Tetrahedron Letters, Vol. 33, No. 19, pp. 2649-2652, 1992
Printed in Great Britain

0040-4039/92 $5.00 + .00
Pergamon Press Ltd

Reactivity of Dehydroaspartic Acid Derivatives Under Peptide Coupling Conditions

Nalin L. Subasinghe and Rodney L. Johnson*

Department of Medicinal Chemistry, University of Minnesota, Minneapolis, Minnesota 55455, USA

Key Words: Dehydroaspartic acid; peptide coupling; active ester; oxazolone

*Abstract: The reactivity of α-carboxy-activated dehydroaspartic acid derivatives with various N- and O-nucleophiles is described for the first time. Reaction of amino acid derivatives with Ac-Δ^ZAsp(OBu^t)-OH (**4a**) under a variety of coupling conditions gave very poor yields of the resulting dehydroaspartyl dipeptides, while the coupling of **4a** with oxygen nucleophiles gave very good yields of the dehydroaspartyl esters. In contrast, the coupling of amino acids with Cbz-Δ^ZAsp(OBu^t)-OH (**4b**) gave good yields of dehydroaspartyl dipeptides. The formation of a highly reactive oxazolone intermediate is proposed to explain the low yields of peptide coupling between **4a** and amino acids.*

α,β-Dehydroamino acids are known to impose unique conformational constraints when incorporated into peptides.[1,2] Thus, they have been used to study the structure-activity relationships of a number of biologically active peptides.[2] The most common dehydroamino acid residues employed in such studies have been dehydrophenylalanine and the aliphatic residues dehydroleucine and dehydroalanine.[1-3] Dehydroaspartic acid (ΔAsp) residues, however, have not been utilized in a similar manner even though many biologically active peptides contain aspartic acid residues. This is most likely due to the absence of convenient methods for incorporating the dehydroaspartyl residue into peptides.[4] We previously reported the synthesis of a dehydroaspartyl containing dipeptide via a Horner-Emmons olefination of a phosphonoglycine containing peptide.[5] In this communication we wish to report on (1) the synthesis of dehydroaspartyl containing peptides by the coupling of N-protected β-*tert*-butyl esters of dehydroaspartic acid with either amino acid or peptide residues and (2) the differing reactivity of α-carboxy-activated dehydroaspartyl residues with N- versus O-nucleophiles.

The dehydroaspartic acid esters **2a,b** and **3a,b** were synthesized as shown in Scheme 1 via the general route of Schmidt et al.[6] The ratio of **2a**:**3a** and **2b**:**3b** was 1:3 and 1:2.5, respectively. The *E*- and *Z*-isomers in each case were distinguished from each other on the basis of their NMR spectra.[7] The *(Z)*-dehydroaspartic acid methyl esters **3a** and **3b** were hydrolyzed to **4a** and **4b**, respectively, with LiOH. Subsequently, **4a** and **4b** were coupled to various amino acid residues under a variety of coupling reaction conditions (Scheme 1, Table 1). Although no attempt was made to hydrolyze **2b**, a single attempt at hydrolyzing the methyl ester of **2a** resulted only in its decomposition. Furthermore, a previous study[8] that showed that *E*-dehydroamino acids tend to isomerize to the *Z*-isomers under peptide coupling conditions dissuaded us from making any attempts at coupling the *E*-dehydroaspartyl derivatives.

Scheme 1.

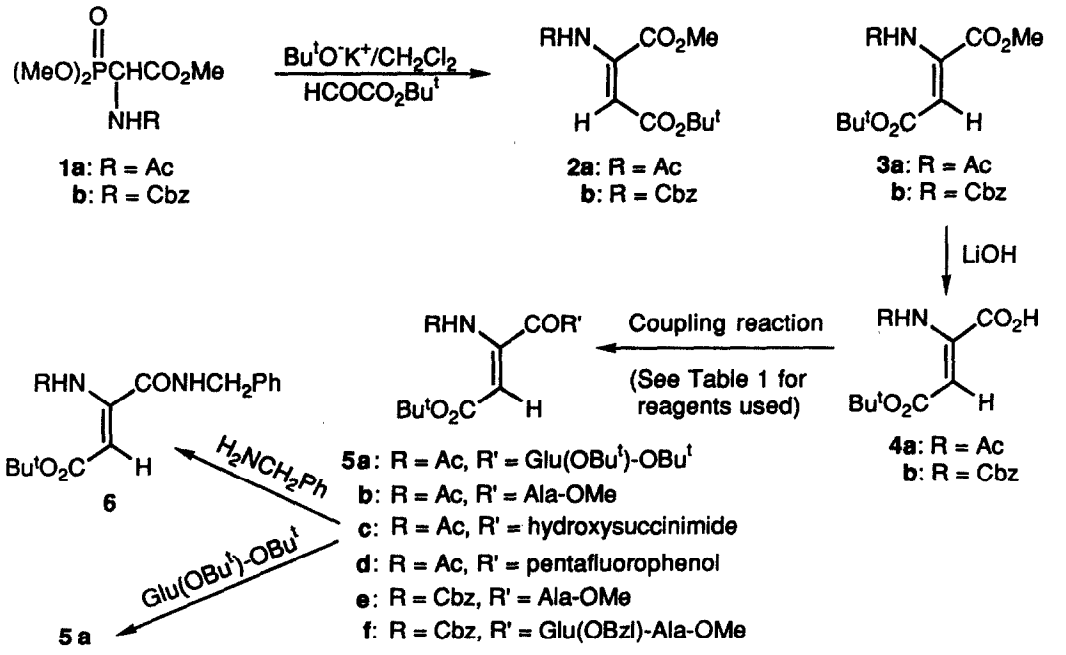


Table 1. Coupling Reactions Carried Out with the Dehydroaspartic Acid Derivatives **4a**, **4b**, and **5c**.

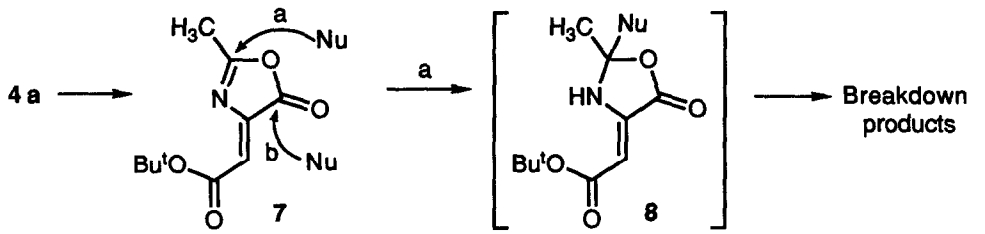
Δ^ZAsp	Coupling reagent (base, equiv)	Nucleophile (base, equiv)	Product (% yield)
4a	EDC[a]/HOBt[b] (NMM[c], 1)	Glu(OBu^t)-OBu^t·HCl (NMM, 1)	**5a** (5)
4a	$ClCO_2Bu^i$ (NMM, 1)	Glu(OBu^t)-OBu^t·HCl (NEt_3, 1.2)	**5a** (12)
4a	$ClCO_2Bu^i$ (NMM, 1)	Ala-OMe·HCl (NEt_3, 1.1)	**5b** (8)
4a	BOP[d]	Ala-OMe·HCl (NEt_3, 2)	**5b** (5)
4a	DCC[e]	N-Hydroxysuccinimide	**5c** (83)
4a	DCC	Pentafluorophenol	**5d** (78)[f]
4b	DCC/HOBt	Ala-OMe·HCl (NMM, 1)	**5e** (53)
4b	DCC/HOBt	Glu(OBzl)-Ala-OMe·CF_3CO_2H (NMM, 1)	**5f** (43)
5c		Glu(OBu^t)-OBu^t	**5a** (27)
5c		H_2NCH_2Ph	**6** (22)

[a]1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide·HCl. [b]1-Hydroxybenzotriazole. [c]N-Methylmorpholine. [d]Benzotriazol-1-yloxy-tris-(dimethylamino)-phosphonium hexafluorophosphate. [e]N,N'-Dicyclohexylcarbodiimide. [f]Based on HPLC analysis.

The coupling of the N-acyl Δ^ZAsp derivative **4a** with a number of amino acid derivatives employing a variety of coupling reagents gave a complex mixture of unstable products from which very poor yields of the dehydroaspartyl dipeptides **5a** and **5b** were obtained. In contrast, the coupling of **4a** with the oxygen nucleophiles N-hydroxysuccinimide and pentafluorophenol gave very good yields of esters **5c** and **5d**, respectively. Although the reaction of active ester **5c** with N-nucleophiles gave higher yields of the coupled products than were obtained when **4a** was activated with either EDC, isobutylchloroformate, or BOP, the formation of unstable products still predominated. The coupling of **4b**, on the other hand, with either an amino acid or dipeptide derivative gave respectable yields of peptides **5e** and **5f**.

Previously, it has been reported that peptides containing either a C-terminal *(Z)*-dehydrophenylalanine or a dehydroalanine residue are coupled with glycine esters in good to very good (48-86%) yields.[8] The very low coupling yields we obtained with the N-acyl Δ^ZAsp residue **4a** indicates that this residue behaves quite differently in terms of its reactivity under peptide coupling conditions than the N-acyl Δ^ZPhe or ΔAla residues. We postulate that the basis of this difference is the ready formation of the highly reactive oxazolone intermediate **7** when **4a** is activated and the subsequent reaction of **7** with N-nucleophiles to give unstable products (Scheme 2). The amide hydrogen of a N-acyl Δ^ZAsp derivative such as **4a** would be expected to be more acidic than that of either a saturated acyl amino acid or an acyl aliphatic dehydro amino acid due to the conjugation through the π-system of the amide nitrogen with the electron withdrawing β-carboxy group. Since the formation of oxazolones is base catalyzed,[9] the increased acidity of the N-H in **4a** should facilitate oxazolone formation even in the presence of a mildly basic N-nucleophile such as an amino acid. Oxazolone **7** could give rise to a peptide if the amino acid nucleophile would attack the oxazolone ring by pathway b. However, **7** offers a second reactive electrophilic site at the acyl carbonyl carbon due to the extended conjugation of this carbon with the β-carboxy group. We postulate that amino acids predominantly undergo 1,6-addition (pathway a) with **7** thereby giving rise to a number of possible unstable intermediates like **8** instead of the desired dipeptides. The higher yields obtained in the formation of N-hydroxysuccinimide ester **5c** and peptides **5e** and **5f** are consistent with such a hypothesis since it is known that hydroxysuccinimide esters[10] and N-Cbz protected amino acids are less likely to form oxazolones under peptide coupling conditions.[9] It is also possible that in the case of **5c** and **5d**, **7** is being formed, but that the O-nucleophiles react preferentially by pathway b.

Scheme 2.



In order to determine whether an oxazolone intermediate was being formed under peptide coupling conditions, **4a** was treated with DCC in CH_3CN at 0 °C and infrared spectroscopic studies were carried out on this reaction mixture at various time intervals. Almost as soon as DCC is added to **4a**, a peak at 1823 cm^{-1} appears. The same absorption band at 1823 cm^{-1} was also observed when **5d** was treated with one equivalent

of NEt_3 in CH_3CN at 0 °C. Since Nujol mulls of some known unsaturated oxazolones[11] have shown carbonyl absorption bands in the 1770 - 1800 cm^{-1} region and saturated oxazolones[12] have shown bands around 1832 cm^{-1}, the results support the proposition that an oxazolone is being formed.

In summary, Ac-Δ^ZAsp(OBu^t)-OH (**4a**) is poorly coupled to other amino acid residues due to its unfavorable reactivity under peptide coupling conditions. However, Cbz-Δ^ZAsp(OBu^t)-OH (**4b**) behaves favorably under these conditions giving good yields of the coupled products.

REFERENCES AND NOTES

1. Singh, T. P.; Narula, P.; Patel, H. C. *Acta Crystallogr., Sect. B: Struct. Sci.* **1990**, *46*, 539-545.
2. Stammer, C. H. Dehydroamino Acids and Peptides. In *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*, Vol. 6; Weinstein, B. Ed.; Marcel Dekker: New York, 1982; pp. 33-74.
3. Spatola, A. F. Peptide Backbone Modifications: A Structure-Activity Analysis of Peptides Containing Amide Bond Surrogates, Conformational Constraints, and Related Backbone Replacements. In *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*, Vol. 7; Weinstein, B. Ed.; Marcel Dekker: New York, 1983; pp. 267-357.
4. Shin, C.; Yonezawa, Y.; Tokuumi, S. *Chem. Lett.* **1988**, 1473-1474. This paper reports the synthesis of β-protected dehydroaspartyl dipeptides wherein the ΔAsp residue occupies the C-terminus.
5. Subasinghe, N. L.; Schulte, M; Chan, Y. M.; Roon, R. J.; Koerner, J. F.; Johnson, R. L. *J. Med. Chem.* **1990**, *33*, 2734-2744.
6. Schmidt, U.; Lieberknecht, A.; Wild, J. *Synthesis* **1984**, 53-60.
7. All isolated compounds had satisfactory analytical and/or spectroscopic data. Selected physical data:
   2a: mp 103-103.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (s, 9 H, $C(CH_3)_3$), 2.08 (s, 3 H, CH_3CO), 3.84 (s, 3 H, OCH_3), 6.65 (s, 1 H, C=CH), 8.03 (br s, 1 H, NH).
   2b: mp 97-98 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46 (s, 9 H, $C(CH_3)_3$), 3.82 (s, 3 H, OCH_3), 5.12 (s, 2 H, $PhCH_2$) 6.52 (s, 1 H, C=CH), 7.07 (s, 1 H, NH), 7.34 (s, 5 H, Ph H's).
   3a: mp 57-58 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.45 (s, 9 H, $C(CH_3)_3$), 2.13 (s, 3 H, CH_3CO), 3.80 (s, 3 H, OCH_3), 5.36 (s, 1 H, C=CH), 10.20 (br s, 1 H, NH).
   3b: mp 55 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.46 (s, 9 H, $C(CH_3)_3$), 3.82 (s, 3 H, OCH_3), 5.16 (s, 2 H, $PhCH_2$) 5.39 (s, 1 H, C=CH), 7.36 (s, 5 H, Ph H's), 9.75 (br s, 1 H, NH).
   4a: mp 125-126 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (s, 9 H, $C(CH_3)_3$), 2.23 (s, 3 H, CH_3CO), 5.78 (s, 1 H, C=CH), 9.71 (br s, 1 H, NH), 10.45 (s, 1 H, CO_2H).
   4b: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.48 (s, 9 H, $C(CH_3)_3$), 5.19 (s, 2 H, $PhCH_2$), 5.64 (s, 1 H, C=CH), 7.37-7.38 (m, 5 H, Ph H's), 9.72 (s, 1 H, NH), 10.2-10.8 (br, 1 H, CO_2H).
8. Makowski, M.; Rzeszotarska, B.; Kubica, Z.; Pietrzynski, G.; Hetper, J. *Liebigs Ann. Chem.* **1986**, 980-991.
9. Bodanszki, M.; Clausner, Y. S.; Ondetti, M. A. *Peptide Synthesis*; John Wiley and Sons, Inc.: New York. 1976; pp. 137-153.
10. Zimmerman, J. E.; Anderson, G. W. *J. Am. Chem. Soc.* **1967**, *89*, 7151-7152.
11. Breitholle, E. G.; Stammer, C. H. *J. Org. Chem.* **1976**, *41*, 1344-1349.
12. Goodman, M.; McGahren, W. J. *J. Am. Chem. Soc.* **1965**, *87*, 3028-3029.

(Received in USA 10 January 1992)