Total Synthesis of a Human Breast Tumor Associated Antigen

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Received May 10, 1995

Antigens which are selective or, ideally, specific for cancer cells could prove useful in fostering active immunity. Novel carbohydrate patterns are often presented by transformed cells.[2] In principle, well-chosen synthetic glycoconjugates which stimulate antibody production could confer active immunity against cancers which present equivalent structural types on their cell surfaces.[3]

From this perspective, we became quite interested in a glycosphingolipid which was isolated by Hakomori and colleagues from breast cancer cell line MCF-7 and immunocharacterized by monoclonal antibody MBr1.[4,5] The novel glycosphingolipid structure **1** (Figure 1) was proposed for this breast tumor associated antigen. While individual sectors of the proposed structure are certainly not unfamiliar, the full structure was described only from the breast cancer line. It should be noted however, that MBr1 does bind to normal human mammary gland tissue and ovarian cancer cell lines. This may be taken to indicate that **1** as a total entity is not restricted to the transformed breast cells. Alternatively, it might reflect that smaller subsections of **1** are adequate for antibody recognition and binding.[6]

Only experimentation will reveal whether this breast tumor antigen is clinically useful in promoting active immunity. At the present time, only synthesis can confirm glycosphingolipid **1** as the antigen recognized by MBr1 and provide sufficient quantities for immunization studies.

We therefore set for ourselves the goal of synthesizing **1** as well as artificial protein conjugates of the oligosaccharide which might be more immunogenic than the smaller glycolipid. The novel array of carbohydrate residues added to the chemical interest of the problem.[7,8] We now report (i) a total synthesis of **1**, (ii) rigorous proof that the Hakomori antigen does, in fact, correspond to **1**, and (iii) the synthesis of a bioconjugatable version of **1** which brings the problem to the point where immunological and preclinical investigations have begun. The conciseness of the solution attests to the usefulness of glycal assembly methods, now augmented by a much more powerful method for sulfonamidoglycosylation (see **14** → **15** → **16**, Scheme 3) in such complex undertakings.

We commenced with galactal **2** (Scheme 1), which was selectively protected to provide **3**. This material was then converted to fluorosugar donor **4** under established conditions.[9] Preparation of the appropriate disaccharide acceptor commenced with **5**,[10] itself obtained from a glycal coupling. Protecting-group manipulations afforded disaccharide **6**. Acceptor **6** was reacted with fluorosugar **4** under modified Mukaiyama condi-

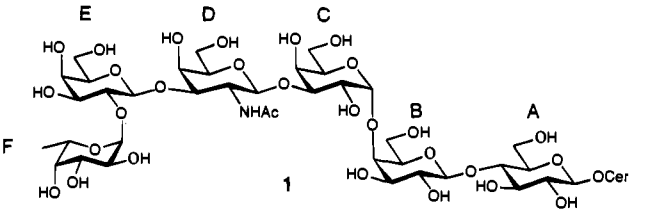
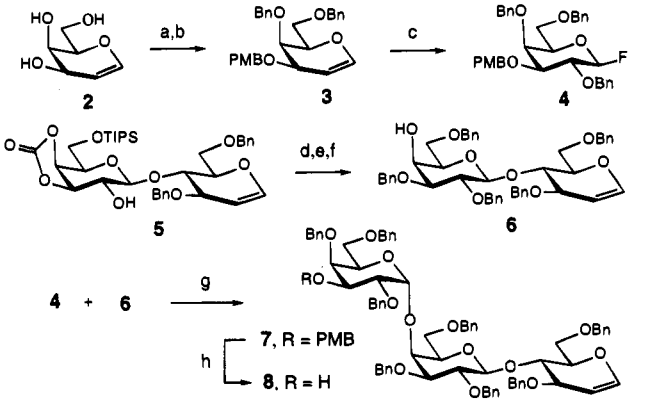


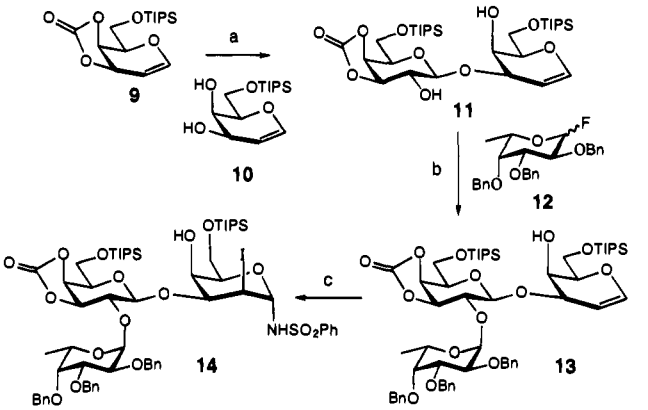
Figure 1. Structure of the MBr1 antigen.

Scheme 1[a]



[a] Reagents: (a) *n*-Bu_2SnO, PMBCl, TBABr, PhH, 70%; (b) NaH, BnBr, DMF, 95%; (c) (i) 3,3-dimethyldioxirane, CH_2Cl_2; (ii) TBAF, THF; (iii) NaH, BnBr, DMF, 40% (three steps); (d) NaH, BnBr, DMF, 80%; (e) (i) TBAF, THF; (ii) NaOMe, MeOH, 93% (two steps); (f) *n*-Bu_2SnO, $(n\text{-}Bu_3Sn)_2O$, BnBr, TBABr, PhH, 90%; (g) $SnCl_2$, $AgClO_4$, DTBP, 4 Å molecular sieves, Et_2O, 54% α (3:1 α:β); (h) DDQ, CH_2Cl_2, H_2O, 84%.

Scheme 2[a]



[a] Reagents: (a) (i) 3,3-dimethyldioxirane, CH_2Cl_2; (ii) **10**, $ZnCl_2$, THF, 87%; (b) $SnCl_2$, $AgClO_4$, Et_2O, 47%; (c) $I(coll)_2ClO_4$, $PhSO_2NH_2$, 4 Å molecular sieves, THF, 47%.

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(6) The syntheses of DEF and CDEF fragments of **1** have been reported recently, and both structures have been shown to bind to MBr1: Lay, L.; Nicotra, F.; Panza, L.; Russo, G. *Helv. Chim. Acta* **1994**, *77*, 509−514. Lay, L.; Panza, L.; Russo, G.; Colombo, D.; Ronchetti, F.; Adobati, E.; Canevari, S. *Helv. Chim. Acta* **1995**, *78*, 533−538.

(7) For the synthesis of a related structure (SSEA-3) which lacks the fucose residue, see: Nunomura, S.; Ogawa, T. *Tetrahedron Lett.* **1988**, *29*, 5681−5684. SSEA-3 is not recognized by MBr1 (see ref 4).

(8) For the synthesis of glycosphingolipid Gb_3, which consists of the ABC trisaccharide domain, see: Nicolaou, K. C.; Caulfield, T.; Kataoka, H.; Kumazawa, T. *J. Am. Chem. Soc.* **1988**, *110*, 7910−7912.

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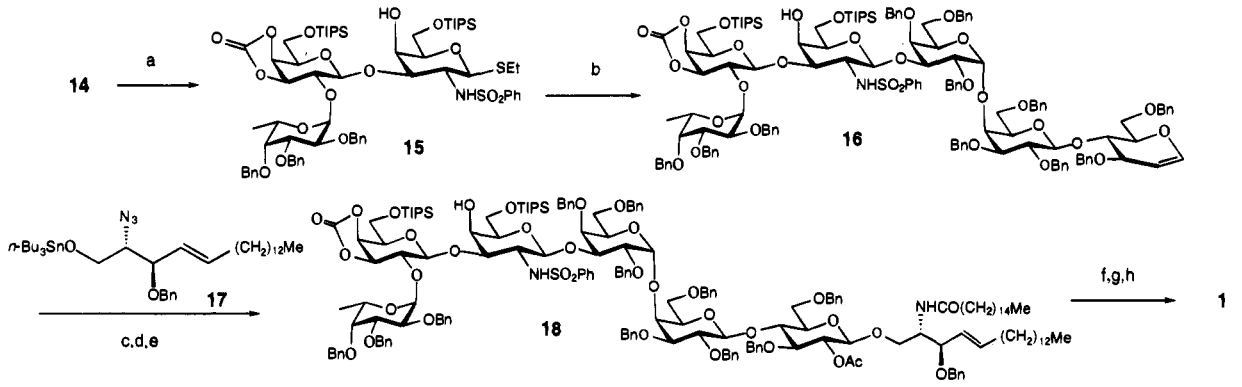
Scheme 3[a]



[a] Reagents: (a) EtSH, LiHMDS, DMF, 75%; (b) **8** (0.5 equiv), MeOTf, 4 Å molecular sieves, 70-85% β (10:1 β:α); (c) (i) 3,3-dimethyldioxirane, CH_2Cl_2; (ii) **17** (5 equiv), $Zn(OTf)_2$, THF, 20%; (d) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 95% (e) Lindlar's catalyst, H_2, palmitic anhydride, EtOAc, 90%; (f) (i) TBAF, THF; (ii) NaOMe, MeOH, 94%; (g) (i) Na, NH_3, THF; (ii) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 80%; (h) NaOMe, MeOH, quantitative.

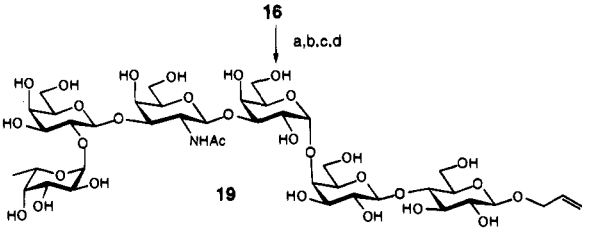
tions[11] to provide trisaccharide glycal **7**. Deprotection of the PMB ether provided ABC trisaccharide **8**, which was poised for coupling with a suitable DEF trisaccharide donor.

The synthesis of the DEF trisaccharide is presented in Scheme 2. Epoxidation of galactal **9** and standard coupling[12] with acceptor **10** afforded, regioselectively, disaccharide **11**. Fucosylation employing fluorofucose **12**[13] provided selectively trisaccharide **13**. This material was treated under the established conditions[14] to afford trans-diaxial iodosulfonamide **14**.

With both trisaccharide structures in hand we then explored coupling conditions. Unfortunately, direct coupling reactions[14,15] employing iodosulfonamides such as **14** with ABC trisaccharide acceptors failed. In response to this conundrum we sought to install a different donor functionality in the trisaccharide. In practice, iodosulfonamide **14** was treated with excess lithium ethanethiolate[14] to afford ethyl thioglycoside **15** (Scheme 3). Precedent established in our laboratories led to the prediction of sulfonamide participation to provide the desired β-linked product from **15**.[16] When donor **15** was treated with MeOTf in the presence of acceptor **8**, a 10:1 mixture of hexasaccharide isomers was obtained. Major product **16** was obtained in 70−85% yield. This work demonstrates that the two-stage formation of *trans*-2-sulfonamido-β-thioglycosides from glycals is very useful for the coupling of complex fragments when direct glycosylation employing the corresponding iodosulfonamide (*e.g.*, **14**) fails.

Ceramide attachment proceeded according to established conditions.[17,18] Following acetylation, reduction of the azide in the presence of palmitic anhydride provided ceramide **18**. Desilylation and saponification were followed by dissolving metal deprotection and MeOH quench. Peracetylation of the crude mixture, followed by saponification, provided glycosphingolipid **1**. Only the chemical shifts and coupling constants of the anomeric protons have been reported for the natural material.[4] The spectrum of synthetic **1** is in complete agreement with this data. Furthermore, the product was characterized by exact mass and ^{1}H NMR.

Scheme 4[a]



[a] Reagents: (a) TBAF, THF, 94%; (b) (i) Na, NH_3, THF; (ii) Ac_2O, Et_3N, DMAP, THF, DMF, 85%; (c) (i) 3,3-dimethyldioxirane, CH_2Cl_2, (ii) allyl alcohol, 66% (+29% of α-manno isomer); (d) NaOMe, MeOH, quantitative.

Synthetic **1** has been shown to bind to monoclonal antibody MBr1 in ELISA and immune thin layer chromatography assays. Also, MBr1 is strongly reactive with human breast cancer cell line MCF-7 by flow cytometry. *Preincubation of MBr1 with glycosphingolipid 1 completely inhibits this reactivity with MCF-7.* These experiments confirm that synthetic glycosphingolipid **1** contains the same antigenic epitope with which MBr1 reacts on breast cancer cells.

In addition, we have constructed the corresponding allyl glycoside (Scheme 4). Deprotection of **16** and acetylation afforded the peracetate of the hexasaccharide glycal. Epoxidation, reaction with allyl alcohol, and saponification provided allyl glycoside **19**.

As in the case of our recently described work on the Le^y determinant,[10] ozonolysis of the allyl group of **19** sets the stage for reductive coupling to lysine residues of proteins.[19] Studies to evaluate the immunogenic properties of glycosphingolipid **1** as well as oligosaccharide−protein conjugates derived from **19** are in progress and will be described in due course.

Acknowledgment. This work was financially supported by the National Institutes of Health (AI 16943). M.T.B. and J.T.R. gratefully acknowledge the NIH for postdoctoral fellowships (GM16291 and GM15240, respectively). Ohyun Kwon is acknowledged for exploratory experiments.

Supporting Information Available: Graph of MBr1 binding assay of **1** and a related isomer and spectral data for **1** (1 page). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

JA9515112

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