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Solid-phase synthesis of oxo(mercaptoacetylglycylglycylglycine)rhenate(v)

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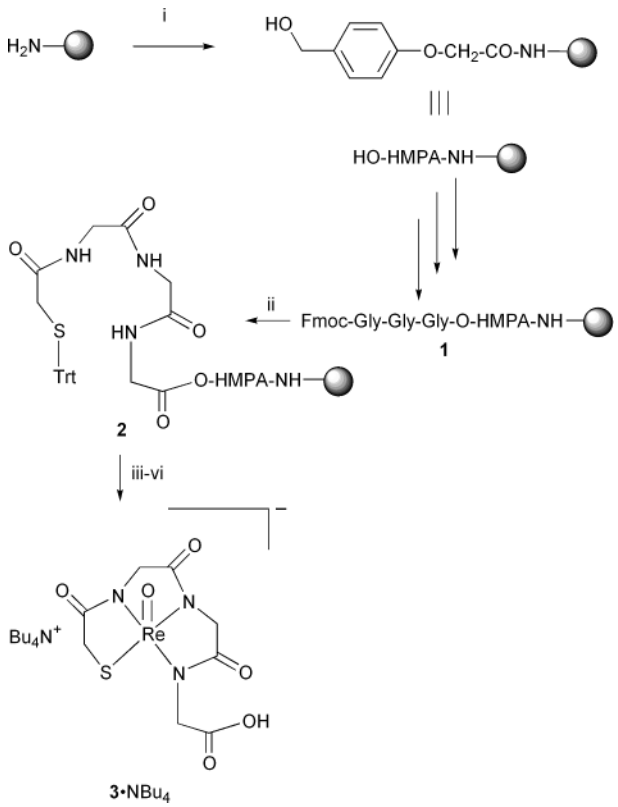
The solid phase synthesis of tetrabutylammonium oxo-(mercaptoacetylglycylglycylglycine) rhenate(v) has been achieved by utilising $ReOCl_3(PPh_3)_2$ as a stable source of Re(v) and provides a synthesis of bi-functional chelates for use in nuclear medicine.

Over the last 10 years, there has been tremendous progress in transferring solution-phase expertise into solid-phase organic synthesis (SPOS). Here, we wish to report the solid-phase synthesis of oxo(mercaptoacetylglycylglycylglycine)rhenate(v) ($ReOMAG_3$)[1] an analogue of ($^{99m}TcOMAG_3$) the widely used renal imaging agent. The advantages of a solid phase approach in this area being the relative ease of synthesis, the ability to directly load the resin with the metal agent of choice, and the prospect of making bead based libraries that can be readily screened using radioactivity to pinpoint the desired ligand. The physical properties of ^{186}Re (1.07 MeV beta max, 3.7 d half life, 137 keV gamma photons (9%) and ^{188}Re (2.12 MeV beta max, 0.9 d half life, 155 KeV gamma photons (15%)) have prompted nuclear medicine chemists to use $^{186/188}Re$-labelled bio-molecules[2] as therapeutic tools in the treatment of cancers. Another important driving force is that Re chemistry can provide a non-radioactive alternative to ^{99m}Tc, the most widely used radionuclide in diagnostic nuclear medicine, when studying the coordination chemistry of Tc.[3] This is due to the lanthanide contraction such that Re and Tc have very similar physical characteristics, although rhenium complexes are harder to reduce and kinetically more inert than those of technetium. In the indirect labelling method, a bifunctional chelate is first metallated and then conjugated to a monoclonal antibody capable of targeting a specific tumour-associated antigen. $ReOMAG_3$ has been the choice of preformed chelate by a number of groups.



Scheme 1 Solid-phase synthesis of $ReOMAG_3$ (**3**). (i) 4-Hydroxymethylphenoxyacetic acid, DIC, HOBt, CH_2Cl_2–DMF (4 : 1), overnight; (ii) (a) 20% piperidine, DMF, (b) Trt-S-CH_2CO_2H, DIC, HOBt, CH_2Cl_2:DMF (4 : 1), 4 h; (iii) 2% TFA, 2% TIPS; (iv) $ReOCl_3(PPh_3)_2$, DBU, DMF, 18 h; (v) 60% TFA, 5% H_2O in CH_2Cl_2, 4 h; (vi) Bu_4NCl, H_2O–CH_2Cl_2.

4-Hydroxymethylphenoxyacetic acid (HMPA) (Scheme 1) was anchored onto TentaGel-S-NH_2 (130 μm, 0.29 mmol g^{-1}, Rapp Polymere) using equimolar amounts of HOBt and DIC.[4] The first Fmoc-Gly residue was attached using a similar procedure but with a catalytic amount of DMAP. Following standard Fmoc chemistry, Fmoc-triglycine (**1**) was synthesised on the solid support. After thoroughly drying the resin was deprotected and subjected to a quantitative ninhydrin test to give a loading of 0.17 mmol g^{-1} (theoretical loading 0.25 mmol g^{-1}). After Fmoc deprotection of **1**, tritylmercaptoacetic acid[5] was coupled to the resin using HOBt and DIC as coupling reagents. The free thiol group was obtained by treating the resin eight times with 2% TFA, 2% TIPS in DCM for 15 min each time. Shorter reaction times were not successful. The resin was then treated with $ReOCl_3(PPh_3)_2$[6] as the most efficient source of Re(v). Optimal conditions were a 1 : 2 molar ratio of Re-$OCl_3(PPh_3)_2$[6] with DBU in DMF for 18 h. The use of higher molar ratios or higher temperatures gave worse results. After

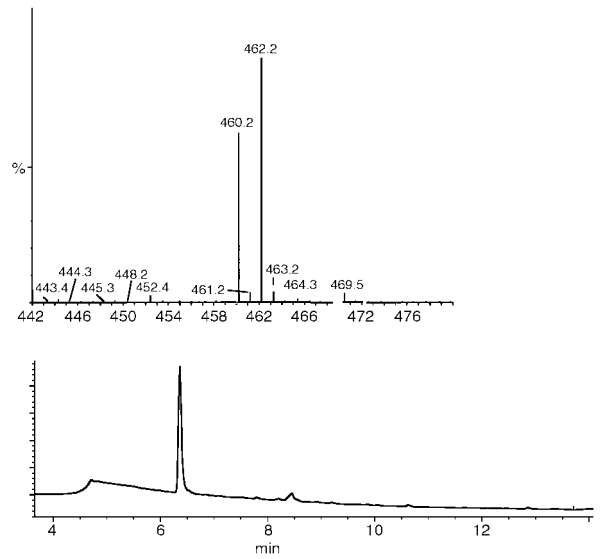


Fig. 1 MS and HPLC analysis of the solid-phase synthesis of $ReOMAG_3$.

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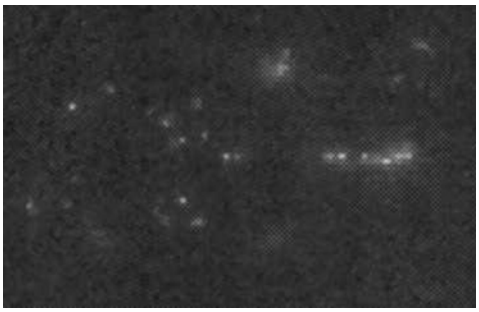

Fig. 2 Phosphoimager scan of resin-bound $^{99m}TcOMAG_3$.

TFA cleavage and counter ion exchange chromatography, $[Bu_4N][ReO(MAG_3)]$ (**3**·NBu_4) was obtained[7] in 91% purity (following RP-HPLC),[8] (Fig. 1). This compound showed identical RP-HPLC retention time, ESMS and IR to a sample prepared in solution following the procedure of Fritzberg *et al*.[9] The same series of reactions was also carried out successfully on aminomethylpolystyrene resin (1% DVB, 1.24 mmol g^{-1}).

To demonstrate the use of resin linked chelates for the screening of resin based libraries of MAG_3 derivatives, a solid phase labelling experiment using MAG_3 as a ligand for ^{99m}Tc was carried-out. In this fashion, Trt-$SMAG_3$ immobilized onto TentaGel-S-NH_2 *via* the HMPA linker was first submitted to trityl deprotection conditions as above and then to $^{99m}TcO_4^{2-}$, sodium gluconate and $SnCl_2$ in saline. After thorough washing of the resin, 45% of the radioactivity was retained. This resin was then swollen in water and carefully plated in a 1% agarose solution poured over a glass surface. Using automatic autoradiography or a Storm Phosphoimager it was possible to localize areas in the gel containing radioactive beads (Fig. 2).

A similar experiment where the thiol deprotection step was intentionally omitted resulted in no ^{99m}Tc complexation, as indicated by the absence of activity in the washed resin. Other controls such as acetylated TentaGel-S-NH_2 submitted to direct labelling conditions ($^{99m}TcO_4$ in saline) or to ligand exchange labelling conditions ($^{99m}TcO_4$, sodium gluconate and $SnCl_2$ in saline) showed no technetium complexation by the polymeric support.

In conclusion the synthesis of tetrabutylammonium oxo-(mercaptoacetylglycylglycylglycine)rhenate(v) has been achieved in high purity by using a preformed Re(v) complex as the source of Re(v). This opens the possibility of screening libraries of ligands for Re affinity and therefore new ligands for binding Tc for medicinal imaging applications.

Notes and references

1 For a solid-phase synthesis of other rhenium(v) oxo complexes see: Y. Shi and S. Sharma, *Bioorg. Med. Chem. Lett*., 1999, **9**, 1469.

2 W. A. Volker and T. J. Hoffman, *Chem Rev*., 1999, **99**, 2269.

3 For reviews see: K. Schwochau, *Angew. Chem., Int. Ed. Engl*, 1994, **33**, 2258; S. Jurisson and J. D. Lydon, *Chem. Rev*., 1999, **99**, 2205; S. Liu and D. S. Edwards, *Chem. Rev*., 1999, **99**, 2235

4 F. Albericio and G. Barany, *Int. J. Peptide Protein Res*., 1985, **26**, 92.

5 D. Brenner, A. Davison, J. Lister-James and A. G. Jones, *Inorg. Chem*., 1984, **23**, 3793.

6 N. P. Johnson, C. J. L. Lock and G. Wilkinson, *Inorg. Synth*., 1967, **9**, 145.

7 Typical procedure: *ca*. 30 mg of resin loaded with Trt-$SMAG_3$ was pre-swollen in CH_2Cl_2 (1mL) for 10 minutes in a peptide vessel. Solvent was removed by applying N_2 pressure. The resin was treated with a solution of 2% TFA, 2% TIPS in CH_2Cl_2 (*ca*. 10 mL × 15 min × 8 times). The resin was washed with CH_2Cl_2 and pre-swollen in DMF for 10 min. After removing excess solvent, a solution of $ReOCl_3(PPh_3)_2$ (23 mg, 28 μmol) and DBU (8.5 μL, 56 μmol) in DMF (3 mL) was added to the resin. After shaking on a mechanical shaker for 12 h at rt, the resin was filtrated and washed thoroughly with DMF, CH_2Cl_2, MeOH and Et_2O. The compound was cleaved from the resin with 60% TFA, 5% H_2O in CH_2Cl_2 for 4 h. TFA was removed *in vacuo* and the residue suspended in H_2O. Bu_4NCl (8 mg, 29 μmol) was added and the compound was extracted into CH_2Cl_2. Negative ESMS: m/z = 462 $[M]^-$. IR: 975 cm^{-1} (Re=O).

8 Analytical HPLC: chromatograms were obtained on a Hewlett Packard HP-1100 system equipped with a Phenomenex Prodigy C18 reverse phase column (3.0 mm × 150 mm). Solvents used were: A: 0.1% TFA in H_2O and B: 0.042% TFA in CH_3CN, gradient 0% B to 100% B over 20 min. The column effluent was monitored using a detector wavelength of 220 nm. The retention time of $ReOMAG_3$ was 6.4 min.

9 T. N. Rao, D. Adhikesavalu, A. Camerman and A. R. Fritzberg, *Inorg. Chim. Acta*, 1991, **180**, 63.