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Synthesis and enzyme-inhibitory activity of methyl acarviosin analogues having the α-*manno* configuration*

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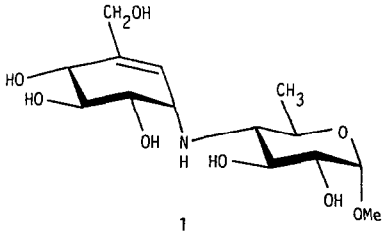
ABSTRACT

Two methyl acarviosin analogues **3a** and **4a**, having the α-*manno* configuration, and their dihydro derivatives **6a** and **7a** were synthesised by coupling the protected pseudo-sugar epoxides with methyl 4-amino-4-deoxy- and -4,6-dideoxy-α-D-mannopyranoside. Similarly, two analogous compounds **5a** and **8a** composed of the 1,6-anhydro-β-D-mannopyranose residues were prepared. Compound **7a** showed mild inhibitory activity against Jack bean α-D-mannosidase, and **3a** was a moderate inhibitor of both α-D-mannosidase and yeast α-D-glucosidase.

INTRODUCTION

The potent α-D-glucosidase inhibitor, methyl acarviosin[2] (**1**) may be considered as an imino-linked carba-maltose analogue, so that, by analogy, similar carba-disaccha-



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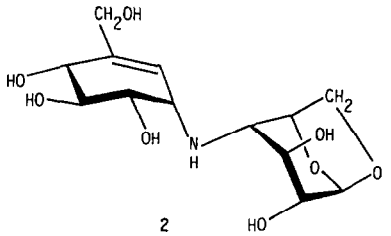

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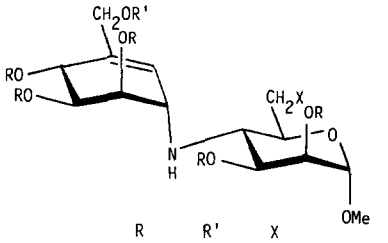
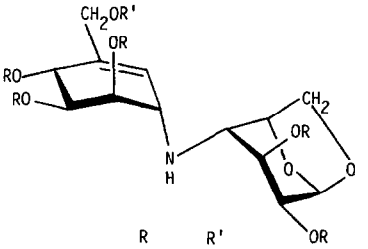


	R	R'	X
3a	H	H	H
3b	Ac	Bz	H
4a	H	H	OH
4b	Ac	Bz	OAc



	R	R'
5a	H	H
5b	Ac	Bz

* Synthesis of Pseudo-oligosaccharidic Glycosidase Inhibitors, Part. XI. For Part X, see ref. 1.
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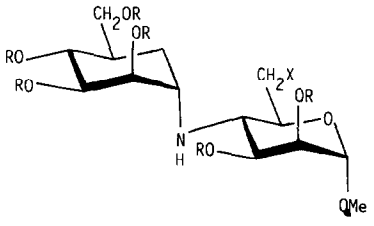
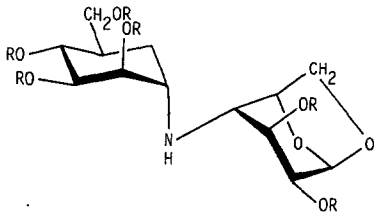
rides having the α-*manno* configuration could be inhibitors of α-D-mannosidase. Therefore, the methyl acarviosin analogues **3a** and **4a**, together with the corresponding saturated derivatives **6a** and **7a**, were synthesised. Since inversion of the conformation of the sugar moiety of **1** by the introduction of the 1,6-anhydro ring (→**2**) enhances[3] the inhibitory activity, the related carba-disaccharides **5a** and **8a**, which contain 1,6-anhydro-β-D-mannopyranose residues, have been synthesised.



	R	X
6a	H	H
6b	Ac	H
7a	H	OH
7b	Ac	OAc



	R
8a	H
8b	Ac

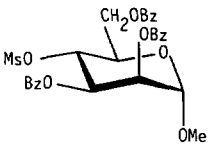
RESULTS AND DISCUSSION

Treatment of methyl 2,3,6-tri-*O*-benzoyl-4-*O*-methanesulfonyl-α-D-mannopyranoside[4] (**9**) with an excess of methanolic sodium methoxide gave the 3,4-anhydride **10**, which reacted with sodium azide to give two azido compounds that were isolated as the 2,3- (**11**, 33%) and the 4,6-*O*-isopropylidene derivatives (**13**, 30%), respectively. Hydrogenation of **11** or **13** in the presence of Raney nickel reduced the azido groups and acetylation of the products afforded the corresponding *N,O*-diacetyl derivatives **12** (73%) or **14** (69%), the structures of which were assigned on the basis of ^{1}H-n.m.r. data.

Compound **11** was tosylated and the product (**15**, 98%) was reduced with lithium aluminum hydride to give, after conventional acetylation, the 6-deoxy compound **16** (64%), which was characterised by hydrolysis and then acetylation to give the *N,O*-triacetyl derivative[5] **17** (78%). Compound **12** was *O*-deisopropylidenated and the product was isolated as the *N,O*-tetra-acetyl derivative **19** (82%).

Hydrazinolysis of **17** and **19** effected deacetylation and afforded methyl 4-amino-4,6-dideoxy- (**18**) and -4-deoxy-α-D-mannopyranoside (**20**), respectively, which were used without purification for the coupling reactions.

4-Amino-1,6-anhydro-4-deoxy-β-D-mannopyranose (**27**) was prepared in five steps from 1,6-anhydro-β-D-mannopyranose (**21**). Thus, **21** was transformed into the 4-*O*-tosyl derivative **24** (23% from **21**), through 2,3-*O*-isopropylidenation[6] (→**22**), tosylation (→**23**), and *O*-deisopropylidenation followed by acetylation (→**24**). Treatment of **24** with an excess of methanolic sodium methoxide gave the 1,6:3,4-dianhydro



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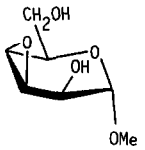

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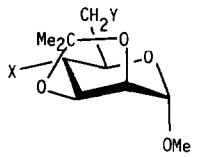
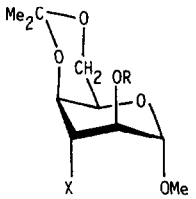
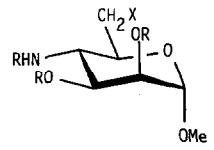


11 X = N_3, Y = OH
12 X = NHAc, Y = OAc
15 X = N_3, Y = OTs
16 X = NHAc, Y = H



13 R = H, X = N_3
14 R = Ac, X = NHAc



17 R = Ac, X = H
18 R = H, X = H
19 R = Ac, X = OAc
20 R = H, X = OH

derivative, which reacted with sodium azide to give, after acetylation, the azide **25** (61%). Hydrogenation of **25** followed by acetylation gave the known[7] *N,O*-triacetyl derivative **26** (65%), hydrazinolysis of which gave **27**.

Coupling of a slight excess of **18** and (1*R*)-(1,2,3/4)-3,4-di-*O*-acetyl-1,2-anhydro-5-(benzoyloxymethyl)-5-cyclohexene-1,2,3,4-tetrol[8] (**28**) in 2-propanol for 3 days at 50° gave, after acetylation and chromatography, **3b** (68%). The structure of **3b** was confirmed by the ^{1}H-n.m.r. spectrum (270 MHz, $CDCl_3$) which contained signals for H-1′,2′ and δ 3.59 (bs) and 5.97 (d, $J_{1',2'}$ 2.2 Hz), respectively. The reaction of **28** occurred at the allylic position to give exclusively the product of diaxial opening of the epoxide ring. Zemplén *O*-deacylation of **3b** then gave the carba-disaccharide glycoside **3a**.

Likewise, coupling of **20** and **28** afforded **4b** (74%), the ^{1}H-n.m.r. spectrum of which was similar to that of **3b** except for the downfield shift of the H-4 signal due to the 6-acetoxymethyl group. Zemplén *O*-deacetylation of **4b** gave the target compound **4a**.

The coupling of **27** and **28** was carried out at 70° for 3 days and yielded **5b** (64%), the structure of which was established by the ^{1}H-n.m.r. spectrum.

The saturated analogue **6a** was prepared by coupling of **18** and the cyclohexene epoxide **30**, prepared from (1*R*)-(1,3/2)-1,2-di-*O*-acetyl-3-bromomethyl-5-cyclohexene-1,2-diol[8] (**29**) by successive reaction with acetate ion, *O*-deacetylation[9], and epoxidation[10] with *m*-chloroperoxybenzoic acid. Reaction of a slight excess of **18** with **30** in 1:2 *N,N*-dimethylformamide–2-propanol at 120° for 24 h afforded, after acetylation, the hexa-*O*-acetyl derivative **6b** (56%).

Similarly, **7b** was obtained (59%) by the reaction of **20** and **30**. The ^{1}H-n.m.r. spectra of **6b** and **7b** contained signals for H-4 (t, *J* 10.6 Hz) and H-1′ (bq, *J* ~ 3.6 Hz) at

TABLE I

Enzyme inhibitory activity of the carba-disaccharides **3a–8a** at 100 μg/mL

Compound	*Inhibition (%)*		
	α-D-*Glucosidase*[a]	β-D-*Glucosidase*[b]	α-D-*Mannosidase*[c]
3a	73.5	7.7	40.7
4a	43.5	0	0
5a	35.1	0	7.7
6a	9.5	9.8	34.7
7a	7.5	5.0	48.5
8a	8.4	2.3	44.3

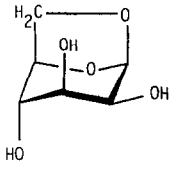
[a] Yeast α-D-glucosidase and *p*-nitrophenyl α-D-glucopyranoside (0.66 mmol) in phosphate buffer (100 mmol) at pH 6.8. [b] Almond β-D-glucosidase and *p*-nitrophenyl β-D-glucopyranoside (0.33 mmol) in acetate buffer (100 mmol) at pH 5.0. [c] Jack bean α-D-mannosidase and *p*-nitrophenyl α-D-mannopyranoside (20 mmol) in acetate buffer (100 mmol) at pH 4.5.

δ 2.70 and 3.15, and 3.02 and 3.12, respectively. In **7b**, H-4 is deshielded by the 6-acetoxymethyl group, and both **6b** and **7b** were formed by diaxial opening of the epoxide ring in **30**.

Reaction of **30** with an excess of **27** required 3 days at 120° and gave, after acetylation, the hexa-*O*-acetyl derivative **8b**.

Compounds **6b–8b** were converted into the respective free carba-disaccharides **6a–8a** by hydrazinolysis.

The activities of the carba-disaccharides **2a–8a** against α- and β-D-glucosidase and α-D-mannosidase are recorded in Table I. Compound **7a** was a moderate inhibitor of Jack bean α-D-mannosidase, being comparable to the mannojirimycin bisulfide adduct, and **3a** had mild inhibitory activity against both α-D-glucosidase and α-D-mannosidase.



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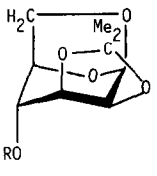

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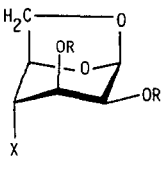
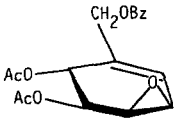
23 R = Ts



24 R = Ac, X = OTs
25 R = Ac, X = N_3
26 R = Ac, X = NHAc
27 R = H, X = NH_2



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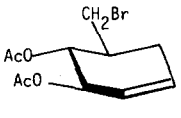

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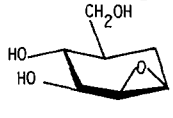

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EXPERIMENTAL

General methods. — Melting points were determined with a MEL-TEMP capillary melting-point apparatus and uncorrected. Optical rotations were measured with a Jasco DIP-370 polarimeter. ^{1}H-N.m.r. spectra were recorded for solutions in $CDCl_3$ (internal Me_4Si) with a Jeol JNM GSX-270 (270 MHz) instrument. T.l.c. was performed on Silica Gel 60 GF (Merck) with detection by charring with H_2SO_4. Column chromatography was conducted on Wakogel C-300 (300 Mesh). Organic solutions were dried over anhydrous Na_2SO_4 and concentrated at $< 50°$ under diminished pressure.

Methyl 4-azido-4-deoxy-2,3- (**11**) *and methyl 3-azido-3-deoxy-4,6-*O*-isopropylidene-*α*-*D*-mannopyranoside* (**13**). — To a solution of **9**[4] (3.0 g, 5.1 mmol) in methanol (50 mL) was added methanolic M NaOMe (15 mL, 15 mmol), and the mixture was stirred for 2 h at room temperature. After neutralisation with Amberlite IR-120 (H^+) resin, the filtrate was concentrated to give crude **10** (0.79 g, ~87%), which was treated with sodium azide (3.0 g, 46 mmol) in *N,N*-dimethylformamide (20 mL) for 12 h at 100°. The mixture was then concentrated and the residue was acetylated with Ac_2O and pyridine overnight at room temperature to give, after column chromatography (1:1 EtOAc–hexane), a mixture of the crude acetates. The acetates were *O*-deacetylated with methanolic M NaOMe, and the resulting crude triols were treated with 2,2-dimethoxypropane (5 mL) and *p*-toluenesuflonic acid monohydrate (0.1 g) in *N,N*-dimethylformamide (25 mL) for 12 h at room temperature. T.l.c. (1:1 EtOAc–hexane) then revealed products with R_F 0.69 and 0.74. Column chromatography (1:5 EtOAc–hexane) afforded, first, **11** (0.41 g, 33%), isolated as a syrup, $[\alpha]_D^{25}$ + 52° (*c* 1.1, chloroform). ^{1}H-N.m.r. data: δ 4.96 (s, 1 H, H-1), 4.23 (dd, 1 H, $J_{2,3}$ 4.8, $J_{3,4}$ 7.7 Hz, H-3), 4.10 (d, 1 H, $J_{1,2}$ ~ 0 Hz, H-2), 3.86 (ddd, 1 H, $J_{5,6a}$ 2.6, $J_{6a,6b}$ 12.1, $J_{6,OH}$ 5.5 Hz, H-6a), 3.76 (ddd, 1 H, $J_{5,6b}$ 2.9, $J_{6b,OH}$ 7.7 Hz, H-6b), 3.58 (dd, 1 H, $J_{4,5}$ 10.6 Hz, H-4), 3.47 (ddd, 1 H, H-5), 3.38 (s, 3 H, OMe), 1.98 (dd, 1 H, OH), 1.57 and 1.38 (2 s, each 3 H, CMe_2).

Eluted second was **13** (0.37 g, 30%), isolated as a syrup, $[\alpha]_D^{26}$ + 104° (*c* 0.3, chloroform). ^{1}H-N.m.r. data: δ 4.81 (s, 1 H, H-1), 4.07 (dd, 1 H, $J_{5,6a}$ 2.6, $J_{6a,6b}$ 12.8 Hz, H-6a), 3.91 (dd, 1 H, $J_{5,6b}$ 2.2 Hz, H-6b), 3.88 (bs, 1 H, H-2), 3.43 (m, 1 H, OH), 3.44 (s, 3 H, OMe), and 1.47 (s, 6 H, CMe_2).

Both **11** and **13** are very hygroscopic and did not give satisfactory elemental analyses.

*Methyl 4-acetamido-6-*O*-acetyl-4-deoxy-2,3-*O*-isopropylidene-*α*-*D*-mannopyranoside* (**12**). — A solution of **11** (0.41 g, 1.7 mmol) in EtOH (5 mL) was hydrogenated in the presence of Raney nickel with an initial pressure of hydrogen of 50 p.s.i.g. for 12 h at room temperature, then filtered, and concentrated. The crude amine was acetylated conventionally to give the *N,O*-diacetyl derivative **12** (0.39 g, 73%) as a syrup, $[\alpha]_D^{26}$ + 31° (*c* 0.26, chloroform). ^{1}H-N.m.r. data: δ 5.68 (bd, 1 H, $J_{4,NH}$ 8.8 Hz, NH), 4.90 (s, 1 H, H-1), 4.23 (bd, 2 H, $J_{5,6}$ 5.1 Hz, H-6,6), 4.20 (dd, 1 H, $J_{2,3}$ 5.9, $J_{3,4}$ 7.3 Hz, H-3), 4.10 (d, 1 H, H-2), 4.02 (ddd, 1 H, $J_{4,5}$ 8.4 Hz, H-4), 3.88 (ddd, 1 H, H-5), 3.41 (s, 3 H, OMe), 2.09 (s, 3 H, OAc), 2.00 (s, 3 H, NAc), and 1.34 and 1.54 (2 s, each 3 H, CMe_2).

Anal. Calc. for $C_{14}H_{23}NO_7$: C, 52.99; H, 7.31; H, 4.41. Found: C, 52.95; H, 7.10; N, 4.40.

Methyl 3-acetamido-2-O-acetyl-3-deoxy-4,6-O-isopropylidene-α-D-idopyranoside (**14**). — A solution of **13** (374 mg, 1.54 mmol) in EtOH (5 mL) was hydrogenated in the presence of Raney nickel for 12 h, then filtered, and concentrated. The residue was acetylated conventionally. Column chromatography (1:10 Me_2CO–toluene) of the product gave **14** (336 mg, 69%), isolated as a syrup, $[\alpha]_D^{20}$ + 51° (*c* 0.54, chloroform). ^{1}H-N.m.r. data: δ 6.49 (d, 1 H, $J_{3,NH}$ 8.4 Hz, NH), 4.86 (s, 1 H, H-1), 4.60 (s, 1 H, H-3), 4.11 (dd, 1 H, $J_{5,6a}$ 1.8, $J_{6a,6b}$ 13.2 Hz, H-6a), 3.95 (dd, 1 H, $J_{5,6b}$ 1.5 Hz, H-6b), 3.83 (s, 1 H, H-4), 3.62 (dd, 1 H, H-5), 3.46 (s, 3 H, OMe), 2.09 and 2.00 (2 s, each 3 H, 2 Ac), and 1.45 and 1.46 (2 s, each 3 H, CMe_2).

Anal. Calc. for $C_{14}H_{23}NO_7$: C, 52.99; H, 7.31; N, 4.41. Found: C, 52.70; H, 7.17; N, 4.31.

Methyl 4-azido-4-deoxy-2,3-O-isopropylidene-6-O-p-toluenesulfonyl-α-D-mannopyranoside (**15**). — A mixture of **11** (509 mg, 1.96 mmol) and *p*-toluenesulfonyl chloride (560 mg, 2.94 mmol) in pyridine (10 mL) was stirred for 12 h at room temperature, then worked-up conventionally. Column chromatography (1:5 EtOAc–hexane) of the product gave **15** (800 mg, 98%), isolated as a syrup, $[\alpha]_D^{20}$ + 59° (*c* 1.2, chloroform). ^{1}H-N.m.r. data: δ 7.83–7.35 (d, 2 H, Ph), 4.65 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.38 (dd, 1 H, $J_{2,3}$ 8.4 Hz, H-2), 3.94 (dd, 1 H, $J_{5,6a}$ 4, $J_{6a,6b}$ 12.1 Hz, H-6a), 3.84–3.82 (m, 2 H, H-3,5), 3.73–3.70 (m, 2 H, H-4,6b), 3.19 (s, 3 H, OMe), 2.45 (s, 3 H, Ts*Me*), and 1.39 (s, 6 H, CMe_2).

Anal. Calc. for $C_{17}H_{23}N_3O_5S$: C, 49.39; H, 5.61; N, 10.16. Found: C, 49.46; H, 5.49; N, 10.00.

Methyl 4-acetamido-4,6-dideoxy-2,3-O-isopropylidene-α-D-mannopyranoside (**16**). — A mixture of **15** (800 mg, 2.1 mmol) and lithium aluminum hydride (860 mg, 2 mmol) in ether (20 mL) was boiled under reflux for 2 h, then diluted with water, and concentrated. The residue was acetylated conventionally to give **16** (335 mg, 64%) as a syrup, $[\alpha]_D^{24}$ + 47° (*c* 0.3, chloroform). ^{1}H-N.m.r. data: δ 5.50 (d, 1 H, $J_{4,NH}$ 9.9 Hz, NH), 5.20 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 9.9 Hz, H-3), 5.12 (d, 1 H, $J_{1,2}$ ~ 0 Hz, H-2), 4.64 (s, 1 H, H-1), 4.21 (q, 1 H, H-4), 3.65 (dq, 1 H, $J_{4,5}$ 9.9, $J_{5,6}$ 6.2 Hz, H-5), 3.38 (s, 3 H, OMe), 2.16, 2.03 and 1.95 (3 s, each 3 H, 3 Ac), and 1.28 (d, 3 H, H-6,6,6).

Anal. Calc. for $C_{12}H_{21}NO_5$: C, 55.58; H, 8.16; N, 5.40. Found: C, 55.81; H, 8.15; N, 5.10.

Methyl 4-acetamido-2,3-di-O-acetyl-4,6-dideoxy-α-D-mannopyranoside (**17**). — A mixture of **16** (335 mg, 1.29 mmol), M hydrochloric acid (1 mL), and tetrahydrofuran (5 mL) was stirred for 2 h at room temperature, then worked-up conventionally. The product was acetylated in the usual way to give **17** (305 mg, 78%), m.p. 153–154.5° (from EtOH), $[\alpha]_D^{20}$ + 113° (*c* 0.1, chloroform); lit.[5] m.p. 159–160, $[\alpha]_D^{23}$ + 95.6° (methanol).

Methyl 4-acetamido-2,3,6-tri-O-acetyl-4-deoxy-α-D-mannopyranoside (**19**). — Compound **12** (393 mg, 1.24 mmol) was *O*-deisopropylidenated and then acetylated, as for **16**, to give **19** (368 mg, 82%) as a syrup, $[\alpha]_D^{24}$ + 78° (*c* 1, chloroform). ^{1}H-N.m.r. data: δ 5.70 (d, 1 H, $J_{4,NH}$ 11 Hz, NH), 5.26 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 11 Hz, H-3), 5.13 (dd, 1 H, $J_{1,2}$ 1.5 Hz, H-2), 4.72 (d, 1 H, H-1); 4.37 (q, 1 H, H-4), 4.25–4.22 (m, 2 H, H-6,6), 3.79 (ddd, 1 H, $J_{4,5}$ 11, $J_{5,6a}$ 1.8 Hz, H-5), 3.38 (s, 3 H, OMe), and 2.16, 2.10, 2.03, and 1.95 (4 s, each 3 H, 4Ac).

Anal. Calc. for $C_{15}H_{23}NO_9$: C, 49.86; H, 6.42; N, 3.88. Found: C, 50.33; H, 6.48; N, 3.70.

*1,6-Anhydro-2,3-*O*-isopropylidene-4-*O*-*p*-toluenesulfonyl-β-*D*-mannopyranose* (**23**). — Compound **22**[6] (7.48 g, 37 mmol) was treated with *p*-toluenesulfonyl chloride (10 g, 5.5 mmol) in pyridine (100 mL) for 12 h at 50°. The mixture was concentrated, the residue was extracted with EtOAc, the extract was concentrated, and the residue was crystallised from EtOH to give **23** (11.1 g, 86%) as prisms, m.p. 144.5–145°, $[\alpha]_D^{25}$ −41° (*c* 0.9, chloroform). ^{1}H-N.m.r. data: δ 7.85 (d, 2 H, aromatic), 5.32 (d, 1 H, $J_{1,2}$ 2.9 Hz, H-1), 4.72 (s, 1 H, H-4), 4.54 (dd, 1 H, $J_{5,6a}$ 1.5, $J_{5,6b}$ 6.2 Hz, H-5), 4.10 (d, 1 H, $J_{2,3}$ 6.2, $J_{3,4}$ ~ 0 Hz, H-3), 4.03 (dd, 1 H, H-2), 3.94 (dd, 1 H, $J_{6a,6b}$ 7.7 Hz, H-6a), 3.72 (dd, 1 H, H-6b), 2.47 (s, 3 H, Ts*Me*), and 1.50 and 1.23 (2 s, each 3 H, CMe_2).

Anal. Calc. for $C_{16}H_{20}O_7S$: C, 53.92; H, 5.66. Found: C, 53.88; H, 5.66.

*2,3-Di-*O*-acetyl-1,6-anhydro-4-*O*-*p*-toluenesulfonyl-β-*D*-mannopyranose* (**24**). — A mixture of **23** (10 g, 28 mmol) and M hydrochloric acid (10 mL) was stirred for 2 h at room temperature, then concentrated, and the residue was acetylated in the usual way. The product was crystallised from EtOH to give **24** (9.0 g, 80%) as needles, m.p. 119.5–121°, $[\alpha]_D^{20}$ −97° (*c* 0.13, chloroform). ^{1}H-N.m.r. data: δ 7.83 and 7.38 (2 d, each 2 H, aromatic), 5.42 (bs, 1 H, H-1), 5.20 (d, 1 H, $J_{1,2}$ ~ 0, $J_{2,3}$ 2 Hz, H-2), 4.93 (d, 1 H, $J_{3,4}$ ~ 0 Hz, H-3), 4.70 (bdd, 1 H, $J_{4,5}$ ~ 2, $J_{5,6a}$ 5.9 Hz, H-5), 4.58 (bs, 1 H, H-4), 4.16 (dd, 1 H, $J_{6a,6b}$ 8.1 Hz, H-6a), 3.84 (dd, 1 H, $J_{5,6b}$ ~ 1 Hz, H-6b), 2.47 (s, 3 H, Ts*Me*), and 2.09 and 2.02 (2 s, each 3 H, 2 Ac).

Anal. Calc. for $C_{17}H_{20}O_9S$: C, 51.00; H, 5.03. Found: C, 50.82; H, 4.88.

*2,3-Di-*O*-acetyl-1,6-anhydro-4-azido-4-deoxy-β-*D*-mannopyranose* (**25**). — Compound **24** (5.0 g, 12.5 mmol) was treated with methanolic M NaOMe (15 mL) in MeOH (10 mL) for 15 h at room temperature. The mixture was neutralised with Amberlite IR-120B (H^+) resin, then concentrated, and the residue was treated with NaN_3 (4.0 g, 62.5 mmol) in aqueous 95% *N,N*-dimethylformamide (10 mL) for 12 h at 120°. The mixture was worked-up in the usual way and the product was acetylated conventionally. Column chromatography (1:10 EtOAc–hexane) gave **25** (2.1 g, 61%), $[\alpha]_D^{24}$ −175° (*c* 1.1, chloroform). ^{1}H-N.m.r. data: δ 5.33 (bs, 1 H, H-1), 5.31 (dd, 1 H, $J_{2,3}$ 5.5, $J_{3,4}$ 1.5 Hz, H-3), 4.98 (dd, 1 H, $J_{1,2}$ 1.5 Hz, H-2), 4.66 (d, 1 H, $J_{4,5}$ ~ 0, $J_{5,6b}$ 5.5 Hz, H-5), 4.21 (d, 1 H, $J_{6a,6b}$ 7.7 Hz, H-6a), 3.94 (dd, 1 H, H-6b), 3.61 (bs, 1 H, H-4), and 2.15 and 2.08 (2 s, each 3 H, 2 Ac).

Anal. Calc. for $C_{10}H_{13}N_3O_6$: C, 44.28; H, 4.83; N, 15.49. Found: C, 44.41; H, 4.84; N, 15.19.

*4-Acetamido-2,3-di-*O*-acetyl-1,6-anhydro-4-deoxy-β-*D*-mannopyranoside* (**26**). — Compound **25** (100 mg, 0.37 mmol) was hydrogenated and then acetylated, as described for synthesis of **14**, to give **26** (69 mg, 65%), m.p. 182.5–183°, $[\alpha]_D^{25}$ −53° (*c* 0.45, chloroform); lit.[7] m.p. 181–183°, $[\alpha]_D$ −53° (chloroform).

Preparation of the free amino sugars **18, 20,** *and* **27**. — The *N,O*-acetyl derivatives **17, 19**, and **26** were each heated in aqueous 80% hydrazine hydrate for 0.5 h at 70°, and each product was eluted from a column of Dowex 50W-X2 (H^+) resin with methanol to give amorphous **18, 20,** and **27**, respectively, which were dried over anhydrous P_2O_5 and NaOH, and used in the coupling reactions.

(1R)-(1,2,3,5/4)-1,2-Anhydro-5-hydroxymethylcyclohexane-1,2,3,4-tetrol (**30**). — (1*R*)-(1,3/2)-1,2-Di-*O*-acetyl-3-bromomethyl-5-cyclohexene-1,2-diol[8] (**29**) was converted into (1*R*)-(1,3/2)-3-hydroxymethyl-5-cyclohexene-1,2-diol following the standard procedure[9]. Without purification, the triol was treated with *m*-chloroperoxybenzoic acid to give the mixture of the epoxides[10], which was crystallised from ethanol, giving **30** (~ 60% from **29**), m.p. 117–118.5° (from EtOH), $[\alpha]_D^{24}$ + 6° (*c* 1, methanol).

Anal. Calc. for $C_7H_{12}O_4$: C, 52.49; H, 7.55. Found: C, 52.30; H, 7.22.

*Methyl 2,3-di-*O*-acetyl-4,6-dideoxy-4-[(1S)-(1,4/5,6)-4,5,6-triacetoxy-4-(benzoyloxymethyl)-2-cyclohexen-1-ylamino]-α-*D*-mannopyranoside* (**3b**). — A mixture of **18** (82 mg, 0.47 mmol) and **28** (125 mg, 0.31 mmol) in 2-propanol (2 mL) was heated in a sealed tube for 3 days at 50°, then concentrated. The residue was acetylated with Ac_2O and pyridine in the usual manner. Column chromatography (1:10 Me_2CO–PhMe) of the product gave **3b** (149 mg, 68%), isolated as a syrup, $[\alpha]_D^{26}$ + 28° (*c* 1.7, chloroform). ^{1}H-N.m.r. data: δ 8.05–7.46 (m, 5 H, Ph), 5.97 (d, 1 H, $J_{1',2'}$ 2.2 Hz, H-2'), 5.59 (d, 1 H, $J_{3',4'}$ ~ 0, $J_{4',5'}$ 5.5 Hz, H-4'), 5.36 (dd, 1 H, $J_{5',6'}$ 2.6, H-5'), 5.14 (dd, 1 H, $J_{1,2}$ 1.5, $J_{2,3}$ 3.7 Hz, H-2), 5.12 (dd, 1 H, $J_{3,4}$ 10 Hz, H-3), 4.98 (dd, 1 H, $J_{1',6'}$ 5.7 Hz, H-6'), 4.79 (s, 2 H, H-7',7'), 4.59 (d, 1 H, H-1), 3.64 (m, 1 H, H-5), 3.59 (bs, 1 H, H-1'), 3.36 (s, 3 H, OMe), 2.92 (t, 1 H, $J_{4,5}$ 10 Hz, H-4), 2.13, 2.08, 2.06, 2.04, and 2.00 (5 s, each 3 H, 5 Ac), and 1.37 (d, 3 H, $J_{5,6}$ 6.2 Hz, C-6,6,6).

Anal. Calc. for $C_{31}H_{39}NO_{14}$: C, 57.31; H, 6.05; N, 2.16. Found: C, 57.19; H, 6.34; N, 1.95.

*Methyl 4,6-dideoxy-4-[(1S)-(1,4/5,6)-4,5,6-trihydroxy-3-(hydroxymethyl)-2-cyclohexen-1-ylamino]-α-*D*-mannopyranoside* (**3a**). — Compound **3b** (38 mg, 0.058 mmol) was treated with methanolic M NaOMe (0.25 mL) in MeOH (2 mL) for 1 h at room temperature. The mixture was neutralised with AcOH, then eluted first from a column of Dowex 50W-X2 (H^+) resin with MeOH→1:25 aqueous 28% NH_4OH–MeOH and then from a column of Amberlite IRA-400 (HO^-) resin with MeOH to give **3a** (15.7 mg, 81%), isolated as a syrup, $[\alpha]_D^{24}$ + 80° (*c* 0.59, methanol).

*Methyl 2,3,6-tri-*O*-acetyl-4-deoxy-4-[(1S)-(1,4/5,6)-4,5,6-triacetoxy-3-(benzoyloxymethyl)-2-cyclohexen-1-ylamino]-α-*D*-mannopyranoside* (**4b**). — A mixture of **20** (70 mg, 0.4 mmol), **28** (126 mg, 0.31 mmol), and 2-propanol (2 mL) was heated in a sealed tube for 3 days at 50°. The mixture was processed, as described for preparation of **3b**, to give syrupy **4b** (149 mg, 74%), $[\alpha]_D^{26}$ + 25° (*c* 1.7, chloroform). ^{1}H-N.m.r. data: δ 8.02–7.45 (m, 5 H, Ph), 5.95 (bs, 1 H, H-2'), 5.59 (d, 1 H, $J_{3',4'}$ ~ 0, $J_{4',5'}$ 5.1 Hz, H-4'), 5.34 (dd, 1 H, $J_{5',6'}$ 2.2 Hz, H-5'), 5.16 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 10.4 Hz, H-3), 5.14 (s, 1 H, $J_{1,2}$ ~ 0 Hz, H-1), 4.96 (dd, 1 H, $J_{1',6'}$ 6.2 Hz, H-6'), 4.78 (s, 2 H, H-7',7'), 4.68 (d, 1 H, H-2), 4.49 (dd, 1 H, $J_{5,6a}$ 2.2, $J_{6a,6b}$ 11.7 Hz, H-6a), 4.34 (dd, 1 H, $J_{5,6b}$ 5.3 Hz, H-6b), 3.71 (ddd, 1 H, $J_{4,5}$ 10.4 Hz, H-5), 3.55 (bs, 1 H, H-1'), 3.38 (s, 3 H, OMe), 3.24 (t, 1 H, H-4), and 2.14, 2.12, 2.08, 2.06, 2.04, and 2.03 (6 s, each 3 H, 6 Ac).

Anal. Calc. for $C_{33}H_{41}NO_{16}$: C, 56.01; H, 5.84; N, 1.98. Found: C, 56.33; H, 6.09; N, 1.83.

*Methyl 4-deoxy-4-[(1S)-(1,4/2,3,5)-2,3,4-trihydroxy-5-(hydroxymethyl)-2-cyclohexen-1-ylamino]-α-*D*-mannopyranoside* (**4a**). — Compound **4b** (52 mg, 0.073 mmol)

was *O*-deacylated and the product was purified as described for the preparation of **3a** to give **4a** (16.4 mg, 64%), $[\alpha]_D^{24}$ + 65° (*c* 0.58, methanol).

2,3-Di-O-acetyl-1,6-anhydro-4-deoxy-4-[(1S)-(1,4/5,6)-2,3,4-trihydroxy-5-(hydroxymethyl-2-cyclohexen-1-ylamino]-β-D-mannopyranose (**5b**). — A mixture of **27** (82 mg, 0.47 mmol), **28** (125 mg, 0.31 mmol), and 2-propanol (2 mL) was heated in a sealed tube for 3 days at 70°, then concentrated. The residue was acetylated and the product was chromatographed as for **3b** to give **5b** (126 mg, 64%), isolated as a syrup, $[\alpha]_D^{25}$ − 50° (*c* 0.27, chloroform). ^{1}H-N.m.r. data: δ 8.05–7.43 (m, 5 H, Ph), 6.02 (bs, 1 H, H-2'), 5.67 (d, 1 H, $J_{4',5'}$ 5.5 Hz, H-4'), 5.46 (dd, 1 H, $J_{5',6'}$ 6.2 Hz, H-5'), 5.39 (s, 1 H, $J_{1,2}$ ~ 0 Hz, H-1), 5.24 (d, 1 H, $J_{2,3}$ 5.1 Hz, H-3), 5.08 (d, 1 H, H-6'), 4.93 (d, 1 H, H-2), 4.80 (s, 2 H, H-7',7'), 4.46 (d, 1 H, $J_{4,5} = J_{5,6endo}$ ~ 0, $J_{5,6exo}$ 5.9 Hz, H-5), 4.20 (d, 1 H, $J_{6endo,6exo}$ 7.7 Hz, H-6endo), 3.85 (dd, 1 H, H-6exo), 3.67 (bs, 1 H, H-1'), 2.98 (s, 1 H, $J_{3,4}$ ~ 0 Hz, H-4), 2.98 (s, 1 H, H-1), and 2.12, 2.10, 2.08, 2.07, and 2.06 (5 s, each 3 H, 6 Ac).

Anal. Calc. for $C_{30}H_{35}NO_{14}$: C, 56.87; H, 5.57; N, 2.21. Found: C, 57.16; H, 5.96; N, 2.33.

1,6-Anhydro-4-deoxy-4-[(1S)-(1,4/5,6)-4,5,6-trihydroxy-3-(hydroxymethyl)-2-cyclohexen-1-ylamino]-β-D-mannopyranose (**5a**). — Compound **5b** (37.5 mg, 0.059 mmol) was *O*-deacylated and the product was purified, as described for the preparation of **3b**, to give **5a** (13.6 mg, 0.043 mmol), isolated as a syrup, $[\alpha]_D^{24}$ − 32° (*c* 0.43, methanol).

Methyl 2,3-di-O-acetyl-4,6-dideoxy-4-[(1S)-(1,4/2,3,5)-2,3,4-triacetoxy-5-(acetoxymethyl)-1-cyclohexylamino]-α-D-mannopyranoside (**6b**). — A mixture of **18** (82 mg, 0.47 mmol), **30** (50 mg, 0.31 mmol), 2 propanol (1 mL), and *N,N*-dimethylformamide (0.5 mL) was heated in a sealed tube for 1 day at 120°, then co-concentrated with 1-butanol. The residue was acetylated in the usual manner and the product was purified by chromatography, as for **3b**, to give **6b** (102 mg, 56%), isolated as a syrup, $[\alpha]_D^{26}$ + 44° (*c* 1.1, chloroform). ^{1}H-N.m.r. data: δ 5.19–5.08 (m, 5 H, H-1,3,2',3',4'), 4.59 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-2), 1.36 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6,6,6), 4.04 (dd, 1 H, $J_{5',7a'}$ 5.5, $J_{7'a,7'b}$ 8.8 Hz, H-7'a), 4.01 (dd, 1 H, $J_{5',7'b}$ 3.3 Hz, H-7'b), 3.63 (dq, 1 H, $J_{4,5}$ 10.1 Hz, H-5), 3.36 (s, 3 H, OMe), 3.15 (q, 1 H, $J_{1',2'} = J_{1',6'ax} = J_{1',6'eq}$ ~ 3.7 Hz, H-1'), 2.70 (t, 1 H, $J_{3,4}$ 10.1 Hz, H-4), and 2.15, 2.11, 2.06, 2.05, 2.03, and 1.97 (6 s, each 3 H, 6 Ac).

Anal. Calc. for $C_{26}H_{39}NO_{14}$: C, 52.97; H, 6.67; N, 2.38. Found: C, 52.79; H, 6.60; N, 2.28.

Methyl 4,6-dideoxy-4-[(1S)-(1,4/2,3,5)-2,3,4-trihydroxy-5-(hydroxymethyl)-1-cyclohexylamino]-α-D-mannopyranoside (**6a**). — Compound **6b** (29 mg, 0.049 mmol) was *O*-deacetylated with methanolic M NaOMe (0.25 mL) in MeOH (2 mL), and the product was purified as described for the preparation of **3a**, to give **6a** (11.3 mg, 65%), isolated as a syrup, $[\alpha]_D^{24}$ + 57° (*c* 0.44, methanol).

Methyl 2,3,6-tri-O-acetyl-4-deoxy-4-[(1S)-(1,4/2,3,5)-2,3,4-triacetoxy-5-(acetoxymethyl)-1-cyclohexylamino]-α-D-mannopyranoside (**7b**). — A mixture of **20** (70 mg, 0.4 mmol), **30** (50 mg, 0.31 mmol), *N,N*-dimethylformamide (0.5 mL), and 2-propanol (1 mL) was heated in a sealed tube for 24 h at 100°. The mixture was co-concentrated with 1-butanol and the residue was acetylated in the usual manner. Column chromatography (1:10 Me_2CO–PhMe) of the product gave **7b** (117 mg, 59%), isolated as a syrup,

$[\alpha]_D^{25}$ + 71° (*c* 0.3, chloroform). ^{1}H-N.m.r. data: δ 5.18–5.10 (m, 5 H, H-1,3,2',3',4'), 4.67 (d, 1 H, $J_{2,3}$ 1.1 Hz, H-2), 4.47 (dd, 1 H, $J_{6a,6b}$ 11.7, $J_{5,6a}$ 1.8 Hz, H-6a), 4.28 (dd, 1 H, $J_{5,6b}$ 5.4 Hz, H-6b), 4.07 (dd, 1 H, $J_{5',7'a}$ 5.9, $J_{7'a,7'b}$ 11.4 Hz, H-7'a), 3.99 (dd, 1 H, $J_{5',7'b}$ 4.4 Hz, H-7'b), 3.71 (ddd, 1 H, $J_{4,5}$ 10.6 Hz, H-5), 3.38 (s, 3 H, OMe), 3.12 (q, 1 H, $J_{1'',2'} = J_{1',6'ax} = J_{1',6'eq}$ 3.6 Hz, H-1'), 3.02 (t, 1 H, H-4), and 2.15, 2.12, 2.11, 2.075, 2.06, 2.04, and 1.98 (7 s, each 3 H, 7 Ac).

Anal. Calc. for $C_{25}H_{35}NO_{14}$: C, 52.35; H, 6.15; N, 2.44. Found: C, 52.73; H, 5.98; N, 2.01.

*Methyl 4-deoxy-4-[(1S)-(1,4/2,3,5)-2,3,4-trihydroxy-5-(hydroxymethyl)-1-cyclohexylamino]-α-*D*-mannopyranoside* (**7a**). — Compound **7b** (49 mg, 0.076 mmol) was treated with methanolic M NaOMe (0.25 mL) in MeOH (2 mL) for 1 h at room temperature, and the product was purified, as described for **3a**, to give **7a** (13.6 mg, 50%), isolated as a syrup, $[\alpha]_D^{24}$ + 65° (*c* 0.49, methanol).

*1,6-Anhydro-4-deoxy-4-[(1S)-(1,4/2,3,5)-2,3,4-triacetoxy-5-(acetoxymethyl)-1-cyclohexylamino]-β-*D*-mannopyranose* (**8b**). — A mixture of **27** (82 mg, 0.47 mmol), **30** (50 mg, 0.31 mmol), 2-propanol (1 mL), and *N,N*-dimethylformamide (0.5 mL) was heated in a sealed tube for 3 days at 120°, the mixture was processed, and the product was acetylated, as described for **7b**, to give **8b** (120 mg, 68%), isolated as a syrup, $[\alpha]_D^{20}$ − 48° (*c* 0.74, chloroform). ^{1}H-N.m.r. data: δ 5.41 (bs, 1 H, H-1), 5.40 (dd, 1 H, $J_{2',3'}$ 3.3, $J_{3',4'}$ 9.9 Hz, H-3'), 5.30 (dd, 1 H, $J_{1',2'}$ 7.0 Hz, H-2'), 5.29 (dd, 1 H, $J_{2,3}$ 5.1, $J_{3,4}$ 1.1 Hz, H-3), 5.22 (t, 1 H, H-4'), 4.92 (dd, 1 H, $J_{1,2}$ 1.8 Hz, H-2), 4.51 (d, 1 H, $J_{5,6exo}$ 5.5 Hz, H-5), 4.24 (d, 1 H, $J_{6endo,6exo}$ 7.3 Hz, H-6endo), 4.09 (dd, 1 H, $J_{5',7'a}$ 5.9, $J_{7'a,7'b}$ 11.4 Hz, H-7'a), 3.95 (dd, 1 H, $J_{5',7'b}$ 4 Hz, H-7'b), 3.88 (dd, 1 H, H-6exo), 3.23 (bdd, 1 H, $J_{1',6'}$ 3.3 Hz, H-1'), 2.95 (bs, 1 H, H-4), and 2.15, 2.13, 2.09, 2.08, 2.05, and 1.98 (6 s, each 3 H, 6 Ac).

Anal. Calc. for $C_{25}H_{35}NO_{14}$: C, 52.35; H, 6.15; N, 2.44. Found: C, 52.73; H, 5.98; N, 2.01.

*1,6-Anhydro-4-deoxy-4-[(1S)-(1,4/2,3,5)-2,3,4-trihydroxy-5-(hydroxymethyl)-1-cyclohexylamino]-β-*D*-mannopyranose* (**8a**). — Compound **8b** (80 mg, 0.14 mmol) was *O*-deacetylated and the product was purified, as described for **3a**, to give **8a** (25 mg, 55%), isolated as a syrup, $[\alpha]_D^{24}$ − 57° (*c* 0.92, methanol).

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