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The synthesis of antibody binding-site probes: a hexasaccharide and two pentasaccharides related to the *Brucella* A antigen and prepared by *in situ* activation of thioglycosides with bromine*,†

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ABSTRACT

Two pentasaccharide analogues and a hexasaccharide fragment of the *Brucella* A antigen [→2)-α-D-Rha*p*4NFo-(1→]$_n$ have been prepared as their methyl glycosides. The pentasaccharide analogues each have two formamido groups replaced by hydroxyl groups. Protected derivatives of the three oligosaccharides were prepared by *in situ* activation with bromine of mono- and di-saccharide thioglycosides of D-rhamnose and 4-azido-4,6-dideoxy-D-mannose in the presence of a glycosyl acceptor and silver triflate as promoter. Reduction of the azido groups with hydrogen sulfide, *N*-formylation with ethyl formate, and hydrogenolysis then gave the target pentasaccharide glycosides.

INTRODUCTION

The *Brucella* A antigen[1], a linear α-(1→2)-linked homopolymer of 4,6-dideoxy-4-formamido-D-mannose (Fig. 1), is bound selectively by monoclonal antibody YsT9-1[2], and its formamido groups have been shown to be essential[3] for formation of the antibody–antigen complex.

In order to probe a model[4], generated by computer-assisted techniques, of the complex between the *Brucella* A antigen and monoclonal antibody YsT9-1, we recently prepared three pentasaccharide analogues[5] of the antigen. Each of these analogues had one 4,6-dideoxy-4-formamido-D-mannose residue replaced by D-rhamnose (6-deoxy-D-mannose), thus substituting a hydroxyl for a formamido group.

In order to investigate further the interactions that occur in the antibody–antigen complex, we have now prepared the two pentasaccharide analogues **15** and **22** of the A antigen, each of which has two formamido groups replaced by hydroxyl groups (in residues b and d, or a and c, respectively, Fig. 1). The antigen has a helical minimum-energy conformation[6] with five residues in the geometrical repeating unit. Thus, the formamido groups replaced in either **15** or **22** are spatially adjacent to each other when the antigen is viewed along its helical axis (Fig. 1). We have also prepared the hexa-

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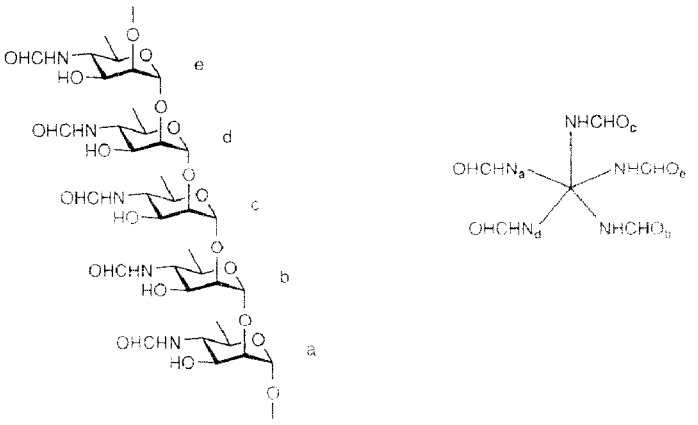
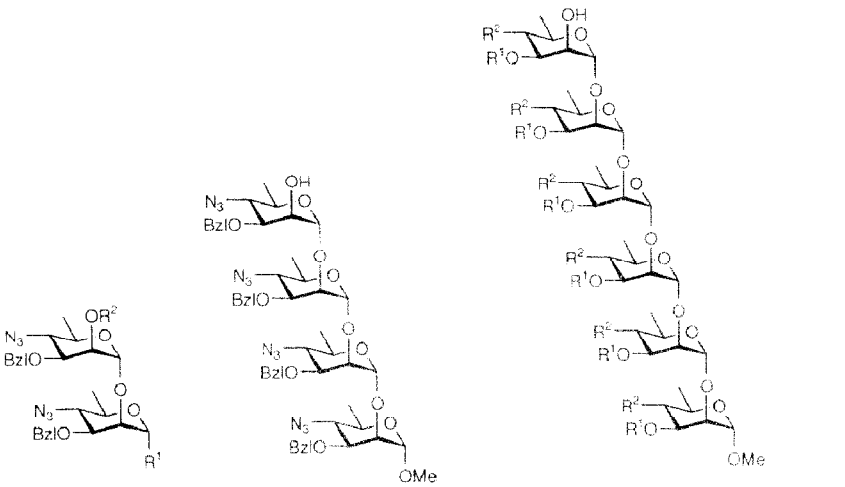


Fig. 1. A pentasaccharide fragment of the *Brucella* A antigen[7] (left) and the orientation of the formamido groups when the antigen is viewed along its helical axis in the minimum energy conformation[8] (right)

saccharide **6** in an effort to confirm further that the combining site of the antibody accommodates at least a pentasaccharide moiety of the *Brucella* A antigen, as suggested by previous inhibition studies[2,3] using synthetic di- to penta-saccharides[5,6].

RESULTS AND DISCUSSION

Activation[9] of thioglycosides *in situ* with bromine in the presence of a glycosyl acceptor and silver triflate as promoter has been used to synthesise the hexasaccharide **6** and the pentasaccharides **15** and **22**.



1 R^1 = OMe, R^2 = H
2 R^1 = SEt, R^2 = Ac

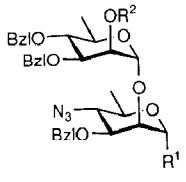
3

2 + **3** ⟶ **4** R^1 = Bzl, R^2 = N_3
5 R^1 = Bzl, R^2 = NHCHO
6 R^1 = H, R^2 = NHCHO

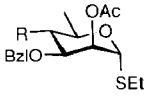
The protected hexasaccharide **4** was synthesised from the glycosyl acceptor **1**[7] and the disaccharide donor **2**[8]. The thioglycoside **2** is composed of two 4-azido-4,6-dideoxy-D-mannose residues and was a key intermediate in the synthesis of tri- to pentasaccharide fragments[8], and pentasaccharide analogues[5], of the *Brucella* A antigen. Glycosylation[9] of disaccharide **1** by activation of the thioglycoside **2** with bromine and promotion by silver triflate gave the tetrasaccharide **3**[8], in 70% yield, after deacetylation. The hexasaccharide **4** (69%) was obtained after glycosylation of **3** with **2** in the same manner, followed by transesterification to remove the acetyl group.

The tetrasaccharide **12**, which contains alternating 4-azido-4,6-dideoxy-D-mannose and D-rhamnose residues, was first prepared in a modest yield (39%) by *in situ* activation[9] of the thioglycoside **8**[5] with bromine and glycosylation of the disaccharide **7**[5], followed by deacetylation. The trisaccharide **11**[5] was prepared previously from **7** in 82% yield and, as the glycosylation of **11** by *in situ* activation of the D-rhamnoside **9**[5] with bromine, and subsequent deacetylation, proceeded in 75% yield, this provided a more efficient route to **12** (61% from **7**). Glycosylation of **12** with the 4-azido-4,6-dideoxy-1-thio-D-mannoside **10**[8], with deacetylation of the product, then gave the protected pentasaccharide **13** (84%) having D-rhamnose residues at positions b and d.

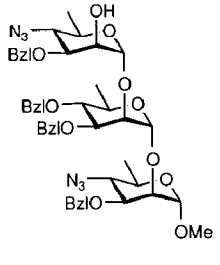
The protected pentasaccharide **20**, which has D-rhamnose residues at positions a and c, was prepared from methyl 3,4-di-*O*-benzyl-α-D-rhamnopyranoside[9] (**16**) and the two disaccharide donors **8**[5] and **2**[8]. Glycosylation[9] of **16** with **8**, in the usual manner, gave the α-glycoside **17** as well as the corresponding β-glycoside **19** (13%). Deacetylation of **17** provided the alcohol **18** (59% from **8**), which was glycosylated with **2** to give, after deacetylation, the α- and β-linked pentasaccharides **20** and **23** in yields of 69 and 11%, respectively.



7 R^1 = OMe, R^2 = H
8 R^1 = SEt, R^2 = Ac

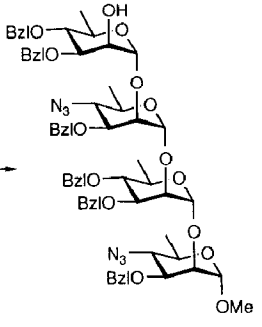


9 R = BzlO
10 R = N_3

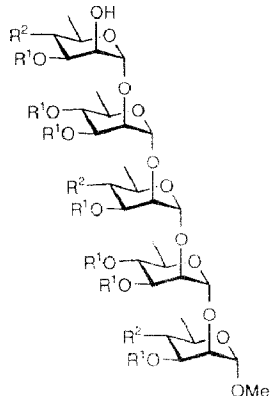


11

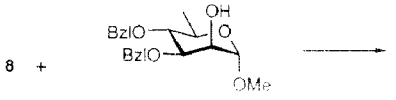
7 + **8**
or
9 + **11**



12

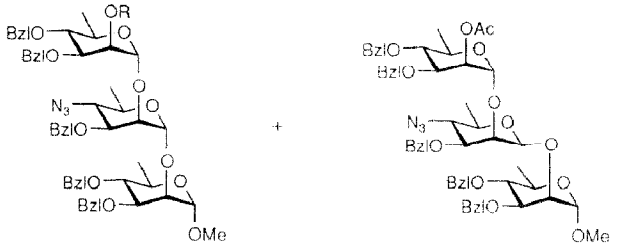


10 + **12** ⟶ **13** R^1 = Bzl, R^2 = N_3
14 R^1 = Bzl, R^2 = NHCHO
15 R^1 = H, R^2 = NHCHO



8 +

16

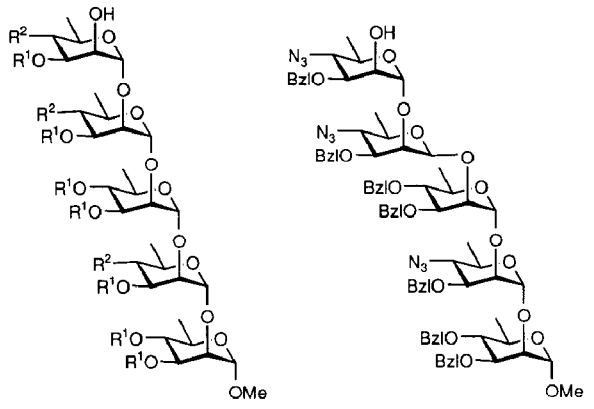


17 R = Ac
18 R = H

19

The synthesis of the protected oligosaccharides **4**, **13**, and **20** further substantiates our previous observations[5,9] that *in situ* activation of thioglycosides with bromine is a convenient glycosylation method that provides α-glycosides with high stereoselectivity when there is a non-participating group at C-2 of the glycosyl donor, and gives exclusively 1,2-*trans*-glycosides when there is a participating group at C-2 of the donor. As shown by several examples[5,9], the method is compatible with a variety of protective groups and, with promotion by silver triflate, gives yields equivalent to, or greater than, those obtained using alternative methods.

The hexasaccharide **4** and the pentasaccharides **13** and **20** were deprotected as described for similar compounds[5,8]. Reduction of the azido groups in **4**, **13**, and **20** with



2 + 18 ⟶ **20** R^1 = Bzl, R^2 = N_3
21 R^1 = Bzl, R^2 = NHCHO
22 R^1 = H, R^2 = NHCHO

23

hydrogen sulfide[10] and *N*-formylation of the resulting amines with ethyl formate–pyridine at elevated temperatures gave the formamides **5**, **14**, and **21** (38, 63, and 85% overall yields, respectively). The low yield of **5** illustrates the difficulties encountered in carrying out simultaneous reaction of several identical functional groups. Finally, hydrogenolysis in formic acid of the benzyl ethers in **5**, **14**, and **21** gave complex mixtures of formates[5], which were converted into the target oligosaccharide glycosides **6**, **15**, and **22** by treatment with methanolic sodium methoxide (70–90% overall yields). The ^{1}H- and ^{13}C-n.m.r. spectra of **6**, **15**, and **22** were complicated by the rotational isomerism[6] of the formamido groups, but could be satisfactorily analysed and were consistent with the assigned structures.

The binding of the oligosaccharide glycosides **6**, **15**, and **22** by monoclonal antibody YsT9-1[2] has been investigated in an enzyme-linked immunosorbent assay (ELISA) and the results have been described in a preliminary report[11]. A detailed discussion of these results, as well as those of microcalorimetry studies, and their application to the further refinement of the model[4] of the complex between the *Brucella* A antigen and monoclonal antibody YsT-91 will be reported separately.

EXPERIMENTAL

General. — The ^{1}H- and ^{13}C-n.m.r. spectra were recorded with a Bruker AM 500 spectrometer at 500 and 125 MHz, respectively, for solutions in $CDCl_3$ [residual $CHCl_3$ (δ_H 7.24) and $CDCl_3$ (δ_C 77.0) as internal standards] or D_2O [internal acetone (δ_H 2.225 and δ_C 30.5)]. First-order chemical shifts and coupling constants were obtained from one-dimensional spectra and assignments of proton resonances were based on COSY and n.O.e. experiments. In oligomeric structures, the resonances are assigned to pyranose rings designated a–f (Fig. 1). Resonances for aromatic and benzylic protons, and proton resonances that could not be assigned, are not reported. The stereochemistry of

the glycosidic bonds was determined from the $^1J_{C-1,H-1}$ coupling constants[12]. Optical rotations were measured with a Perkin–Elmer 243 polarimeter.

T.l.c. was performed on Silica Gel 60 F_{254} (Merck) with detection by u.v. light and charring with sulfuric acid. Silica Gel 60 (Merck, 230–400 mesh) and analytical reagent grade solvents (BDH) were used for column chromatography. Dichloromethane was dried by distillation from phosphorus pentaoxide and was stored over activated molecular sieve (4 Å). Powdered molecular sieve (Aldrich, 4 Å) was used in the glycosylations. Organic solutions were dried over Na_2SO_4.

Methyl 4-azido-2-*O*-(4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside[7] (**1**), ethyl 2-*O*-(2-*O*-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-4-azido-3-*O*-benzyl-4,6-dideoxy-1-thio-α-D-mannopyranoside[8] (**2**), methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-2-*O*-(3,4-di-*O*-benzyl-α-D-rhamnopyranosyl)-α-D-mannopyranoside[5] (**7**), ethyl 2-*O*-(2-*O*-acetyl-3,4-di-*O*-benzyl-α-D-rhamnopyranosyl)-4-azido-3-*O*-benzyl-4,6-dideoxy-1-thio-α-D-mannopyranoside[5] (**8**), ethyl 2-*O*-acetyl-3,4-di-*O*-benzyl-1-thio-α-D-rhamnopyranoside[9] (**9**), ethyl 2-*O*-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-1-thio-α-D-mannopyranoside[8] (**10**), methyl *O*-(4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→2)-*O*-(3,4-di-*O*-benzyl-α-D-rhamnopyranosyl)-(1→2)-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside[5] (**11**), and methyl 3,4-di-*O*-benzyl-α-D-rhamnopyranoside[10] (**16**) were prepared according to literature methods.

Satisfactory elemental analyses could not be obtained for the amorphous compounds **6**, **15**, and **22**, but their purity was established by t.l.c. and n.m.r. spectroscopy.

Methyl O-(4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→2)-O-(4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→2)-O-(4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→2)-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside[8] (**3**). A solution of **1**[7] (68 mg, 0.12 mmol) and **2**[8] (92 mg, 0.15 mmol) in dry dichloromethane (3.0 mL) containing powdered molecular sieve (4 Å, 300 mg) was stirred for 4 h at room temperature. Silver triflate (63 mg, 0.25 mmol) was added, followed after 10 min by bromine (4.7 μL, 92 μmol)[11]. After a further 30 min, more silver triflate (31 mg, 0.12 mmol) was added followed by triethylamine (51 μL, 0.37 mmol) when the reactants had been consumed (~60 min after the addition of bromine, t.l.c.). All operations were carried out under a positive pressure of dry nitrogen and in the dark. The mixture was filtered through Celite, and the flask and Celite were washed with dichloromethane (10 mL). The solution was washed with saturated aqueous sodium hydrogen carbonate (5 mL), dried, and concentrated. Column chromatography (ethyl acetate–hexanes, 1:6) of the residue gave a product (103 mg) that was dissolved in dichloromethane–methanolic 25mM sodium methoxide (1:1, 5 mL). The solution was stirred for 16 h at room temperature, then neutralised [Amberlite IR-120 (H^+) resin], and concentrated. Column chromatography (ethyl acetate–hexanes, 1:4) of the residue gave **3** (93 mg, 70%) with optical rotation and ^{1}H-n.m.r. data as reported[5].

Methyl O-(4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→2)-O-(4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→2)-O-(4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→2)-O-(4-azido-3-O-benzyl-4,6-dideoxy-α-D-man-

*nopyranosyl)-(1→2)-*O*-(4-azido-3-*O*-benzyl-4,6-dideoxy-α-*D*-mannopyranosyl)-(1→2)-4-azido-3-*O*-benzyl-4,6-dideoxy-α-*D*-mannopyranoside* (**4**). — Glycosylation[9] of **3**[8] (88 mg, 82 μmol) with **2**[8] (64 mg, 102 μmol), as described for **3**, and column chromatography (ethyl acetate–hexanes, 1:6) of the residue gave a product (103 mg) which was deacetylated, as described for **3**, and then purified by column chromatography (ethyl acetate–hexanes, 1:4) to give **4** (90 mg, 69%), $[\alpha]_D^{25}$ +97° (*c* 1.2, chloroform). N.m.r. data ($CDCl_3$): ^1H, δ 4.98 (d, 1 H, *J* 1.5 Hz, H-1f), 4.96, 4.89, 4.86, and 4.85 (4 d, each 1 H, each *J* 1.7 Hz, H-1bcde), 4.51 (d, 1 H, *J* 1.7 Hz, H-1a), 3.99 (bs, 1 H, H-2f), 3.71 (dd, 1 H, *J* 9.8 and 3.0 Hz, H-3f), 3.28 (s, 3 H, MeO); ^{13}C, δ 100.4, 100.3, 100.2, 100.1, 100.1, and 99.7 ($^1J_{C,H}$ 170, 172, 172, 172, 172, and 170 Hz, C-labcdef).

Anal. Calc. for $C_{79}H_{94}N_{18}O_{19}$: C, 59.3; H, 5.92; N, 15.8. Found: C, 59.7; H, 6.12; N, 15.8.

Methyl O-*(3-*O*-benzyl-4,6-dideoxy-4-formamido-α-*D*-mannopyranosyl)-(1→2)-*O*-(3-*O*-benzyl-4,6-dideoxy-4-formamido-α-*D*-mannopyranosyl)-(1→2)-*O*-(3-*O*-benzyl-4,6-dideoxy-4-formamido-α-*D*-mannopyranosyl)-(1→2)-*O*-(3-*O*-benzyl-4,6-dideoxy-4-formamido-α-*D*-mannopyranosyl)-(1→2)-*O*-(3-*O*-benzyl-4,6-dideoxy-4-formamido-α-*D*-mannopyranosyl)-(1→2)-3-*O*-benzyl-4,6-dideoxy-4-formamido-α-*D*-mannopyranoside* (**5**). — A solution of **4** (76 mg, 48 μmol) in pyridine–triethylamine (1:1, 8 mL) was saturated with hydrogen sulfide[10] for 1 h at room temperature and then stirred for 18 h. Nitrogen was passed through the solution for 1 h to remove hydrogen sulfide, the solution was concentrated, and toluene was distilled twice from the residue. Column chromatography (methanol–dichloromethane, 1:20 → 1:10 containing 0.1% of triethylamine) of the residue gave a crude product which was taken up in ethyl formate–pyridine (1:1, 4.0 mL). The solution was stirred at 75° for 12 h, then concentrated, and toluene was distilled twice from the residue. Preparative t.l.c. on Silica Gel 60 F_{254} (Merck, 1-mm plate; methanol–dichloromethane, 1:10) of the residue gave **5** (29 mg, 38%), $[\alpha]_D^{25}$ +42° (*c* 0.60, chloroform). N.m.r. data ($CDCl_3$–acetone-d_6, 3:1): ^1H, δ 8.25–7.96 (m, 6 H, NHC*H*O), 3.30 (bs, 3 H, MeO), 1.28–1.06 (m, 18 H, H-6abcdef); ^{13}C, δ 164.3 and 161.1 (2 m, NHCHO), 100.2–98.7 (m, C-labcdef), 50.4–49.4 (m, C-4abcdef-*Z*).

Anal. Calc. for $C_{85}H_{106}N_6O_{25}$: C, 63.3; H, 6.63; N, 5.21. Found: C, 62.8; H, 6.80; N, 5.43.

Methyl O-*(4,6-dideoxy-4-formamido-α-*D*-mannopyranosyl)-(1→2)-*O*-(4,6-dideoxy-4-formamido-α-*D*-mannopyranosyl)-(1→2)-*O*-(4,6-dideoxy-4-formamido-α-*D*-mannopyranosyl)-(1→2)-*O*-(4,6-dideoxy-4-formamido-α-*D*-mannopyranosyl)-(1→2)-*O*-(4,6-dideoxy-4-formamido-α-*D*-mannopyranosyl)-(1→2)-4,6-dideoxy-4-formamido-α-*D*-mannopyranoside* (**6**). — A solution of **5** (17 mg, 10 μmol) in formic acid (3.0 mL) was hydrogenated over Pd/C (10%, 33 mg) at 5 atm and room temperature for 16 h. The mixture was filtered through Celite and concentrated, and methanol was distilled twice from the residue. A solution of the residue in methanolic 25mM sodium methoxide (2 mL) was stirred for 30 min, then neutralized [Amberlite IR-120 (H^+) resin], and concentrated. Column chromatography (water–acetic acid–pyridine, 986:4:10) of the residue on Biogel P4 gave, after freeze-drying, **6** (7.9 mg, 70%), $[\alpha]_D^{25}$ +40° (*c* 0.36,

water). ^{1}H-N.m.r. spectroscopy showed a *Z*/*E*-ratio of ~4.7:1 for the formamido groups. N.m.r. data (D_2O): ^{1}H, δ 8.21–8.19 (m, 4.9 H, NHC*HO*-*Z*), 8.05–8.02 (m, 1.1 H, NHC*HO*-*E*), 5.21–5.16 (m, 4 H, H-1bcde), 5.05 (bs, 1 H, H-1f), 4.80 (bs, 1 H, H-1a), 3.40 (s, 3 H, MeO), 1.29–1.19 (m, 6 H, H-6abcdef); ^{13}C, δ 168.1 (NHCHO-*E*), 165.2 (NHCHO-*Z*), 102.2 (C-1f), 100.8 (C-1bcde), 99.7 (C-1a), 77.6, 77.4, 77.4, 77.3, and 77.3 (C-2abcde), 57.1 and 57.0 (C-4abcdef-*E*), 55.2 (MeO), 52.2, 52.1, 52.1, and 51.9 (C-4abcdef-*Z*).

Methyl O-*(3,4-di*-O-*benzyl*-α-D-*rhamnopyranosyl)*-*(1→2)*-O-*(4-azido-3*-O-*benzyl-4,6-dideoxy*-α-D-*mannopyranosyl)*-*(1→2)*-O-*(3,4-di*-O-*benzyl*-α-D-*rhamnopyranosyl)*-*(1→2)-4-azido-3*-O-*benzyl-4,6-dideoxy*-α-D-*mannopyranoside* (**12**). — (*a*) Glycosylation[9] of **7**[5] (62 mg, 100 μmol) with **8**[5] (87 mg, 130 μmol) at −45° → 0°, as described for **3**, and column chromatography (ethyl acetate–hexanes, 1:6 followed by 1:5) of the residue gave a crude product which was deacetylated as described for **3** and then purified by column chromatography (ethyl acetate–hexanes, 2:9) to give **12** (47 mg, 39%), $[\alpha]_D^{25}$ +56° (*c* 2.8, chloroform). N.m.r. data ($CDCl_3$): ^{1}H, δ 5.06 (d, 1 H, *J* 1.7 Hz, H-1c), 4.99 (d, 1 H, *J* 1.5 Hz, H-1d), 4.90 (d, 1 H, *J* 1.9 Hz, H-1b), 4.54 (d, 1 H, *J* 1.8 Hz, H-1a), 4.05 (bs, 1 H, H-2d), 4.04 (bt, 1 H, *J* 2.4 Hz, H-2c), 3.90 (bt, 1 H, *J* 2.4 Hz, H-2b), 3.85 (1 H, H-2a), 3.84 (dd, 1 H, *J* 9.5 and 3.3 Hz, H-3d), 3.81 (dd, 1 H, *J* 9.2 and 2.8 Hz, H-3b), 3.78 (dq, 1 H, *J* 9.5 and 6.2 Hz, H-5d), 3.72 (dd, 1 H, *J* 10.0 and 3.0 Hz, H-3c), 3.69 (dq, 1 H, *J* 9.4 and 6.2 Hz, H-5b), 3.64 (dd, 1 H, *J* 9.9 and 2.9 Hz, H-3a), 3.46 (dq, 1 H, *J* 9.9 and 6.4 Hz, H-5c), 3.45 (t, 1 H, *J* 9.4 Hz, H-4d), 3.38 (dq, 1 H, *J* 10.0 and 6.1 Hz, H-5a), 3.33 (t, 1 H, *J* 9.2 Hz, H-4b), 3.31 (t, 1 H, *J* 9.9 Hz, H-4c), 3.26 (s, 3 H, MeO), 3.21 (t, 1 H, *J* 10.0 Hz, H-4a), 1.26 (d, 3 H, *J* 6.2 Hz, H-6b), 1.25 (d, 1 H, *J* 6.2 Hz, H-6a), 1.21 (d, 1 H, *J* 6.2 Hz, H-6d), 1.14 (d, 1 H, *J* 6.2 Hz, H-6c); ^{13}C, δ 100.7, 100.4, 100.0, and 99.9 ($^1J_{C,H}$ 170, 170, 173, and 171 Hz, C-1abcd).

Anal. Calc. for $C_{67}H_{78}N_6O_{15}$: C, 66.6; H, 6.51; N, 6.96. Found: C, 66.3; H, 6.49; N, 6.56.

(*b*) Glycosylation[9] of **11**[5] (59 mg, 67 μmol) with **9**[5] (36 mg, 84 μmol) at −45° → 0°, as described for **3**, and column chromatography (ethyl acetate–hexanes, 2:11) of the residue gave a product (69 mg) which was deacetylated as described for **3** and then purified by column chromatography (ethyl acetate–hexanes, 1:4) to give **12** (61 mg, 75%).

Methyl O-*(4-azido-3*-O-*benzyl-4,6-dideoxy*-α-D-*mannopyranosyl)*-*(1→2)*-O-*(3,4-di*-O-*benzyl*-α-D-*rhamnopyranosyl)*-*(1→2)*-O-*(4-azido-3*-O-*benzyl-4,6-dideoxy*-α-D-*mannopyranosyl)*-*(1→2)*-O-*(3,4-di*-O-*benzyl*-α-D-*rhamnopyranosyl)*-*(1→2)*-*4-azido-3*-O-*benzyl-4,6-dideoxy*-α-D-*mannopyranoside* (**13**). — Glycosylation[9] of **12** (57 mg, 47 μmol) with **10**[8] (22 mg, 59 μmol) at 0°, as described for **3**, and column chromatography (ethyl acetate–hexanes, 1:6) of the residue gave a product (65 mg) which was deacetylated as described for **3** and then purified by column chromatography (ethyl acetate–toluene, 1:20) to give **13** (58 mg, 84%), $[\alpha]_D^{25}$ +72° (*c* 0.58, chloroform). N.m.r. data ($CDCl_3$): ^{1}H, δ 5.06 (d, 1 H, *J* 1.6 Hz, H-1c), 4.99 (d, 1 H, *J* 1.6 Hz, H-1c), 4.98 and 4.88 (2 d, each 1 H, *J* 1.9 and 1.8 Hz, H-1bd), 4.52 (d, 1 H, *J* 1.7 Hz, H-1a), 4.04 (bs, 1 H, H-2e), 3.96 (bt, 1 H, *J* 2.2 Hz, H-2c), 3.83 (bt, 1 H, *J* 2.7 Hz, H-2a), 3.73 (dd, 1 H,

J 9.6 and 3.2 Hz, H-3c), 3.68 (dd, 1 H, *J* 10.0 and 2.8 Hz, H-3c), 3.63 (dd, 1 H, *J* 10.0 and 2.9 Hz, H-3a), 3.56 (dq, 1 H, *J* 10.1 and 6.1 Hz, H-5e), 3.42 (dq, 1 H, *J* 10.0 and 6.1 Hz, H-5c), 3.40 (t, 1 H, *J* 9.9 Hz, H-4e), 3.37 (dq, 1 H, *J* 10.0 and 6.0 Hz, H-5a), 3.25 (s, 3 H, MeO), 3.25 (t, 1 H, *J* 10.0 Hz, H-4c), 3.19 (t, 1 H, *J* 10.0 Hz, H-4a), 1.24 (d, 3 H, *J* 6.2 Hz, H-6a), 1.17 (d, 3 H, *J* 6.2 Hz, H-6e), 1.12 (d, 3 H, *J* 6.2 Hz, H-6c); ^{13}C, δ 100.4, 100.4, 100.1, 100.0, and 99.9 (each $^{1}J_{C,H}$ 172 Hz, C-labcde).

Anal. Calc. for $C_{80}H_{93}N_9O_{18}$: C, 65.4; H, 6.38; N, 8.58. Found: C, 65.2; H, 6.33; N, 8.79.

Methyl O-*(3*-O-*benzyl-4,6-dideoxy-4-formamido-*α-D-*mannopyranosyl)-(1→2)*-O-*(3,4-di*-O-*benzyl-*α-D-*rhamnopyranosyl)-(1→2)*-O-*(3*-O-*benzyl-4,6-dideoxy-4-formamido-*α-D-*mannopyranosyl)-(1→2)*-O-*(3,4-di*-O-*benzyl-*α-D-*rhamnopyranosyl)-(1→2)-3*-O-*benzyl-4,6-dideoxy-4-formamido-*α-D-*mannopyranoside* (**14**). — Reduction[10] of **13** (52 mg, 35 μmol), as described for **5**, and *N*-formylation of the residue in refluxing ethyl formate–pyridine (20:1, 5 mL) for 4 h gave, after chromatography (methanol–dichloromethane, 1:40, containing 0.1% of triethylamine) of the residue, **14** (33 mg, 63%), $[\alpha]_D^{25}$ + 50° (*c* 1.9, chloroform). N.m.r. data ($CDCl_3$): ^1H, δ 8.18–7.91 (m, 3 H, NHC*H*O), 3.31 (bs, 3 H, MeO), 1.29–0.97 (m, 15 H, H-6abcde); ^{13}C, δ 165.2–164.7 and 162.0–161.0 (2 m, NH*C*HO), 100.7–99.6 (m, C-labcde), 51.9–49.9 (m, C-4ace-*Z*).

Anal. Calc. for $C_{83}H_{99}N_3O_{21}$: C, 67.6; H, 6.77; N, 2.85. Found: C, 67.2; H, 6.92; N, 2.74.

Methyl O-*(4,6-dideoxy-4-formamido-*α-D-*mannopyranosyl)-(1→2)*-O-α-D-*rhamnopyranosyl-(1→2)*-O-*(4,6-dideoxy-4-formamido-*α-D-*mannopyranosyl)-(1→2)*-O-α-D-*rhamnopyranosyl-(1→2)-4,6-dideoxy-4-formamido-*α-D-*mannopyranoside* (**15**). — Debenzylation of **14** (31 mg, 21 μmol) and column chromatography of the residue on Biogel P4, as described for **6**, gave, after freeze-drying, **15** (16 mg, 90%), $[\alpha]_D^{25}$ + 55° (*c* 0.66, water). ^1H-N.m.r. spectroscopy showed a *Z*/*E*-ratio of ~3.4:1 for the formamido groups. N.m.r. data (D_2O): ^1H, δ 8.19 (s, 2.3 Hz, NHC*H*O-*Z*), 8.04, 8.03, and 8.02 (3 s, 0.7 H, NHC*H*O-*E*), 5.16, 5.12, 5.10, and 5.03 (4 bs, each 1 H, H-1bcde), 4.82 (bs, 1 H, H-1a), 3.50 and 3.48 (2 bt, each 1 H, each *J* 9.0 Hz, H-4bd), 3.40 (s, 3 H, MeO), 1.32–1.18 (m, 15 H, H-6abcde); ^{13}C, δ 168.1 (NH*C*HO-*E*), 165.1 (NH*C*HO-*Z*), 102.4 (C-1e), 101.0 (C-1bcd), 99.8 (C-1a), 78.5, 78.3, 77.4, and 77.2 (C-2abcd), 57.2, 57.2, and 56.9 (C-4ace-*E*), 55.2 (MeO), 52.2, 52.2, and 52.0 (C-4ace-*Z*).

Methyl O-*(3,4-di*-O-*benzyl-*α-D-*rhamnopyranosyl)-(1→2)*-O-*(4-azido-3*-O-*benzyl-4,6-dideoxy-*α-D-*mannopyranosyl)-(1→2)-3,4-di*-O-*benzyl-*α-D-*rhamnopyranoside* (**18**) *and methyl* O-*(2*-O-*acetyl-3,4-di*-O-*benzyl-*α-D-*rhamnopyranosyl)-(1→2)*-O-*(4-azido-3*-O-*benzyl-4,6-dideoxy-β-D-mannopyranosyl)-(1→2)-3,4-di*-O-*benzyl-*α-D-*rhamnopyranoside* (**19**). — Glycosylation[9] of **16**[9] (48 mg, 130 μmol) with **8**[5] (74 mg, 110 μmol) at −45° → 0°, as described for **3**, and column chromatography (ethyl acetate–hexanes, 1:6 followed by 1:3) of the residue gave **19** (14 mg, 13%) and **17** (72 mg); **17** was deacetylated as described for **3** and then purified by column chromatography (ethyl acetate–hexanes, 1:4) to give **18** (60 mg, 59%).

Compound **18** had $[\alpha]_D^{25}$ + 50° (*c* 1.9, chloroform). N.m.r. data ($CDCl_3$): ^1H, δ 5.08 and 4.99 (2 d, each 1 H, *J* 1.5 and 1.2 Hz, H-1bc), 4.53 (d, 1 H, *J* 1.5 Hz, H-1a), 4.05 (bt, 2

H, *J* 2.2 Hz, H-2bc), 3.89 (t, 1 H, *J* 2.4 Hz, H-2a), 3.84 (dd, 1 H, *J* 9.2 and 3.2 Hz, H-3c), 3.81 (dd, 1 H, *J* 9.4 and 2.9 Hz, H-3a), 3.78 (H-5c), 3.76 (dd, 1 H, *J* 10.0 and 2.9 Hz, H-3b), 3.62 (dq, 1 H, *J* 9.4 and 6.2 Hz, H-5a), 3.54 (dq, 1 H, *J* 10.0 and 6.2 Hz, H-5b), 3.45 (t, 1 H, *J* 9.4 Hz, H-4c), 3.35 (t, 1 H, *J* 10.0 Hz, H-4b), 3.32 (t, 1 H, *J* 9.4 Hz, H-4a), 3.28 (s, 3 H, MeO), 1.28 (d, 3 H, *J* 6.1 Hz, H-6a), 1.27 (d, 3 H, *J* 6.3 Hz, H-6b), 1.20 (d, 3 H, *J* 6.4 Hz, H-6c); ^{13}C, δ 100.7, 100.4, and 99.8 ($^{1}J_{C,H}$ 171, 172, and 170 Hz, C-1abc).

Anal. Calc. for $C_{58}H_{67}N_3O_{12}$: C, 68.6; H, 6.71; N, 4.44. Found: C, 68.6; H, 6.84; N, 4.24.

Compound **19** was characterised by n.m.r. spectroscopy ($CDCl_3$): ^{1}H, δ 5.55 (dd, 1 H, *J* 3.3 and 1.5 Hz, H-2c), 5.34 (d, 1 H, *J* 1.3 Hz, H-1c); 4.63 (bs, 1 H, H-1a), 4.45 (bs, 1 H, H-1b), 4.32 (bd, 1 H, *J* 1.8 Hz, H-2b), 4.27 (dd, 1 H, *J* 3.7 and 1.8 Hz, H-2a), 4.24 (H-5c), 4.08 (dd, 1 H, *J* 9.6 and 3.3 Hz, H-3c), 3.76 (dd, 1 H, *J* 9.0 and 3.8 Hz, H-3a), 3.59 (dq, 1 H, *J* 9.5 and 6.1 Hz, H-5a), 3.56 (t, 1 H, *J* 9.8 Hz, H-4b), 3.47 (t, 1 H, *J* 9.2 Hz, H-4a), 3.39 (t, 1 H, *J* 9.6 Hz, H-4c), 3.39 (dd, 1 H; *J* 9.8 and 2.5 Hz, H-3b), 3.30 (s, 3 H, MeO), 3.12 (dq, 1 H, *J* 9.7 and 6.1 Hz, H-5b), 2.06 (s, 3 H, Ac), 1.36 (d, 3 H, *J* 6.3 Hz, H-6c), 1.27 (d, 3 H, *J* 6.1 Hz, H-6b), 1.24 (d, 3 H, *J* 6.1 Hz, H-6a); ^{13}C, δ 98.2 and 97.1 ($^{1}J_{C,H}$ 167 and 177 Hz, C-1ac), 97.2 ($^{1}J_{C,H}$ 154 Hz, C-1b).

Methyl O-(4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→2)-O-(4-azido-3-O-benzyl-4,6-dideoxy-α- (**20**) *and -β-D-mannopyranosyl)-(1→2)-O-(3,4-di-O-benzyl-α-D-rhamnopyransoyl)-(1→2)-O-(4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-(1→2)-3,4-di-O-benzyl-α-D-rhamnopyranoside* (**23**). — Glycosylation[9] of **18** (94 mg, 100 μmol) with **2**[5] (74 mg, 120 μmol) at 0°, as described for **3**, and column chromatography (ethyl acetate–hexanes, 1:5) of the residue gave a crude product that was deacetylated as described for **3** and then purified by column chromatography (ethyl acetate–hexanes, 1:4 followed by 1:3) to give **20** (101 mg, 69%) and **23** (16 mg, 11%).

Compound **20** had $[\alpha]_D^{25}$ +68° (*c* 0.90, chloroform). N.m.r. data ($CDCl_3$): ^{1}H, δ 5.07 and 4.99 (2 d, 1 H and 2 H, each *J* 1.6 Hz, H-1bde), 4.92 (d, 1 H, *J* 1.8 Hz, H-1c), 4.51 (d, 1 H, *J* 1.6 Hz, H-1a), 4.00 (bs, 1 H, H-2e), 3.93 (t, 1 H, *J* 2.4 Hz, H-2c), 3.87 (t, 1 H, *J* 2.6 Hz, H-2a), 3.83 (dd, 1 H, *J* 9.2 and 2.8 Hz, H-3c), 3.80 (dd, 1 H, *J* 9.3 and 2.9 Hz, H-3a), 3.72 (dd, 1 H, *J* 9.7 and 3.1 Hz, H-3e), 3.69 (dq, 1 H, *J* 9.1 and 6.2 Hz, H-5c), 3.60 (dq, 1 H, *J* 9.4 and 6.2 Hz, H-5a), 3.57 (dq, 1 H, *J* 10.1 and 6.2 Hz, H-5e), 3.42 (t, 1 H, *J* 9.9 Hz, H-4e), 3.32 (t, 1 H, *J* 9.0 Hz, H-4c), 3.28 (s, 3 H, MeO), 3.28 (t, 1 H, *J* 9.6 Hz, H-4a), 1.27 (d, 3 H, *J* 6.2 Hz, H-6a), 1.20 (d, 3 H, *J* 6.2 Hz, H-6e), 1.17 (d, 3 H, *J* 6.1 Hz, H-6c); ^{13}C, δ 100.6, 100.3, 100.3, 99.8, and 99.7 (each $^{1}J_{C,H}$ 172 Hz, C-1abcde).

Anal. Calc. for $C_{80}H_{93}N_3O_{18}$: C, 65.4; H, 6.38; N, 8.58. Found: C, 65.1; H, 6.48; N, 8.41.

Compound **23** had $[\alpha]_D^{25}$ +13° (*c* 1.1, chloroform). N.m.r. data ($CDCl_3$): ^{1}H, δ 5.40 (d, 1 H, *J* 1.2 Hz, H-1e), 5.09 (d, 1 H, *J* 1.6 Hz, H-1b), 4.89 (bs, 1 H, H-1c), 4.54 (d, 1 H, *J* 1.6 Hz, H-1a), 4.18 (t, 1 H, *J* 2.3 Hz, H-2b), 4.16 (dd, 1 H, *J* 3.7 and 1.8 Hz, H-2c), 4.07 (bd, 1 H, *J* 2.5 Hz, H-2d), 4.05 (dq, 1 H, *J* 10.3 and 6.3 Hz, H-5e), 4.03 (m, 1 H, H-2e), 3.94 (t, 1 H, *J* 2.4 Hz, H-2a), 3.88 (dd, 1 H, *J* 9.8 and 3.1 Hz, H-3e), 3.83 (dd, 1 H, *J* 9.4 and 2.9 Hz, H-3a), 3.81 (dd, 1 H, *J* 9.5 and 3.2 Hz, H-3c), 3.81 (bs, 1 H, H-1d), 3.77 (dd, 1 H, *J* 10.0 and 2.9 Hz, H-3b), 3.72 (dq, 1 H, *J* 9.4 and 6.2 Hz, H-5c), 3.64 (dq, 1 H, *J* 9.4

and 6.2 Hz, H-5a), 3.53 (dq, 1 H, *J* 10.0 and 6.2 Hz, H-5b), 3.49 (t, 1 H, *J* 9.4 Hz, H-4c), 3.45 (t, 1 H, *J* 10.0 Hz, H-4e), 3.39 (t, 1 H, *J* 9.4 Hz, H-4a), 3.33 (t, 1 H, *J* 10.0 Hz, H-4b), 3.32 (t, 1 H, *J* 9.8 Hz, H-4d), 3.29 (s, 3 H, MeO), 2.99 (dd, 1 H, *J* 9.8 and 2.5 Hz, H-3d), 2.51 (dq, 1 H, *J* 9.8 and 6.2 Hz, H-5d), 1.36 (d, 3 H, *J* 6.2 Hz, H-6e), 1.30 (d, 3 H, *J* 6.2 Hz, H-6a), 1.27 (d, 3 H, *J* 6.2 Hz, H-6b), 1.23 (d, 3 H, *J* 6.4 Hz, H-6c), 1.22 (d, 3 H, *J* 6.4 Hz, H-6d); ^{13}C, δ 100.1, 99.9, 98.8, and 98.0 ($^{1}J_{C,H}$ 173, 169, 166, and 175 Hz, C-labce), 97.0 ($^{1}J_{C,H}$ 155 Hz, C-1d).

Anal. Calc. for $C_{80}H_{93}N_9O_{18}$: C, 65.4; H, 6.38; N, 8.58. Found: C, 65.9; H, 6.73; N, 8.55.

Methyl O-*(3*-O-*benzyl-4,6-dideoxy-4-formamido-*α-D-*mannopyranosyl)-(1→2)*-O-*(3*-O-*benzyl-4,6-dideoxy-4-formamido-*α-D-*mannopyranosyl)-(1→2)*-O-*(3,4-di*-O-*benzyl-*α-D-*rhamnopyranosyl)-(1→2)*-O-*(3*-O-*benzyl-4,6-dideoxy-4-formamido-*α-D-*mannopyranosyl)-(1→2)-3,4-di*-O-*benzyl-*α-D-*rhamnopyranoside* (**21**). — Reduction[10] of **20** (66 mg, 45 μmol), as described for **5**, and *N*-formylation of the residue in refluxing ethyl formate–pyridine (20:1, 10 mL) for 18 h gave, after column chromatography (methanol–dichloromethane, 1:40, containing 0.1% triethylamine) of the residue, **21** (56 mg, 85%), $[\alpha]_D^{25}$ +37° (*c* 1.1, chloroform). N.m.r. data ($CDCl_3$): ^{1}H, δ 8.22–7.99 (m, 3 H, NHC*H*O), 3.31 (bs, 3 H, MeO), 1.40–1.10 (m, 15 H, H-6abcde); ^{13}C, δ 164.8 and 161.5 (2 m, NH*C*HO), 100.9–99.5 (m, C-labcde), 51.1–50.1 (m, C-4bde-*Z*).

Anal. Calc. for $C_{83}H_{99}N_3O_{21}$: C, 67.6; H, 6.77; N, 2.85. Found: C, 68.0; H, 6.84; N, 2.58.

Methyl O-*(4,6-dideoxy-4-formamido-*α-D-*mannopyranosyl)-(1→2)*-O-*(4,6-dideoxy-4-formamido-*α-D-*mannopyranosyl)-(1→2)*-O-α-D-*rhamnopyranosyl-(1→2)*-O-*(4,6-dideoxy-4-formamido-*α-D-*mannopyranosyl)-(1→2)*-α-D-*rhamnopyranoside* (**22**). — Debenzylation of **21** (39 mg, 26 μmol) and column chromatography of the residue on Biogel P4, as described for **6**, gave, after freeze-drying, **22** (18 mg, 80%), $[\alpha]_D^{25}$ +45° (*c* 0.29, water). ^{1}H-N.m.r. spectroscopy showed a *Z*/*E*-ratio of ~3.5:1 for the formamido groups. N.m.r. data (D_2O): ^{1}H, δ 8.20 (s, 2.3 H, NHC*H*O-*Z*), 8.04, 8.03, and 8.02 (3 s, 0.7 H, NHC*H*O-*E*), 5.18, 5.16, 5.10, and 5.05 (4 bs, each 1 H, H-1bcde), 4.75 (bs, 1 H, H-1a), 3.79 and 3.69 (2 dq, each 1 H, each *J* 9.4 and 6.3 Hz, H-5ac), 3.49 and 3.47 (2 t, each 1 H, each *J* 10.1 Hz, H-4ac), 3.40 (s, 3 H, MeO), 1.32–1.19 (m, 15 H, H-6abcde); ^{13}C, δ 168.1 (NH*C*HO-*E*), 165.1 (NH*C*HO-*Z*), 102.2 (C-1e), 101.0, 101.0, and 100.9 (C-1bcd), 99.8 (C-1a), 78.5, 78.2, 77.4, and 77.2 (C-2abcde), 57.1 and 56.9 (C-4bde-*E*), 55.1 (MeO), 52.3, 52.2, and 51.9 (C-4bde-*Z*).

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