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Coordination Chemistry in Water of a Free and a Lipase-Embedded Cationic NCN-Pincer Platinum Center with Neutral and Ionic Triarylphosphines

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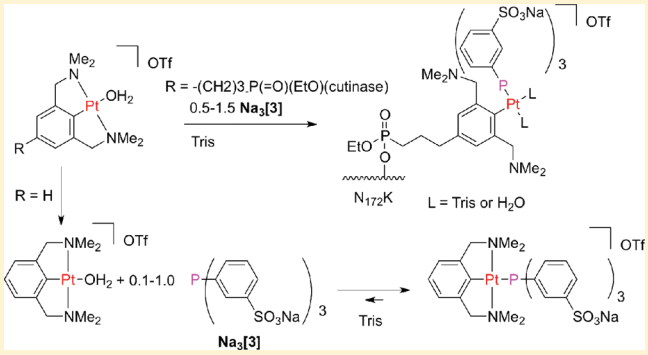
Supporting Information

ABSTRACT: The coordination chemistry in aqueous media was studied for the platinum center of low-molecular-weight cationic NCN-pincer platinum complexes $[RC_6H_2(CH_2NMe_2)_2\text{-}3,5\text{-}Pt(H_2O)\text{-}4]^+$ (R = $-(CH_2)_3P(=O)(OEt)(OC_6H_4NO_2\text{-}4)$ (**1(OH$_2$)**), H (**2(OH$_2$)**)) as well as of the platinum center of the NCN-pincer platinum cation embedded in the lipase cutinase (**cut-1**; molecular weight 20 619.3) with various anionic, neutral, and cationic triarylphosphines. A ^{31}P NMR study of the coordination of triarylphosphines to the cationic NCN-pincer platinum center in low-molecular-weight [**2(OH$_2$)**][**OTf**] in both D_2O and Tris buffer (Tris = tris(hydroxylmethyl)aminomethane) showed that the phosphine−platinum coordination is strongly affected by Tris buffer molecules. Two crystal structures of a NCN-pincer platinum−phosphine and a NCN-pincer platinum−ethanolamine coordination complex with ethanolamine as a functional model of Tris with hydrogen bridges, provoking a dimeric supramolecular structure, confirmed that the coordination observed in solution occurred in the solid state as well. A ^{31}P NMR and ESI-MS study of the lipase **cut-1** showed that the coordination of various triarylphosphines to the enzyme-embedded platinum center is affected by the surrounding protein backbone, discriminating between phosphines on the basis of their size and charge. By using ^{31}P NMR spectroscopy and ESI-MS spectrometry, study of the coordination of triarylphosphines to **cut-1** was possible, thereby avoiding the need for the application of laborious biochemical procedures. To the best of our knowledge, this is the first example of a study involving the selective binding of organic ligands to the metal center of a semisynthetic metalloprotein, unequivocally demonstrating that the well-established coordination chemistry for small-molecule complexes can be transferred to biological molecules. This initial study allows future explorations in the field of selective protein targeting and identification, as in protein profiling or screening studies.



INTRODUCTION

The coordination chemistry of metal ions embedded in proteins plays a crucial role in living systems; e.g., the typical 3D structures and catalytic activities of metalloproteins are determined by the coordination of the protein backbone to one or more transition-metal ions.[1−5] By modifying specific amino acid fragments in existing metalloproteins, the exact positioning of the metal center in the protein backbone and its reactivity can be elucidated. These studies showed that the nature of both the metal ions (e.g., Cu(I), Zn(II), Ni(II)) and the amino acids involved can dramatically influence the tertiary and quaternary structures and the functionalities of the metalloprotein.[6−15]

By a combination of protein engineering techniques with metal coordination chemistry, different artificial metalloproteins have been developed. Pioneering work was reported by Whitesides in 1978[16] and later by Ward et al.[6,17−20] using the biotin−(strept)avidin system. Different metal (e.g., rhodium) coordination complexes were noncovalently embedded into (strept)avidin, thus creating an enantioselective hybrid catalyst; i.e., the stereochemical properties of the protein backbone are transferred to the embedded metal center. In addition to the biotin−(strept)avidin system, also other proteins,[21−24] such as *apo*-myoglobin and heme-oxygenase,[7−11,25,26] papain,[27] other Cys-containing enzymes,[28−30] and carbonic anhydrases, were used as scaffolds to construct artificial metalloenzymes.[21,31] When a transition-metal catalyst is strategically embedded into a chiral protein, the influence of the protein backbone can be

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optimally exploited to tune the activity of the metal center, as e.g. in catalytic applications. In addition to catalysis, protein-embedded metal centers have mainly been used in anticarcinogenic and biomedical studies[32−36] and have been applied as redox systems,[37,38] as probes,[39−42] or in arrays and assemblies,[43−46] illustrating the great potential of these protein−metal hybrid systems.

Recently, our group reported on the covalent anchoring of an ECE-pincer metal (Pd or Pt) moiety to a lipase,[47] where E is a neutral, two-electron heteroatom donor, such as NMe_2 or SMe. In these ECE-pincer metal complexes, the bis ortho chelation of the metal ion by the two donating E groups provides further stability to the central M−C_{pincer} bond, making the ECE-pincer metal complexes compatible with aqueous solvent media and biological molecules, such as proteins. Recently, we were able to obtain X-ray crystal structures of a number of these pincer−cutinase hybrids, which allowed us to obtain a detailed picture of the orientation of the pincer groups in the protein pocket.[47] It appeared that in the case of the NCN-pincer platinum complex $RC_6H_2(CH_2NMe_2)_2$-3,5-PtCl-4 (R= $-(CH_2)_3P(=O)(OEt)(O-)$ (**1Cl**) embedded in cutinase, depending on the type of buffer used (either chloride-rich or chloride-poor), either the monomeric NCN-pincer platinum chloride-lipase hybrid [**cut-1Cl**] (Figure 1a) or the dimeric hybrid [**cut-1-Cl-cut-1**]$^+$, in which two cationic NCN-pincer platinum−lipase hybrids are bridged through a single chloride ion, was formed (Figure 1b).[47] From the structural features of the two hybrids it was obvious that, in the case of the dimer, the two protein-embedded NCN-pincer platinum cations apparently are exposed enough to allow bridge formation by a small halide atom and thus the assembly of two NCN-pincer platinum chloride−lipase hybrids to one cationic dimer. This shows that the metal center is available for further reaction: e.g., coordination chemistry and catalysis.

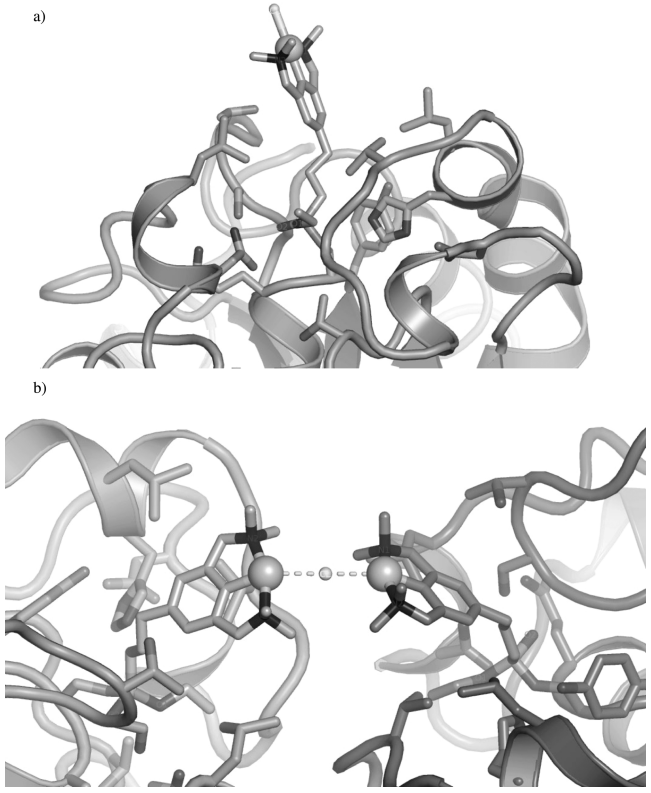

Figure 1. (a) Part of the molecular structure of [**cut-1Cl**] in the solid state, showing the covalent attachment of the NCN-pincer platinum moiety. (b) Dimeric structure with the chloride ion bridging the two platinum(II) cations to form [**cut-1-Cl-cut-1**]$^+$, as obtained from crystallization in chloride-poor buffer.[47,56]

As a follow-up of this work, we decided to explore the coordination properties of the protein-embedded NCN-pincer platinum center with a series of cationic, neutral, and anionic triarylphosphine ligands (Figure 2; vide infra). It was anticipated that classical coordination chemistry[48−55] could be expanded toward artificial biological hybrid systems, thereby adding novel, largely unexploited properties to biological molecules. The various phosphines that have been used in this model study had been selected for their solubility properties in water and for their differences in bulkiness. Furthermore, we set out to develop protocols allowing us to monitor the coordination reactions in a straightforward manner, e.g. by ^{31}P NMR and ESI-MS methods, thereby avoiding laborious biochemical analysis protocols. To evaluate the influence of the protein backbone on the binding of these triarylphosphines to the platinum center, we also performed the same coordination chemistry on the parent low-molecular-weight NCN-pincer platinum complexes lacking the protein para substituent.

RESULTS AND DISCUSSION

As cationic ECE-pincer metal complexes possessing non-coordinating anions, such as triflate or tetrafluoroborate, are ideal building blocks in supramolecular coordination chemistry studies,[57−66] it was decided to synthesize the ionic NCN-pincer platinum complexes [**1(OH$_2$)**][**OTf**] and [**2(OH$_2$)**][**OTf**][65,67] from their corresponding halide analogues ([$RC_6H_2(CH_2NMe_2)_2$-3,5-PtCl-4] (R= $-(CH_2)_3P(=O)(OEt)(OC_6H_4NO_2$-4) (**1Cl**),[56] or H (**2Cl**),[65,67] respectively; Scheme 1a) by reaction with AgOTf. Filtration of the resulting reaction mixture over Celite resulted in the removal of AgCl. [**1(OH$_2$)**][**OTf**] was characterized by elemental analysis, ^{1}H, ^{13}C, ^{19}F, and ^{31}P NMR spectroscopy, and MALDI-TOF MS.

The cutinase-pincer hybrid molecule [**cut-1(OH$_2$)**][**OTf**] was obtained by inhibition of cutinase (125 mM Tris, pH 8, acidified with HBF_4, i.e. in the absence of chloride anions) with a solution of [**1(OH$_2$)**][OTf] (4 equiv in MeCN); the *p*-nitrophenolate leaving group of [**1(OH$_2$)**][**OTf**] was replaced by the nucleophilic oxygen atom of the Ser_{120} residue in the active side of cutinase. After inhibition, excess inhibitor [**1(OH$_2$)**][**OTf**] and *p*-nitrophenolate were removed by dialysis (Scheme 1b). Subsequent analysis of [**cut-1(OH$_2$)**]-[**OTf**] by ESI-MS (*m*/*z* 21134.8, corresponding to [**cut-1**]$^+$ (calcd 21 137.5); note the *m*/*z* value of [**cut-1Cl**] is 21 173.0) and ^{31}P NMR (phosphonate-P δ 28.7 ppm, Scheme 1) showed that complete and irreversible inhibition of cutinase had occurred.

Coordination Studies of [2(OH$_2$)][OTf] with Triarylphosphine Ligands in Aqueous Media Monitored by ^{31}P NMR Spectroscopy. To explore the coordination chemistry of cationic NCN-pincer platinum complexes with various phosphines (Figure 2) by ^{31}P NMR in aqueous media, a model study was carried out first with the pincer complex [**2(OH$_2$)**]-[**OTf**] and anionic triarylphosphine **Na$_3$[3]**[68] (**TPPTS** = $P(C_6H_4(SO_3Na)$-3)$_3$), whereby both [**2(OH$_2$)**][**OTf**] and **TPPTS** were soluble under the aqueous conditions described. Two aqueous solvent systems were selected for our studies: (1) neat D_2O and (2) a halide ion free Tris buffer (Tris =

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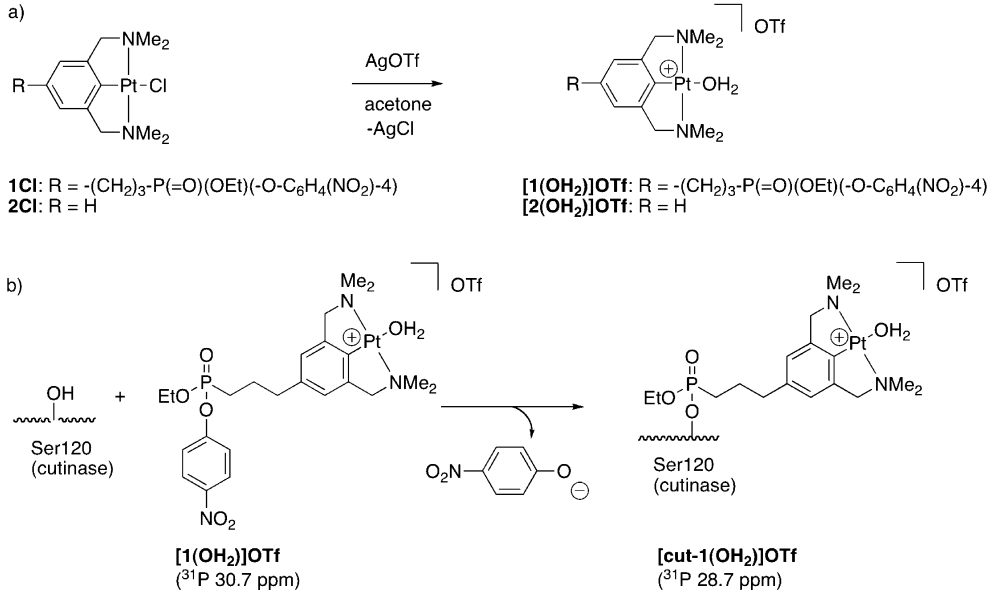
Scheme 1. (a) Synthesis of the Cationic NCN-Pincer Platinum Complexes [1(OH$_2$)][OTf] and [2(OH$_2$)][OTf] and (b) Covalent Anchoring of [1(OH$_2$)][OTf] to Cutinase[a]



[a]The respective ^{31}P NMR values are given in parentheses.

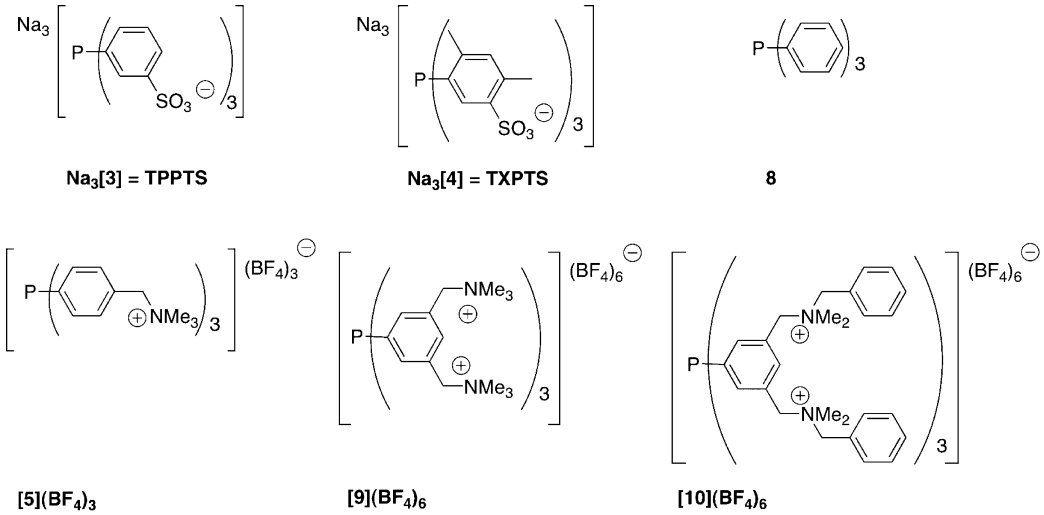


Figure 2. The various anionic, neutral, and cationic triarylphosphines used in the coordination studies with cationic NCN-pincer platinum triflate complexes [**1**(OH$_2$)][**OTf**] and [**cut-1**(OH$_2$)][**OTf**], respectively. Note that [**3**] = TPPTS *minus* three Na^+ and [**4**] = TXPTS *minus* three Na^+.

tris(hydroxylmethyl)aminomethane, pH 8.0), as it is a standard buffer for many enzyme studies. The solvent system with Tris allowed us to study the influence of Tris on the coordination chemistry of the phosphines.

For the study in D_2O, various amounts (0.1−5.0 equiv) of a solution of **TPPTS** (δ(^{31}P) −5.7 ppm) in D_2O were added to a solution of [**2**(**OH$_2$**)][**OTf**][65,67] in D_2O. The resulting solutions were analyzed by ^{31}P NMR spectroscopy 5 min after mixing as well as after 2 days (Scheme 2 and Table S1 (Supporting Information)). Upon addition of 0.1−1.0 equiv of **TPPTS** the only signal observed in the ^{31}P NMR spectra was a sharp resonance at 30.6 ppm with ^1J(^{31}P−^{195}Pt) = 2100 Hz (Scheme 2). No resonance pointing to the presence of free phosphine was observed, indicating that all phosphine molecules had coordinated to platinum. The spectrum revealed no change after 2 days, clearly indicating the formation of a stable 1:1 coordination product. The ^1J(^{31}P−^{195}Pt) value (2100 Hz) is indicative of a trans coordination of the phosphine with respect to C_{ipso} (the carbon atom directly bound to platinum),[69−71] i.e. of the presence of a complex with a phosphine coordination mode as depicted for the complex [**2**(**TPPTS**)][**OTf**] (Scheme 2).

When more than 1.0 equiv of phosphine **TPPTS** was added to a solution of [**2**(**OH$_2$**)][**OTf**], the coordination of a second phosphine to platinum was observed. At 22.9 and 10.7 ppm, doublet resonances (^2J(^{31}P−^{31}P) = 13−15 Hz) appeared with ^1J(^{31}P−^{195}Pt) = 1600 and 3900 kHz, respectively (Table S1, Supporting Information). These signals are attributed to a species in which two phosphines are coordinated to the platinum center and where the two phosphines hold a mutual cis position (*cis*-[**2**(**TPPTS**)$_2$(**D$_2$O**)]$^+$, Scheme 2). The ^1J(^{31}P−^{195}Pt) = 1600 and 3900 Hz coupling constants of *cis*-[**2**(**TPPTS**)$_2$(**D$_2$O**)]$^+$ are attributed to the phosphines bound

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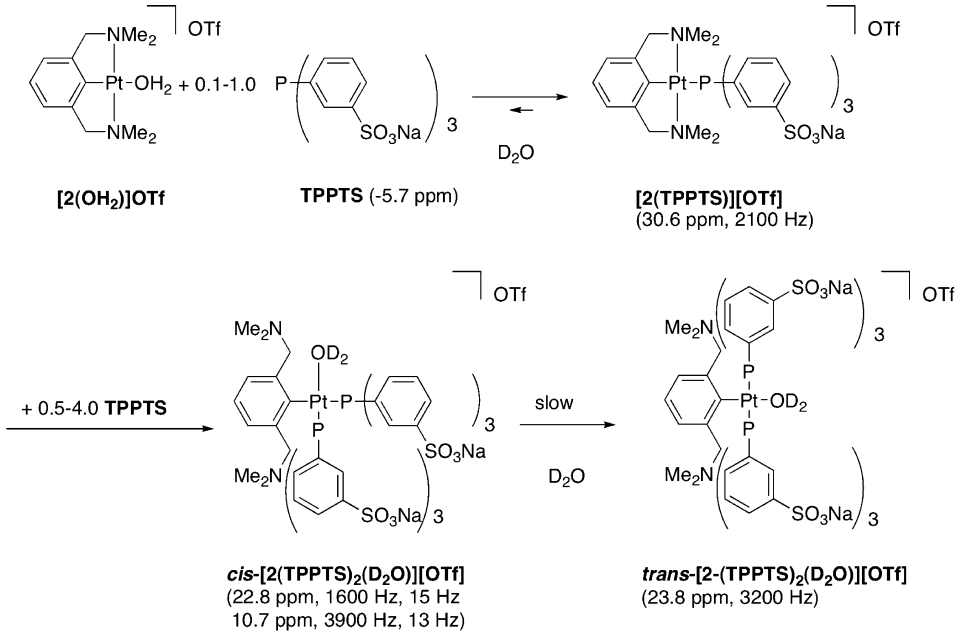
Scheme 2. Observed Resonances in ^{31}P NMR upon the Addition of 0.1−5.0 equiv of TPPTS to $[2(OH_2)][OTf]$ in D_2O and the Proposed Species Formed, $[2(TPPTS)]^+$, *cis*-$[2(TPPTS)_2(D_2O)]^+$, and *trans*-$[2(TPPTS)_2(D_2O)]^+$ [a]



[a]The ^{31}P NMR resonances and the $^1J(^{31}P-^{195}Pt)$ and $^2J(^{31}P-^{31}P)$ values (for *cis*-$[2(TPPTS)_2(D_2O)]^+$), respectively, are given in parentheses.

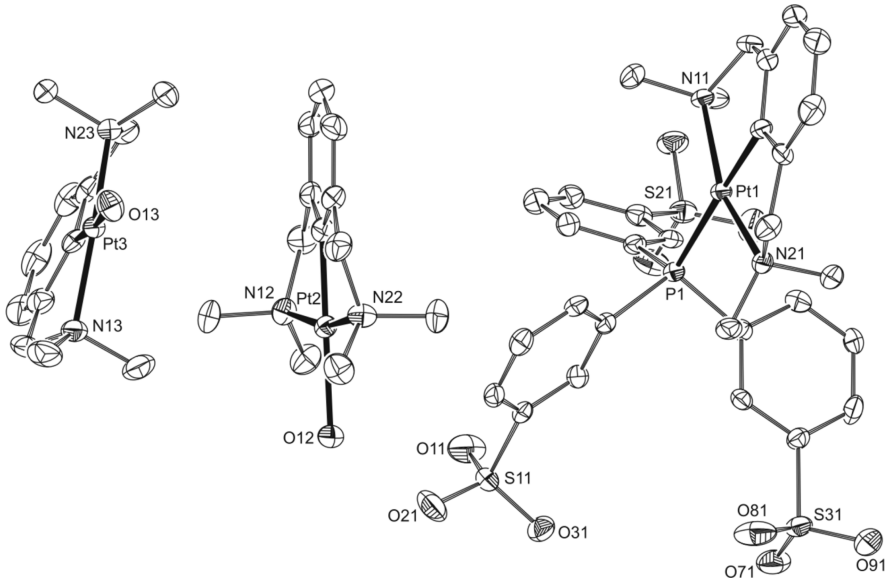


Figure 3. The two cations and the dianion of $[2(OH_2)]_2[2(3)]$ (note that **(3)** = **TPPTS** *minus* three Na^+; residues 1−3) in the asymmetric unit (three cocrystallized water molecules are omitted for clarity). Hydrogen atoms are omitted for clarity, and displacement ellipsoids are drawn at the 50% probability level. See also Figure S1 (Supporting Information), showing the infinite 2D network by intermolecular hydrogen bonding.

trans[69−71] and cis[69,72−74] to C_{ipso}, respectively, which is in good agreement with literature values for related complexes (e.g., 1600−2000 Hz for trans coordination and 3100−4400 Hz for cis coordination). It is noteworthy that previous studies have shown[67,72,75] that free *o*-CH_2NMe_2 groupings, as depicted in *cis*-$[2(TPPTS)_2(D_2O)]^+$ (**Scheme 2**), cannot coordinate to the platinum metal center. Decoordination of *o*-CH_2NMe_2 groupings in $[2(OH_2)]^+$ followed by cis coordination of stronger ligands causes the now *monodentate* C-bonded NCN-pincer ligand to turn its pincer arene plane perpendicular to the platinum coordination plane, thus releasing the steric constraint imposed by the cis-coordinated stronger ligand, in this case the **TPPTS** ligand.

When these solutions were left standing for another 2 days, a singlet signal grew into the spectrum at 23.8 ppm ($^1J(^{31}P-^{195}Pt)$ = 3200 Hz), pointing to the copresence of a Pt complex with two chemically and magnetically identical phosphines. These resonances suggested that on standing an isomerization of *cis*-$[2(TPPTS)_2(D_2O)]^+$ to *trans*-$[2(TPPTS)_2(D_2O)]^+$ had occurred (Scheme 2). The formation of *trans*-$[2(TPPTS)_2(D_2O)]^+$ is supported by $^1J(^{31}P-^{195}Pt)$ values from the literature ranging from 2980 to 3060 Hz[75−78] and a crystal structure of a PCP-pincer platinum complex.[75]

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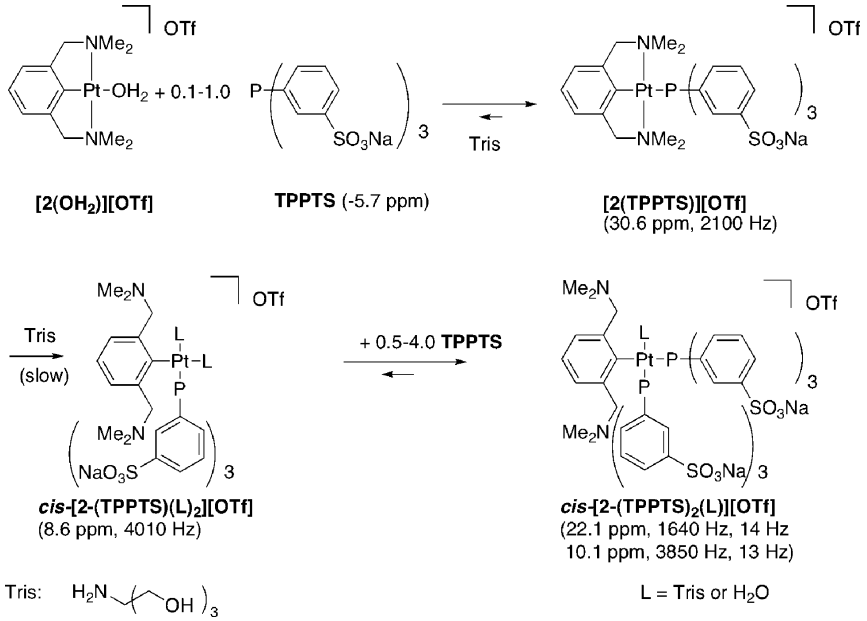
Scheme 3. Observed Resonances in ^{31}P NMR upon the Addition of 0.1−5.0 equiv of TPPTS to [2(OH$_2$)][OTf] in Tris Buffer (125 mM, pH 8.0) and the Different Species Formed[a]



[a]The ^{31}P NMR resonances and the $^{1}J(^{31}P-^{195}Pt)$ and $^{2}J(^{31}P-^{31}P)$ values (for *cis*-[**2**(**TPPTS**)$_2$(**L**)]$^+$ and *cis*-[**2**(**TPPTS**)(**L**)$_2$]$^+$ with L = Tris, H$_2$O) values are given in parentheses. Tris is also shown.

When 1 equiv of phosphine **TPPTS** was added to a solution of independently prepared [**2**(**TPPTS**)]$^+$ (vide infra), the same isomerization behavior was observed, with the initial formation of the coordination complex *cis*-[**2**(**TPPTS**)$_2$(**D$_2$O**)]$^+$, which ultimately converted into *trans*-[**2**(**TPPTS**)$_2$(**D$_2$O**)]$^+$. Also here, no free **TPPTS** was detected after 1 and 2 days, respectively, indicating once more that the phosphine present had coordinated quantitatively to the NCN-pincer platinum complex.

Isolation and Crystallographic Studies. To confirm the formation of at least one of the in situ observed coordination complexes, we decided to isolate and fully analyze the coordination complex [**2**(**TPPTS**)]$^+$. Mixing of [**2**(**OH$_2$**)][**OTf**] and **TPPTS** (1:1) in H$_2$O and subsequent workup yielded a white powder. Elemental analysis and mass spectrometry of this powder clearly showed that a 1:1 phosphine-NCN-pincer platinum complex was formed. The ^{31}P NMR resonance (30.6 ppm) and coupling data ($J(^{31}P-^{195}Pt)$ = 2100 Hz) were identical with those observed during the titration experiment (vide supra and Scheme 2). The ^{1}H NMR resonances, especially the aromatic and aliphatic signals of the pincer moiety, indicated a symmetric molecule with trans coordination of the phosphine with respect to C$_{ipso}$ (triplet and doublet for the aromatic pincer proton signals with $^{3}J_{H-H}$ = 7.2 Hz, two singlets for −CH$_2$ and −NMe$_2$ proton signals at 4.12 and 2.37 ppm, respectively).

To confirm the proposed structure of [**2**(**TPPTS**)]$^+$, we tried to obtain single crystals suitable for an X-ray crystal structure determination. As initial attempts to crystallize [**2**(**TPPTS**)]$^+$ from pure water failed, we added several droplets of CH$_2$Cl$_2$, after which some crystals were formed at the CH$_2$Cl$_2$/H$_2$O interface. The X-ray crystal structure determination of these crystals revealed the unexpected formation of an ionic coordination complex with an interesting molecular structure (Figure 3; relevant bond distances and angles are given in Table S2 in the Supporting Information). In the asymmetric unit, three pincer platinum(II) cations are present, one of which is coordinated to the trianionic triarylphosphine, thus forming the dianionic NCN-pincer phosphine entity [**2**(**3**)]$^{2-}$ (residue 1; note that (**3**) = **TPPTS** *minus* three Na$^+$). The remaining two NCN-pincer platinum cations are coordinated to water, [**2**(**OH$_2$**)]$^+$ (residues 2 and 3), and act as counter-cations to yield a charge-neutral unit cell. The sodium and triflate ions present in the original aqueous solution of **TPPTS** apparently did not turn up in the crystallized material. The phosphine in residue 1 (Figure 3) binds to the NCN-pincer platinum cation trans with respect to C$_{ipso}$ (Pt1−P1 = 2.3627(12) Å). The orientation of the phosphine and the Pt−P bond distance (Table S2, Supporting Information) are comparable to the crystal structure data of other ECE-pincer platinum phosphine complexes, which contain a monodentate phosphine that is coordinated trans to C$_{ipso}$.[70,71] Selected bond lengths and angles of [**2**(**OH$_2$**)]$_2$[**2**(**3**)] are given in Table S2 (Supporting Information). An interesting additional structural feature of [**2**(**OH$_2$**)]$_2$[**2**(**3**)] is that it forms an infinite 2D network by intermolecular hydrogen bonding. This network is aligned parallel to the crystallographic *ab* plane (see Figure S1). The coordinated and noncoordinated water molecules act as hydrogen bond donors. Nine hydrogen bonds are accepted by the oxygen atoms of sulfonate groups and noncoordinated water, respectively; the hydrogen bond of H26O is accepted by the π system of the "pincer" phenyl ring C13−C63.

The reason for this surprising crystallization behavior might originate from the use of CH$_2$Cl$_2$ as cosolvent during crystallization. As phosphine **TPPTS** is insoluble in CH$_2$Cl$_2$ and the crystals were formed at the H$_2$O/CH$_2$Cl$_2$ interface, the different solubilities of the coordination complex [**2**(**TPPTS**)][**OTf**] in the H$_2$O and CH$_2$Cl$_2$ phases might have provoked the observed crystallization.

NMR Studies with [2(OH$_2$)][OTf] and Phosphine TPPTS in Tris Buffer. A slightly different coordination behavior was

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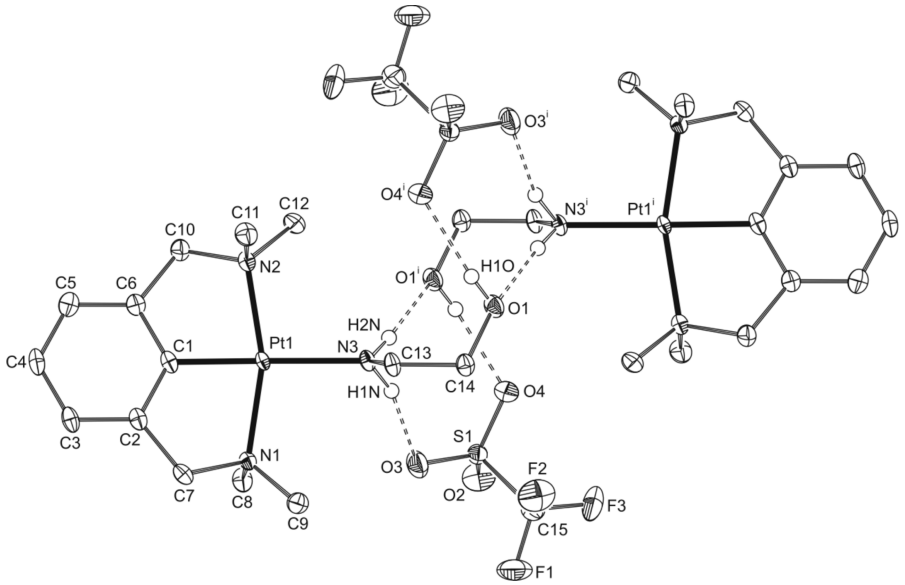

Figure 4. Displacement ellipsoid plot (50% probability level) of the 1:1 coordination complex [**2**(**NH$_2$**(**CH$_2$**)$_2$**OH**)][**OTf**]. C−H hydrogen atoms are omitted for clarity. Symmetry operation: (i) 2 − *x*, −*y*, 1 − *z*.

observed when, instead of D_2O, Tris buffer (chloride free) was used as a solvent for the study of the coordination behavior of phosphine **TPPTS** to the cationic pincer platinum complex [**2**(**OH$_2$**)][**OTf**]. Again for this study, various amounts of **TPPTS** (0.1−5.0 equiv in D_2O) were added to [**2**(**OH$_2$**)]-[**OTf**] (in 125 mM Tris buffer, pH 8.0, acidified with HBF_4; final concentration of [**2**(**OH$_2$**)][**OTf**] in Tris 19 mM). Subsequently, the different solutions were analyzed by ^{31}P NMR spectroscopy after 5 min and 1, 2, and 19 days.

Upon the addition of up to 1 equiv of phosphine **TPPTS**, initially the appearance of a ^{31}P resonance peak at 30.6 ppm ($^1J(^{31}P-^{195}Pt)$ = 2100 Hz) was observed (Table S3, Supporting Information), which is the same signal as was observed in the coordination study in D_2O: i.e., assigned to the phosphine bound trans with respect to C_{ipso} (see complex [**2**(**TPPTS**)]$^+$, Schemes 2 and 3). However, when the same mixture was analyzed after 1 day, a second signal at 8.6 ppm ($^1J(^{31}P-^{195}Pt)$ = 4010 Hz) had appeared (Table S3, Supporting Information), which gradually became more intense over time and ultimately after 19 days was the only resonance present in the spectrum. Its $^1J(^{31}P-^{195}Pt)$ value of 4010 Hz is indicative of phosphine coordination cis with respect to C_{ipso},[70,72−74] which in this case would imply an isomerization of the coordinated phosphine from trans to cis. As the occurrence of the resonance at 8.6 ppm with $^1J(^{31}P-^{195}Pt)$ = 4010 Hz was only observed in the presence of Tris buffer, the formation of the cis-coordination complex (i.e. *cis*-[**2**(**TPPTS**)(**Tris**)(**L**)]$^+$ with L = Tris, H_2O) from the trans coordination complex is most probably assisted by the presence of Tris molecules. Ample evidence[84,85,79,80] is available that an important prerequisite for the trans to cis isomerization to occur is the dissociation of at least one Pt−N_{pincer} bond. The subsequent cis coordination of an external ligand causes severe steric interference with the decoordinated *o*-CH_2NMe_2 grouping of the pincer ligand, making decoordination of the second *o*-CH_2NMe_2 substituent with concomitant rotation of the aryl ring out of the Pt coordination plane a favorable process, as it is releasing the steric strain of the ligands in the platinum coordination plane, vide supra.

In a control experiment, where independently synthesized [**2**(**TPPTS**)][**OTF**] (vide infra) was dissolved in d_{11}-Tris buffer and subsequently analyzed by ^1H NMR and ^{31}P NMR spectroscopy, exactly the same trans to cis isomerization of the coordinated phosphine was observed, as described above. This observation supports the formation of the coordination complex *cis*-[**2**(**TPPTS**)(**Tris**)$_2$]$^+$ from the complex [**2**-(**TPPTS**)][**OTF**], as depicted in Scheme 3.

When more than 1 equiv of phosphine **TPPTS** was added, two additional signals were observed, in addition to the signals of the monocoordinated cis and trans species in its ^{31}P NMR spectrum. These two signals at 22.1 ppm ($^1J(^{31}P-^{195}Pt)$ = 1640 Hz, $^1J(^{31}P-^{31}P)$ = 14 Hz) and 10.1 ppm ($^1J(^{31}P-^{195}Pt)$ = 3850 Hz, $^1J(^{31}P-^{31}P)$ = 13 Hz) were both doublets (Table S3, Supporting Information), indicating the coordination of two phosphines to the same platinum center in a cis orientation (complex *cis*-[**2**(**TPPTS**)$_2$(**Tris**)]$^+$, Scheme 3). An isomerization of the bis-coordinated product, as observed for the study in D_2O (*cis*-[**2**(**TPPTS**)$_2$(**D$_2$O**)]$^+$ → *trans*-[**2**-(**TPPTS**)$_2$(**D$_2$O**)]$^+$, Scheme 2) did not occur when Tris buffer was used as a solvent.

Interestingly, even with an excess of 5.0 equiv of phosphine **TPPTS** present, we could still observe a small amount of the monocoordinated product, in addition to the bis-coordinated product. Furthermore, the ratio between mono- and bis-coordinated product did not change over time. This observation, which is different from the observations in D_2O, is most probably due to the competitive coordination of Tris to the cationic NCN-pincer metal center. Apparently, the large excess of Tris (125 mM Tris to 19 mM [**2**(**OH$_2$**)][**OTf**]) and its competitive coordination to platinum could only allow for the partial formation of the bis-coordinated complex *cis*-[**2**(**TPPTS**)$_2$(**Tris**)]$^+$.

Coordination of Ethanolamine to [2(OH$_2$)][OTf]. The coordination of **TPPTS** to [**2**(**OH$_2$**)][**OTf**] was obviously influenced by the coordinating properties of Tris. Accordingly it was attempted to study the various coordination modes of the potentially tetradentate Tris molecule to [**2**(**OH$_2$**)][**OTf**] by ^1H NMR spectroscopy. As the coordination pattern turned out

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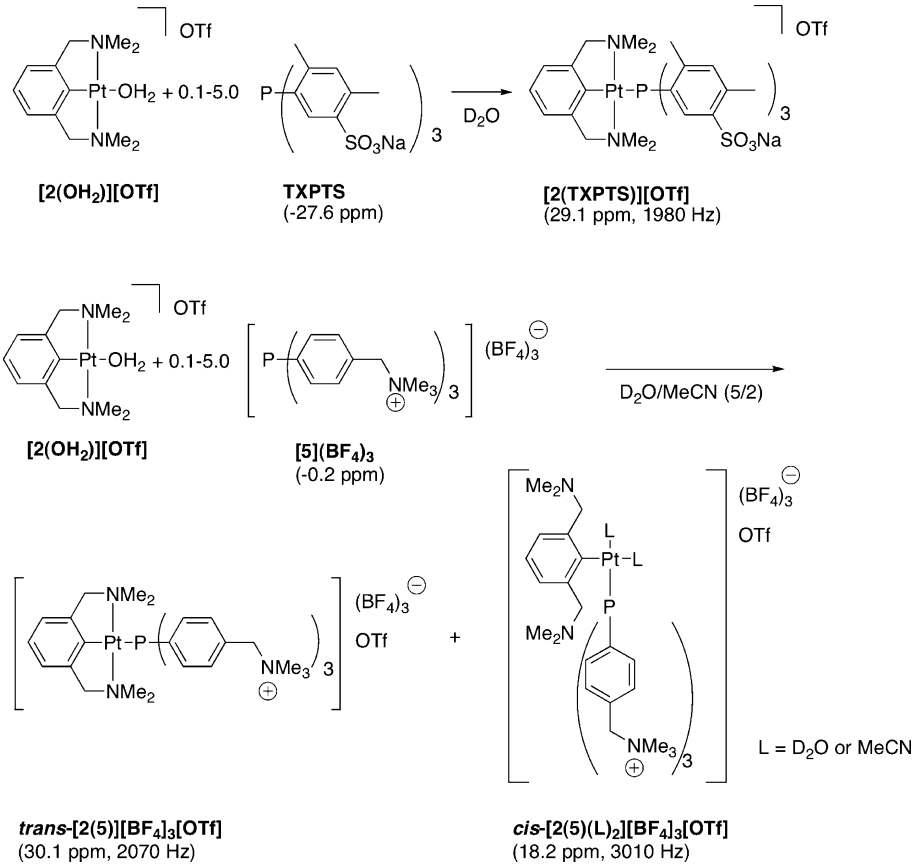
Scheme 4. Coordination of Phosphines TXPTS and $[5](BF_4)_3$, Respectively, to $[2(OH_2)][OTf]$



to be very complex (see Figure S2 for the spectra, Supporting Information), it was very difficult to deduce from these spectra the actual coordination mode of Tris to the cationic NCN-pincer platinum moiety. Therefore, we chose to study the coordination of ethanolamine (2-hydroxyethylamine) (pK_a = 9.3) to $[2(OH_2)][OTf]$ in D_2O at pH 7. As ethanolamine possesses only one hydroxyl group as compared to three for Tris, ethanolamine was considered to be a suitable, though less complex, model for Tris.

When a 1:1 mixture of ethanolamine and $[2(OH_2)][OTf]$ in D_2O was analyzed by ^1H NMR, it was observed that the original multiplet signals of uncoordinated ethanolamine (3.47 and 2.59 ppm) shifted to 3.73 and 2.98 ppm. The aliphatic pincer signals shifted equally from 4.02 and 2.82 ppm to 4.00 and 2.88 ppm, respectively (all values in D_2O). These data pointed to the formation of a coordination complex between ethanolamine and $[2(OH_2)][OTf]$.

When a 1:1 mixture of $[2(OH_2)][OTf]$ and ethanolamine was crystallized from H_2O, single crystals were obtained. An X-ray crystal structure determination of these crystals revealed the molecular structure of the 1:1 coordination complex $[2(NH_2(CH_2)_2OH)][OTf]$ in the solid state (Figure 4; relevant bond distances are given in Table S4). The ORTEP plot of $[2(NH_2(CH_2)_2OH)][OTf]$ shows that the NH_2 group of ethanolamine coordinates to the platinum ion of the pincer moiety trans to C_{ipso}, while the hydroxyl group remains free. Two $[2(NH_2(CH_2)_2OH)]^+$ cations are arranged into a unique dimeric structure via mutual N–H···O hydrogen bonding of one $[2(NH_2(CH_2)_2OH)]^+$ cation with the OH grouping of the other $[2(NH_2(CH_2)_2OH)]^+$ cation; i.e., the structure can be considered as comprising a central 10-membered diaza ring to which two NCN-pincer platinum cations are N bonded. Each of the OTf anions are H bonded via two of its O atoms to one of the two H atoms of the H–N–H···O–H bonding motif of the central 10-membered diaza ring. It must be noted that the intramolecular N–Pt coordination of the *o*-NMe_2 groups is retained. These findings indicate that coordination of Tris molecules to the NCN-pincer platinum cations in both the low-molecular-weight complex [**2**] and [**cut-1**] (vide infra) will be rather complex because of the distinct role the formation of H-bonding patterns will play in the resulting species. Nevertheless, the experiments with ethanolamine suggest that the coordination of Tris and the NCN-pincer platinum cations will occur via its amine N donor atom, while the OH groups will most likely be involved in hydrogen atom bonding patterns.

Coordination of Phosphines TXPTS and $[5](BF_4)_3$ to $[2(OH_2)][OTf]$ in D_2O. To investigate the influence of the steric requirements of the selected neutral, cationic, and anionic triarylphosphines on their coordination to the NCN-pincer platinum cation, the reactions of trianionic triarylphosphine **TXPTS** (note that trianionic (**4**) = **TXPTS** minus three Na cations) and tricationic triarylphosphine [**5**] to $[2(OH_2)][OTf]$ were studied. To a solution of $[2(OH_2)][OTf]$ were added various amounts of a solution of either **TXPTS** or $[5](BF_4)_3$, and the resulting solutions were analyzed by ^{31}P NMR spectroscopy (Scheme 4).

For trianionic phosphine **TXPTS** the coordination of only one phosphine to the NCN-pincer platinum cation was observed in D_2O, even when up to 5 equiv was added. Both the titration study and the preparative synthesis of $[2(TXPTS)][OTF]$ in D_2O gave in the ^{31}P NMR spectra a chemical shift of 29.1 ppm with $^1J(^{31}P-^{195}Pt)$ = 1940 Hz,

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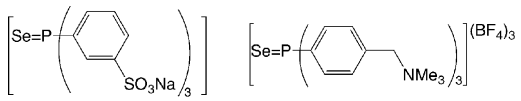
which pointed to a structure with the phosphine grouping coordinating trans to C_{ipso} (Scheme 4).

When various amounts of tricationic $[\mathbf{5}](\mathbf{BF_4})_3$ were added to $[\mathbf{2(OH_2)}][\mathbf{OTf}]$ dissolved in a water/acetonitrile mixture (5/2 v/v), again the 1:1 coordination of one phosphine to the metal center was observed. However, in this case the coordination of a phosphine cis with respect to C_{ipso} was observed as well, with 20% trans ($^1J(^{31}P-^{195}Pt)$ = 2070 Hz) and 80% cis ($^1J(^{31}P-^{195}Pt)$ = 3010 Hz) coordination complex (*trans*-$[\mathbf{2(5)}][\mathbf{BF_4}]_3[\mathbf{OTf}]$ and *cis*-$[\mathbf{2(5)(L)_2}][\mathbf{BF_4}]_3[\mathbf{OTf}]$; Scheme 4). Also for this phosphine, coordination of a second phosphine molecule was not observed upon addition of more than 1 equiv of $[\mathbf{5}][\mathbf{BF_4}]_3$. Complexes $[\mathbf{2(TXPTS)}][\mathbf{OTf}]$ and $[\mathbf{2(5)}][\mathbf{BF_4}]_3[\mathbf{OTf}]$ were synthesized, and the pure white products were fully analyzed by NMR spectroscopy and high-resolution ES mass spectrometry (see the Supporting Information for details).

In addition to steric constraints, also the σ-bond donating properties of the phosphorus are a determining factor for the coordination properties of phosphine ligands (such as PR_3). It has been established that the σ-bond donating properties of the phosphorus atom in substituted triarylphosphines are influenced by the charge of the different R groups.[81,82] It appeared that the nature of the σ-bond donating properties can be measured by converting the respective phosphines into the corresponding Se=PR_3 derivatives with selenium and subsequent measurement and comparison of the $^1J(^{31}P-^{77}Se)$ coupling constant of these compounds.[81,82]

It appeared that the $^1J(^{31}P-^{77}Se)$ coupling values of sodium 3-phosphoroselenoylbenzene sulfonate ($\mathbf{Na_3[6]}$) and *N,N,N*-trimethyl-1-(4-phosphoroselenoylphenyl)methanaminium ($[\mathbf{7}][\mathbf{BF_4}]_3$) (Figure 5) are nearly identical, indicating similar σ-bond donating properties of **TPPTS** and $[\mathbf{5}][\mathbf{BF_4}]_3$, which could indicate similar coordination properties to the platinum cation. These observations indicate that a difference in σ-donation properties cannot be a reason for the different coordination behavior of $[\mathbf{5}][\mathbf{BF_4}]_3$ (vs **TXPTS**) and $[\mathbf{2(OH_2)}][\mathbf{OTf}]$.



Na_3[6]: δ^{31}P 35.9 ppm, $^1J(^{31}P-^{77}Se)$ 707 Hz

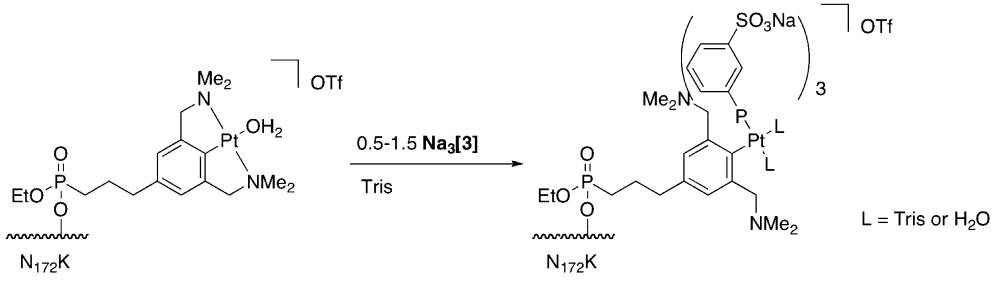
[7]$(BF_4)_3$: δ^{31}P 35.0 ppm, $^1J(^{31}P-^{77}Se)$ 701Hz

Figure 5. ^{31}P NMR data of phosphine selenides $\mathbf{Na_3[6]}$ and $[\mathbf{7}][\mathbf{BF_4}]_3$ in D_2O.[84]

^{31}P NMR Study with [cut-1(OH$_2$)][OTf] and Phosphine TPPTS. From the crystal structures it became apparent that the cutinase-embedded pincer metal center was exposed to the solvent (cf. Figure 1a).[47] Therefore, it was anticipated that the pincer metal head group could be available for the coordination to various phosphines. The coordination of trianionic triarylphosphine **TPPTS** to the cationic pincer cutinase hybrid $[\mathbf{cut\text{-}1(OH_2)}][\mathbf{OTf}]$ was studied by adding different amounts of **TPPTS** (0.5, 1.0, and 1.5 equiv in D_2O) to a solution of $[\mathbf{cut\text{-}1(OH_2)}][\mathbf{OTf}]$ (2 mM) in Tris buffer (125 mM, pH 8.0, acidified with HBF_4, 10% D_2O) (Figure 6). The resulting solution was analyzed by ^{31}P NMR spectroscopy. Interestingly, we observed only one resonance at about 8.8 ppm ($^1J(^{31}P-^{195}Pt)$ = 3970 Hz) in the spectra, which points to the presence of one coordinated **TPPTS** ligand per embedded platinum, regardless of the number of equivalents of **TPPTS** present in solution. See Figure S3 in the Supporting Information for the spectrum that could be obtained for $[\mathbf{cut\text{-}1(OH_2)}][\mathbf{OTf}]$ in the presence of **TPPTS** (1.5 equiv).

The position of the peak and the observed $^1J(^{31}P-^{195}Pt)$ value (3970 Hz; ^{195}Pt satellite signals are marked in Figure S3) indicate that in the case of the embedded pincer platinum the **TPPTS** might be coordinated cis to C_{ipso}.[69,73−75,78,83] However, due to the fact that one of the satellite signals coincides with the internal standard resonance (phosphoric acid at 0 ppm), caution should be taken assigning the **TPPTS** coordination mode solely on the basis of the $^1J(^{31}P-^{195}Pt)$ values. In contrast to what has been observed for the parent low-molecular-weight NCN-pincer platinum compound, i.e. the formation of the bis-coordination product *trans*-$[\mathbf{2(3TPPTS)_2(D_2O)}]^+$ (Scheme 2), the presence of an excess of **TPPTS** (1.5 equiv) in solution did not lead to the formation of a bis-**TPPTS** cutinase pincer hybrid species.

ESI-MS Studies: Titration of [cut-1(OH$_2$)]$^+$ with Various Amounts of TPPTS. To investigate and quantify the coordination of phosphines to $[\mathbf{cut\text{-}1(OH_2)}]^+$ further, we decided to study the coordination of **TPPTS** to $[\mathbf{cut\text{-}1(OH_2)}]^+$ by ESI-MS. For this purpose we added different portions of a solution of **TPPTS** (1.0−5.0 equiv) to a solution of $[\mathbf{cut\text{-}1(OH_2)}]^+$. In order to obtain high-resolution mass spectra, we had to perform these experiments in NH_4Ac instead of Tris buffer. More importantly, a separate coordination study in NH_4Ac buffer showed results similar to those obtained in Tris, which justified the buffer switch required for this ESI-MS study.



[cut-1(OH$_2$)][OTf]
(28.7 ppm for phosphonate)

***cis*-[cut-1(TPPTS)][OTf]**
(28.5 ppm for phosphonate;
8.8 ppm, 3970 Hz for coordinated **Na_3[3]**

Figure 6. Proposed molecular structure of pincer-phosphine coordination complex *cis*-$[\mathbf{cut\text{-}1(TPPTS)}]^+$ formed upon addition of 0.5−1.5 eq. of **TPPTS** to $[\mathbf{cut\text{-}1(OH_2)}]^+$. See also Figure S3 for the recorded ^{31}P NMR spectrum.

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After addition of 1.0 equiv of **TPPTS**, mostly monocoordinated [**cut-1(TPPTS)**]$^+$ was observed (Figure 7), along with

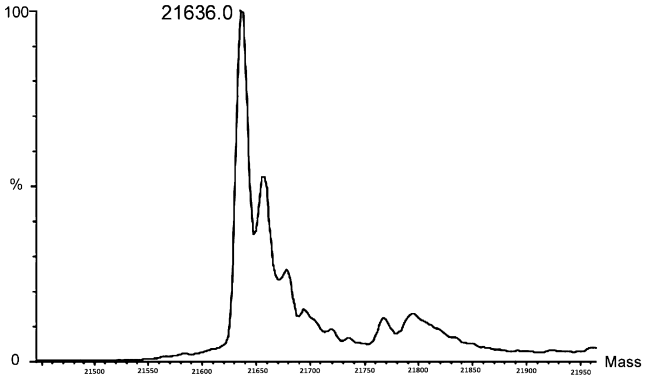

Figure 7. Deconvoluted ESI-MS spectrum of a 1:1 mix of [**cut-1(OH$_2$)**]$^+$ and **TPPTS**. The highest peak corresponds to (**H$_3$**[**cut-1(3)**]$^+$); the shoulder peaks at higher masses correspond to the association of one or two sodium ions (**H$_2$Na**[**cut-1(3)**]$^+$ and **HNa$_2$**[**cut-1(3)**]$^+$, respectively). See also Table S5.

traces of [**cut-1**]$^+$ and traces of bis-**TPPTS** coordinated **H$_6$**[**cut-1(3)$_2$**]$^+$ (vide infra). The latter result was surprising, as this seemed to contrast with the earlier conclusions from the ^{31}P NMR spectroscopic study (vide supra). It appeared that after further dilution of the solution (5× and 10×) these peaks assigned to bis coordination had disappeared and thus turned out to be aspecific. A control experiment showed that under concentrated conditions aspecific phosphine binding to free **cut** (that lacks the NCN-pincer platinum moiety) indeed occurred but disappeared upon diluting the solution.

The mass spectrum in Figure 7 showed a characteristic pattern of three shoulder peaks, with the peak with the highest intensity corresponding to the coordinated phosphine having all three Na$^+$ ions replaced by H$^+$ (**H$_3$**[**cut-1(3)**]$^+$, Table S5), i.e. displaying a +1 charged phosphine metallopincer cutinase coordination complex. The shoulder peaks correspond to the substitution of two and one Na$^+$ ions by H$^+$, respectively, i.e. of **H$_2$Na**[**cut-1(3)**]$^+$ and **HNa$_2$**[**cut-1(3)**]$^+$.

ESI-MS Studies of Reactions of [cut-1(OH$_2$)]$^+$ with Trianionic TPPTS and TXPTS, Neutral Triphenylphosphine, Tricationic [5]$^{3+}$, and Hexacationic [9]$^{6+}$ and [10]$^{6+}$. Encouraged by these results, we also studied the coordination behavior of the bulkier and differently charged triarylphosphines with [**cut-1(OH$_2$)**][**OTf**], as this potentially could be an approach to probe whether the observed binding of the **TPPTS** to the embedded platinum center could be the result of the presence of the negative charge in the **TPPTS**: i.e., by mutual lipase phosphine interactions. Therefore, to [**cut-1(OH$_2$)**][**OTf**] (1 equiv) were added different phosphines (1 equiv) and these samples were subsequently analyzed by ESI-MS. In Table S5, the results for different water-soluble cationic and anionic phosphines (**Na$_3$**[**4**], [**5**][**BF$_4$**]$_3$, [**9**][**BF$_4$**]$_6$, [**10**][**BF$_4$**]$_6$) and water-insoluble, neutral phosphine (**8**) are given.

With compound **TXPTS** we observed partial coordination of the phosphine to [**cut-1(OH$_2$)**]$^+$ when 1.0 equiv of **TXPTS** was added. In this case around 50% of free [**cut-1**]$^+$ was still observed according to mass spectrometry.

Triphenylphosphine **8** lacks charged groups and is *insoluble* in the aqueous buffer used. When **8** was added to [**cut-1(OH$_2$)**]$^+$, again partial formation of [**cut-1(8)**]$^+$ was observed: i.e., a buffer-soluble complex was formed. In order to test whether the coordination of **8** to [**cut-1(OH$_2$)**]$^+$ might be aspecific, a dilution experiment with ESI-MS analysis of the solution was carried out. These experiments indicated that the coordination was indeed selective. Thus, even though **8** itself is water insoluble, selective coordination to the embedded platinum to give water-soluble [**cut-1(8)**]$^+$ does occur.

Subsequently, we also investigated the coordination of the water-soluble tri- and hexacationic phosphines [**5**][**BF$_4$**]$_3$, [**9**][**BF$_4$**]$_6$, and [**10**][**BF$_4$**]$_6$.[84] Most interestingly, after addition of 1.0 equiv of [**5**][**BF$_4$**]$_3$, [**9**][**BF$_4$**]$_6$, or [**10**][**BF$_4$**]$_6$, we did not see any evidence for the presence of [**cut-1(phosphine)**]$^+$ coordination complexes in the ESI-mass spectra (Table S5), showing that no detectable binding of these phosphines to the protein-embedded platinum center had occurred.

CONCLUSIONS

This study has shown that the coordination of various phosphines to an artificial metal center in a semisynthetic metalloenzyme is feasible. We demonstrated that the well-established coordination chemistry for low-molecular-weight NCN-pincer platinum complexes can be transferred to novel semisynthetic metalloprotein systems having these pincer complexes embedded. However, it is apparent that in this semisynthetic protein hybrid system the pincer platinum head group exerts its own unique coordination behavior dictated by the specific influence of the protein backbone on the cationic pincer metal center. The results suggest that these effects are of both kinetic (cf. effect on the Pt−N dissociation−association process of the NCN-pincer platinum moiety and the influence of the charge of the phosphine ligand) and thermodynamic nature (cf. influence of the size of the phosphine in its complex with the embedded Pt center on its stability). An intriguing question arises when the volume and shape of the starting NCN-pincer platinum moiety, which is rather two-dimensional, is compared with those of the cis phosphine coordination product that is clearly three-dimensional with the decoordinated *o*-CH_2NMe_2 groups oriented perpendicular to the Pt's coordination plane and the bulky, negatively charged phosphine. It is obvious that sterics play a role, because coordination of two of these phosphines to the protein-embedded platinum center seems impossible (in contrast with the case for the bis-phosphine complexes obtained with the low-molecular-weight NCN-pincer platinum compound). Further studies are required to understand how the shapes, volumes, and polarity of the phosphine ligands used in the present study and the protein surface surrounding the pincer metal group affect each other. A first indication for this interplay can be found on comparing the different orientations of a NCN-pincer platinum (see Figure 1a) and a SCS-pincer palladium halide group (SCS represents the [$C_6H_3(CH_2SMe)_2$] anion), respectively, in the corresponding solid-state structures [**cut-NCN-pincer PtCl**] and [**cut-SCS-pincer PdBr**] (see overlay of their structures in the solid state in Figure 5a of ref 47). Moreover, the coordination studies described here were all performed in a strongly polar medium, in water or in aqueous Tris, respectively. Due to the fact that all phosphines except **8** are charged, polar effects influencing the interaction of the coordinated phosphines with the hydrophilic and hydrophobic areas of the protein backbone surrounding the embedded Pt center and the coordinated, charged phosphines must play a role as well. It seems likely that the occurrence of intramolecular, interligand Coulombic repulsion

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can explain the coordination of just one phosphine molecule to the platinum center. It must be noted that phosphines **TPPTS** and **TXPTS** and $[\mathbf{5}][\mathbf{BF_4}]_3$ carry opposite charges (anionic versus cationic), which can be expected to affect their coordination behavior in different ways.[85]

Notably, we demonstrated for the first time that the coordination properties of complex metal protein hybrid systems can be studied using standard chemical analysis techniques (^{31}P NMR spectroscopy and ESI-MS spectrometry), thereby creating straightforward analysis protocols without the need for laborious biochemical analysis procedures. This proof of principle study opens the door to various potential new applications in which the unique properties of synthetic metal complexes and biomolecules are combined. For example, we are currently studying the catalytic properties of similar pincer metal protein hybrids[86,88] as well as the use of luminescent NCN-pincer platinum complexes as site-selective protein labels.[87] The latter study can serve as a starting point in assaying the accessibility and the chemical properties of protein-embedded ECE-pincer metal centers.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

CIF files giving crystallographic data for $[\mathbf{2(OH_2)}]_2[\mathbf{2(3)}]$ and $[\mathbf{2(NH_2(CH_2)_2OH)}][\mathbf{OTf}]$ and text, tables, and figures giving experimental procedures and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org. The crystallographic data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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Notes

The authors declare no competing financial interest.

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■ DEDICATION

Dedicated to the memory of Prof F. Gordon A. Stone.

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