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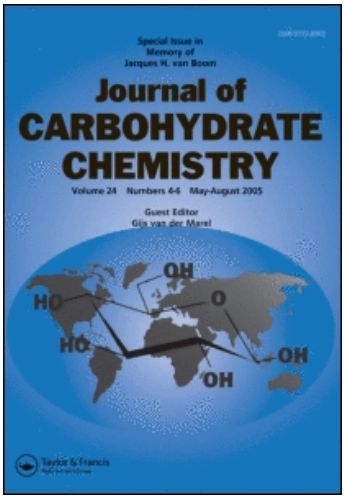


## Journal of Carbohydrate Chemistry
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# Synthetic Studies on Sialoglycoconjugates 72: Synthesis of Sulfo-, Phosphono- and Sialyl-Lewis × Analogs Containing the 1-Deoxy- and 1,2-Dideoxy-hexopyranoses in Place of *N*-Acetylglucosamine Residue

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**To cite this Article** Maeda, Hirkazu , Ito, Kenichi , Ishida, Hideharu , Kiso, Makoto and Hasegawa, Akira(1995) 'Synthetic Studies on Sialoglycoconjugates 72: Synthesis of Sulfo-, Phosphono- and Sialyl-Lewis × Analogs Containing the 1-Deoxy- and 1,2-Dideoxy-hexopyranoses in Place of *N*-Acetylglucosamine Residue', Journal of Carbohydrate Chemistry, 14: 3, 387 — 406
**To link to this Article: DOI:** 10.1080/07328309508002079
**URL:** http://dx.doi.org/10.1080/07328309508002079

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J. CARBOHYDRATE CHEMISTRY, 14(3), 387-406 (1995)

# SYNTHETIC STUDIES ON SIALOGLYCOCONJUGATES 72: SYNTHESIS OF SULFO-, PHOSPHONO- AND SIALYL-LEWIS X ANALOGS CONTAINING THE 1-DEOXY- AND 1,2-DIDEOXY-HEXOPYRANOSES IN PLACE OF *N*-ACETYLGLUCOSAMINE RESIDUE

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*Received November 1, 1994 - Final Form January 4, 1995*

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## ABSTRACT

Sialyl-, sulfo- and phosphono-Le$^x$ analogs containing 1-deoxy and 1,2-dideoxy-D-glucose in place of *N*-acetylglucosamine have been synthesized. Glycosylation of *O*-(2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1→4)-1,5-anhydro-2,6-di-*O*-benzoyl-D-glucitol (**5**), prepared from per-*O*-acetyl-lactose in five steps, or *O*-(2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1→4)-1,5-anhydro-6-*O*-benzoyl-2-deoxy-D-*arabino*-hexitol (**9**), prepared from per-*O*-acetyllactal (**2**) in four steps, with the methyl 1-thioglycoside derivative of L-fucose, gave the corresponding trisaccharides **11** and **18**. These are transformed into the glycosyl acceptors **12** and **19** for the sialyl Le$^x$ analogs, and **15** and **22** for the sulfo- and phosphono Le$^x$ analogs. Selective hydrolysis of the isopropylidene group of **11** and **18** yielded **12** and **19**, respectively. The latter compounds were then converted to **15** and **22**, respectively, by selective levulinylation, reductive removal of the benzyl groups followed by acetylation and removal of the levulinyl group. **15** and **22** were then converted in good yields, by sulfonation followed by *O*-deacylation, into the target compounds **17** and **24**. Treatment of **22** with diphenyl chlorophosphate, and subsequent hydrogenolysis of the phenyl groups with Adams Platinum followed by *O*-deacylation afforded the phosphono-Le$^x$ analog. Glycosylation of **12** or **19** with



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methyl (phenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**27**), using *N*-iodosuccinimide (NIS)-trifluoromethanesulfonic acid (TfOH), afforded the desired tetrasaccharides, which were converted in good yields, *via* hydrogenolysis of the benzyl groups, *O*-acetylation, *O*-deacylation and deesterification into the sialyl $Le^x$ analogs.

## INTRODUCTION

In the previous papers[1-8] we have discussed the importance of synthetic studies on sialyl $Le^x$, sialyl $Le^a$ and various types of analogs for progress toward the goal of elucidating the structural features of the carbohydrate ligand required for selectin recognition.[9-11] Based on our demonstrated, three-dimensional structures[12] of $sLe^a$ and $sLe^x$ epitopes, various types of sialyl $Le^x$ analogs containing chemically modified sialic acid, fucose, galactose and *N*-acetylglucosamine moieties have been synthesized and their binding activities to the selectins were examined[12-16] in order to clarify the carbohydrate structure required for the recognition. As a part of our continuing efforts along these line, we describe here the synthesis of sulfo-, phosphono-, and sialyl $Le^x$ analogs containing 1-deoxy- and 1,2-dideoxy-glucose in place of *N*-acetylglucosamine residue.

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## RESULTS AND DISCUSSION

For the synthesis of the desired $Le^x$ analogs we have selected *O*-(2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-1,5-anhydro-2,6-di-*O*-benzoyl-D-glucitol (**12**) and *O*-(2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-1,5-anhydro-6-*O*-benzoyl-2-deoxy-D-*arabino*-hexitol (**19**) as the suitably protected intermediates. Compounds **12** and **19** could be converted into sulfo- and phosphono-$Le^x$ analogs by further processing according to our procedures.[17]

In addition, the trisaccharide acceptors **12** and **19** were then glycosylated with methyl (phenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-2-(nonulopyranosid)onate[18] (**27**) using NIS-TfOH[19] as a promoter, to afford

the corresponding tetrasaccharides **28** and **31**. According to our usual procedures[20] compound **28** and **31** could be transformed into the $sLe^x$ analogs.

Treatment of *O*-(β-D-galactopyranosyl)-(1→4)-1,5-anhydro-D-glucitol[21] (**3**), prepared from per-*O*-acetyllactose (**1**) by bromination and subsequent reductive debromination with tributyltin hydride followed by *O*-deacylation according to the conventional procedures, followed by isopropylidenation with 2,2-dimethoxypropane in *N,N*-dimethylformamide (DMF) in the presence of *p*-toluenesulfonic acid monohydrate gave the 3',4'-*O*-isopropylidene derivative **4** in 78% yield. This latter compound on selective benzoylation with benzoyl chloride in pyridine at -50 °C was converted in 70% yield into the desired glycosyl acceptor **5**, which has a free hydroxyl group at C-3 of the deoxyglucose residue. Significant signals in the $^1H$ NMR spectrum of **5** were a one-proton multiplet at δ 5.18 (H-2a), a one-proton triplet at δ 5.36 (H-2b), and a multiplet at δ 7.20-8.08 (20H, 4Ph), indicating the structure assigned. Other $^1H$ NMR data are consistent with the structure.

Catalytic hydrogenation (10% Pd-C) of *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,6-di-*O*-acetyl-1,5-anhydro-2-deoxy-D-*arabino*-hex-1-enitol[21] (**2**) in ethanol, prepared from per-*O*-acetyllactosyl bromide according to the literature,[22] afforded the 1,2-dideoxy-derivative **6** in 84% yield, which on Zemplen *O*-deacylation, 3',4'-*O*-isopropylidenation and selective benzoylation as described for **5** gave *O*-(2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1→4)-1,5-anhydro-6-*O*-benzoyl-2-deoxy-D-*arabino*-hexitol (**9**) in good yield; compound is **9** another glycosyl acceptor having a free hydroxyl at C-3 of the 1,2-dideoxyglucose residue. Significant signals in the $^1H$ NMR spectrum of **9** were a one-proton triplet at δ 5.37 (H-2b) and a multiplet at δ 7.25-8.19 (15H, 3Ph), indicating the structure assigned.

Glycosylation of **5** with methyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside[23] (**10**), in dry benzene in the presence of DMTST and 4Å molecular sieves, gave exclusively the α-glycoside **11** in 79% yield; significant signals of the L-fucose residue in the $^1H$ NMR spectrum were a three-proton doublet at δ 1.35 ($J_{5,6}$ = 6.4 Hz, H-6b) and a one-proton doublet at δ 5.59 ($J_{1,2}$ = 3.7 Hz, H-1b). In essentially the same way, glycosylation of **9** with **10** gave the desired trisaccharide **18** in 96% yield.

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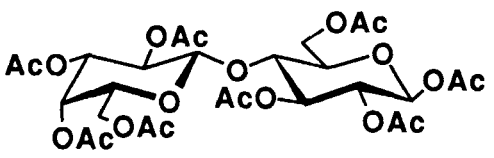

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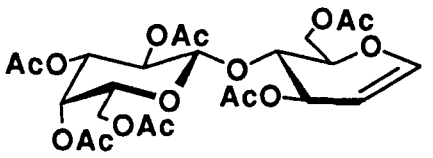

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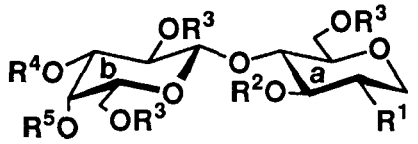

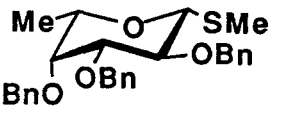

10

| | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ |
|---|---|---|---|---|---|
| 3 | OH | H | H | H | H |
| 4 | OH | H | H | ipd | |
| 5 | OBz | H | Bz | ipd | |
| 6 | H | Ac | Ac | Ac | Ac |
| 7 | H | H | H | H | H |
| 8 | H | H | H | ipd | |
| 9 | H | H | Bz | ipd | |

Bn = benzyl
Bz = benzoyl
ipd = isopropylidene

Hydrolysis of the isopropylidene group in **11** or **18** with aq 80% acetic acid gave the desired intermediates **12** and **19** respectively. Selective levulinylation of **12** or **19**, with levulinic anhydride in pyridine-dichloromethane at -50 °C in the presence of 4-dimethylaminopyridine gave the 3-*O*-levulinyl derivatives **13** (84%) and **20** (quantitative). Catalytic hydrogenolysis of the benzyl groups in **13** or **20** in ethanol-acetic acid and subsequent *O*-acetylation gave the corresponding per-*O*-acyl compounds **14** (76%) and **21** (76%), respectively. Treatment of **14** or **21** with hydrazine monoacetate in ethanol at room temperature afforded the expected 3-hydroxy compounds **15** and **22** quantitatively.

Treatment of **15** or **22** with sulfur trioxide pyridine complex in DMF for one h at room temperature gave the protected 3-sulfo-$Le^x$ analogs **16** (85%) and **23** (quantitative) as their pyridine salts, which on deprotection with sodium methoxide in methanol and subsequent, column chromatography on Sephadex LH-20 yielded the sulfo-$Le^x$ analogs **17** and **24** as their sodium salts in high yields, respectively.

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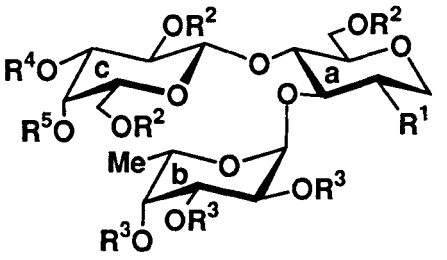


| | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ |
|---|---|---|---|---|---|
| 11 | OBz | Bz | Bn | ipd | |
| 12 | OBz | Bz | Bn | H | H |
| 13 | OBz | Bz | Bn | Lev | H |
| 14 | OBz | Bz | Ac | Lev | Ac |
| 15 | OBz | Bz | Ac | H | Ac |
| 16 | OBz | Bz | Ac | $SO_3 \cdot$ pyr. | Ac |
| 17 | OH | H | H | $SO_3Na$ | H |
| 18 | H | Bz | Bn | ipd | |
| 19 | H | Bz | Bn | H | H |
| 20 | H | Bz | Bn | Lev | H |
| 21 | H | Bz | Ac | Lev | Ac |
| 22 | H | Bz | Ac | H | Ac |
| 23 | H | Bz | Ac | $SO_3 \cdot$ pyr. | Ac |
| 24 | H | H | H | $SO_3Na$ | H |
| 25 | H | Bz | Ac | $PO(OPh)_2$ | Ac |
| 26 | H | H | H | $PO_3Na_2$ | H |

Lev = $CH_3COCH_2CH_2CO$—

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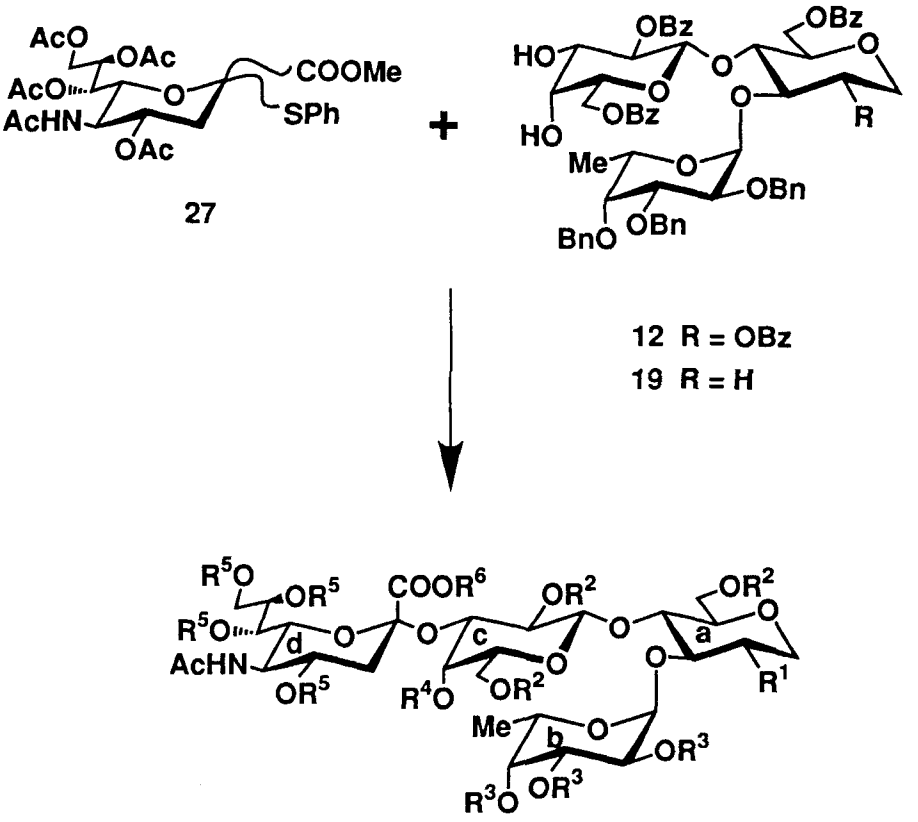


| | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ | $R^6$ |
|---|---|---|---|---|---|---|
| 28 | OBz | Bz | Bn | H | Ac | Me |
| 29 | OBz | Bz | Ac | Ac | Ac | Me |
| 30 | OH | H | H | H | H | H |
| 31 | H | Bz | Bn | H | Ac | Me |
| 32 | H | H | H | H | H | H |

Furthermore, treatment of **22** with diphenyl chlorophosphate in pyridine for 12 h at room temperature gave the 3-*O*-diphenylphosphono derivative **25**, which on hydrogenolysis of the phenyl groups in the presence of pre-reduced Adams platinum catalyst and subsequent treatment with sodium methoxide in methanol gave the 3-*O*-phosphono-$Le^x$ analog **26** in 89% yield, after chromatography on a column of Sephadex LH-20.

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Glycosylation[19,24] of **12** or **19** with **27** in acetonitrile-dichloromethane overnight at -40 °C in the presence of NIS-TfOH and 4Å molecular sieves, gave exclusively the α-glycosides **28** (42%) and **31** (45%), respectively. The structures of **28** and **31** were unambiguously proved by 270 MHz $^1$H NMR spectroscopy. The observed chemical shifts and coupling constants for H-7 of Neu5Ac residue [δ 5.23 (dd, $J_{6,7}$ = 2.4, $J_{7,8}$ = 10.7 Hz) for **28**; 5.23 (dd, $J_{6,7}$ = 2.2, $J_{7,8}$ = 10.0 Hz) for **31**] and H-8 (δ 5.48) are characteristic of α-glycosidically linked[17,20,25] sialic acid analogs.

Catalytic hydrogenolysis of the benzyl groups in **28** in ethanol-acetic acid and subsequent *O*-acetylation gave the per-*O*-acyl compound **29** in 73% yield, which on *O*-deacylation and subsequent saponification of the methyl ester group furnished the 1-deoxyglucose-containing sialyl Le$^x$ analog **30** in quantitative yield. In a similar way, **31** was transformed by reductive removal of the benzyl groups followed by *O*-deacylation and saponification of the methyl ester into the target, 1,2-dideoxyglucose-containing sialyl Le$^x$ analog **32**.

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## EXPERIMENTAL

**General Procedures.** Optical rotations were determined with a Union PM-201 polarimeter at 25 °C, and IR spectra were recorded with a Jasco IRA-100 spectrophotometer. $^1$H NMR spectra were recorded at 270 MHz with a JEOL JNM-GX 270 spectrometer. An electroscopy mass spectrometer (Perkin-Elmer Sciex Instrument, Thornhill, Canada) fitted with an atmospheric pressure ionization source. Preparative column chromatography was performed on silica gel (Wako Chemical Co., 200 mesh) with the solvent systems specified. Concentrations were conducted *in vacuo*.

***O*-(3,4-*O*-Isopropylidene-β-D-galactopyranosyl)-(1→4)-1,5-anhydro-D-glucitol (4).** To a solution of *O*-(β-D-galactopyranosyl)-(1→4)-1,5-anhydro-D-glucitol[21] (**3**, 2.13 g, 6.53 mmol), prepared from per-*O*-acetyllactose (**1**) by C-1-bromination, subsequent reduction with tributyltin hydride and *O*-deacetylation according to the conventional procedures, in *N,N*-dimethylformamide (DMF, 20 mL)

were added 2,2-dimethoxypropane (2.5 mL) and *p*-toluenesulfonic acid monohydrate (100 mg). The mixture was stirred for 4 h at room temperature then neutralized with $Na_2CO_3$. The precipitate was collected and washed with MeOH, and the combined filtrate and washings were concentrated. Column chromatography (20:1 MeOH-$CH_2Cl_2$) of the residue on silica gel (100 g) gave **4** (1.87 g, 78.2%) as an amorphous mass: $[\alpha]_D$ +48.5° (*c* 1.5, 1:1 MeOH-$CH_2Cl_2$); $^1H$ NMR ($D_2O$) δ 1.41, 1.57 (2s, 6H, $Me_2C$), 3.30 (t, 1H, $J_{1ax,2} = J_{gem}$ = 10.8 Hz, H-1a*ax*), 3.54 (t, 1H, $J_{3,4} = J_{4,5}$ = 7.9 Hz, H-4a), 4.38 (dd, 1H, $J_{2,3}$ = 5.4 Hz, $J_{3,4}$ = 1.8 Hz, H-3b), and 4.51 (d, 1H, $J_{1,2}$ = 8.2 Hz, H-1b).

Anal. Calcd for $C_{15}H_{26}O_{10}$ (366.4): C, 49.17; H, 7.15. Found: C, 49.06; H, 7.33.

***O*-(2,6-Di-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1→4)-1,5-anhydro-2,6-di-*O*-benzoyl-D-glucitol (5).** To a solution of **4** (100 mg, 0.27 mmol) in pyridine (0.5 mL) and $CH_2Cl_2$ (1 mL), cooled to -50 °C, was added dropwise a solution of benzoyl chloride (0.16 mL) in $CH_2Cl_2$ (1.6 mL) and the mixture was stirred for 3 h at -50 °C; the course of the reaction was monitored by TLC. After completion of the reaction, MeOH (0.5 mL) was added to the mixture, and this was concentrated then extracted with $CH_2Cl_2$. The extract was successively washed with 2 M HCl, M $Na_2CO_3$ and water, dried ($Na_2SO_4$) and concentrated. Column chromatography (400:1 $CH_2Cl_2$-MeOH) of the residue on silica gel (30 g) gave **5** (150 mg, 70%) as crystals. Recrystallization from ether-hexane gave needles: mp 180 °C; $[\alpha]_D$ +55.5° (*c* 0.9, $CHCl_3$); IR (KBr) 3400 (OH), 1730 and 1230 (ester), 850 ($Me_2C$), and 740 and 710 $cm^{-1}$ (Ph); $^1H$ NMR ($CDCl_3$) δ 1.43, 1.63 (2s, 6H, $Me_2C$), 3.32 (t, 1H, $J_{1ax,2} = J_{gem}$ = 10.9 Hz, H-1a*ax*), 3.98 (t, 1H, $J_{3,4} = J_{4,5}$ = 8.8 Hz, H-4a), 4.08 (dd, 1H, $J_{1eq,2}$ = 5.5 Hz, H-1a*eq*), 4.66 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1b), 5.18 (m, 1H, H-2a), 5.36 (t, 1H, $J_{2,3}$ = 7.9 Hz, H-2b), 7.20-8.08 (m, 20H, 4Ph).

Anal. Calcd for $C_{43}H_{42}O_{14}$ (782.8): C, 65.97; H, 5.41. Found: C, 65.71; H, 5.53.

***O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-acetyl-1,5-anhydro-2-deoxy-D-*arabino*-hexitol (6).** A solution of *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,6-di-*O*-acetyl-1,5-anhydro-2-

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deoxy-D-*arabino*-hex-1-enitol[21] (**2**, 1.0 g, 1.78 mmol), prepared from hepta-*O*-acetyl lactosyl bromide according to the literature,[22] in EtOH (10 mL) was stirred in the presence of 10% Pd-C (700 mg) for 1.5 h at room temperature under hydrogen, then filtered and concentrated. Column chromatography (1:1 EtOAc-hexane) of the residue on silica gel (100 g) gave **6** (837 mg, 84%) as an amorphous mass: $[\alpha]_D$ +18.0° (*c* 1.0, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 1.67 (m, 1H, $J_{1eq,2ax}$ = 5.1 Hz, $J_{1ax,2ax} = J_{gem} = J_{2ax,3}$ = 12.8 Hz, H-2a*ax*), 3.45 (m, 1H, $J_{1ax,2eq}$ = 2.0 Hz, $J_{gem}$ = 12.4 Hz, H-1a*ax*), 3.63 (t, 1H, $J_{3,4}$ = 8.1 Hz, H-4a), 3.95 (m, 1H, H-1a*eq*), 4.58 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1b), 4.98 (dd, 1H, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.5 Hz, H-3b), 4.99 (m, 1H, H-3a), 5.15 (dd, 1H, H-2b), and 5.36 (dd, 1H, H-4b).

Anal. Calcd for $C_{24}H_{34}O_{15}$ (562.5): C, 51.24; H, 6.09. Found: C, 51.08; H, 6.17.

***O*-(β-D-Galactopyranosyl)-(1→4)-1,5-anhydro-2-deoxy-D-*arabino*-hexitol (7).** To a solution of **6** (837 mg, 1.49 mmol) in MeOH (10 mL) was added NaOMe (10 mg) and the mixture was stirred for 3 h at room temperature then neutralized with Amberlite IR-120 ($H^+$) resin. The resin was collected and washed with MeOH, and the combined filtrate and washings was concentrated. The crystalline residue was crystallized from EtOH-ether to give needles: mp 210 °C; $[\alpha]_D$ +26.0° (*c* 0.9, MeOH) [lit.[21] mp 190-193 °C, $[\alpha]_D$ +27.5° ($H_2O$)].

Anal. Calcd for $C_{12}H_{22}O_9$ (310.3): C, 46.45; H, 7.15. Found: C, 46.38; H, 7.21.

***O*-(3,4-*O*-Isopropylidene-β-D-galactopyranosyl)-(1→4)-1,5-anhydro-2-deoxy-D-*arabino*-hexitol (8).** To a solution of **7** (1.0 g, 3.22 mmol) in DMF (100 mL) were added 2,2-dimethoxypropane (0.78 mL, 6.44 mmol) and *p*-toluenesulfonic acid monohydrate (50 mg), and the mixture was stirred for 6 h at 80 °C. After cooling, the mixture was neutralized with $Et_3N$ then concentrated. Column chromatography (25:1 $CH_2Cl_2$-MeOH) of the residue on silica gel (100 g) gave **8** (713 mg, 63%) as crystals. Recrystallization from EtOAc gave needles: mp 198 °C; $[\alpha]_D$ +47.0° (*c* 0.9, MeOH); $^1H$ NMR ($CDCl_3$) δ 1.25, 1.40 (2s, 6H, $Me_2C$), 1.41 (m, 1H, $J_{1eq,2ax}$ = 5.0 Hz, $J_{1ax,2ax} = J_{gem} = J_{2ax,3}$ = 13.0 Hz, H-2a*ax*), 1.86 (m, 1H, H-2a*eq*), 3.68 (d, 1H, $J_{3,4}$ = 2.4 Hz, H-4b), 3.80 (m, 1H, $J_{1eq,2eq}$ = 1.8 Hz, $J_{1eq,2ax}$ = 5.2 Hz, $J_{gem}$ = 11.7 Hz, H-1a*eq*), 3.98 (dd, 1H, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 7.7 Hz, H-2b), 4.12 (dd, 1H, H-3b), and 4.28 (d, 1H, H-1b).

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Anal. Calcd for $C_{15}H_{26}O_9$ (350.4): C, 51.42; H, 7.48. Found: C, 51.33; H, 7.49.

***O*-(2,6-Di-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1→4)-1,5-anhydro-6-*O*-benzoyl-2-deoxy-D-*arabino*-hexitol (9).** To a solution of **8** (502 mg, 1.43 mmol) in pyridine (4 mL) and $CH_2Cl_2$ (2 mL), cooled to -50 °C, was added dropwise a solution of benzoyl chloride (0.55 mL, 4.73 mmol) in $CH_2Cl_2$ (6 mL), and the mixture was stirred for 2 h. Methanol (1 mL) was added to the mixture and concentrated. Column chromatography (1:4 EtOH-hexane) of the residue on silica gel (100 g) gave **9** (520 mg, 55%) as crystals. Recrystallization from EtOH gave needles: mp 167 °C; $[\alpha]_D$ +60.0° (*c* 0.9, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 1.36, 1.65 (2s, 6H, $Me_2C$), 1.65 (m, 1H, $J_{1ax,2ax} = J_{gem} = J_{2ax,3} = 13.1$ Hz, H-2a*ax*), 2.03 (m, 1H, H-2a*eq*), 3.75 (m, 1H, H-1a*eq*), 3.91 (dd, 1H, $J_{2,3} = 12.0$ Hz, $J_{3,4} = 3.7$ Hz, H-3b), 4.53 (dd, 1H, $J_{5,6} = 8.6$ Hz, $J_{6,6'} = 12.0$ Hz, H-6b), 4.63 (d, 1H, $J_{1,2} = 7.9$ Hz, H-1b), 4.84 (dd, 1H, $J_{5,6'} = 3.6$ Hz, H-6'b), 5.37 (t, 1H, $J_{2,3} = 7.7$ Hz, H-2b), and 7.25-8.19 (m, 15H, 3Ph).

Anal. Calcd for $C_{36}H_{38}O_{12}$ (662.7): C, 65.25; H, 5.78. Found: C, 65.08; H, 5.78.

***O*-(2,6-Di-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-1,5-anhydro-2,6-di-*O*-benzoyl-D-glucitol (11).** To a solution of **5** (1.0 g, 1.27 mmol) and **10** (890 mg, 1.91 mmol) in benzene (5 mL) were added molecular sieves 4Å (MS-4Å, 1.9 g) and the mixture was stirred overnight at room temperature then cooled to 0 °C. Dimethyl(methylthio)sulfonium triflate (DMTST, 2.64 g, 5.1 mmol) was added to the mixture which was stirred for 3 h at 7 °C and then neutralized with $Et_3N$. The precipitate was collected and washed with $CH_2Cl_2$, the combined filtrate and washings were concentrated and then extracted with $CH_2Cl_2$. The extract was washed with water, dried ($Na_2SO_4$) and concentrated. Column chromatography (1:5 EtOAc-hexane) of the residue on silica gel (130 g) gave **11** (1.21 g, 79%) as an amorphous mass: $[\alpha]_D$ +8.4° (*c* 1.7, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 1.31, 1.51 (2s, 6H, $Me_2C$), 1.35 (d, 3H, $J_{5,6} = 6.4$ Hz, H-6b), 3.18 (t, 1H, $J_{1ax,2} = J_{gem} = 10.8$ Hz, H-1a*ax*), 4.51 (d, 1H, $J_{1,2} = 8.6$ Hz, H-1c), 5.25 (m, 1H, H-2a), 5.59 (d, 1H, $J_{1,2} = 3.7$ Hz, H-1b), and 7.01-8.16 (m, 35H, 7Ph).

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Anal. Calcd for $C_{70}H_{70}O_{18}$ (1199.3): C, 70.10; H, 5.88. Found: C, 70.22; H, 6.02.

***O*-(2,6-Di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-1,5-anhydro-2,6-di-*O*-benzoyl-D-glucitol (12).** A solution of **11** (1.2 g, 1.0 mmol) in aq 80% AcOH (20 mL) was heated for 48 h at 55 °C then concentrated. Column chromatography (1:2 EtOAc-hexane) of the residue on silica gel (80 g) gave **12** (870 mg, 80%) as an amorphous mass: $[\alpha]_D$ -4.7° (*c* 1.0, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 1.33 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6b), 3.18 (t, 1H, $J_{1ax,2} = J_{gem}$ = 10.6 Hz, H-1a*ax*), 3.54 (t, 1H, $J_{2,3} = J_{3,4}$ = 7.0 Hz, H-4a), 5.24 (t, 1H, $J_{1,2} = J_{2,3}$ = 8.1 Hz, H-2c), 5.59 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1b), and 7.01-8.10 (m, 35H, 7Ph).

Anal. Calcd for $C_{67}H_{66}O_{18}$ (1159.3): C, 68.34; H, 5.74. Found: C, 68.32; H, 5.86.

***O*-(2,6-Di-*O*-benzoyl-3-*O*-levulinyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-1,5-anhydro-2,6-di-*O*-benzoyl-D-glucitol (13).** To a solution of **12** (502 mg, 0.433 mmol) and 4-dimethylaminopyridine (5 mg) in pyridine (9 mL) and $CH_2Cl_2$ (2 mL), cooled to -50 °C, was added dropwise a solution of levulinic anhydride (190 mg) in $CH_2Cl_2$ (2 mL), and the mixture was stirred for one h. After completion of the reaction, MeOH (0.5 mL) was added to the mixture which was concentrated and then extracted with $CH_2Cl_2$. The extract was successively washed with 2 M HCl and M $Na_2CO_3$, dried ($Na_2SO_4$) and concentrated. Column chromatography (1:2 EtOAc-hexane) of the residue on silica gel (60 g) gave **13** (460 mg, 84%) as an amorphous mass: $[\alpha]_D$ +9.5° (*c* 0.9, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 1.40 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6b), 2.14 (s, 3H, Ac), 2.43, 3.68 (2m, 4H, MeCOC$H_2$C$H_2$CO), 3.17 (t, 1H, $J_{1ax,2} = J_{gem}$ = 10.6 Hz, H-1a*ax*), 3.45 (t, 1H, $J_{3,4} = J_{4,5}$ = 6.7 Hz, H-4a), 5.25 (m, 1H, H-2a), 5.57 (d, 1H, $J_{1,2}$ = 4.0 Hz, H-1b), 5.60 (dd, 1H, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 10.0 Hz, H-2c), and 6.81-8.10 (m, 35H, 7Ph).

Anal. Calcd for $C_{72}H_{72}O_{20}$ (1257.4): C, 68.77; H, 6.17. Found: C, 68.59; H, 6.23.

***O*-(4-*O*-Acetyl-2,6-di-*O*-benzoyl-3-*O*-levulinyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-1,5-an-**

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**hydro-2,6-di-*O*-benzoyl-D-glucitol (14).** A solution of **13** (302 mg, 0.24 mmol) in EtOH (15 mL) and AcOH (4 mL) was stirred overnight at 40 °C in the presence of 10% Pd-C (300 mg) under hydrogen. The mixture was then filtered and the filtrate concentrated. The residue was acetylated with $Ac_2O$ (0.7 mL)-pyridine (1 mL) in the presence of 4-dimethylaminopyridine (10 mg) overnight at 45 °C. Methanol (0.5 mL) was added to the mixture and the mixture was concentrated. Column chromatography (200:1 $CH_2Cl_2$-MeOH) of the residue on silica gel (50 g) gave **14** (235 mg, 76%) as an amorphous mass: $[\alpha]_D$ -4.7° (*c* 0.8, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 1.44 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6b), 1.72-2.26 (5s, 15H, 4AcO, Ac), 2.48, 2.57 (2m, 4H, MeCOC*H*$_2$C*H*$_2$CO), 3.24 (t, 1H, $J_{1ax,2} = J_{gem}$ = 9.9 Hz, H-1a*ax*), 5.11 (dd, 1H, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 3.1 Hz, H-3c), 5.54 (d, 1H, H-4c), and 7.21-8.10 (m, 15H, 3Ph).

Anal. Calcd for $C_{59}H_{62}O_{24}$ (1155.1): C, 61.34; H, 5.41. Found: C, 61.29; H, 5.58.

***O*-(4-*O*-Acetyl-2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-1,5-anhydro-2,6-di-*O*-benzoyl-D-glucitol (15).** To a solution of **14** (55 mg, 0.047 mmol) in EtOH (0.5 mL) was added hydrazine monoacetate (5.4 mg) and the mixture was stirred for 3 h at room temperature then concentrated. Column chromatography (100:1 $CH_2Cl_2$-MeOH) of the residue on silica gel (30 g) gave **15** (52 mg, quantitative) as an amorphous mass: $[\alpha]_D$ -17.5° (*c* 0.9, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 1.38 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6b), 1.70-2.24 (4s, 12H, 4AcO), 3.24 (t, 1H, $J_{1ax,2} = J_{gem}$ = 10.6 Hz, H-1a*ax*), 5.32 (dd, 1H, $J_{2,3}$ = 11.1 Hz, $J_{3,4}$ = 4.0 Hz, H-3b), 5.47 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1b), and 7.26-8.16 (m, 20H, 4Ph).

Anal. Calcd for $C_{54}H_{56}O_{22}$ (1057.0): C, 61.36; H, 5.34. Found: C, 61.33; H, 5.49.

***O*-(4-*O*-Acetyl-2,6-di-*O*-benzoyl-3-*O*-sulfo-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-1,5-anhydro-2,6-di-*O*-benzoyl-D-glucitol pyridine salt (16).** To a solution of **15** (126 mg, 0.12 mmol) in DMF (1 mL) was added sulfur trioxide pyridine complex (95 mg) and the mixture was stirred for one h at room temperature. Methanol (0.2 mL) was added to the mixture and the mixture was concentrated. Column chromatography (20:1

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$CH_2Cl_2$-MeOH) of the residue on silica gel (60 g) gave **16** (121 mg, 85%) as an amorphous mass: $[\alpha]_D$ -2.0° (*c* 0.9, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 1.29 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6b), 1.85-2.15 (4s, 12H, 4AcO), 3.24 (t, 1H, $J_{1ax,2} = J_{gem}$ = 10.6 Hz, H-1a*ax*), 5.14 (dd, 1H, $J_{2,3}$ = 11.1 Hz, $J_{3,4}$ = 4.0 Hz, H-3b), 5.45 (d, 1H, $J_{3,4}$ = 3.4 Hz, H-4c), and 7.13-8.06 (m, 25H, 4Ph, $C_5H_5N$).

Anal. Calcd for $C_{59}H_{61}NO_{25}S$ (1216.1): C, 58.27; H, 5.56; N, 1.15. Found: C, 58.34; H, 5.73; N, 1.18.

***O*-(3-*O*-Sulfo-β-D-galactopyranosyl)-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)]-1,5-anhydro-D-glucitol sodium salt (17).** To a solution of **16** (120 mg, 0.1 mmol) in MeOH (2 mL) was added NaOMe (20 mg) and the mixture was stirred overnight at room temperature then concentrated at 25 °C. Column chromatography (MeOH) of the residue on Sephadex LH-20 (60 g) gave **17** (56 mg, 98%) as an amorphous mass: $[\alpha]_D$ -9.7° (*c* 1.1, 1:1 MeOH-$H_2O$); $^1H$ NMR ($D_2O$) δ 1.53 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6b), 3.63 (t, 1H, $J_{1ax,2} = J_{gem}$ = 10.6 Hz, H-1a*ax*). The mass spectrum of **17** (negative ion mode) showed the base peak at *m/z* 550.5 $(M-1)^-$.

***O*-(2,6-Di-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,6-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-1,5-anhydro-6-*O*-benzoyl-2-deoxy-D-*arabino*-hexitol (18).** To a solution of **9** (200 mg, 0.3 mmol) and **10** (67 mg, 0.15 mmol) were added MS-4Å (1.0 g) and the mixture was stirred for 5 h at room temperature and then cooled to 5 °C. DMTST (531 mg) was added to the mixture which was stirred for 10 h at 5 °C. A workup similar to that described for **11** gave **18** (312 mg, 96%) as an amorphous mass: $[\alpha]_D$ -23.0° (*c* 1.0, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 1.31, 1.51 (2s, 6H, $Me_2C$), 4.51 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1c), 4.85 (d, 1H, $J_{1,2}$ = 4.4 Hz, H-1b), 5.23 (t, 1H, $J_{2,3}$ = 8.0 Hz, H-2c), and 7.16-8.14 (m, 30H, 6Ph).

Anal. Calcd for $C_{63}H_{66}O_{16}$ (1079.2): C, 70.12; H, 6.16. Found: C, 69.87; H, 6.15.

***O*-(2,6-Di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-1,5-anhydro-6-*O*-benzoyl-2-deoxy-D-*arabino*-hexitol (19).** A solution of **18** (305 mg, 0.28 mmol) in aq 80% AcOH (10 mL) was heated for 10 h at 55 °C and concentrated. Column

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chromatography (1:2 EtOAc-hexane) of the residue on silica gel (80 g) gave **19** (236 mg, 80%) as an amorphous mass: $[\alpha]_D$ -24.0° (*c* 1.0, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 1.25 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6b), 4.50 (d, 1H, $J_{1,2}$ = 2.8 Hz, H-1b), 5.17 (dd, 1H, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 9.5 Hz, H-2c), and 7.15-8.07 (m, 30H, 6Ph).

Anal. Calcd for $C_{60}H_{62}O_{16}$ (1039.1): C, 69.35; H, 6.01. Found: C, 69.30; H, 5.80.

***O*-(2,6-Di-*O*-benzoyl-3-*O*-levulinyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-1,5-anhydro-6-*O*-benzoyl-D-*arabino*-hexitol (20).** To a solution of **19** (600 mg, 0.577 mmol) and 4-dimethylaminopyridine (5 mg) in pyridine (5 mL) and $CH_2Cl_2$ (2 mL), cooled to -50 °C, was added dropwise a solution of levulinic anhydride (247 mg, 1.15 mmol) in $CH_2Cl_2$ (2.5 mL), and the stirring was continued for 3 h at -50 °C. A similar workup to that described for **13** gave **20** (679 mg, quantitative) as an amorphous mass: $[\alpha]_D$ -6.6° (*c* 1.0, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 1.32 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-1b), 1.71 (s, 3H, Ac), 2.43, 2.68 (2m, 4H, $MeCOCH_2CH_2CO$), 2.98 (d, 1H, OH), 4.68 (d, 1H, $J_{1,2}$ = 2.9 Hz, H-1b), 5.54 (d, 1H, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 10.1 Hz, H-2c), and 7.18-8.08 (m, 30H, 6Ph).

Anal. Calcd for $C_{65}H_{68}O_{18}$ (1137.2): C, 68.65; H, 6.03. Found: C, 68.57; H, 5.92.

***O*-(4-*O*-Acetyl-2,6-di-*O*-benzoyl-3-*O*-levulinyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-1,5-anhydro-6-*O*-benzoyl-2-deoxy-D-*arabino*-hexitol (21).** A solution of **20** (679 mg, 0.6 mmol) in EtOH (8 mL) and AcOH (2 mL) was stirred for 12 h at 45 °C in the presence of 10% Pd-C (700 mg) under hydrogen. The precipitate was collected and washed with EtOH, and the combined filtrate and washings were concentrated. Acetylation of the residue with $Ac_2O$ (2 mL)-pyridine (4 mL) in the presence of 4-dimethylaminopyridine (10 mg) for 10 h at room temperature, and a workup as described for **14** gave **21** (470 mg, 76%) as an amorphous mass: $[\alpha]_D$ -51.0° (*c* 1.0, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 1.36 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6b), 1.96-2.26 (5s, 15H, 4AcO, Ac), 2.48, 2.57 (2m, 4H, $MeCOCH_2CH_2CO$), 4.74 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1c), 5.11 (dd, 1H, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 3.1 Hz, H-3c), 5.22 (d, 1H, $J_{1,2}$ =

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4.0 Hz, H-1b), 5.32 (dd, 1H, $J_{2,3}$ = 10.6 Hz, $J_{3,4}$ = 2.8 Hz, H-3b), 5.43 (dd, 1H, H-2c), 5.49 (d, 1H, H-4b), 5.54 (d, 1H, H-4c), and 8.27-8.10 (m, 15H, 3Ph).

Anal. Calcd for $C_{52}H_{58}O_{22}$ (1035.0): C, 60.34; H, 5.65. Found: C, 60.22; H, 5.42.

***O*-(4-*O*-Acetyl-2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-1,5-anhydro-6-*O*-benzoyl-2-deoxy-D-*arabino*-hexitol (22).** To a solution of **21** (383 mg, 0.37 mmol) in EtOH (4 mL) and tetrahydrofuran (1 mL) was added hydrazine monoacetate (51 mg, 0.56 mmol), and the mixture was stirred for 10 h at room temperature then concentrated. Column chromatography (2:3 EtOAc-hexane) of the residue on silica gel (70 g) gave **22** (346 mg, quantitative) as an amorphous mass: $[\alpha]_D$ -66.0° (*c* 0.9, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 1.32 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6b), 1.64 (m, 1H, $J_{1ax,2ax} = J_{gem} = J_{2ax,3}$ = 11.7 Hz, H-2a*ax*), 1.96-2.22 (4s, 12H, 4AcO), 4.67 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1c), 5.32 (dd, 1H, $J_{2,3}$ = 10.7 Hz, $J_{3,4}$ = 3.4 Hz, H-3b), 5.45 (d, 1H, $J_{3,4}$ = 3.9 Hz, H-4c), 5.47 (d, 1H, $J_{1,2}$ = 3.4 Hz, H-1b), and 7.27-8.13 (m, 15H, 3Ph).

Anal. Calcd for $C_{47}H_{52}O_{20}$ (936.9): C, 60.25; H, 5.59. Found: C, 59.96; H, 5.54.

***O*-(4-*O*-Acetyl-2,6-di-*O*-benzoyl-3-sulfo-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-1,5-anhydro-6-*O*-benzoyl-2-deoxy-D-*arabino*-hexitol pyridine salt (23).** To a solution of **22** (150 mg, 0.16 mmol) in DMF (0.3 mL) was added sulfur trioxide pyridine complex (127 mg, 0.26 mmol), and the mixture was stirred for one h at room temperature. A workup similar to that described for **16** gave **23** (173 mg, quantitative) as an amorphous mass: $[\alpha]_D$ -23.0° (*c* 1.0, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 1.29 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6b), 1.85-2.15 (4s, 12H, 4AcO), and 7.23-8.57 (m, 20H, 3Ph, $C_5H_5N$).

Anal. Calcd for $C_{52}H_{57}NO_{23}S$ (1096.1): C, 56.98; H, 5.24; N, 1.28. Found: C, 56.81; H, 5.36; N, 1.26.

***O*-(3-*O*-Sulfo-β-D-galactopyranosyl)-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)]-1,5-anhydro-2-deoxy-D-*arabino*-hexitol sodium salt (24).** To a

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solution of **23** (173 mg, 0.16 mmol) in MeOH (4 mL) and tetrahydrofuran (2 mL) was added NaOMe (10 mg) and the mixture was stirred for 24 h at room temperature. A workup similar to that described for **17** gave **24** (89.5 mg, quantitative) as an amorphous mass: $[\alpha]_D$ -92.5° (*c* 0.8, MeOH); $^1$H NMR ($D_2O$) δ 1.03 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6b), 4.41 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1c), 4.44 (m, 1H, H-5b), and 4.71 (d, 1H, $J_{1,2}$ = 3.4 Hz, H-1b). The mass spectrum of **24** (negative mode) showed the base peak at *m/z* 534.9 $(M-H)^-$.

***O*-(4-*O*-Acetyl-2,6-di-*O*-benzoyl-3-*O*-diphenylphosphono-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-1,5-anhydro-6-*O*-benzoyl-2-deoxy-D-*arabino*-hexitol (25).** To a solution of **22** (150 mg, 0.16 mmol) in pyridine (1 mL), cooled to 0 °C, was added diphenyl chlorophosphate (130 mg, 0.48 mmol) and the mixture was stirred for 12 h at room temperature. Methanol (0.5 mL) was added to the mixture which was concentrated and then extracted with $CH_2Cl_2$. The extract was successively washed with 2 M HCl, M $Na_2CO_3$ and water, dried ($Na_2SO_4$) and concentrated. Column chromatography (1:2 EtOAc-hexane) of the residue on silica gel (60 g) gave **25** (187 mg, quantitative) as an amorphous mass: $[\alpha]_D$ -19.5° (*c* 1.0, $CHCl_3$); $^1$H NMR ($CDCl_3$) δ 1.38 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6b), 1.94-2.20 (4s, 12H, 4AcO), 4.72 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1c), 5.11 (dd, 1H, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.8 Hz, H-3c), 5.21 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1b), 5.31 (dd, 1H, $J_{2,3}$ = 10.7 Hz, $J_{3,4}$ = 3.4 Hz, H-3b), 5.47 (d, 1H, H-4b), 5.55 (dd, 1H, H-2c), 5.73 (d, 1H, H-4c), and 6.79-8.14 (m, 25H, 5Ph).

Anal. Calcd for $C_{59}H_{61}O_{23}P$ (1169.1): C, 60.61; H, 5.26. Found: C, 60.48; H, 5.14.

***O*-(3-*O*-Phosphono-β-D-galactopyranosyl)-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)]-1,5-anhydro-2-deoxy-D-*arabino*-hexitol sodium salt (26).** A solution of **25** (187 mg, 0.16 mmol) in EtOH (8 mL) was stirred for 6 h at room temperature in the presence of pre-reduced Adams platinum catalyst (200 mg) under hydrogen. The catalyst was collected and the solution was concentrated. To a solution of the residue in dry MeOH (5 mL) was added NaOMe (20 mg) and the mixture was stirred for 24 h at room temperature then concentrated at 25 °C. Column chromatography (1:1 MeOH-$H_2O$) of the residue on Sephadex LH-20 (80 g) gave **26**

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(80.5 mg, 89%) as an amorphous mass: $[\alpha]_D$ -23.5° (*c* 1.1, 1:1 MeOH-$H_2O$); $^1H$ NMR ($D_2O$) δ 1.37 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6b), 4.67 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1c), 4.80 (m, 1H, H-5b), and 5.19 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1b). The mass spectrum (negative ion mode) showed the base peak at *m/z* 534.9 $(M-2H)^{2-}$.

***O*-(Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-*O*-(2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-1,5-anhydro-2,6-di-*O*-benzoyl-D-glucitol (28).** To a solution of **12** (123 mg, 0.11 mmol) and **27**[18] (150 mg, 0.26 mmol) in $CH_3CN$ (1 mL) and $CH_2Cl_2$ (0.3 mL) were added MS-4Å (600 mg), and the mixture was stirred for 6 h at room temperature then cooled to -40 °C. *N*-iodosuccinimide (NIS, 170 mg) and TfOH (4 μL) were added to the mixture, and this was stirred overnight at -40 °C then neutralized with $Et_3N$. The precipitate was collected and washed with $CH_2Cl_2$, and the combined filtrate and washings were concentrated. Column chromatography (80:1 $CH_2Cl_2$-MeOH) of the residue on silica gel (40 g) gave **28** (75 mg, 42%) as an amorphous mass: $[\alpha]_D$ +12.0° (*c* 0.8, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 1.38 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6b), 1.51-1.99 (5s, 15H, 4AcO, AcN), 2.51 (dd, 1H, $J_{gem}$ = 13.4 Hz, $J_{3eq,4}$ = 2.9 Hz, H-3d*eq*), 3.15 (t, 1H, $J_{1ax,2} = J_{gem}$ = 10.6 Hz, H-1a*ax*), 3.64 (s, 3H, MeO), 5.23 (dd, 1H, $J_{6,7}$ = 2.4 Hz, $J_{7,8}$ = 10.7 Hz, H-7d), 5.36 (t, 1H, $J_{1,2} = J_{2,3}$ = 8.3 Hz, H-2c), 5.48 (m, 1H, H-8d), 5.59 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1b), and 7.20-8.15 (m, 35H, 7Ph).

Anal. Calcd for $C_{87}H_{93}NO_{30}$ (1632.7): C, 64.00; H, 5.74; N, 0.86. Found: C, 64.11; H, 5.91; N, 0.98.

***O*-(Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-*O*-(4-*O*-acetyl-2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-1,5-anhydro-2,6-di-*O*-benzoyl-D-glucitol (29).** A solution of **28** (98 mg, 0.06 mmol) in EtOH (10 mL) and AcOH (2 mL) was stirred for 2 days at 40 °C in the presence of 10% Pd-C (100 mg) under hydrogen. The precipitate was collected and the solution was concentrated. Treatment of the residue with $Ac_2O$ (1 mL) and pyridine (2 mL) in the presence of 4-dimethylaminopyridine (5 mg) overnight at 45 °C and a workup similar to that

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described for **14** gave **29** (66 mg, 73%) as an amorphous mass: $[\alpha]_D$ +14.5° (*c* 1.0, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 1.44 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6b), 1.45-2.13 (9s, 27H, 8AcO, AcN), 2.52 (dd, 1H, $J_{gem}$ = 12.4 Hz, $J_{3eq,4}$ = 4.4 Hz, H-3d*eq*), 3.19 (t, 1H, $J_{1ax,2} = J_{gem}$ = 10.6 Hz, H-1a*ax*), 3.68 (s, 3H, MeO), and 7.27-8.17 (m, 20H, 4Ph).

Anal. Calcd for $C_{74}H_{83}NO_{34}$ (1529.8): C, 58.06; H, 5.47; N, 0.92. Found: C, 58.15; H, 5.53; N, 0.88.

***O*-(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-*O*-(β-D-galactopyranosyl)-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)]-1,5-anhydro-D-glucitol (30).** To a solution of **29** (53 mg, 0.033 mmol) in MeOH (2 mL) was added sodium methoxide (10 mg) and the mixture was stirred overnight at room temperature. Water (0.5 mL) was added to the mixture which was stirred for a further 12 h at room temperature, neutralized with Amberlite IR-120 ($H^+$) resin, and filtered. The resin was washed with MeOH, and the combined filtrate and washings were concentrated. Column chromatography (MeOH) of the residue on Sephadex LH-20 (30 g) gave **30** (26 mg, quantitative) as an amorphous mass: $[\alpha]_D$ +20.5° (*c* 0.5, MeOH); $^1H$ NMR ($Me_2SO$-*d*6) δ 0.97 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6b), 1.84 (s, 3H, AcN), 2.45 (dd, 1H, $J_{gem}$ = 13.2 Hz, $J_{3eq,4}$ = 3.2 Hz, H-3d*eq*), 2.46 (t, 1H, $J_{1ax,2} = J_{gem}$ = 10.5 Hz, H-1a*ax*), 4.25 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1c), and 5.14 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1b).

Anal. Calcd for $C_{29}H_{49}NO_{22}$ (763.7): C, 45.61; H, 6.47; N, 1.83. Found: C, 45.41; H, 6.70; N, 1.80.

***O*-(Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-*O*-(2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-1,5-anhydro-6-*O*-benzoyl-2-deoxy-D-*arabino*-hexitol (31).** To a solution of **19** (200 mg, 0.19 mmol) and **27** (225 mg, 0.32 mmol) in $CH_3CN$ (1.6 mL) and $CH_2Cl_2$ (0.4 mL) were added MS-4Å (1.0 g) and the mixture was stirred for 6 h at room temperature then cooled to -40 °C. NIS (130 mg, 0.48 mmol) and TfOH (14 μL) were added to the mixture which was stirred for 10 h at -40 °C. A workup similar to that described for **28** gave **31** (130 mg, 45%) as an amorphous mass: $[\alpha]_D$ -5.0° (*c* 1.0, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 1.32 (d, 3H, $J_{5,6}$

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= 6.4 Hz, H-6b), 1.53-1.99 (5s, 15H, 4AcO, AcN), 2.51 (dd, 1H, $J_{gem}$ = 13.4 Hz, $J_{3eq,4}$ = 2.9 Hz, H-3d*eq*), 3.70 (s, 3H, MeO), 4.17 (dd, 1H, $J_{2,3}$ = 10.3 Hz, $J_{3,4}$ = 3.8 Hz, H-3c), 4.70 (d, 1H, $J_{1,2}$ = 2.4 Hz, H-1b), 5.23 (dd, 1H, $J_{6,7}$ = 2.2 Hz, $J_{7,8}$ = 10.0 Hz, H-7d), 5.48 (m, 1H, H-8d), and 7.20-8.14 (m, 30H, 6Ph).

Anal. Calcd for $C_{80}H_{89}NO_{28}$ (1512.6): C, 63.53; H, 5.93; N, 0.93 Found: C, 63.31; H, 6.05; N, 0.99.

***O*-(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-*O*-(β-D-galactopyranosyl)-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)]-1,5-anhydro-2-deoxy-D-*arabino*-hexitol (32).** A solution of **31** (130 mg, 0.086 mmol) in EtOH (15 mL) and AcOH (5 mL) was hydrogenolyzed in the presence of 10% Pd-C (130 mg) for 48 h at 40 °C, then filtered and concentrated. To a solution of the residue in MeOH (5 mL) was added NaOMe (20 mg) and the mixture was stirred for 24 h at room temperature. Potassium hydroxide (0.2 M, 3 mL) was added to the mixture, and this was stirred for 6 h at room temperature, neutralized with Amberlite IR-120 ($H^+$) resin, and filtered. A workup similar to that described for **30** gave **32** (64 mg, quantitative) as an amorphous mass: $[\alpha]_D$ -23.0° (*c* 1.5, MeOH); $^1H$ NMR ($Me_2SO$-*d*6) δ 1.03 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6b), 1.87 (s, 3H, AcN), 2.57 (dd, 1H, $J_{gem}$ = 13.2 Hz, $J_{3eq,4}$ = 3.2 Hz, H-3d*eq*), 4.37 (d, 1H, $J_{1,2}$ = 7.2 Hz, H-1c), and 4.80 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1b).

Anal. Calcd for $C_{29}H_{49}NO_{21}$ (747.7): C, 46.59; H, 6.61; N, 1.87. Found: C, 46.31; H, 6.83; N, 1.59.

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## ACKNOWLEDGMENT

This work was supported in part by a Grant-in-Aid (No. 05274102 and No. 06281227) for the Scientific Research on Priority Areas from the Ministry of Education, Science and Culture of Japan.

## REFERENCES

1. A. Hasegawa, T. Ando, M. Kato, H. Ishida and M. Kiso, *Carbohydr. Res.*, **257**, 67 (1994).

2. T. Terada, M. Kiso and A. Hasegawa, *Carbohydr. Res.*, **259**, 201 (1994).
3. M. Yoshida, A. Uchimura, M. Kiso and A. Hasegawa, *Glycoconjugate J.*, **10**, 3 (1993).
4. H. Furui, M. Kiso and A. Hasegawa, *Carbohydr. Res.*, **229**, c1 (1992).
5. A. Hasegawa, T. Ando, A. Kameyama and M. Kiso, *J. Carbohydr. Chem.*, **11**, 645 (1992).
6. A. Kameyama, H. Ishida, M. Kiso and A. Hasegawa, *J. Carbohydr. Chem.*, **10**, 729 (1991).
7. A. Kameyama, H. Ishida, M. Kiso and A. Hasegawa, *Carbohydr. Res.*, **209**, c1 (1991).
8. A. Kameyama, H. Ishida, M. Kiso and A. Hasegawa, *J. Carbohydr. Chem.*, **13**, 641 (1994).
9. J. B. Lowe, L. M. Stoolman, R. P. Nair, R. D. Larsen, T. L. Berhend and R. M. Marks, *Cell*, **63**, 475 (1990).
10. G. Walz, A. Aruffo, W. Kolanus, M. P. Bevilacqua and B. Seed, *Science*, **250**, 1132 (1990).
11. M. L. Phillips, E. Nudelman, F. C. A. Gaeta, M. Perez, A. K. Singhal, S. Hakomori and J. C. Paulson, *Science*, **250**, 1130 (1990).
12. D. Tyrrell, P. James, N. Rao, C. Foxall, S. Abbas, F. Dasgupta, M. Nashed, A. Hasegawa, M. Kiso, D. Asa and B. K. Brandley, *Proc. Natl. Acad. Sci. USA*, **88**, 10372 (1991).
13. C. Foxall, S. R. Watson, D. Dowbenko, C. Fennie, L. A. Lasky, M. Kiso, A. Hasegawa, D. Asa and B. K. Brandley, *J. Cell Biol.*, **117**, 895 (1992).
14. B. K. Brandley, M. Kiso, S. Abbas, P. Nikrad, O. Srivasatava, C. Foxall, Y. Oda and A. Hasegawa, *Glycobiology*, **3**, 633 (1993).
15. M. Larkin, T. J. Ahern, M. S. Stoll, M. Shaffer, D. Sako, J. O'Brien, C.-T. Yuen, A. M. Lawson, R. A. Childs, K. M. Barone, P. R. Langer-Safer, A. Hasegawa, M. Kiso, G. R. Larsen and T. Feizi, *J. Biol. Chem.*, **267**, 13661 (1992).
16. C.-T. Yuen, K. Bezouska, J. O'Brien, M. Stoll, R. Lemonie, A. Lubineau, M. Kiso, A. Hasegawa, N. J. Bockovich, K. C. Nicolaou and T. Feizi, *J. Biol. Chem.*, **269**, 1595 (1994).
17. A. Hasegawa, K. Ito, H. Ishida and M. Kiso, *J. Carbohydr. Chem.*, in press.
18. A. Marra and P. Sinaÿ, *Carbohydr. Res.*, **187**, 35 (1989).
19. a) P. Konradsson, U. E. Udodong and B. Fraser-Reid, *Tetrahedron Lett.*, **31**, 4313 (1990); b) G. H. Veeneman, S. H. van Leevwen and J. H. van Boom, *Tetrahedron Lett.*, **31**, 1331 (1990).
20. T. Murase, H. Ishida, M. Kiso and A. Hasegawa, *Carbohydr. Res.*, **188**, 71 (1989).
21. K. Bock and K. Adelhorst, *Acta. Chem. Scand.*, **46**, 186 (1992).
22. L. Somsák and I. Németh, *J. Carbohydr. Chem.*, **12**, 679 (1993).
23. A. Kameyama, H. Ishida, M. Kiso and A. Hasegawa, *J. Carbohydr. Chem.*, **10**, 549 (1991).
24. a) A. Hasegawa, T. Nagahama, H. Ohki, H, Ishida and M. Kiso, *J. Carbohydr. Chem.*, **10**, 493 (1991); b) A. Hasegawa, In *Synthetic Oligosaccharides-Indispensable Probes for the Life Sciences*, P. Kovác, Ed.; ACS Symposium Series 560, American Chemical Society, Washington, DC, p 184 (1994).
25. K. Okamoto and T. Goto, *Tetrahedron*, **46**, 5835 (1990).

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