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Lipase Active-Site-Directed Anchoring of Organometallics: Metallopincer/Protein Hybrids

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Abstract: The work described herein presents a strategy for the regioselective introduction of organometallic complexes into the active site of the lipase cutinase. Nitrophenol phosphonate esters, well known for their lipase inhibitory activity, are used as anchor functionalities and were found to be ideal tools to develop a single-site-directed immobilization method. A small series of phosphonate esters, covalently attached to ECE "pincer"-type d^8-metal complexes through a propyl tether (ECE = $[C_6H_3(CH_2E)_2$-2,6$]^-$; E = NR_2 or SR), were designed and synthesized. Cutinase was treated with these organometallic phosphonate esters and the new metal-complex/protein hybrids were identified as containing exactly one organometallic unit per protein. The organometallic proteins were purified by membrane dialysis and analyzed by ESI-mass spectrometry. The major advantages of this strategy are: 1) one transition metal can be introduced regioselectively and, hence, the metal environment can potentially be fine-tuned; 2) purification procedures are facile due to the use of pre-synthesized metal complexes; and, most importantly, 3) the covalent attachment of robust organometallic pincer complexes to an enzyme is achieved, which will prevent metal leaching from these hybrids. The approach presented herein can be regarded as a tool in the development of regio- and enantioselective catalyst as well as analytical probes for studying enzyme properties (e.g., structure) and, hence, is a "proof-of-principle design" study in enzyme chemistry.

Keywords: inhibitors · lipases · metallopincer complexes · organometallic phosphonate · protein modifications

Introduction

Chemical modification of proteins is a fascinating approach for altering protein function by the introduction of non-natural fragments into proteins.[1] This strategy allows one to combine the specific properties of proteins and enzymes (substrate and stereoselectivity) with those of man-made molecules. Inspired by the pioneering work of Kaiser[2] and Whitesides,[3] this "hybrid" strategy has recently led to new developments in the field of homogeneous catalysis.[4] Here, this strategy relies on the idea of using proteins as scaffolds for transition-metal catalysts and, as such, it represents a promising method to transfer the properties of a protein (water solubility, size, chiral environment) to a transition-metal moiety. One of the first examples, developed by Whitesides[3] in the 1970s and recently optimized by Ward and co-workers,[4e] is based on biotin–avidin technology. In this noncovalent anchoring approach, the hydrogenation of N-protected dehydroamino acid derivatives by a biotinylated rhodium–diphosphine catalyst resulted in catalytic conversions with up to 96% *ee*.[4e]

Regioselective anchoring of stable transition metal probes to proteins may allow the controlled "tagging" of the protein of interest and can serve as biomarker[5] or as an additional tool in protein studies.[6] A classic example is the hy-

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bridization of mercurybenzenesulfonyl fluoride derivatives with the active site of proteases, for example, chymotrypsins.[7] These covalently bound labels were applied for the multiple isomorphous replacement method, used by crystallographers to accelerate phase determination in protein structure elucidation.

Evidently, controlled and site-directed protein modification can become of great importance when constructing transition metal complex/protein hybrid systems. Several requirements to construct a structurally defined metal complex/protein hybrid have to be considered. First of all, the linkage of the metal complex to the protein has to be stable under all future conditions (e.g., during purification and application) and at the same time metal leaching from the ligand has to be prevented. Furthermore, the protein needs to have specific and accessible positions amenable toward functionalization and the anchoring must preferably be in a regioselective manner. These requirements intrinsically demand three careful selections that have to be made: the right protein, the proper anchor unit, and a chemically stable metal complex.

In this study, we have employed *p*-nitrophenol (PnP) phosphonates esters as anchoring units.[8] PnP phosphonate esters are well known as lipase suicide inhibitors, which can irreversibly bind to the active site of various lipases (the γ-OH group of the serine residue),[9] and thereby provide a simple method to functionalize lipases selectively at a pre-established position.[10] Recently, Reetz and co-workers have investigated a similar approach by immobilizing a dinitrophenol phosphonate diphosphine ligand as a potential precursor for the synthesis of hybrid catalysts.[4d,11] However, by using this dinitrophenol phosphonate, the obtained hybrids were unstable due to basic hydrolysis and the catalytic activity of the native lipase was restored in 24 h.

The protein we have chosen to use in our studies is the enzyme cutinase, a 21 kDa lipase isolated from the fungi *Fusarium solani pisi*.[12] Because this lipase does not contain a "lid" covering the active site, it exhibits no interfacial activation, and, hence, the active site is accessible to hydrophilic as well as hydrophobic substrates in aqueous media.[13]

Metallopincer complexes, containing a terdentate monoanionic $[C_6H_3(CH_2E)_2\text{-}2,6]^-$ "pincer"-type ligand (ECE, E = NR_2, SR, or PR_2), were selected as metal-containing building blocks. These organometallic complexes possess several interesting and useful characteristics, which include an exceedingly stable M–C bond,[14] and the potential applicability in sensoring[15] and catalytic systems.[14,16] In recent years, we have reported on the facile modification of the pincer system with numerous functionalities.[17] Together with the high chemical and thermal stability, these properties should make the pincer unit a very suitable candidate for the construction of metal complex/protein hybrids by means of an active-site-directed approach.

Three active-site-directed compounds (ASDC) were developed in which PnP phosphonate esters and metallopincer moieties are combined. The design of these complexes (**1**–**3**) is based on space-filling representations of metallopincer cutinase hybrids (Figure 1).[18] Here we report on the synthesis of these organometallic ASDCs, on their inhibition reaction with wild type cutinase, and on the characterization of the resulting organometallic cutinase hybrids.

Abstract in Dutch: *Een eenvoudige methode voor het regioselectief verankeren van organometaalcomplexen aan het actieve centrum van het lipase cutinase is ontwikkeld. Hierbij is gebruik gemaakt van nitrofenolfosfonaatesters als verankeringsgroep. Deze geactiveerde fosfonaatesters zijn in staat om de enzymatische katalytische activiteit van serine-hydrolasen irreversibel te remmen en blijken daardoor ideale verankeringsfunctionaliteiten te zijn. Door zogeheten pincer–metaalcomplexen te functionaliseren met een dergelijke fosfonaatester is het mogelijk om het enzym cutinase te modificeren met palladium en platina bevattende organometaalcomplexen. De vorming van deze nieuwe hybride materialen zijn bestudeerd met UV/Vis spectrometrie en de producten zijn gezuiverd door middel van dialyse. De geïsoleerde hybriden zijn gekarakteriseerd met behulp van ESI massaspectrometrie. De voordelen van deze strategie zijn: 1) de regioselectieve verankering van een enkel overgangsmetaalcomplex aan een enzym, wat de gecontroleerde modificatie van de omgeving van het metaal mogelijk maakt, 2) zuiveringsprocedures zijn eenvoudig doordat een kompleet metaalcomplex aan het enzym gehecht wordt en bovenal, 3) de tridentate ligandmodulus van het pincerligand zorgt ervoor dat het metaal niet van het eiwit kan dissociëren. Deze strategie kan beschouwd worden als een protocol voor de ontwikkeling van regio- en enantioselectieve katalysatoren, alsmede voor de ontwikkeling van nieuwe analytische detectoren voor het bestuderen van eiwiteigenschappen en -structuren.*

Results and Discussion

Synthesis of complexes 1–3: The connection between the pincer ligand and the phosphonate ester framework was achieved by means of a Suzuki cross-coupling reaction (Scheme 1).[4g] The allyl function of phosphonamidate **4**[19] was hydroborated in situ by using 9-borobicyclononane (9-BBN) and reacted with either 2-bromo-1,3-bis[(dimethylamino)methyl]-5-iodobenzene, 2-bromo-1,3-bis[(methylthio)methyl]-5-iodobenzene or 2-bromo-1,3-bis[(phenylthio)methyl]-5-iodobenzene[19] in the presence of $[PdCl_2(dppf)]$ as catalyst.[20]

The C–C bond formation proceeded with high selectivity at the iodo-position of the pincer units to yield **5**, **6**, and **7** in high yields. Treatment of **6** and **7** with anhydrous HCl (1 M in Et_2O) generated the corresponding chlorophosphonate derivatives in situ, by replacement of the dimethylamino functionality on the phosphorous atom.[21] These intermediates were immediately treated with *p*-nitrophenol furnishing **8** and **9**. The palladium atom could readily be introduced into SCS-pincer ligands **8** and **9**. Oxidative addition by using

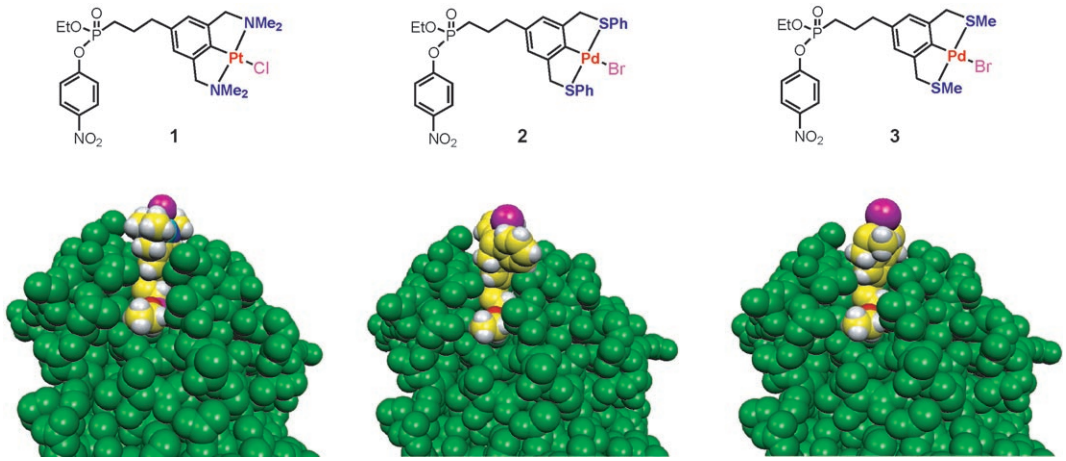
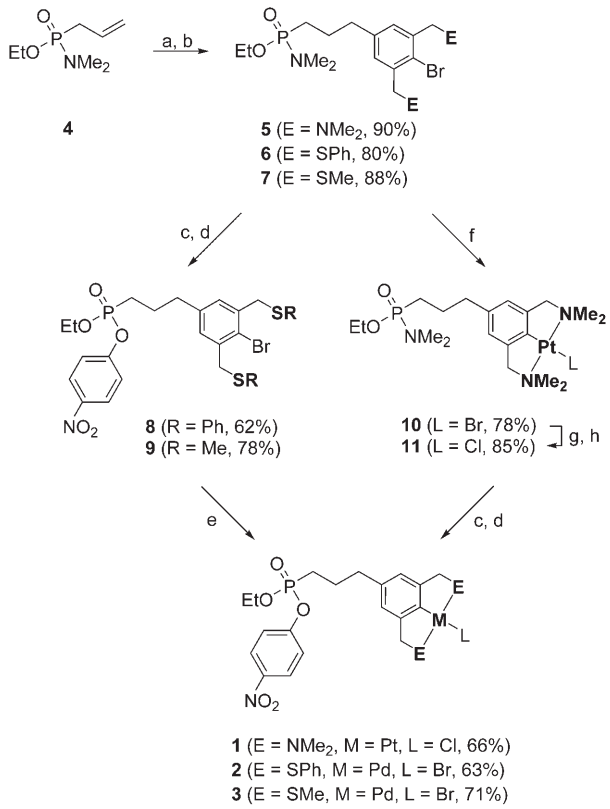


Figure 1. Upper: organometallic ASDCs **1**–**3**. Lower: close-up of space-filling presentations of the ASDCs hybridized with cutinase.[18]



Scheme 1. Synthetic Scheme for the preparation of organometallic ASDCs **1**–**3**. Reagents and conditions: a) 9-BBN, THF, reflux, 3 h; b) I-4-$C_6H_2(CH_2NMe_2)_2$-2,6-Br, I-4-$C_6H_2(CH_2SMe)_2$-2,6-Br or I-4-$C_6H_2(CH_2SPh)_2$-2,6-Br, DMF, $[PdCl_2(dppf)]$, K_3PO_4 (aq.), reflux 3 h; c) 1 M Et_2O/HCl, RT, 2 h; d) *p*-nitrophenol, NEt_3, C_6H_6, RT, 2 h; e) $[Pd_2(dba)_3]$·$CHCl_3$, C_6H_6, RT, 16 h; f) $[\{Pt(tol\text{-}4)(SEt_2)\}_2]$, C_6H_6, reflux, 2 h; g) $AgBF_4$, acetone, H_2O, RT, 1 h; h) NaCl, CH_2Cl_2, RT, 1 h.

$[Pd_2(dba)_3]$·$CHCl_3$ resulted in the desired complexes **2** and **3**, respectively.[16b,c]

The same reaction sequence was also envisaged to synthesize platinum complex **1**. However, reaction of NCN compound **5** with anhydrous HCl resulted in the formation of an inseparable mixture of phosphonic acid, phosphonic acid anhydride, and desired product, as well as some unidentified compounds. This is most probably caused by the basic NMe_2 functionalities of the pincer moiety. For this reason ligand **5** was first cyclometallated to give **10** by using $[\{Pt(tol\text{-}4)_2(SEt_2)\}_2]$[22] as the platinum source. This makes the pincer's NMe_2 groups less basic and non-nucleophilic due to coordination of the nitrogen atoms to the platinum center. To avoid halogen scrambling in the subsequent chlorination of the phosphonate, the bromide ligand of the NCN platinum moiety was first exchanged for chloride. This was achieved by treatment of **10** with $AgBF_4$ in wet acetone, followed by addition of NaCl to obtain **11** as an off-white solid.[23] Analogous to **5** and **6**, the $P(O)NMe_2$ functionality of **9** was treated with anhydrous HCl, affording the desired P(O)Cl-derivative without decomposition of the NCN-pincer platinum complex. This clearly demonstrates the high stability of the Pt–C bond in organometallic NCN-pincer platinum complexes. Reaction of the phosphonate chloride compound with *p*-nitrophenol resulted in the desired complex **1** (Scheme 1). Unfortunately, the corresponding palladium complex could not be prepared. This is due to the incompatibility of the NCN pincer palladium chloride unit with the anhydrous HCl conditions required to generate the P(O)Cl functionality.

Anchoring of the metal complexes to cutinase: We made use of PnP phosphonates for three main reasons: 1) they are very active compounds which selectively react with the active site, that is, serine residue 120 of cutinase; 2) the reaction profile can be followed spectrometrically due to the high UV-visible absorption of the *p*-nitrophenolate anion ($\varepsilon = 16230\ M^{-1}cm^{-1}$, 400 nm, pH 8);[24] and 3) the PnP phosphonates are more stable towards hydrolysis compared to their chloro and dinitrophenol phosphonate counterparts.

The reactions of **1**, **2**, and **3** with the active site of cutinase were monitored over time by measuring the increase of the absorbance at 400 nm (pH 8.0, RT, 25 or 50 μM ASDC, and 25 μM cutinase). When two equivalents of **1** were used with

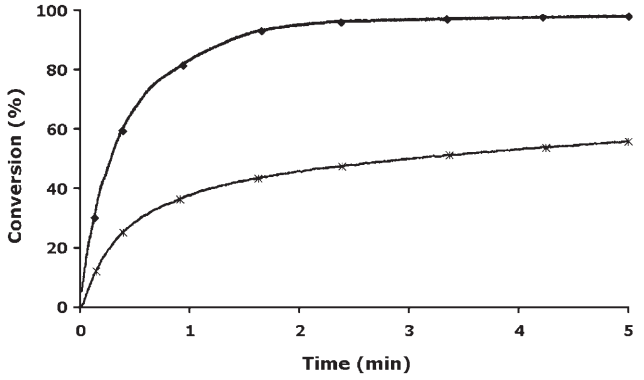


Figure 2. Conversion of cutinase upon reaction with **1**. Release of nitrophenolate was monitored by following the absorbance increase at 400 nm. Conditions: 25 μM cutinase; 25 μM (stars) and 50 μM (diamonds) of ASDC **1**. Buffer: 50 mM Tris-HCl, 0.1 % Triton X-100 at pH 8.0.

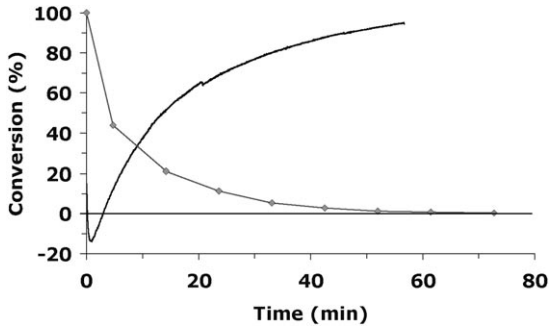


Figure 3. Conversion of cutinase upon reaction with **2**. Unmarked line: Release of nitrophenolate was monitored by following the absorbance increase at 400 nm. Decay line: decrease of enzymatic activity monitored by using a spectrometric assay containing *p*-nitrophenolbutyrate. Reagents and conditions: 25 μM cutinase, 50 μM ASDC **2**, buffer: 50 mM Tris-HCl, 0.1 % Triton X-100 at pH 8.0.

respect to cutinase (Figure 2, upper line, diamonds), a rapid time-dependent conversion of **1** to form the **Cut-1** hybrid was found to occur with a concomitant release of *p*-nitrophenolate anion. After five minutes, a full conversion of cutinase to the hybrid was accomplished.

Earlier studies have shown that the reaction of cutinase with phosphonates proceeds with high enantioselectivity.[25] For this reason we also carried out an experiment by using a stoichiometric amount of **1** with respect to cutinase (Figure 2, bottom line, stars). In this experiment we observed an initial and fast reaction in which 50 % of the racemic mixture of **1** (one enantiomer) and 50 % of the enzyme are converted to the **Cut-1** hybrid, followed by a slower reaction of the remaining and less reactive enantiomer of **1**. These observations imply a 1:1 ASDC/cutinase stoichiometry and in addition, that a chiral preference of cutinase exists for the organometallic ASDCs, much alike other phosphonate inhibitors.[25] Unfortunately, we have not been able to recover the slower reacting enantiomer of **1** after completion of the 2:1 **1**/cutinase reaction. In addition, neither NMR shift reagents nor the use of chiral HPLC was successful in discriminating the enantiomers of **1**. For these

Figure 4. Reaction mixture of **2** with cutinase. Left: start of reaction. Right: end of reaction containing the **Cut-2** hybrid.

reasons we were unable to determine which enantiomer reacts faster and which was left unreacted in solution.

The same reaction was performed with the SCS-pincer complex **2** (with phenyl substituents on the sulfur donor atom). In this reaction also a full conversion was observed (25 μM cutinase:50 μM **2**). Remarkably, the reaction proceeded approximately 12 times slower than the corresponding reaction with NCN ASDC **1** (5 min vs 60 min, Figure 3, unmarked line).

This difference in reaction kinetics might be attributed to the different solubility properties of **2** as compared to **1**. Whereas complex **1** was completely soluble in the buffer solution, addition of **2** to the buffered cutinase solution resulted in a turbid solution, causing the negative absorption evident in Figure 3.[26] During the reaction the turbidity gradually disappeared, resulting in a clear yellowish solution (Figure 4), which illustrates the solubility effect of metallopincer cutinase hybrid formation on the metallopincer complex.

The reaction between **2** and cutinase was also followed in time by measuring the residual enzymatic catalytic activity using a *p*-nitrophenyl butyrate based spectrometric assay (decay line in Figure 3). The same reaction profile was found confirming the results obtained by following the release of *p*-nitrophenolate by UV-visible spectroscopy.

When ASDC **3**, bearing methyl substituents on the sulfur donor atoms, was reacted with cutinase under the same reaction conditions as for complexes **1** and **2** (i.e., in a 2:1 ASDC/cutinase ratio), the same total conversion time was observed as for the reaction of **1** with cutinase (Figure 5, stars). In this experiment **3** was completely soluble in the buffer solution at the start of the reaction.

To examine the reaction rate effect during our previous experiment in which we used ASCD **2**, a final control experiment was performed. Complexes **1** and **2** were incubated with cutinase at concentrations at which clear solutions were obtained (6 μM cutinase and 12 μM of ACSD). For these reactions, lower initial reaction rates were observed for each reaction, but with the same rate ratio difference as in the

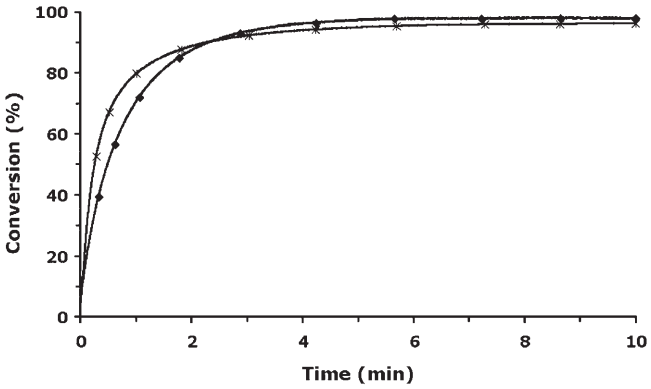


Figure 5. Conversion of cutinase upon reaction with **1** (diamonds) and **3** (stars). Release of nitrophenolate was monitored by following the absorbance increase at 400 nm. Conditions: 25 μM cutinase, 50 μM ASDC, buffer: 50 mM Tris-HCl, 0.1 % Triton X-100 at pH 8.0.

previous experiments with higher reactant concentrations. This observation strongly suggests that steric factors determine the rate of inhibition to a greater extent than the solubility of the ASDCs.

Isolation, purification and characterization of cutinase metallopincer hybrids: A series of ESI-MS samples were prepared by reacting cutinase with two equivalents of **1**, **2**, or **3** over a period of 48 h (Tris buffer, pH 8.0). Prior to purification and analysis of the product, the residual enzymatic catalytic activity was determined by using a *p*-nitrophenyl butyrate based spectrometric assay. No release of *p*-nitrophenolate was observed in all reactions after 48 h of incubation of cutinase; a result that indicates that no esterase activity is left and no wild-type cutinase is present in solution. The crude materials were then purified by dialysis[27] (2×12 h) and analyzed by ESI mass spectrometry.[28] The organometallic complexes are permeable through the dialysis membrane in the set-up used for sample preparation, whereas the native enzyme and the hybrids are not. The dialysis set-up does not seem to degrade the organometallic moieties and, therefore, is ideal for the purification of the metallopincer/protein hybrids.

The native enzyme was used as calibration material for the ESI analysis and a mass of 20603.9 ± 0.2 Da was found (calculated: 20604.1 Da; Table 1). All cutinase metallopincer hybrids showed a somewhat lower mass in the ESI mass spectra than the calculated value. This difference originates from the release of a halide ion from the metal center during the measurement; a result typically found in ESI mass spectra of metallopincer complexes. In the mass spectra of hybrid materials no mass signals corresponding to 20604 Da were observed, indicating complete conversion of wild-type cutinase to the hybrids.

Table 1. ESI-MS results of ASDC-cutinase hybrids.[28]

Compound	Calculated [Da]	Calculated −halide [Da]	Found [Da]
cutinase	20603.1	–	20603.9 ± 0.2
Cut-1	21158.0	21122.5[a]	21120.9 ± 0.4
Cut-2	21244.0	21164.1[b]	21163.2 ± 0.4
Cut-3	21119.8	21039.9[b]	21039.1 ± 0.7

[a] $[M^+-\mathrm{Cl}]$. [b] $[M^+-\mathrm{Br}]$.

The results of the MS measurements show the high integrity of the metallopincer cutinase hybrids. Not only is a 100 % conversion obtained, but also is a precise 1:1 metallopincer/cutinase stoichiometry achieved. The metallopincer/cutinase hybrids can thus be prepared in a single and selective step by specific modification of one amino acid residue of the lipase and, hence, exactly one metal can be introduced in the chiral cavity of cutinase.

Conclusion

This study demonstrates the use of irreversible enzyme inhibitors as anchoring moieties for the site-directed attachment of organometallic complexes to enzymes. Suicide inhibitors are excellent tools for this purpose since they often react chemo-, regio-, and enantioselectively, as well as stoichiometrically with enzymes. Especially the last point is important in constructing a structurally well-defined hybrid. The results from the ESI-mass analysis nicely confirm that discrete 1:1 hybrids are obtained between cutinase and the organometallic ASDCs **1**–**3** by using PnP phosphonate esters as anchoring groups. Moreover, the ESI-mass results also indicate that the metallopincer units of the ASDCs are stable under the applied reaction conditions, 48 h in an aqueous buffer solution at pH 8.0, and withstand aqueous dialysis purification conditions (2×12 h). The stability of the organometallic unit is evidently of great importance to maintain its functionality and to prevent metal leaching. Noteworthy is that, due to the covalent bond formed during the hybridization reaction of the ASDCs with the lipase, dissociation of the phosphonate from the lipase will not occur ($\mathrm{pH}<11$). This feature permits the use of polar and apolar solvents in future applications. The terdentate pincer ligand allows the introduction of a variety of transition metals and in this respect provides a unique method to merge organometallics with the properties found in biology (selectivity, solubility, and size) by using the hybridization protocol outlined in this study. One other major advantage of this approach is the positional precision with which the transition metal unit is introduced in the active site of the enzyme. This positioning promises a fine-tuning of the organometallic/enzyme hybrids on the atomic level. Our current research efforts indeed focus on the hybridization of a variety of transition-metal complexes with cutinase and related proteases, and on the use of such hybrids in aqueous homogeneous catalysis.

Experimental Section

General comments: All organic and organometallic reactions were conducted under a dry dinitrogen atmosphere by using standard Schlenk

techniques. Organic solvents were dried over appropriate materials and distilled prior to use. All reagents were obtained commercially and used without further purification unless otherwise stated. Triton X-100 was purchased from Serva and tris(hydroxymethyl)aminomethane from J. T. Baker. Buffer solutions were prepared using Milli-Q grade water. Purification of water (18.2 MΩ cm) was performed with the Milli-Q Synthesis system (Millipore, Quantum Ultrapure). ^{1}H and ^{13}C{^{1}H} NMR spectra were recorded at 298 K on a Varian Inova 300 spectrometer at 300 and 75 MHz, respectively, and ^{31}P{^{1}H} NMR spectra were recorded at 298 K on a Bruker AC200 at 81 MHz. All NMR chemical shifts are in ppm referenced to residual solvents (^{31}P{^{1}H} NMR shifts to H_3PO_4). The MALDI-TOF mass spectra were acquired using a Voyager-DE Biospectrometry Workstation (PerSeptive Biosystems Inc, Framingham, MA, USA) mass spectrometer. Sample solutions with an approximate concentration of 20–30 mg mL^{-1} in CH_2Cl_2 or THF were prepared. The matrix was 5-chlorosalicylic acid (CSA) with an approximate concentration of 20–30 mg mL^{-1}. A 0.2 μL of the sample and 0.2 μL of the matrix solution were combined and placed on a golden MALDI target plate and analyzed after evaporation of the solvents. [{Pt(tol-4)$_2$(SEt$_2$)}$_2$],[22] [Pd$_2$(dba)$_3$]·CHCl$_3$[29] and 2-bromo-1,3-bis[(dimethylamino)methyl]-5-iodobenzene[30] were prepared according to described procedures. Microcoanalyses were performed by Kolbe, Mikroanalystisches Laboratorium (Müllheim a/d Ruhr, Germany). UV-visible experiments were performed at room temperature using a Carey-100 spectrometer. Electrospray ionization mass spectra of the wild-type cutinase and modified cutinase were recorded on a Finnigan LC-Q ion-trap mass spectrometer. All samples were introduced using a nanoflow electrospray source (Protana, Odense, Denmark).

Inhibition experiments: A solution of **1**, **2**, or **3** in CH_3CN (5–10 μL; 50 mmol, 10 μL á 5 mM) was added to a buffer solution (0.1 % Triton X-100, 50 mM Tris-HCl, pH 8.0) containing wild-type cutinase (25 μM, 25 μmol). The reaction was followed by UV-visible spectroscopy until the reaction was complete, which was confirmed by a plateauing of the intensity of the UV absorption at 400 nm.

Hydrolysis activity of cutinase: The catalytic activity of cutinase was determined spectrometrically on 0.25 mM *p*-nitrophenol butyrate in the presence of 100 mM Triton X-100 and 10 mM Tris-HCl at pH 8.0. Aliquots of 5–10 μL were taken and added to the assay. Activities were calculated from the increase of absorbance at 400 nm.

Chemical stability experiments: The chemical stability of ACDC **1** in the buffer solution (0.1 % Triton X-100, 50 mM Tris-HCl, pH 8.0) was examined over 48 h using UV-visible spectroscopy (400 nm). The compound did not show any hydrolysis of PnP phosphonate ester functionality during this time. Naturally, the stability of cutinase was also tested under the same conditions. Aliquots were taken and added to a spectrometric assay containing 0.25 mM *p*-nitrophenyl butyrate. No decrease of ester hydrolysis activity, and thus no catalytic deactivation, was observed during a 3 day period.

Preparation of samples for mass spectrometry analysis: Cutinase (25 μM) in a 10 mM Tris-HCl buffer solution (pH 8.0) was modified during 48 h by adding an excess of **1**, **2**, or **3**. Completion of the reaction was confirmed by determining the residual catalytic hydrolyses activity of cutinase. The clear yellow solutions were subsequently dialyzed for 2 × 12 h using the same buffer as dialysis solvent. Prior to ESI mass measurements the solutions were diluted to 2–5 μM with CH_3CN.

Preparation of ethyl *P*-{3-{4-bromo-1,3-bis[(dimethylamino)methyl]phenyl}propyl}-*N*,*N*-dimethylaminophosphinate (5):[4g] A solution of allyl ethyl *N*,*N*-dimethylaminophosphinate **4**[19] (1.53 g, 8.65 mmol) in THF (15 mL) was treated with 9-BBN (34.4 mL a 0.5 M in THF, 17.20 mmol). The prepared mixture was stirred for 3 h at reflux temperature. The colorless solution was allowed to cool to room temperature and a degassed aqueous solution of K_3PO_4 (3 M, 7.2 mL, 21.60 mmol) was added, followed by a solution of 2-bromo-1,3-bis[(dimethylamino)methyl]-5-iodobenzene (3.26 g, 8.21 mmol) and [PdCl$_2$(dppf)] (0.5 g, 0.68 mmol) in DMF (15 mL). The solution immediately turned dark red after addition of the palladium catalyst. The mixture was heated to reflux for 3 h and during this period a clear orange solution was formed. THF was evaporated in vacuo and the mixture diluted with Et_2O (60 mL) and water (40 mL). The organic phase was separated and the aqueous phase extracted with Et_2O (2 × 50 mL). The organic fractions were combined, washed with 1 N NaOH (2 × 50 mL) and brine, dried over $MgSO_4$, and concentrated in vacuo to obtain a dark brown oil. The crude was dissolved in Et_2O (5 mL) and purified by column chromatography (silica, eluting with Et_2O, then CH_2Cl_2 and finally with MeOH/5 % NEt_3) to obtain the product as a yellowish oil (3.20 g, 90 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.15 (s, 2H; ArH), 3.90 (m, 2H; CH_2O), 3.52 (s, 4H; CH_2N), 2.63 (d, ^3J(H,P) = 8.79 Hz, 6H; PNMe$_2$ and 2H; CH_2Ar), 2.29 (s, 12H; NMe$_2$), 1.80 (m, 2H; PCH_2), 1.75 (m, 2H; PCH_2CH_2), 1.26 ppm (t, ^3J(H,H) = 6.96 Hz, CH_3; 3H); ^{31}P NMR (81 MHz, $CDCl_3$): δ = 37.25 ppm (s); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 140.43, 139.05, 129.74, 124.27 (s; ArC), 63.85 (s; CH_2N), 59.06 (d, ^2J(C,P) = 6.71 Hz; CH_2O), 45.40 (s; NMe$_2$), 36.09 (d, ^2J(C,P) = 15.26 Hz; CH_2CH_2P), 35.73 (d, ^2J(C,P) = 4.27 Hz; PNMe$_2$), 26.41 (d, ^1J(C,P) = 131.23 Hz; CH_2P), 24.27 (d, ^3J(C,P) = 3.62 Hz; $ArCH_2$), 16.23 ppm (d, ^3J(C,P) = 6.72 Hz; CH_3); elemental analysis calcd (%) for $C_{19}H_{35}BrN_3O_2P$ (448.38): C 50.89, H 7.87, N 9.37; found: C 50.96, H 7.97, N 9.31.

Preparation of ethyl *P*-{3-{4-bromo-1,3-bis[(phenylthio)methyl]phenyl}propyl}-*N*,*N*-dimethylaminophosphinate (6): A similar procedure as for **5** was applied. Compound **4** (0.50 g, 2.85 mmol), THF (10 mL), 9-BBN (0.41 M, 15.3 mL, 6.27 mmol). K_3PO_4 (3 M, 2.0 mL, 6 mmol), [PdCl$_2$(dppf)] (0.18 g, 0.23 mmol), 2-bromo-1,3-bis[(phenylthio)methyl]-5-iodobenzene[19] (1.50 g, 2.85 mmol), DMF (20 mL). The product was purified by column chromatography eluting with Et_2O and acetone. After concentration of the product containing fractions (acetone), a yellow oil was obtained. Yield: 1.31 (80 %); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.34–7.18 (br m, 10H; SArH), 6.89 (s, 2H; ArH), 4.22 (s, 4H; CH_2S), 3.90 (m, 2H; CH_2O), 2.65 (m, 2H; CH_2Ar), 2.63 (d, ^3J(H,P) = 8.79 Hz, PNMe$_2$; 6H), 2.29 (s, 12H; NMe$_2$), 1.80 (m, 2H; PCH_2), 1.75 (m, 2H; PCH_2CH_2), 1.26 ppm (t, ^3J(H,H) = 6.96 Hz, CH_3; 3H); ^{31}P NMR (81 MHz, $CDCl_3$): δ = 37.09 ppm (s); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 142.03, 137.99, 136.48, 130.19, 129.06, 128.16, 127.35, 126.62, 59.37 (d, ^2J(C,P) = 6.72 Hz; CH_2O), 40.88 (s; CH_2S), 36.36 (d, ^2J(C,P) = 3.62 Hz; PCH_2CH_2), 36.01 (d, ^3J(C,P) = 16.53 Hz; PN(CH$_3$)$_2$), 24.86 (d, ^1J(C,P) = 131.24 Hz; PCH_2), 23.78 (d, ^3J(C,P) = 3.70 Hz; CH_2Ar), 16.56 ppm (d, ^3J(C,P) = 6.72 Hz; CH_3); elemental analysis cald (%) for $C_{27}H_{33}BrNO_2PS_2$ (578.56): C 56.05, H 5.75, N 2.42, S 11.08; found: C 56.18, H 5.83, N 2.37, S 11.16.

Preparation of ethyl *P*-{3-{4-bromo-1,3-bis[(methylthio)methyl]phenyl}propyl}-*N*,*N*-dimethylaminophosphinate (7): A similar procedure as for **5** was applied. Compound **4** (0.88 g, 5.00 mmol), THF (20 mL), 9-BBN (1.34 g, 10.10 mmol, 0.5 M in THF), K_3PO_4 (3 M, 5.5 mL, 17.50 mmol), 2-bromo-1,3-bis[(methylthio)methyl]-5-iodobenzene[19] (2.01 g, 5.00 mmol), [PdCl$_2$(dppf)] (0.33 g, 0.43 mmol), DMF (15 mL). The crude was dissolved in 5 mL Et_2O and purified by column chromatography (silica, eluting with CH_2Cl_2/acetone 3:1) to obtain the product as a yellowish oil 2.02 g (88 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.06 (s, 2H; ArH), 4.01 (m, 1H; CH_2O), 3.88 (m, 1H; CH_2O), 3.83 (s, 4H; CH_2S), 2.66 (d, ^3J(H,P) = 9.00 Hz, 6H; NMe$_2$ and 2H; CH_2Ar), 2.07 (s, 6H; SMe), 1.80 (br m, 4H; CH_2), 1.28 ppm (t, ^3J(H,H) = 7.20 Hz, 3H; CH_3); ^{31}P NMR (81 MHz, $CDCl_3$): δ = 37.07 ppm (s); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 140.40, 138.68, 129.83, 124.30 (s; ArC), 59.41 (d, ^2J(C,P) = 6.68 Hz, CH_2O; 2H), 39.56 (s; CH_2S), 36.37 (d, ^2J(C,P) = 16.43 Hz; PCH_2CH_2), 36.11 (d, ^2J(C,P) = 3.68 Hz; NMe$_2$), 24.87 (d, ^1J(C,P) = 132.83 Hz; PCH_2), 24.03 (d, ^3J(C,P) = 3.62 Hz; CH_2Ar), 16.54 (d, ^3J(C,P) = 6.68 Hz; CH_3CH_2O), 15.64 ppm (s; SMe); elemental analysis cald (%) for $C_{15}H_{24}BrNOPS_2$ (454.43): C 44.93, H 6.43, N 3.08, S 14.11; found: C 45.10, H 6.51, N 3.03, S 14.06.

Preparation of ethyl 4-nitrophenyl *P*-{3-{4-bromo-1,3-bis[(phenylthio)methyl]phenyl}propyl}phosphonate (8): Compound **6** (0.49 g, 0.85 mmol) was dissolved in dry benzene (10 mL) and treated with a solution of anhydrous HCl (4.25 mL, 4.25 mmol, 1 M in Et_2O). After stirring for 2 h at room temperature under a nitrogen atmosphere, more benzene (10 mL) was added and the yellow suspension was filtered. A solution of *p*-nitrophenol (0.12 g, 0.85 mmol) and NEt_3 (0.6 mL, 4.24 mmol) in benzene (20 mL) was added dropwise to the filtrate and stirring was continued for 2 h. All volatiles were removed in vacuo and Et_2O (30 mL) was added followed by a saturated solution of K_2CO_3 (30 mL). The organic phase

was separated and washed with a saturated solution of K_2CO_3 (30 mL), water (30 mL), and brine (30 mL). The solution was dried on $MgSO_4$ and concentrated to give a pale yellow solid. Yield: 0.35 g (62%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.21 (d, ^3J(H,H) = 8.70 Hz, 2H; ArH), 7.35–7.17 (br m; 10H; SArH), 6.89 (s, 2H; ArH), 4.22 (s, 4H; CH_2S), 4.16 (m, 2H; CH_2O), 2.51 (t, ^3J(H,H) = 6.30 Hz, 2H; CH_2Ar), 1.81 (br m, 4H; PCH_2, PCH_2CH_2), 1.30 ppm (t, ^3J(H,H) = 6.90 Hz, CH_3; 3H); ^{31}P NMR (81 MHz, $CDCl_3$): δ = 30.56 ppm (s); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.82 (d, ^2J(C,P) = 8.48 Hz; ArC), 144.78, 139.58, 138.03, 135.83, 131.18, 130.02, 129.14, 127.12, 126.30, 124.49 (s; ArC), 121.19 (d, ArC, ^3J(C,P) = 4.28 Hz), 63.25 (d, ^2J(C,P) = 7.32 Hz; CH_2O), 40.82 (s; CH_2S), 35.54 (d, ^2J(C,P) = 17.06 Hz; PCH_2CH_2), 25.47 (d, ^1J(C,P) = 140.97 Hz; PCH_2), 23.77 (d, ^3J(C,P) = 4.91 Hz; CH_2Ar), 16.64 ppm (d, ^3J(C,P) = 5.51 Hz; CH_3CH_2O); elemental analysis cald (%) for $C_{31}H_{31}BrNO_5PS_2$ (672.59): C 55.36, H 4.65, N 2.08, S 9.53; found: C 55.36, H 4.77, N 2.02, S 9.58.

Preparation of ethyl 4-nitrophenyl *P*-{3-{4-bromo-1,3-bis[(methylthio)methyl]phenyl}propyl}phosphonate (9): A similar procedure as for **8** was applied. Compound **7** (0.41 mg, 0.90 mmol), anhydrous HCl (4.50 mL, 4.50 mmol, 1.0 M in Et_2O); triethylamine (0.14 mL), *p*-nitrophenol (125.5 mg, 0.90 mmol) in benzene (15 mL). The product was obtained as a yellowish solid (0.39 g, 78%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.16 (d, ^3J(H,H) = 7.20 Hz, ArH; 2H), 7.31 (d, ^3J(H,H) = 8.10 Hz, ArH; 2H), 7.03 (s, 2H; ArH), 4.20–4.10 (br m, 2H; CH_2O), 3.76 (s, 4H; CH_2), 2.66 (t, ^3J(H,H) = 6.90 Hz, 2H; PCH_2CH_2), 2.02–1.86 (m, 4H; CH_2Ar, PCH_2), 2.01 (s, 6H; SMe), 1.26 ppm (t, ^3J(H,H) = 7.20 Hz, 3H; CH_3); ^{31}P NMR (81 MHz, $CDCl_3$): δ = 30.50 ppm (s); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.84 (d, ^2J(C,P) = 7.93 Hz; ArC), 144.72, 139.60, 138.87, 129.70, 125.87, 124.58 (s; ArC), 121.21 (d, ^3J(C,P) = 4.30 Hz; ArC), 63.56 (d, ^2J(C,P) = 7.31 Hz; CH_2O), 39.50 (s; CH_2S), 35.60 (d, ^2J(C,P) = 17.67 Hz; PCH_2CH_2), 25.58 (d, ^1J(C,P) = 140.10 Hz; PCH_2), 24.11 (d, ^3J(C,P) = 4.80 Hz; CH_2Ar), 16.60 ppm (d, ^3J(C,P) = 5.50 Hz; CH_3); elemental analysis cald (%) for $C_{21}H_{27}BrNO_5PS_2$ (548,45): C 45.99, H 4.96, N 2.55, S 11.69; found: C 46.15, H 5.08, N 2.48, S 11.52.

Preparation of ethyl *N,N*-dimethylamino-*P*-{3-{4-(bromoplatino)-1,3-bis[(dimethylamino)methyl]phenyl}propyl}phosphinate (10):[4g] Solid [{Pt-(tol-4)$_2$(SEt$_2$)}$_2$] (1.06 g, 1.14 mmol) was added to a solution of **5** (1.02 g, 2.27 mmol) in dry toluene (45 mL) and heated to reflux for 2 h. The solvent was removed in vacuo and the remaining dark brown oil dissolved in CH_2Cl_2 (15 mL) and filtered through a short path of Celite. The filtrate was concentrated under reduced pressure leaving a brown oil, which was subsequently washed with pentane (3×30 mL, stirring 30 min) and Et_2O (2×30 mL, stirring 30 min). The resulting mixture was dried in vacuo to obtain the product as an off-white solid (1.11 g, 78%). ^{1}H NMR (300 MHz, C_6D_6): δ = 6.52 (s, 2H; ArH), 3.91 (m, 2H; CH_2O), 3.33 (s, ^3J(H,Pt) = 44.56 Hz, 4H; CH_2N), 2.77 (s, ^3J(H,Pt) = 37.54 Hz, 12H; NMe_2), 2.60 (t, ^3J(H,H) = 7.32 Hz, 4H; CH_2Ar), 2.43 (d, ^3J(H,P) = 8.85 Hz, 6H; $PNMe_2$), 2.02 (m, 2H; CH_2), 1.71 (m, 2H; PCH_2CH_2), 1.06 ppm (t, ^3J(H,H) = 7.02 Hz, 3H; CH_3,); ^{31}P NMR (81 MHz, C_6D_6): δ = 36.26 ppm (s); ^{13}C NMR (75 MHz, C_6D_6): δ = 145.52, 143.77, 136.38, 134.52, 119.54 (s; ArC), 77.13 (s, ^2J(C,Pt) = 32.96 Hz; CH_2N), 58.82 (s; CH_2O), 54.62 (s; NMe_2), 37.44 (d, ^2J(C,P) = 14.65 Hz; CH_2CH_2P), 35.81 (d, ^2J(C,P) = 3.70 Hz; $PNMe_2$), 25.33 (d, ^1J(C,P) = 130.64 Hz; CH_2P), 24.27 (d; $ArCH_2$), 16.23 ppm (d, ^3J(C,P) = 6.72 Hz; CH_3); MS (MALDI-TOF, CSA): *m*/*z*: 561.1 [M^+−Br], 467.2 [M^+−Br−Pt]; elemental analysis cald (%) for $C_{19}H_{35}BrN_3O_2PPt$ (643.46): C 35.47, H 5.48, N 6.53; found: C 35.48, H 5.41, N 6.38.

Preparation of ethyl *N,N*-dimethylamino-*P*-{3-{4-(chloroplatino)-1,3-bis[(dimethylamino)methyl]phenyl}propyl}phosphinate (11): Compound **10** (0.76 g, 1.20 mmol) in wet acetone (10 mL) was treated with $AgBF_4$ (0.25 g, 1.26 mmol) for 1 h. The reaction mixture was filtered over Celite and NaCl (0.74 g, 12.60 mmol) was added to the filtrate. The solvent was removed in vacuo after 1 h stirring of the reaction mixture, and subsequently water (20 mL) was added. The product was extracted with CH_2Cl_2 (4×20 mL), the combined fractions dried on $MgSO_4$ and concentrated under reduced pressure leaving an off-white solid (0.61 g, 85%). ^{1}H NMR (300 MHz, C_6D_6): δ = 6.54 (s, 2H; ArH), 3.93 (m, 1H; CH_2O), 3.69 (m, 1H; CH_2O), 3.38 (s, ^3J(H,Pt) = 44.69 Hz, 4H; CH_2N), 2.75 (s, ^3J(H,Pt) = 38.46 Hz, 12H; NMe_2), 2.60 (t, ^3J(H,H) = 7.33 Hz, 2H; CH_2Ar), 2.43 (d, ^3J(H,P) = 8.79 Hz, 6H; $PNMe_2$), 2.03 (m, 2H; PCH_2), 1.71 (m, 2H; PCH_2CH_2), 1.06 ppm (t, ^3J(H,H) = 7.01 Hz, 3H; CH_3); ^{31}P NMR (81 MHz, C_6D_6): δ = 36.13 ppm (s); ^{13}C NMR (75 MHz, C_6D_6): δ = 144.62, 143.79, 136.21 (s; ArC), 119.55 (s, ^3J(C,Pt) = 31.74 Hz; ArC), 77.69 (s, ^3J(C,Pt) = 31.13 Hz; CH_2N), 58.89 (d, ^2J(C,P) = 6.71 Hz; CH_2O), 53.98 (s; NMe_2), 37.48 (d, ^2J(C,P) = 15.32 Hz; CH_2CH_2P), 35.83 (d, ^2J(C,P) = 3.70 Hz; $PNMe_2$), 24.97 (d, ^1J(C,P) = 130.64 Hz; CH_2P), 24.87 (s, ^3J(C,P) = 3.70 Hz; $ArCH_2$), 16.38 ppm (d; CH_3); elemental analysis cald (%) for $C_{19}H_{35}ClN_3O_2PPt$ (599.00): C 38.10, H 5.92, N 7.02; found: C 37.95, H 5.76, N 6.88.

Preparation of ethyl 4-nitrophenyl *P*-{3-{4-(chloroplatino)-1,3-bis[(dimethylamino)methyl]phenyl}propyl}phosphonate (1): A similar procedure as for **8** and **9** was applied. Compound **11** (246.1 mg, 0.41 mmol), anhydrous HCl (2.05 mL, 2.05 mmol, 1.0 M in Et_2O); triethylamine (0.3 mL), *p*-nitrophenol (57.2 mg, 0.41 mmol) in benzene (15 mL). The product was obtained as a yellowish solid (199 mg, 66%). ^{1}H NMR (300 MHz, C_6D_6): δ = 7.76 (d, ^3J(H,H) = 7.63 Hz, 2H; ArH), 7.05 (d, ^3J(H,H) = 8.24 Hz, 2H; ArH), 6.45 (s, 2H; ArH), 3.88 (m, 2H; CH_2O), 3.35 (s, ^3J(H,Pt) = 43.94 Hz, 4H; CH_2N), 2.73 (s, ^3J(H,Pt) = 32.00 Hz, 12H; NMe_2), 2.51 (t, ^3J(H,H) = 7.63, 2H; $ArCH_2$), 2.02 (m, 2H; PCH_2CH_2), 1.79 (m, 2H; CH_2P), 0.95 ppm (t, ^3J(H,H) = 7.02 Hz, 3H; CH_3,); ^{31}P NMR (81 MHz, C_6D_6): δ = 30.52 ppm (s); ^{13}C NMR (75 MHz, C_6D_6): δ = 155.85 (d, ^2J(C,P) = 7.92 Hz; ArC), 144.65, 144.00, 135.25, 125.51 (s; ArC), 120.81 (d, ^3J(C,P) = 4.88; ArC), 119.41 (s; ArC), 77.68 (s; CH_2N), 62.52 (d, ^2J(C,P) = 6.71 Hz; CH_2O), 53.99 (s; NMe_2), 37.00 (d, ^2J(C,P) = 16.48 Hz; CH_2CH_2P), 25.70 (s, ^1J(C,P) = 141.00 Hz; PCH_2), 24.66 (d, ^3J(C,P) = 4.88 Hz; $ArCH_2$), 16.23 ppm (d, ^3J(C,P) = 6.10 Hz; CH_3); MS (MALDI-TOF, CSA): *m*/*z*: 656.3 [M^+−Cl], 462.2 [M^+−Cl−Pt], 374.6 [M^+−Cl−Pt−NO_2−OEt], 343.1 [M^+−Cl−Pt−NO_2−OEt−2Me]; elemental analysis cald (%) for $C_{23}H_{33}ClN_3O_5PPt$ (693.03): C 39.86, H 4.80, N 6.06; found: C 40.05, H 4.72, N 5.93.

Preparation of ethyl 4-nitrophenyl *P*-{3-{4-(bromopallado)-1,3-bis[(phenylthio)methyl]phenyl}propyl}phosphonate (2): Solid [Pd_2(dba)$_3$]·$CHCl_3$ (0.17 g, 0.16 mmol) was added to a solution of **8** (0.22 g, 0.32 mmol) in benzene (30 mL) and stirred for 16 h at room temperature. All volatiles were removed in vacuo and the residue dissolved in THF (30 mL). Then Et_3N (0.5 mL) was added and the mixture was stirred for 2 h. The color of the mixture turned from purple-black to dark green indicating the formation of Pd black. The Pd black was then removed by filtration over a short path of Celite leaving the filtrate as a clear yellow solution. The filtrate was concentrated under reduced pressure to obtain the crude product as a yellow solid. Pure product was obtained by purification using column chromatography, eluting with Et_2O to remove dba, and acetone to obtain the product as a pale yellow solid. Yield: 0.16 g (63%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.23 (d, ^3J(H,H) = 9.30 Hz, 2H; ArH), 7.83 and 7.36 (m, 12H; ArH), 6.82 (s, 2H; ArH), 4.60 (br s, 4H; CH_2S), 4.16 (m, 2H; CH_2O), 2.62 (t, ^3J(H,H) = 6.90 Hz, 2H; CH_2Ar,), 1.96 (m, 4H; PCH_2 and PCH_2CH_2), 1.30 ppm (t, ^3J(H,H) = 6.90 Hz, 3H; CH_3); ^{31}P NMR (81 MHz, $CDCl_3$): δ = 30.74 ppm (s); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.84 (d, ^2J(C,P) = 8.53 Hz; ArC), 149.75, 144.79, 137.76, 132.63, 131.90, 130.12, 129.85, 125.91, 122.40 (s; ArC), 121.17 (d, ^3J(C,P) = 4.91 Hz; ArC), 63.27 (d, ^2J(C,P) = 8.53 Hz; CH_2O), 52.99 (s; CH_2S), 36.07 (d, ^2J(C,P) = 17.06 Hz; PCH_2CH_2), 25.80 (d, ^1J(C,P) = 141.65 Hz; PCH_2), 24.11 (d, ^3J(C,P) = 4.83 Hz; CH_2Ar), 16.63 ppm (d, ^3J(C,P) = 6.04 Hz CH_3); elemental analysis cald (%) for $C_{31}H_{31}BrNO_5PPdS_2$ (779.01): C 47.80, H 4.01, N 1.80, S 8.23; found: C 47.98, H 4.07, N 1.70, S 8.29; MS (MALDI-TOF, CSA): *m*/*z*: 700.6 [M^+−Br].

Preparation of ethyl 4-nitrophenyl *P*-{3-{4-(bromopallado)-1,3-bis[(methylthio)methyl]phenyl}propyl}phosphonate (3): A similar procedure as for **2** was applied. Compound **9** (0.40 g, 0.73 mmol), [Pd_2(dba)$_3$]·$CHCl_3$ (0.38 g, 0.36 mmol). The product was purified by column chromatography (silica, eluting with Et_2O (dba) and acetone (product)) to leave the product as a yellow solid after removal of the eluent in vacuo. Yield: 0.3 g (71%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.11 (d, ^3J(H,H) = 9.32 Hz, 2H; ArH), 7.28 (d, ^3J(H,H) = 9.60 Hz, 2H; ArH), 6.73 (s, 2H; ArH), 4.30 (br s, 2H; CH_2S), 4.20–3.99 (br m, 6H; CH_2S, CH_2O), 2.71 (s, 6H; SMe), 2.52 (t, ^3J(H,H) = 6.60 Hz, 2H; CH_2Ar), 1.85 (m, 4H; PCH_2, PCH_2CH_2),

1.21 ppm (t, ^3J(H,H) = 6.90 Hz, 3H; CH_3); ^{31}P NMR (81 MHz, $CDCl_3$): δ = 30.74 ppm (s); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 160.11 (s; ArC), 155.84 (d, ^2J(C,P) = 8.48 Hz; ArC), 148.90, 144.58, 137.78, 125.84, 122.80 (s; ArC), 121.21 (d, ^3J(C,P) = 4.20 Hz; ArC), 63.21 (d, ^2J(C,P) = 6.68 Hz; CH_2O), 49.76 (s; CH_2S), 35.93 (d, ^2J(C,P) = 17.63 Hz; PCH_2CH_2), 25.58 (d, ^1J(C,P) = 140.10 Hz; PCH_2), 24.11 (d, ^3J(C,P) = 4.80 Hz; CH_2Ar), 16.62 ppm (d, ^3J(C,P) = 6.08 Hz; CH_3); MS (MALDI-TOF, CSA): m/z: 575.0 [M^+−Br], 452 [M^+−Br−Pd−Me], 303.7 [M^+−Br−Pd−2Me−$NO_2C_6H_4O$]; elemental analysis cald (%) for $C_{21}H_{27}BrNO_5PPdS_2$ (654,87): C 38.52, H 4.16, N 2.14, S 9.79; found: C 38.65, H 4.22, N 1.98, S 9.65.

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