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[AlCl$_3$(BnMe$_2$-tacn)] – a new metal chelate scaffold for radiofluorination by Cl/F exchange

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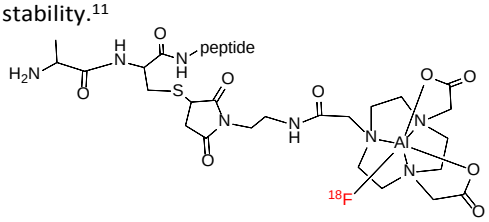
Radiofluorination of a 2.63 μM solution (pH 4, NaOAc buffer) of [AlCl$_3$(BnMe$_2$-tacn)] *via* treatment with 2.99 mol. equiv. of [^{19}F]KF doped with cyclotron-produced [^{18}F]F$^-$ target water, with heating to 80–100 °C for 1 h, gives up to 24% ^{18}F incorporation. SPE purification of the [Al^{19}F$_2$^{18}F(BnMe$_2$-tacn)] radio-product gives >99% RCP, with excellent stability (>99% RCP after 3 h).

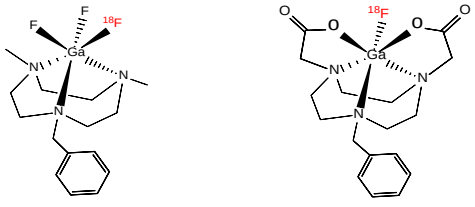
Positron emission tomography (PET) is a non-invasive technique for imaging internal tissues and organs in patients. Amongst the options available for this application, the positron-emitting isotope fluorine-18 offers a number of characteristics that make it particularly attractive. These include a short, but manageable half-life of *ca.* 110 mins., a short positron linear range in tissue (*ca.* 2 mm), dominant positron emission (97%), low energy positron ($E_{\beta max}$ = 635 keV) and its ease of production *via* a cyclotron.

While current ^{18}F-based imaging agents available in the clinic are invariably based on organofluorine moieties, the need for alternatives that offer higher selectivity for target organs, and greater convenience for clinical production, has been recognised.[1] It has been demonstrated that an inorganic approach for the development of new radiotracers can provide options beyond C–F based ^{18}F-PET tracers.[1] With regards to metal chelate-based agents, a key development was the work of McBride *et al.* who reported a two-step, one-pot radiofluorination method where 'Al^{18}F' (obtained by treating $AlCl_3$ with [^{18}F]KF) is chelated to a derivative of 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) in which one of the acetic acid functions is used to conjugate to a peptide, leading to pentadentate N_3O_2 coordination, with the [^{18}F]F$^-$ completing a distorted octahedral geometry at Al(III) (Scheme 1). The complexation/radiofluorination is accomplished at pH 4.1.[2-5] The Al^{18}F-chelate moiety has subsequently been conjugated to several other peptides and been used to image different tumour types *in vivo*.[6-10] Recently, open chain polydentate mixed N/O-donor ligands, attached to temperature-sensitive peptides, have also been used to radiolabel Al^{18}F under milder conditions (< 40 °C) and show good stability.[11]



Scheme 1. The 'Al^{18}F' system reported by McBride *et al.*

In our previous work, the concept of replacing chloride ligands bound to a Ga(III) complex with [^{18}F]F$^-$ was demonstrated by the radiolabelling of the pre-formed [GaCl$_3$(BnMe$_2$-tacn)][12] (BnMe$_2$-tacn = 1-benzyl-4,7-dimethyl-1,4,7-triazacyclononane) and [GaCl(Bn(CH$_2$COO)$_2$-tacn)][13] (Bn(CH$_2$COO)$_2$-tacn = 1-benzyl-4,7-diacetate-1,4,7-triazacyclononane), exploiting the higher bond energy of Ga–F over Ga–Cl as the major thermodynamic driving force for the reaction (Scheme 2). Good [^{18}F]F$^-$ incorporation (*ca.* 30 %) was achieved under very mild radiolabelling conditions (unbuffered aqueous MeCN solution and room temperature) for the former, while buffering to pH 4 (NaOAc) and heating to 80 °C was required for the latter.



Scheme 2. Ga-^{18}F complexes based on BnMe$_2$-tacn (left) and Bn(CH$_2$COO$^-$)$_2$-tacn (right).

The aim of the work in this communication was to investigate the viability of the pre-formed [AlCl$_3$(BnMe$_2$-tacn)] complex as a potential scaffold for next-generation PET imaging agents through radiofluorination by Cl/F halide exchange and to compare the results with the corresponding gallium complex[12] and the 'AlF' system described by McBride et al.[3-5] We describe

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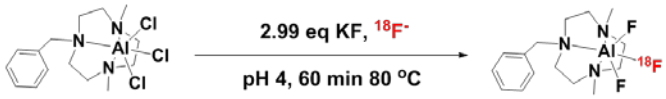
Electronic Supplementary Information (ESI) available: preparative method for [AlCl3(BnMe2tacn)], ^{1}H and ^{27}Al NMR spectroscopic data for [AlCl$_3$(BnMe$_2$tacn)] and [Al^{19}F$_3$(BnMe$_2$tacn)]; the radio-trace from the radiofluorination experiment described using Method 1. See DOI: 10.1039/x0xx00000x

here the radiofluorination on a 1 mg scale (μM concentration) through Cl/^{18}F halide exchange.

Preparative scale experiments established that the Al analogues, [$AlCl_3$(RMe_2-tacn)] (R = Bn or Me), do not undergo Cl/F exchange with [Bu_4N]F or [Me_4N]F in anhydrous MeCN at room temperature. On heating the reaction mixture, the macrocyclic ligand was displaced and [AlF_4]$^-$ was formed.[12] However, fluorination (Cl/F ligand exchange) was achieved upon addition of aqueous KF to a MeCN suspension of the complex at room temperature.[12] We postulated that the difference in reactivity between the two metals might be due to the smaller ionic radius of Al^{3+}, which would disfavour an associative (A) or associative interchange (I_a) ligand substitution mechanism and that the more polar H_2O solvent is involved in a solvent-assisted substitution mechanism.[14]

Treatment of a 2.63 μM MeCN solution of [$AlCl_3$($BnMe_2$-tacn)] (1.0 mg) with 2.99 mol. equiv. of K^{19}F doped with [^{18}F]F$^-$ (50 MBq) in unbuffered water with heating to 80°C for 30 mins. resulted in some ^{18}F incorporation (<10%, determined by radio-HPLC; Fig. S6, ESI). ^{18}F incorporation was increased significantly when the labelling experiment was performed in an aqueous buffered solution at pH 4 (NaOAc) with heating (80-100 °C, 60-90 mins.), leading to ≤24% incorporation (Scheme 3). The identity of the radio-product as the distorted octahedral *fac*-[AlF_3($BnMe_2$tacn) was confirmed by comparison of its UV-trace against that of the reference compound. In the radiolabelling experiments we used 2.99 equiv K^{19}F (per one molecule of [$AlCl_3$($BnMe_2$tacn)]) and doped this with ^{18}F$^-$, so that the final product would likely incorporate a maximum of one ^{18}F atom per molecule.



Scheme 3. Radiolabelling protocol for [$AlCl_3$($BnMe_2$-tacn)] (Method 2).

Fig. 1 shows the analytical HPLC chromatogram of the crude product, along with the UV trace, confirming that [Al^{18}F^{19}F$_2$($BnMe_2$-tacn)] was the only radio-product in addition to unreacted [^{18}F]F$^-$The [Al^{18}F^{19}F$_2$($BnMe_2$-tacn)] was successfully purified through a HLB solid phase extraction (SPE) cartridge to leave the metal chelate as the single product, Rt = 6.92 min. in the radio chromatogram. This product showed very high stability in a 50% EtOH/phosphate buffered saline (PBS) solution at pH 7.4 over 180 min., the RCP being >99% over this period (Fig. 2).

Since the [AlF_3($BnMe_2$tacn)] was originally prepared under hydrothermal conditions (15 h at 180 °C in water),[12] the complex is extremely stable in water. Further stability tests against potentially competitive anions in water have been performed using ^{19}F and ^{27}Al NMR spectroscopy. Solutions of [Al^{19}F$_3$($BnMe_2$tacn)] with a 10-fold excess of NaCl, NaOAc or NaF (close to pH 7) showed no changes even after several days, while it is clear from the radiolabelling experiments that the complex is stable at pH 4 (NaOAc buffer). However, the [Al^{19}F$_3$($BnMe_2$tacn)] is completely decomposed by the addition of a 10-fold excess of Na_2CO_3 (which has pH ~10), with conversion to [$Al(OH)_4$]$^-$ (δ^{27}Al = +80).

We reported previously that the corresponding [$GaCl_3$($BnMe_2$-tacn)] undergoes radiofluorination (with *ca*. 30% [^{18}F]F$^-$ incorporation) at room temperature in unbuffered aqueous MeCN.[12] Thus, a significant difference in the behaviour of the Al(III) *vs*. Ga(III) analogues is evident. Likely factors contributing to the differences observed are the higher Al–F bond dissociation energy (664 kJ mol^{-1}) compared to Ga–F (577 kJ mol^{-1}) and the higher Lewis acidity of the Al system.[15] The pH is an important factor for the radiolabelling of the Al system; in this case it may be that competition with hydroxide anions for the more Lewis acidic Al(III) is important.

This work has demonstrated that [$AlCl_3$($BnMe_2$-tacn)] can be radiolabelled readily in buffered pH 4 solution in the presence of 2.99 equiv. of KF doped with ^{18}F$^-$ at 80 °C/10 mins. and that cyclotron-produced [^{18}F]F$^-$ target water can be added directly to the buffered solution. The radiolabelled Al(III) complex shows excellent stability in PBS buffered ethanolic solution over several hours.

Comparing the results presented here with our earlier [GaF_3($BnMe_2$-tacn)] and the 'AlF' system described by McBride *et al.* suggests that the size of the metal ion, its Lewis acidity and the specific ligand donor set are important considerations in determining the conditions necessary for effective radiofluorination at micromolar concentrations or lower.

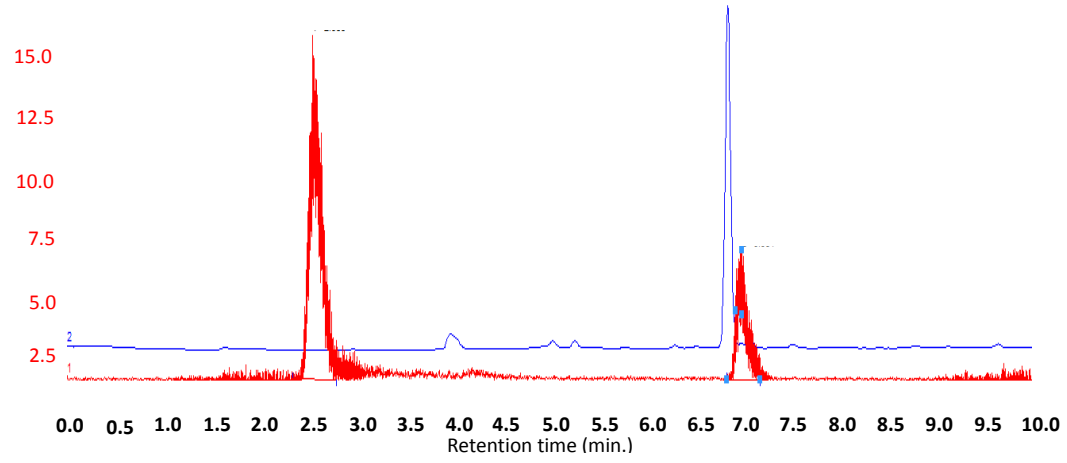


Figure 1 Analytical HPLC chromatogram of the crude product from reaction of [$AlCl_3$($BnMe_2$-tacn)] (1 mg, 2.63 μmol) at pH 4 (NaOAc buffer) with 2.99 eq of KF doped with 0.1 mL of aqueous [^{18}F]F$^-$ at 80 °C for 90 mins. Radio (red) and UV (blue). Peak 1: Rt = 2.51 min 76% (^{18}F$^-$). Peak 2: Rt = 6.95 min 24% ([Al^{18}F^{19}F$_2$($BnMe_2$tacn)]).

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Further, McBride et al. were able to radio-fluorinate their 'AlF' system using a lower Al concentration. We have found that our [$AlCl_3$($BnMe_2$tacn)] is successfully radiofluorinated using 2.63 µM concentration (1 mg of complex in 1 mL), whereas ^{18}F incorporation was not observed using 0.1 mg of the metal precursor.

The results described here also provide strong encouragement that pre-formed Group 13 metal trihalide complexes of the type [MCl_3($BnMe_2$-tacn)] bearing neutral triaza-macrocycles are promising candidates as alternatives to organofluorine compounds for incorporation into next generation imaging agents for PET applications. Future work will aim to rationalise how the choice of the metal ion influences the radiolabelling so that the optimum candidates for bioconjugation with peptides can be identified. We thank GE Healthcare and the EPSRC for support through EP/ L505651/1.

Notes and references

‡ **Synthetic procedure and method.** [$AlCl_3$($BnMe_2$-tacn)] was prepared according to literature method (ESI).[12] Experiments were analysed on an Agilent 1290 HPLC system with an Agilent 1260 DAD UV detector (G4212B). Dionex Chromeleon 6.8 Chromatography data recording software was used to integrate the UV and radiochemical peak areas.

Analytical HPLC method: Column: Phenomenex Luna 5 um C18(2) 250 x 4.6 mm. Mobile phase A: 10 mM ammonium acetate. B: MeCN. Flow rate: 1 mL min^{-1}. Gradient: 0-15 min (10-90 % B), 15-20 min (90 % B), 20-21 min (90-10 % B), 21-26.5 min (10 % B).

Cl/^{18}F Exchange Radiolabelling Procedure: Method 1: [$AlCl_3$($BnMe_2$-tacn)] (0.001 g, 2.63 µmol) was dissolved in MeCN (0.6 mL). 2.99 equiv. of KF in cyclotron target [^{18}F]F^- water (0.4 mL, 50 MBq) was added. The mixture was heated to 80 °C for 30 mins. Analytical HPLC analysis of the crude reaction solution showed *ca.* 9% ^{18}F incorporation.

Method 2: In a typical experiment, [$AlCl_3$($BnMe_2$-tacn)] (0.001 g, 2.63 µmol) was dissolved in pH 4 sodium acetate buffer solution (1 mL). 2.99 eq of KF in cyclotron target [^{18}F]F^- water (0.1-1 mL, 20-280 MBq) was added. The mixture was heated to 80-100 °C for 60-90 mins. Analytical HPLC analysis of the crude reaction solution showed up to 24% ^{18}F incorporation (n = 7).

Peak 1: Rt = 2.51 min ($^{18}F^-$). Peak 2: Rt = 6.95 min ([$Al^{18}F^{19}F_2$($BnMe_2$tacn)] complex).

SPE purification procedure: The crude product was trapped on a HLB cartridge, washed with water (5 mL) to remove the [^{18}F]F^- and eluted from the cartridge with 1 mL of ethanol. pH 7.4 PBS solution was used to dilute the product to give a 50% ethanolic formulation. The purified product was analysed by analytical HPLC, giving a pure product Rt = 6.92 min (RCP >99%). The product was stable for at least three hours (RCP = >99% at t = 180 min.).

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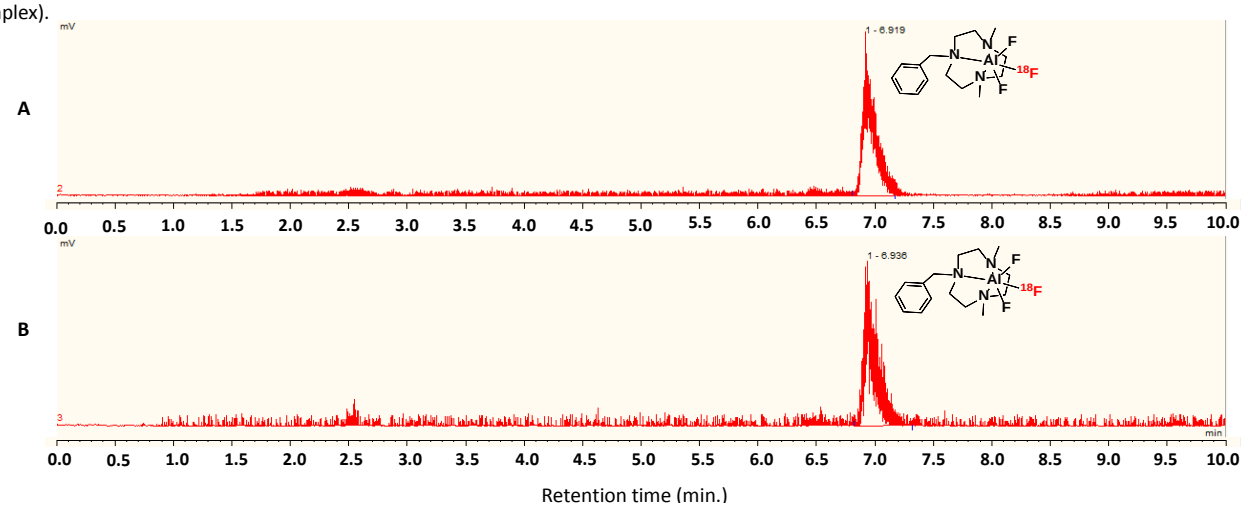

Figure 2 Analytical radio-HPLC chromatogram of: **A**: SPE purified product at t = 20 min. Peak: Rt = 6.92 min >99% ([$Al^{18}F^{19}F_2$($BnMe_2$tacn)]); **B**: SPE purified product at t = 180 min. Peak: Rt = 6.92 min >99% ([$Al^{18}F^{19}F_2$($BnMe_2$tacn)]).