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"Customizable" Units in Di- and Tripeptides: Selective Conversion into Substituted Dehydroamino Acids

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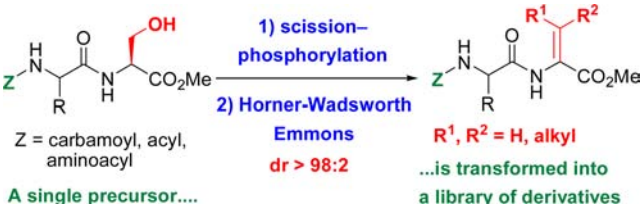
ABSTRACT



The selective conversion of serine or threonine units of di- and tripeptides into substituted dehydroamino acids is reported. Thus, these common α-amino acids undergo a scission–phosphorylation process to give α-amino phosphonate residues. A Horner–Wadsworth–Emmons reaction with aldehydes or ketones follows to afford the final products with excellent *Z*-stereoselectivity (*Z*:*E* > 98:2). In this way, a single peptide precursor can selectively be transformed into a variety of derivatives.

Dehydroamino acids can be found in a variety of bioactive peptides, such as the cyclic peptide tentoxin,[1] the lantibiotics[2] and thiopeptide antibiotics,[3] the protease inhibitor somamide,[4a] the cytotoxic kahalalide F,[4] yaku'amides,[5] and dolastatin,[6] the fungicide pseudomycin,[7] and many others.

In addition, the introduction of dehydroamino acids into synthetic analogues of bioactive peptides can increase their resistance to enzymatic degradation and allow the modulation of their biological properties.[8,9] Several drug analogues with improved properties have been developed,[9] such as gramicidin analogues with potent antibiotic but much lower hemolytic activity[9a] and endorphine analogues for pain control[9b] with high μ opioid receptor selectivity.

The rigidity provided by dehydroamino acids could also be useful to generate folded conformations for new materials or peptide catalysts.[10]

In order to generate libraries of peptides with dehydroamino acid units, each peptide is usually prepared de novo from the starting amino acids. Herein, we report an alternative strategy where a single parent peptide is

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transformed into a variety of derivatives by selective conversion of certain α-amino acid units (serine or threonine) into β-substituted dehydroamino acids.

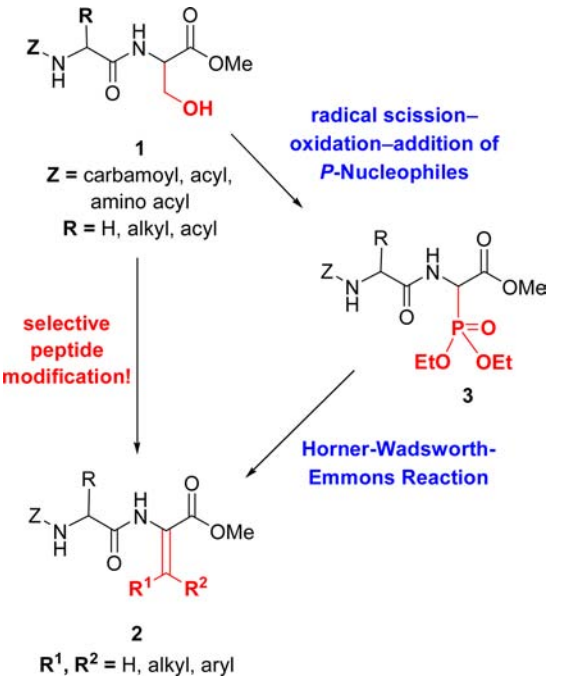
Recently, the use of "customizable" (or "tunable") amino acids in the site-selective modification of peptides has elicited much interest.[11,12] This selective approach requires less time and materials than the conventional de novo synthesis. For instance, Seebach has reported the selective alkylation of enolates from *N*-alkylglycines,[12a] while Kazmaier has achieved the stereoselective allylation and alkylation of glycine residues in dipeptides.[12b,c] Klok has described the addition of *S*-radicals to allylglycines in peptides with 4–16 residues,[12d] and Skrydstrup has generated enolates in "tunable" residues of di- to tetrapeptides, which were trapped by electrophiles.[11a,12e]

Despite these advances, the site-selective modification of peptides remains difficult,[11] even for small peptides, because of the similar reactivity of the amino acid units. The task is particularly difficult when several units of the "tunable" amino acid (glycine, dehydroamino acids, etc) are present in the peptide. The use of serine (or threonine) residues as customizable units solves this problem, since the lateral chains of different serine units can be protected with orthogonal groups. Thus, free serine residues would be selectively transformed, while the protected ones would remain unchanged.

To determine the feasibility of this approach to obtain a variety of peptides with dehydroamino acid units, we used the strategy shown in Scheme 1 (conversion **1**→**2**). Thus, peptide **1** would undergo the radical scission of serine (or threonine) to give a glycyl radical, which would be oxidized in situ to a cation, and the latter would be trapped by phosphorus nucleophiles to give the aminophosphonate **3**. Then, a Horner–Wadsworth–Emmons reaction with different aldehydes or ketones would afford peptides with dehydroamino acids **2**.

For the first step, we used a variation of our reported amino acid decarboxylation–phosphorylation process.[13]

Scheme 1. Site-Selective Scission of Serine Residues and Addition of *P*-Nucleophiles



Since the decarboxylation is much more favored than the radical scission of alcohols (in particular, primary alcohols such as serine), there were concerns that the scission–phosphorylation process would not work as desired or that side reactions (*H*-abstraction, oxidation of the alcohol, cleavage of the peptide chain) would take place.[14,15]

The selective radical scission–oxidation was studied with peptides **4** and **5** (Scheme 2), which present two serine residues or a serine/threonine pair. Using the reported procedure [(diacetoxyiodo)benzene (DIB)/I_2, *hν*, 26 °C, 2–4 h, then 0 °C, Lewis acid, nucleophile],[13,15] a complex mixture of compounds was formed, due either to side reactions or to the formation of unstable scission products, such as a peptide with an α-acetoxyglycine unit.

In order to determine whether the low yields were due to the generation of unstable N,O-intermediates or to other causes, the scission–oxidation was followed by addition of methanol, since this nucleophile usually adds in good to excellent yields, providing stable methoxy acetals.[16] Therefore, peptide **4** was treated with $PhI(OAc)_2$ (DIB) and iodine under irradiation with visible light, affording the methoxy derivative **6** in improved but still moderate yield (< 40%).

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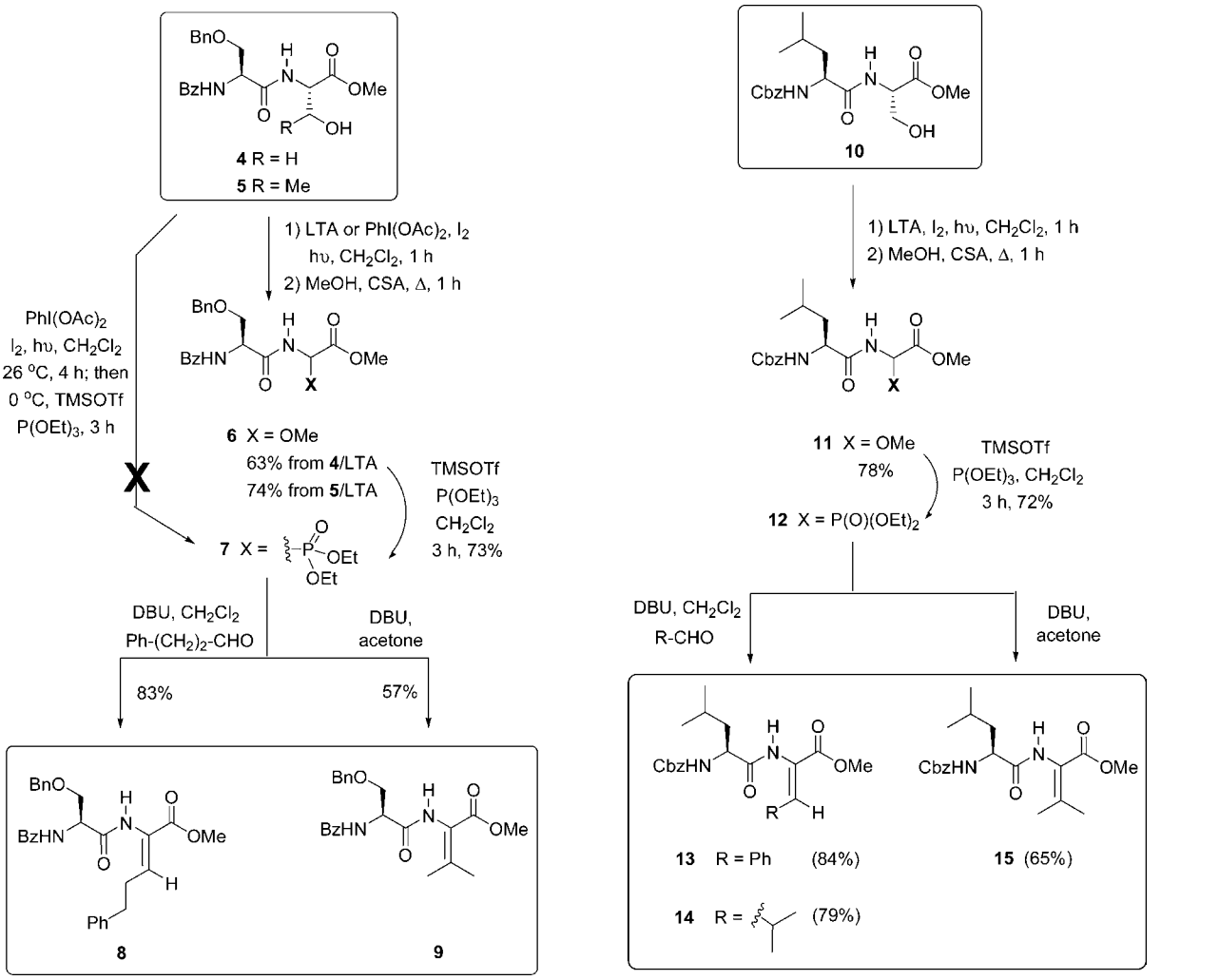
Scheme 2. Formation of a Set of Compounds from a Peptide Precursor by Selective Conversion of Ser and Thr Units into Dehydroamino Acids



Fortunately, when the system DIB–iodine was replaced by lead tetraacetate (LTA) and iodine, a single product was formed. After purification by chromatography, the dipeptide **6** was isolated in 63% yield. Interestingly, the scission of the threonine analogue **5** resulted in increased yield (74%). To account for this result, both the scission and the addition of methanol must have proceeded in good to excellent yields.

The conversion of dipeptide **6** into the aminophosphonate **7** was studied under several conditions, using different phosphorus nucleophiles and Lewis acids. The best results were obtained with $P(OEt)_3$ and TMSOTf, affording compound **7** in 73% yield.

The aminophosphonate **7** underwent the Horner–Wadsworth–Emmons reaction with dihydrocinnamaldehyde to give the dehydro(phenyl)norvaline **8** in good yield and excellent *Z*-stereoselectivity. The reaction also proceeded with ketones (acetone) to give the dehydrovaline derivative **9**. It should be noticed that in both cases the protected *N*-terminal serine unit remained unaffected.

In a similar way, the Cbz-protected peptide **10** (Scheme 2) underwent the scission–addition of methanol process, affording the methoxyglycine derivative **11** in 78% yield. Then compound **11** was converted into the aminophosphonate **12** in good yield.

Product **12** was treated with different aryl and akyl aldehydes[17] to provide compounds **13** and **14**, which present units of dehydrophenyl alanine and dehydroleucine, respectively. The reaction also proceeded with acetone to give the dehydrovaline derivative **15** in good yield.

In the case of compounds **13** and **14**, the process took place with complete stereoselectivity to give the *Z*-isomers. An important concern was that the basic reaction conditions would produce the epimerization of the adjacent amino acid(s). We compared the optical activity of compound **13** and a sample formed by coupling the leucine and

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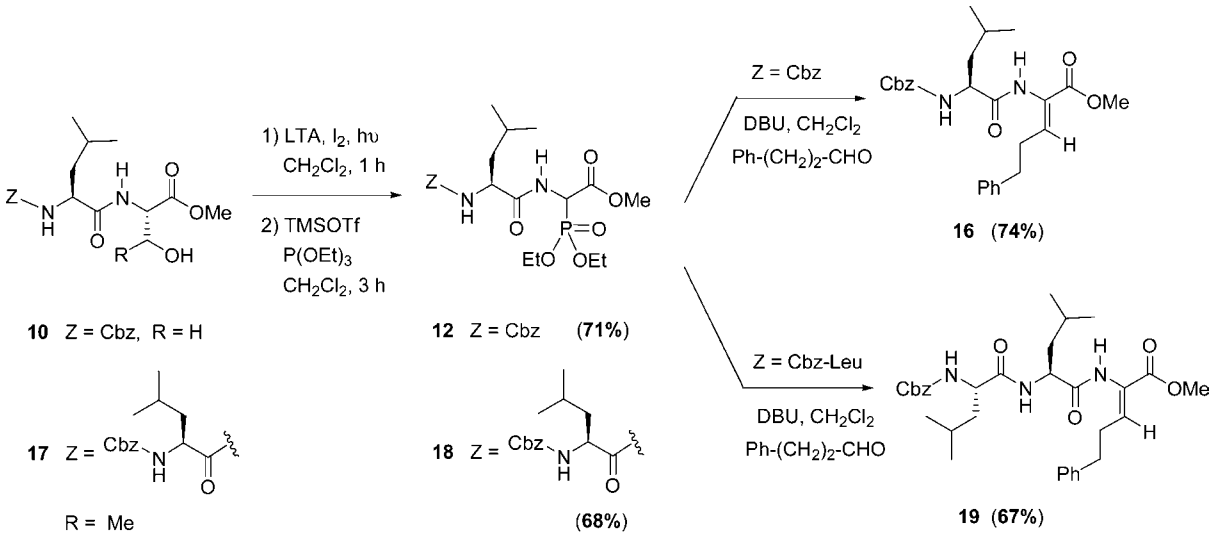
Scheme 3. Simplified Scission–Phosphorylation Procedure and Application to the Synthesis of Modified Peptides



the *Z*-dehydrophenylalanine units;[18a] to our satisfaction, both activities matched completely.

We reasoned that a simplification of the previous procedure could allow the direct transformation of peptide **10**[18] into the phosphonate **12**. Thus, the dipeptide **10** (Scheme 3) underwent scission with LTA/I_2 followed by aqueous workup. The intermediate was not purified but treated with the phosphorylation reagents to give compound **12** in 54% yield (the global yield for the two-step procedure was 56%). The aminophosphonate **12** was treated with dihydrocinnamaldehyde to give the dehydro(phenyl)norvaline derivative **16** in 74% yield.

Finally, we studied the process with tripeptide **17** where the customizable unit is threonine. We are interested in polyleucine-substituted peptides where one of the residues is replaced by a dehydroamino acid since these derivatives present interesting conformational and biological properties.[19]

The tripeptide **17** underwent the simplified scission–phosphorylation process to give the aminophosphonate **18** in good yield. Then, compound **18** was treated with dihydrocinnamaldehyde to give the dehydro(phenyl)norvaline derivative **19**.

In summary, a scission of serine/threonine units–phosphorylation process was developed, which is suitable for the selective modification of peptides; other alternative procedures reported in the literature do not work with these substrates. The resulting aminophosphonates underwent a Horner–Wadsworth–Emmons reaction with aldehydes or ketones to give the corresponding dehydroamino acids with excellent (*Z*) stereoselectivity, and no epimerization of other positions was observed.

This methodology allows for the preparation of a variety of peptide derivatives from a single precursor. The process takes place under mild conditions in good yields.

The use of the serine (or threonine) units to generate diversity is particularly interesting. Since its hydroxymethylene group can be protected with different orthogonal groups, the starting peptide could contain several serine residues, but only the unprotected one(s) would be modified. For further modifications, the orthogonal protecting groups could be sequentially removed. The application of this methodology to the synthesis of other peptides of different sizes will be reported in due course.

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Supporting Information Available. Procedures for the synthesis of substrates **4**, **5**, and **17**. Formation of the α-methoxyglycine derivatives **4**, **5**, and **11**, the amino phosphonates **7**, **12**, and **18**, and the dehydroamino acid-containing peptides **8**, **9**, **13**–**16**, and **19**. ^{1}H and ^{13}C NMR spectra of products **4**–**9** and **11**–**19**. NOE experiments for compounds **8**, **13**, **14**, **16**, and **19**. This material is available free of charge via the Internet at http://pubs.acs.org

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The authors declare no competing financial interest.