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Solid Phase Synthesis of Peptides Containing Novel L-Phenylalanine Derivatives Substituted with Vicinal Tricarbonyl Moieties

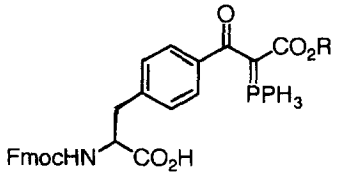
Heinz Fretz

Pharmaceuticals Division, Cancer and Bone Metabolism Research Department, Ciba-Geigy Ltd., CH-4002 Basel, Switzerland

Abstract: Novel Fmoc-L-phenylalanine derivatives **1** substituted with masked vicinal tricarbonyl moieties were incorporated into a peptide sequence using solid phase peptide chemistry. The oxidative cleavage of the peptide phophoranylidene intermediates to the trioxo compounds **3** was achieved in solution and on solid support with Oxone® as oxidant. Copyright © 1996 Elsevier Science Ltd

In our preceding paper, we presented a synthetic route towards novel Nα-Fmoc-phenylalanine derivatives **1a** and **1b** substituted with a keto ylide group as a masked vicinal tricarbonyl moiety.[1] We also demonstrated the oxidative cleavage of the phosphoranylidene double bond with Oxone® yielding amino acid **2a** and **2b**, respectively.

Herein, we give a protocol for the incorporation of **1** into a short peptide sequence as well as for the subsequent oxidative cleaving step of the carbon-phosphorous double bond leading to peptide **3** with the desired vicinal tricarbonyl moiety.



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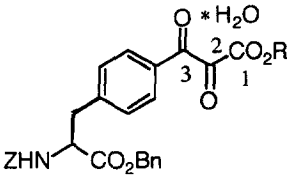
1b R= tBu



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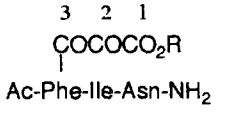
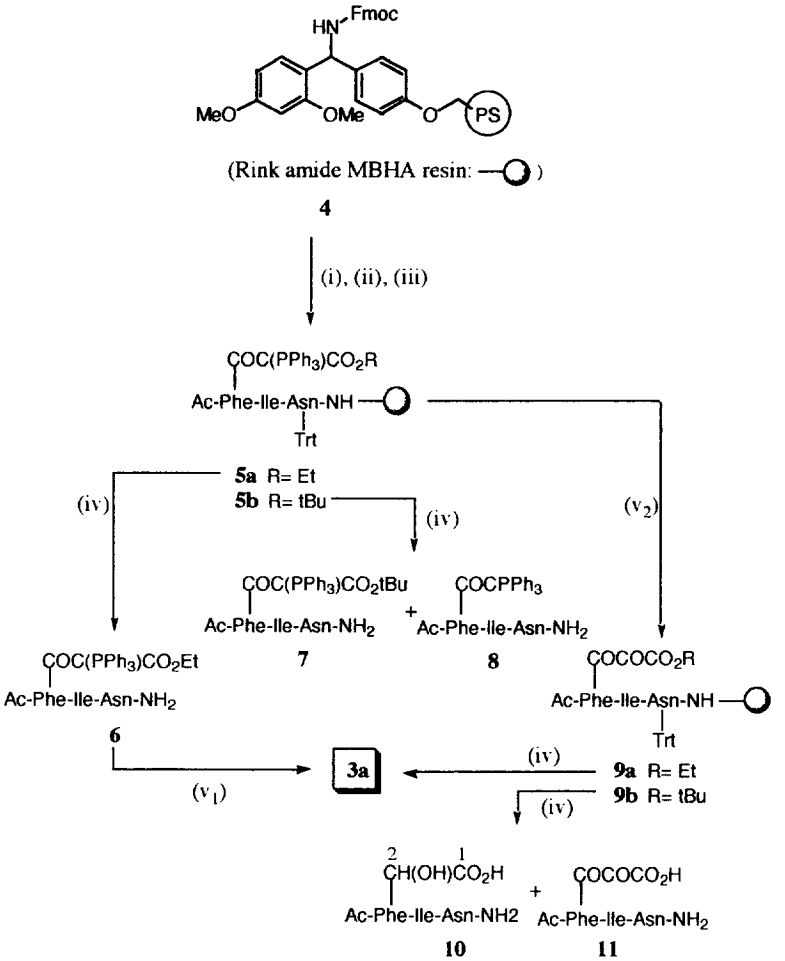


3a R= Et
3b R= tBu

Peptide **5a** and **5b**, respectively, were assembled with a semi-automated instrument on a Rink amide MBHA resin (4-(2',4'-dimethoxyphenyl-Fmoc-aminophenyl)-phenoxyacetamido-norleucyl-MBHA resin (**4**); 0.5 mmol/g),[2] following a standard Fmoc/ *tert*-butyl chemistry protocol. Scheme 1 details the reaction sequences and conditions. Fmoc-Asn(Trt)-OH and Fmoc-Ile-OH (3 equiv. each) were coupled with TPTU (3 equiv.) as coupling reagent in the presence of diisopropylethylamine (DIEA, 3.3 equiv.) with a reaction time of 1h.[3] A minimal excess of the building block **1** (1.5 equiv.) was used for its likewise TPTU-mediated incorporation. The coupling was complete after 6h reaction time as checked by Kaiser test, performed on a resin for the detection of free amino groups.[4] Fmoc cleavage was achieved with 20% piperidine or with 2.5% of the non-nucleophilic 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dimethylacetamide (DMA).[5] After each coupling cycle, a capping step was performed with acetic anhydride/ pyridine in DMA (1:1:8, v/v/v) in order to prevent deletion sequences. Finally, the complete peptide resins **5a** and **5b** were obtained.

Scheme1



Reagents and conditions: (i) Deprotection: 20% Piperidine or 5% DBU in DMA); (ii) Coupling: N^{α}-Fmoc-amino acid, TPTU, DIEA, N-methyl-2-pyrrolidinone (NMP); (iii) Capping: Ac_2O, pyridine in DMA (1:1:8, v/v/v); (iv) TFA/ H_2O (95:5, v/v), 3h at rt; (v_1) Oxone® (1.5 equiv.), dioxane/ H_2O 1:1, 6h at rt; (v_2) Oxone® (10 equiv.), H_2O/ THF 4:1, 8h at rt.

The peptide-resin cleavage of **5a** and the simultaneous removal of side-chain protecting groups were achieved by using 95% trifluoroacetic acid (TFA)/ 5% H_2O for 3h at room temperature (rt). The crude product was precipitated in *tert*-butyl-methyl ether/ petroleum ether (1:1, v/v) at 0°C and collected by centrifugation to give peptide **6** with >95% purity (single peak at t_R= 7.07 min; FAB MS: *m/z* 808 $[M+H]^+$).[6] The oxidative cleavage of the carbon-phosphorous double bond of compound **6** by means of 1.5 equiv. Oxone® in dioxane/ H_2O (1:1, v/v) was complete after 6 h reaction time at room temperature. The reaction mixture was then lyophilized and the desired peptide **3a** was isolated by medium-pressure liquid chromatography (MPLC).[7] The identity of **3a** was assessed by ^1H and ^{13}C NMR spectroscopy and mass spectrometric analyses.[8]

In order to cleave the ylide double bond on the solid support prior to TFA treatment, the oxidation conditions were adapted slightly.[9] A ten-fold excess of Oxone® was added in two portions to the resin suspended in H_2O/ THF (4:1, v/v). After a total of 8h reaction time at room temperature, the transformation of keto ylide peptide resin **5a** to a

resin bound vicinal tricarbonyl peptide (**9a**) was found to be completed. The subsequent cleavage and deprotection step gave a compound that was shown to be identical with peptide **3a** (HPLC, MS, NMR).[6,8]

When peptide resin **5b** was treated with 95% TFA/ 5% H_2O solution, the desired peptide **7** (t_R=8.25 min, MALDI-TOF MS: m/z 836.8 $[M+H]^+$) was obtained in a 1:1 mixture with an accompanying side-product (t_R= 7.13 min) which showed an $[M+H]^+$ peak at *m/z* 736.8 Da in the MALDI-TOF MS. A TFA-mediated cleavage of the *tert*-butyl group and a subsequent decarboxylation step is consistent with the structure assigned to peptide **8**.

Furthermore, when peptide resin **5b** was first subjected to Oxone® oxidation and then followed by the cleavage reaction, a product was obtained consisting of two components in a 1:10 ratio with t_R= 4.23 min and 4.53 min as determined by HPLC analysis. Mass spectrometric analysis (MALDI-TOF) of the crude product revealed $[M+H]^+$ peaks at *m/z* 508.8, 534.4 and 552.9 Da with the positive-ion mode, $[M-H]^-$ peaks at *m/z* 531.8 and 507.0 Da with the negativ-ion mode. The major compound (t_R= 4.53 min) could be isolated by MPLC. The ^1H NMR spectrum (DMSO-d_6) was lacking the typical signal pattern of the phenyl tricarbonyl moiety. Due to the chemical shifts of the aromatic protons as well as due to the additional signal at 4.97 ppm (br.s) for a hydroxy-methylene proton, which shows a weak coupling with an exchangeable proton, we assigned a hydroxy-acetic acid substituent at the para position of phenylalanine. FAB MS (*m/z* 508 $[M+H]^+$) and ESI MS (*m/z* 506 $[M-H]^-$) are in agreement with proposed structure **10** (calc. for $C_{23}H_{33}N_5O_8$: 507.5Da).[10] For the minor compound (t_R= 4.23 min) the assigned structure **11** would be consistent with the MS data obtained (calc. for $C_{24}H_{31}N_5O_9$: 533.53Da, for the hydrate: 551.55Da).

In conclusion, we have synthesized peptides containing phenylalanine derivatives substituted at the para position with α,β-dioxopropionic acid moieties. In addition, the oxidative unmasking step of the phosphoranylidene intermediate to the desired vicinal tricarbonyl was performed in solution as well as on solid support. Besides the biological potential mentioned in the introduction, the synthetic potential of this compound class seems to be very promising. The synthesis of various natural products and their analogues starting from vicinal trioxo systems have already been demonstrated.[11] The formation of such an electrophilic centre on solid support may open up new opportunities for solid phase and eventuelly for combinatorial chemistry approaches as well.

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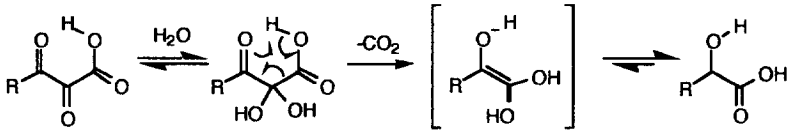
References and Notes

1. Fretz, H. *Tetrahedron Lett.* **1996**, foregoing paper.
2. Rink, H. *Tetrahedron Lett.* **1987**, *28*, 3787.
3. Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. *Tetrahedron Lett.* **1989**, *30*, 1927.
4. Kaiser, E. T.; Colescott, R.L.; Bossinger, C. D.; Cock, P. I. *Anal. Biochem.* **1970**, *54*, 595.
5. Wade, J. D.; Bedford, J.; Sheppard, R. C., Tregaer, G. W. *Peptide Research* **1991**, *4 (3)*, 194.
6. The purity of the peptides was verified by reversed-phase analytical HPLC on a Nucleosil C_{18} column (250x4 mm, 5 μm (AB), 100Å, from Macherey-Nagel): linear gradient of H_2O/ 0.1% TFA (eluent A) and acetonitrile/ 0.09% TFA (eluent B) from 2% to 100% B over 10 min; flow rate 1.5 ml/min, detection at 215 nm.
7. The crude peptide was purified by medium-pressure liquid chromatography (MPLC) using a C_{18} reversed phase column (Merck ®LICHROPREP RP-18, 15-25 μm bead diameter, reversed phase HPLC column material based on C_{18}-derivatized silicagel, Merck, Darmstadt, FRG; column length 46 cm, diameter 3.6 cm; flow rate 60 ml/min; detection at 215nm), eluted with an acetonitrile-water gradient containing 0.1% of TFA.
8. Data for **3a**: Analytical HPLC analyses revealed a single peak at t_R=4.89 min. NMR spectra were measured on a Varian Unity 500 K instrument. ^1H NMR (500MHz, DMSO-d_6, 25°C: 8.14 (d, 1H); 8.08 (d, 1H); 8.04 (d, 1H); 7.91 (d, 2H); 7.38 (d, 2H); 7.32 (s, 1H); 7.04 (s, 1H); 6.98 (s, 1H); 6.86 (s, 1H); 4.61 (m, 1H); 4.42 (dd, 1H); 4.10 (dd, 1H); 4.05 (q, 2H); 3.09 (dd, 1H); 2.75 (dd, 1H); 2.45 (m, 2H); 1.74 (m, 1H); 1.69 (s, 3H); 1.42 (m, 1H); 1.07 (m, 1H); 0.98 (t, 3H); 0.83 (d, 3H); 0.80 (t, 3H). ^{13}C NMR (126 MHz, DMSO-d_6, 25°C): 192.9 (C-3); 172.7; 171.7; 171.6; 170.4; 169.7; 169.2; 144.5; 130.9; 129.3; 129.2; 93.9 (C-2); 61.0; 57.4; 53.3; 49.4; 37.2; 36.6; 36.5; 24.3; 22.3; 15.3; 13.6, 11.2. Mass spectrometric analyses (MS): Electrospray ionisation (ESI; positive-ion mode): 562 $[M+H]^+$, 580 $[M+H+H_2O]^+$; ESI (negative-ion mode): 560 $[M-H]^-$; matrix-assisted laser-desorption ionization time-of-flight (MALDI-TOF; positive-ion mode): 562.6 $[M+H]^+$, 580.1 $[M+H+H_2O]^+$; MALDI-TOF (negative-ion mode): 560.1 $[M-H]^-$; (calc. for $C_{26}H_{35}N_5O_9P$: 561.59 Da; for the hydrate: 579.60 Da).

9. Dimethyldioxirane (DMD) has been proven to be an effective reagent for the selective conversion of phosphoranylidene intermediates to the corresponding vicinal tricarbonyls: see referenc 11. Its applicability as reagent for oxidations on solid support is currently under investigation. In a preliminary experiment, the peptide resin **5a** was treated with a ten-fold excess of DMD in acetone (ca. 0.08M) for 2h at room temperature and then washed with acetone, DMA and CH_2Cl_2. The subsequent cleavage and deprotection step by means of 95% TFA/ 5% H_2O (v/v) gave a compound that was shown to be identical with peptide **3a** (HPLC, MS).
10. The rearrangement and cleavage of α,β-dioxopropionic acid derivatives under alcaline conditions was investigated earlier: Rodé-Gowal, H.; Dao, H. L.; Dahn, H. *Helv. Chim. Acta* **1974**, *57*, 240.
For the decarboxylation under acidic conditions we propose the following mechanism:



"hydrate"

The proposed mechanism is consistent with the product isolated.
11. Wasserman, H. H.; Baldino, C. M.; Coats, S. J. *J. Org. Chem.* **1995**, *60*, 8231 and references cited therein.

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