Preparation and Evaluation of Carborane-Derived Inhibitors of Prostate Specific Membrane Antigen (PSMA).

Mohamed E. El-Zaria,[†‡] Afaf R. Genady,[†‡] Nancy Janzen,[†] Christina I. Petlura,[†] Denis R. Beckford Vera,[†] and John F. Valliant[†,*]

[†]Department of Chemistry and Chemical Biology, McMaster University, 1280 Main St. West, Hamilton, Ont. Canada L8S 4M1.

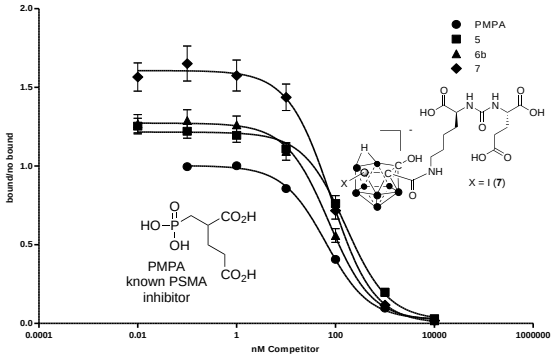
[‡]Department of Chemistry, Faculty of Science, Tanta University, 31527 Tanta, Egypt.

Abstract

A series of C-hydroxy carborane derivatives of (*S*)-2-(3-((*S*)-5-amino-1-carboxypentyl)ureido)-pentanedioic acid were prepared as a new class of boron rich inhibitors of prostate specific membrane antigen (PSMA), which is overexpressed on prostate cancer tumours and metastases. *Closo*-, *nido*- and iodo-carborane conjugates were prepared and screened *in vitro* where the water soluble iodinated cluster had the highest affinity with an IC_{50} value (73.2 nM) that was comparable to a known PSMA inhibitor 2-(phosphonomethyl)-pentanedioic acid (PMPA, 63.9 nM). The radiolabeled analogue was prepared using ^{123}I and the biodistribution determined in a prostate cancer model derived from a PSMA positive cell line (LNCaP) at 1, 2, 4, 6 and 24 h post injection (n = 4 per time point). The results showed good initial tumour uptake of 4.17% at 1 hr, which remained at that level only decreasing somewhat at 6 hr (3.59%). At the latter time point tumour-to-blood and tumour-to-muscle ratios peaked at 3.47 at 25.52 respectively. There was significant off-target binding particularly in the liver and gall bladder and a surprising amount of deiodination in vivo. Notwithstanding, this work

demonstrates that carboranes can be used to prepare potent ligands for PSMA creating the opportunity to develop a new class of BNCT agents for prostate cancer.

Graphical Abstract



Carborane-derived inhibitors of prostate specific membrane antigen are reported. Compounds were prepared from C-hydroxy-carboranes and screened *in vitro* and *in vivo*.

1. Introduction

Boron neutron capture therapy (BNCT) is a binary treatment strategy that involves irradiating boron-enriched tumours with thermal or epi thermal neutrons.[1] The resulting $^{10}B(n,\alpha)^{7}Li$ reaction is a type of focal therapy in that the resultant high linear energy transfer particles ($^{4}He^{2+}$ and $^{7}Li^{3+}$) travel less than 10 μm thereby depositing ionizing radiation predominantly within the tumour.[2] The success of BNCT is reliant on the large capture cross section of boron-10 (3838 barns) and the ability to deliver high concentrations of boron selectively to tumours.

Polyhedral boranes and in particular carboranes are attractive synthons from which to develop new BNCT agents because of their high boron content. In addition their versatile chemistry can be used to develop boron rich small molecules and nanomaterials that can selectively target tumours. [3] Furthermore, the hydrophobic nature of carboranes can be used to develop inorganic analogues of known drugs and potent and metabolically stable ligands for targets overexpressed on the surface of tumours.[4]

There have been reports where BNCT agents were developed and evaluated for the treatment of prostate cancer.[5] The interest in evaluating therapeutic strategies that can deliver alpha emitters (or that generate high linear energy transfer, LET, particles) to prostate cancer tumours has been renewed with the recent approval of radium-223 chloride (Xofigo) for the treatment of bone metastases associated with castration resistant prostate cancer.[6] While initial BNCT work has promise for primary (as opposed to metastatic) prostate cancer treatment, further advancement would be enabled through access to a new generation of boron compounds that can selectively localize polyhedral boranes within prostate cancer tumours.

Prostate specific membrane antigen (PSMA) is a transmembrane amino-acid type II glycoprotein that is primarily expressed in normal human prostate epithelium and is overexpressed in primary prostate cancer and metastatic disease.[7] Several radiolabeled antibodies, including polyhedral borane conjugates,[8] and small molecule inhibitors of PSMA have been reported and been used successfully to visualize prostate cancer in both preclinical and clinical studies.[9] Small molecule constructs have shown to be particularly effective at delivering SPECT, PET and radiotherapy isotopes to PSMA positive tumours and more recently have been used to deliver drug payloads.[10] Building on these successes,

our goal was to prepare the first examples of carborane-derived inhibitors of PSMA and assess their binding *in vitro* and the distribution of radiolabeled derivatives *in vivo* as a means of evaluating the feasibility of the strategy for delivering boron to prostate cancer cells.

2. Results and Discussion

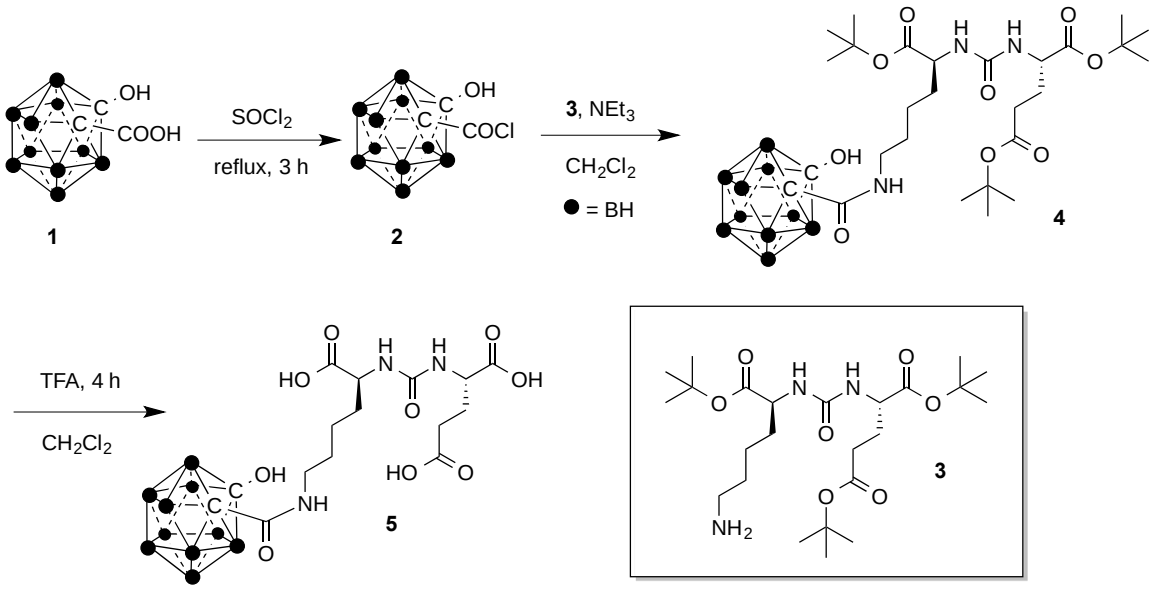
2.1 Synthesis

The urea-linked dipeptide (*S*)-2-(3-((*S*)-5-amino-1-carboxypentyl)ureido)-pentanedioic acid has been used to prepare potent ligands for PSMA. This includes iodo-aryl derivatives, which have been shown to be effective at targeting PSMA expressing tumours in preclinical[11] and clinical studies.[12] Structure-activity relationship (SAR) studies indicate that PSMA binding affinity is greatly impacted by the nature of the linkage between the inhibitor and prosthetic groups and the length of the spacer between the two components.[13] With this in mind, two classes of carborane-PSMA derivatives were prepared in which the cluster was linked directly to the inhibitor via a simple amide bond and a related derivative that contained a short spacer group.

The carborane component consisted of *ortho*-carborane bearing a hydroxyl group on one of the carbon vertices and a carboxylic acid on the other where the latter was used as the site for conjugation. C-hydroxy-carboranes have been used to create inorganic analogues of salicyclic acid,[14] and have the advantage that they are more water-soluble than simple *ortho*-carborane derivatives and the corresponding *nido*-carboranes can be readily labelled with isotopes of iodine.[15] These features are designed to minimize non-specific binding and create the opportunity to use quantitative biodistribution studies to

evaluate lead candidates in preclinical models and in the future to help with treatment planning in clinical trials.

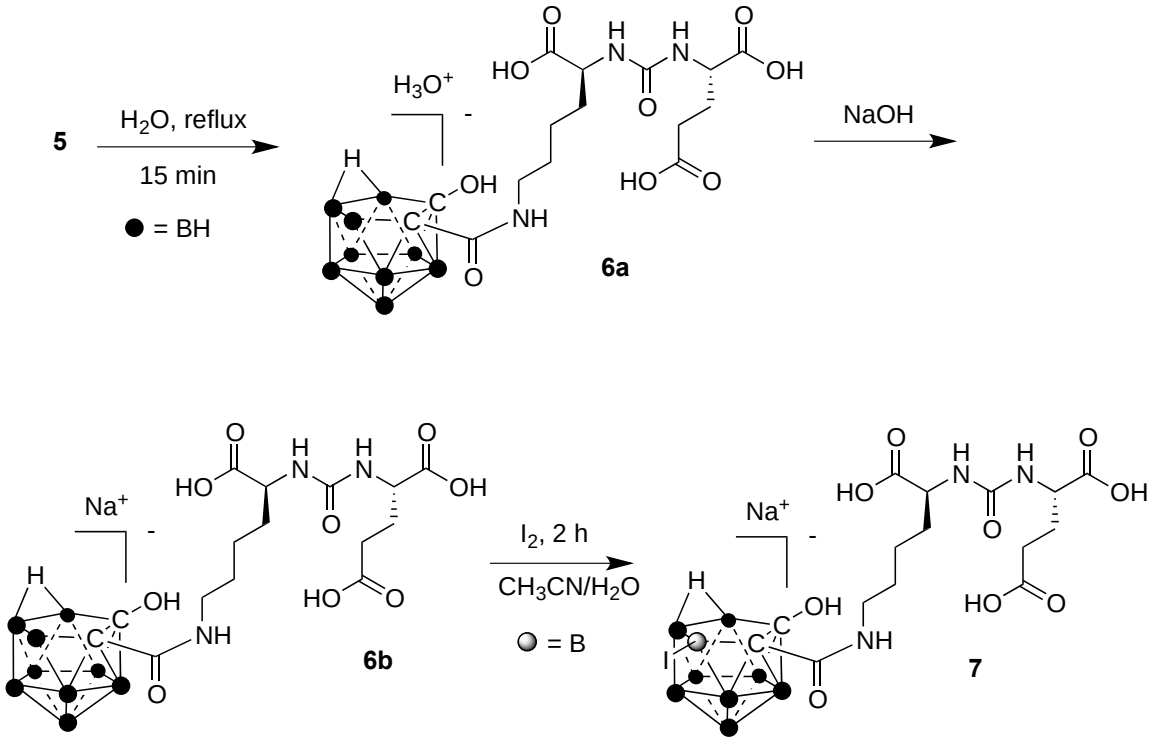
One of the desired ligands was prepared by first converting the *ortho*-carborane acid **1** to the corresponding acid chloride using thionyl chloride[16] and then adding the product to the known t-butyl protected ester **3** (**Scheme 1**).[11a] After six hours the desired product was isolated in high yield (95%) by HPLC. Formation of the desired amide was evident in the ^{1}H NMR, where the NH signal appeared as a broad singlet at 6.98 ppm and the corresponding carbonyl signal appeared at 165.2 ppm in the ^{13}C NMR spectrum. The ^{11}B NMR and HRMS data were consistent with isolation of the *closo*-cluster.



Scheme 1. Synthesis of C-hydroxy-carborane derivatives of the urea-linked dipeptide (*S*)-2-(3-((*S*)-5-amino-1-carboxypentyl)ureido)-pentanedioic acid.

Deprotection was achieved by the treatment of **4** with trifluoroacetic acid (TFA) at room temperature over four hours. The product was isolated in quantitative yield where

the loss of the signals associated with the ^{t}Bu protecting groups was evident in the ^{1}H and ^{13}C NMR spectrum. *Closo*-carboranes can be readily converted to the more water soluble *nido*-carboranes which provides an opportunity to explore the impact of charge, cluster size and polarity on PSMA binding. Consequently compound **5** was heated in water for 15 min. to produce the desired *nido*-carborane **6a** (**Scheme 2**). The electron withdrawing nature of the amide group promoted *nido*-carborane formation[17] consequently there was no need to use more commonly employed methods involving strong bases.[18]



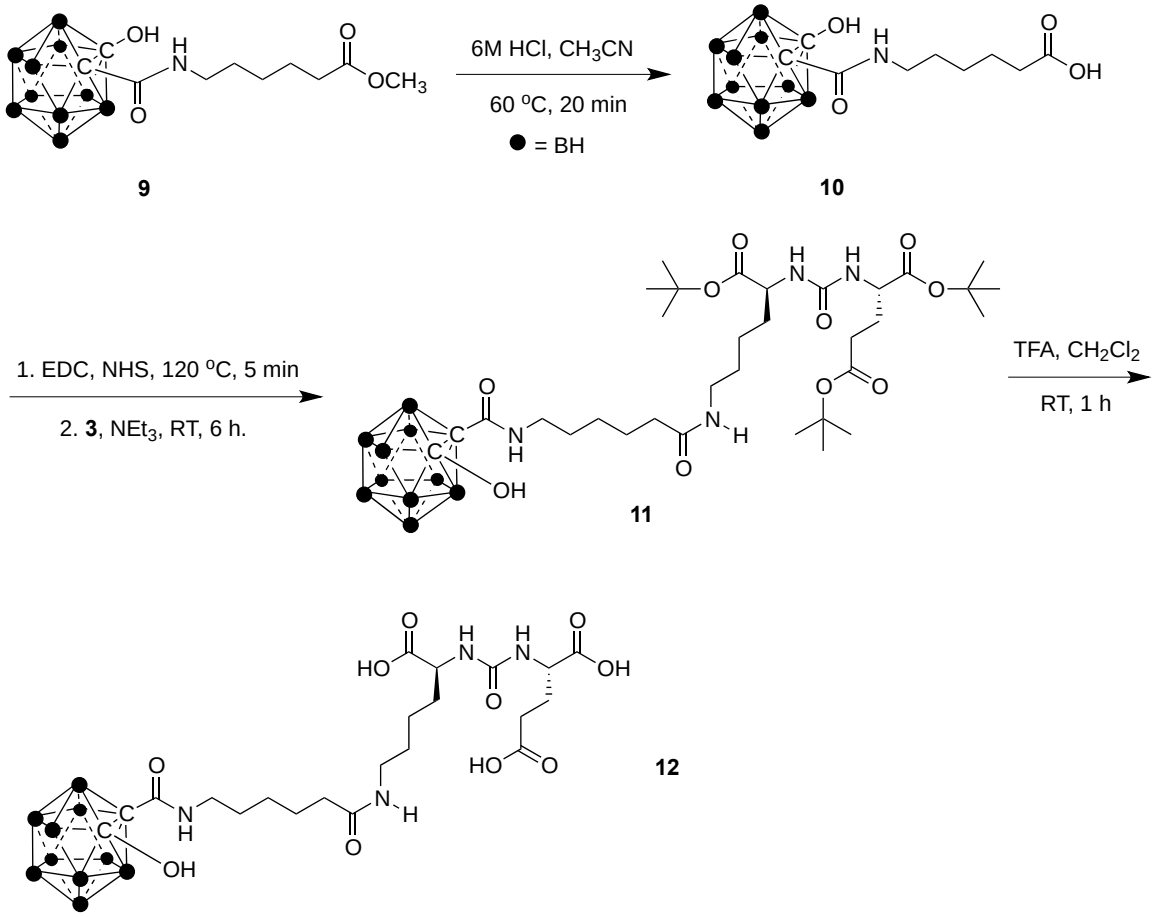
Scheme 2. Preparation and subsequent iodination of the *nido*-carborane derivative of **5**.

MS data indicated the loss of a boron vertex and the ^{11}B NMR was consistent with the reduced symmetry of the molecule and the formation of the *nido*-carborane **6a**. The bridging hydrogen on the *nido*-cluster was evident in the ^{1}H NMR appearing at -2.80 ppm. Interestingly when **6a** was dissolved in water the solution was highly acidic which suggested that the product was isolated as the hydronium ion salt. When the ^{1}H NMR of **6a** was run in D_2O as opposed to DMSO, the peaks associated with the C-hydroxycarborane, acid and amide groups, but not the bridging hydrogen atom on the carborane, readily exchanged (see supporting information). To minimize the influence of the acidic counter ion on the radiolabeling and screening experiments, **6a** was neutralized by the dropwise addition of 1 M NaOH and the water soluble sodium salt isolated by lyophilization.

The iodo-carborane **7**, which was needed as a reference standard for the radioiodinated analogue (*vide infra*), was prepared by treating **6b** with I_2 in aqueous acetonitrile.[19] After two hours the reaction was terminated by the addition of sodium thiosulfate and the product isolated by HPLC in 93% yield. For monosubstituted carboranes it is known that iodine reacts with the boron atom adjacent to the unsubstituted C-atom however the proton coupled and decoupled ^{11}B NMR spectra of **7** were broad making it challenging to identify the peak specifically associated with the B-I bond. Based on previous work[15] there is likely a mixture of products containing iodine attached to either one of the boron atoms adjacent to the C-atoms of the cluster. What was clearly evident was the change in intensity of the peaks and the chemical shifts in the ^{11}B{^{1}H} of **7** (δ_B -8.6, -12.6, -18.5, -19.3, -29.7, -30.9, -35.9) and the *nido*-precursor (δ_B -

10.6, -14.0, -21.1, -27.8, -33.5, -36.4) were significant and the MS data supported the presence of the desired product containing a single iodine atom.

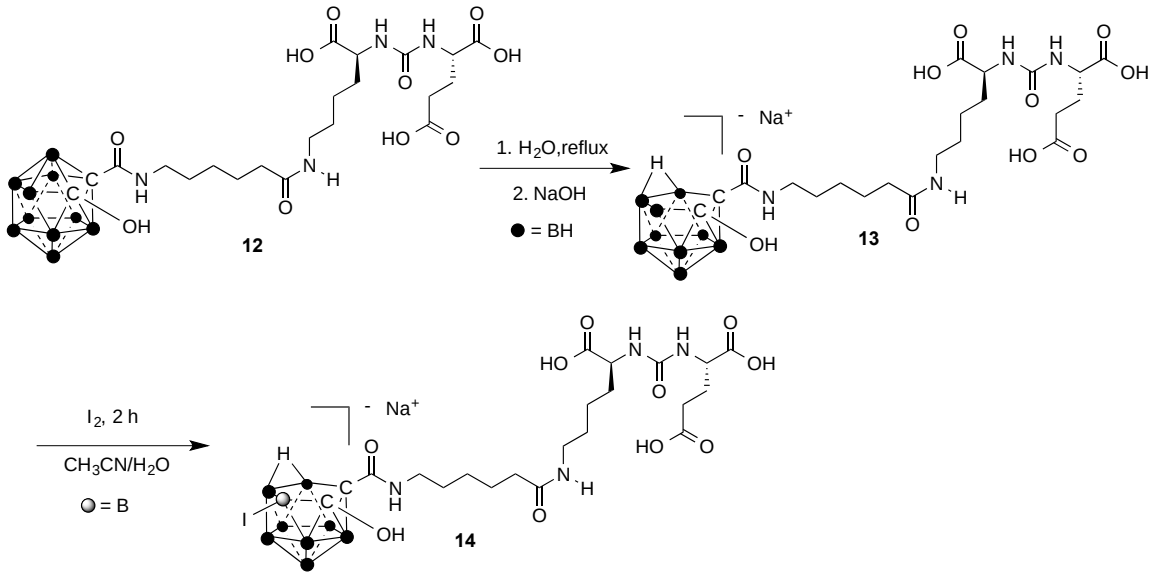
To introduce a space group, methyl 6-aminohexanoate hydrochloride was coupled to the acid chloride **2** and the product **9** isolated in 95% yield (**Scheme 3**). This particular spacer was selected based on other SAR studies of radiolabeled compounds targeting PSMA derived from the same inhibitor. The free acid **10** was generated using acid catalyzed hydrolysis of the methyl ester, which occurred in high (>90%) yield. Synthesis of the acid chloride of **10** proved to be problematic consequently the active ester was produced by heating the acid, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide in a microwave at 120^{o}C for 5 min. This highly efficient procedure generated the NHS ester, which was combined with **3** to give **11** in good yield (92%). Compound **12** was obtained in nearly quantitative yield by treating **11** with TFA.



Scheme 3. Introduction of a spacer group between the carborane and the PSMA binding ligand.

Deboronation was accomplished by dissolving **12** in water and heating to reflux for 30 min. whereupon the product was neutralized using 1M NaOH prior to freeze-drying (**Scheme 4**). The $^{11}B\{^{1}H\}$ NMR of **13** looked nearly identical to that for **6a** and the MS spectra confirmed the loss of one boron atom which is consistent with the formation of the desired *nido*-carborane. The ^{1}H and ^{13}C NMR spectrum of **13** was similar to that for **6b** with the exception of the additional signals associated with the spacer group and that in the ^{13}C NMR spectrum the carborane carbon atom bearing the

amide group upon deboronation shifted upfield to 55.1 ppm from 82.3 ppm. The identical procedure used to prepare **7** was employed to generate the iodo-carborane **14**, which was obtained in 89% yield. As would be expected, the $^{11}B\{^{1}H\}$ NMR spectrum of **14** (δ_B -8.5, -12.5, -18.3, -19.3, -29.6, -30.9, -35.9) was similar to that for **7** with respect to the chemical shifts and peak shapes.



Scheme 4. Preparation and subsequent iodination of the *nido*-carborane derivative of **12**.

2.2 In vitro screening

The *in vitro* competitive binding assays were run using LNCaP prostate cancer cells, which are known to express PSMA, and a known high affinity ^{125}I-labeled PSMA inhibitor.[20] Briefly, the radioligand and increasing concentrations (0 nM – 10,000 nM) of **5**, **6b**, **7**, **12**, **13**, and **14** were combined and allowed to incubate for 1 h following literature protocols. Cells were washed three times and counted in a gamma counter and the resulting data used to calculate IC_{50} values (**Figures 1** and **2**). 2-(Phosphonomethyl)-

pentanedioic acid (PMPA), a known PSMA inhibitor,[21] was run in parallel as a positive control.

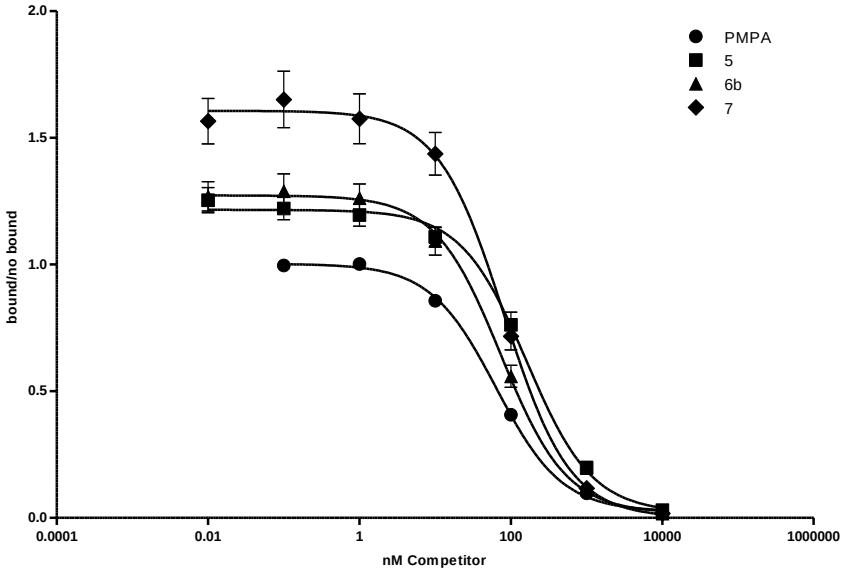


Figure 1. IC_{50} curves for compounds **5**, **6b**, **7** and the positive control (PMPA).

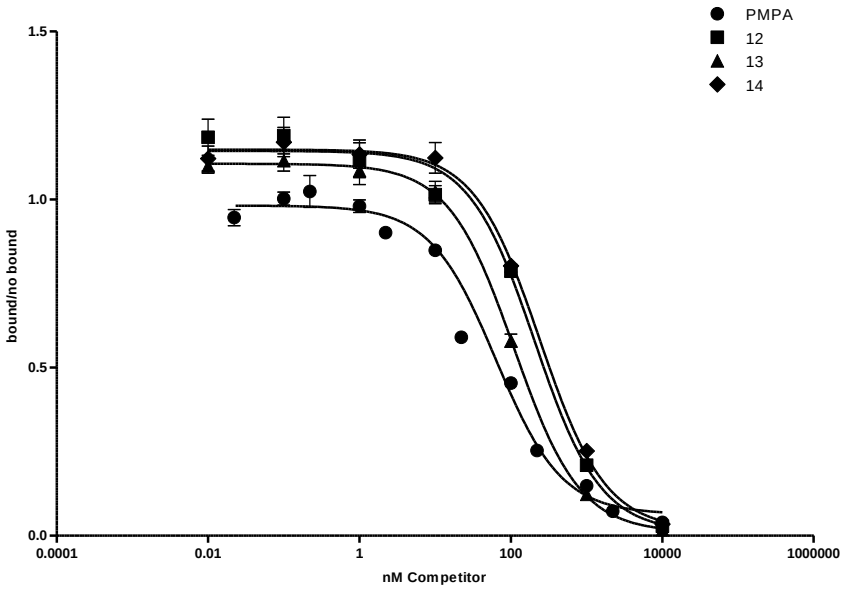


Figure 2. IC_{50} curves for compounds **12**, **13**, **14** and the positive control (PMPA).

Table 1. IC_{50} values for a known PSMA inhibitor and carborane ligands **5**, **6b**, **7**, **12**, **13** and **14**.

Compound	**IC_{50} (nM)**
PMPA (control)	63.9
5	161.0
6b	73.2
7	80.7
12	206.6
13	109.7
14	240.8

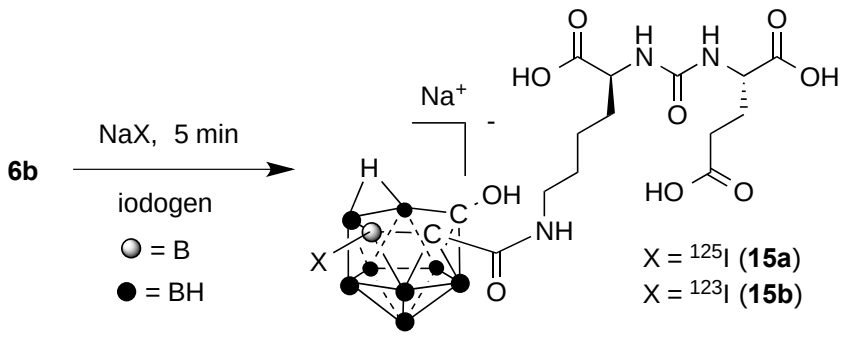
All compounds tested had nanomolar IC_{50} values (**Table 1**) where the *nido*-carborane **6b** and iodinated derivative **7** had the highest affinities for PSMA and that it was comparable to the positive control PMPA. The *closo*-carborane showed over a two-fold increase in IC_{50} value which suggests the anionic charge and open face of the cluster enhances the interaction with the protein. [22] The spacer group had a detrimental impact on affinity where the *closo* and iodinated derivatives had IC_{50} values above 200 nM. Here again the *nido*-caborane showed higher affinity. The IC_{50} for **7** was sufficiently close to PMPA to warrant evaluating the biodistribution of the ^{123}I labeled analogue.

2.3 Radiochemistry

New BNCT agents are often assessed initially by measuring how much boron they can localize in cancer cells. A complementary strategy is to use radiolabeled carboranes to assess not only tumor uptake but also the extent of binding to non-target

tissues.[23] *Nido*-carboranes can be radiolabeled in a single step via oxidative halogenation providing a convenient way of assessing tumour uptake and distribution in preclinical tumour models.[24]

With compound **7** showing <100 nM affinity for PSMA, a method to synthesize the radiolabeled analogue was developed with iodine-125 prior to performing biodistribution studies with the more costly iodine-123 analogue. Ultimately the optimal method involved the treatment of the *nido*-ligand **6b** (100 μg) with Na[^{125}I] in the presence of iodogen (**Scheme 5**) followed by quenching with sodium metabisulfite after 5 minutes. The product was isolated by HPLC in 79% radiochemical yield free from any residual ligand. The gamma HPLC of **15a** was compared to the UV-HPLC chromatograms for the reference standard where the retention times were in agreement (**Figure 3**). The log P values for **15a** measured using the "shake-flask" method[25] was -0.95 ± 0.3 which is significantly higher than an iodo-triazole analogue of **15a** which had a log P of -3.23 ± 0.05 but was less that the log P for p-iodo-benzyl and iodo-phenylurea derivatives of **3** (0.26 and 0.53).[11a] For biodistribution studies, the ^{123}I analogue **15b** was produced using an identical method and the product isolated in 76% yield.



Scheme 5. Radioiodination of **6b** with ^{125}I and ^{123}I.

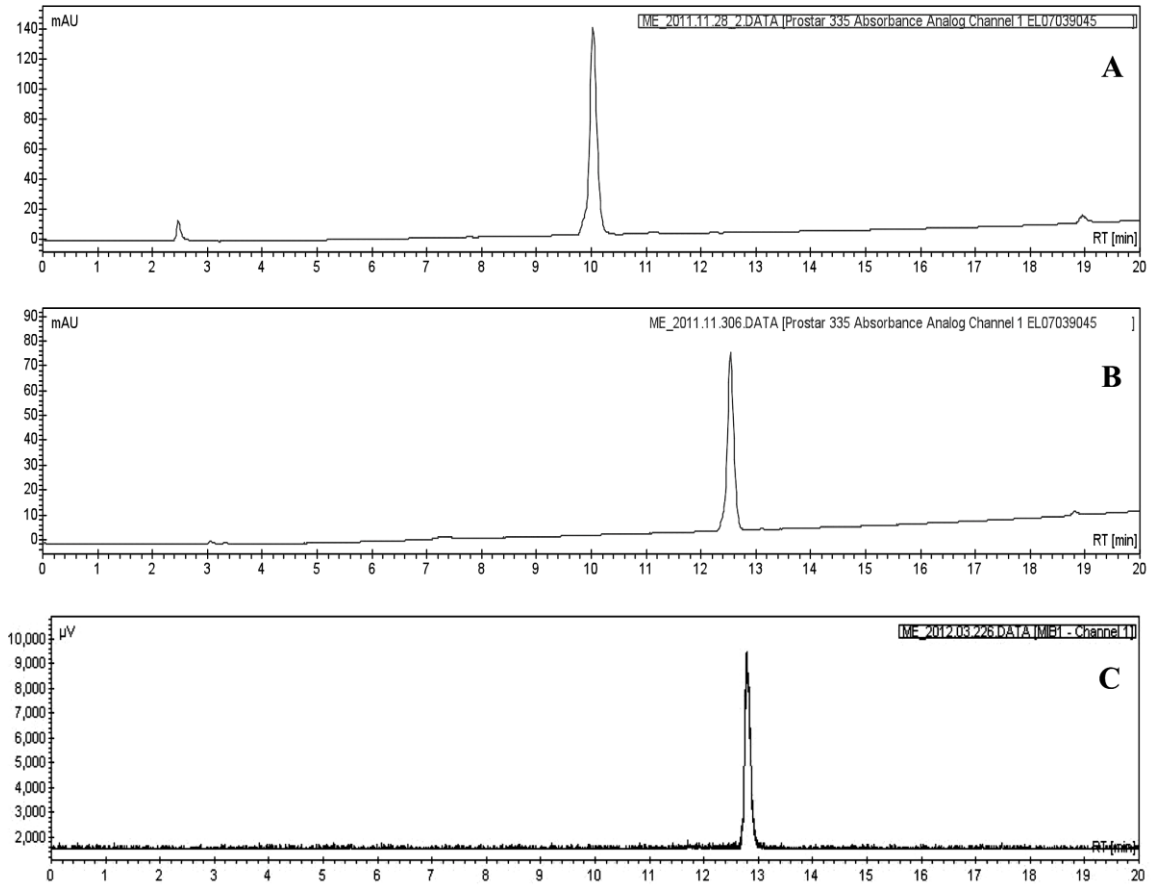


Figure 3. (A) UV-HPLC-trace of **6b** (B) UV-HPLC trace of **7** and (C) γ-HPLC trace of **15a**. Note that the UV and γ-detectors are connected in series.

2.4 Biodistribution Studies

Biodistribution studies on **15b** were performed in LNCaP CD1 nude mice with LNCaP tumour xenografts three weeks post inoculation. Approximately 0.44 MBq of **15b** was administered and animals sacrificed and tissues collected and counted at t=1, 2,

4, 6 and 24 h post injection (n = 4 per time point). The results (**Figure 4** and **Table 2**) showed initial tumour uptake of 4.17% at 1 h, which remained at that level and only decreasing somewhat at 6 h (3.59%). Tumour-to-blood ratios were below one at 1 h (0.96) increasing to 1.27 at 2 h and peaking at 3.47 at 6 h. Tumour-to-muscle ratios were 9.14 at 1 h and reached a maximum of 25.52 at 6 h (**Table 3**). High uptake was observed in the gallbladder, intestines and liver. The gall bladder uptake and large intestine uptake is likely driven by the hydrophobic nature of carboranes even with the addition of the C-hydroxy group.

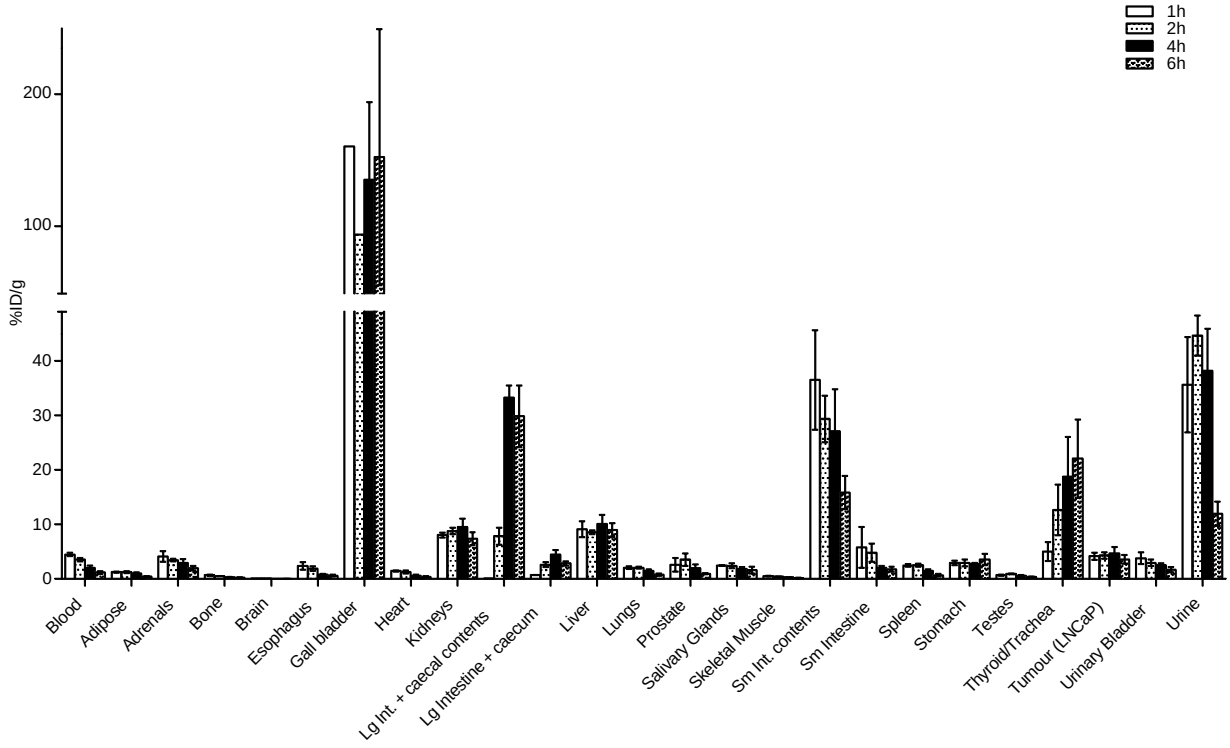


Figure 4. *In vivo* biodistribution of **15b** in LNCaP xenograft mice. Mice were injected with approximately 0.44 MBq and sacrificed at the time points indicated. Data are expressed as percent injected dose per gram (%ID/g).

Table 2. Tissue distribution of **15b** in CD1 nude mice bearing LNCaP xenografts. Data are %ID/g and expressed as mean ± SEM.* Only one gall bladder was collected for these time points.

	Time (h)					
Organs	**1h**	**1h + block**	**2h**	**4h**	**6h**	**24h**
Blood	4.48 ± 0.32	4.34 ± 0.13	3.54 ± 0.30	2.06 ± 0.41	1.17 ± 0.27	0.09 ± 0.01
Adipose	1.25 ± 0.11	0.39 ± 0.05	1.26 ± 0.17	1.00 ± 0.21	0.44 ± 0.11	0.08 ± 0.02
Adrenals	4.13 ± 0.95	2.10 ± 0.48	3.47 ± 0.24	2.94 ± 0.67	1.99 ± 0.41	0.23 ± 0.03
Bone	0.69 ± 0.13	0.62 ± 0.04	0.54 ± 0.02	0.29 ± 0.07	0.22 ± 0.06	0.01 ± 0.005
Brain	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.01 ± 0.002
Esophagus	2.38 ± 0.67	2.54 ± 0.54	1.88 ± 0.41	0.78 ± 0.12	0.63 ± 0.19	0.08 ± 0.04
Gall bladder	160.56 ± n/a*	750.28 ± 534.90	93.54 ± n/a*	135.25 ± 58.83	152.34 ± 97.14	5.69 ± 1.07
Heart	1.48 ± 0.10	1.21 ± 0.09	1.28 ± 0.28	0.63 ± 0.16	0.40 ± 0.13	0.03 ± 0.01
Kidneys	8.07 ± 0.46	1.78 ± 0.08	8.78 ± 0.58	9.58 ± 1.47	7.37 ± 1.18	3.35 ± 0.37
Lg Int. + caecal contents	0.09 ± 0.01	0.12 ± 0.02	7.84 ± 1.57	33.28 ± 2.19	29.84 ± 5.63	2.84 ± 0.79
Lg Intestine + caecum	0.71 ± 0.01	0.78 ± 0.10	2.62 ± 0.43	4.49 ± 0.81	2.86 ± 0.38	0.24 ± 0.03
Liver	9.14 ± 1.43	7.88 ± 0.25	8.59 ± 0.35	10.08 ± 1.64	8.96 ± 1.27	2.83 ± 0.47
Lungs	2.05 ± 0.24	2.20 ± 0.04	2.08 ± 0.20	1.49 ± 0.24	0.82 ± 0.22	0.13 ± 0.03
Prostate	2.60 ± 1.27	3.51 ± 0.75	3.53 ± 1.15	1.98 ± 0.66	0.95 ± 0.11	0.04 ± 0.01
Salivary Glands	2.47 ± 0.04	2.05 ± 0.18	2.43 ± 0.42	1.87 ± 0.31	1.61 ± 0.63	0.10 ± 0.03
Skeletal Muscle	0.50 ± 0.07	0.51 ± 0.04	0.42 ± 0.01	0.28 ± 0.05	0.16 ± 0.03	0.01 ± 0.002
Sm Int. contents	36.50 ± 9.13	27.55 ± 4.88	29.35 ± 4.30	27.07 ± 7.77	15.86 ± 3.07	1.17 ± 0.21
Sm Intestine	5.79 ± 3.74	4.87 ± 0.52	4.82 ± 1.65	2.04 ± 0.32	1.78 ± 0.43	0.16 ± 0.02
Spleen	2.49 ± 0.22	0.75 ± 0.04	2.52 ± 0.28	1.50 ± 0.28	0.67 ± 0.23	0.10 ± 0.02
Stomach	2.95 ± 0.41	2.94 ± 0.32	2.97 ± 0.62	2.73 ± 0.25	3.56 ± 1.05	0.31 ± 0.07
Testes	0.71 ± 0.09	0.60 ± 0.03	0.91 ± 0.07	0.59 ± 0.10	0.38 ± 0.07	0.04 ± 0.01
Thyroid/Trachea	5.00 ± 1.74	7.98 ± 2.30	12.68 ± 4.64	18.76 ± 7.32	22.10 ± 7.17	28.98 ± 7.88
Tumour (LNCaP)	4.17 ± 0.66	3.78 ± 0.62	4.31 ± 0.61	4.71 ± 1.15	3.59 ± 0.81	0.04 ± 0.02
Urinary Bladder	3.80 ± 1.07	5.68 ± 1.14	2.97 ± 0.56	2.51 ± 0.36	1.70 ± 0.47	0.02 ± 0.01
Urine	35.61 ± 8.76	49.80 ± 8.92	44.63 ± 3.67	38.22 ± 7.64	11.96 ± 2.23	1.40 ± 0.54

Table 3. Select tumour-to-blood/tissue ratios ± SEM.

	Time (h)					
	1h	**1h+ block**	**2h**	**4h**	**6h**	**24h**
Tumour/Blood	0.96 ± 0.18	0.88 ± 0.16	1.27 ± 0.23	2.18 ± 0.18	3.47 ± 1.15	0.63 ± 0.10
Tumour/Skeletal Muscle	9.14 ± 2.18	7.70 ± 1.57	10.32 ± 1.62	15.89 ± 2.53	25.52 ± 8.52	8.62 ± 2.59

To evaluate the ability to block uptake, PMPA (5 mg/mL) in saline was administered to a cohort of animals immediately followed by 0.44 MBq of **15b**. Tumour

uptake decreased only a small amount (10%) suggesting that some proportion of the observed uptake was via a different mechanism. The blocking study did have a marked impact on kidney uptake which decreased from 8.07 to 1.78 %ID/g. Unfortunately the PMPA could not be administered concurrently with the agent as we noted that the products would co-precipitate when mixed which could have impacted the observed results. An additional interesting observation was the high thyroid uptake, which was 5% at 1 h increasing to over 22% at 6 h. Iodinated carboranes are an attractive alternative to more commonly used aryl iodides because the B-I bond dissociation energy (381 ± 21 kJ/mol) is substantially greater than for the C-I bond (209 ± 21 kJ/mol).[26] Despite this the tracer is clearly being metabolized and releasing iodide which may be a consequence of the presence of the hydroxyl group on the carborane. We are currently studying the stabilities of iodinated carboranes bearing a range of different substitutents to test this hypothesis.

The results demonstrate that potent PSMA ligands can be prepared from carboranes however ways to optimize the distribution *in vivo* and in particular decrease uptake in non-target organs like the gall bladder are needed. Strategies to further reduce the log P of the reported compounds and decrease non-specific binding are needed to create an effective BNCT agent. The results do however show that it is possible to prepare carboranes with high affinity for PSMA and that radiolabelling C-hydroxy-carboranes with ^{123}I is a convenient method for assessing uptake and specificity of new BNCT agents in animal tumour models.

3. Conclusion

A series of C-hydroxy-carborane derivatives that show high affinity for PSMA were prepared. The compounds reported included *closo*, *nido* and *iodo*-carborane small molecule inhibitors where the ^{123}I labelled analogue of the latter offered the means to use quantitative biodistribution studies to evaluate this new class of BNCT candidates in preclinical tumour models. The influence of the carborane cluster was significant where ways to maximize tumour uptake and further reduce non-specific binding are needed to optimize target-to-non-target ratios.

4. Experimental Section

4.1 Materials and general methods

All chemicals and reagents were purchased from Aldrich, except for *o*-carborane, which was purchased from Katchem (Czech Rep.). Solvents were purchased from Caledon and dried using a PureSolv drying apparatus (Innovative Technology). No-carrier-added, iodine-125 was obtained from the McMaster Nuclear Reactor as Na[^{125}I] in 0.1 N NaOH. Iodine-123 in 0.1 NaOH was purchased from Nordion Inc. (Vancouver, Canada). ^{1}H, ^{13}C, and ^{11}B NMR spectra were measured on a Bruker Avance AV-600 (^{1}H = 600.13 MHz, ^{13}C = 150.90 MHz, ^{11}B = 192.55 MHz) spectrometer. ^{1}H NMR and ^{13}C NMR chemical shifts are expressed in parts per million (ppm, δ units), and coupling constants are expressed in Hertz (Hz). The chemical shifts of ^{11}B NMR were reported relative to an external standard of $BF_3 \cdot Et_2O$ in $CDCl_3$. IR spectra were recorded on a Nicolet 6700 FT-IR spectrometer. Low-resolution mass spectra were obtained on an Agilent 630 ion trap electrospray ionization (ESI) instrument, using a 1200 series LC system and H_2O:MeOH (1:1). High-resolution mass spectra (HRMS) were obtained using a Waters Micromass

Global Ultima Q-TOF in ESI mode. Radioactivity measurements were made using either a Capintec CRC-15W dose calibrator or a Perkin-Elmer Wizard gamma counter. For ^{125}I and ^{123}I high- performance liquid chromatography (HPLC) was performed with a Varian ProStar HPLC system fitted with an IN/US γ-RAM Model 3 detector. A Phenomenex Gemini column (5 µm, 4.6×250 mm, C18) at a flow rate of 1.0 mL/min and monitoring at 254 nm was used for analytical runs. For semi-preparative HPLC, a phenomenex Luna column (5 µm, 10.0×250 mm, C18) at a flow rate of 4.0 mL/min and monitoring at 254 nm was employed. HPLC protocols were as follows: *Analytical* and *semi*-preparative HPLC: Solvent A = 0.1% trifluoroacetic acid (TFA) in water; Solvent B = 0.1% TFA in acetonitrile: Method A: gradient elution, 30% B (0-20 min), 100%B (20-22 min), 30%B (22-25 min); Method B: gradient elution, 20% B (0-20 min), 100% B (20-22 min), 20% B (22-25 min). Analytical thin layer chromatography (TLC) was performed on glass plates of silica gel 60 GF254 (Merck). Development of plates was performed using a 0.1 M $PdCl_2$ in 3 M HCl spray.

4.2 Cell cultures

LNCaP cells were purchased from ATCC (CRL-1740) and maintained in RPMI 1640 with 2 mM glutamine supplemented with 10% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate, 10 mM Hepes, 1% Penicillin Streptomycin and 0.25% D-glucose. All experiments were performed using cells between passage numbers 10-30.

4.3 Synthesis and characterization data

Synthesis of 4: Compound **1** (0.50 g, 2.45 mmol) and $SOCl_2$ (25 mL) were placed under nitrogen atmosphere into a three neck 100 mL round bottom flask equipped with a condenser, and the mixture was heated to reflux for 3 h. Excess $SOCl_2$ was then removed under reduced pressure and the resulting oil dissolved in CH_2Cl_2 (10 mL) and the solution added slowly *via* cannula to a solution of **3** (1.19 g, 2.44 mmol) in CH_2Cl_2 (10 mL) cooled to 0 ^oC under nitrogen. Triethylamine (1.70 mL, 5.00 mmol) in CH_2Cl_2 (10 mL) was added slowly, and the mixture stirred at room temperature for 6 h. The mixture was then extracted with NH_4Cl (2 × 10 mL) and the organic layer dried with sodium sulphate. The solution was concentrated under reduced pressure and the product isolated by column chromatography (CH_2Cl_2:MeOH, 10:1), affording a yellow solid, which was further purified by *semi*-preparative HPLC to yield **4** as a white solid (1.56 g, 95%). HPLC (method B): t_r = 9.25 min, TLC R_f = 0.28 (CH_2Cl_2:MeOH, 10:1), MP = 100-102 ^oC; ^1H NMR ($CDCl_3$, 600 MHz) δ_H 6.98 (1H, s, CONH), 5.78, 5.65 (2H, m, NH), 4.29 (1H, m, CH), 4.17 (1H, m, CH), 3.35 (2H, bs, CH_2), 2.33 (2H, bs, CH_2), 2.06 to 1.60 (m, CH_2), 1.49 to 1.45 (m, *t*-Bu), 2.90-0.91 (bm, BH); ^{13}C NMR ($CDCl_3$, 150 MHz) δ_C 173.2, 173.0, 171.9, 165.2, 157.5, 82.7, 82.4, 81.5, 53.4, 52.9, 39.3, 39.2, 31.4, 28.4, 28.1, 28.0, 22.1; ^{11}B NMR ($CDCl_3$, 192 MHz) δ_B -4.0 (bs, 1B), -12.2 (bs, 9B); IR ν_{max} (KBr)/cm^{-1} 3359, 2577, 1728; HRMS-ESI: *m/z*: calcd for $C_{27}H_{55}N_3O_9B_{10}$: 674.5034, found: 674.5030 $[M + H]^+$.

Synthesis of 5: To a solution of **4** (500 mg, 0.74 mmol) in CH_2Cl_2 (10 mL) was added TFA (10 mL) and the solution stirred at room temperature for 4 h. The solution was subsequently evaporated under vacuum and CH_2Cl_2 (10 mL) added to the residue and the

mixture stirred for 10 min. whereupon the CH_2Cl_2 was decanted. This process was repeated three times. The oily residue was dried under vacuum to yield **5** as a white solid (375 mg, >99%). MP = 56-58 ^oC; ^1H NMR (DMSO-d_6, 600 MHz) δ_H 7.99 (bs, CONH), 6.55 (bs, NH), 4.34 (`H, m, CH), 4.28 (1H, m, CH), 3.38 (2H, m, CH_2), 2.76 (bs, CH_2), 2.17 (m, CH_2), 3.85 to 1.25 (bm, BH, CH_2); ^{13}C NMR (DMSO-d_6, 150 MHz) δ_C 174.4, 174.1, 173.7, 162.7, 157.3, 83.6, 55.0, 52.2, 51.6, 48.6, 39.1, 31.6, 29.8, 28.4, 27.5, 22.3; ^{11}B NMR (DMSO-d_6, 192 MHz) δ_B -6.3 (bs, 1B), -8.0 (bs, 2B), -13.5 (bs, 3B), -16.2 (d, J_{BH} = 130.6, 2B), -19.1 (d, J_{BH} = 130.6, 2B); IR ν_{max} (KBr)/cm^{-1} 3350, 2596, 1718; HRMS-ESI: *m/z*: calcd for $C_{15}H_{31}N_3O_9B_{10}Na$: 529.2939, found 529.2902 $[M + Na]^+$.

Synthesis of 6a/b: A solution of **5** (100 mg, 0.20 mmol) in water (10 mL) was heated to reflux for 15 min. The solution was allowed to cool at room temperature and the solution lyophilized to yield a white solid which was purified by *semi*-preparative HPLC to yield **6a** as a white solid (98 mg, 97%). HPLC (method A): t_r = 9.75 min, MP = 77-79 ^oC; ^1H NMR (DMSO-d_6, 600 MHz) δ_H 7.17 (bs, CONH), 6.30 (2H, m, NH), 4.09 (1H, m, CH), 4.02 (1H, m, CH), 3.62 (m, OH), 2.97 (bs, CH_2), 2.24 (2H, m, CH_2), 1.91 to 1.23 (m, CH_2), 2.45 to -0.15 (bm, BH), -2.80 (bs, μ-H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ_C 174.5, 174.1, 173.7, 173.2, 157.3, 97.9, 54.8, 52.3, 51.6, 48.6, 38.7, 31.7, 29.8, 28.7, 27.5, 22.4; ^{11}B NMR (DMSO-d_6, 192 MHz) δ_B -10.9 (bs, 2B), -14.0 (bs, 2B), -20.8 (bs, 2B), -27.8 (d, J_{BH} = 147.8, 1B), -33.6 (bs, 1B), -36.4 (d, J_{BH} = 145.9, 1B); IR ν_{max} (KBr)/cm^{-1} 3403, 2538, 1696; HRMS-ESI: *m/z*: calcd for $C_{15}H_{31}N_3O_9B_9$: 495.2946, found 495.2945 $[M]^-$. The sodium salt **6b** can be obtained by neutralizing an aqueous solution of **6a** with 1 M NaOH followed by lyophilization, which produced the desired product as a white solid.

Synthesis of 7: To a solution of **6b** (50.0 mg, 0.1 mmol) in water (0.5 mL) was added iodine (24.5 mg, 0.1 mmol) in acetonitrile (2 mL) dropwise over 1 h. The reaction was stirred at room temperature for an additional hour and quenched with 0.1 M sodium thiosulphate (1.5 mL). After evaporation to dryness, the desired product was isolated by semi-preparative HPLC to yield **7** as a white solid (58.0 mg, 93%). HPLC (method A): t_r = 12.25 min, MP = 83-85 ^{o}C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ_H 10.85 (bs, COOH), 7.39 (1H, t, J_{CH} = 6.0, CONH), 6.31 (bs, NH), 4.09 (m, CH), 4.02 (m, CH), 3.00 (2H, bs, CH_2), 2.24 (m, CH_2), 1.92 (m, CH_2), 1.71 (m, CH_2), 1.63 (m, CH_2), 1.51 (m, CH_2), 1.38 (m, CH_2), 1.23 (m, CH_2), 2.45 to 0.25 (bm, BH), -2.89 (bs, μ-H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ_C 174.5, 174.1, 173.7, 172.8, 157.2, 98.7, 52.3, 51.6, 41.36, 40.0, 38.9, 31.7, 29.8, 28.6, 27.5, 22.4; ^{11}B NMR (DMSO-d_6, 192 MHz) δ_B -8.7 (bs, 1B), -12.7 (bs, 1B), -18.5 (bs, 4B), -30.8 (d, J_{BH} = 134.4, 2B), -35.8 (d, J_{BH} = 134.4, 1B); IR ν_{max} (KBr)/cm^{-1} 3317, 2547, 1735; HRMS-ESI: *m/z*: calcd for $C_{15}H_{30}B_9IN_3O_9$: 620.1945, found 620.1927 $[M]^-$.

Synthesis of 9: A solution of **2** (0.30 mg, 1.34 mmol) in CH_2Cl_2 (10 mL) was added slowly *via* cannula to a solution of methyl 6-aminohexanoate (**8**) (0.24 g, 1.32 mmol) in CH_2Cl_2 (10 mL) cooled to 0 ^{o}C under nitrogen. A solution of triethylamine (0.38 mL, 2.77 mmol) in CH_2Cl_2 (10 mL) was added slowly, and the mixture stirred at room temperature for 6 h. The reaction mixture was extracted with 1 M HCl, water, brine and the organic layer dried with sodium sulphate. The solution was concentrated under reduced pressure and the desired product purified by column chromatography (CH_2Cl_2:MeOH, 17:3) affording **9** as a white solid (0.42 g, 95%). TLC R_f = 0.45

(CH_2Cl_2:MeOH, 17:3), MP = 113-115 ^oC; ^1H NMR ($CDCl_3$, 600 MHz) δ_H 6.54 (bs, CONH), 3.70 (3H, s, CH_3), 3.39 (2H, m, CH_2), 2.35 (2H, t, J = 6.0 Hz,CH_2), 1.66 (2H, m, CH_2), 1.61 (2H, m, CH_2), 1.37 (2H, m, CH_2), 3.2 to 1.2 (bm, BH); ^{13}C NMR ($CDCl_3$, 150 MHz) δ_C 174.1, 165.0, 103.2, 69.1, 51.7, 40.4, 33.6, 28.3, 25.8, 23.9; ^{11}B NMR ($CDCl_3$, 192 MHz) δ_B -4.29 (d, J_{BH} = 145.9, 1B), -9.2 (bs, 1B), -10.2, -10.7 (m, overlap, 2B), -11.8, -12.7 (m, overlap, 5B), -14.0 (bs, 1B); IR ν_{max} (KBr)/cm^{-1} 3346, 2599, 1724, 1656; HRMS-ESI: *m/z*: calcd for $C_{10}H_{24}NO_4B_{10}$: 330.2714, found 330.2743 $[M-H]^-$.

Synthesis of 10: A solution of **9** (0.30 mg, 0.91 mmol) was combined with a 1:1 (v/v) mixture of 6 N HCl and CH_3CN (10 mL) and the mixture stirred at 60 ^oC for 20 min. After cooling to room temperature, the solution was diluted with CH_2Cl_2 (30 mL), and extracted with water (30 mL) and saturated sodium carbonate (3x 30 mL). The aqueous layers were combined, acidified with 6 N HCl and the solution extracted with CH_2Cl_2 (3 × 50 mL). The organic extracts were combined, dried over sodium sulfate and concentrated to dryness to give **10** as a colorless crystalline solid (0.27 g, 93%). TLC R_f = 0.35 (CH_2Cl_2:MeOH, 4:1), MP = 83-85 ^oC; ^1H NMR (CD_2Cl_2, 600 MHz) δ_H 10.11 (bs, COOH), 6.53 (1H, t, J = 6.0 Hz, CONH), 3.31 (2H, m, CH_2), 2.35 (t, J = 6.0 Hz, CH_2), 1.64 (2H, m, CH_2), 1.57 (2H, m, CH_2), 1.34 (2H, m, CH_2), 2.95 to 1.20 (bm, BH); ^{13}C NMR (CD_2Cl_2, 150 MHz) δ_C 180.2, 164.2, 105.2, 71.6, 41.0, 34.0, 28.7, 26.2, 24.3; ^{11}B NMR (CD_2Cl_2, 192 MHz) δ_B -4.3 (d, J_{BH} = 109.4, 1B), -10.6 (bs, 3B), -12.3, -13.0 (m, overlap, 6B); IR ν_{max} (KBr)/cm^{-1} 3350, 2594, 1700, 1668; HRMS-ESI: *m/z*: calcd for $C_9H_{22}NO_4B_{10}$: 316.2557, found 316.2556 $[M-H]^-$.

Synthesis of 11: Compound **10** (0.250 g, 0.79 mmol) was combined with EDC-HCl (0.21 g, 1.09 mmol) and N-hydroxysuccinimide (0.13 g, 1.09 mmol) in a microwave vial (5 mL). Dry CH_3CN was added, the vial crimp-sealed, and the reaction mixture heated at 120 ^oC for 5 min in a Biotage microwave reactor. This reaction mixture was added to a solution of **3** (0.38 g, 0.79 mmol) in CH_2Cl_2 (10 mL), and then triethylamine (0.15 mL, 1.08 mmol) in CH_2Cl_2 (5 mL) added slowly, and the mixture stirred at room temperature for 6 h. The mixture was extracted with NH_4Cl, water, brine and the organic layer dried with sodium sulphate. The resulting solution was concentrated under reduced pressure which was isolated by column chromatography affording **11** as a white solid (0.57 g, 92%). TLC R_f = 0.37 (CH_2Cl_2:MeOH, 17:3), MP = 47-49 ^oC; ^1H NMR (CD_2Cl_2, 600 MHz) δ_H 7.85 (bs, CONH), 6.72 (1H, t, *J* = 6.0 Hz, CONH), 5.77 (2H, m, CH), 4.24 (2H, m, NH), 3.29-3.02 (m, CH_2), 2.68 (s, CH_2), 2.30 (m, CH_2), 2.22 (m, CH_2), 2.05 (m, CH_2), 1.87 (m, CH_2), 1.76 (m, CH_2), 1.62 to 1.32 (m, CH_2), 1.47 to 1.42 (m, *t*-Bu's), 2.68 to 1.32 (bm, BH); ^{13}C NMR (CD_2Cl_2, 150 MHz) δ_C 174.9, 173.4, 173.0, 172.7, 172.6, 158.0, 82.7, 82.2, 82.0, 80.8, 40.5, 39.6, 36.5, 32.6, 31.9, 28.9, 28.6, 28.4, 28.2, 27.3, 25.9, 22.9; ^{11}B NMR (CD_2Cl_2, 192 MHz) δ_B -6.1 (bs, 1B), -9.1 (bs, 1B), -13.0 (d, J_{BH} = 99.8, 3B), -15.3 (bs, 3B), -19.0 (bs, 2B); IR ν_{max} (KBr)/cm^{-1} 3387, 2926, 2570, 1739; HRMS-ESI: *m/z*: calcd for $C_{33}H_{65}N_4O_{10}B_{10}$: 787.5679, found 787.5697 $[M-H]^-$.

Synthesis of 12: To a solution of **11** (500 mg, 0.64 mmol) in CH_2Cl_2 (10 mL) was added TFA (10 mL) and the solution was stirred at room temperature for 1 h. The solution was subsequently evaporated under vacuum and CH_2Cl_2 (10 mL) added to the residue and the mixture stirred for 10 min, whereupon the CH_2Cl_2 was decanted. This process was

repeated three times. The residue was dried under vacuum to yield **12** (390 mg, 99%) as a white solid. MP = 52-54 ^{o}C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ_H 8.01 (1H, t, J = 6.0 Hz, CONH), 7.72 (1H, t, J = 6.0 Hz, CONH), 6.31 (bs, NH), 4.08 (m, CH), 4.03 (m, CH), 3.10 (2H, m, CH_2), 3.0 (2H, m, CH_2), 2.23 (m, CH_2), 1.90 (m, CH_2), 1.70 (m, CH_2), 1.62 (m, CH_2), 1.50 (m, CH_2), 1.44 (m, CH_2), 1.37 (m, CH_2), 1.25 (m, CH_2), 1.17 (m, CH_2), 2.50 to 1.17 (bm, BH); ^{13}C NMR (DMSO-d_6, 150 MHz) δ_C 174.5, 174.1, 173.7, 171.9, 171.8, 157.2, 82.3, 52.2, 51.6, 41.2, 38.6, 38.3, 35.3, 31.8, 29.8, 28.8, 28.3, 27.5, 25.8, 24.9, 22.6; ^{11}B NMR (DMSO-d_6, 192 MHz) δ_B -6.0 (bs, 1B), -9.2 (bs, 1B), -13.1 (bs, 4B), -15.6 (d, J_{BH} = 107.5, 4B); IR ν_{max} (KBr)/cm^{-1} 3285, 2935, 2575, 1715; HRMS-ESI: *m/z*: calcd for $C_{21}H_{41}B_{10}N_4O_{10}$: 619.3789, found 619.3787 [M-H].

Synthesis of 13: A solution of **12** (0.10 g, 0.16 mmol) in water (10 mL) was heated to reflux for 30 min. The solution was allowed to cool at room temperature, neutralized with 1 M NaOH and lyophilized to yield a white solid. The compound was purified by *semi*-preparative HPLC to yield **13** (98 mg, >99%) as a waxy solid. HPLC (method A): t_r = 10.0 min; ^{1}H NMR (DMSO-d_6, 600 MHz) δ_H 8.27 (bs, COOH), 7.79 (t, J = 6.0 Hz, CONH), 7.17 (t, 1H, J = 6.0 Hz, CONH), 6.33 (bs, NH), 4.09 (1H, m, CH), 4.04 (1H, m, CH) 3.00 (4H, m, CH_2), 2.24 (m, CH_2), 1.97 (m, CH_2), 1.72 (m, CH_2), 1.64 (m, CH_2), 1.52 (m, CH_2), 1.45 (m, CH_2), 1.37 (m, CH_2), 1.27 (m, CH_2), 1.17 (m, CH_2), 2.50 to -0.21 (bm, BH), -2.77 (bs, μ–H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ_C 174.7, 174.3, 173.9, 173.3, 172.2, 157.5, 98.0, 55.1, 52.4, 51.8, 38.8, 38.5, 35.5, 31.9, 30.0, 28.9, 28.8, 27.6, 26.0, 25.2, 22.7; ^{11}B NMR (DMSO-d_6, 192 MHz) δ_B -10.6 (d, J_{BH} = 99.8, 2B), -13.9 (bs, 2B), -20.7 (bs, 2B), -27.6 (d, J_{BH} = 129.0, 1B), -33.5 (bs, 1B), -36.4 (d, J_{BH} = 136.3, 1B);

IR ν_{max} (KBr)/cm^{-1} 3544, 2533, 1690, 1646; HRMS-ESI: *m/z*: calcd for $C_{21}H_{42}B_9N_4O_{10}$: 609.3771, found 609.3777 [M$^-$].

Synthesis of 14: To a solution of **13** (50.0 mg, 0.08 mmol) in water (0.5 mL) was added iodine (20.9 mg, 0.08 mmol) in acetonitrile (2 mL) dropwise over 1 h. The reaction mixture was stirred at room temperature for an additional hour and quenched with 0.1 M sodium thiosulphate (1.5 mL). After evaporation to dryness the desired product was isolated by semi-preparative HPLC to yield **14** as a white solid (54.0 mg, 89%). HPLC (method A): t_r = 12.55 min, MP = 73-75 ^{o}C; ^{1}H NMR (DMSO-*d*$_6$, 600 MHz) δ_H 7.35 (bs, COOH), 7.74 (t, *J* = 6.0 Hz, CONH), 7.34 (t, *J* = 6.0 Hz, CONH), 6.30 (bs, 2H, NH), 4.08 (m, 1H, CH), 4.03 (m, 1H, CH), 2.99 (m, 4H, CH_2), 2.22 (m, CH_2), 2.01 (m, CH_2), 1.70 (m, CH_2), 1.63 (m, CH_2), 1.50 (m, CH_2), 1.44 (m, CH_2), 1.36 (m, CH_2), 1.25 (m, CH_2), 1.16 (m, CH_2), 2.50 to 0.06 (bm, BH), -2.91 (bs, μ–H); ^{13}C NMR (DMSO-*d*$_6$, 150 MHz) δ_C 174.5, 174.1, 173.7, 172.8, 171.9, 157.3, 98.7, 52.2, 51.6, 40.0, 38.8, 38.3, 35.3, 31.7, 29.8, 28.8, 28.6, 27.5, 25.9, 25.0, 22.6; ^{11}B NMR (DMSO-*d*$_6$, 192 MHz) δ_B -8.6 (bs, 1B), -12.6 (bs, 1B), -18.4 (bs, 4B), -30.9 (d, J_{BH} = 126.7, 2B), -35.9 (d, J_{BH} = 138.2, 1B); IR ν_{max} (KBr)/cm^{-1} 3293, 2546, 1724, 1624; HRMS-ESI: *m/z*: calcd for $C_{21}H_{41}B_9IN_4O_{10}$: 734.2758, found 734.2735 [M$^-$].

Synthesis of 15a: A 1 mg/mL solution of Iodogen® in $CHCl_3$ was prepared shortly before use and 25 μL of the solution added to an eppendorf tube. The solvent was removed using a rotary evaporator leaving a film of Iodogen®. To this tube, 100 μL of a 1 mg/mL solution of **6b** in acetonitrile/water (1:1 v/v, 5% acetic acid) was added,

followed by 92.5 MBq solution of $Na^{125}I$ in sodium hydroxide (10 µL, pH 8-11). The reaction was allowed to stand for 5 min. whereupon 0.1 M $Na_2S_2O_5$ was added (20 µL). The reaction mixture was diluted with water (100 µL) and the product isolated by HPLC. **15a** was characterized by correlation of the HPLC retention time of the product with that of the non-radioactive analogue **7**. HPLC: t_r = 12.75 min; radiochemical yield = 79%.

Synthesis of 15b: Compound **15b** was prepared from **6b** and $Na^{123}I$ (111 MBq) following the method used to synthesize **15a**. The reaction mixture was diluted with water (100 µL) and the product isolated by *semi*-preparative HPLC. **15b** was characterized by correlation of the HPLC retention time of the product with that of the non-radioactive analogue **7**. HPLC: t_r = 12.75 min; radiochemical yield = 76%.

Log *P*

Log *P* measurements were made using the "shake-flask" method. Compounds **15a** was concentrated to dryness and reconstituted in 1 mL of PBS. The PBS solutions were added to nine vials each containing 1 mL phosphate buffer (pH = 7.4) and 1 mL of 1-octanol (the activity in each vial was 0.35 MBq). The vials were shaken on a vortex mixer for 20 min, followed by centrifugation for a further 20 min. Aliquots (0.1 mL) of each layer were transferred to pre-weighed vials, counted for activity in a Perkin Elmer Wizard 1470 Automatic Gamma Counter, and counts/mL calculated from the weight of solution transferred. Log *P* was calculated as {[counts/mL(1-octanol)]/[counts/mL(buffer)]}. The reported values correspond to the average of nine measurements ± standard deviation. Log *P* **15a** = -0.95 ±0.3.

PSMA Binding Assay

LNCaP cells were plated in 24 well plates for confluency at time of use. Growth media was removed and 300 µL of binding buffer, [RPMI 1640 with 2mM glutamine + 0.5% BSA containing a fixed amount of ^{125}I-TAAG-PSMA (0.45 nM) and increasing concentrations of competitor (0 nM - 10 000 nM)]. After 1h, binding buffer and cells were transferred to microcentrifuge tubes (1.5 mL), binding buffer was removed and cells were washed 3 times with wash buffer (RPMI 1640 with 2mM glutamine + 0.5% BSA) using centrifugation for 30s at max speed. After washing, cells were lysed in 500 µL RIPA buffer (100 mM Tris pH 8, 50 mM NaCl, 1% NP40, 0.5% Na deoxycholate, 0.1% SDS). A 400 µL aliquot of lysate was transferred to gamma counting tubes and counted for 10 min. in a Perkin Elmer Wizard 1470 Automatic Gamma Counter. The resulting CPM values were used to calculate the relative binding with respect to the control (no competitor present). The assay was repeated four times for compounds **5**, **6b** and **7** and three times for compounds **12**, **13** and **14** with each experiment done in triplicate. For each repeat the positive control, PMPA was also measured in triplicate. GraphPad Prism 5 software was used to determine the IC_{50} values.

Biodistribution Study

CD1 nude homo male mice (Charles River, Senneville, QC, Canada) were injected with 2.0 x 10^6 LNCaP cells in Matrigel:DPBS (1:1) subcutaneously into the right flank. Biodistribution studies was performed on mice at 3 weeks post innoculation (n = 4 per time point at t=1h, 1h + block, 2h, 4h, 6h and 24h. The t=1h cohort was injected with

50μL of saline immediately followed with a second injection containing approximately 0.44 MBq of **15b** in saline via the tail vein. The t=1h + block cohort received 50μL of 5mg/mL PMPA (a specific PSMA inhibitior) in saline (equivalent to a 10 mg/kg dose) immediately followed by a second injection containing approximately 0.44MBq of **15b**. All other groups received one injection containing approximately 0.44 MBq of **15b** in saline via the tail vein. At the specified time points, animals were anesthetized with 3% isoflurane and euthanized by cervical dislocation. Blood, adipose, adrenals, bone, brain, esophagus, gall bladder, heart, kidneys, contents of large intestine and caecum, large intestine and caecum, liver, lungs, prostate, salivary glands, skeletal muscle, contents of small intestines, small intestine, spleen, stomach (with contents), testes, thyroid/trachea, tumour, urinary bladder, urine and tail were collected, weighed and counted in a Perkin Elmer Wizard 1470 Automatic Gamma Counter. Decay correction was used to normalize organ activity measurements to time of dose preparation for data calculations with respect to injected dose (i.e. %ID/g).

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