

Chemical Synthesis of Disaccharides which are Partial Structures of the Glycosaminoglycan Heparan Sulfate

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A specific tetradecasaccharide sequence (oligo-H, **1**) of the proteoglycan heparan sulfate has recently been identified as being responsible for binding and activation of the basic fibroblast growth factor (bFGF), a potent mitogen. We present here the first synthetic study towards partial structures of oligo-H and analogues. Our first target compound is the disaccharide **2**, which corresponds to the dimeric repeat of the central decameric sequence of oligo-H.

Heparan sulfates are a family of cell-surface proteoglycans which are directly involved in the regulation of cell growth and development.¹ Like heparin, a closely related glycosaminoglycan, they are biosynthesized *via* homopolymers consisting of the β 1-4-linked dimeric units of GlcNAc α 1-4GlcA which are then further modified within the polysaccharide chain by N-deacetylation, N-sulfation, C-5 epimerisation of GlcA to the IdoA and O-sulfation of hydroxy groups.² Each of these secondary modifications occurs only partially, leading to highly poly-morphic polysaccharide structures.

Owing to their sulfate ester and carboxy groups heparin and heparan sulfate are highly negatively charged and can interact with polar or charged protein surfaces. Although the oligo-saccharide sequences of proteoglycans are generally heterogeneous, careful sequence analysis has shown that the polysaccharide chains contain some regions which can have particular biological functions.³ Highly specific proteoglycan-protein interactions have indeed been demonstrated, examples being the pentameric antithrombin binding site of heparin,⁴ and more recently a heparan sulfate sequence (oligo-H, **1**) which binds and activates the basic fibroblast growth factor (bFGF) and its receptor tyrosine kinase.^{5,6} The synthetic studies on the antithrombin binding sequence of heparin have shown that valuable information can be obtained on the detailed molecular sugar-protein interactions by studying the binding of chemically synthesized oligosaccharides.⁷

As the three-dimensional structure of bFGF has recently been published,⁸ and the active site of heparan sulfate has been narrowed down to oligo-H, this system seems to be highly suitable for structural and functional studies of these protein-carbohydrate interactions. We have therefore started a synthetic programme with the aim of synthesizing the partial or total structure of oligo-H (**1**).

Despite its size, oligo-H (**1**) should be accessible to chemical synthesis, since it consists of five dimeric repeats of the disaccharide GlcNSO₃-IdoA-2-SO₃. This suggests the use of a block synthesis approach from suitably protected disaccharides. We chose the dimer **2** as our first target compound because it has the same spacial arrangement of negatively charged groups as the repeated dimeric unit **3** in oligo-H and only differs from unit **3** in having an -O-SO₃⁻ instead of an -NH-SO₃⁻ substituent at the C-2 position of the glucose unit. Studies with the antithrombin binding sequence have shown that this substitution does not affect the affinity of the sugar to the protein.⁹ However, this substitution greatly simplifies the synthesis.

The dimer **2** or a suitably protected precursor could act as a building block for the synthesis of larger oligosaccharides related to oligo-H (**1**). Furthermore, we were interested in studying the strength of specific binding of disaccharide **2** itself

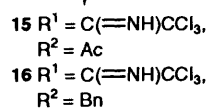
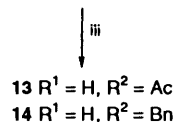
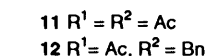
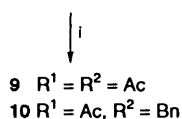
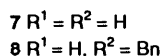
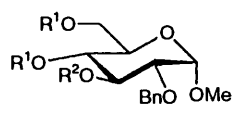
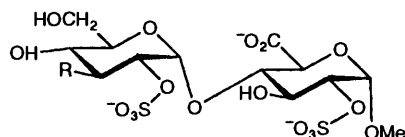
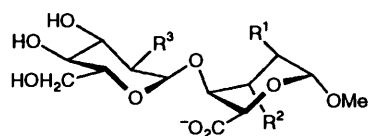
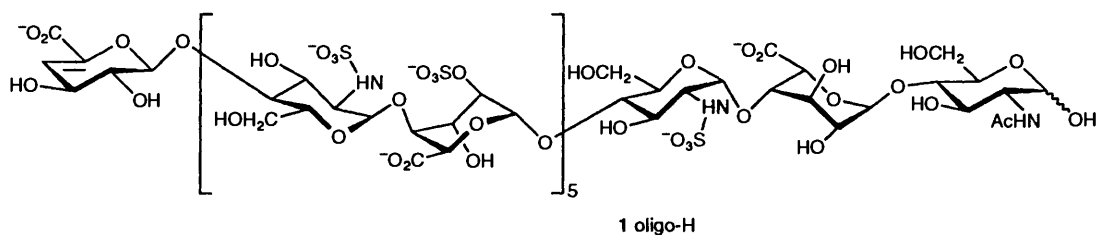
to bFGF. Therefore, we have also synthesized analogues of compound **2**. Analogue **4** contains an O-SO₃⁻ at the C-3 position of the iduronic acid, which might tell us how important regioselective sulfation at C-2, C-3 is for recognition. The other two analogues contain an α -glucuronic instead of the iduronic acid (**5**) and an extra sulfate ester in the C-3' position of the glucose unit (**6**). 3-O-Sulfation was found to be essential for binding of the antithrombin pentasaccharide, but is otherwise a structural feature that is rarely observed in heparan sulfate. None of these dimers can be generated by the commonly used degradation methods of heparan sulfate, either enzymic (*e.g.*, using heparinases¹⁰) or chemical.¹¹

Results and Discussion

The synthetic strategy to compounds **2-6** was as follows: the disaccharides **2-6** were synthesized by coupling of the appropriately protected monosaccharides followed by deprotection and sulfation. Two different alcohol-protecting groups were used; benzyl ethers for those hydroxy groups which would ultimately carry sulfate esters, and acetate groups for those hydroxy groups that were to remain free. This provided a nonparticipating group at the C-2 positions, facilitating the formation of the central α anomeric linkage. The coupling step was achieved by using trichloroacetimidates.¹²

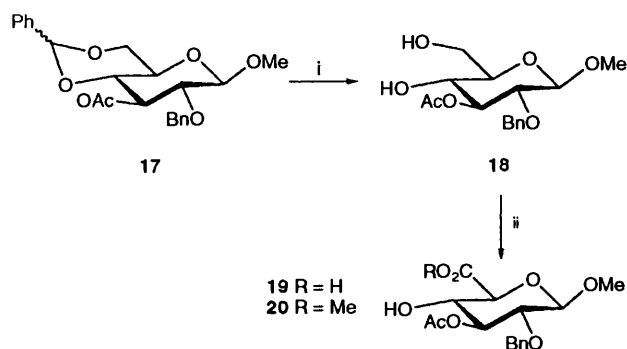
In a lengthy synthetic route such as that envisaged for oligo-H it is particularly important that the number of steps be kept to a minimum, and, where possible, regioselective reactions be used instead of protection groups. The work described here illustrates the particular advantage of a novel oxidation method using oxyammonium ions as oxidants,¹³ which has recently been adopted in our laboratory to carbohydrate chemistry.¹⁴ Using this procedure we show here that partially protected hexose derivatives (*e.g.*, containing both free C-4 and C-6 hydroxy groups) can be directly converted into uronic acids in one step without the need to protect the secondary alcohol groups.

Synthesis of Suitably Protected Monosaccharides.—The benzylation at the C-2 or both C-2 and C-3 positions of the glucose units was achieved from the methyl 4,6-O-benzylidene- α -glucopyranoside to give compounds **7** and **8** (Scheme 1) after removal of the benzylidene group.¹⁵⁻¹⁷ These were acetylated to compounds **9** and **10**.¹⁸ Introduction of the activating group at C-1 was achieved by cleavage of the methyl glycoside using acetolysis,¹⁹ to give compounds **11** and **12**, followed by selective 1-O-deacetylation using hydrazine acetate to give compounds **13** and **14** in 51–60% yield.²⁰ These were activated to the trichloroacetimidates **15** and **16** by treatment with potassium carbonate and trichloroacetonitrile.¹²



Scheme 1 Reagents: i, Ac_2O , pyridine; ii, Ac_2O , AcOH , H_2SO_4 ; iii, $\text{H}_2\text{NNH}_2 \cdot \text{HOAc}$, DMF ; iv, CCl_3CN , K_2CO_3

The synthesis of the protected glucuronic acid **20** is shown in Scheme 2. The methyl benzylidene- β -glucoside **17** was syn-

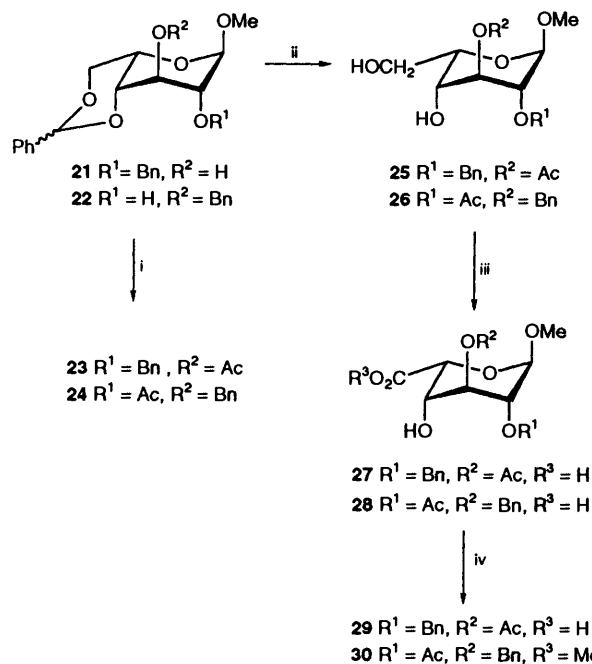


Scheme 2 Reagents: i, PTSA, MeOH , dichloromethane; ii, TEMPO, KBr , $\text{Bu}_4\text{N}^+\text{Cl}^-$, NaHCO_3 , NaOCl ; Dowex 50 H^+ , MeOH

thesized as described for compound **7** by selective benzylation,¹⁵ followed by acetylation.¹⁸ Treatment with toluene-*p*-sulfonic acid (PTSA) cleaved the benzylidene group to give compound **18**.²¹ The selective oxidation of alcohol **18** to acid **19** was

achieved in good yield (83% crude) by using the oxyammonium ion-catalysed oxidation of primary alcohols with sodium hypochlorite.¹⁴ This step shows the advantage of this oxidation method in that the C-4 hydroxy group does not need to be temporarily protected during the synthesis, which saves about four steps in the synthetic route and also prevents the use of difficult protection groups which have to be orthogonal to the acetate and the benzyl ether. The carboxy group was protected as the methyl ester **20**.

The iduronate methyl esters **29** and **30** were synthesized in similar fashion (Scheme 3). Benzylation of methyl 4,6-*O*-



Scheme 3 Reagents: i, Ac_2O , pyridine; ii, PTSA, MeOH , dichloromethane; iii, TEMPO, KBr , $\text{Bu}_4\text{N}^+\text{Cl}^-$, NaHCO_3 , NaOCl ; iv, Dowex 50 H^+ , MeOH

benzylidene- α -L-idopyranoside²² resulted in a 1:2 mixture of the two benzyl ethers **21** and **22**, respectively.¹⁵ The two isomers could be separated by column chromatography and were both used for further synthetic studies. Acetylation gave compounds

23 and **24**, then mild acid hydrolysis gave the C-4- and C-6-unprotected idopyranosides, **25** and **26**, which were oxidised (to the acids **27** and **28**) and esterified as before to give compounds **29** and **30**.^{14,18,21}

Disaccharide Synthesis.—The trichloroacetimidates **15** and **16** were coupled with the partially protected nucleophiles **20**, **29** and **30** as shown in Table 1, with trimethylsilyl triflate as the catalyst, in moderate yields (41–53%) to give the α -anomers.¹² The anomeric configuration of the disaccharides was confirmed by the coupling constants observed in the proton NMR spectrum (see Table 2).

The benzyl ether groups of the fully protected disaccharides **31**, **32**, **37** and **38** were removed by catalytic hydrogenation (Pd–C in methanol), to give compounds **33**, **34**, **39** and **40** in good yields (85–99%).²³ The free hydroxy groups were then sulfated by treatment with sulfur trioxide–trimethylamine complex in dimethylformamide (DMF) to give oligo sulfates **35**, **36**, **41** and **42** in varying yields (48–99%; Scheme 4).²⁴ The number of sulfate groups introduced in each case was confirmed by electrospray mass spectrometry and the position of the sulfates was confirmed from the proton NMR chemical shifts, since sulfation causes a downfield shift of the corresponding NMR signals (Table 3). Final deprotection was achieved by treatment with aq. sodium hydroxide in tetrahydrofuran (THF) to give the target compounds **2**, **4**, **5** and **6**, which were purified by anion-exchange chromatography.²⁵ The final products were characterised by ¹H NMR spectroscopy (Table 4) and electrospray mass spectrometry.

These sulfated disaccharides are currently being tested for binding and activation of the human bFGF. The results of these studies will be reported elsewhere.

Table 1 Coupling reactions between the activated trichloroacetimidate and the corresponding nucleophiles. For a general procedure see the Experimental section of this paper.

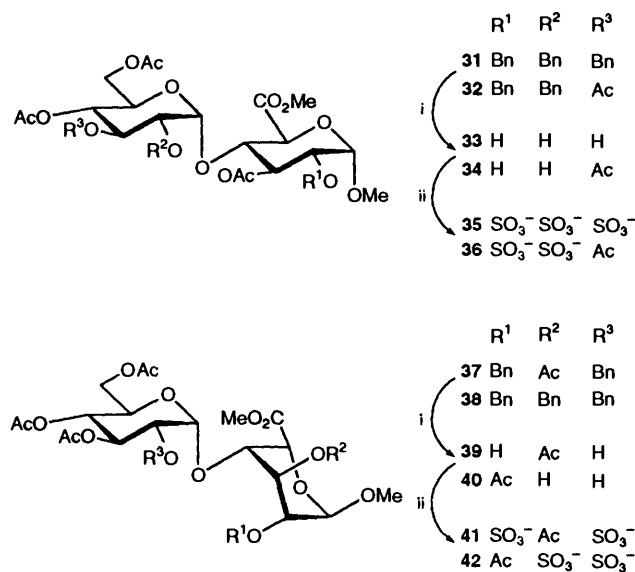
Trichloroacetimidate	Nucleophile	Product (% yield)
16	20	31 (52)
15	20	32 (53)
15	29	37 (46)
15	30	38 (41)

Table 2 Selected ¹H NMR chemical shifts (δ_{H}) and coupling constants (J/Hz) of the fully protected disaccharides **31**, **32**, **37** and **38**

	1-H	$J_{1,2}$	1'-H	$J_{1',2'}$
31	4.68	3.6	4.89	3.3
32	4.67	3.4	5.06	3.3
37	4.98	0	5.09	3.5
38	4.91	0	4.76–4.79	3.4

Table 3 Selected ¹H NMR chemical shifts (δ_{H}) of compounds **33**, **35**, **39**, **40**, **41** and **42** and chemical-shift differences of the sulfated compounds **35**, **41** and **42** relative to the unsulfated compounds **33**, **39** and **40**

	δ_{H}						$\Delta\delta$		
	33	39	40	35	41	42	35 – 33	41 – 39	42 – 40
2-H	3.61	3.71–3.78	4.95	4.30	4.35	4.79–4.84	0.69	0.61	–0.14
3-H	5.33	5.15	4.11–4.17	5.48	5.32	4.73	0.15	0.17	0.59
2'-H	3.38	3.71–3.78	3.67	4.12–4.27	4.50–4.53	4.40	0.82	0.78	0.73
3'-H	3.64	5.15	5.15	4.62	5.15–5.22	5.33–5.38	0.98	0.04	0.21



Scheme 4 Reagents: i, H₂, Pd/C, MeOH; ii, SO₃·Me₃N, DMF

Experimental

General.—Reactions were carried out in solvents distilled from standard drying agents. M.p.s are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter, and are given in units of 10⁻¹ deg cm² g⁻¹. IR were recorded on a Perkin-Elmer 1750 Fourier Transform spectrometer or a Perkin-Elmer 781 spectrometer as a solution in the solvents stated. ¹H NMR spectra were recorded on Bruker AM 500 MHz or Varian Gemini 200 MHz spectrometers using the solvents stated. ¹³C NMR spectra were recorded on a Bruker AM 500 MHz spectrometer operating at 125.78 MHz or a Varian Gemini 200 MHz spectrometer operating at 50 MHz. ¹³C spectra run in D₂O were referenced to 1,4-dioxane (δ_{C} 67.0) as an internal standard. Low-resolution mass spectra were recorded on VG Analytical Ltd, ZABIF or BIO-Q mass spectrometers using the techniques stated. High-resolution mass spectra were recorded by the SERC mass spectrometry service centre at Swansea. TLC was conducted on Merck Kieselgel 60 F₂₅₄ 0.2 mm precoated plates. Flash silica chromatography was conducted using Merck silica gel C60 (40–60 μ). HPLC was carried out using a Waters automated gradient controller, two Waters 501 HPLC pumps, a Waters 486 tunable absorbance detector and a Waters 740 data module. Desalting was carried out on a Bio-Gel P2 column (length 100 cm, internal diameter 1.6 cm) run at 0.2 cm³ min⁻¹ in 0.2 mol dm⁻³ aq. ammonium hydrogen carbonate. Elemental analyses were performed at the Dyson Perrins Laboratory, Oxford.

Methyl 2-O-Benzyl- α -D-glucopyranoside 7—A solution of methyl 2-O-benzyl-4,6-O-benzylidene- α -D-glucopyranoside¹⁵ (2.5 g, 6.7 mmol) and PTSA (10 g, 0.05 mol) in methanol (60

Table 4 ^1H NMR chemical shifts (δ_{H}) of the purified products **2**, **4**, **5** and **6**

	2	4	5	6
1-H	4.83	4.76	4.98	5.00
2-H	4.10	3.69–3.71	4.10	4.11–4.15
3-H	4.10	4.58	3.93	3.97
4-H	4.03	4.22	3.78	3.80
5-H	4.35	4.36	3.88	3.91
1'-H	5.36	5.31	5.71	5.72
2'-H	4.00	4.07	4.00	4.11–4.15
3'-H	3.73–3.81	3.77	3.68–3.71	4.43
4'-H	3.37	3.44	3.39	3.59
5'-H	3.73–3.81	3.69–3.71	3.58–3.60	3.67–3.71
6'-H ₂	3.66, 3.73–3.81	3.65, 3.69–3.71	3.68–3.71	3.67–3.71

cm^3 –water (25 cm^3) was refluxed for 2 h, then was cooled, and the solvent was removed under reduced pressure. The residue was dissolved in chloroform (60 cm^3) and washed with water (3 \times 50 cm^3). The combined aqueous layers were neutralised with sodium hydrogen carbonate and concentrated under reduced pressure. The residue was extracted with ethyl acetate (2 \times 100 cm^3), dried (Na_2SO_4), filtered and the solvent was removed under reduced pressure. The crude product was purified by flash silica chromatography [ethyl acetate–methanol (9 : 1)] to give compound **7** as a solid (1.41 g, 74%), R_f 0.34 [ethyl acetate–methanol (9 : 1)]; m.p. 115–116 °C; $[\alpha]_{\text{D}}^{22} + 85.7$ (c 1, CHCl_3); $\nu_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$ 3600 (OH); δ_{H} (500 MHz; CDCl_3) 3.34 (1 H, dd, J 3.6 and 9.6, 2-H), 3.36 (3 H, s, OMe), 3.55 (1 H, m, 4-H), 3.63 (1 H, m, 5-H), 3.80 (1 H, dd, J 4.2 and 11.7, 6-H), 3.84 (1 H, dd, J 3.5 and 11.8, 6-H), 3.92 (1 H, dd, J 9.1 and 9.3, 3-H), 4.63 (1 H, d, J 3.5, 1-H), 4.67 (1 H, d, J 12.0, PhCH_2), 4.70 (1 H, d, J 12.0, PhCH_2) and 7.31–7.38 (5 H, m, Ph); m/z (DCI, NH_3) 302 (MNH_4^+ , 31%), 285 (MH^+ , 7%), 270 (30), 163 (13), 108 (29) and 91 (100).

Methyl 2,3-Di-O-benzyl- α -D-glucopyranoside 8.—A solution of methyl 2,3-di-O-benzyl-4,6-O-benzylidene- α -D-glucopyranoside **16** (2.5 g, 5.4 mmol) and PTSA (8 g, 42 mmol) in methanol (48 cm^3)–water (20 cm^3) was refluxed for 2 h, then was cooled, and concentrated under reduced pressure. The residue was redissolved in chloroform (100 cm^3) and washed with water until neutral (pH = 7). The aqueous layer was neutralised with sodium hydrogen carbonate and extracted with chloroform (3 \times 50 cm^3). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give a mixture of products. The mixture was purified by flash silica chromatography (ethyl acetate) to give compound **8** as a solid (1.66 g, 82%) R_f 0.32 (EtOAc); m.p. 66–68 °C; $[\alpha]_{\text{D}}^{22} + 13.4$ (c 1, CHCl_3); $\nu_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$ 3590 (OH); δ_{H} (500 MHz; CDCl_3) 3.39 (3 H, s, OMe), 3.49–3.54 (2 H, m, 2- and 6-H), 3.63 (1 H, m, 5-H), 3.73–3.83 (3 H, m, 3-, 4- and 6-H), 4.62 (1 H, d, J 3.5, 1-H), 4.67 (1 H, d, J 12.1, PhCH_2), 4.71 (1 H, d, J 11.6, PhCH_2), 4.77 (1 H, d, J 12.1, PhCH_2), 5.03 (1 H, d, J 11.5, PhCH_2) and 7.29–7.39 (10 H, m, Ph); m/z (DCI, NH_3) 392 (MNH_4^+ , 11%), 374 (MH^+ , 1%), 360 (13), 181 (11), 100 (19) and 91 (100).

Methyl 3,4,6-Tri-O-acetyl-2-O-benzyl- α -D-glucopyranoside 9.—A solution of compound **7** (1.24 g, 4.37 mmol) in pyridine (130 cm^3)–acetic anhydride (80 cm^3) was stirred for 2 h at room temperature. The solvent was removed under reduced pressure and the product was purified by flash silica chromatography (ethyl acetate) to give compound **9** as a pale yellow gum (1.5 g, 98%), R_f 0.63 (EtOAc); $[\alpha]_{\text{D}}^{22} + 51.7$ (c 0.3, CHCl_3); $\nu_{\text{max}}(\text{CDCl}_3)/\text{cm}^{-1}$ 1740 (C=O); δ_{H} (500 MHz; CDCl_3) 1.99, 2.01 and 2.07 (9 H, 3 s, Ac), 3.40 (3 H, s, OMe), 3.57 (1 H, dd, J 3.6 and 10.0, 2-H), 3.94 (1 H, m, 5-H), 4.05 (1 H, dd, J 2.4 and 12.3,

6-H), 4.26 (1 H, dd, J 4.7 and 12.3, 6-H) 4.60 (1 H, d, J 12.4, PhCH_2), 4.65 (1 H, d, J 12.4, PhCH_2), 4.68 (1 H, d, J 3.6, 1-H), 4.96 (1 H, dd, J 9.5 and 10.1, 4-H), 5.45 (1 H, dd, J 9.6 and 9.7, 3-H) and 7.29–7.36 (5 H, m, Ph); δ_{C} (125.78 MHz; CDCl_3) 20.32, 20.39 and 20.50 (COMe), 55.26 (OMe), 61.88 (C-6), 66.95, 68.58, 71.67, 72.95 and 76.79 (C-2, -3, -4, -5 and PhCH_2), 97.79 (C-1), 127.91, 128.08, 128.30 and 128.56 (Ph), 137.92 (q-Ph), 169.94, 170.09 and 170.64 (C=O); m/z (DCI, NH_3) 428 (MNH_4^+ , 50%), 411 (MH^+ , 1%), 379 (14), 319 (19), 259 (21), 205 (10), 186 (11), 169 (13), 152 (53), 139 (20), 100 (20) and 91 (100) (Found: MNH_4^+ , 428.1921. $\text{C}_{20}\text{H}_{26}\text{O}_9\cdot\text{NH}_4^+$ requires m/z , 428.1921).

Methyl 4,6-Di-O-acetyl-2,3-di-O-benzyl- α -D-glucopyranoside 10.—Compound **8** (1.5 g, 4 mmol) was acetylated as described for compound **9** to give compound **10** as a pale yellow gum (1.71 g, 93%), R_f 0.66 (EtOAc); $[\alpha]_{\text{D}}^{22} - 0.8$ (c 1.25, CHCl_3); $\nu_{\text{max}}(\text{CDCl}_3)/\text{cm}^{-1}$ 1740 (C=O); δ_{H} (500 MHz; CDCl_3) 1.92 and 2.07 (6 H, 2 s, Ac), 3.40 (3 H, s, OMe), 3.60 (1 H, dd, J 3.6 and 9.6, 2-H), 3.85 (1 H, qd, J 2.7, 5.0 and 10.3, 5-H), 3.93 (1 H, t, J 9.4, 3-H), 4.02 (1 H, dd, J 2.4 and 12.3, 6-H), 4.21 (1 H, dd, J 5.0 and 12.3, 6-H), 4.62 (1 H, d, J 3.6, 1-H), 4.64–4.98 (4 H, m, PhCH_2), 5.00 (1 H, t, J 9.3, 4-H) and 7.28–7.41 (10 H, m, Ph); δ_{C} (125.78 MHz; CDCl_3) 20.55 (COMe), 55.30 (OMe), 62.24 (C-6), 67.51, 69.67, 73.45, 75.32, 79.06 and 79.65 (C-2, -3, -4, -5 and PhCH_2), 98.24 (C-1), 127.80, 128.02, 128.19, 128.29, 128.56 and 128.67 (Ph), 138.13 and 138.71 (q-Ph), 169.90 and 170.85 (C=O); m/z (Probe CI, NH_3) 476 (MNH_4^+ , 2%), 459 (MH^+ , 1%), 444 (2), 427 (6), 100 (32) and 91 (100) (Found: MNH_4^+ , 459.2019. $\text{C}_{25}\text{H}_{30}\text{O}_8\cdot\text{NH}_4^+$ requires m/z 459.2019).

1,3,4,6-Tetra-O-acetyl-2-O-benzyl- α - and β -D-glucopyranose 11.—A solution of compound **9** (300 mg, 0.73 mmol) in acetic acid (0.3 cm^3), acetic anhydride (0.4 cm^3) and conc. sulfuric acid (6 mm^3) was stirred at 0 °C for 8 h. The mixture was diluted with water, extracted with chloroform (3 \times 50 cm^3), and the extracts were dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give compound **11** as a pale yellow gum (310 mg, 97%), used directly in the next reaction; R_f 0.45 [ethyl acetate–light petroleum (boiling range 40–60 °C) (1 : 1)]; $[\alpha]_{\text{D}}^{22} + 113.2$ (c 0.5, CHCl_3); $\nu_{\text{max}}(\text{CDCl}_3)/\text{cm}^{-1}$ 1700 (C=O); δ_{H} (200 MHz; CDCl_3) 2.01, 2.03, 2.07 and 2.19 (12 H, 4 s, Ac), 3.70 (1 H, m, 2-H), 4.00–4.35 (3 H, m, 5-H and 6-H), 4.52 (1 H, d, J 12.7, PhCH_2), 4.66 (1 H, d, J 12.7, PhCH_2), 4.95–5.12 (1 H, m, 4-H), 5.19–5.43 (1 H, m, 3-H), 5.68 and 6.35 (1 H, 2 d, J 3.4 and 7.8, 1-H) and 7.24–7.37 (5 H, m, Ph); m/z (Probe CI, NH_3) 456 (MNH_4^+ , 72%), 428 (5), 380 (13), 379 (9), 319 (11), 259 (21), 186 (28), 168 (18), 152 (52), 139 (31), 110 (35), 108 (88) and 91 (100) (Found: MNH_4^+ , 456.1870. $\text{C}_{21}\text{H}_{26}\text{O}_{10}\cdot\text{NH}_4^+$ requires m/z 456.1870).

3,4,6-Tri-O-acetyl-2-O-benzyl- α - and β -D-glucopyranose 13.—A solution of compound **11** (310 mg, 0.71 mmol) and hydrazine acetate (105 mg, 1.23 mmol) in DMF (24 cm^3) was stirred at 50 °C under argon for 3 h. The reaction mixture was cooled, diluted with ethyl acetate (72 cm^3), washed with brine (3 \times 24 cm^3), dried (MgSO_4), filtered, and concentrated under reduced pressure. The mixture was purified by flash silica chromatography [ethyl acetate–light petroleum (1 : 1)] to give gummy compound **13** as a mixture of anomers (α : β , 2 : 1) (170 mg, 60%), R_f 0.28 [ethyl acetate–light petroleum (1 : 1)]; $[\alpha]_{\text{D}}^{22} + 31.7$ (c 0.6, CHCl_3); $\nu_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$ 3590 (OH) and 1750 (C=O); δ_{H} (500 MHz; CDCl_3) (α anomer) 2.01, 2.03 and 2.08 (9 H, 3 s, Ac), 3.09 (1 H, br s, OH), 3.61 (1 H, dd, J 3.6 and 9.7, 2-H), 4.07 (1 H, dd, J 2.0 and 12.1, 6-H), 4.22–4.31 (2 H, m, 5- and 6-H), 4.65 (2 H, s, PhCH_2), 4.97–5.02 (1 H, m, 4-H), 5.26 (1 H, d, J 3.5, 1-H), 5.44 (1 H, t, J 9.6, 3-H) and 7.27–7.38 (5 H, m, Ph); (β anomer) 1.95, 2.02 and 2.09 (9 H, 3 s, Ac), 3.29 (1 H, d, J 5.2, OH), 3.42 (1 H, dd,

J 7.7 and 9.5, 2-H), 3.72 (1 H, ddd, *J* 2.4, 5.0 and 10.1, 5-H), 4.14 (1 H, dd, *J* 2.4 and 12.3, 6-H), 4.22–4.31 (1 H, m, 6-H), 4.68 (1 H, d, *J* 11.8, PhCH₂), 4.84 (1 H, dd, *J* 4.4 and 7.7, 1-H), 4.87 (1 H, *J* 11.8, PhCH₂), 4.97–5.02 (1 H, m, 4-H), 5.19 (1 H, t, *J* 9.5, 3-H) and 7.27–7.38 (5 H, m, Ph); *m/z* (Probe CI, NH₃) 414 (MNH₄⁺, 40%), 396 (M⁺, 1%), 379 (13), 354 (25), 259 (21), 217 (17), 187 (37), 186 (29), 169 (21), 168 (18), 109 (19), 108 (72), 91 (100) and 79 (69) (Found: MNH₄⁺, 414.1764. C₁₉H₂₄O₉·NH₄⁺ requires *m/z* 414.1764).

1,4,6-Tri-O-acetyl-2,3-di-O-benzyl- α - and β -D-glucopyranose 12.—A solution of compound **10** (600 mg, 1.31 mmol) in acetic anhydride (5.16 cm³) was cooled to –4 °C. It was treated with a solution of conc. sulfuric acid in acetic anhydride (2.5 cm³ of a 0.8% solution) cooled to the same temperature. After 40 min the reaction was quenched by being poured into ice-water, neutralised with sodium hydrogen carbonate, extracted with chloroform (3 × 25 cm³), and the extract was dried (Na₂SO₄), filtered, and concentrated under reduced pressure to give compound **12** as a pale yellow gum (560 mg, 88%), used directly in the next reaction; *R*_f 0.43 [ethyl acetate–light petroleum (1:1)]; [α]_D²² +42.8 (*c* 0.5, CHCl₃); ν_{\max} (CDCl₃)/cm⁻¹ 1750 (C=O); δ_{H} (200 MHz; CDCl₃) 1.95, 2.08 and 2.20 (9 H, 3 s, Ac), 3.74 (1 H, dd, *J* 3.6 and 9.4, 2-H), 3.90 (1 H, t, *J* 9.3, 3-H), 4.00–4.22 (3 H, m, 5-H and 6-H₂), 4.63–4.94 (4 H, m, PhCH₂), 5.10 (1 H, t, *J* 9.5, 4-H), 6.34 (1 H, d, *J* 3.6, 1-H) and 7.28–7.33 (10 H, m, Ph); *m/z* (Probe CI, NH₃) 504 (MNH₄⁺, 45%), 462 (20), 444 (18), 427 (20), 259 (27), 198 (30), 187 (37), 108 (99) and 91 (100) (Found: MNH₄⁺, 504.223. C₂₆H₃₀O₉·NH₄⁺ requires *m/z* 504.2233).

4,6-Di-O-acetyl-2,3-di-O-benzyl- α - and β -D-glucopyranose 14.—A solution of compound **12** (560 mg, 1.15 mmol) and hydrazine acetate (210 mg, 2.28 mmol) in DMF (50 cm³) was stirred under argon at 50 °C for 3 h. The reaction mixture was cooled, diluted with ethyl acetate (150 cm³), washed with brine (3 × 50 cm³), dried (MgSO₄), filtered, and concentrated under reduced pressure. The residue was purified by flash silica chromatography [ethyl acetate–light petroleum (1:1)] to give a mixture of anomers of compound **14** as a gum (420 mg, 51%), *R*_f 0.24 [ethyl acetate–light petroleum (1:1)]; ν_{\max} (CHCl₃)/cm⁻¹ 3500 (OH) and 1750 (C=O); δ_{H} (200 MHz; CDCl₃) 1.95, 2.08, 1.94 and 2.09 (6 H, 4 s, Ac), 3.60–3.67 (1 H, m, 2-H), 3.94–4.22 (4 H, m, 3- and 5-H and 6-H₂), 4.65–5.00 (5.3 H, m, PhCH₂, 4- and 1-H), 5.21 (0.7 H, d, *J* 3.5, 1-H) and 7.28–7.35 (10 H, m, Ph); *m/z* (DCI, NH₃) 462 (MNH₄⁺, 32%), 444 (MH⁺, 8%), 427 (19), 247 (23), 187 (23), 108 (55) and 91 (100).

O-(3,4,6-Tri-O-acetyl-2-O-benzyl- α - and β -D-glucopyranosyl)trichloroacetimidate 15.—Dried potassium carbonate (176 mg, 1.28 mmol) and trichloroacetonitrile (0.37 cm³) were added to a solution of compound **13** (0.5 g, 1.26 mmol) in dry dichloromethane (6.4 cm³) and the mixture was stirred for 6 h under argon. The mixture was filtered, and concentrated under reduced pressure. The residue was purified by flash silica chromatography [ethyl acetate–light petroleum (1:1)] to give gummy compound **15** as a mixture of anomers (α : β , 8:5) (0.60 g, 88%), *R*_f 0.36 and 0.45 [ethyl acetate–light petroleum (1:1)]; [α]_D²² +68.0 (*c* 1.5, CHCl₃); ν_{\max} (CDCl₃)/cm⁻¹ 1750 (C=O); δ_{H} (500 MHz; CDCl₃) (α anomer) 2.02, 2.04 and 2.06 (9 H, 3 s, Ac), 3.76–3.80 (1 H, m, 2-H), 4.09 (1 H, dd, *J* 2.2 and 12.4, 6-H), 4.18–4.22 (1 H, m, 5-H), 4.27 (1 H, dd, *J* 4.3 and 12.4, 6-H), 4.60–4.89 (2 H, m, PhCH₂), 5.06–5.13 (1 H, m, 4-H), 5.51 (1 H, t, *J* 9.7, 3-H), 6.50 (1 H, d, *J* 3.6, 1-H), 7.26–7.36 (5 H, m, Ph) and 8.67 (1 H, s, NH); (β anomer) 1.92, 2.03 and 2.08 (9 H, 3 s, Ac), 3.76–3.80 (1 H, m, 2-H), 3.86–3.89 (1 H, m, 5-H), 4.13 (1 H, dd, *J* 2.5 and 12.4, 6-H), 4.31 (1 H, dd, *J* 4.6 and 12.4, 6-H), 4.60

(2 H, m, PhCH₂), 5.06 (1 H, m, 4-H), 5.26 (1 H, t, *J* 9.1, 3-H), 5.86 (1 H, d, *J* 7.8, 1-H), 7.26–7.36 (5 H, m, Ph) and 8.8 (1 H, s, NH); *m/z* (FAB⁺, *m*-nitrobenzoic acid–NaOAc) 566 (MNa⁺, 9%), 564 (MNa⁺, 25%), 562 (MNa⁺, 24%), 554 (33), 530 (25), 517 (6), 379 (7), 169 (35) and 91 (100) (Found: MNa⁺, 562.0407. C₂₁H₂₄Cl₃NO₉·Na⁺ requires *m/z* 562.0414).

O-(4,6-Di-O-acetyl-2,3-di-O-benzyl- α - and β -D-glucopyranosyl)trichloroacetimidate 16.—Compound **14** was activated as described for compound **15** to give a mixture of anomers (α : β , 1:4) of compound **16** as a gum (0.46 g, 83%), *R*_f 0.44 [ethyl acetate–light petroleum (1:1)]; [α]_D²² +4.6 (*c* 0.65, CHCl₃); ν_{\max} (CDCl₃)/cm⁻¹ 1740 (C=O); δ_{H} (200 MHz; CDCl₃) (α anomer) 1.97 and 2.06 (6 H, 2 s, Ac), 3.69–4.33 (5 H, m, 2-, 3- and 5-H and 6-H₂), 4.62–4.99 (4 H, m, PhCH₂), 5.11–5.20 (1 H, m, 4-H), 6.48 (1 H, d, *J* 3.5, 1-H), 7.17–7.46 (10 H, m, Ph) and 8.68 (1 H, s, NH); (β anomer) 1.94 and 2.08 (6 H, 2 s, Ac), 3.69–4.32 (5 H, m, 2-, 3- and 5-H and 6-H₂), 4.62–4.99 (4 H, m, PhCH₂), 5.11–5.20 (1 H, m, 4-H), 5.83 (1 H, d, *J* 7.4, 1-H), 7.17–7.46 (10 H, m, Ph) and 8.74 (1 H, s, NH).

Methyl 3-O-Acetyl-2-O-benzyl-4,6-O-benzylidene- β -D-glucopyranoside 17.—A solution of methyl 2-O-benzyl-4,6-O-benzylidene- β -D-glucopyranoside¹⁵ (5.1 g, 14 mmol) in pyridine (204 cm³)–acetic anhydride (102 cm³) was stirred for 1.5 h. The solvent was removed under reduced pressure and the residue was coevaporated with toluene to give compound **17** as a powdery solid (5.72 g, 100%), *R*_f 0.56 [ethyl acetate–light petroleum (1:1)]; m.p. 125–127 °C; [α]_D²² –20.5 (*c* 0.55, CHCl₃); ν_{\max} (CDCl₃)/cm⁻¹ 1740 (C=O); δ_{H} (500 MHz; CDCl₃) 1.98 (3 H, s, Ac), 3.42 (1 H, dd, *J* 7.6 and 9.2, 2-H), 3.51 (1 H, td, *J* 4.9 and 9.7, 5-H), 3.58 (1 H, t, *J* 9.5, 4-H), 3.61 (3 H, s, OMe), 3.78 (1 H, t, *J* 10.2, 6-H), 4.37 (1 H, dd, *J* 4.9 and 10.5, 6-H), 4.51 (1 H, d, *J* 7.6, 1-H), 4.64 (1 H, d, *J* 11.9, PhCH₂), 4.86 (1 H, d, *J* 11.9, PhCH₂), 5.31 (1 H, t, *J* 9.4, 3-H), 5.49 (1 H, s, PhCH) and 7.30–7.45 (10 H, m, Ph); δ_{C} (50 MHz; CDCl₃) 20.82 (COMe), 57.55 (OMe), 66.07, 72.51, 78.78 and 79.75 (C-2, -3, -4 and -5), 68.73 and 74.41 (C-6 and PhCH₂), 101.45 (PhCH), 105.34 (C-1), 126.34, 127.94, 128.20, 128.43, 128.57 and 129.25 (Ph), 137.19 and 138.35 (q-Ph) and 170.24 (C=O); *m/z* (DCI, NH₃) 432 (MNH₄⁺, 11%), 415 (MH⁺, 20%), 383 (12), 309 (20), 205 (23), 121 (58), 108 (29) and 91 (100).

Methyl 3-O-Acetyl-2-O-benzyl- β -D-glucopyranoside 18.—A solution of compound **17** (5.7 g, 14 mmol) and PTSA (0.143 g, 0.75 mmol) in methanol (95 cm³)–dichloromethane (24 cm³) was stirred for 8 h at room temperature. The reaction mixture was neutralised with solid sodium hydrogen carbonate, filtered, and concentrated under reduced pressure. The residue was purified by flash silica chromatography (ethyl acetate) to give compound **18** as a solid (4.25 g, 95%), *R*_f 0.30 (EtOAc); m.p. 89–91 °C; [α]_D²² +25.8 (*c* 0.4, CHCl₃); ν_{\max} (CDCl₃)/cm⁻¹ 3610 (OH); δ_{H} (200 MHz; CDCl₃) 2.04 (3 H, s, Ac), 2.93 (1 H, br s, OH), 3.29–3.42 (2 H, m, 2- and 4-H), 3.59 (3 H, s, OMe), 3.60 (1 H, m, 5-H), 3.88 (2 H, m, 6-H), 4.42 (1 H, d, *J* 7.7, 1-H), 4.53 (1 H, d, *J* 11.9, PhCH₂), 4.86 (1 H, d, *J* 11.7, PhCH₂), 4.96 (1 H, dd, *J* 9.3 and 9.4, 3-H) and 7.29–7.35 (5 H, m, Ph); δ_{C} (50 MHz; CDCl₃) 20.69 (COMe), 57.34 (OMe), 62.09 and 74.31 (C-6 and PhCH₂), 69.60, 75.40, 77.16 and 78.92 (C-2, -3, -4 and -5), 104.67 (C-1), 127.91, 128.09 and 128.53 (Ph), 138.42 (q-Ph) and 172.26 (C=O); *m/z* (DCI, NH₃) 344 (MNH₄⁺, 23%), 327 (MH⁺, 14%), 312 (50), 295 (46), 222 (21), 163 (22), 154 (23), 108 (97) and 91 (100).

Methyl 3-O-Acetyl-2-O-benzyl- β -D-glucopyranosiduronic Acid 19.—To a solution of compound **18** (800 mg, 2.45 mmol) in dichloromethane (7.2 cm³) containing 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO) (4 mg) was added saturated aq.

sodium hydrogen carbonate (4.8 cm³) containing potassium bromide (26.4 mg) and tetrabutylammonium chloride (35.2 mg). The mixture was cooled to 0 °C and aq. sodium hypochlorite (1.3 mol dm⁻³; 6 cm³), saturated aq. sodium hydrogen carbonate (2.64 cm³) and brine (5.28 cm³) were added dropwise over a period of 45 min. The two layers were separated and the organic phase was washed with water (3 × 20 cm³). The combined aqueous extracts were acidified with 4 mol dm⁻³ hydrochloric acid and extracted with ethyl acetate (5 × 100 cm³), and the organic phases were dried (Na₂SO₄), filtered, and concentrated under reduced pressure to give crude compound **19** as a gum (760 mg, 83%), used directly in the next reaction.

Methyl (Methyl 3-O-Acetyl-2-O-benzyl-β-D-glucopyranosid)-uronate 20.—Compound **19** (720 mg, 2.21 mmol) and Dowex 50H⁺ (2.09 g) were stirred in dry methanol (70 cm³) at room temperature under argon for 48 h. The mixture was filtered, and concentrated under reduced pressure. The residue was purified by flash silica chromatography [ethyl acetate–light petroleum (1 : 1)] to give compound **20** as a gum (450 mg, 60%), *R*_f 0.29 [ethyl acetate–light petroleum (1 : 1)] (Found: C, 57.7; H, 6.2. C₁₇H₂₂O₈ requires C, 57.62; H, 6.26%); [α]_D²² +4.0 (c 0.1, CHCl₃); *v*_{max}(CDCl₃)/cm⁻¹ 3520 (OH) and 1750 (C=O); δ_H(500 MHz; CDCl₃) 2.03 (3 H, s, Ac), 3.08 (1 H, br s, OH), 3.39 (1 H, dd, *J* 7.7 and 9.5, 2-H), 3.60 (3 H, s, OMe), 3.84 (3 H, s, CO₂Me), 3.83–4.00 (2 H, m, 4- and 5-H), 4.43 (1 H, d, *J* 7.6, 1-H), 4.61 (1 H, d, *J* 11.8, PhCH₂), 4.88 (1 H, d, *J* 11.8, PhCH₂), 5.05 (1 H, t, *J* 9.3, 3-H) and 7.31–7.36 (5 H, m, Ph); δ_C(50 MHz; CDCl₃) 20.79 (COMe), 52.81 and 57.52 (OMe and CO₂Me), 70.55, 74.29, 74.40, 75.60 and 78.29 (C-2, -3, -4, -5 and PhCH₂), 104.87 (C-1), 127.94, 128.12, 128.35 and 128.53 (Ph), 138.25 (q-Ph) and 171.55 (C=O); *m/z* (Probe CI, NH₃) 372 (MNH₄⁺, 24%), 355 (MH⁺, 4%), 340 (31), 323 (82), 245 (22), 132 (29), 108 (37), 91 (100) and 60 (33).

Methyl 2-O-Benzyl-4,6-O-benzylidene-α-L-idopyranoside 21 and Methyl 3-O-Benzyl-4,6-O-benzylidene-α-L-idopyranoside 22.—Methyl 4,6-O-benzylidene-α-L-idopyranoside²² (1.37 g, 4.86 mmol), tetrabutylammonium hydrogen sulfate (329 mg, 0.96 mmol) and benzyl bromide (1 cm³, 8.3 mmol) were dissolved in dichloromethane (82 cm³). Aq. sodium hydroxide (6.85 cm³ of a 5% solution) was added and the mixture was refluxed for 48 h. The two layers were separated, and the chloroform layer was washed with water (3 × 25 cm³), dried (Na₂SO₄), filtered, and concentrated under reduced pressure. The mixture was purified by flash silica chromatography [ethyl acetate–light petroleum (1 : 1)] to yield compound **22** as a solid (392 mg, 22%), compound **21** as a gum (172 mg, 10%), and starting material (0.86 g, 63% recovery). Compound **21**: *R*_f 0.33 [ethyl acetate–light petroleum (1 : 1)]; [α]_D²² –81.0 (c 0.3, CHCl₃); *v*_{max}(CHCl₃)/cm⁻¹ 3600 (OH); δ_H(500 MHz; CDCl₃) 3.46 (3 H, s, OMe), 3.53 (1 H, dd, *J* 2.6 and 4.7, 2-H), 3.82 (1 H, d, *J* 1.8, 5-H), 4.07–4.10 (2 H, m, 3- and 4-H), 4.15 (1 H, dd, *J* 2.3 and 12.8, 6-H), 4.34 (1 H, dd, *J* 1.3 and 12.7, 6-H), 4.65 (1 H, d, *J* 11.6, PhCH₂), 4.69 (1 H, d, *J* 11.6, PhCH₂), 4.96 (1 H, d, *J* 2.6, 1-H), 5.53 (1 H, s, PhCH) and 7.28–7.52 (10 H, m, Ph); *m/z* (Probe CI, NH₃) 390 (MNH₄⁺, 50%), 373 (MH⁺, 13%), 341 (62), 323 (31), 108 (37), 91 (100) and 75 (41) (Found: MNH₄⁺, 390.1917. C₂₁H₂₄O₆·NH₄⁺ requires *m/z* 390.1917).

Compound **22**: *R*_f 0.46 [ethyl acetate–light petroleum (1 : 1)]; [α]_D²² –83.0 (c 0.3, CHCl₃); *v*_{max}(CHCl₃)/cm⁻¹ 3590 (OH); δ_H(500 MHz; CDCl₃) 3.25 (1 H, d, *J* 11.0, OH), 3.49 (3 H, s, OMe), 3.77–3.82 (2 H, m, 2- and 3-H), 3.98 (1 H, d, *J* 1.5, 5-H), 4.08–4.11 (2 H, m, 4- and 6-H), 4.33 (1 H, dd, *J* 1.7 and 12.5, 6-H), 4.62 (1 H, d, *J* 12.0, PhCH₂), 4.76 (1 H, d, *J* 12.0, PhCH₂), 4.89 (1 H, s, 1-H), 5.53 (1 H, s, PhCH) and 7.29–7.49 (10 H, m, Ph); *m/z* (Probe CI, NH₃) 390 (MNH₄⁺, 28%), 373 (MH⁺, 12%),

341 (100), 252 (29), 108 (43) and 91 (59) (Found: MNH₄⁺, 390.1917).

Methyl 3-O-Acetyl-2-O-benzyl-4,6-O-benzylidene-α-L-idopyranoside 23.—A solution of compound **21** (300 mg, 0.81 mmol) in pyridine (11.6 cm³)–acetic anhydride (5.8 cm³) was stirred at room temperature for 2 h. The mixture was concentrated under reduced pressure and the residue was coevaporated with toluene to give compound **23** as a pale yellow gum (310 mg, 93%), *R*_f 0.62 (EtOAc); [α]_D²² –91.7 (c 0.65, CHCl₃); *v*_{max}(CHCl₃)/cm⁻¹ 1740 (C=O) δ_H(500 MHz; CDCl₃) 2.09 (3 H, s, Ac), 3.43 (3 H, s, OMe), 3.52 (1 H, dd, *J* 2.7 and 4.7, 2-H), 3.84 (1 H, d, *J* 1.7, 5-H), 4.04 (1 H, dd, *J* 2.3 and 2.4, 4-H), 4.14 (1 H, dd, *J* 2.1 and 12.7, 6-H), 4.33 (1 H, dd, *J* 1.3 and 12.6, 6-H), 4.67 (1 H, d, *J* 11.9, PhCH₂), 4.74 (1 H, d, *J* 11.9, PhCH₂), 4.92 (1 H, d, *J* 2.6, 1-H), 5.22 (1 H, dd, *J* 3.0 and 4.6, 3-H), 5.54 (1 H, s, PhCH) and 7.25–7.53 (10 H, m, Ph); δ_C(125.78 MHz; CDCl₃) 20.97 (COMe), 55.29 (OMe), 60.57 (C-6), 69.01, 70.18, 72.22, 74.07 and 74.16 (C-2, -3, -4, -5 and PhCH₂), 100.96 and 101.62 (C-1 and PhCH), 126.49, 127.44, 127.66, 128.07, 128.19 and 128.87 (Ph), 137.96 and 138.19 (q-Ph) and 169.57 (C=O); *m/z* (Probe CI, NH₃) 432 (MNH₄⁺, 8%), 383 (87), 187 (41), 149 (39), 108 (25), 105 (28) and 91 (100) (Found: MNH₄⁺, 432.2022. C₂₃H₂₆O₇·NH₄⁺ requires *m/z* 432.2022).

Methyl 2-O-Acetyl-3-O-benzyl-4,6-O-benzylidene-α-L-idopyranoside 24.—Compound **22** (662 mg, 1.8 mmol) was acetylated as described for compound **23** to give compound **24** as gum (730 mg, 99%), *R*_f 0.54 [ethyl acetate–light petroleum (1 : 1)]; [α]_D²² –84.3 (c 0.6, CHCl₃); *v*_{max}(CDCl₃)/cm⁻¹ 1730 (C=O); δ_H(500 MHz; CDCl₃) 2.07 (3 H, s, Ac), 3.46 (3 H, s, OMe), 3.74 (1 H, dd, *J* 3.0 and 3.3, 3-H), 3.94 (1 H, d, *J* 1.6, 5-H), 4.03 (1 H, dd, *J* 1.9 and 2.0, 4-H), 4.13 (1 H, dd, *J* 1.9 and 12.6, 6-H), 4.32 (1 H, dd, *J* 1.5 and 12.6, 6-H), 4.68 (1 H, d, *J* 12.2, PhCH₂), 4.81 (1 H, d, *J* 12.2, PhCH₂), 4.88 (1 H, s, 1-H), 5.02 (1 H, dd, *J* 2.3 and 3.6, 2-H), 5.53 (1 H, s, PhCH) and 7.29–7.53 (10 H, m, Ph); *m/z* (Probe CI, NH₃) 432 (MNH₄⁺, 14%), 383 (100), 187 (33), 149 (22), 108 (21) and 91 (46) (Found: MNH₄⁺, 432.2022).

Methyl 3-O-Acetyl-2-O-benzyl-α-L-idopyranoside 25.—PTSA (7.8 mg, 0.045 mmol) was added to a solution of compound **23** (310 mg, 0.75 mmol) in methanol (5.2 cm³)–dichloromethane (1.3 cm³) and the mixture was stirred at room temperature under argon for 24 h. The mixture was neutralised with solid sodium hydrogen carbonate, filtered, and concentrated under reduced pressure. The residue was purified by flash silica chromatography (ethyl acetate) to give compound **25** as a gum (172 mg, 70%), *R*_f 0.46 (EtOAc); [α]_D²² –58.8 (c 1.6, CHCl₃); *v*_{max}(CDCl₃)/cm⁻¹ 3360 (OH) and 1740 (C=O); δ_H(500 MHz; CDCl₃) 2.08 (3 H, s, Ac), 3.39 (3 H, s, OMe), 3.61 (1 H, m, 2-H), 3.70 (1 H, br s, 4-H), 3.82 (1 H, dd, *J* 4.4 and 11.8, 6-H), 3.96 (1 H, dd, *J* 6.6 and 11.8, 6-H), 4.09 (1 H, m, 5-H), 4.67 (1 H, d, *J* 11.7, PhCH₂), 4.76 (1 H, d, *J* 11.6, PhCH₂), 4.77 (1 H, s, 1-H), 5.02 (1 H, td, *J* 1.2 and 3.2, 3-H) and 7.31–7.39 (5 H, m, Ph); δ_C(125.78 MHz; CDCl₃) 20.93 (COMe), 55.55 (OMe), 63.21 (C-6), 66.98, 67.93, 68.09, 71.93 and 74.88 (C-2, -3, -4, -5 and PhCH₂), 99.80 (C-1), 127.73, 127.86 and 128.43 (Ph), 137.69 (q-Ph) and 169.13 (C=O); *m/z* (Probe CI, NH₃) 344 (MNH₄⁺, 35%), 327 (MH⁺, 17%), 312 (80), 295 (100), 108 (63) and 91 (67) (Found: MNH₄⁺, 344.1709. C₁₆H₂₂O₇·NH₄⁺ requires *m/z* 344.1709).

Methyl 2-O-Acetyl-3-O-benzyl-α-L-idopyranoside 26.—The benzylidene group of compound **24** (720 mg, 1.7 mmol) was removed as described for compound **25** to give compound **26** as a gum (500 mg, 88%), *R*_f 0.4 (EtOAc); [α]_D²² –62.3 (c 0.3, CHCl₃); *v*_{max}(CDCl₃)/cm⁻¹ 3480 (OH), 1750 (C=O); δ_H(200 MHz; CDCl₃) 2.12 (3 H, s, Ac), 3.45 (3 H, s, OMe), 3.62 (1 H, m, 3-H), 3.72 (1 H, br s, 4-H), 3.83 (1 H, dd, *J* 4.2 and 11.9, 6-H), 3.97

(1 H, dd, J 6.0 and 12.4, 6-H), 4.21 (1 H, m, 5-H), 4.57–4.82 (3 H, m, 1-H and PhCH_2), 5.00 (1 H, m, 2-H) and 7.27–7.35 (5 H, m, Ph); δ_{C} (125.78 MHz; CDCl_3) 20.91 (COMe), 55.53 (OMe), 63.27 (C-6), 67.14, 68.07, 68.21, 71.99 and 75.09 (C-2, -3, -4, -5 and PhCH_2), 99.90 (C-1), 127.69, 127.74, 127.80, 127.87 and 128.45 (Ph), 137.79 (q-Ph) and 169.09 (C=O); m/z (Probe CI, NH_3) 344 (MNH_4^+ , 17%), 312 (15), 295 (100), 270 (23), 108 (64) and 91 (98) (Found: MNH_4^+ , 344.1709).

Methyl 3-O-Acetyl-2-O-benzyl- α -L-idopyranosiduronic Acid 27.—Compound **25** (170 mg, 0.52 mmol) was oxidised as described for compound **19** to give crude compound **27** as a gum (127 mg, 72%), used directly in the next reaction.

Methyl (Methyl 3-O-Acetyl-2-O-benzyl- α -L-idopyranosid)uronate 29.—Crude compound **27** (127 mg, 0.37 mmol) was esterified as described for compound **20** to give compound **29** as a gum (54 mg, 41%), R_f 0.26 [ethyl acetate–light petroleum (1:1)]; $[\alpha]_{\text{D}}^{22} -46.8$ (c 0.4, CHCl_3); ν_{max} (CDCl_3)/ cm^{-1} 3520 (OH) and 1740 (C=O); δ_{H} (200 MHz; CDCl_3) 2.08 (3 H, s, Ac), 3.41 (3 H, s, OMe), 3.54 (1 H, m, 2-H), 3.84 (3 H, s, CO_2Me), 4.02 (1 H, m, 4-H), 4.72 (3 H, m, 5-H and PhCH_2), 4.88 (1 H, s, 1-H), 5.06 (1 H, m, 3-H) and 7.30–7.35 (5 H, m, Ph); δ_{C} (50 MHz; CDCl_3) 20.86 (COMe), 52.39 and 55.99 (CO_2Me and OMe), 67.51, 67.82, 67.93 and 71.89 (C-2, -3, -4 and -5), 72.77 (PhCH_2), 100.10 (C-1), 128.27, 128.46 and 128.76 (Ph), 136.74 (q-Ph), 170.02 and 170.16 (CO_2Me and COMe); m/z (Probe CI, NH_3) 372 (MNH_4^+ , 9%), 323 (29), 245 (15), 132 (21) and 91 (100) (Found: MNH_4^+ , 372.1658. $\text{C}_{17}\text{H}_{22}\text{O}_8\cdot\text{NH}_4^+$ requires m/z 372.1658).

Methyl 2-O-Acetyl-3-O-benzyl- α -L-idopyranosiduronic Acid 28.—Compound **26** (450 mg, 1.38 mmol) was oxidised as described for compound **19** to give crude compound **28** as a gum (212 mg, 45%), used directly in the next reaction.

Methyl (Methyl 2-O-Acetyl-3-O-benzyl- α -L-idopyranosid)uronate 30.—Crude compound **28** (212 mg, 0.62 mmol) was esterified as described for compound **20** to give compound **30** as a gum (54 mg, 24%), R_f 0.27 [ethyl acetate–light petroleum (1:1)]; $[\alpha]_{\text{D}}^{22} -48.8$ (c 0.4, CHCl_3); ν_{max} (CHCl_3)/ cm^{-1} 3510 (OH) and 1750 (C=O); δ_{H} (200 MHz; CDCl_3) 2.09 (3 H, s, Ac), 2.76 (1 H, br d, J 11.6, OH), 3.47 (3 H, s, OMe), 3.67 (1 H, m, 3-H), 3.82 (3 H, s, CO_2Me), 4.02 (1 H, br d, J 10.2, 4-H), 4.62 (1 H, d, J 12.0, PhCH_2), 4.77 (1 H, d, J 12.1, PhCH_2), 4.83 (2 H, m, 1- and 5-H), 4.97 (1 H, t, J 1.2, 2-H) and 7.28–7.37 (5 H, m, Ph); δ_{C} (50 MHz; CDCl_3) 20.82 (COMe), 52.41 and 56.32 (OMe and CO_2Me), 66.87, 67.45, 67.88 and 73.75 (C-2, -3, -4 and -5), 71.82 (PhCH_2), 99.92 (C-1), 127.98, 128.17 and 128.67 (Ph), 137.52 (q-Ph), 169.37 and 170.24 (CO_2Me and COMe); m/z (Probe CI, NH_3) 372 (MNH_4^+ , 6%), 323 (42) and 91 (100) (Found: MNH_4^+ , 372.1658).

General Procedure for Coupling.—Trichloroacetimidate (0.31 mmol) and uronate (0.28 mmol) were dissolved in dry dichloromethane (2.8 cm^3) containing 4 Å molecular sieves and the mixture was cooled to -20°C under argon. Trimethylsilyl trifluoromethanesulfonate (2.14 cm^3 , 11.07 mmol) in dry dichloromethane (0.3 cm^3) was added dropwise during 5 min. After 20 min the reaction was neutralised by addition of saturated aq. sodium hydrogen carbonate (8.6 cm^3). The two layers were separated, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The mixture was purified by flash silica chromatography [ethyl acetate–light petroleum (1:1)] to give the coupled product.

Methyl [Methyl 3-O-Acetyl-2-O-benzyl-4-O-(4',6'-di-O-acetyl-2',3'-di-O-benzyl- α -D-glucopyranosyl)- α -D-glucopyranosid]-

uronate 31.—Trichloroacetimidate **16** (183 mg, 0.31 mmol) and uronate **20** (100 mg, 0.28 mmol) were coupled as described to give compound **31** as a gum (115 mg, 52%), R_f [ethyl acetate–light petroleum (1:1)]; $[\alpha]_{\text{D}}^{22} +38.7$ (c 1.5, CHCl_3); ν_{max} (CDCl_3)/ cm^{-1} 1740 (C=O); δ_{H} (500 MHz; CDCl_3) 1.84, 1.85 and 2.05 (9 H, 3 s, Ac), 3.43 (3 H, s, OMe), 3.45 (1 H, dd, J 3.4 and 9.8, 2'-H), 3.58 (1 H, dd, J 3.9 and 10.5, 2-H), 3.73 (3 H, s, CO_2Me), 3.82–3.86 (2 H, m, 3'- and 5'-H), 3.95 (1 H, t, J 9.5, 4-H), 3.98 (1 H, dd, J 2.3 and 12.6, 6'-H), 4.12 (1 H, dd, J 3.5 and 12.5, 6'-H), 4.16 (1 H, d, J 9.7, 5-H), 4.59–4.83 (6 H, m, PhCH_2), 4.68 (1 H, d, J 3.6, 1-H), 4.89 (1 H, d, J 3.3, 1'-H), 4.96 (1 H, dd, J 9.6 and 10.1, 4'-H), 5.58 (1 H, dd, J 9.5 and 9.8, 3-H) and 7.27–7.38 (15 H, m, Ph); δ_{C} (50 MHz; CDCl_3) 20.63 and 20.87 (COMe), 52.78 and 55.77 (OMe and CO_2Me), 61.64 (C-6), 69.00, 70.67, 71.12, 76.45, 77.96, 78.11 and 79.98 (C-2, -3, -4, -5, -2', -3', -4' and -5'), 73.13, 73.60 and 75.31 (PhCH_2), 98.53 (C-1 and -1'), 127.89, 128.15, 128.29, 128.41, 128.63 and 128.74 (Ph), 137.88, 138.02 and 138.61 (q-Ph), 169.51, 169.98, 170.42 and 171.17 (COMe and CO_2Me); m/z (DCI, NH_3) 798 (MNH_4^+ , 24%), 187 (25), 108 (89) and 91 (100) (Found: MNH_4^+ , 798.334. $\text{C}_{41}\text{H}_{48}\cdot\text{O}_{15}\cdot\text{NH}_4^+$ requires m/z 798.3337).

Methyl [Methyl 3-O-Acetyl-2-O-benzyl-4-O-(3',4',6'-tri-O-acetyl-2'-O-benzyl- α -D-glucopyranosyl)- α -D-glucopyranosid]-uronate 32.—Trichloroacetimidate **15** (114 mg, 0.21 mmol) and uronate **20** (70 mg, 0.2 mmol) were coupled as described to give compound **32** as a gum (77 mg, 53%), R_f 0.33 [ethyl acetate–light petroleum (1:1)]; $[\alpha]_{\text{D}}^{22} +106.2$ (c 2, CHCl_3); ν_{max} (CDCl_3)/ cm^{-1} 1750 (C=O); δ_{H} (500 MHz; CDCl_3) 1.90, 1.96, 2.00 and 2.07 (12 H, 4 s, Ac), 3.42 (3 H, s, OMe), 3.48 (1 H, dd, J 3.5 and 10.0, 2'-H), 3.54 (1 H, dd, J 3.4 and 10.0, 2-H), 3.79 (3 H, s, CO_2Me), 3.85–3.89 (1 H, m, 5'-H), 4.00–4.07 (2 H, m, 4- and 6'-H), 4.21 (1 H, dd, J 3.6 and 12.6, 6'-H), 4.24 (1 H, d, J 9.7, 5-H), 4.48–4.65 (4 H, m, PhCH_2), 4.67 (1 H, d, J 3.4, 1-H), 4.94 (1 H, dd, J 9.7 and 10.0, 4'-H), 5.06 (1 H, d, J 3.6, 1'-H), 5.29 (1 H, dd, J 9.7 and 9.8, 3'-H), 6.00 (1 H, dd, J 9.4 and 9.7, 3-H) and 7.28–7.36 (10 H, m, Ph); δ_{C} (125.78 MHz; CDCl_3) 20.52, 20.63 and 20.82 (COMe), 52.78 and 55.74 (OMe and CO_2Me), 61.40 (C-6'), 68.11, 68.29, 70.25, 71.38, 71.54, 76.70 and 76.98 (C-2, -3, -4, -5, -2', -3', -4' and -5'), 73.00 (PhCH_2), 97.09 and 98.38 (C-1 and -1'), 128.05, 128.24, 128.49, 128.53 and 128.70 (Ph), 137.73 and 137.83 (q-Ph), 169.97, 170.07 and 171.01 (C=O); m/z (DCI, NH_3) 750 (MNH_4^+ , 12%), 213 (15), 186 (18), 152 (19), 139 (20), 108 (92) and 91 (100) (Found: MNH_4^+ , 750.297. $\text{C}_{36}\text{H}_{44}\text{O}_{16}\cdot\text{NH}_4^+$ requires m/z , 750.2973).

Methyl [Methyl 3-O-Acetyl-2-O-benzyl-4-O-(3',4',6'-tri-O-acetyl-2'-O-benzyl- α -D-glucopyranosyl)- α -L-idopyranosid]-uronate 37. Trichloroacetimidate **15** (134 mg, 0.25 mmol) and uronate **29** (80 mg, 0.23 mmol) were coupled as described to give compound **37** as a gum (76 mg, 46%), R_f 0.24 [ethyl acetate–light petroleum (1:1)]; $[\alpha]_{\text{D}}^{22} +56.5$ (c 0.6, CHCl_3); ν_{max} (CHCl_3)/ cm^{-1} 1750 (C=O); δ_{H} (500 MHz; CDCl_3) 1.95, 2.00, 2.01 and 2.08 (12 H, 4 s, Ac), 3.38 (1 H, dd, J 4.2 and 4.3, 2-H), 3.45 (3 H, s, OMe), 3.53 (1 H, dd, J 3.5 and 10.0, 2'-H), 3.87 (3 H, s, CO_2Me), 4.07–4.10 (3 H, s, 4-, 5'- and 6'-H), 4.25 (1 H, dd, J 4.3 and 12.9, 6'-H), 4.47–4.72 (4 H, m, PhCH_2), 4.69 (1 H, d, J 3.6, 5-H), 4.94–4.98 (2 H, m, 1- and 4'-H), 5.09 (1 H, d, J 3.5, 1'-H), 5.35–5.44 (2 H, m, 3- and 3'-H) and 7.24–7.38 (10 H, m, Ph); δ_{C} (50 MHz; CDCl_3) 20.53, 20.61 and 20.86 (COMe), 52.42 and 56.17 (OMe and CO_2Me), 61.66 (C-6'), 68.23, 68.93, 69.42, 71.58, 74.17, 74.82 and 76.42 (C-2, -3, -4, -5, -2', -3', -4' and -5'), 72.40 and 72.56 (PhCH_2), 97.19 and 101.45 (C-1 and -1'), 127.84, 127.97, 128.27, 128.48, 128.59 and 128.78 (Ph), 137.92 and 138.02 (q-Ph), 169.73, 169.83, 170.20 and 171.00 (C=O); m/z (DCI, NH_3) 750 (MNH_4^+ , 100%), 660 (10) and 91 (35) (Found: MNH_4^+ , 750.297).

Methyl [Methyl 2-O-Acetyl-3-O-benzyl-4-O-(3',4',6'-tri-O-acetyl-2'-O-benzyl- α -D-glucopyranosyl)- α -L-idopyranosid]uronate 38.—Trichloroacetimidate **15** (84 mg, 0.16 mmol) and uronate **30** (50 mg, 0.14 mmol) were coupled as described to give compound **38** as a gum (42 mg, 41%), R_f 0.24 [ethyl acetate–light petroleum (1:1)]; $[\alpha]_D^{22} + 38.0$ (c 0.4, CHCl_3); ν_{max} (CHCl_3)/ cm^{-1} 1740 (C=O); δ_{H} (500 MHz; CDCl_3) 1.94, 1.98, 2.07 and 2.09 (12 H, 4 s, Ac), 3.43 (1 H, dd, J 3.4 and 10.0, 2'-H), 3.46 (3 H, s, OMe), 3.81 (3 H, s, CO_2Me), 3.87 (1 H, td, J 1.2, 2.3 and 3.4, 3-H), 3.98 (1 H, t, J 2.6, 4-H), 4.10–4.15 (2 H, m, 5'- and 6'-H), 4.24 (1 H, dd, J 3.2 and 12.7, 6'-H), 4.28 (1 H, d, J 12.2, PhCH_2), 4.37 (1 H, d, J 12.2, PhCH_2), 4.57 (1 H, d, J 12.2, PhCH_2), 4.76–4.79 (3 H, m, 1'-H, 5-H and PhCH_2), 4.90–4.91 (2 H, m, 1- and 2-H), 4.94 (1 H, dd, J 9.4 and 10.3, 4'-H), 5.35 (1 H, dd, J 9.5 and 9.8, 3'-H) and 7.15–7.37 (10 H, m, Ph); δ_{C} (125.78 MHz; CDCl_3) 20.49, 20.63, 20.67 and 20.84 (COMe), 52.16 and 56.02 (OMe and CO_2Me), 61.57 (C-6'), 67.00, 67.25, 68.21, 68.41, 71.92, 72.00, 72.92, 73.62 and 77.27 (C-2, -3, -4, -5, -2', -3', -4', -5' and PhCH_2), 99.49 and 100.16 (C-1 and -1'), 127.65, 127.80, 127.92, 128.06, 128.18, 128.39 and 128.49 (Ph), 137.49 and 137.75 (q-Ph), 169.73, 169.86 and 170.57 (C=O); m/z (DCI, NH_3) 750 (MNH_4^+ , 60%), 259 (20), 186 (20), 152 (27), 108 (89) and 91 (100) (Found: MNH_4^+ , 750.297).

General Procedure for Hydrogenation.—A solution of the fully protected disaccharide (0.096 mmol) and 10% palladium on activated carbon (44 mg) in methanol (7.4 cm^3) was hydrogenated at room temperature and atmospheric pressure for 7 h. The mixture was filtered, and concentrated under reduced pressure to give the debenzylated disaccharide.

Methyl [Methyl 3-O-Acetyl-4-O-(4',6'-di-O-acetyl- α -D-glucopyranosyl)- α -D-glucopyranosid]uronate 33.—Compound **31** (100 mg, 0.13 mmol) was hydrogenated as described to give compound **33** as a gum (65 mg, 99%), R_f 0 (EtOAc); $[\alpha]_D^{22} + 87.7$ (c 0.3, MeOH); δ_{H} (500 MHz; CD_3OD) 2.05, 2.06 and 2.09 (9 H, 3 s, Ac), 3.38 (1 H, dd, J 3.8 and 9.9, 2'-H), 3.47 (3 H, s, OMe), 3.61 (1 H, dd, J 3.5 and 10.2, 2-H), 3.64 (1 H, dd, J 9.5 and 9.9, 3'-H), 3.69–3.71 (1 H, m, 5'-H), 3.77 (3 H, s, CO_2Me), 3.98 (1 H, dd, J 9.4 and 9.5, 4-H), 4.04 (1 H, dd, J 2.7 and 12.2, 6'-H), 4.12 (1 H, dd, J 3.8 and 12.4, 6'-H), 4.19 (1 H, d, J 9.7, 5-H), 4.76 (1 H, d, J 3.6, 1-H), 4.76 (1 H, dd, J 8.5 and 11.0, 4'-H), 4.99 (1 H, d, J 3.9, 1'-H) and 5.33 (1 H, dd, J 9.5 and 9.6, 3-H); δ_{C} (125.78 MHz; CD_3OD) 20.66, 20.85 and 21.34 (COMe), 53.22 and 56.25 (OMe and CO_2Me), 63.30 (C-6'), 69.84, 71.23, 72.03, 72.93, 73.25, 74.91, 78.09 and 79.38 (C-2, -3, -4, -5, -2', -3', -4' and -5'), 101.43 and 101.49 (C-1 and -1'), 170.66, 171.86, 172.50 and 172.60 (COMe and CO_2Me); m/z (DCI, NH_3) 528 (MNH_4^+ , 100%), 282 (57), 264 (28), 247 (43), 233 (21) and 204 (30) (Found: MNH_4^+ , 528.193. $\text{C}_{20}\text{H}_{30}\text{O}_{15}\cdot\text{NH}_4^+$ requires m/z , 528.1928).

Methyl [Methyl 3-O-Acetyl-4-O-(3',4',6'-tri-O-acetyl- α -D-glucopyranosyl)- α -D-glucopyranosid]uronate 34.—Compound **32** (70 mg, 0.096 mmol) was hydrogenated as described to give compound **34** as a gum (52 mg, 99%), R_f 0.11 [ethyl acetate–light petroleum (1:1)] $[\alpha]_D^{22} + 79.8$ (c 0.9, CHCl_3); ν_{max} (CDCl_3)/ cm^{-1} 3560 (OH) and 1740 (C=O); δ_{H} (500 MHz; CDCl_3) 2.03, 2.07, 2.11 and 2.13 (12 H, 4 s, Ac), 3.53 (3 H, s, OMe), 3.61–3.63 (2 H, m, 2- and 2'-H), 3.77–3.81 (1 H, m, 5'-H), 3.83 (3 H, s, CO_2Me), 4.08–4.12 (2 H, m, 4- and 6'-H), 4.23–4.28 (2 H, m, 5- and 6'-H), 4.84 (1 H, d, J 3.6, 1-H), 5.02 (1 H, dd, J 9.8 and 10.0, 4'-H), 5.10 (1 H, dd, J 9.8 and 9.9, 3'-H), 5.16 (1 H, d, J 4.0, 1'-H) and 5.32 (1 H, dd, J 9.4 and 9.5, 3-H); δ_{C} (125.78 MHz; CDCl_3) 20.52, 20.63, 20.70 and 20.94 (COMe), 52.74 and 56.06 (OMe and CO_2Me), 61.39 (C-6'), 67.56, 68.57, 70.53, 70.72, 70.76, 72.29, 74.40 and 76.41 (C-2, -3, -4, -5, -2', -3', -4' and -5'),

99.67 and 99.91 (C-1 and -1'), 168.79, 169.39, 170.66, 171.09 and 171.55 (C=O); m/z (Probe CI, NH_3) 570 (MNH_4^+ , 90%), 289 (100), 169 (27), 140 (30), 108 (25), 98 (42) and 60 (29) (Found: MNH_4^+ , 570.2034. $\text{C}_{22}\text{H}_{32}\text{O}_{16}\cdot\text{NH}_4^+$ requires m/z , 570.2034).

Methyl [Methyl 3-O-Acetyl-4-O-(3,4,6-tri-O-acetyl- α -D-glucopyranosyl)- α -L-idopyranosid]uronate 39.—Compound **37** (70 mg, 0.096 mmol) was hydrogenated as described to give compound **39** as a gum (49 mg, 93%), R_f 0.35 (EtOAc); $[\alpha]_D^{22} + 61.7$ (c 0.9, CHCl_3); ν_{max} (CHCl_3)/ cm^{-1} 3500 (OH) and 1750 (C=O); δ_{H} (500 MHz; CDCl_3) 2.02, 2.06, 2.10 and 2.11 (12 H, 4 s, Ac), 3.47 (3 H, s, OMe), 3.71–3.78 (2 H, m, 2- and 2'-H), 3.82–3.86 (1 H, m, 5'-H), 3.86 (3 H, s, CO_2Me), 4.09 (1 H, dd, J 2.3 and 12.4, 6'-H), 4.14 (1 H, dd, J 2.8 and 5.5, 4-H), 4.24 (1 H, dd, J 3.9 and 12.5, 6'-H), 4.77 (1 H, d, J 2.3, 5-H), 4.93 (1 H, s, 1-H), 5.00 (1 H, dd, J 9.8 and 10.0, 4'-H), 5.15 (2 H, m, 3- and 3'-H) and 5.22 (1 H, d, J 3.6, 1'-H); δ_{C} (125.78 MHz; CDCl_3) 20.53, 20.63, 20.77 and 20.84 (COMe), 52.49 and 56.17 (OMe and CO_2Me), 61.73 (C-6'), 66.77, 67.26, 67.32, 67.91, 68.54, 70.33, 72.01 and 73.26 (C-2, -3, -4, -5, -2', -3', -4' and -5'), 97.53 and 102.04 (C-1 and -1'), 169.52, 169.63, 169.72, 170.64 and 171.03 (CO_2Me and COMe); m/z (DCI, NH_3) 570 (MNH_4^+ , 49%), 528 (17), 331 (37), 289 (100), 264 (34), 247 (53), 229 (50), 204 (36), 187 (52), 169 (41), 127 (30), 98 (34) and 60 (38) (Found: MNH_4^+ , 570.203. $\text{C}_{22}\text{H}_{32}\text{O}_{16}\cdot\text{NH}_4^+$ requires m/z 570.2034).

Methyl [Methyl 2-O-Acetyl-4-O-(3,4,6-tri-O-acetyl- α -D-glucopyranosyl)- α -L-idopyranosid]uronate 40.—Compound **38** (39 mg, 0.053 mmol) was hydrogenated as described to give compound **40** as a gum (25 mg, 85%), R_f 0.37 (EtOAc); $[\alpha]_D^{22} + 42.2$ (c 0.5, CHCl_3); ν_{max} (CHCl_3)/ cm^{-1} 3510 (OH) and 1750 (C=O); δ_{H} (500 MHz; CDCl_3) 2.02, 2.05, 2.10 and 2.12 (12 H, 4 s, Ac), 3.49 (3 H, s, OMe), 3.67 (1 H, dd, J 3.8 and 9.8, 2'-H), 3.87 (3 H, s, CO_2Me), 3.88–3.90 (1 H, m, 5'-H), 4.11–4.17 (3 H, m, 3-, 4- and 6'-H), 4.23 (1 H, dd, J 4.0 and 12.4, 6'-H), 4.77–4.83 (1 H, m, 5-H), 4.82 (1 H, d, J 2.4, 1-H), 4.95 (1 H, s, 2-H), 5.00 (1 H, dd, J 9.7 and 10.1, 4'-H), 5.08 (1 H, d, J 3.8, 1'-H) and 5.15 (1 H, t, J 9.7, 3'-H); δ_{C} (125.78 MHz; CDCl_3) 20.54, 20.66, 20.70 and 20.77 (COMe), 52.56 and 56.45 (OMe and CO_2Me), 61.63 (C-6'), 65.52, 66.77, 67.74, 68.48, 68.55, 70.60, 73.20 and 74.00 (C-2, -3, -4, -5, -2', -3', -4' and -5'), 97.51 and 99.32 (C-1 and -1'), 169.33, 169.48, 170.06 and 170.68 (C=O); m/z (DCI, NH_3) 570 (MNH_4^+ , 42%), 289 (100), 233 (36), 229 (43), 187 (42), 169 (40), 127 (44), 98 (33), 81 (23) and 60 (56) (Found: MNH_4^+ , 570.203).

General Procedure for O-Sulfation.—A solution of the debenzylated disaccharide (0.096 mmol) and the sulfur trioxide–trimethylamine complex (5 mol equiv. for every hydroxy group to be sulfated) in DMF (1.5 cm^3) was stirred at 50 °C for 48 h and then cooled. Methanol (0.74 cm^3) was added and the mixture was layered onto a column of Sephadex LH-20 and eluted with chloroform–methanol (1:1) to give the crude O-sulfated disaccharides.

Methyl [Methyl 3-O-Acetyl-4-O-(4',6'-di-O-acetyl-2',3'-di-O-sulfo- α -D-glucopyranosyl)-2-O-sulfo- α -D-glucopyranosid]uronate, Trisodium Salt 35.—Compound **33** (49 mg, 0.096 mmol) was sulfated as described. The product was further purified by flash silica chromatography [ethyl acetate–pyridine–acetic acid–water (8:5:1:3)] and then by ion-exchange chromatography (Dowex Na^+) in water to give compound **35** as a gum (38 mg, 48%), R_f 0.27 [ethyl acetate–pyridine–acetic acid–water (8:5:1:3)]; $[\alpha]_D^{22} + 56.5$ (c 1.1, MeOH); δ_{H} (500 MHz; CD_3OD) 2.06 and 2.21 (9 H, 2 s, Ac), 3.47 (3 H, s, OMe), 3.79 (3 H, s, CO_2Me), 3.81 (1 H, m, 5'-H), 4.12–4.27 (5 H, m, 2', 4-, 5-H and 6'-H₂), 4.30 (1 H, dd, J 3.6 and 10.1, 2-H), 4.62 (1 H,

dd, *J* 9.5 and 10.0, 3'-H), 5.02 (1 H, dd, *J* 9.5 and 10.1, 4'-H), 5.09 (1 H, d, *J* 3.5, 1-H), 5.35 (1 H, d, *J* 3.5, 1'-H) and 5.48 (1 H, dd, *J* 8.1 and 10.1, 3-H); δ_{C} (500 MHz; CD₃OD) 20.62, 21.08 and 21.94 (COMe), 53.38 and 56.40 (OMe and CO₂Me), 62.88 (C-6'), 69.97, 71.81, 72.48, 75.76, 75.85, 76.46 and 77.86 (C-2, -3, -4, -5, -2', -3', 4' and 5'), 99.79 and 99.26 (C-1 and -1'), 170.61, 172.14, 172.47 and 174.13 (C=O); *m/z* (Es⁻) 793 [(M - Na)⁻, 21%], 385 [(M - 2 Na)²⁻, 44] and 334 (100).

Methyl [Methyl 3-O-Acetyl-2-O-sulfo-4-O-(3,4,6-tri-O-acetyl-2-O-sulfo- α -D-glucopyranosyl)- α -D-glucopyranosid]-uronate, Disodium Salt 36.—Compound 34 (42 mg, 0.076 mmol) was sulfated as described. The residue was purified by ion-exchange chromatography (Dowex Na⁺) in water to give compound 36 as a gum, used directly in the next reaction (57 mg, 99%).

Methyl [Methyl 3-O-Acetyl-2-O-sulfo-4-O-(3',4',6'-tri-O-acetyl-2'-O-sulfo- α -D-glucopyranosyl)- α -L-idopyranosid]-uronate, Bistrimethylammonium Salt 41.—Compound 39 (40 mg, 0.072 mmol) was sulfated as described to give compound 41 as a gum (59 mg, 99%), *R*_f [ethyl acetate-pyridine-acetic acid-water (8:5:1:3)]; $[\alpha]_{\text{D}}^{22} + 22.2$ (*c* 1, MeOH); ν_{max} (CHCl₃)/cm⁻¹ 1750 (C=O); δ_{H} (200 MHz; CDCl₃) 2.03, 2.08 and 2.09 (12 H, 3 s, Ac), 2.96 (18 H, m, NMe₃), 3.42 (3 H, s, OMe), 3.78 (1 H, m, 5'-H), 3.85 (3 H, s, CO₂Me), 3.98 (1 H, br s, 4-H), 4.12–4.16 (2 H, m, 6'-H₂), 4.35 (1 H, br s, 2-H), 4.50–4.53 (1 H, m, 2'-H), 4.83 (1 H, d, *J* 2.5, 5-H), 5.15–5.22 (3 H, m, 3', 4'- and 1-H), 5.32 (1 H, br d, *J* 2.3, 3-H) and 5.43 (1 H, d, *J* 3.5, 1'-H); δ_{C} (125.78 MHz; CDCl₃) 18.21, 20.39, 20.49 and 20.82 (COMe), 45.60 (NHMe₃), 52.13 and 55.86 (OMe and CO₂Me), 61.80 (C-6'), 65.51, 66.87, 67.88, 68.12, 68.43, 69.54, 70.43 and 73.86 (C-2, -3, -4, -5, -2', -3', -4' and -5'), 94.02 and 99.77 (C-1 and -1'), 168.99, 169.17, 169.28, 170.53 and 171.11 (C=O); *m/z* (Es⁻) 711 [(MH - 2NHMe₃)⁻, 26%], 631 [(MH - 2NHMe₃ - SO₃)⁻, 100] and 355 [(M - 2NHMe₃)²⁻, 40].

Methyl [Methyl 2-O-Acetyl-3-O-sulfo-4-O-(3',4',6'-tri-O-acetyl-2'-O-sulfo- α -D-glucopyranosyl)- α -L-idopyranosid]-uronate, Bistrimethylammonium Salt 42.—Compound 40 (20 mg, 0.036 mmol) was sulfated as described to give compound 42 as a gum (29 mg, 96%), *R*_f 0.6 [ethyl acetate-pyridine-acetic acid-water (8:5:1:3)]; $[\alpha]_{\text{D}}^{22} + 27.1$ (*c* 1.1, MeOH); ν_{max} (CHCl₃)/cm⁻¹ 1740 (C=O); δ_{H} (500 MHz; CDCl₃) 1.98, 2.03, 2.08 and 2.19 (12 H, 4 s, Ac), 2.94 (18 H, s, NMe₃), 3.40 (3 H, s, OMe), 3.80 (3 H, s, CO₂Me), 4.13–4.19 (3 H, m, 5'-H and 6'-H₂), 4.37 (1 H, m, 4-H), 4.40 (1 H, dd, *J* 3.4 and 10.2, 2'-H), 4.73 (1 H, m, 3-H), 4.79–4.84 (2 H, m, 2- and 5-H), 5.03 (1 H, dd, *J* 1.0 and 1.1, 1-H), 5.13 (1 H, dd, *J* 7.4 and 9.5, 4'-H) and 5.33–5.38 (2 H, m, 1'-H and 3'-H); δ_{C} (125.78 MHz; CDCl₃) 20.44, 20.64 and 20.85 (COMe), 45.52 (NHMe₃), 52.22 and 55.69 (OMe and CO₂Me), 61.60 (C-6'), 66.24, 67.36, 68.19, 68.33, 70.31, 71.20, 74.62 and 75.47 (C-2, -3, -4, -5, -2', -3', -4' and -5'), 98.99 and 99.97 (C-1 and -1'), 169.19, 169.63, 170.58 and 170.69 (C=O); *m/z* (Es⁻) 711 [(MH - 2NHMe₃)⁻, 25%], 669 [(M - COCH₃)⁻, 4], 631 [(MH - 2NHMe₃ - SO₃)⁻, 100], 355 [(M - 2NHMe₃)²⁻, 48] and 334 [(M - COCH₃)²⁻, 32].

General Procedure for Deacetylation/Deesterification.—Sodium hydroxide (1 mol dm⁻³; 90 mm³) was added at 0 °C to a solution of the sulfated disaccharide (0.015 mmol) in THF (0.46 cm³) and the mixture was stirred for 8–30 h. The mixture was neutralised with 10% acetic acid and concentrated under reduced pressure. The crude residue was purified by ion-exchange chromatography (Dowex Na⁺) in water and was then purified by HPLC [Spherisorb SAX; NaCl gradient of 0–1 mol dm⁻³ at pH 3.5] to give the pure, unprotected, sulfated disaccharides.

Methyl 4-O-(2',3'-Di-O-sulfo- α -D-glucopyranosyl)-2-O-sulfo- α -D-glucopyranosiduronic acid, Tetrasodium Salt 6.—Compound 35 (5 mg, 0.006 mmol) was deprotected and purified as described to give compound 6 as a gum (4 mg, 94%), *R*_f 0 [ethyl acetate-pyridine-acetic acid-water (8:5:1:3)]; $[\alpha]_{\text{D}}^{22} + 78.9$ (*c* 0.27, water); δ_{H} (500 MHz; D₂O) 3.34 (3 H, s, OMe), 3.59 (1 H, dd, *J* 9.2 and 9.6, 4'-H), 3.67–3.71 (3 H, m, 5'-H and 6'-H₂), 3.80 (1 H, dd, *J* 9.6 and 10.0, 4-H), 3.91 (1 H, d, *J* 9.9, 5-H), 3.97 (1 H, d, *J* 8.9 and 9.6, 3-H), 4.11–4.15 (2 H, m, 2- and 2'-H), 4.43 (1 H, t, *J* 9.5, 3'-H), 5.00 (1 H, d, *J* 3.7, 1-H) and 5.72 (1 H, d, *J* 3.8, 1'-H); δ_{C} (125.78 MHz; D₂O) 55.99 (OMe), 60.79 (C-6'), 69.41, 72.09, 72.23, 72.86, 75.87, 77.59, 77.80 and 79.34 (C-2, -3, -4, -5, -2', -3', -4' and -5'), 97.22 and 98.05 (C-1 and -1' and 175.93 (C=O); *m/z* (Es⁻) 695 [(M - 3H)⁻, 6%], 675 [(M - Na)⁻, 13], 653 [(MH - 2Na)⁻, 70] and 551 [(M - 3Na - SO₃ + 2H)⁻, 100].

Methyl 2-O-Sulfo-4-O-(2-O-sulfo- α -D-glucopyranosyl)- α -D-glucopyranosiduronic Acid, Trisodium Salt 5.—Crude compound 36 (12 mg, 0.014 mmol) was deprotected and purified as described to give compound 5 as a gum (1.5 mg, 17%), *R*_f 0.05 [ethyl acetate-pyridine-acetic acid-water (8:5:1:3)]; $[\alpha]_{\text{D}}^{22} + 22.0$ (*c* 0.15, water); δ_{H} (500 MHz; D₂O) 3.32 (3 H, s, OMe), 3.39 (1 H, dd, *J* 9.4 and 10.7, 4'-H), 3.58–3.60 (1 H, m, 5'-H), 3.68–3.71 (3 H, m, 3'-H and 6'-H₂), 3.78 (1 H, dd, *J* 8.7 and 8.9, 4-H), 3.88 (1 H, d, *J* 9.2, 3-H), 4.00 (1 H, dd, *J* 3.8 and 10.0, 2'-H), 4.10 (1 H, dd, *J* 3.7 and 9.6, 2-H), 4.98 (1 H, d, *J* 3.5, 1-H) and 5.71 (1 H, d, *J* 3.7, 1'-H); *m/z* (Es⁻) 264.2 [(MH - 3Na)²⁻, 100].

Methyl 2-O-Sulfo-4-O-(2'-O-sulfo- α -D-glucopyranosyl)- α -L-idopyranosiduronic Acid, Trisodium Salt 2.—Compound 41 (14 mg, 0.017 mmol) was deprotected and half of the product was purified as described to give compound 2 as a gum (2.6 mg, 52%), *R*_f 0.08 [ethyl acetate-acetic acid-water (8:5:1:3)]; $[\alpha]_{\text{D}}^{22} + 28.1$ (*c* 0.26, water); δ_{H} (500 MHz; D₂O) 3.32 (3 H, s, OMe), 3.37 (1 H, dd, *J* 9.4 and 10.0, 4'-H), 3.66 (1 H, dd, *J* 4.4 and 12.3, 6'-H), 3.73–3.81 (3 H, m, 3', 5'- and 6'-H), 4.00 (1 H, dd, *J* 2.9 and 10.4, 2'-H), 4.03 (1 H, m, 4-H), 4.10 (2 H, br s, 2- and 3-H), 4.35 (1 H, s, 5-H), 4.83 (1 H, s, 1-H) and 5.36 (1 H, d, *J* 2.9, 1'-H); *m/z* (Es⁻) 573.0 [(M - Na)⁻, 30%], 567.1 (33), 551.1 [(MH - 2Na)⁻, 72], 529.2 [(M - 3Na + 2H)⁻, 9], 471.2 [(MH - 2Na - SO₃)⁻, 100] and 449.1 [(M - 3Na - SO₃ + 2H)⁻, 48].

Methyl 3-O-Sulfo-4-O-(2-O-sulfo- α -D-glucopyranosyl)- α -L-idopyranosiduronic Acid, Trisodium Salt 4.—Compound 42 (3 mg, 0.0036 mmol) was deprotected and purified as described to give compound 4 as a gum (1.1 mg, 52%), *R*_f 0.08 [ethyl acetate-pyridine-acetic acid-water (8:5:1:3)]; $[\alpha]_{\text{D}}^{22} + 24.5$ (*c* 0.11, water); δ_{H} (500 MHz; D₂O) 3.31 (3 H, s, OMe), 3.44 (1 H, dd, *J* 9.6 and 9.6, 4'-H), 3.65 (1 H, dd, *J* 2.8 and 10.1, 6'-H), 3.69–3.71 (3 H, m, 2- 5'- and 6'-H), 3.77 (1 H, dd, *J* 9.4 and 9.7, 3'-H), 4.07 (1 H, dd, *J* 3.8 and 9.9, 2'-H), 4.22 (1 H, s, 4-H), 4.36 (1 H, d, *J* 1.4, 5-H), 4.58 (1 H, m, 3-H), 4.76 (1 H, s, 1-H) and 5.31 (1 H, d, *J* 3.8, 1'-H); *m/z* (Es⁻) 573.0 [(M - Na)⁻, 91%], 567.1 (50), 551.1 [(MH - 2Na)⁻, 41], 471.2 [(MH - 2Na - SO₃)⁻, 25], 431.2 [(M - 3Na - OSO₃)⁻, 100], 325.1 (33), 311.3 (28), 260.9 (48) and 255.0 (37).

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