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C-Branched Iminosugars: α-Glucosidase Inhibition by Enantiomers of isoDMDP, isoDGDP, and isoDAB—L-isoDMDP Compared to Miglitol and Miglustat

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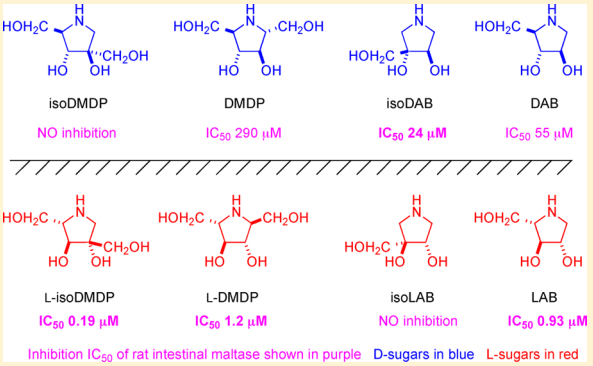
Supporting Information

ABSTRACT: The Ho crossed aldol condensation provides access to a series of carbon branched iminosugars as exemplified by the synthesis of enantiomeric pairs of isoDMDP, isoDGDP, and isoDAB, allowing comparison of their biological activities with three linear isomeric natural products DMDP, DGDP, and DAB and their enantiomers. L-IsoDMDP [(2*S*,3*S*,4*R*)-2,4-bis-(hydroxymethyl)pyrrolidine-3,4-diol], prepared in 11 steps in an overall yield of 45% from D-lyxonolactone, is a potent specific competitive inhibitor of gut disaccharidases [K_i 0.081 μM for rat intestinal maltase] and is more effective in the suppression of hyperglycaemia in a maltose loading test than miglitol, a drug presently used in the treatment of late onset diabetes. The partial rescue of the defective F508del-CFTR function in CF-KM4 cells by L-isoDMDP is compared with miglustat and isoLAB in an approach to the treatment of cystic fibrosis.



INTRODUCTION

The two drugs derived from iminosugars presently on the market are *N*-alkyl derivatives of deoxynojirimycin (DNJ, **1**). Miglitol (**2**) is well established for the treatment of late onset diabetes.[1] Miglustat (*N*-butyl DNJ, Zavesca, **3**) is licensed for substrate reduction therapy (SRT) in Gaucher's disease[2] with potential for the management of Niemann–Pick disease[3] and for the chemotherapeutic treatment of cystic fibrosis (CF).[4] The *N*-acetylhexosaminidase inhibitor siastatin B (**4**),[5,6] isolated from *Streptomyces verticillus* var. *quintum*,[7] is the sole example of a naturally occurring branched *C*-iminosugar; all the iminosugars yet isolated from plants have a linear carbon chain.[8] Usually, introduction of a carbon branch into the pyrrolidine or piperidine ring of an iminosugar leads to a loss of glycosidase inhibition;[9] however, a number of synthetic analogues with carbon branches have significant bioactivity.[10] DADMe-immucillinH **5** and its enantiomer are both nanomolar inhibitors of purine nucleoside phosphorylase (Figure 1).[11] 4-*C*-MethylDAB (**6**) and its enantiomer are specific micromolar inhibitors of α-glucosidases.[12] Both isofagomine (**7**)[13] and noeuromycin (**8**)[14] were designed as β-glucosidase inhibitors; isofagomine (**7**) has been studied as a potential chemical pharmacological chaperone for Gaucher's disease[15] and as an inhibitor of glycogen phosphorylase for the treatment of diabetes.[16]

This paper reports the syntheses of isoDMDP (**9D**), isoDGDP (**10D**), and isoDAB (**11D**) and their enantiomers **9L**, **10L**, and **11L**, respectively, as branched analogues of the natural products DMDP (**12**) (the most widely naturally occurring iminosugar[17]), DGDP (**13**) [from the Thai traditional drug "Non tai yak" (*Stemona tuberosa*)[18]], and DAB (**14**) (isolated from *Arachniodes standishii* and *Angylocalyx boutiqueanus*[19]) (Figure 2). The branched iminosugars have been studied

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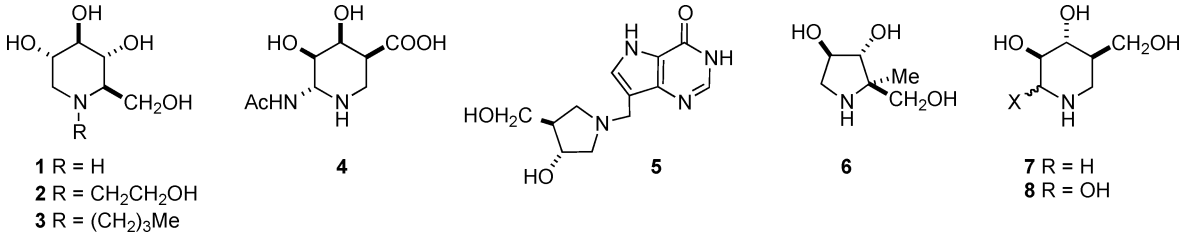


Figure 1. Structures of iminosugars.

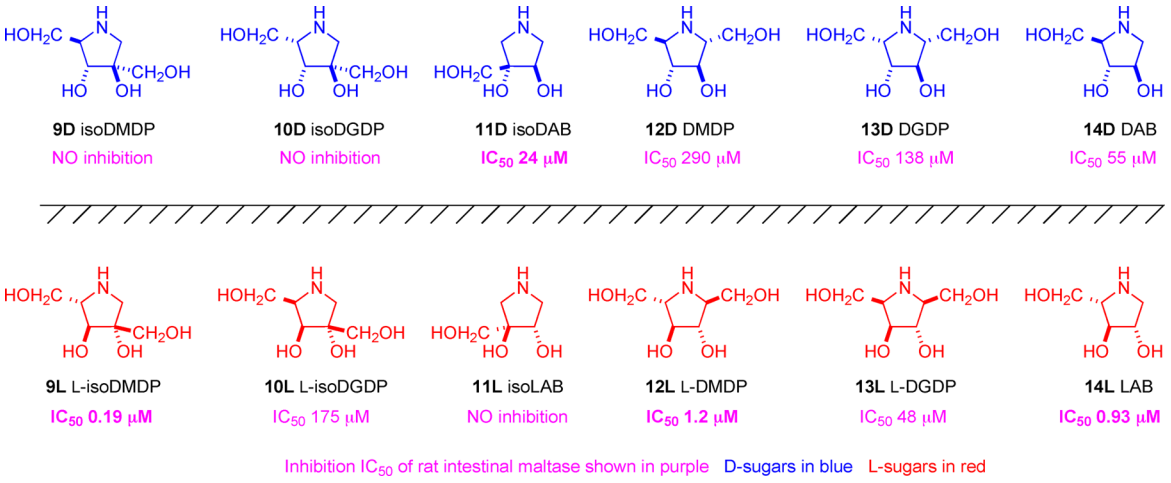
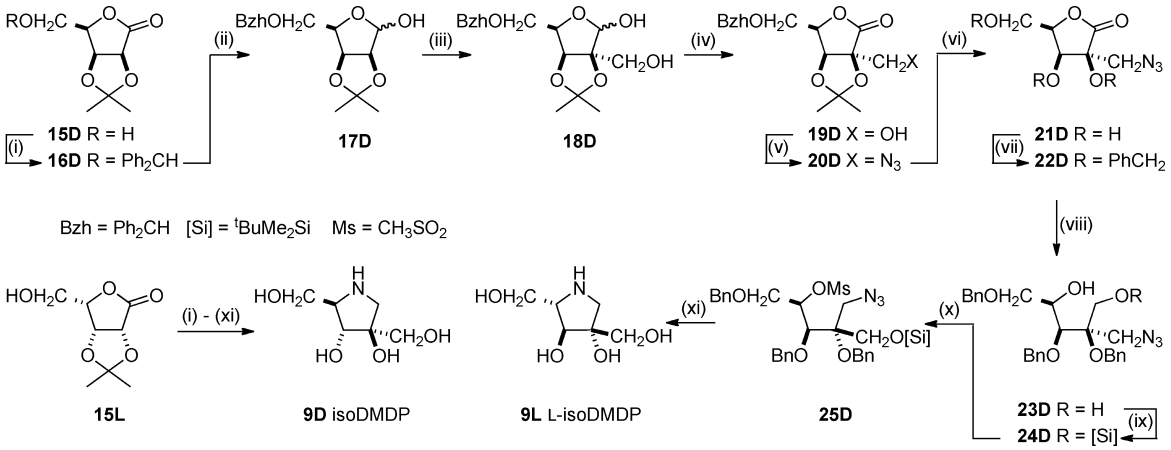


Figure 2. Synthetic targets and inhibition (IC_{50}) of a digestive α-glucosidase (rat intestinal maltase) by pyrrolidines.

Scheme 1[a]



[a]Reagents and conditions: (i) Ph_2CN_2, PhMe, reflux, 2 h, 92%; (ii) DIBALH, CH_2Cl_2, −78 °C, 1 h, 99%; (iii) CH_2O, K_2CO_3, MeOH, H_2O, reflux, 4 h, 74%; (iv) I_2, K_2CO_3, *t*-BuOH, reflux, 1 h, 85%; (v) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2, −30 °C, 1 h; then NaN_3, DMF, rt, 18 h, 100%; (vi) AcCl, MeOH, reflux, 18 h, 97%; (vii) BnBr, NaH, DMF, 0 °C, 4.5 h, 86%; (viii) $NaBH_4$, EtOH, rt, 3 h, 100%; (ix) *t*-$BuMe_2SiCl$, imidazole, DMF, 0 °C to rt, 6 h, 94%; (x) MsCl, Et_3N, CH_2Cl_2, 0 °C to rt, 2 h, 99%; (xi) H_2, Pd (10% on C), NaOAc, H_2O, 1,4-dioxane, rt, 24 h; then H_2, Pd (10% on C), HCl, H_2O, 1,4-dioxane, rt, 48 h, 100%.

in comparison to their linear carbon chain counterparts as inhibitors of a range of glycosidases; the inhibition of a digestive α-glucosidase (rat intestinal maltase) is summarized in Figure 2. L-isoDMDP (**9L**), one of the most potent and specific inhibitors of α-glucosidases, was compared to miglitol (**2**) in mice for the control of blood sugar levels and may have value in the study of late onset diabetes. Iminosugars have been identified as pharmacological chaperones which can stabilize or correct the structure of proteins; the partial rescue by L-isoDMDP (**9L**) of the defective F508del-CFTR function in CF-KM4 cells is compared with miglustat (**3**)[20] and isoLAB (**11L**) as potential agents for the treatment of CF.[21]

RESULTS AND DISCUSSION

1. Synthesis. Usually, treatment of an unprotected sugar with aqueous base gives complicated mixtures arising from reverse aldol reactions, dehydration, epimerization, and Lobry de Bruyn rearrangements; treatment of glucose with aqueous calcium hydroxide for 30 min produces more than 50 identifiable compounds.[22] However, the Ho crossed aldol[23,24]

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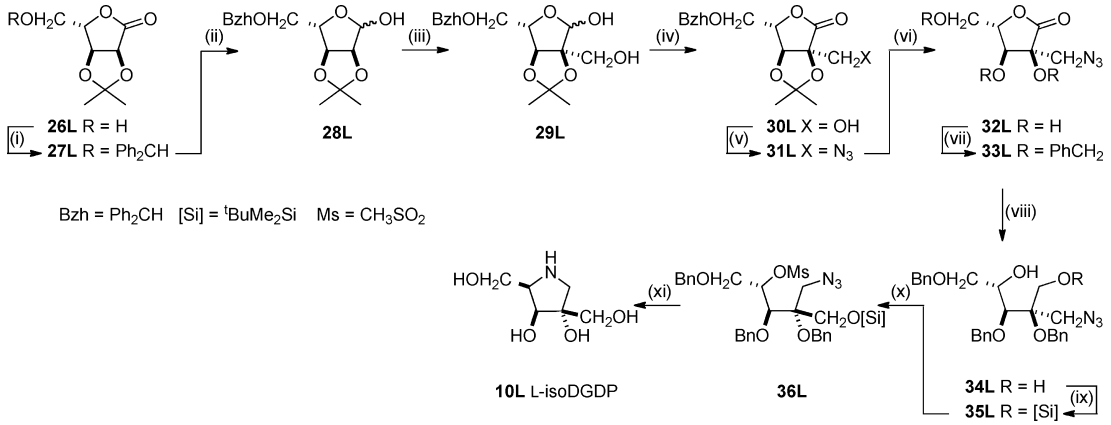
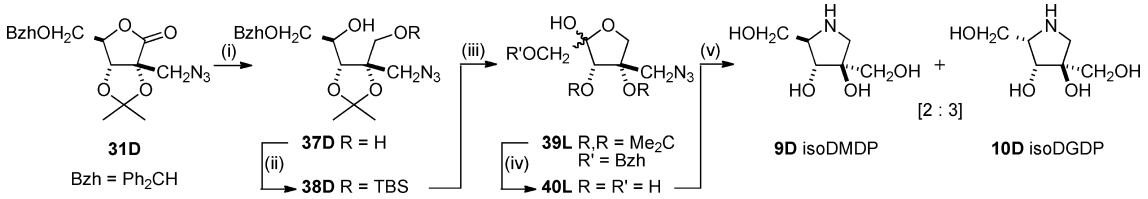
Scheme 2[a]



[a]Reagents and conditions: (i) Ph_2CN_2, PhMe, reflux, 1 h, 94%; (ii) DIBALH, CH_2Cl_2, −78 °C, 3 h, 98%; (iii) CH_2O, K_2CO_3, MeOH, H_2O, reflux, 2 h, 75%; (iv) I_2, K_2CO_3, *t*-BuOH, reflux, 1 h, 86%; (v) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2, −30 °C, 1 h; then NaN_3, DMF, rt, 18 h, 91%; (vi) AcCl, MeOH, reflux, 18 h, 66%; (vii) BnBr, NaH, DMF, 0 °C, 3.5 h, 65%; (viii) $NaBH_4$, EtOH, rt, 3 h, 93%; (ix) *t*-$BuMe_2SiOTf$, 2,6-lutidine, DCM, −78 °C, 6 h, 93%; (x) MsCl, pyridine, CH_2Cl_2, 0 °C to rt, 15 h, 76%; (xi) H_2, Pd (10% on C), NaOAc, H_2O, 1,4-dioxane, rt, 5 h; then H_2, Pd (10% on C), HCl, H_2O, 1,4-dioxane, rt, 54 h, 100%.

Scheme 3[a]



[a]Reagents and conditions: (i) $NaBH_4$, EtOH/^{t}BuOH 13:2, rt, 1.5 h, 97%; (ii) *t*-$BuMe_2SiCl$, imidazole, DMF, 0 °C, 5 h, 98%; (iii) Dess−Martin periodinane, CH_2Cl_2, rt, 2 h; then TBAF, THF, 0 °C to rt, 2 h, 92%; (iv) *p*-TsOH, H_2O, 1,4-dioxane, 85 °C, 18 h, 63%; (v) H_2, Pd (10% on C), H_2O, rt, 18 h, 83%.

of aqueous formaldehyde in the presence of a base with an aldose protected at C2 and C3 by an isopropylidene group reliably introduces a branching hydroxymethyl group at C2 in good to excellent yield. For all the iso-iminosugars **9**−**11**, the branching hydroxymethyl group is introduced by a Ho reaction.

The acetonide **15D** of D-lyxonolactone, readily available from alkaline oxygenation of D-galactose,[25] was the starting material for the synthesis of L-isoDMDP (**9L**) (Scheme 1). The primary alcohol in **15D** was protected under neutral conditions by treatment with diphenyldiazomethane in refluxing toluene as the benzhydryl ether **16D** (92%);[26] it was not possible to convert base-sensitive **15D** to the corresponding benzyl ether, and silyl protection was inappropriate because of instability on later treatment with aqueous base.

The lactol **17D**, formed by reduction of **16D** with DIBALH in dichloromethane in a 5:1 ratio of anomers (99%), underwent the Ho reaction with aqueous formaldehyde in the presence of potassium carbonate in aqueous methanol to afford the branched lyxose derivative **18D** in an anomeric 3:1 ratio in 74% yield. Oxidation of the lactol **18D** by bromine was not compatible with a benzhydryl protecting group; however, iodine in *tert*-butyl alcohol in the presence of potassium carbonate[27] formed the lactone **19D** (85%). Esterification of the highly hindered neopentyl alcohol in **19D** with triflic anhydride in dichloromethane in the presence of pyridine gave the corresponding triflate which, on treatment with sodium azide in DMF, afforded the azide **20D** (100%). It was necessary to exchange the isopropylidene protecting group since pyrrolidine ring closure was not possible with the formation of a *trans*-fused acetonide. The protecting groups were removed from **20D** by hydrogen chloride in methanol to give the deprotected azidolactone **21D** (97%). The unprotected azidolactone **21D**, with no acidic proton at C2, was treated with benzyl bromide and sodium hydride in DMF to afford the tribenzyl azide **22D** (86%). Reduction of **22D** by sodium borohydride in ethanol formed the diol **23D** (100%) which was then protected as the primary silyl ether **24D** (94%). The remaining free hydroxyl group in **24D** was esterified with mesyl chloride in dichloromethane in the presence of triethylamine to afford the mesylate **25D** (99%). Hydrogenation of **25D** by palladium on carbon in aqueous dioxane in the presence of sodium acetate caused reduction of the azide to the amine and subsequent cyclization; further hydrogenation after addition of hydrochloric acid resulted in removal of both the silyl and benzyl protecting groups to give L-isoDMDP (**9L**) in quantitative yield. The overall yield for the synthesis of **9L** from the protected lyxonolactone **15D** was 45%. The enantiomer isoDMDP (**9D**) was prepared from L-lyxonolactone (**15L**), available on a kilogram scale from D-ribose,[28] by an identical procedure (overall yield 30%).

The same strategy provided access to L-isoDGDP (**10L**) from L-ribonolactone (**26L**),[29] epimeric at C4 with D-lyxonolactone (**15D**) (Scheme 2). A sequence of protection **27L**, reduction **28L**, introduction of the branching hydroxyl methyl group by the Ho crossed aldol reaction **29L**, oxidation **30L**, and introduction of the azide afforded the isopropylidene

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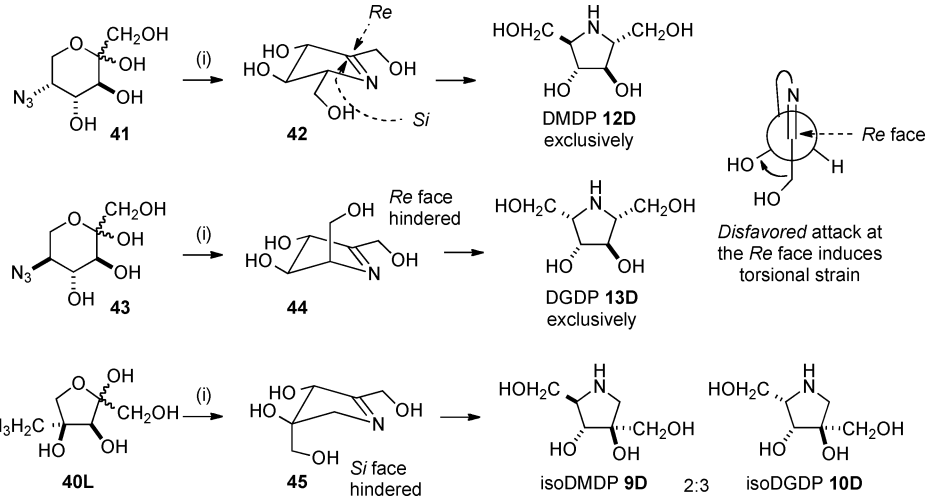
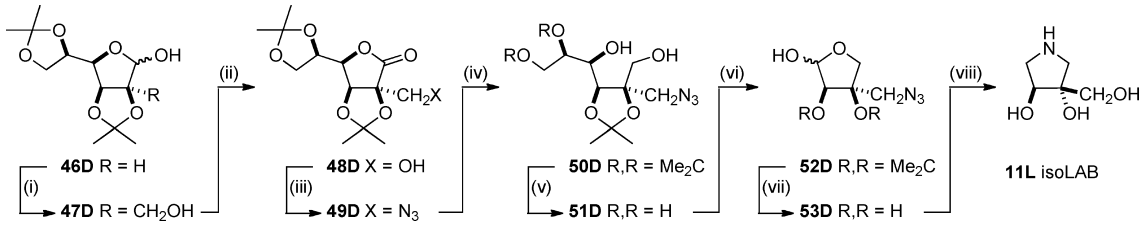
Scheme 4. Diastereoselectivity in the Hydrogenation of Azidoketoses[a]



[a]Reagents: (i) H_2, Pd/C, H_2O.

Scheme 5[a]



[a]Reagents and conditions: (i) CH_2O, K_2CO_3, MeOH, H_2O, reflux, 5 h, 74%; (ii) Br_2, $BaCO_3$, H_2O, 0 °C to rt, 32 h, 80%, or I_2, K_2CO_3, ^{t}BuOH, 100 °C, 81%; (iii) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2, −30 °C; 2.5 h then NaN_3, DMF, rt, 3 h, 96%; (iv) DIBALH, CH_2Cl_2, −78 °C, 2 h; then $NaBH_4$, MeOH, 0 °C to rt, 2.5 h, 68%; (v) H_2O/AcOH 1:1, rt, 16 h, 78%; (vi) $NaIO_4$, H_2O, rt, 18 h, 85%; (vii) Dowex (50W-X8 H^+ form) H_2O/1,4-dioxane 4:1, 60 °C, 36 h, 91%; (viii) Pd/C (10%), H_2, H_2O/AcOH 9:1, rt, 24 h, 81%.

protected lactone **31L** in an overall yield of 54%. Subsequent functional group manipulation analogous to the synthesis of **9L** via **32L**, **33L**, **34L**, **35L**, and **36L** provided L-isoDGDP (**10L**) in an overall yield of 15% in 11 steps from **26L**.

A shorter synthesis, in which the pyrrolidine ring was formed by intramolecular reductive amination of an azido ketone **40L**, avoided the need for the switching of protecting groups but required separation of isoDMDP (**9D**) and isoDGDP (**10D**) as the final step (Scheme 3). Reduction of **31D**, prepared from D-ribonolactone **26D** as in Scheme 2, by sodium borohydride in ethanol/*tert*-butyl alcohol afforded diol **37D** (97%) in which the primary alcohol was selectively protected as the TBDMS ether **38D** (98%). Oxidation of **38D** with Dess−Martin periodinane gave the corresponding ketone which with TBAF formed the lactols **39L** (92%). Reaction of **39L** with *p*-toluenesulfonic acid in aqueous dioxane removed both the benzhydryl and acetonide protecting groups to give the unprotected azide **40L** (63%). Hydrogenation of **40L** in water in the presence of 10% palladium on carbon caused reduction of the azide to the corresponding amine and intramolecular reductive amination to produce a mixture of isoDMDP (**9D**) and isoDGDP (**10D**) in a ratio of 2:3 and a yield of 83% (46% from **31D**; 26% from **26D**). The crude reaction mixture of **9D** and **10D** was separated by ion exchange chromatography using Dowex 1 × 2 (OH^- form).[30]

The lack of diastereoselectivity in the hydrogenation of **40L** was in contrast to the selectivity found in the hydrogenations of the azido-fructose **41** to give only DMDP (**12D**)[31] and of the azido-sorbose **43** to form solely DGDP (**13D**) (Scheme 4).[32] In the case of intermediate imine **42**, neither the *Re* nor *Si* face of the C═N bond is more clearly sterically hindered. The exclusive formation of DMDP (**12D**), as rationalized by Wong, is due to the accumulation of torsional strain associated with addition to the *Re* face.[33] In the case of imine **44**, the cooperative combination of torsional strain and steric hindrance gives DGDP (**13D**) exclusively. In contrast, for branched imine **45** these steric and torsional factors oppose one another, resulting in a mixture of **9D** and **10D** with little stereoselectivity.

IsoLAB (**11L**) was prepared from diacetone mannose **46D**[34] in eight steps with an overall yield of 19% (Scheme 5). The Ho aldol reaction of **46D** introduced the hydroxymethyl branch to form **47D** (74%), which on bromine or iodine oxidation under basic conditions gave the lactone **48D** (80%, Br_2, 81%, I_2). In the early preparations of **48D** the mp was 130−132 °C.[35] On later syntheses, the mp was 107−108 °C as reported by Ho.[24] Both the ^{1}H and ^{13}C NMR samples from all procedures were identical; it is likely there are two different crystalline forms of **48D**. Esterification of the neopentyl alcohol in **48D** by triflic anhydride, followed by sodium azide in DMF, afforded the azide **49D** (96%). Reduction of **49D** by DIBALH in dichloromethane, followed by sodium borohydride in methanol afforded the diol **50D** (68%). The terminal acetonide in **50D** underwent selective hydrolysis with aqueous acetic acid to give

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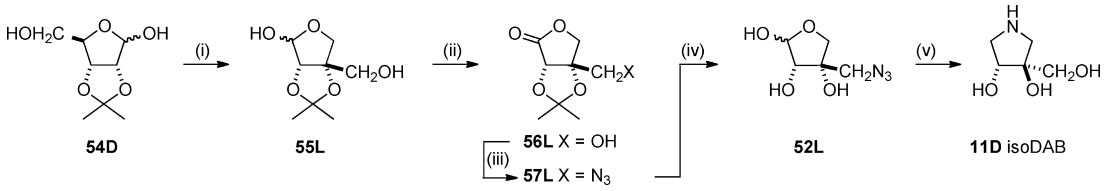
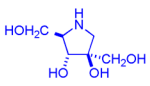
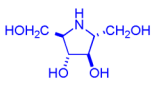
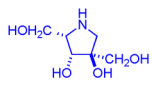
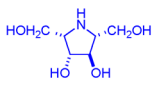
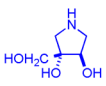
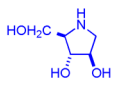
Scheme 6[a]



[a]Reagents and conditions: (i) CH_2O, K_2CO_3, MeOH, H_2O, reflux, 6 h; then $NaBH_4$, H_2O, rt, 1.33 h; then $NaIO_4$, H_2O, rt, 1 h, 84% (from **54D**); (ii) Br_2, $BaCO_3$, H_2O, 0 °C to rt, 3 h, 90%; (iii) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2, −30 °C, 1 h; then NaN_3, DMF, rt, 24 h, 67%; (iv) DIBALH, CH_2Cl_2, −78 °C, 1 h, 93%; (v) Dowex (50W-X8 H^+ form) H_2O/1,4-dioxane 4:1, 75 °C, 18 h; then Pd/C (10%), H_2, H_2O/AcOH 9:1, 24 h, 71%.

Table 1. Concentration of D-Iminosugars Giving 50% Inhibition of Various Glycosidases

		IC_{50} (μM)					
							
	enzyme	isoDMDP (9D)	DMDP (12D)	isoDGDP (10D)	DGDP (13D)	isoDAB (11D)	DAB (14D)
α-glucosidase							
	yeast	NI[a]	0.71	NI	167	NI	0.15
	rice	NI	214	NI	131	41	250
	rat intestinal maltase	NI	290	NI	138	24	55
	rat intestinal isomaltase	NI	91	NI	320	20	5.8
	rat intestinal sucrase	NI	40	639	143	15	16
β-glucosidase							
	almond	NI	10	NI	256	NI	250
	bovine liver	NI	9.7	NI	523	NI	638
α-galactosidase							
	coffee beans	NI	NI	NI	NI	NI	NI
β-galactosidase							
	bovine liver	NI	3.3	NI	361	NI	NI
	rat intestinal lactase	NI	7.9	791	41	NI	323
α-mannosidase							
	jack beans	NI	NI	NI	NI	NI	320
β-mannosidase							
	snail	206	721	NI	NI	NI	NI
α-L-fucosidase							
	bovine kidney	NI	NI	NI	NI	NI	NI
α,α-trehalase							
	porcine kidney	NI	200	NI	379	NI	4.8
α-L-rhamnosidase							
	Penicillium decumbens	NI	NI	NI	NI	NI	NI

[a]NI: No inhibition (less than 50% inhibition at 1000 μM).

the tetraol **51D** (78%). Oxidative cleavage of the terminal diol of **51D** with sodium periodate gave the protected azido-apiose **52D** (85%). Hydrolysis of the acetonide in **52D** with Dowex 50W-X8, H^+ form, formed the lactol **53D** (91%), hydrogenation of which in aqueous acetic acid in the presence of palladium on carbon afforded isoLAB (**11L**) (81%). The preparation of **11L** from D-mannose is experimentally easier than that previously reported from D-tagatose.[21]

For isoDAB (**11D**), the acetonide of D-ribose **54D** was treated sequentially in water with formaldehyde in the presence of potassium carbonate, sodium borohydride and sodium periodate to provide the acetonide of L-apiose (**55L**) on a multigram scale in an overall yield of 84% (Scheme 6). Oxidation of **55L** with bromine in the presence of barium carbonate gave **56L** (90%) which was converted to the triflate and treated with sodium azide in DMF to form the azidolactone **57L** (67%). Reduction of **57L** with DIBALH in dichloromethane gave the lactol **52L** (93%), which on acid hydrolysis and hydrogenation in the presence of palladium on carbon formed isoDAB (**11D**) in 71% yield (34% from ribose **54D**).

2. Biological Assays. Glycosidase inhibition of the iso-D-iminosugars (**9D**, **10D**, and **11D**) was compared with that of their naturally occurring unbranched analogues (DMDP (**12D**), DGDP (**13D**), and DAB (**14D**)) (Table 1); a similar study on the corresponding L-enantiomers is shown (Table 2).[36] The natural products DMDP (**12D**), DGDP (**13D**), and DAB (**14D**) are modest, and not very specific, competitive inhibitors of many α-glucosidases; DMDP (**12D**) and DAB (**14D**) show rare potent inhibition of yeast α-glucosidase. DMDP (**12D**)is a much more potent inhibitor of β-glucosidase and β-galactosidase. IsoDMDP (**9D**) showed weak but specific inhibition against β-mannosidase, whereas isoDGDP (**10D**) did not inhibit any glycosidase.

Unnaturally configured enantiomers of iminosugars frequently inhibit the same enzymes as their natural product analogues.[37] L-DMDP (**12L**), L-DGDP (**13L**), and LAB (**14L**) are much more potent and specific inhibitors of α-glucosidases than their natural D-enantiomers.[36] For the iso-iminosugars, both enantiomers of isoDGDP (**10D** and **10L**) showed only weak inhibition toward rat intestinal lactase. IsoDAB (**11D**) is a better and more specific α-glucosidase inhibitor than DAB

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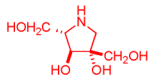
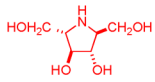
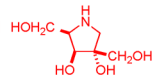
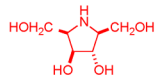
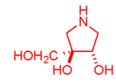
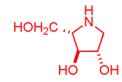
Table 2. Concentration of L-Iminosugars Giving 50% Inhibition of Various Glycosidases

		IC_{50} (μM)					
							
	enzyme	L-isoDMDP (9L)	L-DMDP (12L)	L-isoDGDP (10L)	L-DGDP (13L)	isoLAB (11L)	LAB (14L)
α-glucosidase							
	yeast	NI[a]	NI (34.9%)	NI	NI	NI	**70**
	rice	**2.0**	**5.8**	NI	**60**	NI	**3.2**
	rat intestinal maltase	**0.19** (Ki = 0.081)	**1.2**	175	**48**	NI	**0.93**
	rat intestinal isomaltase	**8.8**	**0.1**	961	**9.9**	NI	**0.36**
	rat intestinal sucrase	**0.38**	**1.4**	127	**11**	NI	**1.0**
β-glucosidase							
	almond	NI	NI	NI	NI	NI	NI
	bovine liver	NI	NI	NI	NI	NI	NI
α-galactosidase							
	coffee beans	NI	NI	NI	216	NI	NI
β-galactosidase							
	bovine liver	NI	NI	NI	NI	NI	NI
	rat intestinal lactase	NI	NI	743	652	NI	415
α-mannosidase							
	jack beans	NI	NI	NI	NI	NI	NI
β-mannosidase							
	snail	NI	NI	NI	NI	NI	NI
α-L-fucosidase							
	bovine kidney	NI	NI	NI	NI	NI	NI
α,α-trehalase							
	porcine kidney	NI	**48**	NI	NI	NI	131
α-L-rhamnosidase							
	Penicillium decumbens	NI	NI	NI	NI	NI	NI

[a]NI: No inhibition (less than 50% inhibition at 1000 μM).

(**14D**), but its enantiomer isoLAB (**11L**) did not inhibit any glycosidase unlike the potent inhibition shown by LAB (**14L**.) In startling contrast, L-isoDMDP (**9L**) is the most potent inhibitor of α-glucosidases by a parent iminosugar yet reported, and is completely specific; L-isoDMDP (**9L**) showed strong inhibition of rat intestinal maltase with IC_{50} value of 0.19 μM. This value is significantly better than clinically available miglitol (**2**). The kinetic analysis showed that L-isoDMDP (**9L**) is a competitive inhibitor of rat intestinal maltase with a K_i of 0.081 μM. Of the 12 iminosugars, only DAB (**14D**) (IC_{50} = 0.15 μM) and DMDP (**12D**) (IC_{50} = 0.71 μM) show potent inhibition of yeast α-glucosidase; as is true for most iminosugars, none of the branched iso-iminosugars have any significant inhibition of yeast α-glucosidase.

To confirm that L-isoDMDP (**9L**) had significant effects on blood glucose levels in vivo, a maltose loading test was conducted, using miglitol (**2**) as a positive control (Figure 3). The animal experimental protocols in this study were approved by the Animal Experiments Committee of the University of Toyama. Male ddY mice (29−33 g) after an overnight fast were used for acute disaccharide loading tests. The blood glucose levels were measured by the StatStrip Xpress kit (Nova Biochemical Co. Ltd.). A control group was loaded with saline only. Administration of maltose (2.5 g/kg body weight p.o.) to fasted mice resulted in a rapid increase in blood glucose concentrations from 95 ± 12 to a maximum of 253 ± 24 mg/dL after 15 min. Thereafter, blood glucose levels recovered to the pretreatment level at 120 min. A significant suppressive effect of the blood glucose level was achieved with 1.0 mg/kg body weight of L-isoDMDP (**9L**) after 15 and 30 min; 1.0 mg/kg body weight miglitol (**2**) also significantly decreased the blood glucose concentrations at 15 min after maltose-loading. However, the suppression effects were clearly weaker than L-isoDMDP (**9L**). These results suggested that L-isoDMDP (**9L**) is worthy of study as a potential therapeutic agent for the treatment of diabetes.

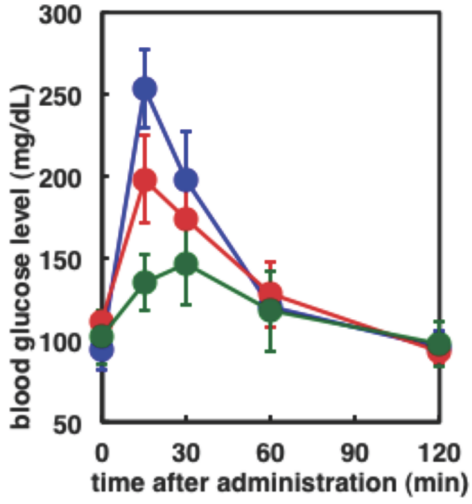


Figure 3. Effect of L-isoDMDP (**9L**) and miglitol (**2**) on blood glucose levels. Blood glucose concentrations of male ddY mice after an oral load with maltose, 2.5 g/kg body weight, with 1.0 mg/kg body weight L-isoDMDP **9L** (green circle). 1.0 mg/kg body weight miglitol **2** (red circle) was used as positive control. A control group was loaded with saline (blue circle). Each value represents the mean ± SEM (n = 5).

None of isoDMDP (**9D**), L-isoDMDP (**9L**), isoDAB (**11D**), or isoLAB (**11L**) showed any significant effect on endoplasmic reticulum (ER)-resident α-glucosidase I and II activity in cells at 0.5 mM using a free oligosaccharide assay.[38] IsoDMDP (**9D**) had no effect at the highest concentration tested (100 μM) whereas L-isoLDMDP (**9L**) at the same concentration showed

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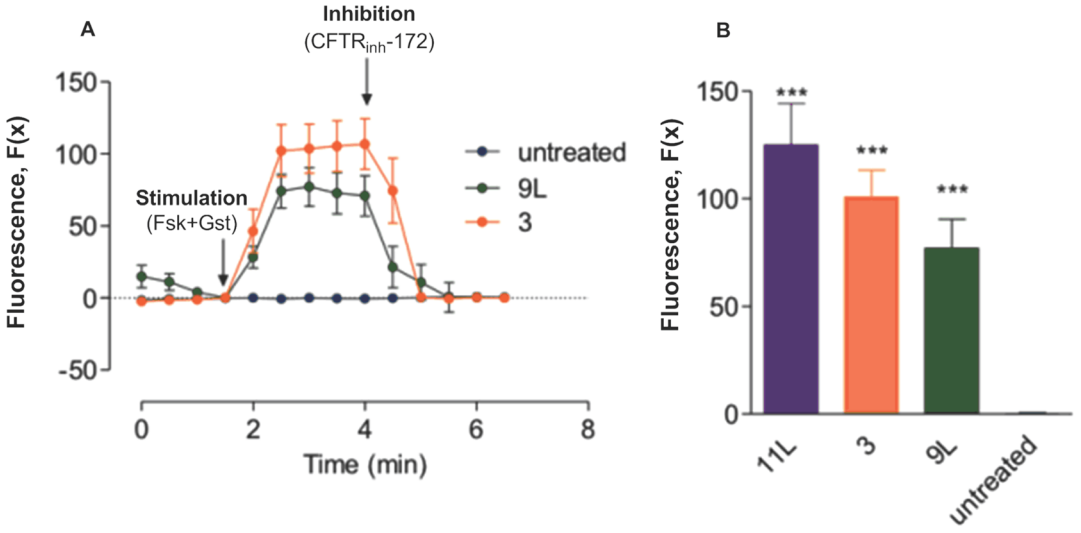

Figure 4. Effect of L-isoDMDP (**9L**), isoLAB (**11L**), and miglustat (**3**) on the activity of F508del-CFTR assessed by single-cell fluorescence imaging using the potential-sensitive fluorescent probe bis(1,3-diethylthiobarbituric acid) trimethine oxonol and its sensitivity to the CFTR selective inhibitor $CFTR_{inh}$-172 using the human airway epithelial CF-KM4 cell line. (A) Cells were incubated with the given iminosugar for 4 h at 100 μM, and the activity of CFTR channels were assessed by stimulation with 10 μM forskolin (Fsk) and 30 μM genistein (Gst), followed by inhibition with 10 μM $CFTR_{inh}$-172. In cells with rescued CFTR function, stimulation results in chloride ion transport and a change in cell potential that translates to a change in fluorescence in the presence of the potential-sensitive fluorescent probe. Subsequent treatment with CFTR-selective inhibitor $CFTR_{inh}$-172 results in a return to the base potential, suggesting that active CFTR is responsible for the change in potential following stimulation. Untreated cells with inactive CFTR do not respond to inhibition or stimulation. Each value represents the mean ± SEM (n = 48). See ref 21 for analogous assessment of **11L**. (B) Histograms summarizing the results collected from separate experiments; comparison of **11L**, **3**, and **9L**.

very modest inhibition (20- to 100-fold less effective than miglustat, **3**). At 1 mM, isoDAB (**11D**) showed weak inhibition of α-glucosidase II. These data suggest that the lack of significant inhibition of glucosidase processing enzymes is due to the inability to administer sufficient concentrations of these weak inhibitors to cells to observe any effects. DMDP (**12D**) and L-DMDP (**12L**) are not inhibitors of α-glucosidase I at 1 mM using in vitro assays. DAB (**14D**) and LAB (**14L**) are presumed inhibitors of processing glucosidases;[39] their *N*-butyl analogues are also weak inhibitors (IC_{50}, 319 μM and 769 μM, respectively) of α-glucosidase I[40] and are consequently ineffective at inhibiting glucosidase activity in cellular assays at concentrations of 1 mM or less.

Pharmacological chaperones (PC) are small molecules that target protein misfolding.[41] In particular, iminosugars have shown promise in the treatment of lysosomal storage diseases (LSD).[42] Fabry's disease is due to a deficiency of the α-galactosidase, α-GAL A.[43] Deoxygalactonojirimycin (DGJ), a competitive inhibitor of α-GAL A, is the C4 epimer of DNJ (**1**) and is in phase 3 clinical trials for the treatment of Fabry's disease.[44] The enantiomer of DGJ, a noncompetitive inhibitor of α-GAL A, showed additive benefits with DGJ for stabilization of α-GAL A; thus, it is not necessary in iminosugar PC therapy for the agent to bind to the active site of the enzyme.[45] This implies that there are other sites distinct from the active site where small molecules, such as iminosugars, can bind and increase thermal stabilization of the protein. The protein responsible for cystic fibrosis transmembrane conductance regulator (CFTR) function in CF is glycosylated, even though it does not involve any sugar metabolism; CFTR is an ABC transporter-class protein and ion channel that transports chloride ions across the apical membrane of epithelial cells. Mutations of the *CFTR* gene affect folding and/or functioning of the chloride channels in these cell membranes, causing CF. The most common CF mutation F508del causes misfolding of the protein and intracellular retention by the endoplasmic reticulum quality control and premature degradation; iminosugars may help in the correction of structure of the misfolded CFTR protein.

Inhibition of glycosyl transferase by miglustat (**3**) is the basis for its use in substrate reduction therapy (SRT) for the treatment of Gaucher's disease.[46] Both **3**[47] (an α-glucosidase inhibitor) and isoLAB (**11L**)[21] have been found to show significant rescue of the defective F508del-CFTR function as assessed by single-cell fluorescence imaging and sensitivity to the CFTR selective inhibitor $CFTR_{inh}$-172,[48] L-isoDMDP (**9L**) was compared with miglustat (**3**) and isoLAB (**11L**) for their corrector effect on CFTR function in CF-KM4 cells[49] using single-cell fluorescence imaging (Figure 4).[50] The correcting effect of L-isoDMDP (**9**) on F508del-CFTR function in CF-KM4 cells was tested by similar experiments; CFTR activity was first stimulated by a cocktail of forskolin (Fsk) + genistein (Gst) and subsequently inhibited by the selective inhibitor CFTRinh-172. As expected, the panel on the left (Figure 4) shows an absence of Fsk + Gst induced response on untreated cells (blue circles). In contrast, after 4 h of treatment with **3** (orange circles) or **9L** (green circles), a significant increase of the fluorescence after addition of the cocktail was observed, demonstrating the restoration of CFTR activity to the plasma membrane in CF-KM4 cells. The histograms in Figure 4 report the variation of fluorescence notes F(x) induced by CFTR stimulation in CF-KM4 after treatment by **11L**, **3**, or **9L** and compare the relative effectiveness of their correction. Although L-isoDMDP (**9L**) shows significant rescue of F508del-CFTR activity, it has less effect than either isoLAB (**11L**) (the most potent corrector) or miglustat (**3**). The majority of iminosugars which are glucosidase inhibitors, for example, miglitol (**2**) shows no rescue of F508del-CFTR activity. The mechanism by which iminosugars show such effects are not clear but may involve binding to the glycosylated site of the protein; although

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α-glucosidase inhibitors such as **3** and **9L** are effective, it is noteworthy that isoLAB (**11L**) is the most effective iminosugar corrector so far described, even though it is not an α-glucosidase inhibitor.

CONCLUSION

The value of the Ho crossed-aldol condensation for the controlled introduction and functional group manipulation of incipient diastereomeric hydroxymethyl groups for the reliable synthesis of *C*-branched iminosugars is firmly established. The S_N2 displacement of triflate derived from a neopentyl alcohol by azide consistently proceeds in high yield.

The inhibition profile of α-glucosidases by branched iminosugar pyrrolidines is complex. L-isoDMDP (**9L**) is the most potent parent iminosugar of digestive enzymes yet described, whereas there is no inhibition by isoDMDP (**9D**); this parallels, though exceeds, the comparative behavior of L-DMDP (**12L**) and DMDP (**12D**). In contrast, isoDAB (**11D**) is a good inhibitor whereas isoLAB (**11L**) shows no inhibition of the digestive enzymes; this is counter to the potent glucosidase inhibition by LAB (**14L**) and the relatively weak inhibition of glucosidases − other than yeast − shown by DAB (**14D**). DMDP (**12D**) and DAB (**14D**) are the only iminosugars to show any significant inhibition of yeast α-glucosidase. None of the iso-iminosugars show any inhibition of ER glucosidases, indicating that in the treatment of CF and diabetes unwanted side effects would be avoided. Miglitol (**2**) is a well-established drug for the treatment of late-onset diabetes. The specificity and potency of L-isoDMDP (**9L**), together with the demonstration of superior control of blood glucose levels, indicate there may be a second generation of iminosugars for the treatment of the disease. Although it is not clear by what mechanism iminosugars are effective in the partial rescue of the defective F508del-CFTR function in CF-KM4 cells, it may well be that binding to other glycosylated sites of the protein not associated with enzyme activity may provide effective PC control. Further studies of iminosugars that have no glycosidase activity, and therefore potentially fewer side effects, may have a significant role in the treatment of diseases involving misfolded proteins. Such bioactivity by small five- or six-carbon pyrrolidines indicate iminosugars will have a major role in disease therapy that is not associated with glycosidase inhibition.

EXPERIMENTAL SECTION

General Experimental Methods. All commercial reagents were used as supplied. Thin-layer chromatography (TLC) was performed on aluminum sheets coated with 60 F_{254} silica. Plates were visualized using a spray of 0.2% w/v cerium(IV) sulfate and 5% ammonium molybdate solution in 2 M aqueous sulfuric acid. Flash chromatography was performed on Sorbsil C60 40/60 silica. Melting points were recorded on a Kofler hot block and are uncorrected. Optical rotations are quoted in 10^3 deg·cm^2·g^{-1} at concentrations (*c*) in g·100 mL^{-1}. ^{1}H and ^{13}C NMR spectra were assigned by utilizing 2D COSY and HSQC spectra. All chemical shifts (δ) are quoted in ppm and coupling constants (*J*) in Hz. Residual signals from the solvents were used as an internal reference.[51] For solutions in D_2O acetonitrile was used as an internal reference. For anomeric mixtures, superscript A denotes the major anomer and superscript B the minor. HRMS measurements were made using a microTOF mass analyzer.

A. Synthesis of L-Iso-DMDP (9L) and IsoDMDP (9D). *5-O-Benzhydryl-2,3-O-isopropylidene-D-lyxono-1,4-lactone (16D).* Diphenyldiazomethane (2.80 g, 14.4 mmol) was added to a refluxing solution of lactone **15D** (1.81 g, 9.63 mmol) in toluene (180 mL). The reaction mixture was stirred at reflux for 1 h, after which no purple color remained and TLC analysis (1:1 EtOAc/cyclohexane) revealed the presence of a small amount of unreacted starting material (R_f 0.10) and one major product (0.70). A further portion of diphenyldiazomethane (0.93 g, 4.8 mmol) was added, and after a further 1 h, the reaction mixture was concentrated in vacuo and the residue purified by flash column chromatography (1:50 → 1:10 → 1:8 EtOAc/cyclohexane) to afford the title lactone **16D** (3.12 g, 92%) as a colorless oil: HRMS (ESI+ve) found 377.1363 [M + Na]$^+$, $C_{21}H_{22}NaO_5$ requires 377.1359; $[\alpha]^{25}_D$ +35.7 (*c* 1.00, $CHCl_3$); ν_{max} (thin film) 1789 (s, C═O); δ_H (400 MHz, $CDCl_3$) 1.38 (3H, s, CH_3), 1.42 (3H, s, CH_3), 3.82 (1H, dd, H5a, J_{gem} 10.7, $J_{5a,4}$ 6.9), 3.88 (1H, dd, H5b, J_{gem} 10.9, $J_{5b,4}$ 5.1), 4.72 (1H, ddd, H4, $J_{4,5a}$ 7.1, $J_{4,5b}$ 4.7, $J_{4,3}$ 3.0), 4.80−4.84 (2H, m, H2, H3), 5.48 (1H, s, $CHPh_2$), 7.25−7.39 (10H, m, ArH); δ_C (100.6 MHz, $CDCl_3$) 25.9 (CH_3), 26.7 (CH_3), 66.8 (C5), 75.9 (C2), 76.0 (C3), 78.2 (C4), 84.4 ($CHPh_2$), 114.2 ($C(CH_3)_2$), 126.9, 127.2, 127.7, 127.7, 128.4 (ArCH), 141.4, 141.5 (ArC), 173.6 (C1); *m*/*z* (ESI +ve) 731 ([2M + Na]$^+$, 100), (ESI−ve): 391 ([M + ^{37}Cl]$^-$, 35), 389 ([M + ^{35}Cl]$^-$, 100), 371 ([M + OH]$^-$, 70).

Enantiomer **16L**: $[\alpha]^{25}_D$ −37.9 (*c* 1.80, $CHCl_3$).

5-O-Benzhydryl-2,3-O-isopropylidene-D-lyxofuranose (17D). DIBALH (1.5 M in toluene, 0.87 mL, 1.3 mmol) was added to a solution of lactone **16D** (417 mg, 1.18 mmol) in DCM (6 mL) at −78 °C. The reaction was stirred at −78 °C for 1 h after which time TLC (1:3 EtOAc/cyclohexane) showed the complete consumption of the starting material (R_f 0.24) and the formation of one major product (R_f 0.29). The reaction was quenched with methanol (0.5 mL), diluted with CH_2Cl_2 (5 mL), and stirred with sodium potassium tartrate (satd aq, 15 mL) at rt until two layers formed. The aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL), and the combined organics were dried ($MgSO_4$), filtered, and concentrated in vacuo to give the lactol **17D** as a colorless, viscous oil (416 mg, 99%) in a 5:1 ratio of anomers, which was used without further purification: HRMS (ESI+ve) found 379.1510 [M + Na]$^+$, $C_{21}H_{24}NaO_5$ requires 379.1516; $[\alpha]^{25}_D$ −4.6 (*c* 1.34, $CHCl_3$); ν_{max} (thin film) 3383 (s, br, OH); δ_H (400 MHz, $CDCl_3$) 1.30 (3H, s, CH_3^A), 1.37 (3H, s, CH_3^B), 1.40 (3H, s, CH_3^A), 1.48 (3H, s, CH_3^B), 2.76 (1H, d, OH1^A, $J_{OH,1}$ 2.3), 3.73 (1H, dd, H5a^A, J_{gem} 10.4, $J_{5a,4}$ 7.3), 3.71−3.76 (1H, m, H5a^B), 3.80−3.83 (2H, m, H5b^B, H4^B), 3.82 (1H, dd, H5b^A, J_{gem} 10.4, $J_{5b,4}$ 4.1), 3.89 (1H, d, OH1^B, $J_{OH,1}$ 12.1), 4.46 (1H, dt, H4^A, $J_{4,5}$ 7.5, $J_{4,3}$ 4.0), 4.50 (1H, dd, H2^B, $J_{2,3}$ 6.1, $J_{2,1}$ 3.5), 4.60 (1H, d, H2^A, $J_{2,3}$ 5.8), 4.71 (1H, dd, H3^B, $J_{3,2}$ 6.1, $J_{3,4}$ 2.8), 4.76 (1H, dd, H3^A, $J_{3,2}$ 5.8, $J_{3,4}$ 3.8), 5.02 (1H, dd, H1^B, $J_{1,OH}$ 12.1, $J_{1,2}$ 3.5), 5.41 (1H, d, H1^A, $J_{1,OH}$ 2.0), 5.46 (2H, s, $CHPh_2^{A+B}$), 7.16−7.41 (20H, m, ArH^{A+B}); δ_C (100.6 MHz, $CDCl_3$) 24.8 (CH_3^A), 25.0 (CH_3^B), 25.8 (CH_3^B), 26.0 (CH_3^A), 66.7 (C5^B), 67.4 (C5^A), 75.0 (C4^B), 78.5 (C2^B), 79.4 (C4^A), 79.7 (C3^B), 80.1 (C3^B), 84.2 (×2) ($CHPh_2^{A+B}$), 85.4 (C2^A), 96.8 (C1^B), 101.3 (C1^A), 112.5 ($C(CH_3)_2^A$), 113.1 ($C(CH_3)_2^B$), 127.1, 127.2, 127.4 (×2), 128.2, 128.3 (×2), 128.6, 129.0 (ArCH^{A+B}), 137.9, 141.9, 142.0 (ArC^{A+B}); *m*/*z* (ESI+ve) 735 ([2M + Na]$^+$, 100), 379 ([M + Na]$^+$, 72).

Enantiomer **17L**: mp 68−71 °C; $[\alpha]^{25}_D$ +3.1 (*c* 1.56, $CHCl_3$).

5-O-Benzhydryl-2-C-hydroxymethyl-2,3-O-isopropylidene-D-lyxofuranose (18D). Potassium carbonate (496 mg, 3.59 mmol) was added to a solution of lactol **17D** (810 mg, 2.28 mmol) in methanol (8 mL). Aqueous formaldehyde (39.5%, 5.1 mL, 68 mmol) was added slowly, and the reaction was heated to reflux. After 4 h, TLC (1:1 EtOAc/cyclohexane) showed the formation of one major product (R_f 0.60) and only a trace of starting material (R_f 0.87) remaining. The reaction mixture was concentrated in vacuo, and the residue was partitioned between ethyl acetate (10 mL) and sodium bicarbonate (satd aq, 10 mL). The aqueous layer was extracted with ethyl acetate (2 × 10 mL), and the combined organics were washed with brine (20 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude was purified by column chromatography (1:4 → 1:2 EtOAc/cyclohexane) to give the lactol **18D** as a colorless oil (650 mg, 74%) in a 3:1 ratio of anomers: HRMS (ESI+ve) found 409.1621 [M + Na]$^+$, $C_{22}H_{26}NaO_6$ requires 409.1622; $[\alpha]^{25}_D$ −6.0 (*c* 0.77, $CHCl_3$); ν_{max} (thin film) 3424 (s, br, OH); δ_H (400 MHz, $CDCl_3$) 1.36 (3H, s, CH_3^A), 1.39 (3H, s, CH_3^A), 1.45 (3H, s, CH_3^B), 1.50 (3H, s, CH_3^B), 1.97 (1H, dd, OH2′^{B}, *J* 6.6, *J* 5.3), 2.61 (1H, dd, OH2′^{A}, *J* 7.8, *J* 6.3), 3.71 (1H, d, OH1^A,

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$J_{OH,1}$ 2.5), 3.69−3.82 (7H, m, H5a^{A+B}, H5b^{A+B}, H2′a^{A+B}, H2′b^{B}), 3.84 (1H, d, OH1^{B}, $J_{OH,1}$ 11.9), 3.84−3.88 (1H, ddd, H4^{B}, *J* 6.6, *J* 5.3, $J_{4,3}$ 3.0), 3.92 (1H, dd, H2′b^{A}, J_{gem} 11.9, $J_{2'b,OH}$ 6.1), 4.48 (1H, a-quin, H4^{A}, *J* 3.7), 4.60 (1H, d, H3^{B}, $J_{3,4}$ 2.8), 4.61 (1H, d, H3^{A}, $J_{3,4}$ 3.0), 4.87 (1H, d, H1^{B}, $J_{1,OH}$ 11.9), 5.41 (1H, d, H1^{A}, $J_{1,OH}$ 2.3), 5.44 (2H, s, $CHPh_2^{A+B}$), 7.22−7.39 (20H, m, ArH^{A+B}); δ_C (100.6 MHz, $CDCl_3$) 26.9 (CH_3^{B}), 27.1 (CH_3^{B}), 27.3 (CH_3^{A}), 27.5 (CH_3^{A}), 62.7 (C2′^{B}), 63.5 (C2′^{A}), 66.6 (C5^{B}), 67.2 (C5^{A}), 75.0 (C4^{B}), 80.0 (C4^{A}), 82.1 (C3^{B}), 83.2 (C3^{A}), 84.2 ($CHPh_2^{B}$), 84.3 ($CHPh_2^{A}$), 89.5 (C2^{B}), 93.6 (C2^{A}), 97.4 (C1^{B}), 103.7 (C1^{A}), 113.7 ($C(CH_3)_2^{A}$), 113.9 ($C(CH_3)_2^{B}$), 127.0 (×2), 127.1, 127.5 (×2), 127.6, 128.3, 128.4 (×2) (ArCH^{A+B}), 141.7, 141.8 (ArC^{A+B}); *m*/*z* (ESI+ve) 795 ([2M + Na]$^+$, 100), 409 ([M + Na]$^+$, 18).

Enantiomer **18L**: $[\alpha]^{25}_D$+8.3 (*c* 0.54, $CHCl_3$).

*5-O-Benzhydryl-2-C-hydroxymethyl-2,3-O-isopropylidene-D-lyxono-1,4-lactone (**19D**).* Potassium carbonate (354 mg, 2.56 mmol) and iodine (651 mg, 2.56 mmol) were added to a hot solution of lactol **18D** (495 mg, 1.29 mmol) in *tert*-butyl alcohol (10 mL). The reaction was stirred at reflux for 1 h after which time TLC (1:1 EtOAc/cyclohexane) showed complete conversion of the starting material (R_f 0.60) to one major product (R_f 0.75). Sodium thiosulfate (satd aq, ∼5 mL) and ethyl acetate (10 mL) were added, and the mixture was stirred at rt until colorless. The aqueous layer was extracted with ethyl acetate (3 × 10 mL), and the combined organics were dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude lactone was purified by column chromatography (1:8 → 1:2 EtOAc/cyclohexane) to give the lactone **19D** as a white crystalline solid (420 mg, 85%): HRMS (ESI +ve) found 407.1464 [M + Na]$^+$, $C_{22}H_{24}NaO_6$ requires 407.1465; mp 126−129 °C; $[\alpha]^{25}_D$ +26.7 (*c* 1.07, $CHCl_3$); ν_{max} (thin film) 3475 (m, br, OH), 1786 (s, C═O); δ_H (400 MHz, $CDCl_3$) 1.39 (3H, s, CH_3), 1.41 (3H, s, CH_3), 2.48 (1H, dd, OH, $J_{OH,2'b}$ 7.3, $J_{OH,2'a}$ 4.3), 3.82 (1H, dd, H5a, J_{gem} 10.6, $J_{5a,4}$ 6.8), 3.86 (1H, dd, H5b, J_{gem} 10.7, $J_{5b,4}$ 4.7), 3.90 (1H, dd, H2′a, J_{gem} 11.4, $J_{2'a,OH}$ 4.1), 3.97 (1H, dd, H2′b, J_{gem} 11.4, $J_{2'b,OH}$ 7.6), 4.73−4.78 (1H, m, H4), 4.78 (1H, d, H3, $J_{3,4}$ 3.5), 5.47 (1H, s, $CHPh_2$), 7.24−7.40 (10H, m, ArH); δ_C (100.6 MHz, $CDCl_3$) 26.4 (CH_3), 26.9 (CH_3), 61.2 (C2′), 66.6 (C5), 78.5 (C4), 78.7 (C3), 84.3 ($CHPh_2$), 86.0 (C2), 113.7 ($C(CH_3)_2$), 127.0, 127.2, 127.7 (×2), 128.4 (ArCH), 141.4, 141.5 (ArC), 175.5 (C═O); *m*/*z* (ESI+ve) 791 ([2M + Na]$^+$, 100), 407 ([M + Na]$^+$, 78).

Enantiomer **19L**: mp 132−134 °C; $[\alpha]^{25}_D$ −28.3 (*c* 0.98, $CHCl_3$).

*2-C-Azidomethyl-5-O-benzhydryl-2,3-O-isopropylidene-D-lyxono-1,4-lactone (**20D**).* Trifluoromethanesulfonic anhydride (0.28 mL, 1.6 mmol) was added dropwise to a solution of lactone **19D** (395 mg, 1.03 mmol) and pyridine (0.25 mL, 3.1 mmol) in CH_2Cl_2 (10 mL) at −30 °C. The reaction was stirred between −30 and −10 °C for 1 h after which time TLC (2:3 EtOAc/cyclohexane) showed complete conversion of the starting material (R_f 0.35) to one major product (R_f 0.72). The reaction mixture was diluted with CH_2Cl_2 (10 mL) and washed with HCl (2 M, 10 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 5 mL), and the combined organics were washed with brine (20 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude triflate (531 mg, 1.0 mmol) was dissolved in DMF (11 mL), and sodium azide (94 mg, 1.4 mmol) was added. The reaction was stirred at rt for 18 h after which TLC (2:3 EtOAc/cyclohexane) showed the complete conversion of the triflate (R_f 0.72) to one major product (R_f 0.78). The reaction mixture was diluted with ethyl acetate (20 mL) and washed with 1:1 brine/water (2 × 15 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude azide was purified by column chromatography (1:20 → 1:8 EtOAc/cyclohexane) to give the azide **20D** as a colorless oil (421 mg, quant): HRMS (ESI+ve) found 432.1531 [M + Na$^+$], $C_{22}H_{23}NaN_3O_5$ requires 432.1530; $[\alpha]^{25}_D$ −2.6 (*c* 0.93, $CHCl_3$); ν_{max} (thin film) 2110 (s, N_3), 1788 (s, C═O); δ_H (400 MHz, $CDCl_3$) 1.41 (3H, s, CH_3), 1.45 (3H, s, CH_3), 3.55 (1H, d, H2′a, J_{gem} 12.9), 3.81 (1H, d, H2′b, J_{gem} 12.9), 3.82 (1H, dd, H5a, J_{gem} 10.7, $J_{5a,4}$ 7.0), 3.86 (1H, dd, H5b, J_{gem} 10.7, $J_{5b,4}$ 5.1), 4.67−4.72 (1H, ddd, H4, $J_{4,5a}$ 6.8, $J_{4,5b}$ 5.1, $J_{4,3}$ 3.4), 4.73 (1H, d, H3, $J_{3,4}$ 3.5), 5.47 (1H, s, $CHPh_2$), 7.27−7.39 (10H, m, ArH); δ_C (100.6 MHz, $CDCl_3$) 26.1 (CH_3), 26.9 (CH_3), 50.4 (C2′), 66.5 (C5), 78.2 (C4), 78.4 (C3), 84.4 ($CHPh_2$), 84.9 (C2), 114.3 ($C(CH_3)_2$), 126.9, 127.1, 127.7 (×2), 128.5 (ArCH), 141.3, 141.4 (ArC), 174.0 (C═O); *m*/*z* (ESI+ve) 841 ([2M + Na]$^+$, 100), 432 ([M + Na]$^+$, 16).

Enantiomer **20L**: $[\alpha]^{25}_D$+3.5 (*c* 1.04, $CHCl_3$).

*2-C-Azidomethyl-D-lyxono-1,4-lactone (**21D**).* A solution of acetyl chloride (0.8 mL) in methanol (16 mL) was added to azide **20D** (864 mg, 2.11 mmol), and the solution was stirred at reflux for 18 h. After this time, TLC (1:1 EtOAc/cyclohexane) indicated the complete consumption of the starting material and the formation of one major product (R_f 0.11). The reaction was concentrated in vacuo and coevaporated with CH_2Cl_2. The solid was triturated at rt with ether (×3) to give the lactone **21D** as a white crystalline solid (417 mg, 97%): HRMS (ESI+ve) found 226.0433 [M + Na]$^+$, $C_6H_9NaN_3O_5$ requires 226.0434; mp 124−128 °C; $[\alpha]^{25}_D$ +42.3 (*c* 0.78, MeOH); ν_{max} (thin film) 3406 (s, br, OH), 2114 (s, N_3), 1774 (s, C═O); δ_H (400 MHz, MeOD) 3.48 (1H, d, H2′a, J_{gem} 13.1), 3.56 (1H, d, H2′b, J_{gem} 13.0), 3.88 (2H, a-d, H5, *J* 5.1), 4.34 (1H, d, H3, $J_{3,4}$ 4.7), 4.58 (1H, a-q, H4, *J* 5.0); δ_C (100.6 MHz, MeOD) 54.2 (C2′), 61.2 (C5), 71.4 (C3), 78.0 (C2), 82.7 (C4), 177.3 (C═O); *m*/*z* (ESI−ve) 405 ([2M − H]$^-$, 65), 240 ([M + ^{37}Cl]$^-$, 50), 238 ([M + ^{35}Cl]$^-$, 97), 202 ([M − H]$^-$, 100%).

Enantiomer **21L**: mp 130−132 °C; $[\alpha]^{25}_D$ −43.9 (*c* 0.77, MeOH).

*2-C-Azidomethyl-2,3,5-tri-O-benzyl-D-lyxono-1,4-lactone (**22D**).* Benzyl bromide (1.1 mL, 9.3 mmol) and sodium hydride (60% in mineral oil, 150 mg, 3.75 mmol) were added to a solution of azide **21D** (190 mg, 0.94 mmol) in DMF (4 mL) at −5 °C. The reaction was stirred at 0 °C for 3.5 h after which time TLC (1:3 EtOAc/cyclohexane) showed almost complete conversion of the starting material (R_f 0.07) to one major product (R_f 0.50). A further portion of NaH (38 mg, 0.95 mmol) was added and the reaction stirred for a further 1 h. The reaction was quenched with acetic acid, diluted with ethyl acetate (10 mL), and washed with 1:1 brine/water (3 × 10 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude residue was purified by column chromatography (1:99 → 1:9 EtOAc/cyclohexane) to give the tribenzyl lactone **22D** as a colorless oil (379 mg, 86%): HRMS (ESI+ve) found 496.1843 [M + Na]$^+$, $C_{27}H_{27}NaN_3O_5$ requires 496.1843; $[\alpha]^{25}_D$ −11.7 (*c* 1.61, $CHCl_3$); ν_{max} (thin film) 2106 (s, N_3), 1779 (s, C═O); δ_H (400 MHz, $CDCl_3$) 3.42 (1H, d, H2′a, J_{gem} 12.9), 3.70 (1H, d, H2′b, J_{gem} 12.9), 3.82 (1H, dd, H5a, J_{gem} 10.9, $J_{5a,4}$ 6.8), 3.86 (1H, dd, H5b, J_{gem} 10.9, $J_{5b,4}$ 4.8), 4.34 (1H, d, H3, $J_{3,4}$ 5.6), 4.54 (1H, d, CH_2Ph^a, J_{gem} 11.9), 4.59 (1H, d, CH_2Ph^a, J_{gem} 11.9), 4.60 (1H, d, CH_2Ph^b, J_{gem} 11.6), 4.65 (1H, a-dt, H4, $J_{4,5a}$ 6.7, $J_{4,3}$ = $J_{4,5b}$ 5.3), 4.78 (1H, d, CH_2Ph^b, J_{gem} 11.6), 4.92 (2H, a-s, CH_2Ph^c), 7.20−7.24 (2H, m, ArH), 7.28−7.40 (13H, m, ArH); δ_C (100.6 MHz, $CDCl_3$) 53.0 (C2′), 68.1 (C5), 69.7 (CH_2Ph^c), 73.7 (CH_2Ph^a), 74.3 (CH_2Ph^b), 77.9 (C3), 79.6 (C4), 81.4 (C2), 127.5, 127.7, 127.9 (×2), 128.1, 128.2, 128.3, 128.5 (×2) (ArCH), 136.8, 137.5, 137.6 (ArC), 171.8 (C═O); *m*/*z* (ESI +ve) 969 ([2M + Na]$^+$, 60), 496 ([M + Na]$^+$, 100).

Enantiomer **22L**: $[\alpha]^{25}_D$+10.6 (*c* 1.73, $CHCl_3$).

*2-C-Azidomethyl-2,3,5-tri-O-benzyl-D-lyxitol (**23D**).* Sodium borohydride (37 mg, 0.96 mmol) was added to a solution of lactone **22D** (181 mg, 0.383 mmol) in ethanol (3 mL). The reaction was stirred at rt for 2 h after which a further portion of sodium borohydride (22 mg, 0.58 mmol) was added. After a further 1 h, TLC analysis (1:3 EtOAc/cyclohexane) showed complete consumption of the starting material (R_f 0.64). The reaction was quenched with ammonium chloride (satd aq), diluted with ethyl acetate (10 mL), and washed with brine (10 mL). The aqueous layers were extracted with ethyl acetate (2 × 5 mL), and the combined organics were dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude was purified by column chromatography (1:9 → 7:13 EtOAc in cyclohexane) to afford the diol **23D** as a colorless oil (184 mg, quant.) (R_f 0.17, 1:3 EtOAc/cyclohexane): HRMS (ESI+ve) found 500.2156 [M + Na]$^+$, $C_{27}H_{31}NaN_3O_5$ requires 500.2156; $[\alpha]^{25}_D$ −15.2 (*c* 1.1, $CHCl_3$); ν_{max} (thin film) 3418 (m, br, OH), 2103 (s, N_3); δ_H (400 MHz, $CDCl_3$) 2.82 (1H, d, OH4, $J_{OH,4}$ 8.1), 2.99 (1H, a-br-t, OH1, *J* 5.3), 3.44 (1H, dd, H5a, J_{gem} 9.4, $J_{5a,4}$ 6.6), 3.54 (1H, dd, H5b, J_{gem} 9.4, $J_{5b,4}$ 5.8), 3.72 (1H, d, H2′a, J_{gem} 13.9), 3.79 (1H, d, H2′b, J_{gem} 13.7), 3.82 (1H, dd, H1a, J_{gem} 12.4, $J_{1a,OH}$ 7.3), 3.99 (1H, s, H3), 4.02 (1H, dd, H1b, J_{gem} 12.4, $J_{1b,OH}$ 3.3), 4.05−4.09 (1H, m, H4), 4.50 (1H, d,

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CH_2Ph^a, J_{gem} 11.9), 4.55 (1H, d, CH_2Ph^a, J_{gem} 11.9), 4.58 (1H, d, CH_2Ph^c, J_{gem} 11.1), 4.69 (1H, d, CH_2Ph^b, J_{gem} 11.1), 4.72 (1H, d, CH_2Ph^b, J_{gem} 10.9), 4.78 (1H, d, CH_2Ph^c, J_{gem} 10.9), 7.21−7.39 (15H, m, ArH); δ_C (100.6 MHz, $CDCl_3$) 51.7 (C2′), 61.5 (C1), 65.3 (CH_2Ph^b), 68.1 (C4), 71.9 (C5), 73.4 (CH_2Ph^a), 75.9 (CH_2Ph^c), 78.6 (C3), 81.7 (C2), 127.5, 127.6, 127.9, 128.0, 128.1, 128.2, 128.4, 128.5 (×2) (ArCH), 137.7 (×2), 138.3 (ArC); *m*/*z* (ESI+ve) 977 ([2M + Na]$^+$, 100), 500 ([M + Na]$^+$, 100), 495 ([M + NH_4]$^+$, 32).

Enantiomer **23L**: $[\alpha]^{25}_D$ +13.6 (*c* 0.97, $CHCl_3$).

2-C-Azidomethyl-2,3,5-tri-O-benzyl-1-O-tert-butyldimethylsilyl-D-lyxitol (24D). Imidazole (168 mg, 2.47 mmol) and TBSCl (248 mg, 1.65 mmol) were added to a solution of diol **23D** (392 mg, 0.822 mmol) in DMF (8 mL) with 3 Å powdered molecular sieves. The reaction was stirred at rt for 4 h after which a further portion of imidazole (84 mg, 1.2 mmol) and TBSCl (124 mg, 0.823 mmol) were added. After a further 2 h, TLC (1:2 EtOAc/cyclohexane) showed almost complete conversion of the starting material (R_f 0.23) to one major product (R_f 0.77). The reaction was diluted with ethyl acetate (20 mL) and washed with 1:1 brine/water (3 × 20 mL), the organic layer was then concentrated in vacuo, and the residue was purified by column chromatography (1:99 → 3:17 EtOAc/cyclohexane) to give the silyl ether **24D** as a colorless oil (455 mg, 94%): HRMS (ESI+ve) found 614.3023 [M + Na]$^+$, $C_{33}H_{45}NaN_3O_5Si$ requires 614.3021; $[\alpha]^{25}_D$ +3.4 (*c* 1.0, $CHCl_3$); ν_{max} (thin film) 3533 (w, br, OH), 2103 (s, N_3); δ_H (400 MHz, $CDCl_3$) 0.09 (3H, s, CH_3Si), 0.11 (3H, s, CH_3Si), 0.94 (9H, s, $(CH_3)_3CSi$), 3.02 (1H, d, OH4, *J* 6.6), 3.48 (1H, dd, H5a, J_{gem} 9.4, $J_{5a,4}$ 7.1), 3.53 (1H, dd, H5b, J_{gem} 9.4, $J_{5b,4}$ 5.8), 3.57 (1H, d, H2′a, J_{gem} 13.1), 3.84 (1H, d, H2′b, J_{gem} 13.1), 3.91 (1H, d, H1a, J_{gem} 11.1), 4.00 (1H, d, H1b, J_{gem} 11.1), 4.12 (1H, d, H3, $J_{3,4}$ 1.3), 4.21 (1H, ddt, H4, $J_{4,5a}$ 6.9, $J_{4,5b}$ 5.7, $J_{4,3}$ 1.3), 4.49 (1H, d, CH_2Ph^a, J_{gem} 11.9), 4.56 (1H, d, CH_2Ph^a, J_{gem} 11.9), 4.63 (1H, d, CH_2Ph^b, J_{gem} 11.1), 4.71 (1H, d, CH_2Ph^c, J_{gem} 11.1), 4.72 (1H, d, CH_2Ph^b, J_{gem} 11.1), 4.82 (1H, d, CH_2Ph^c, J_{gem} 11.1), 7.25−7.37 (15H, m, ArH); δ_C (100.6 MHz, $CDCl_3$) −5.7, −5.6 (CH_3Si), 18.1 ($(CH_3)_3CSi$), 25.9 ($(CH_3)_3CSi$), 52.1 (C2′), 63.0 (C1), 66.4 (CH_2Ph^c), 67.8 (C4), 71.9 (C5), 73.3 (CH_2Ph^a), 75.3 (CH_2Ph^b), 76.9 (C3), 82.0 (C2), 127.4, 127.7 (×2), 127.8, 128.3, 128.4 (×2) (ArCH), 138.0 (×2), 138.6 (ArC); *m*/*z* (ESI+ve) 614 ([M + Na]$^+$, 100), 609 ([M + NH_4]$^+$, 48), 592 ([M + H]$^+$, 12).

Enantiomer **24L**: $[\alpha]^{25}_D$ −2.9 (*c* 1.11, $CHCl_3$).

2-C-Azidomethyl-2,3,5-tri-O-benzyl-1-O-tert-butyldimethylsilyl-4-O-methanesulfonyl-D-lyxitol (25D). Method 1. Triethylamine (0.11 mL, 0.81 mmol) and mesyl chloride (0.06 mL, 0.7 mmol) were added to a solution of alcohol **24D** (267 mg, 0.452 mmol) in CH_2Cl_2 (5 mL) at 0 °C. The reaction was stirred at rt for 1.5 h after which time a further portion of triethylamine (0.03 mL, 0.2 mmol) and mesyl chloride (0.02 mL, 0.2 mmol) were added, and the reaction was stirred for a further 30 min. After this time, TLC (19:1 toluene/acetone) showed the complete conversion of the starting material (R_f 0.67) to one major product (R_f 0.74). The solution was diluted with CH_2Cl_2 (10 mL) and washed with water (3 × 5 mL), and the organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude was purified by column chromatography (0:1 → 3:97 acetone/toluene) to give the mesylate **25D** as a colorless oil (300 mg, 99%).

Method 2. Pyridine (0.13 mL, 1.6 mmol) and mesyl chloride (0.05 mL, 0.6 mmol) were added to a solution of alcohol **24D** (188 mg, 0.318 mmol) in CH_2Cl_2 (3 mL) at rt. The reaction was stirred at rt for 6 h after which time a further portion of pyridine (0.07 mL, 0.9 mmol) and mesyl chloride (0.013 mL, 0.16 mmol) were added, and the reaction was stirred for a further 30 min at rt and then at −10 °C for 16 h. After this time, TLC (19:1 toluene/acetone) showed the complete conversion of the starting material (R_f 0.67) to one major product (R_f 0.74). The reaction mixture was diluted with CH_2Cl_2 (15 mL) and washed with HCl (2 M, aq, 10 mL), the aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL), and the combined organic layers were washed with brine (20 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude was purified by column chromatography (toluene) to give the mesylate **25D** as a colorless oil (198 mg, 93%): HRMS (ESI+ve) found 692.2797 [M + Na^+], $C_{34}H_{47}NaN_3O_7SSi$ requires 692.2796; $[\alpha]^{25}_D$ +7.0 (*c* 1.01, $CHCl_3$); ν_{max} (thin film) 2104 (s, N_3); δ_H (400 MHz, $CDCl_3$) 0.08 (3H, s, CH_3Si), 0.10 (3H, s, CH_3Si), 0.92 (9H, s, $(CH_3)_3CSi$), 2.92 (3H, s, CH_3S), 3.67 (1H, d, H2′a, J_{gem} 13.1), 3.76 (1H, dd, H5a, J_{gem} 11.1, $J_{5a,4}$ 4.6), 3.79 (1H, dd, H5b, J_{gem} 11.1, $J_{5b,4}$ 6.4), 3.83 (1H, d, H1a, J_{gem} 11.5), 3.84 (1H, d, H2′b, J_{gem} 13.1), 3.92 (1H, d, H1b, J_{gem} 11.4), 4.09 (1H, d, H3, $J_{3,4}$ 5.0), 4.40 (1H, d, CH_2Ph^a, J_{gem} 11.6), 4.44 (1H, d, CH_2Ph^a, J_{gem} 11.6), 4.62 (1H, d, CH_2Ph^c, J_{gem} 11.3), 4.70 (1H, d, CH_2Ph^b, J_{gem} 10.9), 4.74 (1H, d, CH_2Ph^b, J_{gem} 11.1), 4.79 (1H, d, CH_2Ph^c, J_{gem} 11.1), 5.21 (1H, a-dt, H4, $J_{4,5b}$ 6.4, $J_{4,3}$ = $J_{4,5a}$ 4.8), 7.16−7.39 (15H, m, ArH); δ_C (100.6 MHz, $CDCl_3$) −5.6 (2 x CH_3Si), 18.1 ($(CH_3)_3CSi$), 25.8 ($(CH_3)_3CSi$), 38.7 (CH_3S), 51.1 (C2′), 62.5 (C1), 66.5 (CH_2Ph^b), 70.2 (C5), 73.2 (CH_2Ph^a), 75.2 (CH_2Ph^c), 77.0 (C3), 80.4 (C4), 81.5 (C2), 127.5, 127.6, 127.8 (×2), 127.9 (×2), 128.3, 128.4 (×2) (ArCH), 137.5, 137.6, 138.3 (ArC); *m*/*z* (ESI+ve): 771 [M + Et_3N + H]$^+$, 100), 692 ([M + Na]$^+$, 98), 687 ([M + NH_4]$^+$, 50).

Enantiomer **25L**: $[\alpha]^{25}_D$ −8.3 (*c* 0.99, $CHCl_3$).

1,4-Dideoxy-2-C-hydroxymethyl-1,4-imino-L-arabinitol (9L) (L-isoDMDP). To a solution of mesylate **25D** (198 mg, 0.296 mmol) in dioxane (2.5 mL) and water (0.5 mL) were added NaOAc (83 mg, 1.0 mmol) and 10% Pd/C (10 mol %, 32 mg), and the reaction was degassed, flushed with argon, degassed, and flushed with hydrogen. After 24 h, low-resolution mass spectrometry (LRMS) showed some formation of the intermediate protected iminosugar and no remaining starting material. The reaction was acidified with HCl (2 M, aq, 1.0 mL) and stirred under hydrogen for 48 h, after which time LRMS indicated only the desired fully deprotected compound. The reaction was degassed, purged with argon, filtered (glass microfiber), and purified directly by ion-exchange chromatography (Dowex 50W-X8 H^+) to give L-isoDMDP (**9L**) (49 mg, quant) as a light orange oil which crystallized on standing.

Data for free base: mp 135−138 °C; $[\alpha]^{25}_D$ +9.0 (*c* 1.01, H_2O); δ_H (400 MHz, D_2O) 2.89 (1H, d, H1a, J_{gem} 12.6), 2.91 (1H, d, H1b, J_{gem} 12.4), 3.00 (1H, dt, H4, $J_{4,5a}$ 6.1, $J_{4,3}$ = $J_{4,5b}$ 5.1), 3.66 (1H, d, H2′a, J_{gem} 12.1), 3.68 (1H, dd, H5a, J_{gem} 11.6, $J_{5a,4}$ 6.2), 3.74 (1H, dd, H5b, J_{gem} 11.6, $J_{5b,4}$ 5.2), 3.75 (1H, d, H2′b, J_{gem} 12.1), 3.83 (1H, d, H3, $J_{3,4}$ 5.1); δ_C (100.6 MHz, D_2O) 53.6 (C1), 62.6 (C5), 63.8 (C2′), 67.8 (C4), 80.0 (C3), 82.8 (C2).

Data for HCl salt: HRMS (ESI+ve) found 164.0921 [M + H]$^+$, $C_6H_{13}NO_4$ requires 164.0917; $[\alpha]^{25}_D$ −7.8 (*c* 1.05, H_2O); ν_{max} (thin film, Ge) 3346 (s, br, OH/NH); δ_H (400 MHz, D_2O) 3.38 (1H, d, H1a, J_{gem} 12.4), 3.43 (1H, d, H1b, J_{gem} 12.4), 3.67−3.70 (1H, m, H4), 3.77 (1H, d, H2′a, J_{gem} 12.2), 3.80 (1H, d, H2′b, J_{gem} 12.1), 3.86 (1H, dd, H5a, J_{gem} 12.1, $J_{5a,4}$ 8.6), 3.98 (1H, dd, H5b, J_{gem} 12.2, $J_{5b,4}$ 4.8), 4.03 (1H, d, H3, $J_{3,4}$ 3.3); δ_C (100.6 MHz, D_2O) 51.9 (C1), 60.2 (C5), 62.3 (C2′), 69.1 (C4), 76.1 (C3), 81.4 (C2); *m*/*z* (ESI−ve) 198 ([M + Cl]$^-$, 100), 162 ([M − H]$^-$, 52).

Enantiomer **9D** (free base): mp 138−140 °C; $[\alpha]^{25}_D$ −9.3 (*c* 0.96, H_2O).

B. Synthesis of L-IsoDGDP (10L). *5-O-Benzhydryl-2,3-O-isopropylidene-L-ribonolactone (27L).* Diphenyldiazomethane (3.00 g, 15.5 mmol) was added to a solution of lactone **26L** (1.83 g, 9.73 mmol) in toluene (200 mL), and the reaction mixture was stirred at reflux. After 1 h, TLC analysis (1:1 EtOAc/cyclohexane) revealed the consumption of starting material (R_f 0.33) and the formation of one major product (R_f 0.73). The reaction mixture was allowed to cool to rt and concentrated in vacuo. The crude mixture was purified by flash column chromatography (1:49 to 17:83 EtOAc/cyclohexane) to afford the benzhydryl ether **27L** as a colorless oil (3.24 g, 94%): HRMS (ESI +ve) found 377.1355 [M + Na]$^+$, $C_{21}H_{22}NaO_5$ requires 377.1359; $[\alpha]^{25}_D$ +23.0 (*c* 0.77, $CHCl_3$) [lit.[36b] $[\alpha]^{21}_D$ +20.8 (*c* 1.00 in $CHCl_3$)]; ν_{max} (thin film) 1786 (s, C═O); δ_H (400 MHz, $CDCl_3$) 1.39 (3H, s, CH_3), 1.49 (3H, s, CH_3), 3.65 (1H, dd, H5a, J_{gem} 10.6, $J_{5a,4}$ 1.8), 3.77 (1H, dd, H5b, J_{gem} 10.6, $J_{5b,4}$ 2.3), 4.67 (1H, a-t, H4, $J_{4,5a}$ = $J_{4,5b}$ 2.0), 4.76 (1H, d, H3, $J_{3,2}$ 5.3), 4.86 (1H, d, H2, $J_{2,3}$ 5.3), 5.37 (1H, s, $CHPh_2$), 7.22−7.38 (10H, m, ArH); δ_C (100.6 MHz, $CDCl_3$) 25.7 (CH_3), 26.9 (CH_3), 68.0 (C5), 75.8 (C2), 78.5 (C3), 81.1 (C4), 84.9 ($CHPh_2$), 113.2 ($C(CH_3)_2$), 126.7, 126.8, 127.9, 128.0, 128.6, 128.7 (ArCH), 140.6, 141.0 (ArC), 174.3 (C1); *m*/*z* (ESI +ve) 731 ([2M + Na]$^+$, 100%), 377 ([M + Na]$^+$, 92).

Enantiomer **27D**: $[\alpha]_D^{23}$ −27.9 (*c* 0.82, $CHCl_3$).

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*5-O-Benzhydryl-2,3-O-isopropylidene-L-ribofuranose (**28L**).* DIBALH solution (1.5 M in toluene, 2.4 mL, 3.6 mmol) was added dropwise to a solution of lactone **27L** (0.99 g, 2.8 mmol) in CH_2Cl_2 (14 mL), and the mixture was stirred at −78 °C. After 2 h, TLC analysis (1:3 EtOAc/cyclohexane) revealed the formation of one major product (R_f 0.41) and some remaining starting material (R_f 0.45). A further portion of DIBALH solution (1.9 mL, 2.8 mmol) was added and the reaction mixture was stirred for a 1 h, after which time TLC analysis revealed the complete consumption of starting material (R_f 0.45). The reaction was quenched with methanol (1 mL), diluted with CH_2Cl_2 (10 mL), and stirred with sodium potassium tartrate (satd aq, 30 mL) at rt until two layers had formed. The aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL), and the combined organic fractions were dried ($MgSO_4$), filtered, and concentrated in vacuo to afford the title furanose **28L** (0.97 g, 98%) as a colorless oil in a 3:1 ratio of anomers which was used without further purification: HRMS (ESI +ve) found 379.1502 $[M + Na]^+$; $C_{21}H_{24}NaO_5$ requires: 379.1516; $[\alpha]^{25}_D$ +0.1 (*c* 0.81, $CHCl_3$); ν_{max} (thin film) 3425 (br, s, OH); δ_H (400 MHz, $CDCl_3$)1.35 (3H, s, CH_3^A), 1.41 (3H, s, CH_3^B), 1.50 (3H, s, CH_3^A), 1.57 (3H, s, CH_3^B), 3.50 (1H, dd, H5a^B, J_{gem} 10.1, $J_{5a,4}$ 2.5), 3.62 (1H, dd, H5a^A, J_{gem} 10.1, $J_{5a,4}$ 2.5), 3.64 (1H, dd, H5b^B, J_{gem} 10.1, $J_{5b,4}$ 2.5), 3.71 (1H, dd, H5b^A, J_{gem} 10.1, $J_{5b,4}$ 2.5), 3.96 (1H, d, OH^B, $J_{OH,1}$ 11.4), 4.24 (1H, a-t, H4^B, $J_{4,5a} = J_{4,5b}$ 2.5), 4.41 (1H, a-t, H4^A, $J_{4,5a} = J_{4,5b}$ 2.3), 4.45 (1H, d, OH^A, $J_{OH,1}$ 11.4), 4.59 (1H, d, H2^A, $J_{2,3}$ 5.8), 4.66 (1H, dd, H2^B, $J_{2,3}$ 6.1, $J_{2,1}$ 4.0) 4.80 (1H, d, H3^B, $J_{3,2}$ 6.3), 4.85 (1H, d, H3^A, $J_{3,2}$ 5.8), 5.32 (1H, d, H1^A, $J_{1,OH}$ 11.4), 5.34 (1H, s, $CHPh_2^B$), 5.45 (1H, s, $CHPh_2^A$), 5.59 (1H, dd, H1^B, $J_{1,OH}$ 11.4, $J_{1,2}$ 4.0), 7.17−7.40 (20H, m, ArH); δ_C (100.6 MHz, $CDCl_3$) 24.7 (CH_3^B), 25.0 (CH_3^A), 26.1 (CH_3^B), 26.5 (CH_3^A), 70.6 (C5^A), 75.0 (C5^B), 79.4 (C2^B), 79.7 (C4^B), 82.1 (C3^{A+B}), 84.8 ($CHPh_2^B$), 85.5 ($CHPh_2^A$), 85.7 (C4^A), 87.6 (C2^A), 98.0 (C1^B), 103.9 (C1^A), 112.2 ($C(CH_3)_2^A$), 113.0 ($C(CH_3)_2^B$), 126.6, 126.8, 127.6, 127.7, 128.5, 128.6 (ArCH^B), 126.9 (×2), 128.2 (×2), 128.8 (×2) (ArCH^A), 140.1, 140.2 (ArC^A), 141.5, 141.6 (ArC^B); *m*/*z* (ESI +ve) 735 ($[2M + Na]^+$, 100), 379 (99, $[M + Na]^+$).

Enantiomer **28D**: mp 68−70 °C; $[\alpha]^{25}_D$ −2.1 (*c* 0.66, $CHCl_3$).

*5-O-Benzhydryl-2-C-hydroxymethyl-2,3-O-isopropylidene-L-ribonofuranose (**29L**).* Aqueous formaldehyde (39.5%, 7.0 mL, 93 mmol) was added dropwise to a solution of furanose **28L** (1.02 g, 2.87 mmol) and potassium carbonate (600 mg, 4.35 mmol) in methanol (10 mL), and the mixture was stirred at reflux. After 2 h, TLC analysis (1:1 EtOAc/cyclohexane) revealed the formation of one major product (R_f 0.56), trace starting material (R_f 0.73), and one minor product (R_f 0.13). The reaction mixture was cooled to rt, neutralized with glacial acetic acid, and concentrated under reduced pressure. The crude residue was partitioned between ethyl acetate (50 mL) and 1:1 satd aq sodium bicarbonate/brine (50 mL). The aqueous was discarded and the organic washed sequentially with 1:1 satd aq sodium bicarbonate/brine (50 mL) and brine (50 mL). The organic layer was dried (MgSO4), filtered, and concentrated under reduced pressure. Purification by flash column chromatography (1:9 → 2:3 EtOAc/cyclohexane) afforded the title furanose **29L** (832 mg, 75%) as a colorless oil in a 1:1 ratio of anomers: HRMS (ESI +ve) found 409.1614 $[M + Na]^+$, $C_{22}H_{26}NaO_6$ requires 409.1622; $[\alpha]^{25}_D$ −14.2 (*c* 0.98, $CHCl_3$); ν_{max} (thin film) 3424 (br, s, OH); δ_H (400 MHz, CD_3CN) 1.41, 1.44, 1.45, 1.53 (4 × 3H, s, CH_3^{A+B}), 2.90 (1H, t, OH2′^{A}, $J_{OH,2'a} = J_{OH,2'b}$ 6.6), 3.08 (1H, t, OH2′^{B}, $J_{OH,2'a} = J_{OH,2'b}$ 6.1), 3.53−3.66 (8H, m, H2′$^{A+B}$, H5^{A+B}), 4.00 (1H, d, OH1^A, $J_{OH,1}$ 10.1), 4.16−4.18 (1H, m, H4^A), 4.25−4.28 (1H, m, H4^B), 4.57−4.61 (3H, m, OH1^B, H3^{A+B}), 5.09 (1H, d, H1^A, $J_{1,OH}$ 10.1), 5.21 (1H, d, H1^B, $J_{1,OH}$ 6.8), 5.46 (1H, s, $CHPh_2^A$), 5.53 (1H, s, $CHPh_2^B$), 7.26−7.43 (20H, m, ArH^{A+B}); δ_H (100.6 MHz, CD_3CN) 26.9, 27.1, 27.6, 27.7 (CH_3^{A+B}), 62.3, 62.5 (C2′$^{A+B}$), 69.9, 70.4 (C5^{A+B}), 80.9 (C4^A), 83.9 ($CHPh_2^B$), 84.4 ($CHPh_2^A$), 84.5 (C4^B), 85.0, 85.3 (C3^{A+B}), 91.7, 94.8 (C2^{A+B}), 98.9 (C1^A), 104.5 (C1^B), 113.3, 114.3 ($C(CH_3)_2^{A+B}$), 127.1 (×3), 127.2, 127.9, 128.0, 128.1 (×2), 128.9 (×2), 129.0 (×2) (ArCH^{A+B}), 142.3, 142.7 (ArC^{A+B}); *m*/*z* (ESI +ve) 795 ($[2M + Na]^+$, 100), 409 ($[M + Na]^+$, 99).

Enantiomer **29D**: $[\alpha]^{25}_D$ +15.2 (*c* 0.68, $CHCl_3$).

*5-O-Benzhydryl-2-C-hydroxymethyl-2,3-O-isopropylidene-L-ribono-1,4-lactone (**30L**).* Potassium carbonate (742 mg, 5.37 mmol) and iodine (1.36 g, 5.37 mmol) were added to a hot solution of furanose **29L** (1.04 g, 2.69 mmol) in *tert*-butyl alcohol (25 mL), and the mixture was stirred at reflux. After 1 h, TLC analysis (1:1 EtOAc/cyclohexane) revealed the formation of a single product (R_f 0.69) and trace starting material (R_f 0.56). The reaction mixture was allowed to cool to rt, satd aq sodium thiosulfate solution (∼30 mL) added dropwise, the mixture diluted with EtOAc (30 mL) and stirred until the iodine was visibly quenched. The aqueous layer was extracted with EtOAc (3 × 25 mL), and the combined organic fractions were collected, dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude mixture was purified by flash column chromatography (1:9 → 1:4, EtOAc/cyclohexane) to afford the title lactone **30L** as a colorless gum (880 mg, 86%): HRMS (ESI +ve) found 407.1453 $[M + Na]^+$, $C_{22}H_{24}NaO_6$ requires 407.1465; $[\alpha]^{25}_D$ −9.8 (*c* 1.01, $CHCl_3$); ν_{max} (thin film) 3504 (br, s, OH), 1779 (s, C═O); δ_H (400 MHz, C_6D_6) 1.33 (3H, s, CH_3), 1.43 (3H, s, CH_3), 1.71 (1H, dd, OH, $J_{OH,2'a}$ 8.6, $J_{OH,2'b}$ 4.0), 3.23 (1H, dd, H5a, J_{gem} 10.6, $J_{5a,4}$ 3.3), 3.42 (1H, dd, H5b, J_{gem} 10.6, $J_{5b,4}$ 3.8), 3.87 (1H, dd, H2′a, J_{gem} 11.9, $J_{2'a,OH}$ 4.0), 4.01 (1H, dd, H2′b, J_{gem} 11.9, $J_{2'b,OH}$ 8.8), 4.44 (1H, a-dd, H4, $J_{4,5b}$ 3.8, $J_{4,5a}$3.3), 4.80 (1H, d, H3, $J_{3,4}$ 0.8), 5.13 (1H, s, $CHPh_2$), 7.09−7.33 (10H, m, ArH); δ_C (100.6 MHz, C_6D_6) 26.8 (CH_3), 27.1 (CH_3), 60.9 (C2′), 68.3 (C5), 79.7 (C3), 82.5 (C4), 84.9 ($CHPh_2$), 86.5 (C2), 113.1 ($C(CH_3)_2$), 127.3, 127.5, 128.0 (×2), 128.7 (×2), (ArCH), 141.2, 141.3 (ArC), 174.4 (C1); *m*/*z* (ESI +ve) 791 ($[2M + Na]^+$, 72), 407 ($[M + Na]^+$, 100).

Enantiomer **30D**: $[\alpha]^{25}_D$+10.3 (*c* 1.01, $CHCl_3$).

*2-C-Azidomethyl-5-O-benzhydryl-2,3-O-isopropylidene-L-ribono-1,4-lactone (**31L**).* Trifluoromethanesulfonic anhydride (0.53 mL, 3.2 mmol) was added dropwise to a solution of lactone **30L** (756 mg, 1.97 mmol) in CH_2Cl_2 (20 mL) and pyridine (0.49 mL, 5.9 mmol) and the mixture was stirred at −30 °C. After 1 h TLC analysis (2:3 EtOAc/cyclohexane) revealed the complete consumption of starting material (R_f 0.58) and the formation of a single product (R_f 0.81). The reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with 2 M HCl (20 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL), and the combined organic fractions were washed with satd brine (50 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo to afford the triflate (954 mg) which was used without further purification. Sodium azide (179 mg, 2.76 mmol) was added to a solution of crude triflate in DMF (20 mL), and the mixture stirred at rt. After 18 h, TLC analysis (2:3 EtOAc/cyclohexane) revealed complete consumption of starting material (R_f 0.81) and the formation of a single product (R_f 0.84). The reaction mixture was diluted with EtOAc (40 mL), washed with 1:1 brine/water solution (3 × 30 mL) and then brine (40 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. Purification by flash column chromatography (1:19 → 1:12 EtOAc/cyclohexane) afforded the azidolactone **31L** as a colorless oil (732 mg, 91%), which crystallized on standing: HRMS (ESI +ve) 432.1528 $[M + Na]^+$; $C_{22}H_{23}N_3NaO_5$ requires: 432.1530; mp 71−74 °C; $[\alpha]^{25}_D$ −71.6 (*c* 0.94, $CHCl_3$); ν_{max} (thin film) 2106 (s, N_3), 1783 (s, C═O); δ_H (400 MHz, C_6D_6) 1.38 (3H, s, CH_3), 1.48 (3H, s, CH_3), 3.01−3.14 (1H, m, H5a), 3.28−3.31 (1H, m, H5b), 3.40 (1H, d, H2′a, J_{gem} 13.6), 3.60 (1H, d, H2′b, J_{gem} 13.6), 4.25 (1H, dd, H4, $J_{4,5a}$ 2.0, $J_{4,5b}$ 1.5), 4.72 (1H, d, H3, $J_{3,4}$ 0.8), 5.04 (1H, s, $CHPh_2$), 7.09−7.26 (10H, m, ArH); δ_H (100.6 MHz, C_6D_6) 26.6 (CH_3), 27.1 (CH_3), 50.1 (C2′), 68.3 (C5), 79.8 (C3), 82.6 (C4), 85.1 ($CHPh_2$), 86.1 (C2), 113.8 ($C(CH_3)_2$), 127.4, 127.6, 127.9, 128.1, 128.8 (×2) (ArCH), 140.7 (×2) (ArC), 173.4 (C1); *m*/*z* (ESI +ve) 841 ($[2M + Na]^+$, 73), 432 ($[M + Na]^+$, 100).

Enantiomer **31D**: mp 74−76 °C; $[\alpha]^{25}_D$+58.4 (*c* 1.13, $CHCl_3$).

*2-C-Azidomethyl-L-ribono-1,4-lactone (**32L**).* A premixed solution of methanol/acetyl chloride (20:1, 15 mL) was added to azidolactone **31L** (700 mg, 1.71 mmol), and the mixture was stirred at reflux. After 18 h, TLC analysis (1:1 EtOAc/cyclohexane) revealed the complete consumption of starting material (R_f 0.89) and the formation of one major product (R_f 0.11). The reaction mixture was concentrated in vacuo and coevaporated with CH_2Cl_2 (3 × 5 mL). The crude mixture was purified by flash column chromatography (1:3 → 3:1 EtOAc/

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cyclohexane) to afford the title lactone **32L** as a white crystalline solid (228 mg, 66%): HRMS (ESI +ve) found 226.0438 $[M + Na]^+$, $C_6H_9N_3NaO_5$ requires 226.0434; mp 92−94 °C; $[\alpha]^{25}_D$ −90.5 (*c* 0.92, $(CH_3)_2CO$); ν_{max} (thin film) 3383 (br, s, OH), 2113 (s, N_3), 1771 (s, C═O); δ_H (400 MHz, $(CD_3)_2CO$) 3.58 (1H, d, H2′a, J_{gem} 12.6), 3.64 (1H, d, H2′b, J_{gem} 12.6), 3.76 (1H, ddd, H5a, J_{gem} 12.6, $J_{5a,OH}$ 6.3, $J_{5a,4}$ 3.8), 3.98 (1H, ddd, H5b, J_{gem} 12.9, $J_{5b,OH}$ 5.1, $J_{5b,4}$ 2.5), 4.27 (1H, dd, OH5, $J_{OH,5a}$ 5.6, $J_{OH,5b}$ 5.3), 4.32 (1H, ddd, H4, $J_{4,3}$ 7.1, $J_{4,5a}$ 3.8, $J_{4,5b}$ 2.3), 4.38−4.42 (1H, m, H3), 5.05 (1H, d, OH3, $J_{OH,3}$ 6.3), 5.18 (1H, s, OH2); δ_C (100.6 MHz, $(CD_3)_2CO$) 52.8 (C2′), 60.2 (C5), 68.6 (C3), 74.8 (C2), 83.7 (C4), 173.6 (C1); *m*/*z* (ESI −ve) 405 ($[2M - H]^-$, 100), 202 ($[M - H]^-$, 74).

*2-C-Azidomethyl-2,3,5-tri-O-benzyl-L-ribono-1,4-lactone (**33L**).* Sodium hydride (60% in mineral oil, 183 mg, 4.57 mmol) was added to a solution of azidolactone **32L** (232 mg, 1.14 mmol) and benzyl bromide (1.36 mL, 11.4 mmol) in DMF (4 mL) in the presence of 3 Å molecular sieves. The mixture was stirred at −5 °C, and after 2.5 h TLC analysis (1:3 EtOAc/cyclohexane) revealed the formation of one major product (R_f 0.58) and remaining starting material (R_f 0.00). A further portion of sodium hydride (46 mg, 1.2 mmol) was added, and the reaction mixture was stirred for 1 h, after which time TLC analysis revealed one major product (R_f 0.58) and trace starting material (R_f 0.00). The pH of the reaction mixture was adjusted to ∼5 with glacial acetic acid, diluted with EtOAc (10 mL) and filtered (glass microfibre). The filtrate was washed with 1:1 brine/water (3 × 10 mL) and brine (1 × 10 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude mixture was purified by flash column chromatography (1:99 → 1:10 EtOAc/cyclohexane) to afford the title tribenzylated lactone **33L** as a colorless oil (351 mg, 65%): HRMS (ESI +ve) found 496.1829 $[M + Na]^+$, $C_{27}H_{27}N_3NaO_5$ requires 496.1843; $[\alpha]^{25}_D$ −107.7 (*c* 1.32, $CHCl_3$); ν_{max} (thin film) 2105 (s, N_3), 1776 (s, C═O); δ_H (400 MHz, C_6D_6) 3.22 (1H, dd, H5a, J_{gem} 10.9, $J_{5a,4}$ 3.0), 3.37 (1H, d, H2′a, J_{gem} 12.6), 3.42 (1H, dd, H5b, J_{gem} 10.9, $J_{5b,4}$ 2.4), 3.46 (1H, d, H2′b, J_{gem} 12.9), 4.17 (1H, d, CH_2Ph^a, J_{gem} 12.1), 4.25 (1H, d, CH_2Ph^a, J_{gem} 11.9), 4.36−4.40 (2H, m, H3, H4), 4.48 (1H, d, CH_2Ph^b, J_{gem} 11.9), 4.74−4.77 (2H, m, CH_2Ph^{b+c}), 4.92 (1H, d, CH_2Ph^c, J_{gem} 10.4), 7.15−7.28 (15H, m, ArH); δ_C (100.6 MHz, C_6D_6) 53.5 (C2′), 68.2 (C5), 69.5 (CH_2Ph^c), 73.3 (CH_2Ph^b), 73.5 (CH_2Ph^a), 76.6 (C4), 80.6 (C2), 82.3 (C3), 127.9, 128.1, 128.2, 128.3, 128.6 (×2), 128.7 (ArCH), 137.8 (×2), 137.9 (ArC), 171.8 (C1); *m*/*z* (ESI +ve) 969 ($[2M + Na]^+$, 55), 496 ($[M + Na]^+$, 100).

*2-C-Azidomethyl-2,3,5-tri-O-benzyl-L-ribitol (**34L**).* Sodium borohydride (64 mg, 1.7 mmol) was added to a solution of tribenzyl lactone **33L** (322 mg, 0.68 mmol) in EtOH (6 mL). TLC analysis (1:3 EtOAc/cyclohexane) after 2 h revealed the formation of one major product (R_f 0.00−0.20 streak) and one minor component (R_f 0.20−0.32 streak). A further portion of sodium borohydride (39 mg, 1.0 mmol) was added and the reaction mixture was stirred for a further 1 h, after which time TLC analysis (1:3 EtOAc/cyclohexane) revealed the presence of one major product (R_f 0.00−0.20 streak) and the near complete consumption of the minor component (R_f 0.20−0.32 streak). The reaction mixture was quenched with satd ammonium chloride solution, diluted with EtOAc (15 mL) and washed with brine (15 mL). The aqueous layer was extracted with EtOAc (2 × 10 mL) and the combined organic fractions were dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude mixture was purified by flash column chromatography (1:9 → 13:37 EtOAc/cyclohexane) to afford the diol **34L** as a colorless oil (302 mg, 93%): HRMS (ESI +ve) found 500.2142 $[M + Na]^+$, $C_{27}H_{31}N_3NaO_5$ requires 500.2156; $[\alpha]^{25}_D$ −21.3 (*c* 0.75, $CHCl_3$); ν_{max} (thin film) 3447 (br, s, OH), 2103 (s, N_3); δ_H (400 MHz, C_6D_6) 3.43 (1H, d, H2′a, J_{gem} 12.9), 3.59 (1H, d, H2′b, J_{gem} 12.9), 3.67−3.73 (2H, m, H5), 3.87 (1H, d, H1a, J_{gem} 12.1), 3.80 (1H, d, H1b, J_{gem} 12.1), 4.03 (1H, d, H3, $J_{3,4}$ 7.1), 4.22−4.26 (1H, m, H4), 4.34 (1H, d, CH_2Ph^a, J_{gem} 11.9), 4.40 (1H, d, CH_2Ph^a, J_{gem} 11.9), 4.64 (1H, d, CH_2Ph^b, J_{gem} 10.9), 4.70 (1H, d, CH_2Ph^b, J_{gem} 11.1), 4.66 (1H, d, CH_2Ph^c, J_{gem}11.2), 4.67 (1H, d, CH_2Ph^c, J_{gem} 11.2), 7.17−7.31 (15H, m, ArH); δ_C (100.6 MHz, C_6D_6) 51.5 (C2′), 62.5 (C1), 65.9 (CH_2Ph^b), 71.0 (C4), 71.7 (C5), 73.5 (CH_2Ph^a), 75.6 (CH_2Ph^c), 81.0 (C3), 81.7 (C2), 127.9 (×2), 128.1, 128.2, 128.6 (ArCH), 138.5, 138.6, 138.9 (ArC); *m*/*z* (ESI +ve) 978 ($[2M + Na]^+$, 35), 500 ($[M + Na]^+$, 100).

*2-C-Azidomethyl-2,3,5-tri-O-benzyl-1-O-tert-butyldimethylsilyl-L-ribitol (**35L**).* *tert*-Butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (0.023 mL, 0.10 mmol) was added dropwise to a solution of diol **34L** (43 mg, 0.090 mmol) and 2,6-lutidine (0.021 mL, 0.18 mmol) in CH_2Cl_2 (1 mL) in the presence of 3 Å molecular sieves, and the mixture was stirred at −78 °C. After 3 h, TLC analysis (1:3 EtOAc/cyclohexane) revealed the formation of one major product (R_f 0.69), one minor product (R_f 0.86), and remaining starting material (R_f 0.20). Further TBSOTf (0.01 mL, 0.05 mmol) and 2,6-lutidine (0.01 mL, 0.09 mmol) were added, and the mixture was stirred for 2 h, followed by addition of further portions of TBSOTf (0.01 mL, 0.05 mmol) and 2,6-lutidine (0.01 mL, 0.09 mmol) with an additional 1 h of stirring. The reaction mixture was diluted with CH_2Cl_2 (5 mL) and washed with water (5 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 5 mL), and the combined organic fractions were dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude mixture was purified by flash column chromatography (1:99 → 1:11 EtOAc/cyclohexane) to afford the silyl ether **35L** as a colorless oil (50 mg, 93%): HRMS (ESI +ve) found 614.3021 $[M + Na]^+$, $C_{33}H_{45}N_3NaO_5Si$ requires 614.3021; $[\alpha]^{25}_D$ −9.6 (*c* 0.89, $CHCl_3$); ν_{max} (thin film) 3473 (br, s, OH), 2102 (s, N_3); δ_H (400 MHz, $CDCl_3$) 0.10 (3H, s, CH_3Si), 0.12 (3H, s, CH_3Si), 0.95 (9H, s, $(CH_3)_3CSi$), 3.30 (1H, d, OH, $J_{OH,4}$ 2.8), 3.64 (1H, d, H2′a, J_{gem} 13.1), 3.71−3.78 (2H, m, H5), 3.87 (1H, d, H1a, J_{gem} 11.1), 3.90 (1H, d, H2′b, J_{gem} 13.1), 3.94 (1H, d, H1b, J_{gem} 11.1), 4.01 (1H, d, H3, $J_{3,4}$ 6.6), 4.20 (1H, m, H4), 4.54 (1H, d, CH_2Ph^a, J_{gem} 11.9), 4.59 (1H, d, CH_2Ph^a, J_{gem} 11.9), 4.64 (1H, d, CH_2Ph^b, J_{gem} 11.4), 4.72 (1H, d, CH_2Ph^b, J_{gem} 11.4), 4.73 (1H, d, CH_2Ph^c, J_{gem} 10.9), 4.76 (1H, d, CH_2Ph^c, J_{gem} 10.9), 7.21−7.40 (15H, m, ArH); δ_C (100.6 MHz, $CDCl_3$) −5.6 (CH_3Si), −5.5 (CH_3Si), 18.1 ($(CH_3)_3CSi$), 25.9 ($(CH_3)_3CSi$), 51.4 (C2′), 62.7 (C1), 66.6 (CH_2Ph^a), 71.2 (C4), 71.4 (C5), 73.5 (CH_2Ph^c), 74.8 (CH_2Ph^b), 79.1 (C3), 82.3 (C2), 127.5, 127.6, 127.7, 127.8, 128.4 (15 × ArCH), 138.1, 138.2 (3 × ArC); *m*/*z* (ESI −ve) 626 ($[M + Cl]^-$, 100).

*2-C-Azidomethyl-2,3,5-tri-O-benzyl-1-tert-butyldimethylsilyl-4-O-methanesulfonyl-L-ribitol (**36L**).* Methanesulfonyl chloride (mesyl chloride) (27 μL, 0.35 mmol) was added dropwise to a solution of silyl ether **35L** (102 mg, 0.173 mmol) and pyridine (70 μL, 0.87 mmol) in CH_2Cl_2 (2 mL), and the mixture was stirred at rt. After 5 h, TLC analysis (1:19 acetone/toluene) revealed the formation of a single product (R_f 0.50) and remaining starting material (R_f 0.37). A further portion of mesyl chloride (13 μL, 0.17 mmol) and pyridine (35 μL, 0.43 mmol) were added. After 30 min, TLC analysis (1:19 acetone/toluene) revealed the persistence of starting material (R_f 0.37). 4-Dimethylaminopyridine (DMAP) (2 mg, 10 mol %) and a further portion of mesyl chloride (13 μL, 0.17 mmol) and pyridine (35 μL, 0.43 mmol) were added, followed by three subsequent additions of mesyl chloride (13 μL, 0.17 mmol) and pyridine (35 μL, 0.43 mmol) after 5 h, 6.5 and 9.5 h respectively, due to the continued persistence of starting material (R_f 0.37). After this time TLC analysis revealed the presence of a single product (R_f 0.50) and significant consumption of starting material (R_f 0.37). The reaction mixture was diluted with CH_2Cl_2 (15 mL) and washed with 2 M HCl (10 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL) and the combined organic fractions were dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude mixture was purified by flash column chromatography (0:1 → 3:47 acetone/toluene) to afford the mesylate **36L** as a colorless oil (88 mg, 76%): HRMS (ESI +ve) found 692.2792 $[M + Na]^+$, $C_{34}H_{47}N_3NaO_7SSi$ requires 692.2796; $[\alpha]^{25}_D$ −9.9 (*c* 0.96, $CHCl_3$); ν_{max} (thin film) 2105 (s, N_3), 1358, 1175 (s, SO_2); δ_H (400 MHz, $CDCl_3$) 0.10 (3H, s, CH_3Si), 0.12 (3H, s, CH_3Si), 0.92 (9H, s, $(CH_3)_3CSi$), 2.98 (3H, s, CH_3S), 3.52 (1H, d, H2′a, J_{gem} 12.6), 3.82 (1H, d, H1a, J_{gem} 11.1), 3.83 (1H, d, H2′b, J_{gem} 12.9), 3.82 (1H, dd, H5a, J_{gem} 11.4, $J_{5a,4}$ 8.6), 3.85 (1H, d, H1b, J_{gem} 11.1), 3.96 (1H, dd, H5b, J_{gem} 11.6, $J_{5b,4}$ 2.0), 4.17 (1H, d, H3, $J_{3,4}$ 1.5), 4.34 (1H, d, CH_2Ph^a, J_{gem} 11.6), 4.41 (1H, d, CH_2Ph^a, J_{gem} 11.6), 4.59 (1H, d, CH_2Ph^b, J_{gem} 11.1), 4.70 (1H, d, CH_2Ph^c, J_{gem} 11.0), 4.73 (1H, d, CH_2Ph^c, J_{gem} 11.0), 4.86 (1H, d, CH_2Ph^b, J_{gem} 11.1), 5.27 (1H, ddd,

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H4, $J_{4,5a}$ 8.6, $J_{4,5b}$ 2.1, $J_{4,3}$ 1.4), 7.23−7.40 (15H, m, ArH); δ_C (100.6 MHz, $CDCl_3$) −5.7 (×2) (CH_3Si), 18.1 ($(CH_3)_3CSi$), 25.9 ($(CH_3)_3CSi$), 38.5 (CH_3S), 51.2 (C2′), 62.6 (C1), 66.6 (CH_2Ph^c), 69.9 (C5), 73.2 (CH_2Ph^a), 74.8 (CH_2Ph^b), 81.2 (C2), 81.5 (C3), 84.1 (C4), 127.4, 127.5, 127.7, 127.8, 128.0 (×2), 128.3, 128.4, 128.5 (ArCH), 137.4, 137.6, 138.3 (ArC); *m/z* (ESI −ve) 706 ($[M + {}^{37}Cl]^-$, 50), 704 ($[M + {}^{35}Cl]^-$, 100).

*1,4-Dideoxy-2-C-hydroxymethyl-1,4-imino-D-xylitol (**10L**) (L-isoDGDP).* Pd (10% on C, 8 mg, 10 mol %) and sodium acetate (19 mg, 0.23 mmol) were added to a solution of mesylate **36L** (52 mg, 0.080 mmol) in 5:1 1,4-dioxane/water (1 mL). The reaction vessel was evacuated and flushed with argon, followed by hydrogen. After 5 h, LRMS revealed the presence of the fully protected form of L-isoDGDP (*m/z* 548, $[M + H]^+$) and absence of starting material. The reaction mixture was acidified with 2 M HCl (0.10 mL) and the reaction vessel recharged with hydrogen. After 54 h, the reaction mixture was filtered (glass microfibre) and concentrated in vacuo. The crude mixture was loaded onto a column of Dowex (50W-X8, H^+) and the resin washed with water before elution of the product with 2 M NH_3 (aq). The ammoniacal fractions were concentrated in vacuo to afford L-isoDGDP (**10L**) as a light orange oil (14 mg, quant.).

Data for HCl salt: HRMS (ESI +ve): found 186.0742 $[M + Na]^+$; $C_6H_{13}NNaO_4$ requires 186.0737; $[\alpha]^{25}_D$+33.5 (*c* 0.65, H_2O); ν_{max} (neat) 3300 (br, s, OH, NH); δ_H (500 MHz, D_2O) 3.30 (1H, d, H1a, J_{gem} 12.6), 3.43 (1H, d, H1b, J_{gem} 12.6), 3.77 (1H, d, H2′a, J_{gem} 12.3), 3.87 (1H, d, H2′b, J_{gem} 12.0), 3.91 (1H, dd, H5a, J_{gem} 12.0, $J_{5a,4}$ 8.5), 4.01 (1H,dd, H5b, J_{gem} 12.1, $J_{5b,4}$ 4.9), 4.09 (1H, ddd, H4, $J_{4,5a}$ 8.5, $J_{4,5b}$ 5.0, $J_{4,3}$ 3.5), 4.23 (1H, d, H3, $J_{3,4}$ 3.5); δ_C (125 MHz, D_2O) 51.6 (C1), 58.2 (C5), 62.2 (C2′), 64.7 (C4), 74.3 (C3), 83.0 (C2); *m/z* (ESI +ve) 164 ($[M + H]^+$, 100).

C. Shorter Synthesis of a Mixture of IsoDMDP (9D) and IsoDGDP (10D). *2-C-Azidomethyl-5-O-benzhydryl-2,3-O-isopropylidene-D-ribitol (**37D**).* Sodium borohydride (180 mg, 4.76 mmol) was added to a solution of lactone **31D** (652 mg, 1.59 mmol) in 13:2 EtOH/^{t}BuOH (7.5 mL) and stirred at rt for 90 min. TLC analysis (1:2 EtOAc/cyclohexane) revealed the complete consumption of starting material (R_f 0.65) and a major product (R_f 0−0.40 streak). The reaction mixture was quenched with satd ammonium chloride, diluted with brine (20 mL) and extracted with EtOAc (3 × 30 mL). TLC analysis (1:2 EtOAc/cyclohexane) of the combined organic fractions at this stage revealed a single product (R_f 0.35). The organic layer was dried ($MgSO_4$), filtered, concentrated in vacuo, and purified by flash column chromatography (1:9 → 7:13 EtOAc/cyclohexane) to give the diol **37D** (637 mg, 97%) as a colorless gum: HRMS (ESI +ve) found 436.1843 $[M + Na]^+$, $C_{22}H_{27}N_3NaO_5$ requires 436.1843; $[\alpha]^{25}_D$+48.4 (*c* 0.73, $CHCl_3$); ν_{max} (thin film) 3385 (s, br, OH); 2103 (s, N_3); δ_H (400 MHz, C_6D_6) 1.19 (3H, s, CH_3), 1.28 (3H, s, CH_3), 2.42 (1H, t, OH1, $J_{OH,1}$ 5.7), 3.07 (1H, d, OH4, $J_{OH,4}$ 3.9), 3.13 (1H, d, H2′a, J_{gem} 13.1), 3.46 (1H, d, H2′b, J_{gem} 13.1), 3.49−3.53 (2H, m, H1a, H5a), 3.62 (1H, dd, H1b, J_{gem} 11.4, $J_{1b,OH}$ 5.2), 3.72 (1H, dd, H5b, J_{gem} 9.6, $J_{5b,4}$ 2.3), 3.94−4.01 (1H, m, H4), 4.02 (1H, d, H3, $J_{3,4}$ 9.5), 5.25 (1H, s, $CHPh_2$), 7.01−7.06 (2H, m, ArH), 7.10−7.16 (4H, m, ArH), 7.28−7.32 (4H, m, ArH); δ_C (100.6 MHz, C_6D_6) 26.1 (CH_3), 28.5 (CH_3), 54.7 (C2′), 62.7 (C1), 69.6 (C4), 71.6 (C5), 77.8 (C3), 84.7 ($CHPh_2$), 85.0 (C2), 109.0 ($C(CH_3)_2$), 127.4, 127.8, 128.3, 128.7 (ArCH), 142.3, 142.4 (ArC); *m/z* (ESI +ve) 849 ($[2M + Na]^+$, 100), 436 ($[M + Na]^+$, 93%).

*2-C-Azidomethyl-5-O-benzhydryl-1-O-tert-butyldimethylsilyl-2,3-O-isopropylidene-D-ribitol (**38D**).* TBSCl (330 mg, 2.19 mmol) was added to a solution of diol **37D** (461 mg, 1.12 mmol) and imidazole (300 mg, 4.41 mmol) in DMF (6 mL) at 0 °C. The mixture was stirred at this temperature for 5 h after which TLC analysis (1:3 EtOAc/cyclohexane) revealed the complete consumption of starting material (R_f 0.20) and the formation of a major product (R_f 0.70). The reaction mixture was diluted with EtOAc (20 mL), washed with 1:1 brine/water (3 × 20 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. Purification by flash column chromatography (1:99 → 1:19 EtOAc/cyclohexane) afforded the silyl ether **38D** (576 mg, 98%) as a colorless oil: HRMS (ESI +ve) found 550.2708 $[M + Na]^+$, $C_{28}H_{41}N_3NaO_5Si$ requires 550.2708; $[\alpha]^{25}_D$+12.7 (*c* 0.90, $CHCl_3$); ν_{max} (thin film) 3474 (m, br, OH), 2103 (s, N_3); δ_H (400 MHz, $CDCl_3$) 0.14 (3H, s, CH_3Si), 0.15 (3H, s, CH_3Si), 0.93 (9H, s, $(CH_3)_3CSi$), 1.38 (3H, s, $C(CH_3)_2$), 1.41 (3H, s, $C(CH_3)_2$), 3.43 (1H, d, H2′a, J_{gem} 12.9), 3.60 (1H, d, H1a, J_{gem} 10.4), 3.61 (1H, dd, H5a, J_{gem} 10.4, $J_{5a,4}$ 5.0), 3.65 (1H, d, H2′b, J_{gem} 12.9), 3.66 (1H, d, OH, $J_{OH,4}$ 4.0), 3.75 (1H, d, H1b, J_{gem} 10.4), 3.78 (1H, dd, H5b, J_{gem} 10.4, $J_{5b,4}$ 2.3), 4.01 (1H, dddd, H4, $J_{4,3}$ 9.6, $J_{4,5a}$ 5.0, $J_{4,5b}$ 2.1, $J_{4,OH}$ 4.0), 4.07 (1H, d, H3, $J_{3,4}$ 9.6), 5.49 (1H, s, $CHPh_2$), 7.22−7.39 (10H, m, ArH); δ_C (100.6 MHz, $CDCl_3$) −5.8 (CH_3Si), −5.7 (CH_3Si), 18.2 ($(CH_3)_3CSi$), 25.8 ($(CH_3)_3CSi$), 25.9 ($C(CH_3)_2$), 28.3 ($C(CH_3)_2$), 55.2 (C2′), 62.7 (C1), 69.4 (C4), 70.8 (C5), 78.0 (C3), 83.8 (C2), 84.2 ($CHPh_2$), 108.5 ($C(CH_3)_2$), 127.0, 127.1, 127.3, 127.3, 128.2, 128.3 (ArCH), 142.1, 142.2 (ArC); *m/z* (ESI +ve) 550 ($[M + Na]^+$, 100), 545 ($[M + NH_4]^+$, 91).

*4-C-Azidomethyl-1-O-benzhydryl-2,3-O-isopropylidene-L-ribulofuranose (**39L**).* Dess−Martin periodinane (675 mg, 1.59 mmol) was added to a solution of ribitol **38D** (576 mg, 1.09 mmol) in DCM (10 mL) and the mixture stirred at rt. TLC analysis (toluene) after 2 h revealed the complete consumption of starting material (R_f 0.20) and the formation of a single product (R_f 0.25). The reaction mixture was diluted with EtOAc (30 mL) and stirred with thiosulfate−bicarbonate solution (30 mL, 8% w/v aqueous sodium thiosulfate saturated with sodium bicarbonate) for 30 min. The aqueous was discarded and the organic washed with thiosulfate-bicarbonate solution (3 × 20 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo to give the crude ketone (573 mg, 100%) as a colorless oil. TBAF (1 M in THF, 0.80 mL, 0.80 mmol) was added to a solution of the crude ketone (361 mg, 0.688 mmol) in THF (5 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h and then rt for 1 h after which TLC analysis (1:3 EtOAc/cyclohexane) revealed the complete consumption of starting material (R_f 0.70) and the formation of a major product (R_f 0.50). The reaction mixture was concentrated in vacuo, and purification by flash column chromatography (1:49 → 1:4 EtOAc/cyclohexane) afforded the protected ribulose **39L** (262 mg, 92%) as a colorless, partially crystalline material, in an 11:2 ratio of anomers: HRMS (ESI +ve) found 434.1684 $[M + Na]^+$, $C_{22}H_{25}N_3NaO_5$ requires 434.1686; $[\alpha]^{25}_D$+79.3 (*c* 1.06, $CHCl_3$); ν_{max} (thin film) 3474 (m, br, OH), 2103 (s, N_3); δ_H (400 MHz, $CDCl_3$) 1.43 (3H, s, CH_3^A), 1.44 (3H, s, CH_3^A), 1.54 (3H, s, CH_3^B), 1.60 (3H, s, CH_3^B), 3.35 (1H, d, H4′a^B J_{gem} 13.0), 3.42 (1H, d, H4′b^B, J_{gem} 12.9), 3.50 (1H, s, OH^A), 3.50 (1H, d, H4′a^A, J_{gem} 12.9), 3.60 (1H, d, H1a^B, J_{gem} 10.2), 3.62 (1H, d, H4′a^B, J_{gem} 12.9), 3.67 (1H, d, H1b,^{B} J_{gem} 10.2), 3.72 (1H, d, H1a^A, J_{gem} 10.4), 3.76 (1H, d, H1b^A, J_{gem} 10.5), 3.85 (1H, d, H5a^B, J_{gem} 9.9), 3.95 (1H, d, H5a^A, J_{gem} 10.1), 3.96 (1H, d, H5b^B, J_{gem} 9.9), 4.02 (1H, d, H5b^A, J_{gem} 10.1), 4.26 (1H, s, OH^B), 4.27 (1H, s, H3^A), 4.51 (1H, s, H3^B), 5.42 (1H, s, $CHPh_2^B$), 5.54 (1H, s, $CHPh_2^A$), 7.24−7.38 (20H, m, ArH); δ_C (125 MHz, $CDCl_3$) 27.4 (CH_3^A), 27.5 (CH_3^A), 27.5 (CH_3^B), 27.9 (CH_3^B), 53.6 (C4′^{B}), 55.0 (C4′^{A}), 69.6 (C1^A), 72.6 (C1^B), 74.0 (C5^B), 75.1 (C5^A), 83.0 (C3^B), 84.6 ($CHPh_2^A$), 84.9 ($CHPh_2^B$), 87.0 (C3^A), 91.9 (C4^A), 91.9 (C4^B), 103.5 (C2^B), 105.2 (C2^A), 114.4 ($C(CH_3)_2^A$), 115.5 ($C(CH_3)_2^B$), 126.5, 126.9, 127.0, 127.1, 127.5, 127.6, 127.8, 127.8, 128.4, 128.5, 128.5, 128.6 (ArCH), 141.1, 141.2, 141.2, 141.4 (ArC); *m/z* (ESI +ve) 845 ($[2M + Na]^+$, 77), 434 ($[M + Na]^+$, 100).

*4-C-Azidomethyl-L-ribulofuranose (**40L**).* *p*TSA (100 mg, 0.526 mmol) was added to a solution of ribulofuranose **39L** (203 mg, 0.494 mmol) in 2:3 water/1,4-dioxane (5 mL) at 85 °C. The reaction mixture was stirred at this temperature for 18 h after which TLC analysis (1:9 acetone/toluene) revealed the complete consumption of starting material (R_f 0.60) and the formation of benzhydrol (R_f 0.55) and a major product (R_f 0.00; R_f 0.50 in 45:5:1 EtOAc/EtOH/H_2O). The mixture was neutralized with satd aq sodium bicarbonate, preadsorbed on silica gel and purified by flash column chromatography (1:2 → 1:0 EtOAc/cyclohexane, then 45:5:1 → 7:2:1 EtOAc/IPA/H_2O) to give the unprotected ribulose **40L** (64 mg, 63%) as a colorless oil. NMR analysis revealed a 5:1 ratio of anomers in aqueous solution: HRMS (ESI +ve) found 228.0593 $[M + Na]^+$, $C_6H_{11}N_3NaO_5$ requires 228.0591; $[\alpha]^{25}_D$ equilibrium −18.8 (*c* 0.97, H_2O); ν_{max} (thin film, Ge): 3399 (s, br, OH), 2111 (s, N_3); δ_H (400 MHz, D_2O) 3.43 (1H, d, H4′a^A, J_{gem} 12.9), 3.55 (1H, d, H4′a^B, J_{gem}

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13.1), 3.56 (1H, d, H1a^A, J_{gem} 12.1), 3.58 (1H, d, H4′b^A, J_{gem} 13.0), 3.59 (1H, d, H1b^A, J_{gem} 12.0), 3.61 (1H, d, H4′b^B, J_{gem} 13.0), 3.67 (1H, d, H1a^B, J_{gem} 12.0), 3.70 (1H, d, H1b^B, J_{gem} 12.0), 3.89 (1H, d, H5a^B, J_{gem} 10.2), 3.92 (1H, d, H5a^A, J_{gem} 10.2), 3.98 (1H, s, H3^A), 3.99 (1H, d, H5b^A, J_{gem} 10.2), 4.02 (1H, s, H3^B), 4.03 (1H, d, H5b^B, J_{gem} 10.2); δ_C (125 MHz, D_2O) 55.8 (C4′^{B}), 56.3 (C4′^{A}), 63.2 (C1^A), 63.4 (C1^B), 72.2 (C3^A), 73.8 (C5^B), 74.1 (C5^A), 78.6 (C4^A), 79.7 (C3^B), 80.0 (C4^B), 103.8 (C2^A), 106.3 (C2^B); *m*/*z* (ESI +ve) 228 ([M + Na]$^+$, 100).

*1,4-Dideoxy-2-C-hydroxymethyl-1,4-imino-L-xylitol (**10D**) (isoDGDP) and 1,4-Dideoxy-2-C-hyroxymethyl-1,4-imino-D-arabinitol (**9D**) (isoDMDP).* Pd (10% on C, 30 mg, 28 μmol) was added to a solution of the unprotected ribulose **40L** (64 mg, 0.31 mmol) in water (5 mL) and the vessel degassed and flushed with Ar before charging with hydrogen. The mixture was stirred under hydrogen at room temperature for 18 h before removal of the catalyst by filtration (glass microfibre). The filtrate was concentrated in vacuo and the crude mixture loaded onto a column of Dowex (50W-X8, H^+). The resin was washed with EtOH and then water until neutral fractions were obtained, and the product was then liberated with 2 M NH_3(aq). The ammoniacal fractions were concentrated in vacuo, assisted by coevaporation with EtOH, to give a mixture of isoDGDP (**10D**) and isoDMDP (**9D**) (43 mg, 84%) in a ratio of approximately 3:2, as judged by relative integrations in the ^{1}H NMR spectrum, as a brown foam. Separation of a sample of the isomeric mixture (32 mg, 0.20 mmol) was achieved by ion exchange chromatography. The crude reaction mixture was applied to a Dowex 1 × 2 column (200 mL, OH^- form) and eluted with water (fraction size 5 mL). This water elute was divided two pools, A (fractions 16−23) and B (fractions 24−31). Each Pool was rechromatographed in the same column with water as eluents to give isoDMDP **9D** (5.6 mg, 18%) from Pool B and isoDGDP **10D** (6.6 mg, 21%) from A as colorless oils.

Data for isoDGDP **10D**: HRMS (ESI +ve) found 164.0916 [M + H]$^+$, $C_6H_{14}NO_4$ requires 164.0917; $[\alpha]^{25}_D$ −16.6 (*c* 0.47, H_2O); ν_{max} (thin film, Ge) 3331 (s, br, OH); δ_H (500 MHz, D_2O) 2.95 (1H, d, H1a, J_{gem} 12.6), 3.11 (1H, d, H1b, J_{gem} 12.6), 3.67−3.71 (1H, m, H4), 3.70 (1H, d, H2′a, J_{gem} 12.0), 3.74 (1H, dd, H5a, J_{gem} 11.0, $J_{5a,4}$ 7.3), 3.85 (1H, d, H2′b, J_{gem} 12.0), 3.86 (1H, dd, H5b, J_{gem} 11.0, $J_{5b,4}$ 6.0), 4.09 (1H, d, H3, $J_{3,4}$ 3.8); δ_C (125 MHz, D_2O) 52.4 (C1), 60.0 (C2′), 63.1 (C5), 63.1 (C4), 75.9 (C3), 84.1 (C2); *m*/*z* (ESI +ve) 164 (M + H$^+$, 100).

D. Synthesis of IsoLAB (11L) and IsoDAB (11D). *2-C-Hydroxymethyl-2,3:5,6-di-O-isopropylidene-D-mannofuranose (**47D**).* Aqueous formaldehyde (39.5%, 44.6 mL, 589 mmol) was added to a stirred solution of diacetone mannose **46D** (9.1 g, 35 mmol) and potassium carbonate (6.5 g, 47 mmol) in methanol (150 mL), and the mixture was stirred for 5 h at reflux under an atmosphere of argon. TLC analysis (1:1 EtOAc/cyclohexane) showed a small amount of starting material (R_f 0.65), a major product (R_f 0.35) and a minor product (R_f 0.10). The mixture was filtered through Celite and concentrated in vacuo. The crude residue was suspended in EtOAc (100 mL), filtered, concentrated in vacuo, and then purified by flash column chromatography (1:3 EtOAc/cyclohexane) affording branched lactol **47D** (7.51 g, 74%) as a white crystalline solid in a 3:2 ratio of anomers: HRMS (ESI +ve) found 313.1253 [M + Na]$^+$, $C_{13}H_{22}NaO_7$ requires 313.1258; mp 86−88 °C; $[\alpha]^{25}_D$ +9.1 (*c* 1.2, MeOH) [lit.[24] $[\alpha]^{25}_D$ +11 (*c* 1.2, MeOH)]; ν_{max} (thin film) 3449 (w, br, OH); δ_H (400 MHz, $CDCl_3$) 1.37 (3H, s, CH_3^B), 1.37 (3H, s, CH_3^A), 1.41 (3H, s, CH_3^A), 1.44 (3H, s, CH_3^B), 1.45 (3H, s, CH_3^A), 1.46 (3H, s, CH_3^A), 1.49 (3H, s, CH_3^B), 1.56 (3H, s, CH_3^B), 2.57 (2H, br, s, OH), 3.51 (1H, dd, H4^B, $J_{4,5}$ 8.3, $J_{4,3}$ 2.8), 3.77 (1H, d, H2′a^B, J_{gem} 11.6), 3.79 (1H, d, H2′b^B, J_{gem} 11.8), 3.85 (1H, d, H2′a^A, J_{gem} 11.9), 3.99 (1H, d, H2′b^A, J_{gem} 11.9), 4.00−4.14 (4H, m, H6^A, H6^B), 4.14 (1H, dd, H4^A, $J_{4,5}$ 7.7, $J_{4,3}$ 2.9), 4.35−4.43 (2H, m, H5^A, H5^B), 4.65 (1H, d, H3^B, $J_{3,4}$ 3.0), 4.65 (1H, d, H3^A, $J_{3,4}$ 2.8), 4.91 (1H, br, s, H1^B), 5.36 (1H, s, H1^A); δ_C (100.6 MHz, $CDCl_3$) 25.1, 25.2, 26.8 26.9, 27.0 (×2), 27.3 (8 x CH_3), 62.7 (C2′^{B}), 63.6 (C2′^{A}), 66.6 (C6^A), 67.1 (C6^B), 72.9 (C5^B), 73.1 (C5^A), 76.4 (C4^B), 81.0 (C4^A), 81.9 (C3^B), 82.8 (C3^A), 89.4 (C2^B), 93.7 (C2^A), 97.6 (C1^B), 103.8 (C1^A), 109.3 (C(CH$_3$)$_2^A$), 109.4 (C(CH$_3$)$_2^B$), 113.8 (C(CH$_3$)$_2^A$), 114.1 (C(CH$_3$)$_2^B$); *m*/*z* (ESI +ve): 603 ([2M + Na]$^+$, 100), 313 ([M + Na]$^+$, 100).

*2-C-Hydroxymethyl-2,3:5,6-di-O-isopropylidene-D-mannono-1,4-lactone (**48D**). Method 1.* Barium carbonate (8.9 g, 45 mmol) was slowly added to a stirred solution of lactol **47D** (8.72 g, 30.0 mmol) in water (80 mL) at 0 °C. Bromine (2.4 mL, 46 mmol) was added dropwise, and the mixture was left to stir for 32 h, after which time TLC (EtOAc) showed complete consumption of starting material (R_f 0.60) and the formation of one product (R_f 0.80). Nitrogen was bubbled through the reaction mixture for 30 min until pale yellow and then the mixture quenched with sodium thiosulfate (satd aq). The reaction mixture was extracted with EtOAc (12 × 60 mL) and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude residue was purified by flash column chromatography (1:3 EtOAc/cyclohexane) to afford lactone **48D** (6.89 g, 80%).

Method 2. Potassium carbonate (6.38 g, 46.2 mmol) and iodine (11.7 g, 46.2 mmol) were added to a stirred solution of lactol **47D** (6.7 g, 23.1 mmol) in *tert*-butyl alcohol (67 mL) at 100 °C. After 1 h, TLC (1:1 EtOAc/cyclohexane) showed complete consumption of starting material (R_f 0.40) and the formation of one product (R_f 0.60). The mixture was stirred with sodium thiosulfate (satd aq) and EtOAc until the solution was colorless and the mixture was extracted with EtOAc (3 × 100 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo, and the crude residue was purified by flash column chromatography (1:4 EtOAc/cyclohexane) to afford lactone **48D** (5.36 g, 81%): HRMS (ESI +ve) found 311.1097 [M + Na]$^+$, $C_{13}H_{20}NaO_7$ requires 311.1101; mp 107−108 °C [lit.[24] mp 106 °C]; $[\alpha]^{25}_D$+32.3 (*c* 1.0, $CHCl_3$) [lit.[35] $[\alpha]^{25}_D$+34.7 (*c* 1.0, $CHCl_3$)]; ν_{max} (thin film) 3488 (m, br, OH), 1782 (s, C═O); δ_H (400 MHz, $CDCl_3$) 1.39 (3H, s, CH_3), 1.44 (3H, s, CH_3), 1.48 (3H, s, CH_3), 1.48 (3H, s, CH_3), 2.38 (1H, dd, OH, $J_{OH,2'a}$ 7.6, $J_{OH,2'b}$ 4.3), 3.93 (1H, dd, H2′a, J_{gem} 11.4, $J_{2'a,OH}$ 4.29), 4.00 (1H, dd, H2′b, J_{gem} 11.4, $J_{2'b,OH}$ 7.6), 4.07 (1H, dd, H6a, J_{gem} 9.1, $J_{6a,H5}$ 3.7), 4.15 (1H, dd, H6b, J_{gem} 9.1, $J_{6b,H5}$ 5.8), 4.40 (1H, dd, H4, $J_{4,5}$ 8.1, $J_{4,3}$ 3.3), 4.44 (1H, H5, ddd, $J_{5,4}$ 8.1, $J_{5,6b}$ 5.8, $J_{5,6a}$ 3.7), 4.83 (1H, d, H3, $J_{3,4}$ 3.3); δ_C (100.6 MHz, $CDCl_3$) 25.1 (CH_3), 26.4 (CH_3), 26.9 (CH_3), 26.9 (CH_3), 61.3 (C2′), 66.5 (C6), 72.5 (C5), 78.5 (C4), 78.5 (C3), 86.1 (C2), 109.0 (C(CH$_3$)$_2$), 114.0 (C(CH$_3$)$_2$), 175.2 (C1); *m*/*z* (ESI +ve): 599 (2M + Na]$^+$, 100), 311 ([M + Na]$^+$, 65).

*2-C-Azidomethyl-2,3:5,6-di-O-isopropylidene-D-mannono-1,4-lactone (**49D**).* Trifluoromethanesulfonic anhydride (6.3 mL, 38 mmol) was added dropwise to a solution of lactone **48D** (6.85 g, 23.8 mmol) and pyridine (5.84 mL, 72.4 mmol) in CH_2Cl_2 (120 mL) at −30 °C. TLC analysis (1:1 EtOAc/cyclohexane) after 2.5 h indicated the conversion of starting material (R_f 0.41) into one major product (R_f 0.77). The reaction mixture was diluted with CH_2Cl_2 (120 mL) and washed with HCl (1 M, 100 mL). The organic residue was dried ($MgSO_4$), filtered, and concentrated in vacuo to afford the triflate, which was used without further purification. Sodium azide (2.3 g, 36 mmol) was added to a solution of the crude triflate in dry DMF (100 mL) at rt. TLC analysis (1:1 EtOAc/cyclohexane) after 3 h showed the conversion of starting material to one major product (R_f 0.73). The reaction mixture was concentrated in vacuo, and the residue was partitioned between EtOAc (100 mL) and brine (60 mL). The aqueous layer was extracted with EtOAc (40 mL) and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo. The resulting residue was purified by flash column chromatography (1:6 → 1:2 EtOAc/cyclohexane) to afford the azidolactone **49D** (7.19 g, 96% over two steps) as a white crystalline solid: HRMS (ESI +ve) found 336.1166 [M + Na]$^+$, $C_{13}H_{19}NaN_3O_6$ requires 336.1166; mp 77−79 °C [lit.[24] mp 77 °C)]; $[\alpha]^{25}_D$ −126 (*c* 0.7, $CHCl_3$) [lit.[52] $[\alpha]^{25}_D$ −130 (*c* 0.7, $CHCl_3$)]; ν_{max} (thin film) 2110 (s, N_3), 1791 (s, C═O); δ_H (400 MHz, $CDCl_3$) 1.40 (3H, s, CH_3), 1.48 (3H, s, CH_3), 1.49 (3H, s, CH_3), 1.49 (3H, s, CH_3), 3.61 (1H, d, H2′a, J_{gem} 12.9), 3.82 (1H, d, H2′b, J_{gem} 12.9), 4.08 (1H, dd, H6a, J_{gem} 9.4, $J_{6a,5}$ 3.8), 4.15 (1H, dd, H6b, J_{gem} 9.4, $J_{6b,5}$ 5.9), 4.43 (1H, H5, ddd, $J_{5,6a}$ 3.8, $J_{5,6b}$ 5.9, $J_{5,4}$ 8.2), 4.33 (1H, dd, H4, $J_{4,5}$ 8.2, $J_{4,3}$ 3.5), 4.77 (1H, d, H3, $J_{3,4}$ 3.5); δ_C (100.6 MHz, $CDCl_3$) 25.1 (CH_3), 26.1 (CH_3), 26.9 (CH_3), 27.0 (CH_3), 50.5 (C2′), 66.5 (C6), 72.4 (C5), 78.1 (C4), 78.4

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(C3), 85.0 (C2), 110.0 ($C(CH_3)_2$), 114.6 ($C(CH_3)_2$), 173.8 (C1); *m/z* (ESI +ve): 649 (2M + Na]$^+$, 100), 336 ([M + Na]$^+$, 75).

*2-C-Azidomethyl-2,3:5,6-di-O-isopropylidene-D-mannitol (**50D**).* DIBALH (1.5 M in toluene, 1.6 mL, 2.4 mmol) was added dropwise to a solution of the lactone **49D** (553 mg, 1.76 mmol) in CH_2Cl_2 (7 mL) at −78 °C. The reaction mixture was stirred for 2 h at −78 °C, after which time TLC analysis (1:2 EtOAc/cyclohexane) revealed the conversion of starting material (R_f 0.54) into one major product (R_f 0.42). The reaction was quenched with methanol (5.7 mL) and allowed to warm to rt. Potassium sodium tartrate solution (satd aq, 48 mL) was added and the reaction was stirred for 16 h at rt. The aqueous phase was extracted with CH_2Cl_2 (3 × 30 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude lactol was dissolved in methanol (7 mL) and stirred with sodium borohydride (63 mg, 1.7 mmol) at 0 °C for 30 min and allowed to warm to rt. TLC analysis (1:2 EtOAc/cyclohexane) after a total of 2.5 h revealed the formation of one major product (R_f 0.21). The reaction was neutralized with Dowex (50W-X8, H$^+$), filtered, and concentrated in vacuo. The resulting residue was purified by flash column chromatography (1:5 → 1:1 EtOAc/cyclohexane) to afford the diol **50D** as a colorless oil (375 mg, 68% over two steps): HRMS (ESI +ve) found 340.1479 [M + Na]$^+$; $C_{13}H_{23}N_3NaO_6$ requires 340.1479; $[\alpha]^{25}_D$ −3.4 (*c* 1.0, $CHCl_3$); ν_{max} (thin film) 3413 (w, br, OH), 2111 (s, N_3); δ_H (400 MHz, $CDCl_3$) 1.37 (3H, s, CH_3), 1.43 (3H, s, CH_3), 1.49 (3H, s, CH_3), 1.50 (3H, s, CH_3), 2.92 (2H, a-br-s, 2 × OH), 3.43 (1H, d, H2′a, J_{gem} 13.1), 3.58 (1H, d, H2′b, J_{gem} 13.1), 3.59 (1H, d, H1a, J_{gem} 12.1), 3.73 (1H, a-s, H4), 3.76 (1H, d, H1b, J_{gem} 12.1), 4.04−4.10 (3H, m, H5, H6), 4.28 (1H, d, H3, $J_{3,4}$ 1.0); δ_C (100.6 MHz, $CDCl_3$) 25.5 (CH_3), 26.3 (CH_3), 26.8 (CH_3), 28.1 (CH_3), 53.5 (C2′), 62.9 (C1), 66.8 (C6), 68.8 (C4), 76.4 (C5), 77.0 (C3), 83.1 (C2), 108.9 ($C(CH_3)_2$), 109.6 ($C(CH_3)_2$); *m/z* (ESI +ve) 657 ([2M + Na]$^+$, 100), 340 ([M + Na]$^+$, 100).

*2-C-Azidomethyl-2,3-O-isopropylidene-D-mannitol (**51D**).* A solution of the diacetonide **50D** (215 mg, 1.06 mmol) in AcOH:water (1:1, 7 mL) was stirred for 16 h at rt. TLC analysis (EtOAc) showed complete conversion of starting material (R_f 0.78) to a single product (R_f 0.22). The reaction mixture was concentrated in vacuo, coevaporated with toluene (3 × 15 mL), and purified by flash column chromatography (EtOAc) to afford the tetraol **51D** as a colorless oil (147 mg, 78%): HRMS (ESI +ve) found 300.1167 [M + Na]$^+$, $C_{10}H_{19}N_3NaO_6$ requires 300.1166; $[\alpha]^{25}_D$+54.1 (*c* 1.2, $CHCl_3$); ν_{max} (thin film) 3383 (s, br, OH), 2106 (s, N_3); δ_H (400 MHz, $(CD_3)_2CO$) 1.41 (3H, s, CH_3), 1.42 (3H, s, CH_3), 3.40 (1H, d, H2′a, J_{gem} 13.1), 3.53 (1H, dd, H6a, J_{gem} 11.6, $J_{6a,5}$ 6.3), 3.59 (1H, d, H2′b, J_{gem} 13.1), 3.62−3.73 (3H, m, H1, H5), 3.73−3.80 (3H, m, H6b, H4, OH1), 3.96 (1H, a−d, OH5, *J* 5.8), 4.26 (1H, t, OH6, *J* 6.3), 4.42 (1H, d, OH4, $J_{OH,4}$ 7.3), 4.44 (H3, d, $J_{3,4}$ 0.7); δ_C (100.6 MHz, $(CD_3)_2CO$): 26.7 (CH_3), 28.5 (CH_3), 54.4 (C2′), 63.4 (C1 or C6), 64.9 (C1 or C6), 69.9 (C4), 72.9 (C5), 78.0 (C3), 84.5 (C2), 108.7 ($C(CH_3)_2$); *m/z* (ESI +ve) 577 ([2M + Na]$^+$, 100), 300 ([M + Na]$^+$, 98).

*3-C-Azidomethyl-2,3-O-isopropylidene-D-erythrose (**52D**).* Sodium periodate (2.0 g, 0.94 mmol) was added to a solution of tetraol **51D** (1.28 g, 0.462 mmol) in water (30 mL) at rt and stirred for 18 h after which time TLC analysis (EtOAc) showed the complete conversion of the starting material (R_f 0.22) to one major product (R_f 0.88). The reaction mixture was extracted with EtOAc (3 × 15 mL), the organic layers were combined, dried ($MgSO_4$), and concentrated in vacuo. The resulting residue was purified by flash column chromatography (1:4 → 1:2 EtOAc/cyclohexane) to afford azidolactol **52D** (840 mg, 85%) in a 6:1 ratio of anomers: HRMS (ESI −ve) found 238.0798 [M + Na]$^+$, $C_8H_{13}N_3NaO_4$ requires 238.0798; $[\alpha]^{25}_D$ −102.1 (*c* 1.0, $CHCl_3$); ν_{max} (thin film) 3425 (m, br, OH), 2106 (s, N_3); δ_H (400 MHz, $CDCl_3$) major anomer only: 1.47 (3H, s, CH_3), 1.50 (3H, s, CH_3), 2.78 (1H, d, OH1, $J_{OH1,1}$ 3.0), 3.54 (1H, d, H3′a J_{gem} 12.9), 3.62 (1H, d, H3′b, J_{gem} 12.9), 3.98 (1H, d, H4a, J_{gem} 10.1), 4.04 (1H, d, H4b, J_{gem} 9.9), 4.34 (1H, s, H2), 5.44 (1H, d, H1, $J_{1,OH1}$ 3.0); δ_C (100.6 MHz, $CDCl_3$) major anomer only 27.2 (CH_3), 27.6 (CH_3), 55.0 (C3′), 75.0 (C4), 87.1 (C2), 91.2 (C3), 101.8 (C1), 114.1 ($C(CH_3)_2$); *m/z* (ESI −ve) 214 ([M − H]$^-$, 100); *m/z* (ESI +ve) 472 ([M + CH_3CN + H]$^+$, 100), 238 ([M + Na]$^+$, 70).

Enantiomer **52L**: $[\alpha]_D^{21}$ +109.9 (*c* 0.94, $CHCl_3$).

*3-C-Azidomethyl-D-erythrose (**53D**).* Dowex (50W-X8, H$^+$) (1.73 g) was added to a solution of azidolactol **52D** (770 mg, 3.58 mmol) in 1:1 water/1,4-dioxane (18 mL) and stirred at 60 °C. After 36 h TLC analysis (1:1 EtOAc/cyclohexane) showed conversion of starting material (R_f 0.64) to a single product (R_f 0.12). The crude mixture was filtered and concentrated in vacuo to afford triol **53D** (572 mg, 91%) as a pale brown oil, in a 3:2 ratio of anomers: HRMS (ESI −ve): found 174.0518 [M-H]$^-$; $C_5H_8N_3O_4$ requires 174.0520; $[\alpha]^{25}_D$+1.7 (*c* 1.3, MeOH); ν_{max} (thin film, Ge) 3356 (m, br, OH), 2110 (s, N_3); δ_H (400 MHz, $(CD_3)_2CO$): 3.16 (1H, br-s, OH), 3.36 (1H, d, H3′a^B, J_{gem} 12.6), 3.43 (1H, d, H3′b^B, J_{gem} 12.9), 3.45 (2H, s, H3′^{A}), 3.74 (1H, d, H4a^A, J_{gem} 9.4), 3.76 (1H, d, H2^A, $J_{2,1}$ 2.3), 3.79 (1H, d, H4a^B, J_{gem} 9.4), 3.82 (1H, d, H2^B, $J_{2,1}$ 4.5), 3.84 (1H, d, H4b^B, J_{gem} 9.6), 3.93 (1H, d, H4b^A, J_{gem} 9.6), 4.31 (1H, br-s, OH), 4.90 (1H, br-s, OH), 5.19 (1H, d, H1^A, $J_{1,2}$ 2.3), 5.24 (1H, d, H1^B, $J_{1,2}$ 4.6), 5.54 (1H, br-s, OH); δ_c (100.6 MHz, $(CD_3)_2CO$); 56.9 (C3′^{B}), 57.0 (C3′^{A}), 73.6 (C2^B), 74.0 (C4^B), 74.6 (C4^A), 78.6 (C3^B), 79.1 (C2^A), 79.8 (C3^A), 97.5 (C1^B), 104.1 (C1^A); *m/z* (ESI −ve) 174 ([M − H]$^-$, 100).

Enantiomer **53L**: $[\alpha]_D^{18}$ −1.9 (*c* 1.37, MeOH).

*1,4-Dideoxy-2-C-hydroxymethyl-1,4-imino-L-threitol (**11L**) (isoLAB).* Palladium on activated carbon (10%, 112 mg) was added to a solution of triol **53D** (545 mg, 3.11 mmol) in 9:1 water/acetic acid (70 mL) and the reaction was purged with argon then hydrogen. The reaction mixture was stirred under hydrogen at rt for 24 h. The reaction was monitored by LRMS until no starting material was detected. The reaction mixture was filtered through Celite and concentrated in vacuo to approximately 5 mL and absorbed onto a column of Dowex (50W-X8, H$^+$). The resin was washed with water before elution of the amine with 2 M aqueous ammonia. The ammoniacal fractions were concentrated in vacuo to afford pyrrolidine **11L** (isoLAB) (333 mg, 81%) as a brown oil.

Data for free base: HRMS (ESI +ve) found 134.0806 [M + H]$^+$, $C_5H_{12}NO_3$ requires 134.0812; $[\alpha]^{25}_D$ +35.6 (*c* 0.27, H_2O); ν_{max} (thin film, Ge) 3326 (w, br, OH); δ_H (400 MHz, D_2O) 2.88 (1H, a−d, H4a, J_{gem} 13.0), 2.89 (1H, d, H1a, J_{gem} 12.6), 2.98 (1H, d, H1b, J_{gem} 12.6), 3.40 (1H, dd, H4b, J_{gem} 12.9, $J_{4,3}$ 4.9), 3.71 (1H, d, H2′a, J_{gem} 12.0), 3.83 (1H, d, H2′b, J_{gem} 12.0), 4.10 (1H, a-dd, H3, $J_{3,4}$ 4.8, *J* 1.7); δ_C (100.6 MHz, D_2O) 53.1 (C4), 53.3 (C1), 63.3 (C2′), 76.9 (C3), 83.8 (C2); *m/z* (ESI +ve) 134 ([M + H]$^+$, 100).

Data for HCl salt: $[\alpha]^{25}_D$ +26.9 (*c* 0.67, H_2O); δ_H (500 MHz, D_2O) 3.30 (1H, d, H1a, J_{gem} 12.6), 3.35 (1H, d, H4a, J_{gem} 12.6), 3.38 (1H, d, H1b, J_{gem} 12.6), 3.73 (1H, dd, H4b, J_{gem} 12.8, $J_{4,3}$ 4.1), 3.78 (1H, d, H2′a, J_{gem} 12.3), 3.87 (1H, d, H2′b, J_{gem} 12.3), 4.28 (1H, d, H3, $J_{3,4}$ 4.1); δ_c (125 MHz, D_2O) 51.7 (C1), 52.8 (C4), 62.0 (C2′), 74.0 (C3), 82.7 (C2).

Enantiomer (**11D**) (IsoDAB): data for free base: $[\alpha]^{25}_D$ −39.7 (*c* 0.17, H_2O); data for HCl salt: $[\alpha]^{23}_D$ −27.8 (*c* 0.93, H_2O).

*2,3-O-Isopropylidene-L-apiose (**55L**).* A solution of lactol **54D** (9.68 g, 50.9 mmol) in methanol (100 mL) was stirred with potassium carbonate (7.74 g, 56.0 mmol) and aqueous formaldehyde solution (39.5%, 50 mL, 660 mmol) at reflux for 6 h. TLC analysis (EtOAc) showed the complete consumption of starting material (R_f 0.64), and the formation of major (R_f 0.33) and minor products (R_f 0.10). The reaction mixture was cooled to rt, neutralized with 2 M HCl, filtered through Celite, and concentrated in vacuo to afford crude 2,3-*O*-isopropylidene-D-hamamelose (11.2 g). Without further purification, the mixture was dissolved in water (400 mL) and stirred with sodium borohydride (3.86 g, 102 mmol) at rt. TLC analysis (EtOAc) after 80 min showed the complete consumption of the hamamelose derivative (R_f 0.33). The solution was neutralized with glacial acetic acid and stirred with sodium metaperiodate (13.4 g, 56.0 mmol) at rt for 1 h, after which TLC analysis (EtOAc) showed the formation of a major product (R_f 0.62). The solution was concentrated to dryness in vacuo and triturated exhaustively with EtOAc. The organic extracts were concentrated in vacuo and purified by flash column chromatography (1:1 → 4:1 EtOAc/cyclohexane) to afford lactol **55L** (8.09 g, 84%) as a white crystalline solid, in a 7:1 ratio of anomers: HRMS (ESI +ve) found 213.0725 [M + Na]$^+$, $C_8H_{14}NaO_5$ requires 213.0733; mp 70−72 °C [lit.[24] mp 74 °C]; $[\alpha]^{19}_D$ +37.8 (*c* 1.70, $CHCl_3$) [lit.[24] $[\alpha]_D^{rt}$

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+39 (*c* 1.7, $CHCl_3$)]; ν_{max} (thin film) 3423 (s, br, OH); δ_H (400 MHz, $CDCl_3$) 1.40 (3H, s, CH_3^A), 1.47 (3H, s, CH_3^B), 1.49 (3H, s, CH_3^A), 1.57 (3H, s, CH_3^B), 2.48 (1H, t, OH3′^{B}, $J_{OH,3'}$ 6.1), 2.84 (1H, t, OH3′^{A}, $J_{OH,3'}$ 5.6), 3.56 (1H, d, H4a^B, J_{gem} 10.6), 3.72 (2H, d, H3′^{B}, $J_{3',OH}$ 6.3), 3.81 (2H, d, H3′^{A}, $J_{3',OH}$ 5.3), 3.84 (1H, d, OH1^A, $J_{OH,1}$ 4.3), 3.90 (1H, d, H4b^B, J_{gem} 10.6), 3.97 (1H, d, H4a^A, J_{gem} 10.1), 4.04 (1H, d, H4b^A, J_{gem} 10.1), 4.08 (1H, d, OH1^B, $J_{OH,1}$ 11.4), 4.35 (1H, s, H2^A), 4.37 (1H, d, H2^B, $J_{2,1}$ 3.5), 5.07 (1H, dd, H1^B, $J_{1,OH}$ 11.2, $J_{1,2}$ 3.3), 5.41 (1H, d, H1^A, $J_{1,OH}$ 4.0); δ_C (100.6 MHz, $CDCl_3$) 27.3 (CH_3^A), 27.5 (CH_3^A), 27.6 (CH_3^B), 27.8 (CH_3^B), 63.6 (C3′^{B}), 64.1 (C3′^{A}), 70.5 (C4^B), 74.2 (C4^A), 81.0 (C2^B), 86.8 (C2^A), 91.6 (C3^A), 91.7 (C3^B), 97.8 (C1^B), 101.5 (C1^A), 113.4 ($C(CH_3)_2^A$), 114.7 ($C(CH_3)_2^B$); *m*/*z* (ESI −ve) 249 ([M + AcO]$^-$, 100).

*2,3-O-Isopropylidene-L-apiono-1,4-lactone (**56L**).* Bromine (0.79 mL, 15.3 mmol) was added dropwise to a suspension of barium carbonate (3.02 g, 15.3 mmol) and lactol **55L** (1.92 g, 10.2 mmol) in water (25 mL) at 0 °C in a covered flask. The mixture was stirred at 0 °C for 1 h and then at rt for a further 2 h. TLC analysis (EtOAc) showed the complete consumption of starting material (R_f 0.62) and formation of a major product (R_f 0.70). The reaction was quenched with saturated sodium thiosulfate solution and the aqueous extracted with EtOAc (6 × 25 mL). The combined organic fractions were concentrated in vacuo. Purification of the crude by flash column chromatography (1:1 EtOAc/cyclohexane) afforded lactone **56L** (1.72 g, 90%) as a white crystalline solid: HRMS (ESI +ve) found 211.0576 [M + Na]$^+$, $C_8H_{12}NaO_5$ requires 211.0577; mp 90−92 °C; $[\alpha]^{22}_D$ +70.5 (*c* 0.95, $CHCl_3$); ν_{max} (thin film) 3500 (br, s, OH), 1774 (s, C═O); δ_H (400 MHz, $CDCl_3$) 1.44 (3H, s, CH_3), 1.49 (3H, s, CH_3), 2.53 (1H, br, s, OH), 3.75 (1H, d, H3′a, J_{gem} 11.4), 3.80 (1H, d, H3′b, J_{gem} 11.4), 4.42 (1H, d, H4a, J_{gem} 10.6), 4.48 (1H, d, H4b, J_{gem} 10.6), 4.69 (1H, s, H2); δ_C (100.6 MHz, $CDCl_3$) 27.4 (CH_3), 28.1 (CH_3), 62.9 (C3′), 73.3 (C4), 76.4 (C2), 86.4 (C3), 114.5 ($C(CH_3)_2$), 174.6 (C1); *m*/*z* (ESI +ve) 211 ([M + Na]$^+$, 100). Anal. Calcd for $C_8H_{12}O_5$: C, 51.06; H, 6.43. Found: C, 50.79; H, 6.33;

*3-C-Azidomethyl-2,3-O-isopropylidene-L-erythrono-1,4-lactone (**57L**).* Trifluoromethanesulfonic anhydride (1.75 mL, 10.3 mmol) was added dropwise to a solution of the acetonide **56L** (1.29 g, 6.86 mmol) in CH_2Cl_2 (30 mL) and pyridine (1.66 mL, 20.6 mmol) at −30 °C. TLC analysis (1:1 EtOAc/cyclohexane) after 1 h showed the complete consumption of starting material (R_f 0.20) and formation of a major product (R_f 0.77). The crude mixture was partitioned between 2 M aqueous HCl (60 mL) and CH_2Cl_2 (60 mL) and the aqueous layer extracted with CH_2Cl_2 (3 × 60 mL). The combined organic fractions were concentrated in vacuo to yield the triflate derivative which was dissolved in DMF (15 mL) without further purification and stirred with sodium azide (670 mg, 10.3 mmol) at rt for 24 h. TLC analysis (1:1 EtOAc/cyclohexane) showed a major product (R_f 0.77). The reaction mixture was concentrated under reduced pressure and partitioned between water (60 mL) and EtOAc (60 mL), and the aqueous extracted with EtOAc. The combined organic fractions were dried ($MgSO_4$), filtered, and concentrated in vacuo. Purification of the crude by flash column chromatography (1:4 EtOAc/cyclohexane) afforded azidolactone **57L** (982 mg, 67%) as a white crystalline solid: HRMS (ESI −ve) found 230.0774 [M + OH]$^-$, $C_8H_{12}N_3O_5$ requires 230.0771; mp 62−64 °C; $[\alpha]^{17}_D$ +104.5 (*c* 1.18, $CHCl_3$); ν_{max} (thin film) 2103 (s, N_3), 1778 (s, C═O); δ_H (400 MHz, $CDCl_3$) 1.44 (3H, s, CH_3), 1.53 (3H, s, CH_3), 3.56 (1H, d, H3′a, J_{gem} 12.9), 3.60 (1H, d, H3′b, J_{gem} 12.9), 4.34 (1H, d, H4a, J_{gem} 10.6), 4.45 (1H, d, H4b, J_{gem} 10.9), 4.62 (1H, s, H2); δ_C (100.6 MHz, $CDCl_3$) 27.3 (CH_3), 28.0 (CH_3), 53.6 (C3′), 73.3 (C4), 76.7 (C2), 85.4 (C3), 115.2 ($C(CH_3)_2$), 173.5 (C1); *m*/*z* (ESI −ve) 230 ([M + OH]$^-$, 100).

*3-C-Azidomethyl-2,3-O-isopropylidene-L-erythrose (**52L**).* DIBALH (1.5 M in toluene, 5.0 mL, 7.5 mmol) was added dropwise to a solution of azidolactone **57L** (1.28 g, 6.01 mmol) in CH_2Cl_2 (10 mL) at −78 °C and stirred for 1 h. TLC analysis (1:1 EtOAc/cyclohexane) showed the complete consumption of starting material (R_f 0.77) and formation of a major product (R_f 0.70). Excess DIBALH was quenched with methanol, and the mixture allowed to warm to rt. CH_2Cl_2 (10 mL) and potassium sodium tartrate solution (satd aq, 20 mL) were added, and the mixture stirred until a biphasic system was obtained (4 h). The organic phase was collected and the aqueous layer extracted with CH_2Cl_2 (2 × 20 mL). The combined organic fractions were dried ($MgSO_4$), filtered, and concentrated in vacuo. Purification by flash column chromatography (1:4 → 1:2 EtOAc/cyclohexane) afforded azidolactol **52L** (1.21 g, 93%) in a 10:1 ratio of anomers as a colorless oil.

Spectroscopic data as reported above.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Copies of ^1H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) (a) Scott, L. J.; Spencer, C. M. *Drugs* **2000**, *59*, 521−549. (b) Aoki, K.; Kamiyama, H.; Yoshimura, K.; Shibuya, M.; Masuda, K.; Terauchi, Y. *Acta Diabetol.* **2012**, *49*, 225−230.

(2) (a) Machaczka, M.; Klimkowska, M.; Hagglund, H. *Adv. Med. Sci.* **2012**, *57*, 169−173. (b) Lieberman, R. L.; Wustman, B. A.; Huertas, P.; Powe, A. C.; Pine, C. W.; Khanna, R.; Schlossmacher, M. G.; Ringe, D.; Petsko, G. A. *Nature Chem. Biol.* **2007**, *3*, 101−107.

(3) (a) Patterson, M. C.; Vecchio, D.; Prody, H.; Abet, L.; Wraith, J. E. *Lancet Neurol.* **2007**, *6*, 765−772. (b) Di Rocco, M.; Dardis, A.; Madeo, A.; Barone, R.; Fiumara, A. *Pediatr. Neurol.* **2012**, *47*, 40−43.

(4) Lubamba, B.; Lebacq, J.; Lebecque, P.; Vanbever, R.; Leonard, A.; Wallemacq, P.; Leal, T. *Am. J. Respir. Crit. Care Med.* **2009**, *179*, 1022−1028.

(5) Kudo, T.; Nishimura, Y.; Kondo, S.; Takeuchi, T. *J. Antibiot.* **1992**, *45*, 1662−1668.

(6) (a) Shitara, E.; Nishimura, Y.; Kojima, F.; Takeuchi, T. *Bioorg. Med. Chem.* **2000**, *8*, 343−352. (b) Nishimura, Y. *J. Antibiot.* **2009**, *62*, 407−423. (c) Birch, N. J.; Parsons, P. J.; Scopes, D. I. C. *Synlett* **2004**, 2776−2778. (d) Wrodnigg, T. M.; Steiner, A. J.; Ueberbacher, B. J. *Anti-cancer Agents Med. Chem.* **2008**, *8*, 77−85.

(7) Umezawa, H.; Aoyagi, T.; Komiyama, T.; Morishim., H.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1974**, *27*, 963−969.

(8) (a) Stuetz, A. E.; Wrodnigg, T. M. *Adv. Carbohydr. Chem. Biochem.* **2011**, *66*, 187−298. (b) Horne, G.; Wilson, F. X.; Tinsley, J.; Williams, D. H.; Storer, R. *Drug Dis. Today* **2011**, *16*, 107−118. (c) Winchester, B. G. *Tetrahedron: Asymmetry* **2009**, *20*, 645−651. (d) Compain, P.; Martin, O. R. *Iminosugars from Synthesis to Therapeutic Applications*; Wiley-VCH: New York, 2007.

(9) (a) Simone, M. I.; Soengas, R. G.; Jenkinson, S. F.; Evinson, E. L.; Nash, R. J.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2012**, *23*, 401−408. (b) Soengas, R. G.; Simone, M. I.; Hunter, S.; Nash, R. J.; Evinson, E. L.; Fleet, G. W. J. *Eur. J. Org. Chem.* **2012**, 2394−2402. (c) Soengas, R. G.; Estévez, A. M. *Eur. J. Org. Chem.* **2010**, 5190−5196.

(10) (a) Pawar, N. J.; Parihar, V. S.; Chavan, S. T.; Joshi, R.; Joshi, P. V.; Sabharwal, S. G.; Puranik, V. G.; Dhavale, D. D. *J. Org. Chem.* **2012**, *77*, 7873−7882. (b) Otero, J. M.; Soengas, R. G.; Estévez, J. C.;

Estévez, R. J.; Watkin, D. J.; Evinson, E. L.; Nash, R. J.; Fleet, G. W. J. *Org. Lett.* **2007**, *9*, 623−626.
(11) Clinch, K.; Evans, G. B.; Fleet, G. W. J.; Furneaux, R. H.; Johnson, S. W.; Lenz, D.; Mee, S.; Rands, P. R.; Schramm, V. L.; Ringia, E. A. T.; Tyler, P. C. *Org. Biomol. Chem.* **2006**, *4*, 1131−1139.
(12) da Cruz, F. P.; Newberry, S.; Jenkinson, S. F.; Wormald, M. R.; Butters, T. D.; Alonzi, D. S.; Nakagawa, S.; Becq, F.; Norez, C.; Nash, R. J.; Kato, A.; Fleet, G. W. J. *Tetrahedron Lett.* **2011**, *52*, 219−223.
(13) (a) Jespersen, T. M.; Dong, W.; Sierks, M. R.; Skrydstrup, T.; Lundt, I.; Bols, M. *Angew. Chem., Int. Ed.* **1994**, *33*, 1778−1779. (b) Li, R. J; Bols, M.; Rousseau, C.; Zhang, X. G.; Wang, R. W.; Qing, F. L. *Tetrahedron* **2009**, *65*, 3717−3727.
(14) (a) Deschamp, J.; Mondon, M.; Nakagawa, S.; Kato, A.; Alonzi, D. S.; Butters, T. D.; Zhang, Y.; Sollogoub, M.; Bleriot, Y. *Bioorg. Med. Chem.* **2012**, *20*, 641−649. (b) Liu, H.; Liang, X.; Sohoel, H.; Bulow, A.; Bols, M. *J. Am. Chem. Soc.* **2001**, *123*, 5116−5117.
(15) (a) Sun, Y.; Liou, B.; Xu, Y.-H.; Quinn, B.; Zhang, W. J.; Hamler, R.; Setchell, K. D. R.; Grabowski, G. A. *J. Biol. Chem.* **2012**, *287*, 4275−4287. (b) Khanna, R.; Benjamin, E. R.; Pellegrino, L.; Schilling, A.; Rigar, B. A.; Soska, R.; Nafar, H.; Ranes, B. E.; Feng, J.; Lun, Y.; Powe, A. C.; Palling, D. J.; Wustman, B. A.; Schiffmann, R.; Mahuran, D. J.; Lockhart, D. J.; Valenzano, K. J. *FEBS J.* **2010**, *277*, 1618−1638. (c) Steet, R. A.; Chung, S.; Wustman, B.; Powe, A.; Do, H.; Kornfeld, S. A. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 13813−13818. (d) Yu, Z.; Sawkar, A. R.; Whalen, L. J.; Wong, C.-H.; Kelly, J. W. *J. Med. Chem.* **2007**, *50*, 94−100.
(16) (a) Somsak, L.; Nagy, V.; Hadady, Z.; Docsa, T.; Gergely, P. *Curr. Pharmaceut. Design* **2003**, *9*, 1177−1189. (b) Oikonomakos, N. G.; Tiraidis, C.; Leonidas, D. D.; Zographos, S. E.; Kristiansen, M.; Jessen, C. U.; Norskov-Lauritsen, L.; Agius, L. *J. Med. Chem.* **2006**, *49*, 5687−5701.
(17) Wrodnigg, T. M. *Monatsh. Chem.* **2002**, *133*, 393−426.
(18) Asano, N.; Yamauchi, T.; Kagamifuchi, K.; Shimizu, N.; Takahashi, S.; Takatsuka, H.; Ikeda, K.; Kizu, H.; Chuakul, W.; Kettawan, A.; Okamoto, T. *J. Nat. Prod.* **2005**, *68*, 1238−1242.
(19) (a) Furukawa, J.; Okuda, S.; Saito, K.; Hatanaka, S.-I. *Phytochemistry* **1985**, *24*, 593−594. (b) Jones, D. W. C.; Nash, R. J.; Bell, E. A.; Williams, J. M. *Tetrahedron Lett.* **1985**, *26*, 3125−3126.
(20) (a) Norez, C.; Noel, S.; Wilke, M.; Bijvelds, M.; Jorna, H.; Melin, P.; DeJonge, H.; Becq, F. *FEBS Lett.* **2006**, *580*, 2081−2086. (b) Norez, C.; Bilan, F.; Kitzis, A.; Mettey, Y.; Becq, F. *J. Pharmacol. Expt. Therapeut.* **2008**, *325*, 89−99.
(21) Best, D.; Jenkinson, S. F.; Saville, A. W.; Alonzi, D. S.; Wormald, M. R.; Butters, T. D.; Norez, C.; Becq, F.; Bleriot, Y.; Adachi, I.; Kato, A.; Fleet, G. W. J. *Tetrahedron Lett.* **2010**, *51*, 4170−4174.
(22) Yang, B. Y.; Montgomery, R. *Carbohydr. Res.* **1996**, *280*, 27−45.
(23) Ho, P.-T. *Tetrahedron Lett.* **1978**, *19*, 1623−1626.
(24) Ho, P.-T. *Can. J. Chem.* **1979**, *57*, 381−383.
(25) Humphlett, W. J. *Carbohydr. Res.* **1967**, *4*, 157−164.
(26) Best, D.; Jenkinson, S. F.; Rule, S. D.; Higham, R.; Mercer, T. B.; Newell, R. J.; Weymouth-Wilson, A. C.; Fleet, G. W. J.; Petursson, S. *Tetrahedron Lett.* **2008**, *49*, 2196−2199.
(27) Mori, N.; Togo, H. *Tetrahedron* **2005**, *61*, 5915−5925.
(28) Batra, H.; Moriarty, R. M.; Penmasta, R.; Sharma, V.; Stanciuc, G.; Staszewski, J. P.; Tuladhar, S. M.; Walsh, D. A. *Org. Process Res. Dev.* **2006**, *10*, 484−486.
(29) (a) Godskesen, M.; Lundt, I.; Robert Madsen, R.; Winchester, B. *Bioorg. Med. Chem.* **1996**, *4*, 1857−1865. (b) Kold, H.; Lundt, I.; Pederson, C. *Acta Chem. Scand.* **1994**, *48*, 675−678.
(30) Kato, A.; Kato, N.; Adachi, I.; Hollinshead, J.; Fleet, G. W. J.; Kuriyama, C.; Ikeda, K.; Asano, N.; Nash, R. J. *J. Nat. Prod.* **2007**, *70*, 993−997.
(31) Card, P. J.; Hitz, W. D. *J. Org. Chem.* **1985**, *50*, 891−893.
(32) (a) Legler, G.; Korth, A.; Berger, A.; Ekhart, C.; Gradnig, G.; Stutz, A. E. *Carbohydr. Res.* **1993**, *250*, 67−77. (b) Liu, K. K. C.; Kajimoto, T.; Chen, L. R.; Zhong, Z. Y.; Ichikawa, Y.; Wong, C. H. *J. Org. Chem.* **1991**, *56*, 6280−6289.
(33) Kajimoto, T.; Chen, L.; Liu, K. K. C.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 6678−6680.
(34) Beacham, A. R.; Bruce, I.; Choi, S.; Doherty, O.; Fairbanks, A. J.; Fleet, G. W. J.; Skead, B. M.; Peach, J. M.; Saunders, J.; Watkin, D. J. *Tetrahedron: Asymmetry* **1991**, *2*, 883−900.
(35) Hotchkiss, D. J.; Soengas, R.; Simone, M. I.; van Ameijde, J.; Hunter, S. J.; Cowley, A. R.; Fleet, G. W. J. *Tetrahedron Lett.* **2004**, *45*, 9461−9464.
(36) Procedures given for the determination of glycosidase inhibition are as follows: (a) Hakansson, A. E.; van Ameijde, J.; Guglielmini, L.; Horne, G.; Nash, R. J.; Evinson, E. L.; Kato, A.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2007**, *18*, 282−289. (b) Mercer, T. B.; Jenkinson, S. F.; Bartholomew, B.; Nash, R. J.; Miyauchi, S.; Kato, A.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2009**, *20*, 2368−2373.
(37) (a) D'Alonzo, D.; Guaragna, A.; Palumbo, G. *Curr. Med. Chem.* **2009**, *16*, 473−505. (b) Jenkinson, S. F.; Fleet, G. W. J.; Nash, R. J.; Koike, Y.; Adachi, I.; Yoshihara, A.; Morimoto, K.; Izumori, K.; Kato, A. *Org. Lett.* **2011**, *13*, 4064−4067. (c) Rountree, J. S. S.; Butters, T. D.; Wormald, M. R.; Boomkamp, S. D.; Dwek, R. A.; Asano, N.; Ikeda, K.; Evinson, E. L.; Nash, R. J.; Fleet, G. W. J. *ChemMedChem* **2009**, *4*, 378−392. (d) Kato, A.; Kato, N.; Kano, E.; Adachi, I.; Ikeda, K.; Yu, L.; Okamoto, T.; Banba, Y.; Ouchi, H.; Takahata, H.; Asano, N. *J. Med. Chem.* **2005**, *48*, 2036−2044. (e) Li, Y.-X.; Huang, M.-H.; Yamashita, Y.; Kato, A.; Jia, Y.-M.; Wang, W.-B.; Fleet, G. W. J.; Nash, R. J.; Yu, C.-Y. *Org. Biomol. Chem.* **2011**, *9*, 3405−3414. (f) Concia, A. L.; Gomez, L.; Bujons, J.; Parella, T.; Vilaplana, C.; Cardona, P. J.; Joglar, J.; Clapes, P. *Org. Biomol. Chem.* **2013**, *11*, 2005−2021. (g) Blériot, Y.; Gretzke, D.; Kruelle, T. M.; Butters, T. D.; Dwek, R. A.; Nash, R. J.; Asano, N.; Fleet, G. W. J. *Carbohydr. Res.* **2005**, *340*, 2713−2718. (h) Stocker, B. L.; Jongkees, S. A. K.; Win-Mason, A. L.; Dangerfield, E. M.; Withers, S. G.; Timmer, M. S. M. *Carbohydr. Res.* **2013**, *367*, 29−32. (i) Smith, S. W. *Toxicol. Sci.* **2009**, *110*, 4−30.
(38) For assay methods, see: Alonzi, D. S.; Neville, D. C.; Lachmann, R. H.; Dwek, R. A.; Butters, T. D. *Biochem. J.* **2008**, *409*, 571−580.
(39) Fleet, G. W. J; Karpas, A.; Dwek, R. A.; Fellows, L. E.; Tyms, A. S.; Petursson, S.; Namgoong, S. K.; Ramsden, N. G.; Smith, P. W.; Son, J. C.; Wilson, F. X.; Witty, D. R.; Jacob, G. S.; Rademacher, T. W. *FEBS Lett.* **1988**, *237*, 128−132.
(40) Butters, T. D.; van den Broek, L. A. G. M.; Fleet, G. W. J.; Krulle, T. M.; Wormald, M. R.; Dwek, R. A.; F., M. *Tetrahedron: Asymmetry* **2000**, *11*, 113−125.
(41) (a) Lori, K. G.; Rajiah, A. D.; Eddine, S. *J. Med. Chem.* **2012**, *55*, 10823−10843. (b) Parenti, G.; Pignata, C.; Vajro, P.; Salerno, D. *Int. J. Mol. Dis.* **2013**, *31*, 11−20. (c) Makley, L. N.; Gestwicki, J. E. *Chem. Biol. Drug Design* **2013**, *81*, 22−32. (d) Porto, C.; Ferrara, M. C.; Meli, M.; Acampora, E.; Avolio, V.; Rosa, M.; Cobucci-Ponzano, B.; Colombo, G.; Moracci, M.; Andria, G.; Parenti, G. *Mol. Ther.* **2012**, *20*, 2201−2211.
(42) (a) Parenti, G. *EMBO Mol. Med.* **2009**, *1*, 268−279. (b) Fan, J. Q. *Biol. Chem.* **2008**, *389*, 1−11. (c) Benito, J. M.; Garcia Fernandez, J. M.; Ortiz Mellet, C. *Expert Opin. Ther. Pat.* **2011**, *21*, 885−903. (d) Glawar, A. F. G.; Best, D.; Ayers, B.; Miyauchi, S.; Nakagawa, S.; Aguilar-Moncayo, M.; Garcia Fernandez, J. M.; Mellet, C. O.; Crabtree, E. V.; Butters, T. D.; Wilson, F. X.; Kato, A.; Fleet, G. W. J. *Chem.—Eur. J.* **2012**, *18*, 9341−9359. (e) Clark, N. E.; Metcalf, M. C.; Best, D.; Fleet, G. W. J.; Garman, S. C. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 17000−17405.
(43) (a) Nicholls, K.; Castelli, J.; Winkler, R.; Sitaraman, S. A.; Benjamin, E. R.; Wu, X.; Duke, C.; Boudes, P. *J. Inherited. Metab. Dis* **2012**, *35*, S95−S95. (b) Benjamin, E. R.; Khanna, R.; Schilling, A.; Flanagan, J. J.; Pellegrino, L. J.; Brignol, N.; Lun, Y.; Guillen, D.; Ranes, B. E.; Frascella, M.; Soska, R.; Feng, J.; Dungan, L.; Young, B.; Lockhart, D. J.; Valenzano, K. J. *Mol. Ther.* **2012**, *20*, 717−726. (c) Lieberman, R. L.; D'Aquino, J. A.; Ringe, D.; Petsko, G. A. *Biochemistry* **2009**, *48*, 4816−4827. (d) Pan, X.-L.; Liu, W.; Liu, J.-Y. *J. Phys. Chem. B* **2013**, *117*, 484−489.
(44) (a) Boyd, R. E.; Lee, G.; Rybczynski, P.; Benjamin, E. R.; Khanna, R.; Wustman, B. A.; Valenzano, K. J. *J. Med. Chem.* **2013**, *56*, 2705−2725. (b) Germain, D. P.; Giugliani, R.; Hughes, D. A.; Mehta, A.; Nicholls, K.; Barisoni, L.; Jennette, C. J; Bragat, A.; Castelli, J.;

Sitaraman, S.; Lockhart, D. J.; Boudes, P. F. *Orphanet J. Rare Dis.* **2012**, *7*, 91.
(45) Jenkinson, S. F.; Fleet, G. W. J.; Nash, R. J.; Koike, Y.; Adachi, I.; Yoshihara, A.; Morimoto, K.; Izumori, K.; Kato, A. *Org. Lett.* **2011**, *13*, 4064−4067.
(46) Machaczka, M.; Hast, R.; Dahlman, I.; Lerner, R.; Klimkowska, M.; Engvall, M.; Hagglund, H. *Upsala J. Med. Sci.* **2012**, *117*, 28−34.
(47) (c) Antigny, F.; Norez, C.; Becq, F.; Vandebrouck, C. *Cell Calcium* **2008**, *43*, 175−183. (d) Dechecchi, M. C.; Nicolis, E.; Norez, C.; Bezzerri, V.; Borgatti, M.; Mancini, I.; Rizzotti, P.; Ribeiro, C. M. P.; Gambari, R.; Becq, F.; Cabrini, G. *J. Cystic Fibrosis* **2008**, *7*, 555−565.
(48) Norez, C.; Antigny, F.; Noel, S.; Vandebrouck, C.; Becq, F. *Am. J. Respir. Cell Mol. Biol.* **2009**, *41*, 217−225.
(49) For details of the human tracheal gland serous epithelial cell line CF-KM4 derived from a CF patient homozygous for the F508del mutation, see: Kammouni, W.; Moreau, B.; Becq, F.; Saleh, R.; Pavirani, A.; Figarella, C.; Merten, M. D. *Am. J. Respir. Cell Mol. Biol.* **1999**, *20*, 684−691.
(50) CFTR Cl^- channel activity was assayed by single-cell fluorescence imaging using the potential-sensitive probe, bis-(1,3-diethylthiobarbituric acid)trimethine oxonol. The results of fluorescence imaging are presented as transformed data to obtain the percentage signal variation (F_x) relative to the time of addition of the stimulus, according to the equation: $F_x = ([F_t - F_0]/F_0] \times 100$ where F_t and F_0 are the fluorescent values at the time t and at the time of addition of the stimulus, respectively. For histogram representation, the values correspond to the level of stable variation of fluorescence induced by each drug.
(51) Gottlieb, H. E.; Koplyar, V.; Nudelman, A. *J. Org. Chem.* **1997**, *62*, 7512−7515.
(52) Mitchell, D. A.; Jones, N. A.; Hunter, S. J.; Cook, J. M. D.; Jenkinson, S. F.; Wormald, M. R.; Dwek, R. A.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2007**, *18*, 1502−1510.