Synthesis and in vitro anti-tumor activity of carboranyl levodopa

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\textbf{A R T I C L E   I N F O}

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\textbf{A B S T R A C T}

Reactions of closo-1-Me-2-Iodobutyl-1,2-closo-dicarborane, 1-Me-2-I(CH\textsubscript{2})\textsubscript{4}C\textsubscript{6}B\textsubscript{11}H\textsubscript{10}, with \textit{l}-dopa methyl ester can produce carboranyl \textit{l}-dopa methyl esters in 54% yield in the presence of sodium hydroxide. The appended closo-carboranes can be decapitated with sodium hydroxide in a mixed solvent of ethanol and deionized water to produce highly water-soluble carboranyl levodopa in 64% yield. All the new compounds were characterized by \textit{H}, \textit{13C}, \textit{11B} NMR, FT-IR spectroscopy and elemental analysis. The highly water soluble carboranyl levodopa shows promising efficacy of anti-tumors in vitro in the presence of slow neutron beams.

\section{1. Introduction}

Gliomas are a broad category of brain tumors that arise from the brain tissue itself. Based on histological characteristics, gliomas can be classified as World Health Organization (WHO) grades I-IV. Glioblastoma multiforme (GBM) is a WHO grade IV glioma, which is well recognized as the most malignant grade [1]. Although the causes of gliomas are not so far clear, it is believed that a prior radiation to the brain is one established environmental risk factor identified for the majority of malignant gliomas [2]. Thus, the GBM remained virtually untreatable and it is, therefore, inevitably lethal. These tumors tend to grow and infiltrate into the normal brain tissues. They infiltrate the brain so aggressively that surgeons are rarely able to remove all the cancerous tissues. These types of tumors are resistant to standard radiation treatment and chemotherapy and that complicates the treatment [3]. Therefore, a significant development of new effective drugs and therapy technologies are needed urgently for the treatment of malignant gliomas.

Boron Neutron Capture Therapy (BNCT) is a binary form of cancer treatment, in which a compound containing B-10 is selectively delivered to tumor tissues prior to irradiation by neutrons. Upon irradiation with thermal neutrons, interaction of a boron-10 atom with thermal neutron produces an \textit{α}-particle, high energy Li-7 ion and low energy gamma \textit{γ} rays (Eq. (1)). The linear energy transfer (LET) of these heavily charged particles has a range of one cell diameter which confines the radiant energy to the cell within which they arise, hence minimizing the damage to surrounding tissue. The capture cross section of boron for neutrons is more than three orders of magnitude higher than for other nuclei common to living tissue, so the target region can be dosed with neutrons at a sizeable flux and still have only minimal effect on boron-free regions in the beam path. Additional advantages of using B-10 compared to other nuclides (\textit{235}U, \textit{6}Li) are that the fission effect on boron-free regions in the beam path. Additional advantages of using B-10 compared to other nuclides (\textit{235}U, \textit{6}Li) are that the fission products have high LET and the boron compounds can be synthesized having hydrolytically stable linkages between boron and other elements such as C, O and N. If boron can be transported to the target tissue with sufficient specificity using tumor selective agents and sufficient concentration of \textit{~10}^{11}10B atoms (natural abundance 19.9%) per cell, which translates to approximately 35 μg \textit{10B} per gram of tissue. To prevent damage to healthy tissue in the path of the neutron beam, the surrounding tissue should contain no more than 5 μg of boron-10/g of tissue.

\begin{equation}
\text{10B} + \text{n} \rightarrow [\text{11B}] \rightarrow 4\text{He}^{2+}(\alpha) + 7\text{Li}^{3+} + 2.31 \text{Mev}
\end{equation}

The BNCT is an innovative treatment that should provide opportunities and hope to patients and their doctors. Besides the treatment of difficult brain tumors, it has been found that BNCT treatment has an excellent response with patients’ recurrent head and neck cancers, for whom there is no other treatment option available [6,7]. The BNCT is a potentially powerful form of radiotherapy involving the preferential incorporation of boron-10 containing compounds into tumor cells, followed by irradiation of the tumor by thermal neutrons. Several requirements must be met in order for this therapy to be effective: (i) a concentration of \textit{~20-50} μg \textit{10B} atoms/g of tumor must be achieved; (ii) a tumor/normal tissue (T/N) ratio of the boron delivery agent greater than 3 and tumor/blood (T/N) ratio of greater than 5 are

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nido-C_{2}B_{10}H_{10})-phenylalanine methyl ester (3) in 54% yield. The closo-carborane cage in (3) can be decapitated by NaOH in 90% ethanol under reflux condition. After neutralization, the nido-carborane-appended \( \text{i-DOPA} \) methyl acetate, \( L,3,4-\text{Di}(1′-\text{Me}-2′-(\text{CH}_{2})_{2}-\text{closo-C}_{2}B_{10}H_{10})\)-phenylalanine methyl ester (3) in 54% yield. The closo-carborane cage in (3) can be decapitated by NaOH in 90% ethanol under reflux condition. After neutralization, the nido-carborane-appended \( \text{i-DOPA} \), \( L,3,4-\text{Di}(7′-\text{Me}-8′-(\text{CH}_{2})_{4}-\text{nido-C}_{2}B_{10}H_{10})\)-phenylalanine (4) is obtained as an off-white solid in 64% yield.

The compounds 3 and 4 were characterized by elemental analysis, NMR spectra and FT-IR spectra. All data are consistent with the formulations shown in Scheme 1. The \(^{1}H\) and \(^{13}C\) NMR spectra of compounds 3 and 4 show normal absorptions of methyl, butylene, ester, carboxylic functional groups and carborane cages. The \(^{13}C\) NMR spectra of 3 show the absorptions of the carbons of carborane cage at \( \delta = 75.66 \) and 74.25 ppm, which are in the range of the reported \( C_{cage} \) resonances of the \( C_{2}B_{10} \) systems [19,20]. The \(^{11}B\) NMR spectra are also consistent with the reported absorptions of the carborane ring.

2. Results and discussion

The carboranyl \( \text{i-DOPA} \) compounds were synthesized according to the reaction routes shown in Scheme 1. Following literature procedures, commercially available closo-carborane, 1-H-2-Me-closo-C_{2}B_{10}H_{10} (1) is deprotoned by \( n \)-butyllithium to form a carboranyl anion, and the \( \text{insitu} \) generated anion subsequently reacts with 1,4-diiodobutane to form the precursor, iodoethyl carboranes, 1-Me-2-\( \text{I}(\text{CH}_{2})_{4}-\text{closo-C}_{2}B_{10}H_{10} \) (2) [19]. The carboranyl iodides react with \( \text{i-DOPA} \) methyl acetate hydrochloride, in a molar ratio of 2:1, to produce closo-carborane-appended \( \text{i-DOPA} \) methyl acetate, \( L,3,4-\text{Di}(1′-\text{Me}-2′-(\text{CH}_{2})_{2}-\text{closo-C}_{2}B_{10}H_{10})\)-phenylalanine methyl ester (3) in 54% yield. The closo-carborane cage in (3) can be decapitated by NaOH in 90% ethanol under reflux condition. After neutralization, the nido-carborane-appended \( \text{i-DOPA} \), \( L,3,4-\text{Di}(7′-\text{Me}-8′-(\text{CH}_{2})_{4}-\text{nido-C}_{2}B_{10}H_{10})\)-phenylalanine (4) is obtained as an off-white solid in 64% yield.

Scheme 1. Syntheses of carboranyl \( \text{i-DOPA} \) agents.
cells-killing agent. These results indicate that such compounds should be further investigated as effective boron carriers for BNCT treatment. Since boron-10 isotope is active for BNCT only, the $^{11}$B-enriched compound 4 will be investigated in the future for clinic tests. Additional bioassessments such as biodistribution and cytotoxicity studies, are currently being investigated in our laboratories.

4. Experimental section

4.1. General

All reactions were carried out under an argon atmosphere using standard Schlenk techniques. Tetrachlorofuran and diethyl ether were heated over sodium/benzophenone until a blue color was sustained, and distilled under nitrogen just before use. The 1,4-Diodobutane, N-butyllithium (1.6 M in hexanes), L-DOPA methyl ester hydrochloride, and other reagents and organic solvents, were used as received. The 1-Methyl-closo-1,2-C$_2$B$_9$H$_{11}$ was purchased from KatChem Ltd. and used as received. The FT-IR spectra were measured using a BIO-RAD spectrophotometer with KBr pellets or organic solvent films. Elemental analyses were measured using a EURO EA equipment. The $^1$H, $^{13}$C, and $^{11}$B NMR spectra were recorded using a Bruker 400 analyzer at 400.13, 100.62, and 128.38 MHz, respectively. Inductively coupled plasma-optical emission spectroscopy (ICP-OES) measurements were made using a VISTA-MPX machine.

4.2. Synthesis of 2

A literature procedure [19] was adopted to synthesize compound 2. Accordingly, 1.05 g (6.64 mmol) of 1-Me-closo-C$_2$B$_9$H$_{11}$ was added to a 250-mL three-necked round-bottom flask equipped with a magnetic stirring bar, and the compound was dissolved in 60 mL of THF. The solution was cooled to $-78^\circ$C, and then 4.35 mL (6.96 mmol) of n-Buli (1.60 M in hexanes) solution was added dropwise. The resulting reaction mixture was continuously stirred for 30 min at $-78^\circ$C before allowing to warm to room temperature. After 4-h, the mixture was cooled to 0°C, and then 0.98 mL (~7.0 mmol) of 1,4-diiodobutane was carefully added using a syringe. After this addition, the reaction mixture was stirred for 30 min at 0°C, slowly allowed to warm to room temperature, stirred for an additional 1 h, and then refluxed for 3 h. The mixture was then cooled to 0°C and quenched with sufficient quantity of deionized water. The reaction mixture was dried in vacuum and the residue was purified by thin-layer chromatography (using silica gel), developed with n-pentane/ethyl acetate (v/v = 6/1) solvent mixture to isolate product (2) as a colorless oil. The product was identified by comparing its $^1$H NMR spectrum with that published in the literature [19].

4.3. Synthesis of 3

Carboranyl butyl groups were appended to L-DOPA molecule using a classical Williamson ether synthesis between iodide (2) and L-DOPA salts. To prepare compound 3, L-DOPA methyl ester hydrochloride (123.8 mg, 0.50 mmol) and sodium hydroxide powder (62.0 mg, 1.55 mmol) were added to a 100-mL three-necked round-bottom flask containing 60 mL dimethylacetamide (DMA) and the resulting mixture was stirred for 0.5 h before adding 1-methyl-2-iodobutyl-o-carborane (423.3 mg, 1.00 mmol) to the flask. The reaction mixture was heated to 90°C and allowed to react for 40 hrs with continuous stirring. The solvent was then removed under reduced pressure and the residue was purified by flash chromatography (SiO$_2$, DCM/THF/Hexane) to isolate the off-white powder product (3). L-3,4-Di(1-Me-C$_2$B$_9$H$_{11}$)-phenylalanine methyl ester (172.4 mg) in 54% yield. Elemental Anal: Calcd for C$_{32}$H$_{75}$B$_{9}$O$_4$: C, 45.33; H, 8.40; N2.20. Found: C, 45.10; H, 8.52; N 2.15. $^1$H NMR (CDCl$_3$, relative to SiMe$_4$, ppm): δ 7.31–7.22 (m, 3H, $-C_6H_3$), 3.95 (t, 4H, 2 CH$_2$O), 3.82 (s, 3H, $-C_6H_3$).
OCH$_3$), 3.64 (m, 1H, CHN), 3.41–1.18 (m, 42H, CH$_2$)

[38x467]waxy solid (135.4 mg) in 64% yield. Elemental Anal: Calcd for

[38x519]was heated to reflux for 12 hrs and allowed to cool to 0 °C and then

[38x530]3


[38x593]br), 733 (s, s), 673 (m, s), 646 (s, w), 591 (s, s).

[38x614]1265 (m, s), 1191 (m, s), 1159 (s, s), 1024 (m, br), 827 (m, s), 796 (w,

[38x331]1291 (m, s), 1258 (s, s), 1228 (m, s), 1180 (m, s), 1144 (s, s), 1113 (s, s),

[38x352]KBr, cm$^{-1}$) 3584 (s, br), 3356 (s, s), 3215 (s, s), 2921 (s, s), 2887 (s, s),

[38x436]38H, CH$_2$

[38x457]boron agent (4, BSH and l-BPA) were added and continued culturing for

[38x478]to give compound 4 as pale yellow solid (135.4 mg) in 64% yield. Elemental Anal: Calcd for

[38x498]4.5.3. Uptake of boron by tumor cells

[38x519]Cell Proliferation Assay. The IC$_{50}$ for compound

[38x530]were detected at 490 nm optical absorbance by AQueous One Solution

[38x551]plates with various concentrations of the samples. The damaged cells

[38x561]exposure, was determined. Suspensions of 10$^8$ C6 cells/200 μL MEM

[38x582]M$^+_e$ for the fast-neutron region. The neutron absorbed dose (Gy)

[38x603]yellow blue solution and then counted microscopically.

4.5.2. Cytotoxicity

The IC$_{50}$ (moles/liter, M), that is the concentration that inhibited

the growth of C6 gliosarcoma cells by 50% after 3 days of continuous

exposure, was determined. Suspensions of 10$^8$ C6 cells/200 μL MEM

containing 20% FCS were incubated for 3 days using 96-well culture

plates with various concentrations of the samples. The damaged cells

were detected at 490 nm optical absorbance by AQueous One Solution

Cell Proliferation Assay. The IC$_{50}$ for compound 4 is 8.93 × 10$^{-4}$ M.

4.5.3. Uptake of boron by tumor cells

The 1.7 × 10$^7$ rat glioma cells (C6) were seeded and cultured at

room temperature (37 °C, 5% CO$_2$) for 24 hrs. This culture fluid was

removed therefrom by suction, culture fluids containing 1.5 mM of each

boron agent (4, BSH and l-BPA) were added and continued culturing for

24 hrs under same conditions. These culture fluids were removed by

suction and the cells were washed three times with PBS and treated

with trypsin to recover the cells. The number of the cells recovered

was counted, and HNO$_3$ (2 N, 1.5 mL) was added and the resulting mixture

was heated at 80 °C for 12 hrs. After filtering with a membrane filter, the

boron concentration was determined by ICP-AES.

4.5.4. Statistics

The in vivo BNCT was carried out in triplicate. Values are the mean ± SEM from three independent experiments. The significance of differences in survival was assessed by Student's t test. The statistical analyses were performed using Prism 3.0 (GraphPad Software Inc., CA, USA).

Declaration of Competing Interest

The authors declare that they have no conflict of interest to disclose.

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