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Sequencing Hydroxyethylamine-Containing Peptides via Edman Degradation

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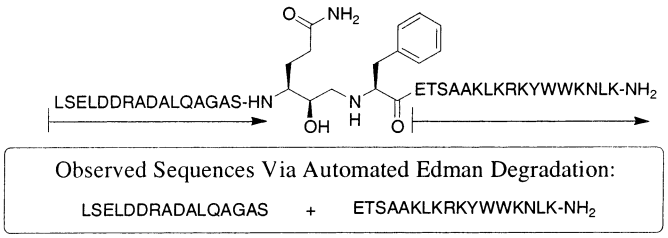
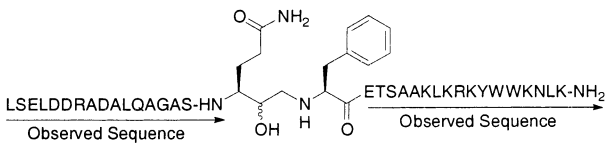
ABSTRACT



Hydroxyethylamine-containing peptides can be sequenced by automated Edman degradation to provide sequence information for peptide segments on either side of the peptide backbone modification.

Modification of a peptide backbone through the incorporation of peptide bond isosteres has proven to be a successful approach to the development of potent enzyme inhibitors.[1−3] Of these modifications, hydroxyethylamine[4] dipeptide isosteres have found particular success in the development of inhibitors for an array of enzymes including renin,[5] HIV protease,[6−9] and more recently β- secretase.[10,11] We have observed that automated Edman degradation[12] of peptides containing the hydroxyethylamine moiety provides sequence information for peptide segments on both sides of the peptide backbone modification.We first noted this during Edman degradation of epimeric hydroxyethylamines **1a** and **1b**, which were prepared as potential substrate-based inhibitors[3,13] of botulism neurotoxin-B metallopeptidase (BoNT-B).[14] The hydroxyethylamine moiety is known to inhibit the thermolysin class of metallopeptidases.[4]



1a/b

The desired hydroxyethylamines **1a/b** were synthesized by on-resin reductive amination of the resin-bound

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(1) Leung, D.; Abbenante, G.; Fairlie, D. P. *J. Med. Chem.* **2000**, *43*, 305.

(2) Babine, R. E.; Bender, S. L. *Chem. Rev.* **1997**, *97*, 1359.

(3) Rich, D. H. In *Comprehensive Medicinal Chemistry. The Rational Design, Mechanistic Study and Theraputic Application of Chemical Compounds*; Hansh, C. S., Sammes, P. G., Taylor, J. B., Eds.; Pergamon Press: New York, 1990; p 391.

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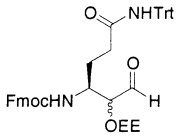
C-terminal sequence with an epimeric mixture of 1-ethoxy ethyl (EE)-protected α-hydroxy aldehyde **2a**/**b**.[15] Chain



2a/b

elongation,[16] followed by final deprotection and cleavage from resin provided a diastereomeric mixture of hydroxyethylamine analogues **1a**/**b**, which were readily separable by preparative reverse-phase HPLC. Although mass spectrometry indicated the desired product, the lower than expected biological activity of these compounds prompted us to more fully characterize them to rule out the possibility that rearrangements had occurred during peptide synthesis.

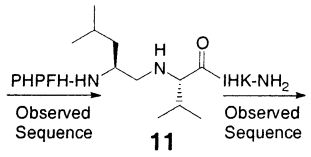
As a means of characterization, hydroxyethylamines **1a** and **1b** were submitted for protein sequencing via automated Edman degradation. We expected to observe the N-terminal residues up to the point of modification. However, degradation provided both the expected N-terminal sequence, and also a sequence commencing after the dipeptide isostere (Table 1). To rule out sample decomposition as the cause of this result, MALDI-TOF mass spectrometry was performed on each sample. In each case only the full-length peptides were observed.

Table 1. Peptide Sequencing Results[a]

sequencing	1a		1b		12		13		14		15	
step	N[b]	C[c]	N	C	N	C	N	C	N	C	N	C
1	L	E	L	E	L	E	S	E	S	E	L	
2	S	T	S		S	T	N	A	N	A	S	
3	E	S	E	S	E		K	N	K	N	E	
4	L	A	L		L	A	T	Q	T	Q	L	
5	D		D	A	D	A	R	R	R	R	D	
6	D	K	D	A	D	K		A			D	
7	R	L	R	K	R	L		T			R	
8	A	K	A	L	A						A	
9	D	R	D	K	D						D	
10	A	K	A	R	A						A	
11	L	Y	L	K	L	Y					L	
12	Q	W	Q	Y	Q						Q	
13	A	W	A	W	A						A	
14	G	K	G	W	G						G	
15	A	N			A						A	

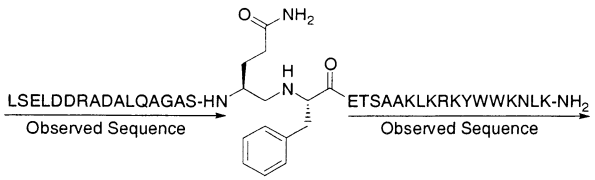
[a] Sequencing was performed on a Beckman Coulter Porton LF-3000 sequencer using the standard sequencing program. [b] N = observed sequence commencing at the N-terminus of the peptide. [c] C = observed sequence commencing after peptide backbone modification.

One report by Jörnvall et al.[17] documents a similar observation for Edman degradation of reduced amide containing peptide **11**. To test the generality of their observation,

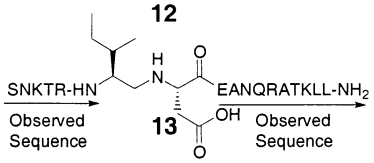


11

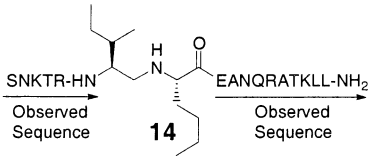
we prepared reduced amides **12**, **13**,[18] and **14**.[19] Reduced amide **12** was synthesized by on-resin reductive amination[20] of aldehyde Fmoc-Gln(Trt)-H[15] with the amino group of the resin-bound C-terminal sequence.[16] Chain elongation,[16] followed by final deprotection and cleavage from resin, provided the desired reduced amide **12**, which was purified by preparative reverse-phase HPLC. Reduced amides **13** and **14** were synthesized in a similar fashion. Degradation again provided both the N-terminal sequence and a secondary sequence starting adjacent to the reduced amide modification (Table 1).



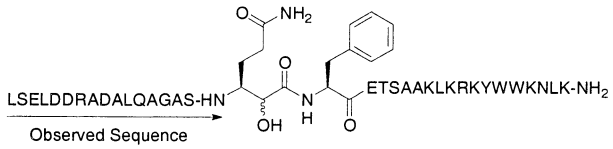
12



13



14



15

Edman degradation of α-hydroxy amide **15**,[15] however, provided only the expected N-terminal sequence up to the

(5) (a) Ryono, D. E.; Free, C. A.; Neubeck, R.; Samaniego, S. G.; Godfrey, J. D.; Petrillo, E. W., Jr. *Proc. Am. Pept. Symp., 9th.* **1985**, 739. (b) Dann, J. G.; Stammers, D. K.; Harris, C. J.; Arrowsmith, R. J.; Davies, D. E.; Hardy, G. W.; Morton, J. A. *Biochem. Biophys. Res. Commun.* **1986**, *134*, 71. (c) Cooper, J. B.; Foundling, S. I.; Blundell, T.; Arrowsmith, R. J., Harris, C. J.; Champness; *Topics in Medical Chemistry, Fourth SCI-RSC Medicinal Chemistry Symposium*; Leening, P. R., Ed.; Special Pub. No. 65, The Royal Society of Chemistry: London, 1988; p 309.

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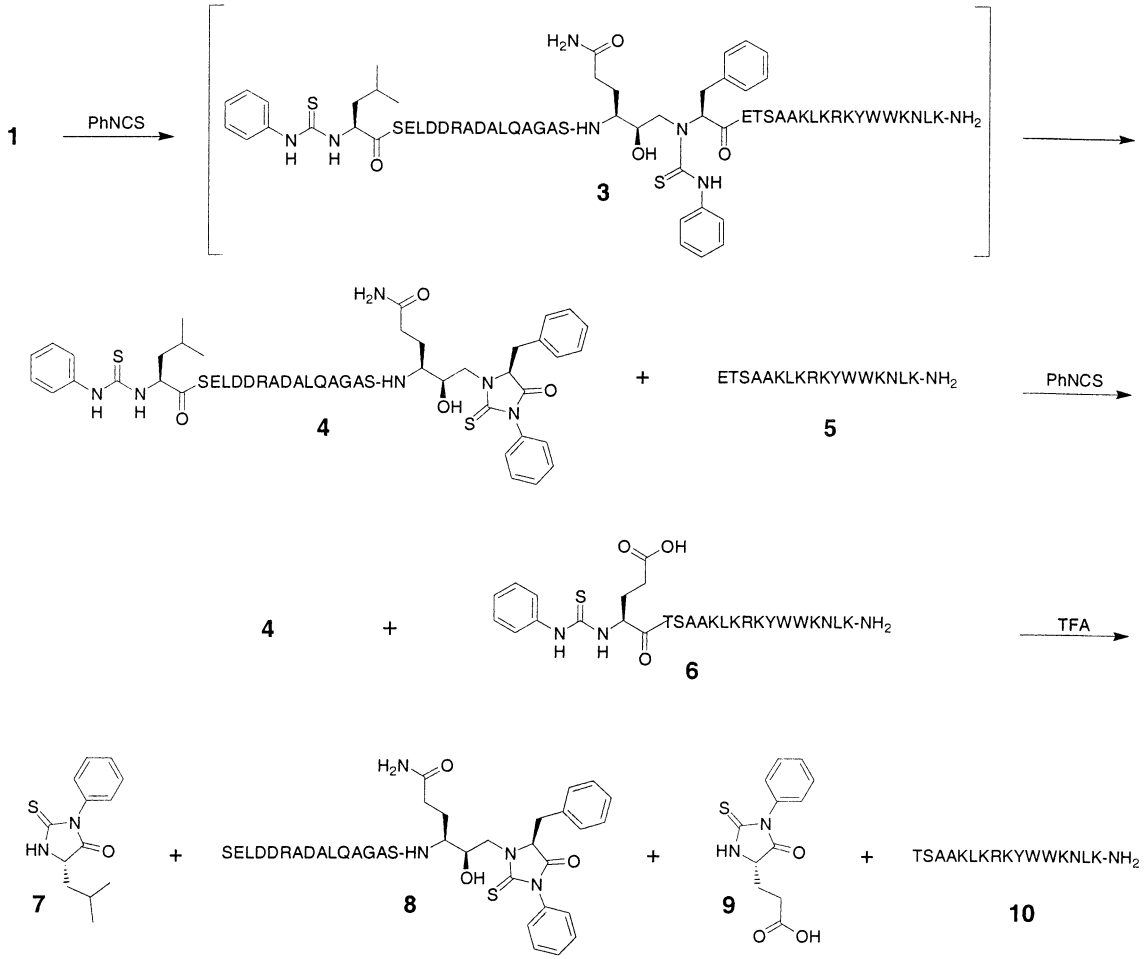
(10) Wolfe, M. S.; Selkoe, D. J. In *PCT Int. Appl.*; The Brigham and Women's Hospital, Inc., USA; WO 0214264 A2, 2002, p 59.

Scheme 1



point of modification (Table 1). Although hydroxy amide **15** has a secondary alcohol that could be a site for acylation by phenyl isothiocyanate, no chain cleavage was observed. This indicates the key role played by the secondary amine present in the reduced amide and hydroxyethylamine derivatives during the degradation process.[21] We hypothesize that under Edman degradation conditions the secondary amine of the hydroxyethylamine reacts in a fashion similar to the N-terminal primary amine and thus provides a second site for peptide bond cleavage. It should be noted that for each of the hydroxyethylamine and reduced amide substrates examined the first residue of the C-terminal sequence (glutamic acid, Table 1, row 1, columns labeled C-term) was observed during the first round of degradation. This was also noted by Jörnvall for reduced amide **11** and can be explained by spontaneous cyclization and cleavage of the internal thiourea to liberate the C-terminal peptide fragment while still in the presence of phenyl isothiocyanate.[17] As shown in Scheme 1, phenyl isothiocyanate reacts with secondary amine **1** to provide bis-thiourea **3**. The internal thiourea of **3** cyclizes under the reaction conditions to provide phenylthiohydantoin **4** and C-terminal peptide **5**. Peptide **5** reacts with another equivalent of phenyl isothiocyanate to provide thiourea **6**. Acid-induced cyclization and cleavage of the primary thioureas provides the expected phenylthiohydantoins **7** and **9** and peptide fragments **8** and **10** available for the next round of sequencing. Formation of bis-thiourea **3** is not quantitative;

(11) Beck, J. P.; Gailunas, A.; Hom, R.; Jagodzinska, B.; John, V.; Maillaird, M. In *PCT Int. Appl.*; Elan Pharmaceuticals, Inc., USA.; Pharmacia + Upjohn Company; WO 0202518 A2, 2002, p 286.

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(14) For a review of BoNT structure and function, see: Montecucco, C.; Schiavo, G. *Q. Rev. Biophys.* **1995**, *28*, 423.

(15) Manuscript in preparation.

(16) Peptides were synthesized on a Pioneer Peptide Synthesis System using standard Fmoc protocol. For reviews on the practice of solid-phase peptide synthesis, see: (a) Fields, G. B.; Lauer-Fields, J. L.; Liu, R.-q.; Barany, G. In *Synthetic Peptides: A User's Guide*; Grant, G. A., Ed.; Oxford University Press: New York, NY, 2002. (b) Atherton, E.; Sheppard, R. C. *Solid-Phase Peptide Synthesis: A Practical Approach*; IRL Press: Oxford, U.K., 1989.

(17) Hempel, J.; Nilsson, K.; Larsson, K.; Jornvall, H. *FEBS Lett.* **1986**, *194*, 333. This reference was particularly difficult to locate because the terminology "reduced amide" was not used in the text or abstract.

(18) MALDI-MS: calcd for $[C_{81}H_{147}N_{29}O_{26}]^+$ 1943.1150, found 1943.1.

(19) MALDI-MS: calcd for $[C_{83}H_{154}N_{29}O_{24}]^+$ 1941.1722, found 1941.2.

(20) Sasaki, Y.; Coy, D. H. *Peptides* **1987**, *8*, 119.

(21) For an examples of secondary amines participating in Edman degradation, see: (a) Miranda, L. P.; Meldal, M. *Angew. Chem., Int. Ed.* **2001**, *40*, 3655. (b) Boeijen, A.; Liskamp, R. M. J. *Tetrahedron Lett.* **1998**, *39*, 3589.

depending on the amino acid sequence, some secondary amine remains and undergoes cleavage in the next cycle of Edman degradation.

The discovery that Edman degradation chemistry can be used to sequence both the N-terminal and C-terminal segments of hydroxyethylamine-derived peptides expands the utility of this important reaction. This finding should be useful for characterizing an important class of compounds often used in the development of enzyme inhibitors.

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Supporting Information Available: Experimental procedure for the synthesis of compound **12**. This material is available free of charge via the Internet at http://pubs.acs.org.

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