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Creating Diversity by Site-Selective Peptide Modification: A Customizable Unit Affords Amino Acids with High Optical Purity

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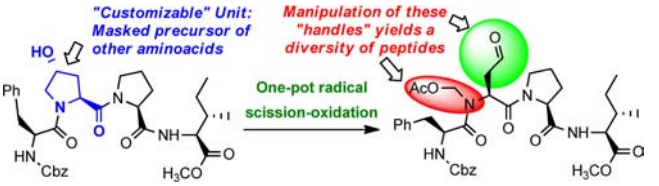
ABSTRACT



The development of peptide libraries by site-selective modification of a few parent peptides would save valuable time and materials in discovery processes, but still is a difficult synthetic challenge. Herein natural hydroxyproline is introduced as a "convertible" unit for the production of a variety of optically pure amino acids, including expensive *N*-alkyl amino acids, and to achieve the mild, efficient, and site-selective modification of peptides.

The modification of peptides has elicited much interest, in order to provide new peptide drugs,[1] probes for molecular imaging,[2] and peptide catalysts for green chemistry.[3] During the discovery process of bioactive or catalytic peptides, libraries of analogues of a lead compound are prepared. Their activities are studied next, in order to determine structure−activity relationships and then design new generations of modified peptides.

In the traditional approach to obtain these peptides, each compound is synthesized *de novo*; in each case, a modification with respect to the parent peptide is introduced.[1−3] This serial procedure is costly in time and materials. When the peptides are particularly difficult to obtain (*e.g.*, long or difficult sequences, macrocycles) or when only part of the peptide requires modification, a site-selective transformation would be preferable.[4,5] In this alternative strategy, a single (or a few) starting peptide is used to generate the other library members, by selective conversion of a "customizable" (or "tunable") residue.

This strategy has recently attracted much attention in academia and industry, and customizable units such as glycine, dehydroalanine, 4-azidoproline, allylglycine, cysteine, and aziridine carboxylic acids have been reported[6−8] and used for the selective modification of small peptides.[7,8]

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Despite these advances, the site-selective modification of peptides remains difficult,[4] particularly when several units of the "tunable" residue are present in the peptide. The introduction of serine (or threonine) residues as customizable units[8] represented considerable progress: the serine residues with free hydroxy groups were transformed, while protected serine units remained unchanged (e.g., conversion **1**→**3**, Scheme 1). Orthogonal protecting groups allow further residue differentiation.

In most of the reported procedures,[8] the serine (or threonine) lateral chain was completely removed and replaced by other chains using scission−addition processes. However, the addition step (either of nucleophiles or electrophiles) usually took place with low stereoselectivity, providing low diastereomeric excesses.

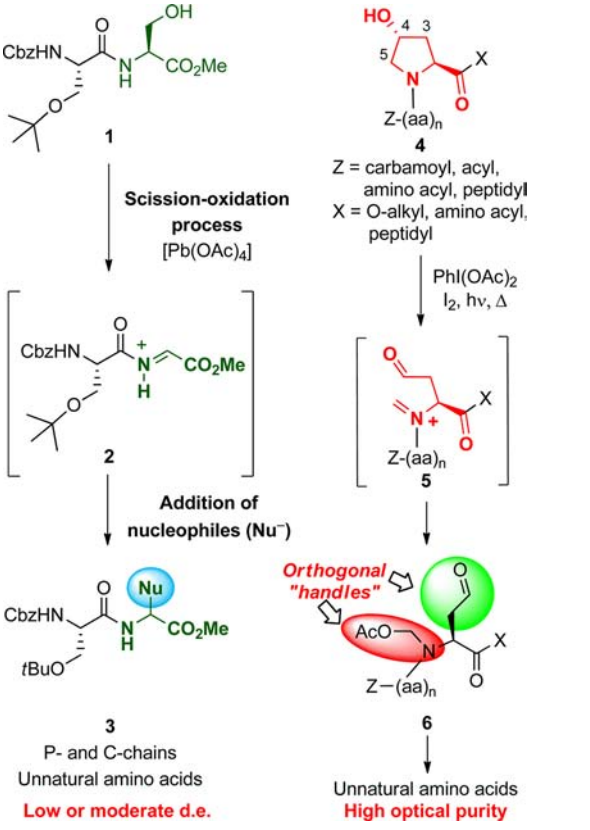
Therefore, the ultimate goal is the development of new "customizable" units which could be transformed selectively, even if several tunable units were present, and which could be converted into unnatural chains with high stereoselectivity. In addition, the customizable units should come from commercial, readily available amino acids, preferably natural ones. This paper reports the use of natural L-hydroxyproline derivatives as customizable units in tandem radical scission−oxidation processes and the feasibility of this approach for the site-selective peptide modification (conversion **4**→**6**, Scheme 1).

The regioselectivity of the scission was an initial concern. Although the fragmentation could take place via the C_4-C_5 or the C_3-C_4 bond, it was expected that the first case would be favored,[9] since the resulting primary *C*-radical would be stabilized by the adjacent nitrogen and then readily oxidized to an acyliminium ion **5**.[10] The scission of the C_3-C_4 bond would yield a nonstabilized primary *C*-radical, which would be disfavored.

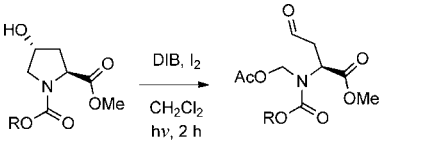
The initial studies of the transformation of hydroxyproline derivatives under oxidative radical fragmentation conditions were performed with methyl carbamate **7** (Scheme 2).[7f]

On treatment with (diacetoxyiodo)benzene and iodine, and under irradiation with visible light, the *N*-alkyl 4-oxo-L-homoalanine derivative **8** was obtained in good yield;

Scheme 1. Introduction of Hyp as "Customizable" Unit (Right) Presents Important Advantages over Use of Ser (Left)



Scheme 2. Study of the Conversion of Hydroxyproline Derivatives into *N*-Alkyl 4-Oxohomoalanine Derivatives



no C_3-C_4 scission products were detected. In order to check that no epimerization took place in this step, the (−)-menthyl carbamate **9** underwent the reaction, affording exclusively compound **10**.

A prerequisite for a high-quality "customizable unit" is that it can be transformed into a diversity of amino acids while keeping the stereochemical integrity. Thus, the transformation of compounds **8** and **9** into other unnatural amino acids, by transformation of the aldehyde and *N,O*-acetal "handles", was studied next (Scheme 3). For instance, the reductive amination of compound **8** with a chiral amine followed by intramolecular lactamization afforded compounds (**1′*R*)-11** or (**1′*S*)-11** in high optical purity; only traces of a minor diastereomer coming from epimerization could be detected (dr ≥ 96:4).[11]

(7) (a) For other interesting approaches, see: Saavedra, C. J.; Boto, A.; Hernández, R.; Miranda, J. I.; Aizpurua, J. M. *J. Org. Chem.* **2012**, *77*, 5907–5913. (b) Saavedra, C. J.; Boto, A.; Hernández, R. *Org. Lett.* **2012**, *14*, 3542–3545. (c) Uhlig, N.; Li, C.-J. *Org. Lett.* **2012**, *14*, 3000–3003. (d) Monfregola, L.; Leone, M.; Calce, E.; De Luca, S. *Org. Lett.* **2012**, *14*, 1664–1667. (e) Saavedra, C. J.; Boto, A.; Hernández, R. *Org. Biomol. Chem.* **2012**, *10*, 4448–4461. (f) Saavedra, C.; Hernández, R.; Boto, A.; Alvarez, E. *J. Org. Chem.* **2009**, *74*, 4655–4665. (g) Chapman, C. J.; Hargrave, J. D.; Bish, G.; Frost, C. G. *Tetrahedron* **2008**, *64*, 9528–9539. (h) Lin, Y. A.; Chalker, J. M.; Floyd, N.; Bernardes, G. J. L.; Davis, B. G. *J. Am. Chem. Soc.* **2008**, *130*, 9642–9643. (i) Wan, Q.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2007**, *46*, 9248–9252 and references cited therein.

(8) (a) Ricci, M.; Madariaga, L.; Skrydstrup, T. *Angew. Chem., Int. Ed.* **2000**, *39*, 242–246. (b) Dialer, H.; Steglich, W.; Beck, W. *Tetrahedron* **2001**, *57*, 4855–4861. (c) Saavedra, C. J.; Boto, A.; Hernández, R. *Org. Lett.* **2012**, *14*, 3788–3791.

(9) (a) Boto, A.; Hernández, D.; Hernández, R.; Montoya, A.; Suárez, E. *Eur. J. Org. Chem.* **2007**, 325–334 and references cited therein.

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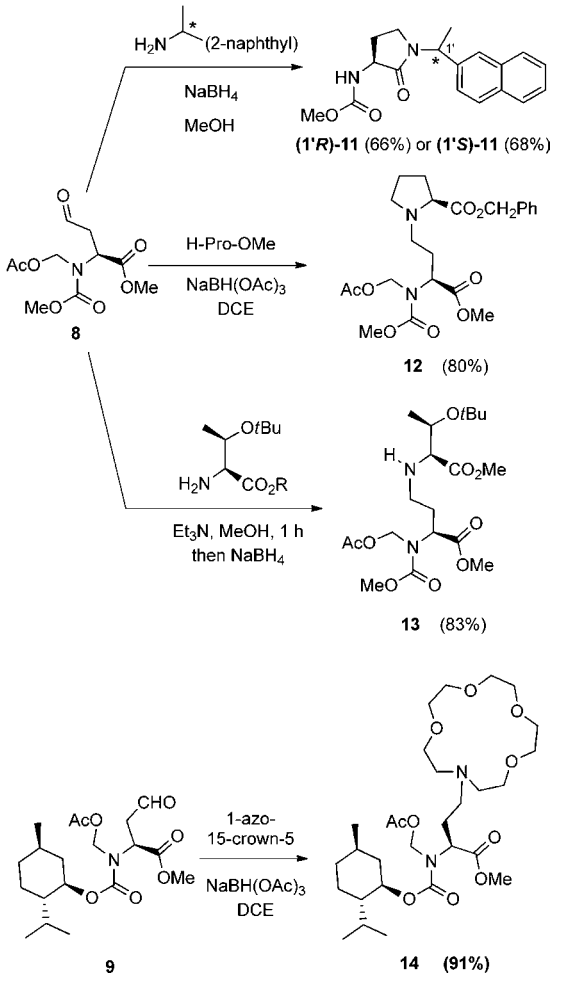
(11) Jamieson, A. G.; Boutard, N.; Sabatino, D.; Lubell, W. D. *Chem. Biol. Drug Des.* **2013**, *81*, 148–165.

Scheme 3. Study of the Conversion of *N*-Alkyl 4-Oxohomoalanine **8** into Other Amino Acids



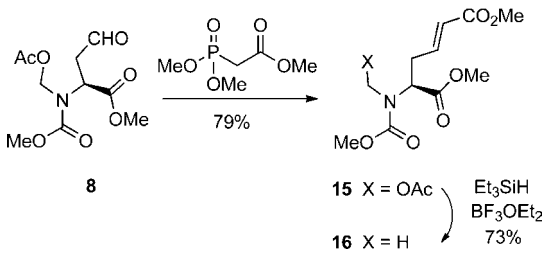
When a secondary amine (proline benzyl ester) was used, the 4-aminohomoalanine derivative **12** was isolated (80% yield). The reductive amination also gave good results using amino acids with primary amino groups. For instance, the aldehyde **8** was converted into the dipeptide **13** on treatment with a threonine derivative, followed by reduction with inexpensive methanolic $NaBH_4$. The preparation of dipeptides such as **12** and **13** would be valuable to synthesize branched peptides.

The reductive amination could also allow the preparation of medical probes.[12] For instance, complexes of crown ethers or cyclenes with different metals have been used in molecular imaging to detect tumors.[2,11] The ready transformation of aldehyde **9** (Scheme 3) into the 15-aza-crown-5 derivative **14** in excellent yield and optical purity provides a valuable probe component.

The aldehyde can also be transformed into alkenyl chains, as shown by treatment of aldehyde **8** (Scheme 4) under Horner–Wadsworth–Emmons conditions to give the expensive dehydrohomoglutamic acid **15**. The reduction of alkenyl chains could then afford unusual alkyl chains.

The derivatization of the amino acid lateral chain can be accompanied by transformation of the *N,O*-acetal function, by saponification, reduction, allylation, or alkylation.[13] For instance, by reduction of the acetal, compound **15** is converted into the *N*-methyl dehydrohomoglutamate **16** (Scheme 4). The *N*-alkylated amino acids usually reach high market values, since the introduction of *N*-methyl amino acids into peptides can affect their conformation and activity. Despite this, not many *N*-methyl amino acids are commercially available. The present method affords an efficient route to these compounds.

Scheme 4. Synthesis of High-Profit *N*-Methyl Amino Acid **16**



Once the conversion of the hydroxyproline unit into new, optically pure residues had been studied, its application for site-selective peptide modification was explored. For instance, the tripeptide **17** (Scheme 5) afforded the aldehyde derivative **18** in good yields. In a similar way, the dipeptide **19** underwent scission to give the aldehyde derivative **20**, which was converted into the leucine-dehydrohomoglutamate derivative **21**; no other isomers were isolated.

Finally, the tetrapeptide **22** (Scheme 6) which contains two tunable residues (a threonine and a hydroxyproline units) underwent cleavage of the unit with the free hydroxy group, while the other tunable residue, whose lateral chain was protected, remained unchanged. The resulting tetrapeptide **23** was reduced to the lactone derivative **24**. By careful control of the conditions, the *N,O*-acetal was removed, releasing the NH group.

The scission of hydroxyproline units in internal positions in the peptide (for instance, conversion **25**→**26**, Scheme 7) is particularly interesting for conformation–activity studies. Such cleavage would decrease the system rigidity, producing conformational or even secondary structure changes which could be used to modulate the biological or catalytic activity. Besides, by removal of the *N,O*-acetal groups in the scission products, new H-bonds could be formed, allowing other conformational changes.

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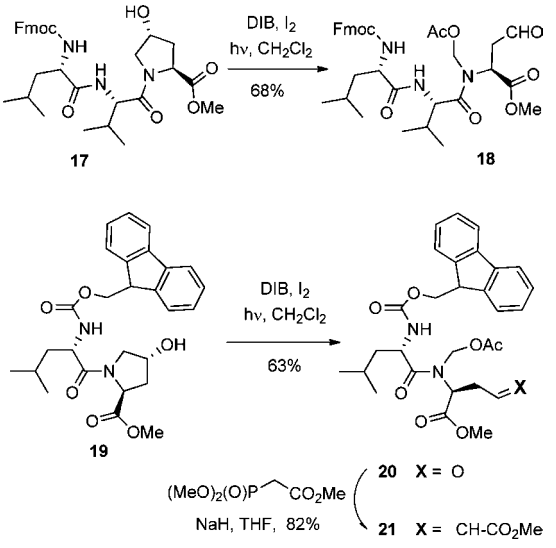
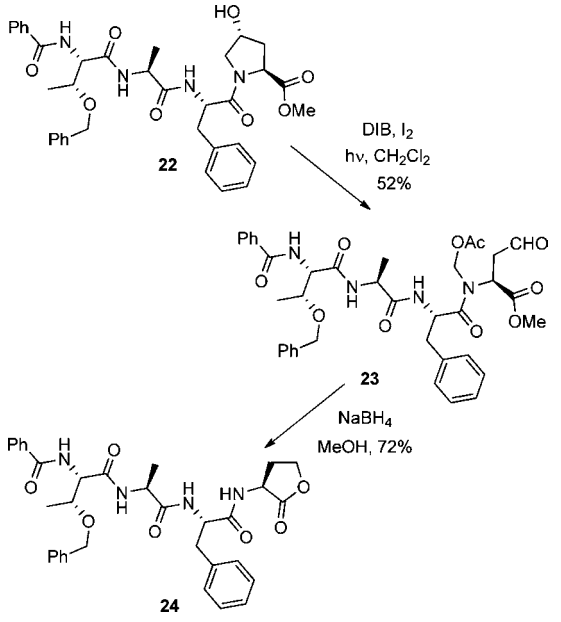
Scheme 5. Selective Modification of the Hyp Residue in Peptides



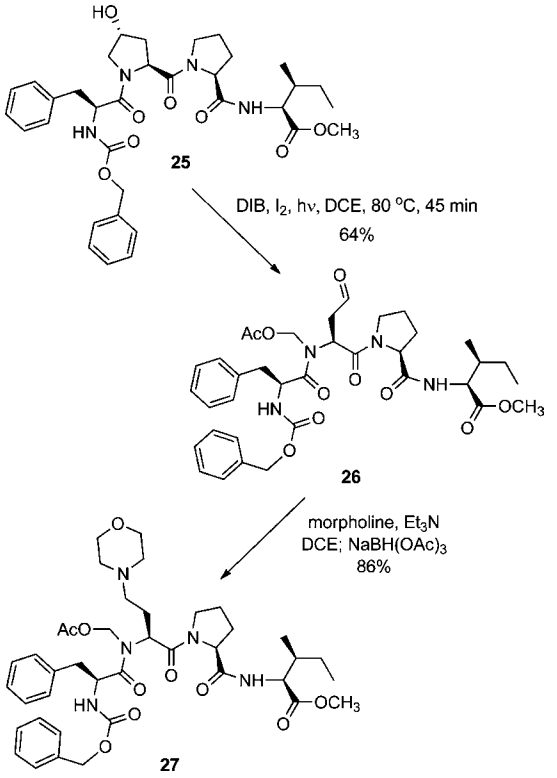
Scheme 6. Selective Converison of Only One Customizable Unit



The functionalization of the scission products would be useful to create libraries of bioactive compounds. For instance, the generation of 4-amino homoalanines could be interesting for the development of antibiotic peptides,[1a,b] where a balance of nonpolar and cationic residues is necessary for pharmacological utility.[14] The conversion of **26** into **27** in very good yield highlights the feasibility of this approach. The chosen morpholino-containing unit can be found in commercial antimicrobials such as Cobicistat.[15]

(14) Savage, P. B. *Eur. J. Med. Chem.* **2002**, 759–768.
(15) De Clercq, E. *Med. Chem. Rev.* **2013**, *33*, 1278–1303.

Scheme 7. Selective Scission of Internal Hyp Residues, and Generation of Cationic Residues for Peptide Antimicrobials



In summary, the use of natural and commercial hydroxyproline as a "customizable" unit allows the production of a diversity of unnatural amino acids with high optical purity, including expensive *N*-alkyl amino acids, many α-alkylglycines, components of medical probes, and branched peptides. The initial one-pot scission−oxidation process generates a residue with two reactive handles which can be manipulated independently.

This "tunable" unit can also be used for the mild, efficient, and site-selective modification of peptides, generating different derivatives from a single parent peptide. Unlike other site-selective strategies, it is possible to discriminate between several "tunable" units. The applications of this method to create diversity in the search for antimicrobial peptides and for conformation/activity studies will be reported in due course.

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Supporting Information Available. Experimental details, and ^{1}H and ^{13}C NMR for all new products. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.