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Note

Synthesis of 1-thio-β-cellobiosides

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1-Thioglycopyranosides are known substrate analog inhibitors of glycosidases[1–3]. Thus, thio-D-galactopyranosides with long aliphatic aglycons (hexyl and octyl)[2] and *p*-aminophenyl 1-thio-β-D-galactopyranoside[3] competitively inhibit the hydrolysis of *o*-nitrophenyl β-D-galactopyranoside by β-D-galactosidase of *Escherichia coli*. Competitive inhibitors of this type have been covalently attached to agarose and polyacrylamide beads, and the resulting adsorbents used for the purification in high yields of glycosidases by affinity chromatography[1,4]. Thioglycosides have also been applied to investigate other biological interactions[5], as well as in glycoside synthesis[6].

The enzymic degradation of cellulose by cellulases [(1→4)-β-D-glucan 4-glucanohydrolases, EC 3.2.1.4] has received considerable attention, as this process is essential to the carbon cycle, beneficial to ruminants, and results in the destruction of timber and manufactured cellulosic goods. Cellulases have also found application in the controlled modification of foodstuffs and in the ecologically important saccharification of waste cellulose. However, structural characterization of these enzymes has been hindered by the small quantities isolated by the gel electrophoretic and isoelectric focusing methods generally used to purify these proteins[7]. 1-Thio-β-cellobiosides are potential competitive inhibitors of cellulases, and, in particular, of exocellobiohydrolases, and derivatives appropriately substituted in the aglycon moiety could be bound to insoluble supporting polymers for the purpose of purifying, by affinity chromatography, these environmentally important enzymes. In this work, the synthesis of a number of potentially useful alkyl, aryl, and aralkyl 1-thio-β-cellobiosides is described.

6-(Trifluoroacetamido)hexyl (**3**), heptyl (**5**), benzyl (**7**), and *p*-nitrobenzyl (**9**) hepta-*O*-acetyl-1-thio-β-cellobioside, as well as methylene bis(hepta-*O*-acetyl-1-thio-β-cellobioside) (**16**), were synthesized by reaction of hepta-*O*-acetyl-1-thio-β-cellobiose (**2**) with the appropriate alkyl or aralkyl halide in the presence of potassium carbonate. *p*-Aminophenyl (**12**) and *p*-chlorophenyl (**14**) hepta-*O*-acetyl-1-thio-β-cellobioside were obtained by condensation of hepta-*O*-acetyl-α-cellobiosyl bromide[8] with the appropriate thiophenol in the presence of sodium hydroxide. The β-D configuration was confirmed by examination of the 300-MHz n.m.r. spectrum in

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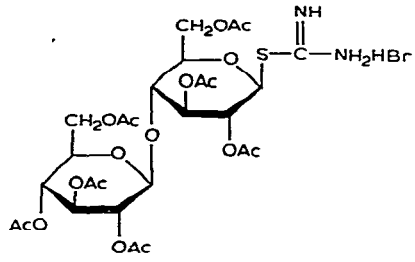

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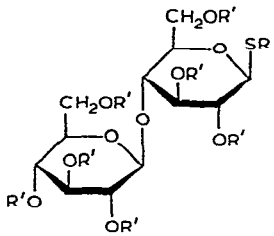
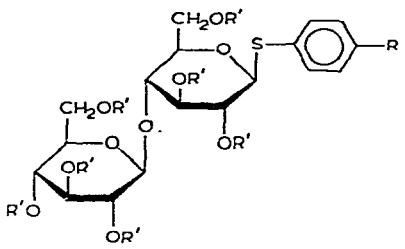
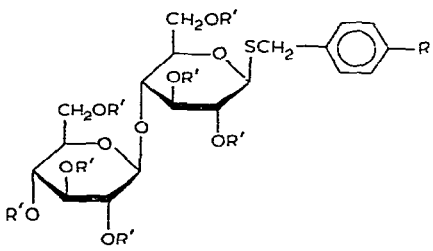


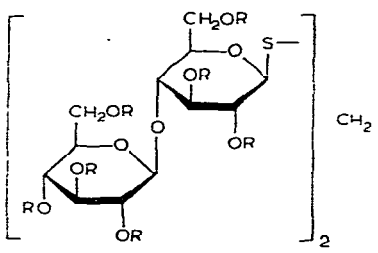
2 R = H, R′ = Ac
3 R = $(CH_2)_6NHCOCF_3$, R′ = Ac
4 R = $(CH_2)_6NH_2$, R′ = H
5 R = $(CH_2)_6CH_3$, R′ = Ac
6 R = $(CH_2)_6CH_3$, R′ = H



12 R = NH_2, R′ = Ac
13 R = NH_2, R′ = H
14 R = Cl, R′ = Ac
15 R = Cl, R′ = H



7 R = H, R′ = Ac
8 R = R′ = H
9 R = NO_2, R′ = Ac
10 R = NO_2, R′ = H
11 R = NH_2, R′ = H



16 R = Ac
17 R = H

chloroform ($J_{1,2} \sim 10$ Hz) (see Experimental). 6-Aminohexyl (**4**) and *p*-aminophenyl (**13**) 1-thio-β-cellobioside were prepared by saponification of the corresponding heptaacetates with strong anion-exchange resin[9], and *n*-heptyl (**6**), benzyl (**8**), *p*-nitrobenzyl (**10**), and *p*-chlorophenyl (**15**), as well as methylene bis(1-thio-β-cellobioside) (**17**), by catalytic deacetylation (sodium methoxide). Catalytic hydrogenation of **10** afforded *p*-aminobenzyl 1-thio-β-cellobioside (**11**).

EXPERIMENTAL

General methods. — Solutions were evaporated below 50° under diminished pressure. Melting points were determined with a Thomas–Hoover "Unimelt" apparatus and are uncorrected. Optical rotations were measured with a Zeiss

polarimeter. N.m.r. spectra were recorded at 60, 100, and 300 MHz with Varian T-60, HA-100 and SC-300 n.m.r. spectrometers respectively; chemical shifts are given on the τ scale; unless otherwise stated, spectra were recorded at ambient temperatures on solutions in chloroform-*d*, with tetramethylsilane (τ 10.00) as the internal standard; spectra were analyzed on a first-order basis. T.l.c. was performed on 250-μm Silica Gel GF_{254} (Analtech) plates, and indication was effected with a ceric sulfate (1%)–sulfuric acid (10%) spray. Column chromatography was conducted with Silica Gel No. 7734 (70–230 mesh) (E. Merck).

2-S-(2,3,6,2′,3′,4′,6′-Hepta-O-acetyl-β-cellobiosyl)-2-thiopseudourea hydrobromide (**1**). — A solution of 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-α-cellobiosyl bromide[8] (8.0 g) and thiourea (1.0 g) in dry acetone (15 ml) was boiled under reflux for 45 min and then cooled. The precipitate was filtered off and dried; yield 7.2 g, (79%). An analytical sample was obtained by recrystallization from isopropyl alcohol, m.p. 182–183° (dec.), $[\alpha]_D^{27}$ −26.2 ±0.9° (*c* 0.5, water).

Anal. Calc. for $C_{27}H_{39}BrN_2O_{17}S \cdot H_2O$: C, 40.86; H, 5.21; Br, 10.07; N, 3.53; S, 4.04. Found: C, 41.05; H, 5.20; Br, 10.41; N, 3.40; S, 4.07.

2,3,6,2′,3′,4′,6′-Hepta-O-acetyl-1-thio-β-cellobiose (**2**). — A mixture of **1** (8.0 g) and potassium pyrosulfite (4.0 g) in 5:3 (v/v) chloroform–water (40 ml) was stirred at reflux temperature for 30 min and cooled. The organic layer was separated, dried (sodium sulfate), and evaporated to a crystalline solid. Recrystallization from methanol afforded pure **2** (yield 4.6 g, 70%), m.p. 209–212°, $[\alpha]_D^{27}$ −10.3 ±0.5° (*c* 1, chloroform) {Lit.[10] m.p. 197°, $[\alpha]_D^{20}$ −12.4° (*c* 2.4, chloroform)}.

Anal. Calc. for $C_{26}H_{36}O_{17}S$: C, 47.85; H, 5.56; S, 4.91. Found: C, 47.60; H, 5.34; S, 4.89.

6-(Trifluoroacetamido)hexyl 2,3,6,2′,3′,4′,6′-hepta-O-acetyl-1-thio-β-cellobioside (**3**). — To a solution of **2** (1.5 g 2.3 mmol) in acetone (10 ml) was added 1-iodo-6-(trifluoroacetamido)hexane[9] (0.74 g 2.3 mmol) and a solution of potassium carbonate (0.32 g 2.3 mmol) in water (4 ml). The reaction mixture was stirred at room temperature overnight and concentrated, the residue partitioned between dichloromethane and water, and the organic layer washed with water and concentrated. The residue was applied to a column of silica gel, and the product was eluted with 8:1 (v/v) chloroform–ethyl acetate. The solid obtained was recrystallized from ethanol to afford pure **3** (yield 1.5 g, 77%), m.p. 143–144°, $[\alpha]_D^{27}$ −27.0 ±0.5° (*c* 1.1, chloroform); n.m.r.: τ 5.47 (d, $J_{1',2'}$ 8 Hz, H-1′), 5.56 (d, $J_{1,2}$ 10 Hz, H-1), 6.22 (t, $J_{3',4'} = J_{4',5'}$ 9.5 Hz H-4′) 6.62 (q $-CH_2NHCOCF_3$) 7.36 (m $-SCH_2-$) and 3.35 (broad s $-NHCOCF_3$).

Anal. Calc. for $C_{34}H_{48}F_3NO_{18}S$: C, 48.17; H, 5.71; F, 6.72; N, 1.65; S, 3.78. Found: C, 48.16; H, 5.65; F, 6.62; N, 1.50; S, 3.85.

6-Aminohexyl 1-thio-β-cellobioside (**4**). — A mixture of **3** (0.40 g) in 50% ethanol (44 ml) was stirred overnight with Bio-Rad AG 1-X2 (OH^-) anion-exchange resin (22 ml). The resin was filtered off and washed with methanol. The combined filtrate and washings were concentrated to a syrup that crystallized after several days (yield 0.14 g, 64%), m.p. 129–140°, $[\alpha]_D^{27}$ −36.2 ±1.1° (*c* 0.45, water).

Anal. Calc. for $C_{18}H_{35}NO_{10}S \cdot 0.5H_2O$: C, 46.34; H, 7.78; N, 3.00; S, 6.87. Found: C, 46.70; H, 7.83; N, 2.85; S, 7.05.

Heptyl 2,3,6,2',3',4',6'-hepta-O-acetyl-1-thio-β-cellobioside (**5**). — To a solution of **2** (1.1 g, 1.7 mmol) in acetone (10 ml) were added heptyl bromide (0.34 g, 1.9 mmol) and a solution of potassium carbonate (0.24 g, 1.7 mmol) in water (5 ml). The mixture was boiled under reflux for 15 min, cooled, and concentrated. The residue was extracted with dichloromethane, and the combined extracts washed with water, dried (sodium sulfate), and evaporated to a crystalline solid. Recrystallization from ethanol gave pure **5** (yield 1.0 g, 79%), m.p. 162.5–163.5°, $[\alpha]_D^{27}$ −31.8 ±0.5° (*c* 1, chloroform); n.m.r.: τ 5.49 (d, $J_{1',2'}$ 8 Hz, H-1'), 5.54 (d, $J_{1,2}$ 10 Hz, H-1), 6.23 (t, H-4'), 7.36 (m, $-SCH_2-$), and 9.12 (t, $(CH_2)_6CH_3$).

Anal. Calc. for $C_{33}H_{50}O_{17}S$: C, 52.79; H, 6.71; S, 4.27. Found: C, 52.42; H, 6.86; S, 4.28.

Heptyl 1-thio-β-cellobioside (**6**). — To a mixture of **5** (0.9 g) in methanol (10 ml) was added 0.1M methanolic sodium methoxide (1 ml). The reaction mixture was stirred at room temperature until solution, and then kept overnight at 0°. Neutralization with Bio-Rad AG 50W-X4 (H^+) cation-exchange resin, filtration, and concentration gave a solid that was recrystallized from methanol–diethyl ether to afford pure **6** (yield 0.53 g, 95%), m.p. 155–156.5°, $[\alpha]_D^{27}$ −39.7 ±0.5° (*c* 1, methanol).

Anal. Calc. for $C_{19}H_{36}O_{10}S \cdot 0.5H_2O$: C, 49.02; H, 8.01; S, 6.88. Found: C, 49.16; H, 7.85; S, 7.05.

Benzyl 2,3,6,2',3',4',6'-hepta-O-acetyl-1-thio-β-cellobioside (**7**). — This compound was prepared, in a similar fashion as **5**, from **2** (1.0 g, 1.5 mmol), benzyl bromide (0.28 g, 1.6 mmol), and potassium carbonate (0.21 g, 1.5 mmol). The solid obtained was recrystallized from ethanol to give pure **7** (yield 0.85 g, 75%), m.p. 191–193°, $[\alpha]_D^{27}$ −70.5 ±0.5° (*c* 1, chloroform); n.m.r.: τ 5.49 (d, $J_{1',2'}$ 8 Hz, H-1'), 5.73 (d, $J_{1,2}$ 10.2 Hz, H-1), 6.13 (AB q, $SCH_2C_6H_5$), 6.22 (t, $J_{3',4'} = J_{4',5'}$ 9.5 Hz, H-4'), and 2.67 (m, C_6H_5).

Anal. Calc. for $C_{33}H_{42}O_{17}S$: C, 53.36; H, 5.70; S, 4.32. Found: C, 53.33; H, 5.76; S, 4.33.

Benzyl 1-thio-β-cellobioside (**8**). — Compound **7** (0.40 g) in methanol (10 ml) was treated with catalytic sodium methoxide for 3 h at room temperature. Usual processing gave a syrup that was crystallized from isopropyl alcohol (yield 0.24 g, 97%), m.p. 185–186°, $[\alpha]_D^{27}$ −128 ±0.5° (*c* 1, methanol).

Anal. Calc. for $C_{19}H_{28}O_{10}S \cdot 0.5H_2O$: C, 49.88; H, 6.40; S, 7.01. Found: C, 49.84; H, 6.33; S, 7.11.

p-*Nitrobenzyl 2,3,6,2'3',4',6'-hepta-O-acetyl-1-thio-β-cellobioside* (**9**). — This compound was prepared, in a similar manner as **5**, from **2** (2.2 g, 3.4 mmol), *p*-nitrobenzyl bromide (0.81 g, 3.7 mmol), and potassium carbonate (0.47 g, 3.4 mmol). The resulting solid was recrystallized from chloroform–diethyl ether to afford pure **9** (yield 2.1 g, 79%), m.p. 213.5–214.5°, $[\alpha]_D^{27}$ −67.4 ±0.5° (*c* 1, chloroform); n.m.r.: τ 5.49 (d, $J_{1',2'}$ 7.8 Hz, H-1'), 5.70 (d, $J_{1,2}$ 10 Hz, H-1), 6.08 (AB q, $SCH_2C_6H_5$), 6.24 (t, $J_{3',4'} = J_{4',5'}$ 9.6 Hz, H-4'), 1.80 and 2.51 (2 d, $-C_6H_4-NO_2$).

Anal. Calc. for $C_{33}H_{41}NO_{19}S$: C, 50.31; H, 5.25; N, 1.78; S, 4.07. Found: C, 50.45; H, 5.22; N, 1.69; S, 4.08.

p-*Nitrobenzyl 1-thio-β-cellobioside* (**10**). — Compound **9** (2.0 g) in methanol (15 ml) was treated with catalytic sodium methoxide. The resulting precipitate was filtered off and recrystallized from water to afford pure **10** (yield 1.2 g, 96%), m.p. 236–238° (dec.), $[\alpha]_D^{27}$ −131 ±1.6° (*c* 0.6, *N*,*N*-dimethylformamide).

Anal. Calc. for $C_{19}H_{27}NO_{12}S$: C, 46.24; H, 5.52; N, 2.84; S, 6.50. Found: C, 46.16; H, 5.55; N, 2.76; S, 6.26.

p-*Aminobenzyl 1-thio-β-cellobioside* (**11**). — A solution of **10** (0.40 g) in 2:1 (v/v) methanol–water (150 ml) was hydrogenated in the presence of 5% palladium-on-barium sulfate (0.3 g) overnight at 2.5 atm. The catalyst was then removed by filtration over Celite, the filtrate concentrated, and the residue applied to a column of silica gel that was eluted with 40:10:1 and subsequently with 6:4:1 (v/v) chloroform–methanol–water. The product was crystallized from hot ethanol–methanol (yield 0.25 g, 67%), m.p. 206–208°, $[\alpha]_D^{27}$ −116 ±1° (*c* 0.5, water).

Anal. Calc. for $C_{19}H_{29}NO_{10}S$: C, 49.24; H, 6.31; N, 3.02; S, 6.92. Found: C, 48.95; H, 6.22; N, 2.90; S, 6.70.

p-*Aminophenyl 2,3,6,2',3',4',6'-hepta*-O-*acetyl-1-thio-β-cellobioside* (**12**). — To a solution of *p*-aminobenzenethiol (0.41 g, 3.3 mmol) in M aqueous sodium hydroxide (4.6 ml, 4.6 mmol) was added with stirring under an atmosphere of nitrogen a solution of hepta-*O*-acetyl-α-cellobiosyl bromide[8] (1.6 g, 2.3 mmol) in acetone (10 ml). Stirring was continued for 2 h under nitrogen, and then the reaction mixture was concentrated and the residue extracted with dichloromethane. The combined organic extracts were washed with water, dried (sodium sulfate), and concentrated to give a solid that was dissolved in the minimal volume of dichloromethane and applied to a column of silica gel. After elution with 8:1 (v/v) dichloromethane–diethyl ether, the fractions containing the product were combined and evaporated to give a solid that was recrystallized from ethanol. A second recrystallization from ethanol gave pure **12** (yield 1.4 g, 82%), m.p. 207–208°, $[\alpha]_D^{27}$ −37.3 ±0.5° (*c* 1, chloroform); n.m.r.: τ 5.52 (d, $J_{1',2'}$ 7.8 Hz, H-1'), 5.55 (d, $J_{1,2}$ 9.8 Hz, H-1), 6.21 (broad s, NH_2), 6.33 (t, $J_{3',4'} = J_{4',5'}$ 9.5 Hz, H-4'), 2.71 and 3.39 (2 m, S-C_6H_4-NH_2).

Anal. Calc. for $C_{32}H_{41}NO_{17}S$: C, 51.68; H, 5.56; N, 1.88; S, 4.31. Found: C, 51.49; H, 5.61; N, 1.61; S, 4.48.

p-*Aminophenyl 1-thio-β-cellobioside* (**13**). — To a solution of **12** (1.3 g) in acetone (15 ml) was added a slurry of Bio-Rad AG 1-X2 (OH^-) anion-exchange resin (50 ml) in 50% ethanol (50 ml). The reaction mixture was stirred overnight at room temperature and then filtered. The filtrate was concentrated to a solid that was recrystallized from ethanol–water to afford pure **13** (yield 0.73 g, 93%), m.p. 258–260° (dec.), $[\alpha]_D^{27}$ −50.9 ±2.0° (*c* 0.5, water).

Anal. Calc. for $C_{18}H_{27}NO_{10}S$: C, 48.10; H, 6.06; N, 3.12; S, 7.13. Found: C, 47.88; H, 6.26; N, 2.87; S, 7.16.

p-*Chlorophenyl 2,3,6,2',3',4',6'-hepta*-O-*acetyl-1-thio-β-cellobioside* (**14**). — To a solution of *p*-chlorobenzenethiol (0.77 g, 5.3 mmol) in M aqueous sodium hydroxide

(8.6 ml, 8.6 mmol) was added a solution of hepta-*O*-acetyl-α-cellobiosyl bromide[8] (3.0 g, 4.3 mmol) in acetone (20 ml). The reaction mixture was boiled under reflux for 30 min, cooled, and concentrated. The residue was partitioned between dichloromethane and water, and the organic layer dried (sodium sulfate) and evaporated to give a crystalline solid. Recrystallization from chloroform–diethyl ether gave pure **14** (yield 2.3 g, 70%), m.p. 231–232.5°, $[\alpha]_D^{27}$ −32.6 ±0.5° (*c* 1, chloroform); n.m.r.: τ 5.40 (d, $J_{1,2}$ 10 Hz, H-1), 5.51 (d, $J_{1',2'}$ 8 Hz, H-1'), 6.30 (t, $J_{3',4'} = J_{4',5'}$ 9.5 Hz, H-4'), 2.56 and 2.70 (2 d, S–C_6H_4–Cl).

Anal. Calc. for $C_{32}H_{39}ClO_{17}S$: C, 50.36; H, 5.15; Cl, 4.65; S, 4.20. Found: C, 50.47; H, 5.32; Cl, 4.66; S, 4.43.

p-*Chlorophenyl 1-thio-β-cellobioside* (**15**). — A solution of **14** (1.5 g) in chloroform (20 ml) was diluted with methanol (25 ml) and treated with 0.1M methanolic sodium methoxide (2 ml). The reaction mixture was stirred overnight at room temperature, neutralized with Bio-Rad AG 50W-X4 (H^+) cation-exchange resin, and filtered. The filtrate was concentrated to a syrup that was applied to a column of silica gel and eluted with 40:10:1 (v/v) chloroform–methanol–water. Pure **15** was obtained as a syrup that crystallized after several days (yield 0.78 g, 83%), m.p. 191–194°, $[\alpha]_D^{27}$ −52.4 ±1.1° (*c* 0.5, water).

Anal. Calc. for $C_{18}H_{25}ClO_{10}S \cdot 0.5H_2O$: C, 45.24; H, 5.48; Cl, 7.42; S, 6.71. Found: C, 45.27; H, 5.34; Cl, 7.45; S, 6.62.

Methylene bis(2,3,6,2'3',4',6'-hepta-O-acetyl-1-thio-β-cellobioside) (**16**). — To a solution of **2** (3.0 g, 4.6 mmol) in acetone (15 ml) was added diiodomethane (0.62 g, 2.3 mmol) and a solution of potassium carbonate (0.64 g, 4.6 mmol) in water (5 ml). The reaction mixture was boiled under reflux temperature for 15 min, cooled, and concentrated. The residue was partitioned between dichloromethane and water, and the organic layer dried (sodium sulfate) and evaporated to give a solid that was recrystallized from chloroform–ethyl acetate–ethanol (yield 2.6 g, 86%), m.p. 266–269°, $[\alpha]_D^{27}$ −98.8 ±0.5° (*c* 1, chloroform); n.m.r.: τ 5.32 (d, $J_{1,2}$ 10 Hz, H-1), 5.49 (d, $J_{1',2'}$ 8 Hz, H-1'), 6.12 (s, –SCH_2S–), and 6.20 (t, $J_{3',4'} = J_{4',5'}$ 10 Hz, H-4').

Anal. Calc. for $C_{53}H_{72}O_{34}S_2$: C, 48.33; H, 5.51; S, 4.87. Found: C, 48.06; H, 5.47; S, 4.84.

Methylene bis(1-thio-β-cellobioside) (**17**). — A solution of **16** (2.0 g) in chloroform (20 ml) was diluted with dry methanol (50 ml) and treated with 0.1M methanolic sodium methoxide (2 ml) overnight at room temperature. The precipitated amorphous solid was filtered off and dried; yield 0.80 g (70%), $[\alpha]_D^{27}$ −121 ±1.1° (*c* 0.9, methanol).

Anal. Calc. for $C_{25}H_{44}O_{20}S_2 \cdot 1.5H_2O$: C, 39.73; H, 6.27; S, 8.48. Found: C, 39.77; H, 6.32; S, 8.54.

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REFERENCES

1 E. STEERS, JR., P. CUATRECASAS, AND H. B. POLLARD, *J. Biol. Chem.*, 246 (1971) 196–200.
2 V. PAČES, J. FRGALA, AND J. ŠATAVA, *Collect. Czech. Chem. Commun.*, 38 (1973) 2983–2988.
3 M. L. CHAWLA AND O. P. BAHL, *Carbohydr. Res.*, 32 (1974) 25–29; C. S. JONES, R. H. SHAH, D. J. KOSMAN, AND O. P. BAHL, *ibid.*, 36 (1974) 241–245.
4 M. CLAEYSSENS, H. KERSTERS-HILDERSON, J. P. VAN WAUWE, AND C. K. DEBRUYNE, *FEBS Lett.*, 11 (1970) 336–338; P. CUATRECASAS, *Annu. Rev. Biochem.*, 40 (1971) 259–278; *Adv. Enzymol.*, 36 (1972) 29–89; A. A. ARTYUKOV AND N. V. MOLODTSOV, *Bioorg. Khim.*, 1 (1975) 1577–1582; R. S. JAIN, R. L. BINDER, C. WALZ, C. A. BUCK, AND L. WARREN, *J. Chromatogr.*, 136 (1977) 141–146.
5 G. STARK (ed.), *Biochemical Aspects of Reactions on Solid Supports*, Academic Press, New York, 1971; S. CHIPOWSKY, Y. C. LEE, AND S. ROSEMAN, *Proc. Natl. Acad. Sci. U.S.A.*, 70 (1973) 2309–2312; Y. C. LEE, C. P. STOWELL, AND M. J. KRANTZ, *Biochemistry*, 15 (1976) 3956–3963; M. J. KRANTZ, N. A. HOLTZMAN, C. P. STOWELL, AND Y. C. LEE, *ibid.*, 15 (1976) 3963–3968.
6 P. J. PFÄFFLI, S. H. HIXSON, AND L. ANDERSON, *Carbohydr. Res.*, 23 (1972) 195–206.; R. J. FERRIER, R. W. HAY, AND N. VETHAVIYASAR, *ibid.*, 27 (1973) 55–61.
7 E. K. GUM, JR. AND R. D. BROWN, JR., *Biochim. Biophys. Acta*, 446 (1976) 371–386.
8 G. ZEMPLÉN, *Ber.*, 53 (1920) 996–1006.
9 S. CHIPOWSKY AND Y. C. LEE, *Carbohydr. Res.*, 31 (1973) 339–346.
10 F. WREDE AND O. HETTCHE, *Hoppe-Seyler's Z. Physiol. Chem.*, 172 (1927) 169–178.