Research Article

Rapid Communications in Mass Spectrometry

Received: 8 April 2015 Revised: 3 September 2015 Accepted: 6 September 2015 Published online in Wiley Online Library

Rapid Commun. Mass Spectrom. **2015**, *29*, 2272–2278
(wileyonlinelibrary.com) DOI: 10.1002/rcm.7386

Complexation of phosphates by 1,3-bis(3-(2-pyridylureido)propyl)-1,1,3,3-tetramethyldisiloxane

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RATIONALE: Compounds containing a urea or thiourea moiety form complexes with anions thanks to the ability to form quite strong hydrogen bonds. We have synthesized 1,3-bis(3-(2-pyridylureido)propyl)-1,1,3,3-tetramethyldisiloxane (**1**). Compound **1** contains two urea moieties connected by a long flexible linker; thus, it should be able to adopt a structure suitable for formation of quite stable complexes with anions.
METHODS: The ability to form complexes of compound **1** with phosphates was tested by electrospray ionization mass spectrometry (ESI-MS). Full scan ESI mass spectra and collision-induced dissociation tandem mass (CID-MS/MS) spectra of the ions of interest were obtained on a quadrupole time-of-flight (QTOF) mass spectrometer.
RESULTS: It has been found that compound **1** is not only much more prone to form complexes with the phosphate anion than with other inorganic anions, but it is also able to form complexes with organic phosphates, namely nucleotides and phospholipids. However, compound **1** is not able to form complexes with organic compounds not containing a phosphate group (e.g. nucleosides, sugars, glycerolipids).
CONCLUSIONS: Compound **1** can be regarded as selective towards phosphate-containing organic compounds. Formation of such complexes may have some interesting applications for identification of organic phosphates in crude extracts from biological materials. Copyright © 2015 John Wiley & Sons, Ltd.

Compounds containing a urea or thiourea moiety form complexes with anions thanks to the ability to form quite strong hydrogen bonds.[1–10] Some urea (or thiourea) conjugates were prepared in order to form stable complexes with the phosphate anion (usually with dihydrogen phosphate $H_2PO_4^-$).[11–16] From the biological point of view, complexation of organic phosphates is more important than complexation of phosphate anions, Therefore, the literature gives many examples of synthesized organic compounds capable of formation of complexes with nucleotides.[17–22] Formation of such complexes may be useful for nucleotide analysis. Besides nucleotides, another important group of organic phosphates are phospholipids. There are a vast number of papers devoted to the analysis of phospholipid, e.g. by mass spectrometric techniques.[23–26] However, to the best of our knowledge, formation of complexes between phospholipids and any organic compounds has not been demonstrated yet.

In this paper, it is demonstrated, by using electrospray ionization mass spectrometry (ESI-MS), that 1,3-bis(3-(2-pyridylureido)propyl)-1,1,3,3-tetramethyldisiloxane (compound **1**, Scheme 1) is able to form complexes not only with the phosphate anion, but also with organic phosphates, namely nucleotides and phospholipids. Compound **1** contains two urea moieties connected by a long flexible linker, thus it should be able to adopt a structure suitable to form quite a stable complex with the anion. We also have prepared an analogous compound containing a thiourea moiety (**2**, Scheme 1); however, compound **2** was found to be less prone to form complexes with the phosphate anion (see Supporting Information).

As demonstrated further, formation of a complex between **1** and an organic phosphate may be useful for identification of organic phosphates in crude extracts from biological materials.

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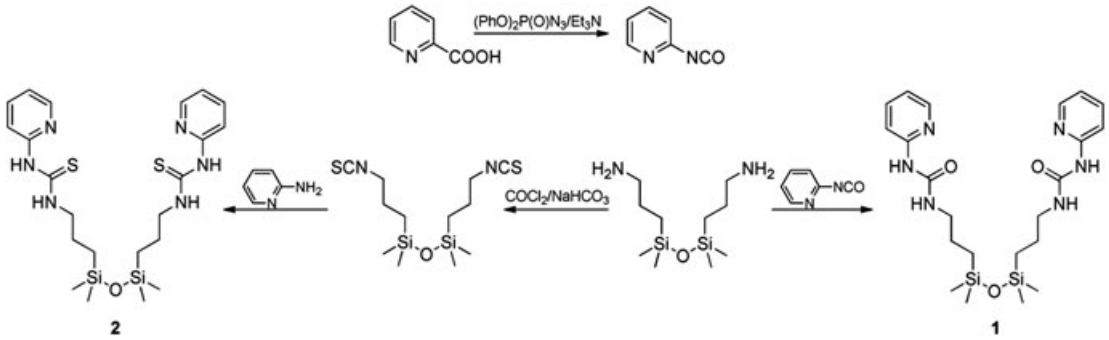
EXPERIMENTAL

Besides **1** and **2**, other compounds used (e.g. Na_2HPO_4, nucleotides, phospholipids) were obtained from commercial sources (mainly Sigma-Aldrich, Poznań, Poland) and used without purification.

Synthesis of 1 and 2

*1,3-Bis(3-(2-pyridylureido)propyl)-1,1,3,3-tetramethyldisiloxane (**1**)*

Portions of picolinic acid (1 g; 8 mmol) and 2.34 g of diphenylphosphoryl azide (8.5 mmol) were dissolved in dry toluene (40 mL). Then 0.85 g of triethylamine (8.5 mol) was added and the mixture obtained was stirred at room temperature for 1 h. After that the solution was heated to 80°C over 2 h. Then the solvent was evaporated and the semisolid residue was dissolved in dry chloroform. To a solution of crude 2-pyridyl isocyanate, 1.06 g of 1,3-bis(3-



Scheme 1. Synthesis of compounds **1** and **2**.

aminopropyl)-1,1,3,3-tetramethyldisiloxane (4.05 mmol) was added and the resulting mixture was heated under reflux over 48 h. After solvent evaporation, the formed urea was purified by chromatography on SiO_2 (diethyl ether/methanol; gradient from 10:0 to 9:1 v/v). The product was obtained as a white, crystalline solid (980 mg; 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.86 (bs, 2H), 8.20 (ddd, J = 5.1, 1.9, 0.9 Hz, 2H), 8.17 (bs, 2H), 7.61 (m, 2H), 7.35 (bs, 2H), 6.88 (dd, J = 6.7, 5.6 Hz, 2H), 3.34 (m, 4H), 1.64 (m, 4H), 0.59 (m, 4H), 0.10 (s, 12H).^{13}C NMR (100 MHz, $CDCl_3$): δ 156.3, 153.8, 146.0, 138.4, 116.8, 112.3, 42.7, 24.1, 15.6, 0.3.

1,3-Bis(3-isothiocyanatopropyl)-1,1,3,3-tetramethyldisiloxane

To a solution of 1 g of 1,3-bis(3-aminopropyl)-1,1,3,3-tetramethyldisiloxane (4 mmol) in dichloromethane (30 mL), a 5% aqueous solution of $NaHCO_3$ was added (30 mL). The mixture was stirred vigorously and a solution of 1.15 g of thiophosgene (10 mmol) in 20 mL of dichloromethane was added dropwise over 0.5 h at room temperature. Then the solution was refluxed for 2 h, the organic phase was separated, washed with water and dried with Na_2SO_4. After solvent evaporation, the isothiocyanate was obtained as a brownish liquid (1.4 g; 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.49 (t, J = 6.7 Hz, 4H), 1.70 (m, 4H), 0.60 (m, 4H), 0.08 (s, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 129.4, 112.3, 47.8, 24.5, 15.4, 0.2.

*1,3-Bis(3-(2-pyridylthioureido)propyl)-1,1,3,3-tetramethyldisiloxane (**2**)*

A portion of 1 g of 1,3-bis(3-isothiocyanatopropyl)-1,1,3,3-tetramethyldisiloxane (2.8 mmol) was dissolved in dry toluene (100 mL) and 0.55 g of 2-aminepyridine (5.9 mmol) was added. The solution obtained was refluxed for 14 days. After that the solvent was evaporated and the product was purified by chromatography on silica (diethyl ether/hexane 9:1 v/v). The thiourea was obtained as a yellowish red, viscous oil (660 mg; 45%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.69 (bt, 2H), 8.93 (bs, 2H), 8.11 (dd, J = 5.2, 2.0 Hz, 2H), 7.57 (m, 2H), 6.90 (m, 2H), 6.79 (d, J = 8.3 Hz, 2H), 3.68 (m, 4H), 1.70 (m, 4H), 0.60 (m, 4H), 0.05 (s, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 179.3, 153.4, 145.7, 138.6, 117.8, 112.1, 48.5, 22.8, 15.7, 0.3.

Isolation of egg lecithin

Egg yolk lipids were extracted with ethanol/diethyl ether (1:2 v/v; 75 ml). The precipitate was filtered off and the solvent was evaporated. The oily residue was dissolved in diethyl ether (50 mL) and the solution was dried with anhydrous Na_2SO_4. The crude lipid fraction, obtained after solvent evaporation, was used for mass spectrometric experiments (concentration of the sample solution was 5 μg/mL).

Isolation of hen liver lipids

A portion of 5 g of fresh chicken liver was frozen in liquid nitrogen and carefully ground. The material obtained was extracted with 25 mL of chloroform/methanol (2:1 v/v). The semisolid mass was filtered off and then the solvents were evaporated in vacuum. The mixture of lipids, obtained as an oily residue, was used without further purification for mass spectrometric experiments (concentration of the sample solution was 5 μg/mL).

Electrospray ionization mass spectrometry

Full scan ESI mass spectra and ESI-CID-MS/MS spectra were recorded on a Q-tof Premier mass spectrometer (Waters/Micromass, Manchester, UK) (software MassLynx version 4.1). The sample solutions, containing 5×10^{-6} mol/dm^3 (unless indicated otherwise) of the compound analyzed in methanol, were infused into the ESI source by a syringe pump at a flow rate of 5 μL/min. The electrospray voltage was 2.7 kV and the cone voltage −30 V. The source temperature was 80°C and the desolvation temperature was 250°C. Nitrogen was used as the cone gas and desolvating gas at flow rates of 50 and 400 L/h, respectively. Argon was used as a collision gas at a flow rate of 0.5 mL/min in the T-wave collision cell. The applied collision energy (CE, laboratory frame), the most important parameter for CID-MS/MS experiments, is indicated in each CID-MS/MS spectrum shown.

RESULTS AND DISCUSSION

At first it had to be demonstrated that **1** forms more stable complexes with the phosphate anion than with other inorganic anions. Figure 1(a) shows the ESI mass spectrum (for clarity in a narrow *m/z* range of interest) obtained for

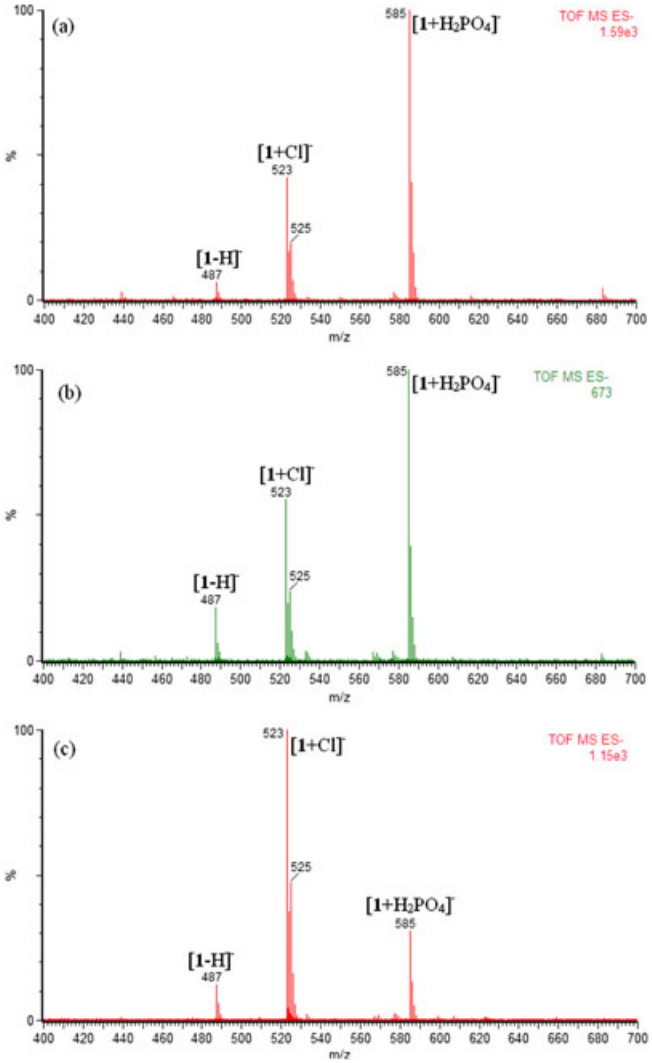


Figure 1. ESI mass spectrum (for clarity in the narrow *m/z* range of interest) obtained for a solution containing **1** and phosphate (Na_2HPO_4) at the same concentrations of 5×10^{-6} mol/dm^3 (a); **1** and phosphate at the same concentrations of 5×10^{-6} mol/dm^3 and chloride (NaCl) added also at a concentration of 5×10^{-6} mol/dm^3 (b); **1** and phosphate at the same concentrations of 5×10^{-6} mol/dm^3 and chloride added at a concentration 5×10^{-5} mol/dm^3 (c).

solution containing **1** and phosphate at the same concentrations of 5×10^{-6} mol/dm^3. The most abundant ion is $[\mathbf{1}+H_2PO_4]^-$ (complex between **1** and dihydrogen phosphate anion), while the $[\mathbf{1}–H]^-$ ion occurs at a low abundance. Although chlorides were not added to the solution analyzed, there is also a quite abundant ion $[\mathbf{1}+Cl]^-$. Chlorides are common contaminants and, at the level 10^{-5} to 10^{-6} mol/dm^3, it is difficult to control their concentration (or to remove them). Figure 1(b) shows the ESI mass spectrum obtained for a solution containing **1** and phosphate at the same concentrations of 5×10^{-6} mol/dm^3 and added chloride also at a concentration of 5×10^{-6} mol/dm^3 (obviously total concentration of Cl^- is higher). As shown in Fig. 1(b), the ion $[\mathbf{1}+H_2PO_4]^-$ is twice as abundant as the $[\mathbf{1}+Cl]^-$ ion. Figure 1(c) shows the ESI mass spectrum obtained for a solution containing **1** and phosphate at the same concentrations of 5×10^{-6} mol/dm^3 and chloride added also at a concentration of 5×10^{-5} mol/dm^3. Although there was a high excess of chloride, the $[\mathbf{1}+H_2PO_4]^-$ ion is still quite abundant.

Attachment of the chloride anion to organic compounds is a well-known phenomenon in negative ESI-MS[27–29] and it is not surprising that chloride attachment is also observed for **1**. However, as clearly seen in Fig. 1, **1** is more prone to form an adduct with $H_2PO_4^-$ than with Cl^-. Obviously, it would be better if the binding of Cl^- by **1** could be avoided. However, it seems to be rather difficult. If we add a compound which binds chlorides, it would also at least slightly bind phosphates.

Obviously, we also compared the ability of **1** to form complexes with $H_2PO_4^-$ with that to form complexes with other inorganic ions. The respective mass spectra are shown in the Supporting Information. Adducts with other inorganic ions were less abundant than the $[\mathbf{1}+H_2PO_4]^-$ ion,

At the next step of the research we tested the ability of **1** to form complexes with organic phosphates, at first with simple nucleotides. Figure 2 shows the ESI mass spectra obtained for a solution containing **1** and uridine monophosphate – UMP. As follows from the spectra, the ion $[\mathbf{1}+UMP–H]^-$ is not very abundant (not more abundant than $[\mathbf{1}+Cl]^-$), but gives a clearly seen signal; the CID MS/MS spectrum of the $[\mathbf{1}+UMP–H]^-$ ion confirmed its composition, as the fragment ion $[UMP–H]^-$ was formed (Fig. 2). It has to be stressed that the complex between **1** and uridine was not detected, as demonstrated in the Supporting Information. Thus, a conclusion can be drawn that the complex between **1** and UMP is formed thanks to the presence of the phosphate group.

Next, we extended the studies to more complex nucleotides, namely nicotinamide adenine dinucleotide – NAD. As presented in Fig. 3, the adduct between **1** and NAD was formed; the CID MS/MS spectrum of the ion $[\mathbf{1}+NAD–H]^-$

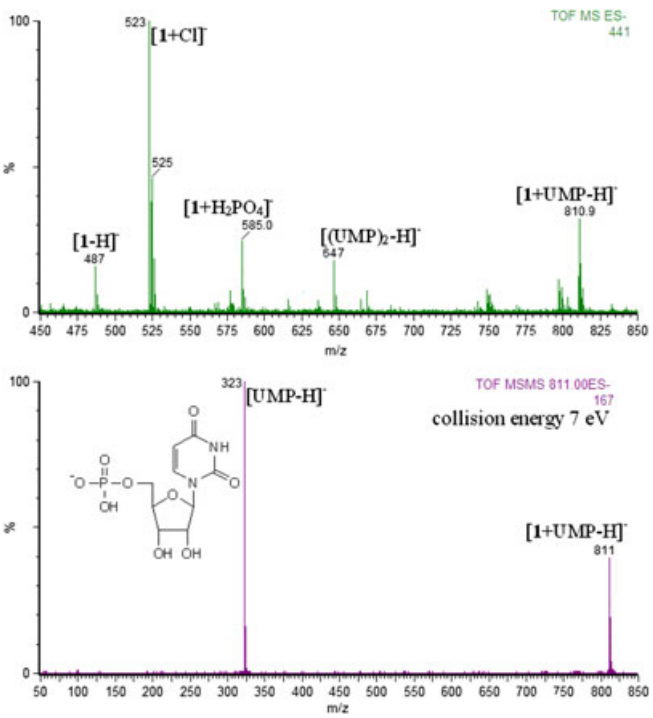


Figure 2. ESI mass spectra obtained for solution containing **1** and uridine monophosphate (UMP) (top: full scan mass spectrum, bottom: CID MS/MS spectrum).

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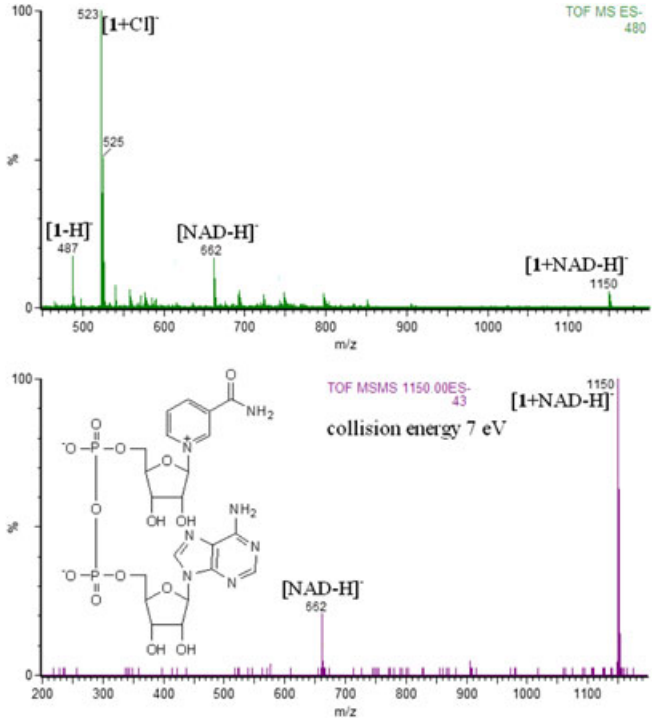

Figure 3. ESI mass spectra obtained for a solution containing **1** and nicotinamide adenine dinucleotide (NAD) (top: full scan mass spectrum, bottom: CID MS/MS spectrum).

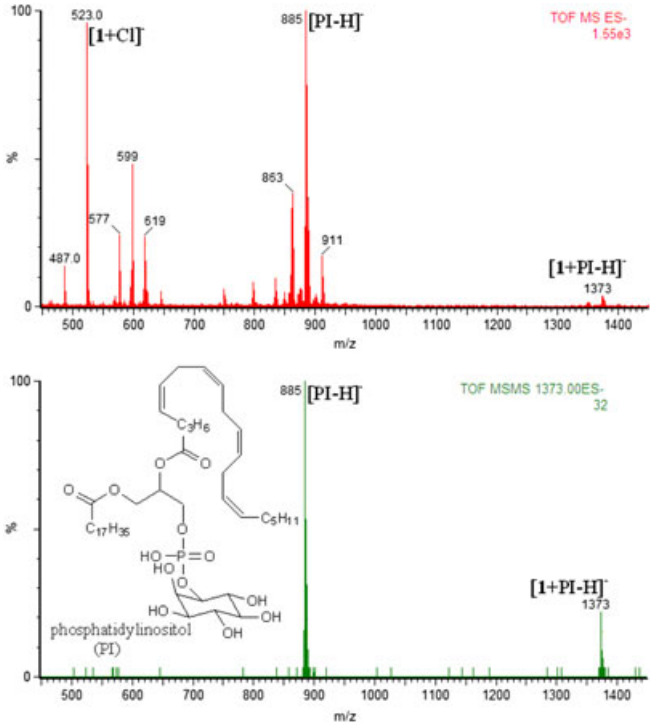

Figure 4. ESI mass spectra obtained for a solution containing **1** and phosphatidylinositol (PI) (top: full scan mass spectrum, bottom: CID MS/MS spectrum).

confirmed its composition as the fragment ion $[NAD–H]^-$ was formed (Fig. 3). It has to be stressed that the respective adducts between **1** and adenosine, or between **1** and nicotinamide, were not detected (Supporting Information). Thus, analogous with the UMP adduct, ion [**1**+NAD–H] was formed thanks to the presence of the phosphate group (in this compound it was a diphosphate group).

It is worth adding that, theoretically, it is possible to form a number of hydrogen bonds between **1** and uridine, between **1** and adenosine, as well as between **1** and nicotinamide. However, the respective adducts were not detected (Supporting Information). Thus **1** is prone to form complexes with organic phosphates but not with the related organic compounds.

At the next stage we studied phospholipids. It is clear that for these compounds access of **1** to the phosphate group is hampered, thus it is expected that respective adducts will not be abundant.

Figure 4 shows the full scan ESI mass spectrum obtained for a solution containing **1** and phosphatidylinositol (PI). Besides the peaks originated from **1** and/or phosphatidylinositol there are other intense peaks which are difficult to rationalize. However, the complex in question was detected (ion $[\mathbf{1}+PI–H]^+$) and its CID MS/MS spectrum (Fig. 4) confirmed that we really deal with the complex between **1** and phosphatidylinositol.

It should be stressed that the complex between glycerol 1-monooleate and **1**, as well as between sugar molecules and **1**, was not detected, as shown in the Supporting Information. Thus, the complex between **1** and PI is formed thanks to the presence of the phosphate group.

A very common group of phospholipids are those containing the choline moiety, i.e. the $-OCH_2CH_2-N^+\equiv(CH_3)_3$ moiety. Complexes between **1** and phospholipids containing a choline moiety were detected in both negative and positive ion mode. In the positive ion mode the respective sodium-containing adducts were observed and these signals were more abundant than the signals observed in the negative ion mode. Figure 5(a) shows the full scan ESI mass spectrum obtained for a solution containing **1** and lysophosphatidylcholine (LPC). Similarly as for phosphatidyl-inositol, there are a number of signals which are difficult to rationalize. However, the complex in question was detected and its CID MS/MS spectrum (Fig. 5(b)) confirmed that we really deal with the complex between **1** and lysophosphatidylcholine.

One can argue that the complex between **1** and lysophosphatidylcholine is formed thanks to the presence of a quaternary nitrogen atom (electrostatic attraction). However, as shown in the Supporting Information, the complex between $C_{14}H_{29}-N^+\equiv(CH_3)_3$ and **1** was not detected.

At the next stage of our study, we isolated phospha tidylcholines from the yolk of hen egg (these compounds are sometimes called egg lecithins). It is known that phosphatidylcholine (PC) of mass 759 is the most common in yolk, the protonated and sodiated adducts were observed at *m/z* 760 and 782, respectively.[30–32] As shown in Fig. 6 the complex of this phosphatidylcholine was detected in the full scan mass spectrum and the CID MS/MS spectrum confirmed the presence of egg lecithin.

Upon analysis of the yolk we knew which phospholipid was dominant in it, thus we knew what we were looking for (the published data). Hen liver was analyzed in terms of fatty acids present in phospholipids;[33–36] however, to the best of our knowledge, there is no data concerning the specific phospholipid present in hen liver. Therefore, at the final stage of our study, we decided to analyze a crude extract from hen liver.

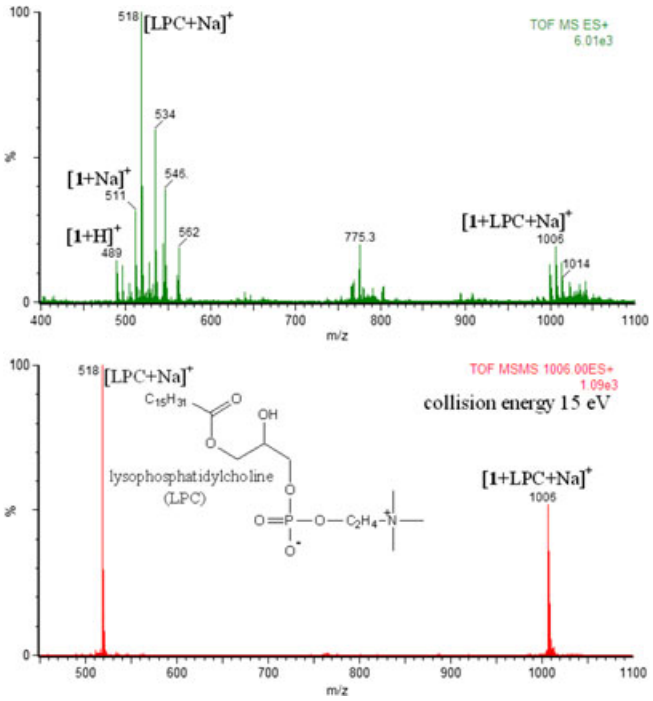

Figure 5. ESI mass spectra obtained for a solution containing **1** and lysophosphatidylcholine (LPC) (top: full scan mass spectrum, bottom: CID MS/MS spectrum).

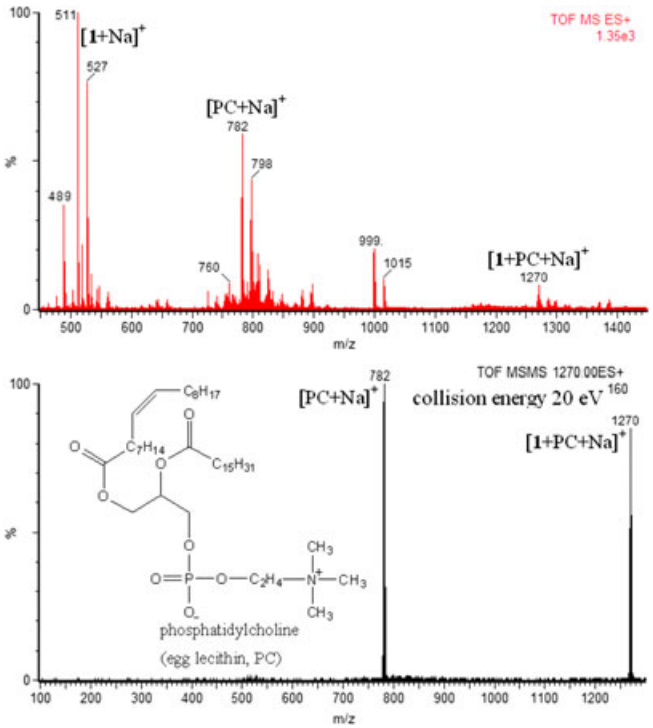

Figure 6. ESI mass spectra obtained for a solution containing **1** and phosphatidylcholine (PC) isolated from egg yolk (top: full scan mass spectrum, bottom: CID MS/MS spectrum).

Figure 7 shows the full scan ESI mass spectrum (negative ion mode) obtained for a solution containing **1** and the crude extract.

In the *m/z* range where the signals of the complex between **1** and phospholipids were expected, we detected a small signal at

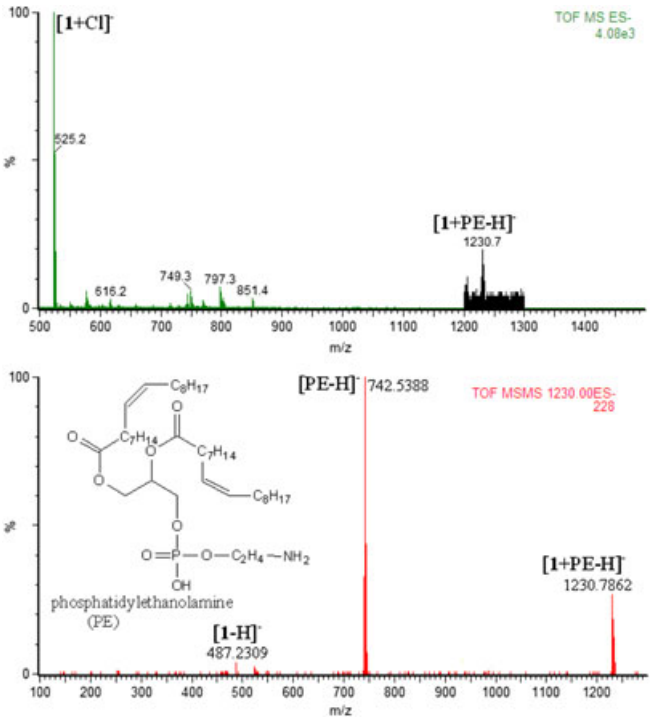

Figure 7. ESI mass spectra obtained for a solution containing **1** and crude extract from hen liver (top: full scan mass spectrum, bottom: CID MS/MS spectrum of ion at *m/z* 1230).

m/z 1230. The CID MS/MS spectrum of the ion at *m/z* 1230 (Fig. 7) revealed a fragment ion at *m/z* 742 and a small fragment ion [**1**–H]$^-$ (*m/z* 487). The ion at *m/z* 742 may correspond to the deprotonated molecule of phosphatidylethanolamine (PE) containing two oleic acid residues (Fig. 7). By using the ion [**1**–H]$^-$ as a lock mass (*m/z* 487.2309), we obtained the exact mass of the ion at *m/z* 742, namely 742.5388, which is in good agreement with the deprotonated molecule of phosphatidylethanolamine (PE) containing two oleic acid residues (composition $C_{41}H_{77}NPO_8$).

CONCLUSIONS

It has been demonstrated by using ESI-MS that compound **1** (1,3-bis(3-(2-pyridylureido)propyl)-1,1,3,3-tetramethyldisiloxane) is very prone to form complexes with the phosphate anion. Compound **1** forms complexes with other inorganic anions; however, its complexation ability toward other inorganic anion is definitely lower than toward the phosphate anion. The high ability of **1** to form complexes with the phosphate anion resulted in the ability of **1** to form complexes with organic phosphates. In this work we tested nucleotides and phospholipids. It has to be stressed that **1** does not form complexes with organic compounds containing no phosphate group (e.g. **1** forms a complex with uridine monophosphate but not with uridine). Thus, it can be concluded that **1** is selective towards phosphate-containing organic compounds.

We are aware that the methodology described here does not allow performance of comprehensive, qualitative and quantitative, phospholipid analysis as for example the method described by Liu *et al.*[37] However, sometimes, relatively time-

consuming comprehensive phospholipid analysis may be not necessary. Our method allows in less than half an hour (obviously provided that compound **1** has been earlier synthesized) to identify the main phospholipids present in biological samples, which sometimes may be enough.

Formation of a complex between **1** and organic phosphates may be applied in initial fast qualitative analysis, by using direct-inlet ESI/MS, of organic phosphates in crude extracts from biological materials (Figs. 6 and 7). When we deal with crude extracts, there are often a vast number of signals in the ESI mass spectra. Therefore, it may be difficult to identify those derived from phosphates. As demonstrated in Fig. 7, formation of a complex between **1** and organic phosphates (even with low abundance) may be useful for identification of signals derived from organic phosphates, as a consequence allowing the identification of organic phosphates in biological samples (obviously MS/MS analysis of uncomplexed phosphate may be also desirable).

Acknowledgement

This work was supported by the Polish National Science Centre (NCN; Grant No. N N204 155840).

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