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Unprotected Oligosaccharides as Phase Tags: Solution-Phase Synthesis of Glycopeptides with Solid-Phase Workups

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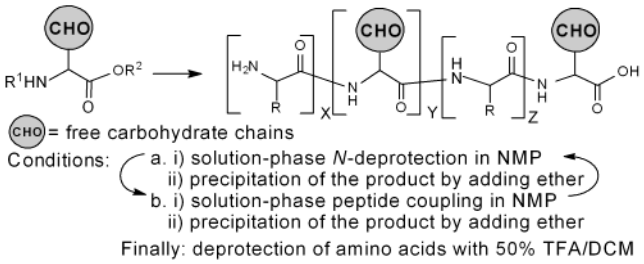
ABSTRACT



N-Linked glycopeptides were synthesized from glycosyl asparagines containing unprotected oligosaccharides and other simple amino acids by an Fmoc method. The free oligosaccharide chains were used as phase tags to facilitate the product isolation by a precipitation method. Thus, while the elongation of glycopeptides was achieved in a solution of *N*-methylpyrrolidinone (NMP), the product of each step could be precipitated by adding ether to the reaction mixtures. The strategy also eliminated the final step of carbohydrate deprotection in glycopeptide synthesis.

The microheterogeneity of biosynthesized glycoproteins almost prohibits the accessibility to their homogeneous glycoforms from biological systems. Thus, glycopeptides, the synthetic analogues of glycoproteins, with well-defined carbohydrate structures, which may be prepared by chemical synthesis, are in high demand from various glycobiological studies.[1,2]

Chemical synthesis of glycopeptides has been a major research focus in organic chemistry during the past two decades. To circumvent the incompatibility of carbohydrate chemistry and peptide chemistry in glycopeptide synthesis, preassembled glycosyl amino acids are usually used as building blocks in glycopeptide constructions[3−13] or enzymatic methods are employed for the elongation of glycopeptide glycans.[10,14−17] Among various synthetic designs investigated, solid-phase synthesis is one of the most attractive because of its convenience and efficacy.[3−5,7,8,12,17−21] Its protocol is to assemble the glycopeptide chain on a polymer support based on glycosylated and simple amino acids. Once the assembly is finished, the glycopeptide will

(1) Varki, A. *Glycobiology* **1993**, *3*, 97−130.
(2) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683−720.
(3) Large, D. G.; Warren, C. D. *Glycopeptides and Related Compounds*; Marcel Dekker: New York, 1997.
(4) Arsequell, G.; Valencia, G. *Tetrahedron: Asymmetry* **1997**, *8*, 2839−2876.
(5) Osborn, H. M. I.; Khan, T. H. *Tetrahedron* **1999**, *55*, 1807−1850.
(6) Paulsen, H. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 823−838.
(7) Meldal, M.; Bock, K. *Glycoconjugate J.* **1994**, *11*, 59−63.
(8) Kunz, H. *Pure Appl. Chem.* **1993**, *65*, 1223−1232.
(9) Wong, C.-H.; Schuster, M.; Wang, P.; Sears, P. *J. Am. Chem. Soc.* **1993**, *115*, 5893−5901.
(10) Witte, K.; Seitz, O.; Wong, C.-H. *J. Am. Chem. Soc.* **1998**, *120*, 1979−1989.
(11) Ogawa, T. *Chem. Soc. Rev.* **1994**, 397−407.
(12) Roberge, J. Y.; Beebe, X.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1998**, *120*, 3915−3927.
(13) Cohen-Anifeld, S.-T.; Lansbury, P. T. J. *J. Am. Chem. Soc.* **1993**, *115*, 10531−10537.

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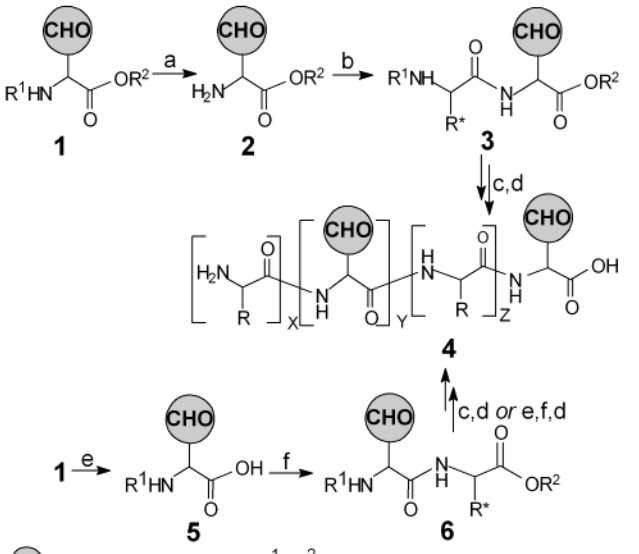
be retrieved from the solid-phase support, and the carbohydrate chains will be finally deprotected to afford the target. However, there are two concerns about the process. One is associated with the retrieval of glycopeptides from the solid-phase support, which is usually achieved under strong acidic conditions such as a 95% aqueous solution of trifluoroacetic acid (TFA) that may affect some labile glycosidic linkages and protecting groups.[22] The other concern is associated with the deprotection of carbohydrates, which could potentially affect the glycopeptides and amino acid residues.[18,23] Thus, chemical synthesis of glycopeptides still remains an important challenge, despite the great progress in the field.

To deal with this synthetic problem, we introduce herein a new strategy using free glycosyl amino acids as building blocks and the "phase tags" (Scheme 1). First, the glycosyl amino acids will be prepared, in which the carbohydrates are unprotected. Then, a glycopeptide can be assembled starting from one glycosyl amino acid, e.g., **1**, with the others as well as the commercially available simple amino acids as incoming units for peptide elongation. Finally, the side chains of amino acids will be deprotected by moderate acids such as 25−50% TFA in dichloromethane (DCM) to afford the free glycopeptide target **4**.

Scheme 1



One important property of this design is the use of free carbohydrates as phase tags to facilitate the isolation of product of each step during the assembly of glycopeptides. As free sugars and their conjugates are only soluble in very polar organic solvents, not in nonpolar ones, peptide elongation can be conducted in homogeneous solutions of a polar solvent, but product isolations can be achieved via a *precipitation method*, i.e., via addition of a nonpolar solvent to the reaction mixtures, with the side products and excess reagents remaining in solution. The precipitates can be further purified by washing with proper solvents. This separation procedure is similar to that of soluble polymer-supported and other phase-tagged organic syntheses.[24−30] However, after the glycopeptide assembly is accomplished in our design, it is not required to cut off the phase tags since they are a part of the final targets.

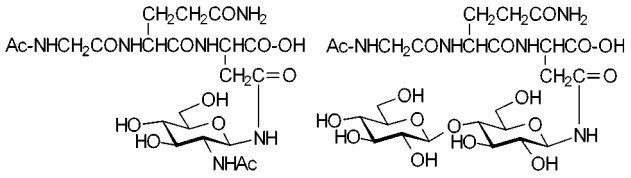
Thus, the new synthetic strategy will take the advantages of homogeneous reactions in solution-phase synthesis and the convenient workup in solid-phase synthesis. Moreover, it can circumvent the two problematic steps involved in traditional methods, namely, retrieval of glycopeptides from the polymer support and deprotection of the carbohydrates. Finally, as all the intermediates should be soluble in proper solvents, it will be simple to monitor the reactions by TLC and other conventional methods such as NMR and MS.

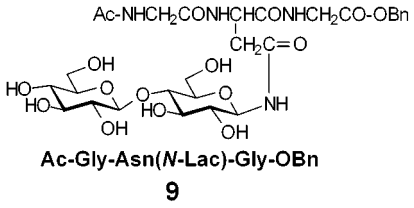
For the strategy to work, we have to find an appropriate solvent that is able to dissolve glycopeptides bearing free oligosaccharides and is compatible with reactions involved in glycopeptide elongation. *N*-Methylpyrrolidone (NMP) is a promising choice for this purpose, for NMP can dissolve a variety of compounds, including free sugars. In addition, NMP is a common solvent in peptide synthesis, which should be compatible to the reactions involved in the new strategy. On the other hand, it is also necessary to ensure that the

(14) Mizuno, M.; Haneda, K.; Iguchi, R.; Muramoto, I.; Kawakami, T.; Aimoto, S.; Yamamoto, K.; Inazu, T. *J. Am. Chem. Soc.* **1999**, *121*, 284−290.
(15) Deras, I. L.; Takegawa, K.; Kondo, A.; Kato, I.; Lee, Y. C. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1763−1766.
(16) Witte, K.; Sears, P.; Martin, R.; Wong, C.-H. *J. Am. Chem. Soc.* **1997**, *119*, 2114−2118.
(17) Schuster, M.; Wang, P.; Paulson, J. C.; Wong, C.-H. *J. Am. Chem. Soc.* **1994**, *116*, 1135−1136.
(18) Nakahara, Y.; Nakahara, Y.; Ogawa, T. *Carbohydr. Res.* **1996**, *292*, 71−81.
(19) Bielfeldt, T.; Peters, S.; Meldal, M.; Bock, K.; Paulsen, H. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 857−859.
(20) Guo, Z.; Nakahara, Y.; Nakahara, Y.; Ogawa, T. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1464−1467 and references therein.
(21) Klich, G.; Paulsen, H.; Meyer, B.; Meldal, M.; Bock, K. *Carbohydr. Res.* **1997**, *299*, 33−48.
(22) Guo, Z.; Nakahara, Y.; Nakahara, Y.; Ogawa, T. *Bioorg. Med. Chem.* **1997**, *5*, 1917−1924 and references therein.
(23) Guo, Z.; Nakahara, Y.; Nakahara, Y.; Ogawa, T. *Carbohydr. Res.* **1997**, *303*, 373−378.
(24) Douglas, S. P.; Whitfield, D. M.; Krepinsky, D. M. *J. Am. Chem. Soc.* **1991**, *113*, 5095−5097.
(25) Gravert, D.; Janda, K. D. *Chem. Rev.* **1997**, *97*, 489−510.
(26) Bosanac, T.; Yang, J.; Wilcox, C. S. *Angew. Chem., Int. Ed.* **2001**, *40*, 1875−1879.
(27) Ley, S. V.; Massi, A.; Rodriguez, F.; Horwell, D. C.; Lewthwaite, R. A.; Pritchard, M. C.; Reid, A. M. *Angew. Chem., Int. Ed.* **2001**, *40*, 1053−1055.
(28) Perrier, H.; Labelle, M. *J. Org. Chem.* **1999**, *64*, 2110−2113.
(29) Hay, A. M.; Hobbs-Dewitt, S.; MacDonald, A. A.; Ramage, R. *Tetrahedron Lett.* **1998**, *39*, 8721−8724.
(30) (a) Studer, A.; Hadida, S.; Ferritto, R.; Kim, S.; Jeger, P.; Wipf, P.; Curran, D. P. *Science* **1997**, *275*, 823−826. (b) Curran, D. P. *Angew. Chem., Int. Ed.* **1998**, *37*, 1174−1196.

free hydroxyl groups of carbohydrates will not interfere with the peptide coupling reactions.

To establish the principles of the new strategy, we first applied it to several simple *N*-glycopeptides **7**−**9**. Glycopeptide **7** has an N-linked monosaccharide, *N*-acetyl D-glucosamine, and **8** and **9** have an N-linked disaccharide, lactose. The peptide structure of **7** and **8** is the N-terminal-glycosylated sequence of CD52, an important glycoprotein in the human immune system.[31,32]

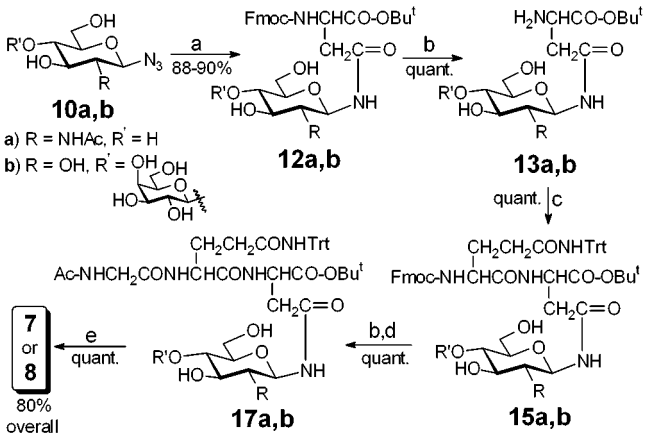




Our specific synthetic design for *N*-glycopeptides was to assemble the glycopeptide chains by a conventional Fmoc method using glycosyl asparagines with free carbohydrates as the key building blocks. The glycosyl asparagines would be in turn prepared from glycosyl azides. The azido group at the reducing ends of the oligosaccharides could serve as a protecting group in the syntheses of oligosaccharides,[22] but in the meantime, it can be conveniently transformed to the free amino group via selective reduction, which will facilitate the conjugation between the oligosaccharides and amino acids. The amines thus formed will probably keep their stereochemistry under anhydrous conditions, which will enable the predominant formation of one anomeric isomer during amino acid−oligosaccharide conjugation.

The synthesis of **7** and **8** is outlined in Scheme 2. For **7**, *N*-acetyl β-D-glucosaminyl azide **10a** was prepared first according to a reported procedure.[22] Then, it was reduced to the glycosylamine by catalytic hydrogenation in NMP. TLC showed a clean reaction. The catalyst was removed by filtration, and to the resulting solution were added 2 equiv of the active ester (**11**) of aspartic acid. After reaction overnight, the product was precipitated by addition of diethyl ether (8 times the NMP volume). We found that the product was soluble in NMP, dimethyl sulfoxide, or dimethylformamide, but not in ethyl acetate, diethyl ether, tetrahydrofuran (THF), or DCM. It was also soluble in hot methanol but only slightly soluble in cold methanol or water. Thus, the product was washed successively by cold water, THF, and ethyl acetate and then dried under vacuum to afford **12a**

Scheme 2



a. i) 10% Pd/C, H_2, NMP, r.t., 4hr, ii) Fmoc-Asp(OBt)-OBu^t (**11**), NMP, r.t., overnight; b. 20% piperidine, NMP, r.t., 40min; c. Fmoc-Glu(Trt)-OBt (**14**), NMP, r.t., overnight; d. Ac-Gly-OBt (**16**), NMP, r.t., overnignt; e. 50% TFA, DCM, r.t., 2hr

(90%). The ^{1}H NMR spectrum of **12a** proved its β-glycosidic linkage ($^3J_{1,2}$ = 9.1 Hz) as well as its purity. The less than perfect yield of this reaction may be caused by decomposition of the glycosylamine, which resulted in a free sugar that was unable to react with the active ester and could be washed away during the workup. Glycosyl amino acid **12a** was then subjected to 20% piperidine in NMP (room temperature for 40 min) to remove the Fmoc group. The product was once again isolated, purified by precipitation with ether, and washed with methanol−ethyl acetate, ethyl acetate, and ether to afford **13a** in quantitative yield. Coupling reaction of **13a** with the active ester of glutamine (**14**, 2 equiv) was achieved in NMP to give the glycodipeptide **15a**. Similarly, N-deprotection of **15a** followed by coupling with active ester **16** afforded the glycotripeptide **17a**, which was finally treated with 50% TFA in DCM to remove the side chain protections of Asn and Gln. After condensation under vacuum, washing of the residue with solvents, and drying, pure glycopeptide **7** (by TLC and NMR) was obtained in almost quantitative yield. Chromatography of **7** on a silica gel column proved to be difficult because of its high polarity, which also caused serious loss of the product (isolated overall yield: 80%). All products were characterized by NMR and MS.

The synthesis of glycopeptide **8** was conducted along the same line shown in Scheme 2. Reduction of **10b** followed by coupling with active ester **11** in NMP gave **12b** in 88% yield. ^{1}H NMR proved the product's β-glycosidic linkages ($^3J_{1,2}$ = 9.3, 7.7 Hz). N-Deprotection of **12b** and then coupling with active ester **14** afforded **15b**, of which the peptide chain was further elongated by the same protocol to result in **17b**. Finally, deprotection of **17b** with 50% TFA in DCM gave the target **8**. These reactions were clean, and each gave only one product (TLC and NMR). The overall yield after purification on a silica gel column was 80%.

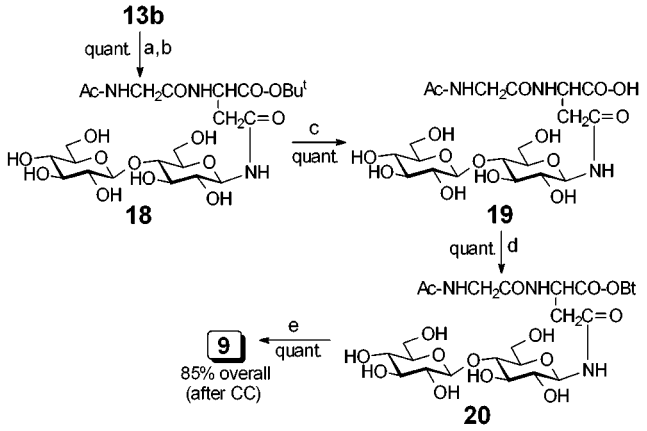
It was observed that working with the disaccharide was more convenient than working with the monosaccharide. For example, in the synthesis of **8**, only 5 volumes of ether,

(31) Xia, M.-Q.; Hale, G.; Lifely, M. R.; Ferguson, M. A. J.; Capmbell, D.; Packman, L.; Waldmann, H. *Biochem. J.* **1993**, *293*, 633−640.
(32) Treumann, A.; Lifely, M. R.; Schneider, P.; Ferguson, M. A. J. *J. Biol. Chem.* **1995**, *270*, 6088−6099.

relative to that of NMP, were required for complete precipitation of the products. This may be because the polarity of the disaccharide is higher than that of the monosaccharide and makes the glycopeptides containing disaccharides more easily precipitated.

We further explored the synthesis of glycopeptides on the basis of elongation of the peptide chain at both of its ends (Scheme 3). Thus, after a glycine was introduced to the

Scheme 3



a. 20% piperidine, NMP, r.t., 40min; b. **16**, NMP, r.t., overnignt;
c. 50% TFA, DCM, r.t., 2hr; d. DCC, HOBt, NMP, r.t., 1.5hr;
e. H-Gly-OBn (**21**), NMP, r.t., overnight

N-terminal of **13b** to give **18**, the *tert*-butyl group at its C-terminus was removed by 50% TFA. For activation of the resulting free carboxyl in **19**, we were at first concerned whether DCC could selectively promote the reaction of the carboxyl and HOBt in the presence of many free carbohydrate hydroxyls. The experimental results proved that the reaction gave only one product that was characterized as active ester **20**. Isolation and purification of the intermediates followed the protocols described above. Finally, reaction of **20** with glycine benzyl ester (**21**) gave the expected glycopeptide **9** with an isolated overall yield of 85%.

In conclusion, this report has demonstrated a new strategy for glycopeptide synthesis utilizing the glycosyl asparagines as the building blocks and the unprotected oligosaccharide chains as the phase tags. Three N-linked glycopeptides have been synthesized accordingly. Our preliminary results indicate that the free hydroxyl groups of carbohydrates do not affect the glycopeptide elongations, which is consistent with the previous observations.[20,33] It is necessary to point out that the philosophy of our use of unprotected carbohydrates in glycopeptide synthesis is fundamentally different from that of the previous solid-phase synthesis.[20,33] Our results also indicate that the isolation and purification of the reaction intermediates and products could be conveniently achieved by a precipitation method via changing the solvent compositions and washing the precipitates with proper solvents. The precipitation method could essentially replace column chromatography in these syntheses. The peptide elongation was very efficient since all the synthetic transformations were performed in homogeneous NMP solutions. Moreover, the actual progress of each step of the reaction could be easily monitored by TLC, and the reaction products could be conveniently characterized by NMR and MS. Therefore, the new strategy possesses the major advantages of both solution-phase and solid-phase syntheses, and it can be a useful alternative to existing methods. The new strategy should be applicable to a variety of structures, including complex glycopeptides. Actually, we anticipate that larger oligosaccharides and/or multiple oligosaccharide chains could be more effective phase tags. The new strategy is now pursued in this regard for the synthesis of glycopeptides containing more complex oligosaccharides, as well as O-linked glycopeptides.

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Supporting Information Available: Experimental procedures and full characterization of compounds **7**−**9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(33) Otvos, L. J.; Urge, L.; Hollosi, M.; Wroblewski, K.; Graczyk, G.; Fasman, G. D.; Thurin, J. *Tetrahedron Lett.* **1990**, *31*, 5889−5892.