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Journal of Molecular Structure 738 (2005) 179–182
Journal of MOLECULAR STRUCTURE
www.elsevier.com/locate/molstruc

Structural characterization of *N*-phosphoryl branched peptides

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Received 26 August 2004; revised 2 December 2004; accepted 2 December 2004
Available online 28 January 2005

Abstract

The structural characteristics of *N*-phosphoryl branched peptides **2–5** in solution were investigated by NMR spectroscopy. We observed diastereotopic phenomena, interactions between hydrogen bonds and lack of regular secondary structure in these peptides. We also conducted conformational analysis on the amino acids binding phosphoryl groups in compounds **2–5**.

Keywords: *N*-phosphoryl branched peptide; Conformational analysis; NMR

1. Introduction

It is well established that many *N*-phosphoryl peptides and their analogues are biologically active and have the potential of becoming antineoplastic drugs. For example, (*N*-[(α-rh-aminopyranosyloxy)hydroxyphosphinyl]-L-Leu-L-Trp) and its analogues are inhibitors of endothelin converting enzyme. They can be used to treat a variety of diseases including hypertension and acute renal failure or cerebral vasospasm caused by overproduction of endothelin [1–3]. It is also worthwhile to note that the dendritic branched peptides containing L-lysine or its oligomers as matrix of their backbones are of pharmaceutical interests. These compounds are widely used in the development of immunodiagnosticreagents [4–6]. We have synthesized a series of *N*-phosphoryl branched peptides **2–5**. ^{31}P-, ^{1}H-, ^{13}C-, and 2D-NMR spectroscopy, MS, and elemental analysis were employed to confirm the structures of these peptides. Results from cell biological tests indicated that these compounds have anti-virus activities [7]. Because of the importance of molecular conformation in determining biological activities, it is of great interest to investigate the structural characteristics of these compounds. In this report, we carried out NMR studies on *N*-phosphoryl branched peptides to gain insights on the structure–activity relationship of these class of compounds.

2. Experimental procedures

Compound **1** was prepared using literature procedures [8].

Compounds **2–5** were synthesized according to the published methods [7].

All the NMR experiments were carried out on either a Bruker AC-200p or a Bruker AM-500 PFT-NMR spectrometer. ^{1}H-and ^{13}C-NMR chemical shifts are referred to TMS and $CDCl_3$, respectively. ^{31}P-NMR spectra were obtained using 85% phosphoric acid as external reference. Measurements were generally performed at room temperature. Variable temperature NMR experiments were performed between 20 and 60 °C using glycol as temperature calibrator.

3. Results and discussion

The syntheses of *N*-phosphoryl branched peptides **2–5** were accomplished by coupling *N*-phosphoryl amino acids

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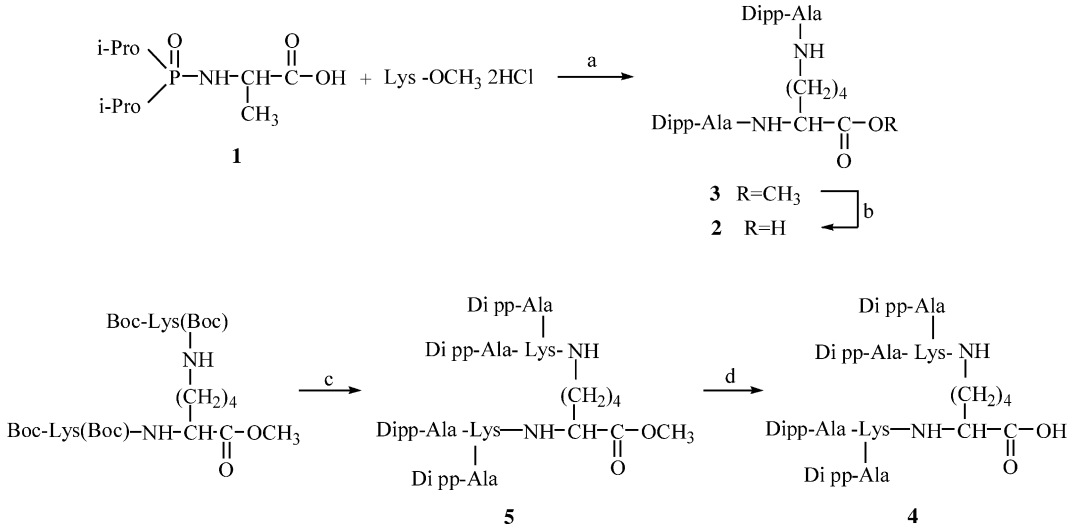
doi:10.1016/j.molstruc.2004.12.003



Scheme 1. Synthetic route of *N*-phosphoryl branched peptides **2–5**. Reagents and conditions: (a) *N*, *N*-dicyclohexylcarbodiimide (DCC), 1-hydroxybenzotriazole (HOBt), *N*-methylmorpholine (NMM), THF, r. t. overnight, 77%; (b) i. 1 mol/L NaOH, r. t. 2 h., ii. HCl, 72%; (c) i. trifluoroacetic acid, CH_2Cl_2, r. t. 1 h., ii. Dipp-Ala, NMM, DCC, HOBt, THF, r. t., overnight, 79%; (d) i. 1 mol/L NaOH, r. t. 5 h., ii. HCl, 66%.

to L-Lysine methyl ester [7]. A brief outline of the syntheses is shown in Scheme 1.

3.1. Diastereotopic phenomena

The phosphorus atom in *N*-phosphoryl branched peptides is a diastereotopic center, thus diastereotopic phenomena were observed in the NMR spectra of compounds **2–5**. The two $-CH_3$'s on the isopropyl group of the diisopropyl phosphoryl alanine **1** (viz. Dipp-Ala) are not chemical equivalents on NMR scale; the chemical shift difference ($\Delta\delta$) between their absorptions is evident. For instance, $\Delta\delta_H$ is 0.02 ppm while $\Delta\delta_c$ is 0.09 ppm in compound **3** (see Table 1). Since all the *N*-phosphoryl branched peptides in this work are diastereisomers, their ^{31}P-NMR appeared as doublets at around 7.00 ppm. The chemical shift difference of the two signals in the doublet ($\Delta\delta_P$) of compound **1** is only 0.02 ppm. However, the $\Delta\delta_P$ values are increased to 0.11–0.14 ppm in the spectra of compounds **2–5**. It is observed that the existence of branched peptide chain affect the diastereotopic phenomena. On the other hand, the ^{1}H-and ^{13}C-NMR resonance signals from the Lysine in the *N*-phosphoryl group are almost identical to those of the un-phosphorylated L-lysine. It is obvious that the phosphoryl group only affects amino acid bound to the phosphoryl group.

3.2. The conformation of amino acids binding phosphoryl groups

The chemical shifts of the ^{1}H-and ^{13}C-NMR resonance signals from the Dipp-Ala in the *N*-phosphoryl branched peptides **2–5** are very similar to those of the *N*-phosphoryl alanine **1**. However, there are obvious differences in the coupling constants. $^3J_{P-C}$ and $^2J_{P-C}$, the observed coupling constants from the ^{13}C-NMR

Table 1
The chemical shifts of Dipp-Ala from compounds **2–5** in $CDCl_3$ (ppm)

Compound	ipro-CH_3		ipro-CH		α-CH		β-CH_3	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	1.31	23.56	4.54	71.18	3.03	49.66	1.44	20.76
	1.33	23.65	4.60	71.46				
2	1.29	23.64	4.57	71.13	3.75	50.23	1.37	20.36
	1.30	23.64	4.58	71.41		51.94	1.39	20.49
3	1.31	23.63	4.57	71.28	3.72	50.91	1.40	20.35
	1.33	23.72	4.58	71.56		51.29	1.41	20.44
4	1.26–1.32	23.83	4.58	71.45, 71.45	3.76	50.80, 50.80	1.38	20.60, 20.60
		23.90		71.90, 71.90		51.88, 51.88		20.60, 20.60
5	1.27–1.32	23.56	4.59	70.54, 70.54	3.75	50.77, 50.77	1.38	20.40, 20.75
		23.61		71.04, 71.04	3.81	51.14, 51.14		20.67, 20.75