

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

Joseph A. Ioppolo^a, Nicolas Giboureau^b, Mohan Bhadbhade^c, Daniel Morrison^a,
Michael Kassiou^{a,b}, and Louis M. Rendina^{*a}

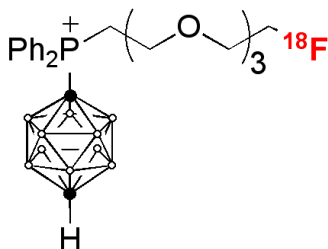
^a *School of Chemistry, The University of Sydney, Sydney, NSW 2006, Australia.*

^b *Brain and Mind Centre, The University of Sydney, NSW 2050, Australia.*

^c *Solid State and Elemental Analysis Unit, The University of New South Wales, Kensington,
NSW 2052, Australia.*

* Correspondence to Prof. Louis M. Rendina (lou.rendina@sydney.edu.au)

Graphical Abstract



An efficient synthesis of an ¹⁸F-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 ¹⁹F 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}

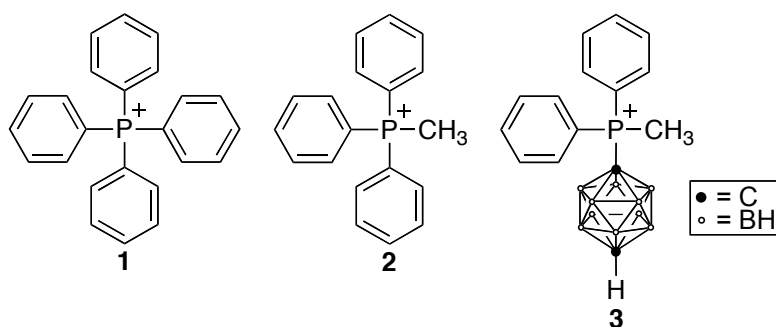


Figure 1: Lipophilic phosphonium agents tetraphenylphosphonium (TPP, **1**), triphenylmethylphosphonium (TPMP, **2**) and *closo*-carborane containing analogue **3**.

Radiolabelled phosphonium agents incorporating ¹⁸F, ¹¹C and ⁶⁴Cu 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 ⁶⁴Cu (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 [¹¹C]-**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 [^{18}F]-fluorodeoxyglucose ([^{18}F]-FDG) or [^{11}C]-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 than clinically used [^{18}F]-FDG (1.03:1).²

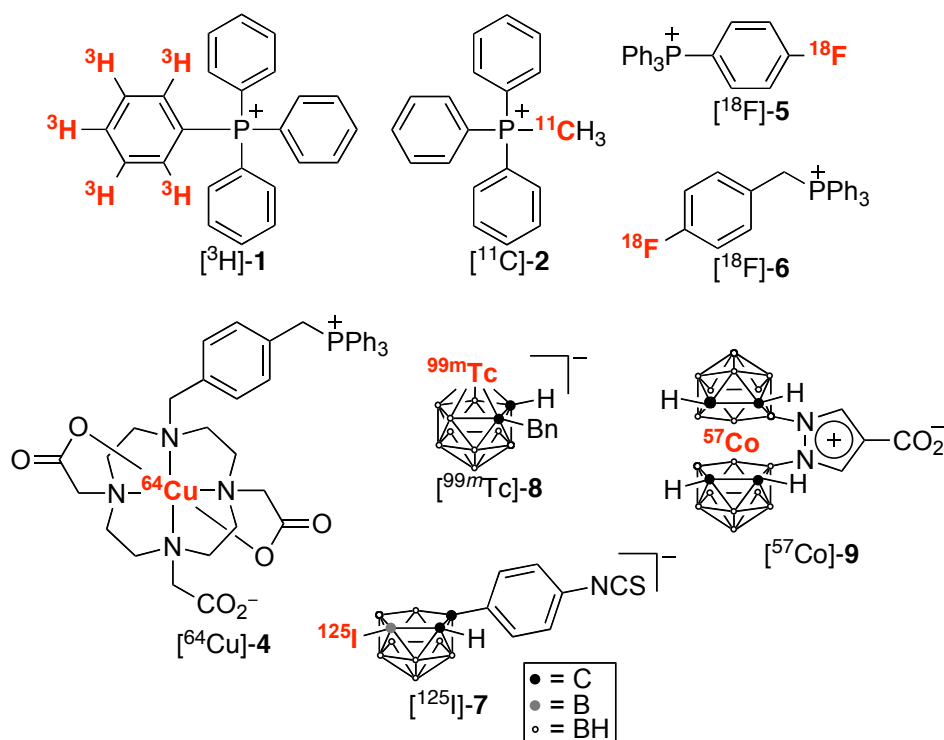


Figure 2: Selected examples of radiolabelled phosphonium agents used in PET imaging.

Single-¹² and multi-step¹⁴ preparations of phosphonium cations incorporating ^{18}F from the $^{18}\text{F}^-$ ion have been reported (e.g. **5** and **6**). The phosphonium cation [^{18}F]-**6** has proved to be quite versatile as an imaging agent. The selective uptake of [^{18}F]-**6** in H345 lung carcinoma *in vivo* was confirmed.¹³ This agent has also been used for *in vivo* myocardium imaging in healthy dogs¹⁶ 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*-carboranes²³ (*e.g.* **7**) or *nido*-carboranes facially bound to radioactive metals such as ^{99m}Tc(I) and ⁵⁷Co(III) (*e.g.* **8** and **9**, respectively).^{24,25} An ¹⁸F-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 ¹⁸F⁻ 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 ¹⁸F-phosphonium salt containing *closo*-carborane ([¹⁸F]-**11**·I, Fig. 3) whereby the radiolabel is not incorporated into the carborane cage.

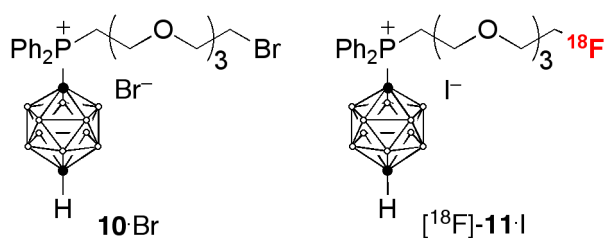
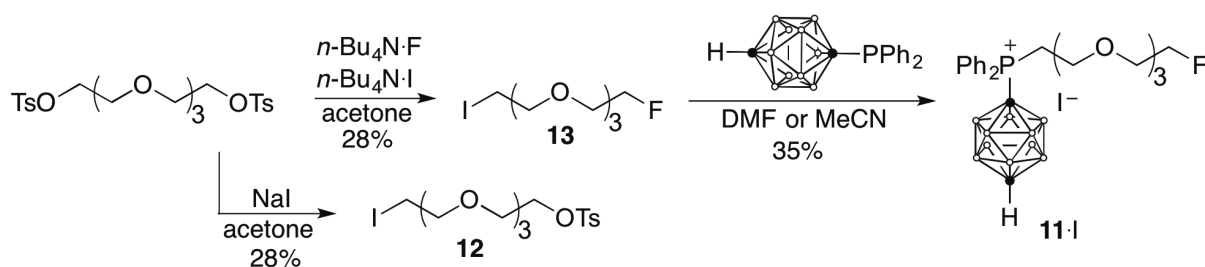


Figure 3: Previously reported glycol-containing phosphonium-carborane agent **10·Br** and the ^{18}F -labelled analogue [^{18}F]-**11·I**.

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 Et₂O 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 CF₃COO⁻ in the HPLC mobile phase, yielding the final product as the **11·CF₃COO** salt after purification. The iodide salt was then recovered by treatment of **11·CF₃COO** with a conc. NaI solution, followed by extraction of the salt into CH₂Cl₂.



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 ³¹P, there was also coupling by the ¹⁹F in the ¹H and ¹³C NMR spectra. For example, in the ¹H NMR spectrum there were two doublets of

triplets that were assigned to the CH₂CH₂F group, (δ 4.55, $^2J_{\text{FH}} = 47.9$ Hz and δ 3.68, $^3J_{\text{FH}} = 25.2$ Hz) and the splitting was consistent with reported ^{19}F - ^1H coupling constants.^{30,31} In the $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum, there were two doublets assigned to the same group, (δ 83.2, $^1J_{\text{FC}} = 169$ Hz and δ 70.4, $^2J_{\text{FC}} = 19$ Hz) with splitting consistent with reported ^{19}F - ^{13}C coupling constants.^{30,31} The $^{19}\text{F}\{^1\text{H}\}$ NMR spectrum displayed a singlet at δ -223 which was consistent with the ^{19}F chemical shifts for alkyl fluorides.^{32,33} The ^1H NMR spectra of analytically pure samples of **11**·CF₃COO obtained by HPLC before treatment with NaI were slightly different to the ^1H NMR spectra of samples after treatment. In particular, the chemical shifts of the PCH₂CH₂ component were shifted slightly upfield ($\Delta\delta$ ca. 0.3) whereas all other signals in the ^1H 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 CF₃COO⁻, which in CDCl₃ would be closely associated with the phosphonium centre due to ion pairing. The presence of CF₃COO⁻ was confirmed by a sharp resonance at δ -72 in the $^{19}\text{F}\{^1\text{H}\}$ NMR spectrum, which agrees with the literature data for the CF₃ environment.³² Furthermore, quartet resonances in the $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum (δ 161.9, $^2J_{\text{FC}} = 34$ Hz and δ 117.8, $^1J_{\text{FC}} = 293$ Hz) were also in agreement with literature data for the CF₃COO⁻ anion.³⁴

Crystals suitable for X-ray diffraction were grown by slow diffusion of Et₂O into a solution of **11**·I in CDCl₃, and an ORTEP representation of the salt is presented in Fig. 4.

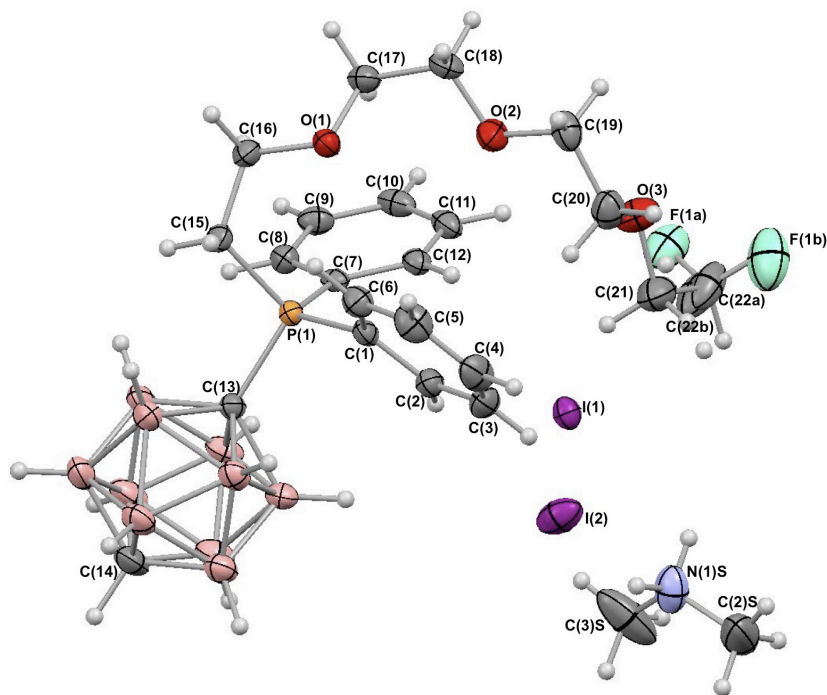
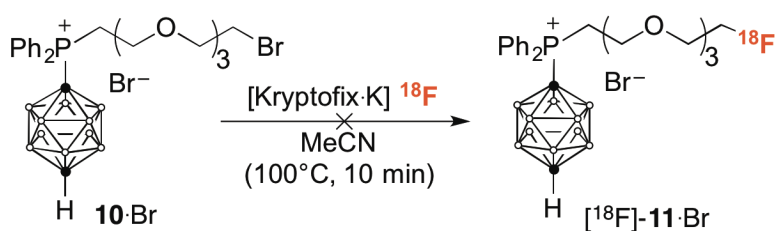


Figure 4: An ORTEP depiction and atomic numbering of **11**·2I·NH₂Me₂ with 50% displacement ellipsoids.

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 NH₂Me₂⁺ 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 NH₂Me₂⁺ 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

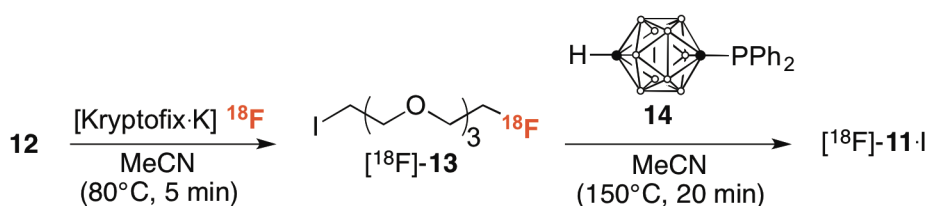
The preparation of [^{18}F]-**11**·Br from **10**·Br in a single step was attempted as shown in Scheme 2. The radiofluorination of a related phosphonium salt by using this method has been achieved previously.¹² The fractions corresponding to each radiopeak in the semi-preparative HPLC γ -trace were collected 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 ($t_{\text{R}} = 1.6$ 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 ($t_{\text{R}} = 4.8$ 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 $[^{18}\text{F}]\text{-11-I}$.

The target phosphonium salt $[^{18}\text{F}]\text{-11-I}$ 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 ($t_{\text{R}} = 4.3$ min, Fig. S5) was symmetrical and is consistent with a single ^{18}F -product. The $[^{18}\text{F}]\text{-11-I}$ 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 $[^{18}\text{F}]\text{-11-I}$ 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 $[^{18}\text{F}]\text{-11-I}$ 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

We thank Dr Ian Luck and Dr Nick Proschogo for assistance with the NMR and ESI-MS experiments, respectively. We also thank the Australian Research Council for funding.

References

1. Madar I, Anderson JH, Szabo Z, et al. Enhanced uptake of [¹¹C]TPMP in canine brain tumor: a PET study. *J Nucl Med.* 1999;40(7): 1180-1185.
2. Calabrese G, Daou A, Rova A, Tseligka E, Vizirianakis I, Fatouros DG, Tsibouklis J. Boron-containing delocalised lipophilic cations for the selective targeting of cancer cells. *MedChemComm.* 2017; 8(1): 67-72.
3. Morrison DE, Issa F, Bhadbhade M, et al. Boronated phosphonium salts containing arylboronic acid, closo-carborane, or nido-carborane: synthesis, x-ray diffraction, in vitro cytotoxicity, and cellular uptake. *J Biol Inorg Chem.* 2010;15(8): 1305-1318.
4. Chen LB. Mitochondrial membrane potential in living cells. *Annu Rev Cell Biol.* 1988;4: 155-181.
5. Calabrese G, Gomes ACNM, Barbu E, Nevell TG, Tsibouklis J. Carborane-based derivatives of delocalised lipophilic cations for boron neutron capture therapy: synthesis and preliminary in vitro evaluation. *J Mater Chem.* 2008;18(40): 4864-4871.
6. Ioppolo JA, Clegg JK, Rendina LM. Dicarba-closo-dodecaborane(12) derivatives of phosphonium salts: easy formation of nido-carborane phosphonium zwitterions. *Dalton Trans.* 2007(20): 1982-1985.
7. Crossley EL, Ziolkowski EJ, Coderre JA, Rendina LM. Boronated DNA-binding compounds as potential agents for boron neutron capture therapy. *Mini-Rev Med Chem.* 2007;7(3): 303-313.
8. Crossley EL, Ching HYV, Ioppolo JA, Rending LM. Boron and gadolinium in the neutron capture therapy of cancer. In: Alessio E, ed. *Bioinorg. Med. Chem.:* Wiley-VCH; 2011:283-305.
9. Kim Y-S, Yang C-T, Wang J, et al. Effects of Targeting Moiety, Linker, Bifunctional Chelator, and Molecular Charge on Biological Properties of ⁶⁴Cu-Labeled Triphenylphosphonium Cations. *J Med Chem.* 2008;51(10): 2971-2984.
10. Wang J, Yang C-T, Kim Y-S, et al. ⁶⁴Cu-labeled triphenylphosphonium and triphenylarsonium cations as highly tumor-selective imaging agents. *J Med Chem.* 2007;50(21): 5057-5069.
11. Yang C-T, Li Y, Liu S. Synthesis and Structural Characterization of Complexes of a DO3A-Conjugated Triphenylphosphonium Cation with Diagnostically Important Metal Ions. *Inorg Chem.* 2007;46(21): 8988-8997.

12. Cheng Z, Subbarayan M, Chen X, Gambhir SS. Synthesis of (4-[¹⁸F]fluorophenyl)triphenylphosphonium as a potential imaging agent for mitochondrial dysfunction. *J Labelled Compd Radiopharm.* 2005;48(2): 131-137.
13. Madar I, Ravert H, Nelkin B, et al. Characterization of membrane potential-dependent uptake of the novel PET tracer ¹⁸F-fluorobenzyl triphenylphosphonium cation. *Eur J Nucl Med Mol Imaging.* 2007;34(12): 2057-2065.
14. Ravert HT, Madar I, Dannals RF. Radiosynthesis of 3-[¹⁸F]fluoropropyl and 4-[¹⁸F]fluorobenzyl triarylphosphonium ions. *J Labelled Compd Radiopharm.* 2004;47(8): 469-476.
15. Herholz K, Holzer T, Bauer B, et al. ¹¹C-methionine PET for differential diagnosis of low-grade gliomas. *Neurology.* 1998;50(5): 1316-1322.
16. Madar I, Ravert HT, Du Y, et al. Characterization of uptake of the new PET imaging compound ¹⁸F-fluorobenzyl triphenyl phosphonium in dog myocardium. *J Nucl Med.* 2006;47(8): 1359-1366.
17. Higuchi T, Fukushima K, Rischpler C, et al. Stable delineation of the ischemic area by the PET perfusion tracer ¹⁸F-fluorobenzyl triphenyl phosphonium after transient coronary occlusion. *J Nucl Med.* 2011;52(6): 965-969.
18. Madar I, Ravert H, DiPaula A, Du Y, Dannals RF, Becker L. Assessment of severity of coronary artery stenosis in a canine model using the PET agent ¹⁸F-fluorobenzyl triphenyl phosphonium: comparison with ^{99m}Tc-tetrofosmin. *J Nucl Med.* 2007;48(6): 1021-1030.
19. Madar I, Huang Y, Ravert H, et al. Detection and quantification of the evolution dynamics of apoptosis using the PET voltage sensor ¹⁸F-fluorobenzyl triphenyl phosphonium. *J Nucl Med.* 2009;50(5): 774-780.
20. Madar I, Isoda T, Finley P, Angle J, Wahl R. ¹⁸F-fluorobenzyl triphenyl phosphonium: a noninvasive sensor of brown adipose tissue thermogenesis. *J Nucl Med.* 2011;52(5): 808-814.
21. Issa F, Kassiou M, Rendina LM. Boron in drug discovery: carboranes as unique pharmacophores in biologically active compounds. *Chem Rev.* 2011;111(9): 5701-5722.
22. Nichols TL, Kabalka GW, Miller LF, Khan MK, Smith GT. Improved treatment planning for boron neutron capture therapy for glioblastoma multiforme using fluorine-¹⁸ labeled boronophenylalanine and positron emission tomography. *Med Phys.* 2002;29(10): 2351-2358.
23. Hawthorne MF, Maderna A. Applications of Radiolabeled Boron Clusters to the Diagnosis and Treatment of Cancer. *Chem Rev.* 1999;99(12): 3421-3434.

24. Paxton RJ, Beatty BG, Hawthorne MF, et al. A transition metal complex (Venus flytrap cluster) for radioimmuno-detection and radioimmunotherapy. *Proc Natl Acad Sci U S A*. 1991;88(8): 3387-3391.
25. Valliant JF, Morel P, Schaffer P, Kaldis JH. Carboranes as Ligands for the Preparation of Organometallic Tc and Re Radiopharmaceuticals. Synthesis of $[M(CO)_3(\text{C}\Sigma^5\text{-}2,3\text{-C}_2\text{B}_9\text{H}_{11})]$ - and *rac*- $[M(CO)_3(\text{C}\Sigma^5\text{-}2\text{-R-}2,3\text{-C}_2\text{B}_9\text{H}_{10})]$ - (M = Re, ⁹⁹Tc; R = CH₂CH₂CO₂H) from $[M(CO)_3Br_3]_2$. *Inorg Chem*. 2002;41(4): 628-630.
26. Gona KB, Gomez-Vallejo V, Padro D, Llop J. [¹⁸F]Fluorination of o-carborane via nucleophilic substitution: towards a versatile platform for the preparation of ¹⁸F-labelled BNCT drug candidates. *Chemical Communications*. 2013;49(98): 11491-11493.
27. Ioppolo JA, Kassiou M, Rendina LM. Water-soluble phosphonium salts containing 1,12-dicarba-closo-dodecaborane(12). *Tetrahedron Lett*. 2009;50(47): 6457-6461.
28. Aced G, Moeckel HJ, Nelsen SF. Ion-pair chromatographic separation of onium salts on an ODS column. *J Liq Chromatogr*. 1989;12(16): 3201-3218.
29. Jandik P, Deschler U, Schmidbaur H. Separation of mono- and bisphosphonium salts by preparative HPLC. *Fresenius' Z Anal Chem*. 1981;305(5): 347-354.
30. Holland HL. Long range carbon-13-fluorine-19 and proton-fluorine-19 coupling in fluorosteroids. *Tetrahedron Lett*. 1978(10): 881-882.
31. Ocampo R, Dolbier WR, Jr., Abboud KA, Zuluaga F. Catalyzed Reformatskii Reactions with Ethyl Bromofluoroacetate for the Synthesis of $\text{C}\Sigma^\pm$ -Fluoro- $\text{C}\Sigma^\leq$ -hydroxy Acids. *J Org Chem*. 2002;67(1): 72-78.
32. Weigert FJ. Fluorine magnetic resonance spectra of monofluoro- and difluoro-substituted hydrocarbons. *J Org Chem*. 1980;45(17): 3476-3483.
33. Dungan CH, Van Wazer JR. *Compilation of Reported ¹⁹F NMR Chemical Shifts: 1951 to Mid-1967* 1970.
34. Katayama H, Kamigaito M, Sawamoto M, Higashimura T. Living cationic polymerization of isobutyl vinyl ether by the CF₃CO₂H-SnCl₄-n-Bu₄NCl system: in situ direct analysis of the growing species by ¹H, ¹³C and ¹⁹F NMR spectroscopy. *J Phys Org Chem*. 1995;8(4): 282-292.
35. Kahrovic E, Orioli P, Bruni B, Di Vaira M, Messori L. Crystallographic evidence for decomposition of dimethylformamide in the presence of ruthenium(III) chloride. *Inorg Chim Acta*. 2003;355: 420-423.
36. Nunez R, Vinas C, Teixidor F, Sillanpaa R, Kivekas R. Contribution of the o-carboranyl fragment to the chemical stability and the ³¹P-NMR chemical shift in closo-

carboranylphosphines. Crystal structure of bis(1-yl-2-methyl-1,2-dicarba-closo-dodecaborane)phenylphosphine. *J Organomet Chem.* 1999;592(1): 22-28.

37. Teixidor F, Vinas C, Abad MM, Nunez R, Kivekaes R, Sillanpaea R. Procedure for the degradation of 1,2-(PR₂)₂-1,2-dicarba-closo-dodecaborane(12) and 1-(PR₂)-2-R'-1,2-dicarba-closo-dodecaborane(12). *J Organomet Chem.* 1995;503(2): 193-203.