A Series of Halogenated Heterodimeric Inhibitors of Prostate Specific Membrane Antigen (PSMA) as Radiolabeled Probes for Targeting Prostate Cancer

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Prostate specific membrane antigen (PSMA) is a validated molecular marker for prostate cancer. A series of glutamate−urea (Glu-urea-X) heterodimeric inhibitors of PSMA were designed and synthesized where X = ε-*N*-(*o*-I, *m*-I, *p*-I, *p*-Br, *o*-Cl, *m*-Cl, *p*-Cl, *p*-F, H)-benzyl-Lys and ε-(*p*-I, *p*-Br, *p*-Cl, *p*-F, H)-phenylureido-Lys. The affinities for PSMA were determined by screening in a competitive binding assay. PSMA binding of the benzyllysine series was significantly affected by the nature of the halogen substituent (IC_{50} values, Cl < I = Br ≪ F = H) and the ring position of the halogen atom (IC_{50} values, *p*-I < *o*-I ≪ *m*-I). The halogen atom had little affect on the binding affinity in the para substituted phenylureido-Lys series. Two lead iodine compounds were radiolabeled with ^{123}I and ^{131}I and demonstrated specific PSMA binding on human prostate cancer cells, warranting evaluation as radioligands for the detection, staging, and monitoring of prostate cancer.

Introduction

At least 250 000 men in the U.S. are diagnosed with prostate cancer annually, and it is estimated that the disease will affect one in six men between the ages of 60 and 80, leading to approximately 30 000 deaths per year. The cancer-related mortality from the disease is second only to lung cancer.[1,2] The global costs associated with prostate cancer are estimated at $15−20 billion with about half of the spending in the U.S. alone.

The management of the prostate cancer patient is challenging, as there are numerous clinical factors and treatment options to consider in deciding on the optimal therapy for a given patient. Since men are living much longer with the disease because of early detection, clinical decision making may have long-term consequences. Accurately defining the extent of disease burden and accurately defining the aggressiveness of the disease at diagnosis are important factors in treatment selection. Hence, the ability to visualize disease is increasingly important for informing therapeutic selection and treatment planning. New imaging agents that will more accurately detect and stage the disease, as well as monitor response to therapy, will enable improved disease management allowing better patient outcome and quality of life. Current imaging techniques offer some opportunity to visualize disease in various parts of the body, but none of them provide both highly specific and sensitive detection of metastatic prostate cancer. A sensitive and specific means of imaging tumor burden throughout the body, in both soft tissue and bone, is the goal of our current effort.

PSMA is an attractive target for the detection of primary and metastatic prostate cancer. PSMA, also known as folate hydrolase I or glutamate carboxypeptidase II, is a transmembrane, 750 amino acid type II glycoprotein that is primarily expressed in normal human prostate epithelium and is up-regulated in prostate cancer, including metastatic disease.[3] PSMA is a unique exopeptidase with reactivity toward poly γ-glutamated folates capable of sequential removal of the poly γ-glutamyl termini.[4,5] It has been reported that overexpression of PSMA in primary prostate cancer correlates with other adverse traditional prognostic factors and independently predicts disease outcome.[6] Since PSMA is expressed by virtually all prostate cancers and its expression is further increased in poorly differentiated, metastatic, and hormone-refractory carcinomas,[7−9] it is a very attractive target for developing radiopharmaceuticals for the diagnosis, staging, and treatment of the disease.

Currently, an indium-111 (^{111}In) radiolabeled anti-PSMA monoclonal antibody (mAb) 7E11-C5.3 (Capromab Pendetide, Cytogen Corporation) is used to detect soft tissue metastasis and recurrence of prostate cancer. This antibody targets the intracellular domain of PSMA and is thought to bind mostly to necrotic cells of prostate tumors.[10] More recently, Bander et al. have developed and radiolabeled monoclonal antibodies that bind to the extracellular domain of PSMA which have entered into various clinical trials including targeted radiotherapy of metastatic prostate cancer.[11]

While monoclonal antibodies offer potential for tumor targeting, long circulating half-life and poor tumor penetrability limit their effectiveness as diagnostic and therapeutic radiopharmaceuticals.[12] For this reason, there have been only limited clinical successes to date, mostly in the treatment of blood-borne cancers such as non-Hodgkin's lymphoma.[13] Small molecules offer significant advantages over antibodies for targeting solid tumors.[14] They can be designed with affinities similar to those of monoclonal antibodies. Small molecules exhibit enhanced diffusibility to the extravascular space and faster blood clearance than antibodies, thus resulting in lower background signal. In addition, the opportunity to synthesize analogues exhibiting diverse chemical properties allows alteration of binding affinity as well as pharmacokinetics. Thus, radiolabeled small molecule radiotracers that bind PSMA may offer the preferred approach.[15−19]

PSMA is highly homologous to N-acetylated α-linked acidic dipeptidase (NAALADase), a neuropeptidase that produces the

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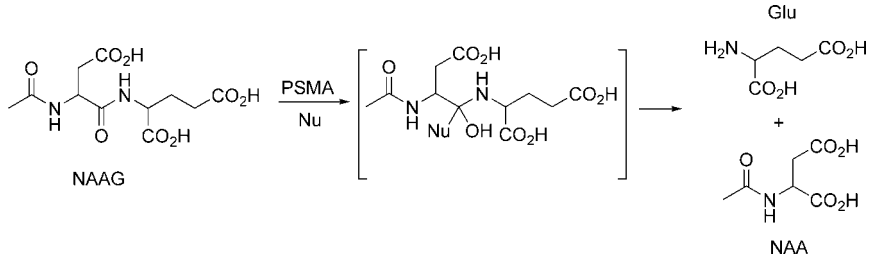


Figure 1. Hydrolytic cleavage of NAAG by NAALADase.

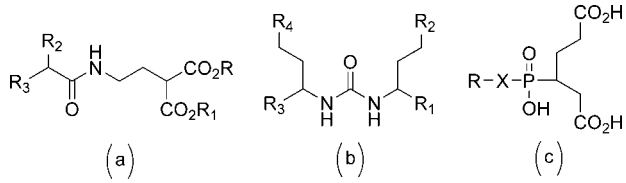


Figure 2. Substrate (a, b) and transition state (c) analogues of NAALADase based on the structure of NAAG.

neurotransmitter glutamate and *N*-acetylaspartate (NAA) through the hydrolysis of *N*-acetylaspartylglutamate (NAAG) (Figure 1).[20]

Analysis of the recently reported crystal structure of PSMA has aided in the understanding of the critical interactions of potent inhibitors within the active site of the enzyme and has led to the design and synthesis of several classes of NAALADase inhibitors that are substrate or transition state analogues,[21] including the amide (Figure 2a), urea (Figure 2b) substrate analogues, phosphinate (Figure 2c, where X = CH_2), and phosphonate (Figure 2c, where X= O) transition state analogues.

Our attention focused on analogues of the urea-linked dipeptide NAALADase inhibitors described by Kozikowski and co-workers (Figure 2b).[22,23] The original structures were developed in which two amino acids were joined through the α-amino groups by a urea linkage, with the rationale that the urea group would serve as a suitable replacement for the central phosphinate/phosphonate backbone present in the early lead structures described by Kozikowski and Barinka.[23−26] On the basis of the inhibitory potency of the different isomers of the glutamate−C(O)−glutamate core and subsequent structure−activity relationship (SAR) studies with other amino acids, information was obtained regarding structural modifications to the pharmacophore and the effect on binding to the enzyme active site.[27] Recently, two radiolabeled urea-based analogues have been reported, *N*-[*N*-[(*S*)-1,3-dicarboxypropyl]carbamoyl]-(*S*)-[^{11}C]methyl-L-cysteine ([^{11}C]DCMC) and *N*-[*N*-[(*S*)-1,3-dicarboxypropyl]carbamoyl]-(*S*)-3-iodo-L-tyrosine ([^{125}I]DCIT).[28,29] These compounds exhibit specific uptake in human prostate cancer xenografts.

Here, we describe the synthesis and in vitro evaluation of novel Glu-urea-X heterodimers as PSMA inhibitors, where X is a derivatized lysine (Lys). These molecules are prepared through the modification of the ε-amino group of the Lys residue of a protected Glu-urea-Lys heterodimer by conversion to halogen substituted benzylamines, benzamides and phenylureas. These in vitro studies explored the electronic, steric and regiochemistry properties of the halogen atoms on the phenyl ring. In addition, these experiments probed the functionality of the linker bridge from the lysine nitrogen to the aromatic ring. Consequently, a preliminary understanding of the structural requirements for PSMA binding of these novel halogenated Glu-urea-X heterodimers was developed. The study resulted in the identification of several potent inhibitors of PSMA, including four iodine-containing molecules that may be applicable for radioimaging or radiotherapy of prostate cancer. Two of the lead iodine containing compounds have been prepared as the radioiodinated (^{123}I and ^{131}I) analogues in high radiochemical yields, high radiochemical purity, and high specific activity and have demonstrated prolonged radiochemical stability at elevated temperature.

Results and Discussion

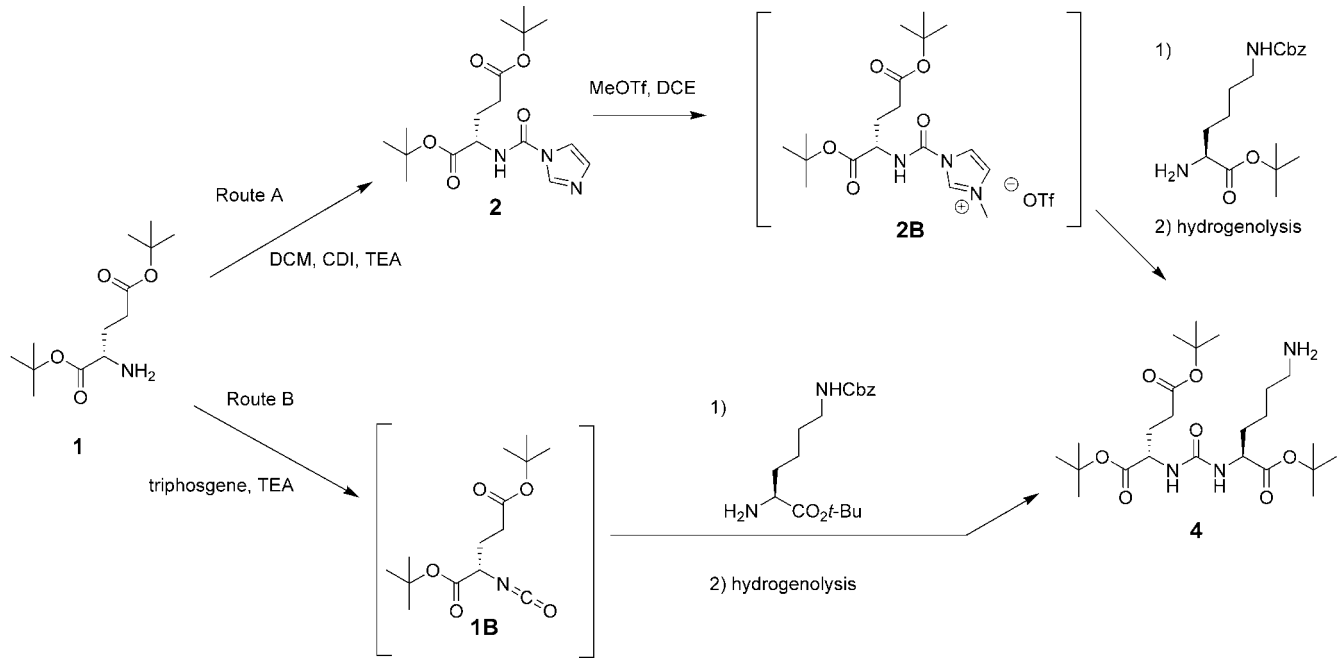
Chemistry. Synthesis of Glu-Urea-Lys (4). The key intermediate (**4**), utilized to prepare the PSMA inhibitors described, was prepared by either route A or route B as depicted in Scheme 1. In route A, **1**, the di-*tert*-butyl ester of glutamic acid, was reacted with carbonyldiimidazole (CDI[a]) under anhydrous conditions in the presence of triethylamine (TEA) to form the intermediate acylimidazole derivative **2**. This intermediate was activated with methyl triflate (MeOTf) to afford the activated methylimidazolium salt, **2B**, which reacted readily with (*S*)-*tert*-butyl 2-amino-6-(benzyloxycarbonylamino)hexanoate (Cbz-Lys-O*t*-Bu) to accomplish formation of the heterodimer. The removal of the carboxybenzyloxy (Cbz) protecting group via hydrogenolysis afforded **4** in moderate yield. Alternatively, **4** was prepared via the isocyanate (**1B**), which was generated in situ from **1** upon treatment with triphosgene. Subsequent reaction with Cbz-Lys-O*t*-Bu followed by removal of the Cbz protecting group by hydrogenolysis afforded **4** in good yield.

Synthesis of Glu-Urea-Lys(*N*-benzyl-X) Analogues (6). Compounds **11**−**19**, of the general structure **6**, were prepared utilizing the route depicted in Scheme 2. The key synthetic intermediate (**4**) was reacted with the appropriate aldehyde to form the intermediate Schiff base, which was not isolated but directly reduced in situ with sodium triacetoxyborohydride to afford the desired tri-*tert*-butyl ester protected benzylamines (**5**). The *tert*-butyl ester protecting groups were removed using TFA. Following deprotection, the samples were purified by high pressure liquid chromatography (HPLC) or recrystallized to afford the desired products (**6**) in overall yields of 20−90%.

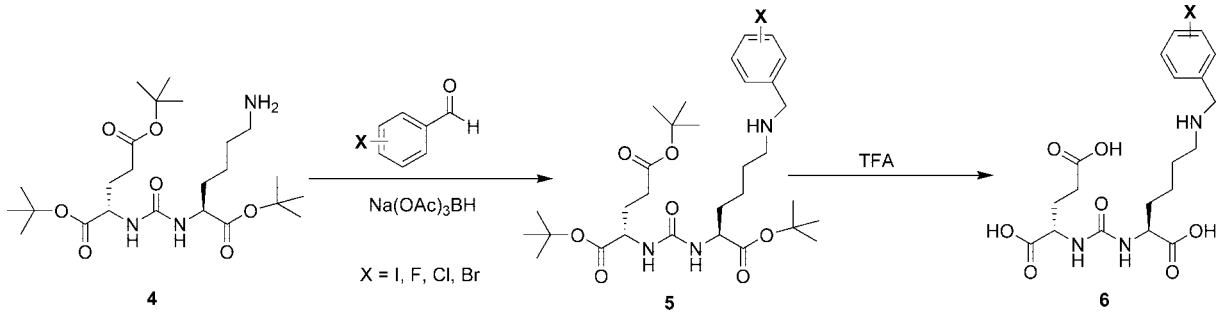
Synthesis of Glu-Urea-Ureido(Phenyl-X) Analogues. Compounds of the general structure **8** were prepared by the route depicted in Scheme 3. The key synthetic intermediate (**4**) was reacted with the appropriate phenyl isocyanate at room temperature to afford the desired protected intermediates (**7**) in good yields. The *tert*-butyl ester protecting groups were removed with TFA. Following deprotection, the sample was purified by column chromatography or recrystallized to afford the desired products (**8**) in overall yields of 40−90%.

[a] Abbreviations: DCM, dichloromethane; EA, ethyl acetate; Hex, hexanes; DCE, dichloroethane; DMF, dimethylformamide; TFA, trifluoroacetic acid; THF, tetrahydrofuran; CDI, carbonyldiimidazole; DMAP, dimethylaminopyridine; TEA, triethylamine; DIPEA, diisopropylethylamine; MeOTf, methyl trifluoromethanesulfonate; Glu, glutamic acid; Boc, *tert*-butoxycarbonyl; Cbz, benzyloxycarbonyl; SWFI, sterile water for injection; RCY, radiochemical yield; RCP, radiochemical purity; Lys, Lysine.

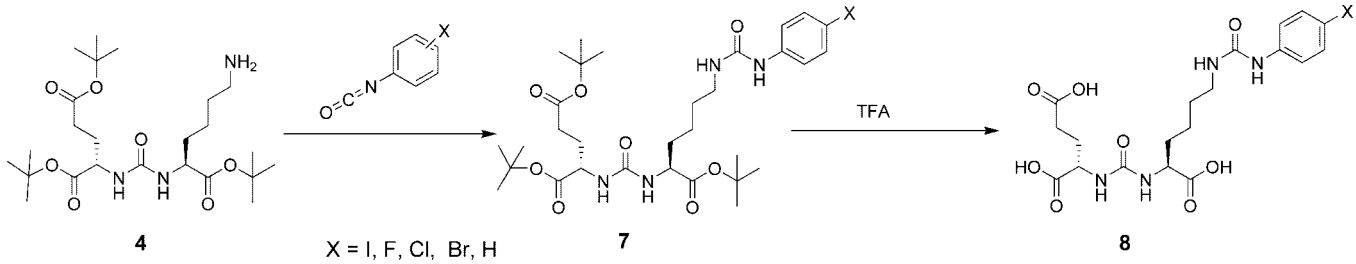
Scheme 1. General Pathway for the Synthesis of the Key Intermediate (**4**) Utilized in the Synthesis of Glu-Urea-Lys Analogues, via the Acyl Imidazole Intermediate (Route A) and the Isocyanate Intermediate (Route B)



Scheme 2. General Pathway for the Synthesis of Halogenated Glu-Urea-Lys (*N*-benzyl-X) Analogues (**6**)



Scheme 3. General Pathway for the Synthesis of Halogenated Glu-Urea-Ureido (Phenyl-X) Analogues

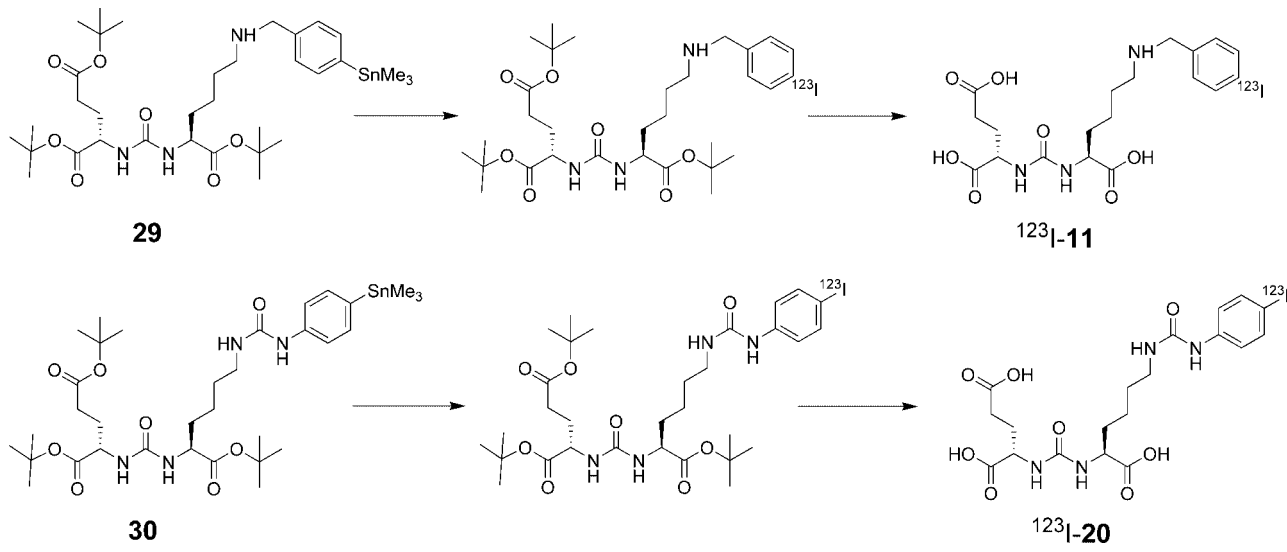


Radiochemistry. ^{131}I-DCIT was used as a radioligand in the competitive binding studies and was prepared following the published literature procedure on the radioiodination of the tyrosine precursor (*S*)-2-(3-((*S*)-1-carboxy-2-(4-hydroxyphenyl)-ethyl)ureido)pentanedioic acid (DCT),[28] utilizing Na^{131}I as the radioiodine source and iodogen (Pierce) as the oxidant.[30] The 8 day half-life of ^{131}I permits the screening of multiple compounds over several days utilizing the same preparation of the radioligand.

The radioiodine labeled compounds (*S*)-2-(3-((*S*)-1-carboxy-5-(4-iodobenzylamino)pentyl)ureido)pentanedioic acid (**11**) and (*S*)-2-(3-((*S*)-1-carboxy-5-(3-(4-iodophenyl)ureido)pentyl)ureido)pentanedioic acid (**20**) were prepared by iododestannylation of the trimethylstannyl precursors (*S*)-di-*tert*-butyl 2-(3-((*S*)-1-*tert*-butoxy-1-oxo-6-(4-(trimethylstannyl)benzylamino)hexan-2-yl)ureido)pentanedioate (**29**) and (*S*)-di-*tert*-butyl 2-(3-((*S*)-1-*tert*-butoxy-1-oxo-6-(3-(4-(trimethylstannyl)phenyl)ureido)hexan-2-yl)ureido)pentanedioate (**30**), respectively, to form both [^{123}I]**11** and [^{123}I]**20** as depicted in Scheme 4. The synthesis of **29** followed the chemistry previously described to prepare compounds **11**−**19** in Scheme 2. Compound **30** was prepared from *tert*-butyl protected **20** by palladium catalyzed deiodostannylation utilizing hexamethylditin as the stannane source. Radioiododestannylation afforded the ^{123}I labeled tri-*tert*-butyl ester which was deprotected with TFA to afford the desired radioiodinated inhibitors.

Binding of Glu-Urea-X Analogues. Two series of Glu-urea-X heterodimers were prepared. The first was benzylamines

Scheme 4. Iododestannylation To Form [^{123}I]**11** and [^{123}I]**20**



where X = ε-*N*-(*o*-I, *m*-I, *p*-I, *p*-Br, *o*-Cl, *m*-Cl, *p*-Cl, *p*-F, H), series structure **A**. The second was phenylureido analogues where X = ε-(*p*-I, *p*-Br, *p*-Cl, *p*-F, H), series structure **B**. All of the compounds, as shown in Figure 3, comprised a Glu linked through a urea to the α-amine of a modified Lys derivative. The Glu residue contributes two of the three carboxylic acids essential for binding to the PSMA active site.[22] The modified Lys residue contributes the third free carboxylic acid functionality and the ε-amine which was used as the synthetic handle that led to the rapid synthesis of the two series of compounds to establish the initial SAR.

The compounds were evaluated in a competitive binding assay using ^{131}I-DCIT as the radioligand for binding to PSMA on LNCaP cells. This method of screening compounds was selected rather than the conventional NAALADase enzymatic assay used previously[21] for several reasons: the throughput is greater, the

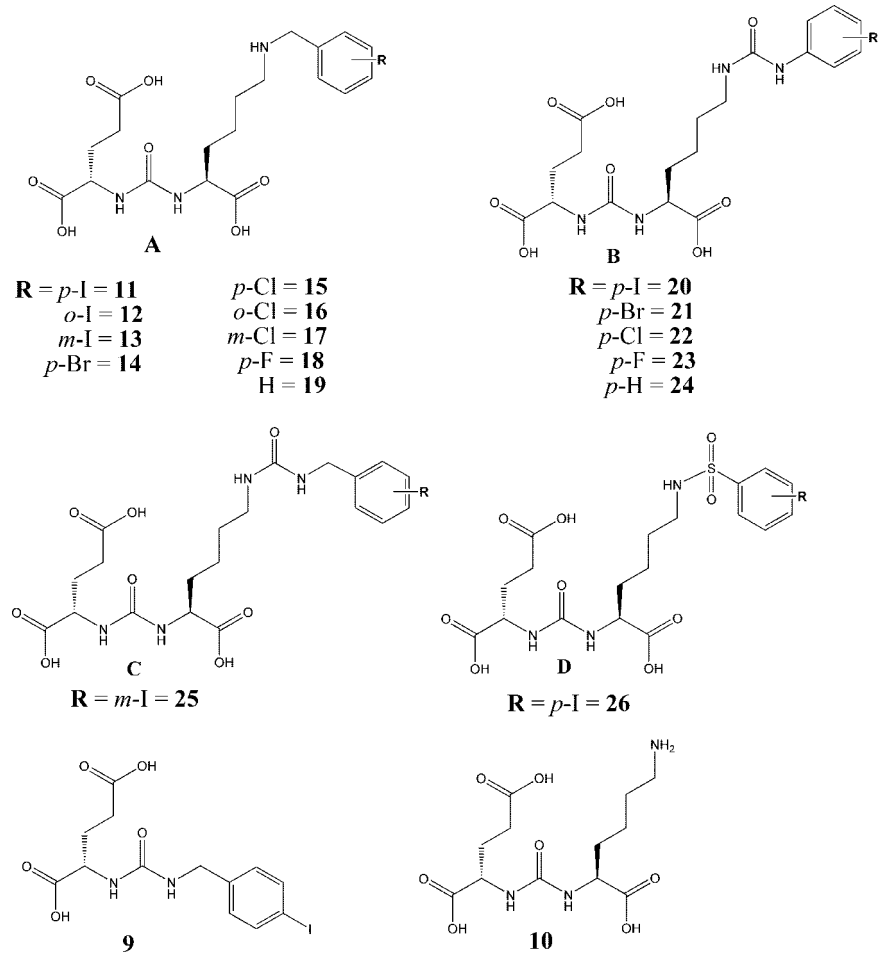


Figure 3. Structures of the Glu-urea-X heterodimers.

Table 1. Summary of the in Vitro Cell Binding Data of the Glu-Urea-X Derivatives

compound	structure series	R	IC_{50} (nM)
9		NA	>3000
10		NA	498
11	A	*p*-I	22
12	A	*o*-I	37
13	A	*m*-I	443
14	A	*p*-Br	43
15	A	*p*-Cl	2
16	A	*o*-Cl	245
17	A	*m*-Cl	277
18	A	*p*-F	1200
19	A	H	2960
20	B	*p*-I	10
21	B	*p*-Br	2
22	B	*p*-Cl	4
23	B	*p*-F	3
24	B	H	12
25	C	*m*-I	18
26	D	*p*-I	10
27	A	2-naphthyl	154

assay assesses binding to native protein on intact prostate cancer cells as opposed to the activity of solubilized protein in a cell lysate, and lead compounds can be subsequently radiolabeled and retested in the same assay prior to proceeding to tumor uptake studies in rodents. In general, this method of in vitro screening may better reflect the in vivo setting.

One of the first Glu-urea-X heterodimers prepared and analyzed was (*S*)-2-(3-(4-iodobenzyl)ureido)pentanedioic acid (**9**). The poor affinity (IC_{50} > 3000 nM) shown in Table 1 confirms earlier reports of the necessity of the third carboxyl group for high affinity binding to the PSMA active site.

In the case of the benzylamine Glu-urea-X heterodimers series, compounds **11**−**19**, binding was dependent upon the halogen substituent and their position on the aryl ring (Table 1). For the para-substituted analogues, the IC_{50} values followed the trend Cl < I = Br ≪ F = H. This relationship appears to favor the larger less electron negative halogen atoms, with the exception of (*S*)-2-(3-((*S*)-1-carboxy-5-(4-chlorobenzylamino)-pentyl)ureido)pentanedioic acid (**15**) where an intermediate size coupled with the electron poor nature of the chlorine atom is the preferred substituent in the para-position of this series. The binding affinity was also strongly influenced by the position of the halogen atom on the aryl ring. For the iodine substituted benzylamine series, the para- and ortho-substituted analogues **11** and **12** exhibited IC_{50} values of 22 and 37 nM, respectively, whereas the meta-substituted analogue **13** was much less active (IC_{50} value of 443 nM). For the chlorine substituted compounds, the para analogue (**15**) was the most potent in the series (IC_{50} value of 2 nM), while the activities of both the ortho (**16**) and the meta (**17**) analogues were significantly decreased (IC_{50} of 245 and 277 nM, respectively).

In the case of the para-substituted phenylureido series **B**, compounds **20**−**24**, the results demonstrated that the type of halogen atom had little effect on binding. Whether the lack of a clear relationship between the halogen atom and the affinity for PSMA is due to the hydrogen bonding ability of the urea functionality that has been introduced into the analogues or simply the increase in tether length due to the additional atom that exists between the ε-lysine amine and the aromatic ring is still to be determined.

The relationship between the halogen substituent and affinity in the substituted phenylureido series **B** was further investigated with the addition of a methylene spacer (series **C**). In this case, the additional methylene group further extends the phenyl ring away from the ε-lysine amine and appears to be well tolerated, as evidenced by the *m*-iodo analogue (9*S*,13*S*)-1-(3-iodophenyl)-3,11-dioxo-2,4,10,12-tetraazapentadecane-9,13,15-tricarboxylic acid (**25**) (IC_{50} = 18 nM).

The replacement of the urea moiety with a sulfonamide as shown by (S)-2-(3-((S)-1-carboxy-5-(4-iodophenylsulfonamido)pentyl)ureido)pentanedioic acid (**26**) was also tested. The *para* iodo analogue **26** was prepared and represents a slightly less lipophilic analogue of **20** (calculated log *P* = 0.63 and 0.81 for **26** and **20**, respectively). This demonstrated that the replacement of the urea functionality with a sulfonamide moiety is an allowed substitution that did not alter the potency of the compound (IC_{50} value of 10 nM).

To test the tolerance of bulk off of the ε-lysine amine in the benzylamine Glu-urea-X series, the 2-naphthyl analogue (*S*)-2-(3-((*S*)-1-carboxy-5-(naphthalen-1-ylmethylamino)pentyl)ureido)pentanedioic acid (**27**) was prepared. The addition of the fused unsubstituted napthalene ring afforded an increase in affinity when compared to the simple phenyl substituted analogue (*S*)-2-(3-((*S*)-5-(benzylamino)-1-carboxypentyl)ureido)pentanedioic acid (**19**), resulting in a 20-fold increase in binding affinity (IC_{50} = 154 nM). The 2-naphthyl substituent apparently contributes detrimental bulk as well as potential interference with the presumed hydrogen bond formed with the ε-nitrogen of the lysine.

Four compounds that contain an aryl iodide moiety, **11**, **20**, **25**, and **26**, hold significant potential as radiopharmaceuticals for imaging (^{123}I-labeled) and therapy (^{131}I-labeled) of PSMA positive prostate cancer. Iodine-123, with its relatively short half-life (13.2 h) and 159 keV γ photons, is an ideal radionuclide for imaging by single-photon emission computed tomography (SPECT).[31,32] In contrast, iodine-131, with an 8 day half-life and both γ and β emissions, offers the potential for therapy.[33,34] Two of the lead iodine-containing compounds, **11** and **20**, were selected based on favorable in vitro activity for further study and were radiolabeled with ^{123}I and ^{131}I using the trimethylstannane precursors **29** and **30**, respectively.

The synthesis of the radioiodine labeled compounds used standard literature procedures starting with the trimethylstannyl precursors.[35,36] The lead compounds were radioiodinated in ≥60% radiochemical yield (RCY) with >95% radiochemical purity (RCP) following purification by reverse phase high pressure liquid chromatography (RP-HPLC) with a specific activity of >4000 mCi/μmol. The identities of the radioiodinated products were confirmed by RP-HPLC through correlation of the retention time of the radioiodinated products with that of the corresponding nonradioiodinated material following co-injection, as shown for **20** in Figure 4.

The specificity for binding of the most potent radioiodinated compound **20** to PSMA on LNCaP cells was tested in a direct binding assay. The compound was incubated with LNCaP cells or the PSMA deficient prostate cancer cell line, PC3, in the presence or absence of homologous nonradiolabeled **20** or 2-(phosphonomethyl)pentanedioic acid (PMPA), a structurally unrelated NAALADase inhibitor. Approximately 400 fmol per million cells of **20** bound to LNCaP cells, but little binding was observed with PC3 cells. Binding was blocked by either unlabeled **20** or PMPA (Figure 5), demonstrating the specificity of the interaction.

The ^{123}I radiolabeled compounds (**11** and **20**) demonstrated prolonged stability at elevated temperature, an important characteristic for potential radiopharmaceuticals. Following radiolabeling and purification, the product was stored at 40 °C for 48 h, after which HPLC analysis was performed to assess

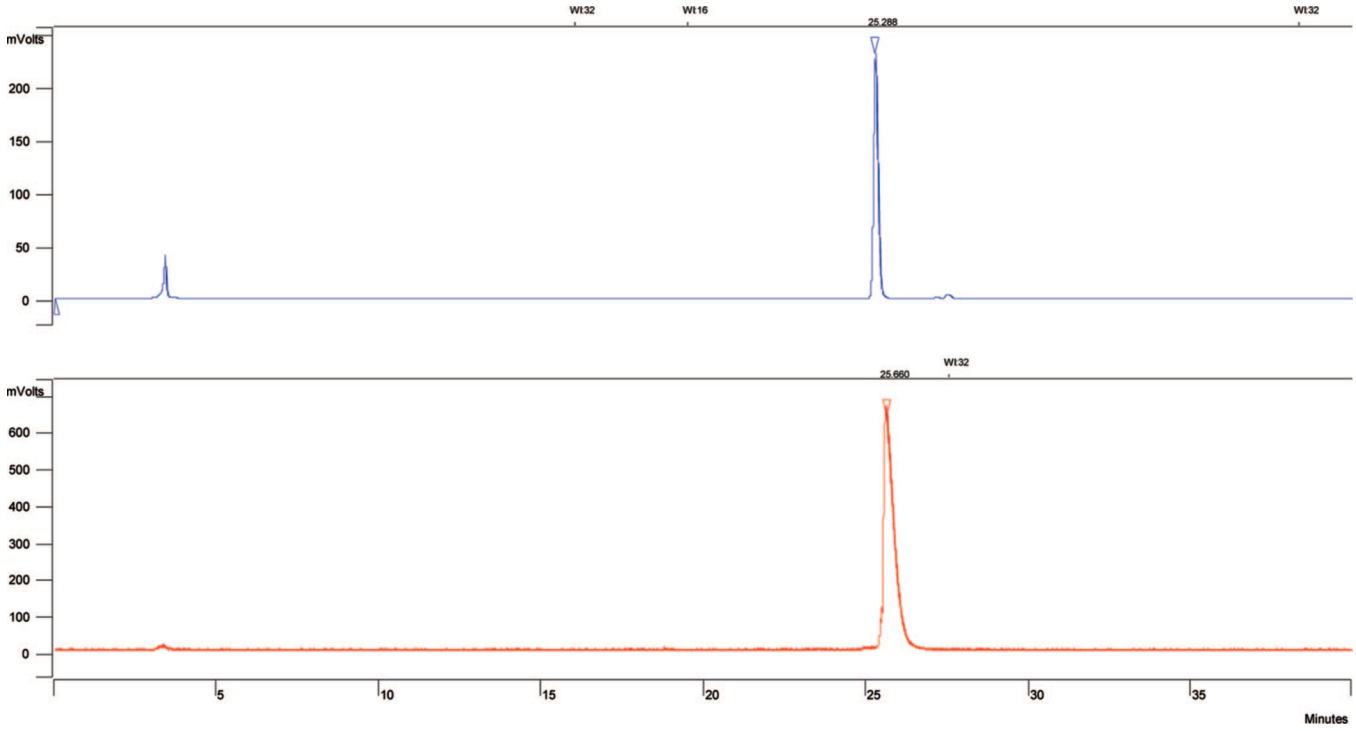


Figure 4. UV−visible chromatogram (upper trace) and radiochromatogram (lower trace) of **20** after RP-HPLC purification demonstrating >95% RCP of the eluted product, upon co-injection.

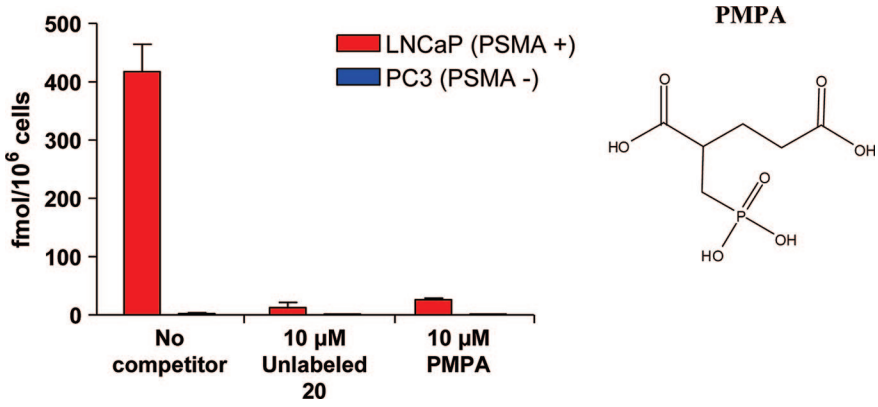


Figure 5. Direct binding analysis of radioiodinated **20**.

the retention of the radiolabel to the molecules, as well as any product degradation. The results of the stability study demonstrated no significant degradation of the products at room or elevated temperature over time, as shown in Table 2.

Conclusions

In summary, a series of novel Glu-urea-X heterodimers for targeting PSMA were successfully synthesized using the Glu-urea-Lys building block (*S*,*S*)-2-[3-(5-amino-1-carboxypentyl)ureido]pentanedioic acid (**10**). The molecules were evaluated in vitro, and a preliminary understanding of the structural requirements for high affinity PSMA binding was established by systematically substituting halogen atoms on the aryl ring, thereby demonstrating the impact of both the nature of the halogen atom and its position on the aryl ring. Altering the halogen substituent produced changes in the molecule's overall hydrophobicity, steric bulk, and electronic properties that potentially influenced the interactions with the binding pocket of PSMA. The study resulted in the identification of several potent inhibitors of PSMA. Two of the lead iodinated compounds, (*S*)-2-(3-((*S*)-1-carboxy-5-(4-iodobenzylamino)pentyl)ureido)pentanedioic acid (**11**) and (*S*)-2-(3-((*S*)-1-carboxy-5-(3-(4-iodophenyl)ureido)pentyl)ureido)pentanedioic acid (**20**), were radiolabeled with both ^{123}I and ^{131}I using the trimethylstannane precursors in high radiochemical yield. The radioiodinated molecules demonstrated high affinity and selectivity for PSMA on LNCaP cells. These data have led to the advancement of these compounds as clinical development candidates that may eventually permit the more accurate detection of metastatic disease, improve staging, and enable the monitoring of response to systemic therapy.

Experimental Methods

General Methods. All reactions were carried out in dry glassware under an atmosphere of argon unless otherwise noted. Reactions were purified by column chromatography under medium pressure using a Biotage SP4 or by preparative high pressure liquid chromatography using a Varian Prostar 210 preparative HPLC system equipped with a semipreparative Vydac C18 reverse-phase column (250 mm × 10 mm × 5 μm) connected to a Varian Prostar model 320 UV−visible detector and monitored at a wavelength of

Table 2. Stability of ^{123}I Radiolabeled **11** and **20**

	11		20	
time (h)	retention (%)	retention at 40 °C (%)	retention (%)	retention at 40 °C (%)
0	94.5	94.2	94.4	94.4
24	94.0	93.6	93.9	93.6
48	94.1	93.2	93.4	92.7

254 nm. The final product purifications were achieved using a binary solvent gradient of 5−50% B over 21 min (A = water + 0.1% TFA, B = acetonitrile + 0.1% TFA). Analytical HPLC of the radioiodinated compounds was performed using the same method with an analytical Vydac C18 reverse-phase column (250 mm × 4.6 mm × 5 μm). ^{1}H NMR spectra were obtained on a Bruker 400 MHz instrument. Spectra are reported as ppm δ and are referenced to the solvent resonances in $CDCl_3$, DMSO-d_6, or methanol-d_4. Elemental analysis was performed by Prevalere Life Sciences, Inc. High-resolution mass spectra were determined by M-Scan Inc. using positive ion electrospray with a Q-Tof API US hybrid quadrupole/time-of-flight mass spectrometer. All solvents were purchased from Sigma-Aldrich. Reagents were purchased from Sigma Aldrich, Bachem, Akaal, or Anaspec.

(*S*)-2-[(Imidazole-1-carbonyl)amino]pentanedioic Acid Di-*tert*-butyl Ester (2). To a suspension of L-di-*tert*-butyl glutamate hydrochloride (15.0 g, 51 mmol) in DCM (150 mL) cooled to 0 °C was added TEA (18 mL) and DMAP (250 mg). After the mixture was stirred for 5 min, CDI (9.0 g, 56 mmol) was added and the mixture was stirred overnight with warming to room temperature. The mixture was diluted with DCM (150 mL) and washed with saturated sodium bicarbonate (60 mL), water (2 × 100 mL), and brine (100 mL). The organic layer was dried over sodium sulfate and concentrated to afford the crude product as a semisolid, which slowly solidified upon standing. The crude material was triturated with hexane/ethyl acetate to afford a white solid which was filtered, washed with hexane (100 mL), and dried to afford the desired product (15.9 g, 45 mmol, 88%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.63 (s, 1H), 7.00 (br, 2H), 6.31 (d, J = 8.00 Hz, 1H), 4.02 (m, 1H), 2.19 (m, 2H), 1.86 (m, 1H), 1.67 (m, 1H), 1.39 (s, 9H), 1.38 (s, 9H). ESMS *m/z*: 354 $(M + H)^+$.

(*S*)-2-[3((*S*)-(5-Benzyloxycarbonylamino)-1-*tert*-butoxycarbonylpentylureido]pentanedioic Acid Di-*tert*-butyl Ester (3). Route A. To a solution of **2** (1 g, 2.82 mmol) in DCE (10 mL) at 0 °C was added MeOTf (0.47 g, 2.85 mmol) and TEA (0.57 g, 5.65 mmol). After the solution was stirred for 30 min, Cbz-Lys-O*t*-Bu (1.06 g, 2.82 mmol) was added in one portion and allowed to stir for 1 h at 40 °C. The mixture was concentrated to dryness and purified by column chromatography (SiO_2) to afford the desired product as a white solid (1.37 g, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (m, 5H), 5.33−5.28 (m, 3H), 5.08 (d, J = 7.4 Hz, 2H), 4.38−4.29 (m, 2H), 3.15 (m, 2H), 2.32−2.01 (m, 2H), 1.90−1.50 (m, 8H), 1.43−1.40 (m, 27H, *t*-Bu's). ESMS *m/z*: 622 $(M + H)^+$.

Route B. In a round-bottom flask triphosgene (2.9 g, 10 mmol) was suspended in DCM (50 mL) and stirred at 0 °C. A solution of Cbz-Lys-O*t*-Bu (9.1 g, 27 mmol) and DIPEA (10.4 mL, 60 mmol) in DCM (50 mL) was added dropwise to the triphosgene solution over 2.5 h. A solution of L-glutamic acid di-*tert*-butyl ester hydrochloride (8.0 g, 27 mmol) containing DIPEA (10.4 mL, 60 mmol) and DCM (50 mL) was then added in one portion and allowed to stir for 45 min. The mixture was concentrated to dryness, diluted with 150 mL of ethyl acetate, washed with 2 N $NaHSO_4$ (2 × 200 mL), brine (150 mL), and dried over sodium sulfate to yield a yellow oil. Purification by column chromatography (SiO_2) afforded the desired product as clear oil which upon standing solidifies to a white solid (12.0 g, 19.4 mmol, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (m, 5H), 5.33−5.28 (m, 3H), 5.08 (d, J = 7.4 Hz, 2H), 4.38−4.29 (m, 2H), 3.15 (m, 2H), 2.32−2.01 (m, 2H), 1.90−1.50 (m, 8H), 1.43−1.40 (m, 27H, *t*-Bu's). ESMS *m/z*: 622 $(M + H)^+$.

2-[3-(5-Amino-1-*tert*-butoxycarbonylpentyl)ureido]pentanedioic Acid Di-*tert*-butyl Ester (4). To a solution of (*S*)-2-[3-(5-benzyloxycarbonylamino-1-*tert*-butoxycarbonylpentyl)ureido]-pentanedioic acid di-*tert*-butyl ester **(3)** (630 mg, 1.0 mmol) in ethanol (20 mL) was added ammonium formate (630 mg, 10 eqv) followed by 10% Pd−C, and the suspension was allowed to stand with occasional agitation overnight until complete. The mixture was filtered through Celite and concentrated to afford the desired product (479 mg, 0.98 mmol, 98%) as a waxy solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15−6.0 (bm, 4H, NHs), 4.29 (m, 2H), 3.02 (m, 2H), 2.33 (m, 2H), 2.06−1.47 (m, 8H), 1.45−1.40 (m, 27H, *t*-Bu's). ESMS *m/z*: 488 $(M + H)^+$. Anal. ($C_{24}H_{45}N_3O_7 \cdot 0.35$TFA) C, H, N.

(*S*)-2-(3-(4-Iodobenzyl)ureido)pentanedioic Acid (9). To a suspension of (*S*)-2-[(imidazole-1-carbonyl)amino]pentanedioic acid di-*tert*-butyl ester **(2)** (0.13 g, 0.37 mmol) in DCE (10 mL) cooled to 0 °C was added methyl triflate (MeOTf) (0.061 g, 0.37 mmol) and TEA (0.11 g, 1.1 mmol). After the mixture was stirred for 30 min, 4-iodobenzylamine (110 mg, 0.40 mmol) was added and the mixture was stirred for 3 h at 50 °C. The mixture was evaporated to dryness and purified via the Biotage column using a gradient method with 5−50% B where solvent A was methylene chloride and solvent B was methanol. The purified compound (56 mg, 0.11 mmol) was deprotected using 1:1 TFA/water mixture with the mixture stirring overnight. Following aqueous extraction, the compound was purified by HPLC using a Vydac C18 column employing a gradient method of 5−50% acetonitrile containing 0.1% TFA and water containing 0.1% TFA resulting in the desired product (67 mg, 0.002 mmol, 15%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.6 (s, 2H), 7.1 (br, 2H), 6.5 (m, 1H), 6.3 (d, 1H), 4.15 (m, 2H), 4.06 (m, 1H), 2.22 (m, 2H), 1.9 (m, 1H), 1.68 (m, 1H). Anal. ($C_{13}H_{15}IN_2O_5 \cdot 0.2$TFA) C, H, N.

(*S*)-2-(3-((*S*)-5-Amino-1-carboxypentyl)ureido)pentanedioic Acid (10). The compound (*S*)-2-[3-(5-amino-1-*tert*-butoxycarbonylpentyl)ureido]pentanedioic acid di-*tert*-butyl ester (**4**) (68 mg, 0.140 mmol) was deprotected using the previously described methods to yield the desired product (31 mg, 0.098 mmol, 70%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.4 (bs, 3H), 6.35 (s, 2H), 4.1 (m, 2H), 3.6 (m, 2H), 2.7 (d, 2H), 2.1 (m, 2H), 1.9 (m, 2H), 1.65 (m, 2H), 1.45 (m, 2H), 1.35 (m, 2H). Anal. ($C_{24}H_{45}N_3O_7 \cdot 0.35TFA\cdot$3 *tert*-butyl) C, H, N.

(*S*)-2-(3-((*S*)-1-Carboxy-5-(4-iodobenzylamino)pentyl)ureido)-pentanedioic Acid (11). To a solution of (*S*)-2-[3-(5-amino-1-carboxypentyl)ureido]pentanedioic acid di-*tert*-butyl ester **(4)** (90 mg, 0.185 mmol) dissolved in DCE (2 mL) was added 4-iodobenzaldehyde (36 mg, 0.155 mmol). The mixture was heated at 50 °C for 1 h whereupon sodium triacetoxyborohydride (36 mg, 0.185 mmol) was added. The mixture was heated for 12 h and was subsequently evaporated to dryness and purified utilizing a Biotage SP4 with a gradient method of 5−50% methanol in DCM. The purified compound (24 mg, 0.034 mmol) was deprotected by treatment with TFA/DCM 1:1 (1 mL), and the mixture was stirred at room temperature for 18 h. Following aqueous extraction the compound was purified by HPLC using a Vydac C18 column employing a gradient method of 5−50% acetonitrile containing 0.1% TFA and water containing 0.1% TFA resulting in the desired product (12 mg, 66%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.4 (bs, 3H), 8.8 (s, 2H), 7.8 (d, 2H), 7.27 (d, 2H), 6.35 (s, 2H), 4.1 (m, 4H), 2.89 (s, 2H), 2.2 (d, 2H), 1.90 (m, 1H), 1.65 (m, 4H), 1.35 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.2, 173.0, 172.5, 156.2, 136.6, 131.3, 131.0, 95.1, 51.9, 51.6, 49.3, 46.4, 31.7, 30.0, 27.7, 25.2, 22.4. ESMS *m/z*: 536 $(M + H)^+$. Anal. ($C_{19}H_{26}IN_3O_7 \cdot 1.0$TFA) C, H, N.

(*S*)-2-(3-((*S*)-1-Carboxy-5-(2-iodobenzylamino)pentyl)ureido)-pentanedioic Acid (12). Following the same procedure as utilized in the preparation of **11**, the target compound was prepared from **4** reacted with 2-iodobenzaldehyde to yield the desired product (108 mg, 35%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.4 (s, 3H), 8.8 (s, H), 7.94 (m, 1H), 7.5 (m, 1H), 7.16 (t, H), 6.38 (m, 2H), 4.15 (m, 5H), 3.06 (s, 2H), 2.85 (s, 1H), 2.2 (m, 2H), 1.90 (m, 1H), 1.70 (m, 2H), 1.50 (s, 2H), 1.35 (m, 2H). ESMS *m/z*: 536 $(M + H)^+$. Anal. ($C_{19}H_{26}IN_3O_7 \cdot 0.8$TFA) C, H, N.

(*S*)-2-(3-((*S*)-1-Carboxy-5-(3-iodobenzylamino)pentyl)ureido)-pentanedioic Acid (13). Following the same procedure in the preparation of **11**, the target compound was prepared from **4** reacted with 3-iodobenzaldehyde to yield the desired product (4 mg, 53%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.4 (s, 3H), 8.7 (s, 2H), 7.9 (s, 1H), 7.8 (d, 1H), 7.44 (d, 1H), 7.22 (t, 1H), 6.25 (s, 2H), 4.09 (m, 5H), 2.89 (s, 1H), 2.75 (s, 1H), 2.2 (d, 2H), 1.90 (m, 2H), 1.65 (m, 2H), 1.40 (m, 2H). Anal. ($C_{19}H_{26}IN_3O_7 \cdot 1.0$TFA) C, H, N.

(*S*)-2-(3-((*S*)-1-Carboxy-5-(4-bromobenzylamino)pentyl)ureido)pentanedioic Acid (14). Following the same procedure as utilized in the preparation of **11**, the target compound was prepared from **4** reacted with 4-bromobenzaldehyde to yield the desired product (100 mg, 45%) as an off-white solid. Di-*tert*-butyl ester: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, 2H), 7.32 (d,2H), 6.28 (m, 2H), 3.98 (m, 2H), 2.55 (t, 2H), 2.48 (t, 2H), 2.22 (m, 2H), 1.85 (m, 1H), 1.62 (m, 2H), 1.45 (m, 2H), 1.37 (s, 27H), 1.28 (m, 2H). ESMS *m*/*z*: 642(M + H)$^+$. The compound was deprotected using the previously described methods. ESMS *m*/*z*: 474 (M + H)$^+$. Anal. ($C_{19}H_{26}BrN_3O_7 \cdot 1.3$TFA) C, H, N.

(*S*)-2-(3-((*S*)-1-Carboxy-5-(4-chlorobenzylamino)pentyl)ureido)pentanedioic Acid (15). Following the same procedure as utilized in the preparation of **11**, the target compound was prepared from **4** reacted with 4-chlorobenzaldehyde to yield the desired product (10 mg, 66%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.3 (bs, 3H), 7.5 (m, 4H), 6.30 (s, 2H), 4.1 (m, 4H), 2.89 (s, 2H), 2.23 (d, 2H), 1.90 (m, 1H), 1.65 (m, 4H), 1.32 (m, 2H). ESMS *m*/*z*: 444 (M + H)$^+$. Anal. ($C_{19}H_{26}ClN_3O_7 \cdot 0.8$TFA) C, H, N.

(*S*)-2-(3-((*S*)-1-Carboxy-5-(2-chlorobenzylamino)pentyl)ureido)pentanedioic Acid (16). Following the same procedure as utilized in the preparation of **11**, the target compound was prepared from **4** reacted with 2-chlorobenzaldehyde to yield the desired product (100 mg, 45%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.0 (br, 3H), 7.63 (d, 1H), 7.2 (m, 2H), 7.15 (d, 1H), 6.30 (d, 2H), 4.1 (m, 4H), 2.9 (br, 2H), 2.2 (m, 2H), 1.90 (m, 2H), 1.60 (m, 4H), 1.35 (m, 2H). ESMS *m*/*z*: 444 (M + H)$^+$. Anal. ($C_{19}H_{26}ClN_3O_7 \cdot 0.8$TFA) C, H, N.

(*S*)-2-(3-((*S*)-1-Carboxy-5-(3-chlorobenzylamino)pentyl)ureido)pentanedioic Acid (17). Following the same procedure as utilized in the preparation of **11**, the target compound was prepared from **4** reacted with 3-chlorobenzaldehyde to yield the desired product (200 mg, 90%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) 8.9 (br, 3H), 7.6 (s, H), 7.43 (m, 3H), 6.39 (br, 2H), 4.1 (m, 4H), 2.9 (br, 2H), 2.2 (m, 2H), 1.90 (m, 2H), 1.60 (m, 4H), 1.35 (m, 2H). ESMS *m*/*z*: 444 (M + H)$^+$. Anal. ($C_{19}H_{26}ClN_3O_7 \cdot 0.8$TFA) C, H, N.

(*S*)-2-(3-((*S*)-1-Carboxy-5-(4-fluorobenzylamino)pentyl)ureido)pentanedioic Acid (18). Following the same procedure as utilized in the preparation of **11**, the target compound was prepared from **4** reacted with 4-fluorobenzaldehyde to yield the desired product (100 mg, 45%) as an off-white solid. The compound was deprotected using the previously described methods. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.4 (s, 2H), 8.7 (s, 1H), 7.5 (m, 2H), 7.3 (m, 2H), 6.35 (m, 2H), 5.2 (bs, H), 4.1 (m, 3H), 3.4 (s, 4H), 2.9 (s, 1H), 2.2 (d, 2H), 1.90 (m, 1H), 1.60 (m, 4H), 1.35 (m, 2H). ESMS *m*/*z*: 428 (M + H)$^+$, 450 (M + Na). Anal. ($C_{19}H_{26}FN_3O_7 \cdot 3$TFA) C. Calcd H, 3.80; found 4.51. Calcd N, 5.46; found 6.12.

(*S*)-2-(3-((*S*)-5-(Benzylamino)-1-carboxypentyl)ureido)pentanedioic Acid (19). Following the same procedure as utilized in the preparation of **11**, the target compound was prepared from **4** reacted with benzaldehyde to yield the desired product (5 mg, 47%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.4 (bs, 2H), 7.5 (m, 5H), 6.3 (m, 2H), 4.1 (m, 2H), 2.9 (s, 1H), 2.2 (d, H), 1.90 (m, 1H), 1.60 (m, 4H), 1.35 (m, 2H). ESMS *m*/*z*: 410 (M + H)$^+$. Anal. ($C_{19}H_{27}N_3O_7 \cdot 0.8$TFA) C, H. Calcd N, 10.58; found 7.89.

(*S*)-2-(3-((*S*)-1-Carboxy-5-(3-(4-iodophenyl)ureido)pentyl)ureido)pentanedioic Acid (20). In a round-bottom flask 4-iodophenyl isocyanate (100 mg, 0.41 mmol) is dissolved in DCM (10 mL) containing TEA (0.057 mL, 0.4 mmol). (*S*)-2-[3-(5-Amino-1-carboxypentyl)ureido]pentanedioic acid di-*tert*-butyl ester (**4**)(200 mg, 0.41 mmol) was added and stirred for 3 h. The reaction mixture was evaporated to dryness and the crude mixture dissolved in methanol (5 mL) and added dropwise to rapidly stirring water (20 mL) to afford a white precipitate which was collected and washed with water (20 mL) and dried to afford the desired tri-*tert*-butyl ester as a white solid. The purified compound was treated with TFA/DCM 1:1 (1 mL) and stirred at room temperature for 18 h. Following aqueous extraction the compound was purified by HPLC using a Vydac C18 column employing a gradient method of 5−50% acetonitrile containing 0.1% TFA and water containing 0.1% TFA, resulting in the desired product (158 mg, 53%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.51 (s, 1H), 7.5 (d, 2H), 7.22 (d, 2H), 6.3 (t, 2H), 6.16 (t, 1H), 4.05 (m, 2H), 3.05 (m, 2H), 2.24 (m, 2H), 1.9 (m, 1H), 1.68 (m, 2H), 1.52 (m, 1H), 1.38 (m, 2H), 1.28 (m, 2H), (3 CO_2H not seen). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.3, 173.0, 172.5, 156.2, 153.9, 139.6, 136.2, 119.2, 83.0, 52.2, 51.5, 39.8, 31.9, 30.0, 29.6, 27.6, 22.8. ESMS *m*/*z*: 565 (M + H)$^+$. Anal. ($C_{19}H_{25}IN_4O_8 \cdot 0.7$TFA) C, H, N.

(*S*)-2-(3-((*S*)-1-Carboxy-5-(3-(4-bromophenyl)ureido)pentyl)ureido)pentanedioic Acid (21). Following the same procedure as utilized in the preparation of **20**, the target compound was prepared from **4** reacted with 4-bromophenyl isocyanate to afford the desired product. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.5 (s, 3H), 8.55 (s, 1H), 7.35 (d, 4H), 6.30 (t, 2H), 6.18 (t, 1H), 4.08 (m, 2H), 3.05 (m, 2H), 2.22 (m, 2H), 1.90 (m, 1H), 1.68 (m, 2H), 1.52 (m, 1H), 1.40 (m, 2H), 1.30 (m, 2H). ESMS *m*/*z*: 518 (M + H)$^+$. Anal. ($C_{19}H_{25}BrN_4O_8 \cdot 0.25H_2O$) C, H, N.

(*S*)-2-(3-((*S*)-1-Carboxy-5-(3-(4-chlorophenyl)ureido)pentyl)ureido)pentanedioic Acid (22). Following the same procedure as utilized in the preparation of **20**, the target compound was prepared from **4** reacted with 4-chlorophenyl isocyanate to afford the desired product (147 mg, 51%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.5 (s, 3H), 8.35 (s, 1H), 7.40 (dd, 2H), 7.19 (dd, 2H), 6.30 (t, 2H), 6.10 (t, 1H), 4.08 (m, 2H), 3.05 (m, 2H), 2.32 (m, 2H), 1.90 (m, 1H), 1.68 (m, 2H), 1.52 (m, 1H), 1.40 (m, 2H), 1.30 (m, 2H). ESMS *m*/*z*: 474 (M + H)$^+$. Anal. ($C_{19}H_{25}ClN_4O_8 \cdot 0.25$TFA) C, H, N.

(*S*)-2-(3-((*S*)-1-Carboxy-5-(3-(4-fluorophenyl)ureido)pentyl)ureido)pentanedioic Acid (23). Following the same procedure as utilized in the preparation of **20**, the target compound was prepared from **4** reacted with 4-fluorophenyl isocyanate to afford the desired product. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.5 (s, 3H), 8.40 (s, 1H), 7.40 (dd, 2H), 7.05 (dd, 2H), 6.30 (t, 2H), 6.10 (t, 1H), 4.1 (m, 2H), 3.05 (m, 2H), 2.20 (m, 2H), 1.90 (m, 1H), 1.70 (m, 2H), 1.52 (m, 1H), 1.40 (m, 2H), 1.30 (m, 2H). HRMS (ES$^+$) calcd for $C_{19}H_{25}FN_4O_8$ (M + H)$^+$ 457.1656, found 457.1735. Anal. ($C_{19}H_{25}FN_4O_8$) C, H, N.

(*S*)-2-(3-((*S*)-1-Carboxy-5-(3-phenylureido)pentyl)ureido)pentanedioic Acid (24). Following the same procedure as utilized in the preparation of **20**, the target compound was prepared from **4** reacted with phenyl isocyanate to afford the desired product (124 mg, 52%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.5 (s, 3H), 8.54 (s, 1H), 7.40 (dd, 2H), 7.26 (dd, 2H), 6.30 (t, 2H), 6.17 (t, 1H), 4.05 (m, 2H), 3.05 (m, 2H), 2.44 (m, 2H), 1.90 (m, 1H), 1.68 (m, 2H) 1.52 (m, 1H), 1.40 (m, 2H), 1.29 (m, 2H). ESMS *m*/*z*: 439 (M + H)$^+$. Anal. ($C_{19}H_{26}N_4O_8 \cdot 0.2$TFA) C, H, N.

(9*S*,13*S*)-1-(3-iodophenyl)-3,11-dioxo-2,4,10,12-tetraazapentadecane-9,13,15-tricarboxylic Acid (25). In a round-bottom flask, triphosgene (55 mg, 0.19 mmol) was dissolved in DCM (10 mL) and cooled to 0 °C. To the solution was added **4** (300 mg, 0.62 mmol) in DCM (50 mL) followed by TEA (0.14 mL, 1.4 mmol). The mixture was stirred for 3 h whereupon the mixture was evaporated to dryness and the crude mixture dissolved in methanol (5 mL) and purified via column chromatography (SiO_2) using an increasing amount of methanol in methylene chloride. The purified compound was treated with TFA/DCM 1:1 (1 mL) and stirred at room temperature for 18 h. Following aqueous extraction the compound was purified by HPLC using a Vydac C18 column employing a gradient method of 5−50% acetonitrile containing 0.1% TFA and water containing 0.1% TFA, resulting in the desired product (14 mg, 32%) as a yellow oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.4 (bs, 3H), 7.6 (m, 2H), 7.22 (m, H), 7.12 (m, 1H), 6.31

(m, 3H), 6.01 (s, 1H), 4.15 (s, 2H), 4.06 (m, 2H), 2.95 (s, 2H), 2.22 (m, 2H), 1.93 (m, 1H), 1.75 (m, 2H), 1.52 (m, 1H), 1.42 (m, 2H), 1.31 (m, 2H). ESMS *m*/*z*: 579 $(M + H)^+$. Anal. ($C_{20}H_{27}IN_4O_8 \cdot 0.8TFA$) C, H, N.

(*S*)-2-(3-((*S*)-1-Carboxy-5-(4-iodophenylsulfonamido)pentyl)-ureido)pentanedioic Acid (26). In a round-bottom flask 2-[3-(5-amino-1-carboxypentyl)ureido]pentanedioic acid di-*tert*-butyl ester (**4**) (300 mg, 0.62 mmol) was suspended in water (10 mL) and 1,4 dioxane (10 mL), and TEA (1.75 mL, 1.25 mmol) was added followed by 4-iodobenzenesulfonyl chloride. The mixture stirred overnight at 50 °C. The reaction mixture was evaporated to dryness, taken up in DCM, and purified via column chromatography (SiO_2) using an increasing amount of methanol in methylene chloride to afford the desired product (375 mg, 80%) as a clear oil. The purified compound was deprotected using 1:1 TFA/methylene chloride mixture stirring for 18 h. Following aqueous extraction, the compound was purified by HPLC using a Vydac C18 column employing a gradient method of 5−50% acetonitrile containing 0.1% TFA and water containing 0.1% TFA to afford the desired product (270 mg, 90%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.97 (d, 2H), 7.68 (t, 1H), 7.53(d, 2H), 6.35 (dd, 2H), 4.10 (m, 1H), 4.00 (m, 1H), 2.65 (m, 2H), 2.22 (m, 2H), 1.91 (m, 1H), 1.71 (m, 1H), 1.55 (m, 1H), 1.45 (m, 1H), 1.35 (m, 2H), 1.25 (m, 2H), (3 CO_2H not seen). HRMS (ES^+) calcd for $C_{18}H_{24}N_3O_9S$ $(M + H)^+$ 586.03, found 586.0356.

(*S*)-2-(3-((*S*)-1-Carboxy-5-(naphthalen-1-ylmethylamino)pentyl)-ureido)pentanedioic Acid (27). Following the same procedure as utilized in preparation of **11**, the target compound was prepared from **4** reacted with 2-naphthaldehyde to yield the desired product (51 mg, 70%) as a light tan solid. ^{1}H NMR (400 MHz, DMSO-d_6) 8.9 (br, 3H), 7.95 (m, 5H), 7.62 (m, 2H), 6.35 (br, 2H), 4.11 (m, 4H), 2.91 (br, 2H), 2.55 (m, 2H), 2.25 (m, 2H), 1.70 (m, 4H), 1.35 (m, 2H). ESMS *m*/*z*: 460 $(M + H)^+$. Anal. ($C_{23}H_{29}N_3O_7 \cdot 1.0TFA$) C, H, N.

4-Trimethylstannanylbenzaldehyde (28). To a solution of 4-iodobenzaldehyde (1.92 g, 8.27 mmol) in dry dioxane (60 mL) was added hexamethylditin (4.1 mL, 19.8 mmol) followed by $Pd(Ph_3P)Cl_2$ (150 mg), and the reaction mixture was heated for 3 h under reflux until judged complete. The mixture was filtered through Celite and purified by column chromatography (SiO_2) using hexanes/ethyl acetate (9/1) as eluent to afford the product (2.24 g, 98%) as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.97 (s, 1H), 7.81 (d, J = 7.8 Hz, 2H), 7.72 (d, J = 7.8 Hz, 2H), 0.29 (s, 9H). ESMS *m*/*z*: 268 (Sn cluster).

(*S*)-Di-*tert*-butyl 2-(3-((*S*)-1-*tert*-Butoxy-1-oxo-6-(4-(trimethylstannyl)benzylamino)hexan-2-yl)ureido)pentanedioate (29). Following the same procedure as utilized in preparation of **11**, the target compound was prepared from **4** reacted with **28** to afford the desired product (88 mg, 38%) as a thick syrup which solidifies on standing. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.48 (d, J = 7.4 Hz, 2H), 7.30 (d, J = 7.4 Hz, 2H), 6.27 (m, 2H, NH's), 3.96 (m, 4H), 2.74 (bm, 2H), 2.21 (m, 2H), 1.87 (m, 2H), 1.65−1.19 (m, 7H), 1.35 (m, 27H, *t*-Bu's), 0.23 (s, 9H). ESMS *m*/*z*: 742 (Sn cluster). Anal. ($C_{34}H_{59}N_3O_7Sn$) C, H, N.

(*S*)-Di-*tert*-butyl 2-(3-((*S*)-1-*tert*-Butoxy-1-oxo-6-(3-(4-(trimethylstannyl)phenyl)ureido)hexan-2-yl)ureido)pentanedioate (30). To a solution of *tert*-butyl protected **20** (227 mg, 0.31mmol) dissolved in anhydrous dioxane (20 mL) was added hexamethylditin (256 mg, 0.78 mmol) at room temperature. Dichlorobis(triphenylphosphine)palladium(II) (23 mg, 0.031mmol) was added in one portion to the mixture, and the mixture was heated to 80 °C and stirred for 1.5 h. The reaction mixture was cooled to room temperature and the dioxane removed in vacuo. The residue was dissolved in DCM (25 mL) and filtered through a pad of Celite. The volume of the filtrate was reduced and the crude product purified by column chromatography (SiO_2) utilizing ethyl acetate/hexane (9:1) as the eluent to afford the desired product (94 mg, 41%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.48 (d, J = 7.4 Hz, 2H), 7.30 (d, J = 7.4 Hz, 2H), 6.27 (m, 2H, NHs), 3.96 (m, 2H), 3.69 (bs, 2H), 2.25 (m, 2H), 1.92−1.75 (m, 4H), 1.71−1.15 (m, 7H), 1.35 (m, 27H, t-Bu's), 0.23 (s, 9H). ESMS *m*/*z*: 771 (Sn cluster). Anal. ($C_{34}H_{58}N_4O_8Sn$) C, H, N.

General Radiochemistry. All reactions were carried out in a laboratory designed and licensed for use of radioactivity. All iodine isotopes were purchased from MDS Nordion as NaI in mild sodium hydroxide solutions or in dry form concentrated from aqueous sodium hydroxide. All solvents (JT Baker) were purchased from Fisher Scientific.

The radioiodinated compounds were purified on a Varian Prostar 210 HPLC system using an analytical Vydac C18 column (250 mm × 4.6 mm × 5 μm) connected to a Varian Prostar model 320 UV−visible detector monitoring at 254 nm. The final products were purified using a binary solvent gradient of 5−50% buffer B in buffer A over 21 min (A = water containing 0.1% TFA, B = acetonitrile containing 0.1% TFA). Analytical HPLC of the compounds was performed using the same method.

Radiolabeling Procedure for the Synthesis ^{123}I-DCIT. The DCIT, utilized as a reference standard, was radiolabeled following standard techniques[30] employing the tyrosine precursor (*S*)-2-(3-((*S*)-1-carboxy-2-(4-hydroxyphenyl)ethyl)ureido)pentanedioic acid (DCT) and either $Na^{131}I$ or $Na^{123}I$. The reactions utilized Iodogen tubes as the source of the oxidant for the radioiodination.

Preparation of the iodine-131 labeled compound (^{131}I-DCIT) was achieved by adding 100 μL of $Na^{131}I$ in 0.1 N NaOH to a phosphate buffered solution (pH 7.2) containing DCT (1 mg/mL) in an Iodogen tube (Pierce). The mixture was vortexed for 3 min and allowed to stand at room temperature for 20 min. Upon completion of the reaction, the solution was purified by HPLC using a gradient method of water, 0.1% TFA, and methanol, RCY > 80%, RCP > 95%).

Radiolabeling Procedure for the Synthesis ^{123}I-(*S*)-2-(3-((*S*)-1-carboxy-5-(4-iodobenzylamino)pentyl)ureido)pentanedioic Acid (11). Into a 5 mL vial containing [^{123}I]NaI (300 mCi) was added 100 μL of sterile water for injection (SWFI), followed by 305 μL of an acid solution [acetic acid (300 μL) and sulfuric acid (5 μL)], followed by 300 μL of oxidant [acetic acid (0.2 mL) and 30% hydrogen peroxide (0.335 mL) brought to a final volume of 5 mL with SWFI], to which was added 150 μL of (*S*)-di-*tert*-butyl 2-(3-((*S*)-1-*tert*-butoxy-1-oxo-6-(4-(trimethylstannyl)benzylamino)hexan-2-yl)ureido)pentanedioate (**29**) (1 mg/mL solution in ethanol). The mixture was vortexed for 2 min and allowed to incubate at room temperature for an additional 30 min. The reaction was quenched with 200 μL of 0.1 M sodium thiosulfate. The product was then diluted in 18 mL of SWFI and loaded onto a C18 Sep Pak Plus column. The column was washed with 60 mL of SWFI to remove unreacted radioiodine and inorganic and organic salts. The ^{123}I-(*S*)-2-(3-((*S*)-1-carboxy-5-(4-iodobenzylamino)pentyl)ureido)pentanedioic acid (**11**) ester was eluted from the column using 6 mL of ethanol. The resulting solution, containing the ester of ^{123}I-**11**, was evaporated to dryness under a stream of nitrogen and the residue dissolved in DCM (0.5 mL). TFA (2 mL) was added to remove the ester protecting groups. The solution was then incubated at room temperature for 40 min. After the deprotection was complete, the solution was evaporated to dryness under a stream of nitrogen and the residue was dissolved in the formulation matrix of 2% gentisate and 5% ascorbate, pH 5. The radiochemical yields ranged from 50% to 70%, RCP > 90%, specific activity of ≥4000 mCi/μmol.

Radiolabeling Procedure for the Synthesis ^{123}I-(*S*)-2-(3-((*S*)-1-Carboxy-5-(3-(4-iodophenyl)ureido)pentyl)ureido)pentanedioic Acid (20). In a 5 mL vial containing [^{123}I]NaI (300 mCi) was added sterile water for injection (SWFI) (50 μL), 50% sulfuric acid in SWFI (50 μL), oxidant (100 μL) [which was prepared fresh via the incubation of acetic acid (0.2 mL) and 30% hydrogen peroxide (0.335 mL) followed by dilution to a final volume of 5 mL with SWFI], acetonitrile (0.5 mL), and (*S*)-di-*tert*-butyl 2-(3-((*S*)-1-*tert*-butoxy-1-oxo-6-(3-(4-(trimethylstannyl)phenyl)ureido)hexan-2-yl)ureido)pentanedioate (**30**) (100 μL of a 1 mg/mL solution in acetonitrile). The mixture was vortexed for 1 min and allowed to incubate at room temperature for an additional 10 min. The reaction was quenched with 200 μL of 0.1 M sodium thiosulfate. The product was then diluted in 18 mL of SWFI and loaded onto a

C18 Sep Pak Plus column. The column was washed with 60 mL of SWFI to remove unreacted radioiodine and inorganic and organic salts. The ^{123}I-(*S*)-2-(3-((*S*)-1-carboxy-5-(3-(4-iodophenyl)ureido)-pentyl)ureido)pentanedioic acid (**20**) ester was eluted from the column using 4 mL of ethanol. The resulting solution, containing the tri-*tert*-butyl ester of ^{123}I-**20**, was evaporated to dryness under a stream of nitrogen. The residue was dissolved in DCM (0.5 mL), and TFA (2 mL) was added to cleave the *tert*-butyl ester protecting groups. The deprotection solution was incubated at room temperature for 45 min. After the deprotection was complete, the solution was evaporated to dryness under a stream of nitrogen. The product was dissolved in 50% acetonitrile/water and purified on a C18 Sep Pak Plus column using a gradient of SWFI containing 0.1% acetic acid and ethanol, and the product was obtained upon elution with 4 mL of 100% ethanol. The solution was evaporated to dryness under a stream of nitrogen, and the residue was dissolved in a formulation matrix of 2% gentisate and 5% ascorbate/ascorbic acid, pH 5. The radiochemical yields ranged from 50% to 70%, RCP > 90%, specific activity of ≥4000 mCi/μmol.

Preparation of the Ascorbate/Ascorbic Acid/Gentisate Excipient Solution. The solution was prepared by adding 1.79 g of sodium ascorbate, 0.3936 g of ascorbic acid, 1.09 g of gentisic acid sodium salt to 17.0 mL of SWFI. The mixture was vortexed for 3 min to dissolve all solids. The pH of the solution measured as 5.0 ± 0.5 using pH paper (Fisher Scientific).

Binding of Analogues to Prostate Cancer Cells. Human prostate cancer cell lines, LNCaP and PC3, were obtained from the American Type Culture Collection. LNCaP cells were maintained in RPMI-1640 medium (Invitrogen) supplemented with 10% fetal bovine serum in a humidified incubator at 37 °C/5% CO_2. PC3 cells were maintained in Dulbecco's modified Eagles medium (Invitrogen) supplemented with 10% fetal bovine serum in a humidified incubator at 37 °C/5% CO_2. Cells were removed from flasks for passage or for transfer to 12-well assay plates by incubating them with 0.25% trypsin/EDTA (Invitrogen).

For competition binding analysis, LNCaP cells were plated in 12-well plates at approximately 4×10^5 cells/well and incubated for 48 h in a humidified incubator at 37 °C/5% carbon dioxide prior to addition of compound. Each Glu-urea-X derivative was diluted in serum-free cell culture medium containing 0.5% bovine serum albumin (BSA). Diluted compounds were added to the cells in the presence of 3 nM ^{131}I-DCIT for 1 h at room temperature. Cells were removed from the plates by gently pipeting and transferred to 1.5 mL Eppendorff tubes. Samples were microcentrifuged for 30 s at 15K × *g*. The medium was aspirated, and the pellet was washed twice by dispersal in fresh assay medium followed by microcentrifugation. Cell binding of ^{131}I-DCIT was determined by counting the cell pellet in a Wallac 1282 automated γ counter.

For direct binding studies, LNCaP and PC3 cells were incubated in RPMI-1640 medium supplemented with 0.5% bovine serum albumin for 1 h with 3 nM ^{123}I labeled test compound alone or in the presence of 10 μM unlabeled test compound or 10 μM 2-(phosphonomethyl)pentanedioic acid (PMPA), a structurally unrelated NAALADase inhibitor. Cells were washed and counted in a γ counter.

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Note Added in Proof. During the review of this manuscript, a relevant paper entitled "Radiohalogenated Prostate-Specific Membrane Antigen (PSMA)-Based Ureas as Imaging Agents for Prostate Cancer" by Chen et al. has been published, *J. Med. Chem.* **2008**, *51*, 7933−7943.

Supporting Information Available: Elemental analysis results and HRMS data for compounds **9**−**27**. This material is available free of charge via the Internet at http://pubs.acs.org.

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