Syntheses and bio-activities of the L-enantiomers of two potent transition state analogue inhibitors of purine nucleoside phosphorylases

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(1*R*)-1-(9-Deazahypoxanthin-9-yl)-1,4-dideoxy-1,4-imino-L-ribitol [(+)-**5**] and (3*S*,4*S*)-1-[(9-deazahypoxanthin-9-yl)methyl]-4-(hydroxymethyl)pyrrolidin-3-ol [(−)-**6**] are the L-enantiomers of immucillin-H (D-ImmH) and DADMe-immucillin-H (D-DADMe–ImmH), respectively, these D-isomers being high affinity transition state analogue inhibitors of purine nucleoside phosphorylases (PNPases) developed as potential pharmaceuticals against diseases involving irregular activation of T-cells. The C-nucleoside hydrochloride D-ImmH [(−)-**5**)·HCl], now "Fodosine™" is in phase II clinical trials as an anti-T-cell leukaemia agent, while D-DADMe–ImmH is a second generation inhibitor with extreme binding to the target enzyme and has entered the clinic for phase I testing as an anti-psoriasis drug. Since the enantiomers of some pharmaceuticals have revealed surprising biological activities, the L-nucleoside analogues (+)-**5**·HCl and (−)-**6**, respectively, of D-ImmH and D-DADMe–ImmH, were prepared and their PNPase binding properties were studied. For the synthesis of compound (−)-**6** suitable enzyme-based routes to the enantiomerically pure starting material (*3S*,*4S*)-4-(hydroxymethyl)pyrrolidin-3-ol [(−)-**16**] and its enantiomer were developed. The L-enantiomers (+)-**5**·HCl and (−)-**6** bind to the PNPases approximately 5- to 600-times less well than do the D-compounds, but nevertheless remain powerful inhibitors with nanomolar dissociation constants.

Introduction

Chiral components of natural products occur predominantly in one of their enantiomeric forms, these being, in particular, the L- and D-modifications, respectively, of the α-amino acids and sugars. Work in the area of synthetic drug discovery is usually restricted to the investigation of compounds in their natural enantiomeric forms and, in this context, it has been unusual until recently for such substances as peptides and nucleosides to be made with D-amino acids or L-sugars, respectively. It appears, however, from many new observations, that this practice has been too restrictive.

Recent work has revealed that derivatives of the unusual L-enantiomers of natural sugars and nucleosides can show considerable potential as pharmaceuticals, and such is the perceived value of L-nucleoside analogues that the chemistry and biological activities of these compounds have been studied extensively and reviewed on several occasions.[1–3] 2′-Deoxy-L-cytidine (**1**) and several other L-nucleosides show potent and specific activity against hepatitis B virus replication, and related unnatural nucleosides have been tested as potential anti-viral and anti-cancer compounds.[1–4] More specifically, Ribavirin® and its L-enantiomer Levovirin® (**2**) have widely different anti-viral activities and, notably, the latter shows a much more favourable safety profile than does the D-ribofuranose-based isomer.[5] Lamivudine® (**3**) was the first L-nucleoside derivative to be approved by the FDA for use in combination therapy against HIV and hepatitis B virus.[4] In an entirely different sphere of fundamental biochemical interest some L-sugars, particularly α-L-arabinopyranose, have attracted attention since, when incorporated into (4′-2′)-linked oligonucleotides, they lead to unusually strong Watson–Crick base-pairing.[6]

In our laboratories we have turned attention to the bio-activities of L-enantiomers of sugar-based specific inhibitors of glycoside- and nucleoside-processing enzymes, and have encountered L-compounds with inhibitory activities quite different from those of the D-analogues. For example, the L-enantiomer **4** (L-DMDP) of 2,5-dideoxy-2,5-imino-D-mannitol is several times more active against some α-D-glucosidases than is its enantiomer, while it is inactive against some β-D-glucosidases which are inhibited by D-DMDP.[7] These studies have been extended to comparisons of the inhibitory effects of the enantiomers of 5- and 6-membered analogues of "nitrogen-in-the-ring compounds" on D-glycohydrolases,[8] and other workers have investigated a range of both D- and L-isomers of pyranoid analogues, *i.e.* of 1-deoxynojirimycin, as glycosidase inhibitors.[9]

The structures of the family of immucillins, which are high affinity transition state analogue inhibitors of PNPases, are based on "nitrogen in the ring" D-ribofuranosyl *C*-glycosidic analogues of natural nucleosides (and closely related compounds), and

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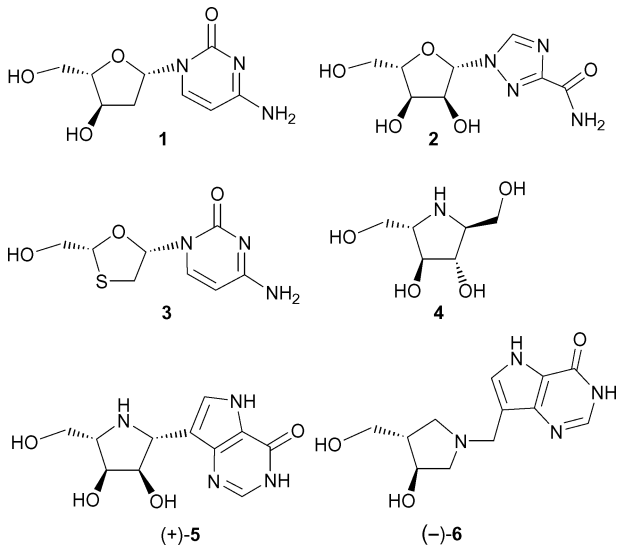
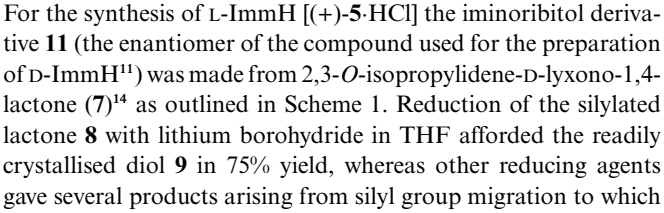
their enzymic inhibitory actions have been studied in connection with their development as pharmaceuticals, active in particular, against T-cell-mediated disorders.[10] We report now the synthesis of the L-enantiomers (+)-**5**·HCl and (−)-**6** of two of the most biologically potent members of the group, D-ImmH[11] and D-DADMe-ImmH,[12,13] and a brief comparison of the activities as inhibitors of PNPases of these four compounds.



Results and discussion

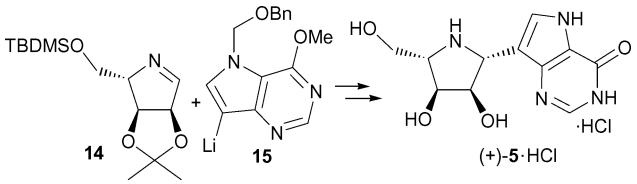
Preparation of (1*R*)-1-(9-deazahypoxanthin-9-yl)-1,4-dideoxy-1,4-imino-L-ribitol hydrochloride [L-ImmH, (+)-5·HCl]

For the synthesis of L-ImmH [(+)-**5**·HCl] the iminoribitol derivative **11** (the enantiomer of the compound used for the preparation of D-ImmH[11]) was made from 2,3-*O*-isopropylidene-D-lyxono-1,4-lactone (**7**)[14] as outlined in Scheme 1. Reduction of the silylated lactone **8** with lithium borohydride in THF afforded the readily crystallised diol **9** in 75% yield, whereas other reducing agents gave several products arising from silyl group migration to which diol **9** is particularly prone. Mesylation of **9** afforded diester **10** which, with one equivalent of sodium azide in hot DMF, gave mainly the product **12** by displacement of the primary ester group (42%), together with the diazide **13** (15%), and the starting material **10** (34%). Hydrogenation of compound **12** in 1,4-dioxane in the presence of palladium black and sodium acetate gave the iminoribitol derivative **11** (94%) required for the synthesis of L-*C*-nucleoside analogues. That the ring-closure reaction **12** → **11** occurred with inversion of configuration is evidenced by the structure of compound **11** which is the enantiomer of the D-iminoribitol derivative used to make ImmH.[11] All comparable ^{1}H NMR signals of these two enantiomers resonated within 0.02 ppm of each other, and the specific rotations of the D- and L-compounds were (+)- and (−)-15.5, respectively. The former figure is apparently recorded for the first time.[11,15,16] The structure and absolute configuration of the D-enantiomer, and hence of compound **11**, follow from the X-ray diffraction analysis of D-ImmH[17] and also from the method of synthesis of this compound from D-gulono-lactone.[11]



Scheme 1 Reagents and conditions: (a) TBDMSCl, imidazole, DMF, N_2, 20 °C, 4 h (91%); (b) $LiBH_4$, THF, N_2, −30 to 20 °C, 24 h, (75%); (c) $(MeSO_2)_2O$, pyridine, N_2, 0 to 20 °C, 18 h (88%); (d) NaN_3, DMF, N_2, 100 °C, 2 h (42% **12**, 15% **13**); (e) H_2, Pd black, NaOAc, 1,4-dioxane, 20 °C, 72 h, (94%).

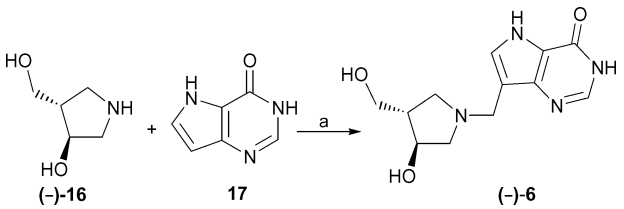
Conversion of the iminoribitol **11** to the imine **14** was effected, as for its enantiomer, by *N*-chlorination with *N*-chlorosuccinimide in pentane followed by elimination of HCl by treatment of the chloro derivative with lithium tetramethylpiperidide in THF.[11,15,16] Coupling of **14** with lithiated deazapurine derivative **15**, and modification of the product in the way described for making D-ImmH,[11b] afforded the required L-enantiomer (+)-**5**·HCl (Scheme 2). This product gave NMR spectral data that identified it as the enantiomer of D-ImmH·HCl. While the L-salt (+)-**5**·HCl had $[\alpha]_D^{25}$ +51.5, the literature value for the D-salt is recorded as −0.50.[11a] Remeasurement of the latter figure during the present work gave −53.6, the discrepancy being identified as being due to a spurious factor of 100 mistakenly used in the calculation of the first value.



Scheme 2 Reagents and conditions: See ref. 11*b*.

Preparation of (3*S*,4*S*)-1-[(9-deazahypoxanthin-9-yl)methyl]-4-(hydroxymethyl)pyrrolidin-3-ol [L-DADMe-Imm H, (−)-6]

Our extensive studies in the immucillin field[10–13] have led to the preparation of D-DADMe–ImmH [(+)-**6**] which is an even more potent inhibitor of human PNPase than is D-ImmH [(−)-**5**]·HCl.[12] The L-enantiomer [(−)-**6**], therefore, was of interest within the present study, and we prepared it using the second method previously adopted for making the D-compound,[13] *i.e.* by Mannich coupling of amine (−)-**16** with deazahypoxanthine **17** in the presence of formaldehyde as illustrated in Scheme 3. For this purpose ready access to pyrrolidine (−)-**16** was required, and the opportunity was taken to develop efficient syntheses of both the D- and L-isomers of this amine which were isolated as their respective hydrochlorides [(+)- and (−)-**16**·HCl]. These salts have been known as components of mixed stereoisomers since 1965,[18] and for a few years, as the "carbohydrate-parts" of racemic *N*–*C*′[19] and *N*–*N*′[20] linked nucleoside analogues.



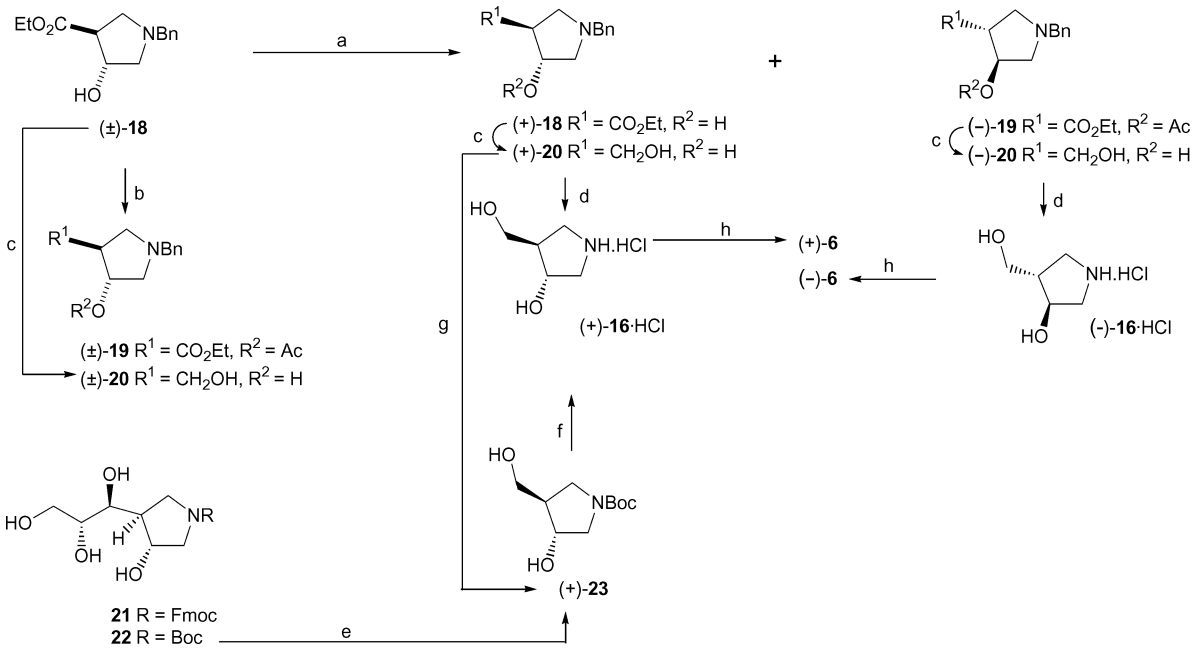
Scheme 3 Reagents and conditions: (a) HCHO (30%, aq.), H_2O, 85 °C, 15 h (48%).

As for the individual enantiomers of pyrrolidine **16**·HCl, the D-compound (3*R*,4*R*) has been made in high enantiomeric excess by methods involving application of the asymmetric Sharpless epoxidation reaction, followed by cyanide opening of the epoxide, applied to an unsymmetrically substituted but-2-en-1,4-diol,[21] and by addition of (*S*)-1-phenylethylamine to an ethyl 2-silyloxy-3-methoxycarbonyl-but-3-enoate.[22] Both enantiomers have also been obtained by a key 1,3-dipolar cycloaddition of chiral azomethine ylides to 3-benzyloxy-substituted alkenoylcamphorsultams,[23] and this procedure has been adapted to afford a large scale synthesis of (+)-**16**.[24] Otherwise, the D-enantiomer has been obtained exclusively by multi-step, low yielding routes from D-xylose[25] and D-glucose.[26]

Encouraged by the appreciable success reported for the lipase-catalysed enantioselective acetylation of *tert*-butyl *trans*-(±)-3-hydroxy-4-(hydroxymethyl)pyrrolidine-1-carboxylate,[27] and especially by the highly efficient resolution of cyclopentyl, cyclohexyl and cycloheptyl *cis*- and *trans*-β-hydroxy esters permitted by enantioselective acylation catalysed by lipase B of *Candida antarctica* (Novozyme® 435),[28] we have applied this lipase to the resolution of racemic ethyl *trans*-1-benzyl-4-hydroxypyrrolidine-3-carboxylate[18] [(±)-**18**, Scheme 4]. This approach to the preparation of the enantiomers (+)- and (−)-**16** is very efficient. Before this enantioselective work was commenced, however, the racemic hydroxyester (±)-**18** was converted by standard methods to diester (±)-**19** and diol (±)-**20** which were required as reference materials.

The enzyme-catalysed *trans*-acetylation of racemic hydroxyester (±)-**18** from vinyl acetate was readily followed by ^{1}H NMR monitoring of the ring proton on the hydroxyl-bearing ring carbon atom (C-4) which underwent deshielding from δ 4.5 to 5.4 on esterification. When the acetylation was performed in *tert*-butyl methyl ether at 40 °C, 50% conversion occurred within 2 h, and after another 5 h insignificant further reaction took place. Chromatographic separation gave the crystalline unreacted alcohol (+)-**18** and the ester (−)-**19** in 85 and 97% yields, with their specific rotations being +16.9 and −41.5, respectively.

These products proved to be of surprisingly high enantiomeric purity. Conversion of the alcohol (+)-**18** to (+)-**16**·HCl was achieved by $LiAlH_4$ reduction of the ester to give diol (+)-**20** followed by de-*N*-benzylation (Scheme 4), and afforded a hydrochloride salt with $[\alpha]_D^{21}$ +19.1. Alternatively, the enantiospecific sequence (+)-**18** → (+)-**20** → (+)-**23** → (+)-**16**·HCl was employed, (+)-**23** being useful for preparing D-DADMe–ImmH derivatives. Made by this route compound (+)-**23** had $[\alpha]_D^{21}$ +15.9, whereas a sample prepared from 1,2:5,6-di-*O*-isopropylidene-α-D-glucose *via* *N*-Boc-carbamate **22** (made in an analogous way to that used previously to make the *N*-Fmoc carbamate **21**[26]) had $[\alpha]_D^{21}$ +16.2, and that of the salt (+)-**16**·HCl derived from (+)-**23** had $[\alpha]_D^{21}$ +18.9. This figure is in good agreement with that given by (+)-**16**·HCl derived directly from diol (+)-**20**, and also with literature data.[23] The specific rotation results show that the alcohol (+)-**18** was of high enantiomeric purity, and attention turned to that of the ester (−)-**19**. Conversion of this product of selective enzymic acetylation to the corresponding hydrochloride (−)-**16**·HCl *via* (−)-**20** afforded the salt with $[\alpha]_D^{21}$ −18.9, indicating that it too was of high enantiomeric purity. As expected, the ^{1}H NMR spectra of



Scheme 4 Reagents: (a) Novozyme® 435, *tert*-BuOMe, CH_2=CHOAc; (b) Ac_2O, Py; (c) $LiAlH_4$, THF, Et_2O; (d) (i) HCO_2H, MeOH, Pd/C; (ii) HCl, MeOH, H_2O; (e) (i) $NaIO_4$, H_2O; (ii) $NaBH_4$; (f) HCl, MeOH, H_2O; (g) (*tert*-BuOCO)$_2$O, MeOH, H_2, Pd/C; (h) as Scheme 3.

the various samples of the enantiomeric hydrochlorides **16**·HCl, made by the different routes described above, were identical, and were consistent with the spectrum described in the literature.[23]

It was not possible to determine the selectivity of the enzymic acetylation reaction by direct measurement. However, the specific rotations of several samples of each enantiomer of the hydrochlorides **16**·HCl prepared during the present work, were all 19.0 ± 0.1. In consequence, it was concluded that the enzyme had acted, when presented with racemate (±)-**18**, efficiently and also specifically with the (−)-enantiomer to within 3%, *i.e.* the estimated error of measurement of the specific rotation of the products. To test this conclusion, a further enzymic acetylation of the substrate was conducted and stopped before completion when the **18** : **19** ratio was approximately 1.2 : 1.0 (^{1}H NMR determination). Consistent with expectations, the sample of the ester **19** obtained by chromatographic separation in this case had $[\alpha]_D^{21}$ −41.8, indicating it was the pure (−)-enantiomer. Further, the alcohol **18** that remained had $[\alpha]_D^{21}$ +14.0 showing that it contained mainly the unreacted (+)-isomer, but was contaminated with roughly 10% of the unreacted (−)-enantiomer.

Conversion of (+)-**16**·HCl to (+)-**6** has been reported previously;[12,13] and the alternative synthesis of (+)-**16** now described opened an alternative pathway to this nucleoside analogue. The sample of (+)-**6** obtained in this way had $[\alpha]_D^{21}$ +16.9. Likewise, the target compound (−)-**6**, derived from pure (−)-**16** (Scheme 3), gave a ^{1}H NMR spectrum identical to that reported and observed for the D-enantiomer,[12,13] and had $[\alpha]_D^{21}$ −16.8.

Biodata of compounds

Kinetic studies of the interactions between compounds (+)-**5**·HCl, (−)-**5**·HCl, (+)-**6** and (−)-**6** and human, plasmodial and bovine PNPases were carried out by the methods previously reported,[29,30] and the results are given in Table 1. The inhibition constants K_i are the dissociation constants for the enzyme–inhibitor complex measured from initial reaction rates. For many, but not all, immucillin inhibitors, a slow onset of inhibition then occurs consequent upon a time-dependent conformational change in the enzyme that leads to tighter binding characterised by the constant K_i*.[31]

To ensure that the inhibition observed with the L-DADMe–ImmH [(−)-**6**] was not due to small proportions of residual, more active, D-compound, samples of the L-enantiomer were pretreated with 0.01 to 1.0 molar equivalents of human PNPase and the mixtures were subjected to ultrafiltration. In this way, L-DADMe ImmH [(+)-**6**] gave a sample that inhibited PNPases with kinetic parameters unchanged relative to those of the original preparation. Accordingly, based on the error limits of the kinetic constant for inhibition, it was concluded that no more than 2% of the D-enantiomer could have been present as a contaminant in the initial inhibitor (−)-**6**.

The L-enantiomer (+)-**5**·HCl of ImmH is revealed to be a slow onset tight binding inhibitor of the PNPases of human, bovine and *Plasmodium falciparum* (the protozoan parasite responsible for malaria) origins. While it is, approximately, 15- to 600-fold less active than the D-enantiomer against the enzymes studied, it still shows surprising potency, and it seems remarkable that it is more active by factors of >10 as an inhibitor of the human enzyme than are the 3′- and 5′-monodeoxy analogues of D-ImmH.[30] The L-DADMe–ImmH [(−)-**6**] shows similar tendencies being from 5- to 160-times less active than its D-enantiomer against the three enzymes. Nevertheless, it is still a sub-nanomolar inhibitor against both the human and bovine enzymes, and in other circumstances could be considered potent.

Experimental

General

Imidazole was recrystallised from CH_2Cl_2. All other reagents were used as supplied; anhydrous solvents were obtained commercially. Air sensitive reactions were carried out under argon unless otherwise stated. Organic solutions were dried over $MgSO_4$ and the solvents were evaporated under reduced pressure. Chromatography solvents were distilled prior to use. Thin layer chromatography (t.l.c.) was performed on glass or aluminium sheets coated with 60 F_{254} silica. Organic compounds were visualised under uv light or by use of a spray or dip of cerium(IV) sulfate (0.2%, *w*/*v*) and ammonium molybdate (5%) in sulfuric acid (2 M), one of I_2 (0.2%) and KI (7%) in H_2SO_4 (1 M) or, for nitrogen-containing compounds, *p*-(*N*,*N*-dimethylamino)benzaldehyde (1%) in HCl

Table 1 Kinetic data for the inhibition of human, plasmodial and bovine PNPases by the enantiomers of ImmH [(−)-**5**·HCl and (+)-**5**·HCl] and DADMe–ImmH [(+)-**6** and (−)-**6**]

Compound	Enzyme source	K_i/nM	K_i*/nM	Reference
D-ImmH [(−)-**5**]·HCl	*H. sapiens*		0.072 ± 0.026	29
		3.3 ± 0.2	0.056 ± 0.015	10, 30
	Plasmodium falciparum	29 ± 8	0.6 ± 0.1	32
			0.86	33
	B. taurus	41 ± 8	0.023 ± 0.005	29
L-ImmH [(+)-**5**]·HCl	*H. sapiens*	18 ± 6	0.9 ± 0.5	This work
	Plasmodium falciparum	32 ± 9	9 ± 6	This work
	B. taurus	360 ± 60	14 ± 4	This work
D-DADMe–ImmH [(+)-**6**]	*H. sapiens*	1.1 ± 0.1	0.016 ± 0.001	12
	Plasmodium falciparum	0.50 ± 0.04	Not observed	34
	B. taurus	2.1 ± 0.3	0.110 ± 0.014	This work
L-DADMe–ImmH [(−)-**6**]	*H. sapiens*	1.5 ± 0.1	0.68 ± 0.26	This work
	Plasmodium falciparum	1700 ± 300	80 ± 7	This work
	B. taurus	19 ± 5	0.5 ± 0.1	This work

(37%)–MeOH, 1 : 3 (100 ml) (Erlich reagent). Flash column chromatography was performed on Sorbsil C60 40/60 silica, Scharlau or Merck silica gel 60 (40–60 μm). Melting points were recorded on a Köfler hot block or a Reichert hot stage microscope and are uncorrected. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter with a path length of 1 dm and are in units of 10^{-1}deg cm^2 g^{-1}; concentrations are in g per 100 ml.

NMR spectra were recorded on a Bruker AC300E or Bruker DPX 400 spectrometer. ^{1}H spectra at 300 or 400 MHz were measured in $CDCl_3$, CD_3OD or CD_3CN (internal reference Me_4Si, δ 0), and ^{13}C spectra at 75.5 or 100.6 MHz in $CDCl_3$ (reference, solvent centre line, δ 77.0), CD_3OD (reference, solvent centre line δ 49.0) or CD_3CN (reference, solvent centre line δ 118.7, CN). Assignments of ^{1}H and ^{13}C resonances were based on 2D (^{1}H–^{1}H DQF-COSY, ^{1}H–^{13}C HSQC) spectra, and DEPT experiments gave unambiguous data on the numbers of protons bonded to each carbon atom. The assignments of the ^{13}C resonances were consistent with the multiplicities observed. Coupling constants (J) are quoted in Hz. Infrared spectra were recorded on a Perkin-Elmer 1750 IR Fourier Transform, or Perkin-Elmer Paragon 1000 spectrophotometer using thin films on NaCl plates (thin film). Only characteristic absorptions are quoted. Electrospray ionisation (ES) low resolution mass spectra (m/z) were measured on a Micromass BioQ II-ZS mass spectrometer. For high resolution mass spectra (HRMS), ES data were collected on a Waters 2790-Micromass LCT mass spectrometer operated at a resolution of 5000 full width half height. Positive ion electrospray ionisation (ES+) spectra were calibrated relative to PEG with tetraoctylammonium bromide as the internal lock mass. Negative ion ES spectra were calibrated relative to poly-DL-alanine with Leu-enkephalin as the internal lock mass. Chemical ionization (CI, NH_3) HRMS were recorded on a Micromass 500 OAT spectrometer. Positive ion fast atom bombardment (FAB+) HRMS were measured on a VG 7070 instrument in a glycerol matrix, and positive ion electron impact (EI+) HRMS were measured on a VG 70SE instrument. Microanalyses were carried out by the Campbell Microanalytical Laboratory, University of Otago or in the Department of Chemistry, University of Oxford.

5-*O*-*tert*-Butyldimethylsilyl-2,3-*O*-isopropylidene-D-lyxono-1,4-lactone (8)

The isopropylidene lactone **7**[14] (3.84 g, 20.4 mmol), derived from the unsubstituted lactone,[35] was added to a solution of *tert*-butyldimethylsilyl chloride (4.61 g, 30.6 mmol) and imidazole (2.78 g, 30.6 mmol) in DMF (25 ml). The solution was stirred at 20 °C under an atmosphere of nitrogen. After 4 h, t.l.c. analysis (EtOAc–cyclohexane, 1 : 1) indicated complete conversion of starting material (R_f 0.12) into a major product (R_f 0.52). The reaction mixture was concentrated and coevaporated with toluene. The residue was then purified by flash chromatography (EtOAc–cyclohexane, 1 : 2) to give the fully protected lactone **8** (5.69 g, 91%) as a white crystalline solid, mp 90–91 °C, $[\alpha]_D^{22}$ +54.9 (c, 1.03, $CHCl_3$); ν_{max} (thin film) 1773 (C=O); NMR δ_H (400 MHz; $CDCl_3$) 0.10 (6 H, s, $SiMe_2$), 0.91 (9 H, s, Si^{t}Bu), 1.40, 1.47 [2 × 3 H, 2s, $C(CH_3)_2$], 3.94 (1 H, dd, $J_{5,4}$ 6.5, $J_{5,5'}$ 10.4, H-5), 3.99 (1 H, dd, $J_{5',4}$ 6.1, H-5′), 4.53 (1 H, m, H-4), 4.82 (2 H, m, H-2,3); δ_C (100.6 MHz; $CDCl_3$) −5.5, −5.4 [$Si(CH_3)_2$], 18.3 [$C(CH_3)_3$], 25.8 [$C(CH_3)_3$], 25.9 [$C(CH_3)_2$], 26.8 [2 × $C(CH_3)_2$], 60.9 (C-5), 75.7 (C-3), 76.0 (C-2), 79.4 (C-4), 114.1 [$C(CH_3)_2$], 173.7 (C-1); HRMS (CI+) m/z 303.1625; $C_{14}H_{27}O_5Si$ [(M + H)$^+$] requires 303.1628. (Found: C, 55.7; H, 8.2%; $C_{14}H_{26}O_5Si$ requires C, 55.6; H, 8.7%).

5-*O*-*tert*-Butyldimethylsilyl-2,3-*O*-isopropylidene-D-lyxitol (9)

Lithium borohydride (4 ml, 2 M in THF, 8 mmol) was added dropwise to a stirred solution of the TBDMS ether **8** (1.21 g, 4.00 mmol) in THF (4 ml) and the stirring was continued at −30 °C under an atmosphere of nitrogen. After 5 h, t.l.c. analysis (EtOAc–cyclohexane, 1 : 1) showed residual starting material as well as a major product (R_f 0.35). More lithium borohydride (2 ml, 2 M in THF 4 mmol) was added and the mixture was left stirring at −30 °C for a further 2 h. The reaction mixture was then allowed to assume room temperature. After a further 16 h, t.l.c. analysis (EtOAc–cyclohexane, 1 : 2) indicated most of the starting material had reacted. A solution of aqueous ammonium chloride (4 ml, saturated) was then added dropwise to the reaction mixture which was partitioned between EtOAc (20 ml) and brine (20 ml). The organic layer was then collected, washed with water (3 × 10 ml) and dried. The solvent was removed and the residue was crystallized from THF to afford the diol **9** (910 mg, 75%), mp 67–68 °C, $[\alpha]_D^{23}$ −9.2 (c, 0.08, $CHCl_3$); ν_{max} (thin film) 3400 (broad, O–H); NMR δ_H (400 MHz; $CDCl_3$) 0.08 (6 H, s, $SiMe_2$), 0.91 (9 H, s, Si^{t}Bu), 1.38, 1.50 [2 × 3 H, 2s, $C(CH_3)_2$], 2.78–2.91 (2 H, br s, OH), 3.63 (1 H, dd, $J_{5,5'}$ 10.0, $J_{5,4}$ 6.8, H-5), 3.73 (1 H, dd, $J_{5',4}$ 6.2, H-5′), 3.77–3.82 (3 H, m, H-1,1′,4), 4.82 (2 H, m, H-2,3); δ_C (100.6 MHz; $CDCl_3$) −5.5, −5.4 [$Si(CH_3)_2$], 18.3 [$C(CH_3)_3$], 25.0 [$C(CH_3)_2$], 25.8 [$C(CH_3)_3$], 27.1 [$C(CH_3)_2$], 61.4 (C-1), 64.6 (C-5), 69.2 (C-4), 75.7, 77.3 (C-2, C-3), 110.4 [$C(CH_3)_2$]; HRMS (ES–) m/z 305.1796; $C_{14}H_{29}O_5Si$ ([M–H]$^-$) requires 305.1784. (Found: C, 54.75; H, 10.0; $C_{14}H_{30}O_5Si$ requires C, 54.9; H, 9.9%).

5-*O*-*tert*-Butyldimethylsilyl-2,3-*O*-isopropylidene-1,4-di-*O*-methanesulfonyl-D-lyxitol (10)

A solution of the diol **9** (2.40 g, 8.16 mmol) in CH_2Cl_2 (3.5 ml) was added dropwise to a mixture of 4-(*N*,*N*-dimethylamino)pyridine (0.50 g, 4.08 mmol) and methanesulfonic anhydride (6.36 g, 36.5 mmol) in dry pyridine (13 ml) at 0 °C under an atmosphere of nitrogen. The reaction mixture was stirred for 30 min at 0 °C, then at 20 °C for 18 h, after which time t.l.c. analysis (EtOAc–cyclohexane, 1 : 1) indicated the presence of a major product (R_f 0.54). The reaction mixture was then diluted with CH_2Cl_2 (100 ml) and aqueous HCl (0.1 M, 50 ml) was added dropwise. The organic layer was washed with aqueous $NaHCO_3$ (50 ml, saturated), water (50 ml), dried and evaporated. The residue was purified by flash chromatography (EtOAc–cyclohexane, 1 : 4) to give the dimesylate **10** (3.17 g, 88%) as a colourless oil, $[\alpha]_D^{24}$ +5.0 (c, 0.14, $CHCl_3$); ν_{max} (thin film) 1360, 1178 (S=O); NMR δ_H (400 MHz; CD_3CN) 0.12 (6 H, s, $SiMe_2$), 0.92 (9 H, s, Si^{t}Bu), 1.37, 1.49 [2 × 3 H, 2s, $C(CH_3)_2$], 3.09, 3.14 (2 × 3 H, 2s, $2SO_2Me$), 3.86 (1 H, dd, $J_{5,5'}$ 11.4, $J_{5,4}$ 5.3, H-5), 3.93 (1 H, dd, $J_{5',4}$ 5.3, H-5′), 4.33–4.39 (2 H, m, H-1,1′), 4.42–4.46 (1 H, m, H-2), 4.47 (1 H, dd, $J_{3,2}$ 6.1, $J_{3,4}$ 9.7, H-3), 4.72 (1 H, dt, $J_{4,3}$ 9.7, H-4); δ_C (100.6 MHz; CD_3CN) −6.0 [$Si(CH_3)_2$], 18.3 [$C(CH_3)_3$], 25.1 [$C(CH_3)_2$], 25.6 [$C(CH_3)_3$], 26.9 [$C(CH_3)_2$], 37.0, 38.8 (2 × SO_2Me), 63.4 (C-5), 69.2 (C-1), 74.7 (C-2), 75.5 (C-3), 80.1 (C-4), 109.6 [$C(CH_3)_2$]; HRMS (ES+) m/z 463.1490; $C_{16}H_{35}O_9S_2Si$ (M + H)$^+$

requires 463.1492. (Found C, 41.6; H, 7.4; $C_{16}H_{34}O_9S_2Si$ requires C, 41.5; H, 7.4%).

1-Azido-5-*O*-*tert*-butyldimethylsilyl-1-deoxy-2,3-*O*-isopropylidene-4-*O*-methanesulfonyl-D-lyxitol (12) and 1,4-diazido-5-*O*-*tert*-butyldimethylsilyl-1,4-dideoxy-2,3-*O*-isopropylidene-L-ribitol (13)

Sodium azide (0.133 g, 2.04 mmol) was added to a solution of the dimesylate **10** (0.942 g, 2.04 mmol) in DMF (5 ml) and the reaction mixture stirred at 100 °C under an atmosphere of nitrogen. The formation of the monoazide **12** (R_f 0.58, EtOAc–cyclohexane, 1 : 2) after 20 min was indicated by t.l.c., but much of the starting material **10** (R_f 0.27) remained. After 1 h, a minor second product (**13**) (R_f 0.74) started to form. The mixture was stirred at 100 °C for another hour, allowed to cool to room temperature and the solvent was removed. The resulting yellow oil was purified by flash chromatography (EtOAc–cyclohexane, 1 : 4) to give the azido mesylate **12** (0.35 g, 42%), $[\alpha]_D^{25}$ +27.7 (*c*, 1.43, $CHCl_3$); ν_{max} (thin film) 2104 (N_3), 1358, 1174 (S=O); NMR δ_H (400 MHz; $CDCl_3$) 0.09, 0.10 (2 × 3H, 2s, $SiMe_2$), 0.90 (9 H, s, Si^{t}Bu), 1.40, 1.53 [2 × 3 H, 2s, $C(CH_3)_2$], 3.12 (3 H, s, SO_2Me), 3.42 (1 H, dd, $J_{1,1'}$ 12.8, $J_{1,2}$ 3.6, H-1), 3.50 (1 H, dd, $J_{1',2}$ 7.6, H-1′), 3.82 (1 H, dd, $J_{5,5'}$ 11.2, $J_{5,4}$ 6.4, H-5), 3.93 (1 H, dd, $J_{5',4}$ 4.8, H-5′), 4.31 (1 H, m, H-2), 4.36 (1 H, dd, $J_{3,4}$ 13.2, $J_{3,2}$ 6.0, H-3), 4.73 (1 H, ddd, H-4); δ_C (100.6 MHz; $CDCl_3$) −5.6 [$Si(CH_3)_2$], 18.2 [$C(CH_3)_3$], 25.4 [$C(CH_3)_2$], 25.8 [$C(CH_3)_3$], 27.6 [$C(CH_3)_2$], 38.7 (SO_2Me), 51.0 (C-1), 63.3 (C-5), 75.8 (C-3), 76.1 (C-2), 79.4 (C-4), 109.2 [$C(CH_3)_2$]; HRMS (CI+) *m*/*z* 427.2052; $C_{15}H_{35}N_4O_6SSi$ ($[M + NH_4]^+$) requires 427.2047. (Found: C, 44.35; H, 7.4; N, 10.1; $C_{15}H_{31}N_3O_6SSi$ requires C, 44.0; H, 7.6; N, 10.3%).

This was followed by the *bis*-azide (**13**), (0.11 g, 15%), $[\alpha]_D^{25}$ +87.1 (*c*, 0.56, $CHCl_3$); ν_{max} (thin film) 2102 (N_3); NMR δ_H (400 MHz; CD_3CN) 0.15, 0.16 (2 × 3H, 2s, $SiMe_2$), 0.94 (9 H, s, Si^{t}Bu), 1.33, 1.46 [2 × 3 H, 2s, $C(CH_3)_2$], 3.47 (2 H, d, $J_{1,2}$ 13.2, H-1,1′), 3.53 (1 H, m, H-4), 3.84 (1 H, dd, $J_{5,5'}$ 10.8, $J_{5,4}$ 6.0, H-5), 4.06 (1 H, dd, $J_{5',4}$ 2.8, H-5′), 4.11 (1 H, dd, $J_{3,2}$ 6.4, $J_{3,4}$ 11.6, H-3), 4.35 (1 H, dd, H-2); δ_C (100.6 MHz; $CDCl_3$) −6.0 [$Si(CH_3)_2$], 18.2 [$C(CH_3)_3$], 25.0 [$C(CH_3)_2$], 25.5 [$C(CH_3)_3$], 25.7 [$C(CH_3)_2$], 51.0 (C-1), 61.0 (C-4), 64.5 (C-5), 74.4 (C-3), 76.7 (C-2), 109.4 [$C(CH_3)_2$]; (ES+) *m*/*z* 329 ($[M–N_2 + H]^+$), (75%), 301 ($[M - 2N_2 + H]^+$), (20%). The starting dimesylate (**10**) (0.317 g, 34%) was also recovered from the reaction.

5-*O*-*tert*-Butyldimethylsilyl-1,4-dideoxy-1,4-imino-2,3-*O*-isopropylidene-L-ribitol (11)

The azidomesylate **12** (0.34 g, 0.84 mmol) in 1,4-dioxane (10 ml) was stirred at 20 °C under an atmosphere of hydrogen in the presence of palladium black (0.07 g) and sodium acetate (0.07 g, 0.825 mmol) for 3 d after which time t.l.c. analysis (EtOAc–cyclohexane, 1 : 1) indicated complete conversion of starting material (R_f 0.35) to a major product (R_f 0.17). The suspension was then filtered through Celite®, and the 1,4-dioxane removed. The resulting yellow oil was dissolved in CH_2Cl_2 (20 ml) and washed with water (3 × 20 ml), dried and the solvent was removed to afford the protected L-iminoribitol **11** (0.224 g, 94%) as a yellow oil, $[\alpha]_D^{25}$ −15.5 (*c*, 0.56, $CHCl_3$); ν_{max} (thin film) 3332 (sharp, N–H); NMR δ_H (400 MHz; $CDCl_3$) 0.02, 0.03 (6 H, 2s, $SiMe_2$), 0.91 (9 H, s, Si^{t}Bu), 1.34, 1.48 (2 × 3 H, 2s, $[C(CH_3)_2]$, 2.54 (1 H, br s, NH), 3.02 (2 H, d, $J_{1,2}$ 2.7, H-1,1′), 3.23 (1 H, dt, $J_{4,5}$, $J_{4,5'}$ 5.4, H-4), 3.56 (1 H, dd, $J_{5,5'}$ 10.4 $J_{5,4}$ 5.8, H-5), 3.65 (1 H, dd, H-5′), 4.65 (1 H, d, $J_{3,2}$ 5.8, H-3), 4.70 (1 H, dt, H-2); δ_C (100.6 MHz, $CDCl_3$) −5.5 [$Si(CH_3)_2$], 18.1 [$C(CH_3)_3$], 24.1 [$C(CH_3)_2$], 25.9 [$C(CH_3)_3$], 26.4 [$C(CH_3)_2$], 53.4 (C-1), 63.8 (C-5), 66.3 (C-4), 82.2 (C-2), 83.4 (C-3), 110.9 [$C(CH_3)_2$]; HRMS (ES+) *m*/*z* 288.1995; $C_{14}H_{30}NO_3Si$ ($[M + H]^+$) requires 288.1995.

(1*R*)-1-(9-Deazahypoxanthin-9-yl)-1,4-dideoxy-1,4-imino-L-ribitol hydrochloride [(+)-5·HCl]

The L-iminoribitol derivative **11** (500 mg) was converted to the imine **14** (186 mg, 39%) and hence to L-immucillin-H [(+)-**5**·HCl] (75 mg, 29%), $[\alpha]_D^{25}$ +51.5 (*c* 0.8, H_2O) as was described for the analogues of the D-series.[11] The enantiomeric products (+)- and (−)-**5**·HCl gave ^1H and ^{13}C NMR spectra with corresponding chemical shifts within 0.1 and 0.01 ppm of each other and $^3J_{H,H}$ values within 0.2 Hz. The specific rotation of the D-enantiomer was found in the present work to be −53.6 (*c* 2.7, H_2O).

Ethyl (*R*,*S*/*S*,*R*)-1-benzyl-4-hydroxypyrrolidine-3-carboxylate [(±)-18]

This compound was prepared by the method described by Jaeger and Biel,[18] but ethyl-*N*-benzyl-*N*-(2-carbethoxyethyl)glycinate, as prepared by the method of Pinto *et al.*,[36] was used as well as the Dieckmann cyclization conditions described by Deshmukh *et al.*[37] The racemic *trans*-isomer was purified by chromatography (EtOAc–hexanes, 1 : 2 → 1 : 1 → EtOAc) and the resulting gum crystallized at −20 °C (44% from the glycinate on the 5 mmol scale). A small sample was recrystallised at −20 °C from EtOAc–hexanes to give colourless needles, mp 52–53 °C, NMR δ_H (300 MHz; $CDCl_3$): 1.26 (3 H, t, *J* 7.1, CH_2CH_3), 2.32 (1 H, br. s, OH, exchanged to D_2O), 2.55 (1 H, dd, $J_{2,2'}$ 9.4, $J_{2,3}$ 7.4, H-2), 2.65 (1 H, dd, $J_{5,5'}$ 10.0, $J_{5,4}$ 5.5, H-5), 2.76 (1 H, dd, $J_{5',4}$ 2.8, H-5′), 2.95 (1 H, dt, $J_{3,2} = J_{3,2'}$ 8.0, $J_{3,4}$ 3.3, H-3), 3.12 (1 H, t, *J* 9.0, H-2′), 3.64 (2 H, s, $PhCH_2$), 4.16 (2 H, q, *J* 7.1 CH_2CH_3), 4.51 (1 H, m, H-4), 7.22–7.37 (5 H, m, Ar); δ_C (75.5 MHz; $CDCl_3$) 14.2 (Me), 53.1 (C-3), 55.3 (C-2), 59.7 ($PhCH_2$), 60.8 (CH_3CH_2), 61.9 (C-5), 74.1 (C-4), 127.1 (ArH), 128.3 (ArH), 128.8 (ArH), 138.2 (Ar), 173.3 (CO); HRMS (EI+) *m*/*z* 249.1365; $C_{14}H_{19}NO_3$ (M^+) requires 249.1365. (Found: C, 67.6; H, 7.5; N, 5.6; $C_{14}H_{19}NO_3$ requires C, 67.5; H, 7.7; N, 5.6%).

Ethyl (*R*,*S*/*S*,*R*)-4-(acetyloxy)-1-benzylpyrrolidine-3-carboxylate [(±)-19]

Racemate **18** (100 mg, 0.4 mmol) was dissolved in a mixture of pyridine (4 ml) and Ac_2O (2 ml) and left at 20 °C overnight. The solvent was evaporated and the resulting oil dissolved in EtOAc and washed with aqueous $NaHCO_3$ (saturated), dried and the solvent was again evaporated. The residue was chromatographed (EtOAc–hexanes, 15 : 85) to afford diester (±)-**19** as a colourless oil (111 mg, 95%) which was stored at −20 °C, NMR δ_H (300 MHz; $CDCl_3$) 1.25 (3 H, t, *J* 7.1, CH_2CH_3), 2.04 (3 H, s, $COCH_3$), 2.50 (1 H, t, $J_{2,2'} = J_{2,3}$ 8.3, H-2), 2.74–2.87 (2 H, m, H-5,5′), 3.06 (1 H, dt, $J_{3,2} = J_{3,2'}$ 7.9, $J_{3,4}$ 3.9, H-3), 3.15 (1 H, t, *J* 8.5, H-2′), 3.59 (1 H, d, *J* 12.9, $PhCHH$), 3.65 (1 H, d, $PhCHH$), 4.16 (2 H, q, *J* 7.1, CH_2CH_3) 5.40 (1 H, m, H-4), 7.22–7.38 (5 H,

m, Ar); δ_C (75.5 MHz; $CDCl_3$) 14.1 (CH_2CH_3), 21.0 ($COCH_3$), 50.1 (C-3), 56.0 (C-2), 59.5 (PhCH_2 or C-5), 59.6 (PhCH_2 or C-5), 61.0 (CH_2CH_3), 76.0 (C-4), 127.2 (ArH), 128.3 (ArH), 128.7 (ArH), 138.0 (Ar), 170.5 (CO), 172.3 (CO); HRMS (FAB+) *m/z* 292.1563; $C_{16}H_{22}NO_4$ $(M + H)^+$ requires 292.1549.

(*R*,*R*/*S*,*S*)-1-Benzyl-4-(hydroxymethyl)pyrrolidin-3-ol [(±)-20]

Racemate **18** (500 mg, 2.01 mmol) was dissolved in dry Et_2O–dry THF, (10 ml : 5 ml) and cooled in an ice bath. Lithium aluminium hydride in Et_2O (4.2 ml, 1 M, 4.2 mmol) was added, and the mixture warmed to 20 °C and stirred for 1 h. After cooling of the solution in an ice bath excess hydride was quenched by the dropwise addition of water (0.50 ml) and the mixture was extracted with EtOAc. The organic extract was washed with aqueous $NaHCO_3$ (saturated), dried and evaporated to give an oily residue that was chromatographed [CH_2Cl_2–MeOH–NH_4OH (0.88), 95 : 5 : 0.5 → 90 : 10 : 0.5] to give racemic diol **20** as a colourless gum (364 mg, 88%), NMR δ_H (300 MHz; CD_3OD) 2.18 (1 H, m, H-4), 2.34 (1 H, dd, $J_{5,5'}$ 9.6, $J_{5,4}$ 6.6, H-5), 2.55 (1 H, dd, $J_{2,2'}$ 10.0, $J_{2,3}$ 4.1, H-2), 2.72 (1 H, dd, $J_{2',3}$ 6.3, H-2′), 2.89 (1 H, t, $J_{5',4} = J_{5',5}$ 8.8, H-5′), 3.47–3.68 (4 H, m, PhCH_2, CH_2O), 4.00 (1 H, m, H-3), 7.20–7.42 (5 H, m, Ar); δ_C (75.5 MHz; CD_3OD) 51.2 (C-4), 57.3 (C-5), 61.5 (PhCH_2 or CH_2O), 63.1 (C-2), 64.2 (PhCH_2 or CH_2O), 74.1 (C-3) 128.3 (ArH), 129.3 (ArH), 130.2 (ArH), 139.4 (Ar); HRMS (FAB+) *m/z* 208.1346; $C_{12}H_{18}NO_2$ $(M + H)^+$ requires 208.1338.

Ethyl (3*S*,4*R*)-1-benzyl-4-hydroxypyrrolidine-3-carboxylate [(+)-18] and ethyl (3*R*,4*S*)-4-(acetyloxy)-1-benzylpyrrolidine-3-carboxylate [(−)-19]

Vinyl acetate (6.66 ml, 72.21 mmol) and Novozyme® 435 lipase from *Candida antarctica* (4.2 g, Novozymes Australia Pty. Ltd, batch LC200207) were added sequentially to a solution of (±)-**18** (6.00 g, 24.1 mmol) in *tert*-butyl methyl ether (200 ml). The mixture was stirred at 40 °C for 2.5 h, filtered through Celite®, the solids were washed with a little ethyl acetate and the combined filtrates were washed with aqueous $NaHCO_3$ (saturated), dried and evaporated. ^{1}H NMR analysis indicated that the residue consisted of alcohol **18** and acetate **19** in equimolar proportions. It was chromatographed (EtOAc–hexanes, 6 : 4) to give first (−)-**19** as a colourless gum (3.44 g, 97%) that was stored at −20 °C, $[\alpha]_D^{21}$ −41.5 (*c* 0.74, $CHCl_3$). The ^{1}H NMR spectrum was identical to that for compound (±)-**19** above. Further elution of the column with EtOAc gave (+)-**18** also as a colourless gum which crystallized at −20 °C (2.53 g, 85%), mp 51–52 °C, $[\alpha]_D^{21}$ +16.9 (*c* 0.71, $CHCl_3$). The ^{1}H NMR spectrum was identical to that for compound (±)-**18** above.

Repetition of the enzymic acetylation with (±)-**18** (0.80 g, 3.21 mmol) under the same conditions, but for 100 min, gave a mixture of **18** and **19** in the approximate ratio of 1.2 : 1 (^{1}H NMR determination). After chromatographic separation, pure (−)-**19** (406 mg, 96%), $[\alpha]_D^{21}$ − 41.8 (*c* 0.895, $CHCl_3$) and impure (+)-**18** (0.393 g, 89%), $[\alpha]_D^{21}$ +14.0 (*c* 0.81 $CHCl_3$) were isolated. The latter contained about 10% of the unreacted (−)-enantiomer.

(3*R*,4*R*)-1-Benzyl-4-(hydroxymethyl)pyrrolidin-3-ol [(+)-20]

Compound (+)-**18** (2.53 g, 10.15 mmol) was reduced, as indicated for the racemic compound, to give (+)-**20** as a colourless gum (1.54 g, 73%), $[\alpha]_D^{21}$ +33.0 (*c* 0.75, MeOH). The ^{1}H NMR spectrum was identical to that of compound (±)-**20**.

***tert*-Butyl (3*R*,4*R*)-3-hydroxy-4-(hydroxymethyl)pyrrolidine-1-carboxylate [(+)-23]**

A. From diol (+)-**20**. Pd/C (300 mg, 10%) was added to a stirred solution of the diol (+)-**20** (1.49 g, 7.19 mmol) and di-*tert*-butyl dicarbonate (1.63 g, 7.47 mmol) in MeOH (30 ml), and hydrogen was added from a balloon over 24 h. The mixture was filtered through Celite®, the solvent was evaporated and the residue was chromatographed (EtOAc–MeOH, 19 : 1) to afford the *N*-Boc protected pyrrolidine (+)-**23** as a colourless gum (1.56 g, 100%), $[\alpha]_D^{21}$ +15.9 (*c* 1.09, MeOH), in good agreement with the value derived from the sample made by method B.

B. From 1,2:5,6-di-*O*-isopropylidine-α-D-glucose. 3-*C*-Azidomethyl-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucose (42.6 g, 142 mmol), which was made from 1,2:5,6-di-*O*-isopropylidine-α-D-glucose, hydrolysed and reduced as previously described,[26] gave the unprotected pyrrolidine from which, in MeOH, (500 ml), compound **22** was obtained by treatment with di-*tert*-butyl dicarbonate (40 g, 185 mmol) and Et_3N (25.7 ml, 185 mmol). The volatiles were removed and the residue was adsorbed on silica gel and chromatographed to give crude carbamate **22** (26.7 g, 68%). The product was dissolved in EtOH (500 ml), cooled in an ice bath and oxidised by the dropwise addition of $NaIO_4$ (47 g, 0.22 mol) in water (500 ml). After recooling of the products in an ice-bath the product was reduced with $NaBH_4$ (7.3 g, 0.19 mmol) added portion-wise. The mixture was warmed to room temperature, the solids were removed by filtration, the volatiles by evaporation and the residue was purified by chromatography ($CHCl_3$–MeOH, 9 : 1). Compound (+)-**23** was obtained as a light yellow syrup (17 g, 81%) which gave ^{1}H and ^{13}C NMR data in agreement with those of the sample made by method A and with literature data.[12] A sample of compound (+)-**23** (50 mg), prepared in this way, in EtOAc was further purified by washing with water and then brine to give a colourless syrup (28 mg) after solvent evaporation, $[\alpha]_D^{21}$ +16.2 (*c* 0.795, MeOH).

(3*R*,4*R*)-4-(Hydroxymethyl)pyrrolidin-3-ol [(+)-16] and its hydrochloride [(+)-16·HCl]

A. From carbamate **23**. A sample of compound (+)-**23** (28 mg) was dissolved in MeOH (2 ml) and HCl (37%, 1 ml) and after a few mins the solvent was evaporated to give (+)-**16**·HCl, $[\alpha]_D^{21}$ +18.9 (*c* 0.92, MeOH). The ^{1}H NMR spectrum was identical to that of the sample made from diol (+)-**20** (method B).

B. From (+)-**20**. Diol (+)-**20** (52 mg, 0.25 mmol) was dissolved in MeOH, HCOOH (98%) (9 : 1, 8 ml) and Pd/C (10%, 80 mg) was added.[38] The mixture was heated under reflux for 30 min, filtered through Celite® and the solvent evaporated. Chromatography [CH_2Cl_2–MeOH–NH_4OH (0.88)–H_2O, 4 : 3 : 0.5 : 0.5] gave the unprotected pyrrolidine as a colourless gum (16 mg, 55%) which darkened slowly on standing. The ^{1}H NMR spectrum (CD_3OD) was in agreement with literature spectral data.[25] The product was dissolved in MeOH (2 ml), HCl (5%, 1 ml) and the solvents were evaporated to give the hydrochloride (+)-**16**·HCl (21 mg, 55%)

as a colourless gum, $[\alpha]_D^{21}$ +19.1 (*c* 1.05, MeOH), lit.[23] $[\alpha]_D^{25}$ +19.0 (*c* 1.0, MeOH). The ^{1}H NMR spectrum (D_2O) was in agreement with the literature spectral data[23] and was identical to that of the compound made by method A.

(3*S*,4*S*)-1-Benzyl-4-(hydroxymethyl)pyrrolidin-3-ol [(−)-20]

Compound (−)-**19** (400 mg, 1.37 mmol) was dissolved in Et_2O (9 ml) and THF (4 ml) and treated with lithium aluminium hydride in Et_2O (5.62 ml, 1 M, 5.62 mmol) as described for the preparation of compound (±)-**20** above to afford (−)-**20** as a colourless gum (190 mg, 67%), $[\alpha]_D^{21}$ −33.4 (*c* 0.805, MeOH). The ^{1}H NMR spectrum was identical to that of (±)-**20**.

(3*S*,4*S*)-4-(Hydroxymethyl)pyrrolidin-3-ol [(−)-16] and its hydrochloride [(−)-16·HCl]

Compound (−)-**20** (189 mg, 0.91 mmol) was de-*N*-benzylated[38] as for the (+)-enantiomer to give the unprotected amine (−)-**16** as a colourless gum (107 mg, 100%), a portion of which (30 mg) was converted to the hydrochloride salt (−)-**16**·HCl (39 mg), $[\alpha]_D^{21}$ −18.9 (*c* 0.74, MeOH), lit.[23] $[\alpha]_D^{25}$ −18.7 (*c* 1.2, MeOH). The ^{1}H NMR spectrum (D_2O) was in agreement with the literature data[23] and was identical to that of (+)-**16**·HCl.

(3*S*,4*S*)-1-[(9-Deazahypoxanthin-9-yl)methyl]-4-(hydroxymethyl)pyrrolidin-3-ol [L-DADMe–ImmH, (−)-6]

To a solution of (3*S*,4*S*)-4-(hydroxymethyl)pyrrolidin-3-ol free base (−)-**16**, (77 mg, 0.66 mmol) in H_2O (1.5 ml) were added 9-deazahypoxanthine (**17**)[39] (81 mg, 0.60 mmol) and aqueous formaldehyde (53 µl, 12.3 M, 0.65 mmol). The mixture was heated at 85 °C for 15 h (a small amount of precipitate formed), silica gel was added to absorb the solvent, the solvent was evaporated and the granular residue added to a column of silica gel and eluted with CH_2Cl_2–MeOH–NH_4OH (0.88), 5 : 4.5 : 0.5 to afford the nucleoside analogue (−)-**6** as a colourless solid (82 mg, 48%) after washing with a little cold MeOH, $[\alpha]_D^{21}$ −16.8 (*c* 0.71, H_2O). A sample of the (3*R*,4*R*)-enantiomer (+)-**6**, prepared during the present work, and ultimately derived from D-glucose *via* the sequence (+)-**22** → (+)-**23** → (+)-**16** → (+)-**6**, had $[\alpha]_D^{21}$ +16.9 (*c* 0.935, H_2O). The ^{1}H NMR spectrum of compound (−)-**6** was in agreement with the literature data for (+)-**6**[12] and with the spectrum of the latter isomer made during the present work.

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