Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 1443–1446

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Phosphofurylalanine, a Stable Analog of Phosphohistidine

Caroline Schenkels, Bernhard Erni* and Jean-Louis Reymond*

Department of Chemistry & Biochemistry, University of Bern, Freiestrasse 3, 3012 Bern, Switzerland

Received 13 January 1999; accepted 9 April 1999

Abstract: *Phosphorylated histidine residues occur in a number of signal-transduction pathways in bacteria as well as in eukaryotes. Phosphohistidine is hydrolytically labile and therefore difficult to study, this by contrast to stable phosphoserine, phosphothreonine or phosphotyrosine. Here we report the design and enantioselective synthesis of (4'-phospho-2'-furyl)alanine* ***1****, a non-hydrolyzable analog of 1-phosphohistidine. This novel amino-acid should be useful to synthesize peptides incorporating a stable analog phosphohistidine.*

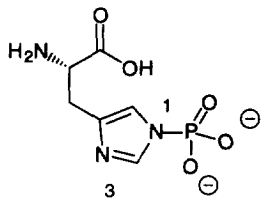
Keywords: Amino acids and derivatives / Phosphopeptides / Phosphorylation / Mimetics

Signal transduction pathways involving series of phosphorylated proteins are central in modulating gene expression in response to environmental and chemical signals.[1] Signal transduction pathways involving phosphoserine, phosphothreonine and phosphotyrosine intermediates have been extensively studied. These investigations have been greatly facilitated by the fact that these phosphorylated intermediates are hydrolytically relatively stable. This stability has allowed not only the isolation of many phosphorylated proteins, but also the production of monoclonal antibodies that selectively recognize these phosphorylated residues specifically. In the last few years there has been a growing awareness that some phosphorylation pathways involve much less stable intermediates such as phosphohistidine, phosphoaspartate, phospholysine, phosphoarginine and phosphocysteine. These intermediates can remain largely undetected due to their hydrolytic lability.[2]

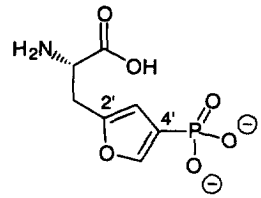
In relation with our interest in bacterial phosphotransferase systems,[3] we have become interested in studying the mode of action of peptides that become phosphorylated at histidine.[4] To achieve this goal, we need a hydrolytically stable analog of phosphohistidine. In vivo histidine residues may be phosphorylated to either 1- or 3-phosphohistidine, but 3-phosphohistidine rearranges spontaneously to 1-phosphohistidine.[5] For mimicking the latter, we substituted simultaneously a carbon atom for the phosphorylated nitrogen atom N_1 and an oxygen atom for the second heterocyclic nitrogen N_3, giving (4'-phospho-2'-furyl)alanine **1**. In this compound a stable phosphorus-carbon bond replaces the hydrolytically labile phosphorus-nitrogen bond of 1-phosphohistidine.

* e-mail: jean-louis.reymond@ioc.unibe.ch; Fax: ++41 31 631 80 57

0960-894X/99/$ - see front matter
PII: S0960-894X(99)00209-7

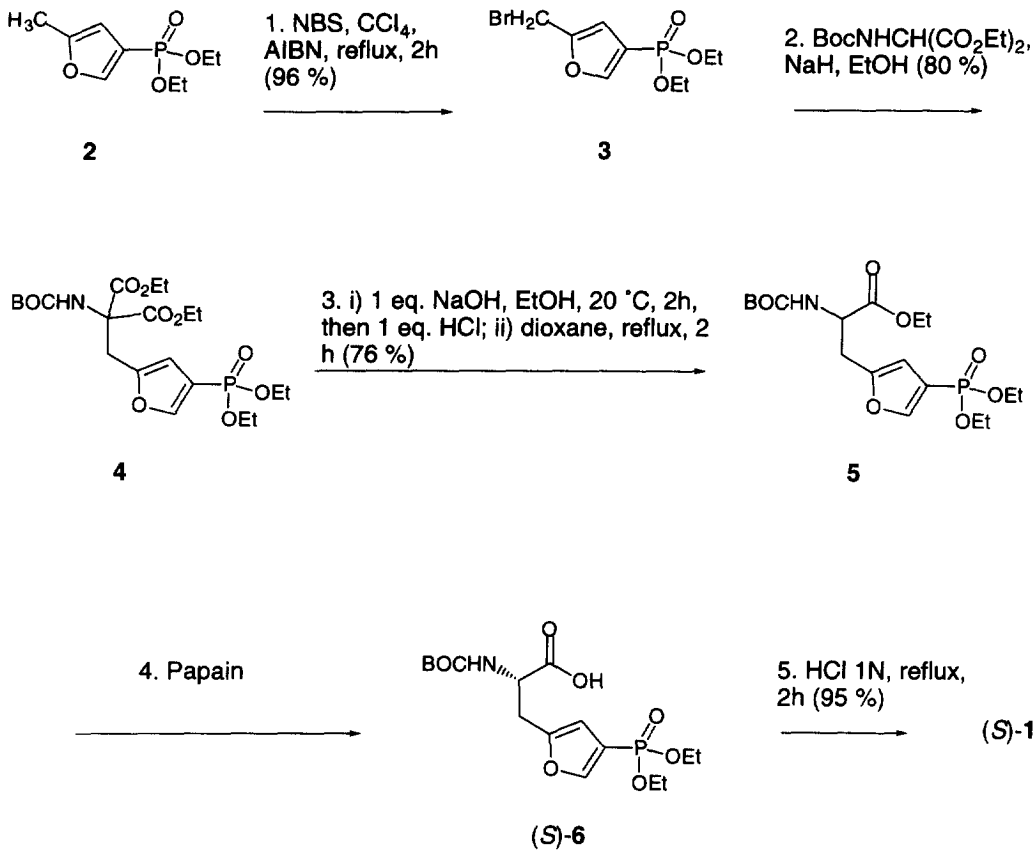


1-phosphohistidine



(4'-phospho-2'-furyl)alanine **1**

(4'-phospho-2'-furyl)alanine **1** was synthesized from furane **2**, which is accessible in four steps from triethyl phosphite, methyl vinyl ketone and ethyl formiate using a recently reported procedure.[6] Bromination gave the sensitive bromide **3**, which was alkylated with BOC-protected diethylaminomalonate to give **4**.[7] Selective saponification of one of the carboxylic ester functions followed by acidic decarboxylation gave **5**.



2 **3** **4** **5**

(*S*)-**6**

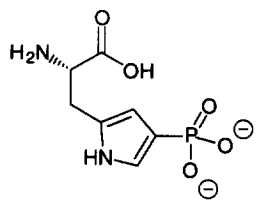
Kinetic resolution of **5** was carried out enzymatically (table 1). Kinetic resolution was achieved using the enzyme papain, which is known to hydrolyze selectively L-configured BOC-protected amino-acid esters.[8] The unreacted ester (*R*)-**5** was recovered by extraction with ethyl acetate.[9] BOC-protected amino-acid (*S*)-**6** was then separated from the enzyme by dialysis of the reaction mixture, acidification and extraction.[10] This procedure allowed to efficiently recycle the enzyme of several reaction cycles.[11] Finally complete acidic hydrolysis of either (*S*)-**6** or (*R*)-**5** gave the fully deprotected amino-acids (*S*)-**1** and (*R*)-**1**.[12]

Table 1. Kinetic resolution of racemic (4'-phospho-2'-furyl)-alanine ethyl ester **5**.

Enzyme[a]	time	%*ee* unreacted (*R*)-**5**[b]	isol. yield of (*S*)-**6**	%ee (*S*)-**6**[c]
α–chymotrypsine, 375 U.mL^{-1}	72 h	0	0	-
pig liver esterase, 240 U.mL^{-1}	48 h	70	30	60
horse liver esterase, 3 U.mL^{-1}	48 h	60	29	50
papain, 170 U.mL^{-1}	24 h	>96[d]	40	75

[a] Assay conditions: 1 mL 100 mM aq. phosphate, pH 7.5, 10 mg/mL **5**, 10 % v/v ethanol, 37 °C. Enzymes were from Sigma or Fluka. The pH was maintained by periodical addition of 2N NaOH. [b] The reaction was followed by HPLC using a chiral column (chiralpak AS from Daicel, 0.45 x 22 cm, 1 mL.min^{-1} hexane/isopropanol 9:1, t_R((*R*)-**5**) = 15.3 min, t_R((*S*)-**5**) = 17.7 min). [c] The enantiomeric purity of **6** was established by comparison of its optical rotation with its enantiomer (*R*)-**6** obtained by saponification of unreacted **5** from the papain reaction (LiOH, THF/H_2O, 25 °C, 3h, 100 %).[d] No remaining (*S*)-**5** detected by chiral phase HPLC.

Molecular modeling shows that 1-phosphohistidine and (4'-phospho-2'-furyl)alanine **1** are almost exactly superimposable and display essentially identical electrostatic potential surfaces. The oxygen atom of the furan replaces the nitrogen atom in position 3 of phosphohistidine and similarly displays an in plane sp^2 lone pair with hydrogen-bond acceptor properties. It should be noted that replacing only nitrogen 1 by a carbon would give a phosphopyrrole compound with an N-H group featuring hydrogen bond donor properties.



phosphopyrrole

In summary we have reported the design and enantioselective synthesis of phosphofurylalanine, a novel amino-acid that is a stable analog of 1-phosphohistidine. BOC-protected acid **5** can be used in peptide synthesis. Peptides incorporating phosphofurylalanine in place of histidine should greatly facilitate the study of phosphohistidine signal transduction pathway.

Acknowledgment. This work was supported by the University of Bern, the Swiss National Science Foundation, the Koordinationsgruppe für Forschungsfragen der basler chemischen Industrie (KGF) and the Wander Stiftung.

References and Footnotes

[1] Hafen, E. *Science* **1998**, *280*, 1212 and ref. cited therein.
[2] Matthews, H. R. *Pharmacol. Ther.* **1995**, *67*, 323.
[3] a) Potsma, P. W.; Lengeler, J. W.; Jacobson, G. R. *Microbiol. Rev.* **1993**, *57*, 543; b) McEvoy, M. M.; Dahlquist, F. W. *Curr. Opin. Struct. Biol.* **1997**, *7*, 793.
[4] Mukhija, S.; Erni, B. *Mol. Microbiol.* **1997**, *25*, 1159.
[5] Hultquist, D.E.; Moyer, R.W.; Boyer, P.D. *Biochemistry* **1966**, *5*, 322-331.
[6] Truel, I.; Mohamed-Hachi, A.; About-Jaudet, E.; Colligoron, N. *Syn. Commun.* **1997**, *27*, 1165.
[7] Schneider, H.; Sigmund, G.; Schricker, B.; Thirring, K.; Berner, H. *J. Org. Chem.* **1993**, *58*, 683.
[8] a) Schricker, B.; Thirring, K.; Berner, H. *Bioorg. Med. Chem. Lett.* **1992**, *5*, 387; b) Druckhammer, D.G.; Barbas III, C.F.; Nozaki, K.; Wong, C.-H. *J. Org. Chem.* **1988**, *53*, 1607; c) Cantacuzene, D.; Pascal, F.; Guerreiro, C. *Tetrahedron* **1987**, *43*, 1823.

[9] Data for (*R*)-**5**: ^{1}H-NMR (300 MHz, $CDCl_3$): 7.71 (s, 1H, H-C(5')), 6.25 (s, 1H, H-C(3')), 5.12 (d, J = 8 Hz, 1H, NH), 4.55-4.50 (dt, 1H, H-C(α)), 4.20-4.12 (m, 2H, CH_3-CH_2O_2C), 4.10-4.0 (m, 4H, $(CH_3CH_2O)_2PO$), 3.15-3.12 (m, 2H, H_2-C(β)), 1.40 (s, 9H, $C(CH_3)_3$), 1.33-1.27 (t, J = 7Hz, 6H, $(CH_3CH_2O)_2PO$), 1.26-1.22 (t, J = 5Hz, 3H, $CH_3CH_2O_2C$). ^{13}C-NMR (50 MHz, $CDCl_3$) 171.10 (CO (Boc)), 153.04 (COOH), 149.22 (C(2')), 148.73 (C(5')), 108.96 (C(4')), 108.73 (C(3')), 80.16 ($C(CH_3)_3$), 62.69 ($(CH_3CH_2O)_2PO$), 61.69 ($CH_3CH_2O_2C$), 52.45 (C(β)H_2), 30.92 (C(α)H), 28.28 ($C(CH_3)_3$), 16.36 (CH_3CH_2OP), 14.11 ($CH_3CH_2O_2C$). $[\alpha]_D^{25} = -19°$ (c = 0.8, $CHCl_3$). FABMS: 364.2 (MH^+ -56).

[10] Preparative procedure: a solution of racemic **5** (1.78 g, 4.25 mmol) and papain (100 mg, 400 U/mmol substrate) in aqueous phosphate buffer (20 mL, 100 mM, pH = 7.5) and ethanol (1 mL) was vigorously stirred during 16 h at 37°C. The pH was maintained to 7.5- 8 by periodical addition of 2N NaOH (total added: 1mL). Extraction of the reaction mixture with ethyl acetate gave (*R*)-**5** (830 mg, 50 %). The product was separated from the enzyme by dialysis into 2 x 500 mL aq. phosphate pH 7.5. The enzyme solution was then used as such for the next cycle. Acidification of the dialysate to pH = 2 with H_3PO_4 and extraction with ethyl acetate (2 x 400 mL) gave, after drying (Na_2SO_4) and evaporation, (*S*)-**6** (665 mg, 40 %) as a yellow oil.

Data for (*S*)-**6**: ^{1}H-NMR (300 MHz, $CDCl_3$): 7.79 (s, 1H, H-C(5')), 6.22 (s, 1H, H-C(3')), 5.29-5.26 (d, J = 8Hz, 1H, NH), 4.54-4.49 (dt, 1H, H-C(α)), 4.12-3.99 (m, 4H, (CH_3-CH_2)$_2$OP), 3.24-3.19 (m, 2H, H_2C(β)), 1.37 (s, 9H, $C(CH_3)_3$), 1.31-1.26 (t, J = 7 Hz, 6H, $(CH_3CH_2)_2OP$). ^{13}C-NMR (50 MHz, $CDCl_3$): 173.34 (CO (Boc)), 155.80 (COOH), 150.73 (C(2')), 150.39 (C(5')), 109.31(C(4')), 109.15 (C(3')), 80.55 ($C(CH_3)_3$), 63.33 (CH_3CH_2OP), 52.76 (C(β)H_2), 31.09 (C(α)H), 28.88 ($C(CH_3)_3$), 16.84 (CH_3CH_2OP). $[\alpha]_D^{25} = +34°$ (c = 0.6, $CHCl_3$). FABMS 336.1 (MH^+ -56).

[11] Reymond, J.-L.; Reber, J.-L.; Lerner, R. A. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 475.

[12] Data for (*S*)-**1** (as HCl salt): ^{1}H-NMR (300 MHz, D_2O): 7.64 (d, 1H, H-C(5')), 6.38 (d, 1H, H-C(3')), 4.29-4.25 (t, J = 5.5Hz, 1H, H-C(α)), 3.30-3.28 (d, J = 5.5Hz, 2H, H-C(β)). ^{13}C-NMR (75 MHz, D_2O) 152.13 (COOH), 150.07 (C(2')), 149.75 (C(5')), 112.66 (C(4')), 112.51 (C(3')), 54.45 (C(β)H_2), 30.52 (C(α)H). FABMS: 236 (MH^+); $[\alpha]_D = -16°$ (c = 0.1, H_2O).