

VIP Very Important Paper

Synthetic Approaches towards Peptide-Conjugates of Pt(II) Compounds with an (O,S) Chelating Moiety

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Metal-containing peptide (bio-)conjugates have received continuous interest due to their enormous potential for bioinorganic and medicinal research. In many bioconjugates the chemical inertness of the metal-containing units facilitates synthesis e.g. by copper-catalyzed alkyne-azide cycloaddition or the formation of peptide bonds. However, when the metal complex contains labile ligands, which are often critical to their biological activity, the synthetic proceeding requires careful planning. Here, we report on the synthesis of a set of peptide bioconjugates with a platinum(II) core, coordinated through widely variable (O,S) chelating β -hydroxydithiocinnamic ester and two monodentate ligands. We have evaluated the synthetic applicability of metal-peptide bioconjugation techniques between the model peptide Leu⁵-enkephalin and differently functionalized (O,S)Pt units. Within this, the type and position of anchor used at the β -hydroxydithiocinnamic unit proved to be crucial for success, but equally important was the synthetic order of conjugation and complexation. In this work, synthetic approaches for the linkage of metal complexes that are coordinated by functionalized ligands towards peptides were explored. Two general methods to link the studied (O,S)Pt

pharmacophore to the model peptide, Leu⁵-enkephalin, have been applied, namely the most prominent "click" reaction, CuAAC, and the linking *via* amide bonds. Overall, it could be shown that the structural motif of the (O,S)Pt compounds presented here offers multiple possibilities of derivatization, so that bioconjugation towards peptides can be made possible. Depending on the demands made on the resulting metal bioconjugate, both the dithioester and the aromatic unit of β -hydroxydithiocinnamic esters can be used as anchor for peptides. However, from our results here, it can be concluded that anchoring at the aromatic subsite seems preferable from a synthetic point of view as the spatial distance of the reactive terminal functional group (alkyne or azide) to the metal center may avoid intramolecular reactions. Also, for using azide-alkyne click chemistry as a conjugation strategy, the (O,S) unit should contain the azide function and not the alkyne function, as triple bond hydration may occur. Classical amide-bond conjugation also proved to be a suitable method in our hands when performed in solution. A coupling of the β -hydroxydithiocinnamic unit to resin-bound peptide would not be possible due to the typically harsh cleavage conditions.

Introduction

Metal-peptide bioconjugates have gained considerable interest over the last decade. Especially in the field of targeted drugs, the combination of different units, such as a metal-containing

pharmacophore and a targeting peptide unit, is an elegant approach to achieve tissue-specific bioactive compounds. In our ongoing interest in transition metal coordination chemistry based on sulfur-containing ligands, the structural motif of β -hydroxydithiocinnamic esters has proven to be a versatile

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starting point for multifaceted derivatizations.^[1] This substance class bears several moieties which can, in principle, easily be modified to fine-tune its properties and to allow the conjugation to diverse biomolecules. In this respect, their aromatic and aliphatic subunits have been modified to a reasonable extent, enabling e.g. the production of defined polymer materials.^[2] Incorporation of functional groups that would allow for the formation of metal complexes as bioconjugates has to date not been reported, but represents a desirable aim. Such bioconjugates could improve e.g. the compounds' selectivity towards certain biological targets through specific receptor targeting. They could also lead to "chimeric" compounds that bear several biologically relevant functionalities for complex and directed pharmacological applications.^[3]

A plethora of methods and techniques are used to form bioconjugates.^[4] Metal-complex bioconjugates resemble a small, yet exciting, portion of this research, and are explored for different biological/medical applications.^[5] Especially metal–peptide bioconjugates have emerged as promising research focus.^[6] A number of recent review articles cover the formation of peptide conjugates with organometallic^[7] or coordination compounds.^[8] Others have focused on particular elements or compound classes such as ferrocene,^[9] Au,^[10] Re,^[11] or Pt.^[12] Applications addressed were antimicrobial activity,^[13] anticancer activity,^[7c,8a,14] or theragnostic applications.^[15] A recent trend goes to organelle-specific targeting.^[16]

The first Pt(II)–peptide bioconjugate was reported by Reedijk, van Boom, and coworkers in the year 2000. Here, a tripeptide was prepared, tethered to ethylenediamine and coordinated towards a Pt^{II}Cl₂ unit under solid phase conditions.^[17] Also a dinuclear Pt(II) complex with two *trans*-(NH₃)₂PtCl moieties, bound in monodentate fashion to a short peptide, has been reported by the same groups.^[18] Other researchers obtained Pt(II) conjugates with octreotide derivatives from a solid-phase approach.^[19] An organometallic variant of a Pt–peptide bioconjugate was prepared by using a (*N,C,N*)-binding pincer ligand which was readily attached to different peptide sequences.^[20] In two independent reports, Pt(II) peptide bioconjugates were described utilizing a malonate-based ligand and a long spacer to connect the coordination site with a peptide.^[21] In addition, the variability of the axial position in Pt(IV) complexes was exploited by several research groups, including ours.^[22] It was thus only a logical consequence to use the well-established structural motif of β -hydroxydithiocinnamic esters available from our previous studies^[1a,b] and strive to combine (*O,S*) chelating Pt(II) complexes with peptides.

Enkephalins are known neurotransmitters and ligands for opioid receptors.^[23] Starting with the first organometallic Mo conjugate in 2002,^[24] the pentapeptides are proven model peptides for the preparation of bioconjugates, and good representatives for subsequent conjugation experiments with further, larger, peptides.^[6a,22a,23c,24,25] On this basis, Leu⁵–Enk was chosen to establish suitable reaction conditions to form (*O,S*)Pt-derived peptide–bioconjugates with β -hydroxydithiocinnamic esters as Pt-coordinating unit.

One approach to obtain metal complex-peptide bioconjugates which has frequently found application is the binding of

suitable metal units *via* amide bonds.^[7b] In most reported cases, the metal unit carries a carboxylic group which can then be coupled *N*-terminally to the desired peptide unit. In this context, both solid-phase coupling during peptide synthesis and solution-phase coupling after the entire peptide synthesis are viable options that have been used previously. Coupling in solid phase or in solution can in principle occur by the same methods,^[26] but it is generally considered that coupling in solid phase is more efficient and leads to less unwanted side reactions.^[27] However, deprotection and cleavage from resin usually require harsh conditions (e.g. high concentrations of trifluoroacetic acid, TFA) which some target compounds, especially metal complexes with highly functionalized ligands, might not subsist. Hence, for coupling in solid phase, it is advantageous if the ligand, whether metal-bound or free, can withstand deprotection/cleavage conditions without undergoing decomposition. Alternatively, if coupled after deprotection and/or cleavage from the resin, it should remain unreactive towards other functional groups within the peptide. With these considerations in mind, introducing a carboxylic group into the overall structure of the β -hydroxydithiocinnamic ester was the first tactic to enable its coupling towards the model peptide Enk *via* amide bonds.

For a second synthetic approach, the use of "click" chemistry as a versatile and bioorthogonal method was considered. The prototype of classical "click" chemistry, Huisgen-type 1–3 dipolar azide–alkyne cycloaddition,^[28] has become widely applied for bioconjugate formation in its Cu(I) mediated application scheme over the last decades (Cu-catalyzed azide alkyne coupling, CuAAC).^[29] Azides and alkynes are both stable towards a variety of reaction conditions and at the same time can conveniently be linked to chemoselectively form 1,4-disubstituted 1,2,3-triazoles under mild conditions. This allows even the modification of peptides with diverse unprotected functional groups. The resulting 1,2,3-triazoles have found wide-spread applications, e.g. as pharmacophores,^[30] bioisosteres to peptide bonds^[31] or as linking function of building blocks.^[29c] The second synthetic tactic was thus the complementary incorporation of both functional groups, azides and alkynes, into the (*O,S*)Pt unit and Enk to finally enable bioconjugation via CuAAC.

In this study, we compare the systematically different possibilities for linking peptides to Pt(II) complexes with β -hydroxydithiocinnamic ester ligands with view on synthetic hurdles and side reactions.

Results and Discussion

Introduction of a carboxy group

The synthesis of β -hydroxydithiocinnamic esters as ligands for metal coordination has been described on the basis of several methods^[1a,32] and has already led to a plethora of structural features that could be incorporated into the system.

To introduce a carboxy group into the metal-coordinating unit, 4-bromo acetophenone **1** was converted with CS₂ and

bromoacetic acid to form the β -carboxyethane derived β -hydroxy-dithiocinnamic ester **2** in good yields (Scheme 1). The successful synthesis of this compound was proven by standard analytical methods as well as by X-ray crystallographic data, showing **2** in a catamer-type^[33] array through intermolecular hydrogen bonds of the molecules' carboxy groups ($d(\text{O} \cdots \text{O}') = 2.675 \text{ \AA}$), leading to band-like secondary structures in the unit cell (Figure 1). The molecule is flat, with only the carboxy function protruding from the molecular plane. The stabilization of the *cis*-conformation in the (O,S) unit through intramolecular hydrogen bonding is clearly demonstrated by the structural data.

The respective monofunctional (O,S) complex **3** (Scheme 1) was successfully prepared in 70% yield. Analytical data of the isolated product proved the selective coordination of the ligand *via* the (O,S) unit and not through oxygen atoms of the carboxy function. NMR spectroscopic and mass spectrometric data showed the chlorido and DMSO ligands to be bound according to the desired binding mode, *i.e.* DMSO binding through the sulfur atom in *cis* position to the dithioester sulfur donor.

Alkyne-functionalized β -hydroxydithiocinnamic esters and their Pt(II) complexes

Terminal alkynes can in principle be introduced either at the aromatic subunit or the dithioester moiety of β -hydroxy-dithiocinnamic esters. Both structural modifications were performed in order to explore the best synthetic approach (Scheme 2).

Compound **5** was prepared in good yields (81%) by Williamson-ether synthesis, using 4-hydroxyacetophenone **4** and propargyl bromide as starting materials and potassium carbonate as base.^[34] Elongating the spacer by one methylene group proved to be unsuccessful, possibly due to elimination

processes within the butynyl bromide caused by activation of the β -CH₂ group (data not shown). A pentynyl group, however, could be introduced by usage of Cs₂CO₃ and KI as base/catalyst pair^[35] in an excellent yield of 92%.

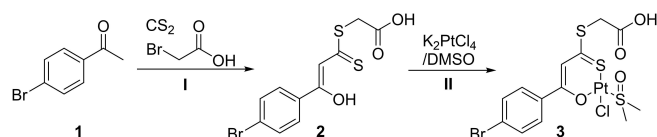
The alkynyl-functionalized acetophenones **5** and **6** were then converted into β -hydroxydithiocinnamic esters by using an established one-pot approach which relies on the acidity of the α -methyl group.^[32] As apparently, the terminal alkyne group is acidic enough to compete for deprotonation, excess base (potassium *tert*-butoxide, KO^tBu) was required to obtain reasonable yields (**7**: 36% with 3 equiv. vs. 14% with 2 equiv. KO^tBu). The acetophenone derivative **6** was also successfully converted to the respective β -hydroxydithiocinnamic ester **8** in 42% yield, using 2.5 equiv. of KO^tBu and ethyl iodide as alkylating agent.

To introduce an alkyne functionality at the dithioester moiety, a two-step reaction has been reported.^[36] Following this procedure, **10** was produced in overall yield of 24% in two steps (**56**: 56% yield, **10**: 43% yield). Attempts to isolate **10** through the one-pot method was not successful, mainly due to the occurrence of rearrangement processes (cf. supplemental material). On the contrary, no rearrangement or other significant side reactions were observed in the preparation of **11** *via* the one-pot synthetic procedure from 4-bromo acetophenone **1** (22% yield).

The obtained alkyne-functionalized compounds **7**, **8**, **10**, **11** were subsequently used to prepare the respective (O,S) chelate complexes (Scheme 2). To activate the β -hydroxydithiocinnamic esters for coordination, deprotonation is necessary. Opposed to earlier methods, which relied on sodium hydride,^[1] sodium acetate (NaOAc) proved to be the base of choice here since it avoids the deprotonation of the terminal alkyne. Owing to the remarkable stability of the (O,S)Pt chelate unit, dark-red bischelates **12**, **13** and **14** were readily obtained in moderate yields (**12**: 11%, **13**: 28% **14**: 12% yield).

Using a slight molar excess (1.1 equiv.) of K₂PtCl₄, compounds **8** and **11** were used to produce monochelate (O,S)Pt(II) complexes **15** and **16**. Interestingly, spectroscopic data of the resulting complexes **15** and **16** showed the presence of a methyl ketone function due to hydration of the terminal triple bond. Platinum-catalyzed alkyne hydration has been known since the early 1960's^[37] and described in detail with Zeise's dimer during the 1990's.^[38] Today, a great variety of alkyne-hydrating catalysts from platinum or other metals are well-documented for their efficient and selective hydration of both terminal and internal alkynes.^[39] Such hydration effects were not observed when preparing the homologous bischelate complexes. Neither in presence nor in absence of DMSO, to rule out any influence from that additive, NMR spectroscopic data of the crude material gave evidence for even partial hydration of the alkyne functionality. Obviously, the fast formation of the inert bischelate structure efficiently eliminates catalytically active Pt(II) species from the reaction mixture.

It was not possible to produce any (O,S)Pt complex of propargyl-containing **10**, the monochelate complex of **7** could also not be isolated. Decomposition and rearrangement processes, probably induced by the highly reactive propargylic group, seem to account for these results and deemed the



Scheme 1. Preparation of compounds **2** and **3** with a carboxylic group at the dithioester unit. Reaction conditions: I [i] 2 equiv. KO^tBu, 1.4 equiv. CS₂, Et₂O, –78 °C to r.t., 4 h, [ii] 0.9 equiv. bromoacetic acid, r.t. 15 h, [iii] H⁺/H₂O. II, 1.1 equiv. K₂PtCl₄/DMSO, THF/H₂O, r.t.

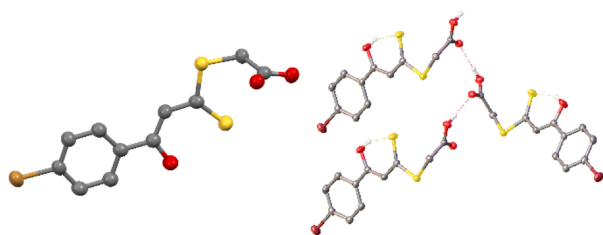
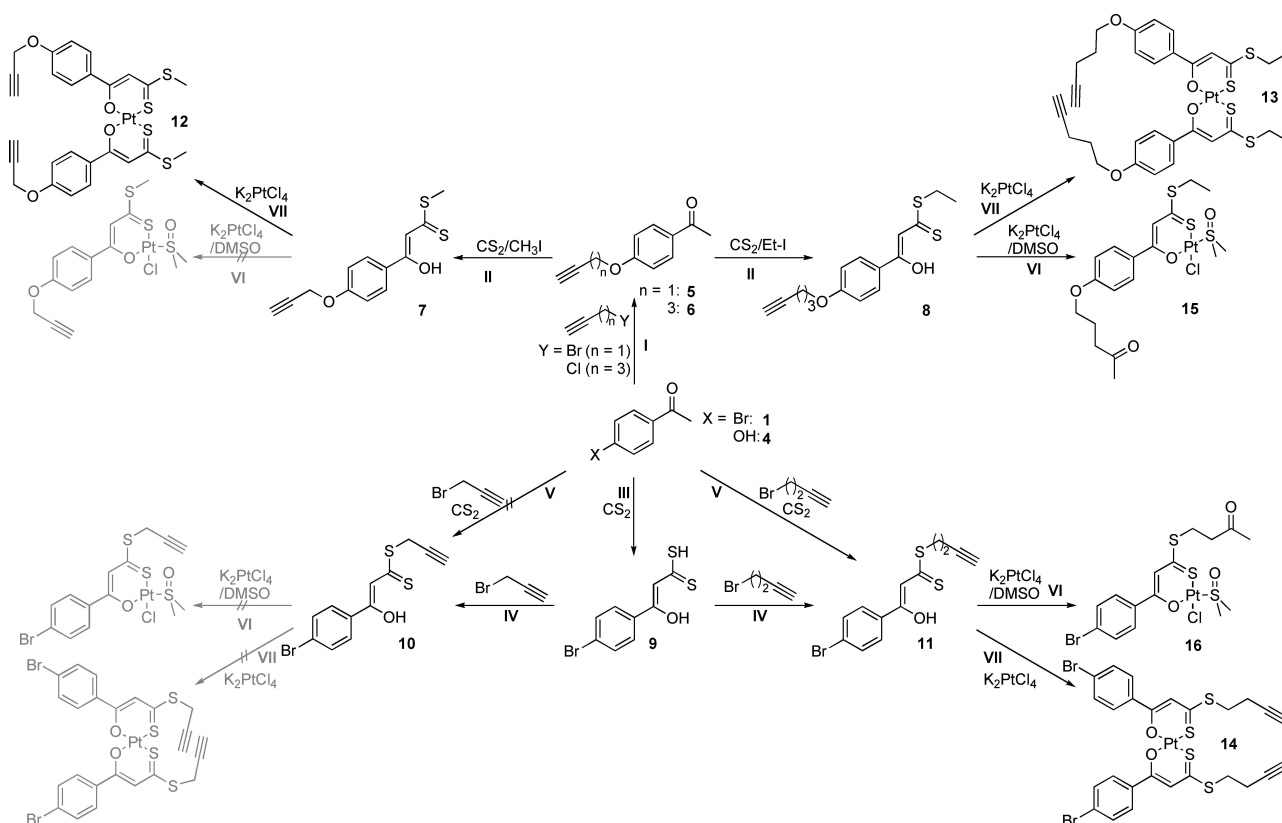


Figure 1. Molecular structure (left) and catamer-type arrangement (right) in single crystals of carboxy-derived compound **2**. The structure is depicted in ORTEP thermal ellipsoids at 50% probability level, all (top)/non H-bonding (bottom) hydrogen atoms were omitted for clarity.



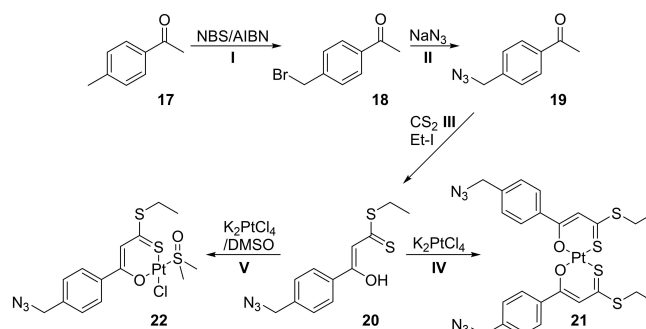
Scheme 2. Synthesis of β -hydroxydithiocinnamic esters with a terminal alkyne at the aromatic (7, 8) or dithio subsite (10, 11) via functionalized acetophenones as well as coordination reactions towards mono- and bisfunctional Pt(II) complexes. Reaction conditions: I, 1.5 equiv. base (K_2CO_3 or CS_2CO_3/KI), 1.1 equiv. alkynyl halide (propargyl bromide or pentynyl chloride), acetonitrile, reflux, 20 h. II, [i] 2.5–3 equiv. KO^tBu , 1.4 equiv. CS_2 , $-78^\circ C$ to r.t., 4 h, [ii] 1 equiv. alkyl iodide, r.t. 15 h, [iii] H^+/H_2O . III, [i] 2 equiv. KO^tBu , 1.4 equiv. CS_2 , Et_2O , $-78^\circ C$ to r.t., 15 h [ii] H^+/H_2O . IV, 1 equiv. nBu_4NOH , 1 equiv. alkynyl bromide, $CHCl_3$, r.t., 2 h. V, [i] 2 equiv. KO^tBu , 1.4 equiv. CS_2 , $-78^\circ C$ to r.t., 4 h, [ii] 0.9 equiv. alkynyl bromide, r.t. 15 h, [iii] H^+/H_2O . VI [i] $NaOAc \cdot 3 H_2O$, [ii] 0.5 equiv. K_2PtCl_4 , THF/ H_2O , r.t. VII [i] base ($NaOAc \cdot 3 H_2O$ or NaH), [ii] 1.1 equiv. K_2PtCl_4 /DMSO, THF/ H_2O , r.t.

propargyl-containing β -hydroxydithiocinnamic esters unsuitable for further synthetic processes.

Azide-containing β -hydroxydithiocinnamic esters and their Pt(II) complexes

In parallel, the preparation of a β -hydroxydithiocinnamic ester with an azide group at the aromatic subsite was attempted (Scheme 3). Introducing an N_3 group in aryllic^[40] or benzylic^[42] positions is well documented. In this proof-of-principle work, the azidomethyl group was chosen as preferential lead structure since it is expected to least alter the electronic structure of the chelating system (e.g. by mesomeric effects).

The preparation of 4-(azidomethyl) acetophenone **19** in two steps was achieved in good yields from 4-methyl acetophenone **17** via 4-(bromomethyl) acetophenone **18** by modified literature procedures.^[41] It was then used to prepare β -hydroxydithiocinnamic ester derivative **20** in moderate yield (33%) through the one-pot synthetic approach. Preparation of the corresponding Pt(II) complexes **21** and **22** was successful for both mono- and bis-functional complex types. Importantly and in contrast to experiences with alkynyl-derived compounds, this synthetic



Scheme 3. Sequential synthesis of compound **20** with a benzyl-azide functionality and coordination reactions towards platinum. Reaction conditions: I, *N*-bromosuccinimide/azobisisobutyronitrile (NBS/AIBN), acetonitrile, $90^\circ C$, 1.5 h. II, NaN_3 , DMF, $100^\circ C$ to r.t., overnight. III, [i] 2 equiv. KO^tBu , 1.4 equiv. CS_2 , $-78^\circ C$ to r.t., 4 h, [ii] 1 equiv. ethyl iodide, r.t. 15 h, [iii] H^+/H_2O . IV [i] $NaOAc \cdot 3 H_2O$, [ii] 0.5 equiv. K_2PtCl_4 , THF/ H_2O , r.t. V [i] $NaOAc \cdot 3 H_2O$, [ii] 1.1 equiv. K_2PtCl_4 /DMSO, THF/ H_2O , r.t.

route proved to yield the target compounds without rearrangement processes or major impeding side-products.

The monochelate **22** was isolated in an appreciable yield (82%) as orange needles. The molecular structure of **22** was confirmed by single-crystal X-ray analysis (Figure 2, left). In the structural motif, all desired functional groups, especially the

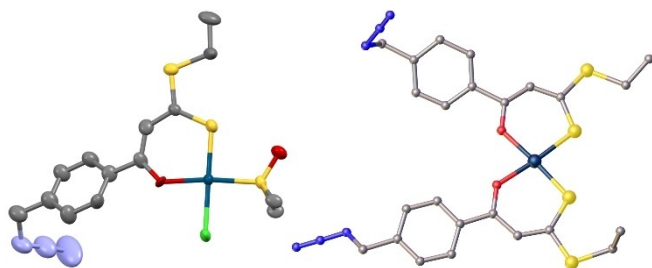


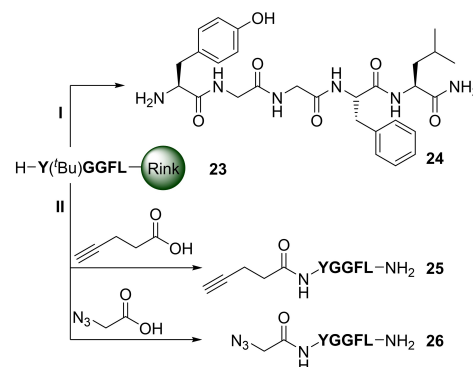
Figure 2. molecular structure of Pt(II) complexes **22** (space group $P2_1/n$, left) and structural motif **21** (space group $P-1$, right) obtained from platination of benzylazide-based compound **20**. The structure of **22** is depicted in thermal ellipsoids at 50% probability level, structural motif **21** is given by ball and stick model. Hydrogen atoms were omitted for clarity.

intact benzyl–azide unit and the monodentate chlorido and DMSO-*S* ligands were found, and the typical square-planar coordination environment clearly demonstrated. Furthermore, the respective bischelate **21** was obtained from the conversion of two equivalents of **20** with K_2PtCl_4 (Figure 2, right). The square-planar coordination environment with the (*O,S*) units in *cis* position and the intact benzyl–azide units are unambiguously shown within the structural motif. For both complexes, twinning effects which could not be overcome, and the flexibility of the azido unit deter a detailed discussion of bond lengths and -angles.

Interestingly, it was noted that addition of DMSO, usually only done for the synthesis of monochelates, leads to significantly increased yields also of the bischelate (*O,S*)Pt complexes: Low yields of ca. 12% **21** were obtained without DMSO, and ca. 70% of **21** could be isolated when K_2PtCl_4 was pre-activated with 2 equiv. of DMSO. Even though the formation of bis-functional (*O,S*)₂Pt complexes is strongly favored overall (which can be seen e.g. by the fact that bischelates are frequently formed as a side product in monochelate syntheses), incomplete conversion alongside with decomposition have been a problem in purposeful bischelate syntheses. DMSO obviously accelerates $PtCl_4^{2-}$ solvolysis and thus leads to neater conversion in subsequent steps.

Preparation and derivatization of Leu⁵–Enkephalin

The model peptide Leu⁵–Enk (H–YGGFL–NH₂) was prepared by Fmoc-based solid phase peptide synthesis (SPPS) on Rink amide resin,^[22a,24,25c] using the TBTU/HOBt/DiPEA activation method (TBTU: *N,N,N',N'*-tetramethyl-*O*-(benzotriazol-1-yl)uronium tetrafluoroborate, HOBt: 1-hydroxybenzotriazole, DiPEA: diisopropylethylamine). In preparation for CuAAC reactions, H–Y(^tBu)GGFL–Rink **23** required suitable derivatization. Before deprotection of the Tyr¹–OH group and cleavage from the resin, **23** was therefore derivatized using either pentynoic acid or azido-acetic acid to introduce a terminal alkyne or azide, respectively (Scheme 4). Both acids were coupled to H–Y(^tBu)GGFL–Rink **23** on solid phase using standard coupling procedures. After deprotection and cleavage from the resin, the functionalized peptides were obtained in good yields (59% and 84% for **25**



Scheme 4. Solid-phase peptide syntheses of Leu⁵–Enk derivatives **24–26**. Reaction conditions: I, TFA/H₂O/TES (95:2.5:2.5 v/v), r.t., 2 h. II, [i] 4 equiv. acid (pentynoic acid or azidoacetic acid), 4 equiv. 1-hydroxybenzotriazole (HOBt), 3.8 equiv. *N,N,N',N'*-tetramethyl-*O*-(benzotriazol-1-yl)uronium tetrafluoroborate (TBTU), 8 equiv. diisopropylethylamine (DiPEA), DMF, r.t., 2 h, [ii] TFA/H₂O/TES (95:2.5:2.5 v/v), r.t., 2 h.

and **26**, respectively) and were used for coupling reactions without further purification.

The identity of the functionalized peptides was established from their ESI MS data, in all cases yielding the $[M+H]^+$ signal. Furthermore, resonance signals in ¹H and ¹³C NMR spectra could, assisted by HSQC and HMBC experiments, unambiguously be identified.

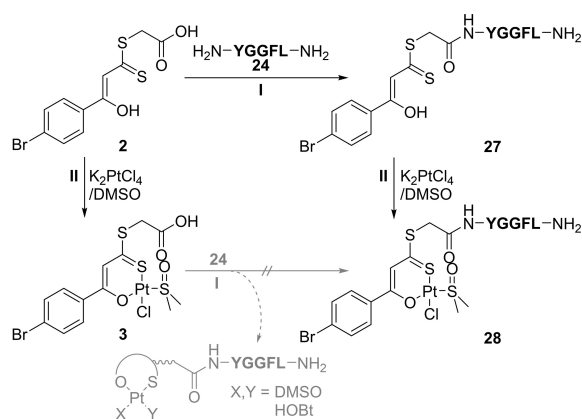
Bioconjugation via formation of amide bonds

To create an (Enk–*O,S*)Pt bioconjugate via amide bonds, both approaches – amide bond formation prior to or after Pt(II) coordination – were explored. In general, coupling in solution with the TBTU/DiPEA method was used.^[42] After 2 h of coupling, compound **27** was isolated in good yields (38%) and purity (> 95%) after preparative *rp*-HPLC purification. Its identity, purity and structure could be established through HPLC, ESI MS and ¹H NMR spectroscopic analysis (*vide infra*).

27 was subsequently converted with K_2PtCl_4 /DMSO by adapting the established protocol for small-molecule complex formation (Scheme 5). By precipitation from water and applying repeated steps of washing with water and diethyl ether, the desired complex **28** was successfully isolated in 52% yield.

Conversely, coupling of the (*O,S*)Pt monochelate **3** in solution was successful in retaining the Pt(II) center within the coordination sphere (Scheme 5). The monodentate DMSO and chlorido ligands were, however, partially substituted and a mixture of different complexes containing DMSO, HOBt^[26] or CH₃CN as monodentate ligands was obtained after HPLC purification. In a control experiment, it was shown that HOBt can in principle substitute both the monodentate chlorido and DMSO ligands (cf. supplementary information).

Compounds **27** and **28** were analyzed by ESI MS analysis and NMR spectroscopy and their identity confirmed. The resulting data can be found in Figure 3 and the supplemental information. Furthermore, ¹H NMR spectra of **27** and **28** in THF-*d*₈ gave all expected signals of Leu⁵–Enk. Interestingly, a high



Scheme 5. Synthetic approaches towards (O,S)Pt bioconjugates through amide bond formation. When subjecting **3** to standard coupling conditions, **28** could not be isolated. Instead, (Enk–O,S)Pt complexes are formed with a mixture of monodentate ligands X/Y. Reaction conditions: I, acid (**2** or **3**)/**24** (1.1:1 equiv.), 1.1 equiv. TBTU, 2.3 equiv. DiPEA, DMF, r.t., 2 h. II [i] NaOAc·3 H₂O, [ii] 1.1 equiv. K₂PtCl₄/DMSO, THF/H₂O, r.t.

selectivity for the desired 1:1 Pt–peptide complex was observed despite competing coordination sites within the peptide moiety.

Amide bond formation of the initially terminal NH₂ group was demonstrated by appearance of a sharp doublet assignable

to the Leu⁵–NH signal at 8.20 ppm in spectra of **27** (a in Figure 3), together with significant shifts of all other NH protons compared to those in **24**. Furthermore, all characteristic signals for the β -hydroxydithiocinnamic unit were found, alongside with a splitting of the S–CH₂ signal at 4.11 ppm into a doublet of doublets (4 in Figure 3). This signal is indicative of the formation of an amide bond in close proximity to this CH₂ group, and of a significant steric crowding, inhibiting rotation and therefore creating diastereotopic effects. Upon binding of **27** towards the Pt(II) ion to give **28**, typical signal shifts characteristic for the (O,S)Pt unit are observed. From HSQC experiments, the presence of Pt-bound DMSO could also be proven from the characteristic ¹J_{CH} cross-peak at 3.63/46.4 ppm despite an overlay with the intrinsic THF-d₈ signal.

Bioconjugates via CuAAC

In initial experiments to form (Enk–O,S)Pt bioconjugates *via* CuAAC, the (O,S)₂Pt bischelate compounds **14** and **21** were used to establish whether a Cu(I)-catalyzed formation of triazole rings would in principle be possible in the presence of the essential functional groups of the (O,S) unit (Scheme 6).

In general, CuAAC was performed as introduced by Sharpless and coworkers, using the combination of copper(II) sulfate (CuSO₄) and sodium ascorbate to generate the Cu(I)

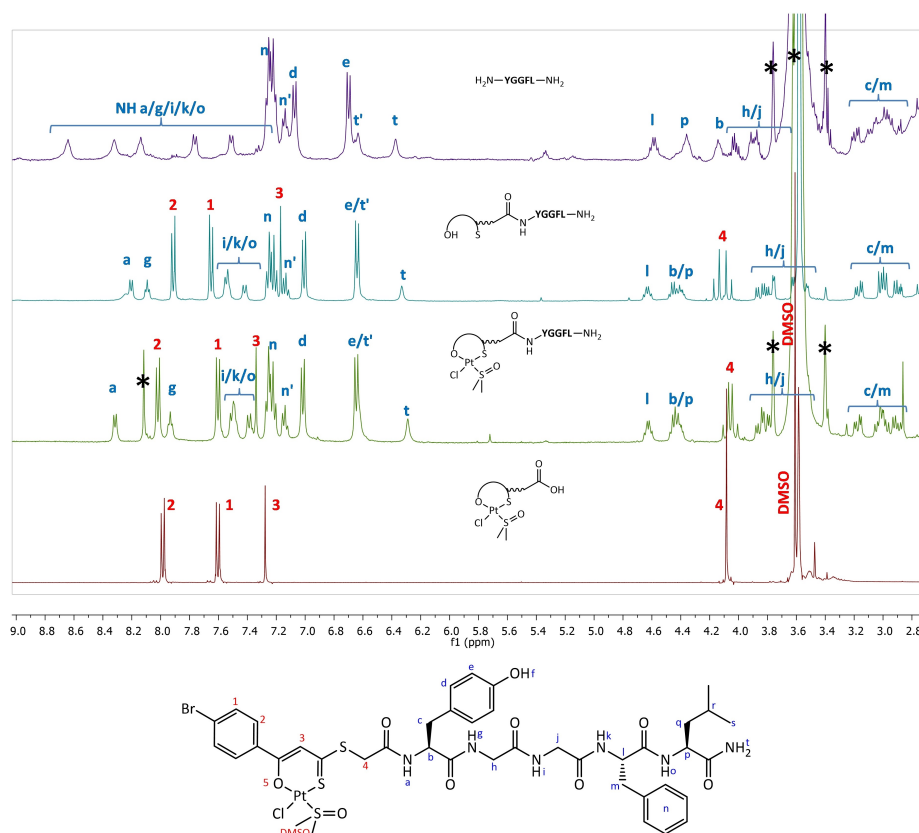
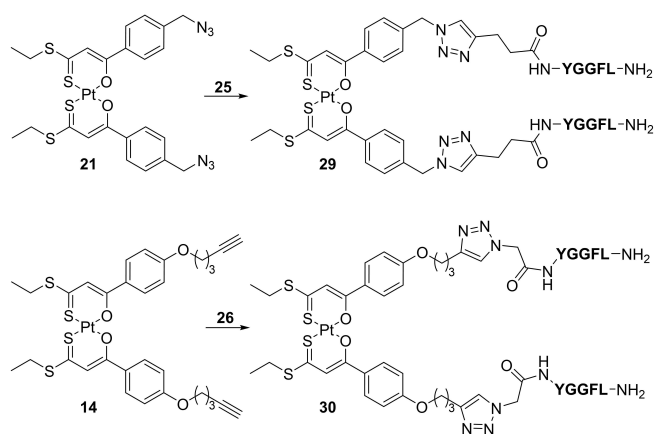


Figure 3. Comparison of ¹H NMR spectra of Leu⁵–Enk **24** (top/violet), ligand **27** (second, petrol), complex **28** (third, green) and complex **3** (bottom, red). Spectral range of 3–9 ppm given, the full spectral range can be found in Figure S4. All spectra were recorded in THF-d₈. Asterisks mark residual signals. Below: representative signal assignment, demonstrated on compound **28**.

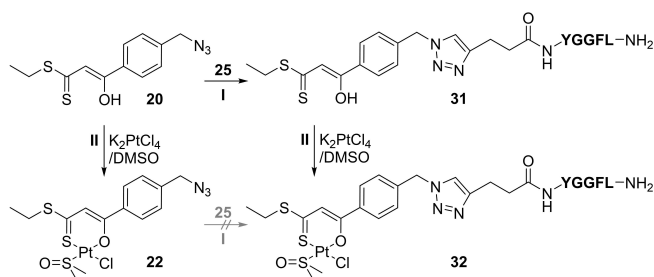


Scheme 6. Bioconjugation of azide and alkyne containing (O,S)₂Pt bischelates 21 and 14 with the complementary Enk derivatives 25 resp. 26 by CuAAC. Reaction conditions: azide/alkyne (1 : 1 molar ratio), 0.2 equiv. CuSO₄·5H₂O, 0.4 equiv. sodium ascorbate, THF/H₂O, r.t.

species *in situ*.^[43] The course of the reaction was monitored by analytical HPLC; conversion was complete after two days of stirring at room temperature. Pure 78 was isolated in 31% yield from coupling of azide-complex 21 and alkyne-Enk 25 and preparative HPLC purification. Furthermore, alkyne-complex 14 and azide-Enk 26 were successfully combined under similar conditions to give pure 30 in 24% yield. The conversion was, however, markedly lower and more side products were formed, as was established from analytical HPLC of the crude material. This combination was excluded from further experiments, primarily because of the aforementioned challenges associated with alkyne-derived (O,S) compounds.

Subsequently, bioconjugation to produce the monofunctional Pt(II) complex 32 was attempted, keeping in mind potential interferences of the Cu(I/II) catalyst (Scheme 7). Indeed, when the monofunctional Pt(II) complex 22 was combined with 25 under influence of CuSO₄ and ascorbate, it was not possible to isolate a pure compound. CuAAC should thus occur prior to Pt(II) complex formation.

When linking alkyne-Enk 25 with the azide-derived ligand 20 through CuAAC in solution, it was necessary to use more than 1 mol-equivalent of CuSO₄ for a successful conversion of the reactants – the usage of catalytical amounts of CuSO₄ did



Scheme 7. Pathways towards (Enk-O,S)Pt complex 32 via CuAAC. Reaction conditions: I, [i] azide/alkyne (1 : 1 molar ratio), 1.2 equiv. CuSO₄·5H₂O, 2.4 equiv. sodium ascorbate, [ii] 2.5 equiv. Na₂EDTA, THF/H₂O, r.t. II [i] NaOAc·3 H₂O, [ii] 1.1 equiv. K₂PtCl₄/DMSO, THF/H₂O, r.t.

not lead to sufficient conversion of the starting materials. Obviously, one equivalent of copper was indeed coordinated towards the (O,S) chelating unit. The starting materials were consumed after two days of stirring, and the addition of excess Na₂EDTA (disodium ethylenediamine tetraacetate) lead to reversal of Cu-(O,S) chelation. The water-soluble Cu-EDTA complex was conveniently removed from the mixture through liquid-liquid extraction. Purification by *rp*-HPLC lead to pure bioconjugated β -hydroxydithiocinnamic ester 31 in appreciable yields (42%). Compound 31 was then successfully converted with K₂PtCl₄ and DMSO. Crude 32 was purified by repeated extraction and precipitation steps and obtained in quantitative yield. According to ESI MS analysis, the presence of monodentate DMSO and chlorido ligands was confirmed.

¹H NMR spectra of 31 and 32 in DMSO-d₆ are shown comparatively in Figure 4 with the starting material 25 as reference. Signal assignment was confirmed through HSQC and HMBC experiments. Upon formation of the triazole by CuAAC, the signals belonging to pentynoic acid in 25 are shifted characteristically for the triazole linker in 31. Upon coordination towards the Pt(II) unit, a typical shift of the methine proton belonging to the (O,S) unit (4 in Figure 4) by ca. +0.05 ppm is witnessed. Even though ESI mass spectral data proved the sum formula to include DMSO and chloride, the signal of Pt-coordinated DMSO is not detected in the ¹H NMR spectrum of 32 due to gradual exchange with its deuterated equivalent.

Discussion

For the preparation of (Enk-O,S)Pt bioconjugates, several synthetic strategies were explored and evaluated for their practical applicability. Besides the general method of conjugation, *i.e.* via amide bonding or CuAAC, the order of preparing the complex, namely by coupling of a functional Pt(II) complex to Enk or conjugation of the β -hydroxydithiocinnamic esters to Enk prior to Pt(II) coordination, were critical variations to the synthetic procedure.

It was demonstrated that a direct conjugation of the (O,S) chelating unit through an amide bond is possible. Carboxy-derived precursor molecules were prepared and both the free 2 and the (O,S)Pt monochelate 3 were coupled to Leu⁵-Enk in solution. Since coupling of 3 under standard TBTU-based coupling conditions resulted in exchange of the chlorido and DMSO ligands by intermediately formed HOBt, the desired (Enk-O,S)Pt(II) complex 28 was prepared by introduction of the Pt center *after* bioconjugation, and appreciably high yields of the monochelate were obtained.

It was furthermore shown that the formation of (Enk-O,S)Pt bioconjugates through CuAAC is in principle possible. Both azide/alkyne combinations were successfully clicked to yield peptide-conjugated (Enk-O,S)₂Pt bischelates 29 and 30. One approach should however be preferred: When introducing the azide group into the (O,S) unit (21) and deriving the peptide with pentynoic acid to give a terminal alkyne (25), far less synthetic obstacles were encountered along the way to compound 29 as opposed to the complimentary combination

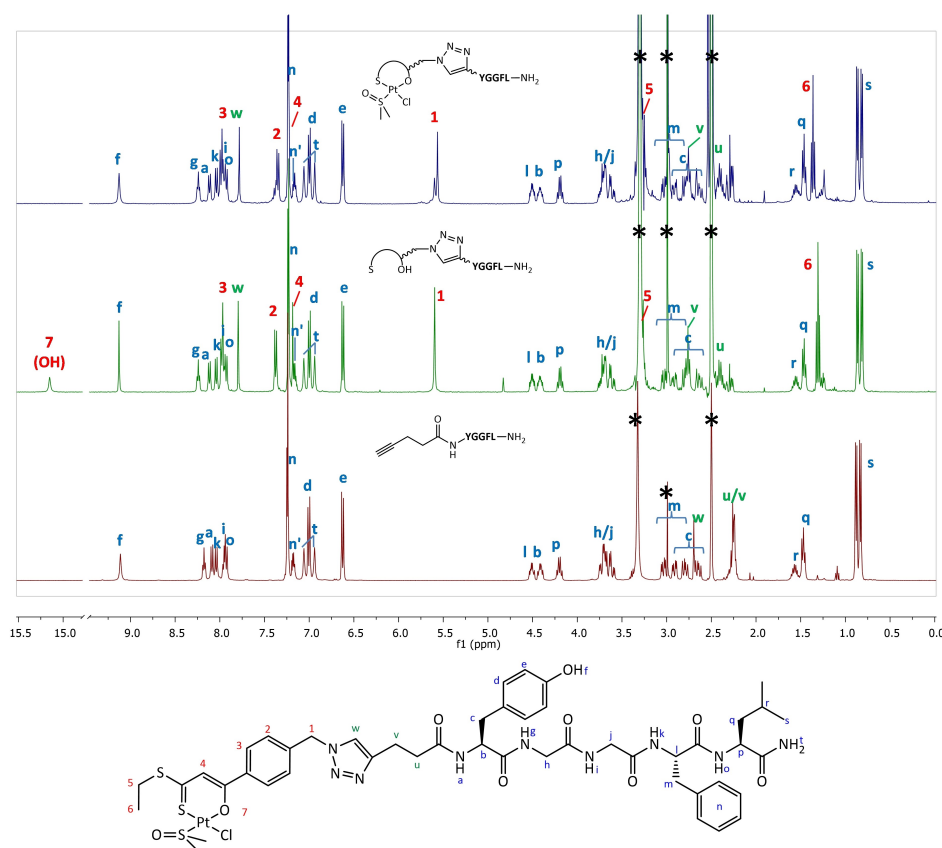


Figure 4. ^1H NMR spectra (400 MHz, DMSO-d_6) of Pt(II) complex **32** (top), the ligand with triazole linker **31** (middle) and the starting material, alkyne-derived Leu⁵–Enk **26** (bottom). The signal of Pt-coordinated DMSO is not witnessed in the spectrum of **32** due to a gradual exchange by DMSO-d_6 .

to give (Enk–O,S)Pt bioconjugate **30**. Alkyne-derived β -hydroxydithiocinnamic esters **7**, **8**, **10**, and **11** could be isolated but coordination towards platinum resulted in a variety of side reactions such as hydrogenation of the $\text{C}\equiv\text{C}$ triple bond or uncontrolled rearrangements. Only bischelates of compounds **7**, **8** and **11** were obtained, no monochelate complex was isolated that still contained the alkynyl group. On the contrary, the synthesis of the benzylazide-derived series **20**, **22** and **21** was straight-forward and yields were generally higher than for the alkyne complex series. CuAAC of the two units **21** and **25** furthermore lead to a cleaner reaction (less side products) and a higher yield of purified bioconjugate **29** than the opposite combination (*i.e.* **14** + **24** to give **30**).

In efforts to obtain the respective (Enk–O,S)Pt monochelate **32**, first CuAAC was performed with the ligand **20** and Enk derivative **25**, followed by quantitative removal of copper through addition of EDTA. Subsequently, the Pt(II) unit could be introduced into the system, and monodentate DMSO and chlorido ligands were retained by purification via precipitation protocols.

All-in-all, this study showed that for our system, it is preferred to first connect the peptide and the (O,S)-unit, followed by its coordination to the metal center. In addition, this work showed a highly selective introduction of a Pt(II) center into a peptide functionalized ligand for the first time.

The results presented here demonstrate that a careful choice of the synthetic strategy is required to obtain metal–bioconjugates with highly functionalized and potentially reactive ligands. We propose that our insights obtained from this work are not restricted to Pt(II) complexes, but are of general applicability to other transition metal complexes with labile ligands. With the proper combination of chelating unit and defined and carefully chosen peptide sequences, such compounds could serve *e.g.* as bioactive pharmacophores with enhanced drug targeting or solubility properties.

Experimental Section

Syntheses: Syntheses were carried out under argon or nitrogen atmosphere using conventional Schlenk techniques when stated in the general procedures, otherwise standard air-exposed methods were used. Most **starting materials and solvents** were purchased from common suppliers (ABCR, Acros, Aldrich, Fluka, Merck, SigmaAldrich, and TCI) and obtained in the highest available purity grade. Solvents were dried according to known procedures^[44] and distilled prior to use when required. For air-exposed syntheses, they were used as received. For column chromatography, silica gel of the type VWR Kieselgel 60 was used. All chemicals and solvents used for **peptide syntheses** were of analytical reagent grade or better and purchased from Novabiochem (Bad Soden, Germany), or Iris Biotech (Marktredwitz, Germany). Dimethyl formamide (DMF) was purchased from Biosolve (Valkenswaard, NL) or Fisher scientific

(Schwerte, D) and of peptide synthesis grade. HPLC-grade acetonitrile was purchased from VWR or Fisher Scientific. Water was purified by a milliQ purification system. A Fmoc–Rink–Amid AM resin^[45] (Iris Biotech Br-1330.0025, in 100–200 mesh) was used with loadings of 0.59 or 0.71 mmol/g. All solid phase syntheses were carried out according to standard solid phase peptide synthesis procedures (SPPS) using the Fmoc protection approach with HOBt/TBTU as coupling reagents.^[42,46] Leu⁵–Enk was synthesized using an automated peptide synthesizer (CEM Liberty microwave assisted peptide synthesizer) in a 250 μ mol scale. Further derivatization was performed manually using plastic syringes with a filter disc made of polypropylene as reaction vessels following standard SPPS procedures.

High Performance Liquid Chromatography: HPLC was performed by using reversed phase (rp) columns of the type Knauer EurospherII 100–5 C18 A (5 μ m, 250 \times 4 mm), for analytical and Dr. Maisch Reprosil–Pur C18–AQ (5 μ m, 250 \times 10 mm) for preparative runs. Eluents consisted of Millipore Water (mQ–H₂O), HPLC-grade acetonitrile (CH₃CN), and trifluoroacetic acid (TFA). Buffer A consisted of 94.95 % mQ–H₂O/4.95 % CH₃CN/0.1 % TFA, Buffer B consisted of 4.95 % mQ–H₂O/94.95 % CH₃CN/0.1 % TFA. All analytical measurements were performed at a flow rate of 1 mL/min, semi-preparative HPLC was performed at a flow rate of 5 mL/min. Linear gradient setups with the following isocratic plateaus were applied: Gradient 1: 0–5 min: 100 % A, 25–30 min: 100 % B, 35–40 min: 100 % A; Gradient 2: 0–5 min: 40 % B, 20–30 min: 100 % B, 35–40 min: 40 % B; Gradient 3: 0–5 min: 100 % A, 45–50 min: 100 % B, 55–60 min: 100 % A; Gradient 4: 0–5 min: 30 % B, 45–50 min: 100 % B, 55–60 min: 30 % B.

Crystallographic data: (excluding structure factors) have been deposited with the Cambridge Crystallographic Data Centre. Experimental details can be found in the provided supplementary material.

4-(Prop-2-ynyloxy)acetophenone **5**^[34] azidoacetic acid^[47] and 4-Bromo- β -hydroxydithiocinnamic acid propargylic ester **10**^[36] were prepared according to literature procedures. The preparation of acetophenone derivatives (**6**, **19**) and other precursors, including full analytical data, are described in detail in the supporting information.

β -Hydroxydithiocinnamic acid esters (2,7,8,10,11**, **20**):** The desired acetophenone derivative (ca. 2 g, 1 equiv.) is dissolved in dry diethyl ether (40 mL) and transferred into a precooled suspension of potassium-*tert*-butoxylate (KO^tBu, 2 equiv.) in dry diethyl ether (40 mL) at –78 °C under inert conditions. Carbon disulfide (CS₂, 1.4 equiv.) is added dropwise under vigorous stirring, the mixture maintained at –78 °C for 3 hours and allowed to warm up to room temperature. After adding the alkyl halide (0.9–1 equiv.), the mixture is stirred for further 15 hours. Subsequently, the suspension is acidified with sulfuric acid (50 mL 2 M aqueous solution), leading to a two-phased system which is then separated. The aqueous phase is extracted with dichloromethane (DCM, 3 \times 5 mL), the combined organic solutions washed with water (3 \times 5 mL), and dried with sodium sulfate. After filtration, the crude product is obtained which is then purified individually.

Monofunctional Pt(II) complexes (3**, **15**, **16**, **22**):** The β -hydroxydithiocinnamic acid ester (1 equiv., dissolved in 30–50 mL THF), is stirred for 30 min with sodium acetate (NaOAc, 1 equiv. in ca. 9 mL THF/water (7:2)). The solution is slowly added to a mixture of potassium tetrachloroplatinate(II) (K₂PtCl₄, 1.1 equiv., typically 200 mg), and dimethyl sulfoxide (DMSO; 2 equiv.) in 2 mL water. The mixture is stirred at room temperature overnight, the resulting red-orange solution concentrated and extracted with DCM (3 \times 4 mL). The combined organic phases are washed with water

(2 \times 3 mL) and dried over sodium sulfate, yielding the crude material after filtration.

Bischelate complexes (12**, **13**, **14**, **21**):** The desired β -hydroxydithiocinnamic acid ester (2 equiv., in 30 mL THF), is deprotonated by addition of NaOAc (1 equiv. in 7 mL THF and 2 mL water). After 30 min of stirring at room temperature, K₂PtCl₄ (1 equiv., typically 100 mg dissolved in 2 mL water) is added and the mixture stirred overnight. The crude product is obtained by evaporation of the solvent *in vacuo*, followed by extraction with DCM (3 \times 4 mL), washing with water (3 \times 3 mL) and drying with sodium sulfate.

Solid phase peptide synthesis (SPPS): In general, peptide synthesis occurred by the same steps irrespective of manual or microwave-assisted coupling (μ W). Before synthesis, the resin is swollen in DMF or DCM for at least 30 min. *N*-terminal Fmoc protection groups are removed by a solution of 20 % piperidine in DMF (μ W: (i) P=30 W, t=35 s, T=38 °C; (ii) P=50 W, t=180 s, T=77 °C). After each deprotection and each coupling step, the resin is washed excessively with DMF to remove any residual activation or deprotection reagents. For coupling of an acid, Fmoc protected amino acids (4 equiv., 0.2 mmol/mL in DMF) resp. functionalized acids (4 equiv., 0.1 mmol/mL in DMF) are mixed with 1-hydroxybenzotriazol (HOBt, 4 equiv. 0.5 M in DMF) and *N,N,N',N'*-Tetramethyl-*O*-(benzotriazol-1-yl)uronium tetrafluoroborate (TBTU, 3.8 equiv. 0.5 M in DMF). Coupling is initiated by addition of diisopropylethylamine (DPEA, 8 equiv. 2 M in DMF). Conditions for manual coupling reactions: shaking at r.t. on a laboratory shaker (350 rpm); for μ W-assisted coupling: P=24 W, t=300 s, T=78 °C. For manual coupling reactions, a Kaiser test is performed to ensure complete conversion of all terminal amines.^[48] After deprotection of the last amino acid, the peptide-loaded resin is transferred into a filter syringe, washed with DMF and DCM (3 \times 5 mL, 1–2 min each) and dried *in vacuo*. When needed, the dried resin is split into smaller portions at this point and manual coupling performed equally as described above. After coupling of all (amino) acids, the peptidyl resin is treated with a mixture of TFA/H₂O/TES (trifluoroacetic acid/water/triethylsilane) (95:2.5:2.5 v/v; 1200 μ L/100 μ M peptide on resin) and shaken on a laboratory shaker at r.t. for approx. 2 h to achieve cleavage from the resin. The cleaved peptide is precipitated by introduction into ca. 80 mL of a 1:1 mixture of *n*-hexane and diethyl ether at –80 °C and the resin washed with a minimum amount of cleavage mixture. The precipitate is centrifuged (8000 rpm, 10 min, r.t.), decanted and washed with diethyl ether (2–3 \times 5–10 mL). When needed, the peptide is purified by HPLC as described for the individual compounds. Purified peptides are lyophilized from H₂O/CH₃CN solutions and obtained as amorphous solids.

Leu⁵–Enkephalin bound to Rink Amide AM Resin (H–Y–(Bu)GGFL–Rink, **23):** Leu⁵–Enkephalin bound to Rink Amide AM Resin **23** was prepared by automated SPPS in a 250 μ mol scale according to the described procedure, using Rink resin with 0.59 or 0.71 mmol/g loading. Fmoc-protected amino acids (Fmoc–Leu–OH, Fmoc–Phe–OH, 2 \times Fmoc–Gly–OH, Fmoc–Tyr(Bu)–OH) were used in 4-fold excess (1 mmol). After drying of the peptidized resin, it was split into two equal portions equivalent to 125 mmol peptide and treated separately for further reactions.

Free Leu⁵–Enkephalin amide (H–Y(Bu)GGFL–NH₂, **24):** One 125 μ mol equivalent of **23** was cleaved (90 min/r.t.) and precipitated from Et₂O/*n*-hexane. Repeated washing and lyophilization according to the described protocol for SPPS yielded Leu⁵–Enk **25** as C-terminal amide (58 mg; 84 %) in > 90 % purity.

Alkyne-functionalized Leu⁵–Enkephalin (25**):** Peptide-loaded rink resin **23**, equivalent to 125 μ mol, was coupled with pentynoic acid (PA, 49 mg, 0.5 mmol) under addition of HOBt/TBTU (4/3.8 eq) and

D'PEA (8 equiv.) at room temperature for 2 h by manual SPPS. After cleavage and precipitation, a white powder of **25** (50 mg; 63% yield) was obtained which was >85% purity according to analyt. HPLC and thus used without further purification.

rp-HPLC (C18, gradient 1): t_R = 16.35 min (>85%).

MS (ESI+): m/z = 635.0 $[M+H]^+$ (calcd. for $C_{33}H_{43}N_6O_7$: 635.3).

1H NMR (400 MHz, DMSO- d_6) δ = 9.11 (s, 1H, Y¹: Ar–OH), 8.18 (t, $^3J_{HH}$ = 5.6 Hz, 1H, G²: –NH–), 8.09 (d, $^3J_{HH}$ = 8.1 Hz, 1H, Y¹: –NH–), 8.04 (d, $^3J_{HH}$ = 8.1 Hz, 1H, F⁴: –NH–), 7.94 (m, 2H, G³: –NH–, L⁵: –NH–), 7.24 (m, 4H, F⁴: Ar–H_{2,3,5,6}), 7.18 (m, 1H, F⁴: Ar–H₄), 7.00 (d, $^3J_{HH}$ = 8.4 Hz, 2H, Y¹: Ar–H_{2,6}), 7.00 (d, $^2J_{HH}$ = 47.3 Hz, 2H, L⁵: –NH₂), 6.63 (d, $^3J_{HH}$ = 8.4 Hz, 2H, Y¹: Ar–H_{3,5}), 4.51 (dt, $^3J_{HH}$ = 8.9, 4.6 Hz, 1H, F⁴: CH^a), 4.41 (dt, $^3J_{HH}$ = 9.0, 4.8 Hz, 1H, Y¹: CH^a) 4.21 (dt, $^3J_{HH}$ = 15.2, 7.9 Hz, 1H, L⁵: CH^a), 3.80–3.52 (m, 4H, G²/G³: –CH₂^a–), 3.04/2.79 (2 m, 2H, F⁴: –CH₂ ^{β} –Ar), 2.91/2.65 (2 m, 2H, Y¹: –CH₂ ^{β} –Ar), 2.70 (t, $^3J_{HH}$ = 2.1 Hz, PA⁰: \equiv CH), 2.31–2.23 (m, 4H, PA⁰: CH₂^{a β}), 1.61–1.52 (m, 1H, L⁵: –CH^aMe₂), 1.47 (m, 2H, L⁵: –CH₂ ^{β} –), 0.89–0.82 (m, 6H, L⁵: 2 \times –CH₃) ppm.

^{13}C NMR (100 MHz, DMSO- d_6) δ = 173.9 (L⁵: C=O), 171.7 (Y¹: C=O), 170.6 (F⁴: C=O), 170.3 (PA⁰: C=O), 169.0 (G²: C=O), 168.6 (G³: C=O), 155.7 (Y¹: Ar–C₄), 137.7 (F⁴: Ar–C₁), 130.0 (2 C, Y¹: Ar–C_{2,6}), 129.2 (2 C, F⁴: Ar–C_{3,5}), 128.0 (2 C, F⁴: Ar–C_{2,6}), 127.9 (Y¹: Ar–C₁), 126.2 (F⁴: Ar–C₄), 114.8 (2 C, Y¹: Ar–C_{3,5}), 83.7 (PA⁰: –C \equiv), 71.2 (PA⁰: \equiv CH δ), 54.4 (Y¹: CH^a), 54.0 (F⁴: CH^a), 51.0 (L⁵: CH^a), 42.0 (G: CH^a), 41.9 (G: CH^a), 40.8 (L⁵: CH₂ ^{β}), 37.3 (F⁴: CH₂ ^{β} –Ar), 36.6 (Y¹: CH₂ ^{β} –Ar), 34.0 (PA⁰: –CH₂^a–), 24.2 (L⁵: CH^a), 23.0 (L⁵: CH₃), 21.6 (L⁵: CH₃), 14.0 (PA⁰: –CH₂ ^{β} –) ppm.

Azide-functionalized Leu⁵–Enkephalin (26**)**: Peptide-loaded rink resin **23**, equivalent to 125 μ mol, was coupled with azidoacetic acid (AA, 50.5 mg, 0.5 mmol) under addition of HOBt/TBTU (4/3.8 eq) and D'PEA (8 equiv.) at room temperature for 2 h by manual SPPS. After cleavage and precipitation, a white powder of **26** (47 mg, 59%) was obtained which was of 90% purity according to analyt. HPLC and thus used without further purification.

rp-HPLC (C18, gradient1): t_R = 16.3 min (90%).

MS (ESI+): m/z = 638.0 $[M+H]^+$ (calcd. for $C_{30}H_{40}N_9O_7$: 638.3).

1H NMR (400 MHz, DMSO- d_6) δ = 9.14 (s, 1H, Y¹: Ar–OH), 8.35 (t, $^3J_{HH}$ = 5.7 Hz, 1H, G²: –NH–), 8.26 (d, $^3J_{HH}$ = 8.2 Hz, 1H, Y¹: –NH–), 8.04 (d, $^3J_{HH}$ = 8.1 Hz, 1H, F⁴: –NH–), 7.98 (t, $^3J_{HH}$ = 5.7 Hz, 1H, G³: –NH–), 7.94 (d, $^3J_{HH}$ = 8.3 Hz, 1H, L⁵: –NH–), 7.24 (m, 4H, F⁴: Ar–H_{2,3,5,6}), 7.18 (m, 1H, F⁴: Ar–H₄), 7.01 (d, $^3J_{HH}$ = 8.5 Hz, 2H, Y¹: Ar–H_{2,6}), 7.00 (d, $^2J_{HH}$ = 50 Hz, 2H, L⁵: –NH₂), 6.63 (d, $^3J_{HH}$ = 8.5 Hz, 2H, Y¹: Ar–H_{3,5}), 4.61–4.40 (m, 2H, Y¹/F⁴: CH^a), 4.20 (dt, $^3J_{HH}$ = 15.1, 8.0 Hz, 1H, L⁵: CH^a), 3.84–3.58 (m, 6H, AA⁰/G²/G³: –CH₂^a–), 3.03/2.79 (2 m, 2H, F⁴: –CH₂ ^{β} –Ar), 2.93/2.65 (2 m, 2H, Y¹: –CH₂ ^{β} –Ar), 1.62–1.52 (m, 1H, L⁵: –CH^aMe₂), 1.47 (m, 2H, L⁵: –CH₂ ^{β} –), 0.89–0.82 (m, 6H, L⁵: 2 \times –CH₃) ppm.

^{13}C NMR (100 MHz, DMSO- d_6) δ = 173.8 (L⁵: C=O), 171.3 (Y¹: C=O), 170.6 (F⁴: C=O), 168.9 (G²: C=O), 168.6 (G³: C=O), 167.2 (AA⁰: C=O), 155.8 (Y¹: Ar–C₄), 137.7 (F⁴: Ar–C₁), 130.0 (2 C, Y¹: Ar–C_{2,6}), 129.2 (2 C, F⁴: Ar–C_{3,5}), 128.0 (2 C, F⁴: Ar–C_{2,6}), 127.6 (Y¹: Ar–C₁), 126.2 (F⁴: Ar–C₄), 114.9 (2 C, Y¹: Ar–C_{3,5}), 54.3 (Y¹: CH^a), 54.0 (F⁴: CH^a), 51.0 (L⁵: CH^a), 50.5 (AA⁰: CH^a), 42.1 (G: CH^a), 41.9 (G: CH^a), 40.8 (L⁵: CH₂ ^{β}), 37.3 (F⁴: CH₂ ^{β} –Ar), 36.7 (Y¹: CH₂ ^{β} –Ar), 24.2 (L⁵: CH^a), 23.0 (L⁵: CH₃), 21.6 (L⁵: CH₃) ppm.

Bioconjugates via amide bonds: Liquid phase peptide synthesis (LPPS). In a microreaction tube, the free carboxylic acid (1.1 equiv.) and peptide (Enk, 1 equiv.) are mixed with TBTU (1.1 equiv.) in DMF (600 μ L). Coupling is initiated by addition of D'PEA (2.3 equiv.) which can be seen through a dark discoloration of the reaction mixture. The mixture is vigorously shaken for 30 sec, centrifuged to

collect all solvents and then continuously shaken on a laboratory shaker (350 rpm) at r.t. for 2 h. Workup is performed individually for the respective compounds.

Coupling of **2 with Leu⁵–Enk **24** in solution (**27**):** By LPSS, compound **2** (6.6 mg, 19.8 μ mol) and Enk **24** (10 mg, 18.0 μ mol) were coupled using TBTU (6.3 mg, 19.8 μ mol) and D'PEA (6.7 μ L, 41 μ mol) in DMF (600 μ L). The reaction mixture was diluted with CH₃CN/water and directly subjected to semiprep. HPLC (gradient 1) in several portions. Pure **84** was obtained as yellow powder (6 mg, 38%).

rp-HPLC (C18, gradient 1): t_R = 20.5 min (>95%).

MS (ESI+): m/z = 906.7/908.7 $[M+K]^+$, 890.8/892.8 $[M+Na]^+$, 868.8/870.8 $[M+H]^+$ (calcd. for $[C_{39}H_{46}BrN_6O_8PtS_2]^+$: 869.2), 851.8/853.8 $[M-NH_2]$.

1H NMR (400 MHz, THF- d_8) δ = 14.97 (s, 1H, (O,S): C–OH), 10.74 (s, 1H, Y¹: Ar–OH), 8.20 (d, $^3J_{HH}$ = 6.9 Hz, Y¹: –NH–), 8.09 (t, $^3J_{HH}$ = 5.5 Hz, G²: –NH–), 7.91 (d, $^3J_{HH}$ = 8.7 Hz, 2H, (O,S): Ar–H_{2,6}), 7.65 (d, $^3J_{HH}$ = 8.7 Hz, 2H, (S,O): Ar–H_{3,5}), 7.54 (m, 2H, G³/F⁴: –NH–), 7.42 (d, $^3J_{HH}$ = 7.3 Hz, L⁵: –NH–), 7.23 (m, 4H, F⁴: Ar–H_{1,2,4,5}), 7.17 (s, 1H, (O,S): –CH–), 7.13 (m, 1H, F⁴: Ar–H₃), 7.01 (d, $^3J_{HH}$ = 8.4 Hz, 2H, Y¹: Ar–H_{2,6}), 6.64 (d + s, $^3J_{HH}$ = 8.4 Hz, 3H, Y¹: Ar–H_{3,5}, L⁵: –NH₂), 6.33 (s, 1H, L⁵: –NH₂), 4.63 (dd, $^3J_{HH}$ = 13.5, 7.9 Hz, 1H, F⁴: CH), 4.43 (m, 2H, Y¹/L⁵: CH), 4.11 (dd, 2H, $^1J_{HH}$ = 34.0 Hz, $^3J_{HH}$ = 15.2 Hz, (O,S): S–CH₂–), 3.88–3.62 (m, 4H, G²/G³: –CH₂–), 3.17, 3.00, 2.89 (m, 4 H, Y¹/F⁴: –CH₂–Ar), 1.59 (m, 3H, L⁵: –CH₂–CHMe₂), 0.88 (m, 6H, L⁵: 2 \times –CH₃) ppm.

CuAAC of (O,S)Pt(II) bischelat complexes and Enk derivatives:

The complimentary compounds, one containing the alkyne and one containing the azide group (1:1 ratio of functional groups, 1 equiv. = 1 functional group pair; FGP), are dissolved in a THF/water mixture (ca. 7 mL 5:2 ratio) and degassed for at least 30 min by an Ar-stream. Sodium ascorbate (0.4 equiv./FGP, 0.1 or 1 M aqueous solution) and CuSO₄·5 H₂O (0.2 equiv./FGP, 0.1 M aqueous solution) are added to initiate the reaction. The mixture is stirred at r.t. until no further conversion of the starting materials was observed according to HPLC analysis. Work-up is performed individually for each compound and includes precipitation from diethyl ether and/or preparative HPLC.

Enkephalin-modified bischelat of (azide–O,S)₂Pt (29**):** The reaction was carried out according to the general procedure for CuAAC, using **25** (10 mg, 15.7 μ mol) and **21** (5.92 mg, 7.88 mmol). CuSO₄·5 H₂O (31 μ L 0.1 M soln.) and sodium ascorbate (63 μ L 0.1 M soln.) was used as catalyst. After 3 days of stirring, THF was removed *in vacuo*, water decanted. The residue was re-dissolved in THF/water, precipitation achieved with cold Et₂O. After centrifugation, the water phase was removed and the precipitate repeatedly washed with Et₂O. After lyophilization, 22 mg of crude material were obtained which were purified in several fractions by semiprep. *rp*-HPLC using gradient 3. Pure (>95%) **29** was obtained in 31% yield (5 mg).

rp-HPLC (C18, gradient 1): t_R = 23.6 min (>95%).

MS (ESI+): m/z = 2020.8 $[M]^+$ (calcd. for $[C_{90}H_{108}N_{18}O_{16}PtS_4]^+$: 2020.7/highest peak), 1010.9 $[M+H]^2+$ (calcd. for $[C_{90}H_{109}N_{18}O_{16}PtS_4]^+$: 1010.8/highest peak).

1H NMR (400 MHz, DMSO- d_6) δ = 9.13 (s, 1H, Y¹: Ar–OH), 8.25 (t, $^3J_{HH}$ = 5.5 Hz, 1H, G²: –NH–), 8.12 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Y¹: –NH–), 8.08–7.92 (m, 2H, F⁴: –NH–, G³: –NH–, L⁵: –NH–, (O,S): Ar–H_{2,6}), 7.81 (s, 1H, PA⁰: ta–CH=), 7.41 (d, $^3J_{HH}$ = 8.4 Hz, (O,S): Ar–H_{3,5}), 7.23–7.22 (m, 4H, F⁴: Ar–H_{2,3,5,6}), 7.21 (s, 1H, (O,S): –CH–), 7.17–7.14 (m, 1H, F⁴: Ar–H₄), 7.00 (d, $^3J_{HH}$ = 8.4 Hz, 2H, Y¹: Ar–H_{2,6}), 7.00 (d, $^2J_{HH}$ = 47.7 Hz, 2H, L⁵: –NH₂), 6.62 (d, $^3J_{HH}$ = 8.3 Hz, 2H, Y¹: Ar–H_{3,5}), 5.58 (s, 2H, (O,S):

Ph-CH₂-N^{1a}), 4.51 (dt, ³J_{HH}=8.8, 4.7 Hz, 1H, F⁴: CHⁿ), 4.45–4.40 (m, 1H, Y¹: CHⁿ), 4.19 (dt, ³J_{HH}=15.3, 7.8 Hz, 1H, L⁵: CHⁿ), 3.77–3.58 (m, 4H, G²/G³: -CH₂^a-), 3.25 (m, 2H, (O,S): S-CH₂-, obscured by SRS), 3.03/2.79 (2 m, 2H, F⁴: -CH₂^β-Ar), 2.92/2.65 (2 m, 2H, Y¹: -CH₂^β-Ar), 2.77 (m, 2H, PA⁰: -CH₂^β-), 2.44–2.37 (m, 4H, PA⁰: -CH₂^a-), 1.59–1.51 (m, 1H, L⁵: -CHⁿMe₂), 1.46 (m, 2H, L⁵: -CH₂^β-), 1.35 (t, ³J_{HH}=7.3 Hz, 3H, (O,S): -CH₃), 0.87–0.80 (m, 6H, L⁵: 2x-CH₃) ppm. Some signals are partially obscured by solvent residual signals (SRS).

Enkephalin-modified bischelatate of (alkyne-O,S)₂Pt (30): The reaction was carried out according to the general procedure for CuAAC, using **26** (10 mg, 7.8 μmol) and **14** (3.2 mg, 3.9 μmol). CuSO₄·5 H₂O (16 μL 0.1 M soln.) and sodium ascorbate (31 μL 0.1 M soln.) was used to enable the conversion. The mixture was stirred for 5 days, upon which the THF was removed *in vacuo*, water decanted, the solid re-dissolved in THF/water, precipitated from ice-cold Et₂O and washed. Preparative *rp*-HPLC (gradient 4) yielded **30** (2 mg, 24 % yield).

rp-HPLC (C18, gradient 1): t_R=25.9 min (>90%).

MS (ESI+): *m/z*=2080.3 [M]⁺ (calcd. for [C₉₂H₁₁₂N₁₈O₁₈PtS₄]⁺: 2080.7/highest peak).

CuAAC of β-hydroxydithiocinnamic esters and Enk derivatives: The complimentary compounds, one containing the alkyne and one containing the azide group (1:1 ratio of functional groups, 1 equiv.=1 FGP), are dissolved in a THF/water mixture (ca. 7 mL 5:2 ratio) and degassed for at least 30 min by an Ar-stream. Sodium ascorbate (2.4 equiv./FGP, 1 M aqueous solution) and CuSO₄·5 H₂O (1.2 equiv./FGP, 0.5 M aqueous solution) are added to initiate the reaction. The mixture is stirred at r.t. until HPLC control shows consumption of the starting materials. To remove the copper salts, sodium ethylenediamine tetraacetate (Na₂EDTA, 2.5 equiv./FGP, 0.1 M aqueous solution) is added and the mixture stirred overnight. Work-up is performed individually for each compound and includes removal of Cu(EDTA), precipitation from diethyl ether and/or preparative HPLC.

Enkephalin-modified triazol-(O,S) compound (31): The reaction was carried out with **25** (10 mg, 15.8 μmol), **20** (4.4 mg, 15.8 μmol), sodium ascorbate (38 μL 1 M soln) and CuSO₄·5 H₂O (35 μL 0.5 M soln) according to the general procedure for CuAAC of ligands. After stirring for 24 h, Na₂EDTA (435 μL 0.1 M soln.) was added and the mixture stirred overnight. THF was removed *in vacuo* upon which a brownish oil was formed. Cu-EDTA containing water was decanted, the residue washed with water and Et₂O. The oil was dissolved in acetonitrile/water and lyophilized to give ca. 13 mg of crude material. This was purified by prep. *rp*-HPLC (gradient 3) to give pure **31** (6 mg, 42%; >95% purity) as yellow amorphous solid after lyophilization.

rp-HPLC (C18, gradient 1): t_R=20.4 min (>95%).

MS (ESI+): *m/z*=914.1 [M+H]⁺ (calcd. for [C₄₅H₅₆N₉O₈S₂]⁺: 914.4).

¹H NMR (400 MHz, DMSO-d₆) δ=15.15 (s, 1H, (O,S): C-OH), 9.13 (s, 1H, Y¹: Ar-OH), 8.24 (t, ³J_{HH}=5.6 Hz, 1H, G²: -NH-), 8.12 (d, ³J_{HH}=8.1 Hz, 1H, Y¹: -NH-), 8.04 (d, ³J_{HH}=8.1 Hz, 1H, F⁴: -NH-), 7.99–7.95 (m, 3H, G³: -NH-, (O,S): Ar-H2,6), 7.93 (d, ³J_{HH}=8.4 Hz, 1H, L⁵: -NH-), 7.79 (s, 1H, PA⁰: ta-CH=), 7.38 (d, ³J_{HH}=8.4 Hz, (O,S): Ar-H3,5), 7.23 (m, 4H, F⁴: Ar-H2,3,5,6), 7.18 (m, 2H, F⁴: Ar-H4, (O,S):=CH-), 7.00 (d, ³J_{HH}=8.4 Hz, 2H, Y¹: Ar-H2,6), 7.00 (d, ²J_{HH}=48.3 Hz, 2H, L⁵: -NH₂), 6.62 (d, ³J_{HH}=8.4 Hz, 2H, Y¹: Ar-H3,5), 5.60 (s, 2H, (O,S): Ph-CH₂-N^{1a}), 4.51 (dt, ³J_{HH}=8.9, 4.6 Hz, 1H, F⁴: CHⁿ), 4.42 (dt, ³J_{HH}=9.5, 4.7 Hz, 1H, Y¹: CHⁿ), 4.19 (dt, ³J_{HH}=15.2, 7.9 Hz, 1H, L⁵: CHⁿ), 3.82–3.54 (m, 4H, G²/G³: -CH₂^a-), 3.27 (m, 2H, (O,S): S-CH₂-, obscured by SRS), 3.03/2.81 (2 m, 2H, F⁴: -CH₂^β-Ar), 2.92/2.63 (2 m, 2H, Y¹: -CH₂^β-Ar), 2.78 (t, ³J_{HH}=8.2 Hz, PA⁰: -CH₂^β-), 2.46–2.35 (m, 2H, PA⁰: -CH₂^a-), 1.63–1.50 (m, 1H, L⁵: -CHⁿMe₂), 1.46 (m, 2H, L⁵: -CH₂^β-), 1.32 (t, ³J_{HH}=

7.4 Hz, 3H, (O,S): -CH₃), 0.89–0.82 (m, 6H, L⁵: 2x-CH₃) ppm. Some signals are partially obscured by solvent residual signals (SRS).

Bioconjugates of monochelate-platinum(II) complexes: In a microreaction tube, K₂PtCl₄ (2 equiv. 0.1 M aqueous solution) is activated by addition of excess DMSO (50 μL). In parallel, the Enk-conjugated β-hydroxydithiocinnamic ester (1 equiv., dissolved in 600 μL THF) is deprotonated by NaOAc (10 equiv., 0.1 M aqueous solution) and added to the Pt-mixture in portions of 50 μL; 100 μL water are added to facilitate mixing of the solvents. After overnight shaking on a laboratory shaker, water and THF are removed *in vacuo*. By addition of 500 μL water the compound is precipitated, then centrifuged and the solvents decanted. The solid residue is washed repeatedly with water and Et₂O and dried under vacuum to give the desired complex.

(Enk-O,S)Pt(DMSO)Cl from 31 (32): Compound **31** (1.3 mg, 1.4 μmol) was reacted with K₂PtCl₄ (28 μL 0.1 M soln.) in presence of DMSO (50 μL) and NaOAc (14 μL 0.1 M soln.) according to the general procedure. The desired compound was obtained as yellow-orange solid (1.8 mg, 100%).

MS (ESI+): *m/z*=1259.8 [M+K]⁺ (calcd. for [C₄₇H₆₀ClKN₉O₉PtS₃]⁺: 1260.3/highest peak), 1243.9 [M+Na]⁺ (calcd. for [C₄₇H₆₀ClN₉O₉PtS₃]⁺: 1244.3/highest peak), 1222.0 [M+H]⁺ (calcd. for [C₄₇H₆₁ClN₉O₉PtS₃]⁺: 1222.3/highest peak).

¹H NMR (400 MHz, DMSO-d₆) δ=9.13 (s, 1H, Y¹: Ar-OH), 8.24 (t, ³J_{HH}=5.5 Hz, 1H, G²: -NH-), 8.12 (d, ³J_{HH}=8.1 Hz, 1H, Y¹: -NH-), 8.05 (d, ³J_{HH}=8.1 Hz, 1H, F⁴: -NH-), 8.00–7.95 (m, 4H, G³: -NH-, L⁵: -NH-, (O,S): Ar-H2,6), 7.78 (s, 1H, PA⁰: ta-CH=), 7.35 (d, ³J_{HH}=8.3 Hz, (O,S): Ar-H3,5), 7.24–7.23 (m, 5H, F⁴: Ar-H2,3,5,6, (O,S):=CH-), 7.17 (m, 1H, F⁴: Ar-H4), 7.00 (d, ³J_{HH}=8.4 Hz, 2H, Y¹: Ar-H2,6), 7.00 (d, ²J_{HH}=48.0 Hz, 2H, L⁵: -NH₂), 6.62 (d, ³J_{HH}=8.3 Hz, 2H, Y¹: Ar-H3,5), 5.56 (s, 2H, (O,S): Ph-CH₂-N^{1a}), 4.51 (dt, ³J_{HH}=8.9, 4.7 Hz, 1H, F⁴: CHⁿ), 4.42 (dt, ³J_{HH}=9.3, 4.6 Hz, 1H, Y¹: CHⁿ), 4.19 (dt, ³J_{HH}=15.2, 7.8 Hz, 1H, L⁵: CHⁿ), 3.77–3.49 (m, 4H, G²/G³: -CH₂^a-), 3.26 (m, 2H, (O,S): S-CH₂-, obscured by SRS), 3.03/2.79 (2 m, 2H, F⁴: -CH₂^β-Ar), 2.92/2.65 (2 m, 2H, Y¹: -CH₂^β-Ar), 2.76 (m, 2H, PA⁰: -CH₂^β-), 2.46–2.35 (m, 4H, PA⁰: -CH₂^a-), 1.60–1.50 (m, 1H, L⁵: -CHⁿMe₂), 1.46 (m, 2H, L⁵: -CH₂^β-), 1.32 (t, ³J_{HH}=7.3 Hz, 3H, (O,S): -CH₃), 0.87–0.81 (m, 6H, L⁵: 2x-CH₃) ppm. Some signals are partially obscured by solvent residual signals (SRS). DMSO signal not detected due to substitution by DMSO-d₆.

(Enk-O,S)Pt(DMSO)Cl from 27 (28): **27** (2 mg, 2.3 μmol) was reacted with K₂PtCl₄ (46 μL 0.1 M soln.) in presence of DMSO (50 μL) and NaOAc (23 μL 0.1 M soln.) according to the general procedure. The desired compound was obtained as orange solid (1.4 mg, 52%).

rp-HPLC (C18, gradient 1): rt=18.9 min (under solvolysis of Cl/ DMSO ligands).

MS (ESI+): *m/z*=1214.9 [M+K]⁺ (calcd. for [C₄₁H₅₀BrClKN₆O₉PtS₃]⁺: 1215.1/first high peak), 1198.8 [M+Na]⁺ (calcd. for [C₄₁H₅₀BrClN₆O₉PtS₃]⁺: 1199.1/first high peak).

¹H NMR (400 MHz, THF-d₈) δ=10.80 (s, 1H, Y¹: Ar-OH), 8.32 (d, 1H, ³J_{HH}=6.9 Hz, Y¹: -NH-), 8.02 (d, ³J_{HH}=8.6 Hz, 2H, (O,S): Ar-H2,6), 7.93 (t, ³J_{HH}=5.6 Hz, G²: -NH-), 7.60 (d, ³J_{HH}=8.6 Hz, 2H, (O,S): Ar-H3,5), 7.50 (m, 2H, G³/F⁴: -NH-), 7.39 (d, ³J_{HH}=7.3 Hz, L⁵: -NH-), 7.34 (s, 1H, (O,S):=CH-), 7.24 (m, 4H, F⁴: Ar-H1,2,4,5), 7.14 (m, 1H, F⁴: Ar-H3), 7.02 (d, ³J_{HH}=8.3 Hz, 2H, Y¹: Ar-H2,6), 6.65 (d + s, ³J_{HH}=8.4 Hz, 3H, Y¹: Ar-H3,5, L⁵: -NH₂), 6.29 (s, 1H, L⁵: -NH₂), 4.63 (dd, ³J_{HH}=13.4, 7.9 Hz, 1H, F⁴: CH), 4.43 (m, 2H, Y¹/L⁵: CH); 4.06 (dd, 2H, ¹J_{HH}=28.0 Hz, ³J_{HH}=16.0 Hz, (O,S): S-CH₂-), 3.83 (m, 4H, G²/G³: -CH₂-), 3.76, 3.62 (CH₃ (DMSO), obscured by SRS but observed through HSQC) 3.17, 3.12–2.87 (m, 4 H, Y¹/F⁴: -CH₂-Ar), 1.62 (L⁵: -CH₂-CHMe₂, obscured by SRS), 0.89 (m, 6H, L⁵: 2x-CH₃) ppm.

Supporting information

Deposition Number 2260478 (for 21), 2260479 (for 2) contain the supplementary crystallographic data for this paper. These data are provided free of charge by the joint Cambridge Crystallographic Data Centre and Fachinformationszentrum Karlsruhe Access Structures service.

Additional data, graphs and figures, extensive experimental details, have been deposited within the Supporting Information that also include additional references.^[49]

Author Contributions

C. M.: Conceptualization, Investigation, Funding Acquisition, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing. N. N.: Investigation, Writing – Review & Editing. M. S.: Investigation, Writing – Review & Editing. N. M.-N.: Conceptualization, Funding Acquisition, Supervision, Writing – Review & Editing. W. W.: Conceptualization, Funding Acquisition, Supervision, Writing – Review & Editing.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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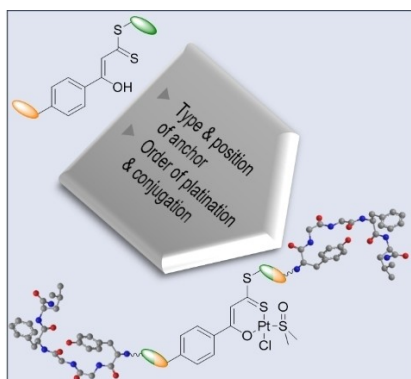
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RESEARCH ARTICLE

The synthesis of peptide bioconjugates featuring a platinum(II) core, coordinated with structurally variable (*O,S*) bidentate β -hydroxy dithiocinnamic esters and labile monodentate ligands, bears numerous opportunities and challenges. Key to success was a proper selection of the type and position of anchors as well as of the synthetic order, providing a panel of bioconjugates with Leu⁵-enkephalin as the model peptide.



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Synthetic Approaches towards Peptide-Conjugates of Pt(II) Compounds with an (*O,S*) Chelating Moiety

