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SYSTEMATIC CHEMICAL SYNTHESIS AND N.M.R. SPECTRA OF METHYL α-GLYCOSIDES OF ISOMALTO-OLIGOSACCHARIDES AND RELATED COMPOUNDS

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ABSTRACT

Acid-catalyzed thiophenolysis of 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose and acetylation of the resulting phenyl 2,3,4-tri-*O*-benzyl-1-thio-α-D-glucopyranoside (**4**) gave phenyl 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-1-thio-α-D-glucopyranoside (**5**). Reaction of **5** with chlorine gave, stereospecifically, the corresponding β-glycosyl chloride, which was treated with **4** in the presence of silver perchlorate and 2,4,6-trimethylpyridine to afford phenyl *O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-1-thio-α-D-glucopyranoside (**17**). Crystalline *O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl chloride, readily obtainable in a stereospecific manner from **17** by treatment with chlorine, was used as the key glycosyl (isomaltosyl) donor in the blockwise synthesis of methyl glycosides of isomalto-oligosaccharides, up to and including the octasaccharide. The methyl α-glycoside of isomaltotetraose fluorinated at C-6 of the terminal D-glucopyranosyl group was prepared by using $SnCl_2$-activated 2,3,4-tri-*O*-benzyl-6-deoxy-6-fluoro-α,β-D-glucopyranosyl fluoride as the glycosyl donor, a suitably protected methyl α-isomaltotrioside as the nucleophile, and silver perchlorate as the promoter. The n.m.r. spectra (^1H- and ^{13}C-) of numerous synthetic intermediates were analyzed and completely assigned by a variety of two-dimensional homo- and hetero-nuclear n.m.r.-spectroscopic techniques, and the final deprotected title oligosaccharides were characterized by ^{13}C-n.m.r. data. Silver perchlorate-mediated glycosylation reactions involving β-glycosyl chlorides were high-yielding and showed high stereoselectivity for the formation of an α-(*cis*)-glycosidic linkage. The practical limitation of obtaining high isomalto-oligosaccharides in this way appears to lie solely in the separation technique applied for the resolution of the crude products formed.

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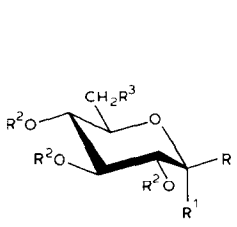
INTRODUCTION

This laboratory has extensively studied binding of antigens to carbohydrate-specific monoclonal antibodies[1–5]. Ongoing investigations involving dextran-specific antibodies require a large number of derivatives of methyl α-D-gluco-pyranoside. Among these, most important are the methyl α-glycosides of α-linked (1→6)-D-gluco-oligosaccharides (isomalto-oligosaccharides). Their specifically fluorinated analogs are important for evaluating the possible role of hydrogen bonding in the binding process. Although several approaches to the synthesis of isomaltose and its methyl glycosides have been described (*cf.*, ref. 6 and papers cited therein), only two chemical syntheses of higher oligosaccharides in this series have been reported[7,8]. Here, we describe an alternative approach to the chemical synthesis of methyl α-glycosides of isomalto-oligosaccharides, and a synthesis of the methyl α-glycoside of isomaltotetraoside fluorinated at C-6 of the terminal D-glucosyl group. Definitive and complete assignment of ^{1}H- and ^{13}C-n.m.r. spectra of key synthetic intermediates, as well as characterization of the final products by ^{13}C-n.m.r. spectroscopy, is also described.

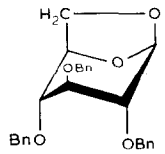
RESULTS AND DISCUSSION

Synthesis. — Blockwise synthesis of isomaltose, isomaltotetraose, and isomaltooctaose were described by Koto *et al.*[7], who used ethyl 2^1,3^1,4^1,2^2,3^2,4^2-hexa-*O*-benzyl-6^2-*O*-(*p*-nitrobenzoyl)-1^1-thio-α-isomaltoside as the key intermediate. Very small amounts of the final products were obtained (18, 4.5 and 1.8 mg of the di-, tetra-, and octa-saccharide, respectively). The most comprehensive work on the chemical synthesis of isomalto-oligosaccharides was conducted and described by Eby and Schuerch[8]. They used 2,3,4-tri-*O*-benzyl-6-*O*-(*N*-phenylcarbamoyl)-D-glucopyranosyl *p*-toluenesulfonate as the glycosyl donor and, in a stepwise manner, obtained fully protected α-glycosides of isomalto-oligosaccharides up to and including the hexasaccharide. They also prepared deprotected methyl α-isomalto-pentaoside, and reported the (unassigned) ^{13}C-n.m.r. spectrum of the crystalline material obtained after seeding the solution of their product with an authentic[9] sample of the independently synthesized compound. In their very carefully executed work, the authors[8] recognized the lack of stereospecificity in formation of the α-(*cis*) interglycosidic linkage (*cf.* ref. 7), and critically assessed the difficulties involved in the synthesis of higher (1→6)-linked D-gluco-oligosaccharides. It is a fact that, in addition to problems generally associated[8,10] with the syntheses of such α-(*cis*)-linked oligosaccharides, the preparation of higher α-(1→6)-linked D-gluco-oligosaccharides suffers from some specific problems. Two such complications are the poor crystallizing properties of the target molecules and the similar chromatographic properties of the α- and β-linked products, formed due to non-stereospecificity of the coupling reaction. Thus, because a stereo*specific* synthesis of an α-(*cis*)-glycosidic linkage has yet to be developed, the critical importance of careful verifi-



	R	R^1	R^2	R^3
1	H	Cl	Bn	OAc
2	SPh	H	Bn	OAc
3	SPh	H	Bn	OH
4	H	SPh	Bn	OH
5	H	SPh	Bn	OAc
6	Cl	H	Bn	OAc
7	H or F	F or H	Bn	F

Bn = $PhCH_2$



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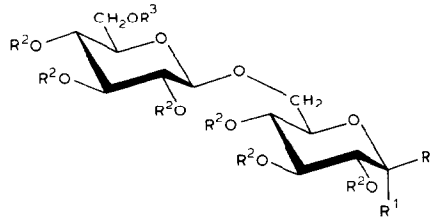
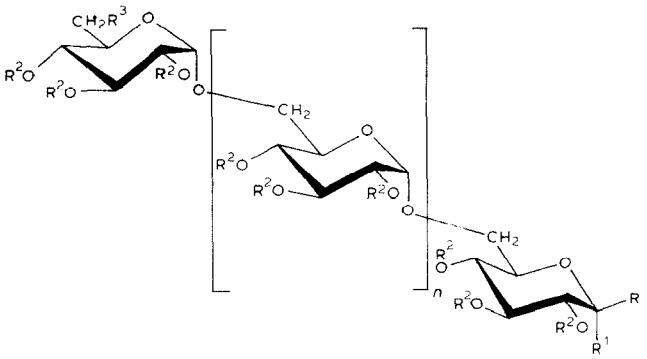


	R	R^1	R^2	R^3
9	SPh	H	Bn	Ac
10	SPh	H	Bn	H
11	H	SPh	Bn	H
12	H	SPh	Bn	Ac

cation of the optical purity of the products obtained cannot be overemphasized. In our effort to comply with this requirement, the desired α-linked (major) products were carefully examined by t.l.c., and ^{1}H- and ^{13}C-n.m.r. spectroscopy for the possible presence of by-products. Only material that showed optical purity, as established by these methods, was carried to the next synthetic step.

There is an unquestionable advantage in the blockwise synthesis of higher oligosaccharides, as compared to the stepwise assembly of an oligosaccharide chain. In the present work, we used chromatographically pure[6] 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl chloride (**1**) to synthesize the di- and the tri-saccharide of the title series in a stepwise manner, and the crystalline disaccharide building-block **19** to prepare higher oligosaccharides by a blockwise extension of an oligosaccharide chain. Methyl α-isomaltoside (**14**) was obtained by simple debenzylation of the crystalline derivative **13**, obtained[6] in connection with the synthesis of the corresponding 6^2-deoxy-6^2-fluoro derivative. To obtain methyl α-isomaltotrioside (**22**), compound **13** was condensed with the α-glycosyl halide **1**, to give the fully protected trisaccharide derivative **20**. Compound **20** was deacetylated, and the product debenzylated, giving the target trisaccharide **22**. The compound was characterized *via* the hitherto unknown, crystalline deca-*O*-(*p*-nitrobenzoyl) derivative **24**.

To synthesize higher oligosaccharides in a blockwise manner, a suitably protected derivative of isomaltose, to be used as a glycosyl (isomaltosyl) donor in the glycosylation reactions, had to be prepared. It appeared to us that a versatile compound for this purpose could be phenyl 6^2-*O*-acetyl-2^1,3^1,4^1,2^2,3^2,4^2-hexa-*O*-benzyl-1^1-thio-β-isomaltoside (**15**). 1-Thioglycosides of sugars can themselves be used as glycosyl donors[11–14], or they can be readily converted into glycosyl halides[15–17]. Compound **15** was previously obtained by Pfäffli *et al.*[18] as a component of a di-



	n	R	R^1	R^2	R^3
13	0	H	OMe	OBn	OH
14	0	H	OMe	H	OH
15	0	SPh	H	Bn	OAc
16	0	SPh	H	Bn	OH
17	0	H	SPh	Bn	OAc
18	0	H	SPh	Bn	OH
19	0	Cl	H	Bn	OAc
20	1	H	OMe	Bn	OAc
21	1	H	OMe	Bn	OH
22	1	H	OMe	H	OH
23	1	H	OMe	Bz	OBz
24	1	H	OMe	$COC_6H_4NO_2-p$	$O-COC_6H_4NO_2-p$
25	2	H	OMe	Bn	OAc
26	2	H	OMe	Bn	OH
27	2	H	OMe	H	OH
28	2	H	OMe	Bn	F
29	2	H	OMe	H	F
30	2	H	SPh	Bn	OAc
31	2	Cl	H	Bn	OAc
32	3	H	OMe	Bn	OAc
33	3	H	OMe	Bn	OH
34	3	H	OMe	H	OH
35	4	H	OMe	Bn	OAc
36	4	H	OMe	Bn	OH
37	4	H	OMe	H	OH
38	6	H	OMe	Bn	OAc
39	6	H	OMe	Bn	OH
40	6	H	OMe	H	OH

saccharide mixture, isolated in 62% yield, formed by condensation of phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (**3**) with the α-glycosyl chloride **1**. Although a preponderance of the α-linked product **15** in this mixture was implied[18] by the authors, no quantitative data could be obtained because **15** and the corresponding β-linked disaccharide **9** could not be separated by chromatography. Our preparation, obtained from the same synthons in 90% yield on employing a more powerful condensation technique[19], was homogeneous according to t.l.c. in various solvents and showed $[\alpha]_D$ +35°, a value close to that of the previously described material[18] ($[\alpha]_D$ +38.1°). Its 300-MHz, ^{1}H-n.m.r. spectrum, however, showed the presence of two COCH_3 signals (at δ 1.99 and 1.96, in the ratio of ~5:1) indicating the presence of two disaccharides. [Pfäffli *et al.*[18] reported the presence of only one COCH_3 signal (δ 1.98) in the ^{1}H-n.m.r. spectrum of their material. The spectrum was, however, recorded at 60 MHz, at which frequency the two COCH_3 signals may not have been resolved]. The lower-intensity signal was tentatively attributed to the COCH_3 of **9**, which was later confirmed, as will be shown. The formation of a relatively large proportion of the β-linked product **9** was unexpected, as we had previously[6,20] observed much better stereoselectivity of formation of the α-glycosidic linkage when **1** was used as the D-glucosyl donor and silver perchlorate as the promoter. In anticipation that, after partial deprotection, the α- and β-linked disaccharides **10** and **16** might be separable by chromatography, the mixture of **9** and **15** was deacetylated. Two products were formed (t.l.c.), and the mixture was resolved by column chromatography. The n.m.r. spectra of the individual compounds showed that the faster-migrating, isolated in 12% yield, was the β-linked product **10**, and that the main product was the disaccharide **16**. Acetylation of **10** and **16** gave the pure, fully protected compounds **9** and **15**, respectively.

In the search for a building block in the isomaltose series which would be equally versatile but easier to isolate in the pure state, a reaction pathway to the disaccharide 1-thio-α-glycoside **17** was explored. 1,6-Anhydro-β-D-glucopyranose[21] was benzylated[22], and the corresponding tri-*O*-benzyl derivative[23] **8** was mercaptolyzed with thiophenol under acid catalysis. Of the several acid catalysts tried [reactions involving zinc chloride[7], boron trifluoride etherate, sulfuric acid, hydrogen chloride, tetrafluoroboric acid, diethylaluminum chloride, and tin(IV) chloride are not described in the Experimental section], the best results were obtained when anhydrous *p*-toluenesulfonic acid was employed. The main product of this conversion, crystalline phenyl 2,3,4-tri-*O*-benzyl-1-thio-α-D-glucopyranoside (**4**), was obtained in a yield of 61% (*cf.* 40% yield of the corresponding ethyl α-glycoside obtained[7] in a similar conversion). Acetylation of **4** with acetic anhydride–pyridine readily gave the crystalline 6-*O*-acetyl derivative **5**.

Next, the glycosyl halide-generating reactions of phenyl 1-thioglycosides **2** and **5** with chlorine were explored. The preparative utility of the reaction of chlorine with 1-thioglycosides of sugars was demonstrated by Wolfrom *et al.*[16,24,25]. On the other hand, the reaction of bromine with ethyl 1-thioglycosides of 2,3,4,6-tetra-*O*-benzyl-α- and β-D-glucopyranose in ether was studied by Weygand and

Ziemann[15]. Based on optical rotation data, they concluded[15] that, while the 1-thio-β-glycoside reacts with an equimolar amount or an excess (2 molar equivalents) of bromine with complete inversion of configuration, to give 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl bromide, the corresponding 1-thio-α-glycoside reacts in a more-complex manner. First, with the inversion of configuration, the thermodynamically less stable 2,3,4,6-tetra-*O*-benzyl β-D-glucopyranosyl bromide was formed. It anomerized slowly, but completely, within ~30 min, to the thermodynamically more stable α-bromide. We have treated phenyl 1-thio-α- (**5**) and -β- (**2**) glycosides in CCl_4 with 2 molar equivalents of chlorine, and monitored the course of the reaction by ^{1}H-n.m.r. spectroscopy. The intensities of the well separated, downfield signals of H-1 of the formed glycosyl chlorides **1** (ref. 6; δ 6.02, $J_{1,2}$ 3.6 Hz) and **6** (δ 5.20, $J_{1,2}$ 7.8 Hz; *cf.* Experimental) were diagnostic for the composition of the reaction mixture. The reaction of the thio-α-glycoside **5** under these conditions was very fast. The ^{1}H-n.m.r. spectrum 1 min after the addition of a solution of chlorine in carbon tetrachloride showed complete conversion of **5** into the β-glycosyl chloride **6**. The composition of the reaction mixture remained unchanged (n.m.r., ambient temperature) for a period of 24 h. Also, when the reaction mixture was then concentrated at 40°/133 Pa, to remove organic solvents and the non-carbohydrate by-products, and the residue was dissolved in $CDCl_3$, a clean spectrum of **6** was recorded. Its stock solution in anhydrous ether could be kept for several weeks at 0° without noticeable change (n.m.r.). Even in the presence of 2,4,6-trimethylpyridine, the anomerization **6**→**1** was very slow; noticeable anomerization was not observed (n.m.r.) within ~15 min, and, after 24 h at room temperature, the mixture contained glycosyl chlorides **1** and **6** in the ratio of ~1:1. On the other hand, when the thio-β-glycoside **2** was treated with chlorine under the conditions of the conversion **5**→**6**, glycosyl chlorides **1** and **6** were formed practically instantaneously (^{1}H-n.m.r.), and in the ratio of ~1:2. At room temperature, this ratio remained unchanged for a period of 24 h. When treated with the phenyl 1-thio-β-glycoside **3**, under the conditions of the conversion **1** + **3**→**9** + **15**, the β-glycosyl chloride **6** gave a practically identical mixture of **9** and **15**. This confirmed the observation made by Igarashi *et al.*[19,26] that the same reactive intermediate is formed in the glycosylations mediated by silver perchlorate, regardless of the configuration of the starting glycosyl chloride.

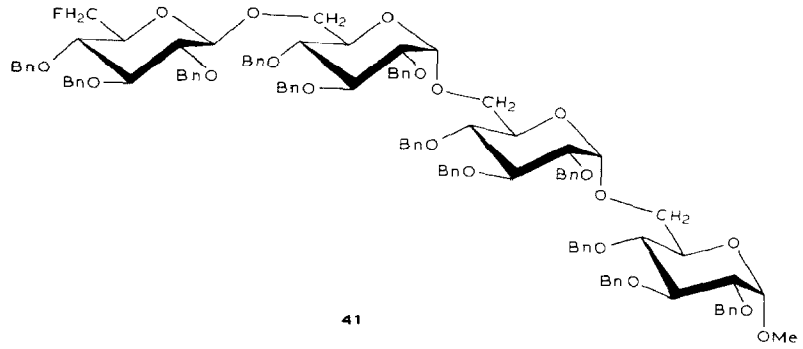
The condensation of the phenyl 1-thio-α-glycoside **4** was performed separately with both α- (**1**) and β- (**6**) glycosyl chloride. The two reactions gave, again, practically identical results. However, in contrast to the case of the crude product originating from the condensation of the thio-β-glycoside **3**, examination of these reaction mixtures by t.l.c. revealed that the disaccharide zone consisted of two compounds, one strongly preponderating. Although some of the α-linked disaccharide **17** could be isolated in the pure state by resolution of the crude product by chromatography, most of the material was eluted from the column in admixture with the very slightly slower-moving β-linked product **12**. For large-scale preparation, it was more advantageous to separate the α- and β-linked disaccharides

(formed by the condensation of either **1** or **6** with **4**) after deacetylation. More importantly, compounds **11** and **18** differed markedly in chromatographic mobility and, thus, their clean separation was readily achieved. Also, both disaccharides (**11** and **18**) were obtained crystalline; this was particularly important in the case of **18**, as it further assured the optical purity of the disaccharide **17** and, consequently, of the key disaccharide building-block **19**.

Monitoring by ^{1}H-n.m.r. spectroscopy of the reaction of the thio-α-glycoside **17** with an excess of chlorine showed that, as in the case of the conversion of the analogous monosaccharide derivative **5**, the formation of the corresponding β-glycosyl halide **19** was practically instantaneous, as indicated by the complete disappearance of the doublet for H-1^1 of **17** at δ 5.53. Noticeable anomerization was not observed during 24 h at room temperature. Compound **19** readily crystallized, and it constituted thus a convenient, pivotal disaccharide building-block for making isomalto-oligosaccharides and related compounds, allowing further extension at O-6 of the terminal α-D-glucosyl group. Accordingly, condensation of **19** with the nucleophiles **13** and **21** gave, respectively, the isomaltotetraose (**25**) and isomaltopentaose (**32**) derivatives. Similarly, condensation of the tetrasaccharide nucleophile **26**, readily obtained from **25** by deacetylation, gave methyl 6^6-*O*-acetyl octadeca-*O*-benzyl-α-isomaltohexaoside (**35**). The target methyl α-glycosides of isomaltooligosaccharides **14, 22, 27, 34,** and **37** were obtained by catalytic hydrogenolysis of their precursors **13, 21, 26, 33,** and **36,** respectively. To prepare the highest oligosaccharide in this series, namely, the methyl α-isomalto-octaoside (**40**), the 1-thio-α-glycoside **18** was first condensed with the versatile glycosyl donor **19**, to give the 1^1-thioisomaltotetraose derivative **30**. Its reaction with chlorine produced the tetrasaccharide glycosyl β-chloride **31**, which was then allowed to react with the tetrasaccharide nucleophile **26**. The octasaccharide derivative **38** formed was deacetylated, and the product debenzylated, to give methyl α-isomalto-octaoside (**40**). Compound **29**, the fluorinated analog of **27**, was prepared by condensation[27] of 2,3,4-tri-*O*-benzyl-6-deoxy-6-fluoro-α,β-D-glucopyranosyl fluoride[28] (**7**) with the trisaccharide nucleophile **21**, followed by catalytic hydrogenolysis of the benzyl groups from the fully protected, tetrasaccharide derivative **28** formed.

N.m.r. spectroscopy. — Complete ^{1}H- and ^{13}C-n.m.r. chemical-shift assignments for compounds listed in Tables I–III were made by a combination of several two-dimensional methods, namely, conventional, homonuclear chemical-shift correlation (COSY)[29] or double quantum-filtered COSY[30], one-bond ^{1}H–^{13}C-chemical-shift correlation[31], and relayed ^{1}H–^{13}C-chemical-shift correlation[32]. For convenience, the reverse-detected versions of the last two methods were used, although sample concentration would have sufficed for direct observation of ^{13}C nuclei.

It should be noted that the assignments given in Tables I–III were obtained independently for each compound, and not by comparison of the spectra of similar compounds. To make unambiguous assignments, it was usually necessary to identify remotely bonded, ^{1}H–^{13}C pairs by relaying magnetization from a ^{13}C



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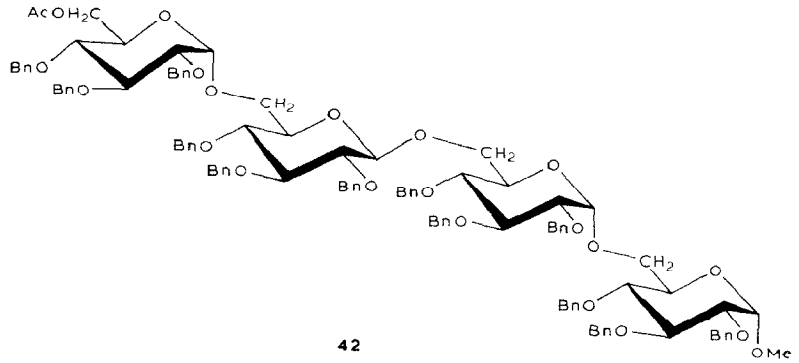

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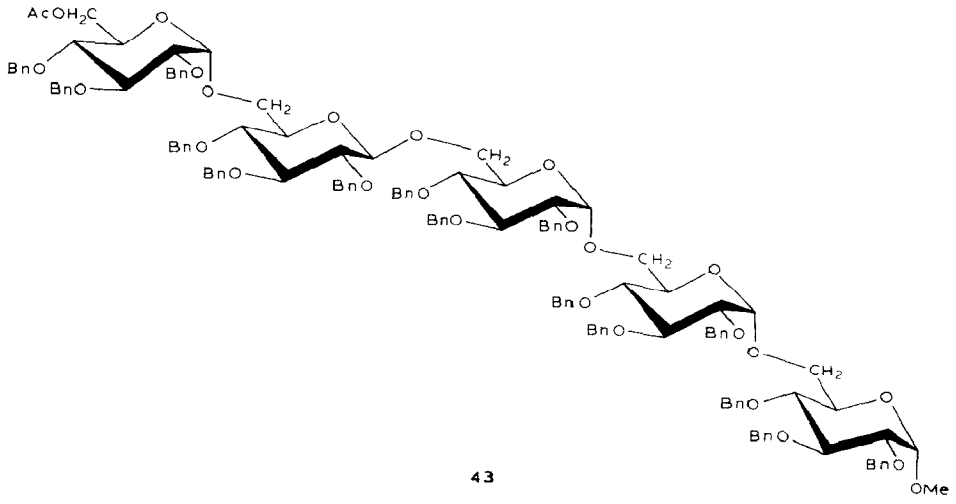

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TABLE I

^{1}H-N.M.R. CHEMICAL SHIFTS (δ)[a]

Compound	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6a*	*H-6b*	$COCH_3$
2	4.66	3.48	3.77	3.53	3.52	4.37	4.22	2.15
3	4.83	3.60	3.85	3.70	3.50	3.97	3.82	
4	5.58	3.84	3.93	3.56	4.20	3.70	3.70	
5	5.60	3.88	3.94	3.50	4.42	4.29	4.18	1.94
9	4.77	3.46	3.71	3.47	3.60	4.14	3.69	
	4.40	3.46	3.62	3.53	3.42	4.33	4.18	1.96
10	4.68	3.46	3.69	3.54	3.60	4.10	3.78	
	4.44	3.44	3.64	3.59	3.35	3.86	3.71	
15	4.64	3.25	3.66	3.73	3.47	3.84	3.77	
	5.03	3.55	4.00	3.49	3.89	4.25	4.21	2.00
16	4.63	3.26	3.65	3.69	3.48	3.84	3.76	
	5.00	3.52	3.99	3.54	3.74	3.76	3.65	
17	5.53	3.76	3.90	3.69	4.39	3.87	3.62	
	4.85	3.48	3.97	3.46	3.82	4.21	4.17	2.00
19	5.15	3.35	3.62	3.85	3.50	3.85	3.85	
	5.09	3.58	4.01	3.50	3.85	4.26	4.21	1.96
23[b]	5.11	5.05	6.08	5.62	4.20	3.94	3.66	
	5.27	5.01	6.16	5.62	4.32	3.96	3.58	
	5.37	5.34	6.30	5.68	4.50	4.50	4.35	

[a]Data in the 1st row of each entry refer to sugar residue 1; data in the 2nd and 3rd row, if present, refer to sugar residues 2 and 3, respectively. [b]The signal of OCH_3 appears at 3.42 p.p.m.

TABLE II

^{1}H–^{1}H COUPLING CONSTANTS[a,b]

Compound	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{6a,6b}$ *(Hz)*
2	9.8	8.2	8.2	[d]	4.1	<1	11.8
3	9.7	9.2	9.4	9.4	4.6	5.0	11.7
4	5.3	9.4	9.4	9.9	3.4	3.4	[c]
5	4.9	9.7	8.2	10.0	5.1	2.3	11.9
9	8.4	[d]	[d]	[d]	1.4	4.8	11.0
	7.8	[d]	9.3	9.3	2.0	4.8	11.9
10[e]	10.0	9.3	8.6	8.6	<1	5.5	11.9
	8.3	8.8	8.9	9.2	<1	4.0	11.3
15	9.9	9.2	9.2	9.4	4.4	1.3	11.8
	3.5	9.4	9.4	10.0	3.8	2.4	12.0
16	9.3	9.3	[d]	[d]	4.4	[d]	11.9
	3.3	9.3	9.3	9.7	<1	3.0	[d]
17	5.4	9.3	9.5	9.5	4.0	<1	11.0
	3.4	9.2	9.4	9.4	4.1	2.0	12.0
19	8.7	8.9	9.0	[d]	[d]	[d]	[d]
	3.5	9.4	9.2	[d]	3.9	2.2	12.0
23	3.3	10.0	10.0	9.9	5.3	<1	11.4
	3.4	9.8	9.9	10.0	[d]	<1	11.3
	3.5	9.8	9.8	9.9	[d]	[d]	[d]

[a]Data determined by 1st-order analysis of spectra. [b]Data in the 1st row of each entry refer to sugar residue 1; data in the 2nd and 3rd row, if present, refer to sugar residues 2 and 3, respectively. [c]First-order coupling constant not observed. [d]Not determined due to overlap of signals. [e]Determined by z-filtered, MLEV pulse sequence[33].

TABLE III

^{13}C-N.M.R. CHEMICAL SHIFTS (δ)[a]

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*COCH$_3$*
2[b]	87.46	80.92	86.88	76.90	77.54	63.26	20.79
3[c]	87.59	81.21	86.60	77.67	79.38	62.18	
4[d]	86.93	79.96	82.39	77.20	71.88	61.84	
5[e]	86.76	79.73	82.41	77.19	69.58	63.09	20.76
9[f]	87.39	80.98	86.70	78.13	78.90	68.88	
	103.81	82.17	84.65	77.45	72.88	63.12	20.80
10[g]	87.91	80.98	86.74	77.88	78.95	68.91	
	103.85	82.33	84.49	77.72	75.00	62.13	
15[h]	88.17	81.20	86.66	77.46	78.87	66.21	
	97.24	80.18	81.66	77.31	68.80	63.05	20.81
16[i]	88.04	81.09	86.60	77.43	78.90	66.10	
	97.24	80.24	81.62	77.43	70.90	61.85	
17[j]	87.24	80.10	82.48	77.51	71.52	66.57	
	97.34	79.94	81.65	77.26	68.73	62.99	
19[k]	90.44	84.61	85.15	76.67	77.10	65.34	
	97.17	80.01	81.48	78.57	68.76	62.96	20.79
23[l]	96.82	72.17	70.68	69.03[m]	68.73	66.00	
	95.96	71.94	70.68	68.92[m]	68.73	65.83	
	96.17	71.89	70.60	69.44	68.01	62.83	

[a]Assignments are based on 2 D methods, as described in the text; actual chemical shifts were obtained from 1 D ^{13}C-spectra (75 MHz), recorded with a digital resolution of 1 Hz; data in the 1st row of each entry refer to sugar residue 1; data in the 2nd and 3rd row, if present, refer to sugar residues 2 and 3, respectively. [b]CH_2Ph: 75.80, 75.43, 75.04. [c]CH_2Ph: 75.79, 75.52, 75.10. [d]CH_2Ph: 75.73, 75.09, 72.63. [e]CH_2Ph: 75.80, 75.07, 72.51. [f]CH_2Ph: 75.71 (2 C), 75.42, 74.98, 74.91, 74.82. [g]CH_2Ph: 75.72, 75.65, 75.41, 75.16, 74.93, 74.88. [h]CH_2Ph: 75.63 (2 C), 75.46, 74.96, 74.91, 72.37. [i]CH_2Ph: 75.60 (2 C), 75.46, 74.96, 74.92, 72.41. [j]CH_2Ph: 75.73, 75.61, 75.01 (2 C), 72.45 (2 C), 71.52, [k]CH_2Ph: 75.60 (2 C), 75.53, 75.03, 74.91, 72.14. [l]The signal of OCH_3 appears at 55.41 p.p.m. [m]The assignments may have to be reversed.

nucleus to its directly attached proton, and then, *via* homonuclear Hartmann–Hahn[32] transfer, to a vicinal proton.

The spectra for the disaccharide **9**, obtained at 500 MHz, will be used to illustrate the assignment strategy and the usefulness of the described methods to deal with spectra that cannot otherwise be analyzed because of extreme overlap of signals. Fig. 1 shows a region of the conventional COSY spectrum of **9**; Fig. 2 shows the chemical-shift correlation map for directly bonded ^{1}H–^{13}C pairs. Some protons, such as H-1^1, H-1^2, H-2^1, H-2^2, H-6^1, and H-6^2, could be assigned directly from the COSY spectrum, but the overlap of proton resonances in the region between 3.4 and 3.8 p.p.m. precluded assignment based only on homonuclear correlation. By itself, the directly bonded ^{1}H–^{13}C map does not provide additional assignments. However, in conjunction with the relay map (see Fig. 3), it can be used to "disentangle" the overlapped region. For example, C-1^1 is readily identified in the directly bonded map, which then marks H-3^1 and H-2^1 in the relay map. For complete assignment, it is necessary to go back and forth between the direct and the relay map until the assignments are complete and consistent.

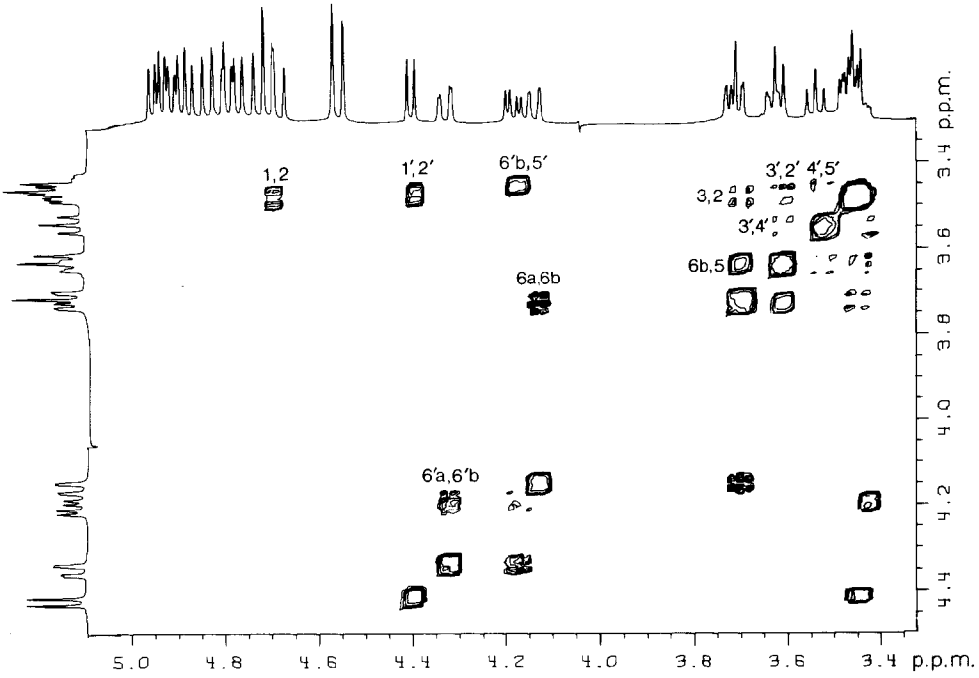

Fig. 1. 500-MHz, ^{1}H-n.m.r. COSY spectrum of compound **9** plotted in the absolute value mode. Sixteen scans per t_1 value were collected, with a delay of 750 ms between scans. The spectral window was ±1302 Hz in both dimensions. The data were collected in a 512 × 1024 matrix, and multiplied by a sine-bell function in both dimensions. The corresponding regions of the one-dimensional, ^{1}H-n.m.r. spectrum are plotted along the axes. Unprimed numbers designate positions in the D-glucose residue, and primed numbers are used for the D-glucosyl group.

The unambiguous assignment of the ^{1}H-n.m.r. spectrum of **19** was critical for establishing the configuration of the glycosyl halide in question. The two anomeric protons (H-1^1 and H-1^2) appear in the one-dimensional, ^{1}H-n.m.r. spectrum as well separated doublets at chemical shifts of 5.15 ($J_{1^1,2^1}$ 8.7 Hz) and 5.09 p.p.m. ($J_{1^2,2^2}$ 3.5 Hz), respectively. The large value of $J_{1^1,2^1}$, typical for diaxial protons, clearly identifies compound **19** as a β-glycosyl chloride. Essentially the same values are observed for H-1 in the spectrum of **6**.

^{13}C-N.m.r. data for higher oligosaccharides, and some other compounds, are listed in the text and in the Experimental section, as needed. These assignments were arrived at by comparison of spectra of related compounds. Taken into consideration were close similarities of chemical shifts for equivalent carbon atoms in compounds belonging to the same homologous series. The individual assignments were made in a manner similar to that described in detail[34].

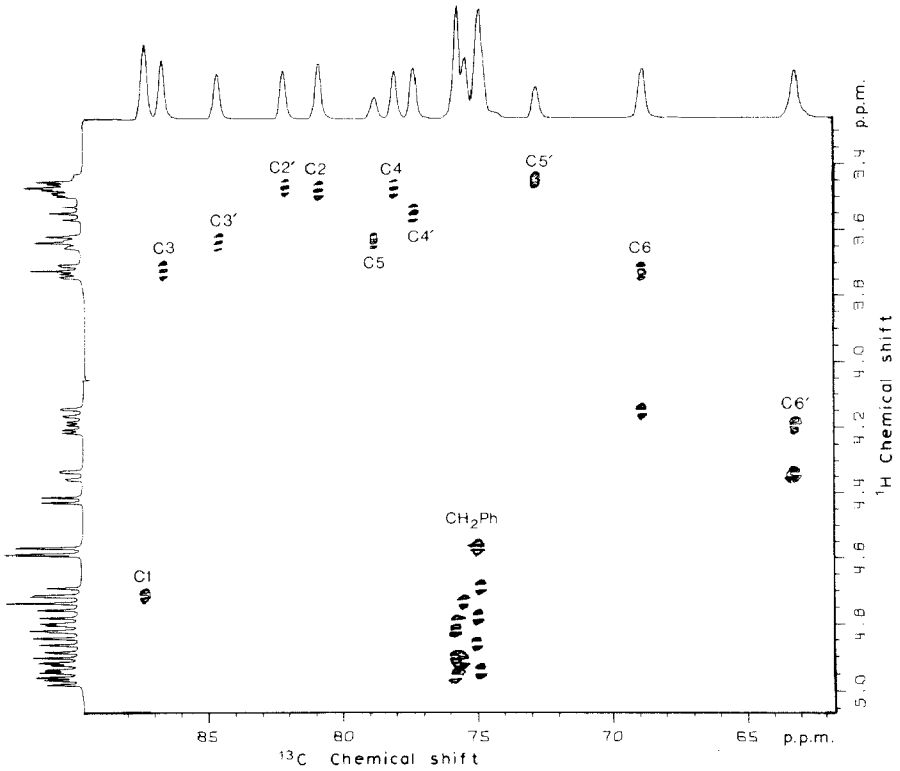

Fig. 2. Region of the absorption-mode chemical-shift correlation map for directly bonded ^1H–^{13}C pairs in compound **9**, obtained at 500 MHz by using the pulse sequence described in ref. 31. The delay between acquisitions was 730 ms. The frequency of the decoupler was 125.76 MHz. The data matrix was 2 × 256 × 1024; 64 scans per t_1 value were collected. The spectral window was ±3628 Hz in the F_1 (^{13}C) dimension, and ±1302 Hz in the F_2 (^1H) dimension. 25- and 2-Hz Gaussian line-broadenings were used in the t_1 and t_2 dimensions, respectively. The 0° projection of the spectrum is plotted along the horizontal (F_1) axis; the corresponding region of the one-dimensional ^1H-n.m.r. spectrum is plotted along the vertical (F_2) axis. The numbering of positions is as in Fig. 1.

CONCLUSIONS

We have shown that methyl α-glycosides of (1→6)-α-D-gluco-oligosaccharides can be efficiently synthesized by using, as glycosyl donors, β-glycosyl chlorides of mono- or oligo-saccharides bearing at O-2 a group incapable of anchimeric assistance. The latter compounds can be readily obtained from the corresponding phenyl 1-thio-α-glycosides, whose reaction with chlorine in carbon tetrachloride is fast, and simple to perform. The β-glycosyl chlorides formed are very reactive, and yet stable enough to allow convenient isolation, handling, and storage. The stereo-*specificity* in the formation of β-glycosyl chlorides from 1-thio-α-glycosides, although not important for the stereochemical outcome of the subsequent glycosylation, is advantageous when a crystalline glycosyl chloride is formed (*e.g.*, **19**), which can then be isolated pure in high yield. Preparation of glycosyl chlorides for oligo-

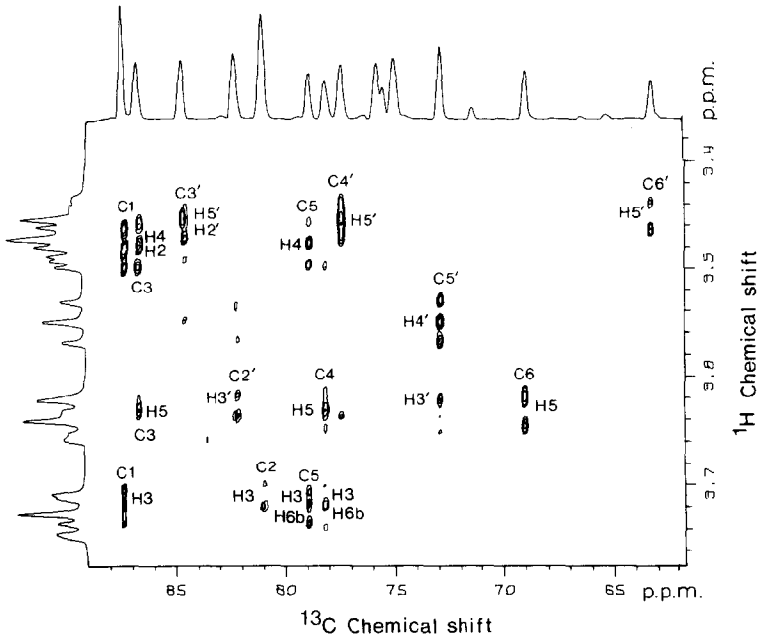

Fig. 3. Region of the absorption-mode chemical-shift correlation map for relayed connectivities between remotely bonded ^1H–^{13}C pairs of compound **9**, obtained at 500 MHz by using the pulse sequence incorporating MLEV17 described in ref. 32. Data matrix size, acquisition delay, spectral windows, and line-broadenings were the same as for Fig. 2. An MLEV17 mixing time of 33 ms flanked by 1 ms trim pulses was used to relay magnetization between protons. The numbering of positions is as in Fig. 1.

saccharide synthesis in this way has a special advantage when glycosyl donors are to be generated from higher oligosaccharides. Although cleavage of substituents at the anomeric position of sugars by means of 1,1-dihalogenomethyl methyl ethers[35] or boron trichloride[36], to generate glycosyl chlorides, can be done chemo*selectively*, side reactions do occur with these procedures. For example, we have found[37] to be critical the proportion of Lewis acid employed to catalyze the conversion of a tetrasaccharide into the corresponding glycosyl chloride by use of 1,1-dichloromethyl methyl ether–$ZnCl_2$ as the chlorinating reagent. On the contrary, no cleavage of the interglycosidic linkages, or any other side reaction, could be detected by n.m.r. spectroscopy during the reaction of the tetrasaccharide 1-thio-α-glycoside **30** with an excess of chlorine, or during subsequent isolation of the corresponding β-glycosyl chloride **31**. The overall strategy applied here for the synthesis of 1,2-α-(*cis*)-linked oligosaccharides, namely, the conversion of a 1-thioglycoside into a glycosyl *chloride*, and use of the latter as a glycosyl donor, appears to be more effective than other means of activation of the anomeric center of 1-thioglycosides. Glycosylations mediated with methyl(methylthio)sulfonium triflate[13,38] or with $CuBr_2$–Bu_4NBr (ref. 39), in conjunction with 1-thioglycosides, do not seem to give good 1,2-α-(*cis*) stereoselectivity, or tend to be sluggish. The approach[40] comprising

the conversion of a disaccharide 1-thioglycoside into the corresponding glycosyl *bromide* resulted in a noticeably low yield (33%) of the trisaccharide produced, presumably due to side reactions of the much too reactive ("unstable") glycosyl bromide.

EXPERIMENTAL

General methods. — Melting points were determined on a Kofler hot stage. Unless otherwise stated, optical rotations were measured at 25° for solutions in chloroform with a Perkin–Elmer automatic polarimeter, Model 241 MC. Preparative chromatography was performed by gradient elution from columns of Silica Gel 60 (Merck, Cat. Nos. 9385 and 15111). Thin-layer chromatography (t.l.c.) was performed on glass slides (Analtech or Whatman) coated with silica gel. Chromatograms were developed with solvent mixtures of appropriately adjusted polarity, consisting of A, toluene–acetone; B, carbon tetrachloride–acetone; C, ethyl acetate–1-propanol–water; D, hexane–ethyl acetate; E, toluene–ethyl acetate; and F, 1-propanol–acetone–water. Detection was effected by charring with 5% (v/v) sulfuric acid in ethanol and, where applicable, by u.v. light. Reactions requiring anhydrous conditions were performed under argon, using common laboratory glassware, and solvents and reagents were handled with Hamilton Series 1000 gas-tight syringes. Compound **2** (m.p. 69–71°; lit.[18] m.p. 69–73°) and compound **3** (m.p. 124–125°; lit.[18] m.p. 121.5–122°) were prepared as described[18]. The solution of chlorine in carbon tetrachloride (0.15 g/mL, determined by weighing) was obtained by passing chlorine gas through dry carbon tetrachloride at 0°. The solution was stored at 5° in a dark-colored, screw-capped, glass container. Palladium-on-charcoal (5%) catalyst was a product of Engelhardt Industries. To obtain anhydrous stannous chloride, the commercial product (Aldrich Chemical Co.) was dried for 8 h at 190°/133 Pa. Powdered 4 A molecular sieves (Fluka Chemical Company) were activated by heating for 8 h at 150°/133 Pa. Organic solvents were purified as described by Perrin *et al.*[41]. Solutions in organic solvents were dried with anhydrous sodium sulfate, and concentrated at 40°/2 kPa. One-dimensional, ^{13}C- and ^{1}H-n.m.r. spectra were routinely recorded at 25° with a Varian XL-300 spectrometer. Solvents are listed as necessary. In these cases, proton-signal assignments were made by first-order analysis of the spectra, supported by selective homonuclear decoupling experiments; ^{13}C-signal assignments were made by mutual comparison of the spectra and by comparison of the spectra with those of related compounds[6]. The signals of secondary carbon atoms were assigned by DEPT experiments. For the complete assignments listed in Tables I–III, two-dimensional proton–proton and proton–^{13}C correlation n.m.r. spectra were recorded at 26° for solutions in $CDCl_3$ at either 270 or 500 MHz (^{1}H frequencies) on modified Nicolet NT-270 and NT-500 spectrometers, equipped with a 5-mm probe designed for observing ^{1}H signals while decoupling heteronuclei (Cryomagnet Systems, Inc., Indianapolis, Indiana). Typical 90° pulse-widths were 30 μs for ^{1}H at 270.07 MHz

and 500.09 MHz, and 100 μs for ^{13}C at 67.89 and 125.76 MHz. Acquisition and data-processing parameters are listed in the Figure captions. For solutions in $CDCl_3$, ^{13}C-n.m.r. chemical-shifts are reported relative to Me_4Si as an internal standard, and for solutions in D_2O, methanol (δ_C 49.0) was the internal standard. The superscripts used in reporting the n.m.r. data for oligosaccharides denote the D-glucosyl group or residue, or D-glucose residue, containing the designated proton or carbon atom. These are serially numbered, beginning with the reducing residue. For example, C-1^2 refers to C-1 of the second D-glucose unit. Desorptive c.i. mass spectra, obtained with ammonia as the reagent gas, were recorded with a Finnigan 4500 spectrometer.

Silver perchlorate. — The promoter used throughout this work was prepared by a simplification of previously described procedures[42,43]. Perchloric acid (70%) was added with stirring at 100° to a suspension of silver carbonate (22 g) in water until the effervescence ceased. After filtration through a medium-porosity sintered-glass funnel, to remove a small amount of insoluble material, the filtrate was concentrated to dryness, and the residue was transferred, with the aid of the minimal volume of benzene (two layers may form) to a beaker. After addition of pentane, the precipitate resulting was collected on a coarse sintered-glass funnel and washed with pentane. It was quickly transferred to a storage container, and dried for 16 h at 105°/133 Pa. The lumps were broken up with a stainless-steel spatula*, and the product (a white powder) was finally dried for 2 h at 160°/133 Pa; yield, 30 g (90%). During these operations, protection against direct light is not necessary.

1,6-Anhydro-2,3,4-tri-O-benzyl-β-D-glucopyranose (**8**). — Benzyl chloride (100 mL) was slowly added, with stirring, to a suspension of 1,6-anhydro-β-D-glucopyranose[21] (25 g) and powdered potassium hydroxide (80 g) in Me_2SO (625 mL). After stirring for 2 h at room temperature, t.l.c. (solvent *B*) showed that the reaction was complete. The mixture was partitioned between dichloromethane and water, the organic phase was dried, and concentrated at 70°/133 Pa, and the residue crystallized from ethanol. Recrystallization from the same solvent gave pure **8** (51.3 g, 77%); m.p. 91–92°, lit.[23] m.p. 90°.

Methyl O-α-D-glucopyranosyl-(1→6)-α-D-glucopyranoside (methyl α-isomaltoside; **14**). — A solution of crystalline[6] **13** (0.7 g) in 2-methoxyethanol (70 mL) was stirred in a hydrogen atmosphere, in the presence of 5% palladium-on-charcoal catalyst (0.3 g), until the uptake of hydrogen ceased (~3 h). The suspension was filtered through a layer of Celite, and the solids were washed with 50% methanol (3 × 50 mL). The filtrate and washings were combined and concentrated, the residue was eluted from a column of silica gel (solvent *C*), and the appropriate fractions were combined and concentrated. A solution of the residue in aqueous methanol was centrifuged for 15 min at 10,000 r.p.m., the clear solution was concentrated, and

*__Caution!__ Although no difficulties have been experienced in this laboratory on at least 15 different occasions, silver perchlorate has been reported[42] to detonate spontaneously. Therefore, this operation should be carried out behind a safety shield.

the concentrate freeze-dried, to give compound **14** as an amorphous, hygroscopic solid (0.21 g, 78%); $[\alpha]_D$ +165° (*c* 0.6, H_2O); lit.[9] $[\alpha]_D$ +177.4°; ^{13}C-n.m.r. (75 MHz, D_2O): δ 99.50 (C-1^1), 98.00 (C-1^2), 73.52 (C-3^1), 73.23 (C-3^2), 71.85 (C-5^2), 71.64 (C-2^2), 71.33 (C-2^1), 70.19 (C-5^1), 69.69 (C-4^1), 69.58 (C-4^2), 65.63 (C-6^1), 60.64 (C-6^2), and 55.26 (OCH_3).

Methyl O-*(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-*D*-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzyl-α-*D*-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-*D*-glucopyranoside* (**20**). — A solution of silver perchlorate (1.035 g, 5 mmol) in ether (85 mL) was slowly added, with stirring, at −30°, to a solution of the nucleophile **13** (3.14 g, 3.5 mmol), glycosyl donor **1** (2.14 g, 4.2 mmol), and 2,4,6-trimethylpyridine (0.65 mL, 4.9 mmol) in ether (35 mL). Cooling was terminated and, after 15 min, t.l.c. (solvent *A*) showed that all of the **1** had reacted, and that very little of the **13** remained. After filtration, the solid was washed with ether, and the ethereal solutions were combined, washed with aqueous sodium thiosulfate solution, dried, and concentrated. The residue was chromatographed, to give the major product **20** (4.56 g, 95%) as a glassy solid; $[\alpha]_D$ +80° (*c* 1); ^{13}C-n.m.r. (75 MHz, $CDCl_3$): δ 98.02 (C-1^1), 97.08, 96.97 (C-1^2,1^3), 82.12 (C-3^1), 81.62 (2 C, C-3^2,3^3), 80.31, 80.22, 80.11 (3 C, C-2^1,2^2,2^3), 77.78, 77.57, 77.21, (3 C, C-4^1,4^2,4^3), 75.67, 75.54, 75.44, 75.00, 74.91 (2 C), 73.37, 72.37, 72.22 (CH_2Ph), 70.68, 70.53 (C-5^1,5^2), 68.72 (C-5^3), 65.28 (2 C, C-6^1,6^2), 63.03 (C-6^3), 55.13 (OCH_3), and 20.79 ($COCH_3$).

Anal. Calc. for $C_{88}H_{90}O_{17}$: C, 73.55; H, 6.61. Found: C, 73.35; H, 6.68.

Methyl O-*(2,3,4-tri-*O*-benzyl-α-*D*-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzyl-α-*D*-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-*D*-glucopyranoside* (**21**). — Methanol (100 mL) was added at 70° to a solution of **20** (4.3 g) in toluene (10 mL), followed by methanolic sodium methoxide, until a strongly alkaline solution was formed. After 6 h at room temperature, t.l.c. (solvent *A*) showed that the reaction was complete. The solution was made neutral with Amberlite IR-120 (H^+) ion-exchange resin, the suspension filtered, and the filtrate concentrated, to give **21** as a colorless glass (4 g, 96%); $[\alpha]_D$ +89° (*c* 0.6); ^{13}C-N.m.r. (75 MHz, $CDCl_3$): δ 97.99 'C-1^1), 97.08, 97.00 (C-1^2,1^3), 82.08 (C-3^1), 81.56 (C-3^2), 81.45 (C-3^3), 80.18 (2 C), 80.14 (C-2^1,2^2,2^3), 77.73, 77.55, 77.47 (C-4^1,4^2,4^3), 75.63, 75.40 (2 C), 74.94, 74.88 (2 C), 73.27, 72.37, 72.24 (CH_2Ph), 70.86, 70.64, 70.46 (C-5^1,5^2,5^3), 65.83, 65.74 (C-6^1,6^2), and 55.10 (OCH_3).

Anal. Calc. for $C_{82}H_{88}O_{16}$: C, 74.07; H, 6.67. Found: C, 73.99; H, 6.71.

Methyl O-α-*D*-*glucopyranosyl-(1→6)-*O*-α-*D*-glucopyranosyl-(1→6)-α-*D*-glucopyranoside (methyl α-isomaltotrioside;* **22**). — Catalytic hydrogenolysis of **21** (0.6 g), under the conditions described for the preparation of **14**, gave **22** as a white amorphous solid (0.2 g, 85%); ^{13}C-n.m.r. (75 MHz, D_2O): δ 99.50 (C-1^1), 97.93 (2 C, C-1^2,1^3), 73.53 (2 C, C-3^1,3^2), 73.25 (C-3^3), 71.95 (C-5^3), 71.65, 71.57, 71.33 (C-2^1,2^2,2^3), 70.38 (C-5^2), 70.13 (C-5^1), 69.65 (3 C, C-4^1,4^2,4^3), 65.68 (2 C, C-6^1,6^2), 60.62 (C-6^3), and 55.29 (OCH_3).

The corresponding per-*O*-benzoyl derivative (**23**) was prepared in the usual

manner, and the crude product was purified by chromatography. The amorphous solid had $[\alpha]_D$ +144° (*c* 1.23).

Anal. Calc. for $C_{89}H_{74}O_{24}$: C, 68.54; H, 4.78. Found: C, 68.54; H, 4.80.

The corresponding per-*O*-(*p*-nitrobenzoyl) derivative (**24**) of **22** was prepared conventionally, and obtained crystalline after purification of the crude product by chromatography; m.p. 278–279°, $[\alpha]_D$ +161° (*c* 1.1, pyridine); ^{13}C-n.m.r. (75 MHz, Me_2SO-d_6): δ 96.04 (C-1^1), 95.12 (2 C, C-1^2,1^3), 71.79, 71.70, 71.56 (3 C), 71.40 (C-2^1,2^2,2^3,3^1,3^2,3^3), 69.63 (C-4^3), 69.03 (2 C, C-4^1,4^2), 68.14 (2 C, C-5^1,5^2), 66.92 (C-5^3), 64.91 (2 C, C-6^1,6^2), 63.36 (C-6^3), and 55.18 (OCH_3).

Anal. Calc. for $C_{89}H_{64}N_{10}O_{46}$: C, 53.19; H, 3.21; N, 6.97. Found: C, 53.26; H, 3.25; N, 6.91.

Phenyl O-*(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-* (**15**) *and -β-*D*-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-1-thio-β-*D*-glucopyranoside* (**9**). — (*a*) *Using 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-*D*-glucopyranosyl chloride*[6] (**1**) *as the glycosyl donor.* To a solution of phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside[18] (**3**; 2.9 g, 5.34 mmol) and 2,4,6-trimethylpyridine (0.865 mL, 6.5 mmol) in dichloromethane (35 mL) was added a solution of **1** (3 g, 5.9 mmol) in ether (40 mL). The solution was cooled to −25°, and, with stirring, a solution of silver perchlorate in ether (0.77M; 85 mL, 6.5 mmol) was added. The cooling bath was removed, and, after 30 min, t.l.c. (solvent *B*) showed that all of the **1** had been consumed, and that only a trace of **3** was present. The mixture was worked up as described for the preparation of **20**, and chromatography yielded the disaccharide fraction as an amorphous material (4.9 g, 90%); ^{1}H-n.m.r. spectroscopy showed the product to be a mixture of **9** and **15** in the ratio of ~1:5; $[\alpha]_D$ +34.8° (*c* 2), lit.[18] $[\alpha]_D$ +38.1° for an independently synthesized, closely related disaccharide mixture resulting from condensation of **1** with **3**.

Anal. Calc. for $C_{62}H_{64}O_{11}S$: C, 73.20; H, 6.34; S, 3.15. Found: C, 72.64; H, 6.25; S, 3.04.

(*b*) *Using 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-β-*D*-glucopyranosyl chloride* (**6**) *as the glycosyl donor.* Compound **6**, prepared from **5** (701 mg, 1.2 mmol) as described, was treated with **3** (542 mg, 1 mmol) in the presence of silver perchlorate and 2,4,6-trimethylpyridine, as described in (*a*). After processing and chromatography, ^{1}H-n.m.r. spectroscopy of the disaccharide fraction isolated (0.87 g, 85.5%) showed that the ratio of compound **9** to **15** was ~1:5.

(*c*) Compound **16** (110 mg) was conventionally acetylated with acetic anhydride–pyridine. The crude product was eluted from a column of silica gel (solvent *B*), to give pure, amorphous **15** (107 mg, 93%); $[\alpha]_D$ +39.6° (*c* 1).

Anal. Calc. for $C_{62}H_{64}O_{11}S$: C, 73.20, H, 6.34; S, 3.15. Found: C, 73.45; H, 6.41; S, 3.11.

When treated as described in (*c*) for the preparation of **15**, compound **10** (120 mg) yielded **9** (110 mg, 88%); m.p. 82–92° (from dichloromethane–isopropyl ether; no change after recrystallization), $[\alpha]_D$ +26.3° (*c* 0.6).

Anal. Calc. for $C_{62}H_{64}O_{11}S$: C, 73.20, H, 6.34; S, 3.15. Found: C, 73.27; H, 6.35; S, 3.10.

Phenyl O-*(2,3,4-tri-*O*-benzyl-α-* (**16**) *and -β-*D*-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-β-*D*-glucopyranoside* (**10**). — A mixture (3.6 g) containing **9** and **15** in the ratio of ~1:5, obtained as already described, was treated as described for the preparation of **21**. T.l.c. (solvent *D*) showed that two products were formed, the one showing the lower R_F greatly preponderating. The crude product was chromatographed, to give, first, **10** (404 mg, 11.7%); $[\alpha]_D$ +17° (*c* 0.7). The compound is polymorphous: crystallization from ether–hexane gave material melting at 114–115.5°. Occasionally, material melting at 134–135°, or 125–126°, was obtained, all products showing identical n.m.r.-spectral characteristics.

Anal. Calc. for $C_{60}H_{62}O_{10}S$: C, 73.89; H, 6.40; S, 3.28. Found: C, 73.94; H, 6.42; S, 3.21.

Continued elution gave amorphous **16** (2.3 g, 66.6%); $[\alpha]_D$ +46.5° (*c* 0.6).

Anal. Calc. for $C_{60}H_{62}O_{10}S$: C, 73.89; H, 6.40; S, 3.28. Found: C, 73.80; H, 6.42; S, 3.23.

An intermediate, mixed fraction was also obtained.

*Phenyl 2,3,4-tri-*O*-benzyl-α-*D*-glucopyranoside* (**4**). — Anhydrous *p*-toluenesulfonic acid (0.5 g) was added to a solution of **8** (4.32 g, 10 mmol) in thiophenol (25 mL), and the mixture was stirred at 55–60°. Several products were formed, one largely preponderating, as shown by t.l.c. (solvent *B*). A minor component (that moved only slightly faster than the major product) co-chromatographed with the phenyl 1-thio-β-glycoside **2**, obtained[18] independently. After 2 h, when very little starting-material remained, the mixture was made neutral with pyridine (0.3 mL), and concentrated to dryness at 60°/133 Pa. The residue was chromatographed, to give the major product **4** (3.3 g, 61%); m.p. 103–104° (from ethanol), $[\alpha]_D$ +169° (*c* 1).

Anal. Calc. for $C_{33}H_{34}O_5S$: C, 73.03; H, 6.32; S, 5.91. Found: C, 72.94; H, 6.36; S, 5.87.

*Phenyl 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-1-thio-α-*D*-glucopyranoside* (**5**). — Compound **4** (2.5 g) was dissolved in pyridine (10 mL), and acetic anhydride (1 mL) was added. The mixture was kept overnight at room temperature, and concentrated to dryness, and the residue crystallized from dichloromethane–ethanol, to afford **5** (2.55 g, 94.6%). Recrystallization of a portion gave material having m.p. 114–115°, $[\alpha]_D$ +167° (*c* 1).

Anal. Calc. for $C_{35}H_{36}O_6S$: C, 71.89; H, 6.21; S, 5.48. Found: C, 71.95; H, 6.22; S, 5.54.

6-O-*Acetyl-2,3,4-tri-*O*-benzyl-β-*D*-glucopyranosyl chloride* (**6**). — A solution of chlorine in carbon tetrachloride (1 mL, ~2 mmol) was added to a solution of phenyl α-glycoside **5** (0.585 g, 1 mmol) in carbon tetrachloride (5 mL). After 5 min, the solution was concentrated under anhydrous conditions, with several additions of toluene to remove all solvents and non-carbohydrate by-products. After drying for 30 min in a rotary evaporator at 40°/15 Pa, the pale-yellow, amorphous **6** had $[\alpha]_D$ +33° (*c* 0.8); *cf.* $[\alpha]_D$ +90° for the corresponding α-glycosyl chloride[44] **1**. Compound **6** decomposes during t.l.c. but its ammonia c.i. mass spectrum contains a

peak at *m/z* 528 ($[M + 18]^+$); ^{1}H-n.m.r. (300 MHz, $CDCl_3$): δ 5.20 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1; *cf.* ref. 6, δ 6.02, d, 1 H, $J_{1,2}$ 3.6 Hz, H-1, in the ^{1}H-n.m.r. spectrum of **1**), 4.97–4.54 (6 d, 6 H, ^2J ~11 Hz, CH_2Ph), 4.33 (bd, 1 H, $J_{6a,6b}$ 11.8 Hz, $J_{6a,5}$ not observed, H-6a), 4.18 (dd, 1 H, $J_{6b,5}$ 3.8 Hz, H-6b), 3.66–3.58 (m, 4 H, H-2,3,4,5), and 2.03 (s, 3 H, OCH_3); ^{13}C-n.m.r. (75 MHz, $CDCl_3$): δ 90.26 (C-1), 85.22 (C-3), 84.51 (C-2), 76.66, 76.52, 75.61 (2 C), 75.07 (C-4,5, CH_2Ph), 62.78 (C-6), and 20.81 ($COCH_3$).

Phenyl O-*(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-* (**17**) *and -β-*D*-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-1-thio-α-*D*-glucopyranoside* (**12**). — (*a*) *Using 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-*D*-glucopyranosyl chloride* (**1**) *as the glycosyl donor.* A solution of silver perchlorate (0.518 g, 2.5 mmol) in ether (35 mL) was added with stirring at −20° to a solution of the nucleophile **4** (1.08 g, 2 mmol), glycosyl chloride **1** (1.02 g, 2 mmol), and 2,4,6-trimethylpyridine (0.3 mL, 2.25 mmol) in ether (15 mL). Cooling was terminated, and, after 30 min, when t.l.c. (solvent *A*) showed that all of the **1** was consumed and that only a small amount of **4** remained, the mixture was processed as described for the preparation of **15**. Chromatography yielded the major, amorphous product **17** (0.65 g, 32%); $[\alpha]_D$ +113° (*c* 0.6).

Anal. Calc. for $C_{62}H_{64}O_{11}S$: C, 73.20; H, 6.34; S, 3.15. Found: C, 73.08; H, 6.36; S, 3.10.

Continued elution gave an unresolved mixture of **17** and **12**, which was processed as described later.

(*b*) *Using 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-β-*D*-glucopyranosyl chloride* (**6**) *as the glycosyl donor.* Reaction of the nucleophile **4** (1.08 g, 2 mmol) with the β-glycosyl chloride **6** [freshly prepared from the phenyl α-thioglycoside **5** (1.46 g, 2.5 mmol)] under the conditions described in (*a*), followed by resolution of the crude product by chromatography, gave practically the same result as described in (*a*).

(*c*) Conventional acetylation of chromatographically pure **18** obtained as described later, with acetic anhydride–pyridine, gave **17**, indistinguishable from the material already described. Similar acetylation of **11** afforded **12**; m.p. 105–107° (from isopropyl ether, twice), $[\alpha]_D$ +98° (*c* 0.9).

Anal. Calc. for $C_{62}H_{64}O_{11}S$: C, 73.20; H, 6.34; S, 3.15. Found: C, 73.26; H, 6.39; S, 3.10.

Phenyl O-*(2,3,4-tri-*O*-benzyl-α-* (**18**) *and -β-*D*-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-1-thio-α-*D*-glucopyranoside* (**11**). — The unresolved mixture of disaccharides **17** and **12** already described (1 g) was deacetylated as described for the preparation of **21**. Two products were formed, which could be well separated by t.l.c. in solvent *D*. After conventional processing, the crude product was chromatographed to give, first, the β-linked disaccharide **11** (0.32 g, 33%); m.p. 105–106° (from isopropyl ether, twice), $[\alpha]_D$ +96° (*c* 0.8).

Anal. Calc. for $C_{60}H_{62}O_{10}S$: C, 73.89; H, 6.40; S, 3.28. Found: C, 73.98; H, 6.44; S, 3.29.

Subsequently eluted was the α-linked disaccharide **18** (0.58 g, 60.5%). Thus, compounds **12** and **17** show reversed chromatographic mobility, compared to the

pair of disaccharides **11** and **18**. Compound **18** solidified on standing, and, after recrystallization from isopropyl ether (twice), had m.p. 100–101°, $[\alpha]_D$ +113° (*c* 0.7).

Anal. Calc. for $C_{60}H_{62}O_{10}S$: C, 73.89; H, 6.40; S, 3.28. Found: C, 73.94; H, 6.43; S, 3.24.

O-(6-O-Acetyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranosyl chloride (**19**). — A solution of chlorine in carbon tetrachloride (1 mL, 2 mmol) was added to a solution of **17** (1 g, 1 mmol) in the same solvent (5 mL). After 5 min, the solution was evaporated at 40–50°/13 Pa under anhydrous conditions, with several additions of toluene, to give pure **19** (^{1}H- and ^{13}C-n.m.r.). Crystallization of the residue from ether–isopropyl ether gave **19** (0.7 g, 82%) which, after recrystallization, had m.p. 135–140° (sint. 115°), $[\alpha]_D$ +47° (*c* 1); NH_3-c.i.m.s.: *m/z* 960 $[M + 18]^+$, 924 $[M + 18 - HCl]^+$, based on monoisotopic mass.

Anal. Calc. for $C_{56}H_{59}ClO_{11}$: C, 71.28; H, 6.03; Cl, 3.75. Found: C, 71.20; H, 6.31; Cl, 3.80.

Methyl O-(6-O-acetyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyl)-(1→6)-bis[O-(2,3,4-tri-O-benzyl-α-D-glucopyranosyl)-(1→6)]-2,3,4-tri-O-benzyl-α-D-glucopyranoside (**25**). — The nucleophile **21** (1.12 g, 1.25 mmol) was treated with the crystalline glycosyl chloride **19** (1.18 g, 1.25 mmol) in the presence of silver perchlorate (1.4 mmol, in the form of a 77mM ethereal solution) and 2,4,6-trimethylpyridine (200 μL), under the conditions described for the preparation of **15**. Chromatography (solvent *B*) gave the major, amorphous product **25** (1.42 g, 63%); $[\alpha]_D$ +90° (*c* 0.7); ^{13}C-n.m.r. (75 MHz, $CDCl_3$): δ 98.02 (C-1^1), 97.10 (2 C), 97.04 (C-1^2,1^3,1^4), 82.10 (C-3^1), 81.59 (3 C, C-3^2,3^3,3^4), 80.37 (2 C), 80.19, 80.05 (C-2^1,2^2,2^3,2^4), 77.74 (C-4^1), 77.52 (2 C, C-4^2,4^3), 77.15 (C-4^4), 75.66, 75.54, 75.42 (2 C), 74.92 (4 C), 73.37, 72.35, 72.23, 72.11 (CH_2Ph), 70.81, 70.74, 70.59 (C-5^1,5^2,5^3), 68.71 (C-5^4), 65.67 (2 C), 65.53 (C-6^1,6^2,6^3), 63.00 (C-6^4), 55.12 (OCH_3), and 20.79 ($COCH_3$).

Anal. Calc. for $C_{111}H_{118}O_{22}$: C, 73.89; H, 6.59. Found: C, 73.66; H, 6.60.

Eluted next was a further amount of **25** contaminated with a mixture of minor by-products. The material eluted at the end of this zone consisted mainly of the β-linked tetrasaccharide **42**, as shown by ^{13}C-n.m.r. spectroscopy (75 MHz, $CDCl_3$): δ 103.67 (C-1^3), 97.91 (C-1^1), 97.22, 96.96 (C-1^2,1^4), 84.60 (C-3^3), 82.86 (2 C), 81.49, 81.36 (C-2^3,3^1,3^2,3^4), 80.08 (2 C), 80.01 (C-1^1,2^2,2^4), 77.77, 77.60, 77.22, 76.98 (C-4^1,4^2,4^3,4^4), 75.58, 75.51 (2 C), 75.27, 74.85 (3 C), 74.78 (2 C), 74.72, 73.30, 72.19, 71.98 (C-5^3, CH_2Ph), 70.62, 70.03, 68.72, 68.14 (C-5^1,5^2,5^4,6^2), 65.54 (C-6^1,6^3), 63.04 (C-6^4), 55.01 (OCH_3), and 20.78 ($COCH_3$).

Methyl O-(2,3,4-tri-O-benzyl-α-D-glucopyranosyl)-(1→6)-bis[O-(2,3,4-tri-O-benzyl-α-D-glucopyranosyl)-(1→6)]-2,3,4-tri-O-benzyl-α-D-glucopyranoside (**26**). — Compound **25** (0.69 g) was deacetylated, as described for the preparation of **21**, to give glycoside **26** as a colorless glass in theoretical yield; $[\alpha]_D$ +81° (*c* 0.7); ^{13}C-n.m.r. (75 MHz, $CDCl_3$): δ 97.97 (C-1^1), 97.12, 97.02 (2 C) (C-1^2,1^3,1^4), 82.04 (C-3^1), 81.54 (2 C, C-3^2,3^3), 80.15 (C-3^4), 80.36, 80.25, 80.15 (2 C) (C-2^1,2^2,2^3,2^4),

77.68 (C-4^1), 77.54 (2 C, C-4^2,4^3), 77.41 (C-4^4), 75.61, 75.36 (3 C), 74.95, 74.88 (3 C), 73.30, 72.29, 72.24, 72.14 (CH_2Ph), 70.85, 70.79, 70.72, 70.53 (C-5^1,5^2,5^3,5^4), 65.80, 65.64, 65.48 (C-6^1,6^2,6^3), 61.79 (C-6^4), and 55.08 (OCH_3).

Anal. Calc. for $C_{109}H_{116}O_{21}$: C, 74.29; H, 6.63. Found: C, 74.11; H, 6.68.

Methyl O-α-D-*glucopyranosyl-(1→6)-bis*[O-α-D-*glucopyranosyl-(1→6)*]-α-D-*glucopyranoside (methyl α-isomaltotetraoside*, **27**). — Glycoside **26** (600 mg) was deprotected by catalytic hydrogenolysis as described for the preparation of **14**. The crude product was eluted from a small column of silica gel (solvent *F*), and freeze-dried, to afford amorphous **27** (190 mg, 84%); ^{13}C-n.m.r. (75 MHz, D_2O): δ 99.46 (C-1^1), 97.80 (3 C, C-1^2,1^3,1^4), 73.47 (3 C, C-3^1,3^2,3^3), 73.18 (C-3^4), 71.92 (C-5^4), 71.49 (3 C, C-2^2,2^3,2^4), 71.26 (C-2^1), 70.29 (2 C, C-5^2,5^3), 70.08 (C-5^1), 69.58 (4 C, C-4^1,4^2,4^3,4^4), 65.67 (3 C, C-6^1,6^2,6^3), 60.57 (C-6^4), and 55.28 (OCH_3).

Methyl O-*(6-O-acetyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyl)-(1→6)-tris*[O-*(2,3,4-tri-O-benzyl-α-D-glucopyranosyl)-(1→6)*]-*2,3,4-tri-O-benzyl-α-D-glucopyranoside* (**32**). — The crude product resulting from condensation of equimolar amounts (0.75 mmol) of the nucleophile **21** and the glycosyl chloride **19** in the presence of silver perchlorate and 2,4,6-trimethylpyridine, as described for the preparation of **20**, was chromatographed. First eluted was the major product **32** (1.04 g, 62.5%), which was obtained as a glassy solid after drying at 105°/133 Pa; $[\alpha]_D$ +90.5° (*c* 1.1); ^{13}C-n.m.r. (75 MHz, $CDCl_3$): δ 98.06 (C-1^1), 97.28, 97.22, 97.16, 97.10 (C-1^2,1^3,1^4,1^5), 82.12 (C-3^1), 81.61 (4 C, C-3^2,3^3,3^4,3^5), 80.45 (3 C), 80.30, 80.16 (C-2^1,2^2,2^3,2^4,2^5), 77.80 (C-4^1), 77.43 (3 C, C-4^2,4^3,4^4), 77.32 (C-4^5), 75.64, 75.52, 75.39 (3 C), 74.92 (5 C), 73.36, 72.38, 72.26, 72.20, 72.13 (CH_2Ph), 70.94 (2 C), 70.84, 70.66 (C-5^1,5^2,5^3,5^4), 68.79 (C-5^5), 65.70, 65.57 (3 C) (C-6^1,6^2,6^3,6^4), 63.06 (C-6^5), 55.12 (OCH_3), and 20.78 ($COCH_3$).

Anal. Calc. for $C_{138}H_{146}O_{27}$: C, 74.01; H, 6.58. Found: C, 74.17; H, 6.64.

Eluted next was a mixture containing a further amount of **32**, contaminated with minor by-products. The material eluted at the end of this zone contained slightly contaminated, β-linked pentasaccharide **43**, as shown by ^{13}C-n.m.r. spectroscopy (75 MHz, $CDCl_3$): δ 103.82 (C-1^4), 98.02 (C-1^1), 97.23, 97.08 (2 C) (C-1^2,1^3,1^5), 84.65 (C-3^4), 82.08 (2 C), 91.59 (2 C), 81.42 (C-2^4,3^1,3^2,3^3,3^5), 80.21 (2 C), 80.08 (2 C) (C-2^1,2^2,2^3,2^5), 77.75 (2 C), 77.38 (2 C), 77.16 (C-4^1,4^2,4^3,4^4,4^5), 75.65, 75.55 (2 C), 75.36 (2 C), 74.95 (3 C), 74.90 (3 C), 74.74, 74.37, 72.19, 72.03 (2 C, C-5^4, CH_2Ph), 70.70, 70.03, 68.75, 68.23 (C-5^1,5^2,5^3,6^3), 65.47 (3 C, C-6^1,6^2,6^4), 63.06 (C-6^5), 55.13 (OCH_3), and 20.81 ($COCH_3$).

Methyl O-α-D-glucopyranosyl-(1→6)-tris[O-α-D-*glucopyranosyl-(1→6)*]-α-D-*glucopyranoside (methyl α-isomaltopentaoside*, **34**). — Compound **32** (0.91 g) was deacetylated as described for the preparation of **21**, to give the product **33**; ^{13}C-n.m.r. (75 MHz, $CDCl_3$): δ 98.06 (C-1^1), 97.28, 97.22, 97.16, 97.10, (C-1^2,1^3,1^4,1^5), 82.12 (C-3^1), 81.61 (4 C, C-3^2,3^3,3^4,3^5), 80.45, 80.30, 80.16 (3 C) (C-2^1,2^2,2^3,2^4,2^5), 77.80 (C-4^1), 77.45 (3 C, C-4^2,4^3,4^4), 77.32 (C-4^5), 75.64, 75.52, 75.39 (3 C), 74.92 (5 C), 73.36, 72.38, 72.26, 72.20, 72.13 (CH_2Ph), 70.94 (2 C), 70.84, 70.66 (C-5^1,5^2,5^3,5^4), 68.79 (C-5^5), 65.70, 65.57 (3 C) (C-6^1,6^2,6^3,6^4), 63.06 (C-6^5), 55.12 (OCH_3), and 20.78 ($COCH_3$).

Compound **33** was hydrogenolyzed as described for the preparation of **14**, and, after elution from a column of silica gel (solvent *F*) and freeze-drying of the eluate, compound **34** was obtained as a white, amorphous solid (0.276 g, 82%); ^{13}C-n.m.r. (75 MHz, D_2O): δ 99.45 (C-1^1), 97.90, 97.83, 97.77 (2 C) (C-1^2,1^3,1^4,1^5), 73.48 (4 C, C-3^1,3^2,3^3,3^4), 73.09 (C-3^5), 71.91 (C-5^5), 71.57, 71.49 (3 C) (C-2^2,2^3,2^4,2^5), 71.26 (C-2^1), 70.33, 70.27 (3 C) (C-5^2,5^3,5^4,5^5), 70.06 (C-5^1), 69.62 (5 C, C-4^1,4^2,4^3,4^4,4^5), 65.66 (4 C, C-6^1,6^2,6^3,6^4), 60.58 (C-6^5), and 55.28 (OCH_3).

Methyl O-*(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-*D*-glucopyranosyl)-(1→6)-tetrakis-*[O-*(2,3,4-tri-*O*-benzyl-α-*D*-glucopyranosyl)-(1→6)]-2,3,4-tri-*O*-benzyl-α-*D*-glucopyranoside* (**35**). — A mixture of the nucleophile **26** (0.9 g, 0.51 mmol) and the isomaltosyl donor **19** (0.566 g, 0.6 mmol) was treated with silver perchlorate and 2,4,6-trimethylpyridine as described for the preparation of **20**. The mixture was processed as already described, and the crude product was chromatographed (solvent *B*), to give the major product **35**; $[\alpha]_D$ +92° (*c* 0.6); ^{13}C-n.m.r. (75 MHz, $CDCl_3$): δ 98.02 (C-1^1), 97.30 (2 C), 97.19, 97.11, 97.04 (C-1^2,1^3,1^4,1^5,1^6), 82.08 (C-3^1), 81.57 (5 C, C-3^2,3^3,3^4,3^5,3^6), 80.43, 80.21 (4 C), 80.08 (C-2^1,2^2,2^3,2^4,2^5,2^6), 77.70 (C-4^1), 77.38 (4 C, C-4^2,4^3,4^4,4^5), 77.20 (C-4^6), 75.65, 75.53, 75.38 (4 C), 74.92 (6 C), 73.36, 72.34, 72.27, 72.15 (2 C), 72.09 (CH_2Ph), 70.92 (2 C), 70.85, 70.78, 70.62 (C-5^1,5^2,5^3,5^4,5^5), 68.72 (C-5^6), 65.51 (5 C, C-6^1,6^2,6^3,6^4,6^5), 63.02 (C-6^6), 55.11 (OCH_3), and 20.79 ($COCH_3$).

Anal. Calc. for $C_{165}H_{174}O_{32}$: C, 74.24; H, 6.57. Found: C, 74.30; H, 6.62.

Methyl O-α-*D*-glucopyranosyl-(1→6)-tetrakis[O-α-*D*-glucopyranosyl-(1→6)]-α-*D*-glucopyranoside (methyl α-isomaltohexaoside,* **37**). — Compound **35** (0.95 g) was deacetylated, as described for the preparation of **21**, to give **36**; ^{13}C-n.m.r. (75 MHz, $CDCl_3$): δ 98.03 (C-1^1), 97.31 (2 C), 97.19, 97.11 (2 C) (C-1^2,1^3,1^4,1^5,1^6), 82.08 (C-3^1), 81.57 (4 C, C-3^2,3^3,3^4,3^5), 81.46 (C-3^6), 80.41 (4 C), 80.21 (2 C) (C-2^1,2^2,2^3,2^4,2^5,2^6), 77.72 (C-4^1), 77.43 (5 C, C-4^2,4^3,4^4,4^5,4^6), 75.65, 75.40, 75.38 (4 C), 74.92 (6 C), 73.37, 72.35, 72.19 (4 C) (CH_2Ph), 70.88 (5 C), 70.62 (C-5^1,5^2,5^3,5^4,5^5,5^6), 65.63 (2 C), 65.52 (2 C), 65.44 (C-6^1,6^2,6^3,6^4,6^5), 61.88 (C-6^6), and 55.12 (OCH_3).

Compound **36** was treated as described for the preparation of **14**. The product was isolated in the usual manner; the solid residue was dissolved in water (~1 mL) and methanol (~1 mL) was added. After filtration, methanol was added to incipient turbidity, and compound **37** crystallized. The material was collected, washed successively with 80% methanol, methanol, and ether, and dried at 105°/13 Pa, to afford pure **37** as the dihydrate (305 mg, 89%); m.p. 204–206° (sint. 202°), $[\alpha]_D$ +181.4° (*c* 0.6, H_2O); ^{13}C-n.m.r. (75 MHz, D_2O): δ 99.54 (C-1^1), 97.86 (5 C, C-1^2,1^3,1^4,1^5,1^6), 73.56 (5 C, C-3^1,3^2,3^3,3^4,3^5), 73.29 (C-3^6), 71.99 (C-5^6), 71.59 (5 C, C-2^2,2^3,2^4,2^5,2^6), 71.36 (C-2), 70.35 (4 C, C-5^2,5^3,5^4,5^5), 70.15 (C-5^1), 69.66 (6 C, C-4^1,4^2,4^3,4^4,4^5,4^6), 65.74 (5 C, C-6^1,6^2,6^3,6^4,6^5), 60.67 (C-6^6), and 55.34 (OCH_3).

Anal. Calc. for $C_{37}H_{64}O_{31}\cdot 2\ H_2O$: C, 42.69; H, 6.58. Found: C, 42.67; H, 6.62.

Methyl O-*(2,3,4-tri-*O*-benzyl-6-deoxy-6-fluoro-α-* (**28**) *and -β-*D*-glucopyrano-*

syl)-(1→6)-bis[O-*(2,3,4-tri*-O-*benzyl*-α-D-*glucopyranosyl)-(1→6)*]-*2,3,4-tri*-O-*benzyl*-α-D-*glucopyranoside* (**41**). — A solution of silver perchlorate (77mM; 10.4 mL, 0.8 mmol) was added with stirring at 0° to a suspension of the nucleophile **21** (0.685 g, 0.5 mmol), finely powdered $SnCl_2$ (0.15 g, 0.8 mmol), molecular sieves 3A (1 g), and 2,3,4-tri-*O*-benzyl-6-deoxy-6-fluoro-α,β-D-glucopyranosyl fluoride[28] (**7**), 0.33 g, 0.72 mmol. Cooling was terminated, and, after 1 h, when t.l.c. (solvent *A*) showed that some unchanged glycosyl donor **7** was still present, 3 mL of the same solution of silver perchlorate was added. After an additional 1 h, t.l.c. showed that the reaction was practically complete. The mixture was made neutral with 2,4,6-trimethylpyridine, the suspension filtered, and the filtrate processed as described for the preparation of **15**. Chromatography gave, first, the faster-moving, α-linked product **28** (a glass; 0.56 g, 64%); $[\alpha]_D$ +81.5° (*c* 0.9); ^{13}C-n.m.r. (75 MHz, $CDCl_3$): δ 98.01 (C-1^1), 97.18, 97.08 (2 C) (C-1^2,1^3,1^4), 82.08 (C-3^1), 81.87 ($J_{F,C}$ 172.1 Hz, C-6^4), 81.59 (2 C), 81.36 (C-3^2,3^3,3^4), 80.39, 80.32, 80.18, 79.99 (C-2^1,2^2,2^3,2^4), 77.74, 77.55, 77.45 (C-4^1,4^2,4^3), 76.65 ($J_{F,C}$ 5.7 Hz, C-4^4), 75.65, 75.45, 75.39 (2 C), 75.08, 74.98, 74.87 (2 C), 73.34, 72.35, 72.22 (2 C) (*C*H_2Ph), 70.78, 70.68, 70.59 (C-5^1,5^2,5^3), 69.90 ($J_{F,C}$ 17.6 Hz, C-5^4), 65.67 (3 C, C-6^1,6^2,6^3), and 55.11 (O*C*H_3)

Anal. Calc. for $C_{109}H_{115}FO_{20}$: C, 74.21; H, 6.57; F, 1.07. Found: C, 74.11; H, 6.60; F, 1.21.

Continued elution afforded the β-linked product **41** (a glass; 185 mg, 21%); $[\alpha]_D$ +57° (*c* 0.7); ^{13}C-n.m.r. (75 MHz, $CDCl_3$): δ 103.75 (C-1^4), 97.96 (C-1^1), 96.96 (2 C, C-1^2,1^3), 84.53 (C-3^4), 81.78 ($J_{F,C}$ 173.9 Hz, C-6^4), 82.07, 81.84, 81.59, 81.47 (C-2^4,3^1,3^2,3^3), 80.26, 80.17, 80.03 (C-2^1,2^2,2^3), 77.83, 77.71, 77.47 (C-4^1,4^2,4^3), 76.67 ($J_{C,F}$ 5.7 Hz, H-4^4), 75.62 (2 C), 75.35 (2 C), 75.03, 74.97, 74.85 (2 C), 74.72, 73.32, 72.28, 72.10 (*C*H_2Ph), 74.00 ($J_{F,C}$ 18.6 Hz, C-5^5), 70.70, 70.55, 69.80 (C-5^1,5^2,5^3), 68.58 (C-6^3), 65.55 (2 C, C-6^1,6^2), and 55.09 (O*C*H_3).

Anal. Calc. for $C_{109}H_{115}FO_{20}$: C, 74.21; H, 6.57; F, 1.07. Found: C, 74.58; H, 6.60; F, 1.55.

Methyl O-*(6-deoxy-6-fluoro*-α-D-*glucopyranosyl)-(1→6)-bis*[O-α-D-*glucopyranosyl-(1→6)*]-α-D-*glucopyranoside (methyl 6⁴-deoxy-6⁴-fluoro-α-isomaltotetraoside,* **29**). — Compound **28** (0.47 g) was treated as described for the preparation of **14**. The product was eluted from a column of silica gel (solvent *C*), to give **29** (170 mg, 94%) as a colorless amorphous solid; ^{13}C-n.m.r. (75 MHz, D_2O): δ 99.48 (C-1^1), 97.95 (2 C), 97.80 (C-1^2,1^3,1^4), 82.25 ($J_{F,C}$ 167.7 Hz, C-6^4), 73.51 (3 C, C-3^1,3^2,3^3), 73.04 (C-3^4), 71.52 (3 C, C-2^2,2^3,2^4), 71.29 (C-2), 70.75 ($J_{F,C}$ 17.5 Hz, C-5^4), 70.29 (2 C, C-5^2,5^3), 70.09 (C-5), 69.67 (2 C, C-4^2,4^3), 69.56 (C-4), 68.53 ($J_{F,C}$ 5.9 Hz, C-4^4), 65.88 (C-6), 65.69 (2 C, C-6^2,6^3), and 55.27 (O*C*H_3).

Phenyl O-*(6-O-acetyl-2,3,4-tri*-O-*benzyl*-α-D-*glucopyranosyl)-(1→6)-bis*[O-*(2,3,4-tri*-O-*benzyl*-α-D-*glucopyranosyl)-(1→6)*]-*2,3,4-tri*-O-*benzyl-1-thio*-α-D-*glucopyranoside* (**30**). — The crude product obtained from reaction of the nucleophile **18** (1.04 g, 1.06 mmol) with the crystalline glycosyl chloride **19** (1.13 g, 1.2 mmol) in the presence of silver perchlorate (1.4 mmol) and 2,4,6-trimethylpyridine (1.4 mmol), performed as described for the preparation of **20**, was chromatographed

(solvent *A*). The main, amorphous product **30** (1.65 g, 82.7%) had $[\alpha]_D$ +101.5° (*c* 1.7); ^{13}C-n.m.r. (75 MHz, $CDCl_3$): δ 97.49, 97.21, 97.03 (C-1^2,1^3,1^4), 87.38 (C-1^1), 82.43 (C-3^1), 81.67, 81.57 (2 C) (C-3^2,3^3,3^4), 80.46, 80.36, 80.01 (2 C) (C-2^1,2^2,2^3,2^4), 77.63, 77.46, 77.38, 77.20 (C-4^1,4^2,4^3,4^4), 75.65, 75.52, 75.44 (2 C), 75.00 (2 C), 74.90 (2 C), 72.50, 72.41, 72.18, 72.10 (CH_2Ph), 71.63 (C-5^1), 70.83, 70.76 (C-5^2,5^3), 68.72 (C-5^4), 66.61 (C-6^1), 65.60, 65.52 (C-6^2,6^3), 63.02 (C-6^4), and 20.78 ($COCH_3$).

Anal. Calc. for $C_{116}H_{120}O_{21}S$: C, 74.01; H, 6.42; S, 1.70. Found: C, 73.50; H, 6.47; S, 1.64.

A mixture (0.40 g) containing **30** contaminated with slower-moving, unidentified by-products was also obtained.

Methyl O-*(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-*D*-glucopyranosyl)-(1→6)-hexakis-[*O*-*(2,3,4-tri-*O*-benzyl-α-*D*-glucopyranosyl)-(1→6)]-tri-*O*-benzyl-α-*D*-glucopyranoside* (**38**). — A solution of the 1-thioglycoside **30** (1.15 g, 0.61 mmol) in carbon tetrachloride (5 mL) was treated with a solution of chlorine in the same solvent, in the manner described for the preparation of **6**. After the usual processing, ^{1}H- and ^{13}C-n.m.r. spectroscopy showed that practically pure β-glycosyl chloride **31** had been obtained; ^{13}C-n.m.r. (75 MHz, $CDCl_3$): δ 97.23, 97.15, 97.03 (C-1^2,1^3,1^4), 90.54 (C-1^1), 85.13 (C-3^1), 84.65 (C-2^1), 81.66 (C-3^2), 81.59, 81.50 (C-3^3,3^4), 80.42, 80.36, 80.09 (C-2^2,2^3,2^4), 78.91 (C-4^4), 77.46 (2 C), 77.22 (C-4^2,4^3,5^1), 76.60 (C-4^1), 75.69, 75.45, 75.48 (3 C), 75.05, 74.91 (3 C), 72.32, 72.11 (2 C) (CH_2Ph), 70.90 (C-5^2), 70.77 (C-5^3), 68.72 (C-5^4), 65.70, 65.55, 64.97 (C-6^1,6^2,6^3), 63.05 (C-6^4), and 20.81 ($COCH_3$).

To a solution of compound **31** in ether (10 mL) was added the hydroxy derivative **26** (0.8 g, 0.45 mmol) and 2,4,6-trimethylpyridine (73 μL, 0.6 mmol). The solution was cooled to −20°, and a solution of silver perchlorate in ether (77mM; 10 mL, 0.77 mmol) was added with stirring. Cooling was terminated, and, after 1 h, when t.l.c. (solvent *B*) showed that the reaction was complete, the mixture was worked-up as usual. Chromatography gave the major product **38** (1.11 g, 69.7%) as a colorless, hard gum; $[\alpha]_D$ +97° (*c* 0.9); ^{13}C-n.m.r. (75 MHz, $CDCl_3$): δ 98.03 (C-1^1), 97.42, 97.35 (2 C), 97.28, 97.18, 97.11, 97.04, (C-1^2,1^3,1^4,1^5,1^6,1^7,1^8), 82.08 (C-3^1), 81.56 (7 C, C-3^2,3^3,3^4,3^5,3^6,3^7,3^8), 80.42 (6 C), 80.21, 80.08 (C-2^1,2^2,2^3,2^4,2^5,2^7,2^8), 77.68 (C-4^1), 77.37 (6 C, C-4^2,4^3,4^4,4^5,4^6,4^7), 77.20 (C-4^8), 75.64, 75.52, 75.36 (6 C), 74.91 (8 C), 73.36, 72.33, 72.20, 72.12 (5 C) (CH_2Ph), 70.94 (3 C), 70.86 (3 C), 70.63 (C-5^1,5^2,5^3,5^4,5^5,5^6,5^7), 68.72 (C-5^8), 65.56 (7 C, C-6^1,6^2,6^3,6^4,6^5,6^6,6^7), 63.02 (C-6^8), 55.11 (OCH_3), and 20.79 ($COCH_3$).

Anal. Calc. for $C_{219}H_{230}O_{42}$: C, 74.42; H, 6.50. Found: C, 74.49; H, 6.57.

Methyl O-*(2,3,4-tri-*O*-benzyl-α-*D*-glucopyranosyl)-(1→6)-hexakis*[O-*(2,3,4-tri-*O*-benzyl-α-*D*-glucopyranosyl)-(1→6)]-2,3,4-tri-*O*-benzyl-α-*D*-glucopyranoside* (**39**). — Compound **38** (1 g) was deacetylated as already described. The ion-exchange resin used to make the reaction mixture neutral was finally washed with dichloromethane, and, after concentration of the solution, the crude product was eluted from a short column of silica gel (solvent *B*), to remove some colored

material. After drying at 50°/13 Pa, compound **39** was obtained in almost theoretical yield as a colorless gum; $[\alpha]_D$ +96° (*c* 0.9); ^{13}C-n.m.r. (75 MHz, $CDCl_3$): δ 98.03 (C-1^1), 97.35 (4 C), 97.19, 97.10 (2 C) (C-1^2,1^3,1^4,1^5,1^6,1^7,1^8), 82.08 (C-3^1), 81.57 (6 C, C-3^2,3^3,3^4,3^5,3^6,3^7), 81.46 (C-3^8), 80.43 (5 C), 80.22 (3 C) (C-2^1,2^2,2^3,2^4,2^5,2^6,2^7,2^8), 75.65, 75.38 (7 C), 74.92 (8 C), 73.37, 72.34, 72.18 (6 C) (*C*H_2Ph), 70.95 (2 C), 70.87 (5 C), 70.64 (C-5^1,5^2,5^3,5^4,5^5,5^6,5^7,5^8), 65.58 (6 C), 65.46 (C-6^1,6^2,6^3,6^4,6^5,6^6, 6^7), 61.88 (C-6^8), and 55.19 (O*C*H_3).

Anal. Calc. for $C_{217}H_{228}O_{41}$: C, 74.63; H, 6.58. Found: C, 74.51; H, 6.62.

Methyl O-α-D-*glucopyranosyl-(1→6)-hexakis*[O-α-D-*glucopyranosyl-(1→6)*]-α-D-*glucopyranoside (methyl α-isomalto-octaoside*, **40**). — The benzylated octasaccharide **39** (0.8 g) was hydrogenolyzed as described for the preparation of **14**. Crystallization from water–methanol gave pure **40** as the trihydrate; m.p. 285–290° (sint. 255°), $[\alpha]_D$ +182.6° (*c* 0.6, H_2O); ^{13}C-n.m.r. (75 MHz, D_2O): δ 99.48 (C-1^1), 97.94, 97.81 (6 C) (C-1^2,1^3,1^4,1^5,1^6,1^7,1^8), 73.51 (7 C, C-3^1,3^2,3^3,3^4,3^5,3^6,3^7), 73.22 (C-3^8), 71.94 (C-5^8), 71.52 (7 C, C-2^2,2^3,2^4,2^5,2^6,2^7,2^8), 71.29 (C-2^1), 70.29 (6 C, C-5^2,5^3,5^4,5^5,5^6,5^7), 70.09 (C-5^1), 69.66 (8 C, C-4^1,4^2,4^3,4^4,4^5,4^6,4^7,4^8), 65.68 (7 C, C-6^1,6^2,6^3,6^4,6^5,6^6,6^7), 60.61 (C-6^8), and 55.29 (O*C*H_3).

Anal. Calc. for $C_{49}H_{84}O_{41} \cdot 3\ H_2O$: C, 42.54; H, 6.55. Found: C, 42.52; H, 6.48.

Monitoring the reactions of phenyl 1-thioglycosides **2, 5,** *and* **17** *with chlorine by* ^{1}*H-n.m.r. spectroscopy*. — A solution of the respective glycoside (0.1 mmol) in $CDCl_3$, contained in an n.m.r. tube, was treated with a solution of chlorine (0.2 mmol) in carbon tetrachloride. The ^{1}H-n.m.r. spectrum (300 MHz) was recorded after ~1 min, 15 min, 1 h, and 24 h, and then again after concentration to dryness with several additions of toluene (to remove organic solvents and non-carbohydrate by-products of the conversion), and redissolution in $CDCl_3$. The results are discussed in the text.

Monitoring the reaction **6→1** *by* ^{1}*H-n.m.r. spectroscopy*. — 2,4,6-Trimethylpyridine (13 μL, 0.1 mmol) was added to a solution of **6** (51 mg, 0.1 mmol) in $CDCl_3$ contained in an n.m.r. tube. The ^{1}H-n.m.r. spectrum (300 MHz) was recorded after ~1 min, 15 min, and 24 h. The results are discussed in the text.

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