# Supporting Information Available

Increasing Time on Target: Utilization of Inhibitors of Cysteine Cathepsins to Enhance the Tumor Retention of Receptor-Targeted Agents

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# Table of Contents

1.	General	Experimental Information	
	1.	Materials	
	2.	Instrumentation	1-2
2.	Chemis	try	
	1.	Synthesis of AOMKs with Linkers (CCTAs)	3-6
	2.	Synthesis of Inactive Controls with Linkers	7-8
	3.	Synthesis of CCTA-incorporated, NTSR1-Targeted Peptides	9-10
	4.	Synthesis of Inactive Control, NTSR1-Targeted Peptides	11
	5.	Synthesis of Cy5-lableled, NTSR1-Targeted Peptides	
	6.	Radiolabeling of NTSR1-Targeted Peptides with 177LuCl <sub>3</sub>	13
3.	In Vitro	Study Figures and Procedures	
	1.	Distribution Coefficient (LogD <sub>7.4</sub> )	14
	2.	Competitive Receptor Binding Studies	
	3.	Cathepsin B Inhibition (IC50) Studies	14-15
	4.	Determining the Cathepsin B Inhibition Constants	
	5.	Cellular Internalization Studies	
	6.	Experimental Procedures of Cellular Efflux Studies	
	7.	Confocal Microscopy	20-21
	8.	Adduct Formation Investigated by SDS-PAGE and GPC	
	9.	Serum Stability Studies	24
4.	In Vivo	Studies	
	1.	Biodistribution Studies	
	2.	Evaluation of Tissue Adduct Formation of the Radioconjugates	
	3.	Investigation of Cysteine Proteases Trapping of the Cy 5-labeled Conjugates	27
5.	NMR ar	nd Mass Spectra	28-60
6.	Referer	ice made op oon all	

## 1 General Experimental Information

## 1.1 Materials

N,N-dimethylformamide (DMF), dichloromethane (DCM), petroleum ether (PE), methanol, ethyl acetate, acetonitrile, formic acid, acetone, diethyl ether, trifluoroacetic acid (TFA), pyridine, piperidine and N-methylpyrrolidone (NMP) were purchased from Fisher Scientific (Fair Lawn, NJ). Fluorenylmethyloxycarbonnyl (Fmoc)-protected natural amino acids, N-(Carbobenzyloxy)-Lphenylalanine, H-Lys(Boc)-OH, Fmoc-Tle-OH, Fmoc-L-Gly(Propargyl)-OH and N/Ndisopropylethylamine (DIEA) were purchased from Chem-Impex International (Wood Dale, IL). Isobutyl chloroformate (IBCF), 4-methylmorpholine (NMM), hydrobromic acid (48 wt. % in H<sub>2</sub>O), 2azidoacetic acid, 1-butanol, ascorbic acid, triethylamine (TEA), Ethylenediaminetetraacetic acid (EDTA) Brij®35 and Diazald® were obtained from Sigma-Aldrich (St Louis, MO). The diazomethane was prepared from Diazald<sup>®</sup> according to the reported method<sup>1</sup>. Potassium fluoride (KF), 2,4,6-Trimethylbenzoic acid, phenethylamine were purchased from Alfa Aesar (Haverhill, MA). Fmoc-D-Ser(t-Bu)-OH was purchased from NovaBiochem (Hoherbrunn, Germany). (1-Cyano-2-ethoxy-2oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU) was purchased from AK Scientific (Union City, CA), Fmoc-Leu-SASRIN™ resin (200-400 mesh), Fmoc-Gly-SASRIN™ resin (200-400 mesh), Z-Phe-Arg-AMC and N-(3-Dimethylaminopropyl)-N'ethylcarbodiimide hydrochloride (EDC) were obtained from Bachem (Bubendorf, Switzerland). Fmoc-N-Me-Arg(Pbf)-OH was produced by ChemPep. (Wellington, FL). Fmoc-2,6-dimethyl-L-tyrosine (Dmt) was purchased from Key Organics (Camelford, UK). Cyanine5 carboxylic acid (Cy 5) was obtained from Lumiprobe (Hunt Valley, MD). N<sub>3</sub>-PEG-COOH was purchased from PurePEG (San Diego, CA). DOTA-NHS ester was produced by Macrocyclics (Plano, TX). Lutetium-177 chloride (177LuCl<sub>3</sub>) was obtained from Oak Ridge National Laboratory (Oak Ridge, TN). CA-074 was purchased from ApexBio (Houston, TX). McCoy's 5A medium (1×; Iwakata & Grace Modification) with L-glutamine was obtained from Mediatech, Inc. (Manassas, VA). Human serum was obtained from MP Biomedicals (Santa Ana, CA). TrypLE Express was obtained from Invitrogen (Grand Island, NY). Penicillin-streptomycin solution and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were procured from HyClone Laboratories, Inc. (Logan, UT). Fetal Bovine Serum (FBS) was purchased from Gibco by Life Technologies Corporation (Grand Island, NY). BD Cytofix Fixation buffer was obtained from BD Biosciences (San Jose, CA). Novex<sup>™</sup> Tris-Glycine SDS sample buffer, Pierce<sup>™</sup> RIPA buffer, PageRuler<sup>™</sup> Prestained protein ladder, Halt™ Protease inhibitor cocktail, LysoTracker Green DND-26, NucBlue® Live ReadyProbe®, Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (Alexa Fluor 488), Immobilon<sup>™</sup>-P PVDF transfer membranes, Pierce<sup>™</sup> western blotting filter papers, NuPAGE<sup>®</sup> sample reducing reagent (10×), Tween<sup>M</sup> 20, and transfer or electro blotting buffer (10X) were purchased from Thermo Fisher Scientific (Waltham, MA). Cathepsin B (D1C7Y) XP® Rabbit mAb and animal-free blocking solution (5X) were purchased from Cell Signaling Technology (Danvers, MA). Amicon Ultra-4 centrifugal filter (10 kDa) was purchased from Merck Millipore (Burlington, MA). Five weeks old female SCID mice were purchased from Charles River Laboratories. The human colon cancer cell line HT-29 was obtained from American Type Culture Collection and cultured under vendorrecommended conditions. All procedures utilizing animals conform to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, and were approved by the Institutional Animal Care and Use Committee at the University of Nebraska Medical Center.

#### 1.2 Instrumentation:

Peptides were synthesized by solid phase peptide synthesis (SPPS) on a Liberty microwave peptide synthesizer from CEM. A Waters e2695 system equipped with a Waters 2489 absorption detector and a Waters Qtof Micro electrospray ionization mass spectrometer was used to perform high performance liquid chromatography/mass spectrometry analyses. <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectrums were recorded on a Bruker Avance-III HD 600 MHz instrument using deuterium oxide as the solvent. A Phenomenex

Jupiter C12 Proteo 250 × 10 mm semi-prep column was used for the purification of bulk amounts of peptides. Evaluation and purification of radiolabeled conjugates were performed on a Waters 1515 binary pump equipped with a Waters 2489 absorption detector and a Bioscan Flow Count radiometric detector system. The Gel Permeation Chromatography (GPC) analysis was carried out in an Agilent PL aquagel-OH MIXED-H Gel column equipped with Radiomatic<sup>™</sup> 150TR flow scintillation analyzer. The radioactivity of the cell samples and tissue homogenates was quantified by Multi-Wiper<sup>™</sup> multi-well wipe test counter. Gamma decay detection of <sup>177</sup>Lu-labeled conjugates for biodistribution studies was accomplished using a NaI (TI) well detector constructed by AlphaSpectra Inc. Fluorescence intensities were measured by a SpectraMax<sup>®</sup> M5 multimode plate reader. Lab-Tek chambered #1.0 borosilicate coverglass disks (4 well) were used for confocal cell imaging. Confocal microscopy images were taken on a Leica LSM510 META Microscope equipped with an argon laser. The fluorescent images were acquired and quantified on the IVIS<sup>®</sup> Spectrum in vivo imaging system. Autoradiography was recorded via BAS storage phosphor screens and scanned by GE Lifesciences Typhoon FLA 9500 variable mode imager.

# 2 Chemistry

2.1 Synthesis of AOMK electrophiles with different linkers



Scheme S1. Synthesis of compounds 9a-9e

Compound 5 was synthesized according to the reported method<sup>2</sup>

Compound 6: This compound was prepared by published procedure<sup>3</sup> with slight modification. Compound **5** (1.1 g, 2 mmol) and NMM (330  $\mu$ l, 3 mmol) were dissolved in anhydrous THF (50 mL) and stirred under nitrogen at 0 °C. Isobutylchloroformate (IBCF) (400  $\mu$ L, 3 mmol) in THF (5 ml) was added and the solution was stirred for another 30 min. To this mixture at -15 °C, a freshly prepared solution of diazomethane (150 mmol) in 200 ml ether was carefully dropped in during 30 min and stirred for 2 h at room temperature. A solution of 47 wt. % HBr and acetic acid (6 ml, v/v =1:2) was added to the yellowish mixture in 5 min and stirred for additional 20 min at 0 °C. Brine (200 ml) was poured into the flask and the organic phase was separated and washed twice with saturated NaHCO<sub>3</sub> (100 mL), water (100 mL) and dried over Na<sub>2</sub>SO<sub>4</sub>. The organic layer was evaporated to dryness and was purified by flash column chromatography (silica gel, PE/acetone = 10:3) to afford as a yellow powder (1.05 g, 83%). <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.34-7.18 (m, 10H), 6.47 (m, 1H), 5.37 (s, 1H), 5.09 (s, 2H), 4.73 (br s, 1H), 4.65 (br s, 1H), 4.44 (br s, 1H), 3.87 (s, 2H), 3.08 (m, 4H), 1.85 (s, 1H), 1.63 (br s, 1H), 1.55-1.42 (m, 11H), 1.25-1.20 (m, 2H). <sup>13</sup>C-NMR (125 MHz; CDCl<sub>3</sub>):  $\delta$  199.9, 171.1, 156.1, 136.0, 129.3, 128.8, 128.6, 128.3, 128.1, 127.3, 79.3, 67.2, 56.2, 56.1, 39.8, 38.1, 31.8, 30.8, 29.4, 28.4, 22.1. LRMS-ESI (m/z): [M+H]+ calcd. for C<sub>29</sub>H<sub>38</sub>BrN<sub>3</sub>O<sub>6</sub>H+ 604.2, found 604.2.

Compound 7: Compound 6 (750 mg, 1.24 mmol), 2,4,6-trimethylbenzoic acid (225 mg, 1.36 mmol) and KF (215 mg, 3.72 mmol) were suspended in anhydrous DMF (7 ml) under nitrogen at room temperature. The mixture was kept stirring for overnight before adding in water (50 ml). The product was extracted with ethyl acetate (70 ml). The organic layer was washed twice with brine (50 ml) and dried over Na<sub>2</sub>SO<sub>4</sub>. The solvent was removed by rotary evaporation and the product was purified by flash column chromatography (silica gel, PE/acetone = 4:1) to give the product as a white powder (744 mg, 87%). <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.32-7.18 (m, 10H), 6.87 (s, 2H), 6.55 (br s, 1H), 5.40 (br s, 1H), 5.06 (s, 2H), 4.90-4.72 (dd, *J* = 47.2, 12.8 Hz, 2H), 4.72 (br s, 1H), 4.63 (m, 1H), 4.46 (m, 1H), 3.09 (d, *J* = 5.2 Hz, 2H), 3.06 (br s, 2H), 2.36 (s, 6H), 2.29 (s, 3H), 1.89 (m, 1H), 1.65-1.60 (m, 2H), 1.42 (s, 10H), 1.25 (m, 2H). <sup>13</sup>C-NMR (125 MHz; CDCl<sub>3</sub>):  $\delta$  171.3, 171.0, 170.6, 170.0, 167.2, 74.8, 74.7, 74.2, 61.2, 61.1, 54.6, 53.7, 53.1, 52.5, 41.5, 27.5, 27.4, 27.3. LRMS-ESI (m/z): [M+H]<sup>+</sup> calcd. for C<sub>39</sub>H<sub>49</sub>N<sub>3</sub>O<sub>8</sub>H<sup>+</sup> 688.4, found 688.3.

Compound 1a: To a solution of compound 7 (500 mg, 0.73 mmol) in DCM (15 mL), TFA (5 mL) was added dropwise at 0 °C. The solution was stirred at room temperature for 2 h. The mixture was concentrated by rotary evaporation to a volume of about 5 ml and precipitated in ice cold ether (45 ml). The solid was collected by filtration, washed three times with cold ether (30 mL) and dried under vacuum for overnight to yield a white powder (407 mg, 95%). <sup>1</sup>H-NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO):  $\delta$  8.55 (d, J = 6.0 Hz, 1H), 7.67 (s, J = 6.4 Hz, 1H), 7.35-7.18 (m, 10H), 4.97 (s, 2H), 4.91-4.77 (dd, *J* = 45.2, 13.6 Hz, 2H), 4.37-4.31 (m, 2H), 3.05-3.01 (m, 1H), 2.86-2.81 (m, 1H), 2.74 (d, *J* = 5.6 Hz, 1H), 2.27 (s, 6H), 2.25 (s, 3H), 1.82 (m, 1H), 1.57-1.52 (m, 3H), 1.35-1.31 (m, 2H). <sup>13</sup>C-NMR (125 MHz; (CD<sub>3</sub>)<sub>2</sub>SO):  $\delta$ 202.6, 172.0, 168.3, 163.0, 155.9, 139.1, 137.7, 136.9, 134.9, 130.0, 129.3, 128.3, 128.2, 128.1, 127.7, 127.6, 127.5, 126.4, 66.6, 65.3, 56.1, 55.6, 38.6, 37.2, 28.8, 26.5, 21.8, 20.7, 19.4, 19.3. LRMS-ESI (m/z): [M+H]<sup>+</sup> calcd. for C<sub>34</sub>H<sub>41</sub>N<sub>3</sub>O<sub>6</sub> H<sup>+</sup> 588.3, found 588.3.

Compound 8: This compound was obtained by SPPS. Fmoc-Gly-SASRIN™ resin (250 mg, 0.2 mmol) was deprotected by 20% piperidine in DMF (7 mL) to expose the primary amine. Fmoc-D-Ser(t-Bu)-OH (384 mg, 1 mmol) was coupled to the resin in the presence of COMU (428 mg, 1 mmol) and DIEA (180 µl, 2.0 mmol) in DMF (5 mL). This process of deprotection and conjugation was repeated for the further conjugation of Fmoc-D-Ser(t-Bu)-OH (384 mg, 1 mmol) and 2-azidoacetic acid (76 μl, 1 mmol) until the desired peptide was synthesized. Cleavage of the peptide from resin was achieved by shaking the resin with 1% TFA in dry DCM (5 × 3 mL) for 2 min. The filtrates were immediately neutralized with 5% pyridine in methanol (1 mL) and evaporated to dryness which was redissolved in methanol (1 mL) and precipitated in cold water (50 mL) to yield the crude peptides. The peptide was purified by a semipreparative Proteo C12 HPLC column with a 15 min gradient and a flow rate of 5.0 mL/min (40% - 90% ACN in water containing 0.1% formic acid) to give compound 5 as a white powder (85 mg, 71%). 1H-NMR (400 MHz, CDCl<sub>3</sub>): δ 7.69 (d, J = 5.2 Hz, 1H), 7.39 (t, J = 4.4 Hz, 1H), 7.28 (m, 1H), 7.15 (d, J = 6.4 Hz, 1H), 4.56 (dt, J = 4.4, 2.0 Hz, 1H), 4.50 (q, J = 3.2 Hz, 1H), 4.42 (dt, J = 5.2, 3.2 Hz, 1H), 4.13 (m, 1H), 4.05-3.97 (m, 3H), 3.91-3.79 (m, 3H), 3.50-3.43 (m, 3H), 1.25 (s, 9H), 1.22 (s, 9H), 1.18 (s, 9H). <sup>13</sup>C-NMR (125 MHz; CDCl<sub>3</sub>): δ 171.3, 171.0, 170.6, 170.0, 167.2, 74.8, 74.7, 74.2, 61.2, 61.1, 54.6, 53.7, 53.1, 52.5, 41.5, 27.5, 27.4, 27.3. LRMS-ESI (m/z): [M+H]<sup>+</sup> calcd. for C<sub>25</sub>H<sub>45</sub>N<sub>7</sub>O<sub>9</sub>H<sup>+</sup> 588.3, found 588.2.

General procedure for synthesis of compounds 9b-9e: To a solution of the azido-linker (0.1 mmol) and NHS (17 mg, 0.15 mmol) in DMF (1 mL) was added EDCI (38 mg, 0.2 mmol) at 0 °C. The mixture was kept stirring for 2 h at room temperature after which a solution of compound 1a (50 mg, 85  $\mu$ mol) and DIEA (54  $\mu$ L, 0.3 mmol) in DMF (500  $\mu$ L) and was added at 0°C. The mixture was allowed to warm up to room temperature and was stirred overnight. The crude product was partitioned in ethyl acetate (50 mL) and water (50 mL) and the organic layer was separated and dried over Na<sub>2</sub>SO<sub>4</sub>. The product was concentrated in vacuum and purified by silica gel chromatography.

Compounds 9b: Chromatography solvent system (silica gel, PE/acetone = 4:1), white powder (37 mg, 65%). <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.35-7.20 (m, 10H), 6.87 (s, 2H), 6.81 (d, *J* = 5.2 Hz, 1H), 6.49 (br s,

1H), 5.53 (d, J = 5.2 Hz, 1H), 5.07 (s, 2H), 4.99-4.73 (dd, J = 30.4, 13.6 Hz, 2H), 4.53 (m, 2H), 3.99-3.88 (dd, J = 28.8, 13.2 Hz, 1H), 3.36 (m, 1H), 3.24-3.15 (m, 1H), 3.08 (d, J = 5.2 Hz, 2H), 2.36 (s, 6H), 2.29 (s, 3H), 1.90-1.88 (m, 1H), 1.72-1.69 (m, 1H), 1.63-1.45 (m, 2H), 1.28 (br s, 2H). <sup>13</sup>C-NMR (125 MHz; CDCI<sub>3</sub>):  $\delta$  201.5, 201.4, 171.5, 169.2, 167.5, 156.0, 139.8, 136.2, 136.0, 129.4, 129.3, 129.2, 128.8, 128.7, 128.6, 128.3, 128.1, 128.0, 127.2,67.1, 66.4, 56.1, 55.7, 55.4, 52.6, 38.4, 38.0, 29.7, 28.9, 21.5, 21.4, 21.2, 20.0. LRMS-ESI (m/z): [M+H]<sup>+</sup> calcd. for C<sub>36</sub>H<sub>42</sub>N<sub>6</sub>O<sub>7</sub>H<sup>+</sup> 671.3, found 671.2.

Compounds 9c: Chromatography solvent system (silica gel, PE/acetone = 3:1), white powder (34 mg, 53%). <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.34-7.22 (m, 10H), 7.12 (br s, 1H), 7.05 (d, *J* = 5.2 Hz, 1H), 6.86 (s, 2H), 5.67 (d, *J* = 6.0 Hz, 1H), 5.07 (q, *J* = 4.0 Hz, 2H), 4.82 (s, 2H), 4.57 (d, *J* = 5.6 Hz, 1H), 4.48 (br s, 1H), 3.96 (q, *J* = 12.4 Hz, 1H), 3.66-3.61 (m, 12H), 3.87 (s, 2H), 3.37 (t, *J* = 3.6, 2H), 3.18-3.09 (m, 2H), 2.36 (s, 6H), 2.28 (s, 3H), 1.89 (m, 1H), 1.76 (m, 1H), 1.66-1.51 (m, 2H), 1.31 (br s, 2H). <sup>13</sup>C-NMR (125 MHz; CDCl<sub>3</sub>):  $\delta$  201.7, 171.7, 170.9, 169.1, 155.9, 139.7, 136.4, 136.3, 136.0, 129.6, 129.4, 128.7, 128.5, 128.2, 128.0, 127.1, 71.0, 70.7, 71.5, 70.3, 70.0, 67.0, 66.5, 56.0, 55.9, 50.7, 38.6, 37.2, 29.4, 29.3, 21.5, 21.2, 20.0. LRMS-ESI (m/z): [M+H]<sup>+</sup> calcd. for C<sub>42</sub>H<sub>54</sub>N<sub>6</sub>O<sub>10</sub>H<sup>+</sup> 803.4, found 803.1.

Compounds 9e: Chromatography solvent system (silica gel, DCM/methanol = 10:1), white powder (55 mg, 56%). <sup>1</sup>H-NMR (400 MHz, CDCI<sub>3</sub>):  $\delta$  7.69 (d, *J* = 4.4 Hz, 1H), 7.46 (br s, 1H), 7.33-6.98 (m, 12H), 6.98 (d, *J* = 5.2 Hz, 1H), 6.86 (s, 2H), 5.98 (d, *J* = 6.4 Hz, 1H), 5.05 (s, 2H), 4.80 (s, 2H), 4.62-4.57 (m, 2H), 4.56 (br s, 1H), 4.42 (br s, 1H), 4.36 (m, 1H), 3.96 (s, 2H), 3.94-3.77 (m, 6H), 3.51-3.41 (m, 2H), 3.27 (m, 1H), 3.19-3.16 (m, 2H), 3.05 (m, 1H), 1.87 (m, 1H), 1.65 (m, 1H), 1.55-1.49 (m, 2H), 1.31 (m, 2H), 1.24 (s, 9H), 1.21 (s, 9H), 1.13 (s, 9H). <sup>13</sup>C-NMR (125 MHz; CDCI<sub>3</sub>):  $\delta$  201.7, 171.9, 171.0,170.9, 169.1, 167.3, 156.2, 139.7, 136.3, 135.9, 129.6, 129.4, 128.6, 128.5, 128.1, 128.0, 126.9, 74.8, 74.0, 67.0, 66.6, 61.1, 60.9, 56.1, 56.0, 55.0, 54.7, 53.2, 52.4, 43.3, 38.4, 38.3, 29.8, 27.5, 27.4, 21.4, 21.1, 20.0 LRMS-ESI (m/z): [M+H] + calcd. for C<sub>59</sub>H<sub>84</sub>N<sub>10</sub>O<sub>14</sub>H + 1157.6, found 1157.3.

Compound 9d: To a solution of compound 6e (25 mg, 22  $\mu$ mol) was deprotected with 50% TFA in DCM (200  $\mu$ L) for 3 h. The solvent was removed under nitrogen flow. The residue was purified by a semi-preparative Proteo C12 HPLC column with a 15 min gradient and a flow rate of 5.0 mL/min (50%-80% ACN in water containing 0.1% formic acid) to give compound 6d as a white powder (15 mg, 69%). <sup>1</sup>H-NMR (400 MHz, CDCI<sub>3</sub>):  $\delta$  8.50-8.49 (m, 1H), 8.22 (d, *J* = 6.4 Hz, 1H), 8.18 (d, *J* = 6.0 Hz, 1H), 8.08 (m, 1H), 7.98 (d, *J* = 6.0 Hz, 1H), 7.67-7.63 (m, 2H), 7.33-7.26 (m, 10H), 7.19 (m, 1H), 6.92 (s, 2H), 5.10-5.05 (m, 3H), 4.97 (s, 2H), 4.88-4.81 (dd, *J* = 42.4, 13.2 Hz, 2H), 4.44 (q, *J* = 6.4 Hz, 1H), 4.38-4.33 (m, 3H), 4.25 (q, *J* = 6.4 Hz, 1H), 3.89 (s, 2H), 3.67-3.64 (m, 4H), 3.61-3.58 (m, 4H), 3.04-2.95 (m, 3H), 2.87-2.75 (m, 1H), 2.27 (s, 6H), 2.25 (s, 3H), 1.79 (m, 1H), 1.55 (m, 1H), 1.39 (m, 2H), 1.23 (m, 2H). <sup>13</sup>C-NMR (125 MHz; CDCI<sub>3</sub>):  $\delta$  202.8, 172.2, 170.3, 170.1, 168.5, 168.4, 167.6, 156.0, 139.3, 137.9, 137.1, 135.1, 130.1, 129.4, 128.5, 128.4, 127.9, 127.7, 126.5, 66.8, 65.5, 62.0, 61.6, 56.2, 56.0, 55.6, 55.3, 54.9, 50.7, 42.3, 38.5, 29.2, 28.7, 22.5, 20.8, 19.5. LRMS-ESI (m/z): [M+H]+ calcd. for C<sub>47</sub>H<sub>60</sub>N<sub>10</sub>O<sub>14</sub>H+ 989.4, found 989.3.

Compound 10: To a solution of compound 5 (1.1 g, 2 mmol) in DCM (30 mL), TFA (10 mL) was added dropwise at 0 °C. The solution was stirred at room temperature for 2 h. The mixture was concentrated by rotary evaporation to a volume of about 5 ml and precipitated in ice cold ether (100 ml). The solid was collected by filtration, washed 3 times with cold ether (30 mL), and dried under vacuum. To the deprotected product in methanol (50 mL) was dropped in the solution of CuSO<sub>4</sub> (7 mg, 44  $\mu$ mol) at 0 °C. NaHCO<sub>3</sub> (672 mg, 8 mmol) and imidazole-1-sulfonyl azide hydrochloride (627 mg, 3 mmol) were added to the mixture, and the pH was adjusted to 9 with 0.1N NaOH. The mixture was stirred for overnight at room temperature before the pH was acidified at 1 using 1N HCl. The product was extracted with ethyl acetate (200 mL), washed twice with brine (100 ml), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated in vacuum. Purification of the crude product by flash column chromatography (silica gel, DCM/methanol = 10:1) gave compound **7** as a white powder (707 mg, 78%). <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.30-7.16 (m, 10H), 6.62 (br s, 1H), 5.59 (d, *J* = 5.2 Hz, 1H), 5.06 (dd, *J* = 11.2, 10.0 Hz, 2H), 4.54-4.50 (m, 2H), 3.20 (t, *J* = 5.6 Hz, 2H), 3.06 (s, 2H), 1.85 (m, 1H), 1.66 (m, 1H), 1.53 (m, 2H), 1.26

(br s, 2H). <sup>13</sup>C-NMR (125 MHz; CDCl<sub>3</sub>):  $\delta$  174.9, 171.4, 156.3, 136.0, 129.3, 128.7, 128.6, 128.3, 128.0, 127.2, 67.3, 56.2, 52.1, 51.0, 38.2, 31.4, 28.3, 22.3. LRMS-ESI (m/z): [M+H]<sup>+</sup> calcd. for C<sub>23</sub>H<sub>27</sub>N<sub>5</sub>O<sub>5</sub>H<sup>+</sup> 454.2, found 454.1.

Compound 11: This compound was synthesized by the method for compound 6. The product was purified by flash column chromatography (silica gel, PE/acetone = 6:1) to afford as a yellow powder (519 mg, 89%). <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.38-7.17 (m, 10H), 6.32 (d, *J* = 5.6 Hz, 1H), 5.25 (br s, 1H), 5.10 (d, *J* = 2.0 Hz, 2H), 4.76-4.72 (dt, *J* = 10.4, 6.4 Hz, 1H), 4.41 (dt, *J* = 10.8, 5.2 Hz, 1H), 3.85 (d, *J* = 2.0, 2H), 3.22 (t, *J* = 5.2 Hz, 2H), 3.15-3.02 (m, 2H), 1.86 (m, 1H), 1.60-1.48 (m, 3H), 1.26 (m, 2H). <sup>13</sup>C-NMR (125 MHz; CDCl<sub>3</sub>):  $\delta$  199.7, 171.0, 135.9, 129.3, 128.9, 128.6, 128.4, 128.2, 127.4, 67.3, 56.4, 56.0, 51.0, 38.0, 31.7, 30.9, 28.3, 22.4. LRMS-ESI (m/z): [M+H]<sup>+</sup> calcd. for C<sub>24</sub>H<sub>28</sub>BrN<sub>5</sub>O<sub>4</sub>H<sup>+</sup> 530.1, found 530.0.

Compound 9a: This compound was synthesized by the method for compound 7. The product was purified by flash column chromatography (silica gel, PE/acetone = 3:1) to afford as a yellow powder (375 mg, 81%). <sup>1</sup>H-NMR (400 MHz, CDCI<sub>3</sub>):  $\delta$  7.35-7.18 (m, 10H), 6.87 (s, 2H), 6.38 (d, *J* = 4.4 Hz, 1H), 5.25 (br s, 1H), 5.10 (s, 2H), 4.89-4.74 (dd, *J* = 47.2, 13.2 Hz, 2H), 4.54 (m, 1H), 4.43 (d, *J* = 4.8 Hz, 1H), 3.21 (t, *J* = 5.6 Hz, 2H), 3.15-3.04 (m, 2H), 2.36 (s, 6H), 2.29 (s, 3H),1.92 (m, 1H), 1.58-1.32 (m, 3H), 1.30 (m, 2H). <sup>13</sup>C-NMR (125 MHz; CDCI<sub>3</sub>):  $\delta$  201.2, 171.0, 169.0, 139.9, 136.0, 129.3, 128.9, 128.6, 128.3, 128.1, 127.3, 67.3, 66.3, 56.3, 55.3, 51.0, 38.1, 30.6, 28.4, 22.1, 21.2, 20.0. LRMS-ESI (m/z): [M+H]+ calcd. for C<sub>34</sub>H<sub>39</sub>N<sub>5</sub>O<sub>6</sub>H+ 614.3, found 614.2.

#### 2.2 Synthesis of inactive controls with different linkers



Scheme S2. Synthesis of compounds 13a and 13b

Compound 12: To a solution of compound 1 (0.6 g, 1.1 mmol) and NHS (138 mg, 1.2 mmol) in DMF (5 mL) was added EDCI (276 mg, 1.4 mmol) at 0 °C. The mixture was kept stirring for 2 h at room temperature. The solution of phenethylamine (151  $\mu$ L, 1.2 mmol) and DIEA (522  $\mu$ L, 3 mmol) in DMF (2 mL) and was added at 0°C. The mixture was allowed to warm up to room temperature and was stirred overnight. Water (50 mL) was poured into the mixture and the crude product was extracted twice with in ethyl acetate (50 mL). The combined organic layer was separated, washed twice with brine (50 mL), and dried over Na<sub>2</sub>SO<sub>4</sub>. The product was concentrated and purified by flash column chromatography (silica gel, PE/acetone = 5:1) to give the product as a white powder (596 mg, 86%). <sup>1</sup>H-NMR (400 MHz, CDCI<sub>3</sub>):  $\delta$  7.36-6.14 (m, 15H), 6.34 (d, *J* = 6.0 Hz, 1H), 6.05 (br s, 1H), 5.31 (br s, 1H), 5.07 (s, 2H), 4.62 (br s, 1H), 4.39 (d, *J* = 5.2 Hz, 1H), 4.26 (d, *J* = 4.8 Hz, 1H), 3.50-3.39 (m, 2H), 3.06-3.03 (m, 4H), 2.78 (d, *J* = 5.6 Hz, 2H), 1.78 (m, 1H), 1.49 (m, 1H), 1.52-1.34 (m, 11H), 1.01 (br s, 2H). <sup>13</sup>C-NMR (125 MHz; CDCI<sub>3</sub>):  $\delta$  7.0.9, 170.7, 156.1, 138.7, 136.0, 129.2, 128.8, 128.7, 128.6, 128.5, 128.3, 128.1, 128.0, 127.2, 126.6, 67.3, 56.4, 53.1, 40.7, 35.5, 31.5, 29.4, 28.5, 22.5. LRMS-ESI (m/z): [M+H]+ calcd. for C<sub>36</sub>H<sub>46</sub>N<sub>4</sub>O<sub>6</sub>H+ 631.3, found 631.3.

Compound 1b: The deprotection of compound 12 was carried out according to the same method for compound 1a. The product was recovered in cold ether and obtained as a white solid (314 mg, 93%). <sup>1</sup>H-NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO):  $\delta$  8.11-7.94 (dd, *J* = 64.4, 6.4 Hz, 1H), 7.95 (s, 1H), 7.67-7.52 (dd, *J* = 54.4, 6.0 Hz, 1H), 7.33-7.20 (m, 15H), 5.95 (d, *J* = 10.4 Hz, 2H), 4.29 (br s, 1H), 4.20-4.11 (dd, *J* = 32.8, 4.4 Hz, 1H), 3.31-3.27 (m, 2H), 3.01-2.91 (m, 1H), 2.81-2.66 (m, 5H), 1.57-1.41 (m, 3H), 1.23 (s, 1H), 1.02 (br s, 2H). <sup>13</sup>C-NMR (125 MHz; (CD<sub>3</sub>)<sub>2</sub>SO):  $\delta$ 171.3, 171.1, 171.0, 156.0, 155.8, 139.3, 139.2, 138.0, 137.7, 137.0, 136.9, 129.3, 129.2, 128.7, 128.6, 128.3, 128.0, 127.7, 127.4, 127.3, 126.3, 126.1, 65.3, 65.2, 56.3, 56.1, 52.4, 52.2, 48.6, 38.8, 38.7, 37.5, 35.0, 31.7, 31.2, 27.0, 26.9, 22.1, 22.0. LRMS-ESI (m/z): [M+H]+ calcd. for C<sub>31</sub>H<sub>38</sub>N<sub>4</sub>O<sub>4</sub>H+ 531.3, found 531.2.

Compound 13a: This compound was synthesized according to the method for compound 9a-9c. The product was purified by flash column chromatography (silica gel, PE/acetone = 4:1), white powder (45 mg, 34%). <sup>1</sup>H-NMR (400 MHz, CDCI<sub>3</sub>):  $\delta$  7.34-7.11 (m, 15H), 7.10 (t, *J* = 4.8 Hz, 1H), 6.82 (d, *J* = 5.6 Hz, 1H), 6.18 (br s, 1H), 5.63 (d, *J* = 2.8 Hz, 1H), 5.05 (q, *J* = 6.0 Hz, 2H), 4.49 (d, *J* = 5.6 Hz, 1H), 4.21 (br s, 1H), 3.95 (q, *J* = 6.4 Hz, 2H), 3.66-3.61 (m, 12H), 3.42 (m, 2H), 3.37 (t, *J* = 4.0 Hz, 2H), 3.15-3.06 (m, 2H), 3.04-2.74 (dt, *J* = 118, 4.8 Hz, 2H), 1.82 (m, 1H), 1.70 (m, 1H), 1.51-1.45 (m, 2H), 1.23-1.19 (m, 2H). <sup>13</sup>C-

NMR (125 MHz; CDCl<sub>3</sub>):  $\delta$  171.6, 171.0, 170.8, 156.1, 138.9, 136.4, 136.2, 129.3, 128.8, 128.7, 128.6, 128.5, 128.3, 128.2, 128.0, 127.1, 126.5, 71.0, 70.7, 70.5, 70.4, 70.3, 70.2, 70.1, 67.0, 56.2, 53.4, 50.7, 40.8, 38.4, 37.3, 35.6, 30.2, 29.3, 29.2, 22.1, 21.9. LRMS-ESI (m/z): [M+H]<sup>+</sup> calcd. for C<sub>39</sub>H<sub>51</sub>N<sub>7</sub>O<sub>8</sub>H<sup>+</sup> 746.4, found 746.2.

Compound 13b: This compound was synthesized according to the method for compound 9a-9c. The product was purified by flash column chromatography (silica gel, DCM/methanol = 10:1), white powder (79 mg, 30%). <sup>1</sup>H-NMR (400 MHz, CDCI<sub>3</sub>):  $\delta$  8.21 (d, *J* = 6.4 Hz, 1H), 8.01-7.97 (m, 2H), 7.91 (br s, 1H), 7.86 (d, *J* = 6.0 Hz, 1H), 7.62 (m, 1H), 7.48 (d, *J* = 6.8 Hz, 1H), 7.32-7.19 (m, 15H), 4.94 (s, 2H), 4.44 (q, *J* = 6.0 Hz, 1H), 4.39 (q, *J* = 6.4 Hz, 1H), 4.34-4.25 (m, 2H), 4.17 (m, 1H), 3.87 (s, 1H), 3.68 (d, *J* = 4.0 Hz, 1H), 3.52-3.43 (m, 6H), 3.25 (m, 2H), 3.01-2.92 (m, 3H), 2.75 (m, 1H), 2.70 (t, *J* = 6.4 Hz, 2H), 1.62-1.42 (m, 2H), 1.36 (m, 2H), 1.19 (m, 2H), 1.11-1.05 (m, 27H). <sup>13</sup>C-NMR (125 MHz; CDCI<sub>3</sub>):  $\delta$  171.2, 169.5, 169.3, 168.0, 167.4, 155.8, 139.3, 137.0, 129.2, 128.6, 128.3, 128.0, 127.6, 127.4, 126.2, 126.0, 73.1, 73.0, 65.2, 61.7, 61.6, 53.5, 53.1, 52.5, 50.6, 42.1, 38.5, 37.4, 35.0, 28.9, 27.1, 22.6. LRMS-ESI (m/z): [M+H]<sup>+</sup> calcd. for C<sub>56</sub>H<sub>81</sub>N<sub>11</sub>O<sub>12</sub>H<sup>+</sup> 1100.6, found 1100.6.

#### 2.3 Synthesis of AOMK-nerotensin peptide conjugates



Scheme S3. Synthesis of compounds 2a - 2f

General procedure for synthesis of peptides 14a and 14b: The peptides were obtained by SPPS. Briefly, Fmoc-Leu-SASRIN<sup>™</sup> resin (150 mg, 0.1 mmol) was deprotected by 20% piperidine in DMF (7 mL) to expose the primary amine. Fmoc-L-Tle-OH (177 mg, 0.5 mmol) was coupled to the resin in the presence of COMU (214 mg, 0.5 mmol) and DIEA (90  $\mu$ l, 1 mmol) in DMF (5 mL). This process of deprotection and conjugation was repeated until the desired peptide was synthesized. Cleavage of the peptide from resin was achieved by shaking the resin with 1% TFA in dry DCM (5 × 3 mL) for 2 min. The filtrates were immediately neutralized with 5% pyridine in methanol (1 mL) and evaporated to dryness which was redissolved in methanol (1 mL) and precipitated in cold water (50 mL) to yield the crude peptides. The peptides were purified by a semi-preparative Proteo C12 HPLC column with a 15 min gradient and a flow rate of 5.0 mL/min to give the target peptides, see Table S1 for chromatography and characterization details.

General procedure for synthesis of compounds 15a-15f: To the mixture of compound 14 (2  $\mu$ mol) and compound 9 (5  $\mu$ mol) in water/n-butanol/DMF (200 uL, v/v/v=1:1:2) was added CuSO<sub>4</sub> (200  $\mu$ g, 1.25  $\mu$ mol) in water (50  $\mu$ L). After stirring for 5 min, a solution of ascorbic acid (1 mg, 6  $\mu$ mol) in water (50  $\mu$ L) was added to the mixture. The reaction mixture was stirred for 1 h at room temperature under nitrogen. The product was obtained by the purification via a semi-preparative Proteo C12 HPLC column with a 15 min gradient and a flow rate of 5.0 mL/min to give the target compound, see Table S1 for chromatography and characterization details.

General procedure for synthesis of compounds 2a-2f: Compound 15 (1  $\mu$ mol) and DOTA-NHS ester (2.3 mg, 3  $\mu$ mol) were dissolved in DMF (5 mL). The solution was basified with DIEA (0.081 mL, 0.47 mmol) and stirred at room temperature for overnight. The completion of the conjugation reaction was confirmed by HPLC before the removal of the solvent under nitrogen flow. A 90% TFA in DCM (300  $\mu$ L) solution was added and the mixture was stirred at room temperature for 5 h under nitrogen. The solvent was removed by nitrogen flow and the residue was redissolved in DMF (300  $\mu$ L) for the purification via a semi-preparative Proteo C12 HPLC column with a 15 min gradient and a flow rate of 5.0 mL/min to give the target compound, see Table S1 for chromatography and characterization details.

Entity	HPLC purification condition <sup>a</sup>	Calculated Mass <sup>b</sup>	Mass found <sup>c</sup>	Yields (%)
14a	30%-70% ACN in water	928.0	927.8	16.2 <sup>d</sup>
14b	40%-80% ACN in water	1107.1	1106.9	14.5 <sup>d</sup>
15a	40%-90% ACN in water	1234.6	1234.6	38.1
15b	40%-80% ACN in water	1263.2	1263.1	49.1
15c	40%-90% ACN in water	1329.2	1329.2	41.1
15d	40%-80% ACN in water	1506.3	1506.5	31.9
15e	40%-90% ACN in water	1508.3	1508.3	31.9
15f	40%-90% ACN in water	1685.4	1685.4	28.1
2a	20%-50% ACN in water	1125.6	1125.6	67.9
2b	20%-50% ACN in water	1154.1	1153.9	55.7
2c	20%-50% ACN in water	1220.2	1220.0	51.6
2d	20%-50% ACN in water	1315.2	1315.2	49.5
2e	20%-50% ACN in water	1313.2	1313.4	46.6
2f	20%-50% ACN in water	1408.2249 <sup>e</sup>	1408.2242 <sup>e</sup>	63.4

Table S1. HPLC purification condition and mass information of compounds 14a-14b, 15a-15f, and 2a-2f.

a. The eluent contained 0.1% formic acid and was with 15 min gradient.

b. Mass calculated for [M+2H]<sup>2+</sup>

c. Mass spectrum peak found for [M+2H]<sup>2+</sup>

d. Total yields of the SPPS for compound 14

e. The mass were calculated and determined by high resolution mass spectrometry (HRMS)

## 2.4 Synthesis of control nerotensin peptide conjugates



Scheme S4. Synthesis of compounds 3a and 3b

General procedure for synthesis of compounds 16a and16b: These compounds were obtained according to the procedure for synthesizing 15a-15f. The product was purified by the same HPLC system with a 15 min gradient and a flow rate of 5.0 mL/min to give the target compound, see Table S2 for chromatography and characterization details.

General procedure for synthesis of compounds 3a and 3b: These compounds were obtained according to the procedure for synthesizing 2a-2f. The product was purified by the same HPLC system with a 15 min gradient and a flow rate of 5.0 mL/min to give the target compound, see Table S2 for chromatography and characterization details.

Table S2. HPLC p	ourification condition and	I mass information of co	mpounds 16a, 16b	, 3a, and 3b.
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Entity	HPLC purification condition <sup>a</sup>	Calculated Mass <sup>b</sup>	Mass found <sup>c</sup>	Yields (%)
16a	40%-90% ACN in water	1300.7	1300.6	51.3
16b	20%-40% ACN in water	1656.9	1156.9	46.7
3a	40%-90% ACN in water	1191.7	1194.6	56.1
3b	20%-50% ACN in water	1379.7197d	1379.7188 <sup>d</sup>	44.6

a. The eluent contained 0.1% formic acid and was with 15 min gradient.

b. Mass calculated for [M+2H]<sup>2+</sup>

c. Mass spectrum peak found for [M+2H]<sup>2+</sup>

d. The mass were calculated and determined by high resolution mass spectrometry (HRMS)

2.5 Synthesis of Cy5 labeled nerotensin peptide conjugates



Scheme S5. Synthesis of compounds 4a and 4b

General procedure for synthesis of compounds 4a and 4b: To the solution of Cyanine 5 carboxylic acid (1.6 mg, 3  $\mu$ mol) and NHS (1 mg, 9  $\mu$ mol) in 50  $\mu$ L of DMF was added EDCI (2mg, 10  $\mu$ mol). The mixture was stirred at room temperature for 2 h and was added to the solution of 15f or 16b (1  $\mu$ mol) and DIEA (5  $\mu$ L, 28  $\mu$ mol) in DMF (100  $\mu$ L) which was stirred for overnight in the dark at room temperature. The solvent was removed by nitrogen flow before adding in a 90% TFA in DCM (200  $\mu$ L) solution. After 3 h of stirring, the mixture was concentrated to dryness and dissolved in DMF (300  $\mu$ L) for the purification by a semi-preparative Proteo C12 HPLC column with a 15 min gradient and a flow rate of 5.0 mL/min to give the target compound, see Table S3 for chromatography and characterization details.

Table S3. HPLC	purification condition a	nd mass information of	compounds 4a and 4b
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Entity	HPLC purification condition <sup>a</sup>	Calculated Mass <sup>b</sup>	Mass found	Yields (%)
4a	30%-70% ACN in water	724.1419 <sup>c</sup>	724.1420 <sup>c</sup>	37.4
4b	30%-70% ACN in water	709.8893 <sup>c</sup>	709.8894 <sup>c</sup>	42.8

a. The eluent contained 0.1% formic acid and was with 15 min gradient.

b. Mass calculated for [M+3H]<sup>4+</sup>

c. The mass were calculated and determined by high resolution mass spectrometry (HRMS)

#### 2.6 General procedure for the radiolabeling of the conjugates with 177LuCl<sub>3</sub>

An aliquot of the conjugate (50  $\mu$ g) in 0.5 M ammonium acetate buffer (pH 5.5, 100  $\mu$ L) was mixed with a predetermined amount of <sup>177</sup>LuCl<sub>3</sub> (37 MBq (1 mCi)) and incubated at 90 °C for 60 min. Subsequently, CoCl<sub>2</sub> (5 mg, 38.5  $\mu$ mol) was added and incubated for 5 min at 90 °C in order to complex to the unlabeled conjugate and enhance separation. The mixture was purified by HPLC system and the radiolabeling efficiency (RE) was calculated based on the analysis of the chromatograms. To remove organic eluent, the radioactive conjugate was loaded onto an Empore (Eagan, MN) C18 high-performance extraction cartridge followed by washing with water (3 × 3 mL) and elution by ethanol/saline solution (v/v = 6:4, 200  $\mu$ L) to obtain the <sup>177</sup>Lu-labeled radioconjugate for further biological experiments. See Figure S1 and S2 for results.



Figure S1. Radio-HPLC traces of the crude mixture after <sup>177</sup>Lu-labeling of 2a-c and 3a. The purifications were carried by an analytical Proteo C12 HPLC column with a 15 min gradient (20%-40% ACN in water containing 0.1 formic acid) and a flow rate of 1.5 mL/min to give the target compound.



Figure S2. Radio-HPLC traces of the crude mixture after <sup>177</sup>Lu-labeling of 2d-f and 3b. The purifications were carried by an analytical Proteo C12 HPLC column with a 15 min gradient (20%-40% ACN in water containing 0.1 formic acid) and a flow rate of 1.5 mL/min to give the target compound.

# 3 In vitro study

## 3.1 Distribution coefficient (Log D<sub>7.4</sub>) of the conjugates

The distribution coefficient was determined for each <sup>177</sup>Lu-labeled conjugate. In a 1.5 mL centrifuge tube, 0.5 mL of 1-octanol was added to 0.5 mL of PBS (pH 7.4) containing the radiolabeled peptide (500, 000 cpm). The solution was vigorously vortexed for 2 min at room temperature and subsequently centrifuged to yield two immiscible layers. The radioactivity of the aliquots (100  $\mu$ L) taken from each layer were quantified by the gamma counter and the LogD<sub>7.4</sub> for each conjugates was calculated. See Table 1 and Table S4 for resulting values.

## 3.2 In vitro competitive neurotensin receptor binding studies

The IC<sub>50</sub> for the unlabeled conjugates binding to the neurotensin receptors was determined using the HT-29 human colon cancer cell line. In these studies, <sup>177</sup>Lu–N1 (<sup>177</sup>Lu-DOTA- $\beta$ -Ala-[N- $\alpha$ -Me<sup>8</sup>, Dmt<sup>11</sup>, Tle<sup>12</sup>]NT(6–13)), which was synthesized according to our previous publication<sup>4</sup>, served as the competitive radioligand for comparing the relative binding affinities of the conjugates. HT-29 cells (~1 × 10<sup>6</sup>) suspended in 100 µL of McCoy's 5A medium (pH 7.4, 4.8 mg/mL HEPES, and 2 mg/mL BSA) were incubated with <sup>177</sup>Lu–N1 (100,000 cpm, 100 µL) at 37 °C for 45 min in the presence of the conjugates with predetermined concentrations (0.5 nM – 1 µM) in 100 µL of medium. At the end of the incubation, the cells were centrifuged, aspirated, and washed with fresh medium (5 × 500 µL). The cell-associated radioactivity was measured using gamma counter and the IC<sub>50</sub> values determined by nonlinear regression using GraphPad Prism 5. All measurements were in biological triplicate. See Table 1 and Table S4 for resulting values.

## 3.3 The inhibition of cathepsin B activity by the conjugates

The phosphate buffer (0.1 M, pH = 5.8) containing EDTA (1 mM), DTT (2.7 mM), and Brij<sup>®</sup> 35 (0.03%) was prepared before the assay. The solution of Z-Arg-Arg-AMC in the assay buffer (50  $\mu$ L, 1.3 mM) was mixed with the conjugate dissolved in assay buffer at predetermined concentrations (100  $\mu$ L, 0.2 nM – 40  $\mu$ M). The solution of cathepsin B (human Liver) in assay buffer (50  $\mu$ L, 0.544 nM) was added to the mixture which was further incubated at 37 °C for 20 min. The fluorescence of the liberated aminomethylcoumarin at 460 nM using 355 nM excitation was measured and the IC<sub>50</sub> of the cathepsin B inhibition versus the samples without the inhibitor was determined by nonlinear regression using GraphPad Prism 5. All measurements were in biological triplicate. See Table 1 and Table S5 for resulting values.

entry	Log D <sub>7.4</sub>	CatB Ki (nM)	NTR1 IC50 (nM)
$N_1^{[a]}$	$-3.20 \pm 0.02^{[b]}$	-	31 ± 5
9a	$2.56 \pm 0.08$ <sup>[c]</sup>	$54000\pm2300$	-
9b	$1.85 \pm 0.01$	$15000 \pm 34000$	-
9c	$1.74 \pm 0.02$	$6020\pm780$	-
9d	$1.45 \pm 0.03$	$5020 \pm 160$	-

Table S4. The log D7.4, CatB inhibition constant an	d competitive binding	J (IC₅0) to NTR1 of N1 and 9a-d
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[a] The nerotensin peptide N1 (DOTA-β-Ala-[N-α-Me<sup>8</sup>, Dmt<sup>11</sup>, Tle<sup>12</sup>] NT (6–13)) was synthesized as reported<sup>4</sup>

[b] The Log D values were obtained as for the  $^{177}\mbox{Lu-labelled}$  forms of  $N_1.$ 

[c] The Log D was determined by HPLC for 9a-d.

Entity Cathepsin <b>B</b> inhibition IC <sub>50</sub> (nM)		
N <sub>1</sub> <sup>[a]</sup>	>200000[b]	
1a	11±2	
1b	>200000[b]	
9a	2600±500	
9b	2200±700	
9d	290±30	
9f	3500±700	
2a	15±1	
2b	16±1	
2c	46±1	
2d	55±5	
2e	40±3	
2f	38±7	
3a	>200000[b]	
3b	>200000[b]	

Table S5. The inhibition ( $IC_{50}$ ) of cathepsin B (human Liver) activity by the conjugates

[a] The nerotensin peptide N<sub>1</sub> (DOTA- $\beta$ -Ala-[N- $\alpha$ -Me<sup>8</sup>, Dmt<sup>11</sup>, Tle<sup>12</sup>] NT (6–13)) was synthesized as reported<sup>4</sup>

[b] No inhibition was observed in the test range

#### 3.4 Determination of cathepsin B binding constants of the compounds

3.4.1 Determination of the K<sub>m</sub> and V<sub>max</sub> of cathepsin B for Z-Arg-Arg-AMC

The solution of cathepsin B (human Liver) (50  $\mu$ L, 2 nM) in assay buffer used in section 3.3 was mixed with 50  $\mu$ L of solution of substrate Z-Arg-Arg-AMC in the assay buffer at different concentrations ([S]) (25  $\mu$ M, 50  $\mu$ M, 100  $\mu$ M, 500  $\mu$ M and 1mM). The mixture was incubated at 37 °C and the fluorescence of the liberated aminomethylcoumarin at 460 nM using 355 nM excitation was measured at predetermined time points (0, 2, 4, 6, 8, and 10 min). The fluorescence intensity was plotted versus time and the reaction rates (v<sub>0</sub>) were calculated as the slope of the trend lines obtained by liner regression. Km and maximum reaction rate (V<sub>max-obs</sub>) was determined from the equation v<sub>0</sub> = V<sub>max</sub>[S]/(K<sub>m</sub> + [S]) and solved by nonlinear regression using GraphPad Prism 5. All measurements were in biological triplicate. See Figure S3 for data.



Figure S3. Determination of the  $K_m$  of cathepsin B (human Liver) to the substrate Z-Arg-Arg-AMC. (A) Time course of substrate hydrolysis. (B) The nonlinear regression of reaction rate (t = 0 - 10 min) versus the concentration of Z-Arg-Arg-AMC.

## 3.4.2 Determination of the inhibition constant (K<sub>i</sub>) of the compounds to cathepsin B

The cathepsin B (human Liver) in assay buffer (25  $\mu$ L) was mixed with the conjugate (25  $\mu$ L) in 96well plate. After the solution was mixed, the Z-Arg-Arg-AMC in assay buffer (50  $\mu$ L) was added to the well, yielding a final cathepsin B concentration of 1 nM, conjugate concentration ([C]) of 15 nM or 5  $\mu$ M, and substrate concentration ([S]) of 25  $\mu$ M, 50  $\mu$ M, 100  $\mu$ M, 500  $\mu$ M and 1mM. The mixture was incubated at 37 °C and the fluorescence of the liberated aminomethylcoumarin at 460 nM using 355 nM excitation was measured at predetermined time points (0, 2, 4, 6, 8, and 10 min). The fluorescence intensity was plotted versus time with observed reaction rates (v<sub>0-obs</sub>) calculated as the slope of the trend lines obtained by liner regression. The observed rate constant (K<sub>obs</sub>) and observed maximum reaction rate (V<sub>max-obs</sub>) was determined from the equation v<sub>0-obs</sub> = V<sub>max-obs</sub> [S]/(K<sub>obs</sub> + [S]) and solved by nonlinear regression using GraphPad Prism 5. The K<sub>i</sub> was calculated from the equation K<sub>obs</sub> = K<sub>m</sub> (1 + [C]/K<sub>i</sub>). All measurements were in biological triplicate. See Figure S4, S5 and S6 for results.



Figure S4. Representative example for determination of the K<sub>i</sub> of 2f to cathepsin B. (A) Time course of substrate hydrolysis by cathepsin B in the presence of conjugate 2f. (B) The nonlinear regression of reaction rate (t = 0 - 10 min) versus the concentration of Z-Arg-Arg-AMC. The K<sub>i</sub> was calculated from the equation  $K_{obs} = K_m (1 + [C]/K_i)$ .



Figure S5. The nonlinear regression of hydrolysis reaction rate (t = 0 - 10 min) by cathepsin B versus the concentration of Z-Arg-Arg-AMC in the presence of compound 9a (A), 9b (B), 9c (C), and 9d (D). The conjugate concentration was 5  $\mu$ M. The K<sub>i</sub> was calculated from the equation K<sub>obs</sub> = K<sub>m</sub> (1 + [C]/K<sub>i</sub>).



Figure S6. The nonlinear regression of hydrolysis reaction rate (t = 0 - 10 min) by cathepsin B versus the concentration of Z-Arg-Arg-AMC in the presence of conjugate 1a (A), 2a (B), 2b (C), 2c (D), 2d (E), and 2e (F). The conjugate concentration was 15 nM. The K<sub>i</sub> was calculated from the equation  $K_{obs} = K_m (1 + [C]/K_i)$ . All measurements were in triplicate.

#### 3.5 Cell internalization studies

HT-29 cells (~1 × 10<sup>6</sup>) suspended in cell culture medium (100 µL) were incubated with each <sup>177</sup>Lu radioconjugate (100 000 cpm) at 37 °C for 4 h. At 15, 30, 60, 120 and 240 min time points, the culture medium was removed and the cells were washed with fresh medium (5 × 500 µL) to remove the unbound conjugates. The fraction of surface-bound radioactivity was removed by washing the cells twice with an acidic buffer (200 µL, 50 mM glycine–HCl/0.1 M NaCl buffer, pH 2.8). The amount of radioactivity for each fraction was measured by gamma counter. The cellular uptake of the radioconjugates were presented as a percentages of the surface-bound and internalized radioactivity relative to the total activity added to the tube. See Figure S7 for results.



Figure S7. The internalization and surface bound of  $^{177}Lu-2c$  (A) and  $^{177}Lu-2f$  (B) by HT-29 cells. Values are means ± SD (n = 3).

## 3.6 The HT-29 cell efflux studies

The <sup>177</sup>Lu radioconjugate was added to a sterilized 1.5 mL microcentrifuge tube containing HT-29 cells (~1 × 10<sup>6</sup>) suspended in 300 µL of cell culture medium, yielding a final radioactivity concentration of 100 000 cpm/100 µL, and was incubated for 2 h at 37 °C. After the removal of the culture medium, the cells were washed with fresh medium (5 × 500 µL) followed by the addition of 500 µL of fresh medium for the efflux assay. At 0, 1, 2, 4, 8, and 24 h time interval, fresh medium (500 µL) was added to the tube to replace the old medium which was harvested for quantitative analysis of the effluxed radioactivity using a gamma counter. The cells were lysed with a 10% aqueous SDS solution at 24 h to quantify the remaining internalized radioactivity. The effluxed fraction is expressed as a percentage of the total radioactivity added to the tube, which is the sum of the effluxed and internalized fractions obtained from the study. See Figure 2A for results.

#### 3.7 Confocal microscopy

3.7.1 The uptake and cell trafficking studies of the Cy 5 labelled conjugates

The HT-29 cells  $(1.25 \times 10^5 / \text{well})$  in Lab-Tek chambered #1.0 borosilicate coverglass disk (four-well) were pre-incubated with the medium (500 µL) with (blocking) or without nerotensin peptide N1 (10 µM) at 37 °C for 2 h. The conjugate was added to the wells to a concentration of 5 µM and incubated for 2 and 12 h. For the last hour, Lysotracker-green (100 nM) was added to the cells. DAPI was added in the media (15 µg/ml) to stain the nuclei for 5 min prior to imaging. The cells were washed with PBS (400 µL) and fixed with formaldehyde (400 µL) prior to imaging. The images were obtained using an excitation wavelength of 405 nm (blueexcitation), 488 nm (green excitation) and 646 nm (red excitation). ImageJ software was used for the quantifying the fluorescence of Cy5 and the colocalization efficiency. Mean pixel intensities in each image were normalized to the total cell number by counting the number of DAPI-labeled nuclei. See Figure S8 and Figure S9 for results and analysis.



Figure S8. Uptake of conjugate 4a into HT-29 cells at 2 h. (A) Representative confocal images of HT-29 cells incubated with 4a in the absence or presence of NTR1 peptide N1. Scale bar = 50  $\mu$ m. (B) Quantitative analysis of co-localization of the red fluorescence (Cy 5) with the green fluorescence (Lysotracker). (C) The fluorescence uptake of 4a as quantified from the confocal images. All the analysis was performed in 6 random images and the results were presented as mean ± SD, \*\*\*p < 0.001.



Figure S9. Uptake of conjugate 4b into HT-29 cells at 2 h. (A) Representative confocal images of HT-29 cells incubated with 4b in the absence or presence of NTR1 peptide N1. Scale bar = 50  $\mu$ m. (B) Quantitative analysis of co-localization of the red fluorescence (Cy 5) with the green fluorescence (Lysotracker) (C) The fluorescence uptake of 4b as quantified from the confocal images. All the analysis was performed in 6 random images and the results were presented as mean ± SD, \*\*\*p < 0.001.

#### 3.7.2 The cell trapping studies of the Cy 5 labelled conjugates

The HT-29 cells  $(1.25 \times 10^5 / \text{well})$  in Lab-Tek chambered #1.0 borosilicate coverglass disk (four-well) were incubated with the conjugate (5  $\mu$ M) in 500  $\mu$ L of medium for 4 h. The cells were washed with fresh medium and cultured for up to 24 h. At 2 and 22 h time points, Lysotracker-green (100 nM) was added to the cells and incubated for 2 h. DAPI was added in the media (15  $\mu$ g/ml) to stain the nuclei for 5 min. The cells were washed with PBS (400  $\mu$ L) and fixed with formaldehyde (400  $\mu$ L) prior to imaging. The images were obtained using an excitation wavelength of 405 nm (blue excitation), 488 nm (green excitation), and 646 nm (red excitation). ImageJ software was used for the quantifying the fluorescence of Cy5 and the co-localization efficiency. Mean pixel intensities in each image were normalized to the total cell number by counting the number of DAPI-labeled nuclei. The analysis was performed in 6 random images. See Figure 2B and 2C for results and analysis.

## 3.8 The cathepsin B binding studies of the radioconjugates

3.8.1 Competitive cathepsin B binding of the radioconjugates with CA-074 and NTR1 ligand N1

The cathepsin B (human Liver) (3 nM, 10  $\mu$ L) in storing buffer (50mM sodium acetate and 1mM EDTA, pH 5.0) was pre-incubated with the commercial cysteine proteases inhibitor CA-074 (10  $\mu$ M, 10  $\mu$ L) or NTR1 ligand N1 (20  $\mu$ M, 10  $\mu$ L) for 30 min. Then the solution of radioconjugate (500,000 cpm) in 30  $\mu$ L of binding assay buffer (5 mM Tris, 5 mM MgCl<sub>2</sub>, and 2 mM DTT, pH = 5.5) was added to the mixture and incubated on ice for 2 h. Aliquot (30  $\mu$ L) of the solution was mixed with Novex Tris-Glycine SDS sample buffer (2×) (30  $\mu$ L) and incubated for further 10 min. The mixtures (20  $\mu$ L) were loaded onto a Novex 16% tris-glycine gel and analyzed by SDS–PAGE at 110 V for 90 min. After shaking in shrinking buffer (50 mL, 65% methanol, and 0.5% glycerol in water) at 4 °C for overnight, the gel was dried for 6 h at room temperature and the ladders were painted with small amount of radioactivity. The gel was then exposed to a phosphor plate for 72 h which was subsequently scanned by a Typhoon FLA 9500 imaging system at a 25  $\mu$ m resolution to achieve the autoradiograph. See Figure 3A and Figure S10A for results.



Figure S10. (A) The autoradiography of the SDS-PAGE indicating the minimal interference of the NTR1 ligand N1 on cathepsin B binding of the conjugates. (B) The autoradiography of SDS-PAGE of cathepsin B (human Liver) and live HT-29 cells samples after incubation with <sup>177</sup>Lu-2c and <sup>177</sup>Lu-3a. The incubation times for cathepsin B and cells samples were 2 h and 4 h, respectively.

#### 3.8.2 Cathepsin B binding of the radioconjugates

This procedure use the same solutions as described in 3.8.1. The cathepsin B (human Liver)(3 nM, 10  $\mu$ L) in storing buffer was added to the solution of the radioconjugate (500,000 cpm) in 40  $\mu$ L of binding assay buffer and kept on ice for 2 h. An aliquot (25  $\mu$ L) of the solution was mixed with Novex Tris-Glycine SDS sample buffer (2×) (25  $\mu$ L) and incubated for a further 10 min. The mixtures (20  $\mu$ L) were loaded onto a Novex 16% tris-glycine gel and analyzed by SDS-PAGE at 110 V for 90 min. The autoradiograph of the SDS-PAGE was performed according to the same method in section 3.8.1. See Figure 3B and S10B for results.

3.8.3 Intracellular trapping of the radioconjugates in living HT-29 cells

To the HT-29 cells (1 × 10<sup>6</sup> / well) seeded in 6-well plates was added the radioconjugates (0.74 MBq, 20  $\mu$ Ci) in 1 mL of cell culture medium. The cells were incubated at 37 °C for 4 h and the medium was removed by vacuum followed washing with PBS (2 × 2 mL). The cells (~3 × 10<sup>6</sup>) were trypsinized and combined in microcentrifuge. The RIPA buffer (100  $\mu$ L) containing Halt<sup>m</sup> protease inhibitor (100 ×, 1  $\mu$ L) was added to the cell pellet and vigorously vortexed for 1 min. The suspension was incubated on ice for 15 min and centrifuged to remove the pellet. The supernatant (80  $\mu$ L) was equally divided into two portions. The first portion (40  $\mu$ L) was directly analyzed by radioactive-GPC with a flow rate of 0.6 mL/min (40% ACN in PBS containing 0.1% sodium azide). See Figure 3D and Figure S11 for results. The second portion (40  $\mu$ L) of the cell lysate was mixed with Novex Tris-Glycine SDS sample buffer (2×) (40  $\mu$ L) and incubated for another 15 min. The mixture (30  $\mu$ L) was loaded onto a Novex 16% tris-glycine gel and analyzed by SDS–PAGE at 110 V for 90 min. The autoradiograph of the SDS-PAGE was performed according to the same method in section 3.8.1. See Figure 3B for results.



Figure S11. The GPC profiles of <sup>177</sup>Lu-2c and HT-29 cells samples after incubation with <sup>177</sup>Lu-2c and <sup>177</sup>Lu-3a for 4 h

3.8.4 Competitive intracellular cathepsin B binding of the radioconjugates with NTR1 ligand N1

To the HT-29 cells (1 × 10<sup>6</sup> / well) seeded in 6-well plates was added the radioconjugates (0.74 MBq, 20  $\mu$ Ci) in 1 mL of cell culture medium with or without competitive NTSR1 ligand N1 (20  $\mu$ M). The cells were incubated at 37 °C for 4 h and the medium was removed by vacuum followed washing with PBS (2 × 2 mL). The cells were lysed and the autoradiograph of the SDS-PAGE was performed according to the same method in section 3.8.3. See Figure 3C for results.

3.8.5 Investigation of the time-dependent retention of CatB-conjugate adducts in HT-29 cells

To the HT-29 cells (1 × 10<sup>6</sup> / well) seeded in 6-well plates was added the radioconjugate (0.74 MBq, 20  $\mu$ Ci) in 1 mL of cell culture medium. The cells were incubated at 37 °C for 4 h and the medium was removed by vacuum followed washing with fresh medium (2 × 2 mL). The fresh medium (1 mL) was added to the wells and the cells were lysed at 2 h, 4h, and 24 h. The autoradiograph of the SDS-PAGE was performed according to the same method in section 3.8.3. See Figure 3E for results.

### 3.9 The metabolic stability of the radioconjugates in human serum

The radioconjugates (11.1 MBq, 300  $\mu$ Ci) was added to 300  $\mu$ L human serum and incubated at 37 °C for 24 h. At predetermined time points (0, 4, and 24 h), acetonitrile (50  $\mu$ L) was added to the mixture (50  $\mu$ L) was centrifuged at 12,000×g for 5 min. The supernatant was collected and dried with nitrogen flow. The sample was reconstituted in water (100  $\mu$ L) and analyzed by radio-HPLC using the gradient described above. See Figures S12 and S13 for data.



Figure S12. Human serum stability of 177Lu-2c examined by radio-HPLC



Figure S13. Human serum stability of 177Lu-2f examined by radio-HPLC

## 4 In vivo evaluation of the cysteine proteases trapping effect of the conjugates

## 4. 1 Biodistribution study

Female SCID mice (5 weeks of age) received subcutaneous injections of HT-29 cells (5 × 10<sup>6</sup>) suspended in Matrigel<sup>®</sup> into the flanks. When the tumor size reached 80 mm<sup>3</sup> (two weeks after injection), the mice were randomized into three groups and intravenously injected with 10  $\mu$ Ci (0.37 MBq) of the purified <sup>177</sup>Lu labeled conjugates via tail vein. The mice were sacrificed and the tissues were excised at 4, 24, and 72 h post-injection time points. The blood, tumor, and excised tissues were weighed. The radioactivity for each sample was measured using a gamma counter. The percentage injected dose per gram (%ID/g) and the radioactivity ratios between tumor and non-targeted tissues were calculated. See Table S6 for data.

<b>Tiggue</b> (ID $\frac{0}{2}$	4 h		24	24 h		72 h	
<b>HISSUE</b> (ID %/g)	2f	<b>3</b> b	<b>2f</b>	3b	<b>2f</b>	3b	
Blood	$1.22\pm0.14$	$0.83\pm0.11$	$0.17\pm0.05$	$0.14\pm0.09$	$0.01\pm0.18$	n.d.	
Heart	$1.40\pm0.36$	$1.10\pm0.22$	$0.89 \pm 0.23$	$0.63\pm0.24$	$0.43\pm0.57$	$0.27\pm0.18$	
Lung	$4.45 \pm 1.54$	$4.26\pm0.61$	$6.14\pm3.78$	$2.25\pm1.05$	$4.69 \pm 2.77$	$1.22\pm0.42$	
Liver	$38.62 \pm 4.24$	$9.24 \pm 1.16$	$25.55\pm8.18$	$8.31 \pm 1.80$	$11.30\pm4.71$	$5.28 \pm 1.70$	
Pancreas	$0.89 \pm 0.15$	$0.84\pm0.16$	$0.64\pm0.18$	$0.77\pm0.18$	$0.65\pm0.29$	$0.68\pm0.13$	
Stomach	$1.74 \pm 1.06$	$1.57\pm0.59$	$0.92\pm0.17$	$0.89\pm0.37$	$0.83\pm0.24$	$0.51\pm0.08$	
Spleen	$16.05\pm2.82$	$18.58 \pm 4.56$	$17.08 \pm 10.12$	$18.32\pm6.72$	$17.11 \pm 14.60$	$18.54\pm12.39$	
Small int.	$6.06\pm0.58$	$7.55\pm0.80$	$5.48 \pm 1.70$	$5.61 \pm 1.16$	$4.26 \pm 1.40$	$3.22\pm0.77$	
Large int.	$2.91\pm0.38$	$4.28\pm2.06$	$2.05\pm0.86$	$2.55\pm0.58$	$1.64\pm0.50$	$1.62\pm0.17$	
Kidney	$58.18 \pm 1.10$	$63.59 \pm 9.91$	$61.96 \pm 4.91$	$55.41 \pm 7.79$	$44.77\pm6.67$	$28.23 \pm 6.75$	
Muscle	$0.53\pm0.04$	$0.44\pm0.04$	$0.35\pm0.06$	$0.38\pm0.13$	$0.15\pm0.12$	$0.12\pm0.07$	
Bone	$1.58\pm0.20$	$2.56\pm0.57$	$1.54\pm0.39$	$1.79\pm0.21$	$1.07\pm0.29$	$1.05\pm0.23$	
Brain	$0.09\pm0.08$	$0.11\pm0.03$	$0.04\pm0.\ 03$	$0.04\pm0.03$	$0.02\pm0.09$	$0.01\pm0.02$	
Tumor	$7.82 \pm 1.82$	$8.35 \pm 1.92$	$9.83 \pm 2.52$	$5.06 \pm 1.01$	$6.34 \pm 1.94$	$3.42\pm0.46$	
Excretion (ID%)	$16.55 \pm 1.39$	$36.64\pm3.92$	$34.94 \pm 6.26$	$56.02\pm3.00$	$57.09 \pm 3.67$	$72.74\pm3.66$	

Table S6. Biodistribution data of the 177Lu-2f and 177Lu-3b in a HT-29 xenograft mouse model. Data are represented as mean  $\pm$  SD. (n = 5).

#### 4.2 Evaluation of tissue adduct formation of the radioconjugates.

The radioconjugates (800  $\mu$ Ci/mouse) were intravenously injected to the tumor bearing mice. The mice were sacrificed and the tumor, liver, and kidney were excised at 24 and 72 h post-injection time points. The tumor and organs were homogenized in RIPA buffer (50 mg / 100  $\mu$ L) containing Halt<sup>tm</sup> protease inhibitor (100 ×, 1  $\mu$ L) on ice and centrifuged to remove the pellet. An aliquot (20  $\mu$ L) of the supernatant of the sample at 24 h was mixed with Novex Tris-Glycine SDS sample buffer (2×, 20  $\mu$ L) and incubated for further 10 min. The mixtures (20  $\mu$ L) were loaded onto a Novex 16% tris-glycine gel and analyzed by SDS-PAGE at 110 V for 90 min. The autoradiograph of the SDS-PAGE was performed according to the same method in section 3.8.1. On the other hand, the aliquots (100  $\mu$ L) of supernatants of all the tumor samples at 24 h and 72 h were individually centrifuged with Pierce<sup>™</sup> protein concentrators (MWCO = 10kDa) to separate the low molecular weight radioactivity. The radioactivity in each fraction was quantified using a gamma counter to calculate the percentage of the cysteine proteases trapped radioconjugate in the total counts. See Table S14 for data.



Figure S14. The autoradiography of SDS-PAGE of the liver and kidney at 24 h postinjection of <sup>177</sup>Lu-2f and <sup>177</sup>Lu-3b in tumor bearing mice.

## 4.3 Evaluation of cysteine proteases trapping of the Cy 5-labeled conjugates.

The Cy 5-labeled conjugates (40 nmol) were intravenously injected to the tumor bearing mice. The mice were sacrificed at 24 post-injection time points, and the heart, lung, liver, spleen, pancreas, kidney, intestine, brain, and tumor were excised and imaged using an IVIS spectrum system. The tumor and organs were homogenized in RIPA buffer (50 mg / 100  $\mu$ L) containing Halt<sup>III</sup> protease inhibitor (100 ×, 1  $\mu$ L) on ice and centrifuged to remove the pellet. An aliquot (18  $\mu$ L) of the supernatant of the sample was added in NuPAGE<sup>®</sup> sample reducing reagent (10×, 2  $\mu$ L) and denatured at 80 °C for 2 min. The sample was mixed with Novex Tris-Glycine SDS sample buffer (2×, 20  $\mu$ L) and incubated for further 10 min. The mixtures (20  $\mu$ L) were loaded onto a Novex 16% tris-glycine gel and analyzed by SDS-PAGE at 110 V for 90 min, followed by electro transferring onto PVDF membranes. The PVDF membranes were blocked by animal-free blocking buffer for 1h at room temperature and then were incubated with Goat anti-Rabbit IgG (H+L) secondary antibody at room temperature for 1h and visualized using Typhoon FLA 9500. See Figure S15 and S16 for data.



Figure S15. (A) The ex vivo fluorescent image of the tissues after injected with 4a and 4b at 24 h. (B) Quantification of the average fluorescence in each tissue. The excitation filter was 615-665 nm, and the emission filter was 695-770 nm.

# 5 NMR and Mass Spectra



<sup>13</sup>C NMR spectrum (125 MHz) of 6 in CDCI<sub>3</sub>



 $^{13}\text{C}$  NMR spectrum (125 MHz) of 7 in CDCI $_3$ 



<sup>13</sup>C NMR spectrum (125 MHz) of 1a in (CD<sub>3</sub>)<sub>2</sub>SO



 $^{13}\text{C}$  NMR spectrum (125 MHz) of 8 in CDCI\_3



<sup>1</sup>H NMR spectrum (500 MHz) of 9b in CDCI<sub>3</sub>



 $^{13}\text{C}$  NMR spectrum (125 MHz) of 9b in CDCI $_3$ 



 $<sup>^{13}\</sup>text{C}$  NMR spectrum (125 MHz) of 9c in CDCI $_3$ 



 $<sup>^{13}\</sup>text{C}$  NMR spectrum (125 MHz) of 9d in CDCI $_3$ 





 $^{13}\text{C}$  NMR spectrum (125 MHz) of 9e in CDCI $_3$ 



 $^{13}\text{C}$  NMR spectrum (125 MHz) of 10 in CDCI $_3$ 



 $^{13}\text{C}$  NMR spectrum (125 MHz) of 11 in CDCI $_3$ 



 $^{13}\text{C}$  NMR spectrum (125 MHz) of 9a in CDCI $_3$ 



الألالية والمتعالية والمتعار والمرجان والمرجوع والمتعاد والمتعاد والمتعاد 170 160 130 120 ppm

 $^{13}\text{C}$  NMR spectrum (125 MHz) of 12 in CDCI $_3$ 



<sup>1</sup>H NMR spectrum (500 MHz) of 1b in (CD<sub>3</sub>)<sub>2</sub>SO



 $^{13}\text{C}$  NMR spectrum (125 MHz) of 1b in (CD<sub>3</sub>)<sub>2</sub>SO



 $^1\text{H}$  NMR spectrum (500 MHz) of 13a in CDCI $_3$ 



 $^{13}\text{C}$  NMR spectrum (125 MHz) of 13a in CDCI $_3$ 



 $^{13}\text{C}$  NMR spectrum (125 MHz) of 13b in CDCI $_3$ 



LRMS-ESI for 6. [M+H]<sup>+</sup> calcd. 604.2, found 604.2



43



LRMS-ESI for 1a.  $[M\!+\!H]^{\scriptscriptstyle +}$  calcd. 588.3, found 588.3





LRMS-ESI for 9b. [M+H]<sup>+</sup> calcd. 671.3, found 671.2



LRMS-ESI for 9c. [M+H]+ calcd. 803.4, found 803.1



LRMS-ESI for 9d. [M+H]<sup>+</sup> calcd. 989.4, found 989.3



LRMS-ESI for 9d. [M+H]+ calcd. 1157.6, found 1157.3



LRMS-ESI for 10. [M+H]+ calcd. 454.2, found 454.1



LRMS-ESI for 11. [M+H]+ calcd. 530.1, found 530.0



LRMS-ESI for 9a. [M+H]<sup>+</sup> calcd. 614.3, found 614.2



LRMS-ESI for 12.  $[M+H]^+$  calcd. 631.3, found 631.3



LRMS-ESI for 1b. [M+H]<sup>+</sup> calcd. 531.3, found 531.2



LRMS-ESI for 13a. [M+H]+ calcd. 746.4, found 746.2



LRMS-ESI for 13b. [M+H]+ calcd. 1100.6, found 1100.6



LRMS-ESI for 14a. [M+2H]<sup>2+</sup> calcd. 928.0, found 927.8



LRMS-ESI for 14b. [M+2H]<sup>2+</sup> calcd. 1107.1, found 1106.9



LRMS-ESI for 15a. [M+2H]<sup>2+</sup> calcd. 1234.6, found 1234.6



LRMS-ESI for 15b. [M+2H]<sup>2+</sup> calcd. 1263.2, found 1263.1



LRMS-ESI for 15c.  $[M+2H]^{2+}$  calcd. 1329.2, found 1329.2



LRMS-ESI for 15d. [M+2H]<sup>2+</sup> calcd. 1506.3, found 1506.5



LRMS-ESI for 15e.  $[M+2H]^{2+}$  calcd. 1508.3, found 1508.3



LRMS-ESI for 15f. [M+2H]<sup>2+</sup> calcd. 1685.4, found 1685.4



LRMS-ESI for 2a. [M+2H]<sup>2+</sup> calcd. 1125.6, found 1125.6



LRMS-ESI for 2b. [M+2H]<sup>2+</sup> calcd. 1154.1, found 1153.9



LRMS-ESI for 2c. [M+2H]<sup>2+</sup> calcd. 1220.2, found 1220.0



LRMS-ESI for 2d. [M+2H]<sup>2+</sup> calcd. 1313.2, found 1313.4



LRMS-ESI for 2e. [M+2H]<sup>2+</sup> calcd. 1315.2, found 1315.2



HRMS-ESI for 2f.  $[M+2H]^{2+}$  calcd. 1408.2249, found 1408.2242



LRMS-ESI for 16a. [M+2H]<sup>2+</sup> calcd. 1300.7, found 1300.6



LRMS-ESI for 16b. [M+2H]<sup>2+</sup> calcd. 1656.9, found 1656.9





HRMS-ESI for 4a. [M+3H]<sup>4+</sup> calcd. 724.1419, found 724.1420

HRMS-ESI for 3b. [M+2H]<sup>2+</sup> calcd. 1379.7197, found 1379.7188





HRMS-ESI for 4b.  $[M+3H]^{4+}$  calcd. 709.8893, found 709.8894

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