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The design and synthesis of antibody binding site probes: three pentasaccharide analogues of the *Brucella* A antigen prepared by activation *in situ* of thioglycosides with bromine*

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ABSTRACT

Three pentasaccharide analogues of the *Brucella* A antigen [→2)-α-D-Rha*p*4NFo-(1→]$_n$, each with one formamido group replaced by a hydroxyl group, have been prepared as their methyl glycosides. Mono- and di-saccharide thioglycosides of D-rhamnose and 4-azido-4,6-dideoxy-D-mannose were used as glycosyl donors for the preparation of protected pentasaccharide derivatives with trisaccharides as intermediates. Glycosylations were performed by activation *in situ* of the thioglycosides with bromine 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 pentasaccharides.

INTRODUCTION

The *Brucella* A and M antigens are linear homopolymers of the rare sugar 4,6-dideoxy-4-formamido-D-mannose[1,2]. The A antigen contains only α 1,2-glycosidic linkages[1] whereas, for the M antigen, one linkage in five is an α 1,3-linkage[2]. The A antigen, which is found predominantly in *Brucella abortus*, is selectively bound by monoclonal antibody YsT9-1 (ref. 3). The formamido groups of the antigen have been shown to be essential for the formation of the antibody–antigen complex[4], a fact which can be rationalized from studies[2,5] that revealed a helical conformation for the A-antigen, with the formamido groups protruding from the axis of the helix. Viewed along the helical axis, the angle subtended by the formamido groups of adjacent monosaccharide residues is 216° (Fig. 1). In this conformation the three dimensional repeating unit for the antigen contains five residues[5].

A model[6] of the complex between the *Brucella* A antigen and the F_v fragment of monoclonal antibody YsT9-1 has been developed using computer-assisted modelling techniques, in parallel with ongoing X-ray crystallographic studies[4] of the complex. The model[6] indicates that at least a pentasaccharide moiety of the *Brucella* A antigen is accomodated in the groove-type binding site of the antibody, in agreement with

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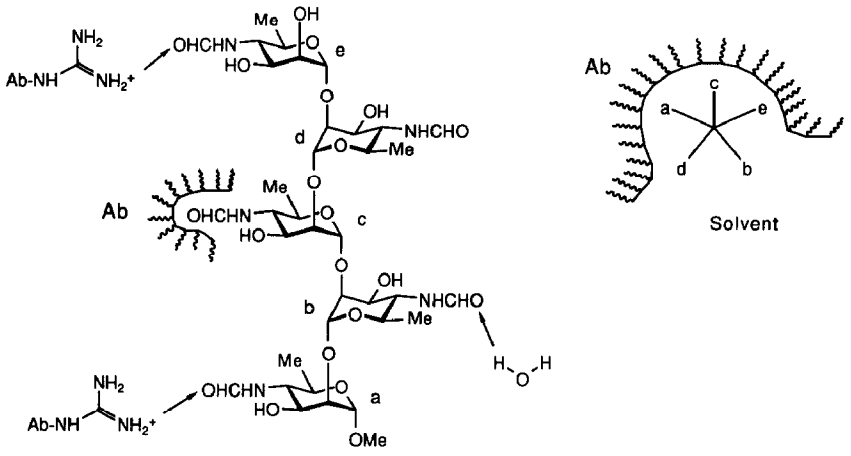


Fig. 1. Proposed interactions in the model[6] of the complex between the *Brucella* A antigen and monoclonal antibody YsT9-1 (left) and orientation of the formamido groups when the antigen is viewed along its helical axis in the minimum-energy conformation (right).

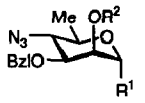
previous inhibition studies[3,4] using synthetic di- to penta-saccharides[7,9]. In the modelled complex[6] the formamido groups of residues a and e of a properly docked pentasaccharide are within hydrogen-bonding distance of the guanidino groups of two arginine residues, which are located symmetrically at either end of the binding site (Fig. 1). The formamido group of residue c is then located in a pocket of the binding site, whereas formamido group b is in contact with the surrounding solution.

In order to evaluate this model of the complex, we have prepared the three pentasaccharide analogues **25**, **30**, and **35** of the *Brucella* A antigen, each of which has one 4,6-dideoxy-4-formamido-D-mannose residue (a, b, or c) replaced by D-rhamnose, thus substituting a formamido group by a hydroxyl group.

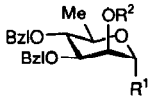
RESULTS AND DISCUSSION

Regioselective benzylation of methyl 4-azido-4,6-dideoxy-α-D-mannopyranoside[7,8], by a modification of the procedure[8] employing an *O*-stannylene acetal as intermediate, gave the 3-*O*-benzyl ether **1** (refs. 7 and 8) (89%) which was then converted to the ethyl thioglycosides **2** (ref. 9) and **3** (ref. 9) as previously described. The rhamnose thioglycoside **5** was obtained (64%), together with the β-anomer **7** (26%), by treatment of 1,2-di-*O*-acetyl-3,4-di-*O*-benzyl-α-D-rhamnopyranose (**4**) in dichloromethane with ethanethiol and boron trifluoride etherate. Similar amounts of ethylthio α- and β-L-rhamnopyranosides have been obtained[10,11] by reaction of tetra-*O*-acetyl-L-rhamnopyranose with ethanethiol under catalysis by Lewis acids. It should also be noted that boron trifluoride etherate and ethanethiol have been found to cause *O*-debenzylation of benzylated carbohydrates under more drastic reaction conditions[12]. Deacetylation of **5** gave the alcohol **6** (97%).

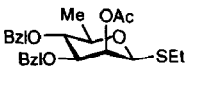
The α 1,2-linked disaccharide thioglycosides **8** (ref. 9), **9**, and **10** were envisaged as



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



4 R^1 = OAc, R^2 = Ac
5 R^1 = SEt, R^2 = Ac
6 R^1 = SEt, R^2 = H



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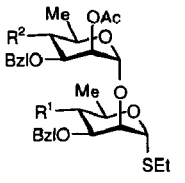
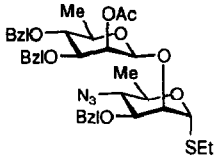


8 R^1 = R^2 = N_3
9 R^1 = N_3, R^2 = BzlO
10 R^1 = BzlO, R^2 = N_3



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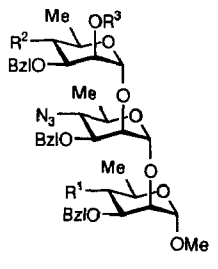
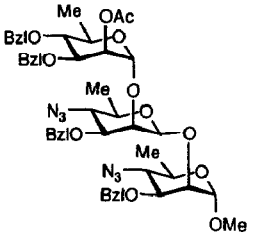


12 R^1 = BzlO, R^2 = N_3, R^3 = Ac
13 R^1 = BzlO, R^2 = N_3, R^3 = H
14 R^1 = N_3, R^2 = BzlO, R^3 = Ac
15 R^1 = N_3, R^2 = BzlO, R^3 = H



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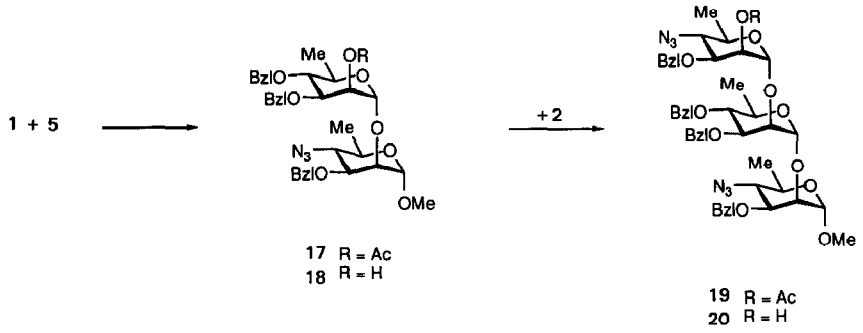
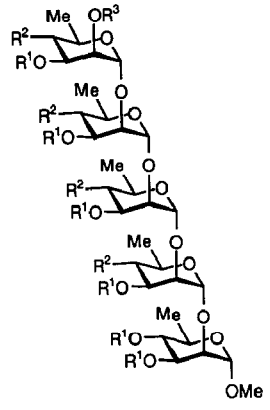


17 R = Ac
18 R = H

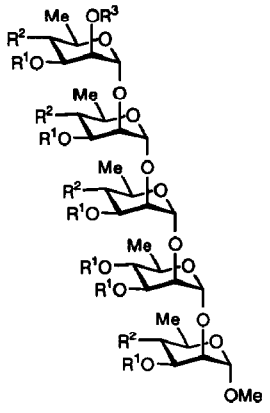
19 R = Ac
20 R = H



8 + 13 → 21 R^1 = Bzl, R^2 = N_3, R^3 = Ac
22 R^1 = Bzl, R^2 = N_3, R^3 = H
23 R^1 = Bzl, R^2 = NH_2, R^3 = H
24 R^1 = Bzl, R^2 = NHCHO, R^3 = H
25 R^1 = R^3 = H, R^2 = NHCHO

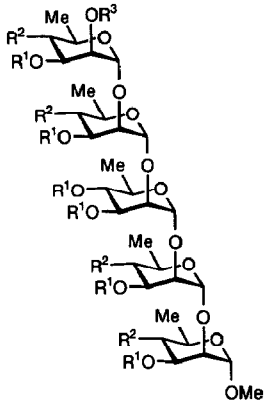
glycosyl donors in a blockwise synthetic approach to the target pentasaccharides **25**, **30**, and **35**. Disaccharide **8** is composed of two 4-azido-4,6-dideoxy-D-mannose residues and was used in the recent synthesis[9] of tri- to penta-saccharide fragments of the *Brucella* A antigen. Disaccharide **9** has a D-rhamnose unit linked to O-2 of a "reducing" 4-azido-4,6-dideoxy-D-mannose residue, whereas **10** has these two residues in the reverse order.

Disaccharide **8** was prepared as previously reported by conversion of ethyl 2-*O*-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-1-thio-α-D-mannopyranoside (**2**) into the glycosyl bromide and glycosylation of the alcohol **3** (75% yield when promoted by silver triflate)[9]. Treatment of the D-rhamnose thioglycoside **5** with bromine, and glycosylation of the 4-azido-4,6-dideoxy-D-mannoside **3** (ref. 9) using mercuric cyanide and mercuric bromide as promoters gave the α- and β-linked disaccharides **9** and **11** in 62 and 20% yields, respectively. When silver triflate was used as a promoter, in combination with 2,4,6-trimethylpyridine, **9** was formed in a lower yield and was contaminated by several



8 + 20 ⟶

26 R^1 = Bzl, R^2 = N_3, R^3 = Ac
27 R^1 = Bzl, R^2 = N_3, R^3 = H
28 R^1 = Bzl, R^2 = NH_2, R^3 = H
29 R^1 = Bzl, R^2 = NHCHO, R^3 = H
30 R^1 = R^3 = H, R^2 = NHCHO



8 + 15 ⟶

31 R^1 = Bzl, R^2 = N_3, R^3 = Ac
32 R^1 = Bzl, R^2 = N_3, R^3 = H
33 R^1 = Bzl, R^2 = NH_2, R^3 = H
34 R^1 = Bzl, R^2 = NHCHO, R^3 = H
35 R^1 = R^3 = H, R^2 = NHCHO

by-products. Surprisingly, attempts to prepare the isomeric disaccharide **10**, by glycosylation of the rhamnoside **6** with the glycosyl bromide prepared from the 4-azido-4,6-dideoxy-D-mannoside **2** (ref. 9), resulted in too low a yield (~17%) for further synthetic purposes, when mercuric cyanide and mercuric bromide or silver triflate were used as promoters. The thioglycoside **2** was one of several by-products formed in this glycosylation, presumably by attack of the glycosyl bromide generated from **2** on the ethylthio group of the glycosyl acceptor **6** (both n.m.r. spectroscopy and t.l.c. analysis showed complete conversion of **2** into the glycosyl bromide on treatment with bromine). The

difference in outcome of the glycosylation giving **8**, **9**, and **10** was not expected, considering the close structural similarity of these disaccharides. We also note the previously reported[9,13] empirical observation that less than one molar equivalent of bromine is required to quantitatively convert thioglycosides, such as **2** and **5**, into their corresponding glycosyl bromides.

The stereochemistry of the newly formed *O*-glycosidic linkage in the disaccharides **9**, **10**, and **11** was determined from the $^1J_{C\text{-}1,H\text{-}1}$ coupling constant, which has been found[14] to be *ca.* 170 Hz for α-glycosides and *ca.* 160 Hz for β-glycosides. The stereochemistry of the products from the glycosylations described below were determined in the same manner.

We recently reported[13] an improved method for glycosylations by activation *in situ* of thioglycosides with bromine in the presence of a glycosyl acceptor and silver triflate as promoter. Application of this method to the glycosylation of methyl 3,4-di-*O*-benzyl-α-D-rhamnopyranoside with the disaccharide donor **8** (ref. 9) was found[13] to give the trisaccharide **12** (68%) and its corresponding β-glycoside (14%). Activation *in situ* of thioglycosides with bromine avoids the use of toxic promoters such as methyl triflate in glycosylations, and provides 1,2-*trans*- or 1,2-*cis*-*O*-glycosides in excellent yields and with high stereochemical control[13]. Thus, glycosylation of methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside[7,8] (**1**) by activation of the disaccharide thioglycoside **9** with bromine and promotion by silver triflate gave the α- and β-linked trisaccharides **14** and **16** in 76 and 7.2% yields, respectively.

Due to the low yield obtained in the synthesis of the disaccharide donor **10**, the trisaccharide **19** was instead prepared from **1** (refs. 7 and 8) by a stepwise approach. Glycosylation of the alcohol **1** by activation *in situ* of the D-rhamnose thioglycoside **5** with bromine was found to give **17** in a significantly higher yield when performed at −45° instead of at room temperature (93 and 59% yields, respectively). Deacetylation of **17** (→**18**, 93%) and glycosylation with **2** (ref. 9) gave the trisaccharide **19** (87%, at −45°).

Deacetylation of the trisaccharides **12** (ref. 13), **14**, and **19** then gave the glycosyl acceptors **13**, **15**, and **20** (~90% yields) which were used in the syntheses of pentasaccharides **21**, **26**, and **31**.

Condensation of the disaccharide donor **8** with the trisaccharide alcohol **13** by activation[13] *in situ* with bromine and promotion by silver triflate gave the pentasaccharide **21** (79%), which was subsequently deacetylated to give **22** (90%). Glycosylation of the alcohols **20** and **15** with **8**, in the same manner, gave pentasaccharides **26** and **31**. Compounds **26** and **31** contained small amounts of impurities (5–10%), but gave pure **27** and **32** on deacetylation (79 and 60% from **20** and **15**, respectively). Pentasaccharides **21** and **32** could also be prepared by glycosylation of **13** and **15** with **8** using methylsulfenyl triflate[15] as promoter in 81 and 59% yields, respectively (*i.e.*, in yields almost identical to those obtained above). It is noteworthy that, in contrast to the syntheses of the trisaccharides **12** and **14**, no β-linked products were obtained in the syntheses of pentsaccharides **21**, **27**, and **32**, even though the disaccharide donor **8** has a non-participating glycosyl residue at O-2.

The pentasaccharides **22**, **27**, and **32** were deprotected in three steps. The azido groups were reduced with hydrogen sulfide[9,16], giving the amines **23**, **28**, and **33** which were directly converted into the formamides **24**, **29**, and **34** using refluxing ethyl formate containing a catalytic amount of pyridine (64–74% overall yields). Reduction of the azido groups with hydrogen sulfide was found to give higher yields in triethylamine–pyridine than in pyridine–water[9], whereas attempted reduction with triphenylphosphine (Ph_3P)[17], lithium aluminum hydride[18], or a tin(II) complex[19] gave either low yields (Ph_3P) or were unsuccessful. *N*-Formylation in the higher boiling point ethyl formate, instead of in methyl formate, permitted significantly shorter reaction times. Hydrogenolysis in formic acid of the benzyl ethers in **24**, **29**, and **34**, gave complex mixtures of *O*-formates which were converted to the target pentasaccharides **25**, **30**, and **35** by treatment with methanolic sodium methoxide (81–89% overall yields).

A detailed analysis of the n.m.r. spectra of the *O*-benzylated formamides **24**, **29**, and **34** was not possible due to population of both rotamers for the formamido groups[5,20]. The structures for **24**, **29**, and **34** were, therefore, further confirmed through chemical transformations. That complete reduction of the azido groups in **22**, **27**, and **32** had been achieved with hydrogen sulfide was confirmed by acetylation of the resulting amines **23**, **28**, and **33** to give acetamides which displayed four *N*-acetates each [δ (CH_3CONH): 1.93–1.86], as well as four AcNH*C*-4 resonances ($\delta \sim 52$). Acetylation of the formamides **24**, **29**, and **34** confirmed that these were indeed tetra-*N*-formylated as each derivative displayed only a single *O*-acetyl resonance [δ(CH_3CO_2): $\sim$2.07]. Although complicated, the ^{1}H- and ^{13}C-n.m.r. spectra of the deprotected pentasaccharides **25**, **30**, and **35** could be satisfactorily analysed and were consistent with the assigned structures.

The binding of the pentasaccharides **25**, **30**, and **35** by monoclonal antibody YsT9-1 is presently being investigated in an enzyme-linked immunosorbent assay (ELISA) and by microcalorimetry. These results, and their use for the further refinement of the model[6] of the complex between the *Brucella* A antigen and monoclonal antibody YsT9-1, will be separately reported.

EXPERIMENTAL

General. — ^{1}H- and ^{13}C-n.m.r. spectra were recorded with Bruker AM 200 and AM 500 spectrometers, 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. Resonances for aromatic and benzylic protons and proton resonances that could not be assigned are not reported. 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 pentoxide and was stored over activated molecular sieves (4 Å). Powdered molecular sieves (Aldrich, 4 Å) were used in the glycosylations. Organic solutions were dried over Na_2SO_4.

Ethyl 2-*O*-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-1-thio-α-D-mannopyranoside[9] (**2**), ethyl 4-azido-3-*O*-benzyl-4,6-dideoxy-1-thio-α-D-mannopyranoside[9] (**3**), 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[9] (**8**), and methyl *O*-(2-*O*-acetyl-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)-3,4-di-*O*-benzyl-α-D-rhamnopyranoside[13] (**12**) were prepared according to the indicated literature methods. 1,2-Di-*O*-acetyl-3,4-di-*O*-benzyl-α-D-rhamnopyranose (**4**) was prepared from methyl 2,3-*O*-isopropylidene-α-D-rhamnopyranoside[7] as described[21] for the preparation of the L-enantiomer of **4**. Compound **4** had $[\alpha]_D^{25}$ +22° (*c* 0.73, chloroform) [lit.[22] $[\alpha]_D^{25}$ −20° (chloroform) for the L-enantiomer of **4**] and ^{1}H- and ^{13}C-n.m.r. data as reported[22] for the L-enantiomer of **4**.

Satisfactory elemental analyses could not be obtained for the amorphous compounds **25**, **30**, and **35**, but their purity was established by t.l.c. and by n.m.r. spectroscopy.

*Methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside*[7,8] (**1**). — Dibutyltin oxide (6.37 g, 25.6 mmol) was added to a solution of methyl 4-azido-4,6-dideoxy-α-D-mannopyranoside[7,8] (5.20 g, 25.6 mmol) in anhydrous methanol (300 mL), and the mixture was boiled under reflux for 2 h. The resulting clear solution was concentrated, and the residue was dried under high vacuum for 2 h. Tetrabutylammonium chloride (7.11 g, 25.6 mmol) and benzyl bromide (3.44 mL, 28.9 mmol) were added to a solution of the residue in dry toluene (125 mL), and the solution was boiled under reflux for 1 h, then concentrated. Column chromatography (1:5 ethyl acetate–hexanes, followed by 1:3) of the residue gave **1** (6.68 g, 89%) with optical rotation and ^{1}H-n.m.r. spectral data as previously reported[7].

*Ethyl 2-*O*-acetyl-3,4-di-*O*-benzyl-1-thio-α-* (**5**) and *β-D-rhamnopyranoside* (**7**). — Ethanethiol (2.46 mL, 33.2 mmol) and boron trifluoride etherate (2.46 mL, 20.0 mmol) were added at room temperature to a stirred solution of **4** (5.68 g, 13.3 mmol) in dry dichloromethane (90 mL) containing molecular sieves (4 Å, 4.0 g). After 8 h, triethylamine (2.97 mL, 21.3 mmol) was added dropwise, and the mixture was filtered and concentrated. Column chromatography (1:8 ethyl acetate–hexanes) of the residue gave **5** (3.69 g, 64%) and **7** (1.48 g, 26%).

Compound **5** had $[\alpha]_D^{25}$ +61° (*c* 2.1, chloroform). N.m.r. data ($CDCl_3$): ^{1}H (200.13 MHz), δ 5.43 (dd, 1 H, *J* 3.3 and 1.6 Hz, H-2), 5.18 (d, 1 H, *J* 1.4 Hz, H-1), 4.08 (dq, 1 H, *J* 9.4 and 6.2 Hz, H-5), 3.86 (dd, 1 H, *J* 9.3 and 3.2 Hz, H-3), 3.45 (t, 1 H, *J* 9.4 Hz, H-4), 2.15 (s, 3 H, Ac), 1.32 (d, 3 H, *J* 6.4 Hz, H-6), and 1.26 (t, 3 H, *J* 7.6 Hz, SCH_2CH_3); ^{13}C (50.32 MHz), δ 82.3 ($^1J_{C,H}$ 166 Hz, C-1).

Anal. Calc. for $C_{24}H_{30}O_5S$: C, 66.9; H, 7.02. Found: C, 66.9; H, 7.07.

Compound **7** had $[\alpha]_D^{25}$ −80° (*c* 1.9, chloroform). N.m.r. data ($CDCl_3$): ^{1}H (200.13 MHz), δ 5.63 (dd, 1 H, *J* 3.5 and 0.9 Hz, H-2), 4.63 (bs, 1 H, H-1), 3.63 (dd, 1 H, *J* 8.9 and 3.4 Hz, H-3), 2.19 (s, 3 H, Ac), 1.36 (bd, 3 H, *J* 5.6 Hz, H-6), and 1.28 (t, 1 H, *J* 7.6 Hz, SCH_2CH_3); ^{13}C (50.32 MHz), δ 81.8 ($^1J_{C,H}$ 151 Hz, C-1).

Anal. Calc. for $C_{24}H_{30}O_5S$: C, 66.9; H, 7.02. Found: C, 67.0; H, 7.37.

Ethyl 3,4-di-O-benzyl-1-thio-α-D-rhamnopyranoside (**6**). — A solution of **5** (779 mg, 1.81 mmol) in dichloromethane–methanolic 25mM sodium methoxide (1:1, 15 mL) was stirred for 16 h at room temperature, then neutralized [Amberlite IR-120 (H^+) resin], and concentrated. Column chromatography (1:3 ethyl acetate–hexanes) of the residue gave **6** (680 mg, 97%), $[\alpha]_D^{25}$ +127° (*c* 0.56, chloroform). ^{1}H-N.m.r. data (200.13 MHz, $CDCl_3$): δ 5.28 (bs, 1 H, H-1), 3.79 (dd, 1 H, *J* 9.0 and 3.3 Hz, H-3), 3.47 (t, 1 H, *J* 9.2 Hz, H-4), 1.30 (d, 3 H, *J* 6.3 Hz, H-6), and 1.26 (t, 3 H, *J* 7.4 Hz, SCH_2CH_3).

Anal. Calc. for $C_{22}H_{28}O_4S$: C, 68.0; H, 7.26. Found: C, 67.8; H, 7.37.

Ethyl 2-O-(2-O-acetyl-3,4-di-O-benzyl-α- (**9**) *and β-D-rhamnopyranosyl)-4-azido-3-O-benzyl-4,6-dideoxy-1-thio-α-D-mannopyranoside* (**11**). — Bromine (9.8 μL, 190 μmol) was added to a stirred solution of **5** (125 mg, 290 μmol) in dry dichloromethane (2.5 mL) at 0°, and after 30 min cyclohexene (29 μL, 290 μmol) was added. The solution was then added to a mixture of **3** (ref. 9) (75 mg, 230 μmol), mercuric cyanide (117 mg, 464 μmol), mercuric bromide (84 mg, 230 μmol), and powdered molecular sieves (4 Å, 200 mg) in dry dichloromethane (2 mL) that had been stirred for 4 h at 0°. After 16 h the mixture was filtered through Celite, and the flask and Celite were washed with dichloromethane (20 mL). The solution was washed with satd. aq. sodium hydrogencarbonate (7.5 mL), water (7.5 mL), and brine (7.5 mL), then dried and concentrated. Column chromatography (1:10 ethyl acetate–hexanes, followed by 1:7) of the residue gave **9** (100 mg, 62%) and **11** (32 mg, 20%).

Compound **9** had $[\alpha]_D^{25}$ +80° (*c* 1.6, chloroform). N.m.r. data ($CDCl_3$): ^{1}H (500.14 MHz), δ 5.44 (dd, 1 H, *J* 3.0 and 2.0 Hz, H-2b), 5.19 (d, 1 H, *J* 1.0 Hz, H-1a), 4.83 (d, 1 H, *J* 1.3 Hz, H-1b), 3.96 (bs, 1 H, H-2a), 3.94 (dd, 1 H, *J* 8.8 and 3.2 Hz, H-3b), 3.84 (dq, 1 H, *J* 9.6 and 6.2 Hz, H-5b), 3.81 (dq, 1 H, *J* 10.0 and 6.2 Hz, H-5a), 3.65 (dd, 1 H, *J* 9.8 and 2.9 Hz, H-3a), 3.43 (t, 1 H, *J* 9.4 Hz, H-4b), 3.38 (t, 1 H, *J* 9.9 Hz, H-4a), 2.11 (s, 3 H, Ac), 1.31 (d, 3 H, *J* 6.2 Hz, H-6b), 1.28 (d, 3 H, *J* 6.2 Hz, H-6a), and 1.25 (t, 3 H, *J* 7.4 Hz, SCH_2CH_3); ^{13}C (125.76 MHz), δ 99.6 ($^1J_{C,H}$ 170 Hz, C-1b) and 83.3 (C-1a).

Anal. Calc. for $C_{37}H_{45}N_3O_8S$: C, 64.2; H, 6.56; N, 6.07. Found: C, 64.3; H, 6.60; N, 6.01.

Compound **11** was characterised by n.m.r. spectroscopy ($CDCl_3$): ^{1}H (500.14 MHz), δ 5.62 (dd, 1 H, *J* 3.2 and 0.6 Hz, H-2b), 5.27 (d, 1 H, *J* 1.3 Hz, H-1a), 4.65 (d, 1 H, *J* 0.8 Hz, H-1b), 4.34 (dd, 1 H, *J* 3.1 and 1.6 Hz, H-2a), 3.80 (dq, 1 H, *J* 10.0 and 6.2 Hz, H-5a), 3.66 (dd, 1 H, *J* 9.8 and 3.1 Hz, H-3a), 3.62 (dd, 1 H, *J* 9.2 and 3.3 Hz, H-3b), 3.47 (t, 1 H, *J* 9.2 Hz, H-4b), 3.39 (t, 1 H, *J* 9.9 Hz, H-4a), 3.35 (dq, 1 H, *J* 9.3 and 6.1 Hz, H-5b), 2.01 (s, 3 H, Ac), 1.34 (d, 3 H, *J* 6.1 Hz, H-6b), 1.30 (d, 3 H, *J* 6.2 Hz, H-6a), and 1.27 (t, 3 H, *J* 7.4 Hz, SCH_2CH_3); ^{13}C (125.76 MHz), δ 95.3 ($^1J_{C,H}$ 156 Hz, C-1b) and 81.1 (C-1a).

Ethyl 2-O-(2-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-3,4-di-O-benzyl-1-thio-α-D-rhamnopyranoside (**10**). — Treatment of **2** (ref. 9) (59 mg, 160 μmol) with bromine, and glycosylation of **6** (50 mg, 130 μmol), as described for **9**, gave after column chromatography (1:10 ethyl acetate–hexanes), **10** (15 mg) which was indicated to be ~90% pure by ^{1}H-n.m.r. spectroscopy. N.m.r. data ($CDCl_3$): ^{1}H (500.14

MHz), δ 5.45 (dd, 1 H, *J* 3.2 and 1.9 Hz, H-2b), 5.13 (d, 1 H, *J* 1.5 Hz, H-1a), 4.92 (d, 1 H, *J* 1.7 Hz, H-1b), 3.99 (dq, 1 H, *J* 9.4 and 6.2 Hz, H-5a), 3.97 (dd, 1 H, *J* 2.7 and 1.9 Hz, H-2a), 3.82 (dd, 1 H, *J* 9.9 and 3.3 Hz, H-3b), 3.77 (dd, 1 H, *J* 9.2 and 2.9 Hz, H-3a), 3.65 (dq, 1 H, *J* 10.1 and 6.2 Hz, H-5b), 3.39 (t, 1 H, *J* 9.3 Hz, H-4a), 3.38 (t, 1 H, *J* 10.0 Hz, H-4b), 2.09 (s, 3 H, Ac), 1.29 (d, 3 H, *J* 6.2 Hz, H-6b), 1.27 (d, 3 H, *J* 6.2 Hz, H-6a), and 1.24 (t, 3 H, *J* 7.4 Hz, SCH_2CH_3); ^{13}C (125.76 MHz), δ 99.4 ($^1J_{C,H}$ 172 Hz, C-1b) and 83.4 (C-1a).

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)-3,4-di-*O*-benzyl-*α*-*D*-rhamnopyranoside* (**13**). — Deacetylation of **12** (ref. 13) (123 mg, 133 μmol) as described for **6**, and column chromatography (1:5 ethyl acetate–hexanes) of the residue gave **13** (102 mg, 87%): $[\alpha]_D^{25}$ +77° (*c* 2.2, chloroform). ^{1}H-N.m.r. data (500.14 MHz, $CDCl_3$): δ 5.05 (d, 1 H, *J* 1.7 Hz, H-1b), 4.93 (d, 1 H, *J* 1.1 Hz, H-1c), 4.52 (d, 1 H, *J* 1.6 Hz, H-1a), 4.01 (t, 1 H, *J* 2.4 Hz, H-2b), 3.99 (bs, 1 H, H-2c), 3.90 (t, 1 H, *J* 2.2 Hz, H-2a), 3.81 (dd, 1 H, *J* 9.4 and 2.9 Hz, H-3a), 3.76 (dd, 1 H, *J* 10.1 and 2.8 Hz, H-3b), 3.71 (dd, 1 H, *J* 9.7 and 3.2 Hz, H-3c), 3.62 (dq, 1 H, *J* 9.4 and 6.2 Hz, H-5a), 3.40 (t, 1 H, *J* 9.9 Hz, H-4c), 3.32 (t, 1 H, *J* 10.0 Hz, H-4b), 3.31 (t, 1 H, *J* 9.5 Hz, H-4a), 3.28 (s, 3 H, MeO), 1.28 (bd, 6 H, *J* 6.0 Hz, H-6ab), and 1.16 (d, 3 H, *J* 6.1 Hz, H-6c).

Anal. Calc. for $C_{47}H_{56}N_6O_{11}$: C, 64.1; H, 6.41; N, 9.54. Found: C, 64.1; H, 6.48; N, 9.34.

Methyl O-*(2-*O*-acetyl-3,4-di-*O*-benzyl-*α*-*D*-rhamnopyranosyl)-(1→2)-*O*-(4-azido-3-*O*-benzyl-4,6-dideoxy-*α*-* (**14**) *and β-*D*-mannopyranosyl)-(1→2)-4-azido-3-*O*-benzyl-4,6-dideoxy-*α*-*D*-mannopyranoside* (**16**). — A solution of **1** (ref. 7) (246 mg, 0.840 mmol) and **9** (465 mg, 0.672 mmol) in dry dichloromethane (14 mL) containing powdered molecular sieves (4 Å, 1.2 g) was stirred for 4 h at room temperature. Silver triflate (345 mg, 1.34 mmol) was added, followed after 10 min by bromine (26 μL, 0.50 mmol)[13]. After a further 30 min, additional silver triflate (173 mg, 0.672 mmol) was added, followed by triethylamine (280 μL, 2.0 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 (30 mL). The solution was washed with satd. aq. sodium hydrogencarbonate (15 mL), dried, and concentrated. Column chromatography (1:6 ethyl acetate–hexanes, followed by 1:4) of the residue gave **14** (472 mg, 76%) and **16** (45 mg, 7.2%).

Compound **14** had $[\alpha]_D^{25}$ +46° (*c* 2.4, chloroform). N.m.r. data ($CDCl_3$): ^{1}H (500.14 MHz), δ 5.43 (dd, 1 H, *J* 3.2 and 1.9 Hz, H-2c), 4.96 (d, 1 H, *J* 1.7 Hz, H-1b), 4.83 (d, 1 H, *J* 1.6 Hz, H-1c), 4.53 (d, 1 H, *J* 2.0 Hz, H-1a), 3.91 (t, 1 H, *J* 2.4 Hz, H-2b), 3.89 (dd, 1 H, *J* 9.4 and 3.3 Hz, H-3c), 3.81 (t, 1 H, *J* 2.4 Hz, H-2a), 3.72 (dq, 1 H, *J* 9.6 and 6.3 Hz, H-5c), 3.70 (dd, 1 H, *J* 9.9 and 3.0 Hz, H-3b), 3.65 (dd, 1 H, *J* 9.9 and 2.9 Hz, H-3a), 3.50 (dq, 1 H, *J* 10.0 and 6.1 Hz, H-5b), 3.39 (dq, 1 H, *J* 10.1 and 6.1 Hz, H-5a), 3.38 (t, 1 H, *J* 9.5 Hz, H-4c), 3.37 (t, 1 H, *J* 10.0 Hz, H-4b), 3.27 (s, 3 H, MeO), 3.22 (t, 1 H, *J* 10.0 Hz, H-4a), 2.11 (s, 3 H, Ac), 1.27 (d, 3 H, *J* 6.2 Hz, H-6a), 1.25 (d, 3 H, *J* 7.1 Hz, H-6b), and 1.20 (d, 3 H, *J* 6.2 Hz, H-6c); ^{13}C (125.76 MHz), δ 100.5, 99.8, and 99.2 ($^1J_{C,H}$ 171, 169, and 171 Hz, C-1abc).

Anal. Calc. for $C_{49}H_{58}N_6O_{12}$: C, 63.8; H, 6.33; N, 9.10. Found: C, 63.4; H, 6.28; N, 8.78.

Compound **16** had $[\alpha]_D^{25}$ +31° (*c* 2.5, chloroform). N.m.r. data ($CDCl_3$): ^1H (500.14 MHz), δ 5.51 (dd, 1 H, *J* 3.3 and 1.5 Hz, H-2c), 5.37 (d, 1 H, *J* 1.2 Hz, H-1c), 4.64 (d, 1 H, *J* 1.3 Hz, H-1a), 4.47 (bs, 1 H, H-1b), 4.31 (m, 2 H, H-2ab), 4.16 (dq, 1 H, *J* 9.6 and 6.2 Hz, H-5c), 4.02 (dd, 1 H, *J* 9.5 and 3.5 Hz, H-3c), 3.72 (dd, AB-type, 1 H, *J* 9.9 and 3.5 Hz, H-3a), 3.67 (t, AB-type, 1 H, *J* 9.8 Hz, H-4a), 3.60 (t, 1 H, *J* 9.8 Hz, H-4b), 3.40 (dd, 1 H, *J* 9.8 and 2.6 Hz, H-3b), 3.38 (t, 1 H, *J* 9.6 Hz, H-4c), 3.31 (s, 3 H, MeO), 3.14 (dq, 1 H, *J* 9.8 and 6.1 Hz, H-5b), 2.07 (s, 3 H, Ac), 1.32 (d, 1 H, *J* 6.2 Hz, H-6c), 1.29 (d, *J* 6.1 Hz, H-6b), and 1.03 (d, 1 H, *J* 6.2 Hz, H-6a); ^{13}C (125.76 MHz), δ 98.0 and 96.7 ($^1J_{C,H}$ 167 and 172 Hz, C-1ac) and 96.6 ($^1J_{C,H}$ 154 Hz, C-1b).

Anal. Calc. for $C_{49}H_{58}N_6O_{12}$: C, 63.8; H, 6.33; N, 9.10. Found: C, 63.7; H, 6.40; N, 8.80.

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)-4-azido-3*-O-*benzyl-4,6-dideoxy*-α-D-*mannopyranoside* (**15**). — Deacetylation of **14** (446 mg, 0.483 mmol) as described for **6** and column chromatography (2:7 ethyl acetate–hexanes) of the residue gave **15** (392 mg, 92%): $[\alpha]_D^{25}$ +56° (*c* 1.4, chloroform). ^1H-N.m.r. data (500.14 MHz, $CDCl_3$): δ 4.96 (d, 1 H, *J* 1.6 Hz, H-1b), 4.95 (d, 1 H, *J* 1.3 Hz, H-1c), 4.53 (d, 1 H, *J* 1.7 Hz, H-1a), 4.03 (bs, 1 H, H-2c), 3.96 (t, 1 H, *J* 2.3 Hz, H-2b), 3.81 (dd, 1 H, *J* 9.2 and 3.1 Hz, H-3c), 3.81 (t, 1 H, *J* 2.4 Hz, H-2a), 3.71 (dq, 1 H, *J* 9.5 and 6.3 Hz, H-5c), 3.70 (dd, 1 H, *J* 9.9 and 2.8 Hz, H-3b), 3.65 (dd, 1 H, *J* 9.9 and 2.9 Hz, H-3a), 3.50 (dq, 1 H, *J* 10.0 and 6.1 Hz, H-5b), 3.43 (t, 1 H, *J* 9.4 Hz, H-4c), 3.39 (dq, 1 H, *J* 10.0 and 6.1 Hz, H-5a), 3.32 (t, 1 H, *J* 10.0 Hz, H-4b), 3.27 (s, 3 H, MeO), 3.22 (t, 1 H, *J* 10.0 Hz, H-4a), 1.27 (d, 3 H, *J* 6.2 Hz, H-6a), 1.25 (d, 3 H, *J* 6.3 Hz, H-6b), and 1.19 (d, 3 H, *J* 6.2 Hz, H-6c).

Anal. Calc. for $C_{47}H_{56}N_6O_{11}$: C, 64.1; H, 6.41; N, 9.54. Found: C, 63.8; H, 6.23; N, 9.39.

Methyl 2-O-*(2*-O-*acetyl-3,4-di*-O-*benzyl*-α-D-*rhamnopyranosyl)-4-azido-3*-O-*benzyl-4,6-dideoxy*-α-D-*mannopyranoside* (**17**). — Glycosylation[13] of **1** (ref. 7) (90 mg, 0.307 mmol) with **5** (165 mg, 0.384 mmol) at −45° as described for **14** and column chromatography (1:8 ethyl acetate–hexanes) of the residue gave **17** (189 mg, 93%): $[\alpha]_D^{25}$ +12° (*c* 1.1, chloroform). N.m.r. data ($CDCl_3$): ^1H (500.14 MHz), δ 5.45 (dd, 1 H, *J* 3.2 and 1.9 Hz, H-2b), 4.88 (d, 1 H, *J* 1.7 Hz, H-1b), 4.60 (bs, 1 H, H-1a), 3.92 (dd, 1 H, *J* 9.4 and 3.4 Hz, H-3b), 3.90 (dd, 1 H, *J* 2.9 and 2.0 Hz, H-2a), 3.79 (dq, 1 H, *J* 9.5 and 6.2 Hz, H-5b), 3.71 (dd, 1 H, *J* 9.8 and 3.0 Hz, H-3a), 3.44 (dq, 1 H, *J* 10.0 and 6.1 Hz, H-5a), 3.41 (t, 1 H, *J* 9.4 Hz, H-4b), 3.36 (t, 1 H, *J* 9.9 Hz, H-4a), 3.29 (s, 3 H, MeO), 2.10 (s, 3 H, Ac), 1.31 (d, 3 H, *J* 6.2 Hz, H-6b), and 1.29 (d, 3 H, *J* 6.1 Hz, H-6a); ^{13}C (125.76 MHz), δ 99.8 and 99.3 ($^1J_{C,H}$ 171 and 172 Hz, C-1ab).

Anal. Calc. for $C_{36}H_{43}N_3O_9$: C, 65.3; H, 6.55; N, 6.35. Found: C, 65.0; H, 6.63; N, 6.12.

Methyl 2-O-*(3,4-di*-O-*benzyl*-α-D-*rhamnopyranosyl)-4-azido-3*-O-*benzyl-4,6-dideoxy*-α-D-*mannopyranoside* (**18**). — Deacetylation of **17** (88 mg, 133 μmol) as described for **6** and column chromatography (1:3 ethyl acetate–hexanes) of the residue gave **18** (77

mg, 93%): $[\alpha]_D^{25}$ +41° (*c* 1.2, chloroform). ^{1}H-N.m.r. data (500.14 MHz, $CDCl_3$): δ 4.96 (d, 1 H, *J* 1.5 Hz, H-1b), 4.61 (d, 1 H, *J* 1.7 Hz, H-1a), 4.04 (bs, 1 H, H-2b), 3.94 (t, 1 H, *J* 2.4 Hz, H-2a), 3.83 (dd, 1 H, *J* 9.2 and 3.2 Hz, H-3b), 3.78 (dq, 1 H, *J* 9.5 and 6.3 Hz, H-5b), 3.71 (dd, 1 H, *J* 9.9 and 3.0 Hz, H-3a), 3.46 (t, 1 H, *J* 9.4 Hz, H-4b), 3.43 (dq, 1 H, *J* 10.1 and 6.2 Hz, H-5a), 3.32 (t, 1 H, *J* 10.0 Hz, H-4a), 3.29 (s, 3 H, MeO), 1.30 (d, 3 H, *J* 6.2 Hz, H-6b), and 1.29 (d, 3 H, *J* 6.2 Hz, H-6a).

Anal. Calc. for $C_{34}H_{41}N_3O_8$: C, 65.9; H, 6.67; N, 6.78. Found: C, 66.0; H, 6.74; N, 6.83.

Methyl O-*(2-*O*-acetyl-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* (**19**). — Glycosylation[13] of **18** (228 mg, 0.368 mmol) with **2** (ref. 9) (168 mg, 0.460 mmol) at −45° as described for **14** and column chromatography (1:8 ethyl acetate–hexanes) of the residue gave **19** (297 mg, 87%): $[\alpha]_D^{25}$ +74° (*c* 1.6, chloroform). N.m.r. data ($CDCl_3$): ^{1}H (500.14 MHz), δ 5.48 (dd, 1 H, *J* 3.1 and 1.9 Hz, H-2c), 4.97 (d, 1 H, *J* 1.8 Hz, H-1b), 4.95 (d, 1 H, *J* 1.5 Hz, H-1c), 4.57 (d, 1 H, *J* 1.5 Hz, H-1a), 3.98 (t, 1 H, *J* 2.4 Hz, H-2b), 3.91 (t, 1 H, *J* 2.3 Hz, H-2a), 3.85 (dd, 1 H, *J* 9.1 and 2.9 Hz, H-3b), 3.80 (dd, 1 H, *J* 9.9 and 3.3 Hz, H-3c), 3.73 (dq, 1 H, *J* 9.3 and 6.2 Hz, H-5b), 3.68 (dd, 1 H, *J* 9.9 and 2.9 Hz, H-3a), 3.53 (dq, 1 H, *J* 10.1 and 6.1 Hz, H-5c), 3.42 (dq, 1 H, *J* 10.1 and 6.4 Hz, H-5a), 3.42 (t, 1 H, *J* 9.1 Hz, H-4b), 3.36 (t, 1 H, *J* 10.0 Hz, H-4c), 3.29 (s, 3 H, MeO), 3.28 (t, 1 H, *J* 9.9 Hz, H-4a), 2.10 (s, 3 H, Ac), 1.29 (d, 6 H, *J* 6.2 Hz, H-6ab), and 1.18 (d, 3 H, *J* 6.2 Hz, H-6c); ^{13}C (125.76 MHz), δ 100.2, 99.9, and 98.7 ($^1J_{C,H}$ 173, 170, and 172 Hz, C-1abc).

Anal. Calc. for $C_{49}H_{58}N_6O_{12}$: C, 63.8; H, 6.33; N, 9.10. Found: C, 63.8; H, 6.48; N, 9.25.

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* (**20**). — Deacetylation of **19** (276 mg, 0.299 mmol) as described for **6** and column chromatography (1:6 ethyl acetate–hexanes) of the residue gave **20** (249 mg, 94%): $[\alpha]_D^{25}$ +80° (*c* 1.1, chloroform). ^{1}H-N.m.r. data (500.14 MHz, $CDCl_3$): δ 5.05 (d, 1 H, *J* 1.3 Hz, H-1c), 4.96 (d, 1 H, *J* 1.9 Hz, H-1b), 4.55 (d, 1 H, *J* 1.7 Hz, H-1a), 4.03 (bs, 1 H, H-2c), 3.99 (t, 1 H, *J* 2.4 Hz, H-2b), 3.88 (t, 1 H, *J* 2.3 Hz, H-2a), 3.83 (dd, 1 H, *J* 9.1 and 2.9 Hz, H-3b), 3.72 (dd, 1 H, *J* 9.6 and 3.2 Hz, H-3c), 3.71 (dq, 1 H, *J* 9.3 and 6.1 Hz, H-5b), 3.66 (dd, 1 H, *J* 9.9 and 2.9 Hz, H-3a), 3.52 (dq, 1 H, *J* 10.1 and 6.2 Hz, H-5c), 3.39 (t, 1 H, *J* 10.0 Hz, H-4c), 3.36 (t, 1 H, *J* 9.2 Hz, H-4b), 3.27 (s, 3 H, MeO), 3.25 (t, 1 H, *J* 10.0 Hz, H-4a), 1.28 (d, 3 H, *J* 6.2 Hz, H-6b), 1.26 (d, 3 H, *J* 6.2 Hz, H-6a), and 1.16 (d, 3 H, *J* 6.2 Hz, H-6c).

Anal. Calc. for $C_{47}H_{56}N_6O_{11}$: C, 64.1; H, 6.41; N, 9.54. Found: C, 63.7; H, 6.50; N, 9.55.

Methyl O-*(2-*O*-acetyl-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*-mannopyranosyl)-(1→2)-3,4-di-*O*-benzyl-*α*-*D*-rhamnopyranoside* (**21**). — Glycosylation[13] of **13** (75 mg, 85 μmol) with **8** (ref. 9) (67 mg, 110 μmol) as described for **14** and column chromatography (1:6 ethyl acetate–hexanes) of the residue gave **21** (97

mg, 79%): $[\alpha]_D^{25}$ +76° (*c* 1.8, chloroform). N.m.r. data ($CDCl_3$): ^1H (500.14 MHz), δ 5.42 (dd, 1 H, *J* 3.0 and 1.9 Hz, H-2e), 4.98, 4.96, and 4.90 (3 d, each 1 H, *J* 1.6, 1.7, and 1.3 Hz, H-1bcd), 4.52 (d, 1 H, *J* 1.4 Hz, H-1a), 3.81 (dd, 1 H, *J* 9.4 and 2.9 Hz, H-3a), 3.78 (dd, 1 H, *J* 10.0 and 3.3 Hz, H-3e), 3.62 (dq, 1 H, *J* 9.4 and 6.1 Hz, H-5a), 3.38 (t, 1 H, *J* 10.0 Hz, H-4e), 3.29 (s, 3 H, MeO), 3.28 (t, 1 H, *J* 9.4 Hz, H-4a), 2.11 (s, 3 H, Ac), 1.29 (d, 3 H, *J* 6.1 Hz, H-6a), and 1.21 (d, 3 H, *J* 6.3 Hz, H-6e); ^{13}C (125.76 MHz), δ 100.2, 100.1, 100.0, 99.7, and 99.1 ($^1J_{C,H}$ 172, 172, 172, 170, and 174 Hz, C-1abcde).

Anal. Calc. for $C_{75}H_{88}N_{12}O_{18}$: C, 62.3; H, 6.14; N, 11.6. Found: C, 62.4; H, 6.11; N, 11.5.

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*-mannopyranosyl)-(1→2)-3,4-di-*O*-benzyl-*α*-*D*-rhamnopyranoside* (**22**). — Deacetylation of **21** (97 mg, 67 μmol) as described for **6** and column chromatography (1:4 ethyl acetate–hexanes) of the residue gave **22** (85 mg, 90%): $[\alpha]_D^{25}$ +88° (*c* 1.4, chloroform). ^1H-N.m.r. data (500.14 MHz, $CDCl_3$): δ 4.97 and 4.91 (bs, 3 H and d, 1 H, *J* 1.2 Hz, H-1bcde), 4.52 (d, 1 H, *J* 1.2 Hz, H-1a), 3.99 (bs, 1 H, H-2e), 3.88 (t, 1 H, *J* 2.2 Hz, H-2a), 3.81 (dd, 1 H, *J* 9.4 and 2.8 Hz, H-3a), 3.62 (dq, 1 H, *J* 9.4 and 6.2 Hz, H-5a), 3.42 (t, 1 H, *J* 9.8 Hz, H-4e), 3.29 (s, 3 H, MeO), 3.28 (t, 1 H, *J* 9.9 Hz, H-4a), 1.29 (d, 3 H, *J* 6.4 Hz, H-6a), and 1.20 (d, 3 H, *J* 6.1 Hz, H-6e).

Anal. Calc. for $C_{73}H_{86}N_{12}O_{17}$: C, 62.8; H, 6.18; N, 12.0. Found: C, 63.1; H, 6.26; N, 12.4.

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)-3,4-di-*O*-benzyl-*α*-*D*-rhamnopyranoside* (**24**). — A solution of **22** (100 mg, 71.2 μmol) in pyridine–triethylamine (1:1, 16 mL) was saturated with hydrogen sulfide[16] for 1 h at room temperature and then stirred for 16 h. Nitrogen was passed through the solution for 1 h to remove hydrogen sulfide, the solution was concentrated, and the residual solvents were codistilled twice with toluene. Column chromatography (methanol–dichloromethane, 1:30 → 1:10 containing 0.1% of triethylamine) of the residue gave the amine **23** which was taken up in ethyl formate–pyridine (50:1, 15 mL). The mixture was boiled under reflux for 24 h, the resulting clear solution was concentrated, and the residual solvents were codistilled with toluene. Column chromatography (1:40 methanol–dichloromethane, followed by 1:30) of the residue gave **24** (65 mg, 65%): $[\alpha]_D^{25}$ +39° (*c* 0.97, chloroform). N.m.r. data ($CDCl_3$): ^1H (500.14 MHz), δ 8.21–7.94 (m, 4 H, NHC*H*O), 3.34 (t, 1 H, *J* 9.5 Hz, H-4a), 3.29 (bs, 3 H, MeO), and 1.30–1.00 (m, 15 H, H-6abcde); ^{13}C (125.76 MHz), δ 164.9 and 161.5 (2 m, NH*C*HO), 100.2–99.6 (m, C-1abcde), 50.8 (m, C-4bcde-*Z*).

Anal. Calc. for $C_{77}H_{90}N_4O_{21}$: C, 65.5; H, 6.71; N, 3.97. Found: C, 65.3; H, 6.78; N, 3.62.

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)-α*-D-*rhamnopyranoside* (**25**). — A solution of **24** (76 mg, 55 μmol) in formic acid (10 mL) was hydrogenated over Pd–C (10%, 150 mg) at 5 atm. and room temperature for 16 h. The mixture was filtered through Celite and concentrated, and the residual solvents were codistilled twice with methanol. The residue was dissolved in methanolic 25mM sodium methoxide (8 mL) and stirred for 1 h, then neutralized [Amberlite IR-120 (H^+) resin], and concentrated. Column chromatography (water–acetic acid–pyridine, 986:4:10) of the residue on Bio-Gel P-4 gave, after freeze drying, **25** (42 mg, 89%): $[\alpha]_D^{25}$ +39° (*c* 0.69, water). ^{1}H-N.m.r. spectroscopy showed a *Z*:*E*-ratio of ~4.3:1 for the formamido groups. N.m.r. data (D_2O): ^{1}H (500.14 MHz), δ 8.20–8.18 (3 s, 3.2 H, NHC*H*O-*Z*), 8.03–8.01 (3 s, 0.8 H, NHC*H*O-*E*), 5.18, 5.16, 5.14, and 5.03 (4 bs, 4 H, H-1bcde), 4.73 (bs, 1 H, H-1a), 4.03 (dd, 1 H, *J* 10.6 and 2.9 Hz, H-3a), 3.67 (dq, 1 H, *J* 9.6 and 6.3 Hz, H-5a), 3.45 (t, 1 H, *J* 9.7 Hz, H-4a), 3.38 (s, 3 H, MeO), 1.29 (d, 3 H, *J* 6.2 Hz, H-6a), and 1.27–1.18 (m, 12 H, H-6bcde); ^{13}C (125.76 MHz), δ 168.1 (NH*C*HO-*E*), 165.2 (NH*C*HO-*Z*), 102.2 (C-1e), 100.9, 100.8, and 100.8 (C-1bcd), 99.8 (C-1a), 78.5 and 77.4 (C-2abcd), 57.1 and 56.9 (C-4bcde-*E*), 55.1 (MeO), 52.2, 52.1, and 51.9 (C-4bcde-*Z*).

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-*(3,4-di*-O-*benzyl-α*-D-*rhamnopyranosyl)-(1→2)-4-azido-3*-O-*benzyl-4,6-dideoxy-α*-D-*mannopyranoside* (**27**). — Glycosylation[13] of **20** (200 mg, 0.227 mmol) with **8** (ref. 9) (178 mg, 0.284 mmol) as described for **14** and column chromatography (1:6 ethyl acetate–hexanes) of the residue gave **26** (290 mg) which was indicated to be 90–95% pure by ^{1}H-n.m.r. spectroscopy. N.m.r. data ($CDCl_3$): ^{1}H (500.14 MHz), δ 5.40 (dd, 1 H, *J* 3.2 and 1.9 Hz, H-2e), 4.97 and 4.96 (2 d, each 1 H, each *J* 1.7 Hz, H-1cd), 4.89 (d, 1 H, *J* 1.8 Hz, H-1b), 4.84 (d, 1 H, *J* 1.7 Hz, H-1e), 4.54 (d, 1 H, *J* 1.9 Hz, H-1a), 3.89 (t, 1 H, *J* 2.5 Hz, H-2b), 3.86 (t, 1 H, *J* 2.4 Hz, H-2a), 3.81 (dd, 1 H, *J* 9.2 and 2.8 Hz, H-3b), 3.77 (dd, 1 H, *J* 9.9 and 3.2 Hz, H-3e), 3.69 (dq, 1 H, *J* 9.3 and 6.0 Hz, H-5b), 3.65 (dd, 1 H, *J* 9.9 and 2.9 Hz, H-3a), 3.51 (dq, 1 H, *J* 10.4 and 6.1 Hz, H-5e), 3.36 (t, 1 H, *J* 9.9 Hz, H-4e), 3.29 (t, 1 H, *J* 9.4 Hz, H-4b), 3.27 (s, 3 H, MeO), 2.10 (s, 3 H, Ac), and 1.26 (d, 3 H, *J* 6.2 Hz, H-6b); ^{13}C (125.76 MHz), δ 100.3, 100.1, 99.9, 99.9, and 99.0 (each $^1J_{C,H}$ 171 Hz, C-1abcde).

Crude **26** (290 mg) was deacetylated as described for **6** and gave, after column chromatography (2:9 ethyl acetate–hexanes) of the residue, **27** (252 mg, 79% from **20**): $[\alpha]_D^{25}$ +87° (*c* 1.3, chloroform). ^{1}H-N.m.r. data (500.14 MHz, $CDCl_3$): δ 4.98–4.97 (m, 3 H, H-1cde), 4.90 (d, 1 H, *J* 1.8 Hz, H-1b), 4.54 (d, 1 H, *J* 1.8 Hz, H-1a), 3.99 (bs, 1 H, H-2e), 3.89 (t, 1 H, *J* 2.4 Hz, H-2b), 3.87 (t, 1 H, *J* 2.4 Hz, H-2a), 3.81 (dd, 1 H, *J* 9.3 and 2.8 Hz, H-3b), 3.71 (dd, 1 H, *J* 9.7 and 3.2 Hz, H-3e), 3.69 (1 H, H-5b), 3.66 (dd, 1 H, *J* 9.9 and 2.9 Hz, H-3a), 3.53 (dq, 1 H, *J* 10.1 and 6.2 Hz, H-5e), 3.41 (t, 1 H, *J* 9.8 Hz, H-4e), 3.39 (dq, 1 H, *J* 10.0 and 6.2 Hz, H-5a), 3.30 (t, 1 H, *J* 9.2 Hz, H-4b), 3.27 (s, 3 H, MeO), 3.22 (t, 1 H, *J* 10.0 Hz, H-4a), 1.27 (d, 3 H, *J* 6.2 Hz, H-6b), 1.26 (d, 3 H, *J* 6.2 Hz, H-6a), and 1.18 (d, 3 H, *J* 6.2 Hz, H-6e).

Anal. Calc. for $C_{73}H_{86}N_{12}O_{17}$: C, 62.5; H, 6.18; N, 12.0. Found: C, 62.2; H, 6.21; N, 11.8.

Methyl O-*(3-O-benzyl-4,6-dideoxy-4-formamido-α-D-mannopyranosyl)-(1→2)-*O-*(3-O-benzyl-4,6-dideoxy-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)-3-O-benzyl-4,6-dideoxy-4-formamido-α-D-mannopyranoside* (**29**). — Reduction[16] of **27** (98 mg, 70 μmol) and *N*-formylation of the intermediate amine **28** as described for **24** gave, after column chromatography (1:40 methanol–ethyl acetate, followed by 1:30) of the residue, **29** (63 mg, 64%): $[\alpha]_D^{25}$ +46° (*c* 3.1, chloroform). N.m.r. data ($CDCl_3$): ^1H (500.14 MHz), δ 8.19–7.94 (m, 4 H, NHC*H*O), 3.31 (bs, 3 H, MeO), and 1.37–0.96 (m, 15 H, H-6abcde); ^{13}C (125.76 MHz), δ 165.0 and 161.4 (2 m, NH*C*HO), 100.5–99.6 (m, C-1abcde), and 50.8 (m, C-4acde-*Z*).

Anal. Calc. for $C_{77}H_{90}N_4O_{21}$: C, 65.5; H, 6.71; N, 3.97. Found: C, 65.3; H, 6.71; N, 3.86.

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-α-D-*rhamnopyranosyl-(1→2)-4,6-dideoxy-4-formamido-α-D-mannopyranoside* (**30**). — Debenzylation of **29** (60 mg, 43 μmol), and column chromatography of the residue on Bio-Gel P-4 as described for **25** gave, after freeze drying, **30** (31 mg, 84%): $[\alpha]_D^{25}$ +49° (*c* 0.73, water). ^1H-N.m.r. spectroscopy showed a *Z*:*E*-ratio of ~3.9:1 for the formamido groups. N.m.r. data (D_2O): ^1H (500.14 MHz), δ 8.21–8.19 (4 s, 3.2 H, NHC*H*O-*Z*), 8.03 (bs, 0.8 H, NHC*H*O-*E*), 5.20, 5.16, 5.11, and 5.05 (4 bs, each 1 H, H-1bcde), 4.82 (bs, 1 H, H-1a), 3.50 (t, 1 H, *J* 9.7 Hz, H-4b), 3.40 (s, 3 H, MeO), and 1.31–1.19 (m, 15 H, H-6abcde); ^{13}C (125.76 MHz), δ 168.1 (NH*C*HO-*E*), 165.1 (NH*C*HO-*Z*), 102.3 (C-1e), 101.0, 100.9, and 100.8 (C-1bcd), 99.8 (C-1a), 78.3, 77.5, 77.4, and 77.3 (C-2abcd), 57.1, and 57.0 (C-4acde-*E*), 55.2 (MeO), 52.2, 52.2, 52.1, and 51.9 (C-4acde-*Z*).

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-*(3,4-di-O-benzyl-α-D-rhamnopyranosyl)-(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* (**32**). — Glycosylation[13] of **15** (140 mg, 0.159 mmol) with **8** (ref. 9) (125 mg, 0.199 mmol) as described for **14** and column chromatography (1:40 ethyl acetate–toluene) of the residue gave **31** (155 mg) which was indicated to be ~90% pure by ^1H-n.m.r. spectroscopy. N.m.r. data ($CDCl_3$): ^1H (500.14 MHz), δ 5.41 (dd, 1 H, *J* 3.1 and 1.9 Hz, H-2e), 5.05 and 4.89 (2 d, each 1 H, each *J* 1.6 Hz, H-1bd), 4.88 (d, 1 H, *J* 1.7 Hz, H-1c), 4.87 (d, 1 H, *J* 1.6 Hz, H-1e), 4.51 (d, 1 H, *J* 1.7 Hz, H-1a), 3.91 (t, 1 H, *J* 2.3 Hz, H-2c), 3.56 (dq, 1 H, *J* 10.1 and 6.2 Hz, H-5e), 3.39 (dq, 1 H, *J* 10.0 and 6.2 Hz, H-5a), 3.37 (t, 1 H, *J* 10.1 Hz, H-4e), 3.30 (t, 1 H, *J* 9.2 Hz, H-4c), 3.27 (s, 3 H, MeO), 3.19 (t, 1 H, *J* 10.0 Hz, H-4a), 2.09 (s, 3 H, Ac), 1.26 (d, 3 H, *J* 6.2 Hz, H-6a), and 1.20 (d, 3 H, *J* 6.2 Hz, H-6e); ^{13}C (125.76 MHz), δ 100.5, 100.3, 99.8, 99.8, and 99.2 ($^1J_{C,H}$ 174, 172, 170, 170, and 172 Hz, C-1abcde).

Crude **31** (155 mg) was deacetylated as described for **6** and gave, after column chromatography (1:4 ethyl acetate–hexanes) of the residue, **32** (133 mg, 60% from **15**): $[\alpha]_D^{25}$ +80° (*c* 0.67, chloroform). ^1H-N.m.r. data (500.14 MHz, $CDCl_3$): δ 4.99 (d, 1 H, *J* 1.4 Hz, H-1e), 5.05 and 4.88 (d and bs, 1 H and 2 H, *J* 1.7 Hz, H-1bcd), 4.50 (d, 1 H,

J 1.7 Hz, H-1a), 3.90 (t, 1 H, *J* 2.4 Hz, H-2c), 3.79 (dd, 1 H, *J* 9.2 and 2.8 Hz, H-3c), 3.78 (t, 1 H, *J* 2.4 Hz, H-2a), 3.72 (dd, 1 H, *J* 9.4 and 3.2 Hz, H-3e), 3.64 (dd, 1 H, *J* 9.9 and 2.9 Hz, H-3a), 3.64 (H-5c), 3.56 (dq, 1 H, *J* 10.1 and 6.2 Hz, H-5e), 3.41 (t, 1 H, *J* 10.0 Hz, H-4e), 3.38 (dq, 1 H, *J* 10.0 and 6.1 Hz, H-5a), 3.26 (s, 3 H, MeO), 3.18 (t, 1 H, *J* 10.0 Hz, H-4a), 1.25 (d, 3 H, *J* 6.2 Hz, H-6a), 1.19 (d, 3 H, *J* 6.0 Hz, H-6e), and 1.16 (d, 3 H, *J* 6.3 Hz, H-6c).

Anal. Calc. for $C_{73}H_{86}N_{12}O_{17}$: C, 62.5; H, 6.18; N, 12.0. Found: C, 62.1; H, 6.09; N, 11.7.

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-O-benzyl-4,6-dideoxy-4-formamido-α-*D*-mannopyranoside* (**34**). — Reduction[16] of **32** (115 mg, 81.9 μmol) and *N*-formylation of the intermediate amine **33** as described for **24** gave, after column chromatography (1:40 methanol–ethyl acetate, followed by 1:30) of the residue, **34** (85 mg, 74%): $[\alpha]_D^{25}$ +35° (*c* 0.69, chloroform). N.m.r. data ($CDCl_3$): ^{1}H (500.14 MHz), δ 8.19–7.95 (m, 4 H, NHC*H*O), 3.29 (bs, 3 H, MeO), and 1.25–1.02 (m, 15 H, H-6abcde); ^{13}C (125.76 MHz), δ 164.8 and 161.3 (2 m, NH*C*HO), 101.3–99.4 (m, C-1abcde), and 51.2–50.0 (m, C-4abde-*Z*).

Anal. Calc. for $C_{77}H_{90}N_4O_{21}$: C, 65.5; H, 6.71; N, 3.97. Found: C, 65.5; H, 6.76; N, 4.04.

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)-4,6-dideoxy-4-formamido-α-*D*-mannopyranoside* (**35**). — Debenzylation of **34** (86 mg, 61 μmol) and column chromatography of the residue on Bio-Gel P-4 as described for **25** gave, after freeze drying, **35** (43 mg, 81%): $[\alpha]_D^{25}$ +44° (*c* 0.61, water). ^{1}H-N.m.r. showed a *Z*:*E*-ratio of ~4.0:1 for the formamido groups. N.m.r. data (D_2O): ^{1}H (500.14 MHz), δ 8.20–8.18 (4 s, 3.2 H, NHC*H*O-*Z*), 8.03–8.00 (4 s, 0.8 H, NHC*H*O-*E*), 5.17, 5.15, 5.09, and 5.03 (4 bs, each 1 H, H-1bcde), 4.79 (bs, 1 H, H-1a), 3.47 (t, 1 H, *J* 9.7 Hz, H-4c), 3.39 (s, 3 H, MeO), and 1.27–1.17 (m, 15 H, H-6abcde); ^{13}C (125.76 MHz), δ 168.1 (NH*C*HO-*E*), 165.1 (NH*C*HO-*Z*), 102.2 (C-1e), 101.0, 100.9, and 100.8 (C-1bcd), 99.8 (C-1a), 78.1, 77.5, 77.4, and 77.2 (C-2abcd), 57.1 and 56.9 (C-4abde-*E*), 55.2 (MeO), and 52.2 and 51.9 (C-4abde-*Z*).

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