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# The Synthesis of Ruthenium and Rhodium Complexes with Functionalized N-Heterocyclic Carbenes and Their Use in Solid Phase Peptide Synthesis

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**Keywords:** Bioorganometallic chemistry / Imidazolium peptides / Metal carbene complexes / NHC ligands / Solid-phase synthesis

While N-heterocyclic carbenes (NHCs) are ubiquitous ligands in catalysts for organic or industrial synthesis, their potential to form stable transition metal complexes has hardly been exploited in metal bioconjugates. In this work, we describe a straightforward synthesis of carboxylato-functionalized imidazolium salts for covalent binding to peptides. Carbene complexes of Ru and Rh were prepared from these imidazolium salts using $Ag_2O$, followed by transmetallation. The neuropeptide [Leu$^5$]-enkephalin (Tyr-Gly-Gly-Phe-Leu) was chosen as a model peptide. Exploratory NMR experiments identified the Ru(*p*-cymene)$Cl_2$ complex of the asymmetrically substituted imidazol-2-ylidene **3b** as the most suitable metal carbene precursor for solid phase peptide synthesis (SPPS). After optimization of the conditions for SPPS, a ruthenium-NHC pseudoenkephalin (dichloro($\eta^6$-*p*-cymene)-[1-methyl-3-(methyl-*p*-benzoyl-Gly-Gly-Phe-Leu-OH)imidazol-2-ylidene]ruthenium(II), **12**) was synthesized from **3b** on solid phase using the 2-Cl-Trt resin and cleavage by 2 % TFA to yield the free carboxylic acid. Peptide **12** was fully characterized by HPLC, $^1$H and $^{13}$C NMR and ESI-MS. Characteristic NMR signals, as well as the isotope pattern of Ru in the ESI-MS, unequivocally confirm the formation of this metal-carbene peptide bioconjugate.



## Introduction

Labelling of biomolecules with organometallic moieties has been used in biochemical and medicinal applications, ranging from biosensors to radioimaging and drugs.[1] One interesting area of the bioconjugate field are organometallic bioconjugates where the metal complex is covalently bound to a biomolecule, for instance DNA, PNA and peptides.[2] In this area, a number of covalent organometallic bioconjugates were synthesized in our group, covering ferrocene- and cobaltocenium peptide and PNA conjugates,[3] as well as cobaltcarbonyl-alkyne peptides[4] and scorpionate derivatives.[5] For such applications, the metalorganic part has to be stable in water and air. Consequently, there is a constant need to explore new ligand systems.

*N*-Heterocyclic carbene (NHC) complexes have been widely investigated in the last decades, mostly due to their potential as catalysts for organic or industrial synthesis.[6] NHCs are isolobal to phosphane ligands but are generally more stable in the presence of oxygen. In view of this, we decided to explore the labelling of bioconjugates with carbene complexes.

Recently, asymmetric silver and gold imidazolium carbene complexes were discovered which show promising antibacterial activity.[7] In spite of the numerous successful application of carbene complexes for organic synthesis, only a few groups have combined small biomolecules with N-heterocyclic carbenes, namely sugars[8] and peptides.[9]

In the work of Xu and Gilbertson,[9] the ligand precursor was an imidazolinium salt which was prepared in an eight-step synthesis and used for solid phase peptide synthesis (SPPS) to form an imidazolinium peptide salt. Complexation with Grubbs' first-generation catalyst could be performed in solution and the formation of a metal carbene was verified by the appearance of characteristic $^1$H NMR resonances. To the best of our knowledge, no further characterisation data were provided and no other metal carbene peptide conjugates have been reported.

In this article, the preparation of functionalized carbene precursors, as well as the successful application of carbene chemistry to SPPS, will be described. Moreover, the synthesis of a ruthenium carbene pseudoenkephalin peptide on solid support and its full characterisation will be presented.

## Results and Discussion

In this work, we focussed on preparing peptide conjugates with a metal carbene moiety using SPPS. We chose the neuropeptide enkephalin as our model peptide, which has the primary sequence Tyr-Gly-Gly-Phe-Leu. Instead of the amino acid tyrosine, we decided to introduce the carbene at the N-terminal position, which resulted in a

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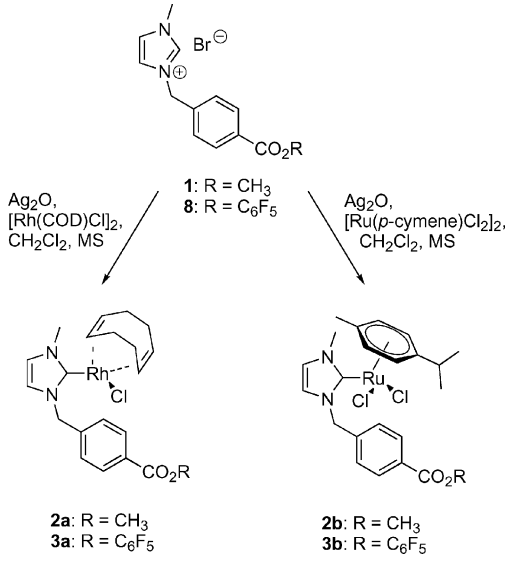
pseudoenkephalin conjugate. For coupling of NHC-type ligands to the N-terminal amino group of a peptide, the ligand has to bear functional groups which can form a bond with amino groups. We therefore concentrated on carboxylate derivatives of the NHC ligand.

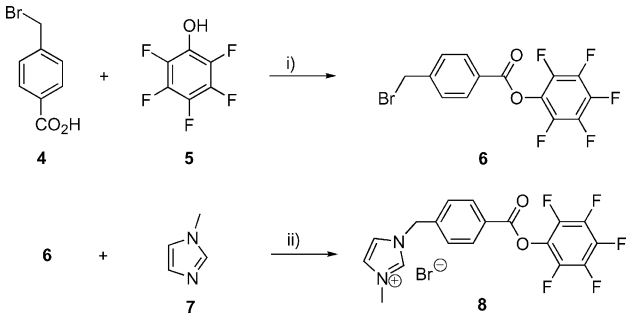
The imidazolium salt **1** (Scheme 1) could be obtained as a white solid in good yields, by refluxing *N*-methylimidazole with methyl *p*-(bromomethyl)benzoate in THF.[10] The 1-{[4-(methoxycarbonyl)phenyl]methyl}-3-methylimidazolium bromide (**1**) was fully characterised. As typical spectroscopic features, the two neighbouring protons of the imidazolium ring (N–C**H**=C**H**–N) show pseudo-triplets due to similar $^3J$ and $^4J$ coupling at $\delta$ = 7.91 and 7.84 ppm in deuterated DMSO. The acidic proton (N–C**H**–N) gives rise to a broad downfield-shifted singlet at $\delta$ = 9.31 ppm. As direct deprotonation of the imidazolium salt failed, the imidazolium salt was first transformed into a silver carbene by using silver(I) oxide ($Ag_2O$), which acts both as base and halide scavenger.[11] The silver NHC complex then easily underwent exchange reactions with binuclear halide-bridged metal complexes of rhodium and ruthenium (Scheme 1).[12] The yellow rhodium dicyclooctadiene chloride carbene complex **2a** and the orange ruthenium *p*-cymene dichloride carbene complex **2b** were thus obtained. Both air-stable compounds were isolated in good yields and fully characterised. A characteristic feature of complexes **2** and **3** is the absence of the downfield-shifted $^1$H NMR signal of the imidazolium ring (N–C**H**–N). The two remaining imidazolium ring protons were now observed as doublets around $\delta$ = 7 ppm with a $^3J$ coupling constant of about 2 Hz, which is in accordance with literature values for similar NHC-carbene complexes.[13]




Scheme 1. Synthesis of the rhodium and ruthenium carbene complexes **2a**, **b** and **3a**, **b**.

With this easy-to-handle procedure for the generation of carbene complexes from functionalized imidazolium salts in hand, we next tried to transfer the conditions to peptide synthesis using imidazolium pseudoenkephalin salts as ligand precursors. Despite several attempts, imidazolium salt **1** could not be hydrolysed to the free carboxylic acid, which would be required for use in SPPS, because it decomposed under the basic conditions necessary for ester hydrolysis. Thus, we set out to synthesise an activated ester imidazolium precursor directly, namely with the pentafluorophenyl group (Pfp).[14]

The carbene precursor **8** was synthesized in a two-step procedure, first starting from *p*-(bromomethyl)benzoic acid (**4**) and pentafluorophenol (**5**) to give the Pfp-ester **6**, then continuing with the same procedure as used for **1** (Scheme 2). A second imidazolium salt was synthesized by refluxing **4** directly with *N*-methylimidazole to yield the imidazolium salt **9** with the free carboxylic acid used in Scheme 3.

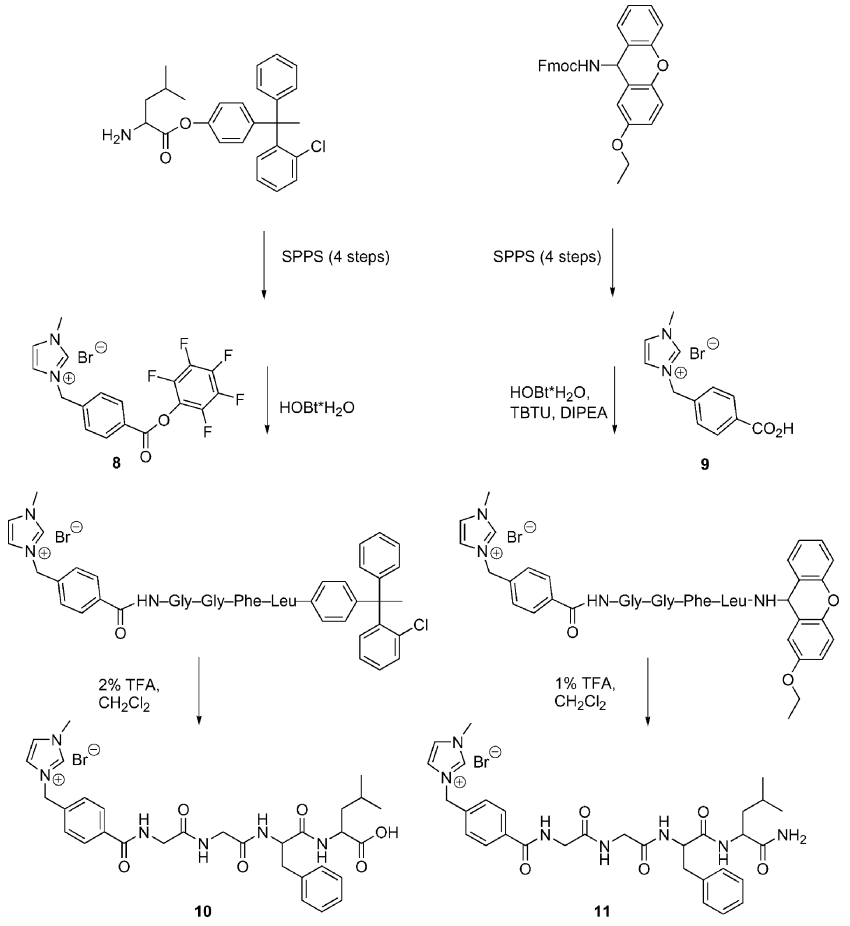



Scheme 2. Synthesis of the Pfp-imidazolium salt **8**. i) DCC, EtOAc/DMF (30:1), 0 °C, 2 h, room temp., 4 h; ii) THF, reflux overnight, $N_2$.

Both imidazolium salts were then employed for solid-phase synthesis. Following a standard SPPS protocol,[3a] the imidazolium pseudoenkephalin salts **10** and **11** were synthesized on two different resins (Scheme 3). Imidazolium salt **8** was coupled to the resin-bound peptide, using HOBt to increase the yield. Imidazolium salt **9** was activated like an amino acid by using HOBt and TBTU in the presence of DIPEA as a base. The 2-chlorotrityl (2-Cl-Trt) and Sieber amide resins were used to yield either the free carboxylic acid or the C-terminal amide after cleavage. In both cases, cleavage from the resin was performed under very mild conditions, taking 1% trifluoroacetic acid (TFA) solution in $CH_2Cl_2$ (v/v) for the Sieber amide or 1% TFA solution in $CH_2Cl_2$ (v/v) for the 2-Cl-Trt resin, respectively. After precipitation with cold diethyl ether and dissolving the crude product in a mixture of acetonitrile and water for lyophilisation, the peptides were purified by preparative HPLC if necessary and fully characterised spectroscopically. The imidazolium peptides **10** and **11** were obtained as ligand precursors, with the free carboxylic acid and amide at the C-terminus.

With these compounds in hand, two different synthetic routes towards metal-carbene peptides were tested. First, the methyl imidazolium peptides were taken as NHC-ligand precursors to be complexed via reaction with $Ag_2O$, followed by transmetallation. Unfortunately, it was impossible to obtain the desired rhodium- and ruthenium carbene




Scheme 3. Synthesis of the 1-methylimidazolium pseudoenkephalins **10** and **11**.

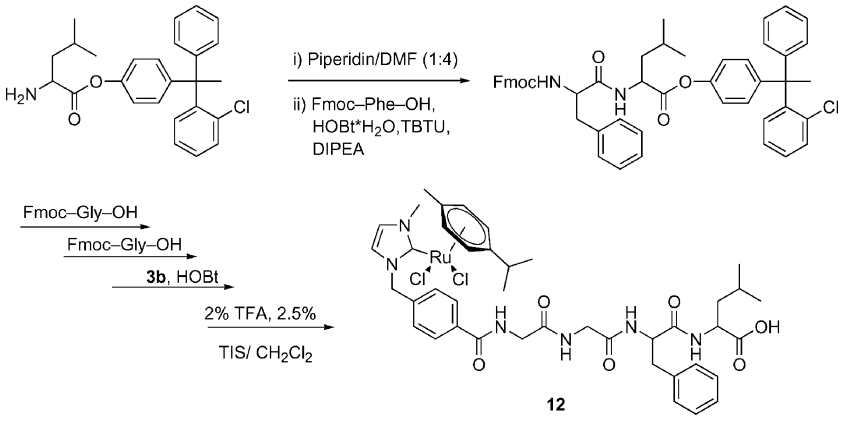
complexes from the NHC-ligand precursors **10** and **11** by this route. No rhodium carbene complexes could be detected, neither for silver carboxylate nor $Ag_2O$ in various solvents at room temperature or under reflux. In the case of the ruthenium carbene pseudoenkephalin, traces of formed carbene complex could be identified with ESI mass spectrometry.

Thus, we focussed on a second entry by direct coupling of Pfp-activated metal carbene complexes to a resin-bound peptide. Thus, the complexes **3a** and **3b** were synthesized following standard procedures (Scheme 1). Both Pfp complexes were fully characterised. The synthesis of metal-carbene pseudoenkephalins was persued by synthesizing the tetrapeptides on the resin, followed by reaction with the rhodium- and ruthenium carbene Pfp-esters **3a** and **3b** (Scheme 1). Cleavage of the peptide from the resin is usually carried out on resins with acid-labile linkers, using concentrated TFA up to 95% (v/v). Therefore, NMR studies in deuterated $CH_2Cl_2$ were performed with the complexes **2a** and **2b** as test systems, increasing the amount of TFA every two hours by 1% (v/v), in order to find conditions suitable for the desired metal conjugates. The rhodium complex **2a** already degraded at the lowest concentration of 1% TFA, giving the initial imidazolium salt precursor **1** as shown by $^1$H NMR spectroscopy. For the ruthenium complex **2b**, 2% TFA showed a degradation of approximately 30% after 4 h which seemed tolerable. Such conditions are well compatible with the 2-Cl-Trt resin. It follows that only the ruthenium carbene complex in combination with the 2-Cl-Trt-resin can be employed in SPPS.

Coupling of the ruthenium complex **3b** to the resin-bound peptide was performed according to the protocol for the imidazolium peptide salts (Scheme 4), using 2.5 equiv. of HOBt and 2.5 equiv. of **3b**. The resin was left on a shaker for 48 h to ensure full conversion. Then, double cleavage was performed with the above-mentioned conditions (compare **2b**) for 2 h each. The combined crude products were precipitated with cold diethyl ether/pentane and dissolved in a mixture of acetonitrile and water for lyophilisation. The purified ruthenium carbene pseudoenkephalin **12** was obtained as a light-orange powder in relatively low yield but in ca. 95% purity as confirmed by analytical HPLC. Thus, it was used without further purifications for spectroscopic measurements.

In order to improve the yield of **12**, NMR experiments were carried out to optimise the cleavage mixture. By leaving the resin for 2 h with 10% TFA in methanol (v/v), followed by a second cleavage with 20% TFA in methanol (v/v), bioconjugate **12** could be obtained in a yield $> 30\%$, and again with $> 95\%$ purity. In our experience, this is a




Scheme 4. Solid-phase synthesis of the ruthenium carbene pseudoenkephalin **12**.

fair yield for organometallic peptide conjugates. In difference to the imidazolium peptide **10**, which has a retention time ($t_R$) of 13.5 min in RP-HPLC, the *p*-cymene ruthenium NHC-pseudoenkephalin **12** has an increased $t_R$ of 16 min. This suggests a higher liphophilicity originating from the organometallic moiety in **12**.

Next, the $^1$H NMR spectra of the imidazolium pseudoenkephalin **10** and the ruthenium carbene pseudoenkephalin **12** were compared. The occurrence of peaks for the *p*-cymene ligand and the absence of the acidic proton of the imidazolium ring (N–C**H**–N) clearly indicates the successful coupling of the ruthenium carbene **3b** to the peptide without decomposition during work-up. Further, the integrals for the *i*Pr group of the first amino acid (Leu) and of the *p*-cymene moiety at the other end of the molecule appear in a 1:1 ratio, which is another proof of the successful synthesis of conjugate **12**.

ESI mass spectrometry (positive ion detection mode) was also performed. For the imidazolium pseudoenkephalin **10**, $[M - Br]^+$ was observed as the major peak at $m/z$ = 591.21 (calculated value $m/z$ = 591.29). The spectrum of conjugate **12** showed two peaks (Figure 1), namely the $[M - Cl]^+$ peak at $m/z$ = 861.15 and the $[M - 2Cl - H]^+$ at $m/z$ = 825.20. These results are in accordance with the calculated values of 861.27 for the $[M - Cl]^+$ and 825.29 for the $[M - 2Cl - H]^+$ peaks. Also, the observed and calculated isotope patterns are in perfect accordance (Figure 1). These cumulated data provide strong support for the formation of ruthenium carbene pseudoenkephalin **12**.

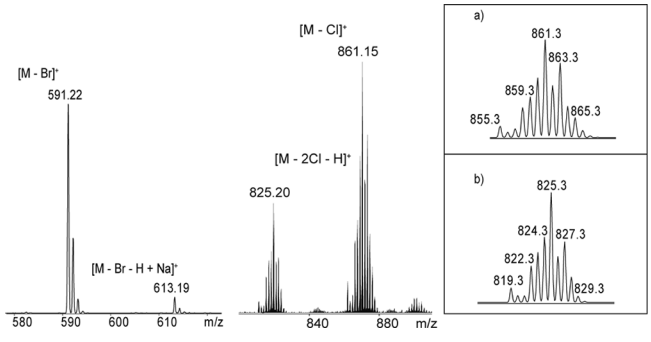



Figure 1. ESI-MS (positive) of the imidazolium pseudoenkephalin salt **10** (left) and the ruthenium carbene pseudoenkephalin **12** (right). a, b): simulated spectra for the peak groups around $m/z$ = 861 ($[M - Cl]^+$) and $m/z$ = 825 ($[M - 2 Cl - H]^+$) of **12**.

## Conclusions

In summary, the present work describes a way to synthesize ruthenium carbene peptide conjugates using solid-phase synthesis without decomposition. The method is exemplified for the *p*-cymene ruthenium NHC pseudoenkephaline derivative **12**. The ESI-MS spectra and NMR spectroscopic data clearly confirm the formation of the ruthenium bioconjugate **12**. Further, the overall yield of the bioconjugate could be increased by careful optimization of the cleavage conditions in preliminary NMR studies. Unfortunately, the successful synthesis for ruthenium conjugate **12** could not be used for the rhodium analogue due to decomposition of the latter complex even under relatively mild cleavage conditions. Future work will concentrate on improving these conditions to make more metal carbene complexes amendable to bioconjugation under SPPS conditions. Given the well-documented cytotoxic activity of *p*-cymene ruthenium compounds such as the RAPTA compounds from Dyson's group,[15] it will be interesting to investigate the biological properties of our new Ru–NHC peptide conjugates.

## Experimental Section

**Abbreviations:** DIPEA = *N*,*N*-diisopropylethylamine, HOBt = 1-hydroxybenzotriazole, Pfp = pentafluorophenyl, SPPS = solid phase peptide synthesis, TBTU = 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate, TFA = trifluoroacetic acid, NHC = N-heterocyclic carbene, 2-Cl-Trt = 2-chlorotrityl, MS = molecular sieves.

**General Remarks:** All reagents were purchased from commercial sources and used as received. H-Leu-2-chlorotrityl-resin (H-Leu-2-Cl-Trt), Sieber amide resin and all amino acid derivatives were purchased from Novabiochem, enantiomerically pure L-amino acids were used throughout. The Fmoc-Leu-Wang resin, DIPEA, HOBt × $H_2O$ and TBTU were purchased from Iris Biotech (Ger-

many). Solvents were distilled from molecular sieves ($CH_2Cl_2$, $CH_3CN$) and over $CaH_2$ (DMF) or taken from the solvent purification system MBraun SPS (THF). Glassware was used oven dry. NMR spectra were recorded on either Bruker DPX 250 ($^1H$ at 250.13 MHz) or on Bruker DRX 400 ($^1H$ at 400.13 MHz) spectrometers. The NMR chemical shifts ($\delta$) are reported in ppm relative to the residual proton chemical shifts of the deuterated solvent set relative to external TMS. Microanalyses were performed on a Analytik Jena multi EA 3100 and on a CE-Instruments EA 1110. – Electrospray ionisation mass spectra (ESI-MS) were recorded with Bruker Esquire 6000. Fast-atom bombardment mass spectra (FAB-MS) were recorded on a Finnigan VG Autospec [glycerol or 3-nitrobenzyl alcohol (NBA) as matrix]. RP-HPLC was performed on a customized HPLC instrument, consisting of Varian ProStar detector (model 330), a Varian solvent delivery system (model 210) and an analytical C-18 Microsorb (4.6 mm × 250, 60 Å/8 μm; Dynamax, Varian) or semipreparative C-18 Microsorb (10 mm × 250 mm, 60 Å/8 μm; Dynamax, Varian) column. Water (A) and acetonitrile (B) were used as solvents, both containing 0.1% TFA. The flow rate was 1 mL min$^{-1}$ (analytical) or 4 mL min$^{-1}$ (semipreparative) and peaks were detected at 254 nm and 220 nm. Typically, gradients were linear from 5% → 95% (B) over 30 min. The test of stability in the presence of TFA was carried out by making a solution of ruthenium and rhodium carbene complex in 500 μL of $CD_2Cl_2$. TFA was then added to the solution starting from 1% to a level of 5% v/v in TFA every 2 h and the $^1H$ spectrum was recorded immediately and subsequently at intervals of 2 h. The ratio of carbene complex to degradation product imidazolium salt was determined by comparison of the integrals. For the use of methanol as cleavage solvent, NMR spectra were measured in $CD_3OD$, adding 1, 3, 5, 10 and 20% (v/v) of TFA every 2 h.

**1-{[4-(Methoxycarbonyl)phenyl]methyl}-3-methylimidazolium Bromide (1):** To a solution of 1-methylimidazole (2.29 g, 10 mmol) in dry THF (10 mL) was added dropwise a solution of methyl *p*-(bromomethyl)benzoate (0.82 g, 10 mmol) in THF (10 mL). After complete addition, the mixture was refluxed for 24 h during which a white precipitate was formed. The solvent was decanted from the precipitate and the solid was washed with THF (3 × 10 mL) and then dried in vacuo to give a white solid (2.42 g, 78% yield). $^1H$ NMR ([D$_6$]DMSO, 200 MHz): $\delta$ = 9.31 (s, 1 H, N-C*H*-N), 7.91 [t, $^3J_{H,H}$ = 1.62 Hz, 1 H, N-C*H*=CH-N], 7.84 (t, $^3J_{H,H}$ = 1.62 Hz, 1 H, N-CH=C*H*-N), 7.99 (AA′*XX*′ N = 8.33 Hz, 2 H, $H_{Ar}$), 7.55 (*AA*′XX′ N = 8.33 Hz, 2 H, $H_{Ar}$), 5.56 (s, 2 H, N-C$H_2$-Ar), 3.88 (s, 3 H, $CO_2CH_3$), 3.85 (s, 3 H, N-C$H_3$) ppm. $^{13}C$ NMR ([D$_6$]-DMSO, 50 MHz): $\delta$ = 165.7 (C=O), 140.0 ($C_{qAr}$), 136.9 (N-*C*H-N), 129.7 ($C_{qAr}$), 129.6 ($C_{Ar}$), 128.4 ($C_{Ar}$), 124.0 (N-*C*H=CH-N), 122.4 (N-CH=*C*H-N), 52.2 ($CO_2CH_3$), 51.2 (N-*C*H$_2$-Ar), 35.9 (N-*C*H$_3$) ppm. MS (FAB pos.): *m*/*z* = 231 [M – Br]$^+$. $C_{13}H_{15}BrN_2O_2$ (311.18): calcd. C 50.18, H 4.86, N 9.00; found C 49.29, H 5.86, N 8.86.

**Rhodium(I) Complex 2a:** To a dried Schlenk tube charged with molecular sieves (4 Å, 100 mg) were added **1** (190 mg, 0.61 mmol) and $Ag_2O$ (74 mg, 0.32 mmol). The mixture was backflashed three times with $N_2$ and then dry $CH_2Cl_2$ was added (30 mL). The flask was closed and shaken for 4 h in the dark. A solution of chloro(1,5-cyclooctadiene)rhodium(I) dimer (150 mg, 0.31 mmol) in $CH_2Cl_2$ was added (10 mL) and the solution was shaken in the dark overnight. The solution was filtered through celite and concentrated in vacuo. The yellow residue was recrystallized from $CH_2Cl_2$/pentane (10 mL/30 mL) at 4 °C. After filtration, a yellow solid was obtained (130 mg, 88% yield). $^1H$ NMR ($CD_2Cl_2$, 400 MHz): $\delta$ = 8.01 (*AA*′XX′, N = 8.29 Hz, 2 H, $H_{Ar}$), 7.47 (AA′*XX*′, N = 8.18 Hz, 2 H, $H_{Ar}$), 6.9 (br. s, 1 H, N-C*H*=CH-N), 6.72 (d, $^3J_{H,H}$ = 1.91 Hz, N-CH=C*H*-N), 5.04 (br. s, N-C$H_2$-Ar), 4.09 (s, 3 H, $CO_2CH_3$), 3.89 (s, 3 H, N-C$H_3$), 3.44–3.20 (m, 2 H, COD), 2.49–1.82 (m, 8 H, COD) ppm. $^{13}C$ NMR ($CD_2Cl_2$, 100 MHz): $\delta$ = 166.6 (*C*OO), 142.1 (N*C*N), 130.1 ($C_{Ar}$), 129.9 ($C_{qAr}$), 128.7 ($C_{Ar}$), 122.9 (N-CH=*C*H-N), 120.5 (N-*C*H=CH-N), 98.7 (N-*C*H$_2$-Ar), 69.2 (COD), 68.2 (COD), 52.1 (CO-O*C*H$_3$), 37.7 (N-*C*H$_3$), 33.2 (COD), 33.1 (COD), 29.3 (COD), 28.9 (COD) ppm. MS (FAB pos.): *m*/*z* = 476 [M]$^+$, 441 [M – Cl]$^+$. $C_{21}H_{26}ClN_2O_2Rh$ (476.81): calcd. C 52.9, H 5.5, N 5.9; found C 48.73, H 5.14, N 5.42.

**Ruthenium(II) Complex 2b:** To a dried Schlenk tube charged with molecular sieves (4 Å, 50 mg) were added **1** (95 mg, 0.31 mmol) and $Ag_2O$ (37 mg, 0.16 mmol). The mixture was backflashed three times with $N_2$ and then dry $CH_2Cl_2$ was added (30 mL). The flask was closed and shaken for 4 h in the dark. A solution of dichloro(*p*-cymene)ruthenium(II) dimer (95 mg, 0.16 mmol) in $CH_2Cl_2$ was added (10 mL) and the solution was shaken overnight in the dark. The solution was filtered through celite and concentrated in vacuo. The orange residue was recrystallized from $CH_2Cl_2$/pentane (10 mL/30 mL) at 4 °C. After filtration, an orange solid was obtained (70 mg, 42% yield). $^1H$ NMR ($CD_2Cl_2$, 250 MHz): $\delta$ = 8.10 (*AA*′XX′, N = 8.46 Hz, 2 H, $H_{Ar}$), 7.36 (*AA*′XX′, N = 8.46 Hz, 2 H, $H_{Ar}$), 7.08 (d, $^3J_{H,H}$ = 2.00 Hz, 1 H, N-C*H*=CH-N), 6.86 (d, $^3J_{H,H}$ = 2.00 Hz, 1 H, N-CH=C*H*-N), 5.35 (br. s, 2 H, $H_{p\text{-cymAr}}$), 4.97 (br. s, 2 H, $H_{p\text{-cymAr}}$), 4.03 (s, 3 H, $CO_2CH_3$), 3.88 (s, 3 H, N-C$H_3$), 2.91 [quint, $^3J_{H,H}$ = 6.90 Hz, 1 H, C*H*(CH$_3$)$_2$], 1.99 (s, 3 H, Ar-C$H_3$), 1.24 [d, $^3J_{H,H}$ = 6.93 Hz, 6 H, CH(C$H_3$)$_2$] ppm. $^{13}C$ NMR ($CD_2Cl_2$, 63 MHz): $\delta$ = 175.5 ($CO_2CH_3$), 166.5 ($C_{qAr}$), 143.0 (N*C*N), 130.0 ($C_{Ar}$), 127.6 ($C_{Ar}$), 124.4 (N-CH=*C*H-N), 122.8 (N-*C*H=CH-N), 109.2 ($C_{p\text{-cymAr}}$), 98.6 ($C_{p\text{-cymAr}}$), 81.8 (N-*C*H$_2$-Ar), 54.5 (CO-O*C*H$_3$), 52.06 (N-*C*H$_3$), 39.68 (*C*H$_3$-Ar), 30.79 [*C*H-(CH$_3$)$_2$], 18.52 [CH(*C*H$_3$)$_2$] ppm. MS (ESI$^+$, $CH_3CN$): *m*/*z* = 501.01 [M – Cl]$^+$. $C_{23}H_{28}Cl_2N_2O_2Ru$ (536.46): calcd. C 51.49, H 5.26, N 5.22; found C 50.22, H 6.19, N 5.11.

**Rhodium(I) Complex 3a:** To a dried Schlenk tube, charged with molecular sieves (4 Å, 100 mg), were added **8** (283 mg, 0.61 mmol) and $Ag_2O$ (74 mg, 0.32 mmol). The mixture was backflashed three times with $N_2$ and then dry $CH_2Cl_2$ was added (30 mL). The flask was closed and shaken for 4 h in the dark. A solution of chloro(1,5-cyclooctadiene)rhodium(I) dimer (150 mg, 0.31 mmol) in $CH_2Cl_2$ was added (10 mL) and the solution was shaken overnight in the dark. The solution was filtered through celite and concentrated in vacuo. The yellow residue was crystallized from $CH_2Cl_2$/pentane (10 mL/30 mL) at 4 °C. After filtration, a yellow solid was obtained (350 mg, 93% yield). $^1H$ NMR ($CD_2Cl_2$, 250 MHz): $\delta$ = 8.19 (*AA*′XX′, N = 8.36 Hz, 2 H, $H_{Ar}$), 7.60 (AA′*XX*′, N = 8.36 Hz, 2 H, $H_{Ar}$), 6.92 (d, $^3J_{H,H}$ = 1.85 Hz, 1 H, N-C*H*=CH-N), 6.73 (d, $^3J_{H,H}$ = 1.85 Hz, 1 H, N-C*H*=CH-N), 4.96 (s, 2 H, N-C$H_2$-Ar), 4.11 (s, 3 H, N-C$H_3$), 3.43–3.43 (m, 2 H, COD), 2.54–2.23 (m, 4 H, COD), 1.96–1.80 (m, 6 H, COD) ppm. $^{13}C$ NMR ($CD_2Cl_2$, 63 MHz): $\delta$ = 162.3 (*C*OO), 144.2 (N*C*N), 131.1 ($C_{Ar}$), 128.6 ($C_{Ar}$), 126.6 ($C_{q,Ar}$), 123.1 (N-CH=*C*H-N), 120.4 (N-*C*H=CH-N), 98.8 (N-*C*H$_2$-Ar), 68.47 (COD), 68.24 (COD), 37.71 (N-*C*H$_3$), 33.11 (COD), 32.71 (COD), 29.01 (COD), 28.69 (COD) ppm. $^{19}F$ NMR ($CD_2Cl_2$, 235 MHz): $\delta$ = –153.1 (d, $^3J_{F,F}$ = 17.1 Hz, 2 F, $Ar_F$), –158.9 (t, $^3J_{F,F}$ = 21.6 Hz, 1 F, $Ar_F$), –163.2 (dd, $^3J_{F,F}$ = 16.9, 21.6 Hz, 2 F, $Ar_F$) ppm. MS (ESI$^+$, $CH_3CN$): *m*/*z* = 593.04 [M – Cl]$^+$. $C_{26}H_{23}ClF_5N_2O_2Rh$ (628.83): calcd. C 49.66, H 3.69, N 4.45; found C 50.13, H 4.42, N 5.11.

**Ruthenium(II) Complex 3b:** To a dried Schlenk tube charged with molecular sieves (4 Å, 100 mg) were added **8** (283 mg, 0.61 mmol) and $Ag_2O$ (74 mg, 0.32 mmol). The mixture was backflashed three

times with $N_2$ and then dry $CH_2Cl_2$ was added (30 mL). The flask was closed and shaken for 4 h in the dark. A solution of dichloro(*p*-cymene)ruthenium(II) dimer (190 mg, 0.31 mmol) in $CH_2Cl_2$ was added (10 mL) and the solution was shaken overnight in the dark. The solution was filtered through celite and concentrated in vacuo. The orange residue was crystallized from $CH_2Cl_2$/pentane (10 mL/30 mL) at 4 °C. After filtration, an orange solid was obtained (256 mg, 61% yield). $^1$H NMR ($CD_2Cl_2$, 250 MHz): $\delta$ = 8.18 (*AA′*XX′, N = 8.51 Hz, 2 H, $H_{Ar}$), 7.48 (AA′*XX′*, N = 8.51 Hz, 2 H, $H_{Ar}$), 7.01 (d, $^3J_{H,H}$ = 2.01 Hz, 1 H, N-C*H*=CH-N), 6.86 (d, $^3J_{H,H}$ = 2.01 Hz, 1 H, N-C*H*=CH-N), 5.39 (AA′*XX′*, N = 4.40 Hz, 2 H, $H_{p\text{-cymAr}}$), 5.03 (*AA′*XX′, N = 4.40 Hz, 2 H, $H_{p\text{-cymAr}}$), 4.04 (s, 2 H, N-C$H_2$-Ar), 2.93 [quint, $^3J_{H,H}$ = 6.92 Hz, 1 H, C*H*(CH$_3$)$_2$], 2.02 (s, 3 H, Ar-C$H_3$), 1.26 [d, $^3J_{H,H}$ = 6.93 Hz, 6 H, CH(C$H_3$)$_2$] ppm. $^{13}$C NMR ($CD_2Cl_2$, 63 MHz): $\delta$ = 175.8 (*C*OO), 162.3 ($C_{qAr}$), 145.1 (N*C*N), 131.0 ($C_{Ar}$), 128.5 ($C_{Ar}$), 126.5 ($C_{qAr}$), 124.6 (N-CH=*C*H-N), 122.6 (N-*C*H=CH-N), 109.7 ($C_{p\text{-cymAr}}$), 98.8 ($C_{p\text{-cymAr}}$), 82.0 (N-*C*H$_2$-Ar), 54.5 (N-*C*H$_3$), 39.7 (*C*H$_3$-Ar), 30.9 [*C*H(CH$_3$)$_2$], 18.6 [CH(*C*H$_3$)$_2$] ppm. $^{19}$F NMR ($CD_2Cl_2$, 235 MHz): $\delta$ = –153.1 (d, $^3J_{F,F}$ = 17.1 Hz, 2 F, Ar$_F$), –158.9 (t, $^3J_{F,F}$ = 21.7 Hz,1 F, Ar$_F$), –163.2 (dd, $^3J_{F,F}$ = 16.3, 21.7 Hz, 2 F, Ar$_F$) ppm. MS (ESI$^+$, $CH_3CN$): *m*/*z* = 653.02 [M – Cl]$^+$. $C_{28}H_{25}Cl_2F_5N_2O_2Ru$ (688.49): calcd. C 48.85, H 3.66, N 4.07; found C 49.24, H 4.76, N 4.29.

**Pentafluorophenyl 4-(Bromomethyl)benzoate (6):** To an ice-cooled solution (0 °C) of 4-(bromomethyl) benzoic acid (2.15 g, 10 mmol) and pentafluorophenol (1.84 g, 10 mmol) in a mixture of ethyl acetate/DMF (30 mL/1 mL) was added *N*,*N*′-dicyclohexylcarbodiimide (2.06 g, 10 mmol). The mixture was stirred at 0 °C for 1 h and then warmed to room temperature. After 2 h, the formed dicyclohexylurea was filtered off and the solvent was removed in vacuo to give **6** as a white solid (1.57 g, 41% yield). $^1$H NMR ([D$_6$]-DMSO, 250 MHz): $\delta$ = 8.17 (*AA′*XX′, N = 8.11 Hz, 2 H, $H_{Ar}$), 7.72 (AA′*XX′*, N = 8.11 Hz, 2 H, $H_{Ar}$), 4.82 (s, 2 H, Br-C$H_2$-Ar) ppm. $^{13}$C NMR ([D$_6$]DMSO, 63 MHz): $\delta$ = 145.7 (*C*OO), 130.8 ($C_{Ar}$), 125.4 ($C_{Ar}$), 32.6 (Br-*C*H$_2$-Ar) ppm. $^{19}$F NMR ([D$_6$]DMSO, 235 MHz): $\delta$ = –153.5 (d, $^3J_{F,F}$ = 19.10 Hz, 2 F, Ar$_F$), –157.5 (t, $^3J_{F,F}$ = 23.2 Hz,1 F, Ar$_F$), –163.2 (dd, $^3J_{F,F}$ = 19.1, 23.2 Hz, 2 F, Ar$_F$) ppm. MS (FAB pos.): *m*/*z* = 383 [M – Br]$^+$. $C_{14}H_6BrF_5O_2$ (381.10): calcd. C 44.12, H 1.59; found C 45.83, H 2.38.

**Imidazolium Bromide 8:** To a solution of 1-methylimidazole (0.25 g, 3 mmol) in dry THF (10 mL) was added dropwise a solution of **6** (1.14 g, 3 mmol) in THF (10 mL). After complete addition, the mixture was refluxed for 24 h during which a white precipitate formed. The solvent was decanted from the precipitate and the solid was washed with THF (3 × 10 mL) and then dried in vacuo to give a white solid (1.09 g, 78% yield). $^1$H NMR ([D$_6$]DMSO, 250 MHz): $\delta$ = 9.31 (s, 1 H, N-C*H*-N), 8.23 (*AA′*XX′, N = 8.54 Hz, 2 H, $H_{Ar}$), 7.85 (s, 1 H, N-C*H*=CH-N), 7.79 (s, 1 H, N-CH=C*H*-N), 7.69 (AA′*XX′*, N = 8.54 Hz, 2 H, $H_{Ar}$), 5.64 (s, 2 H, N-C$H_2$-Ar), 3.89 (s, 3 H, N-C$H_3$) ppm. $^{13}$C NMR ([D$_6$]DMSO, 63 MHz): $\delta$ = 142.3 (*C*OO), 137.0 (N-*C*H-N), 130.9 ($C_{Ar}$), 129.1 ($C_{Ar}$), 125.9 ($C_{qAr}$), 124.1 (N-*C*H=CH-N), 122.4 (N-CH=*C*H-N), 51.2 (N-*C*H$_2$-Ar), 35.9 (N-*C*H$_3$) ppm. $^{19}$F NMR ($CD_2Cl_2$, 235 MHz): $\delta$ = –153.6 (d, $^3J_{F,F}$ = 19.1 Hz, 2 F, Ar$_F$), –157.3 (t, $^3J_{F,F}$ = 23.2 Hz, 1 F, Ar$_F$), –162.2 (dd, $^3J_{F,F}$ = 19.1, 23.2 Hz, 2 F, Ar$_F$) ppm. MS (FAB pos.): *m*/*z* = 847 [2M – Br]$^+$, 383[M – Br]$^+$. $C_{18}H_{12}BrF_5N_2O_2$ (463.20): calcd. C 46.67, H 2.61, N 6.05; found C 46.22, H 3.78, N 6.08.

**Imidazolium Bromide 9:** To a solution of 1-methylimidazole (1.67 mL, 21 mmol) in dry THF (20 mL) was added dropwise a solution of *p*-(bromomethyl)benzoic acid (2.15 g, 10 mmol) in THF (10 mL). After complete addition, the mixture was refluxed for 24 h during which a white precipitate was formed. The solvent was decanted from the precipitate and the solid was washed with THF (3 × 10 mL) and then dried in vacuo to give a white solid (1.96 g, 66% yield). $^1$H NMR ([D$_6$]DMSO, 200 MHz): $\delta$ = 9.39 (s, 1 H, N-C*H*-N), 7.96 (AA′*XX′* N = 8.33 Hz, 2 H, $H_{Ar}$), 7.87 (t, $^3J_{H,H}$ = 1.62 Hz, 1 H, N-C*H*=CH-N), 7.79 (t, $^3J_{H,H}$ = 1.62 Hz, 1 H, N-CH=C*H*-N), 7.54 (*AA′*XX′ N = 8.33 Hz, 2 H, $H_{Ar}$), 5.58 (s, 2 H, N-C$H_2$-Ar), 3.85 (s, 3 H, N-C$H_3$) ppm. $^{13}$C NMR ([D$_6$]DMSO, 50 MHz): $\delta$ = 168.8 (*C*OO), 139.4 ($C_{qAr}$), 136.8 (N-*C*H-N), 131.0 ($C_{qAr}$), 129.7, ($C_{Ar}$), 128.3 ($C_{Ar}$), 124.0 (N-*C*H=CH-N), 122.4 (N-CH=*C*H-N), 51.2 (N-*C*H$_2$-Ar), 35.9 (N-*C*H$_3$) ppm. MS (FAB pos.): *m*/*z* = 217 [M – Br]$^+$. $C_{12}H_{13}BrN_2O_2$ (297.15): calcd. C 48.50, H 4.41, N 9.43; found C 48.02, H 4.92, N 10.85.

**Imidazolium Bromide 10:** The peptide, 2-chlorotrityl-resin-bound GlyGlyPheLeu, was synthesized by standard SPPS methods.[3a] The imidazolium salt **8** was then coupled to the resin-bound peptide as described here. Imidazolium salt **8** (5 equiv.) and HOBt × $H_2O$ (5 equiv.) were combined in 2 mL of DMF, mixed vigorously, and left to stand for 5 min. The activated imidazolium salt solution was shaken with the peptide-loaded 2-Cl-Trt resin for ca. 24 h. After coupling of the salt to the oligopeptide, the reaction mixture was filtered, the resin was washed with DMF (3 × 2 mL), $CH_2Cl_2$ (3 × 2 mL), and methanol (3 × 2 mL), shaken for 30 min in methanol, and dried under reduced pressure for ca. 1 h. The product was cleaved from the resin by treatment with a solution of TFA (2 mL, 2% v/v in dichloromethane) for ca. 4 h. The reaction mixture containing the crude imidazolium salt enkephalin was collected, the resin was extracted again with dichloromethane (2 × 2 mL) and the combined filtrates were concentrated under reduced pressure to a volume of ca. 1 mL, at which point cold diethyl ether (ca. 10 mL, –70 °C) and pentane (10 mL) were added to precipitate the peptide conjugate. The mixture was centrifuged and the solvents decanted to collect a white solid. The solid was taken up in cold ether again and centrifuged (2 times). The supernatant was treated again with pentane and the precipitate collected. The collected solids were combined, dissolved in a minimum volume of acetonitrile and water and lyophilised. The crude product was purified by reversed-phase HPLC giving **10** as a white powder (120 mg, 70% based upon the resin loading of 0.86 mmol/g); $C_{31}H_{39}BrN_6O_6$ (670.21). $^1$H NMR ($CD_3CN$, 400 MHz): $\delta$ = 8.66 (s, 1 H, N-C*H*-N), 8.29 (t, $^3J_{H,H}$ = 5.12 Hz, 1 H, N$H_{ArC=O}$), 7.92 (*AA′*XX′, N = 8.27 Hz, 2 H, $H_{Ar}$), 7.70 (t, $^3J_{H,H}$ = 5.55 Hz, 1 H, N$H_{\alpha,Gly}$), 7.53 (d, $^3J_{H,H}$ = 8.36 Hz, 1 H, N*H*-C$_{\alpha Phe}$), 7.43 (AA′*XX′*, N = 8.27 Hz, 2 H, $H_{Ar}$), 7.39 (t, $^3J_{H,H}$ = 1.73 Hz, 1 H, N-C*H*=CH-N), 7.35 (t, $^3J_{H,H}$ = 1.73 Hz, 1 H, N-CH=C*H*-N), 7.31 (d, $^3J_{H,H}$ = 7.76 Hz, 1 H, N*H*-C$_{\alpha,Leu}$), 7.26–7.14 (m, 5 H, $H_{Ar,Phe}$), 5.37 (s, 2 H, N-C$H_2$-Ar), 4.51 (m, 1 H, C$_{\alpha,Phe}$*H*), 4.25 (m, 1 H, C$_{\alpha,Leu}$*H*), 3.94 (dq, $^3J_{H,H}$ = 5.60, $^2J_{H,H}$ = 16.35 Hz, 2 H, ArCO-NHC$_{\alpha,Gly}H_2$), 3.81 (s, 3 H, N-C$H_3$), 3.65 (d, $^3J_{H,H}$ = 5.90 Hz, 2 H, C$_{\alpha,Gly}H_2$), 3.20 (dd, $^3J_{H,H}$ = 4.34, $^2J_{H,H}$ = 14.02 Hz, 1 H, C$_{\beta,Phe}H_2$), 2.97 (dd, $^3J_{H,H}$ = 10.06, $^2J_{H,H}$ = 14.02 Hz, 1 H, C$_{\beta,Phe}H_2$), 1.63–1.48 (m, 3 H, C$_{\gamma,Leu}$*H*C$_{\beta,Leu}H_2$), 0.80 [dd, $^3J_{H,H}$ = 6.18, $^2J_{H,H}$ = 15.95 Hz, 6 H, CH(C$_{\delta,Leu}H_3$)$_2$] ppm. $^{13}$C NMR ($CD_3CN$, 100 MHz): $\delta$ = 174.5 (*C*OO), 172.4, 171.6, 170.3, 168.4 ($C_{Phe,Gly,Gly,Ar}$ON), 138.9 ($C_{q,p\text{-}Ar}$), 138.4 ($C_{q,p\text{-}Ar}$), 137.5 (N-*C*H-N), 135.2 ($C_{q,Ar,Phe}$), 130.2 ($C_{p\text{-}Ar}$), 129.53 ($C_{p\text{-}Ar}$), 129.2 ($C_{Ar,Phe}$), 127.4 ($C_{Ar,Phe}$), 125.0 (N-*C*H=CH-N), 123.4 (N-CH=*C*H-N), 55.9 ($C_{\alpha,Phe}$), 53.2 (N-*C*H$_2$-Ar), 52.2 ($C_{\alpha,Leu}$), 44.8 (ArCO=NH$C_{\alpha,Gly}$), 43.8 ($C_{\alpha,Gly}$), 40.9 ($C_{\beta,Leu}$), 38.0 ($C_{\beta,Phe}$), 36.9 (N-*C*H$_3$), 25.4 ($C_{\gamma,Leu}$), 23.2, 21.7 ($C_{\delta,Leu}$) ppm. RP-HPLC: $t_R$ = 13.52 min. MS (ESI$^+$, $CH_3CN$): *m*/*z* = 591.22 [M – Br]$^+$.

**Imidazolium Bromide 11:** Sieber amide resin-bound GlyGlyPheLeu was synthesized by standard SPPS methods.[3a] The imidazolium

salt **9** was coupled to the resin-bound peptide as described here. Imidazolium salt **9** and HOBt × $H_2O$ were combined in 2 mL of DMF, mixed vigorously, and left to stand for 5 min. The activated imidazolium salt solution was shaken with the peptide-loaded Sieber amide resin for ca. 24 h. After coupling of the salt to the oligopeptide, the reaction mixture was filtered, the resin was washed with DMF (3 × 2 mL), $CH_2Cl_2$ (3 × 2 mL), and methanol (3 × 2 mL) then shaken for 30 min in methanol and dried under reduced pressure for ca. 1 h. The product was cleaved from the resin by treatment with a solution of TFA (2 mL, 1% v/v in dichloromethane) for ca. 4 h. The reaction mixture containing the crude imidazolium salt peptide product was collected, the resin was extracted again with dichloromethane (2 × 2 mL) and the combined filtrates were concentrated under reduced pressure to a volume of ca. 1 mL at which point cold diethyl ether (ca. 10 mL, –70 °C) and pentane (10 mL) were added to precipitate the peptide conjugate. The mixture was then centrifuged and the solvents decanted to collect a white solid. The solid was taken up in cold ether again and centrifuged (2 times). The supernatant was treated again with pentane and the precipitate collected. The collected solids were combined, dissolved in a minimum volume of acetonitrile and water and lyophilised. The crude product was purified by reversed-phase HPLC giving **11** as a white powder (100 mg, 75% based upon the resin loading of 0.68 mmol/g); $C_{31}H_{40}BrN_7O_5$ (669.23). $^1$H NMR ($CD_3CN$, 400 MHz): $\delta$ = 8.92 (s, 1 H, N-C*H*-N), 8.71 (t, $^3J_{H,H}$ = 5.36 Hz, 1 H, N*H*$_{Ar}$C=O), 8.17 (t, $^3J_{H,H}$ = 5.55 Hz, 1 H, NH$_{\alpha,Gly}$), 8.01 (*AA′*XX′, N = 8.36 Hz, 2 H, $H_{Ar}$), 7.78 (d, $^3J_{H,H}$ = 7.76 Hz, 1 H, N*H*-C$_{\alpha Phe}$), 7.48–7.46 (m, 4 H, $H_{Ar}$, N$H_{\alpha,Gly}$, N-C*H*=CH-N), 7.42 (t, $^3J_{H,H}$ = 1.76 Hz, 1 H, N-CH=C*H*-N), 7.32–7.17 (m, 5 H, $H_{Ar,Phe}$), 6.61 (s, 1 H, CON*H*$_2$), 6.10 (s, 1 H, CON*H*$_2$), 5.42 (s, 2 H, N-C*H*$_2$-Ar), 4.45–4.40 (m, 1 H, C$_{\alpha,Phe}$*H*), 4.16–4.10 (m, 1 H, C$_{\alpha,Leu}$*H*), 3.95 (dq, $^3J_{H,H}$ = 5.5, $^2J_{H,H}$ = 16.16 Hz, 2 H, ArCO-NHC$_{\alpha,Gly}$*H*$_2$), 3.67 (d, $^3J_{H,H}$ = 5.68 Hz, 2 H, C$_{\alpha,Gly}$*H*$_2$), 3.63 (s, 2 H, N-C*H*$_3$), 3.22–3.06 (m, 2 H, C$_{\beta,Phe}$*H*$_2$), 1.60–1.45 (m, 3 H, C$_{\gamma,Leu}$*H*C$_{\beta,Leu}$*H*$_2$), 0.74 [dd, $^3J_{H,H}$ = 6.05, $^2J_{H,H}$ = 21.21 Hz, 6 H, CH(C$_{\delta,Leu}$*H*$_3$)$_2$] ppm. $^{13}$C NMR ($CD_3CN$, 100 MHz): $\delta$ = 176.3 (*C*OO), 172.9, 172.7, 171.8, 168.7 (*C*$_{Phe,Gly,Gly,Ar}$ON), 139.7 (*C*$_{q,p\text{-}Ar}$), 139.2 (*C*$_{q,p\text{-}Ar}$), 138.4 (N-*C*H-N), 135.2 (*C*$_{q,Ar,Phe}$), 130.8 (*C*$_{p\text{-}Ar}$), 130.0 (*C*$_{p\text{-}Ar}$), 129.9 (*C*$_{Ar,Phe}$), 128.1 (*C*$_{Ar,Phe}$), 125.7 (N-*C*H=CH-N), 124.0 (N-CH=*C*H-N), 57.5 (*C*$_{\alpha,Phe}$), 53.7 (N-*C*H$_2$-Ar), 53.5 (*C*$_{\alpha,Leu}$), 45.3 (ArCO=NH*C*$_{\alpha,Gly}$), 44.8 (*C*$_{\alpha,Gly}$), 38.2 (*C*$_{\beta,Phe}$), 37.6 (N-*C*H$_3$), 26.0 (*C*$_{\beta,Leu}$), 25.4 (*C*$_{\gamma,Leu}$), 23.2, 21.7 (*C*$_{\delta,Leu}$) ppm. $t_R$ = 12.91 min. MS (ESI$^+$, $CH_3CN$): *m*/*z* = 590.27 [M – Br]$^+$.

**Ruthenium(II) Complex 12:** 2-Chlorotrityl-resin-bound GlyGlyPheLeu was synthesized by standard SPPS methods.[3a] Carbene complex **3b** (2.5 equiv.) and HOBt × $H_2O$ (2.5 equiv.) were combined in 2 mL of DMF, mixed vigorously, and left to stand for 5 min. The activated carbene complex solution was shaken with the resin-bound peptide for ca. 48 h. After coupling of the complex to the oligopeptide, the reaction mixture was filtered, the resin was washed with DMF (3 × 2 mL), $CH_2Cl_2$ (3 × 2 mL), and methanol (3 × 2 mL) then shaken for 30 min in methanol and dried under reduced pressure for ca. 1 h.

**Method A:** The product was cleaved twice from the resin by treatment with a solution of TFA in $CH_2Cl_2$ (2 mL, 2% v/v) for ca. 2 h each.

**Method B:** The product was cleaved from the resin by treatment with a solution of TFA in methanol (2 mL, 5% v/v) for 2 h, followed by a second cleavage cycle for 2 h with a solution of TFA in methanol (2 mL, 10% v/v).

The reaction mixtures containing the crude ruthenium enkephalin carbene product were collected, the resin was extracted again with $CH_2Cl_2$ (2 × 2 mL) and the combined filtrates were concentrated under reduced pressure to a volume of ca. 1 mL at which point cold diethyl ether (ca. 10 mL, –70 °C) and pentane (10 mL) were added to precipitate the peptide conjugate. The mixture was centrifuged and the solvents decanted to collect an orange solid. The solid was re-dissolved in cold ether again and centrifuged (2 times). The supernatant was treated again with pentane and the precipitate collected. The collected solids were combined, dissolved in a minimum volume of acetonitrile and water and lyophilised. The crude product was purified by reversed-phase HPLC yielding the ruthenium carbene pseudoenkephalin as an orange powder (3 mg, Method A, 8 mg, Method B); $C_{41}H_{52}Cl_2N_6O_6Ru$ (896.86). $^1$H NMR ([D$_6$]DMSO, 400 MHz): $\delta$ = 12.52 (br. s, COO*H*), 8.76 (t, $^3J_{H,H}$ = 5.74 Hz, 1 H, N$H_{ArC=O}$), 8.16 (d, $^3J_{H,H}$ = 7.81 Hz, 1 H, N*H*-C$_{\alpha Phe}$), 8.12 (t, $^3J_{H,H}$ = 5.62 Hz, 1 H, N$H_{\alpha,Gly}$), 7.99 (d, $^3J_{H,H}$ = 8.50 Hz, 1 H, N*H*-C$_{\alpha,Leu}$), 7.86 (*AA′*XX′, N = 8.35 Hz, 2 H, $H_{Ar}$), 7.66 (d, $^3J_{H,H}$ = 2.00 Hz, 1 H, N-C*H*=CH-N), 7.33 (d, $^3J_{H,H}$ = 2.00 Hz, 1 H, N-CH=C*H*-N), 7.28–7.14 (m, 2 H, $H_{Ar}$, 5 H, $H_{Ar,Phe}$), 6.23 (d, $^3J_{H,H}$ = 5.72 Hz, 1 H, $H_{Ar,p\text{-}cymene}$), 6.10 (d, $^3J_{H,H}$ = 10.17 Hz, 1 H, $H_{Ar,p\text{-}cymene}$), 6.10 + 5.87 (2 s, 2 H, N-C*H*$_2$-Ar), 5.84 (d, $^3J_{H,H}$ = 9.48 Hz, 1 H, $H_{Ar,p\text{-}cymene}$), 5.45 (d, $^3J_{H,H}$ = 9.48 Hz, 1 H, $H_{Ar,p\text{-}cymene}$), 4.51 (dt, 1 H, C$_{\alpha,Phe}$*H*), 4.23–4.17 (m, 1 H, C$_{\alpha,Leu}$*H*), 3.91 (s, 3 H, N-C*H*$_3$), 3.88 (m, 2 H, C$_{\alpha,Gly}$*H*$_2$), 3.66 (dq, $^3J_{H,H}$ = 5.65, $^2J_{H,H}$ = 16.78 Hz, 2 H, ArCO-NHC$_{\alpha,Gly}$*H*$_2$), 3.06 (dd, $^3J_{H,H}$ = 4.34, $^2J_{H,H}$ = 14.02 Hz, 1 H, C$_{\beta,Phe}$*H*$_2$), 2.81–2.66 [m, 1 H, C$_{\beta,Phe}$*H*$_2$, 1 H, C*H*(CH$_3$)$_2$], 2.06 (s, 3 H, Ar-C*H*$_3$), 1.67–1.50 (m, 3 H, C$_{\gamma,Leu}$*H*C$_{\beta,Leu}$*H*$_2$), 1.13 [dd, $^3J_{H,H}$ = 6.90, $^2J_{H,H}$ = 10.56 Hz, 6 H, CH(C*H*$_3$)$_2$], 0.84 [dd, $^3J_{H,H}$ = 6.45, $^2J_{H,H}$ = 22.06 Hz, 6 H, CH(C$_{\delta,Leu}$*H*$_3$)$_2$] ppm. $^{13}$C NMR ($CD_3CN$, 100 MHz): $\delta$ = 174.5 (*C*OO), 172.4, 171.6, 170.3, 168.3 (*C*$_{Phe,Gly,Gly,Ar}$ON), 138.9 (*C*$_{q,p\text{-}Ar}$), 138.4 (*C*$_{q,p\text{-}Ar}$), 137.5 (N*C*N), 135.2 (*C*$_{q,Ar,Phe}$), 130.2 (*C*$_{p\text{-}Ar}$), 129.5 (*C*$_{p\text{-}Ar}$), 129.2 (*C*$_{Ar,Phe}$), 127.4 (*C*$_{Ar,Phe}$), 125.0 (N-*C*H=CH-N), 123.4 (N-CH=*C*H-N), 55.9 (*C*$_{\alpha,Phe}$), 53.2 (N-*C*H$_2$-Ar), 52.2 (*C*$_{\alpha,Leu}$), 44.8 (ArCO=NH*C*$_{\alpha,Gly}$), 43.8 (*C*$_{\alpha,Gly}$), 40.9 (*C*$_{\beta,Leu}$), 38.0 (*C*$_{\beta,Phe}$), 36.9 (N-*C*H$_3$), 25.4 (*C*$_{\gamma,Leu}$), 23.2, 21.7 (*C*$_{\delta,Leu}$), 21.4 (Ar-*C*H$_3$), 21.2 [*C*H(CH$_3$)$_2$], 17.3 [CH(*C*H$_3$)$_2$] ppm. RP-HPLC: $t_R$ = 15.97 min. MS (ESI$^+$, $CH_3CN$): *m*/*z* = 861.17 [M – Cl]$^+$, 825.21 [M – 2Cl – H]$^+$.

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