

Automated Synthesis of a Protected *N*-Linked Glycoprotein Core Pentasaccharide

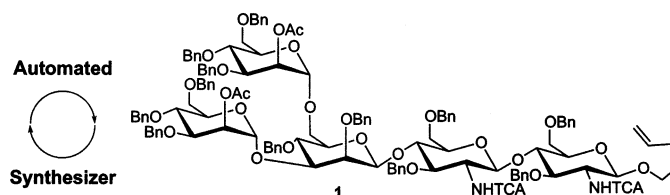
Daniel M. Ratner, Erika R. Swanson, and Peter H. Seeberger*[†]

Department of Chemistry, Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139

seeberger@org.chem.ethz.ch

Received September 27, 2003

ABSTRACT



Described is the first automated solid-phase synthesis of the core *N*-linked pentasaccharide, common to all *N*-linked glycoproteins via stepwise assembly from mono- and disaccharide building blocks. The challenging β -mannosidic linkage was incorporated by the inclusion of a disaccharide trichloroacetimidate. This automated synthesis provides rapid access to an oligosaccharide common to an entire class of glycoconjugates.

Co-translational modification of proteins by glycosylation of asparagine residues includes three classes of *N*-linked oligosaccharides: high-mannose, hybrid, and complex-type mannans.¹ In addition to the many functions of these branched glycans in mammalian cells, they are found on the glycoproteins of a variety of pathogens, including the viral envelope of HIV,² Ebola,³ and some coronaviruses.⁴ Rapid and reliable access to these branched glycans by automated synthesis would facilitate further investigation into the biological role of these glycoconjugates and their potential application as carbohydrate-based vaccines.⁵ Currently, synthetic *N*-glycans are used to study carbohydrate/protein interactions using isothermal calorimetry,⁶ carbohydrate arrays,⁷ and the structural analysis of such complexes (X-ray, NMR).⁸

The three major classes of *N*-linked glycans contain a common core pentasaccharide (Figure 1) that has been a

target of several recent syntheses in solution⁹ and on solid support.¹⁰ This pentasaccharide contains a number of synthetic challenges, including branching, β -(1 \rightarrow 4) glucosamine linkages, and most notably, the daunting β -mannoside.

Described is the first automated solid-phase synthesis of the *N*-linked core pentasaccharide **1**. Retrosynthetic analysis of **1** revealed that the target could be accessed using just three distinct building blocks, two monosaccharides **2**, **3**,¹¹ and one disaccharide **4** (Figure 2). To avoid anomeric mixtures on the solid support, the β -mannosidic linkage was

(5) Calarese, D. A.; Scanlan, C. N.; Zwick, M. B.; Deechongkit, S.; Mimura, Y.; Kunert, R.; Zhu, P.; Wormald, M. R.; Stanfield, R. L.; Roux, K. H.; Kelly, J. W.; Rudd, P. M.; Dwek, R. A.; Katinger, H.; Burton, D. R.; Wilson, I. A. *Science* **2003**, *300*, 2065.

(6) Shenoy, S. R.; Barrientos, L. G.; Ratner, D. M.; O'Keefe, B. R.; Seeberger, P. H.; Gronenborn, A. M.; Boyd, M. R. *Chem. Biol.* **2002**, *9*, 1109.

(7) Adams, E. W.; Uberfeld, J.; Ratner, D. M.; O'Keefe, B. R.; Walt, D. R.; Seeberger, P. H. *Angew. Chem., Int. Ed.* **2003**, *42*, in press.

(8) Barrientos, L. G.; Louis, J. M.; Ratner, D. M.; Seeberger, P. H.; Gronenborn, A. M. *J. Mol. Biol.* **2003**, *325*, 211. Botos, I.; O'Keefe, B. R.; Shenoy S. R.; Cartner, L. K.; Ratner, D. M.; Seeberger, P. H.; Boyd, M. R.; Wlodawer, A. *J. Biol. Chem.* **2002**, *277*, 34336.

(9) For a review of recent solution-phase syntheses of the core pentasaccharide, see ref 18.

(10) Wu, X.; Grathwohl, M.; Schmidt, R. R. *Angew. Chem., Int. Ed.* **2002**, *41*, 4489.

(11) Mayer, T. G.; Kratzer, B.; Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2177.

[†] Current Address: Laboratorium für Organische Chemie, ETH Hönggerberg, Zurich, Switzerland.

(1) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683. Imperiali, B.; O'Connor, S. E. *Curr. Opin. Chem. Biol.* **1999**, *3*, 643.

(2) Feizi, T. Glycobiology of AIDS. *Carbohydrates in Chemistry and Biology*; Wiley-VCH: New York, 2000; Vol. 4, pp 851–863.

(3) Lin, G.; Simmons, G.; Pohlmann, S.; Baribaud, F.; Ni, H. P.; Leslie, G. J.; Haggarty, B.; Bates, P.; Weissman, D.; Hoxie, J. A.; Doms, R. W. *J. Virol.* **2003**, *77*, 1337.

(4) Delmas, B.; Laude, H. *Virus Res.* **1991**, *20*, 107.

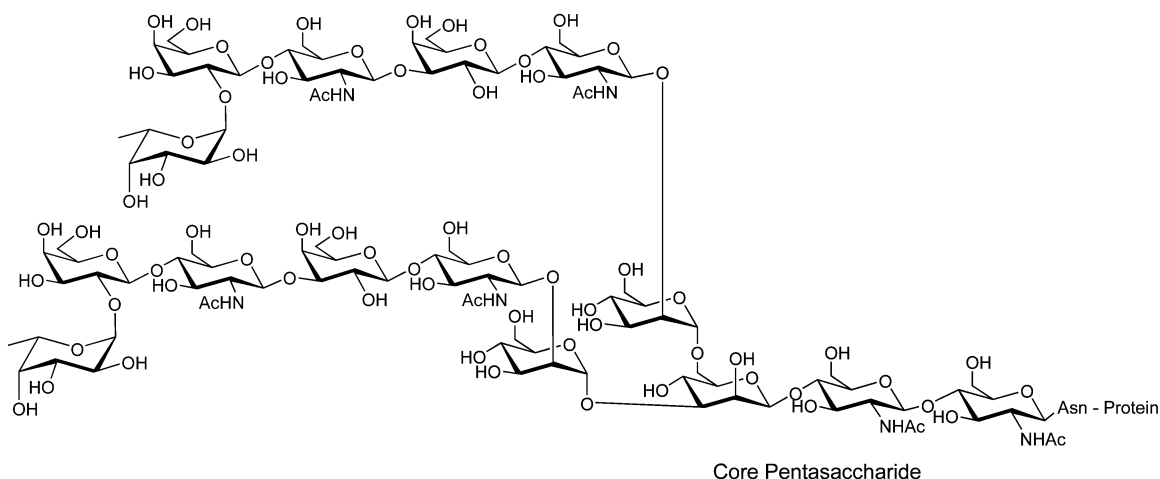


Figure 1. Complex-type *N*-glycan containing the core-pentasaccharide.

to be incorporated during the preparation of disaccharide **4**. Branching would be achieved via the simultaneous dimannosylation of the trisaccharide core by addition of mannosyl trichloroacetimidate **3**.

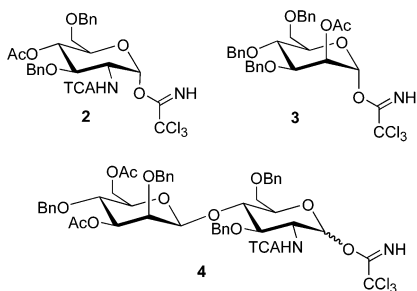


Figure 2. Building blocks used for the automated synthesis of **1**.

Upon identification of the building blocks necessary for the synthesis, glycosyl trichloroacetimidate **2** was prepared from known differentially protected glucosamine **5**¹² (Scheme 1). The 4,6-*O*-benzylidene was opened selectively by treatment with TES/TFA/TFAA to afford **6** in 85% yield. Subsequent acetylation of the C4 hydroxyl (99% yield),

followed by desilylation and treatment with DBU and trichloroacetonitrile, furnished glycosyl trichloroacetimidate **2** in 76% yield.

Disaccharide trichloroacetimidate **4** was prepared via direct β -mannosylation using the Crich method¹³ (Scheme 2). Mannosylation of **6** by treatment of sulfoxide **7**¹⁴ with triflic anhydride and di-*tert*-butyl pyridine furnished the β -linked disaccharide in 68% yield. This procedure efficiently installed the β -mannosidic linkage, without the need for tedious chromatographic separation of an anomeric mixture.

The C3 *p*-methoxy benzyl ether was replaced with the base-labile acetate ester to yield **8** in 79% over two steps. Selective opening of the 4,6-*O*-benzylidene to expose the primary C6 hydroxyl was achieved by treatment with dichlorophenylborane and triethylsilane.¹⁵ Subsequent acetylation yielded differentially protected disaccharide **9** (82%, two steps). Access to the disaccharide glycosyl trichloroacetimidate was readily achieved by desilylation followed by treatment with trichloroacetonitrile and DBU to give donor **4** in 89% yield.

With the necessary building blocks **2**, **3**, and **4** in hand, we proceeded to the automated synthesis using octenediol functionalized Merrifields resin **10** and an automated oligosaccharide synthesizer (Scheme 3).¹⁶ The automated assembly made use of five programmed modules (Table 1): (A) glycosylation, consisting of the addition of 3.5 equiv of

Scheme 1 Synthesis of Glycosyl Trichloroacetimidate **2**

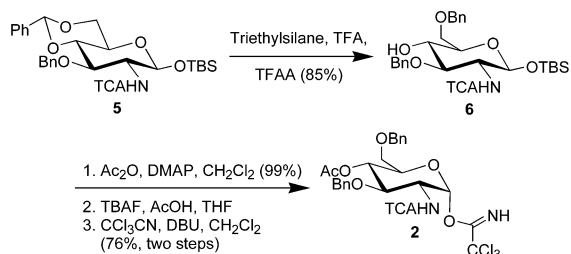
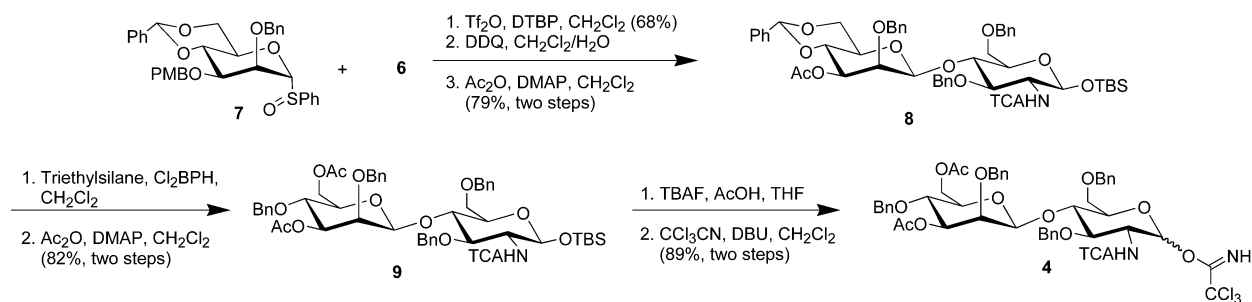


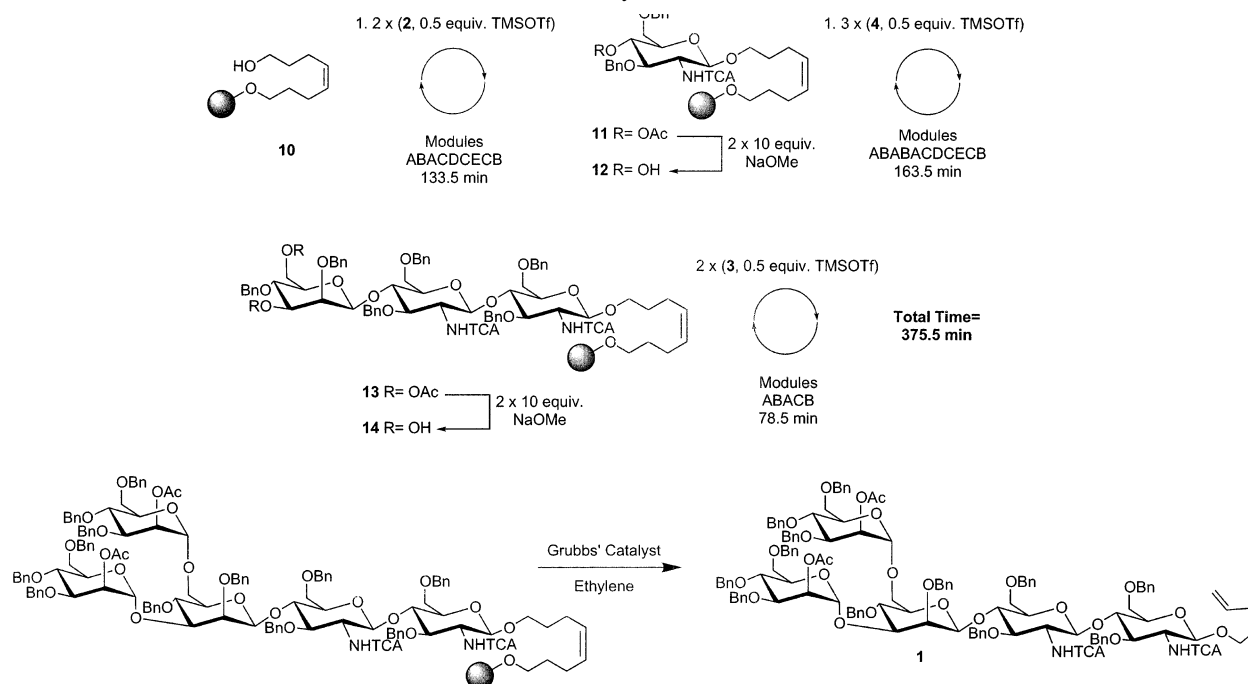
Table 1. Conditions and Reagents Used in the Automated Synthesis of **1**

module	function	reagent	time (min)
A	glycosylation	3.5 equiv of donor and TMSOTf	21
B	wash	CH ₂ Cl ₂	9
C	wash	THF	9.5
D	deprotection	2 × 10 equiv of NaOMe	33
E	wash	0.2 M AcOH/THF	12

Scheme 2. Synthesis of Disaccharide Trichloroacetimidate Building Block 4



Scheme 3. Automated Synthesis of Pentasaccharide 1



donor and catalytic amounts of TMSOTf; (B) methylene chloride wash; (C) THF wash; (D) acetate deprotection by the addition of 10 equiv of sodium methoxide in methanol twice; and (E) pH neutralization with 0.2 M acetic acid in THF.

Glycosylation of the linker with **2** (repeated once) utilized C2-trichloroacetamide participation to ensure anomeric selectivity at the reducing end. Glycosylation with disaccharide donor **4**, determined by solution-phase model studies to be the most challenging step, was repeated three times to ensure complete addition to the support-bound acceptor. Finally,

branching was introduced by glycosylation with mannosyl donor **3** via a simultaneous dimannosylation of the C3 and C6 hydroxyl groups.

Following the final glycosylation, the resin was thoroughly washed and dried. Cleavage of the octenediol linker by olefin cross-metathesis was performed using Grubbs catalyst in an atmosphere of ethylene to furnish the *n*-pentenyl glycoside.¹⁷ The resulting crude product, core pentasaccharide **1**, was purified by semipreparative HPLC. Relative peak area analysis by HPLC showed 27% desired product **1**, with the remainder of isolated side-products consisting of (*n* - 1) and (*n* - 2) deletion sequences.

Starting from the monosaccharide and disaccharide glycosyl donors, the desired pentasaccharide target was assembled and purified in less than 3 days. This and other solid-phase oligosaccharide synthesis studies show that synthetically

(12) Melean, L. G.; Love, K. R.; Seeberger, P. H. *Carbohydr. Res.* **2002**, *337*, 1893.

(13) Crich, D.; Sun, S. X. *J. Am. Chem. Soc.* **1997**, *119*, 11217. Crich, D.; Sun, S. X. *Tetrahedron* **1998**, *54*, 8321.

(14) Crich, D.; Li, H. M.; Yao, Q. J.; Wink, D. J.; Sommer, R. D.; Rheingold, A. L. *J. Am. Chem. Soc.* **2001**, *123*, 5826.

(15) Sakagami, M.; Hamana, H. *Tetrahedron Lett.* **2000**, *41*, 5547.

(16) Plante, O. J.; Palmacci, E. R.; Seeberger, P. H. *Science* **2001**, *291*, 1523.

(17) Andrade, R. B.; Plante, O. J.; Melean, L. G.; Seeberger, P. H. *Org. Lett.* **1999**, *1*, 1811.

challenging and structurally diverse oligosaccharides can be rapidly prepared. While existing methods for the construction of large oligosaccharides have been immensely successful, access to the mono- and disaccharide glycosyl donors

(18) Yamazaki, F.; Kitajima, T.; Nukada, T.; Ito, Y.; Ogawa, T. *Carbohydr. Res.* **1990**, *201*, 15. Unverzagt, C. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1102. Meritt, J. R.; Naisang, E.; Fraser-Reid, B. *J. Org. Chem.* **1994**, *59*, 4443. Dan, A.; Ito, Y.; Ogawa, T. *J. Org. Chem.* **1995**, *60*, 4680. Matsuo, I.; Nakahara, Y.; Ito, Y.; Nukada, T.; Ogawa, T. *Bioorg. Med. Chem.* **1995**, *3*, 1455. Dan, A.; Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1995**, *36*, 7487. Seeberger, P. H.; Cirillo, P. F.; Hu, S. H.; Beebe, X.; Bilodeau, M. T.; Danishefsky, S. J. *Enantiomer* **1996**, *1*, 311. Guo, Z. W.; Nakahara, Y.; Nakahara, Y.; Ogawa, T. *Bioorg. Med. Chem.* **1997**, *5*, 1917. Danishefsky, S. J.; Hu, S.; Cirillo, P. F.; Eckhardt, M.; Seeberger, P. H. *Chem. Eur. J.* **1997**, *3*, 1617. Matsuo, I.; Isomura, M.; Ajisaka, K. *J. Carbohydr. Chem.* **1999**, *18*, 841. Takatani, M.; Nakama, T.; Kubo, K.; Manabe, S.; Nakahara, Y.; Ito, Y.; Nakahara, Y. *Glycoconjugate. J.* **2000**, *17*, 361. Wang, Z. G.; Zhang, X. F.; Live, D.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2000**, *39*, 3652. Dudkin, V. Y.; Miller, J. S.; Danishefsky, S. J. *Tetrahedron Lett.* **2003**, *44*, 1791. Miller, J. S.; Dudkin, V. Y.; Lyon, G. J.; Muir, T. W.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2003**, *42*, 431. Pratt, M. R.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2003**, *125*, 6149.

remains one of the most challenging aspects of synthetic carbohydrate chemistry—leaving room for further advancement in the field.

Acknowledgment. Financial support from Alfred P. Sloan (Fellowship to P.H.S.), GlaxoSmithKline (Fellowship to P.H.S.), Merck & Co. (Academic Development Award to P.H.S.), the NSF (CHE-9808061 and DBI-9729592) for providing NMR facilities, and the NIH (Biotechnology Training Program for D.M.R.) is gratefully acknowledged. We also thank Kerry R. Love for her assistance in the operation of the automated oligosaccharide synthesizer.

Supporting Information Available: Spectral data for all new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

OL035887T