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Automated Synthesis of the Tumor-Associated Carbohydrate Antigens Gb-3 and Globo-H: Incorporation of α-Galactosidic Linkages

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Carbohydrates are displayed on the surface of both normal and tumor cells as glycosphingolipids (GSLs), glycoproteins, and GPI anchors. Cancer cells express altered cell surface glycoconjugates, and some GSLs act as adhesion molecules during tumor cell metastasis.[1]

The GSLs Globo-H (**1**) and Gb-3 (**2**) (Scheme 1) have been identified as antigens of a variety of different cancer types. Globo-H (**1**) is currently being evaluated in clinical trials as an anti-tumor vaccine for the treatment of breast and prostate cancers.[2,3] The trisaccharide glycolipid Gb-3 (**2**) is a receptor for Shiga-like toxins[4] and has recently been implicated in the entry of HIV-1 into cells.[5] Due to their biological importance, these antigens have been the subject of several total syntheses that showcased different methods.[3,6−14]

The Globo series of carbohydrate antigens require the selective installation of a *cis*-galactosidic linkage on the axial C4 hydroxyl of galactose (Scheme 1). The stereochemical outcome of *cis*-glycoside formation cannot be controlled via a C2 participating group. α-Galactosidic linkages have been installed in solution-phase chemistry using a variety of glycosylating agents.[15]

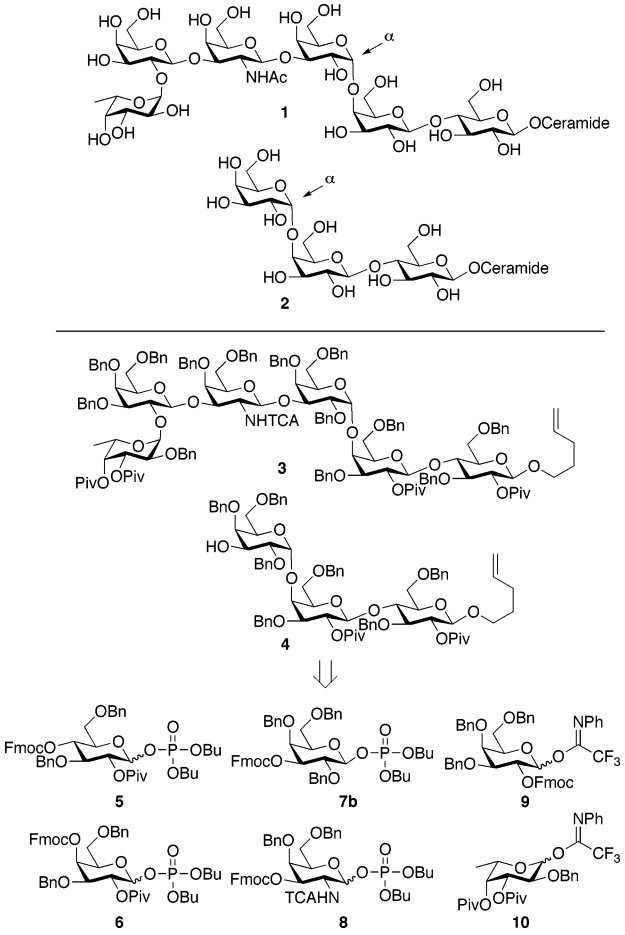
Our initial work on automated oligosaccharide synthesis demonstrated that linear as well as branched carbohydrates can be assembled.[16,18] However, the creation of challenging *cis*-glycosidic linkages such as α-galactosides had not been achieved by automated synthesis.

Here, we describe the automated solid-phase assembly of the protected tumor-associated oligosaccharide antigens Gb-3 (**4**) and Globo-H (**3**). Six building blocks (**5**−**10**) are required for the fully protected Globo-H hexasaccharide **3** (Scheme 1). Each monomer, except the final fucose moiety **10**, contains a temporary protecting group. We chose fluorenylmethoxycarbonyl (Fmoc) that is completely stable under the acidic glycosylation conditions and is readily cleaved by a weak base such as piperidine for temporary protection.[17] The analysis of the Fmoc deprotecting solution provides a quantitative assay for the efficiency of each glycosylation/deprotection cycle during automated assembly.[18]

Installation of the α-galactosidic linkage proved to be crucial for the assembly of Gb-3 trisaccharide **4** in anticipation of the synthesis of the larger oligosaccharide **3**. High α-selectivity is mandatory for the coupling since the solid-phase approach allows for purification only after completion of the synthesis. Investigations aimed at optimizing coupling efficiency and selectivity were performed by automated solid-phase synthesis and are summarized in Table 1. Resin-bound lactose acceptor **11** was assembled by automation using standard building blocks **5** and **6** as detailed in Scheme 2. Support-bound disaccharide **11** was then glycosylated using different galactose building blocks. Coupling efficiency and selectivity were rapidly determined by LC−MS analysis of the cleaved products.

The α- and *β*-glycosyl phosphates containing a C2 participating group differ only in the reaction kinetics.[19] In contrast, the two

Scheme 1. Tumor-Associated Carbohydrate Antigens of the Globo Series **1** and **2** and Their Protected Forms **3** and **4** That Can Be Derived from Monosaccharide Building Blocks **5**−**10**



anomers of building block **7** showed strongly different selectivity (entries 1 and 2). The *β*-anomer **7b** resulted in a significantly better α/*β* ratio on solid support than the corresponding α-anomer **7a** (14:1 vs 4:1). Longer reaction times (3 h) and low temperatures (−50 °C) were required in order to drive the reaction to completion and to achieve the desired selectivity. The *β*-glycosyl trichloroacetimidate **12** showed similar selectivity (entry 3). The presence of a benzoate group in the C4 position (**13**) resulted in poor selectivity and byproduct formation due to migration of the benzoate group during Fmoc deprotection (entry 4). Protected Gb-3 was assembled in 12 h using building blocks **5**, **6**, and **12** (Scheme 2). Cleavage from the solid support by olefin cross-metathesis in the presence of Grubbs' catalyst and HPLC purification yielded pure **4** in 46% yield.[20]

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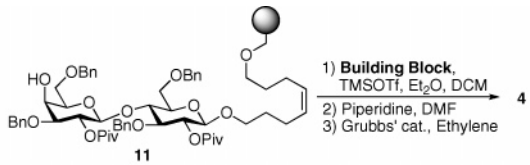
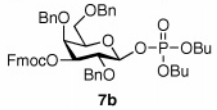
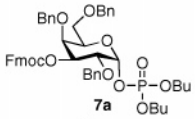
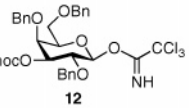
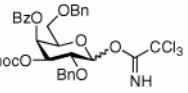
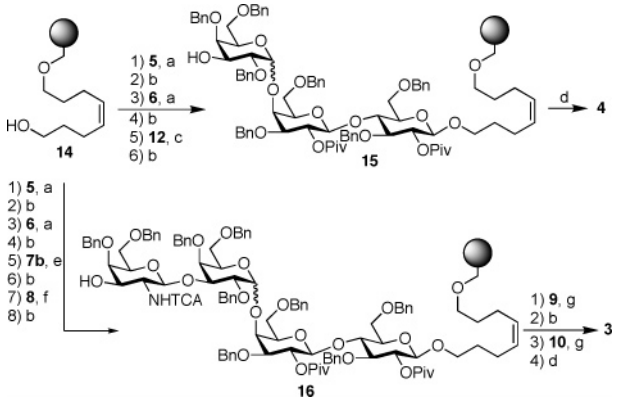
Table 1. Anomeric Selectivity of Gb-3 Using Different Building Blocks



entry	α-galactose building block	α:β selectivity of **4**[a]
1	 **7b**	14:1
2	 **7a**	4:1
3	 **12**	11:1
4	 **13**	4:1

[a] Determined by LC−MC of crude mixtures.

Scheme 2. Automated Synthesis of Trisaccharide **4** and Hexasaccharide **3**[a]



[a] Conditions: (a) building block (5 equiv), TMSOTf (5 equiv), DCM, −15 °C, repeated once for 45 min each; (b) piperidine (20% in 2 mL of DMF), repeated twice for 5 min each; (c) building block (5 equiv), TMSOTf (0.5 equiv), DCM, −30 °C, repeated once for 1 h each; (d) Grubbs' catalyst (first generation), ethylene atmosphere, $CHCl_2$, rt, overnight; (e) building block (5 equiv), TMSOTf (5 equiv), Et_2O, DCM, −50 °C, repeated once for 3 h each; (f) building block (3.3 equiv), TMSOTf (3.3 equiv), DCM, −15 °C, repeated twice for 25 min each; (g) building block (5 equiv), TMSOTf (0.5 equiv), DCM, −10 °C, repeated once for 25 min each.

After establishing a procedure to introduce α-galactosidic linkages with high selectivity, protected Globo-H hexasaccharide was prepared. Building blocks **5**, **6**, **7b**, and **8** were used to assemble the resin-bound tetrasaccharide **16**. This tetrasaccharide was unstable in the presence of stoichiometric amounts of Lewis acid necessary for the activation of glycosyl phosphate building blocks. Glycosyl *N*-phenyl trifluoroacetimidate building blocks **9** and **10** performed well under mild conditions.[21] Assembly of the hexasaccharide required 25 h before cleavage from the polymer support by olefin cross-metathesis yielded the crude product. LC−MS analysis of the crude mixture obtained by cleavage from the support after assembly was employed to assess the outcome of the automated synthesis (Figure 1). The desired product **3** (17.7 min) is the major product along with small amount of the β-anomer (17.2 min) and some deletion sequences. Careful purification by column chromatography afforded **3** in 30% overall yield from resin **16**. Cleavage of all protective groups on hexasaccharide **3** was performed under Birch conditions as established earlier.[14]

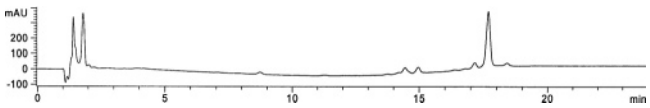


Figure 1. HPLC trace of crude, fully protected Globo-H (**3**) after automated synthesis and cleavage from solid support (UV absorbance at 209 nm).

In conclusion, the automated assembly of two tumor-associated carbohydrate antigens is reported. A solution for the construction of α-galactosidic linkages on solid support is presented in the context of linear syntheses of the complex oligosaccharides Globo-H and Gb-3. LC−MS analysis has become an important tool to monitor rapidly the success and selectivity of oligosaccharide syntheses. Research focusing on the construction of other challenging linkages such as β-mannosidic linkages and the incorporation of sialic acid into automated synthesis protocols is ongoing.

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Supporting Information Available: Detailed procedures for automated assembly, analytical data, and spectra for the compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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