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Mixed-Phase Synthesis of Glycopeptides Using a *N*-Peptidyl-2, 4-dinitrobenzenesulfonamide–Thioacid Ligation Strategy

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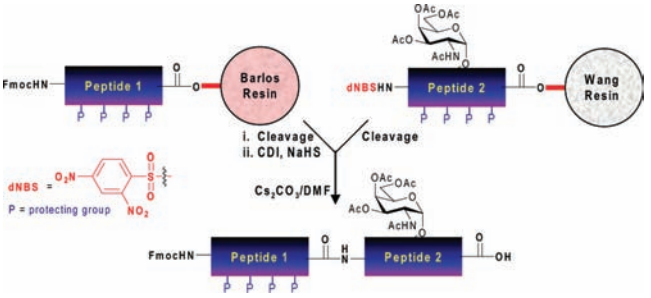
ABSTRACT



A strategy for the solid phase peptide synthesis (SPPS) and coupling of *N*-peptidyl and *N*-glycopeptidyl 2,4-dinitrobenzenesulfonamides (dNBS) with C-terminal peptidyl thioacids has been developed. The resulting *N*-dDNBS peptides were coupled to generate longer peptides. Ligation reactions were complete within 15 to 20 min.

There is significant interest in developing chemoselective amide bond forming reactions for the preparation of peptides and glycopeptides.[1] Many of these amidation reactions involve the use of peptidyl thioesters. However, these reactions are often slow which can lead to hydrolysis of the thioester employed and other side reactions. For example, the direct aminolysis of peptidyl thioesters generally requires reaction times of 48 to 96 h.[2,3]

2,4-Dinitrobenzenesulfonamides (dNBS) were found by Tomkinson et al. to undergo condensation reactions with thioacids to form amides. In addition, dNBSs react with a range of nucleophiles such as hydroxamic acids to yield ureas, dithioic acid to yield thioamides, and dithiocarbamates to yield thioureas.[4] Since those early reports, only a few examples of the use of this chemistry for the chemoselective formation of amide bonds have surfaced. A putaive mechanism for the amidation is shown in Figure 1.[4] More recently, it

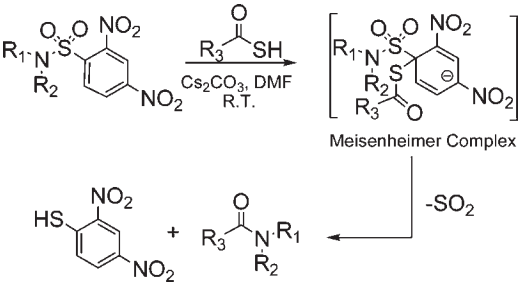


Figure 1. Probable mechanism for the dNBS–thioacid ligation reaction proposed by Tomkinson et al.[4,5]

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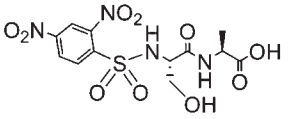
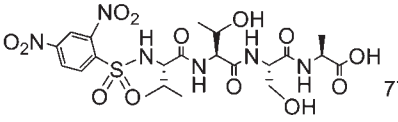
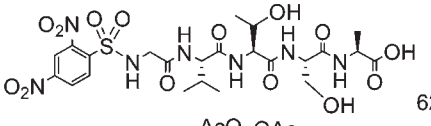
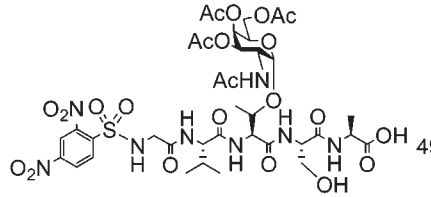
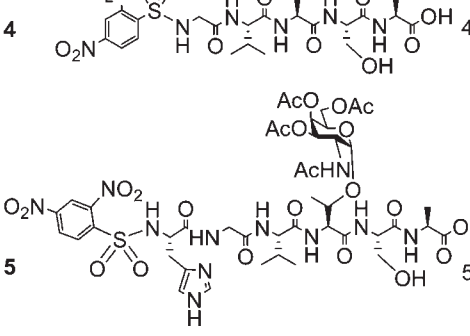
has been suggested the Meisenheimer complex decomposes to an arylthioester which acts as an amine acylating agent.[5]

In the past, thioacids have been used in combination with *N*-terminal dNBS amino acid methyl esters[6] to form glycosyl, peptidoglycosyl, and peptidyl amide linkages. We recently investigated the coupling of thioacids with *N*-glycosyl-2,4-dinitrobenzenesulfonamides to afford *N*-glycosyl amides.[7] Further, Crich et al. demonstrated a solution phase peptide synthesis using combinations of electron-deficient peptidyl benzenesulfonamides capable of reacting with peptidyl thioacids with differential reactivity.[5] Seeking to further expand this amidation reaction involving peptidyl thioacids and electron-deficient peptidyl benzenesulfonamides we were interested in developing an Fmoc-based SPPS of *N*-dNBS peptides to access longer *N*-dNBS peptides. We also desired to ligate the resulting *N*-peptidyl sufonamide with an SPPS-derived peptidyl thioacid to demonstrate a mixed-phase synthesis of peptides and glycopeptides using the chemistry. We chose to work with a glycopeptide sequence based on a MUC1 tandem repeat based on our interest in the target.[8]

MUC1 tandem repeats are a heavily glycosylated sequences that are part of the MUC1 transmembrane protein. Aberrant MUC1 glycoforms are associated with cancers of epithelial origin and are relevant targets for glycopeptide-based anticancer therapeutics.[9−11] The 20 amino acid tandem repeat possesses several possible glycosylation sites at the threonine and serine residues found along the repeating sequence NH_2-PDTRPAPG-STAPPA$_{14}$H$_{15}$G$_{16}$VTSA-COOH, and synthetic access to pure forms of the individual MUC1 glycoforms is desirable. We investigated ligation at the H_{15}-G_{16} and A_{14}-H_{15} sites to evaluate the dNBS−thioacid coupling strategy with the simultaneous presence of multiple unprotected functional groups, such as imidazole, carboxyl, and hydroxyl in the MUC1 fragments to demonstrate the chemoselectivity of the amidation.

SPPS is a common method for procuring medium sized peptide fragments, and the coupling of *o*- and *p*- nitrobenzenesulfonyl groups to the *N*-terminus of a peptide bound to a rink amide MBHA resin has been reported.[12,13] Thus, it seemed feasible that dNBS groups could be introduced into an SPPS as well. To begin our study, we performed a screening of conditions potentially useful for introducing 2,4-dinitrobenzenesulfonyl chloride (dNBS-Cl) on NH_2-Ala-Wang resin. The combination of dichloromethane and pyridine was found optimal for introducing the dNBS group onto peptides in solution phase.[6a] On solid phase however, increased equivalents of dNBS-Cl (4 equiv) were required for completion of the sulfonation. Other conditions that were explored included CH_2Cl_2−DIEA, DMF−pyridine, DMF−DIEA, and CH_2Cl_2−pyridine−DMAP (0.1 equiv), but all appeared unsatisfactory. The sulfonylation took 4 h to reach completion, and the reaction progress was monitored by a Kaiser test.[13] The dNBS-alanine was cleaved from the resin by TFA treatment to give the dNBS-alanine as the primary product. It was notable that the dNBS group was stable to the acidic cleavage condtions. After the optimization of the sufonation conditions, the synthesis of longer 2,4-dinitrobenzenesulfonyl peptides and glycopeptides was attempted. The peptides were manually assembled on preloaded Wang resin using Fmoc chemistry. Coupling of the amino acids was accomplished using PyBOP, HOBt, and DIEA in DMF. The dNBS group was installed in the last coupling step using 4 equiv of dNBS-Cl and 10 equiv of pyridine in dichloromethane.

Table 1. *N*-Peptidyl-2,4-dinitrobenzenesulfonamides

no.	peptides	yield
1		85%
2		77%
3		62%
4		49%
5		56%

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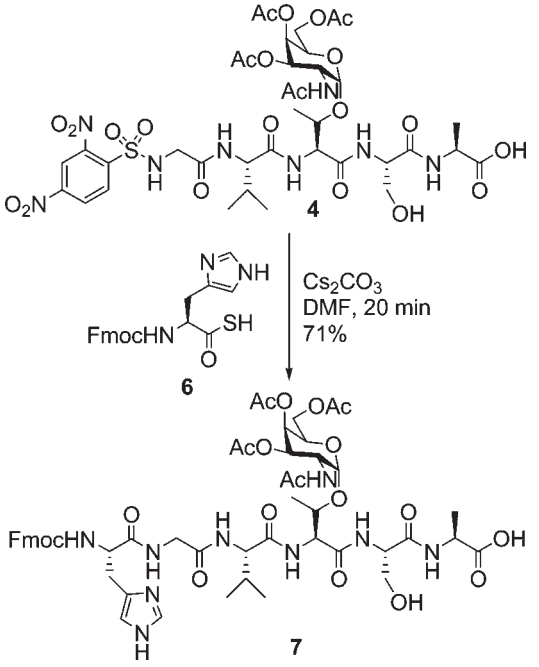
The successful on-resin coupling of dNBS at a variety of amino acids and in the presence of protected glycan provides peptide fragments for ligation at relatively hindered sites (Table 1). Cleavage of peptides from the resin was generally effected by TFA in the presence of various nucleophilic additives to capture highly reactive cationic species.[13] The TFA−TIPS−H_2O (95:2.5:2.5) cleavage mixture[14] not only worked well as a cleavage mixture but also acted as a global deprotecting system for these peptides. The dNBS, which remains attached to the peptide through cleavage from the resin, has not been shown to survive TFA treatment until now. After cleavage, the peptides were isolated by reversed phase HPLC using C-8 column monitoring at a 254 nm wavelength.

Scheme 1. Coupling between Fmoc-Histidinyl Thioacid and dNBS-Glycopeptide **4** Using Thioacid−Sulfonamide Ligation Chemistry



To demonstrate the utility of the *N*-dNBS peptides the solution phase dNBS−thioacid ligation was first executed with dNBS-glycopeptide **4** and Fmoc-histidine thioacid **6** (Scheme 1). The Fmoc-histidine thioacid was obtained from Fmoc-*N*(trityl)-histidine by coupling with tritylthiol in the presence of HATU/DIEA in DMF followed by global deprotection with TFA−TIPS−CH_2Cl_2 (50:5:45). The ligation was performed in the presence of cesium carbonate at 0.5 M total concentration of the reactants in DMF. The reaction reached completion in 20 min (monitored by ESI-MS). Semipreparative reversed phase HPLC purification afforded the hexapeptide **7** in 71% yield (with respect to the dNBS-glycopeptide **4**) (*m*/*z* calculated for $C_{52}H_{68}N_9O_{19}$ is 1122.3 $[M+H^+]$, found: 1122.5).

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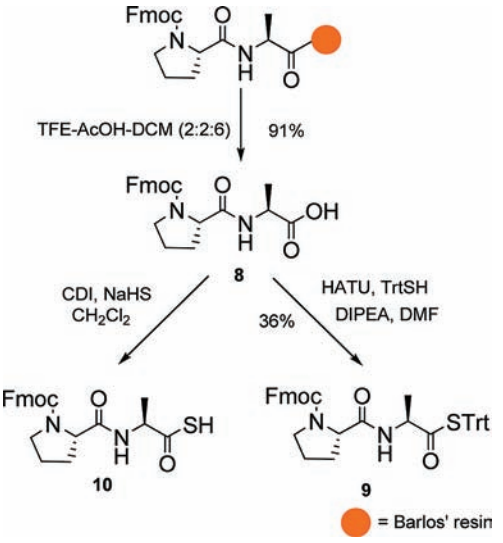
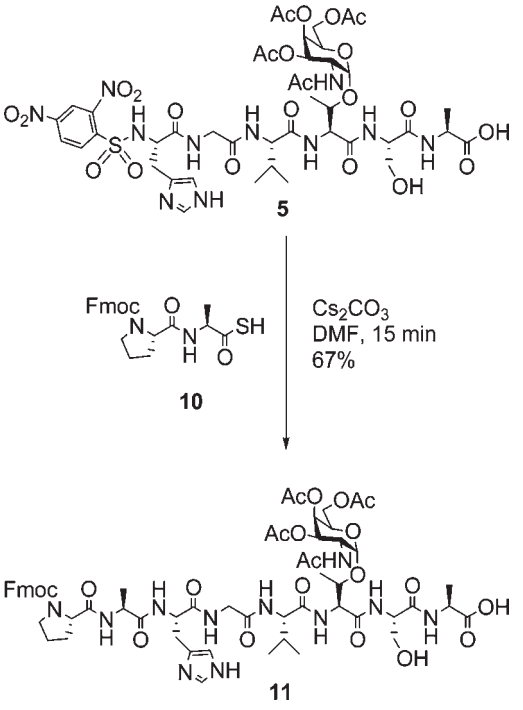
Scheme 2. Access of Peptidyl Acid **8** from Resin and Its Conversion into Corresponding Peptidyl Thioester **9** and Thioacid **10**[a]



[a] Compound **10** was directly taken to the next ligation step without further purification.

Scheme 3. Coupling between Fmoc-Dipeptidyl Thioacid and dNBS-Glycopeptide **5** Using Thioacid−Sulfonamide Ligation Chemistry



Dipeptide **8** was obtained from SPPS by manual assembling of the amino acids on Barlos's (chlorotrityl) resin. Coupling of the amino acids was effected using PyBOP, HOBt, and DIEA in DMF. Cleavage from the resin with

TFE–AcOH–DCM (2:2:6) followed by azeotroping the filtrate with toluene afforded the dipeptidyl acid in considerably pure form. We selected Barlos' resin because the mild conditions required to cleave the peptide chain from this support are compatible with many common side-chain protection groups, and maintaining these protecting groups is necessary prior to conversion of the C-terminal acid group to a thiotrityl ester. However, the attempt to convert dipeptide **8** into the corresponding thiotrityl ester **9** ended with a maximum of a 36% yield. Hence, a more direct route to the thioacid from the peptidyl acid appeared to be necessary.

A one-pot conversion of *N*-terminally protected peptidyl acid into thioacid has recently been reported using CDI and Na_2S in acetonitrile.[15] In our case, a combination of CDI and the less basic NaHS in dichloromethane was superior for obtaining the dipeptide thioacid **10** from **8** (Scheme 2). The peptidyl thioacid **10** was directly used in ligation with peptide **5** without extensive purification, and the reaction completed in 15 min (monitored by ESI-MS). The pure glycosylated MUC1 octapeptide **11** was afforded by reversed phase HPLC with a 67% yield (with respect to the dNBS-glycopeptide **5**) (m/z calculated for $C_{60}H_{80}N_{11}O_{21}$ is 1290.5 $[M+H^+]$, found: 1291.1) (Scheme 3). No epimer of **7** or **11** was identified by mass spectrometry of isolated HPLC peaks.

The successful ligation of an SPPS derived *N*-arylsulfonated MUC1 glycopeptide containing an unprotected amino acid side chain and O-linked glycans using the dNBS–thioacid ligation chemistry suggests that this methodology is efficient and relatively chemoselective. The ease with which we have synthesized the *N*-dNBS peptides on the solid phase and coupled them with peptidyl thioacids, we believe, facilitates the adoption of this chemistry for mixed-phase peptide synthesis.

Acknowledgment. This work was supported by the National Institutes of Health (GM094734).

Supporting Information Available. Experimental details of synthesis, NMR spectra, and HPLCs of compounds **1**–**9** and **11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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