Pergamon

Tetrahedron Letters, Vol. 37, No. 38, pp. 6905-6908, 1996

0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)01512-2

Synthesis of *para*- and *nido*-Carboranyl Phenanthridinium Compounds for Neutron Capture Therapy

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Abstract: The syntheses of two novel *para*-carboranyl and two novel *nido*-carboranyl phenanthridinium compounds as potential boron delivery agents for Neutron Capture Therapy are described

The synthesis of ^{10}B-containing agents that will selectively localize in tumor cells and thereby deliver 15-30 μg of this nuclide to each gram of tumor remains to be one of the most challenging areas of investigation in Neutron Capture Therapy (NCT).[1] Calculations have shown that the radiobiological effectiveness of the boron neutron capture reaction [^{10}B(^{1}n,^{4}He)^{7}Li] is significantly enhanced when it occurs in the cell nucleus rather than in the cytoplasm or the cell membrane.[2] Boronated analogues of DNA binding compounds such as bibenzimidazoles, polyamines, netropsin, and distamycin have been synthesized and their capacity to selectively deliver ^{10}B to tumor cell nuclei is under investigation.[3]

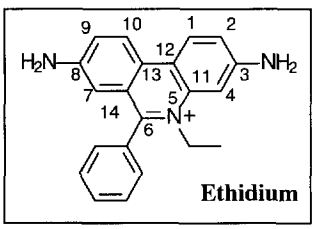

Figure 1

Our own work is focused on the synthesis and biological evaluation of boron-containing analogues of the widely studied DNA intercalator ethidium (**Figure 1**). DNA-binding experiments with numerous ethidium derivatives possessing different substituents at positions 5 and 6 have shown that the size and physico-chemical nature of these groups, probably located in the minor groove[4], have only little influence on the DNA intercalating capacity of the phenanthridine chromophore.[5] Only a negative charge at these groups seems to result in decreased DNA binding constants.[5] Thus, compounds **7** (**Scheme 2**) and **13** (**Figure 2**) with a *para*-carborane moiety in either position 5 or 6 were selected as target compounds. The *nido*-carboranes **8** and **14** were synthesized for comparative biological studies.

Bromination of 1-(3-hydroxypropyl)-*p*-carborane[6] (**1**) (**Scheme 1**) with carbon tetrabromide and triphenylphosphine in dichloromethane at 0 °C for 10 minutes afforded **2** in 91% yield. Iodide **3** was prepared from **2** in 92% yield by Finkelstein reaction with sodium iodide in acetone. Compounds **2** and **3** were purified by silica gel column chromatography with pentane as the mobile phase. *N*-5 Alkylation of 3,8-bis(carbethoxy-amino)-6-phenylphenanthridine[7] (**4**) with a twofold excess of either **3** or 1-(3-iodopropyl)-*o*-carborane[8] (**Scheme 2**) in nitrobenzene for 24 hours at 130 °C afforded 3,8-bis(carbethoxyamino)-5-(3-*p*-carboranyl-propyl)-6-phenylphenanthridinium iodide and the corresponding *ortho*-analogue. The crude products (70%-80% yield) were precipitated by adding diethyl ether and subsequently hexane to the reaction mixtures and

were filtered off. The filtrates were evaporated and excessive iodopropylcarboranes were recovered by column chromatography.

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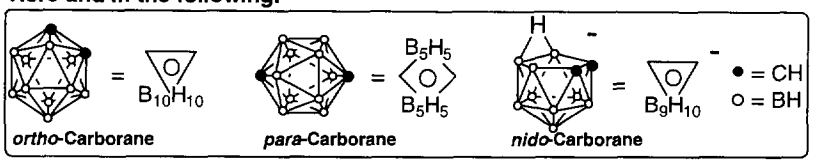
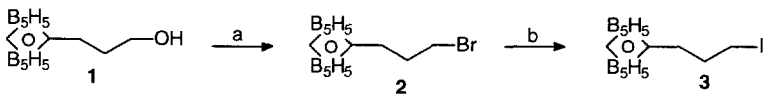
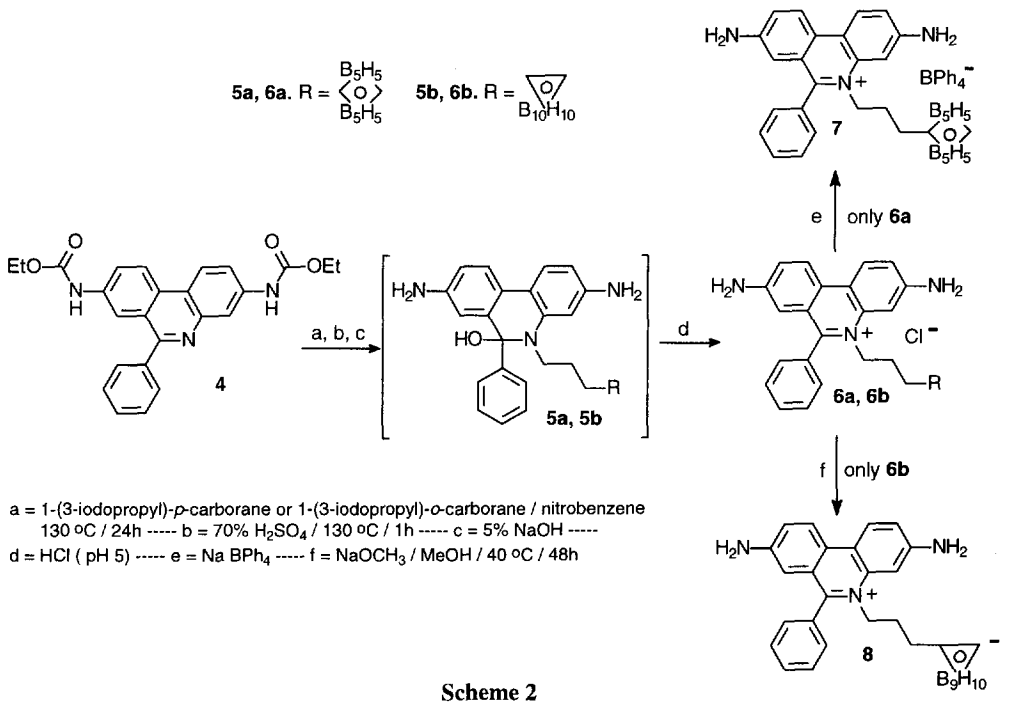




a = CBr_4 / PPh_3 / 0 °C / 10 min / CH_2Cl_2
b = NaI / reflux / 24 h / acetone

Scheme 1

The compounds were directly deprotected by acidic hydrolysis in 70% sulfuric acid for 1 hour at 130 °C. The pHs of the reaction mixtures were carefully raised to 13-14 at 0 °C with 5% aqueous sodium hydroxide and the neutral pseudobase intermediates[7] **5a** and **5b** were extracted with dichloromethane. The organic layers were washed with HCl (pH 5) resulting in the formation of 3,8-diamino-5-(3-*p*-carboranylpropyl)-6-phenylphenanthridinium chloride (**6a**) and its *ortho*-analogue (**6b**), which remained in the organic phases. These salts were roughly purified by silica gel column chromatography with the solvent system dichloromethane/methanol (7/3).



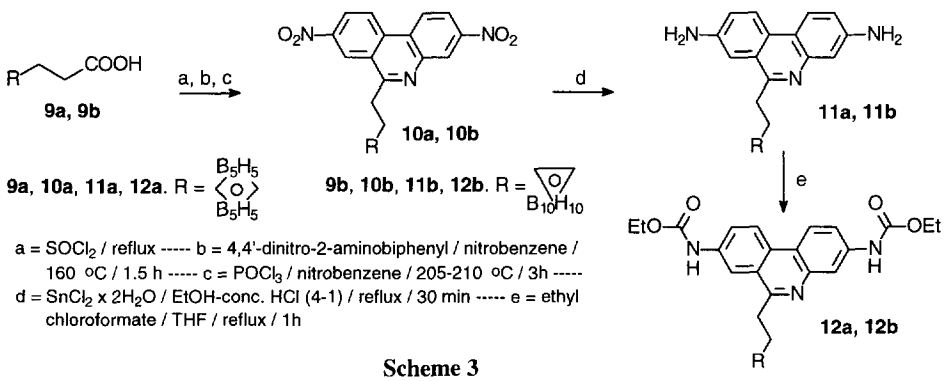
a = 1-(3-iodopropyl)-*p*-carborane or 1-(3-iodopropyl)-*o*-carborane / nitrobenzene 130 °C / 24h ----- b = 70% H_2SO_4 / 130 °C / 1h ----- c = 5% NaOH -----
d = HCl (pH 5) ----- e = Na BPh_4 ----- f = $NaOCH_3$ / MeOH / 40 °C / 48h

Scheme 2

Partial degradation of the *ortho*-carborane compound **6b** to the corresponding *nido*-analogue was already observed by TLC during reaction and work-up. Cage degradation was also found when **6b** was suspended in

methanol/water and left at room temperature for several days. The same was noticed with acetate as the counter ion. It is believed that the weakly basic free amino functions of the molecule and not the counter ions are responsible for the *nido*-formation because we have found that aniline had the same effect on *ortho*-carborane suspended in methanol/water. Basic self degradation of aminoaryl *o*-carboranes has to our knowledge not been reported before.[9] Partial *nido*-formation under aqueous conditions was observed for all *ortho*-carboranyl compounds described herein containing free amino groups.

A small amount of crude **6b** was analyzed by FAB(+)-mass-, and ^{1}H-NMR spectroscopy[10], the rest was directly converted to **8** by treatment with sodium methylate in methanol at 40 °C for 48 hours. Chloride **6a** was dissolved in methanol and a solution of sodium tetraphenylborate in water was added. The resulting precipitate (**7**) as well as **8** were purified by silica gel column chromatography with the dichloromethane/methanol (49/1) as the mobil phase. However, the chromatographic purification proved to be difficult for both compounds because of numerous minor impurities with similar Rf-values. The overall yields from **4** to **7** and **4** to **8** were 43% and 17%, respectively.



Scheme 3

A one-pot procedure, developed for the synthesis of 3,8-dinitro-6-^{14}C-phenylphenanthridine[11], was employed for the preparation of 3,8-dinitro-6-(2-*p*-carboranylethyl)phenanthridine (**10a**) and its *ortho*-analogue (**10b**) (**Scheme 3**). 3-(*p*-Carboranyl)propionic acid[6] (**9a**) and 3-(*o*-carboranyl)propionic acid[12] (**9b**) were refluxed for 1 hour in thionyl chloride to yield the corresponding acid chlorides which were treated with 4,4'-dinitro-2-aminobiphenyl. The resulting amides were cyclized using phosphorous oxychloride to afford **10a** and **10b** in 49% and 53% yield, respectively. Purification of both compounds was accomplished by recrystallization from large volumes of acetone.

Compounds **10a** and **10b** were reduced to the corresponding amines **11a** and **11b** with tin(II) chloride dihydrate in boiling ethanol/conc. HCl (4/1) for 30 minutes. The amines were directly converted to **12a** and **12b** by the action of potassium carbonate and ethyl chloroformate in THF because the formers were found to be light- and air sensitive. Only small amounts of **11a** and **11b** were purified for mass- and ^{1}H-NMR-spectroscopy by silica gel column chromatography with dichloromethane/methanol (23/2).[10] Compounds **12a** and **12b** were purified by recrystallization from ethanol. The overall yields from **10a** to **12a** and **10b** to **12b** were 56% and 49%, respectively.

Phenanthridines **12a** and **12b** were converted to the target compounds **13** and **14** (**Figure 2**) as described for the reactions from **4** to **7** and **4** to **8** using ethyl tosylate as the alkylating agent. The duration of the *N*-5 alkylation was reduced to 4 hours because it had been reported previously that longer reaction times favor the

decomposition of ethyl tosylate to toluenesulphonic acid which in turn causes enhanced decomposition of quaternization products.[7] The low yields found for **13** (7%) and **14** (4%) may already be contributed to the formation of certain quantities of toluensulfonic acid during the 4 hour period.

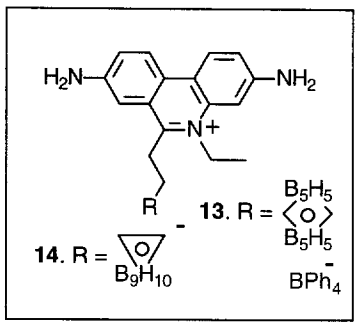


Figure 2

Target compounds **7**, **8**, **13**, **14** were evaluated with respect to their DNA binding capacity using human glioma cells U-1231 as well as calf thymus DNA. The acetate salt of **7** especially showed a strong DNA binding comparable to that of ethidium bromide. A detailed description of the biological evaluation of the target agents will be published elsewhere.

In summary, we have described the syntheses of novel *para- and nido*-carboranyl phenanthridinium compounds which showed promising results as possible NCT agents in initial biological tests. Problems with partially low yields remain to be solved and tumor-selective delivery systems, such as liposomes and protein conjugates, have to be explored.

ACKNOWLEDGMENT

We thank Drs. Soloway, Wyzlic, and Rong from the College of Pharmacy and Dr. Chang from the Campus Chemical Instrumentation Center of The Ohio State University, Columbus, Ohio, for providing us with mass spectra. This work was supported by the Swedish Cancer Society.

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10. Compounds were analyzed by ^{1}H-NMR spectroscopy (**2**, **3**, **6b**, **7**, **8**, **10a**, **10b**, **11a**, **11b**, **12a**, **12b**, **13**, **14**), ^{13}C-NMR spectroscopy (**2**, **3**, **7**, **8**, **12a**, **12b**, **13**, **14**), FAB-mass spectroscopy (**6b**, **8**, **10a**, **10b**, **11a**, **11b**, **12a**, **12b**), ESP-mass spectroscopy (**7**), FAB-high resolution-mass spectroscopy (**13**, **14**), IR (**2**, **3**, **7**, **8**, **10a**, **10b**, **12a**, **12b**, **13**, **14**), and elemental analysis (**2**, **3**, **7**, **8**, **10a**, **10b**, **12a**, **12b**). The obtained results are in accordance with the proposed structures.
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(*Received in UK* 10 *June* 1996; *revised* 29 *July* 1996; *accepted* 1 *August* 1996)