Defining the Molecular Recognition of Globo H (Human Breast Cancer) Antigen through Probe Structures Prepared by Total Synthesis

In Jong Kim,† Tae Kyo Park,† Shuanghua Hu,† Kofi Abrampah,‡ Shengle Zhang,‡ Philip O. Livingston,‡ and Samuel J. Danishefsky*,†,§

Laboratory for Bioorganic Chemistry and Laboratory for Tumor Vaccinology, Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, New York, New York 10021, and Department of Chemistry, Columbia University, Havemeyer Hall, New York, New York 10027

Received August 23, 1995

Recently, we have synthesized the MBr1 antigen (**1**).[1] As its name implies, this novel hexasaccharide, which is a human breast and ovarian cancer antigen, is recognized by the MBr1 antibody.[2] Furthermore, the MBr1 antigen has been shown to be immunogenic in mice.[3] All indications are that the fucose residue is critical for the recognition of antigen by antibody. For instance, the desfucosyl system SSEA-3 (**2**) fails to bind to MBr1 antibody.[4–6] It was of interest to ascertain whether truncated or isomeric versions of **1**, which contain the critical fucose moiety, but lack residues from the potential reducing end of the carbohydrate domain, would be recognized by MBr1 antibody. Toward that end, we set as a goal the synthesis of truncated and stereoisomeric forms of the MBr1 antigen which would deal with this question at a detailed level. In this paper we report the synthesis of four novel analogues of MBr1 antigen (**1**) (Figure 1).

Pentasaccharide **3** contains all of the stereochemical relationships of the intact **1** but lacks the terminal glucose residue. In tetrasaccharide **4**, the key stereochemical relationships of **1** remain intact as the A and B (lactosyl residue) are deleted. In compound **5**, the hexasaccharide motif of **1** is maintained, but the stereochemistry of the glycosidic linkage connecting carbohydrates B and C has been altered to correspond to an α linkage. In compound **6** the hexacyclic motif of **1** is, again, maintained, but the glycosidic bond connecting the rings C and D is altered relative to the natural antigen **1** (Figure 2).

Each acceptor was prepared as shown in Scheme 1. Reactions of compounds **7** and **8** with the previously prepared fluoro sugar **9**[1] followed by deprotection with DDQ gave acceptors **11**, **13a**, and **13b** in the yields and ratios shown, respectively.

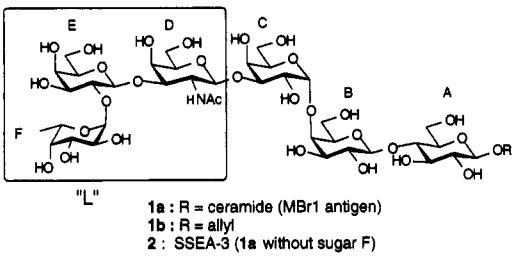


Figure 1.

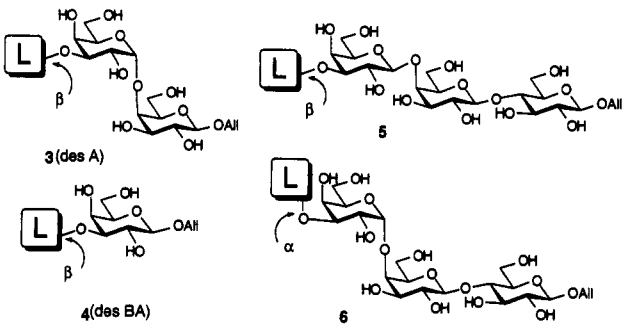
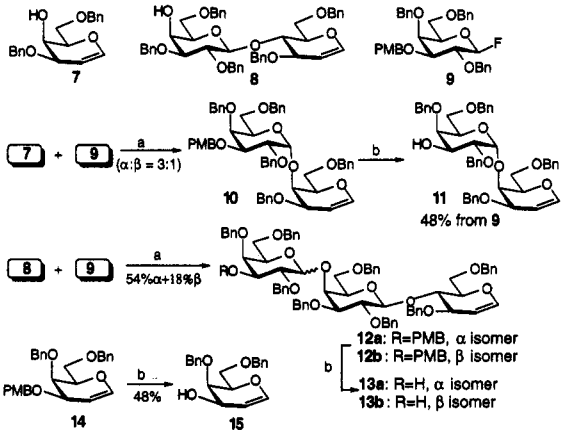


Figure 2. Truncated and isomeric MBr1 epitopes.

Scheme 1[a]



[a] Reagents and conditions: (a) $AgClO_4$, $SnCl_2$, di-*tert*-butylpyridine, 4 Å MS, Et_2O, then TESOTf, Et_3N; (b) DDQ, wet CH_2Cl_2.

The acceptor **11** coupled with donor **16a**[1,7] under mediation by methyl triflate afforded β-glycoside **17**. The fully protected glycal **17** was peracetylated to provide **21** which was converted to allyl glycoside **3** by our standard protocol[1] (Scheme 2). The choice of the allyl glycoside follows from our recently employed[8,9] adaptation of the Bernstein–Hall protocol[10] to prepare BSA and other protein conjugates of oligosaccharides. Hence, any compound shown to be active at the binding level can be quickly converted to a potential immunogenic form of an allyl glycoside.

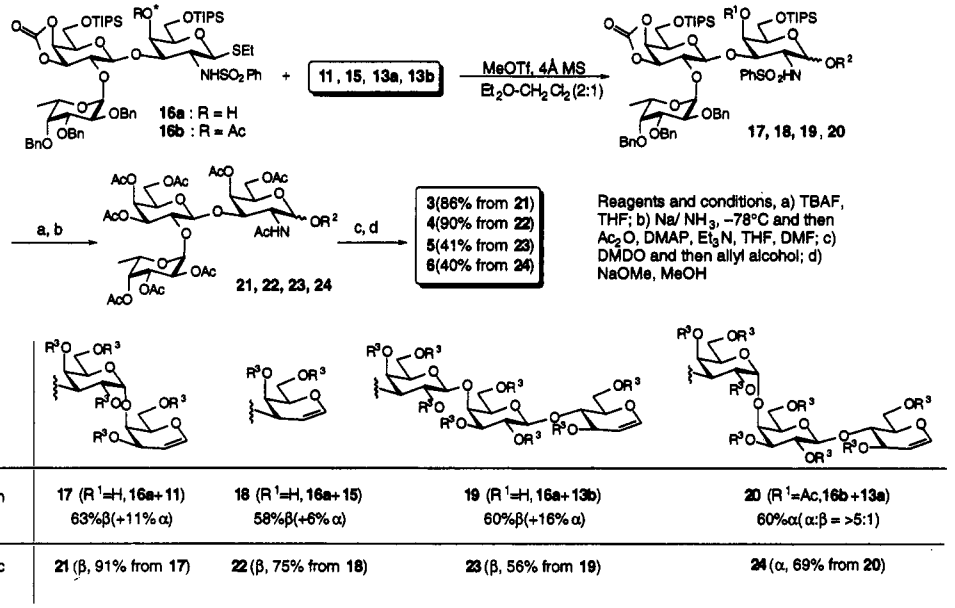
The tetrasachharide **4** was obtained in much the same way. Coupling of acceptor **15** with donor **16a** carrying a

‡ Laboratory for Bioorganic Chemistry.
‡ Laboratory for Tumor Vaccinology.
§ Department of Chemistry.

(1) Bilodeau, M. T.; Park, T. K.; Hu, S.; Randolph, J. T.; Danishefsky, S. J.; Livingston, P. O.; Zheng, S. *J. Am. Chem. Soc.* **1995**, *117*, 7840.
(2) Menard, S.; Tagliabue, E.; Canevari, S.; Fossati, G.; Colnaghi, M. I. *Cancer Res.* **1983**, *43*, 1295.
(3) Bilodeau, M. T.; Park, T. K.; Hu, S.; Randolph, J. T.; Kim, I. J.; Danishefsky, S. J.; Livingston, P. O.; Zheng, S. Unpublished results.
(4) Bremer, E. G.; Levery, S. B.; Sonnino, S.; Ghidoni, R.; Canevari, S.; Kannagi, R.; Hakomori, S.-i. *J. Biol. Chem.* **1984**, *259*, 14773.
(5) The syntheses of the DEF and CDEF fragments of **1** have been reported recently, and both structures have been shown to bind to MBr1, although strict comparison with our compounds have not been carried out yet: Lay, L.; Nicotra, F.; Panza, L.; Russo, G. *Helv. Chim. Acta* **1994**, *77*, 509. Lay, L.; Panza, L.; Russo, G.; Colombo, D.; Ronchetti, F.; Adobati, E.; Canevari, S. *Ibid.* **1995**, *78*, 533.
(6) Park, T. K.; Kim, I. J.; Danishefsky, S. J. A Total Synthesis of a Stage Specific Pentasaccharide Embryogenesis Marker. *Tetrahedron Lett.*, in press.
(7) (a) Griffith, D. A.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1990**, *112*, 5811. (b) Griffith, D. A.; Danishefsky, S. J. *Ibid.* **1991**, *113*, 5863. (c) Danishefsky, S. J.; Gervay, J.; Peterson, J. M.; McDonald, F. E.; Koseki, K.; Oriyama, T.; Marsden, S. J. *Ibid.* **1995**, *117*, 1990. (d) Griffith, D. A. Ph. D. Thesis, Yale University, 1993.
(8) Behar, V.; Danishfefsky, S. J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*(14), 1468.
(9) Randolph, J. T.; Danishefsky, S. J. *Angew. Chem., Int. Ed. Engl.* **1994** *33*(14), 1470.
(10) Bernstein, M. A.; Hall, J. D. *Carbohydr. Res.* **1980**, *78*, C1.

0022-3263/95/1960-7716$09.00/0

Scheme 2



free hydroxyl group at its C4 position gave a high yield of β-glycoside **18**. This compound was converted to tetrasaccharide glycal **22** which, in turn, was transformed to allyl glycoside **4**.

The acceptor **13b** for hexasaccharide **5** was obtained from the deprotection of the minor product **12b**[11] from the coupling reaction of **8** and **9**. The acceptor **13b** coupled with trisaccharide donor **16a** provided hexasaccharide **19** which was converted to allyl glycoside **5** via **23** in the usual way as shown.

A more interesting challenge involved fashioning an α glycosidic bond connecting rings C and D. For this purpose, we returned to donor **16b** which is very similar to **16a**, except that the critical C_4 oxygen on the donor ring was acetylated.[12] *Remarkably, when compound **16b** was coupled with glycosyl acceptor **13a**, a highly selective α glycosidation occurred to provide **20**.* Compound **20** was taken through a very similar sequence to provide **24** and thence **6**.

With the four allyl glycosides in hand, comparative binding studies to MBr1 were carried out. To ensure clinical relevance, human breast cancer cell line MCF7 was used as target, and increasing amounts (0.05–500 μM) of these glycosides were used to inhibit MBr1 binding. The results from an individual inhibition experiment are demonstrated in Table 1.

These studies indicate that the presence of the terminal glucose (see compound **3**) or the terminal lactose (see compound **4**) are not essential to binding. The failure of **2** to bind to MBr1 teaches that the terminal fucose is critical.[13] Thus, the binding domain has been localized in the CDEF ring system.

Table 1. Inhibition of MAb MBr1 Binding to an MCF-7 Cell Line by Synthetic Antigens

	$IC_{50}{}^a$ (μM)		$IC_{50}{}^a$ (μM)
1b	16	**4**	26
2	>500[b]	**5**	27
3	10	**6**	200

[a] 50% inhibitory concentration. [b] IC_{50} not reached.

The stereochemistry of the glycosidic linkages between rings C and D is, indeed, crucial. Thus, **6** binds very poorly to MBr1 antibody with 50% inhibitory concentration of 200 μM. Once this tetrasaccharide domain has been presented, in required length and in suitable stereochemical detail at the exterior hydroxyl groups and in the glycosidic bonds, the nature of the glycosidic linkages joining rings B and C is of little or no consequence. Thus, it is seen that compound **5** is well bound as are the natural version **1** and truncated versions **3** and **4**.

With this information, it seems that shorter versions of the MBr1 antigen, which can be more readily synthesized, stand a good chance of providing comparable biological performance to the hexacyclic domain in terms of immunization against the epitope of the MBr1 antigen recognized by MBr1 antibody.

Acknowledgment. This research was supported by the National Institutes of Health (Grant No. CA 28824 and CA 61422). We gratefully acknowledge Maria I. Colnaghi and Silvana Canevari for providing MAb MBr1.

Supporting Information Available: Experimental procedures, characterization data and NMR spectra (^1H, ^{13}C) for most of the compounds (**7**, **9**, **11**, **15**, **13b**, **17–19**, **21–24**, and **3–6**), and the procedure of Inhibition assay and % Inhibition of MBR1 mAb graph (50 pages).

JO951534Z

(11) When the coupling was conducted on the epoxide derived from **14** (precursor of **9**, cf. Gordon, D.; Danishefsky, S. J. *Carbohydrate Res.* **1990**, *206*, 361), the β-product predominated substantially. At the present writing the product of that coupling had not been fully merged with the series shown here, but this can certainly be accomplished. Since we were primarily interested in the binding result, we conducted the synthesis from the minor β glycoside **12b** derived from coupling of the fluorosugar **9** and the disaccharide **8**.

(12) The trend that with a sulfonamido donor, such as **16a**, the stereochemistry of glycosidation is critically dependent on whether C4 -OH (see asterisk in **16a** and **16b**) is free or acylated is once again manifested. This capacity to control the sense of glycosylation goes beyond the case shown here and the previous precedent[1] but has not yet been fully generalized.

(13) **2** revealed only 14% inhibition even at 500 μM in a non-dose-dependent manner.