[25] A. C. Larson, R. B. v. Dreele, *Los Alamos Laboratory, Rep. No. LA-UR* **1987**, *86*, 748; PC version **1996**.
[26] O. K. Andersen, C. Arcangeli, R. W. Tank, T. Saha-Dasgupta, G. Krier, O. Jepsen, I. Dasgupta, *Tight-Binding Approach to Computational Materials Science*, MRS Symposia Proceedings, no. 491, MRS, Pittsburgh, **1998**; G. Krier, O. Jepsen, A. Burkhardt, O. K. Andersen, *Das TB-LMTO-ASA-Programm*, Version 4.7.
[27] U. von Barth, L. Hedin, *J. Phys. C* **1972**, *5*, 1629.

## A Two-Directional and Highly Convergent Approach for the Synthesis of the Tumor-Associated Antigen Globo-H**

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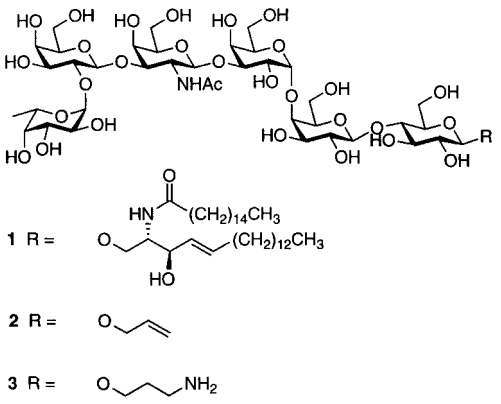
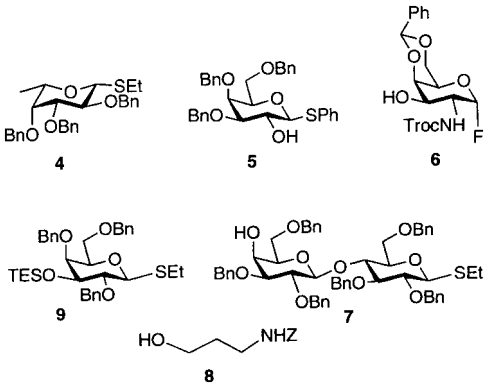
Human cancer cells are often characterized by the presence of tumor-associated glycosphingolipids (GSL).[1] Several GSL antigens have been identified as adhesion molecules that may promote tumor cell metastases. Active immunization with GSL can induce or enhance antibody titers and several studies indicate that these antibodies can suppress metastasis.[2]

Danishefsky and co-workers synthesized the saccharide moiety of the tumor-associated antigen Globo-H[3] (**1**) using the glycal assembly strategy. The final product was substituted with an anomeric allyl moiety (→**2**), which after oxidation to an aldehyde moiety, allowed coupling to the carrier protein




keyhole limpet hemocyanin (KLH, *Megathura crenulata*). High levels of antibodies in patients with progressive and recurrent prostrate cancer could be raised by vaccination with the conjugate.[4] Schmidt and co-workers prepared the protected Globo-H hexasaccharide by employing the trichloroacetimidate methodology.[5]

Herein we report an alternative approach for a highly efficient synthesis of the Globo-H hexasaccharide **3**. For the first time we demonstrate that a hexasaccharide can be assembled in five consecutive glycosylations without the need for any intermediate protecting group manipulations. The new approach gives the readily available building blocks **4, 5**, **6**, **7**, **8**, and **9** (Scheme 1) of the protected hexasaccharide **19**, which was deprotected to yield target compound **3** (Scheme 3). The key feature of the new glycosylation




Scheme 1. Building blocks for **3**.

sequence is a combination of two-directional glycosylation approaches (see below) with chemoselective and orthogonal glycosylations.[6] These strategies exploit both the differences in the reactivities of anomeric leaving groups and the subtle control of nucleophilicities of sugar hydroxyl groups and silyl ethers. The aminopropyl spacer was incorporated for well-defined conjugation to a carrier protein.[7]

Classic strategies for oligosaccharide assembly are characterized by the manipulation of protecting groups between each glycosylation step. Such manipulations increase the linearity and decrease the efficiency of oligosaccharide assembly. Two-directional glycosylation strategies and chemoselective- or orthogonal-glycosylation approaches condense the process of oligosaccharide synthesis by removing the need for unmasking procedures. In a two-directional glycosylation strategy a saccharide derivative first acts as a glycosyl donor and the resulting product is immediately used as a glycosyl acceptor in the next coupling step.[8] This reaction sequence can be performed with glycosyl donors and acceptors that both have a free hydroxyl group. It is critical that the hydroxyl group of the glycosyl acceptor is significantly more reactive than the hydroxyl group of the glycosyl donor to avoid self-condensation of the glycosyl donor. A complementary method exploits the finding that a thioglycoside protected as a silyl ether (for example, triethylsilyl, *tert*-butyldimethylsilyl) can act both as a glycosyl donor and acceptor. In general, silyl ethers are sufficiently stable under *N*-iodosuccinimide/trimethylsilyl trifluoromethanesulfonate (NIS/TMSOTf)[9] or iodonium dicollidine perchlorate (IDCP)[10] mediated glycosylations. Thioglycosides of these derivatives, therefore, can act as glycosyl donors. However, the products of these coupling reactions are suitable acceptors[11] when glycosylated with glycosyl fluorides in the presence of $[Cp_2ZrCl_2]$/AgOTf (Cp=cyclopentadienyl, Tf = trifluoromethanesulfonyl).[12] Chemoselective glycosylation strategies are

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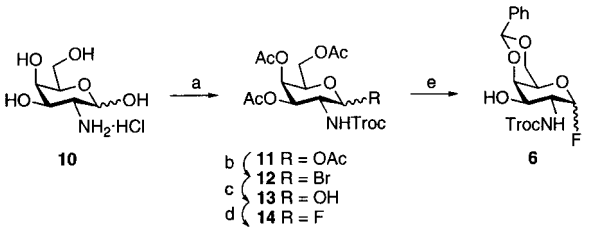
based on the ability to control the reactivity of anomeric leaving groups in such a way that building blocks can be coupled with decreasing anomeric reactivities without the need for any protecting-group manipulations between the glycosylations. Orthogonal-glycosylation approaches use saccharide building blocks containing leaving groups with orthogonal reactivities that can therefore be activated independently.

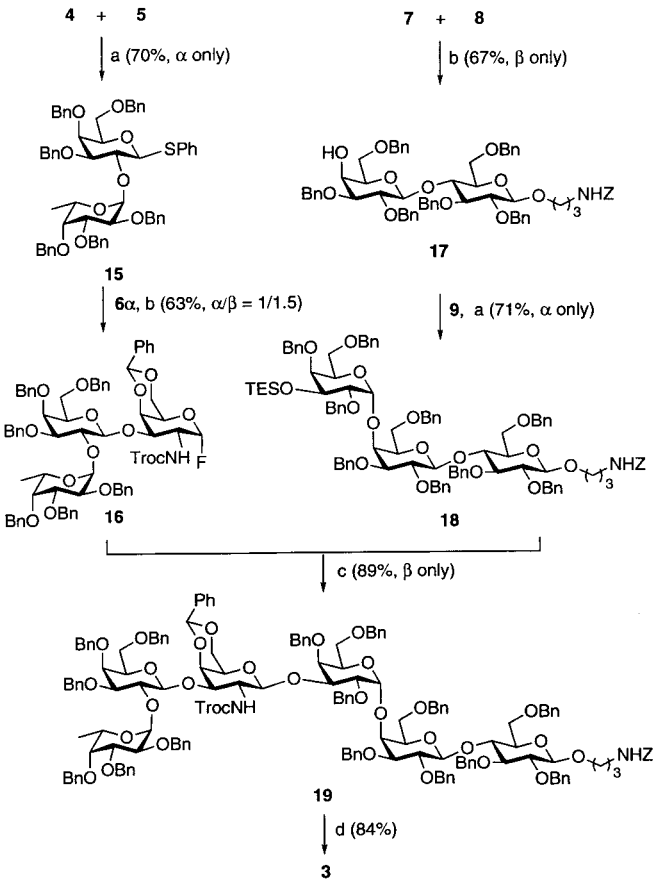
The building blocks **4**,[13] **5**,[14] **7**,[15] and **9**[16] were synthesized by standard manipulations. The preparation of glycosyl fluoride **6** was more complicated (Scheme 2). The synthesis started with the protection of the amino functionality of the




Scheme 2. Synthesis of building block **6**. a) TrocCl, $NaHCO_3$, $H_2O$ then $Ac_2O$, pyridine (78% over two steps); b) HBr/HOAc (33%), $CH_2Cl_2$; c) $Ag_2CO_3$, acetone/water (100% over two steps); d) DAST, $CH_2Cl_2$, $-40\,^{\circ}C$ (92%, $\alpha:\beta = 5:1$); e) $K_2CO_3$, MeOH (75%) then $PhCH(OMe)_2$, CSA, MeCN (78%).

commercially available *N*-acetylgalactosamine hydrochloride (**10**) as a 2,2,2-trichloroethylcarbamate by reaction with 2,2,2-trichlorethyloxycarbonyl chloride (TrocCl) in aqueous sodium bicarbonate.[17] The product obtained was acetylated with acetic anhydride in pyridine to give compound **11**[18] in an overall yield of 78%. The anomeric acetyl moiety of **11** was replaced by a fluoride atom in a three-step procedure. Thus, treatment of **11** with HBr in acetic acid/dichloromethane gave bromide **12**,[17] which was immediately hydrolyzed with $Ag_2CO_3$ in acetone/water to give lactol **13**[19] in a quantitative overall yield. Treatment of **13** with diethylaminosulfur trifluoride (DAST)[20] in dichloromethane gave the glycosyl fluoride **14** as a separable mixture of anomers ($\alpha:\beta = 5:1$). The individual anomers of **14** were converted into the partially-protected derivatives **6**$\alpha/\beta$ (overall yield 78%) by removal of the *O*-acetyl protecting groups with potassium carbonate in methanol followed by treatment with benzaldehyde dimethylacetal in acetonitrile in the presence of a catalytic amount of camphorsulfonic acid (CSA). This reaction sequence demonstrates that glycosyl fluorides can undergo several protecting group manipulations and are stable to mildly acidic and basic reaction conditions.

With significant quantities of building blocks in hand, the assembly of fully protected hexasaccharide **19** could be performed (Scheme 3). Thus, IDCP-mediated glycosylation of the fully protected thioglycosyl donor **4** with semi-protected glycosyl acceptor **5** in dichloromethane/diethyl ether gave **15** as a single anomer in a 70% yield (Scheme 3). The observed chemoselectivity is based on the fact that 6-deoxy-sugars are more reactive glycosyl donors than their 6-hydroxy counterparts. Furthermore, thioethyl glycosides are considerably more reactive than analogous thiophenyl glycosides.[21] The anomeric thiophenyl moiety of **15** could be activated with the more thiophilic reagent NIS/TMSOTf and reaction with the glycosyl fluoride **6**$\alpha$ gave trisaccharide **16** as a separable mixture of anomers.[22] An intractable mixture of products was obtained when the same reaction was performed with **6**$\beta$, probably as a result of the $\beta$-fluoride being much more reactive and activated under the glycosylation conditions. The lower reactivity of the $\alpha$-anomer can be rationalized on the basis of its ground state being stabilized by a strong anomeric effect. Also a low recovery of trisaccharide was obtained when a derivative of **6** was used in which the Troc protecting group was replaced by a phthalimido group. In this case the bulky phthalimido group shields the hydroxyl group at C-3 and thus reduces its nucleophilicity.




Scheme 3. Synthesis of hexasaccharide **3**. a) IDCP, $CH_2Cl_2/Et_2O$ (1/5), 4-Å molecular sieves; b) NIS/TMSOTf, 4 Å molecular sieves, MeCN, $-40\,^{\circ}C$; c) $Cp_2ZrCl_2$, AgOTf, $CH_2Cl_2$, 4-Å molecular sieves; d) 1. Zn, HOAc; 2. $Ac_2O$, pyridine; 3. $Pd(OAc)_2$, EtOH, $H_2$.

Next we focused on the assembly of trisaccharide **18**. Thus, the NIS/TMSOTf-mediated glycosylation of the partially protected lactosyl donor **7** with spacer **8** gave **17** as a pure $\beta$-anomer in a yield of 67%. Mass spectroscopic analyses of the crude reaction mixture revealed no self-condensation or oligomerization of **7**. This expected result is based on the higher reactivity of the primary hydroxyl group of **8** relative to the secondary axial hydroxyl group of **7**. The anomeric selectivity of this glycosylation is based on the formation of an intermediate $\alpha$-nitrilium ion,[21] which is substituted by the alcohol to give a $\beta$-anomer.[23, 24] Compound **17** was immedi-

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ately used in an IDCP-mediated glycosylation with **9** to give the trisaccharide **18** as the only anomer.

The fully-protected hexasaccharide **19** could be obtained by the coupling of trisaccharides **16** with **18** in the presence of $[Cp_2ZrCl_2]$/AgOTf in dichloromethane. The reaction was almost instantaneous and the product was isolated in an 89% yield. No desilylated acceptor was observed and the reaction was significantly slower when the coupling was performed with a trisaccharide acceptor that had a free hydroxyl group. These results indicate that a triethylsilyl (TES) moiety activates the glycosyl acceptor in a $[Cp_2ZrCl_2]$/AgOTf-mediated glycosylation. However, the same protecting group is perfectly stable in an IDCP-mediated glycosylation.

Compound **19** was deprotected in a three-step procedure. Thus, the Troc protecting group was removed by treatment with activated zinc in acetic acid and the resulting amino functionality was acetylated with acetic anhydride in pyridine. Finally, the benzyl ethers, the benzylidene acetal, and the benzyloxycarbonyl moiety were removed by catalytic hydrogenation over palladium to give the target compound **3** in a yield of 84%. Analysis of the product by 1D ($^1$H, gocsy; 800 MHz) and 2D NMR spectroscopy (gCOSY, HSQC, TOCSY, and gHMBC) confirmed the correct anomeric configuration of each glycosidic linkage.[25]

In conclusion, we have described a highly convergent approach for the synthesis of a spacer-modified hexasaccharide derived from the tumor-associated antigen Globo-H. The fully-protected hexasaccharide **19** could be efficiently assembled within five glycosylation steps after the preparation of the readily available building blocks. It is to be expected that similar strategic principles can be used for the synthesis of other oligosaccharides of biological importance.

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[1] S. Hakomori, *Acta Anat.* **1998**, *161,* 79 – 90.
[2] T. Toyokuni, A. K. Singhal, *Chem. Soc. Rev.* **1995**, 231 – 242.
[3] a) M. T. Bilodeau, T. K. Park, S. Hu, J. T. Randolph, S. J. Danishefsky, P. O. Livingston, S. Zhang, *J. Am. Chem. Soc.* **1995**, *117*, 7840 – 7841; the syntheses of fragments of compound **1** have been reported: b) S. Nunomura, T. Ogawa, *Tetrahedron Lett.* **1988**, *29*, 5681 – 5684; c) L. Lay, F. Nicotra, L. Panza, G. Russo, *Helv. Chim. Acta.* **1994**, *77*, 509 – 514; d) L. Lay, L. Panza, G. Russo, D. Colombo, F. Ronchetti, E. Adobati, S. Canevari, *Helv. Chim. Acta.* **1995**, *78*, 533 – 538.
[4] a) G. Ragupathi, T. K. Park, S. Zhang, I. J. Kim, L. Graber, S. Adluri, K. O. Lloyd, S. J. Danishefsky, P. O. Livingston, *Angew. Chem.* **1997**, *109*, 66 – 69; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 125 – 128; b) G. Ragupathi, S. F. Slovin, S. Adluri, D. Sames, I. J. Kim, H. M. Kim, M. Spassova, W. G. Bornmann, K. O. Lloyd, H. I. Scher, P. O. Livingston, S. J. Danishefsky, *Angew. Chem.* **1999**, *111*, 592 – 594; *Angew. Chem. Int. Ed.* **1999**, *38*, 563 – 566.
[5] J. M. Lassaletta, R. R. Schmidt, *Liebigs Ann.* **1996**, 1417 – 1423.
[6] For a review on strategies in oligosaccharide synthesis, see G. J. Boons, *Tetrahedron* **1996**, *52*, 1095 – 1121.
[7] H. J. Jennings, R. K. Snood, in *Neoglycoconjuates, preparation and application* (Eds.: Y. C. Lee, R. T. Lee), Academic, San Diego, **1994**, p. 325.
[8] a) G. J. Boons, T. Zhu, *Synlett* **1997**, *7*, 809 – 811; b) T. Zhu, G.-J. Boons, *Angew. Chem.* **1998**, *110*, 2000 – 2003; *Angew. Chem. Int. Ed.* **1998**, *37*, 1898 – 1900.
[9] a) G. H. Veeneman, S. H. van Leeuwen, J. H. van Boom, *Tetrahedron Lett.* **1990**, *31*, 1331 – 1334; b) P. Konradsson, U. E. Udodong, B. Fraser-Reid, *Tetrahedron Lett.* **1990**, *31*, 4313 – 4316.
[10] G. H. Veeneman, J. H. van Boom, *Tetrahedron Lett.* **1990**, *31*, 275 – 278.
[11] a) T. Ziegler, U. Seidl, *J. Carbohydr. Chem.* **1991**, *10*, 813 – 831; b) T. Ziegler, K. Neumann, E. Eckhardt, G. Herold, G. Pantkowski, *Synlett* **1991**, *10*, 699 – 701; c) T. Zhu, G. J. Boons, *Tetrahedron Lett.* **1998**, *39*, 2187 – 2190.
[12] K. Suzuki, H. Maeta, T. Suzuki, T. Matsumoto, *Tetrahedron Lett.* **1989**, *30*, 6879 – 6882.
[13] H. Lönn, *Carbohydr. Res.* **1985**, 105 – 114.
[14] L. Yan, D. Kahne, *J. Am. Chem. Soc.* **1996**, *118,* 9239 – 9248.
[15] J. M. Lassaletta, R. R. Schmidt, *Synlett* **1995**, 925 – 927.
[16] D. Seebach, H. Chow, R. F. W. Jackson, M. A. Sutter, S. Tahisrivongs, J. Zimmermann, *Liebigs Ann. Chem.* **1986**, 1281 – 1308.
[17] a) T. B. Windlholz, D. B. R. Johnston, *Tetrahedron Lett.* **1967**, *8*, 2555 – 2557; b) M. Imoto, H. Yoshimura, T. Shimamoto, B. Sakaguchi, S. Kusumoto, T. Shiba, *Bull. Chem. Soc. Jpn.* **1987**, *60*, 2205 – 2214.
[18] K. Higashi, H. Susaki, *Chem. Pharm. Bull.* **1992**, *40*, 2019 – 2022.
[19] J. C. Castro-Palomino, G. Ritter, S. R. Fortunato, S. Reinhardt, L. J. Old, R. R. Schmidt, *Angew. Chem.* **1997**, *109*, 2081 – 2085; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1998 – 2001.
[20] G. H. Posner, S. R. Haines, *Tetrahedron Lett.* **1985**, *26*, 5 – 8.
[21] a) H. M. Zuurmond, S. C. van der Laan, G. A. van der Marel, J. H. van Boom, *Carbohydr. Res.* **1991**, *215*, C1 – 3; b) H. M. Zuurmond, G. H. Veeneman, G. A. van der Marel, J. H. van Boom, *Carbohydr. Res.* **1993**, *241*, 153 – 164; c) Z. Zhang, I. R. Ollmann, X. Ye, R. Wischnat, T. Bassov, C. H. Wong, *J. Am. Chem. Soc.* **1999**, *121*, 734 – 753.
[22] The low anomeric selectivity achieved in the synthesis of disaccharide **15** is a drawback of the presented strategy. Compound **16** was obtained in a stereoselective manner and higher overall yield by using building blocks **4**, **6**, and phenyl-3,4,6-tri-*O*-benzyl-2-*O*-levulinoyl-1-thio-$\beta$-D-galactopyranoside. In this synthetic approach, however, two additional synthetic steps (introduction and removal of a levulinoyl group) were required.
[23] a)R. R. Schmidt, J. Michel, *J. Carbohydr. Chem.* **1985**, *4*, 141 – 169; b) R. R. Schmidt, M. Behrendt, A. Toepfer, *Synlett* **1990**, 694 – 696; c) Y. D. Vankar, P. S. Vankar, M. Behrendt, R. R. Schmidt, *Tetrahedron* **1991**, *47*, 9985 – 9988; d) R. R. Schmidt, H. Gaden, H. Jatzke, Tetrahedron Lett. **1990**, *31*, 327 – 330.
[24] Other research groups have also employed glycopyranosyl acetonitrilium ions in carbohydrate chemistry. For example, see a) J. Pougny, P. Sinaÿ, *Tetrahedron Lett.* **1976**, 4073 – 4076; b) R. U. Lemieux, R. M. Ratcliffe, *Can. J. Chem.* **1979**, *57*, 1244 – 1251; c) A. I. Rattcliffe, B. Fraser-Reid, *J. Chem. Soc. Perkin Trans. 1* **1990**, 747 – 750.
[25] Selected NMR data: $^1$H NMR ($D_2O$, 800 MHz): $\delta = 5.14$ (d, $^3J$(H,H) = 2.7 Hz, 1H; H-1‴′), 4.80 (d, $^3J$(H,H) = 3.7 Hz, 1H; H-1″), 4.52 (d, $^3J$(H,H) = 7.7 Hz, 1H; H-1‴), 4.46 (d, $^3J$(H,H) = 7.7 Hz, 1H; H-1‴′), 4.42 (2d, $^3J$(H,H) = 7.3 Hz, 8.1 Hz, 2H; H-1, H-1′); $^{13}$C NMR ($D_2O$, 150 MHz): $\delta = 103.9$ (C-1‴), 103.2 (C-1, C-1′), 101.7 (C-1‴′), 100. 7 (C-1″), 99.5 (C-1‴″).

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