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Incorporation of a Phosphonic Acid Isostere of Aspartic Acid into Peptides Using Fmoc-Solid Phase Synthesis

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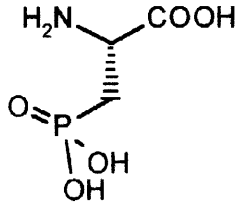
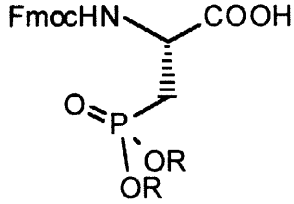
Abstract: A short synthesis of a novel Fmoc-derivative **2b** of the phosphonic acid isostere **1** of aspartic acid is presented. Incorporation of **2b** into peptides was readily achieved using standard Fmoc-solid phase synthesis. Efficient removal of the allyl protecting groups after sequence assembly under mild conditions using Pd(0) catalysis afforded phosphonopeptides **3a** and **3b** in high purity. © 1998 Elsevier Science Ltd. All rights reserved.

We have been interested in exploring the role of aspartic acid isosteres in biologically active peptides. In particular we desired to study the effect of substituting the β-carboxylic acid group of aspartic acid with the more strongly acidic phosphonic acid group. A review of the literature revealed that unprotected 2-amino-3-phosphonopropionic acid (rac.-AP-3) as well as the individual enantiomers L-(R)-AP-3 (**1**) and D-(S)-AP-3[1] have been used for the synthesis of enzyme inhibitors[2] and simple dipeptides.[3] Incorporation of **1** into longer peptides has not been reported. The dimethyl phosphonate **2a** was proposed to be a protected form of **1** suitable for peptide synthesis.[4] However, the use of phosphonic acid dialkylesters in peptide synthesis is limited due to the difficulties connected with cleavage of the alkyl esters after peptide assembly. In particular, incomplete diester cleavage[5] on the one hand and instability of N-terminal formamides and carbamates[6] on the other hand are expected to result in the formation of mixtures of products. We required a derivative of **1** which is compatible with general solid phase peptide synthesis of phosphonopeptides carrying a N-terminal carbamate-functionality.



1: L-(R)-AP-3



2a: R = Methyl
2b: R = Allyl

X-Arg-Gly-(AP-3)-Phe-OH

3

3a: X = Fmoc
3b: X = Ac

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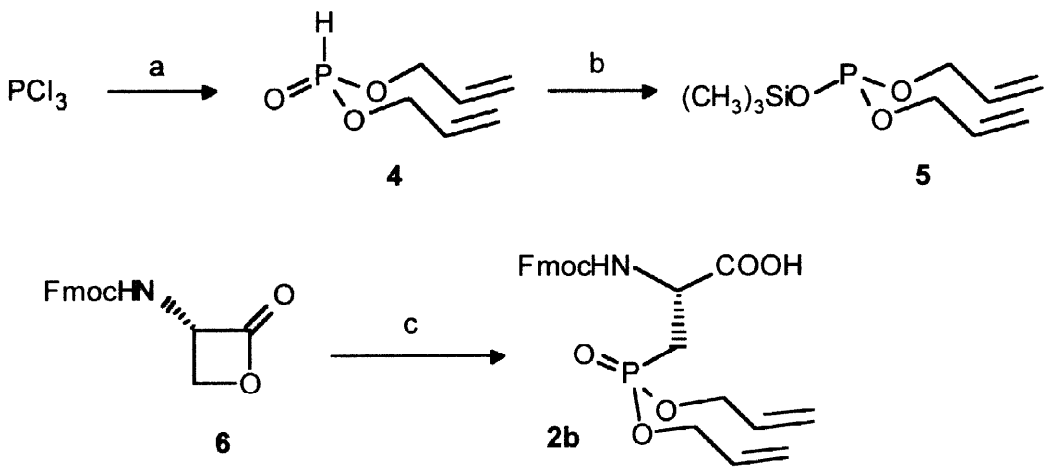
In analogy to a strategy described by Shapiro et al. for the incorporation of the phosphonic acid isostere of glutamic acid into diverse peptide sequences,[7] we chose the protected amino acid phosphonate **2b** as a building block for the introduction of L-(R)-AP-3 (**1**) into peptides. In the following, we will describe a straightforward synthesis of **2b** followed by its successful incorporation into the peptides **3a** and **3b** using solid phase peptide synthesis.

N-protected β-substituted L-alanines can be easily obtained in stereochemically pure form by Mitsunobu-type cyclization of readily available L-serine derivatives followed by nucleophilic ring-opening of the resulting β-lactones.[8] Recently, Hutchinson and Parkes have shown that nucleophilic addition of dimethyl(trimethylsilyl)phosphite to **6** affords the carboxylic trimethylsilyl ester of **2a** by preferential transfer of the trimethylsilyl group.[4] We have prepared phosphite **5** by reaction of allyl alcohol with phosphorous trichloride followed by treatment with trimethylsilylchloride in the presence of triethylamine.[9] Heating of β-lactone **6** in neat phosphite **5** followed by a simple aqueous work up yielded the diallylester **2b** (Scheme).[10]



Scheme: a) 2-propenol, 58 %; b) TMSCl, NEt_3, 79%; c) **5**, 100 °C, aqueous workup, 42 %.[11]

Incorporation of **1** into peptides using building block **2b** was demonstrated by standard Fmoc-solid phase synthesis of the two peptides Fmoc-Arg-Gly-(AP-3)-Phe-OH **3a** and Ac-Arg-Gly-(AP-3)-Phe-OH **3b**. These peptides are N-capped isosteres of the tetrapeptide H-Arg-Gly-Asp-Phe-OH which is known to be a potent inhibitor of fibrinogen binding to the integrin $\alpha_{IIb}\beta 3$ on platelets.[12]

Starting from H-Phe-O-Wang resin, building block **2b** was attached in a single coupling using TPTU followed by Fmoc-cleavage and coupling of the next two amino acids to afford Fmoc-Arg(PMC)-Gly-[AP-3(OAllyl)$_2$]-Phe-O-Wang.[13] The allyl ester protection was cleaved by stirring the resin in a solution of 2 eq. $Pd(PPh_3)_4$ in N-methylmorpholine/ acetic acid/ chloroform 1:2:37 for 5 h at room temperature.[14] Subsequently, the peptide was cleaved from the solid phase using trifluoracetic acid and precipitated from ether to afford peptide **3a**.[15] Peptide **3b** was prepared in analogy.[16] Mass recoveries of **3a** and **3b** were good and the HPLC profiles demonstrate the high purity of the peptide products (Figure).[17] High resolution

electrospray mass spectroscopy showed molecular parent ions in agreement with the constitution $C_{35}H_{42}N_7O_{10}P$ for **3a** and $C_{22}H_{34}N_7O_9P$ for **3b**.

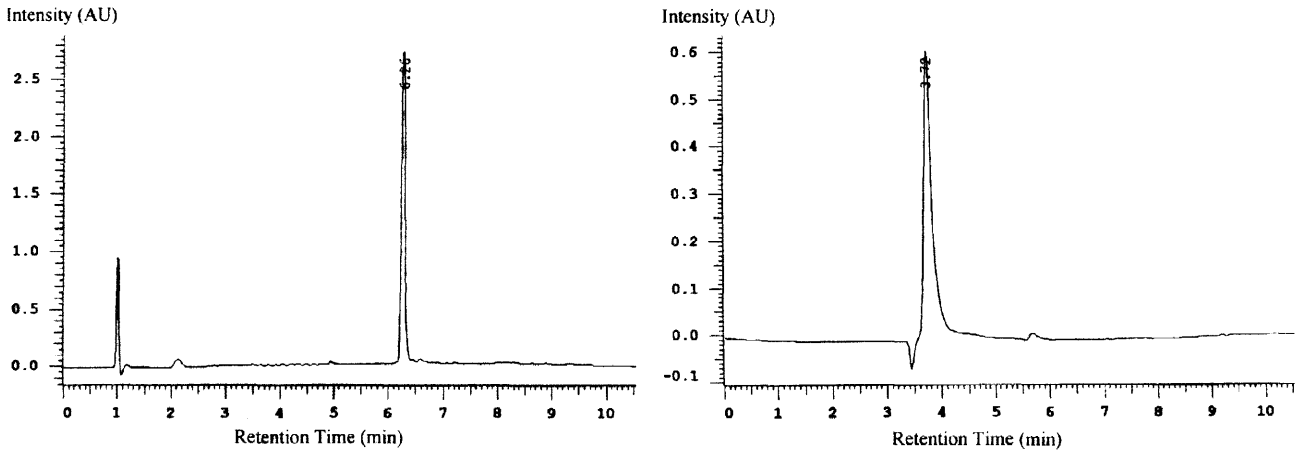


Figure: HPLC profiles of precipitated peptide products **3a** (left) and **3b** (right).[17]

In conclusion, we have demonstrated that L-(R)-AP-3 (**1**), a phosphonic acid isostere of aspartic acid, can be efficiently incorporated into peptides using the building block **2b** and general Fmoc-solid phase peptide synthesis. Synthesis of **2b** is short, easy to perform and allows preparation of **2b** in gram quantities. Efficient cleavage of the phosphonate diallyl esters after peptide assembly using Pd(0) catalysis allowed us to prepare the tetrapeptides **3a** and **3b** in high purity. Our results indicate that **2b** should be well suited for the synthesis of more complicated phosphonopeptides.

Acknowledgments: We thank Drs. G. Shapiro, H. Fretz and S. Veenstra for stimulating discussion, and Mrs. G. M. D'Addio for excellent technical assistance.

References and Notes

1. L-2-Amino-3-phosphonopropionic acid ((L)-AP-3) was referred to as (S)-AP-3 in the literature.[4, 18] In contrast to this assignment and according to the Cahn-Ingold Prelog rules,[19] we think that (L)-AP-3 is identical to (R)-AP-3.
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10. A suspension of 1.2 g (3.9 mmol) **6**[4] in 9 ml freshly distilled **5** was heated under argon at 100 °C. After stirring for 24 h, the reaction had stopped although there was still a significant amount of starting material (**6**) left (TLC analysis on silica gel: methylene chloride/ methanol/ acetic acid 95:5:1, R_f = 0.3). The volatile components were removed under reduced pressure and the residue was resuspended in 9 ml of freshly distilled **5**. After stirring for an additional 14 h at 100 °C the mixture was homogenous and the reaction mixture was concentrated in vacuo. The residue was taken up in ethyl acetate, washed with water, dried over sodium sulfate and purified by flash chromatography on silica gel eluting with methylene chloride/ methanol/ acetic acid (98:2:1) to give 0.77 g product **2b** which crystallized upon cooling. Analytical data for **2b**: ^{1}H-NMR (500 MHz, DMSO-d_6): 12.66 (br., 1H, HOOC), 7.88-7.31 (8H, arom. H(Fmoc)), 7.70 (d, 1H, HN), 5.89 (m, 2H, H(olef.)), 5.30 (m, 2H, H(olef.)), 5.16 (m, 2H, H(olef.)), 4.43 (m, 4H, H_2COP), 4.29 (d, 2H, H_2C(Fmoc)), 4.25 (m, 1H, HC(α)), 4.21 (t, 1H, HC(Fmoc)), 2.30 (m, 2H, H_2C(β)); ^{13}C-NMR (125 MHz, DMSO-d_6): 172.38 (s, COO, d $^3J_{CP}$ = 17), 155.64 (s, OCON), 143.74 (s, Fmoc), 140.67 (s, Fmoc), 133.38 (d, olef., d $^3J_{CP}$ = 7), 127.59 (d, Fmoc), 127.03 (d, Fmoc), 125.18 (d, Fmoc), 120.07 (d, Fmoc), 117.19 (t, olef., d $^4J_{CP}$ = 6), 65.70 (t, Fmoc), 65.56 (t, COP, d $^2J_{CP}$ = 6), 48.85 (d, Cα), 46.56 (d, Fmoc), 26.41 (t, Cβ, d $^1J_{CP}$ = 141); ^{31}P-NMR (202.5 MHz, DMSO-d_6): 29.60; MS (ESpos.): 472 $(M+H)^+$; $[\alpha]^{20}_D$ = -7.4 (c = 0.45, methanol); Mosher analysis of L-(R)-AP-3 (**1**, obtained by exhaustive acid hydrolysis of **2b**) showed 10 % of the enantiomer D-(S)-AP-3.[18]
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15. After washing with dimethylacetamide and methylene chloride, the resin was treated with trifluoracetic acid/ water 95:5 for 3 h at room temperature. The resin was filtered off and the filtrate was added to a 20 fold excess of hexane/ tert. butyl methyl ether 1:9. After standing for 45 min. at 4 °C, the precipitate was collected by centrifugation and dried under high vacuum to yield **3a** as a colorless powder.
16. Synthesis of **3b** started out from Fmoc-Arg(PMC)-Gly-[AP-3(OAllyl)$_2$]-Phe-O-Wang followed by cleavage of the Fmoc protecting group[13] and N-acetylation using dimethylacetamide/ acetic anhydride/ pyridine 8:1:1 for 2 min. at room temperature to give Ac-Arg(PMC)-Gly-[AP-3(OAllyl)$_2$]-Phe-O-Wang. Isolation of the peptide was carried out as described[15] to yield **3b** as a colorless powder.
17. HPLC of **3a** on a 125/3 Nucleosil 120-3 C18 AB column (Marchery-Nagel): Linear gradient of H_2O/ 0.1 % trifluoracetic acid (eluent A) and acetonitrile/ 0.1 % trifluoracetic acid (eluent B) from 2 % to 100 % B over 10 min; flow rate 0.7 ml/ min, detection at 215 nm, retention time: 6.26 min. HPLC of **3b** on a 250/4 Nucleosil 300-5 C4 column (Marchery-Nagel): Linear gradient of H_2O/ 0.1 % trifluoracetic acid (eluent A) and acetonitrile/ 0.1 % trifluoracetic acid (eluent B) from 2 % to 60 % B over 10 min; flow rate 0.7 ml/min, detection at 215 nm, retention time: 3.72 min.
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