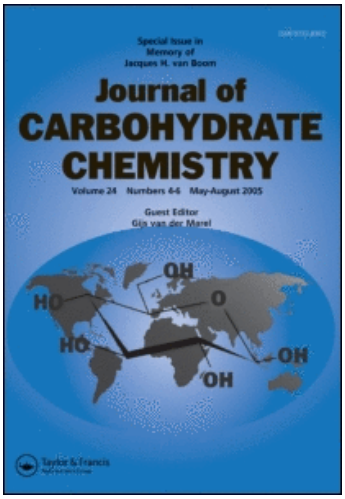

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SYNTHESIS OF 8-METHOXYCARBONYLOCTYL β-GLYCOSIDES OF TRI- AND TETRASACCHARIDES RELATED TO SCHIZOPHYLLAN AND NEOGLYCOPROTEINS THEREFROM

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ABSTRACT

The 8-methoxycarbonyloctyl β-glycosides of the trisaccharides *O*-β-D-Glc*p*-(1→6)-*O*-β-D-Glc*p*-(1→3)-D-Glc*p* and *O*-β-D-Glc*p*-(1→3)-*O*-[β-D-Glc*p*-(1→6)]-D-Glc*p* and of the tetrasaccharide *O*-β-D-Glc*p*-(1→3)-*O*-[β-D-Glc*p*-(1→6)]-*O*-β-D-Glc*p*-(1→3)-D-Glc*p*, corresponding to the fragments of schizophyllan, have been synthesized by using mono- to tetrasaccharide 1-thioglycosides as glycosyl donors, each bearing a participating benzoyl group in the 2-position, and *N*-iodosuccinimide and silver triflate as promoter. Saponification of the tri- and tetrasaccharide β-glycosides, followed by attachment to bovine serum albumin of the resulting sugar derivatives having a carboxyl group at the aglycon terminal, provided neoglycoproteins for immunological studies of the polysaccharide.

INTRODUCTION

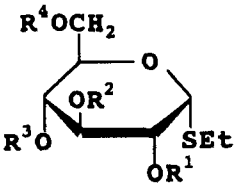
In continuation of our studies of schizophyllan,[1-3] a (1→6)-branched (1→3)-β-D-glucan having antitumor or immunostimulatory activities,[4-6] we required the tri- and tetrasaccharides, representing partial structures of the polysaccharide, as the corresponding β-glycosides containing a linker arm, which makes it possible to attach the sugar sequences to a carrier protein to form neoglycoproteins.[7,8] We now report the synthesis of 8-methoxycarbonyloctyl (8-MCO) *O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→3)-β-

D-glucopyranoside (**26**), 8-MCO *O*-β-D-glucopyranosyl-(1→3)-*O*-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside (**34**), and 8-MCO *O*-β-D-glucopyranosyl-(1→3)-*O*-[β-D-glucopyranosyl-(1→6)]-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside (**45**). Also described is the preparation of the neoglycoproteins by coupling of the derivatives **29**, **37**, and **47**, obtained from **26**, **34**, and **45**, respectively, to bovine serum albumin (BSA), in the hope that the conjugates so prepared can be used as immunizing antigens to produce the antibodies that may recognize partial structures of schizophyllan.

RESULTS AND DISCUSSION

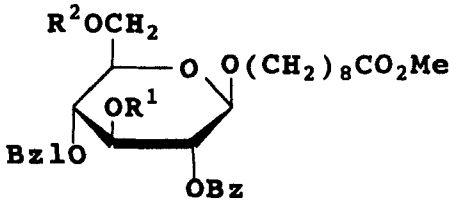
For the synthesis of **26**, **34**, and **45**, two different routes were explored to develop mono- to tetrasaccharide synthons that are not only applicable to the preparation of **26**, **34**, and **45**, but can also serve as versatile intermediates for further synthesis of various (1→6)-branched (1→3)-β-linked D-*gluco*-oligosaccharides related to schizophyllan. Initially, suitably protected 8-MCO β-D-glucopyranosides (**12** and **15**) were prepared, starting from ethyl 2-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-α-D-glucopyranoside[9] (**1**), and then coupled with mono- and disaccharide thioglycosides. By a second route, the tri- (**24** and **32**[3]) and tetrasaccharide thioglycoside **43** were prepared and then condensed with 8-methoxycarbonyloctanol.[7] In both routes, the thioglycoside derivatives, each carrying a benzoyl group at O-2 position, were used as the glycosyl donors to ensure the formation of β-D-glucosidic linkages in the condensations.[10] A combination of *N*-iodosuccinimide (NIS)-silver triflate[11] was used as the promoter for all the glycosylation steps.

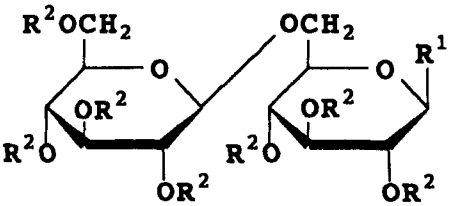
Benzoylation of **1** with benzoyl chloride-pyridine gave the 2,3-di-*O*-benzoyl derivative **2** (93%). The benzylidene ring of **2** was selectively cleaved by treatment with borane-trimethylamine and aluminium(III) chloride in toluene[12] to afford the 2,3-di-*O*-benzoyl-4-*O*-benzyl derivative **3** (80%), the ^{13}C NMR spectrum of which contained a signal for C-6 at 61.4 ppm, confirming[13] that HO-6 was unsubstituted. *O*-Debenzoylation of **3** with methanolic sodium methoxide gave ethyl 4-*O*-benzyl-1-thio-α-D-glucopyranoside (**4**, 93%), which was preferentially benzoylated with 2.4 mol equiv of 1-(benzoyloxy)benzotriazole-triethylamine[14] in dichloromethane to give the 2,6-di-*O*-benzoyl derivative **5** (82%). Occurrence of the benzoyl groups at O-2 and O-6 in **5** was revealed by the presence of a doublet of doublets ($J_{2,3}$ = 9.9 Hz) for H-2 at δ 5.19 in the ^1H NMR spectrum, and the downfield shift of 2.0 ppm exhibited by C-6 compared to that of **4** in the ^{13}C NMR spectrum, respectively. Esterification of **5** with chloroacetyl chloride-pyridine[15] in dichloromethane afforded the 3-*O*-chloroacetyl derivative (**6**, 93%), which was condensed with 8-methoxycarbonyloctanol to give 8-MCO 2,6-di-*O*-benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-β-D-glu-



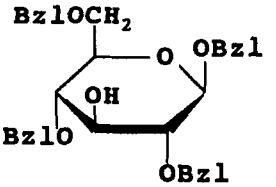
	R^1	R^2	R^3	R^4		R^1	R^2	R^3	R^4
1	H	H	-PhCH-		**6**	Bz	CA	Bzl	Bz
2	Bz	Bz	-PhCH-		**7**	Bz	Bz	Bzl	TBDPS
3	Bz	Bz	Bzl	H	**8**	H	H	Bzl	TBDPS
4	H	H	Bzl	H	**9**	Bz	H	Bzl	TBDPS
5	Bz	H	Bzl	Bz	**10**	Bz	CA	Bzl	TBDPS

CA : $ClCH_2CO$; TBDPS : Bu^tPh_2Si





	R^1	R^2		R^1	R^2
11	CA	Bz	**16**	OAc	Ac
12	H	Bz	**17**	SMe	Ac
13	CA	TBDPS	**18**	SMe	Bz
14	H	TBDPS			
15	H	H			



19

copyranoside (**11**, 87%). *O*-Dechloroacetylation of **11** with thiourea[15] yielded the glucoside derivative **12** (92%) having HO-3 unsubstituted.

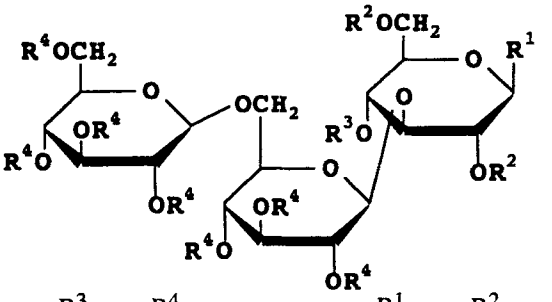
Reaction of β-gentiobiose octaacetate[16] (**16**) with methyl tributyltin sulfide in 1,2-dichloroethane in the presence of tin(IV) chloride[17] gave methyl 1-thio-β-gentiobioside heptaacetate (**17**, 85%), which was successively *O*-deacetylated and benzoylated to afford the heptabenzoate **18**. Condensation of **12** with **18** gave the D-glucotrioside derivative **20** (83%), *O*-debenzoylation of which, followed by hydrogenolysis (Pd-C), afforded the trisaccharide 8-MCO β-glycoside **26**. Acetylation of **26** produced the crystalline decaacetate **27**.

In a second route to **26**, benzyl 2,4,6-tri-*O*-benzyl-β-D-glucopyranoside[18] (**19**) was glycosylated with **18** to afford the D-glucotrioside derivative **21** (87%), which on removal of the protecting groups, as for **20**, gave the crystalline β-undecaacetate **22**. Treatment of **22** with methyl tributyltin sulfide, as for **16**, yielded the trisaccharide thioglycoside **23** (82%), which was converted into the corresponding deca-*O*-benzoyl derivative **24** by successive *O*-deacetylation and benzoylation. Condensation of **24** with 8-methoxycarbonyloctanol gave the D-glucotrioside derivative **25** (86%), which was *O*-debenzoylated to furnish **26**.

Treatment of **3** in *N*,*N*-dimethylformamide (DMF) with *tert*-butyldiphenylsilyl chloride in the presence of imidazole[19] (→ **7**, 92%), followed by *O*-debenzoylation (→ **8**, 93 %), and partial benzoylation with 1.2 mol equiv of 1-(benzoyloxy)benzotriazole-triethylamine, gave the 2-*O*-benzoyl derivative **9** (84%), the ^{1}H NMR spectrum of which showed a doublet of doublets ($J_{2,3}$ = 10.0 Hz) for H-2 at δ 5.18, indicating the location of the benzoyl group in **9**. Chloroacetylation of **9** afforded **10** (92%), which was coupled with 8-methoxycarbonyloctanol to give 8-MCO 2-*O*-benzoyl-4-*O*-benzyl-6-*O*-*tert*-butyl-diphenylsilyl-3-*O*-chloroacetyl-β-D-glucopyranoside (**13**, 86%). *O*-Dechloroacetylation of **13** with thiourea in the presence of 2,6-dimethylpyridine[20] (→ **14**) and *O*-desilylation with tetrabutylammonium fluoride[21] in oxolane afforded 8-MCO 2-*O*-benzoyl-4-*O*-benzyl-β-D-glucopyranoside (**15**).

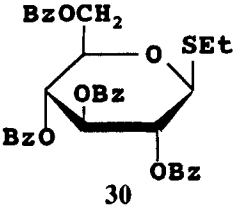
Glucosylation of **15** with 2.6 mol equiv of ethyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside[22] (**30**) afforded the β-(1→6)-branched D-glucotrioside derivative **31** (81 %), which was deprotected, as for **20**, to give the trisaccharide 8-MCO β-glycoside **34**. Acetylation of **34** gave the crystalline decaacetate **35**. In an alternative approach to **34**, coupling of ethyl *O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-*O*-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-2,4-di-*O*-benzoyl-1-thio-α-D-glucopyranoside[3] (**32**) with 8-methoxycarbonyloctanol afforded the D-glucotrioside derivative **33** (85%), which was *O*-debenzoylated to provide **34**.

Condensation of **12** with **10** gave the β-(1→3)-linked D-glucobioside derivative **38** (84%), which was transformed by *O*-dechloroacetylation (→ **39**) and *O*-desilylation into



	R^1	R^2	R^3	R^4		R^1	R^2	R^3	R^4
20	M	Bz	Bzl	Bz	**25**	M	Bz	Bz	Bz
21	OBzl	Bzl	Bzl	Bz	**26**	M	H	H	H
22	OAc	Ac	Ac	Ac	**27**	M	Ac	Ac	Ac
23	SMe	Ac	Ac	Ac	**28**	C	H	H	H
24	SMe	Bz	Bz	Bz	**29**	A	H	H	H

M : $O(CH_2)_8CO_2Me$; C : $O(CH_2)_8CONHNH_2$; A : $O(CH_2)_8CO_2H$



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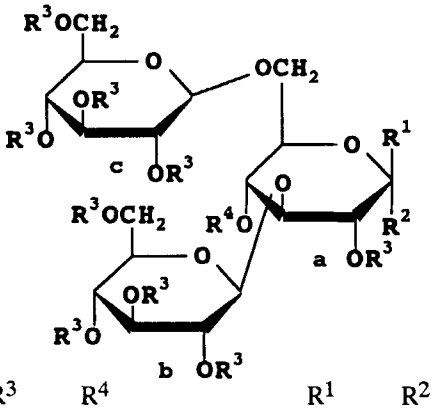


	R^1	R^2	R^3	R^4		R^1	R^2	R^3	R^4
31	M	H	Bz	Bzl	**35**	M	Ac	Ac	Ac
32	H	SEt	Bz	Bz	**36**	C	H	H	H
33	M	H	Bz	Bz	**37**	A	H	H	H
34	M	H	H	H					

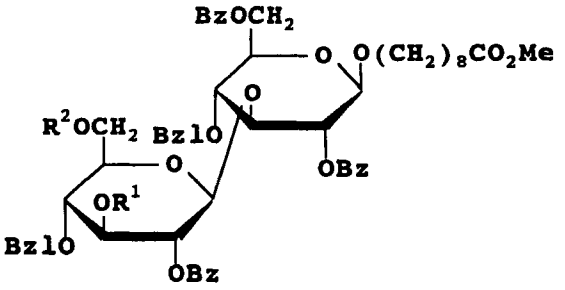
the disaccharide derivative **40** having HO-3' and -6' unsubstituted. Condensation of **40** with 2.6 mol equiv of **30** gave the D-glucotetraoside derivative **41** (80%), which was deprotected, as before, to provide the tetrasaccharide 8-MCO β-glycoside **45**. In an alternate route to **45**, ethyl *O*-(2,3,4,6-tetra- *O*-acetyl-β-D-glucopyranosyl)-(1→3)- *O*-[2,3,4,6-tetra- *O*-acetyl-β-D-glucopyranosyl-(1→ 6)]-*O*-(2,4-di-*O*-acetyl- β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside[3] (**42**) was *O*-deacetylated and then benzoylated to give the trideca-*O*-benzoyl derivative **43**, glycosidation of which with 8-methoxycarbonyloctanol gave the D-glucotetraoside derivative **44** (85%). *O*-Debenzoyla-tion of **44** furnished **45**.

Having obtained the tri- (**26** and **34**) and tetrasaccharide 8-MCO β-glycoside **45**, the preparation of the neoglycoproteins by coupling of these haptens to BSA was next investigated. Attempt to couple **26**, **34** or **45** to BSA by way of a two-step procedure involving hydrazide and acyl azide intermediates[7] was unsuccessful. On treatment with hydrazine hydrate in methanol,[7] both the glycosides **26** and **34** underwent smooth transformation into the hydrazide derivatives **28** and **36**, respectively, but the glycoside **45** could not be converted into the corresponding hydrazide **46**, because of its very low solubility in hydrazine hydrate or a mixture of hydrazine hydrate and alcohol. Furthermore, the trisaccharide hydrazides **28** and **36** were insoluble in DMF to be used for the preparation of the acyl azide.[7] Therefore, an alternative coupling method was sought.

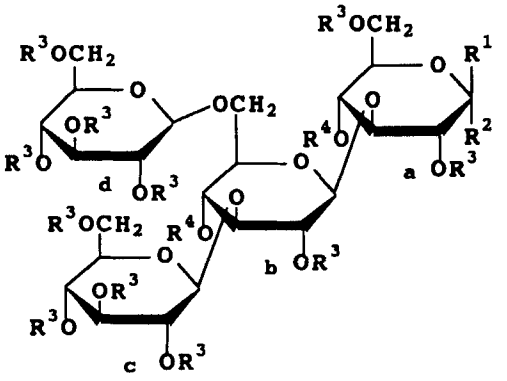
The glycosides **26**, **34**, and **45** were saponified[7] with dilute aqueous sodium hydroxide and then neutralized with dilute aqueous acid to give the tri- (**29** and **37**) and tetrasaccharide derivative **47**, respectively, each having a carboxyl group at the terminal position in the aglycon. Compounds **29**, **37**, and **47** were not purified, but each was coupled to BSA in the presence of 1-ethyl-3-(dimethylaminopropyl)carbodiimide hydrochloride (EDC), according to the procedure employed for the coupling of aldonic acid to BSA.[23] The conjugates obtained were purified by gel permeation chromatography on a column of Bio-Gel P-6. The carbohydrate and protein contents were determined by the phenol-sulfuric acid colorimetric method[24] and the dye-binding assay,[25] respectively. When the molar proportions of the glycosides **26**, **34**, and **45** to BSA [for the sequence involving saponification (→ **29**, **37**, and **47**) and subsequent coupling to BSA] were 300:1, 200:1, and 320:1, the degree of substitution (D.S., number of the oligosaccharide residues per one mole of BSA) was 28, 21, and 30 for the conjugates from **26**, **34**, and **45**, respectively. The immunological studies of schizophyllan using the neoglycoproteins thus prepared are under way.

EXPERIMENTAL

General Procedures. Unless stated otherwise, these were as described.[9] Optical rotations were measured at 20 °C. NMR spectra (^{1}H at 90 MHz, ^{13}C at 22.6MHz) were



	R^1	R^2
38	CA	TBDPS
39	H	TBDPS
40	H	H



	R^1	R^2	R^3	R^4		R^1	R^2	R^3	R^4
41	M	H	Bz	Bzl	**45**	M	H	H	H
42	H	SEt	Ac	Ac	**46**	C	H	H	H
43	H	SEt	Bz	Bz	**47**	A	H	H	H
44	M	H	Bz	Bz					

recorded with a Hitachi R-90H spectrometer for solutions in $CDCl_3$ (internal Me_4Si) or D_2O (internal sodium 4,4-dimethyl-4-silapentanoate-d_4). HPTLC was performed on Silica gel (No. 5628, Merck) in 2:2:1 (v/v) n-BuOH-EtOH-H_2O with detection by charring with H_2SO_4.

Ethyl 2,3-Di-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-α-D-glucopyranoside (2). Benzoyl chloride (9.7 mL) was added dropwise at 0 °C to a stirred solution of **1** (10.0 g)

in pyridine (100 mL). The mixture was kept for 5 h at room temperature, and then poured into ice-H_2O. The precipitate formed was filtered off, washed with H_2O, and dissolved in CH_2Cl_2. The solution was washed successively with aq $NaHCO_3$ and H_2O, dried, and concentrated. The residue was crystallized from $CHCl_3$-EtOH to give **2** (15.5 g, 93%): mp 199-200 °C; $[\alpha]_D$ +139.5° (*c* 1.1, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 165.4 and 165.3 (C=O), 136.8, 133.2, and 132.8 (aromatic C-1), 101.5 (benzylic C), 83.1 (C-1), 79.3, 72.1, 69.9, and 68.6 (C-2,3,4,5), 63.1 (C-6), and 24.5 and 14.5 (SCH_2CH_3).

Anal. Calcd for $C_{29}H_{28}O_7S$: C, 66.91; H, 5.42. Found: C, 66.99; H, 5.50.

Ethyl 2,3-Di-*O*-benzoyl-4-*O*-benzyl-1-thio-α-D-glucopyranoside (3). A mixture of **2** (27.7 g, 53.2 mmol), BH_3·Me_3N complex (15.53 g, 0.213 mol), and 4A powdered molecular sieves (50 g) in dry PhMe (400 mL) was stirred for 1 h at room temperature. Powdered $AlCl_3$ (10.64 g, 79.8 mmol) was added portionwise at room temperature and stirring was continued for 2 h. The mixture was poured into cold M H_2SO_4, filtered through a layer of Celite, and washed with PhMe. The organic layer was separated, washed successively with H_2O, aq $NaHCO_3$, and H_2O, dried, and concentrated. The residue was subjected to column chromatography (PhMe-EtOAc, 20:1) to give **3** (22.3 g, 80%): mp 99-100 °C (from Et_2O-hexane); $[\alpha]_D$ +155.6° (*c* 1.6, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 165.4 (2 C, C=O), 137.3, 133.2, and 132.0 (aromatic C-1), 82.0 (C-1), 75.6, 74.6, 73.0, 72.0, and 71.3 (C-2,3,4,5, $PhCH_2$), 61.4 (C-6), and 24.3 and 14.7 (SCH_2CH_3).

Anal. Calcd for $C_{29}H_{30}O_7S$: C, 66.65; H, 5.79. Found: C, 66.70; H, 5.75.

Ethyl 4-*O*-Benzyl-1-thio-α-D-glucopyranoside (4). A solution of **3** (11.4 g) in dry MeOH (90 mL) was treated with methanolic M NaOMe (5 mL). The mixture was kept overnight at room temperature, made neutral with Amberlite IR-120 (H^+) resin, filtered, and concentrated. The residue was crystallized from petroleum ether-Et_2O to afford **4** (6.38 g, 93%): mp 72-73.5 °C; $[\alpha]_D$ +231.7° (*c* 1.6, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 138.1 (aromatic C-1), 85.8 (C-1), 77.2, 74.9, 74.4, 71.8, and 71.5 (C-2,3,4,5, $PhCH_2$), 61.5 (C-6), and 24.9 and 15.0 (SCH_2CH_3).

Anal. Calcd for $C_{15}H_{22}O_5S$: C, 57.30; H, 7.05. Found: C, 57.21; H, 6.95.

Ethyl 2,6-Di-*O*-benzoyl-4-*O*-benzyl-1-thio-α-D-glucopyranoside (5). To a stirred solution of **4** (9.12 g, 29 mmol) and 1-(benzoyloxy)benzotriazole (16.66 g, 69.6 mmol) in CH_2Cl_2 (130 mL) was added Et_3N (14.57 mL, 0.104 mol). The mixture was stirred overnight at room temperature, washed successively with aq $NaHCO_3$ and H_2O, dried, and concentrated. Column chromatography (PhMe-EtOAc, 50:1→20:1, stepwise) of the product afforded **5** (12.43 g, 82%): mp 63.5-64°C (from hexane); $[\alpha]_D$ +113.1° (*c* 1.5, $CHCl_3$); NMR ($CDCl_3$) δ_H 8.31-6.91 (m, 15 H, 3 Ph), 5.70 (d, 1 H, $J_{1,2}$ = 5.7 Hz, H-1), 5.19 (dd, 1 H, $J_{2,3}$ = 9.9 Hz, H-2), 2.52 (m, 2 H, SCH_2CH_3), and 1.18 (t, 3 H, SCH_2CH_3); δ_C 166.0 and 165.8 (C=O), 137.7, 133.2, and 132.9 (aromatic C-1), 81.8 (C-1),

77.9, 74.8, 73.6, 73.1, and 69.0 (C-2,3,4,5, PhCH$_2$), 63.5 (C-6), and 24.2 and 14.7 (SCH$_2$CH$_3$).

Anal. Calcd for $C_{29}H_{30}O_7S$: C, 66.65; H, 5.79. Found: C, 66.77; H, 5.85.

Ethyl 2,6-Di-*O*-benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-1-thio-α-D-glucopyranoside (6). A solution of **5** (4.48 g) in CH_2Cl_2 (50 mL) containing pyridine (1.52 mL) was cooled to -10 °C, treated with a solution of $ClCH_2COCl$ (0.97 mL) in CH_2Cl_2 (10 mL), and kept for 15 min at 0 °C. The mixture was diluted with CH_2Cl_2, poured into ice-H_2O, and the organic layer was separated, washed successively with dil aq HCl, aq $NaHCO_3$, and H_2O, dried, and concentrated. Column chromatography (hexane-EtOAc, 4:1) of the residue afforded **6** (5.20 g, 93%): $[\alpha]_D$ +121.7° (*c* 1.4, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 166.1, 165.9 and 165.2 (C=O), 137.1, 133.5, and 133.1 (aromatic C-1), 81.7 (C-1), 76.1, 74.7, 74.4, 71.5, and 69.1 (C-2,3,4,5, PhCH$_2$), 63.6 (C-6), 40.3 (COCH$_2$Cl), and 24.2 and 14.6 (SCH$_2$CH$_3$).

Anal. Calcd for $C_{31}H_{31}ClO_8S$: C, 62.15; H, 5.22. Found: C, 62.27; H, 5.35.

Ethyl 2,3-Di-*O*-Benzoyl-4-*O*-benzyl-6-*O*- *tert*-butyldiphenylsilyl-1-thio-α-D-glucopyranoside (7). A mixture of **6** (6.12 g, 11.7 mmol), *tert*-butyldiphenylsilyl chloride (3.65 mL, 14 mmol), and imidazole (1.9 g, 28 mmol) in DMF (30 mL) was stirred for 1 h at 70 °C. The mixture was cooled, poured into ice-brine, and extracted with Et_2O. The extract was washed with brine, dried, and concentrated. Column chromatography (hexane-EtOAc, 9:1) of the product gave **7** (8.19, g, 92%): $[\alpha]_D$ +69.5° (*c* 1.2, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 165.5 and 165.3 (C=O), 81.4 (C-1), 76.2, 74.6, 73.2, 72.1, and 71.8 (C-2,3,4,5, PhCH$_2$), 62.7 (C-6), 26.9 [(*C*H$_3$)$_3$C], 23.9 (S*C*H$_2$CH$_3$) 19.3 [(CH$_3$)$_3$*C*], and 14.5 (SCH$_2$*C*H$_3$).

Anal. Calcd for $C_{45}H_{48}O_7SiS$: C, 71.02; H, 6.36. Found: C, 71.17; H, 6.45.

Ethyl 4-*O*-Benzyl-6-*O*- *tert*-butyldiphenylsilyl-1-thio-α-D-glucopyranoside (8). A solution of **7** (3.37 g) in MeOH (30 mL) and CH_2Cl_2 (5 mL) was treated with M NaOMe (1.3 mL), and the mixture was processed as described for the preparation of **4**. Column chromatography (hexane-EtOAc, 2:1) of the residue gave **8** (2.28 g, 93%), $[\alpha]_D$ +68.8° (*c* 1.4, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 85.9 (C-1), 77.7, 75.6, 74.5, 72.3, and 72.1 (C-2,3,4,5, PhCH$_2$), 62.9 (C-6), 26.8 [(*C*H$_3$)$_3$C], 23.9 (S*C*H$_2$CH$_3$), 19.3 [(CH$_3$)$_3$*C*], and 14.5 (SCH$_2$*C*H$_3$).

Anal. Calcd for $C_{31}H_{40}O_5SiS$: C, 67.36; H, 7.29. Found: C, 67.25; H, 7.45.

Ethyl 2-*O*-Benzoyl-4-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-1-thio-α-D-glucopyranoside (9). A solution of **8** (3.21 g, 5.8 mmol) and 1-(benzoyloxy)benzotriazole (1.67 g, 7 mmol) in CH_2Cl_2 (30 mL) was treated with Et_3N (1.07 mL, 7.7 mml). Processing of the mixture as described for the preparation of **5**, followed by column chromatography (hexane-EtOAc, 4:1) of the product, gave **9** (3.20 g, 84%), $[\alpha]_D$ +73.9° (*c*

1.4, $CHCl_3$); NMR ($CDCl_3$) δ_H 8.12–6.83 (m, 20 H, 4 Ph), 5.74 (d, 1 H, $J_{1,2}$ = 5.9 Hz, H-1), 5.18 (dd, 1 H, $J_{2,3}$ = 10.0 Hz, H-2), 2.52 (m, 2 H, SCH_2CH_3), 1.22 (t, 3 H, SCH_2CH_3), and 1.09 [s, 9 H, $(CH_3)_3C$]; δ_C 165.9 (C=O), 81.3 (C-1), 78.1, 74.8, 73.8, 72.9, and 71.6 (C-2,3,4,5, $PhCH_2$), 62.9 (C-6), 26.8 [$(CH_3)_3C$], 23.9 (SCH_2CH_3), 19.3 [$(CH_3)_3C$], and 14.3 (SCH_2CH_3).

Anal. Calcd for $C_{38}H_{44}O_6SiS$: C, 69.48; H, 6.75. Found: C, 69.55; H, 6.63.

Ethyl 2-*O*-Benzoyl-4-*O*-benzyl-6-*O*- *tert*-butyldiphenylsilyl-3-*O*-chloroacetyl-1-thio-α-D-glucopyranoside (10). Compound **9** (5.0 g) was treated in CH_2Cl_2 (40 mL) containing pyridine (1.24 mL) with a solution of $ClCH_2COCl$ (0.73 mL) in CH_2Cl_2 (10 mL) as described for the preparation of **6**. The residue was subjected to column chromatography (hexane-EtOAc, 4:1) to give **10** (5.13 g, 92%), $[\alpha]_D$ +74.1° (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 166.2 and 165.3 (C=O), 81.3 (C-1), 76.0, 74.6, 74.5, 71.9, and 71.7 (C-2,3,4,5, $PhCH_2$), 62.5 (C-6), 40.4 ($COCH_2Cl$), 26.9 [$(CH_3)_3C$], 23.9 (SCH_2CH_3), 19.3 [$(CH_3)_3C$], and 14.5 (SCH_2CH_3).

Anal. Calcd for $C_{40}H_{45}ClO_7SiS$: C, 65.51; H, 6.18. Found: C, 65.63; H, 6.26.

8-Methoxycarbonyloctyl 2,6-Di-*O*-benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-β-D-glucopyranoside (11). To a stirred mixture of **6** (3.36 g, 5.6 mmol), 8-methoxycarbonyloctanol (1.58 g, 8.4 mmol), and powdered 4A molecular sieves (10 g) in CH_2Cl_2 (60 mL) at -20 °C was added NIS (1.39 g, 6.2 mmol), immediately followed, dropwise, by a solution of silver triflate (0.29 g, 1.1 mmol) in PhMe (15 mL). After 10 min, the mixture was made neutral with Et_3N, diluted with CH_2Cl_2, filtered through a Celite pad, and washed with CH_2Cl_2. The combined filtrate and washings were washed successively with aq $Na_2S_2O_3$, aq $NaHCO_3$, and H_2O, dried, and concentrated. Column chromatography (hexane-EtOAc, 4:1) of the product gave **11** (3.54 g, 87%): mp 77.5-79 °C (from hexane-Et_2O); $[\alpha]_D$ +74.1° (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 173.9 (CO_2Me), 166.3, 165.9, and 165.1 (C=O), 137.0 and 133.1 (2 C) (aromatic C-1), 100.9 (C-1), 76.6, 76.0, 74.5, 73.2, 72.1 and 70.1 [C-2,3,4,5, $PhCH_2$, $OCH_2(CH_2)_7CO_2Me$], 63.0 (C-6), 51.3 (CO_2CH_3), 40.3 ($COCH_2Cl$), and 34.0, 29.3, 28.9, 25.6, and 24.8 [$OCH_2(CH_2)_7$-CO_2Me].

Anal. Calcd for $C_{39}H_{45}ClO_{11}$: C, 64.59; H, 6.25. Found: C, 64.41; H, 6.31.

8-Methoxycarbonyloctyl 2,6-Di-*O*-Benzoyl-4-*O*-benzyl-β-D-glucopyranoside (12). A mixture of **11** (2.79 g, 3.8 mmol) and $(NH_2)_2C=S$ (1.76 g, 23.1 mmol) in MeOH (30 mL) and CH_2Cl_2 (10 mL) was boiled under reflux for 7 h. The mixture was concentrated and the residue was extracted with CH_2Cl_2. The extract was washed with H_2O, dried, and concentrated. Column chromatography (hexane-EtOAc, 4:1) of the residue afforded **12** (2.26 g, 92%); $[\alpha]_D$ -4.8° (*c* 1.4, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 174.0 (CO_2 Me), 166.1 (2 C, C=O), 137.7, 133.1, and 133.0 (aromatic C-1), 100.8 (C-1), 78.0,

76.2, 75.1, 74.8, 73.05, and 69.8 [C-2,3,4,5, Ph*C*H$_2$, O*C*H$_2$(CH$_2$)$_7$CO$_2$Me], 63.5 (C-6), 51.3 (CO$_2$*C*H$_3$), and 34.0, 29.4, 29.0, 25.8, and 24.8 [OCH$_2$(*C*H$_2$)$_7$CO$_2$Me].

Anal. Calcd for $C_{37}H_{44}O_{10}$: C, 68.50; H, 6.84. Found: C, 68.31; H, 6.77.

8-Methoxycarbonyloctyl 2-*O*-Benzoyl-4-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-3-*O*-chloroacetyl-β-D-glucopyranoside (13). A mixture of **10** (2.94 g, 4 mmol), 8-methoxycarbonyloctanol (1.13 g, 6 mmol), and powdered 4A molecular sieves (10 g) in CH_2Cl_2 (60 mL) was treated with NIS (0.99 g, 4.4 mmol), followed by a solution of silver triflate (0.31 g, 1.2 mmol) in PhMe (20 mL), and processed as described for the preparation of **11**. The residue was subjected to a column chromatography (hexane-EtOAc) to give **13** (2.97 g, 86%): $[\alpha]_D$ +2.8° (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 173.9 (*C*O$_2$Me), 166.4 and 165.2 (C=O), 100.6 (C-1), 76.6, 75.8, 75.6, 74.8, 72.3, and 69.45 [C-2,3,4,5, Ph*C*H$_2$, O*C*H$_2$(CH$_2$)$_7$ CO$_2$Me], 62.35 (C-6), 51.3 (CO$_2$*C*H$_3$), 40.4 (CO*C*H$_2$Cl), 34.0, 29.4, 29.0, 25.8, and 24.9 [OCH$_2$(*C*H$_2$)$_7$CO$_2$Me], 26.9 [(*C*H$_3$)$_3$C], and 19.4 [(CH$_3$)$_3$*C*].

Anal. Calcd for $C_{48}H_{59}ClO_{10}Si$: C, 67.07; H, 6.92. Found: C, 67.11; H, 6.81.

8-Methoxycarbonyloctyl 2-*O*-Benzoyl-4-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranoside (14). A mixture of **13** (2.77 g, 3.2 mmol), $(NH_2)_2C{=}S$ (1.23 g, 16.2 mmol), and 2,6-dimethylpyridine (0.37 mL, 3.2 mmol) in MeOH (30 mL) and CH_2Cl_2 (10 mL) was boiled under reflux for 6 h. The mixture was concentrated and the residue was extracted with CH_2Cl_2. The extract was washed successively with cold dil. HCl, aq $NaHCO_3$, and H_2O, dried, and concentrated. Column chromatography (hexane-EtOAc, 4:1) of the product, afforded **14** (2.34 g, 93%): $[\alpha]_D$ -11.0° (*c* 1.2, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 174.0 (*C*O$_2$Me), 166.0 (C=O), 100.5 (C-1), 78.2, 75.8 (2 C), 75.1, 74.8, and 69.15 [C-2,3,4,5, Ph*C*H$_2$, O*C*H$_2$(CH$_2$)$_7$CO$_2$Me], 62.8 (C-6), 51.3 (CO$_2$*C*H$_3$), 34.0, 29.4, 29.0, 25.9, and 24.8 [OCH$_2$(*C*H$_2$)$_7$CO$_2$Me], 26.8 [(*C*H$_3$)$_3$C], and 19.3 [(CH$_3$)$_3$*C*].

Anal. Calcd for $C_{46}H_{58}O_9Si$: C, 70.56; H, 7.47. Found: C, 70.67; H, 7.54.

8-Methoxycarbonyloctyl 2-*O*-Benzoyl-4-*O*-benzyl-β-D-glucopyranoside (15). M Tetrabutylammonium fluoride in oxolane (3.6 mL) was added to a solution of **14** (2.16 g) in oxolane (20 mL) containing AcOH (0.24 mL), and the mixture was stirred for 7 h at room temperature and then concentrated. A solution of the residue in Et_2O was washed with brine, dried, and concentrated. Column chromatography (hexane-EtOAc, 2:1) of the residue gave **15** (1.38 g, 92%); mp 82-83 °C; $[\alpha]_D$ -18.0° (*c* 1.2, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 174.1 (*C*O$_2$Me), 166.0 (C=O), 138.0 and 133.1 (aromatic C-1), 100.9 (C-1), 78.0, 75.7, 75.2, 74.9, 74.8, and 70.0 [C-2,3,4,5, Ph*C*H$_2$, O*C*H$_2$(CH$_2$)$_7$CO$_2$Me], 61.9 (C-6), 51.3 (CO$_2$*C*H$_3$), and 34.0, 29.4, 28.9, 25.7, and 24.8 [OCH$_2$(*C*H$_2$)$_7$CO$_2$Me],

Anal. Calcd for $C_{30}H_{40}O_9$: C, 66.16; H, 7.40. Found: C, 66.20; H, 7.45.

Methyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (17). A solution of $SnCl_4$ (2.03 mL, 17.3 mmol) in 1,2-dichloroethane (20 mL) was added dropwise at 0 °C to a stirred solution of **16** (7.85 g, 11.6 mmol) and Bu_3SnSMe (5.85 g, 17.4 mmol) in 1,2-dichloroethane (70 mL). The mixture was stirred for 4 h at room temperature, poured into ice-aq $NaHCO_3$-aq KF, filtered through a Celite layer, and washed with CH_2Cl_2. The combined filtrate and washings were partitioned, and the organic layer was washed with H_2O, dried, and concentrated. Crystallization of the residue from EtOH gave **17** (6.55 g, 85%): mp 149-151 °C; $[\alpha]_D$ -6.4° (*c* 1.4, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 170.3-169.1 (C=O), 100.8 (C-1'), 82.8 (C-1), 68.35 (C-6), 61.8 (C-6'), 20.6-20.5 ($COCH_3$), and 13.6 (SMe).

Anal. Calcd for $C_{27}H_{38}O_{17}S$: C, 48.65; H, 5.75. Found: C, 48.58; H, 5.69.

Methyl *O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzoyl-1-thio- β-D-glucopyranoside (18). Compound **17** (3.22 g) was treated with M NaOMe (2 mL) in MeOH (40 mL) as described for the preparation of **4**. To a solution of the residue in pyridine (20 mL) at 0 °C was added BzCl (5.5 mL), and the mixture was kept overnight at room temperature and processed as described for the preparation of **2**. Crystallization of the residue from MeOH-CH_2Cl_2 gave **18** (4.84 g, 91%); mp 215-216 °C; $[\alpha]_D$ +17.2° (*c* 1.2, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 165.8-164.9 (C=O), 103.1 (C-1'), 83.1 (C-1), 68.4 (C-6), 62.9 (C-6'), and 11.5 (SMe).

Anal. Calcd for $C_{62}H_{52}O_{17}S$: C, 67.63; H, 4.76. Found: C, 67.70; H, 4.72.

8-Methoxycarbonyloctyl *O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-2,6-di-*O*-benzoyl-4-*O*-benzyl-β-D-glucopyranoside (20). A mixture of **12** (0.61 g, 940 μmol), **18** (1.24 g, 1.1 mmol), and powdered 4A molecular sieves (2 g) in CH_2Cl_2 (20 mL) was treated with NIS (0.28 g, 1.2 mmol), followed by a solution of silver triflate (87 mg, 339 μmol) in PhMe (5 mL), as described for the preparation of **11.** Column chromatography (PhMe-EtOAc, 30:1) of the residue gave **20** (1.33 g, 83%); $[\alpha]_D$ -19.0° (*c* 1.4, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 173.9 (CO_2Me), 166.0-164.0 (C=O), 101.0 (2 C) and 100.1 (C-1,1',1"), 66.55 (C-6'), 63.2 and 62.6 (C-6,6"), 51.3 (CO_2CH_3), and 33.9, 29.4, 28.9, 25.8, and 24.8 [$OCH_2(CH_2)_7CO_2Me$].

Anal. Calcd for $C_{98}H_{92}O_{27}$: C, 69.17; H, 5.45. Found: C, 69.31; H, 5.60.

Benzyl *O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzoyl- β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-glucopyranoside (21). The product obtained by reaction of **19** (2.37 g, 4.4 mmol) with **18** (5.79 g, 5.25 mmol), as described for the preparation of **11**, was subjected to column chromatography (PhMe-EtOAc, 30:1) to afford **21** (6.08 g, 87%): $[\alpha]_D$ +4.3° (*c* 1.7, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 102.2 and 101.0 (C-1',1"), 99.9 (C-1), 66.55 (C-6'), and 63.2 and 62.6 (C-6, 6").

Anal. Calcd for $C_{95}H_{84}O_{23}$: C, 71.60; H, 5.31. Found: C, 71.74; H, 5.42.

***O*-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→3)-1,2,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (22).** Compound **21** (5.88 g) was treated with M NaOMe (2 mL) in MeOH (70 mL) and CH_2Cl_2 (10 mL) as described for the preparation of **4**. A solution of the residue in MeOH (30 mL) and AcOH (10 mL) was hydrogenated in the presence of 10% Pd-C (2 g) at normal pressure overnight at room temperature. Insoluble material was collected on a Celite pad and washed with H_2O, and the combined filtrate and washings were concentrated. The residue was acetylated[26] with Ac_2O (20 mL) and NaOAc (2 g) under reflux for 30 min. Crystallization of the residue from EtOH afforded **22** (3.21 g, 90%): mp 173-174.5 °C; $[\alpha]_D$ -16.0° (*c* 1.2, $CHCl_3$); NMR ($CDCl_3$) δ_H 5.68 (d, 1 H, $J_{1,2}$ = 8.4 Hz, H-1), and 2.11-1.97 (overlapping s, 33 H, 11 OAc); δ_C 170.3-168.8 (C=O), 100.4 (2 C, C-1',1"), 91.7 (C-1), and 20.6 ($COCH_3$).

Anal. Calcd for $C_{40}H_{54}O_{27}$: C, 49.69; H, 5.63. Found: C, 49.72; H, 5.55.

Methyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl- β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (23). A mixture of **22** (3.43 g, 3.5 mmol) and Bu_3SnSMe (1.44 g, 4.3 mmol) in 1,2-di-chloroethane (30 mL) was treated at 0 °C with a solution of $SnCl_4$ (0.5 g, 4.3 mmol) in 1,2-dichloroethane (5 mL). The mixture was stirred overnight at room temperature and processed as described for the prepartion of **17**. Column chromatography (PhMe-EtOAc, 2:1→1:1, stepwise) of the product gave **23** (2.78 g, 82%): $[\alpha]_D$ -36.4° (*c* 1.3, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 170.4-168.8 (C=O), 100.5 and 100.3 (C-1',1"), 82.4 (C-1), 20.9- 20.5 ($COCH_3$), and 11.0 (SMe).

Anal. Calcd for $C_{39}H_{54}O_{25}S$: C, 49.06; H, 5.70. Found: C, 49.88; H, 5.88.

Methyl *O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzoyl- β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzoyl-1-thio-β-D-glucopyranoside (24). The product obtained by *O*-deacetylation of **23** (2.35 g), followed by ben-zoylation, as described for the preparation of **18**, was subjected to column chromatography (PhMe-EtOAc, 30:1) to give **24** (3.45 g, 89%); $[\alpha]_D$ -28.0° (*c* 1.3, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 166.0-164.2 (C=O), 100.7 and 100.4 (C-1',1"), 82.7 (C-1), 67.2 (C-6'), 62.2 and 62.0 (C-6,6") and 11.1 (SMe).

Anal. Calcd for $C_{89}H_{74}O_{25}S$: C, 67.85; H, 4.73. Found: C, 68.02; H, 4.66.

8-Methoxycarbonyloctyl *O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranoside (25). The product obtained by condensation of **24** (1.59 g, 1 mmol) with 8-methoxycarbonyloctanol (0.28 g, 1.5 mmol), as described for the preparation of **11**, was subjected to column chromatography (PhMe-EtOAc, 20:1) to give **25** (1.32 g, 86%);

$[\alpha]_D$ -23.0° (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 174.0 (CO_2Me), 166.0-164.1 (C=O), 100.9, 100.7, and 100.4 (C-1,1',1"), 68.3 (C-6'), 63.3 and 62.9 (C-6,6"), 51.3 (CO_2CH_3), and 34.0, 29.3, 28.9, 25.7, and 24.8 [$OCH_2(CH_2)_7CO_2Me$].

Anal. Calcd for $C_{98}H_{90}O_{28}$: C, 68.60; H, 5.29. Found: C, 68.78; H, 5.12.

8-Methoxycarbonyloctyl *O*-β-D-Glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside (26). (*a*) *O*-Debenzoylation of **20** (1.04 g) followed by hydrogenolysis, as described for **21**, gave **26** (0.37g, 90%): mp 155-157.5 °C (from EtOH); $[\alpha]_D$ -32.6° (*c* 1.1, H_2O); ^{13}C NMR (D_2O) δ 179.3 (CO_2Me), 105.6, 105.1, and 104.5 (C-1,1',1"), 88.45 (C-3), 63.3 (2 C, C-6,6"), 54.5 (CO_2CH_3), and 36.3, 31.5, 31.0, 27.7, and 27.0 [$OCH_2(CH_2)_7CO_2Me$],

Anal. Calcd for $C_{28}H_{50}O_{18}$: C, 49.85; H, 7.47. Found: C, 49.72; H, 7.61.

(*b*) *O*-Debenzoylation of **25** (1.19 g), as described for the preparation of **4**, afforded **26** (0.49 g, 93%), which was identical (mp, $[\alpha]_D$ and ^{13}C NMR) to the compound obtained in *a*.

8-Methoxycarbonyloctyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranoside (27). Acetylation of **26** (0.18 g) with Ac_2O-pyridine (3 mL, 1:1), followed by column chromatography (PhMe-EtOAc, 2:1) of the product, gave **27** (0.27 g, 93%): mp 172-173 °C (from $CHCl_3$-hexane); $[\alpha]_D$ -36.1° (*c* 1.1, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 173.9 (CO_2Me), 170.0-168.4 (C=O), 100.5 (2 C) and 100.2 (C-1,1',1"), 51.3 (CO_2CH_3), and 34.0, 29.3, 29.1, 25.7, and 24.9 [$OCH_2(CH_2)_7CO_2Me$], and 20.6-20.3 ($COCH_3$).

Anal. Calcd for $C_{48}H_{70}O_{28}$: C, 52.65; H, 6.44. Found: C, 52.60; H, 6.51.

8-Hydrazinocarbonyloctyl *O*-β-D-Glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside (28). Compound **26** (109 mg) was stirred with 80% $NH_2NH_2 \cdot H_2O$ (1 mL) in MeOH (8 mL) overnight at room temperature. After concentration and coevaporation with PhMe, the residue was purified by elution from a column of Sephadex LH-20 with 1:1 MeOH-H_2O to give **28** (98 mg, 90%); mp 152-155 °C (from EtOH); $[\alpha]_D$ -16.8° (*c* 0.95, H_2O); ^{13}C NMR (D_2O) δ 178.3 [$O(CH_2)_8CO$-$NHNH_2$], and 105.0, 105.3, and 104.6 (C-1,1',1").

Anal. Calcd for $C_{27}H_{50}N_2O_{17}$: C, 48.07; H, 7.47; N, 4.15. Found: C, 48.22; H, 7.55, N, 4.10.

8-Methoxycarbonyloctyl *O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-*O*-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-2-*O*-benzoyl-4-*O*-benzyl-β-D-glucopyranoside (31). The product obtained by reaction of **15** (0.74 g, 1.35 mmol) with **30** (2.26 g, 3.5 mmol), as described for the preparation of **11**, was subjected to column chromatography (PhMe-EtOAc, 2:1) to afford **31** (1.87 g, 81%); $[\alpha]_D$ +6.3° (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 174.0 (CO_2Me), 165.8-164.2 (C=O),

101.2 (2 C) and 100.4 (C-1a,b,c), 63.1 (2 C, C-6b,c), 51.3 (CO_2CH_3), and 34.0, 28.9, 25.6, and 24.8 [$OCH_2(CH_2)_7CO_2Me$].

Anal. Calcd for $C_{98}H_{92}O_{27}$: C, 69.17; H, 5.45. Found: C, 69.32; H, 5.59.

8-Methoxycarbonyloctyl *O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-*O*-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-2,4-di-*O*-benzoyl-β-D-glucopyranoside (33). The product obtained by condensation of **32** (0.61 g, 384 μmol) with 8-methoxycarbonyloctanol (0.11 g, 584 μmol), as described for the preparation of **11**, was subjected to column chromatography to give **33** (0.56 g, 85%); $[\alpha]_D$ -25.9° (*c* 1.4, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 173.9 (CO_2Me), 166.0-164.0 (C=O), 101.3 and 100.6 (2 C) (C-1a,b,c), 63.2 (2 C, C-6b,c), 51.3 (CO_2CH_3), and 34.0, 28.9, 25.6, and 24.8 [$OCH_2(CH_2)_7CO_2Me$].

Anal. Calcd for $C_{98}H_{90}O_{28}$: C, 68.60; H, 5.29. Found: C, 68.76; H, 5.39.

8-Methoxycarbonyloctyl *O*-β-D-Glucopyranosyl-(1→3)-*O*-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside (34). (*a*) *O*-Debenzoylation of **31** (1.31 g) followed by hydrogenolysis, as described for **21**, afforded **34** (0.47 g, 91%): mp 109 °C (from EtOH); $[\alpha]_D$ -31.1° (*c* 1.2, H_2O); ^{13}C NMR (D_2O) δ 179.7 (CO_2Me), 105.4 (2 C) and 104.5 (C-1a,b,c), 87.3 (C-3a), 63.35 (C-6b,c), 54.6 (CO_2CH_3), and 36.4, 31.4, 30.9, 27.7, and 27.0 [$OCH_2(CH_2)_7CO_2Me$].

Anal. Calcd for $C_{28}H_{50}O_{18}$: C, 49.85; H, 7.47. Found: C, 49.72; H, 7.58.

(*b*) *O*-Debenzoylation of **33** (0.36 g), as described for the preparation of **4**, gave **34** (0.13 g, 93%): mp and mmp 109-112 °C; the ^{13}C NMR spectrum was identical with that of the compound obtained in *a*.

8-Methoxycarbonyloctyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→3)-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→6)]-2,4-di-*O*-acetyl-β-D-glucopyranoside (35). Acetylation of **34** (0.15 g), as described for **26**, afforded **35** (0.23 g, 96%): mp 164-164.5 °C (from $CHCl_3$-hexane); $[\alpha]_D$ -32.8° (*c* 1.4, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 173.9 (CO_2Me), 170.3-168.5 (C=O), 100.8 (2 C) and 100.4 (C-1a,b,c), 61.8 (2C, 6-b,c), 51.3 (CO_2CH_3), and 34.0, 29.3, 29.1, 25.8, and 24.9 [$OCH_2(CH_2)_7CO_2Me$], and 20.8-20.5 ($COCH_3$).

Anal. Calcd for $C_{48}H_{70}O_{28}$: C, 52.65; H, 6.44. Found: C, 52.77; H, 6.32.

8-Hydrazinocarbonyloctyl *O*-β-D-Glucopyranosyl-(1→3)-*O*-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside (36). Compound **35** (102 mg) was treated with 80% $NH_2NH_2 \cdot H_2O$ (1 mL) in MeOH (6 mL) and processed, as described for the preparation of **28**, to give **36** (92 mg, 90%); mp 160-165 °C (from EtOH); $[\alpha]_D$ -23.3° (*c* 0.85, H_2O); ^{13}C NMR (D_2O) δ 178.25 [$O(CH_2)_8CONHNH_2$], and 105.4 (2 C) and 104.6 (C-1a,b,c),

Anal. Calcd for $C_{27}H_{50}N_2O_{17}$: C, 48.07; H, 7.47; N, 4.15. Found: C, 48.15; H, 7.57, N, 4.08.

8-Methoxycarbonyloctyl *O*-(2-*O*-Benzoyl-4-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-3-*O*-chloroacetyl-β-D-glucopyranosyl)-(1→3)-2,6-di-*O*-benzoyl-4-*O*-benzyl-β-D-glucopyranoside (38). The product obtained by condensation of **12** (1.22 g, 1.9 mmol) with **10** (1.79 g, 2.4 mmol), as described for the preparation of **11**, was subjected to column chromatography (PhMe-EtOAc, 30:1) to give **38** (2.09 g, 84%), $[\alpha]_D$ +33.2° (*c* 1.6, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 173.9 (CO_2Me), 166.1-164.3 (C=O), 100.7 (C-1'), 99.2 (C-1), 63.4 and 62.3 (C-6,6'), 51.3 (CO_2CH_3), 40.3 ($ClCH_2CO$), 34.0, 28.9, 26.9, 25.6, and 24.8 [$OCH_2(CH_2)_7CO_2Me$], 26.9 [$(CH_3)_3C$], and 19.3 [$(CH_3)_3C$].

Anal. Calcd for $C_{75}H_{83}ClO_{17}Si$: C, 68.24; H, 6.34. Found: C, 68.44; H, 6.25.

8-Methoxycarbonyloctyl *O*-(2-*O*-Benzoyl-4-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranosyl)-(1→3)-2,6-di-*O*-benzoyl-4-*O*-benzyl-β-D-glucopyranoside (39). *O*-Dechloroacetylation of **38** (1.89 g) as described for the preparation of **14**, followed by column chromatography (PhMe-EtOAc, 25:1) of the product, afforded **39** (1.64 g, 92%): $[\alpha]_D$ +20.5° (*c* 1.1, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 173.9 (CO_2Me), 166.5-166.4 (C=O), 100.8 (C-1'), 99.1 (C-1), 63.4 and 62.7 (C-6,6'), 51.3 (CO_2CH_3), 40.3 ($ClCH_2CO$), 34.0, 29.2, 28.9, 25.6, and 24.8 [$OCH_2(CH_2)_7CO_2Me$], 26.8 [$(CH_3)_3C$], and 19.2 [$(CH_3)_3C$].

Anal. Calcd for $C_{73}H_{82}O_{16}Si$: C, 70.51; H, 6.65. Found: C, 70.69; H, 6.57.

8-Methoxycarbonyloctyl *O*-(2-*O*-Benzoyl-4-*O*-benzyl-β-D-glucopyranosyl)-(1→3)-2,6-di-*O*-benzoyl-4-*O*-benzyl-β-D-glucopyrnoside (40). *O*-Desilylation of **39** (1.43 g) as described for **14**, followed by column chromatography (PhMe-EtOAc, 10:1) of the product, afforded **40** (1.04 g, 90%): $[\alpha]_D$ +6.0° (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 174.0 (CO_2Me), 166.2-166.2 (C=O), 100.9 (C-1'), 100.1 (C-1), 63.3 and 61.8 (C-6,6'), 51.25 (CO_2CH_3), and, 33.9, 29.2, 28.8, 25.6, and 24.8 [$OCH_2(CH_2)_7CO_2Me$].

Anal. Calcd for $C_{57}H_{64}O_{16}$: C, 68.11; H, 6.42. Found: C, 68.22; H, 6.50.

8-Methoxycarbonyloctyl *O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-*O*-[(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-*O*-(2-*O*-benzoyl-4-*O*-benzyl-β-D-glucopyranosyl)-(1→3)-2,6-di-*O*-benzoyl-4-*O*-benzyl-β-D-glucopyranoside (41). The product obtained by reaction of **40** (0.84 g, 835 μmol) with **30** (1.39 g, 2.2 mmol), as described for the preparation of **11**, was subjected to column chromatography (PhMe-EtOAc, 20:1) to give **41** (1.45 g, 80%): $[\alpha]_D$ +5.0° (*c* 1.4, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 174.0 (CO_2Me), 166.0-163.9 (C=O), 100.9 (2 C), 100.25, and 100.1 (C-1a,b,c,d), 63.3, 62.9, and 62.7 (C-6a,c,d), 51.3 (CO_2CH_3), and 34.0, 29.4, 29.0, 25.8, and 24.8 [$OCH_2(CH_2)_7CO_2Me$].

Anal. Calcd for $C_{125}H_{116}O_{34}$: C, 69.44; H, 5.41. Found: C, 69.32; H, 6.56.

Ethyl *O*-(2,3,4,6-Tetra-O-benzoyl-β-D-glucopyranosyl)-(1→3)-*O*-[(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-*O*-(2,4-di-*O*-benzoyl-β-D-glucopy-

ranosyl)-(1→3)-2,4,6-tri-*O*-benzoyl-1-thio-α-D-glucopyranoside (43). *O*-Deacetylation of **42** (0.98 g) and benzoylation, as described for **17**, followed by column chromatography (PhMe-EtOAc, 20:1→10:1, stepwise) of the product, gave **43** (1.48 g, 92%): $[\alpha]_D$ -15.0° (*c* 1.6, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 165.9-163.8 (C=O), 100.9 (2 C) and 100.7 (C-1b,c,d), 80.85 (C-1a), 63.0 (3 C, C-6a,c,d), and 24.3 and 14.7 (SCH_2CH_3).

Anal. Calcd for $C_{117}H_{98}O_{33}S$: C, 68.08; H, 4.79. Found: C, 68.22; H, 4.66.

8-Methoxycarbonyloctyl *O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-*O*-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-*O*-(2,4-di-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranoside (44). The product obtained by reaction of **43** (1.16 g, 562 μmol) with 8-methoxycarbonyloctyl-alcohol (0.16 g, 850 μmol), as described for the preparation of **11**, was subjected to column chromatography (PhMe-EtOAc, 15:1) to give **44** (1.05 g, 85%): $[\alpha]_D$ -39.6° (*c* 1.5, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 174.0 (CO_2Me), 165.9-163.8 (C=O), 100.8 (3 C) and 100.3 (C-1a,b,c,d), 63.3 (2 C) and 62.9 (C-6a,c,d), 51.3 (CO_2CH_3), and 34.0, 29.3, 28.9, 25.6, and 24.8 [$OCH_2(CH_2)_7CO_2Me$].

Anal. Calcd for $C_{125}H_{112}O_{36}$: C, 68.55; H, 5.15. Found: C, 68.62; H, 5.26.

8-Methoxycarbonyloctyl *O*-β-D-Glucopyranosyl)-(1→3)-*O*-[β-D-glucopyranosyl-(1→6)]-*O*-β-D-glucopyranosyl)-(1→3)-β-D-glucopyranoside (45). (*a*) *O*-Debenzoylation of **41** (1.06 g) followed by hydrogenolysis, as described for **21**, gave **45** (0.37 g, 90%): mp 188-191.5 °C (from EtOH), $[\alpha]_D$ -29.3° (*c* 1.5, H_2O); ^{13}C NMR (D_2O) δ 179.2 (CO_2Me), 105.1 (3 C) and 104.4 (C-1a,b,c,d), 88.3 and 86.7 (C-3a,b), 63.3 (3 C, C-6a,c,d), 54.5 (CO_2CH_3), and 36.3, 31.4, 31.0, 27.7, and 26.95 [$OCH_2(CH_2)_7CO_2Me$].

Anal. Calcd for $C_{34}H_{60}O_{23}$: C, 48.80; H, 7.23. Found: C, 48.72; H, 7.36.

(*b*) *O*-Debenzoylation of **44** (0.77 g), as described for the preparation of **4**, afforded **45** (0.27 g, 93%): mp and mmp 187.5-191.5 °C; the ^{13}C NMR spectrum was identical with that of the compound obtained in *a*.

Coupling of 26, 34, and 45 to BSA. The procedure used was essentially the same as that reported by Lönngren et al.[23] A solution of **26** (163 mg, 242 μmol) in 0.1 M aq NaOH (2 mL) was kept overnight at room temperature, at which time HPTLC showed complete disappearance of **26** (R_F 0.67) and the formation of a single product **29** (R_F 0.53). The solution was made neutral with 0.1 M aq HCl, and the pH was adjusted to 4.75 using 0.5 M aq HCl. To the solution was added with stirring a solution of BSA (50 mg; Sigma, A 7638) in H_2O (1 mL) followed, dropwise during 30 min at room temperature, by a solution of EDC (8.7 mg, 45 μmol) in H_2O (0.5 mL); the pH being maintained at 4.75 by addition of 0.5 M aq HCl. The mixture was stirred for 4 h, the reaction was quenched by addition of sodium acetate buffer (1 mL, pH 5.5), applied to a

column of Biogel P-6 (extra fine), and eluted with H_2O. Product-containing fractions were combined and concentrated. Lyophilization then gave the sugar-BSA conjugate (35 mg) having D.S. = 28.

In a similar way, coupling of **34** (98 mg, 145 μmol) with BSA (48 mg) via **37** and that of **45** (202 mg, 241 μmol) with BSA (49 mg) via **47** gave the sugar-BSA conjugates having D.S. = 21 and 30 in yields of 21 mg and 28 mg, respectively.

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REFERENCES AND NOTES

1. B. T. Stokke, A. Elgsaeter, C. Hara, S. Kitamura, and K. Takeo, *Biopolymers*, **33**, 561 (1993).

2. S. Kitamura, K. Hirano, T. Hori, B. T. Stokke, W. Itoh, K. Tabata, and K. Takeo, *Nippon Nogeikagaku Kaishi*, **66**, 305 (1992).

3. K. Takeo, M. Kawaguchi, and S. Kitamura, *J. Carbohydr. Chem.*, **12**, 1043 (1993).

4. R. L. Whistler, A. A. Bushway, P. P. Singh, W. Nakahara, and R. Tokuzen, *Adv. Carbohydr. Chem. Biochem.*, **32**, 235 (1976).

5. T. Yanaki, W. Itoh, K. Tabata, T. Kojima, T. Norisue, N. Takano, and H. Fujita, *Biophys. Chem.*, **17**, 337 (1983).

6. T. Kojima, K. Tabata, W. Itoh, and T. Yanaki, *Agric. Biol. Chem.*, **50**, 231 (1986).

7. R. U. Lemieux, D. R. Bundle, and D. A. Baker, *J. Am. Chem. Soc.*, **97**, 4067 (1975).

8. C. P. Stowell and Y. C. Lee, *Adv. Carbohydr. Chem. Biochem.*, **37**, 225 (1980).

9. K. Takeo, K. Maki, Y. Wada, and S. Kitamura, *Carbohydr. Res.*, **245**, 81 (1993).

10. P. J. Garegg, P. P. K. Konradsson, I. Kvarnström, T. Norberg, S. C. T. Svensson, and B. Wigilius, *Acta Chem. Scand., B*, **39**, 569 (1985).

11. P. Konradsson, U. E. Udodong, and B. Fraser-Reid, *Tetrahedron Lett.*, **31**, 4313 (1990).

12. M. Ek, P. J. Garegg, H. Hultberg, and S. Oscarson, *J. Carbohydr. Chem.*, **2**, 305 (1983).

13. K. Bock and C. Anderson, *Adv. Carbohydr. Chem. Biochem.*, **41**, 27 (1983).

14. S. Kim, H. Chang, and W. J. Kim., *J. Org. Chem.*, **50**, 1751 (1985).

15. M. Bertolini and C. P. J. Glaudemans, *Carbohydr. Res.*, **15**, 263 (1970).

16. E. A. Talley in *Methods Carbohydr. Chem.*, Vol. 2; R. L. Whistler and M. L. Wolfrom, Eds.; Academic Press: New York, 1963, p 337.

17. T. Ogawa and M. Matsui, *Carbohydr. Res.*, **54**, C17 (1977).

18. K. Takeo and S. Tei, *Carbohydr. Res.*, **145**, 293 (1986).

19. S. Hanessian and P. Lavallee, *Can. J. Chem.*, **53**, 2975 (1975).

20. P. Kovác, H. J. C. Yeh, and C. P. J. Glaudemans, *Carbohydr. Res.*, **140**, 277 (1985).

21. E. J. Corey and A. Venkateswarlu, *J. Am. Chem. Soc.*, **94**, 6190 (1972).

22. F. Weygand and H. Ziemann, *Liebigs Ann. Chem.*, 179 (1962).

23. J. Lönngren, I. J. Goldstein, and J. E. Niederhuber, *Arch. Biochem. Biophys.*, **175**, 661 (1976).

24. J. E. Hodge and B. T. Hofreiter in *Methods Carbohydr. Chem.*, Vol. 1; R. L. Whistler and M. L. Wolfrom, Eds.; Academic Press: New York, 1962, p 380.

25. Bio-rad Protein Assay (Dye Reagent Concentrate) was used for determination of the protein contents.

26. M. L. Wolfrom and A. Thompson in *Methods Carbohydr. Chem.*, Vol. 2; R. L. Whistler and M. L. Wolfrom, Eds.; Academic Press: New York, 1962, p 211.