ISO-STRUCTURAL FLUORESCENT AND ^{99m}Tc PROBES FOR LABELING BIOMOLECULES

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The development of novel radiotracers is often impaired by the time required to determine the uptake and ultimate fate of agents at a cellular level. At present, determining cellular uptake and distribution often involves substituting the radioactive entity with a fluorescent prosthetic group, which allows for fluorescence microscopy to be employed. The major drawback of this approach is that the fluorescent probe, which will have a significantly different structure that the radioactive prosthetic group, can alter the distribution profile relative to that of the compound of interest. A system in which the fluorescent probe and radionuclide prosthetic group are similar in terms of structure and reactivity would be particularly advantageous.

To this end, a family of bifunctional chelates that form fluorescent complexes with Re while also possessing the ability to bind ^{99m}Tc were developed. These iso-structural fluorescent and radioactive ligand systems incorporate a metal binding group into a bifunctional construct that enables the conjugation of the chelate to a variety of biomolecules. One system, synthesized from N-α-Fmoc-L-Lysine, employs a tridentate bisquinoline amine for metal binding while enabling bioconjugation to be performed through the use of conventional automated peptide chemistry. Other related ligands allow conjugation to biomolecules featuring amines and thiols through the use of simple active esters and maleimides.

To demonstrate the utility of such a system, the single amino acid chelate- quinoline (SAACQ) ligand was incorporated into the functional domain of the HIV1-Tat peptide in order to develop complementary agents for studying the tracking of cells via fluorescence and radio-imaging. The $Re(CO)_3$-SAACQ-HIV-Tat and $^{99m}Tc(CO)_3$-SAACQ-HIV-Tat were synthesized in parallel and their uptake into neural stem cells monitored. The synthetic aspects of this research along with cell uptake and distribution studies will be presented. The general utility of the SAACQ strategy will also be highlighted.

Keywords: HIV1-TAT Peptide, Fluorescence, SPECT

CARBORANE DERIVATIVES FOR TARGETING ^{99m}Tc TO NEURORECEPTORS

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Neuroreceptors are an attractive target for ^{99m}Tc-radiopharmaceuticals because of their potential use in characterizing neurological disorders such as Parkinson's disease, schizophrenia, Alzheimer's disease, epileptic seizures and drug addiction. The development of ^{99m}Tc agents that are target-specific requires consideration of the size of the agent, its lipophilicity, selectivity and receptor binding affinity. Complexes possessing the ideal combination of these characteristics must also be able to cross the blood-brain-barrier (BBB).

In the case of ^{99m}Tc, the need to use sterically demanding chelators often limits the ability of viable targeting agents from crossing the BBB. There is a need for compact Tc complexes that are stable *in vivo* and that possess facile bioconjugation chemistry. To this end, a method was developed to prepare organometallic derivatives of WAY type biomolecules, which are designed to bind to serotonin receptors. *Closo*-carborane conjugates were synthesized by one-pot biphasic reaction in high yield using the ionic liquid, 1-butyl-3-methylimidazolium chloride. The corresponding Re(I)-metallocarborane complexes, which are small relative to conventional chelate complexes, were synthesized directly from *closo*-carboranes in water under mild conditions in the presence of sodium fluoride. The ligands were also reacted with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ to yield the target Tc-complexes.

The use of carboranes allows for a great deal of synthetic flexibility in that the length of the spacer group can be easily varied and additional substituents can be added to the carborane to help increase brain uptake and affinity for the target receptor. Synthetic aspects and biological evaluation of the carborane-Tc/Re-WAY derivatives will be presented.

Keywords: Technetium-99m, Carboranes, Neuroreceptors

TECHNETIUM(I) PYRAZOLYL BOMBESIN CONJUGATES: A CONCISE *IN VITRO/IN VIVO* STUDY

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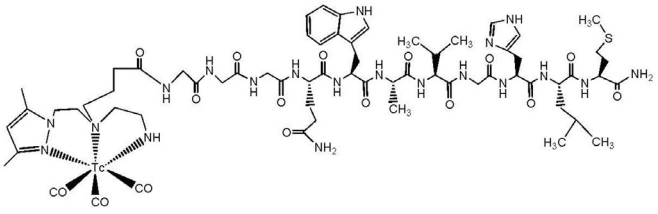
In recent years, our laboratory has focused upon the development of site-directed diagnostic/therapeutic agents based upon bombesin (BBN). Bombesin is a 14 amino acid peptide with very high affinity for the GRP (Gastrin Releasing Peptide) receptor (GRPr). Over-expression of the GRPr on a variety of human cancers (i.e., breast, prostate, pancreatic, small cell lung, ect...) gives some efficacy toward development of radiometallated BBN-derivatives for targetting and, hence, diagnosis/treatment of these specific diseases.

Design and development of specific ligand frameworks for stabilization of the radioactive metal center under *in vivo* conditions continues to be of significant importance toward production of site-directed, peptide-based radiopharmaceuticals. Recently, we have described the synthesis of tridentate, pyrazolyl, bifunctional chelating ligands and their corresponding ^{99m}Tc(I)/Re(I)-tricarbonyl complexes[1]. The versatility of this ligand framework as a novel chelator for the *fac*-M(I)$(CO)_3^+$ moiety was demonstrated utilizing a glycylglycine ethyl ester dipeptide and a derivative of BBN based upon the following structure ([PZ1-G-G-G-Q-W-A-V-G-H-L-M-NH_2], (Figure 1))[1]. The new conjugates demonstrated a degree of *in vitro/in vivo* kinetic inertness suitable for further evaluation in tumor bearing rodent models[1].

Herein, we report the synthesis of new pyazolyl-functionalized bombesin conjugates and subsequent radiolabeling investigations. We have synthesized and characterized (ESI-MS) conjugates of the general type [PZ1-**X**-Q-W-A-V-G-H-L-M-NH_2] where **X** is an aliphatic linking group or a small peptide spacer sequence. A competitive displacement cell binding assay in human PC-3 (Prostate) cancerous cells using ^{125}I-Tyr^4-BBN as the radiolabel demonstrated IC_{50} values in the nanomolar range for all of the new non-metallated conjugates. The new conjugates were radiolabeled with Tc-99m in very high yield (≥90%) *via* the new Isolink® radiolabeling kit. Preliminary *in vivo* evaluation of [^{99m}Tc$(CO)_3$PZ1-**GGG**-Q-W-A-V-G-H-L-M-NH_2] in normal CF-1 mice indicated receptor-specific pancreatic acccumlation of radioactivity (5.80 ± 0.87%ID/g at 1h p.i., n = 5). Tumor accumulation in human PC-3 tumor-bearing rodent models for this new conjugate indicated an average uptake of ~1.76 ± 1.2%ID/g at 1h p.i. (n = 5). A structure activity relationship demonstrating the effectiveness of other linking substituents on *in vivo* tumor accumulation will be reported.

1. Alves, S.; Paulo, A.; Correia, J.D.G.; Gano, L.; Hoffman, T.J.; Smith, C.J.; and Santos, I. "Pyrazolyl Derivatives as Bifunctional Chelators for Labeling Tumor-Seeking Peptides with the *fac*-[M$(CO)_3^+$ Moiety (M = ^{99m}Tc, Re): Synthesis Characterization, and Biological Behavior". *Bioconjugate Chemistry*, (*In Press*, **2005**).

Keywords: Technetium-99m, Pyrazolyl, Bombesin



PN_2S-PEG-BIOMOLECULE DERIVATIVES LABELED WITH TECHNETIUM-99m IN ABSENCE OF AN EXTERNAL REDUCING AGENT

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The PN_2S ligand N-(N-(3-diphenylphosphinopropionyl)glycyl)-S-tritylcysteine was previously conjugated to methoxy-poly(ethylene glycol)-amine, as preface to the use of bifunctional PEGs as linkers. PN_2S-PEGs were found able to easily reduce $^{99m}TcO_4^-$ in absence of an external reducing agent, and to coordinate the reduced species, thanks to a micellar catalysis due to their self-aggregation in water.[1] The labeled compounds exhibited high in vivo stability and a biodistribution dictated by the polymer. These results indicated the new labeling method as an useful tool for the labeling of PN_2S-PEG-biomolecule derivatives.

Based on this, PN_2S(Trt)-PEG-UBI_{29-41} **1**, PN_2S(Trt)-PEG-octreotide **2** and PN_2S(Trt)-HMDA-PEG-biotin **3** were synthesized, to evaluate the labeling method on biomolecules with different solubility and stability. **1 and 2** were obtained using H_2N-PEG-COOH (5 kDa), conjugated first to the ligand through the amino group, and then to the peptide through the carboxylic group (at pH 7 to avoid the conjugation to Lys ε-amino group). **3** was obtained from commercial Biotin-PEG-COOH (5 kDa), bound to the ligand through an hexamethylendiamine (HMDA) moiety. All the couplings were performed by carbodiimide/NHS carboxylic group activation.

The labeling method was evaluated by addition of an acidic $^{99m}TcO_4^-$ solution to detritylated **1-3** in solid form at RT.

Detritylated **1** led to a quantitative yield in a single labeled species in 15 min. The conjugation of PN_2S-PEG to an hydrophilic biomolecule, such as the policationic UBI_{29-41}, just increases its hydrophilic tail, without affecting aggregation and labeling accomplishment. The labeled compound can be recovered as monomer through dilution of the labeling mixture.

Analogously, the labeling of detritylated **2** led to a single species in 20 min. Biodistribution studies showed high accumulation in liver, spleen and lungs, suggesting the presence of stable aggregates seized by the RES. In this case, the conjugate owns two lipophilic heads (PN_2S and biotin) which interact in water giving μm aggregates (light scattering). Though this does not interfere with labeling (eventually favoring it), the lipophilic synergism increases the stability of aggregates, unaffected by dilution.

Concerning **3**, it was supposed that the possible redox reaction between the octreotide S-S bridge and the ligand phosphine could have been disfavored by an in water phosphorous-shielding mediated by the conjugated PEG chain. Also in this case, a single labeled species was obtained, but in a less then 50% yield. It was verified that the P/S-S redox already takes place during the conjugation of PN_2S-PEG-COOH to octreotide, affording the bioconjugate with the phosphorous almost totally oxidized, and therefore not available for TcO_4^- reduction.

These studies showed that the labeling based on a micellar catalysis works in presence of hydrophilic as well as of lipophilic biomolecules. When the biomolecule is lipophilic, stable aggregates can be avoided by replacing the diphenylphosphino moiety with a water soluble analogue. In fact, it was demonstrated that the labeling takes place when the PN_2S is constrained by a supramolecular arrangement, although not included in a "core". On the other hand, peptide bearing a S-S bridge should be replaced with more stable analogues, keeping in mind that the new labeling method is enriched by the possible modulation of pharmacokinetic afforded by PEG.

1- R. Visentin, *et al.* , *Bioconj. Chem.* **2004**, 15: 1046-1054

Keywords: Technetium-99m, BFCA, PEG

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