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CCK8 Peptide Derivatized with Diphenylphosphine for Rhenium Labelling: Synthesis and Molecular Mechanics Calculations

GIANCARLO MORELLI,[a]* STEFANIA DE LUCA,[a] DIEGO TESAURO,[a] MICHELE SAVIANO,[a] CARLO PEDONE,[a] ALESSANDRO DOLMELLA,[b] ROBERTA VISENTIN[b] and ULDERICO MAZZI[b]

[a] Centro Interuniversitario per la Ricerca sui Peptidi Bioattivi (CIRPEB) e Istituto di Biostrutture e Bioimmagini, CNR, 80134 Napoli, Italy
[b] Dipartimento di Scienze Farmaceutiche, Università di Padova, 35131 Padova, Italy

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Abstract: A novel CCK8 derivative bearing a chelating agent at its *N*- end and its oxo-rhenium(V) complex have been synthesized and characterized. The chelating agent *N*-{*N*-[3-(diphenylphosphino)propionyl]glycyl}cysteine (PN_2S) ligand, the coordination set of which is made by the phosphorus atom of phosphine, the nitrogen atoms of the two amido groups and the sulphur atom of cysteine, has been used due to its high affinity towards the oxo-rhenium(V) moiety. Molecular modelling studies indicate that the CCK8 peptide adopts the right conformation for cholecystokinin receptor binding, and that modifications on the *N*-terminal side of CCK8 obtained by introducing chelating agents and its metal complexes should not affect the interaction with CCK_A receptor.

Keywords: CCK8 derivatives; molecular mechanics calculations; rhenium–peptide complex; solid-phase peptide synthesis

INTRODUCTION

Small radiolabelled compounds such as peptides are a very attractive tool for the diagnosis of several different pathologies [1]. Among the possible biological targets for radiolabelled compounds the cholecystokinin receptors CCK_A-R and CCK_B-R are very promising due to their overexpression in many tumours [2]. These receptors belong to the G-protein coupled receptors (GPCRs) superfamily and are localized in the cell membrane. Moreover, CCK_A-R is overexpressed in pancreatic cancer, while CCK_B-R is found in small cell lung cancer, colon and gastric cancers, medullary thyroid carcinomas, astrocytomas and stromal ovarian tumours. Both CCK_A-R and CCK_B-R have been thoroughly investigated with the aim of characterizing the molecular basis of their interaction with the cholecystokinin peptide hormone [3–5]. Most of the studies focus on the binding mode of the *C*-terminal cholecystokinin octapeptide amide (CCK 26–33 or CCK8) that displays high affinity for both receptors, even if the sulphated form of CCK8 (with a sulphate moiety on the Tyr27 side chain) is 1000-fold more active than the non-sulphated CCK8 in binding to CCK_A-R.

A detailed characterization of the interaction between CCK8 and its receptors is crucial for the development of CCK8 derivatives bearing chelating agents able to coordinate, with high stability, radioactive metals for applications in cancer diagnosis by nuclear medicine techniques. This information would allow the choice of the right position to introduce the chelating agent without interfering with its binding properties.

*Correspondence to: Dr Giancarlo Morelli, Centro Interuniversitario di Ricerca sui Peptidi Bioattivi, via Mezzocannone 6-8, 80134 Napoli, Italy; e-mail: morelli@chemistry.unina.it
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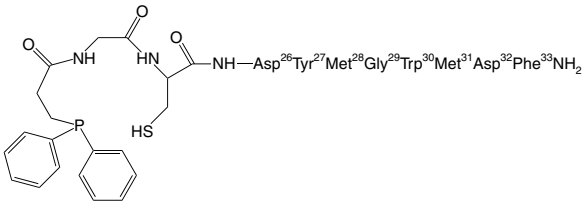
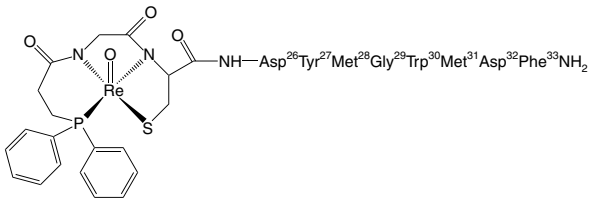
The structural characterization of the bimolecular complex of CCK8 with the 47-residue *N*-terminal extracellular arm of CCK_A-R has been recently achieved by high-resolution NMR and computational refinement [6,7]. The NMR structure of the complex suggests that CCK8 binds to CCK_A-R with the *C*-terminus within the seven-helical bundle of the GPCR and the *N*-terminal side projecting out between the trans-membrane loops 1 and 7 and specifically interacting with the *N*-terminus of CCK_A-R. The structure of the complex also suggests that modifications on the *N*- end of CCK8 obtained by introducing chelating agents and their metal complexes should not affect the interaction with CCK_A-R.

By NMR and fluorescence techniques we have also characterized a CCK8 analogue containing a porphyrin moiety covalently linked through an amide bond to the side-chain of a Lys residue introduced at the *N*-terminus of CCK8 [8]. The results indicate that this CCK8 analogue interacts with the *N*-terminal side of the CCK_A-R 1–47 segment in a fashion similar to that of native CCK8.

Starting from these results we have now synthesized and characterized a novel CCK8 derivative bearing a chelating agent at its *N*- end, and its oxo-rhenium(V) complex (Scheme 1). A chelating agent with high affinity towards the oxo-rhenium(V) moiety is the agent *N*-{*N*-[3-(diphenylphosphino)propionyl]glycyl}cysteine (PN_2S) ligand, the coordination set of which is made by the phosphorus atom of the phosphine, the nitrogen atoms of the two amido groups and the sulphur atom of Cys.



PhosGC-CCK8



ReO-PhosGC-CCK8

Scheme 1

This chelating agent has already been used to complex oxo-rhenium(V) with high stability, as well as for labelling peptides with ^{99m}Tc [9–11]. In published works [9–11] the PN_2S ligand has been coupled to peptides before or after Tc labelling. In the present paper we report the solid-phase synthesis of the CCK8 peptide derivative, modified at its *N*-terminus by introducing a Gly-Cys dipeptide sequence and the diphenylphosphinopropionyl moiety, thus giving a new 10-residue peptide derivative PhosGC-CCK8.

Moreover, in the attempt to investigate the binding of the modified ligand to CCK_A-R, molecular mechanics (MM) and quantum mechanics (QM) calculations were performed on CCK8 and its modified structure ReO-PhosGC-CCK8. In particular, we have tried to estimate the stability of the modified peptide-complex ReO-PhosGC-CCK8, and to evaluate the binding interaction between CCK_A-R and ReO-PhosGC-CCK8.

MATERIALS AND METHODS

Benzotriazole-1-yl-oxy-tris-pyrrolidino phosphonium (PyBop) hexafluorophosphate, 1-hydroxybenzotriazole (HOBt), all Fmoc-amino acid derivatives (Fmoc = 9-fluorenylmethyloxycarbonyl) and the Rink methylbenzhydrylamine (MBHA) resin were purchased from Calbiochem-Novabiochem. All other chemicals were obtained from Aldrich and were used without further purification, unless otherwise stated. Diphenylphosphinopropionylsuccinimide was obtained from Argus Spechem; $(Ph_4P)[ReOCl_4]$ was prepared according to the published procedure [12].

Solid-phase peptide synthesis was performed on a fully automated Shimadzu SPPS-8 synthesizer. Analytical RP-HPLC runs were carried out on a Shimadzu model 10A-LC apparatus using a Phenomenex C_{18} column, 4.6×250 mm, eluted with H_2O/0.1% trifluoroacetic acid (TFA), (A), and CH_3CN/0.1% TFA, (B), linear gradients from 20% to 80% B over 40 min at 1 ml/min flow rate.

Mass spectra were obtained on a MALDI-TOF Voyager-DE Perseptive Biosystem apparatus. Infrared absorption spectra were recorded in the range 4000–500 cm^{-1} on a Nicolet FT-IR mod. 5SXC spectrophotometer as KBr pellets. UV-visible spectra were recorded at room temperature in *N*, *N* dimethylformamide (DMF) on a

Perkin-Elmer Lambda 25 UV/Vis spectrophotometer. Electrospray ionization mass spectra (ESI-MS) were obtained on a Mariner™ API-TOF Biospectrometry Workstation (PerSeptive Biosystem) in the positive-ion mode: the sample was dissolved in DMF (10^{-3} M) and 4 µl of the solution were diluted with 200 µl of 50/50 CH_3CN/H_2O containing acetic acid 1%.

Synthesis of PhosGC-CCK8

The 10-residue GC-CCK8 peptide was synthesized by the solid-phase method under standard conditions using the Fmoc protocol. The Rink MBHA resin (0.54 mmol/g, 54 mmol scale, 0.100 g) was used. Double couplings were performed, by adding each time 4 equivalents of the *N*-protected amino acid activated by PyBop and HOBt and 8 equivalents of *N*, *N*-diisopropylethylamine (DIPEA) in DMF, and stirring for 60 min. When the peptide synthesis was complete, 3-(diphenylphosphinopropionyl) succinimide was coupled at the *N*-terminus by adding 4 equivalents of DIPEA in DMF and stirring for 60 min. For deprotection and cleavage the fully protected phospho-peptide resin was treated with a mixture of trifluoroacetic acid containing tri-isopropylsilane (2.0%), ethandithiol (2.5%) and water (1.5%).

Analytical characterization was carried out by RP-HPLC (PhosGC-CCK8 *R*t: 29.4). Mass spectra carried out on MALDI-TOF confirmed the product identity: 1462 $[M + H]^+$ (m/z) calculated 1461.

Synthesis of Oxo-Rhenium(V) Complex ReO-PhosGC-CCK8

A suspension of PhosGC-CCK8 (4.0 mg, 2.6 µmol) in CH_3CN degassed with argon (2 ml) was added dropwise to a $(Ph_4P)[ReOCl_4]$ solution (2.2 mg, 2.6 µmol) in the same solvent (4 ml) at room temperature. After few minutes, a pale green precipitate separated out and the mixture was stirred for 1 h. The pH of the solution was then increased to 8.5 with triethylamine (Et_3N), thus obtaining a light orange solution. By adding a solution of $NaBPh_4$ (0.9 mg, 2.6 µmol) in CH_3CN (1.5 ml) a precipitate was formed and removed by filtration. The filtrate was then evaporated *in vacuo* and the compound was recrystallized as a brownish powder from CH_3CN/diethyl ether. Yield 73% (3.3 mg, 1.9 µmol). ESI-MS: 851.2345 $[M + H + K]^{2+}$ and 859.2218 $[M + H + K]^{2+}$ (m/z); FT-IR (KBr): $Re = O$ 991.5 cm^{-1}; UV-Vis: λ_{max}: 450 nm.

Molecular Modelling

The main input to such calculation has been the CCK8 structure deposited within the Brookhaven Protein Data Bank [13] under the code 1d6g [6]. The modelling of the $PPh_2(CH_2)_2$-CO-Gly-Cys-modified dipeptide was made using the molecular templates available in the PC Spartan Pro software [14]. The most obvious distortions of the resulting arrangement were removed by running a MM minimization. A full geometry optimization was then performed at the QM level, and the fragment was saved. Next, the $[ReO]^{3+}$ moiety was added by joining (Make Bond utility in PC Spartan Pro) the rhenium atom to the four donor atoms (P, Gly N, Cys N, Cys S_γ) of the modified dipeptide, thus generating the desired rhenium complex. Again, a MM/QM full geometry optimization was performed, and the fragment stored. The latter was linked to CCK8, and the resulting structure saved under the name of ReO-PhosGC-CCK8. The CCK8 arrangement is the one found in the 1d6g structure.

As for the PDB-deposited geometry, the relative positions of CCK_A-R/CCK8 do not match those reported in the original paper [6]. This conclusion was reached by studying the CCK_A-R/CCK8 nonbonding interactions. In the original paper a coulombic 'contact' between Asp^{26} and K37 is quoted; in the Brookhaven structure the Lys N^ζ atom lies 7.5 Å apart from the C^γ atom of the Asp. Also, in the original paper the distance between the C^α atom of Met^{28} and the C^ζ atom of Phe^{33} is reported to be about 5 Å; in the Brookhaven structure this distance is about 10 Å.

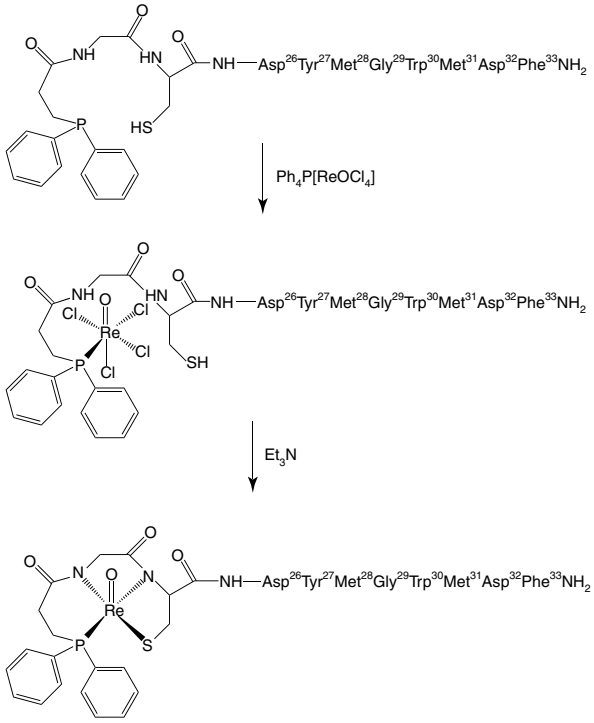
Computational Procedure

All calculations were performed on a Silicon Graphics 320 Workstation powered by two Intel Pentium II 350 MHz processors and operating under Windows NT Version 4.0. Only isolated, 'gas-phase' molecules have been evaluated (*in vacuo*, 'static' calculations). MM calculations were performed with the MMFF94 force field [15]. QM work was made at the PM3-RHF level [16]. All calculations were performed with the PC Spartan Pro Version 1.0.5 program, using software default parameters. We note here that the software lacks the parameters for third-row transition elements at the semiempirical level. Thus, in QM calculations rhenium was replaced by technetium. This approximation is acceptable in our opinion. PC Spartan Pro was also used for all structure visualizations.

RESULTS AND DISCUSSION

Synthesis and Characterization of Peptide Derivative PhosGC-CCK8

The CCK8 peptide analogue functionalized with the phosphine moiety and extended with the two amino acid residues Gly-Cys was synthesized by the solid-phase approach using the Fmoc/tBu chemistry. The final step concerned the covalent binding of the $PPh_2(CH_2)_2$-CO- moiety to the α-amino group of Gly. This reaction is very efficient: only 2.0 equivalents of the phosphine derivative, with its carboxyl function activated as succinimide ester, were sufficient to give a high yield of the conjugated product in a single coupling. To avoid phosphorus oxidation, this coupling step and the final work-up were performed under nitrogen. The peptide derivative PhosGC-CCK8 was isolated in good yield. The analytical data, (MALDI-TOF mass spectrum and HPLC R_t), confirm, respectively, the compound identity and its purity. Due to the good HPLC purity (>90%) of the crude material, the compound was used without further HPLC purification to avoid phosphorus oxidation.



Scheme 2

Synthesis and Characterization of the Oxo-Rhenium(V) Complex ReO-PhosGC-CCK8

The synthesis of the oxo-rhenium(V) complex of PhosGC-CCK8 (Scheme 2) was carried out in acetonitrile at room temperature giving the ReO-PhosGC-CCK8 complex in good yield (>70%). $(Ph_4P)[ReOCl_4]$ was employed as the oxo-rhenium(V) starting material. This compound reacted with 1 equivalent of PhosGC-CCK8 affording, after a few minutes, a pale green precipitate, which could be identified as (Ph_4P)[ReO-PhosGC-CCK8Cl_4]. In this intermediate the phosphine phosphorus is the only atom of the ligand bound to the metal centre. By adding triethylamine (pH 8.5), the solution turns to a light orange colour, due to the formation of the neutral pentacoordinated ReO-PhosGC-CCK8 complex. As the two *N*-terminal amido nitrogens of the decapeptide and the Cys thiol group of the PN_2S chelating system are deprotonated at this pH, a complete coordination of the ligand occurred. The final compound was recovered as a brownish powder by crystallization and characterized by ESI-MS, FT-IR absorption and UV-Vis spectroscopies.

The positive ion ES-MS spectra of the final compound showed a peak exhibiting the characteristic rhenium isotopic pattern at m/z 851.2345, corresponding to the $[M + H + K]^{2+}$ ion, which confirmed the presence of a mononuclear mono-ligand complex. The IR absorption spectrum showed a band at 991.5 cm^{-1} corresponding to the Re = O stretching vibration, within the accepted range for five-coordinate oxo-rhenium(V) complexes (945–1067 cm^{-1}) [10]. FT-IR absorption spectroscopy was also employed for the characterization of the intermediate which exhibits a band at 909 cm^{-1}. The value indicated the presence of a hexa-coordinated complex with a chlorine in *trans* to the oxo oxygen, related to the Ph_4P[ReO-PhosGC-CCK8Cl_4] formulation. The ReO-PhosGC-CCK8 complex exhibited a weak UV-Vis absorption at 450 nm, resulting in the faint brown colour, presumably due to a d → d electronic transition.

The formulation of the coordination type around the oxo-rhenium moiety was conclusively established by comparison with the rhenium complex of the *N*-{*N*-[3-(diphenylphosphino) propionyl]glycyl}-S-benzylcysteine methyl ester ligand which presents the same coordination of the ligand in the equatorial plane [10], and by comparison with the completely characterized ReO-Phos-GC-OMe [17].

The analogous data collected for the rhenium complex with Phos-GC-OMe and Phos-GC-CCK8

confirmed that PhosGC-CCK8 coordinates the oxorhenium(V) moiety throughout the PN_2S set located in the equatorial plane with respect to the Re=O bond, thus leading to a five coordinated complex with the CCK8 moiety not part of the coordination.

Moreover, the ReO-PhosGC-OMe complex exhibited two completely characterized *syn* and *anti* isomers [17] since the methyl ester group can assume a *syn* or *anti* configuration with respect to the oxo-metal group due to the presence of an asymmetric centre on the Re atom.

The ReO-PhosGC-CCK8 complex is expected to present the same two geometrical isomers even though they have not been separated. However, UV-Vis and IR absorption data are in agreement with the results collected from the isomeric mixture of the ReO-PhosGC-OMe complex.

Theoretical Calculations

The theoretical work was carried out to investigate the stability of the complex between the 1–47 *N*-terminal CCK_AR segment and ReO-PhosGC-CCK8. The isomer with the CCK8 in *syn* configuration with respect to the oxo-metal group was selected to perform the theoretical calculations. To simplify the discussion, the amino acids of the natural and modified ligands are termed using the three-letter code (the numbering scheme adopted, 24 → 33, follows that of the 33-residue long full CCK peptide), while the residues of the receptor segment are denoted using the one-letter code. Moreover, the 1–47 *N*-terminal CCK_A-R segment are indicated in the discussion as CCK_A-R. In the first step MM and QM calculations were carried out on the structures of CCK8 and ReO-PhosGC-CCK8. The reason for this effort was to obtain preliminary information a about the stability of the modified ligand. To do this, the MM and QM energies ('potential energies', PE, and heats of formation, H_F) of the two structures before and after a full optimization were studied. The data obtained are not discussed here because a comparison between molecules with a different number of atoms is unwarranted. It is noted, as a mere indication that, as for MM calculations, the energy fall upon optimization is of the same order of magnitude in CCK8 and ReO-PhosGC-CCK8, and that the latter shows starting and final PE higher than those of CCK8. In QM calculations it was found that ReO-PhosGC-CCK8 showed a final H_F much lower than that of CCK8. Our interpretation of these data is that the modified ligand is a stable molecule.

With respect to the arrangements assumed *in vacuo* by CCK8 and ReO-PhosGC-CCK8, the MM-optimized structures were markedly different from that deposited within the PDB (Figure 1). To quantify such differences, the ϕ and ψ values of the PDB-deposited structure were compared with those found in the MM-optimized structures (Table 2). The data indicate that the MM-optimized structure of ReO-PhosGC-CCK8 showed smaller distortions from the experimental values than the MM-optimized structure of CCK8, as witnessed by the average angular deviation in the two structures: 49.6° and 27.6° for CCK8 and ReO-PhosGC-CCK8, respectively. Thus, addition of the rhenium-modified dipeptide to CCK8 narrowed the conformational freedom of the ligand. This finding suggests that even in relatively small peptides an increase in molecular size reduces the tendency to deformation, a circumstance that can positively influence the docking of the modified ligand to CCK_A-R. This observation indicated that modifications of the experimental geometry might be even smaller by performing the MM optimization in the presence of the nonbonding interactions field of CCK_A-R.

To verify such a hypothesis, a MM optimization of the CCK_A-R/CCK8 complex was performed. The optimized structure was manually superimposed at the graphics terminal over the experimental one, looking for the most significant deformations. Although there was a rather large shift of the loop between C18 and C29, and some minor deviations of the domain P35-K37 and of the *C*-terminus of CCK8, the primary binding site of CCK8 (Asp^{26}-Met^{28}) did not appear significantly changed. Encouraged by this result, we proceeded to the optimization of the CCK_A-R/ReO-PhosGC-CCK8 complex.

Again, the ϕ and ψ values were evaluated in the experimental CCK8 structure in comparison with those of ReO-PhosGC-CCK8 in the MM-optimized complex with CCK_A-R (Table 1).

The average angular deviation in the structure of the complexed ReO-PhosGC-CCK8 was slightly smaller than that found in the MM-optimized geometry of ReO-PhosGC-CCK8 alone. Although the size of the effect was smaller than expected, the result showed that our hypothesis was correct. Besides, it was also ascertained that the modified ligand is more rigid and better retains an overall arrangement close to that found experimentally.

This conclusion is also reinforced by comparing the number and the kind of the nonbonding interactions in the experimental structure and in the MM-optimized CCK_A-R/ReO-PhosGC-CCK8

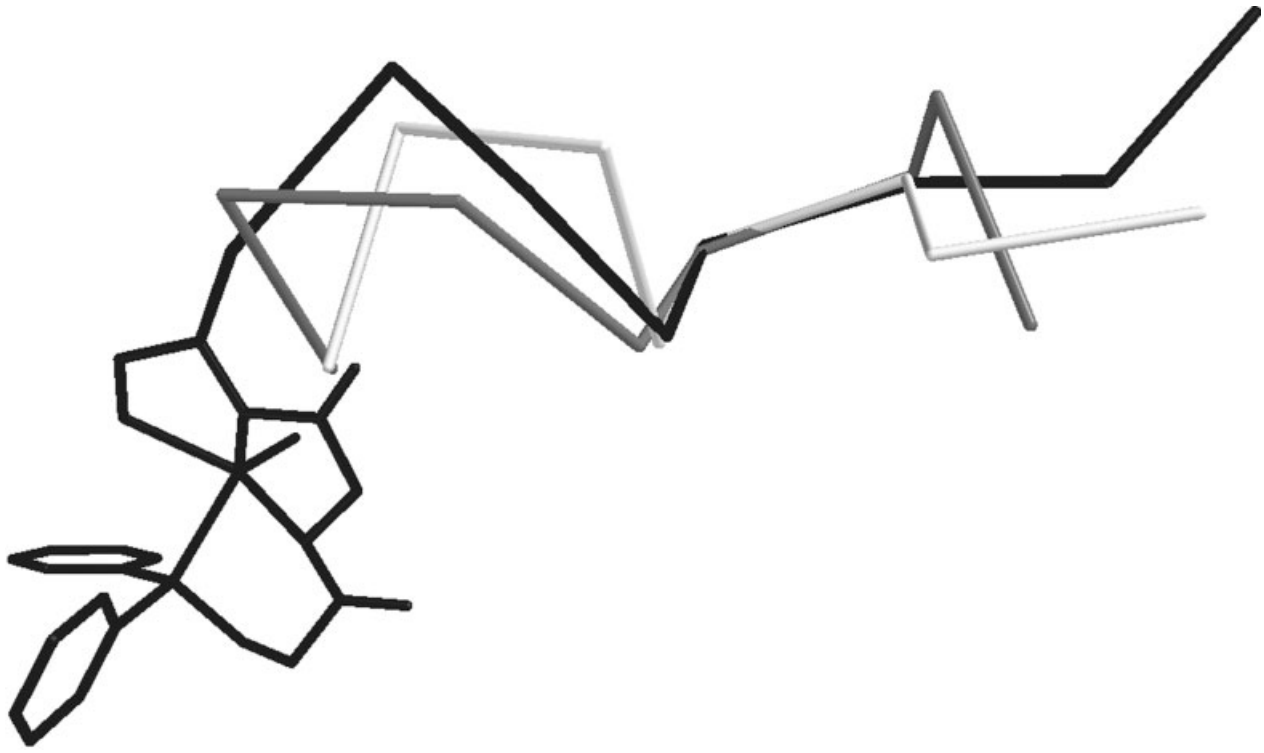


Figure 1 An α-carbon overlay of the experimental CCK8 structure (pale grey) with the MM-optimized arrangements of CCK8 (dark grey) and ReO-PhosGC-CCK8 (black). The rhenium complex position is also shown.

Table 1 Starting (before Optimization) and Final (after Optimization) ϕ and ψ values in the Common Part of the Natural and Modified CCK_A-R Ligands

Torsion angle	CCK8[a]	CCK8-MM[a]	Δ_1[b]	ReO-PhosGC-CCK8-MM[a]	Δ_2[b]	CCK_A-R/ReO-PhosGC-CCK8-MM[a]	Δ_3[b]
$Asp^{26}\psi$	144.4	−124.7	90.9	−178.8	36.8	165.2	20.8
$Tyr^{27}\phi$	−68.6	−74.7	6.1	−85.2	16.6	−81.1	12.5
$Tyr^{27}\psi$	−56.1	−40.1	16.0	−34.8	21.3	−26.7	29.4
$Met^{28}\phi$	−76.1	−116.1	40.0	−82.6	6.5	−72.4	3.7
$Met^{28}\psi$	95.3	−179.2	85.5	65.4	29.9	83.9	11.4
$Gly^{29}\phi$	168.9	78.9	90.0	179.3	10.4	−164.7	26.4
$Gly^{29}\psi$	−53.4	83.5	136.9	−3.5	49.9	−1.2	52.2
$Trp^{30}\phi$	−52.6	−142.9	90.3	−73.6	21.0	−83.3	30.7
$Trp^{30}\psi$	−57.9	−67.4	9.5	−24.7	33.2	−40.0	17.9
$Met^{31}\phi$	−126.0	−152.3	26.3	−154.5	28.5	−165.4	39.4
$Met^{31}\psi$	86.4	22.4	64.0	158.0	71.6	154.1	67.7
$Asp^{32}\phi$	−62.0	−81.3	19.3	−98.0	36.0	−88.2	26.2
$Asp^{32}\psi$	−36.0	−34.8	1.2	−40.4	4.4	−46.7	10.7
$Phe^{33}\phi$	−55.9	−73.6	17.7	−76.6	20.7	−79.0	23.1
Average	—	—	49.6	—	27.6	—	26.6

[a] The columns headed CCK8, CCK8-MM, ReO-PhosGC-CCK8-MM and CCK_A-R/ReO-PhosGC-CCK8-MM report, respectively, the ϕ and ψ values of CCK8 in the experimental structure after MM optimization, ReO-PhosGC-CCK8 after MM optimization, ReO-PhosGC-CCK8 in the MM-optimized complex with CCK_A-R.
[b] The columns headed Δ_1, Δ_2 and Δ_3 report, respectively, the magnitude of change in the ϕ and ψ values upon MM optimization in CCK8, in ReO-PhosGC-CCK8 and in the ReO-PhosGC-CCK8 complexed with CCK_A-R.

complex (Table 2). In the PDB-deposited structure hydrophobic contacts were discovered between Tyr^{27} and P35, W39, Q40; and between Met^{28} and E38, W39, A42, Q43; and between Met^{31} and L46, L47. Coulombic contacts were visible between Q43 and Met^{28}, Gly^{29}, Trp^{30}, Met^{31}; between Met^{28} and L47, and also between Tyr^{27} and Q40. In the CCK_A-R/ReO-PhosGC-CCK8 complex, hydrophobic contacts were seen between Met^{28} and E38, A42, L47; the intermolecular contact between Met^{31} and L46, L47 was modified due to quite strong coulombic interactions involving Phe^{33}, L46, L47. The rather long-ranged coulombic contact between Asp^{26} and K37 (see Materials and Methods) became very tight and induced a marked displacement of the loop P35-K37 with respect to the ligand, thus weakening the hydrophobic contacts between Met^{31} and L46, L47. Instead, Q43 kept its electrostatic interactions with Gly^{29}, Trp^{30}, while Tyr^{27} was shifted half way between W39 and Trp^{30}, where it made a coulombic contact with Q40, but also moved a little farther away from W39.

The above discussion shows that the modified ligand substantially preserves the most important binding interactions, especially those involving Tyr^{27}-Met^{28} and W39-Q40. The overestimation of the charge interactions in the gas phase, although disturbing in principle, allows some contacts to be spotted that have not been mentioned in the reference work [6]. We believe that running an adequately sized molecular dynamics simulation would confirm these 'static' results, i.e. that the modified ligand keeps its ability of docking into the active site because it retains an adequate conformation and because the increased stiffness imposed by modification of the peptide chain seems to favour the interaction with CCK_A-R. It is also noteworthy that in our calculations the *N*-terminus of the modified

Table 2 Analysis of the Nonbonding Interactions between CCK8 and ReO-PhosGC-CCK8 with the *N*-Terminal CCK_A Receptor Segment (CCK_A-R)

CCK_A-R/CCK8		CCK_A-R/ReO-PhosGC-CCK8	
Contact	Distance (Å)	Contact	Distance (Å)
Tyr^{27} $H_{\delta1}$ – P35 $H_{\delta1}$	2.75	Asp^{26} $O_{\delta1}$ – L37 $H_{\zeta2}$	1.83
Tyr^{27} $H_{\varepsilon1}$ – P35 $H_{\delta2}$	2.86	Asp^{26} $O_{\delta2}$ – L37 $H_{\zeta3}$	1.66
Tyr^{27} C_{ζ} – W39 H_{ζ}	2.85	Tyr^{27} $C_{\varepsilon2}$ – Trp^{30} $H_{\varepsilon1}$	2.87
Tyr^{27} C_{γ} – W39 H_{ε}	2.88	Tyr^{27} HH – Q40 O_{ε}	1.68
Tyr^{27} $H_{\varepsilon2}$ – Q40 O_{ε}	2.73	Tyr^{27} $H_{\varepsilon2}$ – Q40 $H_{\beta1}$	2.81
Tyr^{27} $H_{\delta2}$ – Q40 $H_{\beta1}$	2.37	Tyr^{27} $H_{\varepsilon2}$ – Q40 $H_{\gamma2}$	2.81
Met^{28} $H_{\varepsilon2}$ – E38 $H_{\gamma1}$	2.74	Tyr^{27} $H_{\beta2}$ – W39 H_{α}	2.52
Met^{28} $H_{\varepsilon3}$ – E38 $H_{\gamma1}$	2.37	Tyr^{27} $H_{\beta2}$ W39 $H_{\beta1}$	2.34
Met^{28} $H_{\varepsilon2}$ – W39 H_{α}	2.78	Met^{28} $H_{\varepsilon1}$ – L47 $H_{\beta2}$	2.72
Met^{28} $H_{\varepsilon1}$ – A42 $H_{\beta2}$	2.68	Met^{28} $H_{\varepsilon2}$ – A42 $H_{\beta2}$	2.41
Met^{28} $H_{\varepsilon1}$ – Q43 $H_{\varepsilon2}$	2.82	Met^{28} $H_{\varepsilon2}$ – A42 $H_{\beta3}$	2.61
Met^{28} H – Q43 O_{ε}	2.87	Met^{28} $H_{\varepsilon2}$ – E38 $H_{\gamma1}$	2.34
Met^{28} $H_{\varepsilon1}$ – L47 O	2.88	Met^{28} $H_{\varepsilon3}$ – E38 $H_{\gamma1}$	2.80
Gly^{29} H – Q43 O_{ε}	2.56	Met^{28} $H_{\gamma1}$ – E38 $H_{\gamma1}$	2.64
Trp^{30} $H_{\beta1}$ – Q43 O_{ε}	2.92	Gly^{29} H – Q43 O_{ε}	2.58
Met^{31} H – Q43 O_{ε}	2.03	Trp^{30} $H_{\beta1}$ – Q43 O_{ε}	2.60
Met^{31} O – Q43 H_{amide2}	2.20	Trp^{30} $H_{\delta1}$ – Q43 $H_{\gamma2}$	2.64
Met^{31} $H_{\gamma2}$ – Q43 $H_{\varepsilon2}$	2.75	Trp^{30} H – Q43 O_{ε}	1.84
Met^{31} $H_{\varepsilon1}$ – L46 $H_{\beta2}$	2.53	Met^{31} H – Q43 O_{ε}	2.38
Met^{31} $H_{\varepsilon2}$ – L47 OXT	3.00	Met^{31} O – Q43H_{amide2}	1.70
Met^{31} S – L47 H_{α}	2.83	Met^{31} $H_{\varepsilon1}$ – L46 $H_{\delta2}$	2.75
Met^{31} S – L47 H_{γ}	2.73	Met^{31} $H_{\varepsilon2}$ – L46 H_{α}	2.98
Phe^{33} H_{amide1} – L47 OXT	2.91	Met^{31} $H_{\varepsilon2}$ – L46 $H_{\beta2}$	2.65
		Met^{31} $H_{\varepsilon2}$ – L46 H_{γ}	3.04
		Asp^{32} O – L47 HXT	1.67
		Phe^{33} H_{amide1} – L46 O	1.81
		Phe^{33} H_{amide2} – L47 HXT	2.80

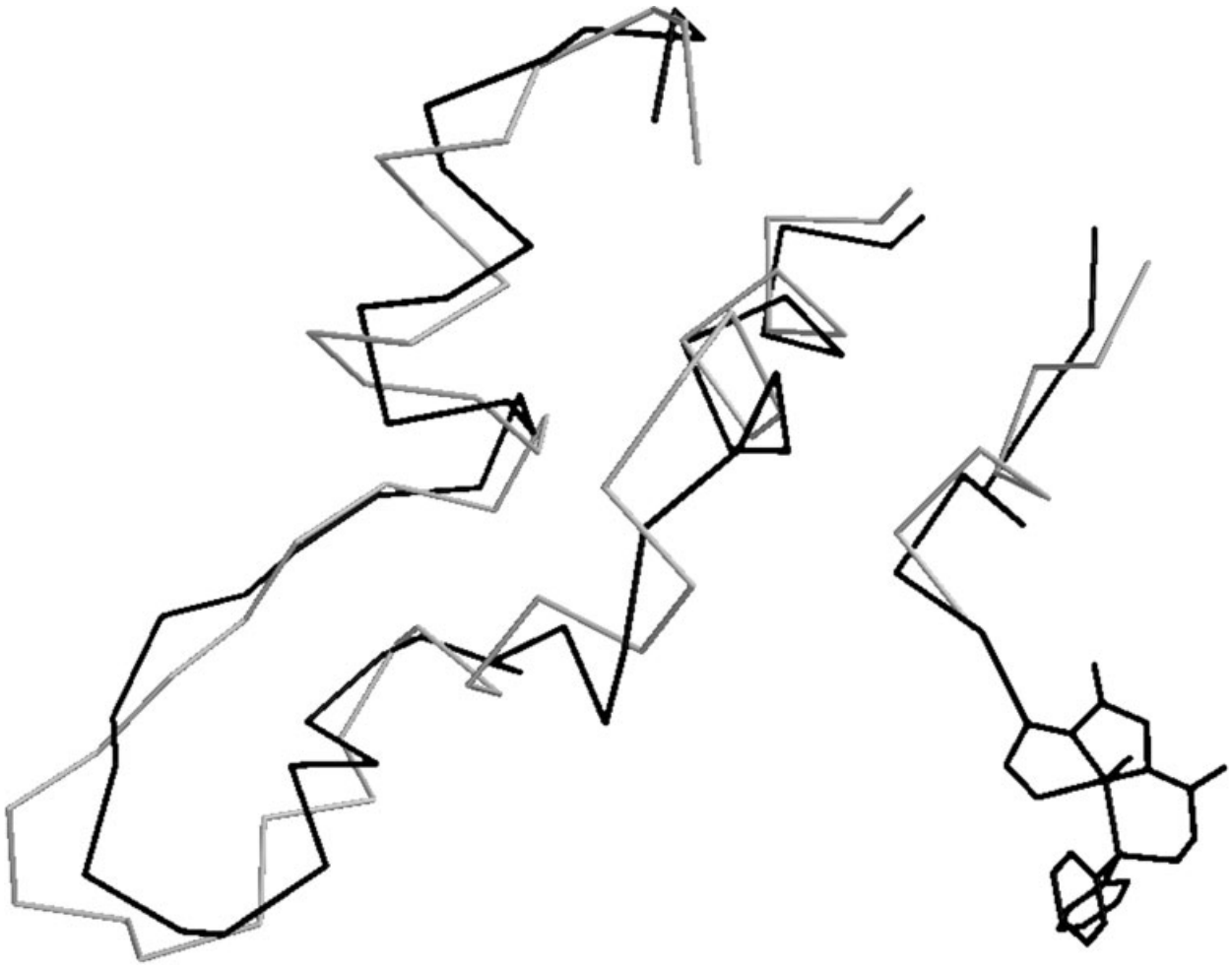


Figure 2 An α-carbon overlay of the PDB-deposited CCK_A-R/CCK8 complex (pale grey) with the MM-optimized arrangement of the CCK_A-R/ReO-PhosGC-CCK8 complex (black). Note that the rhenium complex is pointing away from the receptor.

peptide moves in an opposite direction with respect to the complex CCK_A-R/ReO-PhosGC-CCK8, thus putting the rhenium environment in what would be the aqueous, extracellular domain (Figure 2). This observation would give added value to our system, making easier metal detection in the diagnostic assays.

CONCLUSIONS

A new CCK8 derivative, PhosGC-CCK8, was easily synthesized by solid-phase peptide synthesis. Addition of the phosphine derivative on solid-phase represents a crucial step to obtain a good yield and high purity of the crude material. The spectroscopic data confirmed that PhosGC-CCK8 coordinates the oxo-rhenium(V) moiety through the PN_2S set and that the resulting complex has the same structure of the complex obtained with the chelating system by itself. These results indicate that the peptide derivative could be labelled with the rhenium radioactive isotopes ^{186}Re and ^{188}Re and, in the light of their similar chemical behaviour, also with ^{99m}Tc.

On the other hand, the results of the molecular modelling studies indicate that the CCK8 peptide should adopt the right conformation for CCK-Rs binding, and that modifications on the *N*-terminal side of CCK8, obtained by introducing chelating agents and its metal complexes, should not affect the interaction with CCK_A-R.

The easy synthesis and the theoretical conformational data make the radiolabelled derivatives of PhosGC-CCK8 very attractive for diagnostic (^{99m}Tc) and therapeutic (^{188}Re) applications in tumours overexpressing CCK-Rs. The biological properties of

the radiolabelled derivatives of PhosGC-CCK8 (biological stabilities of the peptide and of the complex) and the preliminary diagnostic tests (*in vitro* studies on cell lines overexpressing the CCK-Rs and *in vivo* studies on nude-mice) are now under evaluation.

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