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Research Article

Radiobromination of *closo*-carboranes using palladium-catalyzed halogen exchange

Karl Johan Winberg[1], Eskender Mume[1], Vladimir Tolmachev[1,2] and Stefan Sjöberg[1,*]

[1] *Department of Chemistry, Organic Chemistry, Uppsala University, Box 599, BMC, S-751 24 Uppsala, Sweden*

[2] *Rudbeck Laboratory, Biomedical Radiation Sciences, Uppsala University, S-751 85, Uppsala, Sweden*

Summary

closo-Carborane derivatives are often proposed as boron carriers for use in boron neutron capture therapy (BNCT) of cancer. A positron emitting radiolabel on a boron atom in such carborane compounds might facilitate pharmacokinetic studies in patients. In this paper the four iodo-*closo*-carboranes, namely 3-iodo-*ortho*-carborane, 9-iodo-*ortho*-carborane, 9-iodo-*meta*-carborane and 2-iodo-*para*-carborane were chosen as model compounds in a study of [^{76}Br]bromine labelling of carboranes using palladium-catalyzed halogen exchange. It was found that within a reaction time of 40 min, the four compounds were all radio-brominated in good to excellent yield, using Herrmann's catalyst (HC) in toluene. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: halogen exchange; carboranes; [^{76}Br]bromide; labelling; palladium; catalyst

Introduction

The development of mild methods for radiohalogenation of polyhedral boron compounds (PBCs) is of great interest in connections with their applications in biomedicine.[1,2] Derivatives of carboranes and carborate anions are frequently proposed to be used in boron neutron capture therapy (BNCT) for cancer.[3,4] Radiolabelling of carboranes with a positron emitting nuclide is an attractive

*Correspondence to: S. Sjöberg, Department of Chemistry, Organic Chemistry, Uppsala University, S-751 24 Uppsala, Sweden. E-mail: ssj@kemi.uu.se

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approach for pharmacokinetic studies, due to the lack of a suitable radioactive isotope of boron and to the fact that the only appropriate carbon isotope would be carbon-11, with a half-life of 20.3 min, which is too short for some applications. It is possible that radiohalogenation of the carborane will affect bio-distribution and pharmacokinetics, especially in smaller molecules, but this can usually be accounted for.

Mild oxidative conditions can be used to radiohalogenate negatively charged PBCs, like mono-*closo*-carborane [$CH_{12}B_{10}^-$], *nido*-carborate [$C_2B_9H_{12}^-$], and *closo*-$B_{12}H_{12}^{2-}$ and their derivates. This method is unfortunately not applicable to *closo*-carboranes, where the cold analogues can be obtained through electrophilic halogenation; however the long and harsh conditions which are required, limit its use for boronated biomolecules.

The first radiolabelling of carboranes using radiohalogens were reported by Stanko and Iroshnikova in 1970.[5] A number of *ortho*-, *meta*-, and *para*-*closo*-carboranes were radiolabelled with ^{131}I, using an isotopic exchange reaction, starting from the cold iodinated analogue and with iron (II) sulfate as a catalyst. A palladium-catalyzed halogen exchange reactions using non-radioactive substances has previously been reported by Marshall *et al.*,[6] and our group recently reported the first palladium-catalyzed radioiodination of *p*-carborane, using an isotopic exchange reaction.[7] The labelling protocol used in Reference[7] was later improved to give a milder and faster method for radioiodination of carboranes, where the palladacycle Herrmann's catalyst (HC) (Figure 1) was used with toluene as reaction media.[8]

^{76}Br is a possible radionuclide for PET studies. This nuclide has a half-life of 16.2 h and emits 54% positrons per decay, properties that are well suited for labelling. In the study reported in this paper, the iodinated *closo*-carboranes **1–4** were subjected to palladium-catalyzed halogen exchange using [^{76}Br]bro-

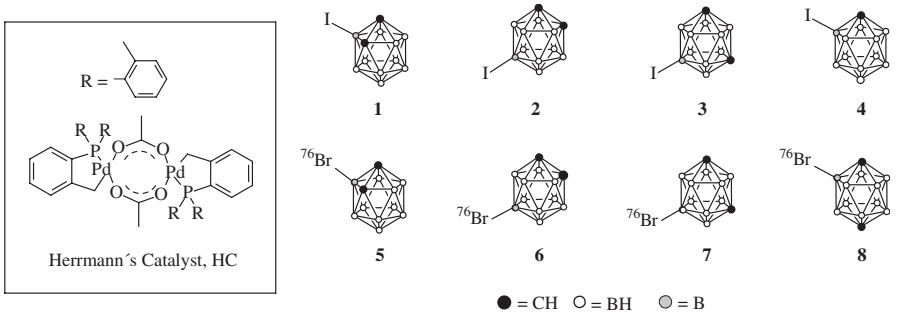


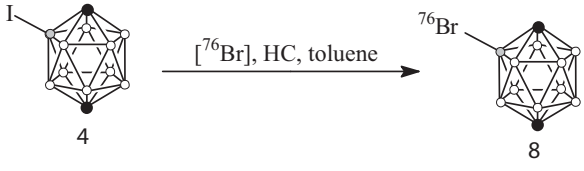
Figure 1. Compounds 1–4: The iodinated *closo*-carboranes subjected to palladium-catalyzed halogen exchange experiments as described in the text. Compounds 5–8: the corresponding radiobrominated analogues of compounds 1–4. The Herrmann's catalyst (HC) was used in the experiments

mide in order to investigate the possibility to obtain the corresponding radiobrominated compounds **5–8** (Figure 1).

Results and discussion

From our previous investigations,[8] we know that the combination of HC (Figure 1) and toluene is a stable and reliable catalytic system for isotopic exchange reaction using isotopes of iodine. Marshall and co-workers[6] have earlier reported bromination of 9-iodo-*m*-carborane by palladium-catalyzed nucleophilic displacement reactions. By using 2-iodo-*p*-carborane as a model substance, we have optimized the conditions for palladium-catalyzed halogen ($[^{76}Br]Br^-$) exchange reaction (see Scheme 1).

Figure 2 displays a typical TLC-result of [^{76}Br]bromide radiolabelling of the model substance, 2-iodo-*p*-carborane (**4**), using the final optimized reaction conditions, 40 min reaction time at 110°C, 2 mg 2-iodo-*p*-carborane in 200 µl of toluene under argon. The small peak at 0 ($R_f = 0.0$) corresponds to non-reacted [^{76}Br]bromide, and the peak at $R_f = 0.5$ corresponds to the labelled 2-[^{76}Br]bromo-*p*-carborane.



Scheme 1. Radiobromination of the model compound 2-iodo-*p*-carborane (4), using HC in toluene

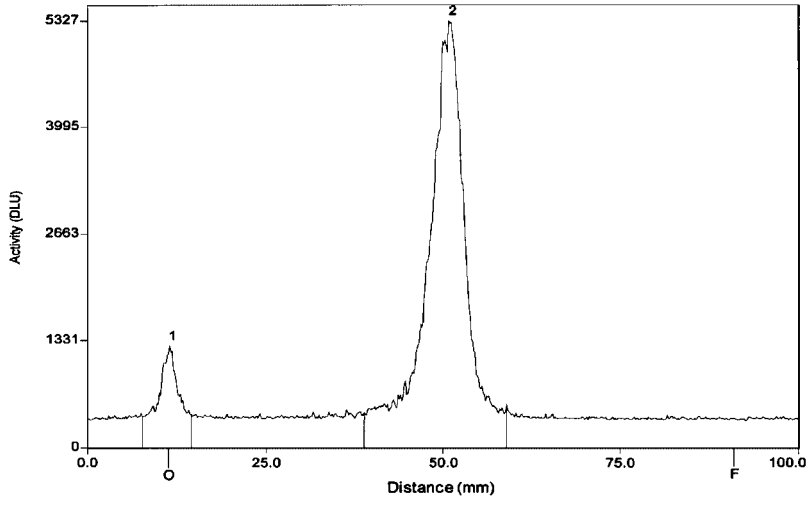


Figure 2. Typical TLC-result for 2-[^{76}Br]bromo-*p*-carborane (8) using the general labelling procedure

Our results from the initial trials, using the same conditions as in our earlier study,[8] namely 0.1 mg/ml 2-iodo-*p*-carborane (**4**) and 5 mol% load of HC in 200 ml toluene for 10 min, was far from acceptable (>20% radiochemical yield). By prolonging the reaction time we could increase the radiochemical yield to 65% after 60 min.

The temperature dependence of the reaction was also examined. It was found that a decrease in reaction temperature resulted in lower yield. However, increasing the temperature to 110°C resulted in an enhanced radiochemical yield: 80% (see Figure 3).

By varying reaction time at this increased reaction temperature we observed that a decrease in reaction time to 40 min, gave approximately the same yield as for 60 min. Decreasing reaction time further resulted in too much drop in yield to be of interest (see Figure 4).

In order to optimize the catalyst concentration, the effect of varying the HC concentration was investigated. In addition to the initial 5% loading, concentrations of 10, 2.5 and 1.25% were investigated. We could not observe

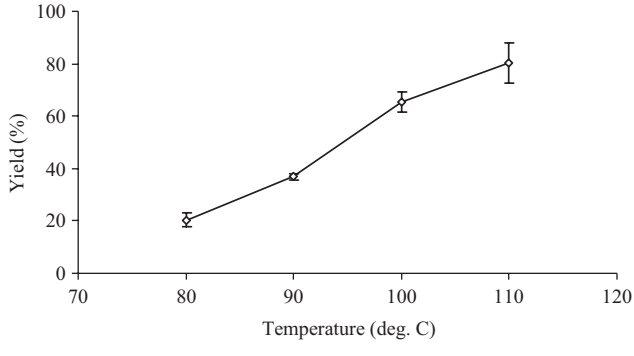


Figure 3. Radiobromination yield as a function of temperature (60 min, 1 mg 2-iodo-*p*-carborane (4), 5 mol% HC, toluene)

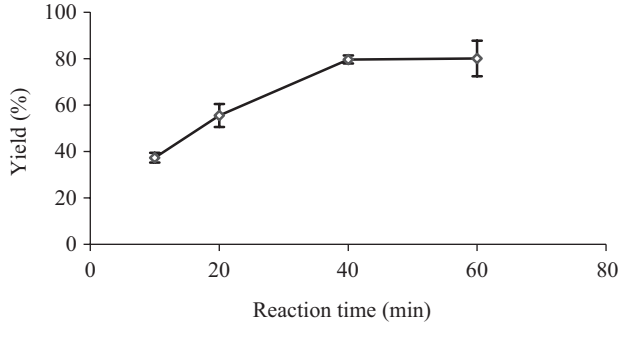


Figure 4. Radiobromination yield as a function of time at 110°C (1 mg 2-iodo-*p*-carborane (4), 5 mol% HC, 40 min, toluene)

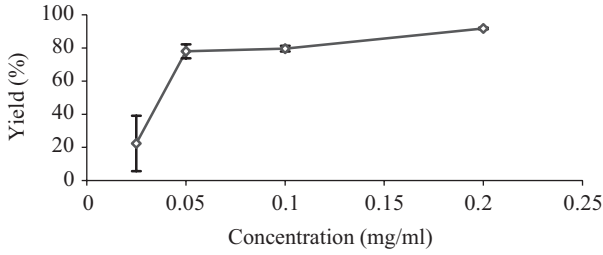


Figure 5. Radiobromination yield as a function of variation of concentration of 2-iodo-*p*-carborane (4) (110°C, 5 mol% HC, 40 min, toluene)

Table 1. Radiochemical yield for the bromo-carboranes (5–8) using 2 mg iodinated carborane, 5 mol% HC in toluene for 40 min at 110°C

Entry	Carborane	Yield (%)
1	2-[^{76}Br]Br-*p*-carborane (**8**)	92 ± 0.5
2	3-[^{76}Br]Br-*o*-carborane (**5**)	99 ± 0.1
3	9-[^{76}Br]Br-*o*-carborane (**6**)	100 ± 0
4	9-[^{76}Br]Br-*m*-carborane (**7**)	64 ± 2.5

any increase of the radiochemical yield by varying the load of the catalyst. Both increase and decrease of the catalyst concentration resulted in lower radiochemical yield.

To investigate the influence of the concentration of 2-iodo-*p*-carborane (**4**) on the radiochemical yield, different concentrations, 0.2, 0.05 and 0.025 mg/ml were compared to the initial concentration of 0.1 mg/ml (37 μmol/ml) (40 min, 5 mol% HC at 110°C). It was found that a concentration of 0.025 mg/ml decreased the outcome of the reaction to a very high extent (yield: 22%). Using a concentration of 0.05 mg/ml was comparable to the standard load, giving 78% yield. However, increasing the concentration of **4**–0.2 mg/ml, the radiochemical yield increased to 92%. See Figure 5.

In order to investigate the range of applicability of the proposed procedure, 3-I-*o*-carborane (**1**), 9-I-*o*-carborane (**2**) and 9-I-*m*-carborane (**3**) were also subjected to this optimized reaction conditions (see Table 1).

Radiolabelling to form the two bromo-*o*-carboranes (**5** and **6**) proceeds smoothly and with excellent yields (Entry 2 and 3), exceeding that of the *p*-compound **8** (Entry 1). Disappointingly, the *meta*-compound (**7**) (Entry 4) did not provide the same radiochemical yield as the three other isomers. Subsequent to the reactions with the two *ortho*-isomers, deposition of palladium black could be observed. This result did not seem to influence the outcome of the reaction, however.

Experimental

Materials

HC (*trans*-di-μ-acetatobis [2-(di-o-tolylphosphino) benzyl] dipalladium) (II) was purchased from Lancaster Ltd. Acetonitrile (HPLC grade) was purchased from Sigma-Aldrich Sweden AB and toluene was distilled from sodium and benzophenone. 2-bromo-*para*-carborane was prepared according to the procedure described by Sieckhaus *et al.*,[9] 9-bromo-*ortho*-carborane and 9-bromo-*meta*-carborane according to procedure described by Zakharkin *et al.*[10] and 3-bromo-*ortho*-carborane according to the procedure described by Li and Jones.[11] The corresponding iodo-carboranes were obtained as described earlier.[8] All iodinated and brominated carborane were characterized by GC-MS, ^{1}H, ^{13}C and ^{11}B NMR. The NMR-spectral data were found to be consistent with those reported in the literature.

The [^{76}B]bromide was produced according to Tolmachev *et al.*[12] A low-energy cyclotron (MC17, Scanditronix, Uppsala, Sweden) at the Uppsala University PET Center was used for bombardment of a ^{76}Se-enriched copper–selenide target with 16 MeV protons. The nuclear reaction utilized was ^{76}Se(p, n)^{76}Br. The water-cooled [^{76}Se]Cu_2Se pellet (0.5 cm^2, 180 mg/cm^2) was bombarded for about 2 h with a beam current of 10–15 μA. The radio-bromine was separated from the target material by dry distillation and finally obtained in high quality (resistance >18 MO/cm) ELGA® water in a total volume of 150–200 μl. Routinely, 150–300 MBq was obtained per production batch.

General ^{76}Br-labelling procedure

The following stock solutions were prepared: iodinated carborane (1 and 2 mg/ml, 37 and 74 μmol/ml) in acetonitrile. HC (6.94 mg) was dissolved in 10 ml degassed toluene (0.74 μmol Pd (II) HC) under argon, prior to use.

In a typical labelling experiment here, 5 MBq, no carrier added (n.c.a) [^{76}Br]bromide was used. 5 μl of an aqueous solution of [^{76}Br]bromide and 100 μl of the acetonitrile solution of iodinated carborane was transferred to a 2 ml Eppendorf tube. The solvents were evaporated under a flow of argon at 100°C. After complete evaporation, 200 μl of stock solution HC in toluene was added, still under a flow of argon, and the reaction vial was sealed. The reaction proceeded at 110°C for 40 min, and subsequently 200 μl of toluene was added to the reaction vial. After mixing, samples for TLC analysis (1–2 μl) were collected. Blank experiments were run using exactly the same conditions, but neat toluene was used instead of the stock solution of HC and neat acetonitrile was used instead of the solution of iodinated carborane. All blank experiments were tested in both TLC-systems.

Analytical techniques

Silica gel 60 F_{254} thin-layer chromatography plates (E. Merck, Darmstadt, Germany) were used for analysis. The reaction mixture (1–2 μl) was applied on a TLC plate, using pure *n*-hexane as eluent for 2-iodo-*p*-carborane (**4**), and a mixture of *n*-hexane and ethyl-acetate (1:1) for the *ortho*- and *meta*- substances (**1–3**). The R_f values for the non-radiolabelled bromo-carboranes were used for comparison and were the same as those of their radiolabelled analogues. For visual detection, an eluated plate of non-labelled carborane was developed by dipping it into an acidified methanol solution of palladium (II) chloride, by subsequent heating. The distribution of radioactivity along the TLC strips (100×50 mm, elution path 80 mm) was measured on the Cyclone™ Storage Phosphor System (Packard Instruments Company Inc., Downers Grove, US) and analyzed using the OptiQuant™ Image Analysis Software. GC-MS analyses were performed with a non-polar column and a Thermquest GCQ mass spectrometer. ^{1}H, ^{13}C, and ^{11}B NMR spectra were recorded in $CDCl_3$ (7.26 and 77.0 ppm, respectively) on a Varian XL spectrometer operating at 400 MHz. Boron trifluoride was used as external standard for the ^{11}B analyses.

Conclusion

By reacting iodinated carboranes with [^{76}Br]bromide in the presence of Herrmann's, catalyst we have demonstrated the feasibility to radiobrominate carboranes. Via halogen exchange reaction we have obtained 2-[^{76}Br]bromo-*p*-carborane (**8**), 3-[^{76}Br]bromo-*o*-carborane (**5**) and 9-[^{76}Br]bromo-*o*-carborane (**6**) in excellent radiolabelled and 9-[^{76}Br]bromo-*m*-carborane (**7**) in good radiochemical yield. The results from the present study may prove to be applicable to pharmacokinetic studies of carboranes and derivates of carboranes.

Acknowledgements

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