JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2003; **46**: 623–631.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.702

Research Article

Feasibility of palladium-catalyzed isotopic exchange between sodium [^{125}I]I and 2-iodo-*para*-carborane

Ludvig Eriksson, Vladimir Tolmachev and Stefan Sjöberg*
Department of Organic Chemistry, Uppsala University, Box 531, Uppsala, S-75121, Sweden

Summary

Many *ortho*-/*meta*-/*para*-*closo*-carborane derivatives have been proposed for boron neutron capture therapy. However, it is difficult to follow their pharmacokinetics in patients, which creates a risk of suboptimal treatment. Adding a radioactive label to *closo*-carboranes may simplify pharmacokinetic studies. This paper reports on a study of the feasibility of palladium-catalyzed isotopic exchange of iodinated *closo*-carborane with radioisotopes of iodine. 2-iodo-*para*-carborane was selected as a model compound. It was shown that such isotopic exchange is possible and provides a high yield ($83 \pm 4.2\%$) after 40 min of reaction time. The reaction conditions were optimized, and it was demonstrated that the presence of tetra *n*-butylammonium hydrogensulfate is important in order to stabilize the catalyst and to give reproducibility of the labeling. Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: isotopic exchange; 2-iodo-*para*-carborane; [^{125}I]iodide; labeling; palladium

Introduction

Boron–neutron capture therapy (BNCT) is a cancer treatment modality based on high cross-section of interaction of thermal neutrons with ^{10}B

*Correspondence to: S. Sjöberg, Department of Organic Chemistry, Uppsala University, Box 531, Uppsala S-75121, Sweden. E-mail: ssj@kemi.uu.se

Contract/grant sponsor: Swedish Cancer Society (Cancerfonden)
Contract/grant sponsor: INTAS; contract/grant number: 99-00806

Received 5 July 2002
Revised 3 February 2003
Accepted 3 February 2003

atoms. The principle is to use a targeting process to deliver a large amount of ^{10}B into a tumor, and then irradiate the tumor area with thermal or epithermal neutrons. The interaction of the ^{10}B and the neutron generates two high-energy and high-linear energy transfer particles, ^{4}He and ^{7}Li, which can efficiently deactivate a cancer cell. Due to the short range of ^{4}He and ^{7}Li, surrounding healthy tissues remain undamaged.

The success of application of BNCT is determined by good timing of treatment. The irradiation should be performed when the tumor-to-non-tumor ratio of boron compound accumulation peaks. Knowledge of the pharmacokinetics of the boron compound is thus of great importance. However, study of the pharmacokinetics of boron compounds is a formidable task. Numerous patient studies employing sampling of tumoral and healthy tissues have demonstrated significant intratumoral and interpatient variability in uptake.[1–4]

It appears that attaching radioactive labels, which emit radiation detectable outside the human body (such as gamma or positron emitters), to boronated compounds for BNCT would facilitate pharmacokinetic study for an individual patient and improve the quality of treatment. The problem is that boron has no isotopes that can be used for pharmacokinetic studies. The only isotope of carbon to emit body-penetrating radiation is carbon-11 with a half-life of 20.3 min, which might enable synthesis of a carbon-11 labeled *closo*-carborane. It is possible that attaching a radioisotope of some other element, such as a radiohalogen, to a *closo*-carborane will not have a great effect on the biodistribution of the carborane and that changes in pharmacokinetics can be taken into account in accumulation studies and corrected for. This is what appears to happen when a carborane is attached to a large tumor-targeting molecule, such as a monoclonal antibody. Mizusawa and co-workers (1985) proposed the use of radioiodine labeling of an anionic boron cluster, *nido*-carborane, to study its pharmacokinetics.[5] Later a 2-nitroimidazole derivative of *nido*-carborane was labeled for the same purpose.[6] Radioiodination and radiobromination of derivatives of *closo*–dodecaborate have also been reported.[7–10]

Two review articles 'Application of Radiolabeled boron Clusters to the Diagnosis and Treatment of Cancer'[11] and 'Polyhedral Boron Compounds as Potential Linkers For Attachment of Radiohalogens to Targeting Proteins and Peptides. A Review'[12] summarize recent work in the area.

Oxidative labeling was used in all these cases. It should be noted that all of the substrates for labeling mentioned above are easily electrophilically iodinated or brominated with non-radioactive halogen. This is not the case for the group of uncharged *ortho*-/ *meta*-/ *para*-/ *closo*-carboranes. Electrophilic halogenation of these *closo*-carboranes is a lengthy process requiring rather harsh conditions for non-radioactive halogens ($AlCl_3/I_2$ in refluxing CH_2Cl_2 for long periods). Our attempts to label *para*-carborane using Chloramine-T as an oxidant were unsuccessful. Although a large number of substances synthesized for BNCT are based on *closo*-carboranes,[11,13–15] no method for radiolabeling uncharged *closo*-carboranes has been reported.

Recently, palladium-catalyzed nucleophilic substitution of 9-iodo-*m*-*closo*-carborane has been reported.[16] We supposed that these mild reaction conditions could be applied in labeling *closo*-carboranes using isotopic exchange. The goal of this study was to examine the feasibility of isotopic exchange in the systemsodium [^{125}I]iodide–iodo-*closo*-carborane, using 2-iodo-*para*-carborane (2-I-*p*-carborane) as a model compound.

Experimental

General

[^{125}I]iodide was obtained from Amersham Pharmacia Biotech UK Ltd, with a specific activity of 644 GBq/mg. Sigma-Aldrich Sweden AB supplied 1,4-*bis*-(diphenylphosphino)-butane (dppb), tris(dibenzylideneacetone)-dipalladium ($Pd_2(dba)_3$), acetonitrile (HPLC grade) and DMF (HPLC grade). DMF was distilled under reduced pressure over barium oxide and stored over 2 Å molecular sieves. Before each labeling experiment, the DMF was degassed by argon gas bubbling. Tetra-*n*-butylammonium hydrogensulfate ($QHSO_4$) was purchased from Lancaster Ltd.

2-I-*p*-carborane was prepared following a procedure analogous to that described by Jones *et al.*[17] for 9-iodo-*o*-carborane and adapted to *p*-carborane by Hawthorne *et al.*[18] The 2-I-*p*-carborane was characterized by ^{1}H, ^{13}C and ^{11}B NMR. Spectral data were found to be consistent with these from earlier reports.

Palladium-catalyzed formation of 2-bromo-para-carborane

One hundred milligram (0.37 mmol) 2-I-*p*-carborane, 358 mg (1.11 mmol) tetra-*n*-butylammonium bromide, 6.8 mg (7.4 μmol)

$Pd_2(dba)_3$, and 6.3 mg (14.8 μmol) dppb were weighed into a 5 ml reaction vial connected to a reflux condenser. The reaction vial was evacuated and back-filled with argon and 2 ml of 1,2-dimethoxyethane was added. The resulting solution was refluxed over night. After cooling, 10 ml diethyl ether was added to precipitate the quaternary salts from the solution. The remaining mixture was filtered and concentrated to give the desired product. GC–MS analysis of the residue showed no remaining 2-I-*p*-carborane and pure 2-Br-*para*-carborane.

General ^{125}I-labeling procedure

The following stock solutions were prepared: 2-I-*p*-carborane (1 mg/ml, 37 μmol/ml) and $QHSO_4$ (12.58 mg/ml, 37 μmol/ml), both in acetonitrile. $Pd_2(dba)_3$ (3.39 mg) and dppb (3.16 mg) were dissolved in 10 ml degassed DMF (0.74 μmol Pd (0) and 0.74 μmol dppb per ml) under argon prior to use.

In a typical labeling experiment, 0.25 MBq or 3.9×10^{-7} mg (3.12×10^{-9} mol) of [$^{125}I^-$] was used. An aqueous solution of sodium [$^{125}I^-$] iodide and acetonitrile-solutions of 2-I-*p*-carborane and $QHSO_4$ was transferred to a 2-ml Eppendorf tube. The solvent was evaporated under a flow of argon at 100°C. After complete evaporation, the stock solution of the Pd-catalyst in degassed DMF was added and the reaction vial was sealed. The reaction proceeded at 100°C. Samples for analysis (1–2 μl) were collected under a stream of argon as the reaction proceeded. Blank experiments were also performed in which the conditions were exactly the same as in the isotopic exchange experiments, but neat acetonitrile was used instead of the solution of 2-I-*p*-carborane in acetonitrile.

Analytical techniques

Silica gel 60 F_{254} thin layer chromatography plates (E. Merck, Darmstadt, Germany) were used in the analysis. The reaction mixture (1–2 μl) was applied to a TLC plate. The plate was subsequently eluted with freshly distilled pentane. R_f values of iodide ($R_f = 0.0$) and 2-iodo-*p*-carborane ($R_f = 0.5$) were determined by using non-labeled authentic samples. An eluted plate of non-labeled 2-I-*p*-carborane and NaI was developed by dipping the silica-coated plate into an acidified methanol solution of palladium (II) chloride and then heating it. The distribution

of radioactivity along the TLC strips (100×100 mm, elution path 80 mm) was measured on the Cyclone™ Storage Phosphor System (Packard Instruments Company Inc., Downers Grove, USA) and analyzed using OptiQuant™ Image Analysis Software. GC–MS analyses were performed on a Finnigan GC with a non-polar column and a Thermquest GCQ mass spectrometer.

Results and discussion

A representative TLC chromatogram of a reaction mixture is shown in Figure 1. Peak 1 with $R_f = 0.0$ corresponds to non-labeled iodide and [^{125}I] iodide. Peak 2 has the same $R_f = 0.5$ as non-labeled 2-I-*p*-carborane. This peak did not appear in the blank experiments, in which no 2-I-*p*-carborane was added. This indicates that isotopic exchange between [^{125}I] iodide and 2-I-*p*-carborane took place, and as a result [^{125}I]-labeled 2-iodo-*p*-carborane was formed. The mechanism of such an exchange could be an oxidative addition of Pd (0) to the I-B bond, followed by iodine exchange on the Pd and reductive elimination, as shown in Figure 2. A blank experiment with no catalyst gives no exchange, which supports this suggested pathway. Furthermore, Grushin and co-workers[10] have shown that 9-iodo-*meta*-

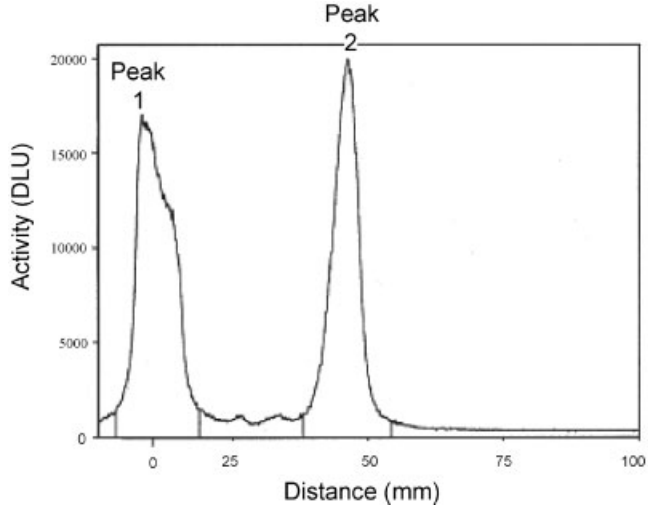

Figure 1. Representative radio-TLC chromatogram of the reaction mixture. Peak 1 ($R_f = 0.0$) corresponds to the peak of non-radioactive iodide, peak 2 ($R_f = 0.5$) corresponds to the peak of non-labeled 2-I-*p*-carborane

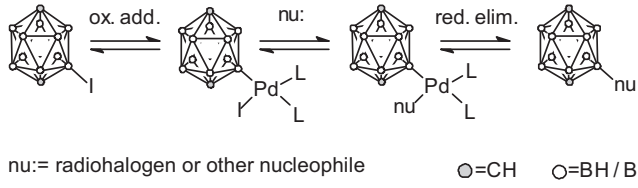

Figure 2. The suggested mechanism of the palladium-catalyzed nucleophilic substitution of 2-I-*p*-carborane

carborane can undergo palladium-catalyzed exchange with tetra-*n*-butylammonium bromide (to give 9-bromo-*meta*-carborane). A similar experiment with 2-I-*p*-carborane performed by our group, using tetra-*n*-butylammonium bromide in the presence of a palladium catalyst, yielded 2-bromo-*para*-carborane almost quantitatively, whereas in a blank experiment in the absence of palladium, no exchange occurred. Since the aqueous solution of [^{125}I]sodium iodide is stabilized with trace amounts of sodium hydroxide, tetra-*n*-butylammonium hydrogensulfate ($QHSO_4$) was added to the reaction. The aim was to prevent nucleophilic attack by hydroxide ions and the formation of 2-hydroxy-*para*-carborane (Figure 2). Quaternary ammonium salts have also been shown to stabilize Pd-catalyzed reactions.[19]

The results from reactions run with and without the $QHSO_4$ were compared, all with fixed amounts of [^{125}I] iodide, 2-I-*p*-carborane and Pd catalyst. The molar ratio between 2-I-*p*-carborane and Pd was 1:5. The reactions performed without $QHSO_4$ sometimes had high initial reaction rates with radiolabeling yield up to 65% after 20 min. The reproducibility was, however, poor and Pd-black precipitation was observed within 25 min in many attempts, with low final yields as a result. Reactions performed with various amounts of $QHSO_4$ generally gave better reproducibility, but at a lower rate. A correlation between the concentration of $QHSO_4$ and the reaction rate was noted, with higher amounts giving lower reaction rates (Figure 3). The best results were obtained with 5 mol equivalents of 2-I-*p*-carborane to 5 mol equivalent of $QHSO_4$ to 1 mol equivalent of Pd catalyst, providing a radiolabeling yield of 81% after 40 min.

We hoped to further increase the rate and yield of the reaction by reducing the reaction volume from 1 ml to 200 µl. At this reduced volume, with the same amounts of substrates as in the most successful series of 1-ml reactions, the concentration of palladium became too high and we once again experienced Pd-black precipitation. By reducing the

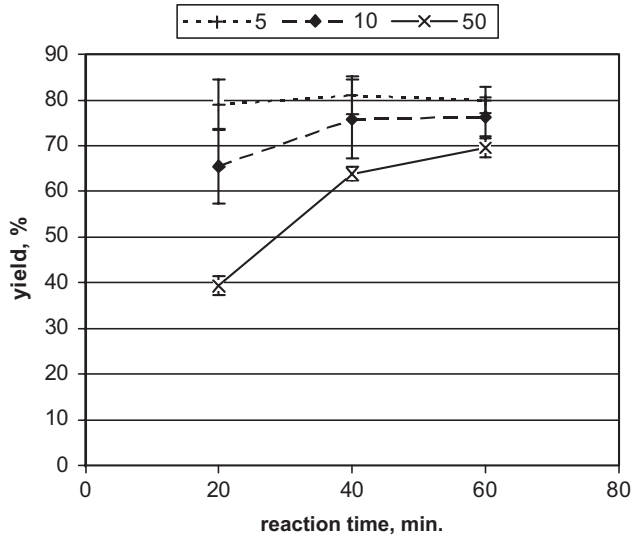

Figure 3. Influence of $QHSO_4$ concentration on the ^{125}I-labeling rate of 2-I-*p*-carborane (5 [+], 10 [◆] and 50 [×] molar equivalents of $QHSO_4$ per equivalent palladium)

amount of Pd stepwise to 0.04 eq and $QHSO_4$ to 0.4 to 2-I-*p*-carborane, the reaction could proceed with a labeling yield of 83% (with a standard deviation of 4.2 from six attempts) after 40 min. Using these optimized reaction conditions decreased the amount of 2-I-*p*-carborane in each reaction (with maintained amounts of [^{125}I]iodide, [Pd-cat.] and with the molar ratio of QHSO4 fixed to 0.4 to 2-I-*p*-carborane). As could be expected, the substitution rate decreased with decreased 2-I-*p*-carborane loading of the reaction, but it still produced a reasonable yield after 40 min reaction time with a loading of 100 μg 2-I-*p*-carborane (Figure 4).

Conclusions

Conditions have been found where the palladium-catalyzed isotopic exchange reaction of [$^{125}I^-$] and 2-I-*p*-carborane proceeds well with a high radiolabeling yield. The use of tetra-*n*-butylammonium hydrogensulfate is needed to prevent undesired side reactions and to stabilize the palladium catalyst, although high amounts will slow down the exchange rate. The reaction could find applications in pharmacokinetic

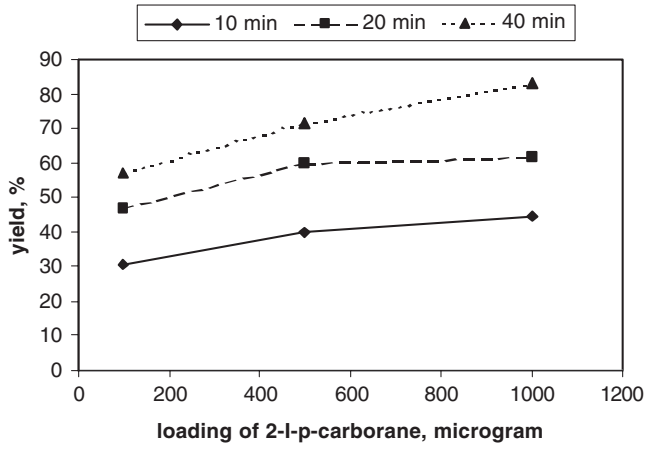

Figure 4. Isotopic exchange between [^{125}I] iodide and 2I-*p*-carborane with fixed volume and varied carborane loading

studies of halogenated *closo*-carborane derivatives and larger *closo*-carborane containing systems. Furthermore, since the oxidative addition of 2-bromo-*para*-carborane is likely to proceed more slowly than the oxidative addition of 2-I-*p*-carborane, this type of reaction could find use in radiobromination reactions with a high specific activity. A further investigation of the scope of the reaction is currently being carried out.

Acknowledgements

This work has been performed with financial support from the Swedish Cancer Society (Cancerfonden) and INTAS (grant 99-00806). We thank the Division of Biomedical Radiation Sciences for permission to use their facilities and equipment for work with radioactive materials.

References

1. Goodman JH, Yang W, Barth RF, *et al*. *Neurosurgery* 2000; **47**: 608–621.
2. Horn V, Pharm D, Slansky J, *et al*. *Int J Radiat Oncol Biol Phys* 1998; **41**: 631–638.
3. Kageji T, Nakagawa Y, Kitamura K, Matsumoto K, Hatanaka H. *J Neurooncol* 1997; **33**: 117–130.

4. Gabel D, Preusse D, Haritz D, *et al. Acta Neurochir* (*Wien*) 1997; **139**: 606–611.
5. Mizusawa EA, Thompson MR, Hawthorne MF. *Inorg Chem* 1985; **24**: 1911–1916.
6. Wilbur DS, Hamlin DK, Livesey JC, Srivastava RR, Laramore GE, Griffin TW. *Nucl Med Biol* 1994; **21**: 601–611.
7. Tolmachev V, Lundqvist H, Sivaev I, *et al. Journal of Lab Comp Radiopharm* 1997; **40**: 122–124.
8. Tolmachev V, Koziorowski J, Sivaev I, *et al. Bioconjugate Chem* 1999; **10**: 338–345.
9. Tolmachev V, Sjöberg S, Lundqvist H. *J Label Compd Radiopharm* 2001; **44**: 962–964.
10. Ul'yanenko SE, Yadrovskaya VA, Savina EP, Bozadzhiev LL, Brattsev VA. *Pharm Chem J* 2000; **34**: 73–75.
11. Hawthorne MF, Madena M. *Chem Rev* 1999; **99**: 3421–3434.
12. Tolmachev V, Sjöberg S. *Collect Czech Chem Commun* 2002; **67**: 913.
13. Hawthorne MF. *Angew Chem Int Ed Engl* 1993; **32**: 950–984.
14. Sjöberg S, Carlsson J, Ghaneolhosseini H, *et al. J Neurooncol* 1997; **33**: 41–52.
15. Soloway AH, Tjarks W, Barnum BA, *et al. Chem Rev* 1998; **98**: 1515–1562.
16. Marshall WJ, Young RJ, Grushin VV. *Organometallics* 2001; **20**: 523.
17. Li J, Logan CF, Jones M. 1991; **30**: 4866.
18. Jiang W, Knobler CB, Curtis CE, Mortimer MD, Hawthorne MF. *Inorg Chem* 1995; **34**: 3491.
19. Beletskaya IP. *Chem Rev* 2000; **100**: 3009–3066.

