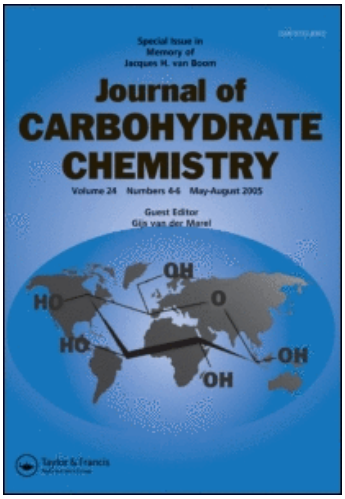

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Synthesis of the Tetrasaccharide Repeating Unit of the Antigen from *Klebsiella* Type 55

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SYNTHESIS OF THE TETRASACCHARIDE REPEATING UNIT OF THE ANTIGEN FROM *KLEBSIELLA* TYPE 55

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ABSTRACT

Starting from D-galactose, D-glucose and L-rhamnose, methyl 2-*O*-acetyl-3-*O*-(3-*O*-allyl-2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (7) and methyl 2-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-3-*O*-[2,4,6-tri-*O*-benzyl-3-*O*-(2,3,4-tri-*O*-benzyl-α-D-glucopyranosyluronic acid)-α-D-galactopyranosyl]-α-L-rhamnopyranoside (19) have been synthesised. Removal of allyl and benzyl groups from 7 and 19 gave the trisaccharide (9) and the tetrasaccharide repeating unit of the antigen from *Klebsiella* type 55 in the form of its methyl glycoside (20), respectively.

INTRODUCTION

Preparation of a synthetic antigen involves synthesis of a corresponding hapten bearing either narrow or wider specificity. Generally, fragments of carbohydrate chains bearing the corresponding determinants, the so-called O-factor[1] or a whole repeating unit itself, are employed as haptens. In the case when wider specificity of an antigen is needed, more complicated combination of the hapten fragments is apparently required. Synthesis of oligosaccharrides,

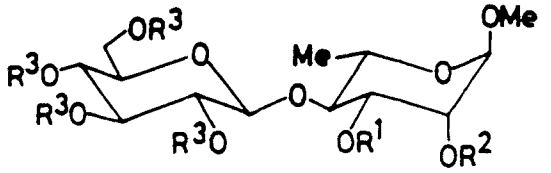
which are such specific fragments, is presently one of the important aspects of carbohydrate chemistry.

The structure of the repeating unit of the capsular polysaccharide from *Klebsiella* type 55 has been established by Bebault and Dutton.[2] As a part of our programme to determine the structure/immunochemical specificity[3] relationship of carbohydrate moieties, it is necessary to synthesise the tetrasaccharide repeating unit of the antigen from *Klebsiella* type 55 together with some related di- and trisaccharides. We have already reported the synthesis of a tetrasaccharide[4] which is basically the same tetrasaccharide repeating unit but without the acetyl group at the 2-position of the L-rhamnose moiety. It was necessary to take recourse to an entirely different strategy in order to have the acetyl groups on the tri- and tetrasaccharides, the syntheses of which are the subject matter of this communication.

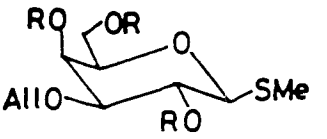
RESULTS AND DISCUSSION

Methyl 4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-2,3-*O*-isopropylidene-α-L-rhamnopyranoside[4] (1) was deisopropylidenated with 85% acetic acid[5] to give 2 which on selective acetylation with triethyl orthoacetate[6] gave methyl 2-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (3). A portion of 3 was hydrogenolysed with 10% Pd-C in ethanol to afford methyl 2-*O*-acetyl-4-*O*-β-D-glucopyranosyl-α-L-rhamnopyranoside (4). The ^{1}H NMR spectrum of the compound confirmed the β-glucosidic and α-rhamnosidic linkages and also the presence of acetyl group at the 2-position as evident from the δ values of H-1, H-2 and H-3 and their couplings. The ^{13}C NMR spectrum of 4 contained signals from 15 carbon atoms including one $\underline{C}H_3CO$ at δ 21.2, two anomeric carbon signals at δ 98.76 (C-1) and δ 103.85 (C-1'), and the carbonyl carbon at δ 174.06 ($CH_3\underline{C}O$).

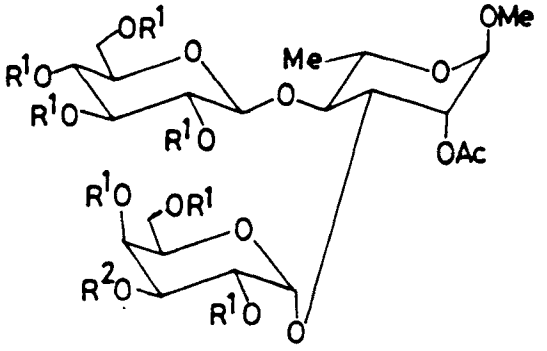
This glycosyl acceptor (3) was allowed to condense with methyl 3-*O*-allyl-2,4,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside[7] (6) in the presence of methyl triflate[8] in ethyl ether to give



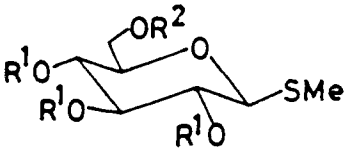
1. $R^1,R^2=CMe_2$, $R^3=Bn$
2. $R^1=R^2=H$, $R^3=Bn$
3. $R^1=H$, $R^2=Ac$, $R^3=Bn$
4. $R^1=R^3=H$, $R^2=Ac$



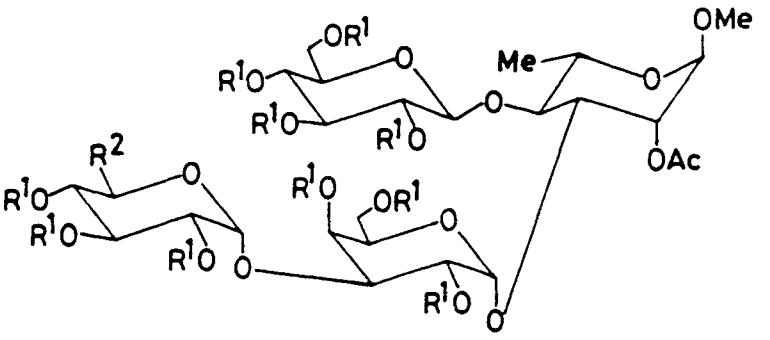
5. R = H
6. R = Bn



7. $R^1=Bn$, $R^2=All$
8. $R^1=Bn$, $R^2=H$
9. $R^1=R^2=H$



10. $R^1=R^2=Ac$
11. $R^1=R^2=H$
12. $R^1=H$, $R^2=Tr$
13. $R^1=Bn$, $R^2=Tr$
14. $R^1=Bn$, $R^2=H$
15. $R^1=Bn$, $R^2=\overset{O}{\overset{\|}{C}}CH_2Cl$



16. $R^1=Bn$, $R^2=CH_2OCOCH_2Cl$
17. $R^1=Bn$, $R^2=CH_2OH$
18. $R^1=Bn$, $R^2=CHO$
19. $R^1=Bn$, $R^2=COOH$
20. $R^1=H$, $R^2=COOH$

methyl 2-*O*-acetyl-3-*O*-(3-*O*-allyl-2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (7) in 91% yield. Deallylation of 7 with $PdCl_2$[9] gave methyl 2-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-3-*O*-(2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-α-L-rhhamnopyranoside (8). Hydrogenolysis of a portion of **8** with 10% Pd-C in ethanol gave methyl 2-*O*-acetyl-3-*O*-α-D-galactopyranosyl-4-*O*-β-D-glucopyranosyl-α-L-rhamnopyranoside (9). The proton NMR spectrum of 9 confirmed the configuration of rhamnopyranose, glucopyranose and galactopyranose moieties as α, β, and α, respectively. The ^{13}C NMR signals at δ 94.00 (C-1"), 98.81 (C-1) and 103.13 (C-1') also supported the presence of α-galactosidic, α-rhamnosidic and β-glucosidic linkages, respectively.

Deacetylation[10] of methyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside[11] (**10**) followed by tritylation[12] of the product **11**, using triphenylmethyl chloride in pyridine, and subsequent benzylation[13] of the product **12** gave methyl 2,3,4-tri-*O*-benzyl-1-thio-6-*O*-trityl-β-D-glucopyranoside (13). The compound **13** was detritylated[14] to give **14** which on subsequent chloroacetylation[15] gave methyl 2,3,4-tri-*O*-benzyl-6-*O*-chloroacetyl-1-thio-β-D-glucopyranoside (15). The trisaccharide **8** was then allowed to condense with the glycosyl donor **15** in the presence of methyl triflate[8] as promoter to afford methyl 2-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-3-*O*-[2,4,6-tri-*O*-benzyl-3-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-chloroacetyl-α-D-glucopyranosyl)-α-D-galactopyranosyl]-α-L-rhamnopyranoside (**16**) in 63% yield. The chloroacetyl group in **16** was removed by treatment with thiourea[15] to produce **17**, as confirmed by the disappearance of the signal from the chloroacetyl group, although the specific rotational values of **16** and **17** were quite close. Compound **17** was then oxidised with dimethyl sulphoxide and oxalyl chloride[16] to give the aldehyde **18** which, without further purification, was treated with $NaClO_2$,[17] in a buffer medium, to give methyl 2-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-3-*O*-[2,4,6-tri-*O*-benzyl-3-*O*-(2,3,4-tri-*O*-benzyl-α-D-glucopyranosyluronic acid)-α-D-galactopyranosyl]-α-L-rhamnopyrano-

side (19). Compound **19** was then hydrogenolysed using 10% Pd-C to give the desired tetrasaccharide methyl 2-*O*-acetyl-3-*O*-(3-*O*-α-D-glucopyranosyluronic acid-α-D-galactopyranosyl)-4-*O*-β-D-glucopyranosyl-α-L-rhamnopyranoside (**20**). The ^{1}H NMR signals at δ 4.75, 5.24, 4.67 and 5.14 indicated the presence of 4 anomeric protons corresponding to α-rhamnosidic, α-galactosidic, β-glucosidic and α-glucuronosidic moieties, respectively. The ^{13}C NMR spectrum exhibited the presence of 27 carbon atoms and the signals at δ 101.34 (C-1), 94.09 (C-1"), 103.34 (C-1'), 97.20 (C-1‴), 171.88 (COOH) and 172.52 ($CH_3\underline{C}O$) also supported the assignment of anomeric linkages.

EXPERIMENTAL

General - Reactions were monitored by TLC on silica gel G (Merck). Column chromatography was performed using silica gel 100-200 mesh (SRL, India), and all solvents were removed below 40 °C under reduced pressure unless stated otherwise. Optical rotations were measured with a Perkin-Elmer 241 MC polarimeter. ^{1}H and ^{13}C NMR spectra were recorded with a Jeol FX-100 or Bruker 300 MHz spectrometer. Chemical shifts are related to $(CH_3)_4Si$ (0 ppm). Melting points were determined using a paraffin oil bath and are reported uncorrected. The organic extracts were dried over anhydrous Na_2SO_4.

Methyl 2-*O*-Acetyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (3). A solution of methyl 4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-2,3-*O*-isopropylidene-α-L-rhamnopyranoside[4] (**1**; 2.5 g, 3.4 mmol) in 85% acetic acid (10.4 mL) was stirred at 90 °C for 2 h. Acetic acid was removed to give **2** (2.35 g, quantitative yield). To a solution of **2** in benzene (71.6 mL), triethyl orthoacetate (17.6 mL) and *p*-toluenesulphonic acid (15 mg) were added and the mixture was stirred for 1.5 h at room temperature. Triethylamine (88 μL) was then added and the mixture was stirred for another 15 min. The solvents and reagents were removed by evaporation and the residue was dissolved in 80% acetic

acid (8 mL) and stirred for 2 h. The reagents were evaporated off and the residue was chromatographed using 6:1 toluene-ether to give **3** (2.14 g, 86%), which was crystallised from ether-petroleum ether (40-60 °C): mp 130-131 °C; $[\alpha]_D^{25}$ -12.86° (*c* 0.65, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.38 (d, 3H, $J_{5,6}$=6.0 Hz, H-6), 2.1 (s, 3H, Ac), 3.36 (s, 3H, OMe), 4.6 (d, 1H, $J_{1',2'}$=6.0 Hz, H-1'), 4.78 (broad s, 1H, H-1), 7.24-7.36 (m, 20H, 4Ph).

Anal. Calcd for $C_{43}H_{50}O_{11}$: C, 69.52; H, 6.78. Found: C, 69.40; H, 6.80.

Methyl 2-*O*-Acetyl-4-*O*-β-D-glucopyranosyl-α-L-rhamnopyranoside (4). Compound **3** (220 mg, 296 μmol) was hydrogenolysed using 10% Pd-C (53 mg) in EtOH for 24 h at room temperature. The mixture was filtered through a celite bed and concentrated to a glass which was crystallised from water to give pure **4** (96 mg, 85%): mp 123-124 °C; $[\alpha]_D^{30}$ -44.6° (*c* 1.0, H_2O); ^1H NMR (D_2O) δ 1.34 (d, 3H, $J_{5,6}$=6.0 Hz, H-6), 2.14 (s, 3H, Ac), 3.37 (s, 3H, OMe), 4.1 (dd, 1H, $J_{2,3}$=3.6 Hz, $J_{3,4}$=8.98 Hz, H-3), 4.685 (d, 1H, $J_{1',2'}$=8.01 Hz, H-1'), 4.7 (broad s, 1H, H-1), 5.05 (dd, 1H, $J_{1,2}$=1.6 Hz, $J_{2,3}$=3.5 Hz, H-2); ^{13}C NMR (D_2O, internal standard 1,4-dioxane) δ 17.71 ($\underline{C}CH_3$), 21.15 ($\underline{C}H_3CO$), 55.80 (OCH_3), 61.50 (C-6'), 67.80, 69.64, 70.41, 73.25, 74.57, 76.60, 76.78, 81.03, 98.72 (C-1), 103.83 (C-1'), 173.98 ($CH_3\underline{C}O$).

Methyl 3-*O*-Allyl-2,4,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (6). Methyl 1-thio-β-D-galactopyranoside (7.77 g, 37.0 mmol), obtained from methyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside,[7] and dibutyltin oxide (9.13 g, 37.2 mmol) were stirred under reflux in benzene (150 mL) with azeotropic removal of water for 15 h. Allyl bromide (4.74 mL, 55.5 mmol) and tetrabutylammonium bromide (14.28 g, 44.4 mmol) were added and the mixture was stirred at 57 °C for 6 h. The solvent was removed, tin compounds precipitated by adding methanol were filtered off and the filtrate was concentrated to dryness. The residue was chromatographed using 5% MeOH in EtOAc to give pure methyl 3-*O*-allyl-1-thio-β-D-galactopyranoside (**5**; 4.7 g, 51%). The product was crystallised from EtOAc-ether: mp 102 °C; $[\alpha]_D^{24}$ +17.4° (*c* 0.6,

$CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.22 (s, 3H, SMe), 4.10 (m, 2H, $C\underline{H}_2$-CH=CH_2), 4.26 (d, $J_{1,2}$=10.0 Hz, H-1), 5.80-6.20 (m, 1H, CH_2-C$\underline{H}$=CH_2).

Anal. Calcd for $C_{10}H_{18}O_5S$: C, 47.98; H, 7.25. Found: C, 47.90; H, 7.31.

To a solution of **5** (4.5 g, 18.0 mmol) in N,N-dimethylformamide (40 mL) was added NaH (3.5 g, 60% oil coated) and BnBr (8.0 mL) and the mixture was stirred at room temperature for 6 h. Excess NaH was then destroyed with methanol and the mixture was diluted with CH_2Cl_2. The organic layer was washed thrice with water, dried and concentrated to dryness. Column chromatography with 9:1 toluene-ether gave syrupy **6** (7.49 g, 80%): $[\alpha]_D^{28}$ -6.65° (*c* 1.5, $CHCl_3$); lit.[7b] $[\alpha]_D$ -7.8° (*c* 0.9); ^1H NMR ($CDCl_3$) δ 2.20 (s, 3H, SMe), 4.3 (d, 1H, $J_{1,2}$=9.0 Hz, H-1), 5.67-6.20 (m, 1H, CH_2-C$\underline{H}$=CH_2), 7.23-7.43 (m, 15H, 3Ph).

Methyl 2-*O*-Acetyl-3-*O*-(3-*O*-allyl-2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (7). To a mixture of **3** (1.6 g, 2.15 mmol) and **6** (1.78 g, 3.43 mmol) in ether (25 mL) containing 4A molecular sieves (3 g) under Ar was injected methyl triflate (2 mL) and the mixture was stirred at room temperature for 98 h. The reaction mixture was filtered through a celite bed and concentrated to dryness. Column chromatography of the product with 6:1 toluene-ether afforded pure **7** (2.32 g, 91%) as syrup: $[\alpha]_D^{31}$ +24.48° (*c* 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.38 (d, 3H, $J_{5,6}$=6.0 Hz, CMe), 1.84 (s, 3H, Ac), 3.22 (s, 3H, OMe), 4.78 (d, 1H, $J_{1',2'}$=7.5 Hz, H-1'), 4.93 (d, 1H, $J_{1,2}$=2.0 Hz, H-1), 5.4 (d, 1H, $J_{1'',2''}$=3.0 Hz, H-1"), 5.78-6.16 (m, 1H, CH_2-C$\underline{H}$=CH_2), 7.20-7.34 (m, 35H, 7Ph).

Anal. Calcd for $C_{73}H_{82}O_{16}$: C, 73.96; H, 6.97. Found: C, 74.10; H, 6.90.

Methyl 2-*O*-Acetyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-3-*O*-(2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-α-L-rhamnopyranoside (8) A mixture of **7** (2.3 g, 1.89 mmol), $PdCl_2$ (462 mg, 2.62 mmol) and sodium acetate trihydrate (1.04 g) in 20:1 acetic acid-water (26 mL) was stirred at room temperature for 18 h. The

reaction mixture was then filtered through a celite bed and washed with EtOAc. The combined organic layer was concentrated to dryness. The residue was dissolved in CH_2Cl_2 (30 mL) and the solution was washed successively with saturated aqueous $NaHCO_3$ solution and water, dried and concentrated to dryness. Column chromatography with 4:1 toluene-ether gave pure **8** (1.47 g, 66%); $[\alpha]_D^{24}$ +34.45° (*c* 1.7, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.38 (d, 3H, $J_{5,6}$=5.5 Hz, H-6), 1.8 (s, 3H, Ac), 1.87 (s, 1H, OH), 3.24 (s, 3H, OMe), 4.8 (d, 1H, $J_{1',2'}$=7.5 Hz, H-1'), 4.96 (broad s, 1H, H-1), 5.3 (d, 1H, $J_{1'',2''}$=3.5 Hz, H-1''), 7.20-7.30 (m, 35H, 7Ph).

Anal. Calcd for $C_{70}H_{78}O_{16}$: C, 71.53; H, 6.69. Found: C, 71.20; H, 6.70.

Methyl 2-*O*-Acetyl-3-*O*-α-D-galactopyranosyl-4-*O*-β-D-glucopyranosyl-α-L-rhamnopyranoside (9). Compound **8** (220 mg) was hydrogenolysed using 10% Pd-C (72 mg) in EtOH (7 mL) at 24 °C for 12 h. The reaction mixture was filtered through a celite bed and concentrated to dryness giving **9** (90.5 mg, 89%): $[\alpha]_D^{30}$ +31.61° (*c* 1.2, H_2O); ^1H NMR (D_2O) δ 1.34 (d, 3H, $J_{5,6}$=6.35 Hz, H-6), 2.12 (s, 3H, Ac), 33.37 (s, 3H, OMe), 4.67 (d, 1H, $J_{1',2'}$=7.81 Hz, H-1'), 5.04 (d, 1H, $J_{1'',2''}$=3.67 Hz, H-1''), 5.39 (broad s, 1H, H-1); ^{13}C NMR (D_2O, internal standard 1,4-dioxane) δ 17.95 ($C\underline{C}H_3$), 21.10 ($\underline{C}H_3CO$), 55.85 (OCH_3), 61.66 (C-6''), 62.24 (C-6'), 67.34, 68.25, 68.57, 70.10, 70.24, 70.63, 71.69, 73.95, 76.51, 76.60, 76.66, 76.87, 94.00 (C-1''), 98.81 (C-1), 103.13 (C-1'), 173.61 ($CH_3\underline{C}O$).

Methyl 2,3,4-Tri-*O*-benzyl-1-thio-6-*O*-trityl-β-D-glucopyranoside (13). Methyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (**10**; 6.5 g), was deacetylated using 0.05 M NaOMe as usual to give methyl 1-thio-α-D-glucopyranoside (**11**). Compound **11** was treated with triphenylmethyl chloride (5.1 g, 1.1 molar equivalent) in pyridine (32 mL) in the dark at room temperature for 48 h. Solvent was evaporated off, traces of pyridine were removed by co-evaporation with toluene and the product was purified by column chromatography to give pure **12**; $[\alpha]_D^{20}$ -22.2° (*c* 1.0, CH_3OH).

Compound **12** was then benzylated as described for the preparation of **6** to give **13** (6.2 g, 52% overall yield from **10**): $[\alpha]_D^{28}$ +3.3° (*c* 2.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.26 (s, 3H, SMe), 4.49 (d, 1H, $J_{1,2}$=9.0 Hz, H-1), 4.85, 4.92, 4.95 (3d, 6H, $3Ph_2C\underline{H}_2$), 7.26-7.40 (m, 30H, 6Ph).

Anal. Calcd for $C_{47}H_{46}O_5S$: C, 81.47; H, 6.69. Found: C, 81.10; H, 6.70.

Methyl 2,3,4-Tri-*O*-benzyl-1-thio-β-D-glucopyranoside (14). The trityl ether **13** (6 g, 8.16 mmol) was stirred in 80% acetic acid (70 mL) at 80 °C for 1 h. The reaction mixture was cooled, filtered, and the filtrate was concentrated to dryness. Column chromatography with 20:1 toluene-ether gave pure **14** (4.12 g, 99%): $[\alpha]_D$ +57.1° (*c* 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.26 (s, 3H, SMe), 4.44 (d, 1H, $J_{1,2}$=9.0 Hz, H-1), 4.84, 4.92, 4.94 (3d, 6H, $3PhC\underline{H}_2$), 7.32-7.40 (m, 15H, 3Ph).

Anal. Calcd for $C_{28}H_{32}O_5S$: C, 69.97; H, 6.71. Found: C, 69.98; H, 6.80.

Methyl 2,3,4-Tri-*O*-benzyl-6-*O*-chloroacetyl-1-thio-β-D-glucopyranoside (15). Compound **14** (1.2 g, 2.5 mmol) was dissolved in CH_2Cl_2 (60 mL) containing pyridine (1.2 mL). The solution was cooled to 0 °C. Chloroacetyl chloride (1.2 mL) in CH_2Cl_2 (10 mL) was added dropwise and the reaction mixture was stirred at room temperature for 4 h. The reaction mixture was washed thrice with cold water, dried and concentrated to dryness. The residue was crystallised from ether-petroleum ether (40-60 °C) to give pure **15** (1.29 g, 93%): mp 80-81 °C; $[\alpha]_D^{26}$ +27.65° (*c* 0.77, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.20 (s, 3H, SMe), 4.00 (s, 2H, $COCH_2Cl$), 4.60 (d, 1H, $J_{1,2}$=10.0 Hz, H-1), 7.24-7.40 (m, 15H, 3Ph).

Anal. Calcd for $C_{30}H_{33}ClO_6S$: C, 64.68; H, 5.97. Found: C, 64.80; H, 5.90.

Methyl 2-*O*-Acetyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-3-*O*-[2,4,6-tri-*O*-benzyl-3-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-chloroacetyl-α-D-glucopyranosyl)-α-D-galactopyranosyl]-α-L-rhamnopyranoside (16). A mixture of **8** (670 mg, 0.57 mmol) and **15** (680 mg, 1.22 mmol) dissolved in ether (10 mL) containing 4A molecular sieves (1 g)

was stirred in the presence of methyl triflate (1 mL) for 46 h at 24 °C as described for the preparation of **7**. The reaction mixture was worked up in the usual way. Column chromatography with 9:1 toluene-ether gave pure **16** (605 mg, 63%): $[\alpha]_D^{25}$ +68.2° (*c* 0.79, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.42 (d, 3H, $J_{5,6}$=6.0 Hz, H-6), 1.88 (s, 3H, Ac), 3.24 (s, 3H, OMe), 3.35 (d, 2H, J=4.0 Hz, $COCH_2Cl$), 4.76 (d, 1H, $J_{1',2'}$=7.5 Hz, H-1'), 4.94 (broad s, 1H, H-1), 5.04 (d, 1H, $J_{1''',2'''}$=3.0 Hz, H-1'''), 5.32 (d, 1H, $J_{1'',2''}$=3.0 Hz, H-1''), 7.16-7.38 (m, 50H, 10Ph).

Anal. Calcd for $C_{99}H_{107}ClO_{22}$: C, 70.60; H, 6.40. Found: C, 70.86; H, 6.61.

Methyl 2-*O*-Acetyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-3-*O*-[2,4,6-tri-*O*-benzyl-3-*O*-(2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-α-D-galactopyranosyl]-α-L-rhamnopyranoside (17). A solution of compound **16** (600 mg, 0.36 mmol) and thiourea (130 mg) in 1:1 benzene-MeOH (65 mL) was refluxed for 12 h. The solvents were evaporated off and the residue was diluted with CH_2Cl_2 (20 mL), washed with water and dried. The residue was chromatographed using 15:1 toluene-ether to give pure **17** (438 mg, 76%): $[\alpha]_D^{26}$ +65.6° (*c* 0.88, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.4 (d, 3H, $J_{5,6}$=6.0 Hz, H-6), 1.74 (s, 3H, Ac), 3.22 (s, 3H, OMe), 4.6 (d, 1H, $J_{1',2'}$=7.75 Hz, H-1'), 4.69 (d, 1H, $J_{1,2}$=2.0 Hz, H-1), 5.18 (d, 1H, $J_{1''',2'''}$=3.5 Hz, H-1'''), 5.3 (d, 1H, $J_{1'',2''}$=3.5 Hz, H-1'').

Anal. Calcd for $C_{97}H_{106}O_{21}$: C, 72.46; H, 6.64. Found: C, 72.40; H, 6.84.

Methyl 2-*O*-Acetyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-3-*O*-[2,4,6-tri-*O*-benzyl-3-*O*-(2,3,4-tri-*O*-benzyl-α-D-glucopyranosyluronic acid)-α-D-galactopyranosyl]-α-L-rhamnopyranoside (19). To a solution of oxalyl chloride (33 μL, 0.37 mmol) in CH_2Cl_2 (2 mL) at -45 °C under N_2, a solution of DMSO (58.5 μL) in CH_2Cl_2 (2 mL) was added with stirring during 5 min. After 20 min, a solution of **17** (400 mg, 0.25 mmol) in CH_2Cl_2 (3 mL) was added during 5 min. Stirring was continued for 15 min and N,N-diisopropylethylamine (0.3 mL) was added during 5 min. The reaction mixture was then allowed to attain room temperature. Water (7 mL)

was then added and the mixture was stirred for another 20 min. The product was extracted with CH_2Cl_2 (3x15 mL) and washed successively with 0.5 M HCl, saturated aqueous $NaHCO_3$ solution and water, dried and concentrated to dryness to obtain **18.** Compound **18** was dissolved in t-BuOH (10 mL) and 2-methyl-2-butene (26.8 mL) was added. The mixture was stirred overnight with a solution of $NaClO_2$ (631 mg, 6.97 mmol) and NaH_2PO_4 (631 mg) in water (6.31 mL). The mixture was concentrated to a solid mass and the residue was dissolved in CH_2Cl_2 (20 mL), washed with M HCl and water in succession, dried and concentrated. Column chromatography with 80:20:1 toluene-ether-AcOH gave **19** (322 mg, 80%): $[\alpha]_D^{28}$ +51.2° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.39 (d, 3H, $J_{5,6}$=6.0 Hz, H-6), 1.83 (s, 3H, Ac), 3.21 (s, 3H, OMe), 4.63 (d, 1H, $J_{1',2'}$=7.5 Hz, H-1'), 4.7 (d, 1H, $J_{1,2}$=2.0 Hz, H-1), 5.18 (d, 1H, $J_{1''',2'''}$=3.5 Hz, H-1'''), 5.29 (d, 1H, $J_{1'',2''}$=3.0 Hz, H-1''), 7.14-7.36 (m, 50H, 10Ph).

Anal. Calcd for $C_{97}H_{104}O_{22}$: C, 71.89; H, 6.46. Found: C, 71.71; H, 6.59.

Methyl 2-*O*-Acetyl-3-*O*-(3-*O*-α-D-glucopyranosyluronic acid-α-D-galactopyranosyl)-4-*O*-β-D-glucopyranosyl-α-L-rhamnopyranoside (20). Compound **19** (300 mg) was hydrogenolysed for 48 h at 26 °C in presence of 10% Pd-C in 1:2 toluene-EtOH in the usual manner. Coolumn chromatography with 100:50:10:1 $CHCl_3$-MeOH-H_2O-AcOH gave pure **20** (115 mg, 86%): $[\alpha]_D^{28}$ +72.1° (*c* 0.6, H_2O); ^1H NMR (D_2O) δ 1.3 (d, 3H, $J_{5,6}$=6.0 Hz, H-6), 1.9 (s, 3H, Ac), 3.41 (s, 3H, OMe), 4.67 (d, 1H, $J_{1',2'}$=8.0 Hz, H-1'), 4.75 (d, 1H, $J_{1,2}$=2.5 Hz, H-1), 5.14 (d, 1H, $J_{1''',2'''}$=3.6 Hz, H-1'''), 5.24 (d, 1H, $J_{1'',2''}$=3.52 Hz, H-1''); ^{13}C NMR (D_2O) δ 17.93 ($C\underline{C}H_3$), 24.10 ($\underline{C}H_3CO$), 55.76 (OCH_3), 61.45 (C-6''), 62.11 (C-6'), 65.59, 65.80, 67.17, 67.49, 68.29, 70.52, 71.79, 72.03, 72.44, 73.01, 73.39, 74.01, 76.35, 76.71, 76.92, 81.92, 94.09 (C-1''), 97.20 (C-1'''), 101.34 (C-1), 103.34 (C-1'), 171.88 (COOH), 172.52 ($CH_3\underline{C}O$).

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