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Note

Synthesis of 6^5- and/or 4^5-*O*-glycosyl *p*-nitrophenyl α-maltopentaoside derivatives for the differential assay of human α-amylases

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Differential and total assays of human pancreatic and salivary α-amylases [α-1,4-glucan 4-glucanohydrolase, EC 3.2.1.1; HPA and HSA] in human serum and urine give accurate diagnostic information on diseases such as pancreatitis and parotitis. A variety of methods have therefore been developed to differentially assay these isozymes. These methods include electrophoresis [1,2], chromatography [3], differential inhibition by a wheat α-amylase inhibitor [4], or by monoclonal antibodies [5,6], immunoassay [7], and assays based on the differing substrate specificities of HPA and HSA [8–13]. Some oligosaccharide substrates modified by the addition of galactose or *N*-acetylglucosamine to the nonreducing-end glucose residue have been prepared by enzymatic transglycosylation [14,15] and used for such differential assays [11]. The systematic synthesis a series of 6^5-*S*-substituted *p*-nitrophenyl 6^5-thio-α-maltopentaoside derivatives has been described [16].

In the present study we developed an alternative, chemical route to sugar-modified *p*-nitrophenyl α-maltopentaoside (G5P) derivatives, namely 6^5-*O*-β-D-glucopyranosyl (pyranosyl substitution) and 4^5- or 6^5-*O*-β-D-ribofuranosyl and 4^5,6^5-di-*O*-β-D-ribofuranosyl (furanosyl substitution). The methods used are applicable to the synthesis of a wide variety of glycosylated substrates.

Treatment of G5P (**1**) with 2,2-dimethoxypropane in the presence of a catalytic amount of *p*-toluenesulfonic acid monohydrate gave the corresponding 4^5,6^5-*O*-

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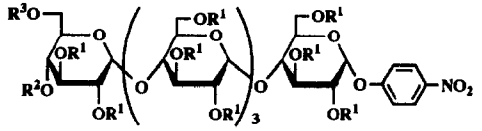
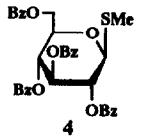


	R^1	R^2	R^3
1	H	H	H
2	Ac	— Ipd —	
3	Ac	H	H

Ipd = isopropylidene



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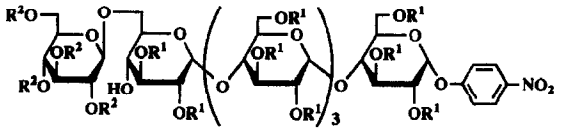
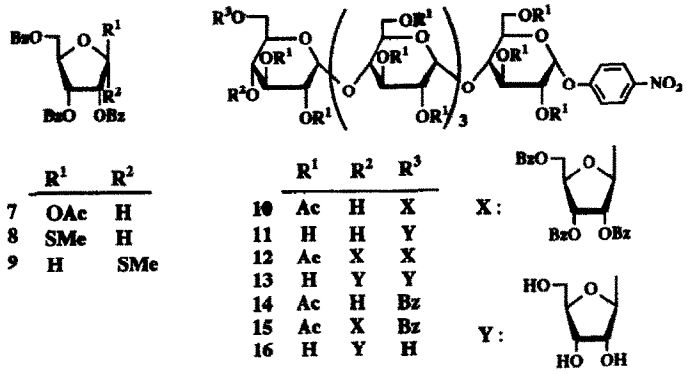


	R^1	R^2
5	Ac	Bz
6	H	H

isopropylidene derivative, which was acetylated in the usual manner to afford **2** (70% in the two steps). Removal of the isopropylidene group from **2** with aqueous 80% acetic acid gave the glycosyl acceptor **3** in a 76% yield.

The glycosyl donor methyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside [17] (**4**) was prepared from methyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside, which was obtained by treatment [18] of 1,2,3,4,6-penta-*O*-acetyl-D-glucopyranose with trimethylmethylthiosilane (Me_3SiSMe) and trimethylsilyl trifluoromethanesulfonate (Me_3SiOTf). The glycosylation of **3** with **4** in dichloromethane in the presence of *N*-iodosuccinimide (NIS)–trifluoromethanesulfonic acid (TfOH) as the glycosyl promoter [19–21] gave **5** in an 86% yield. Characteristic signals in the ^1H NMR spectrum of **5** were a one-proton triplet at high field (δ 3.54, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4^5), indicating that OH-4^5 was free, and a one-proton doublet at δ 4.92 ($J_{1,2}$ 8.1 Hz, H-1^6), showing the configuration of the newly formed glycosidic linkage to be β. *O*-Deacylation of **5** with sodium methoxide in methanol gave *p*-nitrophenyl *O*-β-D-glucopyranosyl-(1 → 6)-*O*-α-D-glucopyranosyl-(1 → 4)-tris[*O*-α-D-glucopyranosyl-(1 → 4)]-α-D-glucopyranoside (**6**), quantitatively.

1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**7**) was treated with Me_3SiSMe and Me_3SiOTf in CH_2ClCH_2Cl at 50°C to give methyl 2,3,5-tri-*O*-benzoyl-1-thio-β-D-ribofuranoside (**8**, 87%) and the corresponding α-glycoside (**9**, 13%). When the glycosylation of **3** with 1.2 molar equivalents of **8** was performed by the same method as described for **5**, the 6^5-*O*-β-D-ribofuranosyl derivative **10** was obtained



in an 82% yield. The use of 6.1 molar equivalents of **8** gave the $4^5,6^5$-di-*O*-β-D-ribofuranosyl derivative **12** in an 88% yield. When 3.0 molar equivalents of **8** was employed, both **10** and **12** were obtained, in a 1:1 ratio (data not shown). Treatment of **10** and **12** with sodium methoxide in methanol at 7°C gave the corresponding *O*-deacylated products **11** and **13**. Compound **14**, obtained by selective 6^5-*O*-benzoylation of **3**, was glycosylated with **8** to give **15** in a 73% yield, and *O*-deacylation of **15** afforded **16** (94%). In the ^{1}H NMR spectra, the H-4^5 and H-6^5 signals in **3** (the 4^5, 6^5-OH derivative) were observed at δ 3.62–3.73. On selectively introducing a benzoyl group to give **14**, the H-6^5 signal was shifted to a significantly lower magnetic field, δ 4.42–4.57, while the H-4^5 signal was observed at the same field as for **3** (δ 3.64). This showed that OH-6^5 of **14** was protected by the benzoyl group, and the only OH-4^5 was free. Significant signals in the ^{1}H NMR spectra of **11**, **13**, and **16** were, respectively, a one-proton triplet at δ 3.22 ($J_{3,4} = J_{4,5} = 9.4$ Hz, H-4^5) and a one proton singlet at δ 4.89 (H-1 of Rib*f*) for **11**, two one-proton singlets at δ 4.91 and δ 4.92 (H-1 of Rib*f*) for **13**, and a one-proton singlet at δ 4.97 (H-1 of Rib*f*) for **16**. For **11**, the H-4^5 signal was observed at high magnetic field, δ 3.22, indicating the C-4^5 position to be free. On the other hand, for **13** and **16** there was no signal at such a high field. So the newly formed glycosidic linkage positions were C-6^5 for **11**, C-4^5 and 6^5 for **13**, and C-4^5 for **16**, and their configurations were β because each Rib*f* H-1 signal was a singlet. Other ^{1}H NMR data are given in the Experimental section and are consistent with the structures assigned.

1. Experimental

General methods.—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. Preparative column chromatography was performed on silica gel (Wako Pure Chemical Industries, 200 mesh) with the solvent systems specified.

Concentrations were conducted in vacuo. G5P was purchased from Boeringer Mannheim GmbH (Mannheim, Germany). 1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**7**) was purchased from Aldrich Chemical Co. Inc. (Milwaukee, WI, USA).

p-*Nitrophenyl* O-*(2,3-di-*O*-acetyl-4,6-*O*-isopropylidene-α-*D*-glucopyranosyl)-(1 → 4)-tris[*O*-(2,3,6-tri-*O*-acetyl-α-*D*-glucopyranosyl)-(1 → 4)]-2,3,6-tri-*O*-acetyl-α-*D*-glucopyranoside* (**2**).—To a stirred solution of G5P (**1**, 3.0 g, 3.16 mmol) in dry MeCN (100 mL) and dry dimethylformamide (DMF) (100 mL) was added Drierite (6 g), *p*-toluenesulfonic acid monohydrate (600 mg), and 2,2-dimethoxypropane (4.1 mL, 33.3 mmol) at 0°C. The mixture was stirred for 45 h at 25°C, and then neutralized with Amberlite IRA-410 (OH^-) resin. The resin was filtered off and washed with MeOH. The combined filtrate and washings was concentrated to a syrup, which was acetylated with Ac_2O (75 mL) and pyridine (150 mL) for two days at room temperature. After completion of the reaction, MeOH (30 mL) was added at 0°C and the mixture was then concentrated. The residue was taken up in CH_2Cl_2, and the solution was successively washed with 2 M HCl and water, dried (Na_2SO_4), and concentrated. Column chromatography (120:1 CH_2Cl_2–MeOH) of the product on silica gel gave **2** (3.49 g, 70%) as an amorphous mass; $[\alpha]_D$ +167° (*c* 0.42, $CHCl_3$); ν 2950 (CH), 1750 and 1220 (ester), 1520 and 1350 (NO_2), 870 (phenyl), and 860 cm^{-1} (isopropylidene); ^1H NMR ($CDCl_3$): δ 1.37, 1.44 (2 s, 6 H, Me_2C), 1.96–2.21 (14 s, 42 H, 14 AcO), 3.62–3.73 (m, 2 H, H-4^5,6^5), 3.80 (dd, 1 H, $J_{5,6'}$, 4.0, J_{gem} 9.2 Hz, H-$6'^5$), 3.93–4.32 (m, 13 H, H-4^{1-4},5^{1-5},6^{1-4}), 4.48–4.58 (m, 4 H, H-$6'^{1-4}$), 4.70, 4.72, 4.74 (3 dd, 3 H, *J* 4.4, 10.3; 4.0 9.9; 4.0, 10.3 Hz, H-2^{2-4}), 4.80 (dd, 1 H, $J_{1,2}$ 4.2, *J* 10.3 Hz, H-2^5), 4.95 (dd, 1 H, $J_{1,2}$ 3.5, $J_{2,3}$ 10.1 Hz, H-2^1), 5.25 (t, 1 H, $J_{2,3} = J_{3,4} = 10.0$ Hz, H-3^5), 5.28 (~ d, 2 H, *J* 4.2 Hz, H-1^3,1^4), 5.31, 5.33 (2 d, 2 H, *J* 4.0, 3.7 Hz, H-1^2,1^5), 5.33–5.45 (m, 3 H, H-3^{2-4}), 5.73 (t, 1 H, $J_{2,3} = J_{3,4} = 9.3$ Hz, H-3^1), 5.74 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1^1), 7.25 (2 d, 2 H, *J* 9.2 Hz, NPh H-2,6), and 8.24 (2 d, 2 H, *J* 9.3 Hz, NPh H-3,5). Anal. Calcd for $C_{67}H_{87}NO_{42}$ (1578.40): C, 50.98; H, 5.56; N, 0.89. Found: C, 51.19; H, 5.69; N, 0.85.

p-*Nitrophenyl* O-*(2,3-di-*O*-acetyl-α-*D*-glucopyranosyl)-(1 → 4)-tris[*O*-(2,3,6-tri-*O*-acetyl-α-*D*-glucopyranosyl)-(1 → 4)]-2,3,6-tri-*O*-acetyl-α-*D*-glucopyranoside* (**3**).— A solution of **2** (3.5 g, 2.22 mmol) in aq 80% AcOH (200 mL) was kept for 30 min at 40°C, and then extracted with CH_2Cl_2 at 0°C. The extract was successively washed with water, M Na_2CO_3 and water, dried (Na_2SO_4), and concentrated. The filtrate and washings were combined and concentrated. Column chromatography (50:1 CH_2Cl_2–MeOH) of the residue on silica gel gave **3** (2.60 g, 76%) as an amorphous mass; $[\alpha]_D$ +160° (*c* 0.22, $CHCl_3$); ν 3500 (OH), 2950 (CH), 1760 and 1240 (ester), 1520 and 1350 (NO_2), and 870 cm^{-1} (phenyl); ^1H NMR ($CDCl_3$): δ 1.97–2.19 (14 s, 42 H, 14 AcO), 3.62–3.65 (m, 2 H, H-4^5,6^5), 3.81–4.08 (m, 9 H, H-4^{2-4},5^{1-5},$6'^5$), 4.10 (t, 1 H, $J_{3,4} = J_{4,5}$ 10.3 Hz, H-4^1), 4.14–4.34 (m, 4 H, H-6^{1-4}), 4.46–4.64 (m, 4 H, H-$6'^{1-4}$), 4.71, 4.73, 4.75, 4.75 (4 dd, 4 H, *J* 4.0, 9.7; 4.0, 10.3; 4.0, 10.1; 4.0, 10.1 Hz, H-2^{2-5}), 4.95 (dd, 1 H, $J_{1,2}$ 3.7, $J_{2,3}$ 10.1 Hz, H-2^1), 5.20 (t, 1 H, $J_{2,3} = J_{3,4} = 9.2$ Hz, H-3^5), 5.27, 5.28 (2 d, 2 H, *J* 3.1, 3.8 Hz, H-1^3,1^4), 5.32, 5.33 (2 d, 2 H, *J* 4.2, 4.2 Hz, H-1^2,1^5), 5.37, 5.38, 5.39 (3 t, 3 H, *J* 9.5, 9.8, 9.3 Hz, H-3^{2-4}), 5.73 (t, 1 H, $J_{2,3} = J_{3,4} = 10.3$ Hz, H-3^1), 5.74 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1^1), 7.25 (2 d, 2 H, *J* 9.0 Hz,

NPh H-2,6), and 8.25 (2 d, 2 H, *J* 9.3 Hz, NPh H-3,5). Anal. Calcd for $C_{64}H_{83}NO_{42}$ (1538.33): C, 49.97; H, 5.44; N, 0.91. Found: C, 50.10; H, 5.72; N, 0.92.

p-*Nitrophenyl* O-*(2,3,4,6-tetra-*O*-benzoyl-β-*D*-glucopyranosyl)-(1 → 6)-*O*-(2,3-di-*O*-acetyl-α-*D*-glucopyranosyl)-(1 → 4)-tris[*O*-(2,3,6-tri-*O*-acetyl-α-*D*-glucopyranosyl)-(1 → 4)]-2,3,6-tri-*O*-acetyl-α-*D*-glucopyranoside* (**5**).–To a solution of **3** (100 mg, 0.065 mmol) and methyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (**4**, 82 mg, 0.13 mmol) in dry CH_2Cl_2 (1 mL) was added powdered 4A molecular sieves (200 mg) and the mixture was stirred for 5 h at 25°C under N_2, then cooled to –35°C. To the cooled mixture was added NIS (60 mg, 0.26 mmol) and TfOH (4.4 μL, 0.052 mmol), and the mixture was stirred for 3 h at –35°C. The precipitate was filtered off and washed with CH_2Cl_2. The filtrate and washings were combined, successively washed with M Na_2CO_3 and M $Na_2S_2O_3$, dried (Na_2SO_4), and concentrated. Column chromatography (50:1 CH_2Cl_2–MeOH) of the residue on silica gel gave **5** (118 mg, 86%) as an amorphous mass; $[\alpha]_D$ +131° (*c* 0.49, $CHCl_3$); ν 3450 (OH), 2980 (CH), 1750 and 1220 (ester), 1520 and 1350 (NO_2), and 860 and 710 cm^{-1} (phenyl); ^1H NMR ($CDCl_3$): δ 1.97–2.18 (14 s, 42 H, 14 AcO), 3.54 (t, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4^5), 3.63–3.67 (m, 1 H, H-6^5), 3.82–4.56 (m, 22 H, H-4$^{1-5}$,5$^{1-6}$,6$^{1-4,6,6'1-6}$), 4.60 (dd, 1 H, $J_{1,2}$ 4.0, $J_{2,3}$ 10.3 Hz, H-2^5), 4.66, 4.70, 4.74 (3 dd, 3 H, *J* 4.4, 10.4; 3.9, 10.0; 3.8, 11.1 Hz, H-2$^{2-4}$), 4.92 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1^6), 4.95 (dd, 1 H, $J_{1,2}$ 3.5,$J_{2,3}$ 10.4 Hz, H-2^1), 5.05 (t, 1 H, $J_{2,3} = J_{3,4} = 9.8$ Hz, H-3^5), 5.25–5.42 (m, 7 H, H-1$^{2-5}$, 3$^{2-4}$), 5.56 (t, 1 H, $J_{1,2} = J_{2,3} = 8.6$ Hz, H-2^6), 5.69 (t, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4^6), 5.72 (t, 1 H, $J_{2,3} = J_{3,4} = 9.6$ Hz, H-3^1), 5.73 (d, 1 H, $J_{1,2}$ 4.2 Hz, H-1^1), 5.92 (t, 1 H, $J_{2,3} = J_{3,4} = 9.6$ Hz, H-3^6), 7.23–8.03 (m, 22 H NPh H-2,6 and 4 PhCO), and 8.23 (2 d, 2 H, *J* 9.2 Hz, NPh H-3,5). Anal. Calcd for $C_{98}H_{109}NO_{51}$ (2116.91): C, 55.60; H, 5.19; N, 0.66. Found: C, 55.40; H, 5.36; N, 0.48.

p-*Nitrophenyl* O-β-*D*-glucopyranosyl-(1 → 6)-O α-*D*-glucopyranosyl-(1 → 4)-tris[O-α-*D*-glucopyranosyl)-(1 → 4)]-α-*D*-glucopyranoside* (**6**).—To a solution of **5** (53 mg, 0.025 mmol) in dry MeOH (5 mL) was added a solution of NaOMe (0.156 mmol) in dry MeOH (1 mL), and the mixture was kept overnight at 25°C, then neutralized with Amberlite IR-120 (H^+) resin. The resin was filtered off and washed with MeOH, and the combined filtrate and washings were concentrated. Column chromatography (MeOH) of the residue on Sephadex LH-20 gave **6** (28 mg, quant.) as an amorphous mass; $[\alpha]_D$ +258° (*c* 0.23, MeOH); ν 3400 (OH), 2940 (CH), 1520 and 1350 (NO_2), and 870 cm^{-1} (phenyl); ^1H NMR (CD_3OD): δ 3.23 (t, 1 H, $J_{3,4} = J_{4,5} = 9.4$ Hz, H-4^5), 4.12 (t, 1 H, $J_{2,3} = J_{3,4} = 9.1$ Hz, H-3^1), 4.30 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1^6), 5.09 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1^5), 5.13, 5.16 (2 d, 2 H, $J_{1,2}$ 3.8 Hz, H-1^3,1^4), 5.22 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1^2), 5.67 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1^1), 7.32 (2 d, 2 H, *J* 9.3 Hz, NPh H-2,6), and 8.23 (2 d, 2H, *J* 9.3 Hz, NPh H-3,5). Anal. Calcd for $C_{42}H_{65}NO_{33}$ (1111.96): C, 45.37; H, 5.89; N, 1.26. Found: C, 45.22; H, 5.62; N, 1.23.

*Methyl 2,3,5-tri-*O*-benzoyl-1-thio-β-*D*-ribofuranoside* (**8**) *and methyl 2,3,5,-tri-*O*-benzoyl-1-thio-α-*D*-ribofuranoside* (**9**).—To a stirred solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**7**; 2.0 g, 3.96 mmol) in CH_2ClCH_2Cl was added Me_3SiSMe (1.68 mL, 11.9 mmol) and Me_3SiOTf (0.23 mL, 1.19 mmol) at 0°C, and

the mixture was stirred for 1 h at 50°C, then extracted with CH_2Cl_2. The extract was successively washed with M Na_2CO_3 and water, dried (Na_2SO_4), and concentrated. Column chromatography (1:20 acetone–hexane) of the product on silica gel gave **8** (1.07g, 87%) and **9** (247 mg, 13%).

Compound **8** had $[\alpha]_D$ + 5.1° (*c* 0.70, $CHCl_3$); ν 3030 (aromatic CH), 2990 (CH), 1720 and 1240 (ester), and 700 cm^{-1} (phenyl); ^1H NMR ($CDCl_3$): δ 2.22 (s, 3 H, MeS), 4.58 (dd, 1 H, $J_{4,5}$ 3.9, J_{gem} 11.5 Hz, H-5), 4.64–4.71 (m, 1 H, H-4), 4.75 (dd, 1 H, $J_{4,5'}$ 3.5, J_{gem} 11.5 Hz, H-5'), 5.39 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 5.74 (t, 1 H, $J_{1,2} = J_{2,3} = 4.6$ Hz, H-2), 5.89 (t, 1 H, $J_{2,3} = J_{3,4} = 5.5$ Hz, H-3), and 7.30–8.13 (m, 15 H, 3 PhCO). Anal. Calcd for $C_{27}H_{24}O_7S$ (492.55): C, 65.84; H, 4.91. Found: C, 65.97; H, 4.86.

Compound **9** had $[\alpha]_D$ + 92° (*c* 0.35, $CHCl_3$); ν 3030 (aromatic CH), 2990 (CH), 1720 and 1240 (ester), and 700 cm^{-1} (phenyl); ^1H NMR ($CDCl_3$): δ 2.25 (s, 3 H, MeS), 4.63 (dd, 1 H, $J_{4,5}$ 4.8, J_{gem} 12.6 Hz, H-5), 4.72–4.78 (m, 2 H, H-4, 5'), 5.74 (d, 1 H, $J_{1,2}$ 5.1 Hz, H-1), 5.75 (t, 1 H, $J_{2,3} = J_{3,4} = 4.4$ Hz, H-3), 5.80 (dd, 1 H, $J_{1,2}$ 6.2, $J_{2,3}$ 4.0 Hz, H-2), and 7.30–8.09 (m, 15 H, 3 PhCO). Found: C, 65.74; H, 5.07.

p-*Nitrophenyl* O-*(2,3,5-tri*-O-*benzoyl-β*-D-*ribofuranosyl)-(1 → 6)*-O-*(2,3-di*-O-*acetyl-α*-D-*glucopyranosyl)-(1 → 4)-tris[*O-*(2,3,6-tri*-O-*acetyl-α*-D-*glucopyranosyl)-(1 → 4)]-2,3,6-tri*-O-*acetyl-α*-D-*glucopyranoside* (**10**).—To a solution of **3** (204 mg, 0.133 mmol) and **8** (79 mg, 0.159 mmol) in dry CH_2Cl_2 (1 mL) was added powdered 4A molecular sieves (300 mg) and the mixture was stirred for 5 h at 25°C under N_2, then cooled to −40°C. To the cooled mixture was added NIS (70 mg, 0.311 mmol) and TfOH (0.0027 mL, 0.0311 mmol), and the mixture was stirred for 2 h at −40°C, then worked up as described for **5** to give **10** (214 mg, 82%) as an amorphous mass; $[\alpha]_D$ + 143° (*c* 0.94, $CHCl_3$); ν 3450 (OH), 2900 (CH), 1750 and 1220 (ester), 1520 and 1350 (NO_2), and 860 and 710 cm^{-1} (phenyl); ^1H NMR ($CDCl_3$): δ 1.98–2.19 (14 s, 42 H, 14 AcO), 3.08 (br s, 1 H, OH) 3.70–4.65 (m, 23 H, H-4^{1-5},5^{1-5},6^{1-5},$6'^{1-5}$, and H-4,5,5' of Rib*f*), 4.70, 4.74, 4.76 (3 dd, 3 H, *J* 4.0, 9.9; 3.7, 9.9; 3.8, 10.3 Hz, H-2^{2-4}), 4.82 (dd, 1 H, $J_{1,2}$ 4.1, $J_{2,3}$ 10.4 Hz, H-2^5), 4.95 (dd, 1 H, $J_{1,2}$ 3.7, $J_{2,3}$ 10.1 Hz, H-2^1), 5.18 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3^5), 5.27, 5.28 (2 d, 2 H, *J* 2.7, 3.8 Hz, H-1^3,1^4), 5.33–5.42 (m, 6 H, H-1^2,1^5,3^{2-4}, and H-1 of Rib*f*), 5.71 (d, 1 H, $J_{2,3}$ 5.3 Hz, H-2 of Rib*f*), 5.73 (t, 1 H, $J_{2,3} = J_{3,4} = 8.6$ Hz, H-3^1), 5.74 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1^1), 5.83 (dd, 1 H, $J_{2,3}$ 5.0, $J_{3,4}$ 6.6 Hz, H-3 of Rib*f*), 7.24–8.07 (m, 17 H, NPh H-2,6 and 3 PhCO), and 8.24 (2 d, 2H, *J* 9.3 Hz, NPh H-3,5). Anal. Calcd for $C_{90}H_{103}NO_{49}$ (1982.77): C, 54.52; H, 5.24; N, 0.71. Found: C, 54.49; H, 4.94; N, 0.63.

p-*Nitrophenyl* O-*β*-D-*ribofuranosyl-(1 → 6)*-O-*α*-D-*glucopyranosyl)-(1 → 4)-tris[*O-*α*-D-*glucopyranosyl-(1 → 4)]-α*-D-*glucopyranoside* (**11**).—To a solution of **10** (214 mg, 0.108 mmol) in dry MeOH (10 mL) was added a solution of NaOMe (0.312 mmol) in dry MeOH (1 mL) at 0°C, and the mixture was stirred overnight at 7°C, then worked up as described for **6** to give **11** (128 mg, quant.) as an amorphous mass; $[\alpha]_D$ + 138° (*c* 0.39, MeOH); ν 3400 (OH), 2940 (CH), 1510 and 1340 (NO_2), and 860 cm^{-1} (phenyl); ^1H NMR (CD_3OD): δ 3.22 (t, 1 H, $J_{3,4} = J_{4,5} = 9.4$ Hz, H-4^5), 4.11 (t, 1 H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3^1), 4.12 (dd, 1 H, $J_{2,3}$ 4.8, $J_{3,4}$ 6.8 Hz, H-3 of Rib*f*), 4.89 (s, 1 H, H-1 of Rib*f*), 5.07 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1^5),

5.13, 5.14, 5.15 (2 d, 2 H, $J_{1,2} = J_{1,2} = 4.2$ Hz, H-1^3,1^4), 5.22 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1^2), 5.66 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1^1), 7.32 (2 d, 2 H, J 9.2 Hz, NPh H-2,6), and 8.22 (2d, 2 H, J 9.2 Hz, NPh H-3,5). Anal. Calcd for $C_{41}H_{63}NO_{32}$ (1081.93): C, 45.52; H, 5.87; N, 1.29. Found: C, 45.49; H, 5.57; N, 1.21.

p-*Nitrophenyl* O-*(2,3,5-tri*-O-*benzoyl-β*-D-*ribofuranosyl)-(1 → 4)*-O-*[(2,3,5-tri*-O-*benzoyl-β*-D-*ribofuranosyl)-(1 → 6)]*-O-*(2,3-di*-O-*acetyl-α*-D-*glucopyranosyl)-(1 → 4)-tris[*O-*(2,3,6-tri*-O-*acetyl-α*-D-*glucopyranosyl)-(1 → 4)]-2,3,6-tri*-O-*acetyl-α*-D-*glucopyranoside* (**12**).—To a solution of **3** (200 mg, 0.130 mmol) and **8** (389 mg, 0.790 mmol) in dry CH_2Cl_2 (2 mL) was added powdered 4A molecular sieves (500 mg) and the mixture was stirred for 5 h at 25°C under N_2, then cooled to −40°C. To the cooled mixture was added NIS (311 mg, 1.38 mmol) and TfOH (0.012 mL, 0.138 mmol), and the mixture was stirred overnight at −40°C, then worked up as described for **5** to give **12** (279 mg, 88%) as an amorphous mass; $[\alpha]_D$ +131° (*c* 1.19, $CHCl_3$); ν 2990 (CH), 1750 and 1220 (ester), 1520 and 1360 (NO_2), and 860 and 710 cm^{-1} (phenyl); ^1H NMR ($CDCl_3$): δ 1.98–2.19 (14 s, 42 H, 14 AcO), 3.70–4.78 (m, 29 H, H-2^{2-4},4^{1-5},5^{1-5},6^{1-5},$6'^{1-5}$, and H-4,5,5′ of Rib*f*), 4.85 (dd, 1 H, $J_{1,2}$ 4.0, $J_{2,3}$ 10.4 Hz, H-2^5), 4.95 (dd, 1 H, $J_{1,2}$ 3.5, $J_{2,3}$ 10.1 Hz, H-2^1), 5.28 (~ d, 2 H, J 3.9 Hz, H-1^3,1^4), 5.33–5.42 [m, 7 H, H-1^2,1^5,3^{2-5}, and H-1 of Rib*f* (C-6^5)], 5.48 {2, 1 H, H-1 of Rib*f*(C-4^5)], 5.50 [d, 1 H, $J_{2,3}$ 5.3 Hz, H-2 of Rib*f* (C-4^5)], 5.58 [dd, 1 H, $J_{2,3}$ 4.8, $J_{3,4}$ 7.1 Hz, H-3 of Rib*f* (C-4^5)], 5.69 [d, 1 H, $J_{2,3}$ 4.9 Hz, H-2 of Rib*f*(C-6^5)], 5.73 (t, 1 H, $J_{2,3} = J_{3,4} = 9.3$ Hz, H-3^1), 5.74 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1^1), 5.83 {dd, 1 H, $J_{2,3}$ 5.1, $J_{3,4}$ 6.4 Hz, H-3 of Rib*f*(C-6^5)], 7.06–8.06 (m, 32 H, NPh H-2,6 and 6 PhCO), and 8.25 (2 d, 2 H, J 9.2 Hz, NPh H-3,5). Anal. Calcd for $C_{116}H_{123}NO_{56}$ (2427.21): C, 57.40; H, 5.11; N, 0.58. Found: C, 57.26; H, 5.28; N, 0.40.

p-*Nitrophenyl* O-*β*-D-*ribofuranosyl-(1 → 4)*-O-*[β*-D-*ribofuranosyl-(1 → 6)]*O-*α*-D-*glucopyranosyl)-(1 → 4)-tris[*O-*α*-D-*glucopyranosyl-(1 → 4)]-α*-D-*glucopyranoside* (**13**).—To a solution of **12** (279 mg, 0.115 mmol) in dry MeOH (28 mL) was added a solution of NaOMe (0.468 mmol) in dry MeOH (1 mL) at 0°C, and the mixture was stirred for 33h at 7°C, then worked up as described for **6** to give **13** (136 mg, 98%) as an amorphous mass; $[\alpha]_D$ +117° (*c* 0.53, MeOH); ν 3400 (OH), 2940 (CH), 1510 and 1340 (NO_2), and 860 cm^{-1} (phenyl); ^1H NMR (CD_3OD): δ 4.11 (t, 1 H, $J_{2,3} = J_{3,4} = 8.8$ Hz, H-3^1), 4.13 [dd, 1 H, $J_{2,3}$ 4.6, $J_{3,4}$ 6.8 Hz, H-3 of Rib*f* (C-6^5)], 4.27 [dd, 1 H, $J_{2,3}$ 4.6, $J_{3,4}$ 7.3 Hz, H-3 of Rib*f*(C-4^5)], 4.91, 4.92 (2 s, 2 H, H-1 of Rib*f*), 5.11 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1^5), 5.15, 5.16 (2 d, 2 H, J 3.8, 3.7 Hz, H-1^3,1^4), 5.23 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1^2), 5.67 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1^1), 7.32 (2 d, 2 H, J 9.3 Hz, NPh H-2,6), and 8.22 (2 d, 2 H, J 9.3 Hz, NPh H-3,5). Anal. Calcd for $C_{46}H_{71}NO_{36}$ (1214.04): C, 45.51; H, 5.90; N, 1.15. Found: C, 45.26; H, 5.86; N, 1.44.

p-*Nitrophenyl* O-*(2,3-di*-O-*acetyl-6*-O-*benzoyl-α*-D-*glucopyranosyl)-(1 → 4)-tris[*O-*(2,3,6-tri*-O-*acetyl-α*-D-*glucopyranosyl)-(1 → 4)]-2,3,6-tri*-O-*acetyl-α*-D-*glucopyranoside* (**14**).—To a solution of **3** (200 mg, 0.130 mmol) in dry CH_2Cl_2 (20 mL) were added pyridine (0.32 mL, 3.90 mmol) and benzoyl chloride (0.046 mL, 0.390 mmol), and the mixture was stirred overnight at 25°C. The mixture was extracted with CH_2Cl_2, and the extract was washed with 2 M HCl and water, dried

(Na_2CO_4), and concentrated. Column chromatography (50:1 CH_2Cl_2–MeOH) of the residue on silica gel gave **14** (185 mg, 87%) as an amorphous mass; $[\alpha]_D$ +165° (*c*0.41, $CHCl_3$); ν 3500 (OH), 2960 (CH), 1750 and 1220 (ester), 1530 and 1350 (NO_2), and 860 and 710 cm^{-1} (phenyl); ^1H NMR ($CDCl_3$): δ 1.98–2.19 (14 s, 42 H, 14 AcO), 3.64 (t, 1 H, $J_{3,5} = J_{4,5} = 9.6$ Hz H-4^5), 3.86–4.09 (m, 9 H, H-4^{1-4},5^{1-5}), 4.13–4.34 (m, 4 H, H-6^{1-4}), 4.42–4.57 (m, 5 H, H-6^5,$6'^{1-4}$), 4.68–4.82 (m, 5 H, H-$6'^5$,2^{2-5}), 4.95 (dd, 1H, $J_{1,2}$ 3.7, $J_{2,3}$ 10.1 Hz, H-2^1), 5.26 (t, 1 H, $J_{2,3} = J_{3,4} = 10.6$ Hz, H-3^5), 5.28 (~ d, 2 H, *J* 3.8 Hz, H-1^3,1^4), 5.32 (d, 1 H, $J_{1,2}$ 4.2 Hz, H-1^2), 5.36 (d, 1 H, $J_{1,2}$ 4.2 Hz, H-1^5), 5.36, 5.37, 5.39 (3 t, 3 H, *J* 10.5, 9.3; 9.3 Hz, H-3^{2-4}), 5.73 (t, 1 H, $J_{2,3} = J_{3,4} = 9.2$ Hz, H-3^1), 5.74 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1^1), 7.25 (2 d, 2 H, *J* 9.2 Hz, NPh H-2,6), 7.45 (t, 2 H, *J* 7.5 Hz, PhCO H-3,5), 7.60 (t, 1 H, *J* 7.4 Hz, PhCO H-4), 8.06 (d, 2 H, *J* 7.1 Hz, PhCO H-2,6) and 8.25 (2 d, 2 H, *J* 9.2 Hz, NPh H-3,5). Anal. Calcd for $C_{71}H_{87}NO_{43}$ (1642.44): C, 51.92; H, 5.34; N, 0.85. Found: C, 51.80; H, 5.47; N, 0.62.

p-*Nitrophenyl* O-*(2,3,5-tri*-O-*benzoyl-β*-D-*ribofuranosyl)-(1 → 4)*-O-*(2,3-di*-O-*acetyl-6*-O-*benzoyl-α*-D-*glucopyranosyl)-(1 → 4)-tris[*O-*(2,3,6-tri*-O-*acetyl-α*-D-*glucopyranosyl)-(1 → 4)]-2,3,6-tri*-O-*acetyl-α*-D-*glucopyranoside* (**15**).—To a solution of **14** (100 mg, 0.0609 mmol) and **8** (135 mg, 0.274 mmol) in dry CH_2Cl_2 (1 mL) was added powdered 4A molecular sieves (150 mg), and the mixture was stirred overnight at 25°C under N_2, then cooled to −35°C. To the cooled mixture were added NIS (123 mg, 0.548 mmol) and TfOH (0.0048 mL, 0.0548 mmol), the mixture was stirred overnight at −35°C, and then worked up as described for **5** to give **15** (87 mg, 73%) as an amorphous mass; $[\alpha]_D$ +148° (*c* 1.7, $CHCl_3$); ν 2960 (CH), 1760 and 1230 (ester), 1530 and 1350 (NO_2), and 860 and 710 cm^{-1} (phenyl); ^1H NMR ($CDCl_3$): δ 1.97–2.21 (14 s, 42 H, 14 AcO), 3.96–4.32 (m, 14 H, H-4^{1-5},5^{1-5},6^{1-4}), 4.47–4.76 (m, 12 H, H-2^{2-4},6^5,$6'^{1-5}$, and H-4,5,5′ of Rib*f*), 4.80 (dd, 1 H, $J_{1,2}$ 4.2, $J_{2,3}$ 10.4 Hz, H-2^5), 4.95 (dd, 1 H, $J_{1,2}$ 3.7, $J_{2,3}$ 10.3 Hz, H-2^1), 5.28, 5.29 (2 d, 2 H, *J* 4.0, 4.4 Hz, H-1^3,1^4), 5.33 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1^2), 5.35–5.48 (m, 6 H, H-1^5,3^{2-5}, and H-1 of Rib*f*), 5.53 (dd, 1 H, $J_{1,2}$ 2.2, $J_{2,3}$ 5.1 Hz, H-2 of Rib*f*), 5.67 (t, 1 H, $J_{2,3} = J_{3,4} = 5.3$ Hz, H-3 of Rib*f*), 5.73 (t, 1 H, $J_{2,3} = J_{3,4} = 9.3$ Hz, H-3^1), 5.74 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1^1), 7.25 (2 d, 2 H, *J* 9.5 Hz, NPh H-2,6), 7.30–8.05 (m, 20 H, 4 PhCO), and 8.24 (2 d, 2 H, *J* 9.2 Hz, NPh H-3,5). Anal. Calcd for $C_{97}H_{107}NO_{43}$ (1974.89): C, 58.99; H, 5.46; N, 0.71. Found: C, 59.21; H, 5.20; N, 0.97.

p-*Nitrophenyl* O-*β*-D-*ribofuranosyl-(1 → 4)*-O-*[α*-D-*glucopyranosyl-(1 → 4)-tris-[*O-*α*-D-*glucopyranosyl)-(1 → 4)]-α*-D-*glucopyranoside* (**16**).—To a solution of **15** (87 mg, 0.044 mmol) in dry MeOH (10 mL) at 0°C was added a solution of NaOMe (0.156 mmol) in dry MeOH (1 mL), the mixture was stirred overnight at 7°C, and then worked up as described for **6** to give **16** (44 mg, 94%) as an amorphous mass; $[\alpha]_D$ +146° (*c* 0.88, MeOH); ν 3300 (OH), 2940 (CH), 1520 and 1350 (NO_2), and 850 cm^{-1} (phenyl); ^1H NMR (CD_3OD): δ 4.11 (t, 1 H, $J_{2,3} = J_{3,4} = 9.2$ Hz, H-3^1), 4.27 (dd, 1 H, $J_{2,3}$ 4.6, $J_{3,4}$ 7.3 Hz, H-3 of Rib*f*), 4.97 (s, 1 H, H-1 of Rib*f*), 5.14, 5.15, 5.17 (3 d, 3 H, *J* 3.5, 3.8, 4.0 Hz, H-1^{3-5}), 5.23 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1^2), 5.67 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1^1), 7.32 (2 d, 2 H, *J* 9.3 Hz, NPh H-2,6), and 8.22 (2 d, 2 H, *J* 9.3 Hz, NPh H-3,5). Anal. Calcd for $C_{41}H_{63}NO_{32}$ (1081.93): C, 45.52; H, 5.87; N, 1.29. Found: C, 45.62; H, 5.81; N, 1.56.

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