Introduction of Phosphine-Gold(I) Precursors into a Cys-modified Enkephalin Neuropeptide as Part of Solid Phase Peptide Synthesis

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Dedicated to Prof. Helgard G. Raubenheimer on the occasion of his 65 th birthday

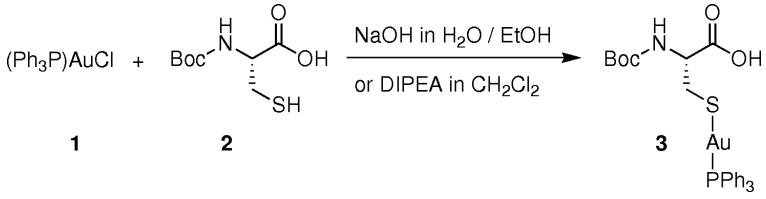
The synthesis and full characterization of a series of (triphenylphosphine)gold complexes with thiol-containing amino acids and peptides is reported. Boc-Cys-Au(PPh_3) was prepared in solution by reaction of Boc-Cys-OH with (Ph_3P)AuCl. The related Ac-Cys-Au(PPh_3) and an Au derivative of the cysteine-modified neuropeptide enkephalin (Enk), [Cys]^5-Au(PPh_3)-Enk, were prepared on the resin, using the orthogonal trityl-protecting group on Cys. For comparison, the metal-free [Cys]^5-Enk and an *S-tert*-butyl-protected [Cys]^5-Enk were also prepared. Most noteworthy, gold complexation works best when carried out on the resin, and the gold thiolate survives cleavage from the resin under optimized conditions. The new conjugates were comprehensively characterized, including 1D and 2D NMR spectroscopy, and mass spectrometry. Along with characteristic changes in the ^{1}H and ^{13}C NMR spectra, ^{31}P NMR spectra reveal a characteristic downfield shift of *ca.* 5 ppm upon complexation of the Au(PPh_3)-fragment to Cys, which is also pertinent in the Au-labelled [Cys]^5-Enk.

Key words: Enkephalin, Gold Compounds, Medicinal Inorganic Chemistry, Peptides

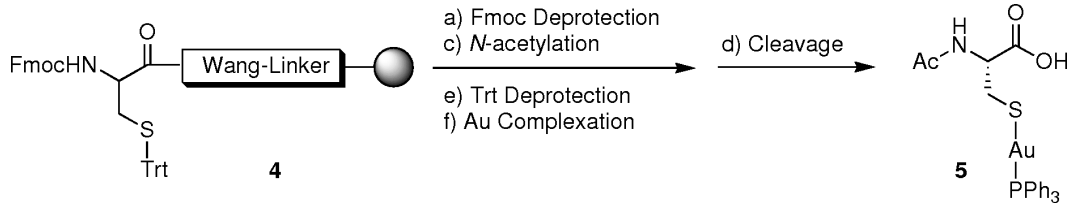
Introduction

Gold complexation to a range of biologically relevant organic molecules is an active field of research. Well known examples include the *anti*-arthritis drugs gold-thiomalate and gold-thioglucose [1]. All these first-generation drugs have polymeric structures in the solid state and only low aqueous solubility, thus limiting their oral applicability. These problems are overcome with the second-generation drug Auranofin, which is a well-defined molecular compound. In Auranofin, a triethylphosphine-gold(I) fragment is complexed to acetylated thioglucose. Following Auranofin's success, many other gold derivatives of carbohydrates were prepared and tested for activity [2, 3]. In relation, far less gold derivatives of amino acids were reported. Ligand exchange reactions of gold-phosphine [4, 5] and gold-cyano [6, 7] complexes with biologically relevant thiols, in particular the amino acid cysteine and the tripeptide glutathione, were investigated. Glutathion in particular is an abundant reductant in cells and is thus an obvious target for gold(I) compounds, as are the thiol groups of cysteine in proteins. The X-ray crystal structure of a gold(I) complex of human glutathione reductase (hGR) has recently been reported and gives interesting insights into the biological function of gold complexes [8]. Earlier on, the first crystallographic investigation of a gold protein complex revealed an unexpected binding of the Au(PEt_3) fragment to the N_ε of a histidine residue in the protein cyclophilin-3 [9]. Decoration of gold nanoparticles with other peptides has also been reported recently [10 – 12]. However, to the best of our knowledge, no

Abbreviations: Bocb – *tert*-butoxycarbonyl; Fmoc – 9-fluorenylmethoxycarbonyl; WANG – *p*-hydroxybenzyl alcohol; TBTU – 2-(1H-benzotriazole-1-yl-)-1,1,3,3-tetramethyluronium tetrafluoroborate; HOBt – hydroxybenzotriazole; DIPEA – diisopropyl(ethyl)amine; DCM – dichloromethane; TIS – triisopropylsilane; TFA – trifluoroacetic acid; HPLC – high-performance liquid chromatography.



Scheme 1. Synthesis of Boc-Cys-Au(PPh_3) **3** in solution.



Scheme 2. Synthesis of Ac-Cys-Au(PPh_3) **4** by solid phase synthesis. See Scheme 3 for reaction numbering order and experimental section for details.

fully characterized 1:1 adduct of biologically relevant peptides to a molecular gold complex fragment has been reported to the present day.

The main source for small peptides is solid phase peptide synthesis (SPPS, also called Merrifield synthesis), in which the peptide is built from suitable amino acid building blocks in a successive manner [13, 14]. The challenge of introducing metal complexes during SPPS is that the complexation reaction must be selective for a given amino acid. In addition, the thus formed complex must survive all subsequent synthetic steps including cleavage of the final product from the resin [15, 16]. In recent work, we have demonstrated that introduction of metal complexes in an SPPS scheme is possible even for relatively sensitive organometallic compounds by a suitable choice of resin, linker and side chain protecting groups [17 – 27]. Very stable Pt(II)-peptide conjugates were also obtained by two Dutch groups [28 – 32]. Much of our work has focussed on the neuropeptide enkephalin (Enk). Enkephalin is the natural ligand for opiate receptors in the central nervous system [33 – 39]. It is a pentapeptide with the sequence H-Tyr-Gly-Gly-Phe-Aaa-OH. The fifth amino acid is indeed variable, with Aaa being leucine in the naturally most abundant example [Leu]^5-Enk. Given the known preference of gold(I) for thiol ligands, we set out to prepare a [Cys]^5-Enk derivative for labelling with phosphine-gold(I) complexes by SPPS. In here, we describe the first preparation of a gold-labelled cysteine-enkephaline *via* solid phase peptide chemistry (SPPS), in which gold-labelling is carried out on the resin.

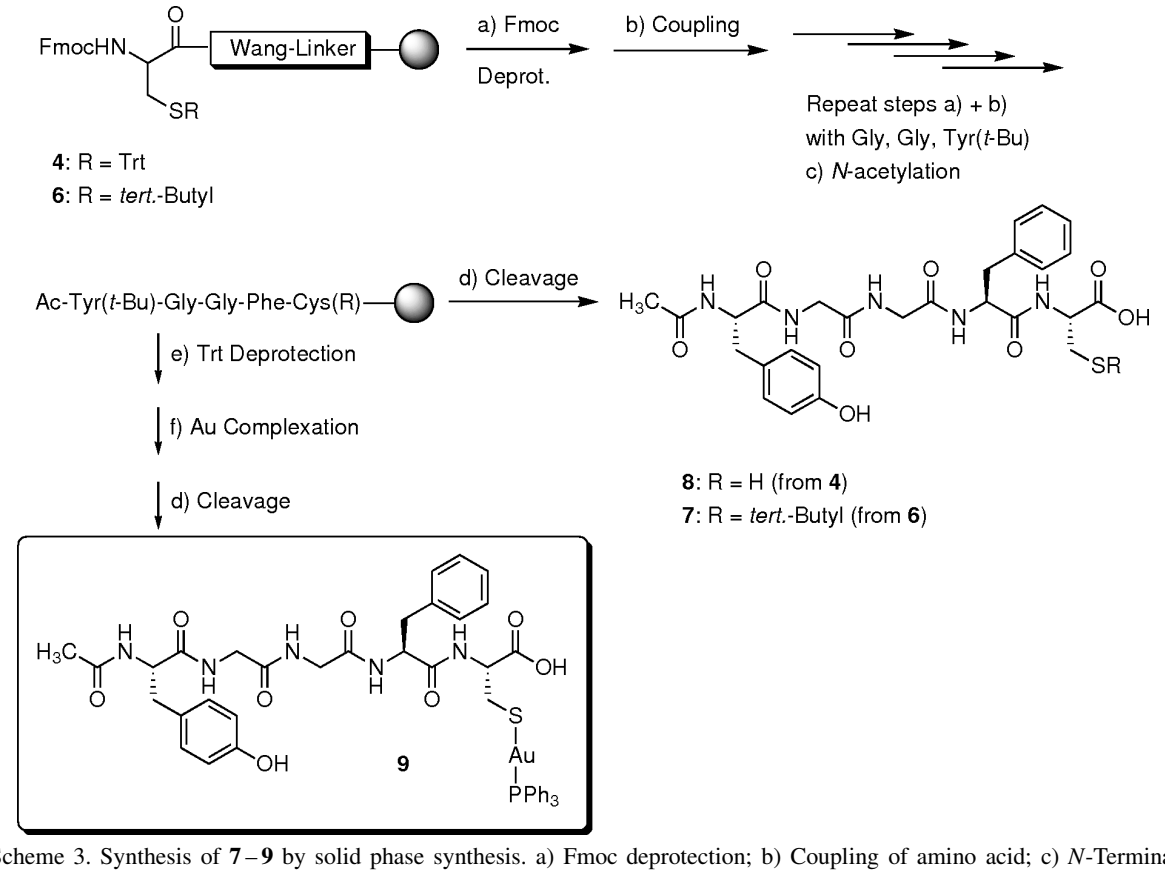
Results and Discussion

Our studies were initiated using (triphenylphosphine)gold chloride **1** and Boc-cysteine **2**, in order to confirm the feasibility of the planned chemistry. The desired Boc-cysteine-gold triphenylphosphine complex **3** was obtained in fair yield according to methods A and B, respectively (Scheme 1).

Complexation was confirmed by a shift in the ^{31}P NMR from 33.3 to 38.3 ppm [40] along with a downfield shift of signals in both the ^{1}H and ^{13}C spectra. The most notable shifts observed in the ^{13}C spectrum were those of the *ipso*-carbon atoms on the triphenylphosphine and the CH_2 group of the cysteine from 29.9 to 30.2 ppm, as well as the CH_2 signal of the cysteine becoming a better defined AB system in the ^{1}H spectrum. These results were further supported by ESI and FAB mass spectrometric analysis.

The combination of solid phase synthesis and gold complexation began by using a trityl protected cysteine precursor, Fmoc-Cys(Trt)-Wang resin **4**. The desired acetyl-cysteine-gold triphenylphosphine **5** was obtained as a white powder, after cleavage and washing (Scheme 2). Spectroscopic data for **5** were similar to that of **3**, thus confirming the integrity of the phosphorus-gold-sulfur unit during cleavage.

Encouraged by the successful preparation of the small molecules **3** and **5**, we set out to prepare the target molecule [Cys]^5-Enk. Suitable choice of protecting groups is crucial for successful SPPS [41]. Therefore, standard Fmoc-solid phase synthesis was carried out *via* two different starting materials, namely Fmoc-Cys(Trt)-Wang resin **4** and Fmoc-Cys(*tert*-butyl)-



Scheme 3. Synthesis of **7**–**9** by solid phase synthesis. a) Fmoc deprotection; b) Coupling of amino acid; c) *N*-Terminal acetylation; d) Cleavage from the resin; e) Trt deprotection; f) Gold complexation. See experimental section for details.

Wang resin **6**, the only difference of the two being the protecting group of the cysteine. Successful preparation of the desired target molecule [Cys]^5-Enk was confirmed in both cases (Scheme 3). However, HPLC analysis of the product **8** obtained from **4** displayed several side products. These were identified to be the result of the free cysteine undergoing disulfide formation. Preparation of the product **7** derived from **6** was of $> 95\,\%$ purity. This finding is in accordance with the different stabilities of sulphur protecting groups. Whereas the trityl group may be removed by as little as 5 % TFA in DCM, the *tert*-butyl group remains intact even in the presence of 85 % TFA as shown in the case of **7**.

Summarizing all of the above findings, the optimal synthesis requires incorporation of the gold complex on the resin from a trityl protected cysteine. The desired gold cysteine-enkephaline complex **9** was prepared by standard Fmoc-solid phase chemistry (Scheme 3) [14]. Trityl deprotection on the resin was effected using 5 % TFA and 5 % TIS in DCM, after which the resin was washed several times with dichloromethane and dried. The resin beads were transferred to a Schlenk tube and the complexation was carried out under an argon atmosphere with (triphenylphosphine)gold chloride and DIPEA in DCM. The complexation was allowed to take place overnight followed by several dichloromethane washings and cleavage. Phenol was added to the cleavage solution as a precaution against oxidation. Analytical HPLC showed $> 90\,\%$ purity of the product. Unfortunately subjection to preparative HPLC resulted in decomposition of the product. Therefore, all further characterization was carried out without HPLC purification. The structure of **9** was confirmed by NMR analysis and mass spectrometry. ^{31}P NMR showed a single broad peak at 37.8 ppm. In addition ^{1}H and ^{13}C NMR spectra displayed similar signatures as for **3**. A peak for the molecular ion at $m/z = 1046$ was observed in both FAB and ESI MS (positive ion detection). Furthermore, the

ESI MS showed a peak at *m/z* = 610 for the metal-free [Cys]^5-Enk **7**.

Conclusions

The synthesis of a cysteine-derived enkephalin was achieved by solid phase Fmoc-chemistry from a Wang resin starting material. Gold complexation to Cys-Enk was successfully achieved as part of the solid phase synthesis scheme by careful selection of protecting groups and linkers. Unambiguous identification of the [Cys]^5-Enk-gold triphenylphosphine conjugate **9** was greatly assisted by data obtained for a series of related smaller molecules. To the best of our knowledge, compound **9** is the first example of a gold peptide conjugate prepared by solid phase synthesis. Further studies of related molecules are underway in our laboratories.

Experimental Section

All peptide synthesis reactions were carried out in ordinary glassware and solvents. Chemicals were obtained from Aldrich, Iris, or Novabiochem. DMF for peptide synthesis (amine-free) was obtained from Roth. Enantiomerically pure L-amino acids were used throughout. (Triphenylphosphine)gold chloride (Ph_3PAuCl, **1**) was prepared by a standard procedure [42].

Mass spectra were recorded on a Finnigan MAT 8200 instrument (EI, 70 eV) or a Finnigan TSQ 700 mass spectrometer (ESI; solvent and detection mode are given in parentheses). Only characteristic fragments with possible composition are given in brackets. For fragments containing metals only the isotopomer with highest intensity was described. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AM 360 (^{1}H at 360.14 MHz, ^{13}C at 90.56 MHz) and Bruker AM 300 spectrometers (^{1}H at 300.16 MHz, ^{13}C at 75.47 MHz). ^{1}H and ^{13}C NMR spectra were referenced to residual signals of the deuterated solvents as internal standards ($CDCl_3$ = 7.24 (^{1}H) and 77.0 (^{13}C); DMSO = 2.49 (^{1}H) and 39.5 (^{13}C); MeOH = 3.31 (^{1}H) and 49.0 (^{13}C)). High-performance liquid chromatography (HPLC) was performed on a customized VarianProStar system on reverse-phase Dynamax Microsorb 60-8 C_{18} columns (analytical: C_{18} 60 Å, diameter 4.5 mm, 250 mm; preparative: C_{18} microsorb 60 Å, diameter 21.4 mm, 250 mm) with water and acetonitrile, both containing 0.1 % TFA, as eluent using the linear gradient 5 to 95 % acetonitrile for 35 min.

*Preparation of (triphenylphosphine)gold-Boc-cysteine-thiolate **3***

To Boc-cysteine (37 mg, 0.167 mmol) (**2**) in ethanol (1 mL) was added (triphenylphosphine)gold chloride (83 mg, 0.167 mmol) (**1**) and a solution of sodium hydroxide in water (6.6 mg in minimal water), under argon. The reaction mixture was allowed to stir overnight. The solvent was removed *in vacuo* to obtain a sticky white crude product, which appeared to oxidize if exposed to air for extended periods of time. The reaction was repeated in dichloromethane, with DIPEA as a base to yield similar results. *Analytical Data for* $C_{26}H_{29}NO_4SAuP$ (679.53). – MS (FAB, pos): *m/z* = 721 $[Ph_3P–Au–PPh_3]^+$, 579 $[M + H – Boc]^+$, 459 $[Au–PPh_3]^+$. – MS (ESI neg., MeOH): *m/z* = 678 $[M – H]^-$. – ^{1}H NMR ($CDCl_3$, 360.1 MHz): δ = 8.26 (br s, 1H, NH), 7.69 – 7.15 (m, 15H, P*Ph*$_3$), 5.77 (br s, 1H, OH), 4.44 (br s, 1H, $C_{\alpha-Cys}H$), 3.51 (unres. dd, 1H, $C_{\beta-Cys}H$), 3.27 (unres. dd, 1H, $C_{\beta-Cys}H$), 1.36 (s, 9H, $C(CH_3)_3$). – ^{13}C NMR ($CDCl_3$, 90.6 MHz): δ = 174.4 (*C*OOH), 155.7 (O*C*NH), 134.2 (d, *J* = 13.8 Hz, *ortho*), 131.6 (d, *J* = 2.17 Hz, *para*), 129.5 (d, *J* = 56.2 Hz, *ipso*), 129.2 (d, *J* = 11.4 Hz, *meta*), 79.6 ($C(CH_3)_3$), 56.9 (C_α-Cys), 30.1 (C_β-Cys), 29.2 ($C(CH_3)_3$). – ^{31}P NMR ($CDCl_3$, 101.26 MHz): δ = 38.3.

*Preparation of (triphenylphosphine)gold-acetyl-cysteine-thiolate **5***

The synthesis was performed manually in a syringe equipped with a porous filter (10 mL, MultiSynTech) using Fmoc-protected Cys(Trt)-Wang resin (50 mg, loading 0.7 mmol/g, Iris Biotech) (**4**). Synthesis: (a) *deprotection* using two times about 3 mL of a 20 % piperidine solution in DMF, first at 5 min and then at 10 min, without washing in between; *washing* 5 times with about 6 mL of DMF; (c) *acetylation* (acetic anhydride/2,6-lutidine/*N*-methyl imidazole/THF = 1/1/1/7, 1 mL, 1 h); *washing* 5 times with about 6 mL of DMF; (e) *trityl-deprotection* 2 min of manual washing with 5 % TFA, 5 % TIS in DCM, followed by a further six 2 min washes on the shaker; *washing* 5 times with about 6 mL of DCM; (f) *gold complexation*: The resin was dried and gold complexation initiated by injection of a solution of (triphenylphosphine)gold chloride (86 mg, 0.015 mmol) and DIPEA (23 mg, 5 equivalents) in DCM, under argon. The reaction was allowed to take place overnight, followed by three DCM washing sessions of 2 min each; (d) *cleavage*: After the resin was successively rinsed with DMF and DCM and dried under reduced pressure (1 h, 10 mbar), final deprotection and cleavage from resin was performed with a TFA mixture (TFA/H_2O/TIS = 95/2.5/2.5, 1 mL, 3 h). The suspension was filtered and the resin washed with TFA (2 × 0.5 mL). The combined TFA solutions were poured into cold ether (10 mL, −30 °C), and the suspension was centrifuged (8000 rpm, 6 min). After decanting the supernatant, the crude product was washed with cold ether (2 × 5 mL), dissolved in water, filtered and lyophilised, yielding a white solid. *Analytical Data for* $C_{23}H_{23}NO_3PSAu$ (621.44). – MS (FAB, pos): *m/z* = 721 $[Ph_3P–Au–PPh_3]^+$, 645 $[M + H – Na]^+$, 459 $[Au–PPh_3]^+$. – ^{1}H NMR ($CDCl_3$,

360.1 MHz): δ = 7.60 – 7.25 (m, 15H, P*Ph*$_3$), 4.43 – 4.37 (m, 1H, CH), 2.74 (unres. dd, 1H, C*H*$_a$CH$_b$), 2.61 (unres. dd, 1H, CH$_a$C*H*$_b$), 1.67 (s, 3H, C$_{\alpha-Ac}$H). – ^{31}P NMR (CDCl$_3$, 101.26 MHz): δ = 37.5.

Preparation of tert-butyl-Cys-enkephalin ***7***

The synthesis was performed manually in a syringe equipped with a porous filter (10 mL, MultiSynTech) using Fmoc-protected Cys(*tert*-butyl)-Wang resin (200 mg, loading 0.77 mmol/g, Iris Biotech GmbH) (**6**), Fmoc-protected amino acid monomers, base, and amine free DMF solvent. The tyrosine side chain protecting group was *tert*-butyl. Synthesis cycle: (a) *deprotection* using two times about 3 mL of a 20 % piperidine solution in DMF, first at 5 min and then at 10 min, without washing in between; *washing* 5 times with about 6 mL of DMF; (b) *coupling* using a solution of activated amino acid, for 30 min; and *washing* 5 times with 3 mL DMF. The coupling mixture contained the protected amino acid monomer (5-fold excess), TBTU (242 mg, 4.9-fold excess), and HOBt (118 mg, 5-fold excess) dissolved in DMF (2.5 mL); then DIPEA (199 mg, 10-fold excess, activation period 2 min) was added. After repeating the synthesis cycle four times all the desired couplings had been carried out. A last Fmoc-deprotection with 20 % piperidine was carried out followed by capping. (c) For our purposes an *acetyl capping* was used (acetic anhydride/2,6-lutidine/*N*-methyl imidazole/THF = 1/1/1/7, 3 mL, 1 h); (d) *cleavage*: After the resin was successively rinsed with DMF and DCM and dried under reduced pressure (1 h, 10 mbar), final deprotection and cleavage from resin was performed with a TFA mixture (TFA/H_2O/TIS = 95/2.5/2.5, 3 mL, 3 h). The suspension was filtered and the resin washed with TFA (2 × 1.5 mL). The combined TFA solutions were poured into cold ether (10 mL, −30 °C), and the suspension was centrifuged (8000 rpm, 6 min). After decanting the supernatant, the crude product was washed with cold ether (2 × 10 mL), dissolved in water, filtered and lyophilised, yielding a white solid. The product was purified by preparative HPLC. Yield: 178 mg (71 %). *Analytical Data for* $C_{31}H_{41}N_5O_8S$ (643.76). MS (FAB, pos): *m/z* = 666 $[M + Na]^+$, 644 $[M + H]^+$, 467 [*], 320 [*]. – MS (ESI pos., MeOH): *m/z* = 666 $[M + Na]^+$, 644 $[M + H]^+$. – ^{1}H NMR (CDCl$_3$, 360.1 MHz): δ = 9.16 (br s, 1H, OH$_{Tyr}$), 8.45 (d, 1H, *J* = 7.9 Hz, NH$_{Cys}$), 8.23 (t, 1H, *J* = 5.8 Hz, NH$_{Gly}$), 8.07 (d, 1H, *J* = 8.3 Hz, NH$_{Tyr}$), 8.03 (d, 1H, *J* = 8.6 Hz, NH$_{Phe}$), 7.92 (t, 1H, *J* = 5.6 Hz, NH$_{Gly}$), 7.24 – 7.14 (m, 5H, ArH$_{Phe}$), 7.01 (d, 1H, *J* = 8.6 Hz, ArH$_{Tyr}$), 6.23 (d, 1H, *J* = 8.6 Hz, ArH$_{Tyr}$), 4.61 – 4.57 (m, 1H, C$_{\alpha-Phe}$H), 4.41 – 4.31 (m, 2H, C$_{\alpha-Tyr}$H and C$_{\alpha-Cys}$H), 3.75 – 3.55 (m, 4H, Gly-CH$_2$ × 2), 3.05 – 2.58 (overlapping m, 6H, C$_{\beta-Phe}$H, C$_{\beta-Tyr}$H and C$_{\beta-Cys}$H), 1.76 (s, 3H, C$_{\alpha-Ac}$H), 1.28 (s, 9H, C(C*H*$_3$)$_3$). – ^{13}C NMR (CDCl$_3$, 90.6 MHz): δ = 171.9 (C=O$_{Cys}$), 171.7 (C=O$_{Phe}$), 171.1 (C=O$_{Tyr}$), 169.3 (C=O$_{Ac}$), 168.9 (C=O$_{Gly}$), 168.3 (C=O$_{Gly}$), 155.6 (*p*-C$_{Tyr}$), 137.6 (*i*-C$_{Phe}$), 129.9/129.2/127.9/128.0 (*o*- and *m*-C$_{Phe}$ and C$_{Tyr}$), 126.2 (*p*-C$_{Phe}$), 114.8 (*o*-C$_{Tyr}$), 54.4 (C$_\alpha$-Phe), 53.4 (C$_\alpha$-Cys), 52.9 (C$_\alpha$-Tyr), 42.0 (*C*(CH$_3$)$_3$), 41.9 (CH$_2$-Gly), 41.6 (CH$_2$-Gly), 37.5 (C$_\beta$-Phe), 36.4 (C$_\beta$-Tyr), 30.44 (C(*C*H$_3$)$_3$), 29.3 (C$_\beta$-Cys), 22.4 (C$_\alpha$-Ac).

[*] Common fragmentation pattern observed with this pentapeptide.

Preparation of Cys-enkephalin ***8***

The synthesis was carried out in analogy to that of *tert*-butyl-Cys-Enkephalin (**7**). Instead of Fmoc-protected Cys(*tert*-butyl)-Wang resin, Fmoc-protected Cys(Trt)-Wang resin (200 mg, loading 0.70 mmol/g, Iris Biotech) (**4**) was used. (e) In addition a *trityl deprotection* step was carried out after capping, accomplished by 2 min of manual washing with 5 % TFA, 5 % TIS in DCM, followed by a further six 2 min washes on the shaker. The trityl-deprotected product was then washed with DCM for three wash sessions, dried and subjected to work up conditions. *Analytical Data for* $C_{27}H_{33}N_5O_8S$ (587.65). MS (FAB, pos): *m/z* = 588 $[M + H]^+$, 467[*], 320[a]. MS (ESI pos., MeOH): *m/z* = 610 $[M + Na]^+$, 588 $[M + H]^+$. MS (ESI neg., MeOH): *m/z* = 587 $[M]^-$, 586 $[M - H]^-$. – ^{1}H NMR (CD$_3$OD, 300.1 MHz): δ = 7.21 – 7.19 (m, 5H, ArH$_{Phe}$), 6.97 (d, 1H, *J* = 8.4 Hz, ArH$_{Tyr}$), 6.63 (d, 1H, *J* = 8.4 Hz, ArH$_{Tyr}$), 4.66 – 4.58 (m, 1H, C$_{\alpha-Phe}$H), 4.51 – 4.48 (m, 1H, C$_{\alpha-Tyr}$H), 4.41 – 4.36 (m, 1H, C$_{\alpha-Cys}$H), 3.80 – 3.60 (m, 4H, Gly-CH$_2$ × 2), 3.19 – 2.66 (overlapping m, 6H, C$_{\beta-Phe}$H, C$_{\beta-Tyr}$H and C$_{\beta-Cys}$H), 1.87 (s, 3H, C$_{\alpha-Ac}$H). – ^{13}C NMR: (CD$_3$OD, 75.4 MHz) δ = 174.8 (C=O$_{Cys}$), 173.7 (C=O$_{Phe}$), 173.4 (C=O$_{Tyr}$), 172.7 (C=O$_{Ac}$), 172.2 (C=O$_{Gly}$), 171.4 (C=O$_{Gly}$), 157.3 (*p*-C$_{Tyr}$), 138.4 (*i*-C$_{Phe}$), 131.3 (*m*-C$_{Tyr}$), 130.4 (*o*-C$_{Phe}$), 129.9 (*m*-C$_{Phe}$), 128.9 (*i*-C$_{Tyr}$), 127.8 (*p*-C$_{Phe}$), 116.3 (*o*-C$_{Tyr}$), 57.1 (C$_\alpha$-Phe), 56.2 (C$_\alpha$-Cys), 56.1 (C$_\alpha$-Tyr), 43.9 (CH$_2$-Gly), 43.4 (CH$_2$-Gly), 38.5 (C$_\beta$-Phe), 37.8 (C$_\beta$-Tyr), 26.6 (C$_\beta$-Cys), 22.5 (C$_\alpha$-Ac).

[*] Common fragmentation pattern observed with this pentapeptide.

Preparation of (triphenylphosphine)gold-cysteine-enkephalin-thiolate ***9***

The synthesis was carried out in analogy to that of Cys-enkephalin **8**, using Fmoc-Cys(Trt)-Wang resin (300 mg, loading 0.70 mmol/g, Iris Biotech) (**4**). After the trityl deprotection (e above) the resin was dried and transferred into a schlenk under argon. (f) *Gold complexation*: To the reaction vessel was added DCM (2 mL) and DIPEA (10 equivalents) followed by the desired gold source (5 equivalents). The reaction vessel was placed on the shaker where it was

allowed to react overnight. All further steps were carried out under argon. Filtration and washing with three DCM washing sessions completes the complexation. (d) *Cleavage*: After complexation the resin was successively rinsed with DMF and DCM and dried under reduced pressure (1 h, 10 mbar), cleavage from resin was performed with a TFA mixture (5 % TIS, 10 % phenol and 85 % TFA, 3 mL, 3 h). The suspension was filtered and the resin washed with TFA (2×1.5 mL). The combined TFA solutions were poured into cold ether (10 mL, −30 °C), and the suspension was centrifuged (8000 rpm, 6 min). After decanting the supernatant, the crude product was washed with cold ether (2×10 mL), dissolved in water, filtered and lyophilised, yielding a peach solid. Unfortunately subjection to HPLC resulted in decomposition of the synthesized product and all further characterization was carried out without HPLC purification. Yield: (73 %). *Analytical Data for* $C_{45}H_{47}N_5O_8PSAu$ (1045.90). – MS (FAB, pos): *m/z* = 1046 $[M]^+$, 721 $[Ph_3P–Au–PPh_3]^+$, 588 $[M + H – AuPPh_3]^+$, 459 $[Au–PPh_3]^+$). – MS (ESI pos., MeOH): *m/z* = 1046 $[M]^+$, 721 $[Ph_3P–Au–PPh_3]^+$, 610 $[M – AuPPh_3 + Na]^+$, 459 $[AuPPh_3]^+$. – ^{1}H NMR (CD_3OD, 360.1 MHz): δ = 7.52 – 7.35 (m, 15H, P*Ph*$_3$), 7.26 – 7.19 (m, 5H, ArH_{Phe}), 7.02 (dd, 1H, $H_{Tyr,ar}$), 6.68 (dd, 1H, $H_{Tyr,ar}$), 4.71 – 4.62 (m, 1H, $C_{\alpha-Phe}H$), 4.45 – 4.38 (m, 2H, $C_{\alpha-Tyr}H$ and $C_{\alpha-Cys}H$), 3.84 – 3.64 (m, 4H, Gly-$CH_2 \times 2$), 2.98 – 2.78 (overlapping m, 6H, $C_{\beta-Phe}H$, $C_{\beta-Tyr}H$ and $C_{\beta-Cys}H$), 1.75 (s, 3H, $C_{\alpha-Ac}H$), 1.29 (s, 9H, $C(CH_3)_3$). – ^{13}C NMR (CD_3OD, 90.6 MHz): δ = 138.4 (*i*-C_{Phe}), 135.3 (d, *J* = 13.9 Hz, *ortho*), 133.4 (d, *J* = 2.4 Hz, *para*), 130.9 (d, *J* = 79.0 Hz, *ipso*), 130.6 (d, *J* = 11.4 Hz, *meta*), 129.9 (*m*-C_{Tyr}), 129.5 (*o*-C_{Phe}), 129.0 (*m*-C_{Phe} and *i*-C_{Tyr}), 127.7 (*p*-C_{Phe}), 116.3 (*o*-C_{Tyr}), 57.0 (C_α-Phe), 56.4 (C_α-Cys), 56.2 (C_α-Tyr), 44.0 ($C(CH_3)_3$), 43.9 (CH_2-Gly), 43.6 (CH_2-Gly), 38.2 (C_β-Phe), 37.8 (C_β-Tyr), 30.7 ($C(CH_3)_3$), 34.5 (C_β-Cys), 22.6 (C_α-Ac). – ^{31}P NMR (CD_3OD, 101.26 MHz): δ = 37.8.

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