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Synthesis of methyl α-isomalto-oligosaccharides specifically deoxygenated at position 2 of the terminal glycopyranosyl unit

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Methyl α-isomaltoside and methyl α-isomaltotrioside specifically deoxygenated at position C-2 of the terminal glucopyranosyl unit were synthesized by trimethylsilyltriflate-mediated condensation of 3,4,6-tri-*O*-benzoyl-1-*O*-*tert*-butyl(dimethyl)silyl-2-deoxy-β-D-*arabino*-hexopyranose with suitably blocked derivatives of methyl α-D-glucopyranoside and methyl α-isomaltoside, respectively.

Keywords: Methyl α-isomalto-oligosaccharides, *tert*-butyl(dimethyl)silyl donor, 2-deoxy-α-D-*arabino*-hexopyranosyl

Introduction

This laboratory has studied the binding of monoclonal, dextran-specific antibodies. In the past we have developed a systematic method of mapping the H-bonding interaction between saccharide immunodeterminants and antibodies [1]. Its application includes the anti-dextran antibodies W3129 and 16.4.12E. These monoclonal antibodies are specific to the non-reducing chain terminal tetrasaccharide sequence of α-(1-6)-dextrans [2, 3]. Presently we are studying IgG 35.8.2H, an anti-(1-6)-dextran capable of binding internal antigenic epitopes [4]. Our preliminary studies have shown that in IgG 35.8.2H there is a perturbable tryptophanyl residue in the general combining area, but that it is removed from the subsite possessing the highest affinity for its glucosyl residue [5]. In order to probe H-bonding interactions [5] we are preparing isomalto di- and trisaccharides deoxygenated at a specific location [5].

We present here a procedure for preparing any methyl α-isomalto-oligosaccharides specifically deoxygenated [6] at C-2 of the terminal glucopyranosyl unit.

Results and discussion

It was shown that 1-*O*-trimethylsilyl [7–14] and 1-*O*-*tert*-butyl(dimethyl)silyl [15–19] derivatives are suitable glycosyl donors in glycosylation reactions which are often mediated by trimethylsilyltriflate [9–19].

For the preparation of methyl α-isomalto-oligosaccharides deoxygenated at C-2 of the terminal glucopyranosyl unit we chose 3,4,6-tri-*O*-benzoyl-1-*O*-*tert*-butyl(dimethyl)silyl-2-deoxy-β-D-*arabino*-hexopyranose (**7**) as a glycosyl donor. Starting with methyl 4,6-benzylidene-α-D-glucopyranoside, treatment with phenoxychlorothiocarbonate in dichloromethane in the presence of pyridine and *N*-hydroxysuccinimide [20–22] gave methyl 4,6-benzylidene-2-phenoxythiocarbonyl-α-D-glucopyranoside (**1**) at a yield of 72.6%. This is in good agreement with the observation [23–25] that partial acylation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside with chlorides of carboxylic, sulfonic, and benzylthiocarboxylic acids preferentially gives a monoester at position 2. After benzoylation of the 2-phenoxythiocarbonyl derivative (**1**) methyl 4,6-benzylidene-2-*O*-phenoxythiocarbonyl-3-*O*-benzoyl-α-D-glucopyranoside (**2**) was obtained (>90%). Compound **2** was deoxygenated smoothly using tributyltinhydride in the presence of 2,2′-azobis(2-methylpropionitrile) [26] giving methyl 4,6-benzylidene-3-*O*-benzoyl-2-deoxy-α-D-*arabino*-hexopyranoside (**3**) at a yield of 91%. The benzylidene group from derivative **3** was removed by 5% HCl in methanol and methyl 3-*O*-benzoyl-2-deoxy-α-D-*arabino*-hexopyranoside (**4**) was isolated by crystallization from ethyl acetate (95%). Benzoylation of **4** gave the fully protected compound **5** in quantitative yield which was treated with 65% trifluoroacetic acid (4 h) affording

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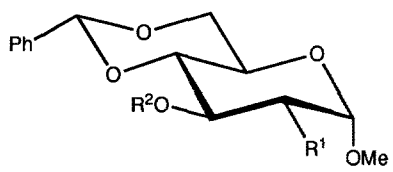
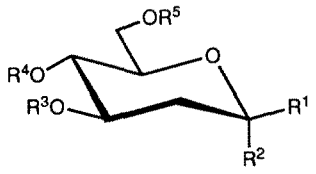


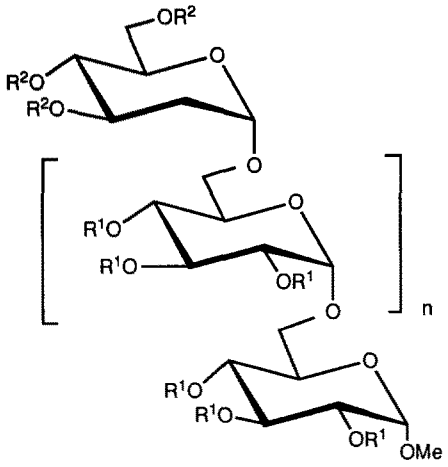


	R^1	R^2
1	OPtc	OH
2	OPtc	OBz
3	H	OBz

	R^1	R^2	R^3	R^4	R^5
4	H	OMe	Bz	H	H
5	H	OMe	Bz	Bz	Bz
6	H,OH		Bz	Bz	Bz
7	OTBS	H	Bz	Bz	Bz

Bz = benzoyl
Me = methyl
Ptc = phenoxythiocarbonyl
TBS = *tert*-butyl(dimethyl)silyl

Scheme 1.



		R^1	R^2
n = 0	**8**	Bn	Bz
	9	Bn	H
	10	H	H
n = 1	**11**	Bn	Bz
	12	Bn	H
	13	H	H

Bn = benzyl

Scheme 2.

3,4,6-tri-*O*-benzoyl-2-deoxy-α,β-D-*arabino*-hexopyranose (**6**) at a yield of 77%. Compound **6** easily underwent reaction with *tert*-butyl(dimethyl)chlorosilane in dichloromethane in the presence of imidazole offering a 95% yield of 3,4,6-tri-*O*-benzoyl-1-*O*-*tert*-butyl(dimethyl)silyl-2-deoxy-β-D-*arabino*-hexopyranose (**7**). Methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside, one of the possible nucleophiles, was prepared as previously described [27]. Its condensation (48 h at −10 °C) with the glycosyl donor **7** was mediated by trimethylsilyl triflate [14–19]. The product, methyl *O*-(3,4,6-tri-*O*-benzoyl-α-D-*arabino*-hexopyranosyl)-(1-6)-*O*-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**8**), was obtained at a yield of 82% from the reaction mixture by chromatograpy. Using Zemplén deacylation, the disaccharide **8** gave compound **9**, which after debenzylation gave methyl *O*-(2-deoxy-α-D-*arabino*-hexopyranosyl)-(1-6)-α-D-glucopyranoside (**10**).

Using the same conditions methyl *O*-(2,3,4-tri-*O*-benzyl-glucopyranosyl)-(1-6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside [28] was condensed with the donor **7**. After debenzoylation and debenzylation, methyl *O*-(2-deoxy-α-D-*arabino*-hexopyranosyl)-(1-6)-*O*-(α-D-glucopyranosyl)-(1-6)-α-D-glucopyranoside (**13**) was obtained.

The structures of all compounds were determined by NMR spectroscopy.

Materials and methods

General methods

Melting points were determined on a Kofler hot stage. Optical rotations were measured at 25 °C with a Perkin

Elmer automatic polarimeter, Model 241 MC. All reactions were monitored by thin-layer chromatography (TLC) on precoated slides of silica gel G F254 (Analtech). Detection was effected by charring with 5% sulfuric acid in ethanol and, when applicable, with UV light. Preparative chromatography was performed by gradient elution from columns of Silica gel 60 (Merck, No. 9385). ^{1}H- and ^{13}C-NMR spectra were measured at ambient temperature using a Varian FX 300 or Varian Gemini spectrometer, operating at 300 MHz for protons and 75 MHz for ^{13}C. Chemical shifts found in the spectra recorded for solutions in C^2HCl_3 and ^2H_2O are reported, respectively, using Me_4Si and methanol (δ_{MeOH} vs. δ_{Me_4Si} 49.0) as internal standards. Proton-signal assignments were done by COSY or homonuclear decoupling experiments. The non-equivalent geminal proton resonating at a lower field is denoted Ha and the one resonating at a higher field is denoted Hb. Carbon-signals assignments were based on heteronuclear shift-correlated 2D experiments (HETCOR). Chemical ionization mass spectra (CIMS) using ammonia as the reactive gas were obtained with a Finigan 1015 D spectrometer. Reactions requiring anhydrous conditions were performed under dry nitrogen using common laboratory glassware, and reagents and solvents were handled with gas-tight syringes. Solutions in organic solvents were dried with anhydrous sodium sulfate, and concentrated at 2 kPa and 40 °C. 2,2-Azobis(2-methylpropionitrile) (99%), from Eastman Kodak Company, was used as supplied.

Methyl 4,6-benzylidene-2-phenoxythiocarbonyl-α-D-glucopyranoside (**1**)

Methyl 4,6-benzylidene-α-D-glucopyranoside (1.4 g, 5 mmol) was dissolved in dichloromethane (30 ml) and pyridine was added (2 ml, 24 mmol) followed by *N*-hydroxysuccinimide (0.375 g). Phenylchlorothiocarbonate (1.25 ml, 6.6 mmol) was added in portions (5) over 2 h. After that no starting material was detected (TLC, 6:1 toluence:acetone). The reaction mixture was washed wtih saturated aqueous sodium bicarbonate, water, dried with sodium sulfate, concentrated and the residue purified on column of silica gel (10:1 toluene:acetone), offering 1.51 g (72.6%) of **1**, m.p. 184–185 °C; $[\alpha]_D$ +53.6 °C (c 0.748, chloroform); ^{1}H-NMR data (C^2HCl_3) δ 7.61–7.21 (m, H, Ph), 5.64 (s, 1H, H-7), 5.41 (dd, 1H, $J_{1,2}$ = 3.8 Hz, $J_{2,3}$ = 9.7 Hz, H-2), 5.31 (bd, 1H, H-1), 4.45 (ddd, 1H, H-3), 4.40 (dd, 1H, $J_{5,6a}$ = 4.7 Hz, $J_{6a,6b}$ = 10.2 Hz, H-6$_a$), 3.99 (ddd, 1H, $J_{4,5}$ = 9.7 Hz, H-5), 3.85 (m, 1H, H-6$_b$), 3.71 (udd, 1H, H-4), 3.54 (s, 3H, O*C*H$_3$); ^{13}C-NMR data (C^2HCl_3) δ 195.06 *C*SOPh), 102.20 (C-7), 96.57 (C-1), 81.85 (C-2), 81.24 (C-4), 68.83 (C-6), 68.73 (C-3), 62.10 (C-5), 55.57 (O*C*H$_3$); CIMS: *m/z* 419 ([M + H]$^+$).

Analytical data. Calculated for $C_{21}H_{22}O_7S$: C, 60.27; H, 5.30; S, 7.66. Found: C, 60.19; H, 5.35; S, 7.72.

Methyl 4,6-benzylidene-3-O-benzoyl-2-O-phenoxythiocarbonyl-α-D-glucopyranoside (**2**)

Compound **1** (4 g, 10 mmol) was dissolved in 25 ml of pyridine and benzoylchloride (3.5 ml, 30.15 mmol) was added. After 30 min the reaction was complete (TLC, 6:1 toluene:acetone). The residue, obtained on concentration, was extracted with dichloromethane, the extract was washed with saturated sodium bicarbonate, water, dried with sodium sulfate and concentrated. Purification on a silica gel column gave 4.7 g (94.2%) of **2**, m.p. 169–170 °C (from 1:1 ethylacetate-hexane); $[\alpha]_D$ –20 °C (c 1.112, chloroform); ^{1}H-NMR data (C^2HCl_3) δ 8.17–8.10 (m, 2H, Ph), 7.68–7.20 (m, 11H, Ph), 6.83 (d, 2H, Ph), 6.05 (udd, 1H, H-3), 5.76 (dd, 1H, $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 5.56 (s, 1H, H-7), 5.22 (d, 1H, H-1), 4.38 (dd, 1H, $J_{5,6a}$ = 4.9 Hz, $J_{6a,6b}$ = 10.3 Hz, H-6a), 4.10 (m, 1H, H-5), 3.90 (m, 1H $J_{4,5}$ = 9.5 Hz, H-4), 3.85 (m, 1H, $J_{5,6b}$ = 10.3 Hz, H-6$_b$), 3.52 (s, 3H, O*C*H$_3$); ^{13}C-NMR data (C^2HCl_3) δ 194.70 (PhO*C*S), 165.09 (Ph*C*O), 101.58 (C-7), 96.99 (C-1), 79.95 (C-2), 79.41 (C-4), 69.71 (C-3), 68.86 (C-6), 62.68 (C-5), 55.64 (O*C*H$_3$); CIMS: *m/z* 523 ([M + NH$_4$]$^+$).

Analytical data. Calculated for $C_{21}H_{26}O_8S$: C, 64.36; H, 5.01; S, 6.14. Found: C, 64.43; H, 5.06; S, 6.23.

Methyl 4,6-benzylidene-3-O-benzoyl-2-deoxy-α-D-arabino-hexopyranoside (**3**)

To 5.3 g (10 mmol) of compound **2** in 150 ml dried toluene, 2,2′ azobis(2-methylpropionitrile) (0.163 g, 1 mmol) was added, followed by 4.6 ml (17 mmol) of tributyltinhydride. The reaction mixture was heated at 100 °C for 1 h when TLC no longer showed starting material (6:1 toluene:acetone). It was concentrated and purified on a silica gel column to give, after crystallization from methanol, 3.4 g (90.7%) of **3**, m.p. 152.5–153.5 °C (from methanol); $[\alpha]_D$ −8 °C (c 0.832 chloroform); ^{1}H-NMR data (C^2HCl_3) δ 7.98–7.96 (m, 2H, Ph), 7.49–7.17 (m, 8H, Ph), 5.59–5.50 (m, 2H, H-3, H-7), 4.78 (bd, 1H, $J_{1,2b}$ = 3.15 Hz, H-1), 4.24 (dd, 1H, $J_{5,6a}$ = 4.78 Hz, $J_{6a,6b}$ = 10.53 Hz, H-6$_a$), 3.92 (ddd, 1H, $J_{4,5}$ = 9.77 Hz, H-5), 3.81–3.72 (m, 3H, H-4, H-6$_b$), 3.31 (s, 3H, O*C*H$_3$), 2.43 (bdd, 1H, $J_{2a,3}$ = 5.75 Hz, $J_{2a\,2b}$ = 13.68 Hz, H-2$_a$), 1.84 (ddd, 1H, H-2$_b$); ^{13}C-NMR data (C^2HCl_3) δ 165.69 (Ph*C*O), 101.68 (C-7), 98.81 (C-1), 80.61 (C-4), 69.15 (C-6), 68.64 (C-3), 63.05 (C-5), 54.88 (O*C*H$_3$), 35.71 (C-2); CIMS: *m/z* 388 ([M + NH$_4$]$^+$).

Analytical data. Calculated for $C_{21}H_{21}O_6$: C, 68.28; H, 5.73. Found C, 68.09; H, 5.84.

Methyl 3-O-benzoyl-2-deoxy-α-D-arabino-hexopyranoside (**4**)

Compound **3** (0.47 g, 1.27 mmol) was stirred in 5% HCl in methanol for 15 min when TLC (6:1 toluene:acetone) showed the absence of starting material. After neutralization with Amberlite IRA-400 (OH), concentration and crystallization from ethylacetate, compound **4** (0.34 g, 94.7%) was

obtained, m.p. 176–177 °C; $[\alpha]_D$ +125 °C (c 0.41, chloroform); ^{1}H-NMR data ($C_5{}^2H_5N$) δ 8.08 (m, 2H, Ph), 7.44 (m, 1H, Ph), 7.25 (m, 2H, Ph), 6.00 (ddd, 1H, H-3), 4.92 (bd, 1H, H-1), 4.40–4.33 (m, 3H, H-6_a, H-6_b, H-4), 4.16 (uddd, 1H, H-5), 3.30 (s, 3H, OCH_3), 2.51 (uddd, 1H, H-2_a), 1.94 (uddd, 1H, H-2_b). ^{13}C-NMR data ($C_5{}^2H_5N$) δ 166.48 (PhCO), 98.58 (C-1), 74.40 (C-5), 74.01 (C-3), 69.24 (C-4), 62.21 (C-6), 54.35 (OCH_3), 35.69 (C-2); CIMS: *m/z* 300 ($[M + NH_4]^+$).

Analytical data. Calculated for $C_{14}H_{18}O_6$: C, 59.57; H, 6.43. Found: C, 59.41; H, 6.44.

*Methyl 3,4,6-tri-*O*-benzoyl-2-deoxy-α-*D*-arabino-hexopyranoside* (**5**)

Benzoyl chloride (1.9 ml, 14.0 mmol) was added to **4** (0.5 g, 2.8 mmol) dissolved in pyridine (15 ml). No starting material was detected (TLC, 6:1 toluene acetone) after 1 h. The reaction mixture was worked up as is mentioned for compound **2** and crystallized from methanol to give **5** (1.3 g, 94.2%), m.p. 108.5–109.5 °C; $[\alpha]$ +60.1 °C (c 0.993, chloroform); ^{1}H-NMR data (C^2HCl_3) δ 8.20–7.96 (m, 10H, Ph), 7.71–7.15 (m, 5H, Ph), 5.80 (m, 1H, H-3), 5.65 (bdd, 1H, H-4), 4.93 (bdd, 1H, H-1), 4.63 (dd, 1H, $J_{5,6a}$ = 2.7 Hz, $J_{6a,6b}$ = 12 Hz, H-6_a), 4.52 (dd, 1H, $J_{5,6b}$ = 5.3 Hz, H-6_b), 4.38 (m, 1H, H-5), 3.40 (s, 3H, OCH_3), 2.53 (bddd, 1H, H-2_a), 2.01 (ddd, 1H, H-2_b); ^{13}C-NMR data (C^2HCl_3) δ 165.31 (PhCO), 164.86 (2C, PhCO), 97.54 (C-1), 70.04 (C-4), 69.59 (C-3), 67.61 (C-5), 63.08 (C-6), 54.33 (OCH_3), 34.81 (C-2); CIMS: *m*/z 508 ($[M + NH_4]^+$).

Analytical data. Calculated for $C_{28}H_{26}O_8$: C, 68.56; H, 5.34. Found: C, 68.63; H, 5.35.

*3,4,6-Tri-*O*-benzoyl-2-deoxy-α,β-*D*-arabino-hexopyranose* (**6**)

Compound **5** (1 g, 0.204 mmol) was stirred in 65% trifluoroacetic acid (7.85 ml) at 75 °C for 4 h when starting material was no longer detected (TLC, 8:1 toluene:acetone). After concentration and purification on a silica gel column derivative **6** (0.76 g, 77.3%) was obtained: $[\alpha]_D$ +51.1 °C (2 min), +46.9 °C (1 h), +35.2 °C (6 h, const.) (c 0.653, chloroform); ^{1}H-NMR data (C^2HCl_3) δ_α 8.15–7.98 (m, 8H, Ph), 7.64–7.18 (m, 7H, Ph), 5.82 (ddd, 1H, $J_{2,3}$ = 5.13 Hz, $J_{3,4}$ = 11.3 Hz, H-3), 5.62 (ddd, 1H, $J_{4,5}$ = 9.7 Hz, H-4), 5.54 (bd, 1H, H-1), 4.65 (m, 1H, H-5), 4.60 (m, 1H, H-6_a), 4.43 (dd, 1H, $J_{5,6b}$ = 4.4 Hz, $J_{6a,6b}$ = 12.4 Hz, H-6_b), 2.55 (bdd, 1H, H-2_a), 2.00 (bdd, 1H, H-2_b); ^{13}C-NMR data (C^2HCl_3) α:β 4.5:1, δ_α 166.25, 165.68, 165.47 (PhCO), 91.84 (C-1), 70.42 (C-3), 69.80 (C-4), 68.39 (C-5), 63.44 (C-6), 35.56 (C-2); δ_β 94.84 (C-1), 72.36 (C-3), 71.44 (C-4), 69.92 (C-5), 63.44 (C-6), 35.56 (C-2); CIMS: *m/z* 494 ($[M + NH_4]^+$), 372 ($[M - BzOH + NH_4]^+$).

Analytical data. Calculated for $C_{27}H_{24}O_8$: C, 68.06; H, 5.08. Found: C, 67.87; H, 5.05.

*3,4,6-Tri-*O*-benzoyl-1-*O*-tert-butyl(dimethyl)silyl-2-deoxy-β-*D*-arabino-hexopyranose* (**7**)

Compound **6** (0.68 g, 1.4 mmol) was dissolved in dried dichloromethane (5 ml), and imidazole (0.26 g, 4 mmol), and *tert*-butyl(dimethyl)chlorosilane (0.31 g, 7 mmol) were added. The reaction mixture was stirred at 40 °C for 1 h when the reaction was complete (TLC, 6:1 toluene:acetone). After extraction with phosphate buffer, water, drying (Na_2SO_4), and concentration, the residue was subjected to column chromatography on silica gel (12:1 toluene:acetone) giving 0.8 g (95.2%) of **7**; $[\alpha]_D$ −38.8° (c 0.86, chloroform); ^{1}H-NMR data (C^2HCl_3) δ 8.06–7.93 (m, 5H, Ph), 7.55–7.16 (m, 10H, Ph), 5.35 (dd, 1H, $J_{4,5}$ = 9.1 Hz, H-4), 5.27 (m, 1H, H-3), 5.08 (dd, 1H, $J_{1,2a}$ = 1.7 Hz, $J_{1,2b}$ = 9.3 Hz, H-1), 4.60 (dd, 1H, $J_{5,6a}$ = 2.9 Hz, $J_{6a,6b}$ = 11.9 Hz, H-6_a), 4.46 (dd, 1H, $J_{5,6b}$ = 6.3 Hz, H-6_b), 4.03 (ddd, 1H, $J_{4,5}$ = 9.3 Hz, H-5), 2.42 (ddd, 1H, $J_{2a,3}$ = 5.0 Hz, $J_{2a,2b}$ = 12.5 Hz, H-2_a), 1.81 (ddd, 1H, $J_{2b,3}$ = 9.4 Hz, H-2_b), 1.03 (s, 3H, C(CH_3)$_3$), 0.89 (s, 6H, C(CH_3)$_3$), 0.14, 0.12 (2s, 6H, Si(CH_3)$_2$); ^{13}C-NMR data (C^2HCl_3) δ 165.97, 165.69, 165.41 (PhCO), 94.69 (C-1), 72.26 (C-5), 71.67 (C-3), 70.27 (C-4), 63.89 (C-6), 39.15 (C-2), 25.55 (C(CH_3)$_3$), −3.98, −4.58, −5.04 (Si(CH_3)$_2$, SiC(CH$_3$)$_3$); CIMS: *m/z* 608 ($[M + NH_4]^+$),

Analytical data. Calculated for $C_{33}H_{38}O_8Si$: C, 67.09; H, 6.48. Found: C, 67.12; H, 6.47.

Methyl O-*(3,4,6-tri-*O*-benzoyl-2-deoxy-α-*D*-arabino-hexopyranosyl)-(1-6)-2,3,4-tri-*O*-benzyl-α-*D*-glucopyranoside* (**8**)

Methyl 2,3,4-tri-*O*-benzyl-α-D-glycopyranoside [27] (0.23 g, 0.5 mmol) and derivative **7** (0.295 g, 0.5 mmol) were dissolved in dry dichloromethane (40 ml). Molecular sieve 4 Å (0.7 g) was added and after 30 min of stirring, the reaction mixture was cooled to −50 °C and trimethylsilyltrifluoromethanesulfonate (0.05 ml, 0.27 mmol) was added. The reaction mixture was kept at −30 °C for 16 h when 0.029 ml trimethylsilylmethanesulfonate was added. No starting material remained after 48 h at −20 °C, and trimethylamine (0.24 ml) was used to quench the reaction. After filtration, the filtrate was washed with 0.1 M citrate buffer, 1 M phosphate buffer and water, dried with sodium sulfate and concentrated. The crude product was purified on a column of silica gel (4:1 hexane:ethylacetate) giving syrupy **8** (0.375 g, 81.87%); $[\alpha]_D$ +58.3 °C (c 1.2, chloroform); ^{1}H-NMR data (C^2HCl_3) δ 8.05–7.92 (m, 6H, Ph), 7.90–7.22 (m, 24H, Ph), 5.70 (uddd, 1H, H-3′), 5.55 (udd, 1H, H-4′), 5.11 (bd, 1H, H-1′), 5.02 (d, 2H, J_{gem} = 11 Hz, CH_2Ph), 4.82 (dd, 2H, J_{gem} = 11.9 Hz, CH_2Ph), 4.69 (d, 2H, J_{gem} = 11.9 Hz, CH_2Ph), 4.64 (d, 1H, J = 3.7 Hz, H-1), 4.50 (m, 1H, H-6′$_a$), 4.32 (m, 2H, H-5′, H-6′$_b$), 4.03 (udd, 1H, H-3), 3.91 (dd, 1H, H-6_a), 3.84 (dd, 1H, H-5), 3.70 (bd, 1H, H-6_b), 3.54 (dd, 1H, H-4), 3.51 (dd, 1H, $J_{1,2}$ = 3.1 Hz, $J_{2,3}$ = 6.3 Hz, H-2), 3.45 (s, 3H, OCH_3), 2.56 (bdd, 1H, H-2′$_a$), 2.00 (uddd, 1H, H-2′$_b$); ^{13}C-NMR data (C^2HCl_3) δ 166.09, 165.77,

165.57 (PhCO), 97.95 (C-1), 97.21 (C-1′), 82.20 (C-3), 80.13 (C-2), 77.86 (C-4), 75.69, 74.99, 73.34 (CH_2Ph), 70.16 (C-3′), 69.96 (C-4′), 69.88 (C-5), 68.25 (C-5′), 66.06 (C-6), 63.24 (C-6′), 55.14 (OCH_3), 35.13 (C-2′); CIMS: *m/z* 940 ($[M + NH_4]^+$), 818 ($[M - BzOH + NH_4]^+$).

Analytical data. Calculated for $C_{55}H_{54}O_{13}$: C, 71.57; H, 5.90. Found: C, 71.33; H, 5.90.

Methyl O-(*2-deoxy*-α-D-arabino-*hexopyranosyl*)-(*1-6*)-*2,3,4-tri*-O-*benzyl*-α-D-*glucopyranoside* (**9**)

Disaccharide **8** (0.226 g, 0.245 mmol) was dissolved in toluene (3 ml), and anhydrous methanol (5 ml) was added. Sodium methoxide in methanol (1 M, 0.1 ml) was added to give an alkaline reaction. After 3.5 h starting material was no longer detected in the reaction mixture and it was neutralized with Amberlite 120 (H^+), concentrated, purified on a column of silica gel (20:1 dichloromethane:methanol), to yield **9** (0.14 g, 93.3%). Compound **9** was crystallized from methanol, m.p. 137–138 °C; $[\alpha]_D$ +67.4 °C (c 0.881, chloroform); ^{1}H-NMR data (C^2HCl_3) δ 7.39–7.25 (m, 15H, Ph), 4.88 (dd, 2H, J_{gem} = 10.8 Hz, CH_2Ph), 4.83 (bd, 1H, H-1′), 4.72 (d, 1H, J_{gem} = 10.8 Hz, 1/2 CH_2Ph), 4.58 (d, 1H, J_{gem} = 12 Hz, 1/2 CH_2Ph), 4.52 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 4.50 (d, 1H, J_{gem} = 12 Hz, 1/2 CH_2Ph), 3.90 (udd, 1H, H-3), 3.82 (m, 1H, H-3′), 3.72–3.61 (m, 2H, H-6′$_a$, H-6′$_b$), 3.54–3.33 (m, 4H, H-5′, H-4, H-2, H-4′), 3.28 (s, 3H, OCH_3); ^{13}C-NMR data (C^2HCl_3) δ 97.81, 97.72 (C-1, C-1′), 82.03 (C-3) 80.03 (C-2), 77.73 (C-4), 75.71, 74.76, 73.29 (CH_2Ph), 71.83 (C-4′), 71.42, 69.69 (C-5, C-5′), 68.96 (C-3′), 65.87 (C-6), 61.63 (C-6′), 55.15 (OCH_3), 37.27 (C-2′); CIMS: *m/z* 629 ($[M + NH_4]^+$), 611 ($[M^+]$).

Analytical data. Calculated for $C_{34}H_{42}O_{10}$: C, 66.87; H, 6.93. Found: C, 66.81; H, 6.96.

Methyl O-*2-deoxy*-α-D-arabino-*hexopyranosyl*-(*1-6*)-α-D-*glucopyranoside* (**10**)

A solution of the benzyl derivative **9** (0.12 g, 0.2 mmol) in 95% ethanol containing a suspension of 5% Pd-C catalyst (0.025 g) was stirred under H_2 overnight. After conventional processing the crude product was chromatographed (2:1:1 2-propanol:EtOAc:water), and lyophilized to yield amorphous, hydroscopic **10** (0.063 g, 94.3%). Neither the ^{1}H- nor the ^{13}C-NMR spectrum revealed signals that indicated the presence of aromatic residues; $[\alpha]_D$ +138.3 °C (c 1.027, water); ^{1}H-NMR data (^2H_2O) δ 4.93 (bd, 1H, H-1′), 4.70 (d, 1H, H-1), 3.85–3.77 (m, 2H, H-3′, H-6$_a$), 3.73–3.64 (m, 2H, H-3, H-6$_b$), 3.60–3.51 (m, 3H, H-5, H-6′$_a$, H-6′$_b$), 3.45 (dd, 1H, $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 9.7 Hz, H-2), 3.40 (dd, 1H, $J_{3,4}$ = 8 Hz, $J_{4,5}$ = 9 Hz, H-4), 3.31 (s, 3H, OCH_3), 3.29 (m, 2H, H-4′, H-5′), 2.08 (ddd, 1H, $J_{1',2'}$ = 0.9 Hz, $J_{2'a,2'b}$ = 13.2 Hz, $J_{2'a,3'}$ = 5.1 Hz, H-2′$_a$), 1.68 (ddd, 1H, $J_{1',2'b}$ = 3.4 Hz, $J_{2'b,3'}$ = 11.9 Hz, H-2′$_b$); ^{13}C-NMR data (^2H_2O): δ 99.72 (C-1), 97.30 (C-1′), 73.67 (C-2), 72.55 (C-3), 71.45 (C-4), 71.21 (C-4′), 70.15 (C-5), 69.70 (C-5′), 68.56 (C-3′), 65.20 (C-6), 60.89 (C-6′), 55.38 (OCH_3), 36.83 (C-2′); CIMS: *m/z* 358 ($[M + NH_4]^+$).

Methyl O-(*3,4,6-tri-O-benzoyl-2-deoxy*-α-D-arabino-*hexopyranosyl*)-(*1-6*)-O-(*2,3,4-tri*-O-*benzyl*-α-D-*glucopyranosyl*)-(*1-6*)-*2,3,4-tri*-O-*benzyl*-α-D-*glucopyranoside* (**11**)

Methyl *O*-(2,3,4-tri-*O*-benzyl-glucopyranosyl)-(1-6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside [28] (0.205 g, 0.218 mmol) and silyl derivative **7** (0.155 g, 0262 mmol) were dissolved in dichloromethane (15 ml) and molecular sieve 4 Å (0.35 g) was added. After 30 min of stirring at −50 °C, trimethylsilyl triflate (0.1 ml, 0.55 mmol) was added dropwise over 3 days (−30 °C). The reaction mixture was quenched, and worked up as mentioned for compound **8**. Purification on a column of silica gel (12:1 toluene:acetone) gave trisaccharide **11** (0.230 g, 75.4%); $[\alpha]_D$ +72.9 °C (c 1.143, chloroform); ^{1}H-NMR data (C^2HCl_3) δ 8.03–7.16 (m, 30H, Ph), 5.69 (bddd, 1H, $J_{3'',4''}$ = 9.7 Hz, H-3″), 5.55 (bdd, 1H, $J_{4'',5''}$ = 9.5 Hz, H-4″), 5.07–4.92 (m, 5H, 5/2 CH_2Ph), 4.86–4.66 (m, 7H, 7/2 CH_2Ph), 5.01, 5.00 (ud, 2H, H-1′, H-1″), 4.75 (od, 1H, H-1), 4.22 (m, 1H, H-5″), 3.95 (dd, 2H, H-3, H-3′), 3.84 (m, 2H, H-5, H-5′), 3.67–3.58 (m, 4H, H-6$_a$, H-6$_b$, H-6′$_b$), 3.56–3.52 (bdd, 2H, H-2, H-2′), 3.46 (dd, 2H, $J_{3,4}$ = 3.4 Hz, $J_{4,5}$ = 9.45 Hz, H-4, H-4′), 3.36 (s, 3H, OCH_3), 2.51 (uddd, 1H, H-2″$_a$), 1.92 (bddd, 1H, H-2″$_b$); ^{13}C-NMR data (C^2HCl_3) δ 165.96, 165.45, 165.33 (COPh), 97.97, 97.29 (C-1′, C-1″), 96.93 (C-1), 82.15, 81.73 (C-3, C-3′), 80.29, 80.12 (C-2, C-2′), 77.82, 77.74 (C-4, C-4′), 75.70, 75.48, 74.97, 74.89, 73.37, 72.44 (CH_2Ph), 70.48, 69.98 (C-5, C-5′), 70.20 (C-3″), 70.05 (C-4″), 68.29 (C-5″), 66.07 (2C, C-6, C-6′), 63.21 (C-6″), 55.20 (OCH_3), 35.26 (C-2″); CIMS: *m/z* 1372 ($[M + NH_4]^+$).

Analytical data. Calculated for $C_{82}H_{82}O_{18}$: C, 72.66; H, 6.10. Found: C, 72.51; H, 6.06.

Methyl O-(*2-deoxy*-α-D-arabino-*hexopyranosyl*)-(*1-6*)-O-(*2,3,4-tri*-O-*benzyl*-α-D-*glucopyranosyl*)-(*1-6*)-*2,3,4-tri*-O-*benzyl*-α-D-*glycopyranoside* (**12**)

Trisaccharide **11** (0.20 g, 0.148 mmol) was dissolved in toluene (3 ml) and anhydrous methanol (5 ml), and sodium methoxide in methanol (1 m, 0.1 ml) was added. After 3 h, when no starting material was detected (TLC, 15:1 dichloromethane:methanol), the reaction mixture was worked up as described for compound **9** yielding trisaccharide **12** (0.148 g, 96.1%); $[\alpha]_D$ +78.1 °C (c 0.896, chloroform); ^{1}H-NMR data (C^2HCl_3) δ 7.30–7.17 (m, 30H, 6Ph), 4.95–4.87 (dd, 4H, 2CH_2Ph), 4.91 (bd, 1H, H-1′), 4.82 (bd, 1H, H-1″), 4.79–4.59 (m, 6H, 3CH_2Ph), 4.55 (d, 1H, H-1), 4.54 (d, 2H, J_{gem} = 11.7 Hz, CH_2Ph), 3.98–3.90 (m, 2H, H-3, H-3′), 3.87–3.82 (m, 1H, H-3″), 3.80–3.70 (m, 2H, H-5, H-5′), 3.68–3.58 (m, 4H, H-6$_a$, H-6$_b$, H-6′$_a$, H-6′$_b$), 3.50–3.37 (m, 5H, H-2, H-4, H-4′, H-2′, H-4″), 3.32 (s, 3H, OCH_3), 2.71 (bd, 1H, OH), 2.31 (bd, 1H, OH), 2.07 (bdd,

1H, H-2″$_a$), 1.79 (m, 1H, O*H*), 1.51 (bdd, 1H, H-2″$_b$); ^{13}C-NMR data (C^2HCl_3) δ 98.03, 97.71, 96.01 (C-1, C-1′, C-1″), 82.13, 81.65 (C-3, C-3′), 80.14, 80.09 (C-2, C-2′), 77.74, 77.66 (C-4, C-4′), 75.75, 75.48, 74.93, 74.63, 73.37, 72.33 (CH_2Ph), 73.01 (C-4″), 71.07, 70.28, 69.85 (C-5, C-5′, C-5″), 69.03 (C-3″), 66.00, 65.75 (C-6, C-6′), 62.51 (C-6″), 55.15 (OCH_3), 37.08 (C-2″); CIMS: *m/z* 1060 ([M + NH_4]$^+$).

Analytical data. Calculated for $C_{61}H_{70}O_{15}$: C, 70.23; H, 6.76. Found: C, 69.94; H, 6.81.

Methyl O-(*2-deoxy*-α-D-arabino-*hexopyranosyl*)-(*1-6*)-O-(α-D-*glucopyranosyl*)-(*1-6*)-α-D-*glucopyranoside* (**13**)

A solution of the trisaccharide **12** (0.14 g, 0.13 mmol) in 95% ethanol (20 ml) containing a suspension of 5% Pd-C catalyst (0.02 g) was stirred under H_2 overnight. After conventional processing the crude product was chromatographed (2:1:1 2-propanol:EtOAc:water), and lyophilized to yield **13** (0.062 g, 92.5%). Neither the ^{1}H- nor the ^{13}C-NMR spectrum revealed signals that indicated the presence of aromatic residues; $[\alpha]_D$ +138.8 °C (c 1.235, water); ^{1}H-NMR data (^2H_2O) δ 4.95 (udd, 1H, H-1″), 4.85 (d, 1H, $J_{1',2'}$ = 3.6 Hz, H-1′), 4.74 (ud, 1H, H-1), 3.91–3.70 (m, 7H, H-3″, H-6′$_a$, H-6′$_b$, H-6$_a$, H-6$_b$, H-5, H-4′), 3.67–3.53 (m, H, H-3, H-6″$_a$, H-6″$_b$), 3.48 (dd, 2H, $J_{1,2}$ = 3.6 Hz, $J_{2,3} = J_{2',3'}$ = 9.7 Hz, H-2, H-2′), 3.41 (bdd, 2H, H-3′, H-4′), 3.33 (s, 3H, OCH_3), 3.36–3.25 (m, 3H, H-5′, H-5″, H-4″), 2.08 (uddd, 1H, $J_{2''a,3''}$ = 5.4 Hz, $J_{2''a,2''b}$ = 13.1 Hz, H-2″$_a$), 1.61 (uddd, 1H, $J_{1'',2''b}$ = 3.5 Hz, H-2″$_b$); ^{13}C-NMR data (^2H_2O): δ 99.61 (C-1), 98.01, 96.96 (C-1′, C-1″), 73.60, 73.55 (C-2, C-2′), 72.47 (C-3), 71.59 (C-3′), 71.40 (C-4), 71.12 (C-4″), 70.17 (C-4′), 70.13 (C-5), 69.74 (C-5′), 69.65 (C-5″), 68.45 (C-3″), 65.66, 65.13 (C-6, C-6′), 60.80 (C-6″), 55.34 (OCH_3), 36.76 (C-2″); CIMS: *m/z* 520 ([M + NH_4]$^+$).

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