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SYNTHESIS FROM PULLULAN OF SPACER-ARM, LIPID, AND ETHYL GLYCOSIDES OF A TETRASACCHARIDE [α-D-Glc-(1→6)-α-D-Glc-(1→4)-α-D-Glc-(1→4)-D-Glc] FOUND IN HUMAN URINE; PREPARATION OF NEOGLYCOPROTEINS*

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ABSTRACT

Enzymic hydrolysis of pullulan, followed by acetylation and chromatography, gave acetylated α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-D-Glc*p* which, with 2-bromoethanol and boron trifluoride etherate in dichloromethane, gave the 2-bromoethyl glycoside. The reactions of the glycoside with methyl 3-mercaptopropionate, methyl 11-mercaptoundecanoate, and octadecanethiol are described, and also its hydrogenolysis to give an ethyl glycoside. The mercaptopropionate-derived, spacer-arm glycoside has been coupled to bovine serum albumin and key-hole limpet haemocyanin.

INTRODUCTION

The tetrasaccharide α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-D-Glc (**1**) is normally excreted in human urine[2]. Increased excretion is observed in patients having type II and III glycogenosis[3], in Duchenne muscular dystrophy[4], and during normal pregnancy[5]. Rapid detection of **1** can be effected by radioimmunoassay[6] using antibodies raised against glycoproteins obtained by reductive amination of **1** (isolated from urine[2]) with *p*-aminophenylethylamine followed by coupling to key-hole limpet haemocyanin (KLH) and bovine serum albumin (BSA).

Reductive amination transforms **1** into a D-glucitol derivative, and it is of interest to prepare a spacer-arm glycoside having all four D-glucose residues intact. We now report the preparation of **1** from pullulan and its transformation, *via* the 2-bromoethyl glycoside **3**, into derivatives (**4**–**12**) of value for the preparation of antibodies.

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*2-Bromoethyl Glycosides, Part 6. For Part 5, see ref. 1.
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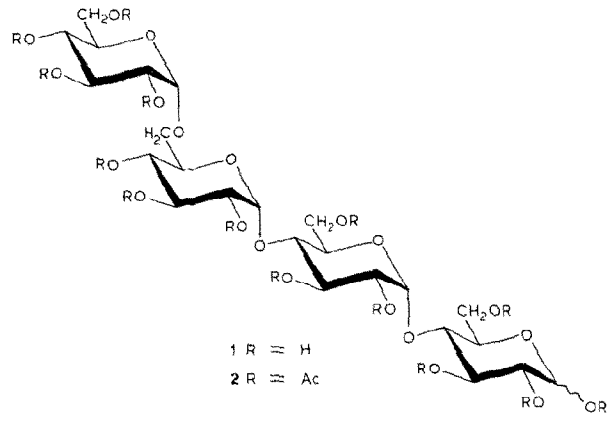

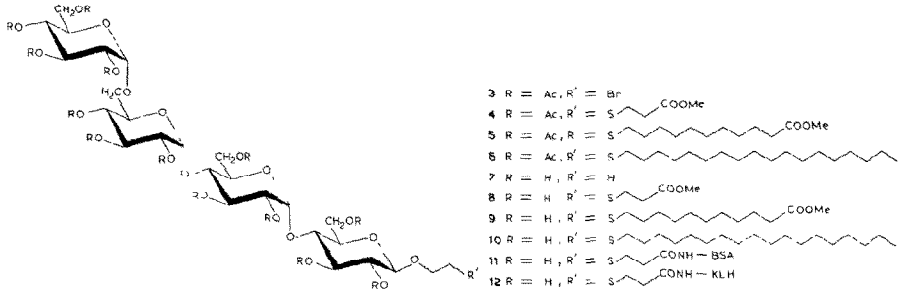

RESULTS AND DISCUSSION

Enzymic hydrolysis[7] (beta-amylase and pullulanase) of pullulan[8], followed by acetylation of the crude product and chromatography on silica gel, gave **2** (12% overall yield, $\alpha\beta$-ratio ~1:1). The structure of **1** was confirmed by methylation analysis[3] and comparison of the mass-spectral data with those of the authentic material[3]. Further evidence to support the assigned structure was provided by the n.m.r. data for **2**–**10** (see Experimental), which also confirmed the anomeric configurations in **1** as proposed by Hallgren *et al.*[2].

The 2-bromoethyl β-glycoside **3** was prepared by boron trifluoride etherate-induced glycosidation[9] of $\alpha\beta$-**2** with 2-bromoethanol in dichloromethane; the α anomer was not detected. Treatment of **3** variously with methyl 3-mercaptopropionate, methyl 11-mercaptoundecanoate (**15**), octadecanethiol, and hydrogen (Pd/C) gave the spacer-arm glycosides **4** and **5**, the neoglycolipid **6**, and the ethyl glycoside **7**, respectively. Deacetylation of **4**–**6** gave **8**–**10**, respectively. These transformations are analogous to those described earlier[1,10].

The neoglycoproteins **11** and **12** were prepared by coupling **8** to BSA and KLH. A modification[10] of the Inman[11]–Lemieux[12] technique was used (see Experimental), where *N,N*-dimethylformamide was substituted by methyl sulfoxide because of the low solubility of **8** in the former solvent. The degree of binding (number of hapten molecules per molecule of protein) of **8** to BSA and KLH was 20 and 245, respectively.

EXPERIMENTAL

Pullulan was prepared[8] and purified by two precipitations from water by addition of 2-propanol (200 g of pullulan, 4 L of water, and 13 L of 2-propanol). Pullulanase and beta-amylase were commercial materials (Sigma). Optical rotations were measured with a Perkin–Elmer 241 polarimeter. N.m.r. spectra were recorded with a Varian XL-200 spectrometer with Me_4Si or sodium 3-(trimethylsilyl)propionate-d_4 (TSP) as internal reference. N.m.r. assignments are based on double-resonance and INEPT[13] experiments.

4-O-[4-O-(6-O-α-D-Glucopyranosyl-α-D-glucopyranosyl)-α-D-glucopyranosyl]-D-glucose (**1**). — Pullulan[8] (100 g) was dissolved in water (1 L) by heating and added to a solution of acetic acid (~15 mL) and sodium acetate (41 g) in water (5 L). The pH of the solution was adjusted to 5, and pullulanase and beta-amylase (200 and 200,000 units, respectively) were added. The reaction mixture was stirred at 37° for 24 h (t.l.c. monitoring, SiO_2; acetone–2-propanol–0.1M lactic acid, 2:2:1) and then heated at 120° for 20 min. The solution was concentrated to 1.5 L and treated with Duolite C26 (H^+) resin (0.5 L). The resin was washed with water, the combined aqueous solutions (~4 L) were ultrafiltered (Millipore PSAC 14205 NMWL 1000), and the colourless solution was freeze-dried, to give **1** (72 g) contaminated with D-glucose, maltose, and maltotriose. A solution of impure **1** (63.7 g) in acetic anhydride–pyridine (1:2, 450 mL) was left at room temperature for 15 h and then co-concentrated with toluene, and the acetylation procedure was repeated twice. A solution of the final product in dichloromethane was washed with M hydrochloric acid and saturated, aqueous sodium hydrogencarbonate, dried (Na_2SO_4), and concentrated. The residue (112.5 g) was subjected to chromatography on silica gel (ethyl acetate–iso-octane, 3:2) to give *αβ*-**2** (20.0 g, 12% from pullulan). Rechromatography of suitable fractions gave *β*-**2** and *α*-**2** (>98% purity), with $[\alpha]_D^{25}$ +114° (*c* 0.2, chloroform) and +148° (*c* 0.73, chloroform), respectively. N.m.r. data ($CDCl_3$, Me_4Si) for *αβ* -**2**: ^1H, *δ* 6.25 and 5.75 (2 d, each ~0.5 H, *J* 3.6 and 8.0 Hz, H-1*α* and H-1*β*); ^{13}C, *δ* 95.8, 95.8, 95.2 (C-1′,1″,1‴), 91.2 (C-1*β*), and 88.8 (C-1*α*). Pure **1** was obtained by treating *αβ*-**2** (1.0 g, see above) with methanolic sodium methoxide (50 mL; from ~1 mg of sodium) at room temperature. The reaction was monitored by t.l.c. (SiO_2; chloroform–methanol–water, 65:35:10, lower phase) and, after 24 h, the mixture was neutralised with dry Duolite (H^+) resin, filtered, and concentrated. The residue was subjected to chromatography (Waters Preppak-500/C_{18}, water), to give **1** (337 mg, 63%, >99.5% pure by

h.p.l.c.), $[\alpha]_D^{25}$ +165° (*c* 0.8, water); lit.[2] $[\alpha]_D^{20}$ +179° (*c* 1.14, water). N.m.r. data (D_2O, TSP): ^1H, δ 5.40, 5.38 (2 d, each 1 H, *J* 3.8 and 3.5 Hz, H-1′,1″), 5.26 (d, ~0.3 H, *J* 3.7 Hz, H-1α), 4.99 (d, 1 H, *J* 3.7 Hz, H-1‴), and 4.68 (d, ~0.7 H, *J* 7.9 Hz, H-1β); ^{13}C, δ 102.6, 102.2, 100.8 (C-1′,1″,1‴), 98.5, 94.7 (C-1), 79.9, 79.8, 79.6, 78.9, 77.3, 76.7, 76.0, 75.97, 75.8, 74.7, 74.6, 74.4, 74.3, 74.2, 74.1, 73.9, 72.7, 72.3, 72.1, 68.6, 63.4, 63.3, and 63.2.

The structure of **1** was confirmed by methylation analysis[3].

*2-Bromoethyl 2,3,6-tri-*O*-acetyl-4-*O*-{2,3,6-tri-*O*-acetyl-4-*O*-[2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl)-α-*D*-glucopyranosyl]-α-*D*-glucopyranosyl}-β-*D*-glucopyranoside* (**3**). — To a solution of $\alpha\beta$-**2** (12.54 g, 9.85 mmol) and 2-bromoethanol (2.5 g, 1.4 mL, 20 mmol) in dichloromethane (50 mL) was added, dropwise, boron trifluoride etherate (7.1 g, 6.3 mL, 50 mmol) at room temperature, and the mixture was stirred (48 h; t.l.c. monitoring, SiO_2; ethyl acetate–iso-octane, 3:1). The mixture was washed with water and saturated, aqueous sodium hydrogencarbonate, dried (Na_2SO_4), and concentrated. The residue (12.3 g) was subjected to chromatography (SiO_2; ethyl acetate–iso-octane, 3:2), to give **3** (4.95 g, 63% based on transformed **2**), $[\alpha]_D^{25}$ +109° (*c* 1.3, chloroform), and $\alpha\beta$-**2** (5.05 g). N.m.r. data ($CDCl_3$, Me_4Si): ^1H, δ 5.35, 5.28, 5.18 (3 d, each 1 H, *J* 3.8, 2.8, and 3.6 Hz, H-1′,1″,1‴), 4.60 (d, 1 H, *J* 7.9 Hz, H-1), and 3.42–3.49 (m, 2 H, CH_2-Br); ^{13}C, δ 100.4 (C-1), 95.9, 95.7, 95.3 (C-1′,1″,1‴), and 29.9 (CH_2Br).

Anal. Calc. for $C_{52}H_{71}BrO_{34}$: C, 47.31; H, 5.42. Found: C, 47.20; H, 5.48.

*2-(2-Methoxycarbonylethylthio)ethyl 2,3,6-tri-*O*-acetyl-4-*O*-{2,3,6-tri-*O*-acetyl-4-*O*-[2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl)-α-*D*-glucopyranosyl]-α-*D*-glucopyranosyl}-β-*D*-glucopyranoside* (**4**). — A mixture of **3** (4.0 g, 3 mmol), methyl 3-mercaptopropionate (0.546 g, 0.49 mL, 4.5 mmol), cesium carbonate (1.17 g, 3.6 mmol), and *N*,*N*-dimethylformamide (25 mL) was stirred at room temperature until **3** had been consumed (7 h; t.l.c., SiO_2; ethyl acetate–iso-octane, 4:1). The solvent was removed and the residue was subjected to chromatography (SiO_2; ethyl acetate–iso-octane, 3:1), to give **4** (3.22 g, 78%), $[\alpha]_D^{25}$ +102° (*c* 1, chloroform). N.m.r. data ($CDCl_3$, Me_4Si): ^1H, δ 5.36, 5.28, 5.19 (3 d, each 1 H, *J* 3.8, 4.1, and 3.7 Hz, H-1′,1″,1‴), 4.57 (d, 1 H, *J* 7.9 Hz, H-1), 3.71 (s, 3 H, MeO), 2.81, 2.71, and 2.61 (3 t, each 2 H, *J* 6.7 Hz, CH_2-S-CH_2 and CH_2-CO); ^{13}C, δ 100.2 (C-1), 95.8, 95.6, and 95.2 (C-1′,1″,1‴).

Anal. Calc. for $C_{56}H_{78}O_{36}S$: C, 49.48; H, 5.78. Found: C, 49.10; H, 5.83.

*2-(10-Methoxycarbonyldecylthio)ethyl 2,3,6-tri-*O*-acetyl-4-*O*-{2,3,6-tri-*O*-acetyl-4-*O*-[2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl)-α-*D*-glucopyranosyl]-α-*D*-glucopyranosyl}-β-*D*-glucopyranoside* (**5**). — A mixture of **3** (431 mg, 0.33 mmol), methyl 11-mercaptoundecanoate (**15**, 114 mg, 0.49 mmol), cesium carbonate (128 mg, 0.39 mmol), and *N*,*N*-dimethylformamide (3 mL) was stirred at room temperature for 15 h, diluted with dichloromethane, washed with water [neutralisation with Duolite (H^+) resin facilitated the phase separation], dried (Na_2SO_4), and concentrated. The residue was subjected to chromatography (SiO_2; ethyl acetate–iso-octane, 2:1), to give **5** (243 mg, 50%), $[\alpha]_D^{24}$ +91° (*c* 4.4,

chloroform). N.m.r. data ($CDCl_3$, Me_4Si): ^1H, δ 5.36, 5.28, 5.19 (3 d, each 1 H, J 3.8, 4.1, and 3.6 Hz, H-1′,1″,1‴), 4.57 (d, 1 H, J 7.9 Hz, H-1), 3.67 (s, 3 H, MeO), 2.68, 2.52, and 2.31 (3 t, each 2 H, J 7 Hz, CH_2-S-CH_2 and CH_2CO); ^{13}C, δ 100.1 (C-1), 95.6, 95.5, and 95.1 (C-1′,1″,1‴).

Anal. Calc. for $C_{64}H_{94}O_{36}S$: C, 52.24; H, 6.44. Found: C, 52.10; H, 6.50.

2-(Octadecylthio)ethyl 2,3,6-tri-O-acetyl-4-O-{2,3,6-tri-O-acetyl-4-O-[2,3,4-tri-O-acetyl-6-O-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-α-D-glucopyranosyl]-α-D-glucopyranosyl}-β-D-glucopyranoside (**6**). — A mixture of **3** (265 mg, 0.2 mmol), octadecanethiol (86 mg, 0.3 mmol), cesium carbonate (79 mg, 0.24 mmol), and *N,N*-dimethylformamide (10 mL) was stirred at room temperature until **3** had been consumed (48 h; t.l.c., SiO_2; ethyl acetate–iso-octane, 4:1). The solvent was removed and the residue was subjected to chromatography (SiO_2; ethyl acetate–iso-octane, 1:1), to give **6** (142 mg, 44%), $[\alpha]_D^{25}$ +93° (*c* 0.5, chloroform). N.m.r. data ($CDCl_3$, Me_4Si); ^1H, δ 5.30, 5.22, 5.12 (3 d, each 1 H, J 4.0, 4.2, and 3.7 Hz, H-1′,1″,1‴), 4.51 (d, 1 H, J 7.9 Hz, H-1), 2.62, and 2.46 (2 t, each 2 H, J 7.0 Hz, CH_2-S-CH_2); ^{13}C, δ 100.2 (C-1), 95.7, 95.6, and 95.2 (C-1′,1″,1‴).

Anal. Calc. for $C_{70}H_{108}O_{34}S$: C, 55.11; H, 7.14. Found: C, 54.90; H, 7.12.

Ethyl 4-O-[4-O-(6-O-α-D-glucopyranosyl-α-D-glucopyranosyl)-α-D-glucopyranosyl]-β-D-glucopyranoside (**7**). — To a solution of **3** (122 mg) in methanol (10 mL) was added methanolic sodium methoxide (5 mL; from 1 mg of sodium) followed by 0.1M sodium hydroxide (5 mL). The mixture was hydrogenated (72 mg of Pd/C; 4 atm.) overnight, neutralised with Duolite (H^+) resin, and filtered. Conc. ammonia (1 drop) in water was added and the solvent was removed. The residue (106 mg) was subjected to chromatography (SiO_2; chloroform–methanol–water, 65:35:10, lower phase), to give **7** (24 mg, 40%), $[\alpha]_D^{24}$ +108° (*c* 1, water). N.m.r. data (D_2O, TSP): ^1H, δ 5.40 (d, 2 H, J 3.8 Hz, H-1′,1″), 4.96 (d, 1 H, J 3.7 Hz, H-1‴), 4.49 (d, 1 H, J 8.0 Hz, H-1), and 1.23 (t, 3 H, J 7.1 Hz, CH_2CH_3); ^{13}C, δ 104.5 (C-1), 102.7, 102.2, and 100.8 (C-1′,1″,1‴).

2-(2-Methoxycarbonylethylthio)ethyl 4-O-[4-O-(6-O-α-D-glucopyranosyl-α-D-glucopyranosyl)-α-D-glucopyranosyl]-β-D-glucopyranoside (**8**). — To a solution of **4** (3.0 g, 2.8 mmol) in methanol (25 mL) was added methanolic sodium methoxide (75 mL from 1 mg of sodium), and the reaction was monitored by t.l.c. (chloroform–methanol–water, 65:35:10, lower phase). After 9 h at room temperature and 30 min at 40°, the solvent was removed and the residue was subjected to chromatography (SiO_2; chloroform–methanol–water, 65:35:10, lower phase), to give **8** (1.50 g, 84%), $[\alpha]_D^{25}$ +113° (*c* 0.7, water). N.m.r. data (D_2O, TSP): ^1H, δ 5.41 (d, 2 H, J 3.5 Hz, H-1′,1″), 4.98 (d, 1 H, J 3.6 Hz, H-1‴), 4.53 (d, 1 H, J 7.9 Hz, H-1), and 3.75 (s, 3 H, MeO); ^{13}C, δ 104.3 (C-1), 102.0, 101.6, and 100.2 (C-1′,1″,1‴).

2-(10-Methoxycarbonyldecylthio)ethyl 4-O-[4-O-(6-O-α-D-glucopyranosyl-α-D-glucopyranosyl)-α-D-glucopyranosyl]-β-D-glucopyranoside (**9**). — Compound **5** (218 mg, 0.148 mmol) was deacetylated and the product purified essentially as described above. Freeze-drying gave **9** (100 mg, 73%), $[\alpha]_D^{24}$ +94° (*c* 0.8, water).

N.m.r. data (D_2O, TSP, 70°): ^1H, δ 5.31 (m, 2 H, H-1′,1″), 4.93 (d, 1 H, J 3.6 Hz, H-1‴), 4.41 (d, 1 H, J 7.9 Hz, H-1), 3.63 (s, 3 H, MeO), 2.76, 2.56 (2 t, 2 H each, J 7.0 Hz, CH_2-S-CH_2), and 2.28 (t, 2 H, J 7.3 Hz, CH_2-CO); ^{13}C (65°), δ 105.9, 103.6, 103.4, and 101.6 (C-1′,1″,1‴).

2-(Octadecylthio)ethyl 4-O-[4-O-(6-O-α-D-glucopyranosyl-α-D-glucopyranosyl)-α-D-glucopyranosyl]-β-D-glucopyranoside (**10**). — Compound **6** (120 mg, 0.079 mmol) was deacetylated essentially as described above. The reaction was monitored by t.l.c. (SiO_2; acetic acid–methanol–water, 2:1:1). Freeze-drying gave **10** (73 mg, 95%). $[\alpha]_D^{24}$ +77° (*c* 0.6, 1:1 chloroform–methanol). N.m.r. data (D_2O, TSP): ^1H, δ 5.35, 5.33 (2 d, each 1 H, J 3.9 Hz, H-1′,1″), 4.96 (d, 1 H, J 3.5 Hz, H-1‴), 4.44 (d, 1 H, J 7.9 Hz, H-1), 2.80, and 2.58 (2 t, each 2 H, J 7.0 Hz, CH_2-S-CH_2); ^{13}C($CDCl_3$, Me_2SO-d_6, Me_4Si), δ 102.8, 101.7, 100.8, and 98.8 (C-1,1′,1″,1‴).

Coupling reactions. — (*a*) Conjugate **11** was prepared[1] by using **8** (56 mg, 0.07 mmol) and bovine serum albumin (BSA; 65 mg, 1 μmol). The degree of binding[1] was 8. With 0.07 mmol of **8** and 0.5 μmol of BSA, the degree of binding was 20.

(*b*) Conjugate **12** was prepared[1] by using **8** (112 mg, 0.14 mmol) and key-hole limpet haemocyanin (KLH; 30 mg, 0.034 mmol; assumed molecular weight, 870,000; Schwarts–Mann). The degree of binding was 65. With 0.7 mmol of **8** and 0.034 mmol of KLH, the degree of binding was 245.

Methyl 11-bromoundecanoate (**13**). — A mixture of 11-bromoundecanoic acid (25.0 g, 94 mmol), Duolite (H^+) resin (10 g), calcium chloride (26.2 g, 94 mmol), and methanol (700 mL) was stirred for 15 h. The methanol was then removed and dichloromethane was added. The mixture was washed with water, dried (Na_2SO_4), and concentrated. Distillation gave pure **13** (22.0 g, 84%), b.p. 103–104°/0.02 Torr. ^1H-N.m.r. data ($CDCl_3$, Me_4Si): δ 3.67 (s, 3 H, MeO), 3.41 (t, 2 H, J 6.8 Hz, Br-CH_2), and 2.31 (t, 2 H, J 7.3 Hz, CH_2CO).

Anal. Calc. for $C_{12}H_{23}BrO_2$: C, 51.62; H, 8.30. Found: C, 51.60; H, 8.24.

Methyl 11-thioacetylundecanoate (**14**). — A mixture of **13** (17.8 g, 64 mmol), thioacetic acid (9.7 g, 9.0 mL, 128 mmol), methyltrioctylammonium chloride (0.5 g), cesium carbonate (25 g, 77 mmol), dichloromethane (95 mL), and water (95 mL) was stirred at room temperature for 15 h. The reaction was monitored by t.l.c. (SiO_2; ethyl acetate–iso-octane, 1:5). The mixture was extracted with dichloromethane, and the combined extracts were dried (Na_2SO_4) and concentrated. Distillation gave **14** (15 g, 89%), b.p. 137–139°/0.1 Torr, m.p. <25° (from iso-octane). N.m.r. data ($CDCl_3$, Me_4Si): ^1H, δ 3.67 (s, 3 H, MeO), 2.86 (t, 2 H, J 7.1 Hz, S-CH_2), 2.32 (s, 3 H, MeCO), and 2.30 (t, 2 H, J 7.7 Hz, CH_2CO); ^{13}C, δ 195.9, 174.1 (CO), 51.3, 34.0, 30.5, 29.4, 29.3, 29.2, 29.1, 29.0, 28.9, 28.7, and 24.8.

Anal. Calc. for $C_{14}H_{26}O_3S$: C, 61.27; H, 9.50. Found: C, 60.80; H, 9.79.

Methyl 11-mercaptoundecanoate (**15**). — To a solution of **14** (15.6 g, 56.9 mmol) in methanol (50 mL) was added saturated, methanolic hydrogen chloride (600 mL). The reaction was monitored by t.l.c. (SiO_2; ethyl acetate–iso-octane,

1:5). After 48 h, excess of sodium hydrogencarbonate was added, and the mixture was diluted with dichloromethane (150 mL), filtered, and concentrated. Distillation of the residue gave **15** (9.0 g, 68%), b.p. 93–94°/0.07 Torr. N.m.r. data ($CDCl_3$, Me_4Si): ^1H, δ 3.68 (s, 3 H, MeO), 2.53 (q, 2 H, J ~7 Hz, H-S-CH_2), and 2.31 (t, 2 H, J 7.5 Hz, CH_2CO); ^{13}C, δ 174.2 (CO), 51.3, 34.0, 33.9, 29.3, 29.2, 29.1, 29.0, 28.9, 28.3, 24.8, and 24.5.

Anal. Calc. for $C_{12}H_{24}O_2S$: C, 62.02; H, 10.41. Found: C, 61.80; H, 10.30.

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REFERENCES

1 J. Dahmén, T. Frejd, G. Magnusson, G. Noori, and A.-S. Carlstrom, *Carbohydr. Res.*, 127 (1984) 15–25.
2 P. Hallgren, G. Hansson, K. G. Henriksson, A. Hager, A. Lundblad, and S. Svensson, *Eur. J. Clin. Invest.*, 4 (1974) 429–433.
3 G. Lennartsson, A. Lundblad, J. Lundsten, S. Svensson, and A. Hager, *Eur. J. Biochem.*, 83 (1978) 325–334.
4 A. Lundblad, S. Svensson, I. Yamashina, and M. Ohta, *FEBS Lett.*, 97 (1979) 249–252.
5 P. Hallgren, B. S. Lindberg, and A. Lundblad, *J. Biol. Chem.*, 252 (1977) 1034–1040.
6 D. A. Zopf, R. E. Levinson, and A. Lundblad, *J. Immunol. Methods*, 48 (1982) 109–119.
7 B. V. McCleary, *Carbohydr. Res.*, 85 (1980) 160–163.
8 B. J. Catley, *J. Gen. Microbiol.*, 78 (1973) 33–38.
9 G. Magnusson, G. Noori, J. Dahmén, T. Frejd, and T. Lave, *Acta Chem. Scand., Ser. B*, 35 (1981) 213–216; J. Dahmén, T. Frejd, G. Magnusson, and G. Noori, *Carbohydr. Res.*, 111 (1982) c1–c4; J. Dahmén, T. Frejd, G. Magnusson, and G. Noori, *ibid.*, 114 (1983) 328–330; J. Dahmén, T. Frejd, G. Gronberg, T. Lave, G. Magnusson, and G. Noori, *ibid.*, 116 (1983) 303–307.
10 J. Dahmén, T. Frejd, G. Gronberg, T. Lave, G. Magnusson, and G. Noori, *Carbohydr. Res.*, 118 (1983) 292–301; J. Dahmén, T. Frejd, G. Magnusson, G. Noori, and A.-S. Carlstrom, *ibid.*, 125 (1984) 237–245.
11 J. K. Inman, B. Merchant, L. Claflin, and S. E. Tacey, *Immunochemistry*, 10 (1973) 165–174.
12 R. U. Lemieux, D. R. Bundle, and D. A. Baker, *J. Am. Chem. Soc.*, 97 (1975) 4076–4083.