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Tetra-2-methoxyethyl phenylene-1,4-di(benzyloaminomethanephosphonate) a new ligand for metal ions and amino acids. Electrospray ionization mass spectrometric and NMR studies

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Abstract

Formation of stable complexes between tetra-2-methoxyethyl phenylene-1,4-di(benzyloaminomethanephosphonate) (receptor) and monovalent (Li^+, Na^+, K^+, Rb^+, and Cs^+) and divalent metal cations (Mg^{2+}, Ca^{2+}, Ba^{2+}) in acetonitrile has been studied by means of ESI-MS technique. Additionally the chelating properties of the phosphonate towards amino acids ($2 \times HCl*Arg$, $2 \times HCl*Lys$) in methanol and water have been evaluated. Crown ether-like esters of the phosphonate do not bind ammonium cations of these basic amino acids and instead of that proton transfer from carboxylic group of amino acids to amino moiety of the receptor, governed by surprisingly low basicity of its amino moiety ($pK = 4$), was observed.

Keywords: Electrospray ionization mass spectrometry; NMR spectroscopy; Ion metal receptor; Metal chelation; Amino acid binding; Host–guest chemistry

1. Introduction

Aminophosphonates and their derivatives were mostly recognized as the biologically active compounds with variety of useful activities ranging from enzyme inhibitors to herbicides. Therefore their coordination properties towards metal ions are of substantial interest [1]. The availability of new derivatives of aminophosphonates, especially these containing substituents able to bind small molecules and/or metal ions may open a gate towards their use in the host–guest chemistry. From the supramolecular chemistry point of view the influence of metal binding on their biological properties is of special interest. Searching for new ligands that are able to form new biologically active complexes with metal ions of the first group of periodic table is considered as one of the most recent trends in biochemistry and supramolecular chemistry [2,3]. General method for obtaining such ligands relays on modification of molecules by introduction of very labile polyethylene glycol (PEG) units [2]. In the last decade organophosphorus compounds were successfully introduced into supramolecular chemistry as hosts for biologically important molecules [4]. In this work we describe preparation of a new ligand for mono- and divalent metal cations and evaluation of its binding properties by means of Electrospray Ionization Mass spectrometry and NMR spectroscopy.

2. Experimental

2.1. Synthesis

2.1.1. Synthesis of di-2-methoxymethyl phosphite (Scheme 1)

2-Methoxyethanol (ethylene glycol monomethyl ether) (5 ml, 63.41 mmol) was dissolved in 20 ml of dry acetonitrile and phosphorous trichloride (3.77 ml, 42.27 mmol)

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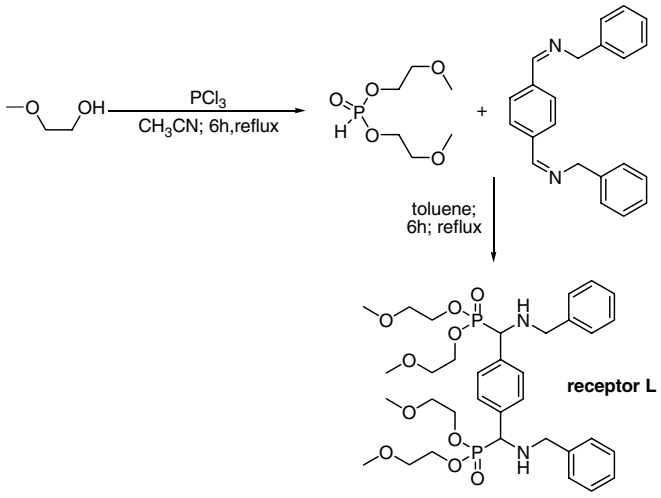

Scheme 1. Route of preparation of tetra-2-methoxyethyl phenylene-1,4-di(benzyloamino-methanephosphonate).

was added. The solution was refluxed for 6 h. The volatile components were evaporated under reduced pressure yielding product of satisfactory purity. Yield: 85% (colorless oil). ^{31}P NMR: δ (ppm): 9.17 (s); ^{1}H NMR ($CDCl_3$): δ (ppm): 6.94 (d, 1H, $J_{H-P} = 717$ Hz, P–**H**), 4.27 and 4.19 (m, 2H each, $OCH_2CH_2OCH_3$), 3.61 (t, 4H, $J = 4.5$ Hz, $OCH_2CH_2OCH_3$), 3.40 (s, 6H, $OCH_2CH_2OCH_3$); ^{13}C NMR: δ (ppm): 71.46 (d, $J = 5.5$ Hz, $OCH_2CH_2OCH_3$), 64.62 (d, $J = 5.6$ Hz, $OCH_2CH_2OCH_3$), 58.95 (s, $OCH_2CH_2OCH_3$).

2.1.2. Synthesis of tetra-2-methoxyethyl phenylene-1,4-di(benzyloaminomethanephosphonate), receptor L

Schiff base was synthesized according to the literature data [5]. The terephthalaldehyde was dissolved in methanol and then two equivalents of benzylamine were added. The mixture was stirred for 4 h at room temperature and the precipitated compound of satisfactory purity was collected by filtration. Obtained Schiff base (0.857 g, 0.0027 mol) was dissolved in toluene and di-2-methoxymethyl phosphite (1 g, 0.0055 mol) was added. The mixture was refluxed for 8 h (Scheme 1). Then the volatile components of reaction mixture were evaporated *in vacuo* and the crude product was purified by column chromatography (silica gel, 70–230 mesh) using hexane/AcOEt/methanol (ratio 4.5:3:1.5) mixture as eluent.

Yield: 21%, oil (colorless). ^{1}H NMR (D_2O): δ (ppm): 7.30 (s, 4H, **Ar**), 7.21–7.03 (m, 10H, **Ar**), 4.16 and 4,17 (d, 1H each, $J = 19.7$ Hz, α-C**H**P) 4.11–4.06 (m, 4H, $OCH_2CH_2OCH_3$) 4.05–3.98 and 3.91–3.84 (m, 2H each, –$OHCHCH_2OCH_3$), 3.74 (d, 2H, $J = 13.5$, $PhCH_2$,), 3.56 (t, 4H, $J = 4.2$, $OCH_2CH_2OCH_3$), 3.502 and 3.498 (d, 1H each, $J = 13.5$ Hz, PhC**H**), 3.50–3.44 and 3.44–3.37 (m, 2H each, $OCH_2HCHOCH_3$), 3.28 and 3.21 (s, 3H each, $OCH_2CH_2OCH_3$) ^{13}C NMR (D_2O): δ (ppm): 50.34 and 50.23 (m, $PhCH_2$), 57.54 (–α-**C**HP, $J = 156$ Hz), 58.06 and 50.03 (s, $OCH_2CH_2OCH_3$), 65.94 and 66.11 (m, $OCH_2CH_2OCH_3$), 70.87 and 70.94 (m, $OCH_2CH_2OCH_3$), 127.68 (s, **Ar**), 128.70 (s, **Ar**), 128.91 (s, **Ar**), 129.45 (s, **Ar**), 134 (d, **Ar**, $J = 8.3$ Hz), 137.91 (d, **Ar**, $J = 3.4$ Hz).

2.2. ESI-MS studies

The ESI (electrospray ionization) mass spectra were recorded on a Waters/Micromass (Manchester, UK) ZQ mass spectrometer equipped with a Harvard Apparatus syringe pump. The measurements were performed for two types of samples: acetonitrile solutions of receptor ($1–5 \times 10^{-3}$ mol dm^{-1}) with a mixture of Li^+, Na^+, K^+, Rb^+ and Cs^+ cations (5×10^{-3} mol dm^{-1}) and the same acetoniotrile solutions of the compound with Li^+, Na^+, K^+, Rb^+ and Cs^+ cations (5×10^{-3} mol dm^{-1}) taken separately. The samples were infused into the ESI source using a Harvard pump at a rate 20 μ dm^3 min^{-1}. The ESI source potentials were: capillary 3 kV, lens 0.5 kV, extractor 4 kV, and the cone voltage 30 V. The source temperature was 120 °C and the desolvation temperature was 300 °C. Nitrogen was used as a nebulizing and desolvation gas at a flow rate of 100 and 300 dm^3 h^{-1}, respectively.

2.3. NMR measurements

NMR spectra were recorded on Bruker Avance DRX300 and Bruker AMX600 instruments in $CDCl_3$, MeOD and D_2O using a variety of 1D and 2D (HMQC, COSY, TOCSY, NOESY, ROESY, T_1-pseudo 2D) correlation spectra techniques. Chemical shifts are given in relation to TMS or TSP, 85% phosphoric acid (phosphorus spectra).

2.4. Theoretical calculations

PM5 semiempirical calculations were performed using the WinMopac 2002 program [6–8]. In all cases full geometry optimalization was carried out without any symmetry constrains.

3. Discussion and results

3.1. Receptor structure by means of theoretical calculations, NMR and ESI-MS studies

The semiempirical PM5 calculations performed for single molecule of receptor carried out in the gas phase show the presence of free rotations of aminomethylphosphonate residues, with the differences between each of the found conformations not exceeding few kJ/mol. In contrary, to the NMR results (*vide supra*), theoretical calculations did not reveal the presence of any intermolecular hydrogen bonds of the N–H–O type neither for *R*,*R* nor *S*,*S* enantiomers.

The ^{1}H NMR signals of the receptor are changing with its concentration, temperature and polarity of the solvent. This fact may be caused by existence of intra- or

intermolecular interactions through formed hydrogen bonds. ^{1}H NMR spectra are more complex when recorded in chloroform and at a low concentration of the aminophosphonate. Their simplification is seen with increase of the polarity of used solvent (from chloroform to methanol) and in aqueous solutions. Upon elevating the temperature from 283 to 298 K visible changes of chemical shifts for following proton signals were recorded: aryl skeleton, α-CH, one of the protons from CH_2 groups of benzyl moiety, one of –CH_2– ester groups and both –OCH_3 groups. This temperature dependence might suggest the presence of inter- and/or intra molecular hydrogen bonding, where each of two esters groups and amine and/or ammonium groups may be involved.

The ESI-MS measurements reveal two kinds of signals in the positive ions area. The first one belongs to the monoprotonated form of the receptor (L) 709 $[L+H]^+$ and its complex with sodium ion 731 $[L+Na]^+$ (Table 1). The second sort of signals arises due to fragmentation of receptor ester groups: 511 $[L-PO(OR)_2]^+$, 313 $[L-2PO(OR)_2]^+$. In the negative ion area fragmentation ions are visible at 649 $[L-R]^-$, 591 $[L-2R]^-$, 451 $[L-PO(OR)_2,R]^-$ (Table 1), whereas $[L-H]^-$ ion was not observed.

3.2. Complexing properties of receptor L

3.2.1. Metal ions complexation

Oxygen atoms deriving from polyethylene glycol chains, due to presence of free electron pairs, are expected to form complexes with metal ions [9]. The NMR titration and ESI-MS measurements of the system receptor L – monovalent metal ions, revealed formation of complexes with 1:1 metal to ligand stoichiometry. This finding is strengthened by semiempirical calculations. The calculated complex structure of the minimal energy is presented in Fig. 1. It is clearly seen that three out of four 2-methoxyethanol moieties are involved in metal ion chelating processes. Heat of formation of this system is 20 kJ/mol lower than in the case when only two 2-methoxyethanol chains are engaged in metal ion coordination. The differences in the heats of formation calculated by PM5 method for various metal ions are as follows: Li 17.15, Na 19.24, K 20.19, Rb 22.43, Cs 28.87 kJ/mol. It is clear that this thermodynamic function calculated for stable complexes rises with the increase of metal ion radius. The average distance between metal ion and oxygen donors is equal to 2.6 Å.

Table 1
The main peaks in positive and negative ion regions of ESI-MS recorded for receptor L ($M_W = 708$) in acetonitrile

Compound	Cone voltage (cv) [V]	Main peaks *m/z*
Positive ions	30	709 $[L+H]^+$, 731 $[L+Na]^+$, 511 $[L-PO(OR)_2]^+$, 313 $[L-2PO(OR)_2]^+$
Negative ions	30	649 $[L-R]^-$, 591 $[L-2R]^-$, 451 $[L-PO(OR)_2,R]^-$

Where R = –$CH_2CH_2OCH_3$.

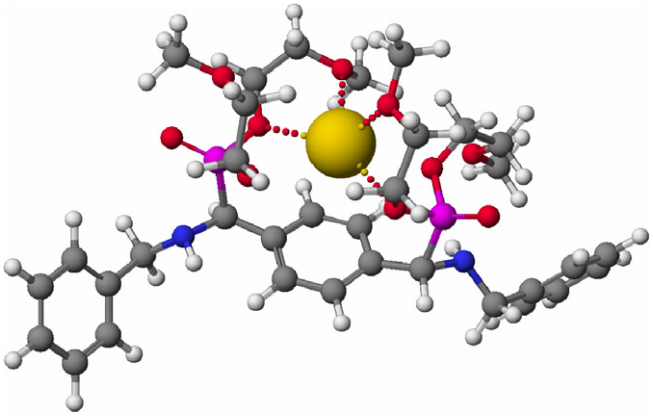

Fig. 1. The structure of metal–ligand complex calculated by PM5 method.

The complexing properties of the receptor L towards divalent metal ions from the second group of periodic table were also investigated. Two biologically important ions Mg(II) and Ca(II) were selected and supplemented by Ba(II) chosen due to its large ion radius. The ESI-MS measurements were performed for the solutions containing equimolar quantities of receptor and metal ions also showed the formation complexes of 1:1 metal to receptor stoichiometry (Table 2). Interestingly the participation of counter ion and formation of complexes of the type of $LM–ClO_4$ were also observed. The studies carried out with an excess of receptor revealed the following order between the ratio of complex stability values: Ca > Mg > Ba.

The formation of complexes between receptor L and metal ions was also confirmed by NMR by observing complexation induced chemical shifts. Although, the complexation was examined in the aqueous and methanol solutions no change of the chemical shifts due to complexation of metal ions was observed in water. Therefore, all chelation studies were performed in $[d_4]$ methanol solution. The presence of monovalent cations does not considerably affect the ^{1}H NMR spectra, and the only change may be noticed in ^{13}P NMR spectra with quite small $\Delta\delta$ value for the M:L ratio 1:1 (up to 0.002 ppm). In contrary, the divalent metal ions change both types of recorded spectra revealing the involvement of receptor in complexation as is seen by shift of δ of –HC**H**NH–, 1 × –CH_2O–, –**H**CHNH–, α-CH– protons with the largest value for methoxy 2 × CH_3O– groups up to 0.01 ppm. The chemical shift, although very small, can be only explained on the basis of interactions between receptor and metal ions. Relatively big shift of proton signals of ester groups may suggest their involvement in metal ion chelation, where at least one group from each phosphorous unit is forced to interact with divalent metal ions. Interestingly, the relatively unexpected shift observed in the case of benzene ring proton signals can be caused by interactions between cation and π electrons kept in a close proximity. The largest change of chemical shifts of aryl protons due to metal ion binding was found for Ba(II) (0.007 ppm) and the smallest for Mg(II) (0.002 ppm) ions.

Table 2
The main peaks (m/z) in the ESI-MS spectra (cone voltage $cv = 10$–50 V) upon formation of the complexes of the studied receptor with various cations (M)

Cation	Cone voltage (V)	Main peaks m (m/z)
Li^+	10	**715** [**L**+**Li**]$^+$, 518 [L−PO(OR)$_2$+Li]$^+$, 511 [L−PO(OR)$_2$]$^+$
	30	715, 518, 313 [L−2PO(OR)$_2$]$^+$, 205
	50	715, 313, **205** [**HPO**(**OR**)$_2$+**Li**]$^+$
Na^+	10	**731** [**L**+**Na**]$^+$, 511 [L−PO(OR)$_2$]$^+$
	30	731, 533 [L−PO(OR)$_2$+Na]$^+$, 511, 313 [L−2PO(OR)$_2$]$^+$, 221
	50	731, 533, **221** [**HPO**(**OR**)$_2$+**Na**]$^+$
K^+	10	**748** [**L**+**K**]$^+$, 511 [L−PO(OR)$_2$]$^+$
	30	748, 511, 313 [L−2PO(OR)$_2$]$^+$
	50	748, 313, **237** [**HPO**(**OR**)$_2$+**K**]$^+$
Rb^+	10	**793**[**L**+**Rb**]$^+$, 511[L−PO(OR)$_2$]$^+$, 313[L−2PO(OR)$_2$]$^+$
	30	793, 511, 313
	50	793, 512, 313, **283** [**HPO**(**OR**)$_2$+**Rb**]$^+$
Cs^+	10	**841** [**L**+**Cs**]$^+$, 511 [L−PO(OR)$_2$]$^+$, 313 [L−2PO(OR)$_2$]$^+$
	30	841, 511, 313
	50	841, 511, 313
Mg^{2+}	10	**313** [**L**−**2PO**(**OR**)$_2$]$^+$, 511 [L−PO(OR)$_2$]$^+$, 831 (L+MgClO$_4$)$^+$
	30	**313** [**L**−**2PO**(**OR**)$_2$]$^+$, 511 [L−PO(OR)$_2$]$^+$, 831 (L+MgClO$_4$)$^+$
	50	**313 L**−**2PO**(**OR**)$_2$]$^+$, 831 (L+MgClO$_4$)$^+$
Ca^{2+}	10	**313** [**L**−**2PO**(**OR**)$_2$]$^+$, 511 [L−PO(OR)$_2$]$^+$, 848 (L+CaClO$_4$)$^+$
	30	**313** [**L**−**2PO**(**OR**)$_2$]$^+$, 511 [L−PO(OR)$_2$]$^+$, 848 (M+CaClO$_4$)$^+$
	50	**313** [**L**−**2PO**(**OR**)$_2$]$^+$, 511 [L−PO(OR)$_2$]$^+$
Ba^{2+}	10	313 [L−2PO(OR)$_2$]$^+$, **511** [**L**−**PO**(**OR**)$_2$]$^+$, 709 [L+H]$^+$, 944 (L+BaClO$_4$)$^+$
	30	**313** [**L**−**2PO**(**OR**)$_2$]$^+$, 511 [L−PO(OR)$_2$]$^+$, 709 [L+H]$^+$
	50	**313** [**L**−**2PO** (**OR**)$_2$]$^+$, 511 [L−PO(OR)$_2$]$^+$

Where R = –$CH_2CH_2OCH_3$ and maximum abundance of peaks (bold), all metal ions were used as ClO_4^- salts.

All these observations are in a good agreement with the calculated structure of the complex shown in Fig. 1. Moreover the NMR relaxation measurements of T_1 performed for these systems show perceivably change of longitudinal relaxation time of phosphorous atom. T_1 values for free receptor were equal to 1.22 s, while in the presence of metal ions such as Ba^{2+}, Ca^{2+} and Mg^{2+} they slightly decreased in the range of 1.08–1.06 s. However, not remarkable change of T_1 values was observed for proton signals.

3.2.2. Complexation of amino acids

Very good affinity of the studied receptor towards alkaline and rare earth metal ions allowed to assume that the same chelating properties might remain preserved towards ammonium cations from amino acid molecules. The adequate examples of ammonium chelation by ether oxygen atoms present in crown ethers have been intensively described in the literature [10]. Therefore, we have studied supramolecular interactions of receptor L with arginine and lysine dihydrochlorides. Upon titration of receptor with both amino acids ^{1}H NMR and ^{31}P NMR spectra revealed change of the chemical shifts of proton as well as phosphorous signals, which are clearly visible in both deuterated methanol and aqueous solutions. The observed changes of chemical shift exhibited the highest value recorded for methylene protons next to amine groups and α-CH protons.

Titration of the receptor in D_2O solution revealed that its both amino groups are surprisingly acidic having pK_a values of 4.0. Comparing ^{1}H and ^{31}P NMR chemical shifts of free receptor to the same shifts in the presence of amino acids versus pH showed no interaction between host and guest molecules. Therefore observed change in chemical shift is caused only by proton transfer from carboxylic group of amino acids (pK about 2) to more basic nitrogen atoms of receptor molecule. The lack of supramolecular interactions is confirmed by addition of dihydrochloride of ethylenediamine, a strong base, which did not influence on proton and phosphorous signals. The phosphorous esters groups are unable to interact with ammonium group of guest molecules most probably because of the bulkiness of compound L molecule, which does not allow optimal arrangement of 2-methoxyethanolic groups. The results presented above were confirmed by ESI-MS studies where lack of complexation was observed as well.

4. Conclusions

The molecule of tetra-2-methoxyethyl phenylene-1,4-di(benzyloaminomethanephosphonate) contains four crown ether-like esteric moieties in its structure and therefore binds monovalent and divalent metal ions with 1:1 stoichiometry, as revealed by ESI-MS and NMR studies. Moreover, the use of ^{31}P NMR T_1 relaxation experiments additionally confirmed the interaction between divalent metal ions and ligand [11]. However, in that case, metal ions bind additionally counter ions ClO_4^- thus forming ternary complexes. Surprisingly, this molecule is unable to bind positively charged functional groups of lysine or arginine in contrary to other phosphono peptide receptor (based on diaminophosphonate skeleton) [12]. This results most likely from rigidity of the receptor L molecule. NMR studies may suggest also the existence of the net of inter- and/or intramolecular interactions in the sole receptor molecules. Observed very low pK_a value of amine groups is most probably due to their interactions with located in a close proximity 2-methoxyethyl esteric groups.

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