46. Oligosaccharides Related to Tumor-Associated Antigens

Part I

Synthesis of the Propyl Glycoside of the Trisaccharide α-L-Fuc*p*-(1 → 2)-β-D-Gal*p*-(1 → 3)-β-D-Gal*p*NAc, Component of a Tumor Antigen Recognized by the Antibody MBr1

by **Luigi Lay, Francesco Nicotra, Luigi Panza**, and **Giovanni Russo***

Dipartimento di Chimica Organica e Industriale,
Centro per lo Studio delle Sostanze Organiche Naturali del CNR, via Venezian 21, I-20133 Milano

and **Elena Adobati**

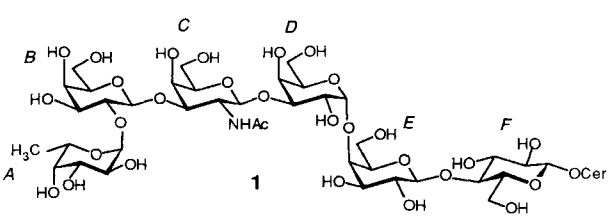
Divisione di Oncologia Sperimentale 'E' – Istituto Nazionale dei Tumori, via Venezian 1, I-20133 Milano

(4.X.93)

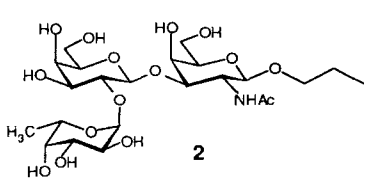
The synthesis of the trisaccharide α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-β-D-Gal*p*NAc-1-OPr (**2**) is described. The *N*-acetylgalactosamine **6** was obtained from **4** by an intramolecular displacement of a (trifluoromethyl)sulfonyloxy by a pivaloyloxy group with its concomitant migration from position 3 to position 4 (*Scheme 1*). The galactosyl donor **9** was obtained from **7** *via* **8** by regioselective opening of the orthoester function with AcOH/pyridine followed by treatment with CCl_3CN and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (*Scheme 2*). Glycosylation of **6** with **9** in the presence of $BF_3 \cdot OEt_2$ gave the disaccharide **10**. Selective deprotection of **10** at *O*–C(2') followed by glycosylation with **12** and by standard deprotection afforded the title trisaccharide **2** (*Scheme 3*). Preliminary biological testing showed that **2** is able to inhibit the binding of the monoclonal antibody MBr1 to the target tumor celles MCF7 in a dose-dependent manner.

Introduction. – Tumor cells show aberrant glycosylation of glycosphingolipids and glycoproteins, and one of the major classes of tumor-associated antigens belongs to the glycosphingolipids of the ganglio or globo series [1].

Breast-cancer cells overexpress a glycosphingolipid antigen as defined by the monoclonal antibody MBr1 [2]. The antigen was isolated from breast-cancer cell-line MCF7, and its structure was determined to be the glycosphingolipid globo-H: α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-β-D-Gal*p*NAc-(1→3)-α-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→1)Cer (**1**) [2]. Due to the highly restricted distribution of this antigen, the elucidation of the properties of the entire epitope, or parts of it, is of great interest. The corresponding defucosylated glycosphingolipid, a stage-specific embryonic antigen (SSEA 3), synthesized by *Ogawa* and *Nunomura* [3], did not show any reaction with the antibody MBr1 [2], demonstrating the importance of the fucose unit.



1



2

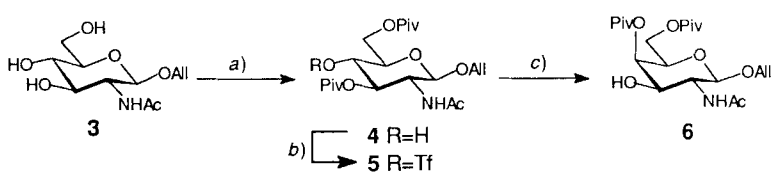
To better characterize the MBr1-defined epitope, we started with the synthesis of fragments of the globo-H oligosaccharide moiety. Since the antibody MBr1 cross-reacted weakly with fucosylasialo-GM1 [2], we chose as first antigen the trisaccharide α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-β-D-Gal*p*NAc-1-OPr (**2**), corresponding to the units *A*–*C* of the parent glycosphingolipid and shared by globo-H and GM1.

As a basic strategy in oligosaccharide synthesis, we decided to avoid, as far as possible, the use of heavy-metal salts as promoters in oligosaccharide synthesis because of the well known disadvantages [4] related to their use.

Results and Discussion. – The first problem in the synthesis of the target oligosaccharide is the availability of a properly protected galactosamine and its glycosylation. As galactosamine is quite expensive, it is usually obtained by azidonitration of tri-*O*-acetylgalactal and reduction of the azido group [5] or by inversion of configuration at position 4 of glucosamine [6][1]). We decided to follow a modification of the second strategy, and we planned to obtain a properly protected allyl 2-acetamido-2-deoxy-β-D-galactopyranoside because the presence of the allyloxy group at the anomeric position allows the transformation of the acetamido group into a dihydrooxazole [8] which can be used for the glycosylation of the unit *D* of globo-H.

Known [9] allyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**3**) was treated with 3 equiv. of pivaloyl chloride in CH_2Cl_2/pyridine 1:2 at 0° to give the 3,6-di-*O*-pivaloyl derivative **4** (76%; *Scheme 1*). Compound **4** was converted into the corresponding trifluoromethanesulfonate (= triflate) **5** with Tf_2O in CH_2Cl/pyridine 20:1 at 0°. Addition of H_2O and refluxing caused the migration of the pivaloyloxy group from position 3 to position 4 with inversion of configuration [10], thus effecting the conversion of the glucosamine unit into the galactosamine derivative **6** and, at the same time, leaving the OH group at C(3) free for the subsequent glycosylation.

Scheme 1



a) 3 Equiv. of PivCl, CH_2Cl_2/Py 1:2, 0°, 76%. *b*) Tf_2O, CH_2Cl_2/Py 20:1, 0°. *c*) H_2O, reflux, 86%.

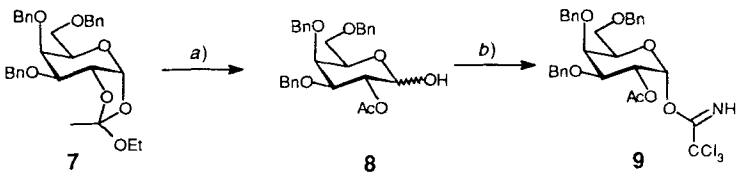
Several syntheses of the disaccharide β-D-Gal*p*-(1→3)-D-Gal*p*NAc are reported (see, *e.g.*, [11–15]). We used the trichloroacetimidate method which was recently applied to the synthesis of some deoxy analogues of this disaccharide [16].

The galactosyl donor **9** [17] was obtained starting from **7** (*Scheme 2*). It is known that opening of the orthoester **7** under acidic conditions gives rise to 1-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-galactopyranose [18]. When the orthoester **7** was treated with AcOH/pyridine (95%) 3:1, compound **8** was obtained (95%) through base-catalyzed migration of the Ac group from position O^1 to position O^2. Reaction of **8** with CCl_3CN in the presence of

[1]) Inversion at position 4 was also achieved in disaccharide β-D-Gal*p*-(1→3)-D-Glc*p*NAc [7].

1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) afforded the trichloroacetimidate **9** [19] in 83% yield. The participation of AcO–C(2) controls the stereochemistry of the reaction (→β-D-glycoside), and this group allows the selective deprotection at O^2 prior to the subsequent fucosylation.

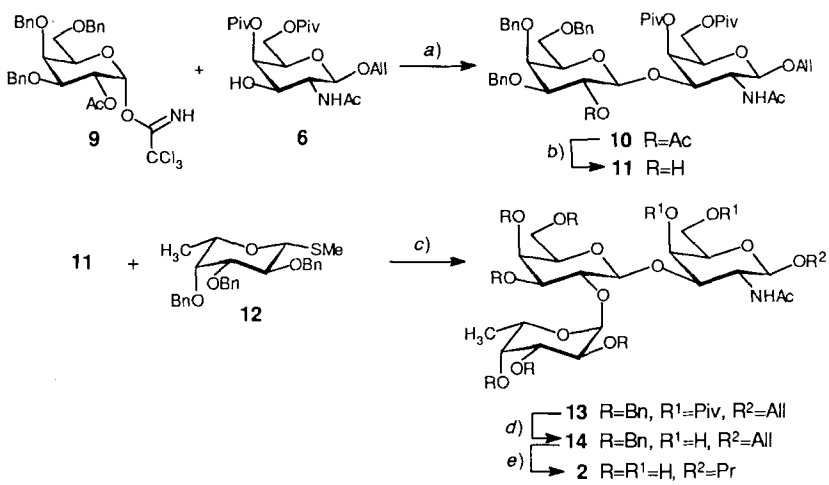
Scheme 2



a) AcOH/Py (95%) 3:1, r.t., 95%. *b*) CCl_3CN, DBU, CH_2Cl_2, 83%.

$BF_3 \cdot OEt_2$-Promoted coupling of **9** with **6** in CH_2Cl_2 at −20° gave the desired disaccharide **10** (52%; *Scheme 3*) which was characterized by homo- and heterocorrelated NMR spectra. The $J(1',2')$ value (7.8 Hz) clearly indicates the β-D-configuration of the formed glycoside. Deacetylation of **10** was quite troublesome. In fact, *Zemplén* deacetylation was almost ineffective at room temperature, and, on warming, a complex mixture was formed. Better results were obtained using a solution of guanidine in EtOH [20] which afforded the desired disaccharide **11** in 51% yield. Although pivaloates are supposed to be inert towards hydrolysis with guanidine, a more polar depivaloylated by-product was obtained.

Scheme 3



a) $BF_3 \cdot OEt_2$, CH_2Cl_2, −20°, 52%. *b*) 8 Equiv. of guanidine, EtOH, r.t., 50%. *c*) NIS, cat. TfOH, CH_2Cl_2. *d*) MeONa in MeOH. *e*) H_2, Pd/C, MeOH, 86%.

Fucosylation of **11** with methyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside (**12**) [21] promoted by *N*-iodosuccinimide (NIS) and catalytic triflic acid [22] gave the trisaccharide **13** (48%; *Scheme 3*). Conventional deprotection of **13** (MeONa in MeOH; H_2,

Pd/C) eventually afforded, *via* **14**, the target trisaccharide **2** (86%). The anomeric configurations of trisaccharide **13** were assigned using the coupling constants between the anomeric C-atom and the corresponding anomeric proton (J(C(1),H–C(1)) = 160.7, J(C(1′),H–C(1′)) = 157.6, and J(C(1″),H–C(1″)) = 170.0 Hz). They were further confirmed by the ^{1}H,^{1}H-coupling constants of the anomeric proton and the corresponding vicinal H-atom of **2** (J(1,2) = 7.6, J(1′,2′) = 7.7 and J(1″,2″) = 3.8 Hz).

Biological Results. – Preliminary biological testing revealed that the oligosaccharide **2** was able to inhibit MBr1 binding to the relevant target cell MCF7 in a dose-dependent manner, with a 50% inhibitory concentration (IC_{50}) of 20 μM. The effect was specific since the unrelated oligosaccharide lactodifucotetraose (LDFT; Lewis^y-like apten: α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→4)-[α-L-Fuc*p*-(1→3)]-D-Glc*p*; *Oxford GlycoSystems Ltd.*, Abingdon, UK) did not affect MBr1 binding, even at the maximum concentration tested (800 μM). These data indicate that the oligosaccharide **2** represents the MBr1-defined epitope or, at least, its more relevant saccharide units.

This work will be extended by the synthesis of other fragments of **1** and by evaluating all the synthesized fragments from the biological and the conformational point of view.

This work was partially supported by *A. I. R. C.* and by the Italian *CNR*, Comitato per le biotecnologie e la biologia molecolare (Progetto speciale oligosaccaridi).

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* instrument.

*Allyl 2-Acetamido-2-deoxy-3,6-di-*O*-pivaloyl-β-*D*-glucopyranoside* (**4**). A mixture of **3** (2.3 g, 8.8 mmol) pivaloyl chloride (= 2,2-dimethyl propanoyl chloride) (3.25 ml, 26.8 mmol; d = 0.98), CH_2Cl_2 (10 ml), and pyridine (20 ml) was stirred for 2 h at 0°. MeOH (1 ml) was added, the mixture diluted with CH_2Cl_2, the org. phase washed with 5% HCl soln., 5% $NaHCO_3$ soln., and H_2O, dried (Na_2SO_4), and evaporated, and the residue crystallized from AcOEt/hexane: 2.87 g (76%) of **4**. White solid. M.p. 135–137°. $[\alpha]_D$ = −42 (c = 1, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$): 5.83 (*m*, CH_2=C*H*CH$_2$); 5.62 *d*, J = 9.1, NH); 5.3–5.1 (2*d*, CH_2=CHCH$_2$); 5.01 (*t*, J = 9.2, H–C(3)); 4.50 (*d*, J = 8.4, H–C(1)); 4.4–4.2 (*m*, 2 H–C(6), 1 H of CH_2=CHCH_2); 4.1–3.9 (*m*, H–C(2), 1 H of CH_2=CHCH_2); 3.6–3.4 (*m*, H–C(4), H–C(5)); 2.93 (br. *s*, OH); 1.92 (*s*, Ac); 1.23, 1.20 (2*s*, 2 *t*-BuCO). Anal. calc. for $C_{21}H_{35}NO_8$ (429.51): C 58.73, H 8.21, N 3.26; found: C 58.55, H 8.44, N 3.19.

*Allyl 2-Acetamido-2-deoxy-4,6-di-*O*-pivaloyl-β-*D*-galactopyranoside* (**6**). Under N_2, Tf_2O (0.45 ml, 2.8 mmol) was added dropwise to a soln. of **4** (1 g, 2.3 mmol) in CH_2Cl_2 (20 ml) and pyridine (1 ml) at −35°. The mixture was allowed to warm to 0° and stirred for 2 h. H_2O (1 ml) was added and the mixture refluxed for 4 h and then diluted with CH_2Cl_2. The org. phase was washed with 5% HCl soln., 5% $NaHCO_3$ soln., and H_2O, dried (Na_2SO_4), and evaporated. FC (SiO_2, hexane/AcOEt 4:6 to 3:7) afforded 860 mg (86%) of **6**. M.p. 48–50°. $[\alpha]_D$ = −32.5 (c = 1.05, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$): 5.91 (*m*, CH_2=C*H*CH$_2$); 5.69 *d*, J = 4.1, NH); 5.29 (*d*, J = 2.8, H–C(4)); 5.4–5.2 (*m*, CH_2=CHCH$_2$); 4.58 (*d*, J = 7.7, H–C(1)); 4.4–4.3 (*m*, 1 H of CH_2=CHCH_2, OH); 4.2–4.0 (*m*, 2 H–C(6), H–C(3), 1 H of CH_2=CHCH_2); 3.87 (*t*, J = 6.7, H–C(5)); 3.67 (*dt*, J = 4.1, 7.7, H–C(2)); 2.04 (*s*, Ac); 1.27, 1.18 (2*s*, 2 *t*-BuCO). Anal. calc. for $C_{21}H_{35}NO_8$ (429.51): C 58.73, H 8.21, N 3.26; found: C 58.50, H 8.05, N 3.13.

*2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-*D*-galactopyranose* (**8**). To a soln. of **7** (2.1 g, 4.0 mmol) in 95% aq. AcOH soln. (21 ml) was added pyridine (7 ml). The mixture was stirred at 22° for 24 h, and then diluted with Et_2O. The org. phase was washed with 5% HCl soln., 5% $NaHCO_3$ soln., and H_2O, dried (Na_2SO_4), and evaporated: 1.89 g (95%) of almost pure **8**. Syrup. $[\alpha]_D$ = +43 (c = 1.05, $CHCl_3$; [12]: $[\alpha]_D$ = +45 (c = 3, $CHCl_3$)). Anal. calc. for $C_{29}H_{32}O_7$ (492.57): C 70.71, H 6.55; found: C 70.90, H 6.74.

*Allyl 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*-galactopyranoside* (**10**). To a mixture of **6** (2.3 g, 5.3 mmol), **9** (3.5 g, 5.5 mmol), and powdered molecular sieves 4 Å in CH_2Cl_2 (150 ml) at −20° under N_2 was added $BF_3 \cdot OEt_2$ (0.8 ml, 6.3 mmol; $d = 1.13$). After 3 h, the mixture was neutralized with 5% aq. $NaHCO_3$ soln. and filtered over *Celite*. The filtrate was washed with 5% $NaHCO_3$ soln. and H_2O, dried (Na_2SO_4), and evaporated. FC (SiO_2, hexane/AcOEt 7:3 to 4:6) afforded 2.44 g (52%) of **10**. White solid. M.p. 58–60°. $[\alpha]_D = +28.3$ ($c = 1.1$, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$): 7.4–7.1 (*m*, arom. H); 6.0–5.8 (*m*, CH_2=C*H*CH_2); 5.84 (*d*, $J = 6.8$, NH); 5.33 (*d*, $J = 3.5$, H–C(4)); 5.3–5.1 (*m*, CH_2=CHCH$_2$, H–C(2′)); 5.03 (*d*, $J = 8.5$, H–C(1)); 4.88 (*d*, $J = 11.3$, PhC*H*); 4.64 (*d*, $J = 12.2$, PhC*H*); 4.58 (*dd*, $J = 10.7$, 3.5, H–C(3)); 4.5–4.4 (*m*, 4 PhC*H*); 4.42 (*d*, $J = 7.8$, H–C(1′)); 4.30 (*dd*, $J = 12.8$, 5.4, 1 H of CH_2=CHCH_2); 4.13 (*dd*, $J = 11.5$, 4.1, 1 H–C(6)); 4.04 (*dd*, $J = 12.8$, 6.2, 1 H of CH_2=CHCH_2); 4.0–3.9 (*m*, 1 H–C(6), H–C(4′)); 3.80 (*dd*, $J = 8.3$, 4.1, H–C(5)); 3.7–3.5 (*m*, 2 H–C(6′), H–C(5′)), 3.42 (*dd*, $J = 10.2$, 2.6, H–C(3′)); 3.21 (br. *dt*, $J = 10.7$, 7.7, H–C(2)); 1.99, 1.91 (2*s*, 2 Ac); 1.18, 1.13 (2*s*, 2 *t*-BuCO). ^{13}C-NMR (75 MHz, $CDCl_3$): 178.8 (*s*, CO); 177.7 (*s*, CO); 171.4 (*s*, CO); 170.0 (*s*, CO); 139.2 (*s*); 138.5 (*s*); 134.5 (*d*); 129.1 (*d*); 128.7 (*d*); 128.5 (*d*); 128.4 (*d*); 128.0 (*d*); 118.4 (*t*); 101.9 (*d*, C(1′)); 98.8 (*d*, C(1)); 80.6 (*d*, C(3′)); 75.4 (*d*, C(3)); 75.0 (*t*); 74.2 (*t*); 73.8 (*d*, C5′)); 72.9 (*d*, C(4′)); 72.4 (*d*, C(5)); 72.3 (*t*); 72.1 (*d*, C2′)); 70.8 (*t*); 69.5 (*d*, C(4)); 68.7 (*t*, C(6′)); 63.6 (*t*, C(6)); 56.1 (*d*, C(2)); 39.6 (*s*); 39.3 (*s*); 27.7 (*q*); 24.3 (*q*); 21.7 (*q*). Anal. calc. for $C_{50}H_{65}NO_{14}$ (904.06): C 66.43, H 7.25, N 1.55; found: C 66.21, H 7.41, N 1.45.

*Allyl 2-Acetamido-2-deoxy-4,6-di-*O*-pivaloyl-3-*O*-(3,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl)-β-*D*-galactopyranoside* (**11**). A soln. of **10** (200 mg, 0.22 mmol) in CH_2Cl_2 (1 ml) was mixed with 0.1M guanidine/EtOH (11 ml; obtained from guanidine hydrochloride (106 mg) and 0.1M NaOEt/EtOH (11 ml)): After 4 h, the mixture was diluted with CH_2Cl_2 and washed with 5% HCl soln., 5% $NaHCO_3$ soln., and H_2O. The org. layer was dried (Na_2SO_4) and evaporated. FC (SiO_2, hexane/AcOEt 1:1) afforded 98 mg (51%) of **11**. Foam. $[\alpha]_D = +14$ ($c = 1$, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$): 7.4–7.1 (*m*, 15 arom. H); 6.01 (*d*, $J = 6.9$, NH); 6.0–5.8 (*m*, CH_2=C*H*CH_2); 5.31 (*d*, $J = 2.5$, H–C(4)); 5.3–5.1 (*m*, CH_2=CHCH_2); 4.86 (*d*, $J = 11.5$, PhC*H*); 4.76 (*d*, $J = 8.4$, H–C(1)); 4.69 (*s*, PhCH_2); 4.6–4.3 (*m*, 4 H); 4.2–4.0 (*m*, 2 H); 4.0–3.8 (*m*, 2 H); 3.7–3.3 (*m*, 5 H); 1.91 (*s*, Ac); 1.16 (*s*, 2 *t*-BuCO). ^{13}C-NMR (75 MHz, $CDCl_3$): 178.5 (*s*, CO); 178.4 (*s*, CO); 172.3 (*s*, CO); 139.2 (*s*); 138.5 (*s*); 134.5 (*d*); 129.1 (*d*); 128.8 (*d*); 128.4 (*d*); 128.0 (*d*); 118.5 (*t*); 104.1 (*d*, C(1′)); 99.8 (*d*, C(1)); 82.2 (*d*); 75.0 (*t*); 74.7 (*d*); 74.1 (*t*); 74.0 (*d*); 73.6 (*d*); 73.0 (*t*); 72.2 (*d*); 71.1 (*d*); 70.7 (*t*); 69.9 (*d*); 69.1 (*t*); 63.3 (*t*); 54.9 (*d*, C(2)); 39.7 (*s*); 39.3 (*s*); 27.7 (*q*); 24.4 (*q*). Anal. calc. for $C_{48}H_{63}NO_{13}$ (862.03): C 66.88, H 7.37, N 1.62; found: C 66.61, H 7.21, N 1.66.

*Allyl 2-Acetamido-2-deoxy-4,6-di-*O*-pivaloyl-3-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-*L*-fucopyranosyl)-β-*D*-galactopyranosyl]-β-*D*-galactopyranoside* (**13**). To a mixture of **11** (100 mg, 0.12 mmol) **12** (66 mg, 0.14 mmol), and powdered molecular sieves 4 Å in CH_2Cl_2/Et_2O 1:1 (1 ml) at 0° under N_2 was added a soln. of NIS (32 mg) and TfOH (3 µl) in CH_2Cl_2/Et_2O 1:1 (4 ml). After 3 h, the mixture was neutralized with 5% aq. $NaHCO_3$ soln. and filtered over *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 to 1:1) afforded 72 mg (48%) of **13**. Syrup. $[\alpha]_D = -17.9$ ($c = 1.2$, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$, 55°): 7.4–7.1 (*m*, 30 arom. H); 6.21 (br. *s*, NH); 5.86 (*m*, CH_2=C*H*CH_2); 5.50 (*d*, $J = 3.5$, H–C(1″)); 5.37 (*d*, $J = 3.3$, H–C(4)); 5.3–5.1 (*m*, CH_2=CHCH_2); 5.0–4.4 (*m*, 13 H, PhCH_2, H–C(1)); 4.38 (*d*, $J = 7.2$, H–C(1′)); 4.30 (*dd*, $J = 13.1$, 5.2, 1 H of CH_2=CHCH_2); 4.2–3.9 (*m*, 2 H–C(6), H–C(5″), H–C(2′), 1 H of CH_2=CHCH_2, H–C(2″), H–C(3″)); 3.86 (*d*, $J = 2.6$, H–C(4′)); 3.8–3.4 (*m*, H–C(5), H–C(2), H–C(4″), H–C(5′), H–C(3), 2 H–C(6′), H–C(3′)); 1.81 (*s*, Ac); 1.19 *d*, $J = 6.2$, H–C(6″)); 1.18, 1.12 (2*s*, 2 ^{t}-BuCO). ^{13}C-NMR (75 MHz, $CDCl_3$): 178.6 (*s*, CO); 177.8 (*s*, CO); 171.4 (*s*, CO); 139.4 (*s*); 138.8 (*s*); 138.6 (*s*); 137.9 (*s*); 134.6 (*d*); 128.9 (*d*); 128.7 (*d*); 128.4 (*d*); 128.1 (*d*); 127.9 (*d*); 127.7 (*d*); 127.5 (*d*); 118.0 (*t*); 104.4 (*d*, ^1J(C,H) = 157.6 C(1′)); 100.9 (*d*, ^1J(C,H) = 160.7, C(1)); 97.7 (*d*, ^1J(C,H) = 170.0, C(1″)); 83.3 (*d*); 79.7 (*d*); 78.6 (*d*); 77.6 (*d*); 77.1 (*d*); 77.0 (*d*); 75.5 (*t*); 75.1 (*t*); 74.2 (*t*); 73.7 (*t*); 73.5 (*d*); 72.9 (*d*); 72.7 (*t*); 70.3, (*t*); 69.9 (*d*); 68.9 (*t*); 67.7 (*d*); 63.9 (*t*); 54.4 (*d*, C(2)); 39.8 (*s*); 39.6 (*s*); 27.8 (*q*); 24.1 (*q*); 17.5 (*q*, C(6″)). Anal. calc. for $C_{75}H_{91}NO_{17}$ (1278.54): C 70.46, H 7.17, N 1.10; found: C 70.61, H 7.42, N 1.01.

*Allyl 2-Acetamido-2-deoxy-3-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4,-tri-*O*-benzyl-α-*L*-fucopyranosyl)-β-*D*-galactopyranosyl]-β-*D*-galactopyranoside* (**14**). A soln. of **13** (63 mg, 0.05 mmol) in MeOH (3 ml) containing a catalytic amount of MeONa under N_2 was kept for 2 days at 28° and then neutralized with *Amberlite IR-120*, filtered, and evaporated. FC (SiO_2, hexane/AcOEt 6:4→1:1) afforded 48 mg (86%) of **14**. Foam. $[\alpha]_D = -38.7$ ($c = 1.1$, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$): 7.4–7.1 (*m*, 30 arom. H); 6.16 (*d*, $J = 7.7$, NH); 5.82 (*m*, CH_2=C*H*CH_2); 5.42 (*d*, $J = 3.8$, H–C(1″)); 5.3–5.1 (*m*, CH_2=CHCH_2); 5.1–4.3 (*m*, 15 H, PhCH_2, H–C(1), H–C(1′), 1 H of CH_2=CHCH_2); 4.2–3.4 (*m*, 16 H); 2.98 (br. *s*, OH); 2.25 (br. *s*, OH); 1.78 (*s*, Ac); 1.18 (*d*, $J = 6.4$, H–C(6″)). ^{13}C-NMR (75 MHz, $CDCl_3$): 171.3 (*d*, CO); 139.4 (*s*); 139.3 (*s*); 139.0 (*s*); 138.3 (*s*); 135.0 (*d*); 128.9 (*d*); 128.8 (*d*); 128.6 (*d*); 128.4 (*d*); 128.1 (*d*); 127.8 (*d*); 127.7 (*d*); 117.4 (*t*); 103.2 (*d*); 101.7 (*d*); 98.6 (*d*, C(1″)); 83.0 (*d*); 79.5 (*d*); 78.4 (*d*); 78.1 (*d*); 76.9 (*d*); 75.4 (*t*); 75.1 (*t*); 74.7 (*d*); 74.4 (*d*); 74.2 (*t*); 74.0 (*d*); 73.8 (*t*); 73.6 (*t*); 73.4 (*t*); 69.9 (*t*);

68.6 (*d*); 68.0 (*d*); 63.2 (*t*); 52.3 (*d*, C(2)); 23.9 (*q*); 17.6 (*q*, C(6″)). Anal. calc. for $C_{65}H_{75}NO_{15}$ (1110.31): C 70.32, H 6.81, N 1.26; found: C 70.11, H 7.02, N 1.20.

*Propyl 2-Acetamido-2-deoxy-3-*O*-[2-*O*-(α-*L*-fucopyranosyl)-β-*D*-galactopyranosyl]-β-*D*-galactopyranoside* (**2**). To a soln. of **14** (32 mg, 0.029 mmol) in MeOH (2 ml), 10% Pd/C (3 mg) was added and the mixture stirred overnight under H_2 (TLC monitoring (CH_2Cl_2/MeOH 2:8)). The mixture was filtered over *Celite*, the solvent evaporated, the residue dissolved in H_2O, and the soln. lyophilized: 17 mg (quant.) of **2**. White solid. M.p. 213–215° (dec.). $[\alpha]_D = -49.1$ (*c* = 1.0, MeOH). ^{1}H-NMR (300 MHz, D_2O): 5.25 (*d*, *J* = 3.8, H–C(1″)); 4.62 (*d*, *J* = 7.6, H–C(1′)); 4.33 (*d*, *J* = 7.7, H–C(1)); 4.25 (br. *q*, *J* = 6.4, H–C(5″)); 4.2–3.4 (*m*, 17 H); 2.06 (*s*, Ac); 1.54 (*m*, Me*CH*$_2$CH$_2$); 1.23 (*d*, *J* = 6.4, H–C(6″)); 0.89 (*t*, *J* = 7.1, *Me*CH$_2$CH$_2$). ^{13}C-NMR (75 MHz, D_2O): 176.6 (*s*, CO); 105.6 (*d*); 105.0 (*d*); 102.1 (*d*); 79.6 (*d*); 78.9 (*d*); 77.9 (*d*); 77.6 (*d*); 76.4 (*d*); 75.3 (*t*); 74.6 (*d*); 72.4 (*d*); 72.0 (*d*); 71.4 (*d*); 70.9 (*d*); 69.7 (*d*); 63.8 (*t*); 54.2 (*d*, C(2)); 25.0 (*t*, Me*C*H$_2$CH$_2$); 18.1 (*q*, C(6″)); 12.5 (*q*, *Me*CH$_2$CH$_2$). Anal. calc. for $C_{23}H_{41}NO_{15}$ (571.58): C 48.33, H 7.23, N 2.45; found: C 48.08, H 7.42, N 2.27.

Evaluation of the Biological Activity. – The effect of **2** on MBr1 binding to live MCF7 cells was tested by an indirect immunofluorescence assay. A fixed amount of MBr1 (1 nM) was incubated with solns. of different concentrations of **2** (800–2 μM) in phosphate-buffered saline +0.03% BSA for 1 h at 0°. The mixture was transferred on suspended MCF7 cells and further incubated for 30 min at 0°. After 3 washes within phosphate-buffered saline + 0.03% BSA, the binding was detected by incubating cells 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*).

REFERENCES

[1] S. Hakomori, *Cancer Cells* **1991,** *3*, 461.
[2] E. G. Bremer, S. B. Levery, S. Sonnino, R. Ghidoni, S. Canevari, R. Kannagi, S. Hakomori, *J. Biol. Chem.* **1984,** *259*, 14773.
[3] S. Nunomura, T. Ogawa, *Tetrahedron Lett.* **1988,** *29*, 5681.
[4] R. R. Schmidt, *Angew. Chem. Int. Ed.* **1986,** *25*, 212.
[5] R. U. Lemieux, R. M. Ratcliffe, *Can. J. Chem.* **1979,** *57*, 1244.
[6] M. A. Nashed, R. I. El-Sokkary, L. Rateb, *Carboyhdr. Res.* **1984,** *131*, 47.
[7] A. Lubineau, H. Bienaymé, *Carbohydr. Res.* **1991,** *212*, 267.
[8] M. A. Nashed, L. Anderson, *J. Chem. Soc., Chem. Commun.* **1982,** 1274.
[9] M. Imoto, H. Yoshimura, M. Yamamoto, T. Shimamoto, S. Kusumoto, T. Shiba, *Bull. Chem. Soc. Jpn.* **1987,** *60*, 2197.
[10] R. W. Binkley, *J. Org. Chem.* **1991,** *56*, 3892.
[11] J.-C. Jacquinet, H. Paulsen, *Tetrahedron Lett.* **1981,** *22*, 1387.
[12] H. Paulsen, J.-C. Jacquinet, W. Rust, *Carbohydr. Res.* **1982,** *104*, 195.
[13] S. S. Rana, K. L. Matta, *Carbohydr. Res.* **1983,** *116*, 71.
[14] H. Paulsen, M. Paal, *Carbohydr. Res.* **1984,** *135*, 53.
[15] W. Kinzy, R. R. Schmidt, *Carbohydr. Res.* **1987,** *166*, 265.
[16] H. Paulsen, V. Rutz, I. Brockhausen, *Liebigs Ann. Chem.* **1992,** 747.
[17] B. Doboszewski, A. Zamojski, *Carbohydr. Res.* **1984,** *132*, 29.
[18] O. Hindsgaul, T. Norberg, J. Le Pendu, R. U. Lemieux, *Carbohydr. Res.* **1982,** *109*, 109.
[19] K. Sakai, Y. Nakahara, T. Ogawa, *Tetrahedron Lett.* **1990,** *31*, 3035.
[20] N. Kunesch, C. Miet, J. Poisson, *Tetrahedron Lett.* **1987,** *28*, 3569.
[21] S. Sato, Y. Ito, T. Nukuda, Y. Nakahara, T. Ogawa, *Carbohydr. Res.* **1987,** *167*, 197.
[22] K. Zegelaar-Jaarsveld, G. A. van der Marel, J. H. van Boom, *Tetrahedron* **1992,** *48*, 10133.