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Access to fluorescent probes via allyl glycosides: the synthesis of a *Brucella* trisaccharide epitope linked to a coumarin*

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Oligosaccharide allyl glycosides are demonstrated to provide a route to fluorescent probes and simple inhibitors. Ethyl 2-*O*-acetyl-4-azido-3-*O*-benzoyl-4,6-dideoxy-1-thio-α-D-mannopyranoside (**6**) was used as glycosyl donor in the preparation of the trisaccharide [α-D-Rha*p*4NFo-(1 → 2)-]$_2$-α-D-Rha*p*4NFo-*O*-allyl (**16**). Thioglycoside **6** was activated with *N*-iodosuccinimide and triflic acid or by bromine in the glycosylations and the inhibitor **16** was obtained after deprotection by transesterification, reduction of the azido groups with hydrogen sulfide, and *N*-formylation with ethyl formate. Ozonolysis of the allyl glycoside in **16** and reductive amination with 7-amino-4-methylcoumarin then gave the target fluorescent trisaccharide conjugate.

Keywords: glycoside synthesis, allyl glycoside, fluorescent probe, *Brucella* common epitope, reductive amination

Allyl glycosides are flexible intermediates [1] that facilitate manipulations at the reducing terminus of synthetic oligosaccharides [1–6]. By ozonolysis of an allyl glycoside and reductive amination of the generated aldehyde functionality we have prepared a fluorescent conjugate (**19**) of 7-amino-4-methylcoumarin and a trisaccharide fragment of the common epitope of the *Brucella* A and M antigens. This common epitope is a linear tetrasaccharide of α(1-2)-linked 4,6-dideoxy-4-formamido-D-mannose residues [7, 8]. Monoclonal antibodies have been generated towards the Brucella A and M antigens and bind specifically to either the A or the M antigen or cross react with both antigens [9]. The structure of the Fab fragment of one of these monoclonal antibodies (YsT9-1) that selectively binds the A antigen has been solved by X-ray crystallography (Rose D, unpublished results) and it has also been independently modelled [10] by computer assisted techniques. So far the position of the antigen in the combining site has yet to be determined.

The fluorescent probe **19** described here was designed to provide insight into specific ligand–amino acid interactions in the oligosaccharide–antibody complexes and to enable determination of the thermodynamics of binding by the sensitive technique of fluorescence quenching.

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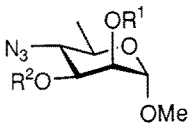
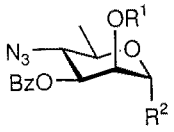
Results and discussion

The thioglycoside **6**, used as glycosyl donor, and the glycosyl acceptor **9** were prepared from methyl 4-azido-4,6-dideoxy-α-D-mannopyranoside [11] (**1**). Regioselective benzoylation of **1** with benzoyl chloride at −45°C gave the 3-*O*-benzoate **2** (62%) as well as the 2-*O*-benzoate **3** (18%) and the dibenzoate **4** (9%). Termination of the reaction by addition of methanol at −45°C was essential in optimizing the yield of **2**. Acetylation and simultaneous acetolysis of **2** gave the 1,2-diacetate **5** (80%) which was converted to the α-thioglycoside **6** (78%) on treatment with ethanethiol and boron trifluoride etherate in dichloromethane.

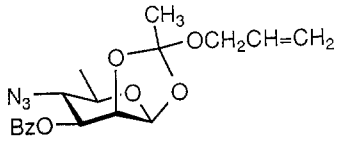
Reaction of the thioglycoside **6** with bromine gave the glycosyl bromide **7** which was directly treated with allyl alcohol and mercuric cyanide to give the α-allyl glycoside **8** (65%) and the 2-hydroxy allyl glycoside **9** (14%), which had lost the 2-*O*-acetyl group during the glycosylation. Orthoesters have been shown to be intermediates in glycosylations of alcohols with 2-*O*-acylated glycosyl halides [12–14]. They have also been shown to give 2-hydroxy glycosides (e.g. **9**) in addition to 2-*O*-acylated 1,2-*trans*-glycosides (e.g. **8**) upon rearrangement catalysed by proton or Lewis acids [12–14]. TLC and NMR did indeed reveal that the allyl glycosides **8** and **9** were formed from the glycosyl bromide **7** with orthoester **10** as intermediate. This orthoester was the only product formed if the mercuric



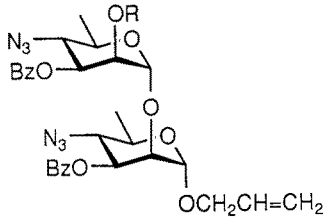
	R^1	R^2
1	H	H
2	H	Bz
3	Bz	H
4	Bz	Bz



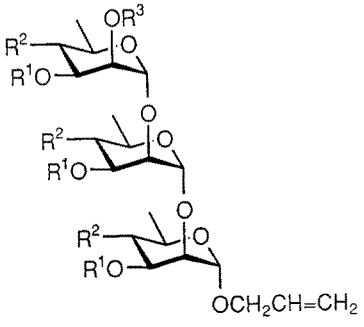
	R^1	R^2
5	Ac	AcO
6	Ac	EtS
7	Ac	Br
8	Ac	O-allyl
9	H	O-allyl



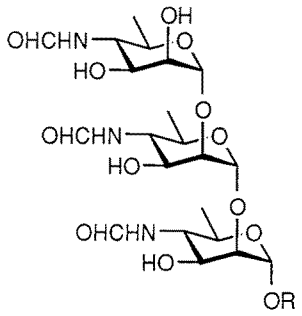
10



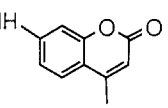
11	R = Ac
12	R = H



	R^1	R^2	R^3
13	Bz	N_3	Ac
14	H	N_3	H
15	H	NH_2	H
16	H	NHCHO	H
17	Bz	NHCHO	Bz



18 R = $-CH_2CHO$

19 R = $-CH_2CH_2NH$ 

cyanide promoted glycosylation of allyl alcohol with the halide **7** was performed in the presence of molecular sieve (which presumably adsorbs the acid required to rearrange the orthoesters **10**). Finally, methanolic hydrogen chloride was used to remove [15] the 2-*O*-acetyl group in **8** without affecting the 3-*O*-benzoyl group and the glycosyl acceptor **9** was obtained in 75% yield.

Glycosylation of the unreactive HO-2 in **9** with the thioglycoside **6** was attempted by several methods. The α-linked disaccharide **11** was obtained in highest yield (77%) when *N*-iodosuccinimide and a catalytic amount of triflic acid were used as promoters [16, 17] of the reaction between **6** and **9**. Activation of **6** with methylsulfenyl triflate [18], or *in situ* with bromine [19], gave lower yields of **11** (31% and 60%, respectively). Deacetylation [15] of **11** with methanolic hydrogen chloride gave the alcohol **12** (68%). Glycosylation of **12** with the thioglycoside **6** under promotion [16, 17] by *N*-iodosuccinimide and triflic acid gave the trisaccharide **13** in 69% yield whereas promotion by methylsulfenyl triflate [18] afforded **13** in only 12% yield.

The trisaccharide **13** was deprotected by transesterification with methanolic sodium methoxide (→ **14**, 89%), reduction of the azido groups with hydrogen sulfide [20] (→ **15**), and *N*-formylation in refluxing ethyl formate and pyridine (→ **16**). Crude **16** could not be satisfactorily purified and was therefore benzoylated to give **17** which was purified by chromatography and then transesterified to afford pure **16** (24% from **14**).

Reductive ozonolysis [21] (O_3, then Me_2S) of the allyl glycoside in **16** afforded the aldehyde **18** in quantitative yield. ^{13}C-NMR showed that the aldehyde group existed in the hydrated form (δ $CH(OH)_2$ at 88.5 ppm) in ^2H_2O. Reductive amination [5, 22, 23] of **18** with 7-amino-4-methylcoumarin using sodium cyanoborohydride as reducing agent in buffered aqueous methanol gave the target fluorescent trisaccharide **19** in 40% yield.

Materials and methods

General methods

Optical rotations were measured with a Perkin-Elmer 243 polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded at 300 K with Bruker AM 200 and AM 500 spectrometers, for solutions in C^2HCl_3 [residual $CHCl_3$ (δ_H 7.24) and C^2HCl_3 (δ_C 77.0) as internal standards] or ^2H_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 NOE experiments. Resonances for aromatic protons and proton resonances that could not be assigned are not reported. The stereochemistry of the glycosidic bonds was determined from the $^1J_{C\text{-}1,H\text{-}1}$ coupling constants [24]. TLC was performed on Silica Gel 60 F_{254} (Merck, Germany) with detection by u.v. light and charring with sulfuric acid. Silica gel 60 (230–400 mesh, Merck, Germany) and analytical reagent grade solvents (BDH, Canada) were used for column chromatography. Dichloromethane was dried by distillation from phosphorus pentoxide. Powdered molecular sieve (4 Å, Aldrich, USA), dried overnight at 300°C under vacuum, was used in the glycosylations. Organic solutions were dried over magnesium sulfate. Methyl 4-azido-4,6-dideoxy-α-D-mannopyranoside (**1**) was prepared as described [11]. The 7-Amino-4-methylcoumarin was from Aldrich, USA.

Methyl 4-azido-3-O-benzoyl-4,6-dideoxy-α-D-mannopyranoside (**2**), *methyl 4-azido-2-O-benzoyl-4,6-dideoxy-α-D-mannopyranoside* (**3**), *and methyl 4-azido-2,3-di-O-benzoyl-4,6-dideoxy-α-D-mannopyranoside* (**4**)

Benzoyl chloride (2.9 ml, 25 mmol) was added dropwise to a stirred solution of **1** [11] (5.0 g, 25 mmol) in dry dichloromethane (200 ml) containing pyridine (15 ml) at −45°C. After 1.5 h methanol (150 µl) was added at −45°C to terminate the reaction. The solution was diluted with dichloromethane (100 ml), washed with saturated aqueous sodium hydrogen carbonate (100 ml), 1 M hydrochloric acid (50 ml), water (100 ml), and brine (100 ml), then dried and concentrated. Column chromatography (ethyl acetate/hexane, 1/6 by vol followed by 1/4) of the residue gave **2** (5.0 g, 62%), **3** (1.4 g, 18%), and **4** (0.72 g, 9%).

Compound **2** had $[\alpha]_D^{25}$ −1° (c = 1.4, chloroform). ^{1}H-NMR data (200.13 MHz, C^2HCl_3): δ 5.32 (m, 1H, H-3), 4.68 (d, 1H, J 1.7 Hz, H-1), 4.19 (bs, 1H, H-2), 3.38 (s, 3H, MeO), 1.40 (d, 3H, J 5.6 Hz, H-6).

Analytical data. Calculated for $C_{14}H_{17}N_3O_5$: C, 54.7; H, 5.57; N, 13.7. Found: C, 54.6; H, 5.63; N, 13.4.

Compound **3** had $[\alpha]_D^{25}$ −48° (c = 0.90, chloroform). ^{1}H-NMR data (200.13 MHz, C^2HCl_3): δ 5.29 (dd, 1H, J 3.4 and 1.7 Hz, H-2), 4.78 (d, 1H, J 1.6 Hz, H-1), 4.12 (ddd, 1H, J 9.5, 6.0, and 3.5 Hz, H-3), 3.63 (dq, 1H, J 9.9 and 6.2 Hz, H-5), 3.37 (t, 1H, J 9.9 Hz, H-4), 3.37 (s, 3H, MeO), 2.31 (d, 1H, J 6.0 Hz, OH), 1.40 (d, 3H, J 6.1 Hz, H-6).

Analytical data. Calculated for $C_{14}H_{17}N_3O_5$: C, 54.7; H, 5.57; N, 13.7. Found: C, 55.0; H, 5.71; N, 13.6.

Compound **4** had $[\alpha]_D^{25}$ −168° (c = 0.94, chloroform). ^{1}H-NMR data (200.13 MHz, C^2HCl_3): δ 5.58–5.52 (m, 2H, H-2,3), 4.82 (d, 1H, J 1.3 Hz, H-1), 3.85–3.68 (m, 2H, H-4,5), 3.43 (s, 3H, MeO), 1.45 (d, 3H, J 5.7 Hz, H-6).

Analytical data. Calculated for $C_{21}H_{21}N_3O_6$: C, 61.3; H, 5.14; N, 10.2. Found: C, 61.0; H, 5.20; N, 10.5.

1,2-Di-O-acetyl-4-azido-3-O-benzoyl-4,6-dideoxy-α-D-mannopyranoside (**5**)

A solution of **2** (4.80 g, 15.6 mmol), in acetic anhydride:acetic acid:sulfuric acid, 50:20:0.5 by vol (35 ml), was stirred for 5.5 h at room temperature, and then poured into ice-cold aqueous potassium carbonate. The product was extracted with dichloromethane, the extract was washed with brine (2 × 100 ml), dried and concentrated. The residue was concentrated with toluene (2 × 100 ml) to give **5** (4.74 g, 80%). $[\alpha]_D^{25}$ +10° (c = 0.9, chloroform).

^{1}H-NMR data (500.14 MHz, C^2HCl_3): δ 6.04 (d, 1H, J 1.7 Hz, H-1), 5.43 (dd, 1H, J 10.2 and 3.5 Hz, H-3), 5.37 (dd, 1H, J 3.4 and 2.0 Hz, H-2), 3.79 (dq, 1H, J 10.0 and 6.1 Hz, H-5), 3.71 (t, 1H, J 10.2 Hz, H-4), 2.16 and 2.11 (2s, each 3H, Ac), 1.41 (d. 3H, J 6.0 Hz, H-6).

Analytical data. Calculated for $C_{17}H_{19}N_3O_7$: C, 54.1; H, 5.07; N, 11.1. Found: C, 53.7; H, 5.13; N, 11.3.

Ethyl 2-O-acetyl-4-azido-3-O-benzoyl-4,6-dideoxy-1-thio-α-D-mannopyranoside (**6**)

Ethanethiol (7.0 ml, 95 mmol) and boron trifluoride etherate (7.0 ml, 57 mmol) were added, at room temperature, to a stirred solution of **5** (4.5 g, 11.9 mmol) in dry dichloromethane (150 ml) containing molecular sieve (4 Å, 10 g). After 18 h, more ethanethiol (1.0 ml, 13.6 mmol) and boron trifluoride etherate (1.0 ml, 8.1 mmol) were added and after a further 18 h, triethylamine (9.3 ml, 66 mmol) was added dropwise and the mixture was filtered and concentrated. Column chromatography (ethyl acetate:hexanes, 1.5:8.5 by vol) of the residue gave **6** (3.52 g, 78%). $[\alpha]_D^{25}$ +103° (c = 0.3, chloroform).

^{1}H-NMR data (500.14 MHz, C^2HCl_3): δ 5.46 (dd, 1H, J 3.4 and 1.4 Hz, H-2), 5.37 (dd, 1H, J 10.3 and 3.3 Hz, H-3), 5.23 (bs, 1H, H-1), 4.10 (dq, 1H, J 9.9 and 6.2 Hz, H-5), 3.68 (t, 1H, J 10.1 Hz, H-4), 2.69–2.58 (m, 2H, SCH_2CH_3), 2.11 (s, 3H, Ac), 1.41 (d, 3H, J 6.1 Hz, H-6), 1.29 (t, 3H, J 7.4 Hz, SCH_2CH_3).

Analytical data. Calculated for $C_{17}H_{21}N_3O_5S$: C, 53.8; H, 5.58; N, 11.1; S, 8.45. Found: C, 53.9; H, 5.59; N, 11.4; S, 8.38.

Allyl 2-O-acetyl-4-azido-3-O-benzoyl-4,6-dideoxy-α-D-mannopyranoside (**8**)

Bromine (246 μl, 4.95 mmol) was added to a stirred solution of **6** (2.5 g, 6.59 mmol) in dry dichloromethane (100 ml) at 0°C and after 3 h cyclohexene (670 μl, 6.59 mmol) was added. Allyl alcohol (2.2 ml, 33 mmol) and mercuric cyanide (1.75 g, 6.92 mmol) were added when the colour of bromine had disappeared and the mixture was stirred for 16 h at room temperature. More mercuric cyanide (350 mg, 1.38 mmol) and allyl alcohol (0.44 ml, 6.6 mmol) were added to convert the intermediate orthoester **10**, and the mixture was stirred for another 24 h. Dichloromethane (100 ml) was added and the mixture was filtered through Celite. The resulting solution was washed with saturated aqueous sodium hydrogencarbonate (2 × 50 ml), water (50 ml), and brine (50 ml), then dried and concentrated. Column chromatography (ethyl acetate:hexane, 1:4 by vol) of the residue gave **8** (1.60 g, 65%) and **9** (0.32 g, 14%).

Compound **8** had $[\alpha]_D^{25}$ +45° ($c = 0.7$, chloroform). NMR data (C^2HCl_3): ^1H (500.14 MHz), δ 5.91–5.83 (m, 1H, =CH-), 5.46 (dd, 1H, J 10.3 and 3.2 Hz, H-3), 5.37 (dd, 1H, J 3.4 and 1.9 Hz, H-2), 5.30 (bdd, 1H, J 15.6 and 1.4 Hz, $=CH_{2trans}$), 5.21 (bdd, 1H, J 10.3 and 1.2 Hz, $=CH_{2cis}$), 4.82 (d, 1H, J 1.7 Hz, H-1), 4.20–4.14 (m, 1H, OCH_2-), 4.03–3.98 (m, 1H, OCH_2-), 3.76 (dq, 1H, J 10.0 and 6.1 Hz, H-5), 3.65 (t, 1H, J 10.1 Hz, H-4), 2.09 (s, 3H, Ac), 1.40 (d, 3H, J 6.1 Hz, H-6); ^{13}C (125.76 MHz), δ 96.4 ($^1J_{C,H}$ 170 Hz, C-1).

Analytical data. Calculated for $C_{18}H_{21}N_3O_6$: C, 57.6; H, 5.64; N, 11.2. Found: C, 57.8; H, 5.83; N, 11.0.

Allyl 4-azido-3-O-benzoyl-4,6-dideoxy-α-D-mannopyranoside (**9**)

A solution of **8** (750 mg, 2.0 mmol) in chloroform (20 ml) was treated [15] with methanolic hydrogen chloride [prepared by addition of acetyl chloride (0.40 ml, 5.6 mmol) to dry methanol (10 ml) at 0°C] at room temperature. The reaction was monitored by TLC and when complete (4 h), the solution was diluted with dichloromethane (10 ml) and treated with an excess of saturated aqueous sodium hydrogen carbonate. The organic solution was washed with water (50 ml) and brine (50 ml), then dried and concentrated. Column chromatography (ethyl acetate:hexane, 1:4 by vol) of the residue gave **9** (0.50 g, 75%). $[\alpha]_D^{25}$ +16° ($c = 1.4$, chloroform).

^1H-NMR data (500.14 MHz, C^2HCl_3): δ 5.93–5.85 (m, 1H, =CH-), 5.36 (dd, 1H, J 9.9 and 3.1 Hz, H-3), 5.30 (dq, 1H, J 17.2 and 1.6 Hz, $=CH_{2trans}$), 5.21 (dq, 1H, J 10.4 and 1.3 Hz, $=CH_{2cis}$), 4.84 (d, 1H, J 1.8 Hz, H-1), 4.21 (m, 1H, H-2), 4.18 (qt, 1H, J 12.9, 5.1, and 1.5 Hz, OCH_2-), 4.01 (qt, 1H, J 12.9, 6.2, and 1.3 Hz, OCH_2-), 3.74 (dq, 1H, J 10.0 and 6.0 Hz, H-5), 3.68 (t, 1H, J 10.0 Hz, H-4), 1.39 (d, 3H, J 6.0 Hz, H-6).

Analytical data. Calculated for $C_{16}H_{19}N_3O_5$: C, 57.6; H, 5.75; N, 12.6. Found: C, 57.5; H, 5.80; N, 12.6.

1,2-O-(1-Allyloxyethylidene)-4-azido-3-O-benzoyl-4,6-dideoxy-α-D-mannopyranoside (**10**)

Compound **6** (70 mg, 180 μmol) in dry dichloromethane (3.0 ml) was converted to the glycosyl bromide **7** as described for **8**. Molecular sieve (200 mg), allyl alcohol (70 μl, 1.0 mmol), and mercuric cyanide (65 mg, 26 μmol) were added and the mixture was stirred for 6 h at room temperature. 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:hexane, 1:4 by vol) of the residue gave **10** (52 mg, 75%) which was characterized by NMR spectroscopy.

NMR data (C^2HCl_3): ^1H (500.14 MHz), δ 5.85–5.77 (m, 1H, =CH-), 5.43 (d, 1H, J 2.4 Hz, H-1), 5.19 (dq, 1H, J 17.2 and 1.6 Hz, $=CH_{2trans}$), 5.16 (dd, 1H, J 10.3 and 4.0 Hz, H-3), 5.05 (dq, 1H, J 10.4 and 1.4 Hz, $=CH_{2cis}$), 4.72 (dd, 1H, J 4.0 and 2.4 Hz, H-2), 3.67 (t, 1H, J 9.9 Hz, H-4), 3.37 (dq, 1H, J 9.6 and 6.1 Hz, H-5), 1.74 (s, 3H, CH_3CO_3), 1.40 (d, 3H, J 6.1 Hz, H-6); ^{13}C (125.76 MHz), δ 124.1 (CH_3CO_3), 25.1 (CH_3CO_3).

Allyl 2-O-(2-O-acetyl-4-azido-3-O-benzoyl-4,6-dideoxy-αD-mannopyranosyl)-4-azido-3-O-benzoyl-4,6-dideoxy-α-D-mannopyranoside (**11**)

A solution of **6** (1.41 g, 3.70 mmol) and **9** (1.35 g, 4.0 mmol) in dry dichloromethane (150 ml) containing powdered molecular sieve (4 Å, 6 g) was stirred for 2 h at room temperature under a nitrogen atmosphere. *N*-Iodosuccinimide (2.07 g, 9.25 mmol) was added and a saturated solution of trifluoromethanesulfonic acid in dichloromethane was then added dropwise in portions (5 × 3 ml) until TLC indicated consumption of the thioglycoside [16, 17]. The mixture was filtered through Celite and the flask and Celite were washed with dichloromethane (50 ml). The solution was washed with saturated aqueous sodium hydrogen carbonate (2 × 50 ml), 10% sodium thiosulphate solution (2 × 50 ml) and brine (50 ml), then dried and concentrated. Column chromatography (ethyl acetate:hexane, 1.5:8.5 by vol) of the residue gave **11** (1.85 g, 77%). $[\alpha]_D^{25}$ −51° ($c = 0.75$, chloroform).

NMR data (C^2HCl_3): ^1H (500.14 MHz), δ 5.92–5.84 (m, 1H, =CH-), 5.52–5.45 (m, 3H, H-3,2′,3′), 5.31 (dd, 1H, J 17.5 and 1.2 Hz, $=CH_{2trans}$), 5.23 (dd, 1H, J 10.3 and 1.2 Hz, $=CH_{2cis}$), 4.83 (d, 1H, J 1.5 Hz, H-1), 4.80 (d, 1H, J 1.2 Hz, H-1′), 4.19 (dd, 1H, J 12.9 and 4.9 Hz, OCH_2-), 4.12 (bt, 1H, J 2.4 Hz, H-2), 4.01 (dd, 1H, J 13.1 and 6.4 Hz, OCH_2-), 3.82 (dq, 1H, J 10.1 and 6.2 Hz, H-5′), 3.76–3.67 (m, 2H,

H-4,5), 3.63 (t, 1H, *J* 10.1 Hz, H-4'), 1.97 (s, 3H, Ac), 1.41 (d, 3H, *J* 5.6 Hz, H-6), 1.35 (d, 3H, *J* 6.2 Hz, H-6'); ^{13}C (125.76 MHz), δ 99.4 and 97.2 ($^{1}J_{C,H}$ 172 and 170 Hz, C-1,1').

Analytical data. Calculated for $C_{31}H_{34}N_6O_{10}$: C, 57.2; H, 5.27; N, 12.9. Found: C, 57.2; H, 5.35; N, 12.9.

Allyl 2-O-(4-azido-3-O-benzoyl-4,6-dideoxy-α-D-mannopyranosyl)-4-azido-3-O-benzoyl-4,6-dideoxy-α-D-mannopyranoside (**12**)

A solution of **11** (1.65 g, 2.53 mmol) in chloroform (75 ml) was treated [15] with methanolic hydrogen chloride [prepared by addition of acetyl chloride (4.5 ml, 63 mmol) to dry methanol (75 ml) at 0°C] and gently refluxed for 7 h under nitrogen. Dichloromethane (100 ml) was added after 16 h at room temperature, and the mixture was washed with saturated aqueous sodium hydrogen carbonate (2 × 100 ml), water (100 ml), and brine (2 × 100 ml), then dried and concentrated. Column chromatography (ethyl acetate:hexane, 1.5:8.5 by vol) of the residue gave **12** (1.05 g, 68%). $[\alpha]_D^{25}$ −102° (*c* = chloroform).

^{1}H-NMR data (500.14 MHz, C^2HCl_3): δ 5.92–5.84 (m, 1H, =CH-), 5.40 (dd, 1H, *J* 9.9 and 3.3 Hz, H-3), 5.36 (dd, 1H, *J* 10.2 and 3.0 Hz, H-3), 5.30 (dd, 1H, *J* 17.2 and 1.5 Hz, $=CH_{2trans}$), 5.21 (dd, 1H, *J* 10.3 and 1.4 Hz, $=CH_{2cis}$), 4.89 (d, 1H, *J* 1.7 Hz, H-1), 4.80 (d, 1H, *J* 1.6 Hz, H-1'), 4.22 (bs, 1H, H-2'), 4.20–4.14 (m, 1H, OCH_2-), 4.12 (dd, 1H, *J* 3.2 and 1.9 Hz, H-2), 4.02–3.96 (m, 1H, OCH_2-), 3.84 (dq, 1H, *J* 10.0 and 6.2 Hz, H-5'), 3.73–3.66 (m, 3H, H-4,4',5), 1.39 (d, 3H, *J* 5.6 Hz, H-6), 1.32 (d, 3H, *J* 6.2 Hz, H-6').

Analytical data. Calculated for $C_{29}H_{32}N_6O_9$: C, 57.2; H, 5.30; N, 13.8. Found: C, 57.4; H, 5.42; N, 13.6.

Allyl O-(2-O-acetyl-4-azido-3-O-benzoyl-4,6-dideoxy-α-D-mannopyranosyl)-(1-2)-O-(4-azido-3-O-benzoyl-4,6-dideoxy-α-D-mannopyranosyl)-(1-2)-4-azido-3-O-benzoyl-4,6-dideoxy-α-D-mannopyranoside (**13**)

Glycosylation [16, 17] of **12** (200 mg, 0.33 mol) with **6** (134 mg, 0.35 mmol), as described for **11**, and column chromatography (ethyl acetate:hexane, 1.5:8.5 by vol) of the crude product gave **13** (210 mg, 69%). $[\alpha]_D^{25}$ −94° (*c* = 0.47, chloroform).

NMR data (C^2HCl_3): ^{1}H (500.14 MHz), δ 5.94–5.86 (m, 1H, =CH-), 5.24 (dd, 1H, *J* 10.4 and 1.2 Hz, $=CH_{2cis}$), 4.88 and 4.82 (2 d, each 1H, each *J* 1.6 Hz, H-1,1'), 4.49 (bs, 1H, H-1''), 4.01 (dd, 1H, *J* 13.0 and 6.3 Hz, OCH_2-), 1.94 (s, 3H, Ac); ^{13}C (125.76 MHz), δ 100.8, 99.2, and 97.2 ($^{1}J_{C,H}$ 167, 171, and 172 Hz, C-1,1'.1'').

Analytical data. Calculated for $C_{44}H_{47}N_9O_4$: C, 57.1; H, 5.11; N, 13.6. Found: C, 57.4; H, 5.31; N, 13.8.

Allyl O-(azido-4,6-dideoxy-α-D-mannopyranosyl)-(1-2)-O-(4-azido-4,6-dideoxy-α-D-mannopyranosyl)-(1-2)-4-azido-4,6-dideoxy-α-D-mannopyranoside (**14**)

Methanolic 1 M sodium methoxide (1.6 ml) was added to a solution of **13** (750 mg, 0.81 mmol) in dry methanol (125 ml). After 16 h at room temperature the solution was neutralized [Amberlite IR-120 (H^+) resin], filtered and concentrated. Column chromatography (ethyl acetate:hexane, 3:7 by vol followed by 4:6) of the residue gave **14** (412 mg, 89%). $[\alpha]_D^{25}$ +74° (*c* = 0.3, chloroform).

^{1}H-NMR data (500.14 MHz, C^2HCl_3)- δ 5.88–5.80 (m, 1H, =CH-), 5.26 (dd, 1H, *J* 17.2 and 1.5 Hz, $=CH_{2trans}$), 5.19 (dd, 1H, *J* 10.5 and 1.2 Hz, $=CH_{2cis}$), 5.01 (bs, 1H, H-1'), 4.99 (bs, 1H, H-1''), 4.82 (bs, 1H, H-1), 4.10 (dd, 1H, *J* 13.0 and 5.0 Hz, OCH_2-), 4.05 (bs, 1H, H-2''), 4.00 (bs, 1H, H-2'), 3.95–3.90 (m, 3H, H-3,3' and OCH_2-), 3.87 (dd, 1H, *J* 10.0 and 3.0 Hz, H-3''), 3.80 (bs, 1H, H-2), 3.72 (dq, 1H, *J* 10.0 and 6.2 Hz, H-5''), 3.66 (dq, 1H, *J* 10.0 and 6.2 Hz, H-5'), 3.53 (dq, 1H, *J* 9.9 and 6.2 Hz, H-5), 3.36 (t, 1H, *J* 10.0 Hz, H-4''), 3.24 (t, 1H, *J* 10.0 Hz, H-4'), 3.19 (t, 1H, *J* 10.0 Hz, H-4), 1.32 (d, 3H, *J* 6.2 Hz, H-6''), 1.28 (d, 3H, *J* 6.2 Hz, H-6), 1.26 (d, 3H, *J* 6.1 Hz, H-6').

Analytical data. Calculated for $C_{21}H_{33}N_9O_{10}$: C, 44.1; H, 5.82; N, 22.1. Found: C, 44.1; H, 5.90; N, 21.6.

Allyl 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 (**16**)

A solution of **14** (390 mg, 0.68 mmol) in pyridine:triethylamine (1:1 by vol, 70 ml) was saturated with hydrogen sulfide [20] for 90 min at room temperature and then stirred for 16 h. Nitrogen was passed through the solution for 1 h to remove excess of hydrogen sulfide and the solution was concentrated and codistilled twice with toluene. The resulting crude amine **15** was taken up in ethyl formate:pyridine (1:1 by vol, 12 ml) and refluxed for 24 h, then concentrated and codistilled with toluene to give **16** that could not be satisfactorily purified by column chromatography. Therefore benzoyl chloride (0.6 ml, 5.2 mmol) was added to a solution of the crude **16** in pyridine (25 ml) at 0°C. After 5 h at room temperature the solution was concentrated and codistilled with toluene. The residue was taken up with dichloromethane (100 ml), washed with 1 M hydrochloric acid (25 ml), saturated aqueous sodium hydrogen carbonate (50 ml), water (50 ml), and brine (50 ml), then dried and concentrated. Column chromatography of the residue (ethyl acetate:hexane:methanol, 10:10:1 by vol) afforded **17** (214 mg), which was debenzoylated as described for **14**. The residue was dissolved in water (20 ml) and washed with hexane (3 × 20 ml) to give, after freeze drying, **16** (96 mg, 24%). $[\alpha]_D^{25}$ +48° (*c* = 0.45, water).

^{1}H-NMR showed a *Z*:*E* ratio of ≈4.2:1 for the formamido groups. NMR data (^2H_2O): ^{1}H (500.14 MHz), δ 8.21 and

8.20 (2 s, 2.4H, NHCHO-*Z*), 8.04 and 8.03 (2 s, 0.6H, NHCHO-*E*), 6.01–5.92 (m, 1H, =CH-), 5.37 (d, 1H, *J* 17.4 Hz, $=CH_{2trans}$), 5.31 (d, 1H, *J* 10.4 Hz, $=CH_{2cis}$), 5.16, 5.05 and 4.96 (3 bs, each 1H, H-1,1′,1″), 1.29–1.18 (m, 9H, H-6,6′,6″); ^{13}C (125.76 MHz), δ 168.1 (NHCHO-*E*), 165.1 (NHCHO-*Z*), 133.36 (=CH-), 119.0 ($=CH_2$), 102.3, 101.0, and 97.5 (C-1,1′,1″), 77.9 and 77.6 (C-2,2′), 57.2, 57.1, and 56.9 (C-4,4′,4″-*E*), 52.2, 52.1, and 51.9 (C-4,4′,4″-*Z*).

2-Oxoethyl 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 (**18**)

Ozone was bubbled through a solution of **16** (50 mg, 86 μmol) in methanol (5 ml) at −78°C until the solution turned blue (≈5 min). Nitrogen was passed through the solution to remove the excess of ozone and dimethyl sulfide (0.2 ml) was added [21]. After a few minutes, nitrogen was again passed through the solution which was then allowed to attain room temperature. Concentration afforded **18** (50 mg, ≈100%). $[\alpha]_D^{25}$ +40° (*c* = 0.93, water).

^1H-NMR showed a *Z*:*E* ratio of ≈4.5:1 for the formamido groups. NMR data (^2H_2O): ^1H (500.14 MHz), δ 8.26 and 8.25 (2 s, 2.5H, NHCHO-*Z*), 8.09 and 8.08 (2 s, 0.5H, NHCHO-*E*), 5.25 (bs, 1H, -CH(OH)$_2$), 5.25, 5.11, and 5.02 (3 bs, 3H, H-1,1′,1″), 3.71 (dd, 1H, *J* 10.4 and 3.9 Hz, OCH_2-), 3.55 (dd, 1H, *J* 10.7 and 5.2 Hz, OCH_2-), 1.34–1.24 (m, 9H, H-6,6′,6″); ^{13}C (125.76 MHz), δ 168.1 (NHCHO-*E*), 165.1 (NHCHO-*Z*), 102.3, 100.8, and 99.1 (C-1,1′,1″), 88.5 (-CH(OH)$_2$), 57.1, 57.1, and 56.9 (C-4,4′,4″-*E*), 52.2, 52.1, and 52.0 (C-4,4′,4″-*Z*).

N-(4-Methyl-7-coumarinyl)-2-aminoethyl 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 (**19**)

7-Amino-4-methylcoumarin (36 mg, 0.20 mmol) was added to a solution of **18** (30 mg, 52 μmol) in methanol (12 ml) followed by sodium acetate (30 mg), aqueous acetic acid (9%, 3.3 ml), and sodium cyanoborohydride (16 mg, 0.25 mmol) [22]. After 1 h the mixture was concentrated, dissolved in water (30 ml), and washed with chloroform (3 × 5 ml). The aqueous phase was freeze dried and the crude product was purified by column chromatography (water: acetic acid:pyridine, 986:4:10 by vol) on Bio-Gel P-4 followed by column chromatography (water) on Bio-Gel P-2. Freeze drying gave **19** (15.3 mg, 40%).

^1H-NMR showed a *Z*:*E* ratio of ≈4.1:1 for the formamido groups. NMR data (^2H_2O): δ 8.19 and 8.18 (2 s, 2.4H, NHCHO-*Z*), 8.02, 7.99, and 7.98 (3 s, 0.6H, NHCHO-*E*), 7.46 (bd, 1H, *J* 8.8 Hz, H-5_{coum}), 6.70 (d, 1H, *J* 8.8 Hz, H-6_{coum}), 6.50 (bs, 1H, H-8_{coum}), 5.97 (bs, 1H, H-3_{coum}), 5.10 (bs, 1H, H-1′), 5.02 (bs, 1H, H-1″), 4.87 (bs, 1H, H-1), 3.45 (bt, 2H, *J* 4.6 Hz, -CH_2N), 2.34 (s, 3H, CH_{3coum}), 1.21–1.07 (m, 9H, H-6,6′,6″); ^{13}C (125.76 MHz), δ 167.0 (NHCHO-*E*), 165.1 (NHCHO-*Z*), 126.5 (C-5_{coum}), 112.0 (C-6_{coum}), 107.5 (C-3_{coum}), 102.2 (*C*-1″), 100.9 (*C*-1′), 98.8 (*C*-1), 98.2 (C-8_{coum}), 77.8 and 77.7 (C-2,2′), 66.2 (OCH_2-), 57.1, 57.1, and 56.9 (C-4,4′,4″-*E*), 52.3, 52.1, and 51.9 (C-4,4′,4″-*Z*), 42.4 (-CH_2N), 18.1 (CH_{3coum}), 17.1, 17.0, and 16.9 (C-6,6′,6″).

References

1. Gigg J, Gigg R, Payne S, Conant R (1987) *J Chem Soc Perkin Trans* **I**:2411–14, and previous parts of the series.
2. Lee RT, Lee YC (1974) *Carbohydr Res* **37**:193–201.
3. Chernyak AY, Antonov KV, Kochetkov NK, Padyukov LN, Tsvetkova NV (1985) *Carbohydr Res* **141**:199–212.
4. Chernyak AY, Levinsky AB, Dimitriev BA, Kochetkov NK (1984) *Carbohydr Res* **128**:269–82.
5. Roy R, Laferrière CA, Gamian A, Jennings HJ (1987) *J Carbohydr Chem* **6**:161–5.
6. Pinto BM, Reimer KB, Morissette DG, Bundle DR (1990) *J Chem Soc Perkin Trans* **I**:293–9.
7. Caroff M, Bundle DR, Perry MB, Cherwonogrodzky JW, Duncan JR (1984) *Infect Immun* **46**:384–8.
8. Bundle DR, Cherwonogrodzky JW, Perry MB (1987) *Biochemistry* **26**:8717–26.
9. Bundle DR, Cherwonogrodzky JW, Gidney MAJ, Meikle PJ, Perry MB, Peters T (1989) *Infect Immun* **57**:2829–36.
10. Oomen R, Young NM, Bundle DR (1990) In ICSU Short Reports, Vol. 10, *Proceedings of the 1990 Miami Bio/Technology Winter Symposia* (Streilein JW *et al.*, eds), p. 56. Oxford: IRL Press.
11. Bundle DR, Gerken M, Peters T (1988) *Carbohydr Res* **174**:239–51.
12. Wallace JE, Schroeder LR (1977) *J Chem Soc Perkin Trans* **II**:795–802.
13. Banoub J, Bundle DR (1979) *Can J Chem* **57**:2091–97.
14. Garegg PJ, Konradsson P, Kvarnström I, Norberg T, Svensson SCT, Wigilius B (1985) *Acta Chem Scand* **B39**:569–77.
15. Byramova NE, Ovchinnikov MV, Backinowsky LV, Kochetkov NK (1983) *Carbohydr Res* **124**:C8–C11.
16. Veeneman GH, van Leeuwen SH, van Boom JH (1990) *Tetrahedron Lett* **31**:1331–4.
17. Konradsson P, Udodong UE, Fraser-Reid B (1990) *Tetrahedron Lett* **31**:4313–6.
18. Dasgupta F, Garegg PJ (1988) *Carbohydr Res* **177**:C13–C17.
19. Kihlberg JO, Leigh DA, Bundle DR (1990) *J Org Chem* **55**:2860–3.
20. Adachi T, Yamada Y, Inoue I, Saneyoshi M (1977) *Synthesis* 45–6.
21. Pappas JJ, Keaveney WP, Gaucher E, Berger M (1966) *Tetrahedron Lett* **36**:4273–8.
22. Borch RF, Bernstein MD, Durst HD (1971) *J Am Chem Soc* **93**:2897–904.
23. Bernstein MA, Hall LD (1980) *Carbohydr Res* **78**:C1–C3.
24. Bock K, Pedersen C (1974) *J Chem Soc Perkin Trans* **II**: 293–7.