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Note

Synthesis of reducing disaccharides bearing a lipophilic chain for the conjugation to proteins*

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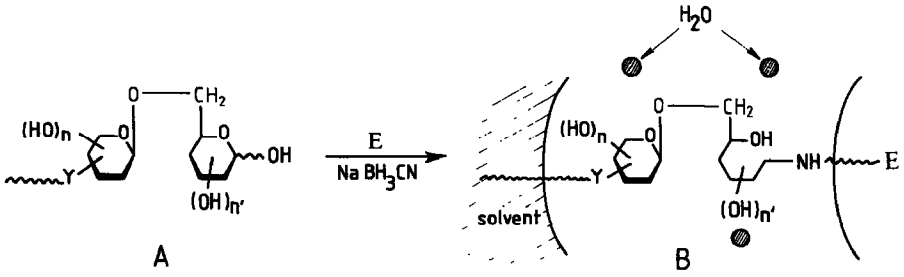
Some native or chemically modified enzymes are active in non-aqueous solvents[1,2]. However, the use of enzymes in organic synthesis is generally limited by thermal and solvent-induced denaturations[3–6].

In mixtures of water and class I solvents (polyols, sugars, etc.), preferential hydration of the proteins occurs and leads to stabilization[7]. Some nonionic and nondenaturing detergents, such as alkyl β-D-glucopyranosides or *N*-(D-*gluco*)-*N*-methylalkanamides are used for the solubilization of membranes[8,9]. Thus, our goal was to synthesize reducing disaccharides bearing an hydrophobic chain (**A**) and to conjugate them with lysine residues of proteins[10], so as to obtain new neoglycoproteins[11]. In organic media having low proportions of water, these conjugates might have the structure **B** shown in Scheme 1. A few layers of water, hydrogen-bonded to the polyol chains, could protect the proteins from the solvent in which the hydrophobic chain is extended.

Two different disaccharides of type **A** have been prepared by following the oxazoline or the modified Koenigs–Knorr procedure of glycosylation[12,13]. The first



Scheme 1

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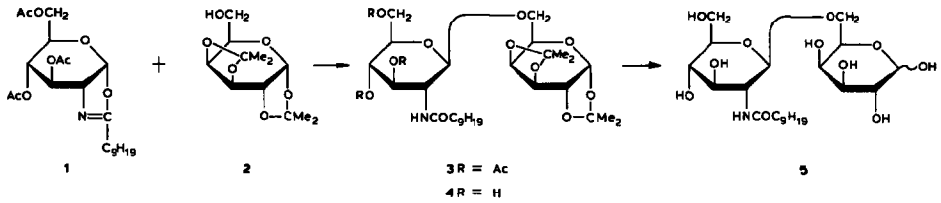
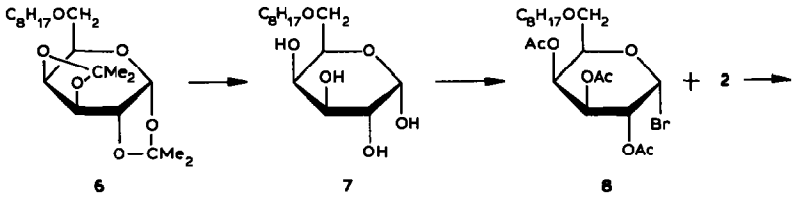
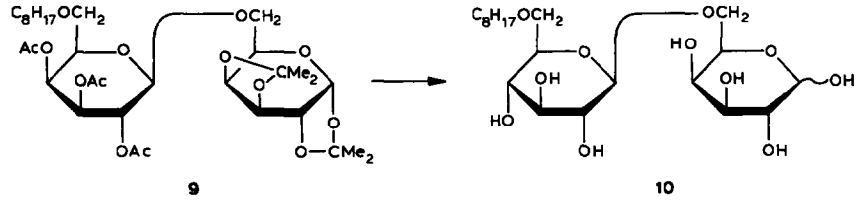


has a long-chain amido substituent, the second a long-chain ether group.

Acylation of 2-amino-2-deoxy-D-glucose with decanoyl chloride gave the known 2-decanamido-2-deoxy-D-glucose. Treatment of this amide with acetyl chloride saturated with dry hydrogen chloride yielded a crude acylated glycosyl chloride[14] which cyclized in the presence of silver nitrate and 2,4,6-trimethylpyridine[15] to give the long-chain oxazoline **1** in 25% overall-yield. Condensation of **1** with 1,2:3,4-di-*O*-isopropylidene-D-galactopyranose (**2**) in the presence of trifluoromethanesulfonic acid[16] led to the protected disaccharide **3** in 54% yield. The cleavages of the acetal groups with 90% trifluoroacetic acid[17] and of acetate groups with methanol–triethylamine gave *O*-(2-decanamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-D-galactopyranose **5** in 80% yield.





Alkylation of **2** with octyl bromide in *N,N*-dimethylformamide containing sodium hydride[18] gave the ether **6** in a 68% yield, and cleavage of the ketal groups the 6-*O*-octyl-D-galactopyranose **7**. An homologous dodecylated surfactant has been prepared under more drastic conditions by an analogous sequence of reactions[19]. Acetylation of **7** and treatment of the resulting tetraacetate with hydrogen bromide led to a glycosyl bromide **8** having a neigboring ester group. Condensation of **8** with **2** in the presence of silver trifluoromethanesulfonate and 1,1,3,3-tetramethylurea[20] gave a mixture from which the protected disaccharide **9** was isolated

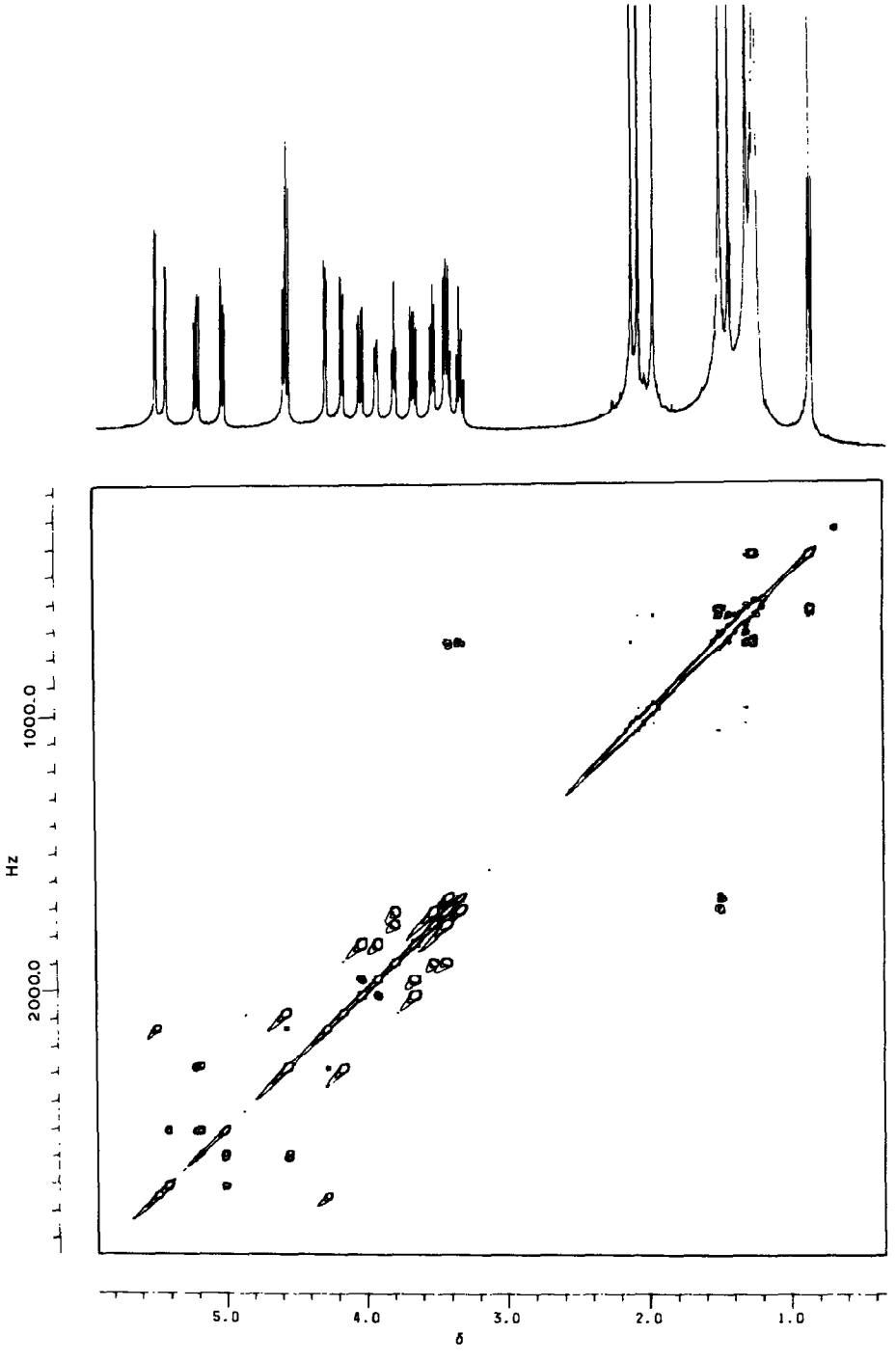


Fig. 1. Contour plot of the 2D-COSY n.m.r. spectrum of **9** in $CDCl_3$. The various cross peaks on either side of the diagonal arose owing to the presence of *J* couplings between various spin multiplets shown in the correpsonding 1D-n.m.r. at the top.

in 40% yield by chromatography. Its structure was confirmed by 2D-COSY n.m.r. spectroscopy[21] (Fig. 1). Cleavages of the protecting groups led to *O*-(6-*O*-octyl-β-D-galactopyranosyl)-(1→6)-D-galactopyranose (**10**).

EXPERIMENTAL

General methods. — Melting points were determined with a Mettler FP61 apparatus. ^{1}H-N.m.r. spectra were recorded at 90 or 500 MHz, ^{13}C-n.m.r. spectra at 20 MHz; unless otherwise stated, the solvent was $CDCl_3$. T.l.c. were developed on Silica gel 60F (Merck) and spots detected with 10% H_2SO_4 in water or with a solution of $(NH_4)_2MoO_4$ (5 g), H_2SO_4 (5 mL), H_3PO_4 (5 mL) in water (100 mL), after heating. Column chromatography were made on Silica gel Merck 60 (70–230 mesh). The compounds described subsequently were homogeneous on t.l.c.

2-Nonyl-(3,4,6-tri-O-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-d]-2-oxazoline (**1**). — A solution of 2-decanamido-2-deoxy-D-glucose[14] (3.33 g, 10 mmol) in acetyl chloride (60 mL) was saturated with dry HCl at −15°. Then, the flask was stoppered and the mixture stirred for 24 h at room temperature. The solution was evaporated, the residue dissolved in dichloromethane (25 mL), quickly washed with cold water, and then with a cold saturated aqueous $NaHCO_3$ solution, dried ($MgSO_4$), and the solvent evaporated. The crude chloride was dissolved in acetone (30 mL) and slowly added with stirring to a suspension of $AgNO_3$ (2 g) in acetone (30 mL) and 2,4,6-trimethylpyridine (5 mL) which was stirred for 2 h at room temperature. Chloroform (50 mL) was added, the suspension filtered, and the filtrate washed with an aqueous $KHCO_3$ solution and dried (K_2CO_3). Solvent removal *in vacuo* left a syrupy residue which was purified by column chromatography in 4:1 pentane–ethyl acetate to give **1** (1.10 g, 25%); ν_{max}^{film} 1725 (OAc) and 1645 cm^{-1} (C=N); ^{1}H-n.m.r. data: δ 6.0 (d, $J_{1,2}$ 7 Hz, H-1), 5.3 (t, H-3), 5.,0 (m, H-5), 4.1 (m, H-4 and -6), 3.6 (m, H-2), 2.4 (t, Hα), 2.2–2.1 (3 s, $COCH_3$), 1.5 [m, $(CH_2)_7$], and 0.9 (t, CH_3). Compound **1** was stable when kept at 4°, but was immediately used for glycosylation reactions.

1,2:3,4-Di-O-isopropylidene-6-O-(3,4,6-tri-O-acetyl-2-decanamido-2-deoxy-β-D-glucopyranosyl)-α-D-galactopyranose (**3**). — A solution of the oxazoline **1** (0.75 g, 1.7 mmol), 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**2**) (0.44 g, 1.7 mmol), and trifluoromethanesulfonic acid (10 μL) in dry dichloroethane (10 mL) was heated under an Ar atmosphere for 20 min at 80°, and then kept for 1 h at room temperature. The solution was washed with cold aqueous $NaHCO_3$ and dried (Na_2SO_4). The filtered solution was evaporated, and the residue applied to a silica gel column and eluted with 2:3 ethyl acetate–pentane to give **3** (0.66 g, 54%), m.p. 151.7°, $[\alpha]_D^{20}$ −38°, $[\alpha]_{546}^{20}$ −45° (*c* 0.3, methanol); ^{1}H-n.m.r. δ; 5.7 (d, $J_{1,2}$ 5 Hz, H-1), 4.72 (d, $J_{1',2'}$ 9 Hz, H-1'), 2.2, 2.1 (s, $COCH_3$), 1.52, 1.42, and 1.27 [s, $C(CH_3)_2$]; the remainder of the spectrum was not interpreted; ^{13}C-n.m.r.: δ 173.6 (CONH), 170.9, 169.6 (3 C, O–C=O), 109.6, 108.8 (2 C, O–C–O), 101.8 (C-1'), 96.4 (C-1), 73.2–62.3 (9 C, cyclic), 54.2 (C-2'), and 36.8–14.2 (16 C).

Anal. Calc. for $C_{34}H_{55}NO_{14}$: C, 58.1; H, 7.9; N, 2.0. Found: C, 57.8; H, 7.9; N, 2.0.

6-O-(2-Decanamido-2-deoxy-β-D-glucopyranosyl)-1,2:3,4-di-O-isopropylidene-α-D-galactopyranose (**4**). — Compound **3** (700 mg, 1 mmol) was deacetylated by stirring for 20 h at room temperature with methanol (15 mL) and triethylamine (2 mL). The solution was evaporated and the residue purified by column chromatography (in 17:3 ethyl acetate–ethanol) to give **4** (520 mg, 90%), m.p. 147°, $[\alpha]_D^{20}$ −33°, $[\alpha]_{546}^{20}$ −39° (*c* 0.3, methanol); ^{1}H-n.m.r.: δ 5.6 (d, $J_{1,2}$ 5 Hz, H-1), 1.53, 1.46, and 1.30 [s, $C(CH_3)_2$]; ^{13}C-n.m.r.: δ 176.9 (CONH), 110.2, 109.7 (2 C, O–C–O), 103.3 (C-1′), 97.6 (C-1), 78.0–62.9 (9 C, cyclic), 57.3 (C-2′), and 37.6–14.4 (remaining C).

Anal. Calc. for $C_{23}H_{49}NO_{11} \cdot H_2O$: C, 56.6; H, 8.6. Found: C, 56.6; H, 8.2.

6-O-(2-Decanamido-2-deoxy-β-D-glucopyranosyl)-D-galactopyranose (**5**). — Compound **4** (290 mg, 0.5 mmol) was dissolved in 90% aqueous trifluoroacetic acid (3 mL) and kept for 1 h at 0°. The solution was evaporated to give 225 mg (90%) of a chromatographically pure product. It crystallized from 90% aqueous methanol to give the disaccharide monohydrate, m.p. 179°, $[\alpha]_D^{20}$ −3.7°, $[\alpha]_{546}^{20}$ +1.9° (*c* 3, pyridine); ^{13}C-n.m.r. [($^2H_5$)-pyridine]: δ 174.6 (CO), 103.0 (C-1′), 99.5 (Cβ-1), 94.5 (Cα-1), 78.5–62.7 (C, cyclic), 57.5 (C-2′), and 37.0–14.3 (remaining C).

Anal. Calc. for $C_{22}H_{41}NO_{11} \cdot H_2O$: C, 51.5; H, 8.4; N, 2.8. Found: C, 51.9; H, 8.2; N, 2.8.

1,2:3,4-Di-O-isopropylidene-6-O-octyl-α-D-galactopyranose (**6**). — A solution of 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**2**) (2.6 g, 10 mmol) in *N,N*-dimethylformamide (15 mL) was slowly added to a suspension of NaH (480 mg, 20 mmol) in dry *N,N*-dimethylformamide (30 mL). After 1 h at room temperature, the suspension was cooled to 0° and bromooctane (2.3 g, 12 mmol) was slowly added. The mixture was stirred for 20 h at room temperature, methanol (5 mL) was added, and the solvent removed. The residue was applied to a silica gel column and eluted with 9:1 pentane–ethyl acetate to give **6** (2.56 g, 68%), $[\alpha]_D^{20}$ −48°, $[\alpha]_{546}^{20}$ −56.6° (*c* 2, methanol); ^{1}H-n.m.r.: δ 5.55 (d, $J_{1,2}$ 5 Hz, H-1), 4.60 (m, $J_{3,4}$ 8 Hz, H-3), 4.30 (m, $J_{2,3}$ 2.3 Hz, H-2), 4.27 (m, $J_{4,5}$ 1.7 Hz, H-4), 4.0 (m, $J_{5,6}$ 6.3 Hz, H-5), 3.6 (d, H-6), 3.5 (t, $J_{\alpha,\beta}$ 6 Hz, H-α), 1.55, 1.46, 1.35 [3 s, $C(CH_3)_2$], 1.3 [m, $(CH_2)_6$], and 0.9 (t, CH_3).

Anal. Calc. for $C_{20}H_{36}O_6$: C, 64.5; H, 9.7. Found: C, 64.5; H, 9.6.

6-O-Octyl-α-D-galactopyranose (**7**). — Compound **6** (2.36 g) was dissolved in 90% aqueous trifluoroacetic acid (10 mL). The solution was kept for 1 h at 0°, and then evaporated to give a chromatographically pure product. Crystallization from ethanol gave **7**, (1.5 g, 81%), m.p. 153°, $[\alpha]_D^{20}$ +32.9°, $[\alpha]_{546}^{20}$ +38.8° (*c* 1.1, methanol).

Anal. Calc. for $C_{14}H_{28}O_6$: C, 57.5; H, 9.6. Found: C, 57.3; H, 9.6.

2,3,4-Tri-O-acetyl-6-O-octyl-α-D-galactopyranosyl bromide (**8**). — Compound **7** (2.9 g) was dissolved in acetic anhydride (30 mL) with $BF_3 \cdot Et_2O$ (0.5 mL) as catalyst. After 3 h, at room temperature, the solvent was removed and the

residue dissolved in a saturated solution of HBr in acetic acid (15 mL). After 3 h at room temperature, HBr and acetic acid were eliminated under reduced pressure. The syrupy residue was purified by column chromatography in 1:4 diethyl ether–pentane to give **8** (3.3 g, 70%); ^{1}H-n.m.r.: δ 6.6 (d, $J_{1,2}$ 5 Hz, H-1), 5.5 (m, H-4), 5.3 and 5.1 (m, H-3 and -2), 4.4 (t, H-5), 3.5 and 3.4 (m, H-6 and -α), 2.0, 2.05 and 2.1 (s, $COCH_3$), 1.2 [m, $(CH_2)_6$], 0.9 (t, CH_3). The bromide **8** was immediately used for glycosylation reactions.

*1,2:3,4-Di-*O*-isopropylidene-6-*O*-(2,3,4-tri-*O*-acetyl-6-*O*-octyl-α-*D*-galacto-pyranosyl)-α-*D*-galactopyranose* (**9**). — To a solution of the bromide **8** (1.28 g, 2.66 mmol) in dichloromethane (15 mL) at −15° under an atmosphere of Ar, were added silver triflate (0.7 g, 2.7 mmol) and a solution of 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (0.7 g, 2.66 mmol) and *N,N,N′,N′*-tetramethylurea (0.31 g, 2.66 mmol) in dichloromethane (10 mL). After 20 min at −15°, the mixture was poured into a cold solution of $NaHCO_3$ and extracted with dichloromethane, washed with water, and dried ($MgSO_4$). The crude product was applied to a silica gel column and eluted with 1:1 diethyl ether–pentane to give **9** (0.72 g, 40%), $[\alpha]_D^{20}$ −46°, $[\alpha]_{546}^{20}$ −54° (*c* 1.6, methanol); ^{1}H-n.m.r.: δ 5.50 (d, $J_{1,2}$ 4.9 Hz, H-1), 5.43 (q, $J_{4',5'}$ 1.1 Hz, H-4′), 5.21 (q, $J_{2',3'}$ 10.5 Hz, H-2′), 5.02 (q, $J_{3',4'}$ 3.4 Hz, H-3′), 4.58 (q, $J_{3,4}$ 7.9 Hz, H-3), 4.56 (d, $J_{1',2'}$ 8.0 Hz, H-1′), 4.29 (q, $J_{2,3}$ 2.4 Hz, H-2), 4.18 (q, $J_{4,5}$ 1.9 Hz, H-4), 4.05 (q, $J_{6A,6B}$ 11.5 Hz, H-6A), 3.93 (m, $J_{5,6A}$ 3.3, $J_{5,6B}$ 7.6 Hz, H-5), 3.81 (m, $J_{5',6'A}$ 6.0, $J_{5',6'B}$ 6.9 Hz, H-5′), 3.67 (q, H-6B), 3.54 (q, $J_{6'A,6'B}$ 9.8 Hz, H-6′A), 3.44 (q, H-6′B), 3.43 (m, H-αA), 3.34 (m, H-αB), 2.13, 2.08, 1.98 (3 s, OAc), 1.51, 1.44, 1.32 [3 s, $C(CH_3)_2$], 1.26 [m, $(CH_2)_6$], and 0.88 (t, CH_3); ^{13}C-n.m.r.: δ 171.8, 171.5 (C=O), 110.5, 109.4 (O–C–O), 103.1 (C-1′), 97.7 (C-1), 72.9–62.9 (C cyclic), and 32.9–14.4 (remaining C).

Anal. Calc. for $C_{32}H_{52}O_{14}$: C, 58.1; H, 7.9. Found: C, 57.9; H, 7.8.

*6-*O*-(6-*O*-Octyl-α-*D*-galactopyranosyl)-*D*-galactopyranose* (**10**). — Compound **9** (690 mg) was dissolved in 90% aqueous trifluoroacetic acid (5 mL). The solution was kept 1 h at 0°, and then evaporated to give a chromatographically pure compound which was deacetylated in methanol (10 mL) with triethylamine (3 mL) for 24 h at room temperature. The solution was evaporated and the residue crystallized from methanol–ethyl acetate to give **10**, m.p. 129°, $[\alpha]_D^{20}$ +7.5°, $[\alpha]_{546}^{20}$ +8.6° (*c* 1, methanol); ^{1}H-n.m.r. [($^2H_4$)-methanol]: δ 5.17 (H-1), 4.5–3.2 (H, cyclic), 1.32 [m, $(CH_2)_6$], and 0.9 (t, CH_3).

Anal. Calc. for $C_{20}H_{38}O_{11}$: C, 52.8; H, 8.4. Found: C, 52.7; H, 8.3.

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