PREPARATION AND REACTIONS OF 2-CHLOROETHYL 1-THIO-β-D-GLYCOPYRANOSIDES DERIVED FROM D-GALACTOSE, D-GLUCOSE, AND 2-ACETAMIDO-2-DEOXY-D-GLUCOSE

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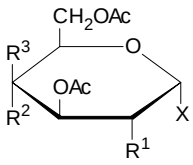
Chloroethyl 1-thio-β-D-glycopyranosides of the D-*galacto* and D-*gluco* configurations **5a–5c** were prepared by alkylation of the corresponding 1-thio-β-D-hexopyranoses **3a–3c** with 1-bromo-2-chloroethane followed by deacetylation. The starting 1-thio-β-D-hexopyranoses were obtained from the acetylated glycopyranosyl halides via isothiouronium salts. It was demonstrated that the chloroethyl thioglycosides **5a–5c** undergo hydrolysis in aqueous solutions to give the 2-hydroxyethyl thioglycosides **6a–6c** and reducing hexoses and that this hydrolysis proceeds via episulfonium salts. The hydrolysis was monitored by ^{1}H and ^{13}C NMR spectroscopy. In 1% aqueous solutions of sodium carbonate containing phenol or aniline, the thioglycosides **5a–5c** provide, in addition to the above hydrolysis products, also the phenoxyethyl and phenylaminoethyl thioglycosides **9a**, **10a** and **9b**, **10b**, respectively.
Key words: 2-Halogenoethyl thioglycosides; Synthesis; Nucleophilic substitution; Mechanism of hydrolysis; ^{1}H and ^{13}C NMR.

Thioglycosides are known to be more reluctant to undergo acid hydrolysis than glycosides and not to be split by normal glycosidases[1]. This property of thioglycosides is utilized in affinity chromatography[2] and in the preparation of enzyme formation inducers. In view of those facts, investigation of the feasibility of functionalizing polymeric carriers, proteins and/or peptides with thioglycosyl groups under mild conditions in aqueous solutions is of interest. Glycosylation agents for this purpose may include 2-haloethyl thioglycosides, containing an atom of chlorine, bromine or iodine in their molecules; such compounds have been examined only scarcely so far. The present work was aimed at developing a suitable procedure for the preparation of such substances and investigating their behaviour as glycosylation agents.

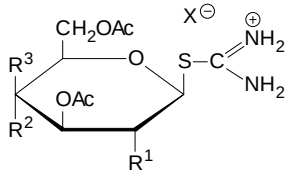
The 2-haloethyl thioglycopyranosides were prepared by a modification of the published procedure[3,4]. The starting compounds, viz. acetylated α-D-glycopyranosyl halides **1a–1c**, were converted to isothiouronium salts **2a–2c** by reaction with thiourea in acetone, and split[3,5] to the corresponding acetylated 1-thio-β-D-glycopyranoses **3a–3c.** Alkyla-

tion of the latter with 1-bromo-2-chloroethane or 1,2-dibromoethane, with subsequent nucleophilic substitution of chlorine with bromine or iodine as appropriate, gave the acetylated 2-haloethyl β-D-thioglycopyranosides **4a–4g**. The corresponding 2-hydroxyethyl thioglycosides **4h**, **4i** were obtained by analogous alkylation with 2-iodoethanol or by reaction with oxirane. Deacetylation of the thioglycosides **4a–4c**, **4h**, **4i** with sodium methoxide in methanol gave the thioglycosides **5a–5c** or **6a**, **6b**, respectively. Attempts at a similar deacetylation of the bromoethyl and iodoethyl thioglycosides **4d–4g** were accompanied by splitting off of the halogen and formation of solvolysis products, mainly 2-methoxyethyl thioglycosides, which were not in the focus of our interest.

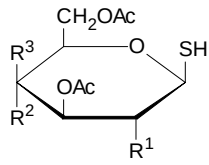
The chloroethyl thioglycosides **5a–5c** are well soluble in water but their aqueous solutions hydrolyze slowly, whereupon the chlorine atom is substituted by a hydroxy group giving rise to the 2-hydroxyethyl thioglycosides **6a–6c**; simultaneously, a side reaction occurs, during which the C(1)–S bond is split and an anomeric mixture of the



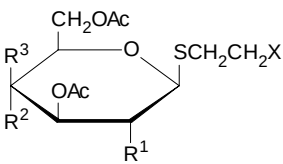
	R^1	R^2	R^3	X
1a	OAc	H	OAc	Br
1b	OAc	OAc	H	Br
1c	NHAc	OAc	H	Cl



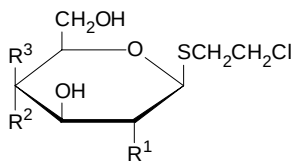
	R^1	R^2	R^3	X
2a	OAc	H	OAc	Br
2b	OAc	OAc	H	Br
2c	NHAc	OAc	H	Cl



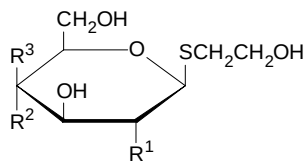
	R^1	R^2	R^3
3a	OAc	H	OAc
3b	OAc	OAc	H
3c	NHAc	OAc	H



	R^1	R^2	R^3	X
4a	OAc	H	OAc	Cl
4b	OAc	OAc	H	Cl
4c	NHAc	OAc	H	Cl
4d	OAc	H	OAc	Br
4e	OAc	OAc	H	Br
4f	OAc	H	OAc	I
4g	OAc	OAc	H	I
4h	OAc	H	OAc	OH
4i	OAc	OAc	H	OH



	R^1	R^2	R^3
5a	OH	H	OH
5b	OH	OH	H
5c	NHAc	OH	H



	R^1	R^2	R^3
6a	OH	H	OH
6b	OH	OH	H
6c	NHAc	OH	H

corresponding hexoses, viz. D-galactose, D-glucose, and 2-acetamido-2-deoxy-D-glucose, is formed. The time development of the hydrolysis and the structure of its products were monitored by ^{1}H and ^{13}C NMR spectroscopy in deuterium oxide solutions. The reaction mixture compositions in 48 h are given in Table I.

The NMR data of the starting chloroethyl thioglycosides **5a–5c** were measured immediately after dissolution. The 2-hydroxyethyl thioglycosides **6a–6c** are the major hydrolysis products, whose NMR data were obtained after allowing the solutions of the compounds **5a–5c** to stand for several hours; authentic samples were also measured. The replacement of the chlorine atom by the hydroxy group manifests itself most markedly by upfield shifts of signals of the S–CH_2 hydrogens (about –0.2 ppm) and the anomeric H-1 hydrogen (about –0.05 ppm). The expected downfield shift of the CH_2X hydrogen signal (about 0.1 ppm), on the other hand, is overlapped to a considerable extent by the signals in the region of δ 3.5–3.9. The ^{13}C NMR spectra exhibit a marked downfield shift for the CH_2X group (approximately 17 ppm) while the signals of the remaining carbons are very little affected (<1 ppm). The ^{1}H and ^{13}C NMR data of substances **5a–5c**, **6a–6c** are given in Tables II and III. The reducing hexopyranoses, which are formed in small amounts, were identified based on the ^{1}H and ^{13}C NMR spectra of authentic samples.

We assumed[6] that the mechanism of hydrolysis of substances **5a–5c** is similar to that for other 2-chloroethyl sulfides[7], i.e. that in the polar aqueous medium, the chloride ion is split off with the participation of the neighbouring sulfur atom giving rise to the unstable episulfonium salts **7**, which give the hydroxyethyl thioglycosides **6a–6c,** thiirane, and hexopyranosyl cations **8** which, in turn, react with water to give the reducing hexoses (Scheme 1).

Since the reaction of the chloroethyl thioglycosides **5a–5c** is accompanied by liberation of the equivalent amount of hydrogen chloride, we verified on authentic samples of

TABLE I
Reaction of chloroethyl thioglycosides **5a–5c** with water. Population of the components of reaction mixture (after 48 h standing)

Starting compound	Population of reaction components after 48 h standing			
	–SCH_2CH_2Cl	–SCH_2CH_2OH	–OH(α)[a]	–OH(β)[b]
5a	8% (**5a**)	78% (**6a**)	5% (Gal-α)	9% (Gal-β)
5b	10% (**5b**)	74% (**6b**)	6% (Glc-α)	10% (Glc-β)
5c	5.5% (**5c**)	86% (**6c**)	5% (GlcNAc-α)	3.5% (GlcNAc-β)

[a] Corresponding α-D-hexoses. [b] Corresponding β-D-hexoses.

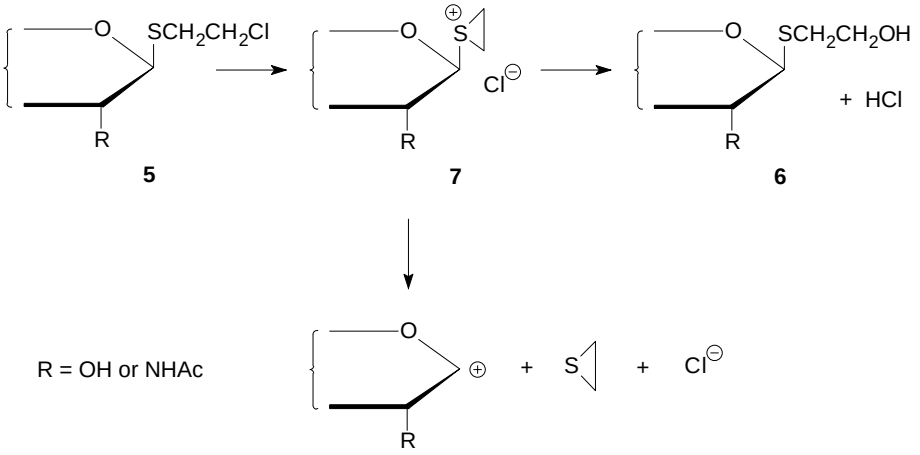
TABLE II
Proton NMR parameters of compounds **3–6, 9, 10**. Chemical shift in ppm and coupling constants in Hz

Compound[a]	Solvent[b]	H-1 *J*(1,2)	H-2 *J*(2,3)	H-3 *J*(3,4)	H-4 *J*(4,5)	H-5 *J*(5,6a)	H-6a *J*(5,6b)	H-6b *J*(6a,6b)	–S–CH_AH_B–	–CH_CH_D–X
									J(A,B); *J*(A,C); *J*(A,D); *J*(B,C); *J*(B,D); *J*(C,D)	
3c	Chl	4.62 dd	4.13 m	5.12 m		3.72 ddd	4.24 dd	4.13 dd	–	–
		(10.1)	([c])	([c])	(9.8)	(4.9)	(2.3)	(12.5)		
4a	Chl	4.56 d	5.23 t	5.06 dd	5.44 dd	3.98 ddd	4.15 dd	4.11 dd	3.13 ddd; 2.93 ddd	3.74 ddd; 3.70 ddd
		(10.0)	(10.0)	(3.4)	(1.2)	(7.1)	(5.9)	(11.5)	(14.0; 10.0; 5.9; 6.1; 9.9; 10.8)	
4b	Chl	4.55 d	5.03 dd	5.23 t	5.07 dd	3.74 ddd	4.20 dd	4.17 dd	3.09 ddd; 2.91 ddd	3.72 ddd; 3.67 ddd
		(10.0)	(9.3)	(9.5)	(10.0)	(5.1)	(2.7)	(12.3)	(13.9; 10.0; 5.9; 6.0; 10.0; 11.0)	
4c	Chl	4.71 d	4.07 dt	5.20 dd	5.07 dd	3.74 ddd	4.19 dd	4.16 dd	3.12 ddd; 2.92 ddd	3.76 ddd; 3.67 ddd
		(10.3)	(10.2)	(9.4)	(10.0)	(5.2)	(2.9)	(12.3)	(14.0; 10.0; 5.8; 5.7; 10.0; 10.7)	
4e	Chl	4.55 d	5.03 dd	5.23 t	5.07 dd	3.74 ddd	4.20 dd	4.17 dd	3.17 ddd; 2.99 ddd	3.57 ddd; 3.53 ddd
		(10.0)	(9.3)	(9.5)	(10.0)	(4.9)	(2.9)	(12.3)	(14.2; 11.0; 5.6; 5.9; 11.0; 9.8)	
4g	Chl	4.56 d	5.03 dd	5.23 t	5.07 dd	3.74 ddd	4.19 dd	4.17 dd	3.21 ddd; 3.03 ddd	3.37 ddd; 3.34 ddd
		(10.0)	(9.3)	(9.5)	(10.0)	(4.9)	(2.9)	(12.2)	(13.9; 11.2; 5.6; 6.0; 11.2; 9.5)	
5a	W	4.53 d	3.54 t	3.63 dd	3.96 d	3.70 ddd	3.74 dd	3.70 dd	3.16 ddd; 3.06 ddd	3.80 ddd; 3.77 ddd
		(9.8)	(9.4)	(3.4)	(1.0)	(7.4)	(4.4)	(12.0)	(14.1; 8.2; 6.5; 6.5; 8.2; 10.9)	
5b	W	4.59 d	3.30 dd	3.47 t	3.39 t	3.45 ddd	3.88 dd	3.68 dd	3.14 ddd; 3.05 ddd	3.80 ddd; 3.76 ddd
		(9.9)	(8.8)	(9.0)	(9.6)	(2.1)	(5.7)	(12.4)	(14.2; 8.1; 6.5; 6.5; 8.1; 10.8)	

TABLE II
(*Continued*)

Compound[a]	Solvent[b]	H-1 *J*(1,2)	H-2 *J*(2,3)	H-3 *J*(3,4)	H-4 *J*(4,5)	H-5 *J*(5,6a)	H-6a *J*(5,6b)	H-6b *J*(6a,6b)	–S–CH_AH_B–	–CH_CH_D–X
									J(A,B); *J*(A,C); *J*(A,D); *J*(B,C); *J*(B,D); *J*(C,D)	
5c	W	4.67 d	3.73 m	3.53 dd	~3.45 m		3.89 dd	3.71 dd	3.13 ddd; 3.01 ddd	3.78 ddd; 3.74 ddd
		(10.5)	(9.8)	(8.8)	([c])	(1.7)	(5.6)	(12.2)	(14.2; 7.8; 6.6; 6.6; 7.8; 11.3)	
6a	W	4.51 d	3.58 t	3.66 dd	3.99 dd	3.70 m–3.82 m			2.98 dt; 2.88 dt	3.70–3.82 m
		(9.8)	(9.5)	(3.4)	(1.0)	([c])	([c])	([c])	(13.9; 6.2; 6.2; 6.5; 6.5; [c])	
6b	W	4.51 d	3.27 dd	3.44 t	3.36 dd	3.42 ddd	3.85 dd	3.65 dd	2.91 dt; 2.81 dt	3.74 m
		(9.9)	(8.8)	(8.9)	(9.6)	(2.2)	(5.8)	(12.5)	(14.0; 6.2; 6.2; 6.5; 6.5; [c])	
6c	W	4.65 d	3.75 m	3.55 t	~3.46 m		3.90 dd	3.73 dd	2.93 dt; 2.82 dt	3.71–3.79 m
		(10.4)	(9.3)	(9.3)	([c])	(1.7)	(5.4)	(12.4)	(13.8; 6.2; 6.2; 6.4; 6.4; [c])	
9a	Me	4.42 d	3.58 dd	3.47 dd	3.89 dd	3.55 ddd	3.74 dd	3.69 dd	3.15 ddd; 2.99 ddd	4.23 ddd; 4.20 ddd
		(9.7)	(9.3)	(3.3)	(1.1)	(6.8)	(5.2)	(11.4)	(13.8; 7.6; 6.3; 6.3; 7.6; 9.9)	
9b	Me	4.36 d	3.57 t	3.46 dd	3.89 dd	3.52 ddd	3.75 dd	3.70 dd	3.01 dt; 2.84 dt	3.38 m
		(9.6)	(9.2)	(3.3)	(1.2)	(6.7)	(5.3)	(11.2)	(13.6; 6.5; 6.5; 6.4; 6.4; [c])	
10a	Me	4.59 d	3.77 dd	3.45 dd	3.36 dd	3.30 ddd	3.86 dd	3.67 dd	3.15 ddd; 2.95 ddd	4.22 ddd; 4.16 ddd
		(10.3)	(9.8)	(8.6)	(9.7)	(2.2)	(5.8)	(12.1)	(13.8; 7.2; 6.3; 6.1; 7.2; 9.8)	
10b	Me	4.54 d	3.75 dd	3.43 dd	3.35 dd	3.29 ddd	3.88 dd	3.68 dd	3.00 dt; 2.78 dt	3.35 m
		(10.4)	(9.9)	(8.6)	(9.7)	(2.2)	(5.8)	(12.0)	(13.6; 7.1; 6.3; 7.0; 7.0; [c])	

[a] Other protons in: **3c** SH: 2.57 d (*J* = 9.4); NH: 5.94 d (J = 9.5); 3 × OAc and NAc: 2.10 s; 2.05 s; 2.03 s; 1.99 s; **4a** 4 × OAc: 2.17 s; 2.09 s; 2.08 s; 1.99 s; **4b** 4 × OAc: 2.11 s; 2.06 s; 2.04 s; 2.02 s; **4c** 3 × OAc and NAc: 2.11 s; 2.05 s; 2.04 s; 1.97 s; NH: 5.81 d (*J* = 9.2); **4e** 4 × OAc: 2.12 s; 2.06 s; 2.04.s; 2.02 s; **4g** 4 × OAc: 2.13 s; 2.06 s; 2.04 s; 2.01 s; **5c** NAc: 2.04 s; **6c** NAc: 2.04 s; **9a** Ph: 6.92 m (*o*-); 7.25 m (*m*-); 6.91 m (*p*-); **9b** Ph: 6.68 m (*o*-); 7.11 m (*m*-); 6.63 m (*p*-); **10a** Ph: 6.91 m (*o*-); 7.25 m (*m*-); 6.91 m (*p*-); NAc: 1.96 s; **10b** Ph: 6.66 m (*o*-); 7.10 m (*m*-); 6.62 m (*p*-); NAc 1.96 s. [b] Chl deuteriochloroform; W deuterium oxide; Me tetradeuteriomethanol. [c] The value of parameter could not be determined.



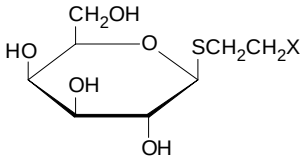
SCHEME 1

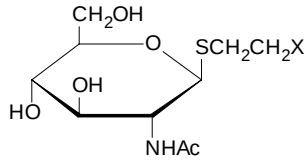
hydroxyethyl thioglycosides **6a–6c** that the decrease in the pH value of the reaction medium does not cause their hydrolysis to the reducing hexoses even in several days of standing at room temperature. In agreement with the expected hydrolysis mechanism, we were able to demonstrate that the thiirane signal (singlet at δ 2.38) is present in the ^{1}H NMR spectra of chloroform extracts of aqueous solutions of the chloroethyl thioglycosides **5a–5c**; addition of the authentic sample of thiirane increases the intensity of that signal. The mechanism of this reaction of aqueous solutions of chloroethyl thioglycosides is also borne out by the facts that water-insoluble thiirane polymers separate from the solutions and that authentic thiirane also polymerizes to poly(thiirane) in similar reaction conditions[8]. The thiirane polymers were characterized by their NMR spectra (see Experimental).

TABLE III
Carbon-13 chemical shifts (ppm) of compounds **5** and **6** in D_2O

Compound	C-1	C-2	C-3	C-4	C-5	C-6	S–CH_2–	–CH_2–X	–NHAc
5a	87.42	80.52	70.86	70.17	75.17	62.52	33.92	45.41	–
5b	88.39	82.68	75.08	72.27	79.96	63.66	35.30	46.76	–
5c	85.62	55.71	80.93	70.72	76.07	61.81	33.74	44.77	175.43; 23.09
6a	87.32	80.30	71.06	70.17	75.25	62.52	33.75	62.52	–
6b	88.23	82.68	75.08	72.27	80.20	63.66	35.06	63.90	–
6c	85.33	55.70	80.81	70.71	76.06	61.80	33.43	61.94	175.40; 23.07

Furthermore, we examined the reactions of phenol and aniline with the thioglycosides **5a–5c** in 1% aqueous solutions of sodium carbonate[9]. We found that in addition to the hydrolysis products of the starting thioglycosides **5a–5c** (see above), the corresponding 2-phenoxyethyl or 2-phenylaminoethyl derivatives **9a**, **10a** and **9b**, **10b**, respectively, are also formed in yields of 27–56%.





9a, X = OC_6H_5
9b, X = NHC_6H_5

10a, X = OC_6H_5
10b, X = NHC_6H_5

The corresponding phenyl glycosides or *N*-phenylglycosylamines failed to be identified in the reaction mixtures; this is consistent with the short lifetime of the hexopyranosyl cations[10], which react rapidly with the surrounding water molecules. Based on these results we developed a method of 1-thioglycosylethylation of some proteins and polymeric carriers in aqueous medium in physiological conditions[11].

The structure of the compounds prepared within this work and their occurrence in the $^4C_1$ conformations was proved by NMR spectroscopy (Table II).

EXPERIMENTAL

The melting points, determined on a Boëtius micro melting-point apparatus, are uncorrected. The optical rotations were measured on a Bendix–Ericsson ETL-NPL 143 A polarimeter at 20 to 22 °C. The ^{1}H and ^{13}C NMR spectra were measured on a Varian UNITY-500 spectrometer (^{1}H at 500 MHz; ^{13}C at 125.7 MHz) in $CDCl_3$, D_2O, or CD_3OD solutions. The 2D-COSY technique was applied to the structural assignment of the proton signals where appropriate. The ^{13}C NMR spectra were measured by using the attached proton test pulse sequence. Mass spectra were measured on a ZAB-EQ (VG Analytical) instrument using the FAB method (TG matrix, Xe ionization). Thin layer chromatography was performed on DC Alufolien plates (Merck) having a layer of Kieselgel 60 F_{254}. Detection consisted in spraying with 50% sulfuric acid and mineralization; an UV lamp was also employed for derivatives of aromatic compounds. Preparative chromatography was carried out in columns containing Kieselgel 60 silica gel (Merck) 70–230 mesh ASTM. The solutions were evaporated at a reduced pressure (water jet pump) at temperatures not exceeding 40 °C. Ethylene sulfide (thiirane, purity >97%) was obtained from Fluka Chemie AG.

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-D-glucopyranose (**3c**)

To a solution of the thiouronium salt[12] **2c** (24.0 g, 54 mmol) in water (55 ml) were added potassium disulfite (10.5 g, 47 mmol) and chloroform (100 ml), and the stirred mixture was heated on a water bath at 80 °C for 20 min. The chloroform layer was separated, washed with water, dried with anhy-

drous Na_2SO_4, and distilled to dryness. The product was recrystallized from ethanol. Yield 18.1 g (91%), m.p. 165–167 °C, $[\alpha]_D$ –19° (*c* 0.75, chloroform) (ref.[13]: m.p. 167–168 °C, $[\alpha]_D$ –14.5° (*c* 0.9, chloroform)). TLC (chloroform–methanol 15 : 1): R_F 0.13. For $C_{14}H_{21}NO_8S$ (363.8) calculated: 46.27% C, 5.82% H, 3.85% N, 8.82% S; found: 46.21% C, 5.83% H, 3.91% N, 8.76% S.

Acetates of 2-Chloroethyl Thioglycosides **4a–4c**. General Procedure

To a solution of the thiohexose **3a–3c** (10 g, 27.5 mmol) in acetone (35 ml) were added a solution of K_2CO_3 (3.85 g, 77 mmol) in water (30 ml) and 1-bromo-2-chloroethane (11 g, 77 mmol). The mixture was agitated under argon at room temperature and the reaction was monitored by TLC using the benzene–acetone 4 : 1 or ethyl acetate–benzene 2 : 1 system. After completion of the reaction (roughly in 1 h), the reaction mixture was extracted twice with chloroform (60 ml in total), the combined extracts were washed with 3% hydrochloric acid and water and dried with anhydrous sodium sulfate, and the solvent was removed by evaporation. The remaining syrup was crystallized from anhydrous methanol or from a dichloromethane–ether–light petroleum mixture.

2-Chloroethyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-*galactopyranoside* (**4a**). The starting thiohexose **3a** was prepared following refs[4,5]. Yield 9.9 g (85%), m.p. 76–78 °C, $[\alpha]_D$ –10° (*c* 1.2, chloroform) (ref.[14]: syrup, $[\alpha]_D$ –6.4° (*c* 0.5, chloroform)). For $C_{16}H_{23}ClO_9S$ (426.9) calculated: 45.02% C, 5.43% H, 8.31% Cl, 7.51% S; found: 45.13% C, 5.46% H, 8.64% Cl, 7.65% S.

2-Chloroethyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-*glucopyranoside*[15] (**4b**). The starting thiohexose **3b** was prepared following ref.[3]. Yield 9.5 g (81%), m.p. 97–98 °C, $[\alpha]_D$ –38° (*c* 0.7, chloroform). For $C_{16}H_{23}ClO_9S$ (426.9) calculated: 45.02% C, 5.43% H, 8.31% Cl, 7.51% S; found: 45.28% C, 5.41% H, 8.06% Cl, 7.33% S.

2-Chloroethyl 2-acetamido-3,4,6-O-acetyl-2-deoxy-β-D-*glucopyranoside* (**4c**). This compound, which was prepared from the thiohexose **3c**, crystallized directly after diluting the reaction mixture with water. Yield 10.1 g (86%), m.p. 196–198 °C, $[\alpha]_D$ –28° (*c* 0.2, chloroform). TLC (ethyl acetate–2-propanol–water 9 : 4 : 2): R_F 0.83. For $C_{16}H_{24}ClNO_8S$ (425.9) calculated: 45.12% C, 5.68% H, 8.32% Cl, 3.39% N, 7.53% S; found: 45.29% C, 5.63% H, 8.17% Cl, 3.25% N, 7.83% S.

2-Bromoethyl 2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (**4d**)

The substance was prepared following ref.[4] and purified for analytical purposes by chromatography on an L 100/250 silica gel column (Lachema); elution with chloroform. Syrup, $[\alpha]_D$ –5.7° (*c* 0.5, chloroform).

2-Bromoethyl 2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (**4e**)

The substance was prepared following ref.[3] and purified by chromatography on a silica gel column; elution with chloroform. M.p. 108–110 °C, $[\alpha]_D$ –32° (*c* 0.5, chloroform).

2-Iodoethyl 2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (**4f**)

Solution of **4d** (5.0 g, 12.5 mmol) in anhydrous acetone (50 ml) was heated with sodium iodide (2.45 g, 61 mmol) to boil under a reflux condenser for 2 h. The separated sodium bromide was filtered off and the solution was evaporated to a syrup. The latter was dissolved in chloroform and washed with water (2 × 25 ml), and the chloroform layer was dried with anhydrous sodium sulfate and evaporated to dryness. Yield 4.9 g (89%), syrup, $[\alpha]_D$ –7.6° (*c* 0.5, chloroform). For $C_{16}H_{23}IO_9S$ (521.3) calculated: 36.86% C, 4.45% H, 24.85% I; found: 37.19% C, 4.51% H, 24.15% I.

2-Iodoethyl 2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (**4g**)

The substance was prepared from the starting compound **4e** by a procedure[15] analogous to that used for the iodo derivative **4f**. The syrup was crystallized from ethanol. Yield 4.9 g (89%), m.p. 125–126 °C, $[\alpha]_D$ –35.8° (*c* 0.5, chloroform). For $C_{16}H_{23}IO_9S$ (521.3) calculated: 36.86% C, 4.45% H, 24.85% I, 6.15% S; found: 37.15% C, 4.69% H, 24.23% I, 6.27% S.

2-Hydroxyethyl 2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-galactopyranoside[14] (**4h**)

A) Thiogalactose **3a** (10.9 g, 30 mmol) in acetone (30 ml) was alkylated by agitation with 2-iodoethanol (5.8 g, 33 mmol) in the presence of K_2CO_3 (4.14 g, 30 mmol). The reaction mixture was treated as reported above for substances **4a–4c**. The syrup obtained was chromatographed on a column of Silica Gel L 100/250 (Lachema); elution with chloroform. Yield 8.1 g (70%), crystalline solidified syrup, $[\alpha]_D$ +23° (*c* 0.5, chloroform). TLC (benzene–ethyl acetate 1 : 1): R_F 0.25. For $C_{16}H_{24}O_{10}S$ (408.4) calculated: 47.05% C, 5.93% H, 7.85% S; found: 47.28% C, 6.02% H, 7.62% S.

B) A gentle stream of oxirane was fed for roughly 2 h into a solution of thiogalactose **3a** (7.2 g, 20 mmol) in chloroform (50 ml). After completion of the reaction (TLC, see above), the reaction mixture was nitrogen purged and extracted consecutively with 5% sodium hydroxide (25 ml), 5% sulfuric acid (25 ml), and water. After drying, the chloroform solution was evaporated and chromatographed (see procedure *A*). Yield 6.7 g (88%), properties identical with those of the product prepared sub *A*).

2-Hydroxyethyl 2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (**4i**)

A) The substance was prepared by alkylation of compound **3b** (10.9 g, 30 mmol) with 2-iodoethanol (5.8 g, 33 mmol) by procedure *A* for substance **4h** above. Yield 10.5 g (86%), m.p. 70–73 °C, $[\alpha]_D$ –14° (*c* 0.5, chloroform). TLC (benzene–ethyl acetate 1 : 1): R_F 0.24. For $C_{16}H_{24}O_{10}S$ (408.4) calculated: 47.05% C, 5.93% H, 7.85% S; found: 47.32% C, 6.21% H, 7.62% S.

B) The substance was obtained by reacting compound **3b** (7.2, 20 mmol) with oxirane as described in procedure *B* for substance **4h** above. Yield 6.6 g (87%).

Preparation of the Free Thioglycosides **5a–5c**, **6a, 6b** by Deacetylation after Zemplén

To a solution of acetylated thioglycoside **4a–4c**, **4h**, **4i** (10 g) in anhydrous methanol (100 ml) was added sodium methoxide prepared from sodium (0.1 g) and methanol (10 ml). The system was allowed to stand for 2 h at room temperature or overnight in a refrigerator at 0 °C, during which the deacetylation was accomplished (TLC monitoring using the benzene–acetone 4 : 1 or ethyl acetate–2-propanol–water 9 : 4 : 2 system). Where the crystalline product failed to separate, the solution was neutralized by adding a catex (Dowex 50 W × 8 in the H^+ cycle) and evaporated to a syrup. Additional fractions were obtained from the mother liquors on crystallization from methanol.

*2-Chloroethyl 1-thio-*β*-D-galactopyranoside* (**5a**). M.p. 93–98 °C (decomposition), $[\alpha]_D$ –23° (*c* 0.8, water), (ref.[4]: m.p. 92–98 °C, $[\alpha]_D$ –24.6° (*c* 0.5, water)), yield 5.5 g (91%). The product crystallizes with an approximately equimolar amount of methanol, which can be removed by drying in a vacuum over P_2O_5 at 64 °C. For $C_8H_{15}ClO_5S$ (258.7) calculated: 37.14% C, 5.84% H, 13.70% Cl, 12.39% S; found: 37.19% C, 5.84% H, 13.81% Cl, 12.37% S.

*2-Chloroethyl 1-thio-*β*-D-glucopyranoside* (**5b**). Yield 5.4 g (90%) of a partly crystallized syrup, whose purification for elemental analysis failed; $[\alpha]_D$ –38° (*c* 0.7, water).

*2-Chloroethyl 2-acetamido-2-deoxy-1-thio-*β*-D-glucopyranoside* (**5c**). Yield 6.24 g (89%), m.p. 174–176 °C (dec.), $[\alpha]_D$ –34° (*c* 0.4, methanol). For $C_{10}H_{18}ClNO_5S$ (299.8) calculated: 40.06% C, 6.05% H, 11.83% Cl, 4.67% N, 10.69% S; found: 37.89% C, 6.10% H, 11.53% Cl, 4.43% N, 10.51% S.

2-Hydroxyethyl 1-thio-β-D-*galactopyranoside* (**6a**). Yield 4.9 g (83%), m.p. 148–150 °C, $[\alpha]_D$ –21° (*c* 0.7, water) (ref.[4]: m.p. 151–152 °C, $[\alpha]_D$ –21.4° (*c* 0.5, water); ref.[16]: m.p. 148.5–150 °C, $[\alpha]_D$ –21.8° (*c* 0.55, water)).

2-Hydroxyethyl 1-thio-β-D-*glucopyranoside* (**6b**). Yield 4.8 g (81%), m.p. 98–99 °C, $[\alpha]_D$ –55° (*c* 0.27, water). For $C_8H_{16}O_6S$ (240.3) calculated: 39.98% C, 6.66% H, 13.33% S; found: 40.31% C, 6.50% H, 13.28% S.

Reactions of Phenol or Aniline with Chloroethyl Thioglycosides **5a** and **5c**. Preparation of Thioglycosides **9a**, **9b**, **10a**, **10b**

Phenol (1.88 g, 20 mmol) or aniline (1.86 g, 20 mmol) and chloroethyl thioglycoside **5a** (1 g, 3.9 mmol) or **5c** (1 g, 3.3 mmol) were added to a solution of sodium carbonate (1 g, 9.4 mmol) in water (100 ml), and the whole was stirred vigorously at room temperature for 24 h. The reaction was monitored by TLC using the ethyl acetate–2-propanol–water 9 : 4 : 2 system. After all the starting chlorothioglycoside had reacted, the reaction mixture was extracted with benzene (3 × 10 ml), the pH of the aqueous solution was adjusted with acetic acid to pH 7, and the solution was filtered with activated carbon and evaporated to dryness. The residue was treated as given below, and chromatographed on a silica gel column (50 g) using the ethyl acetate–2-propanol–water 16 : 2 : 1 system.

2-Phenoxyethyl 1-thio-β-D-*galactopyranoside* (**9a**). The evaporation residue was dissolved in water and the solution was demineralized (Dowex 50 W × 8 in the H^+ cycle) and evaporated to dryness. The residue was chromatographed on a silica gel column. Substance **5a** (1 g) gave the thioglycoside **9a** in a yield of 0.34 g (28%); the product was recrystallized from ethanol. M.p. 103–105 °C, $[\alpha]_D$ –24° (*c* 1.8, methanol). TLC: R_F 0.39. For $C_{14}H_{20}O_6S$ (316.4) calculated: 53.15% C, 6.37% H, 10.13% S; found: 52.83% C, 6.37% H, 10.06% S.

2-Phenylaminoethyl 1-thio-β-D-*galactopyranoside* (**9b**). The evaporation residue was extracted with hot anhydrous methanol, the solvent was removed by evaporation, and the residue was chromatographed on a silica gel column. Thioglycoside **5a** (2.7 g) and aniline afforded thioglycoside **9b** (0.68 g, 56%), which was recrystallized from an ethanol–ether mixture. M.p. 127 – 130 °C, $[\alpha]_D$ –26° (*c* 0.27, ethanol). TLC: R_F 0.34. For $C_{14}H_{21}NO_5S$ (315.4) calculated: 53.32% C, 6.71% H, 4.44% N, 10.17% S; found: 53.04% C, 6.86% H, 4.34% N, 10.16% S.

2-Phenoxyethyl 2-acetamido-2-deoxy-1-thio-β-D-*glucopyranoside* (**10a**). The product crystallized directly from the reaction mixture and was recrystallized from ethanol. Thioglycoside **5c** and phenol gave thioglycoside **10a** in a yield of 0.5 g (42%). M.p. 200–202 °C, $[\alpha]_D$ –31° (*c* 0.23, methanol). TLC: R_F 0.44. FAB MS, *m/z*: 358 (M + H), 380 (M + Na). For $C_{16}H_{23}NO_6S$ (357.0) calculated: 53.77% C, 6.49% H, 3.92% N, 8.97% S; found: 53.24% C, 6.60% H, 3.70% N, 8.60% S.

2-Phenylaminoethyl 2-acetamido-2-deoxy-1-thio-β-D-*glucopyranoside* (**10b**). The evaporation residue was extracted with 2-propanol, the solvent was removed by evaporation, and the residue was chromatographed on a silica gel column. Thioglycoside **5c** (1.0 g) gave substance **10b** in a yield of 0.49 g (41%). M.p. 152–154 °C, $[\alpha]_D$ –31° (*c* 0.11, ethanol). TLC: R_F 0.48. FAB MS, *m/z*: 357 (M + H), 379 (M + Na), for $C_{16}H_{24}N_2O_5S$ calculated *m/z* 356.

NMR Monitoring of Hydrolysis of Chloroethyl Thioglycosides **5a–5c**

A) The chloroethyl thioglycoside **5a**, **5b**, or **5c** (0.1 mmol) was dissolved in deuterium oxide and its NMR spectrum was measured immediately. Subsequently, the solution was allowed to stand at room temperature and stirred occasionally. In 30 or 60 min the solution was extracted with deuteriochloroform (1 ml) and the spectra of the two phases were measured. The aqueous phase contained, in addition to the starting substance, the α and β anomers of the reducing hexose (D-galactose, D-glucose or 2-acetamido-2-deoxy-D-glucose) and the corresponding 2-hydroxyethyl thioglycoside **6a**, **6b**,

or **6c**. The ^{1}H NMR spectrum of the chloroform layer exhibited the thiirane signal (singlet at δ 2.38), whose intensity increased on addition of the authentic sample.

B) Solution of the chloroethyl thioglycoside **5a**, **5b**, or **5c** in deuterium oxide was allowed to stand at room temperature, and the decomposition was monitored by NMR spectroscopy. The reaction mixture composition in 48 h of standing is given in Table I. The fractions of the components were determined based on the intensities of signals of the anomeric carbons C-1, hydrogens H-1, and hydrogens in the CH_2OH or CH_2Cl group in the aglycone part of the glycoside. A small quantity of a white amorphous precipitate separated slowly from the solution due to the polymerization of thiirane. The precipitate was mixed with water, centrifuged, and dried over P_2O_5, and its NMR spectra were measured in hexadeuteriodimethyl sulfoxide. The spectra contained signals identical with those of poly(thiirane) formed from authentic thiirane in aqueous hydrochloric acid in conditions simulating those of hydrolysis of chloroethyl thioglycosides (see later), as well as signals corresponding to the sugar parts. The presence of hexoses and hydroxyethyl glycosides in the reaction mixture after the hydrolysis of the chloroethyl thioglycosides **5a–5c** was also demonstrated by chromatography on a Whatman No. 4 paper using the butanol–acetic acid–water 4 : 1 : 3 system, applying detection with ammoniacal solution of silver nitrate and heating. Before application, the solution samples were neutralized with the anex Dowex 2 in the HO^- cycle.

Polymerization of Authentic Thiirane in 1 M HCl

Thiirane (0.6 g, 10 mmol) was added to 1 M HCl (10 ml), and the mixture was agitated occasionally at room temperature. Gradually, the solution became turbid and a white amorphous substance precipitated. The precipitate was separated by centrifugation, rinsed with distilled water, and dried over P_2O_5. The poly(thiirane) so obtained was partly dissolved in hexadeuteriodimethyl sulfoxide, and its ^{13}C and ^{1}H NMR spectra were measured. The ^{13}C NMR spectrum only displayed signals of carbon atoms in the CH_2–S group (δ 24.51, 27.41, 31.48, 31.64, 31.76. 31.97, 32.02, 34.07, 35.20) and in the CH_2–O group (δ 61.24). Similarly, the ^{1}H NMR spectrum showed signals of hydrogen atoms in the CH_2–S group at δ 2.45–2.91 and in the CH_2–O group at δ 3.53–3.75 at the ratio of approximately 12 : 1 in favour of the former group.

Stability of Hydroxyethyl Thioglycosides **6a**, **6b** in 1 M HCl

Solutions of the substances **6a**, **6b** (10 mg) in 1 M HCl (2 ml) were allowed to stand at room temperature for 7 days, and their composition was monitored by TLC on silica gel using the chloroform–2-propanol–concentrated ammonia–water 10 : 10 : 1 : 1 system. No reducing hexoses were detected with 50% sulfuric acid and mineralization.

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REFERENCES

1. Monod J., Cohn M.: Adv. Enzymol. Relat. Areas Mol. Biol. *13*, 67 (1952).
2. Cuatrecasas P.: Adv. Enzymol. Relat. Areas Mol. Biol. *36*, 29 (1972).
3. Cerny M., Vrkoc J., Stanek J.: Collect. Czech. Chem. Commun. *24*, 64 (1959).
4. Frgala J., Cerny M., Stanek J.: Collect. Czech. Chem. Commun. *40*, 1411 (1975).
5. Cerny M., Stanek J., Pacak J.: Monatsh. Chem. *94*, 290 (1963).
6. Hykl J.: *M.S. Thesis*. Charles University, Prague 1989.

7. Tagaki W. in: *Organic Chemistry of Sulfur* (S. Oae, Ed.), p. 256. Plenum Press, New York 1977; and references therein.
8. Culvenor C. C. J., Davies W., Heath N. S.: J. Chem. Soc. *1949*, 282.
9. Stehlikova I.: *M.S. Thesis*. Charles University, Prague 1995.
10. Sinnot M. L.: Chem. Rev. *90*, 1171 (1990); and references therein.
11. Ticha M., Cerny M., Trnka T.: Glycoconjugate J. *13*, 681 (1996).
12. Horton D., Wolfrom M. L.: J. Org. Chem. *27*, 1794 (1962).
13. Meyer zu Reckendorf W., Bonner W. A.: J. Org. Chem. *26*, 4596 (1961).
14. Frgala J.: *Ph.D. Thesis*. Charles University, Prague 1973.
15. Zbrankova J.: *M.S. Thesis*. Charles University, Prague 1993.
16. Gero S. D., Burstein C.: Biochim. Biophys. Acta *117*, 314 (1966).