Carbohydrate Research, 250 (1993) 1–8
Elsevier Science Publishers B.V., Amsterdam

Synthesis and evaluation of diastereoisomeric alkylating pseudo-disaccharides as potential affinity reagents for trehalase †

Edith Bar-Guilloux [a], Jacques Defaye [b], Jochen Lehmann [c,*], Robert Nardin [b], Daniel Robic [d] and Klaus Urbahns [c]

[a] *Université de Caen, UFR des Sciences Pharmaceutiques, rue Vaubenard, F-14000 Caen (France)*
[b] *Laboratoire de Chimie des Glucides, CNRS and DRFMC / SESAM, Centre d'Etudes Nucléaires de Grenoble, BP 85 X, F-38041 Grenoble (France)*
[c] *Institut für Organische Chemie der Universität, Freiburg i. Br., Albertstrasse 21, D-7800 Freiburg i. Br. (Germany)*
[d] *Université René Descartes, Laboratoire de Chimie Biologique, Avenue de l'Observatoire, F-75006 Paris (France)*

(Received November 24th, 1992; accepted February 20th, 1993)

ABSTRACT

Reaction of (±)-(*3/4,5,6*)-4-bromo-5,6-epoxy-3-hydroxycyclohexene with 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-glucopyranose, followed by treatment of the resulting isolated diastereoisomeric 4-bromo-3,5-dihydroxycyclohexene 1-thioglycoside derivatives with base under phase-transfer conditions, gave (*R*)- and (*S*)-(*3,4,6/5*)-3,4-epoxy-6-*S*-(1-thio-α-D-glucopyranosyl)-5-hydroxycyclohexene. None of them was substrate or inhibitor for cockchafer trehalase.

INTRODUCTION

Alkylating pseudo-disaccharides, comprising a vinyloxirane functionality, part of a 3-hydroxycyclohexene moiety attached to a monosaccharide, have already been used for active-site-directed modifications of sugar-binding proteins. The lactose analog (*3,4,6 / 5*)-3,4-epoxy-6-*S*-(1-thio-β-D-galactopyranosyl)-5-hydroxycyclohexene was thus shown to be a good competitive inhibitor of *Escherichia coli* β-D-galactosidase and also to effect irreversible inhibition of the enzyme[2]. When the glycose moiety was 2-acetamido-2-deoxy-1-thio-β-D-glucopyranose, the resulting pseudo-disaccharide was found to deactivate human β-D-hexosaminidase[3] irreversibly.

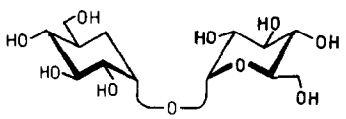
* Corresponding author.
† Part 8 from the Laboratoire de Chimie des Glucides in the series: Specificity and Mechanism of Action of Trehalases. For Part 7, see ref 1.

0008-6215/93/$06.00 © 1993 – Elsevier Science Publishers B.V. All rights reserved
SSDI 0008-6215(93)E0076-D

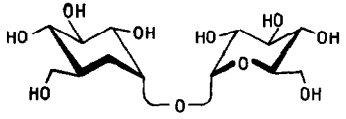
Trehalase (α,α-D-glucoside 1-glucohydrolase, EC 3.2.1.28) is a very specific enzyme which acts on α,α-trehalose and requires at least one intact α-D-glucopyranosyl moiety for its activity, when an overall α,α-trehalose bulk structure is considered[4]. Notable exceptions are with 6,6′-dideoxy-6,6′-difluoro-α,α-trehalose, which behaves as a competitive inhibitor[4] and, more strangely, with monosaccharide derivatives such as β-D-glucopyranosyl fluoride and "D-glucooctenitol" (*Z*-3,7-anhydro-1,2-dideoxy-D-gluco-oct-2-enitol) which have been found to be substrates[5,6]. Replacement of one C-2 equatorial hydroxyl group by hydrogen in the nonreducing disaccharide α,α-trehalose results in a simultaneous decrease in the affinity and in the maximum velocity[7], and its epimerization to an axial orientation, to a competitive inhibition of the enzyme[8], a result which was also obtained with 1-thio-α,α-trehalose[9]. More recently, a number of asymmetric pseudo-α,α-trehaloses which comprise a carbocyclic moiety were prepared[10], and it turned out[11] that the diastereoisomeric pair **1** and **2** (Scheme 1) was substrate for the enzyme with K_m values both in the mM range. Interestingly, it was found that the 1*S* hemihydroxycyclohexyl diastereoisomer **1** was cleaved at a rate ~33% below that of the natural substrate, when its 1*R* counterpart **2** resulted in a V_m ~ 10 times less. This result prompted us to prepare α,α-trehalose analogs, comprising a 1-thioglucopyranose moiety linked to a carbocycle functionalized with a reactive vinyloxirane group, as potential active-site-directed irreversible inhibitors for trehalase.

RESULTS AND DISCUSSION

(±)-(*3/4,5,6*)-4-Bromo-5,6-epoxy-3-hydroxycyclohexene * (**3**, Scheme 2) can be readily prepared from *p*-benzoquinone[12]. The epoxide functionality is opened at the allylic position when good nucleophiles like thio sugars are offered[2,3], resulting in the almost quantitative formation of 1-thioglycosides. As expected, 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-glucopyranose (**4**), prepared[13] from tetra-*O*-acetyl-1,5-anhydro-D-*arabino*-hex-1-enitol via the pentaacetate **5**, reacted slowly but almost quantitatively with a slight excess of the bromo-epoxide **3**, yielding a mixture of diastereoisomeric 1-thioglycosyl bromo-hydroxycyclohexenes **6ab**. These two compounds showed only very slight differences in mobility on TLC, but could be



1

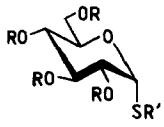


2

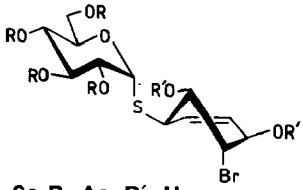
Scheme 1.

* Only one enantiomer is depicted.

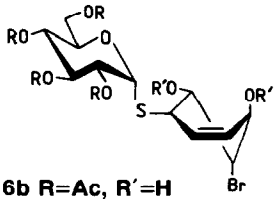
3



4 R=Ac, R'=H
5 R=R'=Ac



6a R=Ac, R'=H
7a R=R'=H
8a R=R'=Ac



6b R=Ac, R'=H
7b R=R'=H
8b R=R'=Ac

Scheme 2.

resolved in mg quantities by preparative HPLC which yielded, according to their ^{13}C NMR spectra, 80% enriched **6b** and diastereoisomerically pure ** **6a**.

Zemplén deacetylation of a small amount of the mixture **6ab** gave the unprotected diastereoisomers **7ab**, which could be resolved more easily by HPLC than the partially acetylated precursors **6ab**. The resulting diastereoisomerically pure compounds **7a** and **7b**, obtained as syrups, could be acetylated to yield the pure acetates **8a** and **8b**. Compound **8a** was obtained crystalline. Diastereoisomers **8a** and **8b** gave well resolved ^{13}C NMR spectra with 12 distinct signals for the heterocyclic and carbocyclic carbon atoms (Table I) confirming their homogeneity. The signal for C-1′ of the glucopyranose moiety was found at rather low field, in agreement with expectations for a 1-thioglycoside. In the ^{1}H NMR spectra, the coupling constant for the anomeric carbon atom ($J_{1',2'}$ 5.9 Hz in both **7a** and **7b**) confirmed the α(D) configuration.

Treatment of diastereoisomerically pure **6a** with powdered sodium hydroxide in dichloromethane, in the presence of tetraethylammonium chloride, resulted in the formation of the vinyloxirane **9a** (Scheme 3), which was rigorously purified to unformity by chromatography on silica gel. Its ^{13}C NMR spectrum gave signals at 56.6 and 46 ppm in agreement with the presence of the vinyloxirane functionality. Shortly before the enzymatic test with trehalase, it was deacetylated to yield equally homogeneous **10a**, the structure of which was proved by reaction with 1-heptanethiol in methanol at room temperature, which resulted in the formation in good yield of the thioether **11a**, characterized as its peracetate **12a**. Compound **12a** gave, in its ^{13}C NMR spectrum, a signal at rather low field (45.7 ppm) in agreement with the C-3 thioheptyl substitution at the carbocyclic moiety.

** Compounds designated "a" have, in chromatographic separation procedures, the higher mobility in all the series of diastereoisomers.

TABLE I

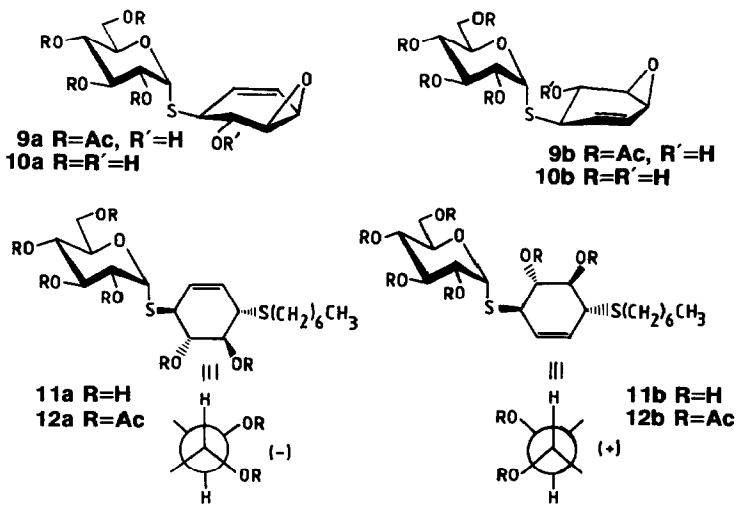
^{13}C NMR data [a] for ring carbon atoms in compounds **6ab–9a** and **12a**

Carbon	6a	6b	7a	7b	8a	8b	9a	12a
C-1	129.9	128.9	130.1	130.1	129.7	128	130	129.3
C-2	127.5	127	128.4	127.4	126	125.50	125	127.1
C-3	70.6	70.5	73.6	74.1	71.3	71.70	56.6	72.3
C-4	58.8	58.3	57.6	57.3	49.05	48.6	46	45.7
C-5	72.1	72.3	74.6	74.8	73.9	73.90	70.6	72.3
C-6	45.8	48.1	45.8	48.6	42.75	44.05	44.1	44.7
C-1′ [b]	81.5	83.2	85.6	88.4	82.45	83.20	83.9	80.8
C-2′ [b]	70.4	70.1	73.2	74	71.25	71.15	70.3	70.4
C-3′ [b]	70.1	68.7	71.7	72	70.85	70.90	69.3	70.4
C-4′ [b]	68.4	68.4	70.4	70.5	68.85	68.60	68	68.6
C-5′ [b]	68.7	68.6	70.5	70.8	69.10	69.40	69.2	68.6
C-6′ [b]	62.2	62.3	61.4	61.4	62.1	61.7	62.6	61.9

[a] At 50.323 MHz. [b] Primed numbers refer to the 1-thio-α-D-Glc*p* moiety.

The same sequence of reactions was applied to the diastereoisomeric mixture containing mainly **6b** yielding, after treatment with base, the 80% enriched epoxide **10b** which was also tested with trehalase. When enriched **10b** was allowed to react with 1-heptanethiol, it gave the thioether **11b**, contaminated with **11a**. A pure crystalline hydrate of **11b** was however obtained when the mixture was dissolved in ethyl acetate–methanol and a small amount of water was added. Diastereoisomeric **11a** and **11b** could also be resolved by flash column chromatography on silica gel.

The absolute configuration for diastereoisomers of the **a** and **b** series can be easily deduced from a comparison of optical properties[14] of compounds **12a** and



Scheme 3.

12b. These two diastereomers differ only in their chiral 1,2-gauche arrangement of two neighbouring acetoxy groups. Obviously the compound with the lower optical rotation (**12a**) must have a *−synclinal* arrangement (compare the Newman projection of the vicinal diacetoxy grouping in **12a** and **12b**, Scheme 3) and the compound with the higher optical rotation a *+synclinal* arrangement. So the formulae for **12a** and **12b** represent the absolute configuration of these compounds. Since all diastereomeric compounds described in this paper are, by unequivocal chemical correlation, either related to **12a** or **12b**, all depicted formulae have the correct absolute configuration.

The 1-thioglycosyl vinyloxiranes **10a** and **10b** turned out to be neither substrate nor competitive or irreversible inhibitors when incubated with cockchafer trehalase at concentrations between 7.81–125 mM.

EXPERIMENTAL

General methods.—All reactions were monitored by TLC on Silica Gel $60F_{254}$ plates (E. Merck) using the eluents indicated and detection was accomplished either by UV irradiation or charring with H_2SO_4. Flash column chromatography was performed on Silica Gel 60 (230–240 mesh, E. Merck). HPLC involved an LKB 2152 controller fitted with three LKB 2150 pumps, a rheodyne 7126 injector, an LKB variable wavelength monitor, and a Shimadzu CR2AX integrator. Preparative HPLC was performed with Knauer equipment comprising three model 64 pumps, a dynamic mixing chamber, an injection valve and a variable wavelength monitor. Columns (Bischoff) were used as indicated. Melting points were determined with a Büchi 535 apparatus and are corrected. Optical rotations were measured either with a Jobin Yvon or a Schmitt and Haensch Polartronic I polarimeter. ^{13}C NMR spectra were recorded either with Bruker AC200, WM 250, or AM400 instruments. For acetylated compounds, solutions in $CDCl_3$ were used, with the central peak of the triplet (76.91 ppm) as internal reference. Spectra of unacylated products were recorded in D_2O and are referenced to external Me_4Si. For spectra at 400 MHz, signals assignment was confirmed using 2D ^{13}C–^1H heteronuclear correlations. ^1H NMR spectra of unacylated products were recorded with a Bruker AM400 instrument in D_2O (external Me_4Si). Signals were assigned using COSY homonuclear correlations.

Trehalase purification.—The purified enzyme from cockchafer (*Melolontha vulgaris*) was prepared according to ref 15, but omitting the last purification step, and was used after chromatography on a column of acrylamide–agarose AcA4/4. The specific activity was 9.2 units per mg of protein (one unit is defined as the amount of enzyme catalyzing the hydrolysis of one μmol of α,α-trehalose per min).

Enzymic tests.—Irreversible inhibition was tested with preincubation media containing various dilutions of **10a** or **10b** (125, 62.5, 31.5, 15.62, and 7.81 mM) in a McIlvaine buffer (20 mM) containing 0.0875 trehalase units per mL. Incubation was maintained at 30°C for 10, 60, and 120 min. Controls consisted of one set of

dilutions without the enzyme protein and one tube of trehalase without the products.

The activity assays were performed by adding the preincubation media to previously prepared solutions of α,α-trehalose in McIlvaine buffer, so that incubation media consisted of McIlvaine buffer (20 mM, pH 6.5), various dilutions of **10a** or **10b** (10, 5, 2, 1, and 0.5 mM), α,α-trehalose (10 mM), and the enzyme protein (0.07 units/mL) in a final volume of 1.25 mL. The incubation time was 30 min at 37°C. D-Glucose was estimated by the Somogyi–Nelson method[16].

Reversible inhibition was tested by direct addition of the enzyme in the incubation media containing one concentration of **10a** or **10b** and various α,α-trehalose concentrations. The experiment was repeated for each concentration of inhibitors. The activity was determined as previously described. Controls consisted of incubation media without **10a,b**.

*(3,6/4,5)- and (4,5/3,6)-4-Bromo-3,5-dihydroxy-6-*S*-(2,3,4,6-tetra-*O*-acetyl-1-thio-α-*D*-glucopyranosyl)-2-cyclohexene* (**6a,b**).—To a solution of crystalline 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-glucopyranose[11] (20 g, 55 mmol) and (±)-*(3,4/5,6)*-diepoxycyclohexene[12] (**3**, 12 g, 66 mmol) in MeOH (100 mL), water (5 mM) was added and the mixture was stored for 2 days at room temperature (TLC control, 1:1 EtOAc–cyclohexane), then concentrated to dryness in vacuo. Flash column chromatography (1:1 EtOAc–cyclohexane), of the residue gave **6a,b** as a colourless foam (22 g, 72%). An aliquot of this mixture (5 g) was submitted to preparative HPLC yielding enantiomerically pure **6a** (0.6 g), and **6b** (0.8 g) which was still contaminated by ~ 20% of the other diastereoisomer. The oxirane **9a** was directly prepared from **6a**.

*(3,6/4,5)- and (4,5/3,6)-4-Bromo-3,5-dihydroxy-6-*S*-(1-thio-α-*D*-glucopyranosyl)-2-cyclohexene* (**7a,b**).—The mixture of diastereoisomers **6a,b** (0.2 g) was conventionally *O*-deacetylated by the Zemplén method. A syrup was obtained which could be resolved into pure (^{13}C NMR) **7a** (29 mg) and **7b** (21 mg) respectively by HPLC (Hypersil ODS, 5 μm, 250 × 20 mm, 1:19 MeOH–H_2O, 15 mL/min). Diastereoisomers **7a** and **7b** had respective R_f values of 0.51 and 0.49.

*(3,6/4,5)- and (4,5/3,6)-4-Bromo-3,5-di-*O*-acetyl-6-*S*-(2,3,4,6-tetra-*O*-acetyl-1-thio-α-*D*-glucopyranosyl)-2-cyclohexene* (**8a,b**).—Purified **7a** (20 mg, 36 μmol) and **7b** (22 mg, 40 mmol) were each acetylated for 2h in 1:0.5 pyridine–Ac_2O (1.5 mL). Concentration to dryness of the reaction mixtures and purification by flash column chromatography (1:2 EtOAc–cyclohexane) yielded respectively **8a** (18 mg, 78%), mp 143°C (EtOH), $[\alpha]_D^{25}$ + 164° (*c* 1.2, EtOAc). Anal. Calcd for $C_{24}H_{31}BrO_{13}S$: C, 45.08; H, 4.89. Found: C, 44.79; H, 4.77, and **8b** (syrup, 19 mg, 82%), $[\alpha]_D^{25}$ + 219° (*c* 1.0, EtOAc).

*(3,4,6/5)- and (5/3,4,6)-3,4-Epoxy-6-*S*-(1-thio-α-*D*-glucopyranosyl)-5-hydroxy-2-cyclohexene* (**10a,b**).—Diastereoisomerically pure **6a** (0.4 g, 0.72 mmol), powdered KOH (60 mg, 1 mmol), and Et_4NCl (3 mg) in CH_2Cl_2 (12 mL) were stirred vigorously at room temperature for 4 h. The mixture was then filtered and concentrated to dryness in vacuo. Flash column chromatography (2:1 EtOAc–

cyclohexane) yielded pure (*3,4,6/5*)- or (*5/3,4,6*)-6-*S*-(2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-glucopyranosyl-5)-hydroxy-2-cyclohexene **9a** (175 mg, 51%). This product was conventionally *O*-deacetylated (Zemplén method, 4 mL soln, 30 min), and the soln was demineralized by elution (MeOH) from a column (2 × 5 cm) of silica gel. Concentration of the eluate yielded amorphous **10a** (101 mg, 91%); TLC (7:2:1 EtOAc–MeOH–H_2O) R_f 0.45, which was used as such in the enzyme investigations.

(3,6/4,5)- or (4,5/3,6)-4,5-Di-O-acetyl-6-S-(2,3,4,6-tetra-O-acetyl-1-thio-α-D-glucopyranosyl)-3-(1-heptylthio)-2-cyclohexene (**12a**).—Epoxide **10a** (60 mg, 0.2 mmol) was dissolved in MeOH (2 mL) containing water (40 mL), and *n*-heptanethiol (100 mg, 0.8 mmol) was added. After 1 h at room temperature, the solution was concentrated in vacuo and the residue was submitted to flash column chromatography (10:3:1 EtOAc–MeOH–H_2O) to yield **11a** (75 mg, 86%). The material was acetylated for 2 h in 1:0.5 pyridine–Ac_2O yielding, after evaporation of the solution to dryness under diminished pressure and flash column chromatography of the residue (1:1 ether–hexane, R_f 0.15), **12a** was obtained as an amorphous solid, $[\alpha]_D$ + 114.1° (*c* 1.4, $CHCl_3$). Anal. Calcd for $C_{31}H_{45}O_{13}S_2$: C, 53.9; H, 6.71; S, 9.28. Found: C, 54.2; H, 4.78; S, 9.43.

(3,6/4,5)- or (4,5/3,6)-4,5-di-O-acetyl-6-S-(2,3,4,6-tetra-O-acetyl-1-thio-α-D-glucopyranosyl)-3-(n-heptylthio)-2-cyclohexene (**12b**).—Enriched compound **10b** (90 mg, 0.3 mmol) was treated under the same conditions as described for **10a** giving, after workup, amorphous crude **11b** (105 mg), which crystallized (70 mg, 53%) on being kept overnight in the solvent mixture used for the flash column chromatography (TLC, R_f 0.55); $[\alpha]_D$ + 118.7° (*c* 1.8, MeOH). Anal. Calcd for $C_{19}H_{33}O_{13}S_2 \cdot 0.5H_2O$: C, 51.0; H, 7.88; S, 14.33. Found: C, 51.1; H, 7.88; S, 14.30 (water content was determined by weight loss after rigorous drying). This material was acetylated for 2 h in 2:1 pyridine–Ac_2O yielding, after the solution had been evaporated to dryness under diminished pressure and flash column chromatography (1:1 ether–hexane, R_f 0.12 in TLC), **12b** as an amorphous solid; $[\alpha]_D$ + 157.7° (*c* 1.1, $CHCl_3$). Anal. Calcd for $C_{31}H_{45}O_{13}S_2$: C, 53.9; H, 6.71; S, 9.28. Found: C, 54.6; H, 4.81; S, 9.11.

Diastereoisomers **12a** (R_f 0.44) and **12b** (R_f 0.39) clearly separated when cochromatographed (4:1 ether–hexane).

ACKNOWLEDGMENTS

One of the authors (J.L.) thanks the CNRS and the EEC for an opportunity to work in Grenoble on this topic. We thank Ms. Corinne Bayle for assistance during part of this work.

REFERENCES

1 J. Defaye, H. Driguez, B. Henrissat, and E. Bar-Guilloux, *Carbohydr. Res.*, 124 (1983) 265–273.
2 J. Lehmann and B. Neumann, *Carbohydr. Res.*, 200 (1990) 355–361.

3 C.-S. Kuhn, J. Lehmann, and K. Sandhoff, *Liebigs Ann. Chem.*, (1992) 407–409.
4 J. Defaye, H. Driguez, B. Henrissat, and E. Bar-Guilloux, in J.J. Marshall (Ed.), *Mechanism of Saccharide Polymerization and Depolymerization*, Academic Press, New York, 1980, pp 331–353.
5 E.J. Hehre, T. Sawai, C.F. Brewer, M. Nakano, and T. Kanda, *Biochemistry*, 21 (1982) 3090–3097.
6 W. Weiser, J. Lehmann, S. Chiba, H. Matsui, C.F. Brewer, and E.J. Hehre, *Biochemistry*, 27 (1988) 2294–2300.
7 J. Defaye, H. Driguez, B. Henrissat, and E. Bar-Guilloux, *Nouveau J. Chim.*, 4 (1980) 59–67.
8 E. Bar-Guilloux, J. Defaye, H. Driguez, and D. Robic, *Carbohydr. Res.*, 45 (1975) 217–236.
9 K. Bock, J. Defaye, H. Driguez, and E. Bar-Guilloux, *Eur. J. Biochem.*, 131 (1983) 595–600.
10 S. Ogawa, S. Yokoi, N. Kimura, Y. Shibata, and N. Chida, *Carbohydr. Res.*, 181 (1988) 57–66.
11 E. Bar-Guilloux, J. Defaye, F. Sichel, S. Ogawa, and D. Robic, in preparation.
12 H.-J. Altenbach, H. Stegelmeier, and E. Vogel, *Tetrahedron Lett.* (1978) 3333–3336; B. Zipperer, K.-M. Müller, B. Gallenkamp, R. Hildebrand, M. Fletschinger, D. Burger, M. Pillat, D. Hunkler, L. Knothe, H. Fritz, and H. Prinzbach, *Chem. Ber.*, 121 (1988) 757–780.
13 A. Gadelle, J. Defaye, and C. Pedersen, *Carbohydr. Res.*, 200 (1990) 497–498.
14 D.H. Whiffen, *Chem. Ind. (London)*, (1956) 964–968.
15 E. Bar-Guilloux, D. Robic, and J.E. Courtois, *Biochimie*, 62 (1980) 719–722.
16 N. Nelson, *J. Biol. Chem.*, 153 (1944) 375–380; M. Somogyi, *J. Biol. Chem.*, 160 (1945) 61–68.