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1,4-Phenylene-di(*N*-L-alanylaminomethylphosphonate) a new diaminophosphonate peptide receptor for lysine and arginine

Piotr Młynarz [a,*], Adriana Olbert-Majkut [b], Sylwia Śliwińska [a], Grzegorz Schroeder [c], Bartosz Bańkowski [a], Paweł Kafarski [a]

[a] *Department of Chemistry, Wrocław University of Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland*
[b] *Faculty of Chemistry, University of Wrocław, F. Joliot-Curie 14, 50-383 Wrocław, Poland*
[c] *Faculty of Chemistry, A. Mickiewicz University, Grunwaldzka 6, 60-780 Poznań, Poland*

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Abstract

New diaminophosphonate peptide receptor composed of two aminomethylphosphonic acid fragments attached to phenyl ring in *para* position was described. Intermolecular interactions between such host molecule and unprotected cationic forms of lysine and arginine (guest molecules) were studied by means of NMR spectroscopy and ESI-MS spectrometry in both methanol and water solutions. Unfortunately, no complexation induced shifts in NMR spectra were observed for these host–guest systems in water solution. However, the performed studies have shown that in methanol complexes in host to guest ratio of 1:1 and 1:2 are formed and they precipitate from the solution, while in water 1:1 complexes are only seen and supramolecular interactions between host and guest discovered using ^{1}H- and ^{31}P NMR T_1 relaxation method suggest that they are present as ionic pairs in aqueous solution. The supramolecular studies were supported by DFT theoretical studies performed for free receptor and its molecular complexes with Lys and Arg.

Keywords: Supramolecular chemistry; Host–guest interaction; Receptor for Arg; Receptor for Lys; NMR spectroscopy; ESI-MS spectrometry; Relaxation time

1. Introduction

At the end of last century interest in supramolecular receptor containing phosphonic acids as binding units has significantly increased, mostly because of works of Schrader who demonstrated their ability to bind cationic organic species [1]. The introduced receptors were designed to bind biologically important molecules such as amino acids, peptides, proteins and neurotransmitters [2–4]. One of the described supramolecular systems composed of suitable receptor for Arg was also the subject of extensive theoretical studies [5]. Undoubtedly contributions to phosphonic receptor development brought Yashima and co-workers by designing polymeric phosphonate receptors able to bind amino acid [6–8].

In contrary to phosphonic acids, aminophosphonate compounds were much rarely of supramolecular chemistry interest. There is a report on application of aminophosphonate functionalized calixarene as carrier for amino acids via liquid organic membrane [9]. In turn, the adduct formation between aminophosphonic acids and their derivatives with arginine and its N- and C-blocked derivatives were also a subject of extensive ESI-MS investigation [10,11].

In this work the supramolecular properties of molecule bearing two *N*-(L-alanyl)aminomethylphosphonic acid fragments attached to phenyl ring (compound **1**, Scheme 1) at *para* position was studied. We have found that molecular complexes, formed between this host molecule and lysine or arginine, spontaneously precipitate from the methanol solution with host to guest ratio of 1:1 and 1:2.

* Corresponding author. Tel.: +48 71 320 3616; fax: +48 71 328 4064.
E-mail address: piotr.mlynarz@pwr.wroc.pl (P. Młynarz).

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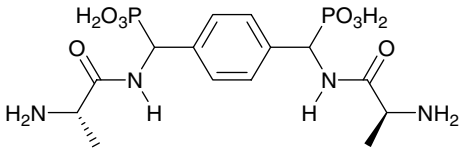


Scheme 1. Structure of the host molecule.

Their structures despite of lack of chemical induced shift were determined with the use of longitudal T_1 relaxation time in ^{1}H- and ^{31}P NMR spectroscopy as a intermolecular interaction indicator. Experimental studies were supported by DFT calculations of the structure and the interaction energies of the studied systems.

2. Experimental

2.1. Synthesis of tetraethyl 1,4-phenylene-di(aminomethylphosphonate)

The reaction was carried out according to the procedure reported earlier [12,13]. Terephthalic aldehyde 0.015 mol (2.01 g) and benzylamine 0.03 mol (3.24 g) were dissolved in 20 ml of methanol and stirred overnight at room temperature. Resulting Schiff base was filtered and analyzed by means ^{1}H NMR ($CDCl_3$, TMS): δ 4.86 (2H, s, CH_2); 7.24–7.42 (10H, m, Ar); 7.86 (4H, s, Ar); 8.42 (2H, s, CH=N); yield 80%, mp 102 °C. This product was of good purity and therefore it was dissolved in 50 ml of toluene and 0.012 mol (1.61 g) of diethylphosphite was added followed by refluxing the reaction mixture for 8 h. Then the volatile components of the solution were evaporated and crude product was dissolved in dry diethyl ether and precipitated as oxalate upon addition of appropriate amount of oxalic acid. The formed precipitate was filtered, dried and suspended in dichloromethane (DCM). The suspension was washed with 1 M NaOH until all solid material was dissolved. After evaporation of the solvent crude product was dissolved in 1.7 M solution of hydrogen chloride gas in methanol and hydrogenated over Pd/C catalyst under atmospheric pressure during 8 h. The progress of hydrogenation reaction was controlled by ^{31}P NMR spectroscopy. When the hydrogenation was completed, reaction mixture was filtered through 4 cm layer of Cellite and solution was evaporated under reduced pressure. The crude hydrochloride was purified by recrystallization from methanol/ethyl acetate mixture.

2.2. Synthesis of 1,4-phenylene-di(N-L-alanylaminomethylphosphonate)

The synthesis of this phosphono peptide was carried out according to the general procedure described earlier [14]. A solution of 0.005 mol (0.946 g) of the Boc-Ala and 0.0052 mol (0.72 ml) of triethylamine in 40 ml of dry chloroform was cooled to about −5 °C and treated with 0.53 ml (0.0055 mol) of ethyl chloroformate. After 40 min 0.0025 mol (1.202 g) of tetraethyl 1,4-phenylene-di(aminomethylphosphonate) was added with stirring and solution was left to reach room temperature, followed by slow warming up to the boiling point. The resulting solution was washed successively with water, 1 M hydrochloride acid, water, saturated solution of sodium bicarbonate, and water. Finally, it was extracted with brine and dried over sodium sulphate. The solvent was distilled off and the oily residue representing crude di-phosphono peptide was dissolved in 20 ml of 40% hydrogen bromide in glacial acetic acid and left overnight. Then acetic acid and excess of hydrogen bromide were removed under reduced pressure, the residue dissolved in absolute ethanol and crude peptide precipitated by addition of propylene oxide. This procedure resulted in crystalline product, which decomposed upon heating over 300 °C. Yield 65%. ESI-MS m/z (−) 437 (for more data see in the paragraph below); ^{1}H NMR (D_2O, TSP): δ 1.38 and 1.50 (3H each, d, $J = 6.0$ Hz, alanyl CH_3); 4.10 (2H, m, J=6.0 Hz, alanyl αCH); 4.99 and 4.96 (1H, d, $J = 19.5$ Hz, each, phosphonate αCH); 7.37 (4H, s, aromatic protons); ^{31}P NMR (D_2O, H_3PO_4): δ 14.51; 14.41. For NMR study in methanol solution 1,4-phenylene-di(*N*-L-alanylaminomethylphosphonic acids) was transformed into its sodium salt by dissolving in equimolar aqueous solution of NaOH followed by evaporation and drying in vacuo.

2.3. Spectroscopic studies

Host and guest concentrations were adjusted to 0.4–10×10^{-3} mol dm^{-3}. ^{1}H and ^{31}P NMR spectra were recorded at 300 MHz DRX Bruker and 600 MHz Bruker Avance instruments at 298 K in D_2O and CD_3OD using coaxial tube filled with TSP [trimethylsilylpropenesulfonic acids sodium salt] or phosphorous acid dissolved in D_2O as external standards. Selective ROESY experiment was recorded applying mixing time ranging from 250 to 400 ms.

2.4. Precipitation studies

Sodium salt of phosphono peptide host was dissolved in methanol and appropriate portion of dihydrochloride salt of guest (in MeOH) was added adjusting stoichiometry to 1:1, 1:3 and 1:20. Resulting precipitates were filtered, washed with methanol and dried. Each precipitate was dissolved in D_2O and examined by means of NMR spectroscopy. pH was controlled using Mettler–Toledo pH-meter supported with semi-micro combined electrode. The host to guest ratios in precipitates were determined on the basis of integration of their 1D ^{1}H NMR spectra after dissolution of the resulting complexes in D_2O.

2.5. ESI spectrometry

The spectroscopic analyses were carried out using Waters/Micromass (Manchester, UK) ZQmass spectrometer equipped with Harvard Apparatus syringe pump. The

measurements were performed in aqueous and methanol solutions when setting up the concentrations of host and guest in the range of 5×10^{-4}– 5×10^{-5} mol dm^{-3}.

2.6. Computational details

The full conformational search was undertaken applying DFT calculations using Gaussian 03 package of computers code [15] and employing the Becke's three parameter hybrid functional with the non-local correlation of Perdew (B3PW91) [16,17]. The structure of receptor and both amino acids as well as their complexes were fully optimized using DGDZVP (Density-Gaussian double-zeta-split-valence plus polarization functions) basis set [18]. The interaction energy was computed as the difference in total energy between the complex and its subunits (receptor and amino acid) at the frozen structures as adopted in the complex according to the following formula: $\Delta E_{\text{interaction}} = E_{\text{complex}} - (E_{\text{receptor}} + E_{\text{aminoacid}})_{\text{frozen}}$. All proton transfer calculations were carried without geometry optimizations at each point on the PES.

3. Results and discussion

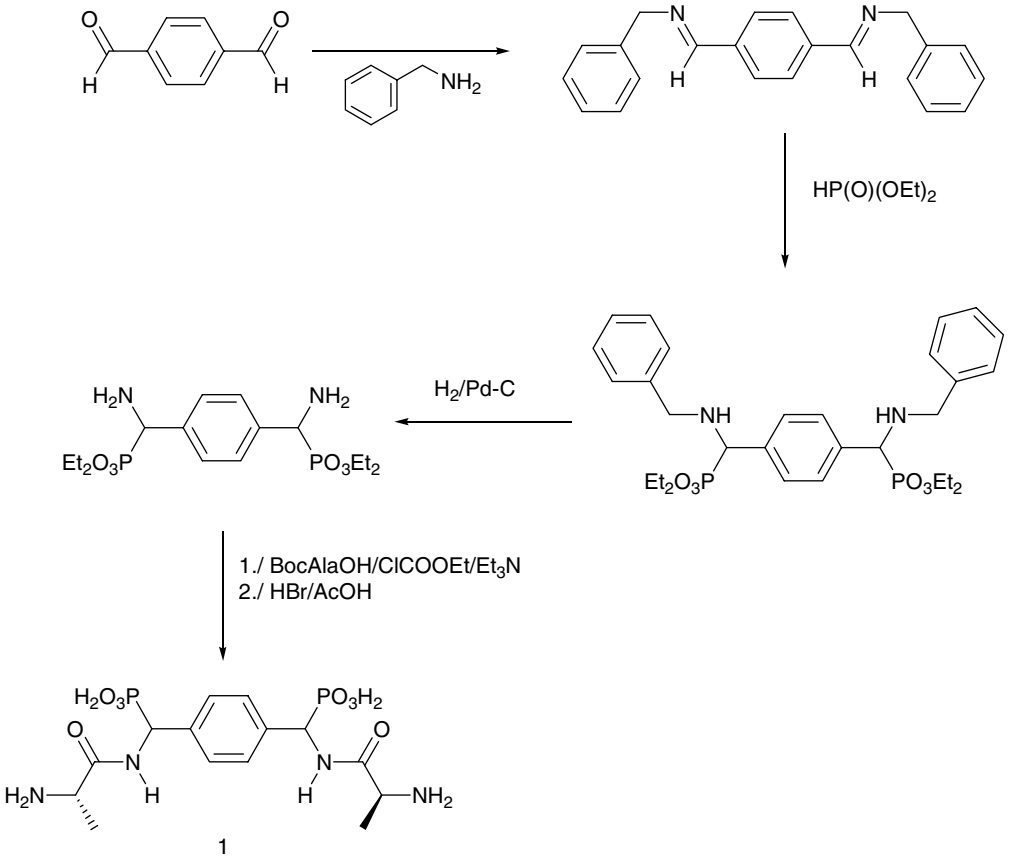
3.1. Synthesis of host molecule 1

Host phosphono peptide **1** was synthesized in a sequence of reactions shown in Scheme 2, according to previously described procedures [14]. Since the starting aminophosphonate was of *meso* configuration this procedure resulted in product of (RS, SS) configuration and thus exhibiting two peaks of 1:1 ratio in ^{31}P NMR.

3.2. Characterization of receptor

The structure of the synthesized host phosphono peptide **1** was proven by ^{1}H and ^{31}P NMR spectroscopy and ESI MS spectrometry. According to the literature the performed synthesis imposes formation of *meso* form of compound **1** [12]. Attachment of two L-alanine (*S*-alanine) residues to amine groups of this compound resulted in differentiation of proton signals of aminophosphonate α-carbon, which appeared as two doublets corresponding to RS and SS fragment of the molecule. Also two sets of signals deriving from two methyl groups of alanine were visible in ^{1}H NMR spectra. The additional evidence for the presence of the two different chiral centers came from ^{31}P NMR spectra where two equipotent singlets of phosphorous signals were observed. The 1D ROESY selective experiments have shown that one of the methyl groups interacts stronger with phenyl protons than the other one (spectrum not shown).

Obtained phosphono peptide **1** exhibits very good solubility in water in the wide range of pH from 1 to 13 revealing four pK_a values: 8.93 and 8.22 (for two NH_2 groups of alanine), and 6.67 and 5.74 (responding to two PO_3H^-groups) [19]. The quite significant divergence in



Scheme 2. Synthetic scheme leading to host molecule **1**.

the respective pK_a values may be caused by different intermolecular interactions between phosphonic and ammonium groups, assumption which was supported by theoretical studies.

ESI-MS studies of the receptor in aqueous solution revealed main *m/z* signals at 437 (−) [R] and 218 (−) [R] and 461 (+) [R+Na]. Its sodium salt in methanol exhibits three main forms 503 (−) [R+3Na], 481 (−) [R+2Na] and 459 (−) [R+Na] and 227 (+) [R+Na], whereas in water only following *m/z* signals: 459 (−) [R+Na], 437 (−) [R], 218 (−) [R] and 191 (+) [R+Na] were observed. This shows that in methanolic solutions the main form of compound **1** is a molecule which binds two sodium ions, while in the aqueous solution only one Na^+ ion is bound. This finding indicates that affinity of compound **1** towards positively charged species is higher in methanol than it is in water.

3.3. Host guest molecular complexes of compound *1* with lysine and arginine

The standard titration of host molecule by basic amino acids in water studied by ^1H and ^{31}P NMR spectroscopy did not demonstrate complexation induced shifts neither in the case of proton nor phosphorous spectra. The only trace of interaction could be deduced from changes in the shape of phosphorous signals during titration. Therefore these systems were additionally studied in less polar solvent – methanol. Addition of the methanolic solution of lysine or arginine dihydrochlorides to phosphono peptide **1** dissolved in the same medium caused precipitation of supramolecular complexes. Systematic studies of precipitates obtained using various host to guest molar ratios have shown the formation of two molecular complexes of host to guest ratio 1:1 and 1:2. This is despite the fact that 20-fold excess of amino acids dihydrochloride caused significant decrease in pD of precipitate (Table 1), which may suggests additional binding of HCl, or more likely the presence of protonated COOH group.

The ESI-MS study of the precipitates dissolved in water revealed formation of molecular complexes of host to guest 1:1 ratio, which is indicated by presence of signals at 583 (−) (peptide **1**–lysine) and 611 (−) (peptide **1**–arginine). Measurements performed in methanolic solution obtained after filtration of precipitate showed the presence of 1:2 complex of compound **1** and lysine, with the signal at *m/z* 731 [R+2Lys].

The T_1 relaxation measurements of obtained precipitates dissolved in water at pD (pH measured in D_2O) of 6.4 were performed. Obtained results were compared with those obtained for individual compound **1** and solutions of lysine and arginine performed in D_2O at the same pD (Table 2). Data presented in Table 2 strongly suggest that decrease of T_1 values observed for proton and phosphorous atoms reflect existence of host–guests interactions. The biggest effect was observed in the case of α- and ε-protons of lysine (decrease in T_1 value from 3.05 to 2.0 s and 1.55 to 1.05 s respectively), α and δ protons of arginine, aryl protons of phenyl ring and α-protons of aminophosphonate, and α-protons of alanyl residues of compound **1**. Interestingly, phosphorous signal is also remarkably affected what confirms participation of phosphonic moieties in supramolecular recognition. As seen from Table 2, the observed effect is stronger when precipitate was freshly dissolved in water than this found for mixtures of phosphono peptide **1** and corresponding amino acids (ratio 1:2). This finding can suggests that complexes precipitated form exhibit in methanol stronger network of binding forces.

The clear decrease in relaxation time T_1 observed for protons as well as for phosphorous atom might be caused by change of correlation time (increase) of both molecules due to complexation processes between relatively small host and guest [20–23]. Also, that example once more shows that in the case when complexation-induced chemical shifts are too small to be significant the studies of relaxation times is very useful [23].

The formed complexes seem to be weakly bound since ROESY and NOESY (2D as well 1D selective) measurements did not equivocally confirm those interactions. The systematic studies of the remaining precipitates, namely those appearing at lower pD values showed that T_1 values of protons as well as phosphorous are very close to these observed in the case of free phosphono peptide. This indicates that the forming supramolecule is most likely stable only in restricted pH range. Anyway, the observed differences in relaxation times suggest that the formation of supramolecules is governed by interactions between phosphonic acid groups and ammonium and/or guanidinium ions of amino acids. Thus, the formed supramolecules might be rather considered as ionic pairs. That observation may confirm the theoretically calculated model for 1:1 complexes (*vide supra*) forming preferentially in methanol as well as in aqueous solutions. The detected involvement of Ala residue of the host molecule can not be easily explained, however there are two options of its interaction with lysine and arginine. They may result either from inter-

Table 1
The dependence of host to guest ratio in the formed complexes from their starting ratio

Molar ratio of peptide **1** to Lys[a] or to Arg	Molar ratio of peptide **1** to Lys in precipitate	pD of precipitate	Molar ratio of peptide **1** to Arg in precipitate	pD of precipitate
1:1	1:1.5	7.80	1:1	7.99
1:3	1:2	6.39	1:1.5	6.17
1:20	1:2.5	2.54	1:2	2.50

[a] concentration of phosphono peptide **1** sodium salt was set at 0.5 mM.

Table 2
T_1 relaxation measurements of proton and phosphorous signals of compound **1**, lysine and arginine in D_2O at the 298 K, pH 6.1–6.4

	T_1[s] for ^1H					T_1[s] for ^{31}P
Free species						
Lys	3.05 (α)	1.55 (ε)				
Arg	2.76 (α)	0.98 (δ)				
Peptide **1**	1.55 (Ar)	1.82 (α)	2.66 (α-Ala)	0.88 (CH_3–Ala)	0.75 (CH_3–Ala)	0.85
Mixed species						
Lys fragment	2.64 (α)	1.34 (ε)				
Peptide **1** fragment	1.20 (Ar)	1.77 (α)	1.96 (α-Ala)	0.76 (CH_3–Ala)	0.74 (CH_3–Ala)	0.74
Arg fragment	2.17 (α)	0.86 (δ)				
Peptide **1** fragment	1.41 (Ar)	1.67 (α)	2.26 (α-Ala)	0.77 (CH_3–Ala)	0.72 (CH_3–Ala)	0.77
Dissolved precipitate						
Lys fragment	2.00 (α)	1.05 (ε)				
Peptide **1** fragment	1.21 (Ar)	1.52 (α)	2.01 (α-Ala)	0.77 (CH_3–Ala)	0.74 (CH_3–Ala)	0.57
Arg fragment	1.63 (α)	0.78 (δ)				
Peptide **1** fragment	1.27 (Ar)	1.58 (α)	2.15 (α-Ala)	0.76 (CH3–Ala)	0.72 (CH3–Ala)	0.70

actions between carboxylic group of amino acid and ammonium group of phosphono peptide yielding 1:1 molecular complexes, or from that amino acid are bridging host phosphonate with ammonium group of the next host by their ammonium and carboxylic groups forming pseudo-polymeric species. This second assumption might be ruled out because polymeric species were not observed in ESI-MS spectrometry.

3.4. Theoretical calculations

3.4.1. Phosphono peptide structure in high pH

In order to obtain the structure analogous to that observed experimentally in methanolic solution, the unprotonated PO_3^{2-} and NH_2 groups in the optimized peptide **1** have been taken into consideration. Two stationary points were found on the Potential Energy Surface of the studied compound. The global minimum was predicted for the conformer exhibiting *anti* relation of the two phosphonate groups with respect to the aryl ring plane (Fig. 1) with the PCCP tortional angle equal to −162°. *Anti* conformer is only by 3.89 kJ/mol more stable than the second conformer that is characterized by *syn* arrangement of the PO_3^{-2} groups (Fig. 2). In this case the PCCP dihedral angle is predicted to be equal +7°. As can be seen from Figs. 1 and 2 both arrangements of the phosphonate groups allowed formation of the internal hydrogen bonds between NH_2 and C═O groups (N─H···O═C) as well as between amide proton (NH) and PO_3^{2-} moiety (N─H···O─P). For both conformers H···O distance in N–H···O═C hydrogen bridge is equal to 2.13 Å for *R*-phosphono peptide fragment and 2.25 Å for *S*-center. In turn, the H···O distance in N–H···O–P interaction is computed to be 1.86 and 1.80 Å for *R* and *S* aminomethylphosphonate fragments, respectively, and is the same in the case of both conformers. This clearly indicates that the rotation of the PO_3^{-2} groups around the C_{ar}–C* bound does not influence the spatial arrangement and internal interactions of both phosphonic acid groups. This fact could explain the experimental observations of the formation of 1:1 and 1:2 host–guest complexes between this peptide and studied amino acids. Hence, the *syn* structure of the studied compound probably allows the formation of the 1:1 complex, while the *anti* conformer favors formation of 1:2 complexes without sterical constrains.

3.4.2. Phosphono peptide structure in acidic solution

For simulation of the structure of compound **1** in acidic environment optimization of the system bearing monoprotonated PO_3^{2-} (PO_3H^-) and protonated NH_2(NH_3^+) groups has been performed. In this case significant changes in the internal arrangement and interactions of the individual parts on the *R* and *S* branches are observed in comparison with non protonated system. In Fig. 3 the DFT optimized structure of the *syn* peptide is presented.

As can be seen the structure shows the presence of the two strong hydrogen bonds in the aminomethylphosphonate group of *R* configuration. The first one is formed between NH_3^+ and C═O groups in alanyl residue. In this case the N–H bond is elongated by ca. 0.77 Å as compared with the non-bonded N–H group. It is interesting to notice that this interaction is characterized by almost linear hydrogen bond with O···H–N of the angle equal to 171°. For the described *R*-moiety formation of additional hydrogen bond between oxygen atom of the phosphonate and H atom of amide group is predicted. This interaction is characterized by H···O distance equal to 1.80 Å.

The different type of interaction is predicted for the *S*-aminomethylphosphonate fragment of compound **1**. In this case very strong hydrogen bond between NH_3^+ and HPO_3^- groups is observed. This interaction is accompanied by the significant elongation of the N–H bond up to 1.72 Å and shortening of the H···O distance up to 1.03 Å. These values clearly indicate that the proton is bounded rather by phosphonate than by amine group.

The DFT predicted inequivalence of NH_3^+ and HPO_3^- groups of each phosphono peptide unit, which is reflected

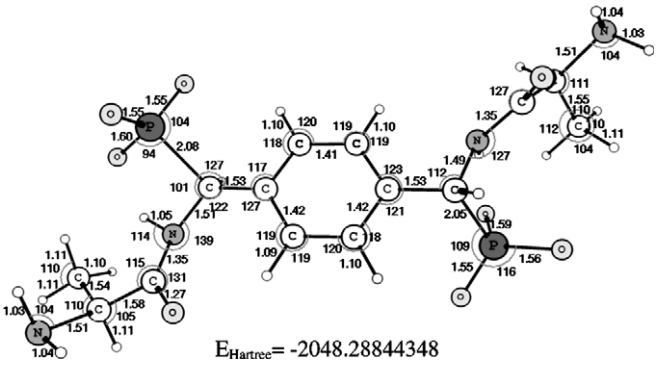


Fig. 1. The optimized structure and calculated energy of compound **1** as *anti* conformer.

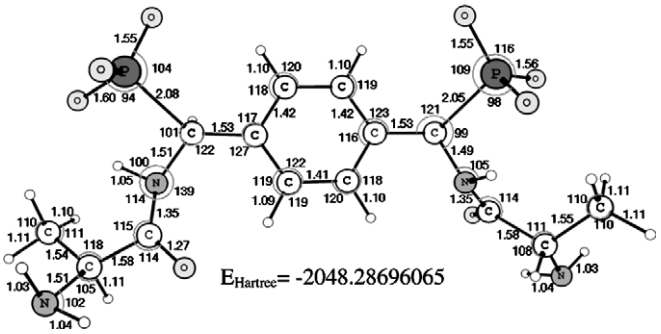


Fig. 2. The optimized structure and calculated energy of compound **1** as *syn* conformer.

in significant difference of the experimentally determinated pK_a values being 0.71 for NH_2 group and 0.93 for HPO_3^-.

3.4.3. Complexes of phosphono peptide *1* with arginine and lysine

The Potential Energy Surface studies performed for the 1:1 receptor-Arg and receptor-Lys systems indicate the existence of only one stable structure in which two hydrogen bonds stabilizing their structure are formed. As the starting point for optimizations the non-protonated *syn* phosphono peptide (Fig. 2) and protonated arginine and lysine has been taken into consideration. In both cases the presence of interactions between negatively charged phosphonic groups and cationic units of amino acid was assumed. As might be seen in Figs. 4 and 5 both arginine and lysine molecules are bidentately bound to phosphono peptide **1**. The spatial arrangement of arginine-peptide 1 complex allows the formation of the hydrogen bonds between the phosphonate moieties of aminomethylphosphonate groups and hydrogen atoms of the α-amine and guanidinium groups of arginine. Analogical pattern of interactions is predicted for complex formed between compound **1** and lysine.

The DFT calculated interaction energy computed for the studied systems after the proton transfer is equal to 104.06 and 99.86 kJ/mol for arginine and lysine complexes, respectively. The energy difference between both molecular complexes is ca. 4 kJ/mol and indicates that the strength of interactions is comparable for complexes of both amino acids.

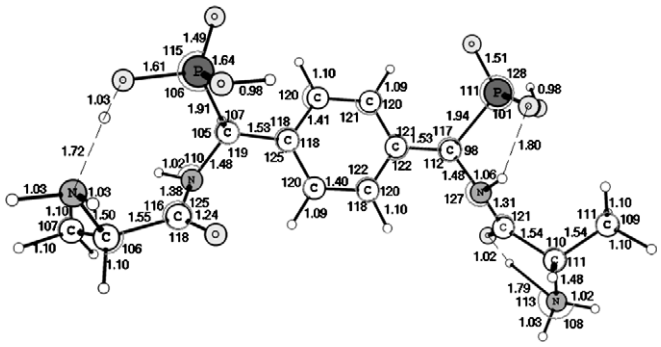


Fig. 3. The optimized structure of the lowest calculated energy for peptide **1**.

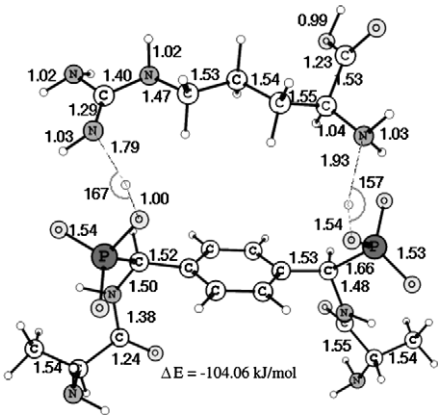


Fig. 4. The optimized structure of the arginine-peptide **1** complex.

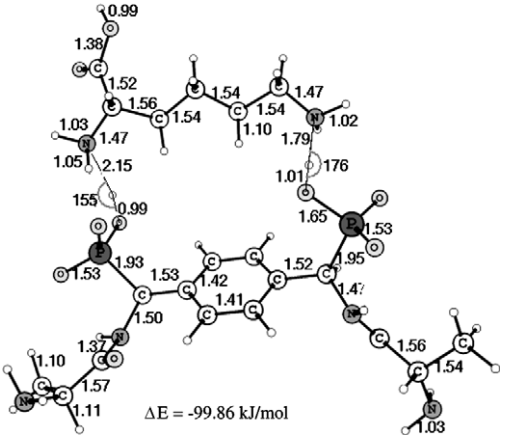
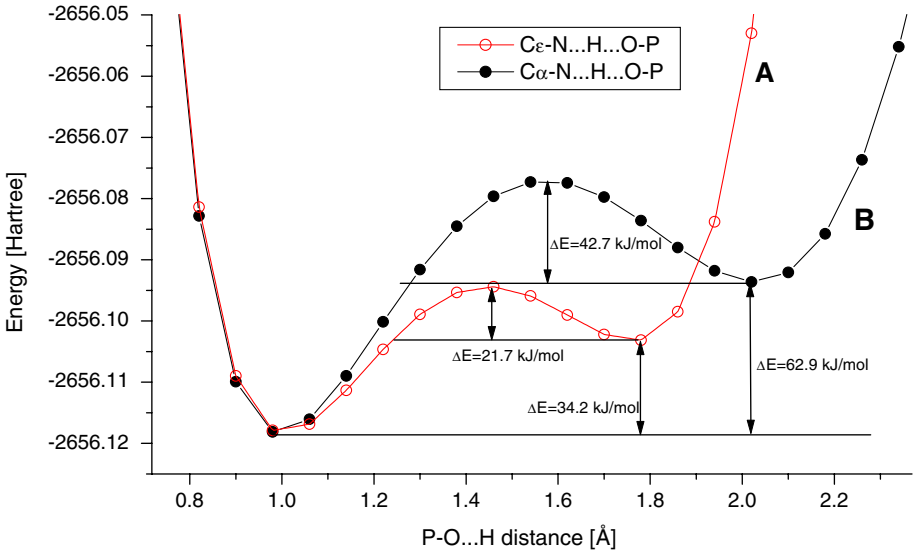


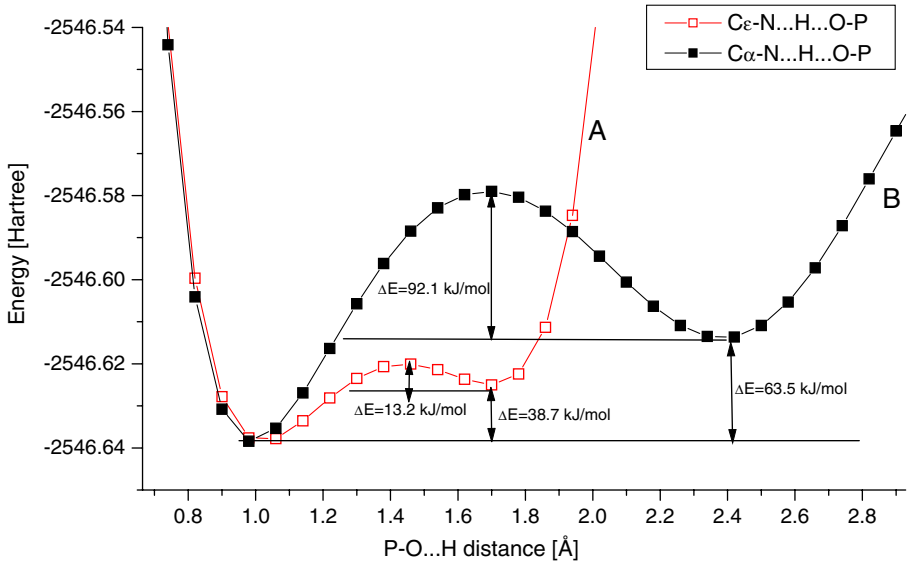
Fig. 5. The optimized structure of the lysine-peptide **1** complex.

For the $PO_3^{2-}\cdots H_3N^+-C_\alpha$ interaction in arginine–peptide **1** complex the calculated hydrogen bridge length and angle are equal to 3.47 Å and 157°, while for the $PO_3^{2-}\cdots H_2N^+-C_\varepsilon$ system the corresponding values are 2.79 Å and 167°.

The same tendency is observed for the lysine–compound **1** system. The calculated length of the hydrogen bridge is



Scheme 3. Energy barriers for proton transfer between arginine and receptor for (A) Cε—N···H···O—P and (B) Cα—N···H···O—P system.



Scheme 4. Energy barriers for proton transfer between arginine and receptor for (A) Cε—N···H···O—P and (B) Cα—N···H···O—P system.

equal to 3.14 Å for $PO_3^{2-}\cdots H_3N^+$—C_α and 2.80 Å for $PO_3^{2-}\cdots H_3N^+$—C_ε. It is clearly indicated that in both studied systems the double proton transfer reaction appears. Schemes 3 and 4 show the results of the Potential Energy Surface studies for the varying N–H bond length in the C_α—N—H···O—P and C_ε—N—H···O—P moieties performed for both arginine and lysine complexes. As one can see for donor–acceptor pair, the second minimum on the right (for proton attached to the ammonium group) is always located higher than the first minimum (for hydrogen atom attached to the PO_3^{2-} moiety) as the proton is more stable when placed on phosphonic group than on a hydrogen bonded arginine or lysine. In Schemes 3 and 4 the significant energy differences between individual minima are observed for the C_α–N···H···O–P hydrogen bridge. For Arg–peptide **1** complex ΔE is calculated to be equal 62.9 kJ/mol, while for Lys–peptide **1** system to be 63.5 kJ/mol. On the other hand, in the case of C_ε–N···H···O–P entities the differences between two minima on the PES are almost twice smaller as the previous ones and are equal to 34.2 kJ/mol (for arginine complex) and 38.7 kJ/mol (for lysine complex).

The predicted energy barriers for proton transfer between amino acids and peptide **1** are distinctly higher in the case of hydrogen bonding between PO_3^{2-} group and C_α—NH_3^+ than these calculated for $PO_3^{2-}\cdots C_\varepsilon$—$H_3N^+$ and $PO_3^{2-}\cdots C_\varepsilon$—$H_2N^+$ systems. The calculated energy barrier for C_α–N···H···O–P system is equal to ca. 43 and 92 kJ/mol for Arg and Lys complex, respectively, while those predicted for C_ε–N···H···O–P hydrogen bond is equal to 22 and 13 kJ/mol for Arg–peptide **1** and Lys–peptide **1** system, respectively. These data clearly indicate

that proton transfer process is much easier in the case of the hydrogen bonds formed between ammonium or guanidinium groups of the side chain of amino acid as compared with the $C_\alpha - NH_3^+$ moieties. This results seems to be slightly surprising since the experimental pK_a values determinated for $C_\alpha - NH_3^+$ entity is always lower than those for guanidinium or ammonium Arg or Lys side chain group.

4. Conclusion

In this work we demonstrated that peptide derivative of diaminophosphonate compound **1** (in the form of sodium salt) is able to bind non-blocked 2HClxArg and 2HClxLys in methanol solution causing formation of molecular complexes of the host-to-guest ratio of 1:1 and 1:2. Moreover, the supramolecular interaction in aqueous solutions between peptide 1-Arg and peptide 1-Lys were revealed by a considerable change in spin-lattice relaxation time T_1 calculated for protons and phosphorous atoms of investigated systems. These studies were confirmed by ESI-MS spectrometry. Theoretical calculations performed for unprotonated form of guest 1 have shown the existence of two conformers (*syn* and *anti*), which may explain the formation of two kinds of complexes in methanolic solutions. On the other hand, in the case of tetraprotonated receptor 1 the net of intramolecular hydrogen bonds was predicted, which might be a cause of difference in pK_a values found for the same kind of dissociable group by potentiometry. Interesting results were obtained by calculating proton transfer energy barrier from α-ammonium group of Arg or Lys to PO_3^{2-} entity in comparison to proton transfer form guanidinium or ammonium side chain group of these amino acids to PO_3^{2-} unit. The calculated binding energy suggests that energy is lower for Arg and Lys side chain group than their α-ammonium groups thus supporting the presumable structures of the formed supramolecular complexes.

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