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APPLICATION OF THE TRICHLOROACETIMIDATE METHOD TO THE SYNTHESIS OF GLYCOPEPTIDES OF THE MUCIN TYPE CONTAINING A β-D-Gal*p*-(1→3)-D-Gal*p*NAc UNIT*,†

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ABSTRACT

Mucin-type *O*-glycopeptides containing the β-D-Gal*p*-(1→3)-D-Gal*p*NAc disaccharide core unit, which is also the T-antigenic determinant, were synthesized from D-galactose, 2-azido-2-deoxy-D-galactose, 2-azido-2-deoxylactose, and L-serine precursors by applying the trichloroacetimidate method. Thus, β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-(1→3)-Ser (**1**) and β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→6)-[β-D-Gal*p*-(1→3)]-α-D-Gal*p*NAc-(1→3)-Ser (**2**) were obtained. A protected precursor of **2** having free OH groups at C-4 and C-6 of the inner sugar unit is a valuable intermediate for the synthesis of further *O*-glycopeptides of this core-unit type.

INTRODUCTION

Epithelial mucous secretions consist mainly of glycoproteins in which the oligosaccharide moiety is *O*-glycosidically linked to the amino acids L-serine or L-threonine through 2-acetamido-2-deoxy-α-D-galactopyranose[2]. In one core type this galactosamine molecule carries an *N*-acetylglucosamine residue, giving a β-D-Glc*p*-NAc-(1→3)-D-Gal*p*NAc disaccharide unit[3]. The trichloroacetimidate method for glycoside-bond formation was recently successfully applied to the synthesis of this disaccharide and derived higher oligosaccharides, for instance the trisaccharide β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-D-GalNAc and the tetrasaccharide β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→6)-[β-D-Glc*p*NAc-(1→3)]-D-GalNAc[4–7]. However, mainly another structural type containing the β-D-Gal*p*-(1→3)-D-Gal*p*NAc core unit was isolated from mucous secretions[3]. This disaccharide is also the determinant of the T-antigen, an *O*-glycoprotein closely related to glycophorin A and considered to be a tumor associated antigen[8].

As part of the synthesis of mucin-related glycopeptides *via* the trichloroacetimidate method we have developed a synthesis of the serine derivative **1** of the Gal-GalNAc disaccharide[9], which has previously been prepared successfully by the

* Dedicated to Burckhardt Helferich in commemoration of the hundredth anniversary of his birth.
† Glycosylimidates, part 24. For part 23, see ref. 1.

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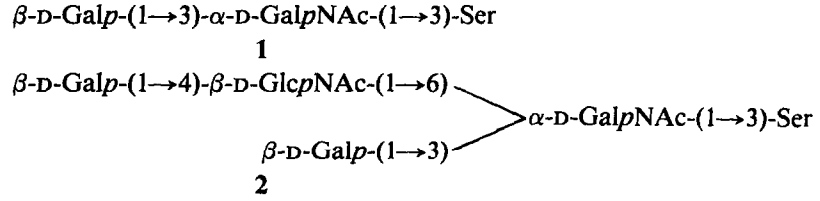
Koenigs-Knorr procedure[10-14]. In addition, we have made the serine-linked tetrasaccharide **2**, the core type of a subclass[9] of mucin oligosaccharides.



The synthesis of these compounds, to be discussed in this paper, demonstrates that β-glycoside bond formation *via* the trichloroacetimidate method can be successfully extended to 2,3,4,6-tetra-*O*-benzylgalactose and to per-*O*-benzylated 2-azido-2-deoxylactose[4-15]. Starting from the corresponding α-glycosyltrichloroacetimidates as donors, inversion of configuration is expected under selected reaction conditions.

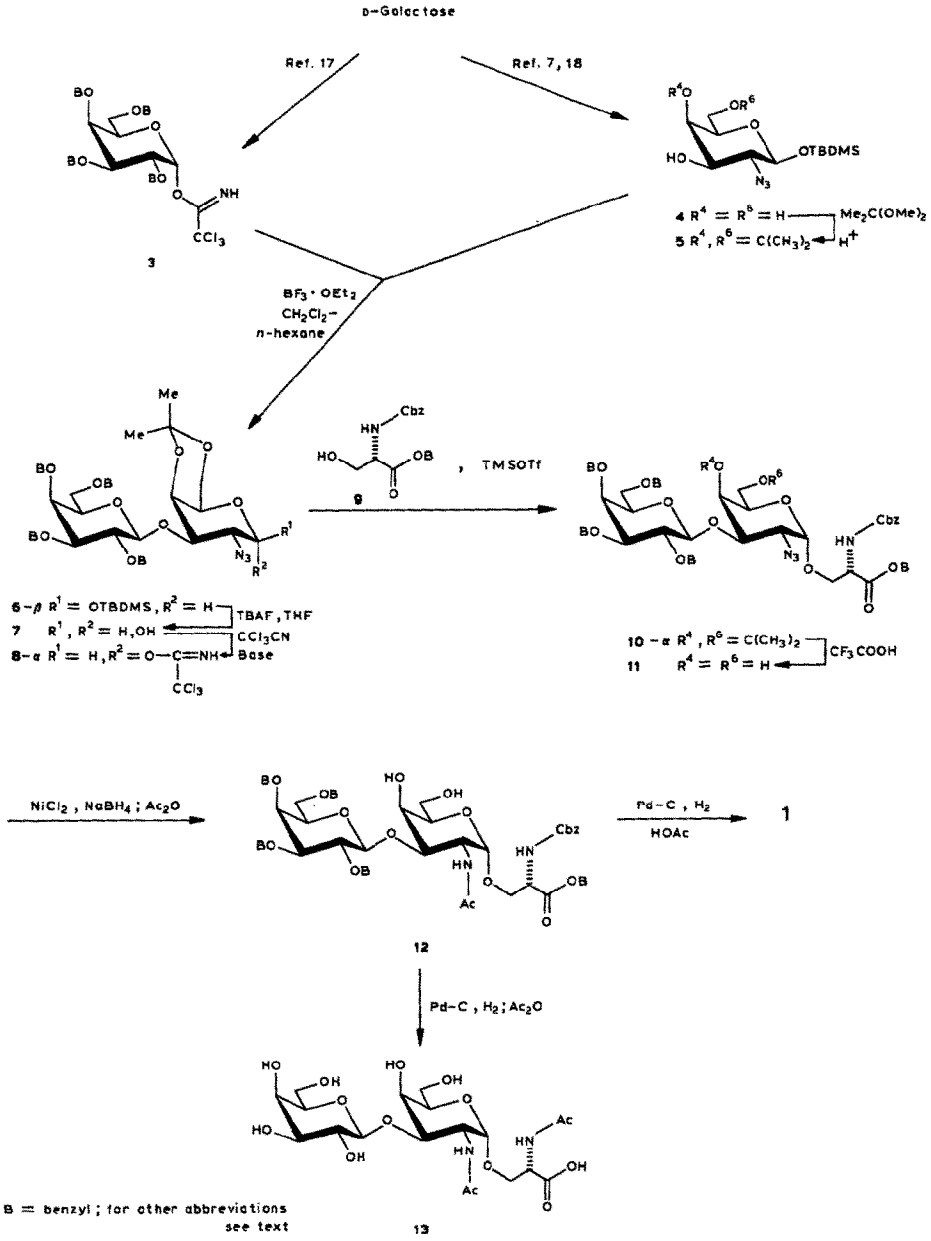
Products **1** and **2** are of importance for the investigation of the conformation of mucin-type oligosaccharides in their natural environment, because for this purpose compounds are required which are α-glycosidically linked to L-serine or L-threonine[16]. Mucin-type oligosaccharides from natural sources are usually isolated after alkaline reductive cleavage from the protein chain as oligosaccharide alditols having a GalNAc-ol end group[3d].

RESULTS AND DISCUSSION

For the synthesis of the disaccharide glycopeptide **1** the α-D-galactosyltrichloroacetimidate **3**, obtained by a known procedure[17], was used as the galactosyl donor. The acceptor was the 3-O-unprotected 2-azido-2-deoxygalactose derivative **5**, obtained from **4** (refs. 7, 18) by treatment with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid as a catalyst. Subsequent disaccharide formation with diethyl ether–boron trifluoride as a catalyst was extremely solvent-sensitive. A high yield (84%) and a good β:α-ratio (**6**-β:**6**-α ≈ 7:1) was obtained with a mixture of dichloromethane–*n*-hexane. The nonpolar solvent strongly favors inversion of configuration at the anomeric center of the galactosyl donor in this reaction. It also became obvious that the isopropylidene protective group in compound **5** gives better results in this reaction than the benzylidene or the *p*-methoxybenzylidene protective group[9].

Compound **6**-β was readily transformed into a disaccharide donor for the synthesis of glycopeptide **1**. Removal of the *tert*-butyldimethylsilyl (TBDMS) protective group with tetra-*n*-butylammonium fluoride (TBAF) gave the anomeric mixture **7**, which was subsequently treated with trichloroacetonitrile in presence of potassium carbonate–sodium hydride as base. From this reaction, exclusively the α-trichloroacetimidate **8**-α was obtained. According to earlier results[6], from di-

saccharide donor **8**-α, serine derivative **9** (ref. 19) as acceptor, and trimethylsilyl triflate (TMSOTf) as a catalyst, exclusive formation of the α-glycoside **10**-α was expected. However, a high yield (86%) of product but only low α:β-selectivity (~2:1) was obtained in this reaction. The desired α-anomer **10**-α was easily



Scheme 1

separated from the reaction mixture. Results with other systems indicated that much better α-selectivities can be obtained with the corresponding β-trichloroacetimidate[9,20].

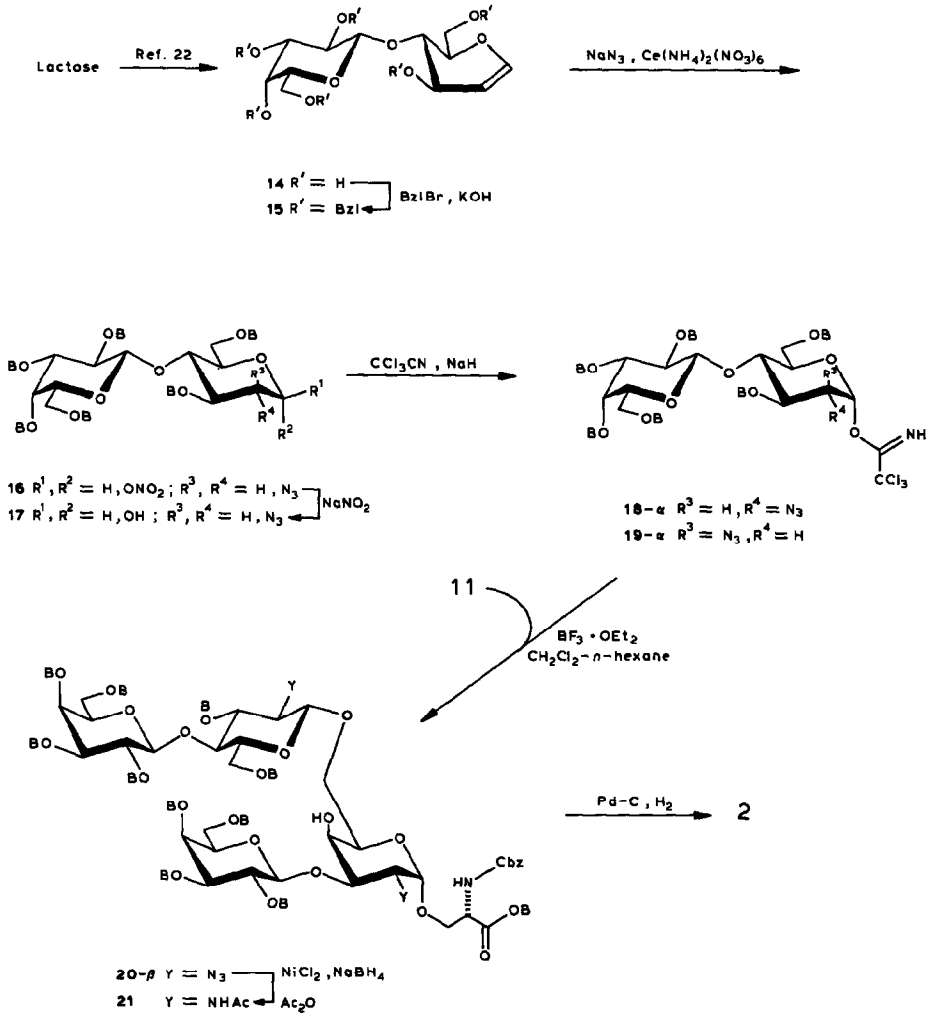
For the initial cleavage of its protective groups, compound **10**-α was treated with trifluoroacetic acid, which led to clean *O*-deisopropylidenation. The product obtained (**11**) was subsequently treated with nickel chloride–sodium borohydride[21] and then immediately with acetic anhydride to provide the acetamido derivative **12**. Hydrogenolytic removal of the benzyl protective groups in the presence of acetic anhydride afforded directly the disaccharide peptide **13** having an *N*-acetyl group on the serine moiety. For the complete removal of all protective groups, hydrogenolytic benzyl-group cleavage in compound **12** had to be carried out in acetic acid, affording cleanly the known[11] glycopeptide **1**.

For synthesis of the tetrasaccharide glycopeptide **2**, a lactosamine donor was required. Towards this end lactal[22] (**14**) was at first *O*-benzylated with benzyl chloride–potassium hydroxide in dioxane. Azidonitration of the resulting compound **15** gave a mixture of epimers (**16**) which could not be separated. The nitrate group was removed with sodium nitrite in aqueous dioxane affording **17**, also a mixture of epimers. Treatment of this mixture with trichloroacetonitrile in the presence of sodium hydride gave, nearly exclusively, the α-*gluco* and the α-*manno* trichloroacetimidates **18**-α and **19**-α, respectively. These could easily be separated; the desired lactosamine derivative **18**-α is a crystalline material.

Compound **18**-α turned out to be an excellent lactosamine donor. With compound **11** as acceptor and diethyl ether–boron trifluoride as a catalyst, the tetrasaccharide peptide **20**-β was obtained as the sole reaction product in high yield (81%). The solvent system dichloromethane–*n*-hexane led to clean inversion of configuration at the anomeric center during product formation. Azido to amino group transformation was again carried out with nickel chloride–sodium borohydride[21]; subsequent acetic anhydride treatment afforded compound **21**. Hydrogenolytic debenzylation gave the desired tetrasaccharide peptide **2**. This reaction sequence indicates that compound **11** is a valuable intermediate for further syntheses of *O*-glycopeptides of other subclasses related to the above-mentioned core unit. The structures of the described compounds were assigned from their ^{1}H-n.m.r. data.

EXPERIMENTAL

General. — Melting points are uncorrected. ^{1}H-N.m.r. and ^{13}C-n.m.r. spectra were recorded in the solvents noted (Me_4Si = δ 0.00), with a Bruker WM 250 Cryospec and a Jeol JNM-GX 400 instrument. R_F values refer to t.l.c. performed on silica gel (Merck) with the solvent systems noted. Column chromatography was carried out on silica gel (Merck 70–230 mesh ASTM and 230–400 mesh ASTM for flash chromatography under normal pressure and Merck "LiChroprep" Si 60, 40–60 μm for medium pressure operation), with the solvent systems noted (petroleum ether, b.p. 40–70° = PE, ethyl acetate = EA). Optical rotations were determined



Scheme 2

with a Perkin-Elmer 241 MC polarimeter.

tert-*Butyldimethylsilyl 2-azido-2-deoxy-4,6-O-isopropylidene-β-D-galactopyranoside* (**5**). — To a solution of compound **4** (refs. 7,18) (53.0 g, 0.166 mmol) in dry *N,N*-dimethylformamide (500 mL) was added 2,2-dimethoxypropane (300 mL, 3.0 mol) and 0.3 g of anhydrous *p*-toluenesulfonic acid at room temperature. After 6 h the reaction mixture was evaporated under reduced pressure (10 torr) at 40°. The residue was treated with ice-water (500 mL), the organic material extracted with ether (3 × 300 mL), and the ether phase was washed with saturated sodium chloride solution (3 × 100 mL), dried (Na_2SO_4), and concentrated. The oily residue was purified by column chromatography with 1:1 PE–EA as eluant. This yielded 44 g

(74%) of colourless oil; $[\alpha]_D^{20}$ +21.7° (*c* 1, CH_2Cl_2); t.l.c. (1:1 PE–EA) R_F 0.29; ^{1}H-n.m.r. (250 MHz, $CDCl_3$): δ 4.48 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1), 4.09 (dd, 1 H, $J_{4,5}$ 1.2, $J_{3,4}$ 3.4 Hz, H-4), 4.05 (dd, 1 H, $J_{5,6a}$ 2.4, $J_{6a,6e}$ 11.3 Hz, H-6a), 3.88 (dd, 1 H, $J_{5,6e}$ 0.5 Hz, H-6e), 3.48 (dd, 1 H, $J_{2,3}$ 7.0 Hz, H-2), 3.43 (ddd, 1 H, H-3), 3.30 (ddd, 1 H, H-5), 2.52 (d, 1 H, $J_{3,OH}$ 8.8 Hz, O*H*-3), 1.46 [s, 6 H, C(CH_3)$_2$], 0.94 [s, 9 H, C(CH_3)$_3$], 0.18, and 0.16 (2s, 6 H, SiCH_3).

Anal. Calc. for $C_{15}H_{29}N_3O_5Si$ (359.5): C, 50.1; H, 8.1; N, 11.7. Found: C, 50.2; H, 8.2; N, 11.4.

tert-*Butyldimethylsilyl 2-azido-2-deoxy-4,6-*O*-isopropylidene-3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-*D*-galactopyranosyl)-β-*D*-galactopyranoside* (**6**-β) *and* tert-*butyldimethylsilyl 2-azido-2-deoxy-4,6-*O*-isopropylidene-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-galactopyranosyl)-β-*D*-galactopyranoside* (**6**-α). — To a solution of compound **5** (9.5 g, 26.4 mmol) and trichloroacetimidate **3** (ref. 17) (16.2 g, 30.0 mmol) in dry dichloromethane (50 mL) at −10° was added *n*-hexane until precipitation of the starting material began (CH_2Cl_2: hexane 4:1). Then 0.1M diethyl ether–boron trifluoride (0.5 mL) in anhydrous dichloromethane diluted with dry hexane (10 mL) was added during 0.5 h under nitrogen. After addition of the solution, precipitation of trichloroacetamide occurred. Stirring was continued for a further 6 h. T.l.c. (4:1 PE–EA) revealed the presence of one major product. Sodium hydrogencarbonate was added and the mixture stirred 15 min before filtration. The filtrate was washed with *n*-hexane, then taken up in CH_2Cl_2 (100 mL). The organic phase was washed with a saturated solution of NaCl in water, dried ($MgSO_4$), and evaporated to dryness. The oily residue was purified by flash chromatography (4:1 PE–EA) yielding 17.0 g (73%) of **6**-β and 2.7 g (10.7%) of **6**-α as colorless oils; $[\alpha]_{578}^{20}$ for **6**-β +27.1° (*c* 1, chloroform), **6**-α +40.5° (*c* 1, chloroform); t.l.c. (1:1 PE–EA): R_F for **6**-β 0.36, for **6**-α 0.27; ^{1}H-n.m.r. (250 MHz, $CDCl_3$): for **6**-α, δ 7.44–7.18 (m, 20 H, 4 C_6H_5), 5.05, 4.98, 4.80, 4.78, 4.73, 4.60 (6 d, 6 H, 3 PhCH_2), 4.58 (d, 1 H, $J_{1',2'}$ 7.94 Hz, H-1'), 4.50 (d, 1 H, $J_{1,2}$ 7.63 Hz, H-1), 4.40 (s, 2 H, PhCH_2), 4.24 (dd, 1 H, $J_{3,4}$, $J_{4,5'}$ 0.5 Hz, H-4), 3.90 (dd, 1 H, H-2'), 3.93–3.80 (m, 2 H, H-6*a*,*e*), 3.74 (dd, 1 H, $J_{2,3}$ 10.6 Hz, H-2), 3.85 (brs, 1 H, H-4'), 3.56–3.45 (m, 3 H,), 3.47 (dd, 1 H, H-3'), 3.38 (dd, 1 H, H-3), 3.14 (brs, 1 H, H-5), 1.47, 1.39 [2 s, 6 H, 2 C(CH_3)$_2$], 0.94 [s, 9 H, C(CH_3)$_3$], 0.18, and 0.16 (2 s, 6 H, SiCH_3); for **6**-α, δ 7.40–7.18 (m, 20 H, 4 C_6H_5), 5.16 (brs, 1 H, H-1'), 4.97–4.47 (m, 8 H, 4 PhCH_2), 4.39 (d, 1 H, $J_{1,2}$ 7.60 Hz, H-1), 4.21 (dd, 1 H, $J_{3,4}$ 3.10, $J_{4,5}$ 0.5 Hz, H-4), 4.16–3.85 (m, 6 H), 3.80 (dd, 1 H, $J_{2,3}$ 10.6 Hz, H-2), 3.62–3.41 (m, 3 H), 3.07 (brs, 1 H, H-5), 1.42, 1.38 [2 s, 6 H, C(CH_3)$_2$], 0.94 [s, 9 H, C(CH_3)$_3$], 0.16, and 0.14 (2 s, 6 H, 2 SiCH_3).

Anal. Calc. for $C_{49}H_{63}N_3O_{10}Si$ (881.70): C, 66.7; H, 7.2. Found: for **6**-β, C, 66.5; H, 7.0; for **6**-α, C, 66.5; H, 7.2.

*2-Azido-2-deoxy-4,6-*O*-isopropylidene-3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-*D*-galactopyranosyl)-α,β-*D*-galactopyranose* (**7**). — A solution of compound **6**-α (15.0 g, 17.0 mmol) in dry tetrahydrofuran (250 mL) was cooled in an atmosphere of dry nitrogen to −20°. After the addition of acetic acid (200 mmol; 20 mL of M solution in tetrahydrofuran) a solution of tetrabutylammonium fluoride (45 mL of M solution

diluted with 50 mL of tetrahydrofuran) was added dropwise over 3 h. The mixture was stirred for a further 3 h at −5°, then diluted with ether (300 mL). The organic phase was washed with water (3 × 100 mL) and brine (2 × 50 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The resulting oily residue was purified by chromatography (1:2 PE–EA), yielding 12.0 g (92%) of **7** as a colorless oil; t.l.c. (1:2 PE–EA); R_F 0.64, 0.48; ^{1}H-n.m.r. (250 MHz, $CDCl_3$): δ 7.45–7.25 (m, 20 H, 4 C_6H_5), 5.45 (brs, 1 H, H-1α), 5.08–3.40 (m, 22 H), 1.48, and 1.40 [2 s, 6 H, $C(CH_3)_2$].

Anal. Calc. for $C_{43}H_{49}N_3O_{10}\cdot H_2O$ (785.89): C, 65.7; H, 6.5; N, 5.4. Found: C, 65.8; H, 6.7; N, 5.6.

*2-Azido-2-deoxy-4,6-*O*-isopropylidene-3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-*D*-galactopyranosyl)-α-*D*-galactopyranosyl trichloroacetimidate* (**8**). — To a solution of **7** (138 mg, 0.18 mmol) in 10 mL of dry dichloromethane was added potassium carbonate (20 g) and trichloroacetonitrile (0.60 mL). The suspension was strongly stirred for 6 h with exclusion of moisture. Then a catalytic amount of sodium hydride was added. After additional stirring for 2 h the mixture was filtered over Celite, washed, the filtrate evaporated under reduced pressure, and the oily brown residue purified by flash chromatography with 1:1 PE–EA as eluant. The yield was 156 mg (95%) of pure **8** as a syrup; $[\alpha]_{578}^{20}$ +80.1° (*c* 1, chloroform); t.l.c. (1:1 PE–EA): R_F 0.35; ^{1}H-n.m.r. (250 MHz, $CDCl_3$): δ 8.71 (s, 1 H, N*H*), 7.42–7.24 (m, 20 H, 4 C_6H_5), 6.55 (d, 1 H, $J_{1,2}$ 3.1 Hz, H-1), 5.04–3.50 (m, 21 H), 1.49, and 1.40 [2 s, 6 H, $C(CH_3)_2$].

Anal. Calc. for $C_{45}H_{49}Cl_3N_4O_{10}\cdot 1.5\ H_2O$ (939.27): C, 57.5; H, 5.6; N, 6.0. Found: C, 57.6; H, 5.6; N, 5.7.

N-*Benzyloxycarbonyl-3-*O*-[2-azido-2-deoxy-4,6-*O*-isopropylidene-3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-*D*-galactopyranosyl)-α-*D*-galactopyranosyl]-*L*-serine benzyl ester* (**10**-α) *and* N-*benzyloxycarbonyl-3-*O*-[2-azido-2-deoxy-4,6-*O*-isopropylidene-3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-*D*-galactopyranosyl)-β-*D*-galactopyranosyl]-*L*-serine benzyl ester* (**10**-β). — A mixture of thoroughly dried **8** (5.00 g, 5.48 mmol) and **9** (ref. 19), dissolved in dry dichloromethane (100 mL), was treated under an atmosphere of dry nitrogen at −30° with 0.2 mL of TMSOTf solution (0.1M in dichloromethane). After 2 h, t.l.c. in 3:2 PE–EA showed that all of the starting material had been converted to **10**α and **10**β. The solution was treated with saturated $NaHCO_3$ in water and diluted with chloroform (200 mL). The organic layer was washed with brine, dried, and evaporated to give, after an easy separation of the anomers by flash chromatography (3:2 PE–EA), 3.1 g (53%) of **10**-α and 2.0 g (34%) of **10**-β as pure oils; $[\alpha]_{578}^{20}$ for **10**α +72.5° (*c* 1, chloroform), for **10**β +12.2° (*c* 1, chloroform); t.l.c. (2:1 PE–EA): R_F for **10**α 0.29, for **10**β 0.08; ^{1}H-n.m.r. (250 MHz, $CDCl_3$): for **10**-α, δ 7.42–7.23 (m, 30 H, 6 C_6H_5), 5.91 (d, 1 H, *J* 8.2, Hz, N*H*), 5.17, 5.10 (2 s, 4 H, 2 PhCH_2), 5.03–3.33 (m, 25 H), 1.43, and 1.35 [2 s, 6 H, $C(CH_3)_2$]; for **10**-β, δ 7.42–7.24 (m, 30 H, 6 C_6H_5), 5.85 (d, 1 H, *J* 8.2 Hz, N*H*), 5.21, 5.12 (2 s, 4 H, 2 PhCH_2), 5.00, 4.95, 4.79, 4.76, 4.70, 4.58 (6 d, 6 H, PhCH_2), 4.57 (d, 1 H, $J_{1',2'}$ 7.4 Hz, H-1'), 4.41 (m, 1 H, CH_2CHCO), 4.40 (s, 2 H, PhCH_2),

4.23 (dd, 1 H, $J_{3,4}$ 3.4, $J_{4,5}$ 0.5 Hz, H-4), 4.20 (d, 1 H, $J_{1,2}$ 7.90 Hz, H-1), 3.92–3.80 (m, 5 H), 3.79 (dd, 1 H, $J_{2,3}$ 10.3 Hz, H-2), 3.60–3.49 (m, 4 H), 3.35 (dd, 1 H, H-3), 3.33 (brs, 1 H, H-5), 1.44, and 1.38 [2 s, 6 H, $C(CH_3)_2$]; ^{13}C-n.m.r. (62.97 Mhz, $CDCl_3$): for **10**-α, δ 169.3 (*C*OOBzl), 155.15 (HN*C*OOBzl), 138.4–134.6 (6 C, C_6H_5), 105.3 (C-1'), 100.2 (C-1), and 98.7 [$C(CH_3)_2$]; for **10**-β, δ 169.3 (*C*OOBzl), 155.7 (HN*C*OOBzl), 138.9–135.3 (6 C, C_6H_5), 105.0 (C-1'), 102.4 (C-1), and 98.9 [*C* $(CH_3)_2$].

Anal. Calc. for $C_{61}H_{66}N_4O_{14}$ (1079.22): C, 67.9; H, 6.2; N, 5.2. Found: for **10**-α, C, 67.7; H, 6.1; N, 5.2; for **10**-β, C, 67.6; H, 6.4; N, 5.2.

*N-Benzyloxycarbonyl-3-O-[2-azido-2-deoxy-3-O-(2,3,4,6-tetra-O-benzyl-β-*D*-galactopyranosyl)-α-*D*-galactopyranosyl]-*L*-serine benzyl ester* (**11**). — A dichloromethane (100 mL) solution of **10**-α (3.00 g, 2.78 mmol) in vigorously stirred was treated with 60% trifluoroacetic acid (0.5 mL). After standing for 2 h at room temperature, the mixture was diluted with chloroform (100 mL), washed repeatedly with water, dried ($MgSO_4$), and evaporated. This yielded 2.60 g (90%) of **11** as colorless crystals (from PE–diethyl ether); m.p. 154–156°, $[\alpha]_{578}^{20}$ +78.1° (*c* 1, chloroform); t.l.c. (1:1 PE–EA): R_F 0.35; ^{1}H-n.m.r. (250 MHz, $CDCl_3$): δ 7.42–7.24 (m, 30 H, 6 C_6H_5), 5.94 (d, 1 H, *J* 8.2 Hz, N*H*), 5.18, 5.11 (2 s, 4 H, PhCH_2), 5.01, 4.93, 4.80, 4.79, 4.71, 4.60 (6 d, 6 H, PhCH_2), 4.89 (d, 1 H, $J_{1,2}$ 4.8 Hz, H-1), 4.58 (m, 1 H, PhCH_2), 4.49 (d, 1 H, $J_{1',2'}$ 7.60 Hz, H-1'), 4.39 (s, 2 H, PhCH_2), 4.18 (dd, 1 H, CH_2CHCO), 4.09 (brs, 1 H, H-4), 3.96 (dd, 1 H, CH_2CHCO), 3.91–3.35 (m, 9 H), 3.89 (dd, 1 H, H-2'), 3.82 (brs, 1 H, H-4'), 3.60 (dd, 1 H, H-2), 3.12 (brs, 1 H, exchangeable by D_2O, O*H*-4), and 2.36 (m, 1 H, exchangeable by D_2O, O*H*-6).

Anal. Calc. for $C_{58}H_{62}N_4O_{14}$ (1039.15): C, 67.0; H, 6.0; N, 5.4. Found: C, 66.9; H, 6.0; N, 5.3.

*N-Benzyloxycarbonyl-3-O-[2-acetamido-2-deoxy-3-O-(2,3,4,6-tetra-O-benzyl-β-*D*-galactopyranosyl)-α-*D*-galactopyranosyl]-*L*-serine benzyl ester* (**12**). — To a solution of the azido compound **11** (1.00 g, 0.96 mmol) in dichloromethane (5 mL) and 200 mL of a solution of $NiCl_2 \cdot 6H_2O$) (40 g) and boric acid (20 g) in ethanol (1000 mL) was added dropwise a suspension of sodium borohydride in ethanol until the black color remained for 1 h. The mixture was kept overnight at room temperature, then treated with acetic anhydride (5 mL) and left overnight at 10°. The suspension was evaporated, diluted with dichloromethane (100 mL), washed repeatedly with cold water and brine, dried ($MgSO_4$), and evaporated to give a crystalline compound. This was recrystallized from ether to yield 1.00 g (98%) of **12** as colorless crystals; m.p. 205–208°, $[\alpha]_{578}^{20}$ +58.3° (*c* 1, chloroform); t.l.c. (1:2 PE–EA): R_F 0.25; ^{1}H-n.m.r. (250 MHz, $CDCl_3$): δ 7.35–7.26 (m, 30 H, 6 C_6H_5), 5.85, 5.49 (2 d, 2 H, N*H*), 5.20–5.08 (m, 4 H, PhCH_2), 4.90 (d, 1 H, PhCH_2), 4.82 (m, 2 H, PhCH_2), 4.81 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 4.70 (s, 2 H, PhCH_2), 4.55 (d, 1 H, PhCH_2), 4.54–4.51 (m, 2 H, H-2, CH_2CHCO) 4.42 (d, 1 H, H-1), 4.40 (s, 2 H, PhCH_2), 4.10 (brs, 1 H, H-4), 3.95–3.40 (m, 12 H), 3.15 (s, 1 H, exchangeable by D_2O, O*H*-4), 2.40 (brs, 1 H, exchangeable by D_2O, O*H*-6), and 1.53 (s, 3 H, CH_3CO).

Anal. Calc. for $C_{60}H_{66}N_2O_{15}$ (1055.19): C, 68.3; H, 6.3; N, 2.6. Found: C, 68.1; H, 6.3; N, 2.7.

N-*Acetyl-3-*O*-(2-acetamido-2-deoxy-3-*O*-β-*D*-galactopyranosyl-α-*D*-galactopyranosyl)-*L*-serine* (**13**). — A solution of **12** (100 mg, 94.7 μmol) in 1:2:1 acetic anhydride–methanol–dioxane (5 mL) was hydrogenolyzed in the presence of 10% palladium-on-carbon (100 mg). After 48 h the suspension was filtered, the filtrate was evaporated, and added toluene was distilled from the residue. The residue was then passed over a short column of silica gel with 6:8:1 chloroform–methanol–water as the eluant. The yield was 44 mg (90%) of amorphous solid; $[\alpha]_D^{20}$ +99.0° (*c* 1, 1:1 methanol–water); t.l.c. (4:8:1 chloroform–methanol–water): R_F 0.55; ^{1}H-n.m.r. (400 MHz, D_2O): δ 4.70 (d, 1 H, $J_{1,2}$ 3.66 Hz, H-1), 4.30 (d, 1 H, $J_{1',2'}$ 7.81 Hz, H-1'), 4.24 (dd, 1 H, CH_2CHCO), 4.15 (dd, 1 H, $J_{2,3}$ 10.99 Hz, H-2), 4.06 (dd, 1 H, $J_{3,4}$ 2.68, $J_{4,5}$ <0.5 Hz, H-4), 3.88 (dd, 1 H, H-3), 3.80 (m, 1 H), 3.75 (dd, 1 H, *J* 3.42 Hz, CH_2CHCO), 3.74 (dd, 1 H, $J_{3',4'}$ 3.4, $J_{4',5'}$ <0.5 Hz, H-4'), 3.64 (dd, 1 H, *J* 10.22, 5.13 Hz, CH_2CHCO), 3.60–3.54 (m, 4 H), 3.50 (m, 1 H, H-5'), 3.45 (dd, 1 H, $J_{3',4'}$ 3.41, $J_{2',3'}$ 9.77 Hz, H-3'), 3.33 (dd, 1 H, H-2'), 1.89, and 1.85 (2 s, 6 H, 2 CH_3CO).

*3-*O*-(2-Acetamido-2-deoxy-3-*O*-β-*D*-galactopyranosyl-α-*D*-galactopyranosyl)-*L*-serine* (**1**). — A solution of **12** (100 mg, 94.7 μmol) in 1:20:10 acetic acid–methanol–dioxane (12 mL) was hydrogenolyzed in the presence of 10% palladium-on-carbon (100 mg). After 16 h the suspension was filtered, the filtrate was evaporated, and toluene was distilled from the residue. The residue was purified on a short silica gel column with 1:2:1 chloroform–methanol–water as the eluant. After lyophilization 45 mg (89%) of a colorless solid was obtained; $[\alpha]_{578}^{20}$ +79.0° (*c* 0.9, 1:1 methanol–water), $[\alpha]_D^{20}$ +77.0° (*c* 0.9, 1:1 methanol–water), lit.[11] $[\alpha]_D^{26}$ +87° (*c* 1, 1:1 methanol–water); t.l.c. (1:2:1 chloroform–methanol–water): R_F 0.48, (4:8:1 chloroform–methanol–water): R_F 0.33; ^{1}H-n.m.r. (400 MHz, D_2O): δ 4.74 (d, 1 H, $J_{1,2}$ 3.42 Hz, H-1), 4.29 (d, 1 H, $J_{1',2'}$ 7.82 Hz, H-1'), 4.18 (dd, 1 H, $J_{2,3}$ 11.23 Hz, H-2), 4.06 (dd, 1 H, $J_{3,4}$ 2.9, $J_{4,5}$ <0.5 Hz, H-4), 3.92 (dd, 1 H, CH_2CHCO), 3.90 (dd, 1 H, H-3), 3.82 (m, 1 H), 3.77 (dd, 1 H, CH_2CHCO), 3.73 (dd, 1 H, $J_{3',4'}$ 3.4, $J_{4',5'}$ <0.5 Hz, H-4'), 3.7 (dd, 1 H, *J* 4.88 Hz, CH_2CHCO), 3.65–3.46 (m, 4 H), 3.44 (dd, 1 H, $J_{2',3'}$ 9.7 Hz, H-3'), 3.34 (dd, 1 H, H-2'), and 1.86 (s, 3 H, CH_3CO).

*1,5-Anhydro-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-*D*-galactopyranosyl)-*D*-arabino-hex-1-enitol* (**15**). — A suspension of **14** (ref. 22) (28.0 g, 91.0 mmol) in dry dioxane (1.4 L) was brought to reflux while being stirred vigorously. Potassium hydroxide (100 g, 1.8 mol) and freshly distilled benzyl chloride (130 mL, 1.1 mol) were alternately added in small portions. After completion of the additions the suspension was heated for 10 h under reflux. The mixture was filtered over Celite, the solvent evaporated, and the residue taken up in ether (700 mL). After standard workup, the crude product was purified by column chromatography (5:1–1:1 PE–diethyl ether) giving 50 g (65%) of yellow oil. The addition of sodium iodide (2.0 g) and dibenzo-18-crown-6 (0.5 g) shortened the reaction time considerably. The product had $[\alpha]_{578}^{20}$ −2.7° (*c* 1, chloroform); t.l.c.

(1:1 PE–diethyl ether): R_F 0.56; ^{1}H-n.m.r. (250 MHz, $CDCl_3$): δ 7.30–7.20 (m, 30 H, 6 C_6H_5), 6.44 (d, 1 H, $J_{1,2}$ 6.1 Hz, H-1), and 4.96–3.40 (cm, 23 H).

Anal. Calc. for $C_{54}H_{56}O_9$ (849.03): C, 76.4; H, 6.6. Found: C, 76.4; H, 6.6.

N-*Benzyloxycarbonyl-3-*O-{*2-azido-6-*O-*[2-azido-3,6-di-*O-*benzyl-2-deoxy-4-*O-*(2,3,4,6-tetra-*O-*benzyl-β-*D-*galactopyranosyl)-β-*D-*glucopyranosyl]-2-deoxy-3-*O-*(2,3,4,6-tetra-*O-*benzyl-β-*D-*galactopyranosyl)-α-*D-*galactopyranosyl*}-L-*serine benzyl ester* (**20**-β). — To a stirred solution of pure **15** (60 g, 70.7 mmol) in dry acetonitrile (1000 mL) a mixture of sodium azide (5.2 g, 80 mmol) and ceric ammonium nitrate (150 g, 283 mmol) was added. The suspension was stirred for 16 h at −40° under nitrogen. Cold ether (1000 mL) was added, and after filtration over Celite the filtrate was washed with water until neutral and then concentrated to dryness yielding a syrup. Following a short filtration over silica gel with 2:1 PE–diethyl ether as eluant we obtained after evaporation *in vacuo* a yellow oil (53 g, 79%), which was dissolved in dioxane (1.5 L). A saturated solution of 70 g sodium nitrite in water was added, and the mixture was heated for 8 h to 80° with stirring. Standard work-up and purification by flash chromatography (1:1 PE–EA) gave 35 g (69%) of a yellow oil which was a mixture (**17**) of *2-azido-3,6-di-*O-*benzyl-2-deoxy-4-*O-*(2,3,4,6-tetra-*O-*benzyl-β-*D-*galactopyranosyl)-α,β-*D-*glucopyranose and 2-azido-3,6-di-*O-*benzyl-2-deoxy-4-*O-*(2,3,4,6-tetra-*O-*benzyl-β-*D-*galactopyranosyl)-α,β-*D-*mannopyranose*; t.l.c. (1:1 PE–diethyl ether): R_F 0.20–0.27. The mixture was used directly for the next step.

To a solution of the epimeric mixture **17** (4.00 g, 4.41 mmol) in dry dichloromethane (5 mL) were added under exclusion of moisture trichloroacetonitrile (4 mL) and a catalytic amount of sodium hydride. The suspension was stirred until t.l.c. showed no more starting material, then more sodium hydride was added to ensure complete anomerization to the α-*manno* and α-*gluco* imidates. After the customary processing as described for **8**, the mixture was purified by flash chromatography using 2:1 PE–diethyl ether as the eluant. On evaporation, the fractions corresponding to the major product yielded a foam, which was crystallized by adding a small volume of PE–diethyl ether. Filtration yielded *2-azido-3,6-di-*O-*benzyl-2-deoxy-4-*O-*(2,3,4,6-tetra-*O-*benzyl-β-*D-*galactopyranosyl)-glucopyranosyl trichloroacetimidate* (**18**-α) (2.5 g, 54%) as a white powder. Evaporation of the fractions corresponding to the minor product yielded 37% of an inseparable mixture of the α-*manno* (**19**-α) and β-*gluco* (**18**-β) isomers. T.l.c. (1:1 PE–diethyl ether): R_F for **18**-α 0.5, for **18**-β/**19**-α 0.55; ^{1}H-n.m.r. (250 MHz, $CDCl_3$): δ 8.69 (s, 1 H, N*H*), 7.39–7.09 (m, 30 H, 6 C_6H_5), 6.35 (d, 1 H, $J_{1,2}$ 3.66 Hz, H-1), 5.21–3.29 (m, 23 H), 3.90 (dd, 1 H, H-3), and 3.60 (dd, 1 H, $J_{2,3}$ 10.38 Hz, H-2). The compound **18**-α was used directly for further reactions.

As described for compound **6**-β, **18**-α (650 mg, 0.62 mmol), **11** (520 mg, 0.50 mmol), and 0.1M diethyl ether· boron trifluoride in dichloromethane (0.2 mL) at −12° gave, after a 3-h reaction time, 838 mg (87%) of crude material. Recrystallization from ether yielded 787 g (81%) of colorless crystals of **20**-β, m.p. 116–121°, $[\alpha]_{578}^{20}$ +25.5° (*c* 1, chloroform); t.l.c. (2:1 PE–EA): R_F 0.51; ^{1}H-n.m.r. (250 MHz,

$CDCl_3$): δ 7.41–7.11 (m, 60 H, 12 C_6H_5), 5.70 (d, 1 H, N*H*), 5.18–3.30 (m, 55 H), 3.00 (brs, 1 H, J <0.5 Hz, exchanged by D_2O, O*H*-4); addition of trichloroacetyl isocyanate gave ^{1}H-n.m.r. (250 MHz, $CDCl_3$): δ 8.55 (s, 1 H, N*H*), 7.50–7.15 (m, 60 H, 12 C_6H_5), 5.65 (d, 1 H, N*H*), 5.28 (dd, 1 H, $J_{3,4}$ 1.7, $J_{4,5}$ <0.5 Hz, H-4), and 5.18–3.30 (m, 54 H); ^{13}C-n.m.r. (62.97 MHz, $CDCl_3$): δ 169.31 (*C*OOBzl), 155.49 (NH*C*OOBzl), 103.6, 102.3, 101.8 (C-1′, C-1″, C-1‴), and 98.84 (C-1).

Anal. Calc. for $C_{112}H_{117}N_7O_{23}$ (1929.19): C, 69.7; H, 6.1; N, 5.1. Found: C, 69.4; H, 6.2; N, 5.1.

N-*Benzyloxycarbonyl-3*-O-{*2-acetamido-6*-O-*[2-acetamido-3,6-di*-O-*benzyl-2-deoxy-4*-O-*(2,3,4,6-tetra*-O-*benzyl-β*-D-*galactopyranosyl)-β*-D-*galactopyranosyl]-2-deoxy-3*-O-*(2,3,4,6-tetra*-O-*benzyl-β*-D-*galactopyranosyl)-α*-D-*galactopyranosyl*}-L-*serine benzyl ester* (**21**). — As described for **12**, from **20**-β (300 mg, 0.155 mmol) after a 48 h sodium-borohydride reduction and an 8 h acylation, a solid residue was obtained which was purified by flash chromatography (1:2 PE–EA) to yield **21** (250 mg; 82%) as a colorless, amorphous solid $[\alpha]_{578}^{20}$ +20.9° (*c* 1, chloroform); t.l.c. (1:2 PE–EA): R_F 0.42; ^{1}H-n.m.r. (250 MHz, $CDCl_3$): δ 7.32–7.15 (m, 60 H), 6.20, 5.85, 5.42 (3 d, 3 H, *J* 8.0 Hz, 3 N*H*), 5.20–3.35 (m, 58 H). 2.85 (br.s, 1 H, O*H*-4), 1.75, and 1.50 (2 s, 6 H, 2 CH_3CO).

Anal. Calc. for $C_{116}H_{125}N_3O_{25}\cdot H_2O$ (1979.27): C, 70.4; H, 6.5; N, 2.1. Found: C, 69.9; H, 6.2; N, 2.1.

3-O-*[2-Acetamido-6*-O-*(2-acetamido-2-deoxy-4*-O-*β*-D-*galactopyranosyl-β*-D-*glucopyranosyl)-2-deoxy-3*-O-*β*-D-*galactopyranosyl-α*-D-*galactopyranosyl]*-L-*serine* (**2**). — As described for the preparation of **1** from **12**, **21** (200 mg; 102 μmol) gave **2** (80 mg; 94%) as an amorphous powder after lyophilization, $[\alpha]_{578}^{20}$ +34.5° (*c* 1, water); t.l.c. (1:2:1 chloroform–methanol–water): R_F 0.42; ^{1}H-n.m.r. (400 MHz, D_2O)*: δ 4.70 (d, 1 H, $J_{1,2}$ 3.66 Hz, H-1), 4.39 (d, 1 H, $J_{1'',2''}$ 7.81 Hz, H-1″), 4.29, 4.28 (2 d, 2 H, *J* 7.57, *J* 7.81 Hz, H-1′, H-1‴), 4.16 (dd, 1 H, $J_{2,3}$ 11.23 Hz, H-2), 4.04 (dd, 1 H, $J_{3,4}$ 2.0, $J_{4,5}$ <0.5 Hz, H-4), 3.91–3.88 (m, 2 H), 3.90 (dd, 1 H, H-3), 3.84–3.81 (m, 2 H), 3.75–3.65 (m, 3 H), 3.72 (m, 2 H, H-4′, H-4″), 3.58–3.40 (m, 11 H), 3.50–3.40 (2 dd, 2 H, H-3′, H-3″), 3.36, 3.32 (2 d, 2 H, H-2′, H-2″), 1.87, and 1.85 (2 s, 6 H, 2 CH_3CO).

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REFERENCES

1 R. R. SCHMIDT AND P. ZIMMERMANN, *Angew. Chem.*, 98 (1986) 722–723; *Angew. Chem., Int. Ed. Engl.*, 25 (1986) 725–726.

* Doubly and triply primed numbers refer to the reducing and nonreducing residues, respectively of the 6-linked branch.

2 (a) N. S. SHARON AND H. LIS, *Chem. Eng. News,* 59, No. 13 (1981) 21–44; (b) J. MONTREUIL, *Adv. Carbohydr. Chem. Biochem.*, 37 (1980) 157–223.
3 (a) A. SLOMIANY AND B. SLOMIANY, *J. Biol. Chem.*, 253 (1978) 7301–7306; (b) G. LAMBLIN, M. LHERMITTE, A. BOERSMA, AND P. ROUSSEL, *J. Biol. Chem.*, 255 (1980) 4595–4598; (c) E. F. HOUNSELL, M. FUKUDA, M. E. POWELL, T. FEIZI, AND S. HAKOMORI, *Biochem. Biophys. Res. Commun.*, 92 (1980) 1143–1150; (d) H. VAN HALBEEK, L. DORLAND, J. F. G. VLIEGENTHART, W. E. HILL, G. LAMBLIN, M. LHERMITTE, A. BOERSMA, AND P. ROUSSEL, *Eur. J. Biochem.*, 127 (1982) 7–20.
4 R. R. SCHMIDT, *Angew. Chem.*, 98 (1986) 213–236; *Angew. Chem., Int. Ed. Engl.*, 25 (1986) 212–235.
5 (a) R. R. SCHMIDT AND G. GRUNDLER, *Angew. Chem.*, 94 (1982) 790–791; *Angew. Chem., Int. Ed. Engl.*, 21 (1982) 781–782; (b) G. GRUNDLER AND R. R. SCHMIDT, *Carbohydr. Res.*, 135 (1985) 203–218.
6 R. R. SCHMIDT AND G. GRUNDLER, *Angew. Chem.*, 95 (1983) 805–806; *Angew. Chem., Int. Ed. Engl.*, 22 (1983) 776–777.
7 G. GRUNDLER AND R. R. SCHMIDT, *Justus Liebigs Ann. Chem.*, (1984) 1826–1847.
8 (a) Z. KIM AND G. UHLENBRUCK, *Z. Immunitätsforsch.*, 130 (1966) 88–99; (b) G. F. SPRINGER, P. R. DESAI, M. S. MURTHY, AND E. F. SCANLON, *A.C.S. Symp. Ser.*, 80 (1978) 311–325; G. F. SPRINGER, P. R. DESAI, M. S. MURTHY, AND E. F. SCANLON, *J. Surg. Oncol.*, 11 (197) 95–106.
9 W. KINZY, unpublished results.
10 H. PAULSEN AND J.-P. HÖLCK, *Carbohydr. Res.*, 109 (1982) 89–107.
11 H. PAULSEN, M. PAAL, AND M. SCHULTZ, *Tetrahedron Lett.*, 24 (1983) 1759–1762; M. PAAL, Dissertation, University of Hamburg, 1983.
12 V. VEREZ BENCOMO AND P. SINAŸ, *Glycoconjugate J.*, 1 (1984) 5–8; V. VEREZ BENCOMO, J.-C. JACQUINET, AND P. SINAŸ, *Carbohydr. Res.*, 110 (1982) C9–C11.
13 B. FERRARI AND A. A. PAVIA, *Tetrahedron*, 41 (1985) 1939–1944.
14 H. KUNZ AND S. BIRNBACH, *Angew. Chem.*, 98 (1986) 354–355; *Angew. Chem., Int. Ed. Engl.*, 25 (1986) 360–361.
15 W. KINZY AND R. R. SCHMIDT, *Justus Liebigs Ann. Chem.*, (1985) 1537–1545.
16 H. VAN HALBEEK, *Abstr. Papers Eur. Symp. Carbohydr.*, 3 (1985) 40.
17 R. R. SCHMIDT, J. MICHEL, AND M. ROOS, *Justus Liebigs Ann. Chem.*, (1984) 1343–1357.
18 R. U. LEMIEUX AND R. M. RATCLIFFE, *Can. J. Chem.*, 57 (1979) 1244–1251; R. U. LEMIEUX AND R. M. RATCLIFFE, *Ger. Offen.*, 2,816,340; *Chem. Abstr.*, 90 (1979) 87,846.
19 E. BAER, D. BUCHNEA, AND H. C. STANCER, *J. Am. Chem. Soc.*, 81 (1959) 2166–2169.
20 B. WEGMANN, University of Konstanz, unpublished results.
21 H. PAULSEN AND V. SINNWELL, *Chem. Ber.*, 111 (1978) 869–889.
22 W. N. HAWORTH, E. L. HIRST, M. M. T. PLANT, AND R. J. W. REYNOLDS, *J. Chem. Soc.*, (1930) 2644–2653; P. W. KENT AND S. D. DIMITRIJEVICH, *J. Fluorine Chem.*, 10 (1977) 455–478.