **5**, the targeted hydroxymethylphosphinic acid **4a** and often with the di-addition product, bis(hydroxymethyl)phosphinic acid **6** as a consequence of the lack of selectivity of the reaction (Scheme 2).[9b]

In the best conditions, the use of 50% aqueous H_3PO_2 **5** with

Keywords: Phosphinic acid; Kabachnik–Fields reaction; Ester group.
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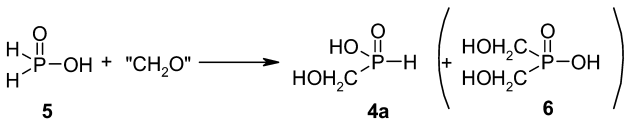


Scheme 2.

an excess (1.5 equiv.) of paraformadehyde in the presence of hydrochloric acid (1.5 equiv.) in refluxing ethanol, after 12 h, afforded the mono-adduct **4a** in 76% yield with only starting H_3PO_2 **5** as side-product. Unfortunately, all attempts to separate **4a** from **5** either by selective precipitation or by separation after chemical modification failed.

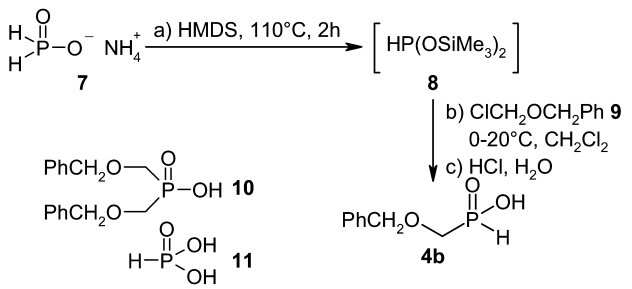
2.2. Synthesis of ethyl benzyloxymethylphosphinate 12 as precursor

Then, we turned to the synthesis of another precursor: ethyl benzyloxymethylphosphinate **12**, in which the acidic function is protected by an ester group and the hydroxymethyl substituent is protected as benzyloxymethyl group, allowing an easier purification step and avoiding retroformylation reaction which can occur in basic media.[10]

Preparation of compound **12** was achieved in two steps: synthesis of benzyloxymethylphosphinic acid **4b**, followed by its esterification.

2.3. Synthesis of benzyloxymethylphosphinic acid 4b

Phosphinic acid **4b** was prepared by a silyl-Arbuzov reaction using benzyloxymethylchloride **9** and in situ generated bis(trimethylsilyl)phosphonite **8**, formed accordingly to the literature (Scheme 3).[11] Unfortunately, besides the desired phosphinic acid **4b**, the symmetric phosphinic acid **10** was obtained as the result of a double silyl-Arbuzov reaction of **9** with phosphonite **8**.

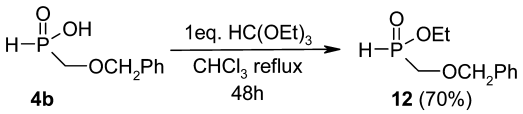


Scheme 3.

The lack of selectivity may probably be attributed to the high reactivity of the benzyloxymethyl chloride **9**. A similar observation was pointed out by Coward and Grobelny for the reaction of the phosphonite **8** with 1 equiv. of *N*-(bromomethyl)phthalimide.[12] Dihydrogenophosphinic acid **5** (10%) and hydrogenophosphonic acid **11** (9%) were also formed as by-products. The latter probably resulted from the oxidation of **8** known to be a very sensitive and pyrophoric compound.[11] Dilution of the reagent **9** in dry dichloromethane and subsequent dropwise addition to the phosphonite **8** solution did not improve the yield of **4b** with a **4b/10** ratio of 82:18. But, the use of a fourfold excess of compound **8** led to a **4b/10** ratio of 96:4, thus allowing the purification by a two-step extraction procedure. Compound **4b** was isolated in 65% yield.

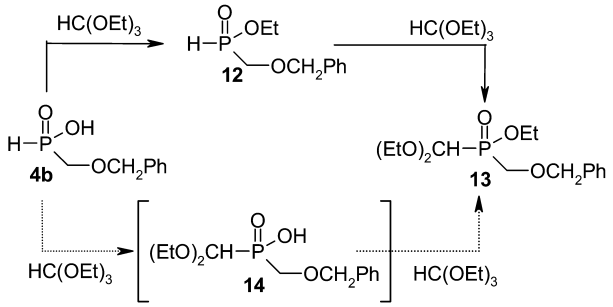
2.4. Synthesis of ethyl benzyloxymethylphosphinate 12

As a final step for the preparation of compound **12**, the esterification of acid **4b** was first accomplished with triethyl orthoformate. Indeed, trialkyl orthoformates have been extensively used to form phosphonates and phosphinates from the corresponding acids.[13] Esterification of **4b** was performed in refluxing chloroform. Thus, reaction of **4b** with 1 equiv. of triethyl orthoformate afforded after 48 h, the ester **12**, isolated in 70% yield after purification by extraction (Scheme 4).



Scheme 4.

The use of an excess of triethyl orthoformate did not improve the yield of compound **12** but lead to the formation of a major by-product exhibiting a ^{31}P signal at δ 38.37 ppm. This compound was identified by its ^{31}P, ^{1}H, ^{13}C, IR and mass spectra as ethyl (diethoxymethyl) phosphinate **13**. Its formation can be rationalized by a S_N reaction of the tricoordinated phosphorus atom of compound **12** to the central atom of triethyl orthoformate as reported by Gallagher et al.[13a] and Schwabacher et al.[13b] for the formation of methyl dimethoxymethylphosphinate in the esterification reaction of bis(hydrogeno)phosphinic acid with trimethylorthoformate. Phosphinate **13** could also be the result of the P-alkylation reaction of acid **4b** with triethyl orthoformate followed by the esterification of the intermediate acid **14**. But no trace of **14** was detected by ^{31}P NMR analysis (Scheme 5).



Scheme 5.

Kinetic monitoring of the reaction, by ^{31}P NMR was performed to determine the best conditions for the formation of phosphinate **12**. Concentrations of **4b**, **12** and **13** are plotted versus time in Figure 1. Indeed, after 16 h, the phosphinate **12** is the only detected product even with an excess of esterification reagent.

Another attempt to improve the formation of phosphinate **12** using another esterification reagent was successfully

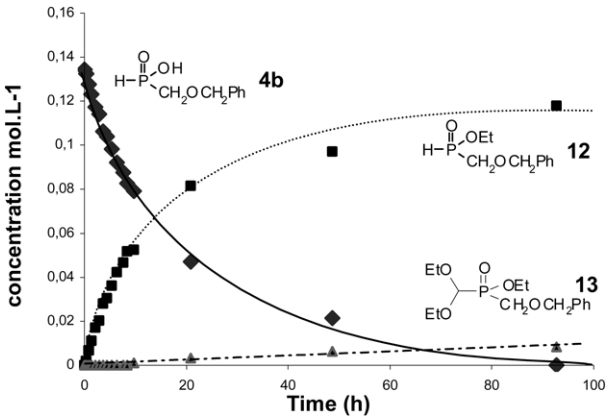
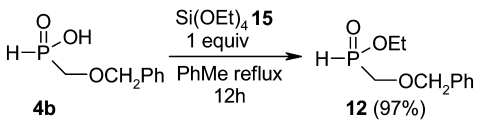


Figure 1.

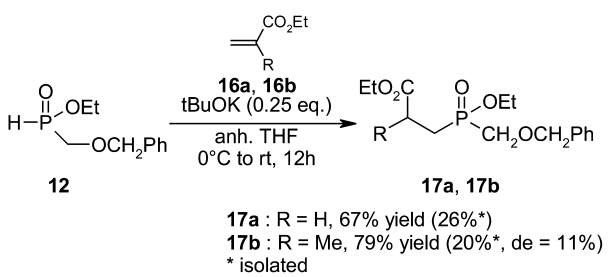
accomplished using tetraethyl orthosilicate **15**, recently employed to esterify phosphinic acids.[14] Acid **4b** was thus treated with 1 equiv. of tetraethyl orthosilicate **15** in refluxing toluene for 12 h to quantitatively afford ester **12**, isolated in 97% yield. (Scheme 6). As reported by Montchamp,[14] simple partioning of the crude product between acetonitrile and hexane is sufficient to provide almost pure phosphinate by elimination of the non-polar silicon derived impurities.



Scheme 6. Ethyl benzyloxymethylphosphinate **12** has thus been prepared in two steps and isolated in 63% overall yield (Schemes 3 and 6).

2.5. Michael addition of phosphinate 12 to α,β-unsaturated esters

Michael addition of phosphinate **12** to α,β-unsaturated esters was performed according to a method previously developed in our laboratory,[4] using a catalytic *tertio* butoxide activation (Scheme 7). Compound **12** was reacted with 1 equiv. of ethyl acrylate **16a** or ethyl methacrylate **16b** in dry THF in the presence of 0.25 equiv. of potassium *tertio*butoxide to afford the corresponding Michael products **17a** and **17b** in 69 and 79% yields, respectively. Phosphinate **17b** was formed as a mixture of two diastereoisomers resulting from the two chiral centers. Diastereoselectivity is poor, with a 11% diastereoisomeric excess. Compounds **17a** and **17b** were purified by column chromatography on silica gel and isolated, respectively in 26 and 20% yields. These low isolated yields result from the



Scheme 7.

presence in the reaction mixture of four unidentified compounds ($^{31P}\delta$=44.36, 49.48, 49.85 and 52.91 ppm in the reaction with **16a** and δ=46.60, 48.52, 48.88 and 48.91 ppm in the reaction with **16b**) which are difficult to separate from the desired Michael adducts.

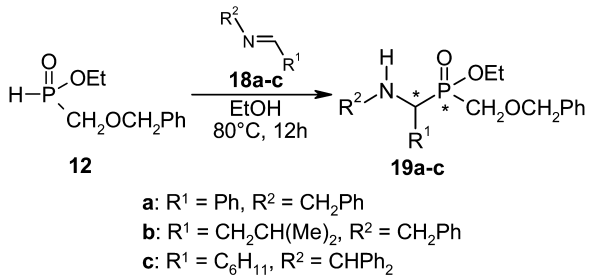
We found that the selectivity of the formation of adducts depended on the quantity of potassium *tertio*butoxide used, as shown in the reaction of **12** with ethyl methacrylate **16b** (Table 1). The use of 0.20 equiv. of potassium *tertio* butoxide afforded phosphinate **17b** as a diastereoisomeric mixture (ed=18%) in 91% yield. No side-product was detected in the reaction mixture and after neutralization, compound **17b** was isolated in 69% yield by column chromatography. However, higher amounts of *t*BuOK induce a notable decrease of the yield (Table 1).

Table 1. Influence of *t*BuOK amount on the Michael addition of phosphinate **12** to **16b**

*t*BuOK (equiv.)	Conversion (**12**) (%)	^{31}P NMR yield (**17b**) (%)	Isolated yield (**17b**) (%)	de (**17b**) (%)
0.20	100	91	69	18
0.25	100	79	20	11
0.30	100	36	—	5

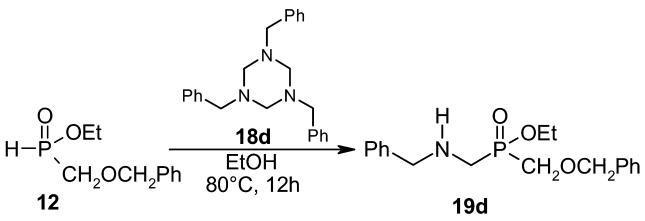
2.6. Aminoalkylation of phosphinate 12 with imines

N-Protected ethyl α-aminoalkyl-benzyloxymethyl-phosphinates **19a–c** were prepared by a Kabachnik–Fields reaction involving the addition of phosphinate **12** to the corresponding aldimines **18a–c** or to the 1,3,5-*N*-benzyl-1,3,5-hexahydrotriazine **18d**. The reaction of phosphinate **12** with 1 equiv. of imines **18a** and **18b** in refluxing ethanol afforded the expected phosphinates **19a** and **19b**, respectively in 81 and 56% yields as an equimolar mixtures of two diastereoisomers resulting from the chirality of the phosphinate group and the α-amino carbon atom (Scheme 8).



Scheme 8.

The ^{31}P NMR analysis of the crude reaction mixture showed the presence of benzyloxymethylphosphinic acid **4b** as side-product (respectively 12% and 29%). A possible explanation for the formation of the acid **4b** is a dealkylation reaction of ethyl phosphinate **12** by nucleophiles present in the reaction mixture such as amines.[15] Theses amines could thus react with phosphinate **12** by a nucleophilic attack of the nitrogen atom on the ethyl ester phosphinic group, leading to the formation of acid **4b** (Scheme 9). This hypothesis is supported by the fact that, in a control experiment, the reaction of phosphinate **12** with



Scheme 9.

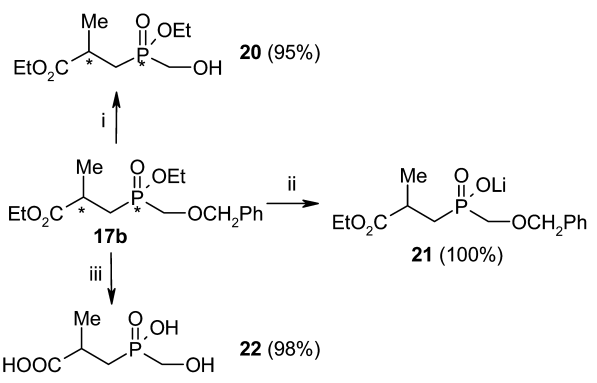
dibenzylamine resulted in the quantitative transformation of ester **12** into acid **4b**.

Consequently, the addition of a slight excess (1.2 equiv.) of imine **18a** afforded the corresponding phosphinate **19a** in 96% yield and **4b** in only 4%. This result implies that the addition reaction of phosphinate **12** to the aldimine is faster than the dealkylation reaction. Phosphinate **19a** was then purified on silica gel by column chromatography and isolated in 70% yield as a mixture of two diastereoisomers. Similar results were obtained with imines **18b** and **18c** which led to the corresponding phosphinates **19b** and **19c** which were isolated after column chromatography in 84 and 69% yields, respectively. Phosphinate **19a** constitutes a precursor of the analog of phosphonoleucine which is known to inhibit leucine aminopeptidase.[16]

Then, we applied the reaction to the 1,3,5-*N*-benzyl-1,3,5-hexahydrotriazine **18d** in order to prepare the analog of phosphonoglycine (Scheme 9).[17] Ethylphosphinate **12** was first treated with 1 equiv. of the triazine **18d** in refluxing ethanol. In contrast to aldimines **18a–c**, total disappearance of **12** in this case needed 48 h. The resulting phosphinate **19d** was formed in 50% yield, but isolated after column chromatography in only 8% yield. This low yield is due to the formation in the reaction mixture of four side-products at δ 49.20 and 49.30 ppm (23%) and at δ 48.84 and 48.94 ppm (5%). In the other hand, **4b** was formed in only 4% yield. Unfortunately, these side-products were very difficult to separate from the targeted phosphinate **19d**. Use of a 1.5 excess of triazine **18d** increased the yield to 60%, and the phosphinate **19d** was isolated in 33% yield.

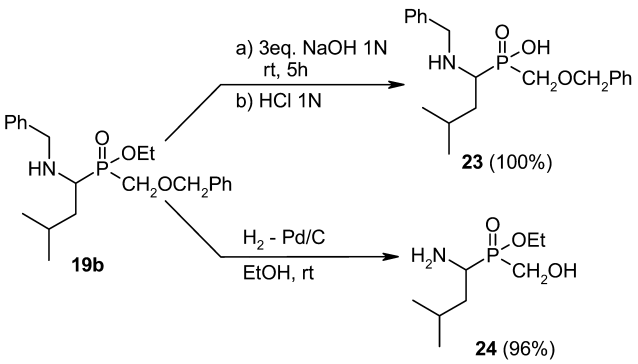
2.7. Total or selective deprotections

Total or selective deprotections have been performed on compound **17b** (Scheme 10). The benzylic group was removed by hydrogenolysis on Pd/C[18] to give the corresponding phosphinate **20** in 95% yield. Selective cleavage of the ester group was achieved by using a two fold excess of lithium bromide in refluxing acetonitrile for 5 days.[19] The corresponding salt **21** was isolated after concentration in 100% yield. Finally, compound **17b** was totally deprotected by an excess of 35% hydrochloric acid at 80 °C for 3 h, affording the phosphinic acid **22** in 98% yield.[6]



Scheme 10. *Conditions and reagents*: (i) H_2-Pd/C, EtOH, Patm, rt; (ii) LiBr (2 equiv.), MeCN, reflux, 3 days; (iii) excess 35% HCl, 80 °C, 3 h.

The same deprotection methodology can be performed on α-aminoalkylphosphinic acid (Scheme 11). The phosphinic acid function of **19b** was selectively deprotected in the presence of an excess of sodium hydroxide to afford the phosphinic acid **23** isolated in quantitative yield (Scheme 11) while total deprotection of both hydroxy and amino groups was achieved by hydrogenolysis on Pd/C leading to the phosphinate **24** isolated in 96% yield.



Scheme 11.

3. Conclusion

A new versatile and stable precursor for the introduction of hydroxymethylphosphinic group, ethyl benzyloxymethyl phosphinate **12**, has been synthesized in two steps and isolated in 65% overall yield. Subsequent reaction with various electrophiles such as α,β-unsaturated esters in the presence of catalytic amount of potassium *tertio*butoxide and imines or triazine afforded the corresponding γ-carboxy- or α-aminoalkylphosphinates. Total or selective deprotections can be performed demonstrating the compatibility and the complementarity of the various protecting groups. The synthetic sequence, described here, affords a reliable and general access to this particular class of functionalized phosphinic acids. Using the same precursor, several aryl or heteroaryl hydroxymethylphosphinic acid derivatives were prepared by palladium (0) catalyzed arylation.[20]

4. Experimental

4.1. General remarks

All reactions involving air or moisture sensitive reagents or intermediates were carried out under dry nitrogen in flame-dried glassware. Reagents and solvents were purified before use and stored under nitrogen atmosphere. All reactions were monitored by TLC (Merk, SIL, G/UV_{254}) or ^{31}P NMR. Merck silica gel (70–200 μm) was used for column

chromatography. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker Ac 200 (^{1}H at 200.13 MHz, ^{13}C at 50.32 MHz and ^{31}P at 81.01 MHz) and on a Brucker AC 250 spectrometers (^{1}H at 250.13 MHz, ^{13}C at 62.89 MHz and ^{31}P at 101.25 MHz). Chemical shifts are expressed in ppm and coupling constants in Hz. IR spectra were obtained with Perkin–Elmer 377 and Nicolet FT-IR 210 spectrometers. Mass spectra were measured with a Jeol JMS DX-300 spectrometer (positive FAB ionisation and High Resolution using glycerol-thioglycerol or *p*-nitrobenzyl alcohol matrix).

4.2. Hydroxymethylphosphinic acid 4a

Reactions conditions for the direct synthesis of hydroxymethylphosphinic acid **4a** using H_3PO_2 **5** or its ammonium salt and the different forms of formaldehyde are listed in Table 2. The best result is observed for entry 9 where **4a** is selectively obtained in 76% yield (Table 2).

4.3. Preparation of ammonium phosphinate 7

Commercially available 50% aqueous phosphinic acid (40 g, 301 mmol) was slowly added to 25% aqueous ammonia (46.6 mL, 301 mmol) at 0 °C. The mixture was allowed to reach room temperature and stirred over a period of 5 h. Removal of water was achieved under reduced pressure and followed by rigorous drying over P_2O_5 under vacuum to obtain ammonium phosphinate **7** as a white solid in 93% yield (23.3 g, 280 mmol).

4.3.1. Benzyloxymethyl-hydrogeno-phosphinic acid 4b. Ammonium phosphinate **7** (10 g, 120.4 mmol) and hexamethyldisilazane (25.6 mL, 120.4 mmol) were heated together under nitrogen at 100–110 °C until all the ammonia by-product has evolved (ca. 2 h) The mixture was then cooled to 0 °C before the addition of dry dichloromethane (100 mL). After 15 min stirring at 0 °C, a solution of benzyloxymethylchloride (4.17 mL, 30.1 mmol) in 50 mL of dry dichloromethane was added dropwise over 15 min. The resulting mixture was allowed to warm to room temperature and stirred for 12 h. Then, 18% HCl (5 mL) was slowly added and the mixture stirred for additional 15 min. The mixture was filtered and the solution was extracted with water (3×5 mL). The organic layer was dried over $MgSO_4$ before the solvent was removed under reduced pressure to afford a colourless oil (4.06 g). This oil was dissolved in water (100 mL) and extracted with ethyl acetate (3×5 mL). The aqueous layer was then continuously extracted with dichloromethane for 5 h. The resulting organic layer was dried over $MgSO_4$ and the solvent was evaporated under reduced pressure to afford benzyloxymethyl-phosphinic acid **4b** as a colourless oil in 65% yield (3.63 g, 19.52 mmol).

^{31}P NMR ($CDCl_3$): 29.40 (dt, $^{1}J_{PH}$=566.0 Hz, $^{2}J_{PH}$=7.4 Hz). ^{1}H NMR ($CDCl_3$): 3.71–3.78 (2dd, ABX system, δ_{HA}=3.73, δ_{HB}=3.77, $^{2}J_{HAHB}$=13.4 Hz, $^{2}J_{PHA}$=2.2 Hz, $^{2}J_{PHB}$=2.2 Hz, 2H, PCH_2), 4.62 (s, 2H, $PhCH_2$), 4.92 (bs, OH), 7.08 (d, $^{1}J_{PH}$=566.0 Hz, $^{3}J_{HH}$=2.2 Hz, 1H, P-H), 7.34–7.39 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$): 66.51 (d, $^{1}J_{PC}$=115.4 Hz, PCH_2), 75.23 (d, $^{3}J_{PC}$=11.9 Hz, $PhCH_2$), 128.15 (s, 2CH), 128.22 (s, CH), 128.58 (s, 2CH), 136,70 (s, C). MS FAB+(NBA) *m/z*=187 (17%) $[M+H]^+$, 91 (100%) $C_7H_7^+$. HRMS calcd for $C_8H_{12}O_3P$: 187.0534, found: 187.0531.

4.3.2. Ethyl benzyloxymethyl-hydrogeno-phosphinate 12 (method 1). Triethyl orthoformate (2.2 mL, 13.20 mmol) was added to a solution of benzyloxymethylphosphinic acid **4b** (2.5 g, 13.20 mmol) in 70 mL of anhydrous chloroform. The reaction mixture was refluxed for 48 h. The conversion yield determined by ^{31}P NMR was 77 whereas, 33% of acid **4b** remained unchanged. As no evolution has been observed after additional 3 h of stirring, the reaction mixture was cooled to room temperature before addition of aqueous $KHCO_3/K_2CO_3$ (1 M). The biphasic mixture was stirred at room temperature for additional 15 min before partition of the organic and aqueous layers. The organic layer was dried over $MgSO_4$ before the solvent was removed under reduced pressure to afford the phosphinate **12** as a colourless oil in 70% yield (2.15 g, 9.30 mmol).

4.3.3. Ethyl benzyloxymethyl-hydrogeno-phosphinate 12 (method 2). Tetraethyl orthosilicate (1.8 mL, 8.26 mmol) was added to a solution of benzyloxymethylphosphinic acid **4b** (1.5 g, 8.26 mmol) in 15 mL of dry toluene. The reaction mixture was refluxed for 12 h. At this time, the ^{31}P NMR

Table 2. Synthesis of hydroxymethylphosphinic acid **4a**

Entry	Formaldehyde source	Phosphorus reagent	Conditions	Reaction mixture (%)		
				6	**4b**	**7**
1	Trioxymethylene (1 equiv.)	aq. H_3PO_2	MeOH, 7d, 20 °C, HCl (1 equiv.)	100	—	—
2	Trioxymethylene (1 equiv.)	aq. H_3PO_2	H_2O, 7d, 20 °C, HCl (1 equiv.)	98	2	—
3	Trioxymethylene (1 equiv.)	aq. H_3PO_2	MeOH, 7d, 60 °C, HCl (1 equiv.)	99	1	
4	Trioxymethylene (1 equiv.)	aq. H_3PO_2	H_2O, 7d, 60 °C, HCl (1 equiv.)	31	66	3
5	37% aq. Formaldehyde (1 equiv.)	aq. H_3PO_2	MeOH, 2.5 days, 20 °C, NH_4Cl (1 equiv.)	73	27	—
6	37% aq. Formaldehyde (1 equiv.)	aq. H_3PO_2	H_2O, 2.5 days, 20 °C, NH_4Cl (1 equiv.)	28	54	18
7	37% aq. Formaldehyde (1 equiv.)	aq. H_3PO_2	MeOH, 2.5 days, 60 °C, NH_4Cl (1 equiv.)	66	34	—
8	Paraformaldehyde (1 equiv.)	aq. H_3PO_2	EtOH, 7 days, 80 °C, HCl (1 equiv.)	46	54	—
9	Paraformaldehyde (1.5 equiv.)	aq. H_3PO_2	EtOH, 7 days, 80 °C, HCl (1.5 equiv.)	24	76	—
10	Paraformaldehyde (1 equiv.)	aq. H_3PO_2	H_2O, 7 days, 80 °C, HCl (1 equiv.)	6	77	17
11	Gazeous Formaldehyde	anh. H_3PO_2	EtOH, 7 h, 80 °C, PTSA (2 equiv.)	46	54	—
12	Paraformaldehyde (1 equiv.)[a]	aq. H_3PO_2	EtOH, 2 days, 110 °C, HCl (1 equiv.)	15	71	14
13	Paraformaldehyde (1.2 equiv.)[a]	aq. H_3PO_2	EtOH, 2 days, 110 °C, HCl (1.2 equiv.)	12	83	5
14	Paraformaldehyde (1.5 equiv.)[a]	aq. H_3PO_2	EtOH, 2 days, 110 °C, HCl (1.5 equiv.)	5	83	12

[a] Reactions were performed in a caped thick-wall tube.

analysis showed that all the acid **4b** had quantitatively been transformed to the phosphinate **12**. The mixture was then allowed to cool to room temperature and the solvent was removed under reduced pressure. According to Montchamp procedure, the oily residue was purified by partition between CH_3CN and hexane. The hexane layer contained the non-polar silicon-derived by-products, while the polar phosphinate remained in the CH_3CN layer. This latter was concentrated under reduced pressure to afford phosphinate **12** as a colourless oil in 97% yield (1.72 g, 8.03 mmol). Further purification can be accomplished using chromatography on silica gel with dichloromeyhane as eluent.

^{31}P NMR ($CDCl_3$): 32.22 (dquint., $^1J_{PH}$=552.5 Hz, $^2J_{PH}$=8.7 Hz). ^1H NMR ($CDCl_3$): 1.33 (t, $^3J_{H-H}$=7.1 Hz, 3H CH_3), 3.73–3.81 (2 dd, ABX system, δ_{HA}=3.76, δ_{HB}=3.80, $^2J_{HAHB}$=−13.8 Hz, $^2J_{PHA}$=4.2 Hz, $^2J_{PHB}$=10.1 Hz, 2H, PCH_2), 4.08–4.19 (m, 2H, $POCH_2$), 4.58 (s, 2H, $PhCH_2$), 7.08 (d, $^1J_{PH}$=552.5 Hz, $^3J_{HH}$=2.3 Hz, 1H, PH), 7.30–7.34 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$): 16.32 (d, $^3J_{PC}$= 6.0 Hz, CH_3), 62.83 (d, $^2J_{PC}$=7.0 Hz, $POCH_2$), 65.82 (d, $^1J_{PC}$=114.2 Hz, PCH_2), 75.28 (d, $^3J_{PC}$=11.9 Hz, $PhCH_2$), 128.13 (s, 2CH), 128.28 (s, CH), 128.57 (s, 2CH), 136.56 (s, C). IR (NaCl): 1200 (PO); 1125 (COC); 1100, 1050 (POC). MS FAB+(NBA) *m*/*z*=215 (21%) $[M+H]^+$; 91 (100%) $C_7H_7^+$. HRMS calcd for $C_{10}H_{16}O_3P$: 251.0837, found: 215.0815.

4.4. General procedure for preparation of phosphinates 17. Synthesis of 17b

A solution of phosphinate **12** (1 g, 4.67 mmol) in 8 mL of anhydrous THF was added dropwise to precooled (0 °C, ice bath) suspension of potassium *tertio*butoxide (105 mg, 0.93 mmol) in 10 mL of anhydrous THF. The resulting mixture was stirred at 0 °C for 15 min. After this time, ethyl methacrylate (0.57 mL, 4.67 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred under nitrogen for 12 h. At this time, ^{31}P NMR analysis of the mixture showed that all the phosphinate **12** had reacted and that phosphinate **17b** had been formed as a mixture of two diastereoisomers in 91% yield. The reaction mixture was then neutralized by addition of aqueous HCl (1N)and diluted with 20 mL of water. The solution was extracted with ethyl acetate (3×30 mL). The organic layer was first washed with brine and then dried over $MgSO_4$. Evaporation of the solvent afforded the crude phosphinate **17b** as a yellow oil which was purified by flash chromatography on silica gel (gradient for elution: from petroleum ether/ethyl acetate, 80:20 to ethyl acetate 100%) to afford the pure phosphinate **17b** as a mixture of two diastereoisomers (59:41) in 69% yield (yellow oil, 1.05 mg, 3.22 mmol).

4.4.1. Ethyl (2-ethoxycarbonyl-1-ethyl)-(benzyloxymethyl)-phosphinate 17a. ^{31}P NMR ($CDCl_3$): 49.88. ^1H NMR ($CDCl_3$): 1.20 (t, $^3J_{HH}$=6.3, 3H, CH_3), 1.27 (t, $^3J_{HH}$= 7.0, 3H, CH_3), 1.24–1.30 (m, 1H, CH_2), 2.02–2.15 (m, 1H, CH_2), 3.67–3.70 (m, 2H, CH_2), 3.91–4.55 (m, 6H, $3CH_2$), 4.55 (s, 2H, CH_2), 7.25–7.33 (m, 5H, CH). ^{13}C NMR ($CDCl_3$): 14.13 (s, CH_3), 16.53 (d, $^3J_{PC}$=5.9 Hz, CH_3), 21.73 (d, $^1J_{PC}$=95.3 Hz, CH_2), 26.,32 (d, $^3J_{PC}$=2.6 Hz, CH_2), 60.86 (s, CH_2), 60.91 (d, $^2J_{PC}$=4.8 Hz, $POCH_2$), 64.92 (d, $^1J_{PC}$=110.2 Hz, PCH_2O), 75.17 (d, $^3J_{PC}$=12.6 Hz, CH_2), 128.05 (CH), 128.,11 (s, CH), 128.51 (s, CH), 136.,67 (s, C), 172.15 (d, $^3J_{PC}$=16.4 Hz, CO). IR (NaCl): 1745 (C═O), 1220 (PO); 1100, 1080, 1040 (O–C). MS FAB+ (NBA) *m*/*z*=315 (100%) $[M+H]^+$, 91 (86%) $C_7H_7^+$.

4.4.2. Ethyl (2-ethoxycarbonyl-1-propyl)-(benzyloxymethyl)-phosphinate 17b. ^{31}P NMR ($CDCl_3$): 48.82 (59%) and 49.87(41%). ^1H NMR ($CDCl_3$): 1.04–1.16 (m, 9H, CH_3, $2CH_3$), 1.68–1.71 (m, 1H, CH), 2.14–2.23 (m, 1H, CH), 2.73–2. 75 (m, 1H, H), 3.54–3.59 (m, 2H, CH_2), 3.84–4.00 (m, 4H, $2CH_2$), 4.42–4.45 (m, 2H, CH_2), 7.11–7.28 (5H, CH). ^{13}C NMR ($CDCl_3$): 16.47 (s, CH_3), 18,90 (d, $^3J_{PC}$=6.0 Hz, CH_3), 21.02 (d, $^3J_{PC}$=6.0 Hz, CH_3), 21.46 (d, $^3J_{PC}$=6.0 Hz, CH_3), 32.13 (d, $^1J_{PC}$=93.8 Hz, CH_2), 32.,22 (d, $^1J_{PC}$=93.8 Hz, CH_2), 35.93 (d, $^2J_{PC}$=3.3 Hz, CH), 35.96 (d, $^2J_{PC}$=2.7 Hz, CH), 63.11 (s, CH_2), 63.,24 (d, $^2J_{PC}$=4.9 Hz, CH_2), 67.65 (d, $^1J_{PC}$=109.8 Hz, CH_2), 67,85 (d, $^1J_{PC}$=109.4 Hz, CH_2), 77.44 (d, $^3J_{PC}$=12.6 Hz, CH_2), 77.48 (d, $^3J_{PC}$=12.6 Hz, CH_2), 130.41, 130.47, 130.49, 130.86: 6-CH, 139.21, 139.29, 177.63, 177.46 (4C). IR (NaCl): 1740 (C═O); 1220 (PO); 1100, 1040 (OC). MS FAB+(NBA) *m*/*z*=329 (100%) $[M+H]^+$, 91 (93%) $C_7H_7^+$.

4.5. General procedure for preparation of phosphinates 19a–d

In a typical procedure, a solution of imine or hexahydrotriazine **18a–d** (1.2 equiv., 1 mol L^{-1}) in anhydrous ethanol is added dropwise to a solution of phosphinate **12** (1 equiv., 0.2 mol L^{-1}) in anhydrous ethanol. The mixture is refluxed under nitrogen until the complete consumption of starting material **12** (generally in 12 h; excepted for **19d**; 48 h were needed). The reaction mixture is then concentrated and chromatographied on silica gel (gradient of elution: from petroleum ether/ethyl acetate, 90:10 to ethyl acetate 100%) to afford the phosphinate **19a–d**.

4.5.1. Ethyl (*N*-benzylamino-phenyl-methyl)-benzyloxymethyl phosphinate 19a. ^{31}P NMR ($CDCl_3$): 45.42 (s, 47%), 46.10 (s, 53%). ^1H NMR ($CDCl_3$): 1.08, 1.31 (2 t, $^3J_{PH}$=7.0, 7.0 Hz, 3H, CH_3), 3.20 (bs, NH), 3.42–4.21 (m, 7H, CH_2, CH_2, CH, CH_2), 4.45, 4.57 (2s, 2H, PCH_2), 7.26–7.40 (m, 15H, Ph). ^{13}C NMR ($CDCl_3$): 16.40, 16.73 (2d, $^3J_{PC}$=5.2, 5.6 Hz, CH_3), 51.17, 51.31 (2d, $^3J_{PC}$=14.9, 16.7 Hz, CH_2), 58.43, 59.99 (2d, $^1J_{PC}$=104.2, 100.1 Hz, CH), 61.70, 62.31 (2d, $^2J_{PC}$=7.1, 7.4 Hz, CH_2), 63.94, 64.15 (2d, $^1J_{PC}$=111.3, 111.0 Hz, CH_2), 74.92, 75.34 (2d, $^3J_{PC}$= 15.1, 15.2 Hz, CH_2), 127–128,93 (Ph), 135,13, 135,20 (2d, $^2J_{PC}$=8.2, 8.6 Hz, C), 136.84, 137.02 (2s, C), 139.30, 139.34 (2s, C). MS FAB+(NBA) *m*/*z*=410 (5%) $[M+H]^+$, 196 (16%) $[(PhCH_2N(H)(Ph)CH]^+$, 91 (100%) $C_7H_7^+$. IR (NaCl): 1220 (PO), 1110 (POC). HRMS calcd for $C_{24}H_{29}O_3$: 410.1885, found: 410.1870.

4.5.2. Ethyl 1-(1-*N*-benzylamino-3-methyl-butyl)-benzyloxy-methyl phosphinate 19b. ^{31}P NMR ($CDCl_3$): 51.29 (s, 52%), 51.56 (s, 48%). ^1H NMR ($CDCl_3$): 0.77–0.95 (m, 3H, CH_3), 1.32–1.37 (m, 3H, CH_3), 2.00–2.04 (m, 2H, CH_2), 3.15 (bs, NH), 3.85–4.15 (m, 8H, $3CH_2$, 2CH), 5.26–5.30 (m, 2H, CH_2), 7.25–7.63 (m, 10H, Ph). ^{13}C NMR ($CDCl_3$): 16.66, 16.76 (2d, $^3J_{PC}$=5.6, 5.2 Hz, CH_3), 21.47 (d, $^3J_{PC}$= 23.1 Hz, CH), 23.43 (d, $^4J_{PC}$=10.4 Hz, CH_3), 24.43, 24.68

(2d, $^4J_{PC}$=11.2, 10.0 Hz, CH_3), 37.68, 38.15 (2d, $^2J_{PC}$=2.2, 4.1 Hz, CH_2), 51.87 (d, $^1J_{PC}$=105.7 Hz, ^{11}CH), 52.39, 52.47 (2d, $^3J_{PC}$=6.6, 3.7 Hz, CH_2), 52.60 (d, $^1J_{PC}$=99.4 Hz, CH), 61.16, 61.19 (2d, $^2J_{PC}$=7.4, 7.8 Hz, CH_2), 63.49, 64.06 (2d, $^1J_{PC}$=104.2, 100.9 Hz, CH_2), 75.25, 75.31 (d, $^3J_{PC}$= 12.3, 12.3 Hz, CH_2), 127.16, 127.19, 127.22, 127.44, 127.69, 128.00, 128.06, 127.06, 127.08, 128.13, 128.15, 128.19, 128.30, 128.38, 128.45, 128.50 (CH_{Ar}), 136.81, 136.85 (2s, C), 140.06, 140.14 (2s, C). MS FAB+(NBA) *m/z*=390 (18%) $[M+H]^+$, 178 (100%) $[(PhCH_2N(H))(Me_2CHCH_2)]CH^+$, 91 (100%) $C_7H_7^+$, 77 (10%) Ph^+. IR (NaCl): 2990, 1240, 1220 (PO), 1110 (POC). HRMS calcd for $C_{22}H_{33}O_3NP$: 390.2207, found 390.2198.

4.5.3. Ethyl benzyloxymethyl-1-(*N*-diphenylmethyl-amino)-1-cyclohexyl-methyl phosphinate 19c. ^{31}P NMR ($CDCl_3$): 50.72 (s, 49%), 51.42 (s, 51%). ^1H NMR ($CDCl_3$): 1.17–1.42 (m, 10H), 1.33–1.40 (2t, $^3J_{HH}$=7.1, 7.2 Hz, 3H, CH_3), 2.23 (bs, 1H, CH), 2.89, 2.93 ppm (2 bs, 1H, NH), 3.78–3.82 (m, 2H, CH_2), 4.00–4.66 (m, 4H, $2CH_2$), 5.20, 5.28 (2 bs, 1H, CH), 7.20–7.40 (m, 15H, Ph). ^{13}C NMR ($CDCl_3$): 16.66, 16.89 (2d, $^3J_{PC}$=5.2, 5.2 Hz, CH_3), 26.18, 26.28, 26.50, 26.67, 26.86, 26.93, 28.11, 28.89, 28.93, 31.37, 31.57, 31.62 ($5CH_2$), 38.39, 38.73 (2d, $^2J_{PC}$=6.3, 6.7 Hz, CH), 56.81, 57.07 (2d, $^1J_{PC}$=99.5, 91.5 Hz, CH), 60.63, 60.78 (2d, $^2J_{PC}$=6.1, 6.3 Hz, CH_2), 65.05, 65.08 (2d, $^1J_{PC}$=101.9, 97.1 Hz, CH_2), 65.44, 65.74 (2d, $^3J_{PC}$=8.2, 11.9 Hz, CH), 75.17, 75.26 (2d, $^3J_{PC}$=11.9, 12.6 Hz, CH_2), 127.16, 127.19, 127.22, 127.44, 127.69, 128.00, 128.06, 128.12, 128.29, 128.34, 128.44, 128.50 (CH_{Ar}), 136.81, 136.88 (2s, C), 143.26, 143.49 (2s, C), 143.59, 143.83 (C). MS FAB+(NBA) *m/z*=492 (5%) $[M+H]^+$, 278 (33%) $[(Ph_2CHN(H))(Cy)]CH^+$, 167 (100%) $C_7H_7^+$. IR (NaCl): 2950, 2870, 1220 (PO); 1105 (POC). MS HR (NBA): HRMS calcd for $C_{24}H_{29}NO_3P$: 410.0385, found 410.0368.

4.5.4. Ethyl 1-(*N*-benzylaminomethyl)-benzyloxymethyl phosphinate 19d. ^{31}P NMR ($CDCl_3$): 48.40 (s). ^1H NMR ($CDCl_3$): 1.34 (t, $^3J_{HH}$=7.0 Hz, 3H, CH_3), 2.15 (bs, NH), 3.61–3.67 (dd, 2H, PCH_2N), 3.76–3.93 (m, 4H, PCH_2, $PhCH_2N$), 4.07–4,66 (m, 2H, $POCH_2$), 4.61 (s, 2H, $PhCH_2O$), 7.27–7.37 (m, 10H, 2Ph). ^{13}C NMR ($CDCl_3$): 16.64 (d, $^3J_{PC}$=5.2 Hz, CH_3), 44.32 (d, $^1J_{PC}$=105.7 Hz, PCH_2N), 54.97 (d, $^3J_{PC}$=15.3 Hz, $PhCH_2N$), 63.99 (d, $^1J_{PC}$=109.8 Hz, PCH_2O), 61.33 (d, $^2J_{PC}$=7.1 Hz, $POCH_2$), 75.25 (d, $^3J_{PC}$=12.3 Hz, $PhCH_2O$), 127.25 (s, CH), 128.08 (s, CH), 128.15 (s, CH), 128.28 (s, CH), 128.45 (s, CH), 136.87 (s, C), 139.13 (s, C). MS FAB+(NBA) *m/z*= 334 (5%) $[M+H]^+$, 120 (30%) $PhCH_2N(H)CH_2^+$,91 (100%) $C_7H_7^+$. MS HR (NBA): HRMS calcd for $C_{18}H_{25}NO_3P$: 334.1572, found 334.1563.

4.5.5. Ethyl (2-ethoxycarbonyl-1-propyl)-(hydroxy-methyl)-phosphinate 20, hygrogenolysis of 17b. Pd/C 10% (212 mg, 0.20 mmol) was added to a solution of phosphinate **17b** (330 mg, 1 mmol) in 10 mL of absolute ethanol. The mixture was placed under hydrogen at atmospheric pressure and room temperature. After consumption of the required volume of nitrogen, the mixture was filtered on celite and the filtrate was concentrated under reduced pressure to afford phosphinate **20** as a yellow oil in 95% yield (227 mg, 0.95 mmol).

^{31}P NMR ($CDCl_3$): 52.22 (59%), 52.85 (41%). ^1H NMR ($CDCl_3$): 1.15–1.25 (m, 9H, $3CH_3$), 1.67–1.89 and 2.15–2.37 (m, 2H, CH_2), 2.80–2.86 (m, 2H, CH), 3.77–3.78 (m, 2H, CH_2), 4.01–4.11 (m, 4H, $2CH_2$), 4.79 (bs, OH). ^{13}C NMR ($CDCl_3$): 14.02 (s, CH_3), 16.47 (d, $^3J_{PC}$=5.6 Hz, CH_3), 19.02, 19.13 (2d, $^3J_{PC}$=8.9, 9.3 Hz, CH_3), 29.15, 29.20 (2d, $^1J_{PC}$=89.7, 89.9 Hz, CH_2), 33.68 (d, $^2J_{PC}$= 3.3 Hz, CH), 58.65, 59.14 (2d, $^1J_{PC}$=106.1, 106.1 Hz, CH_2), 60.80 (s, CH_2), 61.01, 61.14 (2d, $^2J_{PC}$=13.0, 13.0 Hz, CH_2), 175.38, 175.51 (2d, $^3J_{PC}$=8.9, 8.9 Hz, C). IR (NaCl): 3390 (OH); 1730 (C=O); 1210, 1180 (PO); 1030 (OC). MS FAB+(NBA) *m/z*=239 (100%), $[M+H]^+$; 211 (8%), $[M+H-Et]^+$.

4.5.6. Lithium (2-ethoxycarbonyl-1-propyl)-(benzyloxy-methyl)-phosphinate 21. Lithium bromide (81 mg, 0.92 mmol) was added to a solution of phosphinate **17b** (150 mg, 0.46 mmol) in 5 mL of CH_3CN. The reaction mixture was refluxed for 5 days and the solvent is evaporated to quantitatively afford compound **21** (141 mg, 0.46 mmol) as a yellow oil.

^{31}P NMR (D_2O): 51.61. ^{13}C NMR (D_2O): 13.7 (s, C), 19.05 (d, $^3J_{PC}$=8.1 Hz, CH_3), 32.31 (d, $^1J_{PC}$=91.3 Hz, CH_2), 34.89 (d, $^2J_{PC}$=23.0 Hz, CH), 62.34 (s, CH_2), 68.38 (d, $^1J_{PC}$=109.7 Hz, CH_2), 75.13 (d, $^3J_{PC}$=11.5 Hz, CH_2), 128.72, 128.93, 129.10 (s, 5CH), 137.57 (s, C), 185.35 (d, $^4J_{PC}$=9.8 Hz, C). IR (NaCl): 3300 (OH); 1750 (C=O); 1225 (PO); 1105 (OC).

4.5.7. (2-Carboxy-1-propyl)-(hydroxymethyl)-phosphinic acid 22. Phosphinate **17b** (160 mg, 0.49 mmol) was stirred with 35% aqueous HCl (0.5 mL, 15 equiv.) at 80 °C for 5 h. Neutralisation with 2N aqueous NaOH followed by evaporation of the solvent and drying over P_4O_{10} afforded acid **22** in 98% yield (87 mg, 0.48 mmol).

^{31}P NMR (D_2O): 40.88. ^1H NMR (D_2O): 1.61 (br s, 3H, CH_3), 1.85–2.08 (m, 1H, CH), 2.29–2.48 (m, H, CH), 2.85–3.42 (m, 1H, CH), 4.00 (br s, OH), 10.16 (bs, OH). ^{13}C NMR (D_2O): 21.21 (d, $^3J_{PC}$=7.4 Hz, CH_3), 38.44 (d, J_{PC}=3.2 Hz, CH), 47.61 (d, $^1J_{PC}$=91.91 Hz, CH_2), 48.68 (d, $^1J_{PC}$=94.5 Hz, CH_2), 182.33 (d, $^3J_{PC}$=7.8 Hz, C). IR (NaCl): 3350 (OH); 1710 (C=O); 1225 (PO). MS FAB+ (NBA) *m/z*=183 (100%) $[M+H]^+$.

4.5.8. 1-(1-*N*-Benzylamino-3-methyl-butyl)-benzyloxy-methyl phosphinic acid 23. Same procedure as described for 17b. ^{31}P NMR (D_2O): 39.06 (s). ^1H NMR (D_2O): 1.01–1.35 (m, 6H, CH_3), 1.63–1.97 (m, 2H, CH_2), 3.21 (bs, NH), 3.82–4.12 (m, 5H, CH, $2CH_2$), 4.61 (s, 2H, CH_2) 7.26–7.38 (10H, Ph), 8.2 (bs, OH). ^{13}C NMR (D_2O): 21.41 (s, CH_3), 22.15 (s, CH_3), 24.78 (d, $^3J_{PC}$=7.1 Hz, CH), 35.53 (s, CH_2), 49.76 (d, $^1J_{PC}$=92.6 Hz, CH_2), 50.27 (s, CH_2), 65.72 (d, $^1J_{PC}$=118.0 Hz, CH_2), 75.48 (d, $^3J_{PC}$=13.4 Hz, CH_2), 128.18, 128.36, 128.47, 129.04, 129.49, 130.89 (s, CH_{Ar}), 130.03 (s, ^3C), 136.35 (s, ^{19}C). MS FAB+(NBA) *m/z*=362 (21%) $[M+H]^+$, 178 (100%) $PhCH_2N(Me_2CH CH_2)CH^+$, 91 (100%) $C_7H_7^+$, 77 (5%) Ph^+. IR (NaCl) 3320 (OH), 1225 (PO), 1105 (POC). MS HR (NBA); HRMS calcd for $C_{20}H_{28}O_3NP$: 362.1513, found 362.1542.

4.5.9. Ethyl 1-(1-amino-3-methyl-butyl)-hydroxymethyl phosphinate 24. A solution of **19** (140 mg, 0,36 mmol, 1 equiv.) in 5 mL of ethanol was added to a solution of sodium hydroxide (1 N, 3 equiv.). The reaction mixture was stirred 5 h at room temperature and was neutralized by the addition of 1 N hydrochloric acid. After filtration and concentration, 130 mg of a yellowish oil were recovered (100% yield, 0.36 mmol).

^{31}P NMR ($CDCl_3$): 47.94 (s, 51%), 49.30 (s, 49%). ^{1}H NMR ($CDCl_3$): 0.83–0.91 (m, 6H, $2CH_3$), 1.23–1.30 (m, 3H, CH_3), 1.29 (bs, NH_2), 1.92–1.98 (m, 2H, CH_2), 3.29–3.51 (m, 1H, CH) 3.85–4.26 (m, 5H, $2CH_2$, CH), 5.52 (bs, OH). ^{13}C NMR ($CDCl_3$): 16.50 (d, $^{3}J_{PC}$=4.8 Hz, CH_3), 21.09 (d, $^{4}J_{PC}$=6.3 Hz, CH_3), 23.17 (d, $^{4}J_{PC}$=3.72 Hz, CH_3), 24.09 ppm (d, $^{3}J_{PC}$=9.3 Hz, CH), 37.27 (2d, $^{2}J_{PC}$=1.9, 2.2 Hz, CH_2), 45.73, 47.61 (2d, $^{1}J_{PC}$=97.9, 90.8 Hz), 56.61, 56.99 (d, $^{1}J_{PC}$=103.8, 101.4 Hz, CH_2), 62.18 (d, $^{2}J_{PC}$= 117.0 Hz, CH_2). MS FAB+(NBA) m/z=210 (51%) $[M+H]^+$, 86 (100%) $H_2N(Me_2CHCH_2)CH^+$. IR (NaCl) 3310 (OH), 1230 (PO), 1115 (POC). HRMS calcd for $C_8H_{21}O_3NP$ 210.1263, found 210,1259.

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An efficient and stereodivergent synthesis of *threo*- and *erythro*-β-methylphenylalanine. Resolution of each racemic pair by semipreparative HPLC

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Abstract—*threo* and *erythro* diastereoisomers of the constrained amino acid (βMe)Phe can be obtained separately on a multigram scale through a three-step synthesis from the corresponding *Z* and *E* isomers of 2-phenyl-4(α-phenylethylidene)-5(4*H*)-oxazolone. The 5(4*H*)-oxazolones are readily available from acetophenone and hippuric acid. The four enantiomerically pure isomers of β-methylphenylalanine, (2*R*,3*R*)-(βMe)Phe, (2*S*,3*S*)-(βMe)Phe, (2*R*,3*S*)-(βMe)Phe and (2*S*,3*R*)-(βMe)Phe, have been prepared by HPLC resolution of the racemic precursors methyl *threo* (or *erythro*)-2-benzamide-3-phenylbutanoates.

1. Introduction

The incorporation of conformationally constrained α-amino acids into peptides allows the study of structure-activity relationships and the synthesis of peptide analogues with improved pharmacological properties.[1–4]

Special mention must be made of the constrained analogues of phenylalanine, as this naturally occurring α-amino acid is directly involved in a large number of molecular recognition processes.[5–7] In all cases, the three-dimensional arrangement of the side chain moiety of the phenylalanine residue is crucial in eliciting the desired response.

The side-chain conformation can be conformationally constrained by introducing an alkyl group at the β-position of an α-amino acid residue without significantly perturbing the backbone conformation. In particular, β-methyl-α-aromatic amino acids have been incorporated into peptides[5,6,8–10] and confer on these systems a conformational side-chain rigidity that is very valuable for the study of both the specific topochemical arrays of the side chains and topochemical nature of the binding site.

The preparation of all four isomers of β-methylphenylalanine in enantiopure form has been a challenging area of synthetic organic chemistry. Several strategies have been developed and these include classical resolution,[11] the use of threonine as the starting material,[12] enzymatic resolution in conjunction with HPLC,[8,10] asymmetric synthesis,[13,14] the chiral auxiliary approach[15–19] and enantioselective hydrogenation.[20,21] When all stereoisomers are required, it may be more convenient to perform a rapid synthetic route resulting in racemates rather than a stereoselective synthesis for each isomer.

Access to the β-methylphenylalanine amino acid in its *erythro* and *threo* diastereomerically pure forms on a multigram scale is a subject of current interest. Repeated recrystallization of the hydrochloride form of the β-methylphenylalanine mixture[22] is very time consuming and especially difficult for the *threo* racemate, which requires further recrystallizations and even the use of semipreparative RP-HPLC.[10,23,24]

In the course of our work on the synthesis of conformationally restricted aspartame analogues, the availability of a simple, short and efficient method to obtain *threo*- and *erythro*-(βMe)Phe as diastereomerically pure materials was critical. We describe here a convenient route for the multigram-scale preparation of both diastereomers by catalytic hydrogenation of α,β-didehydroamino acid derivatives. *Z*- and *E*-2-Benzamide-3-phenyl-2-butenoates, obtained from *Z*- and *E*-2-phenyl-4(α-phenylethylidene)-5(4*H*)-oxazolones, are the precursors of *threo*- and *erythro*-(βMe)Phe, respectively.

Extensive work on enantiomeric separations of β-methyl amino acids using chiral derivatisations[25,26] and different chiral stationary phases,[27–31] mainly derived from macrocyclic glycopeptide antibiotics, has been reported. To the

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best of our knowledge, separation of all four stereoisomers of (βMe)Phe on HPLC has never been described on a semipreparative scale, so we would like to report here the preparation of the four isomers of β-methylphenylalanine in optically pure form by combining a racemic synthesis with an HPLC resolution procedure.

2. Results and discussion

2.1. Racemic synthesis

The *Z* and *E* isomers of 2-phenyl-4(α-phenylethylidene)-5(4*H*)-oxazolone (*Z*-**1**, *E*-**1**) proved to be convenient starting materials to afford (βMe)Phe, as demonstrated previously by our group.[32–34] The sequence of reactions leading to the amino acid involved the synthesis of pure 5(4*H*)-oxazolones, alkaline hydrolysis to 2-benzoyl-3-phenyl-2-butenoic acids followed by catalytic hydrogenation and, finally, hydrolysis to afford the amino acid. The methodology described here involves some modifications with respect to the originally reported procedure and these are described below. The synthetic route followed for the racemic synthesis of the precursors for the desired α-amino acids is outlined in Scheme 1.

The thermodynamically more stable (*Z*)-isomer, *Z*-**1**, was prepared by fractional crystallisation of an isomeric mixture (*Z*/*E*) obtained from the condensation of hippuric acid and acetophenone.[32] In the same paper, access to the less stable (*E*)-isomer *E*-**1** was effected from *Z*-**1** by treatment with HBr. In our experience, working under similar reaction conditions, isomerization was sometimes completely *E*-selective and sometimes moderately or even poorly *E*-selective. The mechanism of this reaction is apparently the same as that in the isomerization of *cis*-/*trans*-stilbene[35] and it seems to be consistent with the presence of bromine radicals.[36] We proceeded to define new reaction conditions that favour a radical reaction. After several other approaches proved unsuccessful, we were able to obtain the (*E*)-isomer in a totally controlled and reproducible manner by HBr isomerization in the presence of a catalytic amount of benzoyl peroxide.

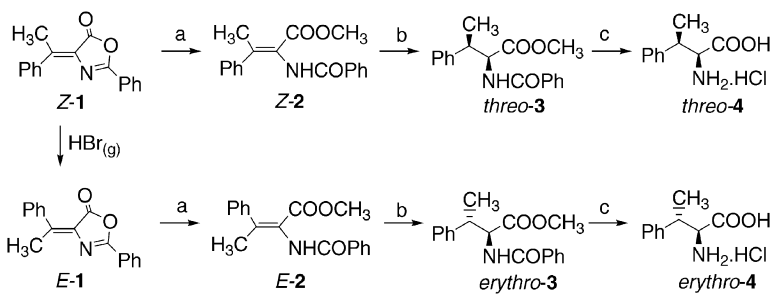
Ester derivatives are more convenient substrates for chromatography than the corresponding carboxylic acids. Thus, with the aim of resolving racemic intermediates into their enantiomers by chiral HPLC, oxazolone ring opening was performed by methanolysis of *Z*-**1** and *E*-**1** in the presence of a catalytic amount of sodium methoxide. This treatment afforded the corresponding benzamido esters *Z*-**2** and *E*-**2** in high yield and with retention of configuration, as reported previously.[37]

The next step in the synthetic route involved hydrogenation of the tetrasubstituted olefin in compounds *Z*-**2** and *E*-**2**. Initial attempts to obtain racemic methyl butanoates *threo*-**3** and *erythro*-**3** involved the use of 10% Pd/C as catalyst. The hydrogenation of *Z*-**2** was achieved by keeping the reaction at 30 °C with vigorous agitation in the presence of a small amount of Pd/C (10% w/w). The diastereomeric purity of *threo*-**3** was confirmed by ^{1}H NMR spectroscopy and HPLC. When the preparation of *erythro*-**3** was attempted under the same conditions, a mixture ranging from 95:5 to 87:13 of *erythro*-**3**:*threo*-**3** was obtained.

It is well known that the factors influencing the hydrogenation of olefins are numerous and include, among others, the nature of the solvent, hydrogen availability and catalyst.[38] These facts prompted us to modify the reaction conditions in an attempt to minimize the isomerization phenomenon.

The use of methanol/benzene mixtures as solvent had been reported to show marked inhibition of isomerization. Nevertheless, this approach did not lead to better results in our experiments. Other catalysts, such as Pt/C and PtO_2/C, did not improve on our previous results. Finally, upon increasing the amount of catalyst up to 40% catalyst, on a weight basis referred to the substrate, we managed to overcome the isomerization problem. The diastereomeric purity of *erythro*-**3** was checked by ^{1}H NMR spectroscopy and HPLC. In fact, it appears that we had indirectly increased the concentration of hydrogen on the catalyst surface. For this reason, we expected that the use of hydrogen pressures of 5–10 atm would lead to the same results, but under these conditions fragmentations occurred.

The last step of the synthetic approach involved removal of the protecting groups in *threo*-**3** and *erythro*-**3** by total hydrolysis. This step has been reported previously[34] and involves very strong conditions: heating under reflux in a mixture of hydrobromic acid and glacial acetic acid. This method led to the formation of 15% of *erythro*-**4** in the reaction mixture on starting from *threo*-**3**. Our subsequent aim was to reduce as much as possible the partial epimerization observed at Cα and, in this respect, milder deprotection conditions were assayed. Finally, the use of a mixture of 2.5 N HCl/HOAc (4:1) gave almost quantitative deprotection with no detectable epimerization by ^{1}H NMR. Nevertheless, diastereomeric purity was assessed (after



Scheme 1. Synthetic route to racemic *threo*-(βMe)Phe and *erythro*-(βMe)Phe. *Reagents and conditions*: (a) MeONa/MeOH. (b) H_2/Pd–C. (c) 2.5 N HCl/HOAc, reflux.

Table 1. Selected chromatographic data for the HPLC resolution of *threo*-**3** and *erythro*-**3** on the amylose-derived chiral stationary phase

Compound	Eluent[a] (A/B/C)	Flow (mL/min)	λ (nm)	k'_1	α	R_s
threo-**3**	95:5:0	1[b]	210	1.59	1.21	0.90
	93:5:2	1[b]	235	1.24	1.15	0.59
	93:5:2	18[c]	270	2.44	1.18	0.81
erythro-**3**	98:2:0	1[b]	210	2.79	1.33	1.67
	96:2:2	1[b]	240	2.98	1.23	1.25
	96:2:2	18[d]	265	2.87	1.19	0.80

For the definition of k', α and R_s see Section 4.2.
[a] A: *n*-hexane, B: 2-propanol, C: chloroform.
[b] Steel column, 150 mm×4.6 mm ID. Temperature: 25 °C. *c*=5 mg/mL.
[c] Steel column, 150 mm×20 mm ID. Temperature: 25 °C. *c*=100 mg/mL.
[d] Steel column, 150 mm×20 mm ID. Temperature: 25 °C. *c*=200 mg/mL.

previous transformation to the free amino acids) using a RP-HPLC protocol analogue to the method described in the literature.[24] It was found that diastereomeric purity for *threo* pair was over 99% whereas for *erythro* pair ranged 96–98% for a wide number of experiments done.

The route described above allows access to diastereomerically pure *threo*-**4** and *erythro*-**4** through a three-step approach with yields of 82–86% from oxazolones and an overall yield of 31–33% from hippuric acid. These results make this methodology particularly appropriate for large-scale preparations of diastereomerically pure *erythro*-(βMe)Phe and *threo*-(βMe)Phe.

2.2. HPLC resolution

Once an efficient racemic route to the target compounds had been developed, we undertook the preparation of the products in enantiomerically pure form by HPLC resolution of a synthetic intermediate using a chiral stationary phase. The utility of polysaccharide-based phases is well documented.[39,40] Recently, a non-commercial stationary phase consisting of a mixed derivative of amylose (10-undecenoate/3,5-dimethylphenylcarbamate) covalently linked to allylsilica gel proved to be very efficient in the semipreparative separation of racemic constrained analogues of phenylalanine.[41–44] The wide applicability shown by this stationary phase together with its chemical stability make it very convenient for the resolution of racemates on a semipreparative scale.

First, analytical separation of derivatives *threo*-**3** and *erythro*-**3** was examined using mixtures of *n*-hexane/2-propanol as the mobile phase. As can be seen from the results in Table 1, chloroform leads to lower selectivity and resolution factors in the analytical assays but its presence is necessary in order to optimize the loading capacity of the column. Thus, mixtures of *n*-hexane/2-propanol/chloroform were also tested as eluents (Fig. 1).

Preparative resolutions of racemates *threo*-**3** and *erythro*-**3** were performed on a 150×20 mm ID column by successive injections using the peak shaving technique. After determination of the optimum cut points, each run was collected into three separate fractions. The first fractions and the last

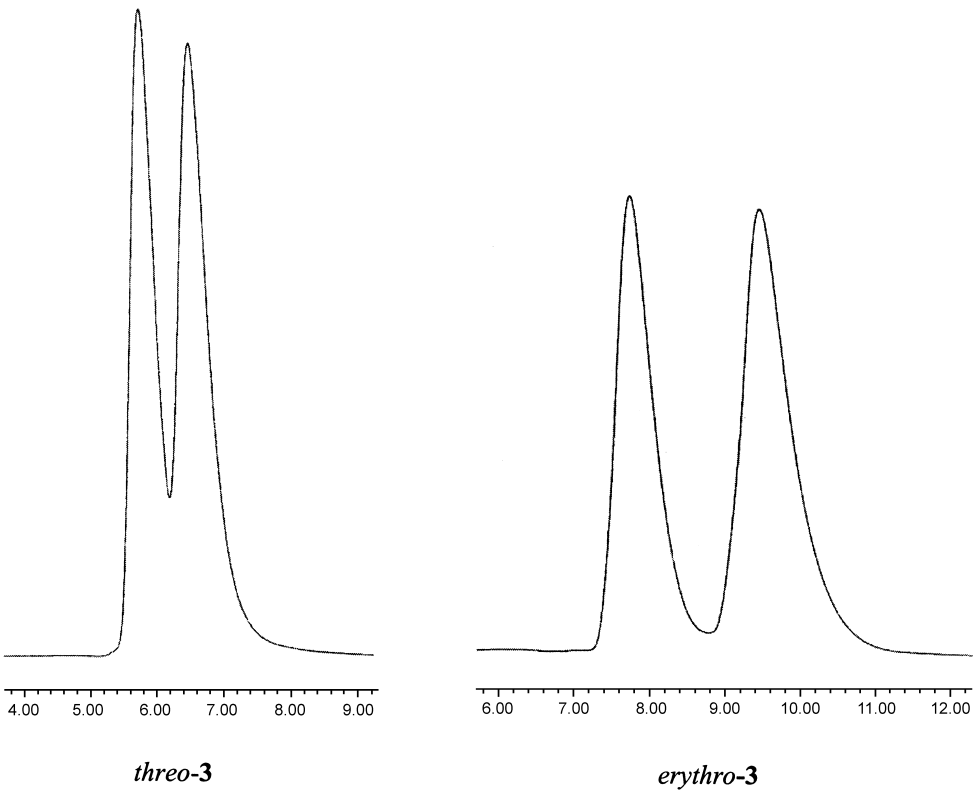


Figure 1. HPLC analytical resolution of *threo*-**3** (eluent: *n*-hexane/2-propanol 95:5) and *erythro*-**3** (eluent: *n*-hexane/2-propanol 98:2). See Table 1 for related chromatographic data.

fractions, each containing one of the enantiomers, were collected at each passage through the column and combined. Therefore, 327 mg of *threo*-**3** dissolved in 3.3 mL of dichloromethane were fractionated to obtain 78 and 67 mg of the less and more strongly retained enantiomers, respectively. A total of 4.4 h were required (33 injections of 100 μL each with 8 min/cycle). Since the preparative column contained about 28 g of chiral stationary phase (CSP), a loading capacity of 2.7 mg racemate per gram CSP/hour was allowed in the *threo*-**3** resolution. As regards racemate *erythro*-**3**, 760 mg dissolved in 3.8 mL of dichloromethane was fractionated to afford 279 and 200 mg of the less and more strongly retained enantiomers, respectively. Six hours were required for total separation (38 injections of 100 μL each with 9.5 min/cycle) and the loading capacity was 4.5 mg racemate/g CSP per hour. Enantiomeric purity of each isomer was checked on the analytical column and only one enantiomer was detected.

2.3. Preparation of enantiomerically pure compounds

Once enantiomerically pure precursors were available, we undertook the deprotection steps under the conditions developed for the racemic material, *threo*-**3** and *erythro*-**3**, with each stereoisomer (Scheme 2). In addition, with the aim of assigning the absolute configurations, the amino acid hydrochlorides obtained after hydrolysis were treated with propylene oxide under reflux in order to obtain the optically pure free amino acids.

2.4. Assignment of absolute configurations

The absolute configurations of all four isomers of β-methylphenylalanine were determined by direct comparison of their optical rotations with the values described in the literature.[10,11,45] This allowed us to assign a (2*S*,3*R*) stereochemistry to the compounds obtained from the first eluted enantiomer of *threo*-**3** and a (2*R*,3*S*) configuration to the derivatives obtained from the last eluted enantiomer of *threo*-**3**. In a similar way, a (2*R*,3*R*) stereochemistry was assigned to the compound obtained from the first eluted enantiomer of *erythro*-**3** and a (2*S*,3*S*) configuration to the derivatives obtained from the last eluted enantiomer of *erythro*-**3**.

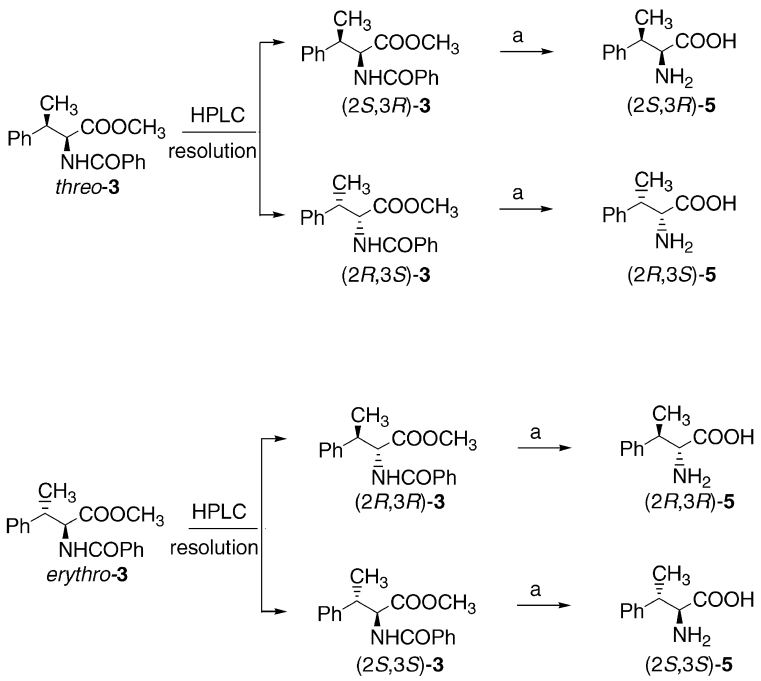
3. Conclusion

The methodology described here allows easy access to the racemic pairs (*erythro* and *threo*) of β-methylphenylalanine with high diastereomeric purity and through high yield transformations. Moreover, combining the racemic synthesis with a resolution by chiral HPLC provides all four individual isomers of β-methylphenylalanine, (2*S*,3*S*)-, (2*R*,3*R*)-, (2*S*,3*R*)- and (2*R*,3*S*)-β-methylphenylalanine, with high optical purity.

4. Experimental

4.1. General

Solvents were purified according to standard procedures. 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 a Varian Unity-300 or a Bruker ARX-300 instrument at room temperature, unless otherwise indicated, using the residual solvent signal as the internal standard; chemical shifts are reported in ppm on the δ scale, coupling constants in Hz. Mass Spectra were obtained on a



Scheme 2. Synthetic route to enantiomerically pure (βMe)Phe stereoisomers. *Reagents and conditions*: (a) (i) 2.5 N HCl/HOAc, reflux; (ii) propylene oxide/EtOH, reflux.

high resolution VG-autospectrometer using either EI or +FAB techniques. Optical rotations were measured on a Perkin–Elmer 241 polarimeter-C in a 1 dm cell of 1 mL capacity at 25 °C. Microanalyses were carried out on a Perkin–Elmer 200 C, H, N, S analyser. Analytical TLC was performed using Merck 60 SI F_{254} precoated silica gel polyester plates and the products were examined by UV fluorescence or developed using iodine vapour. Column chromatography was performed using silica gel 60 (230–400 mesh).

4.2. 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 a mixed 10-undecenoate/3,5-dimethylphenylcarbamate of amylose bonded to allylsilica, was prepared according to a previously described procedure.[46,47] The analytical assays were carried out on a 150 mm×4.6 mm ID column and the semipreparative resolution was achieved on a 150 mm×20 mm ID column. All analytical assays and semipreparative chromatography were performed under the conditions given in Table 1. Diastereomeric purity of the final products was checked 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.

4.3. (*Z*)-2-Phenyl-4-(α-phenylethylidene)-5(4*H*)-oxazolone, *Z*-1

A mixture of acetophenone (36 g, 0.3 mol), hippuric acid (10.26 g, 0.06 mol), acetic anhydride (18.36 g, 0.18 mol), anhydrous lead(IV) acetate (13.29 g, 30 mmol) and dry THF (100 mL) was heated under reflux for 24 h. After cooling, the contents were poured onto crushed ice and extracted with CH_2Cl_2. The combined organic phases were washed with $NaHCO_3$, brine and dried over $MgSO_4$. The solvent and most of the excess acetophenone were removed in vacuo to afford a residue, which was crystallised from EtOH/H_2O to give a mixture of the geometric isomers of the oxazolone. The pure (*Z*)-isomer was obtained after a second recrystallization from EtOH/H_2O as a yellow solid (5.73 g, 38% yield). Mp 109 °C. R_f (hexanes/benzene 1:1)=0.23. IR (nujol) 1784; 1759 cm^{-1}. ^1H NMR ($CDCl_3$ 300 MHz) δ 2.78 (s, 3H); 7.42–7.54 (m, 6H); 7.86 (m, 2H); 8.05 (m, 2H). ^{13}C NMR ($CDCl_3$ 75 MHz) δ 18.37; 125.80; 127.92; 128.13; 128.77; 129.95; 131.20; 132.67; 138.88; 149.26; 160.39; 166.83. MS-EI (*m*/*z*, %) 263 [$(M)^+$, 19]; 105 (100); 77 [$(C_6H_5)^+$, 45]. Anal. calcd for $C_{17}H_{13}NO_2$: C, 77.55; H, 4.98; N 5.32. Found: C, 77.91; H, 5.04; N, 5.19.

4.4. (*E*)-2-Phenyl-4-(α-phenylethylidene)-5(4*H*)-oxazolone, *E*-1

The (*Z*)-isomer (2.5 g) was dissolved in the minimum amount of dry toluene. After adding 10 mg of benzoyl peroxide, the solution was saturated with anydrous hydrogen bromide for 15–30 min or until a precipitate had formed completely, while keeping the reaction in an ice-bath. The solid was filtered off and washed with cold toluene to afford the pure (*E*)-isomer as a pale yellow solid (2.37 g, 94% yield). Mp 115 °C. R_f (hexanes/benzene 1:1)=0.19. IR (nujol) 1788; 1651 cm^{-1}. ^1H NMR ($CDCl_3$ 300 MHz) δ 2.64 (s, 3H); 7.24–7.60 (m, 8H); 8.09 (m, 2H). ^{13}C NMR ($CDCl_3$ 75 MHz) δ 23.02; 125.91; 127.80; 128.09; 128.14; 128.41; 128.80; 129.63; 129.92; 132.68; 137.25; 152.22; 160.83; 164.06. MS-EI (*m*/*z*, %) 263 [$(M)^+$, 93]; 105 (82); 77 [$(C_6H_5)^+$, 100]. Anal. calcd for $C_{17}H_{13}NO_2$: C, 77.55; H, 4.98; N, 5.32. Found: C, 77.33; H, 5.03; N, 5.30.

4.5. Methyl (*Z*)-2-benzamide-3-phenyl-2-butenoate, *Z*-2

A solution of sodium methoxide (50 mg) in absolute MeOH (60 mL) was added to *Z*-**1** (2.5 g, 10 mmol) and the reaction mixture was stirred at room temperature for 30 min (check completion by TLC, hexanes/benzene 1:1). The reaction mixture was filtered and the solvent was removed in vacuo. Cold water was added dropwise to the residue until precipitation was complete. The product was collected by vacuum filtration to give *Z*-**2** as a white solid (2.67 g, 95%). Mp 153 °C. R_f (hexanes/AcOEt 8:2)=0.11. IR (nujol) 3263; 1713; 1638 cm^{-1}. ^1H NMR ($CDCl_3$ 300 MHz) δ 2.31 (s, 3H); 3.86 (s, 3H); 7.19 (brs, 1H); 7.10–7.60 (m, 11H). ^{13}C NMR ($CDCl_3$ 75 MHz) δ 20.52; 52.24; 123.79; 127.09; 127.42; 128.30; 128.61; 128.95; 131.92; 133.10; 137.01; 139.70; 165.40; 165.59. MS-EI (*m*/*z*, %) 295 [$(M)^+$, 8]; 263 [$(M-MeOH)^+$, 16]; 190 (37); 130 (8); 105 (100); 77 [$(C_6H_5)^+$, 73]. Anal. calcd for: $C_{18}H_{17}NO_3$: C, 73.20; H, 5.80; N, 4.74. Found: C, 73.01; H, 5.89; N, 4.71.

4.6. Methyl (*E*)-2-benzamide-3-phenyl-2-butenoate, *E*-2

An identical procedure to that described above was applied to the transformation of *E*-**1** (2.30 g, 8.74 mmol) to *E*-**2** (2.51 g, 97% yield). Mp 210 °C. R_f (hexanes/AcOEt 8:2)=0.11. IR (nujol) 3321; 1721; 1639 cm^{-1}. ^1H NMR ($CDCl_3$ 300 MHz) δ 2.18 (s, 3H); 3.46 (s, 3H); 7.15–7.35 (m, 5H); 7.35–7.60 (m, 4H); 7.87 (m, 2H). ^{13}C NMR ($CDCl_3$ 75 MHz) δ 22.07; 51.86; 123.72; 127.17; 127.34; 127.69; 128.07; 128.71; 132.11; 133.39; 140.87; 141.01; 165.63; 165.78. MS-EI (*m*/*z*, %) 295 [$(M)^+$, 7]; 263 [$(M-MeOH)^+$, 17]; 190 (29); 130 (6); 105 (100); 77 [$(C_6H_5)^+$, 52]. Anal. calcd for $C_{18}H_{17}NO_3$: C, 73.20; H, 5.80; N, 4.74. Found: C, 72.96; H, 5.86; N, 4.76.

4.7. Synthesis of *threo*-3 and *erythro*-3

4.7.1. Methyl *threo*-2-benzamide-3-phenylbutanoate, *threo*-3. A solution of *Z*-**2** (2.2 g, 7.46 mmol) in AcOEt (180 mL) was hydrogenated at 30 °C in the presence of 10% Pd/C (200 mg). After 6 h the catalyst was filtered off and the solvent evaporated to give diastereomerically pure *threo*-**3** as a white solid (2.12 g, 96% yield). Mp 102 °C. R_f (hexanes/AcOEt 8:2)=0.16. IR (nujol) 3327; 1739; 1631 cm^{-1}. ^1H NMR ($CDCl_3$ 300 MHz) δ 1.48 (d, 3H, J=7.1 Hz); 3.34 (q, 1H, J=6.8 Hz); 3.61 (s, 3H); 5.02 (dd, J=6.3, 8.5 Hz); 6.61 (d, 1H, J=8.3 Hz); 7.18–7.34 (m, 5H); 7.40–7.55 (m, 3H); 7.75–7.77 (m, 2H). ^{13}C NMR ($CDCl_3$

75 MHz) δ 17.10; 43.04; 52.11; 57.97; 127.01; 127.29; 127.69; 128.49; 128.63; 131.78; 134.01; 141.03; 166.86; 171.84. MS-EI (*m/z*, %) 298 [(M+1)$^+$, 23]; 238 [(M−MeOH−CO)$^+$, 11]; 193 (23); 161 (9); 105 (100); 77 [$(C_6H_5)^+$, 60]. Anal. calcd for: $C_{18}H_{19}NO_3$: C, 72.71; H, 6.44; N, 4.71. Found: C, 72.54; H, 6.52; N, 4.66.

4.7.1.1. Methyl (2*R*,3*S*)-2-benzamide-3-phenylbutanoate, (2*R*,3*S*)-3. Mp 135 °C. $[\alpha]_D^{20}$=−59.4 (*c*=0.50, $CHCl_3$). Anal. calcd for: $C_{18}H_{19}NO_3$: C, 72.71; H, 6.44; N, 4.71. Found: C, 72.54; H, 6.36; N, 4.68.

4.7.1.2. Methyl (2*S*,3*R*)-2-benzamide-3-phenylbutanoate, (2*S*,3*R*)-3. Mp 135 °C. $[\alpha]_D^{20}$=+58.2 (*c*=0.37, $CHCl_3$). Anal. calcd for: $C_{18}H_{19}NO_3$: C, 72.71; H, 6.44; N, 4.71. Found: C, 72.49; H, 6.42; N, 4.76. Spectroscopic data for both (2*R*,3*S*)-**3** and (2*S*,3*R*)-**3** were the same as those described above for *threo*-**3**.

4.7.2. Methyl *erythro*-2-benzamide-3-phenylbutanoate, *erythro*-3. In a similar way to that described above, hydrogenation of *E*-**2** (2.4 g, 8.14 mmol) using 10% Pd/C (960 mg) for 10 h gave diastereomerically pure *erythro*-**3** as a white solid (82.35 g, 97% yield). Mp 118 °C. R_f (hexanes/AcOEt 8:2)=0.16. IR (nujol) 3345; 1742; 1639 cm^{-1}. ^{1}H NMR ($CDCl_3$ 300 MHz) δ 1.41 (d, 3H, *J*=7.2 Hz); 3.49 (m, 1H); 3.72 (s, 3H); 5.04 (dd, 1H, *J*=5.4, 8.7 Hz); 6.30 (d, 1H, *J*=8.4 Hz); 7.1–7.5 (m, 8H); 7.66 (m, 2H). ^{13}C NMR ($CDCl_3$ 75 MHz) δ 17.63; 42.23; 52.21; 57.47; 126.93; 127.16; 127.63; 128.05; 128.44; 128.57; 128.69; 131.74; 133.80; 140.70; 167.16; 171.96. MS-EI (*m/z*, %) 298 [(M+1)$^+$, 61]; 238 (19); 266 (6); 193 (24); 176 (40); 161 (9); 105 (100); 77 [$(C_6H_5)^+$, 54]. Anal. calcd for: $C_{18}H_{19}NO_3$: C, 72.71; H, 6.44; N, 4.71. Found: C, 72.55; H, 6.49; N, 4.76.

4.7.2.1. Methyl (2*R*,3*R*)-2-benzamide-3-phenylbutanoate, (2*R*,3*R*)-3. Mp 138 °C. $[\alpha]_D^{20}$=−74.7 (*c*=0.51, $CHCl_3$). Anal. calcd for: $C_{18}H_{19}NO_3$: C, 72.71; H, 6.44; N, 4.71. Found: C, 73.03; H, 6.36; N, 4.74.

4.7.2.2. Methyl (2*S*,3*S*)-2-benzamide-3-phenylbutanoate, (2*S*,3*S*)-3. Mp 137 °C. $[\alpha]_D^{20}$=+73.2 (*c*=0.39, $CHCl_3$). Anal. calcd for: $C_{18}H_{19}NO_3$: C, 72.71; H, 6.44; N, 4.71. Found: C, 72.43; H, 6.39; N, 4.85. Spectroscopic data for both (2*R*,3*R*)-**3** and (2*S*,3*S*)-**3** were the same as those described above for *erythro*-*3*.

4.8. Synthesis of *threo*-4 and *erythro*-4

4.8.1. *threo*-β-Methylphenylalanine hydrochloride, *threo*-4. A solution of *threo*-**3** (2 g, 6.73 mmol) in HOAc/2.5 N HCl (90 mL:360 mL) was heated for 42 h at 125 °C. The solvent was removed in vacuo and the residue partitioned between H_2O/CH_2Cl_2. The phases were separated and the aqueous layer was washed with CH_2Cl_2. Removal of water by lyophilization afforded *threo*-**4** as a white solid (1.36 g, 94% yield). Mp 214 °C. Diastereomeric purity: 99%. R_f (CH_2Cl_2/MeOH 8:2)=0. IR (nujol) 3600–2400; 1734 cm^{-1}. ^{1}H NMR (D_2O 300 MHz) δ 1.32 (d, 3H, *J*=6.5 Hz); 3.42 (q, 1H, *J*=6.5 Hz): 4.09 (d, 1H, *J*=6.4 Hz); 7.20–7.34 (m, 5H). ^{13}C NMR (D_2O 75 MHz) δ 14.83; 39.72; 58.68; 127.84; 128.16; 129.21; 139.10; 171.10. MS-FAB (*m/z*, %) 359 [(2M+1)$^+$, 10]; 202 [(M+Na)$^+$, 18]; 180 [(M+1)$^+$, 100]. Anal. calcd for $C_{10}H_{14}ClNO_2$: C, 55.69; H, 6.54; N, 6.49. Found: C, 55.89; H, 6.47; N, 6.55.

4.8.2. *erythro*-β-Methylphenylalanine hydrochloride, *erythro*-4. A similar procedure to that described above for *threo*-**4**, starting from *erythro*-**3** (2 g, 6.73 mmol), gave *erythro*-**4** as a white solid (1.35 g, 93% yield). Mp 200 °C. Diastereomeric purity: 98%. R_f (CH_2Cl_2/MeOH 8:2)=0. IR (nujol) 3600–2500; 1728 cm^{-1}. ^{1}H NMR (D_2O 300 MHz) δ 1.33 (d, 3H, *J*=7.2 Hz); 3.30 (q, 1H, *J*=7.05 Hz); 4.01 (d, 1H, *J*=7.5 Hz); 7.2–7.4 (m, 5H). ^{13}C NMR (D_2O 75 MHz) δ 16.98; 40.32; 59.02; 127.82; 128.22; 129.32; 139.27; 171.63. MS-FAB (*m/z*, %) 359 [(2M+1)$^+$, 13]; 202 [(M+Na)$^+$, 8]; 180 [(M+1)$^+$, 100]. Anal. calcd for $C_{10}H_{14}ClNO_2$: C, 55.69; H, 6.54; N, 6.49. Found: C, 57.21; H, 6.95; N, 6.13.

4.9. Synthesis of enantiomerically pure 5

4.9.1. (2*R*,3*S*)-β-Methylphenylalanine, (2*R*,3*S*)-5. A solution of (2*R*,3*S*)-**3** (50 mg, 0.17 mmol) in HOAc/2.5 N HCl (2.3 mL:9 mL) was heated at 125 °C for 24 h. The solvent was removed in vacuo and the residue partitioned between H_2O/CH_2Cl_2. The phases were separated and the aqueous layer was washed with CH_2Cl_2 and evaporated to dryness to afford the amino acid hydrochloride. This compound was converted into the free amino acid by treatment with EtOH (2 mL) and propylene oxide (0.68 mL) under reflux. Removal of the solvent afforded a residue that was eluted through a C_{18} reverse-phase sep-pak cartridge. Lyophilization of the aqueous phases gave (2*R*,3*S*)-**5** as a white solid (28 mg, 93% yield). Mp 220–222 °C. $[\alpha]_D^{20}$=+6.6 (*c*=0.18, H_2O) lit.[45] (+5.1; *c*=1.1, H_2O). Diastereomeric purity: 99%. R_f (CH_2Cl_2/MeOH 8:2)=0.88. IR (nujol) 1629; 1574 cm^{-1}. ^{1}H NMR (D_2O 300 MHz) δ 1.27 (d, 3H, *J*=7.5 Hz); 3.42 (m, 1H): 3.82 (d, 1H, *J*=4.8 Hz); 7.24–7.25 (m, 5H). ^{13}C NMR (D_2O 75 MHz) δ 13.70; 39.51; 60.73; 127.73; 127.78; 129.09; 140.36; 173.54. MS-FAB (*m/z*, %) 359 [(2M+1)$^+$, 10]; 202 [(M+Na)$^+$, 18]; 180 [(M+1)$^+$, 100]. Anal. calcd for $C_{10}H_{13}NO_2$: C, 67.02; H, 7.31; N, 7.82. Found: C, 66.69; H, 7.39; N, 7.71.

4.9.2. (2*S*,3*R*)-β-Methylphenylalanine, (2*S*,3*R*)-5. An identical procedure to that described above was applied to the transformation of (2*S*,3*R*)-**3** (65 mg, 0.22 mmol) to (2*S*,3*R*)-**5** (36 mg, 92% yield). Mp 200–202 °C. $[\alpha]_D^{20}$=−7.49 (*c*=0.15, H_2O) lit.[45] (−5.3; *c*=0.75, H_2O). Diastereomeric purity: 99.5%. Anal. calcd for $C_{10}H_{13}NO_2$: C, 67.02; H, 7.31; N, 7.82. Found: C, 66.65; H, 7.42; N, 7.70.

4.9.3. (2*R*,3*R*)-β-Methylphenylalanine, (2*R*,3*R*)-5. The procedure described above was applied to the transformation of (2*R*,3*R*)-**3** (95 mg, 0.32 mmol) to (2*R*,3*R*)-**5** (52 mg, 91% yield). Mp 224 °C. $[\alpha]_D^{20}$=+27.2 (*c*=0.10, H_2O) lit.[45] (+21; *c*=1.0, H_2O). Diastereomeric purity: 97.7%. R_f (CH_2Cl_2/MeOH 8:2)=0.88. IR (nujol) 1609 cm^{-1}. ^{1}H NMR (D_2O 300 MHz) δ 1.28 (d, 3H, *J*=7.17 Hz); 3.16 (q, 1H, *J*=7.35 Hz); 3.66 (d, 1H, *J*=7.71 Hz); 7.21–7.34 (m, 5H). ^{13}C NMR (D_2O 75 MHz) δ 17.65; 40.80; 60.98; 127.89; 127.89; 127.96; 129.25; 140.20; 173.79. MS-FAB (*m/z*, %) 180 [(M+1)$^+$, 83]. Anal. calcd for $C_{10}H_{13}NO_2$: C, 67.02; H, 7.31; N, 7.82. Found: C, 66.75; H, 7.39; N, 7.73.

4.9.4. (2*S*,3*S*)-β-Methylphenylalanine, (2*S*,3*S*)-5. The method described above was used for the conversion of

(2*S*,3*S*)-**3** (110 mg, 0.37 mmol) to (2*S*,3*S*)-**5** (60 mg, 91% yield). Mp 224 °C. $[\alpha]_D^{20}=-29$ (c=0.30, H_2O) lit.[45] (−26.7; c=1.0, H_2O). Diastereomeric purity: 99.8%. Anal. calcd for $C_{10}H_{13}NO_2$: C, 67.02; H, 7.31; N, 7.82. Found: C, 66.53; H, 7.24; N, 7.77. Spectroscopic data were the same as those described above for (2*R*,3*R*)-**5**.

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Isolation, structure elucidation, and total synthesis of two new *Chimonanthus* alkaloids, chimonamidine and chimonanthidine

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Abstract—Two new tryptamine-related alkaloids, chimonamidine and chimonanthidine, were isolated from the seeds of *Chimonanthus praecox* Link. and their structures including absolute configuration were elucidated by spectroscopic analysis and biomimetic total synthesis from tryptamine.

1. Introduction

Recently, the potent antinociceptive activity of dimeric or polymeric pyrrolidinoindoline alkaloids that interact with opioid receptors has been reported by Elisabetsky–Verotta's group.[1] In our continuous chemical and pharmacological studies of indole alkaloids possessing analgesic activity,[2] we have been interested in compounds of this type, which have been isolated from plants belonging to genera *Calycanthaceae*, *Idiospermaceae*, and *Rubiaceae*.[3] Overman and co-workers have recently made several important contributions to the synthesis of this family of alkaloids.[4] We started with the investigation of the alkaloidal constituents in *Chimonanthus praecox* Link., which was used as folk medicine for the treatment of rheumatic arthritis in China. (+)-Calycanthine and (±)-chimonanthine were isolated from the roots of this plant by a Chinese group.[5] In the present study, we were able to isolate two new alkaloids, chimonamidine (**1**) and chimonanthidine (**2**), together with (+)-calycanthine, (−)-chimonanthine (**10**), (−)-folicanthine (**11**), and (−)-calycanthidine (**12**) from the MeOH extract of *C. praecox* seeds. In this paper, we report the structure elucidation of these new alkaloids by means of spectroscopic analysis and biomimetic total syntheses.

2. Results and discussion

2.1. Chimonamidine (1)

The new compound **1**, obtained as an amorphous powder, exhibited $[\alpha]_D^{19}=-12.6$ (*c* 0.06, EtOH). High-resolution FABMS analysis gave *m/z* 221.1290 $(M+H)^+$ (Δ±0 mmu) and established the molecular formula as $C_{12}H_{16}N_2O_2$. The splitting mode of the protons in the aromatic region (δ 6.74, d *J*=7.6 Hz; δ 7.24, dd *J*=7.6, 7.6 Hz; δ 6.66, dd *J*=7.6, 7.6 Hz; and δ 6.85, d *J*=7.6 Hz) in the ^{1}H NMR spectrum indicated the presence of an *o*-disubstituted benzene ring. Further, the chemical shift of the carbon (δ 148.3) on this benzene ring as well as the HMBC cross-peak between that carbon and the protons of the *N*-Me group (δ 2.85, 3H, s) suggested that **1** contained an *N*-Me aniline residue in the molecule. In addition, ^{1}H NMR, ^{13}C NMR and COSY spectra (Fig. 1) indicated the presence of one carbonyl carbon (δ 175.1), an isolated ethane fragment, one oxygenated quaternary carbon (δ 79.6), and an *N*-Me group, all of which constituted a γ-lactam ring having a hydroxyl function. These two units could be connected by HMBC cross-peaks between the aromatic proton at δ 6.85 (H4) and the oxygenated quaternary carbon (δ 79.6, C3) and between the protons (δ 2.41 and 2.73, $H_2$8) in the ethane bridge and the aromatic quaternary carbon (δ 124.6, C3a), leading to the construction of the structure of the new alkaloid, now named chimonamidine, to be formula **1**.

To establish the above structure inferred by spectroscopic analysis, we initially performed the total synthesis of (±)-**1**. The synthetic strategy was based on the following biogenetic speculation. As shown in Figure 1, a new alkaloid is expected to be formed from tryptamine (**3**) through conversion into an oxindole derivative, introduction of a

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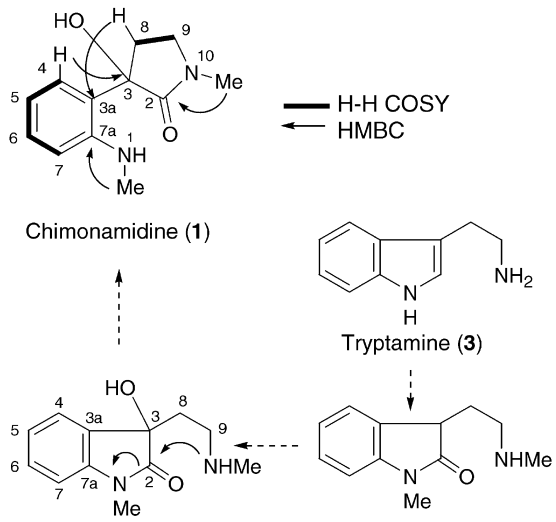
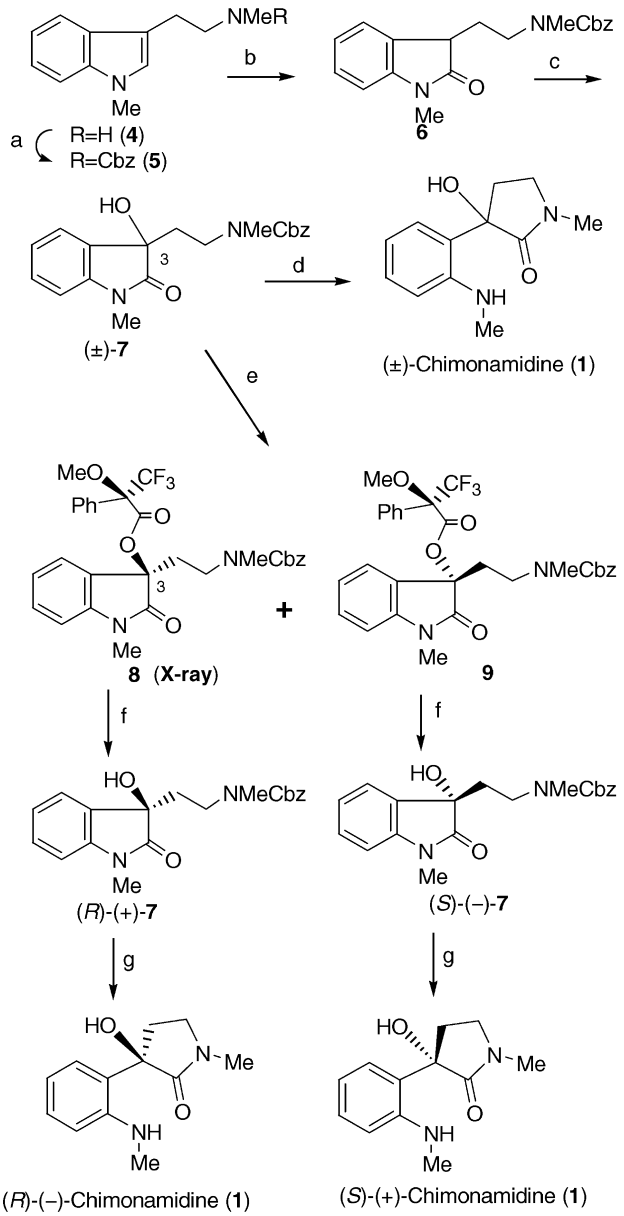


Figure 1. Selected 2D NMR correlations and hypothetical biogenetic route for chimonamidine (**1**).

hydroxyl function to the benzylic position, and transannulation of the lactam ring. Our biomimetic synthesis (Scheme 1) started with the preparation of *N*a,*N*b-dimethyl-*N*b-carbobenzyloxytryptamine (**5**) from a known tryptamine derivative (**4**).[6] The indole ring in **5** was then converted into oxindole (**6**) in 71% yield by oxidation with dimethyl sulfoxide and hydrochloric acid.[7] Next, a hydroxyl group was introduced to the benzylic position (C3) under oxygen atmosphere to give the racemic α-hydroxyketone (**7**) in a quantitative yield. Removal of the protecting group on the nitrogen with trimethylsilyl iodide gave a secondary amine that was gradually and spontaneously converted into the target molecule over two days in 66% isolated yield. The synthetic **1** (mp 213–215 °C) was found to be completely identical with the natural product by comparison of their chromatographic behavior and spectroscopic data including ^{1}H and ^{13}C NMR and MS spectra. Therefore, the structure of chimonamidine was determined to be formula **1**. Next, we synthesized chiral chimonamidine to determine the absolute configuration of the natural product, which exhibited $[\alpha]_D=-12.6$. Initial attempts at the asymmetric hydroxylation of **6** using Davis reagents[8] gave chiral hydroxyketone (**7**) up to 33% enantiomeric excess. Then, we employed an alternative strategy that involved the separation of the diastereomers of chiral ester derivatives. After several attempts, a pair of diastereomeric esters prepared from (+)-MTPA chloride and racemic alcohol (**7**) was found to be separable by SiO_2 column chromatography, and the more polar isomer (**8**) gave a crystal suitable for X-ray crystallographic analysis, revealing that the absolute stereochemistry at C3 in **8** had an *R* configuration. The ester function in **8** and **9** was respectively hydrolyzed with aqueous alkaline solution to afford chiral alcohols, (+)-**7** and (−)-**7**. Their optical purity was confirmed to be 100% ee by chiral HPLC analysis and the CD spectra exhibited antipodal curves, as shown in Figure 2.[9] Finally, the protecting group on the nitrogen in (+)-**7** and (−)-**7** was, respectively, removed with TMSI to give chiral chimonamidines. (*R*)-(−)-Chimonamidine (**1**) obtained from (*R*)-(+)-**7** showed $[\alpha]_D=-178$, whereas the enantiomeric (*S*)-(+)-chimonamidine (**1**) from (*S*)-(−)-**7** exhibited $[\alpha]_D=+171$. As a result, we confirmed that natural chimonamidine



Scheme 1. *Reagents and conditions*: (a) Cbz–Cl, Na_2CO_3, H_2O, CH_2Cl_2, 0 °C, quant. (b) conc. HCl, DMSO, phenol, AcOH, 0 °C, 71%. (c) 1 M NaOH, THF, O_2, rt, quant. (d) TMSI, CH_3CN, 0 °C to rt, 66%. (e) (*S*)-MTPA-Cl, DMAP, CH_2Cl_2, rt; **8**, 43%; **9**, 45%. (f) 0.5 M NaOH, MeOH, rt, quant. (g) TMSI, CH_3CN, 0 °C to rt; 55% from (+)-**7**; 50% from (−)-**7**.

($[\alpha]_D=-12.6$) comprises a mixture slightly enriched with the (*R*)-(−)-enantiomer.[10]

2.2. Chimonanthidine (2)

The new compound **2**, obtained as an amorphous powder, exhibited $[\alpha]_D^{20}=-285$ (*c* 0.05, EtOH). High-resolution FABMS analysis gave *m*/*z* 361.2392 $(M+H)^+$(Δ ±0 mmu) and established the molecular formula as $C_{23}H_{28}N_4$. The UV, ^{1}H and ^{13}C NMR spectra strongly resembled those of known dimeric pyrrolidinoindoline-type alkaloids, chimonanthine (**10**),[3b,11] folicanthine (**11**),[6,12] and calycanthidine (**12**),[13] which were simultaneously isolated from this plant. The ^{1}H NMR spectrum showed the presence of three methyl groups attached to nitrogen atoms. Their chemical shifts, δ 2.36, 2.84, and 2.98, suggested that one methyl group

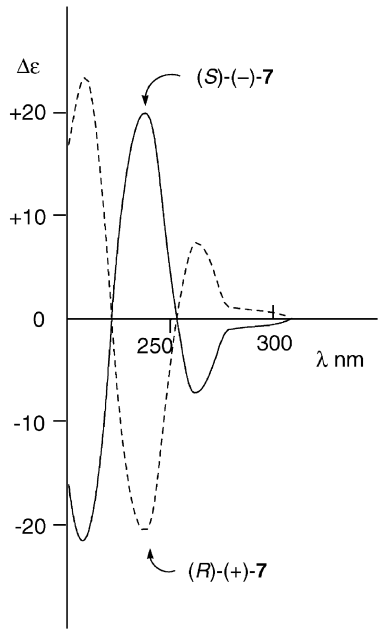


Figure 2. CD spectra of (−)- and (+)-3-hydroxyoxindoles (**7**).

existed on the aliphatic nitrogen atom and the other two were on the nitrogen of aniline function. Therefore, the structure of the new alkaloid, now named chimonanthidine, was deduced to be formula **2** i.e. *N*b-monodemethylfolicanthine (Fig. 3).

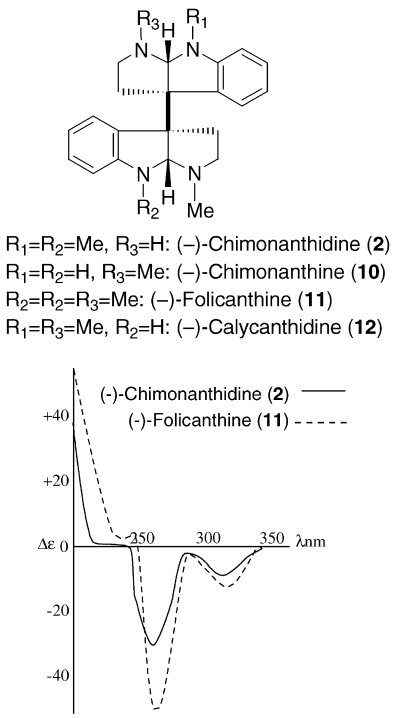


Figure 3. Structures and CD spectra of chimonanthidine (**2**) and folicanthine (**11**).

To establish the above structure inferred by spectroscopic analysis, we attempted the total synthesis of (±)-**2**. Recently, we have developed a new synthetic procedure that uses hypervalent iodine(III) reagents for the dimerization of indole derivatives,[14] and applied it to the concise total synthesis of chimonanthines.[15] We utilized this method for the synthesis of **2** as follows. *N*a-Methyl-*N*b-2-trimethylsilylethoxycarbonyl (Teoc) tryptamine (**14**), prepared from known compound **13**,[16] was treated with 0.5 equiv. phenyliodine (III) bis(trifluoroacetate) (PIFA) in CF_3CH_2OH at −40 °C to give two dimerization products (**15**) and (**16**) in 16 and 21% yields, respectively. To elucidate their relative stereochemistry, those two compounds were transformed into known compounds. On reduction with Red-Al in toluene, the less polar product (**16**) gave *rac*-folicanthine (*rac*-**12**),[6] whereas the more polar one **15** produced *meso*-folicanthine (**17**), demonstrating the relative stereochemistry of the two dimerization products. For the completion of the total synthesis of the new alkaloid, *rac*-(**16**) was employed again for further transformation. Elimination of one of two protecting groups on the nitrogen atoms was carried out by treatment of **16** with 1.0 equiv. tetrabutyl ammonium fluoride in THF at room temperature for 6 h to give the desired monodeprotected amine (**18**) in 33.4% yield together with 51.3% of the recovered starting material. Finally, the remaining carbamate group in **18** was converted into the *N*-methyl function by reduction with Red-Al to furnish target molecule **2** in 73.4% yield. Synthetic **2** was completely identical in all respects (chromatographic behavior; mass; IR; UV; ^1H and ^{13}C NMR) with natural chimonanthidine except for the optical property. The CD spectrum of natural **2** exhibited Cotton curves very similar to those of (−)-folicanthine (**11**), the absolute configuration of which was determined by chemical correlation with (−)-chimonanthine (**10**).[17] The absolute stereochemistry of (−)-chimonanthine has been recently corrected by Overman et al.[18] Therefore, the structure including the absolute configuration of (−)-chimonanthidine was determined to be formula **2** (Scheme 2).

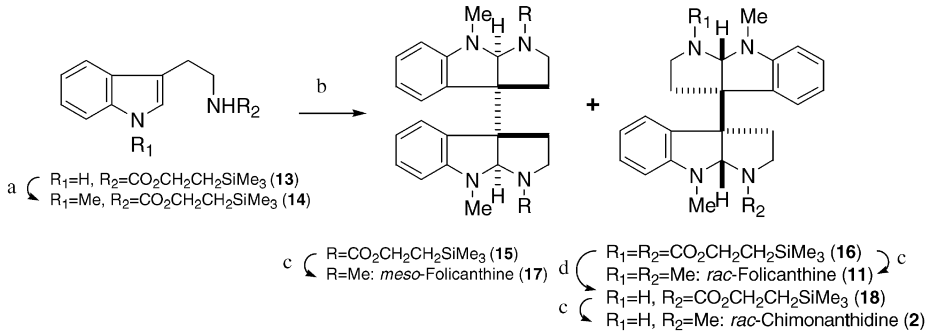
3. Experimental

3.1. General

UV: Recorded in MeOH on a JASCO V-560 instrument. IR: recorded on a JASCO FT/IR-230 spectrophotometer. ^1H and ^{13}C NMR spectra: recorded on JEOL JNM A-400, JNM A-500, JNM ECP-400, or JNM ECP-600 spectrometers, *J* values are given in Hz. EI-MS: direct probe insertion at 70 eV recorded on a JEOL JMS GC-mate spectrometer. FAB-MS: recorded on a JEOL JMS-HX110 mass spectrometer. Optical rotation: measured using a JASCO P-1020 polarimeter. CD: recorded on a JASCO J-720WI spectrometer. TLC: precoated Kieselgel 60 F_{254} plates (Merck, 0.25 mm thick). Column Chromatography: Kieselgel 60 [Merck, 70-230 (for open chromatography) and 230–400 mesh (for flash chromatography)], amino silica gel [Fuji Silysia Chemical, NH-DM1020], medium pressure liquid column chromatography: silica gel prepacked column Kusano CPS-HS-221-05.

3.2. Plant material

The seeds of *Chimonanthus praecox* Link. were collected in June at the medicinal plant garden in the Faculty of Pharmaceutical Sciences, Chiba University, and identified



Scheme 2. *Reagents and conditions*: (a) MeI, NaH, DMF, −20 °C, 94%. (b) 0.5 equiv. PIFA, CF_3CH_2OH, −40 °C; **15**, 16%; **16**, 21%; **14**, 5%. (c) Red-Al, toluene, reflux; *meso*-**17**, 96%. *rac*-**11**, 95%; *rac*-**2**, 73.4%. (d) 1.0 equiv. TBAF, THF, rt, 6 h; **18**, 33.4%; **16**, 51.3%.

by Dr Fumio Ikegami, Graduate School of Pharmaceutical Sciences, Chiba University, Japan. A voucher specimen was deposited at the Herbarium of the Graduate School of Pharmaceutical Sciences, Chiba University.

3.3. Extraction and isolation of alkaloids

The dried powdered seeds (2.5 kg) of *C. praecox* were macerated with MeOH (2.0 L) four times and filtered. The combined filtrates were concentrated under reduced pressure to give a crude extract (97.3 g), which was then dissolved in 10% aqueous acetic acid (2.0 L). The solution was washed with ethyl acetate (600 mL), alkalinized with Na_2CO_3 (pH 10), and then exhaustively extracted with $CHCl_3$. The organic layer was washed with water, dried over $MgSO_4$, and evaporated to give a crude alkaloidal fraction (15.37 g). From this fraction, crude crystalline calycanthine (11.38 g) was directly obtained, a portion (998 mg) of which was recrystallized from ethyl acetate to give pure (+)-calycanthine (632 mg). The mother liquid (3.8 g) of the first crystallization was roughly separated by silica gel flash column chromatography using a $CHCl_3$–MeOH/$CHCl_3$ gradient to give twelve fractions. The 10% MeOH/$CHCl_3$ eluate was recrystallized from ethyl acetate to give 352 mg of (−)-folicanthine (**11**). The 20% MeOH/$CHCl_3$ eluate was purified by SiO_2 medium pressure liquid chromatography (20% MeOH/ethyl acetate) to give 2.6 mg of chimonamidine (**1**) and 22 mg of (−)-calycanthidine (**12**). The 30% MeOH/$CHCl_3$ eluate of the first column chromatography was purified by reverse phase medium pressure liquid chromatography (30% H_2O/MeOH) to give 22 mg of (−)-chimonanthine (**10**) and 13 mg of chimonanthidine (**2**).

3.3.1. Chimonamidine (1). Amorphous powder; $[\alpha]_D^{19}=-12.6$ (*c* 0.06, EtOH); UV (MeOH) λ_{max} 202, 246, 300 nm; IR (neat) ν_{max} 3378, 1695 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.24 (1H, dd, *J*=7.6, 7.6 Hz, H-6), 6.85 (1H, d, *J*=7.6 Hz, H-4), 6.74 (1H, d, *J*=7.6 Hz, H-7), 6.66 (1H, dd, *J*=7.6, 7.6 Hz, H-5), 3.88 (1H, br-s, N_1–H), 3.31 (1H, ddd, *J*=9.8, 9.8, 1.2 Hz, H-9), 3.24 (1H, ddd, *J*=9.8, 9.8, 6.4 Hz, H-9), 2.97 (3H, s, N_{10}–CH_3), 2.85 (3H, s, N_1–CH_3), 2.73 (1H, ddd, *J*=12.8, 6.4, 1.2 Hz, H-8), 2.41 (1H, ddd, *J*=12.8, 9.8, 9.8 Hz, H-8); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 175.1 (C-2), 148.3 (C-7a), 129.4 (C-6), 125.4 (C-4), 124.6 (C-3a), 116.7 (C-5), 111.7 (C-7), 79.6 (C-3), 45.7 (C-9), 33.0 (C-8), 30.2 and 30.1 (*N*-Me×2); FABMS (NBA) *m/z*: 221 $[M+H]^+$; HRFABMS (NBA) *m/z*: 221.1290 (calcd for $C_{12}H_{17}N_2O_2$, 221.1290).

3.3.2. Chimonanthidine (2). Amorphous powder; $[\alpha]_D^{20}=-285$ (*c* 0.05, EtOH); UV (MeOH) λ_{max} 208, 245, 300 nm; IR (neat) ν_{max} 2938, 1603, 1495 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 2.03–2.07 (2H, m, H-3, 3′), 2.24–2.31 (1H, m, H-3′), 2.36 (3H, s, N_1–CH_3), 2.44–2.51 (3H, m, H-2, 3, 2′), 2.61–2.64 (1H, m, H-2), 2.84 (3H, s, $N_{8'}$–CH_3), 2.94–2.97 (1H, m, H-2′), 2.98 (3H, s, N_8–CH_3), 4.18 (1H, br-s, H-8a), 4.58 (1H, br-s, H-8′a), 6.24 (1H, d, *J*=8.1 Hz, H-7′), 6.34 (1H, d, *J*=7.8 Hz, H-7), 6.52 (1H, dd, *J*=7.4, 7.4 Hz, H-5′), 6.56 (1H, dd, *J*=7.6, 7.6 Hz, H-5), 7.00–7.06 (4H, m, H-4, 6, 4′, 6′); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 31.1 ($N_{8'}$–CH_3), 35.0 (C-3), 35.3 (N_8–CH_3), 38.2 (C-3′), 38.3 (N_1–CH_3), 45.6 (C-2′), 53.0 (C-2), 62.4 (C-3a, 3′a), 87.2 (C-8′a), 92.5 (C-8a), 104.7 (C-7′), 106.2 (C-7), 116.2 (C-5′), 116.9 (C-5), 124.3 (C-4′), 124.4 (C-4), 128.4 (C-6, 6′), 131.8 (C-3′b), 132.6 (C-3b), 152.5 (C-7′a), 152.8 (C-7a); FABMS (NBA) *m/z*: 361 $[M+H]^+$; HRFABMS (NBA) *m/z*: 361.2392 (calcd for $C_{23}H_{29}N_4$, 361.2392).

3.3.3. Calycanthidine (12). The ^1H and ^{13}C NMR data of the known alkaloid **12** have not been published so far. Thus, we present them here; ^1H NMR ($CDCl_3$, 500 MHz, VT 50 °C) δ 7.07 (1H, d, *J*=7.3 Hz, H-4), 6.52 (1H, dd, *J*=7.3, 7.3 Hz, H-5), 6.98 (1H, dd, *J*=7.3, 7.6 Hz, H-6), 6.27 (1H, d, *J*=7.6 Hz, H-7), 7.02 (1H, d, *J*=7.3 Hz, H-4′), 6.59 (1H, dd, *J*=7.3, 7.3 Hz, H-5′), 6.92 (1H, dd, *J*=7.3, 7.6 Hz, H-6′), 6.48 (1H, d, *J*=7.6 Hz, H-7′), 4.38 (1H, br-s, 8a-H), 4.42 (1H, br-s, 8a′-H), 2.98 (3H, s, N_8–CH_3), 2.38 (3H, s, N_1–CH_3), 2.33 (3H, s, $N_{1'}$–CH_3), 2.40–2.65 (6H, m, 2-H_2, 2′-H_2, 3-H_1, 3′-H_1), 1.95–2.05 (2H, m, 3-H_1, 3′-H_1); ^{13}C NMR ($CDCl_3$, 125 MHz, VT 50 °C) δ 52.6 (C-2), 35.7 (C-3), 62.8 (C-3a), 132.7 (C-3b), 123.6 (C-4), 116.7 (C-5), 128.1 (C-6), 105.9 (C-7), 152.8 (C-7a), 91.8 (C-8a), 37.9 (N_1–CH_3), 35.4 (N_8–CH_3), 52.6 (C-2′), 35.7 (C-3′), 63.2 (C-3a′), 133.3 (C-3b′), 124.4 (C-4′), 118.2 (C-5′), 127.9 (C-6′), 109.0 (C-7′), 150.8 (C-7a′), 85.0 (C-8a′), 37.0 (N_1–CH_3).

3.4. Synthesis of chimonamidine

3.4.1. *N*a,*N*b-Dimethyl-*N*b-carbobenzyloxytryptamine (5). Under argon atmosphere, a solution of benzyl

chloroformate (2.0 mL, 13.86 mmol) in dry dichloromethane (25 mL) and a solution of Na_2CO_3 (1.336 g) in water (40 mL) were simultaneously added dropwise to a stirred solution of **4** (2.36 g, 12.55 mmol) in dichloromethane (40 mL) at 0 °C. After being stirred at the same temperature for 1 h, the reaction mixture was transferred into a separatory funnel. The organic layer was drawn off and the aqueous layer was extracted with dichloromethane. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated to give a residue that was purified by pre-packed silica gel column chromatography (10% acetone/chloroform) to give 3.48 g (86%) of **5** as a colorless oil; IR ν_{max} ($CHCl_3$) cm^{-1}: 1692; UV λ_{max} (MeOH) nm: 209, 225.5, 289.5; ^1H NMR (DMSO-d_6, 400 MHz, VT 100 °C) δ 2.87 (3H, s), 2.93 (2H, m), 3.51 (2H, m), 3.70 (3H, s), 5.05 (2H, s), 6.98 (1H, ddd, J=7.9, 7.0, 1.0 Hz), 7.03 (1H, s), 7.12 (1H, ddd, J=7.9, 7.0, 1.0 Hz), 7.30–7.37 (6H, m), 7.49 (1H, d, J=7.9 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz, VT 100 °C) δ 22.8, 31.6, 33.7, 49.0, 65.7, 108.9, 110.5, 117.8, 120.6, 126.6, 126.8, 126.9, 127.1, 127.2, 127.8, 136.5, 136.7, 155.0; FABMS (NBA+KI) *m/z*: 361 $[M+K]^+$; HRFABMS (NBA+KI) *m/z*: 361.1309 (calcd for $C_{20}H_{22}N_2O_2K$ $[M+K]^+$, 361.1318).

3.4.2. Preparation of oxindole (6). To a stirred mixture of conc. HCl (3.20 mL), DMSO (0.81 mL, 11 mmol), and phenol (0.16 mL, 1.9 mmol) in acetic acid (40 mL) was added dropwise a solution of **5** (3.05 g, 9.49 mmol) in acetic acid (10 mL) at 0 °C. Stirring was continued for 10 min at the same temperature. The reaction mixture was poured into chilled water and then alkalinized with saturated aqueous Na_2CO_3 solution. The whole mixture was extracted three times with chloroform. The combined extract was washed with brine, dried over $MgSO_4$, and concentrated to give a residue that was purified by silica gel flash column chromatography (40% ethyl acetate in *n*-hexane) to give 2.27 g (71%) of **6** as a colorless oil; IR ν_{max} ($CHCl_3$) cm^{-1}: 1695, 1613; UV λ_{max} (MeOH) nm: 208, 254; ^1H NMR (DMSO-d_6, 400 MHz, VT 100 °C) δ 2.06 (2H, m), 2.81 (3H, s), 3.10 (3H, s), 3.29 (1H, m), 3.43 (2H, m), 5.00 (1H, d, J=12.8 Hz), 5.02 (1H, d, J=12.8 Hz), 6.93 (1H, d, J=7.5 Hz), 6.99 (1H, dd, J=7.5, 7.5 Hz), 7.23–7.36 (7H, m); ^{13}C NMR (DMSO-d_6, 100 MHz, VT 100 °C) δ 25.3, 27.6, 33.4, 42.2, 45.2, 65.7, 107.6, 121.3, 123.0, 125.9, 126.9, 127.1, 127.2, 127.4, 127.7, 128.0, 136.6, 143.7, 154.9, 176.0; FABMS (NBA) *m/z*: 339 $[M+H]^+$; HRFABMS (NBA) *m/z*: 339.1704 (calcd for $C_{20}H_{23}N_2O_3$ $[M+H]^+$, 339.1709).

3.4.3. Preparation of (±)-3-hydroxyoxindole (7). Under oxygen atmosphere, a solution of **6** (992 mg, 3.0 mL) in THF (2 mL) and 1 M aqueous NaOH (3.0 mL) was stirred for 4 h at room temperature. The reaction mixture was diluted with water and then extracted three times with chloroform. The combined extract was washed with brine, dried over $MgSO_4$, and concentrated to give a residue that was purified by silica gel column chromatography (60% ethyl acetate in *n*-hexane) to give 1.21 g (y. quant) of (±)-**7** as colorless prisms; mp 128–129 °C (AcOEt); IR ν_{max} (KBr) cm^{-1}: 3290; UV λ_{max} (MeOH) nm: 209, 258.5; ^1H NMR (DMSO-d_6, 400 MHz, VT 100 °C) δ 2.04 (2H, m), 2.73 (3H, s), 3.08 (3H, s), 3.20 (2H, m), 4.96 (1H, d, J=12.8 Hz), 4.99 (1H, d, J=12.8 Hz), 5.69 (1H, s), 6.93 (1H, dd, J=8.1, 0.9 Hz), 7.02 (1H, ddd, J=7.5, 7.5, 0.9 Hz), 7.26–7.36 (7H, m); ^{13}C NMR (DMSO-d_6, 100 MHz, VT 100 °C) δ 25.2, 33.2, 35.0, 43.0, 65.6, 73.5, 107.8, 118.6, 121.6, 122.9, 126.7, 127.0, 127.7, 128.5, 136.6, 142.7, 154.7, 176.4; FABMS (NBA) *m/z*: 355 $[M+H]^+$. Anal. calcd for $C_{20}H_{22}N_2O_4$: C, 67.78; H, 6.26; N, 7.90. Found: C, 67.48; H, 6.29; N, 7.83.

3.4.4. (±)-Chimonamidine (1). To a stirred solution of (±)-**7** (37.4 mg, 0.11 mmol) in dry CH_3CN (3 mL) was added trimethysilyl iodide (60 μL, 0.42 mmol) at 0 °C and the mixture was stirred at the same temperature for 6 h and then at room temperature for 13 h. The reaction mixture was poured into 10% aqueous HCl solution and then extracted with ether. The acidic aqueous layer was alkalinized with 10% aqueous KOH solution and then extracted three times with chloroform. The combined extract was washed with water, dried over solid K_2CO_3, and concentrated to give a residue that was allowed to stand for two days in a desiccator. The thus obtained crude product was recrystallized from ethyl acetate to give 15.4 mg (66%) of (±)-chimonamidine as colorless needles; mp 213–215 °C (AcOEt). Anal. calcd for $C_{12}H_{16}N_2O_2$: C, 65.43; H, 7.32; N, 12.72. Found: C, 65.21; H, 7.32; N, 12.55. The synthetic compound was found to be completely identical with the natural product by comparison of their chromatographic behavior and spectroscopic data (^1H and ^{13}C NMR, UV, IR and MS spectra).

3.4.5. Preparation and separation of MTPA-esters (8 and 9). A mixture of (±)-**7** (51.9 mg, 0.147 mmol), (*S*)-MTPA-chloride (41 μL, 0.22 mmol), and DMAP (36.4 mg, 0.29 mmol) in dry dichloromethane (1 mL) was stirred for 1.5 h at room temperature under argon atmosphere. Water was added to the reaction mixture, which was then extracted three times with chloroform. The combined extract was washed with brine, dried over $MgSO_4$, and concentrated to give a residue that was purified by pre-packed silica gel column chromatography (acetone/chloroform/*n*-hexane=1:9:10) to give 37.9 mg (y. 45%) of less polar **9** and 35.7 mg (y. 43%) of more polar **8**. **8**; colorless prisms, mp 130–131 °C (MeOH); $[\alpha]_D^{24}$=+9.2 (*c* 0.152, $CHCl_3$); IR ν_{max} (KBr) cm^{-1}: 1729, 1697; UV λ_{max} (MeOH) nm: 214.5, 259; ^1H NMR (DMSO-d_6, 600 MHz, VT 100 °C) δ 2.20 (2H, m), 2.69 (3H, s), 3.19 (3H, s), 3.25 (2H, m), 3.48 (3H, s), 4.98 (1H, d, J=12.6 Hz), 4.99 (1H, d, J=12.6 Hz), 7.07–7.12 (2H, m), 7.26–7.49 (12H, m); ^{13}C NMR (DMSO-d_6, 150 MHz, VT 100 °C) δ 26.9, 34.3, 43.1, 55.8, 66.8, 81.3, 84.8, 109.7, 123.3, 123.5, 124.6, 126.0, 127.85, 127.92, 128.2, 128.8, 129.0, 130.4, 131.1, 131.9, 137.5, 144.4, 155.7, 164.3, 172.8; FABMS (NBA) *m/z*: 571 $[M+H]^+$; HRFABMS (NBA) *m/z*: 571.2103 (calcd for $C_{30}H_{30}N_2O_6F_3$ $[M+H]^+$, 571.2056); CD (*c* 0.195 mmol/L, MeOH, 23 °C) λ nm ($\Delta\varepsilon$): 206 (+12.6), 218 (0), 231 (−15.1), 249 (0), 258 (+2.9), 274 (0). **9**; colorless oil, $[\alpha]_D^{24}$=−5.2 (*c* 0.39, $CHCl_3$); IR ν_{max} (KBr) cm^{-1}: 1728, 1696; UV λ_{max} (MeOH) nm: 214.5, 259; ^1H NMR (DMSO-d_6, 600 MHz, VT 100 °C) δ 2.20 (2H, m), 2.71 (3H, s), 3.20 (3H, s), 3.28 (2H, m), 3.47 (3H, s), 4.97 (1H, d, J=12.6 Hz), 4.98 (1H, d, J=12.6 Hz), 7.07–7.13 (3H, m), 7.25–7.34 (5H, m), 7.41–7.52 (6H, m); ^{13}C NMR (DMSO-d_6, 150 MHz, VT 100 °C) δ 26.9, 34.4, 43.1, 55.7, 66.8, 81.3, 84.9, 109.7, 123.2,

123.3, 124.6, 125.9, 127.7, 127.9, 128.2, 128.8, 129.0, 130.5, 131.1, 132.0, 137.5, 144.5, 155.7, 164.2, 172.9; FABMS (NBA) *m/z*: 571 $[M+H]^+$; HRFABMS (NBA) *m/z*: 571.2048 (calcd for $C_{30}H_{30}N_2O_6F_3$ $[M+H]^+$; 571.2056); CD (*c* 0.195 mmol/L, MeOH, 23 °C) λ nm (Δε): 206 (−21.7), 218 (0), 231 (+24.0), 250 (0), 259 (-5.7), 274 (0).

3.4.6. X-ray crystallographic analysis of 8. All measurements were conducted on a Quantum CCD area detector coupled with a CCD diffractometer with graphite monochromated Mo Kα radiation (λ=0.71069 Å). Crystal data: orthorhombic, $C_{30}H_{29}N_2O_6F_3$ (M_w: 570.56), space group $P2_12_12$ with *a*=9.318(2) Å, *b*=10.628(2) Å, *c*=27.933(5) Å, *V*=2766.1(9) Å^3, *Z*=4, and D_{calc}=1.370 g/cm^3. The structure was solved by direct methods (SIR97) and expanded using Fourier techniques (DIRDIF94). The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement was based on 6504 reflections ($I>0.00\sigma(I)$, $2\theta<57.35°$) and 371 variable parameters and converged with unweighted and weighted agreement factors of R=0.091 and R_w=0.111.

3.4.7. Alkaline hydrolysis of 8. Under argon atmosphere, a mixture of **8** (15.8 mg, 0.028 mmol) in 0.5 M aqueous NaOH solution (0.5 mL) and MeOH (0.5 mL) was stirred at room temperature for 20 h and then heated under reflux for 1.5 h. The reaction mixture was diluted with water and extracted three times with chloroform. The combined extract was washed with brine, dried over $MgSO_4$, and concentrated to give a residue that was purified by silica gel short column chromatography (AcOEt) to give 10.6 mg (y. quant) of (+)-**7** as a colorless amorphous powder; $[\alpha]_D^{25}$=+21.3 (*c* 0.67, $CHCl_3$); CD (*c* 0.458 mmol/L, MeOH, 23 °C) λ nm (Δε): 208 (+23.6), 222 (0), 238 (−20.7), 253 (0), 263 (+7.4), 308 (0). This compound was found to be identical with racemate described above by comparison of their chromatographic behavior and spectroscopic data (^{1}H and ^{13}C NMR, IR, UV, and MS spectra).

3.4.8. Alkaline hydrolysis of 9. Under argon atmosphere, a mixture of **9** (6.9 mg, 0.012 mmol) in 0.5 M aqueous NaOH solution (0.5 mL) and MeOH (0.5 mL) was stirred at room temperature for 90 h. The reaction mixture was diluted with water and extracted three times with chloroform. The combined extract was washed with brine, dried over $MgSO_4$, and concentrated to give a residue that was purified by silica gel short column chromatography (AcOEt) to give 4.7 mg (y. quant) of (−)-**7** as a colorless amorphous powder; $[\alpha]_D^{25}$=−21.3 (*c* 0.58, $CHCl_3$); CD (*c* 0.432 mmol/L, MeOH, 23 °C) λ nm (Δε): 208 (−21.8), 222 (0), 238 (+19.6), 253 (0), 263 (−7.1), 308 (0). This compound was found to be identical with racemate described above by comparison of their chromatographic behavior and spectroscopic data (^{1}H and ^{13}C NMR, IR, UV, and MS spectra).

3.4.9. Preparation of (*R*)-(−)-Chimonamidine. (+)-**7** (185 mg, 0.52 mmol) was treated according to the procedure described above for the synthesis of (±)-**1**. The residue obtained by work-up was allowed to stand for three days in a desiccator and then purified by pre-packed silica gel column chromatography (50% ethyl acetate in *n*-hexane) to give 64 mg (y. 55%) of (−)-chimonamidine **1** as a colorless amorphous powder. $[\alpha]_D^{23}$=−177.8 (*c* 0.17, EtOH); CD (*c* 0.582 mmol/L, MeOH, 23 °C) λ nm (Δε): 201 (−27), 215 (0), 224 (−2.9), 234 (0), 246 (+3.6), 257 (0), 297 (−3.7), 321 (0); HRFABMS (NBA) *m/z*: 221.1308 (calcd for $C_{12}H_{17}N_2O_2$, 221.1290). The synthetic compound was found to be identical with the natural product by comparison of their chromatographic behavior and spectroscopic data (^{1}H and ^{13}C NMR, IR, UV, and MS spectra).

3.4.10. Preparation of (*S*)-(+)-Chimonamidine. (−)-**7** (10 mg, 0.029 mmol) was treated according to the procedure described above. The residue obtained by work-up was allowed to stand for ten days in a desiccator and then purified by pre-packed silica gel column chromatography (50% ethyl acetate in *n*-hexane) to give 3.2 mg (y. 50%) of (+)-chimonamidine **1** as a colorless amorphous powder. $[\alpha]_D^{23}$=+170.7 (*c* 0.18, EtOH); CD (*c* 0.582 mmol/L, MeOH, 23 °C) λ nm (Δε): 202 (+24.4), 215 (0), 226 (+3.1), 237 (0), 251 (−3.2), 257 (0), 298 (+3.5), 321 (0); HRFABMS (NBA) *m/z*: 221.1296 (calcd for $C_{12}H_{17}N_2O_2$, 221.1290). The synthetic compound was found to be identical with the natural product by comparison of their chromatographic behavior and spectroscopic data (^{1}H and ^{13}C NMR, IR, UV, and MS spectra).

3.5. Synthesis of chimonanthidine

3.5.1. *N*a-Methyl-*N*b-2-trimethylsilylethoxycarbonyltryptamine (14). To a stirred solution of **13** (2.2 g, 7.24 mmol) in dry DMF (31 mL) was added sodium hydride (377 mg, 60% dispersion in mineral oil) by portions at −20 °C and the mixture was stirred at the same temperature for 10 min. Iodomethane (0.6 mL, 9.63 mmol) was added to the reaction mixture and stirring was continued for 60 min at 0 °C. The reaction mixture was poured into chilled water and then extracted three times with chloroform. The combined extract was washed with brine, dried over $MgSO_4$, and concentrated to give a residue that was purified by silica gel column chromatography (66% ethyl acetate in *n*-hexane) to give 2.16 g (94%) of **14** as a colorless oil; IR ν_{max} (neat) cm^{-1}: 3341, 2951, 1698, 1250, 740; UV λ_{max} (MeOH) nm: 205.5, 225.5, 289.5; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.03 (9H, s), 0.96 (2H, dd, *J*=8.5, 8.5 Hz), 2.95 (2H, dd, *J*=6.8, 6.8 Hz), 3.49 (2H, d-like, *J*=6.8 Hz), 3.75 (3H, s), 4.15 (2H, dd, *J*=8.5, 8.5 Hz), 4.69 (1H, br-s), 6.88 (1H, s), 7.11 (1H, ddd, *J*=7.8, 7.8, 1.1 Hz), 7.23 (1H, ddd, *J*=7.8, 7.8, 1.1 Hz), 7.30 (1H, d, *J*=7.8 Hz), 7.59 (1H, d, *J*=7.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ −1.5, 17.7, 25.7, 32.5, 41.3, 62.8, 109.2, 111.4, 118.9, 121.7, 126.8, 127.7, 130.7, 156.7; EIMS *m/z* (%): 318 (M^+, 32), 157 (100), 144 (69), 73 (26); HRFABMS (NBA) *m/z*: 318.1755 (calcd for $C_{17}H_{26}N_2O_2Si$, 318.1764).

3.5.2. Dimerization of 14. To a stirred solution of **14** (988 mg, 3.11 mmol) in trifluoroethanol (12.5 mL) was added PIFA (95% purity, 669 mg, 1.55 mmol) at −40 °C and the reaction mixture was stirred at the same temperature for 8 h under argon atmosphere. Aqueous sat. $NaHCO_3$ solution was added to the reaction mixture, which was then extracted three times with chloroform. The combined

extract was washed with brine, dried over $MgSO_4$, and concentrated to give a residue that was purified by silica gel column chromatography (5% acetone in *n*-hexane) and then by pre-packed silica gel column chromatography (7% acetone in *n*-hexane) to give 159 mg (16%) of **15**, 209 mg (21%) of **16**, and 48 mg (5%) of **14**. **15**; colorless oil, IR ν_{max} (neat) cm^{-1}: 2952, 1700, 1698, 746; UV λ_{max} (MeOH) nm: 208.0, 253.0, 309.5; ^1H NMR ($CDCl_3$, 400 MHz, VT 50 °C) δ 0.05 (18H, s), 1.02 (4H, br-s), 2.16–2.20 (4H, m), 2.71 (6H, br), 2.87–2.98 (2H, m), 3.81–3.91 (2H, br), 4.21 (4H, br), 5.17–5.28 (2H, br), 6.31 (2H, d, J=8.1 Hz), 6.52 (4H, m), 7.08 (2H, dd, J=7.6, 7.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz, VT 50 °C) δ −1.5 ($CO_2CH_2CH_2Si(CH_3)_3$), 17.9 ($CO_2CH_2CH_2Si(CH_3)_3$), 33.1 (N_8-CH_3), 34.7 (br, C-3), 45.1 (C-2), 61.7 (br, $CO_2CH_2CH_2Si(CH_3)_3$), 63.5 (C-3a), 83.4 (br, C-8a), 106.4, 117.1, 123.7, 129.1, 129.6, 152.5 (C-3b, 4, 5, 6, 7, 7a), 155.3 (br, $CO_2CH_2CH_2Si(CH_3)_3$); EIMS *m/z* (%): 634 (M^+, 25), 318 (9.5), 144 (65), 73 (100); HRFABMS (NBA) *m/z*: 634.3380 (calcd for $C_{34}H_{50}N_4O_4Si_2$, 634.3371). **16**; colorless oil, IR ν_{max} ($CHCl_3$) cm^{-1}: 2955, 1687, 766; UV λ_{max} (MeOH) nm: 208.5, 253.5, 310.0; ^1H NMR ($CDCl_3$, 400 MHz, VT 50 °C) δ 0.06 (18H, s), 0.96 (4H, dd, J=7.8, 7.8 Hz), 2.01 (2H, dd, J=5.6, 5.6 Hz), 2.32 (2H, m), 2.82 (2H, br-s), 2.94 (6H, br-s), 3.78–3.88 (2H, br), 4.14 (4H, m), 5.14-5.28 (2H, br), 6.31 (2H, d, J=7.6 Hz), 6.59 (2H, dd-like, J=7.1, 7.1 Hz), 7.06 (4H, m); ^{13}C NMR ($CDCl_3$, 100 MHz, VT 50 °C) δ −1.5 ($CO_2CH_2CH_2Si(CH_3)_3$), 17.8 ($CO_2CH_2CH_2Si(CH_3)_3$), 32.1 (br, C-3), 33.6 (N_8-CH_3), 45.0 (C-2), 61.0, 62.0 (br, $CO_2CH_2CH_2Si(CH_3)_3$), 63.3 (C-3a), 83.8 (br, C-8a), 105.7, 116.8, 124.0, 129.0, 129.1, 151.9 (C-3b, 4, 5, 6, 7, 7a), 155.0 (br, $CO_2CH_2CH_2Si(CH_3)_3$); EIMS *m/z* (%): 634 (M^+, 11), 318 (7), 144 (57), 73 (100); HRFABMS (NBA) *m/z*: 634.3315 (calcd for $C_{34}H_{50}N_4O_4Si_2$, 634.3371).

3.5.3. Red-Al reduction of 15. To a solution of **15** (17 mg, 0.027 mmol) in dry toluene (3 mL) was added a solution of Red-Al (65% solution of sodium bis(2-methoxyethoxy)-aluminum hydride in toluene, 0.08 mL) at room temperature under argon atmosphere. The reaction mixture was refluxed at 130 °C for 1.5 h. After cooling, 5% aqueous NaOH solution was added and the mixture was filtered using Celite. The filtrate was extracted three times with chloroform and the combined extract was washed with brine, dried over $MgSO_4$, and concentrated to give a residue that was purified by amino silica gel column chromatography (50% ethyl acetate in *n*-hexane) to give 9.7 mg (96%) of *meso*-folicanthine **17** as pale yellow crystals: mp 176–178 °C (*n*-hexane/AcOEt); IR ν_{max} ($CHCl_3$) cm^{-1}: 2933, 1602, 1491, 669; UV λ_{max} (MeOH) nm: 207.5, 253.5, 308.0; ^1H NMR (Pyridine-d_5, 600 MHz, VT 90 °C) δ 2.01 (2H, dd, J=4.4, 4.4 Hz), 2.43 (10H, m), 2.53 (2H, m), 2.76 (2H, dd, J=8.5, 8.5 Hz), 4.37 (2H, br-s), 6.45 (2H, d, J=7.7, 7.7 Hz), 6.60 (2H, br-s), 7.12 (2H, dd, J=7.7, 7.7 Hz), 7.16 (2H, s); ^{13}C NMR (Pyridine-d_5, 150 MHz, VT 90 °C) δ 36.0 (N_8-CH_3)*, 36.3 (C-3), 36.6 (N_1-CH_3)*, 52.5 (C-2), 63.6 (C-3a), 91.9 (C-8a), 107.3, 117.4, 124.2, 128.4, 133.8, 155.0 (C-3b, 4, 5, 6, 7, 7a) (*interchangeable); EIMS *m/z*: (%): 374 (M^+, 42), 188 (38), 187 (100), 186 (88), 144 (87); HRFABMS (NBA) *m/z*: 375.2560 (calcd for $C_{24}H_{31}N_4$, 375.2549).

3.5.4. Red-Al reduction of 16. To a solution of **16** (16 mg, 0.026 mmol) in dry toluene (3 mL) was added a solution of Red-Al (65% solution of sodium bis(2-methoxyethoxy)-aluminum hydride in toluene, 0.08 mL) at room temperature under argon atmosphere. The reaction mixture was refluxed at 130 °C for 2 h. After cooling, 5% aqueous NaOH solution was added and the mixture was filtered using Celite. The filtrate was extracted three times with chloroform and the combined extract was washed with brine, dried over $MgSO_4$, and concentrated to give a residue that was purified by amino silica gel column chromatography (50% ethyl acetate in *n*-hexane) to give 9.2 mg (95%) of *rac*-folicanthine **11** as pale yellow crystals: mp 172–174 °C (*n*-hexane/AcOEt). The ^1H and ^{13}C NMR and MS spectra were identical with those reported in the literature.[6]

3.5.5. Partial deprotection of carbamates in 16. To a solution of **16** (42 mg, 0.066 mmol) in dry THF (2.5 mL) was added 1.0 M solution of tetrabutylammonium fluoride in THF (66 μL, 0.066 mmol) at 0 °C and the mixture was stirred at room temperature for 6 h. The reaction mixture was poured into chilled water and then extracted three times with chloroform. The combined extract was washed with brine, dried over $MgSO_4$, and concentrated to give a residue that was purified by silica gel column chromatography (3% methanol in chloroform) to give 11 mg (33.4%) of **18** as a colorless oil and 21.5 mg (51.3%) of starting material **16**. **18**. IR ν_{max} (neat) cm^{-1}: 2951, 1697, 1492, 744; UV λ_{max} (MeOH) nm: 212.0, 254.0, 310.5; ^1H NMR (Pyridine-d_5, 400 MHz, VT 90 °C) δ 0.08 (9H, s), 1.06 (2H, dd, J=8.2, 8.2 Hz), 2.12 (1H, dd, J=11.7, 5.2 Hz), 2.18 (1H, dd, J=12.3, 5.7 Hz), 2.42 (1H, ddd, J=11.2, 11.2, 7.5 Hz), 2.58 (1H, ddd, J=10.8, 10.8, 5.5 Hz), 2.67 (1H, ddd, J=11.9, 11.9, 8.1 Hz), 2.86 (3H, s), 2.96–3.03 (2H, m), 3.12 (3H, s), 4.33 (2H, dd, J=8.2, 8.2 Hz), 4.81 (1H, s), 5.67 (1H, br-s), 6.40 (1H, d, J=7.5 Hz), 6.49 (1H, d, J=7.5 Hz), 6.68 (1H, dd, J=7.5, 7.5 Hz), 6.77 (1H, dd, J=7.4, 7.4 Hz), 7.14 (1H, dd, J=7.5, 7.5 Hz), 7.18–7.21 (1H, m), 7.32 (1H, d, J=7.5 Hz), 7.36 (1H, d, J=7.4 Hz); ^{13}C NMR (Pyridine-d_5, 100 MHz, VT 90 °C) δ −1.5, 18.2, 30.9, 32.5, 35.0, 38.3, 45.4, 46.0, 62.8, 63.4, 79.3, 85.0, 87.8, 105.5, 106.2, 116.6, 117.4, 124.5, 124.6, 129.0, 129.3, 131.1, 131.8, 152.8, 153.3, 155.4; EIMS *m/z* (%): 490 (M^+, 26), 316 (100), 272 (46), 244 (49), 173 (55), 144 (91); HRFABMS (NBA) *m/z*: 491.2807 (calcd for $C_{28}H_{39}N_4O_2Si$, 491.2842).

3.5.6. Red-Al reduction of 18. To a solution of **18** (36 mg, 0.073 mmol) in dry toluene (5 mL) was added a solution of Red-Al (65% solution of sodium bis(2-methoxyethoxy)-aluminum hydride in toluene, 0.13 mL) at room temperature under argon atmosphere. The reaction mixture was refluxed at 130 °C for 2 h. After cooling, 5% aqueous NaOH solution was added and the mixture was filtered using Celite. The filtrate was extracted three times with chloroform and the combined extract was washed with brine, dried over $MgSO_4$, and concentrated to give a residue that was purified by amino silica gel column chromatography (75% ethyl acetate in *n*-hexane) to give 19.3 mg (73.4%) of *rac*-chimonanthidine **2** as a colorless amorphous powder. The synthetic compound was found to be completely identical with the natural product by comparison of their chromatographic behavior and spectroscopic data (^1H and ^{13}C NMR, UV, IR and MS spectra).

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Synthesis of β-tosylethylhydrazine and its use in preparation of N-protected pyrazoles and 5-aminopyrazoles

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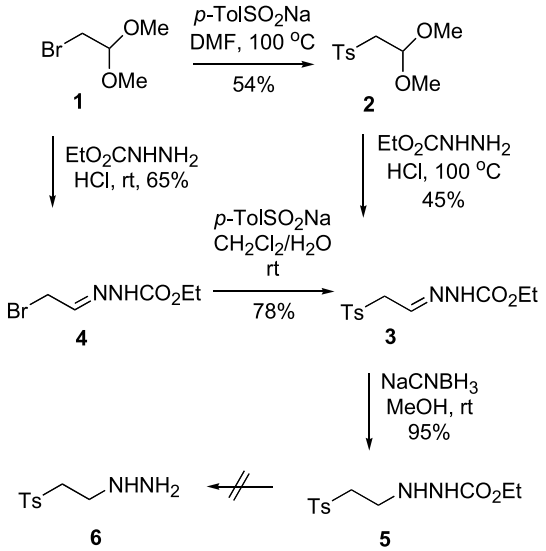
Abstract—β-Tosylethylhydrazine (**6**) can be prepared efficiently in one step from commercially available *p*-tolyl vinyl sulfone (**7**) and hydrazine hydrate. This hydrazine reacts with both 1,3-diketones and conjugated ynones in glacial acetic acid to provide a variety of N-tosylethyl-protected (TSE) pyrazoles in good yields. The TSE group can be removed from the pyrazoles using potassium *t*-butoxide in THF at −30 °C–rt. In addition, hydrazine **6** condenses with β-ketonitriles and β-aminoacrylonitriles to afford 5-aminopyrazoles, which can be deprotected by brief treatment with NaOEt in EtOH/DMSO at 45 °C.

1. Introduction

A few years ago we demonstrated that the β-tosylethyl group (TSE) is useful in N-protection of various types of amides, carbamates and lactams (including β-lactams), and can be removed via a β-elimination upon treatment with potassium *t*-butoxide in THF under mild conditions.[1] This protecting group can be easily introduced using the readily prepared reagent β-tosylethylamine.[1a,b] We subsequently described the synthesis of β-tosylethylhydroxylamine and its use in synthesis of TSE-protected γ-lactams via our amidyl radical cyclization methodology.[1c] In addition, a few scattered examples have appeared on the use of the TSE and β-phenylsulfonylethyl groups for N-protection of heteroaromatics including tetrazoles,[2a] pyrroles,[2b] imidazoles,[2c] and indoles.[3d] In these cases, the protecting groups were usually installed onto a preformed heterocycle. In the latter example, the N-protection could also be effected prior to indole ring construction.[2d] Once again, TSE removal is effected in these cases with various bases. In this article we describe a new extension of our work which involves the synthesis of β-tosylethylhydrazine (**6**)[3] and its application to synthesis of TSE-protected pyrazoles and 5-aminopyrazoles.[4]

2. Results and discussion

The initial approach to this hydrazine was patterned after our synthesis of β-tosylethylhydroxylamine.[2c] Thus, commercially available bromo acetal **1** reacts with sodium *p*-toluenesulfinate to afford sulfone acetal **2** in 54% yield (Scheme 1).[5] Treatment of acetal **2** with ethyl carbazate under aqueous acidic conditions then leads directly to acylhydrazone **3** (45%). A more efficient variation of this route was also developed which involved initial conversion of bromo acetal **1** to acylhydrazone **4** (65%), which reacts with sodium *p*-toluenesulfinate to produce sulfone intermediate **3** in 78% yield. It was then possible to reduce hydrazone **3** with sodium cyanoborohydride to *N*-carbamoylhydrazine **5** in high yield. Unfortunately, despite some



Scheme 1.

Keywords: Protecting groups; Nitrogen heterocycles.
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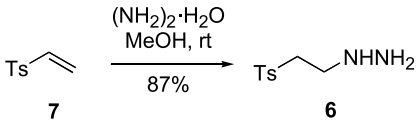
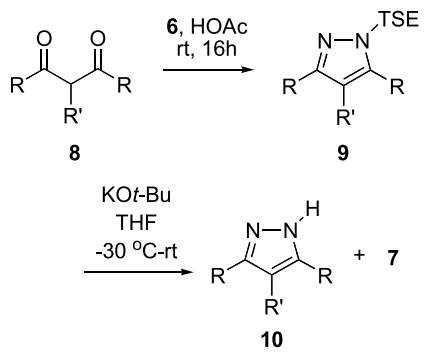
effort the acyl group of **5** could not be removed hydrolytically to afford the desired TSE–hydrazine (**6**).[6]

We therefore turned to an alternative route for preparation of the requisite hydrazine **6** which proved to be simpler and considerably more efficient than the one attempted above. Thus, by analogy with the work of Hill and Vederas,[7] commercially available tosyl vinyl sulfone (**7**)[8] was found to react smoothly with hydrazine hydrate in methanol at room temperature for about 15 min to afford **6** in a single step (Eq. 1).



(1)

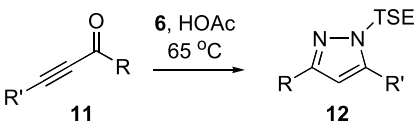
Two equivalents of hydrazine hydrate are used here in order to minimize dialkylation. Hydrazine **6** is rather difficult to purify but the crude material is sufficiently pure for use directly in the condensation reactions.



Scheme 2.

Two well known types of condensations were investigated for construction of the N-TSE-protected pyrazoles.[4] In the first series, TSE-hydrazine (**6**) was found to combine with 1,3-diketones **8** in glacial acetic acid at room temperature to generate the pyrazoles **9** in good yields (Scheme 2). Since this condensation is known to generally produce regioisomeric mixtures of pyrazoles with most unsymmetrical 1,3-diketones,[4a] the reaction was tested with a series of symmetrical substrates as outlined in Table 1.

These pyrazoles can be deprotected by treatment with potassium *t*-butoxide in THF starting at −30 °C and allowing the reaction mixture slowly warm to room temperature. In general, the yields of deprotected pyrazoles **10** were good, as is shown in Table 1. The vinyl sulfone **7** by-product appears to polymerize under these reaction conditions.



(2)

In addition, conjugated ynones are also known to condense with hydrazines, often producing pyrazoles regioselectively depending upon the substrate and reaction conditions.[9] It was found that TSE-hydrazine (**6**) reacts with ynones **11** in glacial HOAc, but the condensations require higher temperatures (65 °C) than the 1,3-diketones to produce the pyrazoles **12** (Eq. 2). This pyrazole synthesis has been explored with a few different ynone systems and the results are listed in Table 2. In the case of ynone **11a**, it was found that a single regioisomeric pyrazole **12a** was formed whose structure was proven by X-ray crystallography.[10] The

Table 1. Preparation of TSE-protected pyrazoles from TSE–$NHNH_2$/1,3-diketones, and deprotection with KO–*t*-Bu/THF

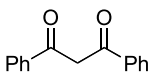
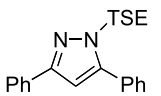
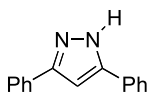
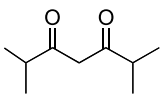
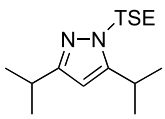
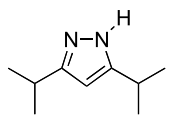
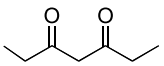
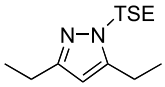
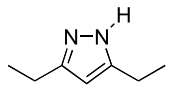
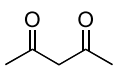
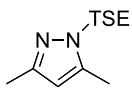
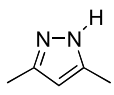
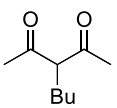
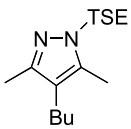
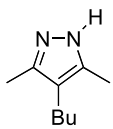
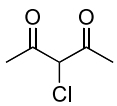
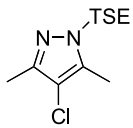
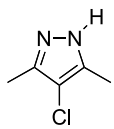
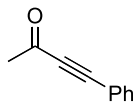
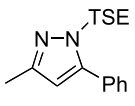
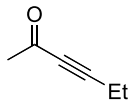
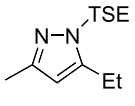
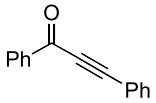
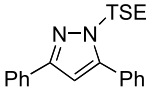
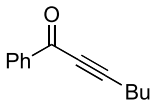
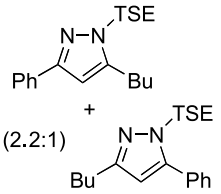
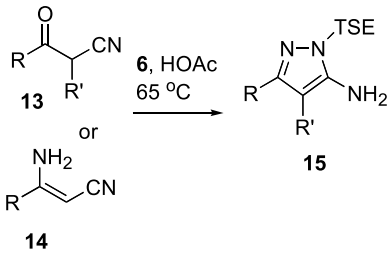
Entry	1,3-Diketone **8**	TSE-protected pyrazole **9**	Isolated yield (%)	Deprotected pyrazole **10**	Isolated yield (%)
a			81		89
b			77		80
c			86		71
d			89		68
e			92		75
f			69		81

Table 2. Preparation of TSE-protected pyrazoles from TSE–$NHNH_2$ and ynones

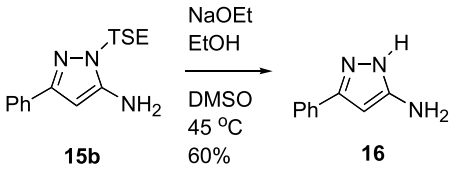
Entry	Ynone **11**	TSE-protected pyrazole **12**	Isolated yield (%)
a			92
b			59
c			62
d			84



Scheme 3.

regiochemistry of the protected pyrazole **12b** was established to be as shown by ^{1}H NMR NOE experiments. With the ynone in entry d, however, an inseparable 2.2:1 mixture of regioisomeric pyrazoles was formed.

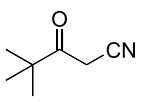
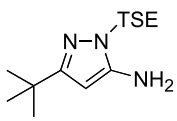
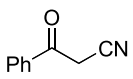
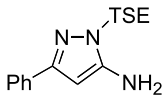
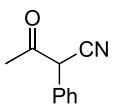
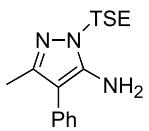
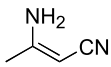
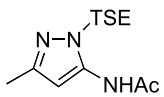
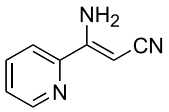
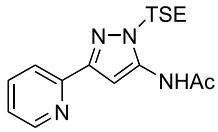
In addition, β-ketonitriles **13** react with hydrazine **6** to produce 5-aminopyrazoles **15** (Scheme 3). Alternatively, β-aminoacrylonitriles **14** can also be used in this reaction. Several substrates were screened in this reaction and the results are listed in Table 3. For the compounds in entries d and e, it was best to N-acetylate the crude, highly polar products with acetyl chloride before chromatographic purification. The deprotection of these products is sluggish, perhaps due to additional acidic protons within the molecules, and does not proceed using the conditions described above for the pyrazoles. However, if the deprotection of TSE-substituted aminopyrazole **15b** is conducted with two equivalents of 1 M NaOEt in ethanol using DMSO as solvent at 45 °C for 30 min, the parent 5-aminopyrazole **16** is produced (Eq. 3).



(3)

In conclusion, a convenient one-step synthesis of β-tosylethylhydrazine (**6**) has been developed from *p*-tolyl vinyl sulfone (**7**). This hydrazine condenses with both β-diketones and alkynyl ketones to directly afford TSE-protected pyrazoles, often with good regioslectivity in the latter case. Similarly, TSE-protected 5-aminopyrazoles can be prepared regioselectively by condensation of **6** with either β-ketonitriles or β-aminoacrylonitriles. The N-TSE protecting

Table 3. Preparation of TSE-protected 5-aminopyrazoles

Entry	β-Ketonitrile **13** or β-aminoacrylate **14**	TSE-protected 5-aminopyrazole **15**	Isolated yield (%)
a			58
b			50
c			70
d			88[a]
e			78[a]

[a] For ease of isolation the condensation product was converted to the *N*-acetyl derivative before purification.

group on the heterocycles produced in these reactions can be removed by exposure to base under mild conditions.

3. Experimental

3.1. Data for compounds

3.1.1. 1-(2,2-Dimethoxyethyl)-(*p*-tolyl)sufone (2). To a solution of sodium *p*-toluenesulfinate (2.00 g, 11.2 mmol) in DMF (32 mL) was added bromoacetaldehyde dimethyl acetal (**1**, 1.06 mL, 9.35 mmol). The resulting solution was stirred overnight at 100 °C, cooled to rt, and diluted with ether (30 mL). The ether layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo to afford the known tosyl acetal **2**[6] as pale yellow solid (1.33 g, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (dd, J=6.6, 1.7 Hz, 2H), 7.34 (d, J=7.9 Hz, 2H), 4.85 (t, J=5.3 Hz, 1H), 3.40 (d, J=5.3 Hz, 2H), 3.23 (s, 6H), 2.44 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.9, 137.1, 129.8, 128.4, 99.3, 59.0, 53.6, 21.8.

3.1.2. *N*′-(2-Bromoethylidene)-hydrazinecarboxylic acid ethyl ester (4). To a solution of ethyl carbazate (9.01 g, 86.6 mmol) in aqueous HCl (1 M, 300 mL) was added bromoacetaldehyde dimethyl acetal (**1**, 5.00 mL, 43.3 mmol). The solution was stirred overnight, and was extracted with CH_2Cl_2. The extract was dried with $MgSO_4$, and concentrated in vacuo to afford the bromide **4** as a white solid (5.87 g, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) (mixture of geometric isomers) δ 8.05 (br s, 1H), 7.24 (br s, 1H), 4.28 (m, 2H), 4.21 (d, J=5.8 Hz, 1.2H), 4.08 (d, J=6.2 Hz, 0.8H), 1.30 (m, 3H); HRMS ($C_5H_9BrN_2O_2$) calcd 208.9926 (MH^+), found 208.9914.

3.1.3. *N*′-[2-(*p*-Tosyl)-ethylidene]-hydrazinecarboxylic acid ethyl ester (3). Sodium *p*-toluenesulfinate (7.45 g, 41.8 mmol) was added in one portion to the bromide **4** (5.83 g, 27.9 mmol) in CH_2Cl_2–H_2O (1:1, 100 mL). The mixture was stirred rapidly at rt overnight, diluted with H_2O, and extracted with CH_2Cl_2. The extract was dried ($MgSO_4$) and concentrated in vacuo to give the hydrazone **3** as a light yellow solid (6.18 g, 78%).

^{1}H NMR (300 MHz, $CDCl_3$) (mixture of geometric isomers) δ 8.35 (br s, 1H), 7.74 (m, 2H), 7.33 (m, 3H), 4.24 (m, 2H), 4.09 (m, 2H), 2.44 (m, 3H), 1.27 (m, 3H).

Alternatively, a solution of sulfone **2** (2.40 g, 10.1 mmol) and ethyl carbazate (3.15 g, 104.1 mmol) in aqueous HCl (1 M, 60 mL) was heated for 6 h at 100 °C. After the solution was cooled the precipitated hydrazone was removed by filtration as a light yellow solid (1.30 g, 45%).

3.1.4. *N*′-[2-(*p*-Tosyl)-ethyl]-hydrazinecarboxylic acid ethyl ester (5). To a solution of hydrazone **3** (1.29 g, 4.54 mmol) and a trace of methyl orange in methanol (17 mL) at 0 °C was added sodium cyanoborohydride (570 mg, 9.08 mmol). The pH was adjusted periodically to the red–yellow transition point (pH 3.2–4.4) by addition of 25% HCl in methanol (prepared from acetyl chloride/MeOH). The reaction was followed by TLC (ethyl acetate/hexanes, 7/3), until the hydrazone was consumed (~1 h). The solution was basified with 1 M NaOH and extracted with Et_2O. The combined organic fractions were dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash silica gel chromatography (ethyl acetate/hexanes, 7/3) to give the hydrazine ethyl ester **5** as a white solid, mp 85–89 °C (1.23 g, 95%). ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.82 (d, J=8.3 Hz, 2H), 7.37 (d, J=8.0 Hz, 2H), 6.20 (br s, 1H), 4.14 (q, J=7.1 Hz, 2H), 3.48 (s, 1H), 3.27 (m, 4H), 2.45 (s, 3H), 1.27 (t, J=7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.7, 145.0, 136.2, 130.0, 128.1, 61.6, 54.7, 45.5, 21.7, 14.6; HRMS ($C_{12}H_{18}N_2O_4S$) calcd 287.1066 (MH^+), found 287.1065.

3.1.5. [2-(*p*-Tosyl)-ethyl]-hydrazine (6). To a rapidly stirred solution of hydrazine monohydrate (107 μL, 2.20 mmol) in methanol (4 mL) was added dropwise a solution of *p*-tolyl vinyl sulfone (**7**) (200 mg, 1.10 mmol) in methanol (4 mL). After stirring for 15 min at rt, the solution was diluted with H_2O and extracted with CH_2Cl_2. The combined organic fractions were dried ($MgSO_4$) and concentrated in vacuo. The resulting thick colorless oil was used immediately without further purification (204 mg, 87%). ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.76 (d, J=8.3 Hz, 2H), 7.34 (d, J=8.3 Hz, 2H), 3.36 (s, 3H), 3.30 (m, 2H), 3.11 (m, 2H), 2.41 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 143.3, 136.6, 130.3, 128.5, 54.4, 47.9, 22.0; HRMS ($C_9H_{14}N_2O_2S$) calcd 215.0842 (MH^+), found 215.0848.

3.2. General procedure for the formation of TSE-protected pyrazoles 9 from 1,3-diketones 8

A solution of TSE–$NHNH_2$ (**6**, 154 mg, 0.719 mmol) and 1,3-diketone **8** (0.497 mmol) in glacial acetic acid (4 mL) was stirred at rt for 16 h. The solution was concentrated in vacuo and the residue was purified by flash silica gel chromatography (ethyl acetate/hexanes, 3/7) to give the corresponding TSE-protected pyrazole **9** (Table 1).

3.2.1. 3,5-Diphenyl-1-[2-(*p*-tosyl)-ethyl]-1*H*-pyrazole (9a/12c). Gummy yellow foam (82% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69–7.17 (m, 14H), 6.48 (s, 1H), 4.51 (m, 2H), 3.78 (m, 2H), 2.27 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.3, 145.4, 145.0, 136.0, 133.0, 130.0, 129.1, 128.9, 128.7, 128.4, 128.0, 127.9, 125.6, 103.8, 55.4, 43.5, 21.6; HRMS ($C_{24}H_{22}N_2O_2S$) calcd 403.1480 (MH^+), found 403.1468.

3.2.2. 3,5-Diphenyl-1-[2-(*p*-tosyl)-ethyl]-1*H*-pyrazole (9b). White solid (77% yield); mp 76–79 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71–7.6 (m, 2H), 7.31–7.28 (m, 2H), 5.72 (s, 1H), 4.38–4.34 (m, 2H), 3.72–3.67 (m, 2H), 2.98–2.87 (m, 1H), 2.81–2.70 (m, 1H), 2.41 (s, 3H), 1.22–1.20 (m, 6H), 1.13–1.11 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.3, 150.4, 145.3, 136.6, 130.3, 128.5, 99.0, 56.2, 42.2, 28.3, 25.6, 23.1, 22.1; HRMS ($C_{18}H_{26}N_2O_2S$) calcd 335.1788 (MH^+), found 335.1770.

3.2.3. 3,5-Diethyl-1-[2-(*p*-tosyl)-ethyl]-1*H*-pyrazole (9c). Yellow oil (86% yield). ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.69 (m, 2H), 7.29 (m, 2H), 5.72 (s, 1H), 4.33 (m, 2H), 3.65 (m, 2H), 2.56 (m, 2H), 2.44 (m, 5H), 1.22 (t, J=7.5 Hz, 3H), 1.11 (t, J=7.6 Hz, 3H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 154.6, 145.6, 145.0, 136.4, 130.0, 127.9, 102.0, 55.8, 42.0,

21.8, 21.5, 18.8, 13.9, 12.9; HRMS ($C_{16}H_{22}N_2O_2S$) calcd 307.1475 (MH^+), found 307.1487.

3.2.4. 3,5-Dimethyl-1-[2-(*p*-tosyl)-ethyl]-1*H*-pyrazole (9d). White solid (89% yield). Mp 65–67 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 (d, J=8.2 Hz, 2H), 7.30 (d, J=8.4 Hz, 2H), 5.68 (s, 1H), 4.33 (m, 2H), 3.65 (m, 2H), 2.42 (s, 3H), 2.23 (s, 3H), 2.05 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.3, 144.9, 139.4, 136.2, 129.9, 127.8, 105.4, 55.6, 41.9, 21.7, 13.4, 10.9; HRMS ($C_{14}H_{18}N_2O_2S$) calcd 279.1167 (MH^+), found 279.1141.

3.2.5. 4-Butyl-3,5-dimethyl-1-[2-(*p*-tosyl)-ethyl]-1*H*-pyrazole (9e). Pale yellow solid (92% yield); mp 81–84 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67 (d, J=8.3 Hz, 2H), 7.28 (d, J=8.3 Hz, 2H), 4.33 (m, 2H), 3.65 (m, 2H), 2.42 (s, 3H), 2.22 (t, J=7.1 Hz, 2H), 2.14 (s, 3H), 2.00 (s, 3H), 1.33–1.26 (m, 4H), 0.90 (t, J=6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 146.9, 144.8, 136.2, 136.1, 129.8, 127.8, 117.0, 55.6, 42.1, 33.1, 23.2, 22.5, 21.7, 14.1, 11.8, 9.5; HRMS ($C_{18}H_{26}N_2O_2S$) calcd 335.1793 (MH^+), found 335.1770.

3.2.6. 4-Chloro-3,5-dimethyl-1-[2-(*p*-tosyl)-ethyl]-1*H*-pyrazole (9f). White solid (69% yield); mp 80–82 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63–7.61 (d, J=8.1 Hz, 2H), 7.29–7.27 (d, J=8.1 Hz, 2H), 4.35–4.31 (m, 2H), 3.71–3.67 (m, 2H), 2.43 (s, 3H), 2.12 (s, 3H), 1.98 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 145.2, 144.9, 135.9, 129.7, 127.6, 107.9, 55.1, 43.0, 21.7, 11.2, 9.3; HRMS ($C_{14}H_{17}N_2O_2SCl$) calcd 313.0757 (MH^+), found 313.0772.

3.3. General procedure for deprotection of TSE-protected pyrazoles

To a solution of a TSE protected pyrazole **9** (0.107 mmol) in THF (5 mL) at −30 °C was added *t*-BuOK (428 μL, 1 M in THF). The solution was warmed slowly to rt, and stirred for an additional 1 h. The mixture was diluted with H_2O and extracted with EtOAc. The combined organic fractions were dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash silica gel chromatography (ethyl acetate/hexanes, 2/3) to give the deprotected pyrazole **10** (Table 1).

3.4. General procedure for the formation of TSE-protected pyrazoles 12 from ynones 11

A solution of TSE–$NHNH_2$ (**6**, 154 mg, 0.719 mmol) and ynone **11** (0.497 mmol) in glacial acetic acid (4 mL) was stirred at 65 °C for 14 h. The solution was concentrated in vacuo and the residue was purified by flash silica gel chromatography (ethyl acetate/hexanes, 3/7) to give the corresponding TSE-protected pyrazole **12** (Table 2).

3.4.1. 5-Phenyl-3-methyl-1-[2-(*p*-tosyl)-ethyl]-1*H*-pyrazole (12a). White solid, mp 89–92 °C (92% yield). A sample for X-ray analysis was crystallized from CH_2Cl_2/hexanes. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57–7.18 (m, 9H), 5.92 (s, 1H), 4.33 (m, 2H), 3.58 (m, 2H), 2.35 (s, 3H), 2.10 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.8, 145.0, 144.9, 136.0, 130.2, 130.0, 129.1, 128.9, 128.8, 128.1, 106.5, 55.6, 43.1, 21.8, 13.6; HRMS ($C_{19}H_{20}N_2O_2S$) calcd 341.1318 (MH^+), found 341.1319.

3.4.2. 5-Ethyl-3-methyl-1-[2-(*p*-tosyl)-ethyl]-1*H*-pyrazole (12b). Yellow oil (59% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (m, 2H), 7.30 (m, 2H), 5.69 (s, 1H), 4.31 (m, 2H), 3.64 (m, 2H), 2.56 (q, J=7.5 Hz, 2H), 2.42 (s, 3H), 2.06 (s, 3H), 1.21 (t, J=7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.7, 145.9, 145.2, 136.4, 130.2, 128.2, 103.9, 55.6, 42.1, 22.0, 19.0, 13.8, 13.1; HRMS ($C_{15}H_{20}N_2O_2S$) calcd 293.1318 (MH^+), found 293.1295.

3.4.3. 5-Butyl-3-phenyl-1-[2-(*p*-tosyl)-ethyl]-1*H*-pyrazole and 3-butyl-5-phenyl-1-[2-(*p*-tosyl)-ethyl]-1*H*-pyrazole (12, entry d). Yellow oil (84% yield), 2.2:1 inseparable mixture of regioisomers. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57–7.18 (m, 37H), 6.11 (s, 1H), 5.93 (s, 2.3H), 4.33 (m, 6.6H), 3.63 (m, 2.4H), 3.57 (m, 4.7H), 3.00 (m, 1.4H), 2.43 (m, 15.9H), 2.18 (s, 3.6H), 1.3 (m, 17.9H), 0.87 (m, 11H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.7, 151.0, 145.0, 144.9, 144.7, 136.1, 135.9, 133.4, 130.3, 130.0, 129.9, 129.1, 128.9, 128.8, 128.6, 128.3, 128.0, 127.7, 127.6, 125.5, 105.4, 101.8, 55.6, 50.8, 43.0, 42.4, 31.8, 30.7, 28.0, 25.2, 22.7, 22.5, 21.8, 21.6, 14.1, 14.0; HRMS ($C_{22}H_{26}N_2O_2S$) calcd 383.1788 (MH^+), found 383.1783.

3.5. General procedure for formation of TSE-protected 5-aminopyrazoles from β-ketonitriles or β-amino-acrylonitriles

A solution of TSE–$NHNH_2$ (**6**, 154 mg, 0.719 mmol) and the β-ketonitrile or β-aminoacrylonitrile (0.497 mmol) in glacial acetic acid (4 mL) was stirred at 65 °C for 14 h. The solution was concentrated in vacuo and the residue was purified by flash silica gel chromatography to give the corresponding TSE-protected 5-aminopyrazole.

3.5.1. 5-*tert*-Butyl-2-[2-(*p*-tosyl)-ethyl]-2*H*-pyrazol-3-ylamine (15a). 58%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, J=8.3 Hz, 2H), 7.25 (d, J=7.9 Hz, 2H), 5.28 (s, 1H), 4.33 (t, J=6.2 Hz, 2H), 3.77 (s, 2H), 3.68 (t, J=6.4 Hz, 2H), 2.40 (s, 3H), 1.12 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.5, 144.9, 144.6, 136.4, 129.8, 127.4, 88.4, 55.7, 40.6, 32.2, 30.4, 21.8; HRMS ($C_{16}H_{23}N_3O_2S$) calcd 322.1589 (MH^+), found 322.1564.

3.5.2. 5-Phenyl-2-[2-(*p*-tosyl)-ethyl]-2*H*-pyrazol-3-ylamine (15b). 50%. ^{1}H NMR, (400 MHz, d_6-DMSO) δ 7.75 (d, J=8.3 Hz, 2H), 7.54 (dd, J=8.1, 1.5 Hz, 2H), 7.36 (d, J=7.9 Hz, 2H), 7.28 (d, J=7.9 Hz, 2H), 7.20 (t, J=7.3 Hz, 1H), 5.64 (s, 1H), 5.25 (s, 2H), 4.18 (t, J=7.0 Hz, 2H), 3.77 (m, 2H), 2.32 (s, 3H); ^{13}C NMR (100 MHz, d_6-DMSO) δ 148.5, 144.9, 136.5, 134.4, 130.3, 128.7, 128.1, 127.5, 125.2, 86.6, 54.2, 41.2, 21.5; HRMS ($C_{18}H_{19}N_3O_2S$) calcd 342.1198 (MH^+), found 342.1257.

3.5.3. 5-Methyl-4-phenyl-2-[2-(*p*-tosyl)-ethyl]-2*H*-pyrazol-3-ylamine (15c). 70%. ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.63 (d, J=8.3 Hz, 2H), 7.39 (t, J=7.5 Hz, 2H), 7.26–7.17 (m, 5H), 4.37 (t, J=6.4 Hz, 2H), 3.96 (s, 2H), 3.72 (t, J=6.2 Hz, 2H), 2.40 (s, 3H), 2.03 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.0, 144.6, 142.5, 136.1, 133.3, 129.7, 128.8, 128.4, 127.4, 125.9, 105.3, 55.3, 40.9, 21.8, 13.0; HRMS ($C_{19}H_{21}N_3O_2S$) calcd 356.1432 (MH^+), found 356.1444.

The following compounds were isolated as acetamides to

simplify purification. After heating for 14 h, the crude reaction mixtures were cooled to rt and 4 equiv. of acetyl chloride were added to the acetic acid solution. After 1 h, the reactions were worked up as above to give the title compounds.

3.5.4. *N*-{5-Methyl-2-[2-(*p*-tosyl)-ethyl]-2*H*-pyrazol-3-yl}-acetamide (15d). 88%. ^{1}H NMR, (400 MHz, $CDCl_3$) δ 8.18 (s, 1H), 7.63 (d, J=7.9 Hz, 2H), 7.30 (d, J=7.9 Hz, 2H), 6.02 (s, 1H), 4.38 (t, J=6.4 Hz, 2H), 3.62 (t, J=6.2 Hz, 2H), 2.43 (s, 3H), 2.19 (s, 3H), 2.10 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.7, 148.7, 145.1, 136.1, 135.8, 129.9, 127.5, 100.1, 55.8, 41.1, 23.7, 21.8, 14.1; HRMS ($C_{15}H_{19}N_3O_3S$) calcd 322.1225 (MH^+), found 322.1207.

3.5.5. *N*-{5-(2-Pyridyl)-2-[2-(*p*-tosyl)-ethyl]-2*H*-pyrazol-3-yl}-acetamide (15e). 78%. ^{1}H NMR, (400 MHz, $CDCl_3$) δ 8.73 (s, 1H), 8.56 (d, J=4.8 Hz, 1H), 7.67–7.61 (m, 4H), 7.24–7.15 (m, 3H), 6.74 (s, 1H), 4.47 (t, J=6.6 Hz, 2H), 3.72 (t, J=6.6 Hz, 2H), 2.28 (s, 3H), 2.17 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.2, 151.4, 150.6, 149.1, 145.1, 137.1, 136.5, 135.6, 129.9, 127.5, 122.6, 120.2, 99.1, 55.5, 41.9, 23.5, 21.7; HRMS ($C_{19}H_{20}N_4O_3S$) calcd 385.1334 (MH^+), found 385.1322.

3.6. Deprotection of 5-phenyl-2-[2-(*p*-tosyl)-ethyl]-2*H*-pyrazol-3-ylamine (15b)

5-Phenyl-2-[2-(*p*-tosyl)-ethyl]-2*H*-pyrazol-3-ylamine (**15b**, 62 mg, 0.18 mmol) was dissolved in 5 mL of DMSO and 0.45 mL of a 1.0 M solution of NaOEt in EtOH was added at rt. The mixture was then heated at 45 °C for 30 min, cooled to rt, and quenched with 0.45 mL of 1.0 N aqueous HCl. The solvent was removed in vacuo, and the residue was subjected to silica gel flash chromatography (9:1 EtOAc/MeOH) to yield 17 mg (60%) of the known 5-phenyl-2*H*-pyrazol-3-ylamine (**16**).

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Total chemical synthesis of large CCK isoforms using a thioester segment condensation approach

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Abstract—Silver-ion mediated thioester segment condensation was applied to the chemical synthesis of high molecular weight isoforms of cholecystokinin (CCK). Three building blocks, a C-terminal Tyr(SO_3H)-containing segment and two partially protected thioester segments having a C-terminal Pro residue, were prepared using Fmoc-based chemistry and 2-chlorotrityl chloride (Clt) resin as a solid support. The entire peptide chain was successfully synthesized by two consecutive silver-ion mediated condensation reactions using these building blocks. A brief TFA treatment of the final condensation product gave highly homogeneous CCK-58 in a satisfactory yield. This peptide exhibited glucose-dependent insulinotropic activity at levels comparable to CCK-33. These results demonstrate the usefulness of the silver-ion mediated segment condensation approach in the preparation of large sulfated peptides.

1. Introduction

Cholecystokinin (CCK) is a peptide hormone that is distributed in both the brain and the gastrointestinal tract, and is reported to be involved in central and pancreatic regulations.[1] This peptide is also known to have several isoforms, such as CCK-8, -22, -33,[1a,b] -39,[1e] and -58[1f] (Fig. 1). The different forms of CCK are generated from the post-translational processing of a common precursor protein, pro-CCK.[2] Although CCK-58 has been shown to be the major circulating isoform in various animal species including humans, the significance of the molecular diversity of CCK remains unclear. Every form has a tyrosine *O*-sulfated residue [Tyr(SO_3H)] at the seventh

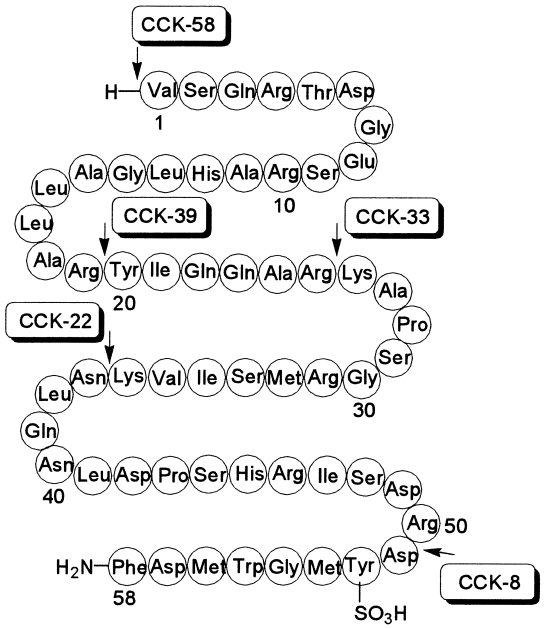

Figure 1. Sequence of CCK peptides.

Keywords: Sulfated peptide; Cholecystokinin (CCK); Peptide thioester; Silver-ion mediated thioester segment condensation; 2-Chlorotrityl chloride resin; Fmoc-based SPPS.

Abbreviations: All, amino acids are of the L-configuration; AcOH, acetic acid; $AcONH_4$, ammonium acetate; Boc, *tert*-butoxycarbonyl; ^{t}Bu, *tert*-butyl; Bum, *tert*-butoxymethyl; CCK, cholecystokinin; Clt, resin, 2-chlorotrityl chloride resin; DIEA, diisopropylethylamine; DIPCDI, *N,N*-diisopropylcarbodiimide; Fmoc, fluoren-9-ylmethoxycarbonyl; HFIP, hexafluoro-2-propanol; HOBT, 1-hydroxybenzotriazole; HOOBt, 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine; LSIMS, liquid secondary-ion mass spectrometry; MALDI-TOFMS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; NMM, *N*-methylmorpholine; Pbf, 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl; Pfp, pentafluorophenyl; PyBOP, benzotriazolyloxytris(pyrrolidino)phosphonium hexafluorophosphate; Su, *N*-hydroxysuccinimidyl; TFA, trifluoroacetic acid; Trt, trityl; WSCDI, water-soluble carbodiimide.

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position from the C-terminus that is crucial for its biological activity. Even though the shortest CCK-peptide, CCK-8, possesses the characteristic pharmacological properties of CCK peptides, it is important to understand the physiological roles of the larger CCK isoforms in order to clarify the purpose of the molecular diversity.

A prerequisite for investigating the biological properties of the larger CCK isoforms is the availability of an efficient method for obtaining sufficient quantities. However, chemical synthesis of CCK peptides has focused on CCK-8 due to the synthetic difficulties caused by the inherent acid lability of Tyr(SO_3H) residues.[3] These difficulties are exemplified by the challenging syntheses of porcine CCK-33 by Sakakibara et al.[4] and human CCK-33 by Yajima et al.[5] Both syntheses were achieved by solution methods adopting a post-assembly sulfation approach. On the other hand, Fmoc-based solid-phase synthesis of porcine CCK-33 using Fmoc-Tyr(SO_3Na)-OH as a building block (a pre-assembly sulfation approach) was reported by Penke et al. in 1991.[6] In their synthesis, a newly developed acid-labile linker-resin was used. Nevertheless, the total yield of the objective peptide was unsatisfactory. Thereafter, two reports on chemical synthesis of larger isoforms of CCK-related peptides were published.[7] In these reports, the acid labile Tyr(SO_3H) residue was replaced by a hydrolysis-resistant analogue Phe(p-CH_2SO_3Na),[8] while the Met and the Trp residues were replaced by norleucine and 2-naphthylalanine residues, respectively. Analogues of CCK-33, -39, and -58 having these unnatural amino acid residues, were reported to exhibit considerably less potent biological activity than CCK-8.[7]

We recently established a facile solid-phase method for the synthesis of Tyr(SO_3H)-containing peptides.[9] This method was successfully applied to the direct solid-phase syntheses of human CCK-33 and -39.[9b] However, a general method for the synthesis of large sulfated peptides having over 50 amino acid residues, such as CCK-58, remains necessary. Therefore, we examined the applicability of a silver-ion mediated thioester segment condensation approach[10] to the synthesis of large Tyr(SO_3H)-containing peptides. Numerous large peptides have been prepared using this approach and it has been successfully applied to the preparation of post-translationally modified peptides, such as glycosylated peptides[11] and phosphorylated peptides.[12] In this paper, we report the synthesis of the larger isoforms of the sulfated peptides CCK-39 and -58, using the silver-ion mediated thioester segment condensation approach.

2. Results and discussion

2.1. Synthetic plan

The amino acid sequence of human CCK peptides is shown in Figure 1. Considering the acid-lability of the Tyr(SO_3H) residue, it is desirable to keep the number of protecting groups to a minimum, as these groups are removed by a final acid treatment. The silver-ion mediated thioester segment condensation approach[10] meets this requirement, because protection of the side-chain functional groups is unnecessary, except for free amino groups. By adopting this approach to construct the entire peptide chain in combination with a brief TFA treatment to remove the acid-labile amino protecting groups, we can expect efficient preparation of a large Tyr(SO_3H)-containing peptide (Fig. 2). For synthesis of the objective CCK-58, the two Pro residues (Pro^{28} and Pro^{43} in Figure 1) were selected as coupling positions in order to circumvent the racemization problem accompanying thioester segment condensation.[10] As shown in Figure 2, we divided the 58-residue peptide into three segments: the C-terminal Tyr(SO_3H)-containing segment, CCK(44–58), and two thioester segments having C-terminal Pro residues, CCK(29–43) and CCK(1–28).

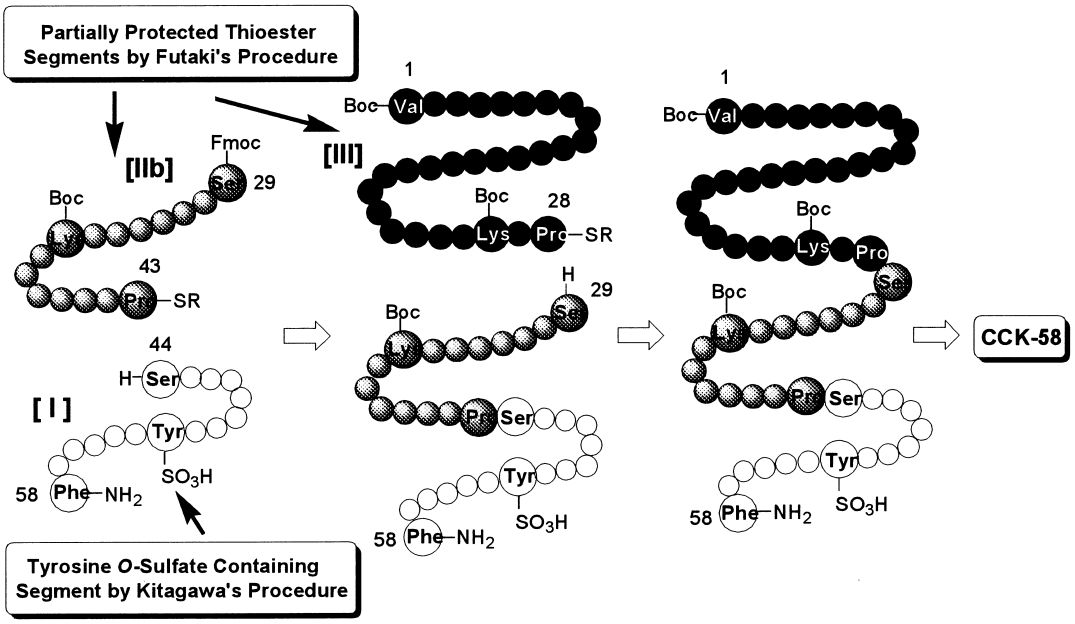


Figure 2. Synthetic plan for CCK-58.

For preparation of the Tyr(SO_3H)-containing segment, the facile solid phase approach for the Tyr(SO_3H)-containing peptides developed by our group[9] is potentially useful. In this protocol, two key features are involved in completing the synthesis in an efficient manner: (i) utilization of a highly acid-sensitive 2-chlorotrityl (Clt) resin[13] to quantitatively detach the sulfated peptide from the solid support, and (ii) TFA deprotection at low temperature to minimize the deterioration of the sulfate. With regard to the peptide thioester segments, preparation of these had been limited to Boc-based chemistry until recently (Boc-strategy in Figure 3). This is due to the nucleophilicity of piperidine, a standard deprotection reagent for the N^{α}-Fmoc group in Fmoc-based SPPS. Resin-bound thioester is generally unstable to repeated exposure to piperidine. However, several groups have reported new methods for preparing peptide thioester using Fmoc-based chemistry,[14] either with a thioester-compatible deprotection reagent for the N^{α}-Fmoc group[14b] or with a special linker, such as an alkanesulfonamide-type 'safety-catch' linker[14c–e] and a backbone amide linker.[14f] In this study, we decided to employ a novel protocol developed by Futaki et al.[14a] in which thioesterification is carried out on a fully protected peptide after Fmoc-based peptide-chain assembly (Fmoc-strategy in Figure 3). Application of Clt resin[13] is critical to prepare such side-chain protected peptides with Fmoc-based SPPS. In addition, the sterically hindered Clt resin is able to minimize the premature detachment of dipeptides having a C-terminal Pro residue (X-Pro) from the resin through diketopiperazine formation. We previously demonstrated the usefulness of Clt resin for Fmoc-based preparation of protected peptide segments having C-terminal Pro residues and the obtained peptides were successfully employed in solid-phase segment condensation aiming to synthesize a 34-residue sulfated peptide, human big gastrin-II.[15] In this way, the preparation of both the Tyr(SO_3H)-containing segment and the thioester segments having a C-terminal Pro residue rely upon the unique characteristics of a Clt resin.

2.2. Segment synthesis

2.2.1. Synthesis of Tyr(SO_3H)-containing segment. The C-terminal sulfated peptide, CCK(44–58): [**I**], was prepared as shown in Figure 4. To overcome the general low recovery of a peptide amide from the amide-offering linker-resin, the C-terminal dipeptide, Fmoc-Asp-Phe-NH_2,[9] was linked with Clt resin via the β-carboxyl group of Asp, and each Fmoc-amino acid including Fmoc-Tyr(SO_3Na)-OH[6] was added to the peptide-resin in a stepwise manner. After completing the peptide chain assembly, cleavage of the peptide from the resin and deprotection were concurrently conducted with 90% aqueous TFA (0 °C, 12 h). Despite this long TFA treatment, desulfated peptide was estimated to account for ca. 10% of total peptide by HPLC analysis (Fig. 4(a)). The crude sulfated peptide was purified by HPLC to give an analytically pure segment [**I**] in 18% yield (Fig. 4(b)). The structural correctness of this peptide was ascertained by amino acid analysis and liquid secondary-ion mass spectrometry (LSIMS), and the fidelity of the sulfate was confirmed by LSIMS in the positive- and negative-ion modes.[16]

2.2.2. Synthesis of thioester segments. The method for peptide thioester synthesis developed by Futaki et al.[14a] consists of the following steps; (i) construction of a peptide chain on Clt resin by Fmoc-based SPPS, (ii) cleavage of the fully protected peptide from the Clt-resin under extremely weak acidic conditions, (iii) thioesterification of the protected peptide by reaction with HS-$(CH_2)_2$COOEt or HS-$(CH_2)_2CONH_2$, and (iv) deprotection of the peptide with TFA. We require thioester segments bearing a TFA-labile amino protecting group in order to construct a large Tyr(SO_3H)-containing peptide sequence, and therefore re-introduction of a Boc protecting group to the functional amino groups (N^{α}- and N^{ε}-amino groups of a peptide) was also conducted as step (v) in this sequential scheme.

We describe here, as an example, the detailed preparation of

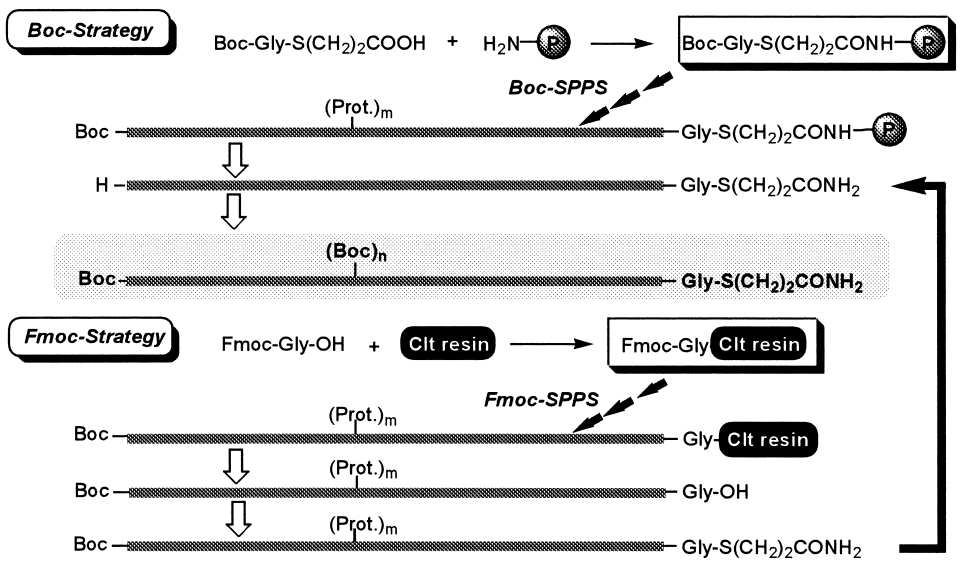


Figure 3. Preparation of peptide thioesters via Boc- or Fmoc-strategy.

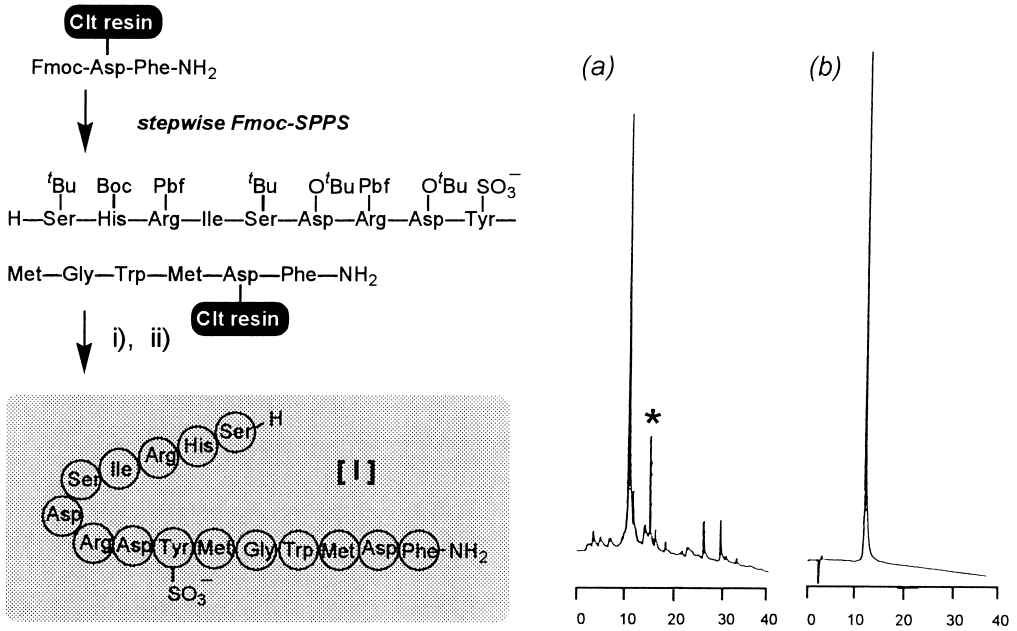


Figure 4. Synthesis of C-terminal Tyr(SO_3H)-containing segment [**I**]. *Reagents*: (i) 90% aqueous TFA, 0 °C, 12 h; (ii) preparative HPLC. *Inlet*: HPLC chromatograms of (a) crude [**I**] after cleavage/deprotection (step i) and (b) HPLC-purified [**I**]. An asterisk in (a) shows the desulfated peptide produced during step (i). [HPLC conditions: column, Cosmosil $5C_{18}$-AR (4.6×150 mm); elution, a linear gradient of B in A (20 to 40% in 40 min); flow rate, 0.8 ml/min].

thioester segment, Boc-[Lys(Boc)^{26,36}]-CCK(20–43)-S$(CH_2)_2$COOEt: [**IIa**], which was used for the synthesis of CCK-39 (Fig. 5, scheme). This 24-mer peptide was constructed on an Fmoc-Pro-Clt resin according to the general procedure of Fmoc-based SPPS. The N-terminal residue was introduced as a Boc-protected derivative, Boc-Tyr(^{t}Bu)-OH. The suppressed diketopiperazine formation and the subsequent dipeptide (Asp-Pro in this case) detachment from the resin were substantiated by the fact that the weight of the peptide-resin increased reasonably

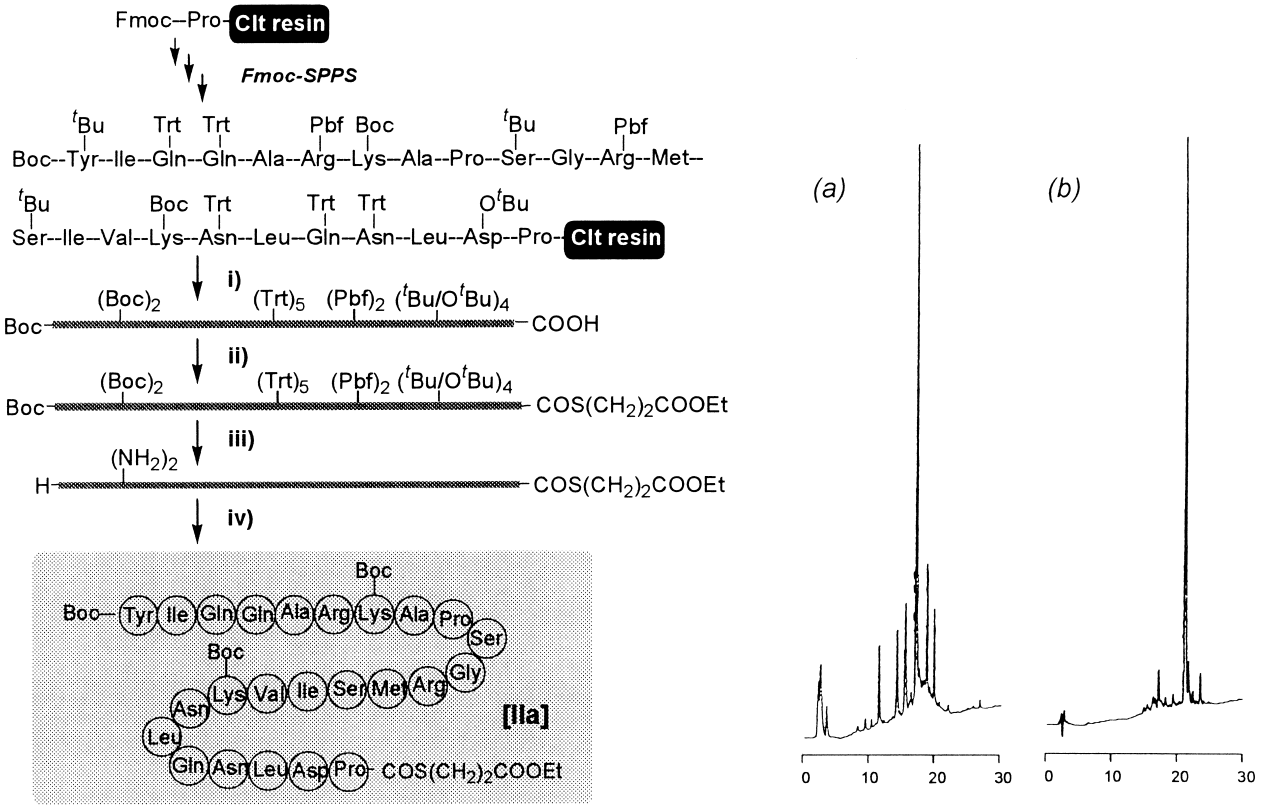


Figure 5. Synthesis of thioester segment [**IIa**]. *Reagents*: (i) HFIP/CH_2Cl_2 (1:4), 25 °C, 30 min; (ii) HS-$(CH_2)_2$COOEt (25 equiv.), WSCDI·HCl (15 equiv.), and HOBt (15 equiv.), 4 °C, 20 h; (iii) 95% aqueous TFA, 25 °C, 3 h; (iv) Boc-OSu (75 equiv.), and NMM (75 equiv.), 25 °C, 20 h. *Inlet*: HPLC chromatograms of (a) crude peptide after step (iii) and (b) crude peptide [**IIa**] after step (iv). [HPLC conditions: column, Cosmosil $5C_{18}$-AR (4.6×150 mm); elution, a linear gradient of D in C (20 to 45% in 30 min for (a) and 30 to 50% in 30 min for (b)); flow rate, 0.8 ml/min].

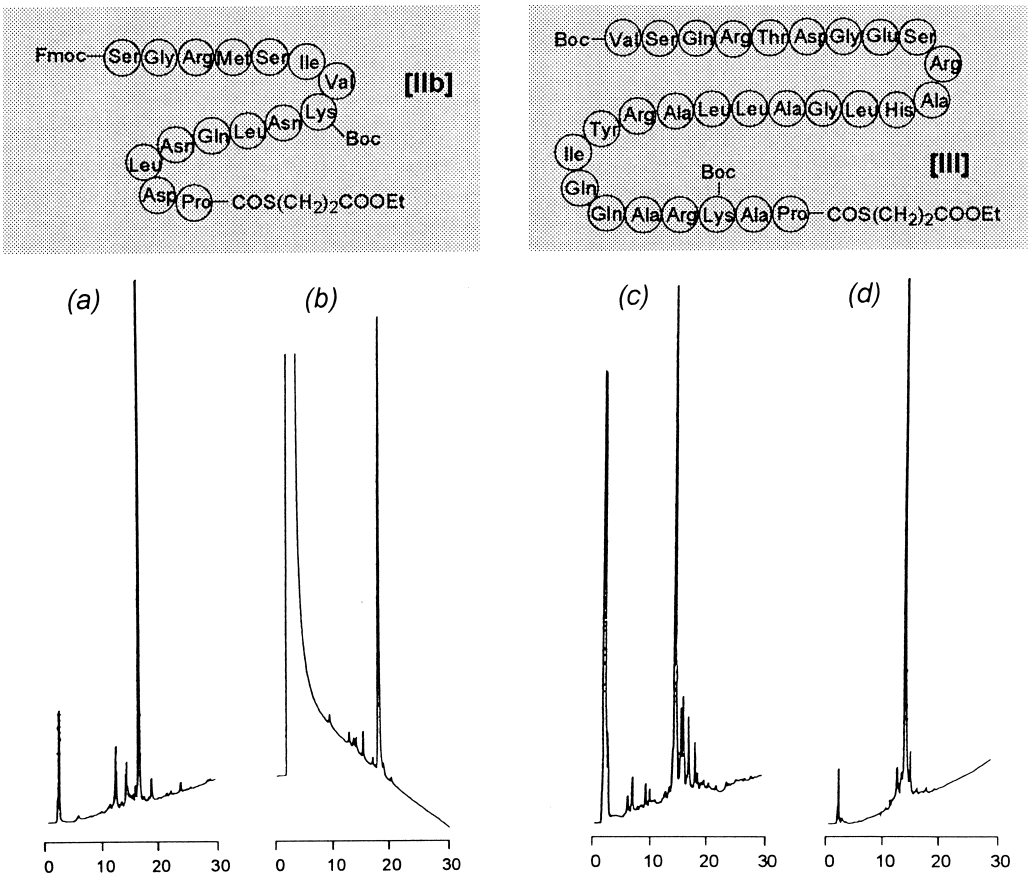


Figure 6. HPLC chromatograms of crude intermediates for preparation of thioester segment [**IIb**] and [**III**]; (a) crude intermediate peptide for [**IIb**] after deprotection (step (iii)), (b) crude intermediate peptide for [**IIb**] after Boc-derivatization (step (iv)), (c) crude intermediate peptide for [**III**] after deprotection (step (iii)), and (d) crude intermediate peptide for [**III**] after Boc-derivatization (step (iv)). Steps (iii) and (iv) correspond to the synthetic steps shown in Figure 5. [HPLC conditions: column, Cosmosil $5C_{18}$-AR (4.6×150 mm); elution, a linear gradient of D in C (30 to 60% in 30 min for (a), 25 to 40% in 30 min for (c), and 30 to 45% in 30 min for (d)), and a linear gradient system of B in A (30 to 65% in 30 min for (b)); flow rate, 1 ml/min for (a), (c), (d) and 0.8 ml/min for (b)].

after incorporation of the third amino acid residue. Detachment of the fully protected peptide from the Clt resin was achieved by treating the peptide-resin with a mixture of hexafluoro-2-propanol (HFIP)/CH_2Cl_2 (1:4, 30 min).[17] We used this cleavage reagent instead of the usual AcOH/trifluoroethanol/CH_2Cl_2[13] cleavage system in order to avoid contamination with trace amounts of AcOH. The fully protected peptide was then directly subjected to thioesterification by reaction with excess ethyl 3-mercaptopropionate (25 equiv.). The reaction was completed with the aid of water-soluble carbodiimide (WSCDI·HCl, 15 equiv.) in the presence of *N*-hydroxybenzotriazole (HOBt, 15 equiv.) in DMF. After overnight stirring at 4 °C, none of the starting material was detected on TLC. The crude product was isolated after washing with ether several times, and then subjected to deprotection with 95% aqueous TFA (25 °C, 3 h). HPLC purification of the crude deprotected peptide (Fig. 5(a)) gave a homogeneous peptide thioester. Finally, Boc groups were introduced to the functional N^{α}- and N^{ε}-amino groups by reacting with excess Boc-OSu (25 equiv. for each amino group) in the presence of *N*-methylmorpholine (NMM) in DMF (25 °C, 3 h). The Boc-protected peptide thioester (Fig. 5(b)) was purified by HPLC to give a homogeneous thioester segment [**IIa**]. The overall yield of thus obtained thioester segment was 11% from the fully protected peptide. Structural correctness of the intermediates at each step and [**IIa**] was confirmed by mass spectrometry.

Two thioester segments used for the synthesis of CCK-58, Fmoc-[Lys(Boc)^{36}]-CCK(29–43)-S$(CH_2)_2$COOEt: [**IIb**] and Boc-[Lys(Boc)^{26}]-CCK(1–28)-S$(CH_2)_2$COOEt: [**III**], were prepared in the essentially same manner. By taking the second segment condensation into account, the N-terminus of segment [**IIb**] was protected with an Fmoc-group. HPLC chromatograms of the crude intermediates obtained after TFA treatment to remove the acid-labile protecting groups (Fig. 6(a) and (c)) were satisfactory. Following preparative HPLC purification, these intermediates were converted to segments [**IIb**] and [**III**] by reacting with Boc-OSu (Fig. 6(b) and (d)). Segment [**III**] was purified by HPLC before segment condensation. On the other hand, segment [**IIb**] was used for the segment condensation without HPLC purification because of the solubility problem. The overall yields of thus obtained thioester segments were 26% for [**IIb**] and 21% for [**III**], respectively, from the fully protected peptides. The structural correctness of the partially protected thioester segments, [**IIb**] and [**III**], was ascertained by amino acid analysis of their acid hydrolysates and LSIMS.

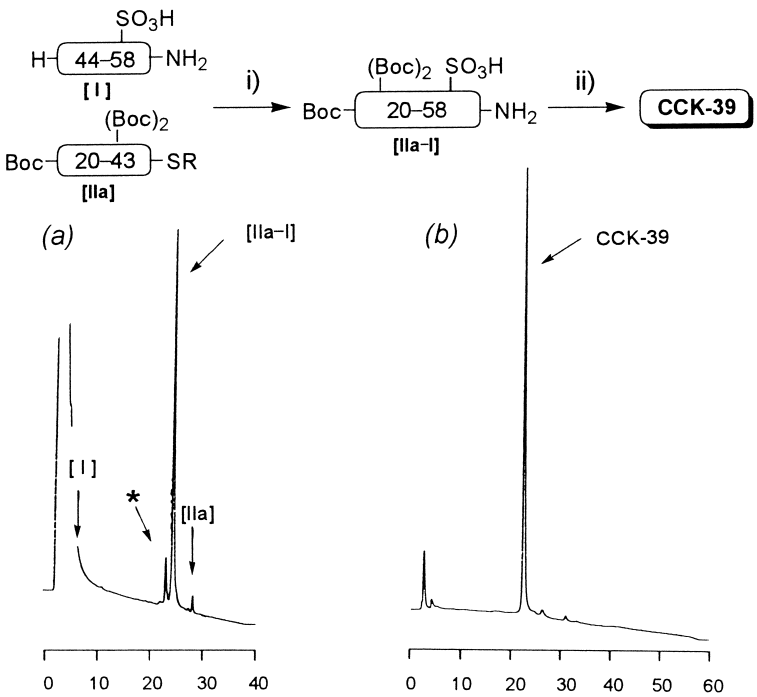


Figure 7. Synthesis of CCK-39 by thioester segment condensation approach. *Reagents*: (i) $AgNO_3$ (3 equiv.), HOOBt (30 equiv.), and DIEA (20 equiv.) in DMSO, 25 °C, 24 h; (ii) 90% aqueous TFA, 0 °C, 2 h. *Inlet*: HPLC chromatograms of (a) a crude condensation product (step (i)) and (b) a crude CCK-39 after the TFA treatment (step (ii)). An asterisk in (a) shows the hydrolyzed product of thioester segment [**IIa**]. The peak of [**I**] is detected as a shoulder after solvent peak in these HPLC conditions. [HPLC conditions: column, Cosmosil $5C_{18}$-AR (4.6×150 mm); elution, a linear gradient of B in A (20 to 50% in 40 min for (a) and 23 to 33% in 60 min for (b)); flow rate, 1 ml/min for (a) and 0.8 ml/min for (b)].

2.3. Synthesis of CCK-39

As a preliminary experiment, we examined the applicability of the silver-ion mediated thioester segment condensation approach to the synthesis of large CCK peptides by preparing CCK-39 (Fig. 7). Segment condensation between [**I**] (1 equiv.) and [**IIa**] (1 equiv.) was performed with the aid of $AgNO_3$ (3 equiv.), 3, 4-dihydro-3-hydroxy-4-oxo-1, 2, 3-benzotriazine[18] (HOOBt, 30 equiv.), and NMM (20 equiv.) in DMSO.[10e] As an additive, HOOBt was used to form a reactive active ester intermediate. The coupling reaction proceeded smoothly without notable side reactions (Fig. 7(a)). After 24 h, the starting two segments were almost completely consumed to form a protected condensation product, Boc-[Lys(Boc)^{26,36}]-CCK(20–58): [**IIa–I**]. Mass spectrometry revealed that the small peak detected before the main peak on the HPLC chromatogram (Fig. 7(a)) was a hydrolyzed product of the thioester segment.[19] The condensation product was isolated by HPLC in 60% yield. This HPLC-purified product was then subjected to TFA treatment (0 °C, 2 h) to remove the three Boc-groups, and CCK-39 was obtained in high purity without production of the desulfated peptide (Fig. 7(b)). The synthetic peptide was found to be identical to authentic CCK-39[9b] with regard to analytical HPLC, mass spectrometry, and lysyl endopeptidase digestion. The results of amino acid analysis after acid

Table 1. Amino acid ratios in acid hydrolysates of synthetic peptides

A.A.	CCK-39 peptides			CCK-58 peptides				
	Residues	[**IIa–I**]	CCK-**39**	Residues	[**Iib–I**]	Residues	[**III–IIb–I**]	CCK-**58**
Asp	6	5.59	6.34	6	6.58	7	6.14	6.75
Thr						1	1.00	0.97
Ser	4	3.67	3.44	4	3.26	6	5.00	5.04
Glu	3	3.25	3.36	1	1.10	5	5.42	5.26
Pro	2	1.92	2.03	1	1.00	2	1.85	2.05
Gly	2	2.25	2.12	2	1.78	4	4.00	3.96
Ala	2	1.90	2.19			5	5.32	4.94
Val	1	0.78	0.79			2	1.83	1.75
Met	3	1.93	2.33	3	2.51	3	2.05	2.16
Ile	3	2.36	2.62	2	1.63	3	2.68	2.60
Leu	2	2.00	2.00	2	2.00	5	5.13	4.84
Tyr	2	2.00	1.95	1	1.05	2	2.03	1.87
Phe	1	0.92	0.85	1	0.87	1	0.97	0.88
His	1	1.06	0.97	1	1.09	2	1.92	1.96
Trp	1	N.D.	N.D.	1	N.D.	1	N.D.	N.D.
Lys	2	2.04	2.05	1	1.00	2	1.75	2.09
Arg	4	3.37	3.69	3	2.86	7	6.52	6.95

hydrolysis of the CCK-39 and the partially protected intermediate are listed in Table 1. From these results, we concluded that the silver-ion mediated thioester segment coupling strategy is promising for the synthesis of longer sulfated peptides, such as CCK-58.

2.4. Synthesis of CCK-58

The synthesis of CCK-58 is outlined in Figure 8. To construct the entire peptide chain, two segments were successively condensed by the silver-ion mediated reaction. The first condensation reaction between segment [**I**] (1 equiv.) and segment [**IIb**] (1 equiv.) was mostly completed after 24 h at 25 °C (Fig. 8(a)), and the condensation product, Fmoc-[Lys(Boc)^{36}]-CCK(29–58): [**IIb**–**I**], was obtained in 67% yield, after HPLC purification. Following removal of the N^{α}-Fmoc group by a brief treatment with piperidine, the resultant N^{α}-free segment [**IIb**–**I**] (1 equiv.) was condensed with segment [**III**] (1.5 equiv.). In this condensation step, significant amounts of the hydrolyzed products of the thioester segment were produced.[20] Nevertheless, the condensed peptide was detected as the main peak on the HPLC chromatogram (Fig. 8(b)). After HPLC purification, the condensation product, Boc-[Lys(Boc)^{26,36}]-CCK(1–58): [**III**–**IIb**–**I**], was obtained in 40% yield. The final TFA treatment (0 °C, 2 h) resulted in a fully deprotected 58-mer peptide, without the accompanying desulfated peptide (Fig. 8(c)). HPLC purification of this peptide gave highly homogeneous CCK-58 in 45% yield. The results of amino acid analysis after acid hydrolysis of the CCK-58 and the partially protected intermediates are listed in Table 1.

The amino acid composition of the purified CCK-58 coincided well with the theoretical value (Table 1), and the sulfate was confirmed to be intact by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS). It is noteworthy that the $[M+H]^+$ was detected without the accompanying desulfated fragment ion $[M+H-SO_3]^+$ on the positive-ion mode MALDI-TOFMS spectrum (Fig. 9(a)). This result reflect the intramolecular stability of the Tyr(SO_3H) residue in CCK-58.[16b] Lysyl endopeptidase digestion of the synthetic CCK-58 resulted in three peptide fragments on the HPLC chromatogram (Fig. 9(b)). These peptide fragments were identified as a 10-mer (positions 27 to 36 of CCK-58, designated as peak 1 in Figure 9(b)), a 22-mer corresponding to CCK-22 (positions 37 to 58, peak 2), and a 26-mer (positions 1 to 26, peak 3), on the basis of the amino acid compositions of their acid hydrolysates. Peptide mass mapping of the digest also supported the structural correctness of the synthetic CCK-58 (Fig. 9(c)).

The insulinotropic activity of the synthetic CCK-58 was investigated using isolated pancreatic islets (male Wister rats) pretreated with glucose (11.1 mmol) as previously

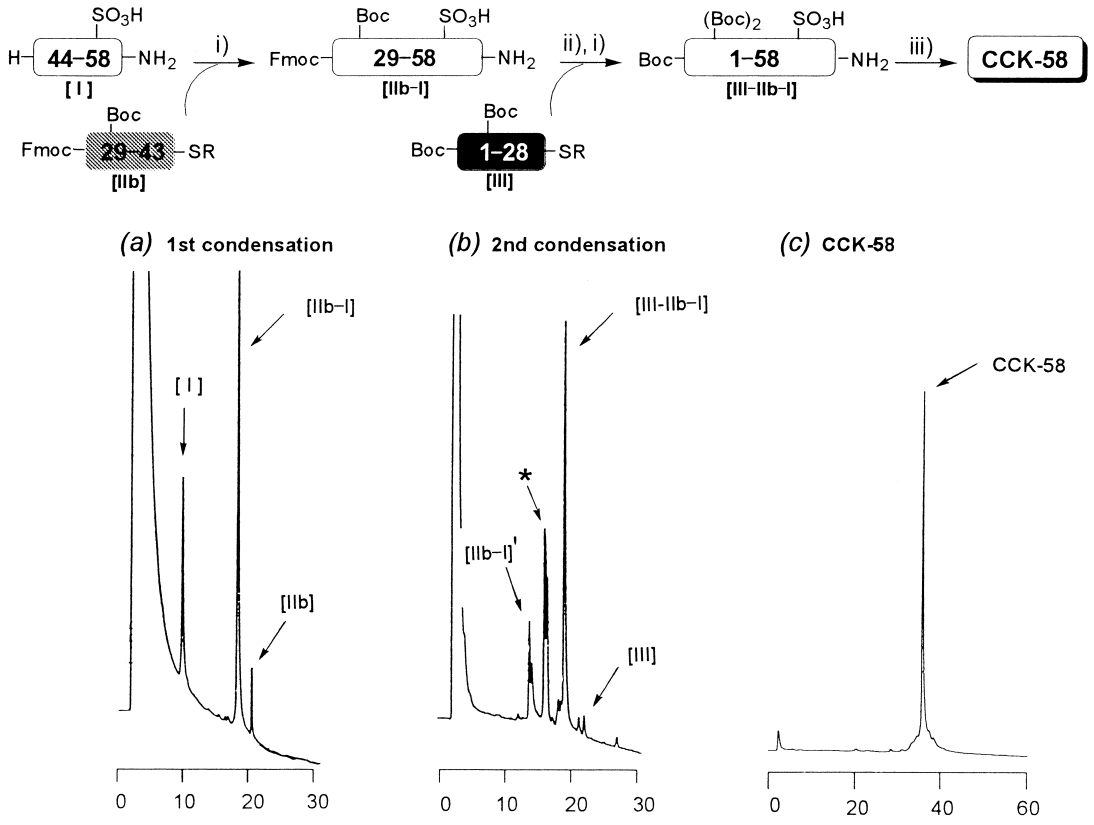
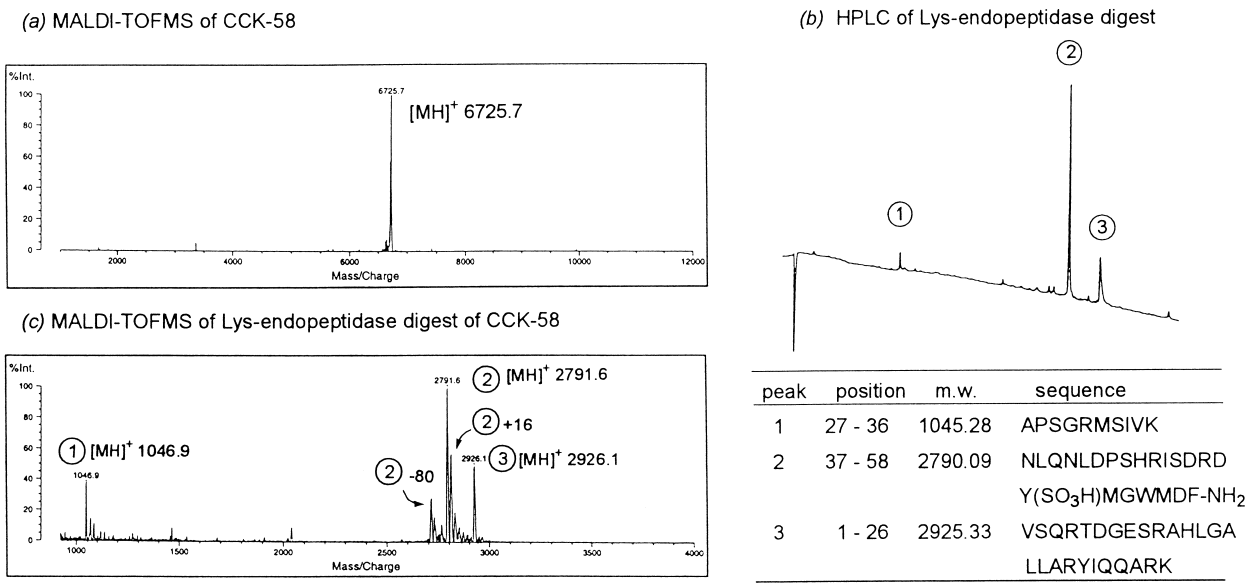


Figure 8. Synthesis of CCK-58 by thioester segment condensation approach. *Reagents*: (i) $AgNO_3$ (3 equiv.), HOOBt (30 equiv.), and DIEA (20 equiv.) in DMSO, 25 °C, 24 h; (ii) 25% piperidine in a mixture of DMF and DMSO, 25 °C, 3 h, then gel-filtration on Sephadex LH-20; (iii) 90% aqueous TFA, 0 °C, 2 h. *Inlet*: HPLC chromatograms of (a) crude product after first condensation reaction, (b) crude product after second condensation reaction, and (c) crude CCK-58 after TFA treatment. An asterisk in (b) shows the hydrolyzed product of thioester segment [**III**] and also [**IIb**–**I**]′ in (b) shows the N^{α}-Fmoc deprotected segment of [**IIb**–**I**]. [HPLC conditions: column, Cosmosil 5C_{18}-AR (4.6×150 mm); elution, a linear gradient of B in A (20 to 65% in 30 min for (a), 25 to 55% in 30 min for (b), and 30 to 45% in 60 min for (c)); flow rate, 0.8 ml/min].



peak	position	m.w.	sequence
1	27 - 36	1045.28	APSGRMSIVK
2	37 - 58	2790.09	NLQNLDPSHRISDRD
			$Y(SO_3H)$MGWMDF-NH_2
3	1 - 26	2925.33	VSQRTDGESRAHLGA
			LLARYIQQARK

Figure 9. Characterization of synthetic CCK-58. (a) MALDI-TOFMS spectrum of CCK-58; (b) HPLC chromatogram of lysyl endopeptidase digested CCK-58; and (c) MALDI-TOFMS spectrum of lysyl endopeptidase digested CCK-58. Assignment of the fragmented peptides is summalized in the inserted table. The sulfated fragment (peak 2) was detected as three mass peaks corresponding to the molecular protonated ion, desulfated peptide ion (-80 Da), and oxidized peptide ion (+16 Da), on the peptide mass map.

described.[9b] Synthetic CCK-58 increased the insulin release from the islets to the same degree as CCK-33.

3. Conclusions

We reported herein the usefulness of the silver-ion mediated thioester segment condensation approach in synthesizing large Tyr(SO_3H)-containing peptides. A large isoform of the CCK-peptide, CCK-58, was successfully prepared for the first time, and in good yield, using this approach. Clt-resin was efficiently employed in preparing the sulfated and thioester segments required for peptide chain construction. Notably the thioester segments were prepared using standard Fmoc-based chemistry without using special N^{α}-Fmoc deprotection reagents or special linkers. This method may contribute to the understanding of the functions of sulfated proteins by facilitating the chemical synthesis of large Tyr(SO_3H)-containing peptides.

4. Experimental

4.1. General

Fmoc-amino acid derivatives, diisopropylcarbodiimide (DIPCIDI), WSCDI·HCl, benzotriazolyloxytris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP)-reagent and Clt resin (substituted level; 1.47 mmol/g, 100–200 mesh) were purchased from Watanabe Chemical Co., Ltd. (Hiroshima, Japan). Fmoc-His(Boc)-OH (cyclohexylamine salt) was obtained from Carbiochem–Novabiochem Japan, Ltd. (Tokyo, Japan). Lysyl endopeptidase from *Achromobacter lyticus* M497-1 (EC 3.4.21.50) was purchased from Wako Pure Chemicals Co., Ltd. (Osaka, Japan). Other chemicals were of analytical grade and used without further purification. Acid hydrolysis was carried out at 110 °C for 24 h with a mixture of propionic acid and 12 M hydrochloric acid (1:1 v/v) for resin-bound peptides or with 6 M hydrochloric acid containing a few drops of phenol for purified peptides. Amino acid ratios were determined with a Shimadzu LC amino acids analyzer system using the *o*-phatalaldehyde protocol. LSIMS were performed on a VG ZAB-2SE double-focusing mass spectrometer using the Opus operating data system. Glycerol, thioglycerol, and *m*-nitrobenzylalcohol were used as the matrix, either neat or in combination. MALDI-TOFMS was performed on a Kratos Kompact MALDI IV. Sinapinic acid was used as the matrix.

4.2. General procedure for Fmoc-based solid-phase peptide synthesis

Fmoc-based solid-phase peptide synthesis (Fmoc-SPPS) was conducted in manually. Side-chain protecting groups used in the synthesis were as follows: ^{t}Bu for Asp, Glu, Ser, Thr, and Tyr; Boc for Lys and His (for segment [**I**]); Trt for Asn and Gln; Pbf for Arg; Bum for His (segment [**III**]). N^{α}-Fmoc protecting groups were cleaved by 1 min treatment with 20% piperidine in DMF followed by a second treatment with the same reagent for 20 min. For deprotection of the N^{α}-Fmoc groups of Gln and Glu(^{t}Bu) residues, the concentration of piperidine was reduced to 10%. After Fmoc cleavage, the peptide-resin was washed with DMF (×6). The next residue was then incorporated using the DIPCDI-HOBt coupling protocol [Fmoc-amino acid (3 equiv.), DIPCDI (3 equiv.), and HOBt (3 equiv.)] or the PyBOP[21]-mediated coupling protocol [Fmoc-amino acid (3 equiv.), PyBOP reagent (3 equiv.), and NMM (9 equiv.)]. After gentle agitation (1.5 h) and washing with DMF (×6), part of the peptide-resin was subjected to the Kaiser test.[22] On completion of the assembly, the peptide-resin was successively washed with DMF (×5), MeOH (×5), and ether (×5), then dried in vacuo.

4.3. General procedure for purification by preparative RP-HPLC and purity assessment by analytical HPLC

Crude peptide was purified by RP-HPLC using a column of Cosmosil $5C_{18}$ AR-300 (20×150 mm) or Cosmosil $5C_{18}$ AR (10×250 mm). Purity of the peptide was assessed using a column of Cosmosil $5C_{18}$ AR (4.6×150 mm). For elution of Tyr(SO_3H)-containing peptides, a solvent system consisting of solvent A (0.1 M $AcONH_4$) and solvent B (CH_3CN) was used. For elution of other peptides, a solvent system consisting of solvent C (0.1% aqueous TFA) and solvent D (CH_3CN containing 0.1% TFA) was used. The absorbance of the eluate was measured at 230 or 275 nm, depending on the peptide.

4.4. Peptide synthesis

4.4.1. CCK(44–58) [I]. Fmoc-Asp-Phe-NH_2 was prepared by the solution method and attached to Clt resin via the β-carboxyl group of Asp, according to the procedure of Barlos et al.[9,13c] The resulting Fmoc-Asp(Clt resin)-Phe-NH_2 (330 mg, 0.15 mmol) was used as the starting dipeptide resin, and each Fmoc-amino acid derivative was added to it, according to the general procedures of Fmoc-based SPPS. Fmoc-Tyr(SO_3Na)-OH was used for incorporation of the Tyr(SO_3H) residue and Fmoc-His(Boc) was incorporated after desalting followed by a brief purification on silica gel column chromatography.[15] After the 15-mer peptide chain was assembled, the N^{α}-Fmoc group was removed. The peptide-resin was then dried in vacuo (620 mg).

The protected peptide-resin, H-Ser(^{t}Bu)-His(Boc)-Arg(Pbf)-Ile-Ser(^{t}Bu)-Asp(O^{t}Bu)-Arg(Pbf)-Asp(O^{t}Bu)-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp(Clt resin)-Phe-NH_2 (100 mg, 17.7 μmol), was treated with pre-cooled 90% aqueous TFA (1.5 ml) at 0 °C for 12 h, then dry ether (50 ml) was added. The formed precipitate was collected by centrifugation and washed twice with ether. The dried product was dissolved in 0.02 M NH_4HCO_3 (10 ml) and applied to a column of Sephadex G-25 (2.5×55 cm). NH_4HCO_3 (0.02 M) was used for elution and the fractions corresponding to the first peak (UV absorbance detected at 280 nm) was pooled. The pooled fractions were lyophilized to give a powder (22.1 mg, 63% on cleavage and deprotection). This sample was purified by preparative HPLC using a linear gradient of B in A (26 to 30% in 40 min) at a flow rate of 2.5 ml/min. After lyophilization, 6.6 mg of pure segment [**I**] was obtained (30% recovery on HPLC purification). This peptide was detected as a sharp single peak at t_R 11.3 min on an analytical HPLC chromatogram [elution, a linear gradient of B in A (20 to 40% in 40 min); flow rate, 0.8 ml/min]. Amino acid ratios in the acid hydrolysate were as follows (theoretical values are given in parentheses): Asp 3.28 (3), Ser 1.91 (2), Gly 1.12 (1), Met 1.61 (2), Ile 1.00 (1), Tyr 1.05 (1), Phe 0.98 (1), Trp not determined (1), His 1.00 (1), Arg 2.01 (2). LSIMS *m/z*: calcd for $C_{83}H_{119}N_{25}O_{27}S_3$ 1995.2 (*M*, average mass); found 1995.9 $[M+H]^+$.

4.4.2. Boc-[Lys(Boc)^{26,36}]-CCK(20–43)-S(CH_2)$_2$COOEt [IIa]. Starting with Fmoc-Pro-Clt resin (255 mg, 0.15 mmol), each amino acid derivative was incorporated to the peptide-resin according to the general Fmoc-SPPS protocol. After the N-terminal Boc-Tyr(^{t}Bu)-OH was incorporated, the peptide-resin was dried in vacuo (630 mg). The fully protected peptide-resin (200 mg, 21.0 μmol) was treated with a mixture of HFIP/CH_2Cl_2 (1:4 v/v, 5 ml) at 25 °C for 30 min, then filtered. The filtrate was concentrated using an N_2 stream, and then dry ether (50 ml) was added to precipitate the peptide. After centrifugation, the collected precipitate was dried in vacuo (105 mg, quantitative).

The obtained fully protected peptide, Boc-Tyr(^{t}Bu)-Ile-Gln(Trt)-Gln(Trt)-Ala-Arg(Pbf)-Lys(Boc)-Ala-Pro-Ser(^{t}Bu)-Gly-Arg(Pbf)-Met-Ser(^{t}Bu)-Ile-Val-Lys(Boc)-Asn(Trt)-Leu-Gln(Trt)-Asn(Trt)-Leu-Asp(O^{t}Bu)-Pro-OH (105 mg, 21.0 μmol), was dissolved in ice-cooled DMF (2 ml), then HS-(CH_2)$_2$$COOC_2H_5$ (70.4 μl, 0.53 mmol), WSCDI·HCl (60.3 mg, 0.31 mmol), HOBt (48.2 mg, 0.31 mmol) were added. After the reaction mixture was stirred at 4 °C for 20 h, the solution was concentrated and the resultant residue was triturated with ether (10 ml) three times, giving a powder. The dried powder was treated with 95% aqueous TFA (3 ml) at 25 °C for 3 h, then ether (50 ml) was added. The resultant precipitate was collected by centrifugation and washed twice with ether. This product was dissolved in H_2O (30 ml) and lyophilized to give the crude peptide thioester (51.1 mg, 86% yield from the protected peptide). The crude peptide (51.0 mg) was purified by preparative HPLC [column, Cosmosil $5C_{18}$-AR (10×250 mm); elution, a linear gradient of D in C (20 to 45% in 60 min); flow rate, 1.5 ml/min]. After lyophilization, 15.8 mg of the pure peptide thioester was obtained (31% recovery on HPLC). LSIMS *m/z*: calcd for $C_{123}H_{207}N_{37}O_{36}S_2$ 2844.4 (*M*, average mass); found 2845.1 $[M+H]^+$.

The peptide thioester (15.3 mg, 5.4 μmol) was dissolved in ice-cooled DMF (2 ml), then Boc-OSu (87 mg, 0.4 mmol) and NMM (45 μl, 0.4 mmol) were added. After the reaction mixture was stirred at 25 °C for 20 h, the solution was concentrated and the resultant residue was triturated with ether (10 ml) twice, giving a powder (11.1 mg, 66% yield). The Boc-protected peptide thioester was purified by preparative HPLC [column, Cosmosil $5C_{18}$-AR (10×250 mm); elution, a linear gradient of D in C (35 to 50% in 60 min); flow rate, 2.0 ml/min]. After lyophilization, 7.1 mg of homogeneous segment [**IIa**] was obtained (64% recovery on HPLC). This peptide was detected as a single peak at t_R 20.8 min on an analytical HPLC chromatogram [column, Cosmosil $5C_{18}$-AR (4.6×150 mm); elution, a linear gradient of D in C (30 to 50% in 30 min); flow rate, 0.8 ml/min]. Amino acid ratios in the acid hydrolysate were as follows: Asp 3.51 (3), Ser 1.72 (2), Glu 3.16 (3), Pro 1.85 (2), Gly 1.00 (1), Ala 2.14 (2), Val 0.72 (1), Met 0.77 (1), Ile 1.59 (2), Leu 1.89 (2), Tyr 0.94 (1), Lys 1.82 (2), Arg 1.78 (2). LSIMS *m/z*: calcd for $C_{138}H_{231}N_{37}O_{42}S_2$ 3144.7 (*M*, average mass); found 3145.5 $[M+H]^+$.

4.4.3. Fmoc-[Lys(Boc)^{36}]-CCK(29–43)-S(CH_2)$_2$COOEt [IIb]. The protected 15-mer peptide was constructed on Fmoc-Pro-Clt resin (255 mg, 0.15 mmol). After incorporation of the *N*-terminal Fmoc-Ser(^{t}Bu)-OH, the peptide-resin was washed and dried in vacuo (603 mg). The protected peptide-resin thus obtained (400 mg, 44.6 μmol) was treated with a mixture of HFIP/CH_2Cl_2 (1:4, 10 ml) at 25 °C for 30 min and filtered. The fully protected peptide,

Fmoc-Ser(^{t}Bu)-Gly-Arg(Pbf)-Met-Ser(^{t}Bu)-Ile-Val-Lys(Boc)-Asn(Trt)-Leu-Gln(Trt)-Asn(Trt)-Leu-Asp(O^{t}Bu)-Pro-OH, was isolated from the filtrate using similar methods as described for the preparation of [**IIa**] (115 mg, 92% yield).

The obtained fully protected peptide (109 mg, 40.0 μmol) was dissolved in ice-cooled DMF (3 ml), then HS-$(CH_2)_2$-$COOC_2H_5$ (134.2 μl, 1.0 mmol), WSCDI·HCl (115 mg, 0.60 mmol), and HOBt (69 mg, 0.60 mmol) were added. After the reaction mixture was stirred at 4 °C for 12 h, the solution was concentrated and the resultant residue was triturated with ether (10 ml) three times to give a powder. The dried powder was then treated with 95% aqueous TFA (4 ml) at 25 °C for 3.5 h, and ether (50 ml) was added to the mixture. The precipitate was collected by centrifugation, washed with ether, dissolved in H_2O (30 ml) and lyophilized to give a crude peptide thioester (58.2 mg, 73% yield from the protected peptide). The crude peptide (51.1 mg) was purified by preparative HPLC [column, Cosmosil 5C_{18}-AR (10×250 mm); elution, an isocratic elution of D in C (38% for 10 min) followed by a linear gradient of D in C (38 to 48% in 20 min); flow rate, 2.0 ml/min]. After lyophilization, 29.6 mg of the pure N^{α}-Fmoc protected peptide thioester was obtained (51% recovery on HPLC). LSIMS *m/z*: calcd for $C_{90}H_{140}N_{22}O_{26}S_2$ 2010.4 (*M*, average mass); found 2010.9 $[M+H]^+$.

The N^{α}-Fmoc protected peptide thioester (40.0 mg, 20 μmol) was dissolved in ice-cooled DMF (4 ml), then Boc-OSu (107.5 mg, 0.5 mmol) and NMM (55 μl, 0.5 mmol) were added. After the reaction mixture was stirred at 25 °C for 10 h, the solution was concentrated and the resultant residue was triturated with ether (10 ml) twice, giving a powder (32.5 mg, 76% yield). The obtained [**IIb**] was detected as a sharp peak at t_R 17.8 min with minor impurities on an analytical HPLC chromatogram [column, Cosmosil 5C_{18}-AR (4.6×150 mm); elution, a linear gradient of B in A (30 to 65% in 30 min); flow rate, 0.8 ml/min]. Because of its solubility, [**IIb**] was used for segment condensation without further purification. Amino acid ratios in the acid hydrolysate were as follows; Asp 3.20 (3), Ser 1.67 (2), Glu 1.05 (1), Pro 1.02 (1), Gly 1.00 (1), Val 0.76 (1), Met 0.49 (1), Ile 0.73 (1), Leu 1.98 (2), Lys 0.98 (1), Arg 0.92 (1). LSIMS *m/z*: calcd for $C_{95}H_{148}N_{22}O_{28}S_2$ 2110.5 (*M*, average mass); found 2111.0 $[M+H]^+$.

4.4.4. Boc-[Lys(Boc)^{26}]-CCK(1–28)-S$(CH_2)_2$COOEt [III]. This protected peptide was constructed on Fmoc-Pro-Clt resin (250 mg, 0.15 mmol). After the incorporation of the N-terminal Boc-Val-OH, the peptide-resin was dried in vacuo (680 mg). The protected peptide-resin (200 mg, 23.0 μmol) was then treated with a mixture of HFIP/CH_2Cl_2 (1:4, 5 ml) at 25 °C for 30 min and filtered. The fully protected peptide, Boc-Val-Ser(^{t}Bu)-Gln(Trt)-Arg(Pbf)-Thr(^{t}Bu)-Asp(O^{t}Bu)-Gly-Glu(O^{t}Bu)-Ser(^{t}Bu)-Arg(Pbf)-Ala-His(Bum)-Leu-Gly-Ala-Leu-Leu-Ala-Arg(Pbf)-Tyr(^{t}Bu)-Ile-Gln(Trt)-Gln(Trt)-Ala-Arg(Pbf)-Lys(Boc)-Ala-Pro-OH, was obtained from the filtrate as described for the preparation of [**IIa**] (140 mg, quantitative).

The fully protected peptide (132 mg, 23.0 μmol) was dissolved in ice-cooled DMF (5 ml), then HS-$(CH_2)_2$-$COOC_2H_5$ (81 μl, 0.60 mmol), WSCDI·HCl (70 mg, 0.36 mmol), and HOBt (42 mg, 0.36 mmol) were added. After the reaction mixture was stirred at 4 °C for 20 h, the solution was concentrated and the resultant residue was triturated with ether (10 ml) three times, giving a powder. The dried powder was then treated with 95% aqueous TFA (5 ml) at 25 °C for 3 h, and ether (50 ml) was added to the mixture. The formed precipitate was collected by centrifugation and washed with ether. This precipitate was dissolved in 5% AcOH (3 ml), applied to a column of Sephadex G-10 (4×55 cm), and eluted with 5% AcOH. The fractions corresponding to the first peak (detected with the UV absorbance at 280 nm) were pooled and lyophilized, giving a fluffy powder (72.5 mg, 94% yield from the protected peptide). This peptide thioester (71.5 mg) was purified by preparative HPLC [column, Cosmosil 5C_{18}-AR (20×150 mm); elution, an isocratic elution of D in C (32% for 10 min) followed by a linear gradient of D in C (32 to 40% in 50 min); flow rate, 3.0 ml/min] (30.3 mg, 42% recovery on HPLC). LSIMS *m/z*: calcd for $C_{137}H_{231}N_{47}O_{40}S$ 3208.7 (*M*, average mass); found 3209.5 $[M+H]^+$.

The peptide thioester (28.9 mg, 9 μmol) was dissolved in ice-cooled DMF (3 ml), then Boc-OSu (97.3 mg, 0.45 mmol) and NMM (24.8 μl, 0.23 mmol) were added. After the reaction mixture was stirred at 25 °C for 10 h, the solution was concentrated and the resultant residue was triturated with ether (10 ml) twice (22.5 mg, 73% yield). The Boc-protected peptide thioester was purified again by preparative HPLC [column, Cosmosil 5C_{18}-AR (4.6×150 mm); elution, a linear gradient of D in C (30 to 45% in 30 min); flow rate, 1 ml/min]. After lyophilization, 16.2 mg of pure [**III**] was obtained (74% recovery on HPLC). This peptide was detected as a single peak at t_R 13.4 min on an analytical HPLC chromatogram. Amino acid ratios in the acid hydrolysate were as follows; Asp 1.01 (1), Thr 0.95 (1), Ser 1.74 (2), Glu 4.45 (4), Pro 1.00 (1), Gly 1.91 (2), Ala 5.76 (5), Val 0.96 (1), Ile 0.94 (1), Leu 3.10 (3), Tyr 1.00 (1), His 1.00 (1), Lys 1.00 (1), Arg 3.98 (4). LSIMS *m/z*: calcd for $C_{147}H_{247}N_{47}O_{44}S$ 3408.9 (*M*, average mass); found 3409.3 $[M+H]^+$.

4.4.5. Synthesis of CCK-39. Silver nitrate (1.02 mg, 6.0 μmol), HOOBt (9.8 mg, 60 μmol), and DIEA (5.2 μl, 40 μmol) were dissolved in DMSO (250 μl) and stirred at 25 °C for 1 h, then a DMSO solution (1 ml) containing segment [**I**] (4.0 mg, 2.0 μmol) and segment [**IIa**] (6.3 mg, 2.0 μmol) was added. After the mixture was stirred at 25 °C for 24 h, the formed insoluble material was precipitated by centrifugation. Portions of the supernatant (ca. 100 μl each) were subjected to RP-HPLC for isolation of the condensation product, Boc-[Lys(Boc)^{26,36}]-CCK(20–58): [**IIa–I**], [HPLC conditions: column, Cosmosil 5C_{18}-AR (10×250 mm); elution, a linear gradient of B in A (25 to 50% in 60 min); flow rate, 2.0 ml/min]. The eluate corresponding to the main peak (t_R 38.2 min) was pooled and lyophilized. The resultant residue was again lyophilized from 0.02 M NH_4HCO_3 (30 ml) to give a fluffy powder of [**IIa–I**] (5.96 mg, 60% yield). MALDI-TOFMS *m/z*: calcd for $C_{216}H_{340}N_{62}O_{67}S_4$ 5005.7 (*M*, average mass); found 5006.9 $[M+H]^+$.

HPLC-purified [**IIa**–**I**] (5.0 mg, 1.0 μmol) was then treated with pre-cooled 90% TFA (1 ml) at 0 °C for 2 h, and dry ether (50 ml) was added. The formed precipitate was collected by centrifugation and washed with ether twice. This precipitate was dissolved in 0.02 M NH_4HCO_3 (25 ml) and lyophilized to give crude CCK-39 (4.25 mg, 90% yield). The obtained crude CCK-39 (Fig. 7(b)) was used for final characterization without further purification. MALDI-TOFMS *m/z*: calcd for $C_{201}H_{316}N_{62}O_{61}S_4$ 4705.3 (*M*, average mass); found 4706.4 $[M+H]^+$. Amino acid ratios in an acid hydrolysate of crude CCK-39 and the condensation product [**IIa**–**I**] were listed in Table 1.

4.4.6. Synthesis of CCK-58. Silver nitrate (1.02 mg, 6.0 μmol), HOOBt (9.8 mg, 60 μmol), and DIEA (5.2 μl, 40 μmol) were dissolved in DMSO (250 μl) and stirred for 1 h. A DMSO (1 ml) solution containing segment [**I**] (4.0 mg, 2.0 μmol) and segment [**IIb**] (4.3 mg, 2.0 μmol) was then added. After the mixture was stirred at 25 °C for 24 h, the resultant insoluble material was precipitated by centrifugation. Portions of the supernatant (ca. 100 μl each) were subjected to RP-HPLC for isolation of the condensation product, Fmoc-[Lys(Boc)^{36}]-CCK(29–58): [**IIb**–**I**], [HPLC conditions: column, Cosmosil 5C_{18}-AR (10× 250 mm); elution, an isocratic elution of B in A (35% for 10 min) followed by a linear gradient of B in A (35 to 55% in 50 min); flow rate, 1.75 ml/min]. The eluate corresponding to the main peak (t_R 31.7 min) was pooled and lyophilized. The resultant residue was again lyophilized from 0.02 M NH_4HCO_3 (30 ml) to give a fluffy powder of [**IIb**–**I**] (5.32 mg, 67% yield). MALDI-TOFMS *m/z*: calcd for $C_{173}H_{257}N_{47}O_{53}S_4$ 3971.5 (*M*, average mass); found 3973.7 $[M+H]^+$.

The obtained condensation product [**IIb**–**I**] (16.3 mg, 4.1 μmol) was dissolved in a mixture of DMSO (1 ml) and DMF (2 ml), and piperidine (1 ml) was then added. After the reaction mixture was stirred at 25 °C for 3 h, the solution was applied onto a column of Sephadex LH-20 (2.5×66 cm) and eluted with DMF. The eluate corresponding to the first peak was pooled and concentrated. The resultant residue was dissolved in 0.02 M NH_4HCO_3 (20 ml) and lyophilized to give a fluffy powder (12.0 mg, 78% yield). This peptide was detected as a sharp single peak at t_R 14.7 min on an analytical HPLC chromatogram [elution, a linear gradient of B in A (25 to 45% in 30 min); flow rate, 0.8 ml/min]. MALDI-TOFMS *m/z*: calcd for $C_{158}H_{247}N_{47}O_{51}S_4$ 3749.2 (*M*, average mass); found 3750.0 $[M+H]^+$.

The second segment condensation was conducted in a similar manner. Silver nitrate (0.76 mg, 4.5 μmol), HOOBt (7.5 mg, 46 μmol), and DIEA (4.0 μl, 31 μmol) were dissolved in DMSO (200 μl) and stirred at 25 °C for 1 h, then a DMSO solution (1 ml) containing the Fmoc-deprotected segment [**IIb**–**I**] (3.76 mg, 1.0 μmol) and segment [**III**] (5.15 mg, 1.5 μmol) was added. After the mixture was stirred at 25 °C for 24 h, the resultant insoluble material was precipitated by centrifugation. Portions of the supernatant (ca. 100 μl each) were subjected to RP-HPLC for isolation of the condensation product, Boc-[Lys-(Boc)^{26,36}]-CCK(1–58): [**III**–**IIb**–**I**], [HPLC conditions: column, Cosmosil 5C_{18}-AR (4.6×150 mm); elution, a linear gradient of B in A (25 to 65% in 40 min); flow rate, 0.8 ml/min]. The eluate corresponding to the main peak (t_R 19.4 min) was pooled and lyophilized. The resultant residue was again lyophilized from 0.02 M NH_4HCO_3 (20 ml) to give a fluffy powder of [**III**–**IIb**–**I**] (2.76 mg, 40% yield). This peptide was detected as a sharp single peak at t_R 19.1 min on an analytical HPLC chromatogram. MALDI-TOFMS *m/z*: calcd for $C_{300}H_{483}N_{93}O_{94}S_4$ 7024.2 (*M*, average mass); found 7025.2 $[M+H]^+$.

HPLC-purified [**III**–**IIb**–**I**] (3.8 mg, 0.54 μmol) was then treated with pre-cooled 90% TFA (0.5 ml) at 0 °C for 2 h, and dry ether (50 ml) was added. The formed precipitate was collected by centrifugation and washed with ether twice. This precipitate was dissolved in 0.02 M NH_4HCO_3 (15 ml) and lyophilized to give crude CCK-58. The crude CCK-58 was further purified by HPLC [HPLC conditions: column, Cosmosil 5C_{18}-AR (4.6×150 mm); elution, a linear gradient of B in A (30 to 45% in 60 min) at a flow rate of 0.8 ml/min]. The eluate corresponding to the main peak (t_R 36.4 min) was pooled and lyophilized. The resultant residue was lyophilized again from 0.02 M NH_4HCO_3 (15 ml) to give a fluffy powder, CCK-58 (1.62 mg, 45% yield). This peptide was detected as a sharp single peak at t_R 36.2 min on an analytical HPLC chromatogram under the same conditions used for purification. MALDI-TOFMS *m/z*: calcd for $C_{285}H_{459}N_{93}O_{88}S_4$ 6724.6 (*M*, average mass); found 6725.7 $[M+H]^+$. Amino acid ratios in an acid hydrolysate of the purified CCK-58 and the condensation products, [**IIb**–**I**] and [**III**–**IIb**–**I**], were listed in Table 1.

4.4.7. Lysyl endopeptidase digestion of CCK-39 and CCK-58. Lysyl endopeptidase digestion of synthetic CCK-39 was carried out in a similar manner as described before.[9b] Briefly, the purified CCK-39 (150 μg) was digested in 0.1 M NH_4HCO_3 (pH 8.2, 300 μl) at 37 °C for 4 h. The weight ratio of the enzyme to the substrate was about 1:200. The enzyme digest of the CCK-39 gave three peaks (t_R 14.2, 18.7 and 43.2 min) on an HPLC chromatogram [HPLC conditions: column, Cosmosil 5C_{18}-AR (4.6×150 mm); elution, a linear gradient of D in C (5 to 35% in 60 min); flow rate, 0.8 ml/min; absorbance was detected at 230 nm]. These peaks were identified as a 7-mer: H-YIQQARK-OH (t_R 14.2 min), a 10-mer: H-APSGRMSIVK-OH (t_R 18.7 min), and a 22-mer corresponding to CCK-22 (t_R 43.2 min) on amino acid analysis of the acid hydrolysates (data not shown) and mass spectrometry. LSIMS: 7-mer, *m/z*: calcd for $C_{40}H_{67}N_{13}O_{11}$ 905.5 (*M*, monoisotopic mass); found 906.5 $[M+H]^+$; 10-mer, *m/z*: calcd for $C_{44}H_{80}N_{14}O_{13}S$ 1044.6 (*M*, monoisotopic mass); found 1045.6 $[M+H]^+$; CCK-22, *m/z* calcd for $C_{117}H_{173}N_{35}O_{39}S_3$ 2790.0 (*M*, average mass); found 2791.0 $[M+H]^+$.

Purified CCK-58 (160 μg) was similarly digested by lysyl endopeptidase, and the digest was analyzed by HPLC under the same conditions used for synthetic CCK-39. Three peaks (peak 1 to peak 3 according to the order of elution, Figure 9(b)) were detected and were identified as H-APSGRMSIVK-OH (peak 1; t_R 18.8 min), CCK-22 (peak 2; t_R 43.3 min), and the N-terminal 26-mer (peak 3; t_R 48.5 min). Amino acid analyses of the acid hydrolysates; peak 1: Ser (2) 1.85, Gly (1) 1.37, Ala (1) 1.00, Pro (1) 0.85, Val (1) 0.75, Ile (1) 0.68, Met (1) 0.57, Lys (1) 0.75, Arg (1)

0.86; peak 2: Asp (6) 6.51, Ser (2) 2.10, Glu (1) 1.39, Gly (1) 1.41, Pro (1) 0.88, Met (2) 1.40, Ile (1) 1.00, Leu (2) 2.00, Tyr (1) 0.97, Phe (1) 1.00, His (1) 1.15, Arg (2) 2.00, Trp (1) N.D.; peak 3: Asp (1) 1.21, Thr (1) 1.00, Ser (2) 2.07, Glu (4) 4.21, Gly (2) 2.14, Ala (4) 3.78, Val (1) 1.00, Ile (1) 0.84, Leu (3) 2.61, Tyr (1) 0.85, His (1) 1.05, Lys (1) 1.13, Arg (4) 3.35. Part of the enzyme digest was then subjected to MALDI-TOFMS and peaks corresponding to the three peptides were detected on the mass spectrum (Fig. 9(c)).

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20. Two peaks in the HPLC chromatogram (t_R 16.40 and 16.50 min in Figure 8(b)) were suspected as hydrolyzed products of thioester segment [**III**] based on the MS determinations (MALDI-TOFMS); *m/z*: found 3293.4 (for peak at t_R 16.40 min) and 3294.9 (for peak at t_R 16.50 min). A calculated *m/z* for Boc-protected peptide carboxylic acid ($C_{142}H_{239}N_{47}O_{43}$) is 3292.7 (*M*). Further characterization for these peaks was not done.
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Host–guest complexes of 3,5-dinitrobenzonitrile: channels and sandwich supramolecular architectures☆

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Abstract—Host–guest type complexes of 3,5-dinitrobenzonitrile, **1**, with some hydrocarbons like benzene, naphthalene, *p*-xylene, *o*-xylene, and aza donor molecules (acridine, phenazine and phenothiazine) have been reported. In all the complexes, **1** forms a host network, yielding channels (in three-dimensional arrangement), which are filled by guest molecules, except in the complex of **1** with *p*-xylene. In this complex, although a host–guest type network is observed, the molecules of **1** and *p*-xylene are arranged in such a manner that the hydrocarbon is embedded between the layers of **1**, like in inorganic clay structures. All the complexes have been characterized by single crystal X-ray diffraction methods.

1. Introduction

In the new era of crystal engineering of supramolecular assemblies, attention is directed towards the synthesis of functional solids with novel tailor-made properties.[1–3] In this direction, noncovalent bonds and dative bonds have been well utilized, because of their directional nature, to create a variety of supramolecular architectures.[4–7] Among the numerous assemblies, host–guest type systems gained special attention for possible applications in the areas of catalysis, separation technology, biomimetics, etc.[8–10] Early success of the synthesis was obtained with carboxylic acid compounds.[11,12] For instance, trimesic acid is a well-known representative example for a class of organic host-structures,[11d–f] while a molecular adduct of trithiocyanuric acid and 4,4′-bipyridyl is one of the binary component hosts, accommodating different types of guest molecules ranging from benzene to anthracene.[13] Other pseudo host–guest type systems, that we reported were adducts of 3,5-dinitrobenzoic acid, and its 4-methyl and 4-chloro derivatives, with anthracene.[14] In these complexes, the acid molecules form a hexagonal ensemble with cavities, which are being occupied by anthracene molecules (see Fig. 1).

The cavities, however, failed to align in a three-dimensional arrangement to yield channels, thus, true host–guest systems could not be obtained. Further, cavity structures were obtained in the presence of hydrocarbon moieties only, while replacement of the anthracene with aza-molecules like acridine, phenazine, 4,4′-bipyridine, etc. gave different assemblies possessing molecular tapes.[15] Nevertheless, aza-molecules could be incorporated as guests upon replacement of the acid group on 3,5-dinitrobenzoic acid with an amide group.[16a] But, surprisingly, hydrocarbons failed to form host–guest systems with the 3,5-dinitrobenzamide.[16b] Thus, both the acid and its amide were able to form only the guest–specific host lattices. In addition, it is understood that, the host-network is created by both strong (O–H···O, N–H···O) and weak (C–H···O) hydrogen bonds.

Hence, we aimed to explore the feasibility of construction of host lattices entirely through weak hydrogen bonds, as such assemblies are not well known in the literature, and also with the hope of creating a universal host rather than one

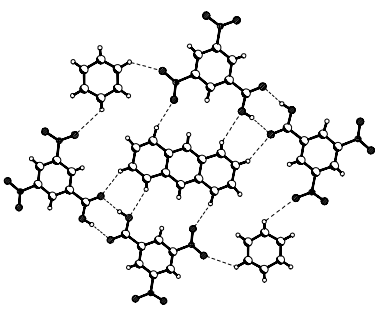

Figure 1. Host–guest complex of 3,5-dinitrobenzoic acid with anthracene in the presence of benzene.

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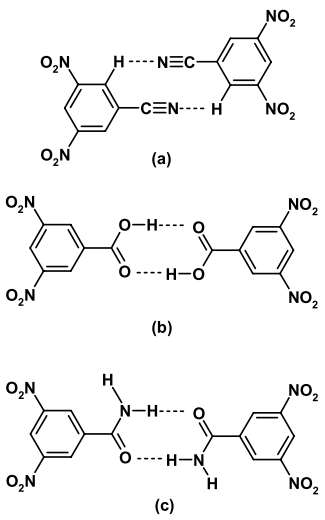


Figure 2. Hydrogen bonding arrangement between the adjacent molecules of (a) 3,5-dinitrobenzonitrile (b) 3,5-dinitrobenzoic acid and (c) 3,5-dinitrobenzamide.

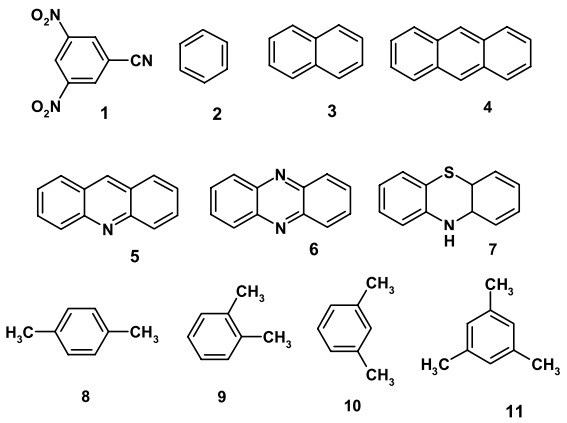


Chart 1.

that is guest specific, utilizing the flexible nature of the weak hydrogen bonds.[17] For this purpose, in our search for a potential molecular entity that provides a topologically similar hydrogen bonding network as that of 3,5-dinitrobenzoic acid and the corresponding amide, we have come across 3,5-dinitrobenzonitrile, **1**, the crystal structure of which was determined by us recently,[18] having the required features. The association of two neighbouring molecules of **1** is shown in Figure 2a along with the similar arrangements for acid and amide analogues in Figure 2b and c, respectively.

Since the topology of the hydrogen bond arrangement is identical, we believed that 3,5-dinitrobenzonitrile, **1** would yield host networks with appropriate guest molecules. Further evidence in this regard is obtained with the reported crystal structure of adduct of **1** and anthracene.[19] Hence, our endeavour started with the crystallization of **1** with benzene, naphthalene, acridine, phenazine, phenothiazine, *-o*, *-m* and *-p* xylenes, mesitylene, etc. Structural aspects of some of these co-crystals, as listed in Chart 1, will be discussed in this article, in a detailed manner.

2. Results and discussion

Crystallization of **1** from a benzene solution gave single crystals, which are quite unstable at ambient conditions suggesting that a solvated complex resulted. Characterization of the single crystals by X-ray diffraction studies reveals a 1:1 molar ratio complex of **1** and benzene. This complex forms channels in its three-dimensional structure along a crystallographic axis. The three-dimensional structure and the arrangement of molecules around the void space are shown in Figure 3a and b, respectively.

The molecules of **1** form a hexagonal network in such a manner that in each hexagon, dimers of **1** (held together by centrosymmetric cyclic C–H···N hydrogen bonds) are connected together by acyclic C–H···O hydrogen bonds (Fig. 3b). The H···N distance in the cyclic pattern is 2.64 Å with the C–H···N angle of 146°, whereas the H···O distance in the acyclic C–H···O hydrogen bonds is 2.94 Å. Other characteristics of the hydrogen bonds are given in Table 1. Thus, a cavity of dimensions 7×15 Å^2 is created

Reactants	Solvent	Product and Composition	
1 + 2	benzene	2a	1:1
1 + 5	methanol	5a	2:1
1 + 6	methanol	6a	2:1
1 + 7	methanol	7a	2:1
1 + 8	methanol	8a	1:1

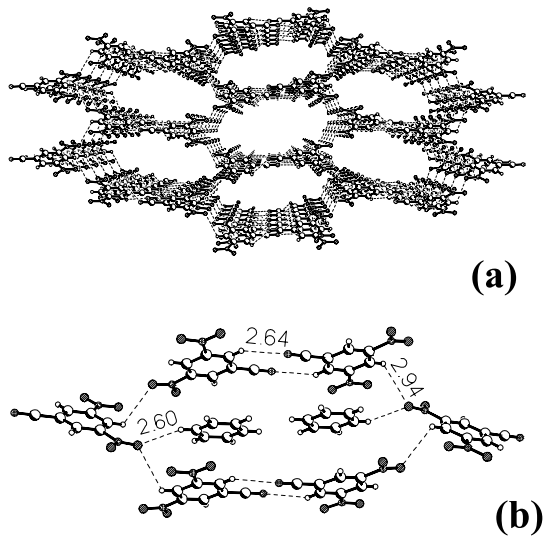


Figure 3. (a) Representation of channels observed in the three-dimensional arrangement of the adduct, **2a**, formed between 3,5-dinitrobenzonitrile, **1** with benzene. (b) Hexagonal arrangement of molecules of **1**, forming a cavity of dimension, (7×15 Å^2).

Table 1. Characteristics of hydrogen bonds[a] distances, Å and angles ° observed in the molecular adducts, **2a**, **5a**, **6a** and **8a**

Hydrogen bonds (donor–H···acceptor)	**2a**			**5a**			**6a**			**8a**		
C–H···O	2.47	3.24	130.0	2.52	3.47	166.2	2.48	3.41	162.7	2.60	3.45	162.9
	2.60	3.46	157.1	2.53	3.35	138.5	2.55	3.37	137.0	2.74	3.44	137.9
	2.61	3.33	125.2	2.59	3.42	139.9	2.57	3.43	142.4	2.78	3.31	120.9
	2.64	3.43	149.5	2.85	3.75	160.6	2.80	3.57	139.6	2.81	3.63	145.0
	2.87	3.42	120.0	2.87	3.63	139.9	2.82	3.72	159.2	2.82	3.57	140.1
	2.94	3.42	113.6							2.85	3.43	125.3
										2.89	3.72	146.6
										2.90	3.75	168.4
										2.90	3.53	124.8
C–H···N	2.64	3.42	145.8	2.45	3.33	158.6	2.48	3.34	161.5	2.65	3.44	143.4
	2.81	3.44	123.9	2.93	3.76	144.1	2.86	3.68	144.1	2.88	3.66	143.5
	2.87	3.60	131.9							2.91	3.58	129.9

[a] In each complex, the three numbers correspond to H···acceptor, donor···acceptor and angle.

and benzene molecules fit in these cavities, interacting with the host network through the formation of C–H···O hydrogen bonds. These hydrogen bonds were formed between the aromatic hydrogens of benzene and $-NO_2$ groups of **1**, with an H···O distance of 2.60 Å (Table 1). Thus, adduct **2a**, as anticipated, gave a host–guest channel structure, entirely made up of weak hydrogen bonds, C–H···N and C–H···O.

Further, a close look at Figure 3b shows that molecules having dimensions equivalent to that of two or three fused benzene moieties can also form similar complexes, replacing the benzene molecules. Hence, we attempted to co-crystallize **1** with naphthalene, **3**. However, the crystals of the adduct of **1** were highly unstable as well as of poor quality to carry out any further analysis. Instead, we obtained stable and good quality single crystals with acridine, **5**, and phenazine, **6**, in a 2:1 ratio. The two-complexes have been labeled as **5a** and **6a**, respectively.

Analysis of X-ray diffraction data of **5a** and **6a** reveal that these two are isomorphous to complex **2a**, with similar unit cell dimensions and crystallizing in the same space group, $P2_1/c$. Further, **2a**, **5a** and **6a** are also iso-structural with the formation of channel structures in the three-dimensional arrangement, similar to the one shown in Figure 3a for **2a**; the only difference being that the channels are occupied by single molecules[20] of either acridine or phenazine molecules in **5a** and **6a** as shown in Figure 4a and b, respectively. In each hexagon the molecules of **1** exist as dimers by forming a centrosymmetric 10-membered ring pattern through C–H···N hydrogen bonds (H···N, 2.45 and 2.48 Å for **5a** and **6a**, respectively, Table 1). Such adjacent dimers are further held together by C–H···O hydrogen bonds (**5a**, H···O, 2.87 and **6a**, 2.80 Å) leading to the formation of cavities (Fig. 4).

A noteworthy and unique nature of the adducts **2a**, **5a** and **6a** is the retention of the host lattice by **1**, irrespective of the nature of the functional groups present on the guest molecules, unlike in the corresponding acid and amide analogues. This is further reflected in the formation of an iso-structural complex between **1** and phenothiazine as confirmed by single crystal[21] as well as X-ray powder diffraction techniques. The powder X-ray diffraction patterns are shown in Figure 5.

The patterns shown in Figure 5, were obtained from the ground mixtures of **1** with **5**, **6** and **7**. It is evident that the intense peak at 25.5°, as well as other minor peaks in Figure 5c match with similar peaks shown in Figure 5a and b. Hence, it could be concluded that **1** and **7** also forms a complex, which is iso-structural to that of **5a** and **6a**. 3,5-dinitrobenzonitrile, **1**, however, forms a different host–guest structure, in an intercalated manner (see Fig. 6a), with *p*-xylene and *o*-xylene.

Crystallization of **1** from *o*-, *m*-, and *p*-xylenes as well as mesitylene gave colorless, block-like single crystals from only *o*-xylene[22] and *p*-xylene, which are also unstable like **2a**, but, *m*-xylene and mesitylene yielded only parent crystals of **1**. The crystals obtained from *p*-xylene have been labeled as **8a**. Structure determination reveals that **8a** crystallizes in a 1:1 ratio of **1** and *p*-xylene, with two molecules of each in the asymmetric unit. The two symmetry independent molecules of **1** are labeled as A and B, while the *p*-xylene molecules are labeled as C and D.

In adduct **8a**, *p*-xylene and **1** form a stacked sheet structure, in the three-dimensional arrangement, shown in Figure 6b,

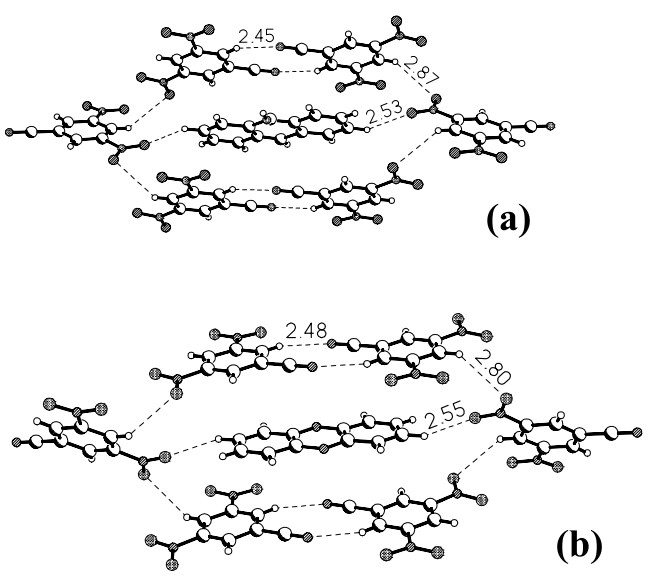


Figure 4. Arrangement of molecules in the adducts (a) **5a** and (b) **6a**, in an hexagonal manner creating voids, which are occupied by guest molecules.

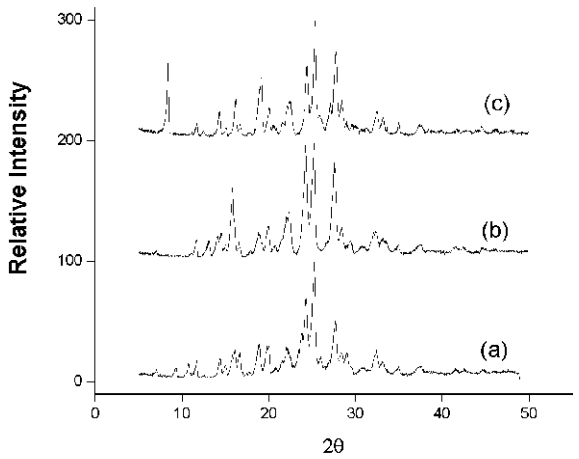
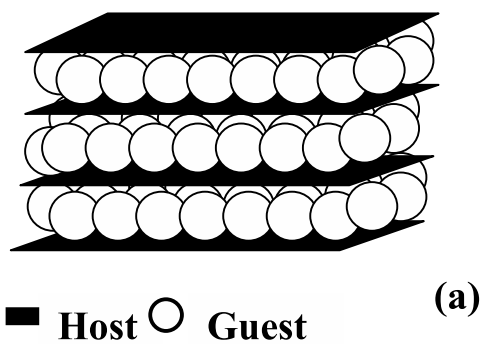


Figure 5. Powder X-ray diffraction patterns of ground mixture of complexes of **1** with (a) acridine (b) phenazine and (c) phenothiazine.



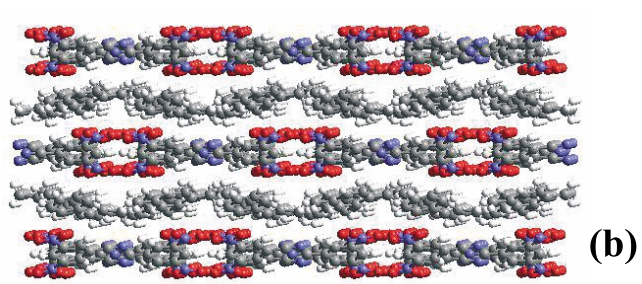
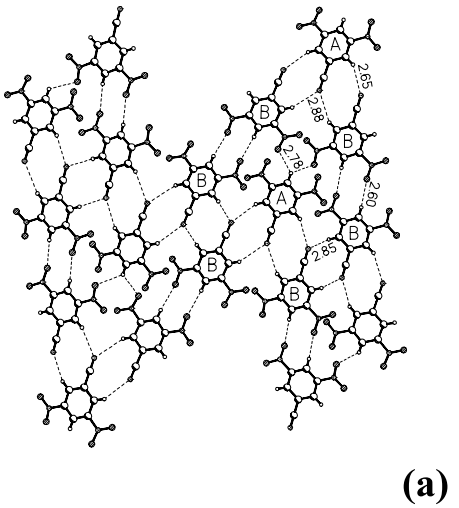


Figure 6. (a) Schematic represenatation of intercalated host–guest complexes. (b) Three-dimensional arrangement of 3,5-dinitrobenzonitrile, **1** and *p*-xylene molecules in the alternate layers in the crystal structure of adduct, **8a**.

such that *p*-xylene molecules are embedded between the layers of **1**, with a close resemblance to inorganic clay structures.[23]

Within the layers of **1**, the molecules are arranged in such a way that interaction between the symmetry independent molecules (A–B) and symmetry dependent molecules (B–B) constitute zig-zag molecular tapes. There is no interaction of the type A–A. This arrangement is shown in Figure 7a. In each tape, molecules A and B are held together by C–H···N hydrogen bond dimers with H···N distances of 2.65 and 2.88 Å (Table 1). However, symmetry dependent molecules (B) form centrosymmetric cyclic C–H···O hydrogen-bonded coupling with an H···O distance of



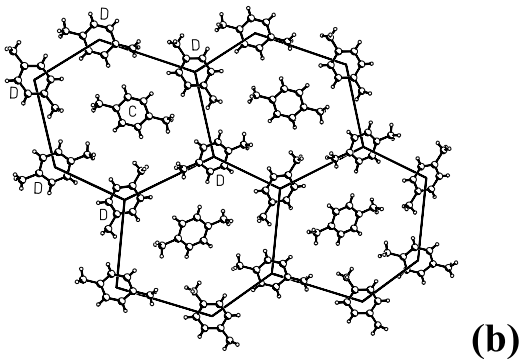


Figure 7. (a) Interaction among the molecules of **1** within a two-dimensional sheet in the complex, **8a**. (b) Two-dimensional sheet arrangement of *p*-xylene molecules constituting a hexagonal arrangement.

2.60 Å. Such adjacent tapes are further connected to each other forming a centrosymmetric cyclic coupling consisting of C–H···N hydrogen bonds, (H···N, 2.85 Å, Table 1). However, in the *p*-xylene layers, the two symmetry independent molecules arrange such that each of six molecules of a particular symmetry (say D) form a hexagonal network around the other molecules (say C), as shown in Figure 7b. This network is stabilized by typical H···H van der Waals interactions, generally known for hydrocarbons.[24]

2.1. Hexagonal versus intercalated host–guest complexation

A comparison of **2a**, **5a**, **6a** and **8a** reveals that the dimensions of the guest molecules (benzene, phenazine and acridine) in adducts, **2a**, **5a** and **6a** is approximately 9 Å.[25] Thus, those molecules could fit into the channels with average dimensions 14×7 Å^2 formed by the hexagonal arrangement of host molecules. However, *p*-xylene with the dimension of ~7 Å appears to be too small to remain in the channels of 14 Å as a single molecule like acridine and phenazine in **5a** and **6a**, respectively, and at the same time it will become big to occupy as two molecules like benzene in **2a**. Hence *p*-xylene failed to yield an iso-structural complex. In general, if the dimension of the guest molecules is inappropriate to the void space that is being created by the hexagonal arrangement of host molecules, the result is often

the formation of a different type of assembly. Nevertheless, still, **1** is able to form a host–guest type complex with *p*-xylene, perhaps, due to the flexibility of the weak C–H···N hydrogen bonds for the reorganization to be commensurate with the dimensions of guest molecules. Thus, it may be concluded that 3,5-dinitrobenzonitrile, **1**, would yield host–guest type complexes, possessing hexagonal channels with the guests of dimension ~9 Å, otherwise, it may form intercalated channel structures. However, if the incoming substrate possesses strong donor/acceptor groups, naturally, **1** would yield different types of molecular adducts.[18]

In conclusion, we have reported host–guest complexes of 3,5-dinitrobenzonitrile, **1**, with various hydrocarbons, as well as aza molecules in which **1** acts as a host lattice accommodating different types of guest molecules. It is also observed that depending upon the dimensions of the guest molecules, different types of host networks are created to yield either hexagonal channels or intercalated channels. These variations appear to be the result of involvement of only weak hydrogen bonds in the formation of either host-network or host–guest interactions. Thus, these examples have demonstrated the utility of the weak hydrogen bonds such as C–H···N, C–H···O, etc. for the creation of flexible supramolecular assemblies, which may have potential applications in the areas of catalysis, separation technology, etc. due to the formation of structures with void space.

3. Experimental

3.1. Synthesis of 2a, 5a, 6a and 8a

All the chemicals were obtained from commercial suppliers (Sigma-Aldrich) and used without further purification. HPLC grade solvents were used for the crystallization experiments. Synthesis of co-crystals was carried out by dissolving the reactants in the appropriate solvents, either at room temperature or by warming on a water bath, and subsequently cooling by a slow-evaporation method. In a typical experiment, 96.5 mg (5 mmol) of 3,5-dinitrobenzonitrile and 45.1 mg of phenazine (2.5 mmol) were dissolved in a boiling methanol solution and then subsequently cooled to room temperature. Yellow colored stable and plate-like single crystals of good quality were obtained over a period of 3 days and were used for X-ray diffraction studies. However, in the case of adducts of hydrocarbons like benzene, *p*-xylene, the obtained complexes were found to be unstable upon removing from the mother liquor. Crystallographic studies on these crystals were carried out following special procedures as described in Section 3.2.

3.2. X-ray crystallography

Good-quality single crystals carefully chosen using a Leica microscope equipped with CCD camera were used to collect X-ray intensity data on a Bruker diffractometer (APEX CCD area detector). The data collection were carried out at 133 K for **2a** and **8a**, whereas for **5a** and **6a** were carried out at room temperature (293 K). The unstable crystals **2a** and **8a** were smeared in paraffin oil as soon as removed from the mother liquor, to protect from decomposition during the data collection period. The data were processed using Bruker suite of programmes (SAINT).[26] Structure determination and refinements were carried out using SHELXTL package.[27] All the intermolecular interactions were computed using PLATON prorgramme.[28] Full details of crystallographic information are deposited at Crystallographic Data Centre as supplementary publication (**2a**, CCDC 219919; **5a**, CCDC 219920; **6a**, 219921; **8a**, 219922). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB12 1EZ, UK [fax: +44-1223-336033 or email: deposit@ccdc.cam.ac.uk].

3.2.1. Crystal data for 2a. $(C_7H_3N_3O_4)$: (C_6H_6), fw=271.23, monoclinic, $P2_1/c$, a=6.836(2) Å, b=7.229(2) Å, c=25.840(1) Å, β=92.44°(1), V=1275.8(5) Å^3, Z=4, D_{calc}=1.412 g cm^{-3}, $F(000)$=560, λ(Mo Kα)=0.71073, T=133 K, reflections collected/unique 5167/1806 (R_{int}=0.0362), final GooF=1.210, R_1=0.0592, wR_2=0.1428, 217 parameters, μ=0.108 mm^{-1}, R indices based on 1633 reflections with $I>2\sigma(I)$ (refinement on F^2), absorption corrections applied.

3.2.2. Crystal data for 5a. $(C_7H_3N_3O_4)$: $(C_{13}H_9N_1)$, fw=283.23, monoclinic, $P2_1/c$, a=6.979(1) Å, b=7.277(1) Å, c=24.807(4) Å, β=90.48°(3), V=1259.8(3) Å^3, Z=4, D_{calc}=1.493 g cm^{-3}, $F(000)$=582, λ(Mo Kα)=0.71073, T=133 K, reflections collected/unique 5201/1803 (R_{int}=0.0212), Final GooF=1.038, R_1=0.0368, wR_2=0.1034, 232 parameters, μ=0.114 mm^{-1}, R indices based on 1482 reflections with $I>2\sigma(I)$ (refinement on F^2), absorption corrections applied.

3.2.3. Crystal data for 6a. $2(C_7H_3N_3O_4)$: $(C_{12}H_8N_2)$, fw=283.23, monoclinic, $P2_1/c$, a=6.873(1) Å, b=7.308(1) Å, c=24.871(5) Å, β=90.68°(1), V=1249.1(4) Å^3, Z=4, D_{calc}=1.506 g cm^{-3}, $F(000)$=580, λ(Mo Kα)=0.71073, T=293 K, reflections collected/unique 5094/1787 (R_{int}=0.0176), Final GooF=1.046, R_1=0.0390, wR_2=0.1036, 219 parameters, μ=0.116 mm^{-1}, R indices based on 1546 reflections with $I>2\sigma(I)$ (refinement on F^2), absorption corrections applied.

3.2.4. Crystal data for 8a. $(C_7H_3N_3O_4)$: (C_8H_{10}), fw=897.85, monoclinic, $C2/c$, a=16.628(5) Å, b= 13.530(4) Å, c=21.080(6) Å, β=112.95°(1), V=4367.0(2) Å^3, Z=4, D_{calc}=1.366 g cm^{-3}, $F(000)$=1872, λ(Mo Kα)=0.71073, T=133 K, reflections collected/unique 9077/3131 (R_{int}=0.0264), Final GooF=1.128, R_1=0.0491, wR_2=0.1137, 378 parameters, μ=0.102 mm^{-1}, R indices based on 2560 reflections with $I>2\sigma(I)$ (refinement on F^2), absorption corrections applied.

4. Supplementary Material

A total of 44 pages, X-ray data with details of refinement procedures (cif files), ORTEP diagrams, lists of bond parameters (bong lengths and angles), structure factors of molecular complexes **2a**, **5a**, **6a** and **8a**.

Acknowledgements

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20. Occupation of cavities by single molecules of acridine and

phenazine, indeed, suggests dense-packing of channels in **5a** and **6a** than in **2a**, which is also reflected in the calculated densities of crystal lattices (**2a**, 1.412; **5a**, 1.493 and **6a**, 1.506 g cm^{-3}).

21. Unit cell dimensions of **1** and **7** are a=7.060(1), b=7.112(9), c=24.850(4), α=90.00, β=90.20(1), γ=90.00°, V=1248.0(3) $Å^3$. Since these parameters are very similar to those for **2a**, **5a** and **6a**, and also the iso-structurality among them is established through powder X-ray diffraction methods, crystal structure determination is not carried out.
22. Single crystals of *o*-xylene adduct of **1** are extremely unstable that we were able to collect data sufficient for unit cell determination only. Unit cell parameters are a=13.86(1), b=16.63(9), c=25.65(2), α=71.82(8), β=82.94(4), γ=66.13(4)°, V=5135(6) $Å^3$. These unit cell parameters are closely similar to that of **8a**, suggesting iso-structurality with **8a**.
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25. The dimension of a benzene molecule is ≈4.5 Å. However, taking into account of two molecules present in the channels, the dimension of the guest is considered to be 9 Å.
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Regioselective acylation of carbohydrate derivatives using lipases leading to a facile two-step procedure for the separation of some α- and β-glucopyranosides and galactopyranosides

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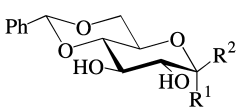
Abstract—The resolution of α- and β-anomers of glucopyranosides and galactopyranosides was achieved via enzyme-catalysed regioselective acylation. This two-step procedure to prepare pure α- and β-anomers of glycopyranosides would be most useful for the cases where glycosidases are not available or expensive to purchase. From a synthetic viewpoint, the regioselective acylation of glycopyranosides provides access to mono- and di-esters with well-defined substitution patterns.

1. Introduction and background information

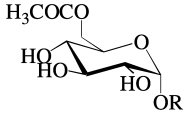
Enzyme-catalysed esterification of carbohydrates and their simple derivatives has been studied extensively.[1] For hexapyranoses, the primary hydroxyl group is esterified preferentially and the preparation of 6-*O*-acylated carbohydrates and glycopyranosides has been a subject of continued interest,[2] especially in connection with the synthesis of novel surfactants[3] and the manufacture of antibacterial agents of potential interest to the food industry.[4]

Following acylation at the 6-position of glycopyranosides, one or more of the secondary hydroxyl groups may be esterified on prolonged reaction.[5] It has also been shown that the configuration of the substituent at the anomeric position can influence the regioselectivity of the acylation of the secondary hydroxyl groups. For example, in a study of the acylation of 4,6-benzylidene glucopyranosides using *Pseudomonas cepacia* lipase as catalyst, acetylation of the α-methoxy compound **1** occurred exclusively at C(2)–OH while the isomer **2** was acetylated with very high selectivity at C(3)–OH.[6] The ethylthio derivative **3** behaves in a similar manner and this regioselective reaction has been used as a key step in the synthesis of the core motif of asparagine-linked glycoprotein oligosaccharides.[7]

Of greatest relevance to the present study is the earlier work by Riva and co-workers.[8] The Italian team showed that α-methyl- and α-benzyl-D-glucopyranoside gave only the 6-acetates **4** and **5** (98% yield) on reaction with vinyl acetate and *Candida antarctica* lipase B in THF/pyridine over 20 h., while β-methyl-D-glucopyranoside furnished the 3,6-di-acetate **6** in 97% yield. Under the same conditions α-methyl-D-galactopyranoside gave a mixture of the 6-acetylated product **7** (74%) and a small amount of the 2,6-diester **8** (24%). In contrast β-methyl-(D)-galacto-pyranoside afforded the 3,6-diacetate **9** as the major product (44%) with lesser amounts of the 2,6-diacetate **10** (31%) and the 6-monoester **11** (24%) after 48 h. Later, it was shown that the benzyl compound **12** was acylated using trifluoro-ethyl butanoate in acetone and in the presence of subtilisin to give the 3,6-diester **13** in 89% yield.[9]



(1) R^1 = OMe, R^2 = H
(2) R^1 = H, R^2 = OMe
(3) R^1 = H, R^2 = SEt



(4) R = Me
(5) R = Bn

Keywords: Regioselective acylation; α- and β-Anomers; Glycopyranosides.
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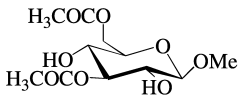
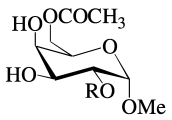
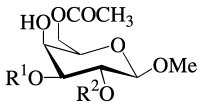


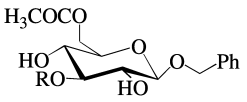
(6)



(7) R = H
(8) R = $COCH_3$



(9) $R^1 = COCH_3$, R^2 = H
(10) R^1 = H, $R^2 = COCH_3$
(11) $R^1 = R^2$ = H

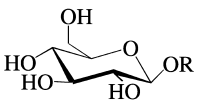


(12) R = H
(13) R = COC_3H_7

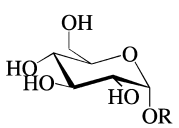
In this paper, we extend the studies of Riva et al.[8] to show that the methodology can lead to the separation of α- and β-glycopyranosides.

2. Results and discussion

Benzyl β-D-glucopyranoside **14** and ethyl β-D-glucopyranoside **15** were synthesised from D-glucose in 43 and 85% yield, respectively, using β-glucosidase from almonds.[10]



(14) R = CH_2Ph
(15) R = Et



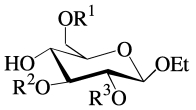
(16) R = CH_2Ph
(17) R = Et

.

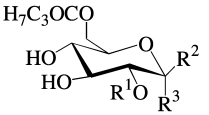
The same enzyme was used selectively to hydrolyse an anomeric mixture of α- and β-benzyl glucoside to afford the unreacted α-anomer **16** in 67% yield. The α-ethoxy compound **17** was prepared from glucose in a similar manner, but in a more moderate yield of 36% over the two steps.

A battery of lipases were inspected for their ability to catalyse the acylation of ethyl β-glucopyranoside **15**. Immobilised *Mucor miehei* lipase (Lipozyme®) and immobilised *Candida cylindracea* B lipase (Novozyme®) were the most efficient catalysts for esterification using vinyl butyrate in THF. Both enzymes gave the 6-*O*-butyrate **18** as the first formed product. Further esterification of the mono-ester **18** with Novozyme® in THF at 60 °C gave the 3,6-diester (50%) **19** and the 2,6-diester (30%) **20**. In contrast, prolonged reaction of the β-benzyl compound **14** with vinyl butyrate in THF containing Novozyme® afforded only the 6-*O*-butanoate **21** (98% isolated yield after a 3 h reaction), in accord with the earlier results documented by Riva et al.[8]

The α-anomers **16** and **17** showed an interesting difference in reactivity, inasmuch as the α-benzyl anomer **16** reacted ca. 5×faster than the β-isomer **14** while the α-ethyl compound **17** reacted ca. 5×slower than the corresponding β-anomer **15** under our standard esterification conditions. In fact, similar patterns of α-/β-reactivity for large[11] and small[12] substituents at the anomeric position have been documented previously. The products from the reactions of **16** and **17** were the appropriate 6-*O*-butanoates **22** and **23**. No further reaction of the latter compounds with vinyl butanoate and Novozyme® could be detected even after prolonged periods of time. Only when a different enzyme was employed (namely *Pseudomonas cepacia* lipase PS-CII) could further acylation of monoester **22** be achieved. By employing this enzyme in vinyl butyrate as solvent, an 86% yield of diester **24** was obtained after 3 days. It was noted that *Ps cepacia* lipase PS CII did not catalyse esterification of monoester **21** under these conditions.



(18) $R^1 = COC_3H_7$, $R^2 = R^3$ = H
(19) $R^1 = R^2 = COC_3H_7$, R^3 = H
(20) $R^1 = R^3 = COC_3H_7$, R^2 = H



(21) $R^1 = R^3$ = H, $R^2 = OCH_2Ph$
(22) $R^1 = R^2$ = H, $R^3 = OCH_2Ph$
(23) $R^1 = R^2$ = H, R^3 = OEt
(24) $R^1 = COC_3H_7$, R^2 = H, $R^3 = OCH_2Ph$
(25) $R^1 = COC_3H_7$, $R^2 = R^3$ = H

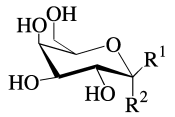
.

From this initial study, it was clear that the different reactivity patterns could be used to separate mixtures of α- and β-anomers of glycopyranosides. Thus the mixture of α- and β-ethyl glucopyranoside, simply prepared from ethanol and glucose under acidic conditions, was reacted with vinyl

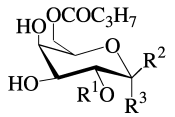
butyrate in THF at 60 °C in the presence of Novozyme® for a period of 4 days. The diesters **19** and **20** (31%) were separated from the monoester **23** (48%). Methanolysis of the diesters gave ethyl β-D-glucopyranoside **15** while similar treatment of the mono-ester gave the α-anomer **17** in quantitative yield.

A combination of Novozyme® and *Ps. cepacia* lipase PS CII could be used to effect a separation of α- and β-benzyl (D)-glucopyranosides. A mixture of the α- and β-benzyl anomers was reacted with the two enzymes in vinyl butyrate for 4 days. The monoester **21** (36%) was readily separated from the diester **24** (28%) by column chromatography. Base-catalysed methanolysis of these esters gave pure β-benzyl-(D)-glucopyranoside and pure α-benzyl-D-glucopyranoside, respectively, in quantitative yields.

It was also clear that this three-step procedure to prepare glycopyranosides would be more useful for the cases where glycosidases are not available, or are expensive to purchase. Thus, we extended the study to galactosides because, while β-benzyl-D-galactoside **26** is available by a modified Königs–Knorr glycosidation procedure,[13] the corresponding α-anomer **27** is not easy to access. Standard Fischer derivatisation of galactose gives a mixture of **26** and **27**. Incubation of this mixture with Novozyme® in THF containing vinyl butyrate over 7.5 h gave the mono ester **28** (46%) and the diester **29** (23%) which were readily separated by chromatography over silica. Methanolysis of the mono ester **28** gave benzyl α-D-galactoside **27** in 91% yield.



(26) $R^1 = OCH_2Ph$, $R^2 = H$
(27) $R^1 = H$, $R^2 = OCH_2Ph$



(28) $R^1 = R^2 = H$, $R^3 = OCH_2Ph$
(29) $R^1 = COC_3H_7$, $R^2 = OCH_2Ph$, $R^3 = H$

.

It is also obvious, but noteworthy from a synthetic viewpoint, that selectively esterified glucose or galactose derivatives are available from some of the compounds in the above portfolio by hydrogenation to remove the anomeric benzyl group. For example, the ester **24** afforded the 2,6-diester **25** (75% yield) on reduction, using palladium on charcoal (10%) as the catalyst.

This work provides further evidence that the regioselective acylation of glycopyranosides can provide access to mono- and di-esters with well-defined substitution patterns. We have shown that the different degrees of reactivity of α- and β-glycosides potentially allows access to a wide range of pure α-anomers which may not be readily accessed by other methodology.

3. Experimental

3.1. General

All reactions were monitored by thin layer chromatography, which was performed on 200–250 μm thickness Merck silica gel plates (60_{F254}). Compounds were detected by ultraviolet light or by staining with ceric ammonium molybdate solution followed by heating. Column chromatography was performed on Merck-60 silica gel (230–240 mesh). Melting points were recorded on a Reichert instrument and are uncorrected. Elemental analyses were performed on a Carlo Erba Elemental Analyser model 1106. IR spectra were recorded on a Perkin–Elmer 883 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded as solutions in deuteriated solvents (Aldrich, Fluorochem) on AVANCE 400 MHz or Varian Gemini300 instruments. ^1H- and ^{13}C-spectra were referenced using TMS as internal standard. Chemical shifts (δ) are quoted in parts per million (ppm) and the coupling constants (J) in Hertz (Hz). The following abbreviations are used to describe the multiplicity: s, singlet; d, doublet; t, triplet; tt, triplet of triplets; q, quartet; dd, doublet of doublets; ddd, doublet of doublets of doublets; dt, doublet of triplets; m, multiplet; br, broad. Optical rotations $[\alpha]_D^T$ (concentration in g/100 cm^3, solvent) were recorded on an Optical Activity A1000 polarimeter at 589 nm, where T is the temperature in °C. Low resolution CI mass spectra were measured on a Fisons TRIO 1000 spectrometer. Accurate mass spectra were obtained on a VG Analytical 7070E double focussing magnetic mass spectrometer. β-Glucosidase from almonds (EC number 3.2.1.21) and Novozyme® 435 (EC number 3.1.1.3) were purchased from Sigma-Aldrich. Lipase PS-C II was purchased from Amano Pharmaceuticals. All solvents and reagents were purchased and used directly from commercial suppliers without purification.

3.1.1. Ethyl β-D-glucopyranoside (15). D-Glucose (0.81 g, 4.48 mmol) was added to a solution of water (2 cm^3) in ethanol (18 cm^3), and heated to 50 °C, with stirring. After 10 min β-glucosidase from almonds (0.11 g) was added to the reaction mixture which was stirred for 3 days. The solution was filtered through Celite® and washed with ethanol (20 cm^3). The excess of solvent was removed under reduced pressure yielding a yellow crude product, which was chromatographed over silica with chloroform–methanol (9:1) yielding compound **15** as a white solid (0.79 g, 85%); mp 82–84 °C (lit.[14] 98–100 °C). (Found: C, 45.88; H, 7.80. $C_8H_{16}O_6$ requires C, 46.15; H, 7.75%); $[\alpha]_D^{23}$ −28.5 (*c* 1.0 in MeOH); ^1H NMR (D_2O): δ 1.11 (3H, t, J=7.1 Hz, CH_3), 3.12 (1H, t, J=9.1 Hz), 3.25 (1H, t, J=9.1 Hz), 3.31–3.38 (2H, m, CH_2), 3.56–3.64 (2H, m), 3.79 (1H, d, J=12.1 Hz), 3.84 (1H, t, J=8.1 Hz,), 4.34 (1H, d, J=8.1 Hz,); ^{13}C NMR (D_2O) δ 14.7, 61.2, 66.6, 70.1, 73.6, 76.3, 76.3, 102.3; *m/z* (CI) $[M+NH_4]^+$226 (100%).

3.1.2. Ethyl 6-*O*-butyryl β-D-glucopyranoside (18). Novozyme® 435 (0.42 g) was added to a solution of ethyl β-D-glucopyranoside **15** (0.42 g, 2.01 mmol) in vinyl butyrate (0.52 cm^3, 4.1 mmol) and THF (10 cm^3). The reaction mixture was stirred for 1 h at 60 °C, after which time the enzyme was filtered off and washed with THF (10 cm^3). The excess THF was evaporated under reduced

pressure to afford a crude residue which was purified by flash chromatography over silica with ethyl acetate–ethanol (95:5) as eluent, yielding compound **18** (0.45 g, 81%) as a white solid; mp 80 °C. (Found: C, 51.92; H, 8.06. $C_{12}H_{22}O_7$ requires C, 51.79; H, 7.97%); $[\alpha]_D^{26}$ −51 (*c* 1.0 in $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 1730 (C═O); ^{1}H NMR ($CDCl_3$) δ 0.96 (3H, t, *J*=8.1 Hz, C*H*$_3$), 1.26 (3H, t, *J*=7.1 Hz, C*H*$_3$), 1.63–1.71 (2H, m, C*H*$_2$CH$_3$), 2.36 (2H, t, *J*=8.1 Hz, C*H*$_2$CO), 3.35–3.41 (2H, m), 3.46 (1H, ddd, *J*=2.0, 5.1, 9.1 Hz), 3.55–3.65 (2H, m), 3.96 (1H, qd, *J*=3.0, 14.2 Hz, C*H*$_2$) 4.29 (1H, d, *J*=7.1 Hz), 4.30 (1H, dd, *J*=12.1, 2.0 Hz), 4.49 (1H, dd, *J*=12.1, 5.1 Hz); ^{13}C NMR ($CDCl_3$), δ 13.9 (C*H*$_3$), 15.4 (C*H*$_3$), 18.7 (C*H*$_2$CH$_3$), 36.4 (C*H*$_2$CO), 63.4, 66.0, 70.4, 74.0, 74.4, 76.3, 102.8, 174.9 (CO); *m*/*z* (CI) $[M+NH_4]^+$ 296 (100%).

3.1.3. Ethyl 3,6-*O*-butyryl β-D-glucopyranoside (19) and ethyl 2,6-*O*-butyryl β-D-glucopyranoside (20). Novozyme® (0.21 g) was added to a solution of ethyl 6-*O*-butyryl β-D-glucopyranoside **18** (0.44, 1.59 mmol) and vinyl butyrate (0.4 cm^3, 3.16 mmol) in THF (10 cm^3). The reaction mixture was heated to 60 °C and stirred for 7 days, after which time the enzyme was filtered off and washed with THF (10 cm^3). The solvent was evaporated under reduced pressure to afford a crude residue which was chromatographed over silica with ethyl acetate–ethanol (95:5) as eluent and re-chromatographed over silica gel with ethyl acetate–petroleum ether (2:1) as eluent affording compound **19** (0.28 g, 50%) and compound **20** (0.17 g, 30%). Compound **19**. (Found: C, 54.97; H, 8.17. $C_{16}H_{28}O_8$ requires C, 55.16; H, 8.10%); $[\alpha]_D^{27}$ −17.6 (*c* 1.1 in $CHCl_3$); mp 36–38 °C; ν_{max} ($CHCl_3$)/cm^{-1} 1730 (C═O); ^{1}H NMR ($CDCl_3$) δ 0.94–0.99 (6H, m, C*H*$_3$), 1.26 (3H, t, *J*=7.1 Hz, C*H*$_3$), 1.62–1.74 (4H, m, 2×C*H*$_2$CH$_3$), 2.35 (2H, t, *J*=8.1 Hz, C*H*$_2$CO), 2.40 (2H, t, *J*=7.1 Hz, $'$C*H*$_2$CO), 3.46–3.55 (3H, m), 3.63 (1H, qd, *J*=2.0, 16.2 Hz), 3.96 (1H, qd, *J*=3.0, 14.2 Hz, C*H*$_2$), 4.34 (1H, d, *J*=8.1 Hz), 4.35–4.44 (2H, m), 4.93 (1H, t, *J*=9.1 Hz); ^{13}C NMR ($CDCl_3$) δ 13.5 (*C*H$_3$), 13.6 (*C*H$_3$), 15.1 (*C*H$_3$), 18.4 (*C*H$_2$CH$_3$), 18.4 (*C*H$_2$CH$_3$), 36.0 (*C*H$_2$CO), 36.2 (*C*H$_2$CO), 63.0 (*C*H$_2$), 65.7 (*C*H$_2$), 69.4, 72.13, 74.4, 77.6, 102.6, 174.0 (C═O), 175.1 (C═O); *m*/*z* (CI) $[M+NH_4]^+$ 366 (88%). Compound **20**. (Found: C, 54.96; H, 8.15. $C_{16}H_{28}O_8$ requires C, 55.16; H, 8.10%); $[\alpha]_D^{26}$ −48.8 (*c* 1.1 in $CHCl_3$); mp 48 °C; ν_{max} $CHCl_3$/cm^{-1} 1760 (C═O); ^{1}H NMR ($CDCl_3$) δ 0.94–0.99 (6H, m, 2×C*H*$_3$), 1.19 (3H, t, *J*=7.1 Hz, C*H*$_3$), 1.63–1.73 (4H, m, 2×C*H*$_2$CH$_3$), 2.36 (4H, t, *J*=7.1 Hz, 2×C*H*$_2$CO), 3.41–3.48 (2H, m), 3.51–3.62 (2H, m), 3.89 (1H, qd, *J*=2.0, 16.2 Hz), 4.32 (1H dd, *J*=2.0, 12.1 Hz), 4.43 (1H, d, *J*=8.1 Hz), 4.44 (1H dd, *J*=2.0, 12.1 Hz), 4.76 (1H, dd, *J*=9.1, 8.1 Hz); ^{13}C NMR ($CDCl_3$) δ 13.8 (*C*H$_3$), 13.9 (*C*H$_3$), 15.4 (*C*H$_3$), 18.7 (*C*H$_2$CH$_3$), 18.8 (*C*H$_2$CH$_3$), 36.4 (*C*H$_2$CO), 36.6 (*C*H$_2$CO), 63.3 (*C*H$_2$), 65.7 (*C*H$_2$), 71.1, 74.1, 74.1, 75.7, 101.0, 174.0 and 174.8 (C═O); *m*/*z* (CI) $[M+NH_4]^+$ 366 (37%).

3.1.4. Ethyl α-D-glucopyranoside (17). A mixture of Amberlite IR-120 (H^+) (3.5 g) and anhydrous D-glucose (3.02 g, 16.76 mmol) in ethanol (25 cm^3) was refluxed for 20 h. After cooling and filtration of the resin, excess ethanol was evaporated under reduced pressure affording a yellow syrup which was dissolved in pH 5.0 buffer (25 cm^3) and heated to 35 °C. β-Glucosidase from almonds (0.047 g) was added to the reaction mixture. After 3 days the solution was filtered through Celite® and the buffer evaporated under reduced pressure. The crude product was chromatographed over silica with chloroform–methanol (9:1) as eluent affording **17** as a syrup (1.02 g, 30%). (Found: C, 45.98; H, 7.88. $C_8H_{16}O_6$ required C, 46.15; H, 7.75%); $[\alpha]_D^{22}$ +147.9 (*c* 0.57 in MeOH) [lit.[15] +150 (*c* 1.0 in MeOH)]; ^{1}H NMR (DMSO) δ 1.12 (3H, t, *J*=7.2 Hz, C*H*$_3$), 3.01–3.09 (1H, m), 3.14–3.21 (1H, m), 3.32–3.48 (5H, m), 3.56–3.65 (2H, m), 4.63 (1H, d, *J*=3.9 Hz); ^{13}C NMR (DMSO) δ 15.2 (*C*H$_3$), 61.1, 62.7, 70.4, 70.5, 72.0, 72.0, 98.4; *m*/*z* (CI) $[M+NH_4]^+$ 226 (100%).

3.1.5. Ethyl 6-*O*-butyryl α-D-glucopyranoside (23). A solution of ethyl α-D-glucopyranoside **17** (0.22 g, 1.04 mmol) and vinyl butyrate (0.2 cm^3, 1.58 mmol) in THF (10 cm^3) was prepared and immersed in a oil bath at 60 °C. After 10 min Novozyme® (0.13 g) was added. The mixture was stirred for 6 h, after which time the enzyme was filtered off and washed with THF (5 cm^3). Excess solvent was evaporated under reduced pressure and the crude product was chromatographed over silica with ethyl acetate–ethanol (95:5) as eluent yielding compound **23** as a white solid (0.18 g, 60%). (Found: C, 51.80; H, 7.99. $C_{12}H_{22}O_7$ required C, 51.79; H, 7.97%); mp 70–72 °C; $[\alpha]_D^{21}$ +81.9 (*c* 1.05 in $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 1730 (C═O); ^{1}H NMR ($CDCl_3$) δ 0.96 (3H, t, *J*=7.1 Hz, C*H*$_3$), 1.25 (3H, t, *J*=7.1 Hz, C*H*$_3$), 1.61–1.73 (2H, m, C*H*$_2$CH$_3$), 2.36 (2H, t, *J*=8.1 Hz, C*H*$_2$CO), 3.45 (1H, t, *J*=9.1 Hz), 3.47–3.60 (2H, m), 3.67 (1H, br s, O*H*), 3.72–3.85 (3H, m), 4.26 (1H, dd, *J*=12.1, 2.0 Hz), 4.47 (1H, dd, *J*=12.1, 4.0 Hz), 4.89 (1H, d, *J*=4.0 Hz); ^{13}C NMR ($CDCl_3$) δ 13.6 (*C*H$_3$), 15.0 (*C*H$_3$), 18.4 (*C*H$_2$CH$_3$), 36.0 (*C*H$_2$CO), 63.1 (*C*H$_2$), 63.9, 69.8, 70.0, 72.0, 74.4, 98.0, 174.4 (*C*═O); *m*/*z* (CI) $[M+NH_4]^+$ 296 (100%).

3.2. Separation of anomers of ethyl D-glucopyranoside

Novozyme 435® (0.53 g) was added to a solution of ethyl D-glucopyranoside (0.88 g, 4.24 mmol) and vinyl butyrate (1.1 cm^3, 8.66 mmol) in THF (20 cm^3). The reaction mixture was immersed in a oil bath at 60 °C and stirred for 4 days, after which time the enzyme was filtered off and washed with THF (10 cm^3). The excess solvent was evaporated under reduced pressure and the crude product was chromatographed over silica gel using a gradient of ethyl acetate–petroleum ether (2:1) to ethyl acetate (100%) as eluent, affording compound **19** (0.27 g, 18%), compound **20** (0.19 g, 13%), and compound **23** (0.57 g, 48%).

3.2.1. Benzyl β-D-glucopyranoside (14). β-Glucosidase from almonds (120 mg) was added to a solution of D-glucose (0.81 g, 4.48 mmol) in distilled water (2 cm^3) and benzyl alcohol (18 cm^3). The solution was stirred for 30 h at 50 °C, after which time the enzyme was filtered off and washed with distilled water (5 cm^3). The excess of benzyl alcohol was removed under reduced pressure at 90 °C and 1 mbar. Flash chromatography of the residue over silica with chloroform–methanol (9:1) as the eluent afforded compound **14** (0.52 g, 43%) as a white solid. (Found: C, 57.73; H, 6.77. $C_{13}H_{18}O_6$ requires C, 57.77; H, 6.71%); mp 104 °C (lit.[16] 120–121 °C); $[\alpha]_D^{27}$ −52 (*c* 0.5 in MeOH) [lit.[16] −55.1 (*c* 1.0 in MeOH)]; ν_{max} ($CHCl_3$)/cm^{-1} 1140 (C═O); ^{1}H NMR (DMSO) δ 3.02–3.19 (3H, m),

3.44–3.52 (2H, m), 3.71 (1H, dd, J=11.6, 4.0 Hz), 4.24 (1H, d, J=7.8 Hz), 4.49 (1H, t, J=6.1 Hz), 4.59 (1H, d, J=12.4 Hz, CH_2), 4.83 (1H, d, J=12.4 Hz, $^{'}$CH_2), 4.88 (2H, dd, J=11.6, 4.8 Hz), 7.26–7.41 (5H, m, Ar); ^{13}C NMR (DMSO) δ 48.9, 61.4, 69.7, 70.4, 73.8, 77.0, 77.3, 102.4, 127.6, 127.9, 128.4, 138.4; *m/z* (CI) $[M+NH_4]^+$ 288 (100%).

3.2.2. Benzyl 6-*O*-butyryl β-D-glucopyranoside (21). Novozyme 435® (0.10 g) was added to a solution of β-benzyl D-glucopyranoside **14** (0.31 g, 1.14 mmol) and vinyl butyrate (0.3 cm^3, 2.36 mmol) in dry THF (10 cm^3) and immersed in a oil bath at 60 °C with stirring. After 3 h the reaction was complete by TLC and was cooled to room temperature; the excess solvent was evaporated under reduced pressure. Flash chromatography of the residue over silica with EtOAc–EtOH (95:5) as eluent gave compound **21** as a white solid (0.38 g, 98%). (Found: C, 59.77; H, 7.17. $C_{17}H_{24}O_7$ requires C, 59.99; H, 7.11%); mp 65 °C; $[\alpha]_D^{23}$ −55.7 (*c* 1.1 in $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 1720 (C═O); ^{1}H NMR ($CDCl_3$) δ 0.96 (3H, t, J=8.1 Hz, CH_3), 1.62–1.71 (2H, m, CH_2CH$_3$), 2.36 (2H, t, J=7.1 Hz, CH_2CO), 3.35–3.43 (2H, m), 3.51 (1H, t, J=8.1 Hz), 4.30–4.32 (1H, m), 4.33 (1H, d, J=7.1 Hz), 4.40 (1H, d, J=4.0 Hz), 4.43 (1H, d, J=4.0 Hz), 4.59 (1H, d, J=12.1 Hz, CH_2Ph) 4.89 (1H, d, J=12.1 Hz, CH_2Ph), 7.27–7.36 (5H, m, Ar); ^{13}C NMR ($CDCl_3$) δ 13.6 (*C*H$_3$), 18.4 (*C*H$_2$CH$_3$), 36.0 (*C*H$_2$CO), 63.1, 70.0, 71.0, 73.5, 73.9, 75.9, 101.4, 128.1, 128.2, 128.5, 136.8, 174.4 (*C*═O); *m/z* (CI) $[M+NH_4]^+$ 358 (100%).

3.2.3. Benzyl α-D-glucopyranoside (16). β-Glucosidase from almonds (0.06 g) was added to a solution of benzyl-D-glucopyranoside (0.42 g, 1.56 mmol) in a citric acid buffer pH 5.0 (20 cm^3). The reaction mixture was stirred for 3 days at 50 °C. The enzyme was filtered off and the buffer was evaporated under reduced pressure. The crude product was chromatographed over silica with chloroform–methanol (9:1) as eluent yielding compound **16** (0.28 g, 67%). (Found: C, 57.49; H, 6.77. $C_{13}H_{18}O_6$ required C, 57.77; H, 6.71%); mp 102 °C (lit.[17] 122 °C); $[\alpha]_D^{22}$ +134.7 (*c* 1.01 in MeOH) [lit.[17]+133.5 (*c* 2.5 in H_2O)]; ^{1}H NMR (DMSO) δ 3.04–3.10 (1H, m), 3.20–3.25 (1H, m), 3.41–3.50 (3H, m), 3.63 (1H, dd, J=6.1, 9.1 Hz), 4.41–4.48 (2H, m), 4.66 (1H, s, O*H*), 4.70 (2H, d, J=6.1 Hz), 4.73 (1H, d, J=3.0 Hz), 4.83 (1H, d, J=5.1 Hz), 7.25–7.38 (5H, m); ^{13}C NMR (DMSO) δ 61.3, 68.2, 70.7, 72.3, 73.4, 73.7, 98.2, 127.6, 127.8, 128.5, 138.5; *m/z* (CI) $[M+NH_4]^+$, 288 (100%).

3.2.4. Benzyl 6-*O*-butyryl α-D-glucopyranoside (22). Vinyl butyrate (0.2 cm^3, 1.58 mmol) was added to a solution of benzyl α-D-glucopyranoside **16** (0.25 g, 0.91 mmol) in THF (20 cm^3) and stirred for 5 min at room temperature. Novozyme 435® (0.10 g) was added and the reaction mixture was heated to 60 °C and stirred for 1 h. The enzyme was filtered and washed with THF (10 cm^3). The solvent was evaporated under reduced pressure and the crude product chromatographed over silica with ethyl acetate–ethanol (95:5) as eluent yielding compound **22** (0.30 g, 97%). (Found: C, 60.20; H, 7.15. $C_{17}H_{24}O_7$ requires C, 59.99; H, 7.11%); mp 72 °C; $[\alpha]_D^{21}$ +77.8 (*c* 1.0 in $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 1729 (C═O); ^{1}H NMR ($CDCl_3$) δ 0.95 (3H, t, J=7.1 Hz, CH_3), 1.62–1.71 (2H, m, CH_2CH$_3$), 2.35 (2H, t, J=7.1 Hz, CH_2CO), 3.33 (1H, t, J=9.1 Hz), 3.5 (1H, dd, J=3.0, 9.1 Hz), 3.74–3.78 (2H, m), 4.19 (1H, dd, J=2.0, 12.1 Hz), 4.42 (1H, dd, J=4.0, 12.1 Hz), 4.53 (1H, d, J=11.1 Hz), 4.72 (1H, d, J=11.1 Hz), 4.95 (1H, d, J=4.0 Hz), 7.28–7.37 (5H, m); ^{13}C NMR ($CDCl_3$) δ 14.0 (*C*H$_3$), 14.5 (*C*H$_2$CH$_3$), 18.7 (*C*H$_2$CO), 36.4, 60.7, 63.3, 70.3, 70.4, 70.5, 72.5, 74.7, 98.0, 128.5, 128.9, 137.2, 174.7 (*C*═O). (Found: $[M+NH_4]^+$, 358.186 $C_{17}H_{28}O_7N$ requires $[M+NH_4]^+$, 358.187); *m/z* (CI) $[M+NH_4]^+$ 358 (100%).

3.2.5. Benzyl 2,6-*O*-butyryl α-D-glucopyranoside (24). Lipase PS-C II (0.054 g) was added to a slurry of benzyl 6-*O*-butyryl α-D-glucopyranoside **22** (0.03 g, 0.09 mmol) in vinyl butyrate (5 cm^3) and immersed in a oil bath at 30 °C and stirred for 3 days. The enzyme was filtered off and washed with THF (5 cm^3), before the solvent was evaporated under reduced pressure. Flash chromatography of the crude residue over silica with ethyl acetate–petroleum ether (2:1) as eluent afforded compound **24** as a yellow syrup (0.032 g, 86%); $[\alpha]_D^{24}$ +46 (*c* 0.5 in $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 1720 (C═O); ^{1}H NMR ($CDCl_3$) δ 0.94 (3H, t, J=8.1 Hz, CH_3), 0.97 (3H, t, J=7.1 Hz, CH_3), 1.59–1.73 (4H, m, 2×CH_2CH_3), 2.39–2.39 (4H, m, 2×CH_2CO), 3.41 (1H, t, J=10.1 Hz), 3.82 (1H, ddd, J=10.1, 4.0, 2.0 Hz), 4.04 (1H, t, J=10.1 Hz), 4.17 (1H, dd, J=12.1, 2.0 Hz), 4.48–4.53 (2H, m), 4.67–4.71 (2H, m), 5.11 (1H, d, J=4.0 Hz), 7.27–7.36 (5H, m, Ar); ^{13}C NMR ($CDCl_3$) δ 13.9, 14.0, 18.5, 18.7, 36.3, 36.4, 63.1, 70.2, 70.2, 70.9, 71.7, 73.3, 95.9, 128.2, 128.3, 128.8, 137.4, 173.8, 174.9. (Found: $[M]^+$411.203 $C_{21}H_{31}O_8$ requires $[M]^+$, 411.202); *m/z* (CI) $[M]^+$ 411 (6%).

3.2.6. 2,6-*O*-Butyryl-D-glucopyranoside (25). Benzyl 2,6-butyryl α-D-glucopyranoside **24** (0.35 g, 0.86 mmol) was dissolved in ethyl acetate, followed by the addition of Pd/C (10%) (0.11 g). The reaction mixture was purged with hydrogen and then placed under hydrogen pressure (0.2 bar), with stirring, for 7 days at room temperature. After completion of the reaction, the catalyst was filtered off and washed with ethyl acetate. The solvent was evaporated under reduced pressure and the crude product was chromatographed over silica using ethyl acetate–petroleum ether (2:1) as eluent affording compound **25** (0.22 g, 75%), as a mixture of α- and β-anomers. (Found: C, 52.63; H, 7.62. $C_{14}H_{24}O_8$ requires C, 52.49; H, 7.55%); $[\alpha]_D^{22}$ +49.6 (*c* 0.6 in $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 1740 (C═O); ^{1}H NMR ($CDCl_3$) δ 0.93–0.99 (12H, m), 1.62–1.72 (8H, m) 2.33–2.41 (8H, m), 3.23 (1H, bs), 3.43 (2H, t, J=9.2 Hz), 3.52 (1H, ddd, J=9.9, 4.8, 2.2 Hz), 3.63–3.71 (3H, m), 3.78 (1H, bs) 4.00–4.05 (3H, m), 4.33 (2H, m), 4.43–4.49 (2H, m), 4.64 (1H, t, J=7.3 Hz), 4.67–4.72 (2H, m), 5.41 (1H, bs); ^{13}C NMR ($CDCl_3$) δ 13.9, 13.9 and 14.0, 18.7, 18.7, 36.3, 36.3, 60.8, 63.3, 63.4, 69.9, 70.9, 71.2 73.5, 74.5, 74.7, 75.8, 90.9, 95.9, 175.2 and 175.2 (*C*═O); *m/z* (CI) $[M+NH_4]^+$ 338 (64%).

3.3. Separation of anomers from benzyl D-glucopyranoside

Novozyme 435® (0.054 g) and lipase PS-C II (0.22 g) was added to benzyl D-glucopyranoside (0.31 g, 1.15 mmol) in vinyl butyrate (5 cm^3). The reaction was stirred for 4 days at

40 °C, after which time the enzymes were filtered off and washed with THF (10 cm^3). Excess solvent was evaporated under reduced pressure and the crude product chromatographed over silica with ethyl acetate–petroleum ether (2:1) as eluent affording compound **21** (0.141 g, 36%) and compound **24** (0.13 g, 28%).

3.3.1. Benzyl 6-*O*-butyryl α-D-galactopyranoside (28) and benzyl 2,6-*O*-butyryl β-D-galactopyranoside (29). Novozyme 435® (0.12 g) was added to a solution of benzyl D-galactopyranoside (0.12 g, 0.43 mmol) and vinyl butyrate (0.1 cm^3, 0.79 mmol) in THF (10 cm^3). The reaction mixture was heated to 60 °C and stirred for 7.5 h; the enzyme was filtered off and washed with THF (10 cm^3). Excess solvent was evaporated under reduced pressure, and the crude product was chromatographed over silica with ethyl acetate–ethanol (95:5) as eluent yielding compound **28** (0.067 g, 46%) and compound **29** (0.041 g, 23%). Compound **28**. (Found: C, 59.94; H, 7.12. $C_{17}H_{24}O_7$ requires C, 59.99; H, 7.11%); $[\alpha]_D^{21}$ +92.5 (*c* 0.56 in $CHCl_3$); mp 106–108 °C; ν_{max} ($CHCl_3$)/cm^{-1} 1735 (C═O); ^{1}H NMR ($CDCl_3$) δ 0.96 (3H, t, *J*=7.6 Hz, C*H*$_3$), 1.62–1.72 (2H, m, C*H*$_2$CH$_3$), 2.34 (2H, t, *J*=7.6 Hz, C*H*$_2$CO), 3.79–3.87 (2H, m), 3.97 (1H, d, *J*=2.0 Hz), 4.01 (1H, t, *J*=6.6 Hz), 4.23 (1H, dd, *J*=11.6, 6.5 Hz), 4.41 (1H, dd, *J*=11.6, 6.1 Hz), 4.54 (1H, d, *J*=11.6 Hz, C*H*$_2$Ph), 4.76 (1H, d, *J*=11.6 Hz), 5.05 (1H, d, *J*=3.5 Hz), 7.31–7.40 (5H, m, Ar); ^{13}C NMR ($CDCl_3$) δ 13.7 (*C*H$_3$), 18.3 (*C*H$_2$), 36.0 (*C*H$_2$), 63.0, 68.3, 68.8, 69.4, 69.8, 70.8, 97.6, 128.1, 128.6, 136.7, 173.7(*C*═O; *m/z* (CI) 358 $[M+NH_4]^+$. Compound **29**. (Found: C, 61.33; H, 7.37. $C_{21}H_{30}O_8$ require C, 61.45; H, 7.37%); $[\alpha]_D^{22}$ −24.2 (*c* 0.8 in $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 1738 (C═O); ^{1}H NMR ($CDCl_3$) δ 0.94 (3H, t, *J*=7.6 Hz, CH$_3$), 0.97 (3H, t, *J*=7.6 Hz, $'$CH$_3$), 1.60–1.72 (4H, m, C*H*$_2$CH$_3$), 2.29–2.36 (4H, m, C*H*$_2$CO), 3.60–3.67 (2H, m), 3.87 (1H, d, *J*=2.9 Hz), 4.32 (1H, dd, *J*=11.4, 6.7 Hz), 4.43 (1H, dd, *J*=11.4, 6.4 Hz), 4.45 (1H, d, *J*=7.9 Hz), 4.62 (1H, d, *J*=12.1 Hz), 4.88 (1H, d, *J*=12.1 Hz), 5.01 (1H, dd, *J*=7.9, 9.5 Hz), 7.26–7.36 (5H, m, Ar); ^{13}C NMR ($CDCl_3$) δ 13.9, 14.0, 18.7, 18.7, 36.4, 36.5, 62.7, 69.1, 70.7, 72.5, 73.1, 73.6, 99.8, 128.2, 128.2, 128.7, 137.3, 174.0 and 174.6 (*C*═O); *m/z* (CI) 428 $[M+NH_4]^+$.

3.3.2. Benzyl α-D-galactopyranoside (27). Benzyl 6-*O*-butyryl α-D-galactopyranoside **28** (0.065 g, 0.19 mmol) was added to a solution of sodium methoxide in methanol (0.08 M 10 cm^3). The reaction mixture was stirred for 3 min after which time the solvent was evaporated under reduced pressure affording a crude product, which was chromatographed over silica with chloroform–methanol (9:1) as eluent, yielding compound **27** as a syrup (0.047 g, 91%); $[\alpha]_D^{24}$ +96.1 (*c* 1.55 in MeOH); ^{1}H NMR (DMSO) δ 3.41–3.56 (2H, m), 3.59–3.69 (3H, m) 3.73 (1H, br s, *O*H), 4.31–4.34 (1H, br s, O*H*), 4.23 (1H, d, *J*=12.1 Hz), 4.49–4.55 (3H, m), 4.68 (1H, d, *J*=12.1 Hz), 4.76 (1H, d, *J*=3.2 Hz), 7.26–7.69 (5H, m, Ar); ^{13}C NMR (DMSO) δ 61.0, 68.2, 68.7, 69.2, 70.0, 71.8, 98.6, 127.6, 127.8, 128.4, 138.5. (Found: $[M+NH_4]^+$, 288.145 $C_{13}H_{18}O_6N$ requires $[M+NH_4]^+$, 288.145); *m/z* (CI) $[M+NH_4]^+$ 288 (100%).

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New access to the 1*H*-pyrazolo[4,3-*c*]pyridine core from bis-acetylenic-*N*-benzoylhydrazones

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Abstract—1*H*-pyrazolo[4,3-*c*]pyridines were obtained from bis-acetylenic-*N*-benzoylhydrazones using aqueous ammonia.

1. Introduction

Bicyclic hetero-aromatic compounds are well known for their wide range of biological activity. For example the 3-furylindazole YC-1 (**1**) (Fig. 1) is now considered as a lead compound in the design of novel indazole derivatives potentially useful for treatment of various diseases linked to smooth muscle relaxation including cardiovascular insufficiency and erectile dysfunction.[1] The related pyrazolopyridines, which comprise five isomers [3,4-*b*], [3,4-*c*], [4,3-*c*], [4,3-*b*] and [1,5-*a*], were shown to display high biological activity; pyrazolo[4,3-*c*]pyridine **2** derivatives have use as angiotensin II antagonists and Bay 41-2272 (**3**) has been introduced as a novel orally available agent which directly stimulates soluble guanylate cyclase (sGC) and sensitizes it to its physiological stimulator, nitric oxide.[2]

The usual synthetic routes towards the pyrazolo[4,3-*c*]pyridine core are ring closure of the pyridine ring of a functionised pyrazole or ring closure of the pyrazole ring of a functionalised pyridine.[3] Herein we report a new synthesis of the pyrazolo[4,3-*c*]pyridine core based on an unusual one step tandem ring closure and rearrangement of bis-acetylenic N-acylated hydrazones using aqueous ammonia.

2. Results and discussion

Our initial aim was the synthesis of the nine membered ring compound **4**, starting from the commercially available 1,4-bis(trimethylsilyl)-1,3-butadiyne **8** as outlined in Scheme 1. The planned synthesis of **4** called for ammonolysis of a bis-acetylenic N-acetylated hydrazone **5** followed by a 9-*endo-dig* cyclisation.[4]

Our synthesis (Scheme 2) began with the commercially

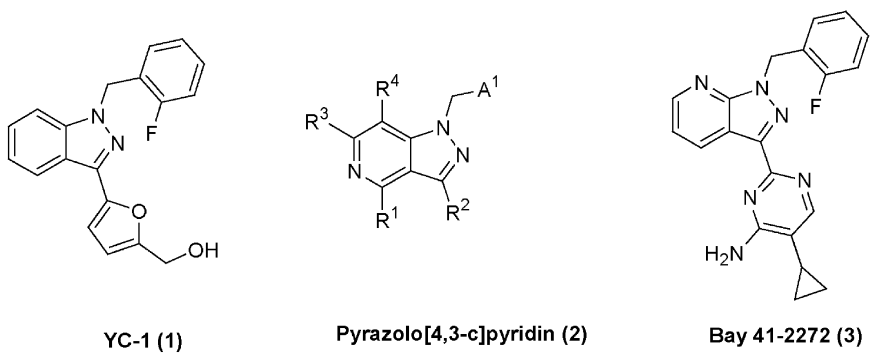

Figure 1.

Keywords: 3-Furylindazole; Angiotensin II antagonist; Pyrazole ring; 1*H*-Pyrazolo[4,3-*c*]pyridine.
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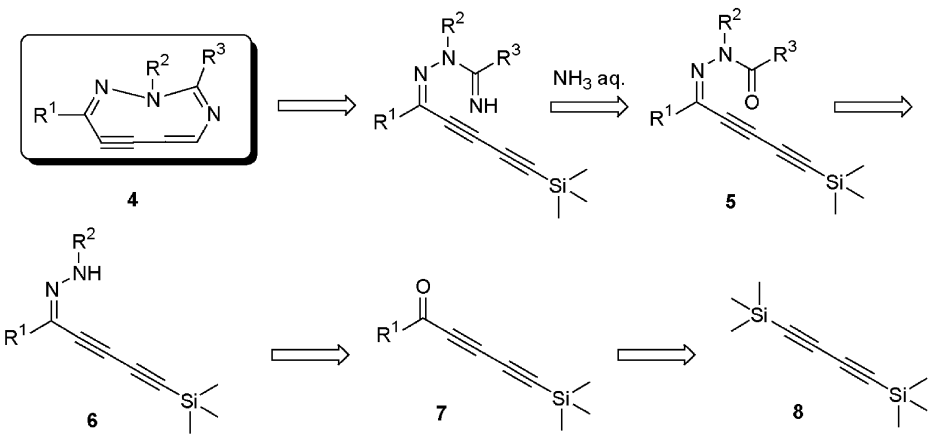
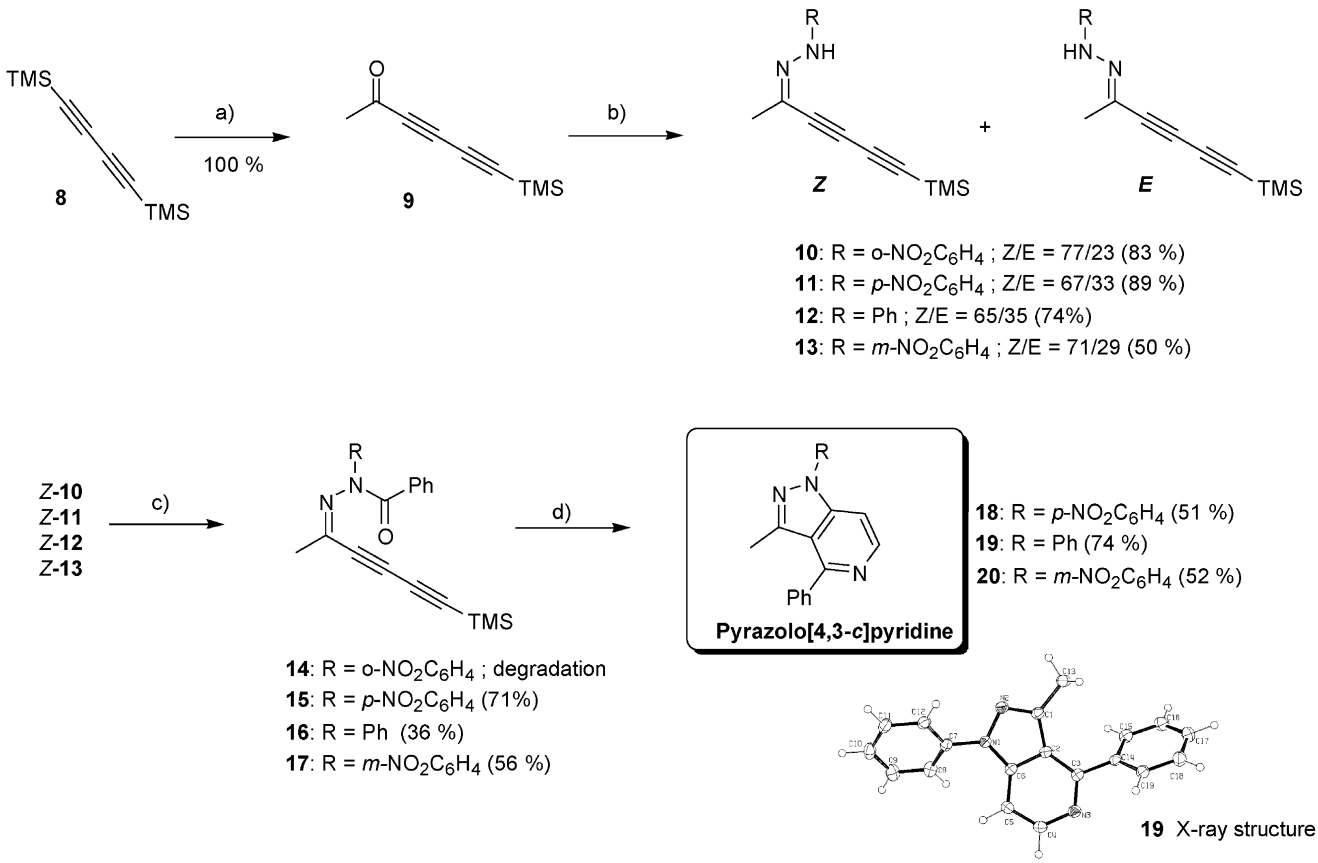


Scheme 1. Retrosynthetic analysis.

available 1,4-bis(trimethylsilyl)-1,3-butadiyne **8**, which upon treatment with acetyl chloride and anhydrous aluminium trichloride in CH_2Cl_2 furnished the corresponding ketone **9** in quantitative yield.[5] The acetylenic ketone **9**, upon reaction with phenylhydrazine or substituted analogues in MeOH was converted into the corresponding hydrazones **10**, **11**, **12** and **13** as separable mixtures of *Z* and *E* isomers. The structure of both of these isomers were confirmed by the intensity of the ^1H NMR NOE interactions between the methyl protons and the N–H proton. However, it was found that the hydrazones *E*-**11**, *E*-**12** and *E*-**13** was relatively unstable, and upon standing in $CDCl_3$ for a few hours, were cleanly converted to their corresponding *Z*-isomer (Fig. 2). *E*-**10** was found to be stable under these conditions and no isomerisation was observed. Thus, the next step of the synthesis was performed with the *Z*-compounds *Z*-**10**, *Z*-**11**, *Z*-**12** and *Z*-**13** which upon treatment with benzoyl chloride and anhydrous aluminium trichloride in refluxing of CH_2Cl_2 gave, in fair yields, the corresponding *N*-benzoyl compounds.[6] Unfortunately hydrazone *Z*-**10** was degraded and no *N*-benzoylated product was observed. However *Z*-**11**, *Z*-**12** and *Z*-**13** gave **15**, **16** and **17** in 71, 36 and 56% yields, respectively.

Next, the ring closure of the *N*-benzoylated hydrazones with aqueous ammonia was attempted.

However, after exposure of compounds **15**, **16** and **17** to



Scheme 2. (a) MeCOCl, $AlCl_3$, DCM, 0 °C; (b) NH_2NHR, MeOH; (c) PhCOCl, $AlCl_3$, DCM, reflux; (d) 33% aq. NH_3, EtOH, 85 °C.

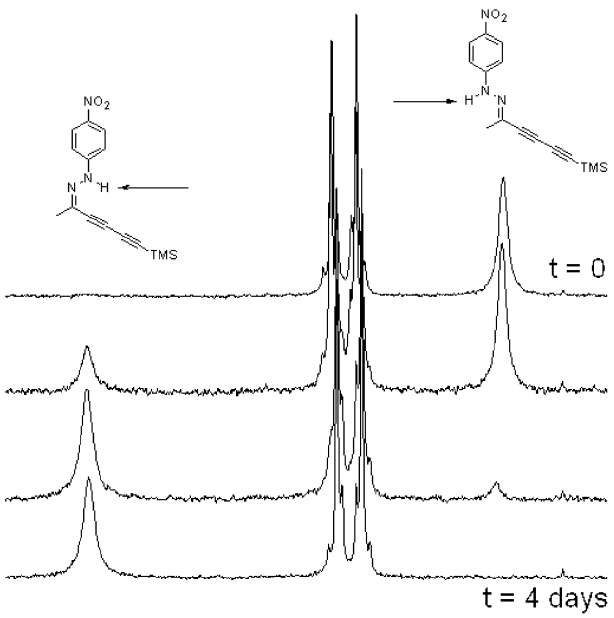


Figure 2.

ethanolic aqueous ammonia,[7] the corresponding pyrazolo[4,3-*c*]pyridines **18**, **19** and **20** were obtained, rather than the nine membered ring **4**. The structure of these compounds was confirmed by X-ray analysis of **19**[8] and by further spectral comparison of **18** and **20** to **19**.

To explain the formation of the pyrazolo[4,3-*c*]pyridines from the corresponding hydrazones, we propose the following mechanistic rationale (Schemes 3 and 4). In Scheme 3, the first step is the formation of the imino-hydrazone **21**[9] followed by 9-*endo-dig* cyclisation of the amidine moiety on the terminal alkyne.[4] However, under the reaction conditions, further reaction of **22** with another equivalent of ammonia presumably gives rise to **23** which undergoes consecutive 5-*endo-dig* pyrazole cyclisation followed by thermal 6πe disrotary ring closure and elimination of ammonia to form the isolated pyrazolo[4,3-*c*]pyridine compounds. An alternative mechanism, which does not require a nine membered ring, is also a possibility (Scheme 4).

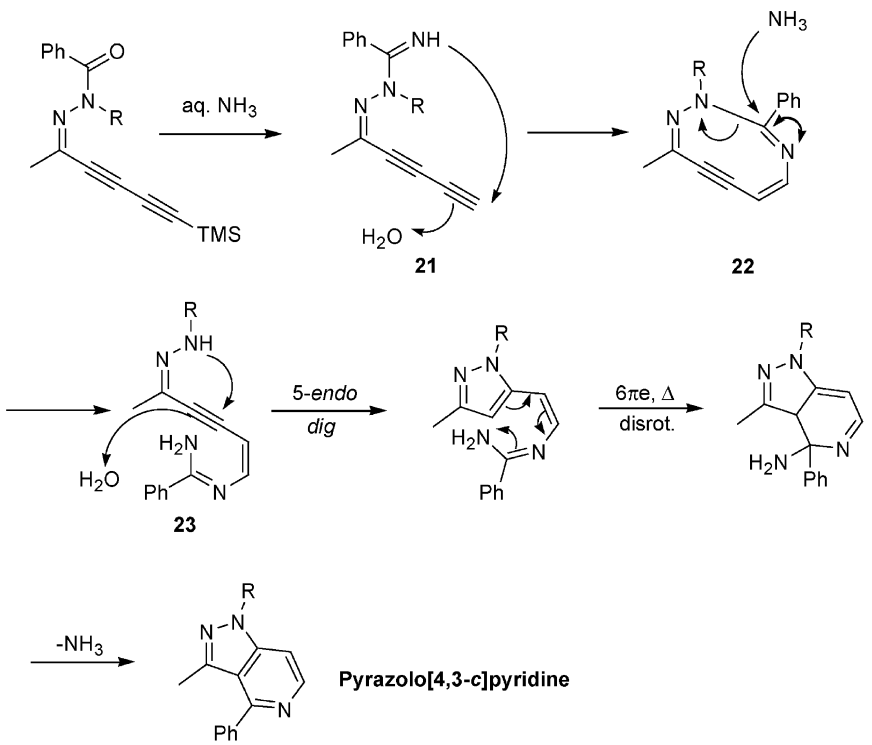
3. Conclusion

A concise novel route to the pyrazolo[4,3-*c*]pyridine core by an unusual mechanistic pathway has been developed.

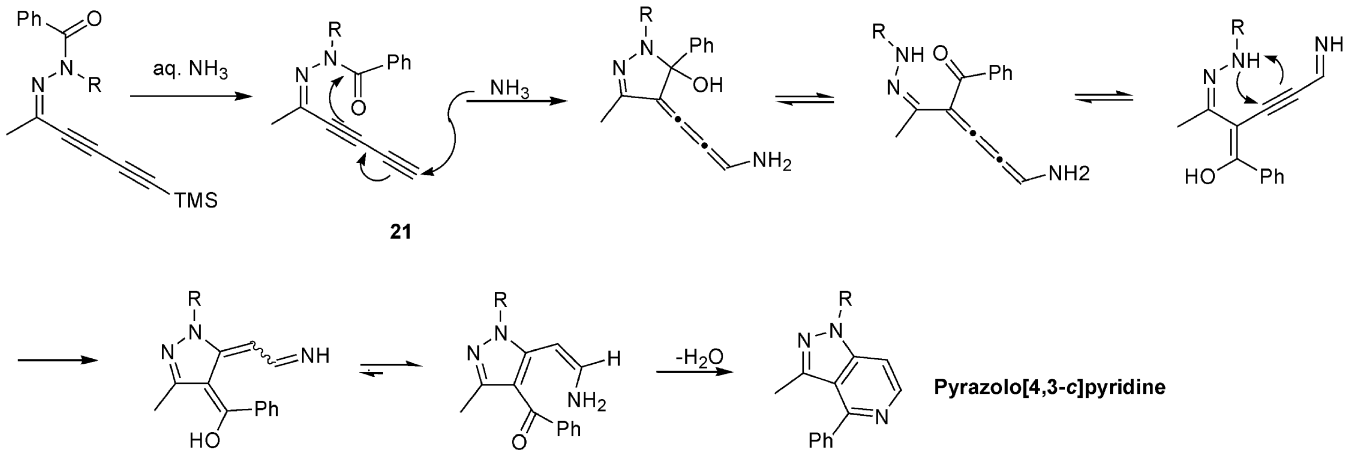
4. Experimental

4.1. General

All solvents and reagents were purified by standard techniques reported in Perrin, D. D.; Amarego, W. L. F. Purification of laboratory chemicals, 3rd ed.; Pergamon: Oxford, 1988 or used as supplied from commercial sources as appropriate. Solvents were removed under reduced pressure using a Buchi R110 or R114 Rotavapor fitted with a water or dry ice condenser as necessary. Final traces of solvent were removed from samples using an Edwards E2M5 high vacuum pump with pressures below 2 mmHg. All experiments were carried out under inert atmosphere unless otherwise stated. ^{1}H NMR spectra were recorded at 400 MHz using Bruker DPX400. For ^{1}H spectra recorded in $CDCl_3$, chemical shifts are quoted in parts per million (ppm) and are referenced to the residual solvent peak. The



Scheme 3.



Scheme 4.

following abbreviations are used: s, singlet; d, doublet; t, triplet; m, multiplet; b, broad. Data are reported in the following manner: chemical shift (integration, multiplicity, coupling constant if appropriate). Coupling constants (*J*) are reported in Hertz to the nearest 0.5 Hz. ^{13}C NMR spectra were recorded at 100 MHz using Bruker DPX400 instrument. Carbon spectra assignments are supported by DEPT-135 spectra, $^{13}C-^{1}H$ (HMQC and HMBC) correlations where necessary. Chemical shifts are quoted in ppm and are referenced to the appropriate residual solvent peak. Flash column chromatography was carried out using Sorbsil™ C60 (40–63 mm, 230–40 mesh) silica gel. Thin layer chromatography was carried out on glass plates precoated with Merck silica gel 60 F_{254} which were visualised by quenching of UV fluorescence or by staining with 10% w/v ammonium molybdate in 2 M sulphuric acid or 1% w/v potassium permanganate in aqueous alkaline solution followed by heat, as appropriate. Melting points were recorded using a Cambridge Instruments Gallen™ III Kofler Block melting apparatus or a Buchi 510 capillary apparatus and are uncorrected. Infrared spectra were recorded either as a thin film between NaCl plates or as a KBr disc (as indicated) on a Perkin–Elmer Paragon 1000 Fourier Transform spectrometer with internal referencing. Absorption maxima are reported in wavenumbers (cm^{-1}). High resolution mass spectrometry was measured on a Waters 2790-Micromass LCT electrospray ionisation mass spectrometer and on a VG autospec chemical ionisation mass spectrometer.

4.1.1. 6-Trimethylsilanyl-hexa-3,5-diyn-2-one (9). To a solution of 1,4-bis(trimethylsily)-1,3-butadiyne (5.30 g, 27.26 mmol) in CH_2Cl_2 (50 mL) at 0 °C was added acetyl chloride (2.14 mL, 29.98 mmol) then anhydrous aluminium trichloride (3.99 g, 29.98 mmol). The reaction was stirred further 30 min at 0 °C and quenched with a mixture of 10% aqueous hydrochloric acid and ice (50 mL, 1/1). The aqueous phase was extracted with CH_2Cl_2 (3×50 mL), and the combined organic extracts were washed with saturated $NaHCO_3$ solution (50 mL), dried over $MgSO_4$, and concentrated under vacuum to afford the crude product. Purification by flash column chromatography (SiO_2, 1:9 to 4:6, CH_2Cl_2/light petroleum) furnished in quantitative yield (4.48 g) the desired ketone **9** as an oil. ν_{max} (film/cm^{-1}) 2963, 2206, 2097, 1678, 1252, 1236, 847; ^{1}H NMR (400 MHz, $CDCl_3$) 0.21 (9H, s), 2.33 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) −0.7, 32.7, 73.6, 74.8, 85.7, 97.5, 183.3; HRMS found 165.0732 (MH^+), $C_9H_{13}OSi$ requires 165.0736.

4.2. General procedure for the preparation of 10–13

4.2.1. *Z*-6-Trimethylsilanyl-hexa-3,5-diyn-2-one, 2-nitrophenylhydrazone (*Z*-10) and *E*-6-trimethylsilanyl-hexa-3,5-diyn-2-one, 2-nitrophenylhydrazone (*E*-10). To a solution of 6-trimethylsilanyl-hexa-3,5-diyn-2-one **9** (1.00 g, 6.09 mmol) in MeOH (16 mL) was added, at 0 °C, 2-nitrophenyl-hydrazine (1.03 g, 6.70 mmol). The solution was stirred at 0 °C and followed by TLC. After disappearance of starting material (approximately 4 h), the mixture was evaporated under vacuum. Water (20 mL) and CH_2Cl_2 (20 mL) were added, the aqueous layer extracted with CH_2Cl_2 (2×20 mL) and the combined organic extracts were washed with saturated aqueous NaCl solution (15 mL), dried over $MgSO_4$ and concentrated under vacuum. The crude product was purified by flash chromatography (SiO_2, 1:9 to 3:7, CH_2Cl_2/light petroleum) to give, in 83% yield (1.52 g), 77% of *Z*-**10** and 23% of *E*-**10**. *Z*-**10**: mp=104 °C; ν_{max} (KBr disc/cm^{-1}) 3300, 2090, 1615, 1503, 1344, 1141, 1075; ^{1}H NMR (400 MHz, $CDCl_3$) 0.26 (9H, s), 2.21 (3H, s), 6.86 (1H, bt, *J*=8.0 Hz), 7.51 (1H, bt, *J*=8.0 Hz), 7.81 (1H, d, *J*=8.5 Hz), 8.17 (1H, d, *J*=8.5 Hz), 11.59 (1H, bs); ^{13}C NMR (100 MHz, $CDCl_3$) −0.5, 22.2, 67.5, 85.9, 86.8, 98.3, 116.4, 118.9, 126.0, 129.1, 131.4, 136.1, 140.9; HRMS found 300.1178 (MH^+), $C_{15}H_{18}N_3O_2Si$ requires 300.1168. *E*-**10**: mp=85 °C; ν_{max} (KBr disc/cm^{-1}) 3304, 2198, 2098, 1613, 1499, 1313, 1275, 1147, 1069; ^{1}H NMR (400 MHz, $CDCl_3$) 0.23 (9H, s), 2.16 (3H, s), 6.92 (1H, bt, *J*=8.0 Hz), 7.55 (1H, bt, *J*=8.0 Hz), 7.89 (1H, bd, *J*=8.5 Hz), 8.16 (1H, bd, *J*=8.5 Hz), 11.02 (1H, bs); ^{13}C NMR (100 MHz, $CDCl_3$) −0.4, 17.3, 74.8, 75.5, 87.4, 93.4, 116.6, 119.8, 125.9, 131.9, 132.1, 136.5, 140.6; HRMS found 300.1168 (MH^+), $C_{15}H_{18}N_3O_2Si$ requires 300.1168.

4.2.2. *Z*-6-Trimethylsilanyl-hexa-3,5-diyn-2-one, 4-nitrophenylhydrazone (*Z*-11) and *E*-6-trimethylsilanyl-hexa-3,5-diyn-2-one, 4-nitrophenylhydrazone (*E*-11). Prepared as above for *E*,*Z*-**10** using **9** (798 mg, 4.86 mmol) in MeOH (13 mL) and 4-nitrophenylhydrazine (818 mg, 5.34 mmol). The crude product was purified by flash chromatography

(SiO_2, 4:6 to 7:3, CH_2Cl_2/light petroleum) to give, in 89% yield (1.30 g), 67% of *Z*-**11** and 33% of *E*-**11**. *Z*-**11**: mp=95 °C; ν_{max} (KBr disc/cm^{-1}) 3288, 2094, 1594, 1498, 1319, 1268, 1140, 1109; ^1H NMR (400 MHz, $CDCl_3$) 0.26 (9H, s), 2.17 (3H, s), 7.09 (2H, d, *J*=9.0 Hz), 8.16 (2H, d, *J*=9.0 Hz), 8.66 (1H, bs); ^{13}C NMR (100 MHz, $CDCl_3$) −0.5, 22.0, 67.0, 85.9, 86.7, 98.4, 112.4, 126.2, 126.6, 141.0, 148.6; HRMS found 298.1005 ($M-H^+$), $C_{15}H_{16}N_3O_2Si$ requires 298.1012. *E*-**11**: mp=153 °C; ν_{max} (film/cm^{-1}) 3306, 2196, 2097, 1595, 1502, 1325, 1260, 1150, 1108; ^1H NMR (400 MHz, $CDCl_3$) 0.22 (9H, s), 2.08 (3H, s), 7.15 (2H, d, *J*=9.0 Hz), 8.04 (1H, bs), 8.15 (2H, d, *J*=9.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) −0.4, 16.4, 74.4, 75.5, 87.4, 93.2, 113.0, 126.1, 129.5, 141.5, 148.4; HRMS found 298.1012 ($M-H^+$), $C_{15}H_{16}N_3O_2Si$ requires 298.1012.

4.2.3. *Z*-6-Trimethylsilanyl-hexa-3,5-diyn-2-one, phenylhydrazone (*Z*-12) and *E*-6-trimethylsilanyl-hexa-3,5-diyn-2-one, phenylhydrazone (*E*-12). Prepared as above for *E*,*Z*-**10** using **9** (291 mg, 1.77 mmol) in MeOH (5 mL) and phenylhydrazine (0.191 mL, 1.95 mmol). The crude product was purified by flash chromatography (SiO_2, 1:9 to 3:7, CH_2Cl_2/light petroleum) to give, in 74% yield (335 mg), 65% of *Z*-**12** and 35% of *E*-**12**. *Z*-**12**: mp=33 °C; ν_{max} (KBr disc/cm^{-1}) 3301, 2199, 2095, 1600, 1504, 1247, 1150, 1088; ^1H NMR (400 MHz, $CDCl_3$) 0.30 (9H, s), 2.17 (3H, s), 6.92 (1H, bt, *J*=7.5 Hz), 7.09 (2H, bd, *J*=8.5 Hz), 7.29 (2H, bdd, *J*=8.5, 7.5 Hz), 8.40 (1H, bs); ^{13}C NMR (100 MHz, $CDCl_3$) −0.4, 21.7, 68.4, 85.7, 86.5, 96.9, 113.2, 120.8, 121.2, 129.4, 143.7; HRMS found 255.1312 (MH^+), $C_{15}H_{19}N_2Si$ requires 255.1318. *E*-**12**: mp=45 °C; ν_{max} (KBr disc/cm^{-1}) 3301, 2195, 2095, 1601, 1504, 1252, 1150, 1073; ^1H NMR (400 MHz, $CDCl_3$) 0.23 (9H, s), 2.03 (3H, s), 6.94 (1H, bt, *J*=7.5 Hz), 7.12 (2H, bd, *J*=8.0 Hz), 7.28 (2H, bt, *J*=8.0 Hz), 7.58 (1H, bs); ^{13}C NMR (100 MHz, $CDCl_3$) −0.3, 15.9, 73.0, 76.8, 88.0, 91.9, 113.7, 121.6, 124.8, 129.4, 143.5; HRMS found 255.1315 (MH^+), $C_{15}H_{19}N_2Si$ requires 255.1318.

4.2.4. *Z*-6-Trimethylsilanyl-hexa-3,5-diyn-2-one, 3-nitrophenylhydrazone (*Z*-13) and *E*-6-trimethylsilanyl-hexa-3,5-diyn-2-one, 3-nitrophenylhydrazone (*E*-13). Prepared as above for *E*,*Z*-**10** using **9** (1.00 g, 6.09 mmol) and 3-nitrophenylhydrazine hydrochloride (1.27 g, 6.70 mmol) at reflux in MeOH (16 mL) for 4 h. The crude product was purified by flash chromatography (SiO_2, 2:8 to 5:5, CH_2Cl_2/light petroleum) to give, in 50% yield (915 mg), 71% of *Z*-**13** and 29% of *E*-**13**. *Z*-**13**: mp=90 °C; ν_{max} (KBr disc/cm^{-1}) 3295, 2206, 2100, 1618, 1529, 1344, 1256, 1143, 1094; ^1H NMR (400 MHz, $CDCl_3$) 0.25 (9H, s), 2.14 (3H, s), 7.31 (1H, bdt, *J*=8.0, 2.0 Hz), 7.34 (1H, bd, *J*=8.0 Hz), 7.67 (1H, bdt, *J*=8.0, 2.0 Hz), 7.88 (1H, bt, *J*=2.0 Hz), 8.49 (1H, bs); ^{13}C NMR (100 MHz, $CDCl_3$) −0.6, 21.8, 67.4, 86.0, 86.3, 97.8, 107.8, 115.1, 118.7, 124.3, 130.0, 144.7, 149.3; HRMS found 300.1179 (MH^+), $C_{15}H_{18}N_3O_2Si$ requires 300.1168. *E*-**13**: mp=205 °C; ν_{max} (KBr disc/cm^{-1}) 3334, 2196, 1622, 1530, 1342, 1253, 1173, 1073; ^1H NMR (400 MHz, $CDCl_3$) 0.22 (9H, s), 2.07 (3H, s), 7.40 (1H, bd, *J*=8.0 Hz), 7.43 (1H, bdt, *J*=8.0, 2.0 Hz), 7.73 (1H, bdt, *J*=8.0, 2.0 Hz), 7.81 (1H, bs), 7.92 (1H, bt, *J*=2.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) −0.4, 16.2, 73.8, 75.9, 87.6, 92.7, 108.4, 115.9, 119.3, 127.7, 130.2, 144.6, 149.3; HRMS found 300.1167 (MH^+), $C_{15}H_{18}N_3O_2Si$ requires 300.1168.

4.3. General procedure for the preparation of 15–17

4.3.1. *Z*-6-Trimethylsilanyl-hexa-3,5-diyn-2-one, *N*-benzoyl-4-nitrophenylhydrazone (15). To a stirred solution of *Z*-**11** (200 mg, 0.67 mmol) in CH_2Cl_2 (4 mL) was added, at 0 °C, benzoyl chloride (0.077 mL, 0.67 mmol) then $AlCl_3$ (89 mg, 0.67 mmol). The solution was stirred at 0 °C for 15 min then heating to reflux and followed by TLC. After disappearance of starting material (approximately 2 h), the reaction was quenched with 10% aqueous HCl. The aqueous layer was extracted with CH_2Cl_2 (3×10 mL), the combined organic layers were dried over $MgSO_4$ and concentrated under vacuum. The crude product was purified by flash chromatography (SiO_2, 3:7 Et_2O/light petroleum) to give **15** (191 mg) in 71% yield. Mp=76 °C; ν_{max} (KBr disc/cm^{-1}) 2189, 2090, 1676, 1520, 1344, 1255, 1227, 1171, 1065; ^1H NMR (400 MHz, $CDCl_3$) 0.17 (9H, s), 2.20 (3H, s), 7.35–7.48 (5H, m), 7.62 (2H, m), 8.21 (2H, bd, *J*=9.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) −0.7, 24.2, 68.3, 85.2, 86.7, 100.1, 124.4, 126.1, 128.1, 129.6, 131.5, 134.4, 145.7, 146.9, 149.9, 169.4; HRMS found 404.1409 (MH^+), $C_{22}H_{22}N_3O_3Si$ requires 404.1430.

4.3.2. *Z*-6-Trimethylsilanyl-hexa-3,5-diyn-2-one, *N*-benzoylphenylhydrazone (16). Prepared as above for **15** using *Z*-**12** (200 mg, 0.79 mmol) in CH_2Cl_2 (5 mL), benzoyl chloride (0.091 mL, 0.79 mmol) and $AlCl_3$ (104 mg, 0.79 mmol). The crude product was purified by flash chromatography (SiO_2, 2:8 Et_2O/light petroleum) to give **16** as an oil (103 mg) in 36% yield. ν_{max} (film/cm^{-1}) 2193, 2097, 1670, 1597, 1490, 1339, 1252, 1148, 1073; ^1H NMR (400 MHz, $CDCl_3$) 0.19 (9H, s), 2.23 (3H, s), 7.20–7.40 (8H, m), 7.61 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) −0.6, 24.4, 68.7, 85.2, 86.1, 98.3, 127.6, 127.8, 128.0, 129.0, 129.4, 130.6, 135.3, 141.4, 168.9; HRMS found 359.1590 (MH^+), $C_{22}H_{23}N_2OSi$ requires 359.1580.

4.3.3. *Z*-6-Trimethylsilanyl-hexa-3,5-diyn-2-one, *N*-benzoyl-3-nitrophenylhydrazone (17). Prepared as above for **15** using *Z*-**13** (210 mg, 0.70 mmol) in CH_2Cl_2 (5 mL), benzoyl chloride (0.081 mL, 0.70 mmol) and $AlCl_3$ (94 mg, 0.70 mmol). The crude product was purified by flash chromatography (SiO_2, 3:7 Et_2O/light petroleum) to give **17** as an oil (159 mg) in 56% yield. ν_{max} (film/cm^{-1}) 2097, 1673, 1531, 1350, 1252, 1078; ^1H NMR (400 MHz, $CDCl_3$) 0.17 (9H, s), 2.17 (3H, s), 7.29–7.47 (3H, m), 7.54 (1H, m, *J*=8.0 Hz), 7.57 (1H, bdt, *J*=8.0, 2.0 Hz), 7.63 (2H, m), 8.10 (1H, bdt, *J*=8.0, 2.0 Hz), 8.15 (1H, bt, *J*=2.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) −0.8, 24.2, 68.3, 85.2, 86.7, 99.9, 122.2, 122.3, 128.0, 129.5, 129.7, 131.3, 132.5, 134.3, 142.3, 147.3, 148.4, 169.6; HRMS found 404.1445 (MH^+), $C_{22}H_{22}N_3O_3Si$ requires 404.1430.

4.4. General procedure for the preparation of 18–20

4.4.1. 3-Methyl-1-(4-nitro-phenyl)-4-phenyl-1*H*-pyrazolo[4,3-*c*]pyridine (18). A solution of **15** (87 mg, 0.22 mmol) in EtOH (9 mL) and 33% aq. NH_3 (9 mL) was heated at 85 °C in a sealed tube for 4 h. The solvent was then removed under vacuum. Water (5 mL) and CH_2Cl_2 (5 mL)

were added, the aqueous layer was extracted with CH_2Cl_2 (3×10 mL), the combined organic layers were dried over $MgSO_4$ and concentrated under vacuum. The crude product was purified by flash chromatography (SiO_2, 5:5 to 10:0 Et_2O/light petroleum) to give **18** (36 mg) in 51% yield. Mp=144 °C; ν_{max} (KBr disc/cm^{-1}) 1523, 1344, 1058; ^1H NMR (400 MHz, $CDCl_3$) 2.33 (3H, s), 7.52–7.54 (3H, m), 7.59–7.63 (2H, m), 7.65 (1H, d, *J*=6.0 Hz), 7.95 (2H, d, *J*=9.0 Hz), 8.42 (2H, d, *J*=9.0 Hz), 8.59 (1H, d, *J*=6.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 15.2, 104.0, 120.5, 121.7, 125.5, 128.4, 129.4, 129.4, 138.8, 143.7, 144.6, 145.5, 145.7, 147.1, 157.4; HRMS found 331.1196 (MH^+), $C_{19}H_{15}N_4O_2$ requires 331.1195.

4.4.2. 3-Methyl-1-phenyl-4-phenyl-1*H*-pyrazolo[4,3-*c*]pyridine (19). Prepared as above for **18** using **16** (100 mg, 0.28 mmol), EtOH (10 mL) and 33% aq. NH_3 (10 mL). The crude product was purified by flash chromatography (SiO_2, 5:5 to 10:0 Et_2O/light petroleum) to give **18** (59 mg) in 74% yield. Mp=68 °C; ν_{max} (KBr disc/cm^{-1}) 1561, 1508, 1443, 1241, 1053; ^1H NMR (400 MHz, $CDCl_3$) 2.34 (3H, s), 7.38 (1H, bt, *J*=7.5 Hz), 7.47–7.56 (5H, m), 7.53 (1H, d, *J*=6.0 Hz), 7.62–7.65 (2H, m), 7.68–7.70 (2H, m), 8.48 (1H, d, *J*=6.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 15.1, 103.9, 119.6, 122.8, 127.2, 128.2, 129.0, 129.4, 129.7, 139.2, 139.3, 143.5, 144.5, 144.8, 156.9; HRMS found 286.1344 (MH^+), $C_{19}H_{16}N_3$ requires 286.1344.

4.4.3. 3-Methyl-1-(3-nitro-phenyl)-4-phenyl-1*H*-pyrazolo[4,3-*c*]pyridine (20). Prepared as above for **18** using **17** (90 mg, 0.22 mmol), EtOH (9 mL) and 33% aq. NH_3 (9 mL). The crude product was purified by flash chromatography (SiO_2, 5:5 to 10:0 Et_2O/light petroleum) to give **20** (38 mg) in 52% yield. Mp=129 °C; ν_{max} (KBr disc/cm^{-1}) 1566, 1534, 1346; ^1H NMR (400 MHz, $CDCl_3$) 2.34 (3H, s), 7.51–7.55 (3H, m), 7.60–7.63 (3H, m), 7.74 (1H, t, *J*=8.0 Hz), 8.10 (1H, bdd, *J*=8.0, 2.0 Hz), 8.21 (1H, bdd, *J*=8.0, 2.0 Hz), 8.58 (1H, d, *J*=6.0 Hz), 8.62 (1H, t, *J*=2.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 15.2, 103.6, 117.0, 120.1, 121.3, 127.6, 128.3, 129.3, 129.4, 130.7, 138.8, 140.5, 143.6, 145.5, 146.4, 149.1, 157.3; HRMS found 331.1187 (MH^+), $C_{19}H_{15}N_4O_2$ requires 331.1195.

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New tetrapyrazolic macrocycle. Synthesis and preliminary use in metal ion extraction

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Abstract—A new macrocycle containing two bipyrazolic units, with a side-arm bearing an attached donor-group is reported. The complexing properties of this compound towards heavy metal ions (Hg^{2+}, Cd^{2+}, Pb^{2+}) and alkaline metal ions (Ca^{2+}, Cs^{+}, K^{+}, Na^{+}, Li^{+}) was studied by a liquid–liquid extraction process and the extracted cation percentage was determined by atomic absorption measurements and UV spectroscopy.

1. Introduction

For many years, the ability of pyrazole and its derivatives to act as ligands with sp^2 hybrid nitrogen donor have been the research subjects of many coordination chemists. This is evident from the large number of articles, several of them being reviews.[1,2] Moreover, polydentate pyrazolic receptors are well known for their ability to complex not only alkali cations[3–7] but also to form stable complexes with transition metal ions.[8–11] These complexes are so stable that it is often difficult to obtain the free macrocycles from them.

For some time, we have been interested in acyclic pyrazole compounds containing one, two, three or four pyrazole rings, which have the ability to extract only the transition metal cations.[12]

In this paper, we describe the synthesis of a new tetrapyrazolic macrocycle (Fig. 1) containing a mobile chain with a donor heteroatom and its binding ability towards alkali and transition metal ions. It has been found that a donor atom in a side chain of lariat ethers increases the binding ability of the macrocycle.[13–15] Furthermore, structures with side arms attached at a nitrogen (N-pivot lariat ethers) instead of a carbon (C-pivot lariat ethers) have stronger binding properties because of greater flexibility, allowing the donor site to have the best binding position.[16]

Keywords: Tetrapyrazolic macrocycle; Liquid–liquid extraction; Cations.
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2. Results and discussion

Our goal was to find a convenient and high yielding procedure, in few steps, to prepare the desired hydroxy-substituted pyrazole macrocycle. Having inexpensive starting materials was another important requirement. The route used by us to prepare this macrocycle is shown in Scheme 1.

The preparation of 1,3-bis(3-chloromethyl-5-methylpyrazole) propane **2** from 3(5)-carboxymethyl-5(3)-methyl pyrazole[17] **1** has been already reported[18] in our laboratory. The reaction of synthon **2** with 3(5)-carboxymethyl-5(3)-methylpyrazole was carried out under solid-liquid phase transfer catalysis to favour the α-isomer.[17] Thus, one isolated major product **3** in 75% as the αα-isomer was formed. Compound **3** was then converted in the presence of lithium aluminium hydride to give a 80% yield of the hydroxy product **4**. This reaction was followed by the addition of thionyl chloride to compound **4** to give **5** in a 80% yield. In the cyclization step we condensed the chlorinated compound **5** with 3-aminopropanol in acetonitrile under high dilution conditions in order to favour the macrocyclic compound, which was formed in 60% yield.

Structures of all compounds were determined on the basis of the corresponding analytical and spectroscopic data.

2.1. Liquid–liquid extraction of individual cations

We used this method in order to compare the relative capabilities of macrocycle **6** in extracting Li^{+}, Na^{+}, K^{+}, Cs^{+}, Ca^{2+}, Cd^{2+}, $Pb^{2+}$and Hg^{2+}cations. Metal picrates

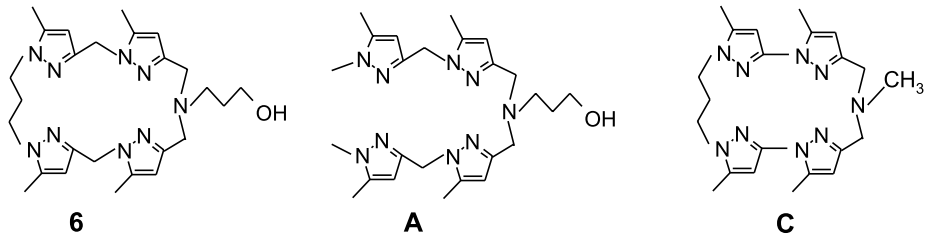


Figure 1. Structures of synthetised tetrapyrazolic macrocycle **6** and of literature compounds **A**[12] and **C**[20].

were extracted into the organic phase by complex formation with the macrocycle, and the decrease in absorbance of the picrate in the aqueous phase was followed by UV spectroscopy. The percentage limits of extraction are given in Table 1.

In order to show that the macrocycle protonation does not occur in the presence of metal picrates, we have determined the extracted cation percentage by atomic absorption measurements, the same results were found.

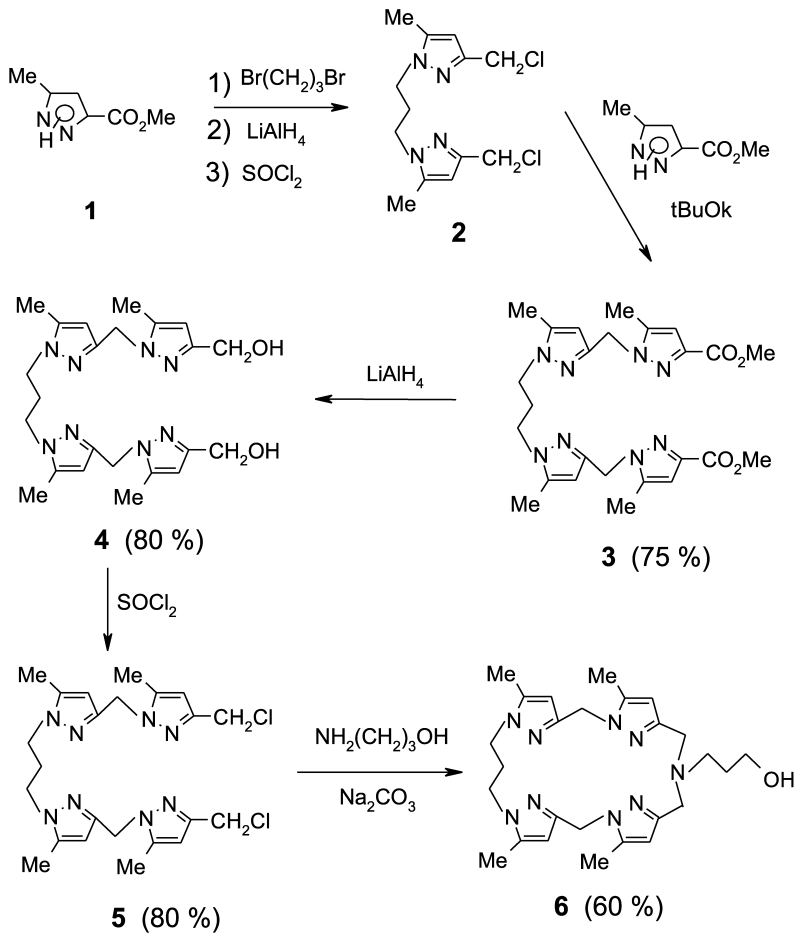
The results in Table 1 show that the comparison with an acyclic pyrazole compound[12] **A** (Fig. 1) which can extract only the transition metal cations and crown-ethers[19] **B** or cryptands which extract only the alkali cations. Macrocycle **6** shows better extraction percentages for alkali cations and for transition metal ions. Only calcium ions are poorly extracted.

We also noticed a high affinity for cesium in the series of alkali cations and a high affinity toward all heavy metal ions. This is undoubtedly related to the size of the cavity possibly enlarged by the junctions between pyrazole units. The ionic radii and the flexibility of the macrocycle also enable cation binding with a possible contribution from the side arm. Moreover, the macrocycle shows high selectivity between Ca^{2+} and other cations.

A possible effect of the lariat arm on the cation binding can be observed by the comparison with a tetrapyrazolic macrocycle[20] **C** (Fig. 1) without donor atoms in the side arm, which shows a different activity.

3. Conclusion

In conclusion, we have prepared a new tetrapyrazolic



Scheme 1.

Table 1. Yields of extraction of various heavy and alkali metal ions

	Mercury (1.10 Å)	Cadmium (0.92 Å)	Lead (1.20 Å)	Calcium (0.99 Å)	Cesium (1.69 Å)	Potassium (1.33 Å)	Sodium (0.98 Å)	Lithium (0.60 Å)
6	60	40	50	6	45	39	39	29
A	55	15	26	0	0	0	0	0
B	0	0	0	0	5	30	2	0
C	—	—	—	—	0	1	25	43

A, acyclic tetra-pyrazole compound[12]; **B**, dibenzo-18-crown-6 ether[19]; **C**, tetrapyrazolic macrocycle compound without donor atoms in the side arm.[20]

macrocycle which has an unusual aptitude for formation of complexes with both alkali and transition metal cations, due to the presence of four donor sp^2 nitrogen atoms in the cavity.

4. Experimental

4.1. General

4.1.1. Syntheses of 3. A mixture of (4.1×10^{-2} mol) of **1** and (4.1×10^{-2} mol) of potassium *tert*-butoxide in 150 ml of THF was stirred under reflux for 30 min. Compound **2** (2×10^{-2} mol) in 100 ml of THF was then added slowly. After stirring under reflux for 6 h, the mixture was filtered, evaporated and the residue was separated on alumina using CH_2Cl_2 as eluant to give a 75% yield of αα-isomer **3** (yellow oil): *Rf*=0.55 (CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ: 2.00 (s, 6H); 2.20 (q, 2H, *J*=7 Hz); 2.30 (s, 6H); 3.83 (s, 6H); 4.00 (t, 4H, *J*=7 Hz); 5.25 (s, 4H); 5.85 (s, 2H); 6.80 (s, 2H). Anal. calcd for $C_{25}H_{32}N_8O_4$: C 59.05, H 6.30, N 22.05. Found: C 59.18, H 6.31, N 22.17; *m/z*: 508 (M^+).

4.1.2. Syntheses of 4. To a solution of $LiAlH_4$ (2.7×10^{-2} mol) in 70 ml of THF was slowly added **3** (1.26×10^{-2} mol) in 100 ml of THF. The mixture was stirred under reflux for 2 h. After cooling, water (1.2 ml), 15% aqueous sodium hydroxide (1.2 ml) and then water (3.6 ml) were added successively to the mixture at 0 °C. The solid material was filtered and the residue was washed with hot THF. The filtrate and THF washings were concentrated under reduced pressure. The residue was passed through a short alumina column (CH_2Cl_2/MeOH, 95:5) to give a 80% yield of **4** (white solid): *Rf*=0.10 (CH_2Cl_2/MeOH, 97:3); mp=69–71 °C (diethyl ether); ^{1}H NMR ($CDCl_3$) δ: 2.10 (s, 6H); 2.25 (bs, 8H); 4.00 (t, 4H, *J*=7 Hz); 4.70 (s, 4H); 5.20 (s, 4H); 5.90 (s, 2H); 6.10 (s, 2H). Anal. calcd for $C_{23}H_{32}N_8O_2$: C 61.06, H 7.08, N 24.78. Found: C 61.08, H 7.11, N 24.77; *m/z*: 452 (M^+).

4.1.3. Syntheses of 5. A solution of thionyl chloride(10 ml) in 15 ml of methylene chloride was slowly added to a compound **4** (3×10^{-2} mol) in 80 ml methylene chloride. This mixture was stirred for 4 h at room temperature. The solvent was removed under reduced pressure and the residue was dissolved in 100 ml of ether. The mixture was then neutralized with about 20 ml of saturated sodium bicarbonate solution and the ether solution was dried over anhydrous sodium sulfate. After evaporating the mixture, the residue was filtred through a short alumina column to give a 80% yield of **5** (yellow oil): *Rf*=0.75 (diethyl ether); ^{1}H NMR ($CDCl_3$) δ: 2.10 (s, 6H); 2.20 (bs, 8H); 4.00 (t, 4H, *J*=7 Hz); 4.50 (s, 4H); 5.20 (s, 4H); 5.90 (s, 2H); 6.10 (s, 2H); *m/z*: 489 (M^+).

4.1.4. Syntheses of macrocycle 6. To a solution of 0.01 mol of sodium carbonate in 1 l of acetonitrile was added slowly and under reflux an equimolar mixture (2×10^{-3} mol) of **5** and 3-aminopropanol in 200 ml of acetonitrile. The mixture was stirred under reflux for 24 h. The solid material was filtered and the filtrate was concentrated under reduced pressure. The residue was purified on alumina using CH_2Cl_2 as eluant to give a 60% yield of **6** (yellow oil): *Rf*=0.42 (CH_2Cl_2/MeOH, 96/4); ^{1}H NMR (DMSO-d_6) δ: 1.65 (m, 2H); 2.10 (m, 4H); 2.15 (s, 6H); 2.20 (s, 6H); 3.20 (s, 4H); 3.35 (m, 2H); 3.85 (t, 4H, *J*=7 Hz); 4.90 (s, 4H); 5.90 (s, 4H). Anal. calcd for $C_{26}H_{37}N_9O$: C 63.54, H 7.53, N 25.66. Found: C 63.58, H 7.51, N 25.67; *m/z*: 520 (MH^+); IR: ν(OH)=3300 cm^{-1}, ν(tertiary nitrogen)=1100 cm^{-1}.

4.2. Extraction experiments

A solution of 7×10^{-5} M of macrocycle in 50 ml of CH_2Cl_2 was stirred for 2 h with an aqueous solution (50 ml) of metal picrates 7×10^{5} M; the complexation was followed first by measuring the picrate anion concentration in the aqueous phase by UV spectroscopy at 355 nm, second by measuring the concentration of cations in the aqueous phase by atomic absorption. The temperature was remained constant during all the experiments at 25 °C and at pH 7 measured by a pH-meter. This was explained by the absence of nitrogen protons in macrocycle and by the low alkalinity and concentration of picrate ions exchanged.

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The synthesis of 7-deazaguanines as potential inhibitors of guanosine triphosphate cyclohydrolase I

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Abstract—Variously substituted 7-deazaguanines are of interest as inhibitors of GTP cyclohydrolase I, the first enzyme in the biosynthetic pathway leading to dihydrofolate and tetrahydrobiopterin. Methods are described for the synthesis of 7-deazaguanines substituted at positions 2, 6 and 9 (purine numbering) such that a wide diversity of compounds can be prepared. These methods supplement our previous work that established routes for the synthesis of 7- and 8-substituted 7-deazaguanines. Emphasis is placed on the properties of 2-thioalkyl pyrimidines as intermediates because they provide the basis for a traceless solid-state synthesis of purines, pteridines, and their analogues. Compounds prepared have been assessed in a primary screen for their ability to inhibit GTPCH I and 8-methyldeazaguanine has been shown to be significantly more potent than any inhibitor yet described. Several compounds appeared to undergo transformation by GTPCH I; with the aid of a model reaction, their behaviour can be interpreted in the context of the mechanism of the hydrolytic phase of GTPCH I.

Guanosine triphosphate cyclohydrolase I (GTPCH I) is the first enzyme in the biosynthetic pathway leading to dihydrofolate and the pathway leading to tetrahydrobiopterin, two essential metabolic cofactors.[1] Recent clarification of the crystal structure of GTPCH I[2] has demonstrated the presence of a zinc cation at the active site and assigned it a role in acid–base catalysis of the hydrolytic opening of the purine ring. This information has strengthened the information for the design of inhibitors of GTPCH I. Such inhibitors may have value in antibacterial therapy or in agrochemistry.[3] It is notable that several antibiotics containing deazaguanines as aglycones have been discovered.[4] In a previous paper in this series[5] we described versatile syntheses of 7-deazaguanines with substituent diversity at positions 7 and 8. Here we describe extensions of these methods to provide diversity at positions 2, 6 and 9 (purine numbering). Taken together, the two papers provide a large part of the basis for a versatile solid phase synthesis methodology for purines, pteridines, and their analogues.[6] Without ability to tolerate a wide range of substituents, these methods would be severely limited. We have therefore studied the capacity of these synthetic methods to prepare a range of highly substituted deazaguanines. The compounds prepared have been assayed against GTP-cyclohydrolase 1. New inhibitors have been identified and unexpected reactivity of several compounds sheds light on the mechanism of action of the enzyme.

1. Synthesis of 9-substituted 7-deazaguanines

In our previous work,[5] N(9)-alkyl groups, alternatives to ribose, were introduced by substitution of 2-amino-6-chloro-4(3*H*)-pyrimidinone **1** (Scheme 1a). It is important for obtaining significant biological activity to be able to include polyfunctional substituents, especially hydroxyl groups that might be phosphorylated in vivo. Consequently, the substitution strategy was extended in this work to include 3-aminopropane-1,2-diol and 4-aminobutan-1-ol as substituents; the corresponding pyrimidines **2b** and **2c** underwent smooth cyclisation with chlorocyanoacetaldehyde to afford the 7-cyano-7-deazaguanines **3b** and **3c** in 60–70% yield. However, this route was shown to be limited to primary amines lacking α-substituents; none of ribosylamine, cyclopentylamine, cyclohexylamine or 1,2-dihydroxy-3-amino-5-hydroxymethylcyclopentane reacted with the 6-chloropyrimidine. We also showed in our previous work[5] that a range of 8-substituted 9-alkyl deazaguanines **4** could be prepared from alkyldiaminopyrimidines such as **2** and the oximes of α-haloketones in the presence of base via intermediate 5-substituted oximes.

Keywords: Purine analogues; Synthesis; Deazaguanines; GTP cyclohydrolase 1; Inhibition; Mechanism.
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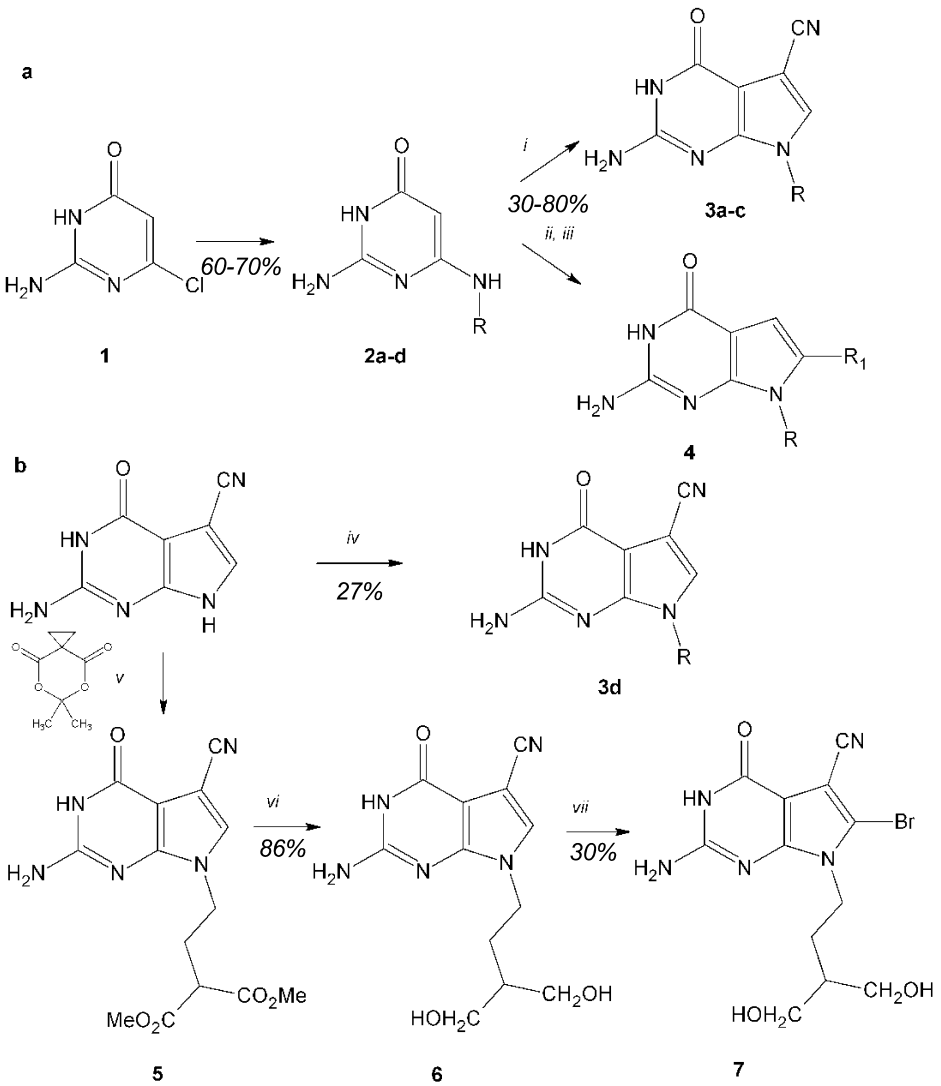
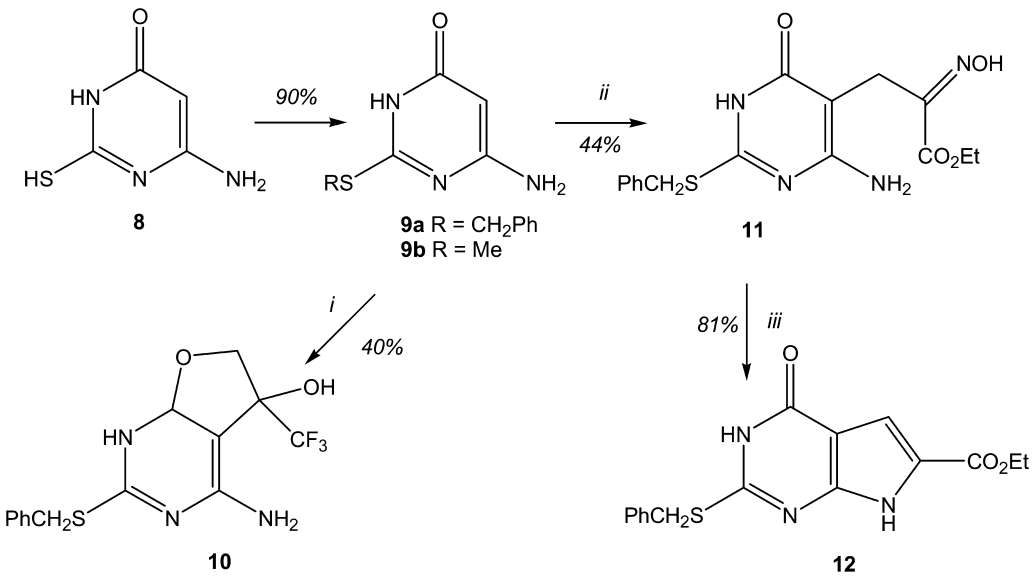


Scheme 1. R=**a** H, **b** $CH_2CH(OH)CH_2OH$, **c** $(CH_2)_4OH$, **d** $CH_2CH{=}CH_2$. *Reagents*: (i) CHCl(CN)CHO; (ii) $R_1C(=NOH)CH_2Br$, Et_3N; (iii) H^+, PhCHO; (iv0 $CH_2{=}CHCH_2Br$, K_2CO_3; (v) 6,6-dimethyl-5,7-dioxaspiro[2.5]octane-4,8-dione, K_2CO_3; (vi) $NaBH_4$, *t*-BuOH; (vii) NBS.

In order to insert substituents more similar to ribose, it was necessary, therefore, to investigate direct alkylation of N-9 as an alternative strategy (Scheme 1b). Whilst this strategy is effective with guanosines,[7] we have found that with deazaguanines there are difficulties associated with the several possible sites of alkylation on nitrogen and oxygen.[4] It was therefore prudent to establish that selective alkylation at N9 was indeed possible. This was done by alkylating the 7-cyano-7-deazaguanine **3a** with allyl bromide in DMF in the presence of potassium carbonate, which afforded a single compound, **3d**, in 68% yield after purification. That alkylation had taken place exclusively at N9 was confirmed by synthesis of **3d** from chloropyrimidine **1** by reaction of the latter with allylamine and subsequent cyclisation. The material thus obtained was identical in all respects to that prepared by direct alkylation. Alkylation with highly functionalised reagents to provide closer analogues to ribose was therefore attempted, based upon published syntheses of the drugs penciclovir and famciclovir.[8] For clean alkylation at N9, a reactive electrophile was required. Triethyl 3-bromopropane-1,1,1-tricarboxylate failed to react on extended reaction times but 6,6-dimethyl-5,7-dioxaspiro[2.5]octane-4,8-dione alkylated 7-cyano-7-deazaguanine **3a** to afford the derivative **5** in 65% yield. Reduction of the diester was accomplished with sodium borohydride affording the corresponding deazaguanine diol, **6**, substituted by an acyclic ribose analogue, in 86% yield. This compound was brominated at C8 with *N*-bromosuccinimide to give **7** and access to the range of sulfur-containing derivatives described by us previously.[5] It is probable that the 7-cyano group is important in controlling the selectivity in these reactions by delocalisation of an anion formed at N9. Similar reactions attempted with 8-ethoxycarbonyl-7-deazaguanine (**4**, R=H, $R_1{=}CO_2Et$) led to complex mixtures; in that case, it seems that introduction of the alkyl group at the pyrimidine stage is essential.

2. Substitution at C2

It is well known that the amino–oxo substitution pattern of the pyrimidine ring of pteridines and guanosines leads not only to ambident reactivity but also to experimental



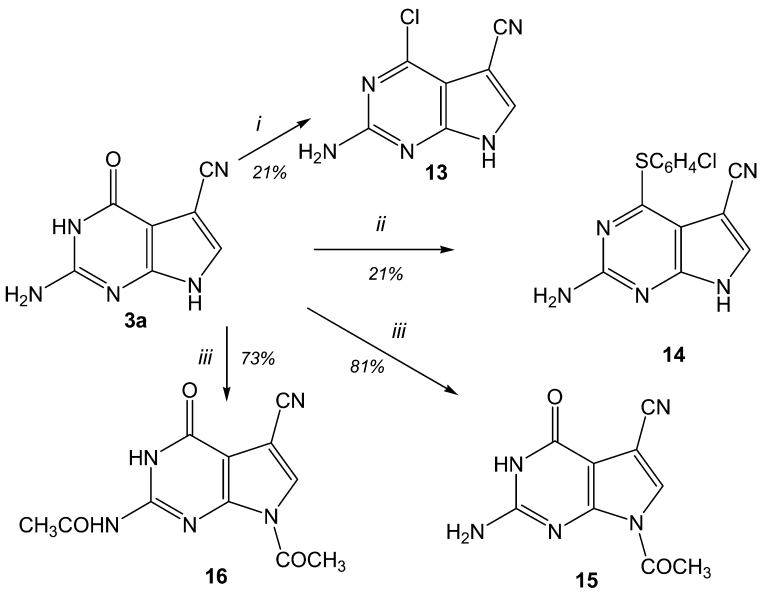
Scheme 2. R=**a** CH_2Ph, **b** Me. *Reagents*: (i) CF_3COCH_2Br, EtOH, 60 °C, 17 h; (ii) $BrCH_2C(=NOH)CO_2Et$, Et_3N, DMF, room temperature, 5 h; (iii) PhCHO, H^+, EtOH, reflux, ~12 h.

problems of low solubility. With a view to extension to solid phase methodologies, the cyclisation chemistry of pyrimidine thioethers was investigated (Scheme 2). 2-Benzylsulfanyl- and 2-methylsulfanyl-6-amino-4(3*H*)-pyrimidinones (**9a**,**b**) were prepared by direct alkylation of the corresponding thiol **8**. Surprisingly, compounds **9a** and **9b** failed to give purines or pyridopyrimidines when treated with C-electrophiles such as chloroacetaldehyde, bromoacetaldehyde diethyl acetal, chlorocyanoacetaldehyde, bromomalonate, ethyl acrylate, acetylacetone, diethyl oxalate, ethyl glyoxylate, and ethyl 2,4-dioxopentanoate, in DMF or ethanol in the presence of base (triethylamine) or acid (4-toluenesulfonic acid) under reflux for periods of up to 4 days.

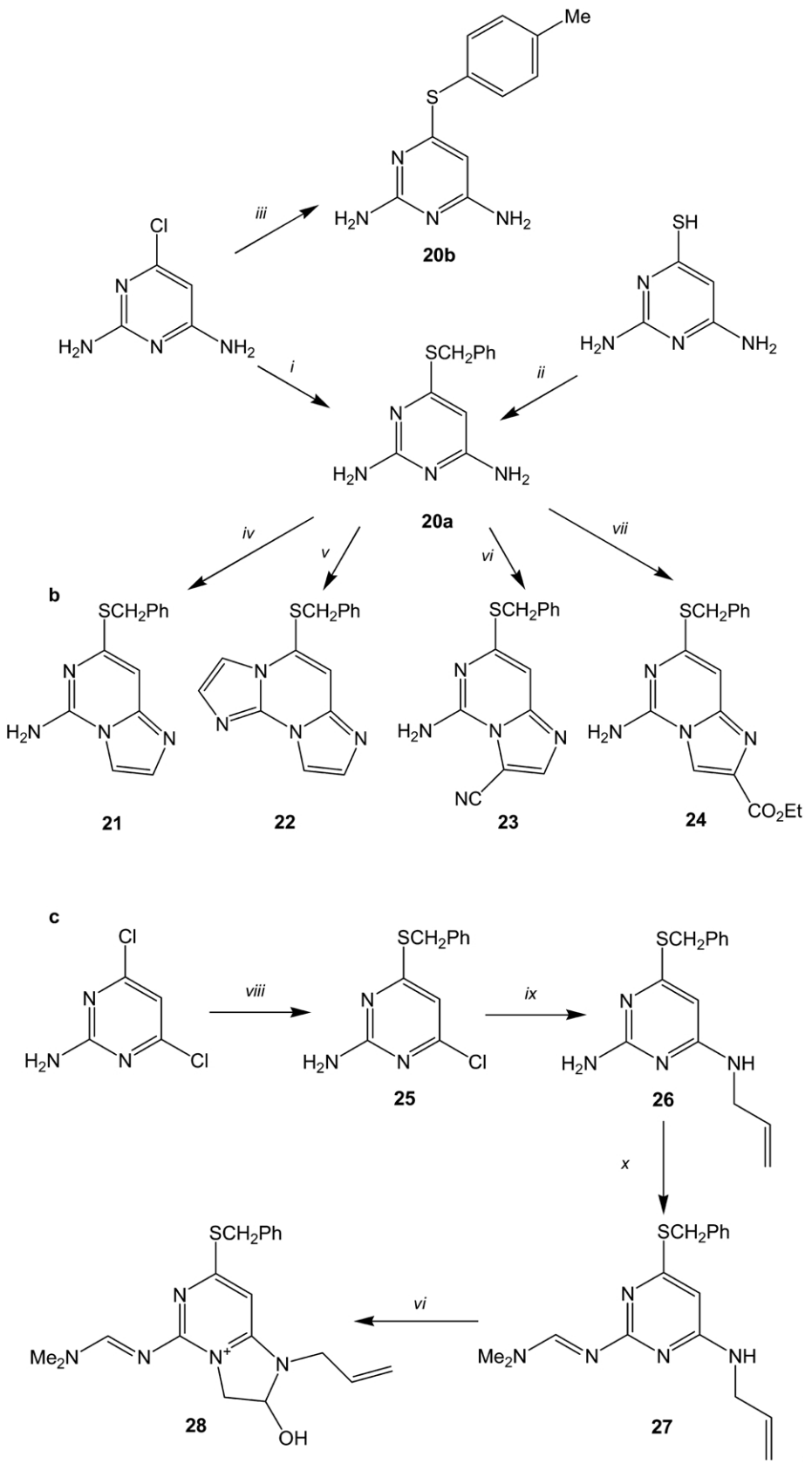
Only two reagents were found to react. Bromotrifluoroacetone reacted with **9a** to afford the furo[2,3-*d*]pyrimidine **10** in 40% yield, a reaction course observed previously with this reagent.[9] Ethyl bromopyruvate oxime, on the other hand, did give the desired C-5 alkylated pyrimidine **11** when reacted with **9a**, and the product **11** was cyclised under our normal conditions by transoximation with benzaldehyde and acid catalysis to afford the 8-ethoxycarbonyl deazaguanine **12** in 81% yield. The influence of the C2 substituent in the reactivity of pyrimidines in this series is striking. Although carbon electrophiles show very limited reactivity, we have shown[5,10] that nitrosation occurs readily leading to effective syntheses of sulfanyl pteridines and purines.

3. Substitution at C6 (C4 in pyrrolo[2,3-*d*] numbering)

Derivatisation of the 4-oxo function in pyrimidines has been found to be a versatile method of controlling reactivity. This



Scheme 3. *Reagents*: (i) $POCl_3$, pyridine, reflux, overnight; (ii) TFAA, pyridine, ClC_6H_4SH, NH_4OH, H_2O_2, 0 °C; (iii) Ac_2O, DMAP, reflux, 6 h.



Scheme 4. *Reagents*: (a) (i) $PhCH_2SH$, NaOH, aq. EtOH, 80 °C, overnight; (ii) $PhCH_2Cl$; (iii) $CH_3C_6H_4SH$, NaOH, aq. EtOH; (b) (iv) $ClCH_2CHO$, 1 equiv. 50 °C, overnight; (v) $ClCH_2CHO$, 2 equiv., 50 °C, 22 h; (vi) Cl(CN)CHCHO, 50 °C, overnight; (vii) bromopyruvate oxime, Et_3N, 80 °C, 10 h, then H^+, PhCHO; (c) (viii) $PhCH_2SH$, NaOH, room temperature, 10 min, (ix) $CH_2{=}CHCH_2Br$, K_2CO_3, 100 °C, 5 days; (x) Bredereck's reagent, 60 °C, 10 min.

modification was applied both to 7-deazaguanines themselves and to the precursor pyrimidines but led to a variety of undesired cyclisations (Schemes 3 and 4) as described below. Conversion of 7-cyano-7-deazaguanine **3a** into more soluble, lipophilic derivatives was approached by chlorination with phosphorus oxychloride to afford **13** in modest yield. Preferably, therefore, **13** was treated sequentially with trifluoroacetic anhydride and pyridine, 4-chlorothiophenol, ammonium hydroxide, and hydrogen peroxide to give the (4-chlorophenylsulfanyl)-deazaguanine **14** in 52% overall yield.[8] Other reagents investigated included acetic anhydride/DMAP; with one equivalent, monosubstitution at N9 giving **15** was observed but used in excess, 3,9-diacetyl-7-deazaguanine **16** was obtained.

In view of the problems associated with C6 substitution described above, it was important to extend the investigation of sulfanyl ethers to C4 in pyrimidines (Scheme 4a). 2,4-Diamino-6-chloropyrimidine readily underwent substitution with benzyl mercaptan to afford the benzyl sulfanyl ether **20a** in 85% yield and the same compound was obtained by alkylation of 2,4-diaminopyrimidine-6-thiol with benzyl chloride. With extension to solid phase synthesis in mind and the evidence that thiophenyl ethers are easier to cleave than thiobenzyl ethers,[11] the (4-methylphenyl)sulfanyl ether **20b** was also prepared. Disappointingly, the 4-sulfanyl ethers introduced yet another reactivity pattern into the pyrimidine ring. Once again, substitution at C5 was not observed. Instead, cyclisation between N3 and 4-NH_2 occurred (Scheme 4b). This was unambiguously demonstrated using chloroacetaldehyde (1 equiv.) which afforded the imidazo[1,2-*c*]-pyrimidine **21** from **20a** in low yield (25%), the structure being confirmed by NMR and X-ray crystallography. Excess chloroacetaldehyde led to dicyclisation and **22**. Chlorocyanoacetaldehyde gave the cyanoimidazo[1,2-*c*]-pyrimidine **23**. Even the highly reactive bromopyruvate oxime in the presence of triethylamine, which had given C-substitution in the 2-sulfanyl series, reacted on nitrogen to form **24**.

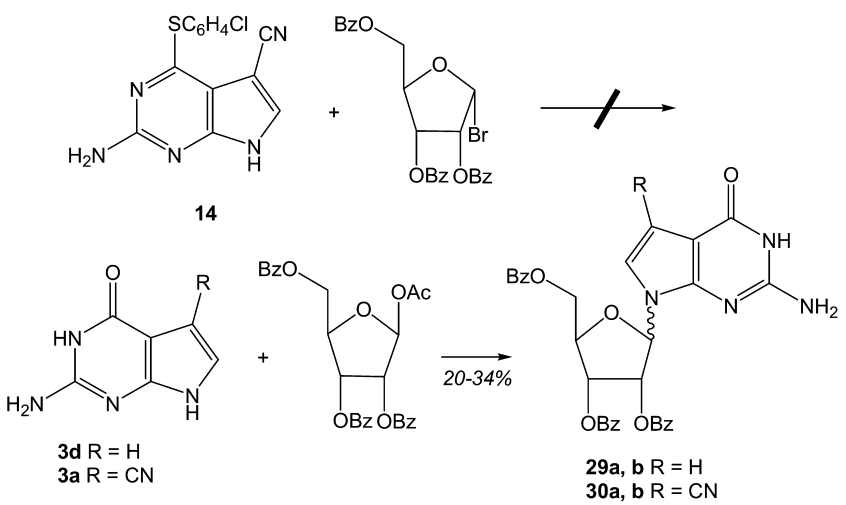
Arguing that this greatly diminished reactivity at C5 was due to a pronounced decrease in electron density and to high nucleophilic reactivity of the 4-amino group, an attempt was made to modify the reactivity to obviate these problems (Scheme 4c). Accordingly 2-amino-4,6-dichloropyrimidine was converted into the benzylsulfanyl ether **25** (96%) and a sterically hindered amino group introduced by substitution of the second chloride with allylamine to give **26** (45%). The remaining amino group was protected with a strongly electron donating dimethylaminoformimino group using Bredereck's reagent to give the required modified pyrimidine **27** (87%). Disappointingly, C5 substitution was not observed with chloracetaldehyde; cyclisation on N3 and the allylamine occurred to give **28**, analogous to the previous reactions but without dehydration. It must therefore be concluded that the synthesis of deazaguanines using 2- and 4-pyrimidine sulfanyl ethers is severely limited to 2-sulfanyl systems with electrophiles derived from α-halo-oximes.

4. Ribosyl derivatives of 7-deazaguanines

A second potential benefit of sulfanyl ethers is the simplification of alkylation chemistry. The synthesis of deazaguanine glycosides in particular might be facilitated by the reduced nucleophilicity of the sulfanyl derivatives compared with their amino and oxo analogues. α-1-Bromoribose-2,3,5-tribenzoate[12] was reacted with the 4-chlorophenyl sulfanyl ether **14** under phase transfer conditions but no riboside formation was observed. However riboside formation was successful using 7-deazaguanine **3d** and the 7-cyano analogue **3a** using 1-acetoxy-ribose-2,3,5-tribenzoate with Lewis acid catalysis[13] affording **29** or **30**, respectively as a mixture of anomers in each case (Scheme 5). It was not found possible to separate the anomers and, since interesting biological activity and structural possibilities were found with simpler compounds, no development work was carried out on glycosylation reactions.

5. Leads from molecular modelling

As part of our international collaboration, Professor Kyuji



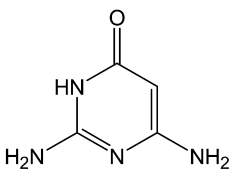
Scheme 5. Ribosyl derivatives of deazaguanines. *Reagents*: hexamethyldisilazane, TMSCl, $SnCl_4$, MeCN, 21 h room temperature.

Ohta in Japan has been investigating the potential binding of deazaguanines and other compounds to the active site of GTPCH I. His studies included the zinc ion and led to the suggestion that deazaguanines with carboxyl or thiol substituents might be very tight binding inhibitors thereby obviating the need for phosphorylated derivatives.[14] Accordingly, target molecules represented by the deazaguanine carboxylates with simplified ribose analogues **31**, and **32** were identified and their synthesis developed (Scheme 6). The precursor pyrimidine **33** was prepared by the conventional substitution methods and cyclised with each of the two established reagents, chlorocyanoacetaldehyde and ethyl bromomalonate oxime. In the former case, a mixture of acetal **34a** and deprotected deazaguanine **34b** in a ratio of 2.5:1 was obtained. Hydrolysis of the cyano group with potassium hydroxide afforded the mixture of carboxylic acids **35a** and **35b** and esterification with thionyl chloride and methanol gave one of the target compounds **31**. In the latter case, C5 substitution occurred without loss of the protecting group to give **36** but we were unable to find conditions under which cyclisation to the deazaguanine **32** could be achieved without cleavage of the protecting group. However, the ester **32** was obtained in 62% yield on cyclisation of **36** thereby confirming the practical utility of the nitroso alkene route for the synthesis of polyfunctional 8,9-substituted deazaguanines.[17]

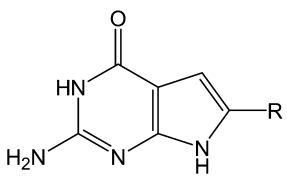
6. 7-Deazaguanines as inhibitors of GTPCH I

We have undertaken assays of the activity of compounds prepared in our studies as inhibitors of GTPCH I using screens designed for rapid assay using small quantities of scarce enzyme. Known inhibitors include guanine, 8-hydroxy-, 8-methyl-, 8-mercapto-, and 8-bromoguanine,[15] 8-azaguanine, and what is probably the most widely used inhibitor, 2,6-diamino-4(3*H*)-pyrimidinone (DAP).[16] GTPCH I is also inhibited naturally by tetrahydrobiopterin mediated by GTPCH I feedback regulatory protein.[15] We have developed two screening assays, one based on UV and the other on HPLC, both of them being suitable for a high throughput of samples and a total of 52 compounds were screened. The compound set included a wide variety of substituted pyrimidines, purines, pteridines, and 7-deazapurines that were synthesised in the present work and in previous studies.[17] The UV assay was carried out by adding a solution of the test compound dissolved in DMSO to a solution of GTPCH I in KCl/Tris buffer, pH 8.5, initiating the reaction by the addition of enzyme. Reaction progress was monitored over 3 min at 1 s intervals by following the absorption at 330 nm. At this wavelength, the product 7,8-dihydroneopterin triphosphate absorbs but the substrate GTP does not. Control experiments were carried out under the same conditions in the absence of test compound and to demonstrate that DMSO had no effect on the reaction in the concentrations used. The HPLC assay monitored the formation of 7,8-dihydroneopterin triphosphate by separation of it on the HPLC column from other reaction components and integration of the product peak. For reasons of availability and activity of GTPCH I, it was not possible to undertake full kinetic assays of the compounds prepared. The available assays, however, functioned together as an effective screen to identify compounds that were significant inhibitors and compounds that underwent reaction with GTPCH I.

The assays showed that 7-deazapurines, substituted at position 8 but unsubstituted at positions 7 and 9, had behaviour consistent with some form of inhibition on GTPCH I. In particular, the HPLC assay showed that in the presence of 8-methyl-7-deazaguanine **37b** (2.9 mM), the amount of 7,8-dihydroneopterin formed from GTP was only 6% of the amount formed in the absence of **37b**, under otherwise identical conditions. The most widely discussed inhibitor, DAP, by comparison showed only 58% inhibition under these conditions. Other related compounds showed similar but smaller decreases: 8-trifluoromethyl-7-deazaguanine **37c** 17%, 8-azaguanine 43%, and 8-ethoxycarbonyl-7-deazaguanine 78%. This general behaviour has been confirmed independently and using a different method (Dr Christian Hesslinger, Frankfurt[18]). 8-Methyl-7-deazaguanine **37b** thus appears to be the most potent inhibitor of GTPCH 1 to have been described so far.



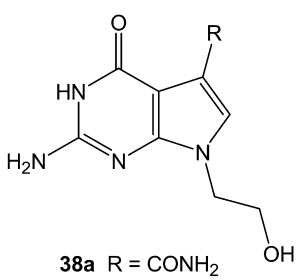
DAP



37a R = H

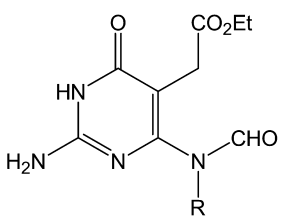
37b R = Me

37c R = CF_3

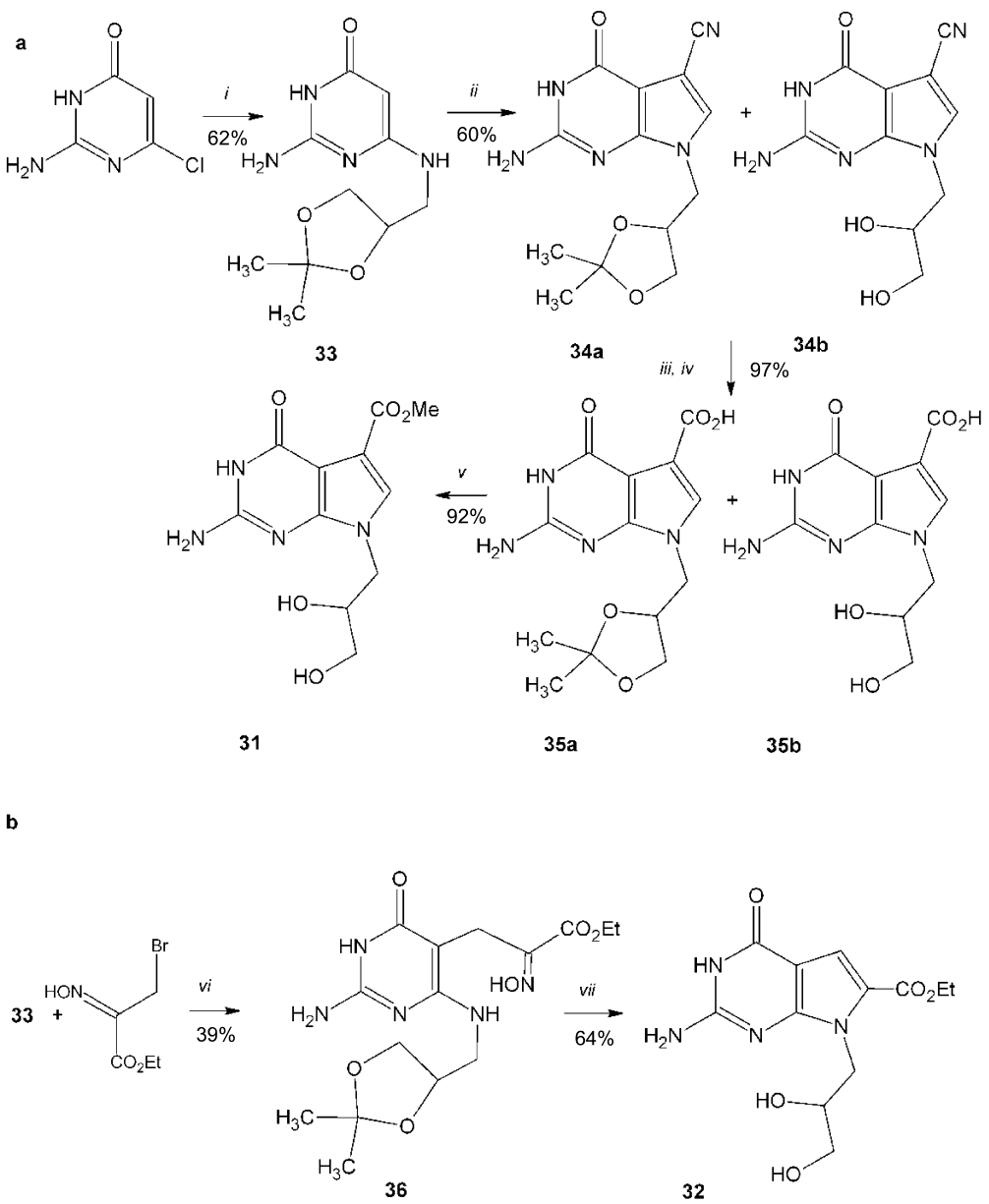


38a R = $CONH_2$

38b R = CO_2Et



39

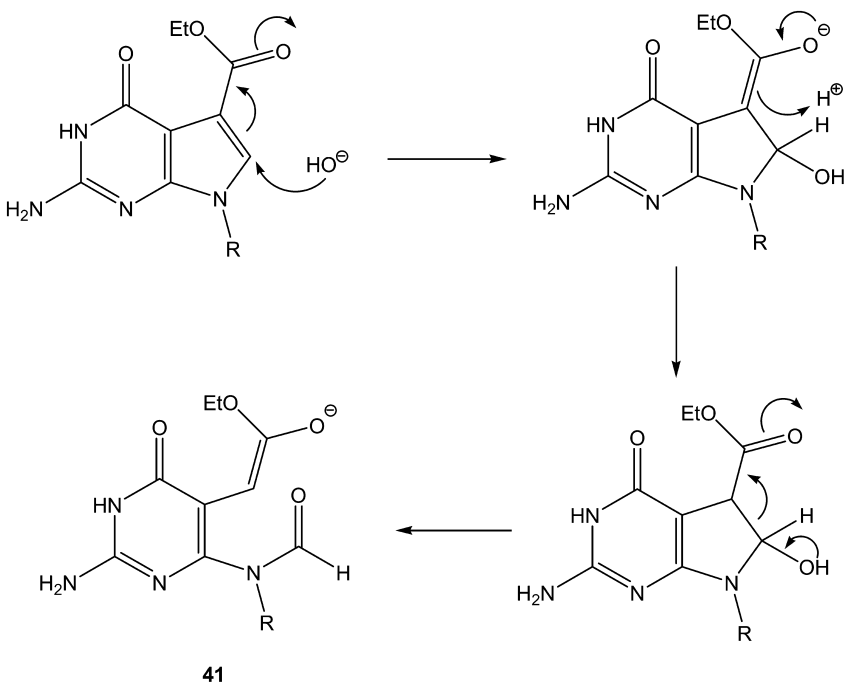


Scheme 6. *Reagents*: (i) RNH_2, Et_3N, EtOH, reflux, 2 days; (ii) Cl(CN)CHCHO, aq. NaOAc, 50 °C, 20 h; (iii) aq. KOH, reflux, 5 h; (iv) AcOH; (v) $SOCl_2$, MeOH, 50 °C, 3 h; (vi) Et_3N, DMF, room temperature, 6 h; (vii) HCl, aq. EtOH, PhCHO, reflux, 2 days.

Observations in the UV assay suggested that **37b** and related compounds may react with the GTPCH I to form a new product, other than 7,8-dihydroneopterin triphosphate. Even in the absence of GTP, the absorption at 330 nm increased over the first minute of assay before slowly decreasing during the remainder of the observation period. Compounds **37a**, **37b**, **37c**, **38a**, and **38b** all showed this effect in the UV assay. A possible explanation of the apparent reaction of these compound with GTPCH I is that, through its normal mechanism, GTPCH I is causing hydrolytic opening of the pyrazole ring. In the case of **38b** this would lead to compound **39**. To determine whether this proposed pathway had a reasonable basis in laboratory chemistry, the ester **38b** was incubated in d_6-DMSO in the presence of sodium ethoxide and the NMR spectrum monitored over many days; special precautions to dry the solvent and reagents were not taken. The use of sodium ethoxide under these conditions provided for the presence of a low concentration of hydroxide thereby avoiding more extensive hydrolytic decomposition. A slow reaction was clearly observed, most notably from the downfield region of the spectrum, where a new peak at δ 8.51 was consistent with the presence of a formamide and a peak at δ 7.05 indicative of the presence of a deshielded alkene (Fig. 1). After 100 days, integration suggested that about 20% conversion into this compound **39** had occurred. A reasonable interpretation of these results (Scheme 7) would be in terms of formation of the ring opened formamide **39**. These preliminary observations encourage further work on the properties and reactivity of deazaguanines with GTPCH I.

7. Conclusions

In this work and in our previous study[5] we have defined the limitations inherent in the ring synthesis of deazaguanines



Scheme 7. Hypothesis for reaction of deazaguanines with GTPCH I.

from substituted pyrimidines and have shown that within these limitations, a wide range of structural diversity can be accessed. We have developed chemistry potentially applicable to solid phase synthesis of a range of related bicyclic heterocyclic compounds.[5] Importantly, we have adduced preliminary evidence that deazaguanines do indeed inhibit and react with GTPCH I in ways that are of interest in the context of the mechanism of action of the enzyme. Moreover our most recent results indicate that deazaguanines are effective in modulating the activity of pteridine biosynthesis in cellular as well as purified enzyme systems.[18]

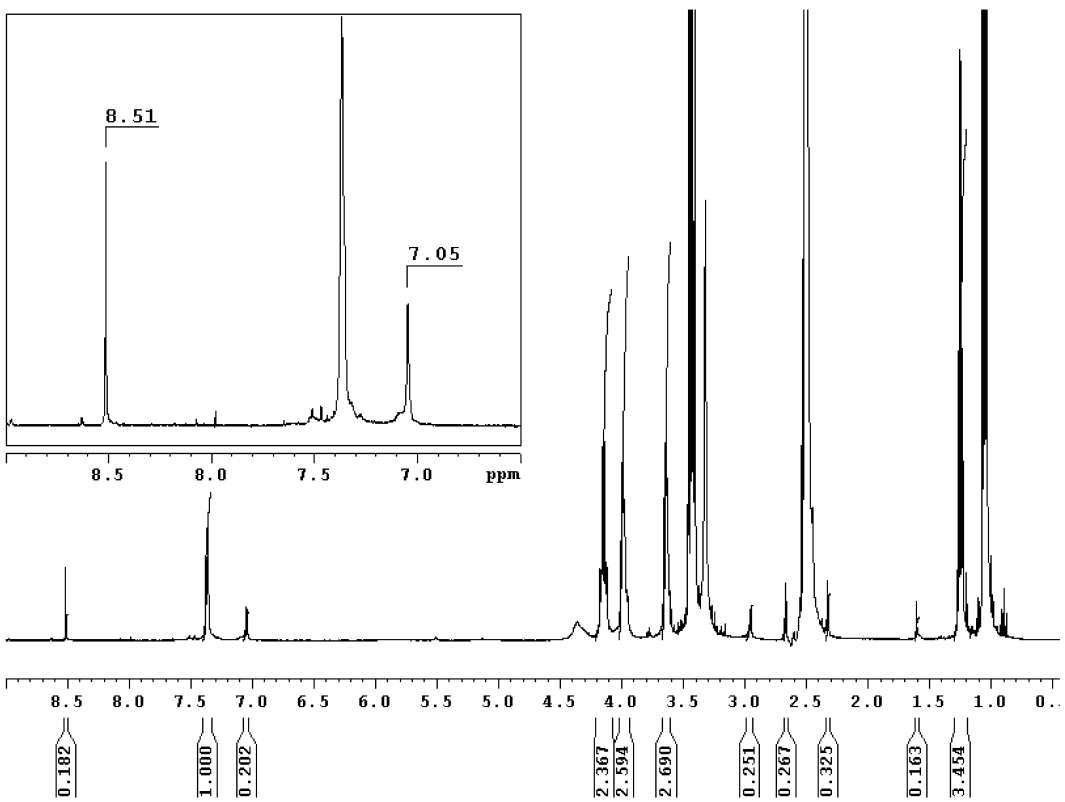


Figure 1. 400 MHz spectrum of **40b** after reacting for 100 days with NaOEt in DMSO solution.

8. Experimental

8.1. Instrumentation and general materials

NMR spectra were determined on a Bruker Spectrospin spectrometer operating at 400 MHz for ^{1}H spectra and 100 MHz for ^{13}C spectra. Chemical shifts are reported as ppm relative to TMS measured from the solvent resonance. IR spectra were determined using a Mattson 1000 FT spectrometer or a Nicolet Impact 400D FT spectrometer. Mass spectra were measured on a Jeol JMS AX505 spectrometer. Microanalyses were carried out using a Perkin–Elmer Series II instrument at the University of Strathclyde. UV spectra were determined using a Perkin–Elmer Lambda 2 spectrometer. Melting points, when measurable, were determined on a Reichert hot stage apparatus and are uncorrected. TLC was carried out on silica (Merck 0.25 mm 60 F_{254}). Column chromatography was carried out using silica gel (230–400 mesh; 40–60 μm).

Reaagents were bought from Aldrich (Gillingham, Dorset, UK).

8.1.1. 2-Amino-6-[(2,3-dihydroxypropyl)amino]-4(3*H*)-pyrimidinone 2b. To a suspension of 2-amino-6-chloropyrimidin-4(3*H*)-one **1** (1.130 g, 6.93 mmol) in water (2.8 mL), was added ethylene glycol dimethyl ether (10 mL) and 1-amino-2,3-propandiol (1.7 g, 18.7 mmol). The mixture was heated to reflux for 6 h and then the solvent was evaporated under reduced pressure to give the required pyrimidinone **2b** as a colourless solid (0.927 g, 4.63 mmol, 68%). After washing successively with water, ethanol and diethyl ether and drying under reduced pressure, the product had mp 204–206 °C. Found: HRMS (FAB) 201.0980; $C_7H_{13}N_4O_3$ (M+1) requires 201.0988. δ_H (DMSO) 2.96 (1H, m, C(9)H), 3.16 (2H, m, C(10)H_2), 3.52 (2H, m, C(8)H_2), 4.47 (1H, s, C(5)H), 4.55 (1H, s, C(10)OH), 4.77 (1H, s, C(9)OH), 6.14 (3H, br s, N(7)H_2, N(6)H), 9.66 (1H, br s, N(3)H). δ_C (DMSO) 44.77 (C-8), 64.23 (C-10), 70.65 (C-9), 75.71 (C-5), 155.33 (C-2), 163.42 (C-4), 164.77 (C-6). ν_{max} (KBr) 3425, 1670, 1537, 1455, 1256, 984, 783 cm^{-1}.

8.1.2. 2-Amino-7-(2,3-dihydroxypropyl)-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile 3b. To a suspension of 2-amino-6-[(2,3-dihydroxypropyl)-amino]-4(3*H*)-pyrimidinone **3b** (0.912 g, 4.56 mmol) and sodium acetate (0.94 g, 10 mmol) in water (30 mL), was added freshly prepared chloro(formyl)-acetonitrile[19] (0.54 g, 5.24 mmol). The mixture was left stirring for 20 h at 50 °C. A precipitate was collected by filtration, washed with water, methanol and diethyl ether to give the required pyrrolopyridine **3b** as a dark solid (0.32 g, 1.3 mmol, 30%; mp >260 °C (lit.[20] 276–278 °C)). Found: HRMS (EI) found 215.0807 (M−2×OH); $C_{10}H_{11}N_5O_3$ requires 249.0862. δ_H (DMSO) 3.26 (2H, m, C(12)H_2), 3.75 (2H, m, C(10)H_2), 4.10 (1H, m, C(11)H), 4.74 (1H, s, C(12)H_2O*H*), 4.99 (1H, s, C(11)HO*H*), 6.50 (2H, s, N(8)H_2), 7.59 (1H, s, C(6)H), 10.73 (1H, s, N(3)H).

8.1.3. 2-Amino-6-[(4-hydroxybutyl)amino]-4(3*H*)-pyrimidinone 2c. To a suspension of 2-amino-6-chloropyrimidin-4(3*H*)-one **1** (1.13 g, 6.93 mmol) in water (3 mL), was added ethanol (20 mL), triethylamine (2 mL) and 1-amino-4-butanol (1.6 g, 18.4 mmol). The mixture was heated to reflux for 2 days, then the solvent was evaporated under reduced pressure to give a colourless solid that was washed with water, ethanol and diethyl ether, dried under reduced pressure, to afford the title compound **2c** (0.95 g, 4.9 mmol, 70%; 206–208 °C (lit.[21] 206 °C)). Found: HRMS (FAB) 199.1194; $C_8H_{15}N_4O_2$ (M+1) requires 199.1195. δ_H (DMSO) 1.38–1.47 (4H, m, C(10)H_2, C(11)H_2), 3.01 (2H, br s, C(12)H_2), 3.34–3.40 (2H, m, C(9)H_2), 4.40 (1H, s, C(5)H), 4.41 (1H, br s, OH), 6.12 (2H, br s, NH_2), 6.33 (1H, br s, N(8)H), 9.66 (1H, br s, N(3)H).

8.1.4. 2-Amino-7-(4-hydroxybutyl)-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile 3c. To a suspension of 2-amino-6-[(4-hydroxybutyl)amino]-4(3*H*)-pyrimidinone **2c** (1.10 g, 5.5 mmol) and sodium acetate (1.03 g) in water (20 mL) was added chloro(formyl)acetonitrile[16] (2 g, 19.4 mmol). Upon addition, the solution became green, turning deep blue immediately. The reaction was stirred at 50 °C for 2 days. The solution was filtered and the colourless solid obtained was washed with water, ethanol, and diethyl ether and dried under reduced pressure to afford the title compound **3c** (1.14 g, 4.7 mmol, 84%; mp >260 °C). Found: HRMS (FAB) 248.1138, $C_{11}H_{14}N_5O_2$ (M+1) requires 248.1147. δ_H (DMSO) 1.30–1.37 (2H, m, C(11)H_2), 1.68–1.76 (2H, m, C(12)H_2), 3.31–3.39 (2H, m, C(13)H_2), 3.92–3.96 (2H, m, C(10)H_2), 4.40–4.43 (1H, m, OH), 6.49 (2H, br s, NH_2), 10.70 (1H, s, N(3)H) δ_C (DMSO) 26.66 (C-11), 29.81 (C-12), 44.85 (C-13), 60.56 (C-10), 85.06 (C-5), 99.21 (C-4a), 116.03 (CN), 130.68 (C-6), 151.07, 154.12, 157.80 (C-4, C-7a, C-2). ν_{max} (KBr) 3343 (NH_2), 3182, 2228 (CN), 1681, 1645, 1599, 1548, 1423, 1343, 1101, 785 cm^{-1}.

8.1.5. 6-(Allylamino)-2-amino-4(3*H*)-pyrimidinone 2d. To a suspension of 2-amino-6-chloropyrimidin-4(3*H*)-one **56** (1.13 g, 6.93 mmol) in water (3 mL), was added ethylene glycol dimethyl ether (10 mL) and allyl amine (1.04 g, 18.4 mmol). The mixture was heated to reflux for 6 h, then the solvent was evaporated under reduced pressure to give a colourless solid that was washed with water, ethanol and diethyl ether and dried under reduced pressure to afford the title compound **2d** (0.68 g, 4.1 mmol, 60%; mp 196–198 °C). Found: HRMS (EI) 166.0860: $C_7H_{10}N_4O$ requires 166.0855. δ_H ($CDCl_3$) 3.68 (2H, s, C(9)H_2), 4.41 (1H, s, C(5)H), 5.04 (1H, d, *J*=9.2 Hz, 1×C(11)H_2), 5.13 (1H, d, *J*=17.2 Hz, 1×C(11)H_2), 5.75–5.84 (1H, m, C(10)H), 6.15 (2H, br s, N(7)H_2), 6.52 (1H, br s, N(8)H), 9.75 (1H, br s, N(3)H). δ_C (DMSO) 45.45 (C-9), 77.89 (C-5), 117.66 (C-11), 134.11 (C-10), 156.23 (C-2), 162.59 (C-4), 165.07 (C-6).

8.1.6. 7-Allyl-2-amino-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile 3d. (a) *Using allyl bromide*. To 7-cyano-7-deazaguanine **3a** (0.5 g, 2.8 mmol) in anhydrous DMF (40 mL) was added allyl bromide (0.35 g, 2.9 mmol) and potassium carbonate (0.45 g, 3.2 mmol). The reaction was left stirring for 4 days at 100 °C, then filtered and evaporated under reduced pressure. The residue was dissolved in water and extracted with dichloromethane, dried over anhydrous sodium sulphate,

and evaporated to give the product **3d** as pale a yellow solid (0.15 g, 0.8 mmol, 27%; mp >260 °C). Found: HRMS (EI) 215.0792, $C_{10}H_9N_5O$ requires 215.0807. δ_H (DMSO) 4.58 (2H, d, J=4.8 Hz, C(10)H_2), 4.95 (1H, d, J=17.2 Hz, 1×C(12)H_2), 5.17 (1H, d, J=10.4 Hz, 1×C(12)H_2), 5.92–6.01 (1H, m, C(11)H), 6.52 (2H, br s, N(8)H_2), 10.76 (1H, s, N(3)H). δ_C (DMSO) 46.85 (C-10), 85.52 (C-5), 99.13 (C-4a), 115.87 (C-9), 117.63 (C-12), 130.49 (C-6), 133.73 (C-11), 151.07, 154.27, 157.82 (C-4, C-7a, C-2). ν_{max} (KBr) 3416, 3325, 2224, 1673, 1626, 1176, 929 cm^{-1}.

(b) *Using allyl amine*. To a suspension of 6-(allylamino)-2-amino-4(3*H*)-pyrimidinone **2d** (0.68 g, 4 mmol) and sodium acetate (0.9 g) in water (20 mL) was added chloro(formyl)-acetonitrile (1.2 g, 11.6 mmol). This was left stirring at 50 °C overnight. After the solution was filtered and the solid obtained was washed with water, ethanol, and diethyl ether to afford the product that was found to be identical with the product prepared by the method (a) above (0.4 g, 1.86 mmol, 45%).

8.1.7. Dimethyl 2-[2-(2-amino-5-cyano-4-oxo-3,4-dihydro-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)ethyl] malonate 5. A mixture of 7-cyano-7-deazaguanine **3a** (0.5 g, 2.8 mmol), 2,2-dimethyl-1,3-dioxaspiro[5.2]octane-4,6-dione (0.65 g, 3.8 mmol), and potassium carbonate (0.75 g) in dry DMF (20 mL) was stirred at 55 °C under an atmosphere of N_2 for 4 days. The resulting mixture was filtered and evaporated under reduced pressure to give a brown solid (the potassium salt). This was dissolved in methanol (35 mL) saturated with HCl and a precipitate formed. A further portion of methanol (35 mL) was added and the mixture was left stirring at room temperature overnight. The solution was then filtered, evaporated under reduced pressure and the residue was dissolved in acetone. Silica gel column chromatography eluting with ethyl acetate/methanol 5/1, gave the product **5** as a colourless solid (0.3 g, 0.83 mmol, 30%; mp >260 °C). Found: HRMS (FAB) 334.1147, $C_{14}H_{16}N_5O_5$ (M+1) requires 334.1151. δ_H (DMSO) 2.26 (2H, q, J=6.9 Hz, C(11)H_2), 3.43 (1H, t, J=6.9 Hz, C(12)H), 3.62 (6H, s, 2×C(16)H_3), 4.01 (2H, t, J=6.9 Hz, C(10)H_2), 6.47 (2H, br s, N(8)H_2), 7.65 (1H, s, C(6)H), 10.73 (1H, s, N(3)H). δ_C (DMSO) 28.81 (C-11), 42.72 (C-10), 48.70 (C-12), 52.92, 52.75 (C-16, C-14), 85.45 (C-5), 99.21 (C-4a), 115.87 (C-9), 130.51 (C-6), 151.22, 154.14, 157.74 (C-4, C-7a, C-2), 169.13 (C-15, C-13). IR ν_{max} (KBr) 3432 (NH_2), 2232 (CN), 1720 (C═O), 1685, 1630, 1427, 787 cm^{-1}.

8.1.8. 2-Amino-7-[4-hydroxy-3-(hydroxymethyl)butyl]-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile 6. Dimethyl 2-[2-(2-amino-5-cyano-4-oxo-3,4-dihydro-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)ethyl]malonate **5** (0.83 g, 2.4 mmol) was partially dissolved in *t*-butanol (30 mL) at 60 °C under an atmosphere of dry nitrogen. Sodium borohydride (0.456 g, 12 mmol) was added and the mixture was heated under reflux while methanol (4 mL) was added dropwise over 1.75 h. The mixture was then cooled, methanol (40 mL) was added, and after the effervescence had ceased, the solvents were evaporated. The residue was dissolved in water (30 mL) and the solution was neutralised with 1 M HCl. Evaporation afforded a colourless solid, which was purified by column chromatography on silica gel eluting with ethyl acetate/methanol 100/0 up to 0/100. The product **6** (0.556 g, 2.0 mmol, 86%) was collected from the last fractions as a pale yellow solid, mp >260 °C. Found: HRMS (FAB) 278.1247, $C_{12}H_{16}N_5O_3$ (M+1) requires 278.1253. δ_H (DMSO) 1.39–1.44 (1H, m, C(12)H), 1.66 (2H, q, J=7.2 Hz, C(11)H_2), 3.30–3.43 (4H, m, C(14)H_2, C(13)H_2), 4.00 (2H, t, J=7.2 Hz, C(10)H_2), 4.50 (2H, s, 2×OH), 6.86 (2H, s, N(8)H_2), 7.71 (1H, s, C(6)H), 11.04 (1H, br s, N(3)H). δ_C (DMSO) 29.05 (C-11), 41.10 (C-12), 43.29 (C-10), 61.57 (C-13, C-14), 85.05 (C-5), 97.14 (C-4a), 116.12 (C-9), 130.49 (C-6), 151.62, 154.41, 157.60 (C-2, C-7a, C-4). ν_{max} (KBr) 3417 (NH_2), 3350, 3233 (NH), 2940 (CH), 2227 (CN), 1683 (C═O), 1637, 1597, 1428, 1031, 778 cm^{-1}.

8.1.9. 2-Amino-6-bromo-7-[4-hydroxy-3-(hydroxymethyl)butyl]-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile 7. To a suspension of 2-amino-7-[4-hydroxy-3-(hydroxymethyl)butyl]-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile **6** (0.556 g, 2.07 mmol) in dry DMF (20 mL), was added *N*-bromosuccinimide (0.398 g, 2.24 mmol). The mixture was stirred at room temperature for 6 h, then evaporated to dryness under reduced pressure and the residue was triturated with water (10 mL). The resulting solid was filtered off, washed with water, ethanol, and then diethyl ether and dried under reduced pressure to afford the product **7** as a microcrystalline off-white solid (0.22 g, 0.61 mmol, 30%; mp 195–199 °C). Found: HRMS (EI) 357.0258, $C_{12}H_{14}N_5O_3^{81}Br$ requires 357.0259. δ_H (DMSO) 1.46–1.51 (1H, m, C(12)H), 1.64 (2H, q, J=7.6 Hz, C(11)H_2), 3.35–3.42 (4H, m, C(13)H_2, C(14)H_2), 4.08 (2H, t, J=7.6 Hz, C(10)H_2), 4.41 (2H, s, 2×OH), 6.61 (2H, br s, N(8)H_2), 10.88 (1H, s, N(3)H). δ_C (DMSO) 28.60 (C-11), 41.10 (C-12), 41.92 (C-10), 61.74 (C-13, C-14), 79.71 (C-5), 107.38 (C-9), 113.95 (C-4a), 125.83 (C-6), 154.46 (C-2), 156.88 (C-7a). ν_{max} (nujol) 3342 (NH_2), 3222, 2942 (CH), 2229 (CN), 1680, 1640, 1562, 1400, 1039, 780 cm^{-1}.

8.1.10. 6-Amino-2-(benzylsulfanyl)-4(3*H*)-pyrimidinone 9a. 6-Amino-2-sulfanyl-4(3*H*)-pyrimidinone monohydrate **8** (8 g, 50 mmol) was suspended in a mixture of water (30 mL) and ethanol (50 mL). Triethylamine (10 g, 0.1 mol) was added and the solution became clear. Benzyl chloride (7 g, 55 mmol) was added to the stirring solution. Within a few minutes, an exothermic reaction started with formation of a colourless precipitate. Stirring was continued for 30 min, the mixture was cooled to 4 °C and the precipitate was filtered and washed with water and diethyl ether to afford the title compound **8a** (10.5 g, 45 mmol, 90%; 248–253 °C (lit.[22] 250–252 °C)). Found: HRMS (EI) 233.0614, $C_{11}H_{11}N_3OS$ requires 233.0623. δ_H (DMSO) 4.33 (2H, s, C(7)H_2), 4.96 (1H, s, C(5)H), 6.54 (2H, br s, N(12)H_2), 7.21–7.32 (3H, m, 2×C(10)H, C(11)H), 7.41–7.43 (2H, m, 2×C(9)H), 11.48 (1H, br s, N(3)H).

8.1.11. 4-Amino-2-(benzylsulfanyl)-5-(trifluoromethyl)-5,6-dihydrofuro[2,3-*d*]pyrimidin-5-ol 10. 6-Amino-2-(benzylsulfanyl)-4(3*H*)-pyrimidinone **9a** (1 g, 4.3 mmol) and bromotrifluoroacetone **224** (1 g, 5.2 mmol) were suspended in ethanol (30 mL) and stirred under nitrogen at 60 °C for 17 h. The solvent was evaporated under reduced

pressure and the residue was dissolved in ethyl acetate, and purified by silica gel column chromatography (1:2 solution of ethyl acetate/*n*-hexane) and then recrystallised from diethyl ether/*n*-hexane. The product **10** was obtained as a white crystalline solid (0.6 g, 1.7 mmol, 40%; mp 145–147 °C). Found: HRMS (EI) 343.0615, $C_{14}H_{12}F_3N_3O_2S$ requires 343.0602. δ_H (DMSO) 4.31 (2H, s, C(8)H_2), 4.33–4.39 (1H, m, 1×C(6)H_2), 4.74–4.77 (1H, m, 1×C(6)H_2), 6.35 (1H, br s, OH), 7.18–7.46 (5H, m, 2×C(10)H, 2×C(11)H, C(12)H). δ_C (DMSO) 34.35 (C-8), 76.12 (C-6), 79.2 (C-13, J=48.2 Hz), 88.42 (C-5), 127.33 (C-4a), 127.35 (C-12), 128.74 (2×C-10), 129.37 (2×C-11), 138.62 (C-9), 160.28 (C-4), 172.46 (C-7a), 174.28 (C-2). ν_{max} (KBr) 3508 and 3305 (NH_2), 3159, 1645, 1606, 1481, 1183, 1172, 713 cm^{-1}.

8.1.12. Ethyl (2*Z*)-3-[4-amino-2-(benzylsulfanyl)-6-oxo-1,6-dihydro-5-pyrimidinyl]-2-(hydroxyimino)-propanoate 11. 6-Amino-2-(benzylsulfanyl)-4(3*H*)-pyrimidinone **9a** (1 g, 4.31 mmol) was dissolved in dry DMF (15 mL). Triethylamine (0.43 g, 4.31 mmol) was added and the mixture was stirred under nitrogen at room temperature. A solution of the ethyl 3-bromopyruvate oxime (1 g, 4.76 mmol) in dry DMF (15 mL) was added over a period of 5 h to the stirred solution with the aid of a syringe pump. Stirring was continued for a further hour after the addition. The solution was evaporated under reduced pressure and the residue was dissolved in ethyl acetate/methanol (4:1 solution, 10 mL). The resulting solution was absorbed on top of a silica gel chromatography column and it was eluted with 100% ethyl acetate, increasing the polarity up to 4:1 ethyl acetate/methanol solution. The product **11** was obtained as a pale yellow solid (0.69 g, 1.90 mmol, 44%; mp 184–186 °C (dec)). Found: HRMS (FAB) 363.1131, $C_{16}H_{19}N_4O_4S$ (M+1) requires 363.1127. δ_H (DMSO) 1.17 (3H, t, J=7.1 Hz, C(18)H_3), 3.44 (2H, s, C(13)H_2), 4.10 (2H, q, J=7.1 Hz, C(17)H_2), 4.33 (2H, s, C(8)H_2), 6.25 (2H, br s, NH_2), 7.22–7.32 (3H, m, 2×C(11)H, C(12)H), 7.42–7.43 (2H, m, 2×C(10)H), 11.66 (1H, br s, NOH), 12.16 (1H, br s, N(1)H). δ_C (DMSO) 14.28 (C-18), 19.67 (C-13), 33.55 (C-8), 60.92 (C-17), 88.56 (C-5), 127.55 (C-12), 128.78 (2×C-11), 129.51 (2×C-10), 138.23 (C-9), 150.66 (C-14), 160.24, 163.05, 163.13, 164.10 (C-2, C-4, C-6, C-15). ν_{max} (KBr) 3498 (NH_2), 3360 (NH), 1731 (C=O), 1575, 1422, 1235, 1128, 770, 705 cm^{-1}.

8.1.13. Ethyl 2-(benzylsulfanyl)-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-6-carboxylate 12. Ethyl (2*Z*)-3-[4-amino-2-(benzylsulfanyl)-6-oxo-1,6-dihydro-5-pyrimidinyl]-2-(hydroxyimino)propanoate **11** (0.5 g, 1.38 mmol) was suspended in a mixture of ethanol (25 mL), water (25 mL) and conc. hydrochloric acid (5 drops). Benzaldehyde (4 mL) was added and the reaction mixture was heated to reflux under nitrogen until reaction was complete as shown by TLC. The solvent was removed under reduced pressure using co-evaporation with toluene/ethanol to remove residual water. The residue was suspended in ethyl acetate/diethyl ether (1:1 solution, 20 mL) and filtered to afford the title compound **12** as an off-white microcrystalline solid (0.37 g, 1.12 mmol, 81%; mp 185–188 °C). Found: HRMS (EI) 329.0849, $C_{16}H_{15}N_3O_3S$ requires 329.0834. δ_H (DMSO) 1.31 (3H, t, J=7.1 Hz, C(15)H_3), 4.29 (2H, q, J=7.1 Hz, C(14)H_2), 4.43 (2H, s, C(8)H_2), 7.04 (1H, s, C(5)H), 7.19–7.51 (5H, m, 2×C(10)H, 2×C(11)H, C(12)H), 12.29 (1H, s, N(7)H), 12.66 (1H, s, N(3)H). δ_C (DMSO) 14.63 (C-15), 34.01 (C-8), 60.71 (C-14), 105.95 (C-4a), 109.53 (C-5), 122.85 (C-6), 127.68 (C-12), 128.79 (2×C-10), 129.71 (2×C-11), 137.71 (C-9), 149.83 (C-7a), 157.29 (C-4), 159.05 (C-2), 160.68 (C-13). ν_{max} (KBr) 3486 (NH), 1717 (C=O), 1624, 1495, 1241, 698 cm^{-1}.

8.1.14. 2-Amino-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile 13. To 2-amino-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile **136** (1.0 g, 5.7 mmol) was added phosphoryl chloride (10 mL, 70 mmol) and *N*,*N*-diisopropylamine (1.0 g, 10 mmol) and the solution was heated to reflux overnight. At the conclusion of this period the mixture was poured in cold-ice water (100 mL) and stirred for 1 h, whereupon, dichloromethane (50 mL) was added. This mixture was filtered and the filtrate was washed with methanol, and ether to afford the crude product. Purification by column chromatography on silica gel (ethyl acetate/methanol—4/1) afforded the title compound **13** as an amorphous solid (0.3 g, 1.55 mmol, 27%; mp >260 °C (lit.[20] >300 °C)). Found: HRMS (EI) found 193.0139, 195.0124. $C_7H_4N_5^{35-37}Cl$ requires 193.0155, 195.0126. δ_H (DMSO) 6.91 (2H, br s, N(8)H_2), 8.10 (1H, s, C(6)H), 12.5 (1H, s, N(3)H). δ_C (DMSO) 83.5 (C-5), 106.5 (C-4a), 115.4 (C-9), 134.5 (C-6), 151.5, 155, 160.8 (C-2, C-7a, C-4).

8.1.15. 2-Amino-4-[(4-chlorophenyl)sulfanyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile 14. Trifluoroacetic anhydride (2.42 mL, 17 mmol) was added dropwise over a period of 15 min to a stirred suspension of 2-amino-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile **3a** (1 g, 5.7 mmol) in dry pyridine (15 mL) at 0 °C (ice-water bath) under a nitrogen atmosphere. After 20 min, solid (4-chloro)thiophenol (2.061 g, 14.2 mmol) was added, and the stirred reactants were allowed to warm up to room temperature. After a further period of 2 h, conc. aqueous ammonia (*d* 0.88, 5.7 mL) was added dropwise over a period of 10 min, followed by 27% aqueous hydrogen peroxide (0.57 mL). After the reaction mixture had been stirred for a further period of 3 h, the products were evaporated to dryness under reduced pressure. The residue was re-evaporated with toluene (20 mL) under reduced pressure, and was then shaken with toluene (15 mL) and water (15 mL). The resulting mixture was filtered and the residue washed first with toluene and then with water. Column chromatography of the residue on silica gel with ethyl acetate/acetone 1/1 gave the title compound **14** as an amorphous, nearly colourless solid (0.90 g, 3 mmol, 52%; mp >260 °C). Found: HRMS (EI) 301.0189, 303.0179 $C_{13}H_8^{35-37}ClN_5S$ requires 301.0189, 303.0159. δ_H (DMSO) 6.39 (2H, s, N(8)H_2), 7.50–7.53 (2H, m, 2×C(11)H), 7.60–7.64 (2H, m, 2×C(12)H), 8.00 (1H, s, C(6)H), 12.27 (1H, s, N(7)H). δ_C (DMSO) 83.08 (C-5), 106.12 (C-4a), 116.43 (C-14), 126.92 (C-13), 129.61 (2×C-11), 133.11 (C-6), 134.44 (C-10), 136.76 (2×C-12), 152.98, 159.76, 160.55 (C-2, C-7a, C-4).

8.1.16. Synthesis of *N*-(7-acetyl-5-cyano-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)acetamide 15. A mixture of 2-amino-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]-pyrimidine-5-carbonitrile **3a** (2 g, 11.4 mmol), acetic

anhydride (30 mL) and a catalytic amount of DMAP were heated to reflux for 6 h. The mixture was then evaporated under reduced pressure to dryness and the residue was washed with acetone and diethyl ether, to yield the title compound **15** (2.406 g, 9.26 mmol, 81%; mp >240 °C). Found: HRMS (FAB) 260.0765, $C_{11}H_{10}N_5O_3$ (M+1) requires 260.0784. δ_H (DMSO) 2.21 (3H, s, C(10)H_3), 2.84 (3H, s, C(13)H_3), 8.34 (1H, s, C(6)H), 11.71 (1H, br s, N(8)H), 12.11 (1H, br s, N(3)H). δ_C (DMSO) 24.33 (C-13), 25.79 (C-10), 90.40 (C-5), 105.10 (C-4a), 113.98 (C-11), 129.51 (C-6), 148.70, 149.17, 155.46 (C-2, C-4, C-7a), 168.31, 174.27 (C-9, C-12). ν_{max} (KBr) 3450, 3136, 2230 (CN), 1729 (C=O), 1707, 1658, 1371, 782 cm^{-1}.

8.1.17. 7-Acetyl-2-amino-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile 16. A mixture of 2-amino-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile **3a** (0.2 g, 1.13 mmol), acetic anhydride (0.159 g, 1.2 mmol) and DMAP (5 mg, catalytic quantity) was gently heated for 5 min with a heating gun. The mixture was then evaporated under reduced pressure, filtered and washed with acetone and diethyl ether and dried under reduced pressure to give the title compound **16** as a colourless solid (0.18 g, 0.83 mmol, 73%; >240 °C). Found: HRMS (FAB) found 218.0669, $C_9H_8N_5O_2$ (M+1) requires 218.0678. δ_H (DMSO) 2.81 (3H, s, C(11)H_3), 6.78 (2H, br s, NH_2), 8.03 (1H, s, C(6)H), 11.11 (1H, s, NH). δ_C (DMSO) 25.77 (C-11), 90.51 (C-5), 100.26 (C-4a), 114.58 (C-9), 126.60 (C-6), 151.88, 154.75, 157.46 (C-2, C-4, C-7a), 168.72 (C-10). ν_{max} (KBr) 3418, 3324, 2228 (CN), 1742 (C=O), 1683, 1635, 1593, 1374, 1310, 780 cm^{-1}.

8.2. General method for the synthesis of benzylsulfanyl- and (4-methylphenyl)sulfanyl-pyrimidines from 6-chloro-2,4-pyrimidinediamine

To a suspension of 6-chloro-2,4-pyrimidinediamine (1 g, 6.94 mmol) and sodium hydroxide (0.35 g, 8 mmol) in ethanol (30 mL) and water (20 mL), the appropriate thiol (1.3 g, 10.46 mmol) was added. The reaction mixture was stirred at 80 °C overnight. The solution was concentrated by evaporation under reduced pressure, and water (20 mL) was added to the residue. The precipitate was collected by filtration, washed with water and diethyl ether, to afford the required product as a white solid.

8.2.1. 6-(benzylsulfanyl)-2,4-pyrimidinediamine 20a. Obtained using benzylthiol in 85% yield (1.37 g, 5.90 mmol), mp 144–146 °C (lit.[23] 146–148 °C). Found: HRMS (FAB) found: 233.0871, $C_{11}H_{13}N_4S$ (M+1) requires 233.0861. δ_H (DMSO) 4.26 (2H, s, C(7)H_2), 5.63 (1H, s, C(5)H), 6.01 (2H, s, NH_2), 6.20 (2H, s, NH_2), 7.20–7.40 (5H, m, 2×C(9)H, 2×C(10)H, C(11)H). δ_C (DMSO) 32.44 (C-7), 90.37 (C-5), 127.26 (C-11), 128.74 (2×C-9), 129.29 (2×C-10), 138.77 (C-8), 162.85 (C-4), 164.14 (C-2), 166.02 (C-6). ν_{max} (KBr) 3440 (NH_2), 3302, 1612, 1562, 1430, 1362, 787, 715 cm^{-1}.

8.2.2. 6-[(4-Methylphenyl)sulfanyl]-2,4-pyrimidinediamine 20b. Obtained using 4-methylbenzenethiol in 94% yield (1.51 g, 6.50 mmol; mp 240–242 °C). Found: HRMS (EI) found: 232.0788, $C_{11}H_{12}N_4S$ requires 232.0783. δ_H (DMSO) 2.35 (3H, s, C(11)H_3), 5.08 (1H, s, C(5)H), 5.97 (2H, br s, NH_2), 6.18 (2H, br s, NH_2), 7.29 (2H, d, *J*=7.8 Hz, 2×C(8)H), 7.44 (2H, d, *J*=7.8 Hz, 2×C(9)H). δ_C (DMSO) 21.71 (C-11), 90.05 (C-5), 126.49 (C-7), 131.26 (2×C-8), 136.36 (2×C-9), 140.16 (C-10), 163.28 (C-4), 164.96 (C-2), 170.12 (C-6) ν_{max} (KBr) 3485, 3371, 1642, 1619, 1558, 1363, 899, 506 cm^{-1}.

8.2.3. 7-(Benzylsulfanyl)imidazo[1,2-*c*]pyrimidin-5-ylamine 21. To a suspension of 6-(benzylsulfanyl)-2,4-pyrimidinediamine **20a** (1 g, 4.31 mmol) and sodium acetate (0.707 g, 8.62 mmol) in water (20 mL) was added chloroacetaldehyde (50% in water) (0.744 g, 4.74 mmol). This was left stirring at 50 °C overnight. After the solution was evaporated to dryness, the residue was suspended in 10 mL of a 4:1 solution of ethyl acetate/methanol and filtered. The solution was purified by silica gel chromatography, using 4:1 ethyl acetate/methanol as eluent. Evaporation of the relevant fractions afforded the title compound **21** as an off-white solid (0.276 g, 1.08 mmol, 25%; mp >240 °C). Found: HRMS (FAB) 257.0851, $C_{13}H_{13}N_4S$ (M+1) requires 257.0861. δ_H (DMSO) 4.32 (2H, s, C(10)H_2), 6.66 (1H, s, C(8)H), 7.21–7.24 (1H, m, C(14)H), 7.28–7.35 (2H, m, 2×C(13)H), 7.39–7.44 (2H, m, 2×C(12)H), 7.40 (1H, s, C(3)H), 7.73 (2H, br s, NH_2), 7.79 (1H, s, C(2)H). δ_C (DMSO) 34.47 (C-10), 95.96 (C-8), 108.37 (C-3), 127.43 (C-14), 128.84 (2×C-13), 129.24 (2×C-12), 133.39 (C-2), 138.10 (C-11), 146.34 (C-7), 147.33 (C-5), 150.93 (C-8a). ν_{max} (KBr) 3435 (NH_2), 3111, 3081, 1670, 1546, 1310, 1156, 931, 742, 704 cm^{-1}.

8.2.4. 5-(Benzylsulfanyl)diimidazo[1,2-*a*:1,2-*c*]pyrimidine 22. To a suspension of 6-(benzylsulfanyl)-2,4-pyrimidinediamine **20a** (0.5 g, 2.15 mmol) and sodium acetate (0.350 g, 4.26 mmol) in water (20 mL) was added chloroacetaldehyde (50% in water) (0.843 g, 5.37 mmol). This was left stirring at 50 °C for 22 h. After the solution was evaporated to dryness, the residue was suspended in 10 mL of a 5:1 solution of ethyl acetate/methanol and filtered. The solution was purified by silica gel chromatography, using 100% ethyl acetate as eluent, and then 4:1 ethyl acetate/methanol. The product was obtained from early fractions as a brown solid (0.144 g, 0.51 mmol, 24%; mp 135–137 °C). Found: HRMS (FAB) 281.0863, $C_{15}H_{13}N_4S$ (M+1) requires 281.0861. δ_H (DMSO) 4.48 (2H, s, C(12)H_2), 7.21 (1H, s, C(6)H), 7.22–7.32 (3H, m, C(16)H, 2×C(15)H), 7.32 (1H, s, C(3)H), 7.36–7.38 (2H, m, 2×C(14)H), 7.55 (1H, s, C(8)H), 7.81 (1H, s, C(2)H), 8.10 (1H, s, C(9)H). δ_C (DMSO) 37.30 (C-12), 104.78 (C-6), 111.90 (C-2), 113.14 (C-9), 128.03 (C-16), 128.48 (C-3), 128.96 (2×C-15), 129.40 (2×C-14), 132.15 (C-13), 132.92 (C-8), 136.15 (C-10a), 136.89 (C-6a), 141.39 (C-5). ν_{max} (KBr) 3125, 3041, 1628, 1568, 1311, 1135, 1030, 858, 707, 695 cm^{-1}.

8.2.5. 5-Amino-7-(benzylsulfanyl)imidazo[1,2-*c*]pyrimidine-3-carbonitrile 23. To a suspension of 6-(benzylsulfanyl)-2,4-pyrimidinediamine **20a** (0.3 g, 1.29 mmol) and sodium acetate (0.212 g, 2.60 mmol) in water (15 mL) was added freshly prepared chloro(formyl)acetonitrile (0.147 g, 4.74 mmol). This was left stirring at 50 °C overnight. After the solution was evaporated to dryness, the residue was suspended in 10 mL of a 4:1 solution of ethyl acetate/methanol and filtered. The solution was

purified by silica gel chromatography, using 4:1 ethyl acetate/methanol as eluent. The title compound **23** was obtained as a brown solid (0.105 g, 0.37 mmol, 29%; mp >240 °C). Found: HRMS (FAB) 282.0819, $C_{14}H_{12}N_5S$ (M+1) requires 282.0813. δ_H (DMSO) 4.45 (2H, s, C(10)H_2), 6.26 (1H, s, C(8)H), 7.22–7.44 (5H, m, 2×C(12)H_2, 2×C(13)H_2, C(14)H), 7.31 (2H, br s, NH_2), 8.37 (1H, s, C(2)H). δ_C (DMSO) 33.23 (C-10), 89.77 (C-8), 92.12 (C-3), 113.42 (C-9), 127.53 (C-14), 128.83 (2×C-12), 129.31 (2×C-13), 137.90 (C-11), 146.26 (C-2), 149.62 (C-8a), 151.70 (C-5), 163.96 (C-7). ν_{max} (KBr) 3340, 3122, 2225 (CN), 1630, 1315, 705 cm^{-1}.

8.2.6. Ethyl 5-amino-7-(benzylsulfanyl)imidazo[1,2-*c*]pyrimidine-2-carboxylate 24. 6-(Benzylsulfanyl)-2,4-pyrimidinediamine **20a** (0.3 g, 1.29 mmol) was dissolved in dry DMF (15 mL). Triethylamine (0.13 g, 1.29 mmol) was added and the mixture was stirred under nitrogen at room temperature. A solution of the ethyl 3-bromopyruvate oxime (0.3 g, 1.42 mmol) in dry DMF (10 mL) was added at room temperature over a period of 5 h to the stirred solution with the aid of a syringe pump. TLC showed no reaction after 2 h, so stirring was continued for a further 10 h at 80 °C. The solution was evaporated under reduced pressure and the residue was dissolved in ethyl acetate/methanol (4:1 solution, 10 mL). The resulting solution was absorbed on top of a silica gel chromatography column and it was eluted with 100% ethyl acetate, increasing the polarity up to 4:1 ethyl acetate/methanol solution. The product **24** was obtained as a brownish solid (0.07 g, 0.21 mmol, 16%; mp >240 °C). HRMS (FAB) found: 329.1064, $C_{16}H_{17}N_4O_2S$ (M+1) requires 329.1072. δ_H (DMSO) 1.30 (3H, t, *J*=7.1 Hz, C(12)H_3), 4.29 (2H, q, *J*=7.1 Hz, C(11)H_2), 4.33 (2H, s, C(14)H_2), 6.66 (1H, s, C(8)H), 7.22–7.25 (1H, m, C(18)H), 7.30–7.33 (2H, m, C(17)H_2), 7.43–7.45 (2H, m, C(16)H_2), 7.92 (2H, br s, NH_2, exchange with D_2O), 8.51 (1H, s, C(3)H). δ_C (DMSO) 14.62 (C-12), 34.41 (C-14), 60.67 (C-11), 95.53 (C-8), 113.99 (C-3), 127.46 (C-18), 128.83 (2×C-16), 129.23 (2×C-17), 136.05 (C-15), 137.84 (C-2), 146.41 (C-8a), 147.42 (C-5), 153.11 (C-9), 162.79 (C-7). ν_{max} (KBr) 3311, 3026, 1716 (C=O), 1626, 1530, 1341, 1014, 787 cm^{-1}.

8.2.7. 4-(Benzylsulfanyl)-6-chloro-2-pyrimidinamine 25. To a suspension of 4,6-dichloro-2-pyrimidinamine (2 g, 12.2 mmol) in 20 mL of ethanol, was added sodium hydroxide (0.48 g, 12.2 mmol) in water (10 mL) with stirring. After 5 min, benzylthiol (1.51 g, 12.2 mmol) was added to the solution and almost immediately a white precipitate formed. Then, water (20 mL) was added to the solution and it was left stirring at room temperature for a further 10 min. The product was collected by filtration, washing extensively with water and diethyl ether, and dried under reduced pressure affording the title compound **25** as a white crystalline solid (2.8 g, 11.06 mmol, 91%; mp 118–120 °C). Found: HRMS (FAB) 252.0367, $C_{11}H_{11}N_3S^{35}Cl$ (M+1) requires 252.0362; found 254.0315, $C_{11}H_{11}N_3S^{37}Cl$ (M+1) requires 254.0333. δ_H (DMSO) 4.39 (2H, s, C(8)H_2), 6.60 (1H, s, C(5)H), 7.20 (2H, s, NH_2), 7.22–7.43 (5H, m, C(12)H, 2×C(11)H, 2×C(10)H). δ_C (DMSO) 32.80 (C-8), 105.18 (C-5), 127.56 (C-12), 128.84 (2×C-11), 129.42 (2×C-10), 137.77 (C-9), 159.36 (C-6), 162.63 (C-2), 171.45 (C-4). ν_{max} (KBr) 3478, 3296, 1633, 1521, 1411, 1215, 816, 785, 696 cm^{-1}.

8.2.8. N^4-Allyl-6-(benzylsulfanyl)-2,4-pyrimidinediamine 26. To a solution of 4-(benzylsulfanyl)-6-chloro-2-pyrimidinamine **25** (2.6 g, 10.3 mmol) in methoxyethanol (30 mL), was added allylamine (2.28 g, 3 mL, 40 mmol) at room temperature. The resulting solution was stirred and heated at 100 °C for 5 days. Then the solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography using as eluent a gradient of ethyl acetate/*n*-hexane (1:1)→100% ethyl acetate. The product **26** was obtained as a white solid (1.25 g, 4.6 mmol, 45%; mp 135–138 °C). Found: HRMS (FAB) found: 273.1185, $C_{14}H_{17}N_4S$ (M+1) requires 273.1174. δ_H (DMSO) 3.84 (2H, br s, C(14)H_2), 4.26 (2H, s, C(8)H_2), 5.05 (1H, dd, *J*=10.3, 1.6 Hz, 1×C(16)H_2), 5.14 (1H, dd, *J*=17.2, 1.6 Hz, 1×C(16)H_2), 5.66 (1H, s, C(5)H), 5.80–5.89 (1H, m, C(15)H), 6.05 (2H, br s, NH_2), 6.83 (1H, br s, N(13)H), 7.20–7.24 (1H, m, C(12)H), 7.27–7.31 (2H, m, C(10)H_2), 7.38–7.40 (2H, m, C(11)H_2). δ_C (DMSO) 32.48 (C-8), 42.61 (C-14), 90.30 (C-5), 115.38 (C-16), 127.23 (C-12), 128.71 (2×C-10), 129.27 (2×C-11), 136.21 (C-9), 138.82 (C-15), 162.62, 163.05, 165.47 (C-2, C-4, C-6). ν_{max} (KBr) 3458, 3136, 1629, 1594, 1559, 1435, 1232, 1166, 788 cm^{-1}.

8.2.9. *N*′-[4-(Allylamino)-6-(benzylsulfanyl)-2-pyrimidinyl]-*N*,*N*-dimethylimidoformamide 27. To a stirring suspension of N^4-allyl-6-(benzylsulfanyl)-2,4-pyrimidinediamine **26** (0.5 g, 1.84 mmol) in anhydrous DMF (5 mL) was added bis(dimethylamino)-*tert*-butoxymethane (Bredereck's reagent) (0.38 g, 2.2 mmol) and the mixture was heated to 60 °C under nitrogen for 20 min. The resulting solution was concentrated under reduced pressure and the residue was dissolved in ethyl acetate (5 mL). Upon addition of diethyl ether a white product crushed out of solution, which was filtered off and washed with diethyl ether and dried under reduced pressure to afford the title compound **27** as a white solid (0.526 g, 1.6 mmol, 87%; mp 133–135 °C). Found: HRMS (FAB) found: 328.1599, $C_{17}H_{22}N_5S$ (M+1) requires 328.1596. δ_H (DMSO) 2.96 (3H, s, C(10)H_3), 3.06 (3H, s, C(10a)H_3), 3.88 (2H, br s, C(12)H_2), 4.32 (2H, s, C(16)H_2), 5.06 (1H, dd, *J*=10.3, 1.6 Hz, 1×C(14)H_2), 5.15 (1H, dd, *J*=17.2, 1.6 Hz, 1×C(14)H_2), 5.86 (1H, m, C(13)H), 5.94 (1H, s, C(5)H), 7.04 (1H, t, *J*=5.7 Hz, N(11)H), 7.21–7.24 (1H, m, C(20)H), 7.28–7.32 (2H, m, 2×C(19)H), 7.38–7.39 (2H, m, 2×C(18)H), 8.53 (1H, s, C(8)H). δ_C (DMSO) 32.73 (C-16), 34.74 and 40.65 (C-10 and C-10a), 42.75 (C-12), 94.47 (C-5), 115.46 (C-14), 127.24 (C-20), 128.74 (2×C-18), 129.11 (2×C-19), 136.16 (C-13), 138.67 (C-17), 158.26 (C-8), 163.15 (C-2), 165.68 (C-4). ν_{max} (KBr) 3436, 3216, 1625, 1592, 1514, 1344, 1111, 796, 713 cm^{-1}.

8.2.10. *N*′-[1-Allyl-7-(benzylsulfanyl)-2-hydroxy-1*H*,2*H*,3*H*-imidazo[1,2-*c*]pyrimidin-4-ium-5-yl]-*N*,*N*-dimethylimidoformamide chloride 28. To a suspension of *N*′-[4-(allylamino)-6-(benzylsulfanyl)-2-pyrimidinyl]-*N*,*N*-dimethylimidoformamide **27** (0.25 g, 0.76 mmol) and sodium acetate (0.125 g, 1.53 mmol) in acetonitrile (10 mL) and water (3 mL) was added chloroacetaldehyde (50% in water) (0.144 g, 0.91 mmol). This was left stirring at 50 °C overnight. After the solution was evaporated to dryness, the residue was taken in 20 mL of dichloromethane, washed with water (10 mL) and purified by silica

gel column chromatography (100% dichloromethane→ dichloromethane/methanol 9:1). Evaporation of the relevant fractions afforded the title compound **28** as its chloride salt, which was recrystallised from acetone/*n*-hexane as a white crystalline solid (0.144 g, 0.35 mmol, 47%; mp 158–160 °C). Found: HRMS (FAB) found: 370.1706, $C_{19}H_{24}N_5OS$ (M+) requires 370.1702. δ_H (DMSO) 3.17 and 3.32 (2×3H, 2×s, C(15)H_3, C(16)H_3), 4.01–4.12 (2H, m, C(9)H_2), 4.18–4.23 (1H, m, 1×C(3)H_2), 4.34–4.39 (1H, m, 1×C(3)H_2), 4.52 (2H, s, C(17)H_2), 5.21 (1H, d, J=10.3, 1.1 Hz, 1×C(11)H_2), 5.38 (1H, dd, J=17.2, 1.1 Hz, 1×C(11)H_2), 5.52–5.57 (1H, m, C(2)H), 5.78–5.87 (1H, m, C(10)H), 6.52 (1H, s, C(8)H), 7.22–7.38 (5H, m, 2×C(19)H, 2×C(20)H, C(21)H), 7.49–7.51 (1H, m, OH), 8.89 (1H, s, C(13)H). δ_C (DMSO) 34.00 (C-17), 35.91 and 41.98 (C-15, C-16), 44.41 (C-9), 53.12 (C-3), 82.07 (C-2), 88.11 (C-8), 118.76 (C-11), 127.63 (C-21), 128.90 (2×C-19), 129.20 (2×C-20), 131.86 (C-10), 137.58 (C-18), 154.03 (C-8a), 154.68 (C-5), 160.02 (C-13), 173.59 (C-7). ν_{max} (KBr) 3435, 3025, 1626, 1541, 1507, 1262, 1132, 1100, 947 cm^{-1}.

8.2.11. 2-Amino-7-(2′,3′,5′-tri-*O*-benzoyl-α-D-ribofuranosyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one 29a and 2-amino-7-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one 29b. To 2-amino-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one **3d** (0.3 g, 1.98 mmol) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (1 g, 1.98 mmol) in 50 mL acetonitrile were added hexamethyldisilazane (0.31 mL, 1.5 mmol), trimethylchlorosilane (0.2 mL, 1.5 mmol) and finally stannic chloride (0.3 mL, 2.5 mmol) in acetonitrile (10 mL) under nitrogen. After a short period of magnetic stirring everything had dissolved and the mixture was stirred for 21 h at room temperature, then dichloromethane (75 mL) was added and the mixture was extracted with aqueous satd $NaHCO_3$ solution. After re-extracting the aqueous phase with dichloromethane, the combined organic phase was washed with satd NaCl solution, dried (Na_2SO_4) and evaporated. The residue was dissolved in dichloromethane and absorbed into the top of a silica gel chromatography column and eluted with dichloromethane (200 mL), then with dichloromethane/methanol—98/2 to give a mixture of two inseparable anomers **29a** and **29b** (unknown ratio) as above (0.40 g, 0.673 mmol, 34%). Found: HRMS (FAB) found 595.1814, $C_{32}H_{27}N_4O_8$ (M+1) requires 595.1829. δ_H($CDCl_3$) 4.58–4.75 (3H, m, C(5′)H_2, C(4′)H), 5.90–6.12 (3H, m, C(3′)H, C(2′)H, C(1′)H), 6.35 (1H, br s, C(5)H), 6.67 (1H, br s, C(6)H), 7.17–7.54 (11H, m, 6×C(9′)H, 3×C(10′)H, NH_2), 7.73–8.14 (6H, m, 6×C(8′)H), 11.20 (1H, br s, N(3)H). δ_C($CDCl_3$) 64.34, 64.65 (C-5′), 70.11, 72.00, 72.91, 74.23, 79.13, 80.25, 81.42, 85.22 (C-1′, C-2′, C-3′, C-4′), 101.57, 101.77 (C-4a), 103.24 (C-5), 118.11, 118.20 (C-6), 128.54–128.80 (C-9′), 128.87–129.74 (C-7′), 129.86–130.26 (C-8′), 133.34–133.85 (C-10′), 150.13, 150.84 (C-7a), 150.57, 151.32 (C-2), 161.09, 161.89 (C-4), 165.31–166.44 (C-6′). ν_{max}(KBr) 3368 (NH_2), 2965 (CH), 1728, (C═O), 1671, 1607, 1580, 1455, 1269, 1125, 712 cm^{-1}.

8.2.12. 2-Amino-7-(2′,3′,5′-tri-*O*-benzoyl-α-D-ribofuranosyl)-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile 30a and 2-amino-7-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile 30b. To 2-amino-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile **3a** (0.7 g, 3.97 mmol) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (2 g, 3.97 mmol) in acetonitrile (50 mL) were added hexamethyldisilazane (0.62 mL, 3 mmol), trimethylchlorosilane (0.4 mL, 3 mmol) and finally $SnCl_4$ (0.6 mL, 5 mmol) in acetonitrile (10 mL). After a short period of magnetic stirring everything had dissolved and the mixture was stirred for 24 h at room temperature, then dichloromethane (75 mL) was added and the mixture was extracted with satd $NaHCO_3$ solution. After re-extracting the aqueous phase with dichloromethane, the combined organic phase was washed with satd NaCl solution, dried (Na_2SO_4) and evaporated. The residue was dissolved in dichloromethane and absorbed onto the top of a silica gel chromatography column and eluted with dichloromethane (200 mL), then with dichloromethane/methanol—98/2 to give a mixture of two inseparable anomers **30a** and **30b** as above (0.50 g, 0.81 mmol, 20%). Found: HRMS (FAB) found 620.1766, $C_{33}H_{26}N_5O_8$ (M+1) requires 620.1781. δ_H ($CDCl_3$) 4.48–4.87 (3H, m, C(5′)H_2, C(4′)H), 5.97–6.12 (3H, m, C(3′)H, C(2′)H, C(1′)H), 7.22–7.34 (9H, m, 6×C(9′)H, C(6)H, NH_2), 7.37–7.48 (3H, m, 3×C(10′)H), 7.84–7.95 (6H, m, 3×C(8′)H), 10.18 and 10.79 (1H, br s, N(3)H of **a** and **b**). δ_C($CDCl_3$) 64.15 (C-5′), 71.77, 74.31, 78.77 (C-2′, C-3′, C-4′), 86.18 (C-1′), 87.77 (C-5), 100.85 (C-4a), 115.46 (CN), 128.54–128.61 (C-9′), 128.97–129.43 (C-7′), 129.84–130.03 (C-8′, C-6), 133.44–133.71 (C-10′), 150.70, 151.09, 159.16 (C-2, C-7a, C-4), 165.27–166.80 (C-6′). ν_{max} (KBr) 3307 (NH_2), 2228 (CN), 1725 (C═O), 1681, 1601, 1492, 1271, 1123, 710 cm^{-1}.

8.2.13. 2-Amino-6-{[(2,2-dimethyl-1,3-dioxolan-4-yl)methyl]amino}-4(3*H*)-pyrimidinone 33. To a suspension of 2-amino-6-chloropyrimidin-4(3*H*)-one (4 g, 27 mmol) in methoxyethanol (80 mL), was added triethylamine (2.7 g, 27 mmol) and (2,2-dimethyl-1,3-dioxolan-4-yl)methylamine (5 g, 38 mmol) with stirring. The mixture was heated at reflux for 2 days, then the solvent was evaporated under reduced pressure and ethyl acetate (50 mL) was added. The resulting solution was left standing overnight at 4 °C and a pale yellow precipitate formed, which was filtered and washed with little ethyl acetate and diethyl ether and dried under reduced pressure to afford the title compound **33** as colourless microcrystals (4 g, 16 mmol, 62%; mp 134–136 °C). Found: HRMS (FAB) found: 241.1301, $C_{10}H_{17}N_4O_3$ (M+1) requires 241.1301. δ_H (DMSO) 1.24 and 1.32 (2×3H, s, 2×C(13)H_3), 3.17–3.40 (2H, m, C(10)H_2), 3.63 (1H, dd, J=8.2, 6.1 Hz, C(8)H), 3.95 (1H, dd, J=8.2, 6.1 Hz, C(8)H), 4.10–4.16 (1H, m, C(9)H), 4.49 (1H, s, C(5)H), 6.23 (2H, br s, NH_2), 6.41 (1H, br s, N(7)H), 9.77 (1H, br s, N(3)H). δ_C (DMSO) 25.71 and 27.21 (2×C-13), 41.93 (C-8), 67.17 (C-10), 72.52 (C-9), 74.6 (C-5), 108.73 (C-12), 155.47 (C-6), 163.18 (C-4), 164.53 (C-2). ν_{max} (KBr) 3351 (NH_2), 2931, 1639, 1213, 787 cm^{-1}.

8.2.14. 2-Amino-7-[(2,2-dimethyl-1,3-dioxolan-4-yl)methyl]-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*] pyrimidine-5-carbonitrile 34a and 2-amino-7-(2,3-dihydroxypropyl)-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile 34b. To a suspension of 2-amino-6-{[(2,2-dimethyl-1,3-dioxolan-4-yl)methyl]amino}-4(3*H*)-pyrimidinone **33** (1.5 g, 6.25 mmol) and sodium acetate

(1.2 g, 14.6 mmol) in water (28 mL), was added freshly prepared chloro(formyl)acetonitrile (1.93 g, 18.75 mmol). The mixture was left stirring for 20 h at 50 °C. A precipitate was collected by filtration, washed with water, methanol and diethyl ether to give a 2.5:1 mixture (by NMR) of **34a** and **34b**, respectively (0.995 g, 3.7 mmol, 60%). Found:(FAB) **a**: 290.1263, $C_{13}H_{16}N_5O_3$ (M+1) requires 290.1253. δ_H (DMSO) 1.23 and 1.31 (2×3H, 2×s, 2×C(13)H_3 of **a**), 3.25–3.38 (2H, m, C(10)H_2 of **b**), 3.71–3.74 (2H, m, C(8)H_2 of **b**), 3.79–3.84 (2H, m, C(8)H_2 of **a**), 3.95–3.99 (2H, m, C(10)H_2 of **a**), 4.08–4.12 (1H, m, C(9)H of **b**), 4.35–4.41 (1H, m, C(9)H of **a**), 4.74 (1H, t, *J*=5.5 Hz, C(10)H_2O*H* of **b**), 5.00 (1H, d, *J*=5.2 Hz, C(9)HO*H* of **b**), 6.50 (2H, s, N(2)H_2 of **b**), 6.52 (2H, s, N(2)H_2 of **a**), 7.59 (1H, s, C(6)H of **b**), 7.65 (1H, s, C(6)H of **a**), 10.72 (1H, s, N(3)H of **b**), 10.75 (1H, s, N(3)H of **a**). δ_C (DMSO) 25.47 and 26.95 (2×C-13 of **a**), 47.37 (C-8 of **a**), 48.23 (C-8 of **b**), 63.89 (C-10 of **b**), 66.40 (C-10 of **a**), 70.16 (C-9 of **b**), 77.93 (C-9 of **a**), 84.77 (C-11 of **b**), 85.27 (C-11 of **a**), 99.48 (C-4a of **b**), 99.99 (C-4a of **a**), 109.36 (C-12 of **a**), 115.85 (C-5 of **a**), 116.05 (C-5 of **b**), 131.32 (C-6 of **a**), 131.77 (C-6 of **b**), 151.22 (C-7a of **b**), 151.32 (C-7a of **a**), 154.06 (C-2 of **b**), 154.23 (C-2 of **a**), 157.73 (C-4 of **a**), 157.76 (C-4 of **b**).

8.2.15. 2-Amino-7-[(2,2-dimethyl-1,3-dioxolan-4-yl)methyl]-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxylic acid 35a and 2-amino-7-(2,3-dihydroxypropyl)-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxylic acid 35b. The obtained 2.5:1 mixture of nitriles **203** and **204** (0.900 g, 3.3 mmol) was dissolved in 5 M aqueous potassium hydroxide (15 mL). The solution was heated to reflux for 5 h. After cooling, the mixture was neutralised with glacial acetic acid and cooled to 5 °C. The precipitate was filtered off, washed with water, ethanol, and diethyl ether, to give the required carboxylic acid as a 1:3 mixture (by NMR) of **35a** and **b**, respectively (0.920 g, 3.2 mmol, 97%). Found: HRMS (FAB) found for **b**: 269.0861, $C_{10}H_{13}N_4O_5$ (M+1) requires 269.0885; for **a**: 309.1217, $C_{13}H_{17}N_4O_5$ (M+1) requires 309.1199. δ_H (DMSO) 1.24 and 1.32 (2×3H, 2×s, 2×C(13)H_3 of **a**), 3.26–3.38 (2H, m, C(10)H_2 of **b**), 3.71–3.88 (2H, m, C(8)H_2 of **b**), 3.72–3.76 (2H, m, C(8)H_2 of **a**), 3.97–4.00 (2H, m, C(10)H_2 of **a**), 4.09–4.17 (1H, m, C(9)H of **b**), 4.40–4.43 (1H, m, C(9)H of **a**), 4.77 (1H, br s, C(10)H_2O*H* of **b**), 5.02 (1H, br s, C(9)HO*H* of **b**), 6.75 (2H, s, N(2)H_2 of **a** and **b**), 7.48 (1H, s, C(6)H of **b**), 7.52 (1H, s, C(6)H of **a**), 11.75 (1H, br s, N(3)H of **a** and **b**), 14.36 (1H, br s, C(14)OOH of **a** and C(11)OOH of **b**). δ_C (DMSO) 25.50 and 26.97 (2×C-13 of **a**), 47.15 (C-8 of **a**), 47.91 (C-8 of **b**), 63.89 (C-10 of **b**), 66.47 (C-10 of **a**), 70.34 (C-9 of **b**), 74.13 (C-9 of **a**), 96.69 (C-4a of **b** and **a**), 109.39 (C-12 of **a**), 109.71 (C-5 of **b**), 110.21 (C-5 of **a**), 129.39 (C-6 of **a**), 130.03 (C-6 of **b**), 152.09 (C-7a of **b**), 152.22 (C-7a of **a**), 154.26 (C-2 of **b**), 154.62 (C-2 of **a**),162.48 (C-4 of **b**), 162.63 (C-4 of **a**), 163.65 (C-14 of **a**, and C-11 of **b**). ν_{max} (KBr) 3436, 3349, 1688, 1646, 1423, 1069, 757 cm^{-1}.

8.2.16. Methyl 2-amino-7-(2,3-dihydroxypropyl)-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxylate 31. To a suspension of the obtained 7:3 mixture of **35a** and **35b** (0.5 g, 1.7 mmol) in methanol (20 mL) was added thionyl chloride (3 mL, 4 mmol). The solution was left stirring for 3 h at 50 °C and then the solvent was evaporated. The resulting residue was taken in water (10 mL), filtered, washed with ethanol and water, and dried under reduced pressure to afford the required ester **31** as an amorphous, off white solid (0.44 g, 1.56 mmol, 92%; mp >240 °C). Found: HRMS (FAB) found: 283.1045, $C_{11}H_{15}N_4O_5$ (M+1) requires 283.1042. δ_H (DMSO) 3.26–3.37 (2H, m, C(10)H_2), 3.69 (3H, s, C(13)H_3), 3.75 (1H, m, C(9)H), 3.86–3.91 (1H, m, C(8)H), 4.13–4.17 (1H, m, C(8)H), 7.05 (2H, br s, N(2)H_2), 7.48 (1H, s, C(6)H). δ_C (DMSO) 48.24 (C-8), 51.33 (C-13), 63.76 (C-10), 70.46 (C-9), 97.90 (C-4a), 109.36 (C-5), 129.89 (C-6), 149.08 (C-7a), 153.14 (C-2), 156.89 (C-4), 163.46 (C-11). ν_{max} (KBr) 3325 (NH_2), 1735 (C=O), 1667, 1311, 1107, 1063, 754 cm^{-1}.

8.2.17. Ethyl 3-(2-amino-4-{[(2,2-dimethyl-1,3-dioxolan-4-yl)methyl]amino}-6-oxo-1,6-dihydro-5-pyrimidinyl)-2-(hydroxyimino)propanoate 36. 2-Amino-6-{[(2,2-dimethyl-1,3-dioxolan-4-yl)methyl]amino}-4(3*H*)-pyrimidinone **33** (1 g, 4.16 mmol) was dissolved in dry DMF (15 mL). Triethylamine (0.5 g, 5 mmol) was added and the mixture was stirred under nitrogen at room temperature. A solution of ethyl 3-bromopyruvate oxime (1 g, 4.76 mmol) in dry DMF (8 mL) was added over a period of 5 h to the stirred solution with the aid of a syringe pump. Stirring was continued for a further hour after the addition. The solution was evaporated under reduced pressure and the residue was dissolved in water (20 mL). The resulting solution was cooled to 4 °C and filtered to give the title compound **36** as a white solid (0.6 g, 1.62 mmol, 39%; mp 233–237 °C). Found: HRMS (FAB) found: 370.1739, $C_{15}H_{24}N_5O_6$ (M+1) requires 370.1727. δ_H (DMSO) 1.17 (3H, t, *J*=7.1 Hz, C(19)H_3), 1.25 and 1.33 (2×3H, 2×s, 2×C(13)H_3), 3.24–3.54 (2H, m, C(10)H_2), 3.39 (2H, s, C(14)H_2), 3.63 (1H, dd, *J*=8.2, 6.1 Hz, C(8)H), 3.93 (1H, dd, *J*=8.2, 6.1 Hz, C(8)H), 4.01–4.15 (1H, m, C(9)H), 4.10 (2H, q, *J*=7.1 Hz, C(18)H_2), 5.87–5.90 (1H, m, N(7)H), 6.18 (2H, s, N(2)H_2), 9.93 (1H, br s, N(1)H), 12.16 (1H, br s, NOH). δ_C (DMSO) 14.28 (C-19), 19.08 (C-14), 25.75 and 27.19 (2×C-13), 43.63 (C-8), 60.92 (C-18), 67.18 (C-10), 74.89 (C-9), 82.47 (C-5), 108.66 (C-12), 151.71 (C-4), 153.87 (C-2), 161.0 (C-15), 162.13 (C-6), 164.37 (C-16). ν_{max} (KBr) 3425, 3335, 1702 (C=O), 1593, 1483, 1326, 827, 778, 701 cm^{-1}.

8.2.18. Ethyl 2-amino-7-(2,3-dihydroxypropyl)-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-6-carboxylate 32. Ethyl 3-(2-amino-4-{[(2,2-dimethyl-1,3-dioxolan-4-yl)methyl]amino}-6-oxo-1,6-dihydro-5-pyrimidinyl)-2-(hydroxyimino)propanoate **36** (0.35 g, 0.95 mmol) was suspended in a mixture of ethanol (50 mL), water (15 mL) and conc. hydrochloric acid (6 drops). Benzaldehyde (4 mL) was added and the reaction mixture was heated under reflux under nitrogen for 2 days. The solvent was removed under reduced pressure using co-evaporation with toluene to remove residual water. The residue was recrystallised from ethanol–water to give the title compound **32** as a white solid (0.18 g, 0.61 mmol, 64%; mp >240 °C). Found: HRMS (FAB) found: 297.1202, $C_{12}H_{17}N_4O_5$ (M+1) requires 297.1199. δ_H (DMSO) 1.28 (3H, t, *J*=7.1 Hz, C(11)H_3), 3.24–3.28 (2H, m, C(14)H_2), 3.75–3.80 (1H, m, C(13)H), 4.21 (2H, q, *J*=7.1 Hz, C(10)H_2), 4.25–4.38 (2H, m, C(12)H_2), 4.53 (1H, t, *J*=5.7 Hz, C(14)H_2O*H*), 4.67 (1H, d, *J*=5.1 Hz, C(13)HO*H*), 6.58 (2H, br s, N(2)H_2), 7.04 (1H,

s, C(5)H), 10.57 (1H, br s, N(3)H). δ_C (DMSO) 15.08 (C-11), 46.58 (C-12), 60.57 (C-10), 64.82 (C-14), 71.46 (C-13), 101.05 (C-4a), 112.51 (C-5), 121.59 (C-6), 154.43 (C-2), 154.85 (C-7a), 159.54 (C-4), 161.47 (C-8). ν_{max} (KBr) 3346 (NH_2), 1698 (C═O), 1603, 1265, 1187, 1098, 752 cm^{-1}.

8.3. Screening for GTPCH inhibitory activity

GTPCH I was kindly supplied by Professor A. Bacher and Dr N. Schramek, of the Technical University, Munich. The enzyme was stored in vials in aqueous solution at −75 °C, at a concentration of 1.6 mg/mL (65 nM/mL of monomer). Individual vials were thawed before use, and centrifuged to remove insoluble material.

The HPLC screening assay was automated using a Shimadzu LC-10ADvp liquid chromatograph linked to a SIL-10ADvp auto-injector and a Shimadzu SPD-M10Avp diode array detector. The column used was a Develosil 5 μm RP Aqueous column, 250×2.0 ID, fitted with a C18 guard column, both from Phenomenex. The mobile phase comprised an aqueous solution containing triethylamine (1%), isopropanol (0.8%) and 85% phosphoric acid (0.3%), as described by Bacher et al.[24] For the assay, a series of vials in the HPLC autosampler contained the test compounds, each vial containing one of the test compounds (4 μL of a 100 mM DMSO solution), GTP (80 μL of a 0.5 mM solution), Tris buffer (20 μL of a 1 M solution, pH 8.5), KCl (20 μL of a 1 M solution) and triply distilled water (16 μL), making a total volume in each vial of 140 μL. The control vial used DMSO (4 μL) containing no test compound. To each of the test vials in turn at time zero, the autosampler added a solution of GTP cyclohydrolase I (60 μL) with mixing, making a total reaction mixture of 200 μL. The final concentration of enzyme in this reaction mixture was 20 μM of active site equivalent (one active site per monomer). The final concentration of GTP in the reaction mixture was 200 μM, thus giving a 10:1 molar ratio of GTP to enzyme active site. Samples of each reaction mixture were injected on to the column after 23, 38, 53 and 68 min reaction time, each chromatogram being run for 13.5 min. Integration of the peak centred at 5.4 min gave a measure of the amount of 7,8-dihydroneopterin triphosphate formed in the reaction.

The UV screening assay used a Shimadzu UV-2401PC ultraviolet–visible spectrophotometer with 400 μL quartz cells of path length 10 mm. For each test compound the following solution was prepared in a vial at 26 °C: GTP (20 μL of a 0.5 mM solution), KCl (20 μL of a 1 M solution), Tris buffer (20 μL of a 1 M solution, pH 8.5), triply distilled water (70 μL) and the test compound (4 μL of either a 50 or100 mM solution in DMSO). At time zero, a solution of GTP cyclohydrolase I at 26 °C (60 μL, 1.6 g/L) was added to the vial, the contents of which were mixed and transferred immediately to the UV cell and placed in the spectrophotometer. Absorption was measured at 330 nm at 1 second intervals over a period of 3 min. Control experiments were carried out using pure DMSO (4 μL) instead of the test compound solution. Data were processed using Microsoft Excel, and slopes were obtained by linear regression on the experimental data points. Percentage inhibition by the test compounds was calculated using the following formula:

$$\% \text{ inhibition} = 100(\text{Control} - \text{Test})/\text{Test},$$

where 'Control' is the rate of reaction in the absence of the test compound and 'Test' is the rate of reaction in presence of the test compound.

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Synthesis of barettin

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Abstract—The indole alkaloid barettin (with bromine in 6-position), isolated from the marine sponge *Geodia Barretti*, has been synthesised via a Horner–Wadsworth–Emmons type reaction from 6-bromoindole-3-carboxaldehyde to introduce the dehydro-functionality. Subsequent deprotection and cyclisation afforded the natural product in *Z*-conformation.

1. Introduction

The structure of the pharmacologically active indole alkaloid barettin, isolated in 1986 from the cold water sponge *Geodia barretti* by Lidgren et al.,[1] has been the subject of debate during several years. The originally proposed structure for barettin, the diketopiperazine **1**, was disproved by an independent total synthesis of **1**, as well as the *E*-isomer **2** a year later (Fig. 1).[2] However, a recent publication by Sölter et al.[3] presented isolation and structure elucidation of a diketopiperazine from *G. barretti*, collected in Norway. The German group found the diketopiperazine to be a condensation product of 6-bromo-Δ-tryptophan[4] and arginine, i.e. compound **3**, which they believed represented the actual structure of barettin. Now we have indeed confirmed these findings after a reinvestigation of isolated material, combined with a total synthesis of compound **3**.

2. Results and discussion

Synthesis of arginine-containing peptides is often very laborious due to problems with the basic guanidino group in the arginine side chain. In our case, the guanidino group was protected by *tert*-butoxycarbonyl groups, via the excellent method by Bernatowicz et al.[5] Thus the Cu(II)-ornithine[6] complex (prepared from L-ornithine·HCl) is guanylated at N^{δ} with a protected derivative of 1-guanylpyrazole, N^{1}-[*N*,*N*′-bis(*tert*-butoxycarbonyl)amidino]pyrazole (**4**) to give the Cu(II)-complex of N^{ω},$N^{\omega'}$-bis(*tert*-butoxycarbonyl)-protected arginine derivative **5** in good yields, prepared as described in the literature.[5] Compound **5** was further N^{α}-protected with di-*t*-butyl dicarbonate in the presence of ethylenediaminetetraacetic acid (EDTA) to afford N^{α}-(*tert*-butoxycarbonyl)-N^{ω},$N^{\omega'}$-bis(*tert*-butoxycarbonyl)-L-arginine (**6**) in a reasonable yield (79%) (Scheme 1).

Using this protected arginine derivative, the saturated analogue of **3** (i.e. **9**) was prepared via standard peptide coupling procedures with 6-bromo-D,L-tryptophan methyl ester (**7**), obtained via esterification of commercially available 6-bromo-D,L-tryptophan. Removal of the protecting groups of the resulting dipeptide **8** with trifluoroacetic acid (TFA) and subsequent cyclisation[7] afforded

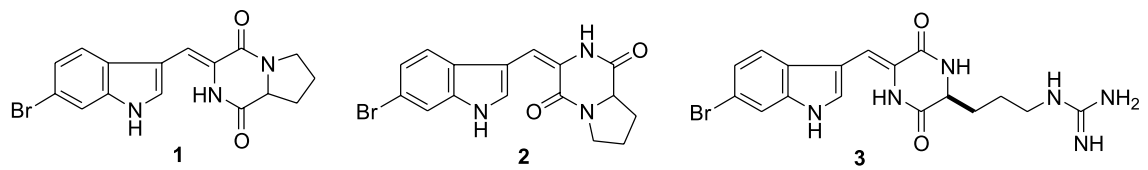

Figure 1.

Keywords: *Geodia Barretti*; Barettin; *N*-Boc protection of arginine; HWE-reaction.
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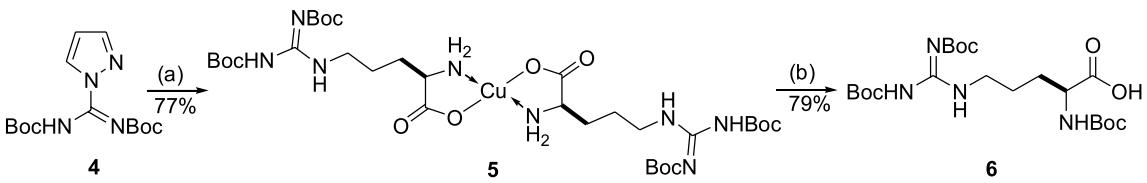
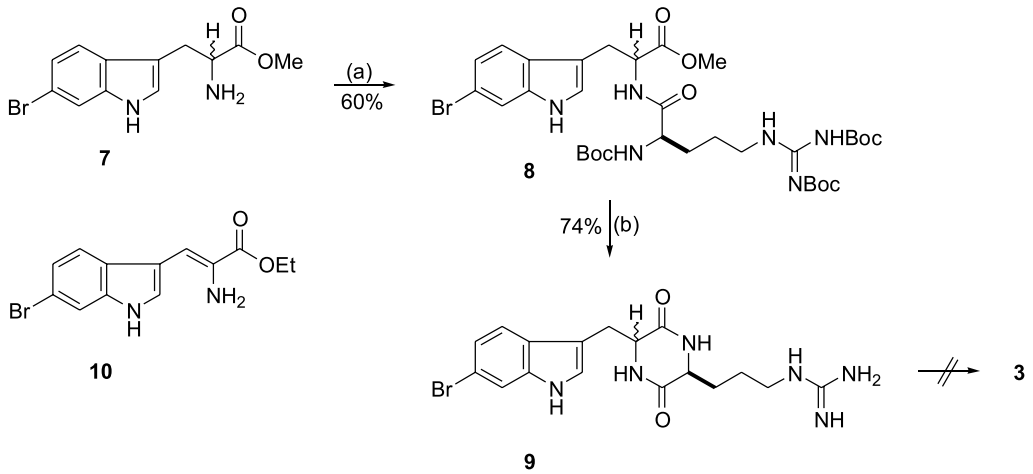


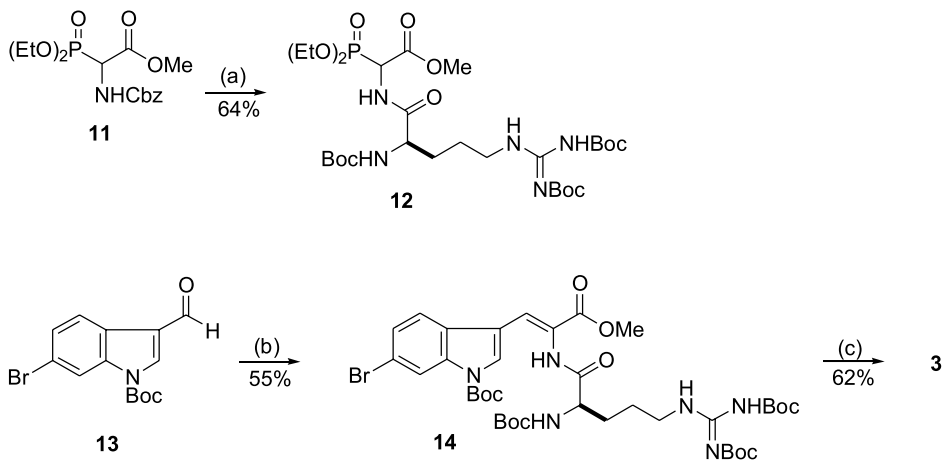
Scheme 1. (a) $Cu(Orn)_2{\cdot}CuCl_2$, DIEA, formamide/dioxane, rt 5 h. (b) $EDTA{\cdot}4Na{\cdot}2H_2O$, $NaHCO_3$, Boc_2O, H_2O/acetone, rt 12 h.

cyclo-6-bromo-D,L-Trp-L-Arg or 8,9-dihydrobarettin (**9**) as a mixture of diastereomers. Although 8,9-dihydrobarettin has previously been identified as a congener of barettin in *G. barretti*, the specific rotation of the natural product still remains to be determined.[8] Here, no efforts were made to separate the diastereomeric mixture. However, despite several attempts, using e.g. 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and trichloroisocyanuric acid (TCCA), we were not able to dehydrogenate **9** to compound **3**. Neither did it prove to be possible to use 6-bromo-Δ-tryptophan ethyl ester[9] (**10**) as starting material, since all attempted peptide coupling with compounds **6** and **10** failed (Scheme 2).

Another route towards functionalised dehydroamino acids involves Horner–Wadsworth–Emmons type reactions. Hydrogenolysis of methyl 2-benzyloxycarbonylamino-2-(diethoxyphosphinyl)-acetate (**11**)[10] afforded the free amine which was immediately reacted with the amino acid derivative **6** in presence of 1-ethyl-3-(3′-dimethylamino-propyl)carbodiimide hydrochloride (EDCI), which gave the dipeptide **12** in 64% yield. The phosphonoglycinate **12** was further condensed with 6-bromo-1-(*tert*-butoxycarbonyl)-indole-3-carboxaldehyde (**13**)[11] using 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) as the base to furnish the desired compound **14**. The yield in this particular reaction has so far been only moderate, usually around 55%. Although the low yield might indicate formation of the *E*-isomer, we were only able to detect the desired *Z*-isomer of **14**. However, there are indications in the literature that *Z*-isomers are formed predominately using DBU as the base in this type of reactions.[12] Using potassium *tert*-butoxide (*t*-BuOK) as



Scheme 2. (a) **6**, EDCI, HOBt, DIEA, CH_2Cl_2, rt 18 h. (b) (i) TFA, CH_2Cl_2, rt 12 h. (ii) 0.1 M HOAc/1-BuOH, NMM, reflux 6 h.



Scheme 3. (a) (i) H_2, Pd/C, EtOH, 4.5 h. (ii) **6**, EDCI, HOBt, DIEA, CH_2Cl_2, rt 18 h. (b) **12**, DBU, CH_2Cl_2, −78° to rt 20 h. (c) (i) TFA, CH_2Cl_2, rt 12 h. (ii) 0.1 M HOAc/1-BuOH, NMM, reflux 6 h.

base in the same reaction resulted in an even lower yield. Several acidic hydrogen atoms present in compound **12** might also account for the poor yield but variation of the amount of base used failed to improve the results.

The four Boc-protecting groups in **14** were removed by treatment with TFA at ambient temperature overnight. The solvent was then removed and the residue in the flask dissolved in 1-butanol containing 0.1 M acetic acid, with addition of *N*-methylmorpholine (NMM). After 5 h at reflux the solvents were removed, giving barettin (**3**) in 62% yield (Scheme 3).

The analytical data of this synthetic material agreed completely with those of the natural product.[8] The NMR data of **3** are in agreement with those reported by Sölter et al.[3,13]

3. Experimental

3.1. General

NMR spectra were recorded at 300 MHz for ^{1}H and 75 MHz for ^{13}C, respectively. NMR spectra were recorded in DMSO-d_6 or $CDCl_3$, using the solvent signal as reference. δ Values are given in ppm, coupling constants are given in Hz. The IR spectra were acquired using a FT-IR instrument. Optical rotation values were determined in a polarimeter equipped with a 1 mL cell measuring 10 cm using the emission wavelength of a sodium lamp; concentrations are given in g/100 mL. High-resolution mass spectroscopic (HRMS) analyses were performed by E. Nilsson, University of Lund, Sweden. Melting points were determined on a capillary melting point apparatus. Chromatographic separations were performed on silica gel 60 (230–400 mesh). All reagents used were purchased from Aldrich, Lancaster, Merck or Biosynth and were used as received. All solvents were purified by distillation or were of analytical grade.

3.1.1. N^{α}-(*tert*-Butoxycarbonyl)-N^{ω},$N^{\omega'}$-bis(*tert*-butoxycarbonyl)-L-arginine (6). To a suspension of $Cu[Arg^{\omega,\omega}(Boc)_2]_2$ (**5**)[5] (4.03 g, 5.00 mmol), EDTA·4Na·2H_2O (2.23 g, 6.00 mmol) and $NaHCO_3$ (1.68 g, 20.0 mmol) in H_2O (30 mL), a solution of Boc_2O (2.40 g, 11.0 mmol) in acetone (30 mL) was added dropwise. The reaction mixture was stirred at room temperature for 12 h when the solvent was evaporated. The aqueous mixture was acidified with 5% $KHSO_4$, until ~pH 3. The resulting gummy precipitate was extracted with EtOAc (3×40 mL) and the combined organic phases were washed with H_2O (100 mL), brine (100 mL) and dried over $MgSO_4$. Evaporation furnished a yellow oil which was purified by column chromatography using hexane/EtOAc (60:40) as eluent, yielding **6** as a colourless glass (3.77 g, 79%): $[\alpha]_D^{21}$ +7° (*c* 0.1, MeOH); IR (KBr): 3332, 2980, 1722, 1634, 1616, 1368, 1332, 1158, 1136, 1052 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 12.53 (s, 1H), 11.49 (s, 1H), 8.29–8.25 (m, 1H), 7.08 (d, *J*=8.0 Hz, 1H), 3.85–3.82 (m, 1H), 3.27–3.25 (m, 2H), 1.57–1.35 (m, 31H); ^{13}C NMR (DMSO-d_6): δ 174.1 (s), 163.1 (s), 155.6 (s), 155.3 (s), 152.1 (s), 82.9 (s), 78.1 (s), 78.0 (s), 53.3 (d), 39.6 (t), 28.2 (q), 28.1 (t), 28.0 (q), 27.6 (q), 25.5 (t). HRMS (FAB+) *m/z* calcd for $C_{21}H_{39}N_4O_8$ $(M+H)^+$ 475.2768, found 475.2767.

3.1.2. 6-Bromo-D,L-tryptophan methyl ester·HCl (7). 6-Bromo-D,L-tryptophan (1.42 g, 5.00 mmol) was suspended in MeOH (18 mL) at 0 °C. $SOCl_2$ (0.37 mL, 5.05 mmol) was added dropwise and the mixture kept at 0 °C for an additional 0.5 h. The solution was refluxed for 1.5 h and thereafter allowed to cool. The solvent was evaporated leaving a quantitative yield of 6-bromo-D,L-tryptophan methyl ester·HCl (**7**) as a pinkish solid: mp 240.0–242.5 °C; IR (KBr): 3274, 2876, 1743, 1590, 1500, 1445, 1246, 1105, 1080, 802 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 11.37 (s, 1H), 8.70 (br s, 2H), 7.57 (d, *J*=1.6 Hz, 1H), 7.50 (d, *J*=8.5 Hz, 1H), 7.30 (d, *J*=2.3 Hz, 1H), 7.15 (dd, *J*=1.7, 8.5 Hz, 1H), 4.22 (t, *J*=6.3 Hz, 1H), 3.63 (s, 3H), 3.38–3.34 (m, 2H); ^{13}C NMR (DMSO-d_6): δ 169.7 (s), 137.1 (s), 126.1 (d), 126.0(s), 121.5 (d), 119.9 (d), 114.1 (d), 113.9 (s), 106.8 (s), 52.7 (q), 52.6 (d), 25.8 (t). HRMS (FAB+) *m/z* calcd for $C_{12}H_{14}N_2O_2^{79}Br$ $(M+H)^+$ 297.0239, found 206.0232.

3.1.3. N^{α}-(Boc)-N^{ω},$N^{\omega'}$-bis(Boc)-L-Arg-6-bromo-D,L-TrpOMe (8). A mixture of 6-bromo-D,L-tryptophan methyl ester·HCl (**7**) (999 mg, 3.00 mmol), arginine derivative **6** (1.42 g, 3.00 mmol), EDCI (690 mg, 3.60 mmol) and HOBt (486 mg, 3.60 mmol) in CH_2Cl_2 (20 mL) was stirred at 0 °C. Et_3N (0.84 mL, 6.00 mmol) was added and the solution was allowed to reach room temperature overnight. The reaction mixture was transferred to a separatory funnel, additional CH_2Cl_2 (15 mL) was added and the organic phase was washed with H_2O (2×20 mL), brine (20 mL) and dried over $MgSO_4$. The solvent was evaporated and the residue purified by column chromatography (hexane/EtOAc 60:40) to give the protected dipeptide **8** as a clear oil (1.36 g, 60%): IR (KBr): 3333, 2979, 2936, 1723, 1647, 1620, 1368, 1163, 1135 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 11.50 (s, 1H), 11.01 (d, *J*=5.0 Hz, 1H), 8.26–8.19 (m, 2H) 7.51 (s, 1H) 7.45 (d, *J*=8.5 Hz, 1H) 7.19 (dd, *J*=2.0, 8.4 Hz, 1H), 7.12 (dd, *J*=1.2, 8.4 Hz, 1H), 6.86–6.76 (m, 1H), 4.53–4.51 (m, 1H), 4.02–3.97 (m, 1H), 3.59–3.54 (m, 3H), 3.25–2.98 (m, 4H), 1.47–1.34 (m, 31H); ^{13}C NMR (DMSO-d_6): δ 172.0 (s), 171.9 (s), 171.8 (s), 163.1 (s), 155.2 (s), 152.1 (s), 136.9 (136.8) (s), 126.1 (126.0) (s), 125.0 (124.9) (d), 121.2 (d), 119.8 (119.7) (d), 113.9 (d), 113.7 (s), 109.7 (109.6) (s), 82.8 (s), 78.1 (s), 78.1 (s), 52.8 (52.7) (d), 51.8 (51.7) (q), 39.7 (39.6) (t), 31.2 (d), 29.3 (t), 28.1 (q), 28.0 (q), 27.6 (q), 27.0 (26.8) (t), 25.1 (24.9) (t). Figures within brackets refer to doublets arising due to the presence of diastereomers. HRMS (FAB+) *m/z* calcd for $C_{33}H_{50}N_6O_9^{79}Br$ $(M+H)^+$ 753.2823, found 753.2835.

3.1.4. 8,9-Dihydrobarettin (9). The dipeptide **8** (1.14 g, 1.52 mmol) was dissolved in CH_2Cl_2 (15 mL) and TFA (2.32 mL, 30.31 mmol) was added at room temperature. The reaction mixture was stirred for 5 h, and then evaporated to dryness. The residue was dissolved in 1-butanol (15 mL) containing 0.1 M AcOH. NMM (0.17 mL, 1.52 mmol) was added and the reaction mixture was refluxed for 12 h and thereafter allowed to cool. The reaction mixture was washed with H_2O (2×20 mL), brine (20 mL) and dried over $MgSO_4$. The solvent was evaporated affording cyclo-6-bromo-D,L-Trp-L-Arg or 8,9-dihydrobarettin (**9**) as a yellowish solid (474 mg 74%): IR (KBr):

3339, 3201, 2959, 2934, 2873, 1668 (br), 1456, 1330, 1202, 1136, 802 cm^{-1}; ^1H NMR (DMSO-d_6): δ 11.08 (s, 1H), 11.02 (s, 1H), 8.15 (br s, 2H), 8.01 (s, 1H), 7.92 (s, 1H), 7.54–7.51 (m, 4H), 7.45 (t, J=5.5 Hz, 1H), 7.24 (t, J=5.3 Hz, 1H), 7.30–6.70 (m, 6H), 7.10–7.05 (m, 4H), 4.13–4.03 (m, 2H), 3.63–3.59 (m, 1H), 3.32–2.97 (m, 3H), 3.03–2.97 (m, 4H), 2.81–2.71 (m, 2H), 1.55–1.27 (m, 4H), 1.10–0.62 (m, 4H); ^{13}C NMR (DMSO-d_6): δ 168.0 (167.4) (s), 167.0 (166.7) (s), 156.9 (156.8) (s), 136.8 (136.7) (s), 126.9 (126.7) (s), 125.7 (125.5) (d), 121.2 (121.1) (d), 120.8 (120.7) (d), 113.8 (113.7) (d), 113.6 (113.5) (s), 109.0 (108.8) (s), 55.4 (55.3) (d), 53.3 (52.9) (d), 40.4 (40.2) (t), 29.2 (t), 28.7 (28.6) (t), 23.5 (23.1) (t). Figures within brackets refer to doublets arising due to the presence of diastereomers. HRMS (FAB+) m/z calcd for $C_{17}H_{22}N_6O_2^{79}Br$ $(M+H)^+$ 421.0988, found 421.0996.

3.1.5. 6-Bromo-Δ-tryptophan metyl ester (10). 2-Nitro-pent-2-enoic acid ethyl ester[14] (1.59 g, 8.40 mmol) was mixed with 6-bromoindole (1.37 g, 7.00 mmol) under a nitrogen atmosphere at ambient temperature. A mixture of Et_2O/hexane (1:1) was added after 12 h and the yellow precipitate formed was collected and washed with further Et_2O/hexane (1:1) to give 3-(6-bromo-1*H*-indol-3-yl)-2-nitro-acrylic acid ethyl ester (808 mg), which was used without further purification. A second crop was collected from the mother liquid (362 mg) to give a total yield of 1.17 g (54%).

$SnCl_2{\cdot}2H_2O$ (2.36 g, 10.5 mmol) was dissolved in 3 M HCl in MeOH (20 mL) at 0 °C. 3-(6-Bromo-1*H*-indol-3-yl)-2-nitro-acrylic acid ethyl ester (1.02 g, 3.00 mmol) was added to the solution in small portions during 0.5 h. The mixture was kept at 0 °C for 1 h. The precipitate formed was collected by filtration and washed with a small amount of ether. The hydrochloride of **10** was obtained as a pinkish solid (560 mg, 54%): mp 189 °C (dec); IR (KBr): 3140, 2996, 2502, 1675, 1653, 1565, 1272, 1145 cm^{-1}; ^1H NMR (DMSO-d_6): δ 12.32 (s, 1H), 8.19 (d, J=2.8 Hz, 1H), 7.75 (d, J=1.7 Hz, 1H), 7.75–7.24 (br, 3H), 7.56 (s, 1H), 7.29 (dd, J=1.7, 8.5 Hz, 1H), 4.33 (q, J=7.1, 14.1 Hz, 2H), 1.39 (t, J=7.1 Hz, 3H); ^{13}C NMR (DMSO): δ 164.0 (s), 136.6 (s), 129.9 (d), 126.0 (s), 123.3 (d), 120.0 (d), 119.0 (d), 118.1 (s), 115.1 (s), 114.7 (d), 107.8 (s), 61.6 (t), 14.2 (q). HRMS (FAB+) m/z calcd for $C_{13}H_{13}N_2O_2^{79}Br$ $(M)^+$308.0160, found 308.0160.

3.1.6. Methyl 2-(N^{α}-(Boc)-N^{ω},$N^{\omega'}$-bis(Boc)-L-arginyl-amino)-2-(diethoxyphosphinyl)-acetate (12). A solution of compound **11**[10] (2.13 g, 5.94 mmol) in EtOH (60 mL) was hydrogenated in the presence of Pd/C (5%; 213 mg) at room temperature for 4.5 h. The reaction mixture was filtered through celite and the filtrate evaporated leaving a clear oil. The free amine was immediately dissolved in CH_2Cl_2 (10 mL) and added to an ice-cold mixture of the arginine derivative **6** (2.56 g, 5.40 mmol), HOBt (803 mg, 5.94 mmol), EDCI (1.14 mg, 5.94 mmol) and DIEA (1.03 mL, 5.94 mmol) in CH_2Cl_2 (15 mL). The reaction mixture was allowed to reach room temperature. After 15 h the solvent was evaporated and the residue was taken up in EtOAc (150 mL) then washed with H_2O (30 mL) and brine (30 mL). The organic phase was dried over $MgSO_4$ and evaporated. Purification by column chromatography with EtOAc/hexane (70:30) as eluent afforded the title compound **12** as a clear oil. Yield: 2.35 g (64%): $[\alpha]_D^{21}$ −7° (c 0.2, MeOH); IR (KBr): 3332, 2978, 2933, 1752, 1719, 1680, 1639, 1617, 1367, 1330, 1252, 1164, 1134, 1050, 1025 cm^{-1}; ^1H NMR ($CDCl_3$): δ 11.45 (s, 1H), 8.34–8.30 (m, 1H), 7.27–7.19 (m, 1H), 5.42–5.32 (m, 1H), 5.19 (d, J=8.9 Hz, 1H), 4.22–4.06 (m, 4H), 3.78 (s, 3H), 3.43–3.39 (m, 2H), 1.86–1.27 (m, 38 H). MS (ESI) m/z 680 $(M-H)^-$; HRMS (FAB+) m/z calcd for $C_{28}H_{53}N_5O_{12}P$ $(M+H)^+$ 682.3428, found 682.3439.

3.1.7. N^{α}-(Boc)-N^{ω},$N^{\omega'}$-bis(Boc)-L-Arg-6-bromo-Δ-(1-Boc)TrpOMe (14). The arginine derivative **12** (710 mg, 1.04 mmol) dissolved in CH_2Cl_2 (5 mL) was added dropwise to a solution of DBU (0.31 mg, 2.09 mmol) in CH_2Cl_2 (5 mL) at −78 °C under a nitrogen atmosphere. After 30 min 6-bromo-1-(*tert*-butoxycarbonyl)-indole-3-carboxaldehyde (**13**)[11] (338 mg, 1.04 mmol) in CH_2Cl_2 (5 mL) was added. The reaction mixture was allowed to reach room temperature. After 18 h the mixture was evaporated to dryness and the residue dissolved in EtOAc (20 mL), washed with H_2O (2×20 mL) and brine (30 mL). The organic phase was dried over $MgSO_4$ and evaporated, leaving a yellow oil which was purified by column chromatography. Elution with hexane/EtOAc (80:20 to 60:40) afforded **14** as a yellow oil (490 mg, 55%): $[\alpha]_D^{21}$+ 74° (c 0.2, MeOH); IR (KBr): 3330, 2978, 2933, 1721, 1641, 1619, 1368, 1333, 1251, 1155, 1135, 1051 cm^{-1}; ^1H NMR ($CDCl_3$): δ 11.46 (s, 1H), 8.52–8.41 (m, 1H), 8.31 (s, 1H), 8.19 (s, 1H), 7.83 (s, 1H), 7.67 (s, 1H), 7.53 (d, J=8.5 Hz, 1H), 7.39 (dd, J=1.7, 8.5 Hz, 1H), 5.72 (d, J=8.1 Hz, 1H), 4.51–4.37 (m, 1H), 3.81 (s, 3H), 3.60–3.55 (m, 1H), 3.49–3.39 (m, 1H), 2.02–1.86 (1H), 1.72–1.25 (m, 39H); ^{13}C NMR ($CDCl_3$): δ 171.0 (s), 165.3 (s), 163.4 (s), 156.8 (s), 155.9 (s), 153.4 (s), 148.9 (s), 135.6 (s), 128.6 (d), 128.3 (s), 126.5 (d), 124.6 (d), 123.2 (s), 120.4 (d), 118.8 (s), 118.7 (d), 114.1 (s), 85.2 (s), 83.4 (s), 80.2 (s), 79.6 (s), 54.4 (d), 52.8 (q), 40.0 (t), 29.1 (t), 28.5 (q), 28.3 (q), 28.2 (q), 26.1 (t). MS (ESI) m/z 849 and 851 $(M-H)^-$; HRMS (FAB+) m/z calcd for $C_{38}H_{56}N_6O_{11}^{79}Br$ $(M+H)^+$ 851.3190, found 851.3199.

3.1.8. Barettin (3). TFA (0.91 mL) was added a solution of compound **7** (500 mg, 0.59 mmol) in CH_2Cl_2 (10 mL) and stirred at room temperature for 8 h. The solvent was evaporated and the residue dissolved in 1-BuOH (10 mL) containing 0.1 M HOAc. After addition of NMM (0.06 mL, 0.59 mmol) the reaction mixture was heated at reflux for 4.5 h. The mixture was allowed to cool and thereafter washed with H_2O (2×15 mL), brine (10 mL) and dried over $MgSO_4$. Evaporation of the solvent under reduced pressure afforded barettin (**3**) as a dark yellow solid (153 mg, 62%). The NMR data of **3** are in agreement with those reported by Sölter et al.[3,13] $[\alpha]_D^{26}$ −32.5° (c 2, MeOH), [lit[1] $[\alpha]_D$ −25° (c 3, MeOH)].

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Epoxidation of chromones and flavonoids in ionic liquids

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Abstract—A convenient and efficient procedure for the epoxidation of chromone, isoflavone, and chalcone derivatives using 1-butyl-3-methyl imidazolium tetrafluoroborate [bmim]BF_4 as solvent and alkaline hydrogen peroxide as oxidant is described. All reactions proceed in good yields and faster than in conventional solvents. No evidence of formation of compounds derived from the opening of the epoxide ring was attained.

1. Introduction

Natural products having a chromonic and flavonoidic structure (chalcones and isoflavones) exhibit important biological properties such as antiviral, cardioprotective, antioxidant, hepatoprotective, antitumoral and antiflammatory activities.[1] Their corresponding epoxides are postulated as biosynthetic intermediates of some natural compounds. Chalcone and isoflavone epoxides are useful building blocks in flavonoid chemistry.[2] A nucleophilic oxidant such as alkaline hydrogen peroxide has been suggested to be the reagent of choice for the direct preparation of these compounds (Weitz–Scheffer epoxidation).[3] Recently, the epoxidation of chalcones and isoflavones by using dimethyldioxirane (DMD), an electrophilic oxidant,[4] has been reported. However, long reaction times (up to 140 h) and a large excess of the reagent (up to 15 equiv.) are required.[5]

Because of the role of chiral epoxides as useful intermediates in the synthesis of natural products and drug molecules,[6] the asymmetric epoxidation of α,β-unsaturated ketones has also been widely investigated in the last few years. An asymmetric Weitz–Scheffer epoxidation of isoflavones mediated by optically active cinchonine catalysts has been reported,[7] as well as the use of the Jacobsen's Mn(III)salen catalyst in the presence of DMD as the source of the oxygen atom.[8] Chalcones have been subjected to asymmetric epoxidation by using a variety of polymer-bound chiral supports.[9–11] All these reactions have been performed in conventional solvents (acetone, toluene, tetrahydrofuran) as well as under phase-transfer conditions.[12–14]

Our interest in the functionalization of natural molecules by benign oxidative methodologies and in the preparation of fine-chemicals as well as bioactive compounds[15] prompted us to investigate the development of an epoxidation protocol for chromenes and flavonoids under environmentally friendly conditions. Particularly, we focused our attention on the use of room temperature ionic liquids. They are considered very promising and attractive substitutes for volatile organic solvents and are widely used in the Green Chemistry area.[16] In fact, having a negligible vapour pressure, their loss into the environment by evaporation is minimal; this property may offer environmental advantages in industrial processes. Ionic liquids possess several other attractive properties including a high flash point, thermal stability, immiscibility with some organic solvents, and capacity to dissolve a wide range of organic, inorganic and organometallic compounds. Cations usually present in room temperature ionic liquids are: tetraalkylammonium, tetraalkylphosphonium, trialkylsulfonium, *N*-alkylpyridinium and 1,3-dialkylimidazolium. Anions are generally polyatomic inorganic species (Fig. 1). They have been employed in a variety of organic reactions such as hydrogenation,[17] olefin dimerization and oligomerization,[18] Heck reactions,[19] hydroformylation,[20] alkoxycarbonylation,[21] and allylic substitution.[22] To the best of our knowledge, however, very little has been done in the oxidation area,[23] in particular in the epoxidation of natural compounds. Recently, the epoxidation of simple cyclohexenone derivatives by alkaline hydrogen peroxide in ionic liquids has been reported.[24]

Keywords: Epoxidation; Chromones; Flavonoids; Chalcones; Isoflavones; Ionic liquids; [bmim]BF_4.
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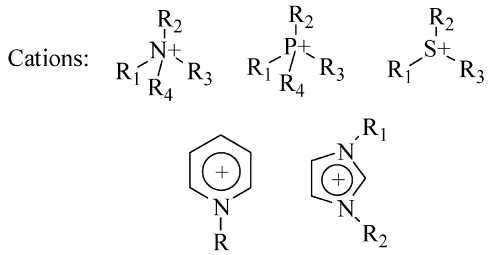


Figure 1. Common cations and anions in room temperature ionic liquids.

We report here that satisfactory results in the epoxidation of the enone moiety of natural compounds such as chromone, chalcone, and isoflavone derivatives can be obtained by hydrogen peroxide in 1-butyl-3-methyl imidazolium tetrafluoroborate, [bmim]BF_4 (Fig. 2).

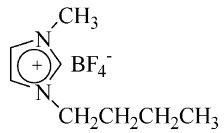


Figure 2. 1-Butyl-3-methyl imidazolium tetrafluoroborate [bmim]BF_4.

2. Results and discussion

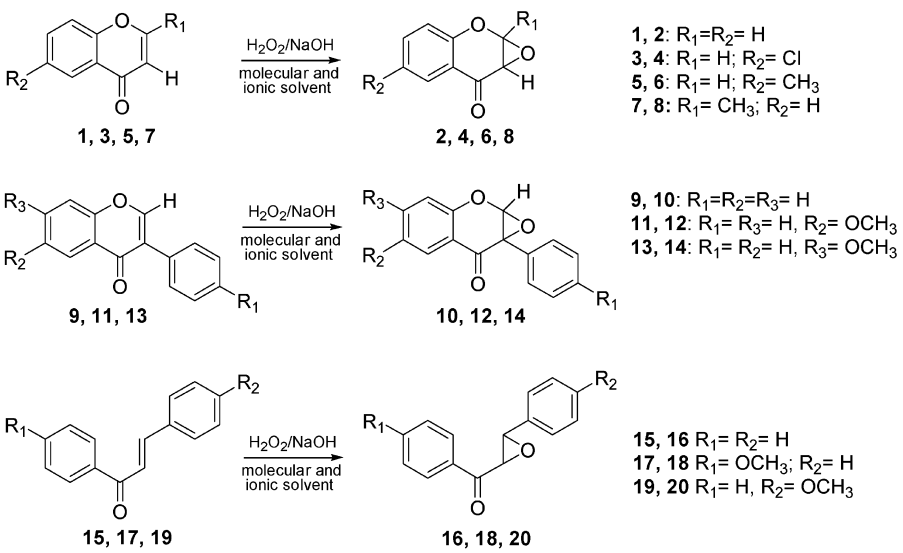
For our initial investigation, the epoxidation of chromone **1** with alkaline hydrogen peroxide was selected as a model reaction. For comparison, reactions were carried out in acetone and [bmim]BF_4. After 2 h, the epoxide derivative **2** was obtained in only 40% yield in acetone and in >98% yield in the ionic liquid (Scheme 1, Table 1, entries 1 and 2). In agreement with the formation of the epoxide ring,[3b] its ^{1}H NMR spectrum shows two doublets at 3.70 and 5.72 ppm (J=2.9 Hz). This structural assignment is further supported by ^{13}C NMR data and GC–MS fragmentation (M^+=162). No evidence of formation of products derived from the opening of the epoxide ring was attained.

Table 1. Epoxidation of chromones **1**, **3**, **5**, **7**, isoflavones **9**, **11**, **13**, and chalcones **15**, **17**, **19** with H_2O_2/NaOH[a] in conventional solvents (dichloromethane or acetone) and [bmim]BF_4

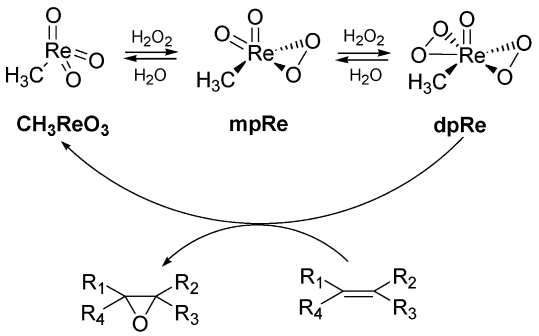
Entry	Substrate	Conditions[a]	Epoxide	Conversion (%)[b]	Yield (%)[b]
1	**1**	CH_3COCH_3, 0 °C, 2 h	**2**	40	>98
2	**1**	[bmim]BF_4, 0 °C, 2 h	**2**	>98	>98
3	**3**	CH_2Cl_2, 0 °C, 2 h	**4**	5	>98
4	**3**	[bmim]BF_4, 0 °C, 2 h	**4**	62	>98
5	**5**	CH_2Cl_2, 0 °C, 2 h	**6**	6	>98
6	**5**	[bmim]BF_4, 0 °C, 2 h	**6**	52	>98
7	**7**	CH_3COCH_3, 0 °C, 2 h	**8**	5[c]	>98[c]
8	**7**	[bmim]BF_4, 0 °C, 2 h	**8**	65[c]	>98[c]
9	**9**	CH_3COCH_3, rt, 2 h	**10**	20	>98
10	**9**	[bmim]BF_4, rt, 2 h	**10**	98	>98
11	**11**	CH_3COCH_3, rt, 2 h	**12**	20	>98
12	**11**	[bmim]BF_4, rt, 2 h	**12**	98	>98
13	**13**	CH_3COCH_3, rt, 2 h	**14**	12	>98
14	**13**	[bmim]BF_4, rt, 2 h	**14**	82	>98
15	**15**	CH_3COCH_3, rt, 0.5 h	**16**	5	>98
16	**15**	[bmim]BF_4, rt, 0.5 h	**16**	98	>98
17	**17**	CH_3COCH_3, rt, 0.5 h	**18**	7	>98
18	**17**	[bmim]BF_4, rt, 0.5 h	**18**	98	>98
19	**19**	CH_3COCH_3, rt, 0.5 h	**20**	5	>98
20	**19**	[bmim]BF_4, rt, 0.5 h	**20**	95	>98

[a] 3 equiv. of H_2O_2 (35% solution in water) and 2 equiv. of NaOH.
[b] Conversions and yields were determined after chromatographic purification of reaction mixtures.
[c] Conversions and yields were determined by ^{1}H NMR analysis of crude reaction mixtures.

Most probably, under our conditions, a nucleophilic mechanism is operating in the oxidation of the α,β-unsaturated fragment. Therefore, for the sake of comparison, we also tested the reactivity of methyltrioxorhenium/hydrogen peroxide in [bmim]BF_4. In fact, the active peroxodicomplexe **dpRe**, generated from methyltrioxorhenium during the reaction course (Scheme 2), has been reported to show some nucleophilic character.[25]



Scheme 1. Epoxidation of chromones **1**, **3**, **5**, **7**, isoflavones **9**, **11**, **13** and chalcones **15**, **17**, **19**.



Scheme 2. Epoxidation of alkenes by the H_2O_2/CH_3ReO_3 catalytic system.

Nevertheless, though the desired epoxide derivative was formed as almost the only product, a very low conversion of the substrate was observed (10–15%), even charging a large excess of the oxidation system (10% of methyltrioxorhenium and up to 12 equiv. of hydrogen peroxide).

Our reaction conditions (H_2O_2/NaOH in [bmim]BF_4 at 0 °C or rt) were next extended to other chromone derivatives such as 6-chlorochromone **3**, 6-methylchromone **5**, 2-methylchromone **7** and to some natural flavonoids such as isoflavone **9**, 6-methoxyisoflavone **11**, 7-methoxyisoflavone **13**, chalcone **15**, 4′-methoxychalcone **17**, and 4-methoxychalcone **19** (Scheme 2). In every case, the use of [bmim]BF_4 resulted to be superior to that of molecular solvents: the corresponding epoxides were isolated in significantly higher yields in all the comparisons (Table 1, compare entries 4, 6, 8, 10, 12, 14, 16, 18, and 20 with entries 3, 5, 7, 9, 11, 13, 15, 17, and 19). In addition, faster reaction times were always observed. Chromone epoxides **4**, **6**, and **8** were obtained in satisfactory yields in 2 h. The new cromone products **4** and **6** were fully characterized by ^{1}H NMR, ^{13}C NMR, IR spectra and GC–MS while **8** was found to be unstable.[3b] After usual work-up with diethyl ether, the reaction mixture could not be purified by chromatography without decomposition of the epoxide. However, ^{1}H NMR analysis of the crude reaction mixture (singlet at 3.64 ppm) confirmed the presence of the epoxide ring[3b] as well as also the GC–MS fragmentation. Isoflavone epoxides **10**, **12**, **14** and chalcone epoxides **16**, **18** and **20** were obtained in satisfactory yields. All products were fully characterized by NMR, IR, and GC–MS (Section 4).

3. Conclusions

We have developed a simple and efficient procedure for the epoxidation of natural compounds containing the α,β-enone moiety in [bmim]BF_4. The method merits attention due to the simplicity of the experimental procedure, the reduced waste production and the high yields in short reaction time.

4. Experimental

Melting points were determined with a Büchi apparatus and are uncorrected. Chromones **1**, **3**, **5**, **7** and chalcones **15**, **17**, **19** are commercially available (Aldrich) and were used as purchased. Isoflavones **9**, **11** and **13** were synthesized according to literature.[26] Acetone and dichloromethane, ACS reagent grade solvents, were redistilled and dried according to standard procedures. Preparation of [bmim]BF_4 was carried out according to Ref. 27. Thin layer chromatography was carried out using Merck platen Kieselgel 60 F254. Reaction products were purified by flash chromatography by using Merck silica gel 60, 230–400 (eluents: hexane/ethyl acetate, 9:1 or 8:2). IR spectra were recorded on a Perkin–Elmer 298 spectrophotometer using NaCl paltes. NMR spectra were recorded on a Bruker (200 MHz) spectrometer and are reported in δ values. Mass spectra were recorded on a VG 70/250S spectrometer with an electron beam of 70 eV. Elementary analyses were performed by a Carlo Erba 1106 Analyser.

4.1. General procedure for the oxidation of chromone and flavonoid derivatives with H_2O_2/NaOH in [bmim]BF_4

The substrate (1.0 mmol) was solubilized in [bmim]BF_4 (1 mL). Then, hydrogen peroxide (35% aqueous solution, 3.0 mmol) and NaOH power (2.0 mmol) were added. Reactions were monitored by thin layer chromatography and by gas-chromatography. Products were extracted with diethyl ether, the organic layer was evaporated under vacuum and the mixture was purified by flash-chromatography.

4.1.1. Chromone epoxide (1). Yellow solid, mp 64–66 °C (lit.[3b] 65–66 °C). Found: C, 66.65; H, 3.73; O, 29.62. $C_9H_6O_3$ requires C, 66.67; H, 3.73; O, 29.60%; ν_{max} (KBr): 3052, 1682; δ_H ($CDCl_3$, 200 MHz): 7.87 (1H, dd, J_1=7.9 Hz, J_2=1.8 Hz, Ph), 7.59–7.50 (1H, m, Ph), 7.18–7.02 (2H, m, Ph), 5.72 (1H, d, J=2.9 Hz, –COC*H*OCH–), 3.70 (1H, d, J=2.9 Hz, –COCHOC*H*–); δ_C ($CDCl_3$, 50 MHz): 188.1, 155.4, 136.3, 127.1, 123.4, 119.8, 118.0, 77.2, 55.3; *m*/*z* (EI) 162 (M^+, 51.1%).

4.1.2. 6-Chloro chromone epoxide (4). Yellow solid, mp 92–94 °C. Found: C, 54.98; H, 2.57; O, 24.40; Cl, 18.05. $C_9H_5O_3Cl$ requires C, 54.99; H, 2.56; O, 24.42; Cl, 18.03%; ν_{max} (KBr): 3049, 1691; δ_H ($CDCl_3$, 200 MHz): 7.85 (1H, d, J=2.6 Hz, Ph), 7.53–7.46 (1H, m, Ph), 7.03 (1H, d, J=8.8 Hz, Ph), 5.68 (1H, d, J=2.8 Hz, –COC*H*OCH–), 3.71 (1H, d, J=2.4 Hz, –COCHOC*H*–), δ_C ($CDCl_3$, 50 MHz): 186.9, 153.8, 136.1, 129.0, 125.6, 120.2, 119.7, 77.4, 55.0; *m*/*z* (EI) 198 (M^++2, 17.0%).

4.1.3. 6-Methyl chromone epoxide (6). Yellow solid, mp 80–82 °C. Found: C, 68.21; H, 4.56; O, 27.23. $C_{10}H_8O_3$ requires C, 68.19; H, 4.57, O, 27.24%; ν_{max} (KBr): 3055, 1679; δ_H ($CDCl_3$, 200 MHz): 7.67 (1H, d, J=1.6 Hz, Ph), 7.38–7.33 (1H, m, Ph), 6.95 (1H, d, J=8.4 Hz, Ph), 5.64 (1H, d, J=2.4 Hz, –COC*H*OCH–), 3.67 (1H, d, J=2.5 Hz, –COCHOC*H*–), 2.31 (3H, s, *Me*); δ_C ($CDCl_3$, 50 MHz): 188.3, 153.5, 138.1, 133.0, 126.7, 119.4, 117.7, 77.2, 55.3, 20.4; *m*/*z* (EI) 177 (M^+, 6.7%).

4.1.4. Isoflavone (9). White solid, mp 129–131 °C (lit.[26] 131–132 °C). Found: C, 81.10; H, 4.53; O, 14.37. $C_{15}H_{10}O_2$ requires C, 81.07; H, 4.53; O, 14.40%; ν_{max} (KBr): 1675, 1638, 1567, 1465; δ_H ($CDCl_3$, 200 MHz): 8.30 (1H, dd, J_1=7.9 Hz, J_2=1.6 Hz, Ph); 7.99 (1H, s, =C*H*), 7.70–7.36 (8H, m, Ph); δ_C ($CDCl_3$, 50 MHz): 176.1, 156.1,

153.0, 136.0, 133.7, 133.5, 131.7, 128.8, 128.4, 128.1, 127.7, 127.1, 126.3, 125.1, 117.9; *m/z* (EI) 222 (M^+, 49.8%).

4.1.5. Isoflavone epoxide (10). Colourless oil. Found: C, 75.60; H, 4.24; O, 20.16. $C_{15}H_{10}O_3$ requires C, 75.62; H, 4.23; O, 20.15%; ν_{max} (KBr): 3043, 1683; δ_H ($CDCl_3$, 200 MHz): 8.01 (1H, dd, J_1=7.9 Hz, J_2=1.7 Hz, Ph), 7.64–7.38 (6H, m, Ph), 7.23–7.08 (2H, m, Ph), 5.51 (1H, s, –OC*H*O); δ_C ($CDCl_3$, 50 MHz): 187.4, 155.0, 136.1, 130.4, 129.0, 128.4, 128.3, 127.7, 127.1, 127.0, 123.3, 120.0, 117.8, 82.9, 63.0; *m/z* (EI) 238 (M^+, 1.0%).

4.1.6. 6-Methoxyisoflavone (11). Yellow solid, mp 137–139 °C. Found: C, 76.21; H, 4.78; O, 19.01. $C_{16}H_{12}O_3$ requires C, 76.18; H, 4.79; O, 19.03%; ν_{max} (KBr): 1670, 1640, 1440; δ_H ($CDCl_3$, 200 MHz): 7.99 (1H, s, =C*H*), 7.65 (1H, d, *J*=3.0 Hz, Ph), 7.57 (2H, dd, *J*=8.2 Hz, Ph), 7.47–7.39 (4H, m, Ph), 7.25 (1H, dd, J_1=9.1 Hz, J_2=3.2 Hz, Ph), 3.89 (3H, s, O*Me*); δ_C ($CDCl_3$, 50 MHz): 175.9, 157.0, 152.9, 151.0, 132.0, 128.9, 128.7, 128.6, 128.4, 128.1, 125.2, 124.5, 123.6, 119.4, 105.4, 55.7; *m/z* (EI) 252 (M^+, 80.7%).

4.1.7. 6-Methoxyisoflavone epoxide (12). Colourless oil. Found: C, 71.60; H, 4.50; O, 23.90. $C_{16}H_{12}O_4$ requires C, 71.63; H, 4.51; O, 23.86%; ν_{max} (KBr) 3020, 1670;?δ_H ($CDCl_3$, 200 MHz): 7.44–7.38 (7H, m, Ph), 7.03 (1H, d, *J*=9.1 Hz, Ph), 5.48 (1H, s, –OC*H*O), 3.81 (3H, s, O*Me*); δ_C ($CDCl_3$, 50 MHz): 187.4, 155.4, 149.4, 130.6, 128.9, 128.7, 128.3, 127.2, 126.1, 125.0, 120.6, 119.1, 108.2, 82.9, 62.8, 55.8; *m/z* (EI) 268 (M^+, 1.7%).

4.1.8. 7-Methoxyisoflavone (13). White solid, mp 140–142 °C. Found: C, 76.15; H, 4.79; O, 19.06. $C_{16}H_{12}O_3$ requires C, 76.18; H, 4.79; O, 19.03%; ν_{max} (KBr): 1680, 1634, 1441; δ_H ($CDCl_3$, 200 MHz): 8.20 (1H, d, *J*=8.9 Hz, Ph), 7.92 (1H, s, =C*H*); 7.56–7.52 (2H, m, Ph), 7.46–7.35 (3H, m, Ph), 6.98 (1H, dd, J_1=8.9 Hz, J_2=2.4 Hz, Ph), 6.84 (1H, d, *J*=2.36 Hz), 3.89 (3H, s, O*Me*); δ_C ($CDCl_3$, 50 MHz): 190.5, 175.5, 164.0, 157.9, 152.5, 131.9, 128.9, 128.7, 128.4, 128.0, 127.8, 125.2, 118.4, 114.5, 100.1, 55.8; *m/z* (EI) 252 (M^+, 69.8%).

4.1.9. 7-Methoxyisoflavone epoxide (14). White solid, mp 121–123 °C (lit.[3b] 123–124 °C). Found: C, 71.62; H, 4.50; O, 23.88. $C_{16}H_{12}O_4$ requires C, 71.63; H, 4.51; O, 23.86%; ν_{max} (KBr) 3005, 1689; δ_H ($CDCl_3$, 200 MHz): 7.94 (1H, d, *J*=8.8 Hz, Ph), 7.48–7.37 (5H, m, Ph), 6.74 (1H, dd, *J*=11.2 Hz, Ph), 6.53 (1H, d, *J*=2.3 Hz, Ph), 5.47 (1H, s, –OC*H*O), 3.86 (3H, s, O*Me*); δ_C ($CDCl_3$, 50 MHz): 186.0, 166.1, 157.1, 130.7, 129.4, 128.9, 128.7, 128.4, 128.3, 127.1, 113.5, 111.6, 101.0, 83.2, 62.3, 55.7; *m/z* (EI) 268 (M^+, 2.5%).

4.1.10. Chalcone epoxide (16). White solid, mp 88–90 °C (lit.[5a] 88–89 °C).

4.1.11. 4′-Methoxychalcone epoxide (18). White solid, mp 74–76 °C (lit.[5a] 75–76 °C).

4.1.12. 4-Methoxychalcone epoxide (20). White solid, mp 82–84 °C (lit.[5a] 82–83 °C).

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Synthesis of cyclopropanes via iodine–magnesium exchange between 3-iodomethyl-1-oxacyclopentanes and organomagnesium reagents

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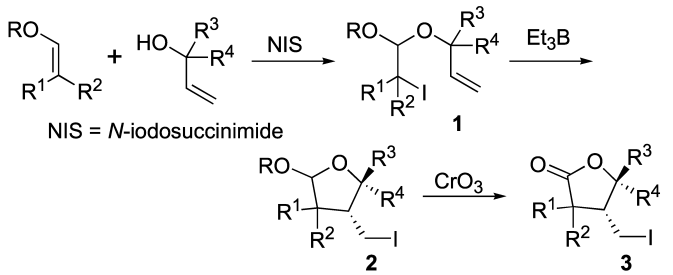
Abstract—Iodine–magnesium exchange occurs upon treatment of 3-iodomethyl-1-oxacyclopentanes with alkyl Grignard reagents or trialkylmagnesate. The resulting organomagnesium compounds undergo intramolecular nucleophilic substitution in ether to afford cyclopropane skeletons in a stereoselective manner.

1. Introduction

Cyclopropane rings are highly strained entities and hence attracts much attention. They are not only structurally challenging targets but also found in a wide variety of naturally occurring useful compounds. While many methodologies are available to construct three-membered rings, synthesis of cyclopropanes still gains in importance.[1] Ring closure of alkyl halide having a leaving group at the third position via halogen–lithium exchange is promising.[2] On the other hand, there are a limited number of halogen–metal exchanges with metal reagents other than organolithium to form cyclopropanes.[3]

Iodine–magnesium exchange between Grignard reagents and organic iodides is a convenient method to prepare new organomagnesium compounds.[4] Aryl and vinyl iodides[5] and alkyl iodides bearing another electronegative groups such as CF_3I, $RCHI_2$, and $ROCH_2I$[6] are usually the choice of precursors. In general, the exchange reaction using alkyl iodides is much less widely used because the exchange is complicated by the possible occurrence of Wultz-type



NIS = *N*-iodosuccinimide

	R	R^1	R^2	R^3	R^4
a	C_2H_5	H (or CH_3)	CH_3 (or H)	n-C_5H_{11}	H
b	CH_3	CH_3	CH_3	n-C_5H_{11}	H
c	TBDMS	CH_3	CH_3	H	H
d	TBDMS	CH_3	CH_3	n-C_5H_{11}	H
e	TBDMS	CH_3	CH_3	CH_3	CH_3

TBDMS = t-$C_4H_9(CH_3)_2Si$

Scheme 1.

Keywords: Cyclopropanes; Iodine–magnesium exchange; Ate complex.
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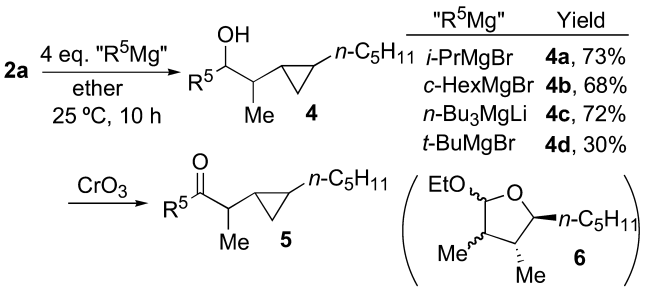
coupling in an ethereal solvent and the fact that complete conversion to product is not achieved.[4c,7] Here we report iodine–magnesium exchange of 3-iodomethyl-1-oxacyclopentane derivatives. Following intramolecular nucleophilic substitution led to the construction of cyclopropane with concomitant opening of the oxacyclopentane ring.

2. Results and discussion

The starting compounds **2a** and **2b** were readily prepared by iodoetherification of vinyl ether with allylic alcohol and *N*-iodosuccinimide and subsequent triethylborane-induced atom transfer radical cyclization (Scheme 1).[8] β-Iodomethyl-γ-lactones **3c**–**3e** were prepared by Jones oxidation of the corresponding cyclic acetals **2c**–**2e**. Radical cyclization of **1a**, **1b** and **1d**, which have R^3=*n*-C_5H_{11} and R^4=H, proceeded with exclusive *trans* selectivity in regard to the pentyl and iodomethyl groups, as is often observed in radical cyclization.[9]

Iodo acetal **2a** (0.50 mmol) was added to an ethereal solution of isopropylmagnesium bromide (2.0 mmol, 4 equiv.) at 0 °C under argon (Scheme 2). The reaction mixture was stirred for 10 h at 25 °C. Usual workup followed by silica gel column purification afforded **4a** in 73% yield. The alcohol **4a** has four stereogenic centers. To simplify the assignment of the stereochemistry, **4a** was subjected to Jones oxidation to provide ketone **5a** in 90% yield. Ketone **5a** consisted of two isomers in a ratio of 71/29. On the other hand, Jones oxidation of **2a** furnished lactone **3a** in a ratio of 70/30. These facts are informative to consider the reaction mechanism (vide infra).

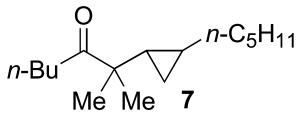
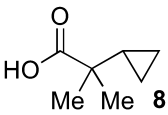
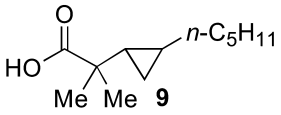
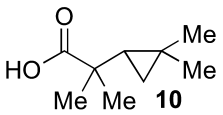


Scheme 2.

Use of three equimolar amounts of isopropyl Grignard reagent resulted in lower yield (58%). An increasing amount of the Grignard reagent (6 equiv.) did not improve the yield of **4a** (75%). It is worth noting that the reaction did not afford **4a** in THF at all, and reduced product **6** was obtained instead as a sole product.[10] *t*-Butyllithium was less effective to give **4d** in 26% yield, in addition to 38% yield of **6**.

Other magnesium reagents effected similar reactions. Reaction with cyclohexyl Grignard reagent furnished the corresponding alcohol **4b** in 68% yield. Use of primary Grignard reagents such as *n*-BuMgBr resulted in the recovery of the starting material. Instead, tributylmagnesate *n*-Bu_3MgLi, prepared from *n*-BuMgBr and 2 equiv. of *n*-BuLi,[11] was suitable to yield the butylated product **4c** (72%).[12] *t*-BuMgBr worked less efficiently, and the corresponding alcohol **4d** was obtained in 30% yield, in addition to remaining starting material (27% yield). Oxidation of **4b**–**4d** proceeded in more than 85% yield.

Table 1. Formation of cyclopropanes from iodomethyl-substituted oxacyclopentanes

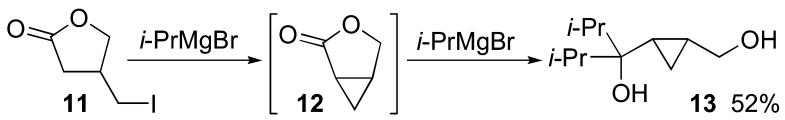
Substrate	Mg reagent	Product	Yield (%)
2b	*n*-Bu_3MgLi		73[a]
3c	*i*-PrMgBr		69
3d	*i*-PrMgBr		79
3e	*i*-PrMgBr		60

[a] Overall yield after Jones oxidation (83% for cyclopropane formation and 88% for oxidation).

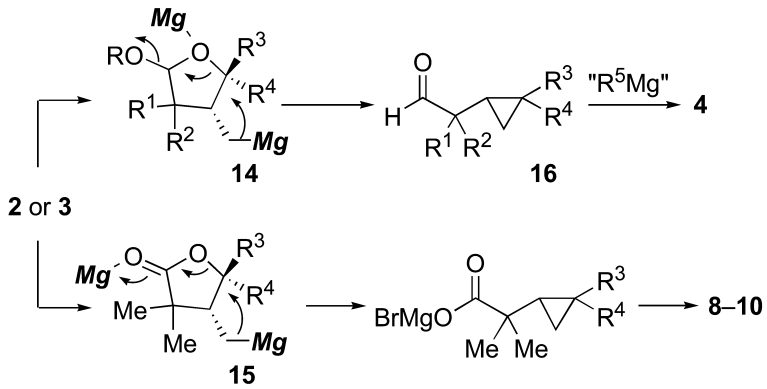
Other substrates were subjected to the transformation (Table 1). Treatment of **2b** with tributylmagnesate afforded **7** in good yield after Jones oxidation. Exclusive formation of the *trans* isomer was deduced by analysis of the coupling constants of the protons on cyclopropane ring,[13] which indicates the stereospecificity of the reaction. Lactones **3c**–**3e** also participated in the reaction to provide 2-cyclopropylisobutyric acids. However, treatment of lactone **11** with isopropyl Grignard reagent did not afford the expected product, instead giving diol **13** (Scheme 3). Formation of the enolate of **11** followed by intramolecular nucleophilic substitution gave bicyclic **12**. Addition of the Grignard reagent to **12** afforded **13**.

We assume the reaction mechanism as shown in Scheme 4. Iodine–magnesium exchange occurs upon treatment of 3-iodomethyl-1-oxacyclopentane **2** or **3** with a Grignard reagent. The resulting organomagnesium species **14** or **15** undergoes intramolecular nucleophilic substitution in ether to afford the cyclopropane skeleton. However, in THF as aforementioned, no cyclopropanes were obtained. This fact implies that the coordination of **14** or **15** to Lewis acidic magnesium species in the substitution step would play a critical role.

To confirm the stereochemistry of the cyclopropanation



Scheme 3.

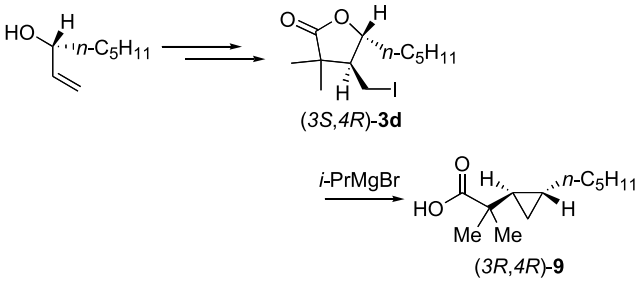


Scheme 4.

step, we prepared optically pure lactone (*3S,4R*)-**3d** from (*R*)-1-octen-3-ol (Scheme 5). Upon treatment of (*3S,4R*)-**3d** with isopropyl Grignard reagent, carboxylic acid **9** was obtained as a single enantiomer (Fig. 1). We assume that its absolute stereochemistry would be (*3R,4R*) and that the cyclopropanation would proceed with inversion of configuration.

3. Conclusion

Iodine–magnesium exchange between 3-iodomethyl-1-oxacyclopentanes and organomagnesium reagents in ether led to the formation of cyclopropane skeleton. The present reaction probably constructs 1,1,2,2-tetrasubstituted cyclopropane, starting from a proper 3-iodomethyl-1-oxacyclopentane derivative. Synthesis of 1,1,2,2,3-penta- or 1,1,2,2,3,3-hexasubstituted cyclopropane through the present method seems difficult because the synthesis requires halogen–magnesium exchange of secondary or tertiary alkyl halide at the initial stage. Although lacking functional group compatiblitity because of the use of organomagnesium reagents, this method can construct highly complex stereodefined cyclopropanes from simple starting materials, vinyl ether, allylic alcohol, and Grignard reagent, in a few steps.

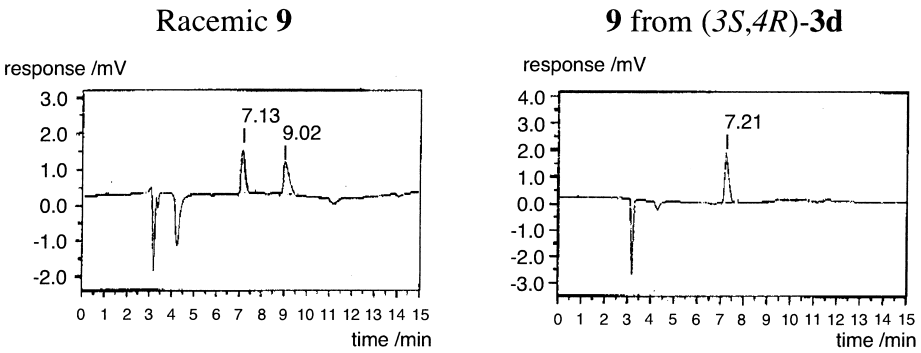


Scheme 5.

4. Experimental

4.1. General

^{1}H NMR (300 MHz) and ^{13}C NMR (75.3 MHz) spectra were taken on a Varian GEMINI 300 spectrometer in $CDCl_3$ as a solvent, and chemical shifts were given in δ value with tetramethylsilane as an internal standard IR spectra were



Retention time /min	7.13	9.02
Area	12756	14276

Figure 1. Chromatograms of racemic **9** and optically pure **9** derived from (*3S,4R*)-**3d**.

determined on a JASCO IR-810 spectrometer. TLC analyses were performed on commercial glass plates bearing a 0.25-mm layer of Merck Silica gel $60F_{254}$. Silica gel (Wakogel 200 mesh) was used for column chromatography. Elemental analyses were carried out at the Elemental Analysis Center of Kyoto University. The optical purity of **9** was established by chiral HPLC analysis (CHIRALCEL® AS-H column 4.6 mm×250 mm Daisel Chemical Industries, Hexane/2-propanol/trifluoroacetic acid=100/1/0.1 eluent, 1.0 mL/min, 25 °C, and RI detector).

Unless otherwise noted, materials obtained from commercial suppliers were used without further purification. Ether was purified over slices of sodium. THF was distilled from sodium benzophenone ketyl. Dichloromethane was stored over molecular sieves 3A. (*R*)-1-Octen-3-ol was purchased from Tokyo Kasei Kogyo Co.

4.2. Preparation of iodo compounds 2 and 11

Iodo acetal **1** was prepared by treatment of an equimolar mixture of the corresponding vinyl ether and allylic alcohol with an equal amount of *N*-iodosuccinimide in dichloromethane at 0 °C for 2 h. Preparation of **1a**–**d** was efficient (more than 90% yield). However, preparation of **1e** resulted in a lower yield (15%, not optimized).

Preparation of **2a** is representative. Iodo acetal **1a** (10.2 g, 30.0 mmol) was dissolved in hexane (25 mL). The solution was flushed with argon in a toy balloon. A solution of triethylborane in hexane (1.0 M, 6.0 mL, 6.0 mmol) was added to iodo acetal. After being stirred for 2 h at ambient temperature, the reaction mixture was evaporated. Silica gel column purification (hexane/ethyl acetate=20/1) provided **2a** (9.31 g, 27.4 mmol) in 91% yield. The diastereomer ratio of **2a** was determined by ^{1}H NMR spectra to be 55/18/15/12.

Iodo compound **11** was prepared according to the literature.[8]

4.3. Synthesis of cyclopropanes 4 from cyclic acetals 2

Iodo acetal **2a** (0.170 g, 0.500 mmol) in ether (2 mL) was added to an ethereal solution of isopropylmagnesium bromide (1.0 M ether solution, 4 equiv., 2.0 mL, 2.0 mmol) at 0 °C under argon. The reaction mixture was stirred at ambient temperature for 10 h. The mixture was poured into a saturated NH_4Cl solution. The product was then extracted with ethyl acetate (20 mL×3). The combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/ethyl acetate=20/1) to furnish alcohol **4a** (0.077 g, 0.36 mmol) in 73% yield.

4.4. Reaction of β-iodomethyl-γ-lactones 3

Under argon, iodo lactone **3c** (0.170 g, 0.500 mmol) in ether (2 mL) was added to isopropylmagnesium bromide (1.0 M ether solution, 2 equiv., 1.0 mL, 1.0 mmol) at 0 °C. The reaction mixture was stirred at ambient temperature for 10 h. The mixture was poured into 1 M HCl solution. The product was extracted with ether (10 mL×3). Sodium hydroxide (2 M, 5 mL) was added to the combined organic layer to transfer the carboxylate into the aqueous layer. After removal of the organic layer, 1 M HCl was then added to the aqueous solution until the solution became acidic. Extraction with ethyl acetate (20 mL×3) and concentration afforded colorless oil. Purification on silica gel with hexane/ethyl acetate=3/1 as an eluent yielded carboxylic acid **8** (0.044 g, 0.34 mmol) in 69% yield.

4.5. Characterization data

Spectral data for **8** was found in the literature.[14]

4.5.1. 2-Ethoxy-4-iodomethyl-3-methyl-5-pentyltetrahydrofuran (2a, diastereomer ratio is 55/18/15/12). IR (neat) 2930, 2860, 1458, 1377, 1188, 1084, 1051, 962 cm^{-1}; ^{1}H NMR ($CDCl_3$) For the most abundant isomer: δ 0.89 (t, *J*=6.6 Hz, 3H), 0.95 (d, *J*=7.2 Hz, 3H), 1.18 (t, *J*=7.2 Hz, 3H), 1.23–1.60 (m, 8H), 2.31–2.40 (m, 1H), 2.57–2.68 (m, 1H), 2.99 (t, *J*=10.5 Hz, 1H), 3.16–3.21 (m, 1H), 3.25–3.45 (m, 1H), 3.62–3.77 (m, 2H), 4.71 (s, 1H); ^{13}C NMR ($CDCl_3$) For the most abundant isomer: δ 2.58, 10.82, 14.12, 15.28, 22.68, 26.07, 31.87, 36.81, 43.60, 48.87, 62.21, 82.34, 108.33. Found: C, 46.13; H, 7.40%. Calcd for $C_{13}H_{25}IO_2$: C, 45.89; H, 7.41%.

4.5.2. 4-Iodomethyl-2-methoxy-3,3-dimethyl-5-pentyltetrahydrofuran (2b, single isomer). IR (neat) 1468, 1369, 1188, 1099, 1020, 978 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.90 (t, *J*=6.6 Hz, 3H), 0.91 (s, 3H), 1.09 (s, 3H), 1.26–1.40 (m, 5H), 1.50–1.60 (m, 2H), 1.78–1.90 (m, 1H), 2.22 (ddd, *J*=6.0, 8.4, 8.4 Hz, 1H), 3.05 (dd, *J*=8.4, 10.2 Hz, 1H), 3.20 (dd, *J*=6.0, 10.2 Hz, 1H), 3.30 (s, 3H), 3.70 (dt, *J*=2.7, 8.4 Hz, 1H), 4.31 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 1.48, 14.04, 20.18, 21.43, 22.61, 26.09, 31.75, 37.82, 47.28, 52.84, 54.28, 85.50, 111.59. Found: C, 45.88; H, 7.12%. Calcd for $C_{13}H_{25}IO$: C, 45.89; H, 7.41%.

4.5.3. 4-Iodomethyl-3,3-dimethyldihydrofuran-2(3*H*)-one (3c). IR (neat) 1757, 1342, 1196, 1115, 1097, 1013 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.10 (s, 3H), 1.30 (s, 3H), 2.66 (dddd, *J*=4.8, 7.2, 9.3, 11.1 Hz, 1H), 3.04 (dd, *J*=9.9, 11.1 Hz, 1H), 3.24 (dd, *J*=4.8, 9.9 Hz, 1H), 3.87 (dd, *J*=9.6, 9.6 Hz, 1H), 4.49 (dd, *J*=7.2, 9.6 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ −0.21, 18.03, 23.90, 43.16, 48.67, 71.01, 181.37. Found: C, 33.09; H, 4.39%. Calcd for $C_7H_{11}IO_2$: C, 33.09; H, 4.36%.

4.5.4. 4-Iodomethyl-3,3-dimethyl-5-pentyldihydrofuran-2(3*H*)-one (3d). IR (neat) 2932, 2860, 1771, 1466, 1389, 1223, 1148, 1117, 1007, 934 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.90 (t, *J*=6.9 Hz, 3H), 1.16 (s, 3H), 1.26–1.50 (m, 5H), 1.37 (s, 3H), 1.50–1.70 (m, 2H), 1.87–2.00 (m, 1H), 2.28 (ddd, *J*=7.2, 7.2, 9.3 Hz, 1H), 3.12 (d, *J*=7.2 Hz, 1H), 3.13 (d, *J*=7.2 Hz, 1H), 4.00 (dt, *J*=3.0, 9.3 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ −2.16, 14.08, 18.56, 22.55, 25.15, 25.42, 31.54, 34.52, 43.93, 52.94, 82.43, 180.68. Found: C, 44.57; H, 6.65%. Calcd for $C_{12}H_{21}IO_2$: C, 44.46; H, 6.53%.

4.5.5. 4-Iodomethyl-3,3,5,5-tetramethyldihydrofuran-2(3*H*)-one (3e). IR (neat) 2341, 1757, 1462, 1377, 1286, 1099, 1065, 941 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.22 (s, 3H), 1.37 (s, 3H), 1.38 (s, 3H), 1.58 (s, 3H), 2.48 (dd, *J*=7.5,

8.1 Hz, 1H), 3.13 (dd, *J*=8.1, 10.5 Hz, 1H), 3.21 (dd, *J*=7.5, 10.5 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ −2.78, 20.32, 22.77, 27.23, 30.28, 44.11, 56.37, 84.19, 180.17. Found: C, 38.26; H, 5.37%. Calcd for $C_9H_{15}IO_2$: C, 38.32; H, 5.36%.

4.5.6. 2-Methyl-4-(2-pentylcyclopropyl)-3-pentanone (5a, diastereomer ratio is 71/29). IR (neat) 2964, 2926, 2874, 2855, 1713, 1468, 1381, 1350, 1092, 1016 cm^{-1}; ^{1}H NMR ($CDCl_3$) For major isomer: δ 0.19–0.32 (m, 1H), 0.34–0.40 (m, 1H), 0.43–0.67 (m, 2H), 0.88 (t, *J*=6.6 Hz, 3H), 1.06 (d, *J*=6.9 Hz, 6H), 1.13 (d, *J*=6.9 Hz, 3H), 1.05–1.14 (m, 2H), 1.20–1.40 (m, 6H), 1.92 (dq, *J*=9.3, 6.9 Hz, 1H), 2.77 (qq, *J*=6.9, 6.9 Hz, 1H), for minor isomer, δ 0.19–0.32 (m, 1H), 0.34–0.40 (m, 1H), 0.43–0.67 (m, 2H), 0.86 (t, *J*=7.2 Hz, 3H), 1.07 (d, *J*=6.9 Hz, 6H), 1.09 (d, *J*=6.9 Hz, 3H), 1.05–1.14 (m, 2H), 1.20–1.40 (m, 6H), 1.95 (dq, *J*=9.3, 6.9 Hz, 1H), 2.77 (qq, *J*=6.9, 6.9 Hz, 1H); ^{13}C NMR ($CDCl_3$) For major isomer: δ 12.14, 14.18, 16.68, 18.19, 18.21, 18.71, 22.11, 22.74, 29.29, 31.77, 34.08, 39.36, 49.25, 217.62. Found: C, 79.88; H, 12.76%. Calcd for $C_{14}H_{26}O$: C, 79.94; H, 12.46%.

4.5.7. 1-Cyclohexyl-2-(2-pentylcyclopropyl)-1-propanone (5b, diastereomer ratio is 72/28). IR (neat) 2928, 2855, 1709, 1450, 1373, 993 cm^{-1}; ^{1}H NMR ($CDCl_3$) For major isomer: δ 0.24–0.31 (m, 1H), 0.36–0.39 (m, 1H), 0.45–0.60 (m, 2H), 0.88 (t, *J*=6.6 Hz, 3H), 1.12 (d, *J*=6.9 Hz, 3H), 1.06–1.15 (m, 1H), 1.17–1.45 (m, 12H), 1.60–1.70 (m, 1H), 1.70–1.84 (m, 4H), 1.90 (dq, *J*=9.6, 6.9 Hz, 1H), 2.49 (m, 1H), for minor isomer, δ 0.24–0.31 (m, 1H), 0.36–0.39 (m, 1H), 0.45–0.60 (m, 2H), 0.88 (t, *J*=6.6 Hz, 3H), 1.07 (d, *J*=6.6 Hz, 3H), 1.06–1.15 (m, 1H), 1.17–1.45 (m, 12H), 1.60–1.70 (m, 1H), 1.70–1.84 (m, 4H), 1.90 (dq, *J*=9.6, 6.9 Hz, 1H), 2.49 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 12.08, 14.18, 16.64, 18.17, 22.00, 22.74, 25.73, 25.94, 28.32, 28.87, 29.30, 31.77, 33.98, 34.08, 49.34, 49.64, 216.92. Found: C, 81.25; H, 11.95%. Calcd for $C_{17}H_{30}O$: C, 81.54; H, 12.08%.

4.5.8. 2-(2-Pentylcyclopropyl)-3-heptanone (5c, diastereomer ratio is 71/29). IR (neat) 2959, 2928, 2856, 1713, 1458, 1373, 1259, 1030 cm^{-1}; ^{1}H NMR ($CDCl_3$): For major isomer δ 0.23–0.35 (m, 1H), 0.38–0.43 (m, 1H), 0.46–0.55 (m, 2H), 0.88 (t, *J*=8.1 Hz, 3H), 0.90 (t, *J*=7.5 Hz, 3H), 1.13 (d, *J*=6.9 Hz, 3H), 1.22–1.40 (m, 10H), 1.49–1.59 (m, 2H), 1.69–1.80 (m, 1H), 2.38–2.57 (m, 2H), for minor isomer, δ 0.23–0.35 (m, 1H), 0.38–0.43 (m, 1H), 0.46–0.55 (m, 2H), 0.88 (t, *J*=8.1 Hz, 3H), 0.90 (t, *J*=7.5 Hz, 3H), 1.09 (d, *J*=6.9 Hz, 3H), 1.22–1.40 (m, 10H), 1.49–1.59 (m, 2H), 1.69–1.80 (m, 1H), 2.38–2.57 (m, 2H); ^{13}C NMR ($CDCl_3$) For major isomer δ 12.23, 14.01, 14.18, 16.23, 18.38, 22.20, 22.49, 22.74, 25.80, 29.29, 31.76, 34.08, 40.87, 51.13, 214.01. HRMS (*m*/*z*) Found: 224.2149. Calcd for $C_{15}H_{28}O$: 224.2140.

4.5.9. 2,2-Dimethyl-4-(2-pentylcyclopropyl)-3-pentanone (5d, diastereomer ratio is 72/28). IR (neat) 2964, 2926, 2855, 1705, 1477, 1466, 1367, 1049, 991 cm^{-1}; ^{1}H NMR ($CDCl_3$) For major isomer: δ 0.16–0.29 (m, 2H), 0.41–0.55 (m, 1H), 0.65–0.78 (m, 1H), 0.87 (t, *J*=6.9 Hz, 3H), 1.10 (s, 9H), 1.12 (d, *J*=6.6 Hz, 3H), 1.18–1.40 (m, 8H), 2.24 (dq, *J*=9.3, 6.9 Hz, 1H), for minor isomer, δ 0.16–0.29 (m, 2H), 0.41–0.55 (m, 1H), 0.65–0.78 (m, 1H), 0.87 (t, *J*=6.9 Hz, 3H), 1.08 (d, *J*=6.9 Hz, 3H), 1.11 (s, 9H), 1.18–1.40 (m, 8H), 2.24 (dq, *J*=9.3, 6.9 Hz, 1H); ^{13}C NMR ($CDCl_3$) For major isomer δ 12.20, 14.20, 18.06, 19.02, 22.76, 22.93, 26.02, 29.33, 31.80, 34.15, 44.00, 44.10, 206.09. Found: C, 80.08; H, 12.58%. Calcd for $C_{15}H_{28}O$: C, 80.29; H, 12.58%.

4.5.10. 2-Methyl-2-(2-pentylcyclopropyl)-3-heptanone (7). IR (neat) 2959, 2928, 2856, 1709, 1468, 1364, 1042 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.17 (ddd, *J*=5.1, 5.1, 8.7 Hz, 1H), 0.44 (ddd, *J*=5.1, 5.1, 8.7 Hz, 1H), 0.58 (ddd, *J*=5.1, 5.1, 8.7 Hz, 1H), 0.63–0.71 (m, 1H), 0.88 (t, *J*=6.9 Hz, 3H), 0.90 (s, 3H), 0.91 (t, *J*=7.5 Hz, 3H), 0.97 (s, 3H), 1.15–1.40 (m, 10H), 1.49–1.59 (m, 2H), 2.54 (t, *J*=7.2 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 8.21, 13.96, 14.05, 14.81, 21.69, 22.47, 22.55, 22.64, 26.00, 26.45, 29.20, 31.72, 34.28, 36.95, 46.18, 215.57. Found: C, 80.32; H, 12.82%. Calcd for $C_{16}H_{30}O$: C, 80.61; H, 12.68%.

4.5.11. 2-Methyl-2-(2-pentylcyclopropyl)propionic acid (9). IR (neat) 2924, 2856, 2569, 1703, 1474, 1412, 1296, 1155, 943 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.17 (ddd, *J*=5.1, 5.1, 8.7 Hz, 1H), 0.47 (ddd, *J*=5.1, 5.1, 8.7 Hz, 1H), 0.62–0.74 (m, 1H), 0.77 (ddd, *J*=5.1, 5.1, 8.7 Hz, 1H), 0.87 (t, *J*=6.6 Hz, 3H), 1.05 (s, 3H), 1.07 (s, 3H), 1.20–1.40 (m, 8H), 11.20 (bs, 1H); ^{13}C NMR ($CDCl_3$) δ 8.23, 14.18, 14.83, 22.77, 22.91, 23.30, 27.23, 29.25, 31.76, 34.29, 41.21, 184.53. Found: C, 72.89; H, 11.03%. Calcd for $C_{12}H_{22}O_2$: C, 72.68; H, 11.18%.

4.5.12. 2-(2,2-Dimethylcyclopropyl)-2-methylpropionic acid (10). IR (neat) 2874, 2571, 1703, 1458, 1412, 1379, 1294, 1175, 1028, 941, 827 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.42 (d, *J*=8.4 Hz, 2H), 0.78 (t, *J*=8.4 Hz, 1H), 1.04 (s, 3H), 1.11 (s, 3H), 1.17 (s, 3H), 1.30 (s, 3H), 11.74 (bs, 1H); ^{13}C NMR ($CDCl_3$) δ 16.51, 16.80, 19.86, 24.79, 27.52, 29.21, 33.43, 41.33, 184.77. Found: C, 68.99; H, 10.11%. Calcd for $C_9H_{16}O_2$: C, 69.19; H, 10.32%.

4.5.13. 3-(2-Hydroxymethylcyclopropyl)-2,4-dimethyl-3-pentanol (13). IR (neat) 3285, 2966, 2878, 1470, 1385, 1367, 1238, 1005, 899 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.64–0.72 (m, 2H), 0.72–0.84 (m, 1H), 0.94–1.02 (m, 1H), 0.99 (d, *J*=6.9 Hz, 6H), 1.00 (d, *J*=6.9 Hz, 6H), 1.92 (qq, *J*=6.9, 6.9 Hz, 1H), 1.98 (qq, *J*=6.9, 6.9 Hz, 1H), 2.33 (bs, 2H), 3.86 (dd, *J*=5.1, 11.4 Hz, 1H), 3.95 (dd, *J*=5.1, 11.4 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 4.42, 17.39, 17.70, 17.87, 17.98, 18.32, 20.20, 36.12, 36.79, 61.34, 76.73. Found: C, 71.16; H, 11.96%. Calcd for $C_{11}H_{22}O_2$: C, 70.92; H, 11.90%.

Acknowledgements

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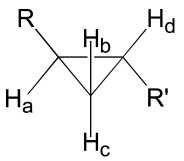
13. The stereochemistry of the cyclopropane was deduced by characteristic coupling constants of *trans*-1,2-disubstituted cyclopropane $J_{ab}=J_{bc}=J_{cd}$=ca. 5 Hz, $J_{ac}=J_{bd}$=ca. 8 Hz. For example, Taylor, R. E.; Risatti, C. A.; Engelhardt, F. C.; Schmitt, M. J. *Org. Lett.* **2003**, *5*, 1377–1379



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A new series of heteroaromatic receptors containing the 1,3-bis(6-oxopyridazin-1-yl)propane unit: their selective transport ability towards NH_4^+ in relation to Na^+, K^+ and Ca^{2+}

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Abstract—The synthesis of a new series of heteroaromatic macrocycles **6**–**9** containing the 1,3-bis(6-oxopyridazin-1-yl)propane and pyridine units is reported. The acyclic compounds **11**–**15** had to be prepared as the intermediates in the synthetic sequence. Evaluation of the ionophoric properties of **6**–**9** and **11**–**15** shows that **8** and **13** behave as good ammonium ion carriers and exhibit a high selectivity for ammonium with respect to spherically symmetric metallic ions like Na^+, K^+, and Ca^{2+}. Molecular modelling of the ammonium complexes suggests that the host's oxyimino groups play a more relevant part in effective complexation than the pyridine unit, and that the high complexating efficiency of **13** might be related to the formation of a pseudocavity by intramolecular hydrogen bonding.

1. Introduction

In the last decades, a considerable interest has been shown in developing selective receptors for the ammonium cation.[1] Such receptors can be used as NH_4^+ sensors in clinical analysis, environmental chemistry, and many other applications.[2] One of the most effective NH_4^+ receptors is nonactin,[3] the natural antibiotic agent commercially used in ion-selective electrodes.[4] However, a serious drawback of nonactin is that it binds only about ten times more tightly to NH_4^+ than to K^+. Similarly, crown ethers show little or no selectivity for binding NH_4^+ against K^+,[3] although the substitution of oxygen atoms by nitrogen increases ammonium selectivity,[5] and pyrido crown ethers have shown to be specially effective in NH_4^+/K^+ discrimination.[6] Achieving selectivity in the complexation of ammonium in relation to metal ions of biological importance like Na^+, K^+ or Ca^{2+} is a significative goal because it could allow some kind of control in transporting the above cations across biological membranes.

Our group has been involved for years in the study of the ionophoric properties of mono- and dinuclear receptors containing sp^2 nitrogen heterocyclic units. We have demonstrated that 36-membered dinuclear ether and ester crowns of 3,5-disubstituted 1*H*-pyrazole containing tetraethyleneglycol units, **1** (Fig. 1), are able to facilitate the NH_4^+ transport, but lack the desired NH_4^+/K^+ selectivity.[7] The introduction of pyridine rings in the flexible oxygenated chains of **1** to give **2** provided better ammonium selectivity by cooperation of the sp^2 heterocyclic nitrogen atoms when they could be included into the macrocyclic cavity.[8] On the other hand, when the macrocyclic ring size was reduced to 26 atoms by linking the heterocyclic moieties with diethyleneglycol units (**3**–**4**), the NH_4^+ complexation ability was improved, because of a better cooperativity of the pyrazole sp^2 nitrogens.[9,10] Subsequently, we introduced the 1,3-bis(1*H*-pyrazol-1-yl)propane unit in the macrocyclic ring, as exemplified in **5**. All the heteroatoms in these compounds have the electron lone pairs oriented inside the cavity, and the propylenic fragment should favour conformational mobility for accommodation of the guest. However, selectivity for the ammonium ion was less than that one obtained in the previous series.[11]

With the aim of improving the ionophoric properties found for **5** and analogues, we have prepared here a new family of heteroaromatic receptors (**6**–**9**). The conformational mobility provided by the 1,3-propylene fragment in **5** has been maintained, and the cooperative effects of the pyrazolic nitrogens substituted by those of two pyridazinone units. The presence of a pyridine ring both modifies the macrocyclic ring flexibility and contributes to a more

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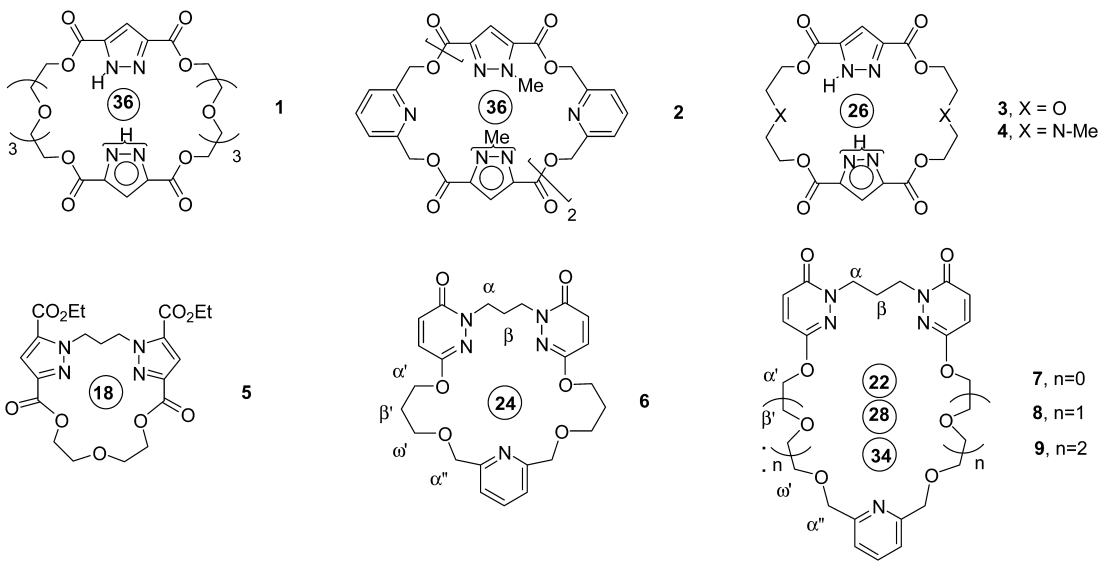


Figure 1.

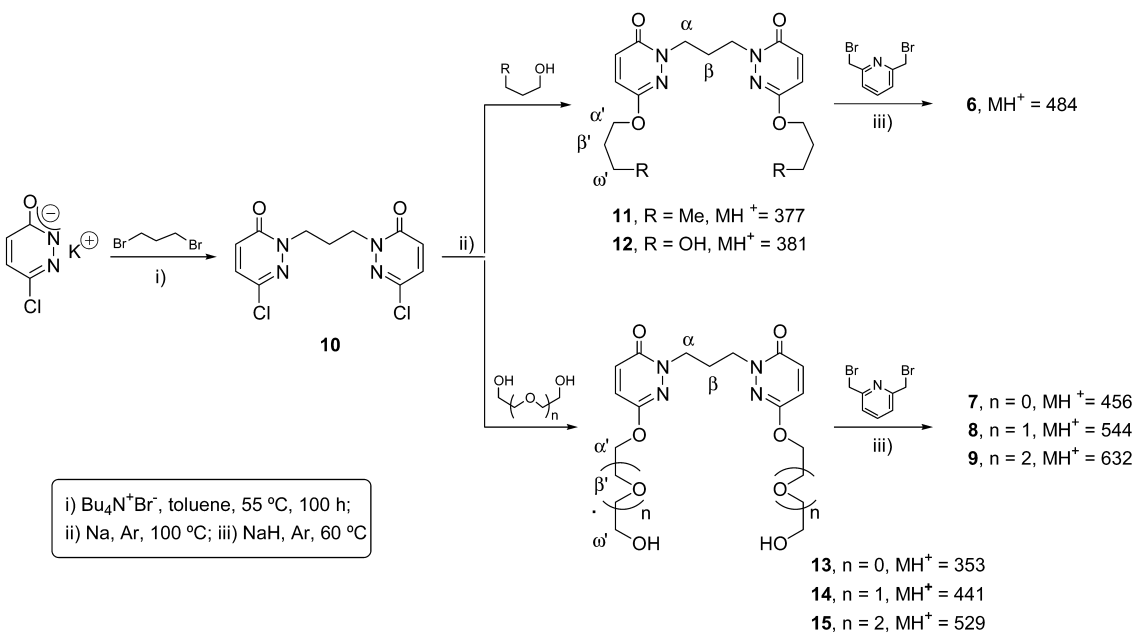
effective complexation through the sp^2 heterocyclic nitrogen, as shown previously for **2**.[8] The respective presence of ethyleneoxy or propyleneoxy units modifies the relative distance between the pyridazine ring and the oxyimino fragments, as presumed main sites for ammonium complexation. The ionophoric properties of compounds **6**–**9** and their open chain precursors in the synthetic process have been evaluated against NH_4^+ and the biologically significative Na^+, K^+, and Ca^{2+} cations.

2. Results and discussion

We have used 1,3-bis(3-chloro-6-oxopyridazin-1-yl)-propane, **10**, as the synthon for the preparation of both the acyclic and cyclic receptors. This compound was in turn obtained from 3,6-dichloropyridazine via the mono-potassium salt of 3-chloro-6(1*H*)-pyridazinone[12] as shown in Scheme 1, in a whole yield of 67%. A side product in the last step is the mono-O-alkylation compound, that must be separated and is formed in 17% yield.

First, the *O*-butyl substituted derivative **11** was prepared in order to set the reaction conditions. It could be formed in a 48% yield by the reaction of **10** with the alkoxide generated in situ from butanol, that was also the solvent, using a stoichiometric 4:1 sodium excess. Following the same procedure, the acyclic compounds **12** and **13**–**15** were synthesized by treatment of the synthon **10** with 1,3-propanediol or polyethyleneglycol units of different lenghts, in yields between 41 and 57%.

In turn, the cyclic derivatives **6** and **7**–**9** were obtained by heating the previously formed diols with 2,6-bis(bromo-methyl)pyridine in the presence of sodium hydride in a dimethoxyethane–dimethylformamide solution at 60 °C.



Scheme 1.

Both the cyclic and acyclic receptors were purified by flash column chromatograpy and usually isolated as stable crystalline solids.

Structures were assigned on the basis of analytical, and spectroscopic mass, IR, and ^{1}H and ^{13}C NMR data. Molecular ions obtained from FAB mass spectra fit the proposed structures and agree in most cases with the base peak (see Scheme 1).

The ^{1}H NMR pyridazinone signals are easily differentiated from those of the pyridine ring, appearing as neat AB systems with coupling constants in the range of 9.6–9.9 Hz. Concerning the aliphatic moieties, Tables 1 and 2 show the ^{1}H and ^{13}C values for signals corresponding to the methylene groups. In all cases, those vicinal to the pyridazine nitrogens are more shielded in the ^{1}H spectra than their counterparts neighbouring the oxyimino groups. Furthermore, both types of methylenes can be differentiated by the shape of the corresponding signals, which are shown as neat triplets coupled to quintuplets in the α units, whereas the α′ ones display variable multiplicities and J values. In the ^{13}C spectra, the CH_2–N signals appear in the vicinity of 50 ppm, but the CH_2–oxyimino are substantially more deshielded (around 65 ppm).

On the other hand, if we compare compounds containing ethylenic and propylenic chains, the methylene groups of **7**–**9** and **13**–**15** are seen in the ^{1}H spectra as the typical multiplet pairs in the AA′BB′ system of the –O–CH_2–CH_2–O– units.[13] However, in **6** and **12** the CH_2 vicinal to oxymino group are always shown as triplets.

As expected, the methylenic hydrogens in the ω′ position of the acyclic derivatives are more shielded than the rest, and in the ^{13}C spectra the CH_2–OH carbons also seem to be the most shielded among those vicinal to oxygen. The protons at the methylene groups vicinal to the pyridine ring appear in the cyclic compounds as sharp singlets between 4.5 and 4.7 ppm, and their carbons are the most deshielded among all the aliphatic ones.

All compounds reported in this paper have been evaluated as carriers of Na^+, K^+, Ca^{2+}, and NH_4^+ picrates in passive transport processes using a classical bulk liquid membrane of chloroform as indicated in Section 3.[11] Picrate anions have been selected on the basis of their lipophilic character and also because they are surface active, favouring the transfer of the cations from the aqueous phase to the interface.[14]

Table 2. ^{13}C NMR [CD_3OD, δ (ppm)] chemical shifts of the most significative methylene carbons in compounds **6**–**9** and their acyclic precursors **12**–**15**

	C_α	C_β	$C_{\alpha'}$	$C_{\beta'}$	$C_{\omega'}$	$C_{\alpha''}$
6	49.84	27.86	65.56	30.12	68.19	74.54
12	50.01	27.27	65.36	32.67	59.36	—
7	49.48	27.56	66.75	—	69.22	73.78
13	49.49	25.93	69.60	—	60.82	—
8	50.88	26.24	67.93	70.17	71.77	75.17
14	50.06	27.41	68.06	70.33	62.51	—
9	52.55	26.95	68.00	70.36	71.70	74.63
15	50.11	27.41	67.96	70.17	62.51	—

The resulting transport rates are gathered in Table 3. It can be observed that, in accordance with our initial design, all the new series of cyclic compounds **6**–**9** transport NH_4^+ better than Na^+, K^+, and Ca^{2+}. In fact, the cyclic receptor **8** in which the two heteroaromatic units are linked by diethylene glycol flexible chains behaves as the most effective carrier of ammonium ions (ν=57 μM h^{-1}). Furthermore, this ligand exhibits an excellent selectivity for NH_4^+ in relation to the three metallic cations evaluated (NH_4^+/Na^+=9.2, NH_4^+/K^+=9.5, NH_4^+/Ca^{2+}=11.8).

It is interesting to point out that the size of the macrocyclic cavity seems to be critical in order to achieve both efficiency and selectivity in the ammonium transport. Taking as the reference the cavity size of **8** (28-membered), it can be observed that smaller or larger cavities lead to both lower transport rates and selectivities. Thus, compounds with 24 (**6**) and 22 (**7**) members are almost five times less effective carriers that **8**, and compound **9** (34-membered) is the worst carrier in this cyclic series.

In relation to the evaluation of the ionophoric properties in the acyclic intermediates **11**–**15**, it is shown that all of them are unable to efficiently transport Na^+, K^+, and Ca^{2+} ions. However, the acyclic intermediate **13**, in which the 1,3-bis(6-oxopyridazin-1-yl)propane unit has been functionalized with shorter flexible chains of ethylene glycol, behaves as an efficient carrier of NH_4^+ ions. Furthermore, compound **13** displays an excellent selectivity of NH_4^+ transport in relation to K^+(NH_4^+/K^+=73), which is almost seven times higher than that one of nonactin.[2] Finally, an impressive selectivity in relation to Ca^{2+}(NH_4^+/Ca^{2+}=146) is also observed. This fact could be related to the hydration grade of the cation inside the complex, as it is dependant of the cation charge.[15]

It is interesting to note that the hydroxy groups of **13**,

Table 1. ^{1}H NMR [CD_3OD, δ (ppm)] chemical shifts of the most significative methylene protons in compounds **6**–**9** and their acyclic precursors **12**–**15**

	H_α	H_β	$H_{\alpha'}$	$H_{\beta'}$	$H_{\omega'}$	$H_{\alpha''}$
6	4.06 (t, 4H)[a]	2.33 (q, 2H)[a]	4.21 (t, 4H)[b]	2.12 (q, 4H)[b]	3.71 (t, 4H)[b]	4.54 (s, 4H)
12	4.03 (t, 4H)[a]	2.24 (q, 2H)[a]	4.16 (t, 4H)[b]	1.87 (q, 4H)[b]	3.61 (t, 4H)[b]	—
7	3.90 (t, 4H)[a]	2.08 (q, 2H)[a]	4.38 (m, 4H)	—	3.84 (m, 4H)	4.63 (s, 4H)
13	4.11 (t, 4H)[a]	2.32 (q, 2H)[a]	4.22 (m, 4H)	—	3.84 (m, 4H)	—
8	4.11 (t, 4H)[a]	2.33 (q, 2H)[a]	4.38 (m, 4H)		3.6–3.8 (m, 4H)	4.67 (s, 4H)
14	4.12 (t, 4H)[a]	2.34 (q, 2H)[a]	4.31 (m, 4H)	3.82 (m, 4H)	3.61 (m, 4H)	—
9	4.10 (t, 4H)[a]	2.32 (q, 2H)[a]	4.23 (m, 4H)	3.89 (m, 4H)	3.71 (m, 4H)	4.61 (s, 4H)
15	4.12 (t, 4H)[a]	2.33 (q, 2H)[a]	4.31 (m, 4H)	3.82 (m, 4H)	3.6–3.8 (m, 4H)	—

[a] J=6.9 Hz.
[b] J=6.3 Hz.

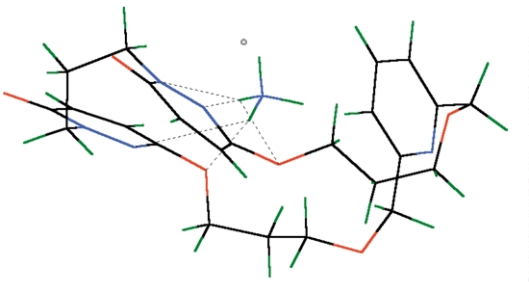
Table 3. Transport rates ($\times\mu M\,h^{-1}$) of alkali, alkaline earth, and ammonium ions through a chloroform phase containing 7×10^{-4} M of cyclic **6**–**9** and acyclic **11**–**15** carriers

	Transport rates				Selectivity		
	Na^+	K^+	Ca^{2+}	NH_4^+	NH_4^+/Na^+	NH_4^+/K^+	NH_4^+/Ca^{2+}
6	4.9	2.6	1.3	11.8	2.4	4.1	9.1
7	2.0	3.8	2.2	10.1	5.0	2.6	4.6
8	6.3	6.9	4.9	**57.9**	**9.2**	**9.5**	**11.8**
9	3.3	4.5	2.0	5.2	1.6	1.1	2.6
11	1.8	0.8	1.7	1.5	0.8	1.8	0.9
12	0.5	1.8	1.7	0.1	0.2	0.05	0.14
13	0.7	0.8	0.4	**58.4**	**83.4**	**73.0**	**146.0**
14	0.6	0.6	0.8	0.1	0.16	0.16	0.13
15	0.5	4.2	0.3	0.7	1.4	0.16	2.3

located at a shorter distance from the heteroaromatic moiety, seem to play a critical role in the complexation process of ammonium, since the butoxy substituted derivative **11** and the hydroxy substituted compounds **12**, **14** and **15** are worse carriers of this ion.

In order to evaluate the possible ammonium-receptor interactions involved in the complexes formed by the most promising carriers found in this work, we have performed molecular modelling of the NH_4^+ complexes formed from ligands **8** and **13**. With the aim of justifying the transport differences observed with the rest of the cyclic series, the complex of the 24-membered compound **6**, which exhibits a macrocyclic cavity relatable to that one of **8** was also included in the modelling studies. The additive AMBER force field has been used because it is specially adequate for describing the complexation processes of our ligands, since it is the best method[16] for reproducing H-bonding and stacking stabilization energies. Methodology and conventions used are summarized in Section 3.

Figures 2 and 3 display the most stable conformations obtained for the cyclic receptor 1:1 complexes **6**-NH_4^+ and



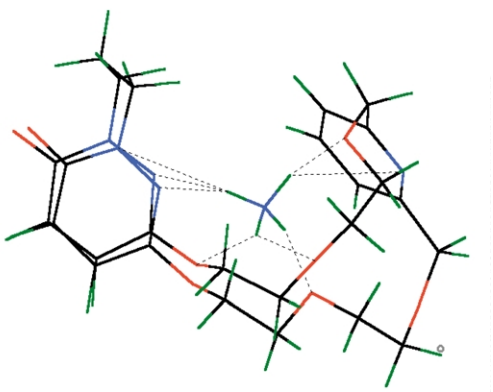
E_c = -15.30 Kcal/mol

NH····N(2)-Pdz A; NH····N(2)-Pdz B; NH·····N(1)-Pdz B
NH·····O-C_3(Pdz A); NH·····O-C_3 (Pdz B)

Figure 3. Molecular modelling, complexation energy, and hydrogen bonding in **6**.

8-NH_4^+. Intermolecular hydrogen bondings between the ligand's heteroatoms and the ammonium ion are also shown, together with the lowest complexation energies found in every case. In **8**, the 28-membered macrocycle has the two pyridazinone rings oriented in a parallel way and has a bowl-shaped conformation around the ammonium ion, that is located outside the macrocyclic cavity in a suitable disposition for allowing simultaneous effective interaction with the nitrogen atoms at the three heterocyclic rings and the oxygens at the aliphtic chains. Therefore, one of the ammonium hydrogens interacts in a trifurcated way with the imine nitrogens of the two pyridazine rings, the second ammonium hydrogen is linked to the nitrogen at the pyridine ring and to one of the oxygens nearest to pyridine. Finally, the other two are bonded to the central oxygens at the aliphatic chains and to the oxyimine oxygen neighbouring one of the pyridazine units.

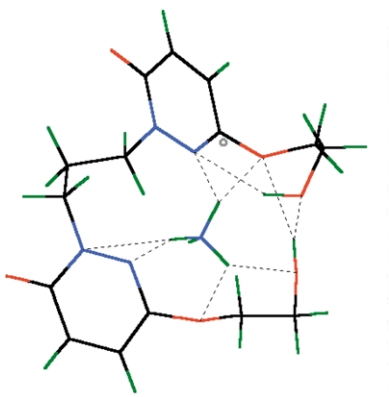
In contrast, the most favoured conformation obtained for the ammonium complex of the smaller 24-membered macrocycle **6** (Fig. 3) implies a more planar disposition of the macrocyclic ring with divergent pyridazine rings, and the ammonium ion is mainly interacting with the oxygen and



E_c = -21.98 Kcal/mol

NH····N(2)-PdzA; NH····N(2)-Pdz B; NH·····N(1)-Pdz B;
NH·····N-Pyr; NH·····O-CH_2 Pyr.
NH·····O-C_3(Pdz A); NH·····O-$CH_{2\,\beta',\,\gamma'}$
NH·····O-$CH_{2\,\beta',\,\gamma'}$

Figure 2. Molecular modelling, complexation energy, and hydrogen bonding in **8**.



E_c = -15.19 Kcal/mol

NH·····N(2)-Pdz A; NH·····N(1)-Pdz A
NH·····N(2)-Pdz B; NH·····O-C_3 (Pdz B)
NH·····O-C_3 (Pdz A); NH·····OH
OH·····O-C_3 Pdz B; OH·····OH; OH····· N(2)-Pdz B

Figure 4. Molecular modelling, complexation energy, and hydrogen bonding in **13**.

nitrogen atoms at the oxyimino groups. As a consequence, the calculated complexation energy is substantially lower in absolute value than that one found for **8** (ΔE_c=6.68 kcal/mol). All this suggests that both the size of the macrocyclic cavity and the participation of the flexible chains oxygens might be decisive in order to achieve an effective transport.

The most favoured conformation for the ammonium complex of the effective acyclic carrier **13** is shown in Figure 4. This complex exhibits strong intramolecular bonding between the terminal OH groups forming a pseudomacrocyclic structure that wraps the guest. On the other hand, the ammonium hydrogens give way to intermolecular interactions with the imine nitrogens at the pyridazine rings, and also with the oxygen atoms at the oxyimino groups. All this is reflected in a complexation energy of −15.90 kcal/mol. The high transport efficiency found for this ligand with respect to the other acyclic compounds tested could be due to its smaller size, that allows the formation of a pseudocavity favouring closeness of the ammonium hydrogens and the oxyimino moieties and giving place to a more effective interaction. Molecular modelling of ligands **12**, **14**, and **15** leads always to bigger pseudocavities and less favourable complexation energies than those calculated for **13**.

Finally, if we compare the complexation energies obtained for **8** and **13**, it should be noted that, although both compounds exhibit similar transport efficiency for the ammonium ion, complexation seems to be clearly more favoured in **8** with respect to **13**. However, it is well known[17] that complexation/decomplexation processes have a great influence on transport rates, and that they are usually much more favoured in acyclic carriers than in cyclic ones.[18]

3. Experimental

3.1. General

Reagents were purchased from commercial suppliers and used without further purification, unless otherwise stated. Ligands were prepared in dry solvents under an argon atmosphere. Reaction courses were routinely monitored by TLC on precoated aluminium sheets of silica gel, and compounds were detected with UV light (245 nm) and/or iodine chamber. Chromatographic separations were performed on columns, using the flash chromatography technique on silica gel (particle size 0.040–0.063 mesh) and as eluent a mixture of toluene/ethyl acetate/methanol/dichloromethane (1:2:1:1, v:v), unless otherwise indicated. Melting points were measured in open capillaries and are uncorrected. The ^{1}H NMR spectra were recorded at 300 MHz and the ^{13}C NMR spectra were recorded at 75 or 100 MHz at room temperature in the solvent indicated in each case. Chemical shifts are given as δ values, using TMS as internal standard, and coupling constants are given in Hz. Mass spectra were recorded by the fast atomic bombardment (FAB) technique using a *m*-nitrobenzyl alcohol matrix. 3-Chloro-1*H*-pyridazin-6-one was obtained following a previously described procedure starting from 3,6-dichloropyridazine.[12]

3.1.1. 1,3-Bis(3-chloro-6-oxopyridazin-1-yl)propane (10). A mixture of the potassium salt of 3-chloro-1*H*-pyridazin-6-one (prepared by reaction with a equimolecular amount of potassium hydroxide in ethanol) (2.28 g, 13 mmol), 1,3-dibromopropane (1.40 g, 7 mmol), and tetrabutylammonium chloride (1.08 g, 3 mmol) in anhydrous toluene (65 mL) was heated at 65 °C for 100 h. After cooling to room temperature, the residual solid was filtered and the solution was evaporated to dryness under reduced pressure. The residue was purified by column chromatography (toluene/ethyl acetate/ethanol, 2:1:0.15). The most retained fraction afforded 3.09 g (67% yield) of **10** as a yellow solid. Mp 124–5 °C. IR (KBr, cm^{-1}) 3040, 1680 (C=O), 1590; ^{1}H NMR ($CDCl_3$) δ 7.18 (d, J=10 Hz, 2H), 6.91 (d, J=10 Hz, 2H), 4.20 (t, J=7 Hz, 4H), 2.33 (q, J=7 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 158.88, 137.53, 133.61, 132.02, 49.13, 26.84. Anal. calcd for $C_{11}H_{10}N_4O_2Cl_2$: C, 43.87; H, 3.35; N, 18.60; Cl, 23.54. Found: C, 44.10; H, 3.58; N, 18.69; Cl, 23.03.

3.2. General procedure for the synthesis of podands 11–15

To a solution of 4–5 mmol of sodium in excess of the appropriate alcohol or glycol 1 mmol of dichloroderivative **10** was added. The mixture was heated to 100 °C for 1 h, diluted with 10 mL of water when cold, and extracted with chloroform. Solvent and excess of glycol was removed under reduced pressure and the crude product was purified by chromatography on silica gel.

3.2.1. 1,3-Bis(3-butoxy-6-oxopyridazin-1-yl)propane (11). Following the general procedure, flash chromatography on silica gel of the residue obtained in the reaction of **10** (0.5 mmol) with 1-butanol (4 mL) and sodium (2.5 mmol) afforded 90 mg (48%) of a pure oil identified as **11**. (R_f=0.33, toluene/ethyl acetate/ethanol, 2:1:0.15). IR ($CHCl_3$, cm^{-1}) 1685 (C=O), 1595, 1225; ^{1}H NMR (CD_3OD) δ 7.09 (d, J=9.6 Hz, 2H); 6.91 (d, J=9.6 Hz, 2H), 4.17 (t, J=7.2 Hz, 4H), 4.12 (t, J=6.6 Hz, 4H), 2.32 (q, J=6.6 Hz, 2H), 1.73 (m,4H), 1.47 (m, 4H), 0.98 (t, J=7.5 Hz, 6H); ^{13}C NMR (CD_3OD) δ 161.28, 155.31, 133.65, 128.85, 68.48, 49.99, 32.13, 27.62, 20.54, 14.56. MS (FAB, *m/z*) 377 (MH^+, 100); HRMS (FAB) calcd for $[MH]^+$ $C_{19}H_{29}N_4O_4$ 377.2188, obsd. 377.2194.

3.2.2. 1,3-Bis[3-(3-hydroxypropoxy)-6-oxopyridazin-1-yl)propane (12). Chromatography on silica gel of the residue obtained in the reaction of **10** (300 mg, 1 mmol) with sodium (100 mg, 4.34 mmol) in 5 mL of 1,3-propanediol afforded 150 mg (41%) of **12** as a white solid (R_f=0.35). Mp 137–8 °C. IR (KBr, cm^{-1}), 3380 (O–H), 1660 (C=O), 1585, 1300; ^{1}H NMR (CD_3OD) δ 7.02 (d, J=9.6 Hz, 2H), 6.82 (d, J=9.6 Hz, 2H), 4.16 (t, J=6.3 Hz, 4H), 4.03 (t, J=6.9 Hz, 4H), 3.61 (t, J=6.3 Hz, 4H), 2.24 (q, J=6.9 Hz, 2H); 1.87 (q, J=6.3 Hz, 4H); ^{13}C NMR (CD_3OD) δ 161.01, 154.96, 133.35, 128.54, 65.36, 59.36, 50.01, 32.67, 27.27. MS (FAB, *m/z*) 381 (MH^+, 32); 136 (100). Anal. calcd for $C_{17}H_{24}N_4O_6$: C, 53.59; H, 6.30; N, 14.71. Found: C, 53.63; H, 6.27; N, 14.35.

3.2.3. 1,3-Bis[3-(2-hydroxyethoxy)-6-oxopyridazin-1-yl)propane (13). Chromatography on silica gel of the residue obtained in the reaction of **10** (500 mg, 1.66 mmol) with sodium (100 mg, 4.34 mmol) in 4 mL of ethylene glycol afforded 244 mg (42%) of **13** (R_f=0.33). Mp 162–4 °C. IR (KBr, cm^{-1}) 1665 (C=O), 1590, 1300; ^1H NMR (CD_3OD) δ 7.14 (d, J=9.6 Hz, 2H), 6.93 (d, J=9.6 Hz, 2H), 4.22 (m, 4H), 4.11 (t, J=6.9 Hz, 4H), 3.84 (m, 4H), 2.32 (q, J=6.9 Hz, 2H); ^{13}C NMR (CD_3OD) δ 160.89, 154.74, 133.33, 128.42, 69.60, 60.82, 49.50, 27.47. MS (FAB, *m/z*) 353 (MH^+, 100). Anal. calcd for $C_{15}H_{20}N_4O_6 \cdot 1/2H_2O$: C, 49.86; H, 5.86; N, 15.50. Found: C, 49.95; H, 5.56; N, 15.21.

3.2.4. 1,3-Bis{3-[2-(2-hydroxyethoxy)ethoxy]-6-oxopyridazin-1-yl}propane (14). Chromatography on silica gel of the residue obtained in the reaction of **10** (300 mg, 1 mmol) with sodium (100 mg, 4.34 mmol) in 5 mL of diethylene glycol afforded 180 mg (41%) of **14** as a white solid (R_f=0.34). Mp 103–4 °C. IR (KBr, cm^{-1}) 3140 (O–H), 1670 (C=O), 1590, 1300; ^1H NMR (CD_3OD) δ 7.13 (d, J=9.6 Hz, 2H), 6.94 (d, J=9.6 Hz, 2H), 4.31 (m, 4H); 4.12 (t, J=6.9 Hz, 4H), 3.82 (m, 4H), 3.68 (m, 4H), 3.61 (m, 4H), 2.34 (q, J=6.9 Hz, 2H); ^{13}C NMR (CD_3OD) δ 161.31, 155.04, 133.83, 128.80, 74.06, 70.33, 68.06, 62.50, 50.06, 27.41. MS (FAB, *m/z*) 441 (MH^+, 100). Anal. calcd for $C_{19}H_{28}N_4O_8 \cdot 1/2H_2O$: C, 50.77; H, 6.50; N, 12.47. Found: C, 50.99; H, 6.24; N, 12.53.

3.2.5. 1,3-Bis(3-{2-[2-(2-hydroxyethoxy)ethoxy]ethoxy}-6-oxopyridazin-1-yl)propane (15). Chromatography on silica gel of the residue obtained in the reaction of **10** (300 mg, 1 mmol) with sodium (100 mg, 4.34 mmol) in 5 mL of triethylene glycol afforded 300 mg (57%) of **15** as a colorless oil (R_f=0.34). IR (KBr, cm^{-1}) 3140 (O–H), 1670 (C=O), 1590, 1300; ^1H NMR (CD_3OD) δ 7.13 (d, J=9.6 Hz, 2H), 6.94 (d, J=9.6 Hz, 2H), 4.31 (m, 4H), 4.12 (t, J=6.9 Hz, 4H), 3.82 (m, 4H), 3.67 (m, 12H), 3.58 (m, 4H), 2.33 (q, J=6.9 Hz, 2H). ^{13}C NMR (CD_3OD) δ 161.34, 155.03, 133.87, 128.83, 74.01, 71.99, 71.72, 70.41, 67.96, 62.51, 50.11, 27.41. MS (FAB, *m/z*) 529 (MH^+, 100). Anal. calcd for $C_{23}H_{36}N_4O_{10}$: C, 52.27; H, 6.82; N, 10.61. Found: C, 52.07; H, 6.91; N, 10.54.

3.2.6. 10,14,22,26-Tetraoxa-1,5,31,32,33-pentaazatetracyclo[25.3.1.1^{5,9}.1^{16,20}]tritriaconta-7,9(32),16,18,20(33),27(31),28-heptaen-6,30-dione (6). To a solution of diol **12** (60 mg, 0.16 mmol) in THF (30 mL), potassium *tert*-butoxide (56 mg, 0.5 mmol) was added and the reaction mixture was kept at 60 °C until the salt of **12** was formed (0.5 h). Then, a solution of 2,6-bis(bromomethyl)pyridine (43 mg, 0.16 mmol) in THF (10 mL) was added slowly. After the addition was complete, the reaction mixture was kept for 4 h at 60 °C, then cooled to room temperature, and stirred overnight. The resulting reaction mixture was filtered, evaporated to dryness, and the residue was chromatographed on silica gel. Fractions of R_f=0.45 afforded 14 mg (18%) of crown **6** as a pure colorless oil. ^1H NMR (CD_3OD) δ 7.78 (t, J=7.8 Hz, 1H), 7.38 (d, J=7.8 Hz, 2H), 7.10 (d, J=9.6 Hz, 2H), 6.92 (d, J=9.6 Hz, 2H), 4.54 (s, 4H), 4.21 (t, J=6.9 Hz, 4H), 4.06 (t, J=6.9 Hz, 4H), 3.71 (t, J=6.0 Hz, 4H); 2.33 (q, J=6.0 Hz, 2H), 2.15 (q, J=6.9 Hz, 4H); ^{13}C NMR (CD_3OD) δ 160.93, 158.78, 154.89, 139.01, 133.35, 128.51, 123.26, 74.54, 68.19, 65.56, 49.84, 30.12, 27.86. MS (FAB, *m/z*) 484 (MH^+, 90); HRMS (FAB) calcd for $[MH]^+$ $C_{24}H_{30}N_5O_6$ 484.219609, obsd. 484.218100.

3.2.7. 10,13,21,24-Tetraoxa-1,5,29,30,31-pentaazatetracyclo[23.3.1.1^{5,9}.1^{15,19}]untriaconta-7,9(30),15,17,19(31),25(29),26-heptaen-6,28-dione (7). To a stirred suspension of NaH (93 mg, 3.88 mmol) in DME (30 mL) a solution of diol **13** (352 mg, 1 mmol) in a mixture of DMF (8 mL) and DME (40 mL) was added and the reaction was kept at 60 °C until the disodium salt of **13** was formed (0.5 h). Then, the mixture was diluted with 100 mL of DME and, after the temperature was again stabilized at 60 °C, a solution of 2,6-bis(bromomethyl)pyridine (265 mg, 1 mmol) in DME (100 mL) was added over a period of 2–3 h. After the addition was completed, the reaction was kept for 4 h at 60 °C, then cooled to room temperature, and stirred overnight. Further treatment with water, filtration, and extraction with dichloromethane afforded and organic layer that was evaporated to dryness and the residue column chromatographed on silica gel. Fractions of R_f=0.36 afforded 57 mg (13%) of crown **7** as a white solid. Mp 174–6 °C. ^1H NMR (CD_3OD) δ 7.63 (t, J=7.8 Hz, 1H), 7.23 (d, J=7.8 Hz, 2H), 7.16 (d, J=9.9 Hz, 2H), 6.95 (d, J=9.9 Hz, 2H), 4.63 (s, 4H), 4.38 (m, 4H), 3.90 (t, J=6.9 Hz, 4H), 3.84 (m, 4H), 2.08 (q, J=6.9 Hz, 2H); ^{13}C NMR (CD_3OD) δ 160.83, 159.06, 154.63, 138.85, 133.52, 128.71, 122.36, 73.78, 69.22, 66.75, 49.48, 27.58. MS (FAB, *m/z*) 456 (MH^+, 100); HRMS (FAB) calcd for [MH^+] $C_{22}H_{26}N_5O_6$ 456.188309, obsd. 456.189090.

3.2.8. 10,13,16,24,27,30-Hexaoxa-1,5,35,36,37-pentaazatetracyclo[29.3.1.1^{5,9}.1^{18,22}]heptatriaconta-7,9(36),18,20,22(37),31(35),32-heptaen-6,34-dione (8). To a stirred suspension of NaH (56 mg, 2.35 mmol) in DME (50 mL) was added a solution of diol **14** (517 mg, 1.18 mmol) in a mixture of DMF (3 mL) and DME (30 mL), and the reaction mixture was kept at 60 °C until the disodium salt of **14** was formed (0.5 h). Then, the mixture was diluted with 70 mL of DME and potassium carbonate (81 mg, 0.59 mmol) was added. After the temperature was again stabilized at 60 °C, a solution of 2,6-bis(bromomethyl)pyridine (312 mg, 1.18 mmol) in DME (100 mL) was added over a period of 2–3 h. After the addition was complete, the reaction mixture was kept for 4 h at 60 °C, then cooled to room temperature, and stirred overnight. The resulting reaction mixture was treated with water, filtered, and extracted with chlorophorm. The organic extracts were evaporated to dryness and the residue was chromatographed on silica gel. Fractions of R_f=0.42 afforded 32 mg (5%) of crown **8** as a solid. Mp 104–6 °C. ^1H NMR (CD_3OD) δ 7.72 (t, J=7.8 Hz, 1H), 7.39 (d, J=7.8 Hz, 2H), 7.00 (d, J=9.6 Hz, 2H), 6.91 (d, J=9.6 Hz, 2H); 4.67 (s, 4H), 4.18 (m, 4H), 4.11 (t, J=6.6 Hz, 4H); 3.79–3.73 (m, 12H), 2.33 (q, J=6.6 Hz, 2H). ^{13}C NMR (CD_3OD) δ 161.29, 159.62, 154.79, 139.19, 133.93, 128.79, 121.98, 75.17, 72.78, 71.71, 70.17; 67.93, 50.88, 26.24. MS (FAB, *m/z*) 544 (MH^+, 100); HRMS (FAB) calcd for [MH^+] $C_{26}H_{34}N_5O_8$ 544.240739, obsd. 544.241300.

3.2.9. 10,13,16,19,27,30,33,36-Octaoxa-1,5,41,42,43-pentaazatetracyclo[35.3.1.1^{5,9}.1^{21,25}]tritetraconta-7,9(42),

18,20,22(37),31(35),32-heptaen-6,34-dione (9). To a stirred suspension of NaH (96 mg, 4 mmol) in DME (50 mL) was added a solution of diol **15** (1056 mg, 2 mmol) in DME (50 mL), and the reaction mixture was kept at 60 °C until the disodium salt of **15** was formed (0.5 h). Then, the mixture was diluted with 200 mL of DME and cesium chloride (337 mg, 2 mmol) was added. After the temperature was again stabilized at 60 °C, a solution of 2,6-bis(bromomethyl)pyridine (530 mg, 2 mmol) in DME (200 mL) was added over a period of 2–3 h. After the addition was complete, the reaction mixture was kept for 4 h at 60 °C, then cooled to room temperature, and stirred overnight. The resulting reaction mixture was treated with water, filtered, and extracted with chlorophorm. The organic extracts were evaporated to dryness and the residue was chromatographed on silica gel. Fractions of R_f=0.42 afforded 170 mg (27%) of crown **9** as an oil. ^{1}H NMR (CD_3OD) δ 7.73 (t, J=7.8 Hz, 1H), 7.41 (d, J=7.8 Hz, 2H), 6.99 (d, J=9.6 Hz, 2H), 6.89 (d, J=9.6 Hz, 2H), 4.61 (s, 4H), 4.23 (m, 4H), 4.10 (t, J=6.6 Hz, 4H), 3.89 (m, 4H), 3.71–3.65 (m, 16H), 2.32 (q, J=6.6 Hz, 2H); ^{13}C NMR (CD_3OD) δ 161.23, 159.48, 154.86, 139.32, 133.90, 128.66, 122.08, 74.63, 72.04, 71.96, 71.70, 70.36, 68.00, 52.55, 26.95. MS (FAB, *m/z*) 632 (MH^+, 49). Anal. calcd for $C_{30}H_{41}N_5O_{10}\cdot 3H_2O$: C,52.55; H,6.91; N, 10.21. Found: C, 52.80; H, 6.14; N, 10.22

3.3. Transport rate measurements

The transport experiments were performed at room temperature following a known procedure[11] in a U-tube (9 mm, i.d.). The membrane phase (3 mL of chloroform, Uvasol, Merck), in which the carrier is dissolved (7×10^{-4} mol L^{-1}), lay below and bridged the two aqueous phases. The first aqueous phase (1 mL) contains 5×10^{-5} mol L^{-1} of LiOH, 10^{-1} mol L^{-1} of alkali or ammonium nitrate, and 2×10^{-3} mol L^{-1} of the corresponding alkali or ammonium picrate. The second aqueous phase contains 1 mL of deionized water. The membrane phase is slowly and constantly stirred by a magnetic bar. The picrate concentration in the second aqueous phase, monitored spectroscopically by UV (λ=355 nm), was confirmed to increase linearly with running time (<12 h) and the initial transport rates were calculated. In each case, a similar experiment was carried out in the absence of carrier. The values indicated in Table 3 were estimated from the differences in the transport rates of carrier-containing systems and blank systems (no carrier present). Dibenzo[18]crown-6 was taken as reference ligand, and it showed the following transport rates (μM h^{-1}): Na^+ 22.5; K^+ 198.2; Ca^{2+} 2.4; NH_4^+ 129.0.

3.4. Molecular modelling

Molecular modelling studies were carried out using the AMBER[19] method implemented in the Hyperchem package.[20] When available, the parameters were extracted from the literature.[21] All others were developed following Kollman[22] and Hopfinger[23] procedures. In order to develop suitable parameters for N–H···N hydrogen bonding, ab initio calculations at STO-3G[24] level were used for calculating atomic charges compatible with the AMBER field ones, as Urban et al.[25] claimed that they gave excellent results for the study of ammonium interactions. In all cases, geometries were minimized to a maximun energy gradient of 0.1 kcal/mol using the Polak–Ribiere algorithm, and simulated annealing procedure was used to cover all conformational space running molecular dynamics at 400 K. To optimize host–guests interactions the cation was moved and/or rotated and docked into the cavity of the ligand in all possible positions and then minimized the energy of the complex with no restraints.

Ions are separated far away and well solvated in water. Consequently, there is no need to use counterions.[26] In the absence of explicit solvent molecules, a distance dependent dielectric factor qualitatively simulates the water saturated chloroform environment, as it takes into account the fact that intermolecular electrostatic interactions should die off fast with distance, as the water environment has a high dielectric constant.[27]

Energy of complexation was calculated for each compound, after minimization of the selected trajectory frames, by simulating a complexation process in chloroform saturated with water molecules. It is known that complexes ligand-cation-picrate always include water molecules until solvent saturation is achieved. It has been shown that results obtained in that case follow the same trend that those found from a pure water environment.[15,28] Therefore, the following relation was applied:

$$E_{\text{complexation}} = E_{\text{complex}} - (E_{\text{ammonium}} - E_{\text{ligand}})$$

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Stereoselective electrogeneration of (*E*)-4-alkoxy-2-phenyl-5-chloro-2-oxazolines by cathodic reduction of *N*-(1-alkoxy-2,2,2-trichloroethyl)benzamides

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Abstract—The first method for the synthesis of the title compounds has been established. Quantitative reactions of benzamides with chloral hydrate provided chloralbenzamides which were efficiently converted to *N*-(1,2,2,2-tetrachloroethyl)amides by treatment with phosphorus pentachloride. These compounds reacted selectively with alcohols under mild conditions to give *N*-(1-alkoxy-2,2,2-trichloroethyl)benzamides in high yields which were stereoselectively transformed to (*E*)-4-alkoxy-2-aryl-5-chloro-2-oxazolines in fair to good yields by electrochemical reduction under constant cathodic potential in an aprotic medium.

1. Introduction

We have recently developed a new heterocyclization methodology based on the cathodic reduction of chloral derivatives. Chloral is an inexpensive, commercially available reagent whose versatile reactivity[1] allows the synthesis of a wide variety of cathodically active polychlorinated derivatives. Some of these compounds seem especially attractive for use as starting materials in electroorganic synthesis, particularly those that have a structural arrangement suitable to undergo a reductive electrochemical heterocyclization process. The key step of this type of transformation involves a migration of charge on the first electrogenerated chlorocarbanion intermediate to a remote heteroatom. In this way, the newly formed anionic intermediate contains two suitably placed centres with opposite nucleophilic–electrophilic activity capable of promoting a ring closure process by internal displacement of a chloride anion. This synthetic strategy was successfully applied for preparing previously unknown 4-amino-2-aryl-2-oxazolines[2,3] which gave access to novel 2-imidazolidinones, 1,3-oxazolidines and 1,3-thiazolidines.[4] 2-Oxazolines are in general compounds of great interest[5] since they are versatile synthetic intermediates.[5e–g] Moreover, their therapeutic potential[5c,6] and many other significant applications[5c] are also important factors in stimulating the research on the chemistry of these substances. Consequently, the synthesis of different classes of 2-oxazolines has received intense attention for many years. A recent upsurge in interest has also been observed.[7]

Given the high potentiality of this electrochemical methodology for expanding the classes of 2-oxazolines available, we attempted the synthesis of the hitherto unknown 4-alkoxy-5-chloro-2-phenyl-2-oxazolines, as is shown in Scheme 1.

2. Results and discussion

Chloralamides **2** were prepared[8] in almost quantitative yields by reaction of chloral hydrate with benzamides **1**. In order to prepare *N*-(1-alkoxy-2,2,2-trichloroethyl)benzamides **4** compounds **2** were firstly converted to *N*-(1,2,2,2-tetrachloroethyl)benzamides **3** in high yields by reaction with phosphorus pentachloride, as described previously.[8b,9] It was found that products **3** undergo selective monoalkoxylation by simple treatment with a mixture of the corresponding alcohol and triethyl amine in a 1:1 ratio to give the targeted intermediates **4** in good to quantitative yields.

Cathodic reductions of compounds **4** in an aprotic medium (acetonitrile–tetrabutylammonium perchlorate) at a mercury pool cathode were carried out under a constant

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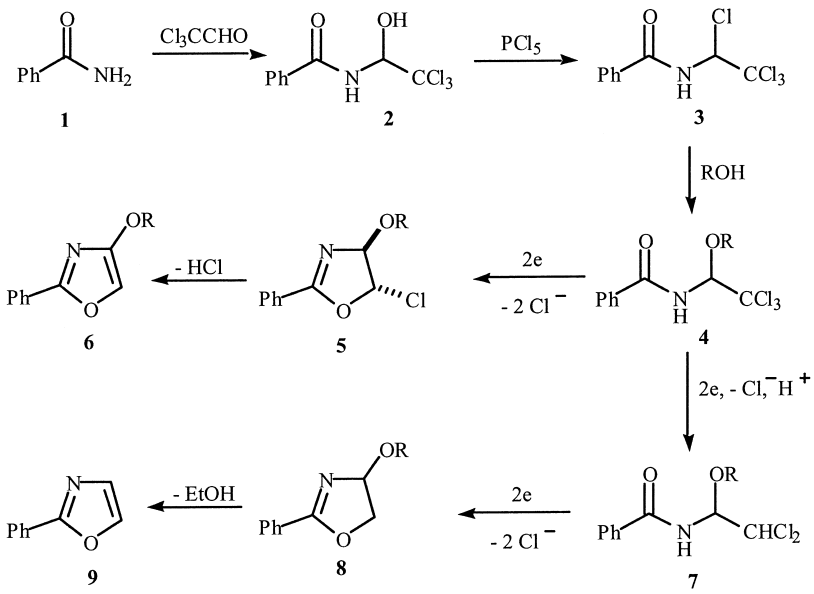


Entry	R	**4** (%)	**5** (%)
a	CH_3CH_2	96	65
b	CH_3	98	79
c	$C_6H_5CH_2CH_2$	65	82
d	$3,4,5\text{-}(CH_3O)_3C_6H_2CH_2$	75	66
e	$4\text{-}CH_3OC_6H_4CH_2$	73	64

Scheme 1.

potential of −1.50 V vs SCE. The electricity consumption was 2 F/mol of **4** in all cases. After electrolyses, the catholyte solutions were checked by TLC and GC, showing a total transformation of starting materials to single products, which were easily isolated and purified. These were oily compounds that were identified by IR, MS, NMR spectroscopy and elemental analysis as (*E*)-4-alkoxy-2-aryl-5-chloro-2-oxazolines **5** which pertain to a new family of 2-oxazoline compounds. Yields ranged from fair to good.

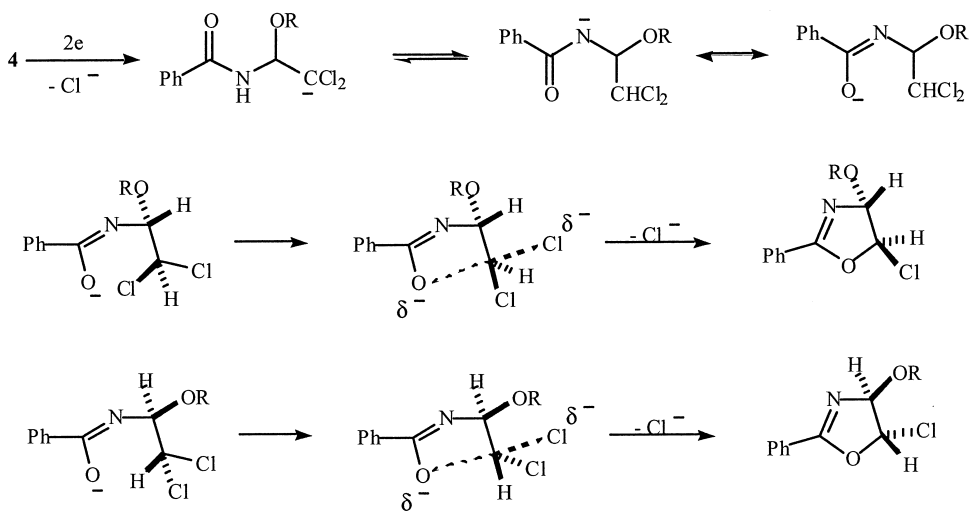
Spectroscopic analyses of products **5** were corroborated by treatment of **5b** with potassium *tert*-butoxide. It caused a total conversion to 2-phenyl-4-methoxyoxazol **6b**, which was conclusively identified by comparison with an authentic sample.[10]

On the other hand, it was found that electrogeneration of products **5** occurs with stereoselectivity towards the formation of (*E*)-isomers. This configurational assignment is firmly supported by categorical ^{1}H NMR studies[11,12] on stereochemistry of substituted five-membered cyclic compounds from which it has been established as a general rule that the arrangement of vicinal protons corresponds to a (*E*)-configuration when they show spin coupling constants of $J<5$ Hz, whereas a (*Z*)-configuration always shows coupling constants with $J>5$ Hz, and ~8 Hz is the value most frequently found. This method leads to the conclusion that the stereochemistry of products **5** corresponds to a (*E*)-configuration since the coupling constants between H-4 and H-5 protons are remarkably small, with *J* values ranging from 1.5 to 1.6 Hz in all cases.

The results of the cathodic reductions of *N*-(1-alkoxy-2,2,2-trichloroethyl)benzamides **4**, along with our previous reports[2,3] on studying electroreductions of *N*-(1-amino-2,2-dichloroethyl)benzamides, provide important insights into the high potential of this heterocyclization methodology, which appears specially suitable to accomplish the synthesis of complex 2-oxazolines.

The formation of oxazolines **5** (Scheme 2) can be reasonably explained on the basis of a two-electron selective cathodic cleavage of one carbon–chlorine bond with generation of a dichlorocarbanionic intermediate which would generate an amide anion. Therefore, the cyclization to yield racemic products **5** would occur by nucleophilic displacement of one of the remaining chlorine atoms. A transition state involving minimal steric interactions between the stationary chlorine atom and a vicinal alkoxyl group would participate.

Given the success in preparing the oxazolines **5**, we also attempted the synthesis of 4-alkoxy-2-phenyl-2-oxazolines **8** by applying a similar methodology. *N*-(1-Ethoxy-2,2-trichloroethyl)benzamide **7a** was used as model compound for this study. It was prepared by cathodic reduction of **4a** in a protic medium. The electrochemical reduction of **7a** was carried out under a similar experimental conditions to those used in the electrolyses of compounds **4**. A considerably more negative potential (−1.90 V versus SCE), however, must be applied in this case. In contrast to that observed with compounds **4**, the passing current did not decrease spontaneously when the charge consumption reached 2 F/mol.



Scheme 2.

At values close to 3 F/mol, the catholyte showed a high basicity; the electrolysis was then stopped and the reaction products present in the catholyte were analyzed by GC/MS. A complex mixture of compounds with 2-phenyloxazol **9** (24%) and 4-ethoxy-2-penyl-2-oxazoline **8a** (17%) as main components was detected. The identity of **9** was corroborated by comparison with an authentic specimen.[13] The progressive conversion of **8a** to **9** in the basic catholyte medium was observed, and even occurred without electricity passing. In the case of shorter electrolyses (1.9 F/mol), a remarkable variety of reaction products was also observed. These adverse results are attributable to the highly negative cathodic potential operating, which causes indiscrimination in electrode process and basicity in the solvent–electrolyte system.

To conclude, a convenient method for the synthesis of (*E*)-4-alkoxy-2-aryl-5-chloro-2-oxazolines, a previously unattainable class of compounds, is reported. Versatility, good yields, easy availability of starting materials, mildness and simple experimental procedure are noteworthy advantages of this approach. However, this method has been found to be of no use in the synthesis of 4-alkoxy-2-aryl-2-oxazolines.

3. Experimental

3.1. General

NMR spectra were determined on Bruker AC-200 or Varian Unity 300 Unity instruments with tetramethylsilane as internal reference. Electron-impact mass spectra were obtained on Hewlett–Packard 5995 and Autospect 5000 VG spectrometers under an ionizing voltage of 70 eV. IR spectra (nujol emulsions) were recorded on a Nicolet Impact 400 spectrophotometer. Microanalyses were performed on a Carlo Erba EA-1108 analyzer. Melting points were determined on a Kofler hot-plate melting point apparatus, and are uncorrected. Electrochemical experiments were performed with an Amel 557 potentiostat coupled to an Amel 558 integrator. Chloralamides **2** and *N*-(1,2,2,2-tetrachloroethyl)benzamides **3** were prepared as described previously.[8b,9]

3.2. Preparation of *N*-(1-alkoxy-2,2,2-trichloroethyl)-benzamides (4)

General procedure. A solution of the corresponding alcohol (5.25 mmol) and triethylamine (5.25 mmol) in dry acetone (6 mL) was added dropwise at room temperature to a stirred solution of the appropriate *N*-(1,2,2,2-tetrachloroethyl)-amide **3** (5.25 mmol) in dry acetone (9 mL) and the stirring was continued for 10 h. The white solid precipitate formed was removed by filtration and the solvent was evaporated under reduced pressure leaving a residue which was washed with cold petroleum ether. The resulting solid product was crystallized in the appropriate solvent.

Preparation of **4a**. *N*-(1,2,2,2-Tetrachloroethyl)benzamide **3a** (7 mmol) was added to dry ethanol (30 mL) and the stirred solution was heated at 40 °C for 1 h. After cooling the crystalline product was isolated by filtration.

3.2.1. *N*-(2,2,2-Trichloro-1-ethoxyethyl)benzamide (4a). 96%. Colourless needles, mp 148 °C (ethanol). (Found: C, 44.64; H, 4.14; N, 4.77. $C_{11}H_{12}Cl_3NO_2$ requires: C, 44.55; H, 4.08; N, 4.72); ^{1}H NMR δ ($CDCl_3$, 200 MHz): 1.31 (t, 3H, J=7.2 Hz), 3.79–3.94 (m, 2H), 5.90 (d, 1H, J=9.8 Hz), 6.75 (d, 1H, J=9.8 Hz), 7.26–7.58 (m, 3H), 7.81–7.86 (m, 2H); ^{13}C NMR δ ($CDCl_3$, 50.4 MHz): 14.91 (CH_3), 66.54 (CH_2), 86.39 (CH), 99.66 (CCl_3), 127.28 (CH), 128.94 (CH), 132.61 (CH), 133.05 (C), 167.46 (CO); MS, *m/z* (%): 216 (29), 178 (17), 105 (100), 77 (91), 51 (36); IR (Nujol): 3306, 1645, 1516, 1462, 1340, 1272, 1108, 915, 821, 791, 720 cm^{-1}.

3.2.2. *N*-(2,2,2-Trichloro-1-methoxyethyl)benzamide (4b). 98%. Colourless needles, mp 99–102 °C (pet ether). (Found: C, 42.48; H, 3.62; N, 5.01. $C_{10}H_{10}Cl_3NO_2$ requires: C, 42.51; H, 3.57; N, 4.96); ^{1}H NMR δ ($CDCl_3$, 200 MHz): 3.63 (s, 3H), 5.82 (d, 1H, J=10.0 Hz), 6.75 (d, 1H, J=10.0 Hz), 7.45–7.59 (m, 3H), 7.82–7.87 (m, 2H); ^{13}C NMR δ ($CDCl_3$, 50.4 MHz): 58.01 (CH_3), 87.89 (CH), 99.22 (CCl_3), 127.29 (CH), 128.95 (CH), 132.69 (CH), 132.85 (C), 167.71 (CO); MS, *m/z* (%): 283 (M^++2, 4), 281 (M^+, 5), 246 (11), 215 (51), 164 (82), 126 (21), 105 (100), 77 (89); IR (Nujol): 3266, 1648, 1523, 1461, 1377, 1103, 796 cm^{-1}.

3.2.3. *N*-[2,2,2-Trichloro-1-(2-phenylethoxy)ethyl]benzamide (4c). 65%. Colourless needles, mp 97 °C (pet ether). (Found: C, 54.89; H, 4.29; N, 3.71. $C_{17}H_{16}Cl_3NO_2$ requires: C, 54.79; H, 4.33; N, 3.76); ^{1}H NMR δ ($CDCl_3$, 200 MHz): 2.96 (t, 2H, J=6.9 Hz), 3.95–4.05 (m, 2H), 5.90 (d, 1H, J=9.8 Hz), 6.53 (d, 1H, J=9.8 Hz), 7.23–7.25 (m, 5H), 7.42–7.57 (m, 3H), 7.68–7.73 (m, 2H); ^{13}C NMR δ ($CDCl_3$, 50.4 MHz): 35.97 (CH_2), 71.33 (CH_2), 86.53 (CH), 99.45 (CCl_3), 126.51 (CH), 127.28 (CH), 128.44 (CH), 128.87 (CH), 129.18 (CH), 132.61 (C), 132.89 (CH), 138.12 (C), 167.49 (CO); MS, *m/z* (%): 371 (M^+0.2), 336 (0.4), 250 (2), 216 (8), 105 (100), 91 (25), 77 (93); IR (Nujol): 3285, 1648, 1528, 1464, 1379, 1277, 1098, 1081, 813, 699 cm^{-1}.

3.2.4. *N*-[2,2,2-Trichloro-1-(3,4,5-trimethoxybenzyloxy)ethyl]benzamide (4d). 75%. Colourless needles, mp 102–103 °C (dichloromethane–hexane). (Found: C, 50.66; H, 5.08; N, 3.26. $C_{19}H_{20}Cl_3NO_5$ requires: C, 50.86; H, 4.49; N, 3.12); ^{1}H NMR δ ($CDCl_3$, 300 MHz): 3.79 (s, 3H), 3.81 (s, 6H), 4.75 (s, 2H), 6.00 (d, 1H, J=9.6 Hz), 6.64 (s, 2H), 6.75 (d, 1H, J=9.6 Hz), 7.44–7.58 (m, 3H), 7.77 (d, 2H, J=6.0 Hz); ^{13}C NMR δ ($CDCl_3$, 75.4 MHz): 56.02 (CH_3O), 60.75 (CH_3O), 72.31 (CH_2), 85.80 (CH), 99.38 (CCl_3), 105.36 (CH), 127.15 (CH), 128.83 (CH), 131.69 (C), 132.61 (CH), 132.74 (C), 137.83 (C), 153.20 (C), 167.37 (CO); MS, *m/z* (%): 447 (M^+, 27), 216 (100), 196 (36), 181 (34), 169 (30), 148 (18), 138 (21), 105 (34), 77 (75); IR (Nujol): 3276, 1681, 1597, 1530, 1465, 1379, 1332, 1270, 1239, 1133, 1085, 1011, 815, 697 cm^{-1}.

3.2.5. *N*-[2,2,2-Trichloro-1-(4-methoxybenzyloxy)ethyl]benzamide (4e). 73%. Colourless needles, mp 105–107 °C (hexane). (Found: C, 52.60; H, 4.09; N, 3.52. $C_{17}H_{16}Cl_3NO_3$ requires: C, 52.53; H, 4.15; N, 3.60); ^{1}H NMR δ ($CDCl_3$, 200 MHz): 3.78 (s, 3H), 4.76 (d, 2H, J=6.4 Hz), 5.95 (d, 1H, J=9.8 Hz), 6.73 (d, 1H, J=9.8 Hz), 6.87 (d, 2H, J=8.6 Hz), 7.34 (d, 2H, J=8.6 Hz), 7.44–7.58 (m, 3H), 7.80 (dd, 2H, J=6.7, 1.2 Hz); ^{13}C NMR δ ($CDCl_3$, 50.4 MHz): 55.30 (CH_3O), 71.93 (CH_2), 85.57 (CH), 99.54 (CCl_3), 113.98 (CH), 127.27 (CH), 128.17 (C), 128.90 (CH), 130.20 (CH), 132.60 (CH), 133.01 (C), 159.78 (C), 167.46 (CO); MS, *m/z* (%): 387 (M^+, 2), 252 (2), 233 (3), 216 (74), 180 (10), 137 (58), 121 (78), 105 (100), 77 (62); IR (Nujol): 3328, 1657, 1520, 1461, 1349, 1258, 1097, 1056, 1034, 998, 808, 716 cm^{-1}.

3.3. Preparation of (*E*)-4-alkoxy-5-chloro-2-phenyl-2-oxazolines (5)

Preparative electrolyses were carried out under a constant cathodic potential in a concentric cylindrical cell with two compartments separated by a circular glass frit (medium) diaphragm. A mercury pool (diameter 5 cm) was used as the cathode and a platinum plate as the anode. The catholyte was magnetically stirred. The temperature was kept at approximately 18 °C by external cooling. The reductions were performed in anhydrous MeCN–Bu_4NClO_4, 0.4 M, of which approximately 35 mL and 15 mL were placed, respectively, in the cathodic and the anodic compartments. Anhydrous sodium carbonate (3 g) was placed in the anode compartment to prevent accumulation of electrogenerated acid. Solutions of compounds **4** (5 mmol) were electrolyzed under a cathodic potential of −1.50 V vs SCE. All electrolysis products were isolated by removing the solvent in vacuo. The residue was then shaken with ether (3×50 mL) over a period of 30 min. The ethereal solutions were combined and concentrated leaving oily crude products that were purified by column chromatography on silica gel (entries a, b, d, e: ethyl acetate or ethyl acetate–hexane 1:3; entry c: petroleum ether–diethyl ether 4:1). The isolated products were viscous oils that gave satisfactory elemental and spectroscopic analyses.

3.3.1. (*E*)-5-Chloro-4-ethoxy-2-phenyl-2-oxazoline (5a). 65%. Pale yellow oil. (Found: C 58.40; H 5.41; N 6.18; $C_{11}H_{12}ClNO_2$ requires C 58.55; H 5.36; N 6.21); ^{1}H NMR δ ($CDCl_3$, 300 MHz): 1.27 (t, 3H, J=7.2 Hz), 3.71–3.77 (m, 1H), 3.93–3.98 (m, 1H), 5.61 (d, 1H, J=1.5 Hz), 6.16 (d, 1H, J=1.5 Hz), 7.44–7.60 (m, 3H), 8.06–8.08 (m, 2H); ^{13}C NMR δ ($CDCl_3$, 75.4 MHz): 15.23 (CH_3), 64.91 (CH_2), 92.44 (CH), 104.91 (CH), 125.77 (C), 128.71 (CH), 129.33 (CH), 133.17 (CH), 165.92 (C=N); MS, *m/z* (%): 225 (M^+, 3), 190 (4), 180 (8), 161 (58), 152 (10), 132 (10), 117 (25), 104 (100), 90 (14), 77 (51), 51 (30); IR (film): 2982, 2933, 1656, 1449, 1337, 1252, 1103, 1032, 979, 895, 749, 693 cm^{-1}.

3.3.2. (*E*)-5-Chloro-2-phenyl-4-methoxy-2-oxazoline (5b). 79%. Pale yellow oil. (Found: C 56.80; H 4.70; N 6.56; $C_{10}H_{10}ClNO_2$ requires C 56.75; H 4.76; N 6.62); ^{1}H NMR δ ($CDCl_3$, 200 MHz): 3.55 (s, 3H), 5.52 (d, 1H, J=1.6 Hz), 6.14 (d, 1H, J=1.6 Hz), 7.40–7.55 (m, 3H), 8.00–8.06 (m, 2H); ^{13}C NMR δ ($CDCl_3$, 50.4 MHz): 56.19 (CH_3O), 91.96 (CH), 106.51 (CH), 126.01 (C), 128.57 (CH), 129.06 (CH), 132.83 (CH), 165.67 (C=N); MS, *m/z* (%): 211 (M^+, 4), 176 (8), 147 (75), 117 (11), 104 (100), 90 (10), 77 (39); IR (film): 2942, 2836, 1659, 1651, 1452, 1337, 1105, 1063, 1033, 981, 968, 849, 746 cm^{-1}.

3.3.3. (*E*)-5-Chloro-2-phenyl-4-(2-phenylethoxy)-2-oxazoline (5c). 82%. Pale yellow oil. (Found: C 67.77; H 5.35; N 4.60; $C_{17}H_{16}ClNO_2$ requires C 67.66; H 5.34; N 4.64); ^{1}H NMR δ ($CDCl_3$, 300 MHz): 2.95 (t, 2H, J=7.2 Hz), 3.85–3.90 (m, 1H), 4.05–4.10 (m, 1H), 5.59 (d, 1H, J=1.5 Hz), 6.08 (d, 1H, J=1.5 Hz), 7.21–7.47 (m, 8H), 8.00–8.04 (m, 2H); ^{13}C NMR δ ($CDCl_3$, 75.4 MHz): 36.34 (CH_2), 69.80 (CH_2), 92.25 (CH), 105.82 (CH), 126.48 (CH), 128.52 (CH), 128.65 (CH), 129.00 (CH), 129.11 (CH), 132.81 (CH), 165.57 (C=N); MS, *m/z* (%): 301 (M^+, 6), 266 (7), 197 (4), 180 (14), 152 (21), 104 (100), 91 (24), 77 (32); IR (film): 3029, 2925, 2870, 1725, 1660, 1498, 1453, 1253, 1099, 1031, 972, 750, 701 cm^{-1}.

3.3.4. (*E*)-5-Chloro-2-phenyl-4-(3,4,5-trimethoxybenzyloxy)-2-oxazoline (5d). 66%. Pale yellow oil. (Found: C 60.29; H 5.40; N 3.63; $C_{19}H_{20}ClNO_5$ requires C 60.40; H 5.34; N 3.71); ^{1}H NMR δ ($CDCl_3$, 300 MHz): 3.64 (s, 3H), 3.66 (s, 6H), 4.46 (d, 1H, J=11.7 Hz), 4.65 (d, 1H, J=11.7 Hz), 5.50 (d, 1H, J=1.5 Hz), 5.99 (d, 1H, J=1.5 Hz), 6.42 (s, 2H), 7.23–7.36 (m, 3H), 7.85 (d, 2H, J=7.2 Hz); ^{13}C NMR δ ($CDCl_3$, 75.4 MHz): 56.08 (CH_3O), 60.81 (CH_3O), 70.97 (CH_2), 92.21 (CH), 105.13 (CH), 126.14 (CH), 128.60 (CH), 129.02 (CH), 130.37 (C), 132.57 (C), 132.83 (CH), 137.76 (C), 153.36 (C), 165.77 (C=N); MS, *m/z* (%): 379 (M^++2, 23), 377 (M^+, 35), 342 (7), 196 (40), 181 (100), 167 (14), 146 (42), 104 (34), 77 (29); IR

(film): 2946, 1738, 1650, 1594, 1506, 1463, 1423, 1336, 1239, 1130, 1083, 1008, 848 cm^{-1}.

3.3.5. (*E*)-5-Chloro-2-phenyl-4-(4-methoxybenzyloxy)-2-oxazoline (5e). 64%. Pale yellow oil. (Found: C 64.13; H 5.11; N 4.34; $C_{17}H_{16}ClNO_3$ requires C 64.26; H 5.08; N 4.41); ^1H NMR δ ($CDCl_3$, 200 MHz): 3.78 (s, 3H), 4.63 (d, 1H, J=11.2 Hz), 4.84 (d, 1H, J=11.2 Hz), 5.66 (d, 1H, J=1.5 Hz), 6.13 (d, 1H, J=1.5 Hz), 6.88 (d, 2H, J=8.6 Hz), 7.31 (d, 2H, J=8.6 Hz), 7.44–7.55 (m, 3H), 8.03 (dd, 2H, J=8.0, 1.2 Hz); ^{13}C NMR δ ($CDCl_3$, 50.4 MHz): 55.33 (CH_3O), 70.49 (CH_2), 92.38 (CH), 104.76 (CH), 114.02 (CH), 126.31 (C), 128.61 (CH), 129.09 (CH), 129.92 (CH), 132.77 (CH), 159.62 (C), 165.67 (C=N); MS, *m/z* (%): 318 (M^++1, 2), 152 (8), 146 (100), 121 (67), 104 (31), 91 (15), 77 (35), 63 (9); IR (film): 2939, 1719, 1651, 1612, 1513, 1451, 1339, 1253, 1178, 1084, 1033, 971, 823, 750 cm^{-1}.

3.3.6. Preparation of *N*-(2,2-dichloro-1-ethoxyethyl)benzamide (7a). Product **7a** was electrochemically prepared by cathodic reduction by using a divided cell as described above. The electrolysis of compound **4a** (3 mmol) was carried out under a constant cathodic potential of −1.60 V vs SCE in DMF–$LiClO_4$ 0.4 M. Acetic acid (3 mmol) was used as proton donor. The electricity consumption was 2 F/mol. The electrolysis product was isolated by dropping the catholyte solution onto ice–water (200 mL). The white solid precipitated was air-dried and crystallized from petroleum ether.

80%. White needles, mp 98–99 °C (pet ether). (Found: C, 50.58; H, 4.93; N, 5.28. $C_{11}H_{13}Cl_2NO_2$ requires: C, 50.40; H, 5.00; N, 5.34); ^1H NMR δ ($CDCl_3$, 200 MHz): 1.26 (t, 3H, J=7.0 Hz), 3.71–3.82 (m, 2H), 5.78 (dd, 1H, J=9.5, 2.4 Hz), 5.89 (d, 1H, J=2.4 Hz), 6.85 (d, 1H, J=9.5 Hz), 7.44–7.57 (m, 3H), 7.84 (d, 2H, J=6.8 Hz); ^{13}C NMR δ ($CDCl_3$, 50.4 MHz): 14.95 (CH_3), 65.23 (CH_2), 72.67 ($CHCl_2$), 81.54 (CH), 127.24 (CH), 128.83 (CH), 132.42 (CH), 133.16 (C), 167.59 (CO); MS, *m/z* (%): 224 (4), 218 (14), 216 (22), 178 (11), 105 (100), 77 (58), 51 (18); IR (Nujol): 3211, 1635, 1524, 1466, 1379, 1085, 791, 696 cm^{-1}.

3.3.7. Reductive electrolysis of *N*-(2,2-dichloro-1-ethoxyethyl)benzamide (7a). Cathodic reductions of **7a** were performed in a divided cell as described above and were carried out under a constant cathodic potential of −1.90 V vs SCE in acetonitrile–tetrabutylammonium perchlorate 0.5 M. The electrolysis products were isolated by removing the solvent in vacuo. The residue was then shaken with ether (3×50 mL) over a period of 30 min. The ethereal solutions were combined and concentrated. These experiments revealed no synthetic utility since the formation of complex mixtures of products was detected by GC/MS. For a 3 F/mol electrolysis, 2-phenyloxazol **9** (24%) and 4-ethoxy-2-penyl-2-oxazoline **8a** (17%) could be identified among other non characterised products.

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$LiClO_4$-Activated stereo- and regioselective alkylation of aldehydes

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Abstract—Aldehydes undergo an unusual and very mild alkylation by $LiClO_4$-activation in the presence of acids. This new methodology enables the inclusion of a broad range of aldehydes as well as tertiary alcohols. Regio- and stereoselectivity observed during this reaction will be discussed.

1. Introduction

C–C bond formation processes are of great interest, in particular regio- and stereoselective transformations. The catalytic execution of these reactions are the focus of attention. The simple alkylation of carbonyl compounds mediated by $LiClO_4$ is not described so far.

Recently we described an unusual addition of aldehydes to tertiary titanium(IV)-alkoxides in the presence of α-hydroxy acids by activation of $LiClO_4$.[1] During our ongoing studies in this field we were able to obtain suitable crystals for X-ray structure analysis of compounds **1d**, **2b** and **4g** (Table 1).[2] Careful comparison of NMR data of these compounds (chemical shifts, coupling constants and NOE-experiments) with the NMR data of other compounds obtained during this work, resulted in a revision of the proposed structure to surprisingly appear as substituted tetrahydropyranols **1**–**4** (Table 1) in contrast to the earlier proposals (*syn*- and *anti*-triols **1a**–**c** and **2a**–**c** in Ref. 1). In addition, the previously described diols **5**[1] were also observed.

2. Results and discussion

In order to explore the scope and limitation of substrates in these reactions, a series of tertiary titanium(IV)-alkoxides (**Tia**–**h**, entries 1–8 in Table 1) were reacted with benzaldehyde using standard conditions (10 equiv. of aldehyde, 10 equiv. dry $LiClO_4$, 1 equiv. tartaric acid, 1 equiv. titanium(IV)-alkoxide). The distribution of products observed in this reaction is given in Table 1.

A high regioselectivity is observed during this reaction by using unsymmetrical substituted tertiary titanium(IV)-alkoxides. The attack of the aldehyde takes place at the highest substituted β-carbon atom of the titanium(IV)-alkoxide used (entries 2, 3 and 6, Table 1). One exception represents the formation of tetrahydropyranols **2e** and **4e** and diol **5e** (entry 5, Table 1). This product arose from an alkylation at the methyl group instead at the expected benzylic carbon atom.

The substituents at C-2 and C-6 are *syn*-configured (diequatorial) in every tetrahydropyranol isolated. They differ only in the configuration at C-4, the tertiary carbon atom. The observed rigid *syn*-configuration of the substituents and the high regioselectivity of the attack of the aldehydes are the sources for the formation of *meso*-configurated tetrahydropyranols during this transformation.

In the reactions of aldehydes with titanium(IV)-alkoxides bearing three or two equivalent substituents the attack of the aldehydes may occur at two equivalent α-carbon atoms. *meso*-Configurated compounds can be formed. This is true for substituted tetrahydropyranols in the a, c, g, and i series (entries 1, 3 (**1c**, **2c**), 7, 9, 11 (**1c**, **2c**), 13 and 15 in Table 1). Diols **5a**, **5b**, **5e** and **5g** were isolated with a high degree of *anti*-selectivity. *syn*-Configurated diols could not be detected.

During our studies we observed this described reaction even when using tertiary alcohols instead of the corresponding tertiary titanium(IV)-alkoxides (**Ha**–**h**, entries 9–15 in Table 1). A comparison of product distribution of these two procedures is shown in Table 1 (entries 1–8 titanium(IV)-

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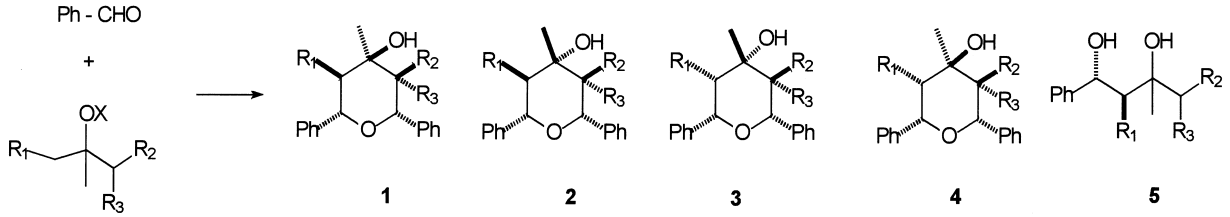
Table 1. Product distribution (in %) in reactions of benzaldehyde with titanium(IV)-alkoxides, respectively tertiary alcohols in the presence of 1 equiv. $LiClO_4$ and 10 mol% *p*-toluenesulfonate

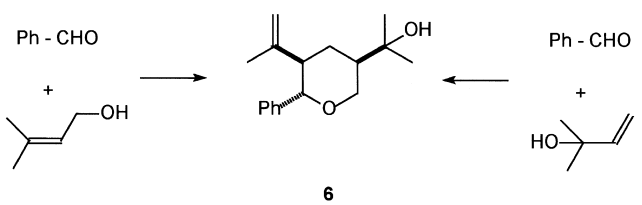


Entry	Compound	X	R_1	R_2	R_3	Overall-yield (%)	**1**	**2**	**3**	**4**	**5**
1	**a**	Ti	H	H	H	46	17	50	—	—	33
2	**b**	Ti	Me	H	H	55	32	17	17	17	17
3	**c**	Ti	Me	Me	H	77	21	32	42	5	—
4	**d**	Ti	H	Me	Me	76	83	17	—	—	—
5	**e**	Ti	H	Ph	H	52	—	8	—	32	60
6	**f**	Ti	*i*Pr	H	H	22	—	100	—	—	—
7	**g**	Ti		H	[a]	58	—	—	33	33	34
8	**h**	Ti	Cl	H	H	—	—	—	—	—	—
9	**a**	H	H	H	H	58	50	50	—	—	—
10	**b**	H	Me	H	H	62	17	66	17	—	—
11	**c**	H	Me	Me	H	61	40	10	20	30	—
12	**e**	H	H	Ph	H	48	—	33	—	67	—
13	**g**	H		H	[a]	53	—	—	40	60	—
14	**h**	H	Cl	H	H	38	—	100	—	—	—
15	**i**	H		H	[b]	46	—	—	100	—	—

[a] R_1=R_3: $-(CH_2)_3-$.
[b] R_1=R_3: $-(CH_2)_2-$.

alkoxides **Tia**–**h** and entries 9–15 tertiary alcohols **Ha**–**h**). Higher stereoselectivities were observed by using tertiary titanium(IV)-alkoxides in these reactions. α-Halogen substituted tertiary alcohols react with aldehydes to give the tetrahydropyranole **2h**. In contrast to that the corresponding titanium(IV)-alkoxide does not react with benzaldehyde under the described conditions (compare entries 8 and 14, Table 1).

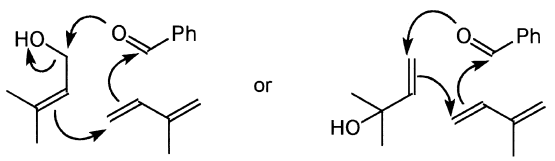
Allyl alcohols react with benzaldehyde under these standard conditions as well. 1-Hydroxy-3-methyl-2-butene and the tertiary alcohol 2-hydroxy-2-methyl-3-butene were transformed into the tetrahydropyranol **6** by a stereoselective and convergent synthesis (Scheme 1).



Scheme 1. *Reaction conditions*: tartaric acid, $LiClO_4$, rt.

There are two explanations for this unexpected result at that time. The formation of isoprene (the elimination product of the two isomeric allyl alcohols) and the subsequent alkylation lead to the product **6** (Scheme 2). Alternatively, a rearrangement of the two isomeric allyl alcohols during this transformation could explain these identical results.

The yields of the products obtained by using these standard conditions (10 equiv. of aldehyde, 10 equiv. dry $LiClO_4$, 1 equiv. tartaric acid, 1 equiv. titanium(IV)-alkoxide) are

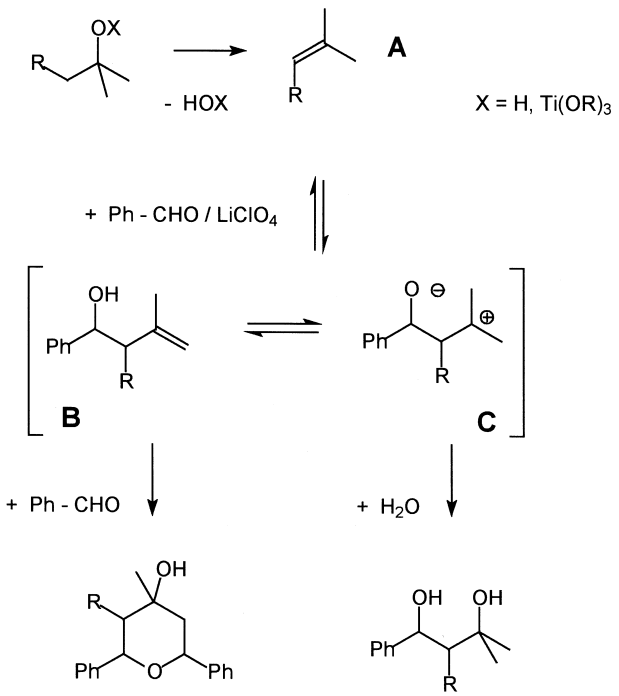


Scheme 2.

low (15–30%). Further investigations in this field led us to a more powerful and catalytic reagent system. Same regio- and stereoselectivities of products were found by using $LiClO_4$ in the presence of 10 mol% ethyl *p*-toluenesulfonate. Under these conditions the products of reaction of tertiary alcohols with aldehydes were isolated in good yields (50–70%, Table 1). It is assumed that under these reactions conditions hydrolysis occurs and toluenesulfonic acid acts as the real agent, as comparative reactions with toluenesulfonic acid showed.

Based on these results, an elimination–addition reaction mechanism can be assumed. This consideration involves an electrophilic attack by a $LiClO_4$-activated aldehyde onto an olefin formed intermediately by elimination from tertiary titanium(IV)-alkoxides or tertiary alcohols. The suggestion of such a *Prins*-type mechanism[7] was supported by the comparative reaction of isobutene (the elimination product of tertiary butanol) with benzaldehyde in the presence of $LiClO_4$ and α-hydroxy acids. The same compounds were isolated as in the corresponding reaction of benzaldehyde with $Ti(OtBu)_4$. A proposed reaction mechanism based on these results is shown in Scheme 3.

Two main products are possible. The one, which predominates, depends on the olefin and the reaction conditions.



Scheme 3. Proposed reaction mechanism.

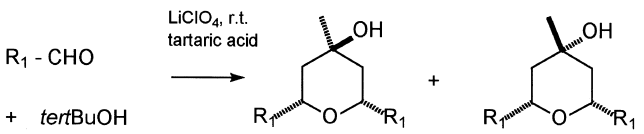
Zwitterionic structure **C** is starting point for a further alkylation of the olefin **B** resulting in the formation of the substituted tetrahydro pyranoles. Diols **5** were formed by hydration of structure **C**.

The real role of $LiClO_4$ is not clear up to now. This reaction is observed only in the presence of dry $LiClO_4$. No reactions were observed with the use of other metal salts and perchlorates (e.g., $NaClO_4$, $Mg(ClO_4)_2$, $Al(ClO_4)_3$, $KClO_4$, Et_4NClO_4). On the other hand $LiClO_4$ does not act as a dehydrating agent only. This alkylation is not observed in comparative experiments with other dehydrating reagents (molecular sieves, Drierite, Na_2SO_4 etc.). No reactions were observed by using $LiClO_4 \cdot 10H_2O$ in these reactions.

In order to demonstrate the broad applicability of this new and promising transformation, aliphatic aldehydes were reacted with *tert*-butanol and 1-methyl-cyclopentanol. The expected tetrahydropyranols **7**, **8** and **9** were isolated in lower yields in comparison with reactions of benzaldehyde. An overview of products observed during these reactions is given in Table 2 and Scheme 4.

Herein we described a very simple and effective alkylation of aldehydes by $LiClO_4$-activation. Nevertheless, there still are several questions remaining, e.g. the role of $LiClO_4$, the enantioselective execution of this reaction, or the extension of this reaction to secondary alcohols. In any event, we are convinced that this extremely mild and easy alkylation is a useful method for the preparation of tertiary alcohols.[8,9]

Table 2. Product distribution in reactions of aliphatic aldehydes with *tert*butanol



7 **8**

Entry	R_1	Compound	Overall-yield (%)	**7**	**8**
1	Me	**a**	36	—	100
2	Et	**b**	26	—	100
3	*n*Pr	**c**	35	50	50
4	*iso*Pr	**d**	36	—	100

3. Experimental

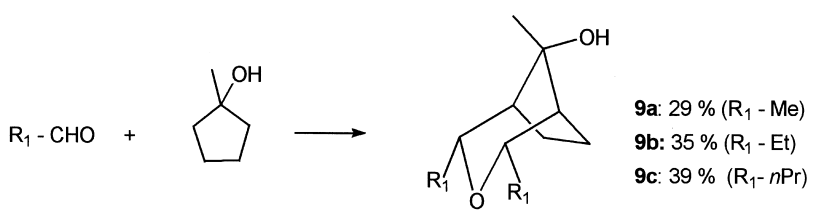
3.1. General procedures

All reactions were performed using oven-dried glassware under an atmosphere of dry argon. Toluene was distilled, dried and stored over molecular sieve (3A). $Ti(OiPr)_4$ purchased from Merck chemical company was used without prior purification. Aldehydes were distilled before use. Purification of products was accomplished using flash chromatography according to the method of Still.[10] $LiClO_4$ was dried at 120 °C in vacuo for 10 h.

^1H NMR and ^{13}C NMR spectra were recorded at 300 and 75 MHz in $CDCl_3$, respectively using a AC-300 spectrometer. Chemical shifts are given in ppm. Thin layer chromatography was performed out using Merck Silica Gel 60 F_{254} TLC plates.

The stereodescriptors a (axial) and e (equatorial) were used for the characterization of configuration. The description of configuration by CIP-rules is not sufficient for the characterization of the *meso*-configurated tetrahydro pyranols.

Yields are related to the amount of titanium(IV)-alkoxides or alcohols used and are not optimized.



Scheme 4. *Reaction conditions*: *p*-toluenesulfonate, $LiClO_4$, rt.

3.2. Preparation of titanium(IV)-alkoxides (Tia–Tih)

The titanium(IV)-alkoxides were prepared by a procedure given in Ref. 11. 100 mmol of the corresponding alcohol were dissolved under inert conditions in 50 ml of anhydrous toluene. 7.5 ml (25 mmol) of $Ti(OiPr)_4$ were carefully added at room temperature. The resulting solution was heated and *iso*propanol was removed by azeotropic distillation of toluene. Resulting residue was dried in vacuo at room temperature and was used without further purification. Using this procedure, the ^1H NMR spectra do not contain any typical signals of the starting $Ti(OiPr)_4$.

3.3. General procedure of alkylation

Procedure A. Alkylation of aldehydes with titanium(IV)-alkoxides (**Tia**–**Tih**): 1.1 g $LiClO_4$ (10 mmol) were dissolved in 1.0 ml benzaldehyde (10 mmol). 1.0 mmol of the corresponding titanium(IV)-alkoxide was added after 10 min stirring at rt. 200 mg ethyl *p*-toluenesulfonate (1 mmol) were added. The reactions were monitored by thin layer chromatography. At the end of the reaction the resulting mixtures were stirred for 24 h at rt and then extracted by diethylether and successively by saturated aq. NH_4Cl- and $NaHCO_3$-solution. The organic layers were separated, dried (Na_2SO_4) and the solvent was removed in vacuo. The residue was purified by column chromatography.

Procedure B. Alcohols (**Ha**–**Hi**): The same procedure as described for titanium(IV)-alkoxides was used with 4 mmol of the corresponding alcohol.

3.3.1. 4e-Methyl-2e,6e-diphenyl-tetrahydropyran-4a-ol (1a). 93 mg **1a** (8.7%) as a colourless oil (procedure A); IR (neat) ν_{max} 3498, 2908, 1454, 1379, 1211, 1137, 1050, 1012 cm^{-1}; ^1H NMR δ=7.41–7.12 (10H, m, CH_{Ph}), 4.88 (2H, dd, *J*=1.9, 11.7 Hz, H2, H6), 1.78 (2H, dd, *J*=1.9, 13.5 Hz, H3, H5), 1.70 (2H, dd, *J*=11.7, 13.5 Hz, H3, H5), 1.21 (3H, s, $-CH_3$); ^{13}C NMR δ=143.0, 128.3, 127.3, 125.9, (C_{Ph}), 75.3, 69.0, (C2, 4, 6), 46.6, (C3, 5), 31.6, ($-CH_3$); HRMS: *m*/*z* calcd for $C_{18}H_{20}O_2$ 268.1463. Found: 268.1463.

3.3.2. 4a-Methyl-2e,6e-diphenyl-tetrahydropyran-4e-ol (2a).[3] 225 mg **2a** (20.1%) as a colourless solid (procedure A); IR (neat) ν_{max} 3483, 2914, 1495, 1455, 1382, 1212, 1115, 1056, 986 cm^{-1}; ^1H NMR δ=7.41–7.18 (10H, m, CH_{Ph}), 4.53 (2H, dd, *J*=1.9, 11.6 Hz, H2, H6), 1.96 (2H, dd, *J*=1.9, 10.5 Hz, H3, H5), 1.70 (2H, dd, *J*=10.5, 11.6 Hz, H3, H5), 1.51 (3H, s, $-CH_3$); ^{13}C NMR δ=142.2, 128.4, 127.5, 125.9, (C_{Ph}), 77.5, 69.8, (C2, C4, C6), 48.2, (C3, C5), 25.8, ($-CH_3$); HRMS: *m*/*z* calcd for $C_{18}H_{20}O_2$ 268.1463. Found: 268.1463.

3.3.3. 3-Methyl-1-phenyl-butan-1,3-diol (5a).[4] 125 mg **5a** (17.3%) as a colourless oil (procedure A); ^1H NMR δ=7.50–7.23 (5H, m, CH_{Ph}), 5.09 (1H, dd, *J*=2.3, 11.3 Hz, H1), 1.99 (1H, dd, *J*=11.3, 14.7 Hz, H2), 1.70 (1H, dd, *J*=2.3, 14.7 Hz, H2), 1.47 (3H, s, $-CH_3$), 1.32 (3H, s, $-CH_3$); ^{13}C NMR δ=144.7, 128.5, 127.5, 125.7, (C_{Ph}), 72.3, 71.7, (C2, C4, C6), 50.4, (C3, C5), 31.9, 27.6 (2×$-CH_3$).

3.3.4. 3e,4e-Dimethyl-2e,6e-diphenyl-tetrahydropyran-4a-ol (1b). 200 mg **1b** (17.7%) as a colourless oil (procedure A); IR (neat) ν_{max} 3788, 3639, 1654, 1544, 1458, 1378, 1217, 1079 cm^{-1}; ^1H NMR δ=7.40–7.10 (10H, m, CH_{Ph}), 4.90 (1H, dd, *J*=2.6, 11.3 Hz, H2), 4.45 (1H, d, *J*=10.2 Hz, H6), 1.89 (1H, dd, *J*=2.6, 13.9 Hz, H5), 1.77 (1H, dd, *J*=11.3, 13.9 Hz, H5), 1.70 (1H, dq, *J*=10.17, 6.8 Hz, H3), 1.23 (3H, s, C(OH)CH_3), 0.68 (3H, d, *J*=6.8 Hz, $-CH_3$); ^{13}C NMR δ=142.8, 141.5, 128.2, 128.2, 128.1, 127.6, 127.6, 125.8, (CH_{Ph}), 81.7, 75.0, 70.4, (C2, C4, C6), 48.2, 45.1, (C3, C5), 29.0, 9.6, (2×$-CH_3$); HRMS: calcd for $C_{19}H_{22}O_2$: 282.1620. Found: 282.1620.

3.3.5. 3e,4a-Dimethyl-2e,6e-diphenyl-tetrahydropyran-4e-ol (2b). 100 mg **2b** (8.9%) as colourless crystals (procedure A); mp 102–103 °C (hexane/ethylacetate); IR (neat) ν_{max} 3413, 2973, 1718, 1603, 1495, 1450, 1381, 1271, 1210, 1100, 1069, 1021 cm^{-1}; ^1H NMR δ=7.40–7.10 (10H, m, CH_{Ph}), 4.56 (1H, dd, *J*=2.3, 10.7 Hz, H6), 4.12 (1H, d, *J*=10.2 Hz, H2), 1.98 (1H, dd, *J*=2.3, 12.8 Hz, H5), 1.83 (1H, dd, *J*=10.7, 12.8 Hz, H5), 1.78 (1H, dq, *J*=6.8, 10.2 Hz, H3), 1.50 (3H, s, $-CH_3$), 0.80 (3H, d, *J*=6.8 Hz, $-CH_3$); ^{13}C NMR δ=140.9, 140.7, 128.4, 128.3, 127.9, 127.5 127.4, 125.9, (CH_{Ph}), 83.7, 77.3, 71.7, (C2, C4, C6), 50.3, 47.8, (C3, C5), 20.7, 10.1, (2×$-CH_3$); HRMS: calcd for $C_{19}H_{22}O_2$ 282.1620. Found: 282.1620.

3.3.6. 3a,4a-Dimethyl-2e,6e-diphenyl-tetrahydropyran-4e-ol (3b). 110 mg **3b** (9.8%) as a colourless oil (procedure A); IR (neat) ν_{max} 3117, 3028, 2971, 2804, 1726, 1398, 1106, 1069 cm^{-1}; ^1H NMR δ=7.40–7.10 (10H, m, CH_{Ph}), 4.83 (1H, d, *J*=2.3 Hz, H2), 4.55 (1H, dd, *J*=3.0, 12.1 Hz, H6), 1.88 (1H, dq, *J*=2.3, 7.2 Hz, H3), 1.84 (1H, dd, *J*=12.1, 13.6 Hz, H5), 1.71 (1H, dd, *J*=3.0, 13.6 Hz, H5), 1.60 (3H, s, $-CH_3$), 0.72 (3H, d, *J*=7.2 Hz, $-CH_3$); ^{13}C NMR δ=142.5, 141.4, 128.3, 128.0, 127.4, 126.6, 125.7, 125.3, (CH_{Ph}), 78.7, 77.6, (C2, C4, C6), 45.5, 42.8, (C3, C5), 27.2, 7.5, (2×$-CH_3$); HRMS: calcd for $C_{19}H_{22}O_2$ 282.1620. Found: 282.1620.

3.3.7. 3a,4e-Dimethyl-2e,6e-diphenyl-tetrahydropyran-4a-ol (4b). 100 mg **4b** (8.8%) as a colourless oil (procedure A); IR (neat) ν_{max} 3416, 2975, 1702, 1603, 1495, 1450, 1379, 1142, 1096, 1056, 1018 cm^{-1}; ^1H NMR δ=7.50–7.20 (10H, m, CH_{Ph}), 5.37 (1H, d, *J*=2.3 Hz, H2), 4.96 (1H, dd, *J*=3.0, 11.3 Hz, H6), 1.90 (1H, dq, *J*=2.3, 7.2 Hz, H3), 1.80 (1H, dd, *J*=11.3, 13.9 Hz, H5), 1.70 (1H, dd, *J*=3.0, 13.9 Hz, H5), 1.40 (3H, s, $-CH_3$), 0.86 (3H, d, *J*=7.2 Hz, $-CH_3$); ^{13}C NMR δ=143.2, 142.0, 128.1, 127.8, 127.0, 126.2, 125.6, 125.4, (CH_{Ph}), 76.4, 75.3, 72.0, (C2, C4, C6), 44.9, 41.9, (C3, C5), 29.4, 9.7, (2×$-CH_3$); HRMS: calcd for $C_{19}H_{22}O_2$ 282.1620. Found: 282.1620.

3.3.8. *anti*-2,3-Dimethyl-1-phenyl-butan-1,3-diol (5b).[5] 80 mg **5b** (10.3%) as a colourless oil (procedure A); ^1H NMR δ=7.30–7.10 (5H, m, CH_{Ph}), 4.47 (1H, d, *J*=10.2 Hz, H1), 1.86 (1H, dq, *J*=6.8, 10.2 Hz, H2), 1.18 (6H, s, 2×$-CH_3$), 0.42 (3H, d, *J*=6.8 Hz, $-CH_3$); ^{13}C NMR δ=143.8, 128.3, 127.6, 125.6, (CH_{Ph}), 78.9, 75.0, (C1, C3) 48.7, (C2), 30.3, 14.2, (2×$-CH_3$); HRMS: calcd for $C_{12}H_{16}O$ (M$-H_2O$) 176.1201. Found: 176.1201 (M$-H_2O$).

3.3.9. 3e,4a,5e-Trimethyl-2e,6e-diphenyl-tetrahydropyran-4e-ol (2c). 300 mg **2c** (25.3%) colourless solid (procedure A); mp 160–162 °C (hexane/ethylacetate); IR (neat) ν_{max} 3478, 2974, 2882, 1495, 1453, 1381, 1312, 1208, 1069, 1026, 984 cm^{-1}; ^1H NMR δ=7.36–7.12 (10H, m, CH_{Ph}), 4.18 (2H, d, J=10.6 Hz, H2, H6), 1.88 (2H, dq, J=7.2, 10.6 Hz, H3, H5), 1.18 (3H, s, $-CH_3$), 0.64 (6H, d, J=7.2 Hz, $-CH_3$); ^{13}C NMR δ=141.0, 128.3, 127.8, 127.3, (CH_{Ph}), 83.4, 73.9, (C2, C4, C6), 49.2, (C3, C5), 14.7, 10.4, (3×$-CH_3$); HRMS: calcd for $C_{20}H_{24}O_2$ 296.17763. Found: 296.17763.

The isomers **1c**, **3c** and **4c** could not be separated by chromatography. The ratio of these isomers was determined by integration of important signals in the ^1H and ^{13}C NMR spectra.

3.3.10. 3e,3′a,4e-Trimethyl-2e,6e-diphenyl-tetrahydropyran-4a-ol (1d). 755 g **1d** (63.7%) as colourless crystals (procedure A); mp 129–130 °C (hexane/ethylacetate); IR (neat) ν_{max} 3505, 2976, 1686, 1454, 1363, 1287, 1201, 1144, 1119, 1091, 1058, 1039, 1007 cm^{-1}; ^1H NMR δ=7.39–7.11 (10H, m, CH_{Ph}), 4.91 (1H, dd, J=2.6, 12.1 Hz, H6), 4.89 (1H, s, H2), 1.95 (1H, dd, J=12.1, 13.9 Hz, H5), 1.64 (1H, dd, J=2.6, 13.9 Hz, H5), 1.14 (3H, s, $-CH_3$), 0.85 (3H, s, $-CH_3$), 0.75 (3H, s, $-CH_3$); ^{13}C NMR δ=143.2, 139.6, 128.3, 128.2, 127.2, 127.1, 126.9, 125.6, (CH_{Ph}), 82.0, 75.4, 73.7, (C2, C4, C6), 44.4, 40.8, (C3, C5), 25.6, 18.6, 17.9, (3×$-CH_3$); HRMS: calcd for $C_{20}H_{24}O_2$ 296.1776. Found: 296.1776.

3.3.11. 3e,3′a,4a-Trimethyl-2e,6e-diphenyl-tetrahydropyran-4e-ol (3d). 150 mg **3d** (12.7%) as a colourless oil (procedure A); IR (neat) ν_{max} 3484, 2975, 1720, 1495, 1453, 1385, 1102, 1081, 1066, 1036 cm^{-1}; ^1H NMR δ=7.43–7.14 (10H, m, CH_{Ph}), 4.65 (1H, dd, J=2.6, 12.1 Hz, H6), 4.50 (1H, s, H2), 2.05 (1H, dd, J=12.1, 13.6 Hz, H5), 1.74 (1H, dd, J=2.6, 13.6 Hz, H5), 1.52 (3H, s, $-CH_3$), 0.93 (3H, s, $-CH_3$), 0.78 (3H, s, $-CH_3$); ^{13}C NMR δ=142.5, 139.1, 128.3, 128.1, 127.4, 127.3, 127.2, 125.7, (CH_{Ph}), 84.1, 77.2, 73.5, (C2, C4, C6), 45.9, 41.8, (C3, C5), 23.3, 19.6, 16.0, (3×$-CH_3$); HRMS: calcd for $C_{20}H_{24}O_2$ 296.1776. Found: 296.1776.

3.3.12. 4e-Methyl-2e-3e,6e-triphenyl-tetrahydropyran-4a-ol (4e). 250 mg **4e** (18.2%) as a colourless oil (procedure A); IR (neat) ν_{max} 3479, 3032, 1719, 1492, 1453, 1116, 1059, 1022 cm^{-1}; ^1H NMR δ=7.43–7.01 (15H, m, CH_{Ph}), 5.17 (1H, d, J=10.6 Hz, H2), 5.12 (1H, dd, J=2.3, 11.3 Hz, H6), 2.88 (1H, d, J=10.6 Hz, H3), 2.05 (1H, dd, J=2.3, 13.6 Hz, H5), 1.88 (1H, dd, J=11.3, 13.6 Hz, H5), 1.03 (3H, s, $-CH_3$); ^{13}C NMR δ=142.8, 141.0, 137.5, 130.6, 130.5, 128.6, 128.3, 128.2, 127.3, 127.0, 126.5, 125.9, (CH_{Ph}), 79.6, 75.1, 70.4, (C2, C4, C6), 58.5, 47.3, (C3, C5), 29.9, ($-CH_3$); HRMS: calcd for $C_{24}H_{24}O_2$ 344.1776. Found: 344.1776.

The isomer **2e** could not be separated by column chromatography. The ratio of the isomers **2e** and **4e** was determined by integration of important signals in the ^1H and ^{13}C NMR spectra.

3.3.13. *syn*-3-Methyl-1,4-diphenyl-butan-1,3-diol (5e). 350 mg **5e** (34.2%) as a colourless oil (procedure A); IR (neat) ν_{max} 2917, 1712, 1602, 1494, 1452, 1103, 1067, 1028 cm^{-1}; ^1H NMR δ=7.40–7.10 (10H, m, CH_{Ph}), 4.88 (1H, dd, J=3.0, 10.9 Hz, H1), 2.72 (2H, d, J=13.7 Hz, H4), 1.75 (1H, dd, J=3.0, 13.2 Hz, H2), 1.69 (1H, dd, J=10.9, 13.2 Hz, H2), 1.18 (3H, s, $-CH_3$); ^{13}C NMR δ=142.9, 137.7, 130.6, 128.7, 128.5, 128.2, 127.3, 126.9, (CH_{Ph}), 75.1, 70.5, (C1, C3), 50.1, 45.1, (C2, C4), 29.1($-CH_3$); HRMS: calcd for $C_{17}H_{16}$ (M-2H_2O) 220.1252. Found: 220.1252 (M−2H_2O).

3.3.14. 3e-Isopropyl-4a-methyl-2e,6e-diphenyl-tetrahydropyran-4e-ol (2f). ^1H NMR δ=7.50–7.10 (10H, m, CH_{Ph}), 4.54 (1H, dd, J=2.6, 11.3 Hz, H6), 4.45 (1H, d, J=10.9 Hz, H2), 2.17 (1H, dqq, J=7.2, 7.2, 9.4 Hz, $-CHMe_2$), 1.91 (1H, dd, J=2.6, 12.8 Hz, H5), 1.88 (1H, dd, J=11.3, 12.8 Hz, H5), 1.81 (1H, dd, J=9.4, 10.9 Hz, H3), 1.51 (3H, s, −Me), 0.88 (3H, d, J=7.2 Hz, −Me), 0.41 (3H, d, J=7.2 Hz, −Me); ^{13}C NMR δ=142.3, 141.4, 128.8, 128.3, 128.2, 128.1, 127.4, 125.9, 80.6, 77.2, 73.1 (C2, C4, C6), 56.4, 51.6 (C3, C5), 24.5, 24.4, 22.7, 18.7.

3.3.15. 9a-Methyl-2e,4e-diphenyl-3-oxa-bicyclo[3.3.1]-nonan-9e-ol (3g). 283 mg **3g** (23%) as a colourless oil (procedure A); IR (neat) ν_{max} 3454, 2960, 1738, 1372, 1215, 1141, 1050 cm^{-1}; ^1H NMR δ=7.50–7.10 (10H, m, CH_{Ph}), 5.09 (1H, m, H2, H4), 2.10–1.00 (11H, m, H1, H5, H6, H7, H8, $-CH_3$); ^{13}C NMR δ=141.8, 128.1, 126.7, 125.2, (CH_{Ph}), 79.0, 71.8, (C2, C4, C6), 45.4, (C3, C5), 26.6, 20.4, (3×$-CH_2$), 19.0 ($-CH_2$); HRMS: calcd for $C_{21}H_{24}O_2$ 308.1776. Found: 308.1776.

3.3.16. 9e-Methyl-2e,4e-diphenyl-3-oxa-bicyclo[3.3.1]-nonan-9a-ol (4g). 230 mg **4g** (18.7%) as colourless crystals (procedure A); mp 132–133 °C (hexane/ethylacetate); IR (neat) ν_{max} 3251, 2925, 1738, 1496, 1449, 1380, 1207, 1139, 1064 cm^{-1}; ^1H NMR δ=7.42–7.11 (10H, m, CH_{Ph}), 5.50 (2H, m, H2, H4), 2.09–1.13 (11H, m, H1, H5, H6, H7, H8, $-CH_3$); ^{13}C NMR δ=142.5, 127.9, 126.3, 125.2, (CH_{Ph}), 76.5, 72.4, (C2, C4, C9), 45.4, (C1, C5), 27.9, 23.0, (C6, C7, C8), 19.3, ($-CH_3$); HRMS: calcd for $C_{21}H_{24}O_2$ 308.1776. Found: 308.1776.

3.3.17. *anti*-2-Hydroxybenzyl-1-methyl-cyclohexan-1-ol (5g).[6] 147 mg **5g** (16.7% yield) as a colourless oil (procedure A); ^1H NMR δ=7.30–7.10 (5H, m, CH_{Ph}), 4.46 (1H, d, J=10.2 Hz, CHOH), 1.72–0.68 (12H, m, H2, H3, H4, H5, H6, $-CH_3$); ^{13}C NMR δ=142.4, 128.3, 127.8, 127.3, (CH_{Ph}), 78.7, 74.5, (C1, CHOH), 52.1, 42.2, (C2, C6), 27.0, 25.5, 23.6, 21.0, (C3, C4, C5, $-CH_3$).

3.3.18. 3e-Chlor-4a-methyl-2e,6e-diphenyl-tetrahydropyran-4e-ol (2h). 470 mg **2h** (38.8%) as a colourless oil (procedure B); IR (neat) ν_{max} 3279, 1690, 1602, 1495, 1449, 1300, 1094, 1065, 1028 cm^{-1}; ^1H NMR δ=7.45–7.12 (10H, m, CH_{Ph}), 4.65 (1H, dd, J=2.3, 11.7 Hz, H6), 4.42 (1H, d, J=10.6 Hz, H2), 3.99 (1H, d, J=10.6 Hz, H3), 2.13 (1H, dd, J=2.3, 13.6 Hz, H5), 2.06 (1H, dd, J=11.7, 13.6 Hz, H5), 1.58 (3H, s, CH_3); ^{13}C NMR δ=140.7, 138.6, 128.6, 128.4, 128.3, 127.9, 127.7, 125.9, (CH_{Ph}), 81.7, 71.9, 71.1, (C2, C3, C4, C6), 47.7, (C5), 21.9 ($-CH_3$); HRMS: calcd for $C_{18}H_{19}ClO_2$ 302.1074. Found: 302.1072.

3.3.19. 8a-Methyl-2e,4e-diphenyl-3-oxa-bicyclo[3.2.1]-octan-8e-ol (3i). 550 mg **3i** (46.7%) as a colourless oil

(procedure B); IR (neat) ν_{max} 3304, 2873, 1495, 1330, 1292, 1120, 1108, 1002 cm^{-1}; ^1H NMR δ=7.40–7.13 (10H, m, CH_{Ph}), 5.00–4.96 (2H, m, H2, H4), 2.02–1.95 (2H, m, H1, H5), 1.73 (3H, s, $-CH_3$), 1.52–1.49 (4H, m, H6, H7); ^{13}C NMR δ=141.7, 128.0, 126.8, 125.7, (CH_{Ph}), 79.9, 77.9, (C2, C4, C8), 51.1, (C1, C5), 21.0, 20.3, (C6, C7, $-CH_3$); HRMS: calcd for $C_{20}H_{22}O_2$ 294.1619. Found: 294.1620.

3.3.20. 5e-(2-Hydroxy-isopropyl)-3e-isopropenyl-2e-phenyl-tetrahydropyran (6). 860 mg **6** (82.6%) were isolated as a colourless oil by the reaction of 1-hydroxy-3-methyl-2-buten (procedure B); IR (neat) ν_{max} 3412, 2970, 1453, 1371, 1191, 1073 cm^{-1}; ^1H NMR δ=7.33–7.12 (5H, m, CH_{Ph}), 4.56 (2H, m, CH_2), 4.17 (1H, ddd, J=2.7, 3.8, 10.9 Hz, H6), 4.03 (1H, d, J=10.2 Hz, H2), 3.38 (1H, dd, J=10.9, 11.3 Hz, H6), 2.29 (1H, ddd, J=3.4, 10.2, 10.2 Hz, H3), 1.91 (1H, ddd, J=3.4, 3.5, 12.6 Hz, H4), 1.78 (1H, dddd, J=3.4, 3.8, 11.3, 12.1 Hz, H5), 1.52 (1H, ddd, J=10.2, 12.1, 12.6 Hz, H4), 1.43 (3H, s, $-CH_3$), 1.22 (6H, s, $-CH_3$); ^{13}C NMR δ=146.0, 140.8, 128.1, 127.8, 127.3,112.3, (CH_{Ph}, C_q, CH_2), 84.2, (C2), 71.3, (–COH), 69.8, ($-CH_2O$), 50.6, 46.8, 31.6, (C3, C4, C5), 27.8, 27.3, 21.5, (3×CH_3); HRMS: calcd for $C_{17}H_{24}O_2$ 260.1776. Found: 260.1776.

3.3.21. 4e-Methyl-2e,6e-di-*n*propyl-tetrahydro-pyran-4a-ol (7c). 160 mg **7c** (20%) as a colourless oil (procedure B); IR (neat) ν_{max} 3117, 2959, 2806, 1735, 1381, 1147, 1124, 1077 cm^{-1}; ^1H NMR δ=3.21 (2H, m, H2, H6), 1.20–1.59 (12H, m, H3, H5, 4×CH_2), 1.24 (3H, s, CH_3), 0.85 (6H, dd, J=6.8, 7.2 Hz, 2×CH_3); ^{13}C NMR δ=74.7 (C2, C4), 69.5 (C4), 46.5 (C3, C5), 38.4 (2×CH_2), 26.2 ($-CH_3$), 19.0 ($-CH_2$), 14.0 (2×CH_3); HRMS: calcd for $C_{12}H_{24}O_2$ 200.1776. Found: 200.1743.

3.3.22. 2e,4a,6e-Trimethyl-tetrahydro-pyran-4e-ol (8a).[12] 210 mg **8a** (36.4%) as a colourless oil (procedure B); ^1H NMR δ=3.43 (2H, ddq, J=1.5, 6.2, 10.7 Hz, H2, H6), 1.58 (2H, dd, J=2.3, 10.7 Hz, H3, H5), 1.33 (2H, dd, J=1.5, 2.3 Hz, H3, H5), 1.25 (3H, s, CH_3), 1.15 (6H, d, J=6.2 Hz, 2×CH_3); ^{13}C NMR δ=77.2 (C2, C5), 70.9 (C4), 47.6 (C3, C5), 26.0, 22.0 (3×CH_3).

3.3.23. 2e,6e-Diethyl-4a-methyl-tetrahydro-pyran-4e-ol (8b). 180 mg **8b** (26.1%) as a colourless oil (procedure B); IR (neat) ν_{max} 3388, 2965, 2936, 1731, 1460, 1371, 1175, 1141, 1125, 1080, 1023 cm^{-1}; ^1H NMR δ=3.18–3.09 (2H, m; H2, H6), 1.64–1.14 (11H, m, H3, H5, 2×CH_2, CH_3), 0.88 (6H, t, J=7.3 Hz; 2×$-CH_3$); ^{13}C NMR δ=76.3, (C2, C6), 69.5 (C4), 46.2, (C3, C5), 29.2 (2×CH_2), 26.2 ($-CH_3$), 10.1 (2×$-CH_3$); HRMS: calcd for $C_9H_{17}O_2$ (M−CH_3) 157.122855. Found: 157.12288.

3.3.24. 4a-Methyl-2e,6e-di-*n*propyl-tetrahydro-pyran-4e-ol (8c). 120 mg **8c** (15%) as a colourless oil (procedure B); IR (neat) ν_{max} 3407, 2958, 2930, 1736, 1459, 1376, 1217, 1142, 1125, 1082 cm^{-1}; ^1H NMR δ=3.54 (2H, dddd, J=1.9, 2.3, 4.5, 15.8 Hz, H2, H6), 1.18–1.48 (12H, m, H3, H5, 4×CH_2), 1.17 (3H, s, CH_3), 0.85 (6H, dd, J=6.8, 7.2 Hz, 2×CH_3); ^{13}C NMR δ=72.6 (C2, C6), 68.8 (C4), 44.7 (C3, C5), 38.3 (2×CH_2), 31.8 (CH_3), 16.9 (2×CH_2), 14.1 (2×CH_3); HRMS: calcd for $C_{12}H_{24}O_2$: 200.1776. Found: 200.1774.

3.3.25. 2e,6e-Di-*iso*propyl-4a-methyl-tetrahydro-pyran-4e-ol (8d). 290 mg **8d** (36.2%) as a colourless oil (procedure B); IR (neat) ν_{max} 2959, 2929, 1733, 1469, 1369, 1154, 1127, 1084, 1015; ^1H NMR δ=2.86 (2H, ddd, J=2.3, 6.8, 11.7 Hz; H2, H6), 1.85–1.76 (4H, m; 2×H_3C-CH_2-CHR), 0.95 (6H, t, J=7.3 Hz; 2×H_3C-CH_2R); ^{13}C NMR δ=79.9 (C2, C4), 43.4 (C3, C5), 33.3 (2×CH), 26.3 (CH_3), 18.7, 18.6 (2×CH_3); HRMS: calcd for $C_{12}H_{23}O_2$ 199.1698. Found: 199.1700.

3.3.26. 2a,4a,8a-Trimethyl-3-oxa-bicyclo-[3.2.1]-octan-8e-ol (9a). 160 mg **9a** (28.5%) as a colourless oil (procedure B); IR (neat) ν_{max} 3317, 2971, 2917, 1738, 1464, 1371, 1348, 1297, 1130, 1089, 1069 cm^{-1}; ^1H NMR: δ=3.78 (2H, q, J=6.4 Hz, H2, H4), 1.55–1.71 (4H, m, H6, H7), 1.45 (3H, s, CH_3), 1.43 (2H, m, H1, H5), 1.03 (6H, d, J=6.4 Hz, 2×CH_3); ^{13}C NMR 80.1 (C8), 72.5 (C2, $C4_l$), 49.8 (C1, C5), 21.0 (C6, C7), 20.0 (CH_3), 19.5 (2×CH_3); HRMS: calcd for $C_{10}H_{18}O_2$ 170.1307. Found: 170.1306.

3.3.27. 2e,4e-Diethyl-8a-methyl-3-oxa-bicyclo[3.2.1]-octan-8e-ol (9b). 280 mg **9b** (35.3%) as a colourless oil (procedure B); IR (neat) ν_{max} 3307, 2970, 2919, 1738, 1365, 1217, 1127, 1070, 989 cm^{-1}; ^1H NMR δ=3.42 (2H, t, J=6.78 Hz; H2, H4), 1.69–1.14 (13H, m, H1, H5, H6, H7, 2×$-CH_2$, $-CH_3$), 0.83 (6H, t, J=7.0 Hz; 2×$-CH_3$); ^{13}C NMR δ=80.4, (C8), 76.9 (2×C2, C4), 49.0, (C1, C5), 26.3, 21.5 (2×CH_2, C6, C7), 20.1 (CH_3), 10.3 (2×CH_3); HMRS: calcd for $C_{12}H_{22}O_2$ 198.1620. Found: 198.1624.

3.3.28. 8a-Methyl-2e,4e-di-*n*propyl-3-oxa-bicyclo[3.2.1]-octan-8e-ol (9c). 350 mg **9c** (38.7%) as a colourless oil (procedure B); IR (neat) ν_{max} 3321, 2954, 2867, 1738, 1464, 1374, 1323, 1217, 1131, 1093 cm^{-1}; ^1H NMR δ=3.52 (2H, t, J=7.16 Hz; H2, H4), 1.69–1.14 (17H, m, H1, H5, H6, H7, 4×CH_2, CH_3), 0.82 (6H, t, J=7.2 Hz; 2×$-CH_3$); ^{13}C NMR δ=80.4 (C8), 78.6 (2×C2, C8), 48.3 (C1, C5), 26.3, 21.5 (2×CH_2, C6, C7), 20.1 (CH_3), 10.3 (2×CH_3); HMRS: calcd for $C_{14}H_{26}O_2$ 226.1933 Found: 226.1933.

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Preparation of *p*-nitrocalix[*n*]arene methyl ethers via *ipso*-nitration and crystal structure of tetramethoxytetra-*p*-nitrocalix[4]arene☆

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Abstract—Nitration of *p-tert*-butylcalix[*n*]arene methyl ethers under a variety of reaction conditions has been examined. It has been determined that amongst different nitration procedures adopted ($AlCl_3/KNO_3$, HNO_3/CH_3COOH, $HNO_3/(CH_3CO)_2O$, cerium(IV) ammonium nitrate/CH_3COOH), *ipso*-nitration with CH_3COOH/HNO_3 gives best yields of *p*-nitrocalixarenes and work up conditions. *ipso*-Nitration of tetramethoxytetra-*p-tert*-butylcalix[4]arene gives tetramethoxytetra-*p*-nitrocalix[4]arene as triclinic crystals with space group $\overline{P1}$, with a=9.102(3) Å, b=11.623(3) Å, c=18.368(3) Å and α=77.99(2)°, β=81.10(2)°, γ=73.37(2)°. Its conformation is partial cone and it forms an exocylic 1:1 complex with DMF.

1. Introduction

Nitration of calix[4]arenes has been attempted by a number of workers using direct and indirect methods.[1–5] Very little work has been reported on nitration of higher calix[*n*]arenes (n>4). There seems to be no reported general methodology to obtain *p*-nitrocalix[*n*]arenes. Some methods are limited to calix[*n*]arene ethers[1] while others are suitable only for calix[*n*]arenes.[2,3] The reported methods are limited by low yields, multiplicity of steps or over oxidation of starting calixarenes by the nitrating mixture.[4] For example, though tetranitrocalix[*n*]arene can be obtained in moderate to good yields by nitration in chloroform medium,[5] it involved an additional step of debutylation of readily available *p-tert*-butyl calix[*n*]arene (Fig. 1).

In view of the importance of calixarenes for diverse applications, we intended to achieve the best workable method for obtaining reasonable yields of *p*-nitrocalix[*n*] arenes at the pilot plant level (for n=4, 6, 8). In pursuance of this objective, we have carried out a comparative study of nitration of calixarene methyl ethers by (i) HNO_3/CH_3COOH, (ii) HNO_3/Ac_2O, (iii) $KNO_3/AlCl_3$ in CH_2Cl_2 and (iv) cerium(IV) ammonium nitrate as against the reported method of using fuming nitric acid.[1,6] Comparative analysis of nitro products obtained under a variety of conditions (Table 1) reveals that pure nitric acid in acetic acid is the best reagent for obtaining nitrocalixarenes in 70–79% yield. *ipso*-Nitration with acetic anhydride/nitric acid leads to good yield of *p*-nitrocalix[*n*]arene methyl ether but the reaction leads to low yields with *p-tert*-butylcalix[*n*]arenes due to acetylation leading to a mixture from which *p*-nitrocalix[*n*]arenes could be separated in lower yields, while cerium(IV)ammonium nitrate/acetic acid gave lower yields, due to oxidation of substrates. $KNO_3/AlCl_3$ has been found to work better for debutylated calix[*n*]arene methyl ether. Our present study also enabled us to obtain good crystals of tetramethoxytetra-*p*-nitrocalix[4]arene. When recrystallized from chloroform/DMF, triclinic crystals in space group $\overline{P1}$ are obtained. Our results are reported in this paper.

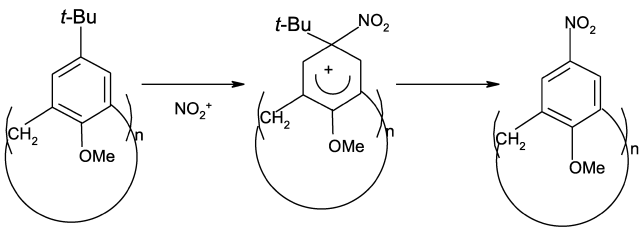

Figure 1.

☆ Supplementary data associated with this article can be found in the online version, at doi: 10.1016/j.tet.2003.11.057

Keywords: Calixarene derivative; Crystal structure; Synthesis; *ipso*-Nitration.

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Table 1. *ipso*-Nitration of *p-tert*-butylcalix[*n*]arene using different nitrating reagents

Calix[*n*]arene	Nitrating mixture	Temperature (°C)	Time (h)	Product	Yield (%)
1[**4**]	CH_3COOH/HNO_3	0–5	4	**2**[**4**]	76
1[**6**]	CH_3COOH/HNO_3	0–5	4	**2**[**6**]	79
1[**8**]	CH_3COOH/HNO_3	0–5	4	**2**[**8**]	70
1[**4**]	Ac_2O/HNO_3	0	5	**2**[**4**]	75
1[**6**]	Ac_2O/HNO_3	0	5	**2**[**4**]	78
1[**8**]	Ac_2O/HNO_3	0	5	**2**[**8**]	76
1[**4**]	CAN/acetone/AcOH	Reflux	8	**2**[**4**]	50
1[**6**]	CAN/acetone/AcOH	Reflux	8	**2**[**4**]	55
1[**8**]	CAN/acetone/AcOH	Reflux	8	**2**[**8**]	55

AcOH, acetic acid, Ac_2O, acetic anhydride, CAN, cerium(IV) ammonium nitrate.

2. Results and discussion

It has been observed that nitration of methoxycalix[*n*]arenes with 100% nitric acid in dichloromethane–acetic acid (0.5:1) gives a good yield of nitro calixarenes (87%) as compared to acetic anhydride–nitric acid methodology.[7] When nitration was carried out with cerium(IV) ammonium nitrate in the presence and absence of acetic acid, it gave moderate yields of nitrocalix[*n*]arenes. Nitration of calix[*n*]arene methyl ethers with $KNO_3/AlCl_3$ in CH_2Cl_2 provides *p*-nitrocalix[*n*]arene methyl ethers in good yield but *ipso*-nitration of *p-tert*-butylcalix[*n*]arenes mainly gave a mixture, which was difficult to separate by usual chromatographic/solvent extraction methods. All the major *p*-nitrocalix[*n*]arene methyl ethers (*n*=4, 6, 8) could be obtained by the method reported in this paper in contrast to other attempted methods reported for the preparation of *p*-nitrocalix[4]arenes.

The synthesized *p*-nitrocalix[*n*]arene methyl ethers showed strong absorption at 1340–1345 and 1518 cm^{-1}. These absorptions can be assigned to NO_2 groups in the products. In ^1H NMR spectra, the tetramethoxytetra-*p*-nitrocalix[4]-arene showed methylene bridge protons at δ 4.45–3.05 and aromatic protons at δ 8.23, 8.13, 7.86, 7.70 indicating that the *p*-nitrocalix[4]arene methyl ether exists in the partial cone conformation. This conclusion was based on comparison of the NMR data with that available in the literature.[6] For parent *p*-nitrocalix[4]arene, variable temperature NMR spectral analysis revealed that the resonance reported[7] for the methylene protons of a tetra-*p*-nitrocalix[4]arene showed a pair of doublets at 23 °C and a broad singlet at 50 °C (Fig. 2) in DMSO-d_6. The pair of double doublets appeared to coalesce at temperatures above 23 °C and completely merge at ca. 42 °C (coalescence temperature). This variable temperature ^1H NMR study therefore indicated that tetra-*p*-nitrocalix[4]arene probably existed in the cone conformation below 23 °C. When the free energy of activation had just been enough to cross the rotational barrier (around 42 °C), the tetra-*p*-nitrocalix[4]arene becomes conformationally mobile in DMSO-d_6. However, in the case of tetramethoxytetra-*p*-nitrocalix[4]arene, the compound existed in the partial cone conformation in $CDCl_3$ and DMSO-d_6 at room temperature (25 °C) and also in the solid state (Fig. 4). Temperature dependent NMR (Fig. 3) indicated that the peaks which appeared at δ 8.23 and 8.13, broadened with the rise in temperature and ultimately appeared as broad singlets at 80 °C. No coalescence temperature could be observed below 80 °C.

3. Results obtained for X-ray crystallography of tetramethoxytetranitrocalix[4]arene

The structure of the compound in its solid state is shown in Figure 4. The torsion angles φ and χ around $ArCH_2Ar$ bonds about C7, C14, C21, C28 are −118.8(5)°, −116.5(4)°, 112.9(5)°, −71.3(5)°, 112.0(4)°, 114.8(4)° and 71.5(5)°, respectively, the −−, ++, −+, −+ sequence is characteristic of the partial cone[8] conformation with a deviation from 90° indicating the deformation from the cone conformation (cf. 88.9(4)°, −89.4(5)° found in *p-tert*-butylcalix[4]arene and its 1:1 complex with toluene).[9] All four aromatic rings A(C1–C6), B(C8–C13), C(C15–C20) and D (C22–C27) were found to be almost planar (maximum deviation being 0.03 Å from a least squares plane). The connecting methylene C atoms (C7, C14, C21, C28) formed an approximate plane where alternate C atoms were between ±0.07 and ±0.05 Å above and below this plane. The interplanar angles found between this plane and rings A–D are 98.0(1), 92.1(1), 79.3(1), and 149.6(1)°. The interplanar angle with ring D is much less than 90°

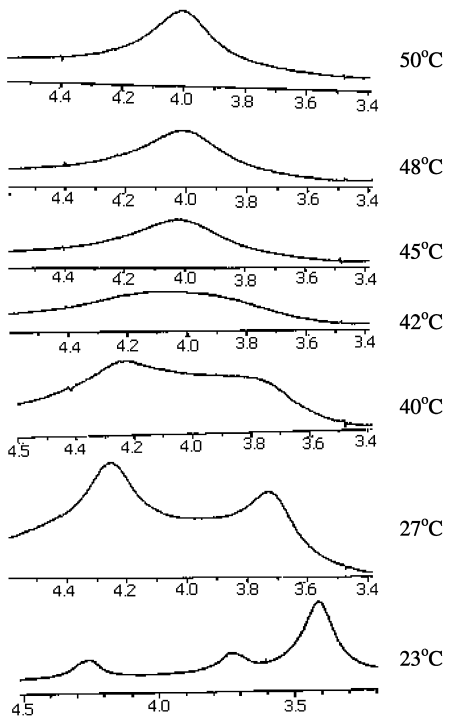

Figure 2. Temperature dependent ^1H NMR spectra of tetra-*p*-nitrocalix[4]-arene in DMSO-d_6 showing bridge protons.

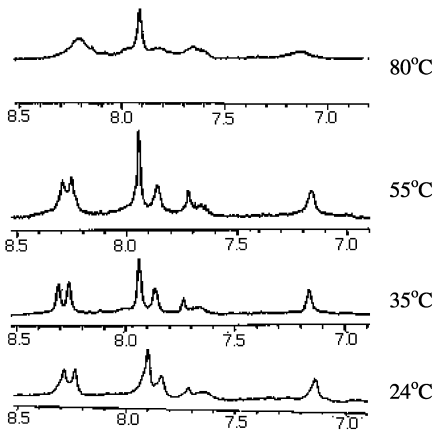

Figure 3. Temperature dependent ^{1}H NMR spectra of tetramethoxytetra-*p*-nitrocalix[4]arene in DMSO-d_6 showing aromatic protons.

indicating that this aromatic ring was tilted in such a way that its NO_2 group was directed away from the calixarene ring cavity. The corresponding methyl substituted O10 is then directed inward to the cavity of calix[4]arene to give an inward flattened partial cone conformation. The inter planar angles between the pairs AC and BD are 18.7(1) and 57.6(1)°, respectively. Thus ring A and C are parallel to each other but ring B and D are not possibly due to flattening of ring D. Both A and C rings are perpendicular to rings B and D (interplanar angles between AB, AD, BC and CD being 90.4(1), 82.0(1), 90.0(1) and 97.6(1)°, respectively). The exocyclic DMF molecule shows a strong OCH···π interaction between the H attached to the amide carbon of DMF C1S and phenyl ring D with OCH···π distance as 2.79(2) Å. There is a CH_3···π interaction with H···π distances being 3.51(1) Å which brings the methoxy oxygen O10 much closer to other two adjacent counterparts. The nonbonding O1···O10 and O7···O10 distances are 2.99(1) and 2.98(1) Å, respectively, which are significantly shorter than a normal O···O nonbonding distances 3.250(1) Å. These nonbonding contacts are 0.3 Å longer than the intramolecular H-bonds observed in other calix[4]arenes[10] but they are similar to nonbonding O···O distances (2.944(15)–3.128(18) Å) found in crowned *p-tert*-butylcalix[4]arene.[11]

There are seven intermolecular C–H···O H-bond interactions[12] between alkyl and aryl hydrogens and oxygens of the nitro groups some of which have been shown in Figure 4. The C···O, H···O and ⟨C–H···O lie within the range found for such interactions. These H-bond interactions form intermolecular cavities suitable for accommodation of solvent molecule that otherwise is difficult to enter into the intramolecular cavity of calix[4]arene being inaccessible due to methoxy groups. At least one nitro group oxygen from each of the four-nitro groups seems to be involved in H-bond interactions. A weak intermolecular C–H···O is also found between the amide oxygen O1S of the solvent molecule and methoxy carbon C29 of a symmetry related molecule.

4. Experimental

NMR spectra were recorded on a 300 MHz Bruker DPX 300 instrument. IR spectra were recorded on a Nicolet Protégé 460 spectrometer in KBr disks while CHN analysis were obtained by using a Perkin–Elmer 240C elemental analyzer. Mass spectra were recorded on a Jeol SX-102 spectrometer. X-ray data was recorded using CAD4 Enraf-Nonius 4-circle automatic diffractometer.

4.1. General procedure for nitration of calixarene ethers with HNO_3/CH_3COOH

Acetic acid (20 mL) was taken in a round bottom flask and HNO_3 (100%, 5.4 mL) was slowly added to it. *p-tert*-Butylcalix[*n*]arene methyl ether (0.5 g) was separately taken in dichloromethane (10 mL) and cooled to 0 °C. This solution was slowly added to the round bottom flask

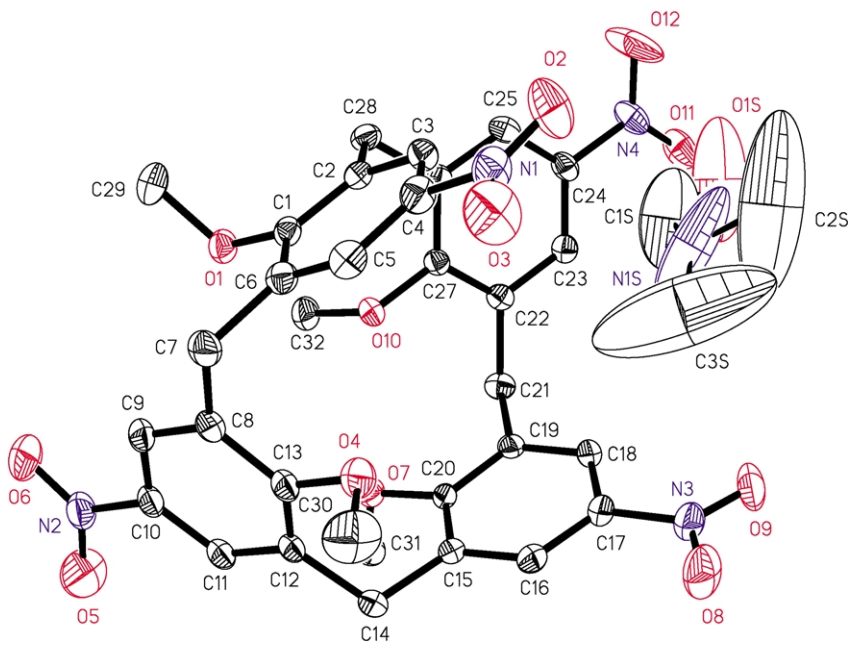

Figure 4. ORTEP diagram showing the labeling of atoms. Hydrogens have been omitted for clarity.

containing nitric acid solution while keeping the mixing temperature below 0 °C. The mixture was stirred at this temperature for 4 h and then added to cold water (300 mL). The product was extracted into dichloromethane and washed with water and concentrated over a water bath. The product was precipitated by adding hexane and recrystallized from chloroform/acetone to give pure *p*-nitrocalix[*n*]arene methyl ether as a pale yellow solid (Table 1).

4.2. General procedure for nitration of calixarene ethers with $HNO_3/(CH_3CO)_2O$

Acetic anhydride (15 mL) was taken in a round bottom flask, it was cooled to 0–5 °C and HNO_3 (100%, 6 mL) was slowly added to it with constant stirring, keeping the temperature below 5 °C. *p-tert*-Butylcalix[*n*]arene methyl ether (0.5 g) was taken in dichloromethane (15 mL) a separate round bottom flask and cooled to 0 °C. The nitrating mixture prepared separately as described above was added slowly to the solution of *p-tert*-butylcalix[*n*]arene methyl ether (highly exothermic reaction, it should be added slowly) and the reaction mixture was stirred at this temperature for 5 h. It was added to 200 mL cold water and worked up as described in the general procedure in reaction with CH_3COOH/HNO_3 to give pale yellow crystalline solid. (Table 1).

4.3. Reaction of *p-tert*-butylcalixarene methyl ether with cerium(IV) ammonium nitrate

p-tert-Butylcalix[*n*]arene methyl ether (1 g) was taken in a round bottom flask and acetic acid (20 mL), THF (50 mL), cerium(IV) ammonium nitrate (20 g) was added to it. The reaction mixture was refluxed for 8 h, concentrated and added to 200 mL water. The reaction mixture was extracted into dichloromethane and washed with water. The organic layer was concentrated and precipitated by adding hexane and the precipitated product was separated by filtration. The product was recrystallized three times from chloroform/hexane to give pure *p*-nitrocalix[*n*] arene as a pale yellow solid (Table 1).

4.4. Reaction of calix[*n*]arene methyl ether with $KNO_3/AlCl_3$

Calix[*n*]arene methyl ether (0.5 g) was taken in 20 mL dichloromethane in a round bottom flask, cooled to 5–10 °C in ice bath and anhydrous $AlCl_3$ (1.00 g) was added to it. To this reaction mixture, potassium nitrate (0.7 g) was added. The reaction mixture was slowly brought to room temperature over a period of 3 h and stirred at this temperature for a period of 24 h. The reaction was quenched by adding it to 200 mL ice cold water and the product was extracted into dichloromethane. The dichloromethane layer was concentrated and product was precipitated by adding hexane and filtered.

4.4.1. Synthesis of tetramethoxytetra-*p*-nitrocalix[4]arene: 2[4]. Yield 88%, mp >300 °C. IR (KBr, ν/cm^{-1}): 1596, 1518, 1452 and 1340. ^1H NMR ($CDCl_3$, δ): 8.23, 8.13, 7.86, 7.70 (8H, s, Ar*H*), 4.45–3.05 (20H, m, ArCH_2Ar and OCH_3). ^{13}C NMR (DMSO-d_6, δ): 29.10, 30.8 (ArCH_2Ar), 60.09, 61.9 (OCH_3), 123.1, 124.3, 125.1, 125.7, 134.6, 137.2, 141.8, 162.7 (Ar). MS-FAB: (*m*/*z*)=661. Anal. Calcd for $C_{32}H_{28}O_{12}N_4$·DMF: C, 57.29; H, 4.77; N, 9.55. Found C, 57.16; H, 4.79; N, 9.54.

4.4.2. Synthesis of hexamethoxyhexa-*p*-nitrocalix[6]arene: 2[6]. Yield 85%, mp >300 °C. IR (KBr, ν/cm^{-1}): 1592, 1518, 1448 and 1345. ^1H NMR ($CDCl_3$, δ): 7.6 (s, 12H, Ar*H*), 4.04 (s, 12H, ArCH_2Ar), 3.74 (s, 18H, OCH_3). ^{13}C NMR ($CDCl_3$, δ): 30.9, 61.3, 124.3, 134.4, 144.0, 161.7. MS-FAB (*m*/*z*): 990. Anal. Calcd for $C_{48}H_{42}O_{18}N_6$: C, 58.18; H, 4.24; N, 8.48. Found C, 58.37; H, 4.15; N, 8.35.

4.4.3. Synthesis of octamethoxyocta-*p*-nitrocalix[8]arene: 2[8]. Yield: 89%, mp >300 °C. IR (KBr, ν/cm^{-1}): 1592, 1518, 1448 and 1345. ^1H NMR ($CDCl_3$, δ): 7.82 (s, 16H, Ar*H*), 4.20 (s, 16H, ArCH_2Ar), 3.80 (s, 24H, OCH_3). ^{13}C NMR (DMSO-d_6, δ): 30.5, 60.6, 125.2, 133.8, 143.6, 161.5. MS-FAB: (*m*/*z*): 1321. Anal. Calcd for $C_{64}H_{56}O_{24}N_8$: C, 58.18; H, 4.24; N, 8.48. Found: C, 57.87; H, 4.01; N, 8.81.

4.5. Crystallography

The crystals were obtained by warming a solution of **2**[**4**] in chloroform and DMF (9:1) up to 60 °C and cooled to room temperature. Yellow crystals of **2**[**4**]. DMF complex were obtained which were found to be of a 1:1 exclusion complex of calix[4]arene and solvent (DMF), with molecular formula $C_{35}H_{35}N_5O_{13}$, M=733.68, triclinic, a=9.102(3) Å, b=11.623(3) Å, c=18.368(3) Å, α=77.99(2)°, β=81.10(2)°, γ=73.37(2)°, V=1811.75(8) A^3, Z=2, D_c=1.345 g cm^{-3}, space group $P\bar{1}$. Intensity diffraction data were calculated up to θ=72.95° by using 2ω step scanning mode with Ni filtered Cu Kα radiation (λ=1.5418 Å) on a 0.2×0.1×0.1 mm^3 crystal at 293 K. A total of 7548 reflections were calculated, 7079 were independent and of which 6748 were considered observed [$I>2\sigma(I)$] and used in the structure analysis and refinement. All the nonhydrogen atoms were refined anisotropically. The solvent was highly disordered and the disorder could be resolved only for the amide nitrogen and amide oxygen of DMF. Solvent could be refined anisotropically using restraints on the bond lengths and thermal parameters. All hydrogen atoms were placed in their geometrical positions and were not refined. The final R index using observed data, refining 497 parameters with 29 restraints was R=0.1285. Relatively higher values of R is due to high degree of disorder in the solvent molecule but low e.s.d.'s for all other atoms suggest that the overall geometry and accuracy of the structure is not compromised to any significant extent. All the calculations involving structure solution, refinement and graphics were performed using SHELXTL-PC.[13] Least square planes and H-bonding was calculated using PARST.[14]

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