

# A glucosamine–dipicolylamine conjugate of $^{99m}\text{Tc}(\text{I})$ and $^{186}\text{Re}(\text{I})$ for use in imaging and therapy†

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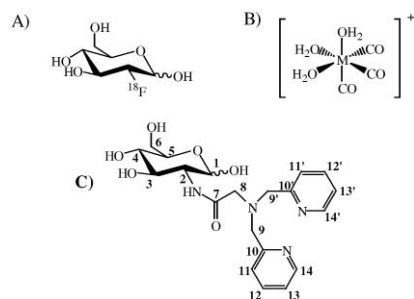
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Received 18th October 2004, Accepted 12th January 2005

First published as an Advance Article on the web 21st January 2005

The synthesis and metal complexation of a glucosamine-appended 2,2'-dipicolylamine ligand to the tricarbonyls of  $^{99m}\text{Tc}$  and  $^{186}\text{Re}$  is described; the ligand was found to bind in a tridentate fashion with the glucosamine function remaining pendant, and the  $^{99m}\text{Tc}$  complex was found to exhibit exceptional stability towards *in vitro* ligand exchange experiments.

Carbohydrates are of primary importance as energy sources for living organisms and thus highly evolved transport and metabolic pathways have been developed for utilization. The use of radiolabelled carbohydrate derivatives takes advantage of these pathways to track energy metabolism as well as imaging regions of interest *in vivo*. Currently 2-deoxy-2-[ $^{18}\text{F}$ ]fluoro-D-glucose (FDG) (Chart 1) is the most widely used carbohydrate-based diagnostic imaging agent. FDG, imaged by positron emission tomography (PET), is utilized for imaging tumours, metastatic tissue, as well as for the assessment of tissue viability in cardiac patients.<sup>1</sup> The short half-life of  $^{18}\text{F}$  (110 min), and the high cost of producing PET isotopes limits the utility of this imaging agent. An effort has therefore been initiated to develop a single photon emission computed tomography (SPECT) analogue utilizing  $^{99m}\text{Tc}$  which has ideal nuclear properties ( $t_{1/2} = 6.01$  h,  $\gamma = 142.7$  keV) and is easily isolated as  $\text{Na}^{99m}\text{TcO}_4$  from a  $^{99}\text{Mo}$  generator. In addition, rhenium, the third row analogue of technetium, has similar chemical properties as well as particle emitting isotopes ( $^{188}\text{Re}/^{186}\text{Re}$ ) for potential use in therapeutic applications.



**Chart 1** A) 2-Deoxy-2-[ $^{18}\text{F}$ ]fluoro-D-glucose (FDG); B) the organometallic precursor  $[\text{M}(\text{H}_2\text{O})_3(\text{CO})_3]^+$  (M = Re,  $^{99m}\text{Tc}$ ); C) Labelling scheme for  $\text{L}^1$ .

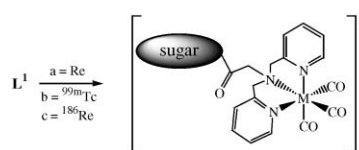
One approach is to attach a chelating ligand to a carbohydrate that, in a subsequent reaction, will bind to the metal center.<sup>2-4</sup>

† Electronic supplementary information (ESI) available: Synthesis and characterisation of  $\text{L}^1$  and characterisation data of  $[\text{Re}(\text{L}^1)(\text{CO})_3]\text{Br}$ . See <http://www.rsc.org/suppdata/dt/b4/b416040a/>

This tactic offers advantages by minimizing the effects of the tracer group and providing a stable and easily characterized complex. Factors that most likely effect recognition *in vivo* include the size of the metal chelate as well as the distance between the chelate and the pendant carbohydrate moiety. In this work we have chosen to use the  $\{\text{M}(\text{CO})_3\}^+$  (M = Tc/Re) core (Chart 1), which has garnered significant interest ever since its development by Jaouen,<sup>5</sup> and Alberto<sup>6</sup> and co-workers. This organometallic core offers advantages in terms of stability, kinetic inertness, and size. In addition, the three labile water molecules are easily exchanged for suitable chelating ligands in a facial arrangement. The tridentate chelator 2,2'-dipicolylamine (containing two pyridines and one tertiary amine) avidly binds to the  $\{\text{Re}(\text{CO})_3\}^+$  core;<sup>7</sup> more recently, this chelate system has been used to attach  $\{\text{Re}(\text{CO})_3\}^+$  as a proof of principle for the preparation of peptide-targeted radiopharmaceuticals,<sup>8</sup> and to prepare molecules to monitor dopamine transporter sites,<sup>9</sup> and image the hepatobiliary system.<sup>10</sup>

In an effort to develop stable carbohydrate-appended imaging agents we have investigated the utility of a glucosamine-appended DPA conjugate  $\text{L}^1$  (Chart 1) as a ligand for the  $\{\text{M}(\text{CO})_3\}^+$  ( $^{99m}\text{Tc}/\text{Re}$ ) core. Glucosamine is an attractive carbohydrate scaffold as the primary amine acts as a site for functionalization. In addition, evidence in the literature increasingly suggests that N-functionalized glucosamines show active transport and accumulation in tumours *in vivo*.<sup>11</sup>

$\text{L}^1$  was synthesized† *via* the amide coupling of glucosamine with bis(2-pyridylmethylamino)acetic acid.<sup>7</sup> As expected,  $\text{L}^1$  was isolated as a mixture of anomers ( $\alpha/\beta$ ) in a ratio of 7 : 3.  $^1\text{H}$  NMR analysis of the crude product of the reaction of  $\text{L}^1$  with  $[\text{NEt}_4]_2[\text{ReBr}_3(\text{CO})_3]$  in MeOH was consistent with the quantitative formation of the proposed structure as well as the presence of the by-product  $\text{NEt}_4\text{Br}$ . The compound  $[\text{Re}(\text{L}^1)(\text{CO})_3]\text{Br}$  was then purified *via* column chromatography in 58% yield (Scheme 1).† The molecular ion was identified as  $[\text{Re}(\text{L}^1)(\text{CO})_3]^+$  by ES-MS and the formulation of the bulk sample confirmed by elemental analysis. As expected, the conductivity value for the complex ( $[\text{Re}(\text{L}^1)(\text{CO})_3]\text{Br} = 145 \Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$ ) corresponds to a 1 : 1 electrolyte.<sup>13</sup> The IR spectra of  $[\text{Re}(\text{L}^1)(\text{CO})_3]\text{Br}$  was



**Scheme 1** Reaction scheme for Re,  $^{99m}\text{Tc}$ , and  $^{186}\text{Re}$  complexes of  $\text{L}^1$ : a)  $[\text{NEt}_4]_2[\text{ReBr}_3(\text{CO})_3]$ , MeOH, 6 h, 58% yield; b)  $^{99m}\text{Tc}(\text{H}_2\text{O})_3(\text{CO})_3^+$ , 70 °C, 40 min, phosphate buffered saline (PBS); c)  $^{186}\text{Re}(\text{H}_2\text{O})_3(\text{CO})_3^+$ , 60 °C, 15 min, PBS buffer.

consistent with the proposed structure as bands attributable to the  $\{\text{Re}(\text{CO})_3\}^+$  core were present between 2100 and 1800  $\text{cm}^{-1}$ .<sup>7</sup>

NMR analysis of both the ligand and the complex was complicated by the presence of the two anomers and the resultant asymmetry due to the chiral centers of the sugar moiety. Resonances were assigned on the basis of 1D ( $^1\text{H}/^{13}\text{C}$ ) as well as by 2D ( $^1\text{H}-^1\text{H}$  COSY, and  $^1\text{H}-^{13}\text{C}$  HMQC) NMR experiments. NMR analysis of the Re compound was indicative of the mode of ligand binding (N-atoms) and illustrated the lower symmetry of the ligand once bound to the  $\{\text{Re}(\text{CO})_3\}^+$  core. Due to the electronic influence of the Re(I) centre a significant downfield shift of the hydrogen resonances in close proximity to the ligating N-atoms was observed. The pyridine hydrogens H-14/H-14' move downfield by approximately 0.4 ppm, while the hydrogens of the methylene link (H-8), adjacent to the tertiary amine, shift downfield by 1.4 ppm upon ligand binding. As well, the hydrogen signals of the methylene groups (H-9a/H-9b and H-9'a/H-9'b) adjacent to the pyridine rings shift downfield ( $\approx 1.1$  ppm) and become non-identical due to a combination of the conformational restriction upon chelation and additional effects exerted by the chiral centres of the glucosamine function.

The hydrogen and carbon resonances of the glucosamine moiety were either unchanged or exhibited very minor shifts compared to those in the free ligand. Coordination-induced shifts<sup>14</sup> (CIS) would suggest carbohydrate ligation to the metal center. The lack of these shifts confirms the pendant nature of the carbohydrate function in solution. The design of a drug molecule exhibiting a pendant carbohydrate offers considerable advantages as this moiety is freely available to interact with carbohydrate transport and metabolic pathways in the body. The potential effect of the tracer group on the biological properties of the radiopharmaceutical is substantially minimized by excluding the glucosamine function from chelating to the metal centre.

From the  $^{13}\text{C}$  NMR of  $\text{L}^1$ , two complete sets of signals are evident due to the presence of the two anomers. The  $^{13}\text{C}$  NMR spectrum becomes more complicated upon ligand binding as the asymmetric effect of the glucosamine moiety is extended due to the increased rigidity upon chelation to the  $\{\text{Re}(\text{CO})_3\}^+$  core. In addition to the effect of the two anomers, the DPA function exhibits further asymmetry so that up to four separate  $^{13}\text{C}$  signals are present for each pyridine carbon. Three sharp Re–CO resonances are present in the  $^{13}\text{C}$  spectrum indicating the low symmetry of the complex.

Labelling of  $\text{L}^1$  with  $^{99\text{m}}\text{Tc}(\text{H}_2\text{O})_3(\text{CO})_3]^{\ddagger}$  and  $^{186}\text{Re}(\text{H}_2\text{O})_3(\text{CO})_3]^{\S}$  was achieved in  $99 \pm 1\%$  and  $85 \pm 5\%$  average radiochemical yields, respectively, as measured by HPLC. The nominally lower yield for labelling with  $^{186}\text{Re}$  is attributed to facile re-oxidation to perrhenate. The identities of the radiolabelled complexes were confirmed to be  $^{99\text{m}}\text{Tc}(\text{L}^1)(\text{CO})_3]^+$  ( $t_{\text{R}} = 13.9$  min) and  $^{186}\text{Re}(\text{L}^1)(\text{CO})_3]^+$  ( $t_{\text{R}} = 13.8$  min) by co-injection with  $[\text{Re}(\text{L}^1)(\text{CO})_3]\text{Br}$  ( $t_{\text{R}} = 13.7$  min). This result confirms that the complexes produced on the tracer level are identical to the Re complex produced and characterised (*vide supra*) on the macroscopic scale.

The *in vitro* stability of the  $^{99\text{m}}\text{Tc}$  complex was assessed *via* cysteine/histidine challenge experiments. In a typical test, the radiolabelled complex was incubated at 37 °C in aqueous phosphate buffer solution (pH = 7.4) containing either 1 mM cysteine or 1 mM histidine (100-fold excess as compared to  $\text{L}^1$ ), and aliquots were removed at 1, 4, and 24 h. The tridentate DPA ligand was expected to form a stable complex resistant to ligand exchange processes and this was indeed the case; the  $^{99\text{m}}\text{Tc}$  complex was  $\geq 94\%$  intact at the 24 h timepoint in the presence of either amino acid. This is in contrast to the  $^{99\text{m}}\text{Tc}$  complexes of a series of bidentate carbohydrate-appended ligands which

exhibited decomposition at the 24 h time point in the presence of excess histidine.<sup>4</sup> This decomposition is most likely due to the substitution of the coordinated water molecule by the functional groups of histidine and subsequent displacement of the carbohydrate ligands. The stability of the  $^{99\text{m}}\text{Tc}$  complex studied herein highlights the utility of the tridentate approach in the design of robust carbohydrate-appended radiopharmaceuticals utilizing the  $\{\text{M}(\text{CO})_3\}^+$  (M = Tc/Re) core.

## Acknowledgements

The authors gratefully acknowledge the Natural Sciences and Engineering Research Council (NSERC) of Canada for a Strategic Grant and a postgraduate scholarship (T. S.), Mallinkrodt Inc. for the Isolink boranocarbonate kits, the UBC Hospital Department of Nuclear Medicine for supplying  $^{99\text{m}}\text{TcO}_4^-$ , and MDS Nordion Inc. for  $\text{Na}^{186}\text{ReO}_4$ .

## Notes and references

$\ddagger$   $^{99\text{m}}\text{Tc}(\text{H}_2\text{O})_3(\text{CO})_3]^+$  was prepared from a saline solution of  $\text{Na}^{99\text{m}}\text{TcO}_4$  (1 mL, 200 MBq) using an Isolink™ kit.<sup>15</sup> Briefly, a 1 mL solution of  $\text{Na}^{99\text{m}}\text{TcO}_4$  was added to an IsoLink™ kit and the vial was heated to reflux for 20 min. Upon cooling, 0.1 mL of a 0.1 M HCl solution was added to adjust the pH to 9–10. Labelling was achieved by mixing an aliquot (200  $\mu\text{L}$ ) of the  $^{99\text{m}}\text{Tc}(\text{H}_2\text{O})_3(\text{CO})_3]^+$  precursor with a 0.1 mM solution of  $\text{L}^1$  in PBS (pH 7.4, 1 mL) and incubating at 75 °C for 30 min.

$\S$   $^{186}\text{Re}(\text{H}_2\text{O})_3(\text{CO})_3]^+$  was prepared by slight alteration of a published procedure.<sup>16</sup> 5 mg of borane ammonia complex was added to a vial, which was then sealed and purged with CO gas for 10 min. To this vial was added a solution containing 4.5  $\mu\text{L}$   $\text{H}_3\text{PO}_4$  (85%) and 0.45 mL of a saline solution containing  $\text{Na}^{186}\text{ReO}_4$  (80–150 MBq). The vial was then equipped with a syringe to compensate for gas evolution and heated to 60 °C for 15 min. 0.2 mL of the resulting solution was added to a 0.1 mM solution of  $\text{L}^1$  in PBS and incubated at 60 °C for 15 min.

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