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Complexing Properties of Synthesized 1,3,5-Triaza-7-Phosphaadamantane Derivatives Towards Some Lanthanides and Transition Metal Cations With Significant Antimicrobial and Antioxidant Activities

Lassaad Baklouti[1], Besma Mellah[2], and Waleed S. Koko[3]

Abstract

The synthesis of new water-soluble N-alkylated derivatives of 1,3,5-triaza-7-phosphaadamantane is presented. $Ru(PPh_3)_2Cl_2$ has been used to react with 1-(4-nitrobenzyl)-3,5-triaza-1-azonia-7-phosphaadamantane bromide (PTAR). By using elemental analysis, NMR, and IR spectroscopy, the obtained compounds were identified. The UV-visible absorption spectroscopy has been used to monitor the complexation of various transition metal cations. Studies on conductivity have been utilized to validate the complexes' stoichiometries. Using the disc diffusion method, five bacteria strains were used for the study of the antimicrobial activity of compounds **1-3**. All tested pathogens, including *M luteus* LB 141107, were found to have strong biologic activity against the compounds tested in this study. Additionally, DPPH (2,2-diphenyl-1-picrylhydrazyl) has been tested for its ability to scavenge hydrogen peroxide and free radicals. According to our results, these compounds exhibit excellent radical scavenging properties.

Keywords

1,3,5-triaza-7-phosphaadamantane, complexing properties, lanthanides, transition metal, water soluble, antimicrobial activity, antioxidant activity

Introduction

Phosphine ligands with sulfonated aryl groups account for the majority of ligands used for water solubilization of organometallic derivatives, but there are other ligands on phosphines that confer water solubility. There is an increasing investigation involving other types of substituents on phosphines which impart water solubility.[1] These include, for example, cationic ammonium groups ($Ph_2PCH_2CH_2NMe_3^+$ and $Cy_2PCH_2CH_2NMe_3^+$)[2-5] and carboxylated aromatic groups (**2**).[6] Nonionic aliphatic phosphine ligands with higher basicity include tris(hydroxymethylphosphine), $P\text{-}(CH_2OH)_3$,[7,8] $(HOCH_2)_2PC_6H_4P(CH_2OH)_2$,[9] and Includes PTA (**3**).[10,11] It is also possible to alkylate or protonate one of the nitrogen bases of the latter ligand to provide the ionic ligands $PTAMe^+$ and $PTAH^+$, respectively, which provide additional water solubility properties. Among the many water-soluble phosphines currently available, the adamantane-like phosphane 1,3,5-triaza-7-phosphaadamantane (PTA) exhibits similar reactivity to other alkylphosphines and is air-stable. It is of particular

[1] Laboratory of Applied Chemistry and Natural Substances Resources and Environment, Faculty of Sciences, University of Carthage, Bizerta, 7021, Tunisia
[2] National Center of Researches in Material Sciences (CNRSM), Soliman, 2050, Tunisia
[3] Department of Science Laboratories, College of Science and Arts, Qassim University, Ar Rass, Saudi Arabia, 51921

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Corresponding Author:
Lassaad Baklouti, Laboratory of Applied Chemistry and Natural Substances Resources and Environment, Faculty of Sciences, University of Carthage, Zarzouna, Bizerta 7021, Tunisia.
Email: bakloutilassaad@yahoo.fr

interest due to its high price and high chemical resistance. It is more resistant to oxidation than other water-soluble phosphines.[12-14] In addition, N-alkyl derivatives of PTA can also be used as ligands for Ru complexes.[15-36] In recent years, the coordination chemistry of PTA has seen a pronounced development justified by the search for water-soluble transition metal complexes.[37] Although Ru, Os, Au, Pt, and Cu compounds bearing PTA and derived ligands are known as rather potent antimicrobial and/or antitumor agents,[38-47] the biological properties of Ru–PTA derivatives remain poorly explored.[48] We describe herein the complexation properties of some functionalized PTA molecules towards some lanthanides and transition metal cations. Quaternization of tertiary phosphine in competition with a tertiary amine group has been often been reported in the litterature.[26,49-51]

In addition, the antimicrobial and antioxidant activities of the obtained compounds were studied.

Results and Discussions

Synthesis of N-alkylated PTA Salt

After adding 4-nitrobenzyl chloride to a suspension of PTA in THF, the quaternary N-alkylated PTA compound **2** was obtained. The reactions were run under reflux under N_2 for 2 h. The intended monoalkylated ammonium salt was obtained as a colorless solid after an appropriate reaction time (Scheme 1).

A bialkylated PTA salt was not observed, even after refluxing in MeOH with 2.5 equivalents of 4-nitrobenzylchloride **2**.

The ^{1}H NMR spectrum of the water-soluble phosphine **2** is slightly more complex than PTA, due to the alkylation of one of the N atoms which reduces the symmetry of the molecule. The methylene NCH_2N protons of all these phosphines are diastereotopic, whereas the methylene protons of NCH_2N^+ groups with alkylated N atoms appear as the AB system. The ^{31}P{1H} NMR spectrum shows a singlet in the range $\delta = -81.7$ ppm.

Signals from methylene units between the quaternary and tertiary nitrogens are at $\delta = 4.90$–5.40 ppm. The methylene moiety adjacent to the quarternary nitrogen and the ester group was detected.

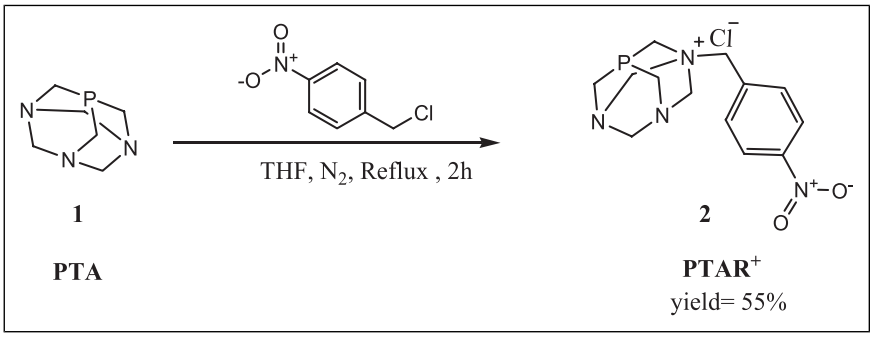
The signals of CH_2-N of PTA were also found in the same range. The solvent effects seem to play a major role in the chemical shifts in the proton NMR. The proton-phosphorus coupling constants varied from JP,H = 10.5 Hz (NCH_2P; 2) to 6.0 Hz (N^+CH_2P; 2) and 6.5 Hz (N^+CH_2P; 2). The carbon–phosphorus coupling constants to the two carbons adjacent to the tertiary nitrogen were also found to be similar for 8, 9, and 10 (JP,C = 21.2 Hz). Also the coupling constants to the quarternary nitrogen were in the same range (JP,C = 34.5–35 Hz).

Scheme 2 depicts the reaction of 1-(4-nitrobenzyl)-1,3,5-triaza-7-phosphaadamantan-1-ium (PTAR) derivatives with $Ru(PPh_3)_3Cl$ precursors to form complex **3**. Synthesis was performed by reacting equimolar amounts of reagents in a mixture of hot methanol and toluene (7:3) under a nitrogen atmosphere for 2 hours. The solid was isolated by filtration, washed with THF and diethyl ether, and dried under reduced pressure. The synthesized complex **3** was characterized by ^{1}H NMR spectroscopy.

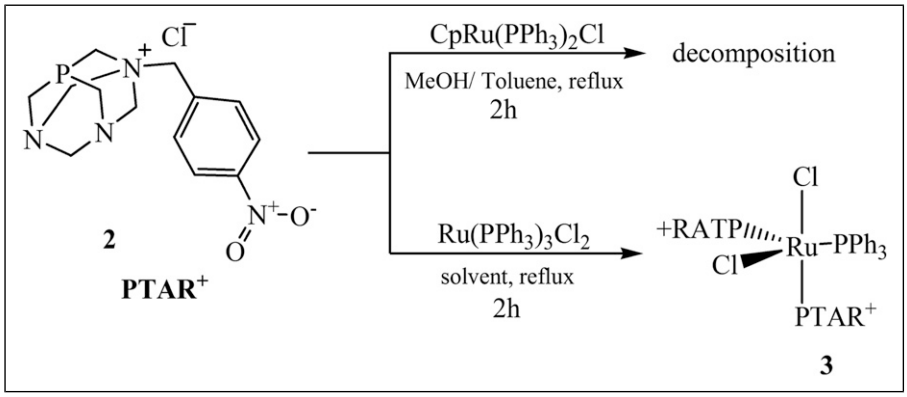
Similarly, reaction of 1-(4-nitrobenzyl)-1,3,5-triaza-7-phosphaadamantan-1-ium (PTAR) with $CpRu(PPh_3)_3Cl$ in a mixture of hot methanol and toluene (7:3) under nitrogen for 2 h results in the formation of rapidly decomposing products.

The complex **3** has been characterized by elemental analysis, IR, ^{1}H, ^{13}C, and ^{31}P NMR spectroscopies. The IR spectra of **3** show a set of characteristic vibrations in the 1450-500 cm^{-1} region, which can be attributed to the coordinated PTA cage. The C-H stretching bands for the alkyl groups as well as for the methylene group of the PTA cage appeared as medium bands around 2985-2899 cm^{-1}. The ^{1}H NMR spectrum of the complex (**3**) shows two types of methylene protons. One of them, P-CH_2-N, occurs as a singlet at 4.54 ppm, respectively. The second type, N-CH_2-N, displays for 1 an AB spin system centered at 4.64 ppm, assigned to the N-CHax-N and the N-CHeq-N protons.

The ^{1}H NMR spectra of the water-soluble phosphanes **2** are rather more complicated than that for PTA since alkylation of one of the N atoms reduces the symmetry in the molecule.



Scheme 1. Synthetic route to compound **2**.



Scheme 2. Synthetic route to compound **3**.

Consequently, the methylene protons are inequivalent and, in some cases, diastereotopic. The methylene NCH_2N protons in all these phosphanes are diastereotopic; although, the methylene protons of the NCH_2N + group with one alkylated N atom appear as an AB system. The $^{31}P\{1H\}$ NMR spectra display a singlet in the range between $\delta = -81.7$ ppm, downfield from that for free PTA.

Complexation of PTAR$^+$ and Complex 3 in Methanol

In methanol at 25°C, complexation investigations of $PTAR^+$ and complex **3** to several lanthanides and transition metals were conducted. These investigations were followed by UV-Vis spectroscopy in the wavelength range of 210–330 nm and the results were processed by the digital program "Letagrop."[52] The UV spectra of $PTAR^+$ (ligand **2**) and Ru complex (ligand **3**) showed absorption maxima at 258 nm and 245 nm, respectively. In addition, a shoulder appears for ligand **3** at 254 nm. The addition of metal cations to the ligand solution changes the shape of the ligand spectrum. In general, spectral changes are manifested as a decrease in absorbance, but copper and chromium with both ligands show an increase in absorbance. Furthermore, the complexation of cobalt, tin (Figure 1 and 2), and samarium is represented by the formation of isosbestic points, indicating the presence of at least two species in solution.

By using letagrop to process UV spectra, it is possible to determine the stoichiometries of each obtained complex as well as the logarithm of their stability constant $\log\beta_{xy}$. All results are given in Tables 1 and 2.

The complex formed by ligand **2** are generally a mixture of mono and binuclear, only in the case of Co_2^+ and Ag^+, the complexes are either M_2L or ML, respectively. The stability constants shift between 2.98 and 4.27 logarithmic units for ML and between 3.70 and 8.47 logarithmic units for M_2L. Although M_2L is produced, it exhibits a low partial stability constant compared to ML (.53 logarithmic units for Sn^{2+}<log β_{21} < 3.60 logarithmic units for Mn^{2+}). This observation indicates the negative cooperative effect of ML for the arrangement of M_2L. Besides, the complexes of Cu^{2+} and Mn^{2+} are more stable than the others.

In the case of the Ru-*complex-as-a-ligand* **3**, only binuclears are formed with transition metal cations and mononuclears with cadmium and silver. The binuclear species exhibit almost same order of stability expect for Co^{2+} where the complex $[Co.\mathbf{3}]^{2+}$ is around 100 times more stable than others as shown on Figure 3. Opposing the Irving–Williams rules,[53] the higher stability of the Co-Ru complex may indicate the easy inclusion of Co^{2+} inside the cavity of PTA and the best fitting between both volumes. Moreover, it is known that the intermetallic cooperativeness between cobalt and ruthenium in the hetero-bimetallic complexes enhances their photophysical, electrochemical, and magnetic properties, which expand their potential applications as catalyst for CO hydrogenation, sensors, and in material science.[54-58]

In the case of lanthanidesLa^{3+}, Ce^{3+}, and Sm^{3+}, complexes ML are formed with ligand 2 and M_2L with ligand **3**. In both cases, higher affinities in favor of Ce^{3+} are observed. The selectivity $S(_{Ce}{}^{3+}/_{Sm}{}^{3+})$ is around 10 for ML and 1500 for M_2L. The complexation of two trivalent cations by ligand **3** could be explained by either the presence of two PTA units or the interesting metal–metal bonding interactions between Ru and lanthanides leading to useful cooperative chemical reactivity.

Stoichiometry of some metals cation complexes with ligands **2** and **3** were confirmed by conductimetric studies (Figures 4 and 5).

Biological Activities

Compounds **1-3** have been shown to exhibit in vitro antibacterial activity against various bacteria utilizing the diffusion method. The obtained results are given in the following Table 3:

According to Table 3, compound **1** is the most active against *M. luteus LB* 141107 with an inhibition zone of 33 mm. Additionally, **1** showed the greatest activity *S. Typhimurium* ATCC 14028 with an inhibitory region of 25 mm.

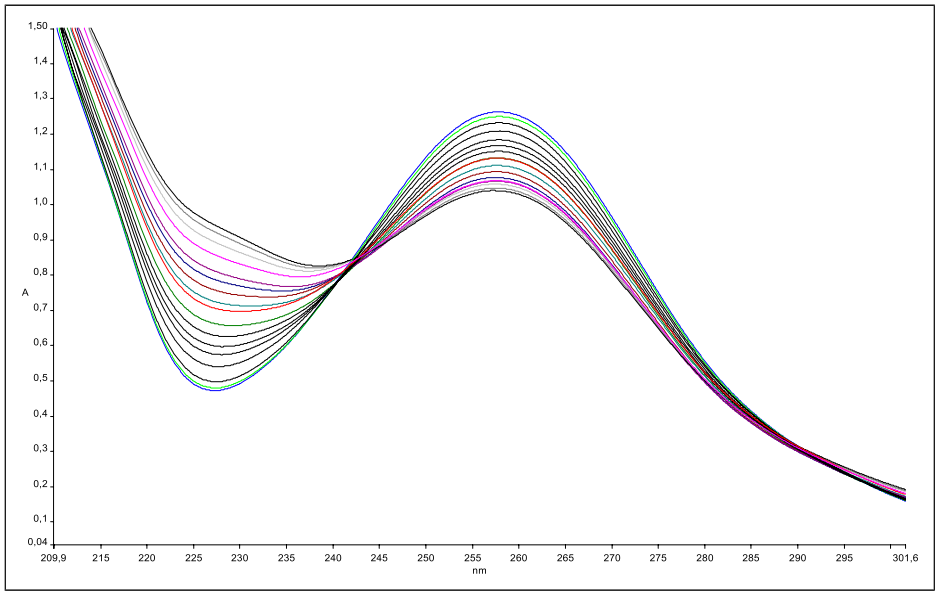


Figure1. Complexation of Co^{2+} by ligand **2**, $C_L = 9.7 \times 10^{-5}$, $C_M = 4.9 \times 10^{-3}$, $R = C_M/C_L = 20$.

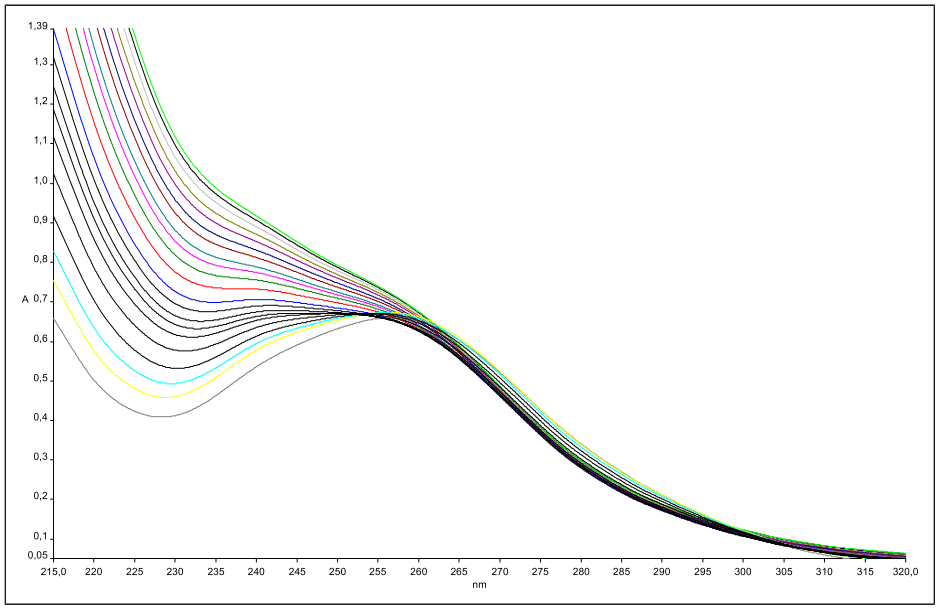


Figure 2. Complexation of Sn^{2+} by ligand **3**, $C_L = 3.9 \times 10^{-5}$, $C_M = 4.9 \times 10^{-3}$, $R = C_M/C_L = 20$.

Table 1. Stability Constants ($\log\beta_{xy} \pm \sigma_{N-1}$) of Complexes **2, 3** With Transition Metal Cations, in Methanol at 25°C, $I = 10^{-2}$ M.

	M:L	Mn^{2+}	Co^{2+}	Ni^{2+}	Cu^{2+}	Sn^{2+}	Cd^{2+}	Ag^{+}
2	**1:1**	4.27 ± .08	—	2.98 ± .08	3.49 ± .03	3.17 ± .11	2.89 ± .05	3.24 ± .05
	2:1	7.87 ± .03	5.36 ± .20	6.00 ± .04	5.66 ± .16	3.70 ± .16	5.91 ± .12	—
3	**1:1**	—	—	—	—	—	5.24 ± .20	5.58 ± .12
	2:1	5.74 ± .11	7.71 ± .19	5.81 ± .11	5.67 ± .02	5.79 ± .11	—	—

Also the compound **2** was found strongly active against *M. luteus LB 141107* with inhibition zone of 26 mm. Tetracycline was used as a benchmark when comparing the obtained results. Actually, in the current experiment, we followed many of the standardization mentioned in both CLSI and EUCAST specially those related to our assay by disc diffusion technique such as temperature, incubation period, media, and standard drugs.[59-63]

Table 4 shows the values of MIC ranging from .142 to .164 µg ml^{-1} for *Listeria monocytogenes* ATCC 1911 and .134 to .156 µg mL^{-1} for *Staphylococcus aureus* ATCC 6538. From .156 to .311 µg mL^{-1} correspond to *Salmonella typhimurium* ATCC 14028.

Table 2. Stability Constants ($log\beta_{xy} \pm \sigma_{n-1}$) of Complexes of Lanthanides, in Methanol at 25°C, I = 10^{-2} M.

	M:L	La^{3+}	Ce^{3+}	Sm^{3+}
2	**1:1**	3.66 ± .06	4.46 ± .22	3.46 ± .23
3	**2:1**	8.12 ± .11	8.54 ± .13	5.38 ± .25

Antioxidant Activity

ABTS (2,20-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay and a DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay were used to evaluate the antioxidant activity of the compounds **1-3**. The IC_{50} (concentration in grams mL^{-1} with 50% effect) was used. Compounds **1-3** from

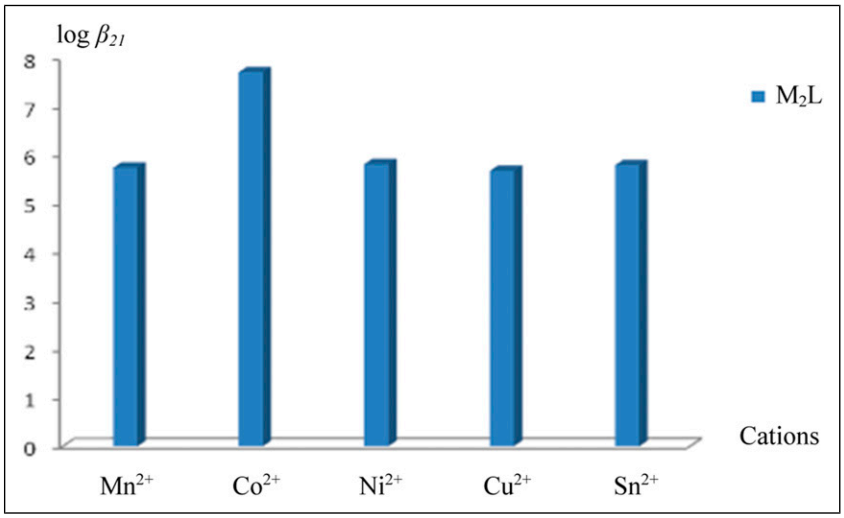


Figure 3. Stability profile of complexes M_2L of ligand **3**.

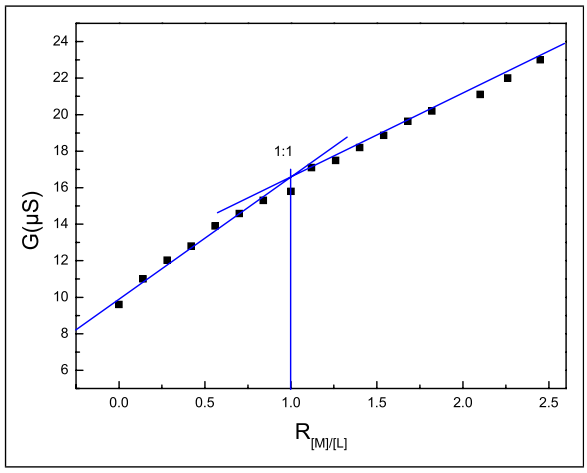


Figure 4. Conductometric titration in the case of ligand **2** with Sm^{3+} (C_L = 6.4 × 10^{-5}mol L^{-1}).

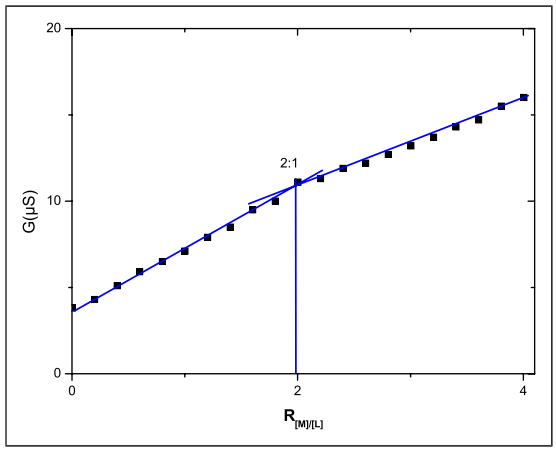


Figure 5. Conductometric titration in the case of ligand **3** with Co^{2+} (C_L = 3.9 × 10^{-5}mol L^{-1}).

Table 3. Antibacterial Inhibition Zones of Compounds **1-3.**

	Microorganisms				
Compounds[a]	*M. luteus* LB 141107	L. monocytogenes ATCC 1911	*S. aureus* ATCC 6538	S. Typhimurium ATCC 14028	*B. cereus* ATCC 14579
1	33 ± .13	23 ± .14	23 ± 1.2	25 ± 1.3	22 ± 0.2
2	26 ± 2.6	22 ± .32	22 ± 0.2	23 ± 1.2	21 ± .11
3	22 ± 1.2	22 ± 0.2	21 ± .21	22 ± 1.1	20 ± .14
Tetracycline	17				

[a]Zone of bacterial inhibition measured in mm.

Table 4. Minimum Inhibitory Concentration (MIC) of Synthesized Compounds Against *L. monocytogenes* ATCC 1911, *S. aureus* ATCC 6538, and *S. typhimurium* ATCC. 14028.

Tested compounds (300 μg/mL)#	*Listeria monocytogenes ATCC 1911*	*Staphylococcus aureus ATCC 6538*	*Salmonella typhimurium ATCC 14028*
1	.142	.134	.156
2	.153	.142	.162
3	.164	.156	.311
Ampicillin	.002	1.1	1.1

Table 5. The Antioxidative Activity of Compounds **1-3** was Assessed by DPPH and ABTS Techniques and Expressed as IC_{50} in g mL^{-1}. BHT was Used as a Control.

IC_{50} in μg mL^{-1}/Compound	DPPH	ABTS
1	49.06	33.23
2	48.15	28.35
3	47.80	29.64
BHT	31.25	17.38

Table 5 show notable DPPH radical scavenging activity. These substances' respective IC_{50} values were 49.06, 48.15, and 47.80 g mL^{-1}. In terms of ABTS free radical activity, the IC_{50} of the produced compounds **1-3** ranged from 28.35 to 33.23 g mL^{-1} (Table 5).

Conclusions

A new water-soluble *N*-alkylated PTA derivative **2** and a complex **3** have been used in this study. Their binding properties towards some metal cations have been investigated by means of spectrophometric and conductometric studies. The titration of ligands **2** and **3** separately by some lanthanides and transition metal cations have shown a modification on UV spectra and on gradients. The treatment of spectral changes by digital program has established the stoichiometries and the stability constants of complexes formed in MeOH. Generally, both complexes ML and M_2L have been formed with ligand **2** and M_2L with **3**. Complexes ML are more stable than M_2L which indicates the negative cooperative effect of ML for the arrangement of M_2L. Moreover, the stability of M_2L with **3** is more important than with **2** unless for Mn^{2+}. This result could be explained by the presence of two PTA units and the inclusion of cations inside their cavities. A significant selectivity $S(_{Ce^{3+}/Sm^{3+}})$ = 1500 was observed with ligand **3**. The compounds **1-3** show selective and moderate efficacy against various microorganisms, and the results for Gram-positive species, which are widespread in the environment, were particularly intriguing. In addition to their studies on microorganisms, these complexes demonstrated substantial free radical scavenging properties that could be applied in medicine.

Experimental Section

General Procedures. All manipulations were performed under an atmosphere of dry nitrogen using vacuum-line and standard Schlenk techniques. All reagents were obtained from commercial suppliers and used without further purification. Solvents were dried by standard methods and distilled under nitrogen before use. IR spectra were recorded on a Perkin-Elmer 398 Spectrophotometer. NMR spectra were recorded on a Bruker AC-400 instrument at 400.1 MHz (^{1}H), 161.9 (^{31}P) or 100.6 MHz (^{13}C) using SiMe4 or 85% H_3PO_4 as standards. DEPT experiments were carried out for all the compounds. Coupling constants *J* are given in hertz.

Materials and Methods. UV spectra were recorded on a Perkin-Elmer Lambda 11 spectrophotometer. Conductance measurements were recorded on a MeterLab CDM210 conductimeter with cell constant k = .93 cm. Methanol (Riedel-deHaën for HPLC) was commercial and

used without further purification. The supporting electrolyte used in the stability constant determinations was Et_4NCl_4 (Acros Organics). The metal salts chosen were chlorides (Fluka, purum).

Conductimetric of 1-(4-nitrobenzyl)-1,3,5 -triaza-7-phosphaadamantan-1-ium (PTAR) and Complex 3 Studies

While complexation by a neutral ligand is not expected to dramatically alter the molar conductivity of a cationic species, the differences can usually be detected and so the measurement of conductance of a solution of a ligand into which a metal ion is added can be a useful rapid means of establishing the stoichiometry of a complex ion species. Thus, this procedure was followed to obtain preliminary estimates of the metal:ligand ratio in the complexes formed by the two ligands **2**, **3**.[64]

Stability Constant Measurements

Stability constant values were determined by fitting to the changes in absorbance resulting when solutions of the ligands initially at concentrations between 2×10^{-5} and 4×10^{-5}mol L^{-1} were titrated with solutions of the various metal ions up to a final M:L ratio of 20. All measurements were made at 25°C using methanol as solvent, with the ionic strength maintained at .01 mol L^{-1} using Et_4NCl_4. Significant changes in absorbance were found in the wavelength range 215–320 nm in most cases and were suitable for analysis using the program "Letagrop."[52]

In principle, multi-step equilibria could have been involved in these reactions, but in all cases where the effects of complexation could be detected, a simple 1:1 model provided a satisfactory fit.

Synthesis of 1-(4-nitrobenzyl)-1,3,5-triaza-7-phosphaadamantan-1-ium (PTAR)

To a solution of PTA (1,3,5-triaza-7-phosphaadamantane (.634 mmol) in THF (20 mL) was added a solution of N-nitrobenzyl chloride (1.585 mmol). Refluxed for 2 hours at rt. The solid was isolated by filtration, washed with THF (3 × 5 mL) and diethyl ether, and dried under reduced pressure. Soluble in polar solvents, slightly soluble in MeOH and EtOH, and insoluble in other medium and low polar solvents. Yield (%) = 80; IR(cm^{-1}): 2935 (CH_2), 1063 (C-N), 1510 (C = Carom), 1207 (C-P), 844 (NO_2), 1H NMR in D_2O (δ): 8.3 (d, 2H, HPh), 7.78 (d, 2H, HPh), 5,14 (q, 4H, NCH2N+), 4,69 (s, 2H, PhCH2N+), 4,3 (s, 2H, PCH2N+), 3,99 (m, 2H, NCH2N), 3.86 (t, 4H, NCH2P) ppm.13 C{1H} NMR in D2O (δ): 149 (s, CNO2), 134 (s, Ar), 132 (s, CPh), 124 (s, Ar), 80 (s, NCH2N+), 70 (s, NCH2N), 65 (d, PhCH2N+), 52 (d, PCH2N+), 45 (s, NCH2P) 31P{1H} NMR in D2O (δ): −81 (s, P)ppm. Anal. Calc. for C13H18PN4O2Cl: C, 47.50%; H, 5.52%; N, 17.04%, Found: C, 47.6; H, 5.6; N, 17.1%.

Synthesis of [$RuCl_2(PTAR)_2\ PPh_3$]. Solid PTAR (20 mmol) was added to a stirred solution of [$RuCl_2$ (PPh_3)$_3$] (4 mmol) in a mixture of toluene and MeOH. The resulting mixture was refluxed under nitrogen for 30 min. The orange solid obtained was filtered off, washed with toluene (3 × 5 mL) and dried under reduced pressure. Yield: 80%. $^{31}P\{^1H\}$ NMR in D_2O (δ): −31.66 (d, PTAR), −27.98 (d, PTAR), 3.55(t, PPh_3), 10.64 (t, PPh_3) ppm

Biological activities

Microorganisms, Media, and Growth Conditions. *Staphylococcus aureus* (*S. aureus*) ATCC6538, *Listeria monocytogenes* (*L monocytogenes*) ATCC 1911, *M lutus* LB 141107, *Salmonella typhimurium* (S. typhimurium) ATCC 14028, and *B cereus* (ATCC 14579) were the bacteria employed as indicators for the antimicrobial determination. All these indicator microorganisms were obtained from International Culture Collections (ATCC). The indicator microorganisms were grown overnight in Luria Bertani (LB) medium (g L^{-1}: peptone 10, yeast extract 5, and sodium chloride (NaCl) 5, pH 7.2) under aerobic condition and constant agitation (200 r/min) at 30°C.

Agar Well Diffusion Method. Agar well diffusion method was employed for the determination of the antimicrobial activity of the synthesized compounds according to Guven et al.[65-69]

Minimum Inhibitory Concentration. (MIC) of the synthesized compounds and the standards ampicillin (stock solutions at 20 mg mL^{-1}) against the five tested bacteria were determined according to Sellem et al.[70-73] The test was performed in sterile 96-well microplates with a final volume in each microplate well of 100 µL. Stock solutions of synthesized compounds and standards were serially diluted with dimethyl sulfoxide (DMSO). To each test well, cell suspension was added to final inoculum concentration of 10^6 a colony-forming unit (CFU) mL^{-1} of indicator microorganism. The plates were then incubated at appropriate growth conditions of the corresponding indicator microorganism. The MIC was defined as the lowest concentration of the synthesized compounds and standards at which the microorganism does not demonstrate visible growth after incubation. 25 µl of Thiazolyl Blue Tetrazolium Bromide (MTT) at .5 mg mL^{-1} were added to the wells and incubated at room temperature for 30 min. The colorless tetrazolium salt acts as an electron acceptor and was reduced to a red-colored formazan product by the indicator microorganisms. When microbial growth was inhibited, the solution in the well remained clear after incubation with MTT.

For the antimicrobial activity determination (inhibition zones and CMIs), each experiment was carried out simultaneously three times under same conditions. The obtained

diameters of inhibition zones reported in mm and the MIC values reported in μg mL^{-1} were quite similar and the reported results are the average of the three experiments.

Antioxidant Activity. Antioxidant activity was expressed as IC_{50} (the concentration that causes 50% of effect). Antioxidant activity of the synthesized compounds was assessed by three different techniques which are the 2.2-diphenyl-1-picrylhydrazyl (DPPH), the 2.2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radicals scavenging, and the β-carotene linoleic acid bleaching assay. Butylated hydroxytoluene (BHT), known as a strong antioxidant compound, was used as control.

(DPPH) Radical Scavenging Activity. The procedure involves measurement of decrease in absorbance of DPPH at its absorption maxima of 517 nm. This assay determines the scavenging of stable radical species according to the method of Kirby and Schmidt.[74] Briefly, synthesized compounds were dissolved in dimethylsulfoxide (DMSO)/water (1/9; v/v) and diluted with ultrapure water at different concentrations (1, .5, .250, .125, .0625, .03125 mg mL^{-1}). Then, 500 μL of a 4% (w/v) solution of DPPH radical in ethanol was mixed with 500 μL of samples. The mixture was incubated for 30 min in the dark at room temperature. The scavenging capacity was determined spectrophotometrically by monitoring the decrease in absorbance at 517 nm against a blank. The percentage of antiradical activity (% ArA) had been calculated as follows:

% ArA = [(absorbance of control−absorbance of test sample)/absorbance of Control] × 100

(ABTS) Radical Scavenging Activity. This determination was carried out according to Re et al[75] protocol. 20 μL of solution of synthesized compounds or control were mixed with 18 μL of ABTS solution, the whole was mixed vigorously for 30 s. A control consisting of ethanol and ultrapure water was prepared (5:5) v/v to which the previous solution was added. White is ethanol and ultrapure water (5:5) v/v. Samples and controls were incubated for 6 min in the dark, then the OD was measured at 734 nm. The calculation is done according to the following formula:

$$\mathrm{AAO} \times \% = [(\mathrm{Ac} - \mathrm{Ae})/\mathrm{Ac}] \times 100$$

Ac: absorbance of the control, Ae: absorbance of the sample.

Author Contributions

L. Baklouti conceptualized the project's primary principles, drafted the analysis methods, conducted the scientific investigation, formal analysis, data curation, and acquired funding. B. Mellah worked on the project's concept, design, and monitoring and evaluation throughout the project. W.S Koko reviewed all the biological part and edited the first draft of the paper.

Declaration of Conflicting Interests

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ORCID iD

Lassaad Baklouti https://orcid.org/0000-0003-3816-4003

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