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Facile Chemoselective Synthesis of Dehydroalanine-Containing Peptides†

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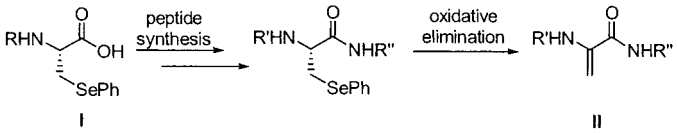
ABSTRACT



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Useful methodology is described for the synthesis of dehydroalanine residues (II) within peptides. The unnatural amino acid (*Se*)-phenylselenocysteine (I) can be incorporated into growing peptide chains via standard peptide synthesis procedures. Subsequent oxidative elimination affords a dehydroalanine at the desired position. The oxidation conditions are mild and tolerate functionalities commonly found in peptides, including variously protected cysteine residues. To illustrate its utility, cyclic lanthionines have been synthesized by this method.

The α,β-unsaturated amino acids dehydroalanine (**1**) and dehydrobutyrine (**2**) are found in a variety of biological

1 **2**

polypeptides and natural products.[1] In nature they impart interesting biological activities, while synthetically they can be versatile precursors to unnatural amino acids[2] and have been used to alter the biological and structural properties of peptides and proteins. The synthesis of dehydroamino acid containing peptides (dehydropeptides) has previously been accomplished by a number of different methods.[3] The most common approach involves incorporation of a masked residue into the peptide and its subsequent conversion to the dehydroamino acid. An abundance of precursors have been used in this fashion to generate **1** and **2** within peptides, such as the activation and elimination of serine or threonine derivatives,[4] Hoffmann elimination from 2,3-diaminopropionic acid or asparagine residues,[5] and oxidative elimination of *S*-alkyl or *S*-aryl cysteines.[6] Although these methodologies have allowed the synthesis of a number of natural products containing dehydroamino acids, most procedures are not

† Abbreviations: Boc, *tert*-butoxycarbonyl; Fmoc, *N*-9-fluorenylmethoxycarbonyl; HBTU, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; NMM, *N*-methylmorpholine.

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(1) (a) Bodanszky, M.; Scozzie, J. A.; Muramatsu, I. *J. Antibiot.* (*Tokyo*) **1970**, *23*, 9−12. (b) Berdy, J. *Adv. Appl. Microbiol.* **1974**, *18*, 309−406. (c) Tori, K.; Tokura, K.; Okabe, K.; Ebata, M.; Otsuka, H.; Lukacs, G. *Tetrahedron Lett.* **1976**, *3*, 185−188 (d) Pascard, C.; Ducruix, A.; Lunel, J.; Prangé, T. *J. Am. Chem. Soc.* **1977**, *99*, 6418−6423. (e) Pearce, C. J.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1979**, *101*, 5069−5070. (f) Pedras, M. S. C.; Taylor, J. T.; Nakashima, T. T. *J. Org. Chem.* **1993**, *58*, 4778−4780. (g) Hamann, M. T.; Scheuer, P. J. *J. Am. Chem. Soc.* **1993**, *115*, 5825−5826. (h) Lau, R. C.; Rinehart, K. L., Jr. *J. Antibiot.* (*Tokyo*) **1994**, *47*, 1466−1472. (i) Sahl, H.-G.; Jack, R. W.; Bierbaum, G. *Eur. J. Biochem.* **1995**, *230*, 827−853. (j) Strohl, W. R.; Floss, H. G. *Biotechnology* **1995**, *28*, 223−238. (k) Dawson, J. F.; Holmes, C. F. *Front. Biosci.* **1999**, *4*, D646−D658.

(2) Ferreira, P. M. T.; Maia, H. L. S.; Monteiro, L. S. *Tetrahedron Lett.* **1999**, *40*, 4099−4102.

(3) (a) For a review, see: Schmidt, U.; Lieberknecht, A.; Wild, J. *Synthesis* **1988**, *3*, 159−172. (b) Rich, D. H.; Tam, J. P. *Tetrahedron Lett.* **1975**, 211−212. (c) Srinivasan, A.; Stephenson, R. W.; Olsen, R. K. *J. Org. Chem.* **1977**, *42*, 2253−2256. (d) Somekh, L.; Shanzer, A. *J. Org. Chem.* **1983**, *48*, 907−908. (e) Paquet, A. *Tetrahedron Lett.* **1990**, *31*, 5269−5272. (f) Zetterstrom, M.; Trogen, L.; Hammarstrom, L. G.; Juhlin, L.; Nilsson, B.; Damberg, C.; Bartfai, T.; Langel, Ü. *Acta Chem. Scand.* **1995**, *49*, 696−700. (g) Yamada, M.; Miyajima, T.; Horikawa, H. *Tetrahedron Lett.* **1998**, *39*, 289−292. (h) Coghlan, P. A.; Easton, C. J. *Tetrahedron Lett.* **1999**, *40*, 4745−4748. (i) Stohlmeyer, M. M.; Tanaka, H.; Wandless, T. J. *J. Am. Chem. Soc.* **1999**, *121*, 6100−6101.

(4) (a) Srinivasan, A.; Stephenson, R. W.; Olsen, R. K. *J. Org. Chem.* **1977**, *42*, 2256−2260. (b) Andruszkiewicz, R.; Czerwinski, A. *Synthesis* **1982**, 968−969. (c) Balsamini, C.; Duranti, E.; Mariani, L.; Salvatori, A.; Spadoni, G. *Synthesis* **1990**, *9*, 779−781. (d) Berti, F.; Ebert, C.; Gardossi, L. *Tetrahedron Lett.* **1992**, *33*, 8145−8148. (e) Ranganathan, D.; Shah, K.; Vaish, N. *J. Chem. Soc., Chem. Commun.* **1992**, *16*, 1145−1147. (f) Shin, C.; Okumura, K.; Ito, A.; Nakamura, Y. *Chem. Lett.* **1994**, 1301−1304.

(5) (a) Nomoto, S.; Sano, A.; Shiba, T. *Tetrahedron Lett.* **1979**, *6*, 521−522. (b) Blettner, C.; Bradley, M. *Tetrahedron Lett.* **1994**, *35*, 467−470.

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Table 1. Oxidation of Model Dehydropeptides

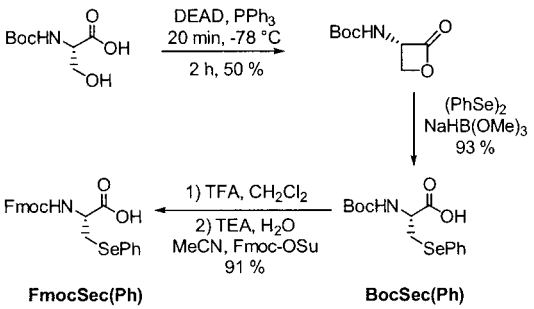
entry	peptide	dehydropeptide[a,b]	solvent	yield (%)[c]
1	Fmoc-Cys(SEt)Sec(Ph)-ODPM	Fmoc-Cys(SEt)Dha-ODPM	CH_2Cl_2/MeOH	70
2	[Boc-Sec(Ph)Cys-OMe]$_2$	[Boc-DhaCys-OMe]$_2$	MeOH	75
3	Boc-Sec(Ph)Cys(Trt)-OMe	Boc-DhaCys(Trt)-OMe	THF	83
4	Fmoc-TrpSec(Ph)-ODPM	Fmoc-TrpDha-ODPM	CH_2Cl_2/MeOH	64[d]
5	AlaMetSec(Ph)Ala	AlaMetDhaAla	MeCN/H_2O	62

[a] Conditions for entries 1−4: aq $NaIO_4$ (4 equiv) was added at 25 °C to solutions of the peptides (final peptide concentrations 7−30 mM). For entry 3, $NaIO_4$ was added at 0 °C and warmed to rt. For entry 5, 1.1 equiv of $NaIO_4$ and 1 equiv of NEt_3 were used. [b] Sec, selenocysteine; Dha, dehydroalanine; DPM, diphenylmethyl. [c] Yields are for products after purification by flash chromatography (entries 1−4) or HPLC (entry 5). [d] The crude yield of this reaction was 98% (95% pure material by ^{1}H NMR).

selective or are incompatible with preexisting amino acid residues in the peptide, thus requiring cumbersome protecting strategies. A facile, site-specific, and chemoselective method of introduction has not been previously described.[7] Our search for a mild method of introducing these amino acids in a manner compatible with standard solid-phase peptide synthesis (SPPS), led us to a previously reported yet unexploited method for the generation of dehydroalanine via oxidative elimination from the unnatural amino acid (*Se*)-phenylselenocysteine (Sec(Ph)).[8,9] In this communication, we report our initial results on the further development and modification of this chemistry to allow the synthesis of dehydropeptides via either solution- or solid-phase peptide synthesis.

To limit the number of possible diastereomeric peptides containing phenylselenocysteine, we desired the optically pure amino acid. The synthesis of (*R*)-FmocSec(Ph) was modified from existing literature reports (Scheme 1). Conversion of Boc-L-serine to its β-lactone,[10] followed by ring opening with phenylselenide anion[11] generated by the reaction of diphenyl diselenide with sodium trimethoxyborohydride,[12] provided BocSec(Ph) in 93% yield. For use in Fmoc peptide synthesis, the Boc protected amino acid was converted to its Fmoc derivative via standard methods. The resulting Boc or Fmoc amino acids were incorporated into growing chains via standard solid- or solution-phase peptide chemistry.

Scheme 1



The short dehydropeptides listed in Table 1 were used to optimize the desired conditions for chemoselective oxidative elimination. These peptides were designed to test the tolerance of oxidation sensitive amino acids, including various protected cysteine residues, methionine and tryptophan, unprotected at the indole moiety. The oxidative eliminations were accomplished with sodium periodate in less than 2 h, except in the case of an *N*-terminal Sec(Ph), which required 12 h (Table 1, entries 2 and 3), reflecting the lower reactivity of a carbamate versus an amide.[13] Cysteine protecting groups that proved to be compatible include mixed (Table 1, entry 1) and symmetrical (entry 2) disulfides, as well as trityl (entry 3). All reaction products were obtained in moderate to good yields after purification without side-chain modification of tryptophan or the protected cysteines.[14] When methionine was present in the peptide, the reaction was carried out with 1.1 equiv of oxidant

(6) (a) Rich, D. H.; Tam, J.; Mathiaparanam, P.; Grant, J. A.; Mabuni, C. *J. Chem. Soc., Chem. Commun.* **1974**, *21*, 897−898. (b) Rich, D. H.; Tam, J. P. *J. Org. Chem.* **1977**, *42*, 3815−3820. (c) Rich, D. H.; Bhatnager, P.; Mathiaparanam, P.; Grant, J. A.; Tam, J. P. *J. Org. Chem.* **1978**, *43*, 296−302. (d) Burrage, S. A.; Raynham, T.; Bradley, M. *Tetrahedron Lett.* **1998**, *39*, 2831−2834. (e) Burrage, S.; Raynham, T.; Williams, G.; Essex, J. W.; Allen, C.; Cardno, M.; Swali, V.; Bradley, M. *Chem.—Eur. J.* **2000**, *6*, 1455−1466.

(7) For example, methods based on activation and elimination of serine, threonine or 2,3-diaminopropionic acid require either that no other Ser, Thr, or Lys residues are present in the peptides or that they are orthogonally protected compared to the residues to be eliminated. Bradley and co-workers recently reported methodology involving incorporation of *S*-methylcysteine into peptides followed by oxidation to the sulfoxide and either pyrolytic or basic (DBU) elimination, which constitutes the most versatile approach to date.[6e] However, this method is not compatible with protected cysteine residues.[6d] Therefore, the authors resorted to segment couplings to prepare peptides containing both cysteine and dehydroalanine (Dha).[6e]

(8) Selenocysteine occurs naturally and is incorporated into proteins via the ribosomal machinery. Hence it has been termed the 21st physiological amino acid and has been given the three-letter abbreviation Sec. The oxidative elimination of Sec(Ph) has been utilized previously by Shirahama and co-workers[9b,c] for the solution phase synthesis of Alternariolide. This 4-residue cyclic peptide does not contain reactive amino acid side chains, necessitating the studies reported in this communication.

(9) (a) Walter, R.; Roy, J. *J. Org. Chem.* **1971**, *36*, 2561−2563. (b) Hashimoto, K.; Sakai, M.; Okuno, T.; Shirahama, H. *Chem. Commun.* **1996**, 1139−1140. (c) Sakai, M.; Hashimoto, K.; Shirahama, H. *Heterocycles* **1997**, *44*, 319−324.

(10) Pansare, S. V.; Arnold, L. D.; Vederas, J. C. *Org. Synth.* **1991**, *70*, 10−17.

(11) Liotta, D.; Markiewicz, W.; Santiesteban, H. *Tetrahedron Lett.* **1977**, *50*, 4365−4368.

(12) (a) Berkowitz, D. B.; Smith, M. K. *Synthesis* **1996**, 39. (b) Pedersen, M. L.; Berkowitz, D. B. *J. Org. Chem.* **1993**, *58*, 6966−6975.

(13) (a) We and others[13b] have noted that selenoxides in amino acids and peptides can be unusually stable. In certain cases they can be isolated and purified and require elevated temperatures to undergo elimination. (b) Woiwode, T. F.; Wandless, T. J. *J. Org. Chem.* **1999**, *64*, 7670−7674.

Table 2. Synthesis of Dehydropeptides

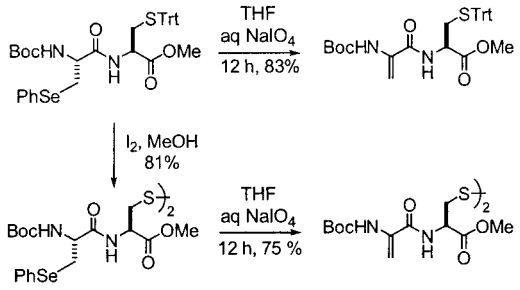
entry	peptide[a,b]	yield (%)[c]	dehydropeptide[d]	oxidant	yield (%)
1	Fmoc-GLPU(Ph)VIA	44	Fmoc-GLPDhaVIA	$NaIO_4$	72
2	Fmoc-ISVU(Ph)RSTS	27	Fmoc-ISVDhaRSTS	$NaIO_4$	67
3	Ac-GLPU(Ph)VIA	40	Ac-GLPDhaVIA	H_2O_2	82
4	Ac−ISVU(Ph)RSTS	34	Ac−ISVDhaRSTS	$NaIO_4$	82
5	Ac-GGC(S*t*Bu)PU(Ph)VIA	33	Ac-GGC(S*t*Bu)PDhaVIA	$NaIO_4$	84
6	LU(Ph)PGC(Trt)VG	15	LDhaPGC(Trt)VG (**3**)	$NaIO_4$	80
7	$[LU(Ph)ANCKI]_2$	30[e]	$[LDhaAECKI]_2$ (**4**)	$NaIO_4$	33
8	RIAU(Ph)IALC(S*t*Bu)K	47	RIADhaIALC(S*t*Bu)K	$NaIO_4$	72

[a] U, selenocysteine; U(Ph), (*Se*)-phenyl selenocysteine. [b] Conditions: Wang resins preloaded with the *C*-terminal amino acids were used. Piperidine was used as deprotectant, HBTU and NMM were used as activators, and DMF was the solvent. [c] Yields are for HPLC purified products after SPPS and are based on the supplier-specified loading of resin. [d] Conditions: the oxidant (4 equiv) was added at 25 °C to solutions of the peptides (final peptide concentrations 1−6 mM). Solvents were MeOH (entries 1, 3, 4) or H_2O/MeCN (entries 2 and 5−8). [e] The symmetrical disulfide was formed by oxidation of the purified free thiopeptide with I_2. The yield is for both SPPS and oxidation.

as partial oxidation of the methionine to the sulfoxide was otherwise observed.[14]

In an alternative approach, trityl-protected or free cysteine residues were chemoselectively oxidized with I_2 to the corresponding symmetrical disulfides without modification of the phenylselenide (Scheme 2). The dehydroalanine was

Scheme 2

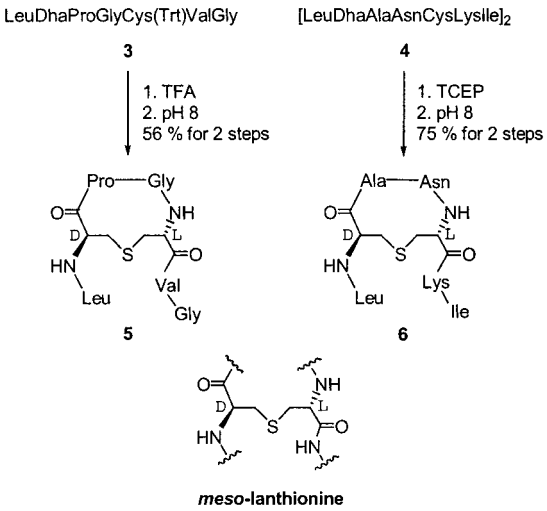


then unmasked chemoselectively in the presence of the new cystine residue (Table 1, entry 2). In general, H_2O_2 could also be used as a suitable oxidant for the reactions in Tables 1 and 2, however *m*-CPBA provided a complex mixture of products.

The versatility of this chemoselective and site-specific method was further demonstrated with longer peptide sequences synthesized by standard SPPS procedures (Table 2). The oxidations were monitored by RP-HPLC, and generally quantitative conversion was observed in as little as 0.5 h and as long as 2 h. Typical functionalities found in peptides such as amines, amides, guanidines, alcohols, and acids did not interfere when left unprotected during the oxidation (Table 2).[14] Cysteine residues protected as symmetrical disulfides were subsequently quantitatively reduced using tris(carboxyethyl)phosphine (TCEP) as monitored by HPLC and mass spectrometry.

Beyond its utility for the synthesis of dehydropeptides, this methodology is well suited for the synthesis of a class of cyclic peptide thioethers known as lanthionines. Lanthionines are structures that are almost exclusively found in lantibiotics, a class of posttranslationally modified antibiotics.[1i] Among these is the promising antibacterial agent nisin, which interacts with lipid II, the physiological target of vancomycin.[15] To illustrate the utility of the current methodology for lanthionine synthesis, we prepared two cyclic thioethers **5** and **6** (Scheme 3). After introduction of dehydroalanines into

Scheme 3



peptides **3** and **4** (Table 2), a clean intramolecular Michael addition of a cysteine onto the dehydroalanines was accomplished by deprotection of the cysteines followed by adjustment of the pH of the reaction. One new species was produced in both cases as monitored by HPLC, consistent

(14) The transformations in Tables 1 and 2 generally showed spot-to-spot TLC or peak-to-peak HPLC conversions. In one case of a methionine-containing peptide we did observe a byproduct containing both a dehydroamino acid and the sulfoxide of methionine. Addition of Et_3N suppressed formation of this side product to 13%. Since no other products were isolated in significant quantities, we believe that the moderate yields obtained in some cases in Tables 1 and 2 reflect losses during purification.

(15) Breukink, E.; Wiedemann, I.; van Kraaij, C.; Kuipers, O. P.; Sahl, H.-G.; de Kruijff, B. *Science* **1999**, *286*, 2361−2364.

with similar observations recently reported by Toogood[16] and Bradley and co-workers.[6e] The single diastereomers were assigned by NMR to the natural (2*S*,6*R*)-lanthionine ring systems ("*meso*"-lanthionines) in the latter studies.

In conclusion, a facile, site-specific, and chemoselective method of incorporating dehydroamino acids into peptides has been developed. This method allows unmasking of the dehydroalanine *after* global deprotection in the ultimate step of the synthesis of dehydropeptides, minimizing possible side reactions. The method is fully compatible with all physiological amino acids including appropriately protected cysteines, and the peptides can be assembled using standard Fmoc SPPS. Its utility is demonstrated by the synthesis of a variety of peptides including the intriguing cyclic lanthionines. Given the high efficiency and yield of the oxidation reaction, we are currently investigating the possibility of on-resin oxidative eliminations to form resin-bound dehydropeptides.

(16) Toogood, P. L. *Tetrahedron Lett*. **1993**, *34*, 7833−7836.

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Supporting Information Available: A general procedure for the oxidation reactions is described, as well as characterization of the peptides. This material is available free of charge via the Internet at http://pubs.acs.org.

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