Solid-Phase Chemical–Enzymatic Synthesis of Glycopeptides and Oligosaccharides

Matthias Schuster,† Peng Wang,† James C. Paulson,†,‡ and Chi-Huey Wong*,†

Department of Chemistry
The Scripps Research Institute
10666 North Torrey Pines Road
La Jolla, California 92037
Cytel Corporation, 3525 John Hopkins Court
San Diego, California 92121

Received December 3, 1993

The fields of protein- and nucleic acid-related chemistry and biochemistry have benefited greatly from the development of solid-phase synthesis of peptides[1] and oligonucleotides.[2] In contrast, the development of solid-phase synthesis of oligosaccharides has been hampered by the lack of effective differential protection/deprotection strategy and high-yield stereoselective coupling of multifunctional carbohydrate donors and acceptors.[3] Only recently, this problem has been addressed by Danishefsky et al.,[4] who have developed a new method based on glycal chemistry. We report here a new strategy which enables a rapid iterative formation of peptide bonds chemically and glycosidic bonds enzymatically with glycosyl transferases on a silica-based solid support compatible with both organic and aqueous solvents. The key element is to attach a proper acceptor–spacer group with a selectively cleavable bond to the solid support so that enzymatic coupling can be effectively achieved and the glycopeptide (or oligosaccharide) products can be released. No protection of the sugar hydroxyl groups is required.

Glycosyltransferases have become valuable reagents for glycosylation due to their high regio- and stereoselectivity and increasing availability via recombinant DNA technology;[5] however, no viable methodology has been developed[6] for solid-phase enzymatic synthesis of oligosaccharides.

Of several solid supports tested,[7] aminopropyl silica was chosen because it (i) is compatible with both aqueous and organic solvents, (ii) has a large surface area accessible to biomolecules, and (iii) has a sufficient density of functional groups (~1.5 mmol/g). In the first step, a hexaglycine spacer was attached to the support (Scheme 1)[8] to give a substitution level of 0.2 mmol/g of dry silica. Excess amino groups were then capped using acetic anhydride. In the second step, a selective cleavage site was implemented for the release of intermediates and final products from the support under mild conditions by introduction of an α-chymotrypsin-sensitive phenylalanyl ester bond.[9] Addition of a glycopeptide gave the glycosyl acceptor **3a**, which upon α-chymotrypsin-catalyzed hydrolysis yielded *N*-Boc-Asn(GlcNAcβ)-Gly-Phe-OH (**3b**) as the exclusive soluble product.

β-1,4-Galactosyl transfer to **3a** was catalyzed by β-1,4-galactosyltransferase (from Sigma) employing UDP-galactose as a glycosyl donor. Reverse-phase HPLC analysis of the supernatant after α-chymotrypsin hydrolysis of an aliquot of washed and dried solid material revealed a galactosylation level of 55% based on the galactosylated tripeptide Boc-Asn-(Galβ1,4GlcNAcβ)-Gly-Phe-OH (**4b**) isolated by semipreparative HPLC. The subsequent solid-phase α-2,3-sialylation of **4a** was performed under similar conditions using α-2,3-sialyltransferase[10] and CMP-sialic acid to give the sialylated product in 65% yield. To illustrate the feasibility of the system for the synthesis of a soluble bioactive glycopeptide, the sialylated tripeptide released from the solid support by chymotrypsin was enzymatically fucosylated to form sialyl Lewis X glycopeptide **7**.[11] The α-1,3-fucosyltransferase-catalyzed[10] reaction with GDP-fucose went to completion within 10 h as monitored by HPLC. Since both **4a** and **5a** are substrates for the enzyme, the product mixture contained 35% of **6**, 20% of **7**, and 45% of the unreacted **3b**.[12]

In summary, the solid-phase system presented here supports both chemical peptide synthesis and glycosyltransferase-catalyzed sugar chain elongation and allows a rapid synthesis and controlled release of glycopeptides.[11] The glycosylation reactions were performed in good yields. The system should also enable the synthesis of oligosaccharides as the peptide moiety can be removed enzymatically using endoglycosidases.[13] In addition, glycopeptides displayed on the solid support may find use in the affinity purification of carbohydrate-binding proteins, the assay of binding, and/or the catalysis of such proteins and the assembly of immobilized oligosaccharide libraries. Work is in progress to improve the yield in each coupling step to avoid the formation

† The Scripps Research Institute.
‡ Cytel Corporation.

(1) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149. For recent advances in solid-phase synthesis, see: Lloyd-Williams, P.; Albericio, F.; Giralt, E. *Tetrahedron* **1993**, *49*, 11065.

(2) Caruthers, M. H. In *Genetic Engineering*; Stelow, J., Holaender, A., Eds.; Plenum: New York, 1982; Vol. 4, Chapter 1.

(3) Eby, R.; Schuerch, C. *Carbohydr. Res.* **1975**, *39*, 151 and references therein. Excoffier, G.; Gagnaire, D.; Utille, J. P.; Vignon, M. *Tetrahedron Lett.* **1972**, *50*, 5065. Chiu, S.-H. L.; Anderson, L. *Carbohydr. Res.* **1976**, *50*, 227. Nilsson, S.; Bengtsson, M.; Norberg, T. *J. Carbohydr. Chem.* **1992**, *11*, 265. Veeneman, G. H.; Notermans, S.; Liskamp, R. M. J.; Van der Marel, G. A.; Van Boom, J. H. *Tetrahedron Lett.* **1987**, *28*, 6695. Frechet, J. M. J.; Schuerch, C. *Carbohydr. Res.* **1972**, *22*, 399. Guthrie, R. D.; Jenkins, A. D.; Stehlicek, J. *J. Chem. Soc., Chem. Commun.* **1971**, 2690. For a polymer-supported solution synthesis, see: Douglas, S. P.; Whitfield, D. M.; Krepinsky, J. J. *J. Am. Chem. Soc.* **1991**, *113*, 5095.

(4) Danishefsky, S. J.; McCure, K. F.; Randolph, J. T.; Ruggeri, R. B. *Science* **1993**, *260*, 1307.

(5) Toone, E. J.; Simon, E. S.; Bednarski, M. D.; Whitesides, G. M. *Tetrahedron* **1989**, *45*, 5365. Ichikawa, Y.; Look, G. C.; Wong, C.-H. *Anal. Biochem.* **1992**, *202*, 215.

(6) Less than a 1% yield of galactosyltransferease-catalyzed galactosylation was reported: Zehavi, U.; Sadeh, S.; Herchman, M. *Carbohydr. Res.* **1983**, *124*, 23. Zehavi, U.; Herchman, M.; Kopper, S. *Carbohydr. Res.* **1992**, *228*, 255.

(7) Styrene- and sugar-based polymers are not suitable, as they tend to swell and lead to low-yield couplings.

(8) Abbreviations: GA, glycolic acid; APS, aminopropyl silica; TFA, trifluoroacetic acid; DIEA, diisopropylethylamine; BOP, benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole.

(9) α-Chymotrypsin is highly specific for substrates containing aromatic amino acid residues in P_1. Moreover, the specificity of serine proteases (expressed as K_{cat}/K_m) for an ester is several orders of magnitude higher than that for the corresponding amide: Liu, T. Y.; Elliot, S. D. In *The Enzymes*; Boyer, P. D., Ed.; Academic Press: New York, 1971; Vol. III, pp 609–647.

(10) For α-2,3-sialyltransferase and α-1,3-fucosyltransferase and the synthesis of sialyl Lewis X and sugar nucleotides, see: Ichikawa, Y.; Lin, Y.-C.; Dumas, D. P.; Shen, G.-J.; Garcia-Junceda, E.; Williams, M. A.; Bayer, R.; Ketcham, C.; Walker, L. E.; Paulson, J. C.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 9283. The E-selectin ligand is a sialyl Lewis X glycolipid. Sialyl Lewis X-containing glycoproteins are ligands for P- and L-selectins. For review, see: Lasky, L. A. *Science* **1992**, *258*, 964.

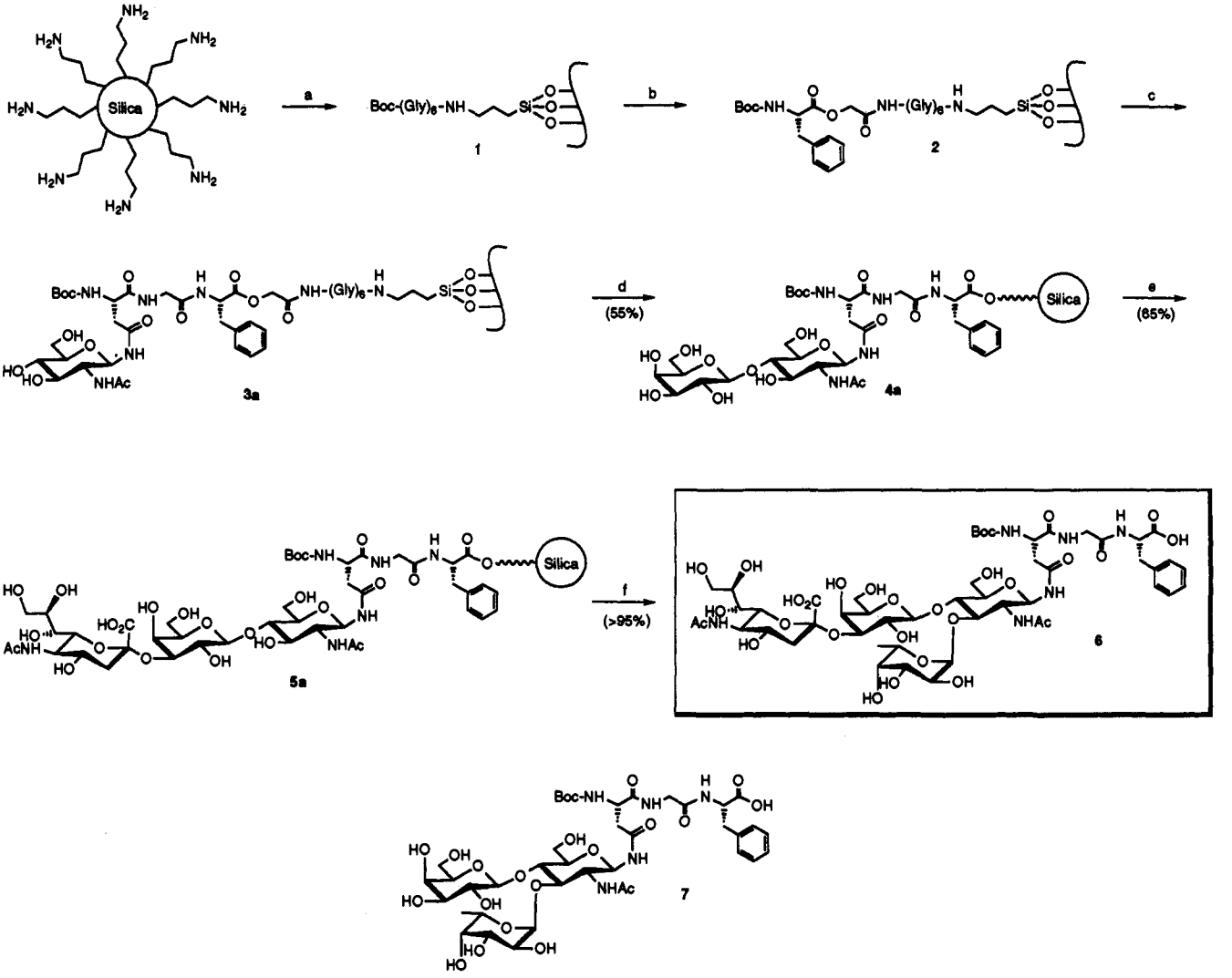
(11) Several methods are available for solid-phase glycopeptide synthesis. None of them involves the use of glycosyltransferase in solid-phase synthesis: Kunz, H. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 294. Paulsen, H.; Merz, G.; Peters, St.; Weichert, U. *Liebigs Ann. Chem.* **1990**, 1165. Christiansen-Brams, I.; Meldal, M.; Bock, K. *J. Chem. Soc., Perkin Trans 1* **1993**, 1461 and references cited. Kunz, H. *Pure Appl. Chem.* **1993**, *65*, 1223. Otvos, L.; Urge, L.; Hollosi, M.; Wroblewski, K.; Grazyk, G.; Fasman, G. D.; Thurin, J. *Tetrahedron Lett.* **1990**, *41*, 5889 and references therein. Chadwick, R. J.; Thompson, J. S.; Tomalin, G. *Biochem. Soc. Trans.* **1991**, *19*, 406S. Unverzagt, C.; Kunz, H.; Paulson, J. C. *J. Am. Chem. Soc.* **1990**, *112*, 9308. Thiem, J.; Wiemann, T. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 80. Schultz, M.; Kunz, H. *Tetrahedron Lett.* **1992**, *33*, 5319. Wong, C.-H.; Schuster, M.; Wang, P.; Sears, P. *J. Am. Chem. Soc.* **1993**, *115*, 5893.

(12) To avoid the formation of **7**, one can use α-1,3-fucosyltransferase VII instead of fucosyltransferase V. John Lowe, personal communication.

(13) Trimble, R. B.; Maley, F. *Anal. Biochem.* **1984**, *141*, 522. Tarentino, A. L.; Gomez, C. M.; Plummer, T. H., Jr. *Biochemistry* **1985**, *24*, 4665.

0002-7863/94/1516-1135$04.50/0

Scheme 1. Solid-Phase Supported Chemical-Enzymatic Synthesis of a Sialyl Lewis X Glycopeptide[a]



[a] Reagents and conditions: (a) (i) Boc-Gly$_3$-OH (0.3 equiv), DCC/HOBt, DIEA, (ii) acetic anhydride, pyridine, (iii) 25% TFA (CH_2Cl_2), (iv) Boc-Gly$_3$-OH (5 equiv), DCC/HOBt, DIEA; (b) (i) 25% TFA (CH_2Cl_2), (ii) O-(*N*-^{t}Boc-phenylalanyl)glycolic acid (7 equiv), BOP/HOBt, DIEA; (c) (i) 25% TFA (CH_2Cl_2), (ii) Boc-Gly-OH (7 equiv), BOP/HOBt, DIEA, (iii) TFA (CH_2Cl_2), (iv) Boc-Asn(GlcNAcβ)-OH (3 equiv), BOP, DIEA; (d) β-1,4-galactosyltransferase, UDP-Gal (1.5 equiv), 0.1 M HEPES (pH 7.0), 10 mM $MnCl_2$; (e) α-2,3-sialyltransferase, CMP-NeuAc (1.5 equiv), 0.1 M HEPES (pH 7.0), 5 mM $MnCl_2$); (f) (i) α-chymotrypsin, H_2O (pH 7.0), (ii) ultrafiltration, (iii) α-1,3-fucosyltransferase, GDP-Fuc (2.5 equiv), 0.1 M HEPES (pH 7.0).

of deleted or truncated products and to develop a new method to extend the peptide chain in aqueous media.

Acknowledgment. This research was partly supported by Cytel Corporation. M.S. is a Deutsche Forschungsgemeinschaft Postdoctoral Fellow.

Supplementary Material Available: Detailed procedures for all synthetic steps, ^{1}H NMR and HRMS data for **3b**, **4b**, **5b**, **6**, and **7** (5 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.