## **Probing the Frontiers of Glycoprotein Synthesis: The Fully Elaborated** β-Subunit of the Human Follicle-Stimulating Hormone\*\*

Pavel Nagorny, Neeraj Sane, Bernhard Fasching, Baptiste Aussedat, and Samuel J. Danishefsky\*

The human follicle-stimulating hormone (hFSH) belongs to a family of hormones responsible for the maintenance of essential reproductive processes (gonadotropins).<sup>[1]</sup> FSH is produced in the anterior pituitary, and the binding of FSH to its receptor stimulates the maturation of follicles and the production of estrogen in females, and maintains spermatogenesis in males.<sup>[2]</sup> Consequently, FSH is clinically used in the treatment of anovulatory disorders associated with infertility.<sup>[3]</sup> Administration is usually in the form of subcutaneous injections, often once a day, over prolonged periods of time. Side effects of this treatment range from allergic reactions and nausea, to mood swings and fatigue.<sup>[4]</sup> Presently, FSH is mainly derived from recombinant technologies, specifically, from Chinese Hamster Ovary (CHO) cells.<sup>[5]</sup> The material so obtained is a complex mixture of hormone glycoforms, that is, highly heterogeneous with respect to the carbohydrates on the peptide backbone.<sup>[6]</sup> In normal adult humans, the FSH receptor (FSH-R) is expressed only on the ovarian granulosa cells of females and the testicular Sertoli cells of males. However, in a recent discovery it was found that the FSH-R is ubiquitously expressed on the endothelial cells of the peripheries of the tumors of the breast, prostrate, colon, pancreas, kidney, stomach, testis, and ovary.<sup>[7]</sup> Earlier studies in mice have indicated that the effect of FSH on the growth of tumors is at least as potent as that of epidermal growth factor (EGF).<sup>[8]</sup> Although there was a concentration dependency of this effect, what might be an interesting study of the relative roles of the various glycoforms is presently stymied by the unavailability of homogeneous forms of FSH.

Structurally, FSH is a heterodimeric glycoprotein composed of two non-covalently associated subunits ( $\alpha$  and  $\beta$ ) (1. Figure 1).<sup>[9]</sup> Each of the subunits contains two N-linked oligosaccharides—the  $\alpha$ -subunit at Asn<sup>52</sup> and Asn<sup>78</sup>, and the

[\*] Dr. P. Nagorny,<sup>[+]</sup> Dr. N. Sane,<sup>[+]</sup> Dr. B. Fasching, Dr. B. Aussedat, Prof. Dr. S. J. Danishefsky Laboratory for Bioorganic Chemistry Sloan-Kettering Institute for Cancer Research 1275 York Avenue, New York, NY 10065 (USA) and Department of Chemistry, Columbia University Havemeyer Hall, 3000 Broadway, New York, NY 10027(USA) E-mail: s-danishefsky@ski.mskcc.org

- [<sup>+</sup>] These authors contributed equally to this work.
- [\*\*] This work was supported by NIH grant CA103823 (to S.J.D.) and NIH grant CA125934-02 (to P.N.). B.F. is grateful to the Austrian Fonds zur Förderung der wissenschaftlichen Forschung for a fellowship. We thank Rebecca Wilson for assistance with the preparation of the manuscript.
  - Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201107482.



Figure 1. a) Structure of hFSH heterodimer (1) with glycans (see inset legend). b)  $\beta$ -Subunit (2) displaying the N-linked consensus sequence oligosaccharide at the wild-type sites. c) Structure of the consensus sequence oligosaccharide. Acm = acetamidomethyl, Ac = acetyl.

 $\beta$ -subunit at Asn<sup>7</sup> and Asn<sup>24</sup>, which are incorporated in the rough endoplasmic reticulum (RER) through co-translational modifications of the peptide backbone. The structures of the oligosaccharides play a crucial role in the proper folding, subunit assembly, secretion, and activation of the target receptor and, ultimately, the metabolic fate of the molecule.<sup>[10]</sup> Clearly, a method for gaining access to homogeneous glycoforms of FSH would be highly desirable for establishing a structure-activity relationship (SAR). Due to a lack of viable techniques for separating such complex mixtures of glycoforms,<sup>[11]</sup> chemical and chemoenzymatic methods<sup>[12]</sup> have emerged as a viable option for the preparation of homogeneous glycoproteins.

The seminal work by Kent and co-workers in developing the native chemical ligation (NCL) reaction<sup>[13]</sup> has led to the synthesis of very challenging protein targets such as a ubiquitin diastereomer,<sup>[14]</sup> RNase,<sup>[15]</sup> integral membrane kinase,<sup>[16]</sup> and tetraubiquitin protein,<sup>[17]</sup> among others, that are inaccessible by conventional peptide synthesis. The scope of the NCL reaction has been vastly expanded now, to enable ligations at Met, Ala, Phe, Ser, Val, Thr, Lys, Leu and Pro.<sup>[18]</sup>

Angewandte nunications

Yet, the ability to gain access to complex glycoproteins through de novo chemical synthesis remains a rather daunting task.<sup>[12a]</sup> The difficulties can be attributed in part to the challenges of obtaining complex carbohydrates, particularly those containing sialic acid, fucose and high mannose residues, by chemical synthesis, in sufficient quantities. With respect to a possible synthesis of FSH, we had recently described an approach to the synthesis of the  $\beta$ -subunit using the disaccharide chitobiose as a model building block for our glycoprotein assembly.<sup>[19]</sup> Herein, we present the results of a rather ambitious undertaking, that is, the synthesis of the βsubunit of FSH (2) containing a consensus sequence oligosaccharide at each of the two N-linkage sites, endowed with high mannose, fucose and sialic acid presentation, using natural motifs in all of the glycosidic linkages. The system has been synthesized with protected cysteines anticipating folding and association with the  $\alpha$ -subunit.<sup>[20]</sup> The particular biantennary consensus dodecasaccharide chosen for this purpose was found to be abundant in batches of recombinant FSH displaying high bioactivity. This oligosaccharide is also found to exist on other glycoprotein hormones such as, Chorionic gonadotropin (hCG), Luteinizing hormone (hLH), and Thyroid-stimulating hormone (hTSH) as well as  $\alpha$ fetoprotein (associated with human hepatocellular carcinoma).<sup>[21]</sup> Consequently, the protocol presented here should also be extendable to the synthesis of these and other glycoproteins.

The  $\beta$ -subunit of FSH consists of 111 amino acids and the N-linked sugars are present at Asn<sup>7</sup> and Asn<sup>24</sup>.<sup>[9a]</sup> The abundance of cysteine residues in the peptide backbone, at fairly regular intervals, speaks to the possibility of assembling the glycoprotein using NCL. The key disconnections are depicted in Scheme 1. The two key ligation sites chosen were Phe<sup>19</sup>–Cys<sup>20</sup> and Trp<sup>27</sup>–Cys<sup>28</sup>. To enable the incorporation of the more precious glycopeptides in the final stages of

assembly, the construction was performed from the C to the N terminus of the subunit. In order to engage in NCL, the C terminus of each individual peptide fragment was functionalized as a thioester by single amino acid coupling. The peptide fragments were obtained by Fmoc-based solid phase peptide synthesis (SPPS) and the protecting groups on the amino acid side chains during SPPS were chosen in a way that the aspartic acid residue that will bear the dodecasaccharide was protected orthogonally to those residues that were likely to interfere during the glycan attachment. Of the twelve cysteines on the protein, the nine that were not required for NCL were protected with acetamidomethyl (Acm) protecting groups. Additionally, these cysteine side chain protections prevent undesirable cross-linkages, by oxidation, during the course of the synthesis. Cleavage from the resin and selective deprotection of the aspartic acid side chain provided a free carboxylic acid, which was then coupled with the glycosylamine 7 by HATU-mediated Lansbury aspartylation.<sup>[22]</sup>

The dodecasaccharide 7 was obtained by chemical synthesis, following the program outlined in Scheme 2.<sup>[19,23]</sup> The strategy exploited the symmetry in the dodecasaccharide, in that a bis-glycosylation reaction on the hexasaccharide acceptor 13 with the trisaccharide donor 12 was executed in the late stages of the synthesis. The hexasaccharide core 13 was obtained through a highly convergent series of glycosylations using the known building blocks 8-11. In each case, the protecting groups were carefully selected to maximize the diastereoselectivity during the glycosylations and to minimize the number of deprotection steps that would need to be performed towards the end of the synthesis. Global deprotection of all the protecting groups present on the sugar moieties provided the dodecasaccharide 14. The anomeric hydroxy group was converted into a primary amine under Kochetkov amination conditions<sup>[19,24]</sup> by treatment with saturated ammonium bicarbonate solution. Excess ammoni-



**Scheme 1.** Retrosynthetic strategy for the construction of the fully elaborated  $\beta$ -subunit of hFSH (2). HATU = O-(7-azabenzotriazol-1-yl)-tetramethyluronium hexafluorophosphate.

976 www.angewandte.org

© 2012 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim



Scheme 2. Schematic representation of the synthesis of the dodecasaccharide 7.

um bicarbonate was removed by repeated lyophilization of the material with minimal exposure to moisture.<sup>[25]</sup>

The protected [ $\beta$ FSH<sup>1-18</sup>] **15** was obtained through Fmocbased SPPS starting from the commerically available Fmoc-Arg(Pbf)-TGT resin. Pseudoproline dipeptides (denoted by  $\psi$ ) were incorporated into the peptide synthesis sequence to improve the yield of this aggregation-prone fragment.<sup>[26]</sup> The C-terminal carboxylic acid of the peptide was coupled to phenylalanine phenylthioester **16** under Sakakibara conditions (Scheme 3),<sup>[27]</sup> which are known to be epimerization-



**Scheme 3.** Synthesis of [ $\beta$ FSH<sup>1-19</sup>] **5.** a) **16**, HOOBt, EDC, TFE/CHCl<sub>3</sub> 1:3; b) Cocktail B; c) **7**, HATU, DIEA, DMSO; d) [Pd(PPh<sub>3</sub>)<sub>4</sub>], PhSiH<sub>3</sub>, NMP. HOOBt = 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one, EDC = *N'*-(3dimethylaminopropyl)-*N*-ethylcarbodiimide, TFE = 2,2,2-trifluoroethanol, DIEA = ethyldiisopropylamine, DMSO = dimethyl sulfoxide, NMP = *N*-methyl-2-pyrrolidone.

Angewandte International Edition Chemi

free. Cocktail B<sup>[28]</sup> treatment removed all the acid-labile protecting groups on the amino acid side chains. This threestep protocol afforded [ $\beta$ FSH<sup>1-19</sup>] **17** in 21 % yield after HPLC purification. The material so obtained was treated with the glycosyl amine **7** under HATU-mediated coupling conditions. The aspartylation proceeded in 73 % conversion as indicated by LC-MS, along with a trace amount of undesired aspartimide formation. Treatment with [Pd(PPh<sub>3</sub>)<sub>4</sub>] and phenylsilane removed all the allyl and alloc protecting groups on the amino acid side chains, in a one-flask procedure. The mixture was purified by HPLC to provide the [ $\beta$ FSH<sup>1-19</sup>] glycopeptide **5** in 17 % yield (averaged over nine trials).

Similarly,  $[\beta FSH^{20-26}]$  **18** was obtained by Fmoc-based SPPS on Fmoc-Thr(*t*Bu)-TGT resin and cleavage from the resin. Treatment of peptide **18** with tryptophan phenyl-thioester **19**, and subsequent deprotection with Cocktail B, provided  $[\beta FSH^{20-27}]$  **6** in 66% yield (3 steps) (Scheme 4).



Scheme 4. Synthesis of  $[\beta FSH^{20-27}]$  4. a) 19, HOOBt, EDC, TFE/CHCl<sub>3</sub> 1:3; b) Cocktail B; c) 7, HATU, DIEA, DMSO.

Lansbury aspartylation of this peptide with the glycosyl amine **7** proceeded in 50% conversion with some aspartimide formation (ca. 8%). The [ $\beta$ FSH<sup>20–27</sup>] glycopeptide **4** was isolated by HPLC purification in 27% yield (averaged over five trials).

The last segment required for the assembly was  $[\beta FSH^{28-111}]$  (3, Scheme 5). Fragment 3 is devoid of glycosylation sites and could have been obtained entirely by SPPS. However, we were unable to obtain useful yields of this peptide on solid support and we therefore opted to accomplish its synthesis through native chemical ligation of two smaller peptide fragments of roughly equal length. Gly<sup>65</sup>-Cys<sup>66</sup> was chosen as the ligation site, for two reasons. First, ligations at glycines are particularly effective, likely due to the lack of steric bulk at the  $\alpha$ -position. Additionally, conversion of terminal glycine carboxylic acids to their thioesters is not subject to epimerization. Thus, two shorter peptide fragments were synthesized. [BFSH<sup>28-65</sup>] was obtained by SPPS on Fmoc-Gly-TGT resin. The terminal glycine residue was converted to glycine phenylthioester and treated with Cocktail B to remove all the acid-labile protecting groups, providing fragment 20 in 48% vield (over three steps). The fully depro-

Angew. Chem. Int. Ed. 2012, 51, 975-979





**Scheme 5.** Final ligation of the glycopeptide fragments. a) **20**, **21**, PhSH, Gnd·HCl, Na<sub>2</sub>HPO<sub>4</sub>, TCEP·HCl, H<sub>2</sub>O (pH 7.4); b) NH<sub>2</sub>OMe·HCl, Gnd·HCl, TCEP·HCl (pH 4.8); c) **4**, PhSH, Gnd·HCl, Na<sub>2</sub>HPO<sub>4</sub>, TCEP·HCl, H<sub>2</sub>O (pH 7.4); d) NH<sub>2</sub>OMe·HCl, Gnd·HCl, TCEP·HCl (pH 4.8); e) **5**, PhSH, Gnd·HCl, Na<sub>2</sub>HPO<sub>4</sub>, TCEP·HCl, H<sub>2</sub>O (pH 7.3). Mass spectrum of compound **2** (inset). Gnd·HCl = guanidine hydrochloride, TCEP·HCl = tris(2-carboxyethyl)phosphine hydrochloride.

tected peptide [ $\beta$ FSH<sup>66-111</sup>] **21** was obtained on solid support using Fmoc-Glu(OtBu)-TGT resin, cleavage and treatment with Cocktail B. The two fragments, **20** and **21**, were coupled under native chemical ligation conditions, using thiophenol as an additive. Upon completion of the reaction as monitored by LC-MS, the terminal Thz protecting group was removed using methoxylamine hydrochloride, in a one-flask procedure, to provide [ $\beta$ FSH<sup>28-111</sup>] **3** in 38 % yield after HPLC purification.

The final assembly of the individual peptide fragments commenced with the coupling of fragment [ $\beta$ FSH<sup>20-27</sup>] **4** with the fragment [ $\beta$ FSH<sup>28-111</sup>] **3** under NCL conditions in a pH 7.3 buffer (Scheme 5). Subsequently, the N-terminal Thz group was cleaved using methoxylamine hydrochloride at pH 4.8, to free the N-terminal cysteine required for the final ligation, in a one-flask procedure. Purification by HPLC provided the desired glycopeptide [ $\beta$ FSH<sup>20-111</sup>] **22** bearing the dodecasaccharide, in 26 % yield (averaged over three trials).

Finally, the glycopeptide [ $\beta$ FSH<sup>1-19</sup>] **5** was coupled to the glycopeptide [ $\beta$ FSH<sup>20-111</sup>] **22** under NCL conditions. Gratifyingly, we obtained the full chain  $\beta$ -subunit of FSH (**2**) containing the two dodecasaccharides in 27 % yield (averaged over three trials) after HPLC purification. Interestingly, the ligation was facile despite the fact that the two bulky dodecasaccharides are merely 16 residues apart during the ligation event. Also noteworthy is the fact that the final

compound **2** represents the largest realistically glycosylated glycoprotein with all natural-type linkages to have been synthesized in a homogeneous state, using strictly chemical methods.

Much encouraged by this demonstration of feasibility, we continue our studies through which we hope to synthesize hFSH itself. There remains deprotection of the Acm protecting groups<sup>[29]</sup> on the cysteine side chains such as to allow for folding and subsequent association with the  $\alpha$ -subunit. The total synthesis of the latter is already well advanced. The results of these ongoing studies will be disclosed shortly.

Received: October 24, 2011 Published online: December 9, 2011

**Keywords:** follicle-stimulating hormone · glycoproteins · native chemical ligation · solid-phase peptide synthesis

- H. L. Fevold, F. L. Hisaw, S. L. Leonard, Am. J. Physiol. 1931, 97, 291–301.
- [2] P. E. Smith, JAMA J. Am. Med. Assoc. 1927, 88, 158-161.
- [3] C. M. Howles, Hum. Reprod. Update 1996, 2, 172–191.
- [4] S. C. Pang, Women's Health 2005, 1, 87-95.
- [5] R. A. Lobo in *Comprehensive Gynecology*, 5th ed. (Eds.: V. L. Katz et al.), MOSBY, Philadelphia, **2007**, pp. 1001–1037.

- [6] A. Gervais, Y.-A. Hammel, S. Pelloux, P. Lepage, G. Baer, N. Carte, O. Sorokine, J.-M. Strub, R. Koerner, E. Leize, A. Van Dorsselaer, *Glycobiology* **2003**, *13*, 179–189.
- [7] A. Radu, C. Pichon, P. Camparo, M. Antoine, Y. Allory, A. Couvelard, G. Fromont, M. T. V. Hai, N. Ghinea, *N. Engl. J. Med.* **2010**, *363*, 1621–1630.
- [8] Y. Li, S. Ganta, C. Cheng, R. Craig, R. R. Ganta, L. C. Freeman, Mol. Cell. Endocrinol. 2007, 267, 26–37.
- [9] a) J. G. Pierce, T. F. Parsons, Annu. Rev. Biochem. 1981, 50, 465 495; b) J. U. Baenziger, E. D. Green, Biochim. Biophys. Acta. 1988, 947, 287 306; c) Q. R. Fan, W. A. Hendrickson, Nature 2005, 433, 269 277.
- [10] a) A. S. Hartree, A. G. Renwick, *Biochem. J.* 1992, 287, 665–679; b) A. Ulloa-Aguirre, C. Timossi, *Hum. Reprod. Update* 1998, 4, 260–283.
- [11] P. M. Rudd, R. A. Dwek, *Crit. Rev. Biochem. Mol. Biol.* **1997**, *32*, 1–100.
- [12] a) For a recent review, see: D. P. Gamblin, E. M. Scanlan, B. G. Davis, Chem. Rev. 2009, 109, 131-163; b) H. Kunz, C. Unverzagt, Angew. Chem. 1988, 100, 1763-1765; Angew. Chem. Int. Ed. Engl. 1988, 27, 1697-1699; c) J. Offer, M. Quibell, T. Johnson, J. Chem. Soc. Perkin Trans. 1 1996, 175-182; d) D. Macmillan, C. R. Bertozzi, Tetrahedron 2000, 56, 9515-9525; e) N. Yamamoto, Y. Ohmori, T. Sakakibara, K. Sasaki, L. R. Juneja, Y. Kajihara, Angew. Chem. 2003, 115, 2641-2644; Angew. Chem. Int. Ed. 2003, 42, 2537-2540; f) K. Hirano, D. Macmillan, K. Tezuka, T. Tsuji, Y. Kajihara, Angew. Chem. 2009, 121, 9721-9724; Angew. Chem. Int. Ed. 2009, 48, 9557-9560; g) R. Chen, T. J. Tolbert, J. Am. Chem. Soc. 2010, 132, 3211-3216.
- [13] P. E. Dawson, T. W. Muir, I. Clark-Lewis, S. B. Kent, Science 1994, 266, 776-779.
- [14] D. Bang, G. I. Makhatadze, V. Tereshko, A. A. Kossiakoff, S. B. Kent, Angew. Chem. 2005, 117, 3920–3924; Angew. Chem. Int. Ed. 2005, 44, 3852–3856.
- [15] a) C. Piontek, D. Varón Silva, C. Heinlein, C. Pöhner, S. Mezzato, P. Ring, A. Martin, F.X. Schmid, C. Unverzagt, *Angew. Chem.* 2009, 121, 1974–1978; *Angew. Chem. Int. Ed.* 2009, 48, 1941–1945; b) C. Piontek, P. Ring, O. Harjes, C. Heinlein, S. Mezzato, N. Lombana, C. Pöhner, M. Püttner, D. Varón Silva, A. Martin, F. X. Schmid, C. Unverzagt, *Angew. Chem.* 2009, 121, 1968–1973; *Angew. Chem. Int. Ed.* 2009, 48, 1936–1940.
- [16] S. Lahiri, M. Brehs, D. Olschewski, C. F. W. Becker, Angew. Chem. 2011, 123, 4074–4078; Angew. Chem. Int. Ed. 2011, 50, 3988–3992.
- [17] K. S. A. Kumar, S. N. Bavikar, L. Spasser, T. Moyal, S. Ohayon, A. Brik, Angew. Chem. 2011, 123, 6261–6265; Angew. Chem. Int. Ed. 2011, 50, 6137–6141.
- [18] a) J. P. Tam, Q. Yu, *Biopolymers* 1998, 46, 319–327; b) L. Z. Yan,
  P. E. Dawson, J. Am. Chem. Soc. 2001, 123, 526–533; c) Q. Wan,
  S. J. Danishefsky, Angew. Chem. 2007, 119, 9408–9412; Angew.
  Chem. Int. Ed. 2007, 46, 9248–9252; d) D. Crich, A. Banerjee, J.
  Am. Chem. Soc. 2007, 129, 10064–10065; e) R. Okamoto, Y.

Kajihara, Angew. Chem. 2008, 120, 5482-5486; Angew. Chem. Int. Ed. 2008, 47, 5402-5406; f) C. Haase, H. Rohde, O. Seitz, Angew. Chem. 2008, 120, 6912-6915; Angew. Chem. Int. Ed. 2008, 47, 6807-6810; g) J. Chen, Q. Wan, Y. Yuan, J. Zhu, S. J. Danishefsky, Angew. Chem. 2008, 120, 8649-8652; Angew. Chem. Int. Ed. 2008, 47, 8521-8524; h) R. Yang, K. K. Pasunooti, F. Li, X. W. Liu, C. F. Liu, J. Am. Chem. Soc. 2009, 131, 13592-13593; i) K. S. Ajish Kumar, M. Haj-Yahya, D. Olschewski, H. A. Lashuel, A. Brik, Angew. Chem. 2009, 121, 8234-8238; Angew. Chem. Int. Ed. 2009, 48, 8090-8094; j) J. Chen, P. Wang, J. Zhu, Q. Wan, S. J. Danishefsky, Tetrahedron 2010, 66, 2277-2283; k) Z. Harpaz, P. Siman, K. S. Kumar, A. Brik, ChemBioChem 2010, 11, 1232-1235; I) Z. Tan, S. Shang, S. J. Danishefsky, Angew. Chem. 2010, 122, 9690-9693; Angew. Chem. Int. Ed. 2010, 49, 9500-9503; m) S. Shang, Z. Tan, S. Dong, S. J. Danishefsky, J. Am. Chem. Soc. 2011, 133, 10784-10786.

- [19] P. N. Nagorny, B. Fasching, X. Li, G. Chen, B. Aussedat, S. J. Danishefsky, J. Am. Chem. Soc. 2009, 131, 5792–5799.
- [20] M. Samaddar, J. F. Catterall, R. R. Dighe, Protein Expression Purif. 1997, 10, 345–355.
- [21] a) S. D. Gharib, M. E. Wierman, M. A. Shupnik, W. W. Chin, *Endocr. Rev.* 1990, 11, 177–199; b) M. R. Sairam, *FASEB J.* 1989, 3, 1915–1926; c) J. H. G. M. Mutsaers, J. P. Kamerling, R. Devos, Y. Guisez, W. Fiers, J. F. G. Vliegenthart, *Eur. J. Biochem.* 1986, 156, 651–654; d) J. B. L. Damm, J. P. Kamerling, G. W. K. van Dedem, J. F. G. Vliegenthart, *Glycoconjugates* 1987, 4, 129– 144; e) C. H. Hokke, A. A. Berwer, G. W. K. van Dedem, J. V. van Ostrum, J. P. Kamerling, J. F. G. Vliegenthart, *FEBS Lett.* 1990, 275, 9–14; f) E. D. Green, J. U. Baenziger, *J. Biol. Chem.* 1988, 263, 25–35; g) K. Yamashita, A. Hitoi, Y. Tsuchida, S. Nishi, A. Kobata, *Cancer Res.* 1983, 43, 4691–4695.
- [22] a) S. T. Cohen-Anisfeld, P. T. Lansbury, Jr., J. Am. Chem. Soc.
   1993, 115, 10531-10537; b) J. S. Miller, V. Y. Dudkin, G. J. Lyon,
   T. W. Muir, S. J. Danishefsky, Angew. Chem. 2003, 115, 447-450;
   Angew. Chem. Int. Ed. 2003, 42, 431-434.
- [23] a) S. J. Danishefsky, S. Hu, P. F. Cirillo, M. Eckhardt, P. H. Seeberger, *Chem. Eur. J.* **1997**, *3*, 1617–1628; b) V. Y. Dudkin, J. S. Miller, S. J. Danishefsky, *Tetrahedron Lett.* **2003**, *44*, 1791–1793; c) B. Wu, Z. Hua, J. D. Warren, K. Ranganathan, Q. Wan, G. Chen, Z. Tan, J. Chen, A. Endo, S. J. Danishefsky, *Tetrahedron Lett.* **2006**, *47*, 5577–5579.
- [24] L. M. Likhosherstov, O. S. Novikova, V. Derevitskaja, N. K. Kochetkov, *Carbohydr. Res.* **1986**, *146*, C1–C5.
- [25] H. S. Isbell, H. L. Frush, J. Org. Chem. 1958, 23, 1309-1319.
- [26] T. Wöhr, F. Wahl, A. Nefzi, B. Rohwedder, T. Sato, X. Sun, M. Mutter, J. Am. Chem. Soc. 1996, 118, 9218–9227.
- [27] S. Sakakibara, Biopolymers 1995, 37, 17-28.
- [28] Cocktail B (2% triisopropylsilane/5% phenol/5%  $\rm H_2O/88\%$  TFA).
- [29] On the chitobiose model system (Ref. [19]), removal of the Acm groups on the nine protected cysteines was smoothly accomplished, producing free glycoprotein.