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Synthesis and Evaluation of Radioiodinated Acyloxymethyl Ketones as Activity-Based Probes for Cathepsin B

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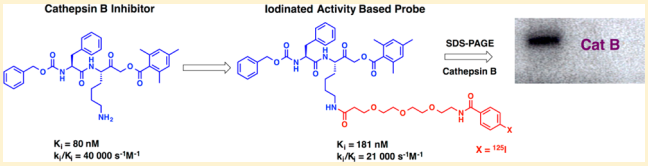
Supporting Information

ABSTRACT: Dipeptidyl (acyloxy)methyl ketones (AOMKs) were functionalized with different iodine-containing prosthetic groups to generate a library of candidate cathepsin B probes. Compound **23a**, (*S*)-20-[(*S*)-2-{[(benzyloxy)carbonyl]-amino}-3-phenylpropanamido]-1-(4-iodophenyl)-1,14,21-trioxo-5,8,11-trioxa-2,15-diazadocosan-22-yl 2,4,6-trimethylbenzoate, was identified as a potential lead through in vitro screening, having a K_i = 181 ± 9 nM and demonstrating the ability to effectively label active cathepsin B in vitro. Its less potent analogue **11a**, (*S*)-3-[(*S*)-2-{[(benzyloxy)carbonyl]amino}-3-phenylpropanamido]-7-[6-(4-iodobenzamido)hexanamido]-2-oxoheptyl 2,4,6-trimethylbenzoate, was also tested as a comparison. Biodistribution studies of the iodine-125-labeled compounds in MDA-MB-231 mouse xenografts exhibited tumor uptake of 0.58% ± 0.06% injected dose per gram (ID/g) for [^{125}I]**11a** and 1.12% ± 0.08% ID/g for [^{125}I]**23a** at 30 min. The tumor-to-blood ratios reached 1.2 for [^{125}I]**23a** and 1.6 for [^{125}I]**11a** after 23 h. The more hydrophilic [^{125}I]**23a** showed an improved clearance profile with a superior tumor-to-muscle ratio of 7.0 compared to 3.4 for [^{125}I]**11a** at 23 h. Iodinated AOMK ligands are suitable in vitro probes for cathepsin B and hold promise as a platform to develop molecular imaging probes.



INTRODUCTION

Cathepsin B is a ubiquitously expressed lysosomal cysteine protease that is involved in normal physiological processes such as protein turnover, wound healing, and cell differentiation and growth.[1] The enzyme is overexpressed in a variety of cancers including breast carcinoma, prostate carcinoma, glioblastoma, and melanoma,[2−5] with higher levels of active cathepsin B residing in tumors having high metastatic potential.[6−8] In normal cells cathepsin B is localized in the lysosomes, yet in tumors it distributes throughout the cytoplasm and cell periphery and is secreted into the extracellular matrix as well.[9−11] These features make cathepsin B an attractive target for developing molecular imaging probes for detecting cancer and assessing metastatic potential.

A number of near-infrared fluorescent (NIRF) probes for cathepsin B have been developed.[12−15] Substrate-based probes such as Cat B 750 FAST and Cat B 680 FAST are commercially available and have been used in a number of cancer models.[13,16−19] Most recently, substrate-based probes incorporating a self-immolative spacer linked to a fluorophore have been developed and used for in vitro and in vivo imaging of cathepsin B.[12,20,21] Irreversible inhibitors have also been employed to develop activity-based probes (ABPs) for cathepsin B imaging.[14,15,22−25]

ABPs for cathepsin B have been prepared from a number of different constructs including dipeptidyl (acyloxy)methyl ketones (AOMKs). The AOMK derivatives are attractive because of their specificity and high affinity for cysteine proteases in vitro and in vivo.[26−29] These inhibitors inactivate the enzyme by reacting with the active-site cysteine, resulting in a thioether linkage between the ABP and cathepsin B.[1,27,30,31] Phe-Lys-AOMKs have been fluorescently labeled at the lysine ε-amine and have shown specific tumor uptake in vivo.[14,15] Radiolabeled variants have also been developed where ^{64}Cu and the macrocyclic ligand 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) was also incorporated at the P1 site.[14,32] This construct exhibited a tumor uptake of 0.35% ± 0.13% injected dose per gram (ID/g) at 30 min postinjection (pi) and the activity remained in the tumor at 24 h pi (0.27% ± 0.05% ID/g). High liver and kidney uptake was also observed, which is likely associated with loss of copper in vivo.[33]

The present study describes the preparation and in vivo evaluation of novel dipeptidyl AOMK cathepsin B inhibitors bearing different iodine-containing prosthetic groups linked to the lysine ε-amine of a Phe-Lys-AOMK derivative. One of the advantages of working with iodine is that a single construct can be used to prepare agents for single photon emission tomography (SPECT), positron emission tomography (PET), or radiotherapy, simply by varying the isotope used (e.g., ^{123}I, ^{124}I, or ^{131}I). This is exemplified by the use of aryl iodides to synthesize diagnostic and therapeutic radiopharmaceuticals for melanoma, glioma, and prostate cancer.[34−38] Radioiodinated AOMKs have been used as ABPs to label diverse families of cysteine proteases in vitro but not cathepsin B, and they employed prosthetic groups that are not stable in vivo.[29] To

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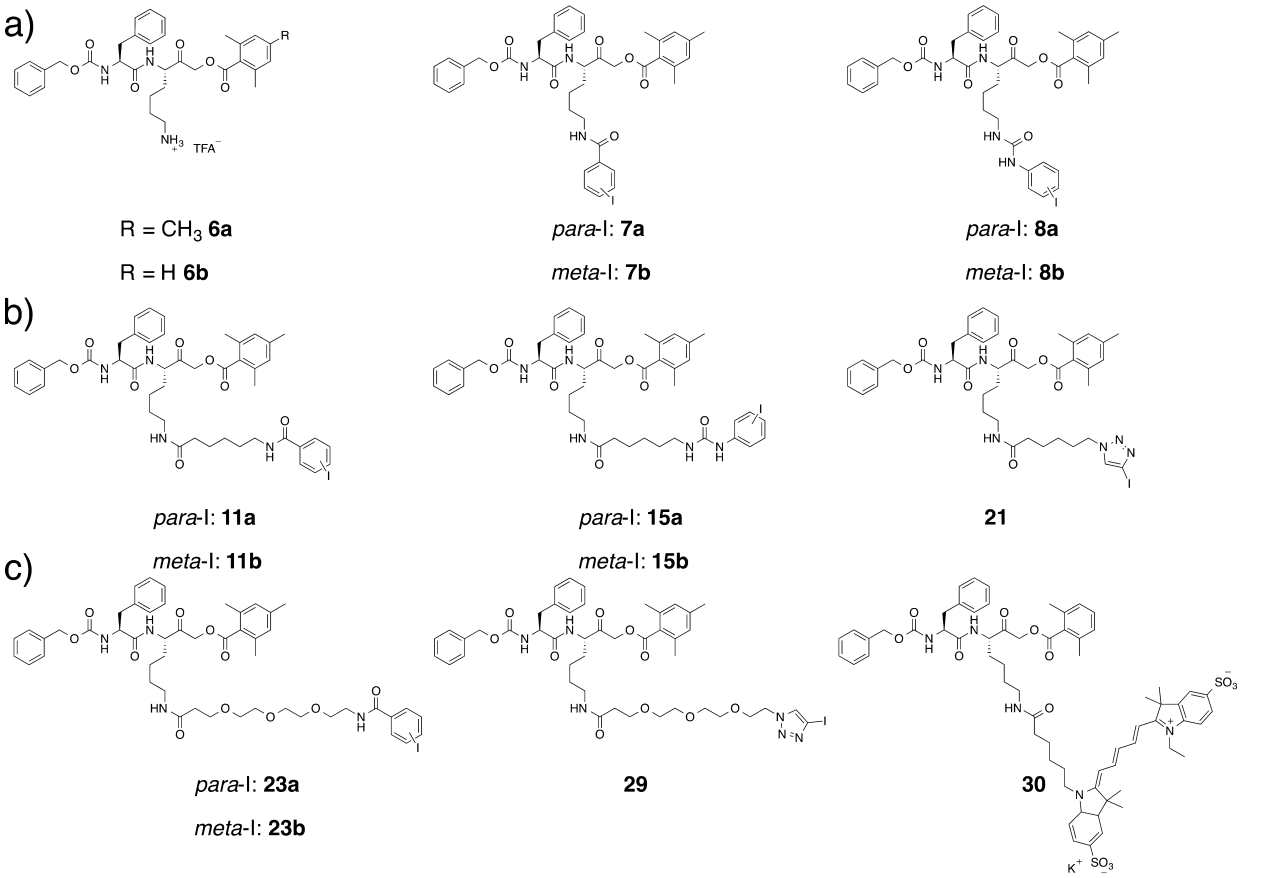


Figure 1. Structures of AOMK cathepsin B inhibitors. (a) Structures of parent AOMKs (**6a**/**b**) and initial iodinated AOMKs: iodobenzamides (**7a**/**b**) and iodophenylureas (**8a**/**b**). (b) Structures of AOMKs with alkyl spacer: iodobenzamides (**11a**/**b**), iodophenylureas (**15a**/**b**), and iodotriazole (**21**). (c) Structures of AOMKs with PEG spacer: iodobenzamides (**23a**/**b**) and iodotriazole (**29**) and the known optical probe GB123 (**30**).

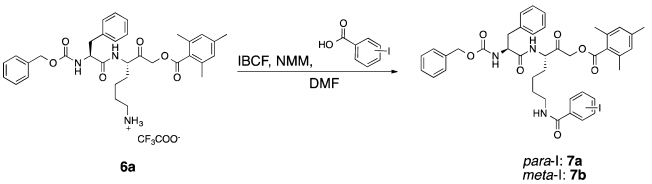
evaluate the potential utility of iodinated AOMKs as molecular imaging probes, a library of candidates derived from robust iodine synthons were prepared and screened as inhibitors of cathepsin B. Two compounds were subsequently radiolabeled and tested in vitro and in vivo.

RESULTS AND DISCUSSION

Synthesis of Parent Inhibitors 6a and 6b. The parent AOMK inhibitors **6a** and **6b** (Figure 1a) were prepared from the protected dipeptide Cbz-Phe-Lys(ε-Boc)-OH (**3**). Following modified published procedures, isobutyl chloroformate (IBCF) (Scheme S1, route A) or benzotriazole (Scheme S1, route B; see Supporting Information) was used to couple the protected phenylalanine and lysine amino acids, giving **3**.[39−41] The dipeptide **3** was activated with IBCF and treated with ethereal diazomethane to generate the diazomethyl ketone in situ. Treatment with HBr gave the bromomethyl ketone **4**, which was then converted to **5a** or **5b**, following the method of Blum et al.[14] The amount of potassium fluoride used in the final step was reduced to help minimize epimerization of the lysine residue, which was evident by both HPLC and ^{1}H NMR in most compounds.[27,4042] The *tert*-butoxycarbonyl (Boc) group was subsequently removed by use of trifluoroacetic acid (TFA), giving **6a** or **6b** as TFA salts.

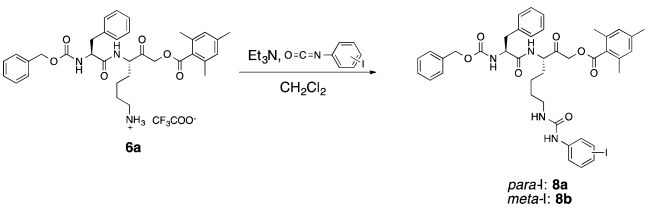
Synthesis of Initial Iodinated AOMKs. The first series of iodinated inhibitors prepared involved linking aryl iodides to the free amine of **6a**, giving iodobenzamides (**7a**/**b**) and iodophenylureas (**8a**/**b**) (Figure 1a). Compound **6a** was coupled to 3- or 4-iodobenzoic acid by use of IBCF in the presence of *N*-methylmorpholine (NMM) (Scheme 1). The

Scheme 1. Synthesis of 7a/b



desired products **7a** and **7b** were isolated following simple extraction in yields of 29% and 45%, respectively. To generate the ureas, **6a** was combined with 3- or 4- iodophenyl isocyanate in the presence of triethylamine, giving **8a** and **8b** in 9% and 14% yields, respectively, following silica gel chromatography (Scheme 2).

Scheme 2. Synthesis of 8a/b



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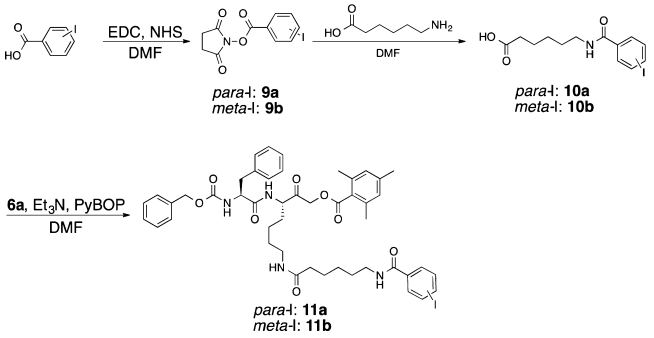
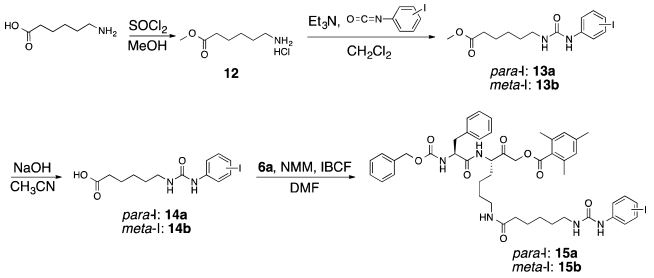
It has been reported that inhibitors containing bulky amino acids at the P1 position exhibit reduced affinity for cathepsin B.[43] To minimize possible steric interactions, a six-carbon spacer was also used to incorporate the aryl iodides (Figure 1b). 6-Aminohexanoic acid was combined with the active esters **9a** and **9b**, giving the aryl iodide spacers **10a** and **10b** in 82% and 87% yields, respectively (Scheme 3). These were linked to

Scheme 3. Synthesis of 11a/b



6a by use of benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) in the presence of triethylamine to generate compounds **11a** and **11b** in 45% and 30% yields, respectively. For the urea derivatives, it was necessary to use the protected methyl 6-aminohexanoate **12** and combine it with 3- or 4-iodophenyl isocyanate (Scheme 4). Following deprotection, **14a** and **14b** were coupled with **6a** by use of IBCF in the presence of NMM to give **15a** and **15b** in 35% and 30% yields, respectively.

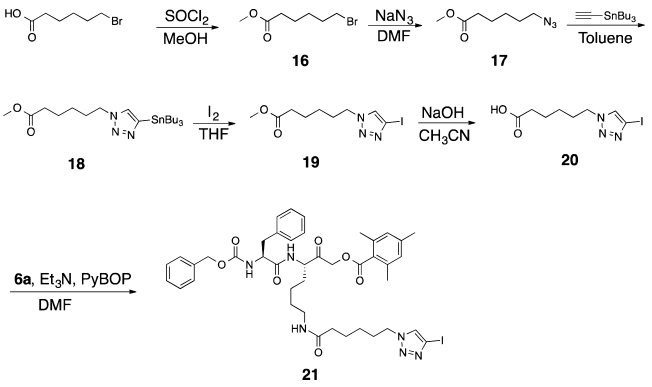
Scheme 4. Synthesis of 15a/b



We have shown that iodinated triazoles are more polar than iodobenzene-based derivatives, have minimal nonspecific binding in vivo, and can clear readily via renal excretion.[44] To prepare a triazole-derived synthon to derivatize **6a**, ethynyl tributyltin was used in a 1,3-dipolar cycloaddition with **17**, giving the triazole **18** (Scheme 5). ^{1}H and ^{13}C NMR spectroscopy confirmed that a single isomer was obtained, which is consistent with the literature.[45] Iododestannylation was achieved by treating **18** with molecular iodine to give **19** in 69% yield.[45] Following hydrolysis, **20** was coupled to **6a** by use of PyBOP in the presence of triethylamine to generate **21** in 33% yield.

Inhibition of Cathepsin B by Iodinated AOMKs. The binding affinity of the iodinated AOMKs was determined by a kinetic assay similar to that reported by Krantz et al.,[27] employing Cbz-Arg-Arg-*p*-nitroanilide (Z-RR-pNA) as the substrate and human liver cathepsin B (Calbiochem). Optimal enzyme concentration range and linearity was determined by monitoring substrate hydrolysis over time in the presence of

Scheme 5. Synthesis of 21



various amounts of enzyme (Figure S1A, Supporting Information). The rate of hydrolysis (ν_0) was linear when the enzyme concentration [E] was between 5 and 15 nM (R^2 > 0.99) (Figure S1B, Supporting Information). The reaction rate (ν_0) was also monitored when varying the substrate concentration in order to determine the Michaelis−Menten constant (K_m) (Figure S2, Supporting Information). A value of 390 ± 20 μM was found, which is lower than the previously reported value of 900 ± 60 μM.[46] The variance is likely due to differences in the amount of dithiothreitol (DTT) used to activate the enzyme and the incubation time for this activation, as well as the enzyme source, which can influence the observed K_m for this substrate.[46,47]

Inhibition constants (K_i) were determined by monitoring the substrate hydrolysis over time in the presence of 0−1 μM inhibitor (Figure S3A, Supporting Information). The observed rate constant (k_{obs}) was determined and plotted against the inhibitor concentration (Figure S3B, Supporting Information). By use of nonlinear regression, K_i values were determined where possible. In cases where full inhibition was not observed, only the second-order rate constant (k_i/K_i) was determined (Table 1).

The known inhibitor **6a** was evaluated as reference point to compare all iodinated derivatives. The measured K_i value was

Table 1. Inhibitory Activities of Iodinated AOMK Derivatives[a]

compd	K_i (nM)	k_i/K_i ($s^{-1}\cdot M^{-1}$)
6a	80 ± 10	40 000 ± 3000
6b	52 ± 6	40 000 ± 6000
7a	2000 ± 1000	810 ± 80
7b	nd[b]	630 ± 10
8a	nd	0.013 ± 0.002
8b	nd	0.0035 ± 0.0002
11a	nd	310 ± 20
11b	nd	280 ± 20
15a	ni[c]	ni
15b	ni	ni
21	1000 ± 50	4430 ± 70
23a	181 ± 9	21 100 ± 300
23b	370 ± 25	12 700 ± 600
29	350 ± 40	9900 ± 200
30	120 ± 20	19 000 ± 2000

[a]Inhibition constants were determined at 37 °C and pH 6.0 with human liver cathepsin B. [b]Full inhibition was not observed; therefore, the value was not determined. [c]No inhibition observed.

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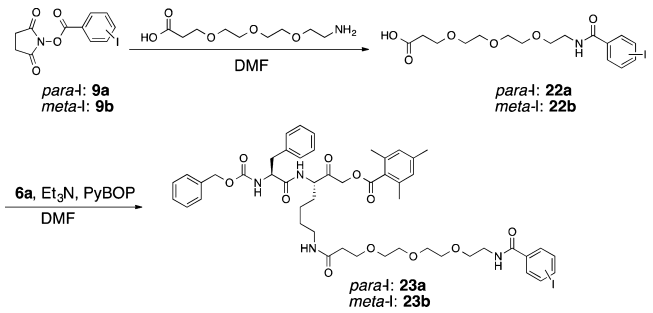
80 ± 10 nM, which differed from the reported value (K_i = 170 ± 50 nM), again likely due to difference in enzyme sources.[27] The simple aryl iodide derivatives (**7a**/**b** and **8a**/**b**) showed poor affinity for the protease, where a K_i value could be determined only for **7a** (K_i = 2 ± 1 μM) and the ureas (**8a**/**b**) exhibited no detectable inhibition. Incorporation of the alkyl spacer did not show an improvement for the iodophenylureas (**15a**/**b**) or the iodobenzamides (**11a**/**b**). These compounds formed heterogeneous solutions when added to the assay buffer, indicating poor solubility, which in turn made it difficult to obtain accurate measures of the binding constants. The iodotriazole derivative **21** had improved solubility and showed improved binding affinity (K_i = 1 ± 0.05 μM) but was over 10 times less potent than the parent compound and was therefore regarded as a poor candidate.

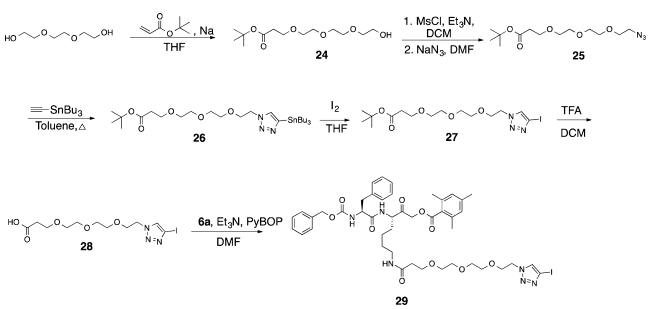
Synthesis of Iodinated AOMKs Containing a Hydrophilic Linker. A more hydrophilic linker was used in an attempt to improve the solubility of the inhibitors and enhance their binding affinities. This was achieved by replacing the six-carbon spacer with a three-unit poly(ethylene glycol) (PEG) linker (Figure 1c). By use of similar methods, **9a** and **9b** were coupled to the tri(ethylene glycol) bifunctional linker to give the iodobenzamide-PEG derivatives **22a** and **22b** (Scheme 6). Compound **6a** was coupled to the new linkers by use of PyBOP in the presence of triethylamine, giving **23a** and **23b** in 22% and 37% yields, respectively.

Scheme 6. Synthesis of 23a/b



To prepare the bifunctional triazole linker, triethylene glycol was combined with *tert*-butyl acrylate, generating the protected carboxylic acid **24** (Scheme 7). Compound **24** was converted to the mesylate and combined with sodium azide to generate **25**. Ethynyl tributyltin was used in a 1,3-dipolar cycloaddition with **25**, producing the triazole **26** as a single isomer. Treatment of **26** with iodine gave the iodinated triazole **27**, which was deprotected to generate **28**. The resultant iodotriazole PEG linker **28** was then coupled to **6a** by use of PyBOP in the presence of triethylamine, giving **29** in 37% yield.

Scheme 7. Synthesis of 29

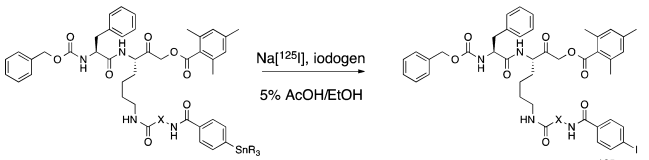


Inhibition of Cathepsin B by Iodinated-PEG-AOMKs. Inhibitors containing the PEG spacer (**23a**, **23b**, and **29**) showed improved binding affinities over their alkyl counterparts (**11a**, **11b**, and **21**) with nanomolar K_i values (Table 1). The *p*-iodobenzamide **23a** exhibited the best affinity with a K_i of 181 ± 9 nM (Table 1). As an additional comparison, a known Cy5-labeled AOMK, GB123 (**30**, Figure 1c), was synthesized and tested. Reports of GB123 have shown good visualization of cathepsin B-positive tumors through optical imaging.[14,48,49] A K_i value of 120 ± 20 nM was determined for **30**, suggesting that the K_i value for **23a** was sufficient to proceed to radiolabeling and biodistribution studies.

Synthesis of Radioiodinated AOMKs. To compare the effect of the hydrophilic spacer in vitro and in vivo, **11a** was selected alongside **23a** for radiolabeling and further testing. We have previously demonstrated that fluorous tin benzamides and phenylureas can be used to radioiodinate small molecules, and the products can be isolated without HPLC via a simple fluorous solid-phase extraction (FSPE).[50,51] To convert the selected cathepsin B inhibitors to their radioactive analogues, the iodobenzamide precursors **10a** and **22a** were converted to the corresponding arylstannanes by use of fluorous distannane and a Pd-catalyzed cross-coupling reaction (Schemes S3 and S4, Supporting Information).[52] Addition of the fluorous tin moiety was performed on the prosthetic group as opposed to the iodinated inhibitors to avoid degradation of the peptide backbone due to the harsh reaction conditions. The fluorous tin compounds **31** and **33** were coupled to **6a** by use of PyBOP in the presence of triethylamine, giving the precursors for radiolabeling **32** and **34** in 14% and 12% yields, respectively.

Radiolabeling was performed by treating **32** and **34** with [^{125}I]NaI in the presence of iodogen (Scheme 8). Following

Scheme 8. Synthesis of [^{125}I]11a and [^{125}I]23a



FSPE purification, isolated radiochemical yields were 32−36% (n = 3) for [^{125}I]**11a** and 26−35% (n = 3) for [^{125}I]**23a**. The modest radiochemical yields can be attributed to nonspecific binding of inhibitors to the reaction vessel and to the FSPE cartridge. The identities of the radiolabeled compounds were confirmed by coinjection with nonradioactive standards and correlation of retention times of peaks in the radiochromatograms and UV−visible chromatograms (Figures 2 and 3). Because of the long reaction times needed to couple the arylstannane spacers to **6a**, precursors **32** and **34** underwent some degree of epimerization. Consequently [^{125}I]**11a** and [^{125}I]**23a** also contained the associated epimers (~7% and 20%, respectively; Figures 2 and 3), similar to what has been observed for other AOMK derivatives.[28]

Activity-Based Labeling of Cathepsin B. The affinity of the radiolabeled inhibitors for cathepsin B was evaluated by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE). Equivalent amounts of cathepsin B were

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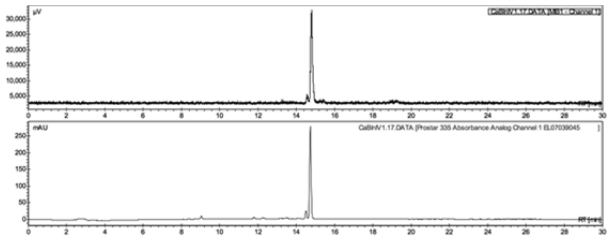

Figure 2. HPLC chromatograms of [^{125}I]**11a** coinjected with the reference standard **11a**. Radiochromatogram (upper trace) and UV−visible (λ = 254 nm) chromatogram (lower trace) are shown.

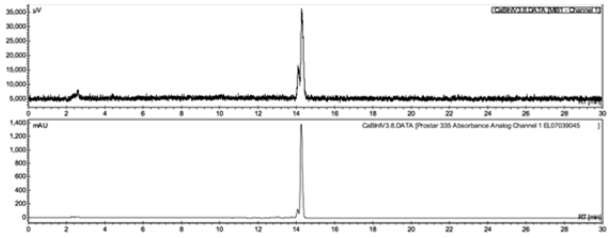

Figure 3. Radiochromatogram (upper trace) and UV−visible (λ = 254 nm) HPLC chromatogram (lower trace) of [^{125}I]**23a** coinjected with the reference standard **23a**.

pretreated with saturating amounts of the known cathepsin B-specific inhibitor L-3-*trans*-(propylcarbamyl)oxirane-2-carbonyl-L-isoleucyl-L-proline (CA-074)[53] or with the vehicle (dimethyl sulfoxide, DMSO). The samples were then treated with [^{125}I]**11a** or [^{125}I]**23** and subjected to autoradiography. Despite different binding affinities, cathepsin B labeling was observed with both [^{125}I]**11a** and [^{125}I]**23** (Figure 4). The activity in

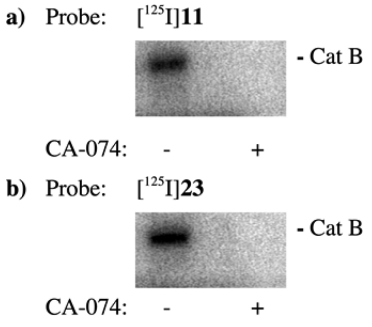

Figure 4. Phosphor images of an SDS−polyacrylamide gel showing the specific labeling of active cathepsin B. Solutions of cathepsin B were pretreated with CA-074 (+) or DMSO (−), followed by (a) [^{125}I]**11a** or (b) [^{125}I]**23a**.

each band was blocked quantitatively by the addition of CA-074, indicating site-specific labeling of cathepsin B. It has be established that Phe-Lys-AOMKs can inactivate other cysteine cathepsins such as cathepsin L and cathepsin S.[14,22,23,32] It is therefore expected that the iodine derivatives described here would show similar inhibition and selectivity.

Biodistributions of Activity-Based Probes in Tumor-Bearing Mice. The labeled probes, [^{125}I]**11a** and [^{125}I]**23a**, were administered intravenously into CD1 nu/nu mice bearing the MDA-MB-231 xenograft. MDA-MB-231 cells are known to express cathepsin B, and the xenografts have been used to evaluate other AOMK-based ABPs.[14,54] After 0.5, 5, and 23 h pi, animals were sacrificed and the percent injected dose per gram (ID/g) in various tissues, organs, and fluids was determined (Table 2).

After 30 min pi, bladder/urine (90% ± 20% ID/g), gall bladder (170% ± 30% ID/g), and liver (16% ± 1% ID/g) showed the highest uptake for [^{125}I]**11a**, which was reduced after 23 h pi (0.9% ± 0.2%, 0.4% ± 0.1%, and 1.6% ± 0.2% ID/g respectively). Areas with the highest uptake at 23 h pi were thyroid/trachea (17% ± 9% ID/g), small intestine (1.6% ± 0.2% ID/g), and liver (1.6% ± 0.2% ID/g). The accumulation in thyroid/trachea is indicative of deiodination, as the sample did not contain any free iodide at the time of administration.[55] The initial tumor uptake was 0.58% ± 0.06% ID/g at 30 min pi and decreased over time. Despite this, the tumor/blood ratios increased from 0.5, reaching a maximum of 1.6 after 23 h pi (Figure 5a). Similarly the tumor/muscle ratios increased from 2.3, reaching a peak value of 4 at 5 h pi.

In the case of the more potent and hydrophilic derivative [^{125}I]**23a**, bladder/urine (400% ± 200% ID/g) and gall bladder (42% ± 7% ID/g) showed the highest uptake after 30 min pi. The liver uptake was lower in comparison to [^{125}I]**11a** (3.4% ± 0.3% vs 16% ± 1% ID/g, 30 min pi), and the radioactivity was cleared from the majority of the tissues after 23 h pi. Again, accumulation in the thyroid/trachea was observed, indicating in vivo deiodination. The observed tumor uptake was higher for [^{125}I]**23a** (1.12% ± 0.08% ID/g) than that of [^{125}I]**11a** at 30 min, and the tumor/blood ratios showed an increase from 0.5 to 1.2 after 23 h pi (Figure 5b). In addition the tumor/muscle ratios increased from 2.1 to 7.3 for **23a**, reaching a maximum at 23 h.

Compound [^{125}I]**23a** showed lower accumulation in non-target tissues in comparison with the previously reported ^{64}Cu AOMK deriviative.[32] When tested in MDA-MB-435 xenografts, it exhibited <0.5% ID/g tumor uptake, a tumor-to-blood ratio of 0.61, and a tumor-to-muscle ratio of 3.03 at 2 h pi. Compound [^{125}I]**23a** exhibited similar tumor uptake at 5 h pi (0.27% ID/g) in the MDA-MB-231 xenografts, which have comparable levels of cathepsin B activity as the MDA-MB-435 model,[14] while the tumor-to-blood (0.98) and tumor-to-muscle (5.31) ratios were higher for [^{125}I]**23a**.

CONCLUSION

This work explored the utility of the AOMK construct as a targeting vector to develop radioiodinated ABPs for cathepsin B. Attachment of bulky aromatic groups to the lysine side chain with and without an aliphatic spacer had a detrimental impact on cathepsin B binding when compared to the parent ligand. However, inhibitors containing a PEG spacer showed high affinity for the enzyme. Despite different binding affinities and lipophilicities, [^{125}I]**11a** and [^{125}I]**23a** were able to label cathepsin B in vitro, which was blocked in the presence of a known cathepsin B inhibitor. Evaluation of [^{125}I]**23a** in a human MDA-MB-231 breast cancer model showed improved tumor-to-nontumor ratios in comparison to other P1 radio-labeled AOMKs but modest total tumor uptake.

EXPERIMENTAL SECTION

Materials and Instrumentation. All chemicals, unless otherwise stated, were purchased from Sigma−Aldrich, Novabiochem, or Bachem and used without further purification. Cy5 succinimidyl ester was obtained from GE Healthcare. Tris(1*H*,1*H*,2*H*,2*H*-perfluorooctyl)tin hydride was obtained from Fluorous Technologies Inc. Diazomethane was prepared by use of the Diazald glassware set with system 45 connections (Aldrich) following the procedures

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Table 2. Biodistribution of [^{125}I]11a and [^{125}I]23a in MDA-MB-231 Tumor-Bearing Mice[a]

	[^{125}I]**11a**[b] (% ID/g)			[^{125}I]**23a**[c] (% ID/g)		
organ	30 min	5 h	23 h	30 min	5 h	23 h
blood	1.14 ± 0.09	0.43 ± 0.05	0.030 ± 0.003	2.3 ± 0.2	0.28 ± 0.03	0.04 ± 0.00
heart	0.68 ± 0.06	0.19 ± 0.02	0.030 ± 0.004	0.9 ± 0.2	0.11 ± 0.01	0.02 ± 0.00
lungs	2.2 ± 0.2	0.84 ± 0.09	0.21 ± 0.02	3.5 ± 0.5	0.59 ± 0.08	1 ± 1
liver	16 ± 1	4.5 ± 0.4	1.6 ± 0.2	3.4 ± 0.3	0.71 ± 0.07	0.29 ± 0.04
gall bladder	170 ± 30	60 ± 20	0.4 ± 0.1	42 ± 7	5 ± 1	0.17 ± 0.09
spleen	8 ± 2	1.3 ± 0.2	0.6 ± 0.1	1.5 ± 0.2	0.37 ± 0.04	0.13 ± 0.01
kidneys	2.9 ± 0.3	0.80 ± 0.05	0.20 ± 0.02	6 ± 1	0.55 ± 0.06	0.4 ± 0.1
stomach	3.8 ± 0.7	4.2 ± 0.6	0.15 ± 0.06	11 ± 3	2.2 ± 0.2	0.09 ± 0.01
small intestine	12 ± 1	10 ± 1	1.6 ± 0.2	5.9 ± 0.2	0.7 ± 0.2	0.07 ± 0.02
large intestine	0.30 ± 0.02	21 ± 2	0.63 ± 0.07	0.7 ± 0.1	4.9 ± 0.1	0.12 ± 0.02
tumor	0.58 ± 0.06	0.5 ± 0.1	0.040 ± 0.002	1.12 ± 0.08	0.27 ± 0.07	0.05 ± 0.01
thyroid/trachea	3.2 ± 0.8	19 ± 5	17 ± 9	11 ± 3	21 ± 9	20 ± 10
adipose	0.22 ± 0.05	0.100 ± 0.004	0.030 ± 0.005	1.1 ± 0.9	0.03 ± 0.01	0.01 ± 0.00
bone	0.52 ± 0.07	0.15 ± 0.01	0.030 ± 0.004	0.55 ± 0.07	0.09 ± 0.01	0.01 ± 0.00
skeletal muscle	0.25 ± 0.03	0.11 ± 0.02	0.010 ± 0.001	0.5 ± 0.2	0.05 ± 0.00	0.01 ± 0.00
brain	0.09 ± 0.01	0.04 ± 0.01	0.003 ± 0.001	0.19 ± 0.02	0.01 ± 0.00	0.00 ± 0.00
bladder and urine	90 ± 20	38 ± 9	0.9 ± 0.2	400 ± 200	31 ± 3	0.7 ± 0.2

[a]Values are the mean % ID/g ± standard error from the mean. [b]n = 5. [c]n = 3.

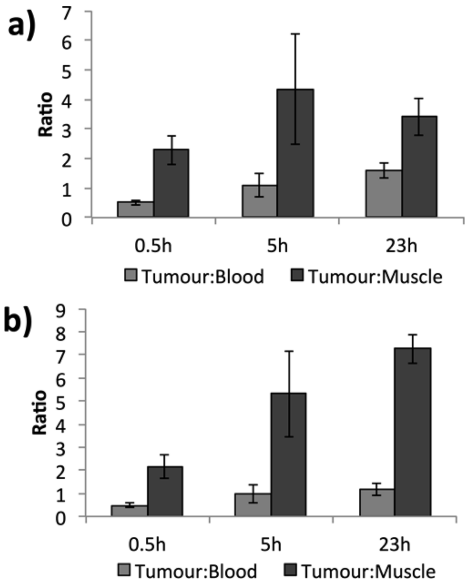

Figure 5. Average tumor/blood and tumor/muscle ratios for (a) [^{125}I]**11a** (n = 5) and (b) [^{125}I]**23a** (n = 3).

provided by the supplier. As this is a toxic and explosive substance, the appropriate precautions should be exercised. 1,3,5,7-Tetramethyl-6-(2,4-dimethoxyphenyl)-2,4,8-trioxa-6-phosphaadamantane was provided by the Capretta research group (McMaster University). [^{125}I]NaI (specific activity 629 GBq/mg) was provided by the McMaster nuclear reactor. As this is a radioactive isotope, appropriate facilities, licenses, and procedures should be in place prior to use.

For screening studies, human liver cathepsin B and Cbz-Arg-Arg-pNA were purchased from Calbiochem and Enzo Life Sciences, respectively. Reagents used in the assay buffer were from Sigma–Aldrich. Inhibitors were dissolved in biological-grade DMSO and diluted in the assay buffer. Black, clear-bottom 96-well plates were obtained from BD Biosciences. L-3-*trans*-(propylcarbamyl)oxirane-2-carbonyl-L-isoleucyl-L-proline (CA-074) was obtained from EMD Biosciences, precast gels were from Bio-Rad, and the gel drying kit was from Promega. 1,3,4,6-Tetrachloro-3a,6a-diphenylglycoluril (iodogen) was obtained from Thermo Scientific. According to HPLC analysis, supported by ^{1}H and ^{13}C NMR spectroscopy and high-resolution mass spectrometry (HRMS), the chemical purity of all compounds screened for cathepsin B inhibition was >95%.

Microwave reactions were performed by use of a Biotage Initiator microwave synthesizer. Automated flash chromatography was performed on a Biotage SP1 flash purification system. Analytical thin-layer chromatography (TLC) was performed on silica gel plates with fluorescent indicator UV_{254} (Macherey-Nagel) and visualized by use of UV light and ninhydrin or vanillin in EtOH. ^{1}H, ^{13}C, and two-dimensional NMR spectra were recorded on either a Bruker AV500 or AV600 spectrometer. ^{1}H NMR signals are reported in parts per million (ppm) measured relative to the residual proton signal of the deuterated solvent. Coupling constants (J) are reported in Hertz (Hz). ^{13}C signals are reported in ppm relative to the carbon signal from the solvent. HRMS was performed on a Waters/Micromass Q-Tof Ultima Global spectrometer. Analytical high-performance liquid chromatography (HPLC) was performed by use of an Agilent/Varian Pro Star model 330 photodiode array (PDA) detector, model 230 solvent delivery system, and Phenomenex Gemini (L × i.d. = 100 × 4.6 mm) column (5 μm C18). Semipreparative HPLC was performed by use of an Agilent/Varian Pro Star model 325 PDA detector, model 24 solvent delivery system, and Phenomenex Gemini (L × i.d. = 250 × 10 mm) column (5 μm C18). The elution conditions were as follows: (Method A) Solvent A = CH_3CN with 0.1% trifluoroacetic acid (TFA), solvent B = H_2O with 0.1% TFA. Gradient: 30% A to 100% A, 0–12 min; 100% A, 12–24 min; 100% A to 90% A, 24–28 min; 90% A to 30% A, 28–30 min. (Method B) Solvent A = CH_3CN, solvent B = H_2O. Gradient: 30% A to 100% A, 0–12 min; 100% A, 12–24 min; 100% A to 90% A, 24–28 min; 90% A to 30% A, 28–30 min. (Method C) Solvent A = CH_3CN, solvent B = H_2O. Gradient: 10% A, 0–2 min; 10% A to 90% A, 2–20 min; 90% A, 20–22 min; 90% A to 10% A, 22–23 min; 10% A, 23–25 min. The flow rate was set at 1 mL/min for analytical methods and 4 mL/min for semipreparative methods; monitoring occurred at 254 nm. Autoradiography was measured on a GE Storm 840 phosphor imager, and absorbance readings were performed on a Bio-Rad EL 808 plate reader. Radioactivity measurements for biodistribution and log P studies were made on a Wizard 1470 automated γ counter (PerkinElmer, Woodbridge, ON, Canada).

Synthetic Procedures for New Compounds. *(S)-3-[(S)-2-{[(Benzyloxy)carbonyl]amino}-3-phenylpropanamido]-7-(3-iodobenzamido)-2-oxoheptyl 2,4,6-Trimethylbenzoate (**7a**).* To a solution of 4-iodobenzoic acid (72 mg, 290 μmol) in *N*,*N*-dimethylformamide (DMF) (12 mL) at −16 °C, NMM (32 μL, 290 μmol) and IBCF (38 μL, 290 μmol) were added and left to stir until

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dissolved. To this, **6a** (204 mg, 290 μmol) was added, and the solution was left to stir overnight while warming to room temperature. The reaction mixture was diluted to 25 mL with water and extracted with EtOAc (3 × 50 mL), and the combined organic layers were extracted with water (5 × 20 mL), saturated Na_2CO_3 (3 × 20 mL), water (20 mL), 5% (w/v) citric acid (3 × 20 mL), water (20 mL), and brine (3 × 20 mL) and then dried over $MgSO_4$. The solvent was removed by rotary evaporation and the product was isolated as a white powder. Yield 68 mg, 29%; mp 163−165 °C; TLC (2:1 EtOAc/Hex) R_f = 0.53; HPLC (method B) ^tR = 18.1 min. ^{1}H NMR [600.13 MHz, $(CD_3)_2SO$] 8.51−8.48 (m, 2H, NHCHCONH and $CH_2CH_2CH_2NH$), 7.79 (d, J = 8.2 Hz, 2H, 2,6-$C_6H_2H_2I$), 7.63−7.60 (m, 1H, $NHCHCOCH_2$), 7.61 (d, J = 8.5 Hz, 2H, 3,5-$C_6H_2H_2I$), 7.33−7.18 (m, 10H, Ph), 6.91 (s, 2H, $Me_3C_6H_2CO$), 4.95 (s, 2H, $PhCH_2OCO$), 4.91 (d, J = 17.4 Hz, 1H, COCHHO), 4.82 (d, J = 17.2 nHz, 1H, COCHHO), 4.38−4.31 (m, 2H, HNCHCO Phe and Lys), 3.25−3.22 (m, 2H, $CH_2CH_2CH_2NH$), 3.01 (dd, J = 13.4, 5.0 Hz, 1H, CHCHHPh), 2.80 (dd, J = 13.1, 10.4 Hz, 1H, CHCHHPh), 2.26 [s, 6H, 2,6-$(CH_3)_3C_6H_2CO$], 2.25 [s, 3H, 4-$(CH_3)_3C_6H_2CO$], 1.84−1.82 (m, 1H, $CHCHHCH_2CH_2$), 1.62−1.56 (m, 1H, $CHCHHCH_2CH_2$), 1.55−1.47 (m, 2H, $CH_2CH_2CH_2NH$), 1.40−1.30 (m, 2H, $CH_2CH_2CH_2NH$). ^{13}C NMR [150.92 MHz, $(CD_3)_2SO$] δ 202.7, 172.0, 168.2, 165.4, 155.8, 139.1, 137.8, 137.0, 136.9, 134.9, 134.1, 129.9, 129.2, 129.1, 128.3, 128.2, 128.1, 127.7, 127.5, 126.3, 98.5, 66.6, 65.3, 56.0, 55.9, 54.9, 37.3, 29.2, 28.6, 22.4, 20.7, 19.3. HRMS calcd for $C_{41}H_{45}N_3O_7I$ $[M + H]^+$, 818.2302; found, 818.2280.

*(S)-3-[(S)-2-{[(Benzyloxy)carbonyl]amino}-3-phenylpropanamido]-7-(3-iodobenzamido)-2-oxoheptyl 2,4,6-Trimethylbenzoate (**7b**).* To a solution of 3-iodobenzoic acid (72 mg, 290 μmol) in DMF (12 mL) at −24 °C, NMM (33 μL, 300 μmol) and IBCF (39 μL, 300 μmol) were added and left to stir until dissolved. To this, **6a** (180 mg, 260 μmol) was added and the solution was left to stir overnight while warming to room temperature. The reaction mixture was diluted to 25 mL with water and extracted with EtOAc (3 × 20 mL), and the combined organic layers were extracted with water (5 × 10 mL), saturated Na_2CO_3 (3 × 10 mL), water (20 mL), 5% (w/v) citric acid (3 × 10 mL), water (20 mL), and brine (3 × 10 mL) and then dried over $MgSO_4$. Yield 95 mg, 45%; mp 135−140 °C; TLC (2:1 EtOAc/Hex) R_f = 0.83; HPLC (method B) ^tR = 18.0 min. ^{1}H NMR [600.23 MHz, $(CD_3)_2SO$] δ 8.52 (m, 2H, NHCHCONH and $CH_2CH_2CH_2NH$), 8.18 (s, 1H, 2-C_6HH_3I), 7.86−7.83 (m, 2H, 4,6-$C_6H_2H_2I$), 7.63 (d, J = 8.2, 1H, $NHCHCOCH_2$), 7.33−7.18 (m, 10H Ph), 6.91 (s, 2H, $Me_3C_6H_2CO$), 4.95 (s, 2H, $PhCH_2OCO$), 4.91 (d, J = 17.2 Hz, 1H, COCHHO), 4.81 (d, J = 17.2 Hz, 1H, COCHHO), 4.39−4.32 (m, 2H, HNCHCO Phe and Lys), 3.24 (m, 2H, $CH_2CH_2CH_2NH$), 3.02 (dd, J = 13.7, 5.3 Hz, 1H, CHCHHPh), 2.81 (dd, J = 13.5, 10.0 Hz, 1H, CHCHHPh), 2.26 (s, 6H, 2,6-$(CH_3)_3C_6H_2CO$), 2.25 (s, 3H, 4-$(CH_3)_3C_6H_2CO$), 1.87−1.78 (m, 1H, $CHCHHCH_2CH_2$), 1.64−1.56 (m, 1H, $CHCHHCH_2CH_2$), 1.56−1.47 (m, 2H, $CH_2CH_2CH_2NH$), 1.41−1.29 (m, 2H, $CH_2CH_2CH_2NH$); ^{13}C NMR (150.92 MHz, $(CD_3)_2SO$) δ 202.6, 172.0, 168.2, 164.5, 155.8, 139.5, 139.1, 137.8, 136.9, 136.6, 135.6, 134.9, 130.4, 129.9, 129.2, 128.2, 128.2, 128.1, 127.7, 127.5, 126.6, 126.3, 94.6, 66.6, 65.2, 56.0, 55.9, 40.0, 37.2, 29.1, 28.6, 22.5, 20.7, 19.3. HRMS calcd for $C_{41}H_{45}N_3O_7I$ $[M + H]^+$, 818.2302; found, 818.2291.

*(S)-3-[(S)-2-{[(Benzyloxy)carbonyl]amino}-3-phenylpropanamido]-7-[3-(3-iodophenyl)ureido]-2-oxoheptyl 2,4,6-Trimethylbenzoate (**8a**).* To a solution of 4-iodophenyl isocyanate (42 mg, 170 μmol) in anhydrous CH_2Cl_2 (5 mL) were added Et_3N (24 μL, 170 μmol) and **6a** (119 mg, 170 μmol), and the solution was left to stir for 3 h under a stream of Ar. The solvent was removed by rotary evaporation and the residue was dissolved in DMF (1 mL). This solution was then added dropwise to rapidly stirring cold water (approximately 100 mL), forming a white powder that was collected by centrifugation. The product was isolated as a white powder following flash chromatography (2:1 EtOAc/Hex). Yield 13 mg, 9%; mp 149−159 °C; TLC (2:1 EtOAc/Hex) R_f = 0.72; HPLC (method B) ^tR = 17.3 min. ^{1}H NMR [600.13 MHz, $(CD_3)_2SO$] δ 8.55 (s, 1H, $CONHC_6H_4I$), 8.51 (d, J = 7.5 Hz, 1H CONHCHCO), 7.63 (d, J = 8.3 Hz, 1H, CONHCHCO), 7.51−7.48 (m, 2H, $CONHC_6H_2H_2I$), 7.33−7.19 (m, 12H, ArH), 6.91 (s, 2H, $Me_3C_6H_2CO$), 6.20 (t, J = 5.4 Hz, 1H, $CH_2CH_2CH_2NH$), 4.96 (s, 2H, $PhCH_2OCO$), 4.91 (d, J = 17.2 Hz, 1H, $COCH_2O$), 4.81 (d, J = 17.2 Hz, 1H, COCHHO), 4.38−4.31 (m, 2H, HNCHCO Phe and Lys), 3.07−3.01 (m, 3H, $CH_2CH_2CH_2NH$, CHCHHPh), 2.81 (dd, J = 13.7, 9.8 Hz, 1H, CHCHHPh), 2.27 [s, 6H, 2,6-$(CH_3)_3C_6H_2CO$], 2.25 [s, 3H, 4-$(CH_3)_3C_6H_2CO$], 1.85−1.78 (m, 1H, $CHCHHCH_2CH_2$), 1.62−1.54 (m, 1H, $CHCHHCH_2CH_2$), 1.46−1.39 (m, 2H, $CH_2CH_2CH_2NH$), 1.39−1.33 (m, 1H, $CHHCH_2CH_2NH$), 1.32−1.25 (m, 1H, $CHHCH_2CH_2NH$). ^{13}C NMR [150.92 MHz; $(CD_3)_2SO$] δ 202.7, 172.0, 168.2, 155.9, 154.9, 140.5, 139.1, 137.8, 137.1, 134.9, 129.9, 129.2, 128.3, 128.3, 128.2, 128.1, 127.7, 127.5, 126.3, 119.9, 83.3, 66.6, 65.3, 56.1, 55.9, 38.9, 37.2, 29.3, 29.2, 22.4, 20.7, 19.3. HRMS calcd for $C_{41}H_{46}N_4O_7I$ $[M + H]^+$, 833.2411; found, 833.2451.

*(S)-3-[(S)-2-{[(Benzyloxy)carbonyl]amino}-3-phenylpropanamido]-7-[3-(4-iodophenyl)ureido]-2-oxoheptyl 2,4,6-Trimethylbenzoate (**8b**).* Synthesized in a similar manner as **8a** with 3-iodophenyl isocyanate in place of 4-iodophenyl isocyanate. Yield 20 mg, 14%; mp 166−169 °C; TLC (2:1 EtOAc/Hex) R_f = 0.85; HPLC (method B) ^tR = 18.0 min. ^{1}H NMR [600.13 MHz, $(CD_3)_2SO$] δ 8.60 (s, 1H, $CONHC_6H_4I$), 8.53 (d, J = 7.4 Hz, 1H, CONHCHCO), 7.96 (dd, J = 1.8, 1.8 Hz, 1H, 5-$NHC_6H_2H_2I$), 7.65 (d, J = 8.2 Hz, 1H, CONHCHCO), 7.34−7.20 (m, 12H, ArH), 6.99 (m, 1H, 2-$NHC_6H_2H_2I$), 6.92 (s, 2H, $Me_3C_6H_2CO$), 6.25 (t, J = 4.6 Hz, 1H, $CH_2CH_2CH_2NH$), 4.96 (s, 2H, $PhCH_2OCO$), 4.92 (d, J = 17.2 Hz, 1H, COCHHO), 4.81 (d, J = 17.2 Hz, 1H, COCHHO), 4.39−4.32 (m, 2H, HNCHCO Phe and Lys), 3.08−3.02 (m, 3H, $CH_2CH_2CH_2NH$ and $CHCH_2HPh$), 2.82 (dd, J = 13.6, 9.8 Hz, 1H, CHCHHPh), 2.27 [s, 6H, 2,6-$(CH_3)_3C_6H_2CO$], 2.25 [s, 3H, 4-$(CH_3)_3C_6H_2CO$], 1.85−1.80 (m, 1H, $CHCHHCH_2CH_2$), 1.63−1.55 (m, 1H, $CHCHHCH_2CH_2$), 1.47−1.40 (m, 2H, $CH_2CH_2CH_2NH$), 1.39−1.33 (m, 1H, $CHHCH_2CH_2NH$), 1.32−1.27 (m, 1H, $CHHCH_2CH_2NH$). ^{13}C NMR [150.92 MHz, $(CD_3)_2SO$] δ 202.7, 172.0, 168.2, 155.9, 154.9, 142.1, 139.1, 137.8, 134.9, 130.6, 129.3, 129.3, 129.2, 128.3, 128.2, 128.1, 127.7, 127.7, 127.5, 126.3, 125.6, 116.8, 94.7, 66.6, 65.3, 56.1, 55.9, 38.9, 37.2, 29.3, 29.2, 22.4, 20.7, 19.4. HRMS calcd for $C_{41}H_{46}N_4O_7I$ $[M + H]^+$, 833.2411; found, 833.2416.

*(S)-3-[(S)-2-{[(Benzyloxy)carbonyl]amino}-3-phenylpropanamido]-7-[6-(4-iodobenzamido)hexanamido]-2-oxoheptyl 2,4,6-Trimethylbenzoate (**11a**).* To a solution of **10a** (49 mg, 137 μmol) in anhydrous DMF (2 mL), benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) (71 mg, 140 μmol) was added and dissolved at 0 °C under a stream of Ar. Et_3N (38 μL, 270 μmol) was then added and allowed to stir for 5 min. Afterward **6a** (96 mg, 140 μmol) and Et_3N (19 μL, 140 μmol) were added and the solution was stirred overnight at room temperature. The reaction mixture was diluted to 25 mL with water and extracted with CH_2Cl_2 (3 × 15 mL), and the organic layers were combined and further extracted with water (5 × 15 mL) and brine (30 mL) and then dried over Na_2SO_4. The solvent was removed by rotary evaporation, leaving an oily residue, which was then purified by flash chromatography (1−10% MeOH/CH_2Cl_2). The product was isolated as a white powder following lyophilization. Yield 57 mg, 45%; mp 190−196 °C; TLC (5% MeOH/CH_2Cl_2) R_f = 0.44; HPLC (method B) ^tR = 17.3 min. ^{1}H NMR [600.13 MHz, $(CD_3)_2SO$] δ 8.48 (m, 2H, NHCHCONH and $CH_2NHCOAr$), 7.83 (d, J = 8.4 Hz, 2H, $COC_6H_2H_2I$), 7.71 (t, J = 5.7 Hz, 1H, $CH_2NHCOCH_2$), 7.63 (d, J = 8.4 Hz, 1H, $NHCHCOCH_2$), 7.61 (d, J = 8.4 Hz, 2H, $COC_6H_2H_2I$), 7.34−7.19 (m, 10H, Ph), 6.91 (s, 2H, $Me_3C_6H_2CO$), 4.97 (s, 2H, $PhCH_2OCO$), 4.91 (d, J = 17.2 Hz, 1H, COCHHO), 4.81 (d, J = 17.2 Hz, 1H, COCHHO), 4.36−4.32 (m, 2H, HNCHCO Phe and Lys), 3.23−3.19 (m, 2H, $CH_2NHCOAr$), 3.04−2.97 (m, 3H, CHCHHPh and $CH_2NHCOCH_2$), 2.84−2.79 (m, 1H, CHCHHPh), 2.27 [s, 6H, 2,6-$(CH_3)_3C_6H_2CO$], 2.25 [s, 3H, 4-$(CH_3)_3C_6H_2CO$], 2.04 (t, J = 7.5 Hz, 2H, $CH_2NHCOCH_2$), 1.82−1.76 (m, 1H, $CHCHHCH_2CH_2$), 1.60−1.54 (m, 1H, $CHCHHCH_2CH_2$), 1.49 (m, J = 7.4 Hz, 5H, $CHCH_2CHHCH_2$ and $NHCOCH_2CH_2$), 1.40−1.30 (m, 3H, $CHCH_2CHHCH_2$ and CH_2CH_2NHCO Lys), 1.28−1.22 (m, 2H, $COCH_2CH_2CH_2$). ^{13}C

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NMR [150.92 MHz, $(CD_3)_2SO$] δ 202.6, 172.0, 171.8, 168.2, 165.3, 155.8, 139.1, 137.8, 137.1, 136.9, 134.9, 134.1, 129.9, 129.2, 129.1, 128.3, 128.2, 128.1, 127.7, 127.5, 126.3, 98.5, 66.6, 65.3, 56.0, 55.9, 38.1, 38.1, 37.2, 35.4, 29.1, 28.8, 28.8, 26.2, 25.1, 22.4, 20.7, 19.3. HRMS calcd for $C_{47}H_{56}N_4O_8I$ $[M + H]^+$, 931.3143; found, 931.3104.

*(S)-3-[(S)-2-{[(Benzyloxy)carbonyl]amino}-3-phenylpropanamido]-7-[6-(3-iodobenzamido)hexanamido]-2-oxoheptyl 2,4,6-Trimethylbenzoate (**11b**).* Synthesized in a similar manner as **11a** with **10a** in place of **10b**. Yield 38 mg, 30%; mp 159−162 °C; TLC (5% MeOH/CH_2Cl_2) R_f = 0.46; HPLC (method B) ^tR = 17.4 min. ^1H NMR [600.13 MHz, $(CD_3)_2SO$] δ 8.54−8.51 (m, 2H, N*H*CHCONH and $CH_2NHCOAr$), 8.17 (s, 1H, $NHCOC_6HH_3I$), 7.87 (d, J = 7.8 Hz, 1H, $NHCOC_6HH_3I$), 7.83 (d, J = 7.9 Hz, 1H, $NHCOC_6HH_3I$), 7.74 (t, J = 5.5 Hz, 1H, $NHCHCOCH_2$), 7.67 (d, J = 8.3 Hz, 1H, $CH_2NHCOCH_2$), 7.33−7.18 (m, 11H, Ph and $NHCOC_6HH_3I$), 6.91 (s, 2H, $Me_3C_6H_2CO$), 4.96 (s, 2H, $PhCH_2OCO$), 4.90 (d, J = 17.3 Hz, 1H, COC*H*HO), 4.80 (d, J = 17.2 Hz, 1H, COC*H*HO), 4.35−4.31 (m, 2H, HNC*H*CO Phe and Lys), 3.22−3.19 (m, 2H, $CH_2NHCOAr$), 3.04−2.99 (m, 3H, CHC*H*HPh and $CH_2NHCOCH_2$), 2.80 (dd, J = 13.6, 9.8 Hz, 1H, CHC*H*HPh), 2.27 [s, 6H, 2,6-$(CH_3)_3C_6H_2CO$], 2.24 [s, 3H, 4-$(CH_3)_3C_6H_2CO$], 2.04 (t, J = 7.4 Hz, 2H, $CH_2NHCOCH_2$), 1.82−1.75 (m, 1H, CHC*H*HCH$_2$CH$_2$), 1.59−1.52 (m, 1H, CHC*H*HCH$_2$CH$_2$), 1.52−1.46 (m, 5H, $CHCH_2CHHCH_2$ and $NHCOCH_2CH_2$), 1.39−1.30 (m, 3H, $CHCH_2CHHCH_2$ and CH_2CH_2NHCO Lys), 1.28−1.22 (m, 2H, $COCH_2CH_2CH_2$). ^{13}C NMR [125.77 MHz, $(CD_3)_2SO$] δ 202.8, 172.15, 172.02, 168.4, 164.7, 156.0, 139.7, 139.3, 138.0, 137.1, 136.8, 135.7, 135.1, 130.6, 130.1, 129.4, 128.41, 128.39, 128.25, 127.86, 127.68, 126.8, 126.5, 94.8, 66.8, 65.5, 56.24, 56.09, 38.28, 38.25, 37.4, 35.5, 29.3, 28.9, 26.3, 25.2, 22.6, 20.8, 19.5, 19.1. HRMS calcd for $C_{47}H_{56}N_4O_8I$ $[M + H]^+$, 931.3143; found, 931.3161.

*(S)-3-[(S)-2-(Benzyloxycarbonyl)-3-phenylpropanamido]-7-{6-[3-(4-iodophenyl)ureido]hexanamido}-2-oxoheptyl 2,4,6-Trimethylbenzoate (**15a**).* To a solution of **14a** (56 mg, 150 μmol) in anhydrous DMF (5 mL) were added NMM (17 μL, 150 μmol) and IBCF (21 μL, 150 μmol), and the mixture was stirred at −10 °C under a stream of Ar. After 1 min, **6a** (95 mg, 140 μmol) and NMM (17 μL, 150 μmol) were added and the solution was left to stir overnight while warming to room temperature. The reaction mixture was diluted to 25 mL with water and extracted with EtOAc (3 × 25 mL). The combined extracts were extracted with saturated Na_2CO_3 (3 × 25 mL), water (25 mL), 5% (w/v) citric acid (3 × 25 mL), water (25 mL), and brine (3 × 25 mL) and then dried over $MgSO_4$. The solvent was removed by rotary evaporation, leaving an oily residue, which was then purified by flash chromatography (1:1 EtOAc/Hex). The product was isolated as a white powder following lyophilization. Yield 45 mg, 35%; TLC (1:1 EtOAc/Hex) R_f = 0.60; HPLC (method B) ^tR = 16.8 min. ^1H NMR [600 MHz, $(CD_3)_2SO$] δ 8.54−8.47 (m, 2H, NHCONHAr and N*H*CHCONH), 7.73−7.70 (m, 1H, $CH_2NHCOCH_2$), 7.65−7.61 (m, 1H, $NHCHCOCH_2$), 7.50 (d, J = 8.7 Hz, 1H $NHCOC_6H_2H_2I$), 7.34−7.18 (m, 12H, Ph and $NHCOC_6H_2H_2I$), 6.91 (s, 2H, $Me_3C_6H_2CO$), 6.20−6.16 (m, 1H, N*H*CONHAr), 4.96 (s, 2H, $PhCH_2OCO$), 4.90 (d, J = 16.7 Hz, 1H, COC*H*HO), 4.80 (d, J = 17.4 Hz, 1H, COC*H*HO), 4.35−4.30 (m, 2H, HNC*H*CO Phe and Lys), 3.04−3.00 (m, 3H, CHC*H*HPh and $CH_2NHCOCH_2$), 2.99−2.93 (m, 1H,), 2.83−2.80 (m, 1H, CHC*H*HPh), 2.27 [s, 6H, 2,6-$(CH_3)_3C_6H_2CO$], 2.25 [s, 3H, 4-$(CH_3)_3C_6H_2CO$], 2.04−2.02 (m, 2H, $CH_2NHCOCH_2$), 1.79−1.78 (m, 1H, CHC*H*HCH$_2$CH$_2$), 1.58−1.54 (m, 1H, CHC*H*HCH$_2$CH$_2$), 1.49−1.47 (m, 2H, $NHCOCH_2CH_2$), 1.40−1.33 (m, 6H, $CHCH_2CH_2CH_2$ and CH_2CH_2NHCO), 1.25−1.21 (m, 2H, $COCH_2CH_2CH_2$). ^{13}C NMR [150.92 MHz, $(CD_3)_2SO$] δ 156.8, 154.9, 142.2, 137.1, 135.4, 134.9, 130.6, 129.3, 129.3, 128.3, 128.2, 128.1, 125.6, 119.8, 116.8, 109.1, 94.7, 65.3, 40.1, 33.99, 33.79, 29.5, 26.0, 24.3, 18.9. HRMS calcd for $C_{47}H_{57}N_5O_8I$ $[M + H]^+$, 946.3252; found, 946.3266.

*(S)-3-[(S)-2-(Benzyloxycarbonyl)-3-phenylpropanamido]-7-{6-[3-(3-iodophenyl)ureido]hexanamido}-2-oxoheptyl 2,4,6-Trimethylbenzoate (**15b**).* To a solution of **14b** (47 mg, 125 μmol) in anhydrous DMF (5 mL) were added NMM (25 μL, 250 μmol) and IBCF (16 μL, 125 μmol), and the mixture was allowed to stir at −10 °C under a stream of Ar. After 1 min, **6a** (106 mg, 150 μmol) and NMM (13 μL, 125 μmol) were added and the solution was left to stir overnight while warming to room temperature. The reaction mixture was made up to 25 mL with water and extracted with EtOAc (4 × 25 mL). The combined extracts were extracted with saturated Na_2CO_3 (3 × 25 mL), water (25 mL), 5% (w/v) citric acid (3 × 25 mL), water (25 mL), and brine (3 × 25 mL) and then dried over $MgSO_4$. The solvent was removed by rotary evaporation, leaving an oily residue, which was purified by flash chromatography (12−100% EtOAc/Hex). The product was isolated as a white powder following lyophilization. Yield 0.047 g, 30%; TLC (1:1 EtOAc/Hex) R_f = 0.60; HPLC (method B) ^tR = 17.0 min. ^1H NMR [600 MHz, $(CD_3)_2SO$] δ 8.54 (s, 1H, NHCON*H*Ar), 8.49 (d, J = 7.3 Hz, 1H, N*H*CHCONH), 7.96 (s, 1H, $CONHC_6HH_3I$), 7.72−7.70 (m, 1H, $CH_2NHCOCH_2$), 7.64−7.62 (m, 1H, $NHCHCOCH_2$), 7.33−7.19 (m, 12H, Ph and $NHCOC_6H_2H_2I$), 7.01−6.98 (m, 1H, $NHCOC_6HH_3I$), 6.92 (s, 2H, $Me_3C_6H_2CO$), 6.20−6.17 (m, 1H, N*H*CONHAr), 4.97 (s, 2H, $PhCH_2OCO$), 4.91 (d, J = 17.2 Hz, 1H, COC*H*HO), 4.81 (d, J = 17.3 Hz, 1H, COC*H*HO), 4.36−4.31 (m, 2H, HNC*H*CO Phe and Lys), 3.07−3.01 (m, 6H, $CH_2NHCONH$, CHC*H*HPh, $CH_2NHCOCH_2$, and $CHCHHCH_2CH_2$), 2.83−2.79 (m, 1H, CHC*H*HPh), 2.28 [s, 6H, 2,6-$(CH_3)_3C_6H_2CO$], 2.25 [s, 3H, 4-$(CH_3)_3C_6H_2CO$], 2.21 (t, J = 7.4 Hz, 2H, $CH_2NHCOCH_2$), 2.06−2.03 (m, 1H, CHC*H*HCH$_2$CH$_2$), 1.52−1.49 (m, 1H, CHC*H*HCH$_2$CH$_2$), 1.43−1.37 (m, 6H, $CHCH_2CH_2CH_2$ and CH_2CH_2NHCO), 1.31−1.25 (m, 2H, $COCH_2CH_2CH_2$). ^{13}C NMR [151 MHz, $(CD_3)_2SO$] δ 174.8, 174.8, 142.5, 139.5, 138.2, 135.3, 133.5, 131.0, 129.7, 129.7, 128.7, 128.6, 128.5, 128.5, 128.1, 127.9, 126.0, 117.2, 95.1, 67.0, 65.7, 65.7, 56.3, 38.5, 37.7, 35.8, 34.1, 29.9, 29.8, 26.5, 26.3, 25.5, 24.7, 22.8, 20.1, 19.8. HRMS calcd for $C_{47}H_{57}N_5O_8I$ $[M + H]^+$, 946.3252; found, 946.3223.

*Methyl 6-[4-(Tributylstannyl)-1H-1,2,3-triazol-1-yl]hexanoate (**18**).* Tributyl(ethynyl)stannane (680 mg, 2.2 mmol) and **16** (310 mg, 1.8 mmol) were combined in toluene (2 mL) and heated to reflux at 120 °C for 14 h. The solvent was evaporated and the product was isolated as a colorless oil following flash chromatography (8−66% EtOAc/hexanes). Yield 680 mg, 78%; TLC (1:2 EtOAc/Hex) R_f = 0.55. ^1H NMR (600.13 MHz, $CDCl_3$) δ 7.41 (s, 1H, triazole-H), 4.36 (t, J = 7.2 Hz, 2H, $NCH_2CH_2CH_2$), 3.63 (s, 3H, $CH_2CH_2COOCH_3$), 2.28 (t, J = 7.4 Hz, 2H, $CH_2CH_2CH_2COOMe$), 1.93−1.88 (m, 2H, $NCH_2CH_2CH_2CH_2$), 1.67−1.60 (m, 2H, $NCH_2CH_2CH_2CH_2$), 1.59−1.47 [m, 6H, $Sn(CH_2CH_2CH_2)_3$], 1.37−1.27 [m, 8H, $NCH_2CH_2CH_2$ and $Sn(CH_2CH_2CH_2)_3$], 1.15−1.06 [m, 6H, $Sn(CH_2CH_2CH_2)_3$], 0.86 [t, J = 7.3 Hz, 9H, $(CH_2CH_2CH_2CH_3)_3$]. ^{13}C NMR (150.92 MHz, $CDCl_3$) δ 173.8, 144.3, 129.7, 51.5, 49.3, 33.7, 30.2, 29.0, 27.2, 26.0, 24.3, 13.7, 9.9. HRMS calcd for $C_{21}H_{42}N_3O_2Sn$ $[M + H]^+$, 488.2303; found, 488.2280.

*Methyl 6-(4-Iodo-1H-1,2,3-triazol-1-yl)hexanoate (**19**).* To a solution of **18** (490 g, 1.0 mmol) in tetrahydrofuran (THF, 8 mL) was added I_2 (280 g, 1.1 mmol), and the mixture was left to stir for 2 h. The solution was diluted to 25 mL with EtOAc, extracted with saturated $Na_2S_2O_3$ (3 × 20 mL) and brine (20 mL), and dried over $MgSO_4$. The solvent was removed by rotary evaporation, and the product was isolated as a white solid following flash chromatography (8−66% EtOAc/hexanes). Yield 220 mg, 69%; mp 49−51 °C; TLC (1:2 EtOAc/Hex) R_f = 0.23. ^1H NMR (600.13 MHz, $CDCl_3$) δ 7.64 (s, 1H, triazole-H), 4.30 (t, J = 7.1 Hz, 2H, $NCH_2CH_2CH_2$), 3.55 (s, 3H, $CH_2CH_2COOCH_3$), 2.21 (t, J = 7.4 Hz, 2H, $CH_2CH_2CH_2COOMe$), 1.85−1.80 (m, 2H, $NCH_2CH_2CH_2CH_2$), 1.58−1.53 (m, 2H, $NCH_2CH_2CH_2CH_2$), 1.28−1.22 (m, 2H, $NCH_2CH_2CH_2$). ^{13}C NMR (150.92 MHz, $CDCl_3$) δ 173.6, 129.1, 86.9, 51.5, 50.4, 33.6, 29.8, 25.8, 24.1. HRMS calcd for $C_9H_{15}N_3O_2I$ $[M + H]^+$, 324.0209; found, 324.0218.

*6-(4-Iodo-1H-1,2,3-triazol-1-yl)hexanoic acid (**20**).* To **19** (190 mg, 600 μmol) dissolved in CH_3CN (30 mL) was added 1 N NaOH (6 mL), and the solution was left to stir overnight. After the solvent was evaporated, the residue was dissolved in water (25 mL), and 1 N HCl (6 mL) was added. The product was then extracted with CH_2Cl_2 (3 × 20 mL), and the combined organic layers were extracted with brine (20 mL) and dried over Na_2SO_4. The product was obtained as a white wax following rotary evaporation. Yield 150 g, 80%; mp 73−77

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°C; TLC (5% MeOH/CH_2Cl_2) R_f = 0.52. ^{1}H NMR (600.13 MHz, $CDCl_3$) δ 7.62 (s, 1H, triazole-H), 4.40 (t, J = 7.1 Hz, 2H, NCH_2CH$_2$CH$_2$), 2.38 (t, J = 7.3 Hz, 2H, CH$_2$CH$_2$CH_2COOH), 1.97−1.92 (m, 2H, NCH$_2$CH_2CH$_2$CH$_2$), 1.72−1.67 (m, 2H, NCH$_2$CH$_2$CH$_2$CH_2), 1.42−1.37 (m, 2H, NCH$_2$CH$_2$CH_2). ^{13}C NMR (150.92 MHz, $CDCl_3$) δ 178.5, 128.8, 86.9, 50.3, 33.4, 29.8, 25.7, 23.8. HRMS calcd for $C_8H_{13}N_3O_2I$ [M + H]$^+$, 310.0052; found, 310.0045.

*(S)-3-[(S)-2-{[(Benzyloxy)carbonyl]amino}-3-phenylpropanamido]-7-[6-(4-iodo-1H-1,2,3-triazol-1-yl)hexanamido]-2-oxoheptyl 2,4,6-Trimethylbenzoate (**21**).* To a solution of **20** (42 mg 140 μmol) in anhydrous DMF (10 mL), PyBOP (71 mg, 140 μmol) was added and allowed to dissolve while the reaction mixture was kept at 0 °C under a stream of Ar. Et_3N (38 μL, 270 μmol) was added and the solution was left to stir for 5 min. Afterward, **6a** and Et_3N (19 μL 140 μmol) were added and the reaction mixture was allowed to warm to room temperature overnight with stirring. The solution was made up to 25 mL with water and extracted with CH_2Cl_2 (5 × 15 mL), and the combined organic layers extracted with water (5 × 15 mL) and brine (3 × 25 mL). The solvent was evaporated and the product was obtained following flash chromatography (1−10% MeOH/CH_2Cl_2) as a white solid. Yield 40 mg, 33%; mp 102−109 °C; TLC (5% MeOH/CH_2Cl_2) R_f = 0.15. ^{1}H NMR [600.13 MHz, $(CD_3)_2SO$] δ 8.48 (d, J = 7.5 Hz, 1H, NHCHCONH), 8.33 (s, 1H, triazole-H), 7.71 (t, J = 5.5 Hz, 1H, CH$_2$NHCOCH$_2$), 7.62 (d, J = 8.3 Hz, 1H, NHCHCOCH$_2$), 7.33−7.18 (m, 10H, Ph) 6.91 (s, 2H, Me$_3$C$_6H_2$CO), 4.96 (s, 2H, PhCH_2OCO), 4.91 (d, J = 17.3 Hz, 1H, COCHHO), 4.80 (d, J = 17.3 Hz, 1H, COCHHO), 4.36−4.31 (m, 4H, CH_2N$_3$C$_2$HI and HNCHCO Phe and Lys), 3.04−2.99 (m, 4H CH_2NHCOCH$_2$ and CHCHHPh), 2.81 (dd, J = 13.8, 9.8 Hz, 1H, CHCHHPh), 2.26 [s, 6H 2,6-(CH_3)$_3$C$_6$H$_2$CO], 2.25 [s, 3H, 4-(CH_3)$_3$C$_6$H$_2$CO], 2.02 (t, J = 7.4 Hz, 2H, CH$_2$NHCOCH_2), 1.79−1.72 (m, 3H, CHCHHCH$_2$CH$_2$ and CH_2CH$_2$N$_3$C$_2$HI), 1.58−1.55 (m, 1H, CHCHHCH$_2$CH$_2$), 1.51−1.46 (m, 2H, NHCOCH$_2$CH_2), 1.40−1.23 (m, 4H CHCH$_2$CH_2CH_2), 1.18−1.12 (m, 2H, COCH$_2$CH$_2$CH_2). ^{13}C NMR [150.91 MHz, $(CD_3)_2SO$] δ 202.6, 172.0, 171.7, 168.2, 155.9, 139.1, 137.8, 136.9, 136.1, 134.9, 129.9, 129.2, 128.26, 128.22, 128.08, 127.69, 127.50, 126.3, 88.3. HRMS calcd for $C_{42}H_{52}N_6O_7I$ [M + H]$^+$, 879.2943; found, 879.2915.

*1-(4-Iodophenyl)-1-oxo-5,8,11-trioxa-2-azatetradecan-14-oic Acid (**22a**).* 3-{2-[2-(2-Aminoethoxy)ethoxy]ethoxy}propanoic acid (332 mg, 1.5 mmol) and **9a** (173 mg, 0.5 mmol) were added to DMF (2 mL) in a 2−5 mL microwave vial. The solution was heated in a microwave at 120 °C for 8 min. Afterward, water (5 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 10 mL); the organic layers were combined and then extracted with water (5 × 10 mL) and brine (3 × 10 mL) and dried over Na_2SO_4. The product was isolated as a white solid following flash chromatography [1−10% MeOH/CH_2Cl_2 (with 0.1% AcOH)]. Yield 150 mg, 67%; mp 56−64 °C; TLC [5% MeOH/CH_2Cl_2 (with AcOH)] R_f = 0.25. ^{1}H NMR (600.13 MHz, $CDCl_3$) δ 7.77 (d, J = 8.3 Hz, 2H, NHCOC$_6H_2$H$_2$I), 7.56 (d, J = 8.4 Hz, 2H, NHCOC$_6H_2$H$_2$I), 7.04 (br s, NHCH$_2$CH$_2$O), 3.74 (t, J = 6.0 Hz, 2H, NHCH_2CH$_2$O), 3.69−3.63 [m, 12H, (CH_2OCH_2)$_3$], 2.59 (t, J = 5.7 Hz, 2H, CH$_2$CH_2COOH). ^{13}C NMR (150.92 MHz, $CDCl_3$) δ 167.3, 137.6, 133.7, 129.0, 98.5, 70.36, 70.26, 70.21, 70.08, 69.9, 66.6, 58.1, 40.0. HRMS calcd for $C_{16}H_{23}NO_6$ [M + H]$^+$, 452.0570; found, 452.0555.

*1-(3-Iodophenyl)-1-oxo-5,8,11-trioxa-2-azatetradecan-14-oic acid (**22b**).* 3-{2-[2-(2-Aminoethoxy)ethoxy]ethoxy}propanoic acid (120 mg 540 μmol) and **9b** (620 mg 180 μmol) were added to DMF (1.5 mL) in a 0.5−2 mL microwave vial. The solution was heated in a microwave at 120 °C for 8 min. Afterward water (5 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 10 mL); the organic layers were combined and then extracted with water (5 × 10 mL) and brine (10 mL) and dried over Na_2SO_4. The solvent was removed by rotary evaporation and the product was isolated as a white wax. Yield 34 mg, 42%; TLC (5% MeOH/CH_2Cl_2) R_f = 0.30. ^{1}H NMR (600.13 MHz, $CDCl_3$) δ 8.16 (dd, J = 1.5, 1.5 Hz, 1H, NHCOC$_6H$H$_3$I), 7.79−7.77 (m, 2H, NHCOC$_6H_2$H$_2$I), 7.24 (br s, NHCH$_2$CH$_2$O), 7.14 (dd, J = 7.8, 7.8 Hz, 1H, NHCOC$_6H$H$_3$I), 3.71 (t, J = 6.1 Hz, 2H, NHCH_2CH$_2$O), 3.67−3.60 [m, 12H, (CH_2OCH_2)$_3$], 2.57 (t, J = 6.1 Hz, 2H, CH$_2$CH_2COOH). ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.5, 166.9, 140.8, 136.97, 136.82, 130.7, 127.0, 94.7, 71.08, 70.92, 70.80, 70.75, 70.4, 67.0, 40.6, 35.5. HRMS calcd for $C_{16}H_{23}NO_6$ [M + H]$^+$, 452.0570; found, 452.0566.

*(S)-20-[(S)-2-{[(Benzyloxy)carbonyl]amino}-3-phenylpropanamido]-1-(4-iodophenyl)-1,14,21-trioxo-5,8,11-trioxa-2,15-diazadocosan-22-yl 2,4,6-Trimethylbenzoate (**23a**).* To a solution of **22a** (36 mg, 80 μmol) in anhydrous DMF (5 mL), PyBOP (42 mg, 80 μmol) was added and dissolved at 0 °C under a stream of Ar. Et_3N (33 μL, 240 μmol) was added and the solution was left to stir for 5 min. Afterward, **6a** (56 mg, 80 μmol) was added and the reaction mixture was stirred while warming to room temperature overnight. The solution was made up to 25 mL with water and extracted with CH_2Cl_2 (3 × 10 mL), and the organic layers were combined and then extracted with water (5 × 15 mL) and brine (5 × 10 mL). The solvent was evaporated and the product was obtained as a white solid following flash chromatography (2−20% MeOH/CH_2Cl_2) as a white wax. Yield 18 mg, 22%; TLC (5% MeOH/CH_2Cl_2) R_f = 0.22; HPLC (method B) ^tR = 16.7 min. ^{1}H NMR [600.13 MHz, $(CD_3)_2SO$] δ 8.58 (t, J = 5.6 Hz, 1H, CH$_2$NHCOAr), 8.50 (d, J = 7.4 Hz, 1H, NHCHCONH), 7.85 (d, J = 8.5 Hz, 2H, NHCOC$_6H_2$H$_2$I), 7.79 (t, J = 5.6 Hz, 1H, CH$_2$NHCOCH$_2$), 7.65−7.62 (m, 3H, NHCOC$_6H_2$H$_2$I and NHCHCOCH$_2$), 7.35−7.20 (m, 10H, Ph), 6.92 (s, 2H, Me$_3$C$_6H_2$CO), 4.98 (s, 2H, PhCH_2OCO), 4.92 (d, J = 17.2 Hz, 1H, COCHHO), 4.81 (d, J = 17.2 Hz, 1H, COCHHO), 4.37−4.32 (m, 2H, HNCHCO Phe and Lys), 3.57 (t, J = 6.5 Hz, 2H, COCH_2CH$_2$O), 3.53−3.39 (m, 12H, CH_2CH$_2$OCH$_2$CH$_2$OCH$_2$CH$_2$), 3.05−3.01 (m, 3H, CHCHHPh and CH_2NHCOCH$_2$), 2.82 (dd, J = 13.6, 9.7 Hz, 1H, CHCHHPh), 2.30 (m, 2H, COCH$_2$CH$_2$O), 2.28 [s, 6H, 2,6-(CH_3)$_3$C$_6$H$_2$CO], 2.26 [s, 3H, 4-(CH_3)$_3$C$_6$H$_2$CO], 1.83−1.77 (m, 1H, CHCHHCH$_2$CH$_2$), 1.61−1.53 (m, 1H, CHCHHCH$_2$CH$_2$), 1.41−1.24 (m, 4H, CHCH$_2$CH_2CH_2). ^{13}C NMR (150.92 MHz; $(CD_3)_2SO$): δ 202.6, 172.0, 169.8, 168.2, 165.6, 155.8, 139.1, 137.8, 137.1, 136.9, 134.9, 133.8, 129.9, 129.2, 129.1, 128.3, 128.2, 128.1, 127.7, 127.5, 126.3, 98.8, 69.7, 69.6, 69.6, 69.5, 68.8, 66.8, 66.6, 65.3, 56.0, 55.9, 40.1, 38.2, 37.2, 36.1, 29.1, 28.7, 22.3, 20.7, 19.3. HRMS calcd for $C_{50}H_{62}N_4O_{11}I$ [M + H]$^+$, 1021.3460; found, 1021.3456.

*(S)-20-[(S)-2-{[(Benzyloxy)carbonyl]amino}-3-phenylpropanamido]-1-(3-iodophenyl)-1,14,21-trioxo-5,8,11-trioxa-2,15-diazadocosan-22-yl 2,4,6-Trimethylbenzoate (**23b**).* To a solution of **22b** (26 mg 57 μmol) in anhydrous DMF (5 mL), PyBOP (30 mg, 57 μmol) was added and dissolved at 0 °C under a stream of Ar. Et_3N (8 μL, 57 μmol) was added and the solution was left to stir for 5 min. Afterward, **6a** (40 mg, 57 μmol) and Et_3N (16 μL, 114 μmol) were added, and the reaction mixture was stirred and allowed to warm to room temperature overnight. The solution was made up to 25 mL with water and extracted with CH_2Cl_2 (3 × 10 mL); the organic layers were combined and then extracted with water (5 × 15 mL) and brine (5 × 10 mL). The solvent was evaporated and the product was obtained as a white solid following flash chromatography (5% MeOH/CH_2Cl_2). Yield 0.216 g, 37%; mp 79−86 °C; TLC (10% MeOH/CH_2Cl_2) R_f = 0.51; HPLC (method B) ^tR = 16.7 min. ^{1}H NMR [600.13 MHz, $(CD_3)_2SO$] δ 8.61 (t, J = 5.5 Hz, 1H, CH$_2$NHCOAr), 8.50 (d, J = 7.4 Hz, 1H, NHCHCONH), 8.19 (m, 1H, NHCOC$_6H$H$_3$I), 7.89−7.87 (m, 1H, NHCOC$_6H$H$_3$I), 7.86−7.85 (m, 1H, NHCOC$_6H$H$_3$I), 7.79 (t, J = 5.7 Hz, 1H, CH$_2$NHCOCH$_2$), 7.64 (d, J = 8.2 Hz, 1H, NHCHCOCH$_2$), 7.34−7.18 (m, 11H, Ph and NHCOC$_6H$H$_3$I), 6.92 (s, 2H, Me$_3$C$_6H_2$CO), 4.97 (d, J = 5.2 Hz, 2H, PhCH_2OCO), 4.91 (d, J = 17.2 Hz, 1H, COCHHO), 4.80 (d, J = 17.2 Hz, 1H, COCHHO), 4.36−4.33 (m, 2H, HNCHCO Phe and Lys), 3.57 (t, J = 6.5 Hz, 2H, COCH_2CH$_2$O), 3.53−3.38 (m, 12H, CH_2CH$_2$OCH$_2$CH$_2$OCH$_2$CH$_2$), 3.05−3.01 (m, 3H, CHCHHPh and CH_2NHCOCH$_2$), 2.82 (dd, J = 13.6, 9.7 Hz, 1H, CHCHHPh), 2.29 (d, J = 6.5 Hz, 1H, COCH$_2$CHHO), 2.27 [s, 6H, 2,6-(CH_3)$_3$C$_6$H$_2$CO], 2.25 [s, 3H, 4-(CH_3)$_3$C$_6$H$_2$CO], 1.82−1.78 (m, 1H, CHCHHCH$_2$CH$_2$), 1.60−1.53 (m, 1H, CHCHHCH$_2$CH$_2$), 1.40−1.24 (m, 4H, CHCH$_2$CH_2CH_2). ^{13}C NMR [150.92 MHz, $(CD_3)_2SO$] δ 202.6, 172.0, 172.0, 169.8, 168.2, 155.8, 139.6, 139.1, 137.8, 136.9, 136.4, 135.6, 134.9, 130.5, 129.9, 129.2, 128.3, 128.2, 128.1, 127.7, 127.5, 126.6, 126.3, 94.6, 69.7,

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69.7, 69.6, 69.5, 68.7, 66.8, 66.6, 65.3, 56.0, 55.9, 40.0, 38.2, 37.2, 36.1, 29.1, 28.7, 22.4, 20.7, 19.3. HRMS calcd for $C_{50}H_{62}N_4O_{11}I$ $[M + H]^+$, 1021.3460; found, 1021.3484.

*tert-Butyl 3-(2-{2-[2-(4-Tributylstannyl-1H-1,2,3-triazol-1-yl)-ethoxy]ethoxy}ethoxy)propanoate (**26**).* Tributyl(ethynyl)stannane (0.432 g, 1.37 mmol) and **25** (0.308 g, 1.80 mmol) were combined in toluene (3 mL) and heated to reflux at 120 °C for overnight. The solvent was evaporated and the product was isolated as a colorless oil following flash chromatography (12−100% EtOAc/hexanes). Yield 0.468 g, 66%; TLC (1:1 EtOAc/Hex) R_f = 0.33. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.60 (s, 1H, triazole-H), 4.57 (t, J = 5.3 Hz, 2H, NCH_2CH_2O), 3.87 (t, J = 5.3 Hz, 2H, NCH_2CH_2O), 3.70 (t, J = 6.6 Hz, 2H, $CH_2CH_2COO^tBu$), 3.59−3.58 [m, 8H, $(OCH_2CH_2)_2$], 2.49 (t, J = 6.7 Hz, 2H, $CH_2CH_2COO^tBu$), 1.58−1.52 [m, 6H, $Sn(CH_2CH_2CH_2)_3$], 1.44 [s, 9H, $(CH_3)_3COOC$], 1.32 [m, 6H, $Sn(CH_2CH_2CH_2)_3$], 1.12−1.09 [m, 6H, $Sn(CH_2CH_2CH_2)_3$], 0.88 [t, J = 7.3 Hz, 9H, $(CH_2CH_2CH_2CH_3)_3$]. ^{13}C NMR (150.92 MHz, $CDCl_3$) δ 171.2, 144.5, 131.3, 80.9, 70.96, 70.89, 70.73, 70.1, 67.3, 49.9, 36.6, 29.4, 28.4, 27.6, 10.2. HRMS calcd for $C_{27}H_{53}N_3O_5Sn$ $[M + H]^+$, 620.3091; found, 620.3077.

*tert-Butyl 3-(2-{2-[2-(4-Iodo-1H-1,2,3-triazol-1-yl)ethoxy]ethoxy}-ethoxy)propanoate (**27**).* To a solution of **26** (0.303 g, 0.49 mmol) in THF (10 mL), iodine (0.137 g, 0.54 mmol) was added and left to stir for 2 h. The solution volume was made up to 25 mL with EtOAc and extracted with saturated $Na_2S_2O_3$ (3 × 20 mL) and brine (20 mL) and dried over $MgSO_4$. The solvent was removed by rotary evaporation, and the product was isolated as a clear colorless oil following flash chromatography (12−100% EtOAc/hexanes). Yield 0.187 mg, 84%; TLC (1:1 EtOAc/Hex) R_f = 0.18. ^{1}H NMR (600.13 MHz, $CDCl_3$) δ 7.90 (s, 1H, triazole-H), 4.57 (t, J = 4.9 Hz, 2H, NCH_2CH_2O), 3.85 (t, J = 4.9 Hz, 2H, NCH_2CH_2O), 3.72 (t, J = 6.5 Hz, 2H, $CH_2CH_2COO^tBu$), 3.62−3.60 [m, 8H, $(OCH_2CH_2)_3$)] 2.50 (t, J = 6.5 Hz, 2H, $CH_2CH_2COO^tBu$), 1.44 [s, 9H, $(CH_3)_3COOC$]. ^{13}C NMR (150.92 MHz, $CDCl_3$) δ 171.2, 130.6, 87.2, 80.9, 70.91, 70.80, 70.79, 69.6, 67.3, 51.0, 36.6, 28.5. HRMS calcd for $C_{15}H_{27}N_3O_5I$ $[M + H]^+$, 456.0995; found, 456.0981.

*3-(2-{2-[2-(4-Iodo-1H-1,2,3-triazol-1-yl)ethoxy]ethoxy}ethoxy)-propanoic Acid (**28**).* To a solution of **27** (0.150 g, 0.33 mmol) in CH_2Cl_2 (10 mL) was added TFA (5 mL) dropwise at 0 °C, and the reaction mixture was left to stir for 3 h. The solvent was coevaporated with Et_2O, giving the product as a clear colorless oil. Yield 0.161 g, >99%; TLC (5% MeOH/CH_2Cl_2) R_f = 0.23. ^{1}H NMR (600 MHz, $CDCl_3$) δ 11.02 (br s, CH_2COOH), 7.94 (s, 1H, triazole-H), 4.60 (t, J = 4.9 Hz, 2H, NCH_2CH_2O), 3.87 (t, J = 4.9 Hz, 2H, NCH_2CH_2O), 3.80 (t, J = 6.1 Hz, 2H, CH_2CH_2COOH), 3.68−3.67 (m, 2H, CH_2), 3.64−3.60 (m, 6H, CH_2), 2.67 (t, J = 6.1 Hz, 2H, CH_2CH_2COOH). ^{13}C NMR (150.92 MHz, $CDCl_3$) δ 176.4, 131.1, 86.0, 70.5, 70.4, 70.4, 70.3, 69.1, 66.4, 50.9, 34.8. HRMS calcd for $C_{11}H_{19}N_3O_5I$ $[M + H]^+$, 400.0370; found, 400.0368.

*(S)-18-[(S)-2-{[(Benzyloxy)carbonyl]amino}-3-phenylpropanamido]-1-(4-iodo-1H-1,2,3-triazol-1-yl)-12,19-dioxo-3,6,9-trioxa-13-azaicosan-20-yl 2,4,6-Trimethylbenzoate (**29**).* To a solution of **28** (0.037 g, 0.091 mmol) in anhydrous DMF (10 mL), PyBOP (0.047 g, 0.091 mmol) was added and dissolved at 0 °C under a stream of Ar. Et_3N (0.020 mL, 0.182 mmol) was added and the solution was left to stir for 5 min. Afterward, **6a** (0.040 g, 0.057 mmol) was added and the reaction mixture was stirred and allowed to warm to room temperature overnight. The solution was made up to 25 mL with water and extracted with CH_2Cl_2 (3 × 20 mL), and the combined organic layers were extracted with water (5 × 10 mL) and brine (3 × 20 mL). The solvent was evaporated and the product was obtained as a white solid following flash chromatography (1−20% MeOH/CH_2Cl_2). Yield 0.22 g, 37%; mp 91−95 °C; TLC (10% MeOH/CH_2Cl_2) R_f = 0.67; HPLC (method B) ^tR = 17.1 min. ^{1}H NMR [600.13 MHz, $(CD_3)_2SO$] δ 8.50 (d, J = 7.5 Hz, 1H, NHCHCONH), 7.79 (t, J = 5.6 Hz, 1H, $CH_2NHCOCH_2$), 7.64 (d, J = 8.2 Hz, 1H, $NHCHCOCH_2$), 7.34−7.18 (m, 10H, Ph), 6.91 (s, 2H, $Me_3C_6H_2CO$), 4.97 (s, 2H, $PhCH_2OCO$), 4.91 (d, J = 17.2 Hz, 1H, COCHHO), 4.80 (d, J = 17.2 Hz, 1H, COCHHO), 4.36−4.32 (m, 2H, HNCHCO Phe and Lys), 3.63−3.56 (m, 4H, $COCH_2CH_2O$), 3.47 (m, 12H, $CH_2CH_2OCH_2CH_2OCH_2CH_2$), 3.03−2.99 (m, 3H, CHCHHPh and $CH_2NHCOCH_2$), 2.82−2.81 (m, 1H, CHCHHPh), 2.27 [s, 6H, 2,6-$(CH_3)_3C_6H_2CO$], 2.25 [s, 3H, 4-$(CH_3)_3C_6H_2CO$], 1.63−1.54 (m, 2H, $CHCH_2CH_2CH_2$), 1.40−1.32 (m, 4H, $CHCH_2CH_2CH_2$). ^{13}C NMR [150.92 MHz, $(CD_3)_2SO$] δ 202.8, 172.1, 169.9, 168.4, 156.0, 139.3, 137.9, 137.1, 135.1, 130.6, 130.1, 128.41, 128.37, 128.23, 128.14, 127.84, 127.66, 126.5, 88.5, 69.85, 69.80, 69.77, 69.71, 69.68, 69.65, 69.62, 67.0, 65.4, 56.20, 56.05, 38.3, 37.4, 36.3, 30.9, 29.2, 28.9, 20.8, 19.5. HRMS calcd for $C_{45}H_{58}N_6O_{10}I$ $[M + H]^+$, 969.3259; found, 969.3243.

*6-{4-[Tris(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)stannyl]-benzamido}hexanoic Acid (**31**).* 1,3,5,7-Tetramethyl-6-(2,4-dimethoxyphenyl)-2,4,8-trioxa-6-phosphaadamantane (8.0 mg, 27 μmol) and $Pd(OAc)_2$ (3.0 mg, 13 μmol), each dissolved in degassed THF (1 mL), were added to a 2−5 mL microwave vial purged with Ar and mixed together for 15 min. Tris(1*H*,1*H*,2*H*,2*H*-perfluorooctyl)tin hydride (850 mg, 730 μmol) dissolved in THF (1 mL) was added, and the solution was stirred for 15 min. A solution of **10a** (120 mg, 332 μmol) dissolved in THF (1 mL) was then added, and the reaction mixture was heated by microwave irradiation at 85 °C for 30 min. The mixture was filtered through silica/KF (3:1) and then further purified by column chromatography [1−5% MeOH/CH_2Cl_2 (with 1% AcOH)]. The desired fractions were then diluted with water and the product, a white wax, was obtained following rotary evaporation. Yield 117 mg, 35%; TLC (5% MeOH/CH_2Cl_2) R_f = 0.57. ^{1}H NMR (600.13 MHz, $CDCl_3$) ^{1}H NMR (600 MHz; $CDCl_3$) δ 7.77 (d, J = 8.1 Hz, 2H ArH), 7.51−7.42 (m, 2H, ArH), 6.25 (t, J = 5.8 Hz, 1H, $NHCH_2CH_2$), 3.48−3.45 (m, J = 6.6 Hz, 2H, $NHCH_2CH_2$), 2.37 (t, J = 7.3 Hz, 2H, CH_2CH_2COOH), 2.62−2.35 [m, 6H, $Sn(CH_2CH_2)_3$], 1.71−1.67 (m, 2H, CH_2CH_2COOH), 1.66−1.62 (m, 2H, $NHCH_2CH_2$), 1.48−1.42 (m, 2H, $CH_2CH_2CH_2COOH$), 1.38−1.33 [m, 6H, $Sn(CH_2CH_2)_3$]. ^{13}C NMR (150.92 MHz; $CDCl_3$) δ 167.6, 141.5, 136.4, 135.8, 127.1, 40.1, 33.9, 29.5, 27.8, 26.6, 24.5, −1.0. HRMS calcd for $C_{37}H_{29}NO_3F_{39}Sn$ $[M + H]^+$, 1396.0554; found, 1396.0468.

*(S)-3-[(S)-2-{[(Benzyloxy)carbonyl]amino}-3-phenylpropanamido]-2-oxo-7-[6-({4-[tris(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)-stannyl]phenyl}amino)hexanamido]heptyl 2,4,6-Trimethylbenzoate (**32**).* To a solution of **31** (42 mg, 30 μmol) in anhydrous DMF (5 mL) was added PyBOP (16 mg, 30 μmol), and the solids were allowed to dissolve at 0 °C under a stream of Ar. Et_3N (4.0 μL, 30 μmol) was added and the solution was left to stir for 5 min. Afterward, **6a** (21 mg, 30 μmol) and Et_3N (8 μL, 60 μmol) were added and the solution was stirred and allowed to warm to room temperature overnight. Water (20 mL) was added and the solution was extracted with CH_2Cl_2 (3 × 10 mL); the combined organic layers were extracted with water (5 × 10 mL) and brine (3 × 10 mL) and then dried over Na_2SO_4. The solvent was evaporated and the product was obtained as a white wax following semipreparative HPLC (method B). Yield 0.008 g, 14%; TLC (5% MeOH/CH_2Cl_2) R_f = 0.36; HPLC (method B) ^tR = 25.1 min. ^{1}H NMR [500.13 MHz, $(CD_3)_2SO$] δ 8.48 (d, J = 7.3 Hz, 1H NHCHCONH), 8.40 (t, J = 4.8 Hz, 1H, $CH_2NHCOAr$), 7.80 (d, J = 7.8 Hz, 2H, $COC_6H_2H_2Sn$), 7.70 (t, J = 5.4 Hz, 1H, $CH_2NHCOCH_2$), 7.63−7.59 (m, 3H, $NHCHCOCH_2$ and $COC_6H_2H_2Sn$), 7.33−7.18 (m, 10H, Ph), 6.90 (s, 2H, $Me_3C_6H_2CO$), 4.96 (d, J = 4.4 Hz, 2H, $PhCH_2OCO$), 4.90 (d, J = 17.5 Hz, 1H, COCHHO), 4.80 (d, J = 17.0 Hz, 1H, COCHHO), 4.35−4.32 (m, 2H, HNCHCO Phe and Lys), 3.24−3.20 (m, 2H, $CH_2NHCOAr$), 3.04−2.99 (m, 3H, CHCHHPh and $CH_2NHCOCH_2$), 2.83−2.79 (m, 1H, CHCHHPh), 2.47−2.36 [m, 6H, $Sn(CH_2CH_2)_2$], 2.26 [s, 6H, 2,6-$(CH_3)_3C_6H_2CO$], 2.24 [s, 3H, 4-$(CH_3)_3C_6H_2CO$], 2.04 (t, J = 7.3 Hz, 2H, $CH_2NHCOCH_2$), 1.82−1.75 (m, 1H, $CHCHHCH_2CH_2$), 1.57−1.46 (m, 4H, $CHCHHCHHCH_2$ and $NHCOCH_2CH_2$), 1.39−1.21 [m, 11H, $CHCH_2CHHCH_2$, CH_2CH_2NHCO, and Sn-$(CH_2CH_2)_3$]. HRMS calcd for $C_{71}H_{68}N_4O_8Sn$ $[M + H]^+$, 1965.3455; found, 1965.2988.

*1-Oxo-1-{4-[tris(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)-stannyl]phenyl}-5,8,11-trioxa-2-azatetradecan-14-oic acid (**33**).* 1,3,5,7-Tetramethyl-6-(2,4-dimethoxyphenyl)-2,4,8-trioxa-6-phosphaadamantane (4 mg, 15 μmol) and $Pd(OAc)_2$ (2 mg, 7 μmol), each

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dissolved in degassed THF (1 mL), were added to a 2−5 mL microwave vial purged with Ar and mixed together for 15 min. Tris(1*H*,1*H*,2*H*,2*H*-perfluorooctyl)tin hydride (470 mg, 400 μmol) dissolved in THF (1 mL) was added, and the solution was stirred for 15 min. A solution of **22a** (82 mg, 182 μmol) dissolved in THF (1 mL) was then added, and the solution was heated by microwave irradiation at 80 °C for 20 min. The mixture was filtered through Celite and the product was isolated as a white wax following reverse-phase flash chromatography (30−100% CH_3CN/H_2O; 100% THF). Yield 0.095 g, 35%; TLC (5% $MeOH/CH_2Cl_2$) R_f = 0.58. ^{1}H NMR (600.13 MHz, $CDCl_3$) δ 7.79 (d, *J* = 8.1 Hz, 2H, Ar*H*), 7.46 (d, *J* = 8.1 Hz, 2H, Ar*H*), 6.91 (t, *J* = 0.5 Hz, 1H, N*H*CH_2CH_2O), 3.76−3.56 [m, 14H NHCH_2CH_2O and $(CH_2OCH_2)_3$], 2.55 (t, *J* = 6.4 Hz, 2H, CH_2CH_2COOH), 2.35−2.29 [m, 6H, Sn$(CH_2CH_2)_3$], 1.32 [t, *J* = 8.2 Hz, 6H, Sn$(CH_2CH_2)_3$]. HRMS calcd for $C_{49}H_{35}NO_6F_{39}Sn$ $[M + H]^+$, 1486.0873; found, 1486.0826.

*(S)-20-[(S)-2-{[(Benzyloxy)carbonyl]amino}-3-phenylpropanamido]-1,14,21-trioxo-1-{4-[tris(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)stannyl]phenyl}-5,8,11-trioxa-2,15-diazadocosan-22-yl 2,4,6-Trimethylbenzoate (**34**).* To a solution of **33** (38 mg, 26 μmol) in anhydrous DMF (5 mL) was added PyBOP (140 mg, 26 μmol), and the solids were allowed to dissolve at 0 °C under a stream of Ar. Et_3N (7 μL, 52 μmol) was added and the solution was left to stir for 5 min. Afterward, **6a** (21 mg, 30 μmol) and Et_3N (4 μL, 26 μmol) were added and the solution was stirred while warming to room temperature overnight. Water (20 mL) was added and the solution was extracted with CH_2Cl_2 (3 × 10 mL); the combined organic layers were extracted with water (5 × 10 mL) and brine (3 × 10 mL) and then dried over Na_2SO_4. The solvent was evaporated and the product was obtained as a white wax following semipreparative HPLC (method B). Yield 6 mg, 12%; TLC (5% $MeOH/CH_2Cl_2$) R_f = 0.09; HPLC (method B) ^tR = 25 min. ^{1}H NMR [600.13 MHz, $(CD_3)_2SO$] δ 8.51−8.47 (m, 2H, CH_2NHCOAr and NHCHCONH), 7.81 (d, *J* = 8.1 Hz, 2H, NHCOC$_6H_2H_2$Sn), 7.77 (t, *J* = 5.6 Hz, 1H, CH_2NHCOCH$_2$), 7.62 (d, *J* = 8.2 Hz, 1H, NHCHCOCH$_2$), 7.60−7.55 (m, 2H, NHCOC$_6H_2H_2$Sn), 7.32−7.18 (m, 10H, Ph), 6.89 (s, 2H, $Me_3C_6H_2CO$), 4.96 (s, 2H, PhCH_2OCO), 4.90 (d, *J* = 17.2 Hz, 1H, COC*H*HO), 4.79 (d, *J* = 17.3 Hz, 1H, COC*H*HO), 4.35−4.31 (m, 2H, HNC*H*CO Phe and Lys), 3.55 (t, *J* = 6.5 Hz, 2H, COCH_2CH_2O), 3.51−3.39 (m, 12H, $CH_2CH_2OCH_2CH_2OCH_2CH_2$), 3.04−3.00 (m, 3H, CHC*H*HPh and CH_2NHCOCH$_2$), 2.81 (dd, *J* = 13.6, 9.5 Hz, 1H, CHC*H*HPh), 2.46−2.37 [m, 6H, Sn$(CH_2CH_2)_3$], 2.28 (d, *J* = 6.5 Hz, 2H, COCH$_2CH_2O$), 2.24 [s, 6H, 2,6-$(CH_3)_3C_6H_2CO$], 2.23 [s, 3H, 4-$(CH_3)_3C_6H_2CO$], 1.80−1.77 (m, 1H, CHC*H*HCH$_2$CH$_2$), 1.59−1.52 (m, 1H, CHC*H*HCH$_2$CH$_2$), 1.38−1.22 [m, 10H, CHCH$_2CH_2CH_2$ and Sn$(CH_2CH_2)_3$]. HRMS calcd for $C_{74}H_{77}F_{39}N_5O_{11}Sn$ $[M + NH_4]^+$, 2072.4038; found, 2072.3989.

Screening Studies. *Optimization of Enzyme Concentration.* Cathepsin B (500 nM) was preincubated for 30 min at room temperature in a solution of 5 mM dithiothreitol (DTT) and 0.01% (v/v) Tween-20. Cathepsin B was added to the wells of a 96-well microplate containing the assay buffer [25 mM K_2PO_4, 1 mM ethylenediaminetetraacetic acid (EDTA), 250 mM NaCl, and 3% (v/v) DMSO, pH = 6.0], and Cbz-Arg-Arg-pNA (50 μL) was added, resulting in enzyme concentrations from 1.25 to 25 nM and substrate concentration of 1 mM. Formation of the *p*-nitroanilide (pNA) product was monitored for 73 min at 405 nm at 37 °C. Measurements were obtained in triplicate. Initial rates (ν_0) were measured from the slope obtained during the first 10 min.

Determination of K_m. Cathepsin B (200 nM) was preincubated for 30 min at 37 °C in a solution of 5 mM DTT and 0.01% (v/v) Tween-20. Cathepsin B (5 μL) was added to the wells of a 96-well microplate containing the assay buffer. Cbz-Arg-Arg-pNA (50 μL) was then added, resulting in an enzyme concentration of 5 nM and substrate concentrations from 125 μM to 3 mM. Formation of the pNA product was monitored for 15 min at 405 nm at 37 °C. Measurements were obtained in triplicate. Initial rates (ν_0) were measured from the slope obtained during the first 10 min. K_m was determined from $\nu_0 = V_{max}[S]/(K_m + [S])$ and solved by use of GraphPad Prism software.

Determination of Inhibition Constants. Cathepsin B (200 nM) was preincubated for 30 min at 37 °C in a solution of 5 mM DTT and 0.01% (v/v) Tween-20. The substrate, Cbz-Arg-Arg-pNA (25 or 40 μL), and the AOMKs (50 μL) were added to the wells of a 96-well microplate containing the assay buffer. The reaction was initiated by addition of cathepsin B, resulting in substrate concentrations of 500 or 800 μM, inhibitor concentrations from 25 nM to 1 μM, and an enzyme concentration of 5 nM. Formation of the pNA product was monitored for 60 min at 405 nm at 37 °C. Measurements were obtained in triplicate. Absorbance versus time measurements were analyzed by nonlinear regression to determine the pseudo-first-order rate constant (k_{obs}) as Abs = $Ae^{-k_{obs}t} + B$. The second-order rate constant (k_i/K_i), the apparent inactivation rate (k_i), and the inhibition constant (K_i) (where possible) were determined from $k_{obs} = k_i[I]/(K_i + [I])$ for hyperbolic relationships or $k_{obs} = (k_i/K_i)[I]$ for linear relationships. Equations were solved by use of GraphPad Prism software.

Radiochemical Methods. *^{125}I-(S)-3-[(S)-2-{[(Benzyloxy)carbonyl]amino}-3-phenylpropanamido]-7-[6-(4-iodobenzamido)hexanamido]-2-oxoheptyl 2,4,6-Trimethylbenzoate ([^{125}I]**11a**).* To a tube containing iodogen (5 μg, 0.01 μmol) in EtOH (25 μL) was added **32** (100 μg, 0.05 μmol) in EtOH (100 μL). AcOH (5 μL) and [^{125}I]NaI (18.5 MBq) in 0.1% NaOH (10 μL) were added, and after 10 min the reaction was quenched with 0.1 M $Na_2S_2O_5$ (25 μL). The reaction mixture was diluted with water (1.5 mL) and loaded onto a FSPE cartridge (1 g). The FSPE cartridge was preactivated by washing with dimethylformamide (1 mL) and water (6 mL). Unreacted [^{125}I]NaI was eluted with water (10 mL) and the pure product was eluted with 80% EtOH (10 mL), while the excess precursor remained on the cartridge. Radiochemical yield (RCY) = 32−36% (*n* = 3); specific activity >23.6 GBq/μmmol; HPLC ^tR = 14.8 min; log *P* (pH 7.4) = 1.05 ± 0.01.

*^{125}I-(S)-20-[(S)-2-{[(Benzyloxy)carbonyl]amino}-3-phenylpropanamido]-1-(4-iodophenyl)-1,14,21-trioxo-5,8,11-trioxa-2,15-diazadocosan-22-yl 2,4,6-Trimethylbenzoate ([^{125}I]**23a**).* To a tube containing iodogen (2.5 μg, 0.005 μmol) in EtOH (25 μL) was added **34** (100 μg, 0.05 μmol) in EtOH (100 μL). AcOH (5 μL) and [^{125}I]NaI (18.5 MBq) in 0.1% NaOH (10 μL) were added, and after 10 min the reaction was quenched with 0.1 M $Na_2S_2O_5$ (25 μL). The reaction mixture was diluted with water (1.5 mL) and loaded onto a FSPE cartridge (1 g). The FSPE cartridge was preactivated by washing with dimethylformamide (1 mL) and water (6 mL). Unreacted [^{125}I]NaI was eluted with water (10 mL) and the pure product was eluted with 50% EtOH (10 mL), while the excess precursor remained on the cartridge. RCY = 26−35% (*n* = 3); specific activity >23.1 GBq/μmmol; HPLC ^tR = 14.1 min; log *P* (pH 7.4) = 0.95 ± 0.01.

Determination of Log P (pH 7.4). A solution of [^{125}I]**23** or [^{125}I]**11** (444 kBq) in 10% EtOH in phosphate-buffered saline (PBS, 300 μL) was added to a 1:1 mixture of *n*-octanol/PBS (pH 7.4) (2 mL); the mixture was vortexed for 20 min and centrifuged for 30 min at 6000 rpm. Aliquots (60 μL) were removed from each layer in triplicate and added to preweighed tubes. The tubes were reweighed and the amount of radioactivity was measured on a γ counter. The partition coefficient, *P*, was calculated as *P* = activity concentration in *n*-octanol/activity concentration in aqueous buffer.

Labeling of Cathepsin B with ABPs. Human liver cathepsin B (1 μg) was incubated in 1× binding buffer (5 mM Tris, pH = 5.5, 5 mM $MgCl_2$, and 2 mM DTT) containing RIPA buffer (100 mM Tris, pH = 8, 50 mM NaCl, 1% IGEPAL CA-630, 0.5% sodium deoxycholate, and 0.1% SDS) in the presence of either of CA-074 or DMSO for 1 h on ice. [^{125}I]**11a** or [^{125}I]**23a** (approximately 1 × 10^6 counts per minute, cpm) was added to each sample to give a total volume of 30 μL, and the mixtures were incubated for 2 h at 0 °C. The assay was stopped by the addition of 6× sample buffer (9% SDS, 60% glycerol, 375 mM Tris, pH 6.8, 0.015% bromophenol blue, and 12% β-mercaptoethanol) to a final concentration of 1× and incubated at room temperature for 30 min. Following incubation, aliquots (30 μL) of the mixtures were loaded onto a 10% Mini-Protean TGX precast gel and analyzed by SDS−PAGE. Gels were incubated overnight in gel-shrinking solution (65% methanol, 0.5% glycerol) while shaking at 4 °C. Gels were dried

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for 2 h at room temperature by use of a commercial gel-drying kit and then exposed to a phosphor imaging screen for 72 h.

Animal Model. MDA-MB-231 cells were purchased from the American Type Culture Collection (ATCC) and cultured in accordance with supplier guidelines. Animal studies were approved by the Animal Research Ethics Board at McMaster University in accordance with Canadian Council on Animal Care (CCAC) guidelines. Female CD1 nu/nu mice (5−6 weeks old) were obtained from Charles River Laboratories (Senneville, QC) and were maintained under specific-pathogen-free (SPF) conditions with 12 h light/dark cycles and given food and water ad libitum. To create the tumor xenograft model, CD1 nu/nu female mice were injected with 2.0×10^6 MDA-MB-231 cells in 100 μL of PBS/Matrigel (1:1; BD Biosciences, Mississauga, ON, and Invitrogen, Burlington, ON, respectively) subcutaneously into the right flank. Tumors were allowed to grow for approximately 2−3 weeks prior to biodistribution studies.

*Biodistribution Studies. [^{125}I]**11a**.* CD1 nu/nu mice (n = 15) were administered approximately 259 kBq of [^{125}I]**11a** (3.7 GBq/mL) in 10% EtOH in sterile PBS via tail vein injection. After 30 min, 5 h, and 23 h, groups of mice (n = 5) were anesthetized with 3% isoflurane and euthanized via exsanguination, and the indicated organs and tissues were collected by dissection and weighed. Activities were measured on a γ counter and reported as percent injected dose per gram (ID/g) of each organ, tissue, or fluid.

*[^{125}I]**23a**.* These studies were performed in a similar manner as for [^{125}I]**11a**, except CD1 nu/nu mice (n = 9) were administered approximately 74 kBq of [^{125}I]**23a** (3.7 GBq/mL).

■ ASSOCIATED CONTENT

Supporting Information

Four schemes depicting synthesis of **6a**/**6b**, **30**, **32**, and **34**; three figures showing optimization of enzyme concentration and determination of K_m, K_i, and k_i/K_i; additional text with synthetic details; and 151 aqdditional figures with ^{1}H NMR, ^{13}C NMR, and HRMS spectroscopic data for **1**−**34**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

AOMK, acyloxymethyl ketone; ABP, activity-based probe; Boc, *tert*-butoxycarbonyl; Cbz, carboxybenzyl; DMSO, dimethyl sulfoxide; DOTA, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid; DTT, dithiothreitol; FSPE, fluorous solid-phase extraction; HPLC, high-pressure (performance) liquid chromatography; IBCF, isobutylchloroformate; ID/g, injected dose per gram; NIRF, near-infrared fluorescent/fluorescence; NMI, nuclear molecular imaging; NMM, *N*-methylmorpholine; PEG, poly(ethylene glycol); PET, positron emission tomography; pi, postinjection; pNA, *p*-nitroanilide; PyBOP, benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; SPECT, single photon emission computed tomography; TFA, trifluoroacetic acid

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