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SYNTHESIS OF 3-(2-AMINOETHYLTHIO)PROPYL GLYCOSIDES†,*

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ABSTRACT

Anomeric pairs of 3-(2-aminoethylthio)propyl D-galactopyranoside (**4**, **4a**), D-glucopyranoside (**5**, **5a**), and 2-acetamido-2-deoxy-D-glucopyranoside (**6**, **6a**) were prepared by addition of 2-aminoethanethiol to the corresponding, anomeric, allyl glycosides. The allyl α-glycosides were prepared by refluxing the sugars with allyl alcohol in the presence of an acid catalyst; the allyl β-glycosides were prepared by the reaction of acetylated glycosyl bromides with allyl alcohol in the presence of mercuric cyanide, followed by *O*-deacetylation. The rate of thiol addition to the allylic group was found to be different for each glycoside.

INTRODUCTION

We have been engaged in the preparation of glycosides having an amino group at the aglycon terminal[1,2] so that these glycosides could be affixed to solid matrices for biological research[3]. In our previous reports[1,2], as well as in reports from other laboratories[4–6], such glycosides were prepared from acetylated 1,2-*cis*-glycosyl halides, and the products always had the 1,2-*trans* configuration, in accordance with the *trans* rule[7]. Both the 1,2-*cis* and 1,2-*trans* glycosides were needed for investigation of specific requirements for anomeric configuration.

Stereospecific synthesis of 1,2-*cis* glycosides has been a subject of considerable interest, and partial solutions have been provided[8,9]. However, the methods used required multistep preparation of glycosylating agents, and were quite time-consuming. We have, therefore, turned to a simpler, more generally applicable method for the preparation of 1,2-*cis* glycosides amenable to ultimate attachment to solid matrices, namely, Michael addition of 2-aminoethanethiol (cysteamine) to 1,2-*cis* allyl glycosides, affording 3-(2-aminoethylthio)propyl glycosides. The corresponding 1,2-*trans* glycosides were prepared by a similar scheme.

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EXPERIMENTAL

Materials. — The following compounds were obtained commercially and were used without purification: allyl alcohol, boron trifluoride etherate (J. T. Baker), D-galactose, D-glucose, 2-acetamido-2-deoxy-D-glucose, 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide, 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (Sigma). 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride was prepared by the method of Horton[10]. Rexyn 101 (cation-exchange resin, Na^+ form, medium porosity, Fisher Scientific Co.) was converted into the ammonium form by successively treating it with 10 volumes each of M sulfuric acid, water, 7M ammonium hydroxide, and water.

2-Aminoethanethiol was purified from a commercial sample* as follows. 2-Aminoethanethiol hydrochloride (98%, Aldrich Chemical; 15 g) was dissolved in 95% ethanol (80 ml) with slight warming, and the solution was kept overnight at 4°. The crystals thus formed (probably cystamine dihydrochloride) showed negative thiol reactions (see General methods), and were removed by filtration. The filtrate was concentrated to ~1/3rd of its volume, and kept overnight at 4°, giving crystalline 2-aminoethanethiol hydrochloride. The crystals were filtered off, dried *in vacuo*, and stored under nitrogen.

General methods. — All evaporations were performed under diminished pressure at 25–40° with a rotary evaporator. Uncorrected melting points were measured with a Fisher–Johns apparatus. Proton magnetic resonance spectra were recorded with a JEOL NMH-100 spectrometer. Optical rotations were measured with a Cary 60 spectropolarimeter. Elementary analyses were performed by Galbraith Laboratories (Knoxville, Tennessee). Thin-layer chromatography (t.l.c.) was performed with layers of silica gel F-258 (Merck) precoated on aluminum sheets. The solvent systems used in elution were: (*A*) 3:2:1 ethyl acetate–glacial acetic acid–water, (*B*) 9:4:2 ethyl acetate–isopropyl alcohol–water, (*C*) 1:1 benzene–ether, and (*D*) 200:31:1 benzene–ethanol–28% ammonia. For detection of the components, t.l.c. sheets were sprayed with (*a*) 10% sulfuric acid in 95% ethanol, and heated for a few minutes at 140° (for carbohydrate), or (*b*) 3% ninhydrin in acetone and heated similarly (for amino group).

Neutral sugars were determined by a modified phenol–sulfuric acid method[11]. Amino group was determined by a modification of the 2,4,6-trinitrobenzenesulfonic acid (TNBS) method[11]. A sample solution (1 ml) containing 0.02–0.2 μmole equivalent of amino group was mixed with 1 ml of 0.2M sodium borate buffer, pH 8.0, and 0.6 ml of 2% TNBS in water. The mixture was kept for 15 min at room temperature, and then the absorbance was determined at 420 nm. (Under these conditions, 2-aminoethanethiol or cystamine form a yellow color having its absorbance peak at 420 nm. The color development was essentially complete within 5–10 min

*Commercial samples of 2-aminoethanethiol hydrochloride often actually contain as little as 50% of the compound.

at room temperature. The Beer–Lambert law was obeyed between 0 and 0.2 μmole equivalence of amino group, yielding an absorbance value of 0–0.8 at 420 nm.) The thiol content was determined by Boyer's method[12] or by a modification[2] of Ellman's method[13].

Allyl α-D-galactopyranoside (**1**). — A suspension of D-galactose (18 g, 0.1 mole) and Dowex-50 X-8 (H^+) (10 g, 100–200 mesh) in anhydrous allyl alcohol (200 ml) was boiled under reflux for 90 min. The resin was filtered off, and washed with absolute ethanol, and the filtrate and washings were combined and evaporated to a syrup. Crystallization from absolute ethanol yielded 9 g of **1** (41% yield), which exhibited a single sugar spot in t.l.c. (solvent *B*), m.p. 143–145°, $[\alpha]_D^{25}$ +181.3° (*c* 1.57, water) (lit.[14] m.p. 145–146°, $[\alpha]_D^{25}$ +185.0° in water); p.m.r. data (methyl sulfoxide-d_6): δ 4.78 (d, 1, *J* 3–4 Hz, anomeric proton), 5.14, 5.24, 5.44 (m, 2, $=CH_2$), and 5.66–6.22 (m, 1, =CH–).

Allyl α-D-glucopyranoside (**2**). — A suspension of D-glucose (18 g, 0.1 mole) and Dowex-50 X-8 (10 g) in allyl alcohol (200 ml) was boiled under reflux for 90 min and then processed as for **1**. As **2** did not crystallize at this stage, and showed presence of some minor impurities in t.l.c., it was fractionated by passing it through a column (5 × 150 cm) of Bio-Gel P-2 (200–400 mesh) in 0.1M acetic acid. Two, partially separated, carbohydrate peaks were obtained. Fractions in the major peak, which contained only **2** (by t.l.c. in solvent *B*), were combined and evaporated. Crystallization from absolute ethanol yielded 5.8 g (26%) of **2**, m.p. 95–97°, $[\alpha]_D^{25}$ +133.8° (*c* 1.65, water) {lit. m.p. 100.5–101.5° (ref. 14), $[\alpha]_D^{25}$ +151.1° (ref. 14), +131.7° (ref. 15)}; p.m.r. data (methyl sulfoxide-d_6): δ 4.69 (d, 1, *J* 3–4 Hz, anomeric proton); other features were similar to those of **1**.

Allyl 2-acetamido-2-deoxy-α-D-glucopyranoside (**3**). — (*a*) A suspension of 2-acetamido-2-deoxy-D-glucose (22.1 g, 0.1 mole) in 400 ml of dry allyl alcohol containing 2 ml of boron trifluoride etherate was refluxed for 2 h. The clear solution was cooled, kept overnight at room temperature, and evaporated to a crystalline mass, which was recrystallized from 95% ethanol and then from ethanol–ether to give 7.83 g (31%) of **3**; it was pure by t.l.c. (solvent *B*), and had m.p. 172–174°, $[\alpha]_D^{25}$ +148.8° (*c* 1.62, water); p.m.r. data (methyl sulfoxide-d_6): δ 4.78 (d, 1, *J* 3–4 Hz, anomeric proton) and 1.82 (s, 3, CH_3CO); other features were similar to those of **1**.

(*b*) A suspension of 2-acetamido-2-deoxy-D-glucose (6.6 g, 30 mmoles) and Dowex-50 X-8 (H^+) (6 g, 100–200 mesh) in allyl alcohol (160 ml) was refluxed for 2 h, and processed as for **1**, but crystals of pure **3** were obtained only after several recrystallizations from 95% ethanol (~10% yield).

Allyl β-D-galactopyranoside (**1a**). — A mixture of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (8.22 g, 20 mmoles) and mercuric cyanide (5.6 g, 22 mmoles) in dry allyl alcohol (180 ml) was stirred for 24 h at room temperature, evaporated to dryness, and the residue dissolved in chloroform (300 ml). The solution was thoroughly extracted with M sodium chloride, dried (sodium sulfate), and evaporated to a syrup, t.l.c. of which (solvent *C*) revealed only a single spot on charring (R_F 0.45). The syrup was dissolved in dry methanol (50 ml), treated overnight

with 1.25M barium methoxide (1 ml), and the solution decationized by mixing it with a suspension of Dowex-50 X-8 (H^+) (5 g, 100–200 mesh) in water (50 ml). The suspension was filtered, and the filtrate was evaporated, giving a crystalline product which was recrystallized from 95% ethanol; yield 3.26 g (74%,) homogeneous by t.l.c. (solvent *B*), m.p. 102–103°, $[\alpha]_D^{25}$ −10.9° (*c* 3.35, water) {lit. m.p. 103–104° (ref. 16), $[\alpha]_D^{25}$ −12.5° in water[17]}; p.m.r. data (methyl sulfoxide-d_6): δ 4.35 (d, 1, *J* 6–7 Hz, anomeric proton); other features were similar to those of **1**.

Allyl β-D-glucopyranoside (**2a**). — 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (8.22 g, 20 mmoles) and mercuric cyanide (5.6 g, 22 mmoles) were stirred in dry allyl alcohol for 24 h, and the mixture was evaporated to dryness. A solution of the residue in dichloromethane (250 ml) was washed with M sodium chloride, dried (sodium sulfate), and evaporated to a syrup which crystallized upon standing overnight. Recrystallization from ether gave 5.79 g (74.5% yield) of acetylated **2a**, m.p. 86° (lit.[14] 88°).

Deacetylation with barium methoxide as for **1a** gave crystalline **2a** (homogeneous by t.l.c. in solvent *B*) in 65% yield, m.p. 100–101°, $[\alpha]_D^{25}$ −40.0° (*c* 3.52, water). The p.m.r. spectrum showed the anomeric proton signal at δ 4.18 (*J* 7–8 Hz).

Allyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**3a**). — A mixture of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (20.3 g, 55.6 mmoles) and mercuric cyanide (13.1 g, 60 mmoles) in dry allyl alcohol (200 ml) was stirred for 24 h, and evaporated to a solid mass, most of which dissolved on shaking with chloroform (250 ml). The suspension was filtered, and the filtrate washed with water, dried (sodium sulfate), and evaporated to a crystalline solid. Recrystallization from absolute ethanol yielded 4.98 g (60.9% yield) of acetylated **3a**, m.p. 162–163° (lit.[18] m.p. 160°); the product was homogeneous by t.l.c. in solvents *C* and *D*. Deacetylation in dry methanol with barium methoxide yielded, in 80% yield, crystalline **3a** which was homogeneous by t.l.c. (solvent *B*); m.p. 171–172°, $[\alpha]_D^{25}$ −33.9° (*c* 4.99, water). The p.m.r. spectrum showed the anomeric proton signal at δ 4.36 (*J* 7–8 Hz).

Michael addition of 2-aminoethanethiol to allyl glycosides. — The addition of 2-aminoethanethiol to allyl glycosides was conducted simply by dissolving the allyl glycoside in a solution of 2-aminoethanethiol hydrochloride (2-fold excess) in water. The reaction was monitored by the decrease in the SH concentration and by t.l.c. The t.l.c. (solvent *A*) showed the formation, at the expense of allyl glycoside (R_F 0.68), of a new compound (R_F 0.29) which contained both carbohydrate and amino group.

All of the allyl glycosides tested reacted with 2-aminoethanethiol to afford a product similar in behavior in t.l.c., but the reactivity of the allyl glycosides differed greatly. The results from one of the comparative studies performed, at a glycoside concentration of 0.5 mmole·ml^{-1}, are tabulated in Table I. The results suggest that the reactivities of the allyl glycosides are as follows: α-D-glucoside ⩾ α-D-galactoside, β-D-galactoside, 2-acetamido-2-deoxy-α-D-glucoside ⩾ β-D-glucoside > 2-acetamido-2-deoxy-β-D-glucoside. For the least reactive glycoside, namely, allyl 2-acetamido-2-deoxy-β-D-glucoside, the yield of addition product was only 30%, even when the

reaction was conducted overnight at the upper limit of solubility of the glycoside (see later).

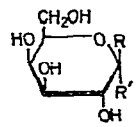
TABLE I

UPTAKE OF SH GROUP BY VARIOUS ALLYL GLYCOSIDES

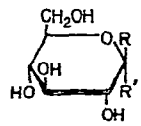
Reaction time (min)	*SH uptake (mole/mole of allyl glycoside)*					
	D-*Galactosides*		D-*Glucosides*		*2-Acetamido-2-deoxy*-D-*glucosides*	
	α	*β*	*α*	*β*	*α*	*β*
0	0	0	0	0	0	0
30	0.20	0.18	0.88	0.06	0.14	0.06
70	0.44	0.40	0.92	0.18	0.38	0.06

The reaction is very sensitive to the concentrations of the reactants. Lowering of the concentrations of both of the reactants by a factor of 0.71 caused a decrease by a factor of ~0.05 in the apparent reaction-rate. On the other hand, the temperature appeared to have little effect on the reaction rate.

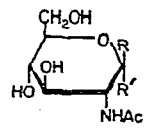
Formation of 3-(2-aminoethylthio)propyl glycosides from allyl glycosides. — The following conditions were used for the Michael addition of 2-aminoethanethiol to glycosides **1**, **1a**, **2**, and **3**. A solution of the allyl glycoside (5 mmoles) in 10 ml of a 2-aminoethanethiol hydrochloride solution that contained 15 meq of thiol groups was kept for 1.5 h at room temperature. For the similar reactions of **2a** and **3a**, the reactant concentrations had to be increased as follows. With some warming, compound **2a** (5 mmoles) was dissolved in 2 ml of a 2-aminoethanethiol hydrochloride



1, 1a, 4, 4a



2, 2a, 5, 5a



3, 3a, 6, 6a

Compound	R	R′
1, 2, 3	H	$-OCH_2CH=CH_2$
1a, 2a, 3a	$-OCH_2CH=CH_2$	H
4, 5, 6	H	$-O(CH_2)_3S(CH_2)_2NH_2$
4a, 5a, 6a	$-O(CH_2)_3S(CH_2)_2NH_2$	H

solution which was 7.7M in thiol, and the solution was kept overnight. Compound **3a** was treated in essentially the same way, except that a small volume (~0.2 ml) of water was added to effect complete dissolution of the glycoside.

The reaction mixture was then fractionated on a column (2.4 × 16 cm) of Rexyn 101 (NH_4^+) (200–400 mesh) by eluting successively with water, 0.5M NH_4OH, and 1M NH_4OH, and collecting 5–6 ml per fraction. A typical elution profile is shown in Fig. 1. Unreacted allyl glycoside appeared in the water washings, and 3-(2-aminoethylthio)propyl glycosides in the 1M NH_4OH elutate; this peak was usually preceded

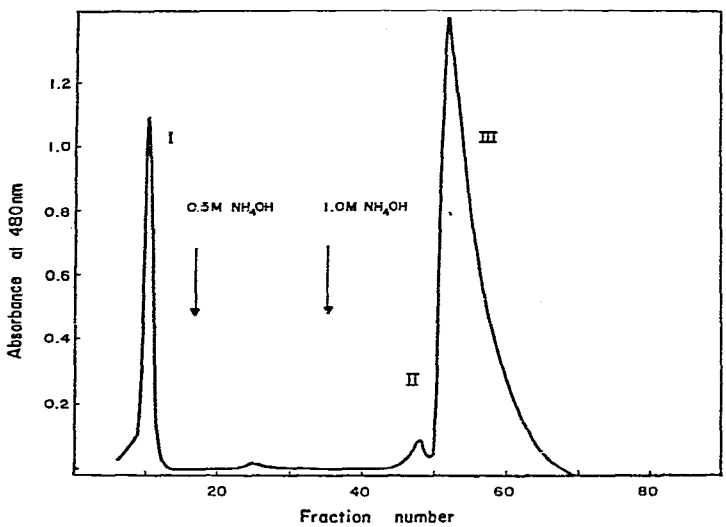


Fig. 1. Fractionation of the allyl α-D-galactopyranoside—2-aminoethanethiol reaction mixture on a column (2.4 × 16 cm) of Rexyn 101 (NH_4^+). [The effluent was analyzed by the phenol—sulfuric acid method. Peak I, allyl α-D-galactopyranoside; peak II, byproduct; peak III, 3-(2-aminoethylthio)propyl α-D-galactopyranoside.]

by a small peak containing a byproduct. T.l.c. (solvent *A*) examination of the fractions in the two peak regions showed a slight overlap in elution of the two products. Elution with 0.5M NH_4OH produced no carbohydrate peak, but a brown color and small quantities of ninhydrin-positive materials were detected. Fractions containing the desired product were combined and evaporated. Further purification and crystallization of the product are described individually in the following sections.

3-(2-Aminoethylthio)propyl α-D-galactopyranoside (**4**). — The effluent (1M NH_4OH) from the Rexyn 101 column was estimated to contain 3.7 mmoles (74% yield) of **4**. The syrup obtained after evaporation of the fractions was co-evaporated twice with absolute ethanol–toluene to remove traces of water. Attempted crystallization of the syrupy product from absolute ethanol, isopropyl alcohol, or ethanol–ether produced either a syrup or hygroscopic crystals. The product was applied to a column (4 × 150 cm) of Sephadex LH-20, and eluted with 95% ethanol; this column

separated **4** from the contaminants preceding it, as well as some colored material that was retarded. Fractions containing pure **4** were combined and evaporated. Syrupy product was fractionally precipitated from absolute ethanol by addition of an increasing concentration of ether. A syrupy precipitate was obtained from the early fractions, but later fractions produced crystals. The yield of crystalline material was 0.5 g (1.72 mmoles, 43% overall yield); it was recrystallized twice from absolute ethanol–ether to give material that was homogeneous in t.l.c. (solvent *A*); m.p. 88–89°, $[\alpha]_D^{25}$ +136.6° (*c* 3.52, water); p.m.r. data (D_2O): δ 2.52 (m, 2, C–CH_2–C), 3.24 (t, 2, C–C–CH_2–S), 3.36 (t, 2, S–CH_2–C–N), and 5.46 (d, 1, *J* 3 Hz, anomeric proton).

Anal. Calc. for $C_{11}H_{23}NO_6S$ (297.37 daltons): C, 44.43; H, 7.80; N, 4.71; S, 10.78. Found: C, 44.65; H, 7.95; N, 4.70; S, 10.77.

3-(2-Aminoethylthio)propyl β-D-galactopyranoside (**4a**). — The amount of **4a** in the eluate (1M NH_4OH) of the Rexyn column was estimated by the phenol–sulfuric acid method to be 3.4 mmoles (69%). Evaporation of fractions produced **4a** as a faintly yellow solid. Recrystallization from 95% ethanol–ether produced 2.9 mmoles (60% overall yield) of **4a**; this was further purified by treatment with activated carbon in absolute ethanol, and recrystallization from absolute ethanol and then from absolute ethanol–ether. The product was homogeneous by t.l.c. (solvent *A*), and had m.p. 115–116°, $[\alpha]_D^{25}$ −6.1° (*c* 3.33, water); p.m.r. data (D_2O): δ 4.95 (d, 1, *J* 8 Hz, anomeric proton); other features were similar to those of **4**.

Anal. Calc. for $C_{11}H_{23}NO_6S$ (297.37 daltons): C, 44.43; H, 7.80; N, 4.71; S, 10.78. Found: C, 44.27; H, 7.86; N, 4.65; S, 10.71.

3-(2-Aminoethylthio)propyl α-D-glucopyranoside (**5**). — The yield of **5** in the eluate from the Rexyn column was estimated to be 4.24 mmoles (85%). The product (**5**) was obtained partly solid upon evaporation. Repeated recrystallization from absolute ethanol and then ethanol–ether afforded 2.45 mmoles of **5** (40%), homogeneous by t.l.c. (solvent *A*); m.p. 93–94°, $[\alpha]_D^{25}$ +113.7° (*c* 3.52, water). The p.m.r. spectrum (in D_2O) showed the anomeric proton signal at δ 5.37 (*J* 4 Hz); other features were similar to those of **4**.

Anal. Calc. for $C_{11}H_{23}NO_6S$ (297.37 daltons): C, 44.43; H, 7.80; N, 4.71; S, 10.78. Found: C, 44.22; H, 7.93; N, 4.63; S, 10.80.

3-(2-Aminoethylthio)propyl β-D-glucopyranoside (**5a**). — The amount of **5a** in the column eluate was estimated to be 4.0 mmoles (80%). As crystallization proved to be difficult, this material was purified by passing it through a column of Sephadex LH-20 as with the α-D-galactoside analog, and was fractionally crystallized from absolute ethanol by adding an increasing volume of ether. Early fractions produced a syrupy precipitate, whereas later fractions gave crystals. The crystals were collected, combined (overall yield 30%), and recrystallized twice from ethanol–ether. The product, **5a**, was homogeneous in t.l.c. (solvent *A*); m.p. 81–82°, $[\alpha]_D^{25}$ −28.2° (*c* 33.8, water). The p.m.r. spectrum (in D_2O) showed the anomeric proton signal at δ 4.96 (*J* 8 Hz); other features were similar to those of **4a**.

Anal. Calc. for $C_{11}H_{23}NO_6S$ (297.37 daltons): C, 44.43; H, 7.80; N, 4.71; S, 10.78. Found: C, 44.22; H, 7.93; N, 4.63; S, 10.80.

3-(2-Aminoethylthio)propyl 2-acetamido-2-deoxy-α-D-glucopyranoside (**6**). — The eluate from the Rexyn column was examined by t.l.c. (solvent *A*), and the fractions containing **6** were combined and evaporated. The white solid thus obtained was recrystallized from 95% ethanol to give 3.7 mmoles (76%) of **6**. Further recrystallization from 95% ethanol gave a specimen of **6** that was homogeneous by t.l.c. (solvent *A*); m.p. 149–151°, $[\alpha]_D^{25}$ +126.0° (*c* 5.02, water); p.m.r. data (D_2O): δ 2.42 (s, 3, $COCH_3$) and 5.36 (d, 1, *J* 2–3 Hz, anomeric proton); other features were similar to those of **4**.

Anal. Calc. for $C_{13}H_{26}N_2O_6S$ (338.43 daltons): C, 46.13; H, 7.74; N, 8.28; S, 9.48. Found: C, 46.38; H, 7.93; N, 8.14; S, 9.59.

3-(2-Aminoethylthio)propyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**6a**). — Fractions containing **6a** were located by the aid of t.l.c. (solvent *A*), combined, and evaporated. The white solid thus obtained was recrystallized from 95% ethanol–ether; yield 1.5 mmoles (30%). Recrystallization from 95% ethanol and then 95% ethanol–ether gave crystals that were homogeneous in t.l.c. (solvent *A*); m.p. 175–177°, $[\alpha]_D^{25}$ −10.8° (*c* 3.97, water); p.m.r. data (D_2O): δ 2.44 (s, 3H, $COCH_3$) and 4.98 (d, 1, *J* 8 Hz, anomeric proton); other features were similar to those of **4a**.

Anal. Calc. for $C_{13}H_{26}N_2O_6S$ (338.43 daltons): C, 46.13; H, 7.74; N, 8.28; S, 9.48. Found: C, 46.25; H, 7.87; N, 8.13; S, 9.60.

DISCUSSION

Allyl α-D-glucopyranoside and α-D-galactopyranoside were obtained in satisfactory yield and purity by the Fischer method with a strong, cation-exchange resin (Dowex-50) as the catalyst. For the preparation of allyl 2-acetamido-2-deoxy-α-D-glucopyranoside, a weaker acid catalyst (BF_3) gave much better results.

Despite the differing reactivities (shown in Table I), each of the glycosides reacted with 2-aminoethanethiol to give one major product that had elemental analyses agreeing with the values calculated for the product expected. The presence of a primary amino group in the product, as well as the almost equivalent decrease in allyl glycoside and SH group during the reaction, indicated that the addition occurred at the SH group. Furthermore, the p.m.r. spectra of all of the products showed that this SH addition yielded the linear aglycon $-(CH_2)_3S(CH_2)_2NH_2$, not the branched $-CH_2CH(CH_3)S(CH_2)_2NH_2$, as a signal for –SMe was totally absent from the p.m.r. spectra of **4**, **4a**, **5**, **5a**, **6**, and **6a**.

A small amount of byproduct was also obtained from all of the allyl glycoside–2-aminoethanethiol reaction-mixtures. The proportion of byproduct formed appeared to be highest for allyl 2-acetamido-2-deoxy-β-D-glucoside, the least reactive of the allyl glycosides studied. In each instance, the byproduct had the following characteristics: (*1*) It moved half as fast in t.l.c. (solvent *A*) as the main product; (*2*) it had a larger molecular size (gel filtration); and (*3*) it was ninhydrin-positive and was

slightly less basic than the main product (as shown by chromatography on Rexyn 101). Based on these facts, plus the observation that there was a small decrease in the content of primary amino group during the reaction, it seems probable that the by-product results from the addition of the amino group of 2-aminoethanethiol to the allyl group, with subsequent dimerization *via* disulfide formation.

Determination of SH groups by Ellman's method[2,13] is more convenient than by Boyer's[12], but, in general, the results from the two methods agreed well. However, reproducible results could not be obtained when Ellman's method was used to monitor the change in concentration of SH group during the allyl glycoside–2-aminoethanethiol reaction, but Boyer's method consistently showed, during the reaction, a decrease in SH that paralleled the increase in the amount of product as indicated by t.l.c.

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