ORGANIC LETTERS

2001
Vol. 3, No. 8
1189−1192

Convergent Synthesis of Peptide Conjugates Using Dehydroalanines for Chemoselective Ligations

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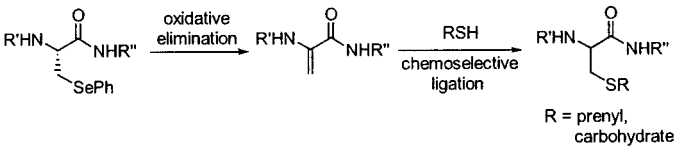
Received February 1, 2001

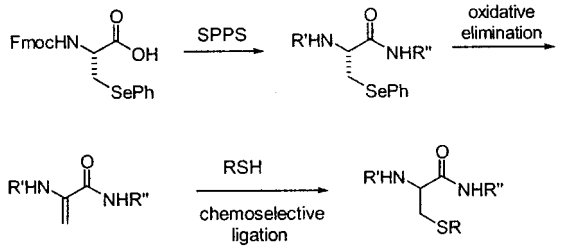
ABSTRACT



Protein and peptide conjugates such as glycopeptides, prenylated peptides, and lipopeptides play essential roles in biology. A rapid and convergent entry into a variety of these compounds is described. The methodology involves the introduction of a dehydroalanine into peptides and subsequent chemoselective conjugate addition of an appropriate thiolate nucleophile, including farnesylthiolate or thioglycosides.

Protein and peptide conjugates such as glycoproteins, prenylated proteins, and lipoproteins play essential roles in numerous cellular processes including cell adhesion, membrane localization, signal transduction, viral infection, and the immune response. Because of the importance of these processes in many human disorders such as cancer metastasis, rheumatoid arthritis, and viral infections, the synthesis of peptide conjugates has been the focus of extensive investigation.[1] We report here a versatile and rapid route toward a variety of peptide conjugates using a chemoselective convergent ligation between nucleophilic reagents and unprotected peptides containing an electrophilic handle. Our general route toward site-specific introduction of functionalities into peptides is shown in Scheme 1. *Se*-Phenylselenocysteine can be incorporated into peptides at pre-selected positions using solid-phase peptide synthesis (SPPS). After global deprotection and cleavage from the resin, chemoselective oxidation yields a dehydroalanine containing peptide.[2] Addition of a suitable nucleophile to this electrophile produces the desired peptide conjugate.

Scheme 1



Most of the methods developed to date for the preparation of peptide conjugates utilize a building block approach in which a derivatized amino acid is incorporated into the peptide during SPPS.[3] The route described above presents a conceptually attractive alternative involving standard SPPS and subsequent site-specific modification of the unprotected peptide in the final step of the assembly. This strategy would circumvent any potential interference of the conjugate of interest with existing optimized protocols of SPPS such as protecting group usage, coupling methods, and cleavage from the support. Furthermore, such a convergent strategy would allow a rapid and versatile entry into libraries of peptide conjugates.[4] Unfortunately, few[5] general methods for the site-specific preparation of peptide conjugates from unprotected peptides exist to date because of the inability to control regioselective introduction of the conjugate in the presence

(1) See, for instance: Hinterding, K.; Alonso-Díaz, D.; Waldmann, H. *Angew. Chem., Int. Ed.* **1998**, *37*, 688−749.

(2) Okeley, N. M.; Zhu, Y.; van der Donk, W. A. *Org. Lett.* **2000**, *2*, 3603−3606.

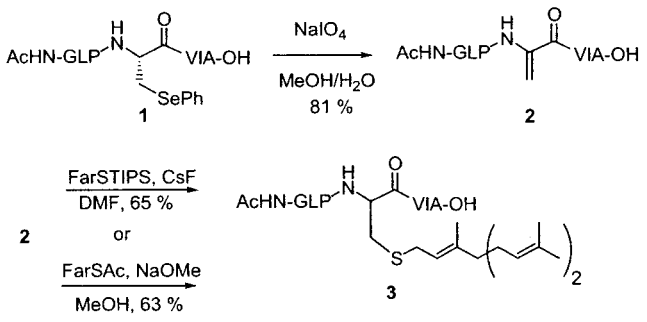
10.1021/ol015648a CCC: $20.00
Published on Web 03/30/2001

of multiple reactive amino acid side chains. The functionalities encountered in natural amino acids include nucleophilic, acidic, and basic groups but no electrophilic moieties. Thus, introduction of an electrophilic group into peptides will provide a handle for the chemoselective conjugation of nucleophiles.[5] We recently reported facile methodology to prepare dehydroalanine containing peptides that is fully compatible with SPPS.[2] Here we describe the utility of such peptides for the rapid preparation of a variety of peptide conjugates including glycopeptides and prenylated peptides.

Our first efforts to test the feasibility of the route focused on prenylated peptides. Many proteins terminating in a CaaX-motif (C = cysteine; a = usually hydrophobic residue; X = various amino acids) are farnesylated or geranylgeranylated on cysteine residues to direct membrane localization.[6] One well-studied example is Ras, which is posttranslationally modified by farnesyl transferase on the cysteine residue of the consensus motif.[7] After farnesylation, proteolytic removal of the three terminal amino acids by a carboxy terminal endoproteinase[8] and transformation of the cysteine carboxylate into its methylester by isoprenylcysteine carboxyl methyltransferase (Icmt)[9] are required for the maturation of Ras. In some cases the prenylated protein is subsequently palmitoylated at a nearby cysteine residue.[10] Small peptides corresponding to the *C*-terminal sequence of Ras and other CaaX-terminated proteins have been shown to function as substrates for farnesyl transferase,[11] and farnesylated peptides can serve as substrates for the protease[8c,12] and methyltransferase.[9b,13] Thus, preparation of small molecular weight substrates for these enzymes is an important goal.[14] Furthermore, substrate analogues may be used as mechanistic tools or inhibitors.[15] Whereas farnesyl transferase has a limited spectrum of substrate tolerance with respect to the prenyl group and can be used only to prepare substrates and certain analogues,[16] chemical synthesis of unnatural analogues is virtually unlimited as long as efficient synthetic routes are available.

Prenylated peptides have been synthesized using a number of strategies and the advantages and drawbacks of the various methods have been reviewed.[14] Waldmann and co-workers have recently developed very useful synthetic routes to isoprenylated peptides that contain both palmitoyl and prenyl attachments using ingenious protecting group strategies to ensure compatibility with the acid sensitive prenyl group and base labile palmitoyl thioester.[17] These methods have relied on solution phase peptide synthesis and fragment couplings and have been used for a variety of applications.[18] Our alternative to previous approaches is shown in Scheme 2.

Scheme 2



We prepared peptide **1** by SPPS with a *Se*-phenylselenocysteine at the position of the prenylated cysteine in the *C*-terminal sequence of N−Ras. Mild oxidation of the selenide and elimination of the selenoxide provided dehydropeptide **2** in good yield. Subsequent addition of either triisopropylsilyl protected farnesylthiol (FarSTIPS) in the

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presence of cesium fluoride or farnesylthiolacetate in combination with NaOMe provided the farnesylated peptide **3** in 65% unoptimized yield.[19]

No asymmetric induction by the chiral backbone of the peptide was observed in the protonation of the enolate intermediate formed by the initial Michael addition.[20] The two diastereomers, however, could be readily separated by reverse phase HPLC. Although the lack of diastereoselectivity in the Michael addition reduces the yield of the desired natural isomer, the ease and versatility of the three step protocol provides an attractive alternative to established synthetic routes. To illustrate this, a number of other lipids as well as thioacetate were linked to peptide **2** (Table 1).

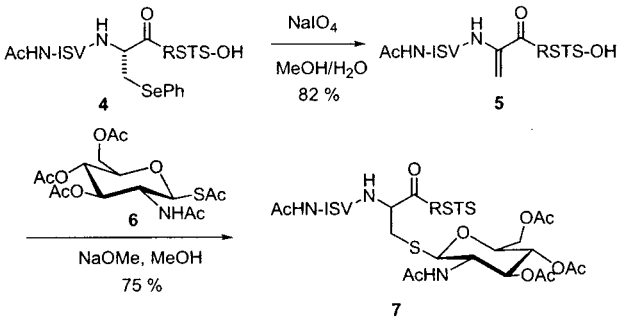
Table 1. Conjugate Addition of Various Thiolate Nucleophiles to Dehydropeptide **2**[a]

entry	nucleophile[b]	yield (%)
1	GerSAc	72
2	GerGerS-TIPS	76
3	Dimethylallylthioacetate	69
4	AcSK	67

[a] See Supporting Information for experimental details. [b] Abbreviations: Ger, geranyl; Far, farnesyl; GerGer, geranylgeranyl; TIPS, triisopropylsilyl.

After establishing the feasibility of the chemoselective conjugation, we turned our attention to glycopeptides. *S*-Glycosylated peptides have been explored as mimetics for *O*-linked glycopeptides.[21] In some of these studies, *S*-glycosylated amino acids were reported to possess a higher chemical stability than their *O*-linked counterparts.[21a,g] Our entry into *S*-linked glycopeptides is presented in Scheme 3. Michael addition of β-2-acetamido-3,4,6-triacetyl-1-*S*-acetyl-2-deoxy-1-thio-D-glucopyranose **6** to dehydropeptide **5** provided the *S*-linked glycopeptide **7**.[22] As in the case of the conjugation of farnesylthiolate to peptide **2**, no diastereoselectivity was observed in the addition of **6**. The product glycopeptide corresponds to an *S*-linked mimic of a sequence of human keratin K18,[23] an intermediate filament (IF) phosphoglycoprotein carrying single β-linked *N*-acetylglucosamines on Ser and Thr residues. Mice knockout studies have shown IF proteins to be essential in fetal development,[24] and inherited blistering skin diseases are associated with mutations in epidermal keratins.[25] The addition of single *N*-acetylglucosamines is not confined to keratins but is in fact a ubiquitous posttranslational modification found for a wide variety of nuclear and cytoplasmic proteins.[26] The rapid preparation of peptides containing this modification may aid in the determination of their functional roles.

Scheme 3



Encouraged by the results in Scheme 3, we explored the scope of the methodology as shown in Table 2. A short dehydroalanine containing tripeptide **8** was used such that the stereoselectivity of the reaction with respect to the glycosidic bond could be determined by NMR spectroscopy. Entries 1 and 2 afforded only the β-linked glycopeptide as determined by COSY experiments (see Supporting Information). Thus, the stereochemical integrity at the anomeric center is maintained during these reactions. The power of this chemoselective conjugation strategy is best illustrated by entry 3, Table 2. Addition of *unprotected* β-*thioglucose* to peptide **8** in aqueous solution provided the desired conjugates in good yield.[27] Combined with our previously developed method for introduction of dehydroalanines into *unprotected* peptides, the overall route confines the use of protecting groups to SPPS. Expansion of the methodology

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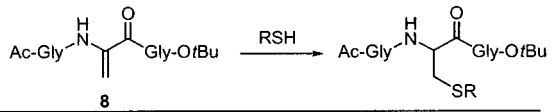
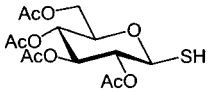
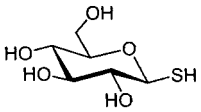
Table 2. Chemoselective Conjugation of Thioglycosides and Dehydropeptide **8**



8

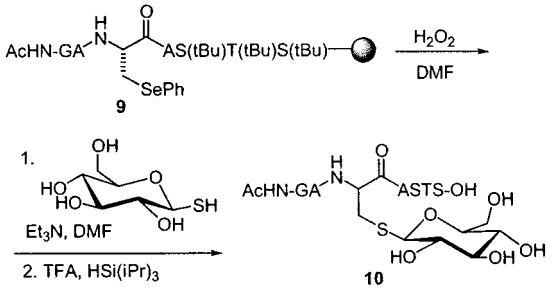
entry	RSH	yield (%)
1		62
2		62
3		74

to α-linked thiogalactosides and tailor-made thiooligosaccharides is in progress.

In principle, both the oxidative elimination and Michael additions should be amenable to solid-phase techniques. We have explored this possibility by using SPPS to prepare peptide **9** on Wang resin, followed by successive treatment with H_2O_2 in DMF and 1-thio-β-glucopyranose. After cleavage of the resulting peptide from the resin and HPLC purification, the desired glycopeptide **10** was obtained in 45% overall yield over 9 steps including SPPS (Scheme 4). This route reduces the number of HPLC purifications from three to one, thereby significantly simplifying the process.

In summary, we have developed a rapid and versatile entry into peptide conjugates using dehydroalanines as an electrophilic handle. The present work describes the preparation of isoprenylated and glycopeptides using conjugation of unprotected carbohydrates and peptides obtained by standard SPPS techniques. The ability to prepare a variety of glycopeptides in a convergent fashion that is readily amenable to combinatorial techniques[28] is particularly useful given

Scheme 4



the heterogeneity found in glycoconjugates in nature. Furthermore, new methods for the rapid synthesis of peptide conjugates are becoming increasingly important with the emergence of powerful ligation techniques to introduce synthetic peptides into larger proteins.[29] It is therefore noteworthy that our method can also be extended to artificial conjugates such as peptides carrying fluorescent probes or spin labels. Our current efforts focus on a diastereoselective version of the methodology.

Acknowledgment. This work was supported by grants from the Burroughs-Wellcome Fund (APP 1920), the National Institutes of Health (GM58822), and the Research Corporation (R10187). Y.Z. acknowledges support from a Zeneca Graduate Fellowship. NMR spectra were obtained in the Varian Oxford Instrument Center for Excellence, funded in part by the W. M. Keck Foundation, NIH (PHS 1 S10 RR10444), and NSF (CHE 96-10502). Mass spectra were recorded on a Voyager mass spectrometer funded in part by the National Institutes of Health (RR 11966).

Supporting Information Available: A general procedure for the addition reactions is described, as well as characterization of the peptide conjugates.

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