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Glycopeptide Synthesis on an Ionic Liquid Support

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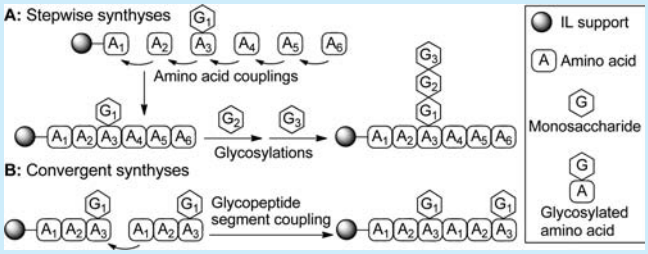
Supporting Information

ABSTRACT: An ionic liquid-supported synthetic method for the construction of glycopeptides in high yields is reported. This method avoids the use of large excesses of reagents and chromatographic purification and, therefore, represents a useful addition to existing approaches for the ionic liquid-supported synthesis of oligosaccharides and peptides.



Efficient methods for the synthesis of glycopeptides are indispensable for both elucidating their biological significance and producing therapeutic reagents of relevant diseases. The challenge associated with the construction of these molecules lies in being able to exercise a high level of control over both carbohydrate and peptide chemistries, which require complex protection and coupling strategies as well as tedious product purification processes. Standard solid-phase peptide synthesis (SPPS) is currently the most commonly used approach for the construction of glycopeptides and involves the use of preprepared glycosylated amino acids as building blocks.[1] The key advantage of SPPS over conventional liquid-phase synthesis is the simple resin-washing process used for product isolation, which allows for the synthesis to be conducted with a high level of automation.[2] Although a number of complex glycopeptides have been successfully constructed by SPPS,[3] the heterogeneous conditions required for SPPS can result in low reaction rates, and it can be particularly difficult to monitor the progress of reactions conducted by SPPS using conventional spectroscopic techniques. Several novel liquid-phase strategies have been developed to address the issues associated with SPPS. According to one such strategy, the reactions themselves can be conducted under homogeneous conditions, whereas the purification of the product can be achieved by a simple phase separation. Prominent examples of this approach include the use of functional supports such as soluble polymers,[4] fluoride,[5] or ionic liquids (ILs)[6] as platforms for conducting the chemical synthesis. A common feature of these supports is their tunable solubility in different solvents, which allows the supported reactants to undergo efficient homogeneous reactions in one solvent and simple product purification processes in another solvent without the need for column chromatography. ILs, in particular, possess several attractive properties in terms of their use as functional supports for chemical synthesis, including good chemical stability, low molecular weight, and well-defined structures, and the versatility of IL supports in this regard has recently been demonstrated by their use for the rapid construction of various molecules including peptides[7] and oligosaccharides.[8]

Herein, we report for the first time the synthesis of glycopeptides using an IL support. In a similar manner to SPPS, our approach involved the use of a solid−liquid phase product separation step to increase the overall efficiency of the synthetic process. However, our approach avoided the use of large excesses of the reagents, which is one of the major problems associated with SPPS, especially for the large-scale synthesis of glycopeptides involving expensive glycosylated amino acid building blocks. We have demonstrated the feasibility of our method for the synthesis of several *O*-glycopeptides and shown that this strategy can be used for not only the stepwise linear elongation of both peptide and glycan chains but also the convergent assembly of glycopeptide fragments.

The design of our method for the IL-based synthesis of glycopeptides was based on three essential considerations. First, the overall synthetic strategy was based on the use of a 9-fluorenylmethoxycarbonyl (Fmoc) for the temporary protection of the hydroxyl or amine groups of the building blocks, which were used in conjunction with glycosyl trichloroacetimidate donors (**2** in Scheme 1; **20** and **23** in Scheme 2). The reaction required for the elongation of the peptide and glycan chains would involve the repeated deprotection of these reactive groups, and the sequence of Fmoc-removal reaction could be achieved under mildly basic conditions without affecting the acid-labile *O*-glycosidic bonds.[9] It was envisaged

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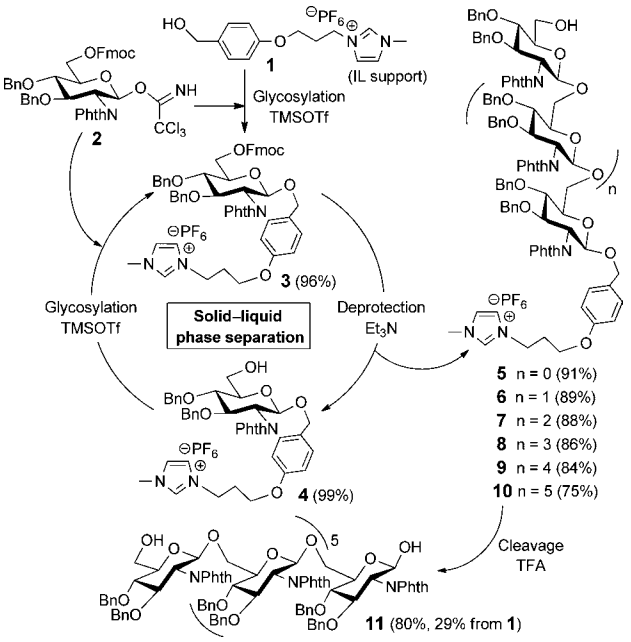
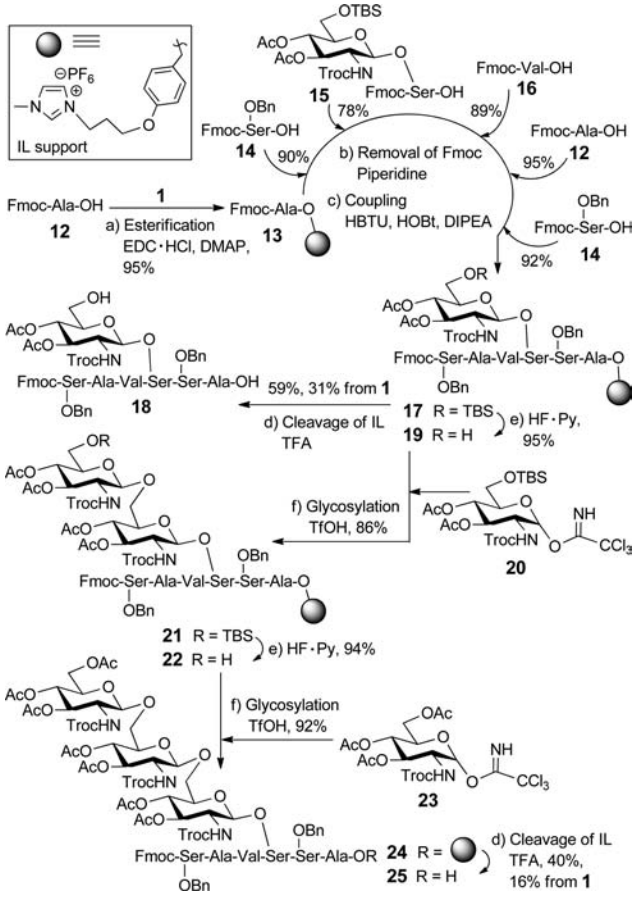
Scheme 1. IL-Supported Synthesis of Oligosaccharides Using the Fmoc-Protection and Trichloroacetimidate-Based Glycosylation Strategies[a]



[a]The yields for compounds **5**–**10** represent the overall yields for the glycosylation and deprotection reactions (2 steps). *Glycosylation conditions:* donor **2** (2.5 equiv), TMSOTf (0.15 equiv), and MS (4 Å) in DCM (a DCM/MeCN mixture was used for the synthesis of **3**) at −20 °C for 1 h. *Deprotection conditions:* Et_3N in DCM at rt for 2 h. *Cleavage of the IL support:* TFA and H_2O in DCM at rt for 6 h to give oligosaccharide **11**, which was purified by gel filtration chromatography (eluent: 1:1 DCM/MeOH). *Phase separation procedure:*[8e] Upon completion of reaction, the mixture was concentrated under vacuum to give a residue, which was dissolved in a DCM/IPE mixture (1:4 v/v). The polar DCM was then removed by rotary evaporation, which allowed for the IL-supported substrate to precipitate from the nonpolar IPE. The precipitate was collected by filtration and washed with diethyl ether to yield the product. *Abbreviations:* TMSOTf, trimethylsilyl trifluoromethanesulfonate; DCM, dichloromethane; MS, molecular sieve; rt, room temperature; IPE, isopropyl ether.

Scheme 2. IL-Supported Stepwise Synthesis of Glycopeptides[a]



[a]The product isolation steps were achieved by phase separation, unless otherwise noted. *Reaction conditions:* (a) amino acid **12** (2.0 equiv), EDC·HCl (2.0 equiv), and DMAP (0.2 equiv) in MeCN at rt for 7 h; (b) piperidine (10.0 equiv) in DCM at rt for 30 min; (c) Fmoc-protected amino acid (2.0 equiv) or glycosylated serine **15** (1.5 equiv), HOBt (2.0 equiv), HBTU (2.0 equiv), and DIPEA (2.5 equiv) in MeCN at rt for 8 h; (d) TFA and H_2O in a mixture of DCM and anisole at rt for 1 h gave glycopeptides **18** and **25**, which were isolated by HPLC; (e) HF·Py in Py at 0 °C to rt for 12 h; (f) donor **20** or **23** (2.5 equiv), TfOH (0.4 equiv), and MS (4 Å) in DCM at −30 °C for 1 h. *Abbreviations:* EDC, 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide; DMAP, 4-dimethylaminopyridine; HOBt, 1-hydroxybenzotriazole; HBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; DIPEA, *N*,*N*-diisopropylethylamine; TfOH, trifluoromethanesulfonic acid; Py, pyridine.

that the use of highly reactive trichloroacetimidates,[10] with an ester protecting group on their C2-amino moiety, would enable the glycosylation reaction to occur in high yield and in a stereospecific manner to form the β-isomer exclusively (i.e., neighboring group participation).[8e,f] Second, benzyl ethers and acetyl esters can be used as permanent protecting groups and would be compatible with the chemistries required for the sequential Fmoc-deprotection steps, as well as the glycosylation and amino acid coupling strategies. Finally, we designed the IL support **1** bearing a *p*-alkoxybenzyl alcohol spacer (Supporting Information Scheme S1) so that it could be tethered to the growing glycan or peptide chain. This spacer was structurally very similar to the Wang linker,[11] which has been used extensively in SPPS and is stable under all of the reaction conditions required during the synthesis of glycopeptides. The Wang linker can be selectively cleaved with trifluoroacetic acid (TFA) when needed.

The construction of glycan chains is generally considered to be technically more challenging than that of peptides. With this in mind, we focused initial efforts on exploring the compatibility of the IL support **1** with the conditions required for the trichloroacetimidate-based glycosylation and the Fmoc-protection strategies by synthesizing a series of β1,6-linked glucosamine oligomers (compounds **5**–**11** in Scheme 1). Structures of this type are very similar to those of the major components of many microbial surface antigens,[12] and considerable synthetic efforts have been directed toward the development of strategies capable of providing access to these biologically important molecules.[13] In this study, we used an "IL-supported glycosyl acceptor" strategy[8e,f] for the oligosaccharide synthesis. The IL support **1** was attached to the reducing end of the growing glycan chain at the anomeric center via a glycosylation reaction with imidate **2**. The triethylamine-mediated removal of the Fmoc group followed by the glycosylation of the resulting alcohol with donor **2** in the presence of the promoter TMSOTf resulted in the formation of

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the target oligosaccharides **5**−**10**. All of these reactions were conducted in dichloromethane under homogeneous conditions and afforded the desired products in good yields without using a large excess of the donor. Importantly, the IL-supported reactants were highly soluble in polar solvents, such as dichloromethane, acetonitrile, and methanol, which allowed for the progress of these reactions to be readily monitored by TLC, NMR, and MS analyses. The product purification steps were performed in accordance with a previously reported phase separation procedure,[8e] which was based on the poor solubility of the IL-bound glycans in nonpolar solvents such as isopropyl ether and diethyl ether. The resulting intermediates **3**−**10** exhibited sufficient purity to be progressed directly into the subsequent reactions. Finally, the IL support was cleaved from compound **10** with TFA to give the partially protected oligosaccharide **11** in an overall yield of 29% following gel filtration purification. The structure of **11** was verified by NMR spectroscopies (^{1}H, ^{13}C, COSY, and HSQC). The ^{1}H NMR spectrum of **11** (400 MHz, in $CDCl_3$) showed characteristic signals for the anomeric protons (δ = 5.5−4.9 ppm) with coupling constants around 8.0 Hz, clearly indicating that **11** contained seven monomeric glycosyl units that were linked to each other with β-configurations. HR-MALDI-TOF-MS analysis of **11** provided further confirmation of the heptaglucosamine structure. Collectively, these results demonstrate that relatively large and complex oligosaccharides can be efficiently constructed using our IL-based method.

Although several IL-based approaches have been developed for the construction of oligosaccharides,[8] the glycan-assembly strategy outlined above for the IL-supported synthesis of glycopeptides warranted further investigation. To highlight the potential of this particular strategy, we applied it to synthesize the glycopeptide fragment of the serum response factor[14] (**18** in Scheme 2) as well as its glycosylated products **21** and **25**. Use of the Fmoc protocol was particularly preferable for these IL-supported glycopeptide constructions, because the corresponding *tert*-butoxycarbonyl (Boc) protocol[7a,b] requires the use of strongly acidic conditions (TFA) for the recurring cleavage of the Boc groups, and these conditions could have an adverse effect on the acid-labile *O*-glycosidic bond and the Wang linker. The esterification of the Fmoc-protected alanine **12** with the IL support **1** afforded the IL-bound amino acid **13**, which was used as a starting material for the glycopeptide synthesis. In addition to the preprepared glycosylated serine **15** (Scheme S3), several commercially available amino acid building blocks (**12**, **14**, and **16**) were incorporated in the reaction cycle and assembled step by step using piperidine, for the cleavage of the Fmoc-protecting groups, and a mixture of HBTU and HOBt, as the coupling activator. The homogeneous liquid-phase conditions allowed for these deprotection and coupling reactions (each amino acid was added in only minor excess) to be accomplished in good to excellent yields. Similar to the IL-supported glycan synthesis, purification by phase separation afforded the intermediates in high purity, and these compounds were subsequently characterized by NMR and MS analyses. Furthermore, HPLC analysis revealed that no detectable racemization or epimerization had occurred during the synthetic process. The desired glycopeptide **18** was obtained in 31% yield following the removal of the IL support from **17** by TFA. This acidic condition also resulted in the concomitant cleavage of the *tert*-butyldimethylsilyl (TBS) protecting group from the C6 hydroxyl of the glucosamine residue. The NMR and HR-ESI-MS analyses of **18** suggested that the hexapeptide backbone was monoglycosylated with a β-linkage. These results therefore confirmed that this method allowed for the successful construction of a glycopeptide with a shorter glycan branch via the incorporation of a glycosylated amino acid to the IL-supported stepwise peptide assembly process.

To demonstrate the utility of our IL-based method for the construction of more complex glycopeptides bearing multiple sugar units, we conducted two consecutive glycosylation reactions using the IL-bound glycopeptide acceptor **19** with the glycosyl donors **20** and **23**. Glycopeptide **19** was obtained from **17** following the selective cleavage of the TBS group by treatment with a HF·pyridine complex. The *N*-2,2,2-trichloroethoxycarbonyl (Troc)-protected imidates **20** and **23** were selected as the donors instead of the *N*-phthaloyl (Phth)-protected imidate **2**, because the Troc group is more amenable to the conditions required for the glycopeptide synthesis than the Phth group.[15] The TBS group on the C6 hydroxyl of donor **20**, which is orthogonal to the Fmoc group used to protect the amines, was employed for temporary protection that could be removed as necessary to allow for the introduction of additional sugar units at this position. Both glycosylation reactions proceeded smoothly in the presence of a TfOH promoter to afford the desired glycopeptide **25** in 16% yield, following the removal of the IL support and purification of the cleavage product by HPLC. The ^{13}C NMR spectrum of **25** (125 MHz, in DMSO-d_6) contained three unique anomeric C signals (δ around 100 ppm) and three CCl_3 peaks characteristic of the Troc group (δ around 96 ppm), confirming that the trisaccharide side chain had been successfully grafted onto the peptide backbone. HPLC analysis indicated that there had been no discernible isomerization of the product during the glycosylations and the IL-support removal process. This convenient and high-yielding synthesis of **25** on an IL support demonstrates that our method is highly effective for glycopeptide construction.

Convergent synthesis represents an efficient way by which to generate peptides with a repetitive sequence and is generally employed in SPPS for the construction of glycopeptides.[16] To determine whether our IL-based method was compatible with this strategy, we investigated the block coupling of the glycopeptide segments **27** and **28** using the method (Scheme 3). The monoglycosylated tripeptide **26**, which was synthesized as an intermediate in the construction of glycopeptide **18**, was used as a precursor to produce **27** and **28**. Cleavage of the IL support from **26** exposed the carbonyl group of the peptide backbone (**27**), whereas removal of the Fmoc-protecting group revealed the amino terminus (**28**). The block coupling of the two precursors in the presence of a PyBOP activator afforded glycopeptide **30** in 29% yield following the removal of the IL support and purification of the crude product by HPLC (the HBTU−HOBt activating conditions were ineffective for this block coupling reaction because they led to the formation of a guanidine side product[17]). The NMR and HR-ESIMS data for **30** corresponded well with the proposed bis-glycosylated hexapeptide structure. This synthesis therefore verified the feasibility of synthesizing glycopeptides by segment coupling on an IL support.

In summary, we have developed a new approach for the construction of glycopeptides, based on the IL-supported synthesis methodology. The use of an IL support bearing a Wang linker, combined with the glycosyl trichloroacetimidate donor and Fmoc-protection strategies, facilitated the synthesis

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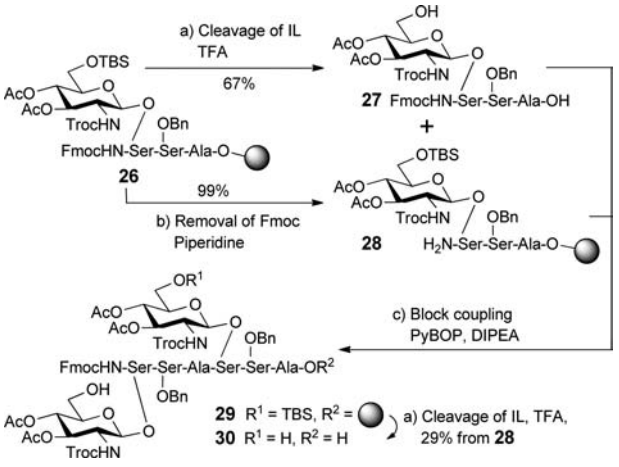
Scheme 3. IL-Supported Convergent Synthesis of a Glycopeptide[a]



[a]The product isolation steps were carried out by phase separation, unless otherwise noted. *Reaction conditions:* (a) TFA and H_2O in a mixture of DCM and anisole at rt for 1 h gave the glycopeptides **27** and **30**, which were purified by HPLC; (b) piperidine (10.0 equiv) in DCM at rt for 30 min; (c) glycopeptide **27** (2.0 equiv), PyBOP (2.0 equiv), and DIPEA (2.5 equiv) in DCM at rt for 24 h. *Abbreviations:* PyBOP, benzotriazole-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate.

of both oligosaccharides and glycopeptides in terms of rapidity and efficiency. The versatility of this approach was exemplified by the construction of the oligosaccharides **5**−**11** and the glycopeptides **18**, **25**, and **30**, which contained a mono- or trisaccharide branched structure or multiple glycosylation sites, respectively. The homogeneous liquid reaction conditions provided easy access to these glycans and glycopeptides in high yields without the need to use large excesses of the reagents. Furthermore, the heterogeneous phase separation for the product purifications avoided the need to use chromatography. The examples described in this study represent a step forward in the development of methods for the facile synthesis of glycopeptides for biological and pharmaceutical applications.

■ ASSOCIATED CONTENT

Supporting Information

Schemes S1−S3; Figures S1; Experimental Section; spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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