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Review

Incorporating ECE-pincer metal complexes as functional building blocks in semisynthetic metalloenzymes, supramolecular polypeptide hybrids, tamoxifen derivatives, biomarkers and sensors

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Dedicated to Prof. Gérard Jaouen for his excellent and imaginative contribution to bioorganometallic research on the occasion of his 65th birthday.

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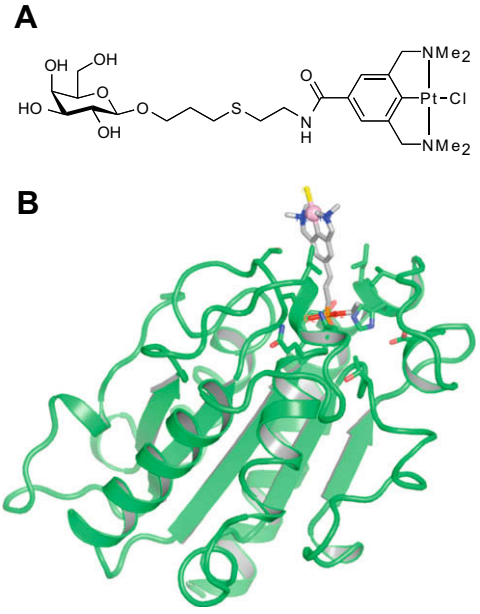
ABSTRACT

ECE-pincer metal compounds often have excellent thermal and chemical stability, which makes these organometallics attractive for use as building blocks in bioorganometallic chemistry. This account highlights different applications of hybrids involving covalent or non-covalent assemblies of ECE-pincer building blocks as, in anticarcinogenic agents (*e.g.* tamoxifen derivatives), carbohydrates (surface plasmon resonance enhancers), polypeptides (supramolecular synthons) and solid supports (organometallic peptide-labels) or lipases (biocatalysts). The molecular structures of a typical surface plasmon resonance enhancer (**A**) and a lipase (**B**), both containing a covalently attached NCN-pincer platinum complex, are shown. The design, synthesis, structural analysis and potential applications of semisynthetic pincer-metalloenzymes is also discussed.



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Contents

1. Introduction

The field of bioorganometallic chemistry comprises the synthesis and application of organometallics, *i.e.* of metal complexes with at least one covalent metal–carbon bond, in biological systems and in chemical biology [1,2]. The properties of the resulting bioorganometallic systems and compounds have been studied in a variety of applications as potential anticarcinogenics [3–8], antibiotic or antiviral drugs [9,10], as *in vitro* and *in vivo* diagnostics or radiopharmaceutical agents [11,12], as biosensors [13] and electrochemical probes [14–16], as metallo-immunoassays [17–19], as active site mimics of metalloenzymes [19–22], as structural probes [23,24] or as semisynthetic metalloenzymes for catalytic purposes [5,25,26].

In naturally occurring bioorganometallics, for example Vitamine B_{12} [27], the steric and electronic properties of the transition metal ion are tuned to realize preferential geometrical arrangements, folding of the protein or porphyrin backbone, or to tune the accessibility and activity of the metal centre, *e.g.* for catalytic reactions [28,29]. The synthetic chemistry of transition metal complexes in biological media is often hampered by the sensitivity and instability of these complexes and/or their ligands in aqueous or aerobic media. Furthermore, lability of bioorganometallics towards biomolecules other than the targeted ones or aspecific binding to the metal centre is often observed. Several anticancer drugs, for instance, cause severe and sometimes toxic side effects when administered to patients, which is often due to the decomposition of the original organometallic or coordination complexes [30]. Recent advances in bioorganometallic chemistry concerning the design of more stable organometallic building blocks have led to the synthesis and application of a whole range of bioorganometallic complexes which are compatible with biological media [1]. Some metallocenes, like substituted ferrocene complexes, are an interesting example of a class of stable, biocompatible organometallic building blocks, which possess interesting redox and electrochemical properties [2,31,32].

Another well-studied class of organometallics, some members of which have considerable thermal and chemical stability, are the so-called ECE-pincer metal complexes. These contain a terdentate, monoanionic ligand of the general formula $[2,6\text{-}(ECH_2)_2C_6H_3]^-$, where E is a neutral, two-electron heteroatom donor, like $N(R)_2$, PR_2, or SR. Fig. 1 shows the general structure of an ECE-pincer metal complex in which M(II) is a divalent transition metal ion, for example from the d^8 metal series, and X is an anion [33]. In these complexes, the bis-*ortho*-chelation of the metal ion by the two E-donating groups provides further stability to the central M–C bond, making some ECE-pincer metal complexes even compatible with aqueous solvent media (acidic, neutral, basic), aerobic conditions and elevated temperatures.

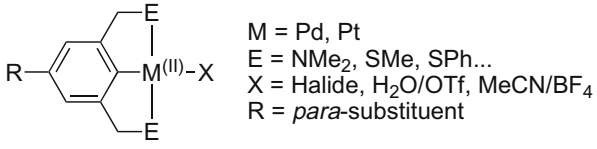


Fig. 1. General structure of an ECE-pincer metal(II) complex [33].

Due to their specific structural features [34,35] and remarkable stability, ECE-pincer metal-complexes have found numerous applications, ranging from their uses such as catalysts, organometallic switches, and heavy atom probes to sensoring applications [33]. Different types of ECE-pincer metal complexes have been attached as building blocks to polymers [36], dendrimers [37] and solid supports (*e.g.* silica surfaces) [38]. Those hybrid materials were successfully used as catalytic materials in both homogeneous and immobilised "homogeneous" (*i.e.,* heterogeneous) catalysis. Due to their robustness, ECE-pincer metal complexes are suitable building blocks for the modification of biomolecules, as they are known to be compatible with a variety of operations common in synthetic (bio)-organic and -inorganic chemistry working under aqueous and aerobic conditions.

The use of ECE-pincer metal complexes as potential anticarcinogenic agents, as Surface Plasmon Resonance enhancers and as supramolecular synthons will be described. In addition, pincer substituted oligopeptides have been successfully applied as peptide biomarkers and bioorganometallic peptide catalysts. Finally, the design, structural analysis and potential applications of semisynthetic pincer-metalloenzymes will be illustrated.

2. Pincercifen as a new tamoxifen derivative

The pioneering work of Jaouen et al. [39–43] demonstrated unambiguously that organometallic complexes can be successfully combined with selective estrogen receptor modulators (SERM) [41] affording organometallic tamoxifen derivatives like ferrocifen (**1**) and hydroxyferrocifen (**2**, Fig. 2) [39,40,42,43] with good cytotoxic and antiestrogenic (for **2**) activities. In both **1** and **2** it is the phenyl group originally present in hydroxy-tamoxifen, which is replaced by a ferrocenyl group. The cytotoxic activities of **1** and **2** were attributed to the redox properties of the ferrocene functionality. In comparison to hydroxy-tamoxifen, the binding affinity of hydroxy-ferrocifen **2** to the estrogen receptor protein was slightly lower, which is most probably caused by the increased steric bulk of the ferrocene moiety when compared to the phenyl ring [43]. Other tamoxifen derivatives containing an oxaliplatin-derived coordination complex (**3** and **4**, Fig. 2) have been developed by Jaouen and co-workers as well [44]. Interestingly, the (poor) stability of platinum coordination complexes under physiological conditions can be used to selectively deliver the cytotoxic metal moiety to its target, however also toxic side effects due to aselective decomposition of the coordination complex can occur [30].

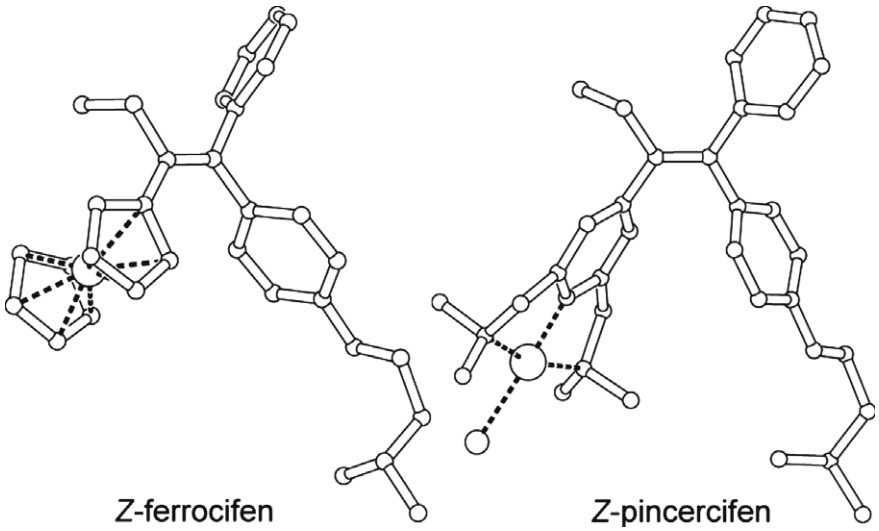
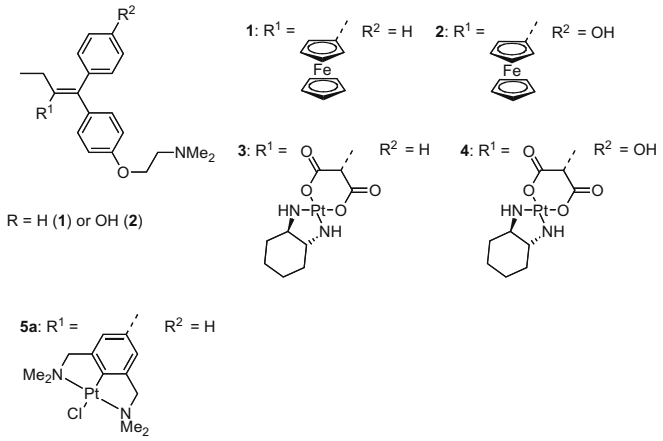


Fig. 2. Different metal-containing tamoxifen analogues and the crystal structures of *Z*-ferrocifen **1** and *Z*-pincercifen **5** [39–46].



Inspired by these elegant studies, our group designed NCN-pincer platinum(II) containing analogues (coined 'pincercifens' **5**, Fig. 2) [45,46]. As the NCN-pincer platinum halide unit possesses a covalent platinum–carbon bond, it was anticipated that compound **5** would be more stable under physiological conditions and show less toxic side-effects upon transport and delivery towards the cancer cells than common commercial anticancer drugs. Comparison of the crystal structures of *Z*-ferrocifen **1** and *Z*-pincercifen **5** (Fig. 2) shows that the steric bulk of pincercifen **5** is different from ferrocifen **1**, which might result in different binding affinities and strengths of the two complexes to the target molecules. Furthermore, the redox properties of ferrocenyl and NCN-pincer platinum(II) units are different, which could result in altered cytotoxic activities of **5** when compared to **1**. Currently, the biological activities of pincercifen **5** are under investigation [46,47].

3. NCN-pincer platinum(II) complexes as SPR enhancers for the study of carbohydrate–protein interactions

For the studies of biomolecular interactions in real time, biosensors based on surface plasmon resonance (SPR) have become an increasingly popular tool [48,49]. SPR has been proven to be an emerging technique for the qualitative and quantitative study of nucleic acid–protein, protein–protein, carbohydrate–carbohydrate and carbohydrate–protein interactions [50–55]. As the SPR response is proportional to the accumulation of mass on the sensor surface, a serious constraint of SPR concerns the dimensions of the analyte-molecules. Especially in the study of the generally weak carbohydrate–protein interactions, the low availability of high-molecular mass oligosaccharides hampers the wide applicability of this technique.

As NCN-pincer platinum complexes are small in size and contain a platinum ion as heavy metal, these complexes were suitable candidates for the enhancement of the SPR signal [56]. For this purpose different low-molecular mass mono- and disaccharides labelled with a NCN-pincer platinum unit were used to assay the carbohydrate–protein interactions with the immobilized lectins RCA_{120} and ConA, being standard proteins for the study of carbohydrate–protein interactions (Fig. 3) [56].

The studies showed that specific binding of the NCN-pincer platinum unit labelled saccharides led to a strong detectable SPR signal enhancement as compared to the pincer-free saccharides, even at low analyte concentrations (9 μM). This interaction was only specific for the intact protein and not for its denatured species (Fig. 4).

As saccharides without NCN-pincer platinum labels did not show any detectable binding affinities in the SPR studies, despite their well-known selectivity for the used lectins, the signal enhancement is purely attributed to the NCN-pincer platinum label. Control studies showed that signal enhancement occurred exclusively upon incorporation of the NCN-pincer platinum(II) label and not in the presence of the NCN-pincer ligand only (*i.e.* without platinum), which proves that the sensitivity increase is only due to the heavy atom effect of the platinum(II) ion [56]. It is believed that not only the mass increase by the Pt(II) ion contributes to the observed beneficial labelling properties, but that also a significant interaction between the platinum electrons and the evanescent wave produced in proximity of the sensorchip surface is responsible for the observed phenomenon [56].

Interestingly, variation of the linker length between the NCN-pincer platinum label and the carbohydrate, as for complexes **6** and **10** (Fig. 4), did not influence the response significantly, which indicates that the signal enhancement is not influenced by the bond distance between the label and the saccharide. In comparison to other SPR enhancers, the NCN-pincer platinum labels described here are small in size, possess a lower molecular weight and do not have a negative influence on the carbohydrate–protein interaction, making them unique, powerful detection tools for SPR. It also highlights the full biocompatibility of the NCN-pincer platinum moiety.

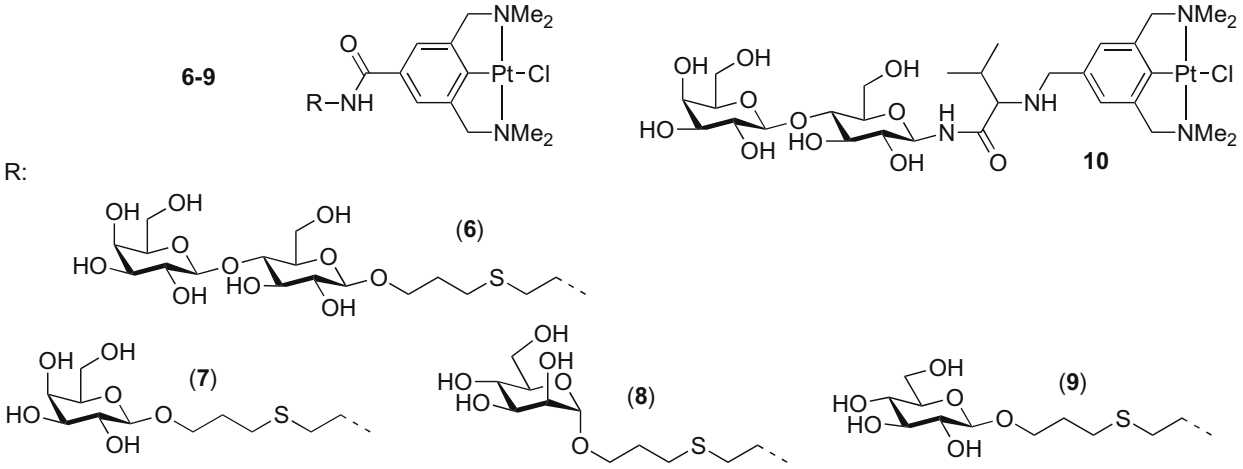


Fig. 3. NCN-pincer platinum unit labelled lactose (**6**, **10**), D-galactose (**7**), D-mannose (**8**), D-glucose (**9**), which was used as SPR sensitizers in carbohydrate–protein interaction studies [56].

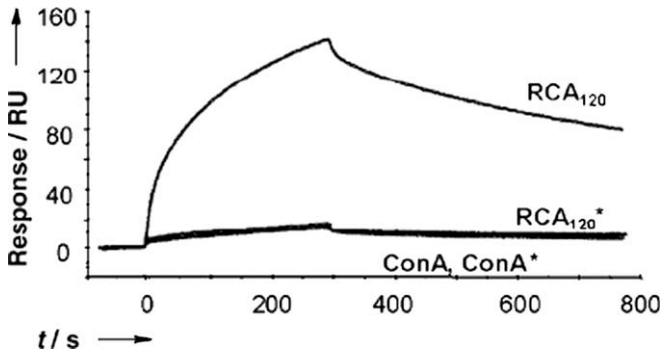


Fig. 4. Sensorgrams of NCN-pincer platinum unit labelled **6** (9 μM) flowing across RCA_{120}, denatured RCA_{120}^{*}, ConA and denatured ConA* (RCA_{120} is known to be specific for galactose/lactose, ConA specific for mannose) [56].

4. Supramolecular assemblies involving ECE-pincer palladium(II) complexes and biomolecules

The groups of Weck and coworker [57] and Reinhoudt and coworkers [58] elegantly used the well-established properties of cationic ECE-pincer metal(II) complexes coordinating to (substituted) pyridines [59–72] to construct supramolecular assemblies involving these pincer cations and pyridine-modified peptides [57] and carbohydrates [58], thereby constructing bio-coordination complexes with special properties.

For this purpose, Weck et al. investigated the coordination strengths of different pyridyl glycine (**12**, **13**) and pyridyl alanine (**14**, **15**) tripeptides with a 3-pyridyl (**13**, **15**) or 4-pyridyl (**12**, **14**) functionality to the *p*-methoxy-substituted SCS-pincer palladium(II) cation **11** (Fig. 5). The formation of the Pd–N coordination bonds was studied by ^{1}H NMR spectroscopy, ES-MS and isothermal

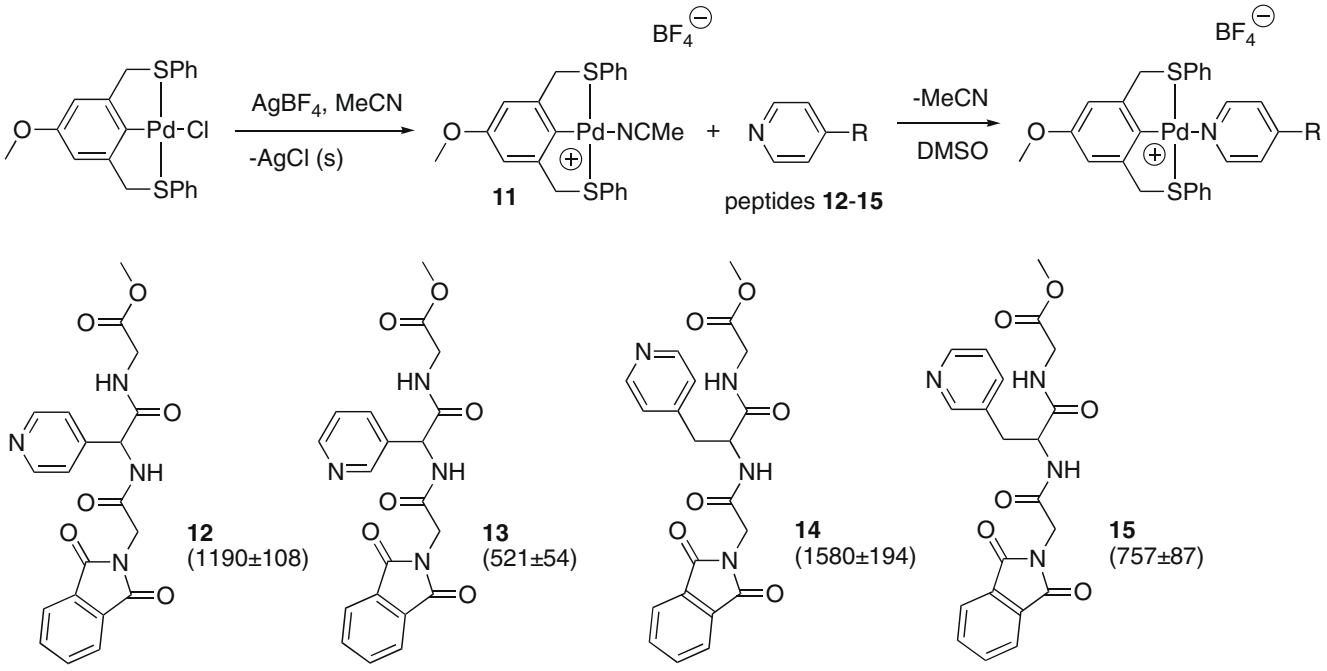


Fig. 5. Coordination of different pyridyl-modified peptides to SCS-pincer palladium(II) cations. The ITC K_a values of peptides 12–15 binding to the cationic pincer complex **11** are given in brackets [57].

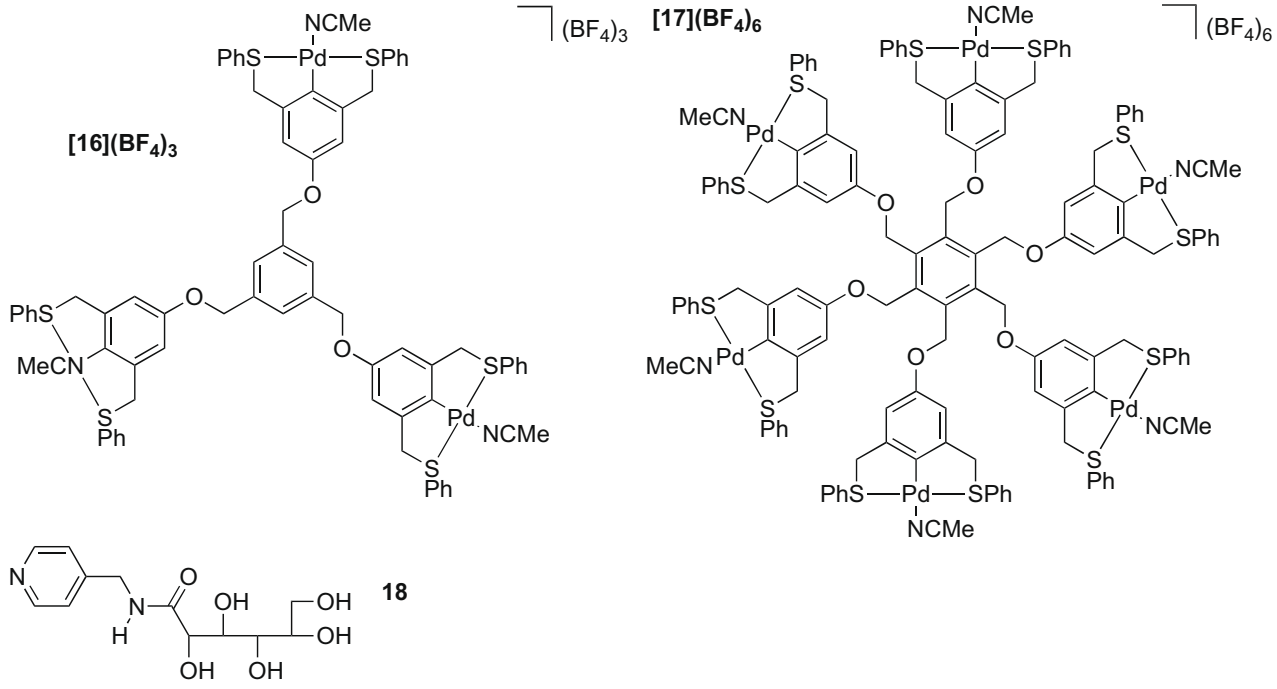


Fig. 6. Tri- and hexa-cationic SCS-pincer palladium(II) dendrimers [16] $(BF_4)_3$ and [17] $(BF_4)_6$ to which 4-pyridyl-substituted carbohydrate **18** was coordinated [58].

titration calorimetry (ITC). In this case, ITC also allowed for the determination of K_a's for the binding of peptides **12**–**15** to the cationic pincer complex **11**.

According to the different analysis methods used, the metal–pyridine coordination to peptides **12**–**15** was quantitative in all cases and no competitive coordination by other heteroatoms present in the peptide backbone was observed. The latter preference for monodentate coordinating ligands underlines the unique features of the tridentate binding of the SCS-manifold to the central d^8 metal atom. The investigation of the bond strength with ITC (Fig. 5) showed that the 4-pyridyl peptides **12** and **14** form a stronger coordination bond with the SCS-pincer palladium centre than the 3-pyridyl peptides **13** and **15**, which was attributed to steric reasons. The stronger coordination of the alanyl derived peptides **14** and **15** in comparison to the glycyl derived peptides **12** and **13** was explained by electronic activation of the pyridyl moiety via a methylene spacer [34,66].

The bioorganometallic coordination complexes described here can be used as biological synthons for the design of biocompatible metal-coordination materials. The next step in these studies will be the design of cyclic peptides and their self-assembly with bifunctional metallated pincer complexes for the creation of novel supramolecular systems [57].

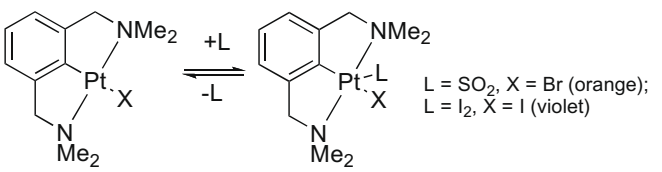
The group of Reinhoudt has held a long tradition in studying the formation of non-covalent metal-induced self-assemblies, for which they also used ECE-pincer metal complexes immobilized onto dendrimers as coordination scaffolds [66,73–75]. In one example [58], they tried to mimic the solubility properties of water-soluble globular proteins, where the hydrophobic core of the protein is decorated with hydrophilic amino acids on the surface. As core-mimics they used hydrophobic dendrimers, which were functionalized with cationic SCS-palladium(II) pincer groups at the periphery (Fig. 6).

To these hydrophobic tri- and hexa-pincer cationic dendrimers $[\mathbf{16}]^{3+}$ and $[\mathbf{17}]^{6+}$ (Fig. 6) dissolved in MeCN were added three (for $[\mathbf{16}]^{3+}$) or six (for $[\mathbf{17}]^{6+}$) equivalents of a linear 4-pyridine-functionalized carbohydrate **18**. Solution NMR analysis showed that the resulting coordination complexes **[16 · (18)3](BF$_4$)$_3$** and **[17 · (18)6](BF$_4$)$_6$** were formed quantitatively in d_6-DMSO and CD_3CN/d_3-MeOD, respectively. However, complex **[16 · (18)3](BF$_4$)$_3$** appeared to be insoluble in D_2O. The hexanuclear coordination complex **[17 · (18)6](BF$_4$)$_6$** dissolved well in hot D_2O, but upon cooling to room temperature an aqueous gel was formed. This study shows that the coordination of functionalized sugar molecules to dendritic cationic SCS-pincer palladium molecules is successful in polar solvents, showing the suitability of SCS pincer palladium dendrimers as building blocks for the construction of new bioorganometallic hybrid materials.

5. Pincer platinum(II) complexes as peptide biomarkers

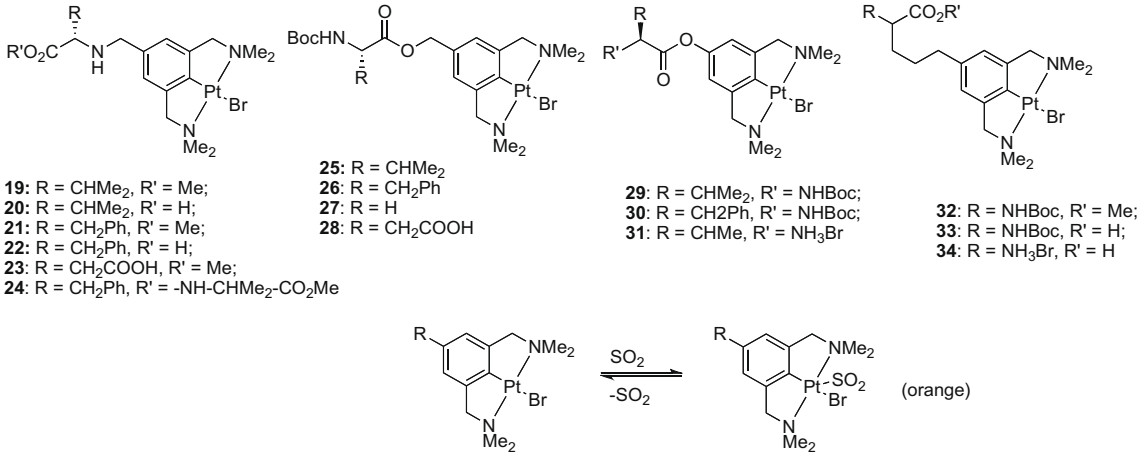
Organometallic complexes attached to peptides have been successfully used in immunoassay [19] and in biomedical studies due to their anti-bacterial and anti-proliferative properties [10]. Ferrocene amino acids [23,24] have also been used as structural probes, e.g. as turn inducers. NCN-pincer platinum(II) complexes are well known for their properties as sensor materials, *e.g.* for the sensing of SO_2 and diiodine [33,76]. The formation of the pentacoordinate SO_2 or I_2 adducts induces a change in the visible part of the UV–VIS spectrum, which enables detection of the coordination complexes by the naked eye (Scheme 1).

The exceptionally high stability of NCN-pincer platinum(II) complexes in aqueous media and in air enabled Van Koten and



Scheme 1. Reversible formation of the pentacoordinated SO_2 and I_2 NCN-pincer platinum(II) coordination complexes [33,76].

Table 1
NCN-pincer platinum complexes bonded to the N- (**19**–**24**) and C- (**25**–**31**) termini and to the α-carbon atom (**32**–**34**) of different diprotected amino acids; the ^{195}Pt NMR data for pincer diprotected amino acids **19**–**34** and the corresponding SO_2^- coordination complexes are given.



Compound	R-group	Solvent	δ(Pt)	δ(Pt/SO_2-adduct)	Δδ
19	CH_2-L-Val-OMe	$CDCl_3$	−1982	−751	1231
20	CH_2-L-Val-OH	CD_3OD	−1972	−1170	802
21	CH_2-L-Phe-OMe	$CDCl_3$	−1973	−732	1241
22	CH_2-L-Phe-OMe	CD_3OD	−1973	−1170	803
23	CH_2-L-Asp-OMe	$CDCl_3$	−1983	−954	1029
24	CH_2-L-Phe-L-Val-OMe	$CDCl_3$	−1983	−954	1029
25	CH_2-L-Val-Boc	$CDCl_3$	−1964	−870	1094
26	CH_2-L-Phe-Boc	$CDCl_3$	−1963	−1219	744
27	CH_2-Gly-Boc	$CDCl_3$	−1964	−776	1188
28	CH_2-L-Asp-Boc	$CDCl_3$	−1964	−1152	812
29	L-Val-Boc	$CDCl_3$	−1967	−790	1177
30	L-Phe-Boc	$CDCl_3$	−1967	−883	1084
31	L-Val-NH_3Br	CD_3OD	−1982	–	–
32	BocHN{$CH(CH_2)_3$}CO_2Me	$CDCl_3$	−1997	−1078	919
33	BocHN{$CH(CH_2)_3$}CO_2H	$CDCl_3$	−1982	−998	984
34	BrH_3N{$CH(CH_2)$}CO_2H	DMSO	−1980	–	–

co-workers to investigate their use as organometallic peptide-labels. For this purpose, NCN-pincer platinum(II) units were covalently bonded to the N-[77,78] and C-termini [78] and to the α-carbon atom [78,79] of different amino acids (Table 1). The pincer sensing moieties were introduced at different stages during the syntheses using various organic transformations. In all cases the covalent platinum–carbon bond remained intact, which allowed the use of this NCN-pincer platinum unit as a low-molecular weight biomarker for peptides [77,78].

Due to the characteristic NMR/MRI activity of the ^{195}Pt nucleus (natural abundance 33.8%, $I = 1/2$), the NCN-pincer platinum units can also be used as biomarkers for peptides. The chemical shifts for the N-terminus derivatives **19**–**24** range from $\delta = -1983$ to -1972 ppm, for the C-terminus derivatives **25**–**31** from $\delta = -1963$ to -1967 ppm and for the α-carbon atom derivatives **32**–**34** from $\delta = -1997$ to -1980 ppm. The characteristic range of the resonances suggests that a distinction between N-, C- and α-carbon labelled peptides based on the resonance of the ^{195}Pt nucleus is possible, making the NCN-pincer platinum(II) moiety a site-specific metal probe.

SO_2 binds instantaneously and reversibly to NCN-pincer platinum(II) halides with a concomitant diagnostic colour change from colourless to orange (Scheme 1, Table 1), resulting in detection limits in the ppm range. This SO_2–Pt bond formation, which can occur in the solid state as well [80], is also reflected in large ^{195}Pt NMR downfield shifts for the modified peptides **19**–**34**. These shifts range from $\delta = 744$–1241 ppm and are fully reversible. As soon as the SO_2 atmosphere is removed, the original pincer peptides are recovered unchanged. Moreover, the formation of the SO_2 adduct is insensitive to other atmospheric gases, like CO, HCl and to humidity [33]. As SO_2 is a physiologically important gas [81], the use of these very stable NCN-pincer platinum(II) halide labels as sensitive sensors in biochemical and medicinal applications is open for further studies, both *in vitro* and *in vivo*.

The reversible coordination of I_2 to NCN-pincer platinum(II) halide complexes could be used to label peptides in solid-phase synthesis. The two different NCN-pincer platinum(II) iodide complexes **35** and **36** were used as colour biomarkers in the solid-phase peptide synthesis of the oligopeptides **35**-GPPFPF and **36**-XGPPFPF (with X being any of the naturally occurring α-amino acids, Fig. 7). Firstly, both NCN-pincer platinum labelled oligopeptides on resin showed the characteristic reversible orange coloration upon exposure to SO_2, which disappeared after removal of the beads from the SO_2 containing atmosphere. Secondly, when the oligopeptides **35**-GPPFPF and **36**-XGPPFPF on resin were treated with an aqueous solution of KI_3, a persistent purple/black coloration of the beads was observed. This coloration was due to the formation of an η^1–I_2 coordination complex [76] (Fig. 7) from which the I_2 could easily be removed (decoordinated) by washing the beads with a DMF/Et_3N solution.

As biomarker **36**, lacking the labile secondary amine function present in **35**, was more stable towards acidic peptide deprotection protocols, the deprotection, photocleavage and analysis of the peptides labelled with **36** was investigated in detail. For **36**-XGPPFPF

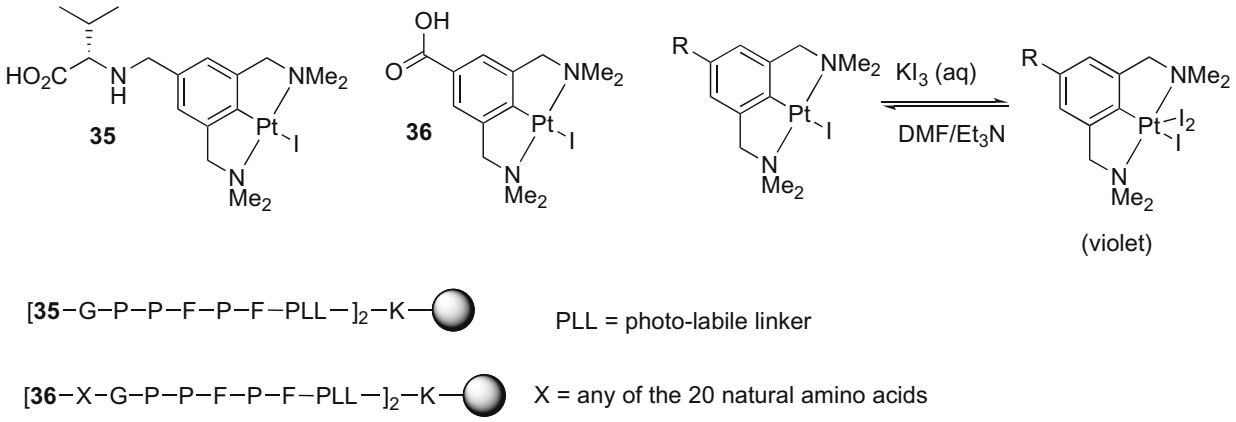


Fig. 7. The solid-phase peptide biomarkers **35** and **36**, their attachment onto oligopeptides and the reversible colouring of the NCN-pincer platinum halide labels by KI_3 [82].

on resin first the side chain protecting groups *t*-Bu, Boc and Trt were removed by treatment with TFA/tri-isopropylsilane/H_2O (90/5/5), after which the metal-labelled peptides were removed from the support by UV-irradiation. Subsequent mass spectrometric analysis of the 20 different **36**-XGPPFPF metal-labelled oligopeptides showed that in all cases the biomarker **36** had been introduced quantitatively (the characteristic Pt pattern was well-visible in the MALDI-TOF mass spectra in all cases) and that no degradation of the NCN platinum moiety had occurred under the acidic deprotection conditions applied, again emphasizing the remarkable stability of this moiety under many reaction conditions.

As the mild coloration method with KI_3 was reversible and easily detectable, but more persistent (removal of coloration only after treatment with DMF/Et_3N or DMF/morpholine) than the coloration with SO_2, it appeared to be a very convenient assay for the analysis of different polypeptides. An investigation of the sensitivity of the coloration process on beads also showed that only capping of 6% of the amine termini with **36** already resulted in differently coloured resins, which could be distinguished by the eye [82]. The coloration process did not interfere with the polypeptide backbone and as the coloration was stable in water and could repeatedly be switched on and off, the NCN-pincer platinum(II) moiety proved to be a very useful solid-phase peptide label.

6. Pincer palladium(II) complexes as catalytically active artificial peptides

Peptides substituted by phosphines have been used successfully as chiral backbones for transition metal catalysts in asymmetric hydrogenation, alkylation and allylic substitution reactions [83–87]. ECE-pincer palladium complexes, which have been broadly applied as homogeneous catalysts, *e.g.* in Suzuki, Heck and aldol condensation reactions [33], could also be attached to different peptides and applied as organometallic peptide catalysts (Fig. 8) [79,88].

For the purpose of preparing catalytically active artificial peptides, protocols were developed to couple appropriately functionalized NCN-ligands to peptide chains. This was done by coupling the N- or C-termini or the α-carbon atom of the respective mono- or di-peptides to the NCN-bromide ligand and subsequent metallation with $[Pd_2(dba)_3 \cdot CHCl_3]$. Interestingly, palladation of the NCN-bromide peptide ligands was relatively straightforward, and even the hydrolysis of the protected NCN-pincer palladium(II) peptide **37** to the free acid **38**, which was performed using LiOH, could be pursued without any problems, as the Pd–C bond remained intact under the deprotection conditions applied.

Subsequently, the NCN-pincer palladium(II) substituted amino acids **37**–**40** were used as catalysts in the aldol condensation reaction of methyl isocyanate and benzaldehyde to give either the *cis* or the *trans* product. To do so, the organometallic pincer peptides **37**–**40** were first activated with $AgBF_4$ to form their cationic analogues, and after thorough removal of the residual silver salt by filtration, they were used in catalysis.

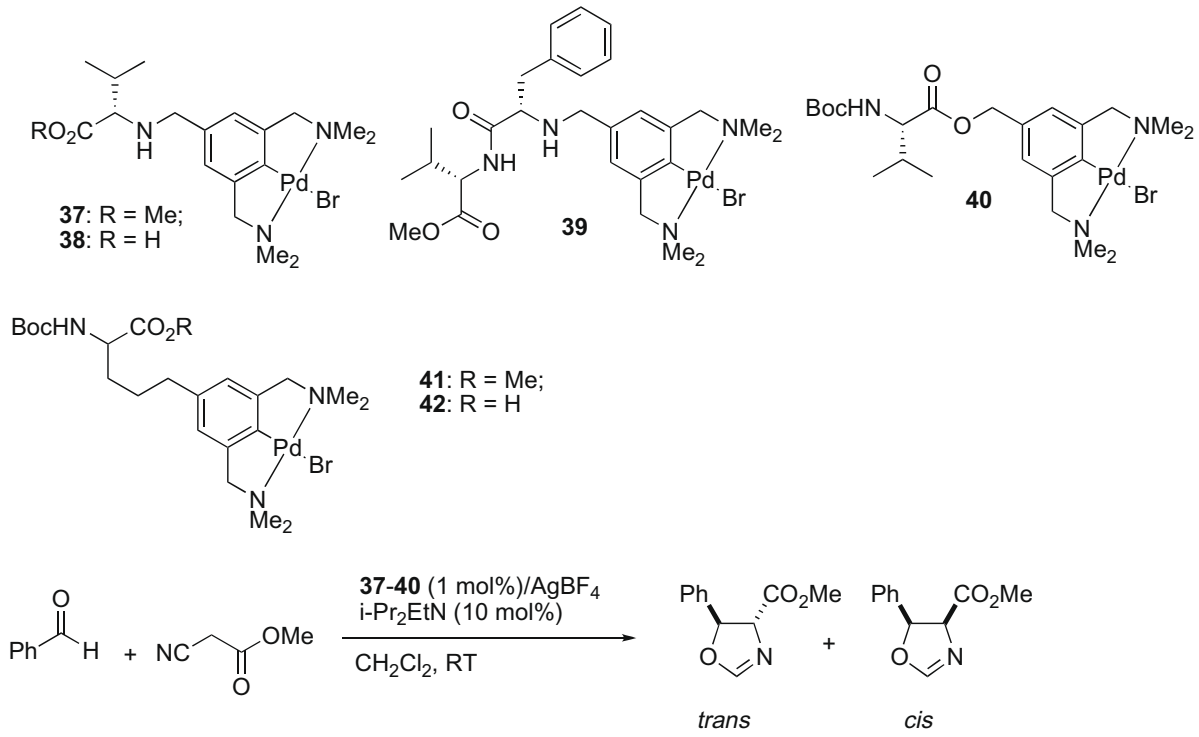
All activated analogues of **37**–**40** were catalytically active and gave ⩾94% conversion after the indicated time (Fig. 8). However, no significant stereoselective influence of the pincer peptide catalysts on the product formation was observed. This is probably due to the short lengths of the peptides used. As the stereocentres of the peptides are too far away from the catalytically active Pd(II) centre, the chiral induction of the peptide backbone is too low. To induce chirality, the introduction of NCN-pincer palladium(II) complexes into longer peptide chains is necessary.

7. ECE-pincer metal(II) protein hybrids

Recently, the bioorganometallic research of the Van Koten group has gradually shifted its focus from the modification of carbohydrates and oligopeptides to the modification of entire proteins by ECE-pincer metal complexes. As illustrated for sugars and oligopeptides (*vide infra*), pincer complexes could be successfully applied as biosensors or artificial bio-catalysts. Therefore, it was interesting to explore whether the obtained knowledge could be applied to more complex biological systems, *i.e.* proteins. Since the pioneering work of Whitesides et al. [89], different groups have been working on the modification of proteins with transition metal complexes already, with very popular examples being the non-covalent biotin-(strept)avidin [26,90–93] system and apo-myoglobin, which had been modified through coordination by *e.g.* salen complexes [25,94].

For the development of a general and selective anchoring strategy of organometallic ECE-pincer complexes to proteins it was decided to not only address one specific type of protein, but a whole protein class. As the serine hydrolases are a very large and well-studied class of proteins with a very pronounced and broad activity and substrate profile [95], we focused on the site-selective modification of serine hydrolases. A common feature of serine hydrolases is their irreversible and covalent inhibition by phosphonate inhibitors, which is due to a covalent bond formation between the catalytically active serine residue and the phosphorous atom of the phosphonate (Fig. 9).

Because of these interesting features, new synthesis protocols were developed to couple various pincer palladium and platinum complexes to reactive phosphonate moieties. The designed ECE-



Entry	Catalyst	Time (h)	TOF	Conversion (%)	*trans:cis*
1	None	5	0	0	-
2	[PdCl(NCN)]	7	43	83	62:38
3	BnValOH	24	1	18	80:20
4	**37**	7	52	96	62:38
5	**38**	3	62	>99	64:36
6	**39**	7	40	97	68:32
7	**40**	7	30	94	62:38

Fig. 8. NCN-pincer palladium complexes bonded to the N- (**37**–**39**) and C- (**40**) termini and to the α-carbon atoms (**41**–**42**) of different peptides; **37**–**40** were used as catalysts in the aldol condensation of methyl isocyanate and benzaldehyde [88].

pincer metal phosphonate inhibitors **43**–**46** all possess one *para*-nitrophenolate leaving group, which makes the inhibitors less susceptible to autohydrolysis in aqueous media when compared to other phosphonate inhibitors [98]. Furthermore, the release of the *para*-nitrophenolate anion (PNP) during inhibition allowed us to follow the inhibition reaction profile spectrophotometrically, since the PNP anion is brightly yellow coloured in solution and thus easily detectable with UV–VIS spectroscopy at 400 nm. After irreversible binding, the active site of the serine hydrolase is blocked and therefore no residual hydrolytic activity of the serine hydrolase is observed anymore. This feature can be used to verify and quantify the inhibition, by assaying the remaining hydrolytic activity of a serine hydrolase after (partial) inhibition with a test substrate (should be lacking upon full inhibition) [96,97]. In a preliminary study, it was found that several different serine hydrolases, *e.g.* CALB, patatine, Chromobacterium Viscosum lipase and cutinase could be inhibited with different phosphonates [95].

Using this methodology, the serine hydrolase cutinase was successfully modified with phosphonate-pincer adducts **43**–**46**. Comparing the inhibition reactions of the various phosphonates (**43**–**46**) a large difference in reaction speed between the C0-tethered **43** [96], and the C3-tethered pincer phosphonates **44**–**46** [97] was observed. For pincer phosphonates **44** and **45** for instance, the inhibition with 2 equiv. of inhibitor was complete after 5 min, whereas the complete inhibition of cutinase with **43** took overnight. The slow inhibition of cutinase by **43** causes most probably

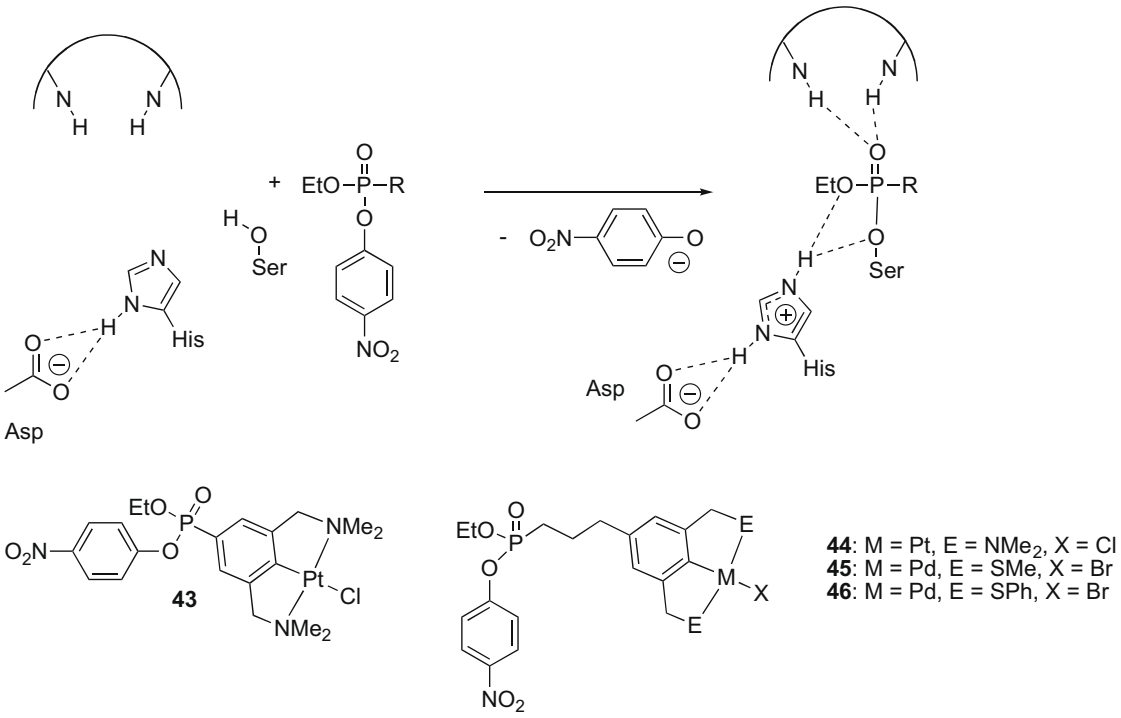


Fig. 9. Irreversible inhibition of a serine hydrolase by a phosphonate inhibitor and the different ECE-pincer metal phosphonate inhibitors developed with a C0- (**43**) and a C3-tether (**44**–**46**) [96,97].

more steric constraints by the bulk of the pincer head group than for **44** and **45** when it approaches the active site of the protein.

Recently, several high resolution protein crystal structures of pincer metal complexes **44** and **45** bound to cutinase were resolved (Fig. 10) [99]. These crystal structures clearly show that the pincer metal unit is situated at the surface of the enzyme and can be accessed by solvent molecules or other ligands.

When cutinase inhibited by **44** was crystallized under halide-poor conditions, a halide-bridged dimeric structure was found (Fig. 10b), where two pincer-platinum ions coordinated to the same μ-bonded chloride anion [99]. These halide-bridged dimeric structures have been observed before for NCN-pincer metal(II) cations in the presence of low chloride anion concentrations [100,101]. These results show that coordination chemistry at the protein-embedded metal centre is still possible, even with a protein as a gigantic *para*-substituent.

The crystal structures illustrate unequivocally that the NCN-pincer platinum(II) centre is accessible for coordinating ligands or substrate molecules and that coordination chemistry, like shown for the dimeric structure, is possible. Currently, our group is pursuing this by performing different coordination studies with organic ligands to determine the influence of the protein backbone.

The asymmetric scattering properties of the platinum ion in **44** could also be instrumental to phase the raw protein diffraction data and could serve as a phasing tool for the determination of the final crystal structure [99]. As the incorporation of asymmetric scatterers, *e.g.* selenium atoms, into proteins is often limited to bacterial protein expression systems only [102], the post-translational site-selective modification of proteins with NCN-pincer platinum phosphonate complexes as asymmetric scatterering devices can be a very interesting and powerful approach for phasing raw protein diffraction data. Due to their high stability, *e.g.* unlike selenomethionine and –cysteine [102], NCN-pincer platinum complexes are insensitive to oxidation under physiological

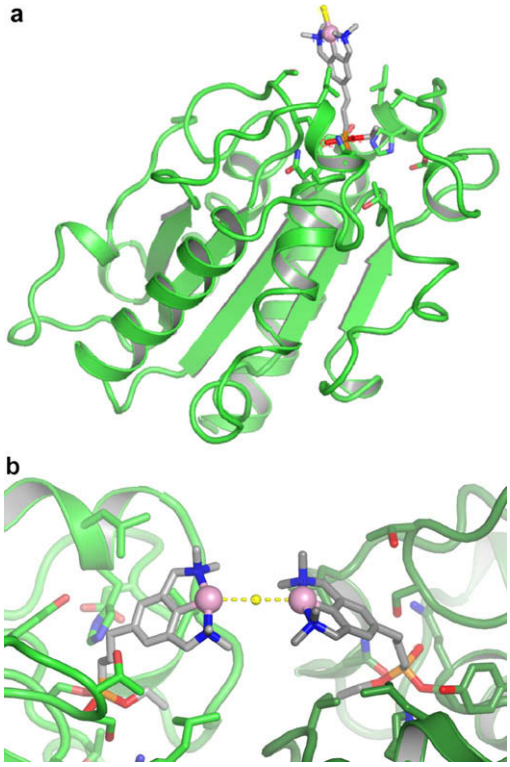


Fig. 10. (a) Crystal structure of the serine hydrolase cutinase inhibited by NCN-pincer platinum phosphonate **44** and (b) halide-bridged dimer formed upon crystallization under halide-poor conditions [99].

conditions, and due to their versatile properties as heavy metal atom labels, NCN-pincer platinum phosphonate complexes can in principle be used for the modification and phasing of very many different serine hydrolases. Due to their specificity, identification

of active site topology is obtained as well. Currently, we are investigating, whether we can use complex **44** as asymmetric scatterer for the phasing of other protein crystal diffraction data and use the pincer metal complex as a structural probe for other serine-hydrolase proteins.

Due to the known catalytic activities of SCS-pincer palladium(II) complexes, different semisynthetic SCS-pincer palladium(II) metalloenzymes with **45** and **46** as phosphonate inhibitor can be used as hydrophilic catalysts. By screening different serine-hydrolases with **45** and **46**, the influence of the different protein backbones on product (enantio)selectivity can be assayed. Currently, we are performing catalytic studies in aqueous media, to investigate the influence of different protein backbones on the selectivity and the activity of the palladium(II) metal centres.

8. Conclusions

The many different examples of the use of ECE-pincer metal complexes in bioorganometallic chemistry, highlighted in this review, show clearly that pincer complexes are versatile building blocks with diverse and broad applicabilities. Moreover, these ECE-pincer metal complexes display exceptional stability and robustness in biological media, which enables their use in the study of biomolecules and biological interactions. Their applications range from anticarcinogenic agents, SPR enhancers, supramolecular assemblies for the generation of new hybrid-biomaterials, peptide biomarkers and catalysts to building blocks for new semisynthetic metalloenzymes. The recent shift of the research focus towards the modification of whole proteins by ECE-pincer metal complexes shows that larger systems can be addressed, which will enable us to study the behaviour of more complex biological systems with metal complexes further and provide us with a useful tool in chemical biology.

References

[1] G. Jaouen, Bioorganometallics: Biomolecules, Labeling, Medicine, Wiley-VCH Verlag, Weinheim, 2005, p. 266.
[2] H.-B. Kraatz, N. Metzler-Nolte, Concepts and Models in Bioinorganic Chemistry, Wiley-VCH Verlag, Weinheim, 2006.
[3] G.E. Atilla-Gokcumen, D.S. Williams, H. Bregman, N. Pagano, E. Meggers, ChemBioChem 7 (2006) 1443–1450.
[4] D.S. Williams, P.J. Carroll, E. Meggers, Inorg. Chem. 46 (2007) 2944–2946.
[5] S.J. Dougan, A. Habtemariam, S.E. McHale, S. Parsons, P.J. Sadler, Proc. Natl. Acad. Sci. USA 105 (2008) 11628–11633.
[6] R.E. Aird, J. Cumminds, A.A. Ritchie, M. Muir, R.E. Morris, H. Chen, P.J. Sadler, D.I. Jodrell, Br. J. Can. 86 (2002) 1652–1657.
[7] P. Pigeon, S. Top, A. Vessieres, M. Huche, E.A. Hillard, E. Salomon, G. Jaouen, J. Med. Chem. 48 (2005) 2814–2821.
[8] E. Allard, C. Passirani, E. Garcion, P. Pigeon, A. Vessieres, G. Jaouen, J.-P. Benoit, J. Control. Release 130 (2008) 146–153.
[9] C.S. Allardyce, P.J. Dyson, Top. Organomet. Chem. 17 (2006) 177–210.
[10] N. Metzler-Nolte, Chimia 61 (2007) 736–741.
[11] R. Alberto, Radiopharmaceuticals, Wiley-VCH Verlag, Weinheim, 2006.
[12] R. Alberto, Top. Curr. Chem. 252 (2005) 1–44.
[13] R.H. Fish, Supramolecular host recognition processes with biological compounds, organometalli pharmaceuticals, and alkali-metal ions as guests, Wiley-VCH Verlag, Weinheim, 2006.
[14] A. Maurer, H.-B. Kraatz, N. Metzler-Nolte, Eur. J. Inorg. Chem. 16 (2005) 3207–3210.
[15] U. Schatzschneider, N. Metzler-Nolte, Angew. Chem., Int. Ed. 45 (2006) 1504–1507.
[16] C.-Z. Li, Y.-T. Long, T. Sutherland, J.S. Lee, H.-B. Kraatz, Front. Biochip Technol. (2006) 274–291.
[17] N. Fischer-Durand, M. Salmain, B. Rudolf, A. Vessieres, J. Zakrzweski, G. Jaouen, ChemBioChem 5 (2004) 519–525.
[18] J.-M. Heldt, N. Fischer-Durand, M. Salmain, A. Vessieres, G. Jaouen, J. Organomet. Chem. 689 (2004) 4775–4782.
[19] K. Severin, R. Bergs, W. Beck, Angew. Chem., Int. Ed. 37 (1998) 1635–1654.
[20] I. Aguirre de Carcer, A. Di Pasquale, A.L. Rheingold, D.M. Heinekey, Inorg. Chem. 45 (2006) 8000–8002.
[21] M.V. Rampersad, S.P. Jeffery, J.H. Reibenspies, C.G. Ortiz, D.J. Darensbourg, M.Y. Darensbourg, Angew. Chem., Int. Ed. 44 (2005) 1217–1220.
[22] K.N. Green, S.P. Jeffery, J.H. Reibenspies, M.Y. Darensbourg, J. Am. Chem. Soc. 128 (2006) 6493–6498.
[23] L. Barisic, M. Dropucic, V. Rapic, H. Pritzkow, S. Kirin, N. Metzler-Nolte, Chem. Commun. (2004) 2004–2005.
[24] L. Barisic, M. Mojca, A. Khaled, Y. Liu, H.-B. Kraatz, H. Pritzkow, S.I. Kirin, N. Metzler-Nolte, V. Rapic, Chem. Eur. J. 12 (2006) 4965–4980.
[25] T. Ueno, T. Koshiyama, M. Ohashi, K. Kondo, M. Kono, A. Suzuki, T. Yamane, Y. Watanabe, J. Am. Chem. Soc. 127 (2005) 6556–6562.
[26] M. Creus, A. Pordea, T. Rossel, A. Sardo, C. Letondor, A. Ivanova, I. LeTrong, R.E. Stenkamp, T.R. Ward, Angew. Chem., Int. Ed. 48 (2008) 1400–1404.
[27] L. Randaccio, S. Geremia, J. Wuerges, J. Organomet. Chem. 693 (2007) 1198–1215.
[28] F. Arnesano, L. Banci, I. Bertini, S. Ciofi-Baffoni, Eur. J. Inorg. Chem. 8 (2004) 1583–1593.
[29] F. Arnesano, L. Banci, I. Bertini, F. Capozzi, S. Ciofi-Baffoni, S. Ciurli, C. Luchinat, S. Mangani, A. Rosato, P. Turano, M.S. Viezzoli, Coord. Chem. Rev. 250 (2006) 1419–1450.
[30] C.S. Allardyce, A. Dorcier, C. Scolaro, P.J. Dyson, Appl. Organomet. Chem. 19 (2005) 1–10.
[31] L. Tebben, K. Bussmann, M. Hegemann, G. Kehr, R. Frohlich, G. Erker, Organometallics 27 (2008) 4269–4272.
[32] G. Gasser, N. Husken, S.D. Koster, N. Metzler-Nolte, Chem. Commun. (2008) 3675–3677.
[33] M. Albrecht, G. van Koten, Angew. Chem., Int. Ed. 40 (2001) 3750–3781.
[34] M.Q. Slagt, G. Rodriguez, M.M.P. Grutters, R.J.M. Klein Gebbink, W. Klopper, L.W. Jenneskens, M. Lutz, A.L. Spek, G. van Koten, Chem. Eur. J. 10 (2004) 1331–1344.
[35] S.-E. Stiriba, M.Q. Slagt, H. Kautz, R.J.M. Klein Gebbink, R. Thomann, H. Frey, G. van Koten, Chem. Eur. J. 10 (2004) 1267–1273.
[36] M.Q. Slagt, S.-E. Stiriba, R.J.M. Klein Gebbink, H. Kautz, H. Frey, G. van Koten, Macromolecules 35 (2002) 5734–5737.
[37] A.W. Kleij, R.A. Gossage, J.T.B.H. Jastrzebski, J. Boersma, G. van Koten, Angew. Chem., Int. Ed. 39 (2000) 176–178.
[38] N.C. Mehendale, J.R.A. Sietsma, K.P. de Jong, C.A. van Walree, R.J.M. Klein Gebbink, G. van Koten, Adv. Synth. Catal. 349 (2007) 2619–2630.
[39] G. Jaouen, S. Top, A. Vessieres, R. Alberto, J. Organomet. Chem. 600 (2000) 23–26.
[40] S. Top, B. Dauer, J. Vaissermann, G. Jaouen, J. Organomet. Chem. 541 (1997) 355–361.
[41] S. Top, J. Tang, A. Vessieres, D. Carrez, C. Provot, G. Jaouen, Chem. Commun. (1996) 955–956.
[42] S. Top, A. Vessieres, G. Leclercq, J. Quivy, J. Tang, J. Vaissermann, M. Huche, G. Jaouen, Chem. Eur. J. 9 (2003) 5223–5236.
[43] A. Vessieres, S. Top, W. Beck, E. Hillard, G. Jaouen, J. Chem. Soc., Dalton Trans. (2006) 529–541.
[44] S. Top, E.B. Kaloun, A. Vessieres, G. Leclercq, I. Laios, M. Ourevitch, C. Deuschel, M.J. McGlinchey, G. Jaouen, ChemBioChem 4 (2003) 754–761.
[45] As Presented at the IVth International Symposium on Bioorganometallic Chemistry, 2008.
[46] G.D. Batema, T.J. Korstanje, G. Guillena, G. Rodriguez, M. Lutz, H. Kooijman, A.L. Spek, G.P.M. van Klink, G. van Koten (in preparation).
[47] The cytotoxic studies are currently performed.
[48] R.J. Green, R.A. Frazier, K.M. Shakesheff, M.C. Davies, C.J. Roberts, S.J.B. Tendler, Biomaterials 21 (2000) 1823–1835.
[49] R.L. Rich, D.G. Myszka, Curr. Opin. Biotechnol. 11 (2000) 54–61.
[50] R.L. Rich, D.G. Myszka, J. Mol. Recognit. 16 (2003) 351–382.
[51] R.L. Rich, D.G. Myszka, J. Mol. Recognit. 15 (2002) 352–376.
[52] R.L. Rich, D.G. Myszka, J. Mol. Recognit. 14 (2001) 273–294.
[53] R.L. Rich, D.G. Myszka, J. Mol. Recognit. 13 (2000) 388–407.
[54] M.E. Lopper, J.L. Keck, Handbook Prot. 2 (2007) 1259–1265.
[55] W. Huber, F. Mueller, Curr. Pharm. Des. 12 (2006) 3999–4021.
[56] D. Beccati, K.M. Halkes, G.D. Batema, G. Guillena, A. Carvalho de Souza, G. van Koten, J.P. Kamerling, ChemBioChem 6 (2005) 1196–1203.
[57] W.W. Gerhardt, M. Weck, J. Org. Chem. 71 (2006) 6333–6341.
[58] H.-J. van Manen, R.H. Fokkens, F.C.J.M. van Veggel, D.N. Reinhoudt, Eur. J. Org. Chem. (2002) 3189–3197.
[59] H. Jude, J.A. Krause Bauer, W.B. Connick, Inorg. Chem. 43 (2004) 725–733.
[60] J. Hall, S.J. Loeb, G.K.H. Shimizu, G.P.A. Yap, Angew. Chem., Int. Ed. 37 (1998) 121–123.
[61] H. Jude, J.A. Krause Bauer, W.B. Connick, J. Am. Chem. Soc. 125 (2003) 3446–3447.
[62] C.R. South, M. Weck, Langmuir 24 (2008) 7506–7511.
[63] W.W. Gerhardt, A.J. Zucchero, J.N. Wilson, C.R. South, U.H.F. Bunz, M. Weck, Chem. Commun. 20 (2006) 2141–2143.
[64] H.-J. van Manen, T. Auletta, B. Dordi, H. Schonherr, G.J. Vancso, F.C.J.M. van Veggel, D.N. Reinhoudt, Adv. Funct. Mater. 12 (2002) 811–818.
[65] H.-J. van Manen, R.H. Fokkens, N.M.M. Nibbering, F.C.J.M. van Veggel, D.N. Reinhoudt, J. Org. Chem. 66 (2001) 4643–4650.
[66] H.-J. van Manen, K. Nakashima, S. Shinkai, H. Kooijman, A.L. Spek, F.C.J.M. van Veggel, D.N. Reinhoudt, Eur. J. Inorg. Chem. 12 (2000) 2533–2540.
[67] W.T.S. Huck, L.J. Prins, R.H. Fokkens, N.M.M. Nibbering, F.C.J.M. van Veggel, D.N. Reinhoudt, J. Am. Chem. Soc. 120 (1998) 6240–6246.
[68] B.M.J.M. Suijkerbuijk, B.N.H. Aerts, H.P. Dijkstra, M. Lutz, A.L. Spek, G. van Koten, R.J.M. Klein Gebbink, Dalton Trans. 13 (2007) 1273–1276.
[69] A.V. Chuchuryukin, P.A. Chase, A.M. Mills, M. Lutz, A.L. Spek, G.P.M. van Klink, G. van Koten, Inorg. Chem. 45 (2006) 2045–2054.

[70] C.H.M. Amijs, A. Berger, F. Soulimani, T. Visser, G.P.M. van Klink, M. Lutz, A.L. Spek, G. van Koten, Inorg. Chem. 44 (2005) 6567–6578.
[71] A.V. Chuchuryukin, H.P. Dijkstra, R.J.M. Klein Gebbink, G.P.M. van Klink, G. van Koten, Polym. Prepr. (Am. Chem. Soc. Div. Polym. Chem.) 45 (2004) 343–344.
[72] A.V. Chuchuryukin, H.P. Dijkstra, B.M.J.M. Suijkerbuijk, R.J.M. Klein Gebbink, G.P.M. van Klink, A.M. Mills, A.L. Spek, G. van Koten, Russ. J. Org. Chem. 39 (2003) 422–429.
[73] W.T.S. Huck, F.C.J.M. van Veggel, B.L. Kropman, D.H.A. Blank, E.G. Keim, M.M.A. Smithers, D.N. Reinhoudt, J. Am. Chem. Soc. 117 (1995) 8293–8294.
[74] W.T.S. Huck, B. Snellink-Rueel, F.C.J.M. van Veggel, D.N. Reinhoudt, Organometallics 16 (1997) 4287–4291.
[75] W.T.S. Huck, L.J. Prins, R.H. Fokkens, N.M.M. Nibbering, F.C.J.M. van Veggel, D.N. Reinhoudt, J. Am. Chem. Soc. (1998) 6240–6246.
[76] R.A. Gossage, A.D. Ryabov, A.L. Spek, D.J. Stufkens, J.A.M. van Beek, R. van Eldik, G. van Koten, J. Am. Chem. Soc. 121 (1999) 2488–2497.
[77] M. Albrecht, G. Rodriguez, J. Schoenmaker, G. van Koten, Org. Lett. 2 (2000) 3461–3464.
[78] G. Guillena, G. Rodriguez, M. Albrecht, G. van Koten, Chem. Eur. J. 8 (2002) 5368–5376.
[79] G. Guillena, C.A. Kruithof, M.A. Casado, M.R. Egmond, G. van Koten, J. Organomet. Chem. 668 (2003) 3–7.
[80] M. Albrecht, M. Lutz, A.M.M. Schreurs, E.T.H. Lutz, A.L. Spek, G. van Koten, Dalton Trans. 21 (2000) 3797–3804.
[81] C.M. Okpodu, R.G. Alscher, E.A. Grabau, C.L. Cramer, J. Plant Physiol. 148 (1996) 309–316.
[82] G. Guillena, K.M. Halkes, G. Rodriguez, G.D. Batema, G. van Koten, J.P. Kamerling, Org. Lett. 5 (2003) 2021–2024.
[83] S.J. Greenfield, A. Agarkov, S.R. Gilbertson, Org. Lett. 5 (2003) 3069–3072.
[84] A. Agarkov, S.J. Greenfield, T. Ohishi, S.E. Collibee, S.R. Gilbertson, J. Org. Chem. 69 (2004) 8077–8085.
[85] A. Agarkov, S.J. Greenfield, D. Xie, R. Pawlick, G. Starkey, S.R. Gilbertson, Biopolymers 84 (2006) 48–73.
[86] J.M. Benito, C.A. Christensen, M.A. Meldal, Org. Lett. 7 (2005) 581–584.
[87] C.A. Christensen, M.A. Meldal, Chem. Eur. J. 11 (2005) 4121–4131.
[88] G. Guillena, G. Rodriguez, G. van Koten, Tetrahedron Lett. 43 (2002) 3895–3898.
[89] M.E. Wilson, G.M. Whitesides, J. Am. Chem. Soc. 100 (1978) 306–307.
[90] C. Letondor, N. Humbert, T.R. Ward, Proc. Natl. Acad. Sci. USA 102 (2005) 4683–4687.
[91] J. Pierron, C. Malan, M. Creus, J. Gradinaru, I. Hafner, A. Ivanova, A. Sardo, T.R. Ward, Angew. Chem., Int. Ed. 47 (2008) 701–705.
[92] J. Steinreiber, T.R. Ward, Coord. Chem. Rev. 252 (2008) 751–766.
[93] A. Pordea, M. Creus, J. Panek, C. Duboc, D. Mathis, M. Novic, T.R. Ward, J. Am. Chem. Soc. 130 (2008) 8085–8088.
[94] Y. Satake, S. Abe, S. Okazaki, N. Ban, T. Hikage, T. Ueno, H. Nakajima, A. Suzuki, T. Yamane, H. Nishiyama, Y. Watanabe, Organometallics 26 (2007) 4904–4908.
[95] H.P. Dijkstra, H. Sprong, B.N.H. Aerts, C.A. Kruithof, M.R. Egmond, R.J.M. Klein Gebbink, Org. Biomol. Chem. 6 (2008) 523–531.
[96] C.A. Kruithof, H.P. Dijkstra, M. Lutz, A.L. Spek, R.J.M. Klein Gebbink, G. van Koten, Eur. J. Inorg. Chem. (2008) 4425–4432.
[97] C.A. Kruithof, M.A. Casado, G. Guillena, M.R. Egmond, A. van der Kerk-van Hoof, A.J.R. Heck, R.J.M. Klein Gebbink, G. van Koten, Chem. Eur. J. 11 (2005) 6869–6877.
[98] M.T. Reetz, A. Rentzsch, M. Pletsch, M. Maywald, Chimia 56 (2002) 721–723.
[99] L. Rutten, B. Wieczorek, J.-P.B.A. Mannie, C.A. Kruithof, H.P. Dijkstra, M.R. Egmond, M. Lutz, R.J.M. Klein Gebbink, P. Gros, G. van Koten, Chem. Eur. J. 15 (in press).
[100] J. Terheijden, G. van Koten, D.M. Grove, K. Vrieze, J. Chem. Soc., Dalton Trans. (1987) 1359–1366.
[101] J. Van den Broeke, J.J.H. Heeringa, A.V. Chuchuryukin, H. Kooijman, A.M. Mills, A.L. Spek, J.H. Van Lenthe, P.J.A. Ruttink, B.-J. Deelman, G. van Koten, Organometallics 23 (2004) 2287–2294.
[102] S.E. Ealick, Curr. Opin. Chem. Biol. 4 (2000) 495–499.