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Research Article

Synthesis and radioiodination of 7-(3′-ammoniopropyl)-7,8-dicarba-*nido*-undecaborate(-1), (ANC)

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Summary

Derivatives of *nido*-carborate have potential use in tumour targeting as hydrophilic boron-rich compounds for boron neutron capture therapy (BNCT) and as pendant groups for attachment of radiohalogens to tumour-seeking molecules. For this purpose, functionalized derivatives of *nido*-carborates that can be conjugated to biomolecules should be synthesized and evaluated. In this study, racemic **1**, 7-(3′-ammoniopropyl)-7,8-dicarba-*nido*-undecaborate(-1) (acronym **ANC**) was obtained by degradation of the corresponding aminopropyl-*o*-carborane, which was synthesized in three steps from 1-*tert*-butyldimethylsilyl-2-(3-bromopropyl)-*o*-carborane, with sodium hydroxide in absolute ethanol. The racemate **1** was radioiodinated (^{125}I) using the Chloramine-T method. Radio-TLC results showed that radiolabelling with ^{125}I was achieved in a yield greater than 95%. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: 7-(3′-Ammoniopropyl)-7,8-dicarba-*nido*-undecaborate(-1); radioiodination; chloramine-T; ^{125}I; ANC

Introduction

Iodine radionuclides play an important role both in biomedical research and in clinical practice.[1] A variety of decay schemes allow a wide range of applications in the field of nuclear medicine. The selection of radioiodine

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label can be made in accordance with the objectives of individual biomedical experiments and the 'time window' required. Some of the most important iodine radioisotopes are listed below:

- ^{125}I ($t_{1/2} = 60$ days, 100% electron capture (EC)) is a convenient radionuclide for laboratory studies and radioimmunoassay,
- ^{131}I ($t_{1/2} = 8$ days, β^-) is the most frequently used isotope for systemic radionuclide therapy,
- ^{123}I ($t_{1/2} = 13.3$ h, 100% EC) is widely used for diagnostic purposes in gamma-scintigraphy and single-photon emission computerized tomography,
- ^{124}I ($t_{1/2} = 4.2$ days, 23% β^+) is used for labelling of macromolecules as positron emission tomography tracer.

One of the most commonly used methods of protein radioiodination is the so-called direct iodination, when the *in situ* oxidized radioiodide electrophilically reacts with the tyrosine residues of the proteins. However, a serious problem for the medical application of radioiodine is the relatively rapid release of radioactivity from the cells after intracellular processing of the labelled compound. It has been demonstrated that leakage of radioactivity occurs in the form of monoiodotyrosine, which is relatively easily dissolved through the cellular membranes due to its lipophilicity.[2–4] One possible way of improving intracellular retention is to use as linkers for radiohalogen compounds that are charged at lysosomal pH, and thus, cannot diffuse through the cellular membranes. For example, *N*-succinimidyl-5-halo-3-pyridinecarboxylate was iodinated[5] and astatinated for this purpose.[6] The results of *in vitro* evaluation of antibodies labelled with this method were promising, but, unfortunately, an animal study has not demonstrated any better tumour accumulation compared to the results obtained with directly labelled antibodies.[7] There is a good possibility that this problem can be solved by use of polyhedral boron anions, such as *closo*-dodecaborate anion $[B_{12}H_{12}]^{2-}$ or the *nido*-carborate anion $[C_2B_9H_{12}]^-$ as prosthetic groups for labelling macromolecules. The rationale behind this view is that these compounds should be charged at lysosomal pH, in addition, boron-halogen bond e.g. the B–I bond (bond dissociation energy = 381 ± 21 kJ/mol) is substantially stronger than the C–I bond (209 ± 21 kJ/mol)[8] and that no enzymatic system *in vivo* is known to cleave boron-halogen bond. More details concerning the use of polyhedral boron anions for radiohalogenation are give in recent reviews by Hawthorne and Maderna,[9] and Tolmachev and Sjöberg.[10]

The first report of a radioiodinated carborate species was by Hawthorne *et al.*[11,12] using the Chloramine-T method with $Na^{125}I$. These authors labelled a *nido*-carborate derivative [7-(4′-C_6H_4NCS)-*nido*-7,8-$C_2B_9H_{11}^-$] and investigated its use as a carrier for radioiodine in biodistribution studies. The labelling chemistry of *nido*-carborate anions has later been investigated by Wilbur *et al.*[13–16]

The possibility to use *closo*-dodecaborate $[B_{12}H_{12}]^{2-}$ derivatives as linkers for the attachment of radioactive bromine, iodine, and astatine to biomolecules has recently been demonstrated in our laboratory.[17–19] It was shown that the radiohalogen label on the *closo*-dodecaborate cage is easily introduced under mild labelling conditions, that the label is stable *in vivo* and can be used in nuclear medicine.

An important potential advantage of the use of *nido*-carborate as a prosthetic group for attachment of radiohalogens to targeting proteins is the possibility to produce derivatives, which could be attached to side groups (amino, carboxyl, hydroxyl) of proteins. As previously mentioned 7-(4′-C_6H_4NCS)-*nido*-7,8-$C_2B_9H_{11}^-$ is a good example of such compounds, which can be coupled to an amino terminus of lysine residues. It gives greater impetus to develop methods for synthesis of *nido*-carborate derivatives with other side groups in order to give increased flexibility to conjugation chemistry. For example, amino compounds can be conjugated to carbohydrate parts of the targeting molecules by a variety of methods.

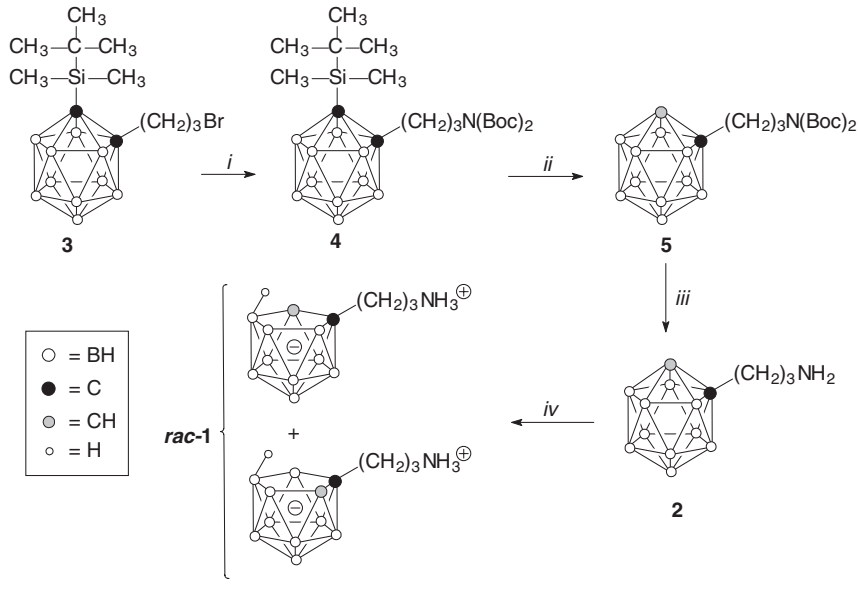
We have shown[20,21] that racemic 7-(3′-ammoniopropyl)-7,8-dicarba-*nido*-undecaborate (−**1**) (**1**), (**ANC**) can be conjugated to human epidermal growth factor (hEGF), and subsequently labelled in high yield with ^{211}At at pH 7.2, using the Chloramine-T method. The method was shown to give a higher yield than the standard method using *N*-succinimidyl-4-astatobensoate for conjugation to hEGF. We have also studied ANC with regard to binding and toxicity in cultured human glioma and mouse melanoma cells.[22]

Here we report synthesis of the *nido*-carborate **1** (ANC), its corresponding hydrochloride **1 · HCl** as well as electrophilic iodination/radioiodination (with ^{125}I) of racemic **1**.

Results and discussion

The synthesis of racemic **1** is outlined in Scheme 1. 1-*tert*-Butyldimethylsilyl-2-(3-bromopropyl)-*o*-carborane (**3**) was previously prepared in our laboratory.[23] This compound (**3**) was converted to di-Boc-protected amine **4** in 66% yield by reaction with di-*tert*-butyl iminodicarboxylate $[HN(Boc)_2]$ in a mixture of Bu_4NHSO_4 and NaOH in dichloromethane/water. The protective silyl group in **4** was removed by reaction with tetrabutylammonium fluoride in THF solution at low temperature ($\approx -35^{\circ}C$) to give the di-Boc derivative **5**. The hydrochloride **2 · HCl** of the amine **2** was obtained in 97% yield by removal of the Boc groups in **5** with hydrogen chloride gas in dry diethylether, as described previously.[24]

The *nido*-carborate **1** was obtained by degradation of the *o*-carborane cage of the free amine **2** with sodium hydroxide (molar ratio **1**/NaOH; 1/2, thus a 100% excess)[25,26] in absolute ethanol. The work-up procedure required



Scheme 1. (i) Bu_4NHSO_4, NaOH, $HN(Boc)_2$, CH_2Cl_2; (ii) Bu_4NF, THF (iii) a: HCl/ether; b: Na_2CO_3 (aq); (iv) a: NaOH, MeOH; b: CO_2

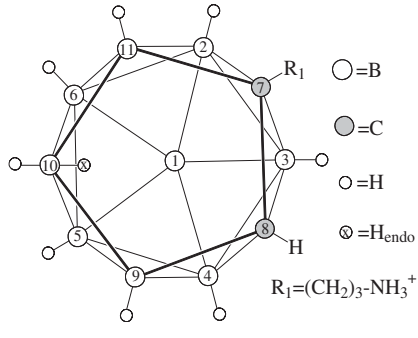


Figure 1. Numbering of the boron and carbon atoms in the *nido*-cage

precipitation of excess sodium hydroxide, as the carbonate, with carbon dioxide and chromatographic purification of the product.

A sample of the *nido*-carborate **1** was converted to the corresponding *nido*-carborate hydrochloride **1 · HCl** by addition of dilute hydrochloric acid.

The *nido*-compound structures of **1** and **1 · HCl** were analysed by 1D and 2D NMR spectroscopy (for numbering the *nido*-cage atoms see Figure 1.). Typical ^{11}B NMR spectra (CD_3OD solution) are shown in Figure 2. The spectra reveals the typical large coupling constants to the exo-protons for both compounds and for **1 · HCl** also an additional smaller coupling to the bridging H_{endo}. The reason for its non-appearance in the spectrum of **1** is that it

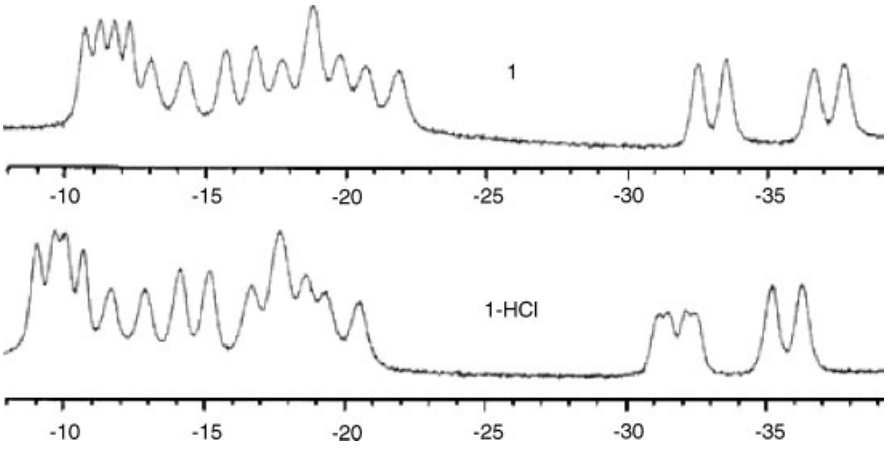


Figure 2. ^{11}B NMR-spectra (CD_3OD solution) for compound 1 (for a sample with complete deuterium exchange of the *endo*-proton) and 1 · HCl, with coupling to ^{1}H (128.3 MHz)

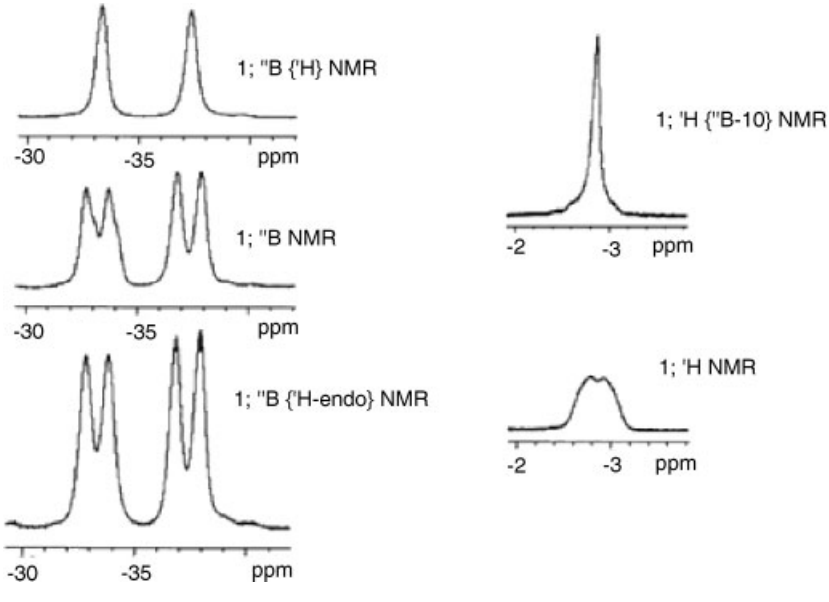


Figure 3. NMR spectra (CD_3OD solution) of 1 (with partial deuterium exchange of the *endo*-proton): left, ^{11}B NMR-spectra (128.3 MHz), right, ^{1}H NMR spectra (400 MHz)

undergoes fast deuterium exchange with the solvent. This view is verified by the information from the NMR spectra (Figure 3) of a freshly prepared solution of **1**. In the ^{11}B spectrum a shoulder appears, at the signal around

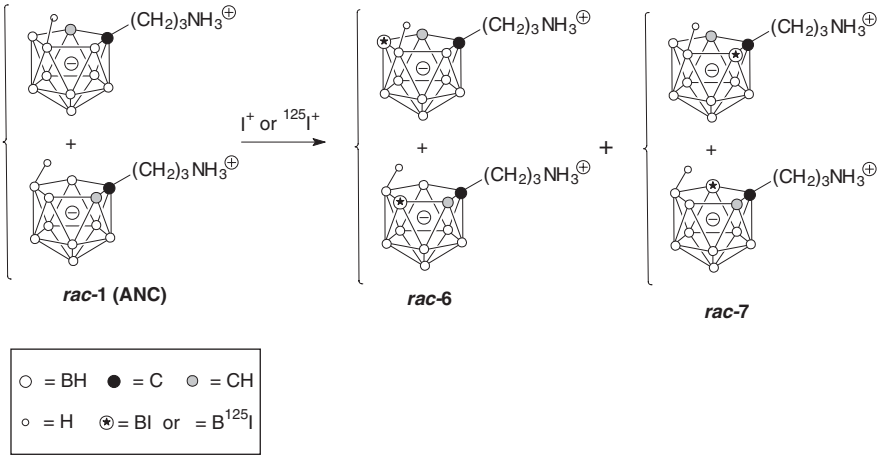
−33 ppm, which disappears in the $^{11}B\{^1H_{endo}\}$ NMR spectrum. Further support is obtained from the ^1H and NMR spectra. The doublet of the signal in the typical region of the $^1H_{endo}$ signal diminishes with time and disappears in the $^1H\{^{11}B\text{-}10\}$ spectrum.

Assignment of the other boron atoms was carried out starting from B-10 because of its known shift, and the fact that B-10 couples to boron atoms B-5, B-6, B-9 and B-11, that B-1 couples to B-2–B-6, and that H-8 couples to H-3, H-4 and H-9.

Iodination of the *nido* compound **1** was obtained by electrophilic substitution using NaI with *N*-chlorosuccinimide as oxidizing agent. The resulting crude product was analysed by electrospray ionisation mass spectrometry. It was found that besides mono-iodinated **1** (major product), di-iodo-, mono-chloro- and mono-chloro-mono-iodo-substituted **1** were formed.

Purification of the crude reaction mixture by flash chromatography gave a mixture of two mono-iodinated compounds in a molar ratio of 2:1. The two compounds are in the Experimental section described as **1-Ia** and **1-Ib** with **1-Ia** being the major component. Attempts to obtain a total separation of the two compounds by preparative HPLC was unsuccessful. However, small pure fractions of each isomer could be collected and analysed. It is suggested that the two compounds are the two regioisomerc pairs *rac*-**6** and *rac*-**7** in Scheme 2, and that *rac*-**6** due to sterical reasons should be formed in largest amounts, thus corresponding to **1-I-a**.

Radiolabelling of the *nido*-carborate (**1**) was performed using $Na^{125}I$ and Chloramine-T as oxidizing agent. The iodinated *nido*-carborate mixture (**1-Ia** and **1-Ib**) and non-radioactive sodium iodide were used as references



Scheme 2. Iodination of *rac*-1

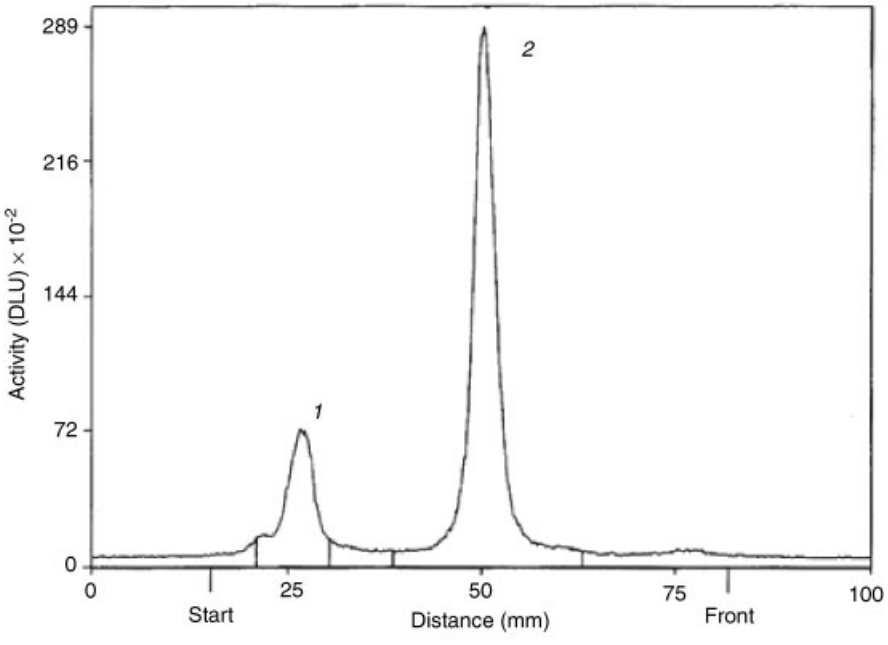


Figure 4. Typical TLC chromatograms of the radioiodination of *nido*-carborate 1. System: silica-coated aluminium plate, using ethyl acetate:acetone (1:1) as eluent. Peaks: peak 1 ($R_f = 0.2$) 125-iodide, peak 2 ($R_f = 0.5$) radioiodinated 1

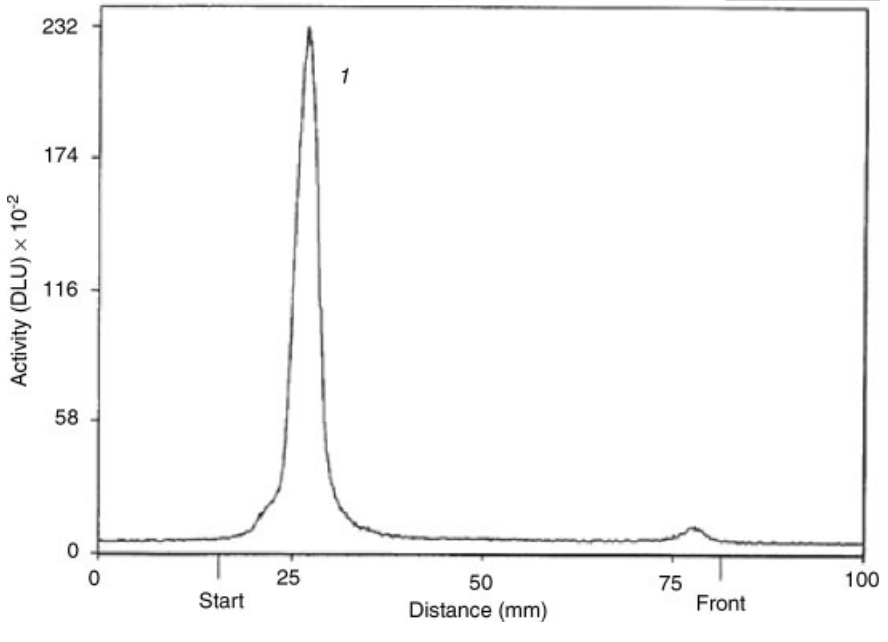


Figure 5. Radio-TLC chromatogram from a blank experiment. System: silica-coated plate, ethyl acetate:acetone (1:1) as eluent. Peak 1 has the same R_f-value as peak 1 in Figure 4 and non-radioactive iodide

during the thin layer chromatography separation. Figures 4 and 5 show the radio-TLC diagrams of the radiolabelled **1** and the blank experiment, respectively. Peak 1 ($R_f = 0.2$) in Figure 4 has the same position as non-radioactive sodium iodide. Peak 2 ($R_f = 0.5$) is co-eluted with non-radioactive mono-iodinated compound mixture (**1-Ia** and **1-Ib**). This peak is associated with the presence of **1** and an oxidant in the reaction mixture, and has never appeared in those blank experiments where either Chloramine-T (CAT) or **1** was not added (Figure 5). For this reason, we assumed that this peak represents radioiodinated **1-Ia** and **1-Ib**. A labelling efficiency of 96.2 $\pm$ 0.1% was obtained according to radio-TLC analysis.

Experimental

General details

The ^{1}H, ^{13}C and ^{11}B NMR spectra were recorded in $CDCl_3$ (7.26 ppm, ^{1}H, 77 ppm ^{13}C) or CD_3OD (3.35 ppm, ^{1}H, 49.0 ppm ^{13}C) on a Varian Unity-400 spectrometer operating at 400, 100.6 and 128.3 MHz, respectively. Boron trifluoride etherate was used as an external standard for the boron spectra. ^{1}H and ^{11}B connectivity in the carborane cage was accessible from ^{1}H-^{1}H TOCSY and ^{11}B-^{11}B TOCSY with ^{1}H decoupling using short mixing times (0.5 ms).

Merck Silica Gel 60 (230–400 mesh) or C_{18}-silica (230–400 mesh) was used for flash chromatography. Merck Silica 60 F_{254} or C_{18}-silica (230–400 mesh) was used for TLC. The following chromatographic systems were used for preparative HPLC: (A) a 250 $\times$ 10 Spherisorb 10 µ column (Column I) on a Waters 501 HPLC Pump equipped with a Waters R401 Differential Refractometer detector using 50% methanol; or (B) a Spherisorb ODS1 column (Column II) using a waters HPLC system equipped with a Waters 991 photodiodearray detector operating in isocratic mode (solvent system 60% 5 mM Bu_4NHSO_4 in H_2O and 40% MeOH as eluent). FAB mass spectra were recorded on a SX/SX 102A (JEOL) mass spectrometer and low resolution mass spectra was performed on an AQA mass spectrometer with electrospray negative ionization using the direct infusion method. Melting points are uncorrected and were obtained using a Büchi capillary melting point apparatus. Radiolabelling analysis was performed using Merck Silica 60 F_{254} gel TLC plates. The radioactivity distribution along TLC strips was measured on a Cyclone™ Storage Phosphor System and analysed on an OptiQuant™ Imager Analysis Software. The spots of non-radioactive reference substances, sodium iodide and monoiodinated **1** (the mixture of **1-Ia** and **1-Ib**) were visualized using an acidified solution of palladium chloride in methanol for R_f measurement. The acronym 'cb' is used for carborane and *nido*-carborate cages.

N,N-Di-tert-Butyloxycarbonyl-1-(tert-butyldimethylsilyl)-2-(3′-amino propyl)-o-carborane (4)

To a stirred mixture of Bu_4NHSO_4 (8.4 g, 24.8 mmol) and 2 M NaOH (24.8 ml, 49.6 mmol) were added CH_2Cl_2 (60 ml) and $HN(Boc)_2$ (5.39 g, 24.8 mmol). Compound **3** (9.0 g, 23.8 mmol) in methylene chloride (30 ml) was added to the mixture. The resulting mixture was heated to reflux for 2.5 h, then stirred at room temperature overnight. The reaction was quenched with water (29 ml) and extracted with methylene chloride (3×60 ml). The combined organic layer was washed with water (30 ml) and the solvent was evaporated. Ca. 115 ml of dry ether was added and the residue was stirred, the precipitate (Bu_4NBr) was removed by filtration and the solvent was stripped off. The crude product was purified by flash chromatography on silica (15:1, pentane:ethylacetate), giving 8.0 g of compound **4** in 66% yield. $R_f = 0.42$; ^{1}H NMR ($CDCl_3$) δ: 3.5 (t, 2H, CH_2-N), 2.13 (m, 2H, CH_2), 1.7 (m, 2H, CH_2) 1.4 (s, 18H, Boc), 1.0 (s, 9H, CH_3), 0.28 (s ,6H, CH_3); ^{11}B{1H} NMR ($CDCl_3$) δ: 0.33 (1B), −3.8 (1B), −7.36 (2B), −10.35 (6B).

N,N-Di-tert-Butyloxycarbonyl-1-(3′-aminopropyl)-o-carborane (5)

The silylated compound **4** (5.78 g, 11.2 mmol) was dissolved in anhydrous THF (100 ml) and the solution was cooled to ca. −35°C under N_2 gas. A solution of Bu_4NF (1 M, 1.5 eq.) in THF was added to the reaction flask and the mixture was stirred for about 3 h. The reaction was allowed to reach room temperature and was then quenched with water (100 ml). THF was evaporated and the aqueous layer was extracted with diethylether (3×60 ml). The combined organic extract was dried over $MgSO_4$, filtered and the solvent was then evaporated under reduced pressure. The crude product was purified by column chromatography on silica (4:1 pentane : ethyl acetate) to give 4.4 g (98%) of the title compound. The physical data of the product agreed with those previously published by us.[24]

1-(3′-Aminopropyl)-o-carborane hydrochloride (2 · HCl)

1-(3′-Aminopropyl)-*o*-carborane hydrochloride (2 · HCl) was prepared in 97% yield from **5** by deprotection with HCl in ether solution as described by Malmquist and Sjöberg, and the physical data were in accord with their observation.[24]

7-(3′-Ammoniopropyl)-7,8-dicarba-nido-undecaborate(-1) (1)

7-(3′-Aminopropyl)-*o*-carborane hydrochloride **2 · HCl** (1.0 g, 4.2 mmol) was mixed with an excess of Na_2CO_3 in water. The liberated amine was extracted with ether (3×20 ml), and the combined extracts were washed with brine (3×5 ml) and were dried over sodium sulphate. The extract was concentrated

in vacuo and dissolved in absolute ethanol (46 ml) and finely ground sodium hydroxide (0.34 g, 8.4 mmol) was added. After reflux for 28 h, CO_2 was bubbled through the cool solution. The solution was filtered through celite and concentrated *in vacuo* while heating at 65°C. The crude product was purified by flash column chromatography (C-18) using MeOH:H_2O (1:1) as the eluent to give 89% (0.712 g) of **1**. (Alternatively, the crude product can be purified by flash chromatography on silica (CH_2Cl_2:MeOH, 5:1), $R_f = 0.49$). mp = 284−285°C dec. Solubility in water at RT: 40 g/l H_2O. Anal. Calcd for $C_5H_{20}B_9N$: C, 31.36; H, 10.53; N, 7.31; Found: C, 31.07; H, 10.22; N, 7.49; ^{1}H NMR (CD_3OD): δ 2.86 (t, 2H, $J = 7.6$ Hz, CH_2-N), 1.80 (m, 2H, -CH_2-CH_2-CH_2), 1.70 (m, 1H, cb-CH_a-), 1.66 (broad s, 1H, cage-CH), 1.54 (m, 1H, cb-CH_b), −2.76 (bd, 1H, H-10_{endo}); ^{13}C NMR (CD_3OD): δ 61.0 (cage-C-CH_2), 47.6 (cage-CH), 41.6 (CH_2-N), 37.4 (cb-CH_2-CH_2), 31.7 (CH_2-CH_2-CH_2); ^{1}H{^{11}B} NMR (CD_3OD): (only cage protons are listed) δ 1.88 (s, 2H, H-9 and H-11), 1.66 (broad s, 2H, cb-CH and H-3), 1.37 (s 1H, H-2), 1.24 (s, 2H, H-4 and H-6), 1.06 (s, 1H, H-5), 0.50 (s, 1H, H-1), 0.06 (s, 1H, H-10_{exo}), −2.76 (bs, 1H, H-10_{endo}); ^{11}B{^{1}H} NMR (CD_3OD): δ −11.3 (B-11), −11.9 (B-9), −13.8 (B-3), −16.3 (B-6), −18.5 (B-2), 19.4 (B-5), −21.4 (B-4), −33.1 (B-10), −37.3 (B-1); IR (KBr-disc): 3209, 3159, 3124, 2923, 2527, 2482, 1585, 1560, 1467, 1420, 1395, 1065, 1029, 1004 cm^{-1}. Negative-ion FAB-MS (*m*/*z*) calcd for [C_5H_{19} $^{11}B_9N$]: 192.2364, found: 192.2350 (within the isotopic boron cluster envelope).

Titration of 7-[3′-Ammoniopropyl]-nido-carborate with hydrochloride (1 · HCl)

nido-carborate **1** was titrated with hydrochloric acid (0.011 M). The product was concentrated *in vacuo*, dissolved in methanol, filtered and finally concentrated *in vacuo* to give **1 · HCl** in quantitative yield. ^{1}H NMR (CD_3OD): δ 2.94 (t, 2H, $J = 7.6$ Hz, CH_2-N), 1.80 (m, 2H -CH_2-CH_2-CH_2), 1.74 (cb-CH_a-), 1.67 cage-CH), 1.54 (m, 1H, (cb-CH_b), −2.76 (bd, 1H, H-10_{endo}); ^{1}H{^{11}B} NMR (only cage protons are listed): δ 1.88 (s, 2H, H-9 and H-11), 1.70 (s, 1H, H-3), 1.33 (s, 1H, H-2), 1.26 (s, 2H, H-4 and H-6), 0.93 (s, 1H, H-5), 0.42 (s, 1H, H-1), 0.05 (s, 1H, H-10_{exo}), −2.76 (bs, 1H, H-10_{endo}); ^{11}B{^{1}H} NMR (CD_3OD): δ −9.4 (B-11), −10.0 (B-9), −12.2 (B-3), −14.6 (B-6), −17.2 (B-2), −17.9 (B-5), −19.8 (B-4), −31.7 (B-10), −35.6 (B-1).

Iodination of the nido-carborate (1)

The *nido*-carborate **1** (0.18 g, 0.95 mmol) was dissolved in methanol (18 ml). NaI (0.16 g, 1.05 mmol) in methanol containing 1% acetic acid was added. *N*-Chlorosuccinimide (NCS) (0.190 g, 1.43 mmol) was added in four portions and stirred at room temperature for 15 min. The reaction was quenched with aqueous $Na_2S_2O_5$ and filtered. Evaporation gave a residue of 471 mg. This

crude mixture was analysed by electro spray (ES) negative ionization mass spectroscopy. The sample was prepared in methanol solution containing a few drops of ammonia. The mass spectrum showed the presence of four boron containing peaks with the following mass spectral data:

Molecular ion	Cald (*m*/*z*)	Found (*m*/*z*)
$[C_5H_{18}B_9ClN]^-$	225.0	225.1
$[C_5H_{18}B_9IN]^-$	317.1	317.1
$[C_5H_{17}B_9ClIN]^-$	351.1	351.1
$[C_5H_{17}B_9I_2N]^-$	443.0	443.0

All peaks showed the expected isotopic pattern.

The crude product was purified by flash chromatography (4/1; CH_2Cl_2/CH_3OH; v/v) to give 156 mg (52%) of monoiodinated product. This mixture was shown to contain two components, denoted **1-Ia** and **1-Ib**. ^1H and ^{11}B NMR spectra showed overlap of most signals from the two compounds but in the ^1H NMR spectrum the cage-CH protons were different enough to allow their integration, and the molar ratio of the two compounds were found to be 2:1 (**1-Ia**/**1-Ia**). Small amounts of pure components could be isolated using HPLC (system B).

*NMR data for component **1 − Ia***. ^1H NMR (CD_3OD): δ 2.90 (m, 2H, CH_2N), 2.23 (bs, 1H, cage-CH), 1.90 −1.73 (m, 4H, -CH_2-CH_2-). $^{11}B\{^1H\}$ NMR: δ −7.0 (1B), −14.5 (2B), −16.8 (1B), −18.5 (1B), −21.4 (1B), −25.10 (1B), −30.1 (1B), −37.3 (s, 1B).

*NMR data for component **1 − Ib***. ^1H-NMR (CD_3OD): δ 2.87 (t, -CH_2N), 2.30 (bs, cage-CH), 1.84-1.71 (m, 4H, cb-CH_2-CH_2-).

^{125}I Labelling of 7-[3′-ammoniopropyl]-nido-carborate (1)

To an aqueous solution of compound **1** (10 µl, 0.1 mg/ml) was added a phosphate buffered saline, pH 7.4 (PBS) (30 µl), Chloramine-T (10 µl, 4 mg/ml) and $Na^{125}I$ (5 µl). The mixture was vortexed and left to react for 5 min. The reaction was quenched with aqueous $Na_2S_2O_5$ (20 µl, 20 mg/ml). NaI (5 µl, 20 mg/ml) was finally added as a carrier to stabilize the unreacted radioiodide from oxidation. A blank experiment was performed by applying the same procedure without adding compound **1**. The reaction product was analysed by radio-TLC (solvent system 1:1 ethyl acetate:acetone). As a TLC-reference for the radioiodinated **1**, a sample of the non-radioactive mono-iodination of **1** was used.

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