Carbohydrate Research, 252 (1994) 69–84
Elsevier Science B.V., Amsterdam

Chemical synthesis and NMR spectra of a protected branched-tetrasaccharide thioglycoside, a useful intermediate for the synthesis of branched oligosaccharides

Mohammed Saddik Motawia [a], Carl Erik Olsen [b], Birger Lindberg Møller [a,*] and Jan Marcussen [c]

[a] *Plant Biochemistry Laboratory, Department of Plant Biology, The Royal Veterinary & Agricultural University, 40 Thorvaldsensvej, DK-1871 Frederiksberg C, Copenhagen (Denmark)*
[b] *Department of Chemistry, The Royal Veterinary & Agricultural University, 40 Thorvaldsensvej, DK-1871 Frederiksberg C, Copenhagen (Denmark)*
[c] *DANISCO A / S, MARIBO Seed Biotechnology, 1 Langebrogade, PO Box 17, DK-1001 Copenhagen K (Denmark)*

(Received January 4th, 1993; accepted June 11th, 1993)

ABSTRACT

Acid-catalyzed thiophenolysis of per-*O*-acetylated 1,6-anhydromaltose (**3**) gave phenyl 2,3-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-1-thio-β-D-glucopyranoside (**4**) in quantitative yield. Phenyl 4-*O*-α-D-glucopyranosyl-1-thio-β-D-glucopyranoside (**5**) was obtained by acid-catalyzed thiophenolysis of maltose octaacetate (**2**), using trimethylsilyl triflate as catalyst, and subsequent deacetylation. Standard benzylation of **5** gave phenyl 2,3-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-1-thio-β-D-glucopyranoside (**6**) which upon treatment with *N*-bromosuccinimide in aqueous acetone gave 2,3,6-tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-D-glucopyranose (**8**). Compound **8** was treated with trichloroacetonitrile in the presence of anhydrous potassium carbonate to give 2,3,6-tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-α,β-D-glucopyranosyl trichloroacetimidate (**9**), which was effectively used as the glycosyl donor in the condensation reaction with compound **4**, using trimethylsilyl triflate as catalyst, to obtain the branched tetrasaccharides phenyl *O*-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1 → 4)]-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1 → 6)-*O*-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-(1 → 4)-2,3-di-*O*-acetyl-1-thio-β-D-glucopyranoside (**10**) and phenyl *O*-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1 → 4)]-*O*-(2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl)-(1 → 6)-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-(1 → 4)-2,3-di-*O*-acetyl-1-thio-β-D-glucopyranoside (**11**) in 67 and 21% yield, respectively. A complete NMR interpretation of **10** is presented. Alternative methodologies for the synthesis of the branched tetrasaccharides were investigated. Chemical synthesis of the phenyl thioglycoside **5** was achieved by deacetylation of **4**. Reaction of **6** with diethylaminosulfur trifluoride in the presence of *N*-bromosuccinimide gave 2,3,6-tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-α,β-D-glucopyranosyl fluoride (**7**) in 78% yield. Subsequent condensation of **7** and **4**, using the combination silver perchlorate–stannous chloride as catalyst, gave the corresponding branched tetrasaccharides **10** and **11** in 55 and 10% yield, respectively.

* Corresponding author.

SSDI 0008-6215(93)E0226-Q

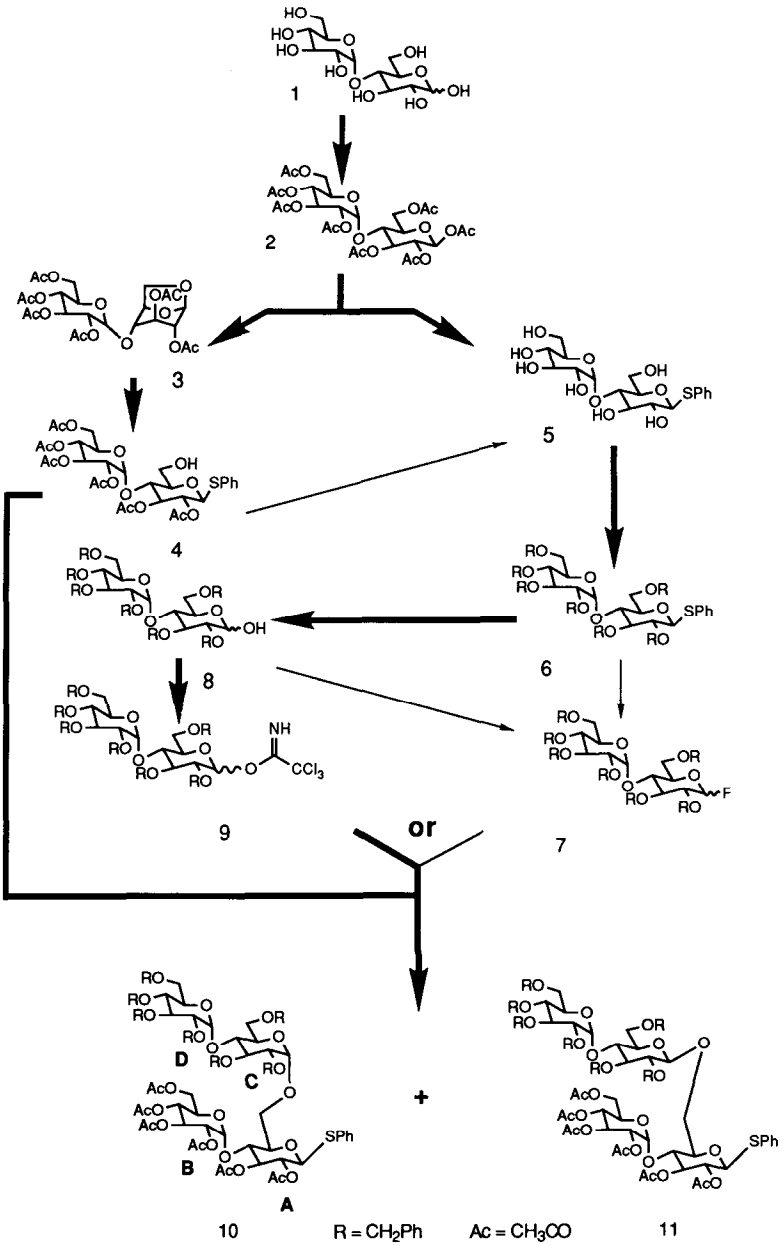
INTRODUCTION

Soluble and granule-bound starch synthases and branching enzymes catalyze the synthesis of starch[1]. Each of these enzymes occurs as a number of isozymes. The isozymes may exhibit different substrate specificity with respect to the structure of preferred primers and glycosyl side chains[1–6], but the lack of proper, chemically defined oligosaccharide substrates has prevented detailed analyses. Consequently, the biological importance of different isozymes in controlling and defining the chemical structure of starch during its synthesis remains largely unresolved. Starch structure has been studied mainly by characterizing the oligosaccharides formed by the action of starch-degrading enzymes[7–9]. If complex oligosaccharides with a defined number of glucose residues between the branch points were available, these could be tested as specific substrates for the degradative enzymes and a more precise analysis of starch structure obtained. These considerations prompted us to initiate a research program based on the chemical synthesis of complex oligosaccharides of interest for studies of starch biosynthesis and degradation. Chemical synthesis of oligosaccharides in which the identity of the constituent sugars, their anomeric configurations, and the positions of the intersugar linkages vary from residue to residue are dependent on the use of different types of blocking groups. Finally, the blocking groups should be chosen as being readily removable by a minimal number of otherwise nondestructive operations at the end of the synthetic sequence. To achieve the synthesis of a large number of complex oligosaccharides, a systematic approach based on a limited number of building blocks needs to be developed. In this paper, we report the chemical synthesis of a branched-tetrasaccharide thioglycoside derivative which comprises the branch point of starch and which can be used as a suitable building block in the chemical synthesis of higher branched oligosaccharides. The NMR spectra (^{1}H, ^{13}C, and ^{19}F) of the intermediates were analyzed and a complete NMR assignment of **10** is presented based on two-dimensional homonuclear and heteronuclear chemical-shift correlations.

RESULTS AND DISCUSSION

The synthetic strategy for obtaining the target molecule **10** is outlined in Scheme 1 and involves the conversion of maltose (**1**) into a suitable glycosyl acceptor molecule **4** as well as into a glycosyl donor **7** or **9**.

To synthesize the desired glycosyl acceptor **4**, maltose (**1**) was acetylated[10], and converted[11–13] into 1,6-anhydro-β-maltose hexaacetate (**3**). 1,6-Anhydro derivatives of D-glucopyranose and of di- and tri-saccharides, including maltose, cellobiose, and maltotriose, are readily available from the parent mono- and oligo-saccharides[14–16] and are used as versatile synthons in carbohydrate chemistry[17–21]. The 1,6-anhydro ring of compound **3** was cleaved selectively using Hanessian's reagent system[22], as originally proposed by Sakairi et al.[23], but modified to



Scheme 1. Chemical synthesis of the branched thioglycosides **10** and **11**. The favored route is marked with heavy arrows.

accomplish quantitative conversion of **3** into **4**, using (phenylthio)trimethylsilane–zinc iodide at room temperature for thiophenylation and subsequent deprotection with tetrabutylammonium fluoride. Crystallization provided **4** in 96% yield without chromatography.

Chemical synthesis of the glycosyl donor **9** required a number of intermediates (Scheme 1). Synthesis of **5** was accomplished by treating maltose octaacetate **2**[10] with thiophenol, using trimethylsilyl triflate as catalyst, and subsequent deacetylation using sodium methoxide in methanol. After purification by silica gel chromatography, the yield was 69%. Compound **5** was benzylated with benzyl bromide in DMF–NaH to give the phenyl thioglycoside derivative **6**, which was isolated in 94% yield after column chromatography. The thioglycoside **6** was converted into 2,3,6-tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-D-glucopyranose (**8**) by treatment with *N*-bromosuccinimide in aqueous acetone[24]. Treatment of **8** with trichloroacetonitrile in the presence of anhydrous potassium carbonate as catalyst[25,26] gave an anomeric mixture of the glycosyl trichloroacetimidate derivative **9**, the α:β ratio of which was 2:3 as estimated from ^{1}H and ^{13}C NMR spectra. Since the β-trichloroacetimidate and the corresponding α anomer give similar results in glycosylations under comparable reaction conditions, probably due to the participation of the solvent diethyl ether[26], the anomeric mixture of **9** was used for the glycosylation step without prior chromatographic separation. By treating **9** with the glycosyl acceptor **4** in diethyl ether, using trimethylsilyl triflate as catalyst, the desired branched-tetrasaccharide derivative **10** (67%) and its related β analogue **11** (21%) were obtained after chromatographic separation. The α and β configurations at C-1^C (Scheme 1) in compounds **10** and **11** were apparent from the ^{13}C NMR signals at 96.0 and 104.3, respectively[27]. Both compounds could be crystallized from 96% ethanol.

During the development of the strategy outlined above, a number of alternative procedures were examined. Deacetylation of compound **4** by standard methods afforded phenyl 1-thio-β-maltoside (**5**) in quantitative yield. However, it was more convenient to synthesize **5** via the trimethylsilyl triflate route. The glycosyl trichloroacetimidate procedure[25] used for the synthesis of **10** is well documented for the synthesis of complex glycosides and oligosaccharides[26,28,29]. Glycosyl trichloroacetimidates and fluorides offer advantages in oligosaccharide synthesis because of the difficulties encountered in handling and purifying the more labile and readily hydrolyzed chlorides and bromides. On the contrary, glycosyl fluorides have not been considered sufficiently reactive to be useful as glycosyl donors. However, it has recently been shown that glycosyl fluorides activated with stannous chloride can be useful glycosyl donors in stereocontrolled α-(1,2-*cis*)-glycosylations catalyzed with silver perchlorate[30]. The phenyl thioglycoside derivative **6** was therefore converted into the corresponding glycosyl fluoride **7** by treatment with diethylaminosulfur trifluoride and *N*-bromosuccinimide[31]. Compound **7** was obtained in 78% yield after column chromatography on silica gel. As an alternative, **8** was converted into the glycosyl fluoride **7** by reaction with diethylaminosulfur trifluoride[32,33]. Coupling of **7** with **4** in diethyl ether by using the combination stannous chloride–silver perchlorate as an activator[30,31] afforded the desired α-(1 → 6)-linked tetrasaccharide thioglycoside derivative **10** (55%) and its related β-(1 → 6)-linked anomer **11** (10%) after separation by column chromatography

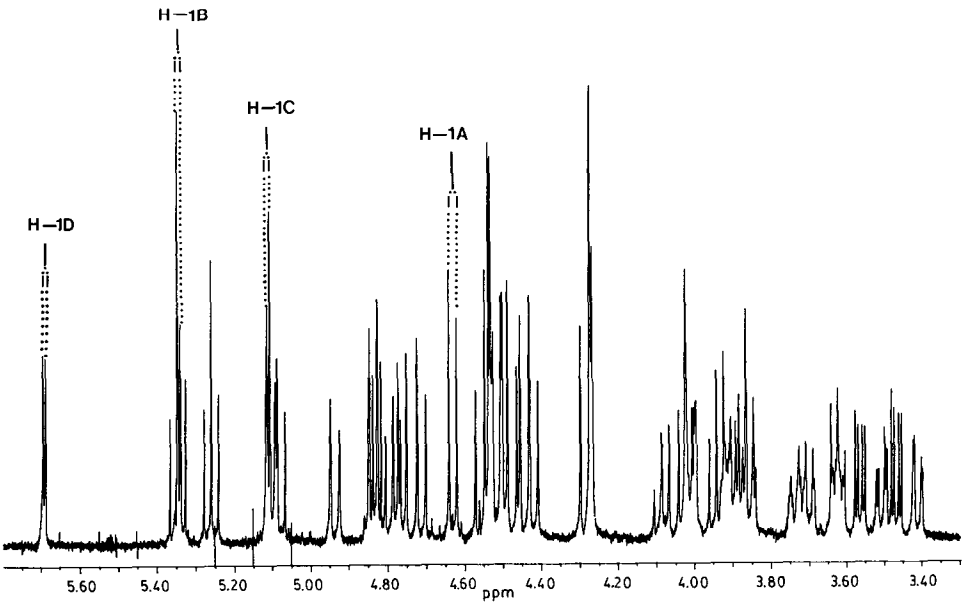


Fig. 1. 500-MHz ^{1}H NMR spectrum of **10** in $CDCl_3$.

using silica gel. The yield of the target compound **10** is lower using this approach but the α-stereoselectivity is increased. The explosive nature and expense of silver perchlorate argue in favor of the trichloroacetimidate method.

NMR Spectroscopy studies.—NMR spectra of **2** and **3** are available from the literature[13,34–37] and identical data were obtained in the present study. The ^{1}H NMR spectrum of **4** has also been reported[23] and is identical with the spectrum obtained in the present study. The ^{13}C NMR spectrum of **4** has not been reported earlier. The COSY spectrum of **4** reveals that the H-4 proton resonating at δ 4.10 (lit.[23], δ 4.07) is coupled with the proton resonating at δ 3.54 (J 9.4 Hz). This dictates that the proton resonating at δ 3.54 is H-5 and not H-6 as assigned in the literature[23]. The proton resonating at δ 3.80 shows no coupling with H-4, whereas it is coupled with H-6b resonating at δ 3.96 (lit.[23], δ 3.97–3.91) (J 12.3 Hz, a typical geminal coupling) and with the proton resonating at δ 3.54 (J 2.1 Hz). This dictates that the signal at δ 3.96 ppm must be assigned to H-6a and not H-5 as reported in the literature[23]. Apart from the interchanged assignments of the chemical shifts reported[23] for H-5 and H-6 and the resolution of some of the multiplets in the present study, the remaining assignments are identical to those previously reported[23]. The ^{1}H NMR spectrum of **10** is shown in Fig. 1 and the ^{13}C NMR spectrum in Fig. 2. The glucopyranosyl units of the branched tetrasaccharide **10** are labelled **A** to **D** (Scheme 1). The complete ^{1}H and ^{13}C NMR chemical-shift assignments for **10** and **11** listed in Tables I and II were obtained using homonuclear (COSY)[38] (Fig. 3) as well as ^{1}H–^{13}C-heteronuclear (HETCOR)[39] (Fig. 4) chemical shift correlation. The ^{13}C-DEPT spectrum of **10** (Fig. 5) was recorded to

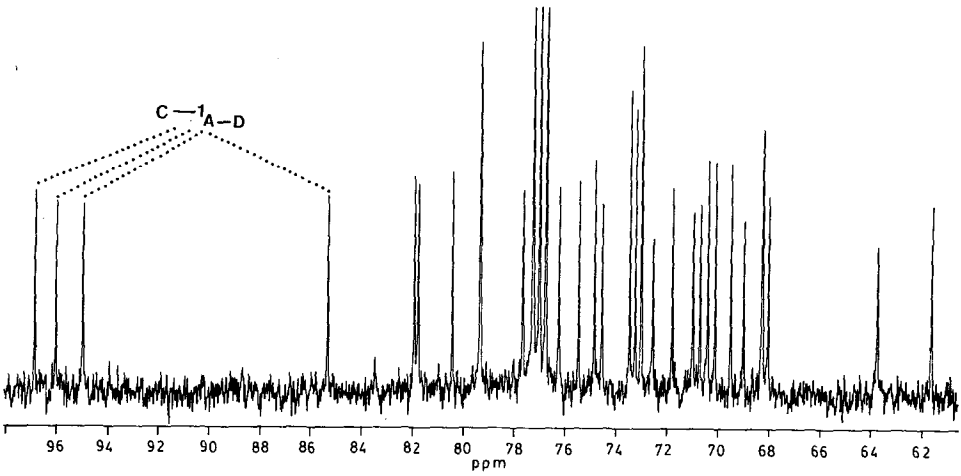


Fig. 2. 126-MHz ^{13}C NMR spectrum of **10** in $CDCl_3$.

extract more easily the ^{13}C resonances corresponding to the four C-6 carbons of the glucopyranosyl residues **A**, **B**, **C**, and **D** and the methylenes of the benzyl-protecting groups. The spectrum clearly reveals the resonances for these eleven methyleneoxy carbons.

The ^1H NMR spectrum of **10** (Fig. 1) showed the diagnostic signals for the four anomeric protons at δ 5.69 (d, 1 H, $J_{1,2}$ 3.6 Hz), 5.34 (d, 1 H, $J_{1,2}$ 3.8 Hz), 5.11 (d,

TABLE I

^1H NMR and ^{13}C NMR data[a,b] for the tetrasaccharide derivative **10**

Residue		Proton or carbon						
		1 ($J_{1,2}$)	2 ($J_{2,3}$)	3 ($J_{3,4}$)	4 ($J_{4,5}$)	5 ($J_{5,6a}$, $J_{5,6b}$)	6a	6b ($J_{6a,6b}$)
A	H	4.63d	4.78t	5.26t	3.94t	3.64–3.60m	4.11–3.99m	3.92–3.84m
β-D-Glc*p*SPh		(10.1)	(9.1)	(9.4)	(9.4)	(–)	(–)	
	C	85.3	70.4	77.0	72.5	77.6	63.7	
B	H	5.34d	4.84dd	5.35t	5.08t	4.11–3.99m	4.30–4.26m	4.30–4.26m
α-D-Glc*p*-(1 → 4)		(3.8)	(9.9)	(9.8)	(10.3)	(–)	(–)	
	C	95.0	70.7	69.5	68.0	68.3	61.7	
C	H	5.11d	3.36dd	4.02t	3.89t	3.75–3.68m	4.11–3.99m	3.92–3.84m
α-D-Glc*p*-(1 → 6)		(3.5)	(9.4)	(9.6)	(9.6)	(–)	(–)	
	C	96.0	80.4	81.8	70.1	71.8	69.0	
D	H	5.69d	3.47dd	3.86t	3.62t	3.75–3.68m	3.51dd	3.41dd
α-D-Glc*p*-(1 → 4)		(3.6)	(9.9)	(9.4)	(9.4)	(1.9, 2.8)	(10.8)	
	C	96.9	79.3	81.9	79.3	71.0	68.3	

[a] $CDCl_3$; Chemical shifts in ppm, J in Hz. [b] Chemical shifts for CH_3CO and $PhCH_2O$ are given in the Experimental.

TABLE II

^{1}H NMR and ^{13}C NMR data[a,b] for the tetrasaccharide derivative **11**

Residue		Proton or carbon						
		1 ($J_{1,2}$)	2 ($J_{2,3}$)	3 ($J_{3,4}$)	4 ($J_{4,5}$)	5 ($J_{5,6a}$, $J_{5,6b}$)	6a ($J_{6a,6b}$)	6b
A	H	4.68d	4.83t	5.24t	3.98t	3.53–3.48m	4.10–4.06m	3.84–3.79m
β-D-Glc*p*SPh		(10.1)	(9.3)	(9.0)	(9.2)	(–)	(–)	
	C	85.3	70.9	76.5	72.5	74.4	70.1	
B	H	5.38d	4.86dd	5.40t	5.06t	4.34–4.31m	4.34–4.31m	4.10–4.06m
α-D-Glc*p*-(1 → 4)		(4.0)	(9.9)	(10.0)	(9.6)	(–)	(–)	
	C	95.3	70.1	69.6	68.2	67.8	61.5	
C	H	4.50d	3.56t	3.77t	4.02t	3.53–3.48m	3.84–3.79m	3.74–3.70m
β-D-Glc*p*-(1 → 6)		(7.7)	(8.4)	(8.8)	(9.1)	(–)	(–)	
	C	104.2	83.1	84.7	73.4	77.8	69.2	
D	H	5.67d	3.46dd	3.88t	3.64t	3.74–3.70m	3.53–3.48m	3.38–3.36m
α-D-Glc*p*-(1 → 4)		(3.7)	(8.8)	(9.4)	(9.4)	(–)	(–)	
	C	96.6	79.3	81.9	77.6	71.0	68.1	

[a,b] See footnotes for Table I.

1 H, $J_{1,2}$ 3.5 Hz), and 4.63 (d, 1 H, $J_{1,2}$ 10.0 Hz). The ^{1}H-decoupled ^{13}C NMR spectrum showed the corresponding anomeric carbons resonating at δ 96.9, 96.0, 95.0, and 85.3. These spectra clearly document the identity of **10** as a tetrasaccharide with the reducing end as a phenyl β-thioglycoside, and containing two α-(1 → 4)-linked and one α-(1 → 6)-linked glucopyranosyl units. The resonances for the anomeric protons, labelled H-1A (4.63), H-1B (5.34), H-1C (5.11), and H-1D (5.69 ppm) as shown in Fig. 3, served as the starting points from which the seven ^{1}H-resonances (H-1 → H-6) associated with each of the four glucopyranosyl residues were assigned from the appropriate cross-peaks (Table I). The ^{13}C resonances for the glucopyranosyl residues **A**, **B**, **C**, and **D** were assigned by comparing the ^{1}H-chemical shift data with the ^{1}H–^{13}C correlation data obtained from the HETCOR[39] experiment (Fig. 4 and Table I).

CONCLUSIONS

The chemical synthesis of two branched-tetrasaccharide thiglycoside derivatives phenyl *O*-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1 → 4)]-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1 → 6)-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-(1 → 4)-2,3-di-*O*-acetyl-1-thio-β-D-glucopyranoside (**10**) and phenyl *O*-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1 → 4)]-*O*-(2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl)-(1 → 6)-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-(1 → 4)-2,3-di-*O*-acetyl-1-thio-β-D-glucopyranoside (**11**) has been accomplished. The method outlined in Scheme 1 for the synthesis of **10** via **4** and **9** results in overall yields of 40, 59, and 40% for the synthesis of **4**, **9**, and **10**, respectively, from maltose octaacetate (**2**). Considering the large number of steps involved, these yields are highly acceptable

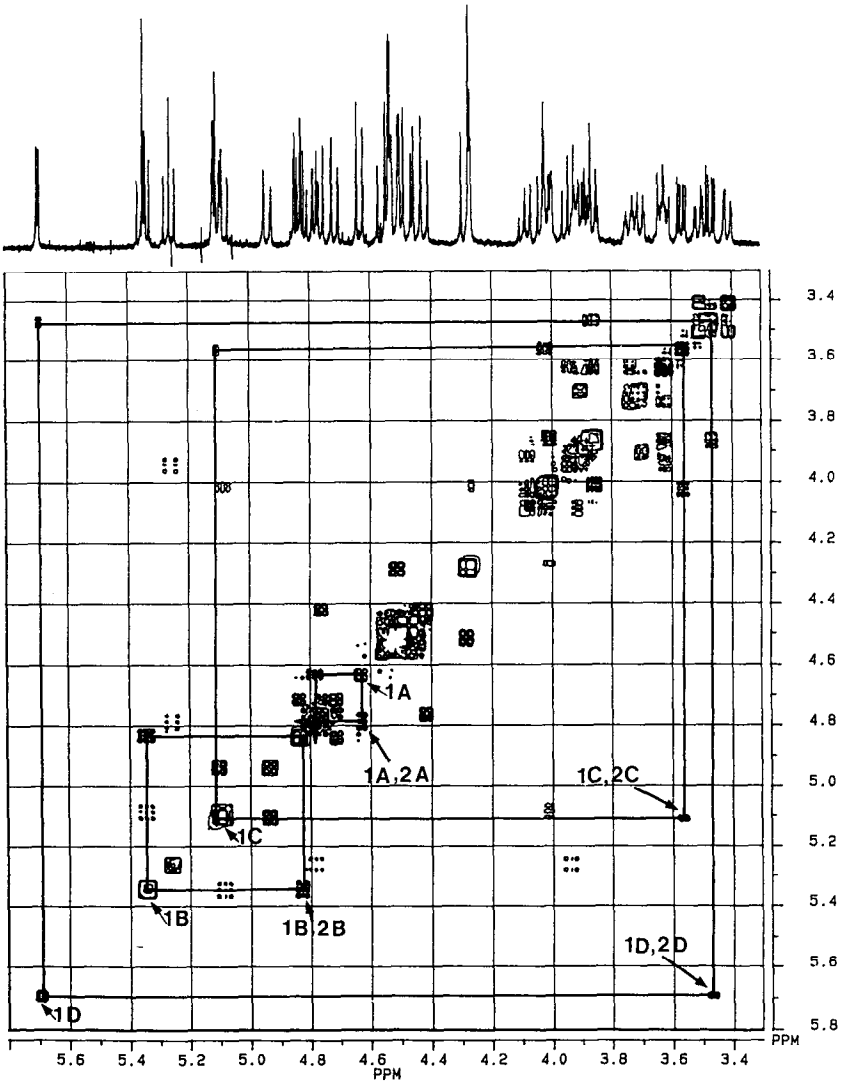


Fig. 3. 500-MHz COSY spectrum of **10** in $CDCl_3$.

and reflect the efforts made to optimize the yield of each individual synthetic reaction.

Thioglycosides are easily converted into glycosyl halides and have therefore received attention as versatile intermediates for glycoside synthesis[31,40–43]. Thioglycosides may also serve as enzyme substrates[44] and as potential enzyme inhibitors[45–47] which modulate the enzymatic activity of specific isozymes. β-Thioglycosides have been shown to bind to the active site of alpha-amylases and to be retained in crystals of the enzyme[48]. After deprotection, the branched tetrasaccharide reported in the present study as well as more complex oligosaccharides

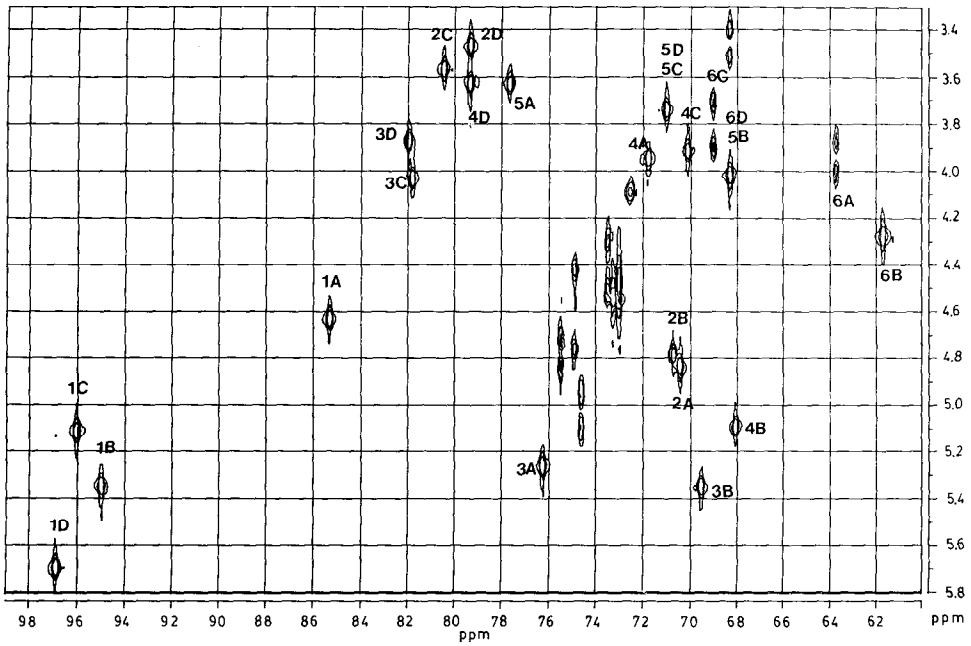


Fig. 4. Partial $^{1}H-^{13}C$ correlation (HETCOR) spectrum of **10**.

synthesized from it may possibly, in a similar manner, be used for unambiguous identification of the active sites of other enzymes involved in polysaccharide synthesis or degradation. If linked to Sepharose, the thioglycosides have the potential for easy and specific purification of such enzymes by affinity chromatography. The chemically synthesized and spectroscopically characterized glycosides reported in the present study might thus be used for purposes other than those directly related to starch analysis.

EXPERIMENTAL

General methods.—Melting points were determined with a Mettler FP81 MBC Cell connected to a Mettler FP80 Central Processor unit and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker AM 500 or AC250P instrument, using Me_4Si as internal standard, unless otherwise indicated. The ^{19}F NMR spectra were recorded with an AC250P spectrometer operated at 235 MHz. The δ_C ($CDCl_3 = 77.0$), δ_H (internal $Me_4Si = 0$), and δ_F (external $CF_3CO_2H = -78.5$) values were measured in $CDCl_3$ unless otherwise stated.

Reactions were monitored by TLC on aluminium sheets coated with silica gel $60F_{254}$ (0.2-mm thickness, E. Merck, Darmstadt, Germany). Column chromatography was carried out using Silica Gel 60 (particle size 0.040–0.063, 230–400 mesh ASTM, E. Merck, Darmstadt, Germany).

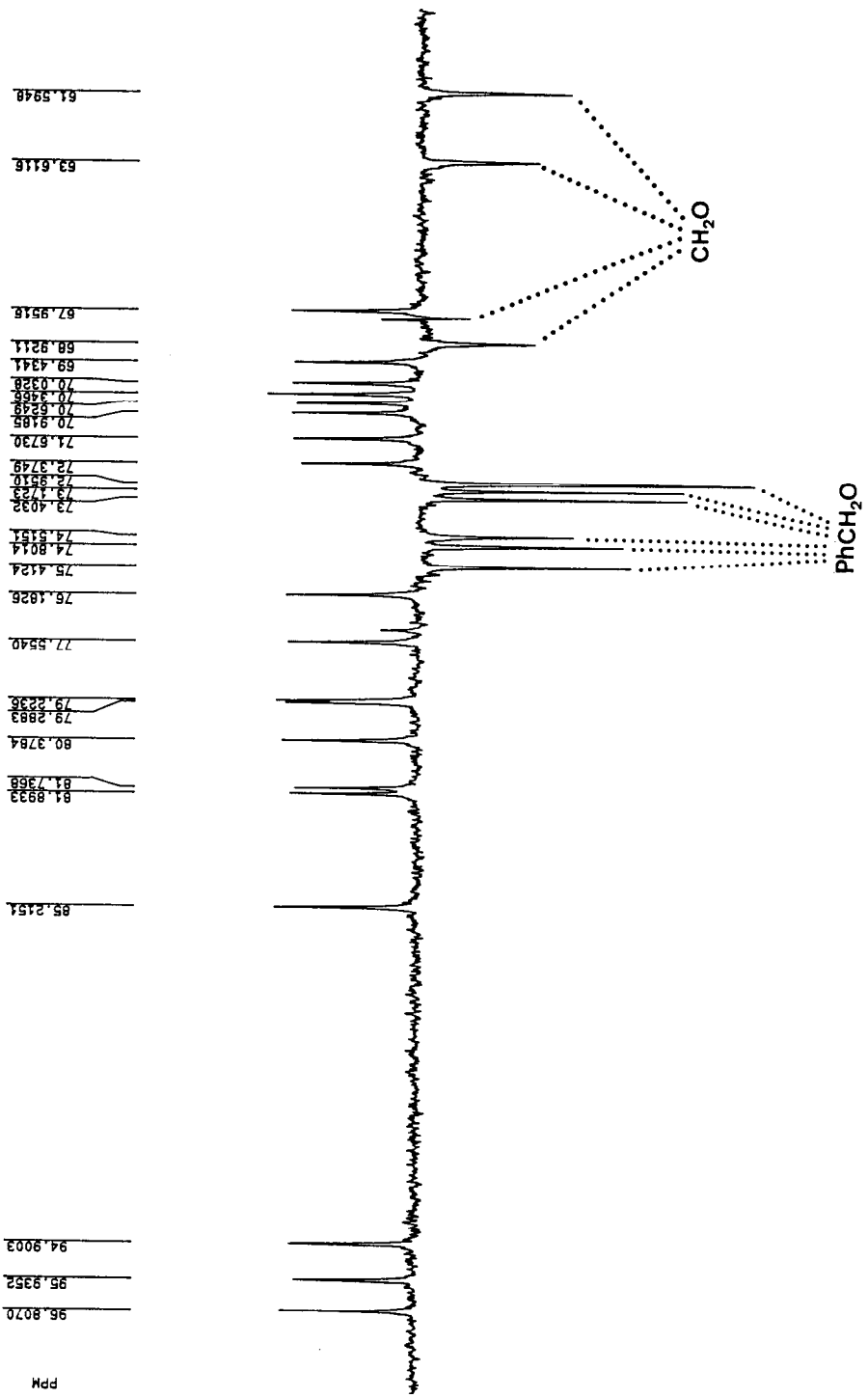


Fig. 5. Partial ^{13}C-DEPT spectrum of **10** recorded at 63 MHz.

Phenyl 2,3-di-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-1-thio-β-D-glucopyranoside (**4**).—A mixture of 2,3-di-*O*-acetyl-1,6-anhydro-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-β-D-glucose (**3**; 10 g, 17.3 mmol), (phenylthio)trimethylsilane (10 mL, 53 mmol), and ZnI_2 (17 g, 53.3 mmol) in dry CH_2Cl_2 (100 mL) was stirred at room temperature for 21 h. The mixture was diluted with EtOAc (400 mL) and washed successively with satd aq $NaHCO_3$ (500 mL), water (3 × 250 mL), and brine (250 mL), dried (Na_2SO_4), and evaporated. The residue was dissolved in THF (50 mL), 1.1 M tetrabutylammonium fluoride in THF (20 mL) was added dropwise with stirring at room temperature, and the mixture was stirred for an additional 20 min. The solvent was evaporated, and a solution of the residue in EtOAc (200 mL) was washed with water (3 × 100 mL), satd aq $NaHCO_3$ (2 × 100 mL), and brine (100 mL), and dried (Na_2SO_4). The solvent was evaporated, the residue was triturated with boiling *n*-pentane (5 × 100 mL), and the resulting paste was crystallized from 96% EtOH to give **4** (11.42 g, 96%, needle-shaped crystals); $[\alpha]_D^{23}$ +53° (*c* 1.00, $CHCl_3$) {lit.[23] $[\alpha]_D^{21}$ +53° ($CHCl_3$)}; mp 171°C (lit.[23], 169–170°C); ^{1}H NMR data (500 MHz, $CDCl_3$): δ 1.99 (s, 4 H, $COCH_3$ and OH), 2.00 (s, 3 H, $COCH_3$), 2.02 (s, 6 H, 2 $COCH_3$), 2.05 (s, 3 H, $COCH_3$), 2.09 (s, 3 H, $COCH_3$), 3.54 (ddd, 1 H, $J_{5,6a}$ 2.1, $J_{5,6b}$ 3.4, $J_{4,5}$ 9.7 Hz, H-5), 3.80 (ddd, 1 H, $J_{5,6b}$ 3.5, $J_{6b,OH}$ 3.7, $J_{6a,6b}$ 12.3 Hz, H-6b), 3.96 (dd, 1 H, $J_{5,6a}$ 2.1, $J_{6a,6b}$ 12.2 Hz, H-6a), 3.99 (dd, 1 H, $J_{5',6'a}$ 2.2, $J_{5',6'b}$ 4.2, $J_{4',5'}$ 10.1 Hz, H-5′), 4.1 (t, 1 H, $J_{3,4} = J_{4,5} = 9.4$ Hz, H-4), 4.11 (dd, 1 H, $J_{5',6'a}$ 2.3, $J_{6'a,6'b}$ 12.5 Hz, H-6′a), 4.27 (dd, 1 H, $J_{5',6'b}$ 4.1, $J_{6'a,6'b}$ 12.5 Hz, H-6′b), 4.78 (d, 1 H, $J_{1,2}$ 9.4 Hz, H-1), 4.82 (dd, 1 H, $J_{1',2'}$ 4.0, $J_{2',3'}$ 10.5 Hz, H-2′), 4.83 (t, 1 H, $J_{1,2} = J_{2,3} = 9.2$ Hz, H-2), 5.03 (dd, 1 H, $J_{3',4'}$ 9.5, $J_{4',5'}$ 10.2 Hz, H-4′), 5.32 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 5.36 (dd, 1 H, $J_{3',4'}$ 9.5 Hz, $J_{2',3'}$ 10.5 Hz, H-3′), 5.44 (d, 1 H, $J_{1',2'}$ 4.0 Hz, H-1′), 7.31 (m, 3 H, H-arom), 7.46 (m, 2 H, H-arom); ^{13}C NMR data (125 MHz, $CDCl_3$): δ 170.7, 170.5, 170.3, 169.9, 169.5, 169.5 (6 $COCH_3$), 132.8 (*o*-C_{arom}), 131.7 (*ipso*-C_{arom}), 129.1 (*m*-C_{arom}), 128.4 (*p*-C_{arom}), 95.1 (C-1′), 85.6 (C-1), 78.6 (C-5), 76.5 (C-3), 70.9 (C-2), 70.2, 70.2 (C-4 and C-2′), 69.4 (C-3′), 68.2 (C-4′), 68.2 (C-5′), 61.8 (C-6′), 61.4 (C-6), 20.9, 20.7, 20.7, 20.6, 20.6, 20.5 (6 $COCH_3$).

Phenyl 2,3-di-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-1-thio-β-D-glucopyranoside (**6**).—Trimethylsilyl trifluoromethanesulfonate (1.25 mL, 6.9 mmol) was added in one portion at room temperature to a stirred solution of maltose octaacetate[10] (10 g, 14.74 mmol) and thiophenol (2 mL, 19.50 mmol) in dry CH_2Cl_2 (50 mL) under Ar. Stirring was continued for 2 h at room temperature. The mixture was diluted with CH_2Cl_2 (200 mL), washed with satd aq $NaHCO_3$ (3 × 100 mL) and water (3 × 100 mL), and dried (Na_2SO_4), and the solvent evaporated. The residue was dissolved in MeOH (100 mL) and treated with 1 M NaOMe in MeOH (2 mL). The solution was stirred for 1 h at room temperature, neutralized with Dowex 50W-X8 (H^+ form, 200–400 mesh) resin, and evaporated to dryness. The residue was then chromatographed on silica gel (150 g) with 4:1 CH_2Cl_2–MeOH as eluent to give amorphous phenyl 1-thio-β-maltoside (**5**; 4.42 g, 69%); $[\alpha]_D^{23}$ +36.3° (*c* 1.24, $CHCl_3$); mp 68–70°C; ^{1}H NMR data (250 MHz, D_2O):

δ 3.64 (d, 1 H, $J_{1,2}$ 10.0 Hz, H-1), 5.37 (d, 1 H, $J_{1',2'}$ 3.8 Hz, H-1'), 7.46 (m, 3 H, H-arom), 7.58 (m, 2 H, H-arom); ^{13}C NMR data (62.50 MHz, D_2O): δ 132.9 (*ipso*-C_{arom}), 132.5 (*o*-C_{arom}), 130.2 (*m*-C_{arom}), 129.0 (*p*-C_{arom}), 100.6 (C-1'), 88.0 (C-1), 79.3 (C-4), 78.5, 77.6 (C-3 and C-5), 73.2, 73.6 (C-3' and C-5'), 72.6, 72.4 (C-2 and C-2'), 70.2 (C-4'), 61.6 (C-6'), 61.3 (C-6). Anal. Calcd for $C_{18}H_{26}O_{10}S \cdot 1.5H_2O$: C, 46.85; H, 6.33; S, 6.95. Found: C, 47.03; H, 6.17; S, 6.78.

A solution of **5** (4 g, 9.20 mmol) in DMF (80 mL) was stirred with NaH (7.75 g; 50% in mineral oil) for 3 h at room temperature and then cooled to 0°C. Benzyl bromide (16 mL) was added dropwise and the mixture was stirred overnight at room temperature. Methanol was added to decompose excess of NaH. The concentrated solution was partitioned between EtOAc and water, and the organic phase was washed several times with water to neutrality, dried (Na_2SO_4), and evaporated. The residual syrup was chromatographed on silica gel (130 g) with 9:1 *n*-pentane–EtOAc as eluent to give **6** (9.26 g, 94%) as a colorless syrup; $[\alpha]_D^{23}$ +27.3° (*c* 0.53, $CHCl_3$). An analytical sample crystallized from *n*-pentane as fiber crystals on standing for three weeks at room temperature; mp 81–83°C; ^{1}H NMR data (250 MHz, $CDCl_3$): δ 7.59 (m, 2 H, SPh), 7.19 (m, 38 H, H-arom), 5.62 (d, 1 H, $J_{1',2'}$ 3.6 Hz, H-1'), 4.69 (d, 1 H, $J_{1,2}$ 9.7 Hz, H-1); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 97.1 (C-1'), 87.7 (C-1), 86.7 (C-3), 82.0 (C-3'), 80.9 (C-2), 79.3 (C-4'), 78.7 (C-2'), 77.7 (C-5'), 75.5, 75.2, 74.9, 74.3, 73.5, 73.3, 73.3 (7 CH_2Ph), 72.7 (C-5), 71.1 (C-4), 69.1 (C-6), 68.2 (C-6'). Anal. Calcd for $C_{67}H_{68}O_{10}S$: C, 75.54; H, 6.43; S, 3.01. Found: C, 75.78; H, 6.57; S, 2.82.

2,3,6-Tri-O-*benzyl*-4-O-(*2,3,4,6-tetra*-O-*benzyl*-α-D-*glucopyranosyl*)-α,β-D-*glucopyranosyl fluoride* (**7**).—Diethylaminosulfur trifluoride (1.8 mL, 13.62 mmol) was added at −15°C to a stirred solution of **6** (8 g, 7.51 mmol) in CH_2Cl_2 (80 mL) under Ar. After 5 min, *N*-bromosuccinimide (2.08 g, 11.69 mmol) was added and the mixture brought to room temperature over a 45-min period. The mixture was diluted with CH_2Cl_2 (50 mL) and filtered through silica gel. The silica gel was washed several times with CH_2Cl_2 (5 × 25 mL), and the collected filtrate was washed thoroughly with satd aq $NaHCO_3$ (2 × 100 mL), water (3 × 100 mL), and brine (100 mL), dried (Na_2SO_4), and evaporated to dryness. The residue was chromatographed on silica gel (150 g) with 4:1 *n*-pentane–diethyl ether as eluent to give **7** (5.69 g, syrup, 78%); $[\alpha]_D^{23}$ +35.6° (*c* 0.54, $CHCl_3$); ^{1}H NMR data (250 MHz, $CDCl_3$): δ 5.67 (d, 1 H, $J_{1',2'}$ 3.8 Hz, H-1', α anomer), 5.59 (d, 1 H, $J_{1',2'}$ 3.8 Hz, H-1', β anomer), 5.55 (dd, 1 H, $J_{1,2}$ 2.8, $J_{H\text{-}1,F}$ 54.8 Hz, H-1, α anomer), 5.36 (dd, 1 H, $J_{1,2}$ 5.5, $J_{H\text{-}1,F}$ 55.0 Hz, H-1, β anomer); ^{13}C NMR data (62.5 MHz, $CDCl_3$): δ 109.4 (C-1, d, $J_{C\text{-}1,F}$ 217.1 Hz, β anomer), 105.0 (C-1, d, $J_{C\text{-}1,F}$ 226.8 Hz, C-1 α anomer), 97.0 (C-1', α anomer), 96.8 (C-1', β anomer); ^{19}F NMR (235 MHz, $CDCl_3$, CF_3CO_2H as external standard): δ 243.8 (dd, $J_{H\text{-}1,F}$ 53.6, $J_{H\text{-}2,F}$ 25.6 Hz, α anomer), 227.1 (dd, $J_{H\text{-}1,F}$ 54.2 Hz, β anomer).

2,3,6-Tri-O-*benzyl*-4-O-(*2,3,4,6-tetra*-O-*benzyl*-α-D-*glucopyranosyl*)-D-*glucopyranose* (**8**).—*N*-Bromosuccinimide (1.8 g, 10.11 mmol) was added to a solution of **6** (9 g, 8.45 mmol) in 9:1 acetone–water (130 mL) and stirred at room temperature

for 45 min. The solvent was evaporated at room temperature until turbidity, and a solution of the residue in EtOAc (200 mL) was washed with satd aq $NaHCO_3$ (3 × 50 mL) and water (3 × 50 mL), dried (Na_2SO_4), and evaporated. The residue was chromatographed on silica gel (150 g) with 3:2 diethyl ether–*n*-pentane as eluent to give **8** (7.88 g, syrup, 96%); $[\alpha]_D^{23}$ +39.3° (*c* 1.35, $CHCl_3$); lit.[49] $[\alpha]_D^{26}$ +39.5° ($CHCl_3$); ^{13}C NMR data (62.5 MHz, $CDCl_3$): δ 97.3 (C-1, β anomer), 96.9 (C-1′, α anomer), 96.8 (C-1′, β anomer), 90.7 (C-1,α anomer)

Preparation of **7** from **8**.—Diethylaminosulfur trifluoride (0.8 mL, 6.06 mmol) was added to a stirred solution of **8** (5 g, 5.14 mmol) in dry CH_2Cl_2 (50 mL) at −15°C under Ar. Stirring was continued and the temperature allowed to rise to room temperature during a 30-min period. The mixture was diluted with CH_2Cl_2 (50 mL) and worked-up as described above to give **7** (4.92 g, 98%).

*2,3,6-Tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-glucopyranosyl)-α,β-*D*-glucopyranosyl trichloroacetimidate* (**9**).—A solution of **8** (7 g, 0.72 mmol) and trichloroacetonitrile (4 mL) in dry CH_2Cl_2 (40 mL) was stirred vigorously with anhyd K_2CO_3 (3.60 g) for 12 h at room temperature under N_2. The mixture was diluted with dry CH_2Cl_2 (20 mL) and filtered through a Celite pad and a silica gel layer. After thorough washing with CH_2Cl_2 (5 × 20 mL), the combined filtrates were evaporated to give **9** (7.64 g, syrup, 95%) in a chromatographically pure form; ^1H NMR data (250 MHz, $CDCl_3$): δ 8.69 (s, 1 H, NH, β anomer), 8.58 (s, 1 H, NH, α anomer), 6.56 (d, 1 H, *J* 3.5 Hz, H-1, α anomer), 5.90 (d, 1 H, *J* 7.0 Hz, H-1, β anomer), 5.70 (d, 1 H, *J* 3.5 Hz, H-1′, α anomer), 5.61 (d, 1 H, *J* 3.6 Hz, H-1′, β anomer); ^{13}C NMR data (62.5 MHz, $CDCl_3$): δ 163.7 (C=NH, β anomer), 161.3 (C=NH, α anomer), 98.1 (C-1, β anomer), 96.9 (C-1′, β anomer), 96.8 (C-1′, α anomer), 94.1 (C-1, α anomer), 91.8 (CCl_3, β anomer), 91.3 (CCl_3, α anomer).

Phenyl O-*[(2,3,4,6-tetra-*O*-benzyl-α-*D*-glucopyranosyl)-(1 → 4)]-*O*-(2,3,6-tri-*O*-benzyl-α-*D*-glucopyranosyl)-(1 → 6)-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl)-(1 → 4)-2,3-di-*O*-acetyl-1-thio-β-*D*-glucopyranoside* (**10**) *and phenyl* O-*[(2,3,4,6-tetra-*O*-benzyl-α-*D*-glucopyranosyl)-(1 → 4)]-*O*-(2,3,4-tri-*O*-benzyl-β-*D*-glucopyranosyl)-(1 → 6)-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl)-(1 → 4)-2,3-di-*O*-acetyl-1-thio-β-*D*-glucopyranoside* (**11**).—*Method A*. A solution of **4** (5.60 g, 8.16 mmol) and **9** (7.50 g, 6.71 mmol) in dry 2:1 diethyl ether–CH_2Cl_2 (150 mL) was stirred for 30 min at room temperature in the presence of 4A molecular sieves (3 g, activated powder) under Ar. The stirred mixture was then cooled to −20°C and trimethylsilyl trifluoromethanesulfonate (0.4 mL, 2.20 mmol) was added. The temperature was raised to room temperature over a period of 2 h. After dilution with diethyl ether (250 mL), solid $NaHCO_3$ (10 g) was added and stirring was continued for 10 min. The mixture was filtered through Celite. The filtrate was washed thoroughly with satd aq $NaHCO_3$ (2 × 100 mL), water (2 × 100 mL), and brine (50 mL), dried (Na_2SO_4), and evaporated to dryness. The residue was chromatographed on silica gel (150 g) with 3:2 diethyl ether–*n*-pentane as eluent to give pure **10** (7.42 g, white powder after recrystallization from 96% EtOH, 67%); $[\alpha]_D^{21}$ +60.9° (*c* 0.45, $CHCl_3$); mp 68–69°C; and pure **11** (2.35 g, white powder after recrystallization

from 96% EtOH, 21%); $[\alpha]_D^{23}$ +46.7° (*c* 0.54, $CHCl_3$); mp 64–66°C; ^1H NMR data (500 MHz, $CDCl_3$) for **10**: δ 1.92 (s, 3 H, $COCH_3$), 1.98 (s, 3 H, $COCH_3$), 1.98 (s, 3 H, $COCH_3$), 1.99 (s, 3 H, $COCH_3$), 2.04 (s, 3 H, $COCH_3$), 2.08 (s, 3 H, $COCH_3$), 4.29, 4.51 (2 d, 2 H, *J* 12.2 Hz, PhCH_2), 4.42, 4.76 (2 d, 2 H, *J* 10.9 Hz, PhCH_2), 4.47 (d, 1 H, *J* 12.3 Hz, PhC*H*), 4.48, 4.56 (2 d, 2 H, *J* 12.0 Hz, PhCH_2), 4.52 (d, 1 H, *J* 11.7 Hz, PhC*H*), 4.55 (m, 2 H, PhCH_2), 4.71, 4.84 (2 d, 2 H, *J* 10.9 Hz, PhCH_2). 4.94, 5.08 (2 d, 2 H, *J* 12.1 Hz, PhCH_2), 7.52–7.09 (m, 40 H, H-arom); ^{13}C NMR data (125 MHz, $CDCl_3$): δ 170.3, 170.3, 170.2, 169.8, 169.5, 169.5, (6 $COCH_3$), 139.2, 138.9, 138.6, 138.3, 138.0, 137.9, 137.8 (7 *ipso*-C_{arom}, benzyl), 134.2 (*o*-C_{arom}, SPh), 130.8 (*ipso*-C_{arom}, SPh), 129.1 (*m*-C_{arom}, SPh), 128.7 (*p*-C_{arom}, SPh), 128.4, 128.2, 128.2, 128.2, 128.2, 128.0, 127.9 (7 *o*-C_{arom}, benzyl), 127.9, 127.8, 127.8 127.7, 127.7, 127.6, 127.5 (7 *m*-C_{arom}, benzyl), 127.5, 127.4, 127.4, 127.3, 127.3, 126.9, 126.6 (7 *p*-C_{arom}, benzyl), 76.2, 75.4, 74.8, 74.5, 73.5, 73.2, 73.0 (7 PhCH_2O), 20.9, 20.8, 20.7, 20.7, 20.6, 20.4 (6 CH_3CO). For **11**, ^1H NMR data (500 MHz, $CDCl_3$): δ 1.93 (s, 3 H, $COCH_3$), 1.96 (s, 3 H, $COCH_3$), 1.98 (s, 3 H, $COCH_3$), 2.01 (2 s, 6 H, 2 $COCH_3$), 2.06 (s, 3 H, $COCH_3$), 4.29, 4.51 (2 d, 2 H, *J* 12.4 Hz, PhCH_2), 4.44, 4.78 (2 d, 2 H, *J* 10.9 Hz, PhCH_2), 4.52 (d, 1 H, *J* 12.2 Hz, PhC*H*), 4.58, 4.59 (2 d, 2 H, *J* 12.0 Hz, PhCH_2), 4.53, 4.73 (2 d, 2 H, *J* 11.6 Hz, PhCH_2), 4.75, 4.87 (2 d, 2 H, *J* 10.8 Hz, PhCH_2), 4.76 (d, 1 H, *J* 12.5 Hz, PhC*H*), 4.88, 4.97 (2 d, 2 H, *J* 11.5 Hz, PhCH_2), 7.46–7.09 (m, 40 H, H-arom); ^{13}C NMR data (125 MHz, $CDCl_3$): δ 170.4, 170.4, 170.1, 169.7, 169.5, 169.5 (6 $COCH_3$), 138.8, 138.7, 138.7, 138.4, 138.2, 138.0, 137.9 (7 *ipso*-C_{arom}, benzyl), 132.8 (*o*-C_{arom}, SPh), 131.8 (*ipso*-C_{arom}, SPh), 129.0, 128.3, 128.3, 128.2, 128.2, 128.2, 128.1, 128.1, 128.0 (9 C, *m*-C_{arom}, SPh, *p*-C_{arom}, SPh, and 7 *o*-C_{arom}, benzyl), 127.9, 127.8, 127.8, 127.7, 127.6, 127.6, 127.5 (7 *m*-C_{arom}, benzyl), 127.5, 127.4, 127.4, 127.4, 127.4, 127.1, 126.7 (7 *p*-C_{arom}, benzyl), 75.4, 74.9, 74.9, 74.0, 73.5, 73.2, 73.2 (7 PhCH_2O), 20.8, 20.7, 20.6, 20.6, 20.5, 20.5 (6 CH_3CO) (for signals not related to the protecting groups, and their assignment, see Tables I and II) Anal. Calcd for $C_{91}H_{100}O_{26}S$: C, 66.57; H, 6.14; S, 1.95. Found for **10**: C, 66.20; H, 6.09; S, 2.07, Found for **11**: C, 66.20; H, 6.19, S, 1.89

Method B. A solution of **4** (4.81 g, 7 mmol) in dry CH_2Cl_2 (50 mL) was added at −15°C to a stirred suspension of $AgClO_4$ (2.66 g, 12.88 mmol), $SnCl_2$ (2.44 g, 12.88 mmol), and 4A molecular sieves (3 g, activated powder) in dry diethyl ether (200 mL) under Ar with protection from light. After 5 min, a solution of the fluoride **7** (6.83 g, 7 mmol) in dry diethyl ether (100 mL) was added. Stirring was continued and the temperature was raised to room temperature over a period of 2 h, after which the mixture was worked-up essentially as in Method A. After purification by silica gel chromatography, pure **10** (6.32 g, white powder after recrystallization from 96% EtOH, 55%); $[\alpha]_D^{21}$ +60.9° (*c* 0.28, $CHCl_3$); mp 68–69°C; and pure **11** (1.15 g, white powder after recrystallization from 96% EtOH, 10%); $[\alpha]_D^{23}$ +46.7° (*c* 0.71, $CHCl_3$); mp 64–66°C, were obtained. Anal. Calcd for $C_{91}H_{100}O_{26}S$: C, 66.57; H, 6.14; S, 1.95. Found for **10**: C, 66.31; H, 6.14; S, 1.93. Found for **11**: C, 66.37; H, 6.20; S, 1.94.

ACKNOWLEDGMENTS

Part of this work was supported by the Nordic Industrial Foundation, Center for Plant Biotechnology, the Food Technology Programme, and the Danish Agricultural and Veterinary Research Council.

REFERENCES

1 J. Preiss, in *Biochem. Plants,* 16 (1988) 181–254.
2 J.L. Ozbun, J.S. Hawker, and J. Preiss, *Plant Physiol.*, 48 (1971) 765–769.
3 C. Pollock and J. Preiss, *Arch. Biochem. Biophys.*, 204 (1980) 578–588.
4 A.S. Ponstein, K. Oosterhaven, W.J. Feenstra, and B. Witholt, *Staerke*, 43 (1991) 208–220.
5 F.D. MacDonald and J. Preiss, *Plant Physiol.*, 78 (1985) 849–852.
6 C.D. Boyer and J. Preiss, *Biochem. Biophys. Res. Commun.*, 80 (1978) 169–175.
7 M. Steup, in *Biochem. Plants*, 14 (1988) 255–296.
8 E. Bertoft, *Carbohydr. Res.*, 189 (1989) 195–208.
9 E. Bertoft and S.-E. Kulp, *J. Inst. Brew., London*, 92 (1986) 69–72.
10 M.L. Wolfrom and A. Thompson, *Methods Carbohydr. Chem.*, 1 (1962) 334–335.
11 L. Asp and B. Lindberg, *Acta Chem. Scand.*, 6 (1952) 941–946.
12 I. Fujimaki, Y. Ichikawa, and H. Kuzuhara, *Carbohydr. Res.*, 101 (1982) 148–151.
13 K. Bock and H. Pedersen, *Acta Chem. Scand., Ser. B,* 42 (1988) 75–85.
14 M.V. Rao and M. Nagalajan, *Carbohydr. Res.*, 162 (1987) 141–144.
15 M. Georges and B. Fraser-Reid, *Carbohydr. Res.*, 127 (1984) 162–164.
16 D. Lafont, P. Boullanger, O. Cadas, and G. Descotes, *Synthesis*, (1989) 191–194.
17 H. Paulsen, *Angew. Chem. Int. Ed. Engl.*, 21 (1982) 155–173.
18 M. Černý and J. Stanék, Jr., *Adv. Carbohydr. Chem. Biochem.*, 34 (1977) 23–177.
19 F. Dasgupta and P.J. Garegg, *Synthesis*, (1988) 626–628.
20 M. Georges, D. Mackay, and B. Fraser-Reid, *J. Am. Chem. Soc.*, 104 (1982) 1101–1103.
21 T. Uryu, K. Hatanaka, K. Matsuzaki, and H. Kuzuhara, *Macromolecules*, 16 (1983) 853–858.
22 S. Hanessian and Y. Guindon, *Carbohydr. Res.*, 86 (1980) c3.
23 N. Sakairi, M. Hayashida, A. Amano, and H. Kuzuhara, *J. Chem. Soc., Perkin Trans. 1,* (1990) 1301–1313.
24 M.S. Motawia, J. Marcussen, and B. Møller, *J. Carbohydr. Chem.*, submitted.
25 R.R. Schmidt and J. Michel, *Tetrahedron Lett.*, 25 (1984) 821–824, and references therein.
26 B. Wegmann and R.R. Schmidt, *J. Carbohydr. Chem.*, 6 (1987) 357–375.
27 K. Bock and C. Pedersen, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 27–66; K. Bock, C. Pedersen, and H. Pedersen, *ibid.*, 42 (1984) 193–225.
28 R.R. Schmidt, *Angew. Chem.*, 98 (1986) 213–236, *Angew. Chem. Int. Ed. Engl.*, 25 (1986) 21–235.
29 B.A. Silwanis, R.I. El-Sokkary, M. Nashed, and H. Paulsen, *J. Carbohydr. Chem.*, 10 (1991) 1067–1078.
30 T. Mukaiyama, Y. Murai, and S. Shoda, *Chem. Lett.*, (1981) 431–432.
31 K.C. Nicolaou, R.E. Dolle, D.P. Papahatjis, and J.L. Randall, *J. Am. Chem. Soc.*, 106 (1984) 4189–4192.
32 W.J. Middleton, *J. Org. Chem.*, 40 (1975) 574–578.
33 G.H. Posner and S.R. Haines, *Tetrahedron Lett.*, 26 (1985) 5–8.
34 B. Casu, M. Reggiani, G.G. Gallo, and A. Vigevani, *Carbohydr. Res.*, 12 (1970) 157–170.
35 P.L. Durette, L. Hough, and A.C. Richardson, *J. Chem. Soc., Perkin Trans. 1* (1974) 88–96.
36 P.L. Durette, L. Hough, and A.C. Richardson, *J. Chem. Soc., Perkin Trans. 1* (1974) 97–101.
37 D.Y. Gagnaire, F.R. Taravel, and M.R. Vignon, *Carbohydr. Res.*, 51 (1976) 157–168.
38 K. Nagayama, A. Kumar, K. Wuthrich, and R.R. Ernst, *J. Magn. Reson.*, 40 (1980) 321–334.
39 J.A. Wilde and P.H. Boltor, *J. Magn. Reson.*, 59 (1984) 343–346.
40 T. Norberg and H. Ritzen, *Glycoconjugate J.*, 3 (1986) 135–142.
41 H. Lönn, *J. Carbohydr. Chem.*, 6 (1987) 301–306.

42 K.C. Nicolaou, J.L. Randall, and G.T. Furst, *J. Am. Chem. Soc.,* 107 (1985) 5556–5558.
43 V. Pozsgay and H.J. Jennings, *J. Org. Chem.,* 53 (1988) 4042–4052.
44 M. Blanc-Muesser, J. Defaye, and H. Driguez, *J. Chem. Soc., Perkin Trans. 1,* (1984) 1885–1889.
45 K. Bock, J. Defaye, H. Driguez, and E. Bar-Guilloux, *Eur. J. Biochem.,* 131 (1983) 595–600.
46 O. Kanie, J. Nakamura, Y. Itoh, M. Kiso, and A. Hesegawa, *J. Carbohydr. Chem.,* 6 (1987) 117–128.
47 M. Blanc-Muesser and H. Driguez, *J. Chem. Soc., Perkin Trans. 1,* (1988) 3345–3351.
48 P.J. Stankiewicz, D. Cascio, and A. McPherson, *J. Appl. Biochem.,* 5 (1983) 388–398.
49 K. Takeo and T. Imai, *Carbohydr. Res.,* 165 (1987) 123–128.