Studies on Organophosphorus Compounds; LXXIII: Synthesis of Phosphonopeptides by an in situ Active Ester Method

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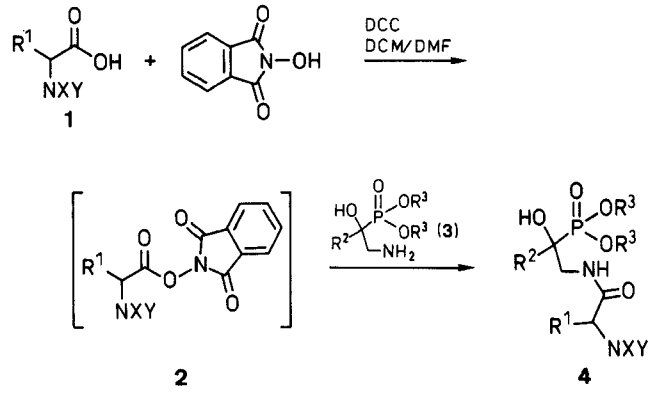
An active ester prepared in situ from an *N*-protected amino acid and *N*-hydroxyphthalimide was successfully employed in the synthesis of phosphonopeptides bearing a free hydroxy group.

Since the discovery of the antibacterial activity of synthetic phosphonopeptides such as alaphospholin,[1] increasing attention has been paid to the synthesis of this type of biologically interesting peptides.[2-10] Among various coupling procedures reported for peptide bond formation,[2,3,11,12] the mixed carboxylic–carbonic acid anhydride method (MCCA) is the most popular.[13] Unfortunately, MCCA is unsuitable for the synthesis of phosphonopeptides bearing a free hydroxy function. Active esters of *N*-protected amino acids are widely used in peptide synthesis. Efforts are still reported for new and effective active esters in peptidic amide bond formation[14-17] but, as far as we are aware, with only limited success for phosphonopeptide formation. Herein we wish to report the use of active esters **2** prepared in situ from *N*-hydroxyphthalimide (HONPht) and *N*-protected amino acid **1** in the synthesis of phosphonopeptides. Since 1-aminoalkylphosphonates, the building block of 1-aminophosphonopeptides, could be conveniently prepared via a facile, three-component condensation reaction followed by selective deprotection,[18,19] it is convenient to synthesize 1-aminophosphonopeptide through this active ester.[20] On the other hand, 2-amino-1-hydroxyethylphosphonic acid (HAEP) is the fifth C–P compound found in nature.[21] The 2-alkyl substituted HAEP and its peptide derivatives were also reported as having potent biological activity, e.g. as an inhibitor of renin.[22,23] We have previously reported the preparation of the 1-substituted derivatives of HAEP, dialkyl 1-alkyl-2-amino-1-hydroxyethylphosphonate tosylates, by an improved "one-pot" catalytic hydrogenation procedure to prevent the alkali labile free amino compounds, namely, dialkyl 1-alkyl-2-amino-1-hydroxyethylphosphonates **3**, from decomposing.[24] Now we report the use of in situ active ester formed by reaction of HONPht and **1** in the synthesis of the new type of phosphonopeptides containing a 1-alkyl-substituted HAEP unit.

It should be noted that if the tosylate of **3** and a tertiary amine were used instead of the free amino ester in the coupling process, the yield of peptide was significantly decreased. In this particular case, apart from its role in accelerating the coupling process via an active ester, HONPht also acted as an acid to prevent the alkali labile amino ester **3** from decomposing.[24] Meanwhile, HONPht can be used as a self-indicator in the coupling process since the color of this reagent in solution is different in acidic and basic conditions.

A similar result was obtained when an isolated active ester, e.g. **2e**, was employed in the coupling procedure.



1

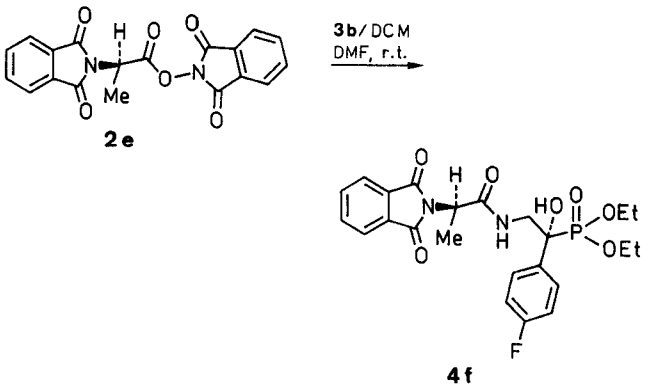
2

4

1, 2	X, Y	R^1
a	Boc, H	H
b	Cbz, H	H
c	Cbz, H	Bn(L)
d	Pht	Bn(L)
e	Pht	Me(D)

3	R^2	R^3
a	Ph	Me
b	*p*-FC_6H_4	Et
c	*m*-FC_6H_4	Et

4	X, Y	R^1	R^2	R^3
a	Boc, H	H	Ph	Me
b	Cbz, H	H	Ph	Me
c	Cbz, H	Bn(L)	*p*-FC_6H_4	Et
d	Cbz, H	Bn(L)	*m*-FC_6H_4	Et
e	Pht	Bn(L)	Ph	Me
f	Pht	Me(D)	*p*-FC_6H_4	Et
g	Pht	Bn(L)	*m*-FC_6H_4	Et



2e

4f

The free peptides **5** could be obtained in good yield after deprotection of the phosphonopeptide **4** in the usual manner.

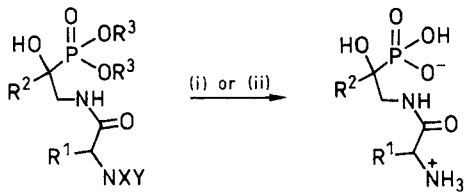
Table 1. Compounds **4** Prepared[a]

Product	Yield (%)	mp (°C)	Mol. Form (Mol. Weight)	$[\alpha]_D^{20}$ (c%, sol.)	^{31}P NMR
4a	88	157–158	$C_{17}H_{27}N_2O_7P$ (402.4)		
4b	85	167–169	$C_{20}H_{25}N_2O_7P$ (436.4)		
4c	53	104–106	$C_{29}H_{34}FN_2O_7P$ (572.6)	−17.9 (1.0, EtOH)	20.65, 20.75
4d	70	99–101	$C_{29}H_{34}FN_2O_7P$ (572.6)	−17.1 (0.11, EtOH)	
4e	89	152–154	$C_{27}H_{27}N_2O_7P$ (522.5)	−85.4 (0.24, $CHCl_3$)	
4f	81	96–98	$C_{23}H_{26}FN_2O_7P$ (492.5)		20.53, 20.63
4g	71	112–114	$C_{29}H_{30}FN_2O_7P$ (568.6)	−82.1 (0.38, $CHCl_3$)	

[a] Compounds **2e**, **4a–g** and **5a–d** gave C, H, N ± 0.4 except **5b–d** C ± 0.5.

Table 2. ^{1}H NMR and IR Spectral Data of Compounds **4**

	^{1}H NMR δ, J (Hz)	IR, ν (cm^{-1}) O–H, N–H, P=O, P–O–C
4a	1.3 (s, 9H), 2.6 (s, 1H, OH), 3.2–3.9 (m, 10H, $2NCH_2 + 2CH_3O$), 4.4 (br, 2H, 2NH), 6.9–7.6 (m, 5H)	3422, 3254, 1202, 1031
4b	2.7 (s, 1H, OH), 3.3–3.8 (m, 10H), 4.3–4.6 (br, 2H), 5.1 (s, 2H), 7.4 (m, 10H)	3600–3200, 1200, 1020
4c	1.0–1.4 (m, 6H), 3.0 (d, 2H, 7), 3.6–4.5 (m, 7H, $2CH_2O + CH_2CP + OH$), 5.0 (s, 2H), 5.5 (t, 1H), 6.8–7.9 (m, 16H)	3650–3100, 1220, 1030
4d	1.0–1.3 (m, 6H), 2.9 (d, 2H), 3.7–4.4 (m, 7H), 5.0 (s, 2H), 5.1 (t, 1H, 8), 6.7 (br, 1H), 6.9–7.4 (m, 15H)	3600–3100, 1230, 1030
4e	2.9 (d, 2H, 7), 3.4 (d, 6H, 11), 3.7 (d, 2H, 5, 11), 3.6–4.1 (br, 1H, OH), 5.5 (t, 1H, 7), 7.0–7.4 (m, 11H), 7.4–8.0 (m, 4H)	3700–3000, 1220, 1030
4f	1.0–1.8 (m, 9H), 2.1 (s, 1H, OH), 3.6–4.4 (m, 5H), 4.6–5.2 (m, 2H), 6.8–7.3 (m, 4H), 7.4–7.7 (m, 1H, NH), 7.8 (s, 4H)	3650–3100, 1225, 1020
4g	0.9–1.4 (m, 6H), 2.0 (s, 1H), 3.4 (d, 2H), 3.6–4.8 (m, 4H), 4.8 (dd, 2H, 8, 15), 5.0 (t, 1H, 7), 6.81 (t, 1H, 7, NH), 7.0–7.4 (m, 9H), 7.7 (s, 4H)	3700–3010, 1240, 1035



4 **5**

5	R^1	R^2
a	H	*m*-FC_6H_4
b	Me(D)	*p*-FC_6H_4
c	Bn(L)	*m*-FC_6H_4
d	Bn(L)	*p*-FC_6H_4

Conditions: (i) 41% HBr/AcOH, r.t., 24h (where X = Cbz or Boc, Y = H); (ii) (a) $N_2H_4 \cdot H_2O$, DMF, 50°C; (b) TMSBr, $CHCl_3$, r.t. 12h (where X, Y = pht)

Melting points were uncorrected. ^{1}H NMR spectra were recorded on a Varian EM-360L or an XL-200 spectrometer with $CDCl_3$ as solvent for the protected peptides and D_2O/NaOD for the free peptides. TMS was used as the standard in every case. ^{31}P NMR spectra were taken on a Varian FX-90Q spectrophotometer employing 85% H_3PO_4 as the external standard. IR spectra were obtained on a Shimaduzu IR-440 or a Perkin-Elmer 983G spectrometer. Optical rotations were obtained on a Perkin-Elmer 241MC polarimeter. HONPht was prepared from *N*-carboethoxyphthalimide and hydroxylamine according to ref. 25. DCC and TMSBr were Fluka reagents, Boc and Cbz masked amino acids were obtained from the Shanghai Institute of Biochemistry, Chinese Academy of Sciences, and were used as received. The Pht-protected amino acids were prepared from the corresponding amino acids and phthaloyl anhydride by conventional methods. Dialkyl 1-alkyl-2-amino-1-hydroxyethylphosphonates **3** were prepared from their tosylates[24] in the following manner: The tosylate (1.2 mmol) was shaken with aq K_2CO_3 (10%, 5 mL) for several min and rapidly extracted with CH_2Cl_2 (3 × 5 mL). The extracts were combined and dried (Na_2SO_4). This dried solution was recommended for use immediately in the coupling process, otherwise decomposition of **3** would occur during long periods of storage.

Peptides of Dialkyl 1-Alkyl-1-hydroxy-2-(Acylamino)ethylphosphonates (4); General Procedure:

DCC (0.22 g, 1.1 mmol) was added to a stirred solution of **1** (1 mmol) and HONPht (0.18 g, 1.1 mmol) in CH_2Cl_2 (10 mL) and DMF (2 mL) at r.t. After being stirred for 30 min, a solution of **3** (ca. 1 mmol) in CH_2Cl_2 (15 mL) was added and the stirring was continued for another 12 .

The mixture was then concentrated to a minimum volume on a rotory evaporator and the residue was triturated with EtOAc (20 mL), the precipitated dicyclohexylurea was filtered off and the filtrate was washed successively with sat. aq K_2CO_3 (5 × 6 mL), H_2O (2 × 15 mL), 5% citric acid (15 mL), H_2O (15 mL) and brine (15 mL), and dried (Na_2SO_4). After removal of the solvent, the products were isolated either by recrystallization from EtOAc/petroleum ether or by column chromatography on silica gel (200–300 mesh) using EtOAc/hexane (1 : 1) as eluents. The yields, physical and spectroscopic data of **4** are listed in Tables 1 and 2.

Preparation and Isolation of Pht-D-Ala-*O*-NPht (2e):

Pht-D-Ala–OH (0.3 g, 1.4 mmol) was dissolved in a mixture of CH_2Cl_2 (10 mL) and DMF (2 mL), and HONPht (0.25 g, 1.5 mmol) and DCC (0.29 g, 1.4 mmol) were then added. After being stirred for 12 h, the mixture was filtered and the filtrate was concentrated. The residue was triturated with EtOAc/petroleum ether (1 : 4 by volume) and the resultant solid was collected; after being dried, 0.38 g of crude **2e** was obtained (80% yield), which was recrystallized from EtOAc, as white powder, mp 199–200°C.

IR (KCl): ν = 1820, 1790, 1740, 1050 cm^{-1}.

^{1}H NMR ($CDCl_3$/TMS): δ = 1.9 (d, 3 H, J = 7.2 Hz), 5.45 (q, 1 H, J = 7.2 Hz), 7.9 (s, 8 H) ppm.

MS (EI, 70 eV): m/z = 365 (M + 1).

Reaction of 2e with 3b:

Compound **3b** (ca. 0.6 mmol) in CH_2Cl_2 (5 mL) was added to a stirred solution of **2e** (0.2 g, 0.55 mmol) in CH_2Cl_2 (5 mL) and DMF (1 mL) at r.t. After 12 h stirring, and workup, **4f** was obtained in 82% yield.

1-(*m*-Fluorophenyl)-2-(glycylamino)-1-hydroxyethylphosphonic Acid (5a):

A mixture of Cbz–Gly–OH (0.21 g, 1 mmol), DCC (0.23 g, 1.1 mmol) and HONPht (0.18 g, 1.1 mmol) in CH_2Cl_2 (5 mL) and DMF (1 mL) were stirred at r.t. for 30 min. A solution of **3c** (ca. 1 mmol) in CH_2Cl_2 (15 mL) was then introduced into the reaction mixture and the stirring was continued at r.t. for 12 h. After workup, the crude protected phosphonodipeptide was obtained as a semi-solid which was treated with 41% HBr/AcOH (10 mL) at r.t. for 24 h. The volatile components were then removed under reduced pressure at a temperature below 70 °C, the residue was dissolved in EtOH (5 mL), and propylene oxide was added dropwise with stirring. The precipitated white solid was collected and recrystallized from H_2O/EtOH. Compound **5a** was obtained as white crystals, 0.23 g (85% yield), mp 250–251 °C (dec.).

IR (KCl): ν = 3365, 3120, 1170, 1020 cm^{-1}.

^{1}H NMR: δ = 3.1 (s, 2 H), 3.8 (AB, 1 H, J = 4.3, 13.7 Hz), 3.9 (AB, 1 H, J = 5.4, 14.4 Hz), 7.0–7.3 (m, 4 H).

2-(D)-(Alanylamino)-1-hydroxy-1-(*p*-fluorophenyl)ethylphosphonic Acid (5b):

An ethanolic solution of **4f** (0.2 g, 0.4 mmol) was refluxed with $N_2H_4 \cdot H_2O$ (10% ethanolic solution, 0.4 mL) for 4 h. After being cooled to r.t., the precipitate was filtered off, and the filtrate was concentrated, the residue was dissolved in dry and EtOH-free $CHCl_3$ (5 mL). TMSBr (1.53 g, 10 mmol) was then introduced via syringe under an atmosphere of dry N_2. After 12 h stirring at r.t., the volatile components were removed under reduced pressure, the residue was stirred with MeOH (5 mL) for 0.5 h, and propylene oxide was added dropwise with stirring until precipitiation occurred. After several days storage in a refrigerator, the product was collected by suction and recrystallized from H_2O–EtOH. **6b** was obtained as shiny crystals in 66% yield, mp 208–210 °C (dec.).

IR (KCl): ν = 3700–3200 (O–H, N–H), 1160 (P=O), 1040 (P–O–H) cm^{-1}.

^{1}H NMR: δ = 0.7–1.2 (m, 3 H, CH_3), 3.0–3.7 (m, 3 H, CHMe + CH_2N), 3.8–4.2 (m, 1 H, CHP), 7.0 (t, 2 H_{aryl}), 7.4 (m, 2 H_{aryl}).

1-(*m*-Fluorophenyl)-1-hydroxy-2-(L)-(phenylalanylamino)ethylphosphonic Acid (5c):

4d (0.19 g, 0.51 mmol) was treated with 41% HBr/AcOH (10 mL) at r.t. for 24 h and the volatile components were removed on a rotory evaporator with heating on an oil bath (70 °C). The resultant semi-solid was dissolved in EtOH (4 mL) with stirring and propylene oxide was then added dropwise until pH 6 was reached. The precipitated product was kept at 0 °C in a refrigerator overnight and then collected. After being recrystallized from H_2O–EtOH, 0.12 g of **5c** was obtained as an amorphous powder, yield 77%, mp 234–234.5 °C. $[\alpha]_D^{20}$ = −44.50 (0.4% in 0.71 N NaOH).

IR (KCl): ν = 3650–2000 (O–H, N–H), 1160 (P=O), 1040 (P–O–H) cm^{-1}.

^{1}H NMR: δ = 2.5 (d, 1 H, J = 2 Hz), 2.6 (s, 1 H), 3.4–3.8 (m, 2 H), 4.1 (dd, 1 H, J = 3.6, 14.4 Hz), 6.9–7.1 (m, 3 H), 7.2–7.4 (m, 6 H).

^{31}P NMR (D_2O/NaOD): 16.72, 19.26.

1-(*p*-Fluorophenyl)-1-hydroxy-2-(L)-(phenylalanylamino)ethylphosphonic Acid (5d):

Prepared from **4c** as described for **5c** with 59% yield; mp 210–211 °C; $[\alpha]_D^{20}$ = −42.00 (0.1% in 0.71 N NaOH).

IR (KCl): ν = 3650–2000 (O–H, N–H), 1160 (P=O), 1040 (P–O–H) cm^{-1}.

^{1}H NMR: δ = 2.5 (d, 2 H, 5.4), 3.3–3.5 (m, 2 H, CH_2N), 3.8–4.2 (m, 1 H, CHP), 6.8–7.5 (m, 9 H).

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