41. Oligosaccharides Related to Tumor-Associate Antigens

Part 3[1])

Synthesis of the Propyl Glycosides of the Trisaccharide β-D-Galp-(1→3)-β-D-GalpNAc-(1→3)-α-D-Galp and of the Tetrasaccharide α-L-Fucp-(1→2)-β-D-Galp-(1→3)-β-D-GalpNAc-(1→3)-α-D-Galp, Components of a Tumor Antigen Recognized by the Antibody MBr1

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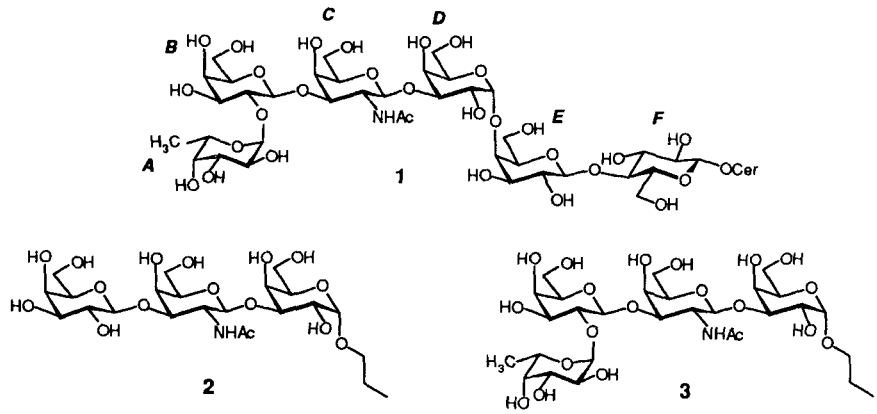
The synthesis of the trisaccharide β-D-Galp-(1→3)-β-D-GalpNAc-(1→3)-α-D-Galp-1-OPr (**2**) and of the tetrasaccharide α-L-Fucp-(1→2)-β-D-Galp-(1→3)-β-D-GalpNAc-(1→3)-α-D-Galp-1-OPr (**3**), starting from the disaccharidic dihydrooxazole donor **5**, is described. Glycosylation of **5** with **6** in the presence of Me_3SiOTf gave the trisaccharide **7** which was deprotected with standard methods to give, *via* **8**, compound **2** (*Scheme 1*). Alternatively, protection of **8** as the 4′,6′-*O*-benzylidene derivative **9** followed by glycosylation with **10** and by standard deprotection afforded the tetrasaccharide **3** (*Scheme 2*). Biological testing showed that trisaccharide **2** is unable to inhibit the binding of the monoclonal antibody MBr1 to the target tumor cells MCF7, while tetrasaccharide **3** inhibits the binding in *ca.* 7-fold extent with respect to the previously tested trisaccharide α-L-Fucp-(1→2)-β-D-Galp-(1→3)-β-D-GalpNAc-1-OPr. These results indicate that the galactose corresponding to the unit *D* of compound **1** plays an important role in defining the MBr1-recognized epitope and confirm the essential role of fucose for MAb recognition.

Introduction. – In a previous paper [2], we described the synthesis of the trisaccharide α-L-Fucp-(1→2)-β-D-Galp-(1→3)-β-D-GalpNAc-1-OPr which corresponds to the units *A*-*B*-*C* of the glycosphingolipid globo-H, α-L-Fucp-(1→2)-β-D-Galp-(1→3)-β-D-GalpNAc-(1→3)-α-D-Galp-(1→4)-β-D-Galp-(1→4)-β-D-Glcp-(1→1)Cer (**1**), overexpressed by breast-cancer cells [3]. Preliminary biological results revealed that the trisaccharide *A*-*B*-*C* was able to inhibit the binding of the monoclonal antibody MBr1 to the target tumor cells (line MCF7) in a specific and dose-dependent manner [2].

To get more detailed information of the MBr1-defined epitope, we decided to synthesize two other fragments of the globo-H hexasaccharide. The first one was the trisaccha-

[1]) Part 2: [1].

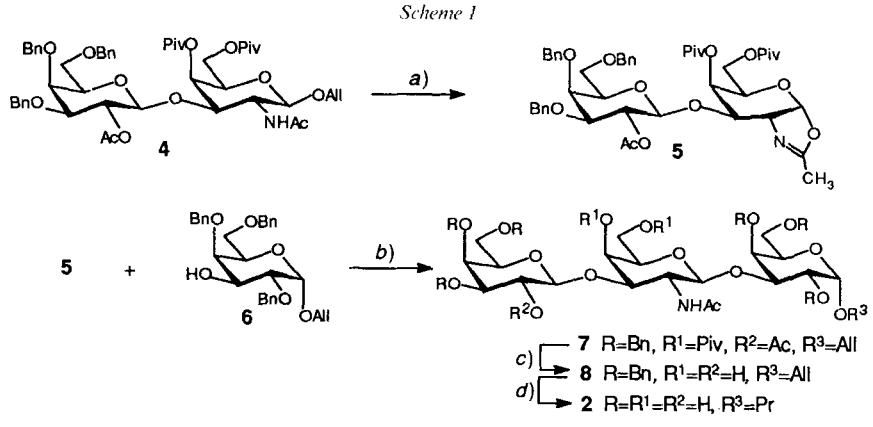


ride β-D-Gal*p*-(1→3)-β-D-Gal*p*NAc(1→3)-α-D-Gal*p*-1-OPr (**2**), corresponding to the units *B-C-D* of **1**, and the second one was the tetrasaccharide α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-β-D-Gal*p*NAc-(1→3)-α-D-Gal*p*-1-OPr (**3**), corresponding to the units *A-B-C-D* of **1**.

Results and Discussion. – The synthesis of the trisaccharide **2** (*Scheme 1*) and of the tetrasaccharide **3** (*Scheme 2*) was effected starting from the disaccharidic dihydrooxazole (oxazoline) donor **5** which, in turn, was derived from the previously obtained disaccharide **4** [2]. Although the dihydrooxazole method was employed successfully only in some cases in oligosaccharide synthesis [4], we decided to follow this approach for two reasons: *1*) the presence of the allyl group in the anomeric position of **4** allows an easy access to the corresponding dihydrooxazole [5]; *2*) the glycosylation affords a product already containing the desired 2-acetamido group on the unit *C*, so avoiding further manipulations.

Thus, 1-*O*-allyl-disaccharide **4** was converted into the dihydrooxazole **5** by catalytic isomerization of the allyl group with (cycloocta-1,5-diene)bis(methyldiphenylphosphine)-iridium hexafluorophosphate [6] followed by treatment with I_2 and 1,8-diazabi-

Scheme 1

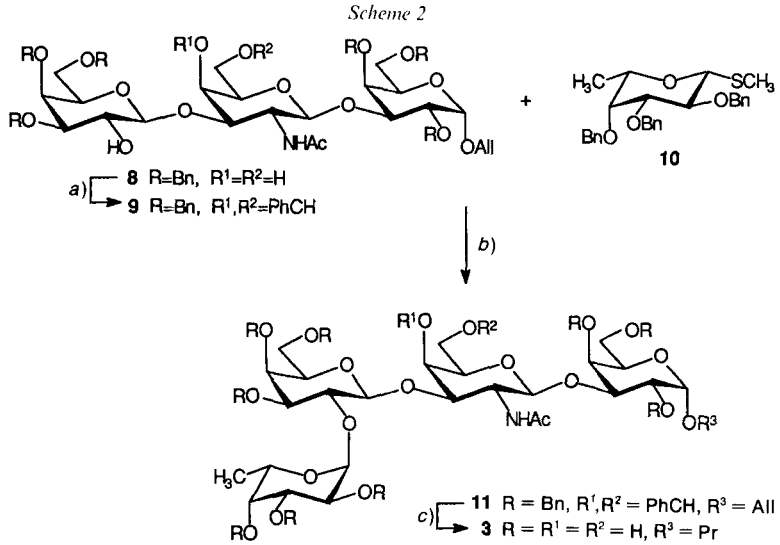


a) [Ir(cod)(PMePH$_2$)$_2$]PF$_6$ cat., H$_2$, THF, r.t., 2 h; then I$_2$, DBU, THF, r.t., 2 h; 53%. *b*) Me$_3$SiOTf, CH$_2$Cl, 40°, 5 days; 63%. *c*) MeONa in MeOH, r.t., 2 days; 78%. *d*) H$_2$, Pd/C, MeOH r.t., 2 days; quant.

cyclo[5.4.0]undec-7-ene (DBU) [5] (*Scheme 1*). Compound **5** was coupled with allyl 2,4,6-tri-*O*-benzyl-α-D-galactopyranoside (**6**) [7] following a modified literature procedure [8]. In fact, the suggested procedure (see [8]) gave a complex mixture; however, the use of an excess of trimethylsilyl trifluoromethanesulfonate (Me_3SiOTf) gave the trisaccharide **7** (63% yield). The $J(1',2')$ value (8.3 Hz) clearly established the β-configuration of the newly formed glycosidic bond. Conventional deprotection of **7** (MeONa in MeOH then H_2, Pd/C) afforded, *via* **8**, the trisaccharide **2** corresponding to the units *B-C-D* of the glycosphingolipid globo-H. The $^1H,^1H$-coupling constants between the anomeric protons and the corresponding vicinal H-atom further confirms the anomeric configurations of **2** ($J(1,2) = 3.5$ Hz, $J(1',2') = 8.5$ Hz, $J(1'',2'') = 7.5$ Hz).

Attempts to selectively de-*O*-acetylate compound **7** with guanidine following a procedure previously described for the preparation of the trisaccharide α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-β-D-Gal*p*NAc-1-OPr (see [2]) gave the desired compound with the free OH group at C(2″) in unsatisfactory yield (< 20%). To overcome this difficulty, we exploited compound **8** in which the positions 4′ and 6′ were first protected as benzylidene derivative by treatment with benzaldehyde and $ZnCl_2 \cdot OEt_2$ complex to give the desired glycosyl acceptor **9** in 71% yield (*Scheme 2*).

Scheme 2



a) PhCHO, $ZnCl_2 \cdot OEt_2$, CH_2Cl_2, r.t., 4 h; 71%. *b*) NIS, TfOH cat., CH_2Cl_2/Et_2O, 0°, 2 h; 83%. *c*) H_2, $Pd(OH)_2/C$, MeOH, r.t., 24 h; quant.

Fucosylation of **9** was effected using an excess of methyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside (**10**) [9], *N*-iodosuccinimide (NIS), and catalytic triflic acid as promoter [10] to give the tetrasaccharide **11** in satisfactory yield (83%). Compound **11** was finally deprotected by catalytic hydrogenolysis using *Pearlman*'s catalyst in MeOH to afford the desired tetrasaccharide **3**. The anomeric configurations were confirmed by the $^1H,^1H$-coupling constants of the anomeric proton and the corresponding vicinal H-atom of **3** ($J(1,2) = 3.7$ Hz, $J(1',2') = 7.5$ Hz, $J(1'',2'') = 7.5$ Hz, $J(1''',2''') = 4.0$ Hz).

Biological Results. – Biological assays revealed that the defucosylated trisaccharide **2** is completely unable to affect MBr1 binding to the relevant target cell MCF7, which confirms the essential role of fucose for MAb recognition [3]. The tetrasaccharide **3** successfully inhibits MBr1 binding to the same cell line in a dose-dependent manner, its 50% inhibitory concentration IC_{50} is 7-fold lower than that of the previously reported [2] trisaccharide α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-β-D-Gal*p*NAc-1-OPr. These data suggest that the inner galactose residue plays an important role in defining the MBr1-recognized epitope.

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Experimental Part

General. Reagents and dry solvents were added *via* oven-dried syringes through septa. Thin-layer chromatography (TLC): *Merck* silica gel *60* F_{254} plates; detection by spraying with a 1:1 mixture of 20% H_2SO_4 soln. and a soln. of I_2 (10 g) and KI (100 g) in H_2O (500 ml) followed by heating. Flash column chromatography (FC): *Merck* silica gel *60* (230–400 mesh). M.p.: *Büchi* apparatus; uncorrected. Specific rotations ($[\alpha]_D$): *Perkin-Elmer-241* polarimeter at 20°. ^{1}H- and ^{13}C-NMR Spectra: *Bruker-AC-300* or *Bruker-AM-500* instrument; δ's for the spectra in D_2O (0.04M soln. at 303 K) are referenced to HDO at 4.55 ppm.

3-O-(2-O-Acetyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-2-amino-2-deoxy-4,6-di-O-pivaloyl-1α-O:2-N,N-(ethan-1-yl-1-ylidene)-D-galactopyranose (**5**). To a soln. of 500 mg (0.55 mmol) of **4** in 20 ml of dry THF, a catalytic amount of (cycloocta-1,5-diene)bis(methyldiphenylphosphine)iridium hexafluorophosphate was added. The soln. was degassed and left *ca.* 1 min under H_2 until the orange colour turned yellow. The soln. was then degassed again and left under N_2 for 2 h. Evaporation of the solvent and filtration through SiO_2 (hexane/AcOEt 4:6) gave 479 mg (96%) of the prop-1-enyl derivative. The obtained compound (479 mg, 0.52 mmol) was dissolved in 20 ml of dry THF under N_2. Powdered 4 Å molecular sieves, 206 mg (0.81 mmol) of I_2, and 162 μl (1.1 mmol) of DBU were added. After 2 h, the mixture was diluted with CH_2Cl_2 and filtered on *Celite*. The filtrate was washed with 10% $Na_2S_2O_3$ soln. and H_2O, dried (Na_2SO_4), and evaporated. FC (SiO_2, hexane/AcOEt 1:1) of the residue afforded 236 mg of **5** (53%, 71% based on recovered starting material). Syrup. $[\alpha]_D = +35.1$ ($c = 2$, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$): 7.4–7.1 (*m*, 15 arom. H); 5.86 (*d*, $J = 5.6$, H–C(1)); 5.4–5.2 (*m*, H–C(4), H–C(2′)); 4.75 (*d*, $J = 7.8$, H–C(1′)); 5.0–4.3 (*m*, 6 H, PhCH_2); 4.2–4.0 (*m*, 3 H); 3.93 (*d*, $J = 2.4$, H–C(4′)); 3.9–3.8 (*m*, 2 H); 3.7–3.5 (*m*, 4 H); 2.02, 1.97 (2*s*, 2 Me); 1.18, 1.16 (2*s*, 2 *t*-BuCO). ^{13}C-NMR (75 MHz, $CDCl_3$): 178.7 (*s*, CO); 177.3 (*s*, CO); 169.8 (*s*, CO); 165.9 (*s*, CN); 139.2 (*s*); 138.6 (*s*, 2 C); 129.1–128.0 (*m*, arom. CH); 101.8 (*d*); 100.7 (*d*); 80.8 (*d*); 76.2 (*d*); 75.0 (*t*); 74.1 (*t*); 73.9 (*d*); 73.3 (*d*); 72.5 (*t*); 72.0 (*d*); 71.4 (*d*); 69.0 (*t*); 67.6 (*d*, 2 C); 62.5 (*t*); 39.6 (*s*, Me_3C); 39.3 (*s*, Me_3C); 27.7 (*q*, Me_3C); 21.6 (*q*, *Me*CO); 15.0 (*q*, *Me*CO). Anal. calc. for $C_{47}H_{59}NO_{13}$ (845.98): C 66.73, H 7.03, N 1.66; found: C 66.51, H 7.27, N 1.49.

Allyl 3-O-[2-Acetamido-3-O-(2-O-acetyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-2-deoxy-4,6-di-O-pivaloyl-β-D-galactopyranosyl]-2,4,6-tri-O-benzyl-α-D-galactopyranoside (**7**). To a mixture of **5** (220 mg, 0.26 mmol), **6** (132 mg, 0.27 mmol), and powdered 4 Å molecular sieves in dry CH_2Cl_2 (20 ml) under N_2, Me_3SiOTf (60 μl, 0.33 mmol) was added. The mixture was refluxed for 5 days and portions of 60 μl each of Me_3SiOTf were added every 24 h. The mixture was neutralized by adding 5% $NaHCO_3$ soln. and filtered through *Celite*. The filtrate was washed with 5% $NaHCO_3$ soln. and H_2O, dried (Na_2SO_4), and evaporated. FC (SiO_2, hexane/AcOEt 6:4) of the residue afforded 221 mg of **7** (63%). Foam. $[\alpha]_D = +30.5$ ($c = 1.5$, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$): 7.5–7.2 (*m*, 30 arom. H); 5.88 (*m*, CH_2=C*H*CH$_2$); 5.48 (*d*, $J = 7.1$, NH); 5.32 (*d*, $J = 3.2$, H–C(4′)); 5.3–5.1 (*m*, 3 H, H–C(2″), CH_2=CHCH$_2$); 5.07 (*d*, $J = 8.3$, H–C(1′)); 4.78 (*d*, $J = 3.7$, H–C(1)); 5.0–4.3 (*m*, 13 H, PhCH_2, H–C(3′)); 4.42 (*d*, $J = 7.9$, H–C(1″)); 4.2–3.8 (*m*, 10 H); 3.7–3.2 (*m*, 7 H); 1.95, 1.63 (2*s*, 2 Ac); 1.19, 1.16 (2*s*, 2 *t*-BuCO). ^{13}C-NMR (75 MHz, $CDCl_3$): 178.5 (*s*, CO); 177.6 (*s*, CO); 171.0 (*s*, CO); 169.9 (*s*, CO); 139.7 (*s*); 139.5 (*s*, 2 C); 139.2 (*s*); 138.5 (*s*, 2 C); 134.5 (*d*, CH_2=*C*HCH$_2$); 129–128 (*m*, arom. CH); 118.4 (*t*, CH_2=CHCH$_2$); 101.9 (*d*, 2 C, C(1′), C(1″)); 96.8 (*d*, C(1)); 80.7 (*d*); 79.9 (*d*); 77.8 (*d*); 76.3 (*d*); 75.1 (*t*); 75.0 (*t*); 74.2 (*t*); 73.9 (*d*); 73.7 (*t*); 73.5 (*t*); 73.0 (*d*); 72.4 (*t*); 72.2 (*d*); 71.9 (*d*); 70.2 (*t*); 70.0 (*d*); 69.6 (*d*); 69.1 (*d*); 68.8 (*t*, 2 C); 63.7 (*t*); 55.9 (*d*, C(2′)); 39.6 (*s*, Me_3C); 39.3 (*s*, Me_3C); 27.7 (*q*, Me_3C); 24.1 (*q*, Me_3CO); 21.7 (*q*, *Me*CO). Anal. calc. for $C_{77}H_{93}NO_{19}$ (1336.58): C 69.19, H 7.01, N 1.05; found: C 68.94, H 7.22, N 1.01.

Allyl 3-O-[2-Acetamido-2-deoxy-3-O-(3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-β-D-galactopyranosyl]-2,4,6-tri-O-benzyl-α-D-galactopyranoside (**8**). Compound **7** (207 mg, 0.155 mmol) was dissolved in 8 ml of MeOH containing a catalytic amount of MeONa under N_2. After 2 days at 28°, the mixture was neutralized with *Amberlite IR-120* and filtered and the solvent evaporated. FC (SiO_2, hexane/AcOEt 2:8) of the residue afforded 135 mg (78%) of **8**. Foam. $[\alpha]_D = +10.1$ ($c = 1.2$, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$): 7.5–7.1 (*m*, 30 arom. H); 5.88 (*m*, CH_2=C*H*CH_2); 5.52 (br. *d*, NH); 5.3–5.1 (*m*, 2H, CH_2=CHCH_2); 5.0–4.3 (*m*, 14H, PhCH_2, H–C(1), H–C(1′)); 4.20 (*d*, $J = 7.5$, H–C(1″)); 4.15–3.70 (*m*, 13H); 3.6–3.3 (*m*, 7H); 3.05 (br. *s*, OH); 2.91 (br. *s*, OH); 2.16 (br. *s*, OH); 1.71 (*s*, Ac). ^{13}C-NMR (75 MHz, $CDCl_3$): 172.2 (*s*, CO); 139.5 (*s*); 138.9 (*s*, 2C); 138.6 (*s*); 134.4 (*d*, CH_2=*C*HCH$_2$); 129.2–128.2 (*m*, arom. CH); 118.7 (*t*, *C*H$_2$=CHCH$_2$); 105.6 (*d*, C(1″)); 102.7 (*d*, C(1′)); 96.5 (*d*, *C*(1)); 82.1 (*d*); 81.5 (*d*); 79.2 (*d*); 77.4 (*d*); 77.0 (*d*); 75.3 (*t*, 2C); 74.5 (*d*, 2C); 74.0 (*t*); 73.4 (*t*); 73.2 (*t*); 71.9 (*d*); 70.1 (*d*, 2C); 69.9 (*t*, 2C); 69.5 (*t*); 69.2 (*d*); 69.0 (*t*); 63.1 (*t*); 53.9 (*d*, C(2′)); 24.3 (*q*, *Me*CO). Anal. calc. for $C_{65}H_{75}NO_{16}$ (1126.31): C 69.32, H 6.71, N 1.24; found: C 69.04, H 6.92, N 1.15.

Propyl 3-O-[2-Acetamido-2-deoxy-3-O-(β-D-galactopyranosyl)-β-D-galactopyranosyl]-α-D-galactopyranoside (**2**). To a soln. of **8** (31 mg, 0.027 mmol) in 2 ml of MeOH, 2 mg of 10% Pd/C were added, and the mixture, after stirring under H_2 for 2 days (TLC CH_2Cl_2/MeOH 2:8), was filtered through *Celite* and the solvent evaporated. The residue was dissolved in H_2O and then lyophilized: 16 mg (quant.) of **2**. White solid. M.p. 158–160°. $[\alpha]_D = +69.0$ ($c = 0.6$ MeOH). ^{1}H-NMR (500 MHz, D_2O): 4.73 (*d*, $J = 3.5$, H–C(1)); 4.52 (*d*, $J = 8.5$, H–C(1′)); 4.26 (*d*, $J = 7.5$, H–C(1″)); 4.00 (br. *d*, $J = 3.0$, H–C(4)); 3.99 (br. *d*, $J = 3.0$, H–C(4′)); 3.86 (*dd*, $J = 1.5$, 3.0, H–C(2′)); 3.8–3.4 (*m*, 15H); 3.35 (*dd*, $J = 10.0$, 7.5, H–C(2″)); 3.31 (*m*, 1H of MeCH$_2$CH_2); 1.85 (*s*, Ac); 1.44 (*m*, MeCH_2CH$_2$); 0.74 (*t*, $J = 7.5$, *Me*CH$_2$CH$_2$). ^{13}C-NMR (125 MHz, D_2O): 176.0 (*s*, CO); 105.7 (*d*, C(1″)); 103.7 (*d*, C(1′)); 99.3 (*d*, C(1)); 80.6 (*d*); 80.1 (*d*); 75.9 (*d*); 75.6 (*d*); 73.5 (*t*); 71.6 (*d*); 71.4 (*d*); 70.9 (*d*); 70.2 (*d*); 69.6 (*d*); 68.9 (*d*); 68.4 (*d*); 62.1 (*t*); 61.9 (*t*, 2C); 52.5 (*d*, C(2′)); 23.3 (*t*, CH$_2$CH_2Me); 23.0 (*q*, *Me*CO); 10.9 (*q*, CH$_2$CH$_2$*Me*). Anal. calc. for $C_{23}H_{41}NO_{16}$ (587.58): C 47.02, H 7.03, N 2.38; found: C 46.86, H 7.23, N 2.21.

Allyl 3-O-[2-Acetamido-4,6-O-benzylidene-2-deoxy-3-O-(3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-β-D-galactopyranosyl]-2,4,6-tri-O-benzyl-α-D-galactopyranoside (**9**). To a soln. of **8** (92 mg, 0.0816 mmol) in 1.5 ml of freshly distilled benzaldehyde, 300 µl of $ZnCl_2 \cdot OEt_2$ complex (2.2M in CH_2Cl_2) were added. After 4 h, the mixture was diluted with CH_2Cl_2, washed with brine and H_2O, dried (Na_2SO_4), and evaporated. The residue was treated with toluene and evaporated many times to eliminate most of the benzaldehyde. FC (SiO_2, hexane/AcOEt 3:7→1:9) afforded 70 mg (71%) of **9**. Foam. $[\alpha]_D = +30.3$ ($c = 1.0$, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$): 7.5–7.1 (*m*, 35 arom. H); 5.88 (*m*, CH_2=C*H*CH$_2$); 5.51 (*d*, $J = 7.3$, NH); 5.49 (*s*, PhC*H*); 5.3–5.1 (*m*, 2H, CH_2=CHCH$_2$); 5.07 (*d*, H–C(1′), 1H of PhCH_2); 5.0–4.3 (*m*, 11 H, PhCH_2); 4.78 (*d*, $J = 3.6$, H–C(1)); 4.3–4.05 (*m*, H–C(1″), H–C(3), H–C(4), H–C(3′), 1H of CH$_2$=CHCH_2, 1H); 4.05–3.8 (*m*, H–C(2), H–C(2′), H–C(2″), H–C(4″), 1H of CH$_2$=CHCH_2, 3H); 3.6–3.4 (*m*, 6H); 3.34 (*dd*, $J = 7.9$, 2.7, H–C(3″)); 2.56 (*s*, OH); 1.68 (*s*, Ac). ^{13}C-NMR (75 MHz, $CDCl_3$): 172.2 (*s*, CO); 139.7 (*s*); 139.2 (*s*, 2C); 139.1 (*s*); 138.8 (*s*); 138.6 (*s*, 2C); 134.6 (*d*, CH_2=*C*HCH$_2$); 129.4–127.2 (*m*, arom. *C*H); 118.5 (*t*, *C*H$_2$=CHCH$_2$); 105.9 (*d*, PhC*H*); 102.3 (*d*); 101.7 (*d*); 96.9 (*d*, C(1)); 82.4 (*d*); 79.9 (*d*); 77.9 (*d*); 77.7 (*d*); 76.4 (*d*, 2C); 75.5 (*t*); 75.2 (*t*); 74.5 (*d*); 74.2 (*d*); 74.1 (*t*); 73.9 (*t*); 73.4 (*t*); 73.3 (*t*); 71.7 (*d*); 70.0 (*d*) ; 69.9 (*t*); 69.7 (*t*, 2C); 68.9 (*t*); 67.0 (*d*); 54.7 (*d*, C(2′)); 24.2 (*q*, *Me*CO). Anal. calc. for $C_{72}H_{79}NO_{16}$ (1214.42): C 71.21, H 6.56, N 1.15; found: C 71.08, H 6.73, N 1.08.

Allyl 3-O-{2-Acetamido-4,6-O-benzylidene-2-deoxy-3-O-[2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-3,4,6-tri-O-benzyl-β-D-galactopyranosyl]-β-D-galactopyranosyl}-2,4,6-tri-O-benzyl-α-D-galactopyranoside (**11**). To a mixture of 61 mg (0.050 mmol) of **9**, 70 mg (0.151 mmol) of **10** and powdered 4 Å molecular sieves in CH_2Cl_2/Et_2O 1:1 (1 ml) at 0° under N_2 was added a soln. of NIS (34 mg) and TfOH (5 µl) in CH_2Cl_2/Et_2O 1:1 (3 ml). After 2 h, the mixture was neutralized by adding 5% $NaHCO_3$ soln. and filtered through *Celite*. The filtrate was washed with 20% $Na_2S_2O_3$ soln., 5% $NaHCO_3$ soln., and H_2O, dried (Na_2SO_4), and evaporated. FC (SiO_2, hexane/AcOEt 7:3→4:6) of the residue afforded 69 mg (83%) of **11**. Syrup. $[\alpha]_D = -27.7$ ($c = 1.0$, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): 7.5–7.1 (*m*, 50 arom. H); 5.85 (*m*, CH_2=C*H*CH$_2$); 5.83 (*d*, $J = 7.2$, NH); 5.59 (*d*, $J = 3.7$, H–C(1‴)); 5.50 (*s*, PhC*H*); 5.3–5.1 (*m*, 2H of CH_2=CHCH$_2$, 1H of PhCH_2); 5.03 (*d*, $J = 8.1$, H–C(1′)); 4.85–4.25 (*m*, 18H, H–C(1)); H–C(1″), PhCH_2); 4.25–4.10 (*m*, H–C(3′), H–C(2″), H–C(4), H–C(5‴), 1H of CH$_2$=CHCH_2, 2H); 4.08 (*dd*, $J = 9.8$, 3.5, H–C(3)); 3.99 (*m*, H–C(2′)); 3.95–3.88 (*m*, H–C(2‴), H–C(2), 1H of CH$_2$=CHCH_2, 2H); 3.86 (*d*, $J = 2.3$, H–C(4″)); 3.81 (*dd*, $J = 10.2$, 2.4, H–C(3‴)); 3.60–3.45 (*m*, H–C(3″), H–C(4‴), 4H); 3.4–3.3 (*m*, H–C(4′), 1H); 1.67 (*s*, Ac); 0.78 (*d*, $J = 6.3$, 3 H–C(6‴)). ^{13}C-NMR (75 MHz, $CDCl_3$): 171.1 (*s*, CO); 139.8 (*s*); 139.6 (*s*); 139.1 (*s*); 138.8 (*s*); 138.7 (*s*); 138.5 (*s*); 134.7 (*d*, CH_2=*C*HCH$_2$); 129.2–127.2 (*m*, arom. CH); 118.4 (*t*, *C*H$_2$=CHCH$_2$); 103.5 (*d*); 103.1 (*d*); 101.7 (*d*); 97.4 (*d*); 97.2 (*d*); 84.5 (*d*); 80.1 (*d*); 79.9 (*d*); 78.6 (*d*); 77.9 (*d*); 76.6 (*d*); 76.4 (*d*); 76.3 (*d*); 75.4 (*t*, 2C); 75.1 (*t*); 74.9 (*d*); 74.3 (*d*); 74.1 (*t*); 73.9 (*t*); 73.7 (*t*); 73.4 (*d*, 2C); 73.1 (*t*); 73.0 (*t*); 72.5 (*t*); 70.1 (*t*); 70.0 (*d*); 69.8 (*t*); 69.6 (*t*); 68.9 (*t*); 67.3 (*d*); 67.0 (*d*); 54.2 (*d*, C(2′)); 24.2 (*q*, *Me*CO); 16.9 (*q*, C(6‴)). Anal. calc. for $C_{99}H_{107}NO_{20}$ (1630.93): C 72.91, H 6.61, N 0.86; found: C 71.68, H 6.76, N 0.83.

Propyl 3-O-{2-Acetamido-2-deoxy-3-O-[2-O-(α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-galactopyranosyl}-α-D-galactopranoside (**3**). To a soln. of **11** (34 mg, 0.021 mmol) in 3 ml of MeOH, 50 mg of 10% $Pd(OH)_2/C$ were added, and the mixture was stirred 24 h under H_2 (TLC AcOEt/MeOH/H_2O 4:2:1). The mixture was filtered through *Celite*, the solvent evaporated, and the residue dissolved in H_2O and then lyophilized: 16 mg (quant.) of **3**. White solid. M.p. 238–240° (dec.). $[\alpha]_D = +27.9$ ($c = 1.0$, H_2O). ^{1}H-NMR (500 MHz, D_2O): 5.05 (*d*, $J = 4.0$, H–C(1‴)); 4.71 (*d*, $J = 3.7$, H–C(1)); 4.44 (*d*, $J = 7.5$, H–C(1″)); 4.37 (*d*, $J = 7.5$, H–C(1′)); 4.06 (br. *q*, $J = 6.5$, H–C(5‴)); 4.01 (br. *d*, $J = 3.5$, H–C(4)); 3.93 (br. *d*, $J = 2.5$, H–C(4′)); 3.8–3.4 (*m*, 20H); 3.31 (*m*, 1H of $MeCH_2CH_2$); 1.86 (*s*, Ac); 1.45 (*m*, $MeCH_2CH_2$); 1.04 (*d*, $J = 6.5$, 3 H–C(6‴)); 0.75 (*t*, $J = 7.5$, $MeCH_2CH_2$). ^{13}C-NMR (75 MHz, D_2O): 177.3 (*s*, CO); 106.9 (*d*); 105.2 (*d*); 102.4 (*d*); 101.5 (*d*); 82.1 (*d*); 79.8 (*d*); 79.4 (*d*); 78.3 (*d*); 77.9 (*d*); 76.9 (*d*); 75.1 (*d*); 73.5 (*d*); 73.2 (*t*, $MeCH_2CH_2$); 72.8 (*d*); 72.6 (*d*); 72.4 (*d*); 71.7 (*d*); 71.4 (*d*); 70.8 (*d*); 70.0 (*d*); 64.2 (*t*, 3C); 54.9 (*d*, C(2′)); 25.6 (*q*, *Me*CO); 25.2 (*t*, $MeCH_2CH_2$); 18.5 (*q*, C(6‴)); 13.1 (*q*, $MeCH_2CH_2$). Anal. calc. for $C_{29}H_{51}NO_{20}$ (733.72): C 47.47, H 7.01, N 1.91; found: C 47.18, H 7.26, N 1.83.

Evaluation of the Biological Activity. The effect of **2** and **3** on MBr1 binding to live MCF7 cells was tested by an indirect immunofluorescence assay as previously described [2]. Briefly, MBr1 (1 nM) was incubated with serial dilution of each oligosaccharide (800 μM to 2 μM) in phosphate-buffered saline +0.03% BSA (bovine-serum albumine) for 1 h at 0°. The mixture was transferred on suspended MCF7 cells and incubated for 30 min at 0°. The cells were then washed 3 times in phosphate-buffered saline +0.03% BSA and further incubated for 30 min at 0° with fluorescein-conjugated goat anti-mouse IgM (*Kpl*, Gaithersburg, Maryland, USA). The bound fluorescence was evaluated by cytofluorimetric analysis using *FacScan* (*Beckton Dickinson*). The amount of **3** required to induce 50% inhibition of MBr1 binding to the target cells (IC_{50}) was 9 μM (mean of 5 experiments), while **2** failed to affect MAb binding to the same cells even at the maximum concentration tested (800 μM). A comparison of **3** and the previously published trisaccharide [2] was performed; the IC_{50} of the trisaccharide was 66 μM (mean of 5 experiments).

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