

A Strategy for a Convergent Synthesis of *N*-Linked Glycopeptides on a Solid Support

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Oligosaccharides and glycopeptides are of considerable importance in molecular biology and pharmacology. However, their synthesis is complicated by the large number of different linking sites between each saccharide unit, the need for stereochemical control, the chemical sensitivity of the glycopeptide bonds, and the need to harmonize diverse protecting groups. Here, an efficient solid-phase synthesis of three *N*-linked glycopeptides based on glycal assembly is presented. The peptide domain can be extended while the ensemble remains bound to the polymer. The glycopeptides synthesized here are among the largest *N*-linked glycopeptides ever accessed by either solution- or solid-phase synthesis.

The surge of interest in glycoproteins (1–3) arises from heightened awareness of their importance in diverse biochemical processes, including cell growth regulation, binding of pathogens to cells (4), intercellular communication, and metastasis (5). Glycoproteins serve as cell differentiation markers and assist in protein folding and transport, possibly by providing protection against proteolysis (6). Improved isolation techniques and structural elucidation methods (7) have revealed high levels of microheterogeneity in naturally produced glycoproteins (8). Single eukaryotic cell lines often produce many glycoforms of any given protein sequence. For instance, erythropoietin, a clinically useful red blood cell stimulant against anemia, is glycosylated by more than 13 known types of oligosaccharide chains when expressed in Chinese hamster ovary cells (9). The efficacy of erythropoietin is heavily dependent on the type and extent of glycosylation (10).

Elucidation of the biological relevance of particular glycoprotein oligosaccharide chains benefits from isolation of pure entities. Glycoprotein heterogeneity renders this process particularly labor intensive. Some relief may be in sight in that particular cell lines can be selected to produce more homogeneous glycoproteins for structure-activity relation studies (11). However, the problem of isolation from natural sources remains daunting.

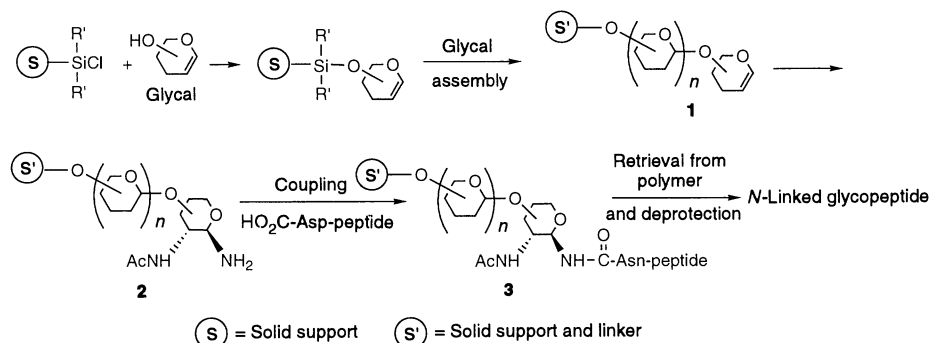
Fortunately, receptors normally recognize only a small fraction of a given macromolecular glycoconjugate. Consequently, synthesis of smaller but well-defined putative glycopeptide ligands could emerge as competitive with isolation as a source of

critical structural information (9). Important progress in glycopeptide synthesis pioneered by Kunz and others allowed synthetic access to homogenous target systems both in solution and in the solid phase (2, 12–15). Cohen-Anisfeld and Lansbury have recently reported a highly convergent solution-based coupling of selected already-available saccharides with peptides (13). In our method, the terminal glycal on the solid phase is linked with a peptide domain to generate an asparagine-linked *N*-acetylglucosamine construct; the whole ensemble is then retrieved and deblocked. This procedure allows one to fashion the carbohydrate domain of choice and also benefits from the

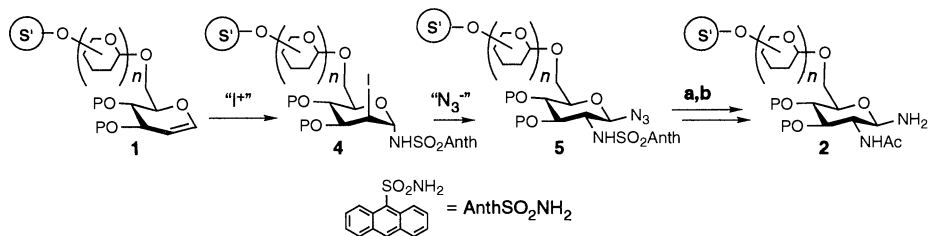
advantages associated with solid-phase synthesis in the critical carbohydrate-peptide coupling step.

The most successful approach to solid-phase *N*-linked glycopeptide synthesis in current practice involves construction of a peptide segment bearing an amine at the terminal residue on a solid support. The amine is then deprotected and coupled with either an appropriate oligosaccharide or small glycopeptide. Cleavage from the solid support and deprotection yields the desired glycopeptide (12).

The method we propose is illustrated in Scheme 1. An oligosaccharide terminating in a glycal is constructed on the solid support (see structure 1), which can be an extended linear structure or can contain branching as desired (16, 17). Through chemistry described below (Scheme 2), 1 is converted to the solid phase-bound 2, bearing a terminal 2-*N*-acetyl-1 β -aminoglucosamine residue (GlcNAc). A peptide is readily assembled through standard solution-phase peptide synthesis methodology or by a solid-phase assembly-retrieval sequence. Coupling of 2 with a suitable aspartic acid-containing peptide affords solid phase-bound glycopeptide 3. Retrieval and full deprotection affords the desired *N*-linked glycopeptide. In addition, deprotection of the carboxyl terminus and addition of a peptide with a free amino terminus allows for elongation of the peptide chain while the glycopep-



Scheme 1. *N*-Linked glycopeptide synthesis through the use of polymer-bound glycals.



Scheme 2. Preparation of polymer-bound 2-*N*-acetyl-1 β -amino glucosylamine by the azasulfonamidation reaction. P = saccharide protecting group; "I⁺" = iodonium bis(collidine) perchlorate; "N₃⁻" = tetrabutylammonium azide (Bu₄NN₃); Anth = anthracene; [a] acylation with acetic anhydride (Ac₂O) and 4-*N,N*-dimethylaminopyridine (DMAP); [b] reduction with 1,3-propanedithiol and *N,N*-diisopropyl-*N*-ethylamine (*i*-Pr₂NEt).

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tide 3 is still bound to the solid support.

A simple sequence was devised to convert 1 to 2 (Scheme 2). The use of the anthracenesulfonamide (18) in the azasulfonamidation sequence (19) was crucial for the addition step (4), the azide-induced rearrangement (5), and the presentation of the solid phase-bound GlcNAc residue bearing a 1 β -amino function (2). Two examples of this design for glycopeptide syn-

thesis are illustrated in Scheme 3, showing the relative simplicity of protecting-group requirements and the high order of convergence of the approach. They result, after deprotection, in 22 or 23.

The principal advantage in using the anthracenesulfonamide is that the nitrogen-sulfur linkage can be cleaved by a variety of mild methods (18). For instance, we developed the use of thiophenol or 1,3-

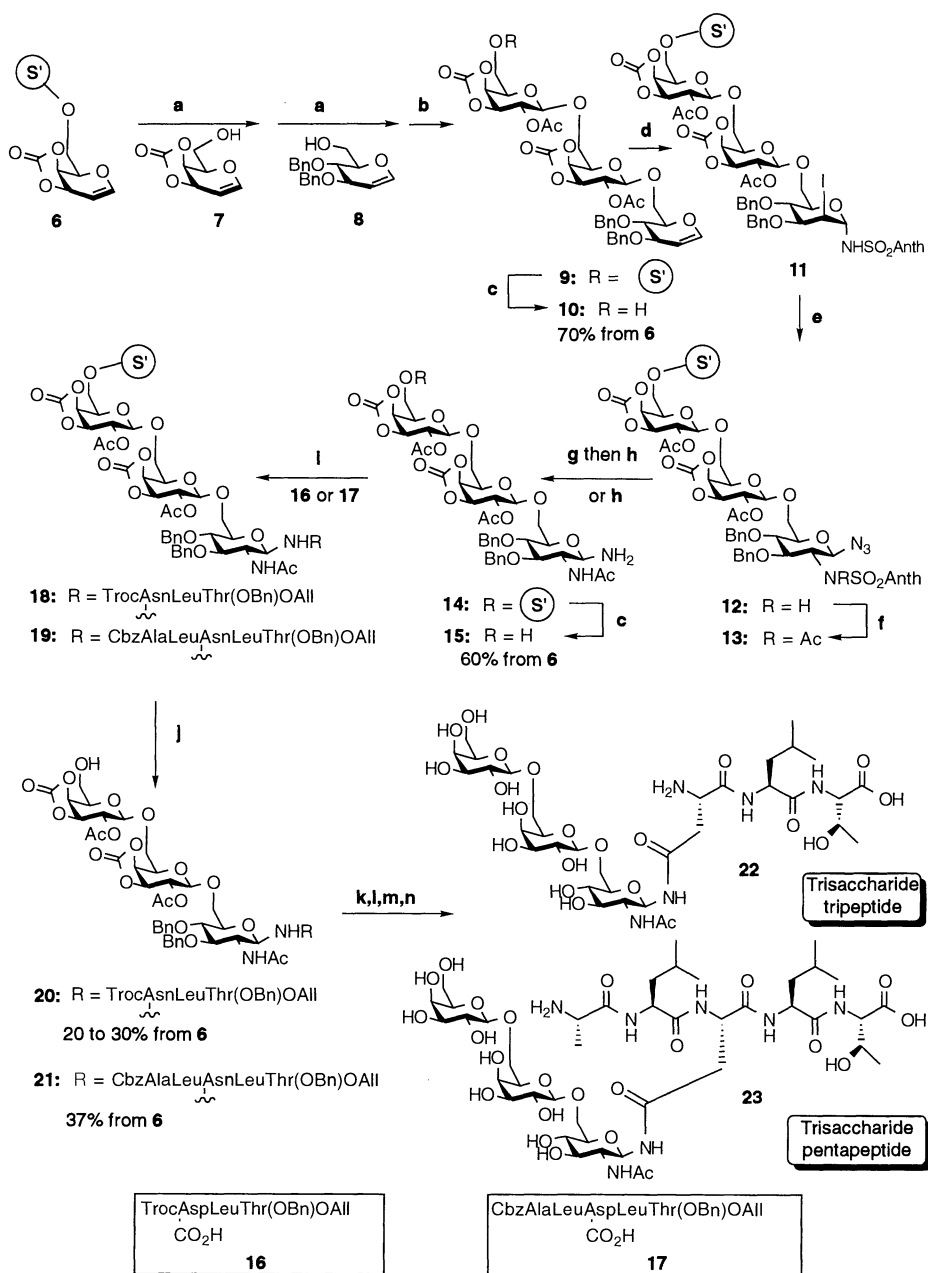
propanedithiol and Hunig's base for the removal of the anthracenesulfonyl group. These protocols are compatible with the solid-supported synthesis. Also, anthracenesulfonamide itself is more soluble than benzenesulfonamide in tetrahydrofuran (THF), which is a good swelling solvent for the polymer-supported work. Thus, the use of the anthracene-based agent results in a more efficient and complete iodosulfonamidation reaction.

In solution-phase coupling of carbohydrates and peptides, the process of separating the unreacted components and by-products is not a trivial matter. Purification is greatly simplified by conducting the coupling reaction on the solid support. Most of the excess peptide is recovered from the washing by chromatography. Small amounts are lost when the activated aspartic residue cyclizes to an aspartimide. In practice, protected trisaccharide pentapeptide 21 was obtained in 37% overall yield on the basis of the initial loading of galactal carbonate. The average yield for each of the 10 steps of the sequence to 21 was 91%. Chromatography on a short column of reverse-phase silica (octadecylsilane) was sufficient to obtain this compound in pure form. This purification capability arises from the previously described "self-policing" feature of the solid-phase glycal assembly method, which avoids deletions through destruction of uncoupled donors before the next coupling cycle (17).

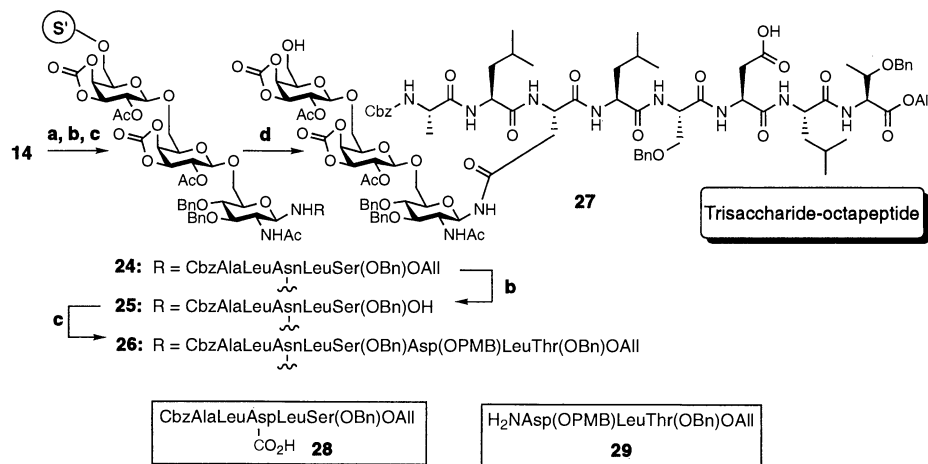
The glycopeptides retrieved from the support were deprotected as shown and the fully deblocked glycopeptides 22 and 23 were obtained in 61% and 48% overall yields from 20 and 21, respectively. Structural characterization of the glycopeptides by nuclear magnetic resonance spectroscopy showed the β configuration of all the anomeric linkages. The structures were further corroborated by mass spectroscopy.

The presence of orthogonal protecting groups on the carboxyl and amino termini of the peptide provides the opportunity to extend the peptide chain while the ensemble is bound to the solid support. Alternatively, after removal from the support, the freed peptide terminus may provide a functionality for linking to a carrier molecule to generate other glycoconjugates (20). Scheme 4 shows how the peptide portion of the glycopeptide was extended while still bound to the polymer support. Solid phase-bound trisaccharide pentapeptide 24 was assembled and the carboxyl terminus deprotected to give the acid, 25. Polymer-bound 25 was then coupled to tripeptide 29 with a free amino terminus to give glycopeptide 26. Retrieval from the solid support afforded trisaccharide octapeptide 27 in an 18% overall yield from polymer-bound galactal carbonate.

Regarding the future of glycopeptide



Scheme 3. Solid-phase synthesis of *N*-linked glycopeptides. All = allyl; Troc = 2,2,2-trichloroethoxycarbonyl; Cbz = benzyloxycarbonyl; Bn = benzyl. [a] Dimethyldioxirane, THF (23), 7 or 8, zinc chloride, and THF; [b] Ac₂O, collidine, DMAP, and THF; [c] tetrabutylammoniumfluoride, acetic acid, and THF at 40°C; [d] iodonium bis(collidine) perchlorate (24) and AnthSO₂NH₂ (9 → 11); [e] Bu₄NN₃ and THF (11 → 12); [f] Ac₂O, DMAP, and THF; [g] thiophenol, *i*-Pr₂NEt, and THF; [h] 1,3-propanedithiol, *i*-Pr₂NEt (14), and THF (13 → 14); [i] 1-isobutoxy-2-isobutoxycarbonyl-1,2-dihydroquinoline (IIDQ), CH₂Cl₂, and 16 or 17 (14 + 16 → 18 and 14 + 17 → 19); [j] HF-pyridine, anisole (25), and THF (18 → 20 and 19 → 21); [k] Pd(PPh₃)₄, dimethylbarbituric acid, and THF (26); [l] Zn, acetic acid, and methanol (20); [m] H₂, Pd(OAc)₂, and methanol (27); [n] KCN and methanol. Yields 22 (61% from 20) or 23 (48% from 21) (28).



Scheme 4. Extension of the peptide portion of the glycopeptide on the solid support. PMB = *p*-methoxybenzyl. [a] **28**, IIDQ, and CH₂Cl₂ (**14** → **24**); [b] Pd(PPh₃)₄, dimethylbarbituric acid, and THF; [c] **29**, IIDQ, and CH₂Cl₂; [d] HF-pyridine, anisole, and CH₂Cl₂ (**26** → **27**).

synthesis, one should also consider the method of Wong, which involves enzymatically mediated elaboration of a solid phase-bound glycosylated peptide construct using glycosyl transferases to append unprotected nucleoside phosphate-activated monosaccharides in the elongation phase (21). By this method, retrieval from the solid support is mediated by protease action. The relief from protecting groups in enzymatically mediated chemistry can be a substantial advantage. Both Wong's method and our method allow the use of unnatural amino acids and non-amino acids. The method described herein is, in principle, totally general in that it does not require the existence of the transferases and the availability of nucleoside-activated hexoses. It can also accommodate the inclusion of unnatural (artificial) sugars in the scheme. Such building blocks are available from the Lewis acid-catalyzed diene-aldehyde cyclocondensation reaction (22). All workable approaches, whether purely chemical or chemoenzymatic, are complementary for reaching the common goal of carefully designed, fully synthetic glycopeptides.

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Inhibitor-Enhanced Electron Transfer: Copper Cytochrome c as a Redox-Inert Probe of Ternary Complexes

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Copper-substituted cytochrome c (CuCc) has been used as a structurally faithful, redox-inert inhibitor to probe the mechanism of electron transfer (ET) between Cc molecules and cytochrome c peroxidase (CcP). This inhibitor enhances photoinduced ET quenching of the triplet excited state of a zinc-substituted protein (ZnCcP or ZnCc) by its iron(III) partner (Fe³⁺Cc or Fe³⁺CcP). These results show that CcP and Cc form a ternary complex in which one Cc molecule binds tightly at a surface domain of CcP having low ET reactivity, whereas the second Cc molecule binds weakly to the 1:1 complex at a second domain with markedly greater (~10³) reactivity. These results also rule out the possibility that Cc bound at the second domain cooperatively enhances ET to Cc at the first domain. The multiphasic kinetics observed for the photoproduced ET intermediate do not reflect electron self-exchange between two Cc molecules within the ternary complex.

Respiration and metabolism depend on the sequential transfer of electrons from one protein to another (1), and this ET in turn

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depends on the recognition and docking, as well as the reaction, of the ET partners (2). Issues of binding specificity and of reactivity in protein-protein reactions are analogous to those in enzyme-substrate reactions, but studies of ET complexes have lacked paral-