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Site-Selective Ser-Hydrolase Labelling with a Luminescent Organometallic NCN–Platinum Complex

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The synthesis, spectroscopic properties and protein-binding studies of a novel luminescent organometallic NCN–platinum complex are described. The luminescent organometallic complex was linked to a serine hydrolase reactive phosphonate group by means of click chemistry, and was exploited in serine hydrolase specific-binding studies using gel electrophoresis. The NCN–platinum protein label was found to be a robust dye with absorbance and emission maxima between 374–380 and 482–493 nm, respectively, and quantum yields between 0.04 and 0.15.

Introduction

Luminescent probes are playing an important role in the study and understanding of biomolecular processes, such as protein functioning within cells,[1] membrane dynamics[2] and tumour cell surface targeting.[3] Various luminescence techniques [e.g., Förster resonance energy transfer (FRET)[4,5] and single molecule spectroscopy][5,6] have become increasingly important tools in the study of these phenomena. The design and development of novel luminescent probes is crucial for the advancement of this field.

Excellent examples that illustrate the power of luminescence in biology are the use of the green,[7] yellow or cyan fluorescent proteins (GFP, YFP, CFP) in the labelling of reporter molecules in cells to study intracellular dynamics.[8–10] Also various commercially available synthetic fluorescent small-molecule organic dyes,[11,12] with different emission wavelengths and various protein reactive groups (e.g., maleimide functional groups for cysteine labelling) are increasingly used in proteomics to screen for different types of proteins and monitor their activity profiles.[13,14] These dyes are generally smaller in size and possess a lower molecular weight than the protein-based fluorescent labels, which enables chemists to more easily alter their spectroscopic and protein-binding properties through chemical synthesis.[13,14]

In the emerging field of activity-based protein profiling, novel small molecular probes are highly desirable (e.g., for the targeting of cancer-inducing mutated proteins).[15] When fluorescent dyes with different emission wavelengths are combined with various protein-reactive functional groups, a wide spectrum of the proteome can potentially be addressed and studied with these fluorescent probes. For instance, Cravatt and co-workers have probed several different enzyme classes like aspartyl proteases, deubiquitinating enzymes, glycosidases and serine hydrolases through the design of a variety of enzyme-targeted probes based on their active-site reactivity and substrate selectivity.[16–18]

Currently, our group is working on the development of protein reactive probes as well, with the aim of modifying serine hydrolases and lipases for protein diagnosis purposes and/or for the construction of novel semisynthetic enzymes.[19–24] By doing so, various phosphonate inhibitors substituted by different organic[22,23] and organometallic moieties[19–21,24] have been used to target different lipases (Figure 1). By substituting organometallic ECE-pincer–metal complexes (in which E is an electron-donating substituent such as nitrogen or sulfur) with a protein-reactive phosphonate group, the special properties of the metal centre can be introduced into a protein. By doing so, novel, unnatural features can be added to the protein, which can be exploited in coordination studies involving the metal centre or in the phasing of protein diffraction data.[21]

Phosphonates have long been known to be substrate analogues for serine hydrolases, and even phosphonates with various organic fluorescent reporter tags have been studied by different groups.[17,22,29–31] So far, phosphonates substituted by organic dyes [e.g., fluorescent dansyl (Figure 1),[22] rhodamine[15] and *p*-nitrobenzofurazan[32] groups] have

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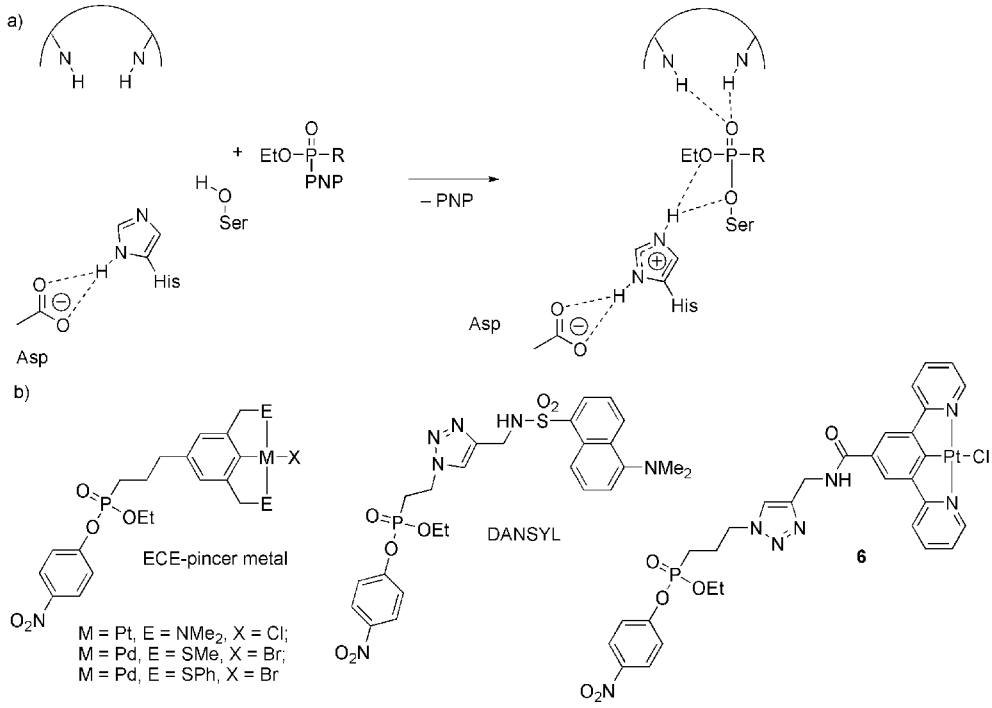


Figure 1. (a) General inhibition reaction of serine hydrolases with phosphonates (PNP = *p*-nitrophenolate anion). (b) Structures of organometallic ECE-pincer–metal phosphonates (left),[20,21] of an organic luminescent dansyl phosphonate inhibitor (middle)[22] and of the organometallic luminescent target inhibitor **6** (right).

been used in fluorescent labelling studies. Due to the exciting spectroscopic properties of certain NCN–metal complexes,[19,20,25–28] we became interested in applying these in the labelling of proteins.

With the organometallic ECE-pincer–metal phosphonate inhibitors[19–21] developed by our group, we have been exploiting the special properties of the heavy-metal atom probe as, for example, coordination target for phosphanes and as phasing tool for the elucidation of protein crystal structures.[21,33]

Interestingly, many different coordination complexes with exciting luminescent properties are known from the literature,[34–36] but only very few of them have been used in the labelling of biomolecules,[37–54] which is mainly caused by the instability (e.g., water or redox sensitivity) of these complexes under biological conditions. Inspired by the high quantum yields of various organoplatinum complexes, as demonstrated by the Williams[25–27,55] and other groups[56] and by our own results on the design and applications of novel organometallic phosphonate inhibitors, we embarked on the modification of one of these highly luminescent NCN–platinum complexes[57] (complex **1**, Scheme 1) to develop a novel luminescent organometallic NCN–platinum phosphonate inhibitor (**6**, Figure 1, Scheme 1). Organometallic pincer complexes possess a covalent metal–carbon bond, which is completed by two *ortho*-chelated metal–heteroatom interactions.[19–21,27,28,57–59] This combination of metal–ligand interactions leads to a high stability in aqueous and biological media, which in turn led us to expect that these complexes would be stable luminescence labels for protein profiling.

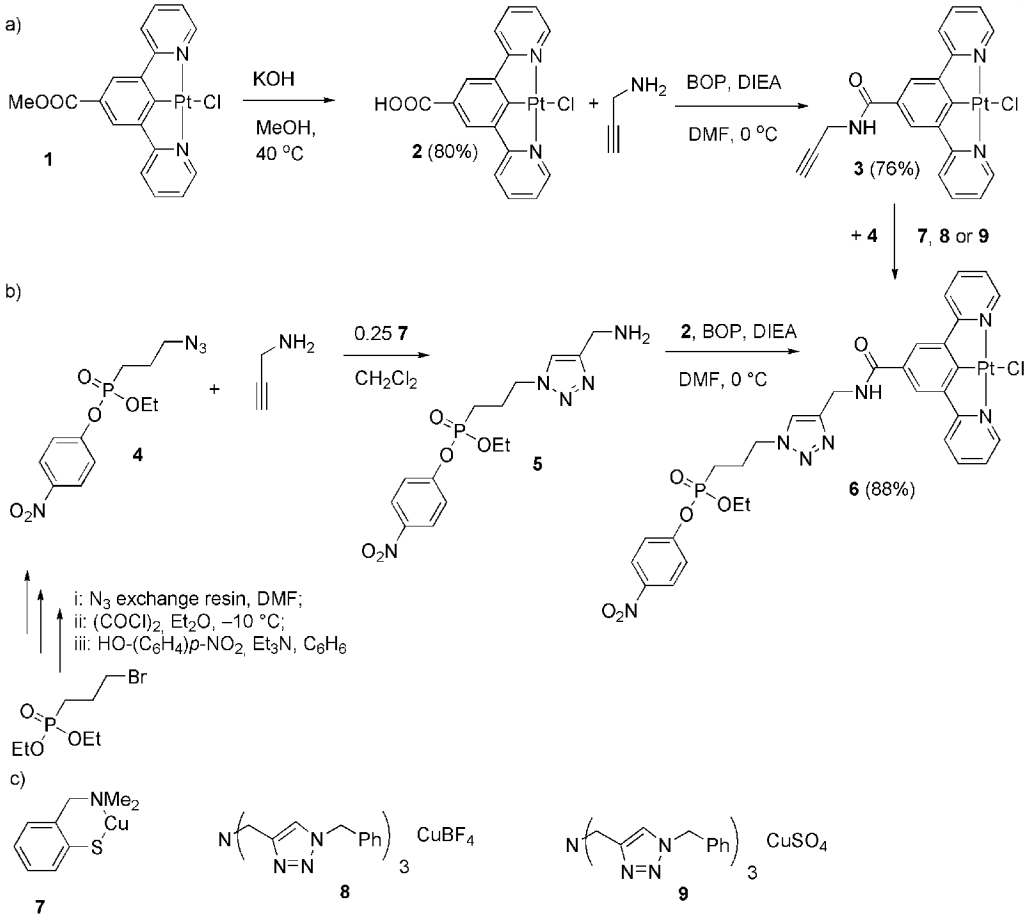
Contrary to common organic fluorescent probes, which generally emit from a singlet excited state, these complexes emit from a low-lying triplet excited state due to the influence of the heavy metal.[27,36,60] In general, the absorbance and the emission maxima display a large Stokes shift, which is different from common organic fluorescent dyes.[27,37,42] These special features of organometallic luminescent complexes can potentially be used in novel spectroscopic techniques [e.g., luminescence resonance energy transfer (LRET) and anisotropy studies on dynamic protein interactions[37,52] or in time-resolved spectroscopy].[27] Additionally, a rich and diverse chemistry is known for similar NCN transition-metal complexes, including chemical sensing, supramolecular coordination chemistry and catalysis.[58]

Here, we report on the synthesis of the novel luminescent NCN-pincer–platinum probe **6** and its photophysical properties in both organic and biological media. Furthermore, we illustrate how this probe can be used for the efficient, diagnostic labelling of several proteins.

Results and Discussion

Synthesis of the Luminescent Pincer Platinum Phosphonate Complex

The synthesis of the luminescent NCN–platinum inhibitor **6** was pursued by means of two different routes (a) and (b), both by means of a Cu^{I}-catalyzed click reaction as a crucial step to introduce the reactive PNP phosphonate group (PNP = *p*-nitrophenolate anion) using a propyl azido



Scheme 1. Explored routes (a) and (b) towards the synthesis of the luminescent pincer platinum phosphonate inhibitor **6**; the different tested click catalysts are shown under (c).

phosphonate reagent (**4**) similar to the ethyl azido phosphonate reagent[22] developed earlier by us (Scheme 1). The Cu^{I}-catalyzed click reaction[61,62] between an azide and an alkyne has been widely studied and is known to be very selective, tolerant to functional groups and can be performed under very mild conditions, even with unactivated alkynes.[63,64] Due to the anticipated sensitive nature of the PNP-phosphonate group (PNP is a very good leaving group), it was initially decided to perform the click reaction as the last step in the synthesis of **6** (Scheme 1, a).

Route (a) started with the hydrolysis of ester **1**[25] to the corresponding acid **2**, which was isolated as a bright yellow powder. Subsequently, an amide coupling was performed with propargylamine to obtain complex **3**.

Contrary to the general ease with which click reactions are performed,[61,62] initial attempts to realize the subsequent Cu^{I}-catalyzed click reaction under standard reaction conditions (acetylene/azide = 1:1, 10–20 mol-% **9** in MeCN or DMF/THF = 1:1) did not work. Therefore, it was decided to screen for optimum reaction conditions by varying the solvent, Cu^{I} catalyst (**7**,[65] **8** and **9**[63]), catalyst loading (10–23 mol-%), concentration, reaction temperature (room temp. to 40 °C) and time (12 h to 5 d). Based on ^{31}P and ^{1}H NMR spectroscopic analysis, none of these screens gave rise to any formation of the desired product. This was rather surprising, since other NCN–platinum-type complexes that contained acetylene moieties were successfully applied under standard click reaction conditions in our laboratory before.[66] Therefore it was decided to attempt an alternative synthesis route to **6**.

In the alternative route (b), the click reaction between phosphonate **4** and propargylamine was performed in the first step, with aminoarenethiolate catalyst **7** giving the highest activity when compared to the commonly used click catalysts **8** or **9**.[61,63] The subsequent amide coupling reaction of **5** with **2** in the presence of benzotriazole-1-yloxytris-(dimethylamino)phosphonium hexafluorophosphate (BOP) gave rise to the formation of target molecule **6**, which was isolated in 77% overall yield starting from **4**. Complex **6** was characterized by ^{1}H, ^{31}P, ^{13}C NMR and IR spectroscopy, high-resolution mass spectrometry and by photophysical methods (vide infra). Notably, both the organometallic complex **2** and phosphonate **5** did not show any signs of decomposition under the applied reaction conditions. The resulting inhibitor **6** possesses only limited solubility in common organic solvents; it was only found to be sufficiently soluble in DMSO, DMF and in a DMF/buffer mixture for the inhibition studies, and therefore these solvents were used in the subsequent enzyme inhibition and spectroscopic studies.

Spectroscopic Characterization of the Various NCN Pincer Platinum Complexes

The various NCN–platinum complexes (**1**–**3**, Scheme 1) and inhibitor **6** were characterized by UV/Vis absorption (Figure 2 and Figures S1–S3 in the Supporting Information) and emission spectroscopy (Figure 3) and the data are collected in Table 1. Due to both the limited solubility of each of these complexes in common organic solvents and their complete insolubility in water or buffer solutions, solvents commonly used for spectroscopic analyses (e.g., CH_2Cl_2, hexane or THF) could not be used. As a result, the solvent used by the Williams group (dichloromethane)[57] was not suitable for the spectroscopic studies of **1**–**3** and **6**. DMF appeared to be the only solvent in which all complexes dissolved readily. Since DMF is a strongly coordinating solvent, it is not the ideal solvent for performing the spectroscopic studies; the use of coordinating solvents renders the studied compounds less emissive (i.e., lower quantum yields are observed). However, it is the only solvent that allows for the comparison of all complexes **1**–**3**, **6**. Note that in cases in which a complex is soluble in another solvent besides DMF, the spectroscopic data in this solvent are reported as well (Table 1).

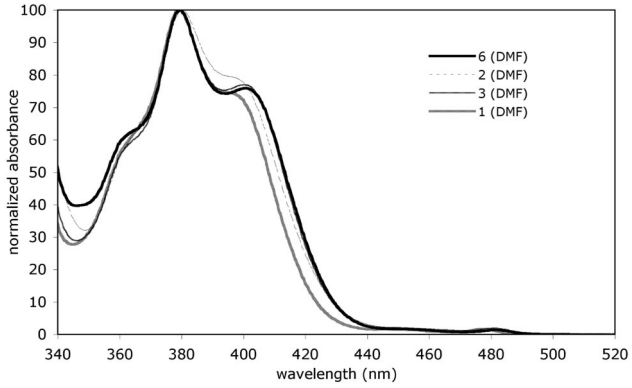


Figure 2. Normalized absorption spectra of NCN–platinum complexes **1**–**3** and inhibitor **6** in DMF.

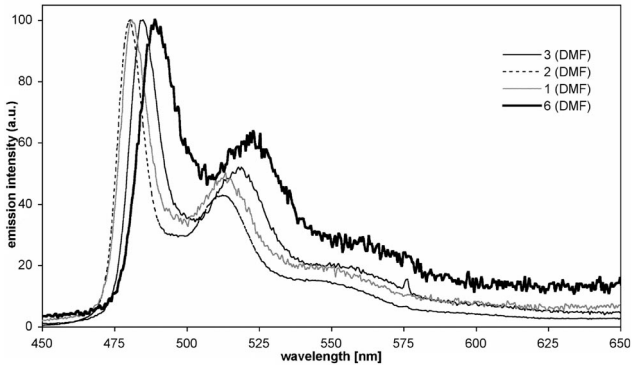


Figure 3. Normalized emission spectra of NCN–platinum complexes **1**–**3** and inhibitor **6** in DMF.

Table 1. Photophysical data of the pincer platinum complexes **1**–**3** and inhibitor **6**.

	Solvent[a]	Absorbance λ_{max} [nm][b,c]	ε [$M^{-1}cm^{-1}$]	Luminescence λ_{max} [nm][b]	Quantum yield[b,d]
1	DMF	380 (399)	14940	481[e]	0.08
	MeCN	376 (401)	10980	480[e]	0.31
	CH_2Cl_2	380 (400)	9840	482[f]	0.38 (0.58)[25][g]
2	DMF	380 (401)	9270	481[f]	0.21
	MeCN	377 (402)	3120	480[e]	0.08
3[h]	DMF	380 (400)	8770	485[f]	0.17
6	DMF	380 (400)	12320	493[e]	0.05
	MeCN	377 (396)	3650	487[e]	0.04
	CH_2Cl_2	380 (402)	8220	490[e]	0.15
	DMF[i]	374 (398)	9470	482[e]	0.04

[a] Sample dissolved in the indicated solvent; the solubility of all complexes in H_2O, 125 mM Tris (pH 8.0), Tris (50 mM), 0.1% (m/m) Triton (pH 8.0) or 150 mM NH_4OAc buffer was too low to allow for appropriate measurements. [b] Measured in deaerated solutions. [c] The shoulders around 400 nm are listed in brackets. [d] Uncertainty ±20%. [e] Value was obtained from measurements using $[Ru(bpy)_3]Cl_2$ in H_2O (ϕ = 0.028) as internal standard. [f] Value was obtained from measurements using $[Ru(bpy)_3](PF_6)_2$ in MeCN (ϕ = 0.062) as internal standard. [g] The quantum yield based on spectroscopic analysis with our equipment was lower than the one reported previously.[25] The observed difference, which is within the uncertainty region, could be due to the different degassing methods or internal standards used. [h] The solubility of complex **3** in MeCN and CH_2Cl_2 was too low to allow for measurements. [i] A 150 mM NH_4OAc buffer/DMF mix (1:1 v/v) was used.

The UV/Vis absorption spectra of **1**–**3** and **6** in DMF display very similar features with a strong absorbance region between 350 and 440 nm (Figure 2). All complexes have an absorbance maximum between 374 and 380 nm, which seems to be almost independent of the nature of the solvent used (Table 1, vide infra). The strong features in the absorbance region from 350 to 440 nm are assigned to $^1\pi$–π^* transitions of the ligands and to charge-transfer transitions.[57]

All complexes are luminescent in solution and the emission spectra display very similar features with maxima between 480 and 493 nm (Figure 3). The quantum yields in different solvents vary from 0.04 to 0.38, with the highest quantum yield for the parent ester complex **1** in dichloromethane and lower quantum yields for inhibitor **6** (Table 1).

The emission maxima are slightly redshifted with a small Stokes shift increasing in the order **1** = **2** < **3** < **6**. The emission energy is also redshifted when changing the *para* substituent of the NCN–platinum complex from –C(O)-OMe (complex **1**) to –C(O)N(H)–R (**3** and inhibitor **6**), which is due to the electronic nature of the different substituents.[25,27,67,68] The quantum yield for **6** in DMF is slightly decreased in comparison to **1**–**3**.

Lipase Labelling Studies with Inhibitor 6

The pincer platinum phosphonate inhibitor **6** was applied as a site-selective inhibitor in labelling studies of different serine hydrolases. Due to the poor solubility of **6**, previous protocols to study artificial enzymes[19–23] were adapted for

protein-labelling studies with luminescent probe **6**. As a model protein for the labelling studies, cutinase from *Fusarium solani pisi* (21 kDa) was chosen. Cutinase is a lipase with high esterase activity that is also known to be very reactive towards phosphonate inhibitors.[19,20,22] Therefore, a solution of phosphonate **6** (6 equiv., 10 mM) in DMF was added to a solution of cutinase in buffer (200 μM, pH 8.0). After incubation overnight, the excess amount of inhibitor was removed by dialysis with buffer (see the Experimental Section for details) and an activity test of the resulting solution was performed. This test showed no residual ester-hydrolysis activity for cutinase, thereby indicating complete inhibition of cutinase by **6** as a result of the formation of **cut-6**.[19–21] Illumination of the inhibited cutinase batches at 257 nm showed that the enzyme solutions were luminescent after inhibition and dialysis (Figure 4, negative photograph), thereby indicating successful protein labelling (**cut-6**) and a high stability of the organometallic label **6** under the described conditions. After exposure of the **cut-6** solution to UV light over 5 min, the solution was still luminescent. Storage of the **cut-6** protein solution in the refrigerator over 6 weeks also did not affect its emission properties.

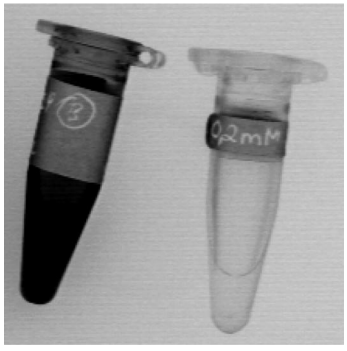


Figure 4. Negative photograph of cutinase solutions (200 μM) under UV light (excitation wavelength 257 nm). Left: after incubation with inhibitor **6** and subsequent dialysis (**cut-6**). Right: cutinase solution without inhibitor.

Encouraged by these results, it was decided to investigate the labelling properties of **6** towards other proteins[22,23] and to assay the formation and stability of the covalent **protein-6** adducts by performing gel electrophoresis. For this study, the serine hydrolases *Bacillus subtilis* lipase A (BSLA)[69] (19 kDa), commercially available *Candida antarctica* lipase B (CALB) (33 kDa) and cutinase as reference protein were chosen.

The solutions of the respective enzymes in buffer (pH 8.0) were incubated with a solution of luminescent inhibitor **6** (in DMF) overnight. Also, a control experiment without **6** was performed simultaneously. These solutions were used (i.e., without any further treatment) in gel electrophoresis using a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel. The luminescence and Coomassie stained negative photograph of the gel are shown in Figure 5.

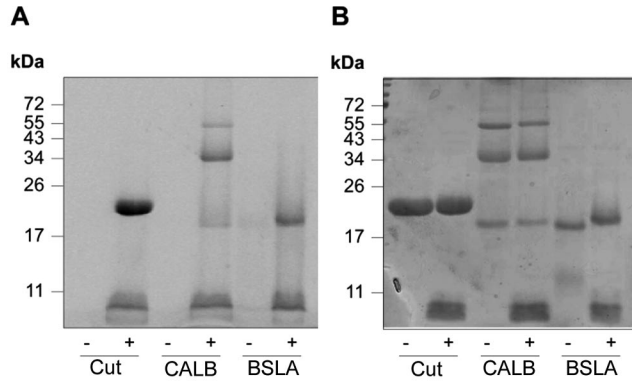


Figure 5. (A) Luminescence (negative photograph) and (B) Coomassie stained gel of cutinase (Cut), *Candida antarctica* lipase B (CALB) and *Bacillus subtilis* lipase A (BSLA) without (–) and after (+) exposure to **6** overnight. As the samples were not dialyzed prior to gel electrophoresis, the excess amount of unreacted **6** at the lower end of the gels (below the 11 kDa line) is visible.

The luminescent gel photograph shows that luminescent labelling of cutinase, CALB and BSLA did indeed occur (Figure 5, A). The Coomassie stained photograph of the same gel shows that protein was also present in the samples without **6** (Figure 5, B). Since SDS-PAGE is run under denatured protein conditions, these experiments clearly prove the covalent labelling of the different proteins by **6**. The different **protein-6** molecules were still luminescent after gel-electrophoresis conditions, thereby showing again the stability of inhibitor **6**.

Spectroscopy with cut-6 at Different pH Values and under Denatured Conditions

Different, commonly used organic dyes like, for example, fluorescein, are not very photostable and their luminescence emission properties often depend on the polarity and pH value of their environment.[13,14] Furthermore, the active site His^{188} residue in cutinase [Figure 1, pK_a(His) ≈ 6.5] could be (de)protonated, thereby potentially exerting an influence on the spectroscopic properties of the luminescent metal dye in **cut-6**. To investigate the chemical robustness and the influence of the environment polarity and of the pH value on the spectroscopic properties of protein label **6**, it was decided to analyze a **cut-6** solution at different pH values in Tris/Triton buffer and under denatured conditions. The emission spectrum of **cut-6** at pH 5, 7 and 9 in Tris/Triton buffer is shown in Figure 6. For all measurements the quantum yield remained constant within the experimental error (0.20–0.12, uncertainty ±20%).

The spectroscopic properties of **cut-6** at pH 5, 7 and 9 are very similar with nearly identical absorbance (376–378 nm) and emission (470–471 nm) maxima (Figure 6, Table 2). The absorbance and emission maxima of **cut-6** in Tris/Triton buffer are blueshifted in comparison to inhibitor **6** in DMF or in a DMF/buffer mixture (Table 1, $\Delta_{\lambda Abs}$ = 2–4 nm, $\Delta_{\lambda Em}$ = 11–12 nm), thereby indicating the difference of the hydrophobic and sterically constraint environ-

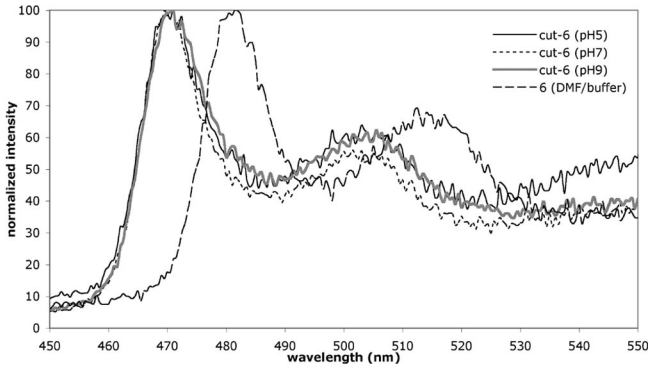


Figure 6. Normalized luminescence spectra of **cut-6** at pH 5, 7 and 9 in Tris/Triton buffer; for comparison, the spectrum of inhibitor **6** in DMF/buffer (1:1 v/v, buffer: Tris/Triton, pH 8.0) has been included.

ment of the protein-active site[21,70–72] compared to the highly polar solvents. The higher quantum yield of **cut-6** at pH 5, 7 and 9 than for **6** in DMF or DMF/buffer (Table 1) was also attributed to the hydrophobic environment of the cutinase active site[21,70–72] into which the metal moiety is embedded in **cut-6**. The emission spectra showed an additional broad maximum around 630 nm for **cut-6** at pH 5, 7 and 9, but not for **6** in DMF/buffer or **1** in DMF (Figure S5 in the Supporting Information), which was attributed to excimer formation of **cut-6** in aqueous solution. This excimer formation can be facilitated by the hydrophobic packing of the cutinase active sites in aqueous media, as it is a common feature observed for lipases.[21,70–72] The aggregate formation of **cut-6** could also be the reason for the observed broadness of the spectra in Figure 6.

Table 2. The absorbance λ_{max}, luminescence λ_{max} and quantum yield values for **cut-6** at pH 5, 7 and 9 in Tris/Triton buffer.

	pH[a]	Absorbance λ_{max} [nm]	Luminescence λ_{max} [nm]	Quantum yield[b]
cut-6	5	376	471	0.20
cut-6	7	378	470	0.13
cut-6	9	377	471	0.12
6	8 (DMF/buffer)	374	482	0.04

[a] Measurements were performed in 0.1% (m/m) Triton, 50 mM Tris buffer at the indicated pH value. [b] Uncertainty ±20%.

The study of **cut-6** at different pH values showed that the dye is remarkably stable in the pH range 5–9, thus making it widely applicable.[21,70–72] The protonated (pH < 6.5) or deprotonated (pH > 6.5) state of the ^{188}His residue in cutinase did not significantly affect the spectroscopic properties of the dye. This spectroscopic behaviour differs from commonly used luminescent organic dyes like fluorescein and rhodamine,[11,42] being pH dependent, and from some other biological transition metal labels like redox-active probes (e.g., ferrocene derivatives),[37] which have been reported to be very sensitive to changes in pH.

Conclusion

The design, syntheses and spectroscopic analyses of luminescent inhibitor **6** and its use in protein labelling studies are demonstrated. It turns out that inhibitor **6** is a very stable, luminescent organometallic complex, which can be selectively used for the labelling of various lipases. Complexes **1**–**3** and **6** display the expected photophysical properties, with the emission energy and quantum yield decreasing after introduction of the amide functionality (for **3** and **6**), but maintaining a clearly luminescent complex. The luminescent properties of **6** can potentially be used in novel spectroscopic applications [e.g., luminescence resonance energy transfer (LRET)][52] with an organic dye and subsequent phosphorescence emission by this dye, thereby expanding the spectroscopic toolbox for studying biomolecules.

Previous studies by our group[22] have shown that phosphonate inhibitors are selective toward serine hydrolases and do not react with other proteins that lack the reactive active-site residue. Furthermore, we illustrated that such inhibitors can even be used to selectively label proteins in living cells and as such have been used for protein isolation and identification. This opens up the possibility to use **6** as a luminescent lipase-specific label in cell lysates and for future proteomic applications. Currently, we are investigating how the properties of organometallic pincer complexes can be used in this area (e.g., by exploiting the unique coordination properties of the platinum metal centre).[33]

In conclusion, this study shows that organometallic pincer–platinum complexes[73] can be used for diagnostic targeting of proteins, which in combination with a serine hydrolase reactive phosphonate inhibitor opens up new activity-based protein profiling possibilities. The proof-of-principle methodology demonstrated here is by no means limited to serine hydrolases; the chemical versatility of pincer complexes also allows attachment to other biochemical probes like, for example, carbohydrates or oligopeptides.[27,28,58,74,75]

Experimental Section

General Comments: All reagents were used as supplied from Acros or Sigma–Aldrich, unless stated otherwise. MeCN and CH_2Cl_2 were distilled from CaH_2. DIEA = *N*,*N*-diisopropylethylamine (Hünig's base). All synthetic procedures were performed under an inert atmosphere using Schlenk techniques and distilled solvents, unless stated otherwise. The enzyme inhibition studies and dialysis experiments were performed in air. Water for the preparation of the buffer solutions was filtered with the Milli-Q filtration system (Millipore, Quantum Ultrapure) prior to use. The dialysis cassettes (Slide-A-Lyzer™, 10,000 MWCO, 0.1–0.5 mL or 0.5–3 mL) for the purification of enzyme NCN–platinum hybrids were purchased from Pierce. Cutinase mutant N172K was provided by Unilever. BSLA was provided by Prof. Wim J. Quax and co-workers.[69] CALB (batch PPW 4534) was purchased from Novo Nordisk A/S, Copenhagen, Denmark. Electrospray ionization mass spectra and high-resolution electrospray ionization mass spectra were recorded with a Micromass LCT spectrometer. All samples were introduced with a nanoflow electrospray source (Protana, Odense, Denmark) and all spectra were calibrated with a CsI solution (50 mg mL^{-1} in MQ/H_2O). Matrix-assisted laser desorption ionization mass spectrometry experiments were performed with a Voyager-DE Pro (Ap-

plied Biosystems) instrument in the reflector mode with trihydroxy anthracene in MeCN as matrix. Photographs of SDS-PAGE gels were taken with a BioRad GelDoc 2000 workstation (exciation wavelength 257 nm). Elemental microanalyses were performed by Dornis and Kolbe, Mikroanalytisches Laboratorium (Mülheim a.d. Ruhr, Germany). NMR spectroscopic measurements were performed with a Varian Inova 300 MHz or Varian Oxford 400 MHz spectrometer at 298 K. The syntheses of **1**[25] and **7**[65] were carried out as reported.

Electronic Spectroscopic Measurements: UV/Vis spectra were recorded with a Varian Cary 50 Scan UV/Vis spectrometer. Luminescence spectra were obtained on a SPEX fluorolog spectrometer with $[Ru(bpy)_3](PF_6)_2$ in degassed MeCN as standard ($\phi_r = 0.062$). Emission quantum yields were measured by the method of Crosby and Demas[76] in degassed solvents and calculated by $\phi_S = \phi_r(B_r/B_s)(n_s/n_r)^2(D_s/D_r)$, in which n is the refractive index of the solvents and D is the integrated intensity. The quantity B is calculated by $B = 1–10^{-AL}$, in which A is the absorbance and L is the optical path length.

Diethyl (3-Azidopropyl)phosphonate: Azide exchange resin (44.88 g, surface loading 3.8 mmol g^{-1}, 0.17 mol, 10 equiv.) was added to a solution of diethyl (3-bromopropyl)phosphonate (4.42 g, 0.017 mol, 1 equiv.) in DMF (250 mL), after which the suspension was stirred at 40 °C for 3 d. The beads were filtered off and washed with Et_2O (2 × 200 mL), and the solution was filtered through Celite (2×), after which all volatiles were removed in vacuo. The crude product was distilled with the second fraction containing the product (5.7 mbar, 105 °C, 2.79 g, 0.013 mol, 74%). The ^{1}H NMR spectroscopic signals were assigned with the aid of COSY spectra. ^{1}H NMR ($CDCl_3$, 298 K, 400 MHz): δ = 1.30 (t, $^3J_{H,H}$ = 4.4 Hz, 6 H, 2 × P-O-CH$_2$CH_3), 1.73–1.89 (m, 4 H, 2 × CH_2), 3.35 (t, $^3J_{H,H}$ = 6.6 Hz, 2 H, CH_2), 4.02–4.12 (m, 4 H, 2 × CH_2CH$_3$) ppm. ^{31}P NMR ($CDCl_3$, 298 K, 162 MHz): δ = 31.84 ppm. ^{13}C NMR ($CDCl_3$, 298 K, 101 MHz): δ = 16.60 (d, $^3J_{C,P}$ = 5.8 Hz, 2 × P-O-CH$_2$CH_3), 22.58 (d, $^3J_{C,P}$ = 4.5 Hz, PCH$_2$CH$_2$CH_2N$_3$) 23.02 (d, $^1J_{C,P}$ = 143.0 Hz, PCH$_2$), 51.61 (d, $^2J_{C,P}$ = 16.6 Hz, PCH$_2$CH$_2$), 61.80 (d, $^2J_{C,P}$ = 6.2 Hz, 2 × P-O-CH$_2$CH$_3$) ppm. MS (ES+, MeCN) for $C_7H_{16}N_3O_3P$ (221.0929) [M]$^+$: *m*/*z* calcd. for [M + H]$^+$ 222.1002; found 222.1007; calcd. for [M + Na]$^+$ 244.0821; found 244.0827. $C_7H_{16}N_3O_3P$: calcd. C 38.01, H 7.29, N 19.00, P 14.00; found C 37.91, H 7.21, N 18.85, P 13.91. IR (liquid): $\tilde{\nu}$ = 2094.7, 1445.2, 1392.3, 1368.5, 1349.2, 1237.7, 1019.0, 952.3, 781.5, 704.0 cm^{-1}.

Ethyl (3-Azidopropyl)phosphonochloridate: Freshly distilled oxalyl chloride (9.9 mL, 14.90 g, 0.12 mol, 10 equiv.) was added to a solution of diethyl (3-azidopropyl)phosphonate (2.60 g, 0.012 mol, 1 equiv.) in Et_2O (100 mL), which had been cooled to –10 °C, after which the solution was stirred at room temp. The progress of the reaction was monitored by ^{31}P NMR spectroscopy. After 3 d, fresh oxalyl chloride (9.9 mL) was added. After 11 additional days all volatiles were evaporated in vacuo, thus yielding a slightly yellowish oil (2.25 g, 0.011 mol, 91%). The ^{1}H NMR spectroscopic signals were assigned with the aid of COSY spectra. ^{1}H NMR ($CDCl_3$, 298 K, 400 MHz): δ = 1.38 (t, $^3J_{H,H}$ = 4.6 Hz, 3 H, P-O-CH$_2$CH_3), 1.90–2.02 (m, 2 H, PCH$_2$CH_2CH$_2$N$_3$), 2.17–2.25 (m, 2 H, PCH_2CH$_2$CH$_2$N$_3$ or PCH$_2$CH$_2$CH_2N$_3$), 3.42 (t, $^3J_{H,H}$ = 6.4 Hz, 2 H, PCH_2CH$_2$CH$_2$N$_3$ or PCH$_2$CH$_2$CH_2N$_3$), 4.15–4.35 (m, 2 H, CH_2CH$_3$) ppm. ^{31}P NMR ($CDCl_3$, 298 K, 162 MHz): δ = 43.82 ppm. ^{13}C NMR ($CDCl_3$, 298 K, 101 MHz): δ = 16.12 (d, $^3J_{C,P}$ = 7.0 Hz, P-O-CH$_2$CH_3), 22.59 (d, $^3J_{C,P}$ = 5.0 Hz, PCH$_2$CH$_2$CH_2N$_3$), 31.00 (d, $^1J_{C,P}$ = 126.4 Hz, PCH$_2$), 51.08 (d, $^2J_{C,P}$ = 19.1 Hz, PCH$_2$CH$_2$), 63.62 (d, $^2J_{C,P}$ = 8.3 Hz, P-O-CH$_2$CH$_3$) ppm. IR (liquid): $\tilde{\nu}$ = 2985.6, 2941.4, 2096.2, 1445.1, 1394.9, 1368.9, 1349.3, 1256.2, 1018.4, 967.2, 848.5, 749.2, 711.2 cm^{-1}. Due to the hydrolysis sensitivity of (3-azidopropyl)-phosphonochloridate, the crude product was used directly in the next synthesis step.

Ethyl 4-Nitrophenyl (3-Azidopropyl)phosphonate (4): A solution of *p*-nitrophenol (0.7152 g, 5.1416 mmol, 1 equiv.) and distilled triethylamine (2.6014 g, 3.57 mL, 0.0257 mol, 5 equiv.) in dry benzene (50 mL) was added dropwise to a solution of (3-azidopropyl)phosphonochloridate (1.0879 g, 5.1416 mmol, 1 equiv.) in dry benzene (20 mL), after which the mixture was stirred at room temp. for 2 h. All volatiles were removed in vacuo, after which the residue was dissolved in Et_2O (150 mL) and washed with 1 M K_2CO_3 (2 × 100 mL), brine (2 × 100 mL) and H_2O (3 × 100 mL). The organic layer was dried with Na_2SO_4 and after filtration and removal of all volatiles in vacuo a slightly orange oil was obtained (1.5998 g, 5.0911 mmol, 99%). The ^{1}H NMR spectroscopic signals were assigned with the aid of COSY spectra. ^{1}H NMR ($CDCl_3$, 298 K, 400 MHz): δ = 1.31 (t, $^3J_{H,H}$ = 7.0 Hz, 3 H, P-O-CH$_2$CH_3), 1.90–2.10 (m, 4 H, PCH$_2$CH_2CH$_2$N$_3$, PCH_2CH$_2$CH$_2$N$_3$ or PCH$_2$CH$_2$CH_2N$_3$), 3.42 (t, $^3J_{H,H}$ = 6.4 Hz, 2 H, PCH_2CH$_2$CH$_2$N$_3$ or PCH$_2$CH$_2$CH_2N$_3$), 4.10–4.31 (m, 2 H, P-O-CH_2CH$_3$), 7.38 (d, $^3J_{H,H}$ = 9.6 Hz, 2 H), 8.22 (d, $^3J_{H,H}$ = 9.6 Hz, 2 H) ppm. ^{31}P NMR ($CDCl_3$, 298 K, 162 MHz): δ = 29.78 ppm. ^{13}C NMR ($CDCl_3$, 298 K, 101 MHz): δ = 16.53 (d, $^3J_{C,P}$ = 5.8 Hz, P-O-CH$_2$CH_3), 22.42 (d, $^3J_{C,P}$ = 5.0 Hz, PCH$_2$CH$_2$CH_2N$_3$), 23.40 (d, $^1J_{C,P}$ = 143.4 Hz, PCH$_2$), 51.38 (d, $^2J_{C,P}$ = 17.4 Hz, PCH$_2$CH$_2$), 63.42 (d, $^2J_{C,P}$ = 7.1 Hz, P-O-CH$_2$CH$_3$), 121.2 (d, $^3J_{C,P}$ = 4.6 Hz, Ar*C*2, Ar*C*6), 125.9 (Ar*C*3, Ar*C*5), 144.8 (Ar*C*4), 155.7 (d, $^2J_{C,P}$ = 8.2 Hz, P-O-Ar*C*1) ppm. MS (ES+, MeCN) for $C_{11}H_{15}N_4O_5P$ (314.0780) [M]$^+$: *m*/*z* calcd. for [M + H]$^+$ 315.0853; found 315.0858; calcd. for [M + Na]$^+$ 337.0672; found 337.0678. $C_{11}H_{15}N_4O_5P$: calcd. C 42.04, H 4.81, N 17.83, P 9.86; found C 41.96, H 4.76, N 17.78, P 9.94. IR (liquid): $\tilde{\nu}$ = 3479.4, 3114.5, 3081.4, 2985.0, 2939.3, 2873.2, 2455.0, 2101.9, 1613.8, 1591.7, 1520.1, 1488.9, 1446.0, 1347.2, 1226.2, 1162.6, 1111.2, 1031.4, 914.8, 861.6, 752.8, 689.1, 636.9, 608.3 cm^{-1}.

Ethyl 4-Nitrophenyl {3-[4-(Aminomethyl)-1*H*-1,2,3-triazol-1-yl]-propyl}phosphonate (5): Propargylamine (127.7 μL, 0.0020 mol, 0.1098 g, 1.4 equiv.), the copper amino arenethiolate catalyst (0.0082 g, 0.0356 mmol, 0.025 equiv.) and more dichloromethane (1 mL) were added to a solution of phosphonate **4** (0.4475 g, 0.0014 mol, 1.0 equiv.) in degassed dichloromethane (1 mL). The solution was stirred overnight, after which all volatiles were evaporated in vacuo and the residue was dissolved in CH_2Cl_2 (50 mL). The organic layer was washed with 1 M K_2CO_3 (2 × 50 mL), brine (2 × 50 mL) and water (2 × 50 mL). After drying with Na_2SO_4 and filtration through Celite, all volatiles were evaporated in vacuo. The oil was dissolved in a minimum of dichloromethane and precipitated with hexane. After drying in vacuo, a yellow oil was obtained (0.4598 g, 1.2450 mmol, 87%). The ^{1}H NMR spectroscopic signals were assigned with the aid of COSY spectra. ^{1}H NMR ($CDCl_3$, 298 K, 400 MHz): δ = 1.28 (t, $^3J_{H,H}$ = 7.0 Hz, 3 H, P-O-CH$_2$CH_3), 1.90–1.98 (m, 2 H, PCH_2CH$_2$CH$_2$N$_3$), 2.24–2.35 (m, 2 H, PCH$_2$CH_2CH$_2$N$_3$), 3.35 (d, $^2J_{H,H}$ = 511.1 Hz, 2 H, NH_2), 4.02–4.27 (m, 2 H, P-O-CH_2CH$_3$), 4.46 (t, $^3J_{H,H}$ = 6.8 Hz, 3 H, PCH$_2$CH$_2$CH_2N$_3$), 7.34 (d, $^3J_{H,H}$ = 9.2 Hz, 2 H, Ar*H*), 7.54 (s, 1 H, triazole*H*), 8.20 (d, $^3J_{H,H}$ = 9.2 Hz, 2 H, Ar*H*) ppm. ^{31}P NMR ($CDCl_3$, 298 K, 162 MHz): δ = 29.00 ppm. ^{13}C NMR ($CDCl_3$, 298 K, 101 MHz): δ = 16.56 (d, $^3J_{C,P}$ = 6.2 Hz, P-O-CH$_2$CH_3), 23.26 (d, $^1J_{C,P}$ = 143.8 Hz, PCH$_2$), 23.66 (d, $^3J_{C,P}$ = 4.6 Hz, PCH$_2$CH$_2$CH_2N$_3$), 49.98 (d, $^2J_{C,P}$ = 17.0 Hz, PCH$_2$CH$_2$), 63.64 (d, $^2J_{C,P}$ = 7.0 Hz, P-O-CH$_2$CH$_3$), 115.9 (triazole *C*=CH), 121.7 (*C*H$_2$-

NH_2), 126.4 (triazole C=*C*H), 121.2 (d, $^3J_{C,P}$ = 4.5 Hz, Ar*C*2, Ar*C*6), 126.0 (Ar*C*3, Ar*C*5), 144.9 (Ar*C*4), 155.5 (d, $^2J_{C,P}$ = 8.6 Hz, P-O-Ar*C*1) ppm. MS (ES+, MeCN) for $C_{14}H_{20}N_5O_5P$ (369.3129) $[M]^+$: *m*/*z* calcd. for $[M + H]^+$ 370.1275; found 370.1280; calcd. for $[M + Na]^+$ 392.1094; found 392.1100. HRMS (ES+, MeCN) for $C_{14}H_{20}N_5O_5P$ (369.3129) $[M]^+$: *m*/*z* calcd. for $[M + H]^+$ 370.1275; found 370.1233. IR (solid): $\tilde{\nu}$ = 2937.8, 1612.4, 1590.2, 1519.6, 1490.0, 1345.4, 1218.6, 1161.5, 1105.8, 1029.4, 911.1, 853.2, 751.0, 688.3 cm^{-1}.

[4-(Methoxycarbonyl)-2,6-bis(pyridin-2-yl)phenyl]platinum(II) Chloride (1): This complex was prepared according to the literature procedure.[25] ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.38 (dd, $^3J_{H,H}$ = 5.6 Hz, $^4J_{H,H}$ = 1.6 Hz, $^2J_{Pt,H}$ = 35.19, 46.79 Hz, 2 H, H^6-py), 8.14 (s, $^4J_{Pt,H}$ = 4.0 Hz, 2 H, H^4 and H^6), 8.00 (dt, $^3J_{H,H}$ = 7.80 Hz, $^4J_{H,H}$ = 1.6 Hz, 2 H, H^4-py) 7.81 (d, $^4J_{H,H}$ = 5.6, 7.8 Hz, 2 H, H^3-py), 7.36 (ddd, $^4J_{H,H}$ = 5.6, 7.8 Hz, $^4J_{H,H}$ = 1.6 Hz, 2 H, H^5-py), 3.94 (s, 3 H, CH_3) ppm.

[4-Carboxy-2,6-bis(pyridin-2-yl)phenyl]platinum(II) Chloride (2): KOH (1.08 g, 19.24 mmol, 100 equiv.) was dissolved in MeOH (5 mL). [4-(Methoxycarbonyl)-2,6-bis(pyridin-2-yl)phenyl]platinum(II) chloride (100 mg, 0.192 mmol, 1 equiv.) was suspended in this solution. The suspension was stirred at 40 °C for 24 h. A 4 M HCl (9.62 mL, 38.48 mmol, 200 equiv.) solution was added to the suspension. The yellow precipitate was washed with water (3 × 20 mL) and MeOH (2 × 20 mL) and the solvents evaporated to dryness. A yellow solid was obtained (78.3 mg, 80%, 0.155 mmol) ^{1}H NMR (400 MHz, $CDCl_3$): δ = 13.00 (s, 1 H), 9.12 (d, $^3J_{H,H}$ = 5.8 Hz, $^3J_{H,Pt}$ = 34.0 Hz, 2 H, py-H^6), 8.28 (m, 4 H, H^2 and H^4 and py-H^3), 8.23 (dt, $^3J_{H,H}$ = 7.4 Hz, $^4J_{H,H}$ = 1.6 Hz, 2 H), 7.60 (ddd, $^3J_{H,H}$ = 5.8, 7.4 Hz, 2 H, py-H^5) ppm. ^{13}C NMR (75 MHz, DMSO): δ = 168.4 (C=O), 167.8 (C^6-py), 166.3 (C^4-py), 156.1 (C^2), 152.0 (quat), 141.3 (quat), 141.3 (C^4 and C^6), 126.7 (C^4-py), 125.5 (quat), 121.52 (quat) ppm. MALDI-TOF MS: calcd. for $C_{20}H_{14}N_3OPt$: 470.0468; found 469.8722 $[M - Cl]^+$. HRMS (ES+, MeCN) for $C_{17}H_{11}ClN_2O_2Pt$ (505.0157) $[M]^+$: *m*/*z* calcd. for $[M - Cl + H_2O]^+$ 488.0574; found 488.0559.

[4-(Prop-2-ynylcarbamoyl)-2,6-bis(pyridin-2-yl)phenyl]platinum(II) Chloride (3): Compound **1** (73.3 mg, 0.145 mmol, 1 equiv.), propargylamine (15.97 mg, 0.290 mmol, 2 equiv.) and DIEA (23.41 mg, 0.181 mmol, 1.25 equiv.) were dissolved in DMF (20 mL) and cooled for 10 min with ice to 0 °C. Subsequently, BOP (67.25 mg, 0.152 mmol, 1.05 equiv.) was added. After 24 h, all volatiles were removed under reduced pressure. The resulting yellow powder was washed with $CHCl_3$ (3 × 20 mL) and Et_2O (2 × 20 mL) and dried under reduced pressure (60.0 mg, 76%, 0.12 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.13 (d, $^3J_{H,H}$ = 5.9 Hz, $^3J_{H-Pt}$ = 35.2 Hz, 2 H, H^6-py), 8.88 (t, $^3J_{H,H}$ = 5.4 Hz, 1 H, N*H*), 8.26 (t, $^3J_{H,H}$ = 8.0 Hz, 2 H, H^4-py), 8.25 (s, 2 H, H^3 and H^6), 8.15, (d, $^3J_{H,H}$ = 8.0 Hz, 2 H, H^3-py), 7.60 (t, $^3J_{H,H}$ = 5.9 Hz, 2 H, H^5-py), 4.12 (dd, $^3J_{H,H}$ = 5.4 Hz, $^4J_{H,H}$ = 2.4 Hz, 2 H, NH–C*H*$_2$), 3.17 (t, $^4J_{H,H}$ = 2.4 Hz, 1 H, C≡C–*H*) ppm. ^{13}C NMR (75 MHz, DMSO): δ = 166.6, 152.2, 141.1, 129.8, 124.8, 121.0 ppm. MALDI-TOF MS: calcd. for $C_{20}H_{14}N_3OPt$: 507.0785; found 507.1562 $[M - Cl]^+$. Elemental analysis calcd. (%) for $C_{18}H_{13}ClN_2O_2Pt$: C 44.25, H 2.60, N 7.74, Pt 35.93; found: C 44.12, H 2.48, N 7.79, Pt 36.10.

Complex 6: Complex **2** (0.0831 g, 0.1643 mmol, 1.0 equiv.) and more DMF (5 mL) was added to phosphonate **5** (0.0607 g, 0.1643 mmol, 1.0 equiv.) dissolved in dry DMF (1 mL). The solution was cooled to 0 °C, after which DIEA (33.9 μL, 0.2054 mmol, 1.25 equiv.) and BOP (0.0763 g, 0.1725 mmol, 1.05 equiv.) were added. After being stirred overnight, all volatiles were evaporated in vacuo. The solid residue was dissolved in CH_2Cl_2 (50 mL) and washed with 1 M K_2CO_3 (2 × 50 mL), brine (2 × 50 mL) and water (2 × 50 mL). After drying with Na_2SO_4 and filtration through Celite, all volatiles were evaporated in vacuo to obtain a yellow solid (0.1239 g, 0.1446 mmol, 88%). The ^{1}H NMR spectroscopic signals were assigned with the aid of COSY and long-range COSY spectra. ^{1}H NMR ([D$_6$]DMSO, 298 K, 400 MHz): δ = 1.20 (t, $^3J_{H,H}$ = 7.0 Hz, 3 H, P-O-CH$_2$C*H*$_3$), 1.98–2.10 (m, 4 H, PC*H*$_2$C*H*$_2$CH$_2$N$_3$), 4.09–4.15 (m, 2 H, P-O-C*H*$_2$CH$_3$), 4.44 (t, $^3J_{H,H}$ = 6.8 Hz, 3 H, PCH$_2$CH$_2$C*H*$_2$N$_3$), 4.57 (d, $^4J_{H,H}$ = 5.6 Hz, 2 H, C*H*$_2$NH), 7.42 (d, $^3J_{H,H}$ = 9.2 Hz, 2 H, PNPAr*H*), 7.59 (t, $^3J_{H,H}$ = 5.9 Hz, 2 H, H^5-py), 8.07 (s, 1 H, triazole*H*), 8.12 (d, $^3J_{H,H}$ = 7.2 Hz, 2 H, H^3-py), 8.24 (d, $^3J_{H,H}$ = 9.2 Hz, 2 H, PNPAr*H*), 8.24–8.27 (m, 4 H, t, H^4-py, H^3, H^6), 8.97 (t, $^3J_{H,H}$ = 7.0 Hz, 1 H, N*H*), 9.12 (d, $^3J_{H,H}$ = 5.9 Hz, 2 H, H^6-py) ppm. ^{31}P NMR ([D$_6$]DMSO, 298 K, 162 MHz): δ = 30.19 ppm. ^{13}C NMR ([D$_6$]DMSO, 298 K, 101 MHz): δ = 16.81 (d, $^3J_{C,P}$ = 5.0 Hz, P-O-CH$_2$*C*H$_3$), 22.89 (d, $^1J_{C,P}$ = 140.8 Hz, P*C*H$_2$), 23.82 (d, $^3J_{C,P}$ = 4.6 Hz, PCH$_2$CH$_2$*C*H$_2$), 49.79 (d, $^2J_{C,P}$ = 19.0 Hz, PCH$_2$*C*H$_2$), 63.45 (d, $^2J_{C,P}$ = 7.2 Hz, P-O-*C*H$_2$CH$_3$), 116.5 (triazole *C*=CH), 121.3 (*C*H$_2$-NH$_2$), 122.1 (Ar*C*2, Ar*C*6), 123.9, 124.4, 124.6, 125.3, 126.5 (triazole C=*C*H), 126.9 (Ar*C*3, Ar*C*5), 130.1, 141.0, 141.4, 144.8, 145.7, 152.1, 156.1 (d, $^2J_{C,P}$ = 8.6 Hz, P-O-Ar*C*1), 166.2, 166.5 ppm. MS (ES+, MeCN) for $C_{31}H_{29}N_7O_6PPt$ (856.1253) $[M]^+$: *m*/*z* calcd. for $[M - Cl]^+$ 821.1559; found 821.1586; calcd. for $[M + Na]^+$ 880.1105; found 880.1238. HRMS (ES+, MeCN) for $C_{31}H_{29}N_7O_6PPt$ (856.1253) $[M]^+$: *m*/*z* calcd. for $[M - Cl]^+$ 821.1565; found 821.1575. IR (solid): $\tilde{\nu}$ = 3071.5, 1644.3, 1609.0, 1591.1, 1519.4, 1475.7, 1344.3, 1219.0, 1159.1, 1110.7, 1027.3, 910.2, 860.0, 751.1, 687.7 cm^{-1}.

Inhibition of Cutinase with Complex 6: A solution of complex **6** (30 μL, 10 mM, 0.3 μmol, 3.0 equiv.) in DMF or MeCN was added to a solution of cutinase (0.5 mL, 200 μM, 0.1 μmol, 1.0 equiv.) in Tris (50 mM) and 0.1% (m/m) Triton buffer (pH 8.0). After 2 h, more inhibitor **6** (30 μL, 10 mM, 0.3 μmol, 3.0 equiv.) was added. After incubation overnight, the solution was dialyzed over 24 h with NH_4OAc buffer (150 mM, 2 × 400 mL) and an activity test was performed.

Activity Tests: A solution of *p*-nitrophenyl butyrate (7.0 μL, 50 mM) in MeCN was added to different batches of Tris (50 mM) and 0.1% (m/m) Triton buffer (1.493 mL, pH 8.0). Portions of inhibited and uninhibited cutinase (0.5 μL, 200 μM, 0.1 nmol) were added to these substrate solutions and the release of *p*-nitrophenolate was measured at 400 nm over 10 min.

Inhibitions for the Gel-Electrophoresis Experiments: A solution of **6** (20.0 mM, 50.0 μL, 1.0 mmol) in DMF was added to solutions of cutinase (1.0 mg/mL), CALA (0.8 mg/mL) and CALB (2.0 mg/mL) in Tris (50 mM) and 0.1% (m/m) Triton buffer (200 μL each). The samples were incubated in the refridgerator overnight, after which the samples were analyzed with SDS-PAGE. For this, the protein samples (20 μL) were loaded onto 12.5% SDS-PA gels and separated by gel electrophoresis.[77] Subsequently, the gel was analyzed under a UV lamp (257 nm, GelDoc), after which the gel was stained with Coomassie.

Supporting Information (see also the footnote on the first page of this article): Additional absorption and emission spectra of compounds **1**, **2**, **6** and **cut-6** in different solvents.

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[73] Complexes like inhibitor **6** can easily be converted into its cationic analogue by abstraction of the halide ion (e.g., by using AgBF4 or AgOTf). By doing so, a vacant coordination site becomes available, which can be used for the coordination of various ligands, proteins or other potential probes. Consequently, the protein-substituted organometallic moiety can be coordinated to other luminescent reporter molecules, or even solid supports, thereby enabling us to apply this system to microarrays. A similar pincer–platinum complex covalently bound to cutinase already revealed that selective coordination of neutral phosphane ligands to the platinum centre is possible. This would make **6** a very versatile probe in studying proteins.
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