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View Article Online
DOI: 10.1039/C6CC10066J

ChemComm

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Rhenium(I) complexes of *N*-heterocyclic carbene ligands that bind to amyloid plaques of Alzheimer's disease†

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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A series of $[Re(I)L(CO)_3]^+$ complexes (where L is a bifunctional *bis*(NHC)-amine ligand) that are analogues of potential Tc-99m diagnostic imaging agents for Alzheimer's disease have been synthesised. One of the complexes bound to amyloid plaques in human frontal cortex brain tissue from subjects with Alzheimer's disease.

Alzheimer's disease (AD) is the most common form of dementia and is unambiguously diagnosed post-mortem by the presence of extracellular senile plaques in the brain, which are comprised of insoluble aggregates of the peptide amyloid-β (Aβ).[1-3] The clinical hallmarks of AD include progressive memory loss and behavioural abnormalities leading to serious social issues.[3-5] The precise pathological mechanism of AD remains to be elucidated, however the formation of Aβ plaques is thought to play a fundamental role in the initial neurodegeneration.[2, 6, 7] Therefore, the early diagnosis of AD may be achieved via detection of Aβ plaques using radiopharmaceutical diagnostic agents that target amyloid.[7] The development of positron emission tomography (PET) radiopharmaceutical agents that target Aβ plaques is currently an area of intense research and much recent progress has been made.[7, 8] For example, the carbon-11 labelled compounds ^{11}C-PiB and ^{11}C-SB-13 (Figure 1) bind to Aβ plaques and are under development as PET diagnostic agents for AD.[3, 9] The structures of these compounds consist of a planar aromatic system (2-phenylbenzothiazole and stilbene respectively) and it is believed that they bind to hydrophobic pockets in the Aβ fibril structure as a result of hydrophobic and π-π stacking interactions.[3]

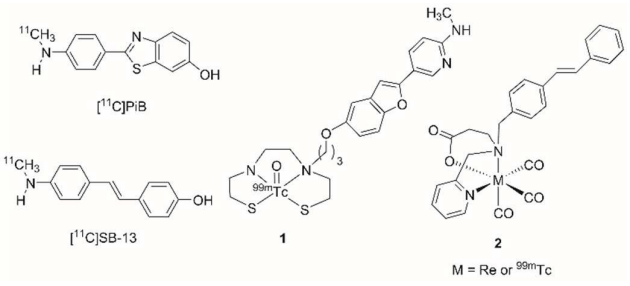

Fig. 1. Chemical structures of [^{11}C]PiB, [^{11}C]SB-13, Tc(V) (**1**) and Tc/Re (**2**) complexes designed to bind to amyloid plaques of AD.

To widen the scope of available radiopharmaceutical diagnostics for AD there is a need for single photon emission tomography (SPECT) agents that incorporate the γ-ray emitting isotope Tc-99m. In contrast to most PET isotopes (e.g. F-18 or C-11) Tc-99m is easily obtained from Mo-99/Tc-99m generators that provide a constant and cheap source of the metallic radionuclide. Previously a ^{99m}Tc(V) complex (**1**) (Figure 1) conjugated to a pyridylbenzofuran group was reported and this compound displayed good brain uptake.[10, 11] Additionally, we[12] (**2**, Figure 1) and others[13] have reported complexes of the $[M(CO)_3]^+$ core (M = Tc(I) or Re(I)) of tridentate NNO ligands that bind to Aβ plaques in AD human brain tissue. However in both cases the ^{99m}Tc complexes showed relatively low brain uptake in animal models.

N-heterocyclic carbene ligands (NHCs) offer significant scope for potential medicinal inorganic and radiopharmaceutical applications and we have recently reported the first example of labelling a NHC ligand with Tc-99m.[14] Herein we describe the preparation of a series of acyclic and cyclic tridentate imidazolium salt, NHC pro-ligands functionalised with amyloid binding moieties and their corresponding $[Re(I)(CO)_3\text{-NHC}]^+$ complexes. The capacity of these molecules to bind to fibrils composed of the $A\beta_{1-42}$ peptide and amyloid plaques present in human frontal cortex brain tissue from subjects with AD has been evaluated.

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† Electronic Supplementary Information (ESI) available: Ligand and complex synthesis, X-ray crystallography details, photophysical and metal complex stability studies additional microscopy images. See DOI: 10.1039/x0xx00000x

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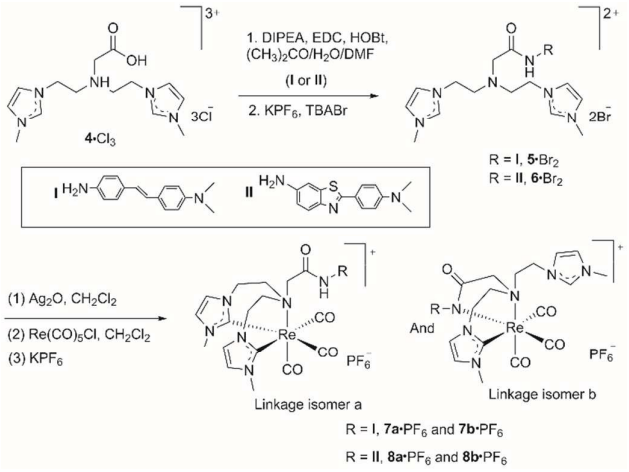
DOI: 10.1039/C6CC10066J

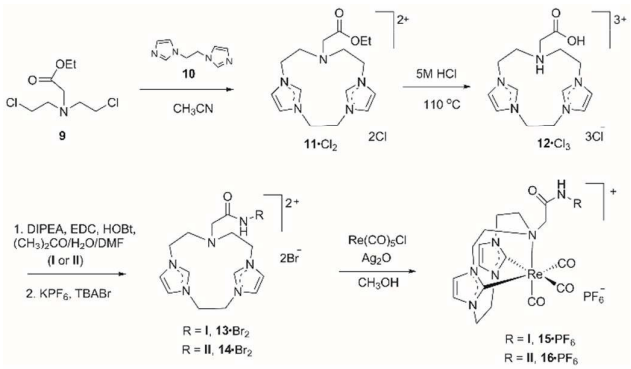
We have previously reported synthesis of the carboxylic acid functionalized *bis*(NHC)-amine pro-ligand **4**·Cl_3 (Scheme 1).[15] To allow for the formation of Re(I) complexes that target amyloid, **4**·Cl_3 was coupled to the amine functionalized amyloid binding moieties: *p*-amino-*p*'-*N*,*N*-dimethylamino-stilbene (**I**) or 6-Amino-2-(4-*N*,*N*-dimethylaminophenyl)benzothiazole (**II**) using the coupling reagent 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), yielding the diimidazolium salts **5**·Br_2 and **6**·Br_2 respectively (Scheme 1). The Re(I) tricarbonyl complexes (**7a**·PF_6, **7b**·PF_6, **8a**·PF_6, **8b**·PF_6) of these pro-ligands were synthesized using silver trans-metalation (Scheme 1) and as noted by us previously for Re(I) complexes of a related ligand system,[15] the Re(I) complexes (**7**·PF_6 and **8**·PF_6) were obtained as mixtures of two linkage isomeric forms that could be separated using semi-preparative HPLC. Analysis of these linkage isomers by ^{1}H NMR spectroscopy was consistent with the ligands being coordinated to the Re(I) centre either via the two NHC units and the amine group (linkage isomer a) or via the NHC, amine and deprotonated amide (amidate) groups (linkage isomer b)



(Scheme 1).

Scheme 1. Synthesis of acyclic *bis*(NHC)-amine pro-ligands conjugated to either *p*-amino-*p*'-*N*,*N*-dimethylamino-stilbene (**I**) or 6-Amino-2-(4-*N*,*N*-dimethylaminophenyl)benzothiazole (**II**) and the two isomeric forms of the Re(I) complexes of these ligands.

The formation of linkage isomers is undesirable for radiopharmaceutical applications so an alternative macrocyclic tridentate ligand system was envisaged, which would allow only the desired *bis*(NHC)-amine coordination mode. Thus the macrocyclic carboxylic acid functionalised diimidazolium salt **12**·Cl_3 was prepared by the reaction between ethyl *bis*(2-chloroethyl)glycinate **9** and 1,2-di(imidazol-1-yl)ethane **10** under high dilution followed by hydrolysis of the ester group with HCl (Scheme 2). This cationic molecule was then coupled to the amyloid binding moieties **I** or **II** using EDC to give the macrocyclic pro-ligands **13**·Br_2 and **14**·Br_2 respectively (Scheme 2). The ^{1}H NMR spectra of these pro-ligands show characteristic downfield resonances for the imidazolium NC*H*N (pro-carbenic) protons and for the amide N-*H* protons. HPLC and NMR analysis of the crude products obtained in the synthesis of the Re(I) complexes **15**·PF_6 and **16**·PF_6 of these macrocyclic pro-ligands showed no evidence for linkage isomers, confirming that incorporation of the three donor groups into a macrocyclic ring structure allows only the desired coordination mode with the NHC units and the tertiary amine group bound to the Re(I) centre. The Re(I) complex (**17**·PF_6) of macrocyclic ligand **11**·Cl_2 was also prepared and this complex was purified by semi-preparative HPLC. The presence of the methyl ester for **17**·PF_6 rather than the enthyl ester was confirmed by NMR spectroscopy, XRD, MS (ESI†) and elemental analysis due to trans-esterification with the



methanol containing mobile phase (Scheme S1, ESI†).

Scheme 2. Synthesis of the macrocyclic *bis*(NHC)-amine pro-ligands conjugated to either *p*-amino-*p*'-*N*,*N*-dimethylamino-stilbene (**I**) or 6-Amino-2-(4-*N*,*N*-dimethylaminophenyl)benzothiazole (**II**) and the Re(I) complexes of these ligands.

The molecular structures of the the cationic Re(I) complexes **15**·PF_6 and **16**·PF_6 (Figure 2) and **17**·PF_6 (Figure S1 and Table S1, ESI†) were confirmed by single crystal X-ray crystallography revealing that the macrocyclic *bis*(NHC)-amine ligand bound to the metal centre as a *facial* tridentate via the two NHC donors and the tertiary amine group. In the case of **15**·PF_6 and **16**·PF_6 the amyloid binding moieties, **I** and **II** respectively, are linked to the macrocyclic ligand via an acetamide linker.

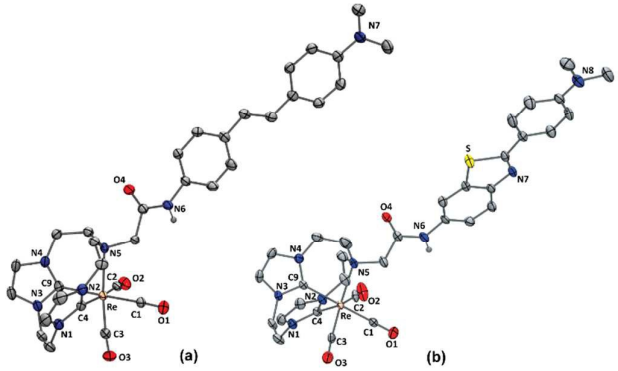


Fig. 2. ORTEP-3[16] structures of the Re(I) complexes (a) **15**$^+$ and (b) **16**$^+$ (PF_6^- counter ions solvent of crystallisation and hydrogen atoms, except the amide hydrogen atom, omitted for clarity). Ellipsoids are shown 50% probability.

The Re(I) complexes prepared here are analogues of potential Tc-99m based imaging agents for AD and a key goal of this research is to evaluate the capacity of these complexes to bind to amyloid plaques in human brain tissue using fluorescence microscopy. As such the UV-visible and electronic emission spectra were recorded for: **I** and **II**, the imidazolium salts (**5**·Br_2, **6**·Br_2, **12**·Br_2 and **13**·Br_2) and the Re(I) complexes (**7**·PF_6,

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8·PF_6, **15**·PF_6 and **16**·PF_6) (Figures S6-S13 and Table 2, ESI†). The emission spectra for these compounds are strongly influenced by the nature of the conjugated amyloid binding moieties (**I** and **II**). In the case of the Re(I) complexes coupled to **I**, emission maxima occurred within the range of λ_{em} = 428 – 454 nm, while for the complexes linked to **II**, emission was red-shifted to λ_{em} = 434 – 467 nm.

Ligand challenge experiments with L-histidine and L-cysteine were conducted to evaluate the stability of the Re(I) complexes **8a**·PF_6, **8b**·PF_6 and **16**·PF_6. These Re(I) complexes were chosen as they represent examples of the two different isomeric forms produced by the acyclic ligand (**8a**·PF_6 and **8b**·PF_6) and a complex of the macrocyclic ligand (**16**·PF_6). The reactions between the Re(I) complexes and the amino acids were monitored by HPLC in combination with ESI-MS and the results of these studies are shown in Figures S14 – S17 (ESI†). The Re(I) complexes bearing the *bis*(NHC)-amine ligand coordination mode (**8a**·PF_6 and **16**·PF_6) showed no sign of decomposition over a 24 h period in the presence of either of these amino acids, while complex **8b**·PF_6 with the NHC/amine/amidate ligand coordination mode was unstable and decomposed within 4 h of mixing (results not shown).

Thioflavin T (ThT) is widely used for the detection of amyloid and for evaluating interactions between molecules of interest and amyloid fibrils.[17, 10, 18, 19] The binding of Re(I) complexes **7a**·PF_6, **8a**·PF_6, **15**·PF_6 and **16**·PF_6 to fibrils formed from synthetic $A\beta_{1-42}$ peptide was evaluated in a ThT fluorescence assay. The formation of fibrils from $A\beta_{1-42}$ was confirmed by increasing fluorescence intensity of ThT at λ = 485 nm in a freshly prepared solution of $A\beta_{1-42}$ over a period of 50 h (control, Figure 3, black line). In contrast the addition of the Re(I) complexes (20 μM) resulted in little or no increase in fluorescence intensity of ThT in the presence of $A\beta_{1-42}$ (Figure 3). These results suggest that the chosen Re(I) complexes bind competitively with ThT to $A\beta_{1-42}$ fibrils or that the Re complexes inhibit fibril formation.

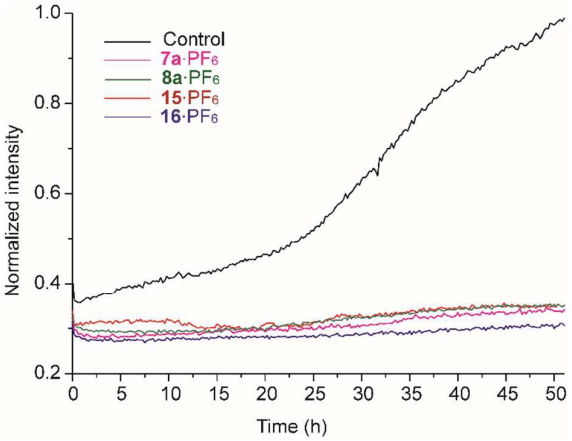

Fig. 3. Time dependent changes in Thioflavin T (ThT) fluorescence over a period of 52 h for solutions of ThT (40 μM) and freshly prepared Aβ(1-42) (10 μM) at 37 °C in the presence of: no addition (control, black), **7a**·PF_6 (Pink), **8a**·PF_6 (Green), **15**·PF_6 (Red) and **16**·PF_6 (Blue) (20 μM in each case).

The capacity complexes **8a**·PF_6 (conjugated **II**) and **15**·PF_6 (conjugated to **I**) to bind to the Aβ plaques in human AD brain tissues was investigated. Frontal cortex brain tissue (7 μm sections) collected from subjects with clinically diagnosed AD and tissue and from an age-matched control were pre-treatment with bovine serum albumin (BSA) to prevent non-

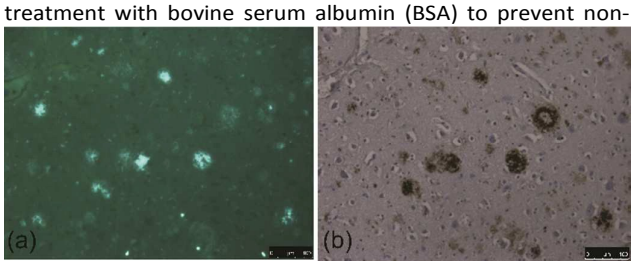

Fig. 4. (a) Epi-fluorescence microscopy image of AD affected frontal cortex brain tissue treated with the Re(I) complex **15**·PF_6 (λ_{ex} = 359 nm, λ_{em} = 461 nm) and (b) microscopy image of the contiguous section immune-stained with an anti-amyloid β peptide antibody 1E8 showing the positions of the amyloid plaques.

The Re(I) complex **8a**·PF_6 (benzothiazole moiety) showed no evidence of Aβ plaque binding but complex **15**·PF_6 showed excellent apparent co-locatisation with the immunostaining (Figure 4a and b) and importantly, the epi-fluorescent image (Figure S18, ESI†) for the age-matched control showed no evidence of non-specific binding of complex **15**·PF_6. The reason for the lack of amyloid binding displayed by **8a**·PF_6 is unknown but it may be the associated with the inefficient binding of the benzothiazole moiety in the presence of the bulky metal complex.[13] In addition, the benzothiazole moiety of **8a**·PF_6 lacks the –OH group present on [^{11}C]PiB (Figure 1), which may also alter its capacity to bind to amyloid.

In conclusion, a series of Re(I) tricarbonyl complexes of acyclic and macrocyclic tridentate NHC ligands coupled to stilbene- or benzothiazole-based amyloid binding groups have been synthesized. Two linkage isomeric forms were obtained for the complexes of the acyclic ligand, while only the desired coordination mode (*bis*-NHC-amine) was observed for the macrocyclic ligands. The capacity of these compounds to bind to amyloid plaques in human AD brain tissue was evaluated and complex **15**·PF_6 bound selectively to the Aβ plaques with low background fluorescence and no evidence of non-specific binding. These results demonstrate the potential for appropriately designed NHC ligands to be incorporated into diagnostic imaging agents for AD. Radiochemical studies are currently being undertaken to allow these *bis*(NHC)-amine ligands to be labelled with Tc-99m. We note at this stage that the cationic nature of these complexes may result in undesirable biodistribution as certain lipophilic cationic Tc-99m complexes e.g. Tc-99m sestamibi (Cardiolite) and Tc-99m tetrofosmin (Myoview) accumulate in myocardial cell mitochondria as a result of increased membrane potentials and are used clinically as heart perfusion imaging agents.[20-22]

This work has been supported by the Australian Research Council (DP150102741) and the La Trobe University Understanding Disease Research Focus Area.

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