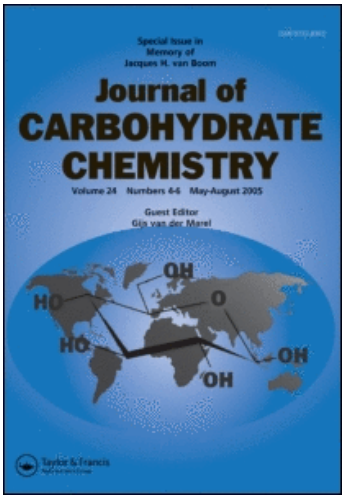

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Synthesis of Tetrasaccharide Repeating Unit of the K-Antigen from *Klebsiella* Type-16

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SYNTHESIS OF TETRASACCHARIDE REPEATING UNIT OF THE K-ANTIGEN FROM *KLEBSIELLA* TYPE-16

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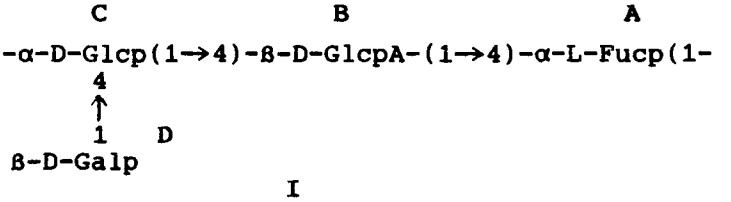
ABSTRACT

Starting from L-fucose, D-glucose and lactose, methyl *O*-[2,3-di-*O*-benzoyl-4,6-*O*-(4-methoxybenzylidene)-ß-D-glucopyranosyl]-(1→4)-2,3-di-*O*-benzoyl-α-L-fucopyranoside and methyl *O*-(2,3,4,6-tetra-*O*-benzyl-ß-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-*O*-(methyl 2,3-di-*O*-benzoyl-ß-D-glucopyranosyluronate)-(1→4)-2,3-di-*O*-benzoyl-α-L-fucopyranoside were synthesized. Removal of protecting groups gave the tetrasaccharide repeating unit of the antigen from *Klebsiella* type-16 in the form of its methyl ester methyl glycoside.

INTRODUCTION

The structure of the repeating unit of the capsular polysaccharide of *Klebsiella* type 16 has been established[1] as the tetrasaccharide I

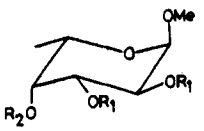


I

In continuation of our efforts to determine the relationship between structure and immunological specificity of various saccharides, we synthesized the tetrasaccharide repeating unit of the antigen from *Klebsiella* type-16 in the form ofe its methyl ester methyl glycoside. The two disaccha-ride blocks AB and CD were first synthesized and were then joined together to give the tetrasaccharide ABCD.

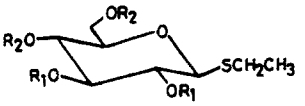
RESULTS AND DISCUSSION

The disaccharide block AB was prepared from L-fucose and D-glucose. Methyl α-L-fucopyranoside (1) obtained from L-fucose, by using Dowex 50 W (H^+) resin as utilised for the preparation of allyl α-L-fucopyranoside,[2] was converted into methyl 2,3-di-*O*-benzoyl-α-L-fucopyranoside[3] (2) in 61.5% yield. In another experiment the 4,6-(4-methoxybenzylidene) derivative[4] (4), prepared from 3, was benzoylated in the usual way to afford ethyl 2,3-di-*O*-benzoyl-4,6-(4-methoxybenzylidene)-1-thio-ß-D-glucopyranoside (5). The donor (5) and the acceptor (2) were then allowed to condense in the presence of methyl triflate to afford the disaccharide (6) in 60% yield together with a small amount (6%) of its α-anomer (6a). Compound 6 gave ^1H NMR peaks for $CH_3OC_6H_4CH$, OCH_3 and $C\text{-}CH_3$ together with signals at δ 4.70 and 5.09 corresponding to ß-glucosidic and α-fucosidic moieties.

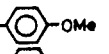


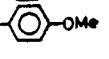
1 $R_1 = R_2 = H$

2 $R_1 = Bz, R_2 = H$



3 $R_1 = R_2 = H$

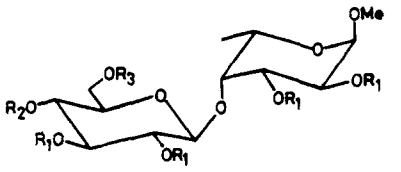
4 $R_1 = H, R_2R_2 =$ 

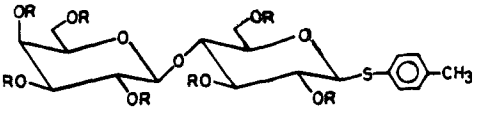
5 $R_1 = Bz, R_2R_2 =$ 

Removal of protecting groups from 6 afforded the disaccharide 7. The ^{13}C NMR spectrum of 7 exhibited the presence of 13 carbon atoms and the signals at δ 104.02 (C-1'), 100.33 (C-1), 61.43 (C-6'), 56.05 (OCH_3) and 16.09 (C-6) supported the assignments of the anomeric linkages.

Regioselective opening of the 4-methoxybenzylidene ring[4] with sodium cyanoborohydride and trifluoroacetic acid gave the 4'-hydroxy compound 8. Compound 8 had ^{1}H NMR signals for $CH_3OC_6H_4CH_2$, OCH_3, CCH_3 and specific anomeric protons but there were no signals at δ 5.64 indicating the absence of a benzylidene group.

The disaccharide fragment CD was prepared from lactose. *p*-Tolyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside 9, prepared from lactose by use of the same reaction conditions as normally used for the preparation of thioglycosides using boron trifluoride etherate,[5] was deacetylated according to Zemplén and the product 10 was benzylated to give perbenzylated thioglycoside donor 11.





6 R_1 = Bz, R_2 R_3 = CH-(p-OMe-Ph)

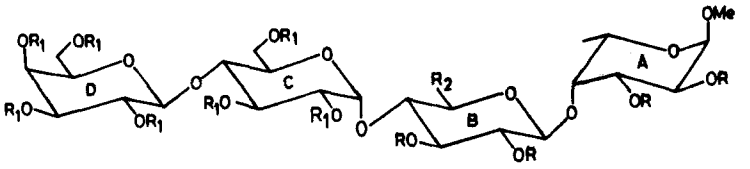
7 R_1 = R_2 = R_3 = H

8 R_1 = Bz, R_2 = H, R_3 = CH_2-(p-OMe-Ph) = MBn

9 R = Ac

10 R = H

11 R = Bn



12 R = Bz, R_1 = Bn, R_2 = CH_2-OMBn

13 R = H, R_1 = Bn, R_2 = CH_2-OMBn

14 R = Ac, R_1 = Bn, R_2 = CH_2-OMBn

15 R = Bz, R_1 = Bn, R_2 = CH_2-OH

16 R = Bz, R_1 = Bn, R_2 = CO_2Me

17 R = Bz, R_1 = H, R_2 = CO_2Me

18 R = R_1 = H, R_2 = CO_2Me

The glycosyl acceptor 8 was allowed to condense with the donor 11 in the presence of methyl triflate using diethyl ether as solvent to give the tetrasaccharide derivative 12 in 77% yield. The ^{1}H NMR spectrum of 12 showed

the presence of four anomeric protons in addition to other characteristic signals. In further characterisation of 12, removal of benzoyl groups from 12 followed by acetylation of the product 13 gave the tetraacetate 14 as confirmed from its ^1H NMR spectrum. Oxidative removal of the 4-methoxybenzyl group from 12 with ceric ammonium nitrate[4] gave 15. Oxidation of the primary hydroxy group of 15 with chromium trioxide[6] followed by treatment of the resulting acid with diazomethane in ether gave the methyl ester 16 in 67% overall yield. The ^1H NMR spectrum of 16 showed a signal for $COOCH_3$ (δ 2.96) together with other characteristic peaks. Hydrogenolysis of 16 with 10% Pd-C followed by removal of benzoyl groups from the product 17 with sodium methoxide gave the tetrasaccharide 18. The ^{13}C NMR spectrum of 18 gave signals for 26 carbons and showed the presence of $COOCH_3$, OCH_3, and CCH_3 together with peaks at δ 100.3, 103.7, 99.4, and 103.9 for 4 anomeric carbon atoms corresponding to α-fucosidic, β-glucosidic, α-glucosidic and β-galactosidic moieties respectively.

EXPERIMENTAL

General. All reactions were monitored by TLC on Silica gel G (E. Merck). Column chromatography was performed using 100-200 mesh silica gel (SRL, India). The weight of silica gel taken for individual separations was approximately 10 to 25 times that of the weight of crude reaction mixture, depending on the extent of separation. All solvents were dried and/or distilled before use, and all evaporations were conducted below 50 °C unless otherwise stated. Optical rotations were measured at 24 °C with a Perkin-Elmer 241 MC polarimeter. ^1H NMR and ^{13}C NMR spectra were recorded (internal standard tetramethylsilane) with a Jeol FX 100 and Bruker 300 MHz spectrometer, using $CDCl_3$ as the solvent unless stated otherwise. The organic extracts were dried over anhydrous Na_2SO_4.

Methyl α-L-Fucopyranoside (1). A solution of L-fucose (4 g, 24.4 mmol), in methanol (60 mL) was refluxed with

Dowex 50 W (H^+) resin (4 g) for 6 h. The mixture was then filtered and the resin washed with methanol. The filtrate and washings were combined and concentrated to a syrup which crystallised from EtOH containing trace of EtOAc to give 1 (3.0 g, 69.1%): mp 146-147 °C; $[\alpha]_D$ -192° (*c* 1.02, H_2O). Lit.[7] mp 154 °C; $[\alpha]_D$ -197.1°.

Methyl 2,3-Di-*O*-benzoyl-α-L-fucopyranoside (2). To a solution of 1 (1.5 g, 8.4 mmol) in dry pyridine (17 mL) at -40 °C, a solution of benzoyl chloride (2.0 mL, 17.7 mmol) in dry pyridine (5.5 mL) was added dropwise. The mixture was stirred at -40 °C for 30 min and then poured into cold water. The suspended solids were extracted with $CHCl_3$; the extract dried and concentrated. Traces of pyridine were removed by coevaporation with toluene. Column chromatography with 7:1 toluene-EtOAc gave syrupy 2 (2 g, 61.5%); $[\alpha]_D$ -175° (*c* 1.1, $CHCl_3$). Lit.[3] $[\alpha]_D$ -187° ($CHCl_3$).

Ethyl 4,6-*O*-(4-methoxybenzylidene)-1-thio-β-D-glucopyranoside (4). To a solution of ethyl 1-thio-β-D-glucopyranoside[5,8] 3 (1.98 g, 8.8 mmol) in CH_3CN (25 mL), 4-methoxybenzaldehyde dimethylacetal[4] (3.0 mL, 17.7 mmol), 3A molecular sieves (3 g) and toluene-4-sulfonic acid (80 mg) were added and the mixture was stirred for 2 h at room temperature. The reaction was then quenched with Et_3N (0.5 mL), the mixture filtered through celite and washed with CH_3CN. The filtrate was concentrated to a syrup which crystallised from EtOH to afford pure 4 (2.3 g, 76%): mp 160-162 °C; $[\alpha]_D$ -22° (*c* 0.81, $CHCl_3$); ^1H NMR δ 7.53-6.95 (m, 4H, aromatic protons), 5.53 (s, 1H, $CHC_6H_4OCH_3$), 4.48 (d, 1H, $J_{1,2}$ = 9.5 Hz, H-1), 3.83 (s, 3H, $CHC_6H_4OCH_3$), 2.78 (q, 2H, SCH_2CH_3), 1.33 (t, 3H, SCH_2CH_3).

Anal. Calcd for $C_{16}H_{22}O_6S$: C, 56.12; H, 6.47. Found: C, 56.01; H, 6.78.

Ethyl 2,3-Di-*O*-benzoyl-4,6-(4-methoxybenzylidene)-1-thio-β-D-glucopyranoside (5). To a solution of 4 (1.7 g, 5.0 mmol) dissolved in pyridine (20 mL), benzoyl chloride (4.1 mL, 3.4 mmol) was added dropwise at 0 °C, and the mixture was stirred for 2 h. Excess benzoyl chloride was then decomposed by the addition of water (1 mL).Stirring was

continued for another 30 min. The mixture was concentrated under vacuum to a small volume, diluted with CH_2Cl_2, washed with aq $NaHCO_3$ and water, dried and concentrated. Crystallisation from EtOH gave 5 (2.4 g, 87%): mp 166-167 °C; $[\alpha]_D$ -5.17° (*c* 0.93, $CHCl_3$); ^1H NMR δ 7.98-6.86 (m, 14H, aromatic protons), 5.51 (s, 1H, $CHC_6H_4OCH_3$), 4.83 (d, 1H, $J_{1,2}$ = 9 Hz, H-1), 3.76 (s, 3H, $CHC_6H_4OCH_3$), 2.78 (q, 2H, SCH_2CH_3), 1.27 (t, 3H, SCH_2CH_3).

Anal. Calcd for $C_{30}H_{30}O_8S$: C, 65.43; H, 5.49. Found: C, 65.88; H, 5.54.

Methyl *O*-[2,3-Di-*O*-benzoyl-4,6-*O*-(4-methoxybenzylidene)-β-D-glucopyranosyl]-(1→4)-2,3-di-*O*-benzoyl-α-L-fucopyranoside (6). A mixture of 2 (0.93 g, 2.40 mmol), 5 (1.83 g, 3.33 mmol) and 4A molecular sieves (4 g) in dry toluene (40 mL) was stirred for 1 h at 22 °C under argon. Methyl triflate (1.85 mL, 16.35 mmol) was then added and the mixture was stirred for 18 h. The reaction was quenched with Et_3N, filtered through celite and concentrated to a syrup. Column chromatography with 10:1 toluene-EtOAc gave 6 (1.27 g, 60%) and 6a (131 mg, 6%). Compound 6 was crystallised from CH_2Cl_2-EtOH: mp 238-240 °C; $[\alpha]_D$ -186.8° (*c* 1.13, $CHCl_3$); ^1H NMR of 6 δ 8.13-6.86 (m, 24H, aromatic protons), 5.64 (s, 1H, $CHC_6H_4OCH_3$), 5.09 (d, 1H, $J_{1,2}$ = 2.0 Hz, H-1), 4.70 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 3.78 (s, 3H, $CHC_6H_4OCH_3$), 3.36 (s, 3H, OCH_3), 1.18 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6). ^1H NMR of 6a δ 7.98-6.84 (m, 24H, aromatic protons), 5.48 (s, 1H, $CHC_6H_4OCH_3$), 5.26 (d, 1H, $J_{1,2}$ = 2.0 Hz, H-1), 4.80 (d, 1H, $J_{1',2'}$ = 1.0 Hz, H-1'), 3.76 (s, 3H, $CHC_6H_4OCH_3$), 3.30 (s, 3H, OCH_3), 1.26 (d, 3H, $J_{5,6}$ = 6.0 Hz, H-6). ^{13}C NMR of 6a δ 165.6-164.9 (4 COC_6H_5), 133.2-127.3 (aromatic carbons), 102.1 ($CHC_6H_4OCH_3$), 101.2 (C-1), 100.5 (C-1'), 84.3, 82.4, 82.2, 78.5, 78.3, 75.7, 72.7, 72.3, 66.4 (C-6'), 55.3 (OCH_3), 54.9 (OCH_3), 17.7 (C-6).

Anal. Calcd for 6, $C_{49}H_{46}O_{15}$: C, 67.26; H, 5.3. Found: C, 67.09; H, 5.49.

Methyl *O*-(β-D-Glucopyranosyl)-(1→4)-α-L-fucopyranoside (7). Compound 6 (200 mg, 0.23 mmol) was debenzoylated

with NaOMe in the usual way. The solution was neutralised with Dowex 50 W (H^+) resin. The solution was further stirred with excess Dowex 50 W (H^+) resin for 1 h, filtered and concentrated to give syrupy 7 (65 mg, 83.5%): $[\alpha]_D$ -108.57° (*c* 1.4, MeOH), ^1H NMR (D_2O) δ 4.80 (d, 1H, $J_{1,2}$ = 2.0 Hz, H-1), 4.48 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 3.40 (s, 3H, OCH_3), 1.30 (d, 3H, $J_{5,6}$ = 6.0 Hz, H-6); ^{13}C NMR (D_2O) δ 104.0 (C-1'), 100.3 (C-1), 81.8, 76.8, 76.4, 74.3, 70.3 (2 C), 69.7, 69.4, 61.4 (C-6'), 56.1 (OCH_3), 16.1 (C-6).

Methyl *O*-[2,3-Di-*O*-benzoyl-6-*O*-(4-methoxybenzyl)-ß-D-glucopyranosyl]-(1→4)-2,3-di-*O*-benzoyl-α-L-fucopyranoside (8). A mixture of 6 (286 mg, 0.33 mmol), sodium cyanoborohydride (67 mg, 2.1 mmol) and 3A molecular sieves (1 g), in dry DMF (3 mL) was stirred for 1 h at 0 °C. A solution of trifluoroacetic acid (0.17 mmol) in dry DMF (0.5 mL) was then added dropwise and the mixture was stirred for 19 h. The solution was filtered through celite and diluted with CH_2Cl_2. The solution was washed with aq $NaHCO_3$ and water, dried and concentrated to a syrup. Column chromatography with 10:1 toluene-EtOAc gave 8 as an amorphous solid (243 mg, 84.7%): $[\alpha]_D$ -152.78° (*c* 1.1, $CHCl_3$); ^1H NMR δ 8.02-6.84 (m, 24H, aromatic protons), 5.02 (d, 1H, $J_{1,2}$ = 2.0 Hz, H-1), 4.68 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 3.78 (s, 3H, $CH_2C_6H_4OCH_3$), 3.32 (s, 3H, OCH_3), 1.14 (d, 3H, $J_{5,6}$ = 7.0 Hz, H-6).

Anal. Calcd for $C_{49}H_{48}O_{15}$: C, 67.11; H, 5.52. Found: C, 67.17; H, 5.56.

***p*-Tolyl *O*-(2,3,4,6-Tetra-*O*-acetyl-ß-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-1-thio-ß-D-glucopyranoside (9).** A solution of ß-lactose octaacetate (2.4 g, 3.63 mmol) and *p*-thiocresol (540 mg, 4.35 mmol) in CH_2Cl_2 was cooled in an ice bath. Boron trifluoride etherate (0.9 mL) was added and the mixture was stirred at room temperature for 3 h. The mixture was then diluted with CH_2Cl_2 (3 x 25 mL) and washed with 5% aq NaOH and water respectively. The solution was dried, filtered and then concentrated. Crystallisation from 20:1 EtOH-EtOAc gave 9 (2.3 g, 85%): mp 148-150 °C; $[\alpha]_D$

-17.27° (*c* 0.19, $CHCl_3$); ^1H NMR δ 7.27 (m, 4H, aromatic protons), 4.80 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 4.53 (d, 1H, $J_{1,2}$ = 7.0 Hz, H-1), 2.37 (s, 3H, $SC_6H_4CH_3$), 2.17-1.97 (7s, 21H, 7 OAc).

Anal. Calcd for $C_{33}H_{42}O_{17}S$: C, 53.36; H, 5.7. Found: C, 53.48; H, 6.13.

p-Tolyl *O*-(2,3,4,6-Tetra-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (11). Compound 9 (5.0 g, 6.7 mmol) was de-O-acetylated with 0.05 M NaOMe in the usual way and the resulting *p*-tolyl *O*-(β-D-galactopyranosyl)-(1→4)-1-thio-β-D-glucopyranoside (10) (2.97 g, 6.6 mmol) was dissolved in DMF (30 mL). NaH (2.85 g, 118 mmol) and benzyl bromide (6.85 mL, 57 mmol) were added. The reaction mixture was stirred at room temperature for 6 h. Excess NaH was destroyed by adding methanol and the mixture was diluted with CH_2Cl_2. The organic layer was washed with water, dried and concentrated to a syrup. Column chromatography with 5:1 toluene-EtOAc gave 11 which crystallised from EtOH (6.87 g, 96%): mp 108-110 °C; $[\alpha]_D$ -6.4° (*c* 0.94, $CHCl_3$); ^1H NMR δ 7.33-7.03 (m, 39H, aromatic protons), 4.86 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 4.30 (d, 1H, $J_{1,2}$ = 6.0 Hz, H-1), 2.26 (s, 3H, $SC_6H_4CH_3$).

Anal. Calcd for $C_{68}H_{70}O_{10}S$: C, 75.67; H, 6.54. Found: C, 76.01; H, 6.86.

Methyl *O*-(2,3,4,6-Tetra-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-*O*-[2,3-di-*O*-benzoyl-6-*O*-(4-methoxybenzyl)-β-D-glucopyranosyl]-(1→4)-2,3-di-*O*-benzoyl-α-L-fucopyranoside (12). A mixture of 8 (162 mg, 0.18 mmol), 11 (300 mg, 0.28 mmol) and 4A molecular sieves (1 g) in dry ether (5 mL) was stirred for 1 h at 22 °C under argon. Methyl triflate (0.125 mL, 1.12 mmol) was then added and the mixture was stirred for 20 h. The reaction was quenched with Et_3N, filtered through celite, and then concentrated to a syrup. Column chromatography with 10:1 toluene-EtOAc afforded 9 as thick glass (261 mg, 77%): $[\alpha]_D$ -59.14° (*c* 1.33, $CHCl_3$); ^1H NMR δ 7.98-6.74 (m, 59H, aromatic protons), 5.04 (d, 1H, $J_{1,2}$ =

2.0 Hz, H-1), 4.9 (d, 1H, $J_{1'',2''}$ = 3.4 Hz, H-1''), 4.70 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 4.42 (d, 1H, $J_{1''',2'''}$ = 8.6 Hz, H-1'''), 3.72 (s, 3H, $CH_2C_6H_4OCH_3$), 3.32 (s, 3H, OCH_3), 1.12 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6).

Anal. Calcd for $C_{110}H_{110}O_{25}$: C, 72.12; H, 6.05. Found: C, 72.34; H, 6.38.

Methyl *O*-(2,3,4,6-Tetra-*O*-benzyl-ß-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-*O*-[2,3-di-*O*-acetyl-6-*O*-(4-methoxybenzyl)-ß-D-glucopyranosyl]-(1→4)-2,3-di-*O*-acetyl-α-L-fucopyranoside (14). Compound 12 (32 mg, 0.017 mmol) was debenzoylated with 0.05 M NaOMe in the usual way and the resulting product, 13, was treated with pyridine (0.2 mL) and acetic anhydride (0.2 mL). The reaction mixture was stirred for 4 h, concentrated and pyridine was removed by coevaporation with toluene. Column chromatography with 5:1 toluene-EtOAc gave 14 (23.4 mg, 87%); ^{1}H NMR δ 7.27-6.76 (m, 39H, aromatic protons), 5.30 (d, 1H, $J_{1,2}$ = 2.0 Hz, H-1), 5.13 (d, 1H, $J_{1'',2''}$ = 3.4 Hz, H-1''), 4.86 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 4.60 (d, 1H, $J_{1''',2'''}$ = 8.5 Hz, H-1'''), 3.73 (s, 3H, $CH_2C_6H_4OCH_3$), 3.33 (s, 3H, OCH_3), 2.06-1.66 (4s, 12H, 4 OAc), 1.26 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6).

Methyl *O*-(2,3,4,6-Tetra-*O*-benzyl-ß-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzoyl-ß-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-α-L-fucopyranoside (15). To a solution of 12 (170 mg, 0.09 mmol) in 9:1 acetonitrile-water (3 mL), ceric ammonium nitrate (240 mg) was added. The mixture was stirred for 2 h at room temperature. The solution was diluted with CH_2Cl_2, and washed with aq $NaHCO_3$. The aqueous layer was again extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layer was dried and concentrated. Column chromatography with 10:1 toluene-ether gave 15 as amorphous solid (138 mg, 86.7%): $[\alpha]_D$ -72.26° (*c* 0.69, $CHCl_3$); ^{1}H NMR δ 7.98-7.26 (m, 55H, aromatic protons), 5.09 (d, 1H, $J_{1,2}$ = 2.0 Hz, H-1), 4.87 (d, 1H, $J_{1'',2''}$ = 3.4 Hz, H-1''), 4.74 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 4.42 (d, 1H, $J_{1''',2'''}$ = 8.6 Hz, H-1'''),

3.35 (s, 3H, OCH_3), 1.18 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6).

Anal. Calcd for $C_{102}H_{102}O_{24}$: C, 71.56; H, 6.01. Found: C, 71.38; H, 6.06.

Methyl *O*-(2,3,4,6-Tetra-*O*-benzyl-ß-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-*O*-(methyl 2,3-di-*O*-benzoyl-ß-D-glucopyranosyluronate)-(1→4)-2,3-di-*O*-benzoyl-α-L-fucopyranoside (16). A solution of CrO_3 (225 mg, 2.25 mmol) in 3.5 M aq H_2SO_4 (1.2 mL) was added to a solution of 15 (366 mg, 0.21 mmol) in 3:2 acetone-CH_2Cl_2 (6 mL) at 0 °C. After 15 min the mixture was allowed to attain room temperature. After 9 h, the reaction was quenched with EtOH and the solid precipitates were filtered off. The filtrate was concentrated under vacuum to a small volume and the remaining aqueous phase was extracted with CH_2Cl_2 (3 x 15 mL). The combined extracts were washed with water and saturated NaCl solution, dried and concentrated under reduced pressure. The resulting syrupy acid was then dissolved in CH_2Cl_2 and treated with CH_2N_2 in ether. The solution was concentrated to dryness and purified by column chromatography with 4:1 toluene-EtOAc to yield pure syrupy 16 (250 mg, 67%): $[\alpha]_D$ -80° (*c* 0.5, $CHCl_3$); ^1H NMR δ 7.94-7.28 (m, 55H, aromatic protons), 5.02 (d, 1H, $J_{1,2}$ = 3.0 Hz, H-1), 4.86 (d, 1H, $J_{1",2"}$ = 3.6 Hz, H-1"), 4.72 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 4.38 (d, 1H, $J_{1"',2"'}$ = 8.0 Hz, H-1"'), 3.30 (s, 3H, OCH_3), 2.96 (s, 3H, $COOCH_3$), 1.14 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6); ^{13}C NMR δ 166.7-164.9 ($COOCH_3$, 4 COC_6H_5), 139.5-126.9 (aromatic carbons), 102.8 (C-1"'), 102.4 (C-1'), 99.0 (C-1), 97.4 (C-1"), 82.6, 79.9, 79.6, 78.7, 78.0, 76.1, 75.6, 75.4, 75.1, 74.7, 73.9, 73.5, 73.2, 72.7, 71.6, 69.9, 69.1 (C-6"'), 67.4 (C-6"), 55.4 ($COOCH_3$), 52.1 (OCH_3), 16.0 (C-6).

Anal. Calcd for $C_{103}H_{102}O_{25}$: C, 71.09; H, 5.91. Found: C, 70.68, H, 5.98.

Methyl *O*-(ß-D-Galactopyranosyl)-(1→4)-*O*-(α-D-glucopyranosyl)-(1→4)-*O*-(methyl ß-D-glucopyranosyluronate)-(1→4)-α-L-fucopyranoside (18). A solution of 16 (150 mg, 86 µmol) in acetic acid (5 mL) was stirred with 10% Pd-C (150 mg)

under hydrogen at 24 °C for 2 days, then filtered through celite and concentrated in vacuum. The resulting product (17) was debenzoylated by the Zemplén method. The residue was purified by column chromatography using 3:1 $CHCl_3$-MeOH to give 18 (36 mg, 60.4%): $[\alpha]_D$ -8.5° (*c* 0.82, H_2O); ^1H NMR (D_2O) δ 5.40 (d, 1H, $J_{1,2}$ = 2.0 Hz, H-1), 5.18 (d, 1H, $J_{1",2"}$ = 3.4 Hz, H-1"), 4.53 (d, 1H, $J_{1',2'}$ = 8.3 Hz, H-1'), 4.41 (d, 1H, $J_{1"',2"'}$ = 8.0 Hz, H-1"'), 3.35 (s, 3H, OCH_3), 3.30 (s, 3H, $COOCH_3$), 1.25 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6); ^{13}C NMR (D_2O, internal standard dioxane) δ 171.5 ($COOCH_3$), 103.9 (C-1"'), 103.7 (C-1'), 100.3 (C-1), 99.4 (C-1"), 81.6, 78.7, 77.4, 76.2 (2 C), 74.4, 73.9, 73.5, 73.4, 72.1, 72.0, 71.8, 71.6, 69.6, 69.4, 69.2, 61.9 (C-6"'), 60.2 (C-6"), 56.0 ($COOCH_3$), 54.4 (OCH_3), 16.0 (C-6).

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