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PREPARATION OF ANOMERIC PAIRS OF 1-THIOGLYCOSIDES: USE OF ANOMERIZATION CATALYZED BY BORON TRIFLUORIDE*

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ABSTRACT

Anomeric pairs of some alkyl 1-thioaldopyranosides of D-galactose, D-glucose, D-mannose, 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-galactose, and L-fucose were prepared. The per-*O*-acetylated, 1,2-*trans* anomers of 6-(trifluoroacetamido)hexyl 1-thioaldopyranosides and 5-(methoxycarbonyl)pentyl 1-thioaldopyranosides were anomerized with boron trifluoride in dichloromethane. The anomeric mixtures were then separated by chromatography, using columns of either silica gel or an ion-exchange resin. De-blocking of the separated compounds provided pure anomers of 6-aminohexyl 1-thioaldopyranosides or 5-carboxypentyl 1-thioaldopyranosides. The aglycons of the latter glycosides were further extended by reaction with aminoacetaldehyde diethyl acetal, which, after deacetalization of the products, provided an ω-aldehydo group. These series of glycosides could be readily coupled to proteins or solid matrices.

INTRODUCTION

Alkyl 1-thioaldopyranosides containing an amino, imido ester, or aldehyde group at the terminal position in the aglycon have previously been synthesized in our laboratory[1–3] for use in affinity chromatography[4], studies of intercellular adhesion[5], and the preparation of neoglycoproteins[6]. These 1-thioglycosides were all of the 1,2-*trans* configuration, but, because many naturally occurring oligosaccharides contain sugars linked in the 1,2-*cis* configuration, the availability of anomeric pairs of 1-thioglycosides is highly desirable.

We now report a method for the preparation of anomeric pairs of alkyl 1-thioglycosides that contain either an amino or aldehyde group in the aglycon. This technique utilizes boron trifluoride-catalyzed anomerization[7] of the readily available 1,2-*trans* anomers, followed by chromatographic separation of the anomers. By this

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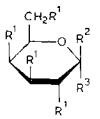
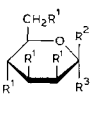
procedure, we have prepared anomeric pairs of the per-*O*-acetylated 6-aminohexyl 1-thioaldopyranosides and 5-(methoxycarbonyl)pentyl 1-thioaldopyranosides of D-glucose, D-galactose, 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-galactose, D-mannose, and L-fucose. In order to introduce an aldehyde group into the aglycon, peracetylated 5-(methoxycarbonyl)pentyl 1-thioaldopyranosides were de-esterified, and the terminal carboxyl group was then allowed to react with aminoacetaldehyde diethyl acetal. The resulting 1-thioglycosides, after deacetalization, could



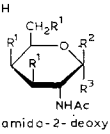
D-Galactose derivatives

	R^1	R^2	R^3
1	Ac	$S(CH_2)_6NHCOCF_3$	H
2	Ac	H	$S(CH_2)_6NHCOCF_3$
3	OH	$S(CH_2)_6NH_2$	H
4	OH	H	$S(CH_2)_6NH_2$
5	Ac	$S(CH_2)_5CO_2Me$	H
6	Ac	H	$S(CH_2)_5CO_2Me$
7	OH	$S(CH_2)_5CONHCH_2CH(OEt)_2$	H
8	OH	H	$S(CH_2)_5CONHCH_2CH(OEt)_2$



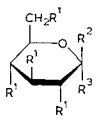
D-Mannose derivatives

	R^1	R^2	R^3
13	Ac	$S(CH_2)_6NHCOCF_3$	H
14	Ac	H	$S(CH_2)_6NHCOCF_3$
15	OH	$S(CH_2)_6NH_2$	H
16	OH	H	$S(CH_2)_6NH_2$
17	Ac	$S(CH_2)_5CO_2Me$	H
18	Ac	H	$S(CH_2)_5CO_2Me$
19	OH	$S(CH_2)_5CONHCH_2CH(OEt)_2$	H
20	OH	H	$S(CH_2)_5CONHCH_2CH(OEt)_2$



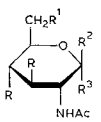
2-Acetamido-2-deoxy-D-galactose derivatives

	R^1	R^2	R^3
29	Ac	$S(CH_2)_5CO_2Me$	H
30	Ac	H	$S(CH_2)_5CO_2Me$
31	OH	$S(CH_2)_5CONHCH_2CH(OEt)_2$	H
32	OH	H	$S(CH_2)_5CONHCH_2CH(OEt)_2$



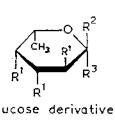
D-Glucose derivatives

	R^1	R^2	R^3
9	Ac	$S(CH_2)_6NHCOCF_3$	H
10	Ac	H	$S(CH_2)_6NHCOCF_3$
11	OH	$S(CH_2)_6NH_2$	H
12	OH	H	$S(CH_2)_6NH_2$



2-Acetamido-2-deoxy-D-glucose derivatives

	R^1	R^2	R^3
21	Ac	$S(CH_2)_6NHCOCF_3$	H
22	Ac	H	$S(CH_2)_6NHCOCF_3$
23	OH	$S(CH_2)_6NH_2$	H
24	OH	H	$S(CH_2)_6NH_2$
25	Ac	$S(CH_2)_5CO_2Me$	H
26	Ac	H	$S(CH_2)_5CO_2Me$
27	OH	$S(CH_2)_5CONHCH_2CH(OEt)_2$	H
28	OH	H	$S(CH_2)_5CONHCH_2CH(OEt)_2$



L-Fucose derivatives

	R^1	R^2	R^3
33	Ac	H	$S(CH_2)_6NHCOCF_3$
34	Ac	$S(CH_2)_6NHCOCF_3$	H
35	OH	H	$S(CH_2)_6NH_2$
36	OH	$S(CH_2)_6NH_2$	H
37	Ac	H	$S(CH_2)_5CO_2Me$
38	Ac	$S(CH_2)_5CO_2Me$	H
39	OH	H	$S(CH_2)_5CONHCH_2CH(OEt)_2$
40	OH	$S(CH_2)_5CONHCH_2CH(OEt)_2$	H

41 $HO(CH_2)_6NHCOCF_3$

42 $I(CH_2)_6NHCOCF_3$

43 $Br(CH_2)_5CO_2Me$

be coupled to proteins by reductive amination[8]. The availability of these 1-thioglycosides should facilitate studies of anomeric specificity in a variety of biological systems involving carbohydrates.

EXPERIMENTAL

Materials. — The following compounds were obtained from the indicated sources, and used without further purification: 1,2,3,4,6-penta-*O*-acetyl-D-galactose (Koch–Light Lab.); 1,2,3,4,6-penta-*O*-acetyl-D-glucose (Pfanstiehl Lab.); D-mannose, L-fucose, 2-acetamido-2-deoxy-D-glucose and -D-galactose (Sigma Chem. Co.); ethyl trifluoroacetate, 6-amino-1-hexanol, and 6-aminohexanoic acid (Aldrich Chem. Co.); aminoacetaldehyde diethyl acetal (Fluka); 3-(dimethylaminopropyl)-1-ethylcarbodiimide · HCl (EDAC; Story Chem. Corp.); Dowex-50 X8 and -1 X8 (Dow Chem. Co.); silica gel, type 60H (E. Merck); and boron trifluoride (Matheson).

General methods. — Evaporations were performed in a rotary evaporator at 20–50° under diminished pressure. Melting points (uncorrected) were measured with a Fisher–Johns apparatus. Proton magnetic resonance (p.m.r.) spectra were recorded with a JEOL NMH-100 spectrometer for solutions in $CDCl_3$ (acetylated sugars) or in D_2O (non-acetylated sugars). Thin-layer chromatography (t.l.c.) was performed on precoated layers (0.25 mm thick) of silica gel Type 60F-254 on aluminum sheets (E. Merck). Spray reagents for t.l.c. were 15% (v/v) sulfuric acid in 50% (v/v) ethanol for carbohydrates; 0.2% (w/v) ninhydrin in acetone for amino groups; 0.4% (w/v) 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) in phosphate buffer, pH 7.5, for sulfhydryl groups, and 0.4% (2,4-dinitrophenyl)hydrazine (DNP-hyd) in 2M HCl for aldehyde groups; u.v. absorption and iodine staining were also used for general detection. Neutral sugar in fractions from preparative-scale column-chromatography was determined by a modification of the phenol–sulfuric acid procedure[9]; effluents from analytical-scale columns were analyzed with an automated, sugar analyzer[10]. Conductivity was measured by use of a Thomas–Serfass Conductance Bridge. Optical rotations (589 nm) of solutions in water at 22° were measured with a Cary 60 spectropolarimeter.

Preparation of 6-(trifluoroacetamido)-1-hexanol (**41**). — A solution of 6-amino-1-hexanol (3 g, 25.6 mmol) in ethyl trifluoroacetate (3.6 mL, 30 mmol) was stirred for 5 h at room temperature, poured into water (50 mL), and stirred overnight at 4°; the crystalline product (3.82 g, 70% yield) was collected by filtration, m.p. 48–49° (lit.[1] m.p. 52–53°); p.m.r. data ($CDCl_3$): δ 1.2–1.8 (m, 8 H, C-CH_2), 2.73 (s, 1 H, OH), 3.3 (q, 2 H, N-CH_2), 3.56 (t, 2 H, O-CH_2), and 7.35 (s, 1 H, NH).

This compound was converted into 1-iodo-6-(trifluoracetamido)hexane (**42**) by the procedure already described[1].

Preparation of methyl 6-bromohexanoate (**43**). — *Method A.* Diazomethane (~25 mmol) in ether (50 mL) was generated as described[11]. To this solution was added a solution of 6-bromohexanoic acid (4.9 g, 25 mmol) in ether (20 mL). After the mixture had been kept on ice for 2 h, the excess of diazomethane was quenched

with acetic acid, and the solution evaporated to a yellow liquid. This was applied to a column (2 × 10 cm) of silica gel Type 60H, and the column eluted with 1:2 (v/v) toluene–ether. Effluent fractions were monitored by t.l.c., and those that contained the u.v.-absorbing, iodine-positive product were pooled, to yield 5.22 g (100% yield) of clear, liquid **43**.

Method B. A solution of 6-bromohexanoic acid in dry methanol (200 mL) was refluxed for 90 min with Dowex-50 X-8 (H^+) resin equilibrated with dry methanol. After filtration, evaporation, and chromatography on silica, as in Method A, **43** (41.8 g, 76% yield) was obtained. Characteristics of **43** were: d_{22} 1.49; p.m.r. data ($CDCl_3$): δ 1.2–2 (m, 6 H, C-CH_2), 2.25 (t, 2 H, CO_2-CH_2), 3.28 (t, 2 H, Br-CH_2), and 6.52 (s, 3 H, O-CH_3).

*Preparation of 1,2-*trans*-6-aminohexyl 1-thioaldopyranosides.* — The per-*O*-acetylated 6-(trifluoroacetamido)hexyl 1,2-*trans*-aldopyranosides of D-galactose (**1**), D-glucose (**9**), 2-acetamido-2-deoxy-D-glucose (**21**), L-fucose (**33**), and D-mannose (**14**) were synthesized, according to methods already described[1], from the corresponding 2-thiopseudourea derivatives and **42**.

*Preparation of 1,2-*trans *5-(methoxycarbonyl)pentyl 1-thioaldopyranosides.* — The title glycosides of D-galactose (**5**), 2-acetamido-2-deoxy-D-glucose (**25**), 2-acetamido-2-deoxy-D-galactose (**29**), L-fucose (**37**), and D-mannose (**18**) were synthesized according to the following general procedure, as exemplified by the D-galactose derivative.

To a solution of 2-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-thiopseudourea hydrobromide[1] (9.74 g, 20 mmol) in a rapidly stirred mixture of chloroform and water (80 mL each) were added potassium carbonate (3.32 g, 24 mmol) and sodium hydrogensulfite (2.08 g, 20 mmol). After being stirred rapidly for 45 min, the layers were separated, the aqueous solution extracted with chloroform (50 mL), and the extracts combined, dried (sodium sulfate), and evaporated. The residue was taken up in dry methanol (10 mL), and potassium carbonate (24 mmol), sodium hydrogensulfite (20 mmol), and **43** (4.6 g, 22 mmol) were added. This suspension was stirred rapidly for ~2 h at room temperature, by which time, all of the 1-thio sugar (DTNB-staining) had been converted into a product having a higher R_F value in t.l.c., developed with 1:1 (v/v) benzene–ether [for the 2-acetamido-2-deoxy-glycosides, the solvent system used was 4:1 (v/v) ethyl acetate–acetone]. The mixture was evaporated, the residue was taken up in chloroform (50 mL), the insoluble materials were removed by suction filtration, and the filtrate was successively washed with 0.05M H_2SO_4 (50 mL, twice) and water (50 mL), dried (sodium sulfate), and evaporated. The resulting 1,2-*trans*-5-(methoxycarbonyl)pentyl 1-thioaldopyranosides were of sufficient purity for use in the next step.

Anomerization. — The fully protected 1-thioaldopyranosides were dissolved in dichloromethane, usually to a concentration of 0.1 mmol/mL, in Teflon-lined, screw-capped, glass culture tubes or in Erlenmeyer flasks fitted with polyethylene caps. Boron trifluoride gas was quickly bubbled into the solution until apparent saturation was achieved (~0.4M at room temperature), and then the vessel was tightly

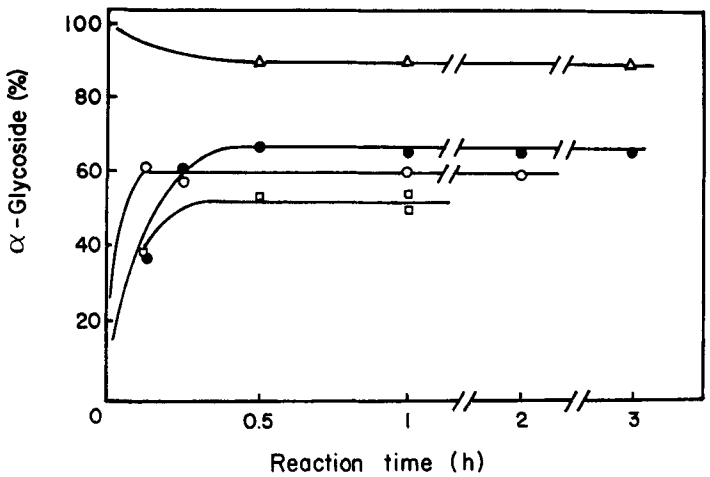


Fig. 1. Time course of boron trifluoride-catalyzed anomerization. [The per-*O*-acetylated 6-(trifluoracetamido)hexyl 1-thioaldopyranosides **1**, **9**, **14**, and **33** were each treated with boron trifluoride for various times, de-acylated, and eluted from a column (0.5 × 63 cm) of Amberlite CG-120 resin to separate the anomers, as described in the Experimental section, and as shown in Fig. 2. □, D-glucopyranoside; ○, L-fucopyranoside; ●, D-galactopyranoside; △, D-mannopyranoside.]

sealed. The course of anomerization was monitored by t.l.c. With the exception of the anomers (**9** and **10**) from D-glucose, all of the anomeric pairs were separable by t.l.c. in 1:1 (v/v) benzene–ether. When anomeric equilibrium was reached (<1–2 h for the glycosides from D-glucose, D-galactose, D-mannose, or L-fucose, but at least 24 h for the 2-amino-2-deoxy-D-glucosides and -galactosides), the solution was washed twice with equal volumes of cold, saturated sodium hydrogencarbonate solution, once with an equal volume of water, dried (sodium sulfate), and evaporated.

The rate of anomerization differed considerably among the different sugars. Fig. 1 shows that anomerization of the 1,2-*trans*-6-(trifluoroacetamido)hexyl per-*O*-acetyl-1-thioaldopyranosides (**33**) of L-fucose, (**1**) of D-galactose, (**9**) of D-glucose, and (**14**) of D-mannose reaches an equilibrium within 30 min after addition of boron trifluoride. Although these solutions became progressively more colored with time, t.l.c. indicated that little degradation of sugar had occurred, even after 24 h. The anomerization of the corresponding glycoside (**21**) of 2-acetamido-2-deoxy-D-glucose, on the other hand, did not reach an apparent equilibrium until elapse of ~24 h.

Separation of the anomeric pairs. — The following chromatographic systems were used for separation of the anomeric pairs.

Method A. By preparative, high-performance, liquid chromatography (h.p.l.c.). Anomeric mixtures, prepared as already described, were dissolved in an appropriate solvent mixture (<20 mL), and chromatographed in a column (4 × 50 cm; Jobin–Yvon Chromatospac Prep 100) packed with silica gel 60H (200 g) and eluted at a column pressure of 9 bar (130 lb. in.$^{-2}$) and a flow rate of 10–12 mL/min. Fractions containing the purified anomers (as determined by t.l.c.) were pooled. Optimal

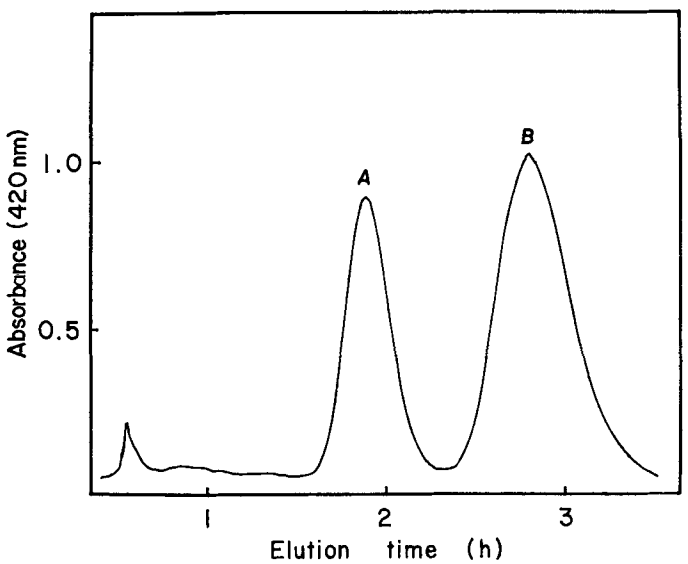


Fig. 2. Separation of anomers using a column of Amberlite CG-120 resin. [The anomers of 6-aminohexyl 1-thio-D-galactopyranoside, **3** and **4**, were prepared as described in the text. The anomers were eluted with 0.5M sodium acetate from a water-jacketed (56°) column (0.5 × 63 cm) of Amberlite CG-120 resin at a flow rate of 0.3 mL/min. Peaks A and B contained the β and α anomers, respectively].

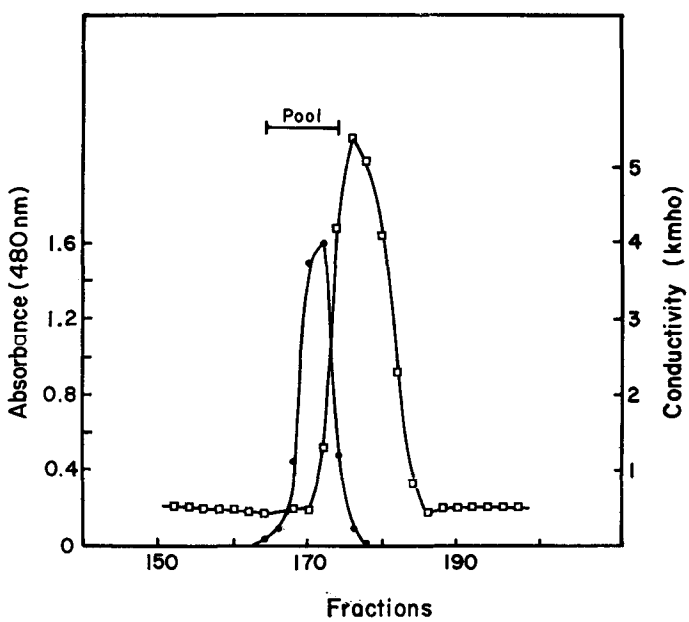


Fig. 3. Separation of 6-aminohexyl 1-thio-α-D-galactopyranoside and sodium acetate. [After separation from the β anomer by chromatography on Amberlite CG-120 resin, 6-aminohexyl 1-thio-α-D-galactopyranoside (**4**) was chromatographed on a column (5 × 196 cm) of Sephadex G-25 to remove sodium acetate. Key: ●, A_{480}; □, conductivity.]

separation of anomers (0.2–7 g) was achieved with solvent mixtures in which the anomers had R_F values of 0.05–0.4 in t.l.c.

Method B. By conventional, silica gel chromatography. Solutions (0.5 mL) of crude, anomeric mixtures of 1-thioglycosides (0.2–0.5 g) were applied to a prepacked column (3.7 × 44 cm) of silica (Lobar, E. Merck), and eluted with an appropriate solvent at a constant flow-rate of 1.7 mL/min. Fractions were monitored by t.l.c., and those containing pure anomers were pooled.

Method C. Chromatography on Amberlite CG-120 resin. Crude, anomeric mixtures containing per-*O*-acetylated 6-(trifluoroacetamido)hexyl 1-thioaldopyranosides were totally deacylated in one step by using Dowex-1 (OH^-) resin in 50% ethanol[1]. The anomeric mixtures of 6-aminohexyl 1-thioaldopyranosides were separable on a column of Amberlite CG-120 (Na^+) resin, as shown in Fig. 2. For preparative separations, up to 1 g of an anomeric mixture was applied to a column (5 × 71 cm) of Amberlite CG-120 (Na^+) resin maintained at 56°, and eluted with 0.5M sodium acetate (pH 6.0). Fractions containing the purified anomers were pooled, and evaporated. The residue was dissolved by warming in 0.1M acetic acid (50 mL), and sodium acetate was allowed to crystallize out overnight at room temperature. The salt was removed by filtration, and the filtrate was applied to a column (5 × 217 cm, or 5 × 196 cm) of Sephadex G-25 in 0.1M acetic acid (see Fig. 3). Two or three passages through the column were necessary in order to effect complete separation of the sugars from sodium acetate.

Although all three procedures were successfully employed for the purification of the 1-thioaldopyranosides, Method A is preferred, because it is the simplest of the three techniques, and consistently gave the best yields. In addition, the capacity of the preparative-scale, h.p.l.c. column (~7 g for the separation of anomers) was greater than that of the columns employed in Methods B and C. Using Method A, the entire procedure, from anomerization to purification, can be performed in one day.

The recovery of glycoside (both anomers) purified by Method A was 70–90%, but it was only 40–70% by Method C. As expected, the yields of the desired, 1,2-*cis* anomers at equilibrium were characteristic for each glycoside, and varied from 6% for the β-D-mannopyranoside **15** to 51% for the α-L-fucopyranoside **36**. As the 1,2-*trans* anomers could be recovered, it is possible to repeat the cycle, in order to obtain more of the 1,2-*cis* anomers.

Preparation of [6-(aldopyranosylthio)hexanoyl]aminoacetaldehyde diethyl acetal. — The purified 5-(methoxycarbonyl)pentyl 1-thioaldopyranoside peracetates (1,2-*cis* or 1,2-*trans*) were dissolved in 70% ethanol (1 mmol/10 mL), and 2.5M sodium hydroxide (0.8 mL) was added. The solutions were stirred for 30 min at room temperature, made neutral (pH 4–5) with Amberlite CG-120 (H^+) resin, the suspensions filtered, and the filtrates evaporated to dryness. A solution of each residue in dry methanol (1 mmol/10 mL) was stirred overnight at room temperature with 2 mmol each of EDAC and aminoacetaldehyde diethyl acetal, and the reaction mixtures were chromatographed on a column (4 × 194 cm) of Sephadex LH-20 in 95% ethanol.

TABLE I

PROPERTIES OF 6-AMINOHEXYL 1-THIOALDOPYRANOSIDES

Parent sugar	*Glycoside*	*Anomer*	*Method of purification*	*Melting point (°C)*	*Elution time[a] (min)*	*P.m.r.*		$[\alpha]_D^{22}$ (c) *(degrees)*	
						Chemical shift (p.p.m.)	$J_{1,2}$ *(Hz)*		
D-Galactose	**4**[b]	α	C	114–116	168	5.81	8	+200	(0.10)
	3[b]	β	C	147–149	114	4.86	12	−22	(0.03)
D-Glucose	**12**[b]	α	C	syrup	166	5.76	7	+219	(0.06)
	11	β	C	syrup	123	4.87	12	−26.7	(0.09)
D-Mannose	**16**	α	A	syrup	302	5.59	<1	+72	(0.01)
	15	β	A	139–141	178	5.15	<1	−21	(0.11)
2-Acetamido-2-deoxy-D-glucose	**24**	α	B	165–167	[c]	5.78	5	+164	(0.10)
	23	β	B	199–200	[c]	4.99	9	−26	(0.10)
L-Fucose	**36**[b]	α	C	114–117	360	5.28	7	−192	(0.06)
	35[b]	β	C	123–124	183	4.33	12	+30	(0.10)

[a]From a column (0.5 × 63 cm) of Amberlite CG-120 resin at a flow-rate of 0.3 mL/min. [b]Isolated as the acetate (salt). [c]Not determined.

TABLE II

PROPERTIES OF PER-*O*-ACETYLATED 5-(METHOXYCARBONYL)PENTYL 1-THIOALDOPYRANOSIDES AND [6(ALDOPYRANOSYLTHIO)HEXANOYL]AMINOACETALDEHYDE DIETHYL ACETALS

Parent sugar	*Glycoside*	*Anomer*	*H.P.L.C. solvent*[a]	*Melting point (°C)*	*P.m.r.*		$[\alpha]_D^{22}$ (c) *(degrees)*	R_F *(in t.l.c.)*
					Chemical shift (p.p.m.)	$J_{1,2}$ *(Hz)*		
D-Galactose	**6**	α	*A*	syrup	5.65	6	[b]	0.52[c]
	5	β	*A*	syrup	4.42	12	[b]	0.45[c]
	8	α	*B*	74–75	5.78	5	+135 (0.11)	0.59[d]
	7	β	*B*	112–114	4.77	8	−6.3 (0.14)	0.54[d]
D-Mannose	**18**	α	*C*	69–70	5.14	<1	[b]	0.44[c]
	17	β	*C*	72–75	4.64	<1	[b]	0.38[c]
	20	α	*B*	syrup	5.50	[b]	+56 (0.07)	0.75[d]
	19	β	*B*	115–117	5.05	[b]	−38 (0.11)	0.66[d]
6-Acetamido-6-deoxy-D-glucose	**26**	α	*D*	109–110	5.24	6	[b]	0.15[e]
	25	β	*D*	syrup	4.50	14	[b]	0.08[e]
	28	α	*B*	105–107	[b]	[b]	+71 (0.11)	0.70[d]
	27	β	*B*	171–173	4.75	[b]	−24 (0.09)	0.61[d]
6-Acetamido-6-deoxy-D-galactose	**30**	α	*D*	syrup	5.34	6	[b]	0.14[e]
	29	β	*D*	syrup	4.51	13	[b]	0.04[e]
	32	α	*E*	152–154	5.40	6	+134 (0.10)	0.60[d]
	31	β	*E*	175–177	4.75	10	−23 (0.06)	0.49[d]
L-Fucose	**38**	α	*F*	syrup	5.46	6	[b]	0.69[c]
	37	β	*F*	syrup	4.28	12	[b]	0.62[c]
	40	α	*B*	61–62	5.97	5	−138 (0.07)	0.79[d]
	39	β	*B*	100–102	4.74	8	+24 (0.10)	0.73[d]

[a]Glycosides were purified by using the following solvents: *A*, 2:1 (v/v) toluene–ether; *B*, 9:4:2 (v/v) ethyl acetate–isopropyl alcohol–water; *C*, 3:1 (v/v) toluene–ether; *D*, 3:2 (v/v) toluene–ethyl acetate; *E*, 18:8:1 (v/v) ethyl acetate–isopropyl alcohol–water; *F*, 6:1 (v/v) toluene–ether. [b]Not determined. [c]1:2 (v/v) toluene–ether. [d]9:4:2 (v/v) ethyl acetate–isopropyl alcohol–water. [e]3:2 (v/v) toluene–ethyl acetate.

Two peaks containing carbohydrate were eluted. When analyzed by t.l.c., it was found that the first peak contained carbohydrate that stained with DNP-hyd [R_F 0.5–0.8, developed with 9:4:2 (v/v) ethyl acetate–isopropyl alcohol–water], and another component (R_F 0–0.1) that stained only with DNP-hyd, but did not readily char with sulfuric acid spray upon heating, indicating that it was not a sugar. The second peak contained carbohydrate that did not stain with DNP-hyd, and was therefore not the desired product. Fractions from the first peak were pooled, and purified further by h.p.l.c., as already described (see Method A). The title compounds were thus obtained in 20–70% yields.

Characterization of compounds. — Table I summarizes the characteristics of the 6-aminohexyl 1-thioaldopyranosides isolated. The characteristics of the 5-(methoxycarbonyl)pentyl 1-thioaldopyranosides and the [6-(aldopyranosylthio)hexanoyl]-aminoacetaldehyde glycosides are shown in Table II. All of the 1-thioglycosides were homogeneous by t.l.c. The R_F values of the α anomers were generally higher than those of the corresponding β anomers. In general, the melting points of the crystalline β anomers were higher than those of the corresponding α anomers. The p.m.r. spectra were, in all cases, consistent with the structures proposed. The signals observed for α-anomeric protons were at lower fields than those for the corresponding β-anomeric protons, and the coupling constants were larger for the β-anomeric protons (12–14 Hz) than for the α-anomeric protons (5–8 Hz). Each 6-aminohexyl 1-thioglycopyranoside had a characteristic elution volume from a column of Amberlite CG-120 resin.

DISCUSSION

The most convenient and versatile method for the synthesis of 1-thioglycosides utilizes 2-thiopseudourea derivatives of sugars, derivatives that can be readily prepared from the respective, peracetylated glycosyl halides[1,17]. However, the known 2-thiopseudourea derivatives of sugars, and the resulting 1-thioglycosides, are all of the 1,2-*trans* configuration. Although several other synthetic methods have been used to prepare anomeric mixtures of simple alkyl[13] and aryl[14,15] 1-thioglycosides, these methods are limited by the availability of suitable thiols, or by the need to introduce nonparticipating protecting groups at O-2 of the sugars[16]. No general method has been proposed for the direct synthesis of 1,2-*cis*-1-thioglycosides.

As an alternative, available 1,2-*trans*-1-thioglycosides can be anomerized, and the 1,2-*cis* anomers can then be isolated from the mixtures. Erbing and Lindberg[7] first described boron trifluoride as an effective and relatively mild catalyst for the anomerization of simple alkyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosides. In the present study, per-*O*-acetylated 1,2-*trans*-6-(trifluoroacetamido)hexyl 1-thioaldopyranosides and 5-(methoxycarbonyl)pentyl 1-thioaldopyranosides were successfully anomerized, without appreciable side-reactions, by treatment with boron trifluoride.

Successful employment of this approach requires effective isolation of the anomers. We have employed three chromatographic systems for this purpose. Chromatography on manually packed columns of silica gel (Method B) had pre-

viously been used in the separation of anomeric pairs of alkyl 2,3,4,6-tetra-*O*-acetyl-1-thio-D-glucopyranosides[7], and, although this method can yield purified anomers, the capacity of conventional columns of silica gel is relatively low. Preparative, high-performance liquid chromatography, on the other hand, allowed separation of gram quantities of anomers within a few hours (Method A).

A third method for such separation (Method C), applicable only to anomers of 6-aminohexyl 1-thioaldopyranosides, is based on a combination of ion-exchange and reversed-phase chromatography. Of the several cation-exchange resins tested, Amberlite CG-120, eluted with sodium acetate, was found to yield the best results. This method of separation was particularly well-suited for analytical scale experiments because column effluents could be readily coupled to an automated sugar-analyzer[10]. Method C was complementary to silica gel chromatography. For example, 6-aminohexyl 1-thio-α,β-D-glucopyranosides could not be separated by silica gel chromatography, but were readily separable on a column of Amberlite CG-120 resin.

Polystyrene-type ion-exchange resins eluted with water have been used before for chromatography of uncharged aryl 1-thioaldopyranosides[14]. Although the mechanism for the separation of anomers under these conditions is still poorly understood, it is considered to arise mainly from hydrophobic interaction with the resin matrix. This method was not, however, successful, as such, for separation of anomeric pairs of 6-(trifluoroacetamido)hexyl 1-thioaldopyranosides, probably because these compounds were not sufficiently hydrophobic and were eluted too quickly from the column. Successful separation was achieved only after amino groups were exposed. A salt solution was, in this case, needed to elute the compounds. As both anomers possess the same ionic group, separation was not likely to be based purely upon ion exchange, but, presumably, upon combined hydrophobic and charge interactions with the resin matrix. Similar conditions have been reported for the separation of anomers of 2-amino-2-deoxy-aldopyranosides and -aldofuranosides[18].

In applying the anomerization technique to the 1-thioglycosides containing a diethyl acetal group at the terminal position of the aglycon, this group was found to be unstable, but this problem was overcome by rearrangement and modification of the reaction sequence previously described[2]. In the present reaction-scheme, the per-*O*-acetylated 1,2-*trans*-6-(methoxycarbonyl)pentyl 1-thioaldopyranosides, prepared from methyl 6-bromohexanoate and the appropriate 2-thiopseudourea derivatives, were anomerized by means of boron trifluoride. The anomers were then separated by h.p.l.c. (Method A). De-protection, followed by coupling to aminoacetaldehyde diethyl acetal, yielded anomeric pairs of the desired glycosides of [6-(aldopyranosylthio)hexanoyl]aminoacetaldehyde diethyl acetal.

Observations involving a number of 1-thioaldopyranosides led to the conclusion that the rate of boron trifluoride-catalyzed anomerization is influenced by the substituents on O-2 of the sugar and the β-position of the aglycon. For instance, the per-*O*-acetylated alkyl 1-thioaldopyranosides of D-glucose and D-galactose reach an anomeric equilibrium within $\sim$15 min, whereas equilibrium is not reached until elapse of $\sim$24 h for the 2-acetamido-2-deoxy counterparts. One explanation for this

difference in rate of anomerization is that the 2-acetamido group, unlike the 2-acetoxyl group, does not readily form the intermediate postulated by Lemieux[12].

An electronegative substituent at the β position of the aglycon can also retard the rate of anomerization. For example, anomerization of cyanomethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside[3] was not detectable within 24 h (as analyzed by t.l.c. and p.m.r. spectroscopy), but the corresponding cyanobutyl β-D-galactopyranoside, which was prepared by a similar procedure, had reached anomeric equilibrium ($\sim$60% of α anomer) within 30 min (data not shown). Similarly, the rate of anomerization of (methoxycarbonyl)methyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside was much lower than that for the corresponding, 5-(methoxycarbonyl)pentyl derivative. With regard to the substituent at the β position in the aglycon, the rate of anomerization is, therefore, methylene > carbonyl > nitrile. For this reason, and because of the ready availability of 6-aminohexanol and 6-aminohexanoic acid, aglycons having C_6 spacer-arms were used in this study.

It is shown here that boron trifluoride-catalyzed anomerization can be of general use in the synthesis of anomeric pairs of 1-thioglycosides. Studies of the anomeric specificity of various biological processes will therefore be facilitated by the use of these derivatives.

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