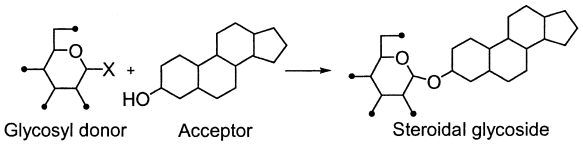
ELSEVIER

Tetrahedron

Tetrahedron Vol. 60, No. 24, 2004

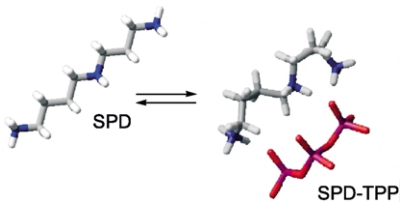
Contents

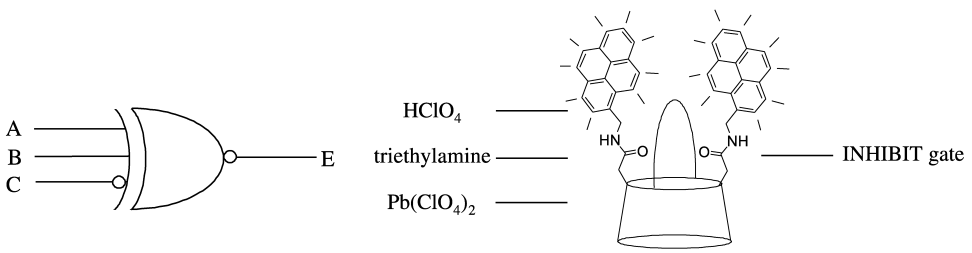
REPORT

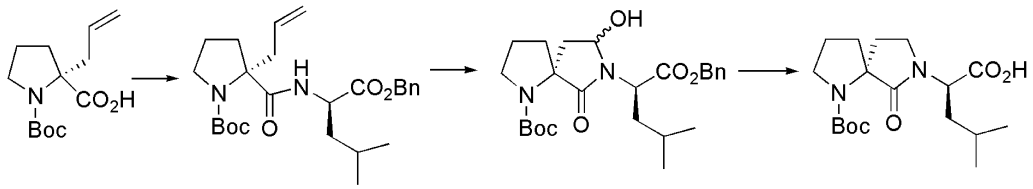


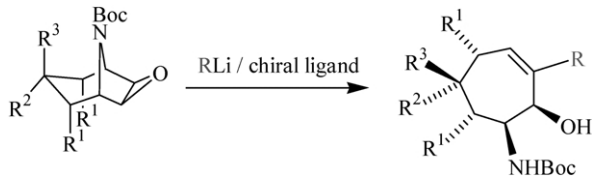
The principal techniques used to prepare steroidal glycosides are reviewed for the first time.

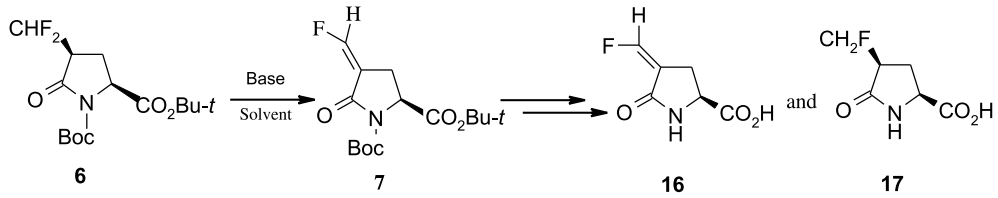
ARTICLES

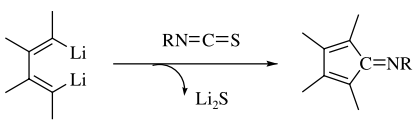


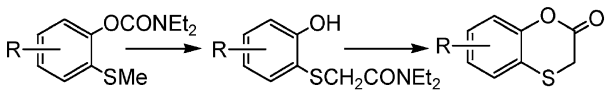


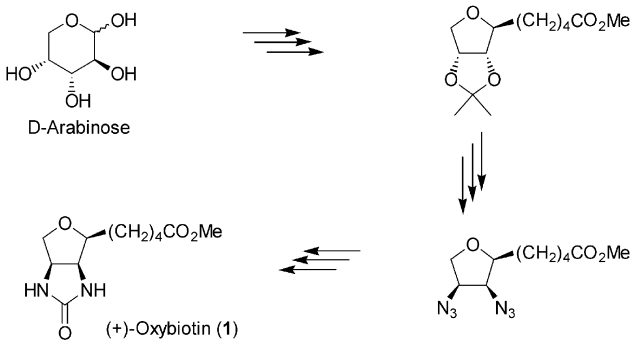


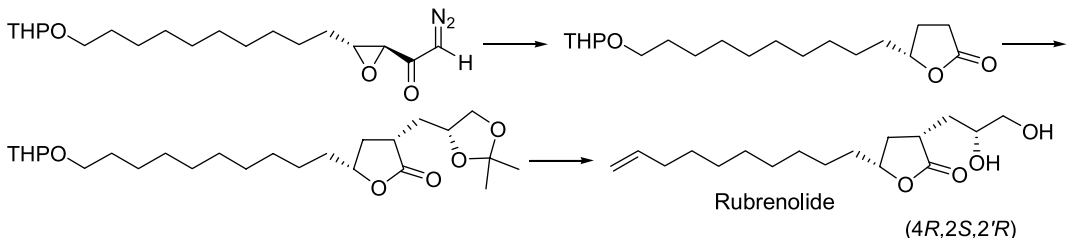


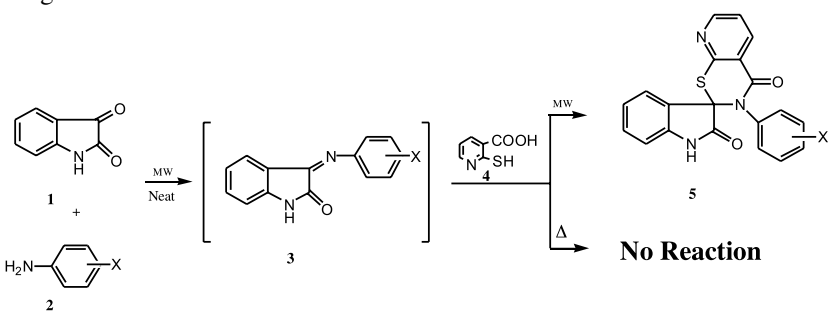


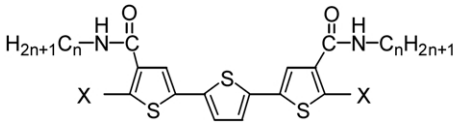












DNC_nDCl3T X = Cl, n = 18
DNC_nDBr3T X = Br, n = 8,16,18
DNC_nDI3T X = I, n = 18

OTHER CONTENTS

*Corresponding author
ⓘ+ Supplementary data available via ScienceDirect

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

ELSEVIER

ISSN 0040-4020

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron
Tetrahedron 60 (2004) 5123–5162

Tetrahedron report number 678

The glycosylation of steroids

Hélène Pellissier*

Laboratoire de Synthèse Organique UMR n° 6180, Faculté des Sciences de Saint-Jérôme, Avenue Esc. Normandie-Niemen, 13397 Marseille Cedex 20, France

Received 22 March 2004

Contents

Keywords: Glycosylation; Steroids; Glycoconjugates.

Abbreviations: Ac, acetyl; Bn, benzyl; Bz, benzoyl; Bu, butyl; $BF_3(Et_2O)$, boron trifluoride etherate; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DCC, *N*,*N*′-dicyclohexylcarbodiimide; DMAP, 4-dimethylaminopyridine; DMF, *N*,*N*-dimethylformamide; DTBMP, 2,6-di-*tert*-butyl-4-methylpyridine; NIS, *N*-iodosuccinimide; Phth, phthalimido; Piv, pivaloyl; PMB, *p*-methoxybenzoyl; pNBz, *p*-nitrobenzoyl; PPTS, pyridinium 4-toluenesulfonate; Pr, propyl; py, pyridine; TBDMS, *tert*-butyldimethylsilyl; TBDPS, *tert*-butyldiphenylsilyl; TES, triethylsilyl; Tf, trifluoromethanesulphonyl; Tips, triisopropylsilyl; TMS, trimethylsilyl; TMSOTf, trimethylsilyl trifluoromethanesulphonate; TMU, *N*,*N*,*N*′,*N*′-tetramethylurea; Tr, triphenylmethyl (trityl); Troc, 2,2,2-trichloroethoxycarbonyl; $TrtClO_4$, triphenylmethyl perchlorate; Ts, 4-toluenesulfonyl (tosyl); UDP, uridine-5′-diphosphate.

* Tel.: +33-4-91-28-27-65; e-mail address: h.pellissier@univ.u-3mrs.fr

0040–4020/$ - see front matter
doi:10.1016/j.tet.2004.04.009

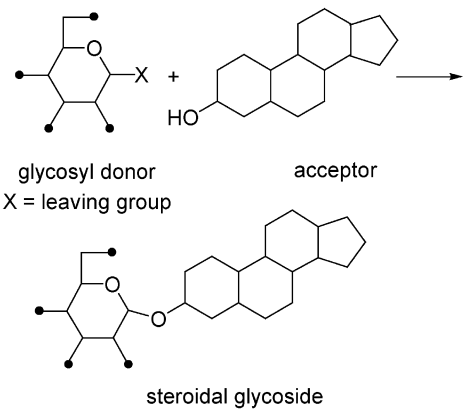
1. Introduction

To the best of the author's knowledge, there is no review in the literature concerning the glycosylation of steroids. Several earlier reviews have reported the more general *O*-glycosylation methods[1] which have sometimes been applied to the synthesis of natural products.[2] Therefore, the present exhaustive article concentrates on the *O*-glycosylation methods applied to the preparation of steroidal glycosides.

A number of important groups of drugs are glycosides. Although the sugars per se seem to have no therapeutic action, it is becoming ever clearer that the very presence of them in naturally occurring structures and their mimetics

has a dramatic effect on their physical, chemical and biological properties.[3] Indeed, their presence greatly modifies the biological activity of all of the drugs. Actually, little research has been devoted to the biological or pharmacological role of the sugar moieties. Clearly, the aglycon itself is not active in most instances, as was demonstrated for many antibiotics and antitumour compounds, with erythromycin, daunomycin or amphotericin B being prominent examples. The sugars seem to play a key role in the interaction of the drugs with their receptors, as well as significantly affecting the pharmacokinetics of the drugs. Traditionally, the glycan chains of these glycoconjugates have been viewed as molecular elements that control the pharmacokinetics of a drug, such as absorption, distribution, metabolism and excretion. For instance, a number of amphiphilic glycosylated bile acid-derived steroids have been demonstrated as effective enhancers of the transport of polar molecules across cellular membranes.[3] Moreover, glucuronides are frequently the final form of a drug or xenobiotic eliminated from the body and often perform an important detoxification role. Analysts, in addition, demand authentic high-purity samples of glucuronides for assay purposes, while potential new drugs are frequently found as their glucuronides during toxicological investigations or clinical trials. Regulatory bodies are now demanding that the glucuronide should be tested thoroughly in its own right before acceptance of the parent drug. The rigid character of the pyran rings, along with the flexibility associated with the glycosidic linkages, give them the ability of preorganisation. Many carbohydrate-bearing antitumour antibiotics are known to act at the DNA level and therefore a contribution of the deoxysugar moiety to the cytostatic activity can be traced either to facilitated cell membrane permeability or to adhesion, i.e. binding to the DNA itself.[4] Structural evidence for a direct interaction between duplex DNA and a carbohydrate moiety of a glycoconjugate came from crystallographic studies.[4] Steroidal glycosides constitute a structurally and biologically diverse class of molecules which have been isolated from a wide variety of both plant and animal species.[5] Members of this class of biomolecules such as the cardenolides or the saponins have received considerable recent attention, due to their physiological and pharmacological activities. Recent developments in glycobiology have revealed the important roles of many glycoconjugates in immune responses, viral and bacterial infections, inflammation and many other inter- and intracellular signal transductions. Among the huge number of glycoconjugates, the steroidal glycosides are often found as the major components in traditional Chinese medicines, because of their remarkable pharmacological activities. Since the chemical syntheses of oligosaccharides and steroids have been studied intensively, the key to synthesising steroidal glycosides turns out to be the construction of the glycosidic bond between the oligosaccharide and the steroid. More exactly, the glycosylation involves the selective reaction of a sterol with the sugar anomeric carbon (C-1 for aldoses and C-2 for ketoses) which generally bears a leaving group (Scheme 1).

Despite considerable progress in carbohydrate chemistry in the past few years, the stereoselective formation of *O*-glycosidic bonds between carbohydrates and steroids is frequently a time-consuming process, with relatively



Scheme 1. Glycosylation of steroids.

low yields of the newly formed glycosides. This is due to the low reactivity of the secondary alcohol functions in the steroid moiety and the necessity to activate the glycosyl donors. From a survey of the general current methods of glycosylation of steroids, glycosyl donors are roughly classified into ten groups, based on the type of anomeric functional group and their activating methods which are discussed in an early part of this review: (1) glycosyl halides, (2) trihalogenoacetimidates, (3) thioglycosides, (4) 1-*O*-sulfonyl glycosides, (5) glycals, (6) 1-*O*-acyl sugars, orthoesters or ethers, (7) phosphate derivatives, (8) 1-hydroxyl sugars, (9) 1-*O*-silylated glycosides, and (10) others.

2. Glycosyl halides

2.1. Glycosylation using glycosyl bromide and chloride

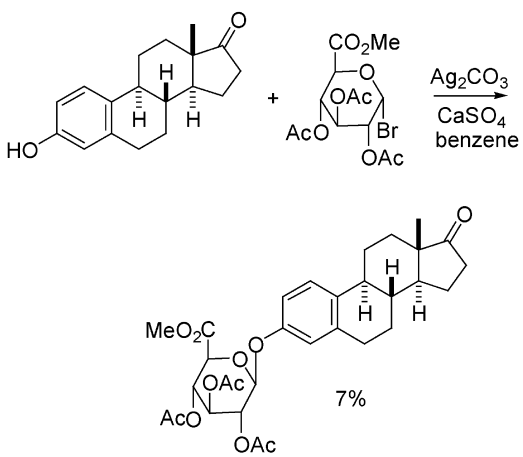
In the classical Koenigs–Knorr method, dating from 1901,[6] and the subsequently developed efficient variants, the activation step is achieved through the formation of glycosyl halides (halogen=bromine and chlorine) and their reaction in the transfer step, in the presence of certain metal salts (preferably those of silver and mercury). This general method of glycosylation has been critically reviewed[7] and still represents the most versatile and generally applicable route. The catalysts used for the Koenigs–Knorr method are typically AgI salts, especially Ag_2CO_3, and $Hg(CN)_2$ or $CdCO_3$. The most common solvents used have been benzene, toluene, dichloromethane, acetonitrile, and ethers and removal of any water produced (molecular sieves, Drierite) is important, in order to prevent its reaction with the sugar. In this way, a huge excess of sugar is needed. Moreover, an additional base such as collidine may be necessary to destroy the HBr formed. The most popular glycosylation of steroids is the glycosylation of the hydroxyl group located at C-3 and is described in Section 2.1.1, which is is organised according to the type of catalyst involved in the Koenigs–Knorr reaction (silver salts, mercuric salts, cadmium carbonate, and other catalysts). Section 2.1.2 deals with the glycosylation occurring at positions other than C-3 of steroids.

2.1.1. Glycosylation of the hydroxyl group at C-3 of steroids

2.1.1.1. Using silver salts as catalysts. The synthesis of

glycosylated steroids was opened up in 1936 by Lettré and Hagedorn,[8] who prepared cholesterol glucoside tetraacetate in 33% yield by the use of calcium hydride combined with silver oxide in refluxing ether. Schapiro[9] in 1938, and Huebner et al.[10] and Meystre et al.[11] in 1944 subsequently reported the synthesis of some other steroid glucosiduronates. The same procedure was applied in 1955 by Schneider et al. to the synthesis of methyl (21-acetoxy-11,20-dioxo-17α-hydroxy-5β-pregnan-3α-yl-2,3,4-tri-*O*-acetyl-β-D-glucosid)-uronate.[12]

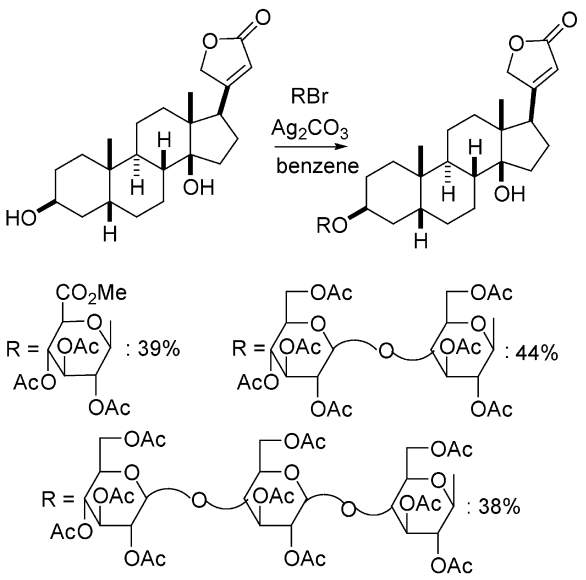
The most popular catalyst used for the glycosylation of steroids, silver carbonate, was involved in the first glycosylation of estrone with methyl 2,3,4-tri-*O*-acetyl-1α-bromo-1-deoxy-D-glucopyranuronate in the presence of calcium sulphate (Scheme 2).[13]



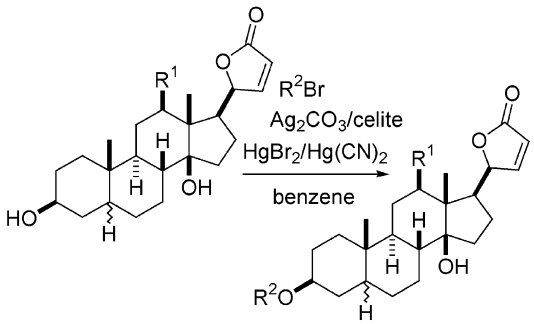
Scheme 2. First glycosylation of estrone.

In 1947, Elderfield et al. prepared the first glucosides of digitoxigenin and digoxigenin which have been widely involved in glycosylation.[14] These authors showed that this reaction was specific to the secondary hydroxyl group of the aglycones. The less reactive tertiary hydroxyl group at C-14 was not glycosylated during the condensation. Instead, this hydroxyl group is extremely sensitive to desiccating agents and the aglycone tends to undergo dehydration, forming anhydrodigitoxigenin derivatives. For this reason, the use of desiccating agents such as anhydrous magnesium sulphate and Drierite should be avoided, and azeotropic distillation was employed by Takiura et al.,[15] with satisfactory results, to facilitate the condensation reaction by eliminating the water formed during condensation from the benzene medium. Thus, the acetates of digitoxigenin 3β-*O*-D-glucopyranoside, -gentiobioside and -gentiotrioside were obtained in fairly good yields minimising the dehydration reaction, by condensation of digitoxigenin with the respective acetobromo sugars in benzene in the presence of silver carbonate (Scheme 3).[15]

A more efficient catalyst was reported in 1981 by Brown et al.,[16] who observed that a combination of mercuric salts and Fetizon's reagent[17] (silver carbonate finely deposited on celite) gave at room temperature the expected glycosylated products in better yields, in most cases, since almost no anhydro product was formed. This glycosylation procedure was found to be generally applicable to a variety of sugars



Scheme 3. Synthesis of digitoxigenin glycosides.



Scheme 4. Synthesis of cardenolide analogues.

Table 1.

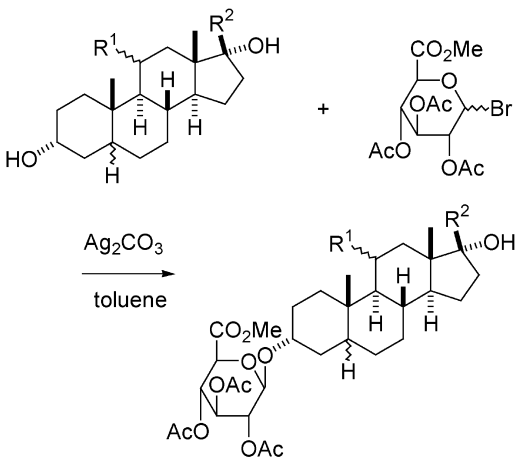
Caption	R^1	5H	R^2	Yield (%)	Ref.
Digitoxigenin	H	β	H	—	—
Digitoxigenin 3β-D-glucoside	H	β	D-glucose	68	18
Digitoxigenin 3β-β-D-galactoside	H	β	D-galactose	57	16
Digitoxigenin 3β-α-L-rhamnoside	H	β	L-rhamnose	79	18
Digoxigenin	OH	β	H	—	—
Digoxigenin 3β-β-D-glucoside	OH	β	D-glucose	14	16
Uzarigenin	H	α	H	—	—
Uzarigenin 3β-β-D-glucoside	H	α	D-glucose	58	16

and to other cardenolide analogues such as digoxigenin or uzarigenin (Scheme 4 and Table 1).[18]

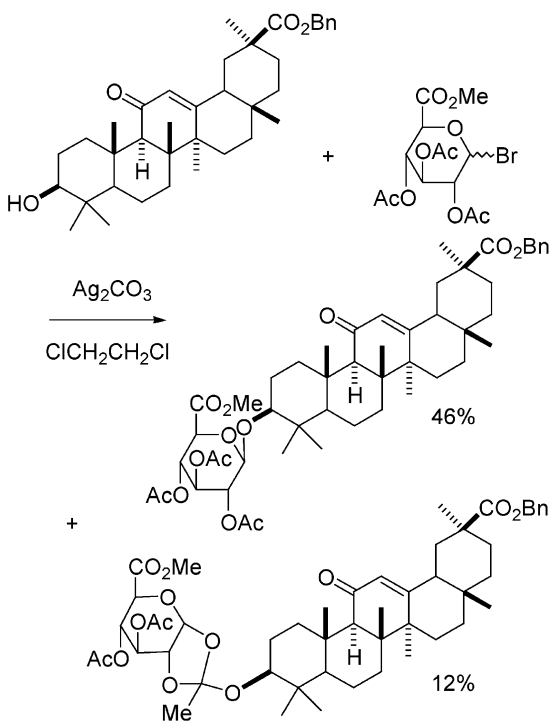
Tetrahydrocortisol and tetrahydrocortisone are major metabolites of cortisol, and are excreted mainly as the 3- and 21-monoglucuronides in human urine. The level of urinary 17-hydroxycorticosteroids has long been an important index of adrenocortical activity. In order to develop specific and sensitive immunoassays, variously substituted monoglucuronides of these corticosteroids were synthesised. The introduction of the glucuronyl residue into the C-3 position was achieved in all cases with methyl 2,3,4-tri-*O*-acetyl-1-deoxy-α-D-glucuronate in the presence of silver carbonate in toluene. The results are collected in Table 2 and Scheme 5.

Table 2.

R^1	R^2	H-5	Yield (%)	Ref.
βOH	$COCH_2OAc$	β	54	19
=O	$COCH_2OAc$	β	45	20
βOH	$CH(OAc)CH_2OAc$	β	30	21
=O	$CH(OAc)CH_2OAc$	β	42	21
=O	$CH(CO_2Me)OAc$	β	81	22
αOH	$CH(CO_2Me)OAc$	β	61	22
H	$COCH_2OAc$	α	60	23
H	$CH(CO_2Me)OAc$	β	29 (20α)	24
H	$CH(CO_2Me)OAc$	β	43 (20β)	24
βOH	$COCH_2OH$	α	28	25
=O	$COCH_2OH$	α	43	25
βOH	$CH(OH)CH_2OH$	α	23 (20α)	26
βOH	$CH(OH)CH_2OH$	α	22 (20β)	26
=O	$CH(OH)CH_2OH$	α	24 (20α)	26
=O	$CH(OH)CH_2OH$	α	34 (20β)	26



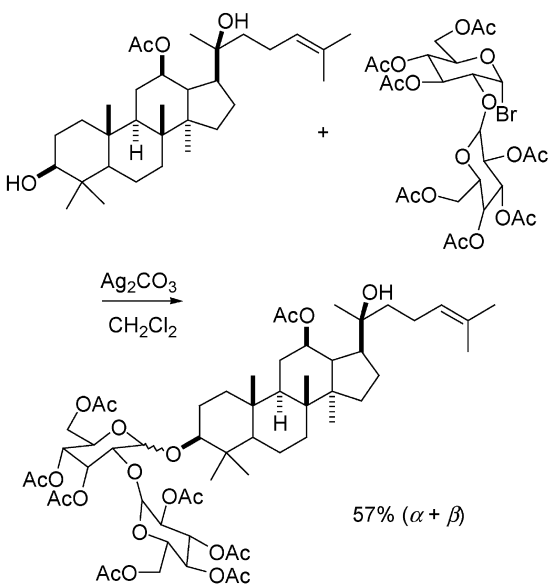
Scheme 5. Synthesis of C-3 glucosiduronates of adrenal steroids.



Scheme 6. Synthesis of glucuronides of 18β-glycyrrhetic acid.

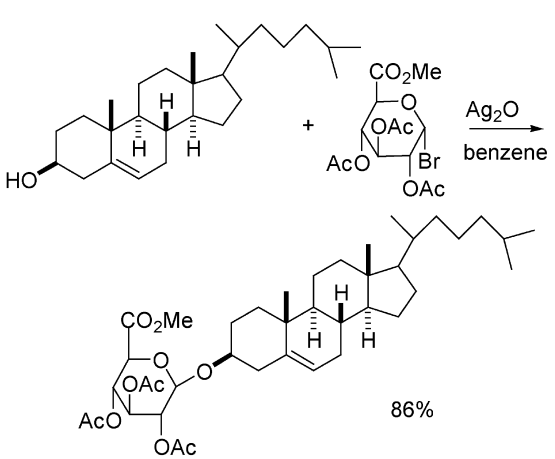
In order to prepare an immunogen for enzyme immunoassay of 3β-(monoglucuron-1′-β-yl)-18β-glycyrrhetic acid (3MGA), which was isolated from a patient with glycyrrhizin-induced pseudoaldosteronisms, benzyl glycyrrhetate was allowed to react with an acetobromosugar in the presence of silver carbonate to give benzyl 3β-(methyl 2′,3′,4′-triacetyl-glucuron-1′β-yl)-glycyrrhetate and unexpected methyl 3′,4′-diacetyl-α-1′,2′-*O*-[1-(benzyl glycyrrhet-3β-yl)-ethylidene]-D-glucuronate (Scheme 6).[27]

More recently, Anufriev et al. applied the Koenigs–Knorr procedure to 12β-acetoxy-dammar-24-en-3β,20(*S*)-diol and hepta-*O*-acetyl-α-sophorosyl bromide, in order to prepare after deacetylation ginsenoside Rg_3, a constituent of ginseng, a well-known plant drug (Scheme 7).[28] The tertiary hydroxyl group at C-20 is sterically hindered by the 12β-acetoxyl group and is therefore resistant towards glycosylation.

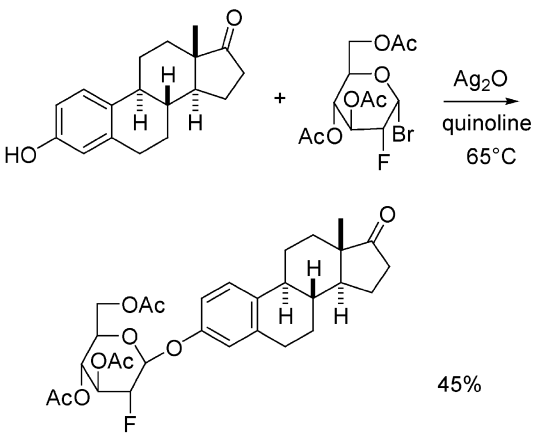


Scheme 7. Synthesis of ginsenoside Rg_3.

Another efficient catalyst often used in the Koenigs–Knorr reaction is silver oxide. This compound was involved in the first glycosylation of cholesterol reported by Schneider et al. in 1969 (Scheme 8).[29]

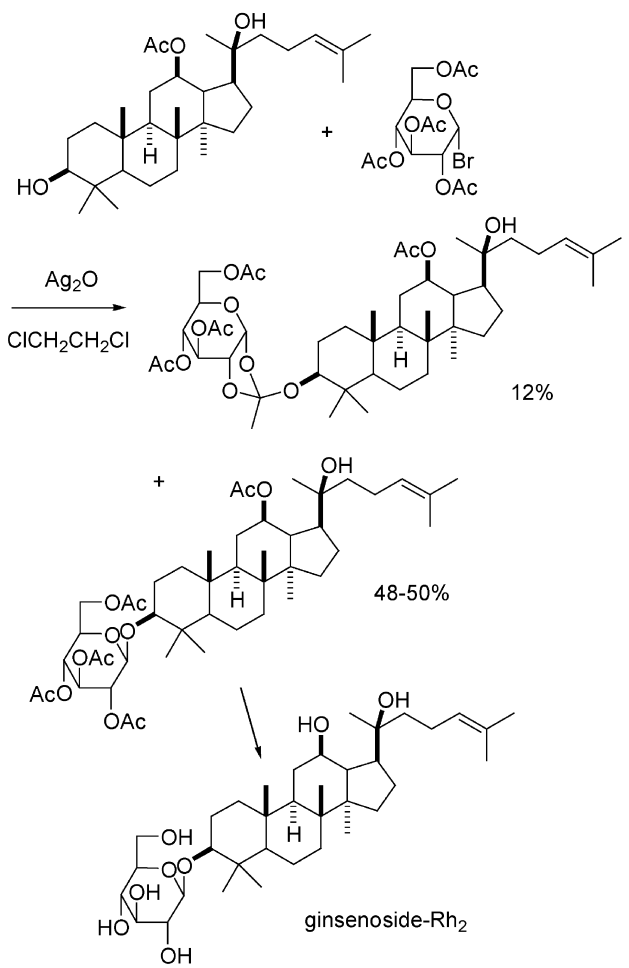


Scheme 8. First glycosylation of cholesterol.



Scheme 9. Synthesis of a 2-deoxy-2-fluoro-D-glucopyranoside derived from estrone.

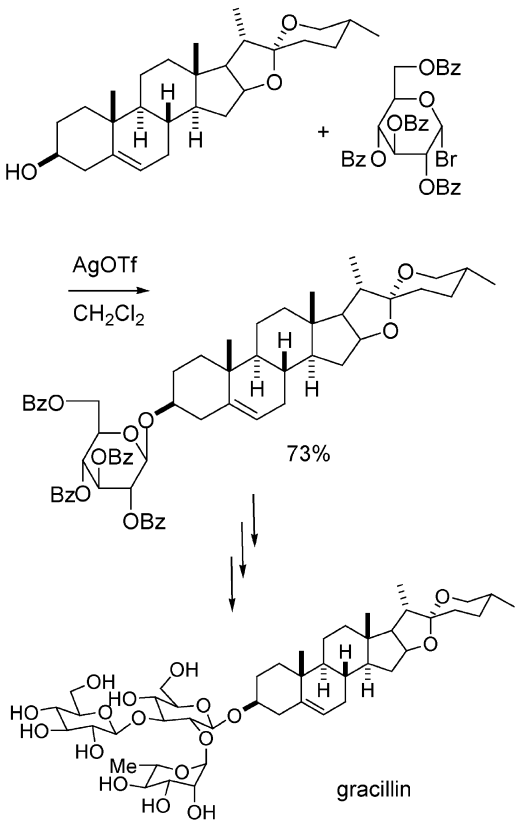
In order to prepare more stable steroidal glycosides, Cerny reported the glycosylation of estrone with 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl bromide catalysed by silver oxide in quinoline at 65 °C (Scheme 9).[30] The difference in the stability of the glycosides and their deoxyfluorinated analogues against acid hydrolysis was then studied and it was found that, in some cases, the first compounds were hydrolysed about 15 times more quickly than their fluorinated derivatives.



Scheme 10. Synthesis of ginsenoside-Rh_2.

In 1997, Elyakov proposed a simplified preparation of natural ginsenoside-Rh_2, exhibiting cytotoxic activity, based on the condensation of the 12-*O*-acetyl derivative of 20(*S*)-protopanaxadiol with tetra-*O*-acetyl-α-D-glucopyranosyl bromide in the presence of silver oxide and molecular sieves in dichloroethane, followed by deacetylation (Scheme 10).[31] Some corresponding orthoester, isomeric with the major compound, was detected in the reaction mixture.

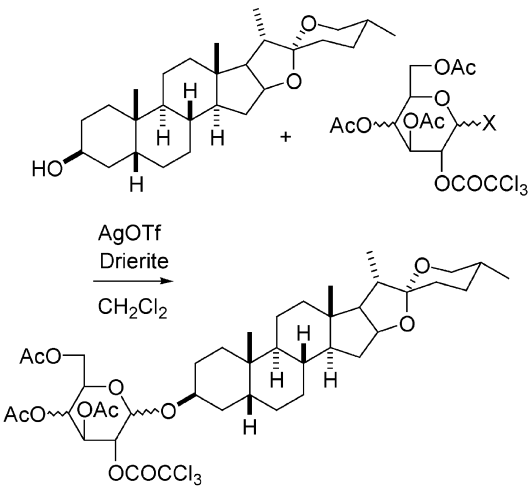
Silver triflate has also been widely used as a catalyst of glycosylation. Li et al. reported in 1998 the glycosylation of diosgenin with benzoyl-protected glucopyranosyl bromide catalysed by silver triflate, producing, after removal of the benzoyl protection, the corresponding diosgenyl saponin (Scheme 11).[32] This latter compound was further converted into gracillin, exhibiting cardiovascular and antitumour activities, by the addition of two other monosaccharide entities.[33]



Scheme 11. Synthesis of diosgenyl saponin intermediate of gracillin.

The synthesis of sarsasapogenin glycosides was reported by Saito et al.,[34] based on the glycosylation of sarsasapogenin with monosaccharides bearing an easily removable trichloroacetyl group at C-2. It was shown that the yields and β-selectivities were both better when pyranosyl bromides were employed instead of the corresponding chlorides (Scheme 12 and Table 3).[34] The trichloroacetyl group was then selectively removed and the resulting OH function could be linked to another pyranose derivative.

Similarly, digitoxigenin glycosides of deoxy sugars could be prepared with the same catalyst and the best yields were

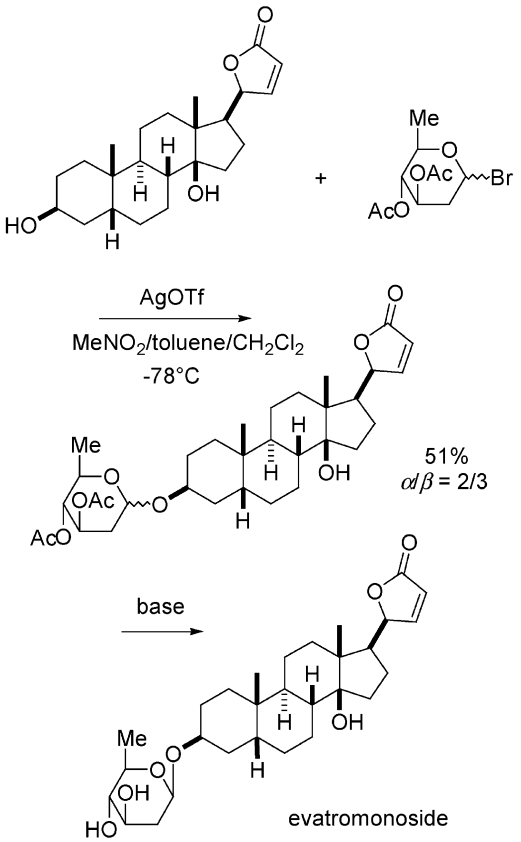


Scheme 12. Synthesis of sarsasapogenin glycosides.

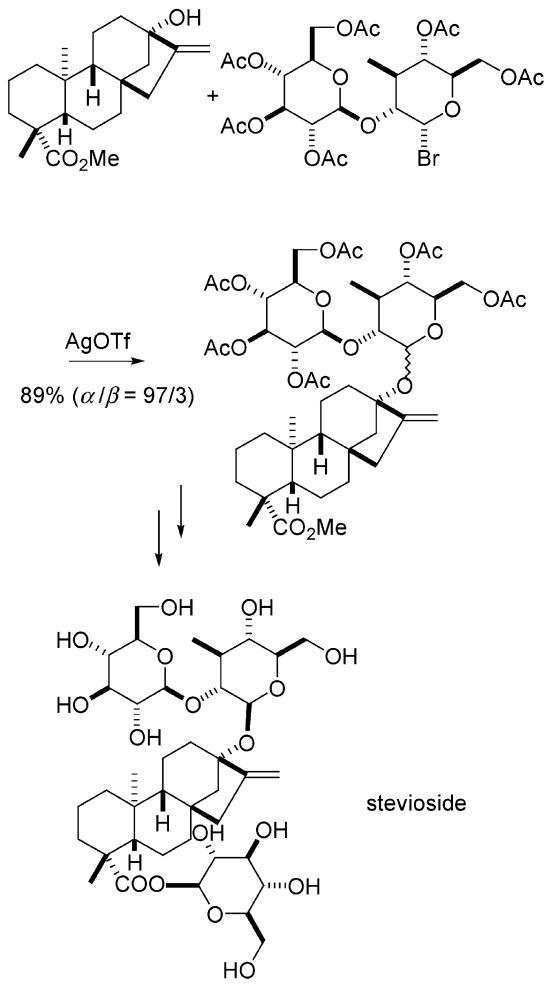
Table 3.

4-OAc	X	Yield (%)	α/β
α	βCl	76	62/38
β	βCl	72	67/33
α	αBr	86	22/78
β	αBr	83	25/75

obtained when a mixture of nitromethane/toluene/dichloromethane (3:7:1) was used as the solvent (Scheme 13).[35] A simple transesterification finally led to the corresponding free glycosides, among which was evatromonoside (β-isomer), a cardiac glycoside.

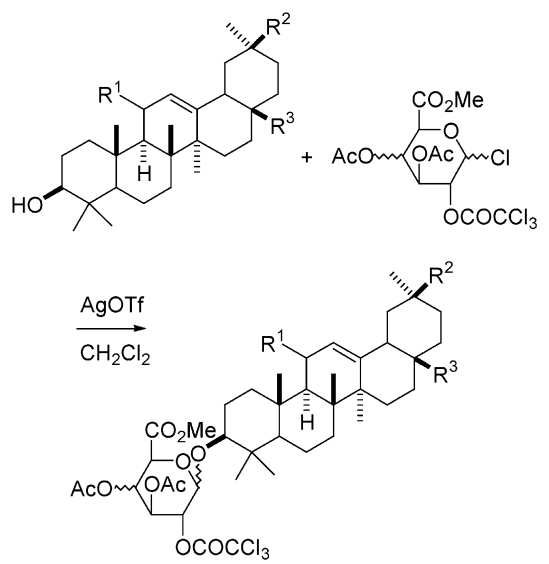


Scheme 13. Synthesis of evatromonoside and its anomer.



Scheme 14. Synthesis of stevioside.

Stevioside is a natural diterpene glycoside 300 times sweeter than sucrose. Its first synthesis was reported by Ogawa et al., starting from steviol methyl ester.[36] When the glycosylation was carried out with 3,4,6-tri-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-glucopyranosyl bromide in the presence of silver triflate and



Scheme 15. Synthesis of glycyrrhetic acid and oleanic acid glycosides.

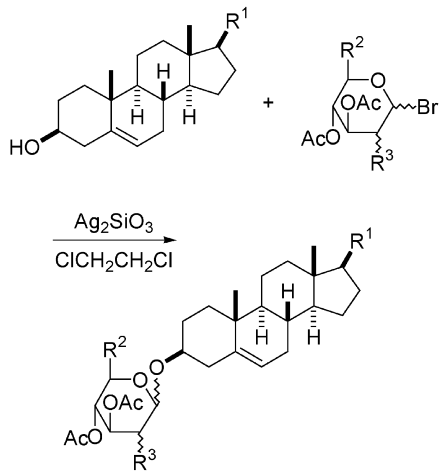
Table 4.

4-OAc	R^1	R^2	R^3	Yield (α/β) (%)
α	=O	CO_2Me	Me	71 (42/58)
β	=O	CO_2Me	Me	70 (38/62)
α	=O	CO_2Me	Me	68 (39/62)
α	H_2	Me	CO_2Me	82 (48/52)

2,4,6-collidine, the desired β-glycoside was formed in only 3% yield, along with the unexpected corresponding α-glycoside obtained in 86% yield (Scheme 14). The orthoester method, however, allowed the stereoselective synthesis of stevioside bearing the β-stereochemistry (see Section 7).

The same procedure was applied by Saito to the preparation of some glycyrrhetic acid and oleanic acid glycosides having cytoprotective activities against hepatic injury (Scheme 15 and Table 4).[37]

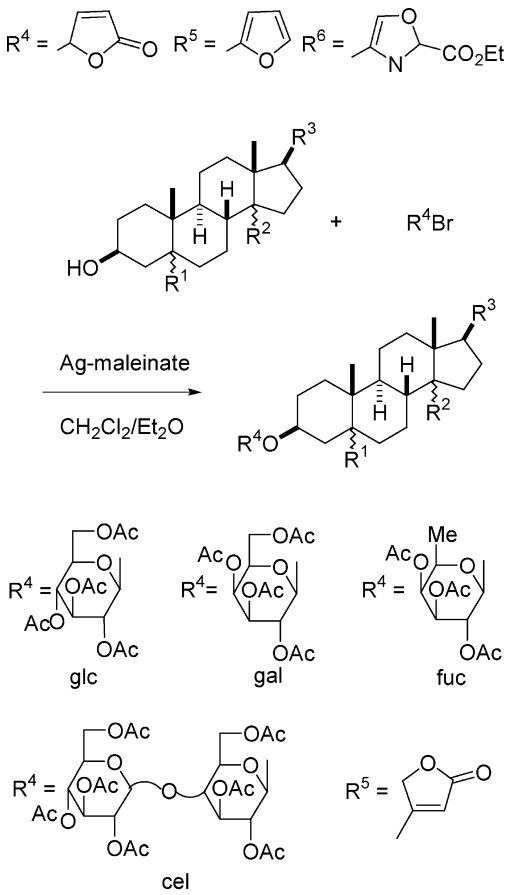
In 1984, Cerny and Pouzar used silver silicate for the first time as a catalyst in the glycosylation of steroids.[39] This new method was particularly well adapted to $\Delta^{5(6)}$ unsaturated and polyfunctionalised steroids (Scheme 16 and Table 5). The corresponding $\Delta^{5(6)}$ saturated steroids were submitted to the same procedure and provided in similar yields the corresponding functionalised steroidal glycosides.[38–43]



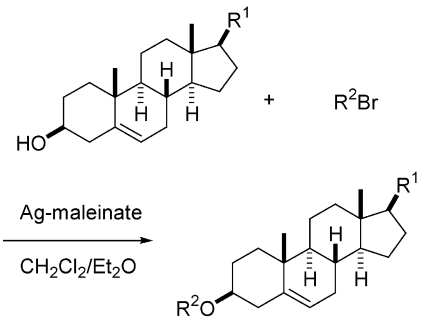
Scheme 16. Glycosylation of $\Delta^{5(6)}$ unsaturated steroids.

Table 5.

R^1	R^2	R^3	Yield (%)	Ref.
OBz	CH_2OAc	αOAc	68 (β)	38
R^4	CH_2OAc	αOAc	78 (β)	38
R^5	CH_2OAc	αOAc	30 (β)	38
CH=CH–CO_2Me	CH_2OAc	αOAc	76 (β)	38
R^6	CH_2OAc	αOAc	50 (β)	39
C(Me)=CH–CO_2Et	CH_2OAc	αF	75 (α/β=1/8)	40
CH=CH–CO_2Me	Me	βOAc	65 (β)	41
CH=CH–CO_2Et	Me	βOAc	61 (β)	41
CH=CH–CN	Me	βOAc	71 (β)	41
CH=CH–CO_2Me	Me	αOAc	65 (β)	42
CH=CH–CO_2Et	Me	αOAc	51 (β)	42
CH=CH–CN	Me	αOAc	45 (β)	42
CH=CH–CO_2Et	Me	βOAc	32 (β)	42
C(Me)=CH–CN	CH_2OAc	αOAc	89 (β)	43
Ac	CH_2OAc	αOAc	80 (β)	43



Scheme 17. Silver maleinate-catalysed glycosylation of steroids.



R^1 = Ac, R^2 = fuc: 33%
R^1 = $COCH_2OH$, R^2 = fuc: 32%
R^1 = C(Me)=CH-CO_2H, R^2 = fuc: 30%

Scheme 18. Silver maleinate-catalysed glycosylation of steroids.

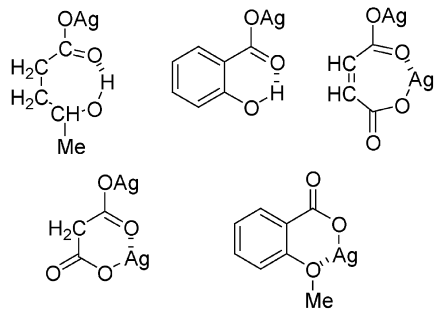
Table 6.

R^1	R^2	R^3	R^4	Yield (%)
βH	βOH	R^5	glc	46
βH	βOH	R^5	gal	49
βH	βOH	R^5	fuc	43
βH	βOH	R^5	cel	39
βH	αH	Ac	fuc	38
αH	αH	Ac	fuc	39

Using the same procedure, Harmatha et al. developed the synthesis of 20-hydroxyecdysone glycosides.[44] Recently, Kreis et al. reported[45] that silver maleinate used as a catalyst showed a superior purity profile for the newly formed glycosides to the other usual catalysts which generally resulted in products with higher impurity profiles, moderate yields or a poor stereoselectivity. On the other hand, silver maleinate (formula depicted in Scheme 19) forced the carbohydrate moiety exclusively into the desired β-configuration. Moreover, this new protocol was shown to be non-destructive, at all stages, to the sugar moiety and the steroidal nucleus.[45] A rapid and efficient procedure for the synthesis of various cardenolide glycosides and putative biosynthetic precursors of cardenolide glycosides was therefore established (Schemes 17 and 18 and Table 6).

In the same way, Helferich et al. reported the silver succinate-promoted glycosylation of cholesterol and cortexon by 3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl chloride, leading to the corresponding steroidal glycosides in respective yields of 50 and 45%.[46]

On the other hand, the silver salts of 2-, 3- and 4-hydroxyalkanoic acids, as well as 1,3- and 1,4-dicarboxylic acids, have been proven to be superior in some cases to the commonly used Ag_2CO_3 or Ag_2O (Scheme 19).[47]



Scheme 19. Silver salts of hydroxyalkanoic acids and dicarboxylic acids.

The silver salt of 4-hydroxyvaleric acid, for example, gave good results with various steroids and sugars such as acetobromoglucose, acetobromogalactose or acetobromoarabinose (Table 7).[47]

Table 7.

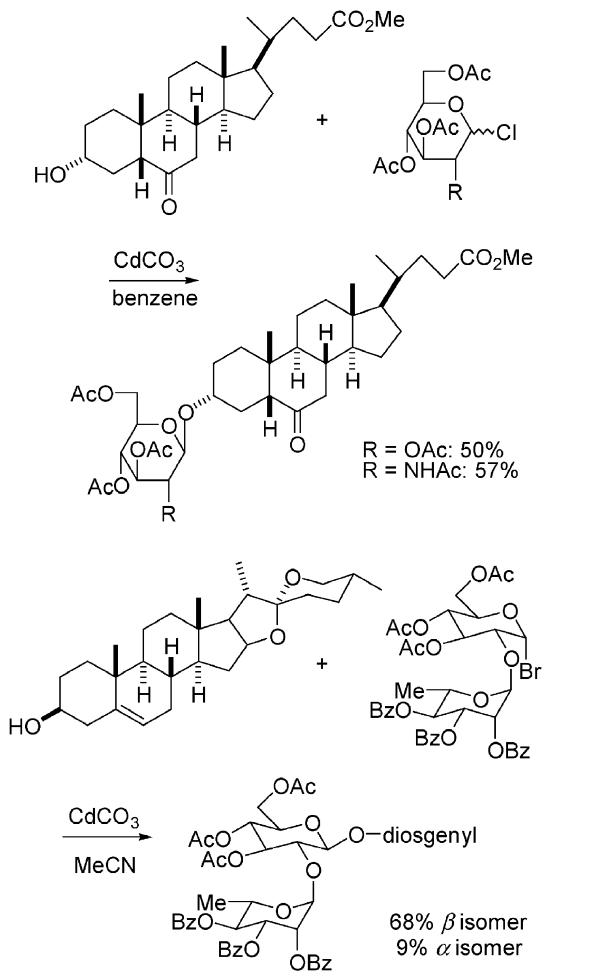
Steroid	Type of acetobromosugar	Yield (%)
Tigogenin	Glucose	65
Tigogenin	Galactose	36
Tigogenin	Arabinose	61
Cholesterin	Glucose	58
Δ^4-Cholestenol	Glucose	48
Digitoxigenin	Glucose	31

2.1.1.2. Using cadmium carbonate as catalyst. In 1971 Bernstein and Conrow proposed a novel catalyst, cadmium carbonate, for the synthesis of steroidal aryl glycosides,[48] and they showed that steroids such as estrone, estradiol derivatives, equilin ($\Delta^{7(8)}$-estrone) or equilenin ($\Delta^{7(8),9(10)}$-estrone) were successfully glucuronidated in the presence of methyl (2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyran)uronate and $CdCO_3$ in toluene (Table 8).

Table 8.

Steroid	Yield (%)
Estrone	71
17β-Formyloxy-estradiol	71
16α,17β-Diformyloxy-estradiol	65
Equilin	68
Equilenin	46

The $CdCO_3$-mediated Koenigs–Knorr reaction was later generalised to other steroids such as hyodeoxycholic acid[49] or diosgenin[50] and to a wide range of sugars as α-acetochloroglucosamine (Scheme 20).

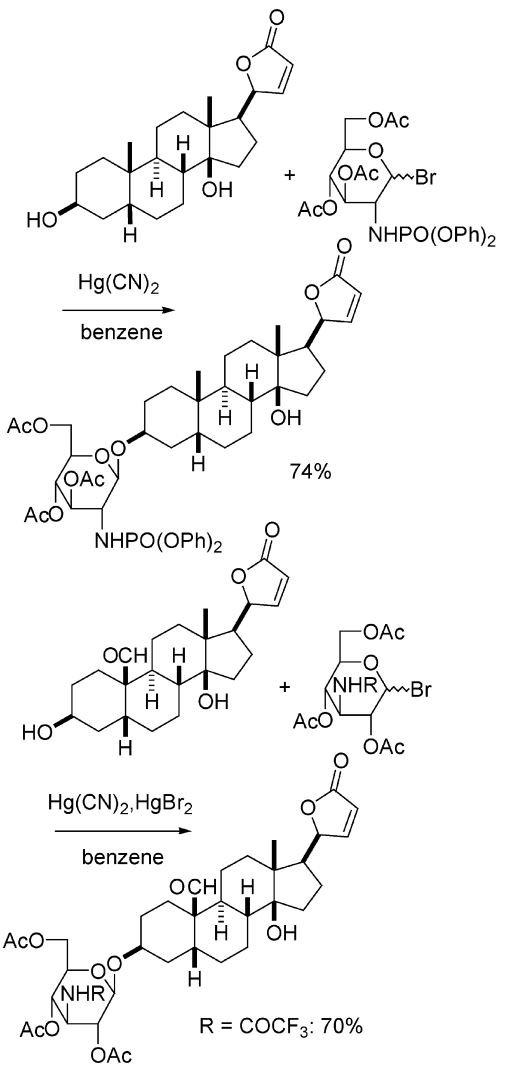


Scheme 20. Synthesis of hyodeoxycholic acid and diosgenyl glycosides via $CdCO_3$.

The exact mechanism of the catalytic action of $CdCO_3$ is unknown. It is believed, however, that the in situ formation of a Cd^{2+} halide is responsible for the heterogeneous catalysis. Conrow and Bernstein have found that commercial anhydrous $CdCl_2$ or $CdBr_2$ was ineffective as a catalyst for the Koenigs–Knorr reaction.[48] They therefore, assumed that the active form of the catalyst, a cadmium halide which is formed in situ, might have different surface properties from the commercial halides.

2.1.1.3. Using mercuric salts as catalysts. Mercuric salts such as mercuric cyanide were introduced as catalysts of oligosaccharide synthesis by Helferich and Wedemeyer in 1949.[51] This latter catalyst was applied for the first time to

steroid glycosylation by Meyer zu Reckendorf et al. in 1971 to produce glucosamine derivatives of digitoxigenin.[52] The same authors used equal parts of mercuric cyanide and mercuric bromide in benzene to prepare other digitoxigenin derivatives (Scheme 21).

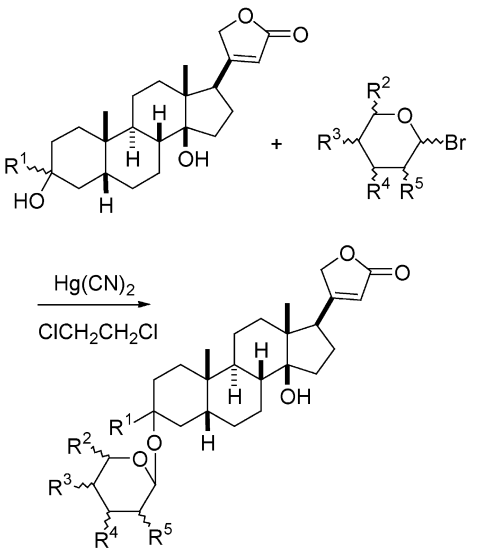


Scheme 21. First mercuric salt-mediated glycosylation of steroids.

In 1977, Albrecht used mercuric cyanide in dichloroethane to synthesise a series of C-3 branched cardenolide glycosides.[53] In 1992, the same conditions were applied by Templeton to digitoxigenin and tribenzoyloxyrhamnopyranosyl bromide, providing the corresponding glycoside in an excellent yield of 93% (Scheme 22).[54]

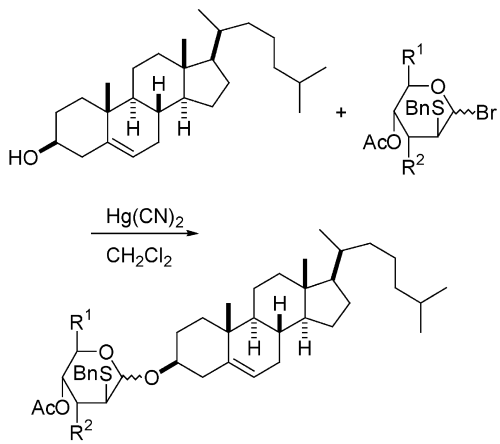
2-Alkylthio- and 2-alkylthio-6-deoxy-hexopyranosyl bromides reacted with cholesterol in the presence of mercuric cyanide, providing a mixture of the corresponding α- and β-glycosides (Scheme 23).[55]

Glycosylation of methyl glycyrrhetinate was performed with 3-*O*-benzyl-1,2,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl bromide in the presence of a mixture of $Hg(CN)_2$ and $HgBr_2$, in order to prepare glycyrrhetic acid diglycosides having cytoprotective activities (Scheme 24).[56]



R^1	R^2	R^3	R^4	R^5	Yield (%)
αMe	βOBz	αOBz	αOBz	αMe	50
βMe	βOBz	αOBz	αOBz	βMe	58
αC_2H	βOBz	αOBz	αOBz	αC_2H	48
βC	βOBz	αOBz	αOBz	βC_2H	61
αMe	αOAc	βOAc	αOAc	αMe	56
βMe	αOAc	βOAc	αOAc	βMe	61
αH	αOBz	βOBz	αOBz	αH	93

Scheme 22. Mercury(II) cyanide-mediated glycosylation of cardenolide steroids.

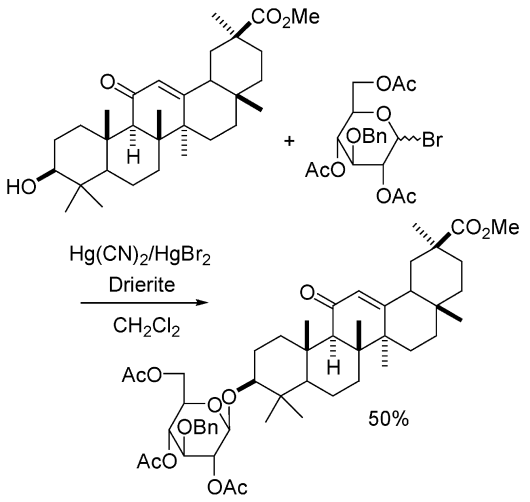


$R^1 = CH_2OAc$, $R^2 = OMe$: 71% ($\alpha/\beta = 55/45$)
$R^1 = Me$, $R^2 = OAc$: 69% ($\alpha/\beta = 26/74$)

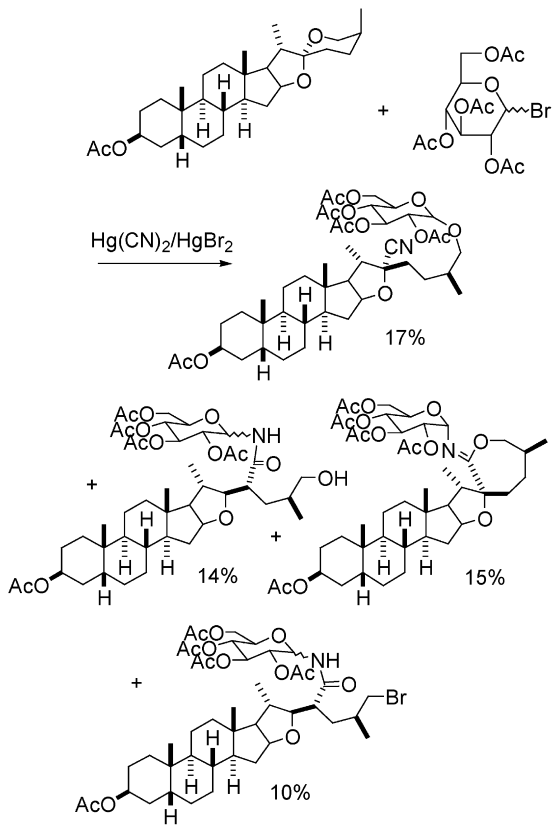
Scheme 23. Synthesis of cholesterin-(2-alkylthio-pyranosides) via $Hg(CN)_2$.

On the other hand, the reaction of 3-*O*-acetylsarsasapogenin with acetylated glucopyranosyl bromide in the presence of the mixed catalyst, $Hg(CN)_2$ and $HgBr_2$, in dry benzene/nitromethane was not selective, since it gave a mixture of four unexpected products which were changed in the structure of the aglycon moiety (Scheme 25).[57]

The proposed mechanisms for the formation of these compounds are depicted in Scheme 26. All of the compounds resulted from cleavage of the F-ring of



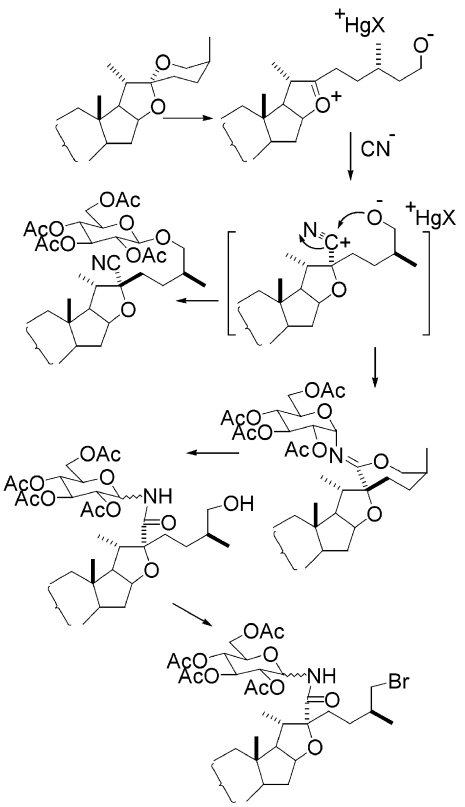
Scheme 24. Synthesis of glycyrrhetic acid glycosides.



Scheme 25. Cyanoglycosylation accompanied by ring opening of spirostanol.

sarsasapogenin accompanied by glycosylation. As a trigger for the cleavage of the F-ring, it was speculated that a cyano anion generated from $Hg(CN)_2$ used as the catalyst attacked at C-22 of the steroid to open the F-ring, and the four products were then produced.

In 1980, Uvarova compared the efficiency of different catalysts for a defined glycosylation. In this study, silver catalysts, mercuric catalysts or cadmium carbonate were successively involved in the glycosylation of cholesterol, β-sitosterol, and 28-*O*-acetylbetulin with acetylated glucopyranosyl bromide.[58] The results, collected in Table 9,



Scheme 26. Proposed mechanisms for the formation of the compounds obtained by glycosylation of 3-*O*-acetylsarsasapogenin.

Table 9.

Steroid	Catalyst	Yield (%)	α/β
Cholesterol	$Hg(CN)_2$	77	2/98
Cholesterol	$Hg(OAc)_2$	77	60/40
Cholesterol	$CdCO_3$	60	8/92
Cholesterol	Ag_2CO_3	60	12/88
Cholesterol	Ag_2O	51	12/88
Cholesterol	$HgO/HgBr_2$	55	10/90
β-Sitosterol	$Hg(OAc)_2$	66	60/40
β-Sitosterol	$Hg(CN)_2$	42	3/97
β-Sitosterol	$CdCO_3$	59	8/92
28-*O*-acetylbetulin	$Hg(OAc)_2$	77	60/40
28-*O*-acetylbetulin	$Hg(CN)_2$	80	2/98
28-*O*-acetylbetulin	$CdCO_3$	70	7/93

showed that the best yields and selectivities were observed with mercuric cyanide in these sterols. The use of cadmium carbonate was also efficient, whereas the α-anomers preponderated when $Hg(OAc)_2$ was the catalyst.

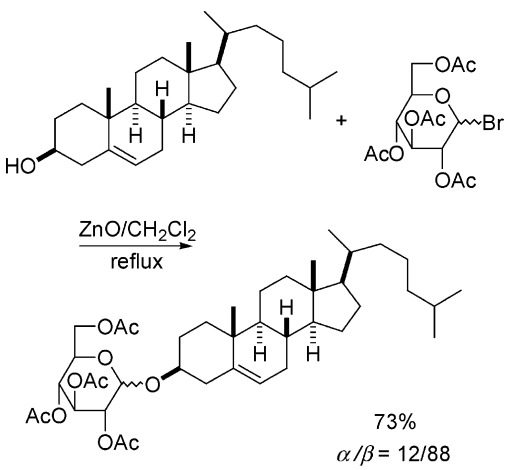
2.1.1.4. Using other catalysts. Classical methods using metals such as mercury or silver, however, have some severe, partly inherent, disadvantages such as the low thermal stability and sensitivity to hydrolysis of many glucosyl halides and the need for stoichiometric quantities of metal salts which are either expensive or hazardous in nature. This becomes a serious limitation, especially in the large-scale preparation of glycosides. Moreover, the use of Hg^{++} salts has been reported to give the product often contaminated with organomercury complexes and Seshadri therefore envisaged the application of a softer, non-oxidising promoter such as Co^{2+}.[59] It was indeed found that sterols reacted with acetobromoglucose in refluxing

benzene (toluene or dichloromethane) in the presence of cobalt carbonate to furnish the corresponding glycoside acetates in good yield. It was noted that no reaction occurred at room temperature. The application of this new method allowed the preparation of several steroid glycosides in 60–68% yields (Table 10).

Table 10.

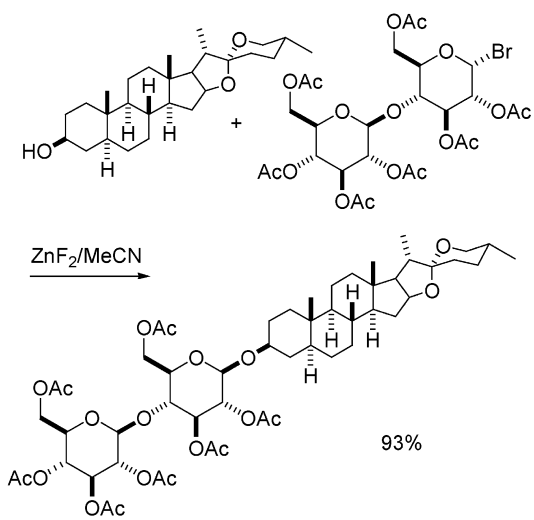
Steroid glycoside	Yield (%)
Cholesterol 2,3,4,6-tetra-*O*-acetyl-β-D-glycoside	68
β-Sitosterol 2,3,4,6-tetra-*O*-acetyl-β-D-glycoside	62
Stigmasterol 2,3,4,6-tetra-*O*-acetyl-β-D-glycoside	60

In 1998, Gurudutt reported a convenient preparative method for the α/β-glucosides of cholesterol based on the use of zinc salts such as zinc oxide (Scheme 27).[60]



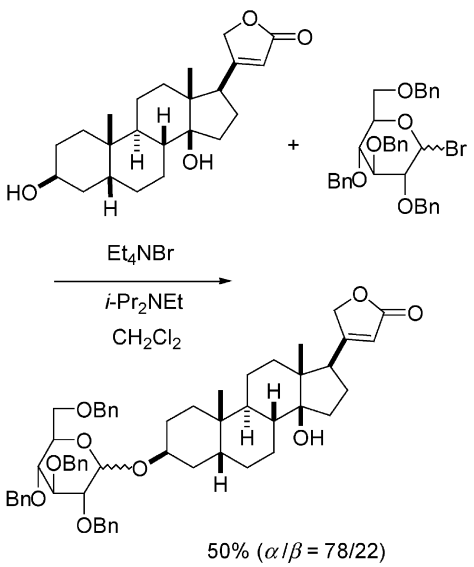
Scheme 27. Zinc oxide-mediated glycosylation of cholesterol.

Similarly, DeNinno reported a very efficient ZnF_2 catalysis in acetonitrile to prepare 11-ketotigogenin cellobioside (after deacetylation), a highly potent cholesterol absorption inhibitor (Scheme 28).[61] The method was applied to other modified cellobiose analogues also having lipid-lowering properties.



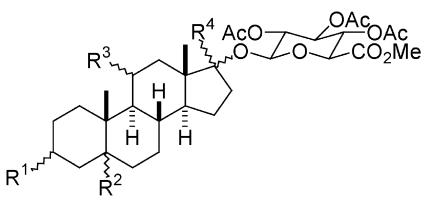
Scheme 28. Synthesis of 11-ketotigogenin peracetate via ZnF_2-mediated glycosylation.

2.1.1.5. Using no catalysts. Finally, other glycosylation methods using glycosyl bromide and chloride in the absence of any metal were studied and Lemieux and co-workers[62] introduced a mild glycosylation in the presence of Et_4NBr and diisopropylethylamine in dichloromethane at 20 °C. This procedure could be applied by Fullerton et al. to the glycosylation of digitoxigenin and 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl bromide, providing the corresponding digitoxigenin α- and β-glucosides (Scheme 29).[93]



Scheme 29. Glycosylation of digitoxigenin without catalyst.

2.1.2. Glycosylation at positions other than C-3 of steroids. Sexual hormones such as estrogens or androgens bear a hydroxy group at position C-17. They are excreted in the urine as conjugates, primarily as glucosiduronates. In order to study the metabolism of these hormones, it was essential to be able to prepare their corresponding glycosides as reference standards and for radioimmunoassays. The first C-17



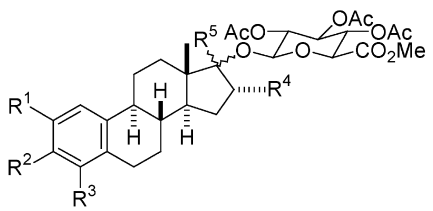
Scheme 30. C-17 androgen glucuronides.

Table 11.

Entry	R^1	R^2	R^3	R^4	Δ	Catalyst	Yield (%)	Ref.
1	=O	—	H	αH	4.5	Ag_2CO_3	30	63
2	βOAc	αH	H	αH	6.7	Ag_2O	51	67
3	αOAc	αH	—	αH	9.10	Ag_2CO_3	37	68
4	αONO_2	βH	H	αH	—	Ag_2SiO_3	65	69
5	βONO_2	βH	H	αH	—	Ag_2SiO_3	60	69
6	βOAc	—	H	αH	5.6	Ag_2SiO_3	54	69
7	βOAc	—	H	βH	5.6	Ag_2SiO_3	45	69
8	αOAc	αH	αOTBDMS	αH	—	Ag_2CO_3	32	64
9	=	—	H	αD	4.5	$CdCO_3$	60	66

glycosylation of steroids dealt with testosterone and was reported in 1959 by Wotiz et al. (Scheme 30 and Table 10, entry 1).[63] Since then, several glucosides of androstanediol have been prepared since some of them show peripheral 5α-reductase activity (Table 11, entry 8).[64] Moreover, high levels of these latter products are also indicative of increased dehydroepiandrosterone or dehydroepiandrosterone sulphate production. On the other hand, it has been suggested that androstanediol-glucuronide might serve as a marker for events occurring in steroid target tissues.[65] More recently, isotopically pure [16,16,17-$^2H_3$]-testosterone and -epitestosterone glucuronides have been prepared to be used in athletics to detect testosterone doping by direct measurements of T- and EpiT-conjugates excreted in urine (Table 11, entry 9).[66] Similarly, several C-17 glucuronides of estriol derivatives have been prepared to be used in enzyme immunoassay systems (Scheme 31 and Table 12). In all cases, the sole sugar involved in glycosylation at C-17 has been acetylated glucopyranosyl bromide (Tables 11 and 12).

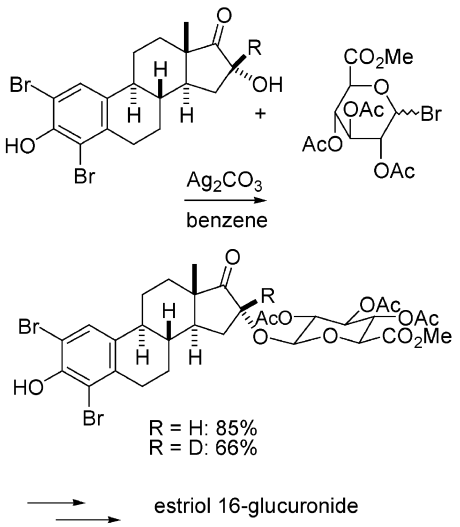
16-Hydroxy estrogens are major estrogens in pregnancy and are mainly found as the 16-glucuronides in the urine and in plasma. The considerable current clinical interest in the correlation of estriol 16-glucuronide concentration during



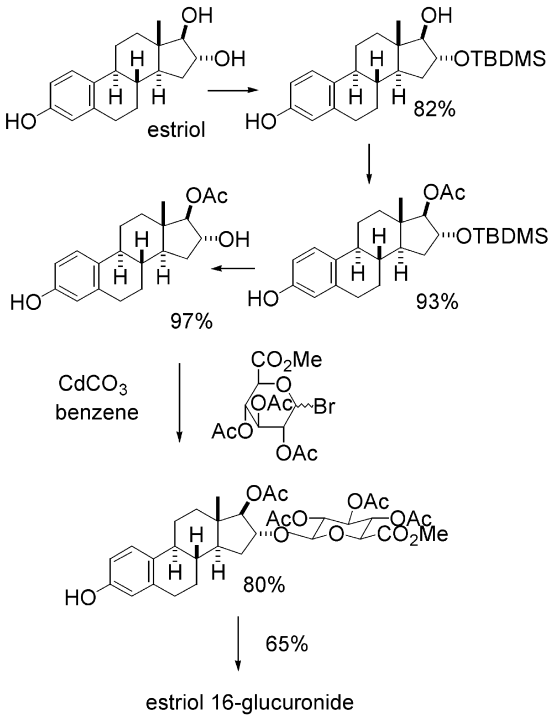
Scheme 31. C-17 estrogen glucuronides.

Table 12.

Entry	R^1	R^2	R^3	R^4	R^5	Catalyst	Yield (%)	Ref.
1	H	OBn	H	OH	αH	Ag_2CO_3	31	70
2	H	OH	H	H	αC_2H	$CdCO_3$	11	71
3	Br	OH	Br	H	αH	Ag_2CO_3	72	72
4	H	OAc	H	H	αOH	Ag_2SiO_3	43	69
5	H	OAc	H	H	βOH	Ag_2SiO_3	72	69
6	H	OH	H	OTBDMS	αH	$CdCO_3$	47	73



Scheme 32. Synthesis of estriol 16-glucuronide.

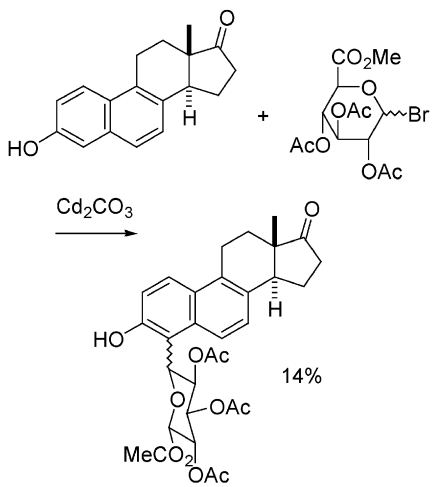


Scheme 33. Synthesis of estriol 16-glucuronide.

pregnancy with foetal maturity has led to a number of approaches to its determination. Numazawa et al. reported in 1981 a synthesis of estriol 16-glucuronide via 2,4,16α-tribromoestrone (Scheme 32).[74] These authors applied the same conditions to the preparation of the corresponding deuterated compound to be used as a marker (Scheme 32).[75]

In 1993, Blackwell et al. reported another method for the synthesis of estriol 16-glucuronide starting from estriol and based on the selective protection and deprotection of the hydroxyl groups (Scheme 33).[73]

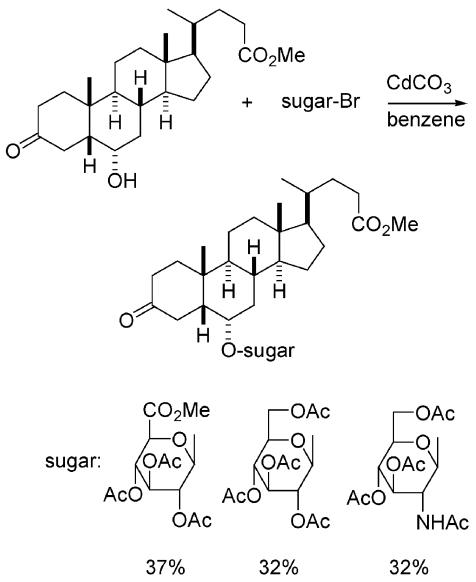
A novel and very unusual by product, obtained in significant yield (14%) from the $CdCO_3$-mediated glucuronidation of equilenin (providing the major C-3 steroidal glycoside in 48% yield), was characterized as C-4-glucuronosyl equilenin (Scheme 34).[48] To the best of the author's



Scheme 34. Synthesis of C-4-glucuronosyl equilenin.

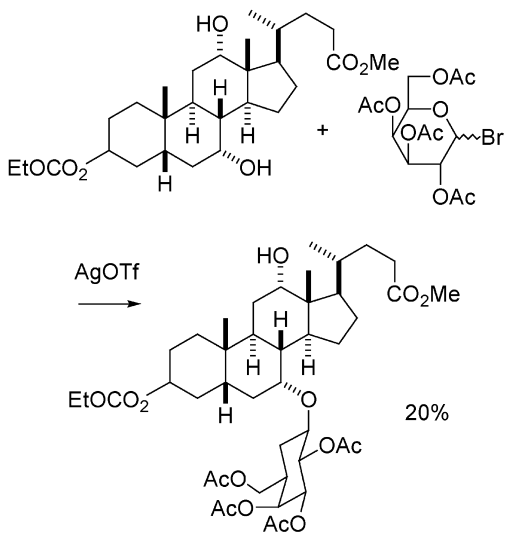
knowledge, this is the sole C-4 steroidal glycoside present in the literature.

In 1994, Iida reported the synthesis of hyodeoxycholic acid 6-glycosides, which are of considerable interest in view of their biosynthetic and metabolic roles (Scheme 35).[49]



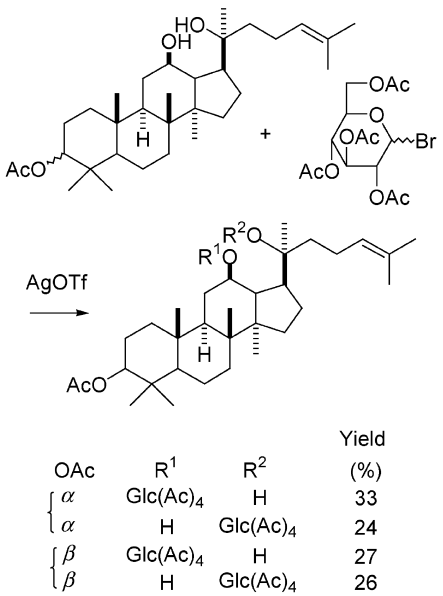
Scheme 35. Synthesis of hyodeoxycholic acid 6-glycosides.

A rare glycosylation reaction at the 7-hydroxyl group of cholic acid methyl ester was reported by Nakanishi.[76] Since the order of reactivity the hydroxyl groups in cholic acid is 3α>7α>12α, only the 3α-hydroxyl group needed to be protected (Scheme 36).



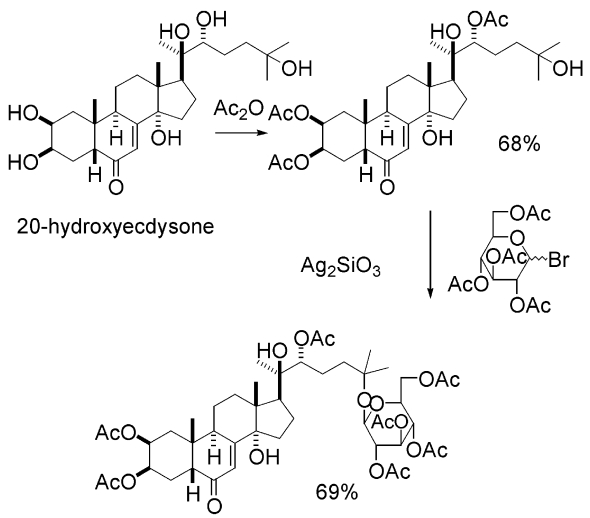
Scheme 36. Galactosidation of 3-protected cholic acid methyl ester.

On the other hand, Uvarova et al. showed that glycosylation of the dammar-24-ene-3,12β,20(*S*)-triols with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide in the presence of silver oxide in dichloromethane was not regioselective, even when the 3-hydroxyl group was acetylated (Scheme 37),[77] and a mixture of C-12 and the corresponding C-20 steroidal glycosides was obtained.



Scheme 37. Non-regioselective glucosylation of dammarane derivatives.

The growing number of ecdysteroid conjugates isolated from both animals and plants suggests an active role in the ecdysteroid metabolism, transport or deactivation. In the course of preparing 20-hydroxyecdysone glycosides, Harmatha et al. achieved a regioselective course of glycosylation of 20-hydroxyecdysone by a combination of hydroxyl and 1,2-diol protecting groups, i.e. acetates, in the aglycone moiety (Scheme 38).[44]

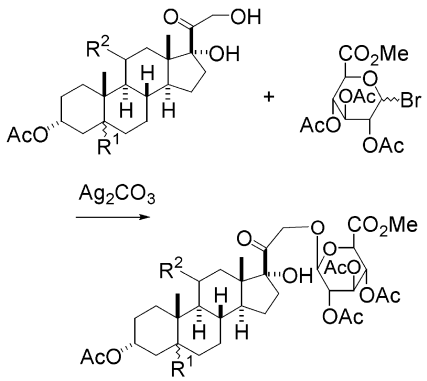


Scheme 38. Regioselective glucosylation of 20-hydroxyecdysone.

When the starting steroid had a free hydroxyl group at C-22 instead of an acetate group, however, the glycosylation carried out using the same conditions was much less regioselective, since a mixture of C-22 and C-25 steroidal glucosides was obtained in 9 and 32% yields, respectively.

Cortisol metabolism in humans involves various transformations such as oxidation at C-11 and reduction of the Δ-3-keto group to form 3α-hydroxy products. The metabolites are tetrahydrocortisol, its 5α-isomer, cortols, cortolic acids and their 11-keto derivatives. These are excreted in the urine as conjugates with glucuronic acid. The level of

urinary 17-hydroxycorticosteroids has long been an important index of adrenocortical activity. For use in metabolic studies and immunoassays of corticosteroids, Nambara developed the syntheses of C-21 glucuronides of cortisol and cortisone derivatives (Scheme 39 and Table 13).



Scheme 39. C-21 glycosylation of cortisol and cortisone derivatives.

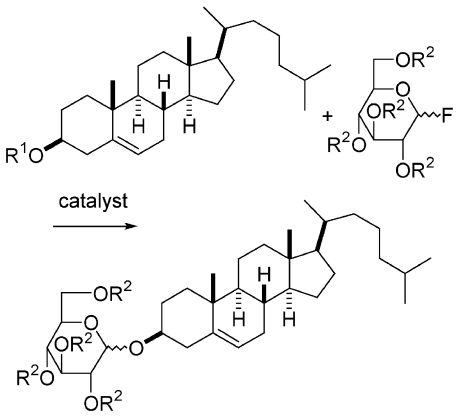
Table 13.

R^1	R^2	Yield (%)	Ref.
βH	βOH	81	20
βH	=O	37	20
αH	βOH	54	25
αH	=O	47	25
αH	H	68	23

2.2. Glycosylation using glycosyl fluorides

The use of a glycosyl fluoride as a glycosyl donor was first introduced by Mukaiyama et al. in 1981.[78] One of the notable advantages of a glycosyl fluoride as a glycosyl donor is its higher thermal and chemical stability as compared to the low stability of the other glycosyl halides. This is due to the higher strength of the carbon–fluorine bond, as compared to the other carbon–halogen bonds. The glycosyl fluoride can therefore be generally purified by an appropriate distillation and even by column chromatography with silica gel. A number of specific fluorophilic reagents have been developed having such favourable synthetic attributes. In 1984, for example, Noyori et al. found that tetrafluorosilane effectively catalysed the condensation of appropriately protected glycopyranosyl fluorides and trimethylsilyl ethers of cholesterol.[79] They observed a dependence of the anomeric configuration of the glycoside formed on the polarity of the medium (Et_2O or MeCN). When the glycosylation was carried out in acetonitrile/ether (1/1), the corresponding β-glycosides were obtained with high stereoselectivity, whereas the reaction in ether afforded the α-anomers predominantly (Scheme 40 and Table 14). In 1985, Kunz et al. reported the use of boron trifluoride etherate as a catalyst in similar reactions. Indeed, this non-hydrolytically sensitive and non-volatile catalyst allowed the avoidance of a heterogeneous reaction, achieving highly stereoselective glycosylations of cholesterol and its corresponding silyl ether (Scheme 40 and Table 14).[80]

Similarly, in the reaction of 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranosyl fluoride, α-D-glycosides were formed

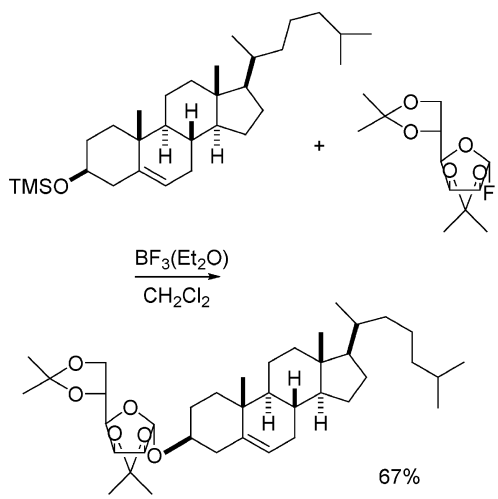


Scheme 40. SiF_4- and $BF_3(Et_2O)$-mediated glycosylations of cholesterol and glucopyranosyl fluorides.

Table 14.

R^1	R^2	F	Catalyst	Solvent	Yield (%)	α/β	Ref.
TMS	Bn	α	SiF_4	$MeCN/Et_2O$ (1/1)	85	19/81	79
TMS	Bn	β	SiF_4	$MeCN/Et_2O$ (1/1)	86	20/80	79
TMS	Bn	β	SiF_4	Et_2O	72	77/23	79
TMS	Bn	α	$BF_3(Et_2O)$	CH_2Cl_2	81	86/14	80
H	Bn	α	$BF_3(Et_2O)$	CH_2Cl_2	77	86/14	80
H	Piv	α	$BF_3(Et_2O)$	CH_2Cl_2	84	0/100	80

highly stereoselectively. This example reveals an additional advantage of the method, namely the complete conservation of the acid-labile acetal protection during the mild glycosylation procedure (Scheme 41).[80]

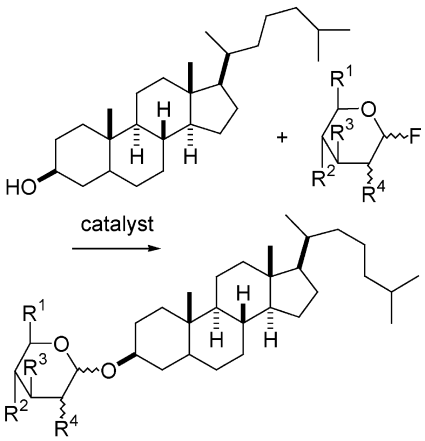


Scheme 41. Synthesis of 3β-cholesteryl 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranoside.

Another activation system for glycosyl fluorides, Cp_2HfCl_2–$AgClO_4$, was involved by Suzuki et al. in the course of preparing mycinamycin macrolide antibiotics.[81] Indeed, this preparation was a challenge because of the existence of the basic Me_2N group at C-3 of the very sensitive glycosyl acceptor, D-desosamine, which may interfere with the glycosidic activation. Finally, 1-fluoro-2-*O*-methoxycarbonyl-D-desosamine could be successfully glycosylated with cholestanol in high yield and selectivity by this method (Scheme 42 and Table 15). The glycosylation of tetra-*O*-benzyl-D-mannopyranosyl fluoride and the same sterol,

Table 15.

R^1	R^2	R^3	R^4	Catalyst	Solvent	Yield (%)	α/β	Ref.
Me	H	NMe_2	αOCO_2Me	$Cp_2HfCl_2-AgClO_4$	CH_2Cl_2	92	0/100	81
Me	H	NMe_2	αOCO_2Me	$SnCl_2-AgClO_4$	CH_2Cl_2	97	0/100	81
CH_2OBn	OBn	OBn	βOBn	$Cp_2ZrCl_2-BF_4$	Benzene	94	96/4	82
CH_2OBn	OBn	OBn	βOBn	$Cp_2ZrCl_2-AgClO_4$	Benzene	91	94/6	82



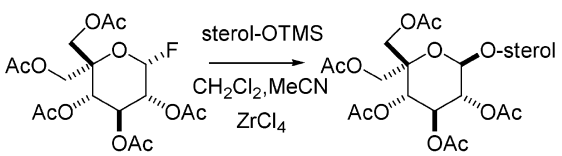
Scheme 42. Cp_2HfCl_2- or Cp_2ZrCl_2-$AgClO_4$-mediated glycosylations of cholestanol.

however, gave better results with the combination $Cp_2ZrCl_2-AgBF_4$ in benzene, yielding highly selectively the α-mannosides (Scheme 42 and Table 15).[82]

More recently, Pikul studied the glycosylation of 2,3,6,6′-penta-*O*-acetyl-5-hydroxymethylgalactosyl fluoride and various trimethylsilyl ethers of sterols and found that the best catalyst was, in this case of this sugar, zirconium tetrachloride (Scheme 43 and Table 16).[83] The method was proven to be specific to this sugar since no glycosylation occurred when it was applied to more common sugars such as peracetylated glucosyl- or galactosyl fluoride. This result was explained by the additional 1,3-neighbouring group assistance of the C-5 acetoxymethylene group.

2.3. Thermal glycosylation

In 1988, Nishizawa et al. described the first thermal

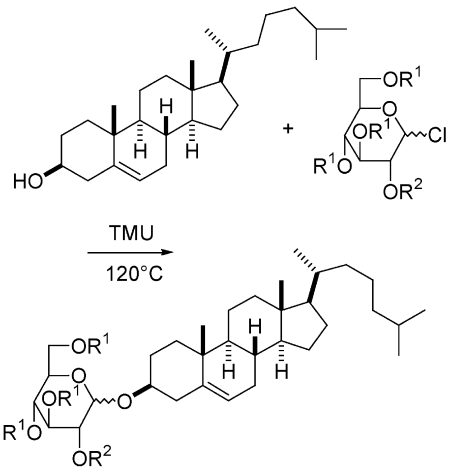


Scheme 43. Glycosylation of sterols with peracetylated 5-hydroxymethylene galactosyl fluoride.

Table 16.

Sterol	Yield (%)
Diosgenin	58
Tigogenin	45
Sarsasapogenin	27
Cholesterol	43
Stigmasterol	62
Stigmastanol	64

glycosidation without using any metal salts or traditional glycosylation promotors or a Lewis acid.[84] This very simple procedure, which consists of heating the starting materials at 120 °C in the presence or absence of tetramethylurea (TMU), was applied for the first time to cholesterol, providing no stereoselectivity and almost equal amounts of the corresponding α- and β-glucosides (Scheme 44).[85]

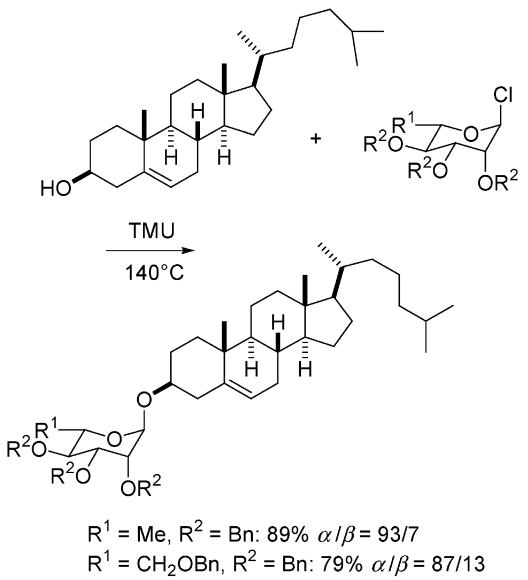


Scheme 44. First thermal glycosylation.

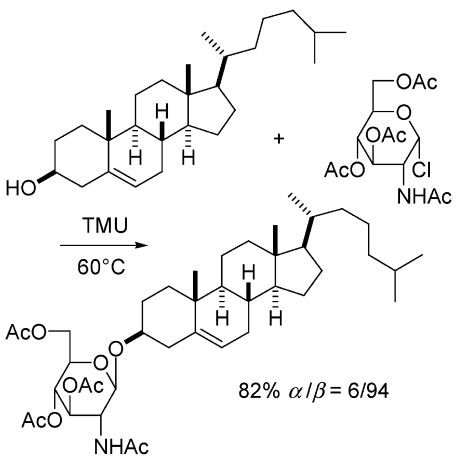
No stereoselectivity was also observed during the glycosylation of dihydrolanosterol with the same sugar, since the two corresponding anomeric glycosides were isolated in 55% yield and an α/β ratio of 59/41. When the procedure was applied with 2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl chloride and 2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl chloride, however, highly α-selective glycosylation resulted (Scheme 45).[86] Total α-stereoselectivity was obtained in the case of glycosylation of dihydrolanosterol with 2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl chloride, but in lower yield (48%).

On the other hand, the same authors found a dramatic stereoselectivity depending upon the reaction temperature in the course of thermal glycosylation involving an aminosugar chloride such as 2-deoxy-2-acetoamino-3,4,6-tri-*O*-acetyl-glucopyranosyl chloride.[87] The thermal glycosylation of this latter halide with cholesterol at 60 °C gave rise to the corresponding β-glycoside selectively, whereas a higher temperature (120 °C) favoured the α.-glycoside (Scheme 46).

The total synthesis of the intensely sweet saponin (3000 times sweeter than glucose), osladin, reported by Nishilving a newly developed β-selective and 2′-hydroxyl group



Scheme 45. α-Stereoselective thermal glycosylation.



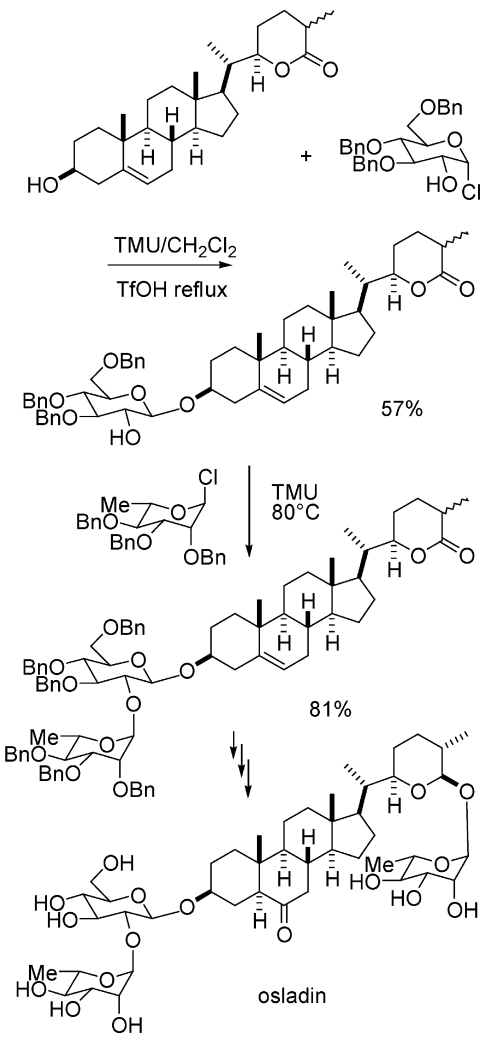
Scheme 46. β-Stereoselective thermal glycosylation.

discriminating glucosylation procedure and an α-selective thermal rhamnosylation reaction (Scheme 47).[88]

Tigogenyl β-cellobioside is a synthetic spirostane glycoside which represents a novel hypocholesterolemic and anti-atherosclerosis agent. The synthesis of this compound was achieved by thermal glycosylation of β-cellobiosyl fluoride heptaacetate with tigogenin followed by deacetylation (Scheme 48).[89]

3. Trihalogenoacetimidates

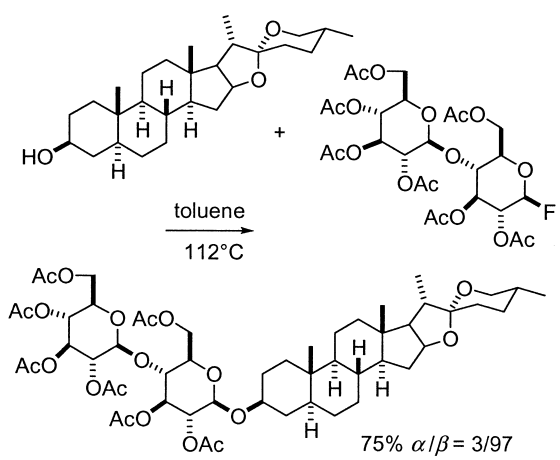
Trichloroimidate-mediated glycosylation was reported by Schmidt and his co-workers[90] in 1980 as an alternative useful method to the classical Koenigs–Knorr procedure and now appears to be one of the most ideal glycosylation protocols. In this case, the glycosylation reaction is smoothly promoted by the catalytic use of $BF_3{\cdot}Et_2O$ or TMSOTf. Some other Lewis acids such as PPTS, $ZnCl_2$ or $SnCl_4$ were also investigated. Some advantages of this reaction are the very mild conditions, its irreversible nature, the thermal stability and ease of preparation of the



Scheme 47. Total synthesis of osladin.

trichloroacetimidates, the usually good chemical yield, the good stereocontrol and the lack of affect on other glycosidic bonds. Various sugar moieties have been readily attached to number of steroids using the trichloroacetimidate method (Scheme 49 and Table 17).

When the starting trichloroacetimidate is a mixture of the two anomers, the stereochemical outcome of the reaction



Scheme 48. Thermal glycosylation of tigogenin.

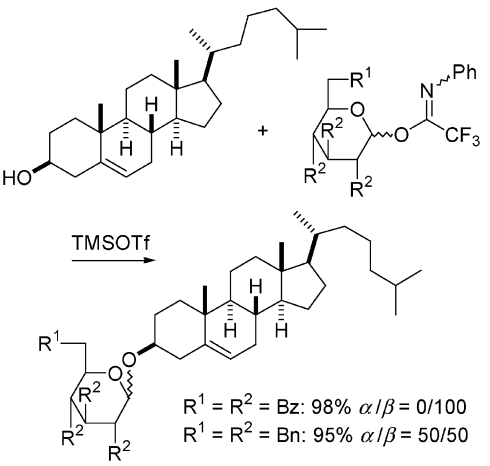
depends on the nature of the neighbouring participating groups. Not surprisingly, donors with neighbouring participating groups give only the corresponding 1,2-*trans* products, whilst donors without a neighbouring group give mixtures of the α- and β-anomeric glycosides.

The corresponding glycosyl trifluoroacetimidates have been readily prepared by Yu et al. and demonstrated to be effective glycosyl donors in the presence of TMSOTf. For instance, they could be condensed with cholesterol in high yield and selectivity (Scheme 50).[98]

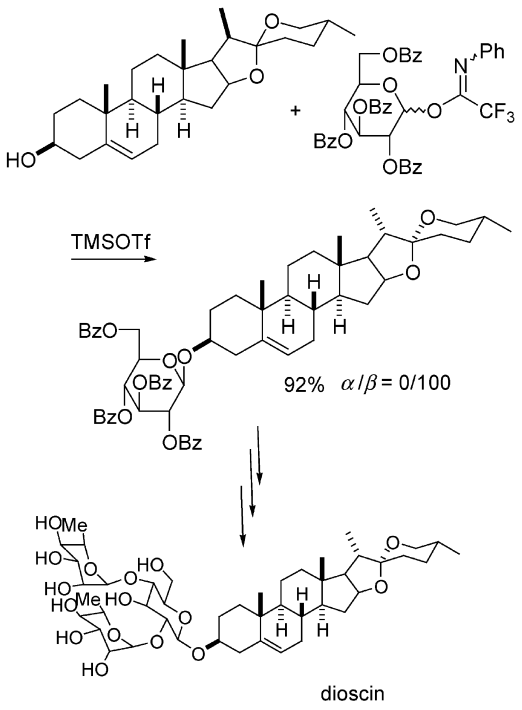
The same methodology was applied to a synthesis of a trisaccharide saponin, dioscin (Scheme 51).[99]

Saponins containing *N*-acetylglucosamine are rare, with their numbers being less than 30 and their structures highly limited. Very recently, Yu and co-workers have reported the synthesis of such a saponin showing significant activity against lung cancer cell lines via glycosylation of oleanic acid with 2-deoxy-2-phthalimido-D-glucopyranosyl trifluoroacetimidate (Scheme 52).[100]

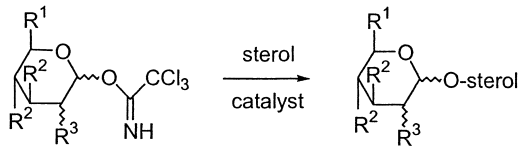
With the aim of preparing aminoglycosides of cardioactive steroids, Finizia studied the stereoselectivity of glycosylation of 3-amino-2-deoxydigitoxose derivatives with digitoxigenin analogues.[101] The lack of functionality at the carbohydrate C-2 made the achievement of the required β-stereoselectivity of the coupling reaction difficult. Moderate stereoselectivities were observed during the glycosylation of digitoxigenin and (23-*R*)-23-methylisodigitoxigenin with a 3-amino-2-deoxy-*a*-D-ribohexopyranosyl trichloroacetimidate derivative (Scheme 53). The best β-stereoselectivity was observed using $MgCl_2$ as the Lewis acid (α/β=38/62).



Scheme 50. Glycosyl trifluoroacetimidates as glycosyl donors.



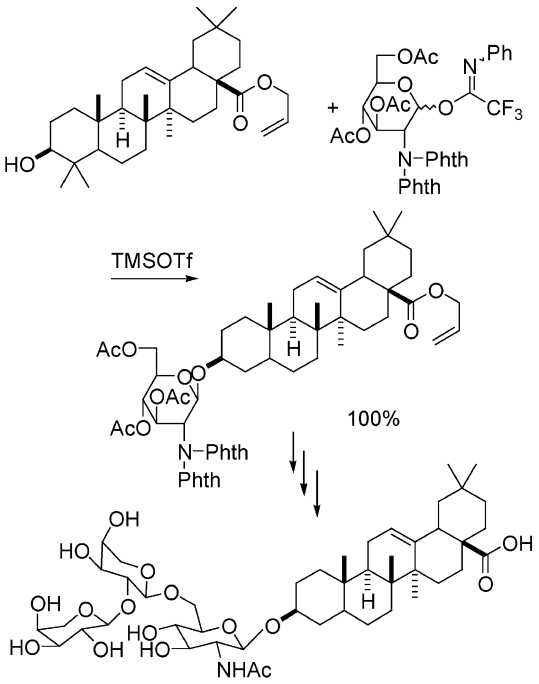
Scheme 51. Synthesis of dioscin.



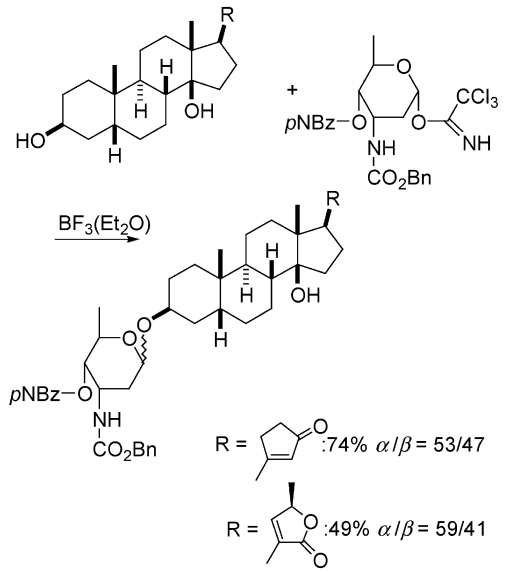
Scheme 49. Trichloroimidate-mediated glycosylation of steroids.

Table 17.

R^1	R^2	R^3	Imidate	Steroid	Catalyst	Yield (%)	α/β	Ref.
CO_2Me	OBn	α OBn	α	Cholesterol	$BF_3(Et_2O)$	91	0/100	91
CO_2Me	OBn	α OBn	α	Sitosterol	$BF_3(Et_2O)$	92	0/100	91
CH_2OBn	OBn	α OBn	α	Cholesterol	$BF_3(Et_2O)$	78	7/93	7
CH_2OBn	OBn	α OBn	α	Cholesterol	PPTS	80	50/50	7
CH_2OBn	OBn	β OBn	α	Cholestanol	$BF_3(Et_2O)$	83	83/17	92
CH_2OBn	OBn	α OBn	α	Digitoxigenin	$BF_3(Et_2O)$	85	24/76	93
CH_2OBn	OBn	α OBn	β	Digitoxigenin	TMSOTf	77	75/25	93
CH_2OBn	OBn	β OBn	β	Digitoxigenin	TMSOTf	89	66/34	94
CH_2OBn	OBn	β OBn	α	Digitoxigenin	$BF_3(Et_2O)$	89	31/69	94
CH_2OBn	OBn	β OBn	β	Digitoxigenin	TMSOTf	90	31/69	94
CH_2OBn	OBn	β OBn	α	Digitoxigenin	$BF_3(Et_2O)$	80	24/76	94
Me	OBn	β OBn	β	Digitoxigenin	TMSOTf	48	90/10	94
Me	OBn	β OBn	α	Digitoxigenin	$BF_3(Et_2O)$	55	20/80	94
CO_2Me	OAc	αOAc	α	Estrone	$BF_3(Et_2O)$	42	0/100	95
CO_2Me	OAc	αOH	α	Estrone	$BF_3(Et_2O)$	94	0/100	95
CH_2OBn	OBn	α OBn	α	Diosgenin	TMSOTf	100	0/100	96
CH_2OBn	OBn	α OBn	α	Cholesterol	TMSOTf	90	0/100	96
CH_2OBn	OBn	α OBn	α	Allyl oleanate	TMSOTf	100	0/100	96
CH_2SPh	OAc	α OBn	α	Cholesterol	TMSOTf	82	0/100	97



Scheme 52. Synthesis of an *N*-acetylglucosamine-containing plant saponin.

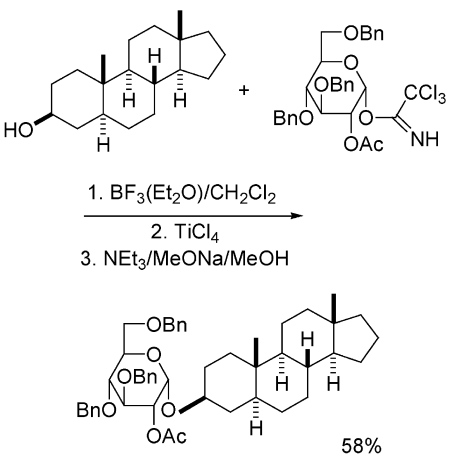


Scheme 53. Synthesis of aminoglycosides of cardioactive steroids.

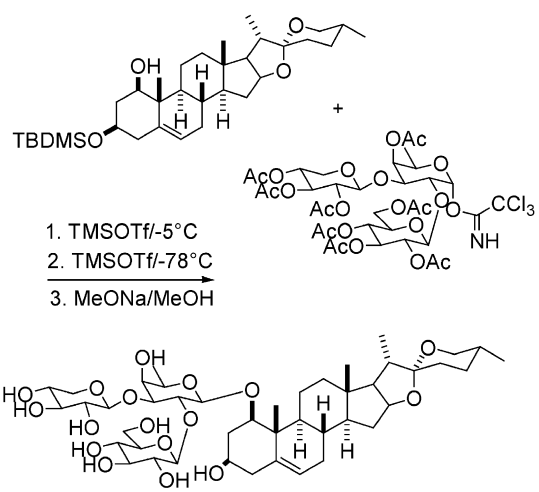
On the other hand, in the presence of $SnCl_4$, the α-anomer was the sole product.

The stereoselective α-glycosylation of sterols was, moreover, possible by using the three-step, one-pot procedure reported by Charette, which involved as the second step an in situ anomerisation upon treatment of the β-glycoside with $TiCl_4$ (Scheme 54).[102]

Steroids bearing a hydroxy group at a position other than C-3 have also been glycosylated by the acetimidate method.[103] For instance, a steroidal trisaccharide saponin possessing strong anti-inflammatory and immunopharmacological activities has been prepared under Schmidt's 'inverse



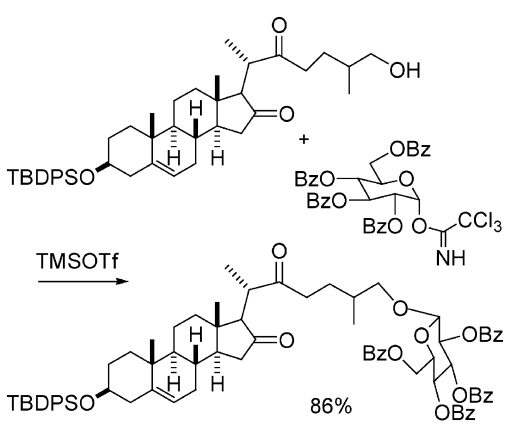
Scheme 54. One-pot synthesis of an α-glycoside.



Scheme 55. Synthesis of a steroidal trisaccharide saponin by an 'inverse procedure'.

procedure',[104] which consists of a previous activation of the alcohol by treatment with TMSOTf before adding the imidate, whilst the normal procedure of glycosylation failed owing to the steric hindrance of 1-OH (Scheme 55).

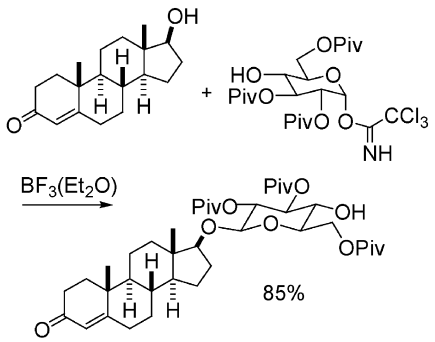
The first synthetic route to furostan saponins exhibiting a β-D-glucopyranose substituent at the 26-OH and with



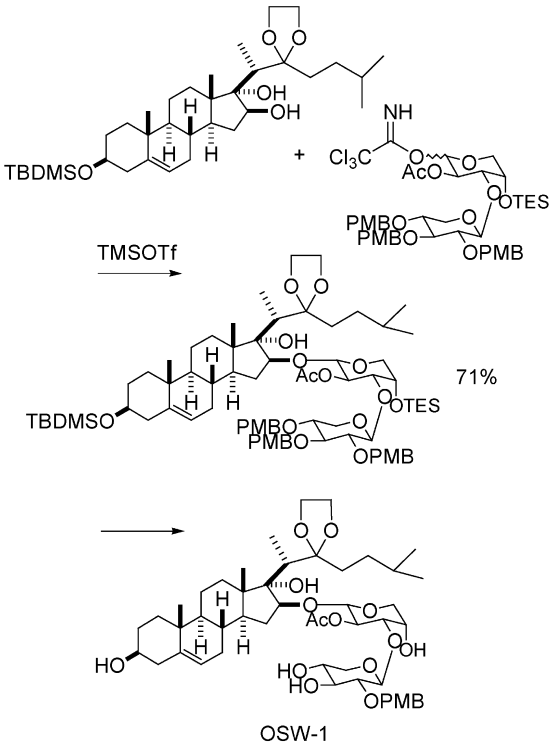
Scheme 56. Synthesis of a furostan saponin.

another sugar chain attached, usually at the 3-OH, was recently reported.[105] The key step of the 11-step synthesis was the glycosylation of a diosgenin derivative by 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate in the presence of TMSOTf (Scheme 56).

Becker et al. demonstrated in 1992 that glucose could be coupled via the imidate to testosterone having a potential site for further reaction at the unprotected 4-hydroxyl group, an option of importance in the synthesis of carbohydrate derivatives (Scheme 57).[106]

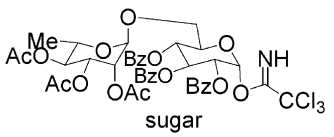


Scheme 57. Glycosylation of testosterone with an unprotected 4-hydroxy-glucopyranosyl imidate.



Scheme 58. Synthesis of OSW-1.

Several disaccharides could be condensed with total β-stereoselectivity on various steroids via Schmidt's trichloroacetimidate method. The results are collected in Table 18.



The highly potent antitumour natural product OSW-1 is an attractive synthetic target. It has been proven that the steroid aglycone and the sugar moiety were both important for the biological activity of OSW-1. Indeed, removal of the acetyl and the 4-methoxybenzoyl groups from the sugar part diminished the cytotoxicity by 1000 times. It was recently proven that direct glycosylation of the aglycon in its hemiketal form could be achieved (Scheme 58).[109,110]

A number of new glycosides bearing the disaccharide of OSW-1 were synthesised, starting from various steroids, in order to test their antitumour activities (Scheme 59).[111,112]

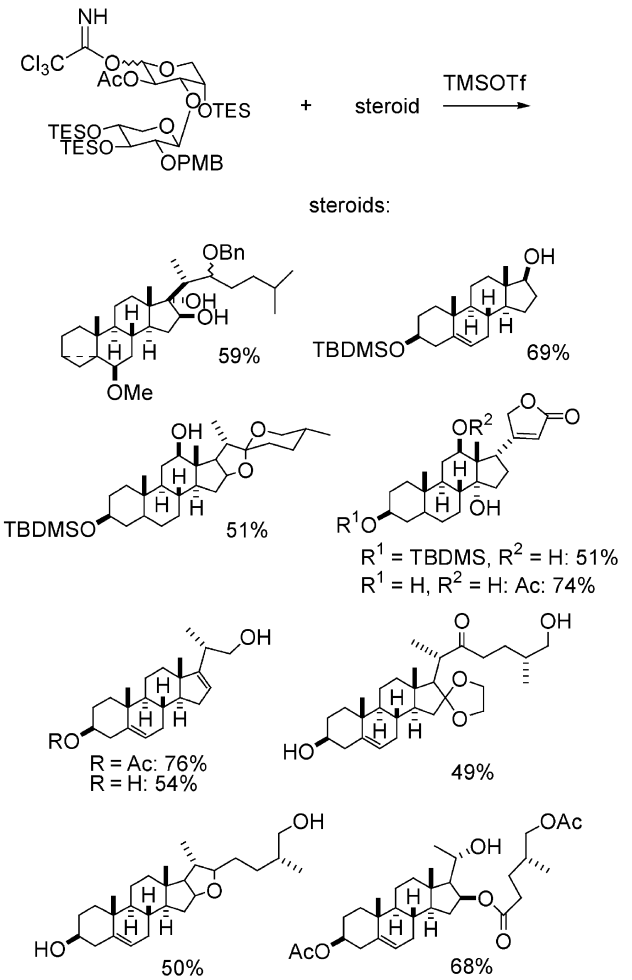
In the same way, the disaccharide moiety of OSW-1 has been attached at position C-3 of seven accessible steroid aglycons (Scheme 60).[113]

Some trisaccharides have also been attached to diosgenin, since diosgenyl glycosides are the most widely existing group of steroid saponins, sharing the multiple bioactivities of saponins, including antitumour, cardioactive, antimicrobial, or mulluscicidal activities, etc. Scheme 61 depicts the various trisaccharides which have been involved in the $BF_3(Et_2O)$-mediated glycosylation of diosgenin and their corresponding yields and preparative references (Table 19).

It is noteworthy that more than half of the triterpene saponins are glycosides of oleanic acid or its derivatives, with one sugar chain attached through an ether linkage at C-3 and another through an ester linkage at C-28 (a bidesmosidic saponin). The first synthesis of such a bidesmosidic triterpene saponin was reported in 1999 by Yu et al. using another basic strategy to build the steroidal glycoside (Scheme 61).[119] This latter procedure consists of beginning by connecting the first monosaccharide to the aglycon, then manipulating the protecting groups of the sugar moiety, and extending the sugar chain sequentially.

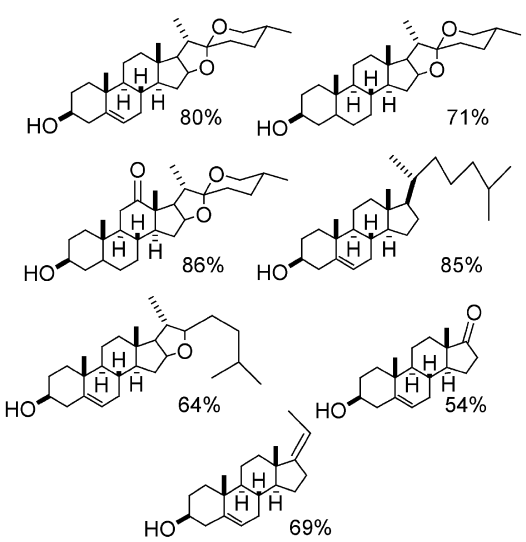
Table 18.

Sterol	Sugar	Catalyst	Yield (%)	Ref.
Tigogenin	Cellobiose heptaacetate	$ZnBr_2$	60	107
[2,2,3α,4,4-D_5]tigogenin	Cellobiose heptaacetate	$BF_3(Et_2O)$	43	108
Diosgenin	Sugar	TMSOTf	100	96
Cholesterol	Sugar	TMSOTf	100	96
Dehydroisoandrosterone	Sugar	TMSOTf	93	96



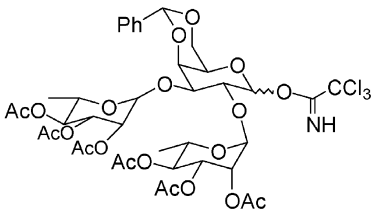
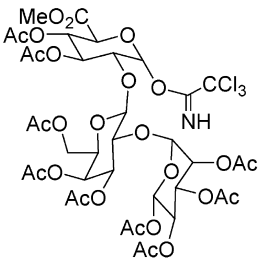
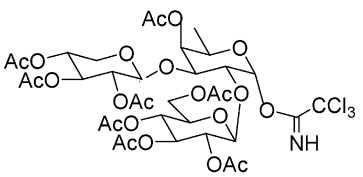
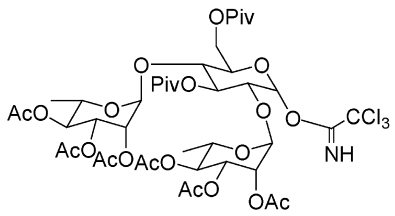
Scheme 59. Synthesis of steroidal glycosides bearing the disaccharide moiety of OSW-1.

The usual strategy was generalised by Nohara to the glycosylation of tetrasaccharides, since the mimosatetraose unit could be transferred to either diosgenin or glycyrrhetinic acid (Scheme 62).[116,120]



Scheme 60. Synthesis of steroidal glycosides bearing the disaccharide of OSW-1 at C-3.

Table 19.

Trisaccharide	Yield (%)	α/β	Ref.
	41	17/83	114,115
	64	0/100	116
	56	18/82	117
	82	74/26	118

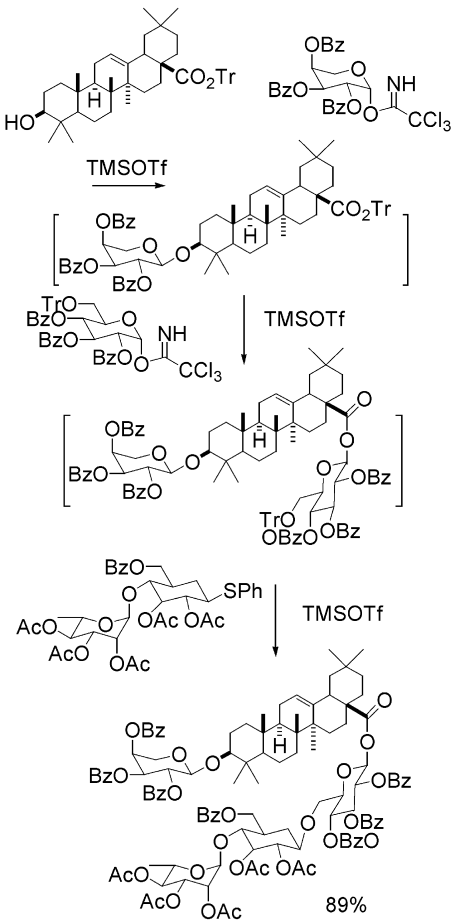
4. Thioglycosides

Glycosylation via phenylthioglycosides was first described by Ferrier[121] and, since this work, thioglycosides have been extensively studied as useful glycosyl donors due to their high stability in many organic operations.[122] Ogura et al. introduced the use of the (1-phenyltetrazol-5-yl)thio group as a new thio functional group activated by AgOTf under mild conditions (Scheme 63).[123]

Some bioactive diosgenyl saponins such as dioscin, polyphyllin D or balanitin 7 have been prepared starting from ethyl thioglycosides activated by NIS-AgOTf or MeOTf and diosgenin.[124,125] Cholesterol was also successfully coupled to a 6-phenylthio-substituted ethylthioglucopyranoside donor under the promotion of MeOTf.[97] Moreover, aminoglycosides of cardioactive steroids were prepared by glycosylation of diosgenin with thioaminodigitoxose derivatives. No stereoselectivity was, however, observed due to the lack of functionality at the C-2 position of the glycosyl donors (Table 20).[101]

Antitumoural steroidal glycosides bearing the disaccharide of OSW-1 were synthesised using a similar strategy (Scheme 64).[113]

In 1998, Crich et al. showed that the activation of



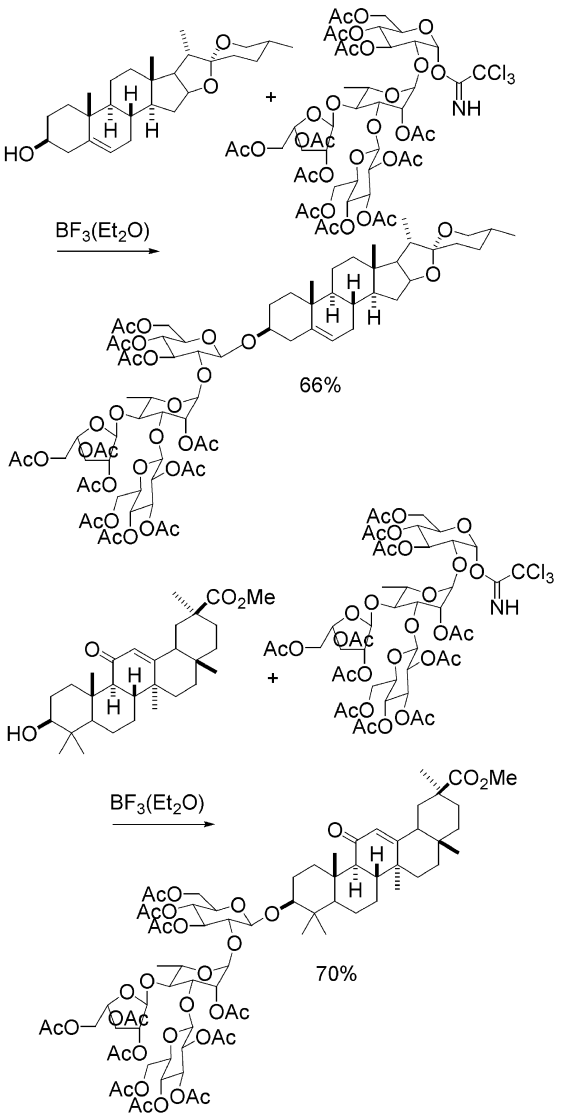
Scheme 61. First synthesis of a bidesmosidic triterpene saponin by successive glycosylation.

thioglycosides with benzenesulphenyl triflate led to the efficient formation (clean and quantitative) of the corresponding triflates in the presence of 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP). These triflates then reacted in an S_N2-like manner with sterols to give the corresponding steroidal glycosides in high yields and selectivities. In particular, the application of this procedure to mannosylthioglycosides allowed the first example of the clean formation of tertiary β-mannopyranosides or glycosides derived from highly hindered sterols (Scheme 65).[126,127] Moreover, the coupling of sterols with thioglycosides at position C-7 or C-20 of steroids is a rare occurrence.

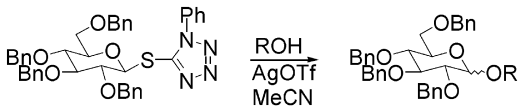
In 2003, a new class of thioglycosides having unprotected 2- and 2,4-hydroxyl groups were investigated under the standard glycosylation conditions.[128] This approach was shown to be generally effective for the synthesis of steroidal glycosides (Scheme 66).

5. 1-*O*-Sulphonyl glycosides

The use of 1-*O*-sulphonyl derivatives generally activated by Tf_2O as glycosyl donors produced major advantages in the period 1970–1980.[129] The preceding reaction was first investigated by Kahne et al.[130] using the corresponding sulphoxides in the presence of Tf_2O and 2,6-di-*tert*-butyl-4-methylpyridine. The stereochemical outcome of the reaction



Scheme 62. Glycosylation of tetrasaccharides.



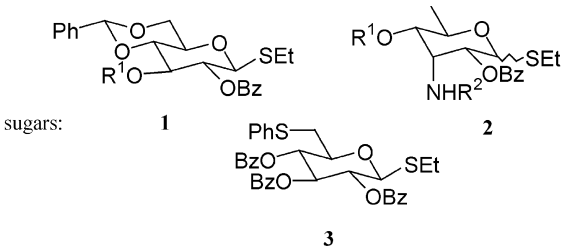
ROH = cholesterol: 95% α/β = 50/50

Scheme 63. The (1-phenyltetrazol-5-yl)thio group as a glycosyl donor in the glycosylation of cholesterol.

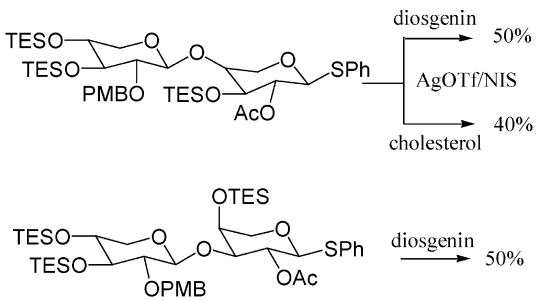
in the absence of neighbouring group participation was strongly influenced by the solvent. In general, the percentage of the β-glycoside produced increased with the solvent polarity.[130] The glycosylation under the same conditions of a bis-α-glucosylate cholic acid derivative was noteworthy, since the C-12 hydroxyl group did not react at −78 °C (Scheme 67).

Methyl 3-β-amino-7α,12α-di(1′α-glucosyl)-5β-cholate (TC-002) is a general delivery reagent facilitating the transport of both nontraditional drugs and hydrophilic small molecule drugs across membrane barriers. The key step of its synthesis developed by Sofia et al. was a glycosylation

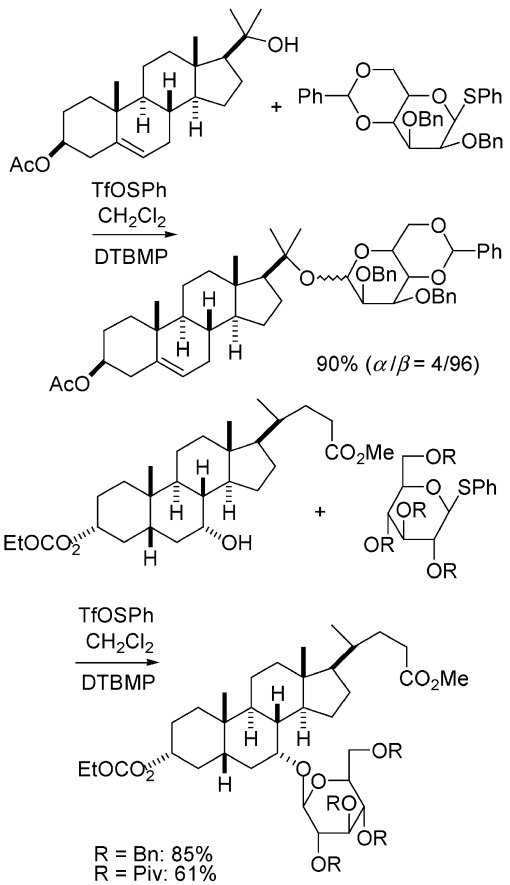
Table 20.



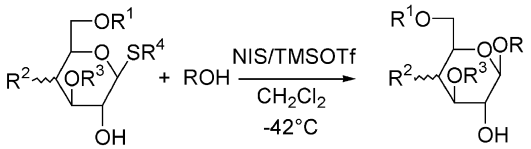
Steroid	Sugar	R^1	R^2	Catalyst	Yield (%)	Ref.
Diosgenin	**1**	Bz	—	AgOTf/NIS	50 (β)	124
Diosgenin	**1**	TBDMS	—	AgOTf/NIS	55 (β)	125
Cholesterol	**3**	—	—	MeOTf	56 (β)	97
Digitoxigenin	**2**	Bz	$COCF_3$	$HgCl_2/CdCO_3$	30 (α/β=47/53)	101
Digitoxigenin	**2**	pNBz	CO_2Bn	$HgCl_2/CdCO_3$	35 (α/β=45/55)	101



Scheme 64. Synthesis of steroidal glycosides bearing the disaccharide of OSW-1.



Scheme 65. Synthesis of highly hindered steroidal glycosides from thioglycosides.



R^1	R^2	R^3	R^4	Yield (%)	α/β
Bz	βOH	Bz	*i*-Pr	54	0/100
TBDMS	βOH	TBDMS	*i*-Pr	59	0/100
Bn	αOBn	Bn	Et	80	0/100

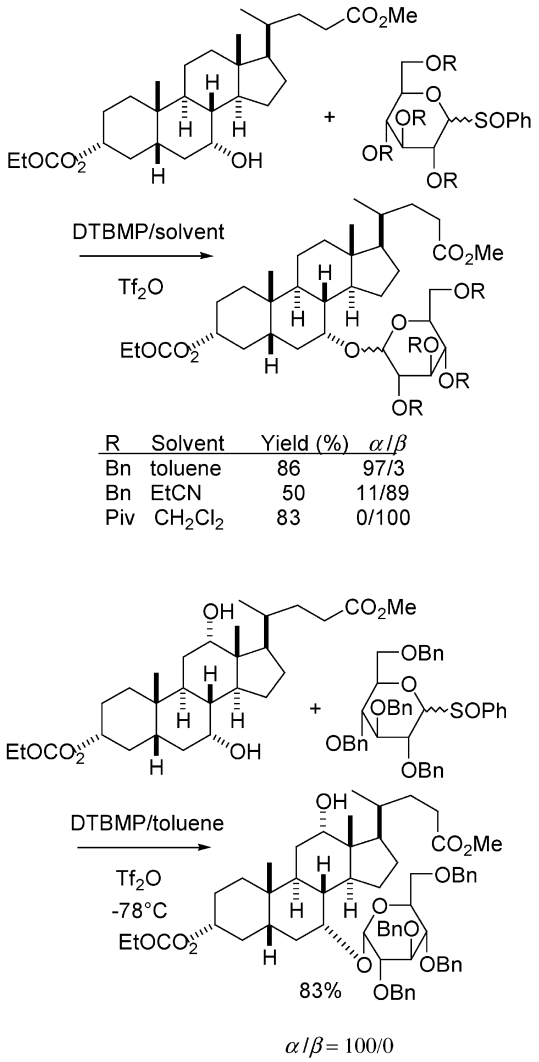
Scheme 66. Synthesis of saponins using partially protected glycosyl donors.

using sulphoxide and a C-3β-azido cholate derivative (Scheme 68).[131]

6. Glycals

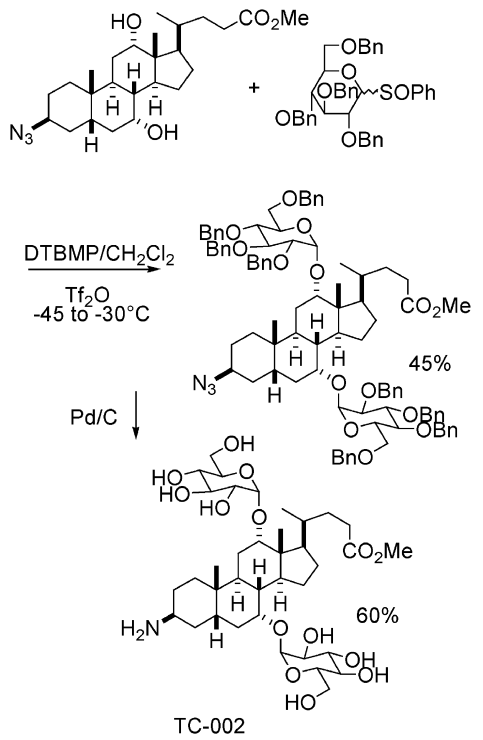
A thorough and useful review of the general glycal methodology has been published by Danishefsky.[132] In this methodology, it appeared that the complexities associated with differential hydroxyl protection would be significantly reduced. Indeed, in a hexose glycal, only three hydroxyl groups need to be distinguished. Furthermore, each hydroxyl moiety could well differ from the others in its expected reactivity, since one is primary, one is allylic, and the other is a more hindered secondary alcohol. It seemed, therefore, that the need for selective protection could be lessened and those protections which are needed could be simplified in the context of glycals. The use of glycals is, moreover, particularly attractive in that glycosidic bond formation as well as C-2-functionalisation of the carbohydrate donor is achieved in the same process. In 1972, Lemieux et al. reported the first demonstration of the utility of glycals in the synthesis of steroidal glycosides by the use of 2-nitrosyl chloride exerting a *cis*-directing effect on glycosylation (Scheme 69).[133]

Thiem et al. subsequently developed the glycosylation of D-digitoxal diacetate with digitoxigenin in the presence of *N*-iodosuccinimide, resulting in the exclusive formation of

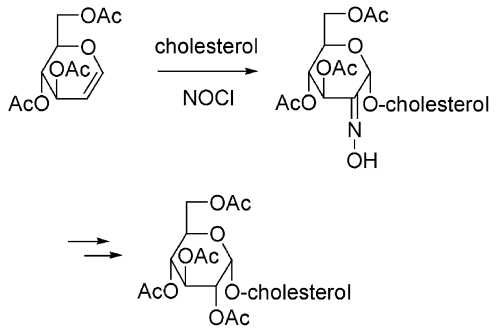


R	Solvent	Yield (%)	α/β
Bn	toluene	86	97/3
Bn	EtCN	50	11/89
Piv	CH_2Cl_2	83	0/100

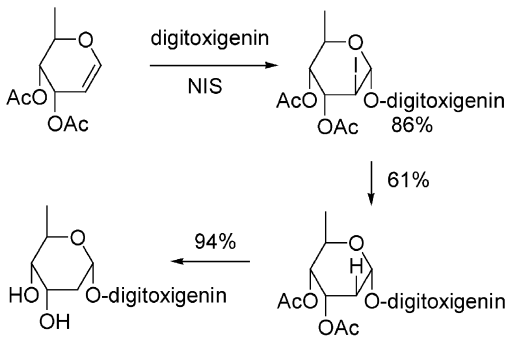
Scheme 67. Synthesis of highly hindered steroidal glycosides from sulphoxides.



Scheme 68. First synthesis of TC-002 via glycosylation.



Scheme 69. First use of glycals in the synthesis of steroidal glycosides.

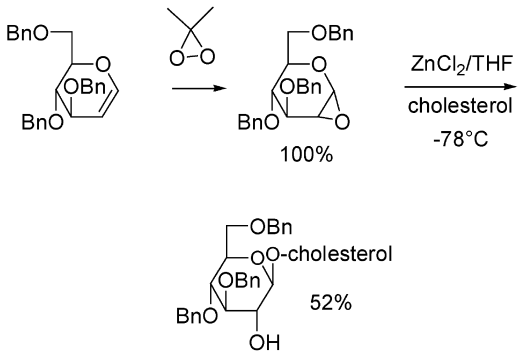


Scheme 70. First synthesis of digitoxigenyl α-D-digitoxoside from a glycal.

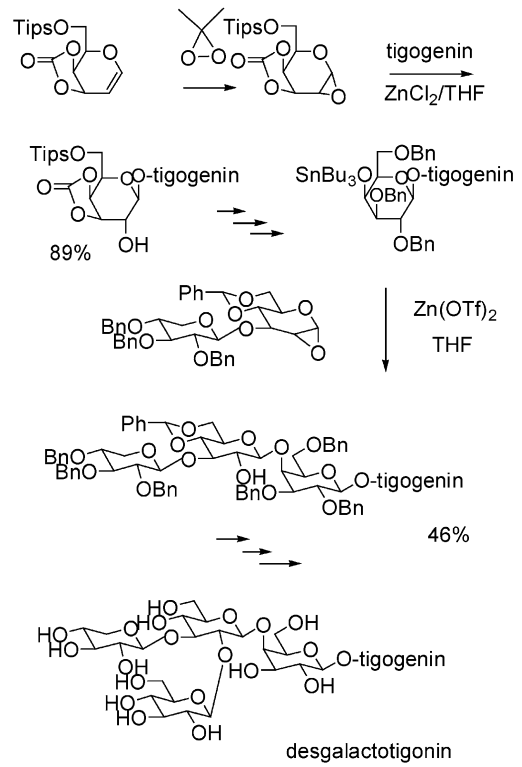
the α-D-glycoside, which could be successively reductively dehalogenated and then deacetylated to give digitoxigenyl α-D-digitoxoside (Scheme 70).[35]

In 1989, Danishefsky described the first general, high-yield, one-step conversion of glycals to 1,2-anhydro sugars in the presence of dimethyldioxirane and demonstrated that the epoxide linkage at the anomeric centre could be displaced with clean inversion of configuration to form glycoconjugates with β-glycosidic linkages.[134] The 1,2-anhydrosugar methodology constitutes a major expansion of the role of glycals in that it leads to fully oxygenated branched glycosides. The first example in the field of steroids was the $ZnCl_2$-mediated reaction of 1,2-anhydro-3,4,6-tri-*O*-benzyl-α-D-glucopyranose with cholesterol, providing stereoselectively the corresponding β-steroidal glycoside (Scheme 71).

Danishefsky applied this glycal assembly strategy to the synthesis of desgalactotigonin, a digitalis saponin.[135,136] In the first step, tigogenin was galactosylated with a D-galactal epoxide derivative giving a 89% yield of the corresponding galactosylated tigogenin. Upon glycosylation, the C-2 hydroxyl group was exposed to serve as the acceptor in the next glycosylation involving a disaccharide epoxide. This zinc triflate-mediated glycosylation was an important step in that it gave access to the β-glycoside of the rather hindered axial hydroxyl centre at C-4 of D-galactose (Scheme 72).



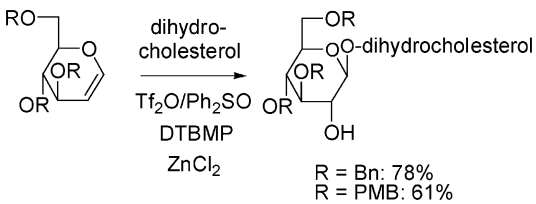
Scheme 71. First synthesis of steroidal glycoside from a glycal epoxide.



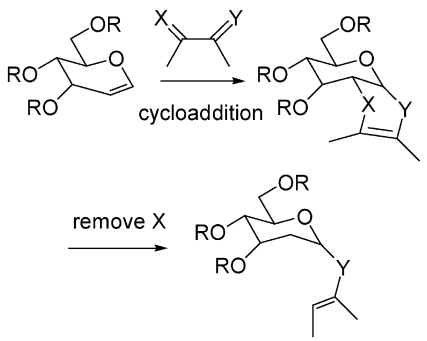
Scheme 72. Synthesis of desgalactotigonin.

In 1998, Gin proposed a new method employing the reagent combination of triflic anhydride (Tf_2O) and diphenylsulphoxide for glycosylation with glucal donors. The key features of the coupling reaction included (1) a novel method for glycal oxidation/activation via oxygen transfer from the sulphoxide reagent to the C-2 of the product glycoside and (2) a one-pot diastereoselective synthesis of C-2-hydroxyglucopyranosides from glucal substrates (Scheme 73).[137]

Win and Franck have explored an alternate approach to



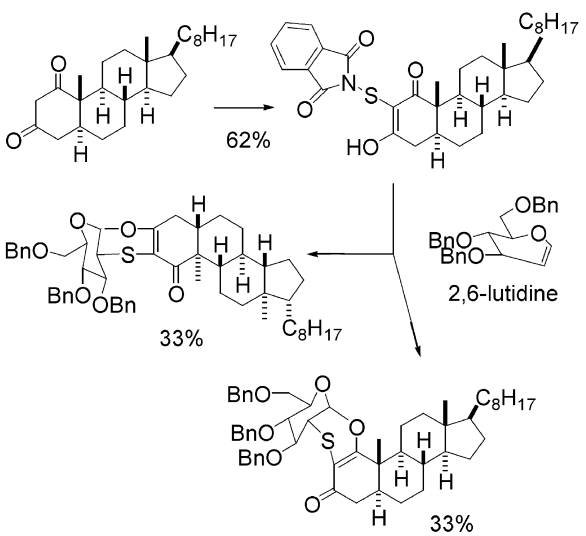
Scheme 73. Direct oxidative glycosylations with glycal donors.



Scheme 74. Glycosyl transfer mediated by heterocycloaddition.

glycosyl transfer, which is shown by the general cycloaddition in Scheme 74.[138]

In order to apply this new concept to the glycosylation of steroids, the first task was to prepare cholestane-1,3-dione selected to be the precursor to the required heterodiene after phthalimidosulphenylation. The heterodiene was generated and trapped in situ in the presence of tribenzylglucal. Unfortunately, a lack of regioselectivity was observed in the cycloaddition step, since two novel steroidal glycosides were obtained in identical yields. Anyway, this original methodology could not in any event be considered as a practical alternative to the classical methods for steroid glycoside synthesis because of the several steps needed to prepare the steroidal heterodiene precursor (Scheme 75).

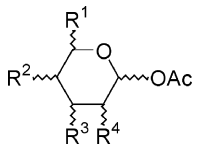


Scheme 75. Synthesis of steroidal glycosides via glycosyl transfer mediated by heterocycloaddition.

7. 1-*O*-Acyl sugars, orthoesters and ethers

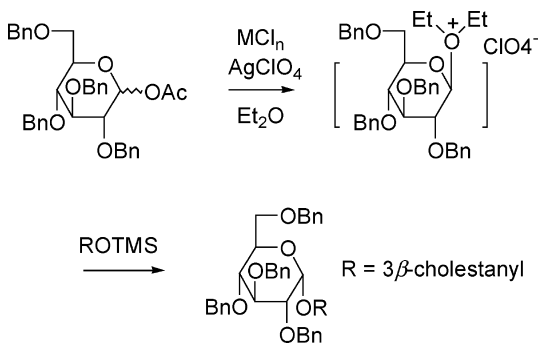
An advantage of the 1-*O*-acylated glycosyl donor in the glycosylation method is undoubtedly the ease of its preparation. The most representative anomeric functional group in this area is the acetyl group. Various promoters have been engaged in this type of glycosylation, such as TsOH or Lewis acids, e.g. $SnCl_4$, $BF_3(Et_2O)$ or TMSOTf, and combinations of $Zn(OTf)_2$ with TMSBr or TMSCl (Table 21).

Table 21.



Steroid	R^1	R^2	R^3	R^4	OAc	Catalyst	Yield (%) (α/β)	Ref.
Estradiol 17-acetate	βCO_2Me	αOAc	βOAc	αOAc	α	$SnCl_4$	49 (0/100)	139
Estradiol-16,17-diacetate	βCH_2OAc	αOAc	βOAc	αOAc	α	$SnCl_4$	45 (0/100)	139
Diosgenin	βCH_2OAc	αOAc	βOAc	αOAc	(α,β)	$BF_3(Et_2O)$	20 (0/100)	117
Digitoxigenin	βMe	αOAc	αOAc	H	β	TsOH	60 (32/68)	140
Methyl glycyrrhetinate	βCO_2Bn	αOBn	βOBn	αOAc	β	TMSOTf	45 (65/35)	141
3β-Cholestanol	αMe	αOBn	αOBn	βOBn	(α,β)	$TMSCl/Zn(OTf)_2$	88 (71/29)	142
3β-Cholestanol	βCH_2OBn	αOBn	βOBn	αOBn	(α,β)	$TMSBr/Zn(OTf)_2$	63 (65/35)	142
3β-Cholestanol	βCH_2OAc	αOAc	βOAc	αNHTroc	(α,β)	$TMSBr/Zn(OTf)_2$	81 (99/1)	142
3β-Cholestanol	βCH_2OAc	βOAc	βOAc	αNHTroc	(α,β)	$TMSBr/Zn(OTf)_2$	82 (99/1)	142

Mukaiyama et al. introduced the combinations of $AgClO_4$ and a Lewis acid ($SnCl_4$, $SiCl_4$, $GeCl_4$, $GaCl_3$, $InCl_3$, $FeCl_3$ or $HfCl_4$) and found that the catalytic use of these promoters was good enough to perform highly stereoselectively the glycosylation reactions of the 1-*O*-acetyl sugar with the trimethylsilylated sterol (Scheme 76 and Table 22).[143]



Scheme 76. Lewis acid/$AgClO_4$-mediated glycosylation of 1-*O*-acetyl-D-glucose.

Table 22.

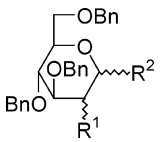
Lewis acid	Yield (%)	α/β
$ZrCl_4$	92	94/6
$HfCl_4$	95	94/6
$FeCl_3$	92	93/7
$GaCl_3$	89	92/8
$InCl_3$	78	94/6
$SiCl_4$	73	93/7
$GeCl_4$	72	93/7
$SnCl_4$	88	93/7
$SbCl_5$	99	89/11

On the other hand, other acyl groups were employed as good anomeric leaving groups in the glycosylation of 3β-cholestanol and could be activated by $TrClO_4$,[144] combinations of $Zn(OTf)_2$ and TMSBr,[145] or other combinations such as $AgClO_4$ with $SnCl_4$ or $SiCl_4$ (Table 23).[146] In this latter case, the combination was used in catalytic amount and, according to the nature of the Lewis acid, the α- or β-glucosides were obtained stereoselectively.

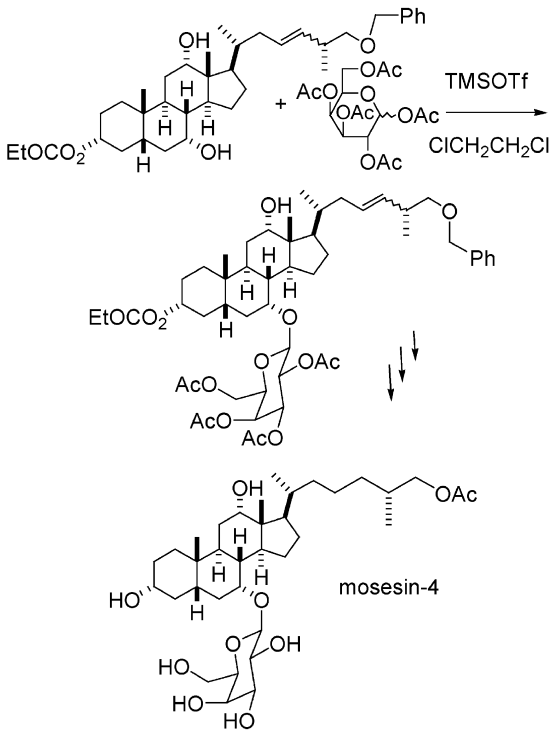
In 1989, Nakanishi reported the first synthesis of mosesin-4, a naturally occurring steroid saponin with shark repellent activity, based on the TMSOTf-mediated glycosylation of a cholic acid derivative with galactose peracetate.[76] The severely hindered 7α-position was selectively glycosylated (Scheme 77).

It has been demonstrated that bile acid synthesis was abnormal in patients with the rare inherited lipid storage disease, cerebrotendinous xanthomatosis, and that a severe neurologic disease developed in these subjects. In order to study the transformations of bile alcohols and evaluate the biological effects on the blood-brain barrier, Williams et al. developed a mild and regiocontrolled method for *O*-glucuronidation of the highly reactive 3-hydroxyl group of 5β-cholestane-3α,7α,12α,25-tetrol using $SnCl_4$ (Scheme 78).[147]

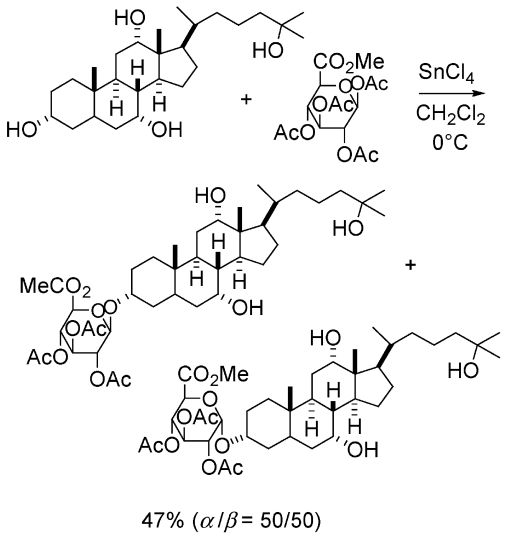
Table 23.



R^1	R^2	Catalyst	Solvent	Yield (%)	α/β	Ref.
αBn	$\beta OCOCHCl_2$	$TrClO_4$	Et_2O	57	91/9	144
αBn	$\beta OCOCH_2Br$	$TrClO_4$	Et_2O	75	96/4	144
αBn	$\beta OCOCH_2I$	$TrClO_4$	Et_2O	89	86/14	144
αBn	$(\alpha,\beta)OCOCH_2O\ (CH_2)_2OMe$	$SiCl_4-2AgClO_4$	MeCN	97	5/95	146
αBn	"	$SnCl_4-2AgClO_4$	Et_2O	98	97/13	146
βBn	αOPNBz	$Zn(OTf)_2-TMSBr$	CH_2Cl_2	91	93/7	145



Scheme 77. Synthesis of mosesin-4.



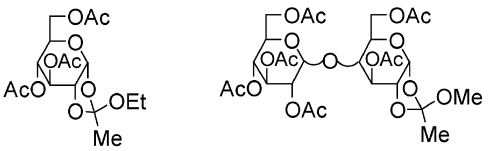
Scheme 78. Regioselective $SnCl_4$-mediated preparation of bile alcohol glucuronides.

The orthoester method has been widely studied by Kochetkov et al. and employed for the construction of 1,2-*trans*-glycosidic linkages and, in particular, they applied this procedure to synthesise glycosides of cholesterol and oleanic acid.[148] In 1973, Uvarova et al. reported the preparation of β-glucosides of β-sitosterol, cholesterol and 16-dehydropregnenolone by the orthoester method in the presence of $HgBr_2$ in nitromethane (Table 24).[149]

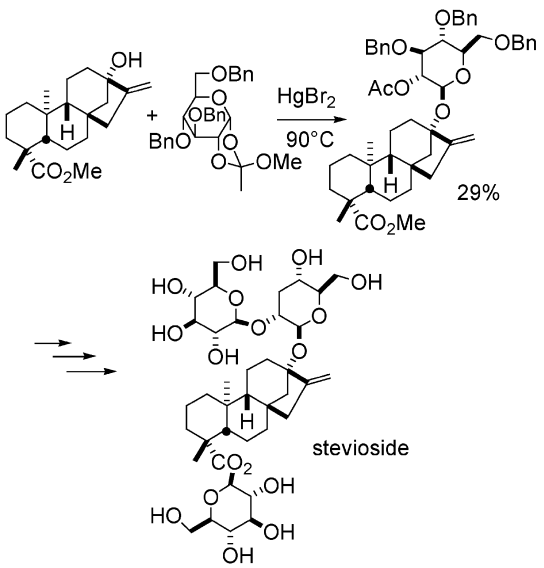
The same methodology allowed the first formal total synthesis of stevioside (Scheme 79).[36]

On the other hand, Kunz et al. reported a very efficient and

Table 24.



Steroid	Orthoester	Yield (%)
Cholesterol	**1**	45
Cholesterol	**2**	43
β-Sitosterol	**1**	26
β-Sitosterol	**2**	47
16-Dehydropregnenolone	**1**	27
16-Dehydropregnenolone	**2**	20

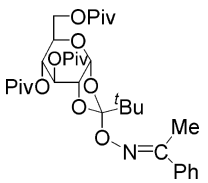


Scheme 79. Total synthesis of stevioside.

β-stereoselective glycosylation using a new glycosyl donor, the oximate orthoester of *O*-pivaloyl glucopyranose, in the presence of $BF_3(Et_2O)$ which provided better yields.[150] Moreover, this method has the advantage that both the glycosyl donor and the aglycon need only be used in equivalent amounts, whereas the normal orthoester procedure requires an excess of the alcohol (Table 25).

As an extension of the studies on glycosylation by the combined use of a trimethylsilyl halide and $Zn(OTf)_2$ as

Table 25.



Steroid	Yield (%)
Cholesterol	82
Androsterone	73
Testosterone	68
Estrone	81

activators, Higashi et al. demonstrated that the glycosylation promoted by this combination could be applied to 1-*O*-alkyl glycopyranosides as glycosyl donors (Scheme 80).[142]

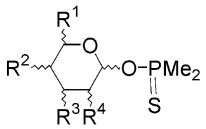
Scheme 80. Glycosylation involving 1-*O*-alkyl glycopyranosides as glycosyl donors.

8. Phosphate derivatives

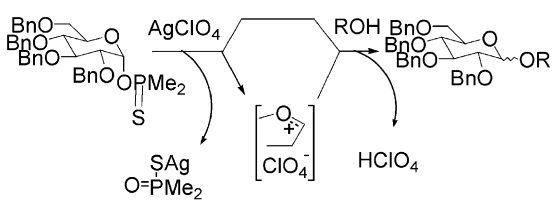
Several glycosyl donors possessing a phosphorus atom in the leaving group at the anomeric centre have been investigated. Since phosphorus compounds can be easily modified by several kinds of other atoms, a wide variety of leaving groups with different properties could be designed. These glycosyl donors were activated by $AgClO_4$, I_2–$TrtClO_4$, $ZnCl_2$, combinations such as $ZnCl_2$–$AgClO_4$, TMSOTf or $BF_3(Et_2O)$. In 1985, Inazu et al. developed the syntheses and glycosylation reactions of the glycopyranosyl dimethylphosphinothioate series having a nonparticipating group at the C-2 position.[151–153] These new glycosyl donors, stable at room temperature, provided, in the presence of $AgClO_4$ in benzene and 3β-cholestanol, the corresponding α-glycosides predominantly (Table 26).

The authors have assumed that this glycosylation would proceed mainly by an S_N1 reaction, as shown in Scheme 81.

Table 26.



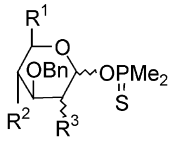
R^1	R^2	R^3	R^4	Yield (%)	α/β	Ref.
βCH$_2$OBn	αOBn	βOBn	αOBn	89	81/19	515
αCH$_3$	αOBn	αOBn	βOBn	75	56/44	152
βCH$_2$OBn	αOBn	βOBn	βOBn	70	100/0	153
βCH$_3$	αOBn	βOBn	βOBn	90	100/0	153



Scheme 81. Postulated mechanism of glycoside formation using $AgClO_4$.

The combined use of iodine and a catalytic amount of triphenylmethyl perchlorate ($TrtClO_4$) was also effective for glycosylation as a new activating system instead of silver perchlorate.[152] The results concerning the glycosylation of 3β-cholestanol are collected in Table 27 and showed that the selectivity of the reaction was related in part to the nature of the solvent.

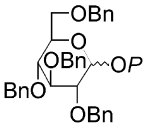
Table 27.



R^1	R^2	R^3	Solvent	Yield (%)	α/β	Ref.
CH$_2$OBn	OBn	αOBn	Benzene	82	72/28	152
Me	OBn	βOBn	Toluene	78	90/10	153
CH$_2$OBn	OBn	βOBn	Benzene	67	30/70	154
Me	OBz	βOBn	Benzene	85	18/82	153

In 1994, Watanabe investigated the use of glycosyl phosphites activated with a Lewis acid such as $ZnCl_2$ or $ZnCl_2$–$AgClO_4$ as glycosyl donors in the glycosylation of 3β-cholestanol and cholesterol.[155] When the reaction was carried out in a solvent such as dichloromethane, the β-anomer was formed predominantly (Table 28).

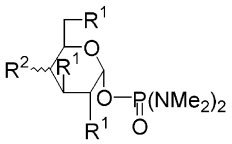
Table 28.



Steroid	P	Catalyst	Yield (%)	α/β
3β-Cholestanol	$(MeO)_2P$	$ZnCl_2$	86	22/78
3β-Cholestanol		$ZnCl_2$–$AgClO_4$	86	32/68
3β-Cholestanol	PPh_2	$ZnCl_2$	77	15/85
Cholesterol	$(MeO)_2P$	$ZnCl_2$	89	25/75

On the other hand, benzyl-protected glycopyranosyl *N*,*N*,*N*′,*N*′-tetramethylphosphoroamidates were demonstrated to be effective glycosyl donors to afford, in the presence of $BF_3(Et_2O)$ or TMSOTf in dichloromethane, stereoselectively the 1,2-*trans*-β-linked steroidal glycosides (Table 29).[156]

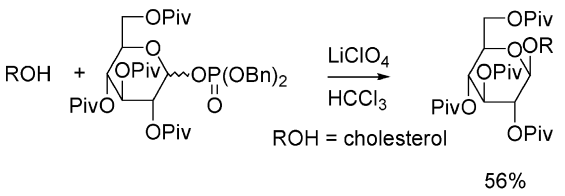
Table 29.



Steroid	R^1	R^2	Catalyst	Yield (%)	α/β
3β-Cholestanol	OBn	αOBn	$BF_3(Et_2O)$	73	10/90
3β-Cholestanol	OBn	αOBn	$BF_3(Et_2O)$	70	9/91
Androsterone	OBn	βOBn	$BF_3(Et_2O)$	76	13/87
Estrone	OBz	αOBz	TMSOTf	80	0/100
Estrone	OBz	βOBz	TMSOTf	72	0/100

Waldmann demonstrated that the synthesis of steroidal glycosides was possible in very mild and neutral conditions in the presence of concentrated solutions of $LiClO_4$ at room temperature without the addition of promoters (Scheme 82).[157]

Other glycosyl donors such as benzoyl- or benzyl-protected glycopyranosyl *P*,*P*-diphenyl-*N*-(*p*-toluenesulphonyl)phosphinimidates promoted by $BF_3(Et_2O)$ in dichloromethane

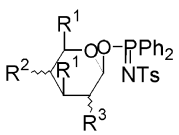


Scheme 82. $LiClO_4$-mediated glycosylation under neutral conditions.

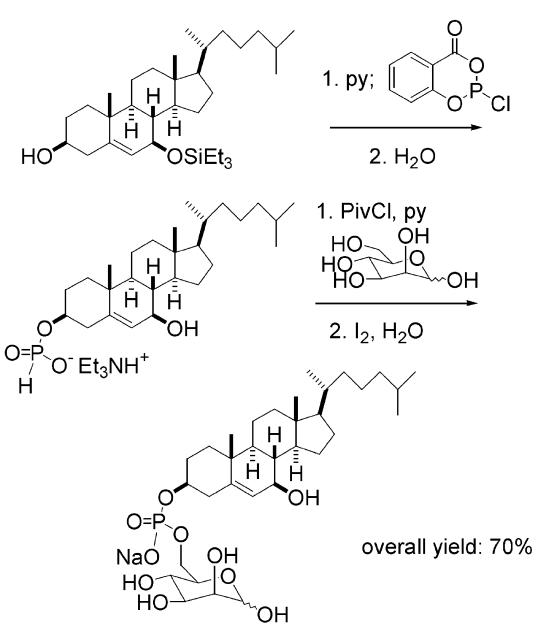
were employed for the efficient construction of 1,2-*trans*-β-glycosidic linkages with sterols (Table 30).[158,159]

In order to make bioactive compounds more available, Luu conjugated sterols with carbohydrates by a phosphodiester linkage, which permitted an increase in the water solubility and/or a targeting of the drug to a specific organ. In this way, a phosphite was reacted with the C-3 hydroxyl group of a sterol according to the hydrogen-phosphonate methodology. After activation by pivaloyl chloride, selective coupling at the primary alcohol of the non-protected carbohydrate yielded the corresponding phosphate (Scheme 83).[160,161]

Table 30.

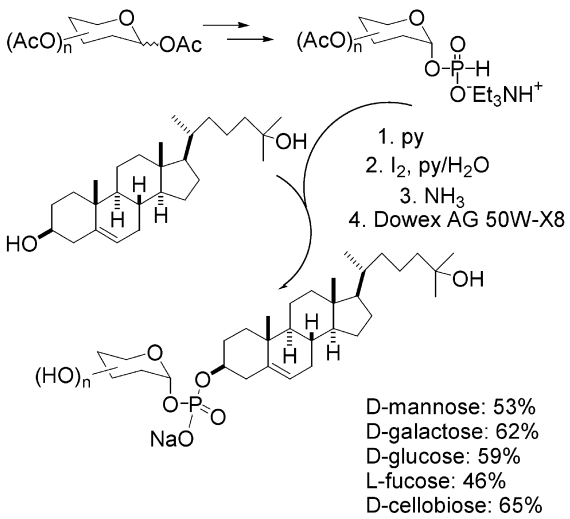


Steroid	R^1	R^2	R^3	Yield (%)	α/β
3β-Cholestanol	CH_2OBn	αOBn	αOBn	74	10/90
3β-Cholestanol	CH_2OBn	βOBn	αOBn	73	11/89
Cholesterol	CH_2OBn	αOBn	αOBn	72	13/87
Androsterone	CH_2OBn	αOBn	αOBn	72	7/93
Tigogenin	CH_2OBn	αOBn	αOBn	70	12/88
Digitoxigenin	CH_2OBz	αOBz	αOBz	51	0/100
Tigogenin	CH_2OBz	αOBz	αOBz	70	0/100
Tigogenin	H	αOBz	αOBz	73	0/100
Digitoxigenin	Me	αOBz	βOBz	76	0/100



Scheme 83. Synthesis of an antitumour agent by coupling a sterol with a carbohydrate via a phosphodiester bond.

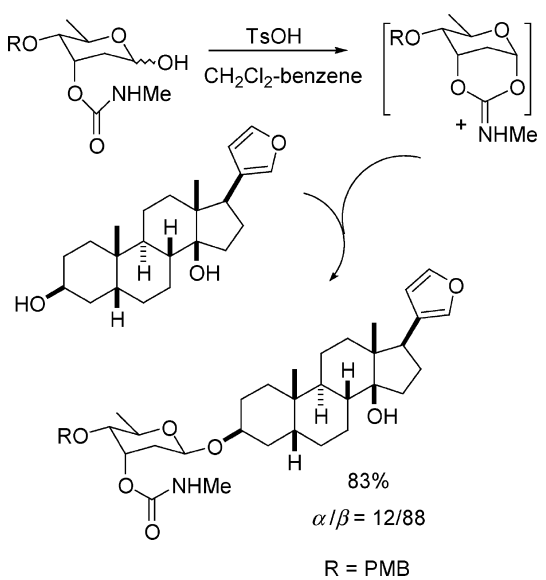
Since the preceding methodology was limited by the narrow range of monosaccharides that could be efficiently phosphorylated, Luu elaborated a different approach based on the use of peracetylated-α-glycosyl H-phosphonates. With the use of this method, various mono- and disaccharides could be coupled to 25-hydroxycholesterol through a diester linkage (Scheme 84).[162]



Scheme 84. Synthesis of phosphodiester derivatives of 25-hydroxycholesterol.

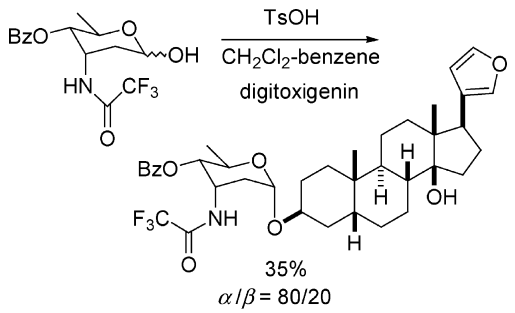
9. 1-Hydroxyl sugars

The Fischer–Helferich method comprises the direct formation of a glycosidic bond from the 1-hydroxyl sugar through an acid-catalysed acetalisation procedure. The first application of this methodology in the field of steroids was the glycosylation, in 10% yield, of digitoxigenin with digitoxose in the presence of hydrogen chloride reported, by Zorbach in 1964.[163] A considerable improvement was achieved by Wiesner et al., who used *p*-toluenesulphonic acid (TsOH) in CH_2Cl_2–benzene as a catalyst. The observed high β-stereoselectivity was presumably due to the intermediacy of the bridged species depicted in Scheme 85.[164,165]



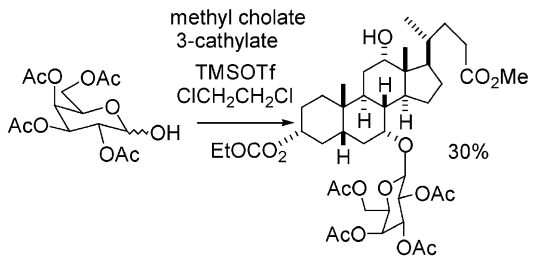
Scheme 85. First efficient direct coupling of digitoxigenin with digitoxose.

More recently, a similar reaction to that depicted in Scheme 84 was performed on a 3-trifluoroacetamido-D-*ribo*-hexopyranoside derivative and led unexpectedly to a large preponderance of the α-anomer.[101] In this reaction, the electron-withdrawing trifluoroacetyl group was believed to not be effective in stabilising the oxonium intermediate via the 1,3-participation of the carbamate group. The stereochemical outcome of the reaction was therefore probably determined by a series of anomerization equilibria,[1b] in which the relative reactivity of the starting glycosyl donors, the relative anomerisation rates and the strength of the catalyst would be the major factors. The reactive intermediate would then favour the formation of the more stable α-product (Scheme 86).



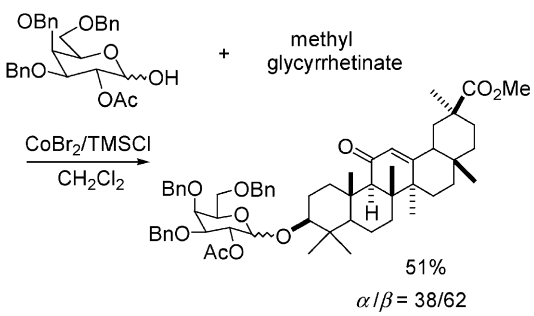
Scheme 86. Unexpected α-stereoselectivity of 3-amino digitoxose derivatives.

The TMSOTf-promoted reaction of β-galactose tetracetate with methyl cholate 3-cathylate was developed by Nakanishi, although the 7α-position of the steroidal aglycon was severely hindered (Scheme 87).[76]



Scheme 87. TMSOTf-mediated glycosylation at C-7 of methyl cholate 3-cathylate.

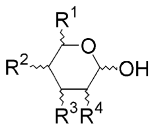
A low stereoselectivity was observed by Saito, who involved a combination of cobalt (II) bromide and TMSCl as the promoter to glycosylate methyl glycyrrhetinate with 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-D-galactopyranose (Scheme 88).[141]



Scheme 88. $CoBr_2$-TMSCl-mediated glycosylation of methyl glycyrrhetinate.

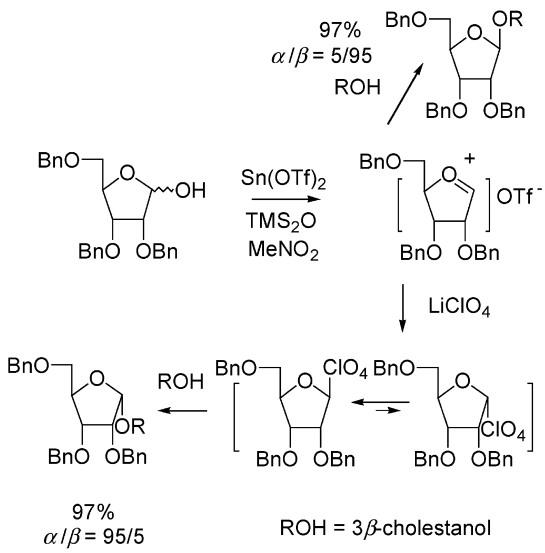
Suzaki demonstrated that another combination comprising a mixture of TMSCl and a catalytic amount of $Zn(OTf)_2$ in CH_2Cl_2 was a more efficient promoter of glycosylation than TMSOTf.[166] The results concerning the glycosylation of 3β-cholestanol with various 1-hydroxy sugars are collected in Table 31.

Table 31.



R^1	R^2	R^3	R^4	Yield (%)	α/β
βCH_2OBn	αOBn	βOBn	αOBn	63	49/51
βCH_2OBn	αOBn	βOBn	βOBn	43	88/12
βCH_2OBn	βOBn	βOBn	αOBn	61	62/38
αCH_3	αOBn	αOBn	βOBn	95	73/27

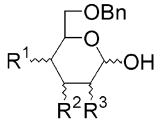
In 1994, Mukaiyama reported that the efficient stereoselective glycosylation of 3β-cholestanol with 2,3,5-tri-*O*-benzyl-D-ribofuranose could be successfully carried out by the combined use of catalytic amounts of hexamethyldisiloxane and tin(II) triflate in polar solvents such as nitromethane.[167] The perfect β-selectivities were probably due to the partial anomerisation of any initially formed α-anomers to the thermodynamically more stable β-anomers under the reaction conditions (Scheme 89). In contrast to these results, the corresponding α-ribosides were obtained predominantly in the presence of $LiClO_4$. The high α-selectivity was probably due to the rapid formation of the corresponding 1-perchlorinated intermediates. The location of the perchlorate on the β-side would be preferred to that on the α-side, because of the reverse anomeric effect and the steric hindrance of the bulky perchlorate group. Subsequently, the β-perchlorinated intermediate was attacked by the alcohol from the α-side of the anomeric carbon, to afford the α-anomer predominantly. In addition, the β-anomer, a byproduct, would probably anomerise to the α-anomer, via a similar process.



Scheme 89. Catalytic, highly α- or β-stereoselective, synthesis of ribosides.

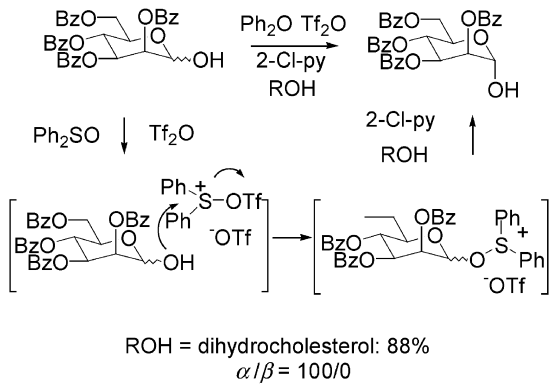
These conditions could be applied to other 1-hydroxy sugars with excellent yields and α-stereoselectivities in the presence of $LiClO_4$. The results for the glycosylation of 3β-cholestanol are collected in Table 32.

Table 32.



R^1	R^2	R^3	Yield (%)	α/β
αOBn	βOBn	αOBn	95	84/16
βOBn	βOBn	αOBn	95	84/16
βOBn	βOBn	N_3	95	90/10

In 2000, Gin et al. developed a new method for direct dehydrative glycosylations with 1-hydroxyglycosyl donors employing a reagent combination of triflic anhydride and diphenyl sulphoxide.[168] Oxygen-18-labelling studies were consistent with the first step in the carbohydrate activation being the formation of an anomeric oxosulphonium intermediate. This reactive species was observable in low-temperature NMR experiments. The new glycosylation procedure could be generalised to a wide range of sugars and alcohols and, in particular, to the efficient synthesis of dihydrocholesteryl-2,3,4,6-tetra-*O*-benzoyl-α-D-manno-pyranoside (Scheme 90).



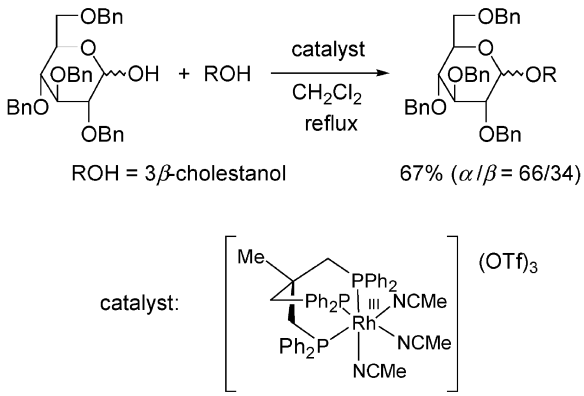
Scheme 90. Triflic anhydride and diphenyl sulphoxide-mediated glycosylation.

Very recently, Ernst et al. reported a new catalytic glycosylation procedure using Rhodium(III)-complexes of the terdentate triphos ligand, which could be applied to 3β-cholestanol (Scheme 91).[169]

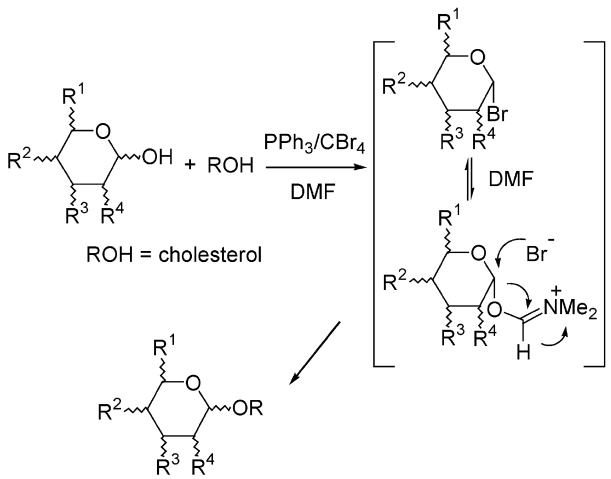
A reagent combination of triphenylphosphine (Ph_3P) and carbon tetrabromide (CBr_4) has been successfully used in DMF to propose a new practical one-pot α-glycosylation method.[170] This reaction, in situ giving the equilibrium of glycosyl bromides and more reactive *O*-glycoside intermediates, has accomplished near-quantitative α-glycosylations of cholesterol (Scheme 92 and Table 33).

10. 1-*O*-Silylated glycosides

Mukaiyama et al. have developed the stereoselective



Scheme 91. Catalytic glycosylation with Rh(III)-complexes.



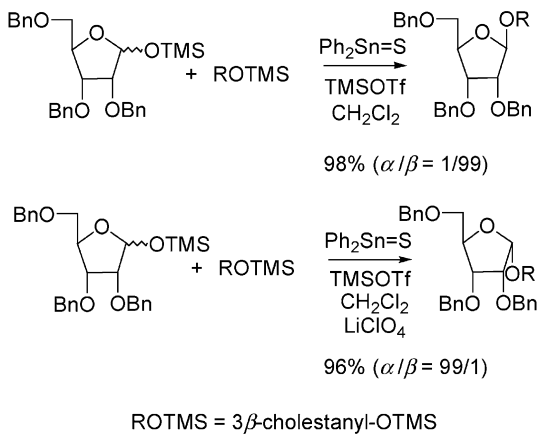
Scheme 92. One-pot α-glycosylation method of cholesterol.

Table 33.

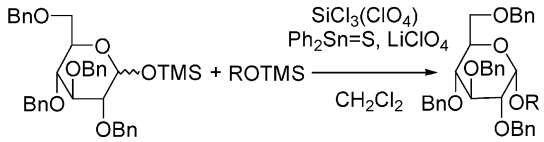
R^1	R^2	R^3	R^4	Yield (%)	*a* /β
βCH_2OAc	α OBn	β OBn	α OBn	96	95/5
βCH_2OBn	α OBn	β OBn	α OBn	95	85/15
βCH_2OAc	β OBn	β OBn	α OBn	92	94/6
βH_2OBn	β OBn	β OBn	α OBn	96	82/18
αMe	α OBn	α OBn	β OBn	95	86/14

glycosylation reactions of 1-*O*-trimethylsilyl sugars. The 1,2-*trans*-ribofuranosides were predominantly synthesised by the glycosylation of 1-*O*-trimethylsilyl ribofuranose and the trimethylsilyl ether of 3β-cholestanol in the presence of a catalytic amount of TMSOTf and diphenyltin sulfide ($Ph_2Sn{=}S$) as an additive, while the 1,2-*cis*-ribofuranosides were selectively prepared by the addition of $LiClO_4$ in the above reaction conditions (Scheme 93).[171]

The above procedure was further applied to the glycosylation reaction of 1-*O*-trimethylsilyl-2,3,4,6-tetra-*O*-benzyl-D-glucopyranose with the same steroid, but, the corresponding glucopyranosides were obtained only in poor yields and with low selectivities. On the other hand, when the reaction was carried out by the use of an active catalyst [$SiCl_3$-(ClO_4)] generated from $SiCl_4$ and $AgClO_4$, the 1,2-*cis*-glucoside was obtained in quantitative yield with good selectivity in the presence of $LiClO_4$ (Scheme 94).



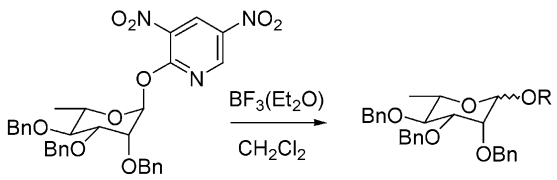
Scheme 93. Stereoselective synthesis of ribofuranosides from 3β-cholestanyl-OTMS.



Scheme 94. Stereoselective synthesis of glucopyranosides from 3β-cholestanyl-OTMS.

11. Other chemical methods

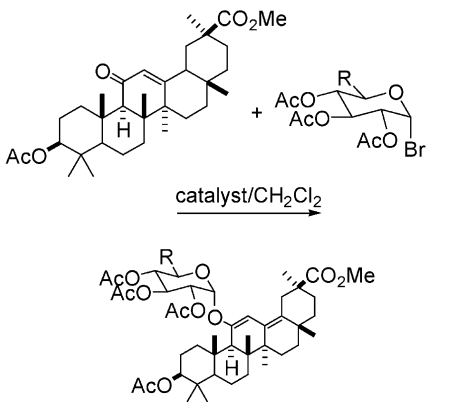
Other donor types such as 1-*O*-(3,5-dinitro-2-pyridyl)-2,3,4-tri-*O*-benzyl-L-rhamnose have also been involved in glycosylations. Fullerton et al. condensed this compound with digitoxigenin in the presence of $BF_3(Et_2O)$ and isolated the corresponding steroidal glycosides (Scheme 95).[94]



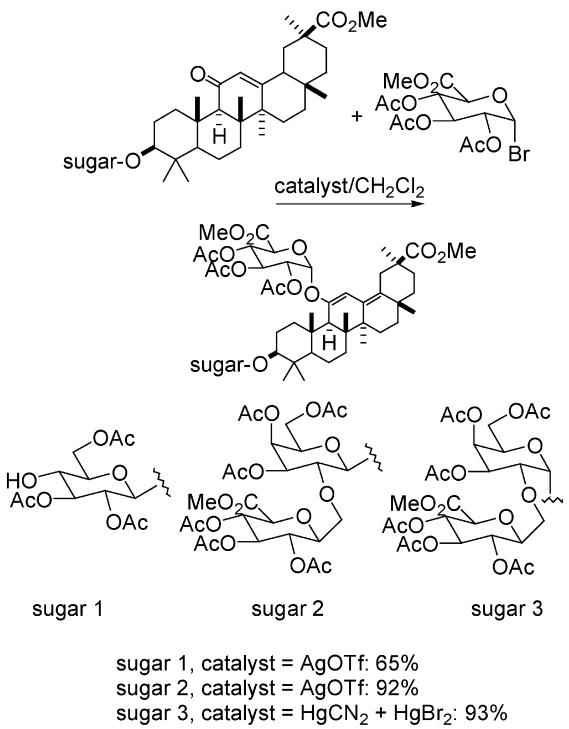
Scheme 95. Use of a special glycosyl donor in the glycosylation of digitoxigenin.

In 1992, Saito et al. reported the first example of the direct enol glycosylation of glycyrrhetic acid derivatives having no OH or slightly reactive OH groups with acetylated pyranose bromides in the presence of AgOTf or $Hg(CN)_2$ and $HgBr_2$ (Scheme 96).[172,37b]

In addition, the glycosylation of methyl 3-*O*-(2′,3′,6′-tri-*O*-acetyl-β-D-glucopyranosyl)glycyrrhetinate was investigated and confirmed the poor reactivity of the 4-OH group, since the corresponding enol diglycoside was obtained in good yield. The same methodology was also applied to diglycoside derivatives, leading quantitatively to the corresponding enol triglycosides (Scheme 97).

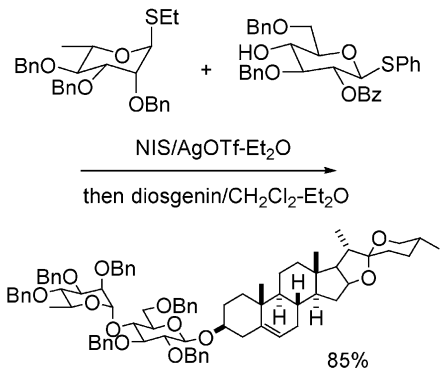


Scheme 96. Enol glycosylation of glycyrrhetic acid derivatives.



Scheme 97. Synthesis of other enol glycosides.

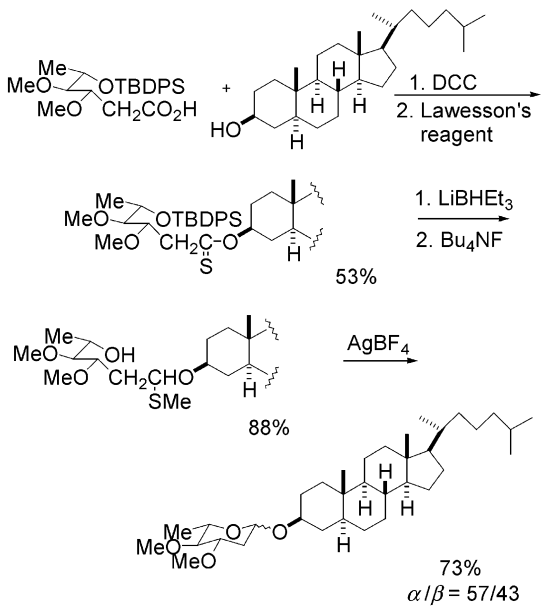
A common strategy for preparing spirostan saponins has been to construct the glycosidic bond between the steroid and a monosaccharide moiety, before extending the sugar part by standard carbohydrate chemistry. Very recently, Oscarson et al. decided to introduce the 4-*O*-substituent and the β-linkage to the steroid in a one-pot coupling sequence (Scheme 98).[173] The main attraction of this approach was to perform the subsequent couplings by just changing the solvent system, using the difference in glycosylation rates between the various solvents. The thioethyl rhamnopyranosyl donor reacted in almost quantitative yield, with the thiophenyl glucopyranosyl acceptor promoted by NIS/AgOTf in Et_2O. The addition of diosgenin alone to the mixture, as well as together with an additional portion of the promoter, did not lead to any further coupling. As soon as



Scheme 98. One-pot glycosylation.

enough CH_2Cl_2 was present in the mixture, however, the next glycosylation occurred. The use of mainly benzyl ethers as protecting groups guaranteed an activated glycosyl donor and, thus, a high yield in the coupling step with the steroid was obtained.

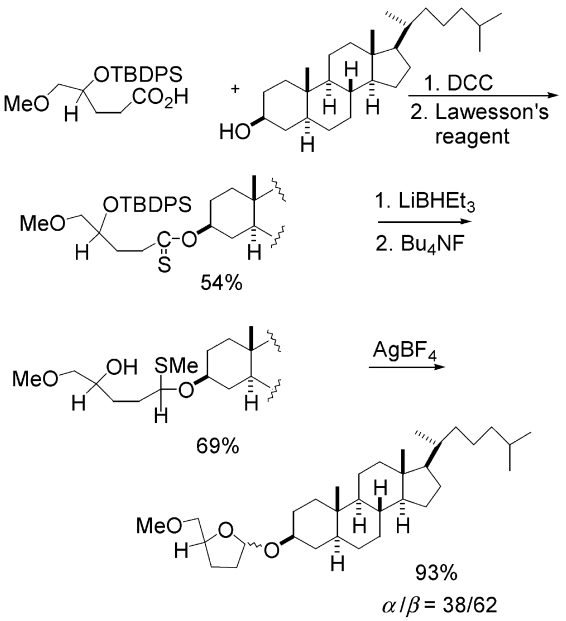
In 1989, Barrett et al. prepared several steroidal glycosides via esterification with aldonic acids, Lawesson thionation, lithium triethylborohydride-mediated reductive methylation and silver(I)-catalysed ring closure,[174] and these results clearly demonstrated that aldonic acid thionoesters were useful intermediates for redox glycosylation (Scheme 99).



Scheme 99. Redox glycosylation via thionoester intermediates.

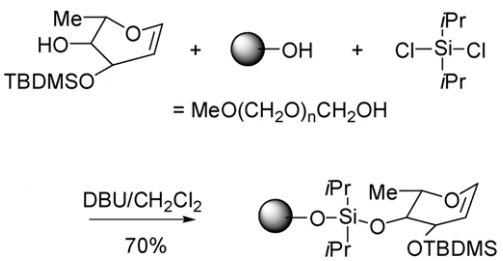
This chemistry was extended to the preparation of the 2,3-dideoxyribofuranoside derivatives of 5α-cholestan-3β-ol (Scheme 100).

In 2000, Kirschning et al. described the first solid-phase synthesis[175] of glycosteroids by two polymer-assisted strategies using two different polymeric supports, along with two appropriate linkers.[176] The first strategy used soluble polyethylene methyl ether (MPEG) as the polymeric support, which has the major advantage of being soluble in CH_2Cl_2 and insoluble in Et_2O. For the construction of the linker, the authors envisaged a bifunctional reagent that



Scheme 100. Synthesis of 2,3-dideoxyribofuranoside derivatives via redox glycosylation.

could be attached to both units, the MPEG-resin as well as the carbohydrate in a one-pot, two-step fashion. Furthermore, the linker had to be introduced without affecting the glycosyl donor properties of the enol ether double bond in the glycal employed. Finally, conditions to liberate the final glycoconjugate from the polymeric support should be accompanied by the deblocking of the remaining protecting groups from the sugar moiety (Scheme 101).

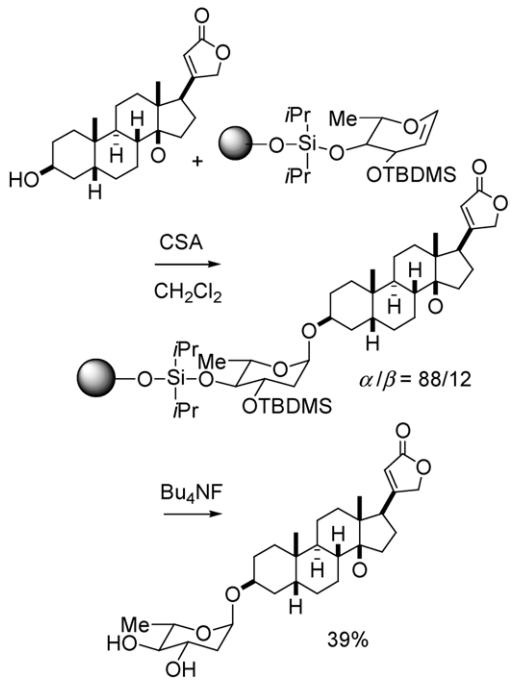


Scheme 101. Preparation of a polymer-bound glycal.

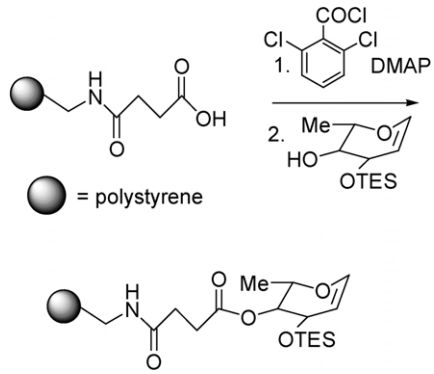
Treatment of this polymer-bound glycal with digitoxigenin in the presence of camphorsulphonic acid yielded the corresponding glycoconjugate, which gave, after cleavage of the silyl linker and deblocking of the TBDMS-protecting group successively, a new glycosteroid in 39% overall yield (Scheme 102).

A second method used a polystyrene as the solid support for glycal coupling via the succinate linker. The partially protected glycal was attached to resin as depicted in Scheme 103.

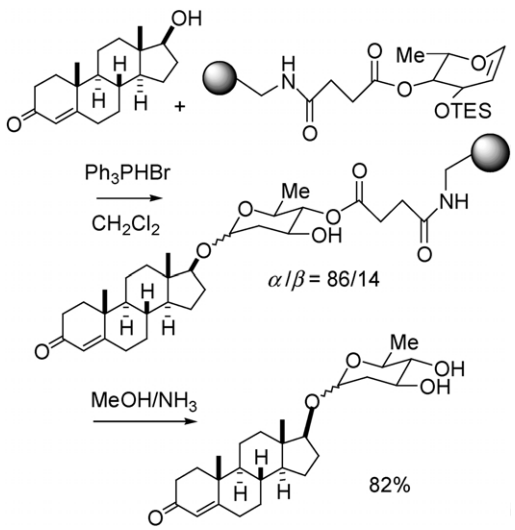
This latter polymer was treated with testosterone in the presence of a catalytic amount of triphenylphosphonium bromide, a mild acidic glycosylation promoter which yielded polymer-bound glycosylated testosterone in high yield (Scheme 104).



Scheme 102. First solid-phase synthesis of a new glycosteroid.



Scheme 103. Preparation of a polystyrene-bound glycal.



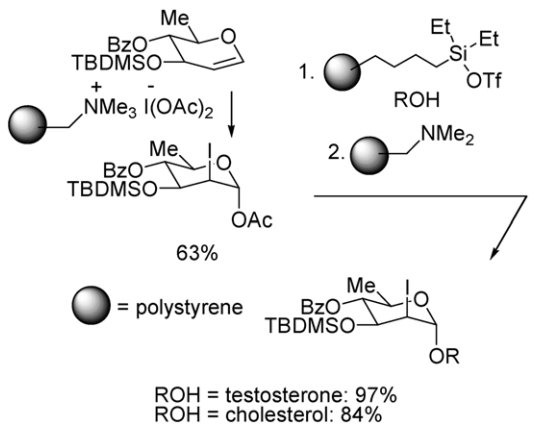
Scheme 104. Solid-phase glycosylation of testosterone.

A major disadvantage of solid-phase synthesis, however, is its requirement of robust linkers which are stable under various reaction conditions. In addition, the target glycoconjugate needs to be cleaved from the linker without affecting the diverse functionalities present in both the saccharide and the aglycon domain. In 2001, Kirschning et al. developed the polymer-assisted solution-phase synthesis of deoxysugar-based glycoconjugates using polymer-bound reagents.[177] The first step was 1,2-iodoacetoxylation of the glycal by treatment with a polymer-bound bis(acetoxy)iodate(I) complex. The glycosyl acetate thus formed was then glycosylated with the sterol in the presence of a polymer-bound silyl triflate (Scheme 105).

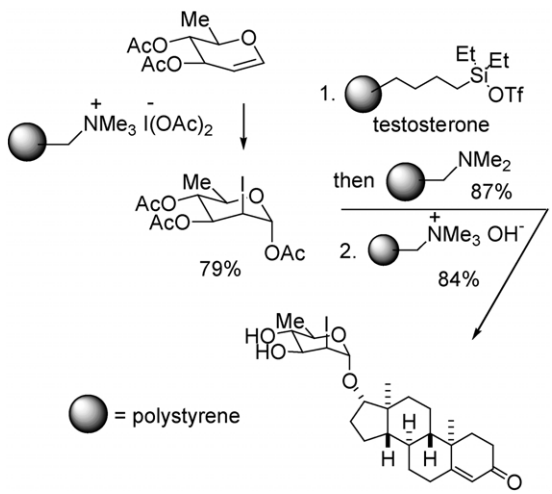
A three-step synthesis of unprotected glycosylated testosterone based on polymer-bound reagents was devised as an extension of the concept of polymer-assisted solution-phase glycosylation (Scheme 106).

12. Enzymatic methods

The examples of syntheses of steroidal glycosides via enzymatic systems are still rare in the literature. Indeed, the

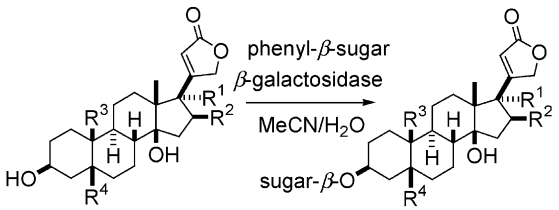


Scheme 105. Polymer-assisted glycosylation of 2-deoxy-2-iodo-α-D-glycosyl acetate.



Scheme 106. Polymer-assisted glycosylation of testosterone.

complexity of enzyme systems, the frequently observed low yields, the stringent specificities or the lack of an available catalyst still often favour chemical systems. In view of the often long-winded protection regimes required for most chemical glycosylations, however, the use of enzyme-catalysed one-step systems is clearly an attractive proposition. Furthermore, enzymatic methods of glycosylation seem to be promising as a means of synthesising unstable glycosides, because they can be carried out under mild conditions with no accompanying decomposition of the starting materials or products.[178] In 1984, Satoh et al. demonstrated that several species from inexpensive industrial β-galactosidase were stable and that they showed a high transglycosylation activity, even in the water–organic solvent media, a β-galactosidase from *Aspergillus oryzae* being involved in the enzymatic synthesis of various chemically unstable (to acid or base) cardiac glycosides.[179] This enzyme was found to be suitable for the synthesis of either β-galactosides or β-glucosides (Scheme 107 and Table 34).



Scheme 107. Enzymatic synthesis of cardiac glycosides by β-galactosidase.

Table 34.

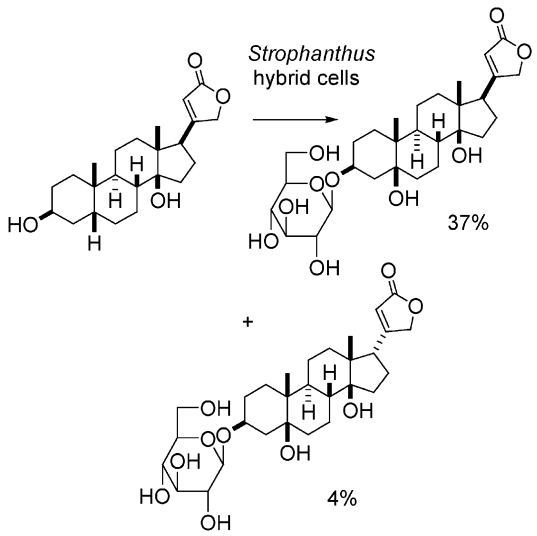
R^1	R^2	R^3	R^4	Sugar	Yield (%)
H	OH	Me	H	Galactose	26
H	OH	Me	H	Glucose	43
H	H	Me	H	Galactose	38
H	H	Me	H	Glucose	74
R^1, R^2=O	R^1, R^2=O	Me	H	Galactose	64
H	H	CHO	OH	Galactose	40

In 1998, Kawaguchi studied the biotransformation of digitoxigenin by cultured *Strophanthus* hybrid cells.[180] The main product of this biotransformation was periplogenin β-D-glucoside, isolated in 37% yield, along with a minor compound arising from isomerisation of the 17β-lactone (Scheme 108).

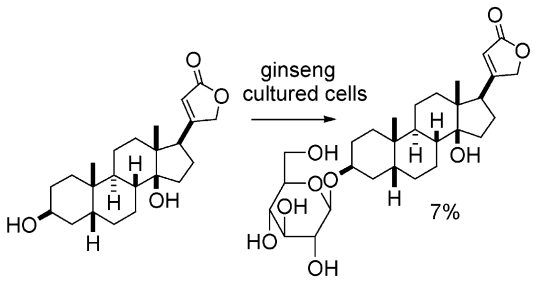
A simple glucosidation at C-3 of digitoxigenin without hydroxylation at C-5 was, however, observed by biotransformation of digitoxigenin by cultured ginseng cells (Scheme 109).[181]

A crude enzyme preparation from the young leaves of *Digitalis lanata EHRH* was shown to catalyse the transfer of D-fucose from synthetic UDP-α-D-fucose to cardenolide aglycons such as digitoxigenin (Scheme 110).[182]

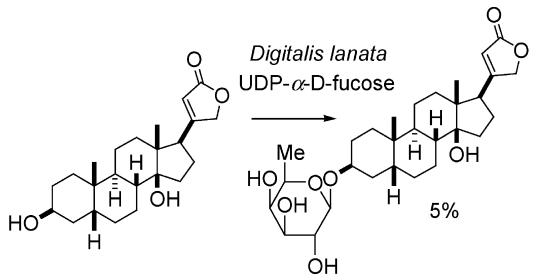
Uridine 5′-diphosphoglucuronyl transferases are a family of isoenzymes, ubiquitous in living organisms, which catalyse the synthesis of β-D-glucuronides from uridine 5′-diphospho-α-D-glucuronic acid and a wide variety of endo- as well as xenobiotic aglycons. In 1998, Thiem et al. developed an enzymatic synthese of the β-glucuronides of estradiol and



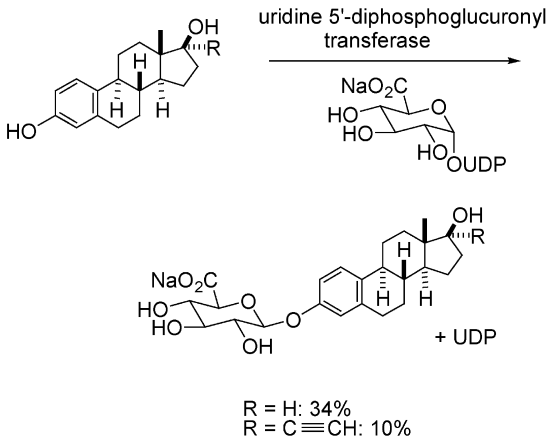
Scheme 108. Biotransformation of digitoxigenin by cultured *Strophanthus* hybrid cells.



Scheme 109. Biotransformation of digitoxigenin by cultured ginseng cells.



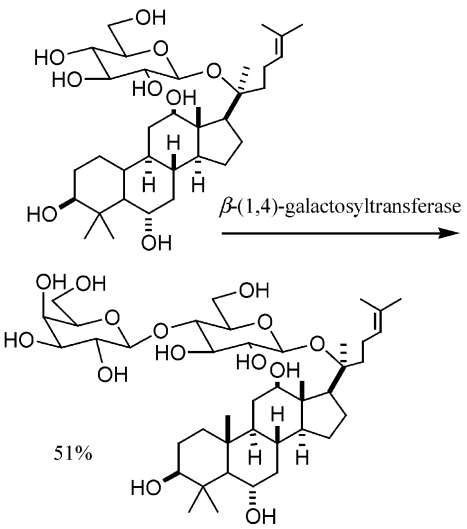
Scheme 110. Biotransformation of digitoxigenin by *Digitalis lanata*.



Scheme 111. Enzymatic synthesis β-glucuronides of estradiol and ethynylestradiol by uridine 5′-diphosphoglucuronyl transferase.

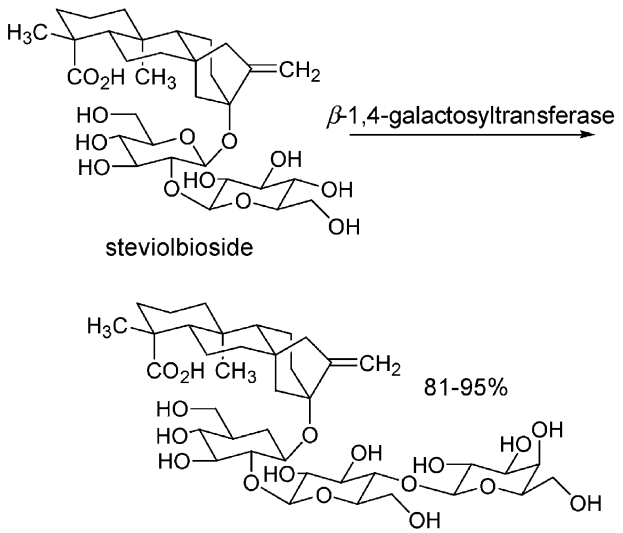
ethynylestradiol on a preparative scale employing uridine 5′-diphosphoglucuronyl transferase (Scheme 111).[183]

Other enzymatic methodologies deal with selective modifications of natural complex steroidal glycosides, Danieli reporting in 2001 the regioselective enzymatic transfer of galactose to ginsenosides Rg_1 by the use of glycosyl transferases (Scheme 112).[184]



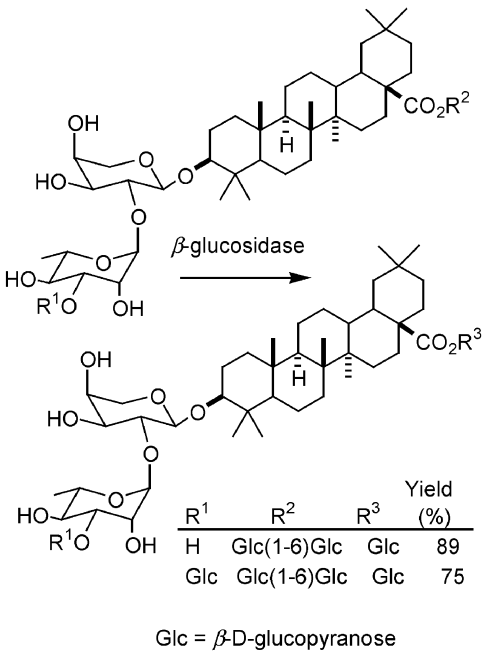
Scheme 112. Enzymatic galactosylation of ginsenosides.

Similarly, in a regioselective synthesis, the galactosyl derivatives of stevioside and steviolbioside were afforded in good yields (Scheme 113).[185]



Scheme 113. Enzymatic galactosylation of steviolbioside.

On the other hand, it is also possible to transform natural steroidal glycosides by enzymatic degradation procedures and natural saponins having terminal β-D-glucopyranose were treated with β-glucosidase, yielding the corresponding hydrolysed compounds (Scheme 114).[186]



Scheme 114. Synthesis of saponins via β-glucosidase.

13. Conclusions

Since sugar is an indispensable biosubstance in our life activity, the study of *O*-glycosylation will be continued for a long time. The possibilities of major biomedical applications based on glycobiology have dramatically increased the demand for glycoconjugates such as steroidal glycosides. In spite of almost 150 years of glycosylation chemistry, the clear fact remains that chemists have singularly failed to develop a general solution and glycosylation chemistry is still not routine, predictable or generally accessible. For this reason, chemists need cataloguing reviews for this type of chemistry. To the best of the author's knowledge, this exhaustive review is the first report offering a detailed analysis of the literature concerning the *O*-glycosylation methods applied to the preparation of steroidal glycosides.

References and notes

1. (a) Wulff, G.; Röhle, G. *Angew. Chem., Int. Ed. Engl.* **1974**, *13*, 157–216. (b) Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212–235. (c) Schmidt, R. R. *Comp. Org. Synth.* **1991**, *6*, 33–64. (d) Whitfield, D. M.; Douglas, S. P. *Glycoconjugate J.* **1996**, *13*, 5–17. (e) Stachulski, A. V.; Jenkins, G. N. *Nat. Prod. Rep.* **1998**, 173–186. (f) Davies, B. G. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2137–2160.
2. (a) Brown, L.; Thomas, R.; Aust, *J. Pharm. Sci.* **1979**, *8*, 1–10. (b) Toshima, K.; Tatsuta, K. *Chem. Rev.* **1993**, *93*, 1503–1531.
3. Davis, B. G. *J. Chem. Soc., Perkin Trans. 1* **1999**, 3215–3237.
4. Kirschning, A.; Bechthold, A. F.-W.; Rohr, J. *Top. Curr. Chem.* **1997**, *188*, 1–84.
5. (a) In *Studies in Plant Science*; *Advances in Plant Glycosides, Chemistry and Biology*; Yang, C.-R., Tanaka, O., Eds.;

Elsevier: Amsterdam, 1999; Vol. 6. (b) In *Encyclopedia of Contemporary Chinese Medical Plants*; Chi, S., Ed.; Kogyo Chosakai: Tokyo, 1980; pp 79–87 translation editor, Sugi, M. (c) *Chinese Drug Dictionary*; Koso New Medical College, Ed.; Shanghai Science and Technology: Shanghai, 1978; Vol. 1, pp 630–631. (d) Nakamura, T.; Komori, C.; Lee, Y.-Y.; Hashimoto, F.; Yahara, S.; Nohara, T.; Ejima, A. *Biol. Pharm. Bull.* **1996**, *19*, 564–566. (e) Ikeda, T.; Ando, J.; Miyazono, A.; Zhu, X.-H.; Tsumagari, H.; Nohara, T.; Yokomiso, K.; Uyeda, M. *Biol. Pharm. Bull.* **2000**, *23*, 364–365. (f) Hsu, S.-H.; Tsai, T.-R.; Lin, C.-N.; Yen, M.-H.; Kuo, K.-W. *Biochem. Biophys. Res. Commun.* **1996**, *229*, 1–5. (g) Chang, L.-C.; Tsai, T.-R.; Wang, J.-J.; Lin, C.-N.; Kuo, K.-W. *Biochem. Biophys. Res. Commun.* **1998**, *242*, 21–25. (h) Hostettmann, K.; Marston, A. *Saponins*; Cambridge University Press: New York, 1995. (i) Waller, G. R.; Yamasaki, K. *Saponins used in Traditional and Modern Medicine*; Plenum: New York, 1996. (j) Waller, G. R.; Yamasaki, K. *Saponins used in Food and Agriculture*; Plenum: New York, 1996. (k) Rouhi, A. M. *Chem. Eng. News* **1995**, *Sept. 11*, 28.

6. Koenigs, W.; Knorr, E. *Chem. Ber.* **1901**, *34*, 957–981.
7. (a) Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212–235. (b) Paulsen, H. *Chem. Soc. Rev.* **1984**, *13*, 15–45. (c) Igarashi, K. *Adv. Carb. Chem. Biochem.* **1977**, *34*, 243–283.
8. Lettré, H.; Hagedorn, A. *Zeitschrift für Physiologische Chemie* **1936**, *242*, 210–214.
9. Schapiro, E. *Biochem. J.* **1939**, *33*, 385–388.
10. Huebner, C. F.; Overman, R. S.; Link, K. P. *J. Biol. Chem.* **1944**, *155*, 615–618.
11. Meystre, C.; Miescher, K. *Helv. Chim. Acta* **1944**, *27*, 231–236.
12. Schneider, J. J.; Lewbart, M. L.; Levitan, P.; Leiberman, S. *J. Am. Chem. Soc.* **1955**, *77*, 4184–4186.
13. Elce, J. S.; Carpenter, J. G. D.; Kellie, A. E. *J. Chem. Soc.* **1967**, 542–550.
14. Elderfield, R. C.; Uhle, F. C.; Fried, J. *J. Am. Chem. Soc.* **1947**, *69*, 2235–2236.
15. Takiura, K.; Yuki, H.; Okamoto, Y.; Takai, H.; Honda, S. *Chem. Pharm. Bull.* **1974**, *22*, 2263–2269.
16. Brown, L.; Boutagy, J.; Thomas, R. *Arzneim. -Forsch.* **1981**, *7*, 1059–1064.
17. Hartenstein, J.; Satzinger, G. *Liebigs Ann. Chem.* **1974**, 1763–1766.
18. Templeton, J. F.; Setiloane, P.; Sashi Kumar, V. P.; Yan, Y.; Zeglam, T. H.; LaBella, F. S. *J. Med. Chem.* **1991**, *34*, 2778–2782.
19. Mattox, V. R.; Goodrich, J. E.; Nelson, A. N. *Steroids* **1982**, *40*, 23–34.
20. Hosoda, H.; Saito, K.; Ito, Y.; Yokohama, H.; Ishii, K.; Nambara, T. *Chem. Pharm. Bull.* **1982**, *30*, 2110–2118.
21. Hosoda, H.; Yokohama, H.; Nambara, T. *Chem. Pharm. Bull.* **1984**, *32*, 4023–4028.
22. Hosoda, H.; Yokohama, H.; Nambara, T. *Chem. Pharm. Bull.* **1984**, *32*, 1359–1364.
23. Hosoda, H.; Takasaki, W.; Miura, H.; Tohkin, M.; Maruyama, Y.; Nambara, T. *Chem. Pharm. Bull.* **1985**, *33*, 4281–4287.
24. Hosoda, H.; Yokohama, H.; Shioya, H.; Nambara, T. *Chem. Pharm. Bull.* **1985**, *33*, 1049–1054.
25. Hosoda, H.; Osanai, K.; Fukasawa, I.; Nambara, T. *Chem. Pharm. Bull.* **1990**, *38*, 1949–1952.
26. Hosoda, H.; Osanai, K.; Nambara, T. *Chem. Pharm. Bull.* **1991**, *39*, 3283–3286.
27. Kanaoka, M.; Kato, H.; Yano, S. *Chem Pharm. Bull.* **1990**, *38*, 221–224.
28. Anufriev, V. P.; Malinovskaya, G. V.; Denisenko, V. A.; Uvarova, N. I.; Elyakov, G. B.; Kim, S.-I.; Baek, N.-I. *Carbohydr. Res.* **1997**, *304*, 179–182.
29. Schneider, J. J.; Bhacca, N. S. *J. Org. Chem.* **1969**, *34*, 1990–1993.
30. Pacak, J.; Köllnerova, Z.; Cerny, M. *Collect. Czech. Chem. Commun.* **1979**, *44*, 933–941.
31. Atopkina, L. N.; Uvarova, N. I.; Elyakov, G. B. *Carbohydr. Res.* **1997**, *303*, 449–451.
32. Li, C.; Yu, B.; Liu, M.; Hui, Y. *Carbohydr. Res.* **1998**, *306*, 189–195.
33. Zhou, J. *Pure Appl. Chem.* **1989**, *61*, 457–460.
34. Saito, S.; Ichinose, K.; Sasaki, Y.; Sumita, S. *Chem. Pharm. Bull.* **1992**, *40*, 3261–3268.
35. Thiem, J.; Köpper, S. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 779–780.
36. Ogawa, T.; Nozaki, M.; Matsui, M. *Tetrahedron* **1980**, *36*, 2641–2648.
37. (a) Saito, S.; Sasaki, Y.; Kuroda, K.; Hayashi, Y.; Sumita, S.; Nagamura, Y.; Nishida, K.; Ishiguro, I. *Chem. Pharm. Bull.* **1993**, *41*, 539–543. (b) Saito, S.; Sumita, S.; Kanda, Y.; Sasaki, Y. *Chem. Pharm. Bull.* **1994**, *42*, 1016–1027. (c) Saito, S.; Sumita, S.; Furumoto, T.; Ebashi, J.; Sasaki, Y.; Nagamura, Y.; Ishiguro, I. *Eur. J. Med. Chem.* **1994**, *29*, 455–470.
38. Cerny, I.; Pouzar, V.; Drasar, P.; Budesinsky, M.; Havel, M. *Collect. Czech. Chem. Commun.* **1983**, *49*, 881–891.
39. Drasar, P.; Pouzar, V.; Cerny, I.; Smolikova, J.; Havel, M. *Collect. Czech. Chem. Commun.* **1984**, *49*, 1039–1050.
40. Cerny, I.; Novotny, P.; Drasar, P.; Havel, M.; Pacak, J. *Collect. Czech. Chem. Commun.* **1989**, *54*, 2775–2783.
41. Chodounska, H.; Pouzar, V. *Collect. Czech. Chem. Commun.* **1990**, *55*, 1639–1646.
42. Chodounska, H.; Pouzar, V. *Collect. Czech. Chem. Commun.* **1993**, *58*, 3000–3008.
43. Cerny, I.; Pouzar, V.; Drasar, P.; Havel, M. *Collect. Czech. Chem. Commun.* **1987**, *52*, 2521–2533.
44. Pis, J.; Hykl, J.; Budesinsky, M.; Harmatha, J. *Tetrahedron* **1994**, *50*, 9679–9690.
45. Luta, M.; Hensel, A.; Kreis, W. *Steroids* **1998**, *63*, 44–49.
46. Helferich, B.; Müller, W. M. *Chem. Ber.* **1973**, *106*, 715–716.
47. Wulff, G.; Röhle, G.; Krüger, W. *Chem. Ber.* **1972**, *105*, 1097–1110.
48. Conrow, R. B.; Bernstein, S. *J. Org. Chem.* **1971**, *36*, 863–870.
49. Iida, T.; Tazawa, S.; Ohshima, Y.; Niwa, T.; Goto, J.; Nambara, T. *Chem. Pharm. Bull.* **1994**, *42*, 1479–1484.
50. Zou, C.-C.; Hou, S.-J.; Shi, Y.; Lei, P.-S.; Liang, X.-T. *Carbohydr. Res.* **2003**, *338*, 721–727.
51. Helferich, B.; Wedemeyer, K. F. *Ann.* **1949**, *563*, 139–145.
52. Meyer zu Reckendorf, M.; Wassiliadou-Micheli, N.; Machleidt, H. *Chem. Ber.* **1971**, *104*, 210–214.
53. Albrecht, H. P. *Liebigs Ann. Chem.* **1977**, 1429–1434.
54. Templeton, J. F.; Ling, Y.; Zeglam, T. H.; Marat, K.; LaBella, F. S. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2503–2517.
55. Thiem, J.; Jürgens, H.-J. *Chem. Ber.* **1977**, *110*, 2852–2861.

56. Saito, S.; Nagase, S.; Kawase, M.; Nagamura, Y. *Eur. J. Med. Chem.* **1996**, *31*, 557–574.
57. Tobari, A.; Miyamae, H.; Nagasawa, A.; Koyanagi, J.; Kawase, M.; Saito, S. *Heterocycles* **2000**, *53*, 1745–1764.
58. Uvarova, N. I.; Atopkina, L. N.; Elyakov, G. B. *Carbohydr. Res.* **1980**, *83*, 33–42.
59. Rajanikanth, B.; Seshadri, R. *Ind. J. Chem.* **1989**, *28B*, 764–765.
60. Nagarajan, S.; Mohan Rao, L. J.; Gurudutt, K. N. *Ind. J. Chem.* **1998**, *37B*, 132–134.
61. DeNinno, M. P.; McCarthy, P. A.; Duplantier, K. C.; Eller, C.; Etienne, J. B.; Zawistoski, M. P.; Wen Bangerter, F.; Chandler, C. E.; Morehouse, L. A.; Sugarman, E. D.; Wilkins, R. W.; Woody, H. A.; Zaccaro, L. M. *J. Med. Chem.* **1997**, *40*, 2547–2554.
62. Lemieux, R. U.; Hendriks, K. B.; Stick, R. V.; James, K. *J. Am. Chem. Soc.* **1975**, *97*, 4056–4062.
63. Wotiz, H. H.; Smakula, E.; Lichtin, N. N.; Leftin, J. H. *J. Am. Chem. Soc.* **1959**, *81*, 1704–1708.
64. Rao, P. N.; Rodriguez, A. M.; Moore, P. H.; Cessac, J. W. *Steroids* **1992**, *57*, 216–221.
65. Chung, B.-C.; Mallamo, J. P.; Juniewicz, P. E.; Shackleton, C. H. L. *Steroids* **1992**, *57*, 530–536.
66. Bowers, S.; Bowers, L. D. *J. Steroid. Mol. Biol.* **1996**, *58*, 225–234.
67. Schneider, J. J. *Carbohydr. Res.* **1971**, *17*, 199–207.
68. Rao, P. N.; Damodaran, K. M. *Steroids* **1984**, *43*, 343–350.
69. Cerny, I.; Pouzar, V.; Drasar, P.; Havel, M. *Collect. Czech. Chem. Commun.* **1992**, *57*, 362–374.
70. Elce, J. S.; Carpenter, J. G. D.; Kellie, A. E. *J. Chem. Soc.* **1967**, 542–550.
71. Hadd, H. E.; Slikker, W.; Helton, E. D. *J. Steroid Biochem.* **1980**, *13*, 1107–1114.
72. Numazawa, M.; Nagaoka, M.; Tsuji, M. *J. Chem. Soc., Perkin Trans. 1* **1983**, 121–125.
73. Wu, Y.; Blackwell, L. F. *Steroids* **1993**, *58*, 452–456.
74. Numazawa, M.; Nagaoka, M.; Tsuji, M. *J. Chem. Soc., Chem. Commun.* **1981**, 383–384.
75. Numazawa, M.; Nagaoka, M.; Ogata, M. *Chem. Pharm. Bull.* **1984**, *32*, 618–622.
76. Gargiulo, D.; Blizzard, T. A.; Nakanishi, K. *Tetrahedron* **1989**, *45*, 5423–5432.
77. Atopkina, L. N.; Denisenko, V. A.; Uvarova, N. I.; Elyakov, G. B. *Carbohydr. Res.* **1988**, *177*, 101–109.
78. Mukaiyama, T.; Murai, Y.; Shoda, S. *Chem. Lett.* **1981**, 431–432.
79. Hashimoto, S.; Hayashi, M.; Noyori, R. *Tetrahedron Lett.* **1984**, *25*, 1379–1382.
80. Kunz, H.; Sager, W. *Helv. Chim. Acta* **1985**, *68*, 283–287.
81. Suzuki, K.; Maeta, H.; Matsumoto, T.; Tsuchihashi, I. G.-i. *Tetrahedron Lett.* **1988**, *29*, 3571–3574.
82. Suzuki, K.; Maeta, H.; Suzuki, T.; Matsumoto, T. *Tetrahedron Lett.* **1989**, *30*, 6879–6882.
83. Pikul, S.; Switzer, A. G. *Tetrahedron: Asymmetry* **1997**, *8*, 1165–1168.
84. Nishizawa, M.; Kan, Y.; Yamada, H. *Tetrahedron Lett.* **1988**, *29*, 4597–4598.
85. Nishizawa, M.; Kan, Y.; Yamada, H. *Chem. Pharm. Bull.* **1989**, *37*, 565–567.
86. Nishizawa, M.; Kan, Y.; Shimomoto, W.; Yamada, H. *Tetrahedron Lett.* **1990**, *31*, 2431–3434.
87. Nishizawa, M.; Shimomoto, W.; Momii, F.; Yamada, H. *Tetrahedron Lett.* **1992**, *33*, 1907–1908.
88. (a) Yamada, H.; Nishizawa, M. *Synlett* **1993**, 54–56. (b) Yamada, H.; Nishizawa, M. *J. Org. Chem.* **1995**, *60*, 386–397.
89. Goggin, K. D.; Lambert, J. F.; Walinsky, S. W. *Carbohydr. Res.* **1997**, *303*, 347–351.
90. Schmidt, R. R.; Michel, J. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 731–732.
91. Schmidt, R. R.; Grundler, G. *Synthesis* **1981**, 885–887.
92. Schmidt, R. R. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21–122.
93. Rathore, H.; Hashimoto, T.; Igarashi, K.; Nukaya, H.; Fullerton, D. S. *Tetrahedron* **1985**, *41*, 5427–5438.
94. Rathore, H.; From, A. H. L.; Ahmed, K.; Fullerton, D. S. *J. Med. Chem.* **1986**, *29*, 1945–1952.
95. Werschkun, B.; Gorziza, K.; Thiem, J. *J. Carbohydr. Chem.* **1999**, *18*, 629–637.
96. Deng, S.; Yu, B.; Xie, J.; Hui, Y. *J. Org. Chem.* **1999**, *64*, 7265–7266.
97. Yu, B.; Zhu, X.; Hui, Y. *Org. Lett.* **2000**, *2*, 2539–2541.
98. Yu, B.; Tao, H. *Tetrahedron Lett.* **2001**, *42*, 2405–2407.
99. Yu, B.; Tao, H. *J. Org. Chem.* **2002**, *67*, 9099–9102.
100. Sun, J.; Han, X.; Yu, B. *Carbohydr. Res.* **2003**, *338*, 827–833.
101. Finizia, G. *J. Carbohydr. Chem.* **1998**, *17*, 75–98.
102. Charrette, A. B.; Turcotte, N.; Côté, B. *J. Carbohydr. Chem.* **1994**, *13*, 421–432.
103. Liu, M.; Yu, B.; Hui, Y. *Tetrahedron Lett.* **1998**, *39*, 415–418.
104. Schmidt, R. R.; Toepfer, A. *Tetrahedron Lett.* **1991**, *32*, 3353–3356.
105. Yu, B.; Liao, J.; Zhang, Y.; Hui, Y. *Tetrahedron Lett.* **2001**, *42*, 77–79.
106. (a) Becker, D.; Galili, N. *Tetrahedron Lett.* **1992**, *33*, 4775–4778. (b) Becker, D.; Galili, N. *Carbohydr. Res.* **1993**, *248*, 129–141.
107. Urban, F. J.; Moore, B. S.; Breitenbach, R. *Tetrahedron Lett.* **1990**, *31*, 4421–4424.
108. Zawistoski, M. P.; Kiplinger, J. P.; McCarthy, P. A. *Tetrahedron* **1993**, *49*, 4799–4808.
109. Yu, W.; Jin, Z. *J. Am. Chem. Soc.* **2001**, *123*, 3369–3370.
110. Deng, S.; Yu, B.; Lou, Y.; Hui, Y. *J. Org. Chem.* **1999**, *64*, 202–208.
111. Morzycki, J. W.; Wojtkielewicz, A. *Carbohydr. Res.* **2002**, *337*, 1269–1274.
112. Ma, X.; Yu, B.; Hui, Y.; Miao, Z.; Ding, J. *Carbohydr. Res.* **2001**, *334*, 159–164.
113. Ma, X.; Yu, B.; Hui, Y.; Xiao, D.; Ding, J. *Carbohydr. Res.* **2000**, *329*, 495–505.
114. Li, C.; Yu, B.; Hui, Y. *J. Carbohydr. Chem.* **1999**, *18*, 1107–1120.
115. Liu, M.; Yu, B.; Guo, Z.; Li, C.; Hui, Y. *Carbohydr. Lett.* **1997**, *2*, 423–428.
116. Ikeda, T.; Kajimoto, T.; Kinjo, J.; Nakayama, K.; Nohara, T. *Tetrahedron Lett.* **1998**, *39*, 3513–3516.
117. Wang, P. G.; Bertozzi, C. R. *Glycochemistry* **2001**, *6*, 163–176.
118. Ikeda, T.; Miyashita, H.; Kajimoto, T.; Nohara, T. *Tetrahedron Lett.* **2001**, *42*, 2353–2356.
119. Yu, B.; Xie, J.; Deng, S.; Hui, Y. *J. Am. Chem. Soc.* **1999**, *121*, 12196–12197.

120. Ikeda, T.; Kinjo, J.; Kajimoto, T.; Nohara, T. *Heterocycles* **2000**, *52*, 775–798.
121. Ferrier, R. J.; Hay, R. W.; Venthaviyasar, N. *Carbohydr. Res.* **1973**, *27*, 55–61.
122. Wiesner, K.; Tsai, T. Y. R.; Jin, H. *Helv. Chim. Acta* **1985**, *68*, 300–314.
123. Tsuboyama, K.; Takeda, K.; Torii, K.; Ebihara, M.; Shimizu, J.; Suzuki, A.; Sato, N.; Furuhata, K.; Ogura, H. *Chem. Pharm. Bull.* **1990**, *38*, 636–638.
124. Deng, S.; Yu, B.; Hui, Y. *Tetrahedron Lett.* **1998**, *39*, 6511–6514.
125. Deng, S.; Yu, B.; Hui, Y.; Yu, H.; Han, X. *Carbohydr. Res.* **1999**, *317*, 53–62.
126. Crich, D.; Sun, S. *J. Am. Chem. Soc.* **1998**, *120*, 435–436.
127. Crich, D.; Sun, S. *Tetrahedron* **1998**, *54*, 8321–8348.
128. Du, Y.; Gu, G.; Wei, G.; Hua, Y.; Linhardt, R. *J. Org. Lett.* **2003**, *5*, 3627–3630.
129. Eby, R.; Schuerch, C. *Carbohydr. Res.* **1974**, *34*, 79–90.
130. Kahne, D.; Walker, S.; Cheng, Y.; Van Engen, D. *J. Am. Chem. Soc.* **1989**, *111*, 6881–6882.
131. Sofia, M. J.; Kakarla, R.; Kogan, N.; Dulina, R.; Hui, Y. W.; Hatzenbuhler, N. T.; Liu, D.; Chen, A.; Wagler, T. *Biorg. Med. Chem. Lett.* **1997**, *7*, 2251–2254.
132. Williams, L. J.; Garbaccio, R. M.; Danishefsky, S. *J. Carbohydr. Chem. Biol.* **2000**, *1*, 61–92.
133. Lemieux, R. U.; Nagabhushan, T. L. *Methods Carbohydr. Chem.* **1972**, *6*, 487–496.
134. Halcomb, R. L.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1989**, *111*, 6661–6666.
135. Randolph, J. T.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1993**, *115*, 8473–8474.
136. Randolph, J. T.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1995**, *117*, 5693–5700.
137. Di Bussolo, V.; Kim, Y.-J.; Gin, D. Y. *J. Am. Chem. Soc.* **1998**, *120*, 13515–13516.
138. Win, W. W.; Franck, R. W. *J. Org. Chem.* **1997**, *62*, 4510–4512.
139. Honma, K.; Nakazima, K.; Uematsu, T.; Hamada, A. *Chem. Pharm. Bull.* **1976**, *24*, 394–399.
140. Boivin, J.; Monneret, C.; Pais, M. *Tetrahedron Lett.* **1978**, *13*, 1111–1114.
141. Saito, S.; Kuroda, K.; Hayashi, Y.; Sasaki, Y.; Nagamura, Y.; Nishida, K.; Ishiguro, I. *Chem. Pharm. Bull.* **1991**, *39*, 2333–2339.
142. Higashi, K.; Susaki, H. *Chem. Pharm. Bull.* **1992**, *40*, 2019–2022.
143. Mukaiyama, T.; Katsurada, M.; Takashima, T. *Chem. Lett.* **1991**, 985–988.
144. Mukaiyama, T.; Kobayashi, S.; Shoda, S.-i. *Chem. Lett.* **1984**, 907–910.
145. Suzaki, H.; Higashi, K. *Chem. Pharm. Bull.* **1993**, *41*, 201–204.
146. Matsubara, K.; Sasaki, T.; Mukaiyama, T. *Chem. Lett.* **1993**, 1373–1376.
147. Dayal, B.; Salen, G.; Padia, J.; Shefer, S.; Tint, G. S.; Sasso, G.; Williams, T. H. *Carbohydr. Res.* **1993**, *240*, 133–142.
148. Kochetkov, N. F.; Bochkov, A. F. *Recent Dev. Chem. Nat. Carbon Compds* **1972**, *4*, 77–191.
149. Uvarova, N. I.; Oshitok, G. I.; Elyakov, G. B. *Carbohydr. Res.* **1973**, *27*, 79–87.
150. Kunz, H.; Pfrengle, W. *J. Chem. Soc., Chem. Commun.* **1986**, 713–714.
151. Inazu, T.; Hosokawa, H.; Satoh, Y. *Chem. Lett.* **1985**, 297–300.
152. Yamanoi, T.; Nakamura, K.; Sada, S.; Goto, M.; Furusawa, Y.; Takano, M.; Fujioka, A.; Yanagihara, K.; Satoh, Y.; Hosokawa, H.; Inazu, T. *Bull. Chem. Soc. Jpn* **1993**, *66*, 2617–2622.
153. Yamanoi, T.; Nakamura, K.; Takeyama, H.; Yanagihara, K.; Inazu, T. *Bull. Chem. Soc. Jpn* **1994**, *67*, 1359–1366.
154. Yamanoi, T.; Nakamura, K.; Takeyama, H.; Yanagihara, K.; Inazu, T. *Chem. Lett.* **1993**, 343–346.
155. Watanabe, Y.; Nakamoto, C.; Yamamoto, T.; Ozaki, S. *Tetrahedron* **1994**, *50*, 6523–6536.
156. Hashimoto, S.-i.; Yanagiya, Y.; Honda, T.; Harada, H.; Ikegami, S. *Tetrahedron Lett.* **1992**, *33*, 3523–3526.
157. Waldmann, H.; Böhm, G.; Schmid, U.; Röttele, H. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1944–1946.
158. Hashimoto, S.-i.; Honda, T.; Ikegami, S. *Chem. Pharm. Bull.* **1990**, *38*, 2323–2325.
159. Hashimoto, S.-i.; Honda, T.; Ikegami, S. *Heterocycles* **1990**, *30*, 775–778.
160. Pannecoucke, X.; Schmitt, G.; Luu, B. *Tetrahedron* **1994**, *50*, 6569–6578.
161. Knerr, L.; Pannecoucke, X.; Schmitt, G.; Luu, B. *Tetrahedron Lett.* **1996**, *37*, 5123–5126.
162. Knerr, L.; Pannecoucke, X.; Schmitt, G.; Luu, B. *Tetrahedron Lett.* **1998**, *39*, 273–274.
163. Zorbach, W. W.; Henderson, N.; Saeki, S. *J. Org. Chem.* **1964**, *29*, 2016–2017.
164. Jin, H.; Tsai, T. Y. R.; Wiesner, K. *Can. J. Chem.* **1983**, *61*, 2442–2444.
165. Wiesner, K.; Tsai, T. Y. R. *Pure Appl. Chem.* **1986**, *58*, 799–810.
166. Suzaki, H. *Chem. Pharm. Bull.* **1994**, *42*, 1917–1918.
167. Mukaiyama, T.; Matsubara, K.; Hora, M. *Synthesis* **1994**, 1368–1373.
168. Garcia, B. A.; Gin, D. Y. *J. Am. Chem. Soc.* **2000**, *122*, 4269–4279.
169. Wagner, B.; Heneghan, M.; Schnabel, G.; Ernst, B. *Synlett* **2003**, *9*, 1303–1306.
170. Nishida, Y.; Shingu, Y.; Dohi, H.; Kobayashi, K. *Org. Lett.* **2003**, *5*, 2377–2380.
171. Mukaiyama, T.; Matsubara, K. *Chem. Lett.* **1992**, 1041–1044.
172. Saito, S.; Sumita, S.; Kanda, Y.; Sasaki, Y. *Tetrahedron Lett.* **1992**, *33*, 7381–7384.
173. Lahmann, M.; Gybäck, H.; Garegg, P. J.; Oscarson, S.; Suhr, R.; Thiem, J. *Carbohydr. Res.* **2002**, *337*, 2153–2159.
174. Barrett, A. G. M.; Bezuidenhoudt, B. C. B.; Howell, A. R.; Lee, A. C. *J. Org. Chem.* **1989**, *54*, 2275–2277.
175. Danishefsky, S. J.; McClure, K. F.; Randolph, J. R.; Ruggeri, R. B. *Science* **1993**, *260*, 1307–1309.
176. Jesberger, M.; Jaunzems, J.; Jung, A.; Jas, G.; Schönberger, A.; Kirschning, A. *Synlett* **2000**, *9*, 1289–1293.
177. Kirschning, A.; Jesberger, M.; Schönberger, A. *Org. Lett.* **2001**, *3*, 3623–3626.
178. Crout, D. H. G.; Vic, G. *Curr. Opin. Chem. Biol.* **1998**, *2*, 98–111.
179. Ooi, Y.; Hashimoto, T.; Mitsuo, N.; Satoh, T. *Tetrahedron Lett.* **1984**, *25*, 2241–2244.
180. Kawaguchi, K.; Koike, S.; Hirotani, M.; Fujihara, M.; Furuya, T.; Iwata, R.; Morimoto, K. *Phytochemistry* **1998**, *47*, 1261–1265.

181. Kawaguchi, K.; Watanabe, T.; Hirotani, M.; Furuya, T. *Phytochemistry* **1996**, *42*, 667–669.
182. Faust, T.; Theurer, C.; Eger, K.; Kreis, W. *Biorg. Chem.* **1994**, *22*, 140–149.
183. Werschkun, B.; Wendt, A.; Thiem, J. *J. Chem. Soc., Perkin Trans. 1* **1998**, 3021–3023.
184. Danieli, B.; Falcone, L.; Monti, D.; Riva, S.; Gebhardt, S.; Schubert-Zsilavecz, M. *J. Org. Chem.* **2001**, *66*, 262–269.
185. Danieli, B.; Luisetti, M. *Helv. Chim. Acta* **1997**, *80*, 1153–1160.
186. Saito, S.; Ebashi, J.; Sumita, S.; Furumoto, T.; Nagamura, Y.; Nishida, K.; Isiguro, I. *Chem. Pharm. Bull.* **1993**, *41*, 1395–1401.

Biographical sketch

Hélène Pellissier was born in Gap, France. She carried out her PhD under the supervision of Dr. G. Gil in Marseille and then entered the Centre National de la Recherche Scientifique in 1988. After a postdoctoral period in Professor K. P. C. Vollhardt's group, she joined the group of Professor M. Santelli in Marseille in 1992, where she focused on the chemistry of BISTRO and its large application in organic synthesis. Thus, she developed several new very short total syntheses of steroids starting from 1,3-butadiene and benzocyclobutenes.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron
Tetrahedron 60 (2004) 5163–5170

Synthesis and conformation of deuterated spermidine for investigating weak interaction with polyanionic biomolecules

Keisuke Maruyoshi, Tetsuo Demura, Takeshi Sagane, Nobuaki Matsumori, Tohru Oishi and Michio Murata*

Department of Chemistry, Graduate School of Science, Osaka University, 1-16 Machikaneyama, Toyonaka, Osaka 560-0043, Japan

Received 23 March 2004; revised 22 April 2004; accepted 23 April 2004

Abstract—Polyamines such as spermidine and spermine are known to interact with polyanionic biomolecules under physiological conditions. Conformation analysis of these highly flexible acycles is virtually unprecedented due to the lack of appropriate methodologies. Spin–spin coupling constants, which are often utilized for acyclic systems, are rather uninformative for polyamines due to unresolved ^{1}H NMR signals of their tri- and tetra-methylene moieties. We attempted to solve this problem by synthesizing diastereospecifically deuterated spermidine, (1*S**,2*S**)-1,8-diamino-4-azaoctane-1,2,3,3,5,5,6,6,7,7,8,8-d_{12}. To evaluate its utility, ^{1}H–^{1}H spin coupling constants were measured as trihydrochloride or tripolyphosphate salt. The relevant coupling constant markedly decreased upon complexation to tripolyphosphate. Under neutral pH, where the net charge of tripolyphosphate became tetravalent, the coupling constant was small; as pH was lowered to 1.4, where the charge was divalent, the constant increased significantly. These data clearly indicate that, when interacting with polyanions, spermidine undergoes conformational changes to increase bent conformation, which could be effectively monitored by the deuterated spermidine.

1. Introduction

The interaction between a bioactive compound and its target molecule is one of the most intriguing subjects in biochemistry and pharmacology. Recently, highly specific recognitions between ligand and receptor have been extensively studied in structural biology, particularly in association with drug–target interactions. The crystal structures of a ligand–protein complex are solved by X-ray crystallography, and partial structures near a ligand binding site are elucidated by NMR-based methodologies. Pieces of the structural information have deepened our understanding about the molecular basis of physiological events such as cellular signal transductions, endocrine systems, and immune reactions. Besides these strict intermolecular recognitions, weak interactions between biomolecules sometimes play a crucial role. For example, neuronal receptors mostly have relatively low affinity for their intrinsic neurotransmitter, which enables the rapid on/off switching of nervous signal transmission. Structural requirements for these compounds to elicit weak interactions, however, remain mostly unelucidated; they are mostly flexible molecules and undergo rapid association/dissociation, which makes the elucidation of 3D structures of a complex exceptionally difficult. To gain a better understanding of molecular recognitions among biomolecules, the structure basis of weak interactions is essential information. In particular, conformation of flexible molecules upon complexation should be the first question to address.

Thus, we aimed at establishing a methodology toward conformation analysis of flexible small molecules implicated in weak interactions. As a model compound, we became interested in polyamines such as putrescine, spermidine and spermine because they distribute ubiquitously over eukaryotes and prokaryotes as major constituents and are considered to regulate diverse cellular events through weak interactions; e.g. polyamines are known to possess affinity to various negatively charged biopolymers, such as nucleic acids, phospholipids, and proteins,[1,2] and regulate their functions, particularly in association with cell growth, differentiation, and proliferation. In contrast to nanomolar/subnanomolar dissociation constants as seen for drug-target and hormone-receptor interactions, the values of spermidine with DNA, 16S rRNA, phospholipid, and ATP are reported to be 15.6, 2.66, 5.56 and 2.24 mM, respectively.[3] Among polyamines, we have focused on spermidine (SPD), which bears three amino groups (Fig. 1) and comparable affinity with polyanions to spermine, a tetramine congener. We are also interested in the difference in conformation between a butanylene (C2–C5) and propanylene (C7–C9) parts. In this study, the conformation change of SPD upon weakly complexating

Keywords: Spermidine; TPP; Weak interaction; Conformation analysis.
* Corresponding author. Tel./fax: +81-6-6850-5774;
e-mail address: murata@ch.wani.osaka-u.ac.jp

0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.04.056

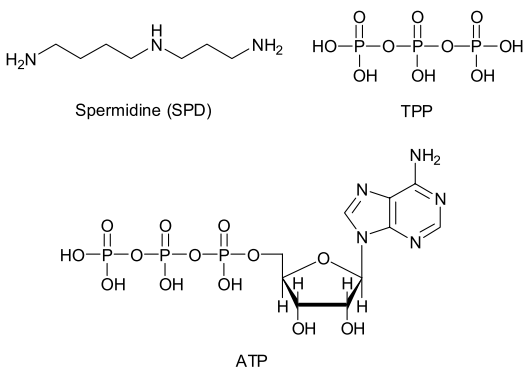


Figure 1. Structure of spermidine, ATP, TPP.

to tripolyphosphoric acid (TPP) was examined to obtain a clue as to how spermidine recognize polyanionic biomolecules (Fig. 2).

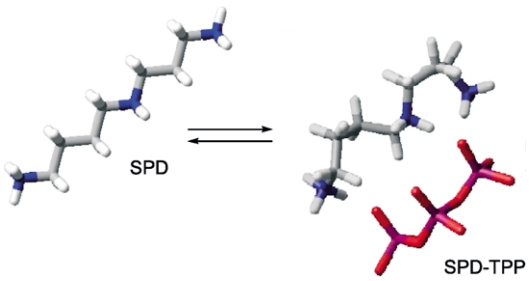
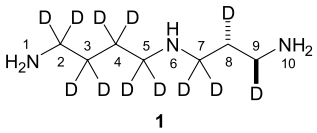


Figure 2. Hypothetical model when spermidine interacts with TPP.

Spin–spin coupling constants are a useful NMR parameter for conformational studies of biomolecules. In particular, proton–proton vicinal coupling constants ($^3J_{H,H}$) are most frequently used for this purpose because of their high sensitivity and a well-established theory known as the Karplus equation. $^3J_{H,H}$ possesses several advantages over NOE in conformation studies of acyclic and flexible systems. For compounds undergoing a conformational alteration, the coupling constants are observed as a weighted average of those due to each conformer, thereby greatly facilitating the determination of population of conformers.[4,5]

The conformation analysis of SPD by using $^3J_{H,H}$, however, is hampered by its acyclic structure, where the absence of asymmetric centers causes unresolved ^{1}H NMR methylene signals. If the spin system can be simplified by the diastereospecific labeling of each one of two geminal protons with deuterium, this problem should be addressed. In compound **1**, the rest of methylene protons are mostly deuterized to avoid the overlap of ^{1}H NMR signals. In this study, we attempted to synthesize a molecular probe (**1**) for measurement of the dihedral angles with respect to the C8–C9 bond and evaluate its conformational alteration when forming a salt with monovalent or multivalent anion.



1

2. Result and discussion

2.1. Synthesis

The deuterated SPD (**1**) would be synthesized through the coupling of bromide (**2**) and 2-nitrobenzenesulfonamide (**3**) by means of Fukuyama method (Fig. 3).[6,7] The crucial steps of the present synthesis involve the diastereoselective construction of the *erythro*-1,2-dideuterio-1,3-diaminopropane moiety, which commenced with 5,6-dihydro-2*H*-pyran-2-one (**4**) as shown in Scheme 1. Although catalytic *cis*-deuteration of alkenes sometimes resulted in scrambled addition of deuterium and polydeuteration through the β-hydride elimination of the intermediate,[8] that of **4** using Wilkinson catalyst [$RhCl(PPh_3)_3$] in benzene successfully afforded **5** as a single diastereomer.[9] The purity of **5** was

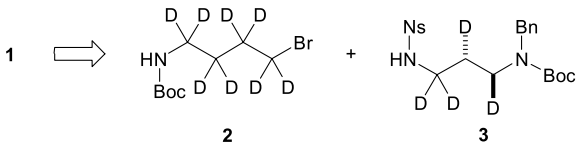
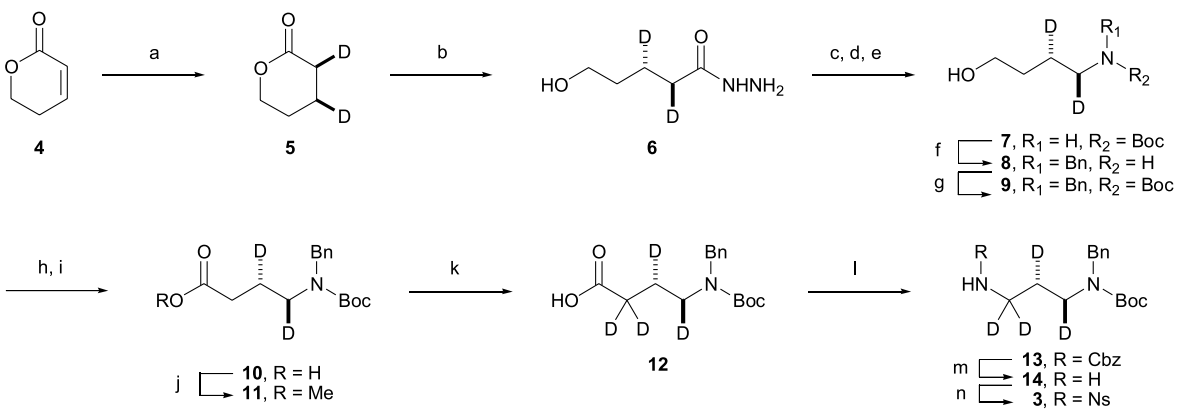


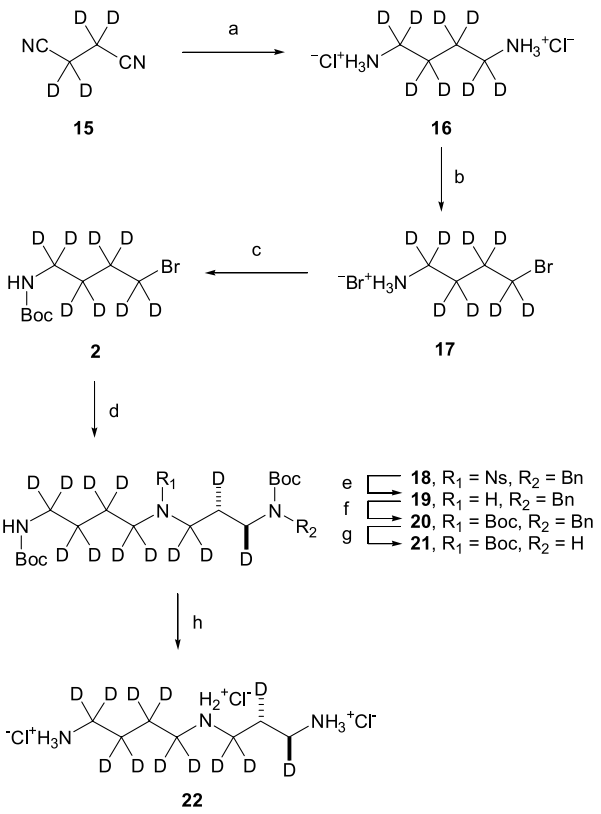
Figure 3. The coupling by Fukuyama's method.



Scheme 1. Reagents and conditions. (a) D_2, $RhCl(PPh_3)_3$, benzene, rt, 40 h (b) hydrazine, MeOH, reflux, 2 h (98% for two steps); (c) $NaNO_2$, 1 M HCl, 0 °C, 30 min; (d) toluene, reflux, 1 h then HCl; (e) $(Boc)_2O$, Et_3N, H_2O–THF, rt, 10 h (15% from **6**); (f) (1) TFA–CH_2Cl_2, rt, 10 min, 2) C_6H_5CHO, Et_3N, MeOH, rt, 1.5 h then $NaBH_4$, 0 °C, 2 h; (g) $(Boc)_2O$, Et_3N, CH_2Cl_2, rt, 2 h (87% from **7**); (h) SO_3-py, DMSO, Et_3N, rt, 30 min; (i) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, ^{t}BuOH–H_2O, rt, 1 h (91% for two steps); (j) CH_3I, $NaHCO_3$, DMF, rt, 14 h (85%); (k) CH_3OD, CH_3ONa, reflux, 50 h (94%); (l) (1) DPPA, Et_3N, reflux, 1 h, (2) benzylalcohol, reflux, 22 h (76% for two steps); (m) H_2, 20% $Pd(OH)_2$-C, MeOH, rt, 14 h (97%); (n) NsCl, Et_3N, CH_2Cl_2, rt, 16 h (99%).

confirmed by ^{13}C NMR measurements. Ring opening reaction of the lactone **5** with hydrazine in methanol at reflux for 2 h yielded hydrazide **6** (98% for two steps). Curtius rearrangement of **6** under the standard conditions[10] proceeded with the complete retention of the configuration to afford (3*S**,4*S**)-4-amino-1-propanol-3,4-d_2, which was isolated as its *N*-Boc derivative **7** in 15% yield. The low yield of **7** was due to the competitive intramolecular nucleophilic attack of the primary alcohol to the acyl azide intermediate giving lactone **5**.

Removal of the Boc group of **7**, followed by reductive amination of the resulting primary amine using benzaldehyde and $NaBH_4$, afforded *N*-benzylamine **8**, which was further protected with Boc group to yield **9** in 87% from **7**. Successive oxidation of the primary alcohol **9** with SO_3·py followed by $NaClO_2$ led to carboxylic acid **10**, which was treated with methyl iodide to afford ester **11**. Hydrogen–deuterium exchange at Cα carbon of **11** was successfully achieved by treatment with sodium methoxide in methanol-*d* at reflux for 50 h, and concomitant hydrolysis of the methyl ester occurred under the reaction conditions to yield carboxylic acid **12**.[11] Modified Curtius rearrangement of **12** using DPPA in benzylalcohol proceeded smoothly to yield benzylcarbamate **11** in 76% for two steps.[12] Cbz group of **13** was converted to 2-nitrobenzenesulfonyl (Ns) group through the hydrogenolysis of **13**, followed by treatment of the resulting primary amine with 2-nitrobenzenesulfonyl chloride[13] in the presence of triethylamine to afford propanylene fragment **3** in high yields.



Scheme 2. Reagents and conditions. (a) D_2, PtO_2, CH_3COOD, rt, 48 h then HCl (42%); (b) $NaNO_2$ (0.9 equiv.), HBr, H_2O, 0 °C, 17 h; (c) $(Boc)_2O$, Et_3N, $CHCl_3$, rt, 3 h (12% for two steps, 84% recovery of **16**); (d) **3**, K_2CO_3, DMF, 80 °C, 14 h; (e) PhSH, K_2CO_3, DMF, rt, 2 h (68% for two steps); (f) $(Boc)_2O$, Et_3N, CH_2Cl_2, rt, 1 h (91%); (g) H_2, 20% $Pd(OH)_2$-C, MeOH, rt, 12 h (41%); (h) TFA–CH_2Cl_2, rt, 15 min, then HCl (78% for two steps).

Synthesis of the butanylene fragment **2** and coupling with **3** was achieved as shown in Scheme 2. Commercially available succinonitrile-d_4 (**15**) was reduced to 1,4-diaminobutane-d_8 by treatment with PtO_2 in acetic acid-*d* under the deuterium atmosphere,[14] which was isolated as hydrochloride salt (**16**). Mono-bromination of the diamine **16** using 0.9 equiv. of $NaNO_2$ followed by protection of the remaining amino group with Boc afforded fragment **2** in 12% yield with the recovery of **16** (84%). Then, next task is the coupling of fragments **2** and **3** and successive removal of the protecting groups.

Treatment of the bromide **2** with 2-nitrobenzenesulfonamide **3** in the presence of K_2CO_3 in DMF resulted in the formation of **18**,[6,7] followed by removal of the Ns group with thiophenol afforded **19** in 68% yield for two steps. Although removal of the *N*-benzyl group of the secondary amine **19** was sluggish, that of corresponding carbamate **20** proceeded smoothly to give **21** in 41% yield. Removal of the Boc groups of **21** with TFA afforded (1*S**,2*S**)-1,8-diamino-4-azaoctane-1,2,3,3,5,5,6,6,7,7,8,8-d_{12}, **1**, which was isolated as trihydrochloride salt **22**. These synthetic strategies will be essentially applicable to preparation of other deuterated spermidine analogues for conformational study of the rest of four C–C bonds although yields in some reactions such as Curtius rearrangement from **6** to **7** and the hydrogenation of **20** should be improved. The deuterium contents were estimated by ^{13}C NMR to be >99% for C2, C3, C4, C5, C8, C9, and by ^1H NMR to be 93% for C7.

2.2. Measurements of $^3J_{H,H}$ values

The spin–spin coupling constant between H-8 and H-9 ($^3J_{H\text{-}8,H\text{-}9}$) was measured for non-labeled SPD and the molecular probe **1**. With use of non-labeled one, $^3J_{H,H}$ values could not be determined better than 0.5 Hz due to the overlap of ^1H NMR signals and the equalization of spin coupling constants, which was further complicated by second order couplings derived from close chemical shifts of H_2-7 and H_2-9. The deuterated SPD–HCl and SPD–TPP at various pH (Fig. 4(B)–(D)) were subjected to NMR measurements (Fig. 4).

As depicted in Figure 4, $^3J_{H\text{-}8,H\text{-}9}$ was clearly determined with the accuracy of 0.1 Hz although signals were broadened partly by the association/dissociation equilibrium of complexation. The value of SPD–HCl gave rise to a large value of 9.8 Hz, which showed that C7–N9 conformation of **1** as a HCl salt was anti-dominant. In acidic pH 1.4 and 3.2, $^3J_{H\text{-}8,H\text{-}9}$ of SPD–HCl was unchanged. Next, the same measurements were carried out for the SPD–TPP complex. As reported previously, the dissociation constant (K_d) of SPD–TPP complexes was calculated to be 1.23±0.07 mM[15], which indicated that 98% of SPD formed complex with TPP under the conditions of NMR measurements, and thus the spin coupling constants obtained from Figure 4(B)–(D) should be those upon complexation with TPP (Table 2). The net charge of TPP was −2, −3, and −4 at pH 1.40, 3.20, and 7.35, respectively, whereas SPD being a trivalent cation

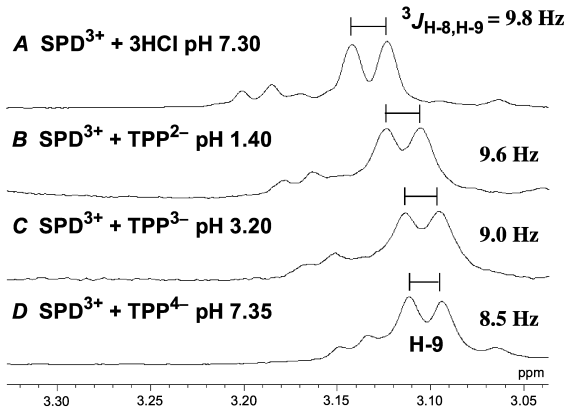


Figure 4. Partial ^{1}H NMR spectra (500 MHz) of **1**. NMR experiments were measured with 25 mM of **1** as 3HCl salt in D_2O at pH 7.30 (A), and in the presence of 100 mM tripolyphosphate (TPP) at pH 1.40 (B), and 100 mM TPP at pH 3.20 (C), and 100 mM TPP at pH 7.35 (D). The spectra of **1** as 3HCl salt at pH 1.4 and 3.2 were virtually identical with (A).

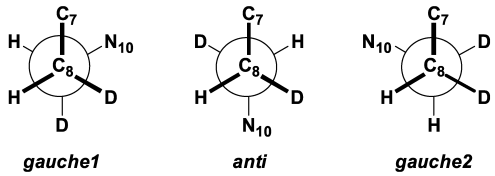
throughout experiments.[16] When compared with the HCl salt, the spin coupling constant $^3J_{H\text{-}8,H\text{-}9}$ of the TPP salt notably decreased. In neutral pH, where the net charge of TPP becomes tetravalent, $^3J_{H\text{-}8,H\text{-}9}$ was the smallest, which clearly indicated that SPD undergoes conformational changes to increase the bent conformation (equal to *gauche* rich).

2.3. Conformation analysis

In flexible or acyclic molecules like SPD, staggered conformers, where H–H dihedral angles are 60, 180 and 300°, are predominant. These *anti* and *gauche* angles are not greatly deviated unless bulky substituents are attached.[4] Thus, the conformation bearing intermediate angles, such as skew conformations, is virtually negligible. When falling on intermediate values, $^3J_{H,H}$ values are not due to non-staggered rotamers but due to the rapidly interconverting *anti* and *gauche* rotamers. Thus, once the spin coupling constants for the rotamers are obtained by using the Karplus relation, the population ratio between *anti* and *gauche* conformers can be determined. We attempted to use a modified Karplus-type equation[17] for this purpose. However, since this equation was derived from rigid cyclic compounds, fluctuation from the staggered angles should be taken into accounts before applying to flexible acyclic systems. First, Boltzmann populations of rotamers around most stable conformation were obtained using force field calculations (OPLSA*[18] force field, MacroModel[19]) in an essentially same manner as those in the previous study.[4] The values of $^3J_{H,H}$ for flexible SPD thus obtained are shown in Table 1. Since the difference between hydrogen and deuterium is negligible for conformational potentials, *gauche1* and *gauche2* should be equally populated. With these values in hand, we estimated the population of *anti* and *gauche* rotamers as shown in Table 2.

Table 1. Calculated $^3J_{H,H}$ for *anti* and *gauche* orientations in fluctuating system corresponding to the C8–C9 bond of SPD

C7–N10 dihedral angle	$^3J_{H\text{-}8,H\text{-}9}$ (Hz)
gauche1 (near 60°)	0.9
anti (near 180°)	11.2
gauche2 (near 300°)	1.7



The population of *anti* conformer was 86% around the C8–C9 bond of SPD 3HCl. With respect to the C2–C3 bond of butane, *anti* is known to be about 70%, while a pair of *gauche* comprising about 30%. These findings indicate that *anti* conformer of SPD C8/C9 is more populated than that of alkane. This can be accounted for by the electrostatic repulsion among three ammonium groups in SPD, which force these cation sites to separate each other, resulting in the extended conformation. On the other hand, when TPP was present, the *anti* conformer decreased significantly (Table 2). These results suggest that SPD tends to take a bent conformation to interact with TPP. Actually, the distance between nitrogen atoms in the propanylene part (N6–N10) is about 0.5 nm in the extended conformation, which is larger than the distance between neighbouring oxygens of TPP (ca. 0.3 nm). Therefore, SPD needs to bend its structure to arrange each ammonium group close to an anionic site of TPP. Furthermore, when pH was raised from 1.40 to 7.35, the population of *gauche* rotamers markedly increased. This may be accounted for by a notion that SPD more firmly interacts with TPP as a tetravalent anion than a divalent or trivalent form.

Table 2. Populations of rotamers obtained from ^{1}H NMR experiments

	$^3J_{H\text{-}8,H\text{-}9}$ (Hz)	*Anti*	*Gauche* (%)
SPD^{3+}–3HCl (pH 1–7)	9.8	86	14
SPD^{3+}–TPP^{2-} (pH 1.40)	9.6	84	16
SPD^{3+}–TPP^{3-} (pH 3.20)	9.0	78	22
SPD^{3+}–TPP^{4-} (pH 7.35)	8.5	73	27

3. Conclusions

We synthesized (1*S**,2*S**)-1,8-diamino-4-azaoctane-1,2,3,3,5,5,6,6,7,7,8,8-d_{12}, as a molecular probe for investigating weak interaction by SPD and utilized it for conformation analysis with polyanionic polytriphosphate. The detailed examinations on proton–proton coupling constants have disclosed that SPD undergoes conformation change upon complexation with polyanion and this interaction becomes firmer as the number of negative charges in the polyphosphate increases from divalent to tetravalent. The present results indicate for the first time that the structure of the flexible molecule with rapidly alternating conformations can be quantitatively analyzed and characterized by using diastereospecifically deuterated analogues. This method would provide a powerful strategy for investigating the molecular basis underlying weak interactions among biomolecules. Currently, we are examining the weak interaction between polyamine and ATP using this method.

4. Experimental

4.1. Method for measuring $^3J_{H,H}$

$^3J_{H,H}$ values were extracted from 1D ^1H NMR spectra. NMR spectra were obtained on a JEOL GSX-500 (500 MHz) spectrometer. Digital resolution for ^1H NMR spectra is 0.076 Hz/point. Hence, measurement of coupling constants can be carried out with an accuracy of ±0.1 Hz. The temperature of the probe was kept at 40 °C. A sample of **1** was dissolved in D_2O as a solvent. The concentration of SPD and TPP was prepared by 25 and 100 mM, respectively. The pH was adjusted to 1.4, 3.2, and 7.3 by deuterium chloride and sodium deuteroxide. The chemical shifts were recorded from 3-(trimethylsilyl)-1-propanesulfonic acid, sodium salt (DSS).

4.2. Determination of anti/gauche populations

$^3J_{H,H}$ for the C8–C9 bond of **1** with conformational fluctuation were calculated as follows. The energies (E_i) of 360 conformers (total number, N) were obtained for each 1° in the dihedral angle along the C8–C9 bond using OPLSA* force field on MacroModel. The calculations were performed with taking account of the solvent effect of water as a dielectric constant. These values were substituted for the formula (1) and Boltzmann population P_i was obtained.

$$P_i = \frac{\exp(-E_i/RT)}{\sum_i^N \exp(-E_i/RT)} \quad (1)$$

A spin coupling constant ^3J_i in conformer i is given by substituting the dihedral angle between H8 and H9 for the modified Karplus equation.[17]

$$^3J_{H,H} = \sum_i^N P_i {}^3J_i \quad (2)$$

The averaged values for *gauche1* 60°, *anti* 180°, and *gauche2* 300° in Table 1 were obtained from formula (2) for the dihedral angles between 0 and 120°, those between 120 and 240°, and those between 240 and 360°, respectively.

4.3. Synthesis of labeled spermidine

All reactions sensitive to air or moisture were carried out under argon atmosphere in dry, freshly distilled solvents under anhydrous conditions, unless otherwise noted. THF was distilled sodium/benzophenone, diethyl ether (Et_2O) from $LiAlH_4$, and DMF, and DMSO from calcium hydride under reduced pressure. Dichloromethane (CH_2Cl_2) and toluene was dried over activated MS4A. All other reagents were used as supplied unless otherwise stated. Analytical thin-layer chromatography (TLC) was performed using E. Merck Silica gel 60 F254 pre-coated plates. Column chromatography was performed using 100–210 μm Silica Gel 60N (Kanto Chemical), and for flash column chromatography 40–63 μm Silica Gel 60N (Merck) was used. ^1H and ^{13}C NMR spectra were recorded on a JEOL GSX-500 (500 MHz) spectrometer. Chemical shifts are reported in δ (ppm) using residual $CHCl_3$ as an internal standard of δ 7.26 and δ 77.00 for ^1H and ^{13}C NMR, respectively. Signal patterns are indicated as s, singlet; d, doublet; t, triplet; q, quartet; quin, quintet; m, multiplet; br, broad peak. Mass spectra (ESI) were recorded on a ThermoQuest LCQ DECA instrument.

4.3.1. (2*S* *,3*S* *)-δ-Valerolactone-2,3-d_2 (5). A solution of 5,6-dihydro-2*H*-pyrane-2-one (**4**) (5.27 g, 53.7 mmol) and Wilkinson catalyst (500 mg, 0.01 equiv.) in benzene (50 ml) was stirred under 1 atm of deuterium atmosphere for 40 h. The resulting precipitates were removed off by filtration through the florisil pad, and the filtrate containing the volatile material was concentrated carefully in vacuo to give lactone **4** as a colorless oil. The purity was estimated to be >95% by ^{13}C NMR analysis, and this material was employed without further purification: R_f 0.16 (hexane–ethyl acetate 3:1); δ_H (500 MHz; $CDCl_3$) 4.27 (2H, t, $J_{5,4}$=5.5 Hz, 5-H), 2.40 (1H, br d, 2-H), 1.82 (1H, br q, 3-H), 1.78 (2H, q, $J_{4,5}$=5.5 Hz, $J_{4,3}$=7.0 Hz, 4-H); δ_C (125 MHz; $CDCl_3$) 171.1 (C1), 69.2 (C5), 29.3 (t, C2), 22.1 (C4), 18.5 (t, C3); MS (ESI) *m/z* 103 $[M+H]^+$; calcd for $C_5H_6D_2O_2$ $[M+H]^+$ 103.

4.3.2. (2*S* *,3*S* *)-5-Hydroxypentanoylhydrazide-2,3-d_2 (6). Hydrazine monohydrate (3.9 ml, 80.6 mmol) was added to a solution of the crude lactone **5** (ca. 53.7 mmol) in methanol (200 ml), and the mixture was heated at reflux for 2 h. The solvent was removed under reduced pressure and the residue was purified by recrystallization from methanol/diethyl ether to give hydrazide **6** (6.92 g, 51.6 mmol, 96% yield from **4**) as colorless prisms: mp 89–91 °C; R_f 0.083 (chloroform–methanol 9:1); δ_H (500 MHz; CD_3OD) 3.54 (2H, t, $J_{5,4}$=6.5 Hz, 5-H), 2.16 (1H, d, $J_{2,3}$=8.0 Hz, 2-H), 1.64 (1H, q, $J_{3,2}$=8.0 Hz, $J_{3,4}$=7.5 Hz, 3-H), 1.52 (2H, q, $J_{4,5}$=6.5 Hz, $J_{3,4}$=7.5 Hz, 4-H); δ_C (125 MHz; CD_3OD) 174.9 (C1), 62.3 (C5), 34.3 (t, C2), 32.9 (C4), 22.8 (t, C3); MS (ESI) *m/z* 135 $[M+H]^+$; calcd for $C_5H_{10}D_2N_2O_2$ $[M+Na]^+$ 135.

4.3.3. (3*S* *,4*S* *)-4-(*tert*-Butoxycarbonylamino)butanol-3,4-d_2 (7). A solution of sodium nitrite (5.34 g, 77.4 mmol) in water (50 ml) was slowly added to a solution of the hydrazide **6** (6.92 g, 51.6 mmol) in 0.5 M HCl (400 ml) at 0 °C over 30 min. The reaction mixture was extracted with diethyl ether, and the combined organic layers were washed with saturated aqueous $NaHCO_3$, dried over Na_2SO_4, concentrated in vacuo to give a yellow oil, which was dissolved in toluene (300 ml) and heated at reflux for 1 h. While still hot, concentrated HCl (10 ml) and 2-propanol (90 ml) were poured into the solution and heated at reflux for 10 h, then toluene was removed in vacuo. Ethanol (50 ml) was added to the flask and then distilled off. This process was repeated twice to obtain crude amino-alcohol as a cream-yellow oil (2.26 g, 17.7 mmol), which was dissolved in tetrahydrofuran (150 ml) and water (50 ml). Triethylamine (4.5 ml, 32.2 mmol) was added to the solution, followed by addition of di-*tert*-butoxydicarbonate (6.3 ml, 26.6 mmol). After stirring for 10 h, the reaction mixture was extracted with diethyl ether, and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. Column chromatography on silica gave **7** (1.48 g, 7.74 mmol, 15% from **6**) as a colorless oil: R_f 0.21 (hexane–ethyl acetate 1:1); δ_H (500 MHz; $CDCl_3$) 4.57 (1H, br s, NH), 3.64 (2H, t,

$J_{1,2}$=6.0 Hz, 1-H), 3.13 (1H, br d, 4-H), 1.55–1.46 (3H, m, 3-H, 2-H), 1.41 (9H, s, Boc); δ_C (125 MHz; $CDCl_3$) 156.0 (Boc), 79.2 (Boc), 62.5 (C1), 40.0 (t, C4), 29.7 (C2), 28.5 (Boc), 26.2 (t, C3); MS (ESI) *m/z* 214 $[M+Na]^+$; calcd for $C_9H_{17}D_2NO_3$ $[M+Na]^+$ 214.

4.3.4. (3*S,4*S**)-4-(Benzylamino)butanol-3,4-d_2 (8).** TFA (8 ml) was added to a solution of **7** (860 mg, 4.50 mmol) in CH_2Cl_2 (16 ml). After stirring for 10 min, the solvent was removed in vacuo. Benzaldehyde (551 μl, 5.4 mmol) and triethylamine (1.9 ml, 13.5 mmol) were added sequentially to a solution of the residue in methanol (10 ml). After stirring for 10 min, anhydrous $MgSO_4$ was added and the resulting suspension was stirred for 1 h. The reaction mixture was cooled to 0 °C, and treated with sodium borohydride (2.04 g, 54 mmol). After stirring for 2 h, the reaction was quenched with water, and methanol was removed in vacuo. The residue was extracted with diethyl ether, and combined organic layers were washed with water, dried over Na_2SO_4, filtered, and concentrated in vacuo to give **8** as a colorless oil. The purity was estimated to be >95% by ^{13}C NMR analysis, and this material was employed without further purification: R_f 0.18 (chloroform–methanol–isopropylamine 45:5:1); δ_H (500 MHz; $CDCl_3$) 7.28–7.13 (5H, m, Bn), 3.71 (2H, s, Bn), 3.52 (2H, t, $J_{1,2}$=4.8 Hz, 1-H), 2.63 (1H, br d, 4-H), 1.61–1.57 (3H, m, 2-H, 3-H); δ_C (125 MHz; $CDCl_3$) 140.4 (Bn), 129.4 (Bn), 129.3 (Bn), 128.0 (Bn), 62.7 (C1), 54.4 (Bn), 49.8 (t, C4), 31.7 (C2), 27.2 (t, C3); MS (ESI) *m/z* 182 $[M+H]^+$; calcd for $C_{11}H_{15}D_2NO$ $[M+H]^+$ 182.

4.3.5. (3*S,4*S**)-4-(Benzyl-*tert*-butoxycarbonylamino) butanol-3,4-d_2 (9).** Triethylamine (1.9 ml, 13.5 mmol) and di-*tert*-butoxy-dicarbonate (1.2 ml, 4.95 mmol) was added sequentially to a solution of **8** (ca. 4.50 mmol) in CH_2Cl_2 (20 ml). After stirring for 2 h, the reaction mixture was poured into water, and extracted with diethyl ether. The combined organic layers were washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated in vacuo. Column chromatography on silica (hexane–ethyl acetate 1:1) gave **9** (1.10 g, 87% from **7**) as a colorless oil: R_f 0.69 (chloroform–methanol 9:1); δ_H (500 MHz; $CDCl_3$) 7.25–7.15 (5H, m, Bn), 4.34 (2H, br, Bn), 3.53 (2H, br, 1-H), 3.16–3.08 (1H, br d, 4-H), 1.41–1.36 (12H, br m, 2-H, 3-H, Boc); δ_C (125 MHz; $CDCl_3$) 155.8 (Boc), 138.3 (Bn), 128.3 (Bn), 127.2 (Bn), 127.0 (Bn), 79.8 (Boc), 62.1 (C1), 50.5 (Bn), 46.0 (t, C4), 29.6 (C2), 28.5 (Boc), 23.8 (t, C3); MS (ESI) *m/z* 304 $[M+Na]^+$; calcd for $C_{16}H_{23}D_2NO_3$ $[M+Na]^+$ 304.

4.3.6. (3*S,4*S**)-4-(Benzyl-*tert*-butoxycarbonylamino) butanoic acid-3,4-d_2 (10).** Pyridine sulfur trioxide complex (3.17 g, 19.9 mmol) was added to a solution of **9** (700 mg, 2.49 mmol) and triethylamine (5.2 ml, 37.4 mmol) in dimethyl sulfoxide (14 ml, 199 mmol). After stirring for 30 min, the reaction mixture was poured into water, and extracted with diethyl ether. The combined organic layers were washed sequentially with saturated aqueous NH_4Cl, saturated aqueous $NaHCO_3$, saturated aqueous NaCl, dried over Na_2SO_4, and concentrated in vacuo to give a yellow oil. The crude aldehyde was immediately subjected to the next reaction. 2-Methyl-2-butene (1.3 ml, 12.5 mmol) and NaH_2PO_4–$2H_2O$ (775 mg, 4.98 mmol) was added to a solution of the crude aldehyde in *tert*-butanol (10 ml) and water (5 ml), subsequently sodium chlorite (990 mg, 9.96 mmol) was added in portionwise. After stirring for 1 h, the reaction mixture was poured into saturated aqueous NaCl, and extracted with ethyl acetate, dried over Na_2SO_4, and concentrated in vacuo. Column chromatography on silica (hexane–ethyl acetate 3:1) gave **10** (670 mg, 91% from **9**) as a colorless oil: R_f 0.080 (hexane–ethyl acetate 3:1); δ_H (500 MHz; $CDCl_3$) 7.25–7.15 (5H, m, Bn), 4.40 (2H, br, Bn), 3.23–3.16 (1H, br d, 4-H), 2.31 (2H, br, 2-H), 1.79 (1H, br, 3-H), 1.48–1.43 (9H, br, Boc); δ_C (125 MHz; $CDCl_3$) 178.3 (C1), 155.8 (Boc), 138.1 (Bn), 128.3 (Bn), 127.6 (Bn), 127.1 (Bn), 80.1 (Boc), 50.1 (br d, Bn), 45.2 (t, C4), 31.1 (C2), 28.4 (Boc), 22.6 (t, C3); MS (ESI) *m/z* 318 $[M+Na]^+$; calcd for $C_{16}H_{21}D_2NO_4$ $[M+Na]^+$ 318.

4.3.7. Methyl(3*S,4*S**)-4-(benzyl-*tert*-butoxycarbonylamino)butanoate-3,4-d_2 (11).** Methyl iodide (226 μl, 3.63 mmol) was added to a mixture of **10** (670 mg, 2.27 mmol) and $NaHCO_3$ (381 mg, 4.54 mmol) in DMF (10 ml). After stirring for 14 h, the reaction mixture was poured into water, and extracted with ethyl acetate. The combined organic layers were washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated in vacuo. Column chromatography on silica (hexane–ethyl acetate 3:1) gave **11** (597 mg, 85%) as a yellow oil: R_f 0.39 (hexane–ethyl acetate 3:1); δ_H (500 MHz; $CDCl_3$) 7.25–7.15 (5H, m, Bn), 4.37 (2H, br d, Bn), 3.59 (3H, s, OMe), 3.19–3.11 (1H, br d, 4-H), 2.21 (2H, br, 2-H), 1.73 (1H, br, 3-H), 1.48–1.43 (9H, br d, Boc); δ_C (125 MHz; $CDCl_3$) 173.2 (C1), 155.8 (Boc), 138.1 (Bn), 128.2 (Bn), 127.4 (Bn), 126.9 (Bn), 79.6 (Boc), 51.4 (OMe), 50.0 (br d, Bn), 45.2 (t, C4), 31.0 (C2), 28.3 (Boc), 22.7 (t, C3); MS (ESI) *m/z* 332 $[M+Na]^+$; calcd for $C_{17}H_{23}D_2NO_4$ $[M+Na]^+$ 332.

4.3.8. (3*S,4*S**)-4-(Benzyl-*tert*-butoxycarbonylamino) butanoic acid-2,2,3,4-d_2 (12).** A solution of **11** (550 mg, 1.78 mmol) sodium methoxide (144 mg, 2.67 mmol) in methanol-d_1 (10 ml, 246 mmol) was heated at reflux for 50 h. The solvent was removed in vacuo and the residue was diluted with 1 M deuterium chloride in D_2O (18 ml, 99 atom % D), extracted with ethyl acetate, dried over Na_2SO_4, and concentrated in vacuo to afford **12** (496 mg, 94%) as a colorless oil. The material was employed without further purification: R_f 0.080 (hexane–ethyl acetate 3:1); δ_H (500 MHz; $CDCl_3$) 7.25–7.15 (5H, m, Bn), 4.40 (2H, br, Bn), 3.23–3.16 (1H, br d, 4-H), 1.79 (1H, br, 3-H), 1.48–1.43 (9H, br, Boc); δ_C (125 MHz; $CDCl_3$) 178.3 (C1), 155.8 (Boc), 138.1 (Bn), 128.3 (Bn), 127.6 (Bn), 127.1 (Bn), 80.1 (Boc), 50.1 (br d, Bn), 45.2 (t, C4), 30.6 (quin, C2), 28.4 (Boc), 22.6 (t, C3); MS (ESI) *m/z* 320 $[M+Na]^+$; Calcd for $C_{16}H_{19}D_4NO_4$ $[M+Na]^+$ 320.

4.3.9. (1*S,2*S**)-1-(Benzyl-*tert*-butoxycarbonylamino)-3-(benzyloxycarbonylamino)propane-1,2,3,3-d_4 (13).** Triethylamine (279 μl, 2.00 mmol) and diphenyl phosphorazidate (431 μl, 2.00 mmol) were added to a solution of **12** (496 mg, 1.67 mmol) in anhydrous toluene (10 ml), and heated at reflux for 1 h. Benzylalcohol (259 μl, 2.51 mmol) was added to the mixture, and heated at reflux for 22 h. The solvent was removed in vacuo, and the residue was dissolved in ethyl acetate. The solution was washed sequentially with 1 M HCl, water, saturated aqueous

$NaHCO_3$, saturated aqueous NaCl, dried over Na_2SO_4, and concentrated in vacuo. Column chromatography on silica (hexane–ethyl acetate 3:1) gave **13** (511 mg, 76%) as a yellow oil: R_f 0.31 (hexane–ethyl acetate 3:1); δ_H (500 MHz; $CDCl_3$) 7.31–7.14 (10H, m, Cbz, Bn), 5.05 (2H, s, Cbz), 4.40 (2H, br, Bn), 3.23–3.11 (1H, br, 1-H), 1.55–1.42 (10H, br, 2-H, Boc); δ_C (125 MHz; $CDCl_3$) 156.4 (Cbz), 155.9 (Boc), 138.0 (Bn), 136.4 (Cbz), 128.3 (Bn, Cbz), 127.3 (Cbz), 127.0 (Bn), 126.9 (Bn), 79.8 (Boc), 66.7 (Cbz), 50.0 (br d, Bn), 43.7 (t, C1), 37.1 (quin, C3), 28.2 (Boc), 26.9 (t, C2); MS (ESI) *m/z* 425 $[M+Na]^+$; calcd for $C_{23}H_{26}D_4N_2O_4$ $[M+Na]^+$ 425.

4.3.10. (1*S* *,2*S* *)-1-(Benzyl-*tert*-butoxycarbonylamino)-3-aminopropane-1,2,3,3-d_4 (14). 20% $Pd(OH)_2$ on charcoal (100 mg) was added to a solution of **13** (511 mg, 1.27 mmol) in methanol (5 ml), and the mixture was stirred under 1 atm of hydrogen atmosphere for 14 h. The catalyst was removed by filtration through a celite pad, and the filtrate was concentrated in vacuo. Column chromatography on silica (chloroform–methanol–isopropylamine 45:5:1) gave **14** (331 mg, 97%) as a colorless oil: R_f 0.18 (chloroform–methanol–isopropylamine 45:5:1); δ_H (500 MHz; $CDCl_3$) 7.25–7.15 (5H, m, Bn), 4.34 (2H, br, Bn), 3.19–3.10 (1H, br d, 3-H), 1.52–1.37 (10H, br m, 2-H, Boc); δ_C (125 MHz, $CDCl_3$) 178.3 (C1), 155.7 (Boc), 138.1 (Bn), 128.2 (Bn), 127.4 (Bn), 126.9 (Bn), 79.7 (Boc), 49.8 (br d, Bn), 43.0 (t, C1), 37.9 (quin, C3), 30.6 (t, C2), 28.3 (Boc); MS (ESI) *m/z* 269 $[M+H]^+$; calcd for $C_{15}H_{20}D_4N_2O_2$ $[M+H]^+$ 269.

4.3.11. (1*S* *,2*S* *)-1-(Benzyl-*tert*-butoxycarbonylamino)-3-[(2-nitrobenzenesulfonyl)amino]-1,3-propane-1,2,3,3-d_4 (3). Triethylamine (78 μl, 0.559 mmol) and 2-nitrobenzenesulfonyl chloride (124 mg, 0.559 mmol) were sequentially added to a solution of **14** (125 mg, 0.466 mmol) in CH_2Cl_2 (5 ml). After stirring for 16 h, the reaction mixture was poured into water, and extracted with CH_2Cl_2. The combined organic layers were washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated in vacuo. Column chromatography on silica (hexane–ethyl acetate 3:1) gave **3** (209 mg, 99%) as a yellow amorphous: R_f 0.24 (hexane–ethyl acetate 3:1); δ_H (500 MHz; $CDCl_3$) 8.01 (1H, m, Ns), 7.80 (1H, m, Ns), 7.65 (2H, m, Ns), 7.24–7.14 (5H, m, Bn), 5.36 (1H, br d, NH), 4.30 (2H, br, Bn), 3.19 (1H, br, 1-H), 1.55–1.36 (10H, br, 2-H, Boc); δ_C (125 MHz; $CDCl_3$) 156.0 (Boc), 147.6 (Ns), 137.8 (Bn), 133.2 (Ns), 132.4 (Ns), 130.3 (Ns), 128.3 (Bn), 127.0 (Bn), 126.9 (Bn), 124.8 (Ns), 80.1 (Boc), 50.3 (br d, Bn), 42.6 (t, C1), 40.1 (quin, C3), 28.2 (Boc), 27.6 (t, C2); MS (ESI) *m/z* 476 $[M+Na]^+$; calcd for $C_{21}H_{23}D_4N_3O_6S$ $[M+Na]^+$ 476.

4.3.12. 1,4-Butyldiammonium chloride-1,1,2,2,3,3,4,4-d_8 (16). Platinum (IV) oxide (200 mg, 0.881 mmol) was added to a solution of succinonitrile-d_4 (**15**) (5.00 g, 59.4 mmol) in acetic acid-*d* (20 ml, 350 mmol, 99.5 atom%), and stirred under 1 atm of deuterium atmosphere for 48 h. The reaction mixture was filtered through a celite pad, and the filtrate was concentrated in vacuo. Concentrated HCl was added to the residue, and then the solvents were distilled off. This process was repeated twice to obtain crude diamine. Recrystallized from ethanol gave **16** (4.91 g, 42%) as colorless prisms: R_f 0 (chloroform–methanol 9:1); δ_C (125 MHz; D_2O) 39.3 (quin, C1, C4), 23.9 (quin, C2, C3); MS (ESI) *m/z* 97 $[M-2Cl-H]^+$; calcd for $C_4H_6D_8Cl_2N_2$ $[M-2Cl-H]^+$ 97.

4.3.13. 4-(*tert*-Butoxycarbonylamino)butylbromide-1,1,2,2,3,3,4,4-d_6 (2). Diammonium chloride (**16**) (4.91 g, 24.8 mmol) was dissolved in 1.5 M HBr (100 ml) at 0 °C. After short stirring, a solution of sodium nitrite (1.54 g, 22.3 mmol) in water (25 ml) was added in dropwise to the reaction mixture with stirring at 0 °C over a period of 1 h. Stirring was continued at room temperature for 16 h. Evaporation of the solvent gave crude bromide **17** as a yellow solid which was used in the next step without further purification. A solution of **17** in methanol (50 ml) was added dropwise to a solution of di-*tert*-butoxy-dicarbonate (7.1 ml, 29.7 mmol) and triethylamine (34 ml, 248 mmol) in $CHCl_3$ (100 ml) over a period of 1 h at 0 °C. After stirring for 2 h at room temperature, the solvent was removed in vacuo. Water was added to the residue, and extracted with $CHCl_3$, dried over Na_2SO_4, and concentrated in vacuo. Column chromatography on silica (hexane–ethyl acetate 3:1) gave **2** (774 mg, 12% for two steps, 84% recover of **16**) as a colorless amorphous: R_f 0.59 (hexane–ethyl acetate 3:1); δ_H (500 MHz; $CDCl_3$) 1.39 (9H, s, Boc); δ_C (125 MHz; $CDCl_3$) 155.8 (Boc), 79.3 (Boc), 38.8 (quin, C4), 32.4 (quin, C1), 28.7 (quin, C3), 28.4 (Boc), 27.6 (quin, C2); MS (ESI) *m/z* 282 $[M+Na]^+$; calcd for $C_9H_{10}D_8BrNO_2$ $[M+Na]^+$ 282.

4.3.14. (1*S* *,2*S* *)-1-(Benzyl-*tert*-butoxycarbonylamino)-8-(*tert*-butoxycarbonylamino)-4-azaoctane-1,2,3,3,5,5,6,6,7,7,8,8-d_{12} (19). Bromide **2** (180 mg, 0.692 mmol) was added to a mixture of diamine **3** (209 mg, 0.461 mmol) and K_2CO_3 (319 mg, 2.31 mmol) in DMF (5 ml), and the mixture was stirred for 14 h at 80 °C. After cooling, K_2CO_3 (127 mg, 0.922 mmol) and thiophenol (141 μl, 1.38 mmol) were added to the reaction mixture, and stirred for 2 h. The reaction mixture was poured into water, and extracted with $CHCl_3$. The combined organic layers were washed with saturated aqueous $NaHCO_3$, saturated aqueous NaCl, dried over Na_2SO_4, and concentrated in vacuo. Column chromatography on silica (chloroform–methanol–isopropylamine 45:5:1) gave **19** (140 mg, 68% for two steps) as a colorless oil: R_f 0.14 (chloroform–methanol–isopropylamine 45:5:1); δ_H (500 MHz; $CDCl_3$) 7.25–7.16 (5H, m, Bn), 4.37 (2H, br, Bn), 3.20–3.09 (1H, br d, 1-H), 1.46–1.35 (19H, br, 2-H, Boc); δ_C (125 MHz; $CDCl_3$) 155.8 (Boc), 155.7 (Boc), 138.1 (Bn), 128.2 (Bn), 127.4 (Bn), 126.9 (Bn), 78.7 (Boc), 78.0 (Boc), 50.0 (br d, Bn), 48.3 (quin, C3), 44.9 (quin, C5), 43.4 (t, C1), 39.5 (quin, C8), 28.3 (Boc), 27.9 (quin, C6), 26.8 (t, C2), 25.8 (quin, C7); MS (ESI) *m/z* 448 $[M+H]^+$; calcd for $C_{24}H_{29}D_{12}N_3O_4$ $[M+H]^+$ 448.

4.3.15. (1*S* *,2*S* *)-1-(Benzyl-*tert*-butoxycarbonylamino)-8-(*tert*-butoxycarbonylamino)-4-(*tert*-butoxycarbonyl)-4-azaoctane-1,2,3,3,5,5,6,6,7,7,8,8-d_{12} (20). Compound **20** was obtained (91%) as a colorless oil from **19** by an analogous procedure described for **9**: R_f 0.77 (chloroform–methanol–isopropylamine 45:5:1); δ_H (500 MHz; $CDCl_3$) 7.25–7.15 (5H, m, Bn), 4.35 (2H, br, Bn), 3.10–3.01 (1H, br d, 1-H), 1.42–1.36 (28H, br, 2-H, Boc); δ_C (125 MHz; $CDCl_3$) 155.8 (Boc), 138.0 (Bn), 128.1 (Bn), 127.2 (Bn), 126.8 (Bn), 78.7 (Boc), 50.1 (br d, Bn), 44.4 (t, C1), 44.2

(quin, C3), 43.0 (quin, C5), 39.5 (quin, C8), 28.2 (Boc), 27.3 (quin, C7), 27.0 (quin, C6), 26.4 (t, C2); MS (ESI) *m/z* 570 $[M+Na]^+$; calcd for $C_{29}H_{37}D_{12}N_3O_6$ $[M+Na]^+$ 570.

4.3.16. (1*S* *,2*S* *)-1,8-Bis(*N*,*N*′-*tert*-butoxycarbonyl-amino)-4-(*tert*-butoxycarbonyl)-4-azaoctane-1,2,3,3,5,5,6,6,7,7,8,8-d_{12} (21). Compound **21** was obtained (41%) from **20** by an analogous procedure described for **14**: R_f 0.17 (hexane–ethyl acetate 3:1); δ_H (500 MHz; $CDCl_3$) 3.12 (1H, br d, 1-H), 1.45–1.36 (28H, br, 2-H, Boc); δ_C (125 MHz; $CDCl_3$) 47.2 (quin, C5), 44.9 (quin, C3), 39.1 (quin, C8), 37.3 (t, C1), 25.1 (quin, C7), 24.0 (t, C2), 22.6 (quin, C6); MS (ESI) *m/z* 480 $[M+Na]^+$; calcd for $C_{22}H_{31}D_{12}N_3O_6$ $[M+Na]^+$ 480.

4.3.17. (1*S* *,2*S* *)-1,8-Diamino-4-azaoctane-1,2,3,3,5,5,6,6,7,7,8,8-d_{12} (SPD-2,2,3,3,4,4,5,5,7,7,8,9-d_{12}) (22). TFA (1 ml) was added to a solution of **21** (53 mg, 0.117 mmol) in CH_2Cl_2 (3 ml). After stirring for 15 min, the solvent was removed in vacuo. The residue was dissolved in 1 M HCl, and the solvent was distilled off. This process was repeated twice to obtain crude product as a yellow solid. Recrystallization from ethanol gave **22** (24 mg, 78%) as a colorless powder: mp 252–254 °C; R_f 0 (chloroform–methanol 9:1); δ_H (500 MHz; D_2O) 3.12 (1H, d, $J_{1,2}$=9.8 Hz, 1-H), 2.11 (1H, d, $J_{2,1}$=9.8 Hz, 2-H); δ_C (125 MHz; D_2O) 47.2 (quin, C5), 44.8 (quin, C3), 39.2 (quin, C8), 37.3 (t, C1), 24.7 (quin, C7), 24.1 (t, C2), 22.6 (quin, C6); MS (ESI) *m/z* 158 $[M-3Cl-2H]^+$; calcd for $C_7H_{10}D_{12}Cl_3N_3$ $[M-3Cl-2H]^+$ 158. Anal. calcd for $C_7H_{10}D_{12}Cl_3N_3 \cdot H_2O$: C, 29.53; H+D, 7.96; N, 14.76. Found: C, 29.12; H+D, 7.75; N, 14.24.

Acknowledgements

We are grateful to Prof. Kazuei Igarashi of Chiba University for creating an opportunity for this polyamine study, to Prof. Tohru Fukuyama, The University of Tokyo, for suggestions; to Yukihiro Yamada and Kaori Nonaka in our laboratory, for their help in synthesizing labeled SPD; and to Prof. Koichi Ute and Iichiro Fukuda, Osaka University, for their help in NMR experiments at Venture Business Laboratory; and to Mr. Seiji Adachi, Osaka University, for measurements of NMR spectra.

References and notes

1. Igarashi, K.; Kashiwagi, K. *J. Biol. Chem. Biophys.* **1991**, *266*, 20803–20809.
2. Igarashi, K.; Kashiwagi, K. *Biochem. Biophys. Res. Commun.* **2000**, *271*, 559–564.
3. McCormack, S. A.; Johnson, L. R. *J. Physiol. Pharmacol.* **2001**, *52*, 327–349.
4. Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem.* **1999**, *64*, 866–876.
5. Murata, M.; Matsumori, N.; Tachibana, K. *Nippon Kagaku Kaishi* **1997**, *11*, 749–757.
6. Fukuyama, T.; Cheung, M.; Jow, C.-K.; Hidai, Y.; Kan, T. *Tetrahedron Lett.* **1997**, *38*, 5831–5834.
7. Fukuyama, T.; Cheung, M.; Kan, T. *Synlett* **1999**, *8*, 1301–1303.
8. Gungor, M.; Jardine, F. H.; Wheatley, J. D. *Polyhedron* **1988**, *7*, 1827–1829.
9. Djerassi, D.; Gutzwiller, *J. Am. Chem. Soc.* **1966**, *88*, 4537–4538. Morandi, J.; Jensen, H. B. *J. Org. Chem.* **1969**, *34*, 1889–1891.
10. Reddy, V. K.; Valansinas, A.; Sarkar, A.; Basu, H. S.; Marton, L. J.; Frydman, B. *J. Med. Chem.* **1998**, *41*, 4723–4732.
11. MacMillan, J.; Willis, C. L. *J. Chem. Soc. Perkin Trans.* **1984**, 351–355. Beale, M. H.; MacMillan, J. *J. Chem. Soc. Perkin 1* **1980**, 877–883.
12. Ninomiya, K.; Shioiri, T.; Yamada, S. *Tetrahedron* **1974**, *30*, 2151–2157.
13. Fukuyama, T.; Jow, C.-K.; Cheung, M. *Tetrahedron Lett.* **1995**, *36*, 6373–6374.
14. Smith, R. G.; Daves, G. D., Jr. *J. Org. Chem.* **1978**, *43*, 2178–2183.
15. Di Paolo, M. L.; Corazza, A.; Scarpa, M.; Stevanato, R.; Rigo, A. *Biochim. Biophys. Acta* **1995**, *1247*, 246–252.
16. Labadi, I.; Jenei, E.; Lahti, R.; Lonnberg, H. *Acta Chemica Scand.* **1991**, *45*, 1055–1059.
17. Haasnoot, C. A. G.; De Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783–2792.
18. Jorgensen, W. L.; Tirado-Rives, *J. Am. Chem. Soc.* **1988**, *110*, 1657–1666.
19. Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron
Tetrahedron 60 (2004) 5171–5176

Pyrene-appended calix[4]crowned logic gates involving normal and reverse PET: NOR, XNOR and INHIBIT

Seoung Ho Lee,[a] Jong Yeol Kim,[b] Sung Kuk Kim,[c] Joung Hae Lee[d] and Jong Seung Kim[c,*]

[a]*Department of Chemistry, Konyang University, Nonsan 320-711, South Korea*
[b]*KT & G Central Research Institute, Taejon 305-345, South Korea*
[c]*Department of Chemistry, Nano-sensor and Biotechnology, Dankook University, Hannam Dong, Seoul 140-714, South Korea*
[d]*Korea Research Institute of Standards and Science, Taejon 305-606, South Korea*

Received 25 February 2004; revised 21 April 2004; accepted 23 April 2004

Abstract—As a novel sensing system, *N*-(1-pyrenylmethyl) amide-appended calix[4]crown-5 (**2**) and crown-6 (**3**) have been newly synthesized. Judging from the fluorescence changes upon the addition of cations, **3** having crown-6 ring showed the Pb^{2+} ion selectivity over other cations tested regarding fluorescence quenching. Upon the Pb^{2+} ion coordination to two amide oxygen atoms with aid of crown ring, a reverse-photo-induced electron transfer (PET) occurs in such a way that electrons transfer from the pyrene groups to the electron deficient amide oxygen atoms to give a quenched fluorescence. By the addition of either $HClO_4$ or triethylamine in the solution of **3**, the fluorescence intensity decreased because of the reverse-PET from pyrene groups to protonated amide oxygen atoms and because of normal PET from the nitrogen anion formed by triethylamine to pyrene groups, respectively. For **3**, NOR logic gate in which the strong fluorescence signal appears at 395 nm (output: 1) is operated only when neither of triethylamine nor $Pb(ClO_4)_2$ (inputs A and B) is added (A=B: 0). XNOR gate is also operated only when both of two inputs are added (triethylamine and $HClO_4$, A=B: 1) or when neither of two inputs is added (A=B: 0). Then, for **3**, new INHIBIT gate system was also designed using such combinational inputs as $HClO_4$, $Pb(ClO_4)_2$ and triethylamine.

1. Introduction

Fluorescence probes have been developed to construct molecular devices such as wires,[1,2] switches,[3–5] and diodes[6,7] since the fluorescence was known to be a useful signaling tool to monitor such operations. As one of the chemical devices, the logic gate based on the principle of Boolean algebra has been investigated by organic chemists as well.[8–13] Thus far, basic logic gates (YES, NOT, AND and OR)[10,11] and rather complicated two-integrated logic functions such as NAND, NOR and XOR[11–13] have been newly documented. Then, more advanced ways of an integration of the relatively simple logic systems into higher-level devices are still being exploited by the chemists. In particular, NOR and XNOR (eXclusive NOR) gates have gained an intense attention as an important logic system in most digital circuits as well as in more complex device. In this point of view, de Silva et al. reported that the NOR gate performs the logic sum between the binary inputs: the output 1 only if both of two inputs are 0 (A=B: 0).[12] In the XNOR gate, there are two independent meanings: 'eXclusive' for NOR or 'NOT' for XOR.[12,14] Accordingly, the XNOR can be represented by the situation where the output is 1 only if none (A=B: 0) or both of two inputs (A=B: 1) are operated.[14] As more complex logic system, INHIBIT logic gate referred to as 'denial (nullifying) logic system' against the AND logic gate, i.e. a combination function of AND and NOT, was also reported.[12,13] All those logic gates mentioned above can be operated by one or more inputs but with a single modular molecule.[8–13]

As a consequence of the rapid development of host–guest chemistry, the supramolecular technology has led us to designing new fluorescent probes capable of selectively sensing metal cations.[15] Calixarenes, one of the supramolecular molecules, with fluorogenic pendent groups have received increasing attention and became promising candidates for the sensing probes because they are in a certain pre-organized framework to easily accommodate metal cations and neutral molecules, exhibiting a selective change in the fluorescence emission.[16–21]

Keeping the fluorogenic calixarene incorporating the logic gate system in mind, we herein report on the syntheses of novel pyrene-armed calix[4]crowns and their sensing behaviors toward metal cations. In application of the fluorogenic calix[4]crown, here we also present the logic circuits of NOR and XNOR (eXclusive NOR) as well as an INHIBIT logic gate which were newly constructed using three combinational inputs.

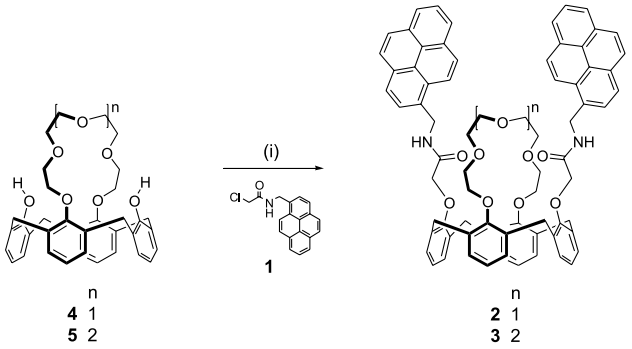
Keywords: Calixarenes; Reverse-PET; Logic gates.
* Corresponding author. Tel.: +82-2-799-1351; fax: +82-2-797-3277; e-mail address: jongskim@dankook.ac.kr

0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.04.061

2. Results and discussion

2.1. Syntheses and sensing behavior

The syntheses of pyrene-appended calix[4]arenes **2** and **3** are outlined in Scheme 1.



Scheme 1. Synthetic scheme for **2** and **3**. (i) K_2CO_3, NaI, CH_3CN, reflux for 3 days.

Compounds **1**, **4** and **5** were prepared by adaptation of the published procedures.[17,22–23] The reaction of calix[4]mono-crown-5 (**4**) and crown-6 (**5**) with 2.2 equiv. *N*-(1-pyrenylmethyl)chloroacetamide in the presence of K_2CO_3 in acetonitrile with a catalytic amount of sodium iodide afforded the desired *N*-(1-pyrenylmethyl) amide-armed calix[4]crowns **2** and **3** in moderate yields, respectively. For both **2** and **3**, the presence of the doublets at around δ 4.2 and 3.1 in the ^{1}H NMR[16a] spectrum as well as of a resonance at about 32 ppm in the ^{13}C NMR[16a] spectrum indicated that they are in the cone conformation.

First of all, we investigated fluorescence intensity changes of **2** (crown-5) and **3** (crown-6) to determine the metal binding ability. It is depicted in Figure 1.[24] It was reported that when the amide oxygen atoms take part in the metal ion complexation, the fluorescence emission of pyrene unit at 395 nm (344 nm excitation) is markedly quenched.[22] The quenching phenomena are ascribed to the reverse-PET (photo-induced electron transfer) mechanism, that is, electron transfer from pyrene unit to electron deficient carbonyl group occurs by metal ion complexation.[12a,25,26] Since heavy metal ions such as Pb^{2+} are known for quenching metal ion,[27] **1** as a reference material absent of the ionophoric cavity was tested for the quenching behavior. But, we rarely observed any change of fluorescence emission toward all metal ions tested including Pb^{2+} ion. For **2** having crown-5 ring, we observed almost no change of the fluorescence emission in spite of addition of 300 equiv. metal ions, indicating that the K^+ or Rb^+ ion might be selectively encapsulated because of the size agreement with crown-5,[28] but it does not reflect on the fluorescence quenching because the two carbonyl oxygen atoms do not interact with the ions. For lead ion, however, rather small loop, the crown-5 ring probably interferes the Pb^{2+}–O=C of **2**, which is sterically disfavored.

On the other hand, **3** having crown-6 ring showed a decreasing intensity with various metal ions, but especially

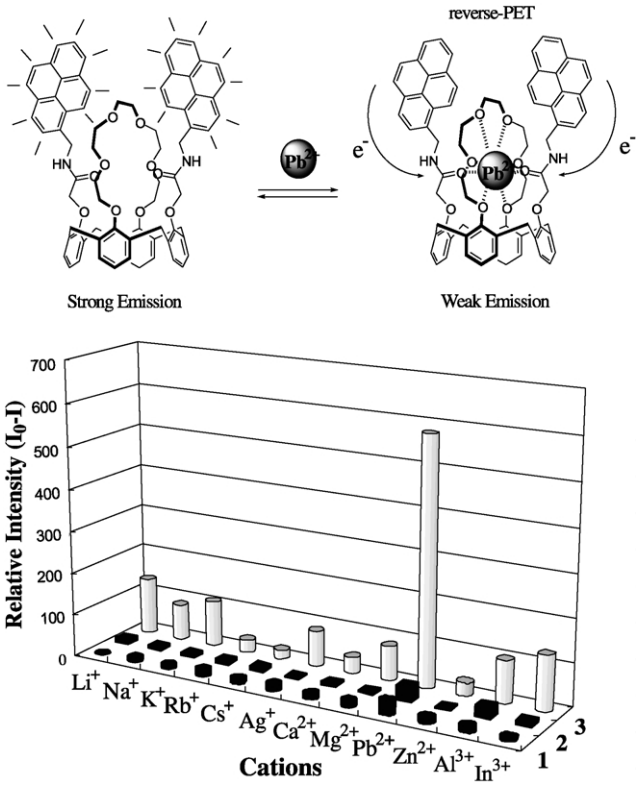


Figure 1. Reverse-PET: fluorescence emission changes of **1**–**3** at 395 nm upon the addition of metal cations. Conditions: **1**–**3** (6 μM, excitation at 344 nm)/CH_3CN; metal cations (300 equiv.)/CH_3CN. I_0: fluorescence emission intensity of free **1**–**3**; *I*: fluorescence emission intensity of metal ion complexed **1**–**3**.

gave Pb^{2+} ion selectivity regarding the fluorescence quenching. The titration profile of fluorescence intensity change of **3** with the lead ion is shown in Figure 2. According to the extent of the fluorescence emission changes, we could obtain the association constants[29] of **3** (K_a=5.5×10^7 M^{-1}) for Pb^{2+} ion. After all, we noticed that in this cone conformation, when carbonyl oxygen atoms of the two amide groups take part in the lead ion complexation, the crown-6 ring plays an important role in the Pb^{2+} ion selectivity in fluorescence quenching. So, considering the crown ring size and lead ion selectivity, it is estimated that unlike **2**, the crown-6 loop (**3**) is spaced enough to assist the complexation of Pb^{2+} ion with two carbonyl amides. In addition, importantly, upon the Pb^{2+} ion coordination, the reverse-PET occurs in such a way that electrons transfer from the pyrene groups to the electron deficient amide oxygen atoms to give a quenching fluorescence as shown in Figure 1.[12a,25,26]

It is well known that π-metal complexation of the fluorescence material is capable of quenching the fluorescence emission, causing the absorbance or the wavelength changes on the UV/Vis spectra.[30] However, for **3**, no absorbance change was observed in the UV/Vis spectra even when 300 equiv. of Pb^{2+} ion was added, implying that the π-metal interaction of the two pyrene units of **3** is ignorable.

There are a number of variable experimental conditions

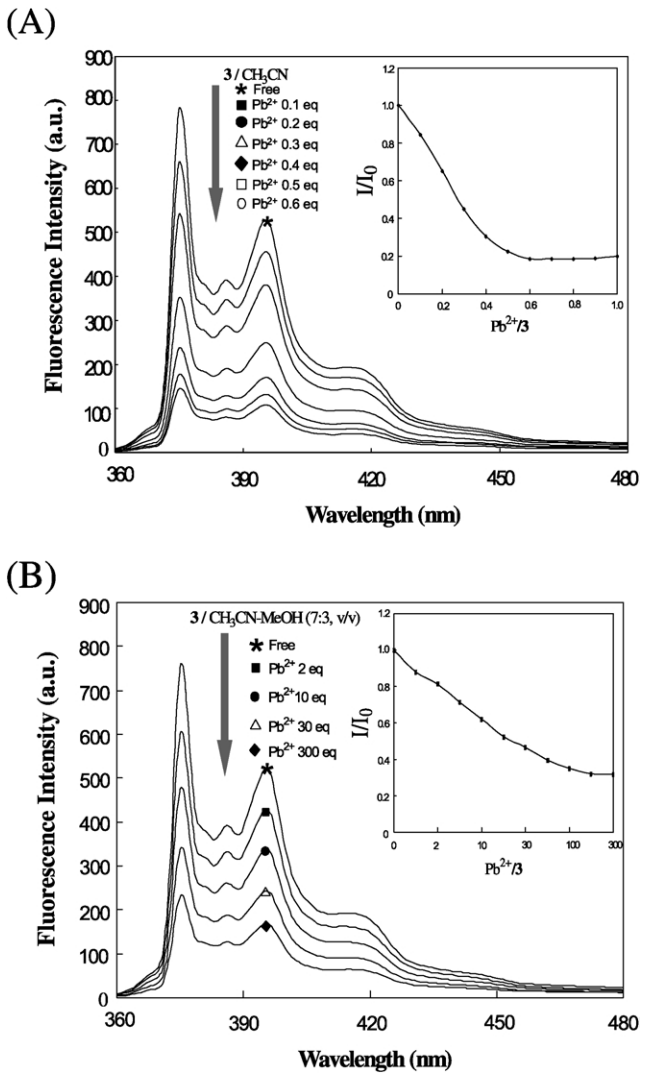


Figure 2. Fluorescence emission spectra of **3** (6 μM, excitation at 344 nm) with variable amounts of $Pb(ClO_4)_2$ along with corresponding changes in I/I_0 ratio represented by the intensity at 395 nm (inset). Solvent: (A) CH_3CN (B) CH_3CN/MeOH (7:3, v/v).

to test the fluorescence changes (intensity or wave length change), such as substrate species, pH (acid or base), and solvent effects.[20] As seen in Figure 2, when a polar co-solvent (MeOH/CH_3CN) was used, the quenching behavior of **3** for Pb^{2+} ion was not as effective as that in only CH_3CN. This is probably because the binding ability between the lead ion and the two carbonyl oxygen atoms become weaker due to an intramolecular hydrogen bonding between amide group and the MeOH solvent.

In order to gain an insight into the PET and reverse-PET effect on the fluorescence quenching, we added $HClO_4$ as an acid and triethylamine as a base independently in the solution of **3**, and also observed remarkably decreased fluorescence intensity in both cases as shown in Figure 3. In the case of using the acid, protonated oxygen atom behaves as a PET-acceptor to give a quenching fluorescence (reverse-PET). On the other hand, in the presence of triethylamine as a base, nitrogen anion was first formed by the base and acts as a PET-donor, that is, electron transfer from the nitrogen anion to the pyrene unit, giving a quenched fluorescence (normal PET). This is in accordance with a concept reported by Hamilton et al. that a coumarin containing macrocyclic amide anion formed by deprotonation influences on an excited-state intermolecular proton transfer (ESPT).[31] This control experiment could explicitly support the reason that fluorescence of **3** is quenched with Pb^{2+} ion through the reverse-PET.

2.2. Logic gate: NOR, XNOR and INHIBIT

As a consequence of the fluorescence emission behavior of the **2** and **3** in the presence of $HClO_4$, triethylamine, or $Pb(ClO_4)_2$, the probe molecule mimics the function of a logic gate. In this study we selected **3** as a probe because **2** rarely altered the fluorescence emission by the addition of the metal cations. The fluorescence logic gate for **3** was operated by combinational inputs of $HClO_4$, triethylamine, and $Pb(ClO_4)_2$ as input signals. As shown in Figure 3, we constructed two logic circuits along with the truth tables for the fluorescence quenching behavior of **3** upon addition of two sets of inputs. NOR (not OR) is performed only when neither of two inputs is present while XNOR (exclusive NOR) is active only if both or neither of two inputs are present.[12,14] As described above, there was an event that strong fluorescence emission appears at 395 nm (output: 1) when neither 10^{-3} M $Pb(ClO_4)_2$ nor 10^{-2} M triethylamine (inputs A and B in the truth table) is added (A: 0 and B: 0). However, when one of the two (A: 1, B: 0 or A: 0, B: 1) or both (A=B: 1) inputs are operated, the fluorescence emission of **3** is quenched at 395 nm. This process can be referred to as NOR logic system which integrates NOT and OR logic gate.

XNOR (eXclusive NOR) can be operated by the situation only where both of two inputs (A=B: 1) or neither of two inputs (A=B: 0) is computed.[12c,14] With combinational inputs of acid ($HClO_4$) and base (triethylamine), the fluorescence emission was present at 395 nm (output: 1) not only when both 10^{-2} M $HClO_4$ and 10^{-2} M triethylamine (inputs A and B) were simultaneously added (A=B: 1, addition of the perchloric acid after the triethylamine or vice versa) but also when neither of two inputs was added (A=B: 0). To the solution of **3**, however, if one of two ($HClO_4$ or triethylamine) was added (A: 1, B: 0 or A: 0, B: 1), the fluorescence emission was extinguished, which is due to the PET effect as mentioned previously. The logic circuit incorporating truth table (inset of Fig. 3) constitutes the XNOR logic gate.

On the basis of this simple logic gate system, we have designed multi-component chemical logic system, which is applicable for more complex logic gate. According to the NOR concept, there was no interference between $Pb(ClO_4)_2$ and triethylamine because both of two inputs operate independently in **3**, that is, the fluorescence emission quenched by the $Pb(ClO_4)_2$ was not revived by the addition of either $HClO_4$ or triethylamine. Therefore, combining both of two logic systems (NOT and XNOR), we could build up a higher-leveled logic system INHIBIT to which an additional input (three inputs in total) is introduced as shown in truth table of Figure 4. In the presence of

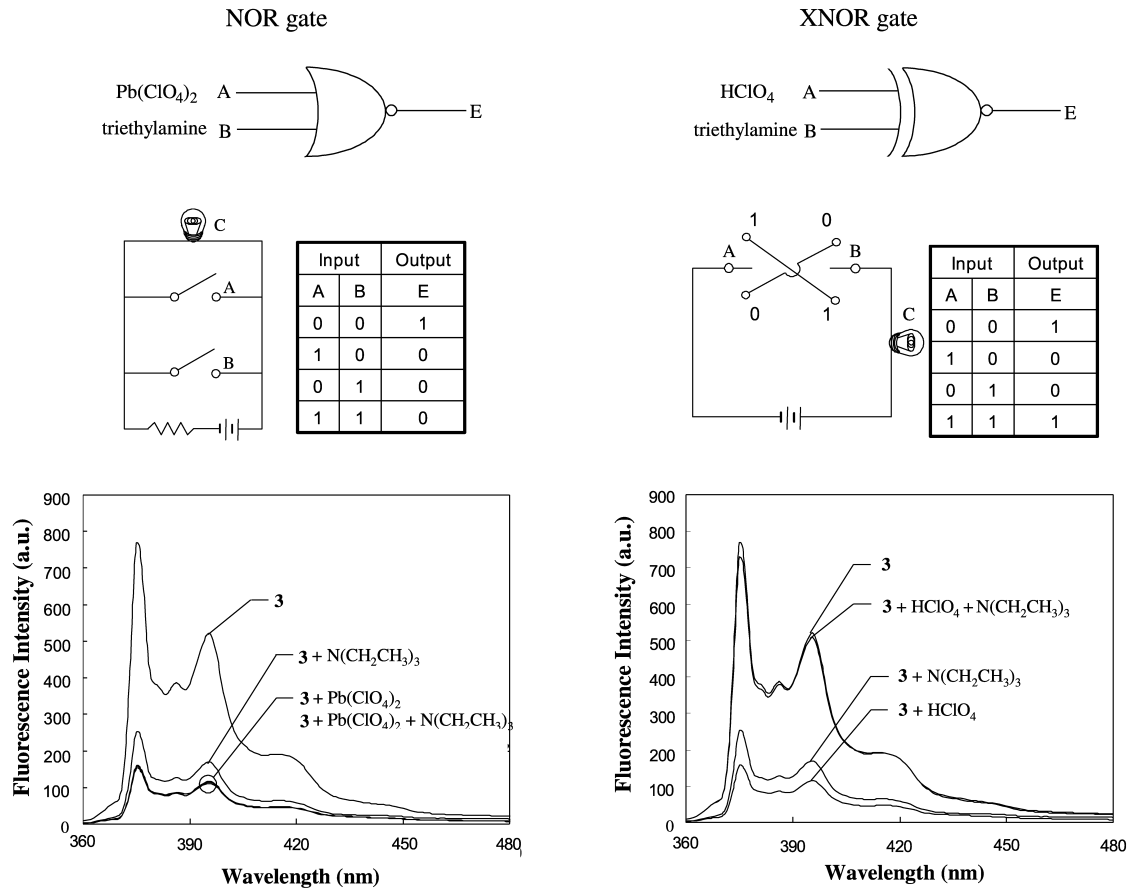
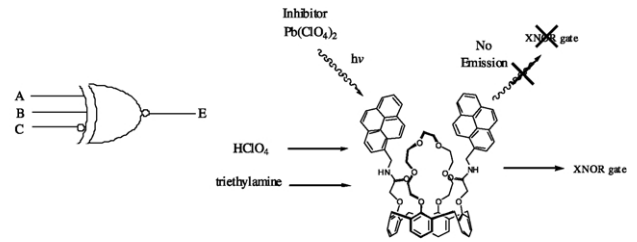


Figure 3. Fluorescence emission spectra of **3** for the NOR and XNOR gates in CH_3CN (6 μM, excitation at 344 nm). Schematic representation of a chemical system (E) which performs the NOR and XNOR logic operations under the action of two chemical inputs (A and B). The truth tables for such operations are also shown, along with their representations based on electric circuit schemes.

$Pb(ClO_4)_2$, the output of the fluorescence emission is inhibited (0) in all cases of the input combinations (A: 1, B: 0; A: 0, B: 1; A=B: 0 and A=B: 1) of acid ($HClO_4$) and base (triethylamine). So, one can conclude that for **3** the $Pb(ClO_4)_2$ as a guest material can be used as an inhibitor against XNOR gate.



$HClO_4$	$N(Et)_3$	$Pb(ClO_4)_2$	Emission
A	B	C	E
0	0	0	1
1	0	0	0
0	1	0	0
1	1	0	1
0	0	1	0
1	0	1	0
0	1	1	0
1	1	1	0

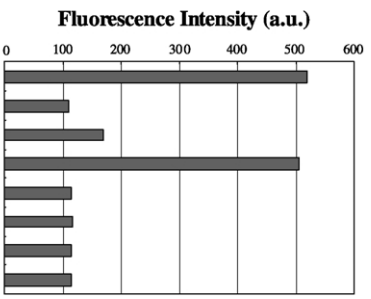


Figure 4. Fluorescence intensities of **3** at 395 nm for the INHIBIT gates in CH_3CN (6 μM, excitation at 344 nm). Output of fluorophore **3** is inhibited by combination of three combinational inputs (acids ($HClO_4$), bases (triethylamine) and $Pb(ClO_4)_2$).

So, we for the first time report here a new synthetic compound **3** capable of computing three different logic gates (NOR, XNOR, and INHIBIT), which will be useful in more complex algorithm system and its interpretation, and can speed up the computing operation, which might be useful in digital computers.

3. Conclusions

We synthesized a Pb^{2+} ion selective novel calixarene molecule appending pyrene amide pendent. Fluorescence intensity of **3** was selectively quenched with Pb^{2+} ion, which can be explained by selective complexation with two amide oxygen atoms followed by the reverse-PET effect. Control experiments using base and acid also supported the PET and reverse-PET behavior in **3**. NOR logic gates can be operated by the combinational inputs of triethylamine and lead perchlorate. In addition, XNOR gate was also operated when neither (triethylamine and $HClO_4$) or both of two inputs are added (A=B: 1). For **3**, we could build up the new INHIBIT gate system computed by such combinational inputs as $HClO_4$, $Pb(ClO_4)_2$ and triethylamine, which is noticeable to apply for the more complex system at the molecular level.

4. Experimental

4.1. Syntheses

Compounds **1**, **4** and **5** were prepared in 80–90% yield as described in the literatures.[17,22,23]

4.1.1. 26,28-Bis[(*N*-(1-pyrenylmethyl)aminocarbonyl) methoxy]-25,27-calix[4]monocrown-5 (2). To a mixture of 1.00 g (1.72 mmol) of calix[4]monocrown-5 and 1.16 g (3.78 mmol) of *N*-(1-pyrenylmethyl)chloroacetamide (**1**) in 50 mL dried acetonitrile, anhydrous potassium carbonate (0.71 g, 5.15 mmol) and a catalytic amount of sodium iodide were added. This reaction mixture was refluxed for 3 days. After removal of the solvent in vacuo, the residue was acidified with 10% aqueous HCl solution (50 mL), and then extracted with $CHCl_3$ (50 mL). The organic layer was separated, and washed with 10% HCl solution, and dried over anhydrous $MgSO_4$, and the solvent was evaporated to give the crude product. Column chromatography using $CHCl_3$:acetone (3:1) as eluent (R_f=0.65) on silica gel gave **1** in 56% yield. Mp: 267–268 °C; (KBr pellet, cm^{-1}): 3300 (–NH), 1650 (CO); ^1H NMR (400 MHz; $CDCl_3$): δ 8.23–7.76 (m, 18 H, pyrene), 6.68 (d, 4 H, Ar-H_{meta}, J=7.1 Hz), 6.60 (s, 2 H, CON*H*CH_2pyrene), 6.56–6.54 (m, 8 H, Ar-H_{meta}, Ar-H_{para}), 5.15 (s, 4 H, NHCH_2pyrene), 4.73 (s, 4 H, ArOCH_2CO), 4.19 (d, 4 H, ArCH_2Ar, J=13.2 Hz), 3.62 (s, 4 H, OCH_2CH_2O), 3.40 (s, 4 H, OCH_2CH_2O), 3.01 (d, 4 H, ArCH_2Ar, J=13.3 Hz), 2.82 (s, 4 H, OCH_2CH_2O), 2.56 (s, 4 H, OCH_2CH_2O); ^{13}C NMR ($CDCl_3$): δ 170, 156, 155, 135.2, 135.1, 132, 131.9, 131.7, 131.4, 129, 128.8, 128.7, 128.2, 128.1, 127, 126.8, 126.1, 125.9, 125.6, 125.3, 125.2, 123 (pyrene*C*H, Ar*C*H, *C*O), 73, 72, 70 (O*C*H_2*C*H_2O, ArO*C*H_2CO), 42 (N*C*H_2pyrene) 32 (Ar*C*H_2Ar) ppm. FAB MS *m*/*z* (M^+): Calcd, 1125.31, found, 1126.00. Anal. Calcd for $C_{74}H_{64}N_2O_9$: C, 78.98; H, 5.73. Found: C, 78.94; H, 5.69.

4.1.2. 26,28-Bis[(*N*-(1-pyrenylmethyl)aminocarbonyl) methoxy]-25,27-calix[4]monocrown-6 (3). The procedures are same as that for **2**. 62% yield. Mp 270–271 °C; (KBr pellet, cm^{-1}): 3300 (–NH), 1650 (CO); ^1H NMR (400 MHz; $CDCl_3$): δ 8.3–7.73 (m, 18 H, pyrene), 6.52–6.49 (m, 8 H, Ar-H_{meta}), 6.48 (s, 2 H, CON*H*CH_2pyrene), 6.38–6.36 (m, 4 H, Ar-H_{para}), 5.27 (s, 4 H, NHCH_2pyrene), 4.65 (s, 4 H, ArOCH_2CO), 4.23 (d, 4 H, ArCH_2Ar, J=13.7 Hz), 4.12 (m, 8 H, OCH_2CH_2O), 3.68 (s, 4 H, OCH_2CH_2O), 3.50 (s, 4 H, OCH_2CH_2O), 3.21–3.10 (m, 8 H, OCH_2CH_2O, ArCH_2Ar); ^{13}C NMR ($CDCl_3$): δ 171, 170, 156, 155, 135, 134, 132, 131.9, 131.6, 131.4, 129, 128.9, 128.6, 128.0, 127, 126, 125.1, 125.6, 125.3, 125.2, 123 (pyrene*C*H, Ar*C*H, *C*O), 74, 73.8, 73.3, 70.8, 70.5, 70.4 (O*C*H_2*C*H_2O, ArO*C*H_2CO), 41 (N*C*H_2pyrene) 31.8, 31.7 (Ar*C*H_2Ar) ppm. FAB MS *m*/*z* (M^+): Calcd, 1169.36; found, 1170.00. Anal. Calcd for $C_{76}H_{68}N_2O_{10}$: C, 78.06; H, 5.86. Found: C, 78.09; H, 5.82.

Acknowledgements

This work was fully supported by the Grant (No. KRF-2003-042-C00044) from the Korea Research Foundation.

References and notes

1. Schliche, B.; Belser, P.; De Cola, L.; Sabbioni, E.; Balzani, V. *J. Am. Chem. Soc.* **1999**, *121*, 4207.
2. Cygan, M. T.; Dunbar, T. D.; Arnold, J. J.; Bumm, L. A.; Shedlock, N. F.; Burgin, T. P.; Jones, L., II; Allara, D. L.; Tour, J. M.; Weiss, P. S. *J. Am. Chem. Soc.* **1998**, *120*, 2721.
3. Ashton, P. R.; Balzani, V.; Becher, J.; Credi, A.; Fyfe, M. C. T.; Mattersteig, G.; Menzer, S.; Nielsen, M. B.; Raymo, F. M.; Stoddart, J. F.; Venturi, M.; Williams, D. J. *J. Am. Chem. Soc.* **1999**, *121*, 3951.
4. Gosztola, D.; Niemczyk, M. P.; Wasielewski, M. R. *J. Am. Chem. Soc.* **1998**, *120*, 5118.
5. Debreczeny, M. P.; Svec, W. A.; Wasielewski, M. R. *Science* **1999**, *274*, 5287.
6. Martin, A. S.; Sambles, J. R. *Nanotechnology* **1996**, *7*, 401.
7. Gao, F. G.; Bard, A. J. *J. Am. Chem. Soc.* **2000**, *122*, 7426.
8. Langford, S. J.; Yann, T. *J. Am. Chem. Soc.* **2003**, *125*, 11198.
9. Ji, H.-F.; Dabestani, R.; Brown, G. M. *J. Am. Chem. Soc.* **2000**, *122*, 9306.
10. de Silva, A. P.; Gunaratne, H. Q. N.; McCoy, C. P. *J. Am. Chem. Soc.* **1997**, *119*, 7891.
11. Credi, A.; Balzani, V.; Langford, S. J.; Stoddart, J. F. *J. Am. Chem. Soc.* **1997**, *119*, 2679.
12. (a) de Silva, A. P.; Dixon, I. M.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Maxwell, P. R. S.; Rice, T. E. *J. Am. Chem. Soc.* **1999**, *121*, 1393. (b) de Silva, A. P.; Gunaratne, H. Q. N.; McCoy, C. P. *Nature* **1993**, *364*, 42. (c) de Silva, A. P.; McClenaghan, N. D. *J. Am. Chem. Soc.* **2000**, *122*, 3965.
13. Saghatelian, A.; Volcker, N. H.; Guckian, K. M.; Lin, V. S. Y.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2003**, *125*, 346.
14. (a) Lee, H.; Sobelman, G. E. *Microelectron J* **1998**, *29*, 509. (b) Bachtold, A.; Hadley, P.; Nakanishi, T.; Dekker, C. *Science* **2001**, *294*, 1317.
15. (a) In *Chemosensors of Ion and Molecule Recognition. NATO ASI Series*; Desvergne, J. P., Czarnik, A. W., Eds.; Kluwer Academic: Dordrecht, 1997. (b) de Silva, A. P.; Gunaratne, H. Q.; Gunnlaugsson, N. T. A.; Huxley, T. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515.
16. (a) Gutsche, C. D. *Calixarenes, Monographs in Supramolecular Chemistry*; Stoddart, J. F., Ed.; Royal Society of Chemistry: Cambridge, UK, 1989; Vol. 1. (b) In *Calixarenes: A Versatile Class of Macrocyclic Compounds*; Vicens, J., Böhmer, V., Eds.; Kluwer: Dordrecht, 1991. (c) Böhmer, V. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 713.
17. (a) Kim, J. S.; Shon, O. J.; Ko, J. W.; Cho, M. H.; Yu, I. Y.; Vicens, J. *J. Org. Chem.* **2000**, *65*, 2386. (b) Kim, J. S.; Lee, W. K.; No, K.; Asfari, Z.; Vicens, J. *Tetrahedron Lett.* **2000**, *41*, 3345. (c) Kim, J. S.; Shon, O. J.; Sim, W.; Kim, S. K.; Cho, M. H.; Kim, J. G.; Suh, I. H.; Kim, D. W. *J. Chem. Soc. Perkin Trans. 1* **2001**, 31. (d) Kim, J. S.; Thuery, P.; Nierlich, M.; Rim, J. A.; Yang, S. H.; Lee, J. K.; Vicens, J. *Bull. Kor. Chem. Soc.* **2001**, *22*(3), 321.
18. Aoki, I.; Sakaki, T.; Shinkai, S. *J. Chem. Soc., Chem. Commun.* **1992**, 730.
19. Jin, T.; Ichikawa, K.; Koyama, T. *J. Chem. Soc., Chem. Commun.* **1992**, 499.
20. (a) Ji, H.-F.; Brown, G. M.; Dabestani, R. *Chem. Commun.* **1999**, 609.
(b) Ji, H.-F.; Dabestani, R.; Brown, G. M.; Sachleben, R. A. *Chem. Commun.* **2000**, 833.
(c) Ji, H.-F.; Dabestani, R.; Brown, G. M.; Hettich, R. L. *J. Chem. Soc., Perkins Trans. 2* **2001**, 585.

21. Leray, I.; O'Reilly, F.; HabibJiwan, J.-L.; Soumillion, J.-Ph.; Valeur, B. *Chem. Commun.* **1999**, 795.
22. van der Veen, N. J.; Flink, S.; Deij, M. A.; Egberink, R. J. M.; van Veggel, F. C. J. M.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **2000**, *122*, 6112.
23. Koh, K. N.; Araki, K.; Shinkai, S.; Asfari, Z.; Vicens, J. *Tetrahedron Lett.* **1995**, *36*, 6095.
24. Fluorescence spectra were recorded with a RF-5301PC SHIMADZU spectrofluorophoto-meter. The perchlorate salts of Li^+, Na^+, K^+, Rb^+, Cs^+, Ag^+, Ca^{2+}, Mg^{2+}, Pb^{2+}, Zn^{2+}, Al^{3+} and In^{3+} ions (stock solutions=1.0 mM in CH_3CN) were tested to evaluate the metal ion binding properties of **1**–**3** (stock solution=0.6 mM in CH_3CN). For all fluorescence measurements, the excitation was made at 344 nm to give fluorescence intensity at 395 nm at room temperature.
25. Ojida, A.; Mito-oka, Y.; Inoue, M.-A.; Hamachi, I. *J. Am. Chem. Soc.* **2002**, *124*, 6256.
26. de Silva, A. P.; Gunarante, H. Q. N.; Lynch, P. M. L. *J. Chem. Soc., Perkins Trans. 2* **1995**, 685.
27. Chae, M.-Y.; Cherian, X. M.; Czarnik, A. W. *J. Org. Chem.* **1993**, *58*, 5797.
28. (a) Kim, J. S.; Lee, W. K.; Suh, I.-H.; Kim, J. G.; Yoon, J.; Lee, J. H. *J. Org. Chem.* **2000**, *65*, 7215. (b) Kim, J. S.; Shon, O. J.; Rim, J. A.; Kim, S. K.; Yoon, J. *J. Org. Chem.* **2002**, *67*, 2348. (c) Kim, S. K.; Sim, W.; Vicens, J.; Kim, J. S. *Tetrahedron Lett.* **2003**, *44*, 805. (d) Kim, S. K.; Vicens, J.; Park, K.-M.; Lee, S. S.; Kim, J. S. *Tetrahedron Lett.* **2003**, *44*, 993. (e) Lee, J. K.; Kim, S. K.; Bartsch, R. A.; Vicens, J.; Miyano, S.; Kim, J. S. *J. Org. Chem.* **2003**, *68*, 6720.
29. (a) Association constants were obtained using the computer program ENZFITTER, available from Elsevier-BIOSOFT, 68 Hills Road, Cambridge CB2 1LA, United Kingdom. (b) Connors, K. A. *Binding Constants*; Wiley: New York, 1987.
30. Kang, J.; Choi, M.; Kwon, J. Y.; Lee, E. Y.; Yoon, J. *J. Org. Chem.* **2002**, *67*, 4384.
31. Choi, K.; Hamilton, A. D. *Angew. Chem, Int. Ed. Engl.* **2001**, *40*, 3912.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
Tetrahedron 60 (2004) 5177–5183

Improved synthesis of a [4.4]-spirolactam β-turn mimetic as surrogate of the didemnin side chain dipeptide Pro-*N*-Me-D-Leu

Marta Gutiérrez-Rodríguez, M. Teresa García-López and Rosario Herranz*

Instituto de Química Médica (CSIC), Juan de la Cierva 3, E-28006 Madrid, Spain

Received 17 February 2004; revised 13 April 2004; accepted 22 April 2004

Abstract—An efficient synthesis of [4.4]-spirolactam restricted derivatives of the didemnin side chain dipeptide L-Pro-*N*-Me-D-Leu is described. This methodology involves: (a) peptide coupling of *N*-Boc-2-allylproline with D-Leu-OBn; (b) $OsO_4/NaIO_4$ mediated allyl oxidation and intramolecular cyclization to the corresponding cyclic hemiaminals; and (c) $NaBH_4$ mediated reduction of an intermediate *N*-acyliminium ion. This synthetic strategy gave significant better results than the previously reported strategies for the synthesis of [4.4]-spirolactam β-turn mimetics.

1. Introduction

The didemnins are a family of macrocyclic depsipeptides, isolated from several tunicates, which exhibit a wide variety of biological activities, including antitumor,[1,2] antiviral,[2] and immunosupresive properties.[2,3] These depsipeptides contain a common 23-membered macrocycle, made up of six subunits [(3*S*,4*R*,5*S*)-isostatine (Ist[1]), (2*S*,4*S*)-3-oxo-4-hydroxy-2,5-dimethylhexanoic acid (Hip[2]), (Leu[3]), (Pro[4]), [N,O-(Me)$_2$-Tyr[5]], and (Thr[6])], and differ in the side chain attached to the threonine NH, through a *N*-Me-D-leucine residue. Among these macrocyclic depsipeptides, didemnin B (Fig. 1, **1**), isolated in 1981 from the Caribbean tunicate *Tridemnum solidum*,[4] and aplidine (**2**), isolated in 1990 from the Mediterranean tunicate *Aplidium albicans*,[5] have stood out because of their potent antitumoral activity. The good antitumoral therapeutic profile of aplidine in pre-clinical and phase I clinical studies has facilitated its recent entry into phase II clinical trials.[1,2,6–9] Analysis of the X-ray crystal structure of didemnin B,[10] as well as conformational studies on didemnin B[11,12] and aplidine,[13,14] have shown that their side chain folds back toward the macrocycle into a type II β-turn conformation. With the aim of studying the contribution of this β II turn conformation to the bioactive conformation responsible for the antitumoral activity, as a first step for the design of didemnin peptidomimetics, we decided to synthesize didemnin B and aplidine analogues, where the side chain dipeptide L-Pro[8]-*N*-Me-D-Leu[7] was replaced by a β II turn mimetic.[15] As shown in Figure 1, a [4.4]-spirolactam was selected for this replacement, because

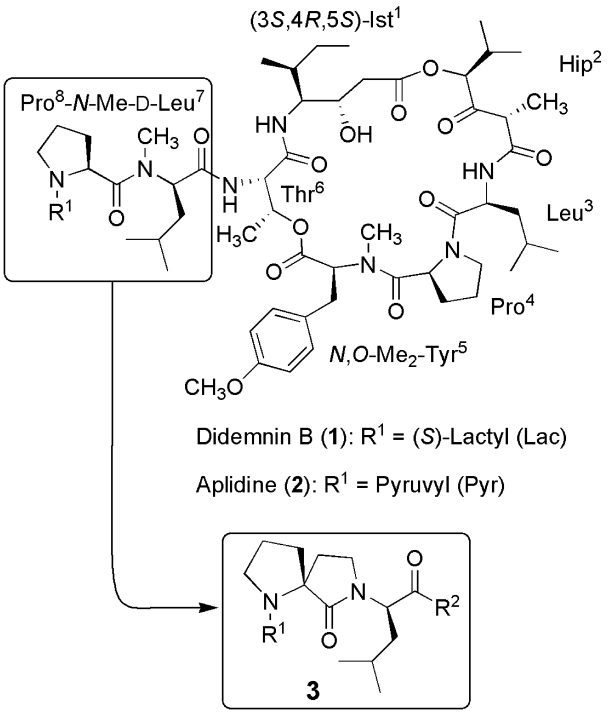


Figure 1.

it introduces only an additional methylene bridge, and allows the essential side chain and configuration of the (D-Leu[7]) residue at the $i+2$ position of the β-turn to be retained.[16,17] Furthermore, according to the described data for this type of β-turn mimetic,[18] it would fix the dipeptide backbone ϕ, ψ torsion angles into values (−50.9, 128.7; 91.1, and −5.4°) very close to those observed in the crystal

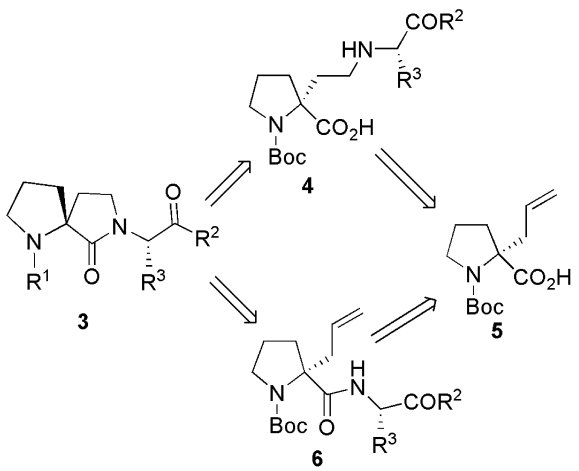
Keywords: Didemnins; Peptidomimetics; β-Turn mimetics; Spirolactams; Mitsunobu type reaction; Hemiaminal reduction.
* Corresponding author. Tel.: +34-91-5622900; fax: +34-91-5644853; e-mail address: rosario@iqm.csic.es

0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.04.052

structure of didemnin B[10] (−65, 125; 103, and −29°) and those calculated for aplidine (−63, 131; 90, and −38°).[13,14] Herein, we described our efforts to develop an efficient synthesis of [4.4]-spirolactam pseudodipeptide derivatives **3**, which would give access to the proposed conformationally restricted didemnin analogues in acceptable yields.

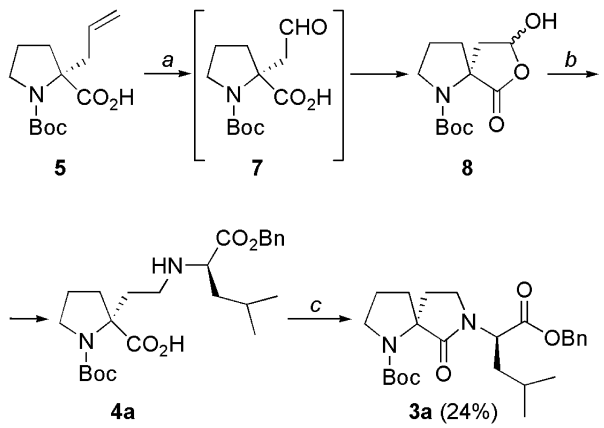
2. Results and discussion

Two alternative strategies have been described for the synthesis of [4.4]-spirolactam structures, both from 2-allylproline derivative **5** (retrosynthetic Scheme 1). The first, reported by Ward et al.[19] for the preparation of derivatives of L-Pro-L-Leu, involves the reductive *N*-alkylation of the C-terminal amino acid with *N*-protected 2-(formylmethyl)-proline, followed by lactamization of the resulting secondary amine **4**. Whereas the second, described for the synthesis of L-Pro-L-Tyr,[20,21] and L-Pro-Gly derivatives,[18,22] involves a first step of peptide coupling (**6**), followed by cyclization via allyl group oxidation–reduction and a Mitsunobu type *N*-alkylation.



Scheme 1. Retrosynthesis of spirolactam pseudodipeptides **3**.

Initially, the building of our spirolactam skeleton was tried by applying the first strategy, using *O*-Bn/*N*-Boc protecting groups. The starting material, *N*-Boc-2-allyl-L-Pro-OH (**5**), was prepared by applying the Khalil et al.[23] procedure of *N*-Boc-protection of sterically hindered amino acid to 2-allyl-L-Pro-OH, which was obtained from L-Pro-OH according to the Annunziata et al.[24] improvement to the Seebach's methodology for amino acid α-alkylation with self-reproduction of chirality.[25] As shown in Scheme 2, the oxidation of the double bond of the allyl group in (**5**), by treatment with OsO_4 and $NaIO_4$, gave a (≈1:1) mixture of epimeric hemiacetals **8** (95%), which was not resolved. The reductive amination of this mixture with H-D-Leu-OBn·PTSA, using $NaBH_3CN/ZnCl_2$ as reducing agent, led to the secondary amine **4a**, whose zwitterionic character hampered its purification. Consequently, the reaction crude was directly used in the next step of γ-lactamization. This was carried out using 2-chloro-1-methylpyridinium iodide as condensing agent,[26] obtaining the desired N- and C-protected spiropseudodipeptide **3a** in a 24% overall yield from **5**.

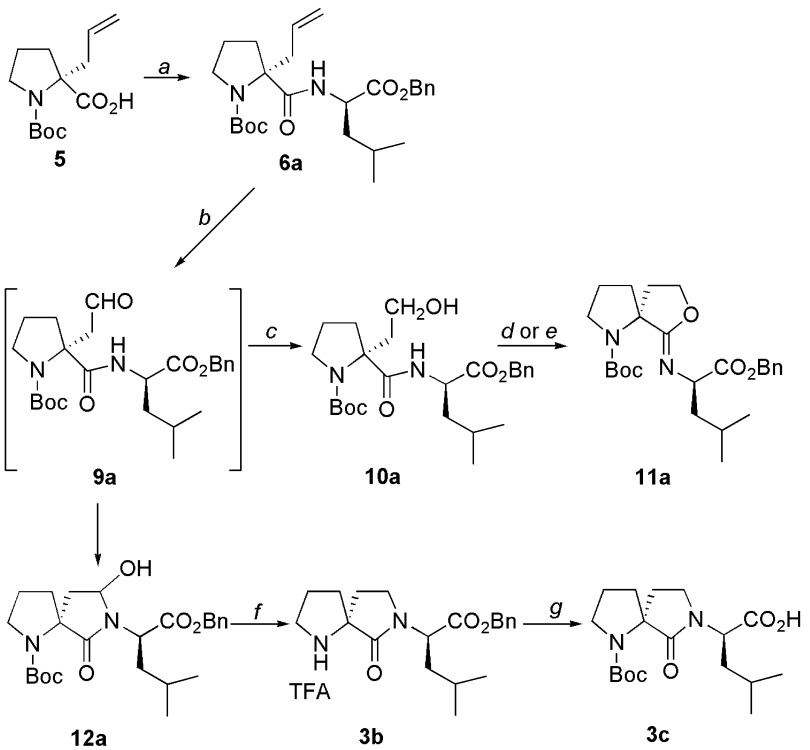


Scheme 2. Reagents: (a) OsO_4, $NaIO_4$, (2:1) MeOH/H_2O; (b) H-D-Leu-OMe·PTSA, TEA, $ZnCl_2$, $NaBH_3CN$, CH_2Cl_2; (c) 2-chloro-1-methylpyridinium iodide, TEA, CH_2Cl_2.

The low yield of the first strategy hampered the preparation of our proposed didemnin analogues in acceptable amounts. Therefore, we studied the application of the second alternative synthetic strategy shown in Scheme 3. This required, as first step, the peptide coupling of **5** with H-D-Leu-OBn·PTSA, which initially was carried out using 1-hydroxy-benzotriazole (HOBt) and *N*,*N*′-dicyclohexylcarbodiimide (DCC) as activating reagent, in the presence of *N*-methylmorpholine (NMM).[27] These reaction conditions led to the dipeptide **6a** in a low yield (28%). Therefore, to optimize yield, we carried out a study of the coupling using other activating reagents and reaction conditions. The results are shown in Table 1. Neither the use of (benzotriazol-1-yloxy)tris(dimethylamino)-phosphonium hexafluoro-phosphate (BOP)/HOBt[27] nor *N*,*N*′-bis(2-oxo-3-oxazolidinyl)phosphinic choride (BOP-Cl),[28,29] activating reagents for sterically hindered amino acids, improved the yield of **6a**. Moreover, when we used DCC/HOBt or BOP/HOBt, the dipeptide was obtained mixed with the 1-hydroxybenzotriazole ester derived from **5**,[†] which had identical R_f in TLC to that of **6a**. Excellent yield (>90%) of this dipeptide was obtained using 1-hydroxy-7-azabenzotriazole (HOAt)[27,30] and DCC or *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluoro-phosphate *N*-oxide (HATU),[27] and 2 equiv. of H-D-Leu-OBn·PTSA.

The oxidation of the allyl group in the dipeptide **6a** was performed with OsO_4 and $NaIO_4$, and the resulting aldehyde **9a** was reduced in situ with $NaBH_4$ to the corresponding alcohol **10a**. Unlike the previously described synthesis of other [4.4]-spirolactam dipeptide mimics,[18,20–22] the treatment of this alcohol with the Mitsunobu reagents, triphenylphosphine and diethyl azodicarboxylate (DEAD),[31] led exclusively to the product of *O*-alkylation, the cyclic imidate **11a**, and the formation of the desired γ-lactam derivative **3a** was not observed. The same result was obtained when we tried to favour the *N*-alkylation

[†] The ^1H NMR spectrum of the mixture showed the presence of ≈30% of this 1-hydroxybenzotriazole ester. The signals corresponding to this side product, as well as its R_f in TLC, were identical to those of the product resulting from the treatment of **5** with HOBt in the presence of DCC, without H-D-Leu-OBn.



Scheme 3. Reagents: (a) H-D-Leu-OMe·PTSA, HOAt, DCC, NMM, CH_2Cl_2; (b) OsO_4, $NaIO_4$, (2:1) MeOH/H_2O; (c) $NaBH_4$, EtOAc, −78 °C; (d) Ph_3P, DEAD, THF; (e) Ph_3P, NBS, CH_2Cl_2; (f) $NaBH_4$, TFA; (g) Boc_2O, $(CH_3)_4NOH·H_2O$, CH_3CN.

product, via the corresponding bromo intermediate, using triphenylphosphine/*N*-bromosuccinimide. In the ^1H NMR spectrum of imidate **11a**, the 8-H proton signals appeared at 0.7 ppm lower field relative to those of γ-lactam isomer **3a**. Similarly, C_8 of **11a** appeared considerably deshielded (79.3 ppm) with respect to C_8 of **3a** (39.57). The structural assignment of imidate **11a** was supported by its HMBC spectrum which did not show correlation between 8-H and C_α(Leu), and between C_8 and α-H(Leu), observed in the γ-lactam **3a** spectrum. Interestingly, none of the previously described syntheses of spirolactams via a Mitsunobu reaction has pointed out the formation of cyclic imidates, resulting from *O*-alkylation.[18,20–22,32] In our case, the preference for the *O*-alkylation versus the *N*-alkylation could be due to a higher steric hindrance of the D-Leu side chain in comparison with those of the Gly[18,22] and Tyr[20,21] spirolactam analogues previously described. The regioselectivity of the Mitsunobu reaction in other substrates where there is also a possible competition between *N*- and *O*-alkylation products, such as in β-hydroxy-α-amino acid-derived peptides, depends on subtle steric effects.[31b,c] Thus, serine and *allo*-threonine derivatives, in general, have shown selectivity for the *O*-alkylation, leading to peptide oxazolines,[33] while threonine derivatives have shown selectivity for *N*-alkylation[31c,33b] or 1,2-dehydration.[33a]

Table 1. Study on the yield optimization for dipeptide **6a**

Activating reagent	Base	H-D-Leu-Obn (equiv.)	Solvent	**6a** (%)
DCC/HOBt	NMM	2.5	CH_2Cl_2	28[a]
BOP/HOBt	NMM	1.6	CH_2Cl_2	30[a]
BOP-Cl	NMM	2.5	CH_2Cl_2	30
HOAt/DCC	NMM	1	CH_2Cl_2	50
HOAt/DCC	NMM	2	CH_2Cl_2	96
HOAt/HATU	DIEA[b]	1	THF	55
HOAt/HATU	DIEA[b]	2	THF	90

[a] Dipeptide **6a** impure with a variable quantity of the 1-hydroxybenzotriazole ester derived from *N*-Boc-2-allylproline (**5**).
[b] *N*-Ethyldiisopropylamine.

To overcome the difficulty of preparing the γ-lactam derivative via a Mitsunobu alkylation, as an alternative, we carried out the cyclization simultaneously with the oxidation, by allowing the intermediate aldehyde **9a** to stand in the osmylation reaction mixture at room temperature for 24 h. Cyclic hemiaminals **12a** were obtained as a (1:1) mixture of epimers,[‡] that were chromatographically resolved; however, their respective ^1H NMR spectra did not show relevant NOE effects for their absolute configuration assignment. The epimeric mixture was reduced via the *N*-acyliminium ion intermediate using $NaBH_4$ in neat TFA, because both epimers showed similar reactivity. Spirolactam **3b** was isolated as its trifluoroacetate in 73% overall yield from **5**. Attempts failed to maintain the Boc protection and **12a** was recovered unchanged by carrying out the reduction in AcOH, and by using $NaBH_3CN$ in the presence of $ZnCl_2$ or AcOH.

Finally, the reaction of trifluoroacetate **3b** with di(*tert*-butyl) dicarbonate in dry acetonitrile, using tetramethylammonium hydroxide as base,[23] led directly to the *N*-Boc acylated and C-deprotected pseudodipeptide **3c** (70%). The ^1H NMR spectra of this pseudodipeptide in $CDCl_3$ and DMSO-d_6 showed the presence of two conformations in a (2:1) ratio, corresponding to the *cis* and *trans* rotamers at the

[‡] Similar hemiaminals were obtained by Genin et al.[18] as unwanted side products from the silica gel chromatography of the Pro-Gly-derived aldehyde analogue of **9a**.

Boc-spirolactam bond. Signals due to both conformations coalesced at 90 °C in DMSO-d_6. The selectively N- and C-protected [4.4]-spirolactam pseudodipeptides **3b** and **3c** give access to backbone C- and N-extensions and, therefore, to the preparation of our proposed conformationally constrained didemnin analogues.

3. Conclusion

The herein reported synthesis of [4.4]-spirolactam restricted L-Pro-D-Leu derivatives, which involves, as key steps, the cyclization of an α-(formylmethyl)-carboxamide, followed by $NaBH_4$ mediated reduction of the resulting hemiaminals, constitutes an advantageous efficient methodology to the described strategies for the synthesis of [4.4]-spirolactams, such as reductive amination of α-(formylmethyl)-proline derivatives, followed by γ-lactamization, or aldehyde reduction, followed by a Mitsunobu type alkylation.

4. Experimental

4.1. General procedures

All reagents were of commercial quality. Solvents were dried and purified by standard methods. Analytical TLC was performed on aluminum sheets coated with a 0.2 mm layer of silica gel 60 F_{254}, Merck. Preparative radial chromatography was performed on 20 cm diameter glass plates coated with a 1-mm layer of silica gel PF_{254} Merck. Silica gel 60 (230–400 mesh), Merck, was used for flash chromatography. Melting points were taken on a micro hot stage apparatus and are uncorrected. Optical rotations were measured on a Perkin–Elmer 141 polarimeter. ^{1}H NMR spectra were recorded at 300, 400 or 500 MHz, using TMS as reference, and ^{13}C NMR spectra were recorded at 50, 75 or 100 MHz. Elemental analyses were obtained on a CH-O-RAPID apparatus. Analytical RP-HPLC was performed on a Waters Novapak C_{18} (3.9×150 mm, 4 μm) or a μBondapak C_{18} (3.9×300 mm, 4 μm) columns, with a flow rate of 1 mL/min, and using a tunable UV detector set at 214 nm. Mixtures of CH_3CN (solvent A) and 0.05% TFA in H_2O (solvent B) were used as mobile phases.

4.1.1. Synthesis of (5*R*,8*RS*)-1-(*tert*-butoxycarbonyl)-8-hydroxy-6-oxo-7-oxa-1-azaspiro[4.4]nonane (8). OsO_4 (2.5%, w/w solution in *tert*-butanol, 1.9 mL, 0.15 mmol) was added to a solution of (*R*)-*N*-Boc-2-allylproline[23] (**5**) (633 mg, 2.48 mmol) in (2:1) MeOH/H_2O (45 mL), under argon, which was stirred at room temperature for 10 min. $NaIO_4$ (1.612 g, 7.43 mmol) was slowly added. After 2 h of stirring at room temperature, H_2O (30 mL) was added, and the mixture was extracted with EtOAc (3×60 mL). The combined organic extracts were washed with brine (50 mL), dried over Na_2SO_4, and evaporated to dryness, to yield the epimeric hemiacetals **8** as white solid (638 mg, 100%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.32 [s, 9H, Me(Boc)], 1.59–1.90 (m, 4H, 3-H, 4-H), 2.35–2.50 (m, 2H, 9-H), 3.20–3.45 (m, 2H, 2-H), 6.03 (m, 1H, 8-H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.11, 28.18, 28.53, 36.22, 39.57, 41.81, 66.65, 80.24, 97.38, 161.21, 174.82. ESI-MS *m/e* 258.3 $[M+H]^+$. Anal. Calcd for $C_{12}H_{19}NO_5$: C, 56.02; H, 7.44; N, 5.44. Found: C, 56.00; H, 7.48; N, 5.43.

4.1.2. Synthesis of (5*R*)-7-[(1*R*)-1-benzyloxycarbonyl-3-methylbutyl]-1-(*tert*-butoxy-carbonyl)-6-oxo-1,7-diaza-spiro[4.4]nonane (3a). Triethylamine (61 μL, 0.43 mmol) was added to a suspension of H-D-Leu-OBn·PTSA (95 mg, 0.43 mmol) in MeOH (10 mL), and the mixture was stirred at room temperature and under argon for 15 min. The epimeric mixture of hemiacetals **8** (470 mg, 1.83 mmol), $ZnCl_2$ (124 mg, 0.91 mmol), and $NaBH_3CN$ (126 mg, 2.01 mmol) were successively added to the mixture, and the stirring was maintained for 6 h. The solvent was removed under vacuum, and the residue was dissolved in EtOAc (20 mL). The solution was washed with H_2O (10 mL) and brine (10 mL), dried over Na_2SO_4, and evaporated to dryness. The residue (716 mg, 1.32 mmol), corresponding to the crude amino acid **4a**, was dissolved in dry CH_2Cl_2 (120 mL), and, to this solution, 2-chloro-1-methylpyridinium iodide (372 mg, 1.52 mmol) and triethylamine (0.41 mL, 2.92 mmol) dissolved in CH_2Cl_2 (3 mL) were successively added, and the mixture was refluxed for 3 days. The solution was successively washed with 10% citric acid solution (2×30 mL), saturated $NaHCO_3$ (2×30 mL), and brine (2×30 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography, using a 10–100% gradient of EtOAc in hexane as eluant. The spirolactam pseudodipeptide **3a** was obtained as a white solid (200 mg, 24%). Mp 91 °C. $[\alpha]_D^{20}$ −1.5° (*c*, 1 in MeOH). HPLC [Novapack C_{18}] (A/B, 50:50) t_R 9.47 min. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.91 and 0. 93 [2d, 6H, *J*=6 Hz, Me (Leu)], 1.32 [s, 9H, Me (Boc)], 1.40 [m, 1H, 4-H (Leu)], 1.60–2.00 [m, 7H, 3-H (Leu), 4-H, 3-H, 9-H], 2.50 [c, 1H, *J*=3, 6 Hz, 9-H], 3.10 [m, 1H, *J*=3, 9 Hz, 8-H), 3.50 (m, 2H, 1-H), 3.73 (t, 1H, *J*=9 Hz, 8-H), 4.92 [dd, 1H, *J*=5, 9 Hz, 2-H (Leu)], 5.10 [s, 2H, CH_2 (Bn)], 7.30 (m, 5H, aromatics). ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.06, 22.48, 23.12, 24.91, 28.24, 30.49, 36.26, 38.28, 39.57, 47.71, 52.17, 66.62, 66.86, 80.21, 128.26, 128.39, 128.57, 135.08, 153.50, 171.24, 174.82. ESI-MS *m/e* 445.5 $[M+1]^+$. Anal. Calcd for $C_{25}H_{36}N_2O_5$: C, 67.54; H, 8.16; N, 6.30. Found: C, 67.42; H, 8.27; N, 6.27.

4.1.3. Synthesis of *N*-[2-allyl-*N*-(*tert*-butoxycarbonyl)-L-prolyl]-D-leucine benzyl ester (6a). HOAt (980 mg, 7.2 mmol), H-D-Leu-OBn·PTSA (3.983 g, 18 mmol), NMM (1.820 g, 18 mmol), and DCC (1.485 g, 7.2 mmol) were successively added under argon to a 0 °C cooled solution of (*R*)-*N*-Boc-2-allylproline[23] (**5**) (1.530 g, 6 mmol) in CH_2Cl_2 (33 mL). The stirring was maintained at 0 °C for 2 h, and subsequently at room temperature for 15 h. The precipitated *N*,*N*′-dicyclohexylurea was filtered off, and the solution was evaporated to dryness. The residue was dissolved in EtOAc (30 mL), and the resulting solution was successively washed with 10% citric acid solution (2×25 mL), saturated $NaHCO_3$ (2×25 mL), and brine (25 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography, using a (6:1) hexane/EtOAc mixture as eluant, to give the dipeptide **6a** as a syrup (2.638 g, 96%). $[\alpha]_D^{20}$ +12.4° (*c*, 1 in MeOH). HPLC [μBondapack C_{18}] (A/B, 40:60) t_R 9.08 min. ^{1}H NMR (300 MHz, DMSO-d_6, 90 °C): δ 0.85 and 0.88 [2d, 6H, *J*=6, 7 Hz, Me (Leu)], 1.37 [s, 9H, Me (Boc)], 1.55–1.72

[m, 5H, 3-H (Leu), 2-H (Leu), 4-H (Pro)], 2.00 [m, 2H, 3-H (Pro)], 2.64 [m, 1H, 1-H (allyl)], 2.86 [m, 1H, 1-H (allyl)], 3.15 [m, 1H, 5-H (Pro)], 3.55 [m, 1H, 5-H (Pro)], 4.41 [m, 1H, 2-H (Leu)], 5.05 [s, 2H, CH_2 (Bn)], 5.10 [dd, 2H, J=14, 6 Hz, 3-H (allyl)], 5.63–5.72 [m, 1H, 2-H (allyl)], 7.28–7.35 (m, 5H, aromatics), 7.46 (bs, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.65, 22.30, 22.87, 24.67, 28.32, 34.71, 38.26, 41.15, 49.41, 50.98, 66.76, 69.73, 80.14, 119.37, 128.34, 128.54, 132.77, 135.63, 153.90, 172.79, 173.92. ESI-MS *m/e* 459.3 $[M+H]^+$. Anal. Calcd for $C_{26}H_{38}N_2O_5$: C, 68.10; H, 8.35; N, 6.11. Found: C, 68.02; H, 8.47; N, 6.08.

4.1.4. Synthesis of *N*-[*N*-(*tert*-butoxycarbonyl)-2-(2-hydroxyethyl)-L-prolyl]-D-leucine benzyl ester (10a). OsO_4 (2.5%, w/w solution in *tert*-butanol, 4.81 mL, 0.38 mmol) was added to a solution of the dipeptide **6a** (2.603 g, 5.68 mmol) in (2:1) MeOH/H_2O (180 mL), under argon, which was stirred at room temperature for 10 min. $NaIO_4$ (3.645 g, 17.11 mmol) was slowly added. After 2 h of stirring at room temperature, H_2O (40 mL) was added, and the mixture was extracted with EtOAc (3×80 mL). The combined organic extracts were washed with brine (50 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was dissolved into dry EtOAc (180 mL), and the solution was cooled to −78 °C. To this solution, $NaBH_4$ (215 mg, 5.68 mmol) dissolved into MeOH (13 mL) was added, the mixture was stirred at −78 °C for 1 h, and then 30 min at room temperature. H_2O (50 mL) was added, and the organic phase was washed with brine (50 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography, using a 20–100% gradient of EtOAc in hexane as eluant, to give the alcohol **10a** as a syrup (1.200 g, 50%). $[\alpha]_D^{20}$ −1.8° (*c*, 1.5 in MeOH). HPLC [μBondapack C_{18}] (A/B, 37:63) t_R 9.98 min. ^{1}H NMR (300 MHz, DMSO-d_6, 90 °C): δ 0.89 and 0.86 [2d, 6H, J=7, 6 Hz, Me (Leu)], 1.38 [s, 9H, Me (Boc)], 1.55–1.73 [m, 5H, 3-H (Leu), 4-H (Leu), 4-H (Pro)], 1.90–2.08 [m, 4H, 3-H (Pro), 1-H (hydroxyethyl)], 3.13–3.63 [m, 4H, 5-H (Pro), 2-H (hydroxyethyl)], 4.35 [m, 1H, 2-H (Leu)], 5.11 [s, 2H, CH_2 (Bn)], 7.34 (m, 5H, aromatics), 7.80 (bs, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.58, 22.77, 24.59, 28.27, 36.44, 38.22, 41.13, 48.57, 51.01, 58.78, 60.29, 66.87, 80.10, 128.21, 128.32, 128.51, 135.01, 153.51, 172.51, 174.54. ESI-MS *m/e* 463.3 $[M+H]^+$. Anal. Calcd for $C_{25}H_{38}N_2O_6$: C, 64.91; H, 8.28; N, 6.06. Found: C, 64.79; H, 8.45; N, 6.03.

4.1.5. Synthesis of (5*R*)-6-[(1*R*)-1-benzyloxycarbonyl-3-methylbutyl]imino-1-(*tert*-butoxycarbonyl)-7-oxa-1-azaspiro-[4.4]nonane (11a). Triphenylphosphine (1.348 g, 5.35 mmol) and DEAD (588 mg, 3.38 mmol) were added to a solution of the alcohol **10a** (1.200 g, 2.6 mmol) in dry THF (20 mL), and the mixture was stirred at room temperature for 14 h. The solvent was evaporated, and the residue was treated with ethyl ether (20 mL), to precipitate the formed triphenylphosphine oxide, which was filtered off. The solution was evaporated to dryness, and the residue was purified by flash chromatography, using a 15–50% gradient of EtOAc in hexane as eluant, to give the cyclic imidate **11a** as a syrup (700 mg, 61%). $[\alpha]_D^{20}$ −38.3° (*c*, 1.4 in MeOH). HPLC [μBondapack C_{18}] (A/B, 37:63) t_R 9.51 min. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.84 and 0.87 [2d, 6H, J=6 Hz, Me (Leu)], 1.41 [s, 9H, Me (Boc)], 1.45–1.78 [m, 5H, 3-H (Leu), 4-H (Leu), 4-H, 3-H), 1.84–1.91 (m, 2H, 9-H), 2.10 (m, 1H, 3-H), 2.20 (m, 1H, 4-H), 2.80 (m, 1H, 9-H), 3.38–3.61 (m, 2H, 2-H), 3.92 (m, 1H, 8-H), 4.23 [m, 1H, 2-H (Leu)], 4.41 (m, 1H, 8-H), 5.10 [m, 2H, CH_2 (Bn)], 7.34 (m, 5H, aromatics). ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.15, 23.32, 23.42, 24.20, 28.40, 34.31, 39.42, 41.01, 42.39, 47.75, 57.58, 65.96, 66.34, 67.08, 79.32, 127.92, 128.00, 128.22, 128.35, 136.41, 153.14, 165.99, 173.80. ESI-MS *m/e* 445.3 $[M+H]^+$. Anal. Calcd for $C_{25}H_{36}N_2O_5$: C, 67.54; H, 8.16; N, 6.30. Found: C, 67.40; H, 8.36; N, 6.27.

4.1.6. Synthesis of (5*R*,8*RS*)-7-[(1*R*)-1-benzyloxy-carbonyl-3-methylbutyl]-1-(*tert*-butoxycarbonyl)-8-hydroxy-6-oxo-1,7-diazaspiro-[4.4]nonane (12a). OsO_4 (2.5%, w/w solution in *tert*-butanol, 2.9 mL, 0.23 mmol) was added to a solution of the dipeptide **6a** (1.567 g, 3.42 mmol) in (2:1) MeOH/H_2O (108 mL), under argon, which was stirred at room temperature for 10 min. $NaIO_4$ (2.195 g, 10.3 mmol) was slowly added. After 24 h of stirring at room temperature, H_2O (40 mL) was added, and the mixture was extracted with EtOAc (3×60 mL). The combined organic extracts were washed with brine (50 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography, using a 20–100% gradient of EtOAc in hexane as eluant, to give the two epimeric hemiaminals **12a**, as white solids, whose absolute configuration at C_8 could not be assigned. *Epimer* **A**. (584 mg, 38%). Mp 140–141 °C. $[\alpha]_D^{20}$ −4° (*c*, 1 in MeOH). HPLC [Novapack C_{18}] (A/B, 40:60) t_R 14.45 min. ^{1}H NMR (300 MHz, acetone-d_6): δ 0.88 and 0.91 [2d, 6H, J=7, 6 Hz, Me (Leu)], 1.29 [s, 9H, Me (Boc)], 1.64–2.30 [m, 9H, 3-H (Leu), 4-H (Leu), 3-H, 4-H, 9-H], 2.68 (dd, 1H, J=6, 13 Hz, OH), 3.37 (m, 2H, 2-H), 4.51 [dd, 1H, J=5, 11 Hz, 2-H (Leu)], 5.13 [d, 2H, J=15 Hz, CH_2 (Bn)], 5.79 (t, 2H, J=5 Hz, 8-H), 7.40 (m, 5H, aromatics). ^{13}C NMR (75 MHz, acetone-d_6): δ 21.80, 23.10, 24.58, 24.87, 28.59, 39.57, 40.79, 41.65, 48.48, 53.42, 66.89, 67.06, 79.41, 81.24, 128.64, 128.99, 129.08, 129.29, 135.26, 153.74, 171.67, 172.01. ESI-MS *m/e* 483.4 $[M+Na]^+$. Anal. Calcd for $C_{25}H_{36}N_2O_6$: C, 65.20; H: 7.88; N, 6.08. Found: C, 65.18; H, 7.92; N, 6.06. *Epimer* **B**. (584 mg, 38%). Mp 134–135 °C. $[\alpha]_D^{20}$ +26° (*c*, 1.2 in MeOH). HPLC [Novapack C_{18}] (A/B, 40:60) t_R 15.97 and 18.75 min. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 and 0.89 [2d, 6H, J=7, 6 Hz, Me (Leu)], 1.40 [s, 9H, Me (Boc)], 1.50–2.60 [m, 9H, 3-H (Leu), 4-H (Leu), 3-H, 4-H, 9-H], 3.40 (m, 2H, 2-H), 4.20–5.40 [m, 5H, 2-H (Leu), CH_2 (Bn), 8-H], 7.40 (m, 5H, aromatics). ^{13}C NMR (75 MHz, acetone-d_6): δ 21.30, 23.47, 24.14, 24.93, 28.28, 38.93, 40.25, 42.55, 47.73, 53.21, 66.82, 67.74, 77.58, 80.66, 128.11, 128.38, 128.54, 128.64, 135.83, 154.14, 171.24, 174.16. ESI-MS *m/e*483.4 $[M+Na]^+$. Anal. Calcd for $C_{25}H_{36}N_2O_6$: C, 65.20; H, 7.88; N, 6.08.

4.1.7. Synthesis of (5*R*)-7-[(1*R*)-1-benzyloxycarbonyl-3-methylbutyl]-6-oxo-1,7-diaza-spiro[4.4]nonane trifluoroacetate (3b). $NaBH_4$ (106 mg, 2.8 mmol) was added to a solution of the epimeric mixture of hemiaminals **12a** (430 mg, 0.93 mmol) in neat TFA (10 mL), and the mixture was stirred at room temperature for 2 h. The solvent was evaporated to dryness, and the residue was dissolved in CH_2Cl_2 (20 mL). This solution was washed with H_2O

(5 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was dissolved in H_2O (3 mL), and the resulting solution was lyophilized, to give the title compound as a yellow syrup (414 mg, 100%). $[\alpha]_D^{20}$ +15° (*c*, 1 in MeOH). HPLC [Novapack C_{18}] (A/B, 60:40) t_R 1.45 min. ^{1}H NMR (300 MHz, acetone-d_6): δ 0.91 and 0.92 [2d, 6H, *J*=6 Hz, Me (Leu)]; 1.50 [m, 1H, 4-H (Leu)]; 1.66–1.94 [m, 2H, 3-H (Leu)]; 2.11–2.72 (m, 6H, 3-H, 4-H, 9-H); 3.35–3.72 (m, 4H, 2-H, 8-H); 4.74 [dd, 1H, *J*=6, 15 Hz, 2-H (Leu)]; 5.18 [s, 2H, CH_2 (Bn)]; 7.37 (m, 5H, aromatics). ^{13}C NMR (75 MHz, acetone-d_6): δ 21.21, 23.26, 23.89, 30.24, 34.61, 30.01, 42.24, 46.65, 53.93, 67.59, 67.99, 69.58, 129.07, 129.35, 129.45, 136.77, 161.12, 161.57, 170.95, 172.65. ESI-MS *m/e* 443.2 $[M+1]^+$. Anal. Calcd for $C_{22}H_{29}F_3N_2O_4$: C, 59.72; H, 6.61; N, 6.33. Found: C, 59.86; H, 6.65; N, 6.29.

4.1.8. Synthesis of (5*R*)-1-(*tert*-butoxycarbonyl)-7-[(1*R*)-1-carboxy-3-methylbutyl]-6-oxo-1,7-diazaspiro[4.4]-nonane (3c). Tetramethylammonium hydroxide pentahydrate (158 mg, 0.87 mmol) and di(*tert*-butyl) dicarbonate (144 mg, 0.66 mmol) were added to a solution of the trifluoroacetate **3b** (150 mg, 0.44 mmol) in acetonitrile (5 mL). After 48 h of stirring at room temperature, the solvent was evaporated to dryness, and the residue was dissolved in CH_2Cl_2 (3 mL). The solution was extracted with H_2O (5 mL), and the extracts were lyophilized. The resulting residue was purified by flash chromatography, using 8–40% gradient of MeOH in CH_2Cl_2 as eluant, to give the title compound **3c** as a white solid (110 mg, 70%). Mp 192–194 °C. $[\alpha]_D^{20}$ −3° (*c*, 1 in MeOH). HPLC [Novapack C_{18}] (A/B, 50:50) t_R 13.41 min. ^{1}H NMR (300 MHz, DMSO-d_6, *T*=90 °C): δ 0.87 and 0.91 [2d, 6H, *J*=7 Hz, Me (Leu)], 1.36 [s, 9H, Me (Boc)], 1.40 [m, 1H, 4-H (Leu)], 1.64 [t, 2H, *J*=8 Hz, 3-H (Leu)], 1.70–1.90 (m, 5H, 3-H, 4-H, 9-H), 2.50 (m, 1H, 9-H), 3.10 (c, 1H, *J*=9 Hz, 8-H), 3.30 (m, 2H, 2-H), 3.70 (t, 1H, *J*=10 Hz, 8-H), 4.39 [t, 1H, *J*=8 Hz, 2-H (Leu)]. ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.05, 22.60, 23.40, 25.24, 28.54, 30.44, 36.87, 38.20, 40.59, 48.19, 54.32, 67.29, 81.37, 154.09, 174.97, 176.01. ESI-MS *m/e* 353.4 $[M-1]^-$. Anal. Calcd for $C_{18}H_{30}N_2O_5$: C, 61.00; H, 8.53; N, 7.90. Found: C, 59.93; H, 8.59; N,7.88.

Acknowledgements

This work has been supported by CICYT (SAF2000-0147) and Pharma Mar, S. A.

References and notes

1. Rinehart, K. L. *Med. Res. Rev.* **2000**, *20*, 1–27.
2. Vera, M. D.; Joullie, M. M. *Med. Res. Rev.* **2002**, *22*, 102–145.
3. Caufield, C. E.; Musser, J. H. *Ann. Rep. Med. Chem.* **1989**, *25*, 195–204.
4. Rinehart, K. L., Jr.; Gloer, J. B.; Hughes, R. G., Jr.; Renis, H. E.; McGovren, J. P.; Swynenberg, E. B.; Stringfellow, D. A.; Kuentzel, S. L.; Li, L. H. *Science* **1981**, *212*, 933–935.
5. Rinehart, K. L.; Lithgow-Bertelloni, A. M. WO 9104985, 1991.
6. Raymond, E.; Paz-Ares, L.; Izquierdo, M.; Belanger, K.; Maroun, J.; Bowman, A.; Anthoney, A.; Jodrell, D.; Armand, J.-P.; Cortes-Funes, H.; Germa-Lluch, J.; Twelves, C.; Celli, N.; Guzman, C.; Jimeno, J. *Eur. J. Can.* **2001**, *37*(Suppl. 6), S32.
7. Raymond, E.; Ady-Vago, N.; V, .; Ribrag, V.; Faivre, S.; Lecot, F.; Wright, T.; López-Lázaro, L.; Guzmán, C.; Jimeno, J.; Armand, J. P. *Ann. Oncol.* **2000**, *11*(Suppl. 4), 134.
8. Izquierdo, M. A.; Bowman, A.; Martínez, M.; Cicchella, B.; López-Lázaro, L.; Guzmán, C.; Germá, J.; Smyth, J. *Ann. Oncol.* **2000**, *11*(Suppl. 4), 134.
9. Maroun, J.; Belanger, K.; Seymour, L.; Soulieres, D.; Charpentier, D.; Goel, R.; Stewart, D.; Eva Tomiak,; Jimeno, J.; Matthews, S. *Ann. Oncol.* **2000**, *11*(Suppl. 4), 134.
10. Hossain, M. B.; van der Helm, D.; Antel, J.; Sheldrick, G. M.; Sanduja, S. K.; Weinheimer, A. J. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 4118–4122.
11. Kessler, H.; Will, M.; Antel, J.; Beck, H.; Sheldrick, G. M. *Helv. Chim. Acta* **1989**, *72*, 530–555.
12. Searle, M. S.; Hall, J. G.; Kyratzis, I.; Wakelin, L. P. G. *Int. J. Pept. Protein Res.* **1989**, *34*, 445–454.
13. Cardenas, F.; Thormann, M.; Feliz, M.; Caba, J. M.; Lloyd-Williams, P.; Giralt, E. *J. Org. Chem.* **2001**, *66*, 4580–4584.
14. Cardenas, F.; Caba, J. M.; Feliz, M.; Lloyd-Williams, P.; Giralt, E. *J. Org. Chem.* **2003**, *68*, 9554–9562.
15. For recent reviews see: (a) Kee, K. S. *Curr. Pharm. Des.* **2003**, *9*, 1209–1224. (b) Souers, A. J.; Ellman, J. A. *Tetrahedron* **2001**, *57*, 7431–7448. (c) Müller, G.; Hessler, G.; Decornez, H. Y. *Angew. Chem., Int. Ed.* **2000**, *39*, 894–896. (d) Hanessian, S.; McNaughton-Smith, G.; Lombart, H.-G.; Lubell, W. D. *Tetrahedron* **1997**, *53*, 12789–12854. (e) Goodman, M.; Ro, S. *Burger's Medicinal Chemistry and Drug Discovery*; Wolf, M. E., Ed.; Interscience: New York, 1995; Vol. 1, pp 803–861.
16. Schmidt, U.; Griesser, H.; Haas, G.; Kroner, M.; Riedl, B.; Schumacher, A.; Sutoris, F.; Haupt, A.; Emling, F. *J. Pept. Res.* **1999**, *54*, 146–161.
17. Jouin, P.; Poncet, J.; Dufour, M.-N.; Aumelas, A.; Pantaloni, A.; Cros, S.; François, G. *J. Med. Chem.* **1991**, *34*, 486–491.
18. Genin, J. M.; Ojala, W. H.; Gleason, W. B.; Johnson, R. L. *J. Org. Chem.* **1993**, *58*, 2334–2337.
19. Ward, P.; Ewan, G. B.; Jordan, C. C.; Ireland, S. J.; Magan, R. M.; Brown, J. R. *J. Med. Chem.* **1990**, *33*, 1848–1851.
20. Hinds, M. G.; Richards, N. G. J.; Robinson, J. A. *Chem. Commun.* **1988**, *22*, 1447–1449.
21. Hinds, M. G.; Welsh, J. H.; Brennand, D. M.; Fisher, J.; Glennie, M. J.; Richards, N. G. J.; Turner, D. L.; Robinson, J. A. *J. Med. Chem.* **1991**, *34*, 1777–1789.
22. Long, R. D.; Moeller, K. D. *J. Am. Chem. Soc.* **1997**, *119*, 12394–12395.
23. Khalil, E. M.; Subasinghe, N. L.; Johnson, R. L. *Tetrahedron Lett.* **1996**, *37*, 3441–3444.
24. Annunziata, R.; Ferrari, M.; Papeo, G.; Resmini, M.; Sisti, M. *Synth. Commun.* **1997**, *27*, 23–38.
25. Seebach, D.; Boes, M.; Naef, R.; Schweizer, W. B. *J. Am. Chem. Soc.* **1983**, *105*, 5390–5398.
26. Khalil, E. M.; Ojala, W. H.; Pradhan, A.; Nair, V. D.; Gleason, W. B.; Mishra, R. K.; Johnson, R. L. *J. Med. Chem.* **1999**, *42*, 628–637.
27. Albericio, F.; Carpino, L. A. *Methods Enzymol.* **1997**, *289*, 104–126.
28. Diago-Meseguer, J.; Palomo-Coll, A. L.; Fernández-Lizarbe, J. R.; Zugaza-Bilbao, A. *Synthesis* **1980**, 547–551.

29. Goodfellow, V. S.; Marathe, M. V.; Kuhlman, K. G.; Fitzpatrick, T. D.; Cuadrado, D.; Hanson, W.; Zuzack, J. S.; Ross, S. E.; Wieczorek, M.; Burkard, M.; Whalley, E. T. *J. Med. Chem.* **1996**, *39*, 1472–1484.
30. Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397–4398.
31. Reviews on the Mitsunobu reaction: (a) Hughes, D. L. *Org. React.* **1992**, *42*, 335–656. (b) Hughes, D. L. *Org. Prep. Proced. Int.* **1996**, *28*, 127–164. (c) Wiśniewski, K.; Kołdziejczyk, A. S.; Falkiewicz, B. *J. Pept. Sci.* **1998**, *4*, 1–14.
32. Genin, M. J.; Gleason, W. B.; Johnson, R. L. *J. Org. Chem.* **1993**, *58*, 860–866.
33. (a) Galéotti, N.; Montagne, C.; Poncet, J.; Jouin, P. *Tetrahedron Lett.* **1992**, *33*, 2807–2810. (b) Wipf, P.; Miller, C. P. *Tetrahedron Lett.* **1992**, *33*, 6267–6270. (c) Wipf, P.; Hayes, G. B. *Tetrahedron* **1998**, *54*, 6987–6998.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron 60 (2004) 5185–5199
Tetrahedron

Studies on organolithium-induced alkylative desymmetrisation of epoxides: synthesis of enantioenriched β-amino cycloheptenols from 6,7-epoxy-8-azabicyclo[3.2.1]octanes☆

David M. Hodgson* and Edyta Paruch†

Department of Chemistry, University of Oxford, Chemistry Research Laboratory, Mansfield Road, Oxford OX1 3TA, UK

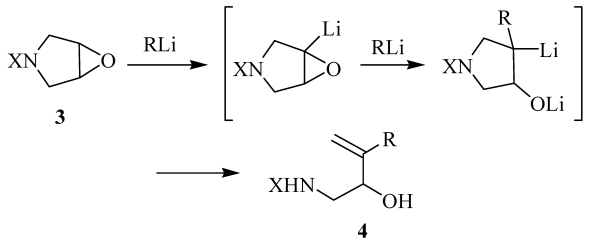
Received 1 March 2004; revised 26 March 2004; accepted 22 April 2004

Abstract—The synthesis and enantioselective α-deprotonation—double ring opening of 6,7-epoxy-8-azabicyclo[3.2.1]octanes **5** using organolithiums in the presence of (−)-sparteine or (4*S*)-2,2′-(1-ethylpropylidene)bis-4-(1-methylethyl)-4,5-dihydrooxazole, giving amino cycloheptenols in up to 85% yield and 82% ee is described. The impact of different reaction variables on reaction profiles has been studied, including the nature of organolithium, solvent, ligand, temperature and epoxide structure. The reactions proved to be dependent on all these variables, in particular on the structure of substrate. A mixed organolithium system ($Pr^iLi/TMSCH_2Li$) has been successfully used to introduce potentially versatile allylsilane functionality.

1. Introduction

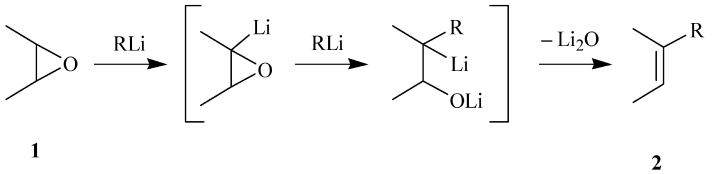
There is continuing need for new synthetic methods that control absolute stereochemistry, and enantioselective desymmetrisation of achiral materials is one of the most attractive and powerful approaches.[1] Asymmetric syntheses which begin with prochiral substrates and involve reactions of organolithium reagents or intermediates under the influence of chiral ligands are being reported at an increasing pace,[2] desymmetrisation of epoxides by enantioselective α-deprotonation being one area under active investigation.[3] The alkylative deoxygenation of epoxides **1** to give substituted alkenes **2** using organolithiums (Scheme 1) was originally discovered by Crandall and Lin,[4] and a number of research groups have subsequently made contributions to this area.[3,5]

In one development of this methodology we have taken advantage of the fact that an amine moiety is a better leaving group than the oxide substituent in the reaction of dihydropyrrole epoxides **3** (X=Boc, Bu^tSO_2) with organolithiums to give acyclic unsaturated 1,2-amino alcohols **4** (Scheme 2).[6] Our interests have now focused on the extension of this chemistry to epoxides derived from azabicyclic compounds and in an enantioselective manner.



3 **4**

Scheme 2.



1 **2**

Scheme 1.

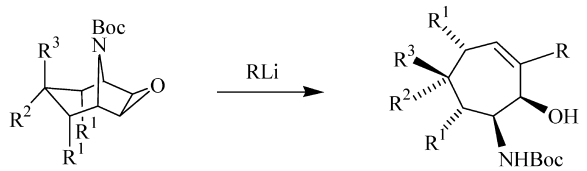
☆ Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.tet.2004.04.047

Keywords: Desymmetrisation; Epoxides; Organolithiums; Amino alcohols; Tropanes; (−)-Sparteine and bisoxazolines.
* Corresponding author. Tel.: +44-01865-275697; fax: +44-01865-275674; e-mail address: david.hodgson@chem.ox.ac.uk
† On leave from the Department of Chemistry, Agricultural University, ul. Norwida 25, 50-375 Wrocław, Poland.

0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.04.047

Vicinal amino alcohol functionality is important, since it is a common structural feature in several naturally occurring compounds and many of these exhibit significant biological activity, for example being immunomodulators, peptide mimetics, drug resistance inhibitors, protein synthesis inhibitors and antifungal agents.[7] Examples of the more important synthetic therapeutics containing the β-amino alcohol motif include saquinavir[8] and crixivan, used as anti-HIV agents.[9] The stereochemistry of the amino alcohol group is important for the precise biological activity of these compounds and, as a consequence, considerable importance is attached to new methods which access this moiety with high stereochemical control, both in a relative and absolute manner.

We recently communicated the first examples of enantioselective alkylative double ring-opening of epoxides derived from dihydropyrrole and azabicyclic alkenes, which led to enantioenriched acyclic and cyclic unsaturated amino alcohols, respectively.[10] Here we present in detail our studies on the desymmetrisation of epoxides of 8-azabicyclo[3.2.1]octenes. Our aim was to investigate whether it is possible to obtain enantioenriched β-amino cycloheptenols **6** (Scheme 3) from 6,7-epoxy-8-azabicyclo[3.2.1]octanes **5** by reaction with different organolithiums in the presence of an external chiral ligand. If successful, this transformation (enantioselective alkylative desymmetrisation) would be very powerful. Up to five stereocentres in an achiral substrate could be translated into an enantioenriched product in one step, along with an intermolecular C–C single bond formation, generation of a double bond, and two functional group reorganisations.



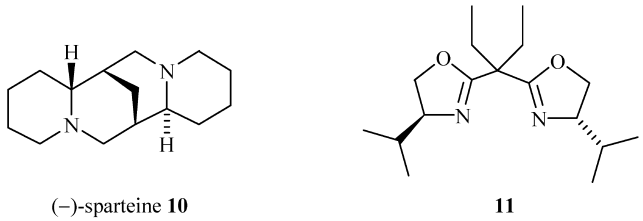
5 **6**

Scheme 3.

2. Results and discussion

The desymmetrisations of three tropane-type *meso*-epoxides were selected for study (Fig. 1). Ethers **7**, **8** and **9** were anticipated to provide insight into steric (and any stereoelectronic) effects on product profile/ee, with the dimethyl-substituted system **9** additionally providing a 'block' against potentially competing transannular C–H insertion (cyclopropane formation)[11] from the α-lithiated epoxide (vide infra). Previous organolithium-induced reactions of epoxides[3] suggested that TBDMS and Boc protection should be compatible with the chemistry we intended to investigate.

For each of the above substrates we planned to study the effects of solvent, temperature, and the nature of the organolithium and chiral ligand on product profile. (−)-Sparteine **10** and bisoxazoline **11** (Fig. 2), two of the best ligands known to mediate enantioselective deprotonations with organolithiums,[2] were chosen as ligands for examination with epoxides **7**–**9**. One advantage of the use of bisoxazolines over sparteine is that they can provide access to either product enantiomer, and are readily available.[12]



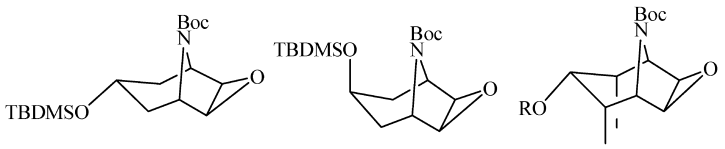
(−)-sparteine **10** **11**

Figure 2. External chiral ligands used.

The framework for epoxides **7**, **8** and **9** was constructed by [4+3] oxyallyl cycloadditions[13] using α,α′-perbromoketones and *N*-Boc pyrrole. Thus, ketone **13**, the precursor of epoxides **7** and **8**, was obtained from 1,1,3,3-tetrabromoacetone **12**[14] (1,3-dibromoacetone is known to be ineffective[13]) and *N*-Boc pyrrole (Scheme 4).

Although [4+3] cycloaddition between tetrabromoacetone **12**[14] and *N*-Boc pyrrole in the presence of Et_2Zn[15] gave very poor yields of the desired cycloadduct, use of activated zinc and $B(OEt)_3$[14] proved to be more efficient, giving, after debromination with Zn/Cu couple, ketone **13** (24% yield over 2 steps, Scheme 4). Reduction of ketone **13** by DIBAL-H gave 4:1 mixture of *endo*- and *exo*- alcohols **14** and **15**, which were separable following silylation. Epoxidation of *endo*-ether **16** with *m*CPBA afforded epoxide **7** in 96% yield. The anticipated *exo*-selection in the epoxidation was supported by NOE studies (see Section 4). *Exo*-alcohol **15** was obtained selectively by reduction of **13** with SmI_2 (65% yield).[16] This demonstration of the *N*Boc group's ability to effect a chelate-controlled reduction analogous to the corresponding oxygen-bridged system[16] is noteworthy. Subsequent silylation of the hydroxyl group and epoxidation gave epoxide **8** in good yield. The relative stereochemistry of the epoxide was determined by ^{1}H NOE studies.

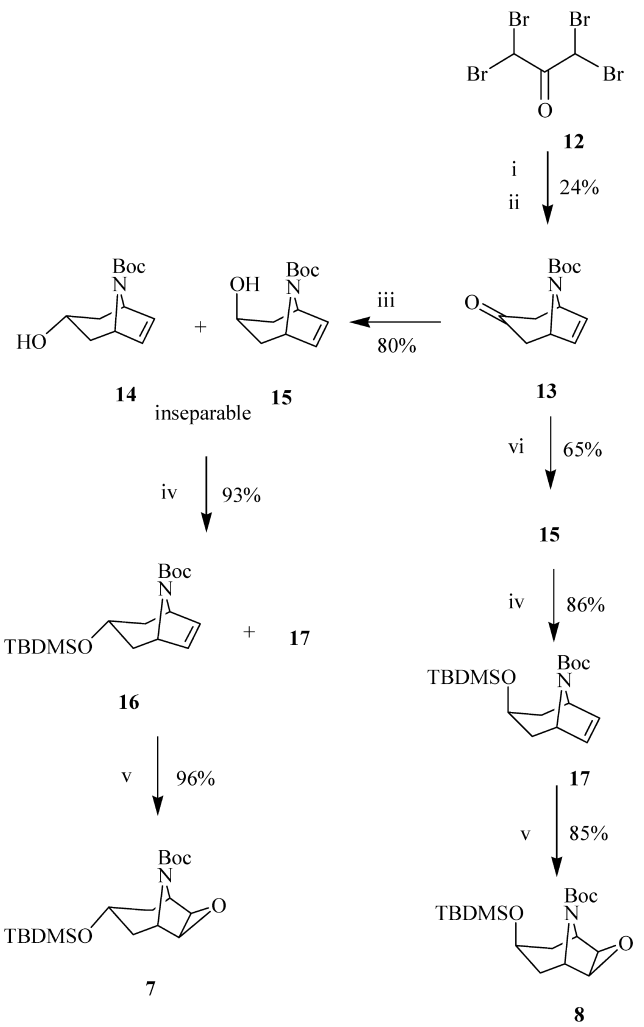
Synthesis of epoxide **9** (Scheme 5) started with the known



7 **8**

9a R = TBDMS
9b R = Me
9c R = MOM

Figure 1. Epoxides selected for desymmetrisation studies.



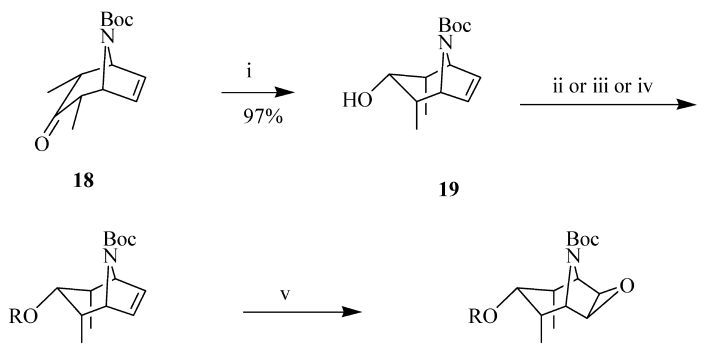
Scheme 4. Reagents and conditions: (i) *N*-Boc-pyrrole, Zn, $B(OEt)_3$, THF, 80 °C, 1.5 h, then 18 °C, 1 h; (ii) Zn/Cu, NH_4Cl, MeOH, 18 °C, 3 h; (iii) DIBAL-H, toluene, −88 to −40 °C, 17 h; (iv) TBDMSCl, DMF, imidazole, 18 °C, 3 h; (v) *m*CPBA, CH_2Cl_2, 0 °C, 2 h, then to 18 °C over 14 h; (vi) SmI_2, Pr^iOH, THF, reflux, 3 h.

ketone **18**, obtained from α,α′-dibromo-3-pentanone and *N-tert*-butoxycarbonyl pyrrole in the presence of diethylzinc, following the procedure of Paparin et al.[17] Reduction of ketone **18** with DIBAL-H gave *endo*-alcohol **19** stereoselectively.

Reaction of the sterically hindered hydroxyl group in **19** with TBDMSCl required strongly basic conditions (KH and 18-crown-6).[18] Attempted protection using TBDMSOTf resulted in loss of the Boc group.[19] Subsequent epoxidation of silyl ether **20** with *m*CPBA afforded epoxide **9a**, whose relative stereochemistry was assigned by NOE studies and later confirmed by X-ray crystallographic analysis of a related derivative (vide infra, Fig. 4). For reasons that are explained later, protecting groups other than TBDMS were also used. Introduction of MOM and methyl protection for the hydroxyl group gave ethers **20b** and **20c**, which were epoxidised with *m*CPBA to give epoxides **9b** and **9c** in high yields.

Desymmetrisation of epoxide **7** was first examined using Bu^nLi and Pr^iLi in the absence of a ligand under our typical conditions for epoxide lithiation [dropwise addition of a solution of epoxide to the organolithium (3.5 equiv.) at −78 °C in Et_2O, followed after 5 h by slow warming to −5 °C over 16 h].[10] This led to the desired amino cycloheptenols **21** (R=Bu^n, Pr^i) in poor yields (31% and 12%, respectively), together with azatricyclic alcohol **22** (19 and 23%, respectively) (Scheme 6, Table 1, entries 1 and 5); the latter likely arising from transannular C–H insertion of the intermediate α-lithiated epoxide.[3,11]

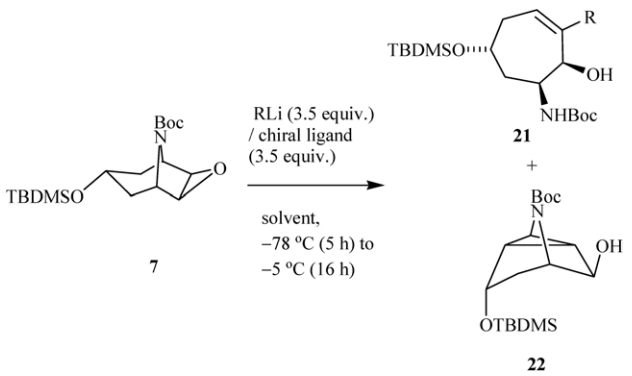
Reaction of epoxide **7** under the typical conditions, but using preformed organolithium/sparteine complex, gave an improvement in the relative ratio of amino cycloheptenol **21**: azatricyclic alcohol **22** compared to the ligand-free reactions [from 1.6:1 to 3.6:1 (for R=Bu^n) and from 0.5:1 to 1:1 (for R=Pr^i), Table 1, entries 2 and 6]. This led to isolation of the amino cycloheptenols **21** in improved yields, and significant asymmetric induction was also observed [58 and 72% ee (R=Bu^n), 39 and 82% ee (R=Pr^i)]. The absolute configuration of the predominant amino cycloheptenol **21** enantiomer obtained with (−)-sparteine **10** is



20a R = TBDMS (94%)
20b R = MOM (96%)
20c R = Me (82%)

9a R = TBDMS (94%)
9b R = MOM (91%)
9c R = Me (92%)

Scheme 5. Reagents and conditions: (i) DIBAL-H, toluene, −78 to −40 °C, 1 h; (ii) TBDMSCl, KH, 18-crown-6, THF, 0 to 18 °C, 4 h; (iii) MOMCl, 18-crown-6, THF, 0 to 18 °C, 14 h; (iv) MeI, KH, 18-crown-6, THF, 0 to 18 °C, 14 h; (v) *m*CPBA, CH_2Cl_2, 0 °C for 2 h, then 14 h at 18 °C for **20a** and **20c**, or 0.5 h at 18 °C for **20b**.



Scheme 6.

Table 1. Asymmetric alkylative double ring opening of epoxide **7**

Entry	RLi	Ligand	21		22	
			Yield (%)[a]	ee (%)[b]	Yield (%)	ee (%)
1	Bu^{n}Li	—	31	—	19	—
2	Bu^{n}Li	**10**	58	+72	16	+33
3[c]	Bu^{n}Li	**10**	68 (92)	+79	5	+10
4	Bu^{n}Li	**11**	9	−53	29	−71
5	Pr^{i}Li	—	12	—	23	—
6	Pr^{i}Li	**10**	39	+82	41	+78
7[c]	Pr^{i}Li	**10**	36 (72)	+83	10	+50
8	Pr^{i}Li	**11**	Trace	—	33	−77

[a] Yields in parentheses based on recovered epoxide **7**.
[b] Determined by chiral HPLC on the 3,5-dinitrobenzoate derivative. A positive value indicates that the major enantiomer was first to elute.
[c] Reaction quenched after 5 h at −78 °C.

likely that shown in Scheme 6. This is based on all our previous observations on organolithium-induced enantioselective α-deprotonations of epoxides[3] [medium-sized (8, 9 and 10-membered) cycloalkene epoxides, silyloxy substituted cyclooctene epoxides,[20] and norbornene epoxide,[21] 3,4-epoxytetrahydrofuran[22] and (*N*-Boc)-7-azanorbornene epoxide[11]] using (−)-sparteine, where proton removal at the *R*-epoxide stereocentre is consistently seen. Products from similar α-deprotonation-alkylation of related epoxides **8** and **9**, discussed later in this paper, are also assigned by analogy as being derived from proton removal at the *R*-epoxide stereocentre using sparteine. If the reactions with epoxide **7** were quenched after 5 h at −78 °C (rather than being allowed to warm up after that time), then this resulted in a further improvement in the ratios of amino cycloheptenol **21**: azatricyclic alcohol **22** [13.6:1 (R=Bu^n), 3.6:1 (R=Pr^i)] (Table 1 entries 3 and 7). Although some unreacted epoxide was also recovered, the isolated yields (and ees) of the amino cycloheptenols **21** were similar to those found on warming. These observations suggest that during the warming process any unreacted epoxide **7** is not converted into the desired aminocycloheptenol **21**, perhaps due to reduced availability of the organolithium. With Pr^{i}Li/sparteine, toluene was briefly examined as an alternative solvent under otherwise typical reaction conditions; however only 3% of **21** (R=Pr^i) was observed, along with azatricyclic alcohol **22** (36 and 62% ee).

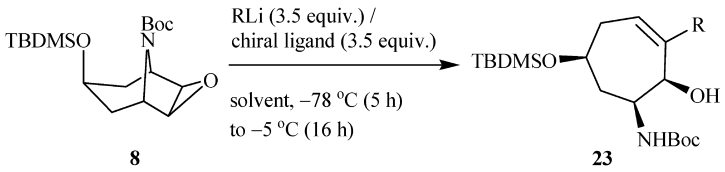
Bisoxazoline **11**, when used in equimolar amounts with organolithiums, appears to retard or prevent insertion of the carbenoid into an RLi bond (Table 1, entries 4 and 8). The major product of these desymmetrisations is the azatricyclic alcohol **21** obtained in about 30% yield and in good ees (above 70%). These results are in accordance with previous observations that 1:1 RLi/bisoxazoline systems tend to favour transannular C–H insertion of lithiated epoxides over reductive alkylation.[11,20] As anticipated on the basis of previous studies, bisoxazoline **11** was found to favour the opposite enantiomers of **21** and **22** compared with sparteine **10**.[3]

We had previously observed with 3,4-epoxytetrahydrofuran that decreasing the quantity of chiral ligand relative to organolithium gave improved yields and ee of the resulting alkene diol (cf. Scheme 2, XN=O).[22] The effect of varying the concentration of (−)-sparteine **10** and bisoxazoline **11** in the reaction of **7** is presented in Table 2. When the quantity of (−)-sparteine used for the reaction with Bu^{n}Li was reduced from 3.5 to 1 equiv. no significant loss in yield or enantioselectivity was observed (Table 2, entries 1 and 2). This suggests that only Bu^{n}Li complexed to sparteine deprotonates the epoxide. When a catalytic amount of the ligand was used, the yield of amino cycloheptenol was reduced in favour of the C–H insertion product **22** (entry 3) and a considerable rise in the ee of the latter was also observed. These results with Bu^{n}Li and epoxide **7** may indicate that, whilst a catalytic amount of sparteine is capable of effecting initial enantioselective deprotonation to a similar level as the stoichiometric ligand-assisted process, it does not assist incorporation of Bu^{n}Li into the intermediate lithiated epoxide as well as when a greater quantity of sparteine is present. It is also worth noting that with Bu^{n}Li and at least 1 equiv. of (−)-sparteine there is significant enantiomeric partitioning[22] (Table 2, entries 1 and 2); that is, in the presence of at least 1 equiv. of chiral ligand the relative proportions of the enantiomeric lithiated epoxides of **7** proceeding to **21** (R=Bu^n) and **22** are different. In the current case, the major lithiated epoxide enantiomer preferentially leads to the amino cycloheptenol **21**, whereas the minor one undergoes mainly C–H insertion leading to **22**. However, with catalytic sparteine the ees of **21** and **22** are similar. For the reaction of **7** with Bu^{n}Li-sparteine, the effect of reducing the quantity of sparteine to

Table 2. Reaction of epoxide **7** with Bu^{n}Li and Pr^{i}Li in the presence of varying quantities of ligand

Entry	RLi[a]	Ligand (equiv.)	21		22	
			Yield (%)	ee (%)[b]	Yield (%)	ee (%)
1	Bu^{n}Li	**10** (3.5)	58	+72	16	+33
2	Bu^{n}Li	**10** (1.0)	63	+61	19	+11
3	Bu^{n}Li	**10** (0.2)	38	+52	32	+57
4	Pr^{i}Li	**10** (3.5)	39	+82	51	+78
5	Pr^{i}Li	**10** (1.0)	28	+48	20	+64
6	Pr^{i}Li	**10** (0.2)	35	+40	17	+67
7	Bu^{n}Li	**11** (3.5)	9	−53	29	−71
8	Bu^{n}Li	**11** (1.0)	42	−39	29	−61
9	Bu^{n}Li	**11** (0.2)	36	−38	21	−55
10	Pr^{i}Li	**11** (3.5)	Trace	—	33	−77
11	Pr^{i}Li	**11** (1.0)	32	−36	19	−55
12	Pr^{i}Li	**11** (0.2)	38	Racemic	20	Racemic

[a] 3.5 equiv. was used.
[b] Determined by chiral HPLC on the 3,5-dinitrobenzoate derivative. A positive value indicates that the major enantiomer was first to elute.



8 23

Scheme 7.

0.2 equiv. on product profile and ee can be explained by postulating that dissociation of the ligand from the lithiated epoxide (so that it may complex with ligand-free Bu^{n}Li) occurs prior to subsequent reaction with Bu^{n}Li or C–H insertion.

In contrast to the results with Bu^{n}Li, the analogous series of experiments carried out with Pr^{i}Li gave results which suggest that this organolithium is basic enough to deprotonate epoxide **7** without the assistance of the ligand and, therefore, reducing the amount of (−)-sparteine resulted in a significant drop in the ee of amino cycloheptenol **21** (R=Pr^i) (Table 2, entries 4–6), with a relatively smaller decrease in the ee of tricyclic alcohol **22**. The yields of both products are not strongly affected. The distribution of products from both enantiomers of the lithiated epoxide seems to be more equal than in case of Bu^{n}Li, with a slight preference for the major enantiomer to undergo transannular C–H insertion when the concentration of the ligand is lower.

With epoxide **7**, decreasing the amount of bisoxazoline **11** gave the expected increase in the proportion of amino cycloheptenols **21**, with yields up to 40% and similar ees (Table 2, entries 7–12). When Bu^{n}Li was used, catalytic amounts of bisoxazoline **11** were sufficient to achieve this level of enantioselectivity (Table 2, entry 9). To the best of our knowledge, this is the first example of catalytic activity of bisoxazolines in this type of epoxide reaction.[3] The ees of tricyclic alcohol **22** observed in reactions with Bu^{n}Li and bisoxazoline **11** are high and drop gradually with the decrease in ligand concentration (entries 7–9). Bisoxazoline **11**, however, provides higher ees of the C–H insertion product **22** than of the amino alcohol **21**.

The effect of reducing the amount of bisoxazoline **11** on the product profile in the reaction of epoxide **7** with Pr^{i}Li was also investigated (Table 2, entries 10–12). It was found that using only 1 equiv. of **11** resulted in an increase in the yield of **21** up to 32%, with only moderate enantiomeric excess however (entries 10 and 11). A drop in both yield and ee of tricyclic alcohol **22** was observed. Reducing the amount of the bisoxazoline further to 0.2 equiv. gave comparable yields of the products, but a complete loss of enantioselectivity (entry 12). In this case it would appear that this ligand cannot be used in a catalytic amount to obtain enantioenriched products; however, unexpectedly, its presence seems to improve the yield of insertion into R-Li (compare Table 1, entry 5 and Table 2, entry 12).

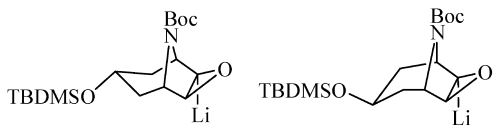
Desymmetrisation of epoxide **8** using Bu^{n}Li and Pr^{i}Li in the absence of a chiral ligand gave the amino cycloheptenols **23** (69 and 34%, respectively) (Scheme 7, Table 3 entries 1 and 4); in contrast to epoxide **7** no azatricyclic alcohol (cf. **22**) was observed as a co-product.

Table 3. Asymmetric alkylative double-ring opening of epoxide **8**

Entry	RLi	Ligand	**23**	
			Yield (%)	ee (%)[a]
1	Bu^{n}Li	—	69	—
2	Bu^{n}Li	**10**	83	+41
3	Bu^{n}Li	**11**	60	−67
4	Pr^{i}Li	—	34	—
5	Pr^{i}Li	**10**	59	+65
6[b]	Pr^{i}Li	**11**	52	−65

[a] Determined by chiral HPLC on the 3,5-dinitrobenzoate derivative. A positive value indicates that the major enantiomer was first to elute.
[b] Reaction quenched at 20 °C.

Also in contrast to epoxide **7**, for epoxide **8** bisoxazoline **11** was found to be at least as good a ligand as sparteine with respect to asymmetric induction using either Bu^{n}Li or Pr^{i}Li (Table 3, entries 2, 3, 5 and 6). The differing results for epoxides **7** and **8** may be rationalised by considering that the *endo*-silyloxy group in lithiated **7** slows (by non-bonded steric interactions) intermolecular insertion by Bu^{n}Li or Pr^{i}Li relative to intramolecular C–H insertion. Alternatively, or in addition, the silyloxy group may prefer to adopt an equatorial-like position in both epimeric epoxides **7** and **8**; intramolecular insertion into the axial C–H bond of lithiated **7** would then be easier than insertion into the equatorial C–H bond of lithiated **8** (Fig. 3).

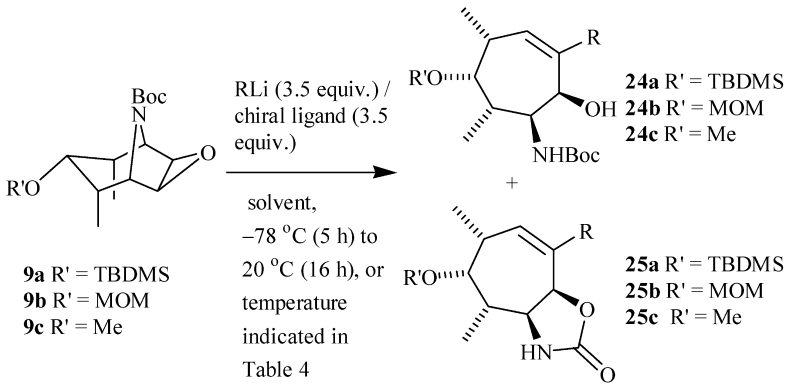


Li-**7** Li-**8**

Figure 3. Possible conformations of lithiated epoxides **7** and **8**.

Desymmetrisation of epoxide **9** with RLi/chiral ligand proved to be an efficient method for synthesising amino cycloheptenols **24** (Scheme 8, Table 4).

It is noteworthy that using (−)-sparteine **10** or bisoxazoline **11** gives access to both enantiomers of amino alcohols **24a** with both good yield and enantioselectivity (Table 4, entries 2, 3, 5 and 6). The side-product oxazolidinones **25** observed in these reactions arise from cyclisation between alkoxide and carbamate groups, a known reaction in basic conditions.[23] Oxazolidinone formation may proceed via isocyanate formation (loss of Bu^{t}OLi). It was found possible to reduce the formation of the oxazolidinones **25** by controlling the reaction conditions, as oxazolidinone generation is slow at low temperatures. The rate of this latter reaction depends on the type of organolithium and ligand used. When Bu^{n}Li/(−)-sparteine complex was used the cyclisation rate was very low (entry 2). Also with **9a**, in



Scheme 8.

Table 4. Asymmetric alkylative double ring opening of epoxide **9**

Entry	Epoxide	RLi	Ligand	24		25
				Yield (%)	ee (%)[a]	Yield (%)
1	**9a**	Bu^{n}Li	—	66	—	8
2	**9a**	Bu^{n}Li	**10**	84	+66	4
3[b]	**9a**	Bu^{n}Li	**11**	71	−67	14
4	**9a**	Pr^{i}Li	—	51	—	34
5[c]	**9a**	Pr^{i}Li	**10**	85	+71	2
6[c]	**9a**	Pr^{i}Li	**11**	72	−75	7
7	**9b**	Pr^{i}Li	**10**	79	+77	—
8	**9b**	Mixed[d]	—	57 (+11, R=Pr^i)	—	15
9	**9b**	Mixed[d]	**10**	49	+68	22
10	**9b**	$TMSCH_2Li$	**10**	30 (46)[e]	+65	13
11	**9c**	Pr^{i}Li	—	61 (76)[e]	—	—
12	**9c**	Pr^{i}Li	**10**	69 (79)[e]	+82	—
13	**9c**	Mixed[d]	—	70 (+2, R=Pr^i)	—	21
14	**9c**	Mixed[d]	**10**	66 (+7, R=Pr^i)	+64	12
15	**9c**	$TMSCH_2Li$	**10**	61 (66)[e]	+57	16

[a] Determined by chiral HPLC on the 3,5-dinitrobenzoate derivative.† A positive value indicates that the major enantiomer was first to elute.
[b] Reaction quenched at −30 °C.
[c] Reaction quenched at −50 °C.
[d] Mixed organolithiums: Pr^{i}Li (1.1 equiv.) and $TMSCH_2Li$ (2.5 equiv.), R=$TMSCH_2$.
[e] Yields in parentheses based on recovered epoxide **9**.

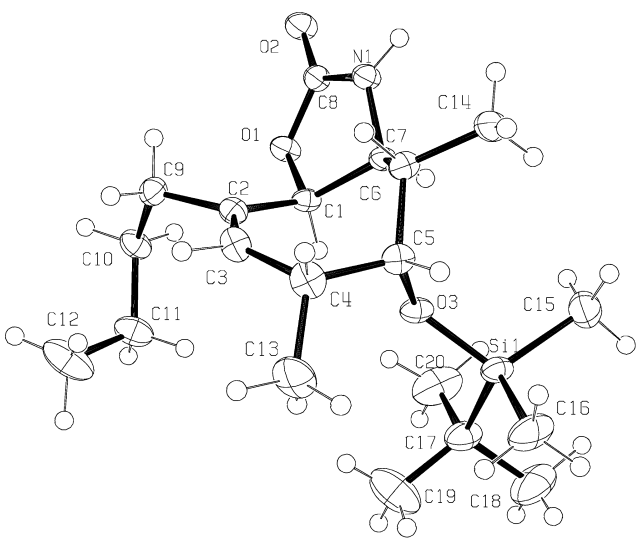
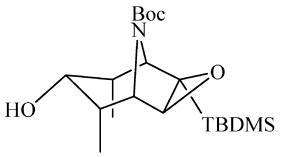


Figure 4. Molecular structure of oxazolidinone **25a** (R=Bu^n) (thermal ellipsoids are at the 40% level).

contrast to using Bu^{n}Li/(−)-sparteine, when the reaction of **9a** with Pr^{i}Li/(−)-sparteine was quenched at 18 °C, we observed 60% of **24a** (R=Pr^i) and 30% **25a** (R=Pr^i) (compare with entry 5 in Table 4). However, reaction using bisoxazoline **11** as a ligand with Bu^{n}Li was best quenched at −30 °C (entry 3) [quenching at 18 °C gave 60% of **24a** (R=Bu^n) and 22% **25a** (R=Bu^n)]. The crystal structure of oxazolidinone **25a** (R=Bu^n, R′=TBDMS) obtained from the reaction of **9a** with Bu^{n}Li (Fig. 4) provides confirmation of the stereochemistry of the amino alcohols, and precursor epoxides **9**.

At this stage we considered broadening the range of organolithiums used with epoxide **9** to include Me_3SiCH_2Li. If successful this would result in the generation of potentially versatile allylsilane functionality[24] in the product amino cycloheptenol. However, introduction of a trimethylsilylmethyl group by the reaction of **9a** with $TMSCH_2Li$/sparteine [3.5 equiv. each, Et_2O, −78 °C (5 h) to 18 °C over 14 h] was not successful—the starting epoxide was recovered in quantitative yield. Me_3SiCH_2Li appears to be too weak a base to deprotonate epoxide **9a**. We now considered whether it was possible to use a mixed organolithium system, containing an additional alkyllithium that was strong enough a base for the deprotonation, to reach our target. We chose Pr^{i}Li as the deprotonating agent, because of its increased basicity over $TMSCH_2Li$, and for its effective complexation with (−)-sparteine to provide good enantioselectivity. We hoped that on formation of the lithiated epoxide, $TMSCH_2Li$ would add to it efficiently. So, epoxide **9a** was subjected to desymmetrisation with Pr^{i}Li (1.1 equiv.) mixed with $TMSCH_2Li$ (2.5 equiv.) in the presence of (−)-sparteine (3.6 equiv.). However under these conditions the TBDMS group present in epoxide **9a** tends to undergo retro [1,5]-Brook migration.[22,25] We obtained an inseparable mixture of **26** (54%, Fig. 5) and the desired allylsilane **24a** (27%, R=CH_2SiMe_3). This result indicated that, although the lithiated epoxide from **9a** was generated using the mixed organolithium system [presumably by the Pr^{i}Li (1.1 equiv.) present], it was trapped only slowly by the excess Me_3SiCH_2Li, relative to retro-Brook rearrangement. We concluded that if we replaced the TBDMS protecting group by one with no tendency to migrate, we could achieve the desired reaction. To check this hypothesis we selected MOM protection (epoxide **9b**). However, we were also concerned at possible complications due to the presence of relatively acidic methylene protons in this protecting group.



26

Figure 5.

So as to investigate this variant of the reaction in the simplest possible system we also prepared methyl ether **9c** (Scheme 5).

Both epoxides **9b** and **9c** on treatment with the mixed organolithium system gave allyl silanes **24b** and **24c** in good yields and enantiomeric excesses (Table 4, entries 9 and 14, R=$TMSCH_2$). Using such a mixture of organolithiums is unprecedented in this type of reaction and provides a way to introduce the CH_2TMS group, leading to valuable allylsilane functionality. OMOM and OMe substituents (more coordinative and less bulky than the OTBDMS group) evidently made the protons attached to the epoxide ring more accessible, and for **9b** and **9c** $TMSCH_2Li$ by itself was basic enough to abstract them (Table 4, entries 10 and 15). However, when the mixed RLi system was used the yields and the ees of the allylsilane-containing amino alcohols **24b** and **24c** were higher. Desymmetrisation of **9b** and **9c** with Pr^iLi/(−)-sparteine gave very good yields and ees of the desired amino alcohols **24b** and **24c** (R=Pr^i) (Table 4, entries 7 and 12).

3. Conclusion

In conclusion, we have demonstrated that organolithium-induced alkylative desymmetrisation of 6,7-epoxy-8-azabicyclo[3.2.1]octanes in the presence of external chiral ligands provides a new and enantioselective route to substituted amino cycloheptenols. The reactions are quite sensitive to the epoxide substitution pattern, the chiral ligand and organolithium used. Also noteworthy is the introduction of versatile allylsilane functionality, best effected using a mixed organolithium system.

4. Experimental

4.1. General

All reactions requiring anhydrous conditions were conducted in flame- or oven-dried apparatus under an atmosphere of argon. Syringes and needles for the transfer of reagents were dried at 140 °C and allowed to cool in a desiccator over P_2O_5 before use. Ethers were distilled over sodium benzophenone ketyl, (chlorinated) hydrocarbons, amines and DMF from CaH_2. Reactions were monitored by TLC using commercially available glass-backed plates, pre-coated with a 0.25 mm layer of silica containing a fluorescent indicator (Merck), which were developed using potassium permanganate or molybdophosphoric acid solutions, and heated. Column chromatography was carried out on Kieselgel 60 (40–63 μm). Light petroleum refers to the fraction with bp 30–40 °C. Melting points were determined using a Leica VMTG apparatus and are uncorrected. Optical rotation values were measured on a Perkin–Elmer 241 Polarimeter. $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. IR spectra were recorded as thin films unless stated otherwise, on a Perkin–Elmer Paragon 1000 Fourier transformation spectrometer. Peak intensities are specified as strong (s), medium (m) or weak (w). Only selected absorbances are reported. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ with Bruker DPX200, Bruker DPX400, Bruker DRX500 or Bruker AMX500 spectrometers. Chemical shifts are reported relative to $CHCl_3$ [δ_H 7.26, δ_C (central line of t) 77.0 ppm]. Enantiomeric excesses of the amino alcohols were established by chiral HPLC analysis of their 3,5-dinitrobenzoates. Chiral stationary phase HPLC was performed using a Daicel Chiralcel OD column (4.6 mm×250 mm) with 10% EtOH in heptane and a flow rate of 1 cm^3 min^{-1} on a Gilson System with 712 Controller Software and a 118 UV/vis detector set at 254 nm. Retention times (t_R) for both enantiomers are given in minutes.

4.1.1. *tert*-Butyl 3-oxo-8-azabicyclo[3.2.1]oct-6-ene-8-carboxylate 13. *Step A*. A flask was charged with activated Zn dust (7.97 g, 0.122 mol), heated and flushed with argon. *N*-Boc pyrrole (50 g, 0.299 mol) in THF (30 mL) was added. A solution of 1,1,3,3-tetrabromoacetone **12**[14] (37 g, 0.1 mol) and triethyl borate (18.6 mL, 18.9 g, 0.13 mol) in THF (30 mL) was added dropwise via a cannula to this suspension, followed by one drop of Br_2. The mixture was heated to 80 °C for 1.5 h, and then allowed to cool to ambient temperature and stirred for 1 h. The black reaction mixture was suction filtered through a Celite pad and the residue was washed with several portions of Et_2O (250 mL in total). The combined organic phases were washed with water and brine. The aqueous layer was re-extracted with Et_2O (3×60 mL). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure.

Step B. Zinc dust was firstly activated by stirring for a few minutes in HCl (1 mol dm^{-3} solution in H_2O). The acid was removed by filtration and the zinc was washed with further portions HCl (1 mol dm^{-3}), water, acetone, Et_2O and finally dried under reduced pressure (17 mbar). This activated zinc (80 g, 1.22 mmol) was added to a hot, rapidly stirred solution of $Cu(OAc)_2H_2O$ (4.1 g, 0.021 mol) in glacial acetic acid (130 mL). After 1 min the blue green colour changed to dark purple. The mixture was then cooled to room temperature and the supernatant liquid was decanted. The Zn/Cu couple was washed with glacial acetic acid (150 mL), water (150 mL), acetone (150 mL), and Et_2O (3×150 mL) and was then dried under reduced pressure (1 mbar).

Step C. The brominated cycloadduct was dissolved in a saturated methanolic solution of NH_4Cl (250 mL), and the freshly prepared Zn/Cu couple (60 g) was added portionwise. The mixture was stirred at room temperature for 3 h and then the solid was removed by filtration through a Celite pad. The filtrate was concentrated under reduced pressure to remove some of MeOH (to approximately 1/3 of its volume), then diluted with CH_2Cl_2 (500 mL) and washed with brine (250 mL) and water (250 mL). The water phases were re-extracted with CH_2Cl_2 (250 mL) and Et_2O

(250 mL). The combined organic phases were dried ($MgSO_4$) and evaporated under reduced pressure to give 35 g of crude product mixture as brown oil. Column chromatography (light petroleum/EtOAc 9:1) afforded cycloadduct **13** (5.3 g, 24%) as orange crystals. Repeated recrystallisation from light petroleum/EtOAc (9:1) gave colourless crystals; R_f 0.13 (light petroleum/EtOAc 9:1); mp 86–87 °C; ν_{max}(KBr)/cm^{-1} 2978s, 2934m, 1713s, 1653s, 1456w, 1434w, 1367m, 1367m, 1327m, 1291m, 1211w and 1147s; δ_H (400 MHz) (two rotamers) 6.20–6.10 (2H, m, 6,7-H), 4.76, 4.69 (2H, two br s, 1,5-H), 2.80, 2.60 (2H, two br d, J=14.9 Hz, 2,4-H$_{exo}$), 2.30 (2H, d, J=16.8 Hz, 2,4-H$_{endo}$), 1.44 (9H, s, CMe_3); δ_c (100 MHz) 205.9 (C3), 152.1 (NC=O), 133.9, 133.7 (C6, C7), 80.4 (*C*Me$_3$), 55.7, 56.2 (C1, C5), 45.6, 45.1 (C2, C4) and 28.3 (C*Me*$_3$); *m*/*z* [CI+(NH_3)] 241 (M+NH_4^+, 20%), 224 (M+H^+, 35), 185 (25) and 124 (100) (Found: M+H^+, 224.1288. $C_{12}H_{18}NO_3$ requires 224.1286).

4.1.2. *tert*-Butyl 3α-hydroxy-8-azabicyclo[3.2.1]oct-6-ene-8-carboxylate 14 and *tert*-butyl 3β-hydroxy-8-azabicyclo[3.2.1]oct-6-ene-8-carboxylate 15. A solution of bicyclic ketone **13** (0.51 g, 2.3 mmol) in toluene (50 mL) was cooled to −88 °C and DIBAL-H (1 mol dm^{-3} in toluene; 3.8 mL, 3.8 mmol) was added dropwise. The reaction mixture was allowed to warm up to −40 °C over 17 h, then diluted with Et_2O (20 mL) and washed with a saturated solution of sodium potassium tartrate (15 mL). The aqueous layer was extracted with CH_2Cl_2 (3×10 mL) and the combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure to give a cream solid. This mixture was purified by column chromatography (light petroleum/EtOAc 2:1) to give an inseparable mixture of *endo*-**14** and *exo*-**15** alcohols (4:1, 0.41 g, 80%) (R_f 0.28); ν_{max}(KBr)/cm^{-1} (mixture of isomers) 3482m, 2972m, 2922m, 1679s, 1602w, 1421s, 1179m, 1105s and 1065s; discernible data for **14**: δ_H (400 MHz) (two rotamers) 6.38–6.23 (2H, m, 6,7-H), 4.49, 4.42 (2H, two br s, 1,5-H), 3.88 (1H, br s, 3-H), 2.47 (1H, br s, OH), 2.23–2.15, 2.13–2.05 (2H, two m, 2,4-H$_{exo}$), 1.69 (2H, br d, J=14.5 Hz, 2,4-H$_{endo}$), 1.40 (9H, s, CMe_3); δ_c (100 MHz) 152.6 (C=O), 136.3, 136.0 (C6, C7), 80.0 (*C*Me$_3$), 65.9 (C3), 57.7, 57.0 (C1, C5), 35.8, 35.1 (C2, C4) and 28.9 (C*Me*$_3$); *m*/*z* [CI+(NH_3)] 226 (M+H^+, 45), 187 (36) and 126 (100) (Found: M+H^+, 226.1445. $C_{12}H_{20}NO_3$ requires 226.1443). Data for pure **15** is listed below.

4.1.3. *tert*-Butyl 3α-(*tert*-butyldimethylsilyloxy)-8-azabicyclo[3.2.1]oct-6-ene-8-carboxylate 16 and *tert*-butyl 3β-(*tert*-butyldimethylsilyloxy)-8-azabicyclo[3.2.1]oct-6-ene-8-carboxylate 17. To a stirred solution of TBDMSCl (0.4 g, 2.6 mmol) and imidazole (0.33 g, 4.8 mmol) in DMF (1 mL) a mixture of alcohols **14** and **15** (0.41 g, 1.82 mmol) was added. The reaction mixture was stirred at room temperature for 3 h, then it was partionated between Et_2O (6 mL) and H_2O (3 mL). The layers were separated, the organic layer was diluted with Et_2O (50 mL), washed with H_2O (10 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The products were separated by column chromatography (light petroleum/EtOAc 18:1) to give *endo*-ether 16 (0.41 g, 66%) as a white solid and a mixture of **16** (R_f 0.25) and **17** (R_f 0.20) (0.15 g). Total yield 93%. Data for **16**: mp 66–69 °C; ν_{max}(KBr)/cm^{-1} 2958s, 2928s, 1690s, 1603w, 1407s, 1315m, 1259m, 1184m, 1103s, 1065s, 837s and 775s; δ_H (200 MHz) (two rotamers) 6.10–5.92 (2H, m, 6,7-H), 4.32, 4.40 (2H, two br s, 1,5-H), 3.93 (1H, t, J=6.0 Hz, 3-H), 2.17–2.05 (2H, m, 2,4-H$_{exo}$), 1.55, 1.48 (2H, two br s, 2,4-H$_{endo}$), 1.45 (9H, s, $OCMe_3$), 0.83 (9H, s, $SiCMe_3$) and −0.05 (6H, s, $SiMe_2$); δ_c (50 MHz) 152.2 (C=O), 134.0, 133.5 (C6, C7), 79.0 (O*C*Me$_3$), 65.0 (C3), 56.5, 57.3 (C1, C5), 35.5, 34.7 (C2, C4), 28.5 (*Me*$_3$CO), 25.6 (SiC*Me*$_3$), 17.6 (Si*C*Me$_3$) and −5.0 ($SiMe_2$); *m*/*z* [CI+(NH_3)] 340 (M+H^+, 60), 240 (100), 132 (18) and 108 (17) (Found: M+H^+, 340.2304. $C_{18}H_{33}NO_3Si$ requires 340.2308).

4.1.4. *tert*-Butyl *exo*−6,7-epoxy-3α-(*tert*-butyldimethylsilyloxy)-8-azabicyclo[3.2.1]octane-8-carboxylate 7. A solution of *m*CPBA (70%, 0.44 g, 1.77 mmol) in CH_2Cl_2 (10 mL) was added dropwise to a stirred, cold (0–5 °C) solution of *endo*-ether **16** (0.40 g, 1.18 mmol) in CH_2Cl_2 (10 mL). The mixture was stirred in an ice bath for 2 h and left stirred for the next 14 h, during which time it warmed up to room temperature. Then it was diluted with CH_2Cl_2 (20 mL) and washed with sat. aq. $Na_2S_2O_3$ (2×10 mL), aq. NaOH (1 mol dm^{-3}, 10 mL), brine (10 mL) and dried ($MgSO_4$). After evaporation of CH_2Cl_2, the residue was purified by column chromatography (light petroleum/Et_2O 3:1) to give epoxide **7** (0.40 g, 96%) as a white solid; R_f 0.39 (light petroleum/Et_2O 3:1); mp 66–68 °C; ν_{max}(KBr)/cm^{-1} 2928s, 1700s, 1364m, 1305m, 1257m, 1177m, 1100m, 1081s, 1022m, 839s and 779s; δ_H (500 MHz) (two rotamers) 4.43, 4.28 (2H, two t, J=2.8 Hz, 1,5-H), 4.03, 4.01 (1H, two d, J=5.0 Hz, 3-H), 3.53, 3.51 (2H, AB, J=3.1 Hz, 6,7-H), 2.10, 2.05 (2H, two ddd, J=15.0, 5.0, 4.0 Hz, 2,4-H$_{exo}$), 1.65, 1.68 (2H, two d, J=15.0 Hz, 2,4-H$_{endo}$), 1.50 (9H, s, $OCMe_3$), 0.92 (9H, s, $SiCMe_3$) and 0.01 (6H, s, $SiMe_2$); δ_c (50 MHz) 156.5 (C=O), 79.7 (O*C*Me$_3$), 63.8 (C1, C5), 53.8, 53.4 (C6, C7), 53.6, 52.9 (C3), 34.9, 34.6 (C2, C4), 28.3 (OC*Me*$_3$), 25.7 (SiC*Me*$_3$), 17.7 (Si*C*Me$_3$) and −5.1 ($SiMe_2$); *m*/*z* (EI) 356 (M+H^+, 10), 355 (M, 41), 296 (30) and 282 (100); *m*/*z* [CI+(NH_3)] 373 (M+NH_4^+, 15), 356 (M+H^+, 57), 317 (55), 300 (45), 256 (70), 240 (100) and 132 (55) (Found: M+H^+, 356.2260. $C_{18}H_{34}NO_4Si$ requires 356.2257); ^{1}H NOE: irradiation at 6,7-H resonance frequency resulted in enhancement of signals of 2,4-H$_{endo}$ and 1,5-H, whereas those of 2,4-H$_{exo}$, 3-H and Boc group remained unaffected (indicating *exo*-orientation of the epoxide ring and *endo*-orientation of the TBDMS ether).

4.1.5. *tert*-Butyl 3β-hydroxy-8-azabicyclo[3.2.1]oct-6-ene-8-carboxylate 15. *Part A*. CH_2I_2 (2.6 g, 9 mmol) was dried under vacuum overnight, then THF (20 mL) was added and argon was bubbled through this solution for 30 min. The solution was then cooled to 0 °C in an ice bath.

Part B. Samarium chips (1.6 g, 10.6 mmol) were transferred under argon to a 3-neck flask equipped with a condenser. The flask was evacuated with a high vacuum pump and filled with argon. The procedure was repeated three times and then the flask was heated with a flame for 10 min under vacuum. After cooling down and filling with argon, the solution of CH_2I_2 in THF was added at 0 °C through a cannula. After stirring the mixture for 15 min at 0 °C and for 1.5 h at room temperature under Ar, a dark blue colour appeared.

Part C. Argon was bubbled through a solution of ketone **13** (1 g, 4.48 mmol) and Pr^{i}OH (0.35 mL, 4.5 mmol) in THF (4.5 mL) for 30 min. This solution was added to a refluxing solution of SmI_2 through a cannula. After 3 h the reaction mixture was cooled in an ice bath, then carefully quenched with water and HCl (2 mol dm^{-3}, 15 mL), while the mixture turned pea green. Et_2O (30 mL) was then added and the layers separated. The organic layer was washed with sat. aq. $Na_2S_2O_3$ solution, dried over $MgSO_4$ and concentrated under reduced pressure to give **15** (0.657 g, 65%) as a yellowish oil; R_f 0.14 (light petroleum/EtOAc 2:5); ν_{max}/cm^{-1} 3408s br, 2975m, 1674s, 1415s, 1367m, 1175m, 1103m and 1049w; δ_H (400 MHz) (two rotamers) 5.99–5.90 (2H, m, 6,7-H), 4.50, 4.40 (2H, two br s, 1,5-H), 3.80–3.70 (1H, m, 3-H), 3.25 (1H, br s, OH), 1.85–1.75 (2H, m, 2,4-H$_{endo}$), 1.50–1.40 (2H, m, 2,4-H$_{exo}$) and 1.35 (9H, s, OCMe$_3$); δ_C (100 MHz) 151.9 (C=O), 130.9, 130.7 (C6, C7), 79.5 (O*C*Me$_3$), 64.1 (C3), 57.3, 56.6 (C1, C5), 34.1, 34.4 (C2, C4) and 28.4 (OC*Me*$_3$); *m/z* (EI) 226 (M+H$^+$, 30%), 210 (45) and 183 (100); *m/z* [CI+(NH$_3$)] 226 (M+H$^+$, 30%), 126 (100) and 80 (10) (Found: M+H$^+$ 226.1447. $C_{12}H_{20}NO_3$ requires 226.1443).

4.1.6. *tert*-Butyl 3β-(*tert*-butyldimethylsilyloxy)-8-azabicyclo[3.2.1]oct-6-ene-8-carboxylate 17. TBDMSCl (0.6 g, 3.98 mmol) was added to a solution of alcohol **15** (0.44 g, 1.96 mmol) and imidazole (0.5 g, 7.35 mmol) in DMF (3 mL). The mixture was stirred at room temperature for 3 h, and then partitioned between Et_2O (6 mL) and H_2O (3 mL). The layers were separated, the organic layer was diluted with Et_2O (50 mL), washed with H_2O (10 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Column chromatography (light petroleum/EtOAc 18:1) afforded *exo*-ether **17** (0.57 g, 86%) as a white solid; mp 44–46 °C; R_f 0.55 (light petroleum/EtOAc 9:1); ν_{max}/cm^{-1} 2955s, 2929s, 2857s, 1699s, 1595w, 1473m, 1392s, 1255m, 1179m, 1096s, 1056m, 953m, 865m, 837m and 775m; δ_H (500 MHz) (two rotamers) 6.03–5.94 (2H, m, 6,7-H), 4.55, 4.46 (2H, two br s, 1,5-H), 3.92–3.82 (1H, m, 3-H), 1.83–1.72 (2H, m, 2,4-H$_{endo}$), 1.68–1.55, 1.55–1.46 (2H, two m, 2,4-H$_{exo}$), 1.45 (9H, s, OCMe$_3$), 0.82 (9H, s, (SiCMe$_3$) and −0.03 (6H, s, SiMe$_2$); δ_C (125 MHz) 152.4 (C=O), 131.6, 131.3 (C6, C7), 79.8 (O*C*Me$_3$), 65.8 (C3), 57.8, 57.1 (C1, C5), 35.2, 34.4 (C2, C4), 28.9 (OC*Me*$_3$), 26.2 (SiC*Me*$_3$), 18.4 (Si*C*Me$_3$) and −4.2 (Si*Me*$_2$); *m/z* [CI+(NH$_3$)] 340 (M+H$^+$, 65%), 284 (20), 240 (100), 226 (15) and 182 (10) (Found: M+H$^+$, 340.2310. $C_{18}H_{34}NO_3Si$ requires 340.2308).

4.1.7. *tert*-Butyl *exo*−6,7-epoxy-3β-(*tert*-butyldimethylsilyloxy)-8-azabicyclo[3.2.1]octane-8-carboxylate 8. A solution of *m*CPBA (70%, 0.5 g, 2 mmol) in CH_2Cl_2 (10 mL) was added dropwise to a stirred, cold (0–5 °C) solution of silyl ether **17** (0.46 g, 1.36 mmol) in CH_2Cl_2 (10 mL). The mixture was stirred in an ice bath for 2 h and left stirring for further 14 h, during which time it warmed to room temperature. The reaction mixture was then diluted with CH_2Cl_2 (20 mL) and washed with sat. aq. $Na_2S_2O_3$ (2×10 mL), aq. NaOH (1 mol dm^{-3}, 10 mL), brine (10 mL) and dried ($MgSO_4$). After evaporation the residue was purified by column chromatography (light petroleum/Et_2O 3:1, then 1:1) to give epoxide **8** (0.41 g, 85%), as a yellowish oil; R_f 0.18 (light petroleum/Et_2O 3:1); ν_{max}/cm^{-1} 2956s, 2930s, 2858m, 1704s, 1473w, 1393s, 1367m, 1300m, 1257m, 1176m, 1104s, 1078m, 1036w and 977w; δ_H (500 MHz) (two rotamers) 4.48–4.42, 4.34–4.27 (2H, two m, 1,5-H), 4.13–4.07 (1H, m, 3-H), 3.37, 3.35 (2H, AB, *J*=3.1 Hz, 6,7-H), 1.97–1.89 (2H, m, 2,4-H$_{endo}$), 1.73–1.64 (2H, m, 2,4-H$_{exo}$), 1.45 (9H, s, OCMe$_3$), 0.86 (9H, s, SiCMe$_3$) and 0.03 (6H, s, SiMe$_2$); δ_c (125 MHz) 155.8 (C=O), 79.8 (O*C*Me$_3$), 64.1 (C3), 53.8, 52.7 (C1, C5), 52.0 51.6 (C6, C7), 35.6, 35.3 (C2, C4), 28.2 (OC*Me*$_3$), 25.6 (SiC*Me*$_3$), 17.9 (Si*C*Me$_3$) and −4.8 (SiMe$_2$); *m/z* (EI) 356 (M+H$^+$, 30%), 296 (25), 282 (100) and 256 (95); *m/z* [CI+(NH$_3$)] 356 (M+H$^+$, 100%), 340 (25) and 300 (50) (Found: M+H$^+$ 356.2256. $C_{18}H_{34}NO_4Si$ requires 356.2257); ^{1}H NOE: irradiation at 6,7-H resonance frequency resulted in enhancement of signals of 1,5-H, 3-H and 2,4-*endo* protons, whereas the rest of the signals remained unaffected.

4.1.8. *tert*-Butyl 3α-hydroxy-2α,4α-dimethyl-8-azabicyclo[3.2.1]oct-6-ene-8-carboxylate 19. A solution of *tert*-butyl 2α,4α-dimethyl-8-azabicyclo[3.2.1]oct-6-ene-3-one-8-carboxylate **18**[17] (0.47 g, 1.88 mmol) in toluene (50 mL) was cooled to −78 °C and DIBAL-H (1 mol dm^{-3} in hexanes; 2.8 mL, 2.8 mmol) was added dropwise. The reaction mixture was stirred and allowed to warm up to −40 °C over 1 h, then diluted with Et_2O (30 mL) and washed with a saturated solution of sodium potassium tartrate (15 mL). The aqueous layer was extracted three times with CH_2Cl_2 (3×10 mL) and the combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure to give alcohol **19** (0.46 g, 97%) as yellowish oil, which was used in the next reaction without purification. When purified by column chromatography (light petroleum/EtOAc 1:1; R_f 0.45) the compound crystallises on exposure to the air; mp 74–76 °C; ν_{max}/cm^{-1} 3481s, 2973s, 1682s, 1599w, 1417s, 1294s, 1178s, 1050m, 979m, 916m and 674m; δ_H (400 MHz) (two rotamers) 6.50–6.39 (2H, m, 6,7-H), 4.33, 4.23 (2H, two br s, 1,5-H), 3.65 (1H, td, *J*=11.0, 5.1 Hz, C*H*OH), 2.29–2.19, 2.18–2.08 (2H, two m, 2,4-H), 1.56 (2H, d, *J*=11.3 Hz, O*H*), 1.47 (9H, s, CMe$_3$) and 1.05 (6H, d, *J*=7.3 Hz, 2,4-Me); δ_C (50 MHz) 151.8 (C=O), 135.9, 135.5 (C6, C7), 79.2 (O*C*Me$_3$), 72.5 (C3), 61.9, 61.1 (C1, C5), 38.0, 37.0 (C2, C4), 28.3 (OC*Me*$_3$), and 14.1, 14.0 (2,4-Me); *m/z* [CI+(NH$_3$)] 271 (M+NH$_4^+$, 5%), 254 (M+H$^+$, 60), 215 (25) and 154 (100) (Found: M+H$^+$, 254.1752. $C_{14}H_{24}NO_3$ requires 254.1756).

4.1.9. *tert*-Butyl 2α,4α-dimethyl-3α-(*tert*-butyldimethylsilyloxy)-8-azabicyclo[3.2.1]oct-6-ene-8-carboxylate 20a. KH in oil (180 mg, 4.5 mmol) was transferred into a preweighed 2-neck round-bottom flask fitted with a nitrogen inlet and septum. The oil was removed by washing KH with dry hexane (3×1.5 mL), removed via syringe. The flask was reweighed after complete evaporation of solvent and 18-crown-6 (10 mg, 0.04 mmol) in THF (4 mL) was added. The suspension was cooled to 0 °C and alcohol **19** (0.455 g, 1.8 mmol) was added in THF (10 mL). Gas evolution occurred and subsided in about 5 min after which time a solution of TBDMSCl (0.475 g, 3.2 mmol) in THF (10 mL) was added. The reaction was then warmed to room temperature and allowed to stirr for 4 h. Then it was quenched with water (5 mL), extracted with CH_2Cl_2

(3×20 mL) and dried ($MgSO_4$). Purification of the residue by column chromatography (light petroleum/EtOAc 18:1) afforded silyl ether **20a** (0.62 g, 94%) as a yellowish oil; R_f 0.17 (light petroleum/EtOAc 18:1); ν_{max}/cm^{-1} 2968s, 2932s, 1690s, 1598w, 1413s, 1265s, 1178s, 1112m, 1066s, 1023s, 876m, 737s and 706s; δ_H (400 MHz) (two rotamers) 6.21–6.07 (2H, m, 6,7-H), 4.18, 4.08 (2H, two br s, 1,5-H), 3.85–3.77 (1H, m, 3-H), 2.18–2.06, 2.05–1.94 (2H, two m, 2,4-H), 1.45 (9H, s, $OCMe_3$), 0.91 (6H, d, J=7.4 Hz, 2,4-Me), 0.89 (9H, s, $SiCMe_3$) and −0.34 (6H, s, $SiMe_2$); δ_C (100 MHz) 152.1 (C=O), 133.8, 133.3 (C6, C7), 79.1 ($OCMe_3$), 72.5 (C3), 61.5, 62.3 (C1, C5), 37.6, 38.6 (C2, C4), 28.5 (Me_3CO), 26.1 (Me_3CSi), 18.5 ($SiCMe_3$), 15.5, 15.4 (2,4-Me) and −3.5 ($SiMe_2$); m/z (EI) 368 ($M+H^+$, 20), 367 (M, 68), 311 (75), 224 (73), 178 (100) and 173 (65); m/z [CI+(NH_3)] 368 ($M+H^+$, 60), 268 (70), 226 (60), 187 (50), 126 (100) and 94 (58) (Found: $M+H^+$, 368.2618. $C_{20}H_{38}NO_3Si$ requires 368.2621).

4.1.10. *tert*-Butyl *exo*−6,7-epoxy-2α,4α-dimethyl-3α-(*tert*-butyldimethylsilyloxy)-8-azabicyclo[3.2.1]octane-8-carboxylate 9a. *m*CPBA (70%, 0.73 g, 2.96 mmol) in CH_2Cl_2 (20 mL) was added dropwise to a solution of silyl ether **20a** (0.72 g, 1.96 mmol) in CH_2Cl_2 (20 mL) at 0 °C. The reaction was mixture was stirred at 0 °C for 2 h and left overnight, during which time it warmed to room temperature. The reaction mixture was then diluted with CH_2Cl_2 (30 mL) and washed with sat. aq. $Na_2S_2O_3$ (2×15 mL), 5% aq. NaOH (10 mL), brine (20 mL) and the organic layer dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by column chromatography (light petroleum/Et_2O 3:1) to give epoxide **9a** (0.71 g, 94%) as a white solid; R_f 0.42 (light petroleum/Et_2O 3:1); mp 102–103 °C; ν_{max}(KBr)/cm^{-1} 2963s, 2932s, 1704s, 1412m, 1366m, 1258m, 1183m, 1125m, 1026s, 881m and 840s; δ_H (500 MHz) (two rotamers): 4.19, 4.02 (2H, two d, J=2.5 Hz, 1,5-H), 3.82, 3.81 (1H, two d, J=4.0 Hz, 3-H), 3.49, 3.47 (2H, AB, J=3.4 Hz, 6,7-H), 2.17–2.07 (2H, m, 2,4-H), 1.50 (9H, s, $OCMe_3$), 1.08 (6H, d, J=7.5 Hz, 2,4-Me), 0.97 (9H, s, $SiCMe_3$) and 0.10 (6H, s, $SiMe_2$); δ_C (50 MHz) 156.3 (C=O), 79.7 ($OCMe_3$), 72.1 (C3), 57.9, 58.5 (C1, C5), 52.5, 52.1 (C6, C7), 39.2, 38.8 (C2, C4), 28.3 (Me_3CO), 26.2 ($SiCMe_3$), 18.4 ($SiCMe_3$), 14.8 (2,4-Me) and −3.4 ($SiMe_2$); m/z (EI) 384 ($M+H^+$, 25), 383 (M, 75), 282 (42), and 226 (100); m/z [CI+(NH_3)] 384 ($M+H^+$, 72), 328 (45), 284 (58), 272 (100), 178 (56), 132 (75) and 90.2 (58) (Found: $M+H^+$, 384.2575. $C_{20}H_{38}NO_5Si$ requires 384.2570); ^1H NOE: irradiation at 6,7-H resonance frequency resulted in enhancement of signals of 2,4-methyl groups and 1,5-protons, whereas 2,4-protons signals and Boc group remained unaffected (indicating *endo*-orientation of 6,7-protons and *exo*-position of the epoxide ring).

4.1.11. *tert*-Butyl 3α-(methoxymethyloxy)-2α,4α-dimethyl-8-azabicyclo[3.2.1]oct-6-ene-8-carboxylate 20b. KH (0.12 g, 3 mmol) in oil was transferred to a dry flask, washed with dry pentane and dried under argon, after which THF (10 mL) and 18-crown-6 (8 mg, 0.03 mmol) was added. The mixture was cooled to 0 °C, then alcohol **19** (0.49 g, 1.9 mmol) was added and the mixture was stirred at 0 °C for 15 min. Then MOMCl (0.2 mL, 2.63 mmol) was added and the mixture was stirred at room temperature for 14 h. The milky cream mixture was diluted with THF (10 mL), carefully quenched with a few drops of H_2O, then poured to 100 mL of H_2O, extracted with Et_2O (3×25 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Column chromatography (light petroleum/EtOAc 4:1) afforded MOM ether **20b** (552 mg, 96%) as a colourless oil; R_f 0.34 (light petroleum/EtOAc 4:1); ν_{max}/cm^{-1} 2973s, 2932m, 2895m, 1698s, 1409s, 1367m, 1292m, 1180m, 1153m, 1098m, 1034s and 917w; δ_H (400 MHz) (two rotamers) 6.22–6.12 (2H, m, 6,7-H), 4.44 (2H, s, CH_2), 4.18, 4.08 (2H, two br s, 1,5-H), 3.55 (1H, t, J=4.9 Hz, 3-H), 3.31 (3H, s, OMe), 2.29–2.18, 2.18–2.04 (2H, two m, 2,4-H), 1.42 (9H, s, $OCMe_3$) and 0.94 (6H, d, J=7.3 Hz, 2,4-Me); δ_C (100 MHz) 152.0 (C=O), 134.0, 133.5 (C6, C7), 99.5 (CH_2), 80.7 (C3), 79.2 ($OCMe_3$), 62.2, 61.0 (C1, C5), 56.2 (OMe), 38.3, 37.4 (C2, C4), 28.5 ($OCMe_3$), and 14.6, 14.4 (2,4-Me); m/z [CI+(NH_3)] 298 ($M+H^+$, 43), 198 (100); m/z (EI) 298 ($M+H^+$, 85%), 297 (M, 100), 272 (60), 271 (92) and 254 (60) (Found: $M+H^+$, 298.2017. $C_{16}H_{28}NO_4$ requires 298.2018).

4.1.12. *tert*-Butyl *exo*−6,7-epoxy-3α-(methoxymethyloxy)-2α,4α-dimethyl-8-azabicyclo[3.2.1]octane-8-carboxylate 9b. A solution of *m*CPBA (70%, 0.87 g, 3.5 mmol) in CH_2Cl_2 (20 mL) was added dropwise to a stirred, cold (0–5 °C) solution of MOM ether **20b** (0.698 g, 2.35 mmol) in CH_2Cl_2 (20 mL). The mixture was stirred in an ice bath for 2 h and at room temperature for 0.5 h. Then it was diluted with CH_2Cl_2 (20 mL) and washed with sat. aq. $Na_2S_2O_3$ (2×20 mL), sat. aq. $NaHCO_3$ (2×15 mL), brine (20 mL) and dried ($MgSO_4$). After evaporation of CH_2Cl_2 the product was purified by column chromatography (light petroleum/Et_2O 3:1) to give epoxide **9b** (0.67 g, 91%) as a white solid; R_f 0.42 (light petroleum/Et_2O 1:1); mp 66–67 °C; ν_{max}/cm^{-1} 2976s, 2934s, 1699s, 1456m, 1367s, 1287m, 1265m, 1168s, 1126m, 1090m, 1034s, 924m, 902w, 879w and 846m; δ_H (400 MHz) (two rotamers) 4.52 (2H, s, CH_2), 4.12, 3.95 (2H, two d, J=2.7 Hz, 1,5-H), 3.50 (1H, t, J=4.0 Hz, 3-H), 3.41, 3.39 (2H, AB, J=3.3 Hz, 6,7-H), 3.34 (3H, s, OMe), 2.16–2.03 (2H, m, 2,4-H), 1.40 (9H, s, $OCMe_3$), and 1.04, 1.03 (6H, two d, J=7.4 Hz, 2,4-Me); δ_C (400 MHz) 156.2 (C=O), 99.1 (CH_2), 80.0 (C3), 79.7 ($OCMe_3$), 58.5, 57.1 (C1, C5), 56.4 (OMe), 52.3, 51.9 (C6, C7), 38.4, 38.1 (C2, C4), 28.3 ($OCMe_3$), 13.9, 13.8 (2,4-Me); m/z (EI) 314 ($M+H^+$, 20%), 313 (M, 100) and 268 (100); m/z [CI+(NH_3)] 314 ($M+H^+$, 27%), 298 (45), 275 (95), 214 (100), 198 (95) and 170 (35) (Found: $M+H^+$, 314.1970. $C_{16}H_{28}NO_5$ requires 314.1967).

4.1.13. *tert*-Butyl 3α-methoxy-2α,4α-dimethyl-8-azabicyclo[3.2.1]oct-6-ene-8-carboxylate 20c. KH (0.023 g, 0.57 mmol) in oil was transferred to a dry flask, washed with dry pentane and dried under argon, after which THF (4 mL) and 18-crown-6 (2 mg, 7.6×10^{-3} mmol) was added. The mixture was cooled to 0 °C, then alcohol **19** (0.118 g, 0.47 mmol) was added and the mixture was stirred at 0 °C for 15 min, during which time it turned red. Then MeI (0.1 mL, 1.6 mmol) was added and the mixture was stirred at room temperature for 14 h. The milky cream mixture was diluted with THF (10 mL), quenched carefully with sat. aq. NH_4Cl (10 mL), extracted with CH_2Cl_2 (3×15 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Column chromatography (light petroleum/EtOAc 4:1) afforded methyl ether **20c** (102 mg, 82%) as a colourless

oil; R_f 0.59 (light petroleum/EtOAc 4:1); ν_{max}/cm^{-1} 2973s, 2931m, 1698s, 1407s, 1367m, 1291m, 1181m, 1090s, 938w, 916w, 898w and 875w; δ_H (400 MHz) (two rotamers) 6.25–6.15 (2H, m, 6,7-H), 4.17, 4.08 (2H, two br s, 1,5-H), 3.27 (3H, s, OMe), 3.15 (1H, t, J=5.0 Hz, 3-H), 2.28–2.17, 2.17–2.06 (2H, two m, 2,4-H), 1.43 (9H, s, $OCMe_3$) and 0.97 (6H, d, J=7.3 Hz, 2,4-Me); δ_C (100 MHz) 152.0 (C=O), 134.1, 133.6 (C6, C7), 83.3 (C3), 79.2 ($OCMe_3$), 63.0 (OMe), 61.9, 61.1 (C1, C5), 38.7, 37.7 (C2, C4), 28.5 ($OCMe_3$), 13.8, 13.7 (2,4-Me); m/z [CI+(NH_3)] 268 ($M+H^+$, 40%), 170 (35), 168 (100), 152 (22) and 94 (24) (Found: $M+H^+$, 268.1913. $C_{15}H_{26}NO_3$ requires 268.1912).

4.1.14. *tert*-Butyl *exo*−6,7-epoxy-3α-methoxy-2α,4α-dimethyl-8-azabicyclo[3.2.1]octane-8-carboxylate 9c. A solution of *m*CPBA (70%, 0.61 g, 2.5 mmol) in CH_2Cl_2 (15 mL) was added dropwise to a stirred, cold (0–5 °C) solution of methyl ether **20c** (0.44 g, 1.65 mmol) in CH_2Cl_2 (15 mL). The mixture was stirred in an ice bath for 2 h and left stirring for 14 h, during which time it warmed up to room temperature. Then the reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with sat. aq. $Na_2S_2O_3$ (2×15 mL), sat. Aq. $NaHCO_3$ (2×15 mL), brine (20 mL) and dried ($MgSO_4$). After evaporation of CH_2Cl_2 the residue was purified by column chromatography (light petroleum/Et_2O 3:1) to give epoxide **9c** (0.427 g, 92%) as a white solid; R_f 0.42 (light petroleum/Et_2O 1:1); mp 95–97 °C; ν_{max}(KBr)/cm^{-1} 2978m, 2934m, 1701s, 1457m, 1368s, 1265m, 1188m, 1165s, 1127m, 1192s, 988m, 914m, 881m and 849m; δ_H (400 MHz) (two rotamers) 4.09, 3.92 (2H, two d, J=3.0 Hz, 1,5-H), 3.39, 3.37 (2H, AB, J=3.4 Hz, 6,7-H), 3.32 (3H, s, OMe), 3.08 (1H, t, J=4.0 Hz, 3-H), 2.10–2.00 (2H, m, 2,4-H), 1.40 (9H, s, $OCMe_3$), 1.05, 1.04 (6H, two d, J=7.4 Hz, 2,4-Me); δ_C (100 MHz) 156.0 (C=O), 82.2 (C3), 79.6 ($OCMe_3$), 62.2 (OMe), 58.4, 57.1 (C1, C5), 52.4, 52.1 (C6, C7), 38.8, 38.5 (C2, C4), 28.3 ($OCMe_3$) and 13.2, 13.1 (2,4-Me); m/z [CI+(NH_3)] 284 ($M+H^+$, 100%) and 281 (15) (Found: $M+H^+$, 284.1859. $C_{15}H_{26}NO_4$ requires 284.1862).

4.1.15. General procedure for the reaction of RLi with epoxides 7, 8 and 9a–c. Distilled ligand (3.5 equiv.) was added to a stirred solution of RLi (3.5 equiv.), or to a solution of mixed organolithiums [Pr^iLi (1.1 equiv.) and $TMSCH_2Li$ (2.5 equiv.), Table 4, entries 8, 9, 13 and 14] in Et_2O (1 mL) at −78 °C under argon and stirred for 1 h. Then epoxide **7**, **8** or **9** (100 mg) in Et_2O (2.5 mL) was added dropwise over 15 min. The reaction mixture was stirred at −78 °C for 5 h and then allowed to gradually warm to the indicated temperature over 14 h. Then H_3PO_4 (0.5 mol dm^{-3}, 5 mL) when (−)-sparteine was used, or HCl (1 mol dm^{-3}, 5 mL) when bisoxazoline **11** was used was added and the aqueous layer was extracted with Et_2O (3×20 mL). The organic extracts were combined, washed with saturated $NaHCO_3$ (10 mL) and brine (10 mL), dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residue was purified by column chromatography (light petroleum/EtOAc 4:1, then 1:1).

4.1.16. *tert*-Butyl [(1*R,2*S**,6*S**)-3-butyl-6-(*tert*-butyldimethylsilyloxy)-2-hydroxycyclohept-3-enyl]carbamate 21 (R=Bu).** Colourless oil; R_f 0.88 (light petroleum/EtOAc 1:1); ν_{max}/cm^{-1} 3440s, br, 2929s, 2858s, 1694s, 1502s, 1472s, 1366s, 1253s, 1171s, 1081s, 910s, 836s, 775s and 735m; δ_H (400 MHz) 5.43 (1H, t, J=6.8 Hz, 4-H), 4.74 (1H, d, J=7.5 Hz, NH), 4.45 (1H, br s, 2-H), 4.01–3.93 (1H, m, 1-H), 3.74–3.64 (1H, m, 6-H), 3.32 (1H, br s, OH), 2.38–2.20 (2H, m, 5-CH_2), 2.20–2.08 (2H, m, 7-H and 1′-H), 2.07–1.93 (1H, m, 1′-H), 1.92–1.82 (1H, m, 7-H), 1.43 (9H, s, $OCMe_3$), 1.42–1.20 (6H, m, 3×CH_2), 0.89 (3H, t, J=8.2 Hz, $MeCH_2$), 0.85 (9H, s, $SiCMe_3$) and 0.03 (6H, s, $SiMe_2$); δ_C (100 MHz) 156.7 (C=O), 144.9 (C3), 120.6 (C4), 79.9 ($OCMe_3$), 77.2 (C2), 75.5 (C6), 53.0 (C1), 41.5 (C7), 36.5 (C5), 34.9 (C1′), 30.7 (C2′), 28.3 ($OCMe_3$), 25.8 ($SiCMe_3$), 22.5 (C3′), 18.0 ($SiCMe_3$), 14.0 ($MeCH_2$) and −4.8 ($SiMe_2$); m/z (EI) 414 ($M+H^+$, 10%), 131 (M, 15), 357 (100) and 342 (50); m/z [CI+(NH_3)] 414 ($M+H^+$, 100%), 357 (38), 340 (35), 296 (25), 164 (28), 149 (82) and 92 (22) (Found: $M+H^+$, 414.3032. $C_{22}H_{44}NO_4Si$ requires 414.3039). The ee was determined by chiral HPLC (t_R 8.0 and 12.4) following derivatisation as the 3,5-dinitrobenzoate.

4.1.17. *tert*-Butyl [(1*R,2*S**,6*S**) 6-(*tert*-butyldimethylsilyloxy)-2-hydroxy-3-isopropylcyclohept-3-enyl]carbamate 21 (R=Pr^i).** Colourless oil; R_f 0.83 (light petroleum/EtOAc 1:1); ν_{max}/cm^{-1} 3401s, br, 2957s, 2929s, 2857s, 1693s, 1503w, 1472w, 1366m, 1255m, 1172m, 1084m, 910m, 837m and 773m; δ_H (400 MHz) 5.42 (1H, t, J=6.6 Hz, 4-H), 4.80 (1H, d, J=8.3 Hz, NH), 4.37 (1H, br s, 2-H), 3.90–3.80 (2H, m, 1-H and 6-H), 2.85 (1H, br s, OH), 2.32–2.5 (2H, m, 5-H and C*H*Me_2), 2.15–2.25 (2H, m, 5-H and 7-H), 1.80–1.86 (1H, m, 7-H), 1.43 (9H, s, $OC(CH_3)_3$), 1.02 (6H, d, J=6.6 Hz, CHMe_2), 0.85 (9H, s, $SiCMe_3$) and 0.02 (6H, s, $SiMe_2$); δ_C (100 MHz) 156.1 (C=O), 149.2 (C3), 120.3 (C4), 79.6 (C2), 75.1 ($OCMe_3$), 67.1 (C6), 51.0 (C1), 40.3 (C7), 35.1 (C5), 34.0 ($CHMe_2$), 28.4 ($OCMe_3$), 25.7 ($SiCMe_3$), 21.7 ($CHMe_2$), 18.0 ($SiCMe_3$) and −4.9 ($SiMe_2$); m/z (EI) 400 ($M+H^+$, 25%), 399 (M, 85), 381 (70) and 355 (100); m/z [CI+(NH_3)] 400 ($M+H^+$, 68%), 196 (42), 135 (100), 94 (93), 84 (62) and 72 (97) (Found: $M+H^+$, 400.2882. $C_{21}H_{42}NO_4Si$ requires 400.2883). The ee was determined by chiral HPLC (t_R 8.3 and 12.6) following derivatisation as the 3,5-dinitrobenzoate.

4.1.18. *tert*-Butyl 3-*endo*−3,6-*trans*−3-(*tert*-butyldimethylsilyloxy)-6-hydroxy-8-azatricyclo[3.2.1.0^{2,7}]-octane-8-carboxylate 22. White solid; R_f 0.53 (light petroleum/EtOAc 1:1); mp 68–71 °C; ν_{max}/cm^{-1} 3387s, br, 2958s, 2930s, 2857s, 1698s, 1434m, 1366m, 1252m, 1170m, 1077s, 836m and 775m; δ_H (400 MHz) 4.18–4.12 (2H, m, 6-H and 3-H), 3.86 (1H, br s, 5-H), 3.82–3.76 (1H, m, 1-H), 2.18–2.08 (1H, m, 4-H_{exo}), 1.98 (1H, br s, OH), 1.87–1.81 (1H, m, 2-H), 1.45 (9H, s, $OCMe_3$), 1.43–1.27 (2H, m, 4-H_{endo} and 7-H), 0.85 (9H, s, $SiCMe_3$), 0.03 and 0.02 (6H, 2s, 2×SiMe); δ_C (100 MHz) 155.2 (C=O), 80.0 ($OCMe_3$), 71.3 (C6), 63.2 (C3), 57.6 (C5), 37.2 (C4), 34.6 (C1), 28.4 ($OCMe_3$), 25.8 ($SiCMe_3$), 23.4 (C7), 23.0 (C2), 18.0 ($SiCMe_3$), −4.6 and −4.8 (2×SiMe); m/z (EI) 356 ($M+H^+$, 12%), 355 (M, 30), 299 (80), 282 (100); m/z [CI+(NH_3)] 373 ($M+NH_4^+$, 22%), 356 ($M+H^+$, 95), 317 (100) and 300 (30) (Found: $M+H^+$, 356.2247. $C_{18}H_{34}NO_4Si$ requires 356.2257). The ee was determined by chiral HPLC (t_R 18.7 and 21.6) following derivatisation as the 3,5-dinitrobenzoate.

4.1.19. *tert*-Butyl [(1*R,2*S**,6*R**)-3-butyl-6-(*tert*-butyldimethylsilyloxy)-2-hydroxycyclohept-3-enyl]carbamate 23 (R=Bu).** Colourless oil; R_f 0.88 (light petroleum/EtOAc 1:1); ν_{max}/cm^{-1} 3404s, 2956s, 2930s, 2859s, 1685s, 1506s, 1366s, 1253s, 1173s, 1085m, 837s and 776s; δ_H (400 MHz) 6.04–5.98 (1H, m, NH), 5.32–5.28 (1H, m, 4-H), 4.32–4.22 (2H, m, 2-H and OH), 4.05–3.99 (1H, m, 6-H), 3.99–3.90 (1H, m, 1-H), 2.42–2.32 (1H, m, one of 5-CH_2), 2.28–2.18 (1H, m, one of 5-CH_2), 2.20–1.90 (4H, m, 7-H and 1′-H), 1.43 (9H, s, $OCMe_3$), 1.44–1.20 (4H, m, 2′-CH_2 and 3′-CH_2), 1.92–1.82 (15H, m, $SiCMe_3$ and CH_2Me) and 0.06 (6H, s, $SiMe_2$); δ_C (100 MHz) 157.0 (C=O), 145.2 (C3), 118.1 (C4), 79.2 ($OCMe_3$), 76.8 (C2), 67.3 (C6), 53.2 (C1), 39.5 (C7), 34.7 (C1′), 33.8 (C5), 30.6 (C2′), 28.3 ($OCMe_3$), 25.6 ($SiCMe_3$), 22.5 (C3′), 17.8 ($SiCMe_3$), 14.0 (CH_2Me) and −4.9 ($SiMe_2$); *m/z* (EI) 414 ($M+H^+$, 20%), 358 (35), 340 (100), 322 (35) and 313 (100); *m/z* [CI+(NH_3)] 414 ($M+H^+$, 100%), 358 (65), 314 (20), 164 (20) and 149 (38) (Found: $M+H^+$, 414.3036. $C_{22}H_{44}NO_4Si$ requires 414.3039). The ee was determined by chiral HPLC (t_R 9.4 and 16.4) following derivatisation as the 3,5-dinitrobenzoate.

4.1.20. *tert*-Butyl [(1*R,2*S**,6*R**) 6-(*tert*-butyldimethylsilyloxy)-2-hydroxy-3-isopropylcyclohept-3-enyl]carbamate 23 (R=Pr^i).** Colourless oil; R_f 0.58 (light petroleum/EtOAc 4:1); ν_{max}/cm^{-1} 3403m, 2957s, 2931s, 2859m, 1715s, 1507s, 1366m, 1253m, 1173m, 1085m, 837s and 776m; δ_H (400 MHz) 5.77–5.65 (1H, m, NH), 5.30 (1H, dd, *J*=7.6 Hz, J_2=4.8 Hz, 4-H), 4.34–4.26 (1H, m, 2-H), 4.05 (1H, br s, OH), 4.02–3.96 (1H, m, 6-H), 3.94–3.85 (1H, m, 1-H), 2.48–2.38 (2H, m, C*H*Me_2 and 5-H), 2.21 (1H, dd, J_{gem}=15.7 Hz, J_2=4.8 Hz, 5-H), 2.11–2.01 (1H, m, 7-H), 1.98–1.86 (1H, m, 7-H), 1.43 (9H, s, $OCMe_3$), 1.06 and 1.04 (6H, two d, *J*=6.6 Hz, Me_2CH), 0.89 (9H, s, $SiCMe_3$) and 0.07 (6H, s, $SiMe_2$); δ_C (100 MHz) 156.4 (C=O), 150.4 (C3), 117.0 (C4), 79.2 ($OCMe_3$), 76.0 (C2), 67.5 (C6), 53.0 (C1), 39.6 (C7), 33.9 (C5), 32.8 ($CHMe_2$), 28.3 ($OCMe_3$), 25.7 ($SiCMe_3$), 23.3 (CH*Me*), 22.0 (CH*Me*), 17.8 ($SiCMe_3$) and − 4.9 ($SiMe_2$); *m/z* (EI) 400 ($M+H^+$, 25%), 344 (35), 326 (100), 308 (30) and 299 (85); *m/z* [CI+(NH_3) 400 ($M+H^+$, 100%), 344 (32), 256 (35), 240 (25) and 135 (25) (Found: $M+H^+$, 400.2877. $C_{21}H_{42}NO_4Si$ requires 400.2883). The ee was determined by chiral HPLC (t_R 8.6 and 13.7) following derivatisation as the 3,5-dinitrobenzoate.

4.1.21. *tert*-Butyl [(1*R,2*S**,5*S**,6*S**,7*R**)-3-butyl-6-(*tert*-butyldimethylsilyloxy)-5,7-dimethyl-2-hydroxycyclohept-3-enyl]carbamate 24a (R=Bu).** White solid; R_f 0.81 (light petroleum/EtOAc 1:1); mp 108–110 °C; ν_{max}(KBr)/cm^{-1} 3443m, 3335m, 2962s, 2928s, 2857s, 1687s, 1664s, 1549m, 1507m, 1367m, 1310m, 1250m, 1175m, 1056m, 1032s, 870m, 836m and 772m; δ_H (400 MHz) (rotamers 3:1) 5.03 (1H, d, *J*=4.3 Hz, 4-H), 4.94 (1H, d, *J*=9.2 Hz, NH), 4.17, 4.03 (1H, 2s, 2-H), 3.72–3.59 (1H, m, 6-H), 3.49 (1H, t, *J*=9.7 Hz, 1-H), 3.02 (1H, br s, OH), 2.95–2.80 (1H, m, 5-H), 2.50–2.35 (1H, m, 7-H), 2.03 (2H, t, *J*=7.2 Hz, 1′-H_2), 1.42 (9H, s, $OCMe_3$), 1.40–1.20 (4H, m, 2′-H_2 and 3′-H_2), 1.05 (3H, d, *J*=7.5 Hz, 5-Me), 1.01 (3H, d, *J*=6.9 Hz, 7-Me), 0.93–0.82 (12H, m, $SiCMe_3$ and CH_2Me) and 0.06 (6H, s, $SiMe_2$); δ_C (50 MHz) 156.2 (C=O), 140.4 (C3), 131.1 (C4), 79.9 (C6), 79.1 ($OCMe_3$), 77.1 (C2), 54.0 (C1), 40.2 (C7), 38.2 (C1′), 37.6 (C5), 30.5 (C2′), 28.4 ($OCMe_3$), 26.2 ($SiCMe_3$), 22.5 (C3′), 20.9 (5-Me), 18.8 (7-Me), 18.6 ($SiCMe_3$), 14.0 (CH_2Me), −3.9 and −3.4 ($SiMe_2$); *m/z* [CI+(NH_3)] 442 ($M+H^+$, 100%), 424 (38), 368 (65), 358 (50), 342 (45), 324 (60) and 309 (50) (Found: $M+H^+$, 442.3344. $C_{24}H_{48}NO_4Si$ requires 442.3352). The ee was determined by chiral HPLC (t_R 8.2 and 13.5) following derivatisation as the 3,5-dinitrobenzoate.

4.1.22. (1*R,4*R**,5*R**,6*S**,7*S**)-2-Butyl-5-(*tert*-butyldimethylsilyloxy)-4,6-dimethyl-8-aza-10-oxabicyclo-[5.3.0]dec-2-en-9-one 25a (R=Bu^n, Fig. 4).** White solid; R_f 0.63 (light petroleum/EtOAc 1:1); ν_{max}/cm^{-1} 3212w, 2956m, 2929m, 2857w, 1747s, 1462w, 1384w, 1242w, 1055w, 1020m, 833m and 773m; δ_H (500 MHz) 5.90 (1H, s, NH), 5.27 (1H, d, *J*=7.9 Hz, 1-H), 5.22–5.19 (1H, m, 3-H), 3.90 (1H, dd, *J*=7.9, 8.5 Hz, 7-H), 3.67 (1H, d, *J*=3.5 Hz, 5-H), 2.40–2.30 (2H, m, 4-H and 1′-H), 2.05–1.90 (2H, m, 6-H and 1′-H), 1.50–1.25 (4H, m, 2′-H_2 and 3′-H_2), 1.10 (3H, d, *J*=7.0 Hz, 6-Me), 1.00 (3H, d, *J*=7.3 Hz, 4-Me), 0.89 (9H, s, CMe_3), 0.89 (3H, t, *J*=7.1 Hz, CH_2Me), 0.09 and 0.05 (6H, two s, $SiMe_2$); δ_C (125 MHz) 159.3 (C=O), 133.3 (C2), 128.0 (C3), 79.0 (C1), 76.6 (C5), 56.0 (C7), 42.6 (C4), 40.0 (C6), 36.7 (C1′), 31.1 (C2′), 26.1 ($SiCMe_3$), 22.1 (C3′), 19.1 (Me), 19.0 (Me), 18.3 ($SiCMe_3$), 13.9 (CH_2Me), −3.4 and −4.5 ($SiMe_2$); *m/z* [CI+(NH_3)] 368 ($M+H^+$, 75%), 324 (25), 192 (40), 177 (42), 132 (61), 124 (76), 112 (83), 110 (100), 98 (72), 96 (68), 84 (45) and 72 (38) (Found: $M+H^+$, 368.2614. $C_{20}H_{38}NO_3Si$ requires 368.2615).

A crystal of compound **25a** was mounted on a glass fibre using perfluoropolyether oil and cooled rapidly to 150 K in a stream of cold N_2. X-ray diffraction data were measured using a Nonius KappaCCD diffractometer (graphite-monochromated Mo K_α radiation).

Crystal data.‡ $C_{20}H_{37}NO_3Si$, *M*=367.61, monoclinic, *a*=15.9226(3) Å, *b*=8.7866(2) Å, *c*=15. 9212(4) Å, *U*=2207.05(9) Å^3, *T*=150 K, space group $P2_1/a$, *Z*=4, μ(Mo Kα)=0.123 mm^{-1}, 15165 reflections measured, 5321 unique (R_{int}=0.028), 3563 observed with $I>3\,3\sigma(I)$ which were used in all calculations. The final *wR*(*F*) was 0.0630 (all observed data).

4.1.23. *tert*-Butyl [(1*R,2*S**,5S*,6*S**,7*R**)-6-(*tert*-butyldimethylsilyloxy)-2-hydroxy-3-isopropyl-5,7-dimethylcyclohept-3-enyl]carbamate 24a (R=Pr^i).** Colourless oil; R_f 0.79 (light petroleum/EtOAc 1:1); ν_{max}/cm^{-1} 3396s, br, 2960s, 2930s, 2858s, 1720s, 1694s, 1500w, 1463m, 1390m, 1366s, 1256s, 1172m, 1120m, 1034m, 874w, 836w and 772m; δ_H (400 MHz) 5.00 (1H, d, *J*=4.2 Hz, 4-H), 4.94 (1H, d, *J*=8.7 Hz, NH), 4.18 (1H, s, 2-H), 3.68–3.58 (1H, m, 6-H), 3.47 (1H, t, *J*=9.7 Hz, 1-H), 3.05–2.95 (1H, m, 5-H), 2.90 (1H, s, OH), 2.50–2.40 (1H, m, 7-H), 2.29 (1H, sept., *J*=6.7 Hz, C*H*Me_2), 1.42 (9H, s, $OCMe_3$), 1.06 (3H, d, *J*=7.5 Hz, 5-Me), 1.02–0.95 (9H, m, 7-Me and $CHMe_2$),

‡ Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary pubilication numbers CCDC 217328. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

0.86 (9H, s, $SiCMe_3$) and 0.05 (6H, s, $SiMe_2$); δ_C (100 MHz) 156.2 (C=O), 145.6 (C3), 129.2 (C4), 80.6 (C6), 79.1 (C2), 76.1 (O*C*Me_3), 53.8 (C1), 39.9 (C7), 36.8 (C5), 35.9 (*C*HMe_2), 28.4 (OC*Me*$_3$), 26.2 (Si*C*Me_3), 21.4 (5-Me), 21.2 (7-Me), 19.1 (Si*C*Me_3), 18.7 (CH*Me*$_2$), −3.3 and −3.9 ($SiMe_2$); *m/z* (EI) 428 ($M+H^+$, 20%), 427 (M, 100) and 409 (90); *m/z* [CI+(NH_3)] 428 ($M+H^+$, 100%), 410 (25), 354 (40), 295 (25), 163 (67), 132 (35), 91 (25) and 72 (32) (Found: $M+H^+$, 428.3196. $C_{23}H_{46}NO_4Si$ requires 428.3196). The ee was determined by chiral HPLC (t_R 8.8 and 13.6) following derivatisation as the 3,5-dinitrobenzoate.

4.1.24. (1*R,4*R**,5*R**,6*S**,7*S**)-5-(*tert*-Butyldimethylsilyloxy)-2-isopropyl-4,6-dimethyl-8-aza-10-oxabicyclo[5.3.0]dec-2-en-9-one 25a (R=Pr^i).** White solid; R_f 0.08 (light petroleum/EtOAc 4:1); ν_{max}(KBr)/cm^{-1} 3247w, 2960s, 2929m, 1764s, 1737m, 1460w, 1380w, 1253m, 1017m, 836m and 774m; δ_H (500 MHz) 6.40 (1H, s, NH), 5.23 (1H, d, *J*=7.8 Hz, 1-H), 5.22–5.19 (1H, m, 3-H), 3.90 (1H, dd, *J*=7.9, 8.4 Hz, 7-H), 2.58 (1H, sept., *J*=7.1 Hz, C*H*Me_2), 2.50–2.40 (1H, m, 4-H), 2.00–1.90 (1H, m, 6-H), 1.09 (3H, d, *J*=7.0 Hz, 6-Me), 1.07–1.00 (9H, m, 4-Me and *Me*$_2$CH), 0.87 (9H, s, CMe_3), 0.07 and 0.04 (6H, two s, 2×SiMe); δ_C (125 MHz) 159.8 (C=O), 138.8 (C2), 126.3 (C3), 79.4 (C1), 76.6 (C5), 59.5 (C7), 41.6 (C4), 40.3 (C6), 32.5 (*C*HMe_2), 26.0 (Si*C*Me_3), 23.2 (*Me*CH), 21.9 (*Me*CH), 19.4 (4-Me), 18.5 (6-Me), 18.3 (*C*Me_3), −3.5 and −4.5 (2×SiMe); *m/z* [CI+(NH_3)] 354 ($M+H^+$, 100%), 310 (85), 178 (60), 163 (75), 136 (83), 122 (78), 121 (58), 91 (59) and 75 (62) (Found: $M+H^+$, 354.2959. $C_{19}H_{34}NO_3Si$ requires 428.3196).

4.1.25. *tert*-Butyl [(1*R,2*S**,5*S**,6*S**,7*R**)-2-hydroxy-3-isopropyl-6-(methoxymethyloxyoxy)-5,7-dimethylcyclohept-3-enyl]carbamate 24b (R=Pr^i).** Colourless oil; R_f 0.48 (light petroleum/EtOAc 1:1); ν_{max}/cm^{-1} 3396s, br, 2962s, 1693s, 1503m, 1455w, 1367m, 1250w, 1171s, 1103m, 1040s, 925w and 874w; δ_H (400 MHz) 5.10 (1H, d, *J*=4.2 Hz, 4-H), 5.00 (1H, d, *J*=9.2 Hz, NH), 4.59 and 4.66 (2H, AB, *J*=6.8 Hz, OCH_2O), 4.19–4.11 (1H, m, 2-H), 3.60–3.50 (1H, m, 1-H), 3.45–3.38 (1H, m, 6-H), 3.33 (3H, s, OMe), 3.05–2.94 (1H, m, 5-H), 2.71 (1H, br s, OH), 2.50–2.40 (1H, m, 7-H), 2.28 (1H, sept., *J*=6.8 Hz, C*H*Me_2), 1.43 (9H, s, $OCMe_3$), 1.13 (3H, d, *J*=7.4 Hz, 5-Me) and 1.09–1.00 (9H, m, 7-Me and CH*Me*$_2$); δ_C (100 MHz) 156.1 (C=O), 146.0 (C3), 129.0 (C4), 98.5 (OCH_2O), 87.7 (C6), 79.2 (O*C*Me_3), 76.0 (C2), 57.1 (OMe), 54.0 (C1), 39.5 (C7), 38.1 (*C*HMe_2), 35.9 (C5), 28.4 (OC*Me*$_3$), 21.3 (Me), 21.1 (Me), 20.8 (Me) and 17.9 (Me); *m/z* [CI+(NH_3)] 358 ($M+H^+$, 100%), 319 (40), 284 (45), 275 (35) and 214 (30); *m/z* (EI) 358 ($M+H^+$, 100%), 257 (M, 40) and 313 (90) (Found: $M+H^+$, 358.2593. $C_{19}H_{36}NO_5$ requires 358.2588). The ee was determined by chiral HPLC (t_R 11.6 and 16.1) following derivatisation as the 3,5-dinitrobenzoate.

4.1.26. *tert*-Butyl [(1*R,2*S**,5*S**,6*S**,7*R**)-2-hydroxy-6-(methoxymethyloxyoxy)-5,7-dimethyl-3-(trimethylsilylmethyl)cyclohept-3-enyl]carbamate 24b (R=CH_2TMS).** Colourless oil; R_f 0.74 (light petroleum/EtOAc 1:1); ν_{max}/cm^{-1} 3391s, br, 2958s, 1682s, 1504m, 1367m, 1249m, 1172s, 1043s and 861s; δ_H (400 MHz) 4.98 (1H, br d, *J*=9.3 Hz, NH), 4.94 (1H, d, *J*=4.4 Hz, 4-H), 4.66 and 4.63 (2H, AB, *J*=6.8 Hz, OCH_2O), 4.05 (1H, br s, 2-H), 3.67 (1H, t, *J*=9.9 Hz, 1-H), 3.48–3.42 (1H, m, 6-H), 3.36 (3H, s, OMe), 2.94–2.83 (1H, m, 5-H), 2.73 (1H, s, OH), 2.43–2.33 (1H, m, 7-H), 1.56 (2H, s, CH_2Si), 1.43 (9H, s, $OCMe_3$), 1.10 (3H, d, *J*=7.4 Hz, 5-Me), 1.06 (3H, d, *J*=7.0 Hz, 7-Me) and 1.02 (9H, s, $SiMe_3$); δ_C (100 MHz) 156.0 (C=O), 138.4 (C3), 128.8 (C4), 98.3 (OCH_2O), 86.9 (C6), 79.2 (O*C*Me_3), 78.5 (C2), 55.6 (OMe), 53.7 (C1), 39.8 (C7), 36.7 (C5), 29.4 (CH_2Si), 28.4 (OC*Me*$_3$), 20.8 (5-Me), 17.7 (7-Me) and −1.46 ($SiMe_3$); *m/z* [CI+(NH_3)] 402 ($M+H^+$, 28%), 346 (60), 302 (100) and 90 (22) (Found: $M+H^+$, 402.2673. $C_{20}H_{40}NO_5Si$ requires 402.2676). The ee was determined by chiral HPLC (t_R 10.8 and 13.2) following derivatisation as the 3,5-dinitrobenzoate.

4.1.27. (1*R,4*R**,5*R**,6*S**,7*S**)-5-(Methoxymethyloxy)-4,6-dimethyl-2-(trimethylsilylmethyl)-8-aza-10-oxabicyclo[5.3.0]dec-2-en-9-one 25b (R=CH_2TMS).** Colourless oil; R_f 0.32 (light petroleum/EtOAc 1:1); ν_{max}/cm^{-1} 3245m, br, 2956s, 1756s, 1383w, 1247m, 1151m, 1092m, 1034s and 924w; δ_H (400 MHz) 6.58–6.38 (1H, m, NH), 5.15 (1H, d, *J*=7.9 Hz, 1-H), 5.08–5.00 (1H, m, 3-H), 4.64, 4.61 (2H, AB, *J*=6.6 Hz, OCH_2O), 3.96 (1H, dd, *J*=7.9, 8.6 Hz, 7-H), 3.50 (1H, d, *J*=6.8 Hz, 5-H), 3.39 (3H, s, OMe), 2.50–2.39 (1H, m, 4-H), 2.05–1.95 (1H, m, 6-H), 1.91 (1H, d, *J*=14.4 Hz, one of CH_2Si), 1.46 (1H, d, *J*=14.4 Hz, one of CH_2Si), 1.17 (3H, d, *J*=7.2 Hz, 6-Me), 1.03 (3H, d, *J*=7.2 Hz, 4-Me) and 0.02 (9H, s, $SiMe_3$); δ_C (100 MHz) 159.6 (C=O), 131.5 (C2), 125.6 (C3), 98.5 (OCH_2O), 83.6 (C5), 79.6 (C1), 60.4 (C7), 56.1 (OMe), 41.6 (C4), 38.8 (C6), 25.7 (CH_2Si), 18.8 (6-Me), 18.5 (4-Me) and −1.4 ($SiMe_3$); *m/z* [CI+(NH_3)] 328 ($M+H^+$, 100%), 284 (20), 153 (20), 123 (48), 109 (30), 90 (55) and 73 (50) (Found: $M+H^+$, 328.1936. $C_{16}H_{30}NO_4Si$ requires 328.1936).

4.1.28. *tert*-Butyl [(1*R,2*S**,5*S**,6*S**,7*R**)-2-hydroxy-3-isopropyl-6-methoxy-5,7-dimethylcyclohept-3-enyl]-carbamate 24c (R=Pr^i).** White solid; R_f 0.54 (light petroleum/EtOAc 1:1); mp 91–93 °C; ν_{max}(KBr)/cm^{-1} 3481s, 3372s, 2958s, 2875m, 1665s, 1533s, 1370m, 1315m, 1270m, 1174s, 1089s, 918w, 872w; δ_H (400 MHz) 5.10 (1H, d, *J*=3.7 Hz, 4-H), 4.98 (1H, br d, *J*=8.6 Hz, NH), 4.18 (1H, br s, 2-H), 3.47 (4H, 1-H and OMe), 3.20 (1H, br s, 6-H), 3.02–2.91 (1H, m, 5-H), 2.89 (1H, br s, OH), 2.59–2.46 (1H, m, 7-H), 2.30 (1H, sept., *J*=6.8 Hz, C*H*Me_2), 1.44 (9H, s, $OCMe_3$), 1.17 (3H, d, *J*=7.5 Hz, 5-Me), 1.09 (3H, d, *J*=6.9 Hz, 7-Me) and 1.03 (6sH, d, *J*=6.8 Hz, CH*Me*$_2$); δ_C (100 MHz) 156.3 (C=O), 146.2 (C3), 128.9 (C4), 89.3 (C6), 79.3 (O*C*Me_3), 75.7 (C2), 62.3 (OMe), 54.3 (C1), 39.2 (C7), 36.4 (C5), 36.0 (*C*HMe_2), 28.4 (OC*Me*$_3$), 21.5 (5-Me), 21.5 (1′-Me), 21.3 (1′-Me) and 18.1 (7-Me); *m/z* (EI) 328 ($M+H^+$, 2%), 227 (30), 210 (40), 178 (50), 136 (45), 123 (100), 100 (97), 97 (70) and 72 (35); *m/z* [CI+(NH_3)] 328 ($M+H^+$, 100%), 271 (30) and 254 (50) (Found: $M+H^+$, 328.2488. $C_{18}H_{34}NO_4$ requires 328.2488). The ee was determined by chiral HPLC (t_R 10.9 and 16.5) following derivatisation as the 3,5-dinitrobenzoate.

4.1.29. *tert*-Butyl [(1*R,2*S**,5*S**,6*S**,7*R**)-2-hydroxy-6-methoxy-5,7-dimethyl-3-(trimethylsilylmethyl)cyclohept-3-enyl]carbamate 24c (R=CH_2TMS).** Colourless

oil; R_f 0.76 (light petroleum/EtOAc 1:1); ν_{max}/cm^{-1} 3396s, br, 2963s, 1682s, 1504s, 1367s, 1249s, 1172s, 1095s and 847s; δ_H (400 MHz) 4.98–4.90 (2H, m, 4-H and NH), 4.05 (1H, br s, 2-H), 3.60 (1H, t, J=9.7 Hz, 1-H), 3.46 (3H, s, OMe), 3.20 (1H, br s, 6-H), 2.95–2.82 (1H, m, 5-H), 2.80 (1H, br s, OH), 2.49–2.36 (1H, m, 7-H), 1.59 and 1.54 (2H, AB, J=13.5 Hz, CH_2Si), 1.43 (9H, s, $OCMe_3$), 1.13 (3H, d, J=7.5 Hz, 5-Me), 1.10 (3H, d, J=7.1 Hz, 7-Me) and 0.04 (9H, s, $SiMe_3$); δ_C (100 MHz) 156.2 (C=O), 138.4 (C3), 128.5 (C4), 88.5 (C6), 79.2 ($OCMe_3$), 78.5 (C2), 61.8 (OMe), 54.1 (C1), 39.6 (C7), 37.1 (C5), 29.3 ($SiCH_2$), 28.4 ($OCMe_3$), 21.3 (5-Me), 17.9 (7-Me) and −1.5 ($SiMe_3$); m/z [CI+(NH_3)] 372 ($M+H^+$, 30%), 316 (100), 272 (90), 96 (83) and 79 (42) (Found: $M+H^+$, 372.2572. $C_{19}H_{38}NO_4Si$ requires 372.2570). The ee was determined by chiral HPLC (t_R 13.2 and 17.5) following derivatisation as the 3,5-dinitrobenzoate.

4.1.30. (1*R,4*R**,5*R**,6*S**,7*S**)-5-(Methyloxy)-4,6-dimethyl-2-(trimethylsilylmethyl)-8-aza-10-oxabicyclo-[5.3.0]dec-2-en-9-one 25c (R=CH_2TMS).** Colourless oil; R_f 0.40 (light petroleum/EtOAc 1:1); ν_{max}/cm^{-1} 3246m, br, 2958m, 1752s, 1382w, 1246m, 1092m, 1018w and 845m; δ_H (400 MHz) 6.40–6.21 (1H, m, NH), 5.13 (1H, d, J=7.9 Hz, 1-H), 5.07–5.00 (1H, m, 3-H), 3.97 (1H, dd, J=7.9, 8.5 Hz, 7-H), 3.48 (3H, s, OMe), 3.18 (1H, d, J=4.2 Hz, 5-H), 2.50–2.39 (1H, m, 4-H), 2.00–1.90 (1H, m, 6-H), 1.88 (1H, d, J=13.0 Hz, one of CH_2Si), 1.45 (1H, d, J=13.0 Hz, one of $SiCH_2$), 1.28 (3H, d, J=6.8 Hz, 6-Me), 1.04 (3H, d, J=6.8 Hz, 4-Me) and 0.02 (9H, s, $SiMe_3$); δ_C (100 MHz) 159.5 (C=O), 131.3 (C2), 125.9 (C3), 86.0 (C5), 79.8 (C1), 61.9 (OMe), 59.8 (C7), 41.5 (C4), 39.2 (C6), 25.8 ($SiCH_2$), 19.9 (6-Me), 18.3 (4-Me) and −1.4 ($SiMe_3$); m/z [CI+(NH_3)] 298 ($M+H^+$, 100%), 254 (21), 164 (11), 132 (12), 90 (11) and 73 (10) (Found: $M+H^+$, 298.1837. $C_{15}H_{28}NO_3Si$ requires 298.1833).

5. Supporting information

Electronic supporting information available: preparation and characterisation of derivatives for ee determinations, tables of specific rotation values and PDB file of the crystal structure of **25a** (R=Bu).

Acknowledgements

We thank European Community for a Marie Curie Fellowship (HPMF-CT-2000-00560 to E.P.) We also thank the EPSRC National Mass Spectrometry Service Centre for mass spectra and Dr A.R. Cowley (Inorganic Chemistry Crystallography Service, University of Oxford) for the X-ray structure analysis.

References and notes

1. (a) Willis, M. C. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1765–1784. (b) Mikami, K.; Yoshida, A. *J. Synth. Org. Chem. Jpn* **2002**, *60*, 732–739.
2. *Topics in Organometallic Chemistry: Organolithiums in Enantioselective Synthesis*; Hodgson, D. M., Ed.; Springer: Berlin, 2003; Vol. 5.
3. For recent reviews, see: (a) Hodgson, D. M.; Gras, E. *Synthesis* **2002**, 1625–1642. (b) Hodgson, D. M.; Tomooka, K.; Gras, E. *Topics in Organometallic Chemistry: Organolithiums in Enantioselective Synthesis*; Hodgson, D. M., Ed.; Springer: Berlin, 2003; Vol. 5, pp 217–250.
4. (a) Crandall, J. K.; Lin, L.-H. C. *J. Am. Chem. Soc.* **1967**, *89*, 4526–4527. (b) Crandall, J. K.; Lin, L.-H. C. *J. Am. Chem. Soc.* **1967**, *89*, 4527–4528. (c) Crandall, J. K.; Apparu, M. *Org. React. (N. Y.)* **1983**, *29*, 345–443.
5. Reviews: (a) Satoh, T. *Chem. Rev.* **1996**, *96*, 3303–3325. (b) Doris, E.; Dechoux, L.; Mioskowski, C. *Synlett* **1998**, 337–343.
6. (a) Hodgson, D. M.; Miles, T. J.; Witherington, J. *Synlett* **2002**, 310–312. (b) Hodgson, D. M.; Miles, T. J.; Witherington, J. *Tetrahedron* **2003**, *59*, 9729–9742.
7. (a) Bergmeier, S. C. *Tetrahedron* **2000**, *56*, 2561–2576. (b) Aoyagi, Y.; Jain, R. P.; Williams, R. M. *J. Am. Chem. Soc.* **2001**, *123*, 3472–3477. (c) Kazmaier, U.; Krebs, A. *Tetrahedron Lett.* **1999**, *40*, 479–482. (d) Davies, S. G.; Smyth, G. D.; Chippindale, A. M. *J. Chem. Soc., Perkin Trans. 1* **1999**, 3089–3104. (e) Sasaki, S.; Hamada, Y.; Shioiri, T. *Tetrahedron Lett.* **1999**, *40*, 3187–3190. (f) Verresha, G.; Datta, A. *Tetrahedron Lett.* **1997**, *38*, 5223–5224.
8. (a) Ohta, Y.; Shinkai, I. *Bioorg. Med. Chem.* **1997**, *5*, 465–466. (b) Florence, E.; Dreezen, C.; Desmet, P.; Smets, E.; Fransen, K.; Vandercam, B.; Pelgrom, J.; Clumeck, N.; Colebunders, R. *Antiviral Therapy* **2001**, *6*, 255–262.
9. LeBlond, C. R.; Rossen, K.; Gortsema, F. P.; Zavialov, I. A.; Cianciosi, S. J.; Andrews, A. T.; Sun, Y. *Tetrahedron Lett.* **2001**, *42*, 8603–8606.
10. Preliminary communication: Hodgson, D. M.; Maxwell, C. R.; Miles, T. J.; Paruch, E.; Stent, M. A. H.; Matthews, I. R.; Wilson, F. X.; Witherington, J. *Angew. Chem., Int. Ed.* **2002**, *41*, 4313–4316, *corrigendum* **2002**, *41*, 4611. See also: Hodgson, D. M.; Maxwell, C. R.; Miles, T. J.; Paruch, E.; Matthews, I. R.; Witherington, J. *Tetrahedron* **2004**, *60*, 3611–3624.
11. Hodgson, D. M.; Maxwell, C. R.; Matthews, I. R. *Tetrahedron: Asymmetry* **1999**, *10*, 1847–1850.
12. Denmark, S. E.; Nakajima, N.; Nicaise, O. J.-C.; Faucher, A.-M.; Edwards, J. P. *J. Org. Chem.* **1995**, *60*, 4884–4892. See, however: Dearden, M. J.; Firkin, C. R.; Hermet, J.-P. R.; O'Brien, P. *J. Am. Chem. Soc.* **2002**, *124*, 11870–11871.
13. Rigby, J. H.; Pigge, F. C. *Org. React. (N. Y.)* **1997**, *51*, 351–478.
14. Kim, H.; Hoffmann, H. M. R. *Eur. J. Org. Chem.* **2000**, 2195–2201.
15. Mann, J.; de Almeida Barbosa, L. C. *J. Chem. Soc., Perkin Trans. 1* **1992**, 787–790.
16. (a) Dunkel, R.; Mentzel, M.; Hoffmann, H. M. R. *Tetrahedron* **1997**, *53*, 14929–14936. (b) Treu, J.; Hoffmann, H. M. R. *J. Org. Chem.* **1997**, *62*, 4650–4652.
17. Paparin, J.-L.; Crévisy, C.; Toupet, L.; Grée, R. *Eur. J. Org. Chem.* **2000**, 3909–3918.
18. Braish, T. F.; Fuchs, P. L. *Synth. Commun.* **1986**, *16*, 111–115.
19. Sakaitani, M.; Ohfune, Y. *Tetrahedron Lett.* **1985**, *26*, 5543–5546.
20. Hodgson, D. M.; Cameron, I. D.; Christlieb, M.; Green, R.; Lee, G. P.; Robinson, L. A. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2161–2174.
21. Hodgson, D. M.; Lee, G. P.; Marriott, R. E.; Thompson, A. J.;

Wisedale, R.; Witherington, J. *J. Chem. Soc., Perkin Trans. 1* **1998**, 2151–2162.
22. Hodgson, D. M.; Stent, M. A. H.; Štefane, B.; Wilson, F. X. *Org. Biomol. Chem.* **2003**, *1*, 1139–1150.
23. (a) Avenoza, A.; Cativiela, C.; Corzana, F.; Peregrina, J. M.; Zurbano, M. M. *Tetrahedron: Asymmetry* **2000**, *11*, 2195–2204. (b) Park, Y. S.; Boys, M. L.; Beak, P. *J. Am. Chem. Soc.* **1996**, *118*, 3757–3758. (c) Ibuka, T.; Suzuki, K.; Habashita, H.; Otaka, A.; Tamamura, H.; Mimura, N.; Miwa, Y.; Taga, T.; Fujii, N. *J. Chem. Soc., Chem. Commun.* **1994**, 2151–2152. (d) Bull, S. D.; Davies, S. G.; Jones, S.; Polywka, M. E. C.; Prasad, R. S.; Sanganee, H. J. *Synlett* **1998**, 519–521.
24. Fleming, I.; Dunoguès, J.; Smithers, R. *Org. React. (N. Y.)* **1989**, *37*, 57–575.
25. (a) Jung, M. E.; Nichols, C. J. *J. Org. Chem.* **1996**, *61*, 9065–9067. (b) Moser, W. H. *Tetrahedron* **2001**, *57*, 2065–2084.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron
Tetrahedron 60 (2004) 5201–5206

Synthesis of 4-monofluoromethylenyl- and *cis*-4-monofluoromethyl-L-pyroglutamic acids via a novel dehydrofluorination

Xiao-Long Qiu,[a] Wei-Dong Meng[b] and Feng-Ling Qing[a,b,*]

[a]*Key Laboratory of Organofluorine Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Science, 354 Fenglin Lu, Shanghai 200032, China*
[b]*College of Chemistry and Chemical Engineering, Donghua University, 1882 West Yanan Lu, Shanghai 200051, China*

Received 31 December 2003; revised 15 April 2004; accepted 16 April 2004

Abstract—Novel dehydrofluorination reactions accidentally found were used to synthesize terminal monofluoro olefin lactam analogues in good yield. The following hydrogenation of the resulting defluorinated product was systematically investigated. Two important fluorinated amino acids: 4-monofluoromethylenyl-L-pyroglutamic acid **16** and *cis*-4-monofluoromethyl-L-pyroglutamic acid **17** were synthesized using the methodology.

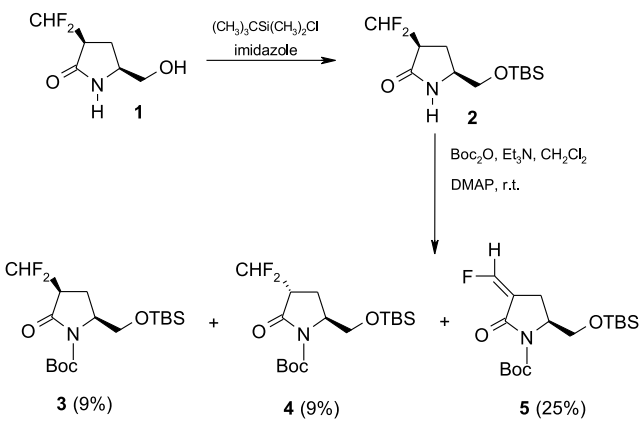
1. Introduction

In recent years, the application of the fluorinated amino acids has been progressed a great deal. As well as being used as the biological tracers and mechanistic probes for investigations on the structures and properties of enzymes, fluorinated amino acids have also played an important role in medicinal chemistry, especially in the control of blood pressure, allergies and tumor growth.[1] In particular, fluorinated amino acids have recently emerged as valuable building blocks for designing hyperstable protein folds, as well as directing highly specific protein–protein interactions.[2] For these reasons, the stereoselective synthesis of novel fluorinated amino acids is of great interest and intensive demand.[1,3,4]

Pyroglutamic acid and its derivatives are important amino acids in many bioactive compounds.[5] The 4-substituted pyroglutamic acid derivatives are important for their conformation and activities. For example, some natural and synthetic 4-substituted glutamic acids have been used to study their structure-–activity relationships of excitatory effects on the nervous system.[6] Although efficient asymmetric synthesis of fluorinated pyroglutamic acids have been reported,[7] to the best of our knowledge, there is no report on the synthesis of monofluoromethyl and monofluoromethylenyl pyroglutamic acids, probably because of the difficulties in stereoselective introduction of monofluoromethyl group into specific position of pyroglutamic acids. Herein reported is the efficient synthesis of two novel fluorinated amino acids: 4-monofluoromethylenyl-L-pyroglutamic acid and *cis*-4-monofluoromethyl-L-pyroglutamic acid via a novel dehydrofluorination reaction.

2. Results and discussion

We accidentally found that the dehydrofluorination reaction occurred when the amino group of 5-*tert*-butyldimethylsilyloxymethyl-3-difluoromethyl-pyrrolidin-2-one **2** was protected with *tert*-butoxycarbonyl group (Boc) (Scheme 1). That was: treatment of compound **2** with di-*tert*-butyl

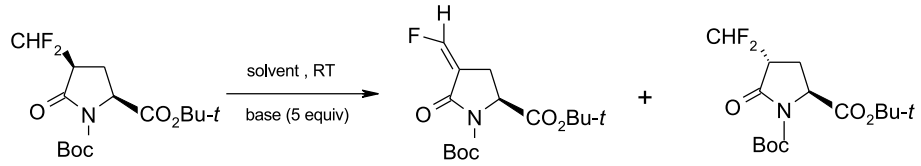


Scheme 1.

Keywords: Fluorinated amino acids; Pyroglutamic acids; Dehydrofluorination.
* Corresponding author. Tel.: +86-21-641-63300; fax: +86-21-64166128; e-mail address: flq@mail.sioc.ac.cn

0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.04.044

Table 1. Influence of base and solvent on dehydrofluorination reaction of **6**



6 **7** **8**

Entry	Base	Solvent	Time (h)	Product **7**[a] (%)	Product **8**[a] (%)	Conversion (%)
1	Pyridine	CH_2Cl_2	24	2.7[b]	0	10
2	*i*-Pr_2NEt	CH_2Cl_2	24	77	5	92
3	Et_3N	CH_2Cl_2	24	90	0	100
4	Et_3N	THF	24	74	15	96
5	Et_3N	DMF	24	81	0	100
6	Et_3N	CH_3CN	18	90	0	100
7	Et_3N	CH_3CN/H_2O	3	70	0	100
8	Et_3N	THF/H_2O	3	85	0	100

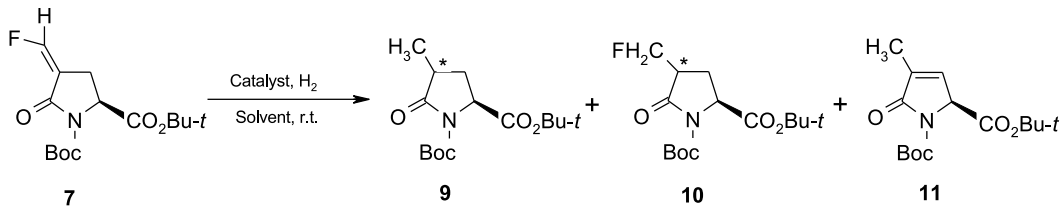
[a] Isolated yield.
[b] Determined by ^1H NMR and ^{19}F NMR.

dicarbonate (Boc_2O) and Et_3N under the catalysis of 4-(dimethylamino)pyridine (DMAP) provided the monofluoro olefin **5** in 25% yield along with desired compound **3** in very low yield (9%). The dehydrofluorination reaction was not observed during the protection of the hydroxy group of 5-hydroxymethyl-3-difluoromethyl-pyrrolidin-2-one **1** with *tert*-butyldimethylsilyl group using imidazole as a base (Scheme 1). In our opinion, dehydrofluorination reaction occurred after the desired product **3** was formed. It was induced by the enhancement of acidity of 4-H as a result of the protection of amino group of **2** by electron-withdrawing groups (Boc). Recently, the synthesis of terminal monofluoro olefins of the general structure $R^1R^2C{=}CHF$ has been summarized by Gen et al.[8] Because loss of HCl, HBr or HI seems sufficiently favorable over dehydrofluorination, only a few syntheses have applied the dehydrofluorination reaction of difluoromethyl groups.[9–11] Among these synthetic methods of terminal monofluoro olefins, all of the dehydrofluorination reactions were carried out in the presence of strong bases (NaOEt, KOH or NaOH) which could cause the racemization of some chiral substrates. The discovery of the dehydrofluorination reaction caused by weak organic amine base (Et_3N) promoted us to improve the yield of this novel dehydrofluorination for the synthesis of 4-monofluoromethylenyl-L-pyroglutamic acid.

We first studied the novel dehydrofluorination reactions with (2*S*, 4*S*)-*tert*-butyl-*N*-*tert*-butoxycarbonyl-4-difluoromethylpyroglutamate **6** as the substrate (Table 1) which was easily prepared from *trans*-4-hydroxy-L-proline.[7a] First, the effect of bases on dehydrofluorination reaction was investigated by conducting the model reaction in CH_2Cl_2 at room temperature (Table 1, entries 1–3). When pyridine was used as the base, the dehydrofluorination reaction proceeded slowly and the defluorinated compound **7** was afforded only in 2.7% yield even after 24 h (entry 1). However, when *i*-Pr_2NEt was used, the reaction rate increased dramatically and the desired compound **7** was isolated in 77% yield along with the isomeric compound **8** in 5% yield (entry 2). The reaction was performed smoothly with Et_3N as the base and compound **7** was obtained in 90% yield after 24 h without isomeric compound **8** formed (entry 3). These experiments showed that the amine bases had a profound influence. Then the investigation of the solvent effect on the reaction was followed (Et_3N as the base, entries 3–8). When the reaction was conducted in THF at room temperature for 24 h, **7** and **8** were isolated in 74 and 15% yields with a little starting material (entry 4). When DMF was used as the solvent, **7** was obtained in 81% yield without the formation of the isomeric compound **8** (entry 5). The reaction was completed in 18 h in CH_3CN to give **7** as the single product in 90% yield (entry 6). Surprisingly, H_2O had not altered the dehydrofluorination reaction, but had enhanced the reaction rate dramatically (entries 7 and 8). When reactions were conducted in a mixed solvent (CH_3CN/H_2O, v/v=2:1; THF/H_2O, v/v=2:1), the dehydrofluorination reaction was finished in 3 h to give **7** in 70 and 85% isolated yields, respectively, without a trace of compound **8** observed. It was easily concluded that Et_3N was the best amine base and THF/H_2O (v/v=2:1) was the most suitable solvent system for the novel dehydrofluorination reaction. The absolute configuration of **7** was confirmed by NOE correlation.

With compound **7** in hand, we turned our attention to synthesize 4-monofluoromethyl pyroglutamic acid via the catalytic hydrogenations of **7** (Table 2). There are only a few reports on the catalytic hydrogenation of α- or β-fluoro-α, β-unsaturated esters and ketones.[12] This may be due to the hydrogenolyic lability of vinylic fluorine compared to the saturated counterpart.[13] The catalytic hydrogenation of **7** was first carried out under usual condition (Table 2, entries 1–3). However, the desired compound **10** was not observed, and only defluorinated compound **9** was isolated in moderate yields and good diastereoselectivities (entry 1, 10% Pd/C, 81% yield, *cis*/*trans*=19.3:1; entry 2, 10% Pt/C, 83% yield, *cis*/*trans*=5.9:1). Even with Ranney Ni as the catalyst in MeOH, compound **10** was still not observed except **9** (entry 3). In our opinion, these three catalysts caused the hydrogenolytic cleavage of carbon–fluorine bonds due to their strong catalytic activities. Thus, some weak catalysts were tried (entries 4–6). Surprisingly, *cis*-**10** was obtained in 12% yield along with **9** (77% yield) when

Table 2. Influence of catalysts and solvents on hydrogenation of **7**



Entry	Catalyst	Solvent	Time (h)	**9** Yield (%) (*cis*:*trans*)	**10** Yield (%) (*cis*:*trans*)	**11** Yield (%)	Conversion (%)
1	10% Pd/C	EtOH	5	81 (19.3:1.0)[a]	—	—	100
2	5% Pd/C	EtOH	0.5	83 (5.9:1.0)[a]	—	—	100
3	Ranney Ni	MeOH	1	96[b]	—	—	100
4	$Pd(OH)_2/C$	EtOH	0.5	77 (25.0:1.0) [a]	12[c]	—	100
5	$Pd-CaSO_4$	EtOH	0.5	74 (36.0:1.0) [a]	19[c]	—	100
6	$Pd-BaSO_4$	EtOH	1	75 (74.0:1.0) [a]	20[c]	—	100
7	$Pd-BaSO_4$	EtOH[d]	24	47[b]	1.5[e]	—	84[f]
8	$Pd-BaSO_4$	THF	24	45[b]	37[c]	9.7[g]	95[f]
9	$Pd-BaSO_4$	CH_2Cl_2	24	8[b]	29[c]	12.0[g]	88[f]
10	$Pd-BaSO_4$	EtOAc	2	20[b]	77 (>38.5:1.0)[h]	—	100
11	$Pd-BaSO_4$	Dioxane	2	13[b]	78 (>78.0:1.0)[h]	—	100

[a] *Cis*and *trans* were isolated by flash chromatography.
[b] No or trace *trans*-**9** was observed or determined.
[c] No *trans*-**10** was isolated or detected.
[d] Several drops of quinoline was added.
[e] Determined by ^1H NMR.
[f] Starting material was recovered.
[g] Isolated yield.
[h] Trace *trans*-**10** was detected or isolated.

$Pd(OH)_2/C$ was used in EtOH (entry 4). *cis*-**10** was also isolated in 19 and 20% yields, respectively, with $Pd-CaSO_4$ and $Pd-BaSO_4$ as catalysts in EtOH (entries 5 and 6). However, when $Pd-BaSO_4$ was poisoned with several drops of quinoline, **10** was observed only in 1.5% yield together with **9** (47%) and starting material even after 24 h (entry 7). Then, different solvents were investigated with $Pd-BaSO_4$ as the catalyst (entries 8–11). The usage of THF

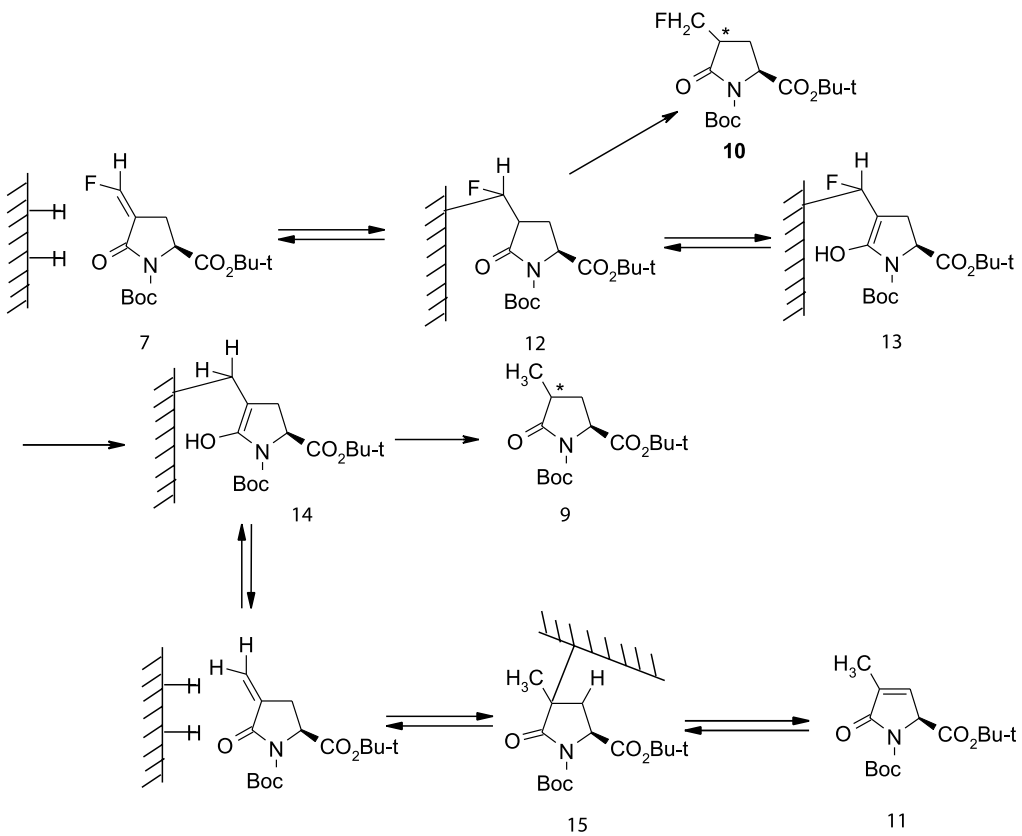


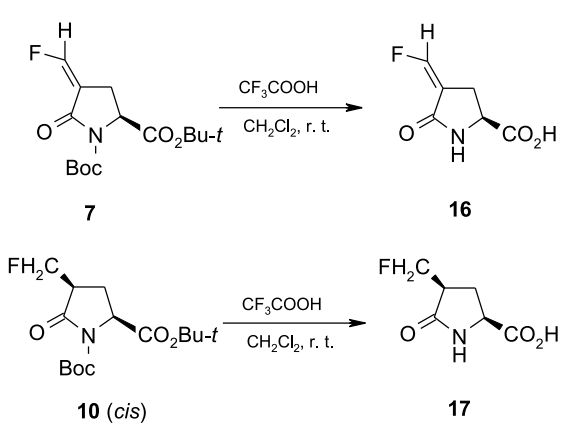
Figure 1. Mechanism of hydrogenation of compound **7**.

and CH_2Cl_2 gave **10** in 37 and 29% yields, respectively, together with **9** (entries 8 and 9), to our surprise, the unexpected defluorinated compound **11** was also observed in 9.7 and 12.0% yields, respectively. The best results were obtained when EtOAc and dioxane were used as the solvents. **10** was isolated in 77 and 78% yields, respectively, with a small amount of **9** formed (entries 10 and 11). It was concluded from these results that the catalysts and solvents had great effects on the hydrogenation of **7**. The absolute configuration of *cis*-**10** was confirmed by X-ray diffraction.

How to explain the hydrogenation mechanism was a challenge for us. According to the different products, the reaction probably proceeded through the following mechanism (Fig. 1). Attachment of **7** to Pd carrier gave the intermediate **12** which could be directly hydrogenated to give the desired compound **10**. **12** could also transform to **13** due to the enolization of lactam. Elimination of HF from **13** by rearranging the enol followed by reattachment of Pd afforded the intermediate **14** that could be reductive eliminated to furnish **9**. **14** could be converted to **15** via reduction elimination followed by re-attachment to Pd carrier. Finally, compound **11** was provided via elimination of 3-H of **15**.

With **7** and *cis*-**10** in hand, one-step removal of protective groups with trifluoroacetic acid in CH_2Cl_2 successfully afforded two important fluorine-containing amino acids: 4-monofluoromethylenyl-L-pyroglutamic acid **16** and *cis*-4-monofluoromethyl-L-pyroglutamic acid **17** in 72 and 66% yields, respectively, (Scheme 2).

In conclusion, we have described a novel dehydrofluorination reaction caused by weak organic amino base and have systematically investigated the effects of the different bases and solvents on the reaction. The process of this reaction was simple operated with readily available and cheap reagents and occurred under very mild condition. We have also described the hydrogenation of the resulting desired compound **7** in view of different catalysts and solvents, and have proposed a corresponding hydrogenation mechanism. Finally, we have synthesized two important fluorine-containing amino acids: 4-monofluoromethylenyl-L-pyroglutamic acid **16** and *cis*-4-monofluoromethyl-L-pyroglutamic acid **17**. We believe that the novel dehydrofluorination reaction and hydrogenation of corresponding defluorinated compounds could be applied to synthesize other α-monofluoromethylenyl amide analogues and corresponding α-monofluoromethyl amide analogues. Studies on detailing the application of this novel reaction to other substrates and incorporation of these two novel important fluorine-containing amino acids into peptides, domimetics and potential bioactive molecules are in intensive progress.



Scheme 2.

3. Experimental

3.1. General procedure for dehydrofluorination of comound 6

To a solution of **6** (around 100 mg) in the different solvent (10 mL), organic base (5.0 equiv.) was added dropwise. Then, the mixture was stirred at room temperature. The reaction was quenched with H_2O (5 mL) and the resulted mixture was extracted with CH_2Cl_2. The combined organic phases were washed with brine and dried over anhydrous Na_2SO_4. Filtration and removal of the solvent gave a residue, which was purified by silica gel chromatography to afford the corresponding products.

3.1.1. (2*S*)-*tert*-Butyl-*N*-*tert*-butoxycarbonyl-4-monofluoromethylenylpyroglutamate 7. White solid; Mp 104–105 °C; $[\alpha]_D^{20}$ −17.1 (*c* 0.5, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.51 (dt, *J*=78.4, 3.0 Hz, 1H), 4.52 (dd, *J*=3.3, 2.7 Hz, 1H), 3.02 (m, 1H), 2.72 (dq, *J*=17.6, 3.0 Hz, 1H), 1.53 (s, 9H), 1.48 (s, 9H); ^{19}F NMR (282 MHz, $CDCl_3$) δ −120.6–120.8 (dq, *J*=78.3, 3.0 Hz, 1F); IR (thin film) ν_{max} 1784, 1772, 1747, 1711, 1690, 1294, 1149 cm^{-1}; MS (ESI) *m*/*z* 338 (M^++Na); Anal. Calcd for $C_{15}H_{22}FNO_5$: C, 57.13; H, 7.03; N, 4.44. Found: C, 57.18; H, 7.12; N 4.33.

3.1.2. (2*S*,4*R*)-*tert*-Butyl-*N*-*tert*-butoxycarbonyl-4-difluoromethylpyroglutamate 8. White solid; Mp 66–68 °C; $[\alpha]_D^{20}$ −21.1 (*c* 0.6, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 6.23 (td, *J*=55.4, 2.1 Hz, 1H), 4.51 (dd, *J*=1.8, 2.1 Hz, 1H), 3.30–3.12 (m, 1H), 2.58–2.47 (m, 1H), 2.12 (ddd, *J*=13.8, 1.8, 1.8 Hz, 1H), 1.52 (s, 9H), 1.49 (s, 9H); ^{19}F NMR (282 MHz, $CDCl_3$) δ −124.0 to −125.2 (dd, *J*=277.5, 54.1 Hz, 1F), −127.3–128.6 (ddd, *J*=286.2, 25.0, 27.6 Hz, 1F); IR (thin film) ν_{max} 1758, 1739, 1717, 1327, 1158 cm^{-1}; MS (ESI) *m*/*z* 353 (M^++NH_4); Anal. Calcd for $C_{15}H_{23}F_2NO_5$: C, 53.72; H, 6.91; N, 4.18. Found: C, 53.92; H, 6.99; N, 4.02.

3.2. General procedure for hydrogenation of compound 7

To a solution of compound **7** in the solvent, Pd-catalyst (5–10% mmol) was added. The mixture was hydrogenated at room temperature. Filtration and removal of the solvent gave a residue that was purified by silica gel chromatography to afford the corresponding products.

3.2.1. (2*S*,4*S*)-*tert*-Butyl-*N*-*tert*-butoxycarbonyl-4-methylpyroglutamate 9 (*cis*). White solid; Mp 58–60 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 4.41–4.36 (m, 1H), 2.67–2.50 (m, 2H), 1.62–2.56 (m, 1H), 1.51 (s, 9H), 1.49 (s, 9H), 1.23 (d, *J*=6.9 Hz, 3H); ^{13}C NMR spectra was identical to that of literature (*J. Chem. Soc., Perkin Trans. 1* **1996**, 507).

3.2.2. (2*S*,4*R*)-*tert*-Butyl-*N*-*tert*-butoxycarbonyl-4-methylpyroglutamate 9 (*trans*). White solid; Mp 63–64 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.43 (dd, J=1.5, 1.8 Hz, 1H), 2.73–2.59 (m, 1H), 2.28–2.20 (m, 1H), 1.95–1.84 (m, 1H), 1.51 (s, 9H), 1.48 (s, 9H), 1.23 (d, J=7.2 Hz, 1H) (literature reported: 4.42 (d, J=9.5 Hz, 1H), 2.65 (m, 1H), 2.24 (ddd, J=13.5, 8.7, 1.0 Hz, 1H), 1.89 (m, 1H), 1.51 (s, 9H), 1.48 (s, 9H), 1.21 (t, J=7.0 Hz, 1H). *J. Chem. Soc., Perkin Trans. 1* **2001**, 2367.)

3.2.3. (2*S*,4*S*)-*tert*-Butyl-*N*-*tert*-butoxycarbonyl-4-monofluoromethylpyroglutamate 10 (*cis*). White solid; Mp 68–70 °C; $[\alpha]_D^{20}$ −35.7 (c 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.73 (ddd, J=21.4, 5.1, 5.0 Hz, 1H), 4.58 (ddd, J=20.9, 5.1, 5.0 Hz, 1H) 4.48 (dd, J=5.4, 5.7 Hz, 1H), 3.01–2.84 (m, 1H), 2.62–2.51 (m, 1H), 2.08–2.00 (m, 1H), 1.52 (s, 9H), 1.48 (s, 9H); ^{19}F NMR (282 MHz, $CDCl_3$) δ −227.9 (m, 1F); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 171.5 (d, J=9.6 Hz), 170.1, 149.2, 83.8, 83.2, 81.7 (d, J=112.6 Hz), 58.0, 44.0 (d, J=21.7 Hz), 29.7, 27.9, 27.8, 23.9 (d, J=3.2 Hz); IR (thin film) ν_{max} 1757, 1740, 1713, 1321, 1156 cm^{-1}; MS (ESI) m/z 657 ($2M^+$ +Na), 652 ($2M^+$+NH_4), 335 (M^++NH_4); Anal. Calcd for $C_{15}H_{24}FNO_5$: C, 56.77; H, 7.62; N, 4.41. Found: C, 56.93; H, 7.69; N 4.12.

3.3. X-ray crystal structures of 10 (*cis*)

A white crystal having approximate dimension of 0.36×0.26×0.20 mm^3 was used for X-ray study. The data were collected on Bruker Smart APEX CCD diffractometer. The crystal structure has been deposited at the Cambridge crystallographic Data center and allocated the deposition number CCDC 236000.

Crystal data: $C_{15}H_{24}FNO_5$, M=317.35, Orthorhombic, Space group $P2(1)2(1)2(1)$, α=8.7047(13), β=11.2204(16), c=17.628 (3) Å, V=1721.7(4) Å^3, Z=4, D_{calc}=1.224 mg cm^{-3}, μ=0.098 mm^{-1}, $F(000)$=680, T=293 K, 2θmax=56.56°, 4036 independent reflections scanned, 1716 reflections observed ($I \geqq 2\sigma(I)$), 296 parameters, $R1$=0.0420, $wR2$=0.0654.

3.3.1. (2*S*)-*tert*-Butyl-*N*-*tert*-butoxycarbonyl-4-methyl-3,4-dehydropyroglutamate 11. White solid; Mp 59–61 °C; $[\alpha]_D^{20}$ −218.1 (c 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.71–6.69 (m, 1H), 4.91–4.89 (m, 1H), 1.92 (t, J=4.8 Hz, 3H), 1.55 (s, 9H), 1.48 (s, 9H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 169.1, 165.9, 148.8, 136.8, 135.9, 83.2, 83.1, 63.2, 28.0, 27.9, 11.0; IR (thin film) ν_{max} 3082, 2982, 1787, 1747, 1721, 1657, 1160 cm^{-1}; MS (ESI) m/z 617 ($2M^+$+Na), 612 ($2M^+$+NH_4); Anal. Calcd for $C_{15}H_{23}NO_5$: C, 60.59; H, 7.80; N, 4.71. Found: C, 60.88; H, 7.78; N, 4.59.

3.3.2. (2*S*)-4-Monofluoromethylenylpyroglutamic acid (16). TFA (0.85 mL) was added to a solution of **7** (205 mg, 0.65 mmol) in CH_2Cl_2 (15 mL) at 0 °C. The mixture was allowed to warm to room temperature and stirred for 24 h. The reaction mixture was concentrated in vacuo and the residue was triturated with Et_2O–petroleum ether to precipitate white solid, which was washed several times with Et_2O–petroleum ether. Remaining of the resulted solid was dried under high vacuum at 60 °C for several hours to give **16** (75 mg, 72%) as an off-white solid. Mp 153–154.5 °C; $[\alpha]_D^{20}$+37.6 (c 1.1, H_2O); ^{1}H NMR (300 MHz, MeOH-d_4) δ 7.44 (dt, J=78.3, 3.0 Hz, 1H), 4.50 (dd, J=4.2, 4.2 Hz, 1H), 3.37–3.26 (m, 1H), 3.04–2.97 (m, 1H); ^{19}F NMR (282 MHz, MeOH-d_4) δ −123.5 to −123.8 (dt, J=78.5, 3.9 Hz, 1F); ^{13}C NMR (75.5 MHz, MeOH-d_4) δ 176.6, 173.6 (d, J=19.5 Hz), 154.2 (d, J=270.3 Hz), 116.1 (d, J=13.4 Hz), 54.7, 26.6; IR (thin film) ν_{max} 3370, 3319, 3101, 2471, 1918, 1726, 1703, 1642, 1257, 1087 cm^{-1}; MS (EI) m/z 159 (M^+, 2), 114 (M^+ −COOH, 100); Anal. Calcd for $C_6H_6FNO_3$: C, 45.29; H, 3.80; N, 8.80. Found: C, 45.17; H, 3.78; N, 8.54.

3.3.3. (2*S*,4*S*)-4-Monofluoromethylpyroglutamic acid (17). Compound **17** (66 mg, 66%) was prepared as an off-white solid from compound **10** (*cis*) (198 mg, 0.625 mmol) following the procedure described for compound **16**. Mp 110–112 °C; $[\alpha]_D^{20}$ −28.4 (c 0.60, H_2O); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.87 (s, 1H), 8.15 (s, 1H), 4.61 (ddd, J=34.9, 4.5, 4.5 Hz, 1H), 4.51 (ddd, J=34.9, 3.0, 3.0 Hz, 1H) 4.11 (t, J=7.2 Hz, 1H), 2.80–2.55 (m, 2H), 1.95–1.85 (m, 1H); ^{19}F NMR (282 MHz, DMSO-d_6) δ −227.8 (m, 1F); ^{13}C NMR (75.5 MHz, MeOH-d_4) δ 189.6, 186.4, 93.2 (d, J=165.6 Hz), 65.2, 53.4 (d, J=20.5 Hz), 37.4 (d, J=4.0 Hz); IR (thin film) ν_{max} 3366, 1716, 1664, 1642, 1265, 948 cm^{-1}; MS (EI) m/z 161 (M^+, 2), 116 (M^+ −COOH, 100); Anal. Calcd for $C_6H_8FNO_3$: C, 44.72; H, 5.00; N, 8.69. Found: C, 44.46; H, 4.95; N, 8.56.

Acknowledgements

We thank the National Natural Science Foundation of China and the Ministry of Education of China for financial support.

References and notes

1. (a) Welch, J. T.; Eswarakrishnan, S. *Fluorine in Bioorganic Chemistry*; Wiley: New York, 1991. (b) Kukhar, V. P.; Soloshonok, V. A. *Fluorine Containing Amino Acids: Synthesis and Properties*; Wiley: New York, 1995. (c) Filler, R.; Kobayashi, Y.; Yagupolski, L. M. *Biomedical Aspects of Fluorine Chemistry*; Elsevier: Amsterdam, 1993.
2. For a recent review, see: Yoder, N. C.; Kumar, K. *Chem. Soc. Rev.* **2002**, *31*, 335–341.
3. For reviews, see: (a) Haufe, G.; Kroger, S. *Amino Acids* **1996**, *11*, 409–424. (b) Tolman, V. *Amino Acids* **1996**, *11*, 15–36. (c) Sutherland, A.; Willis, C. L. *Nat. Prod. Rep.* **2000**, *17*, 621–631.
4. For recent examples, see: (a) Vera-Ayoso, Y.; Borrachero, P.; Cabrera-Escribano, F.; Diánez, M. J.; Estrada, M. D.; Gómez-Guillén, M.; López-Castro, A.; Pérez-Garrido, S. *Tetrahedron: Asymmetry* **2001**, *12*, 2031–2041. (b) Xing, X.; Fichera, A.; Kumar, K. *Org. Lett.* **2001**, *3*, 1285–1286. (c) Fustero, S.; Navarro, A.; Pina, B.; Soler, J. G.; Bartolomé, A.; Asensio, A.; Simon, A.; Bravo, P.; Fronza, G.; Volonterio, A.; Zanda, M. *Org. Lett.* **2001**, *3*, 2621–2624. (d) Abe, H.; Amii, H.; Uneyama, K. *Org. Lett.* **2001**, *3*, 313–315. (e) Lebouvier, N.; Laroche, C.; Huguenot, F.; Brigaud, T. *Tetrahedron Lett.* **2002**, *43*, 2827–2830. (f) Xing, X.; Fichera, A.; Kumar, K. *J. Org. Chem.* **2002**, *67*, 1722–1725. (g) Staas, D. D.; Savage, K. L.; Homnick, C. F.; Tsou, N. N.; Ball, R. G. *J. Org. Chem.*

2002, *67*, 8276–8279. (h) Del Valle, J. R.; Goodman, M. *Angew. Chem., Int. Ed.* **2002**, *41*, 1600–1602. (i) Fustero, S.; Pina, B.; Salavert, E.; Navarro, A.; Ramírez de Arellano, C.; Fuentes, A. S. *J. Org. Chem.* **2002**, *67*, 4667–4679. (j) Anderson, J. T.; Toogood, P. L.; Marsh, E. N. G. *Org. Lett.* **2002**, *4*, 4281–4283. (k) Sani, M.; Bruche, L.; Chiva, G.; Fustero, S.; Piera, J.; Volonterio, A.; Zanda, M. *Angew. Chem., Int. Ed.* **2003**, *42*, 2060–2063. (l) Fustero, S.; Bartolome, A.; Sanz-Cervera, J. F.; Sanchez-Rosello, M.; Soler, J. G.; Ramírez de Arellano, C.; Fuentes, A. S. *Org. Lett.* **2003**, *5*, 2523–2526.

5. (a) Barco, A.; Benetti, S.; Pollini, G. P.; Baraldi, P. G.; Guarneri, M.; Gandolfi, C.; Ceserani, R.; Longiave, D. *J. Med. Chem.* **1981**, *24*, 625–628. (b) Kar, G. K.; Roy, B. C.; Adhikari, S. D.; Ray, J. K.; Brahma, N. K. *Bioorg. Med. Chem.* **1998**, *6*, 2397–2403.
6. Hon, Y.-S.; Chang, Y.-C.; Gong, M.-L. *Heterocycles* **1990**, *31*, 191–195.
7. (a) Qiu, X.-L.; Qing, F.-L. *J. Org. Chem.* **2003**, *68*, 3614–3617. (b) Hudlickú, M. *J. Fluorine Chem.* **1993**, *60*, 193–210. (c) Tolman, V.; Vlasáková, V.; Němeček, J. *J. Fluorine Chem.* **1993**, *60*, 185–191. (d) Tolman, V.; Sedmera, P. *J. Fluorine Chem.* **2000**, *101*, 5–10. (e) Doyle, M. P.; Hu, W.; Phillips, I. M.; Moody, C. J.; Pepper, A. G.; Slawin, A. M. Z. *Adv. Synth. Catal.* **2001**, *343*, 112–117. (f) Vidal-Cros, A.; Gaudry, M.; Marquet, A. *J. Org. Chem.* **1985**, *50*, 3163–3167. (g) Hart, B. P.; Coward, J. K. *Tetrahedron Lett.* **1993**, *34*, 4917–4920. (h) Hart, B. P.; Haile, W. H.; Licato, N. J.; Bolanowska, W. E.; McGuire, J. J.; Coward, J. K. *J. Med. Chem.* **1996**, *39*, 56–65.
8. van Steenis, J. H.; van der Gen, A. *J. Chem. Soc., Perkin Trans. 1* **2002**, 2117–2133.
9. Komatsu, Y.; Sakamoto, T.; Kitazume, T. *J. Org. Chem.* **1999**, *64*, 8369–8374.
10. Bornstein, J.; Blum, M. S.; Pratt, Jr. J. J. *J. Org. Chem.* **1957**, *22*, 1210–1213.
11. McDonald, I. A.; Lacoste, J. M.; Bey, P.; Palfreyman, M. G.; Zreika, M. *J. Med. Chem.* **1985**, *28*, 186–193.
12. (a) Martinet, P.; Sauvetre, R.; Normant, J.-F. *J. Fluorine Chem.* **1991**, *52*, 419–432. (b) Allmendinger, T.; Dandois, C.; Walliser, B. *Tetrahedron Lett.* **1991**, *32*, 2735–2736. (c) Zheng, G.-C.; Yoshihara, K.; Kobayashi, Y.; Taguchi, T. *Chem. Express* **1990**, *5*, 577–580.
13. (a) Hudlicky, M. *J. Fluorine Chem.* **1979**, *14*, 189–199. (b) Hudlickú, M. *J. Fluorine Chem.* **1983**, *23*, 241–259.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron 60 (2004) 5207–5214
Tetrahedron

Reactions of 1,4-dilithiobutadienes with isothiocyanates: preparation of iminocyclopentadiene derivatives via cleavage of the C═S double bond of a RN═C═S molecule

Congyang Wang,[a,b] Qiuling Song[a,b] and Zhenfeng Xi[a,b,*]

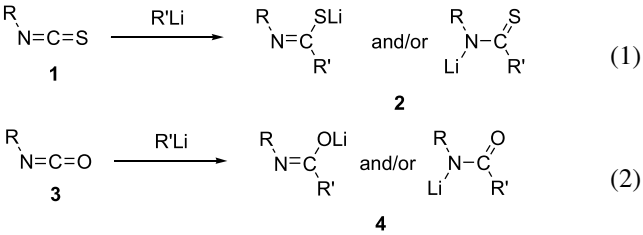
[a]*Key Laboratory of Bioorganic Chemistry and Molecular Engineering of Ministry of Education, College of Chemistry, Peking University, Beijing 100871, China*
[b]*State Key Laboratory of Organometallic Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, China*

Received 4 November 2003; revised 10 April 2004; accepted 14 April 2004

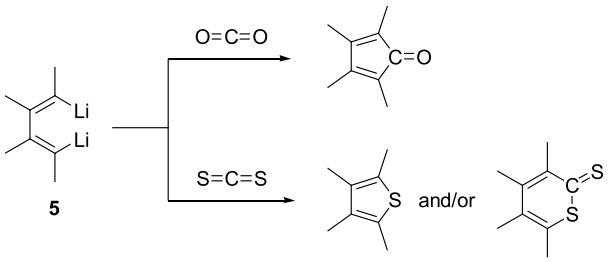
Abstract—Reaction patterns of 1,4-dilithio-butadiene derivatives with isothiocyanates RN═C═S and isocyanates RN═C═O were investigated and synthetically useful preparative methods were developed. Isothiocyanates reacted with 1,4-dilithio-butadienes to afford iminocyclopentadiene derivatives in excellent isolated yields and high selectivity. When aromatic isothiocyanates were used, cleavage of the C═S double bond of a RN═C═S molecule took place via a successive inter-intramolecular carbophilic addition. A number of products were obtained from the reaction of isocyanates with 1,4-dilithio-butadienes, probably due to the high reactivity of isocyanates towards 1,4-dilithio-butadienes.

1. Introduction

Heterocumulenes including isothiocyanates RN═C═S and isocyanates RN═C═O are a class of important synthetic intermediates. Site-selectivities of these unsymmetrical heterocumulenes toward nucleophiles are of great interest in heteroatom chemistry and controlled synthetic chemistry.[1,2] Addition of organolithium reagents to these heterocumulenes has been a fundamental protocol for the preparation of a wide variety of S-, N- and O-containing linear and cyclic compounds.[1,2] Carbophilic addition of organolithium reagents to isothiocyanates RN═C═S (**1**) or isocyanates RN═C═O (**3**) usually takes place to generate lithium thioamidates **2** (Eq. (1))[3–5] or lithium carboxamides **4** (Eq. (2)),[6] respectively. Trapping of these lithiated intermediates with electrophiles provides useful preparation methods for S-, N- and O-containing linear and cyclic compounds. Although interesting reaction patterns and useful preparative methods can be expected from further reactions of these lithiated intermediates with nucleophiles such as a second molecule of organolithium reagent, such investigations are rare.[7]



Recently, we demonstrated that dilithio compounds, such as 1,4-dilithio-1,3-diene derivatives **5**, react with symmetrical heterocumulenes CO_2 and CS_2 in unprecedented reaction patterns (Scheme 1).[7] The C═O bond of CO_2 and the C═S bond of CS_2 were selectively cleaved in these reactions.[7,8] As our continuous interests in the development of synthetically useful methods by taking advantage of the cooperative reaction patterns of dilithio compounds **5**, we



Scheme 1. Reaction of 1,4-dilitho-1,3-dienes with symmetrical heterocumulenes CO_2 and CS_2.

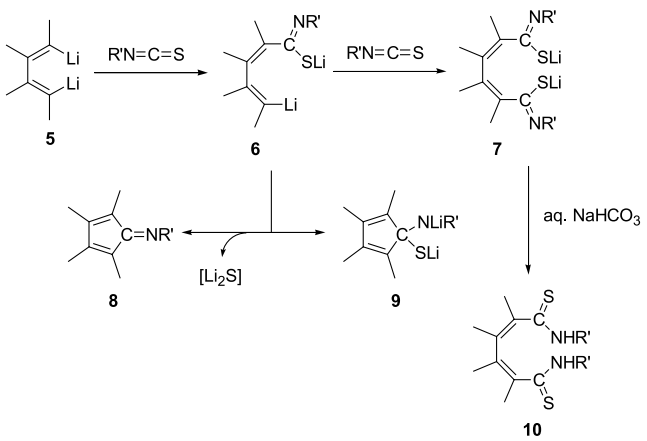
Keywords: Dilithio reagents; Isothiocyanates; Iminocyclopentadienes.
* Corresponding author. Tel.: +86-10-6275-9728; fax: +86-10-6275-1708; e-mail address: zfxi@pku.edu.cn

0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.04.045

investigated reactions of **5** with asymmetrical heterocumulenes such as isothiocyanates RN═C═S and isocyanates RN═C═O. Iminocyclopentadiene derivatives were prepared in excellent yields via cleavage of the C═S double bond of a RN═C═S molecule.

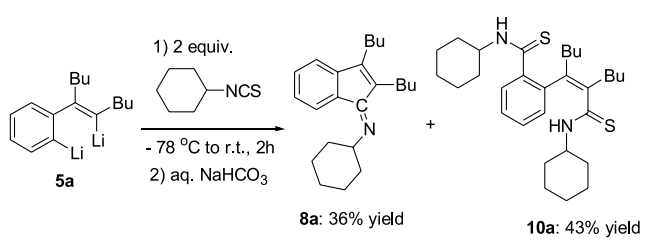
2. Results and discussion

It is known that carbophilic addition of an organolithium reagent to the C═S bond of an isothiocyanate normally takes place.[3–5,9,10] For the reactions of the dilithio reagent **5** with isothiocyanates **1**, the possible reaction pathways is proposed in Scheme 2. Carbophilic addition intermediates **6** are expected in the first step. Further reaction of **6** with a second molecule of isothiocyanate could afford **7**, which gives **10** after hydrolysis, as usually observed for the reactions of monolithio reagents with isothiocyanates.[3–5,9,10] As a novel reaction pattern, an intramolecular carbophilic attack by the remaining alkenyl lithium in **6** may afford direct formation of iminocyclopentadiene derivatives **8** via cleavage of C–S bond, or formation of cyclopentadiene derivatives **9**.



Scheme 2. Proposed reactions of 1,4-dilitho-1,3-dienes with isothiocyanates.

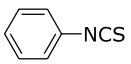
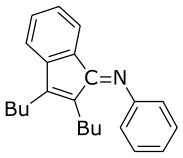
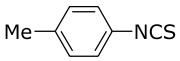
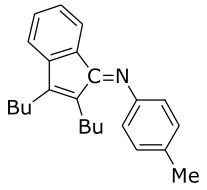
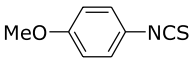
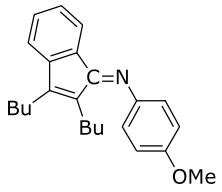
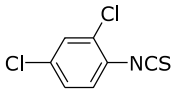
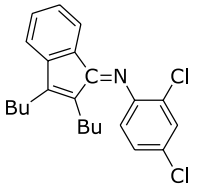
When the dilithio reagent **5a**, prepared in situ from the corresponding diiodo compound and 4 equiv. of *t*-BuLi,[11,12] was treated with two equivalents of cyclohexylisothiocyanate at −78 °C to room temperature for 2 h, quench with saturated aqueous $NaHCO_3$ afforded a mixture of products **8a** and **10a** (Scheme 3). Separation of these two products gave **8a** in 36% isolated yield and **10a** in 43% isolated yield. Interestingly, the reaction of **5a** with aromatic isothiocyanates gave indenimine derivatives exclusively in



Scheme 3.

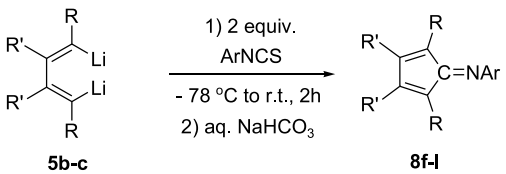
excellent isolated yields (Table 1).[13] No formation of the type of compound **10a** was observed. To the best of our knowledge, this reaction pattern is unprecedented for the reactions of organolithium reagents with isothiocyanates.

Table 1. Formation of 2,3-dibutylindenimine derivative from the reaction of **5a** with isothiocyanates

Entry	Isothiocyanate	Product	8	Yield (%)[a]
1			**8b**	85
2			**8c**	82
3			**8d**	87
4			**8e**	81

[a] Isolated yields.

When 1,4-dilithio-butadienes **5b–c** were treated with aromatic isothiocyanates following a similar procedure as described above, iminocyclopentadiene derivatives **8f–l** were obtained as the only products in excellent isolated yields (Eq. (3)). Listed in Table 2 are the representative results. Iminocyclopentadienes bearing various substituents could be prepared by using this method.



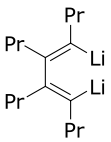
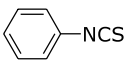
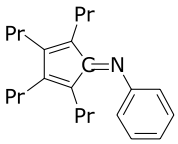
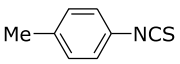
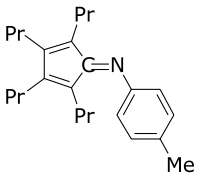
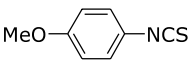
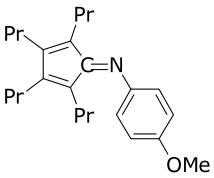
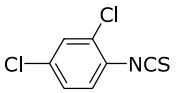
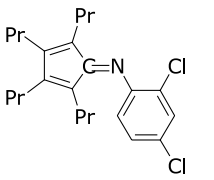
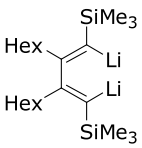
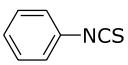
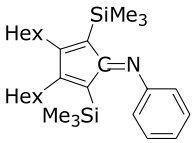
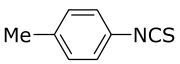
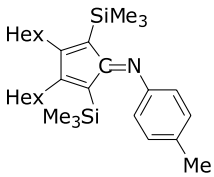
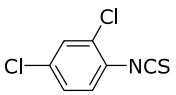
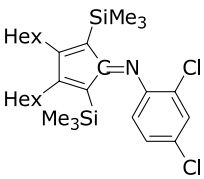
(3)

5b: R = R' = Pr
5c: R = $SiMe_3$, R' = Hex

N-(4,5,6,7-tetrahydro-2*H*-inden-2-ylidene)arylamines **8m–o** were also prepared in a similar procedure when 1,4-dilithio-1,3-diene **5d** was treated with isothiocyanates (Table 3).[13] The molecular structure of **8o** has been determined by single crystal X-ray diffraction analysis (Fig. 1).[13a]

Iminocyclopentadiene, indenimine and tetrahydroindenylimine derivatives are interesting and useful intermediates

Table 2. Formation of iminocyclopentadienes from the reaction of 1,4-dilithio-1,3-dienes with isothiocyanates

1,4-Dilitho-1,3-diene		Isothiocyanate	Product	**8**	Yield (%)[a]
	**5b**			**8f**	84
5b				**8g**	63
5b				**8h**	74
5b				**8i**	79
	**5c**			**8j**	82
5c				**8k**	74
5c				**8l**	72

[a] Isolated yields.

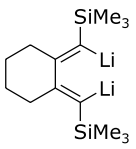
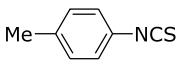
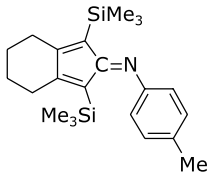
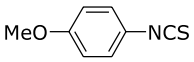
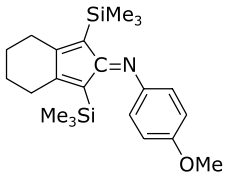
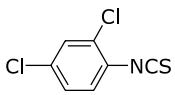
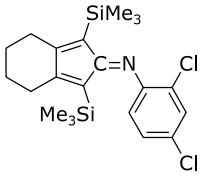
for synthetic and organometallic chemistry.[13,14] The reaction reported here provides a practical alternative for such complex compounds. In addition, these products could be readily transformed in aqueous HCl to their corresponding cyclopentadienone derivatives, which are also important compounds.[7a,13a]

It is noteworthy that the reaction of dilithio compound **5e** with 2,4-dichlorophenyl isothiocyanate afforded a mixture of two double bond positional isomers **8p** and **8p′**, with the 'normal' iminocyclopentadiene derivative **8p** as the minor one (Scheme 4). When **5e** was treated with phenyl isothiocyanate, the double bond positional isomer **8q′** was formed exclusively in 75% isolated yield; no formation of the 'normal' iminocyclopentadiene derivative **8q** was observed in this case.

A plausible reaction course for the formation of iminocyclopentadienes **8** is given in Scheme 5. Intermolecular carbophilic addition to RNCS by one of the two alkenyllithium moieties in a dilithio reagent **5** may be firstly involved,[15] as usually done by a monolithio reagent. A successive intramolecular carbophilic addition by the remaining alkenyllithium moiety is followed to afford an iminocyclopentadiene derivative **8**, along with the elimination of Li_2S. Cleavage of the C═S bonds in CS_2 using transition metal complexes is well-known and the use of organolithium compounds has been observed by Seyferth and us.[7b,c,16] However, as far as we know, the cleavage of the C═S double bond of a RN═C═S molecule using organolithium compounds has not been reported.[17]

It is interesting to reveal that, towards 1,4-dilithio-butadienes,

Table 3. Formation of tetrahydroindenimines from the reaction of 1,4-dilithio-1,3-dienes with isothiocyanates

1,4-Dilitho-1,3-diene		Isothiocyanate	Product	**8**	Yield (%)[a]
	**5d**			**8m**	59
5d				**8n**	68
5d				**8o**	61

[a] Isolated yields.

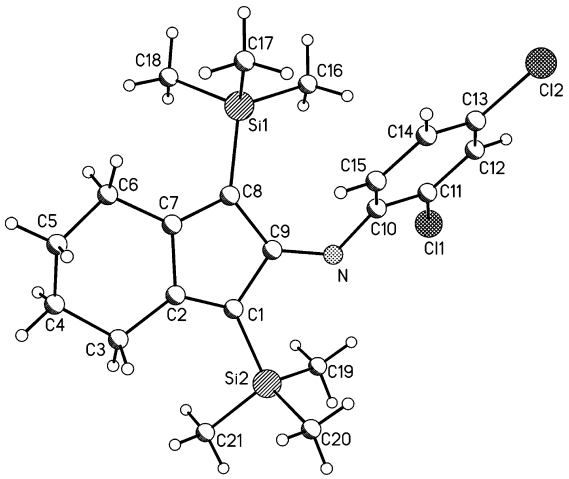


Figure 1. The Molecular Structure of **8o**.

the reactivity of the isocyanates are remarkably different from that of the isothiocyanates. A few products were detected from the reaction of isocyanates with 1,4-dilithio-butadienes, probably due to the high reactivity of the isocyanates. A representative example, the reation of **5f** with tolylisocyanate, is shown in Scheme 6. At least four products were formed in the reaction of **5f** with tolylisocyanate. Three of them were isolated and characterized as cyclopentadienone **11** (17% isolated yield),[7a] butadienyl imide **12a** (5% isolated yield) and butadienyl 1,4-bis-(imides) **13** (28% isolated yield).

3. Conclusions

We have developed a useful synthetic method for the preparation of iminocyclopentadiene derivatives from the reaction of 1,4-dilithio-butadienes with isothiocyanates; The cleavage of the C═S double bond of a RN═C═S molecule took place via a successive inter-intramolecular carbophilic addition.

4. Experimental

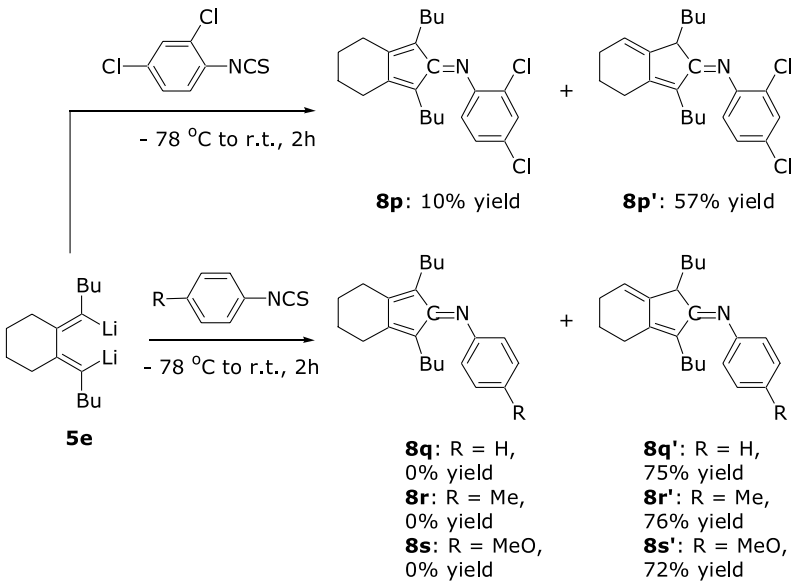
4.1. General methods

All reactions were conducted under a slightly positive pressure of dry, prepurified nitrogen using standard Schlenk line techniques when appropriate. Unless otherwise noted, all starting materials were commercially available and were used without further purification. Diethyl ether was refluxed and distilled from sodium benzophenone ketyl under a nitrogen atmosphere. *t*-BuLi was obtained from ACROS ORGANICS.

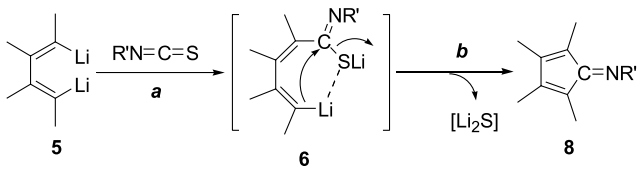
^{1}H and ^{13}C NMR spectra were recorded at 300 and 75.4 MHz, respectively, in $CDCl_3$ or C_6D_6 solution on JEOL JNM-AL300 NMR spectrometer. 1,4-Diiodo-1,3-diene derivatives were synthesized by the reported procedure.[12]

A typical procedure for the preparation of iminocyclopentadiene derivatives from the reactions of 1,4-dilithio-1,3-dienes with isothiocyanates.

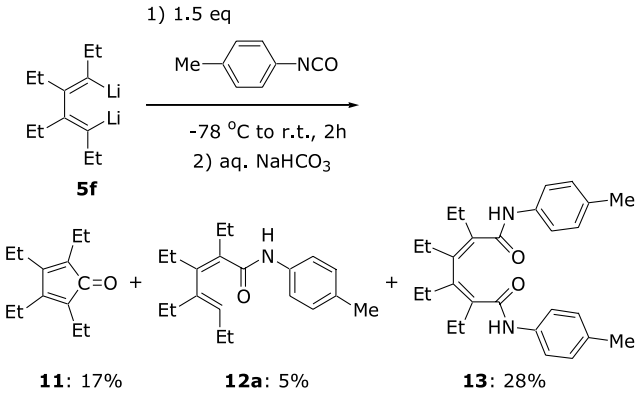
To a diethyl ether (5 mL) solution of 1,4-diiodo-1,3-butadiene (1 mmol) at −78 °C was added *t*-BuLi (4.0 mmol, 1.5 M in pentane). The above reaction mixture was then stirred at −78 °C for 1 h to generate 1,4-dilithio-1,3-diene **5**(**a**–**e**), which was monitored by GC analysis or by TLC. After addition of isothiocyanate (2 mmol) at −78 °C, the mixture was stirred at room temperature for 2 h. The above reaction mixture was then quenched with saturated aqueous $NaHCO_3$ and extracted with diethyl ether. The extract was washed with brine and dried over



Scheme 4.



Scheme 5. (a) Intermolecular carbophilic addition, (b) intramolecular carbophilic addition.



Scheme 6.

$MgSO_4$. The solvent was evaporated in vacuo to give a red-orange oil, which was purified by column chromatography (Al_2O_3, hexane) to afford **8**(**a**–**s**′).

4.1.1. Compound 8a. Column chromatography on Al_2O_3 (hexane) afforded orange liquid, isolated yield 36% (116 mg). ^{1}H NMR ($CDCl_3$, TMS): δ 0.88–0.97 (m, 6H), 1.28–1.89 (m, 18H), 2.40–2.52 (m, 4H), 4.31 (t, J=9.9 Hz, 1H), 7.07–7.26 (m, 3H), 7.55 (d, J=7.8 Hz, 1H); ^{13}C NMR ($CDCl_3$, TMS): δ 14.0, 14.1, 22.8, 23.1, 23.7, 24.7, 25.4, 25.9, 30.7, 32.5, 34.1, 59.9, 118.4, 125.1, 125.8, 128.9, 129.7, 139.3, 144.6, 146.7, 164.0. HRMS calcd for $C_{23}H_{33}N$ 323.2613, found 323.2605.

4.1.2. Compound 10a. Column chromatography on Al_2O_3 (CH_2Cl_2/hexane=1:9) afforded yellow-green liquid, isolated yield 43% (214 mg). ^{1}H NMR ($CDCl_3$, TMS): δ 0.37–2.78 (m, 38H), 4.07 (br, 1H), 4.48 (br, 1H), 7.17–7.28 (m, 4H), 7.76 (s, 1H), 9.46 (s, 1H); ^{13}C NMR ($CDCl_3$, TMS): δ 13.9, 14.0, 22.8, 22.8, 24.6, 24.8, 25.3, 25.4, 29.8, 29.8, 30.0, 31.1, 31.2, 31.3, 31.3, 33.0, 53.6, 54.5, 125.9, 127.4, 129.3, 130.7, 135.7, 139.8, 140.3, 141.1, 198.4, 199.4. HRMS calcd for $C_{30}H_{46}N_2S_2$ 498.3102, found 498.3109.

4.1.3. Compound 8b. Column chromatography on Al_2O_3 (CH_2Cl_2/hexane=1:4) afforded orange liquid, isolated yield 85% (269 mg). ^{1}H NMR ($CDCl_3$, TMS): δ 0.92–1.00 (m, 6H), 1.38–1.61 (m, 8H), 2.48–2.57 (m, 4H), 6.28 (d, J=7.2 Hz, 1H), 6.71–7.36 (m, 8H); ^{13}C NMR ($CDCl_3$, TMS): δ 14.0, 14.1, 23.0, 23.1, 23.8, 25.7, 30.5, 32.5, 118.2, 118.6, 123.6, 125.1, 125.8, 128.7, 129.0, 130.5, 137.7, 146.3, 148.6, 152.2, 166.8. HRMS calcd for $C_{23}H_{27}N$ 317.2144, found 317.2145.

4.1.4. Compound 8c. Column chromatography on Al_2O_3 (CH_2Cl_2/hexane=1:4) afforded orange liquid, isolated yield 82% (271 mg). ^{1}H NMR ($CDCl_3$, TMS): δ 0.82–0.90 (m, 6H), 1.26–1.54 (m, 8H), 2.28 (s, 3H), 2.39–2.47 (m, 4H), 6.28 (d, J=7.8 Hz, 1H), 6.63–6.71 (m, 3H), 6.93–7.06 (m, 4H); ^{13}C NMR ($CDCl_3$, TMS): δ 14.0, 14.1, 20.9, 22.9, 23.1, 23.8, 25.6, 30.5, 32.5, 118.1, 118.5, 125.0, 125.7, 128.7, 129.5, 130.4, 132.9, 137.8, 146.3, 148.3, 149.5, 166.7. HRMS calcd for $C_{24}H_{29}N$ 331.2300, found 331.2306.

4.1.5. Compound 8d. Column chromatography on Al_2O_3 (CH_2Cl_2/hexane=1:4) afforded orange liquid, isolated yield 87% (302 mg). ^{1}H NMR ($CDCl_3$, TMS): δ 0.92–1.00 (m, 6H), 1.35–1.61 (m, 8H), 2.48–2.57 (m, 4H), 3.82 (s, 3H), 6.43 (d, J=7.8 Hz, 1H), 6.74–7.17 (m, 7H); ^{13}C NMR ($CDCl_3$, TMS): δ 14.0, 14.1, 23.0, 23.1, 23.8, 25.6, 30.5, 32.5, 55.4, 114.3, 118.5, 119.6, 124.9, 125.8, 128.7, 130.4, 137.9, 145.4, 146.3, 148.3, 156.4, 167.2. HRMS calcd for $C_{24}H_{29}NO$ 347.2249, found 347.2250.

4.1.6. Compound 8e. Column chromatography on Al_2O_3

(CH_2Cl_2/hexane=1:9) afforded orange liquid, isolated yield 81% (312 mg). ^{1}H NMR ($CDCl_3$, TMS): δ 0.92–1.00 (m, 6H), 1.35–1.65 (m, 8H), 2.48–2.57 (m, 4H), 6.36 (d, J=7.2 Hz, 1H), 6.79–6.85 (m, 2H), 7.05 (d, J=7.8 Hz, 1H), 7.15–7.23 (m, 2H), 7.45 (d, J=2.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, TMS): δ 14.0, 14.1, 22.9, 23.1, 23.7, 25.7, 30.4, 32.3, 118.9, 120.5, 124.0, 124.3, 126.3, 127.5, 129.0, 129.0, 129.6, 131.2, 137.5, 146.1, 147.6, 150.0, 168.9. HRMS calcd for $C_{23}H_{25}NCl_2$ 385.1364, found 385.1362.

4.1.7. Compound 8f. Red-orange liquid, isolated yield 84% (271 mg). ^{1}H NMR ($CDCl_3$, TMS): δ 0.39 (t, J=7.2 Hz, 3H), 0.90–1.03 (m, 11H), 1.37–1.62 (m, 8H), 2.14–2.31 (m, 6H), 6.80 (d, J=7.2 Hz, 2H), 7.01 (t, J=7.5 Hz, 1H)), 7.24 (t, J=7.8 Hz, 2H)); ^{13}C NMR ($CDCl_3$, TMS): δ 14.0, 14.4, 14.6, 23.1, 23.8, 24.1, 26.1, 27.5, 28.2, 28.3, 118.1, 122.9, 124.1, 128.2, 131.4, 146.9, 151.4, 153.8, 170.5. HRMS calcd for $C_{23}H_{33}N$ 323.2613, found 323.2615.

4.1.8. Compound 8g. Red-orange liquid, isolated yield 63% (212 mg). ^{1}H NMR ($CDCl_3$, TMS): δ 0.40 (t, J=7.2 Hz, 3H), 0.90–1.02 (m, 11H), 1.36–1.65 (m, 8H), 2.14–2.31 (m, 9H), 6.70 (d, J=8.4 Hz, 2H), 7.04 (d, J=8.4 Hz, 2H)); ^{13}C NMR ($CDCl_3$, TMS): δ 13.9, 14.4, 14.6, 20.8, 23.1, 23.7, 24.1, 26.1, 27.6, 28.2, 28.3, 118.2, 124.0, 128.7, 131.5, 132.3, 146.7, 148.9, 153.6, 170.7. HRMS calcd for $C_{24}H_{35}N$ 337.2770, found 337.2764.

4.1.9. Compound 8h. Red-orange liquid, isolated yield 74% (261 mg). ^{1}H NMR ($CDCl_3$, TMS): δ 0.44 (t, J=7.2 Hz, 3H), 0.90–1.02 (m, 11H), 1.36–1.69 (m, 8H), 2.14–2.30 (m, 6H), 3.78 (s, 3H), 6.73–6.83 (m, 4H); ^{13}C NMR ($CDCl_3$, TMS): δ 14.0, 14.4, 14.6, 23.1, 23.6, 24.1, 26.1, 27.7, 28.2, 28.3, 55.6, 113.7, 119.6, 123.7, 131.6, 144.9, 146.8, 153.7, 156.1, 171.2. HRMS calcd for $C_{24}H_{35}ON$ 353.2719, found 353.2724.

4.1.10. Compound 8i. Red-orange liquid, isolated yield 79% (309 mg). ^{1}H NMR (C_6D_6, TMS): δ 0.45–0.50 (m, 3H), 0.87–1.03 (m, 11H), 1.40 (br, 4H), 1.77 (br, 4H), 2.14 (br, 4H), 2.50 (br, 2H), 6.39 (d, J=8.4 Hz, 1H), 6.76–6.79 (m, 1H) 7.22 (d, J=2.4 Hz, 1H); ^{13}C NMR (C_6D_6, TMS): δ 14.2, 14.5, 23.2, 23.8, 24.4, 26.5, 27.8, 28.4, 120.5, 124.0, 124.6, 127.0, 128.5, 129.3, 132.1, 147.4, 147.8, 154.6, 173.0. HRMS calcd for $C_{23}H_{31}NCl_2$ 391.1834, found 391.1839.

4.1.11. Compound 8j. Red-orange liquid, isolated yield 82% (383 mg). ^{1}H NMR ($CDCl_3$): δ 0.05 (s, 18H), 0.96 (t, J=6.6 Hz, 6H), 1.38–1.53 (m, 16H), 2.49 (t, J=7.8 Hz, 4H), 6.80–6.83 (m, 2H), 7.07–7.12 (m, 1H), 7.29–7.34 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 1.38, 14.06, 22.64, 28.91, 29.92, 31.33, 31.65, 122.58, 123.99, 128.57, 132.16 (appeared after long accumulation), 151.81, 167.23 (appeared after long accumulation), 179.57. HRMS calcd for $C_{29}H_{49}NSi_2$ 467.3404, found 467.3391.

4.1.12. Compound 8k. Red-orange liquid, isolated yield 74% (356 mg). ^{1}H NMR ($CDCl_3$): δ 0.06 (s, 18H), 0.96 (t, J=6.9 Hz, 6H), 1.38–1.54 (m, 16H), 2.38 (s, 3H), 2.49 (t, J=8.0 Hz, 4H), 6.73 (d, J=8.4 Hz, 2H), 7.13 (d, J=8.4 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 1.3, 14.0, 20.9, 22.5, 28.8, 29.8, 31.3, 31.6, 122.7, 129.1, 133.6, 149.5, 167.3 (appeared after long accumulation), 179.3. HRMS calcd for $C_{30}H_{51}NSi_2$ 481.3560, found 481.3554.

4.1.13. Compound 8l. Red-orange liquid, isolated yield 72% (385 mg). ^{1}H NMR ($CDCl_3$): δ 0.07 (s, 18H), 0.94 (t, J=6.9 Hz, 6H), 1.36–1.54 (m, 16H), 2.45 (br, 4H), 6.42 (d, J=8.4 Hz, 1H), 7.13–7.16 (m, 1H), 7.41 (d, J=2.4 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 1.2, 14.0, 22.6, 28.9, 29.9, 31.2, 31.6, 123.2, 126.9, 128.7, 129.3, 129.5, 131.6 (appeared after long accumulation), 147.6, 168.4 (appeared after long accumulation), 181.3. HRMS calcd for $C_{29}H_{47}NSi_2Cl_2$ 535.2624, found 535.2632.

4.1.14. Compound 8m. Red-orange liquid, isolated yield 59% (217 mg). ^{1}H NMR ($CDCl_3$): δ 0.01 (br, 18H), 1.67–1.71 (m, 4H), 2.37 (s, 3H), 2.71–2.76 (m, 4H), 6.74 (d, J=8.4 Hz, 2H), 7.13 (d, J=8.4 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 0.9, 21.0, 23.3, 28.4, 122.6, 129.2, 133.9, 149.7, 158.7 (appeared after long accumulation), 167.4 (appeared after long accumulation), 178.7. HRMS calcd for $C_{22}H_{33}NSi_2$ 367.2152, found 367.2157.

4.1.15. Compound 8n. Red-orange liquid, isolated yield 68% (260 mg). ^{1}H NMR ($CDCl_3$): δ −0.20 (br, 9H), 0.25 (br, 9H), 1.66–1.71 (m, 4H), 2.72–2.74 (m, 4H), 3.82 (s, 3H), 6.77–6.80 (m, 2H), 6.87–6.90 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 0.9, 23.3, 27.2, 29.6, 55.5, 114.1, 124.2, 135.8 (appeared after long accumulation), 146.1, 157.2, 158.3 (appeared after long accumulation), 167.3 (appeared after long accumulation), 178.7. HRMS calcd for $C_{22}H_{33}NOSi_2$ 383.2101, found 383.2106.

4.1.16. Compound 8o. Red-orange solid, mp: 153–154 °C isolated yield 61% (257 mg). ^{1}H NMR ($CDCl_3$): δ 0.03 (s, 18H), 1.68 (br, 4H), 2.69–2.76 (m, 4H), 6.45 (d, J=8.4 Hz, 1H), 7.14–7.18 (m, 1H), 7.41 (d, J=2.8 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 0.8, 23.1, 28.4, 122.9, 126.9, 128.6, 129.3, 129.5, 149.7, 164.4 (appeared after long accumulation), 180.4. HRMS calcd for $C_{21}H_{29}NSi_2Cl_2$ 421.1216, found 421.1223.

4.1.17. Compound 8p. Red-orange liquid, isolated yield 10% (39 mg). ^{1}H NMR ($CDCl_3$, TMS): δ 0.66–1.62 (m, 21H), 2.28–2.45 (m, 5H), 6.75 (d, J=8.4 Hz, 1H), 7.12–7.15 (m, 1H), 7.36 (d, J=2.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, TMS): δ 13.6, 14.1, 22.8, 23.0, 23.3, 24.4, 24.5, 24.9, 31.2, 32.1, 120.5, 122.2, 123.8, 126.7, 128.4, 128.9, 129.2, 144.2, 147.0, 151.2, 172.7. HRMS calcd for $C_{23}H_{29}NCl_2$ 389.1677, found 389.1662.

4.1.18. Compound 8p′. Red-orange liquid, isolated yield 57% (222 mg). ^{1}H NMR ($CDCl_3$, TMS): δ 0.71–1.82 (m, 18H), 2.21–2.58 (m, 6H), 3.30 (t, J=4.5 Hz, 1H), 5.64 (t, J=4.2 Hz, 1H), 6.77 (d, J=8.4 Hz, 1H), 7.11–7.14 (m, 1H), 7.36 (d, J=2.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, TMS): δ 13.8, 14.1, 22.3, 22.7, 22.7, 23.7, 24.3, 25.2, 26.3, 29.6, 30.5, 42.2, 119.5, 121.3, 125.6, 127.2, 127.8, 129.3, 140.2, 142.6, 148.0, 155.3, 178.8. HRMS calcd for $C_{23}H_{29}NCl_2$ 389.1677, found 389.1662.

4.1.19. Compound 8q′. Red-orange liquid, isolated yield 75% (241 mg). ^{1}H NMR ($CDCl_3$, TMS): δ 0.71 (t, J=6.9 Hz, 3H), 0.87–1.57 (m, 13H), 1.72–1.80 (m, 2H), 2.19–2.57 (m, 6H), 3.46 (t, J=4.2 Hz, 1H), 5.58 (t,

J=4.2 Hz, 1H), 6.83–7.28 (m, 5H); ^{13}C NMR ($CDCl_3$, TMS): δ 13.8, 14.1, 22.4, 22.6, 22.8, 23.7, 24.2, 25.2, 26.3, 29.2, 30.7, 41.3, 118.4, 119.6, 122.6, 128.7, 140.5, 143.1, 152.3, 153.6, 176.6. HRMS calcd for $C_{23}H_{31}N$ 321.2457, found 321.2449.

4.1.20. Compound 8r′. Red-orange liquid, isolated yield 76% (255 mg). ^{1}H NMR ($CDCl_3$, TMS): δ 0.71 (t, J=7.2 Hz, 3H), 0.90–1.55 (m, 13H), 1.72–1.80 (m, 2H), 2.21–2.56 (m, 9H), 3.46 (t, J=4.2 Hz, 1H), 5.57 (t, J=4.2 Hz, 1H), 6.73 (d, J=8.4 Hz, 2H), 7.06 (d, J=8.4 Hz, 2H); ^{13}C NMR ($CDCl_3$, TMS): δ 13.8, 14.1, 20.9, 22.4, 22.7, 22.8, 23.7, 24.2, 25.2, 26.3, 29.3, 30.7, 41.3, 118.2, 119.4, 129.2, 131.8, 140.6, 143.3, 149.7, 153.3, 176.6. HRMS calcd for $C_{24}H_{33}N$ 335.2613, found 335.2613.

4.1.21. Compound 8s′. Red-orange liquid, isolated yield 72% (253 mg). ^{1}H NMR ($CDCl_3$, TMS): δ 0.71 (t, J=7.2 Hz, 3H), 0.90–1.55 (m, 13H), 1.74–1.80 (m, 2H), 2.19–2.56 (m, 6H), 3.47 (br, 1H), 3.79 (s, 3H), 5.58 (t, J=4.2 Hz, 1H), 6.77–6.84 (m, 4H); ^{13}C NMR ($CDCl_3$, TMS): δ 13.8, 14.11, 22.4, 22.7, 22.8, 23.7, 24.2, 25.2, 26.3, 29.2, 30.7, 41.3, 55.4, 114.0, 118.2, 120.5, 140.6, 143.3, 145.7, 153.3, 155.5, 177.0. HRMS calcd for $C_{24}H_{33}NO$ 351.2562, found 351.2570.

4.1.22. Compound 12a. Colorless liquid. ^{1}H NMR ($CDCl_3$, TMS): δ 0.86 (t, J=7.5 Hz, 3H), 0.96–1.12 (m, 9H), 1.97–2.04 (m, 2H), 2.16–2.25 (m, 4H), 2.30 (s, 3H), 2.38–2.45 (m, 2H), 5.44 (t, J=7.2 Hz, 1H), 7.09 (d, J=4.2 Hz, 2H), 7.25 (s, 1H), 7.34 (d, J=4.2 Hz, 2H); ^{13}C NMR ($CDCl_3$, TMS): δ 12.8, 13.6, 13.8, 14.3, 20.8, 21.3, 22.8, 23.3, 24.4, 119.3, 129.4, 132.0, 133.4, 135.8, 135.8, 140.4, 145.1, 169.8. HRMS calcd for $C_{20}H_{29}NO$ 299.2249, found 299.2247.

4.1.23. Compound 13. Column chromatography on silica (CH_2Cl_2/hexane=1:1) afforded colorless solid, mp: 180–181 °C isolated yield 28% (484 mg, 4 mmol). ^{1}H NMR ($CDCl_3$, TMS): δ 0.98–1.03 (m, 12H), 2.17–2.42 (m, 14H), 7.10 (d, J=8.4 Hz, 4H), 7.49 (d, J=8.4 Hz, 4H), 8.93 (s, 2H); ^{13}C NMR ($CDCl_3$, TMS): δ 12.9, 13.5, 20.9, 23.5, 27.3, 119.9, 129.4, 133.7, 135.1, 135.7, 142.6, 170.1. HRMS calcd for $C_{28}H_{36}N_2O_2$ 432.2777, found 432.2787. Anal. calcd for $C_{28}H_{36}N_2O_2$: C, 77.74%; H, 8.39%; N, 6.48%; found: C, 77.40%; H, 8.40%; N, 6.41%.

5. Supporting information available

Experimental details and spectroscopic characterization of all new compounds, molecular structure, tables of crystallographic data, atomic coordinates, thermal parameters, bond lengths and angles for **8o**.

Acknowledgements

This work was partially supported by the Natural Science Foundation of China (29825105, 20172003, 20232010) and the Major State Basic Research Development Program (G2000077502-D). Cheung Kong Scholars Programme, Qiu Shi Science and Technologies Foundation, and Dow Corning Corporation are gratefully acknowledged.

References and notes

1. Brandsma, L. *J. Org. Chem.* **2001**, 4569–4581. (b) Mukerjee, A. K.; Ashare, R. *Chem. Rev.* **1991**, *91*, 1–24. (c) Esmail, R.; Kurzer, F. *Synthesis* **1975**, 301–314.
2. Wakefield, B. J. *Organolithium Methods*; Academic: London, 1988; p. 89 and 103. (b) Guy, G. In *The Chemistry of Cyanates and their Thio Derivatives*; Patai, S., Ed.; Wiley: London, 1977; Chapter 18.
3. Chivers, T.; Downard, A.; Parvez, M. *Inorg. Chem.* **1999**, *38*, 5565–5570. (b) Maeda, H.; Kambe, N.; Sonoda, N. *Tetrahedron* **1996**, *52*, 12165–12176. (c) Ares, J. J. *Synth. Commun.* **1991**, *21*, 625–633. (d) Seyferth, D.; Hui, R. C. *Tetrahedron Lett.* **1984**, *25*, 5251–5254. (e) Kelly, T. R.; Wchavarren, A.; Chandrakumar, N. S.; Koksal, Y. *Tetrahedron Lett.* **1984**, *25*, 2127–2130. (f) Krapcho, A. P.; Stephens, W. P. *J. Org. Chem.* **1980**, *45*, 1106–1109. (g) Ito, Y.; Kobayashi, K.; Saegusa, T. *Tetrahedron Lett.* **1979**, 1039–1042. (h) Gosselin, P.; Masson, S.; Thuillier, A. *Tetrahedron Lett.* **1978**, 2715–2716. (i) Walter, W.; Bode, K. D. *Angew. Chem., Int. Ed. Engl.* **1966**, *5*, 447–461. (j) Gilman, H.; Breuer, F. *J. Am. Chem. Soc.* **1933**, *55*, 1262–1264.
4. Nedolya, N. A.; Schlyakhtina, N. I.; Klyba, L. V.; Ushakov, I. A.; Fedorov, S. V.; Brandsma, L. *Tetrahedron Lett.* **2002**, *43*, 9679–9681. (b) Tarasova, O. A.; Klyba, L. V.; Vvedensky, V. Y.; Nedolya, N. A.; Trofimov, B. A.; Brandsma, L.; Verkruijsse, H. D. *Eur. J. Org. Chem.* **1998**, 253–256. (c) Nedolya, N. A.; Brandsma, L.; Trofimov, B. A. *Tetrahedron Lett.* **1997**, *38*, 6279–6280.
5. Ohno, A.; Nakamura, K.; Uohama, M.; Oka, S.; Yamabe, T.; Nagata, S. *Bull. Chem. Soc. Jpn* **1975**, *48*, 3718–3722. (b) Ohno, A.; Nakamura, K.; Uohama, M.; Oka, S. *Chem. Lett.* **1975**, 983–984. (c) Beak, P.; Worley, J. W. *J. Am. Chem. Soc.* **1972**, *94*, 597–604.
6. Oberhoff, M.; Duda, L.; Karl, J.; Mohr, R.; Erker, G.; Frohlich, R.; Grehl, M. *Organometallics* **1996**, *15*, 4005–4011. (b) Effenberger, F.; Gleiter, R.; Heider, L.; Niess, R. *Chem. Ber.* **1968**, *101*, 502–511.
7. Xi, Z.; Song, Q. *J. Org. Chem.* **2000**, *65*, 9157–9159. (b) Chen, J.; Song, Q.; Xi, Z. *Tetrahedron Lett.* **2002**, *43*, 3533–3535. (c) Wang, C.; Chen, J.; Song, Q.; Xi, Z. *Arkivoc* **2003**(ii), 155–164.
8. Xi, Z.; Song, Q.; Chen, J.; Guan, H.; Li, P. *Angew. Chem., Int. Ed. Engl.* **2001**, *40*, 1913–1916. (b) Chen, J.; Song, Q.; Li, P.; Guan, H.; Jin, X.; Xi, Z. *Org. Lett.* **2002**, *4*, 2269–2271.
9. Wenzel, M.; Beckert, B.; Gunther, W.; Gorls, H. *Eur. J. Org. Chem.* **1998**, 1803–1810.
10. Snieckus, V. *Chem. Rev.* **1990**, *90*, 879–933. (b) Snieckus, V. *Bull. Soc. Chim. Fr.* **1988**, 67–78. (c) Beak, P.; Zajdel, W. J.; Reitz, D. B. *Chem. Rev.* **1984**, *84*, 471–523. (d) Narasimhan, N. S.; Mali, R. S. *Synthesis* **1983**, 957–968. (e) Snieckus, V.; Beak, P. *Acc. Chem. Res.* **1982**, *15*, 305–312.
11. Preparative methods for 1,4-diiodo-1,3-diene derivatives, see: (a) Negishi, E.; Cederbaum, F. E.; Takahashi, T. *Tetrahedron Lett.* **1986**, *27*, 2829–2832. Negishi, E.; Holmes, S. J.; Tour, J. M.; Miller, J. A.; Cederbaum, F. K.; Swanson, D. R.; Takahashi, T. *J. Am. Chem. Soc.* **1989**, *111*, 3336–3346.

(c) Buchwald, S. L.; Nielsen, R. B. *J. Am. Chem. Soc.* **1989**, *111*, 2870–2874. (d) Hill, J. E.; Balaich, G.; Fanwick, P. E.; Rothwell, I. P. *Organometallics* **1993**, *12*, 2911–2924. (e) Yamaguchi, S.; Jin, R.; Tamao, K.; Sato, F. *J. Org. Chem.* **1998**, *63*, 10060–10062.

12. Xi, C.; Huo, S.; Afifi, T. H.; Hara, R.; Takahashi, T. *Tetrahedron Lett.* **1997**, *38*, 4099–4102.
13. Takahashi, T.; Tsai, F.; Li, Y.; Nakajima, K. *Organometallics* **2001**, *20*, 4122–4125. (b) Hill, J. E.; Balaich, G.; Fanwick, P. E.; Rothwell, I. P. *Organometallics* **1993**, *12*, 2911–2924.
14. Tamao, K.; Kobayashi, K.; Ito, Y. *Synlett* **1992**, 539–546. (b) Tamao, K.; Kobayashi, K.; Ito, Y. *J. Org. Chem.* **1989**, *54*, 3517–3519. (c) Eisch, J. J.; Aradi, A. A.; Han, K. I. *Tetrahedron Lett.* **1983**, *24*, 2073–2076. (d) Yamazaki, H.; Wakatsuki, Y. *Bull. Chem. Soc. Jpn* **1979**, *52*, 1239–1240. (e) Suzuki, Y.; Takizawa, T. *J. Chem. Soc., Chem. Commun.* **1972**, 837–838.
15. Crystal structures of analogues dilithio compounds of type **5** have been determined by X-ray analysis, (a) Pauer, F.; Power, P. P. *J. Orgnomet. Chem.* **1994**, *474*, 27–30. see also: (b) Bauer, W.; Feigel, M.; Muller, G.; Schleyer, P.; von, R. *J. Am. Chem. Soc.* **1988**, *110*, 6033–6046. (c) Schubert, U.; Neugebauer, W.; Schleyer, P.; von, R. *J. Chem. Soc., Chem. Commun.* **1982**, 1184–1185. (d) Maercker, A.; Bodenstedt, H.; Brandsma, L. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1339–1341.
16. Seyferth, D.; Hui, R. C. *Tetrahedron Lett.* **1984**, *25*, 2623–2626.
17. Insertion of isothiocyanates into M-X bonds to give their corresponding insertion products, see: (a) Braunstein, P.; Nobel, D. *Chem. Rev.* **1989**, *89*, 1927–1945, and references therein. Adams, R. D.; Huang, M. *Organometallics* **1995**, *14*, 4535–4541. (c) Adams, R. D.; Huang, M. *Organometllics* **1996**, *15*, 3644–3652. (d) Srinivas, B.; Chang, C.; Chen, C.; Chiang, M. Y.; Chen, I. T.; Wang, Y.; Lee, G. *J. Chem. Soc., Dalton Trans.* **1997**, 957–963. (e) Buter, R. N.; Grogan, D. C.; McDonald, P. D.; Burke, L. A. *J. Chem. Soc., Perkin Trans. 1* **1997**, 3587–3590.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron
Tetrahedron 60 (2004) 5215–5224

Application of directed metalation in synthesis. Part 6: A novel anionic rearrangement under directed metalation conditions leading to heteroannulation☆

Tarun Kanti Pradhan, Chandrani Mukherjee, Sukanta Kamila and Asish De*

Department of Organic Chemistry, Indian Association for the Cultivation of Science, Jadavpur, Kolkata 700 032, India

Received 8 January 2004; revised 24 March 2004; accepted 14 April 2004

Dedicated to Professor Victor Snieckus

Abstract—A short and efficient synthesis of condensed 1,4-oxathiin-2-ones from easily available phenols is described. The key step in this synthesis is a hitherto unreported anionic rearrangement under directed metalation conditions. The rearrangement occurs after side chain deprotonation of a methyl sulfanyl group by an *O*-carbamate directed metalating group and the reaction mixture is kept at room temperature for 8–12 h. Acid-mediated cyclisation of the rearranged product affords [1,4]oxathiin-2-one.

1. Introduction

The variety of rearrangements undergone by aromatic or heteroaromatic molecules after *ortho*-deprotonation under standard directed metalation conditions[2] have contributed to the ubiquitous nature of applications of heteroatom directed *ortho*-metalation[2] (Directed Metalation; DoM). When DoM is carried out with *O*-carbamate as the directed metalating group (DMG), the *ortho*-deprotonated species, in the absence of electrophile quench, was found to be susceptible to three types of rearrangements. All of these rearrangements using *O*-carbamate as DMG were reported by Snieckus who was the first to show the possibilities of anionic rearrangements under directed metalation conditions and also demonstrated their usefulness in organic synthesis. The first of these rearrangements is an anionic version of the *ortho*-Fries rearrangement[3,4] involving 1,3-carbamoyl transfer and christened as 'Snieckus rearrangement' by Gawley.[5] This rearrangement takes place after DMG mediated deprotonation at −78 °C, if the deprotonated species is allowed to reach room temperature instead of being quenched with an electrophile.

The second type of anionic rearrangement[6] termed as 'remote anionic *ortho*-Fries rearrangement' involving ring to ring carbamoyl transfer, provides regioselective entry into sterically encumbered biaryls as well as substituted and condensed dibenzo[*b,d*]pyranones and fluorenones.

The third type of anionic rearrangement[7] called 'anionic homologous *ortho*-Fries rearrangement' involves side chain deprotonation of *ortho*-alkyl substituents by an *O*-carbamate DMG followed by intramolecular anionic rearrangement. Subsequent acid mediated cyclisation of the rearranged product results in heteroannulation leading to benzofuranones. All these three types of anionic rearrangements are summarised in Scheme 1.

A fourth variety of anionic rearrangement under directed metalation condition, recently reported by us[1] involves the side chain deprotonation of *ortho*-methylsulfanyl substituents by an *O*-carbamate DMG followed by rearrangement. In common with the rearrangements described above, this rearrangement also provides access to interesting target molecules. Thus acid mediated cyclisation of the rearranged products affords condensed 1,4-oxathiin-2-ones in excellent yields, thereby providing an easy access to this heterocyclic system since the substrate for rearrangement can be obtained from phenols via successive *O*-carbamoylation and directed metalation.

Since publication of our preliminary findings, we have examined the scope of this rearrangement:

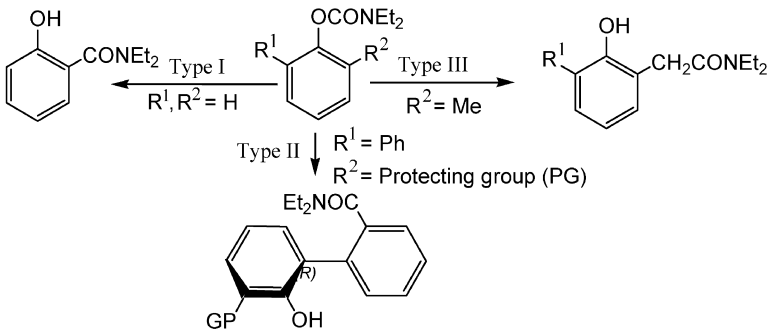
(a) by utilising new phenolic compounds as starting materials in the synthesis of condensed 1,4-oxathiin-2-ones in order to establish the generality of this synthesis

☆ For Part 5 see Ref. 1.

Keywords: Directed metalation; Anionic rearrangement; [1; 4]Oxathiin-2-one.

* Corresponding author. Tel.: +91-33-473-4971x246/253; fax: +91-33-473-2805; e-mail address: ocad@mahendra.iacs.res.in

0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.04.033



Scheme 1. Reagents and conditions: *s*-BuLi/THF/TMEDA/−78 °C to rt.

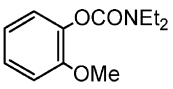
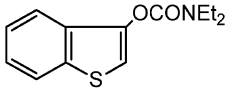
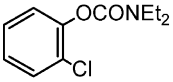
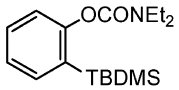
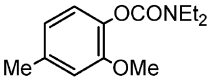
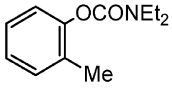
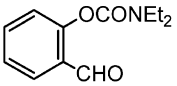
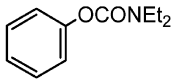
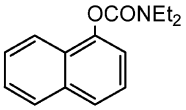
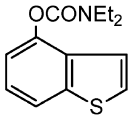
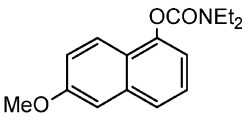
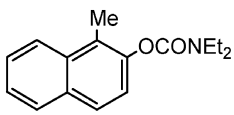
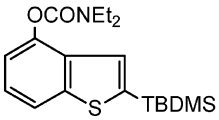
(b) by examining the selectivity of this rearrangement viz a viz other types of rearrangements reported by Snieckus through examining substrates where other types of rearrangement could also take place.

The results which we have obtained so far are presented herein.

2. Results and discussion

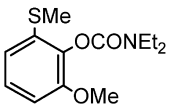
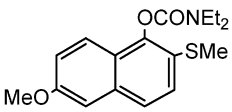
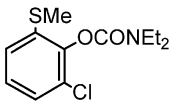
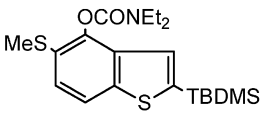
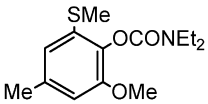
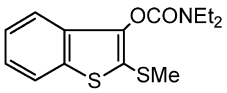
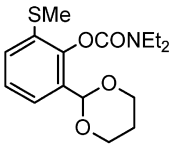
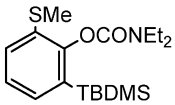
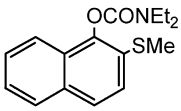
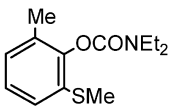
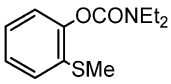
For heteroannulation we have chosen benzene, naphthalene and benzo[*b*]thiophene as basic aromatic cores. The corresponding starting materials were phenols (parent compound as well as substituted phenols), α- and β-naphthol (with and without substituents) and hydroxybenzo[*b*]thiophenes. *O*-Carbamates were prepared in good yields by treating the corresponding hydroxy compounds with *N*,*N*-diethylcarbamoyl chloride in tetrahydrofuran (THF) in the presence of sodium hydride (Table 1). Compound **1h** was prepared from 2,3-dihydrobenzo[*b*]thiophene-3-one which was synthesised via an expedient route reported[8,9] earlier by us. *Ortho*-deprotonation of *O*-carbamates with 2.5 equiv. of *s*-BuLi in THF in the presence of tetramethylethylenediamine (TMEDA) followed by

Table 1. Aryl *O*-carbamate

Entry	Compound	Yield (%)	Reference	Entry	Compound	Yield (%)	Reference
1a		90	2e	**1h**		87	—
1b		94	2e	**1i**		86	—
1c		95	2e	**1j**		93	2e,3,7a
1d		85	—	**1k**		96	2e,3,14
1e		89	2e,13,14	**1l**		95	10
1f		96	15	**1m**		87	2e,7a
1g		73	10				

Note: introduction of TBDMS group in compounds **1g** and **1i** done from **11** and **1k**, respectively using *s*-BuLi/TMEDA/THF/TBDMSC1.

Table 2. *N*,*N*-Diethyl *ortho*-methylsulfanyl aryl carbamates

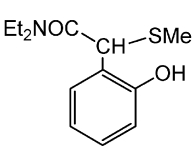
Entry	Compound	Yield (%)	Entry	Compound	Yield (%)
2a		81	**2f**		84
2b		92	**2g**		75
2c		96	**2h**		93
2d		80	**2i**		55
2e		82	**2j**[a]		92
			2k		92

[a] See Scheme 3.

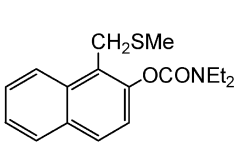
quenching with dimethyl disulfide afforded the corresponding *ortho*-methylsulfanyl derivatives in excellent yields (Table 2).

In the case of parent *N*,*N*-diethyl carbamoyloxy benzene (**1k**) it was necessary to protect one of the two free *ortho*-positions of the *O*-carbamate function as a *t*-butyl dimethyl silyl (TBDMS) derivative, by quenching the deprotonated species with TBDMSCl in order to prevent anionic *ortho*-Fries rearrangement which occurs in preference to the desired rearrangement (vide infra) when a substrate can undergo both. Similar silyl protection of the 2-position of *N*,*N*-diethyl-4-carbamyloxybenzo[*b*]thiophene (**1l**) was needed because it was preferentially deprotonated in the 2-position.[10] Not unexpectedly[2e] attempts to introduce a methylsulfanyl function in the 2-position of *N*,*N*-diethyl-2-methyl carbamoyloxy benzene (**1j**) and the 3-position of *N*,*N*-diethyl-1-methyl-2-carbamyloxy naphthalene (**1m**) by directed lithiation resulted in the formation of **9** and **10**, respectively. It is apparent that DMG induced lateral deprotonation in **9** was followed by electrophile quench and anionic homologus *ortho*-Fries rearrangement because of the use of 2 equiv. of *s*-BuLi and 1 equiv. of dimethyldisulfide.

8

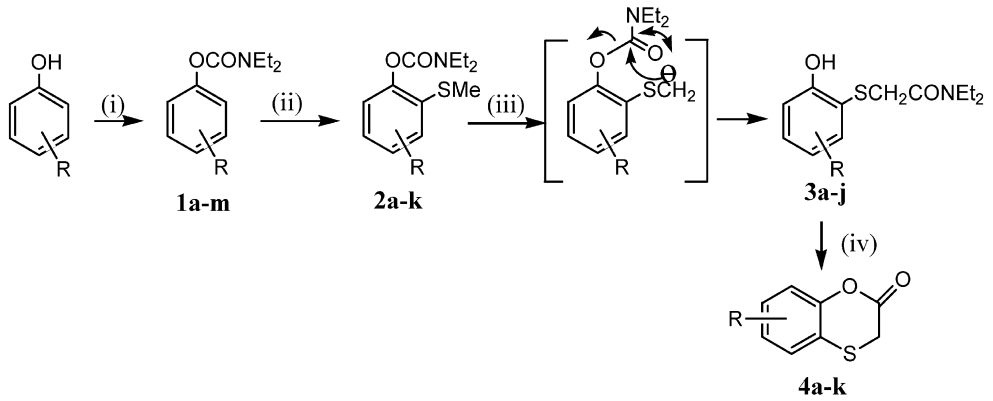


9



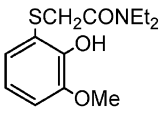
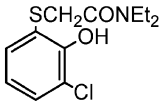
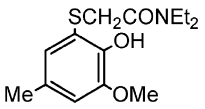
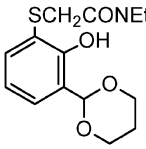
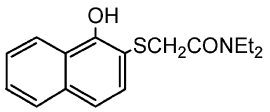
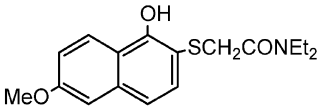
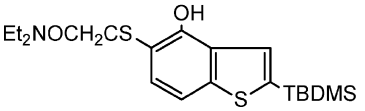
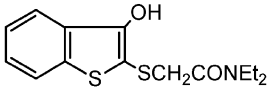
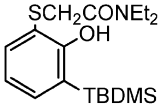
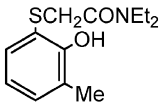
10

While side chain deprotonation of the methylsulfanyl group in the presence of an *ortho*-tertiary amide was earlier accomplished with LDA, all attempts to generate SCH_2^- anion in the presence of the *ortho*-*O*-carbamate DMG by LDA resulted in the recovery of starting materials. Use of *s*-BuLi at −78 °C instead of LDA was however successful in generating the anion. Allowing the reaction mixture to attain room temperature and maintaining that temperature for 8–12 h resulted in anionic rearrangement (Scheme 2) affording *N*,*N*-diethyl-2-hydroxy aryl thioacetamides in good to excellent yields (Table 3). We have reported[1] earlier that when **2k** was subjected to rearrangement conditions, the only product obtained was *N*,*N*-diethyl-2-hydroxy-3-methylsulfanyl benzamide (**8**) from a 'normal' anionic *ortho*-Fries rearrangement. It was conjectured[1] that the preferential ring deprotonation was due to the use of *s*-BuLi instead of LDA for generating the SCH_2^- anion (vide supra). It thus appears that 'normal' anionic *ortho*-Fries rearrangement occurs in preference to the desired rearrangement. After one of the two free *ortho*-positions was protected as a *t*-butyldimethylsilyl (TBDMS) derivative (**2i**), deprotonation and rearrangement afforded *N*,*N*-diethyl-2-hydroxy-3-*t*-butyldimethylsilyl phenyl thioacetamide (**3i**) in 64% yield. The substrate *ortho*-carbamate **2j** needed for examining the selectivity between an anionic homologous *ortho*-Fries rearrangement versus the desired rearrangement in its simplest form could not be obtained in a straightforward way. Compound **2j** was ultimately synthesised from **2d** in several steps as shown in Scheme 3. Under rearrangement conditions (*s*-BuLi/TMEDA/THF/−78 °C to rt/8–10 h) compound **2j** afforded *N*,*N*-diethyl-2-hydroxy-3-methyl phenyl



Scheme 2. Reagents and conditions: (i) NaH/THF/ClCONEt$_2$; (ii) *s*-BuLi/TMEDA/THF/dimethyldisulfide/−78 °C; (iii) *s*-BuLi/TMEDA/THF/−78 °C to rt; (iv) aceticacid/reflux.

Table 3. *N*,*N*-Diethyl-2-hydroxy aryl thioacetamide

Entry	Compound	Yield (%)
3a		93
3b		75
3c		85
3d		72
3e		92
3f		90
3g		83
3h		78
3i		64
3j		63

thioacetamide (**3j**) as the exclusive reaction product indicating that carbamoyl transfer to the methylsulfanyl side chain took place in preference to the methyl substituent.

Heating the rearranged products with glacial acetic acid under reflux for 18–20 h resulted in the annulation of [1,4]-oxathiin ring (Scheme 2) to the existing aromatic core in excellent yields (Table 4). Compound **4k** was obtained after desilylation of **4i** with tetrabutylammonium fluoride in 89% yield.

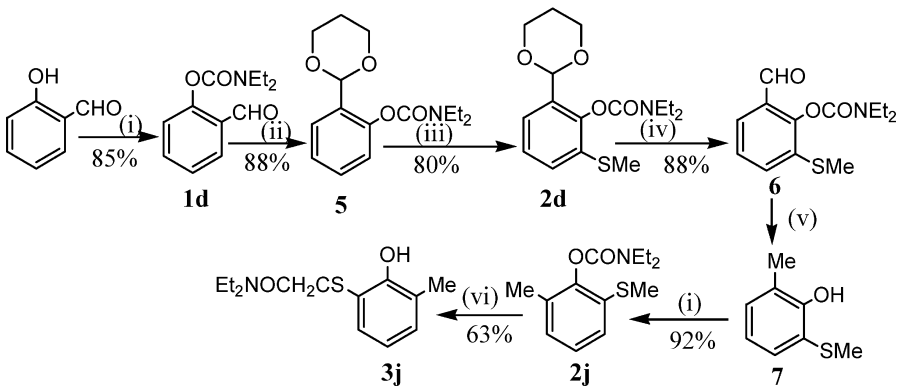
3. Conclusion

The high yielding anionic rearrangement reported above, characterised by its wide scope and selectivity with respect to anionic homologous *ortho*-Fries rearrangement is yet another example of the power of directed metalation as a synthetic tool. This is further corroborated by the key role which this rearrangement plays in the short and efficient synthesis of condensed [1,4]oxathiin-2-ones, which compares favourably with the earlier reported[11,12] synthesis of this class of compounds.

4. Experimental

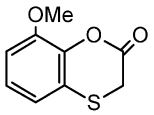
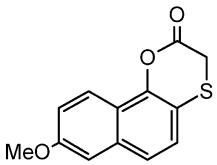
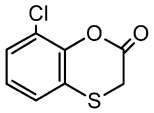
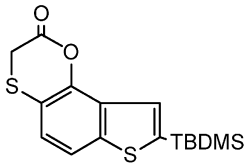
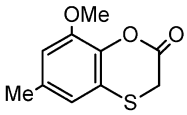
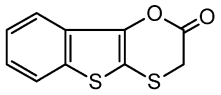
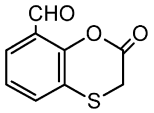
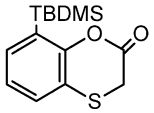
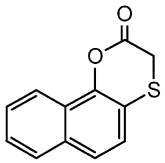
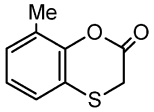
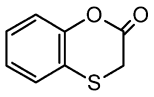
4.1. General

Melting points (uncorrected) were recorded in open capillaries on a hot stage apparatus. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on Bruker DPX-300 spectrometer. Chemical shifts (δ) are expressed in ppm using tetramethylsilane as internal standard. IR spectra were recorded on FTIR-8300, SHIMADZU spectrometer, for solids in KBr discs and for liquids by placing a thin layer of the sample between two KBr discs. Commercially available solvents were purified by distillation. Diethyl ether and tetrahydrofuran, after keeping overnight over potassium hydroxide were further purified by benzophenone ketyl method. Both *n*- and *s*-butyl lithium were prepared by slow addition of the appropriate halide to the freshly prepared dispersion of granular lithium or lithium chips in *n*-hexane (for *n*-butyl lithium) or cyclohexane (for *s*-butyl lithium). Petroleum ether has boiling point 60–80 °C.



Scheme 3. Reagents and conditions: (i) NaH/THF/$ClCONEt_2$; (ii) 1,3-propanediol/C_6H_6/$FeCl_3$; (iii) *s*-BuLi/THF/TMEDA/ dimethyldisulfide/−78 °C; (iv) 1:1 aq. MeOH/$FeCl_3$; (v) hydrazenehydrate/DIGOL/KOH; (vi) *s*-BuLi/TMEDA/THF/−78 °C to rt.

Table 4. Annulated oxathiin-2-one

Entry	Compound	Yield (%)	Entry	Compound	Yield (%)
4a		82	**4f**		84
4b		81	**4g**		90
4c		83	**4h**		91
4d		92	**4i**		81
4e		86	**4j**		89
			4k		89

For compounds **2c**, **3c** and **4c** see Ref. 1.

Silicagel (60–120 mesh) was used for column chromatography.

4.1.1. *N,N*-Diethyl-1-carbamoyloxybenzene-2-carboxaldehyde (1d). Prepared by dropwise addition of the solution of salicylaldehyde (4.88 g, 40 mmol) in THF (15 mL) to the stirred mixture of NaH (60%) (4 g, 100 mmol) in THF (30 mL). Stirring was continued for 1 h at room temperature and *N,N*-diethylcarbamoylchloride (7 mL, 50 mmol) in THF (10 mL) was added. The mixture was left under stirring for 10 h. After removal of most of the solvent, residue decomposed with water and extracted with diethyl ether. The ethereal layer was washed with water, dried over Na_2SO_4 and evaporated to afford the crude product. Purified by column chromatography [eluent: ethyl acetate–light petroleum (1:9)].

Colourless oil, yield 6.36 g, 72%. [Found: C, 65.26; H, 6.91; N, 6.38. $C_{12}H_{15}NO_3$ requires C, 65.14; H, 6.83; N, 6.33%]; ν_{max} (Neat) 1726.2, 1679 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 10.17 (1H, s, C*H*O), 7.84 (1H, d, *J*=7.5 Hz), 7.60–7.54 (1H, m, ArH), 7.32–7.24 (1H, m), 7.19 (1H, d, *J*=8.1 Hz), 3.56–3.34 (4H, m, CH_2CH_3), 1.36–1.17 (6H, m, CH_2CH_3); δ_C (75 MHz, $CDCl_3$) 188.5, 153.4, 153.0, 134.9, 129.4, 128.5, 125.5, 123.4, 42.3, 41.9, 14.1, 13.1.

4.1.2. *N*,*N*-Diethyl-3-carbamoyloxybenzo[*b*]thiophene (1h). Prepared from 2,3-dihydrobenzo[*b*]thiophene-3-one (1.5 g, 10 mmol) in THF (15 mL), NaH (60%) (0.8 g, 20 mmol) in THF (30 mL) and *N*,*N*-diethylcarbamoyl-chloride (2.7 mL, 20 mmol) following the same procedure. Purified by column chromatography [eluent: ethyl acetate–light petroleum (7.5: 92.5)], colourless oil, yield 2.16 g, 87%. [Found: C, 62.83; H, 6.26; N, 5.34. $C_{13}H_{15}NO_2S$ requires C, 62.62; H, 6.06; N, 5.62%]; ν_{max} (Neat) 1720 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.81–7.78 (1 H, m), 7.71–7.68 (1H, m), 7.41–7.34 (3H, m), 3.54–3.40 (4H, m, CH_2CH_3), 1.36–1.22 (6H, m, CH_2CH_3); δ_C (75 MHz, $CDCl_3$) 153.0, 141.2, 136.8, 132.5, 124.8, 124.0, 122.8, 120.2, 110.6, 42.4, 42.1, 14.2, 13.3.

4.1.3. *N*,*N*-Diethyl-2-*t*-butyldimethylsilyl carbamoyloxybenzene (1i). To a well stirred solution of TMEDA (0.33 mL, 2.2 mmol) in anhydrous THF (10 mL) kept under argon, *s*-BuLi (2 M, 1.1 mL, 2.2 mmol) was added through syringe at −78 °C. After 10 min **1k** (0.5 g, 2 mmol) in THF (5 mL) was added in the same way. After stirring for 20 min at that temperature TBDMSCl (0.36 g, 2.4 mmol) in THF (3 mL) was added. The reaction mixture was allowed to attain room temperature and stirred at that temperature for 10 h. After this period saturated ammonium chloride solution was added and the mixture was stirred for 5 min. After removal of most of the THF under reduced pressure, the residue was extracted with diethyl ether. The ethereal extract was washed with water and dried (Na_2SO_4). The residue left after removal of the solvent was purified by column chromatography [eluent: ethylacetate–petroleum (1:9)], colourless oil, yield 0.68 g, 86%. [Found: C, 66.53; H, 9.72; N, 4.42. $C_{17}H_{29}NO_2Si$ requires C, 66.40; H, 9.51; N, 4.55%]; ν_{max} (Neat) 1722 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.22–7.14 (2 H, m), 6.96–6.91 (1H, m), 6.78 (1H, d, *J*=7.2 Hz), 3.60–3.42 (2H, m, CH_2CH_3), 3.23–3.08 (2H, m, CH_2CH_3), 1.24–1.20 (3H, t, *J*=7.1 Hz, CH_2CH_3), 1.01–0.91 (12H, m, CH_2CH_3, $SiCCH_3$), 0.20 (6H, br s, $SiCH_3$); δ_C (75 MHz, $CDCl_3$) 168.9, 150.9, 129.6, 129.4, 127.7, 121.2, 119.1, 42.7, 39.0, 25.5, 17.9, 13.9, 13.0, −4.2, −4.6.

4.1.4. General procedure for the synthesis of *N*,*N*-diethyl-1-carbamoyloxy-2-methylsulfanyl arenes. *N*,*N*-Diethyl-1-carbamoyloxy-2-methylsulfanyl-6-methoxy benzene (2a). To a well-stirred solution of TMEDA (3.4 mL, 22 mmol) at −78 °C *s*-BuLi (14 mL of 1.6 M solution, 22 mmol) was added through needle syringe system. After 5 min **1a** (2 g, 9 mmol) in THF (15 mL) was added and kept at that temperature for 30 min. Then dimethyl disulfide (1.8 mL, 20 mmol) was added and the reaction mixture was allowed to reach room temperature followed by stirring at that temperature for 10 h. Ammonium chloride work up as described before afforded the compound **2a**. Purification by crystallisation (ethyl acetate–petroleum) afforded colourless solid, yield 1.9 g, 81%, mp 65–67 °C. [Found: C, 58.00; H, 7.20; N, 5.30. $C_{13}H_{19}O_3NS$ requires C, 57.99; H, 7.06; N, 5.20%]; ν_{max} (Neat) 1724 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.06 (1 H, dd, *J*=8.0, 8.1 Hz), 6.68 (2H, m), 3.74 (3H s, O*Me*), 3.37 (4H, q, *J*=7.0 Hz, CH_2CH_3), 2.34 (3H, s, S*Me*), 1.19 (6H, t, *J*=7.0 Hz, CH_2CH_3); δ_C (75 MHz, $CDCl_3$) 153.5, 152.6, 137.9, 133.9, 126.5 118.2, 109.6, 56.5, 42.5, 42.4, 15.4, 14.5, 13.8.

4.1.5. *N*,*N*-Diethyl-1-carbamoyloxy-2-chloro-6-methylsulfanyl benzene (2b). Prepared in the same way from *N*,*N*-Diethyl-1-carbamoyloxy-2-chlorobenzene (1.8 g, 8 mmol), using TMEDA (2.6 mL, 18 mmol), *s*-BuLi (9.2 mL of 1.9 M solution, 18 mmol), dimethyl disulfide (1.6 mL, 18 mmol). Purified by column chromatography [eluent: ethyl acetate–petroleum (3:17)] to afforded yellowish gummy material, yield 2 g, 92%. [Found: C, 52.33; H, 6.1; N, 5.3. $C_{12}H_{16}O_2ClNS$ requires C, 52.65; H, 5.85; N, 5.11%]; ν_{max} (Neat) 1732 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.18 (1H, dd, *J*=2.3, 7.3 Hz) 7.12–7.01 (2H, m), 3.52–3.40 (4H, q, *J*=7.1 Hz, CH_2CH_3), 2.39 (3H, s, S*Me*), 1.31 (3H, t, *J*=7.1 Hz, CH_2CH_3), 1.19 (3H, t, *J*=7.1 Hz, CH_2CH_3); δ_C (75 MHz, $CDCl_3$) 152.5, 144.8, 135.5, 128.8, 126.9, 126.7, 126.6, 42.9, 42.6, 15.4, 14.6, 13.7.

4.1.6. 2-(*N*,*N*-Diethyl-1-carbamoyloxy-2-methylsulfanyl-phenyl-6-)-1,3-dioxane (2d). Prepared from 2-(*N*,*N*-diethyl-1-carbamoyloxy phenyl-2-)-1,3-dioxane (**5**) (1.11 g, 4 mmol), THF (10 mL), *s*-BuLi [1.9 M (4 mL, 8 mmol)], TMEDA (1.2 mL, 8 mmol) and dimethyldisulfide (0.7 mL, 8 mmol) following the same procedure. Purified by column chromatography over alumina [eluent: ethyl acetate–petroleum (1:4)], crystallised from ether, yield 1.03 g, 80%, mp 97–99 °C. [Found: C, 59.16; H, 7.23; N, 4.48. $C_{16}H_{23}NO_4S$ requires C, 59.05; H, 7.12; N, 4.30%]; ν_{max} (KBr) 1724.2 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.47–7.44 (1H, m), 7.23–7.17 (2H, m), 5.53 (1H, s, OC*H*O), 4.19–3.86 (4H, m, OCH_2CH_2), 3.47 (2H, q, *J*=7.0 Hz, NCH_2), 3.37 (2H, q, *J*=7.0 Hz, NCH_2), 2.39 (3H, s, S*Me*), 2.39–2.12 (2H, m, $CH_2CH_2CH_2$), 1.30 (3H, t, *J*=7.0 Hz, CH_2CH_3), 1.19 (3H, t, *J*=7.0 Hz, CH_2CH_3); δ_C (75 MHz, $CDCl_3$) 152.8, 146.0, 132.7, 131.7, 127.2, 125.9, 123.8, 97.9, 67.3, 42.5, 41.9, 25.5, 15.3, 14.2, 13.3.

4.1.7. *N*,*N*-Diethyl-2-methylsulfanyl-1-carbamoyloxy naphthalene (2e). Prepared in the same way from **1e** (1.9 g, 8 mmol), *s*-BuLi [2.3 M (4 mL, 10 mmol)] in THF (10 mL), TMEDA (1.5 mL, 10 mmol) and dimethyl-disulfide (1 mL, 12 mmol). Purified by column chromatography [eluent: ethyl acetate–petroleum (1:8)], crystallised from petroleum, white solid, yield 1.8 g, 82%, mp 83–85 °C. [Found: C, 66.61; H, 6.79; N, 4.96. $C_{16}H_{19}NO_2S$ requires C, 66.40; H, 6.62; N, 4.84%]; ν_{max} (KBr) 1708.8 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.97–7.82 (2H, m), 7.70 (1H, d, *J*=7.9 Hz), 7.54–7.40 (3H, m), 3.62 (2H, q, *J*=7.1 Hz, CH_2CH_3), 3.49 (2H, q, *J*=7.1 Hz, CH_2CH_3), 2.52 (3H, s, S*Me*), 1.41 (3H, t, *J*=7.1 Hz, CH_2CH_3), 1.25 (3H, t, *J*=7.1 Hz, CH_2CH_3); δ_C (75 MHz, $CDCl_3$) 153.7, 144.7, 133.0, 128.5, 128.3, 128.2, 127.3, 126.4, 126.0, 125.1, 121.4, 42.9, 42.6, 16.1, 14.9, 13.8.

4.1.8. *N*,*N*-Diethyl-1-carbamoyloxy-2-methylsulfanyl-6-methoxynaphthalene (2f). Prepared in the same way

from **1f** (2 g, 7.3 mmol), TMEDA (2.73 mL, 18 mmol), *s*-BuLi (11.4 mL of 1.6 M solution, 18 mmol) and dimethyl disulfide (1.44 mL, 18 mmol). Purified by crystallisation (ethyl acetate–petroleum). White powder, yield 1.98 g, 84%, mp 64–65 °C. [Found: C, 64.00; H, 6.66; N, 4.27. $C_{17}H_{21}O_3NS$ requires C, 63.90; H, 6.58; N, 4.38%]; ν_{max} (Neat) 1718 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.6 (1H, d, *J*=9.1 Hz), 7.5 (1H, d, *J*=8.6 Hz), 7.35 (1H, d, *J*=8.6 Hz), 7.08 (1H, dd, *J*=2.4, 9.1 Hz), 7.02 (1H, d, *J*=2.4 Hz), 3.82 (3H, s, O*Me*), 3.56 (2H, q, *J*=7.1 Hz, CH_2CH_3), 3.38 (2H, q, *J*=7.1 Hz, CH_2CH_3), 2.4 (3H, s, S*Me*), 1.34 (6H, t, *J*=7.1 Hz, CH_2CH_3); δ_C (75 MHz, $CDCl_3$) 166.3, 158.0, 153.8, 145.6, 134.6, 126.8, 125.2 124.1, 123.2, 120.1, 106.2, 55.7, 42.8 42.6, 16.8, 14.8, 13.7.

4.1.9. *N*,*N*-Diethyl-2-*t*-butyldimethylsilyl-5-methylsulfunyl-4-carbamolyloxy benzo[*b*]thiophene (2g). Prepared in the same way from **1g** (0.363 g, 1 mmol), *s*-BuLi [2 M (1 mL, 2 mmol)] in THF (7 mL), TMEDA (0.3 mL, 2 mmol) and dimethyldisulfide (0.2 mL, 2 mmol). Purified by column chromatography [eluent: ethyl acetate–petroleum ether (7.5:82.5)], yield 0.30 g, 75%, mp 77–79 °C. [Found: C, 58.58; H, 7.60; N, 3.33. $C_{20}H_{31}NO_2S_2Si$ requires C, 58.63; H, 7.63; N, 3.42%]; ν_{max} (Neat) 1728.1 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.52 (1H, d, *J*=8.7 Hz), 7.20–7.15 (2H, m), 3.50–3.30 (4H, m, CH_2CH_3), 2.33 (3H, s, S*Me*), 1.28–1.10 (6H, m, CH_2CH_3), 0.83 (9H, s, CCH_3], 0.20 (6H, s, $SiCH_3$); δ_C (75 MHz, $CDCl_3$) 153.6, 144.7, 143.9, 141.5, 136.3, 127.9, 126.2, 126.0, 42.9, 42.7, 26.7, 17.5, 17.2, 17.1, 13.8, −4.6.

4.1.10. *N*,*N*-Diethyl-2-methylsulfanyl-3-carbamoyloxy-benzo[*b*]thiophene (2h). Prepared in the same way as before starting from **1h** (2.5 g, 10 mmol), *s*-BuLi [2 M (10 mL, 20 mmol), TMEDA (3 mL, 20 mmol) and dimethyldisulfide (2 mL, 22 mmol). Purified by column chromatography [eluent: ethyl acetate–petroleum (1:9)], to afford colorless oil, yield 2.7 g, 93%. [Found: C, 56.84; H, 5.68; N, 4.86. $C_{14}H_{17}NO_2S_2$ requires C, 56.92; H, 5.80; N, 4.74%]; ν_{max} (Neat) 1726 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.67 (1H, dd, *J*=2.0, 6.9 Hz), 7.53 (1H, dd, *J*=2.0, 6.9 Hz), 7.38–7.31 (2H, m), 3.52 (2H, q, *J*=7.0 Hz, NCH_2), 3.40 (2H, q, *J*=7.0 Hz, NCH_2), 2.50 (3H, s, S*Me*), 1.35 (3H, t, *J*=7.0 Hz, CH_2CH_3), 1.23 (3H, t, *J*=7.0 Hz, CH_2CH_3); δ_C (75 MHz, $CDCl_3$) 152.9, 142.6, 137.2, 133.2, 125.0, 124.9, 122.3, 120.3, 42.5, 42.2, 19.9, 14.2, 13.3.

4.1.11. *N*,*N*-Diethyl-2-methylsulfanyl-6-*t*-butyldimethylsilyl carbamoyloxy benzenes (2i). Prepared by the same procedure from **1i** (0.8 g, 2.6 mmol) *s*-BuLi [1.3 M (4 mL, 5 mmol)], in THF (10 mL), TMEDA (0.8 mL, 5 mmol) and dimethyldisulfide (0.5 mL, 5 mmol). Purified by column chromatography [eluent: ethyl acetate–petroleum (12.5:87.5)], colourless oil, yield 0.50 g, 55%. [Found: C, 61.36; H, 8.63; N, 3.81. $C_{18}H_{31}NO_2SSi$ requires C, 61.14; H, 8.84; N, 3.96%]; ν_{max} (Neat) 1726.2 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.03 (1H, dd, *J*=8.4, 7.8 Hz), 6.76 (1H, d, *J*=7.8 Hz), 6.53 (1H, d, *J*=8.4 Hz), 3.31–3.22 (2H, m, NCH_2), 3.10–3.01 (2H, m, NCH_2), 1.16 (3H, t, *J*=7.2 Hz, CH_2CH_3), 0.96 (3H, d, *J*=7.2 Hz, CH_2CH_3), 0.87 (9H, s, CCH_3), 0.17–0.15 (6H, d, $SiCH_3$); δ_C (75 MHz, $CDCl_3$) 166.5, 151.4, 136.6, 126.3, 125.5, 119.1, 115.8, 42.6, 38.9, 25.4, 14.8, 13.9, 13.0, 12.7, −4.1, −4.7.

4.1.12. *N*,*N*-Diethyl-2-methylsulfanyl-6-methyl carbamoyloxybenzene (2j). Compound **6** (0.267 g, 1 mmol) was heated at 160 °C with hydrazenehydrate (0.2 g, 4 mmol) in DIGOL (5 mL) for 30 min. Then KOH (0.392 g, 8 mmol) in DIGOL (3 mL) was added to the reaction mixture and heated at 170 °C for 1 h. The reaction mixture was allowed to attain room temperature and poured in to ice water, extracted with diethyl ether (3×30 mL), organic layer washed with water and dried over Na_2SO_4. Evaporation of the solvent left a gummy mass, which did not show any carbonyl peak in the IR, but showed a broad peak at 3390 cm^{-1} (OH) indicating the hydrolysis of *O*-carbamate function. The crude phenol was reconverted into *O*-carbamate in the same way which was purified by column chromatography [eluent: ethyl acetate–petroleum ether (1:9)]. Colourless liquid, overall yield 0.17 g, 68%. [Found: C, 61.72; H, 7.72; N, 5.59. $C_{13}H_{19}NO_2S$ requires C, 61.63; H, 7.56; N, 5.53%]; ν_{max} (Neat) 1722.3 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.12–6.99 (3H, m), 3.47 (2H, q, *J*=6.6 Hz, NCH_2), 3.36 (2H, q, *J*=6.6 Hz, NCH_2), 2.40 (3H, s, S*Me*), 2.19 (3H, s, $ArCH_3$), 1.30 (3H, t, *J*=6.6 Hz, CH_2CH_3), 1.19 (3H, t, *J*=6.6 Hz, CH_2CH_3); δ_C (75 MHz, $CDCl_3$) 152.8, 146.8, 132.1, 131.4, 127.3, 125.8, 123.7, 42.2, 41.9, 16.2, 14.9, 14.2, 13.3.

4.1.13. *N*,*N*-Diethyl-1-carbamoyloxy-2-methylsulfanyl-benzene (2k). Prepared in the same way starting from **1k** (1.2 g, 6.5 mmol), TMEDA (2.45 mL, 15 mmol), in dry THF (10 mL), *s*-BuLi (8.6 mL of 1.9 M, 15 mmol) and dimethyl disulfide (1.2 mL, 15 mmol). Purified by column chromatography [eluent: ethyl acetate–petroleum (3:17)]. Yellowish oil, yield 1.38 g, 92%. [Found: C, 62.9; H, 7.5; N, 6.3. $C_{12}H_{17}O_2NS$ requires C, 62.88; H, 7.42; N, 6.11%]; ν_{max} (Neat) 1722 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.18–7.03 (4H, m), 3.39–3.34 (4H, m, CH_2CH_3), 2.35 (3H, s, S*Me*), 1.18–1.15 (6H, m, CH_2CH_3); δ_C (75 MHz, $CDCl_3$) 153.9, 148.9, 132.2, 126.8, 126.4, 126.3, 123.2, 42.6, 42.5, 15.4, 14.6, 13.9.

4.1.14. General procedure for the synthesis of substituted *N*,*N*-diethyl-2-hydroxy aryl thioacetamides. *N*,*N*-Diethyl-2-hydroxy-3-methoxy phenyl thioacetamide (3a). To a well stirred solution of dry THF (10 mL), TMEDA (0.75 mL, 4 mmol), *s*-BuLi (2.6 mL of 1.6 M solution, 4 mmol), **2a** (0.45 g, 1.6 mmol) in dry THF (10 mL) was added at −78 °C and kept at that temperature for 30 min. Stirring at room temperature for 10 h and ammonium chloride work up as described before afforded compound **3a** which was purified by column chromatography [eluent: ethyl acetate–petroleum ether (1:8)], oily liquid, yield 0.42 g, 93%. [Found: C, 58.04; H, 7.20; N, 5.31. $C_{13}H_{19}O_3NS$ requires C, 57.90; H, 7.06; N, 5.20%]; ν_{max} (Neat) 3244, 1622 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 6.96 (1H, d, *J*=8.1 Hz), 6.78 (1H, dd, *J*=8.0, 8.1 Hz), 6.67 (1H, d, *J*=8.0 Hz), 3.75 (3H, s, O*Me*), 3.55 (2H, s, SCH_2), 3.36–3.13 (4H, m, CH_2CH_3), 1.16–1.00 (6H, m, CH_2CH_3); δ_C (75 MHz, $CDCl_3$) 168.3, 153.2, 124.8, 124.6, 122.6, 119.2, 111.6, 56.3, 42.9, 40.9, 35.8, 14.3, 12.9.

4.1.15. *N*,*N*-Diethyl-2-hydroxy-3-chloro phenyl thioacetamide (3b). Prepared following the same procedure from **2b** (0.26 g, 1 mmol), *s*-BuLi [2 M (1 mL, 2 mmol)] using THF (8 mL), TMEDA (0.3 mL, 2 mmol). An oily

mass, difficult to separate from the unreacted starting material was obtained. But its formation was confirmed from IR and ^{1}H NMR and was sufficiently pure for the next step; ν_{max} (Neat) 3226, 1623 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.41–7.31 (2H, m), 6.72 (1H, dd, J=7.7, 7.8 Hz), 3.63 (2H, s, SCH_2), 3.44 (4H, m, CH_2CH_3), 1.15 (6H, t, J=6.9 Hz, CH_2CH_3).

4.1.16. 2-(*N*,*N*-Diethyl-1-acetamido-2-hydroxyphenyl-3-)-1,3-dioxane (3d). Prepared by the same procedure from **2d** (0.33 g, 1 mmol), *s*-BuLi (2 M, 1 mL, 2 mmol) in THF (8 mL), TMEDA (0.3 mL, 2 mmol). Purified by column chromatography over alumina; [eluent: ethyl acetate–petroleum (1:3)], yield 0.23 g, 72%, Crystallised from ether, mp 76–77 °C. [Found: C, 59.04; H, 7.32; N, 4.42. $C_{16}H_{23}NO_4S$ requires 59.05; H, 7.12; N, 4.30%]; ν_{max} (KBr) 3072, 1622 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 9.82 (1H, s, O*H*), 7.54–7.45 (2H, m), 6.84–6.79 (1H, m), 5.90 (1H, s, OC*H*O), 4.26–4.20 (2H, m, OCH_2CH_2), 4.05–3.96 (2H, m, OCH_2CH_2), 3.60 (2H, s, SCH_2), 3.33 (2H, q, J=7.1 Hz, CH_2CH_3), 3.21 (2H, q, J=7.1 Hz, CH_2CH_3), 2.28–2.16 (1H, m, $CH_2CH_2CH_2$), 1.45–1.40 (1H, m, $CH_2CH_2CH_2$), 1.20–1.08 (6H, m, CH_2CH_3); δ_C (75 MHz, $CDCl_3$) 169.1, 156.1, 136.9, 129.1, 125.7, 119.7, 98.0, 67.4, 42.3, 41.3, 39.1, 25.7, 14.0, 12.7.

4.1.17. *N*,*N*-Diethyl-1-hydroy naphthyl-2-thioacetamide (3e). Prepared in the same way from **2e** (0.29 g, 1 mmol), *s*-BuLi (2 M, 1 mL, 2 mmol) in THF (8 mL), TMEDA (0.3 mL, 2 mmol). Purified by column chromatography [eluent: ethyl acetate–petroleum ether (1:7)], crystallised from ether–petroleum ether, yield 0.26 g, 92%, mp 88–90 °C. [Found: C, 66.32; H, 6.48; N, 4.89. $C_{16}H_{19}NO_2S$ requires C, 66.40; H, 6.62; N, 4.84%]; ν_{max} (KBr) 3049, 1624 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 10.63 (1H, s, O*H*), 8.36 (1H, m), 7.71 (1H, m), 7.51–7.43 (3H, m), 7.25 (1H, d, J=8.4 Hz), 3.63 (2H, s, SCH_2), 3.36 (2H, q, J=7.2 Hz, NCH_2), 3.18 (2H, q, J=7.2 Hz, NCH_2), 1.13–1.07 (6H, m, CH_2CH_3); δ_C (75 MHz, $CDCl_3$) 170.1, 157.3, 135.9, 133.4, 127.8, 126.6, 125.7, 125.6, 124.0, 119.7, 112.0, 42.8, 41.1, 40.5, 14.5, 13.2.

4.1.18. *N*,*N*-Diethyl-1-hydroxy-6-methoxy naphthyl-2-thioacetamide (3f). Prepared in the same way from **2f** (0.5 g, 1.6 mmol), TMEDA (0.58 mL, 4 mmol), THF (8 mL) and *s*-BuLi (2.4 mL of 1.6 M solution, 4 mmol). Purified by column chromatography [eluent: ethyl acetate–petroleum ether (3:17)], liquid oil, yield 0.45 g, 90%. [Found: C, 64.06; H, 6.66; N, 4.29. $C_{17}H_{21}O_3NS$ requires C, 63.90; H, 6.58; N, 4.38%]; ν_{max} (Neat) 3062, 1730 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.2 (1H, d, J=9.1 Hz), 7.38 (1H, d, J=8.6 Hz), 7.09 (1H, d, J=8.6 Hz), 7.03 (1H, dd, J=2.4, 9.1 Hz), 6.96 (1H, d, J=2.4 Hz), 3.83 (3H, s, O*Me*), 3.56 (2H, s, SCH_2), 3.50–3.15 (4H, m, CH_2CH_3), 1.27–1.03 (6H, m, CH_2CH_3); δ_C (75 MHz, $CDCl_3$) 159.3, 158.0, 137.5, 134.2, 133.0, 121.2, 118.6, 117.9, 105.9, 55.6, 42.8, 42.1, 40.6, 14.6, 13.2.

4.1.19. *N*,*N*-Diethyl-5-(2-*t*-butyldimethylsilyl-4-hydroxy-benzo[*b*]thienyl) thioacetamide (3g). Prepared in the same way from **2g** (0.4 g, 1 mmol), *s*-BuLi [2 M (1 mL, 2 mmol)], THF (10 mL) using TMEDA (0.3 mL, 2 mmol). Purified by column chromatography [eluent: ethyl acetate–petroleum (1:8)], yield 0.33 g, 83%, mp 35–39 °C. [Found: C, 58.78; H, 7.75; N, 3.38. $C_{20}H_{31}NO_2S_2Si$ requires C, 58.63; H, 7.63; N, 3.42%]; ν_{max} (Neat) 3307, 1641.3 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.72 (1H, s), 7.38 (1H, d, J=8.6 Hz), 7.31 (1H, d, J=8.6 Hz), 3.61 (2H, s, SCH_2), 3.04 (2H, q, J=7.2 Hz, NCH_2), 3.23 (2H, q, J=7.2 Hz, NCH_2), 1.69–1.15 (6H, m, CH_2CH_3), 0.95 (9H, s, CCH_3), 0.33 (6H, s, $SiCH_3$); δ_C (75 MHz, $CDCl_3$) 170.2, 155.8, 147.9, 138.5, 133.0, 132.3, 130.2, 113.8, 112.3, 42.8, 42.1, 40.8, 26.8, 17.3, 14.5, 13.2, −4.5.

4.1.20. *N*,*N*-Diethyl-2-(3-hydroxybenzo[*b*]thienyl)thioacetamide (3h). Prepared in the same way from **2h** (0.3 g, 1 mmol), *s*-BuLi [2 M (1 mL, 2 mmol)], THF (10 mL) using TMEDA (0.3 mL, 2 mmol). Purified by column chromatography [eluent: ethyl acetate–petroleum (3:17)], gummy liquid, yield 0.23 g, 78%. [Found: C, 56.89; H, 5.58; N, 4.48. $C_{14}H_{17}NO_2S_2$ requires C, 56.92; H, 5.80; N, 4.74%]; ν_{max} (Neat) 3130, 1605 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 10.74 (1H, s, O*H*), 7.76–7.72 (1H, m), 7.55–7.52 (1H, m), 7.25–7.22 (2H, m), 3.49 (2H, s, SCH_2), 3.28 (2H, q, J=7.2 Hz, NCH_2), 3.12 (2H, q, J=7.2 Hz, NCH_2), 1.05–0.97 (6H, m, CH_2CH_3); δ_C (75 MHz, $CDCl_3$) 169.5, 154.7, 138.4, 135.5, 132.1, 124.6, 123.7, 122.1, 121.9, 42.1, 41.8, 40.6, 13.9, 12.7.

4.1.21. *N*,*N*-Diethyl-2-hydroxy-3-*t*-butyldimethylsilyl-phenyl thioacetamide (3i). Prepared in the same way from **2i** (0.35 g, 1 mmol), *s*-BuLi [2 M (1 mL, 2 mmol) in THF (8 mL), TMEDA (0.3 mL, 2 mmol). Purified by column chromatography [eluent: ethyl acetate–petroleum (1:5)], mp 30–33 °C, yield 0.22 g, 64%. [Found: C, 61.34; H, 8.92; N, 3.88. $C_{18}H_{31}NO_2SSi$ requires C, 61.14; H, 8.84; N, 3.96%]; ν_{max} (Neat) 3325, 1595 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.38 (2 H, m), 7.28 (1H, d, J=8.0 Hz), 3.61 (2H, s, SCH_2), 3.38 (2H, q, J=7.1 Hz, NCH_2), 3.21 (2H, q, J=7.1 Hz, NCH_2), 1.15–1.09 (6H, m, CH_2CH_3), 0.95 (9H, s, CCH_3), 0.33 (6H, s, Si*Me*); δ_C (75 MHz, $CDCl_3$) 170.2, 155.8, 147.9, 133.0, 132.3, 130.3, 113.8, 112.3, 42.8, 42.1, 40.0, 26.8, 17.3, 14.5, 13.2, −4.59.

4.1.22. *N*,*N*-Diethyl-2-hydroxy-3-methylphenylthioacetamide (3j). Prepared in the same way from **2j** (0.25 g, 1 mmol), *s*-BuLi [2 M (0.5 mL, 1 mmol) in THF (8 mL), TMEDA (0.15 mL, 1 mmol). Purified by column chromatography [eluent: ethyl acetate–petroleum (1:5)], mp 30–35 °C, yield 0.16 g, 63%. [Found: C, 61.81; H, 7.38; N, 5.73. $C_{13}H_{19}NO_2S$ requires C, 61.63; H, 7.56; N, 5.53%; ν_{max} (Neat) 3168, 1620 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 10.19 (1H, s, O*H*), 7.18–7.04 (1H, m), 6.84 (1H, d, J=7.4 Hz), 6.77–6.72 (1H, m), 3.63 (2H, s, SCH_2), 3.43–3.29 (4H, m, NCH_2), 2.34 (3H, s, $ArCH_3$), 1.26–1.03 (6H, m, CH_2CH_3); δ_C (75 MHz, $CDCl_3$) 173.6, 159.3, 133.3, 132.4, 123.7, 121.6, 121.3, 54.3, 45.9, 44.3, 17.5, 17.1, 15.2.

4.1.23. General procedure of synthesis of oxathiin-2-ones. 8-Methoxy benz[1,4]oxathiin-2-one (4a). The hydroxy compound **3a** (0.25 g, 9.3 mmol) was heated with 7 mL of glacial acetic acid for 18 h under magnetic stirring condition. Cooling, the reaction mixture was extracted with dichloromethane (2×20 mL). The organic layer was washed with water, dried (Na_2SO_4). Removal of solvent afforded the

crude 8-Methoxy benz[1,4]oxathiin-2-one, which was purified by crystallisation (ethyl acetate–petroleum) to afford colourless crystal, yield 0.15 g, 82%, mp 68–71 °C. [Found: C, 55.03; H, 4.06. $C_9H_8O_3S$ requires C, 55.10; H, 4.08%]; ν_{max} (KBr) 1760 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 6.99 (1H, dd, *J*=7.9, 8.0 Hz), 6.84 (1H, dd, *J*=1.1, 7.9 Hz), 6.78 (1H, dd, *J*=1.1, 8.0 Hz), 3.83 (3H, s, O*Me*), 3.39 (2H, s, SCH_2); δ_C (75 MHz, $CDCl_3$) 162.9, 149.2, 140.6, 124.9, 121.3, 119.8, 111.0, 56.5, 29.0.

4.1.24. 8-Chloro benz[1,4]oxathiin-2-one (4b). The crude product **3b** (0.5 g, 1.8 mmol) was heated with 10 mL glacial acetic acid for 20 h. Purified by crystallisation [ethyl acetate–petroleum ether]. White powder, yield 0.24 g, 81%, mp 94–96 °C. [Found: C, 47.63; H, 2.22. $C_8H_5O_2ClS$ requires C, 47.80; H, 2.50%]; ν_{max} (KBr) 1772 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.34 (1H, dd, *J*=1.1, 8.0 Hz), 7.25 (1H, dd, *J*=1.1, 7.8 Hz), 7.04 (1H, dd, *J*=8.0, 7.8 Hz), 3.49 (2H, s, SCH_2); δ_C (75 MHz, $CDCl_3$) 162.1, 146.9, 129.3, 126.8, 125.2, 124.2, 122.2, 30.1.

4.1.25. 8-Formyl benz[1,4]oxathiin-2-one (4d). Prepared in the same way from **3d** (0.16 g, 0.5 mmol) by heating with acetic acid (5 mL). Simultaneous deprotection of the aldehyde function took place during cyclisation affording **4d** which was purified by crystallisation from diethyl ether, yield 0.09 g, 92%, solid, mp 106–108 °C. [Found: C, 55.66; H, 3.14. $C_9H_6O_3S$ requires C, 55.66; H, 3.11%]; ν_{max} (KBr) 1716.5, 1641.3 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 9.83 (1H, s, C*H*O), 7.62 (1H, dd, *J*=1.5, 6.1 Hz), 7.46 (1H, dd, *J*=1.5, 6.1 Hz), 6.91 (1H, d, *J*=7.68 Hz), 3.63 (2H, s, SCH_2); δ_C (75 MHz, $CDCl_3$) 196.3, 174.8, 160.5, 140.0, 136.5, 133.5, 121.0, 120.4, 34.6.

4.1.26. Naphtho[1,2-*b*][1,4]oxathiin-2-one (4e). Prepared in the same way as stated above from **3e** (0.4 g, 1.3 mmol) acetic acid (10 mL). Purified by column chromatography [eluent: ethyl acetate–light petroleum (1:4)], crystallised from petroleum ether, yield 0.26 g, 86%, mp 62–64 °C. [Found: C, 66.73; H, 3.62. $C_{12}H_8O_2S$ requires C, 66.65; H, 3.73%]; ν_{max} (KBr) 1755 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.20–8.16 (1H, m), 7.82–7.78 (1H, m), 7.60–7.49 (3H, m), 7.36–7.25 (1H, m), 3.55 (2H, s, SCH_2); δ_C (75 MHz, $CDCl_3$) 165.0, 148.1, 135.4, 130.2, 129.7, 129.1, 127.0, 126.9, 126.8, 123.0, 117.0, 31.2.

4.1.27. 8-Methoxy naphtho[1,2-*b*][1,4]oxathiin-2-one (4f). Prepared in the same way from **3f** (0.4 g, 1.25 mmol) and glacial acetic acid (10 mL). Purified by crystallisation (ethyl acetate–petroleum ether). Colourless shinny crystals, yield 0.26 g, 84%, mp 82–84 °C. [Found: C, 63.2; H, 4.09. $C_{13}H_{10}O_3S$ requires C, 63.4; H, 4.06%]; ν_{max} (KBr) 1755 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.02 (1H, d, *J*=9.2 Hz), 7.41 (1H, d, *J*=8.6 Hz), 7.23 (1H, d, *J*=8.6 Hz), 7.14 (1H, dd, *J*=2.5, 9.2 Hz), 7.03 (1H, d, *J*=2.5 Hz), 3.86 (3H, s, O*Me*), 3.46 (2H, s, SCH_2); δ_C (75 MHz, $CDCl_3$) 163.2, 158.7, 146.4, 135.0, 125.7, 123.7, 122.7, 120.3, 120.2, 112.3, 106.4, 55.8, 29.3.

4.1.28. 8-*t*-Butyldimethylsilyl[1]benzo[5,4-*b*]thieno[1,4]-oxathiin-2-one (4g). Prepared by same way from **3g** (0.2 g, 0.5 mmol) in aceteic acid (10 mL). Purified by column chromatography [eluent: ethyl acetate–light petroleum (1:9)], yield 0.15 g, 90%, mp 122–124 °C. [Found: C, 57.23; H, 5.86. $C_{16}H_{20}O_2S_2Si$ requires C, 57.10; H, 5.99%]; ν_{max} (KBr) 1750.7 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.62 (1H, s), 7.57 (1H, d, *J*=8.4 Hz), 7.21 (1H, d, *J*=8.4 Hz), 3.53 (2H, s, SCH_2), 0.95 (9H, s, CCH_3), 0.36 (6H, s, SiCH_3); δ_C (75 MHz, $CDCl_3$) 161.8, 144.6, 143.2, 141.2, 130.7, 125.9, 117.2, 112.5, 112.2, 28.0, 25.3, 15.8, −6.0.

4.1.29. [1]Benzo[3,2-*b*]thieno[1,4]oxathiin-2-one (4h). Prepared by same way from **3h** (0.295 g, 1 mmol) and acetic acid (10 mL). Purified by crystallisation (ethyl acetate–petroleum ether), yield 0.20 g, 91%, mp 72–74 °C. [Found: C, 54.48; H, 2.54. $C_{10}H_6O_2S_2$ requires C, 54.03; H, 2.72%]; ν_{max} (KBr) 1754 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.70–7.67 (1H, m), 7.48–7.43 (1H, m), 7.35–7.32 (1H, m), 7.15–7.09 (1H, m), 3.70 (2H, s, SCH_2); δ_C (75 MHz, $CDCl_3$) 169.5, 154.7, 138.5, 135.5, 132.1, 123.8, 123.7, 122.1, 121.9, 41.8.

4.1.30. 8-*t*-Butyldimethylsilyl benz[1,4]oxathiin-2-one (4i). Prepared from **3i** (0.1 g, 0.3 mmol) and acetic acid (7 mL) in the same way. Purified by column chromatography [eluent: ethyl acetate–light petroleum (1:9)], yield 0.07 g, 86%, solid, mp 60–62 °C. [Found: C, 59.83; H, 7.28. $C_{14}H_{20}O_2SSi$ requires C, 59.96; H, 7.19%]; ν_{max} (KBr) 1768 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.62–7.57 (2H, m), 7.21 (1H, d, *J*=8.4 Hz), 3.53 (2H, s, SCH_2), 0.95 (9H, s, CCH_3), 0.36 (6H, s, SiCH_3); δ_C (75 MHz, $CDCl_3$) 161.8, 143.2, 141.2, 125.9, 122.5, 117.2, 112.2, 28.0, 25.3, 15.8, −6.0.

4.1.31. 8-Methyl benz[1,4]oxathiin-2-one (4j). Prepared from **3j** (0.06 g, 0.24 mmol) and acetic acid (5 mL) in the same way. Purified by column chromatography [eluent: ethyl acetate–petroleum (1:9)], solid, mp 58–50 °C, yield 0.04 g, 89%. [Found: C, 59.73; H, 4.63. $C_9H_8O_2S$ requires C, 59.98; H, 4.47%]; ν_{max} (KBr) 1768 cm^{-1}; δ_H (300MH_Z, $CDCl_3$) 7.59–7.56 (1H, m), 7.18–7.14 (1H, m), 6.94–6.92 (1H, m), 3.69 (2H, s, SCH_2), 2.33 (3H, s, ArCH_3); δ_C (75 MHz, $CDCl_3$) 170.6, 155.0, 147.6, 129.1, 126.7, 126.6, 125.1, 39.8, 22.4.

4.1.32. Benz[1,4]oxathiin-2-one (4k). Compound **4i** (0.166 g, 1 mmol) and tetrabutyl ammonium fluoride (Bu_4NF) trihydrate (0.315 g, 1 mmol) in THF (10 mL) were stirred for 24 h at room temperature. After this period the reaction mixture was diluted with diethyl ether, washed with water (3×30 mL) and dried over Na_2SO_4. Removal of solvent afforded crude **4k**. Purified by column chromatography [eluent: ethyl acetate–petroleum (1:9)], solid, mp 48–50 °C, yield 0.09 g, 89%. [Found: C, 57.88; H, 3.85. $C_8H_6O_2S$ requires C, 57.81; H, 3.64%]; ν_{max} (KBr) 1741 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.70–7.67 (1H, m), 7.46–7.43 (1H, m), 7.35–7.32 (1H, m), 7.15–7.09 (1H, m), 3.70 (2H, s, SCH_2); δ_C (75 MHz, $CDCl_3$) 170.1, 154.1, 135.5, 130.8, 126.5, 124.6, 124.4, 39.1.

4.1.33. 2-(*N*,*N*-Diethyl-1-carbamoyloxyphenyl-2-)-1,3-dioxane (5). Compound **1d** (4.42 g, 20 mmol), 1,3-propanediol (3.04 g, 40 mmol) and anhydrous $FeCl_3$ (10%) in dry C_6H_6 (50 mL) were refluxed with continuous distilling of water for 10 h. Then most of the solvent distilled off, diluted with ether (50 mL), washed with water (3×50 mL) and dried (Na_2SO_4). Evaporation of the solvent left a

viscous liquid which was purified by column chromatography over alumina. [eluent: ethyl acetate–light petroleum (1:9)], colourless oily liquid, yield 4.9 g, 88%. [Found: C, 64.63; H, 4.60; N, 5.11. $C_{15}H_{21}NO_4$ requires C, 64.50; H, 7.58; N, 5.01%]; ν_{max} (Neat) 1720.4 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.67–7.65 (1H, m), 7.36–7.31 (1H, m), 7.23–7.12 (2H, m), 5.64 (1H, s, OC*H*O), 4.26–4.21 (2H, m, OC$H_2$$CH_2$), 3.97–3.89 (2H, m, OC$H_2$$CH_2$), 3.49–3.39 (4H, m, C$H_2$$CH_3$), 2.27–2.13 (2H, m, CH_2C$H_2$$CH_2$), 1.43–1.20 (6H, m, CH_2CH_3); δ_C (75 MHz, $CDCl_3$) 155.7, 148.7, 130.5, 129.4, 126.9, 125.1, 122.6, 97.7, 67.3, 42.3, 34.7, 25.6, 14.2, 13.2.

4.1.34. *N*,*N*-Diethyl-6-formyl-2-methylsulfanyl carbamoyloxybenzene (6). Compound **2d** (0.650 g, 2 mmol) was heated under refluxed with 1:1 aq. MeOH (8 mL) and $FeCl_3$ (0.32 g, 0.2 mmol) for 1 h. After distilling off most of the MeOH the compound extracted with dichloromethane (3×30 mL), washed with water (3×20 mL) and dried over Na_2SO_4. The oily residue was purified by column chromatography [eluent: ethyl acetate–light petroleum (1:4)], oily liquid, yield 0.47 g, 88%. [Found: C, 58.53; H, 6.52; N, 5.38. $C_{13}H_{17}NO_3S$ requires C, 58.40; H, 6.41; N, 5.24%]; ν_{max} (Neat) 1772, 1724 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 10.07 (1H, s, C*H*O), 7.61 (1H, dd, *J*=1.8, 7.5 Hz), 7.41 (1H, dd, *J*=1.5, 6.3 Hz), 7.25 (1H, dd, *J*=7.5, 6.3 Hz), 3.50 (2H, q, *J*=7.2 Hz, NCH_2), 3.49 (2H, q, *J*=7.2 Hz, NCH_2), 2.42 (3H, s, S*Me*), 1.27 (3H, t, *J*=7.2 Hz, CH_2CH_3), 1.18 (3H, t, *J*=7.2 Hz, CH_2CH_3); δ_C (75 MHz, $CDCl_3$) 188.5, 152.7, 149.8 134.4, 131.6, 129.3, 126.2, 125.8, 42.5, 42.1, 14.9, 14.1, 13.1.

4.1.35. *N*,*N*-Diethyl-1-[2-hydroxyphenyl]-1-methylsulfanylacetamide (9). Prepared from **1j** (2.07 g, 10 mmol), *s*-BuLi [2 M, 10 mL, 20 mmol)], TMEDA (3 mL, 20 mmol), THF (15 mL) and dimethyl disulfide (2 mL, 22 mmol) following the same procedure. White crystalline solid. Purified by crystallisation from diethyl ether, mp 130–132 °C, yield 1.71 g, 83%. [Found: C, 61.83; H, 7.64; N, 5.64. $C_{13}H_{19}NO_2S$ requires C, 61.63; H, 7.56; N, 5.53%]; ν_{max} (KBr) 3170, 1620 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 10.41 (1H, s, O*H*), 7.20–7.15 (1H, m), 7.00–6.92 (2H, m), 6.79–6.74 (1H, m), 4.73 (1H, s, C*H*SMeCONEt$_2$), 3.52–3.27 (4H, m, C$H_2$$CH_3$), 1.98 (3H, s, SC$H_3$), 1.23 (3H, t, *J*=6.2 Hz, CH_2CH_3), 1.11 (3H, t, *J*=6.2 Hz, CH_2CH_3); δ_C (75 MHz, $CDCl_3$) 173.6, 159.3, 133.4, 132.4, 123.1, 121.6, 121.3, 54.3, 45.9, 44.3, 17.5, 17.1, 15.20.

4.1.36. *N*,*N*-Diethyl-1-thiomethyl methyl-2-carbamyloxynaphthalene (10). Compound **1m** (2.57 g, 10 mmol) deprotonated with *s*-BuLi [2 M (5.5 mL, 11 mmol)] in THF (5 mL) using TMEDA (1.6 mL, 11 mmol) and dimethyldisulfide (1 mL, 12 mmol) following the general procedure compound **10** was obtained as an oil. Purification by column chromatography [eluent: ethyl acetate–petroleum (12.5:87.5)], afforded a colourless oily liquid, yield 2.3 g, 78%. [Found: C, 64.84; H, 6.83; N, 5.23. $C_{17}H_{21}NO_2S$ requires C, 67.42; H, 6.90; N, 4.48]; ν_{max} (Neat) 1718 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.96 (1H, d, *J*=8.0 Hz), 7.77 (1H, d, *J*=8.0 Hz), 7.71–7.68 (1H, m), 7.54–7.48 (1H, m), 7.37–7.32 (1H, m), 7.13–7.09 (1H, m), 4.23 (2H, s, CH_2SCH_3), 3.58–3.45 (4H, m, C$H_2$$CH_3$), 2.07 (3H, s, S*Me*), 1.36–1.26 (6H, m, CH_2CH_3); δ_C (75 MHz, $CDCl_3$) 159.7, 152.6, 133.0, 129.2, 129.2, 128.6, 126.6, 123.2, 122.3, 118.3, 114.6, 44.6, 44.4, 27.9, 16.8, 15.8, 14.6.

Acknowledgements

Thanks for financial assistance are due to Council of Scientific and Industrial Research (New Delhi) under the project No. 01(1573)/99/EMR-II and Royal Society of Chemistry for a Research Fund Grant to A. D. T. K. P and S. K. thank Council of Scientific and Industrial Research (New Delhi) for Senior Research Fellowships.

References and notes

1. Part 5. Mukherjee, C.; Kamila, S.; De, A. *Synlett* **2003**, 1474.
2. (a) Gschwend, H. W.; Rodriguez, H. R. *Org. React.* **1979**, *26*, 1. (b) Snieckus, V. *Heterocycles* **1980**, *14*, 1649. (c) Beak, P.; Snieckus, V. *Acc. Chem. Res.* **1982**, *15*, 306. (d) Snieckus, V. *Bull. Soc. Chim. Fr.* **1988**, 67. (e) Snieckus, V. *Chem. Rev.* **1990**, *90*, 879.
3. Sibi, M. P.; Snieckus, V. *J. Org. Chem.* **1983**, *48*, 1935.
4. Seebach, D.; Bauer, V. W. *Helv. Chim. Acta* **1984**, *67*, 1972.
5. Zhang, P.; Gawley, R. E. *J. Org. chem.* **1993**, *58*, 3223.
6. Wang, W.; Snieckus, V. *J. Org. Chem.* **1992**, *57*, 424.
7. (a) Kalinin, A. V.; Jalil Miah, M. A.; Chattopadhay, S.; Tsukazaki, M.; Wicki, M.; Nguyen, T.; Coelho, A. L.; Kerr, M.; Snieckus, V. *Synlett* **1997**, 839. (b) Focken, T.; Hopf, H.; Snieckus, V.; Dix, I.; Jones, P. G. *Eur. J. Org. Chem.* **2001**, 2221.
8. Mukherjee, C.; De, A. *Synlett* **2002**, 325.
9. Mukherjee, C.; Kamila, S.; De, A. *Tetrahedron* **2003**, *59*, 4767.
10. Mondal, S. S.; Samanta, S. S.; Deb, C.; De, A. *J. Chem. Soc., Perkin Trans. 1* **1998**, 2559.
11. Greenwood, D.; Stevenson, H. A. *J. Chem. Soc.* **1953**, 1514.
12. Horner, L.; Sturm, K. *Liebigs Ann. Chem.* **1955**, *597*, 1.
13. Beaulieu, F.; Snieckus, V. *Synthesis* **1992**, 112.
14. Sibi, M. P.; Chattopadhay, S.; Dankwardt, J. W.; Snieckus, V. *J. Am. Chem. Soc.* **1985**, *107*, 6312.
15. Kamila, S.; Mukherjee, C.; Mondal, S. S.; De, A. *Tetrahedron* **2003**, *59*, 1339.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron 60 (2004) 5225–5235
Tetrahedron

A total synthesis of (+)-oxybiotin from D-arabinose

Velimir Popsavin,[a,*] Goran Benedeković,[a] Mirjana Popsavin,[a] Vladimir Divjaković[b] and Thomas Armbruster[c]

[a]*Department of Chemistry, Faculty of Sciences, University of Novi Sad, Trg D. Obradovića 3, 21000 Novi Sad, Serbia and Montenegro*
[b]*Department of Physics, Faculty of Sciences, University of Novi Sad, Trg D. Obradovića 4, 21000 Novi Sad, Serbia and Montenegro*
[c]*Laboratory for Chemical and Mineralogical Crystallography, Bern University, Freiestr. 3, CH-3012 Bern, Switzerland*

Received 11 February 2004; revised 23 March 2004; accepted 14 April 2004

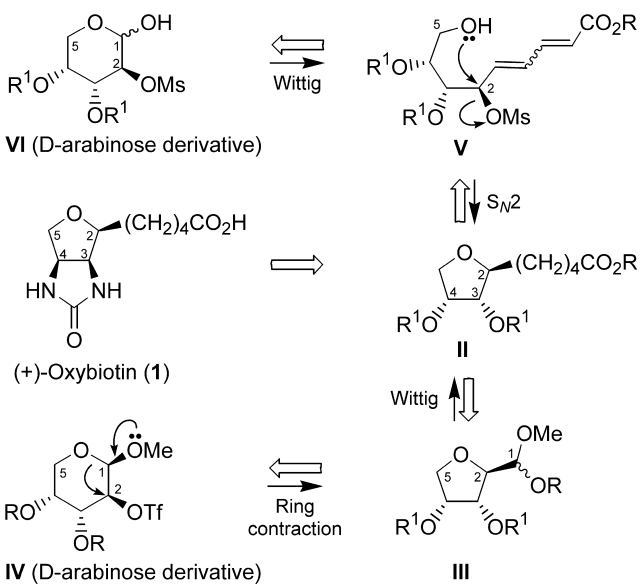
Abstract—A novel ten-step synthesis of (+)-oxybiotin, a biologically active analogue of (+)-biotin, has been achieved starting from D-arabinose.

1. Introduction

The oxygenated analogue of biotin in which an oxygen atom replaces sulphur was synthesized by Hofmann[1] and named oxybiotin.[2] The obtained racemic material showed 50% of biotin like growth-stimulatory activity.[3] Such results implied that the biologically active enantiomer should have the same absolute configuration as naturally occurring (+)-biotin. This assumption was confirmed by a total synthesis of enantiopure (+)-oxybiotin (**1**) that was achieved in 19 steps starting from D-glucose.[4] Recently we have reported a 14-step synthesis of (+)-**1** from D-xylose,[5] and now we describe a new ten-step synthesis of (+)-oxybiotin based on chirality transfer from D-arabinose.[6]

Retrosynthetic analysis of (+)-oxybiotin (**1**) is presented in Scheme 1. An examination of the target molecule **1** reveals a chiral tetrahydrofuran system containing three contiguous substituents including the C-3 and C-4 nitrogen functions incorporated into a *cis*-fused imidazolidinone ring. Our synthetic plan for the assembly of the C_3-C_4 domain involved an introduction of two nitrogen functions at C-3 and C-4 in a derivative of type **II** (with Walden inversion), followed by a subsequent closure of the imidazolidinone ring system. Further disconnection of **II** leads to a protected 2,5-anhydro-D-ribose derivative **III**, which should be accessible from an arabinopyranoside 2-triflate **IV** by a ring contraction process.

An alternative disconnection of the retron **II** leads to the open-chain intermediate **V**, which might be converted to a synthetic precursor of **II** by an intramolecular displacement of the allylic C-2 mesyloxy function by the C-5 hydroxyl group. The structure **V** can be finally correlated with a partially protected D-arabinose derivative **VI** via a simple Wittig reaction. Accordingly, the preparation of the postulated intermediates of type **III** and **VI** was first attempted.



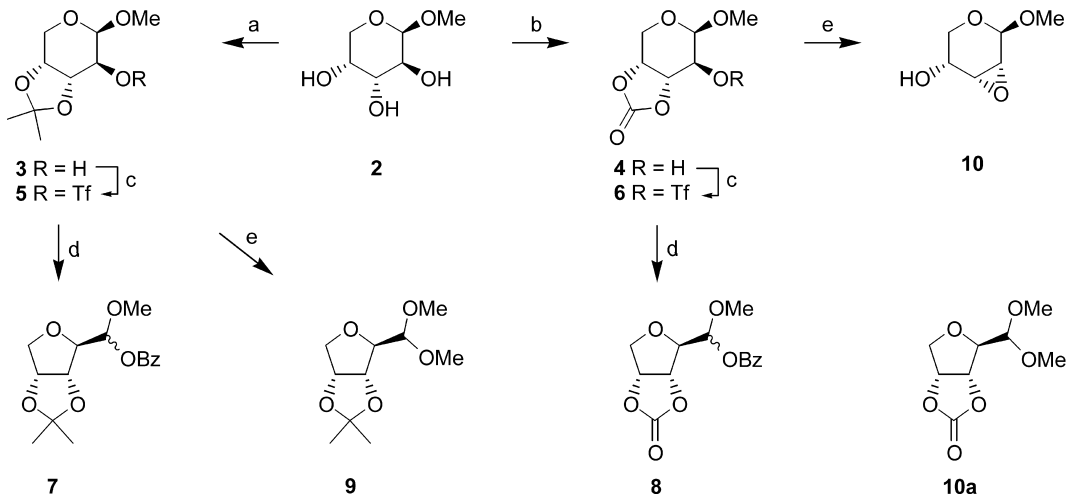
Scheme 1. Retrosynthetic analysis (sugar numbering scheme).

2. Results and discussion

In 1989, Baer et al.[7] reported a facile formation of 2,5-anhydro-6-deoxy-L-talose derivatives by ring contraction

Keywords: 2,5-Anhydro sugars; D-Arabinose; (+)-Oxybiotin; Ring contraction; Triphosgene; Wittig reaction.
* Corresponding author. Tel.: +381-21-350-122; fax: +381-21-454-065; e-mail address: popsavin@ih.ns.ac.yu

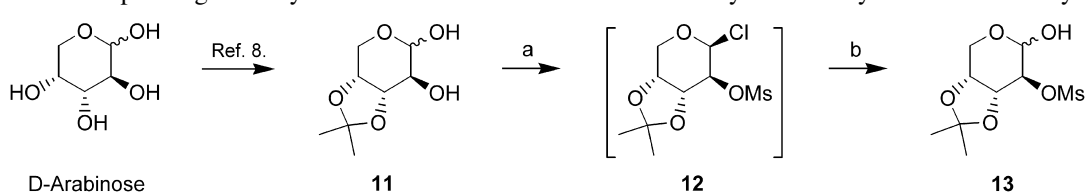
0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.04.040



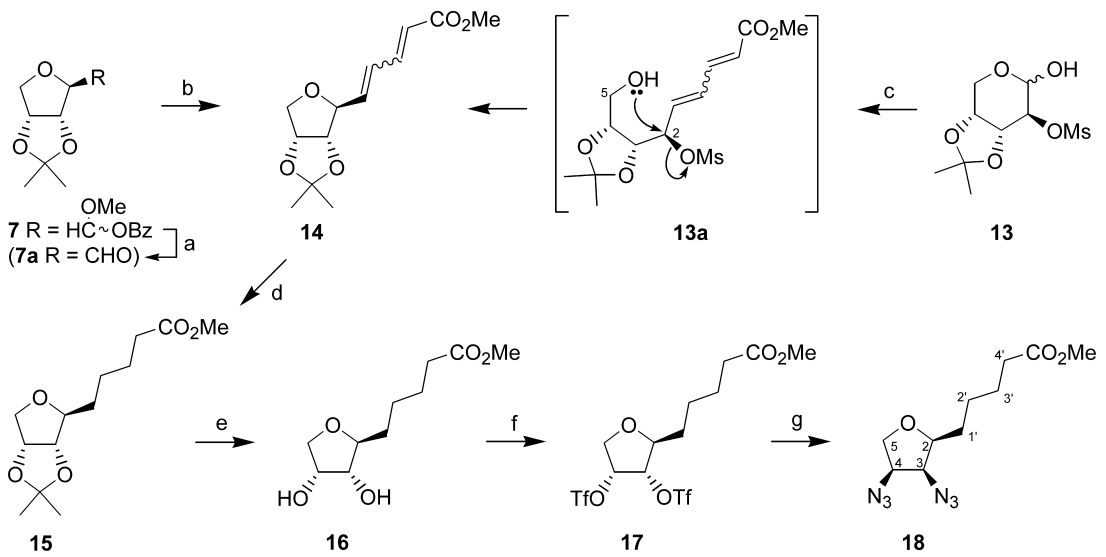
Scheme 2. (a) $Me_2C(OMe)_2$, TsOH, DMF, rt, 3.5 h, 89%; (b) Imd_2CO, C_6H_6, reflux, 1.5 h, 69%; (c) Tf_2O, Py, CH_2Cl_2, −10 °C, 0.5 h; (d) KOBz, DMF, rt, 20 h for **5**, 99% of **7** (two steps), 60–65°, 2 h for **6**, 80% of **8** (two steps); (e) $NaHCO_3$, MeOH, 50 °C, 4 h for **5**, 12% of **9**, 55–60 °C, 3 h for **6**, 77% of **10**.

in methyl 2-*O*-trifluoromethanesulfonyl-β-L-fucopyranoside under solvolytic conditions (KOBz/DMF, $NaHCO_3$/MeOH). It was therefore assumed that utilization of similar methodology in the D-arabinopyranose series would provide the postulated intermediate **III** from the retrosynthetic analysis scheme. The preparation of the 2-triflate esters **5** and **6** was first attempted starting from the 3,4-*O*-protected methyl β-D-arabinopyranosides **3** and **4** (Scheme 2). We adopted the Kiso–Hasegawa acetonation procedure[8] for the conversion of commercially available methyl β-D-arabinopyranoside (**2**) to the known[9] 3,4-*O*-isopropylidene derivative **3**. Compound **4**, in turn, was conveniently prepared by treatment of **2** with 1,1′-carbonyldiimidazole in boiling benzene. The melting point and NMR spectral data of thus obtained intermediate **4** were in reasonable agreement with those earlier reported for the L-configuration counterpart of **4**, obtained by treatment of the corresponding 3,4-*O*-thiocarbonyl derivative with bistributyltin oxide.[10] Both **3** and **4** readily reacted with triflic anhydride to afford the corresponding 2-*O*-triflate esters **5** (74%) and **6** (89%). Compound **5** was partially characterized from NMR spectroscopic data, but was rather unstable on storage similar to its fucopyranoside analogue.[7] It should be therefore used in the next synthetic step immediately after its brief isolation. On the contrary, the triflate **6** is a stable crystalline compound, which was fully characterized by the corresponding spectral (IR, NMR, MS) and analytical data. Similarly to L-fucopyranoside derivatives,[7] both arabinopyranosides **5** and **6** smoothly reacted with potassium benzoate in *N*,*N*-dimethylformamide to give the corresponding 2,5-anhydro-D-ribose derivatives **7** (99% from **2**; 2:1 mixture of C-1 epimers) and **8** (80% from **2**; 1:1 mixture of C-1 epimers). Compound **5** also reacted with sodium hydrogen carbonate in methanol (50 °C for 4 h)[7] to afford a low yield of the corresponding dimethyl acetal derivative **9** (12%), as a ring-contracted product. Conversely, the 3,4-*O*-carbonyl derivative **6**, under the similar reaction conditions, gave the known[11] epoxide **10** (77%) as a product of transesterification of the cyclic carbonate functionality in **6**, followed by a subsequent epoxide ring closure process. The dimethyl acetal derivative **10a**, an expected product of the presumed ring contraction process, could not be detected in the reaction mixture. In the light of their stereochemical and topological features, the 2,5-anhydro derivatives **7**–**9** fully correspond to the intermediate **III** from our retrosynthetic analysis. However, further work was continued with the isopropylidene derivative **7**, since only this intermediate was accessible from the starting material **2** in an almost quantitative yield.

Preparation of the 2-*O*-mesyl derivative **13**, a postulated intermediate in the alternative approach to **1**, started from 3,4-*O*-isopropylidene-D-arabinose (**11**), which was readily available from D-arabinose through a modified literature procedure[8] (Scheme 3). Treatment of **11** with mesyl chloride and triethylamine in dry dichloromethane gave the crystalline glycosyl chloride **12** as the only reaction product. Small vicinal coupling between H-1 and H-2 ($J_{1,2}$=3.7 Hz) is consistent with the *cis* arrangement of these protons and convincingly proved a β-configuration at the anomeric position. Although the compound **12** could be stored at −20 °C for weeks without change, it tended to decompose on prolonged standing at room temperature. Hence, the intermediate **12** was immediately treated with silver oxide in aqueous acetone, in the presence of a catalytic amount of silver triflate, to give the stable lactol **13** (94% from **11**). The synthesis of product **13** from methyl 3,4-*O*-isopropylidene-2-*O*-methanesulfonyl-β-D-arabinopyranoside, has already been described in the literature,[9] but in an overall yield of only 18% from two synthetic steps.



Scheme 3. (a) MsCl, Et_3N, CH_2Cl_2, −10 °C, 1 h; (b) Ag_2O, AgOTf, aq. Me_2CO, rt, 24 h, 95% from **11**.

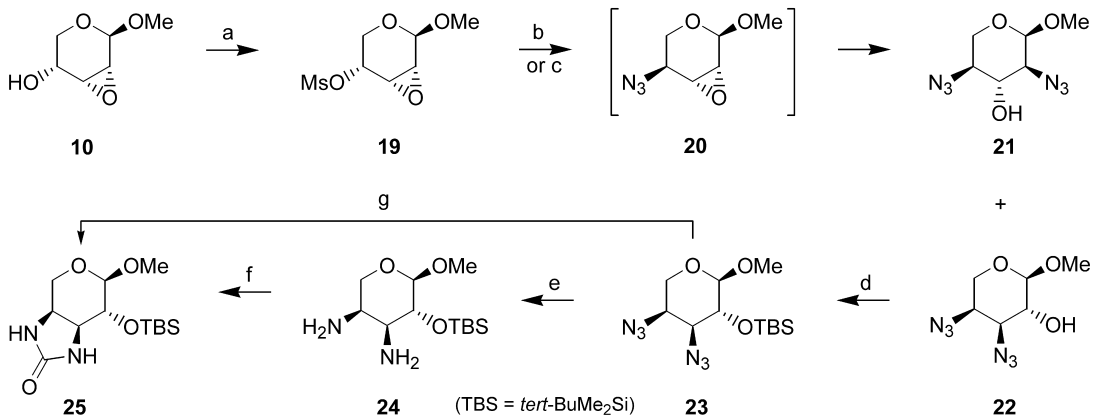


Scheme 4. (a) MeONa, MeOH, rt, 2 h; (b) Ph_3P:CHCH:$CHCO_2Me$, MeOH, rt, 2 h, 41% from **7**; (c) Ph_3P:CHCH:$CHCO_2Me$, Na_2CO_3, DMF, 135 °C, 2 h; (d) H_2, PtO_2, MeOH, rt, 24 h, 36% from **3**, 67% from **13**; (e) 9:1 TFA–H_2O, rt, 0.5 h, 95%; (f) Tf_2O, Py, CH_2Cl_2, 0 °C, 1.5 h; (g) NaN_3, HMPA, rt, 1.5 h, 68% from **16**.

Our sample **13** displayed a value of optical rotation {$[\alpha]_D=-116.5$ (*c* 1.5)} similar to that reported previously {$[\alpha]_D=-118.0$ (*c* 2.06)},[9] but its melting point was significantly lower (116–117 °C) with respect to the reported value (130–131 °C).[9] However, its IR, NMR (^1H and ^{13}C) and HR MS spectral data were fully consistent with structure **13**. The product **13** was mainly the β anomer since its chloroform solution mutarotated to a less negative equilibrium value {$[\alpha]_D=-116.5\rightarrow-106.4$ (24 h)}. The ^1H NMR spectral data also proved that the crystalline sample **13** consists of both α- and β-anomers, as established by integration of the corresponding proton signals [δ 3.83 (dd, $J_{5a,5b}$=14.1 Hz, $J_{4,5a}$=2.5 Hz, H-5aα), 3.97 (d, $J_{5a,5b}$= 13.3 Hz, H-5aβ). The initial 1:5 α/β anomeric ratio, recorded immediately after dissolution of the sample in $CDCl_3$, was changed to 1:3 after storing the solution at room temperature for 48 h.

Having obtained the key intermediates **7** and **13**, we next focused on their C_4-elongation at C-1 in order to elaborate the carboxybutyl (+)-oxybiotin side chain (Scheme 4). O-Debenzoylation of **7** with sodium methoxide in methanol produced the unstable aldehyde **7a**, which was not isolated but was further treated with 3-(carbomethoxy-2-propenylidene)triphenylphosphorane,[12] by using a one-pot procedure. The expected dienoate **14** was thus obtained as an inseparable mixture of corresponding *E*- and *Z*-isomers. Catalytic hydrogenation of **14** over PtO_2 in methanol finally furnished the saturated ester **15** in 36% overall yield with respect to **3**. In a different approach, the 3,4-*O*-isopropylidene-2-*O*-methanesulfonyl-D-arabinose (**13**) was treated with 3-(carbomethoxy-2-propenylidene)triphenylphosphorane in dry DMF, in the presence of sodium carbonate as a proton acceptor, to give directly the dienoates **14** (an inseparable mixture of *E*- and *Z*-isomers), as a result of the sequential Wittig reaction/intramolecular displacement process. Neither the acyclic intermediate **13a** nor the products of the competitive Michael addition could be detected in the reaction mixture. The ^1H and ^{13}C NMR spectra of the mixture of **14** thus obtained displayed essentially the same signals as the sample **14** prepared from the 2,5-anhydride **7**, but indicated somewhat different ratio of *E*- and *Z*-isomers. Conversely, the reaction of **13** with trimethyl-4-phosphono crotonate, in the presence of NaH in THF, at room temperature for 0.5 h, gave pure *E*,*E*-**14** (48%) as the only stereoisomer (not shown in the scheme). Finally, catalytic hydrogenation of **14**, over the Adams catalyst, gave the corresponding saturated ester **15** (67% from **13**). The four-step sequence based on the Wittig reaction of lactol **13** with 3-(carbomethoxy-2-propenylidene)triphenylphosphorane represents a more convenient route towards the key intermediate **15**, since it provided a considerably higher overall yield (63% from **11**) compared to the combined five-step sequence via the 2,5-anhydride **7** (36% from **2**). Hydrolytic removal of the isopropylidene protective group in **15** gave an excellent yield of the expected diol **16** (95%). Reaction of **16** with triflic anhydride in pyridine and dichloromethane gave the corresponding 3,4-ditriflate **17**, isolated by flash column chromatography in 51% yield. Subsequent treatment of **17** with sodium azide in HMPA afforded the corresponding 3,4-diazido derivative **18** as the only reaction product (47% from **16**). However, when the last two-step sequence was carried out without purification of the intermediate **17**, the desired product **18** was obtained in a considerably higher overall yield (68% from **16**).

Diazide **18** represents a final chiral intermediate for the completion of the synthesis of target **1**, since it has the correct absolute configuration at all the stereocentres. Therefore we next focused on the conversion of its vicinal diazido functionality into the imidazolidinone heterocyclic system. This requires previous conversion of **18** into the corresponding diamine **18a** (Scheme 6), followed by subsequent cyclization of the intermediate upon treatment with phosgene or its equivalent. However, in order to avoid wasting of the valuable intermediate **18**, the final imidazolidinone system building was first explored on the diazido derivative **23** as a model compound (Scheme 5).



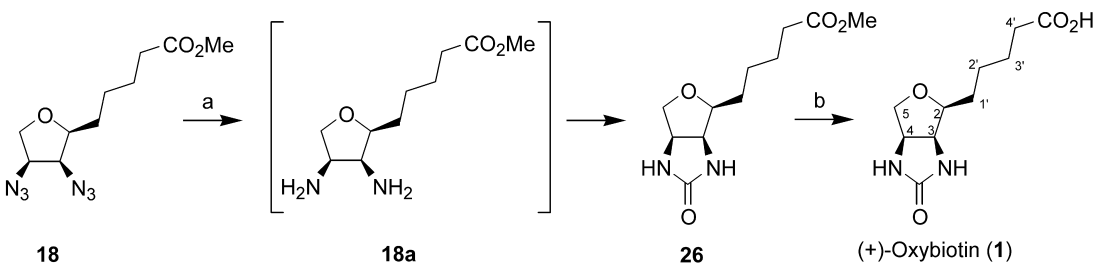
Scheme 5. (a) MsCl, Et_3N, CH_2Cl_2, −10 °C, 40 min, 97%; (b) NaN_3, DMF, 90–95 °C, 0.5 h, then 110–115 °C, 15 min 56% of **20**, 18% of **22**; (c) NaN_3, DMF, 140–145 °C, 3.5 h, **20**, 4% of **21**, 51% of **22**; (d) TBSCl, imidazole, DMF, rt, 24 h, 97%; (e) Ph_3P, THF, rt, 3 h, then aq. $NaHCO_3$, rt, 24 h, 63%; (f) $(Cl_3CO)_2CO$, Et_3N, CH_2Cl_2, 0°C, 2 h, 67%; (g) H_2, PtO_2, CH_2Cl_2, rt, 24 h, then $(Cl_3CO)_2CO$, Et_3N, 0°C, 2 h, then rt 22 h, 69% from **23**.

Methyl 2,3-anhydro-β-D-arabinopyranoside (**10**) was used as a convenient starting compound in this part of the work. Treatment of **10** with mesyl chloride and triethylamine in dichloromethane gave the corresponding 4-*O*-mesyl derivative **19** in an almost quantitative yield. Compound **19** readily reacted with sodium azide in DMF (90–95 °C) to afford the corresponding 4-azido derivative **20** (56%) accompanied with a small amount of the 3,4-diazido derivative **22** (18%). However, when the last reaction was carried out at an elevated temperature (140–145 °C), the desired compound **22** was isolated in 51% yield along with a minor quantity of the 2,4-diazido derivative **21** (4%). Major regioisomer **22** was treated with *tert*-butyldimethylsilyl chloride and imidazole to give the corresponding silyl ether **23** (97%), a convenient model-compound for optimising reaction conditions for the conversion of two vicinal azido functions into the imidazolidinone ring. The Staudinger reaction[13] of **23** provided the corresponding 3,4-diamino derivative **24** (63%), which was subsequently treated with triphosgene, under the conditions similar to those recently applied for the conversion of vicinal amino alcohols to oxazolidinones,[14] to give the imidazolidinone **25** in 67% yield (42% from **23**). However, the one-pot catalytic reduction of **23** followed by subsequent triphosgene treatment provided a significantly higher overall yield of **25** (69% from **23**).

Given this success in the model series, the last one-pot sequence was applied to **18**. The diazide **18** was first reduced over PtO_2 in dichloromethane and after 24 h, when the TLC indicated complete conversion of **18**, the reaction mixture was treated with triphosgene, whereby the imidazolidinone **26** was obtained in 66% yield. Treatment of **26** with an aqueous solution of sodium hydroxide, followed by neutralization with Amberlyst 15, gave an almost quantitative yield of (+)-oxybiotin (**1**, Scheme 6), with physical constants (mp and $[\alpha]_D$) in full agreement with those already reported.[4] Spectroscopic data of the final product thus obtained were consistent with structure **1**.

2.1. X-ray analysis

A single crystal X-ray diffraction analysis of compound **26** (Fig. 1) unambiguously confirmed its structure providing a proof that all intermediates generated by the multistep sequence **7→18** retained the required (*S*)-configuration at the C-2. The values of torsion angles C1′–C2–C3–N3=42.4(1)° and N3–C3–C4–N4=6.4(2)° are consistent with the all-*cis* geometry of **26**. The ureido ring, including the carbonyl oxygen, is essentially planar. The maximum deviation from the best plane of the ureido ring atoms is 0.066(2) Å for C-4. The bond distances [C6–O6=1.241(2), C6–N4=1.348(2) and C6–N3=1.350(2) Å] are comparable to those observed in the ureido system of (+)-biotin.[15] The five membered tetrahydrofuran ring adopts an envelope conformation, with O-1 above the best plane [0.608(1) Å] that contains the C-2, C-3, C-4 and C-5 ring atoms. The bicyclic moiety adopts an *endo* conformation with the O-1 oxygen atom proximal to the ureido ring [the nonbonded distance O1···C6=3.433(2) Å; torsion angles: N3–C3–C2–O1=−79.7(2)° and N4–C4–C5–O1=90.6(1)°]. Similar geometry of the bicyclic moiety was already observed in



Scheme 6. (a) (*i*) H_2, PtO_2, CH_2Cl_2, rt, 22 h, (*ii*) $(Cl_3CO)_2CO$, Et_3N, 0 °C, 2 h, then rt, 21 h, 66%; (b) NaOH, H_2O, rt, 24 h, then Amberlyst 15, rt, 1 h, 99%.

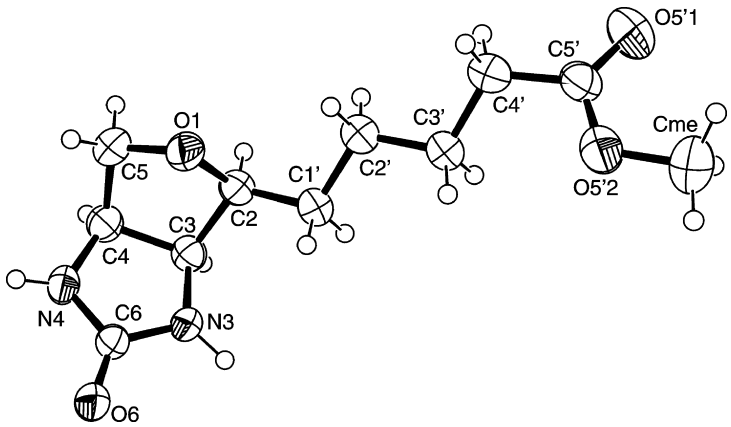


Figure 1. ORTEP drawing of the (+)-oxybiotin methyl ester (**26**) with non-H labelling scheme. The displacement ellipsoids were drawn at 50% probability.

the molecular structure of biotin.[15] The values of torsion angles C2–C1′–C2′–C3′=−176.4(1)°, C1′–C2′–C3′–C4′=179.3(2)° and C2′–C3′–C4′–C5′=171.8(2)° are consistent with an all-*trans* extended conformation of the valeryl side chain.

3. Conclusions

In conclusion, this paper reports a convenient ten-step synthesis of (+)-oxybiotin by chirality transfer from D-arabinose. Two independent routes towards the key intermediate **15** have been developed. The six-step sequence that involves the arabinopyranoside 2-triflate ring contraction process as a key step (Scheme 2) provided **15** in 32% overall yield with respect to the commercially available methyl β-D-arabinopyranoside (**2**). However, the alternative five-step sequence, based on the lactol **13** as a key intermediate (Scheme 3) furnished **15** in considerably higher overall yield (60% from D-arabinose). The intermediate **15** was finally converted to the target **1** by using the four-step sequence, which included the successive introduction of two azido groups at C-3 and C-4, with inversion of configuration at these positions, followed by a newly developed one-pot procedure for construction of the ureido system by using triphosgene, a safe and stable replacement of phosgene.[16] The overall yield of (+)-oxybiotin (**1**) from D-arabinose achieved via the lactol **13** as an intermediate was 22%. Finally, an X-ray diffraction analysis of **26** confirmed that its bicyclic moiety adopts an *endo* conformation, which is thought to be crucial for biological activity of (+)-biotin and analogues.[17]

4. Experimental

4.1. General methods

Melting points were determined on a Büchi 510 apparatus and were not corrected. Optical rotations were measured on a Polamat A (Zeiss, Jena) polarimeter. IR spectra were recorded with a Specord 75 IR spectrophotometer. NMR spectra were recorded on a Bruker AC 250 E instrument and chemical shifts are expressed in ppm downfield from tetramethylsilane. Low resolution mass spectra were recorded on Finnigan-MAT 8230 (CI) and VG AutoSpec (FAB) mass spectrometers. High-resolution mass spectra were taken on a Micromass LCT KA111 spectrometer. TLC was performed on DC Alufolien Kieselgel 60 F_{254} (E. Merck). Flash column chromatography was performed using ICN silica 32–63. All organic extracts were dried with anhydrous Na_2SO_4. Organic solutions were concentrated in a rotary evaporator under diminished pressure at a bath temperature below 35 °C.

4.1.1. Methyl 3,4-*O*-isopropylidene-β-D-arabinopyranoside (3). To a solution of **2** (1.37 g, 8.37 mmol) in dry DMF (12 mL) was added $Me_2C(OMe)_2$ (2.22 mL, 18.08 mmol) and TsOH×H_2O (0.015 g, 0.08 mmol). The mixture was stirred for 3.5 h at room temperature and then neutralized by stirring with Amberlite IRA-400 resin (3 g) at room temperature for 1 h. The mixture was filtered and the resin washed with MeOH. The combined organic solutions were evaporated to give pure **3** (1.52 g, 89%) as a colorless syrup, $[\alpha]_D$=−184.2 (*c*, 2.0 in $CHCl_3$), lit.[9] $[\alpha]_D$=−197.0 (*c*, 1.85 in $CHCl_3$), R_F=0.60 (EtOAc). ^{1}H NMR ($CDCl_3$): δ 1.35 and 1.52 (2×s, 3H each, Me_2C), 3.43 (s, 3H, OMe), 2.41 (d, 1H, exchangeable with D_2O, $J_{2,OH}$=7 Hz, OH), 3.74 (td, 1H, $J_{1,2}$=3.7 Hz, $J_{2,3}$=6.4 Hz, H-2), 3.92 (pseudo d, 2H, $J_{4,5}$=1.7 Hz, 2×H-5), 4.16 (t, 1H, $J_{3,4}$=6 Hz, H-3), 4.21 (m, 1H, H-4), 4.71 (d, 1H, H-1); ^{1}H NOE contact: OMe and H-5. ^{13}C NMR ($CDCl_3$): δ 25.94 and 27.92 (Me_2C), 55.61 (OMe), 59.24 (C-5), 70.12 (C-2), 72.95 (C-4), 76.00 (C-3), 98.84 (C-1), 109.14 (Me_2C).

4.1.2. Methyl 3,4-*O*-carbonyl-β-D-arabinopyranoside (4). A solution of **2** (0.149 g, 0.85 mmol) and *1*,*1*′-carbonyldiimidazole (0.157 g, 0.97 mmol) in dry benzene (3 mL) was stirred for 1.5 h at reflux. The mixture was evaporated and the residue was purified by flash column chromatography (3:2 EtOAc–CH_2Cl_2). Crystallization from CH_2Cl_2–hexane gave pure **4** (0.111 g, 69%) as colorless needles, mp 113–114 °C; lit.[10] mp 115–119 °C (L-enantiomer), $[\alpha]_D$=−142.7 (*c*, 0.79 in $CHCl_3$), R_F=0.31 (Et_2O). IR (KBr): ν_{max} 3410 (OH), 1800 (C=O). ^{1}H NMR (pyridine-d_5+D_2O): δ 3.33 (s, 3H, OMe), 4.18 (dd, 1H, $J_{1,2}$=3.7 Hz, $J_{2,3}$=7 Hz, H-2), 4.27 (s, 2H, 2×H-5), 5.10 (d, 1H, H-1), 5.28 (m, 2H, H-3 and H-4). ^{13}C NMR (pyridin-d_5): δ 55.82 (OMe), 58.30 (C-5), 69.14 (C-2), 76.41 and 78.37 (C-3 and C-4), 99.71 (C-1), 155.38 (C=O). FAB MS: *m*/*z* 191 (M^++H), 173 (M^+−OH), 159 (M^+−OMe).

4.1.3. Methyl 3,4-*O*-isopropylidene-2-*O*-trifluoromethanesulfonyl-β-D-arabinopyranoside (5). To a cooled (−10 °C) and stirred solution of **3** (1.34 g, 6.65 mmol) in dry CH_2Cl_2 (10 mL) and pyridine (2.66 mL, 32.92 mmol) was added a cooled (−10 °C) solution of Tf_2O (2.16 mL, 13.17 mmol) in dry CH_2Cl_2 (10 mL). The mixture was stirred at −10 °C for 0.5 h, then diluted with CH_2Cl_2 (20 mL) and washed successively with aq 5% HCl (2×25 mL) and 1% $NaHCO_3$ (25 mL). The organic solution was dried and evaporated. Flash column chromatography of the residue (1:1 CH_2Cl_2–cyclohexane) gave pure **5** (7.84 g, 70%) as a colorless solid. Recrystallization from CH_2Cl_2–hexane gave colorless crystals, mp 121–123 °C, $[\alpha]_D$=−176.6 (*c*, 0.5 in $CHCl_3$), R_F=0.50 (CH_2Cl_2). IR (KBr): ν_{max} 1415 (as. SO_2), 1225–1210 (sim. SO_2), 1125 (CF_3). ^{1}H NMR ($CDCl_3$): δ 1.37 and 1.53 (2×s, 3H each, Me_2C), 3.44 (s, 3H, OMe), 3.93 (dd, 1H, $J_{5a,5b}$=13.5 Hz, $J_{4,5a}$=2.6 Hz, H-5a), 4.03 (d, 1H, H-5b), 4.31 (dd, 1H, $J_{3,4}$=5.5 Hz, H-4), 4.38 (dd, 1H, $J_{2,3}$=7.6 Hz, H-3), 4.73 (dd, 1H, $J_{1,2}$=3.4 Hz, H-2), 4.88 (d, 1H, H-1). ^{13}C NMR ($CDCl_3$): δ 26.08 and 27.69 (Me_2C), 55.17 (C-5), 72.26 (C-3), 74.22 (C-4), 85.23 (C-2), 97.13 (C-1), 110.05 (Me_2C), 118.43 (q, $J_{C,F}$=319 Hz, CF_3). FAB MS: *m*/*z* 359 (M^++Na), 337 (M^++H).

4.1.4. Methyl 3,4-*O*-carbonyl-2-*O*-trifluoromethanesulfonyl-β-D-arabinopyranoside (6). A solution of **4** (0.326 g, 1.71 mmol) in dry CH_2Cl_2 (15 mL) and pyridine (0.7 mL, 8.66 mmol) was treated with Tf_2O (0.7 mL, 4.27 mmol) in dry CH_2Cl_2 (3 mL) under the above described conditions to give crude **6**. Flash column chromatography (CH_2Cl_2) afforded pure **6** (0.493 g, 89%) that crystallized from CH_2Cl_2–hexane in the form of colorless needles, mp 118–120 °C, $[\alpha]_D$=−148.2 (*c*, 0.89 in $CHCl_3$), R_F=0.47 (CH_2Cl_2). IR (KBr): ν_{max} 1800 (C=O), 1420 (as. SO_2), 1230–1210 (sim. SO_2), 1140 (CF_3). ^{1}H NMR ($CDCl_3$): δ 3.51 (s, 3H, OMe), 3.96 (dd, 1H, $J_{5a,5b}$=14.3 Hz, $J_{4,5a}$=2.7 Hz, H-5a), 4.17 (d, 1H, H-5b), 4.83 (dd, 1H, $J_{1,2}$=3.5 Hz, $J_{2,3}$=7.2 Hz, H-2), 4.89 (dd, 1H, $J_{3,4}$=6.9 Hz, H-4), 4.96 (t, 1H, H-3), 5.03 (d, 1H, H-1). ^{13}C NMR ($CDCl_3$): δ 56.46 (OMe), 56.86 (C-5), 73.12 (C-3), 75.29 (C-4), 81.55 (C-2), 96.03 (C-1), 118.32 (q, $J_{C,F}$= 320.4 Hz, CF_3SO_2), 152.65 (C=O). FAB MS: *m*/*z* 345 (M^++Na), 323 (M^++H). Anal. calcd for $C_8H_9F_3O_8S$: C, 29.82; H, 2.82; S, 9.95. Found: C, 30.06; H, 2.95; S, 10.33.

4.1.5. 2,5-Anhydro-1-*O*-benzoyl-3,4-*O*-isopropylidene-D-ribose methyl hemiacetal (7). *Procedure A*. To a solution of **5** (1.80 g, 5.35 mmol) in dry DMF (25 mL) was added KOBz (2.00 g, 12.49 mmol) and the resulting suspension was stirred at room temperature for 20 h. The solvent was evaporated and the residue partitioned between CH_2Cl_2 (50 mL) and water (50 mL). Organic phase was washed with water (2×50 mL), dried and evaporated. Flash column chromatography of the residue (4:1 CH_2Cl_2–toluene) gave pure **7** (1.31 g, 79%) as a colorless oil (an inseparable mixture of C-1 epimers).

Procedure B. A solution of **3** (5.50 g, 26.93 mmol) in CH_2Cl_2 (70 mL) and pyridine (11 mL, 136.14 mmol) was treated with Tf_2O (10.66 mL, 64.99 mmol) in CH_2Cl_2 (20 mL) according to procedure given in Section 4.1.4. to afford crude **5**. Treatment of crude **5** with KOBz (8.60 g, 53.40 mmol) in dry DMF (100 mL) for 20 h at room temperature, followed by the same workup as described above (Procedure A) gave **7** (8.20 g, 99%), as an inseparable 2:1 mixture of C-1 epimers, $[\alpha]_D$=−42.6 (*c*, 0.5 in $CHCl_3$), R_F=0.44 (CH_2Cl_2). IR (film): ν_{max} 1730 (C=O, ester), 1600 (Ph); ^{1}H NMR ($CDCl_3$): δ 1.38, 1.52 and 1.53 (3×s, 6H, CMe_2), 3.51 and 3.53 (2×s, 3H, OMe), 3.92–4.11 (m, 2H, 2×H-5), 4.21 and 4.29 (partially overlapped 2×dd, 1H, H-2), 4.82–5.05 (m, 2H, H-3 and H-4), 6.00 and 6.09 (2×d, 1H, H-1), 7.40–8.16 (m, 5H, Ph). FAB MS: *m*/*z* 331 (M^++Na), 309 (M^++H). HR MS (ES+): *m*/*z* 331.1157 (M^++Na). Calcd for $C_{16}H_{20}O_6Na$: 331.1158.

4.1.6. 2,5-Anhydro-1-*O*-benzoyl-3,4-*O*-carbonyl-D-ribose methyl hemiacetal (8). *Procedure A*. A mixture of **6** (0.203 g, 0.63 mmol) and KOBz (0.20 g, 1.25 mmol) in dry DMF (5 mL) was stirred for 2 h at 60–65 °C. After workup as described above (preparation of **7**) followed by chromatographic purification on a column of flash silica (9:1 CH_2Cl_2–toluene) gave pure **8** (0.161 g, 87%) as a colorless syrup (an inseparable mixture of C-1 epimers).

Procedure B. A solution of **4** (0.51 g, 2.68 mmol) in a mixture of CH_2Cl_2 (20 mL) and pyridine (2 mL, 24.75 mmol) was allowed to react with a solution of Tf_2O (1.32 mL, 8.05 mmol) in CH_2Cl_2 (5 mL) according to procedure B in Section 4.1.5 to afford crude **6**. A mixture of crude **6** and KOBz (0.861 g, 5.38 mmol) in dry DMF (20 mL), was stirred for 2 h at 60–65 °C. After workup as described above (preparation of **7**) followed by chromatographic purification on a column of flash silica (9:1 CH_2Cl_2–toluene) oily **8** was obtained (0.63 g, 80%), as a 1:1 mixture of C-1 epimers, $[\alpha]_D$=−16.7 (*c*, 0.70 in $CHCl_3$), R_F=0.54 (CH_2Cl_2). IR (film): ν_{max} 1810 (C=O, carbonate), 1730 (C=O, BzO), 1600 (Ph). ^{1}H NMR ($CDCl_3$): δ 3.51 and 3.52 (2×s, 3H, OMe), 4.09–4.23 (m, 2H, 2×H-5), 4.40 and 4.51 (2×d, 1H, $J_{1,2}$=3 Hz, H-2), 5.29 (m, 1H, H-4), 5.41 and 5.49 (2×d, 1H, $J_{3,4}$=7.1, 7 Hz, H-3), 6.06 and 6.13 (2×d, 1H, H-1), 7.40–8.15 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$): δ 57.61 and 57.66 (OMe), 73.76 and 74.04 (C-5), 80.54, 80.66 and 81.00 (C-3 and C-4), 83.91 and 84.04 (C-2), 97.28 and 97.39 (C-1), 128.50, 128.59, 128.71, 129.69, 129.81, 129.94, 133.84 and 133.94 (Ph), 154.00 (C=O, carbonate), 165.42 and 165.58 (C=O, BzO). FAB MS: *m*/*z* 317 (M^++Na), 295 (M^++H). HR MS (ES+): *m*/*z* 317.0640 (M^++Na). Calcd for $C_{14}H_{14}O_7Na$: 317.0637.

4.1.7. 2,5-Anhydro-3,4-*O*-isopropylidene-D-ribose dimethyl acetal (9). A suspension of **5** (3.90 g, 11.60 mmol) and $NaHCO_3$ (1.20 g, 14.29 mmol) in dry MeOH (35 mL), was stirred for 4 h at 50 °C. The mixture was cooled to ambient temperature, diluted with Et_2O (15 mL), filtered and evaporated to an oil. Flash column chromatography (17:3 toluene–Et_2O) of the residue gave pure **9** (0.29 g, 12%) as a colorless oil, $[\alpha]_D$=−21.0 (*c*, 1.02 in $CHCl_3$), R_F=0.36 (CH_2Cl_2). IR (film): ν_{max} 1100–1050 (C–O–C). ^{1}H NMR (1:1 $CDCl_3$–benzene-d_6): δ 1.22 and 1.46 (2×s, 3H each, Me_2C), 3.20 and 3.22 (2×s, 3H each, 2×OMe), 3.88 (dd, 1H, $J_{4,5a}$=4.2 Hz, $J_{5a,5b}$=9.9 Hz, H-5a), 3.94 (dd, 1H, $J_{4,5b}$=1.1 Hz, H-5b), 4.09 (bs, 2H, H-1 and H-2), 4.57 (ddd, 1H, $J_{3,4}$=6.3 Hz, H-4), 4.77 (d, 1H, H-3). ^{13}C NMR (1:1 $CDCl_3$–benzene-d_6): δ 24.60 and 26.42 (Me_2C), 54.68 and 55.66 (2×OMe), 74.06 (C-5), 81.32

(C-4), 81.48 (C-3), 84.46 (C-2), 104.98 (C-1), 112.07 (CMe_2). FAB MS: *m/z* 241 (M^++Na), 225 (M^++Na+H-Me), 217 (M^+−H). HR MS (ES+): *m/z* 241.1054 (M^++Na). Calcd for $C_{10}H_{18}O_5Na$: 241.1052.

4.1.8. Methyl 2,3-anhydro-β-D-ribopyranoside (10). To a solution of **6** (0.218 g, 0.68 mmol) in dry MeOH (5 mL) was added $NaHCO_3$ (0.07 g, 0.83 mmol) and the resulting suspension was stirred at 55–60 °C for 3 h, then filtered and evaporated. Flash column chromatography (9:1 CH_2Cl_2–EtOAc) of the residue gave pure **10** (0.076 g, 77%). Crystallization from CH_2Cl_2–hexane furnished colorless crystals, mp 51 °C, $[\alpha]_D$=−53.2 (*c*, 0.5 in $CHCl_3$); lit.[11] mp 46 °C, $[\alpha]_D$=−35.8 (*c*, 0.6 in $CHCl_3$), R_F=0.47 (Et_2O). IR (KBr): ν_{max} 3440–3300 (OH). ^{1}H NMR ($CDCl_3$): δ 2.64 (d, 1H, exchangeable with D_2O, $J_{4,OH}$=11.3 Hz, OH), 3.21 (d, 1H, $J_{2,3}$=3.8 Hz, H-2), 3.41 (dt, 1H, $J_{5a,5b}$=12.5 Hz, $J_{4,5a}$=1.3 Hz, $J_{3,5a}$=1 Hz, H-5a), 3.46 (s, 3H, OMe), 3.54 (bt, 1H, $J_{3,4}$=4.5 Hz, H-3), 3.77 (dd, 1H, $J_{4,5b}$=3 Hz, H-5b), 3.90 (m, 1H, H-4), 4.84 (s, 1H, H-1). ^{13}C NMR ($CDCl_3$): δ 51.68 (C-2 and C-3), 55.70 (OMe), 61.60 and 61.65 (C-4 and C-5), 95.34 (C-1). FAB MS: *m/z* 147 (M^++H).

4.1.9. 3,4-*O*-Isopropylidene-2-*O*-methanesulfonyl-β-D-arabinopyranosyl chloride (12). To a stirred and cooled (−10 °C) solution of **11**[8] (1.15 g, 6.05 mmol) in a mixture of dry CH_2Cl_2 (12 mL) and Et_3N (2.53 mL, 18.15 mmol) was added dropwise a solution of MsCl (1.17 mL, 15.12 mmol) in CH_2Cl_2 (3.5 mL). The mixture was stirred for 1 h at −10 °C, then diluted with CH_2Cl_2 (20 mL), and washed successively with cold (+4 °C) aq 5% HCl (2×40 mL) and 1% $NaHCO_3$ (20 mL). Organic phase was dried and evaporated to a pale yellow solid. Flash column chromatography (CH_2Cl_2) gave pure **12** (1.52 g, 88%). Recrystallization from CH_2Cl_2–hexane gave colorless crystals, mp 129–131 °C (decomposition), $[\alpha]_D$=−205.3 (*c*, 1.0 in $CHCl_3$), R_F=0.40 (CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 1.39 and 1.59 (2×s, 3H each, Me_2C), 3.19 (s, 3H, $MeSO_2$), 4.18–4.38 (m, 3H, 2×H-5 and H-4), 4.43 (dd, 1H, $J_{2,3}$=7.6 Hz, $J_{3,4}$=4.9 Hz, H-3), 4.75 (dd, 1H, $J_{1,2}$=3.7 Hz, H-2), 6.13 (d, 1H, H-1). ^{13}C NMR ($CDCl_3$): δ 26.13 and 27.86 (Me_2C), 38.75 ($MeSO_2$), 61.12 (C-5), 72.37 (C-3), 73.03 (C-4), 78.11 (C-2), 90.92 (C-1), 110.11 (Me_2C). CI MS: *m/z* 536 ($2M^+$−Cl), 287 (M^++H), 251 (M^+−Cl).

4.1.10. 3,4-*O*-Isopropylidene-2-*O*-methanesulfonyl-D-arabinopiranose (13). To a solution of **11** (3.00 g, 15.77 mmol) in dry CH_2Cl_2 (25 mL) and Et_3N (9.10 mL, 65.29 mmol) was added dropwise a solution of MsCl (4 mL, 51.58 mmol) in dry CH_2Cl_2 (12 mL). The mixture was stirred at −10 °C for 1 h. After workup as described above (procedure in Section 4.1.9), crude **12** was dissolved in Me_2CO (40 mL) and cooled to 0 °C. To the solution were added Ag_2O (3.70 g, 15.97 mmol), AgOTf (0.30 g, 1.17 mmol), and water (2 mL). The mixture was allowed to warm to room temperature and then stirred for the next 20 h. The reaction mixture was diluted with EtOAc (20 mL), filtered through a Celite pad and evaporated. Flash column chromatography (EtOAc) of the residue gave pure **13** (4.00 g, 95%), as a colorless syrup. Crystallization from a mixture of EtOAc–light petroleum furnished colorless crystals, mp 112–114 °C. Recrystallization from Et_2O gave an analytical sample **13**, mp 116–117 °C, $[\alpha]_D$=−116.5 (*c*, 1.5 in $CHCl_3$), lit.[9] mp 130–131 °C, $[\alpha]_D$=−118.0 (*c*, 2.06 in Me_2CO), R_F=0.70 (EtOAc). IR (KBr): ν_{max} 3420 (OH), 1360 (as. SO_2), 1190 (sim. SO_2). ^{1}H NMR ($CDCl_3$): δ 1.38 and 1.58 (2×s, 3H each, Me_2C), 3.18 (s, 3H, $MeSO_2$), 3.83 (dd, $J_{5a,5b}$=14.1 Hz, $J_{4,5a}$=2.5 Hz, H-5aα), 3.97 (d, 1H, $J_{5a,5b}$=13.3 Hz, H-5aβ), 4.14–4.33 (m, 2H, H-4αβ, H-5bαβ), 4.40 (dd, 1H, $J_{2,3}$=7.8 Hz, $J_{3,4}$=5.4 Hz, H-2α and H-3β), 4.56 (dd, 1H, $J_{1,2}$=3.3 Hz, H-2β), 4.61 (d, 1H, $J_{1,2}$=7.9 Hz, H-1α), 5.35 (d, 1H, H-1β). ^{13}C NMR ($CDCl_3$): δ 26.25 and 27.99 (Me_2C, α), 26.24 and 27.80 (Me_2C, β), 38.66 ($MeSO_2$, β), 39.06 ($MeSO_2$, α), 58.4 (C-5, β), 63.28 (C-5, α), 72.98 (C-3), 73.83 (C-4), 80.01 (C-2, β), 83.42 (C-2, α), 90.94 (C-1, β), 93.91 (C-1, α), 109.84 (Me_2C, β), 110.84 (Me_2C, α). FAB MS: *m/z* 518 ($2M^+$−H_2O), 269 (M^++H), 251 (M^+−OH). HR MS (ES+): *m/z* 286.0960 (M^++NH_4). Calcd for $C_9H_{20}NO_7S$: 286.0960.

4.1.11. *E,E*−2*S*-(4′-Methoxycarbonyl-1′,3′-butadienyl)-3*S*,4*R*-*O*-isopropylidene-tetrahydrofuran (14). To a stirred solution of **13** (0.54 g, 2.01 mmol) and trimethyl-4-phosphonocrotonate (0.46 g, 2.21 mmol) in dry THF (20 mL) was added NaH (0.136 g, 5.67 mmol) in portions during 5 min. The mixture was stirred at room temperature for 20 min then filtered, diluted with Et_2O (20 mL) and evaporated. Flash column chromatography (19:1 CH_2Cl_2–EtOAc) of the residue gave pure *E,E*-isomer **14** (0.246 g, 48%) as a colorless solid. Recrystallization from CH_2Cl_2–hexane gave an analytical sample *E,E*-**14** as colorless needles, mp 75 °C, $[\alpha]_D$=−108.2 (*c*, 0.88 in $CHCl_3$), R_F=0.29 (CH_2Cl_2). IR (KBr): ν_{max} 1710 (C=O, ester), 1650 and 1630 (CH=CH–CH=CH). ^{1}H NMR ($CDCl_3$): δ 1.32 and 1.51 (2×s, 3H each, Me_2C), 3.73 (s, 3H, CO_2Me), 3.83 (dd, 1H, $J_{5a,5b}$=10.7 Hz, $J_{4,5a}$=4.2 Hz, H-5a), 3.99 (dd, 1H, $J_{4,5b}$=1 Hz, H-5b), 4.58 (dd, 1H, $J_{2,3}$=1.7 Hz, $J_{3,4}$=6.2 Hz, H-4), 4.62 (ddd, 1H, $J_{2,2'}$=1.8 Hz, $J_{2,1'}$=4.6 Hz, H-2), 4.77 (dd, 1H, H-3), 5.88 (d, 1H, $J_{3',4'}$=15.4 Hz, H-4′), 5.97 (dd, 1H, $J_{1',2'}$=15.5 Hz, H-1′), 6.39 (ddd, 1H, $J_{2',3'}$=11 Hz, H-2′), 7.24 (dd, 1H, H-3′). ^{13}C NMR ($CDCl_3$): δ 24.91 and 26.48 (Me_2C), 51.53 (CO_2Me), 72.53 (C-5), 80.84 (C-4), 83.92 (C-2), 84.76 (C-3), 112.90 (Me_2C), 121.75 (C-4′), 128.83 (C-2′), 138.02 (C-1′), 143.23 (C-3′), 167.09 (CO_2Me). FAB MS: *m/z* 277 (M^++Na), 255 (M^++H). HR MS (ES+): *m/z* 277.1052 (M^++Na). Calcd for $C_{13}H_{18}O_5Na$: 277.1052. Anal. calcd for $C_{13}H_{18}O_5$: C, 61.40; H, 7.14. Found: C, 61.52; H, 6.97.

4.1.12. 2*S*-(4′-Methoxycarbonyl-1′-butyl)-3*S*,4*R*-*O*-isopropylidene-tetrahydrofuran (15). *Procedure A*. To a solution of **7** (0.85 g, 2.76 mmol) in anhydrous MeOH (35 mL) was added 0.15 M NaOMe in MeOH (3.5 mL) and the mixture was stirred for 2 h at room temperature. 3-(methoxycarbonyl-2-propenylidene)triphenylphosphorane[14] (1.49 g, 4.14 mmol) was added to the solution and the reaction mixture was stirred at room temperature for additional 2 h and then evaporated. Chromatographic purification on a column of flash silica (7:3 light petroleum–Et_2O) afforded pure **14** (0.29 g) as an inseparable mixture of *E*- and *Z*-isomers. A solution of **14** (0.29 g, 1.14 mmol) in MeOH (20 mL) was hydrogenated over PtO_2 (0.03 g) for 24 h at room temperature. The mixture was filtered and the catalyst washed with MeOH. The organic

solution was evaporated. and the residue was purified by flash chromatography (9:1 CH_2Cl_2–EtOAc) to afford pure **15** (0.26 g, 36%) as a colorless oil.

Procedure B. To a solution of **13** (0.15 g, 0.56 mmol) in anhydrous DMF (6 mL) was added 3-(methoxycarbonyl-2-propenylidene)triphenylphosphorane[14] (0.28 g, 0.78 mmol) and anh Na_2CO_3 (0.50 g, 4.72 mmol). The mixture was stirred for 2 h at 110–120 °C, then poured in water (100 mL) and extracted with Et_2O (3×30 mL). The extracts were combined and evaporated. Flash column chromatography (7:3 light petroleum–ether) of the residue gave an inseparable mixture of *E*- and *Z*-isomers **14** (0.115 g, 81%). A solution of **14** (0.115 g, 1.14 mmol) in MeOH (6 mL) was hydrogenated over PtO_2 (0.01 g) by using the same methodology as described in the Procedure A, to afford pure **15** (0.127 g, 88%) as a colorless oil, $[\alpha]_D$=−30.7 (*c*, 0.90 in $CHCl_3$), R_F=0.32 (CH_2Cl_2). IR (KBr): ν_{max} 1735 (C=O, ester). ^{1}H NMR ($CDCl_3$): δ 1.24 and 1.41 (2×s, 3H each, Me_2C), 1.28–1.67 (m, 6H, 3×CH_2), 2.23 (t, 2H, C$H_2$$CO_2$Me), 3.58 (s, 3H, CO_2Me), 3.72, (dd, 1H, $J_{5a,5b}$=10.6 Hz, $J_{4,5a}$=4.2 Hz, H-5a), 3.81 (dd, 1H, $J_{4,5b}$=1.7 Hz, H-5b), 3.89 (m, 1H, $J_{2,3}$=1.7 Hz, H-2), 4.32 (dd, 1H, $J_{3,4}$=6.3 Hz, H-3), 4.69 (ddd, 1H, H-4). ^{13}C NMR ($CDCl_3$): δ 24.76 and 26.40 (*Me*$_2$C), 24.44, 25.09 and 30.18 (3×CH_2), 33.64 (*C*$H_2$$CO_2$Me), 51.24 ($CO_2$*Me*), 71.28 (C-5), 80.71 (C-4), 83.87 (C-2), 84.70 (C-3), 112.50 (Me$_2$*C*), 173.68 (*C*O_2Me). FAB MS (ES+): *m*/*z* 281 (M^++Na). HR MS (ES+): *m*/*z* 281.1363 (M^++Na). Calcd for $C_{13}H_{22}O_5Na$: 281.1365.

4.1.13. 2*S*-(4′-Methoxycarbonyl-1′-butyl)-3*S*,4*R*-dihydroxy-tetrahydrofuran (16). A solution of **15** (0.316 g, 1.22 mmol) in aq 90% TFA (2 mL) was stirred for 1 h at room temperature. The mixture was evaporated by co-distillation with toluene (3×5 mL) to a yellow oil. Flash column chromatography (EtOAc) of the residue gave pure **16** (0.253 g, 95%) as a colorless oil, $[\alpha]_D$=−39.4 (*c*, 1.0 in $CHCl_3$), R_F=0.26 (Et_2O). IR (film): ν_{max} 3380 (OH), 1370 (C=O, ester). ^{1}H NMR ($CDCl_3$): δ 1.38–1.65 (m, 6H, 3×CH_2), 2.35 (t, 2H, *C*$H_2$$CO_2$Me), 3.62 (s, 3H, CO_2Me), 3.6–3.8 (m, 5H, 2×OH, H-2, H-3 and H-5a), 4.04 (dd, 1H, $J_{5a,5b}$=9.9 Hz, $J_{4,5b}$=5.2 Hz, H-5b), 4.19 (m, 1H, $J_{4,5a}$=4.6 Hz, H-4). ^{13}C NMR ($CDCl_3$): δ 24.67, 25.19 and 32.74 (3×CH_2), 33.79 (*C*$H_2$$CO_2$Me), 51.52 ($CO_2$*Me*), 70.81 (C-4), 72.44 (C-5), 75.64 (C-2), 81.85 (C-3). CI MS: *m*/*z* 219 (M^++H). FAB MS (ES+): *m*/*z* 241 (M^++Na). HR MS (ES+): *m*/*z* 241.1057 (M^++Na). Calcd for $C_{10}H_{18}O_5Na$: 241.1052.

4.1.14. 2*S*-(4′-Methoxycarbonyl-1′-butyl)-3*S*,4*R*-*bis*-trifluoromethanesulfonyloxi-tetrahydrofuran (17). To a stirred and cooled (−10 °C) solution of **16** (0.43 g, 1.97 mmol) in a mixture of dry CH_2Cl_2 (17 mL) and pyridine (0.80 mL, 9.9 mmol) was added dropwise a cooled (−10 °C) solution of Tf_2O (0.69 mL, 4.21 mmol) in dry CH_2Cl_2 (7 mL). The mixture was stirred at 0 °C for 1.5 h, then diluted with CH_2Cl_2 (20 mL) and washed successively with aq 5% HCl (2×25 mL), 1% $NaHCO_3$ (25 mL) and water (25 mL). The organic solution was separated, dried and evaporated to a yellow oil. Flash column chromatography (CH_2Cl_2) of the residue gave pure **17** (0.48 g, 51%) as a colorless oil, $[\alpha]_D$=−35.9 (*c*, 2.25 in $CHCl_3$), R_F=0.68 (CH_2Cl_2). IR (film): 1730 (C=O, ester), 1420 (as. SO_2), 1240–1205 (sim. SO_2), 1130 (CF_3). ^{1}H NMR ($CDCl_3$): δ 1.37–1.83 (m, 6H, 3×CH_2), 2.31 (t, 2H, C$H_2$$CO_2$Me), 3.65 (s, 3H, CO_2Me), 4.03 (dd, 1H, $J_{5a,5b}$=11.2 Hz, $J_{4,5a}$=4.2 Hz, H-5a), 4.09 (m, 1 H, *J*=6.7 Hz, H-2), 4.32 (dd, 1H, $J_{4,5b}$=5.3 Hz, H-5b), 4.88 (t, 1H, $J_{3,4}$=6.1 Hz, H-3), 5.34 (m, 1H, H-4). ^{13}C NMR ($CDCl_3$): δ 24.40, 24.61 and 31.38 (3×CH_2), 33.59 (*C*$H_2$$CO_2$Me), 51.43 ($CO_2$*Me*), 69.24 (C-5), 79.23 (C-2), 81.42 (C-4), 83.66 (C-3), 118.19 (q, $J_{C,F}$=319.5 Hz, 2×CF_3SO_2), 173.69 (C=O). HR MS (EI): *m*/*z* 482.0163 (M^+). Calcd for $C_{12}H_{16}F_6O_9S_2$: 482.0140.

4.1.15. 2*S*-(4′-Methoxycarbonyl-1′-butyl)-3*S*,4*R*-diazido-tetrahydrofuran (18). *Procedure A*. To a solution of **17** (0.40 g, 0.83 mmol) in HMPA (4 mL) was added NaN_3 (1.50 g, 23.08 mmol) and the resulting suspension was stirred for 1.5 h at room temperature. The mixture was poured in water (30 mL) and extracted with 1:1 benzene–light petroleum (4×20 mL). Organic phase was washed with H_2O (2×20 mL), dried and evaporated to a yellow oil. Flash column chromatography (CH_2Cl_2) of the residue gave pure **18** (0.207 g, 93%) as a pale yellow oil.

Procedure B. A solution of **16** (0.56 g, 2.57 mmol) in dry CH_2Cl_2 (40 mL) and pyridine (2.08 mL, 25.74 mmol) was treated with Tf_2O (2.53 mL, 15.42 mmol) in dry CH_2Cl_2 (10 mL) under the same reaction conditions as described in procedure under Section 4.1.14. The workup as described above yielded crude **17**, which was immediately dissolved in HMPA (20 mL), and treated with NaN_3 (4.70 g, 72.31 mmol) according to the procedure 4.1.15A. Thus obtained crude mixture was purified by flash chromatography (4:1 light petroleum–EtOAc) to afford pure **18** (0.467 g, 68%) as a bright yellow oil, $[\alpha]_D$=+36.0 (*c*, 1.95 in $CHCl_3$), R_F=0.28 (CH_2Cl_2). IR (film): ν_{max} 2100 (N_3), 1740 (C=O, ester). ^{1}H NMR ($CDCl_3$): δ 1.29–1.78 (m, 6H, 3×CH_2), 2.33 (t, 2H, C$H_2$$CO_2$Me), 3.66 (s, 3H, CO_2Me), 3.78 (dd, 1H, $J_{5a,5b}$=9.1 Hz, $J_{4,5a}$=7.2 Hz, H-5a), 3.88 (m, 1H, $J_{1'a,2}$=5.8 Hz, $J_{1'b,2}$=7.3 Hz, $J_{2,3}$=3.4 Hz, H-2), 3.95–4.05 (m, 2H, $J_{3,4}$=7.7, $J_{4,5b}$=4 Hz, H-3 and H-5b), 4.25 (td, 1H, H-4). ^{13}C NMR ($CDCl_3$): δ 24.76, 25.47 and 29.68 (3×CH_2), 33.72 (*C*$H_2$$CO_2$Me), 51.46 ($CO_2$*Me*), 62.96 (C-4), 65.24 (C-3), 68.52 (C-5), 80.78 (C-2), 173.87 (*C*O_2Me). CI MS: *m*/*z* 269 (M^++H). FAB MS (ES+): *m*/*z* 291 (M^++Na). HR MS (ES+): *m*/*z* 291.1174 (M^++Na). Calcd for $C_{10}H_{16}N_6O_3Na$: 291.1182.

4.1.16. Methyl 2,3-anhydro-4-*O*-methanesulfonyl-β-D-ribopyranoside (19). To a stirred and cooled solution (−10 °C) of **10** (0.97 g, 6.65 mmol) in dry CH_2Cl_2 (20 mL) was added Et_3N (1.8 mL, 12.91 mmol) and a solution of MsCl (0.8 mL, 10.32 mmol) in CH_2Cl_2 (3 mL). Stirring was continued for 0.5 h at −10 °C and the mixture diluted with CH_2Cl_2 (10 mL), washed successively with aq 5% HCl (2×30 mL), satd aq $NaHCO_3$ (20 mL) and water (20 mL). The organic solution was dried and evaporated to yellow syrup. Flash column chromatography (19:1 CH_2Cl_2–EtOAc) of the residue gave pure **19** (1.45 g, 97%) as a solid, which upon crystallization from CH_2Cl_2–hexane gave colorless needles, mp 90 °C, $[\alpha]_D$=−19.2 (*c*, 0.5 in $CHCl_3$), R_F=0.52 (Et_2O). IR (KBr): ν_{max} 1350 (as. SO_2), 1190–1170 (sim. SO_2). ^{1}H NMR ($CDCl_3$): δ 3.16 (s, 3H, $MeSO_2$), 3.22 (d, 1H, $J_{2,3}$=3.7 Hz, H-2), 3.46 (s, 3H, OMe),

3.57–3.67 (m, 2H, $J_{3,4}$=4.3 Hz, $J_{4,5a}$=2.4 Hz, $J_{5a,5b}$=13.5 Hz, H-3 and H-5a), 3.92 (dd, 1H, $J_{4,5b}$=4.1 Hz, H-5b), 4.87 (s, 1H, H-1), 4.97 (td, 1H, H-4). ^{13}C NMR ($CDCl_3$): δ 38.72 ($MeSO_2$), 48.88 (C-3), 51.19 (C-2), 56.02 (OMe), 58.41 (C-5), 70.71 (C-4), 95.15 (C-1). FAB MS: *m/z* 247 (M^++Na), 225 (M^++H), 193 (M^+−OMe). Anal. calcd for $C_7H_{12}O_6S$: C, 37.49; H, 5.39; S, 14.30. Found: C, 37.65; H, 5.57; S, 14.56.

4.1.17. Methyl 2,3-anhydro-4-azido-4-deoxy-α-L-lyxopyranoside (20). To a solution of **19** (0.25 g, 1.12 mmol) in dry DMF (10 mL) was added NaN_3 (0.755 g, 11.62 mmol). The mixture was stirred at 90–95 °C for 0.5 h and then at 110–115 °C for additional 15 min. The mixture was evaporated and extracted with EtOAc (30 mL). Organic phase was filtered, washed with water (2×20 mL), dried and evaporated. The residue was purified by flash chromatography (3:2 light petroleum–EtOAc) to give pure **20** (0.107 g, 56%) as a colorless oil, $[\alpha]_D$=−91.8 (*c*, 0.8 in $CHCl_3$), R_F=0.66 (CH_2Cl_2). IR (film): ν_{max} 2120 (N_3). ^{1}H NMR ($CDCl_3$): δ 3.11 (d, 1H, $J_{2,3}$=3.7 Hz, H-3), 3.33 (dd, 1H, $J_{3,5b}\approx$0.7 Hz, H-2), 3.46 (s, 3H, OMe), 3.52 (dd, 1H, $J_{4,5a}$=9.1 Hz, $J_{5a,5b}$=11.4 Hz, H-5a), 3.62 (ddd, 1H, $J_{4,5b}$=5.8 Hz, H-5b), 3.75 (s, 1H, H-1), 4.79 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$): δ 49.98 (C-3), 52.29 (C-4), 52.56 (C-2), 55.91 (OCH_3), 57.44 (C-5), 99.79 (C-1). FAB MS: *m/z* 194 (M^++Na), 172 (M^++H). Further elution of the column gave 3,4-diazido derivative **22** (0.042 g, 18%) as a minor product.

4.1.18. Methyl 3,4-diazido-3,4-dideoxy-α-L-arabinopyranoside (22) and methyl 2,4-diazido-2,4-dideoxy-α-L-xylopyranoside (21). To a solution of **19** (0.63 g, 2.83 mmol) in dry DMF (30 mL) was added NaN_3 (1.84 g, 28.31 mmol) and the resulting suspension was stirred at 140–145 °C for 3.5 h. The workup as described above, followed by flash column chromatography (4:1→3:2 light petroleum–EtOAc), gave two fractions. The first fraction contained pure **21** (0.025 g, 4%), which crystallized from CH_2Cl_2–hexane as colorless crystals, mp 124 °C, $[\alpha]_D$=−202.1 (*c*, 0.5 in $CHCl_3$), R_F=0.55 (9:1 CH_2Cl_2–EtOAc). IR (KBr): ν_{max} 3480 (OH), 2130 (N_3). ^{1}H NMR ($CDCl_3$): δ 3.01 (bd, 1H, exchangeable with D_2O, $J_{3,OH}$=2.4 Hz, OH), 3.26 (dd, 1H, $J_{1,2}$=3.5 Hz, $J_{2,3}$=10.1 Hz, H-2), 3.42 (s, 3H, OMe), 3.46–3.79 (m, 3H, $J_{3,4}$=8.9 Hz, $J_{4,5a}$=7 Hz, $J_{4,5b}$=3.6 Hz, 2×H-5 and H-4), 3.96 (bt, 1H, H-3), 4.78 (d, 1H, H-1). ^{13}C NMR ($CDCl_3$): δ 55.47 (OMe), 59.49 (C-5), 61.90 (C-4), 63.41 (C-2), 71.03 (C-3), 98.76 (C-1). CI MS: *m/z* 215 (M^++H). Anal. calcd for $C_6H_{10}N_6O_3$: C, 33.65; H, 4.71; N, 39.24. Found: C, 33.96; H, 5.06; N, 38.89. Pure **22** (0.306 g, 51%) was then eluted, which crystallized from CH_2Cl_2–hexane as colorless needless, mp 91 °C, $[\alpha]_D$=−17.0 (*c*, 0.5 in $CHCl_3$), R_F=0.37 (9:1 CH_2Cl_2–EtOAc). IR (KBr): ν_{max} 3450 (OH), 2170 and 2120 (N_3). ^{1}H NMR ($CDCl_3$): δ 2.90 (bs, 1H, exchangeable with D_2O, OH), 3.55 (s, 3H, OMe), 3.60 (dd, 1H, $J_{5a,5b}$=12.8 Hz, $J_{4,5a}$=1.6 Hz, H-5a), 3.62 (dd, 1H, $J_{2,3}$=9.8 Hz, $J_{3,4}$=3.8 Hz, H-3), 3.80 (dd, 1H, $J_{1,2}$=7.2 Hz, H-2), 3.81 (m, 1H, $J_{4,5b}$=2.2 Hz, H-4), 4.06 (dd, 1H, H-5b), 4.13 (d, 1H, H-1). ^{13}C NMR ($CDCl_3$): δ 55.47 (OMe), 59.49 (C-5), 61.90 (C-4), 63.41 (C-2), 71.03 (C-3), 98.76 (C-1). FAB MS: *m/z* 215 (M^++H), 172 (M^+−N_3). Anal. calcd for $C_6H_{10}N_6O_3$: C, 33.65; H, 4.72; N, 39.24. Found: C, 34.05; H, 4.72; N, 38.88.

4.1.19. Methyl 3,4-diazido-3,4-dideoxy-2-*O*-*tert*-butyldimethylsilyl-α-L-arabinopyranoside (23). To a stirred solution of **22** (0.27 g, 1.26 mmol) in dry DMF (11 mL) were added *tert*-$BuMe_2SiCl$ (0.81 g, 5.37 mmol) and imidazole (0.373 g, 5.48 mmol). The mixture was stirred for 24 h at room temperature and then evaporated. Flash column chromatography (9:1 light petroleum–EtOAc) of the residue gave pure **23** (0.403 g, 97%) as a colorless syrup, $[\alpha]_D$=−12.8 (*c*, 0.5 in $CHCl_3$), R_F=0.76 (CH_2Cl_2). IR (film): ν_{max} 2120 (N_3). ^{1}H NMR ($CDCl_3$): δ 0.11 and 0.16 (2×s, 3H each, Me_3CSiMe_2), 0.91 (s, 9H, Me_3CSiMe_2), 3.47 (s, 3H, OMe), 3.50 (dd, 1H, $J_{2,3}$=8.6 Hz, $J_{3,4}$=3.6 Hz, H-3), 3.58 (dd, 1H, $J_{4.5a}$=1.6 Hz, $J_{5a,5b}$=12.5 Hz, H-5a), 3.68 (dd, 1H, $J_{1,2}$=6.3 Hz, H-2), 3.88 (m, 1H, $J_{4,5b}$=3.3 Hz, H-4), 4.01 (dd, 1H, H-5b), 4.09 (d, 1H, H-1). ^{13}C NMR ($CDCl_3$): δ −5.08 and −4.48 (Me_3CSiMe_2), 18.09 (Me_3CSiMe_2), 25.64 (Me_3CSiMe_2), 56.69 (OMe), 59.49 (C-4), 63.20 (C-5), 65.66 (C-3), 70.93 (C-2), 104.58 (C-1). FAB MS (ES+): *m/z* 351 (M^++Na). HR MS (ES+): *m/z* 351.1593 (M^++Na). Calcd for $C_{12}H_{24}N_6O_3SiNa$: 351.1577.

4.1.20. Methyl 3,4-diamino-3,4-dideoxy-2-*O*-*tert*-butyldimethylsilyl-α-L-arabinopyranoside (24) and the corresponding oxalate (24×$H_2C_2O_4$). To a solution of **23** (0.229 g, 0.70 mmol) in dry THF (4 mL) was added Ph_3P (0.46 g, 1.75 mmol). The mixture was stirred for 3 h at room temperature. To the reaction mixture was added water (0.3 mL) and $NaHCO_3$ (0.06 g, 0.71 mmol), and the stirring at ambient temperature was continued for the next 24 h. The mixture was evaporated and the residue was purified on a column of flash silica (4:1 EtOAc–MeOH) to give pure **24** (0.121 g, 63%) as a colorless oil, $[\alpha]_D$=−26.6 (*c*, 0.4 in $CHCl_3$), R_F=0.18 (4:1 EtOAc–MeOH). IR (film): ν_{max} 3390–3300 (NH_2). ^{1}H NMR ($CDCl_3$): δ 0.06 and 0.07 (2×s, 3H each, Me_3CSiMe_2), 0.86 (s, 9H, Me_3CSiMe_2) 2.42 (bs, 4H, 2×NH_2), 2.76 (dd, 1H, $J_{2,3}$=7.7 Hz, $J_{3,4}$=3.9 Hz, H-3), 3.05 (m, 1H, $J_{4,5a}$=6 Hz, $J_{4,5b}$=4.5 Hz, H-4), 3.37 (dd, 1H, $J_{1,2}$=5.7 Hz, H-2), 3.41 (s, 3H, OMe), 3.52 (dd, 1H, $J_{5a,5b}$=11.8 Hz, H-5a), 3.71 (dd, 1H, H-5b), 4.06 (d, 1H, H-1). ^{13}C NMR ($CDCl_3$): δ −4.96 and −4.42 (Me_3CSiMe_2), 18.14 (Me_3CSiMe_2), 25.82 (Me_3CSiMe_2), 49.04 (C-4), 55.86 (C-3), 56.22 (OMe), 66.14 (C-5), 73.34 (C-2), 104.44 (C-1). A portion of **24** was converted to the corresponding oxalic acid salt (**24**×$H_2C_2O_4$) by using the following procedure: To a solution of **24** (0.055 g, 0.2 mmol) in dry EtOH (2 mL) was added a solution of oxalic acid (0.02 g, 0.22 mmol) in dry EtOH (1 mL). The mixture was stirred at room temperature for 4 h and then stored at +4 °C for 20 h to yield colorless crystals of pure **24**×$H_2C_2O_4$ (0.051 g, 70%). Recrystallization from EtOH gave an analytical sample as colorless needles, mp 164 °C, $[\alpha]_D$=−24.2 (*c*, 0.2 in H_2O). IR (KBr): ν_{max} 3450–2320 (NH_3^+), 1650 (C=O, oxalate). ^{1}H NMR (D_2O): δ 0.24 (s, 6H, Me_3CSiMe_2), 0.97 (s, 9H, Me_3CSiMe_2), 3.59 (s, 3H, OMe), 3.73 (m, 1H, H-2), 3.93–4.11 (m, 4H, H-3, H-4, and 2×H-5), 4.62 (d, 1H, $J_{1,2}$=4.4 Hz, H-1). Anal. calcd for $C_{14}H_{30}N_2O_7Si$: C, 45.88; H, 8.25, N, 7.64. Found: C, 46.08; H, 8.26, N, 7.32.

4.1.21. Methyl 3,4-dideoxy-3,4-carbonyldiamino-2-*O*-*tert*-butyldimethylsilyl-α-L-arabinopyranoside (25). *Procedure A*. To a stirred and cooled solution (0 °C) of **24** (0.051 g, 0.18 mmol) in dry CH_2Cl_2 (5 mL) was first added

Et_3N (0.08 mL, 0.57 mmol) and then a cooled solution (0 °C) of triphosgene (0.018 g, 0.06 mmol) in dry CH_2Cl_2 (1 mL) was added in three portions. The mixture was stirred for 2 h at 0 °C and evaporated. Flash column chromatography (EtOAc) of the residue, gave pure **25** (0.038 g, 67%), which crystallized from CH_2Cl_2–hexane.

Procedure B. A solution of **23** (0.15 g, 0.46 mmol) in dry CH_2Cl_2 (9 mL) was hydrogenated over PtO_2 (0.015 g, 0.66 mmol) for 24 h at room temperature, and then to the stirred and cooled (0 °C) mixture was added Et_3N (0.2 mL, 1.47 mmol) in one portion. A solution of triphosgene (0.047 g, 0.16 mmol) in dry CH_2Cl_2 (1.5 mL) was added dropwise while stirring the mixture at 0 °C for 1 h. After stirring at room temperature for additional 18 h, the suspension was filtered and the catalyst washed with CH_2Cl_2. The combined organic solution was evaporated and the residue purified by flash chromatography (EtOAc) to afford pure **25** (0.095 g, 69%) as colorless crystals. Recrystallization from CH_2Cl_2–hexane gave an analytical sample **25**, mp 111–112 °C, $[\alpha]_D=-3.2$ (*c*, 1.65 in $CHCl_3$), R_F=0.25 (EtOAc). IR (KBr): ν_{max} 1710 (C=O). ^{1}H NMR ($CDCl_3$): δ 0.08 and 0.10 (2×s, 3H each, Me_3CSiMe_2), 0.88 (s, 9H, Me_3CSiMe_2), 3.41 (s, 3H, OMe), 3.55 (t, 1H, $J_{2,3}=J_{3,4}$=8.3 Hz, H-3), 3.6 (dd, 1H, $J_{1,2}$=6 Hz, H-2), 3.73 (dd, 1H, $J_{4,5a}$=5.7 Hz, $J_{5a,5b}$=12.3 Hz, H-5a), 3.81 (dd, 1H, $J_{4,5b}$=6.4 Hz, H-5b), 4.07 (m, 1H, H-4), 4.20 (d, 1H, H-1), 4.92 (bs, 1H, NH-3), 5.50 (bs, 1H, NH-4). ^{13}C NMR ($CDCl_3$): δ −5.04 and −4.42 (Me_3CSiMe_2), 18.01 (Me_3CSiMe_2), 25.74 (Me_3CSiMe_2), 51.45 (C-4), 55.73 (OMe), 56.13 (C-3), 62.22 (C-5), 73.81 (C-2), 103.13 (C-1), 163.56 (C=O). FAB MS (ES+): *m*/*z* 301 (M^+-H). HR MS (ES+): *m*/*z* 325.1543 (M^++Na). Calcd for $C_{13}H_{26}N_2O_4SiNa$: 325.1560. Anal. calcd for $C_{13}H_{26}N_2O_4Si$: C, 51.66; H, 8.77; N, 9.27. Found: C, 51.94; H, 8.77; N, 9.88.

4.1.22. (+)-Oxybiotin methyl ester (26). A solution of **18** (0.086 g, 0.32 mmol) in dry CH_2Cl_2 (5 mL) was hydrogenated over PtO_2 (0.02 g) for 22 h at room temperature. A solution of triphosgene (0.033 g, 0.11 mmol) in dry CH_2Cl_2 (1 mL), was added dropwise while stirring the mixture at 0 °C for 1 h, and then at room temperature for 3 h. To the solution was added an additional amount of triphosgene (0.012 g, 0.04 mmol) and the mixture was stirred for 1 h at 0 °C and then at room temperature for 18 h. The suspension was filtered and the catalyst washed with CH_2Cl_2. The combined organic solution was concentrated and the residue purified by flash chromatography (9:1 EtOAc–MeOH) to afford pure **26** (0.051 g, 66%) as colorless solid. Recrystallization from CH_2Cl_2–hexane gave colorless crystals, mp 141 °C, $[\alpha]_D=+44.7$ (*c*, 0.5 in $CHCl_3$), R_F=0.29 (Me_2CO). IR (KBr): ν_{max} 3410–3120 (NH), 1750 (*CO*OMe), 1710 (NH*CO*NH). ^{1}H NMR ($CDCl_3$): δ 1.21–1.80 (m, 6H, 3×CH_2), 2.23 (t, 2H, CH_2CO_2Me), 3.40 (m, 1H, $J_{2,3}$=3.6 Hz, $J_{1'a,2}$=6.4 Hz, H-2), 3.49 (dd, 1H, $J_{5a,5b}$=10.1 Hz, $J_{4,5a}$=4.2 Hz, H-5a), 3.63 (s, 3H, CO_2Me), 3.86 (d, 1H, H-5b), 4.17 (dd, 1H, $J_{3,4}$=8.4 Hz, H-3), 4.34 (dd, 1H, H-4), 5.98 and 6.18 (2×bs, 1H each, 2×NH). ^{13}C NMR ($CDCl_3$): δ 24.81, 25.52 and 28.36 (3×CH_2), 33.67 (CH_2CO_2Me), 51.42 (CO_2Me), 57.52 (C-4), 58.98 (C-3), 75.23 (C-5), 82.58 (C-2), 163.62 (NH*C*ONH), 174.14 (*C*O_2Me). FAB MS: *m*/*z* 243 (M^++H). FAB MS (ES+): *m*/*z* 265 (M^++Na). HR MS (ES+): *m*/*z* 265.1168 (M^++Na). Calcd for $C_{11}H_{18}N_2O_4Na$: 265.1164.

4.1.23. (+)-Oxybiotin (1). A solution of **26** (0.068 g, 0.28 mmol) in 1 M aq NaOH (2 mL) was stirred for 24 h at room temperature. The mixture was diluted with water (3 mL) and neutralized by stirring with Amberlist-15 resin (3 g) at room temperature for 1 h. The mixture was filtered and the resin washed with water. The combined aqueous solution was evaporated by co-distillation with a mixture of 1:1 toluene–EtOH to give pure **1** (0.064 g, 99%) as a white powder. Recrystallization from water gave pure **18** as silky crystals, mp 187–188 °C, $[\alpha]_D=+57.8$ (*c*, 0.65 in 1 M NaOH); lit.[4] mp 187–188 °C, $[\alpha]_D=+57.7$. IR (KBr): ν_{max} 3430–2500 (CO*OH*), 1700 (NH*CO*NH), 1670 (*CO*OH). ^{1}H NMR (D_2O): δ 1.41–1.80 (m, 6H, 3×CH_2), 2.46 (t, 2H, CH_2CO_2H), 3.64–3.76 (m, 2H, $J_{2,3}$=4 Hz, $J_{4,5a}$=4.4 Hz, $J_{5a,5b}$=10.4 Hz, H-2 and H-5a), 3.94 (d, 1H, H-5b), 4.42 (dd, 1H, $J_{3,4}$=8.7 Hz, H-3), 4.45 (dd, 1H, H-4). ^{1}H NMR (Me_2SO-d_6): δ 1.18–1.58 (m, 6H, 3×CH_2), 2.20 (t, 2H, CH_2CO_2H), 3.33 (m, 1H, $J_{2,3}$=4 Hz, H-2), 3.39 (dd, 1H, $J_{4,5a}$=4.6 Hz, $J_{5a,5b}$=9.8 Hz, H-5a), 3.65 (d, 1H, H-5b), 4.07 (dd, 1H, $J_{3,4}$=8.7 Hz, H-3), 4.21 (dd, 1H, H-4), 6.36 and 6.40 (2×bs, 1H each, 2×NH). ^{13}C NMR (Me_2SO-d_6): δ 25.29, 25.99 and 28.33 (3×CH_2), 34.35 (CH_2CO_2H), 57.53 (C-4), 59.01 (C-3), 74.34 (C-5), 82.85 (C-2), 163.80 (NH*C*ONH), 176.09 (*C*O_2H). FAB MS: *m*/*z* 229 (M^++H), 211 (M^+-OH). FAB MS (ES+): *m*/*z* 251 (M^++Na). HR MS (ES+): *m*/*z* 251.1007 (M^++Na). Calcd for $C_{10}H_{16}N_2O_4Na$: 251.1008.

4.2. X-ray analysis[18]

A single transparent crystal of compound **26** selected for data collection was mounted on a Bruker PLATFORM three-circle goniometer equipped with SMART 1K CCD detector mounted at a crystal to detector distance of 5.4 cm. The data were collected using graphite monochromated MoKa X-radiation and frame widths of 0.3° in ω, with 10 s used to acquire each frame. More than a hemisphere of three-dimensional data were collected. Additional information regarding data collection and structure refinement is given in Table 1. The data were reduced using the Bruker program SAINT.[19] A semiempirical absorption-correction based upon the intensities of equivalent reflections was applied (program XPREP),[20] and the data were corrected for Lorentz, polarization, and background effects. The Bruker SHELXTL[20] system of programs was used for the refinement of the crystal structure. The positions of all non H-atoms were located by direct methods. The positions of hydrogen atoms were found from the inspection of the difference Fourier maps. The high value of the Flack parameter [1.8 (1.0)] indicates that the absolute configuration cannot reliably be resolved. The final refinement included atomic positional and displacement parameters for all atoms. The non H-atoms were refined anisotropically, while all H sites were refined with isotropic displacement parameters. The refinement converged at a final agreement index (R1) of 0.0376, calculated for 2143 unique observed reflections ($|F_o|>4\sigma F$) and a goodness-of-fit (S) of 0.945 (226 refined parameters).

Table 1. Crystallographic data and structure refinement of **26**

Crystallographic parameter	
Empirical formula	$C_{11}H_{18}N_2O_4$
Formula weight	242.3
Temperature (K)	293
Wavelength (Å)	0.71073
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a=4.5903
	b=7.517
	c=36.0507
Volume (Å^3)	1243.94
Z	4
Density (calculated)	1.354 Mg/m^3
Absorption coefficient (mm^{-1})	0.10
F(000)	520
Crystal size	0.30 mm×0.20 mm×0.20 mm
2Θ max for data collection	56.43°
Index ranges	h: −6+5, k: −9+10, l: −47+27
Reflections collected	7228
Independent reflections	2786 [R(int)=0.0507]
Refinement method	Full matrix l.s. on F^2
Data/restraints/parameters	2786/0/226
Goodness-of-fit on F2	0.945
Final R indices [$F_o>4\sigma F_o$]	R1=0.0376
R indices (all data)	$R1$=0.0512, $wR2$=0.0905
Extinction coefficient	No
Largest diff. peak and hole	0.14 and −0.18 e A^{-3}

Acknowledgements

This work was supported by a research grant from the Ministry of Science, Technologies and Development of the Republic of Serbia (Grant No. 1896). The authors are grateful to Mrs T. Marinko-Covell (Department of Chemistry, University of Leeds, UK) for recording the mass spectra.

References and notes

1. Hofmann, K. *J. Am. Chem. Soc.* **1945**, *67*, 1459–1462.
2. In a review related to the chemistry of biotin dependent enzymes the oxygenated analogue was named *O*-heterobiotin; see: Knowles, J. R. *Annu. Rev. Biochem.* **1989**, *58*, 195–221. However, the original name seems to be still in use according to some more recent references; see for example: De Titta, G. T.; Blessing, R. H.; Moss, G. R.; King, H. F.; Sukumaran, D. K.; Roskwitalski, R. L. *J. Am. Chem. Soc.* **1994**, *116*, 6485–6493.
3. Dyke, S. F. *Chemistry of Natural Products*; Bentley, K. W., Ed.; Interscience: London, 1964; Vol. 6, pp 161–181.
4. Ohrui, H.; Kuzuhara, H.; Emoto, S. *Agric. Biol. Chem.* **1971**, *35*, 752–755.
5. (a) Miljković, D.; Popsavin, V.; Harangi, J. *Tetrahedron Lett.* **1987**, *28*, 5733–5736. (b) Miljković, D.; Popsavin, V.; Harangi, J.; Batta, G. *J. Serb. Chem. Soc.* **1989**, *54*, 163–165. (c) Popsavin, V.; Benedeković, G.; Popsavin, M.; Miljković, D. *Carbohydr. Res.* **2002**, *337*, 459–465.
6. For a preliminary account of this work see: Popsavin, V.; Benedeković, G.; Popsavin, M. *Tetrahedron Lett.* **2002**, *43*, 2281–2284.
7. Baer, H. H.; Mateo, F. H.; Siemsen, L. *Carbohydr. Res.* **1989**, *187*, 67–92.
8. Kiso, M.; Hasegawa, A. *Carbohydr. Res.* **1976**, *52*, 95–102.
9. Jones, J. K. N.; Nicholson, W. H. *J. Chem. Soc.* **1955**, 3050–3055.
10. Tsuda, Y.; Sato, Y.; Kakimoto, K.; Kanemitsu, K. *Chem. Pharm. Bull.* **1992**, *40*, 1033–1036.
11. *Carbohydrates*; Collins, P. M., Ed.; Chapman & Hall: London, 1987; p 58.
12. Buchta, E.; Andree, F. *Chem. Ber.* **1959**, *92*, 3111–3115.
13. For a review on the Staudinger reaction, see: Golobov, Y. G.; Kasukhin, L. F. *Tetrahedron* **1992**, *48*, 1353–1406.
14. Mitchell, S. A.; Oates, B. D.; Razavi, H.; Polt, R. *J. Org. Chem.* **1998**, *63*, 8837–8842. Falb, E.; Bechor, Y.; Nudelman, A.; Hassner, A.; Albreck, A.; Gottlieb, H. E. *J. Org. Chem.* **1999**, *64*, 498–506.
15. DeTitta, G. T.; Edmonds, J. W.; Stallings, W.; Donohue, J. *J. Am. Chem. Soc.* **1976**, *98*, 1920–1926.
16. Eckert, H.; Foster, B. *Angew. Chem. Int. Ed. Engl.* **1987**, *26*, 894–895.
17. Kondo, H.; Miura, K.; Uno, S.; Sunamoto, J. *J. Inorg. Biochem.* **1984**, *21*, 93–102. DeTitta, G. T.; Parthasarathy, R.; Blessing, R. H.; Stallings, W. *Proc. Natl. Acad. Sci. U. S. A.* **1980**, *77*, 333–337.
18. Crystallographic data (excluding structure factors) for the structure **26** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 226377. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].
19. *SAINT*, version 6.2.9; Bruker AXS Inc.: Madison, WI, USA, 2001.
20. *SHELXTL*, version 5.1; Bruker AXS Inc.: Madison, WI, USA, 2001.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron 60 (2004) 5237–5252
Tetrahedron

Rubrenolide, total synthesis and revision of its reported stereochemical structure

L. Thijs and B. Zwanenburg*

Department of Organic Chemistry, NSR Institute, University of Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands

Received 11 July 2003; revised 19 March 2004; accepted 14 April 2004

Abstract—In this paper the synthesis of the natural product rubrenolide is presented. Due to an error in the original proposed stereochemical structure of rubrenolide, the synthesis was not straightforward. Application of the photo-induced rearrangement of an appropriate epoxy diazomethyl ketone gave access to the precursor lactone with an ee of 91%. Coupling of this lactone with (4*S*)-2,2-dimethyl-[1,3]-dioxolane-4-carbaldehyde gave, after some additional steps, the final product that was identical with an authentic sample of the natural product.

1. Introduction

Rubrenolide **1** is a natural product which has been isolated from trunk wood of the Amazonian tree *Nectandra rubra* of the Lauraceae family. Its chemical constitution was first published in 1971.[1] Actually, rubrenolide **1** was isolated as a mixture with an analog—rubrynolide **2**, which only differs in the terminal unsaturated bond in the aliphatic side chain (Fig. 1). The biosynthesis of these compounds was also described.[2] In 1977 the full structure, including assignment of the configuration at the chiral centers, was reported.[3] The assignment of the configuration of rubrenolide was made according to the denotation of the structure in Figure 1 and it was named (2*S*,4*R*)-2-[(2′*S*)-2′,3′-dihydroxypropyl]-4-(dec-9i″-enyl)-γ-lactone.† According to this structure the substituents at C_2 and C_4 have a *cis* relationship. It is important to note here that in assigning the *R*/*S* descriptors great care should be exercised by the correct use of the priority rules,[4] which often are not straightforward. In Figure 2 the priority

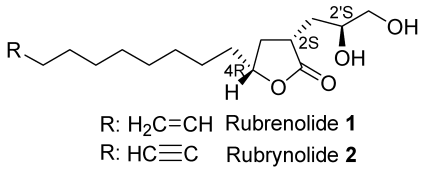


Figure 1.

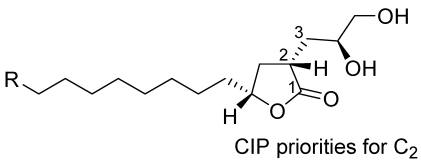


Figure 2.

of the groups attached to the chiral center C_2 is depicted for the sake of clarity.

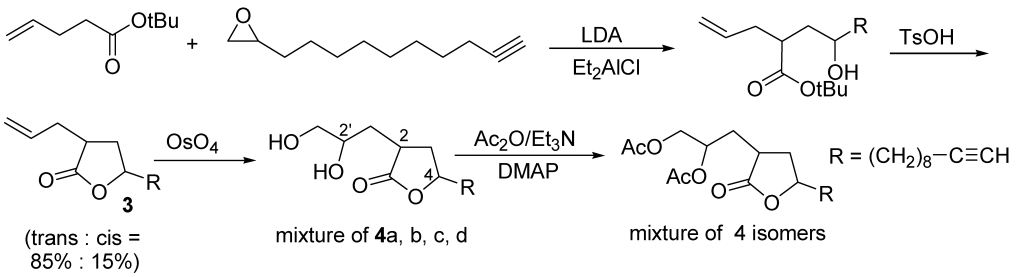
In the paper by Franca et al.[3] concerning the absolute configuration of rubrenolide and rubrynolide the following phrase is puzzling: '*The complete assignment of all proton resonances and coupling constants associated with the γ-lactone showed rubrenolide to possess the 2,4-trans configuration. Comparison with analogous data given by some model cis (**7**) and trans (**8**) 2,4-disubstituted γ-butyrolactones, specially synthesized for this purpose, yielded unequivocal proof of this fact*'.

Because of the very detailed NMR studies that were carried out on rubrenolide and the model compounds mentioned,[5] it must be assumed that an interchange of two figures (denoted there as 7 and 8, see quote) has taken place in the final paper,[3] leading to the incorrect statement that the substituents at C_2 and C_4 are positioned *trans*.

An approach to the synthesis of rubrynolide **2**, reported by Taylor et al.[6] in 1991, was regrettably not noticed by us at that time. This synthesis was based on a reaction of an epoxide with an aluminum enolate, which leads to lactone **3** as a *trans*–*cis* mixture in a ratio of 85:15 (Scheme 1). Dihydroxylation of the olefinic bond with OsO_4 resulted in a mixture of four racemic components, namely *cis R* alcohol

Keywords: Rubrenolide; Epoxy diazomethyl ketone; Lactone.
* Corresponding author. Tel.: +31-24-365-3159; fax: +31-24-365-3393; e-mail address: zwanenb@sci.kun.nl
† Throughout the text the original nomenclature will be used, describing rubrenolide as a γ-lactone. In Section 5 the names generated by the Beilstein Autonom version 2.1 program will be used describing a γ-lactone as dihydrofuran-2-one.

0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.04.037



Scheme 1.

4a, *cis S*-alcohol **4b**, *trans R*-alcohol **4c**, and *trans S*-alcohol **4d**. Their configurations relative to the 4*R* configuration are 2*S*,4*R*,2′*R* for **4a**, 2*S*,4*R*,2′S for **4b**, 2*R*,4*R*,2′*R* for **4c** and 2*R*,4*R*,2′*S* for **4d**. Only one of the minor products was, according to the NMR analysis of the mixture, identical with rubrynolide, which implies either structure **4a** or **4b**. As the diols could not be separated, they were converted into their diacetates, which could be separated by HPLC. One product was identical with the diacetate of rubrynolide. Taylor et al. assumed that the originally assigned *R* configuration of C_4 was correct, that C_2' has the *S* configuration, and that the diacetate derived from **4b** was identical to rubrynolide diacetate, which then should have the 2*S*,4*R*,2′*S* configuration. This is exactly the same configuration as was reported by Franca et al. Notwithstanding, Taylor et al. entitled their paper as 'Synthesis of (±)-rubrynolide and a revision of its reported stereochemistry'.

It seems that Taylor et al. refer to the phrase of Franca, which is cited above in italics, stating that rubrenolide and rubrynolide have the 2,4-*trans* relationship (which is incorrect vide supra). The total synthesis of natural rubrenolide as carried out by the Nijmegen group,[7] unambiguously showed that the correct absolute configuration of this product is 2*S*,4*R*,2′*R*.

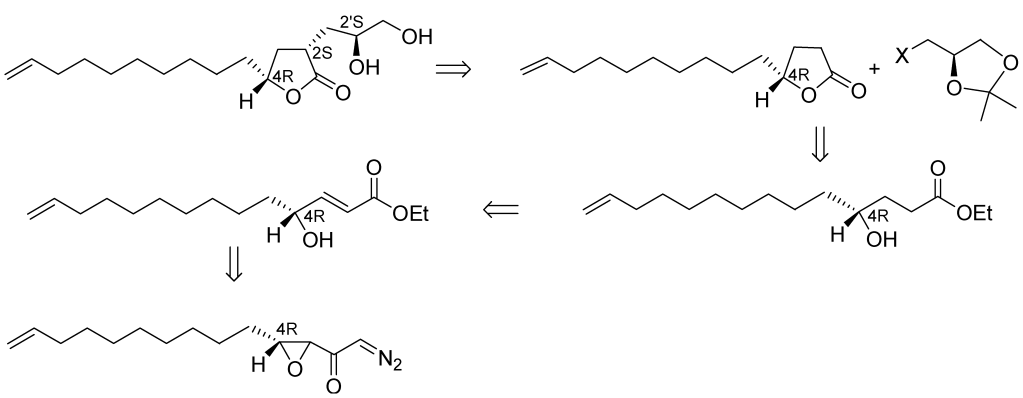
The total synthesis of the naturally occurring functionalized γ-lactone rubrenolide **1** offers an opportunity to test the applicability of the synthetic methodology based on epoxy diazomethyl ketones.[8] The key reaction in this methodology for the synthesis of γ-lactones is the photo-induced rearrangement of epoxy diazomethyl ketones to epoxy ketene followed by a domino reaction with an alcohol to give γ-hydroxy-α,β unsaturated esters.[9] The introduction of the side chain in rubrenolide in a stereocontrolled manner constitutes an extra challenge. The retrosynthesis of rubrenolide with the proposed (2*S*,4*R*,2′*S*)-configuration is outlined in Scheme 2. The stereochemistry at C4 is derived from the epoxy diazomethyl ketone and can be introduced as required by choosing the appropriate chiral inductor during the Sharpless epoxidation that is used in the synthesis of the epoxy unit. The side chain can possibly be introduced by an alkylation reaction, which may give rise to *cis*/*trans* mixtures that hopefully can be separated.

2. Results

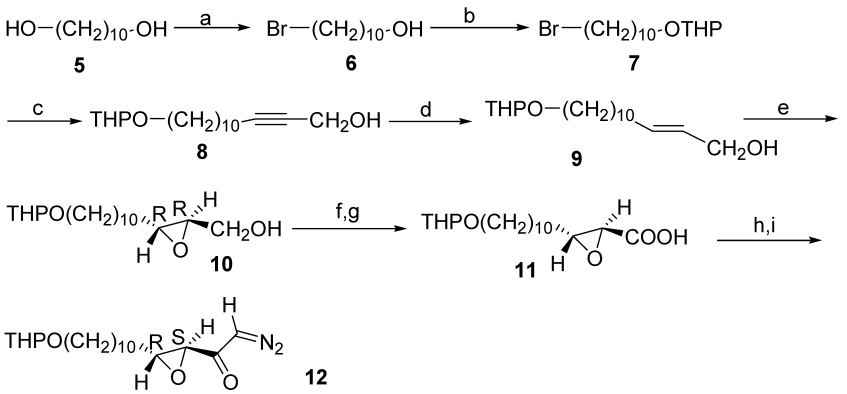
2.1. Synthesis of rubrenolide with the proposed (2*S*,4*R*,2′*S*)-configuration

The synthesis of the required epoxy diazomethyl ketone **12** started with decane-1,10-diol **5** which was treated with hydrobromic acid to give the bromo alcohol **6** (Scheme 3). After protection of the alcohol as a THP ether (compound **7**) a chain elongation with the dianion of propargylic alcohol was carried out. The alkynol **8** was reduced with $LiAlH_4$ to give the *trans* allylic alcohol **9** with high efficiency. In the next step the required chirality had to be installed by choosing the right chiral inductor in the Sharpless epoxidation.[10] In order to obtain the 4*R* configuration in the natural product **1** D-(−) diethyl tartrate was needed for this purpose. The (2*R*,3*R*)-epoxy alcohol **10** thus obtained needed to be oxidized to the corresponding carboxylic acid. The one-step oxidation procedure with RuO_4 was not possible in this case due to the vulnerability of the THP protecting group.

The Swern oxidation to the aldehyde, followed by further oxidation with sodium chlorite[11] to the carboxylic acid was a perfect alternative. The thus obtained carboxylic acid **11**



Scheme 2.



Scheme 3. (a) HBr (48%), pet. ether 80–100 °C (73%); (b) DHP, TsOH (94%); (c) $LiNH_2$ 6 equiv., propyn-2-ol-1, 3 equiv., (99%); (d) $LiAlH_4$, 2 equiv., (quant.); (e) D-(−)-DET, Ti(*i*OPr)$_4$, *t*-BuOOH (69%); (f) Swern oxid.(quant.); (g) $NaClO_2$ (quant.); (h) *i*-butyl chloroformate, Et_3N; (i) CH_2N_2 (68%, calcd on epoxy alcohol **10**).

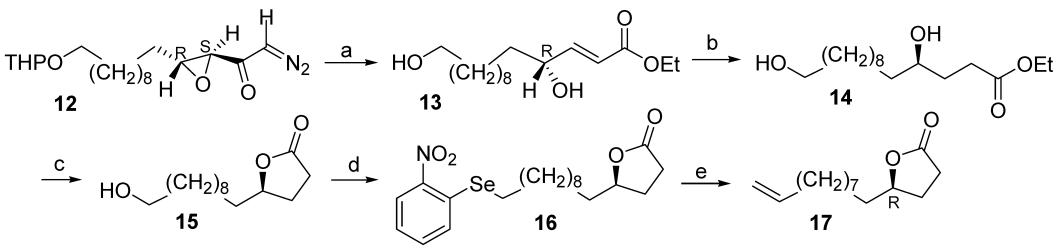
was converted into the epoxy diazomethyl ketone **12** by reaction with iso-butyl chloroformate and triethylamine to give the mixed anhydride and subsequent treatment with ethereal diazomethane. The diazo ketone **12** was purified by chromatography and obtained in an acceptable overall yield (Scheme 3).

The next step in the sequence to the γ-lactone **17** is the photo-induced rearrangement of epoxy diazomethyl ketone **12** in ethanol as the solvent. This reaction was monitored by IR. Unexpectedly, the THP protecting group was lost completely during this reaction (Scheme 4). The product obtained after irradiation, i.e. the γ-hydroxy-α,β-unsaturated ester **13**, preferably is used for further reactions without purification.[12] Thus, product **13** was subjected to reduction with P2–Ni[13] as the catalyst to give the dihydroxy carboxylic ester **14**. Subsequent lactonization to γ-lactone **15** was accomplished by treatment with TsOH in benzene at reflux temperature.

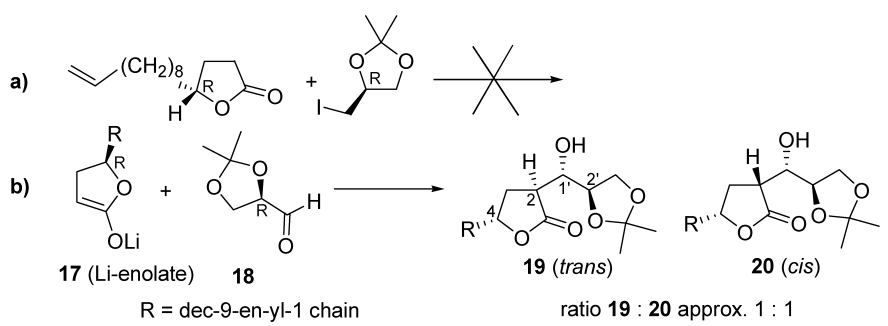
For the next step, the introduction of the terminal olefinic bond, *o*-nitrophenylselenyl cyanide was used as the reagent.[14] Although this ingredient is not very pleasant, the formation of selenide **16** and the subsequent elimination reaction to give lactone **17** took place smoothly (Scheme 4). Product **17** was slightly yellow due to impurities arising from the nitrophenylselenyl reagent.

The planned alkylation step was attempted with (4*R*)-4-iodomethyl-2,2-dimethyl-[1,3]-dioxolane using the Li-enolate of lactone **17** (Scheme 5(a)). Despite the use of a range of reaction conditions, the desired coupling could not be realized. Apparently, this alkylating agent is very reluctant to undergo an S_N2 reaction with an enolate anion. An alternative candidate electrophile for the introduction of the side chain is (4*R*)-2,2-dimethyl-[1,3]dioxolane-4-carbaldehyde **18**.[15]

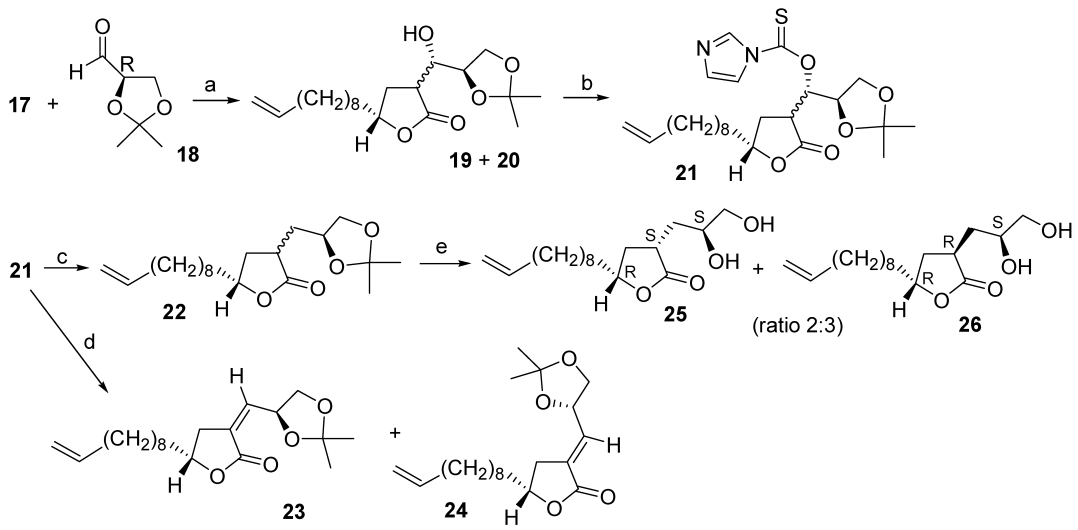
The enolate of **17** reacted smoothly with aldehyde **18** to give



Scheme 4. (a) *hν*, 300 nm, EtOH; (b) $Ni(OAc)_2$, $NaBH_4$ (57% calcd on **12**); (c) TsOH, benzene (quant.); (d) *o*-NO_2PhSeCN, Bu_3P (93%); (e) H_2O_2, Na_2CO_3 (68%).



Scheme 5.



Scheme 6. (a) LDA, THF, HMPA, (80%); (b) thiocarbonyl diimidazole (64%); (c) Bu_3SnH, toluene, gentle warming, (70%); (d) Bu_3SnH, toluene, reflux, (64%); (e) TsOH, MeOH, (61%).

a mixture of two major products (ratio 1:1) as was deduced from a GLC analysis. Tentatively, structures **19** and **20** were assigned to these condensation products (Scheme 5, line b). If the assignment of the Aldol products **19** and **20** is correct, then it will be possible to prepare the rubrenolide type product with the *cis* as well as the *trans* configuration. For the transformation of the Aldol products into the final products the alcohol function at C_1' has to be removed. An attractive approach would be the conversion into a thio-imidazolide **21** and a subsequent reduction with tributyltin hydride[16] (Scheme 6).

The formation of the thio-imidazolide **21** was accomplished in THF at room temperature for 6 h, in a yield of 64%. Some starting material (21%) was recovered and some by-products, mainly a mixture of **23** and **24** were isolated. Repeating the same reaction at reflux temperature for 3 h gave product **21** (55%), starting material (20%) and a mixture of **23**+**24** (22%).

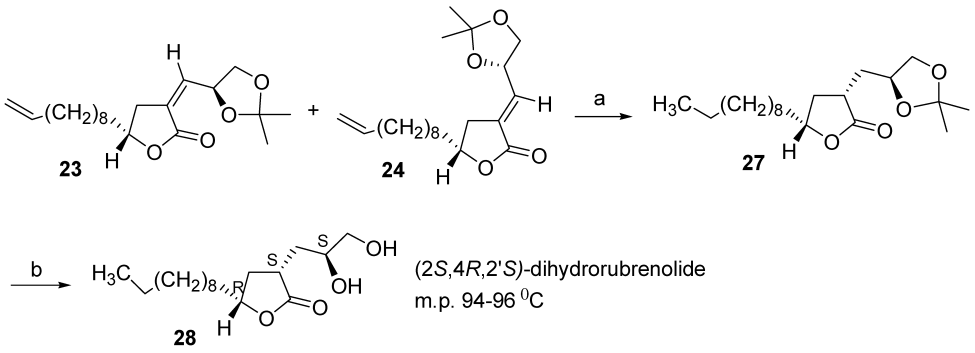
For the reductive removal of the thio-imidazolide group in **21** the method described by Rasmussen et al.[16] was followed, namely reaction with tributyltin hydride in toluene at reflux temperature. A mixture of the unsaturated compounds **23** and **24** was obtained in 64% yield. These geometrical isomers could be separated by chromatography and the structures could be assigned on the basis of the ^1H NMR spectra.[17] Unexpectedly, the product **22** had not been obtained under these conditions. However, repeating the reaction at lower temperature, i.e. after addition of freshly prepared tributyltin hydride, the reaction mixture was gently heated for 30 min, resulted in the desired product **22** in 70% yield and almost no unsaturated product was obtained. According to GLC this product consisted of two isomers (as expected), however, chromatographic separation on silica gel was not possible. Hydrolysis of the product **22** by treatment with TsOH in methanol gave the deprotected product, which according to GLC and TLC, was not a mixture of isomers. However, the presence of the expected two isomers was demonstrated by ^1H NMR spectroscopy (400 MHz). The products were identified as the *cis* and *trans* compounds **25** and **26** (product ratio 2:3). **'Surprisingly, neither of these two products was the expected rubrenolide'.**‡

This conclusion was further substantiated as follows. Hydrogenation of the mixture of the unsaturated compounds **23** and **24** gave a single product as was shown by GLC analysis. Indeed, catalytic hydrogenation should produce the *cis*-disubstituted lactone **27** (Scheme 7). Predictably, the terminal olefin is hydrogenated as well. This saturated compound **27** (mp 38–41 °C) should then be identical to the dihydrorubrenolide derivative obtained by Franca et al.[3] by reaction of dihydrorubrenolide with acetone.

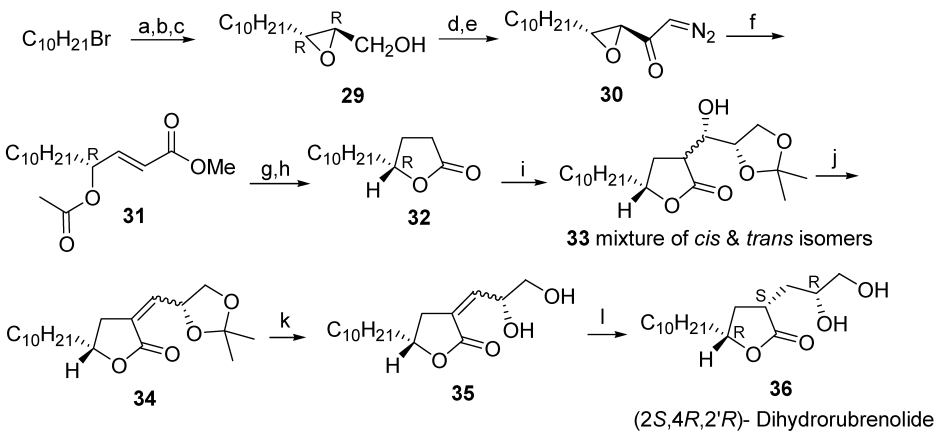
The spectral data however were not in agreement with those reported[3] and the melting points were different as well (lit.[3] mp 47–48 °C). When the acetonide unit was hydrolyzed, product **28** with mp 94–96 °C was obtained, which, according to its mp and NMR spectrum§ clearly was not dihydrorubrenolide as described by Franca et al.[3] (mp dihydrorubrenolide: 106–107 °C). Thus the (2*S*,4*R*,2′*S*) configuration is not in agreement with the true structure of rubrenolide. The unambiguous conclusion is that the proposed absolute configuration of 2*S*,4*R*,2′*S* by Franca et al.[3] for dihydrorubrenolide and rubrenolide is not correct. In addition, the results also indicate that the 2*R*,4*R*,2′*S* configuration, as in *trans* disubstituted lactone **26**, is not dihydrorubrenolide either.

‡ Compound **25** (*cis* substituted lactone) is an epimer of rubrenolide at the C2′ position. The *trans* disubstituted lactone **26** shows ^1H NMR absorptions identical with one of the *trans* dihydroxy-propyl lactones described in Ref. 6. The compound is denoted as *trans*-alcohol **4d** in Scheme 1.

§ The late Professor Ollis, kindly provided us with a free sample of the natural product consisting of a mixture of rubrenolide and rubrynolide. The two products could be separated as reported.[3] Rubrynolide was hydrogenated to give dihydrorubrenolide (see Section 5).



Scheme 7. (a) H2, Pd/C, quant.; (b) TsOH, MeOH, quant.



Scheme 8. (a) Propyn-2-ol-1, 3 equiv., $LiNH_2$ 6 equiv., (90%); (b) $LiAlH_4$, 2 equiv., (85%); (c) (D)-(−)-DET, $Ti(OiPr)_4$, TBHP, (77%); (d) $RuCl_3{\cdot}3H_2O$, $NaIO_4$; (e) *i*-BuO(C═O)Cl, Et_3N, CH_2N_2, (47%); (f) *hν*, 300 nm, MeOH; Ac_2O, pyridine, (50%); (g) $PdCl_2$, $NaBH_4$, H_2, (76%); (h) KOH/EtOH, reflux, 15 min, TsOH, benzene, reflux, quant.; (i) LDA, DMPU, (4S)-2,2-dimethyl-[1,3]-dioxolane-4-carbaldehyde **38**, (63%); (j) MsCl, pyridine, DBU, (96%); (k) TsOH, MeOH, (77%); (l) Pd/C, H_2, EtOH, (84%).

2.2. Synthesis of rubrenolide with the correct (2*S*,4*R*,2′*R*) configuration

As mentioned above it seemed reasonable that the C_2' configuration is opposite to the original assignment. Therefore, the synthesis of dihydrorubrenolide using (4*S*)-2,2-dimethyl-[1,3]-dioxolane-4-carbaldehyde **38** (the antipode of aldehyde **18**) to install the *R*-chirality at C_2', was undertaken.

This synthesis of dihydrorubrenolide (Scheme 8) follows the general sequence for the preparation of lactones. Reaction of decyl bromide with the di-anion of propargyl alcohol, reduction to the *trans*-allylic alcohol, Sharpless epoxidation with D-(−) DET as chiral inductor, RuO_4 oxidation to the glycidic acid, activation of the acid followed by treatment with diazomethane, gave the epoxy diazomethyl ketone **30** in good yield. Irradiation in MeOH and subsequent acetylation gave **31**, reduction of the double bond and ring closure resulted in the desired lactone **32**. The synthesis of this lactone was reported previously as the reduction product of the sex pheromone *cis*-4*R*-4-(1′-decenyl)-γ-lactone[18] of the Japanese beetle.¶ The enantiopurity of the lactone **32** was determined via ^1H NMR of the acetate **31** (by the use of chiral shift reagent) and appeared to be 91%.[19]

The introduction of the side chain was performed by a condensation of the lactone **32** with (4*S*)-2,2-dimethyl-[1,3]-dioxolane-4-carbaldehyde[20] **38**. The thus obtained *cis*/*trans* mixture of alcohol **33** was treated with mesyl chloride to give the corresponding mesylate, which was subjected to an elimination reaction by treatment with DBU giving alkene **34** as a *cis*/*trans* mixture. Catalytic hydrogenation was best performed after deprotection of the vicinal alcohols. Deprotected diol **35** was then hydrogenated using Pd(C) as the catalyst to produce (2*S*,4*R*,2′*R*)-dihydrorubrenolide **36**, which, gratifyingly, was identical to the dihydrorubrenolide prepared from an authentic sample of rubrenolide. The conclusion can be drawn that the absolute configuration of natural rubrenolide must be 2*S*,4*R*,2′*R*.

The total synthesis of natural rubrenolide, having the terminal olefinic bond, was prepared from epoxy diazomethyl ketone **12** as the key starting material, following the synthetic sequence shown in Scheme 3. The photo-induced rearrangement of **12** could be conducted in this case without the loss of the THP protecting group, thus providing **13**-OTHP, which was reduced with P2-Ni/H_2 to the γ-hydroxy ester which was ring closed to the lactone **37**

¶ Although the configuration at the chiral centre of this natural product was assigned correctly as *R*, the reduction product was also given the *R* configuration, which is violating the CIP rules, because the change in priority has not been implemented.

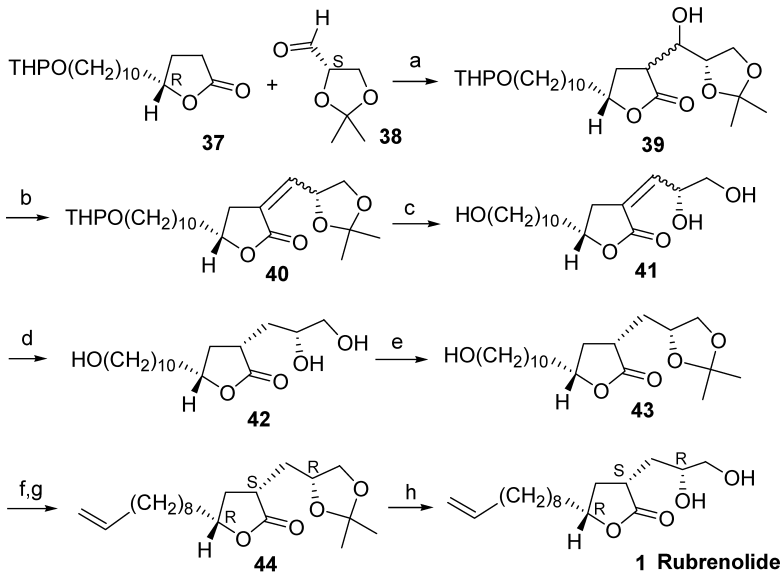
(compare synthesis of lactone **15** shown in Scheme 4). Condensation of lactone **37** with the 4(*S*)-carbaldehyde **38** smoothly led to product **39** (mixture of *cis*/*trans* isomers) (Scheme 9). The *exo* double bond was introduced by elimination of the mesylate using DBU as the base (compare Scheme 8). The thus obtained product **40** (mixture of *cis* and *trans*) was treated with acid to remove both protective groups to give triol **41**, which on catalytic hydrogenation (Pd/C, H_2) afforded the single product **42**, as was evident from its narrow melting range. For the final introduction of the terminal olefinic bond the vic. diol had to be protected as the acetonide **43**. Then the primary hydroxy group was converted into the alkene **44**, following the procedure shown in Scheme 4 using *o*-nitrophenylselanyl cyanide. Finally, removal of the acetonide protecting group gave the target product **1**, whose spectral features and mp were in full agreement with those of the natural product that was kindly provided by (the late) Professor Ollis.

3. Discussion

Having established that rubrenolide does not have the proposed[3] (2*S*,4*R*,2′*S*)-configuration, the question arose what would be the alternative. The original assignment of the configuration at C_4 seemed quite reliable. This was deduced by application of Hudson's lactone rule.[21] In simple terms this rule states that when the lactone is drawn in the Fischer projection (Fig. 3(a)) and the lactone ring lies to the right then the rotation is (+). When the lactone structure is drawn in an alternative way (Fig. 3(b)) then the rule states that if the hydrogen lies below the plane of the lactone ring, the compound will have a (+) rotation. This Hudson lactone rule is supported by numerous examples. It also predicts the rotation and configuration of the simple 4-monosubstituted γ-lactones. When more stereogenic centers are present the situation changes. When the rule is applied to muricatacin (Fig. 3(c)) the predicted optical rotation is not in agreement with the configuration of the chiral center of the lactone.

This problem can be solved by implying the Cotton effect. The contribution of the chiral lactone ring to the optical rotation can be estimated by subtracting the measured rotation of a reference compound without the lactone ring. This, for instance, could be the open form: hydroxy acid or hydroxy ester. The o.r.d. curves of the lactone and a reference compound can be measured and a difference curve can be obtained by subtraction. The sign of the difference curve can now be related to the configuration at the chiral lactone site (a positive sign means that the configuration is *R*). The Cotton effect measures the rotation at the ultraviolet absorption of the lactone function (normally between 225 and 233 nm). The sign of the Cotton effect is in agreement with the sign of the difference curve and thus can be used to determine the contribution of the lactone ring to the optical rotation. Rubrenolide showed a positive Cotton effect at 226 nm (ϕ+1700). This means that rubrenolide has the (*R*)-configuration at C_4.

Accepting this assignment, two possible diastereomers remain, viz. 2*S*,4*R*,2′*R*; and 2*R*,4*R*,2′*R* because the (2*S*,4*R*,2′*S*) and (2*R*,4*R*,2′*S*)-configuration were already excluded. Closer examination of the 400 MHz NMR spectra revealed that the difference between the synthesized product **28** (Scheme 7) and dihydrorubrenolide derived from the natural product[22] is caused by the environment of the hydroxy at C_2'. Franca et al.[3] who reported the complete structure of rubrenolide, determined the configuration at C_2' by applying Horeau's method[23] to the monomethyl ether of the primary alcohol of the natural product. The free secondary alcohol was brought into reaction with α-phenylbutyric anhydride in the presence of pyridine. Hydrolysis of the remaining anhydride gave the acid with a positive rotation. This result then was related to a Fischer projection (Fig. 4), which according to Horeau's method leads to the *S*-configuration at the C_2' chiral center. It is important to note that Horeau and Kagan[24] state in a footnote in their paper of 1964 that when a polar group is close to the secondary alcohol, other rules for the assignment of L and M in the Fischer projection may be necessary.



Scheme 9. (a) LDA, HMPA, THF, −70 °C, 16 h, (26%); (b) MsCl, Et_3N, DBU, (72%); (c) TsOH, MeOH, (70%); (d) Pd/C, H_2, quant.; (e) TsOH, acetone, (73%); (f) *o*-nitro-phenylselanyl cyanide, Bu_3P, (91%); (g) H_2O_2; (h) TsOH, MeOH, (60%).

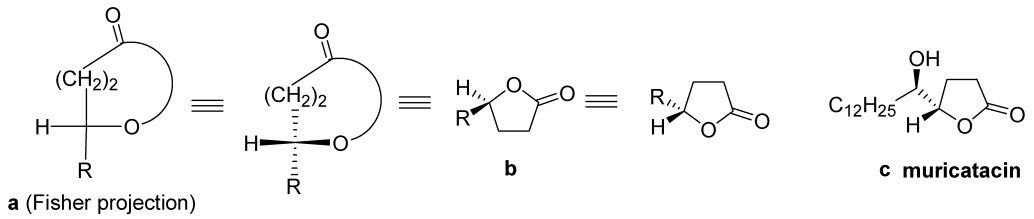


Figure 3.

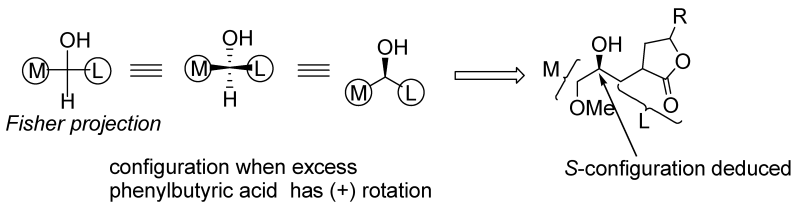


Figure 4.

This special situation is encountered in the case of rubrenolide because of the methoxy group and leads to a deviant priority of M and L, with the ultimate consequence that the assignment of the chirality at C_2' by Franca et al.[3] is not correct.

Concerning Scheme 5 it was assumed that a mixture of *cis* and *trans* substituted lactone products was obtained. A more detailed representation of the possible Aldol condensation products is depicted in Scheme 10. The (*R*)-aldehyde **18** is taken as an example.

It should be noted that the *R* descriptor for the chirality changes to *S* in the final product due to a change in the priority of the substituents flanking this chiral center.

Conclusive information about these structures was obtained by comparison with related condensations reported in the literature and by applying the Felkin–Ahn rule[25] for the reaction of an enolate with a carbonyl compound. The reaction of the Li-enolate of methyl propanoate with carbaldehyde **18** results in the formation of a mixture of *anti*/*syn* and *anti*/*anti* product in a ratio of 3:2 (Scheme 10, line b).[26] Thus this condensation exhibits an exclusive *anti* selectivity with respect to the dioxolane ring. The selectivity with respect to the ester moiety is rather low as is apparent from the *anti*,*syn*/*anti*,*anti* ratio of 3:2. The Felkin–Ahn model for the reaction of aldehyde **18** is shown in Figure 5.[‖] This picture is in agreement with the proposed mechanism described by Jurczak et al.[27] By calculating minimum energy structures of aldehyde **18**, attack of the nucleophile leads to the same result (Fig. 5).[28] Clearly, the *syn* attack is disfavored ruling out structures C and D. It is reasonable to assume that the condensation of the enolate of **17** with aldehyde **18** also proceeds with a high selectivity with respect to the dioxolane ring,[29] implying the preferred formation of the *anti*/*syn* and *anti*/*anti* products shown in Scheme 10, line a. Taking into account that the chirality at C_4 and C_2' is defined by the starting materials, the product mixture A+B is to be expected.

[‖] The representation of the Felkin–Ahn model was misinterpreted in the first draft of this paper as was pointed out by one of the referees.

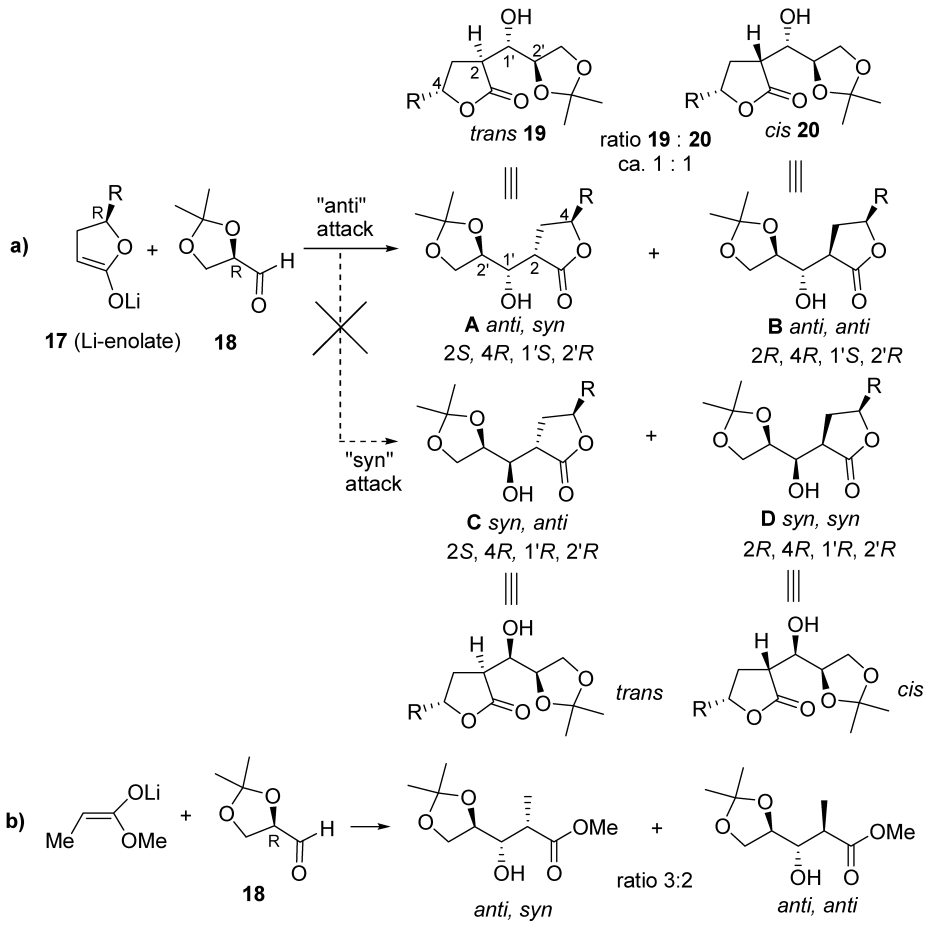
4. Concluding remarks

The absolute configuration of the natural products rubrenolide and rubrynolide has been established by a fully stereoselective total synthesis. The originally assigned (2*S*,4*R*,2′S)-configuration turned out to be incorrect, the configuration at C_2' is opposite. In the original paper of Franca et al.[3] dealing with the absolute stereochemical structure of these natural products, an incorrect and confusing statement concerning the *cis*/*trans* geometry was made. In the synthetic strategy effective use was made of the chemistry of epoxy diazomethyl ketones, in particular the photo-induced rearrangement in an alcoholic solution leading to γ-hydroxy-α,β-unsaturated esters, which conveniently can be converted to γ-lactones with a designed chirality at C_4, when optically active substrates are used.

5. Experimental

5.1. General remarks

The solvents used for synthetic procedures and for chromatography were purified and dried according to conventional methods. Melting points were determined using a Reichert thermopan microscope and are uncorrected. IR spectra were recorded on a Perkin–Elmer 298 spectrophotometer or on a FTIR spectrophotometer of ATI Mattson (Genesis Series). NMR's were measured on different instruments: routinely ^{1}H spectra with a Varian EM-390, or a Bruker AC 100 (100 MHz, FT) instrument. More detailed spectra requiring also ^{13}C data were measured on a Bruker AM-400 (400 MHz, FT) instrument. Optical rotations were measured with a Perkin–Elmer 241 MC automatic polarimeter. Mass spectra were run on a double focusing VG 7070E instrument. Elemental analyses were performed using a Carlo Erba Instruments CHNS–O 1108 element analyzer. GLC's were run using



Scheme 10.

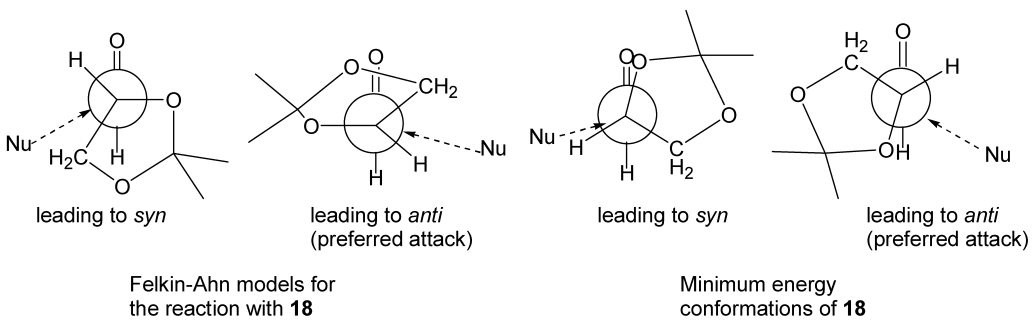


Figure 5.

Hewlett–Packard 5790 or 5890 instruments, equipped with apolar capillary columns. TLC's were performed using glass plates coated with 25 mm silica (Merck 60 F-254). Spots were visualized with a 6.2% H_2SO_4 solution (1 L) containing ammonium molybdate (42 g) and cerium ammonium sulfate (3.6 g), followed by charring. For 'normal' chromatography silica gel 60 (Baker) was used. For flash chromatography Kieselgel 60H (Merck) was applied.

5.1.1. 10-Bromo-decan-1-ol 6.[30] Decane-1,10-diol **5** (47.0 g, 0.27 mol) was mixed with hydrobromic acid (380 mL, 47%) in a Dean–Stark apparatus (1 L size) and the mixture was extracted continuously with petroleum ether (bp 80–100 °C) during 60 h. The organic layer was collected and shaken with solid K_2CO_3. After filtration the filtrate was concentrated in vacuo and the residue was purified by flash chromatography (hexane–diethyl ether 8:1, removal of dibromide, followed by diethyl ether). Yield of **6** (colorless oil) 47.0 g (73%). ^{1}H NMR ($CDCl_3$): δ 3.60 (t, 2H, CH_2OH), 3.35 (t, 2H, CH_2Br), 1.40 (m, 16H) ppm.

5.1.2. 2-(10-Bromo-decyloxy)-tetrahydropyran 7. To a cooled (0 °C) solution of 10-bromo-decan-1-ol **6** (27.5 g 116 mmol) in dichloromethane (100 mL) a small amount of *p*-TsOH was added, followed by dropwise addition of dihydropyran (10 g, 119 mmol). The reaction mixture was stirred for 2 h, diluted with diethyl ether and washed with

aqueous $NaHCO_3$ solution. The organic phase was dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography (hexane–ether 6:1) giving 35.17 g of product **7** (94%). ^{1}H NMR ($CDCl_3$): δ 4.50 (m, 1H, OC*H*O, 3.60 (m, 4H, 2×CH_2–O), 3.35 (t, 2H, CH_2Br), 1.40 (m, 22H) ppm. This material was used in the next step.

5.1.3. 13-(Tetrahydropyran-2-yloxy)-tridec-2-yn-1-ol 8. Ammonia (250 mL) was condensed in a 500 mL 3-necked flask. The temperature was kept between −40 and −50 °C with dry ice/*i*-PrOH and 0.025 g of Li metal was added to the ammonia giving a blue color on dissolution. The reaction mixture was kept under nitrogen and stirred magnetically. After addition of a small amount of $Fe(NO_3)_3{\cdot}9H_2O$ the formation of $LiNH_2$ was catalyzed and could be observed by the change of color (blue to grey). Finely cut pieces of Li (2.17 g, 312 mmol) were added slowly. The initially blue color on addition of the Li, eventually turned into a white-greyish one. A solution of propargyl alcohol (8.74 g, 156 mmol) in THF (40 mL) was then added dropwise. The resulting mixture was stirred for another hour after which a solution of the bromide **7** (16.71 g, 52 mmol) in THF (70 mL) was added. Stirring was continued for 1.5 h at −40 °C and the reaction mixture was left overnight at ambient temperature resulting in complete evaporation of the ammonia. The residue was cautiously treated with water (120 mL) and then extracted with ether (3×80 mL). The organic layer was washed with water and dried ($MgSO_4$). After work-up 15.3 g of crude material (99.3%, purity according to GLC 92.2%) was obtained. This crude material was pure enough for further use. A small amount (5.0 g) was purified further via chromatography over silica gel (eluent hexane–CH_2Cl_2 1:1, followed by CH_2Cl_2–AcOEt 1:1), giving 4.46 g of pure product according to GC. IR (neat): ν_{max} 3390 (OH), 2280, 2220 (C≡C) cm^{-1}. ^{1}H NMR (400 MHz): δ 4.58 (1H, m, O–C*H*–O), 4.24 (2H, s, CH_2OH), 3.87 (1H, m, CH*H*–O, THP-ether ring), 3.72 (1H, m, O–CH*H*, alkyl chain), 3.51 (1H, m, C*H*H–O, THP-ether ring), 3.39 (1H, m, O–C*H*H, alkyl chain), 2.18 (2H, m, CH_2–C≡), 1.90 (1H, br s, O*H*), 1.85 (1H, m), 1.70 (1H, m), 1.61–1.48 (8H, m), 1.36–1.28 (12H, m) ppm. ^{13}C NMR: signals at 98.78, 86.41, 78.37, 67.64, 62.25, 51.26, 30.73, 29.68, 29.43, 29.37, 29.35, 29.02, 28.77, 28.56, 26.16, 25.47, 19.61 and 18.68 ppm. Mass spectrum (EI) peak match for M^+ (abundancy.8%) calcd for $C_{18}H_{32}O_3$, 296.2352; found: 296.2344. Peak match for M^+-CH_2OH (abundancy 3.5%) calcd for $C_{17}H_{29}O_2$ 265.2168; found: 265.2166.

5.1.4. 13-(Tetrahydropyran-2-yloxy)-tridec-2-en-1-ol 9. A mixture of diethyl ether–THF (650 mL, ratio 7:8), was added to LAH (7.6 g, 0.2 mol). The suspension was stirred magnetically, kept under nitrogen and cooled below −65 °C. A solution of the alkynol **8** in diethyl ether–THF (400 mL, 7:8) was added slowly; the temperature was kept below − 60 °C. After the addition the temperature of the reaction mixture was allowed to rise to room temperature, then the temperature was raised to 35–40 °C and maintained at this level during 12 h. The reaction mixture was decomposed by careful addition of water at −10 °C. The colorless $Al(OH)_3$ was filtered off and the filtrate was dried ($MgSO_4$). Work-up gave the product as a slightly colored viscous oil (29.86 g, quant.). This crude product could be used as such in the next step. A small amount was purified by chromatography for further analysis. IR (neat): ν_{max} 3360 (OH), 1662 (C=C) cm^{-1}. NMR (400 MHz) ($CDCl_3$): δ 5.73–560 (2H, m, *H*C=C*H*), 4.57 (1H, m, O–C*H*–O), 4.07 (2H, m, CH_2OH), 3.86 (1H, m, CH*H*–O, THP ring), 3.71 (1H, d t, C*H*H–O, chain), 3.49 (1H, m, C*H*H–O, THP ring), 3.38 (1H, dt, CH*H*–O, chain), 2.03 (2H, m, C–CH_2–C=C), 1.84 (1H, m), 1.70 (1H, m) 1.60–1.27 [21H, m, (CH_2)$_8$+(CH_2)$_2$+OH] ppm. ^{13}C NMR: 133.4, 128.9 (alkene C), 98.8 (acetal C), 67.7, 63.7, 62.3 (*C*–O), further methylene ^{13}C absorptions at 32.16, 30.77, 29.73, 29.51, 29.48, 29.42, 29.36, 29.11, 29.10, 26.20, 25.48, 19.65 ppm. Mass spectrum (EI), peak match at M^+ calcd for $C_{18}H_{34}O_3$: 298.2508; found: 298.25085. Peak match at M−85, calcd for $C_{13}H_{25}O_2$: 213.1855; found: 213.1855.

The by-product (the allene, 2-trideca-11,12-dienyloxy-tetrahydro-pyran) was isolated by chromatography over silica gel (CH_2Cl_2). IR (neat): ν_{max} 3040 (=CH_2), 1950 (C=C=C) cm^{-1}. ^{1}H NMR (90 MHz): δ 5.08 (1H, appearing as quintet, C*H*–C=C), 4.73–4.47 (3H, q+m, O–C*H*–O+C=C=CH_2), 4.03–3.2 (4H, 2×CH$_2$, CH_2–O–C), 2.16–1.07 [24H, m, CH_2–C=+(CH_2)$_8$+(CH_2)$_3$] ppm.

5.1.5. (2*R*,3*R*)-2,3-Epoxy-13-(2-tetrahydropyran-yloxy)-tridecan-1-ol 10. A solution of Ti(O*i*Pr)$_4$ (29.00 g, 102 mmol) in dichloromethane (650 mL) was cooled to −30 to −40 °C. D-(−)-Diethyl tartrate (21.03 g, 102 mmol) dissolved in dichloromethane (100 mL) was added in one portion. The resulting solution was stirred for 5 min at the low temperature and then allylic alcohol **9** (29.80 g, 100 mmol) was added. After stirring for another 5 min a solution of *tert*.-butyl hydroperoxide (50 mL, 4.1 mol) in dichloroethane was added. The reaction mixture was left overnight at −25 °C. A 10% aqueous tartaric acid solution (300 mL) was added slowly and the mixture was stirred at −10 °C for 30 min and for 1 h at room temperature. A clear yellow mixture was obtained, the organic layer was washed with water (3×) and dried over $MgSO_4$. The solvent was evaporated and the residue was dissolved in diethyl ether (400 mL). The solution was cooled to 0 °C and stirred with 1 N NaOH (400 mL) for 30 min. The ether layer was washed once with water and dried over Na_2SO_4. Evaporation of the solvent and removal of remaining *t*-BuOOH in vacuo gave the crude product (28.30 g). This material was stored and purified in portions when needed.

Crude epoxy alcohol **10** (6.30 g) was chromatographed over 130 g of silica gel. Elution with EtOAc–hexane (2:1) gave 0.48 g of allene and 0.22 g of starting material. Elution with EtOAc–hexane (1:1) gave 4. 33 g of pure epoxy alcohol as an oil (68.7%). $[\alpha]_D^{20}$=+18 ($CHCl_3$, *c*=0.5). IR: ν_{max} 3420 (OH) cm^{-1}. ^{1}H NMR (400 MHz) ($CDCl_3$): δ 4.57 (1H, m, O–C*H*–O), 3.92–3.84 (2H, m, C*H*H–OH+C*H*H–O THP-ring), 3.73 (1H, dt, O–C*H*H–CH$_2$, alkyl chain), 3.62 (1H, dd, CH*H*OH), 3.38 (1H, m, CH*H*–O THP-ring), 3.38 (1H, dt, O–CH*H*–CH$_2$, alkyl chain), 2.96–2.90 (2H, m, *H*C–O–C*H*), 1.71 (1H, m), 1.63–1.28 (25H, m) ppm. ^{13}C NMR: δ 98.87 (*C* acetal), 67.69 (O–*C*H$_2$ alkyl chain), 62.34 (*C*H$_2$–O, THP-ring), 61.77 (*C*H$_2$–OH), 58.34 (CH$_2$*C*H, epoxy), 55.99 (CH–O–*C*H–CH$_2$OH), further methylene C absorptions at 31.55, 30.82, 29.76, 29.51, 29.47, 29.44, 29.36, 26.23, 25.92, 25.54, 19.71 ppm, one ^{13}C absorption

was missing due to overlap of two signals. MS, peak match M^+, calcd 314.2457; found: 314.2453.

Another purification of crude epoxy alcohol (13.25 g) gave a product (9.90 g) with a purity of 95.6% according to GC (75.2% yield calcd on allylic alcohol **9**).

5.1.6. (2*S*,3*R*)-2,3-Epoxy-13-(2-tetrahydro-pyran-yloxy)-tridecan-1-al. To a magnetically stirred solution of oxalyl chloride (3.30 g, 26 mmol) in dichloromethane (50 mL), cooled at −60 °C and kept under nitrogen, was added dropwise a solution of DMSO (2.73 g, 35.0 mmol) in dichloromethane (20 mL). A solution of the epoxy alcohol **10** (5.41 g, 17.2 mmol) in dichloromethane (30 mL) was added and the mixture was stirred for 15 min. Then, triethylamine (12 mL) was added and the mixture was allowed to warm-up to room temperature. Water (100 mL) was added and the two layers were separated. The dichloromethane layer was washed once more with water, and dried ($MgSO_4$). Evaporation of the solvent gave crude epoxy aldehyde (5.4 g) as a yellow colored oil. Purity according to GLC: 88.6%. NMR (90 MHz) ($CDCl_3$): δ 9.0 (1H, d, C*H*═O), 4.55 (1H, m, O−C*H*−O), 4.0–3.0 (6H, m, 2×CH_2O+C*H*−O−C*H*), 2.0–1.0 [24H, m, (CH_2)$_9$+(CH_2)$_3$] ppm. This aldehyde was used immediately for the next step.

5.1.7. (3*S*,4*R*)-1-Diazo-3,4-epoxy-14-(2-tetrahydropyran-yloxy)-tetradecan-2-one 12. The crude epoxy aldehyde (5.4 g, assumed 17.2 mmol) was dissolved in *t*-BuOH (200 mL). Isobutene (75 mL) was added as chlorine scavenger. At room temperature a solution of $NaClO_2$ (12 g, 133 mmol) and NaH_2PO_4 (12 g, 100 mmol) in water (120 mL) was added dropwise. The resulting mixture was left overnight at room temperature. Evaporation of the more volatile compounds gave an oily compound in the water layer. The oil was extracted with diethyl ether (4×40 mL) and the solution was dried over $MgSO_4$. Evaporation of the solvent gave the glycidic acid **11** as a crude oil in quantitative yield. This product was used for the next reaction immediately.

To an ice-cooled solution of the crude glycidic acid (5.37 g) in diethyl ether (60 mL) was added iso-butyl chloroformate (2.60 g, 19.0 mmol). This was followed by addition of a solution of Et_3N (2.26 g, 22.4 mmol) in diethyl ether (20 mL). After stirring for 30 min, the precipitated salt was filtered off and the filtrate was added to an ethereal diazomethane solution (143 mL, 0.3 mol) that was cooled at −30 °C. The mixture was left overnight at room temperature and excess diazo methane was removed by a stream of nitrogen. Some additional Et_3N·HCl salt was removed by filtration and the filtrate was concentrated. A yellow oil remained (5.88 g), that was purified by chromatography over 90 g of silica gel. Elution with hexane–EtOAc 4:1 yielded the pure epoxy diazomethyl ketone **12** (4.00 g, 68% calculated on the epoxy alcohol) as a yellow oil. $[\alpha]_D^{20}$=−43.1 ($CHCl_3$, *c*=1). IR (neat): ν_{max} 3115 (CH═), 2940, 2860 (aliph CH_2), 2110 (═N═N), 1640 (C═O) cm^{-1}. ^{1}H NMR (C_6D_6, 400 MHz): δ 4.75 (1H, s, C*H*═N_2), 4.62 (1H, m, appears as t with *J*=3.0 Hz, O−C*H*−O), 3.87–3.82 (2H, m, CH_2−O), 3.44–3.35 (2H, m, CH_2O), 3.04 (1H, d, *J*=2 Hz, O−C*H*−C═O), 2.56 (1H, bs, CH_2−C*H*−O, epoxide), 1.79 (1H, m), 1.65–1.61 (4H, m) 1.42–1.02 (19H, m) ppm. ^{13}C NMR: 190.45 *C*═O, 98.70 O−*C*H−O, 67.56 *C*H_2−O, 61.69 *C*H_2−O, 58.93 *C*−C═O, epoxide, 58.66 *C*−CH_2, epoxide, 50.63 *C*H−N_2, further methylene carbon absorptions at 31.77, 31.15, 30.31, 29.94, 29.86, 29.80, 29.73, 29.63, 29.48, 26.79, 26.00, 19.75 ppm. MS, EI$^+$ 352 (M^+) 0.3%, 351 (M^+-1) 0.7%, 269 (M^+-83) 8.6%, 101 ($C_5H_9O_2^+$) 71%, 85 ($C_5H_9O^+$) 100%.

5.1.8. (4*R*)-4,14-Dihydroxy-tetradec-2-enoic acid ethyl ester 13. A solution of epoxy diazomethyl ketone **12** (3.84 g, 10.9 mmol) in EtOH (1050 mL) was irradiated in a Pyrex tube at 300 nm. The progress of the reaction was followed by IR. After 4 h the diazo absorption had vanished, and the solvent was evaporated leaving 3.12 g of an oil. According to GLC this product was 80% pure. According to NMR the product had lost its THP protecting group. IR (CCl_4): ν_{max} 3620 (sharp, OH), 3500 (br, OH), 2940, 2860 (aliphatic CH_2), 1740 (EtO−C═O), 1660 (C═C), cm^{-1}. ^{1}H NMR (90 MHz) ($CDCl_3$): δ 6.95 (1H, dd, *J*=16 Hz, *J*=5.5 Hz, *H*C═C−C═O), 6.02 (1H, dd, *J*=16, 2 Hz, C═C*H*−C═O), 4.17 (3H, q, *J*=7 Hz, O−C$H_2$$CH_3$ and C*H*−OH), 3.60 (2H, t, *J*=6 Hz, CH_2−OH), 2.92 (2H, m, O*H*), 1.8–1.1 [21H, m, (CH_2)$_9$+CH_3] ppm. The product was used in the next step right away.

5.1.9. (4*R*)-4,14-Dihydroxy-tetradecanoic acid ethyl ester 14. $Ni(OAc)_2$·$4H_2O$ (1.43 g, 5.8 mmol), was suspended in EtOH (50 mL) and transferred to a hydrogenation flask. A 1 mol solution of $NaBH_4$ (6 mL) in EtOH was added, giving a black suspension. A solution of the γ-hydroxy-α,β-unsaturated ester **13** (3.12 g, when pure 10.9 mmol) in EtOH (50 mL) was added. The device was connected to a hydrogen supply and the uptake of hydrogen at normal pressure was measured. After uptake of 225 mL of hydrogen the reaction mixture was filtered through celite. The solvent was evaporated and the residue purified by chromatography over silica gel (100 g). Elution with hexane–EtOAc 3:1, which was gradually changed to 1:1 gave the desired compound **14** as colorless fluffy needles (1.77 g, 57%, calcd on epoxy diazomethyl ketone). After recrystallization from hexane–CH_2Cl_2: mp 48–50 °C. IR ($CHCl_3$): ν_{max} 3600, 3450 (OH), 1765 (sh, C═O), 1720 (C═O) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 4.13 (2H, q, *J*=7.5 Hz, O−C$H_2$$CH_3$), 3.60 (3H, t+m, t *J*=5 Hz, HO−CH_2,+C*H*−OH), 2.43 (2H, t, *J*=7.5 Hz, CH_2C═O), 2.2 (2H, b s, 2×O*H*), 1.8–1.1 [23H, m, (CH_2)$_9$+CH_2+CH_3) ppm. Elemental analysis for $C_{16}H_{32}O_4$ (288.431), calcd, C: 66.63; H 11.18%; found: C: 66.59; H: 11.05%.

5.1.10. (5*R*)-5-(10-Hydroxydecyl)-dihydrofuran-2-one 15. A solution of the dihydroxy ester **14** (1.40 g, 4.85 mmol) in benzene (80 mL) containing a small amount of TsOH, was heated at reflux for 2 h. The reaction was monitored by TLC. The reaction mixture was washed with aq. $NaHCO_3$ and water. Work-up gave compound **15** (1.18 g, quant.) as colorless crystals (mp 80–81 °C). $[\alpha]_D^{20}$=+23.9 ($CHCl_3$, *c*=0.8). IR ($CHCl_3$): ν_{max} 3600, 3470 (OH), 1765 (C═O), cm^{-1}. ^{1}H NMR (400 MHz) δ 4.48 (1H, m, C*H*OC═O), 3.64 (2H, t, *J*=6.5 Hz, CH_2OH), 2.52 (2H, m, CH_2−C═O), 2.32 (1H, m, C*H*H−CH_2−C═O), 1.71 (1H, m, CH*H*−CH_2−C═O)1.60–1.23 [19H, m, (CH_2)$_9$+O*H*] ppm. ^{13}C 177.15 *C*═O, 81.00 *C*H−O, 63.01 *C*H_2OH, *C*H_2 peaks at 35.56, 32.76, 29.52, 29.47,

29.34, 29.26, 27.96, 26.18, 25.50, 25.42 and 25.16 ppm. Elemental analysis for $C_{14}H_{26}O_3$ (242.360): calcd, C: 69.38; H: 10.81%; found: C: 68.66; H: 10.81%.

5.1.11. (5*R*)-5-[10-(2-Tetrahydropyran-yloxy)]-decyl-dihydrofuran-2-one 37. The alcohol function of compound **15** was reprotected as follows: to a solution of alcohol **15** (435 mg, 1.8 mmol) in CH_2Cl_2 (10 mL) cooled at 0 °C, a solution of dihydropyrane (160 mg, 1.9 mmol) in CH_2Cl_2 (2 mL) was added. A small amount of TsOH was added as catalyst. After 1 h at room temperature the solution was washed with satd bicarbonate solution and dried over $MgSO_4$. Work-up and chromatography over silica gel (hexane–EtOAc 2:1) gave product **37** as a slightly yellow oil (500 mg, 85%). $[\alpha]_D^{20}=+20.6$ ($CHCl_3$, $c=0.6$). ^{1}H NMR (400 MHz) ($CDCl_3$): δ 4.57 (1H, m, O–C*H*–O), 4.48 (1H, m, C*H*–O–C=O), 3.87 (1H, m, C*H*H–O THP-ring), 3.73 (1H, m, C*H*H–O, chain), 3.52 (1H, m, CH*H* THP-ring), 3.38 (1H, m, CH*H*–O, chain), 2.52 (2H, m, CH_2–C=O), 2.32 (1H, m, C*H*H–CH_2–C=O), 1.84 (1H, m, CH*H*–CH_2–C=O), 1.8–1.28 [24H, m, $(CH_2)_3$+$(CH_2)_9$)] ppm. ^{13}C NMR: 177.05 *C*=O, 98.84 O–*C*H–O, 80.95 *C*H–O–C=O, 67.64 O–*C*H_2 chain, 62.31 O–*C*H_2 THP-ring, other *C*H_2 peaks at 35.55, 32.76, 30.77, 29.71, 29.47, 29.39, 29.37, 29.27, 28.78, 27.95, 26.18, 25.49, 25.16, 19.67 ppm.

5.1.12. (5*R*)-5-[10-(2-Nitro-phenylselanyl)-decyl]-dihydrofuran-2-one 16. To a magnetically stirred solution of alcohol **15** (754 mg, 3.11 mmol) and *o*-nitrophenyl-selanyl cyanide (1181 mg, 5.20 mmol) in THF (25 mL) was added at room temperature a solution of Bu_3P (1296 μL, 5.20 mmol) in THF (10 mL). The reaction was followed by TLC. After 2 h the reaction was complete and the deeply reddish-brown colored solution was concentrated. The residue was subjected to column chromatography over silica gel, eluent EtOAc–hexane 1:3→1:2. This gave yellow crystals (1186 mg, 93%) of compound **16**. IR ($CHCl_3$): ν_{max}1760 (C=O), 1590 (Ar), 1500, 1335 (NO_2). ^{1}H NMR ($CDCl_3$): δ 8.23 (1H, d, J=8.0 Hz, C*H*=C–NO_2), 7.6–7.0 (3H, m, Ar*H*), 4.48 (1H, m, C*H*–OC=O), 2.90 (2H, t, J=7.5 Hz, CH_2Se), 2.7–1.1 [22H, m, $(CH_2)_9$+$(CH_2)_2$] ppm. This material was processed immediately in the next step.

5.1.13. (5*R*)-5-(Dec-9-enyl)-dihydrofuran-2-one 17. To a solution of the selenide **16** (532 mg, 1.25 mmol) in THF (15 mL) at a temperature of 5 °C was added hydrogen peroxide (1.5 mL 30%, 13 mmol). The reaction mixture was stirred for 3.5 h at room temperature. At this point very little starting material remained. After 5 h the starting material was consumed completely. Then, hexane (50 mL) was added. A satd aqueous solution of $NaHCO_3$ (50 mL) was added and the mixture was stirred vigorously. The aqueous layer was extracted twice with hexane (40 mL). The combined organic layers were dried over $MgSO_4$. Work-up and purification by chromatography over silica gel (30 g), eluent EtOAc–hexane 1:5, gave compound **15** (190 mg, 68%) as a yellow oil. The yellow color is due to a small trace of a selenide contaminant. According to GLC the purity was >95%. IR (neat): ν_{max} 3060 (C=CH), 1770 (C=O), 1640 (C=C) cm^{-1}. ^{1}H NMR: δ 6.23–5.33 (1H, m, H_2C=C*H*), 5.3–4.7 (2H, m, *H*$_2$C=C), 4.47 (1H, m, C*H*–OC=O), 2.7–1.1 [20H, m, $(CH_2)_8$+$(CH_2)_2$] ppm. This material was coupled with **18** without further purification.

5.1.14. (3*R*/*S*,5*R*)-5-Dec-9-enyl-3-{[(4*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl]-hydroxy-methyl}-dihydrofuran-2-one 19/20. To a LDA solution [prepared from *i*Pr_2NH (303 mg, 3 mmol) and BuLi (2 mL of 1.6 molar sol. in hexane)] in THF (5 mL) while kept at −70 °C, was slowly added a solution of the lactone **15** (448 mg, 2 mmol) and DMPU (320 mg, 2 mmol) in THF (5 mL).The reaction mixture was stirred for 15 min at this temperature and then a solution of (4*R*)-2,2-dimethyl-[1,3]-dioxolane-4-carbaldehyde **18** (781 mg, 6.0 mmol) in THF (5 mL) was added in one portion. The reaction mixture was kept at −70 °C for 6 h. The reaction was quenched with satd aqueous NH_4Cl and the mixture was extracted with ether. After drying ($MgSO_4$), work-up gave an oil that was purified by column chromatography over 40 g of silica gel. Elution with CH_2Cl_2 gave starting material (40 mg, 9%). With diethyl ether the product (650 mg, 91%) was obtained as a mixture of two isomers **19/20** (according to GLC). By careful crystallization (diethyl ether–hexane) one of the alcohols was isolated in pure form (mp 60–62 °C). A mass spectrum (CI) showed the M^{+1} peak at 355. IR (CCl_4): ν_{max} 3600 (OH, w), 3500–3400 (OH, m, b), 3080 (=CH, w), 2990, 2930, 2860 (aliphatic hydrogens) 1760 (C=O, s), 1640 (C=C, w), 1380, 1370 (typical absorptions of the dioxolane function, cm^{-1}. ^{1}H NMR (90 MHz FT) ($CDCl_3$): δ 6.2–5.6 (1H, m, *H*C=CH_2), 5.15–4.8 (2H, m,=C*H*$_2$), 4.75–4.4 (1H, m, C*H*OC=O), the remaining part of the spectrum contained several signals, however, the resolution was to poor to allow a reliable assignment. The mixture of **19/20** was used as such in the next step.

5.1.15. Reaction of alcohol 19/20 with *N*,*N*′-thiocarbonyldiimidazole. *At room temperature*. To a solution of the alcohol mixture of **19/20** (350 mg, 1.0 mmol) in THF (5 mL) was added *N*,*N*′-thiocarbonyldiimidazole (350 mg, 2 mmol) at room temperature. The reaction mixture was stirred for 6 h at this temperature. The solvent was evaporated under reduced pressure and the residue was chromatographed over 30 g of silica gel with hexane–EtOAc 2:1. A small amount of conjugated unsaturated product **23**+**24** (20 mg, 6%) was eluted first, followed by fractions with starting material (73 mg, 21%), and the desired thiocarbonyl-imidazolide mixture **21** (an oil, 298 mg, 64. IR (CCl_4): ν_{max} 3160 (w), 3145 (w), 3075(w,=CH), 2995, 2925, 2860 (aliphatic CH_2), 1770 (C=O), 1640 (=CH_2), 1380, 1370 (typical absorptions of dioxolane), cm^{-1}. ^{1}H NMR (CCl_4): δ 8.2, 7.6, 6.9, 3×s of imidazole ring. The proton at the carbon with the imidazolide substituent was present as a multiplet at 5.8 ppm. No further assignments were made.

In THF at reflux temperature. A solution of the alcohol mixture **19/20** (177 mg, 0.5 mmol) and *N*,*N*′-thiocarbonyl-diimidazole (178 mg, 1 mmol) in dry THF (10 mL) was refluxed for 3 h. Still some starting material **19/20** was left. Another portion of *N*,*N*′-thiocarbonyldiimidazole (45 mg, 0.25 mmol) was added and heating at reflux temperature was continued for 1 h. The solvent was evaporated and the residue was chromatographed over silica gel (hexane–EtOAc 2:1). The conjugated unsaturated products **23**+**24** were obtained first (37 mg, 22%), followed by starting material (34 mg, 20%) and the desired product **21** as an oil (128 mg, 55%). The

imidazolides **21** were used in the next step without further characterization.

5.1.16. (3*R*/*S*,5*R*)-5-Dec-9-enyl-3-[(4*S*)2,2-dimethyl-[1,3]-dioxolan-4-yl]-methyl-dihydrofuran-2-one 22. To a solution of thiocarbonyldiimidazoles **21** (90 mg, 0.19 mmol) in toluene (10 mL) was added freshly prepared tributyltin hydride (125 mg, 0.43 mmol). The solution was slowly heated to reflux temperature, at which point the reaction was complete. After removal of the solvent, the residue was purified by flash chromatography (EtOAc–hexane 1:4) over silica gel. This afforded product **22** as an oil (45 mg, 70%). According to GLC two isomers were present. IR (neat): ν_{max} 3070, 2980, 2920, 2850, 1770, 1640, 1380, 1370 cm^{-1}. ^1H NMR (90 MHz) ($CDCl_3$): δ 6.1–6.5 (1H, m, *H*C=CH_2), 5.2–4.85 (2H, m, C*H*$_2$=CH), 4.80–4.0 (2H, m, 2×C*H*–O), 4.0–3.3 (2H, m, C*H*$_2$–O) ppm, the remaining signals could not be assigned. This material was used as such in the next deprotection step.

5.1.17. (3*S*,5*R*,2′*S*)-5-Dec-9″-enyl-3-(2′,3′-dihydroxy-propyl)-dihydrofuran-2-one 25 and (3*R*,5*R*,2′*S*)-5-dec-9″-enyl-3-(2′,3′-dihydroxy-propyl)-dihydrofuran-2-one 26. Product **22** (45 mg) was dissolved in a small amount of MeOH (5 mL) and TosOH (50 mg) was added. After stirring for 1 h at room temperature hydrolysis was complete. The solvent was evaporated and the residue dissolved in diethyl ether. After washing with bicarbonate solution, the organic phase was dried ($MgSO_4$), filtered and the solvent evaporated. The residue was purified by flash chromatography over silica gel (EtOAc–hexane 2:1). This gave 20 mg of product (61%). It was pure according to TLC and GLC. However, a long melting range was observed starting at 65 °C. A 400 MHz ^1H NMR spectrum in $CDCl_3$ showed signals of two products. Compound **25**: δ 5.80 (m), 4.94 (m), 4.38 (m), 3.98 (m), 3.68 (m), 3.49(m), 2.84 (m), 2.54 (m), 2.03–1.28 (m) ppm. Compound **26**: δ 5.80 (m), 4.94 (m), 4.60 (m), 3.82 (m), 3.65 (m), 3.55 (m), 2.91 (m), 2.18 (m), 2.03–1.28 (m) ppm. The ratio of **25**/**26** was 2:3.

5.1.18. Reaction of thiocarbonylimidazolide 21 with tributyltin hydride in toluene at reflux temperature. A solution of the imidazolide **21** (crude 840 mg, 1.8 mmol) in toluene (20 mL) was added dropwise to a solution of tributyl tinhydride (1.16 mL, 4.0 mmol) in toluene (20 mL) which was heated at reflux. The mixture was heated at reflux for another hour. After cooling, the solvent was evaporated under reduced pressure and the residue was chromatographed over silica gel (45 g). Eluting with hexane–EtOAc 4:1, followed by hexane–EtOAc 2:1. The first fraction (50 mg, 8%) was the pure conjugated *Z*-alkene **23**. IR (CCl_4): ν_{max} 3070 (w, =CH), 2990, 2920, 2850 (s, aliphatic CH_2 and CH_3), 1755 (s, C=O), 1675 (w, C=C conjugated), 1640 (w,=CH_2) 1380, 1370 (dioxolane absorptions) cm^{-1}. ^1H NMR: δ 6.20 (1H, dt, J=8 Hz, J=1.5 Hz, O=C–C=C*H*), 6.05–5.50 (2H, m, CH_2=C*H*, C=CH–C*H*–O), 5.1–4.75 (2H, m,=C*H*$_2$), 4.6–4.0 (2H, m, C*H*O–C=O, C*H*H–O), 3.75–3.40 (1H, m, CH*H*–O), 3.15–2.15 (2H, dq, J=18 Hz, J=7.0 Hz, and an allylic coupling of 1.5 Hz, lactone C*H*$_2$), 2.15–1.8 (2H, m,=CH–C*H*$_2$), 1.8–0.6 (rest of protons, m) ppm. The next fraction gave a colorless oil (339 mg, 56%). which consisted of a mixture of the *E* and *Z*-isomers **24** and **23**, in equal amounts according to GC. Another chromatographic purification with hexane–EtOAc as eluent gave also a small amount of *E*-isomer **24**. IR (CCl_4): ν_{max} 3070 (w, =CH), 2990, 2920, 2850 (s, aliphatic CH_2 and CH_3), 1750 (s, C=O), 1670 (w, C=C–C=O), 1640 (w, C=C), 1380, 1370 (dioxolane absorptions) cm^{-1}. ^1H NMR ($CDCl_3$): δ 6.66 (1H, dt, J=8 Hz, J=1.8 Hz, O=C–C=C*H*), 6.1–5.6 (1H, m, H_2C=C*H*), 5.15–4.8 (2H, m,=C*H*$_2$), 4.9–4.3 (2H, *H*C–O–C=O,=C–C*H*–O), 4.3–4.1 (1H, s, H*H*C–O), 3.9–3.6 (1H, m, *H*HC–O), 3.25–2.45 (2H, dq, C*H*$_2$ lactone+allyl coupling), 2.2–1.9 (2H, m, =CH–C*H*$_2$), 1.95–1.0 (the remaining protons as broad multiplet) ppm.

5.1.19. (3*S*,5*R*,4′*S*)-5-Decyl-3-(2′,2′-dimethyl-[1′,3′]-dioxolan-4′-yl)-methyl-dihydrofuran-2-one 27. A mixture of the two isomeric alkenes **23**+**24** (20 mg) was dissolved in EtOH (10 mL) and hydrogenated over Pd/C catalyst at room temperature at normal pressure. After 2 h the mixture was filtered over hyflo and the filtrate concentrated. This gave a single compound **27** (18 mg), according to GC, as an oil that crystallized on standing. Recrystallization from hexane gave a solid, mp 38–41 °C. (mp of acetonide of rubrenolide 47–48 °C). IR (CCl_4): ν_{max} 2995, 2940, 2860 (s, CH_2, CH_3), 1770 (s, C=O), 1470, 1455 (m), 1380, 1370 (s), 1170 (s) cm^{-1}. ^1H NMR (90 MHz, FT) ($CDCl_3$): δ 4.5–4.2 (1H, m, C*H*OC=O), 4.2–4.0 (2H, m, C*H*$_2$O), 3.7–3.4 (1H, m, C*H*–O–CH_2–O), 2.9–2.35 (2H, m), 2.3–2.0 (1H, m), 1.9–1.2 (remaining protons as multiplet, with singlets of C*H*$_3$ at 1.38 and 1.32), 0.88 (3H, broad triplet, CH_2–C*H*$_3$) ppm.

5.1.20. Epi-dihydro-rubrenolide 28. Acetonide **27** (5 mg) was treated with *p*-TsOH in MeOH (1 mL). Almost immediate hydrolysis of the acetonide function took place. A small amount of satd aq. $NaHCO_3$ soln was added and the mixture was extracted with ether. After drying and removal of the solvent a solid was obtained. Recrystallization from ether–hexane gave fine needles, mp 94–96 °C. IR (KBr): ν_{max} 3450 (OH), 2995, 2945, 2860 (CH_2, CH_3), 1745 (C=O) cm^{-1}. ^1H NMR (400 MHz) (CD_3OD): δ 4.46 (1H, m, C*H*O–C=O), 3.88 (1H, m, HO–C*H*–CH_2–OH), 3.51 (2H, m, C*H*$_2$–OH), 2.90 (1H, m, C*H*–C=O), 2.60 (1H, m, lactone ring H*H*C–C–O–C=O), 2.06 (1H, m, C*H*H of dihydroxy-propyl side chain), 1.74 (1H, m, CH*H* of dihydroxy-propyl side chain), 1.7–1.3 (19H, m), 0.9 (3H, t, J=7 Hz, CH_2–C*H*$_3$) ppm.

For comparison the ^1H NMR spectrum of dihydrorubrenolide obtained through hydrogenation of natural rubrynolide is given. ^1H NMR (400 MHz) (CD_3OD): δ 4.40 (1H, m, C*H*–O–C=O), 3.60 (1H, m, HO–C*H*–CH_2–OH), 3.49 (2H, dd, C*H*$_2$–OH), 2.94 (1H, m, C*H*–C=O), 2.54 (1H, ddd, lactone ring H*H*C–C–O–C=O, α H), 1.92 (1H, ddd, C*H*H of dihydroxy-propyl side chain), 1.70 (1H, m, CH*H* of dihydroxy-propyl side chain), 1.60 (1H, m, lactone ring *H*HC–C–O–C=O, βH), 1.6–1.25 (remaining protons), 0.90 (3H, t, J=7 Hz) ppm. This spectrum clearly differs from that of **28**.

5.1.21. Tridec-2-yn-1-ol.[31] 1-Bromotridecane (20.0 g, 91 mmol), propargylic alcohol (14.84 g, 264 mmol) and $LiNH_2$ (from 3.6 g of Li, 514 mgat) in ammonia (100 mL) was brought in reaction as described for compound **8**. After

1.5 h DMSO (15 mL) was added and the reaction mixture was left overnight After work-up the oil obtained was purified by destillation b.p. 94–95 °C (0.05 Torr), yield 16.0 g (90%). The oil solidified on standing, lit. mp 37–38 °C. IR (CCl_4): ν_{max} 3620 (OH), 2280, 2220 (alkyn) cm^{-1}. ^1H NMR ($CDCl_3$): δ 4.2–4.4 (2H, bs, $-CH_2OH$), 2.1–2.3 (2H, m, $CH_2C\equiv$), 1.2–1.7 [16H, m, $(CH_2)_8$)], 0.9 (3H, t, CH_2CH_3) ppm.

5.1.22. *E*-Tridec-2-ene-1-ol. To a suspension of $LiAlH_4$ (10.0 g, 262 mmol) in diethyl ether (75 mL) that was cooled with an ice-salt bath, a solution of tridec-2-yn-1-ol (25.7 g, 130 mmol) in diethyl ether (75 mL) was added over a period of 2.5 h. Then THF (170 mL) was added and the mixture was heated at reflux for 2.5 h. After cooling in ice a few mL of water were added carefully to hydrolyse the Li/Al salts. A white solid was formed and the mixture was dried over $MgSO_4$. Work-up gave a light-yellow oil that solidified on standing at 0 °C (23.45 g). This alkene was contaminated with the allene (15%) according to GC and it was used as such for the next reaction.

5.1.23. (2*R*,3*R*)-2,3-Epoxy-tridecane-1-ol 29.[32] To a magnetically stirred suspension of molsieves (1.51 g, 4 Å) in dichloromethane (175 mL), while kept at −20 °C, D(−)-DET (625 mg, 3.0 mmol) and $Ti(OiPr)_4$ (.72 g, 2.5 mmol) were added. After slow addition (1 h) of TBHP (25 mL of a 4.08 molar solution in dichloroethane, 100 mmol) the slightly yellow solution was stirred at −20 °C for 30 more minutes. A solution of tridec-2-ene-1-ol (10 g, 50.5 mmol) in dichloromethane was added over a period of 1 h. The reaction mixture was kept at −20 °C for 3 h. The temperature was allowed to rise to 0 °C and the reaction mixture was then poured into a solution of ferrosulfate (16 g, 60 mmol) and tartaric acid (5.0 g, 30 mmol) in water (50 mL). After stirring vigorously for 15 min the two layers were separated. The water layer was extracted with ether (2×75 mL) and the organic layers were stirred with a solution of NaOH (30%, 25 mL, satd with NaCl, kept at 0 °C). After stirring at 0 °C for 45 min, water (250 mL) was added and the layers were separated. The water layer was extracted once more with ether (150 mL) and the organic phase was dried over $MgSO_4$. Work-up afforded a colorless solid (8.93 g, 77%). Chromatography (EtOAc–hexane 1:3) over silica gel gave the pure compound (7.5 g, 69%), $[\alpha]_D^{20}$=+25 ($CHCl_3$, c=1). Lit.[32] $[\alpha]_D$ of the 2*S*,3*S* compound −24 ($CHCl_3$). IR (CCl_4): ν_{max} 3600 (OH) cm^{-1}. ^1H NMR (90 MHz) (CCl_4): δ 4.40–3.4 (2H, m, CH_2–OH), 3.0–2.8 (2H, m, *H*C–C*H*), 1.8–1.2 [(18H, m, $(CH_2)_9$], 0.9 (3H, t, CH_3) ppm.

5.1.24. (3*S*,4*R*)-1-Diazo-3,4-epoxy-tetradecan-2-one 30. To a mixture of (2*R*,3*R*)-epoxy-tridecan-1-ol **29** (8.89 g, 41.5 mmol) in CCl_4 (80 mL), acetonitrile (80 mL) and water (120 mL) was added $NaIO_4$ (29.7 g, 138.8 mmol) and $RuCl_3{\cdot}3H_2O$ (240 mg, 2.2 mol%). The brown mixture was stirred for 2.5 h at room temperature. According to TLC the reaction was complete. Dichloromethane (350 mL) and water (200 mL) were added and the layers were separated. The water layer was extracted with dichloromethane (3×100 mL). After drying ($MgSO_4$) and evaporation of the solvent a black residue was residing. This was dissolved in diethyl ether (500 mL). To the cooled (2 °C) solution was added isobutyl chloroformate (5.66 g, 41.5 mmol), followed by triethylamine (6.26 g, 61.8 mmol). After stirring for 1 h at the indicated temperature the precipitate was filtered off under a stream of nitrogen. The filtrate was added to a solution of diazomethane in diethyl ether (300 mL, concentration of CH_2N_2: 0.3 mmol/mL). After one night at room temperature the reaction mixture was flushed with nitrogen to remove excess diazomethane. Residual $Et_3N{\cdot}HCl$ was filtered off and the solvent was evaporated leaving a black residue. Flash chromatography (EtOAc–hexane 8:1) over silica gel gave the epoxy diazomethyl ketone as a yellow solid (4.93 g, yield calcd on epoxy alcohol 47%), mp 41–42 °C (hexane–diethyl ether). Elemental analysis: calcd for $C_{14}H_{24}N_2O_2$ (252.357): C 66.63, H 9.59, N 11.10%; found: C 66.38, H 9.60, N 10.83%; $[\alpha]_D^{20}$=−65.7 ($CHCl_3$, c=1). IR (CCl_4): ν_{max} 3120 (HC=), 2115 (C=N=N), 1645 (C=O) cm^{-1}. ^1H NMR (300 MHz) ($CDCl_3$): δ 5.41 (1H, s, *H*C=N_2), 3.15 (1H, d, J=1.4 Hz, O–C*H*–C=O), 2.90 (1H, m, $-CH_2CH-O$), 1.6–1.2 [(18H, m, $(CH_2)_9$], 0.81 (3H, t, J=6.9 Hz, CH_3) ppm. ^{13}C NMR: 147.80 *C*=O, 59.26 CH_2C–O, 58.75 O–*C*H–C=O, 51.96 *C*H=N_2, CH_2 signals at 31.85, 31.66, 29.53, 29.45, 29.41, 29.27, 29.21, 25.63, 22.64, CH_3 at 14.08 ppm.

5.1.25. Ethyl (4*R*)-4-acetoxy-tetradec-2-enoate 31. A solution of (3*S*,4*R*)-1-diazo-3,4-epoxy-tetradecan-2-one **30** (2.36 g, 9.35 mmol) in abs ethanol (800 mL) was irradiated for 2.5 h. The reaction was monitored by taking IR spectra. Evaporation of the solvent gave a yellow oil (2.50 g). A mixture of thus obtained ethyl (4*R*)-4-hydroxy-tetradec-2-enoate (5.09 g, 18.7 mmol), pyridine (6.9 g, 85 mmol), acetic anhydride (6.0 g, 60 mmol) and a small amount of DMAP was stirred at room temperature for 18 h. The mixture was poured in ice-water (100 mL) and extracted with ether (3×100 mL). After drying ($MgSO_4$), the solvent was evaporated and the residue was purified by flash chromatography (hexane–EtOAc 20:1), giving **31** as a colorless oil (2.89 g, 50%). IR (CCl_4): ν_{max} 1740 (C=O), 1720 (=C–C=O), 1660 (C=C) cm^{-1}. ^1H NMR (90 MHz) (CCl_4): δ 6.8 (1H, dd, J=15, 4.5 Hz, EtO–C(=O)–CH=C*H*–CH), 5.9 (1H, d, J=15 Hz, O=C–C*H*), 5.5–5.2 (1H, m, C*H*–O–C(C=O)–CH_3), 4.1 (2H, q, J=6.7 Hz, O–CH_2CH_3), 2.0 (3H, s, O=C–CH_3), 1.7–1.1 [(21H, m, $(CH_2)_9$+CH_3CH_2–O)], 0.9 (3H, t, CH_2–CH_3) ppm.

A 400 MHz spectrum was run and the ee was determined (91%) with the aid of the optical shift reagent $Eu(hfc)_3$. The chemical shifts found of the 400 MHz spectrum matched approximately with the 90 MHz spectrum; the values were as follows: 6.84 (1H, d d, J=15.7 Hz, J=5.4 Hz), 5.93 (1H, d, J=15.7 Hz), 4.20 (1H, m), 4.20 (2H, q, J=7.2 Hz), 2.09 (3H, s), 1.33–1.25 (21H, m), 0.88 (3H, t, J=7.2 Hz) ppm.

5.1.26. (5*R*)-5-Decyl-tetrahydrofuran-2-one 32. To a suspension of palladium dichloride (31 mg, 0.17 mmol) in EtOH (15 mL), $NaBH_4$ (15 mg, 0.36 mmol) was added. The mixture was stirred for 15 min and ethyl (4*R*)-4-acetoxy-tetradec-2-enoate **31** (1.08 g, 3.46 mmol) was added. In a closed system the mixture was kept in a hydrogen atmosphere applying a small over-pressure. The theoretical amount of hydrogen was absorbed in 90 min. The mixture was filtered over hyflo and the filtrate was evaporated. The

residue was chromatographed over silica gel (EtOAc–petroleum ether 1:20). Ethyl (4*R*)-4-acetoxy-tetradecanoate was obtained as a colorless solid (827 mg, 76%). IR (CCl_4): ν_{max} 1740, 1730 cm^{-1} (C═O). ^1H NMR (90 MHz): δ 4.85 (1H, m, C*H*–O–C═O), 4.1 (2H, q, *J*=6.7 Hz, CH_3CH_2–O), 2.3 (2H, t, CH_2C(═O)–O), 2.0 (3H, s, O–C(═O)–CH_3), 2.0–1.7 (2H, m, CH_2–CH_2–C(C═O)–OEt), 1.7–1.1 [21H, m, $(CH_2)_9$+O–CH_2–CH_3), 0.9 (3H, t, *J*=6.0 Hz, CH_3-alkyl) ppm.

To a solution of (4*R*)-4-acetoxy-tetradecanoate (980 mg, 3.12 mmol) in EtOH (20 mL) was added KOH (0.8 g, 14.2 mmol). The solution was heated at reflux for 15 min. Then the solvent was evaporated and the residue was dissolved in a small amount of water. After one extraction with ether the aqueous layer was acidified and extracted with ether (3×). These ether extracts were concentrated and the residue was dissolved in benzene (80 mL). A small amount of TsOH was added and the solution was heated at reflux and water was removed azeotropically. The benzene solution was washed with aq. $NaHCO_3$ solution. After drying, evaporation of the solvent gave the product as a pale yellow solid (711 mg, quant.). Recrystallization from heptane gave colorless crystals, mp 35–36 °C, $[\alpha]_D^{20}$=+32 ($CHCl_3$, *c*=1.1), lit.[21] mp 36–37 °C, $[\alpha]_D^{26}$=+29.95 ($CHCl_3$, *c*=2.7). Elemental analysis, calcd for $C_{14}H_{26}O_2$ (226.359): C 74.29, H 11.58%; found: C 74.33, H 11.39%. IR (CCl_4): ν_{max} 1780 (C═O) cm^{-1}. ^1H NMR (300 MHz) ($CDCl_3$): δ 4.5 (1H, m, C*H*OC═O), 2.56–2.50 (2H, m, CH_2C═O), 2.37–2.26 (1H, m, C*H*CH–O–C═O), 1.9–1.26 [19H, m, C*H*CH–O–C═O, $(CH_2)_9$)], 0.88 (3H, t, *J*=6.9 Hz, CH_3) ppm. ^{13}C NMR: 177.25 *C*═O, 81.02 *C*H–O, CH_2 peaks at 35.54, 31.85, 29.53, 29.48, 29.42, 29.30, 29.27, 28.83, 27.97, 25.18, 22.64, CH_3 at 14.07 ppm.

5.1.27. (3*R*/*S*,5*R*)-5-Decyl-3-{[(4*R*)-2,2-dimethyl-[1,3]dioxolan-4-yl]-hydroxy-methyl}-dihydrofuran-2-one 33. To a stirred solution of di-isopropyl amine (505 mg, 5.0 mmol) in THF (10 mL) kept at 0 °C, was added a solution of BuLi in hexane (3.12 mL, 1.6 N). The mixture was cooled at −78 °C and a solution of the lactone **32** (567 mg, 2.5 mmol) in THF (5 mL) was added dropwise, followed by DMPU (390 mg, 2.2 mmol) in THF (5 mL). The solution was stirred for 15 min and (4*S*)-2,2-dimethyl-[1,3]-dioxolane-4-carbaldehyde **38** (1.0 g, 7.7 mmol) in THF (5 mL) was added. The reaction mixture was kept at the low temperature for 4 h and a satd aq. solution of ammonium chloride (25 mL) was added. The organic layer was collected and the water layer was extracted with ether (3×25 mL). After drying over Na_2SO_4, the solvents were evaporated to give a pale yellow oil (730 mg). Chromatography over silica gel (EtOAc–petroleum ether 1:5 gave the product consisting of mainly two isomers (0.56 g, 63%). Some starting material (84 mg, 15%) was also obtained. IR (CCl_4): ν_{max} 3600–3300 (OH, broad), 1760 (C═O), 1380, 1370 (dioxolane absorption) cm^{-1}. ^1H NMR: complex due to the presence of two isomers. This mixture was used as such in the next step.

5.1.28. (5*R*)-5-Decyl-3-[(4*R*)-2,2-dimethyl-[1,3]dioxolan-4-ylmethylene]-dihydrofuran-2-one 34. To a stirred solution of the isomeric mixture **33** (800 mg, 2.27 mmol) in dichloromethane (20 mL) and pyridine (10 mL) kept at 0 °C a few crystals of DMAP and methanesulfonyl chloride (2.0 mL) were added. After 1 h at room temperature, the dichloromethane was evaporated, the residue was dissolved in ether (30 mL). The solution was washed with water and an aqueous solution of $CuSO_4$. The ether solution was dried over $MgSO_4$ and the solvent was evaporated leaving a yellow oil (825 mg). To a solution of this crude mesylate (825 mg, 1.9 mmol) in dichloromethane (20 mL) DBU (580 mg, 3.8 mmol) was added. The reaction was monitored by TLC. After 2 h the reaction was completed. The dichloromethane solution was washed with aq. bicarbonate and dried ($MgSO_4$). After evaporation of the solvent a mixture of *cis* and *trans* alkene **34** was obtained (620 mg, 96%). IR (CCl_4): ν_{max} 1760 (C═O), 1685 (C═C), 1380, 1370 (dioxolane absorptions) cm^{-1}. This material was then subjected to hydrolysis.

5.1.29. (5*R*)-5-Decyl-3-[(2*R*)-2,3-dihydroxypropylidene]-dihydro-furan-2-one 35. The alkene mixture **34** (560 mg, 1.66 mmol) was dissolved in MeOH (20 mL) and a small amount of TsOH was added. After stirring for 3 h aq. $NaHCO_3$ solution was added and the methanol was evaporated. The residue was treated with water and the mixture was extracted with ether. Usual work-up gave **35** as a colorless solid (380 mg, 77%) which was hydrogenated right away.

5.1.30. (3*S*,5*R*,2′*R*)-5-Decyl-3-(2′,3′-dihydroxy-propyl)-dihydrofuran-2-one, (dihydro rubrenolide) 36. The lactone **35** (380 mg, 1.28 mmol) was dissolved in EtOH (10 mL) and hydrogenated over Pd/C as catalyst at normal pressure at room temperature. After shaking for 3 h in a hydrogen atmosphere the uptake of hydrogen had stopped. Filtration over hyflo and evaporation of the solvent gave a pure residue **36** (320 mg, 84%), mp 105–107 °C (lit.[3] 106–107 °C), $[\alpha]_D^{20}$=+21.8 ($CHCl_3$, *c*=1) (lit.[3] +22, $CHCl_3$). IR and NMR (400 MHz) spectra of **36** were in complete agreement with the spectra obtained from dihydrorubrenolide that was prepared by hydrogenation of authentic rubrynolide obtained from Professor Ollis.[22]

5.1.31. (3*R*/*S*,5*R*)-5-[10-(Tetrahydropyran-2-yloxy)-decyl]-3-{[(4*R*)-2,2-dimethyl-[1,3]dioxolan-4-yl]-hydroxy-methyl}-dihydrofuran-2-one 39. To a solution of di-isopropylamine (450 mg, 4.5 mmol) in THF (20 mL) at 0 °C a solution of BuLi (2.5 mL, 1.6 N) in hexane was added. The stirred solution was cooled to −70 °C and lactone **37** (1.1 g, 3.4 mmol) dissolved in THF (10 mL) was added drop by drop. After stirring for 15 min excess (4*S*)-2,2-dimethyl-[1,3]-dioxolane-4-carbaldehyde **38** (1.17 g, 9.0 mmol) in THF (5 mL) was added in one portion, followed by HMPA (1 mL). The reaction mixture was left for 16 h at −70 °C and was quenched with satd aq. NH_4Cl solution. The mixture was extracted with diethyl ether, the organic phase was washed with water and dried over $MgSO_4$. The residue obtained after work-up was chromatographed over silica gel (100 g, EtOAc–hexane 3:1, followed by 2:1). This gave starting material (0.41 g, 40.8%) and the desired product **39** (400 mg, 26%) as a mixture of isomers which was processed as such in the next step. IR (neat): ν_{max} 3600, 3480 (OH), 2980, 2920, 2840 aliphatic CH_2, 1765 (C═O), 1380,1370 (dioxolane absorptions) cm^{-1}.

5.1.32. (5*R*)-5-[10-(Tetrahydropyran-2-yloxy)-decyl]-3-{[(4*R*)-2,2-dimethyl-[1,3]-dioxolan-4-yl]-propylidene}-dihydro-furan-2-one 40. The mixture of alcohols **39** (400 mg, 0.88 mmol) was dissolved in CCl_4 (10 mL) and methanesulfonyl chloride (120 mg, 1.04 mmol) and Et_3N (120 mg, 1.2 mmol) were added. The reaction was followed by IR. The alcohol absorption at 3600 and 3480 cm^{-1} disappeared and strong absorptions at 1380 and 1180 cm^{-1} from the sulfonate were emerging. In addition the C=O absorption shifted to 1775 cm^{-1}. The solution was washed with aqueous bicarbonate solution and dried ($MgSO_4$). After evaporation of the solvent, the crude oily mesylate was immediately used in the next step.

To a solution of the mesylate in diethyl ether (10 mL), DBU (200 mg, 1.33 mmol) was added. The amine·HCl salt formed immediately started to precipitate. According to TLC two products had been formed (*cis* and *trans* alkene **40**). After 2 h the reaction mixture was shaken with 10% aq. tartrate solution and the ether phase was dried over $MgSO_4$. After evaporation of the solvent the residue was chromatographed over silica gel (diethyl ether–hexane 1:1). This gave **40** as an oily mixture of isomers (247 mg, 72.5%) that was used as such in the next step. IR (neat): 1755 (C=O), 1680 (C=C) cm^{-1}.

5.1.33. (5*R*)-5-(10-Hydroxy-decyl)-3-[(2*R*)-2,3-dihydroxy-propylidene]-dihydrofuran-2-one 41. The mixture of *cis* and *trans* alkene **40** was dissolved in MeOH (10 mL), and a small amount of TsOH was added with stirring. The protecting groups were removed fast. The reaction was monitored by TLC. A small amount of aq. bicarbonate solution was added and the mixture was concentrated in vacuo. Water was added to the residue and the mixture was extracted with chloroform (4×). After drying and evaporation of the solvent, triol **41** was obtained as a solid (140 mg, 70%). The solid still showed a long melting point range due to the presence of two isomers. IR (KBr): ν_{max} 3500–3100 (OH), 1750 (C=O), 1680 (C=C) cm^{-1}. This material was used as such in the next step.

5.1.34. (5*R*,3*S*,2′*R*)-5-(10-Hydroxy-decyl)-3-(2′,3′-dihydroxy-propyl)-dihydrofuran-2-one 42. Product **41** was hydrogenated quantitatively in MeOH with H_2 Pd/C to give compound **42** as a solid. IR (KBr): ν_{max} 3500–3200 (OH), 2920, 2840 (aliphatic CH), 1740 (C=O) cm^{-1}. This product was used as such in the next step.

5.1.35. (5*R*,3*S*,4′*R*)-5-(10-Hydroxy-decyl)-3-[(2′,2′-dimethyl-[1′,3′]dioxolan-4′-yl)-methyl])-dihydrofuran-2-one 43. A solution of the above mentioned triol **42** in acetone was stirred with a small amount of TsOH. After 30 min aq. bicarbonate solution was added. The reaction mixture was concentrated and the residue was extracted with ether. After work-up the residue was chromatographed over silica gel (diethyl ether–hexane 1:1). Product **43** was obtained as a solid (115 mg, 73%), mp 78–79 °C (hexane). Elemental analysis, calcd for $C_{20}H_{36}O_5$ (356.477) C: 67.41, H: 10.11%; found: C 67.31, H 9.94%. IR (KBr): ν_{max} 3500–3300 (OH), 1770 (C=O), cm^{-1}. ^1H NMR (400 MHz), ($CDCl_3$): δ 4,35 (1H, m, C*H*–O–C=O), 4.13 (1H, m, CH_2–C*H*–O–CMe_2), 4.07 (1H, m, O–CH–CH*H*–O), 3.64 (2H, t, J=6.6 Hz, CH_2–OH), 3.57 (1H, m, O–CH–C*H*H–O), 2.86 (1H, m, C*H*–C=O), 2.54 (1H, m, C*H*H–CH–C=O, lactone ring), 2.20 (1H, m, C*H*H–CH–C=O, side chain), 1.58 (1H, m, CH*H*–CH–C=O, lactone ring), 1.54–1.20 (25H, m, including dioxolane 2×Me at 1.41 and 1.35) ppm. ^{13}C NMR: 178.62 (*C*=O), 109.20 (O–*C*–O), 79.11 (*C*H–O–C=O), 73.49 (OCH_2–*C*H–O), 69.23 (O–*C*H_2–CH–O), 63.07 (*C*H_2–OH), 38.24 (*C*H_2–C=O), 35.52 (*C*H_2–CH–C=O, lactone ring), 34.20 (*C*H_2–CH–C=O, side chain), 26.96 (C–*C*H_3), 25.65 (C–*C*H_3), other *C*H_2 signals at 35.43, 32.80, 29.50, 29.39, 29.38, 29.30, 25.71, and 25.21 ppm. Another *C*H_2 seems to be hidden at 29.28 ppm.

5.1.36. (5*R*,3*S*,4′*R*)-5-Dec-(9″)-enyl-3-(2′,2′-dimethyl-[1′,3′]dioxolan-4′-yl)-methyl)-dihydrofuran-2-one 44. To a solution of alcohol **43** (69 mg, 0.2 mmol) in dry THF (5 mL) kept under argon, *o*-nitrophenylselanylcyanide (68 mg, 0.3 mmol) was added, followed by tributylphosphine (61 mg, 0.3 mmol) in a small amount of THF. Soon a red colored solution appeared. The mixture was stirred for 2 h at room temperature, after which the starting material had disappeared. After evaporation the residue was chromatographed over silica gel (diethyl ether–hexane 1:1). This gave 98 mg (91%) of the selenide, which was processed further immediately.

To a stirred solution of the selenide in THF (5 mL) an excess of H_2O_2 (0.3 mL, 30%) was added. Stirring was continued for 2 h. TLC pointed out that the reaction was complete. Satd aq. bicarbonate solution was added and the mixture was extracted with hexane. The solution was chromatographed over silica gel. Elution with petroleumether–diethyl ether 1:1 gave a yellowishly colored product **44** (68 mg). Some selenide material was apparently sticking to the product. IR (CCl_4): ν_{max} 2980, 2920, 2855 (aliphatic C–H), 1775 (C=O), 1635 (C=C), and the sharp dioxolane absorptions at 1370 and 1380 cm^{-1}. This product was subjected to hydrolysis right away.

5.1.37. (5*R*,3*S*,2′*R*)-5-Dec-9″-enyl-3-(2′,3′-dihydroxy-propyl)-dihydrofuran-2-one 1 (rubrenolide). Compound **44** (crude 68 mg, 0.2 mmol) was dissolved in MeOH (5 mL). A small amount of TsOH was addded and upon stirring a fast deprotection of the diol function occurred (TLC). Aqueous bicarbonate solution was added and the mixture was concentrated in vacuo. The residue was extracted with ether. The ether solution was dried over $MgSO_4$ and the solvent was removed after filtration. The residue was chromatographed over silica gel. Elution with hexane–EtOAc removed the yellow colored by-product. Elution with EtOAc gave pure compound **1** as a colorless solid (36 mg, 60%), mp 99–100 °C, $[\alpha]_D^{20}$=+21.2 (CH_2Cl_2, c=0.3), lit.[3] mp 100 °C, $[\alpha]_D^{20}$=+21 ($CHCl_3$). The 400 MHz ^1H NMR spectrum was in complete agreement with a 400 MHz NMR spectrum that was taken from the authentic product.

5.2. Separation of rubrenolide 1 and rubrynolide 2

Professor Ollis (University of Sheffield) kindly provided a mixture of the natural product (78 mg) as it was isolated from the natural source; 25 mg of this mixture was dissolved in EtOH (0.5 mL, 95%) and 5% $AgNO_3$ solution in EtOH

(95%, 1 mL) was added. A white precipitate formed immediately. The solid was filtered off and washed with EtOH. The filtrate was concentrated and the residue was treated with water. The mixture was extracted with ether. Drying over $MgSO_4$, filtration and evaporation of the solvent gave rubrenolide (7 mg, mp 98 °C), which was used as reference material. The solid that was filtered off, was treated with a 10% NaCN aq. solution and extracted with ether. After work-up 16 mg of rubrynolide was obtained. This compound was hydrogenated in EtOH with Pd/C as catalyst to give dihydrorubrenolide **36** which was used as reference material (for the 400 MHz spectrum, see preparation of epi-dihydrorubrenolide **28**).

References and notes

1. Franca, N. C.; Gottlieb, O. R.; Coxon, D. T.; Ollis, W. D. *J. Chem. Soc., Chem. Commun.* **1972**, 514.
2. Gottlieb, O. R. *Phytochemistry* **1972**, *11*, 1537. Filho, R. B.; Diaz, P. P.; Gottlieb, O. R. *Phytochemistry* **1980**, *19*(1980), 455.
3. Franca, N. C.; Gottlieb, O. R.; Coxon, D. T. *Phytochemistry* **1977**, *16*, 257.
4. Cahn, S. R.; Ingold, C.; Prelog, V. *Angew. Chem.* **1966**, *78*, 413.
5. Hussain, S. A. M. T.; Ollis, W. D.; Smith, C.; Fraser Stoddart, J. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1480.
6. Taylor, S. K.; Hopkins, J. A.; Spangenberg, K. A.; McMillen, D. W.; Grutzner, J. B. *J. Org. Chem.* **1991**, *56*, 5951.
7. Saito, T.; Thijs, L.; Ettema, G.-J.; Zwanenburg, B. *Tetrahedron Lett.* **1993**, *34*, 3589.
8. For other examples see: (a): Waanders, P. P.; Thijs, L.; Zwanenburg, B. *Tetrahedron Lett*. **1987**, *28*, 2409. (b) Dommerholt, F. J.; Thijs, L.; Zwanenburg, B. *Tetrahedron Lett.* **1991**, *32*, 1495. (c) Dommerholt, F. J.; Thijs, L.; Zwanenburg, B. *Tetrahedron Lett.* **1991**, *32*, 1499.
9. Thijs, L.; Dommerholt, F. J.; Leemhuis, F. M. C.; Zwanenburg, B. *Tetrahedron Lett.* **1990**, *31*, 6589.
10. Katsuki, T.; Sharpless, K. B. *J. Am. Chem. Soc.* **1980**, *102*, 5974.
11. Bal, B. S.; Childers, W. E.; Pinnick, H. W. *Tetrahedron* **1981**, *37*, 2091.
12. Thijs, L.; Waanders, P. P.; Stokkingreef, E. H. M.; Zwanenburg, B. *Recl. Trav. Chim. Pays-Bas* **1986**, *105*, 332.
13. Russell, T. W.; Hoy, R. C. *J. Org. Chem.* **1971**, *36*, 2018.
14. Marshall, J. A.; Flynn, G. A. *J. Org. Chem.* **1981**, *44*, 1391. For the preparation of the selenide see: Grieco, P. A.; Gilman, S.; Nishizawa, M. *J. Org. Chem.* **1976**, *41*, 1485.
15. Jackson, D. Y. *Synth. Commun.* **1988**, *18*, 337.
16. Rasmussen, J. R.; Slinger, C. J.; Kordish, R. J.; Newman-Evans, D. D. *J. Org. Chem.* **1981**, *46*, 4843. See also: Rasmussen, J. R. *J. Org. Chem.* **1980**, *45*, 2725.
17. For a related structure see: Tamamura, H.; Bienfait, B.; Nacro, K.; Lewin, N. E.; Blumberg, P. M.; Marquez, V. E. *J. Med. Chem.* **2000**, *43*, 3209.
18. Doolittle, R. E.; Tumlinson, J. H.; Proveaux, A. T.; Heath, R. R. *J. Chem. Ecol.* **1980**, *6*, 473. The assignment *R* and *S* were made opposite to that of the correct structure.
19. An NMR of **31** in the presence of the chiral shift reagent $Eur(hfc)_3$ indicated an ee of 91%.
20. Hubschwerlen, C. *Synthesis* **1986**, 962.
21. Klyne, W.; Scopes, P. M.; Wlliams, A. *J. Chem. Soc.* **1965**, 7237.
22. The late Professor Ollis, kindly provided us with a free sample of the natural product consisting of a mixture of rubrenolide and rubrynolide. The two products could be separated as reported.[3] Rubrynolide was hydrogenated to give dihydrorubrenolide (see Section 5).
23. Horeau, A.; Nouaille, A. *Tetrahedron Lett.* **1971**, 1939.
24. Horeau, A.; Kagan, H. B. *Tetrahedron* **1964**, *20*, 2431.
25. Chérest, M.; Felkin, H. *Tetrahedron Lett.* **1968**, 2205. Anh, N. T.; Eisenstein, O. *Nouv. J. Chim.* **1977**, *1*, 61.
26. Heathcock, C. H.; Young, S. D.; Hagen, J. P.; Pirrung, M. C.; White, C. T.; Van Derveer, D. *J. Org. Chem.* **1980**, *45*, 3846.
27. Jurczak, J.; Pikul, S.; Bauer, T. *Tetrahedron* **1986**, *42*, 447, and references cited herein.
28. Minimum energy structures were generated by semi-empirical MOPAC PM3 calculations and carried out by Dr G.J.A. Ariaans (Synthon, Nijmegen, The Netherlands).
29. There are examples of complete control of stereochemistry when both components in the Aldol condensation are chiral, see: Dongala, E. B.; Dull, D. L.; Mioskowski, C.; Solladié, G. *Tetrahedron Lett.* **1973**, 4983. see also: Masamune, S.; Choy, W.; Petersen, J. S.; Sita, L. R. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 1.
30. Girlanda-Junges, C.; Keyling-Bilger, F.; Schmitt, G.; Luu, B. *Tetrahedron* **1998**, *54*, 7735.
31. Ermilova, E. V.; Remizova, L. A.; Favorskaya, I. A.; Tregubova, N. L. *Zh. Org. Khim.* **1975**, *11*, 520.
32. Kang, S. K.; Kim, Y. S.; Lim, J. S.; Kim, K. S.; Kim, S. G. *Tetrahedron Lett.* **1991**, *32*, 363.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron 60 (2004) 5253–5258
Tetrahedron

Microwave assisted green chemical synthesis of novel spiro[indole-pyrido thiazines]: a system reluctant to be formed under thermal conditions☆

Anshu Dandia,* Kapil Arya, Meha Sati and Sangeeta Gautam

Department of Chemistry, Lab. 215, University of Rajasthan, Jaipur 302 004, India

Received 31 July 2003; revised 15 March 2004; accepted 8 April 2004

Abstract—Spiro [3*H*-indole-3,2′-[4*H*] pyrido [3,2-*e*]-1,3-thiazine]-2,4′ (1H) diones, a class of previously unknown compound which does not form under conventional conditions, can be prepared by treatment of 'in situ' generated 3-indolylimine derivatives with 2-mercaptonicotinic acid under microwave irradiation in absence of any solvent or solid support in 85–92% yields in 3–8 min. The facile one pot reaction is generalized for a variety of ketones and amines to give pure pyrido [3,2-*e*] thiazine derivatives, which do not require further purification processes.

1. Introduction

The spiro indole compounds are of current interest due to their exceptional biological activity.[1–4] Thiazine derivatives have also been reported as well known useful therapeutic agents.[5–12] Thiazines incorporating a pyridine nucleus have shown anticancer,[13] antitumor,[14] antioxidant,[15] and potential CNS activities.[16] They have been patented as antibacterial[17] and thrombin inhibitors,[18] antidiabieties,[19] antiinflammatory agents,[20] analgesics,[21] lipogenase inhibitors in leucocyte cell.[22] The presence of an N–C–S linkage is believed to account for the amoebicidal, anticonvulsant, fungicidal[23] and antiviral[24] activities.

Pyridothiazine derivatives have been synthesized conventionally by tedious multi-step procedures, with prolonged refluxing using volatile and hazardous organic solvents[25] and are patented as pharmacological agents, the synthetic details of which are not available.[17–22]

Inspite of the immense biological activities of pyridothiazines and spiro indoles no report is yet available on the synthesis of spiro [indole-pyridothiazines]. In view of these observations, and in continuation of our earlier interest on the reactions of thioacids with 3-indolylimines to give biologically active spiro compounds,[26] we planned to synthesize novel spiro[indole-pyridothiazines] **5** by reacting 3-arylimino-2*H*-indol-2-one (**3**) with 2-mercaptonicotinic acid (**4**).

There are a few reports on the reactions involving the synthon mercaptonicotinic acid[27] due to its low reactivity. To the best of our knowledge its reaction with arylimines has not been studied. Conventionally, it is reluctant to undergo any reaction with 3-arylimino-2*H*-indol-2-one, even under harsh conditions (reflux for many days) in high boiling solvents, e.g. dry toluene with azeotropic removal of water; or using dehydrating agents like $ZnCl_2$+ dioxane, strong acids toluene+TFA/gl. AcOH, etc.

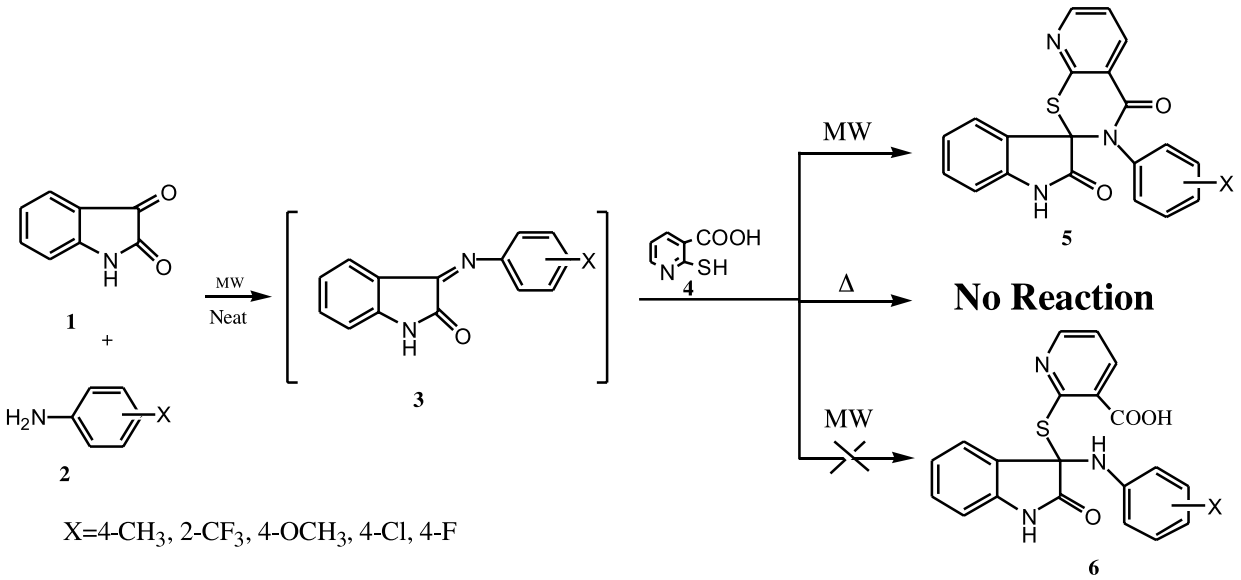
Microwave irradiation is well known to promote the synthesis of a variety of compounds,[28,29] where chemical reactions are accelerated because of selective absorption of microwaves by polar molecules. Recently, the coupling of MWI with solid supports under solvent free conditions has received notable attention.[29] A literature survey reveals examples of specific reactions, which do not occur under conventional heating/sonication, but could be made possible by microwave irradiation coupled with a solid support.[30]

Hence, in continuation of our work on the synthesis of biodynamic heterocycles under microwave irradiation,[31] we report herein for the first time a facile one-pot synthesis of 3′-substituted phenyl spiro [3*H*-indole-3,2′-[4*H*]pyrido [3,2-*e*]-1,3-thiazine]-2,4′ (1*H*) diones] in 88–92% yield in 3–5 min. (Scheme 1)

☆ Presented at the Sixth International Electronic Conference on Microwave Chemistry (ECSOC-6), 1–30 April 2003.

Keywords: Pyrido thiazine; Microwave irradiation.
* Corresponding author. Tel.: +91-98-2914-4056; fax: +91-14-1221-4321; e-mail address: dranshudandia@eth.net

0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.04.018



Scheme 1.

In the present work, we studied the reaction of **3a** and **4** taking different parameters of solid supports like (i) montmorillonite KSF; (ii) neutral alumina; (iii) *p*-toluene sulfonic acid (PTSA); (iv) silica gel for which promising results were obtained.

Encouraged by the recent focus on the green chemical theme of eliminating the use of solvents, we extended our studies to neat reactions, since the reaction using solid support requires an appreciable amount of solvent for adsorption of reactants and elution of products. However, no reaction occurred, but it could be made successful by addition of a few drops of DMF. The role of DMF is to act as energy transfer agent and homogenizer to increase the reaction temperature.[32] It did not lead to any side reactions and no detectable by-product were observed. Consequently, we extended this condition to the synthesis of compounds (**5b**–**e**).

In order to check the general applicability of the reaction for the synthesis of pyrido [3,2-*e*]thiazine derivatives, we extended the reaction of **4** with imine derivatives derived from variety of aryl/heterocyclic amines, ketones/aldehydes and developed facile elegant solvent free method for exclusive synthesis of pyrido [3,2-*e*]thiazine derivatives (**5f**–**k**) with an easier work up procedure.

In order to check the possible intervention of 'specific (non-thermal) microwave effect'[33] the best result obtained under microwave irradiation was extrapolated to conventional heating. In the case of compound **5a** the reaction was carried out using a preheated oil bath under the same reaction conditions (time, temperature, pressure and vessel) for 5 min. It was found that no reaction occurred and that the reactants remain unchanged, even on extended reaction time (8 h), thus demonstrating that the effect of microwaves is not simply thermal[34] (Table 1).

Table 1. Comparative study for the synthesis of **5a** (X=4-CH_3)

(A) Under microwave irradiation

S.No.	Solid support/energy transfer medium	MW power (W)	Time (min)	Temperature[a] (°C)	Yield[b] (%)
1	Montmorillonite KSF	640	12	112	Nil
2	Montmorillonite KSF+ϵgl. AcOH	640	3	122	70
3	Montmorillonite KSF+ϵ DMF	640	6	132	86
4	Neutral alumina	640	30	102	Nil
5	PTSA	275	20	125	65
6	SiO_2	640	12	122	72
7	Neat (without DMF)	640	12	95	Nil
8	Neat+DMF (few drops)	640	3	140	90

(B) By thermal heating

S.No.	Thermal heating	Time (min)	Temperature[a] (°C)	Yield[b] (%)
1	Toluene+TFA	6 days	Reflux	Nil
2	Dry toluene	7 days	Reflux	Nil
3	Glacial AcOH	6 days	Reflux	Nil
4	Dioxane+$ZnCl_2$	8 days	Reflux	Nil
5	Neat+DMF (few drops)	3 min	140	Nil
6	Neat+DMF (few drops)	480 min	140	Nil

[a] Final temperature is measured at the end of microwave irradiation by introducing a glass thermometer in the reaction mixture in the beaker.
[b] Yield of the isolated products.

2. Results and discussion

After analysis of the results summarized in the tables, we found the reaction of 3-arylimino-2*H*-indol-2-one (**3**) with 2-mercaptonicotinic acid (**4**) afforded **5** as white needles. The IR spectrum of **5a** showed a strong absorption band at 3320–3290 cm^{-1} (NH), two sharp bands at 1720–1710 (both C═O) and an absorption due to the C═N functionality appeared at 1625 cm^{-1}.

The ^1H NMR spectrum of **5a** revealed characteristic signals for methyl protons at 2.15 (s, 3H, CH_3) along with aromatic protons at δ 6.83–7.52 (m, Ar-H, 8H), δ 7.62–8.73 (m, 3H, pyridyl-H,) and NH proton at δ 9.01(s, 1H, NH, exchanged with deuterium). Formation of the **5a** was further confirmed on the basis of ^{13}C NMR spectroscopy and Mass spectrometry. In the ^{13}C NMR spectrum of **5a**, sharp signals were observed at 21.08 (CH_3), 82.17(spiro carbon), 120.8–147.86 (aromatic ring carbons), 161.4 (S–C═N), 163.7,168.2 (two C═O). The mass spectrum of **5a** showed molecular ion peak *m*/*z* at 373 [M^+] (32.4%) along with peaks at 345 (M^+-CO, 9.4%) and base peak at 264 (M^+-C_5H_3NS, 100%), 194 (13.2), 186 (31.1), 163 (28.5), 129 (63.7), 76 (50.4), 55 (19.2).

On the basis of spectral data, the products (**5a–k**) have been identified as cyclocondensed product **5** instead of addition product **6**.

3. Conclusion

In conclusion, we have developed an economical, safe and environmentally benign-solvent free synthesis, for spiro and other pyrido [3,2-*e*] thiazine derivatives. The use of microwave conditions is absolutely essential for this transformation, as conventional heating fails.

In all cases, a comparison of the reactions using either thermal or microwave heating under the same conditions shows clearly a specific (non-thermal) microwave effect.

4. Experimental

4.1. Equipment

Melting points were determined in open glass capillaries and are uncorrected. IR spectra were recorded on a Perkin–Elmer (Model 577) infra cord spectrophotometer using KBr pellets ^1H NMR spectra were recorded on model Jeol-FX-90Q, Bruker-DRX-300/DRX-200 using $CDCl_3$ as solvent at 89.95, 300.13 and 200.13 MHz and ^{13}C NMR spectra were recorded on model Bruker-DRX-300 using $CDCl_3$ as solvent at 300.13 MHz. TMS was used as internal reference for ^1H NMR and ^{13}C NMR spectra. Mass spectra of the representative compounds were recorded on Kratos-30 spectrometer. All compounds were homogenous on TLC in various solvent systems. The microwave induced reactions were carried out in an open borosil glass vessel under atmospheric pressure in BMO-700T domestic oven, equipped with magnetic stirrer, manufactured by BPL multimode Sanyo utilities and appliances Ltd. operating at 700 W generating 2450 MHz frequency.

4.1.1. General procedure for the synthesis of 3a–k. A neat equimolar (1 mmol) mixture of indole-2,3-dione/carbonyl compound (**1a–k**) and aromatic amine (**2a–k**) was irradiated inside a microwave oven at 640 W untill completion of the reaction (1–4 min). TLC, showing the formation of a single product, determined complete conversion. The intermediates **3** so obtained in reasonable purity were used as such for the next step without further purification.

The structure of the compounds were confirmed by IR and ^1H NMR spectroscopy and by comparison with authentic samples (**3a–k**) prepared according to literature methods.[26d,35a–f]

4.2. Investigation of the reaction of 3 and mercaptonicotinic acid (4)

4.2.1. Under conventional condition.

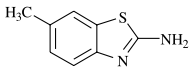
(a) An equimolar (1 mmol) mixture of **3** and mercaptonicotinic acid was refluxed 6–8 days in dry toluene (25 ml) with azeotropic removal of water/toluene (25 ml)+TFA (5 drops)/dioxane (25 ml)+$ZnCl_2$/glacial AcOH (25 ml). TLC indicated the unchanged reactants and no product formation.

(b) An equimolar (1 mmol) mixture of **3** and mercaptonicotinic acid (**4**) was heated on pre heated oil bath under identical conditions of time, temperature, pressure, vessel and medium as under microwaves. TLC indicated the unchanged reactants. Even extended heating for 8–24 h not lead to formation of any product.

4.2.2. Under microwave irradiation (one pot synthesis).

(a) *Using different solid supports such as montmorillonite KSF, neutral alumina, silica gel, p-toluene sulfonic acid (PTSA).* Equimolar quantities of 'in situ' synthesized **3a** (1 mmol, 236 mg) and **4** (1 mmol, 155 mg) were introduced into a beaker and dissolved in methanol (15 ml). Solid support (1 g) (montmorillonite KSF/neutral alumina/*p*-toluene sulfonic acid (PTSA)/silica gel) was then added and swirled for a while followed by removal of the solvent under gentle vacuum. The dry free flowing powder obtained was placed into a pyrex-glass opened vessel and irradiated in the microwave oven at a power output of 90% (640 W), for the specifies time and at the final temperature as indicated in the Table 2. When irradiation was stopped, the final temperature was measured by introducing a glass thermometer into the reaction mixture and homogenizing it, in order to obtain a temperature value representative of the whole mass. After completion of the reaction (monitored by TLC) the recyclable solid support was separated by filtration after eluting the product with methanol and excess solvent was evaporated on a rotary evaporator to give solid, which was recrystallized from methanol to give desired product.

Table 2. Cyclocondensation of arylimines with 2-mercaptonicotinic acid under microwave irradiation

Entry	Product No.	Carbonyl compounds	Amines	Temperature[a] (°C)	MW		Δ		Mp (°C)
					Time[b] (min.)	Yield[c] (%)	Time[d] (hrs.)	Yield (%)	
1	**5a**	Indole-2,3-dione	4-Methyl aniline	140	0.5+3	90	8	Nil	138
2	**5b**	Indole-2,3-dione	2-Trifluoromethyl aniline	138	2+4	92	7	Nil	172
3	**5c**	Indole-2,3-dione	4-Methoxy aniline	138	1+2.5	89	7	Nil	205
4	**5d**	Indole-2,3-dione	4-Chloro aniline	140	0.5+3	90	6	Nil	220
5	**5e**	Indole-2,3-dione	4-Fluoro aniline	142	1+3	92	8	Nil	192
6	**5f**	Cyclopentanone		138	4+8	87	14	Nil	175
7	**5g**	Cyclohexanone	4-Chloro aniline	140	3+7	85	24	Nil	248
8	**5h**	Benzaldehyde	2-Amino 6-methyl pyridine	132	4+6	87	22	Nil	202
9	**5i**	Benzaldehyde	4-Methyl aniline	142	3+7	85	26	Nil	172
10	**5j**	Acetophenone	2-Amino 6-methyl pyridine	145	5+7	89	22	Nil	215
11	**5k**	Acetophenone	4-Methyl aniline	138	3+8	86	23	Nil	245

[a] Final temperature is measured at the end of microwave irradiation by introducing a glass thermometer in the reaction mixture in the beaker.
[b] Compounds **5a**–**k** were synthesiszed in one pot and time is indicated for the condensation of 'in situ' generated arylimines in microwave oven e.g. **5a** 0.5+3 indicates first irradiation for 0.5 min give intermadiate **3a** (100% conversion, TLC) and then further irradiation after adding 2-mercaptonicotinic acid for 3 min.
[c] Isolated yield of purified compounds that exhibited physical and spectral properties in accordance with the assigned structure.
[d] Extended reaction time for reaction of thermally presynthesized arylimines under identical reaction conditions and temperature used under microwaves.

(b) *Neat reaction with few drops of DMF*. An equimolar (1 mmol) mixture of **3** and **4** with few drops of DMF, in an open tall beaker was irradiated inside microwave oven for an appropriate time (monitored by TLC). The solid mass obtained poured in to water to give crystalline product with no need of further purification (TLC) in most cases (**5f**–**k**), while in cases (**5a**–**e**) the solid was recrystallized by methanol to give pure product.

4.2.3. Compound 5a. Mp 138 °C; [Found: C, 67.7; H, 4.0; N, 11.0. $C_{21}H_{15}N_3O_2S$ requires C, 67.54; H, 4.02; N, 11.25%]; ν_{max} (KBr) 3320–3290 (br), 1720, 1710, 1625 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 9.26 (1H, br, N*H*, D_2O exchangeable), 7.93–7.20 (11H, m, Ar-*H* and pyridine *H*), 2.37 (3H, s, CH_3); δ_C (300 MHz, $CDCl_3$) 168.1, 161.4, 159.8, 142.1–109.4, 77.4, 22.4; *m/z* 373 (32, MH^+), 345 (10), 264 (100), 194 (13), 186 (31), 163 (29), 129 (64), 76 (50), 55 (19%).

4.2.4. Compound 5b. Mp 172 °C; [Found: C, 58.8; H, 2.7; N, 9.7; S, 7.43. $C_{21}H_{12}F_3N_3O_2S$ requires C, 59.01; H, 2.81; N, 9.83; S, 7.49%]; ν_{max} (KBr) 3330–3290 (br), 1715, 1705, 1620 cm^{-1}; δ_H (90 MHz, $CDCl_3$) 9.08 (1H, br, N*H*, D_2O exchangeable), 8.75–7.01 (11H, m, Ar-*H* and pyridine *H*); δ_C (22.4 MHz, $CDCl_3$) 168.2, 163.5, 162.4, 140.86–119.2, 82.12, 64.13; *m/z* 427 (20, MH^+), 399 (16), 290 (100), 196 (33), 184 (12), 165 (19), 128 (44), 74 (41%).

4.2.5. Compound 5c. Mp 205 °C; [Found: C, 64.8; H, 3.8; N, 10.8; S, 8.2. $C_{21}H_{15}N_3O_3S$ requires C, 64.77; H, 3.85; N, 10.79; S, 8.22%]; ν_{max} (KBr) 3330–3300 (br), 1730, 1720, 1620, 1110–1050 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 9.4 (1H, br, N*H*, D_2O exchangeable) 7.40–7.03 (11H, m, Ar-*H* and pyridine *H*), 3.84 (3H, s, OCH_3); δ_C (300 MHz, $CDCl_3$)170.2, 164.5, 161.4, 157.6, 145.8–122.0, 81.1, 56.2; *m/z* 389(11, MH^+), 361 (12), 252 (100), 178 (22), 126 (53), 72 (41%).

4.2.6. Compound 5d. Mp 220 °C; [Found: C, 61.2; H, 3.1; N, 10.7; S, 8.1. $C_{20}H_{12}ClN_3O_2S$ requires C, 61.06; H, 3.05; N, 10.68; S, 8.11%]; ν_{max} (KBr) 3340–3310 (br), 1720, 1710, 1625, 770 cm^{-1}; δ_H (90 MHz, $CDCl_3$) 9.04 (1H, br, N*H*, D_2O exchangeable), 8.46–7.14 (11H, m, Ar-*H* and pyridine *H*); δ_C (300 MHz, $CDCl_3$) 168.3, 163.8, 161.8, 140.5–119.2, 81.8; *m/z* 393(15, MH^+), 365 (25), 256 (100), 196 (22), 186 (15), 156 (19%).

4.2.7. Compound 5e. Mp 192 °C; [Found: C, 63.4; H, 3.1; N, 11.0; S, 8.5. $C_{20}H_{12}FN_3O_2S$ requires C, 63.65; H, 3.18; N, 11.13; S, 8.48%]; ν_{max} (KBr) 3330–3290 (br), 1715, 1705, 1620, 720; δ_H (90 MHz, $CDCl_3$) 9.04 (1H, br, N*H*, D_2O exchangeable), 8.56–7.10 (11H,, m, Ar-*H* and pyridine *H*); δ_C (300 MHz, $CDCl_3$) 167.2, 164.7, 161.5, 147.8–121.0, 117.2, 82.1; *m/z* 377(14, MH^+), 349 (14), 240 (100), 192 (43), 74 (51%).

4.2.8. Compound 5f. Mp 175 °C; [Found: C, 62.2; H, 4.6; N, 11.5; S, 17.4. $C_{19}H_{17}N_3O_2S_2$ requires C, 62.11; H, 4.63; N, 11.44; S, 17.43%]; ν_{max} (KBr) 3030–3010, 2920–2850, 1720, 1620 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 7.41–7.03 (6H, m, Ar-*H* and pyridine *H*), 2.72 (4H, m, CH_2–CH_2), 2.29 (3H, s, CH_3), 1.47 (4H, m, CH_2–CH_2); δ_C (300 MHz, $CDCl_3$) 172.5, 162.7, 145.2–120.0, 72.1, 36.3, 20.9, 17.5; *m/z* 367 (40, MH^+), 339 (24), 258 (100), 217 (45), 205 (20), 181 (20), 138 (60), 125 (14%).

4.2.9. Compound 5g. Mp 248 °C; [Found: C, 62.7; H, 4.78; N, 8.0; S, 9.2. $C_{18}H_{17}$ ClN_2OS requires C, 62.59; H, 4.92; N, 8.11; S, 9.27%]; ν_{max} (KBr) 2930–2840, 1720, 1610, 770 cm^{-1}; δ_H (90 MHz, $CDCl_3$) 8.46–7.15 (7H, m, Ar-*H* and pyridine *H*), 1.87 (4H, m, CH_2–CH_2), 1.29 (6H, m, (CH_2)$_3$; *m/z* 347 (4, MH^++2), 345 (12.0), 317 (100), 208 (45), 178 (35), 149 (7), 135 (60), 121 (14%).

4.2.10. Compound 5h. Mp 202 °C; [Found: C, 68.2; H, 4.5; N, 12.5; S, 9.6. $C_{19}H_{15}N_3OS$ requires C, 68.45; H, 4.50; N,

12.60; S, 9.60%]; ν_{max} (KBr) 2840–2810, 1710, 1620 cm^{-1}; δ_H (90 MHz, $CDCl_3$) 8.35–7.10 (11H, m, Ar-*H* and pyridine *H*), 5.64 (1H, s, C*H*), 2.28 (3H, m, CH_3); δ_C (22.4 MHz, $CDCl_3$) 163.6, 159.2, 140.8–118.5, 70.5, 21.3; *m/z* 333 (14, MH^+), 305 (36), 224 (100), 165 (13%).

4.2.11. Compound 5i. Mp 172 °C; [Found: C, 72.1; H, 4.7; N, 8.3; S, 9.5. $C_{20}H_{16}N_2OS$ requires C, 72.26; H, 4.81; N, 8.43; S, 9.63%]; ν_{max} (KBr) 2860–2820, 1715, 1615 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.10–7.26 (12H, m, Ar-*H* and pyridine *H*), 5.91 (1H, s, C*H*), 2.73 (3H, s, CH_3); δ_C (300 MHz, $CDCl_3$) 164.2, 158.5, 140.5–118.2, 71.2, 20.5; *m/z* (%) 332 (25, MH^+), 304 (100), 195 (45), 181(14), 139 (60), 118 (14), 98 (32%).

4.2.12. Compound 5j. Mp 215 °C; [Found: C, 69.3; H, 4.9; N, 12.1; S, 9.2. $C_{20}H_{17}N_3OS$ requires C, 69.14; H, 4.89; N, 12.09; S, 9.21%]; ν_{max} (KBr) 2840–2820, 1720, 1620 cm^{-1}; δ_H (90 MHz, $CDCl_3$) 8.52–7.18 (12H, m, Ar-*H* and pyridine *H*), 2.32 (3H, s, CH_3), 1.87(3H, s, CH_3); δ_C (300 MHz, $CDCl_3$) 164.5, 162.4, 148.2–118.2, 68.2, 27.5, 20.9; *m/z* (%) 347 (15, MH^+), 319 (14), 234 (100), 184 (20%).

4.2.13. Compound 5k. Mp 245 °C; [Found: C, 72.6; H, 5.1; N, 8.1; S, 9.2. $C_{21}H_{18}N_2OS$ requires C, 72.73; H, 5.19; N, 8.08; S, 9.23%]; ν_{max} (KBr) 2850–2820, 1720, 1620 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 7.73–6.71 (11H, m, Ar-*H* and pyridine *H*), 2.32 (3H, s, CH_3), 1.95 (3H, s, CH_3); δ_C (300 MHz, $CDCl_3$) 168.9, 164.6, 145.6–117.8, 66.2, 28.2, 21.5; *m/z* (%) 346 (12, MH^+), 318 (10), 237 (100), 183 (50%).

Acknowledgements

Financial assistance from CSIR 01 (1907)/03 is gratefully acknowledged. We are also thankful to RSIC, CDRI, Lucknow, for the spectral analyses.

References and notes

1. Joshi, K. C.; Jain, R.; Chand, P. *Heterocycles* **1995**, *23*, 957.
2. Joshi, K. C.; Jain, R. *J. Indian Chem. Soc.* **1999**, *76*, 515.
3. Edmondson, S.; Danishefsky, J. S.; Sepp-Lorenzino, L.; Rosen, N. *J. Am. Chem. Soc.* **1999**, *121*(10), 2147.
4. Khjan, M. H.; Tewari, S.; Begum, K.; Nizamudidin, *Indian J. Chem.* **1998**, *37*, 1075.
5. Kono, M.; Nakai, T.; Hamanka, K. Eur. Pat. 115, 28892, 1991. (b) Ohseng, E.; Fujioka, T.; Harada, H.; Nakumura, M.; Maeda, R. *Chem. Pharm. Bull.* **1989**, *37*, 1268. *Chem. Abstr.* **1990**, *112*, 178508r.
6. Reifscheider, W.; Ershadi, B. B.; Dripps, J. E.; Barron, J. B. US Patent 5075293, 1991; *Chem. Abstr.* **1992**, *116*, 129249f.
7. Goto, J.; Sakane, K. Jpn Kokai 121, 6997, 1989; *Chem. Abstr.* **1990**, *112*, 2165541f.
8. Saito, K.; Nakagawa, S.; Yashioka, T.; Hozaka, H. Jpn. Kokai 2160784, 1990; *Chem Abstr.* **1990**, *113*, 206727c.
9. Cavrini, V.; Gatti, R.; Giovanninetti, G.; Roveni, P.; Franchi, L.; Landini, M. P. *Ed. Sci.* **1981**, *36*, 140.
10. Hanefild, W.; Roethlisberger, R. Ger. Offon. 2803783, 1989; *Chem. Abstr.* **1990**, *112*, 55898k.
11. Doi, N. *Ten kan-Kenkyu* **1990**, *8*, 167. *Chem. Abstr.* **1991**, *114*, 114947s.
12. Surrey, A. R.; Webb, W. G.; Gesler, R. M. *J. Am. Chem. Soc.* **1958**, *80*, 3469.
13. Temple, C.; Wheeler, G. P.; Comber, R. N.; Elliot, R. D.; Montgomery, J. A. *J. Med. Chem.* **1983**, *26*(11), 1641.
14. El-Subbagh, H. I.; Abadi, A.; Al-Khawad, I. E.; Al-Pashood, K. A. *Arch. Pharm.* **1999**, *332*(1), 19.
15. Malinka, W.; Kaczmarz, M.; Filipek, B.; Sepa, J.; Gold, B. *Farmco* **2002**, *57*(9), 737.
16. Malinka, W.; Pyng, S.; Sicklucka-Dziuba, M.; Rajtar, G.; Glowniak, A.; Kleinrok, Z. *Farmco* **1994**, *49*, 783.
17. Maeda, T.; Koide, T.; Nakai, H.; Yozota, M.; Araki, T.; Murakai, T.; Nohare, C. Jpn Kokai Tokkyo Koho JP 07, 17, 1995, 980; *Chem. Abstr.* **1995**, *122*, 265167d.
18. Sandersan, P. E.; Cutrona, K. PCT Int. Appl. WO 18, 1998, 762; *Chem. Abstr.* **1999**, *132*, 251163u.
19. Tomaji, N.; Yoshiyuki, M.; Ishikawa, H. PCT Int. Appl WO 00 94, 22, 1996, 875; *Chem. Abstr.* **1995**, *122*, 214086u.
20. Malinka, W.; Zawisza, T.; Gieldenowski, J. Pol. PT PL 139, 1987, 585, Appl. 253, 057, 1985; *Chem Abstr.* **1991**, *114*, 102026n.
21. Malinka, W.; Zawisza, T.; Millimowski, M. Pol. PT PL 143, 1988, 077, Appl. 257, 400, 1986; *Chem. Abstr.* **1989**, *111*, 78013n.
22. Klausener, A.; Fengler, G.; Buysch, H. J.; Pelster, B. Ger. Offen DE 3, 1987, 545; *Chem. Abstr.* **1987**, *107*, 154346n.
23. Joshi, H. D.; Upadhyay, P. S.; Baxi, A. *J. Indian J. Chem.* **2000**, *39B*, 967.
24. Al-Khamees, H. A.; Bayomi, S. M.; Kandil, H. A.; El-Thahir, K. *Eur. J. Med. Chem.* **1990**, *25*, 103.
25. (a) Couture, A.; Grandclaudon, P.; Huguerre, E. *Tetrahedron* **1989**, *45*(13), 4153. (b) Dunn, A. D.; Norrie, R. *Z. Chem.* **1988**, *28*(6), 212.
26. (a) Dandia, A.; Singh, R.; Arya, K. *OPPI* **2003**, *35*(20), 387. (b) Dandia, A.; Saha, M.; Rani, B. *J. Chem. Res. (S)* **1998**, 360. *J. Chem. Res. (M)* **1998**, *1425*. (c) Dandia, A.; Saha, M.; Taneja, H. *Phosphorus, Sulfur Silicon* **1998**, *139*, 77. (d) Dandia, A.; Saha, M.; Shivpuri, A. *Indian J. Chem. Tech.* **1997**, *4*, 201. (e) Joshi, K. C.; Dandia, A.; Sanan, S. *J. Fluorine Chem.* **1989**, *44*, 59–72. (f) Joshi, K. C.; Dandia, A.; Ahmed, N. *Heterocycles* **1986**, *24*, 2479.
27. (a) Cindic, M.; Struken, N.; Kaifer, T.; Giester, G.; Karmenar, B. *Zeitschrift Fuer Anorganische und Allgemeine Chemie* **2001**, *627*(12), 2604. (b) Abu-Baker, M. S.; Rageh, H. M.; Hashen, E. Y.; Moustaga, M. H. *Bull. Fac. Sci.* **1993**, *22*(10), 77. (c) Wright, S. W.; Corbett, R. L. *Org. Prep. Proced. Int.* **1993**, *25*(2), 247. (d) Luo, Y. L.; Yang, Z. X.; Peng, S. X. *Yaoxue Xuebao* **1990**, *25*(5), 374. (e) Matsubara, E.; Miura, T. Jpn. Kokai Tokkyo Koho JP 05, 105,1993, 663; *Chem. Abstr.* **1993**, *119*, 82816c.
28. Hermken, S. P.; Ottenheijm, H.; Ress, D. *Tetrahedron* **1997**, *53*, 5643.
29. (a) Jeselnik, M.; Varma, R. S.; Polanc, S.; Kocevar, M. *Green Chem.* **2002**, *4*, 35. (b) Lidstrom, P.; Tierney, J.; Wathey, B.; Westman, J. *Tetrahedron* **2001**, *57*, 9225. (c) Loupy, A.; Perreux, L. *Tetrahedron* **2001**, *57*, 9199. (d) Hoz, A. De L. A.; D.-Ortis, A.; Moreno, A.; Langa, F. M. *Eur. J. Org. Chem.* **2000**, 3659. (e) Elander, N.; Jones, J. R.; Lu, S.-Y.; Stone-Elender, S. *Chem. Soc. Rev.* **2000**, *29*, 239. (f) Deshayes, S.; Liagre, M.; Loupy, A.; Luche, J.; Petit, A. *Tetrahedron* **1999**, *55*, 10851. (g) Varma, R. S. *Green Chem.* **1999**, *1*(1999), 43.
30. (a) Boruah, B.; Boruah, J.; Prajapati, D.; Sandhu, J. C.; Gosh,

A. C. *Tetrahedron Lett.* **1996**, *37*, 4203. (b) Garringnes, B.; Laporte, C.; Laurnte, R.; Laporterie, A.; Dubac, J. *Leibigs Ann.* **1996**, 739.

31. (a) Dandia, A.; Sati, M.; Arya, K.; Loupy, A. *Heterocycles* **2003**, *60*(3), 563. (b) Dandia, A.; Sati, M.; Loupy, A. *Green Chem.* **2002**(4), 599. (c) Dandia, A.; Singh, R.; Saha, M.; Shivpuri, A. *Die Pharm.* **2002**, *57*, 602. (d) Dandia, A.; Sachdeva, H.; Singh, R. *Synth. Commun.* **2001**, *31*, 1879. (e) Dandia, A.; Sachdeva, H.; Singh, R. *J. Chem. Res. (S)* **2000**, 272. (f) Dandia, A.; Singh, R.; Sachdeva, H.; Arya, K. *J. Fluorine Chem.* **2000**, *111*, 67. (g) Dandia, A.; Taneja, H.; Gupta, R.; Paul, S. *Synth. Commun.* **1999**, *29*, 2323.
32. (a) Marquez, H.; Plutin, A.; Rodriguez, Y.; Perez, E.; Loupy, A. *Synth. Commun.* **2000**, *30*, 1067. (b) Suarez, M.; Loupy, A.; Salfran, E.; Moran, L.; Rolando, E. *Heterocycles* **1999**, *51*, 21. (c) Perez, R.; Perez, E.; Suarez, M.; Gonzalez, L.; Loupy, A.; Jimeno, M. L.; Ochoa, C. *Org. Prep. Proced. Int.* **1997**, *29*, 671. (d) Suarez, M.; Loupy, A.; Perez, E.; Moran, L.; Gerona, G.; Morales, A.; Autie, M. *Heterocycl. Commun.* **1996**, *2*, 275.
33. (a) Gydye, R. N.; Wei, J. B. *Can. J. Chem.* **1998**, *76*, 525. (b) Langa, F.; De la Cruz, P.; Dela Hoz, A.; Diaz-Ortiz, A.; Diez-Barra, E. *Contemp. Org. Synth.* **1997**, *4*, 373. (c) Prrreux, L.; Loupy, A. *Tetrahedron* **2001**, *57*, 9199. (d) Gabriel, C.; Gabriel, S.; Grant, E. H.; Halsted, B. S.; Mingos, D. M. P. *Chem. Soc. Rev.* **1998**, *7*, 213.
34. (a) Syassi, B.; Bougrin, K.; Soufiaoui, M. *Tetrahedron Lett.* **1997**, *38*, 8855. (b) Perez, E. R.; Marrero, A. L.; Perez, R.; Autie, M. A. *Tetrahedron Lett.* **1995**, *36*, 1779. (c) Varma, R. S.; Varma, M.; Chatterjee, A. K. *J. Chem. Soc. Perkin Trans. 1* **1993**, 999. (d) Ben Alloum, A.; Labiad, B.; Villemin, D. *J. Chem. Soc., Chem. Commun.* **1989**, 607.
35. (a) Hassan, K. M.; El-Shafei, A. K.; El-Shafei, H. S. *Z. Naturforsch.* **1978**, *33b*, 1515. (b) Jacini, G. *Gazz. Chim. Ital.* **1943**, *73*, 85. (c) Knoevengal, E. *J. Prakt. Chem.* **1914**, *2*(89), 48. (d) Joshi, K. C.; Patni, R.; Chand, P. *Heterocycles* **1981**, *16*, 1555. (e) Crippa, G. B.; Pietra, S. *Gazz. Chim. Ital.* **1951**, *81*, 195. (f) Piscopo, E.; Diurno, M. V.; Antonicci, M.; Imperadrice, F.; Caliendov, V.; Cataldi, M. T. *Boll. Soc. Ital. Biol. Per.* **1985**, *61*(19), 1327. *Chem. Abstr.* **1984**, *104*, 65713w.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron 60 (2004) 5259–5264
Tetrahedron

Synthesis and liquid crystal properties of a novel family of oligothiophene derivatives

Ping Liu,[a,*] Yamin Zhang,[a] Guiju Feng,[a] Jianhua Hu,[a] Xiaoping Zhou,[a] Qizhong Zhao,[a] Yunhua Xu,[b] Zhen Tong[a] and Wenji Deng[b]

[a]*Polymer Structure and Modification, Research Institute of Materials Science, South China University of Technology, Guangzhou 510640, China*
[b]*Department of Applied Physics, South China University of Technology, Guangzhou 510640, China*

Received 10 September 2003; revised 29 March 2004; accepted 5 April 2004

Abstract—In order to develop novel oligothiophene-based liquid crystals capable of hydrogen bonding, new terthiophene derivatives containing an alkylamide group, *N*,*N*′-dialkyl-5,5″-dichloro-2,2′:5′,2″-terthiophene-4,4″-dicarboxamide (DNC$_n$DCl3T, n=8, 18), *N*,*N*′-dialkyl-5,5″-dibromo-2,2′:5′,2″-terthiophene-4,4″-dicarboxamide (DNC$_n$DBr3T, n=5, 8, 16, 18), or *N*,*N*′-dialkyl-5,5″-diiodo-2,2′:5′,2″-terthiophene-4,4″-dica-rboxamide (DNC$_n$DI3T, n=8, 18), were designed and synthesized, and their thermal behaviour was examined. It was found that DNC$_{18}$DCl3T, DNC$_{18}$DI3T and DNC$_n$DBr3T (n=8, 16, 18) form a smectic A phase and that the alkyl chain length greatly affects liquid crystal phase formation. The absence of liquid crystallinity in the corresponding ester derivatives suggests that intermolecular hydrogen bonding also plays a role in the formation of a liquid crystal phases in the DNC$_n$DBr3T system.

1. Introduction

Oligothiophenes with well defined structures have recently received a great deal of attention not only as model compounds for conducting polythiophenes, but also as a new class of functional π-electron systems. A variety of oligothiophenes have been synthesized[1] and their molecular and crystal structures,[2,3] self ordering,[4,5] electrochemical,[6,7] photophysical,[8,9] optical,[10,11] and electrical properties,[12,13] have all been studied. In addition, their potential application in field-effect transistors,[14] photovoltaic devices,[15] and organic electroluminescent devices[16] has been investigated.

Oligothiophenes are crystalline compounds because of their planar molecular structures and both vacuum-evaporated and spin-coated films of oligothiophenes have been reported to be polycrystalline. Since the properties and functionality of a material are greatly affected by its morphology, it is of both fundamental and technological interest to exert control over the morphology of oligothiophenes in the design of novel functional organic materials.

Recently, several reports regarding the liquid crystalline properties of oligothiophene derivatives have appeared.[17–20] It has been reported that terthiophenes substituted with long alkyl or alkanoyl groups at the terminal α- and α″-positions, for example, 5-alkyloxycarbonyl-5″-alkyl-2,2′:5′,2″-terthiophenes and 5,5″-dialkyl-2,2′:5′,2″-terthiophenes exhibit a smectic liquid crystalline phase. Both 5-substituted and 5‴-disubstituted quarterthiophenes have also been reported to exhibit smectic and nematic liquid crystalline phases. However, examples of oligothiophene-based liquid crystals are still scarce.

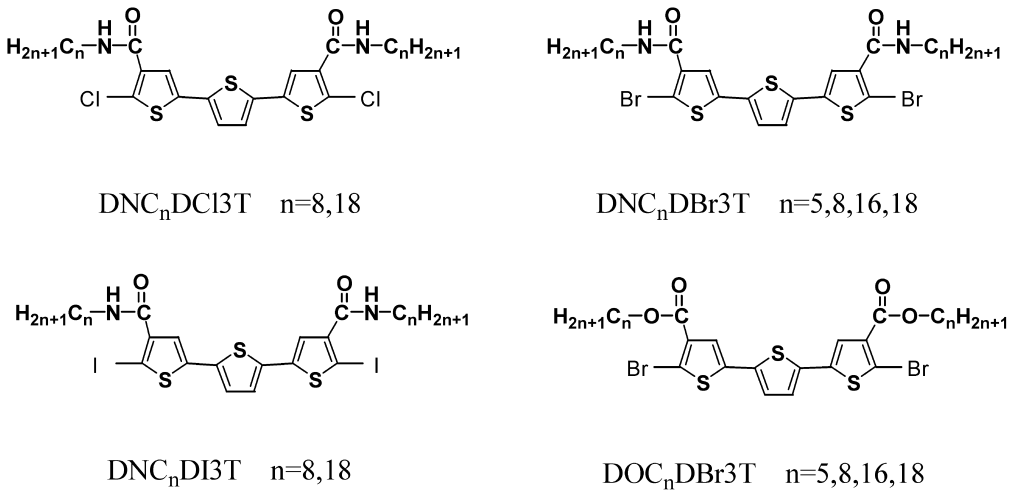
This paper reports on the synthesis of new compounds containing a terthiophene moiety, *N*,*N*′-dialkyl-5,5″-dichloro-2,2′:5′,2″-terthiophene-4,4″-dicarboxamide (DNC$_n$DCl3T, n=8, 18), *N*,*N*′-dialkyl-5,5″-dibromo-2,2′:5′,2″-terthiophene-4,4″-dicarboxamide (DNC$_n$DBr3T, n=5, 8, 16, 18), or *N*,*N*′-dialkyl-5,5″-diiodo-2,2′:5′,2″-terthiophene-4,4″-dicarboxamide (DNC$_n$DI3T, n=8, 18) (Scheme 1), and their liquid crystal properties. The relationship between molecular structure and liquid crystalline behaviour is discussed.

2. Results and discussion

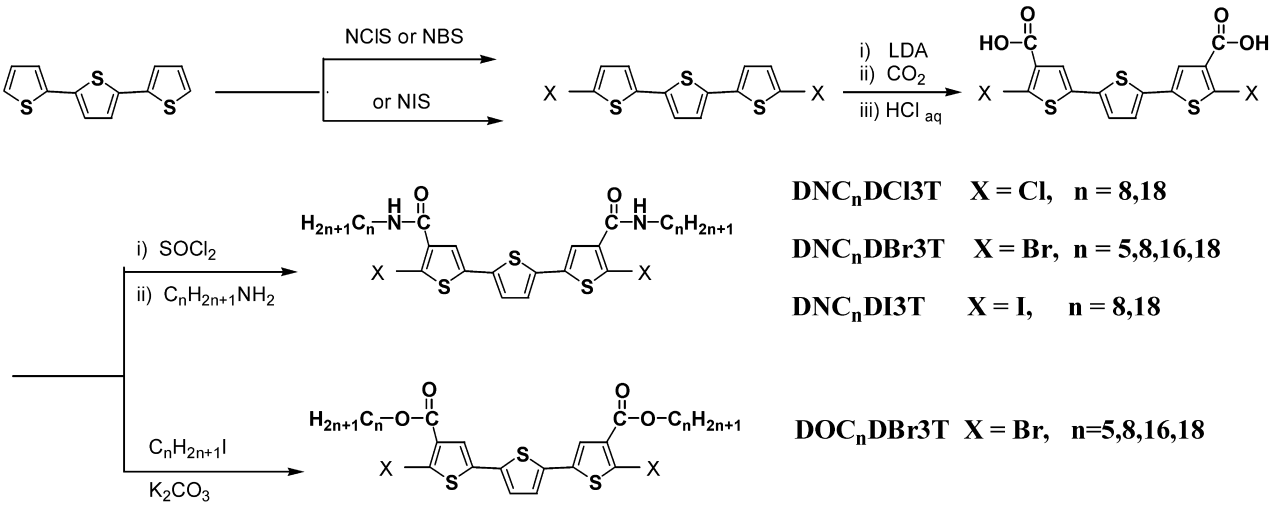
Novel families of oligothiophene derivatives containing an alkylamide group at the β- and β′-position of 2,2′:5′,2″-terthiophene, DNC$_n$DCl3T (n=8, 18), DNC$_n$DBr3T (n=5, 8, 16, 18) and DNC$_n$DI3T (n=8, 18), were synthesized according to the procedures shown in the Scheme 2. All

Keywords: Oligothiophene derivatives; Liquid crystal; Intermolecular hydrogen bonding.
* Corresponding author. Tel.: +86-20-87111686;
e-mail address: mcpliu@scut.edu.cn

0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.04.029



Scheme 1.



Scheme 2.

compounds were characterized by IR and ^{1}H NMR spectroscopy, mass spectrometry, and elemental analysis.

DSC and polarized light microscopy performed on DNC_{18}DCl3T, DNC_nDBr3T (n=8, 16, 18) and DNC_{18}DI3T revealed that these compounds, containing two alkylamide groups at the β- and β′-position of 3T, formed a smectic A (SmA) phase. Figure 1 shows the polarizing micrograph of the DNC_{18}DCl3T, DNC_{18}DBr3T and DNC_{18}DI3T SmA phase formed from the isotropic liquid in the cooling process. A focal-conic fan texture typical of a SmA phase was clearly observed.

The formation of a SmA phase by DNC_{18}DCl3T, DNC_{18}DBr3T and DNC_{18}DI3T was also evidenced by DSC. Figure 2 shows the DSC curves for DNC_{18}DBr3T. When the crystalline sample of DNC_{18}DBr3T was heated, an endothermic peak was observed at 110 °C to give a smectic A phase. Upon further heating, the onset of the transition from a SmA phase into the isotropic liquid was observed at 121 °C. When the isotropic liquid was cooled, the onset of the transition into a SmA phase was observed at approximately 123 °C. The SmA phase crystallized at 107 °C. The trace was reproducible.

The effects of the alkyl chain length in the alkylamido group on liquid crystalline behaviour were investigated. Like DNC_{18}DBr3T, DNC_{16}DBr3T was found to form a SmA phase. In addition, DNC_{16}DBr3T exhibited crystal

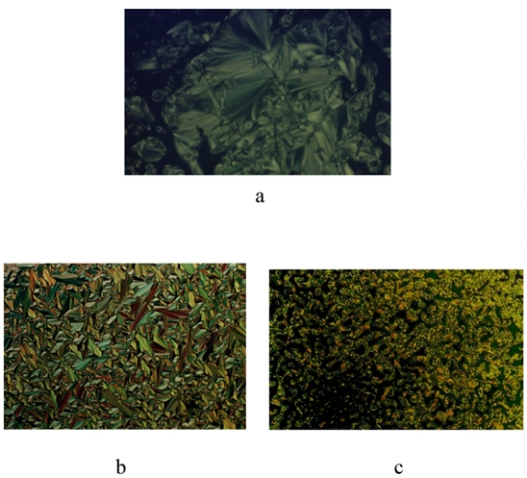


Figure 1. Photomicrograph of the texture of the SmA phase of DNC_{18}DCl3T, DNC_{18}DBr3T and DNC_{18}DI3T obtained on cooling the melt. (a): DNC_{18}DCl3T; (b): DNC_{18}DBr3T; (c): DNC_{18}DI3T (magnification×200).

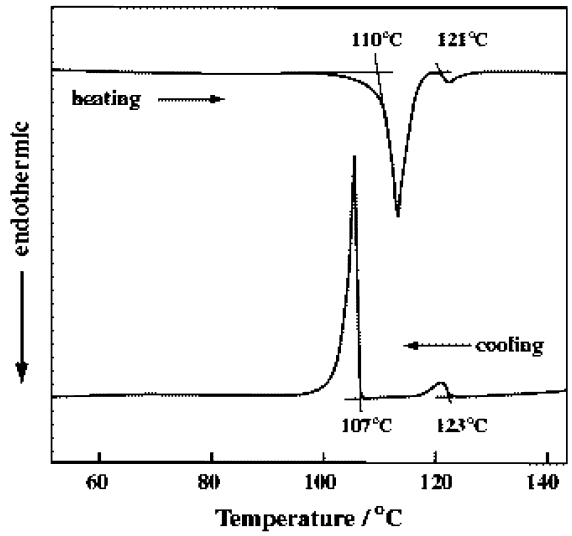


Figure 2. DSC curves for $DNC_{18}DBr3T$.

polymorphism, showing two different crystal forms. In contrast, with DNC_8DBr3T, the formation of a liquid crystalline phase did not occur on heating. However, a SmA phase appeared on cooling in the temperature range from about 126–109 °C, as observed by polarized light microscopy.

It is noteworthy that DNC_8DCl3T DNC_5DBr3T, and DNC_8DI3T did not form liquid crystal phase, and showed only the phase transition between the crystal and the isotropic liquid, as revealed by DSC and polarized light microscopy. The absence of a liquid crystalline phase for DNC_8DCl3T, DNC_5DBr3T and DNC_8DI3T is thought to be because there are strong hydrogen bonding interactions in these molecules, and they thus favor direct crystallization from the isotropic liquid.

In order to gain information on the importance of the amide group in the formation of liquid crystal phases, the thermal behavior of the corresponding ester compounds, DOC_n-DBr3T (n=5, 8, 16, 18) were examined. It was found that DOC_nDBr3T (n=5, 8, 16, 18) did not exhibit a liquid crystalline phase, showing only melting and crystallization behavior upon heating and cooling. These results indicate that the alkylamide groups in the β- and β′-position of 3T play an important role in the formation of liquid crystal phases in the DNC_nDBr3T system. Table 1 summarizes the transition temperatures of $DNC_{18}DCl3T$, DNC_nDBr3T (n=8, 16, 18) and $DNC_{18}DI3T$, together with the enthalpy changes (ΔH) associated with the phase transitions.

Table 1. Transition temperatures and enthalpy changes of $DNC_{18}DCl3T$, DNC_nDBr3T (n=8, 16, 18) and $DNC_{18}DI3T$

Compound	Transition temperature (°C); enthalpy change (in parentheses) (kJ mol^{-1})				
$DNC_{18}DCl3T$[a]	K	114(43)	SmA	121(3.3)	I
DNC_8DBr3T[b]	K	109(—)	SmA	126(—)	I
$DNC_{16}DBr3T$[c]	K	110(46)	SmA	127(3.6)	I
$DNC_{18}DBr3T$[a]	K	107(56)	SmA	123(3.7)	I
$DNC_{18}DI3T$[a]	K	130(62)	SmA	143(4.3)	I

K: crystal, S_A: smectic A liquid crystal, I: isotropic liquid.
[a] Transition temperatures and enthalpy changes were determined by DSC upon cooling.
[b] Transition temperatures and enthalpy changes were determined by polarized light microscopy.
[c] Transition temperatures and enthalpy changes were determined by DSC upon heating.

It is suggested that intermolecular hydrogen bonding between the amido groups is involved in liquid crystal phase formation. The presence of intermolecular hydrogen bonding was evidence by infrared (IR) absorption spectroscopy. Figure 3 shows the IR absorption spectra of $DNC_{18}DBr3T$ in its crystalline and liquid crystalline phases in the wavelength region of the N–H stretching vibration mode. The crystalline sample showed only a broad absorption at around 3300 cm^{-1}, which is attributable to the stretching vibration of hydrogen-bonded N–H groups. The liquid crystalline sample showed both the hydrogen bonded absorption band and a sharp absorption peak at 3419 cm^{-1} that is attributable to the free N–H groups. The spectrum of the isotropic liquid was almost identical to that of the liquid crystal. The changes in the IR absorption spectra between the crystal and the liquid crystal phases during both heating and cooling were reproducible. This indicates that the molecules in the SmA phase only partly forms intermolecular hydrogen bonds, whereas in the crystal phase all the molecules are fixed tightly by the intermolecular hydrogen bonding. Partial intermolecular hydrogen bonding interactions may play a role in the formation of liquid crystal phases in the DNC_nDBr3T system.

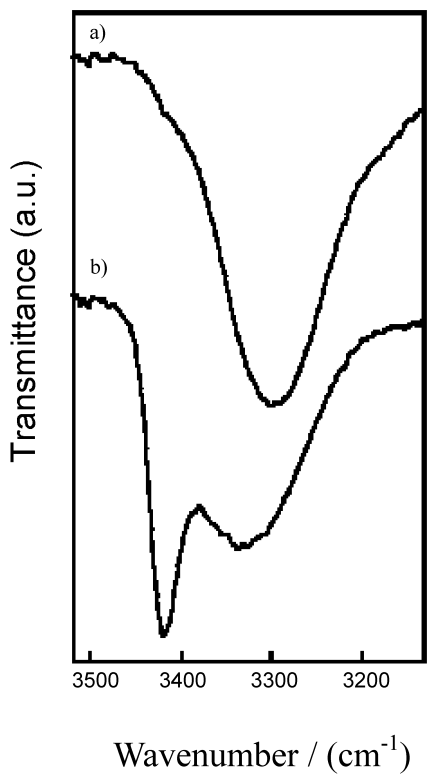


Figure 3. Infrared absorption spectra of $DNC_{18}DBr3T$; (a) crystal at 100 °C; (b) SmA phase at 120 °C formed on heating the crystal.

3. Conclusion

A novel family of terthiophene derivatives containing an alkylamide group, DNC_nDCl3T, (n=8, 18), DNC_nDBr3T, (n=5, 8, 16, 18) or DNC_nDI3T, (n=8, 18), were designed and synthesized. $DNC_{18}DCl3T$, DNC_nDBr3T, (n=8, 16, 18) and $DNC_{18}DI3T$, were found to form a SmA phase, thereby constituting a new class of oligothiophene-based liquid crystals. It was shown that the alkyl chain length greatly affected liquid crystal phase formation. The absence of liquid crystallinity for DOC_nDBr3T and changes in the IR spectra suggest that the alkylamide group is involved in liquid crystal phase formation in the DNC_nDBr3T.

4. Experimental

4.1. Synthesis

2-Bromothiophene,2,5-dibromothiophene,[1,3-bis-(diphenyphosphino) propane] nickel(II) chloride ($NiCl_2$-(dppp), magnesium, diisopropylamine, butyllithium (hexane solution), thionyl chloride, triethylamine, monoalkylamines ($C_nH_{2n+1}NH_2$, n=5, 8, 16, 18), and 1-iodoalkanes ($C_nH_{2n+1}I$, n=5, 8, 16, 18) were commercially available and used without further purification. 2,2′:5′,2″-Terthiophene (3T) was prepared by the Grignard coupling reaction of 2,5-dibromothiophene with 2-bromomagnesiumthiophene in ether at refluxing. Synthetic procedures are illustrated in the Scheme 2.

4.1.1. Synthesis of *N*,*N*′-dialkyl-5,5″-dichloro-2,2′:5′,2″-terthiophene-4,4″-dicarboxamide (DNC_nDCl3T, n=8, 18). 5,5″-Dichloro-2,2′:5′,2″-terthiophene (DCl3T) was prepared by the reaction of 3T (3.0 g, 12.1 mmol) with *N*-chlorosuccinimide (3.2 g, 24.2 mmol) in chloroform (200 mL) at room temperature for 5 h and purified by silica gel column chromatography using chloroform as eluent, followed by recrystallization from a benzene/hexane mixture (1:2 v/v).

A hexane solution of butyllithium was added to a THF solution of diisopropylamine at −78 °C under a nitrogen atmosphere to give lithium diisopropylamine (LDA). DCl3T (3.0 g, 9.46 mmol), was added to a THF solution (50 mL) of LDA (37.84 mmol) at −78 °C and the solution was stirred for 1 h. Excess dry ice was added to the solution and the resulting mixture was stirred for 2 h. The solution was allowed to warm to room temperature and aqueous hydrochloric acid was added. After stirring for 5 h, the resulting solid was collected and washed with hot acetonitrile to give 5,5″-dichloro-2,2′:5′,2″-terthiophene-4,4″-dicarboxylic acid (yield 65% based on DCl3T).

A dichloromethane solution (100 mL) of 5,5″-dichloro-2,2′:5′,2″-terthiophene-4,4″-dicarboxylic acid (1.0 g, 2.47 mmol) and $SOCl_2$ (0.59 g, 4.94 mmol) was heated at reflux for 8 h. After the solvent was removed under reduced pressure, the resulting solid was added to a THF solution (100 mL) of octylamine (0.64 g, 4.94 mmol) in the presence of triethylamine (2.5 mL) at 0 °C and the solution stirred for 5 h at 0 °C. The solvent was removed under reduced pressure to give DNC_8DCl3T. The product was purified by silica gel column chromatography using chloroform as the eluent (yield 62% based on 5,5″-dichloro-2,2′:5′,2″-terthiophene-4,4″-dicarboxylic acid). ^{1}H NMR (400 MHz, THF-d_8): δ (ppm)=7.34 (s, 2H, ArH), 7.30 (t, 2H, NH), 7.29 (s, 2H, ArH), 3.36 (dt, 4H, octyl α-CH_2), 1.60 (tt, 4H, octyl β-CH_2), 1.47–1.22 (m, 20H, octyl CH_2), 0.88 (t, 6H, CH_3). IR (KBr, cm^{-1}): 3301 (ν_{N-H}), 2956, 2922, 2855 (ν_{C-H}), 1623 ($\nu_{C=O}$). MS(EI): m/z 628 (M^+). Elemental analysis: found C 57.49, H 6.40, N 4.38%. Calcd for $C_{30}H_{40}N_2S_3O_2Cl_2$ C 57.40, H 6.42, N 4.47%.

The $DNC_{18}DCl3T$ was synthesized using analogous procedures with stearylamine (yield 55%). ^{1}H NMR (400 MHz, THF-d_8): δ (ppm)=7.33 (s, 2H, ArH), 7.32 (t, 2H, NH), 7.26 (s, 2H, ArH), 3.39 (dt, 4H, stearyl α-CH_2), 1.61 (tt, 4H, stearyl β-CH_2), 1.45–1.23 (m, 60H, stearyl CH_2), 0.88 (t, 6H, CH_3). IR (KBr, cm^{-1}): 3311 (ν_{N-H}), 2958, 2919, 2847 (ν_{C-H}), 1618 ($\nu_{C=O}$). MS(EI): m/z 908 (M^+). Elemental analysis: found C 66.03, H 9.01, N 3.15%. Calcd for $C_{50}H_{80}N_2S_3O_2Cl_2$ C 66.12, H 8.88, N 3.08%.

4.1.2. Synthesis of *N*,*N*′-dialkyl-5,5″-dibromo-2,2′:5′,2″-terthiophene-4,4″-dicarboxamide (DNC_nDBr3T, n=5, 8, 16, 18). 5,5″-Dibromo-2,2′:5′,2″-terthiophene (DBr3T) was prepared by the reaction of 3T (3.5 g, 14.1 mmol) with *N*-bromosuccinimide (5.0 g, 28.2 mmol) in chloroform (200 mL) at room temperature for 5 h and purified by silica gel column chromatography using chloroform as eluent, followed by recrystallization from a benzene/hexane mixture (1:2 v/v).

DBr3T (2.8 g, 6.89 mmol), was added to a THF solution (50 mL) of LDA (27.44 mmol) at −78 °C and the solution was stirred for 1 h. Excess dry ice was added to the solution and the resulting mixture was stirred for 2 h. The solution was allowed to warm to room temperature and aqueous hydrochloric acid was added. After stirring for 5 h, the resulting solid was collected and washed with hot acetonitrile to give 5,5″-dibromo-2,2′:5′,2″-terthiophene-4,4″-dicarboxylic acid (yield 65% based on DBr3T).

A dichloromethane solution (100 mL) of 5,5″-dibromo-2,2′:5′,2″-terthiophene-4,4″-dicarboxylic acid (1.0 g, 2.0 mmol) and $SOCl_2$ (0.48 g, 4.0 mmol) was heated at reflux for 8 h. After the solvent was removed under reduced pressure, the resulting solid was added to a THF solution (100 mL) of amylamine (0.35 g, 4.0 mmol) in the presence of triethylamine (2.3 mL) at 0 °C and the solution was stirred for 5 h at 0 °C. The solvent was removed under reduced pressure to give DNC_5DBr3T. The product was purified by silica gel column chromatography using chloroform as the eluent (yield 71% based on 5,5″-dibromo-2,2′:5′,2″-terthiophene-4,4″-dicarboxylic acid). ^{1}H NMR (600 MHz, THF-d_8): δ (ppm)=7.35 (s, 2H, ArH), 7.32 (t, 2H, NH), 7.28 (s, 2H, ArH), 3.36 (dt, 4H, pentyl α-CH_2), 1.60 (tt, 4H, pentyl β-CH_2), 1.45–1.33 (m, 8H, pentyl CH_2), 0.88 (t, 6H, CH_3). IR (KBr, cm^{-1}): 3388 (ν_{N-H}), 2958, 2927, 2859 (ν_{C-H}), 1625 ($\nu_{C=O}$). MS(EI): m/z 632 (M^+). Elemental analysis: found C 45.65, H 4.44, N 4.47%. Calcd for $C_{24}H_{28}N_2S_3O_2Br_2$ C 45.58, H 4.46, N 4.43%.

The DNC_8DBr3T, $DNC_{16}DBr3T$ and $DNC_{18}DBr3T$ were

synthesized using analogous procedures with octylamine, 1-hexadecylamine, or stearylamine (yields 55–63%).

DNC_8DBr3T: ^1H NMR (600 MHz, THF-d_8): δ (ppm)=7.36 (s, 2H, ArH), 7.31 (t, 2H, NH), 7.28 (s, 2H, ArH), 3.38 (dt, 4H, octyl α-CH_2), 1.61 (tt, 4H, octyl β-CH_2), 1.46–1.23 (m, 20H, octyl CH_2), 0.88 (t, 6H, CH_3). IR (KBr, cm^{-1}): 3302 (ν_{N-H}), 2958, 2923, 2853 (ν_{C-H}), 1625 ($\nu_{C=O}$). MS(EI): *m/z* 716 (M^+). Elemental analysis: found C 50.38, H 5.60, N 3.96%. Calcd for $C_{30}H_{40}N_2S_3O_2Br_2$ C 50.28, H 5.63, N 3.91%.

$DNC_{16}DBr3T$: ^1H NMR (600 MHz, THF-d_8): δ (ppm)=7.35 (s, 2H, ArH), 7.32 (t, 2H, NH), 7.29 (s, 2H, ArH), 3.34 (dt, 4H, cetyl α-CH_2), 1.58 (tt, 4H, cetyl β-CH_2), 1.48–1.15 (m, 52H, cetyl CH_2), 0.88 (t, 6H, CH_3). IR (KBr, cm^{-1}): 3328 (ν_{N-H}), 2948, 2921, 2850 (ν_{C-H}), 1619 ($\nu_{C=O}$). MS(EI): *m/z* 941 (M^+). Elemental analysis: found C 58.74, H 7.64, N 3.01%. Calcd for $C_{46}H_{72}N_2S_3O_2Br_2$ C 58.71, H 7.71, N 2.98%.

$DNC_{18}DBr3T$: ^1H NMR (600 MHz, THF-d_8): δ (ppm)=7.35 (s, 2H, ArH), 7.31 (t, 2H, NH), 7.28 (s, 2H, ArH), 3.38 (dt, 4H, stearyl α-CH_2), 1.60 (tt, 4H, stearyl β-CH_2), 1.45–1.22 (m, 60H, stearyl CH_2), 0.88 (t, 6H, CH_3). IR (KBr, cm^{-1}): 3312 (ν_{N-H}), 2956, 2918, 2849 (ν_{C-H}), 1619 ($\nu_{C=O}$). MS(EI): *m/z* 997 (M^+). Elemental analysis: found C 60.36, H 8.00, N 2.79%. Calcd for $C_{50}H_{80}N_2S_3O_2Br_2$ C 60.22, H 8.09, N 2.81%.

4.1.3. Synthesis of *N,N′*-dialkyl-5,5″-diiodo-2,2′:5′,2″-terthiophene-4,4″-dicarboxamide (DNC_nDI3T, n=8, 18). 5,5″-Diiodo-2,2′:5′,2″-terthiophene (DI3T) was prepared by the reaction of 3T (3.0 g, 12.1 mmol) with *N*-iodosuccinimide (5.4 g, 24.2 mmol) in chloroform (200 mL) at room temperature for 5 h and purified by silica gel column chromatography using chloroform as eluent, followed by recrystallization from a benzene/hexane mixture (1:2 v/v).

DI3T (2.8 g, 5.60 mmol), was added to a THF solution (50 mL) of LDA (22.4 mmol) at −78 °C and the solution was stirred for 1 h. Excess dry ice was added to the solution and the resulting mixture was stirred for 2 h. The solution was allowed to warm to room temperature and aqueous hydrochloric acid was added. After stirring for 5 h, the resulting solid was collected and washed with hot acetonitrile to give 5,5″-diiodo-2,2′:5′,2″-terthiophene-4,4″-dicarboxylic acid (yield 75% based on DI3T).

A dichloromethane solution (100 mL) of 5,5″-diiodo-2,2′:5′,2″-terthiophene-4,4″-dicarboxylic acid (1.0 g, 1.7 mmol) and $SOCl_2$ (0.40 g, 3.4 mmol) was heated at reflux for 8 h. After the solvent was removed under reduced pressure, the resulting solid was added to a THF solution (100 mL) of octylamine (0.43 g, 3.4 mmol) in the presence of triethylamine (2.5 mL) at 0 °C and the solution was stirred for 5 h at 0 °C. The solvent was removed under reduced pressure to give DNC_8DI3T. The product was purified by silica gel column chromatography using chloroform as the eluent (yield 75% based on 5,5″-diiodo-2,2′:5′,2″-terthiophene-4,4″-dicarboxylic acid). ^1H NMR (400 MHz, THF-d_8): δ (ppm)=7.37 (s, 2H, ArH), 7.33 (t, 2H, NH), 7.27 (s, 2H, ArH), 3.38 (dt, 4H, octyl α-CH_2), 1.62 (tt, 4H, octyl β-CH_2), 1.47–1.24(m, 20H, octyl CH_2), 0.88 (t, 6H, CH_3). IR (KBr, cm^{-1}): 3303 (ν_{N-H}), 2957, 2922, 2854 (ν_{C-H}), 1624 ($\nu_{C=O}$). MS(EI): *m/z* 810 (M^+). Elemental analysis: found C 44.18, H 5.05, N 3.43%. Calcd for $C_{30}H_{40}N_2S_3O_2I_2$ C 44.45, H 4.97, N 3.46%.

The $DNC_{18}DI3T$ was synthesized using analogous procedures with stearylamine (yield 72%). ^1H NMR (400 MHz, THF-d_8): δ (ppm)=7.32 (s, 2H, ArH), 7.30 (t, 2H, NH), 7.27 (s, 2H, ArH), 3.36 (dt, 4H, stearyl α-CH_2), 1.60 (tt, 4H, stearyl β-CH_2), 1.43–1.21 (m, 60H, stearyl CH_2), 0.88 (t, 6H, CH_3). IR (KBr, cm^{-1}): 3313 (ν_{N-H}), 2957, 2916, 2850 (ν_{C-H}), 1617 ($\nu_{C=O}$). MS(EI): *m/z* 1091 (M^+). Elemental analysis: found C 54.98, H 7.45, N 2.54%. Calcd for $C_{50}H_{80}N_2S_3O_2I_2$ C 55.04, H 7.39, N 2.57%.

4.1.4. Synthesis of 4,4″-bis-(alkyloxycarbonyl)-5,5″-dibromo-2,2′:5′,2″-terthiophene (DOC_nDBr3T, n=5, 8, 16, 18). 5,5″-Dibromo-2,2′:5′,2″-terthiophene-4,4″-dicarboxylic acid (0.5 g, 1.0 mmol) was reacted with 1-iodopentane (0.40 g, 2.0 mmol) in the presence of potassium carbonate (0.20 g, 1.5 mmol) in hexamethylphosphorotriamide (20 mL) at room temperature for 24 h under a nitrogen atmosphere. The resulting solid was collected to give DOC_5DBr3T. The product was purified by silica gel column chromatography using chloroform as the eluent (yield 91%). ^1H NMR (400 MHz, THF-d_8): δ (ppm)=7.45 (s, 2H, ArH), 7.40 (s, 2H, ArH), 4.29 (t, 4H, pentyl α-CH_2), 1.64–1.27 (m, 12H, pentyl CH_2), 0.93 (t, 6H, CH_3). IR (KBr, cm^{-1}): 2953, 2935, 2862 (ν_{C-H}), 1683 ($\nu_{C=O}$). MS (EI): *m/z* 634 (M^+). Elemental analysis: found C 45.44, H 4.00%. Calcd for $C_{24}H_{26}S_3O_4Br_2$ C 45.43, H 4.13%.

The DOC_8DBr3T, $DOC_{16}DBr3T$ and $DOC_{18}DBr3T$ were synthesized using analogous procedures with 1-iodooctane, 1-iodohexadecane, or 1-iodooctadcane (yields 91–93%).

DOC_8DBr3T: ^1H NMR (400 MHz, THF-d_8): δ (ppm)=7.45 (s, 2H, ArH), 7.40 (s, 2H, ArH), 4.28 (t, 4H, octyl α-CH_2), 1.59–1.13 (m, 24H, octyl CH_2), 0.89 (t, 6H, CH_3). IR (KBr, cm^{-1}): 2958, 2924, 2852 (ν_{C-H}), 1707 ($\nu_{C=O}$). MS(EI): *m/z* 718 (M^+). Elemental analysis: found C 50.19, H 5.32%. Calcd for $C_{30}H_{38}S_3O_4Br_2$ C 50.14, H 5.33%.

$DOC_{16}DBr3T$: ^1H NMR (400 MHz, THF-d_8): δ (ppm)=7.45 (s, 2H, ArH), 7.39 (s, 2H, ArH), 4.20 (t, 4H, cetyl α-CH_2), 1.55–1.05 (m, 56H, cetyl CH_2), 0.86 (t, 6H, CH_3). IR (KBr, cm^{-1}): 2955, 2917, 2848 (ν_{C-H}), 1688 ($\nu_{C=O}$). MS (EI): *m/z* 943 (M^+). Elemental analysis: found C 58.82, H 7.47%. Calcd for $C_{46}H_{70}S_3O_4Br_2$ C 58.59, H, 7.48%.

$DOC_{18}DBr3T$: ^1H NMR (400 MHz, THF-d_8): δ (ppm)=7.45 (s, 2H, ArH), 7.39 (s, 2H, ArH), 4.28 (t, 4H, stearyl α-CH_2), 1.57–0.99 (m, 64H, stearyl CH_2), 0.88 (t, 6H, CH_3). IR (KBr, cm^{-1}): 2956, 2918, 2849 (ν_{C-H}), 1689 ($\nu_{C=O}$). MS(EI): *m/z* 999 (M^+). Elemental analysis: found C 60.82, H 8.06%. Calcd for $C_{50}H_{78}S_3O_4Br_2$ C 60.11, H 7.87%.

4.2. Characterization

Differential scanning calorimetry (DSC) was performed

using a SSC/5200(SEIKO I&E) calorimeter. Polarizing microscopy was carried out with an OPTI-PHOT X2 (Nikon) microscope, fitted with a TH-600PM hot stage (Linkam) and crossed polarizers.

Acknowledgements

This work was supported by Ministry of Education of the People's Republic of China (Research Funds for Chinese Scholars Returning from Abroad), the Ministry of Science and Technology of the People's Republic of China (National Key Program for Basic Research, 2001-CCA03500). We also thank the Natural Science Foundation of South China University of Technology for financial support.

References and notes

1. Bäuerle, P.; Fischer, T.; Bidlingmeier, B.; Stabel, A.; Rabe, J. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 303–306.
2. Yassar, A.; Garnier, F.; Deloffre, F.; Holowitz, G.; Ricard, L. *Adv. Mater.* **1994**, *6*, 660–663.
3. Fichou, D.; Bachet, B.; Demanze, F.; Billy, I.; Horowitz, G.; Garnier, F. *Adv. Mater.* **1996**, *8*, 500–504.
4. Soukopp, A.; Glöckler, K.; Bäuerle, P.; Sokolowski, M.; Umbach, E. *Adv. Mater.* **1996**, *8*, 902–906.
5. Azumi, R.; Götz, G.; Bäuerle, P. *Synth. Met.* **1999**, *101*, 569–572.
6. Bäuerle, P.; Segelbacher, U.; Maier, A.; Mehring, M. *J. Am. Chem. Soc.* **1993**, *115*, 10217–12223.
7. Bäuerle, P.; Segelbacher, U. K.; Gauda, U.; Huttenlocher, D.; Mehring, M. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 76–78.
8. Bennati, M.; Grupp, A.; Mehring, N.; Bäuerle, P. *J. Phys. Chem.* **1996**, *100*, 2849–2853.
9. Bennati, M.; Grupp, A.; Mehring, N.; Nemeth, K.; Surjan, P. R. *Synth. Met.* **1997**, *84*, 607–608.
10. Tour, J. M.; Wu, R. *Macromolecules* **1992**, *25*, 1901–1907.
11. Fichou, D.; Dumarcher, V.; Nunzi, J. M. *Synth. Met.* **1999**, *101*, 610–613.
12. Horowitz, G.; Garnier, F.; Yassar, A.; Hajlaoui, R.; Kouki, F. *Adv. Mater.* **1996**, *8*, 52–54.
13. Nakanishi, H.; Sumi, N.; Aso, Y.; Otsubo, T. *J. Org. Chem.* **1998**, *63*, 8632–8633.
14. Garnier, F.; Hajlaoui, R.; Yassar, A.; Srivastava, P. *Science* **1994**, *265*, 1684–1686.
15. Videlot, C.; Fichou, D. *Synth. Met.* **1999**, *102*, 885–886.
16. Geiger, F.; Stoldt, M.; Schweizer, H.; Bäuerle, P.; Umbach, E. *Adv. Mater.* **1993**, *5*, 922–925.
17. Byron, D.; Matharu, A.; Wilson, R.; Wright, G. *Mol. Cryst. Liq. Cryst.* **1995**, *265*, 61–76.
18. Azumi, R.; Götz, G.; Bäuerle, P. *Synth. Met.* **1999**, *101*, 5544–5545.
19. Yamada, T.; Azumi, R.; Tachibana, H.; Sakai, H.; Abe, M.; Bäuerle, P.; Matsumoto, M. *Chem. Lett.* **2001**, *10*, 1022–1023.
20. Ponomarenko, S.; Kirchmeyer, S. *J. Mater. Chem.* **2003**, *13*, 197–202.