STRUCTURAL ANALYSIS BY NMR TECHNIQUES OF A $Re(CO)_3$ - PYRAZOLYL CONJUGATE OF CYCLO-(ARG-GLY-ASP-D-TYR-LYS)

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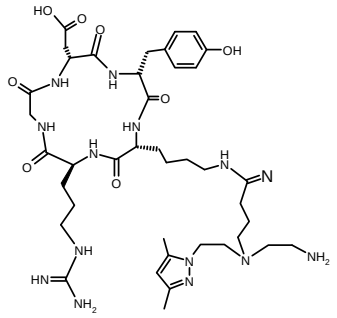
Extensive effort to target integrin receptors upregulated on tumor and neovasculature has been made using radiolabeled peptides containing the Arg-Gly-Asp amino acid sequence (RGD). [1] The $\alpha_V\beta_3$ integrin is known to be overexpressed in many tumor types and is expressed at lower levels in normal tissues. Thus, there is a growing interest to image angiogenesis and tumor formation *in vivo* using RGD-based peptides targeting vectors which selectively binds to the $\alpha_V\beta_3$ receptors. The conjugate ligand PZ-cyclo-(Arg-Gly-Asp-D-Tyr-Lys) (Scheme 1) with high affinity for $\alpha_V\beta_3$ receptors (IC50= 3 nM) has been radiolabeled with the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ and the compound formed, *fac*-$[^{99m}Tc(CO)_3\{\kappa^3$-PZ-cyclo-(Arg-Gly-Asp-D-Tyr-Lys)$\}]^+$ (**1**), still retains biological activity with high specificity and selectivity for the integrin receptors $\alpha_V\beta_3$.[2]



Scheme 1: Conjugate PZ-c(RGDyK)

The main goal of this work is to analyse how the coordination of the metal fragment *fac*-$[M(CO)_3]^+$ (M=Re, Tc) affects the structure of the peptide backbone and to provide more insight into structure-activity relationships. In this communication, we will present the structural characterization of the conjugate PZ-c(RGDyK) and of the complex *fac*-$[Re(CO)_3\{k^3$-PZ-cyclo-(Arg-Gly-Asp-D-Tyr-Lys)$\}]^+$ (**1a**), used as a surrogate of the radioactive compound **1**. These solution studies have been performed using several NMR techniques, namely 2D COSY, 2D TOCSY, 2D HSQC, 2D HMBC and NOESY. Derived NOE constraints and $^3J(H^\alpha,H^N)$ coupling constants were also used to better elucidate the solution peptide conformations.

[1] Liu, S., *Molecular Pharm.,* 3, 473, 2006.
[2] Alves, S; Correia, J.D.G; Gano L.; Rold, T.L.; Prasanphanich, A.; Haubner, R.; Rupprich, M.; Alberto, R.; Decristoforo, C.; Santos, I.; Smith, C.J., *Bioconj. Chem.*, 18, 530, 2007.

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