Efficient Radiosynthesis of a [18F]-Phosphonium Salt Containing *Closo*-Carborane

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Graphical Abstract
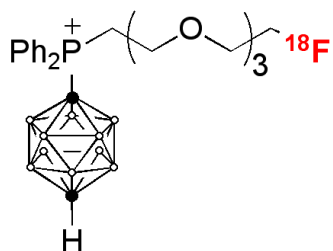
An efficient synthesis of an ^{18}F-labelled phosphonium salt containing *closo*-carborane is described. The preparation of this salt was attempted using both single-step and two-step protocols, with greater success found for the latter. An X-ray structure for the unlabelled ^{19}F salt is also presented.

Lipophilic phosphonium agents (*e.g.* **1** and **2**, Fig. 1) are known to accumulate selectively in the mitochondria of cancerous cells.[1-3] This is attributed to the elevated mitochondrial membrane potential present in a broad range of cancer cells compared to healthy epithelial cells (*ca.* 60 mV *e.g.* −163 mV in colon carcinoma cells *vs.* −104 mV in kidney epithelial cells).[2,4] Very high tumour to normal tissue uptake ratios (*ca.* 40 : 1) have been obtained *in vivo* for **1** and **2**.[1,2] The lipophilic nature of these agents, particularly the reported *closo*-carborane containing salts (*e.g.* **3**, Fig. 1),[3,5,6] may facilitate their diffusion across the lipophilic mitochondrial membrane. Boron-containing phosphonium salts may also be useful in boron neutron capture therapy (BNCT), delivering boron selectively to tumour foci prior to neutron irradiation.[7,8]
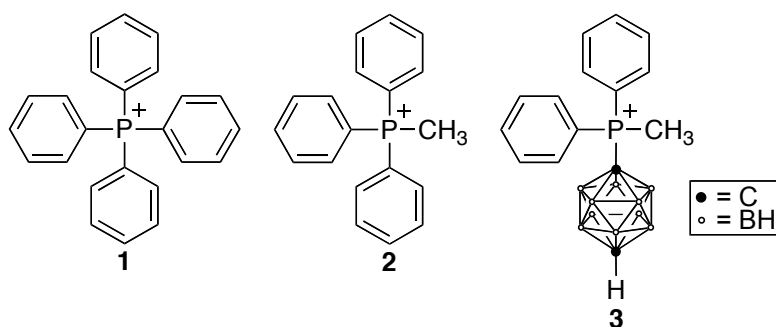

Figure 1: *Lipophilic phosphonium agents tetraphenylphosphonium (TPP, 1), triphenylmethylphosphonium (TPMP, 2) and closo-carborane containing analogue 3.*

Radiolabelled phosphonium agents incorporating ^{18}F, ^{11}C and ^{64}Cu for positron emission tomography (PET) imaging of cancer have been reported (Fig. 2).[2,9-14] *In vivo* and *in vitro* biological distribution studies of phosphonium salts incorporating chelated ^{64}Cu (*e.g.* **4**) have shown good tumour localization and effective imaging of U87MG human glioma xenografts in athymic nude mice.[9-11] The persistent and highly selective *in vivo* uptake of the phosphonium cation [^{11}C]-**2** has also been demonstrated. A very high tumour to healthy brain tissue ratio (47.5 : 1) was observed, which is one order of magnitude more selective than

clinically used [18F]-fluorodeoxyglucose ([18F]-FDG) or [11C]-methionine.[1,15] The long-lived tritium agent [3H]-**1** has shown selective accumulation in C6 glioma in mice *in vivo* (metastatic to normal tissue ratio 8.03 : 1) which was also more selective that clinically used [18F]-FDG (1.03:1).[2]
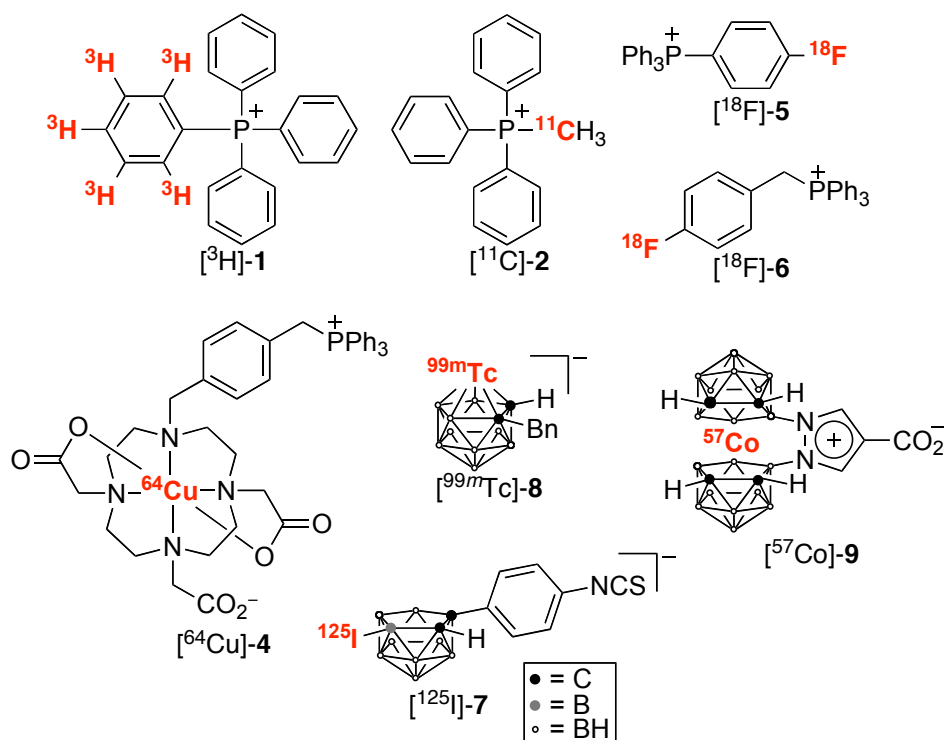

Figure 2: Selected examples of radiolabelled phosphonium agents used in PET imaging.

Single-[12] and multi-step[14] preparations of phosphonium cations incorporating 18F from the 18F$^-$ ion have been reported (*e.g.* **5** and **6**). The phosphonium cation [18F]-**6** has proved to be quite versatile as an imaging agent. The selective uptake of [18F]-**6** in H345 lung carcinoma *in vivo* was confirmed.[13] This agent has also been used for *in vivo* myocardium imaging in healthy dogs[16] and rats,[17] for the assessment of severity of coronary artery stenosis in dogs *in vivo* and *ex vivo*,[18] to quantify the time dependent apoptotic action of the chemotherapeutic agents paclitaxel and docetaxel *in vitro* and *in vivo*,[19] and most recently to detect unstimulated brown adipose tissue and quantify mitochondrial thermogenic activity in rats *in vivo*.[20] The

incorporation of *closo*-carborane, a known lipophilic pharmacophore,[21] into phosphonium cations may facilitate their passage across the mitochondrial membrane, resulting in more selective cell-uptake and improved PET resolution. Biodistribution studies (*e.g.* microPET) would also be useful in assessing such salts for potential application in BNCT.[22] Radiolabelled compounds containing carboranes have been reported previously, with most radiolabelled carboranes comprising of radio-halogenated anionic *nido*-carboranes[23] (*e.g.* **7**) or *nido*-carboranes facially bound to radioactive metals such as ^{99m}Tc(I) and ^{57}Co(III) (*e.g.* **8** and **9**, respectively).[24,25] An ^{18}F-labelled *closo*-carborane was recently described in which the boron cage itself was radiolabelled,[26] although such entities would show compromised receptor binding characteristics if the *closo*-carborane cage is critical to receptor binding, for example, at hydrophobic sites.

We have previously reported the detailed synthesis of the phosphonium salt **10**·Br (Fig. 3).[27] Incorporation of the glycol moiety in **10**·Br was shown to increase water solubility in comparison to **3**·I, a methyl-containing analogue, by more than one order of magnitude (10.8 mM *vs*. 0.9 mM). The covalently-bound bromine atom in **10**·Br may be exchanged for a nucleophilic ^{18}F^{-} ion, and the *in vivo* distribution of the resulting radioproduct may be detected by means of PET. Herein, we report the first efficient synthesis of an ^{18}F-phosphonium salt containing *closo*-carborane ([^{18}F]-**11**·I, Fig. 3) whereby the radiolabel is not incorporated into the carborane cage.
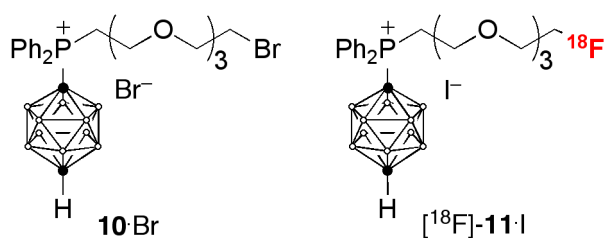
Figure 3: *Previously reported glycol-containing phosphonium-carborane agent **10**·Br and the*
*[18]F-labelled analogue [^{18}F]-**11**·I.*

Synthesis

The glycol chains **12** and **13** were prepared by nucleophilic substitution of
ditosyltetraethylene glycol with the fluoride and iodide ions, respectively (Scheme 1). The
compounds were isolated from a statistical mixture of components. The preparation of **11**·I
from **13** was achieved according to a reported method for **10**·Br[27] (Scheme 1) using either
DMF or MeCN as the solvent, and the compound was purified by trituration of the crude
residue with Et_2O and collection of the precipitate by filtration. Alternatively, small amounts
of analytically pure **11**·I were also obtained by preparative HPLC using adapted elution
protocols.[28,29] The I⁻ counter-ion was readily replaced by CF_3COO^- in the HPLC mobile
phase, yielding the final product as the **11**·CF_3COO salt after purification. The iodide salt was
then recovered by treatment of **11**·CF_3COO with a conc. NaI solution, followed by extraction
of the salt into CH_2Cl_2.
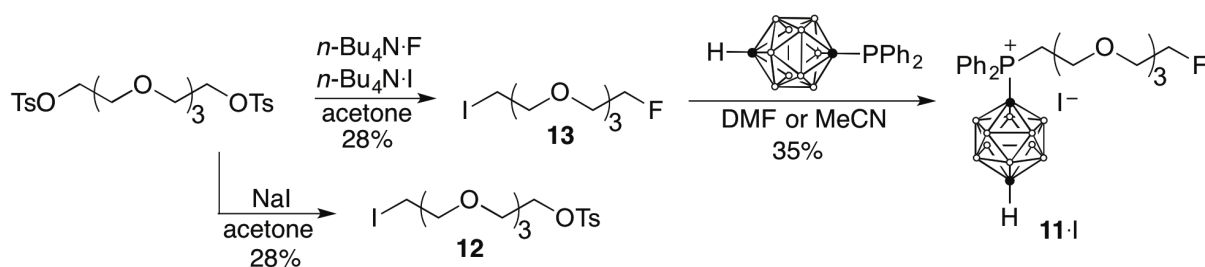
Scheme 1: *Syntheses of 'cold' standard compounds and the precursor glycol chain for the*
radiofluorination protocol shown in Scheme 3.

The phosphonium product **11**·I exhibited similar NMR characteristics to **10**·Br[27] with a few
key differences. In addition to coupling to ^{31}P, there was also coupling by the ^{19}F in the [1]H
and ^{13}C NMR spectra. For example, in the ^{1}H NMR spectrum there were two doublets of

triplets that were assigned to the CH$_2$CH$_2$F group, (δ 4.55, $^2J_{FH}$ = 47.9 Hz and δ 3.68, $^3J_{FH}$ = 25.2 Hz) and the splitting was consistent with reported ^{19}F$-^1$H coupling constants.[30,31] In the ^{13}C{^{1}H} NMR spectrum, there were two doublets assigned to the same group, (δ 83.2, $^1J_{FC}$ = 169 Hz and δ 70.4, $^2J_{FC}$ = 19 Hz) with splitting consistent with reported ^{19}F$-^{13}$C coupling constants.[30,31] The ^{19}F{^{1}H} NMR spectrum displayed a singlet at δ -223 which was consistent with the ^{19}F chemical shifts for alkyl fluorides.[32,33] The ^{1}H NMR spectra of analytically pure samples of **11·CF$_3$COO** obtained by HPLC before treatment with NaI were slightly different to the ^{1}H NMR spectra of samples after treatment. In particular, the chemical shifts of the PCH$_2$CH$_2$ component were shifted slightly upfield ($\Delta\delta$ *ca.* 0.3) whereas all other signals in the ^{1}H NMR spectrum as well as in the ^{11}B, ^{19}F, ^{13}C and ^{31}P NMR spectra were not significantly changed. This shift can be directly attributed to the change in counter-ion from I$^-$ to CF$_3$COO$^-$, which in CDCl$_3$ would be closely associated with the phosphonium centre due to ion pairing. The presence of CF$_3$COO$^-$ was confirmed by a sharp resonance at δ -72 in the ^{19}F{^{1}H} NMR spectrum, which agrees with the literature data for the CF$_3$ environment.[32] Furthermore, quartet resonances in the ^{13}C{^{1}H} NMR spectrum (δ 161.9, $^2J_{FC}$ = 34 Hz and δ 117.8, $^1J_{FC}$ = 293 Hz) were also in agreement with literature data for the CF$_3$COO$^-$ anion.[34]

Crystals suitable for X-ray diffraction were grown by slow diffusion of Et$_2$O into a solution of **11·I** in CDCl$_3$, and an ORTEP representation of the salt is presented in Fig. 4.
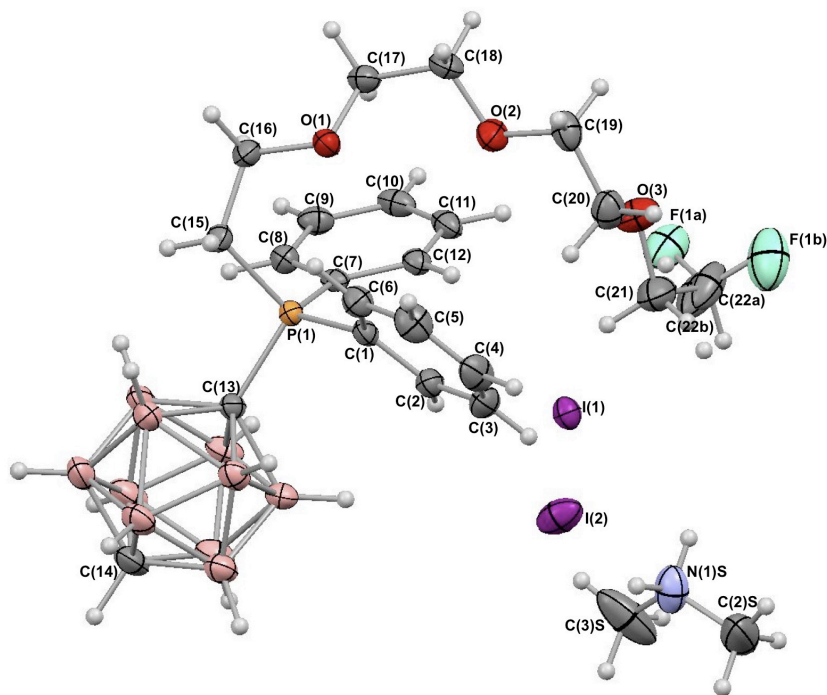
Figure 4: *An ORTEP depiction and atomic numbering of* **11·2I·NH₂Me₂** *with 50%*

displacement ellipsoids.

Interestingly, the solid-state structure was found to contain *two* iodide counter-ions, although

the phosphonium group itself is mono-cationic. The remaining anionic charge is balanced by

the presence of one equivalent of adventitious $NH_2Me_2^+$ ion that had co-crystallised with **11·I**.

There are clear intermolecular contacts between the N-atom and both iodide ions in the crystal

lattice (Fig. S1). These crystals were grown from a batch of **11·I** that had been prepared in

DMF at high temperature (>100°C), and $NH_2Me_2^+$ is a known decomposition product of this

solvent which has been observed in other X-ray structures.[35] Consequently, further syntheses

of **11·I** were carried out using MeCN as the solvent in place of DMF. Despite repeated

attempts, crystallization of pure **11·I** prepared in this manner did not afford X-ray quality

crystals.

Radiochemistry

The preparation of $[^{18}F]$-**11**·Br from **10**·Br in a single step was attempted as shown in Scheme 2. The radiofluorination of a related phosphonium salt by using this method has been achieved previously.[12] The fractions corresponding to each radiopeak in the semi-preparative HPLC γ-trace were collected manually and assessed by analytical HPLC. One fraction appeared to contain the target $[^{18}F]$-**11**·Br product, and when this material was co-injected with the **11**·I standard, the product and the **11**·I standard eluted at exactly the same time, as indicated by the identical retention times of the radio- and UV-peaks (t_R = 1.6 min, Fig. S2). This result was consistent with the target product $[^{18}F]$-**11**·Br being successfully prepared. However, despite this favourable result, the retention time was too early to definitively confirm the identity of the product. The resulting material also possessed very low radioactivity, indicating a very low radiochemical yield (< 1%). Furthermore, the radiolabelling of **10**·Br could not be reproduced in a suitably reliable manner using this single-step method.
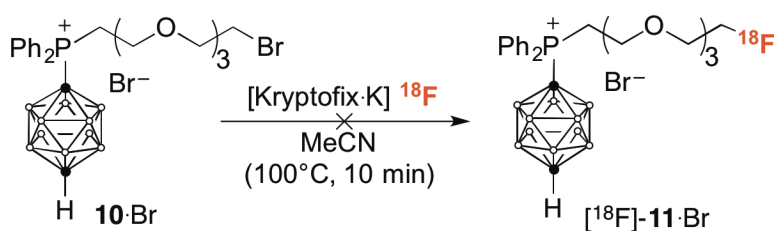
*Scheme 2: Attempted one-step synthesis of $[^{18}F]$-**11**·Br.*

A second route toward $[^{18}F]$-**11**·I is presented in Scheme 3. This radiosynthesis involved a two-step protocol whereby the $[^{18}F]$-**13** precursor was first prepared in good (10%) radiochemical yield from **12** and purified from all Kryptofix and K_2CO_3 residues by means of HPLC (Fig. S3). The single radiopeak (t_R = 4.8 min) was symmetrical and indicated the presence of only one product. The UV- and γ-traces confirmed it was both chemically and

radiochemically pure. The product was then co-injected with 'cold' **13**. The co-incident retention times in the UV- and γ-traces confirm the identity of the product (Fig. S4).
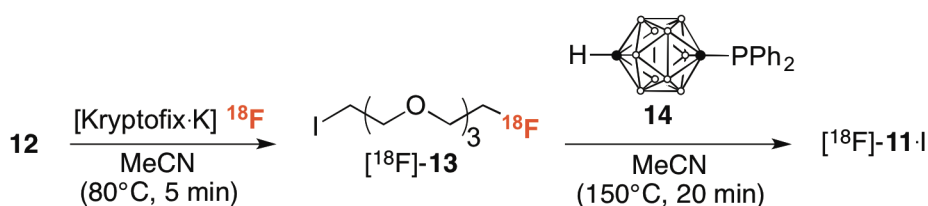
Scheme 3: *Efficient two-step synthesis of [^{18}F]-**11**·I.*

The target phosphonium salt [^{18}F]-**11**·I was first prepared in DMF but the reaction proceeded very slowly and it was found to be increasingly susceptible to side-reactions at temperatures greater than 100 °C. MeCN was then chosen as an alternative solvent. The crude product was purified by semi-preparative HPLC and 50 MBq of product was isolated. A sample of this isolated product was then tested for purity by analytical HPLC. The single radiopeak observed (t_R = 4.3 min, Fig. S5) was symmetrical and is consistent with a single ^{18}F-product. The [^{18}F]-**11**·I was then co-injected with 'cold' **11**·I; co-incident retention times in the UV- and γ-traces confirmed the identity of the tracer (Fig. S6). The target [^{18}F]-**11**·I agent was obtained in *ca.* 3% radiochemical yield over two steps in excellent chemical and radiochemical purity (>99%), as confirmed by the UV- and γ-traces.

Conclusion

The successful synthesis of an ^{18}F-phosphonium agent containing *closo*-carborane [^{18}F]-**11**·I was achieved. We are now in a position to assess the biodistribution of these agents in animal models using PET imaging, and the results of this *in vivo* study will be reported in due course.

Acknowledgements

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