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Synthesis and inhibitory activity of a di- and a trisaccharide corresponding to an erythrocyte glycolipid responsible for the nor polyagglutination

Ulrika Westerlind,[a] Per Hagback,[a] Maria Duk,[b] Thomas Norberg[a,*]

[a]*Department of Chemistry, Swedish University of Agricultural Sciences, PO Box 7015, S-750 07 Uppsala, Sweden*
[b]*Department of Immunochemistry, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, PL-53114 Wroclaw, Poland*

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Abstract

The polyagglutinable erythrocytes NOR contain unusual neutral glycolipids reactive with anti-NOR antibodies. The disaccharide α-D-Gal*p*-(1→4)-D-Gal*p*NAc and the trisaccharide α-D-Gal*p*-(1→4)-β-D-Gal*p*NAc-(1→3)-D-Gal corresponding to the non-reducing end of a NOR glycolipid (NOR1) were chemically synthesized. The syntheses were based on a common (1→4)-β-D-GalNAc precursor, and utilized benzyl glycoside and benzyl ether functions for persistent blocking of hydroxyls. The α-D-Gal*p*-(1→4)-β-D-Gal*p*NAc structural element has been found only recently in Nature, and derivatives thereof have not been synthesized before. Both the synthesized oligosaccharides inhibited specifically human anti-NOR antibodies, the trisaccharide being 300 times more active than the disaccharide.

Keywords: NOR polyagglutination; Erythrocytes; Oligosaccharide synthesis

1. Introduction

The rare NOR polyagglutination was recently shown to be associated with the occurrence on the erythrocytes of unusual neutral glycolipids.[1] The structure of one of them (NOR1) has been recently established[2] as Gal(α1→4)GalNAc(β1→3)Gal(α1→4)Gal(β1→4)Glc-Ceramide.

The non-reducing terminal end of this structure contains the unusual structural element Gal(α1→4)GalNAcβ which has hitherto only been demonstrated in NOR glycolipids and in amphibium oviductal mucins.[3] To our knowledge, compounds with this structural element have not been synthesized before. In order to gain access to material for further immunological work, a chemical synthesis of the non-reducing end di- and trisaccharide structures (**9** and **15**) corresponding to the above glycolipid was carried out. We chose to synthesize the free reducing oligosaccharides, rather than oligosaccharide derivatives with a spacer attached. Should spacer derivatives be needed in the future (e.g. for affinity purification of anti-NOR antibodies), they can be prepared from the free oligosaccharides by several methods. The preparative details of the free oligosaccharide synthesis and the inhibitory activity of the oligosaccharides are hereby reported.

2. Results and discussion

Synthesis of the oligosaccharides.—The syntheses were carried out using compounds **3**, **6** and **10** as monosaccharide building blocks. The terminal Gal and →3Gal building blocks (**6** and **10**, respectively) were prepared by slightly modified literature synthesis procedures,[4,5] whereas the →4GalNAc building block (**3**) was prepared from a corresponding glucosamine derivative using the following modified literature procedure:[6] treatment of **1**[7] with triflic anhydride gave the 4-triflate **2**, which was treated in situ with cesium acetate in *N*,*N*-dimethylformamide to produce the

* Corresponding author. Tel.: +46-18-671578; fax: +46-18-673477
E-mail address: thomas.norberg@kemi.slu.se (T. Norberg).

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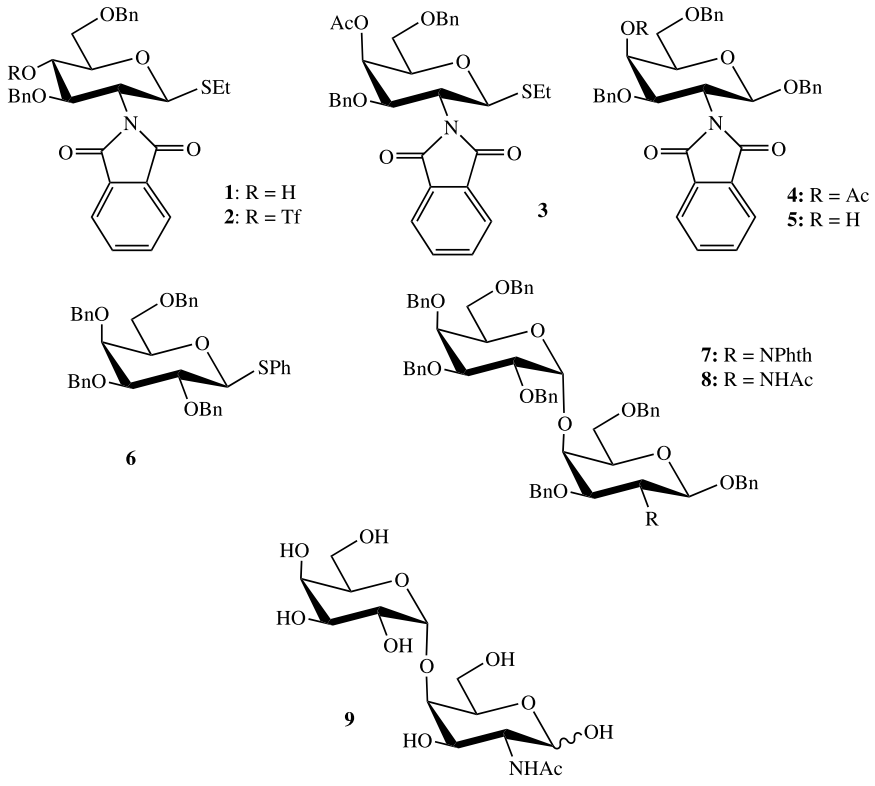


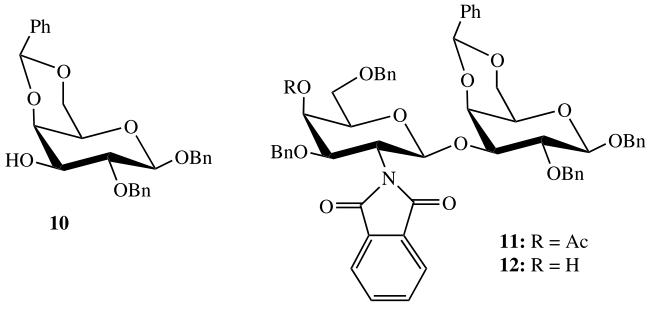
Table 1
^{1}H (upper row) and ^{13}C NMR data for **9** (coupling constants in parentheses). Comments and explanations, see experimental part, prepn. of **9**

	1	2	3	4	5	6a	6b
Gal(α)	4.96 (3.9)	3.81	3.92	3.99	4.25	3.65	3.65
	101.72	69.77	70.13	70.20	72.18	61.76	
Gal(β)	4.94 (3.9)	3.81	3.92	3.99	4.25	3.65	3.65
	101.72	69.77	70.13	70.20	72.18	61.76	
GalNAc(α)	5.22 (3.3)	4.14	3.94	4.06	4.10	3.65	3.65
	92.30	51.75	68.28	79.85	71.97	61.76	
GalNAc(β)	4.66 (8.2)	3.87	3.72	3.99	3.68	3.79	3.79
	96.70	55.95	71.97	78.29	76.09	61.54	

galactosamine derivative **3** (72% yield from **1**). Compound **3** carries a protecting group pattern suitable for 4-substitution, and was subsequently used in β-glycosylation of either benzyl alcohol or the monosaccharide **10** to give monosaccharide **4** (95% yield) or the disaccharide **11**, respectively. The latter compound was obtained only in impure form after chromatography. Final purification was, however, achieved in the next step. Compounds **4** and **11** were both deacylated in the 4-position to give compounds **5** (95%) and **12** (37%, from **3**), respectively. The latter relatively low yield of **12** was partly caused by the difficulties in chromatographic purification of **11**. However, the glycosylation yield in the reaction between **3** and **10** was consistently less than 50% (TLC estimation) during several different runs, and the reason for this is currently not clear. Compounds **5** and **12** were both α-glycosylated with phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**6**)[4] to give the disaccharide **7** and trisaccharide **13** in 66 and 70% yield, respectively. Phthalimido deprotection (hydrazine hydrate) and N-acetylation (acetic anhydride) gave **8** and **14** (93 and 97% yield), catalytic hydrogenation of which produced the free di- and trisaccharides **9** and **15** in 68 and 61% yields, respectively. The structures of both oligosaccharides was confirmed by complete assignment of the proton and carbon NMR spectra (Tables 1 and 2, respectively), as well as by high-resolution mass spectroscopy.

Table 2
^{1}H (upper row) and ^{13}C NMR data for **15** (coupling constants in parentheses). Comments and explanations, see experimental part, prepn. of **15**

	1	2	3	4	5	6a	6b
Gal(α+β)	4.96(3.7)	3.82	3.94	4.01	4.29	3.63	3.68
	101.34	69.46	69.89	69.94	71.80	61.84	
GalNAc(α)	4.70 (8.2)	3.95	3.78	4.02	3.69	3.83	3.86
	103.92	53.71	71.34	78.10	75.75	61.49	
GalNAc(β)	4.66 (8.4)	3.90	3.79	4.02	3.69	3.83	3.86
	103.92	53.71	71.34	78.10	75.75	61.49	
Gal*(α)	5.19 (3.7)	3.84	3.89	4.13	3.64	3.70	3.70
	93.20	68.33	79.83	69.94	70.91	61.67	
Gal*(β)	4.53 (7.8)	3.52	3.68	4.08	3.64	3.70	3.70
	97.34	71.92	82.96	69.32	75.46	61.17	



Inhibitory activity of the oligosaccharides.—The ability of the oligosaccharides **9** and **15** to react with anti-NOR antibodies isolated from human sera was tested by hemagglutination inhibition, using papain-treated NOR erythrocytes (Table 3). The antibodies were weakly inhibited by galactose, and 8- and 2400-times more strongly by the di- and trisaccharide, respectively. They were not inhibited by the reference disaccharide α-D-Gal*p*-(1→3)-D-Gal. To assess the specificity of these reactions, the inhibition of anti-α-D-Gal*p*-(1→3)-D-Gal human antibodies was compared. The latter antibodies were also inhibited weakly by galactose, slightly more strongly by the α-D-Gal*p*-(1→3)-D-Gal disaccharide, but were not inhibited by oligosaccharides **9** and **15** (Table 3). In conclusion, these results show that the disaccharide **9** and trisaccharide **15** are specifically recognized by anti-NOR antibodies and that the galactose reducing end of trisaccharide **15** is important for an effective binding to the antibodies.

3. Experimental

General methods.—Concentrations were performed at reduced pressure (bath temperature < 40 °C). NMR spectra were recorded for solutions in $CDCl_3$ (internal

Table 3
Inhibition of human anti-NOR and anti-Gal(α1-3)Gal antibodies by galactose and oligosaccharides

Inhibitor	Anti-NOR[a] (mM)[b]	Anti-α-D-Gal*p*-(1→3)-D-Gal[a] (mM)[b]
Galactose	25	25
α-D-Gal*p*-(1→4)-D-GalNAc*p* (**9**)	3	>50
α-D-Gal*p*-(1→4)-β-D-GalNAc*p*-(1→3)-D-Gal (**15**)	0.01	>50
α-D-Gal*p*-(1→3)-D-Gal	>100	6

[a] Anti-NOR were tested with papain-treated NOR erythrocytes, and anti-Gal(α1-3)Gal with untreated rabbit erythrocytes.
[b] Minimal concentration of inhibitor required for inhibition of four hemagglutinating units of each antibody

Me_4Si, $\delta = 0.00$) or D_2O (internal acetone, ^1H $\delta = 2.225$, ^{13}C $\delta = 30.7$) at 303 K unless otherwise stated with a Bruker DRX 400 spectrometer. Only selected NMR data are reported. Assignments were corroborated by appropriate 2D experiments. Coupling constants (in Hz) are given within brackets. In oligosaccharide derivatives, sugar protons are denotated as H-1, H-1′ or H-1″, in order of increasing distance from the reducing end. The FAB-MS spectra were recorded with a JEOL JMS-SX/SX-102A instrument. Ions were produced by a beam of Xe-atoms (6 keV), using a matrix of glycerol or *m*-nitrobenzyl alcohol. For HRMS, PEG was used as an internal standard. TLC was performed on Silica Gel F_{254} (E. Merck, Darmstadt, Germany) with detection by UV-light and by staining with 5% H_2SO_4 in EtOH. Column chromatography was performed on Matrex Silica Gel 60 Å (35–70 μm, Amicon). Molecular sieves (powdered 4 Å) were dried at 280 °C/0.5 torr overnight. Dichloromethane was distilled from P_2O_5 when necessary. Dimethyl(thiomethyl)sulfonium triflate (DMTST) was prepared[8] by mixing equimolar amounts of dimethyl disulfide and methyl triflate in dry CH_2Cl_2 and then filtering off the precipitated (fridge) crystals. The material was stored under dry nitrogen at −20 °C. The disaccharide α-D-Gal*p*-(1→3)-D-Gal was purchased from Glycorex (Lund, Sweden).

*Ethyl 4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-*D*-galactopyranoside* (**3**).—Triflic anhydride (1.2 mL, 7.0 mmol) was added dropwise during 5 min at 0 °C to a solution of **1**[7] (1.52 g, 2.85 mmol) in dry 2:1 CH_2Cl_2–pyridine (10 mL). The mixture was stirred for 1 h at 0 °C and 1.5 h at room temperature after which TLC (2:1 petroleum ether–EtOAc) indicated complete conversion. The mixture was diluted with CH_2Cl_2, washed with aq 1 M H_2SO_4, aq 1 M $NaHCO_3$, water, dried and concentrated. The residue, containing the 4-triflate **2**, was dissolved in dry DMF (7 mL) and added to a mixture of CsOAc (2.73 g, 14.2 mmol) and dry DMF (17 mL). The reaction mixture was stirred at room temperature overnight and was then partitioned between toluene and water. The organic layer was washed with water, dried and concentrated. Column chromatography (5:2 petroleum ether–EtOAc) of the residue gave **3** (1.18 g, 2.05 mmol, 72%) as a syrup. The NMR data were as published.[6]

*Benzyl 4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-galactopyranoside* (**4**).—A mixture of **3** (400 mg, 0.695 mmol), benzyl alcohol (114 mg, 1.06 mmol) and powdered 4 Å molecular sieves (2.40 g) in dry CH_2Cl_2–diethyl ether–toluene (5.3–3.8–3.8 mL) was stirred at room temperature for 5 min. Solid DMTST (567 mg, 2.20 mmol) was added in portions over 2 h and then pyridine (0.5 mL) was added to quench the reaction. The mixture was diluted with diethyl ether and the solids were filtered off. The filtrate was then washed with aq 2 M H_2SO_4, aq 1 M $NaHCO_3$, dried and concentrated. Column chromatography (5:2 petroleum ether–EtOAc) of the residue gave **4** (421 mg, 0.677 mmol, 97%) as a colorless syrup. NMR data ($CDCl_3$): ^1H, δ 5.58 (dd, 1 H, $J_{3,4}$ 3.3, $J_{4,5}$ 0.5, H-4), 5.05 (d, 1 H, $J_{1,2}$ 8.6, H-1), 4.73 (d, 1 H, J 12.3, $PhCH_2$), 4.56–4.39 (m, 4 H, $PhCH_2$), 4.36 (dd, 1 H, $J_{2,3}$ 11.0, H-2), 4.21 (dd, 1 H, H-3), 4.15 (d, 1 H, J 12.5, $PhCH_2$), 3.84 (m, 1 H, H-5), 3.59 (dd, 1 H, $J_{6a,6b}$ 9.5, $J_{6a,5}$ 5.9, H-6a), 3.53 (dd, 1 H, $J_{6b,5}$ 7.0, H-6b). [HRMS: Calc. for $C_{37}H_{35}NO_8Na$: 644.2260 ($M + Na^+$). Found: 644.2326 ($M + Na^+$)].

*Benzyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-galactopyranoside* (**5**).—To a solution of **4** (350 mg, 0.563 mmol) in dry MeOH (43 mL) was added 1 M NaOMe (2.15 mL). The mixture was stirred at room temperature for 8 h, then neutralized with Dowex-50 (H^+), and filtered. A drop of conc. aq ammonia was added and the solution was then concentrated and co-evaporated several times with EtOAc and once with toluene to give **5** (309 mg, 0.53 mmol, 95%). NMR data ($CDCl_3$): ^1H, δ 5.02 (d, 1 H, $J_{1,2}$ 8.6, H-1), 4.72 (d, 1 H, $PhCH_2$), 4.54 (s, 2 H, $PhCH_2$), 4.51 (d, 1 H, $PhCH_2$), 4.43 (dd, 1 H, $J_{2,3}$ 11.0, H-2), 4.40 (d, 1 H, $PhCH_2$), 4.20 (d, 1 H, $PhCH_2$), 4.16 (dd, 1 H, $J_{3,4}$ 3.3, H-3), 4.06 (dd, 1 H, $J_{3,4}$ 3.3, $J_{4,5}$ 0.5, H-4). [HRMS: Calc. for $C_{35}H_{33}NO_7Na$: 602.2155 ($M + Na^+$). Found: 602.2134 ($M + Na^+$)].

Benzyl O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-galactopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-galactopyranoside* (**7**).—A mixture of **5** (287 mg, 0.495 mmol), phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside **6**[4] (477 mg, 0.753 mmol) and powdered 4 Å molecular sieves (1.71 g) in dry CH_2Cl_2–diethyl ether–toluene (3.8–2.7–2.7 mL) was stirred at room temperature for 5 min. Solid DMTST (404 mg, 1.56 mmol) was added in portions over 2 h and then pyridine (360 μL) was added to quench the reaction. The mixture was diluted with diethyl ether and the solids were filtered off. The filtrate was then washed with aq 2 M H_2SO_4, aq 1 M $NaHCO_3$, dried and concentrated. Column chromatography (5:2 petroleum ether–EtOAc) of the residue gave **7** (360 mg, 0.327 mmol, 66%) as a colorless syrup. NMR data ($CDCl_3$): ^1H, δ 5.17 (d, 1 H, $J_{1,2}$ 8.6, H-1), 5.08 (d, 1 H, $J_{1,2}$ 3.1, H-1′), 4.67 (1 H, H-2), 4.28 (1 H, H-3′), 4.16 (1 H, H-2′). [HRMS: Calc. for $C_{69}H_{67}NO_{12}Na$: 1124.4561 ($M + Na^+$). Found: 1124.4530 ($M + Na^+$)].

Benzyl O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-*D*-galactopyranoside* (**8**).—Hydrazine hydrate (200 μL, 5.16 mmol) was added to a mixture of **7** (354 mg, 0.321 mmol) in 2:3 toluene–EtOH (15 mL). The mixture was refluxed for 36 h, and then cooled and evaporated. The residue was partitioned between 1:1 CH_2Cl_2–EtOH and water. The organic layer was concentrated and then

dissolved in 1:1 CH_2Cl_2–MeOH (15 mL) and treated with acetic anhydride (0.75 mL) at room temperature. After 2 h, the mixture was concentrated. Column chromatography (2:1 petroleum ether–EtOAc) gave **8** (302 mg, 0.298 mmol, 93%). NMR data ($CDCl_3$): ^{1}H, δ 5.20 (d, 1 H, $J_{NH,2}$ 7.6, NH), 4.99 (d, 1 H, $J_{1,2}$ 2.5, H-1′), 4.83 (d, 1 H, H-1), 4.03 (1 H, H-2′), 4.01 (1 H, H-4), 3.91 (1 H, H-3), 3.54 (1 H, H-2), 1.98 (CH_3CONH). [HRMS: Calc. for $C_{63}H_{67}NO_{11}Na$: 1036.4612 ($M + Na^+$). Found: 1036.4584 ($M + Na^+$)].

O-(α-D-*Galactopyranosyl*)-(1→4)-2-*acetamido*-2-*deoxy*-D-*galactopyranose* (**9**).—A suspension of Pd/C (10%, 150 mg) in EtOH (6 mL) was added to a solution of **8** (200 mg, 0.197 mmol) in EtOH (6 mL). The mixture was flushed with nitrogen and then with hydrogen and stirred overnight at rt under a hydrogen atmosphere. The solution was filtered and the filtrate concentrated and purified on a Biogel P-2 column, using 95:5 water–*n*-butanol as eluant, to give **9** (51 mg, 0.133 mmol, 68%), $[\alpha]_D$ +143° (*c* 0.6, water). [HRMS: Calc. for $C_{14}H_{26}NO_{11}$: 384.1506 ($M + H^+$). Found: 384.1540 ($M + H^+$)].

NMR data (D_2O, 50 °C) are given in Table 1. Each spectrum of **9** consists of two subspectra corresponding to (α) or (β) configurations at the reducing end. The two subspectra are distinguished by the suffixes (α) or (β). The (α + β) *N*-acetyl signals were at 1.99 and 175.80/23.00. In the Gal spin system, the α and β reducing end assignments (if unequal) may be reversed. The (1→4) linkage was evident from the δ_C increase of the substituted position as compared to an unsubstituted reference compound, as well as from 2D HMBC experiments.

Benzyl O-(*3,6-di*-O-*benzyl*-2-*deoxy*-2-*phthalimido*-β-D-*galactopyranosyl*)-(1→3)-2-O-*benzyl*-4,6-O-*benzylidene*-β-D-*galactopyranoside* (**12**).—A solution of DMTST (910 mg, 3.52 mmol) in dry CH_2Cl_2 (5 mL) was added to a stirred mixture of **3** (607 mg, 1.06 mmol), **10** (475 mg, 1.06 mmol) and molecular sieves 4 Å (3.85 g) in dry CH_2Cl_2 (10 mL) at 0 °C. The mixture was stirred for 3 h at rt and then pyridine (0.77 mL) was added. The mixture was diluted with diethyl ether and the solids were filtered off. The filtrate was then washed with aq 2 M H_2SO_4, aq 1 M $NaHCO_3$, dried, and concentrated. Column chromatography (2:1 petroleum ether–EtOAc) of the residue gave **11**, contaminated with an unknown compound. Fractions containing **11** were pooled, concentrated and dissolved in dry MeOH (43 mL), and then 1 M NaOMe (2.15 mL) was added. The mixture was stirred at room temperature for 12 h, then neutralized with Dowex-50 (H^+), and filtered. A drop of conc. ammonia (aq) was added and the solution was concentrated and the residue was purified by column chromatography (4:1 toluene–EtOAc). Appropriate homogenous fractions were pooled and concentrated to give **12** (357 mg, 0.388 mmol, 37% calculated from **10**). NMR data ($CDCl_3$): ^{1}H, δ 5.37 (d, 1 H, $J_{1,2}$ 8.5, H-1′), 5.38 (1 H, $PhCH_2$), 4.47 (1 H, H-2′), 4.16 (1 H, H-6a), 4.15 (1 H, H-H-3′), 3.78 (1 H, H-6a), 3.17 (1 H, H-5). [HRMS: Calc. for $C_{55}H_{53}NO_{12}Na$: 942.3465 ($M + Na^+$). Found: 942.3383 ($M + Na^+$)].

Benzyl O-(*2,3,4,6-tetra*-O-*benzyl*-α-D-*galactopyranosyl*)-(1→4)-O-(*3,6-di*-O-*benzyl*-2-*deoxy*-2-*phthalimido*-β-D-*galactopyranosyl*)-(1→3)-2-O-*benzyl*-4,6-O-*benzylidene*-β-D-*galactopyranoside* (**13**).—A mixture of **12** (185 mg, 0.201 mmol), **6**[4] (199 mg, 0.314 mmol) and powdered 4 Å molecular sieves (0.71 g) in dry CH_2Cl_2–diethyl ether–toluene (1.6–1.1–1.1 mL) was stirred at room temperature for 5 min. Solid DMTST (169 mg, 0.654 mmol) was added in portions for 2 h and then pyridine (150 μL) was added to quench the reaction. The mixture was diluted with diethyl ether and the solids were filtered off. The filtrate was then washed with aq 2 M H_2SO_4, aq 1 M $NaHCO_3$, dried and concentrated. Column chromatography (4:1 toluene–EtOAc) of the residue gave **13** (203 mg, 0.141 mmol, 70%). NMR data ($CDCl_3$): ^{1}H, δ 5.43 (d, 1 H, $J_{1,2}$ 8.4, H-1′), 5.31 (s, 1 H, PhCH), 4.93 (d, 1 H, $J_{1,2}$ 3.3, H-1″), 4.83 (d, 1 H, $PhCH_2$), 4.79 (d, 1 H, $PhCH_2$), 4.77 (d, 1 H, $PhCH_2$), 4.64 (1 H, H-2′), 4.62 (d, 1 H, $PhCH_2$), 4.49 (d, 1 H, $PhCH_2$), 4.45 (d, 1 H, $PhCH_2$), 4.43 (1 H, H-5″), 4.18 (1 H, H-3″), 4.14 (1 H, H-6a), 4.08 (1 H, H-3′), 4.06 (1 H, H-2″), 3.88 (dd, 1 H, $J_{5,6a}$ 5.4, $J_{6a,6b}$ 8.5, H-6′b), 3.71 (dd, 1 H, $J_{5,6a}$ 1.5, $J_{6a,6b}$ 12.1, H-6b), 3.48 (t, 1 H, *J* 9.1, H-6″a), 3.15 (s, 1 H, H-5), 3.10 (dd, 1 H, $J_{5,6a}$ 4.9, $J_{6a,6b}$ 8.8, H-6″b). [HRMS: Calc. for $C_{89}H_{87}NO_{17}Na$: 1464.5872 ($M + Na^+$). Found: 1464.5845 ($M + Na^+$)].

Benzyl O-(*2,3,4,6-tetra*-O-*benzyl*-α-D-*galactopyranosyl*)-(1→4)-O-(*2-acetamido*-3,6-*di*-O-*benzyl*-2-*deoxy*-β-D-*galactopyranosyl*)-(1→3)-2-O-*benzyl*-4,6-O-*benzylidene*-β-D-*galactopyranoside* (**14**).—Hydrazine hydrate (127 μL, 3.27 mmol) was added to a mixture of **13** (228 mg, 0.158 mmol) in 2:3 toluene–EtOH (7 mL). The mixture was refluxed for 36 h, and then cooled and evaporated. The residue was partitioned between 1:1 CH_2Cl_2–EtOH and water. The organic layer was concentrated and then dissolved in 1:1 CH_2Cl_2–MeOH (6 mL) and treated with acetic anhydride (0.35 mL) at room temperature. After 2 h, the mixture was concentrated. Column chromatography (2:1 petroleum ether–EtOAc) gave **14** (207 mg, 0.153 mmol, 97%) as a solid. NMR data ($CDCl_3$): ^{1}H, δ 5.30 (s, 1 H, PhCH), 5.13 (1 H, $J_{2,NH}$ 7.4, NH), 5.09 (1 H, $J_{1,2}$ 8.3, H-1′), 4.93 (d, 1 H, H-1″), 4.27 (1 H, 5″), 4.17 (1 H, H-6a), 4.06 (1 H, H-3′), 4.01 (1 H, H-2″), 3.75 (1 H, H-6b), 3.42 (t, *J* 8.8, H-6″a), 3.42 (1 H, H-2′), 3.17 (s, 1 H, H-5), 3.14 (dd, 1 H, $J_{5,6a}$ 5.1, $J_{6a,6b}$ 8.8, H-6″b), 1.95 (CH_3CONH). [HRMS: Calc. for $C_{83}H_{87}NO_{16}Na$: 1376.5923 ($M + Na^+$). Found: 1376.5935 ($M + Na^+$)].

O-*(α-D-Galactopyranosyl)-(1→4)*-O-*(2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1→3)*-D-*galactopyranose* (**15**).—A suspension of Pd/C (10%, 150 mg) in EtOH (6 mL) was added to a solution of **14** (110 mg, 81.2 μmol) in EtOH (6 mL). The mixture was flushed with nitrogen and then with hydrogen and stirred overnight at rt under a hydrogen atmosphere. The solution was filtered and the filtrate concentrated and purified on a Biogel P-2 column, using 95:5 water–*n*-butanol as eluant, to give **15** (27 mg, 49.5 μmol, 61%), $[\alpha]_D$ +101° (*c* 0.3, water). [HRMS: Calc. for $C_{20}H_{36}NO_{16}$: 546.2034 ($M+H^+$), $C_{20}H_{35}NO_{16}Na$: 568.1854 ($M+Na^+$). Found: 546.2061 ($M+H^+$), 568.1876 ($M+Na^+$)].

NMR data (D_2O, 55 °C) are given in Table 2. Each spectrum of **15** consists of two subspectra corresponding to (α) or (β) configurations at the reducing end. The two subspectra are distinguished by the suffixes (α) or (β). Gal* denotes the reducing end Gal. The (α + β) *N*-acetyl signals were at 1.998 and 175.99/23.06. In spin systems one or more rings away from the reducing end, the α and β reducing end assignments (if unequal) may be reversed. The (1→4), (1→3) linkage pattern was evident from the δ_C increase of the substituted position as compared to unsubstituted reference compounds, as well as from 2D HMBC experiments.

Inhibition assay.—Anti-NOR and anti-α-D-Gal*p*-(1→3)-D-Gal antibodies were isolated from human sera by affinity procedures.[2] The NOR erythrocytes were pretreated with papain to enhance the agglutination.[1] The aliquots of antibodies diluted to a titer 4 were mixed with equal volumes of serially diluted oligosaccharide solutions. The agglutinating activity of these samples was determined in U-shaped microtiter plates, using 2% suspensions of NOR (for anti-NOR) or rabbit erythrocytes (for anti-α-D-Gal*p*-(1→3)-D-Gal), as already described.[1]

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