SYNTHESIS OF 1,6-ANHYDRO-6-THIO-β-LACTOSE (6-THIOLACTOSAN)*

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ABSTRACT

A facile synthesis of lactosan hexaacetate (**4**) is described. Zemplén degradation of **4**, followed by the Koenigs–Knorr reaction, was shown to yield 1,2,3,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactose (**5**) in 62% yield. Compound **5** is a useful intermediate for modification of the hydroxymethyl group of the reducing moiety in lactose. Starting from **5**, via a crystalline 6-sulfonate (methyl, **6**, or *p*-tolyl, **7**), the corresponding 2,3,2′,3′,4′,6′-hexa-*O*-acetyl-6-*O*-methyl- or *p*-tolylsulfonyl-β-lactosyl ethylxanthate (**9** or **10**) was synthesized. Alkaline treatment of **9** or **10**, followed by acetylation of the product gave 2,3,2′,3′,4′,6′-hexa-*O*-acetyl-1,6-anhydro-6-thio-β-lactose (**11**) in good yield. Deacetylation of **11** gave the title compound (**12**), a novel thio analog of lactosan. Crystalline phenyl α-lactoside (**3**), its peracetate (**2**), and 1,2,3,2′,3′,4′,6′-hepta-*O*-acetyl-6-deoxy-6-iodo-β-lactose (**8**) are also described.

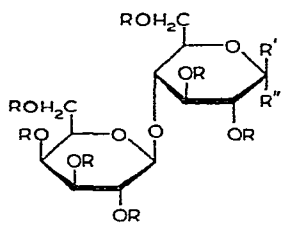
INTRODUCTION

In 1943, Montgomery, Richtmyer, and Hudson[1] reported the first synthesis of lactosan. Generally, when treated with titanium tetrachloride in dry chloroform, peracetylated 1,6-anhydro-β-D-aldohexoses afford the corresponding acetylated glycosyl chloride in which one primary hydroxyl group is free; the degradation was first established by Zemplén and Csürös[2]. Therefore, lactosan hexaacetate (**4**) is a potential starting material for modification of the hydroxymethyl group of the reducing moiety in lactose. Asp and Lindberg[3] described the Zemplén degradation for maltosan hexaacetate; treatment of the product by the Koenigs–Knorr reaction, followed by action of water, yielded the corresponding heptaacetylmaltose. Recently, in order to synthesize some new derivatives of phenyl α-maltoside, Matsushima *et al.*[4] have also utilized the method. However, for lactosan hexaacetate (**4**), no paper concerned with further chemical studies on **4** has been published since the description of the original work[1].

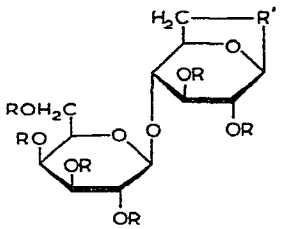
Tejima and co-workers[5] have prepared the thio derivative of 1,6-anhydro-D-glucopyranose that has a sulfur atom in the 1,6-anhydro ring; this is the so-called

*Dedicated to Dr. Nelson K. Richtmyer in honor of his 70th birthday.

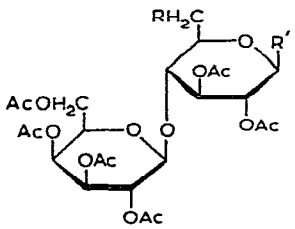
"thiolevoglucosan", presumably the first thio analog of a 1,6-anhydroglycosan reported[6]. Since this publication, as further examples of this series, the preparation of "thiolevogalactosan" and of 2-deoxy-"thiolevoglucosan", respectively, has been reported by Whistler and Seib[7] and Maki and Tejima[8]. The series of compounds is of interest not only as intermediates for the preparation of 6-thiohexoses or 1,6-dideoxyhexoses, but also as substrates having potential biological activities affecting β-D-glycosidases. In order to extend the previous studies on "thiolevoglycosans", the author has now synthesized thiolactosan (**12**). Starting from **4**, the title compound (**12**) was prepared in five steps (with comparatively good yields) as fine needles. In the course of the preparation, valuable information on the properties of lactose derivatives has been acquired, and the preparation of several new compounds is now reported in full detail.



1, R = Ac, R' = OPh, R'' = H
2, R = Ac, R' = H, R'' = OPh
3, R = R' = H, R'' = OPh



4, R = Ac, R' = O
11, R = Ac, R' = S
12, R = H, R' = S



5, R = OH, R' = OAc
6, R = OMs, R' = OAc
7, R = OTs, R' = OAc
8, R = I, R' = OAc
9, R = OMs, R' = SSCOEt
10, R = OTs, R' = SSCOEt

RESULTS AND DISCUSSION

In the original paper[1] on lactosan, Richtmyer *et al.* synthesized compound **4** in six steps, starting from lactose. Therefore, the present author first attempted to decrease the number of steps and simplify the procedure. It has proved feasible to obtain compound **4** in 25% yield in two steps, starting from commercial lactose monohydrate, as follows.

Syrupy octaacetyl-lactose was prepared by acetylation of lactose monohydrate

by the method of Hudson *et al.*[9] Without purification, the acetate was fused with phenol by a slight modification of the procedure originally reported by Asp and Lindberg[3] for the preparation of phenyl hepta-*O*-acetyl-β-maltoside. Crystalline phenyl hepta-*O*-acetyl-β-lactoside (**1**), chromatographically homogeneous, was obtained in a yield of 31%; its physical properties were in agreement with those reported in the literature[10]. Although the yield is low, the method is applicable to large-scale preparation of **1**; recently, Dea[11] has reported the preparation of **1** by the fusion method on a small scale in poor yield (18%).

Little has been reported on the corresponding α anomers; only amorphous phenyl α-lactoside and its peracetate have been described in the literature[12]. Therefore, the author decided to prepare the α anomer by the fusion method.

Crystalline octa-*O*-acetyl-β-lactose, prepared by Hudson and Johnson's method[9], had a specific rotation of −4°, in agreement with the literature value, but the melting point showed a broad range from ~90–135°. The peculiarity of the melting was pointed out by the original authors[9]; Sasaki and Taniguchi[13] recorded a melting point of 140°, but gave no reason for the difference. The present author ascribes it to slow external heating during the determination: rapid heating results in a sharp melting point of 101–103°, and slow heating gives a broad melting point of 85–135°.

To a homogeneous mixture of the octaacetate and phenol was added zinc chloride, and the mixture was fused under vacuum for 2 h at 120°. Crystalline phenyl hepta-*O*-acetyl-α-lactoside (**2**) having $[\alpha]_D^{19}$ +49° was obtained in 55% yield after the resultant mixture had been processed in the usual way. Compound **2** was chromatographically homogeneous and its R_F values were indistinguishable from those of the corresponding β anomer (**1**); it showed a broad melting point of 150–170°. In the n.m.r. spectrum of **2**, the anomeric proton appeared at τ 4.20–4.43 as a broad doublet showing a spacing of 3 Hz, a value of interest in comparison with that of the β anomer (**1**), for which the anomeric proton appeared at τ 4.62 as a sharp doublet having a spacing of 5 Hz. Deacetylation of **2** with sodium methoxide in methanol afforded phenyl α-lactoside (**3**); after recrystallization from methanol, it was obtained as fine needles having m.p. 139–141° and $[\alpha]_D^{19}$ +65°.

In order to obtain **4**, a mixture of **1** with 2.6M potassium hydroxide was heated for 20 h at 100°; mutarotation ceased after 16 h, and thereafter the rotation remained constant, equilibrium being reached much faster than for the maltose series. Isolation of the product and subsequent acetylation were performed as in the maltose series, as reported by Asp and Lindberg[3], to give an 81% yield of **4**, m.p. 205°, used without purification for subsequent experiments. Pure **4**, m.p. 208°, was obtained by one recrystallization from ethyl acetate. In the n.m.r. spectrum of **4**, the signal for the anomeric proton at lowest field (τ 4.55) is a singlet; this was assigned to the anomeric proton (H-1) of the 1,6-anhydro-D-glucose moiety. It is of interest that the anomeric proton appears as a singlet. The same phenomenon is also seen in the n.m.r. spectrum of thiolactosan hexaacetate (**11**), as will be mentioned later. The present author suggests the following reason: owing to the co-existence of a 1,6-anhydro (or

1,6-anhydro-6-thio) ring and the bulky D-galactopyranosyl group on the D-glucose moiety, the molecule may assume a boat form as the favored conformation in **4** or **11**. Thus, the dihedral angle between C-1 and C-2 is near 90°, so that the $J_{1,2}$ coupling is[14] ~ zero. The sharp doublet (τ 4.62), showing a spacing of 5 Hz, which is located nearest the singlet just mentioned, was assigned to the anomeric proton (H-1') of the D-galactose moiety.

Zemplén degradation of **4**, followed by treatment of the product with mercuric acetate (in order to replace the chlorine atom by an acetoxyl group), afforded 1,2,3,2',3',4',6'-hepta-*O*-acetyl-β-lactose (**5**) in 62% yield. In the maltose series[3], 40% of the maltosan hexaacetate was recovered unchanged, and so the yield of heptaacetate obtained was only 25%. In contrast, in the lactose series, only a trace of starting material (**4**) (identified by t.l.c.) remained unchanged. Compound **5** melted at 191–192° and showed $[\alpha]_D^{22}$ −12.5°. In the n.m.r. spectrum of **5**, the signal for the anomeric proton appears as a sharp doublet (τ 4.31) showing a spacing of 8 Hz. By acetylation with pyridine–acetic anhydride, **5** yielded octa-*O*-acetyl-β-lactose. On methanesulfonylation or *p*-toluenesulfonylation in the usual way, **5** yielded 1,2,3,2',3',4',6'-hepta-*O*-acetyl-6-*O*-(methylsulfonyl)-β-lactose (**6**) and 1,2,3,2',3',4',6'-hepta-*O*-acetyl 6-*O*-*p*-tolylsulfonyl-β-lactose (**7**), respectively. Treatment of **6** or **7** with sodium iodide in boiling acetonitrile caused replacement of the sulfonyloxy group by iodine, and crystalline 1,2,3,2',3',4',6'-hepta-*O*-acetyl-6-deoxy-6-iodo-β-lactose (**8**) was obtained in a yield of >80%; this confirmed that the sulfonyl group in **6** and **7** was located on the primary alcohol group. Further proof of this structure was obtained by alkaline degradation of **6** and **7**.

In 1962, Tejima and co-workers[15] reported a new synthesis of levoglucosan; it was prepared from 1,2,3,4-tetra-*O*-acetyl-6-*O*-*p*-tolylsulfonyl-β-D-glucopyranose. By using a similar procedure, 2-acetamido-1,6-anhydro-2-deoxy-β-D-glucose was also synthesized by the same authors[16]. Since the levoglycosans are not obtainable from the corresponding α anomers, our procedure is applicable for chemically distinguishing α from β anomers. Treatment of **6** or **7** with sodium methoxide in methanol, followed by acetylation, afforded **4** in comparatively good yield. From analogy with the levoglycosan formation just mentioned, this result presumably supports the structures assigned to **6** and **7**, in which an acetoxyl group (β configuration) is attached to C-1, and a sulfonyloxy group to C-6.

Treatment of **6** with a brominating agent afforded the corresponding syrupy bromide. In this preparation, a slight modification of the procedure used by Scheurer and Smith[17] for the synthesis of peracetylated glycosyl bromides was employed. By treatment of the bromide with potassium ethylxanthate, crystalline 2,3,2'3',4',6'-hexa-*O*-acetyl-6-*O*-(methylsulfonyl)-β-lactosyl ethylxanthate (**9**) was prepared. The corresponding *p*-toluenesulfonate (**10**) was similarly prepared from **7**, as an amorphous powder, and, as all attempts to crystallize the powder failed, pure **10** was obtained by column chromatography on silica gel. Treatment of compound **9** with sodium methoxide in methanol, followed by acetylation, gave 2,3,2',3',4',6'-hexa-*O*-acetyl-1,6-anhydro-6-thio-β-lactose (6-thiolactosan hexaacetate) (**11**), m.p. 154° and $[\alpha]_D^{19}$

$-37.2°$, in 92% yield. The same product was obtained in 90% yield from **10** by use of a similar procedure.

On comparison of the physical properties of **11** with those of the oxygen analog (**4**), some interesting information was obtained. Compound **11** has a melting point much lower than that of **4**, but its high R_F value (t.l.c.) and its specific rotation are scarcely distinguishable from those of **4**. In their i.r. spectra, only a slight difference between **4** and **11** was found, in a part of the fingerprint region at 915–850 cm^{-1}; **4** shows two strong bands, at 915 and 885 cm^{-1}, whereas **11** shows four bands (of medium intensities) at 910, 895, 875, and 860 cm^{-1}. In the n.m.r. spectra of **4** and **11**, a marked difference was observed at higher field; in the spectrum of **11**, a two-proton multiplet in the region of τ 6.60–7.10 was observed, and assigned to the methylene proton (H-6) which might be shifted to higher field owing to the effect of sulfur. In this region, no signal can be detected in the spectrum of **4**.

Deacetylation of **11** in the usual way gave the title compound (**12**) in 81% yield; it was readily crystallized from boiling ethanol to give fine needles, m.p. 79–80°, $[\alpha]_D^{23}$ $-36.4°$, and was not reducing to boiling Fehling solution. After acid hydrolysis of **12**, galactose was the sole reducing sugar formed; it was identified by preparative paper-chromatography.

Studies on the hydrolysis of lactosan and thiolactosan (**12**) with β-D-galactosidase prepared from swine intestinal mucous membrane, and on the acetolysis of **12**, will be reported in the near future.

EXPERIMENTAL

General. — Melting points are uncorrected. Solutions were evaporated in a rotary evaporator at <40° under diminished pressure. Optical rotations were measured with a Yanagimoto Model OR-10 polarimeter in a 0.5-dm tube. I.r. spectra were recorded for Nujol mulls with a Japan Spectroscopic Co. Model IR-S spectrophotometer. N.m.r. spectra were recorded at 100 MHz with a Japan Electron Optics Lab. Model JNM-PS-100 spectrometer. Tetramethylsilane was used as the internal standard in chloroform-*d*. Chemical shifts are given on the τ scale. T.l.c. on Silica Gel G (E. Merck, Darmstadt, Germany) activated at 110° was performed with solvent systems (A) 6:1 (v/v) chloroform–acetone, (B) 4:1 ether–benzene, and (C) 3:1 ethyl acetate–benzene. Detection was effected with sulfuric acid. Column chromatography was performed on a column (2 × 30 cm) of Wakogel C-100 (Wako Pure Chemical Industries, Ltd., Osaka) as the adsorbent, with 1 g of the mixture to be separated per 15 g of adsorbent, and components were eluted from the column with 6:1 (v/v) dichloromethane–acetone. Preparative paper-chromatography (p.p.c.) was performed on Toyo Filter Paper No. 50 (Toyo Roshi Kaisha, Ltd., Tokyo) by the ascending method, with 6:4:3 (v/v) butyl alcohol–pyridine–water by the procedure of Ueda[18], and detection was effected with aniline hydrogen phthalate[19].

Phenyl hepta-O-acetyl-β-lactoside (**1**). — To a mixture of acetic anhydride (400 ml) and anhydrous sodium acetate (25 g), preheated to 100°, lactose mono-

hydrate (100 g) was added portionwise, with stirring and gentle heating. The external heating and the rate of addition were so controlled as to maintain the reaction temperature at <130°. After all of the sugar had dissolved, the mixture was boiled under reflux for 15 min, cooled to 60°, and poured into ice–water (3 liters). The insoluble, viscous mass precipitated was stirred with the water, and kept overnight to solidify. The supernatant liquor was decanted, the residue was ground and stirred well with fresh ice–water (3 liters), the supernatant liquor was decanted, and the wet residue was extracted with dichloromethane (3 × 100 ml). The extracts were combined, washed successively with chilled, saturated aqueous sodium hydrogen carbonate, and water, dried (calcium chloride), and evaporated to dryness to afford syrupy octa-*O*-acetyl-lactose (190 g).

p-Toluenesulfonic acid (2.5 g) and acetic anhydride (10 ml) were dissolved in molten phenol (190 g), and the mixture was combined with the syrupy acetate. After a homogeneous solution had been obtained by gentle warming, it was heated for 1 h at 100° (oil bath) under vacuum (water pump) and, while still hot, diluted with benzene (400 ml). The solution was washed successively with ice–water (500 ml), chilled 2M sodium hydroxide (10 × 500 ml), and water (500 ml), dried (calcium chloride), and evaporated to dryness to afford a syrup (170 g) which crystallized from warm ethanol (170 ml). After being kept overnight, the crystals (70 g) were collected by filtration, and recrystallized from ethanol (210 ml). The product (65 g, 31%) had m.p. 165° and $[\alpha]_D^{25}$ −22.7° (*c* 1.58, chloroform) (lit.[10] m.p. 161.5°, $[\alpha]_D^{20}$ −23.2°, and was chromatographically homogeneous; R_F 0.62 (solvent A), 0.50 (B), 0.82 (C); n.m.r. data: τ 2.60–3.10 (5-proton multiplet, Ph), 4.62 (1-proton doublet, $J_{1,2}$ 5 Hz, H-1), 7.82, 7.90, 8.00 (21 protons, singlets, 7 OAc).

Anal. Calc. for $C_{32}H_{40}O_{18}$: C, 53.93; H, 5.66. Found: C, 53.81; H, 5.64.

Phenyl hepta-O-acetyl-α-lactoside (**2**). — Crystalline octa-*O*-acetyl-β-lactose, $[\alpha]_D^{19}$ −4° (*c* 3.51, chloroform), was prepared by Hudson and Johnson's method[9]; the melting point showed a broad range of ~90–135°. To a homogeneous mixture of the acetate (40 g) and molten phenol (40 g) was added anhydrous zinc chloride (6 g). The mixture was heated for 2 h at 120° (oil bath) under vacuum (water pump), and then treated as described for the preparation of the β anomer (**1**), to afford a syrup (37 g) which crystallized from warm ethanol (40 ml). The product (24 g) was collected by filtration, and recrystallized twice from 70-ml portions of ethanol to give pure **2** (23 g, 55%); homogeneous by t.l.c. (solvents A, B, and C), and scarcely separable from **1**; began to melt at 150° and completely melted at 170°; $[\alpha]_D^{19}$ +49° (*c* 2.53, chloroform); n.m.r. data: τ 4.20–4.43 (1-proton, broad doublet; $J_{1,2}$ 3 Hz, H-1), 7.81, 7.90, 8.00 (21 protons, singlets, 7 OAc).

Anal. Calc. for $C_{32}H_{40}O_{18}$: C, 53.93; H, 5.66. Found: C, 53.95; H, 5.83.

Phenyl α-lactoside (**3**). — To a chilled suspension of the acetate **2** (7.5 g) in dry methanol (75 ml) was added 0.6M sodium methoxide in dry methanol (9 ml) at 0°; the mixture was stirred, with exclusion of moisture, for 30 min (to effect dissolution), and then kept for 15 h at 5°. Dry Amberlite IR-120 (H^+) ion-exchange resin (6 g) was added, and the suspension was stirred for 30 min, and then filtered. Evaporation of

the filtrate afforded a syrup (6 g), which was dissolved in boiling ethanol (90 ml). On cooling, it crystallized; the gelatinous precipitate was collected by filtration, and dried in a vacuum desiccator; wt. 4.5 g. Recrystallization from dry methanol (45 ml) gave pure **3** (4 g, 91%), m.p. 139–141°, $[\alpha]_D^{19}$ +65° (*c* 2.45, chloroform).

Anal. Calc. for $C_{18}H_{26}O_{11}$: C, 51.65; H, 6.27. Found: C, 51.89; H, 6.22.

Lactosan hexaacetate (**4**). — A mixture of acetate **1** (46 g) with 2.6M aqueous potassium hydroxide (800 ml) was heated for 20 h in an oil bath at 100°. The solution was made neutral at 0° with 2.25M sulfuric acid (~320 ml) and concentrated. The distillation was interrupted thrice so that precipitated salts could be removed by filtration. To the completely dried syrup, obtained by repeated azeotropic distillation with ethanol, were added acetic anhydride (250 ml) and anhydrous sodium acetate (25 g); the mixture was heated for 2 h on a steam bath, and then poured into ice–water (1.5 liters). The resulting precipitate was collected by filtration, and dried in the air. The resultant, light-brown powder (33 g) was dissolved in dichloromethane (100 ml), and the solution was decolorized with carbon, and concentrated to half-volume. Addition of petroleum ether (b.p. 30–60°) caused formation of a precipitate (30 g, 81%), m.p. 205°, which was used for further experiments. One recrystallization from ethyl acetate (600 ml) gave pure **4**, m.p. 208°, $[\alpha]_D^{19}$ −39.5° (*c* 2.33, chloroform) (lit.[1] m.p. 206–208°, $[\alpha]_D^{20}$ −40.8°); R_F 0.38 (solvent A), 0.56 (C); n.m.r. data: τ 4.55 (1-proton singlet, H-1), 4.62 (1-proton doublet, $J_{1',2'}$ 5 Hz, H-1'), 7.85, 7.95, 8.02 (18 protons, singlets, 6 OAc).

Anal. Calc. for $C_{24}H_{32}O_{16}$: C, 50.00; H, 5.59. Found: C, 49.79; H, 5.48.

1,2,3,2',3',4',6'-Hepta-O-acetyl-β-lactose (**5**). — To a chilled solution of compound **4** (25 g) in dry chloroform (300 ml) and ethanol (5 ml) was added titanium tetrachloride (40 g). The mixture, protected from moisture, was warmed gently on an oil bath, whereupon vigorous evolution of hydrogen chloride ensued; this ceased after about 30 min. The mixture was then boiled under reflux for 4 h and cooled. The yellow, supernatant liquor and the brown, gummy precipitate were poured into ice–water (1 liter), traces being transferred with the aid of small amounts of chloroform and ice–water. The chloroform layer was separated, washed with ice–water (3 × 250 ml), dried (calcium chloride), and evaporated to dryness. The residue was dissolved in a solution of mercuric acetate (25 g) in acetic acid (250 ml), and the solution was kept overnight at room temperature, poured into ice–water (1.5 liters), and the mixture extracted with dichloromethane (3 × 100 ml). The extracts were combined, washed successively with saturated aqueous sodium hydrogen carbonate and water, dried (calcium chloride), and evaporated to dryness. The crystalline residue (18 g) was recrystallized from ethanol (70 ml) to give colorless needles (17 g, 62%) of **5** contaminated with a trace of **4**, as shown by t.l.c.; it could not be completely freed of **4** by repeated recrystallization from ethanol. After 4 recrystallizations, the product had m.p. 191–192°, $[\alpha]_D^{22}$ −12.5° (*c* 2.24, chloroform); R_F 0.33 (solvent A); n.m.r. data: τ 4.31 (1-proton doublet, $J_{1,2}$ 8 Hz, H-1), 7.83, 7.88, 7.93, 8.01 (21 protons, singlets, 7 OAc).

Anal. Calc. for $C_{26}H_{36}O_{18}$: C, 49.06; H, 5.70. Found: C, 49.04; H, 5.77.

Acetylation of **5** (2 g) with acetic anhydride (10 ml) in pyridine (10 ml) for 48 h at room temperature, in the usual way, gave octa-*O*-acetyl-β-lactose (2 g).

1,2,3,2',3',4',6'-Hepta-O-*acetyl-6*-O-(*methylsulfonyl*)-β-*lactose* (**6**). — Compound **5** (8 g) was added to a solution of methanesulfonyl chloride (4 g) in pyridine (32 ml) at −10°. After it had been stirred for 1 h, the mixture was kept for 24 h at 5°, diluted with dichloromethane (100 ml), and then processed, in the usual way, to give a syrup which crystallized on adding ethanol and scratching the insides of the flask; yield 9 g. Recrystallization from ethanol (90 ml) at 40° afforded pure **6** (8 g, 89%); rapid cooling caused deposition of **6** as a syrup. Compound **6** had m.p. 110–111°, $[\alpha]_D^{18}$ −5° (*c* 2.11, chloroform); R_F 0.48 (solvent A), 0.21 (B), 0.76 (C); ν_{max}^{Nujol} 1170 and 1350 cm^{-1} ($MeSO_2$).

Anal. Calc. for $C_{27}H_{38}O_{20}S$: C, 45.37; H, 5.35; S, 4.49. Found: C, 45.46; H, 5.55; S, 4.24.

1,2,3,2',3',4',6'-Hepta-O-*acetyl-6*-O-p-*tolylsulfonyl*-β-*lactose* (**7**). — Compound **5** (7 g) was treated with pyridine (28 ml) and *p*-toluenesulfonyl chloride (8 g) for 48 h, and the product isolated as described for the preparation of **6**; yield 9 g. On recrystallization from ethanol (90 ml), **7** separated first as a syrup; this crystallized on being kept for 48 h at 5°; yield 8 g (83%), m.p. 129–131°, $[\alpha]_D^{19}$ −1° (*c* 3.54, chloroform), R_F 0.55 (solvent A), 0.34 (B), ν_{max}^{Nujol} 1170 cm^{-1} (*p*-$C_7H_7SO_2$).

Anal. Calc. for $C_{33}H_{40}O_{20}S$: C, 50.12; H, 5.35; S, 4.05. Found: C, 49.88; H, 5.41; S, 3.79.

1,2,3,2',3',4',6'-Hepta-O-*acetyl-6-deoxy-6-iodo*-β-*lactose* (**8**). — A mixture of compound **6** (3 g) and sodium iodide (1.5 g) in acetonitrile (45 ml) was boiled for 4 h under reflux. Sodium methanesulfonate soon began to precipitate; the starting material (**6**) (R_F 0.48, solvent A) had disappeared after 3.5 h, and had been replaced by a single product having R_F 0.66. The suspension was filtered, and the filtrate was evaporated to dryness. The residue was triturated with ice–water (50 ml), to afford a white solid which was collected by filtration. The air-dried powder (3 g) was recrystallized from ethanol (120 ml), to give pure **8** (2.5 g, 80%), m.p. 220°, $[\alpha]_D^{19}$ −9° (*c* 2.80, chloroform); R_F 0.66 (solvent A), 0.56 (B).

Anal. Calc. for $C_{26}H_{35}IO_{17}$: C, 41.83; H, 4.72. Found: C, 42.02; H, 4.69.

Compound **8** (1 g) was also obtainable starting from the tosylate (**7**) (1 g) by a similar procedure, except for the boiling period, 1 h.

Lactosan hexaacetate (**4**) *from sulfonate* **6** *or* **7**. — A mixture of the methanesulfonate **6** (3 g) with sodium methoxide in dry methanol (1 g of sodium dissolved in 35 ml of dry methanol) was stirred, with exclusion of moisture, for 1 h at room temperature, and then kept for 15 h, and filtered from the precipitated sodium methanesulfonate. The filtrate was made neutral with acetic acid (~1.5 ml), and evaporated to dryness. The residue was acetylated with acetic anhydride (20 ml) and pyridine (20 ml) in the usual way, to afford a pale-brown powder (2 g). After treatment with carbon, and recrystallization from ethyl acetate (40 ml), the product (1.5 g, 62%) had m.p. 206–207°, $[\alpha]_D^{23}$ −39.7° (*c* 2.10, chloroform); it was indistinguishable with authentic **4** (i.r. spectrum and t.l.c.).

Treatment of the *p*-toluenesulfonate **7** (3 g) by the same procedure also gave **4** (1.2 g, 55%).

*2,3,2',3',4',6'-Hexa-*O*-acetyl-6-*O*-(methylsulfonyl)-β-lactosyl ethylxanthate* (**9**). — Bromine (9 ml) was added dropwise at 20° to a suspension of red phosphorus (4.5 g) in glacial acetic acid (45 ml) with external cooling. To this brominating reagent was added finely powdered methanesulfonate **6** (18 g) in one portion, and the suspension was stirred (for 30 min) until a solution resulted; this was kept for 1.5 h at room temperature. Dichloromethane (60 ml) was added, and the mixture was poured into ice–water (1 liter), stirred for 45 min at 0°, and filtered. The organic layer was separated, successively washed with water and aqueous sodium hydrogen carbonate solution, dried (magnesium sulfate), and evaporated, to give a syrupy bromide which was used without further purification.

To the syrupy bromide were added potassium ethylxanthate (7.5 g) and ethanol (150 ml). The mixture was warmed for a few minutes on a steam bath to effect dissolution, kept for 1 h at room temperature, and then poured into ice–water (1 liter) containing acetic acid (10 ml). The mixture was extracted with dichloromethane (3 × 50 ml), and the extracts were combined, washed with water, dried (magnesium sulfate), and evaporated to a syrup (18 g) which crystallized on addition of a small amount of ethanol and scratching of the insides of the flask. The crystals (16 g) were collected by filtration, and recrystallized from ethanol (160 ml), to give pure **9** (15 g, 77%), m.p. 166°, $[\alpha]_D^{18}$ +11° (*c* 2.54, chloroform); R_F 0.58 (solvent A), 0.37 (B).

Anal. Calc. for $C_{28}H_{40}O_{19}S_3$: C, 43.29; H, 5.19; S, 12.38. Found: C, 43.34; H, 5.11; S, 12.52.

*2,3,2',3',4',6'-Hexa-*O*-acetyl-6-*O*-p-tolylsulfonyl-β-lactosyl ethylxanthate* (**10**). — Amorphous 2,3,2',3',4',6'-hexa-*O*-acetyl-6-*O*-*p*-tolylsulfonyl-lactosyl bromide (15 g) was prepared, starting from **7** (18 g), as described for the preparation of **9**; it was treated with potassium ethylxanthate (3 g) in ethanol (60 ml), to afford crude **10** (10 g) as an amorphous powder, contaminated with a small proportion of by-products t.l.c. with solvent A). All attempts to crystallize the compound failed. Part of the powder (2 g) was dissolved in dichloromethane (20 ml), and the solution was chromatographed on a column of silica gel. Evaporation of the eluate afforded a pale-yellow powder, m.p. 78–80°, $[\alpha]_D^{18}$ +12° (*c* 2.51, chloroform); R_F 0.72 (solvent A), 0.63 (B).

Anal. Calc. for $C_{34}H_{44}O_{19}S_3$: C, 47.88; H, 5.20. Found: C, 47.21; H, 5.16.

*2,3,2',3',4',6'-Hexa-*O*-acetyl-1,6-anhydro-6-thio-β-lactose (6-Thiolactosan hexaacetate)* (**11**). — A mixture of compound **9** (10 g) with sodium methoxide in dry methanol (from 2 g of sodium in 125 ml of methanol) was stirred for 30 min at room temperature, and then kept overnight. The mixture was made neutral with acetic acid (~3.8 ml), and filtered, and the filtrate was evaporated to dryness. The residue was acetylated with acetic anhydride (60 ml) and pyridine (60 ml) for 24 h at 0°. The mixture was poured into ice–water (800 ml), and extracted with dichloromethane (3 × 50 ml), and the organic layer was processed in the usual way, to afford a syrup (7.5 g) which crystallized from ethanol (40 ml). The product (7 g, 92%) was collected

by filtration, and recrystallized from ethanol (70 ml) to give pure **11**, m.p. 154°, $[\alpha]_D^{19}$ −37.2° (*c* 1.56, chloroform); R_F 0.56 (solvent A), 0.77 (C); n.m.r. data: 4.52 (1-proton singlet, H-1), 4.62 (1-proton doublet, $J_{1',2'}$ 4 Hz, H-1'), 6.70–7.10 (2-proton multiplet, H-6), 7.85, 7.94, 8.01 (18 protons, singlets, 6 OAc).

Anal. Calc. for $C_{24}H_{35}O_{15}S$: C, 48.64; H, 5.44; S, 5.41. Found: C, 48.87; H, 5.42; S, 5.47.

Compound **11** (1.8 g, 90%) was also obtainable from *p*-toluenesulfonate **10** (3 g) by a similar procedure.

1,6-Anhydro-6-thio-β-lactose (*6-Thiolactosan*) (**12**). — Deacetylation of **11** (7.5 g) with sodium methoxide in methanol, and treatment of the resultant solution as described for the preparation of **3**, afforded a syrup (4 g) which crystallized spontaneously when it was dissolved in warm ethanol (60 ml) and the solution was kept in a refrigerator; fine needles (3.5 g, 81%). Recrystallization from ethanol (50 ml) gave pure **12**, m.p. 79–80°, $[\alpha]_D^{23}$ −36.4° (*c* 3.43, water). The product did not reduce boiling Fehling solution.

Anal. Calc. for $C_{12}H_{20}O_9S$: C, 42.35; H, 5.92; S, 9.42. Found: C, 42.17; H, 5.88; S, 9.50.

A solution of **12** (0.3 g) in 0.5M sulfuric acid (6 ml) was heated for 2 h at 90°. The acid was neutralized with barium carbonate (3 g), the suspension was filtered, and the filtrate was treated with carbon and evaporated to a thin sirup, in which galactose was identified as the sole reducing sugar (R_F 0.36) by p.p.c.

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