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PREPARATION OF 6-AMINOHEXYL D-ALDOPYRANOSIDES*

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ABSTRACT

6-Aminohexyl glycosides covalently linked to solid matrices are effective reagents for the isolation of proteins that bind to carbohydrates [Schnaar and Lee, *Biochemistry*, 14 (1975) 1535–1541], and for the study of interactions between intact cells and immobilized carbohydrates [Weigel *et al.*, *J. Biol. Chem.*, 253 (1978) 330–333]. The preparation of the 6-aminohexyl glycosides of the following D-pyranoses is now described: β-glucose, β-galactose, 2-acetamido-2-deoxy-β-glucose, α-mannose, β-maltose, β-melibiose, β-lactose, and β-cellobiose. These glycosides were prepared by glycosylation of 6-(trifluoroacetamido)hexanol with the appropriate acetylated glycosyl halide in 1 : 1 (v/v) benzene–nitromethane, with mercuric cyanide as the catalyst. Deacylation of the glycosides was achieved in two steps: use of sodium methoxide for *O*-deacetylation, and of an anion-exchange resin for *N*-de(trifluoroacetyl)ation.

INTRODUCTION

Aminoalkyl glycosides have proved to be extremely useful compounds for isolating proteins that interact with glycose residues. In general, these glycosides are coupled to solid matrices by utilizing the free, terminal amino groups in the aglycon. Some 6-aminohexyl glycosides were first synthesized by R. Barker *et al.*[1,2], and additional compounds in this series are now reported by Barker and co-workers in the accompanying paper[3].

We have previously described[4–6] the synthesis of a series of 1-thioaldopyranosides that have an ω-amino group on the aglycon. These compounds have been used on solid matrices to purify lectins[7], and to study the sugar-specific binding of intact

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fibroblasts[8]. The present report describes the preparation and properties of the 6-aminohexyl 1,2-*trans*-pyranosides of the following sugars: β-D-glucose, β-D-galactose, 2-acetamido-2-deoxy-β-D-glucose, α-D-mannose, β-maltose, β-melibiose, β-lactose, and β-cellobiose. These carbohydrate derivatives have been utilized in studies on the specific recognition of immobilized sugars by chicken[9,10] and rat[11] heptocytes.

EXPERIMENTAL

Materials. — The following compounds or materials were obtained from commercial sources: 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide and 6-aminohexanol (recrystallized twice from dry ethanol) from Sigma; Dowex-1 X8 (50–100 mesh) from Bio-Rad; Rexyn 101 (200–400 mesh) from Fisher, and Amberlite CG-50 (200–400 mesh) from Mallinckrodt; mono- and di-saccharides, all of the D series, from Sigma or Pierce; Sephadexes LH-20, G-15, and G-25 from Pharmacia; silica gel 60 from E. Merck; trifluoroacetic anhydride from Matheson, Coleman and Bell; other chemical compounds were of reagent grade. Solvents were dried over Linde Molecular Sieves (type 4A) before use.

General methods. — Melting points (uncorrected) were obtained with a Fisher–Johns or a Thomas–Hoover apparatus. Proton magnetic resonance (p.m.r.) spectra were recorded with a JEOL NMH-100 spectrometer. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tennessee. A Cary 60 spectropolarimeter set at 589 nm was used for optical rotation studies at 25°. Neutral sugars were analyzed by a modified phenol–sulfuric acid procedure[12]. Amino groups were measured with a previously reported fluorometric method using fluorescamine[13] (FLURAM: from Roche Diagnostics, Nutley, N.J.) and also by a modified trinitrobenzenesulfonic acid procedure[14]. The following solvent systems were used to develop thin-layer chromatograms on aluminum-backed plates of silica gel F-254 (Merck): *A*, 1:1 (v/v) benzene–ethyl acetate; *B*, 3:2:1 (v/v) ethyl acetate–acetic acid–water; and *C*, 9:4:2 (v/v) ethyl acetate–isopropyl alcohol–water. In t.l.c., the components were detected by spraying with 10% sulfuric acid in 50% ethanol and charring for 5–10 min at 130° for carbohydrates, and, for free amino groups, by spraying with 0.2% ninhydrin in 95% ethanol and heating for 5 min at 130°. For detection of trifluoroacetylated amino groups, the plate was first sprayed with 2M NaOH, dried for a few minutes at room temperature, and then sprayed with the ninhydrin solution and 2M acetic acid before heating for 5 min at 130°. Evaporations were performed at 40° in a Büchi Rotavapor R with a water aspirator.

Preparation of 6-(trifluoroacetamido)hexanol. — 6-Aminohexanol (20 g, 160.4 mmol) was slowly added, with stirring, to trifluoroacetic anhydride (60 ml, 424 mmol) in a 250-ml, round-bottomed flask during ~20 min at 0°. The flask was stoppered with a drying tube, and the mixture was stirred for 2–3 h, the reaction being monitored by t.l.c. (R_F values in solvent *A*: 6-aminohexanol, 0.00; 6-(trifluoracetamido)hexanol trifluoroacetate, 0.60). When the reaction was complete, water (150 ml) was added, and the mixture was stirred for 1 h while being allowed to warm to room temperature.

The mixture was then extracted three times with equal volumes of chloroform, and the extracts were combined, dried (anhydrous sodium sulfate), and evaporated to a syrup. The syrup was dissolved in a small volume of dry methanol, the solution was evaporated, and the process was repeated. A solution of the syrup in dry methanol (100 ml) was now treated with an equimolar amount (based on the weight of the syrup) of sodium methoxide in methanol, and the reaction was allowed to proceed with stirring at room temperature. When *O*-de(trifluoroacetyl)ation was complete (based on t.l.c. in solvent *A*: R_F of 6-(trifluoracetamido)hexanol, 0.15), methanol-washed Rexyn 101 (H^+) resin (40 ml) was added, and stirring was continued. When the pH reached 6–7, the mixture was filtered, the resin was rinsed with dry methanol, and the combined filtrates were evaporated. The product crystallized from 20% ethanol in a yield of 50–70%, m.p. 51–52° (lit.[6] m.p. 52–53°). Despite the lower yield, this method of synthesis is preferred to previously reported procedures[2,6], as it does not produce the noxious ethanethiol as a by-product.

Preparation of acetylated glycosyl halides. — The mono- or di-saccharide (18–20 g, containing 414–556 meq of hydroxyl group) was dissolved in dry pyridine (150 ml, 1.858 mol) and acetic anhydride (150 ml, 1.591 mol), and the mixture was stirred overnight at room temperature. This treatment was sufficient to acetylate all of the sugars used, except for cellobiose, which was acetylated by using 50% more acetic anhydride–pyridine and heating overnight at 60°. The mixture was then concentrated to half its volume by rotary evaporation, and the concentrate was added dropwise to 1.5 liters of ice-cold water with vigorous stirring. The precipitate was collected by filtration, washed extensively with cold water, and dried (83–95% yield). Per-*O*-acetylated saccharides were usually at least 95% pure by t.l.c. (R_F values in solvent *A*: monosaccharides, ~0.6; disaccharides, 0.50–0.64), and were used without further purification.

The per-*O*-acetylglycosyl bromides from cellobiose, galactose, lactose, maltose, mannose and melibiose were prepared at room temperature by using HBr in glacial acetic acid[15]. To optimize the extent of reaction and the purity of the product, trial reactions were performed with each sugar by varying the concentrations of the sugar and the HBr, and the reaction times. Reactions were monitored by t.l.c. (R_F in solvent *A*: monosaccharides, 0.75–0.85; disaccharides, 0.65–0.75). Typical reaction-conditions were as follows. To dry glacial acetic acid (50 ml) and ~30% (w/v) HBr (50 ml) at room temperature was added maltose octaacetate (20 g, 29.5 mmol). After the octaacetate had dissolved, the solution was stirred for 30 min, chloroform (100 ml) was added, and the mixture was poured into a 2-liter, separatory funnel containing 1.2 liters of ice–water. After vigorous shaking, the chloroform layer was removed, and washed twice with half-volumes of water and then with 0.2M $NaHCO_3$ until the pH of the aqueous phase was ~7, dried (anhydrous sodium sulfate), concentrated by rotary evaporation to ~30 ml, and the concentrate added dropwise with stirring to petroleum ether (b.p. 30–60°; 350 ml) at 4°. The precipitate was collected, and vacuum-dried. The corresponding halides of galactose and mannose were also prepared by the method of Lemieux[16], using red phosphorus and bromine

in acetic anhydride. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride was prepared by the method of Horton[17].

The yields of the glycosyl halides, by any of these procedures, were 80–90%, and all of the products were ~95% pure by t.l.c. (solvent *A*).

Preparation of 6-(trifluoroacetamido)hexyl glycoside acetates. — Each per-*O*-acetylglycosyl halide was coupled to 6-(trifluoroacetamido)hexanol as follows. Equimolar amounts (12–20 mmol) of acetylated glycosyl halide, mercuric cyanide, and the alcohol were mixed with 1:1 (v/v) benzene–nitromethane (100 ml), and the mixture was vigorously stirred. After 24–48 h at room temperature, the glycosyl halide became depleted, as determined by t.l.c. in solvent *A* (R_F values for the glycosides produced: 0.7–0.8 for those of monosaccharides and 0.3–0.4 for those of disaccharides; the R_F values for all products in solvent *B* or *C* were ~0.9). The mixture was filtered, the solids were washed with a small volume of the benzene–nitromethane mixture, and the combined filtrates were evaporated, the residue taken up in chloroform (~150 ml), and the suspension filtered. The filtrate was washed 3 times with half-volumes of 10% NaCl and once with 0.01M (ethylenedinitrilo)tetraacetic acid, dried (anhydrous sodium sulfate), and evaporated to dryness. A solution of the residue in 1:1 (v/v) pyridine–acetic anhydride (50 ml) was stirred overnight at room temperature, to ensure complete acetylation of the hydroxyl groups. The mixture was evaporated, the residue dissolved in chloroform (50 ml), and the solution was washed successively with half-volumes of water (twice) and 0.2M $NaHCO_3$ until the pH of the aqueous phase reached 7. The chloroform layer was evaporated, and the residue was dissolved (once) in dry toluene and the solution evaporated, and (twice) in 100% ethanol and the solution evaporated.

Chromatographic purification of the glycosides. — One of the following chromatographic methods was used for purification of the glycosides, as needed.

(*a*) *Dry-column chromatography on silica gel.* Dry, powdery silica gel (50–100 g/g of material to be purified) was packed into a Nylon bag (Walter Coles and Co.; 2.54 × 50 cm). The crude glycoside was dissolved in the minimal volume of 1:1 (v/v) benzene–ether, and the solution was transferred to the column. The column was eluted by slow addition of the same solvent until the solvent front reached the bottom of the column. The bag was then cut into 1-cm sections by using an electric carving-knife. A small portion of silica gel from each section was directly assayed by the phenol–sulfuric acid procedure[12], and the sugar-containing segments were extracted with 50 volumes of 1:1 (v/v) benzene–ether. The eluted products were identified by t.l.c. (solvent *A*), and the desired fractions were combined, and evaporated to dryness.

(*b*) *Chromatography on Sephadex LH-20.* The sample to be purified (2–3 g) was dissolved in 95% ethanol (10–20 ml), and the solution was applied to a Sephadex LH-20 column (4 × 150 cm) equilibrated with 95% ethanol. The same solvent was used for elution. Fractions were assayed for carbohydrate by the phenol–sulfuric acid procedure[12] and by t.l.c. (solvent *A*), and fractions containing the desired glycoside were combined and evaporated. This column gave excellent separation of

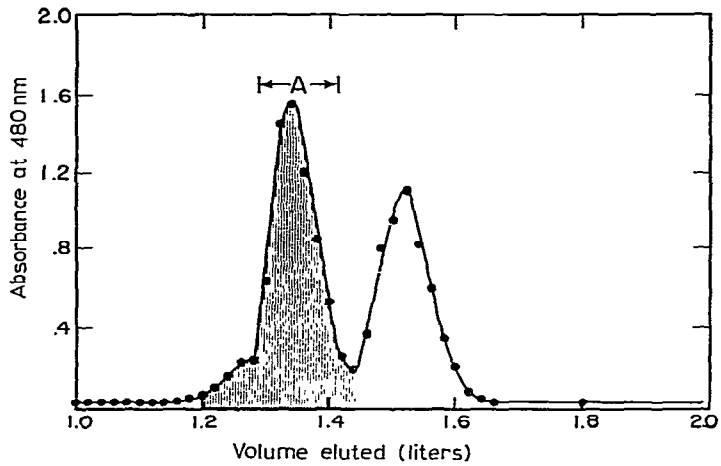

Fig. 1. An example of the chromatographic purification of the glycosylation products. [6-(Trifluoroacetamido)hexyl hepta-*O*-acetyl-β-cellobioside was synthesized as described in the text. A 3-g sample of the syrup obtained from the reaction mixture was dissolved in 95% ethanol (10 ml), and the solution was applied to a column (4 × 150 cm) of Sephadex LH-20 equilibrated with the same solvent. The column was eluted with 95% ethanol, and 20-ml fractions were collected. Portions (10 μl) of each fraction were analyzed for neutral sugar by the phenol–sulfuric acid procedure[12], and samples of the carbohydrate-containing fractions were examined by t.l.c. in solvent *A*. The shaded region represents fractions containing compounds having trifluoroacetamido groups. The unshaded region contained unreacted hepta-*O*-acetylcellobiosyl bromide. The region denoted by A contained the purified glycoside, as shown by t.l.c. Unreacted 6-(trifluoroacetamido)hexanol was eluted with ~1.6 liters of the solvent.]

glycosidic product from starting materials and from byproducts of larger molecular weight. A typical fractionation is shown in Fig. 1.

After purification by either of the column procedures, the products were obtained in 40–60% yield, and the 6-(trifluoroacetamido)hexyl glycoside acetates of the following sugars were crystallized from ethanol or ethanol/water: cellobiose (m.p. 130–135°), maltose (m.p. 177–178°), D-glucose (m.p. 76–79°), and 2-acetamido-2-deoxy-D-glucose (m.p. 168–170°; lit.[2] m.p. 165.5–166.5°). The remaining products were isolated as amorphous materials.

Deacylation of 6-(trifluoroacetamido)hexyl glycoside acetates. — Deacylation was accomplished in two stages; first, by *O*-deacetylation with sodium methoxide, and second, by *N*-de(trifluoroacetyl)ation with an anion-exchange resin. *O*-Deacetylation was conducted by dissolving 5–6 mmol of acetylated glycoside in anhydrous methanol (25 ml), treating with sodium methoxide (equivalent to 5 mole% of the total *O*-acetyl groups), and stirring at room temperature. The reaction was usually complete within a few hours, as judged by t.l.c. with solvent *C*. (R_F values of *O*-deacetylated compounds were 0.68–0.75 for monosaccharides, and 0.42–0.60 for disaccharides. R_F values of these compounds in solvent *B* were 0.75–0.85.) Rexyn 101 (H^+) resin (2 ml), prewashed with anhydrous methanol, was added, and the mixture was gently stirred for 10 min (to remove sodium ions), filtered, and the filtrate evaporated. 6-(Trifluoroacetamido)hexyl 2-acetamido-2-deoxy-β-D-glucopyranoside

crystallized (m.p. 189–192°), but the other deacetylated glycosides were obtained as syrups or amorphous materials.

To remove *N*-trifluoroacetyl groups, the material was dissolved in 50% ethanol (2–4 g per 25 ml), and the solution was treated with Dowex-1 X8 (OH^-) resin (20–50 mesh; 2 ml of resin per meq of trifluoroacetyl group) pre-equilibrated with 50% ethanol. The mixture was stirred at room temperature, and the de(trifluoroacetyl)ation was monitored by t.l.c. with solvent *B* (R_F values of the products: monosaccharides, 0.25–0.33; disaccharides, 0.10–0.15) or solvent *C* (R_F values of all products, essentially zero). The reaction was usually complete within 3 h. The mixture was filtered, the resin rinsed twice with 50% ethanol (20 ml), and twice with water (20 ml), and the filtrates were combined and evaporated. The overall yields for deacetylation were almost quantitative. However, if *O*- and *N*-deacylation were performed using only[6] Dowex-1 (OH^-), without prior *O*-deacetylation with sodium methoxide, the yields were only ~70%.

Purification of 6-aminohexyl D-*aldopyranosides.* — Some of the 6-aminohexyl glycosides crystallized from 95% ethanol after treatment with Dowex-1 (OH^-) (see later). If crystals were not obtained, the product was further purified by passage through a column (2.5 × 142 cm) of Sephadex G-15, or a column (5 × 212 cm) of Sephadex G-25 equilibrated with 0.1M acetic acid. The fractions containing product were combined, evaporated to dryness, the residue dissolved in water and evaporated (several times), and a solution in water lyophilized, to give the acetate (salt). In some cases, further steps were needed, in order to remove minor contaminants.

TABLE I

ELEMENTAL ANALYSES OF 6-AMINOHEXYL ALDOPYRANOSIDES[a]

Parent sugar	*Molecular weight*	*C (%)*	*H (%)*	*N (%)*
D-Glucose	339.39[b]	49.64	8.71	4.08
		(49.55)	(8.61)	(4.13)
D-Galactose	339.39[b]	49.51	8.62	4.17
		(49.55)	(8.61)	(4.13)
2-Acetamido-2-deoxy-D-glucose	320.39	52.14	8.90	8.67
		(52.48)	(8.81)	(8.74)
D-Mannose	348.39[b,c]	48.01	8.71	3.92
		(48.27)	(8.68)	(4.02)
Cellobiose	501.53[b]	47.40	7.74	2.78
		(47.89)	(7.83)	(2.79)
Maltose	450.48[c]	48.26	8.20	3.05
		(48.00)	(8.06)	(3.11)
Melibiose	501.53[b]	47.79	7.92	2.72
		(47.89)	(7.83)	(2.79)
Lactose	501.53[b]	47.76	7.84	2.89
		(47.89)	(7.83)	(2.79)

[a]Calculated values are given in parentheses. [b]Isolated as acetates (salts). [c]Calculated as the hemihydrate.

TABLE II

SOME PHYSICAL CONSTANTS OF 6-AMINOHEXYL ALDOPYRANOSIDES

Parent sugar	*P.m.r. data for anomeric proton*[a]		*Optical rotation* *deg.cm².mol⁻¹*
	Chemical shift (δ)	*Coupling constant (Hz)*	
D-Glucose[b]	4.90	7	−1,341
D-Galactose[b]	4.86	7	−1,462
D-Mannose	5.26	<2	+14,484
2-Acetamido-2-deoxy-D-glucose	4.88	7.5	−8,587
Cellobiose[b]	4.90	8	+6,249[c]
Melibiose[b]	4.92	8	+35,043
Lactose	4.82	7	+11,294[c]
Maltose	4.86	8	+27,981

[a]In D_2O. [b]Isolated as acetate (salts). [c]P.m.r. spectroscopy revealed ~5–10% contamination by the α-glycoside.

Several preparations of 6-aminohexyl α-D-mannopyranoside were purified by ion-exchange chromatography using Amberlite CG-50 (H^+) resin (200–400 mesh) in water. The aminohexyl glycoside was retained on the column, but contaminants lacking free amino groups were eluted; the pure glycoside was then eluted with 0.1M acetic acid, and isolated as already described. A different cation-exchange resin, namely, Rexyn 101 (NH_4^+) (200–400 mesh), was used to purify a preparation of 6-aminohexyl β-D-maltopyranoside; this product was eluted with ammonium hydroxide, the eluate evaporated, the residue rinsed several times with absolute methanol, and the rinse lyophilized.

Characterization of 6-aminohexyl D-*aldopyranosides.* — All of the glycosides showed a single spot (with ninhydrin and sulfuric acid sprays) in t.l.c. in solvent *B*. Elemental analyses of the glycosides, many of which were obtained as acetates (salts), are given in Table I; they agreed with the theoretical values. Many of these compounds are extremely hygroscopic, and difficult to keep as dry solids. Crystals (of the free amine) were obtained for the 6-aminohexyl β-D-pyranosides of the following sugars: D-glucose (m.p. 99–101°), D-galactose (m.p. 133–135°), maltose (m.p. 137–141°), lactose (m.p. 146–149°), and 2-acetamido-2-deoxy-D-glucose (m.p. 179–180°). Overall yields of final product, based on the amount of initial, free sugar, ranged from 5 to 15%.

Some physical constants of the glycosides are presented in Table II. All p.m.r. data were obtained for solutions of samples (pre-exchanged three times with D_2O) in D_2O. All of the spectra show the characteristic signals of the 6-aminohexyl group that were reported previously[1,3]. The 8 internal methylene protons appear as a broad, double peak (δ 1.7–2.4) and the protons of $-CH_2ND_2$ appear as a well-resolved triplet (δ 3.09–3.48).

In most cases, the optical rotation of the glycoside was in good agreement with the values reported[18] for the appropriate methyl glycoside. The exceptions noted

in Table II (the cellobioside and the lactoside) were judged by p.m.r. spectroscopy to be contaminated with 5–10% of the α-glycoside. From these results, we conclude that the mannose derivative was the α anomer, whereas the corresponding β anomers were the products obtained for 2-acetamido-2-deoxy-D-glucose, D-galactose, D-glucose, maltose and melibiose. The glycosides of lactose and cellobiose were isolated as mixtures containing ~90–95% of the β anomer and ~5–10% of the α anomer.

The acetates (salts) of the 6-aminohexyl glycosides are very stable, and cannot be exchanged easily with other acid groups, for example by evaporation from solvents. That the acetate present in the final glycoside was present as a salt (and not as a residual, acetic ester) was demonstrated by exchange of acetate for *p*-toluenesulfonate by passage through Dowex-1 (p-$MeC_6H_4SO_3^-$); p.m.r. spectroscopy then revealed the loss of the acetyl signal at δ 1.7, and the acquisition of characteristic aromatic signals between δ 7.0 and 7.5.

DISCUSSION

A general and powerful method for the study of such interacting molecules as enzymes and substrates, or lectins and carbohydrates, is to link covalently one of the two interacting molecules to a solid matrix. This method (*i.e.*, affinity chromatography) has been widely used to purify antigens, enzymes, immunoglobulins, lectins, and other substances[19], and for the study of molecular interactions between the fixed molecules and the second component in solution[20].

We have adapted this technique in order to study the specific recognition by intact cells[6,9–11] of glycose residues immobilized on surfaces. The solid matrix of choice for these experiments appears to be poly(acrylamide), and procedures have recently been developed[21,22] to immobilize, on flat poly(acrylamide) surfaces, molecules containing amino groups. These experiments require ligands that contain, at one end of the molecule, an amino group that is separated from the glycose residue by a "spacer arm". The present report describes the preparation of 6-aminohexyl glycosides, namely, ligands containing substituted hexyl groups as the spacer arm.

As reported elsewhere[9–11,23,24], chicken hepatocytes bind specifically to poly(acrylamide) gels containing 6-aminohexyl 2-acetamido-2-deoxy-β-D-glucopyranoside, whereas rat hepatocytes bind specifically to poly(acrylamide) gels containing 6-aminohexyl β-D-galactopyranoside. More-systematic studies are needed in order to determine the effect of the length of the spacer arm on the ability of cells to bind to these glycoside gels, but, thus far, the hexyl group is the most active of those tested. However, should longer or shorter aglycon groups be required in some cases, it should be noted that the procedures described herein may be readily adapted for the synthesis of the appropriate glycosides.

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REFERENCES

1 R. BARKER, K. W. OLSEN, J. H. SHAPER, AND R. L. HILL, *J. Biol. Chem.*, 247 (1972) 7135–7147.
2 R. BARKER, C. CHIANG, I. P. TROYER, AND R. L. HILL, *Methods Enzymol.*, 34 (1974) 317–328.
3 C. K. CHIANG, M. MCANDREW, AND R. BARKER, *Carbohydr. Res.*, 70 (1979) 93–102.
4 R. T. LEE AND Y. C. LEE, *Carbohydr. Res.*, 34 (1974) 151–160.
5 R. T. LEE AND Y. C. LEE, *Carbohydr. Res.*, 37 (1974) 193–201.
6 S. CHIPOWSKY AND Y. C. LEE, *Carbohydr. Res.*, 31 (1973) 339–346.
7 R. L. SCHNAAR AND Y. C. LEE, *Biochemistry*, 14 (1975) 1535–1541.
8 S. CHIPOWSKY, Y. C. LEE, AND S. ROSEMAN, *Proc. Natl. Acad. Sci. USA*, 70 (1973) 2309–2312.
9 R. L. SCHNAAR, Ph.D. Dissertation, Johns Hopkins University, 1976.
10 P. H. WEIGEL, R. L. SCHNAAR, AND M. S. KUHLENSCHMIDT, *Fed. Proc.*, 36 (1977) 653.
11 P. H. WEIGEL, E. SCHMELL, Y. C. LEE, AND S. ROSEMAN, *J. Biol. Chem.*, 253 (1978) 330–333.
12 J. F. MCKELVY AND Y. C. LEE, *Arch. Biochem. Biophys.*, 132 (1969) 99–110.
13 M. NAOI AND Y. C. LEE, *Anal. Biochem.*, 57 (1974) 640–644.
14 Y. C. LEE, *Carbohydr. Res.*, 67 (1978) 509–514.
15 H. G. FLETCHER, JR., *Methods Carbohydr. Chem.*, 2 (1963) 226–228.
16 R. U. LEMIEUX, *Methods Carbohydr. Chem.*, 2 (1963) 221–222.
17 D. HORTON, *Methods Carbohydr. Chem.*, 6 (1972) 282–285.
18 J. CONCHIE, G. A. LEVVY, AND C. A. MARSH, *Adv. Carbohydr. Chem.*, 12 (1957) 157–187.
19 M. WILCHEK AND W. B. JAKOBY, *Methods Enzymol.*, 34 (1974) 3–10.
20 V. HOŘEJŠÍ, M. TICHÁ, AND J. KOCOUREK, *Biochim. Biophys. Acta*, 499 (1977) 290–300.
21 P. H. WEIGEL, R. L. SCHNAAR, S. ROSEMAN, AND Y. C. LEE, *Methods Carbohydr. Chem.*, 8 (1978) in press.
22 R. L. SCHNAAR, P. H. WEIGEL, S. ROSEMAN, AND Y. C. LEE, *Methods Carbohydr. Chem.*, 8 (1978) in press.
23 R. L. SCHNAAR, P. H. WEIGEL, M. S. KUHLENSCHMIDT, Y. C. LEE, AND S. ROSEMAN, *J. Biol. Chem.*, in press.
24 P. H. WEIGEL, R. L. SCHNAAR, E. SCHMELL, M. S. KUHLENSCHMIDT, R. T. LEE, Y. C. LEE, AND S. ROSEMAN, manuscript in preparation.