

Supporting Information - New covalent capture probes

New covalent capture probes for imaging and therapy, based on a combination of binding affinity and disulfide bond formation.

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Materials & Methods

Reagents: Aminobenzyl-DOTA (ABD) was purchased from Macrocylics. Triethylamine (Et₃N), diisopropylethylamine (DIPEA), acetonitrile (CH₃CN) and plastic backed thin layer silica (TLC) plates were purchased from EMD Chemicals. Thioglycolic acid (mercaptoacetic acid, MAA), 3-mercaptopropionic acid (MPA), N-hydroxysuccinimide (NHS), tributylamine, yttrium chloride (YCl₃) and piperidine were purchased from Sigma Aldrich. Methylmethanethiosulfanate (MMTS) was purchased from Pierce. Biotechnical grade glucose, peptide grade tetrahydrofuran and methanol were purchased from Fisher Scientific. 2, 2'-Dithiodipyridine (DTP), trisopropylsilane (TIS), dimethylformamide (DMF), dichloromethane (CH₂Cl₂) and DTPA were purchased from Acros Organics. Boc-aminoxy acetic acid (Boc-AOA), *N,N'*-Dicyclohexylcarbodiimide (DCC) and Fmoc-Cys(trt)-Opfp were purchased from Novabiochem. 18 mΩ-cm water was used where HPLC or mass spec grade water was not required. ⁹⁰YCl₃ and ¹¹¹InCl₃ solutions in 0.05 M HCl were purchased from PerkinElmer Life Sciences, Boston, MA.

Syntheses:

(S)-2-(4-acrylamidobenzyl)-1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid (*AABD*) **1** (Figure 1). This was prepared by a modification of the previous method.(1-2) ABD (40 mg, 0.056 mmol) was dissolved in triethylammonium acetate (Et₃N•HOAc), pH 8.0 (0.1 M, 200 μL). The pH was adjusted to 9 with neat Et₃N. Acryloyl chloride (187 μL, 2.24 mmol) was dissolved in CH₂Cl₂ (213 μL). 200 μL of the acryloyl chloride/CH₂Cl₂ mixture (0.56 mmol) was added to 200 μL of the ABD and vortex mixed for 30 sec. The pH was adjusted to 8, while stirring with Et₃N until a stable pH 8 was achieved. The mixture was vortex mixed for another 15 min. A negative test to fluorescamine confirmed complete acylation of the amino group on ABD. The CH₂Cl₂ was removed and reaction mixture extracted 20× with Et₂O (1 mL each time). The pH

Supporting Information - New covalent capture probes

was maintained at ~ 2 with 6 M HCl during the Et₂O extraction. The last two extractions tested negative to 4-(p-nitrobenzyl)-pyridine confirming complete removal of the alkylating agent, acryloyl chloride (3). Residual Et₂O was removed by blowing Argon through the crude mixture. The crude mixture was purified by reverse phase chromatography and the fractions analyzed by TLC and mass spec. HPLC gradient: solvent A, water with 0.1% CF₃COOH; solvent B, CH₃CN, 0-5 min, 3-18% B, 5-45 min, 18-38%, 45-55 min, 38-100%, 55-60 min, 100% B, 60-62 min, 100-3% B, 62-65 min 3% B, 3 mL/min. Desired peak was collected between 21 and 23 min. (ESI-MS *m/z* calc'd (MH⁺): 564.27. Observed: (MH⁺): 564.4 and (M-H)⁻: 562.4, Figure S1 and S2). TLC solvent: equal volumes of aqueous 0.1 M Et₃N•HOAc and methanol.

(S)-2-(4-*N*_α-Fmoc-*S*-trityl-*L*-cysteinylamidobenzyl)-1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid (fmoc-Cys(Trt)-ABD) **6** (Scheme 3). ABD (100 mg, 0.138 mmol, FW 705) was dissolved in 5 mL of H₂O, to which 20 mL of tributylamine was added. The solution was stirred for 4 h at room temperature. The aqueous layer was separated from the organic layer and taken to dryness under high vacuum. The dried product was completely dissolved in 3 mL of DMF. Fmoc-Cys(Trt)-OpFp (420 mg, 4 molar equiv, 0.55 mmol, FW 751.76) was added to the ABD-tributylamine and mixed until complete dissolution was achieved. The reaction mixture was stirred for 48 h at room temperature. Mass spec analysis shows approximately 90% conversion to product. The reaction was quenched with twelve 50 μL aliquots of water and stirred for an additional 30 min. The crude product was dried under high vacuum to an oily brownish-yellow paste. (ESI-MS *m/z* calc'd (MH⁺): 1077.44, (MNa⁺): 1099.42. Found: 1077.5, 1099.2 and 1100.3, Figure S3).

Removal of fmoc from 6. The crude fmoc-Cys(Trt)-ABD was fmoc deprotected with 6 mL of 33% piperidine in DMF for 18-24 h at room temperature. The crude mixture was dried under vacuum, re-dissolved in 3 mL of DMF and concentrated again to give an oily paste. Purification of the crude Cys(Trt)-ABD was accomplished using preparative reverse phase chromatography, adding small portions of the paste to 0.8 mL of CH₃OH (0.15 mL of DMF was added to obtain complete dissolution). Yield: 58 mg, 68 μmol (49%). HPLC gradient: solvent A, water with 0.1% CF₃COOH; solvent B, CH₃CN with 0.075% CF₃COOH; 0-37 min, 3-45% B; 37-42 min, 45-100% B; 42-62 min, 100% B; 62-67 min, 100-3% B, 7mL/min. (ESI-MS *m/z* calc'd (MH⁺): 855.37, (MNa⁺): 877.36. Found: 855.5 and 877.2, Figure S4).

(S)-2-(4-*L*-cysteinylamidobenzyl)-1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid (Cys-ABD) **7**. HPLC-purified Cys(Trt)-ABD (58 mg, 68 μmol by UV, ε₂₈₀=17,800 M⁻¹cm⁻¹) was deprotected in 2 mL of CF₃COOH:CH₂Cl₂:TIS (50:50:2). The mixture was stirred at room temperature until positive to DTNB test (about 2.5 h). The resulting **7** was confirmed by mass spec analysis and taken to complete dryness under high vacuum. (ESI-MS *m/z* calc'd (MH⁺): 613.27, (MNa⁺): 635.68 Found: 613.5 and 635.4, Figure S5). Crude yield: 62 μmol by UV, (91%). A portion of the crude **7** to be used as a positive control was purified using preparative reverse phase chromatography. HPLC gradient: solvent A, water with 0.1% CF₃COOH; solvent B, CH₃CN with 0.075%

Supporting Information - New covalent capture probes

CF₃COOH, 0-37 min, 3-45% B; 37-42 min, 45-100% B; 42-62 min, 100% B; 62-67 min, 100-3% B, 7 mL/min.

(S)-2-(4-*L*-Cysteinyl(methyldisulfide)-amidobenzyl)-1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid (CH₃SSCys-ABD) **3** (R¹=CH₃). The dried, crude **7** (62 μmol) was dissolved in 1 mL of CH₃OH and the pH carefully adjusted with DIPEA with alternating additions of MMTS (40 μL total volume, 420 μmol) to a final pH between 5 and 6. The reaction mixture was stirred at room temperature for 1 h. The crude **3** (R¹=CH₃) was taken to dryness and left under high vacuum for another 2 h. (ESI-MS m/z calc'd (MH⁺): 659.25, (MNa⁺): 681.24 Found: 659.0 and 681.4, Figure S6).

(S)-2-(4-*L*-cysteinyl-(mercaptopropionic acid-disulfide)amidobenzyl)-1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid (MPASSCys-ABD) **3b** (Scheme 1). Vacuum-dried **7** (28 μmol) was dissolved in 400 μL of CH₃OH and 2 equiv of 2, 2'-Dithiodipyridine (DTP, 56 μmol, 120 μL of 0.5M DTP stock in CH₃OH, 220.31g/mol) was added. The pH of the mixture was approximately 4 due to residual CF₃COOH from the HPLC purification. The reaction mixture was stirred at room temperature for 5 h. Mass spec analysis after 5 h shows complete conversion of the free cysteine thiol group to the disulfide pyridine. (ESI-MS m/z calc'd (MH⁺): 722.26, (MNa⁺): 744.24 Found: 722.5 and 744.3, Figure S7). The crude **3** (R=2-pyridyl) was taken to dryness. A disulfide exchange was done with 3-mercaptopropionic acid (MPA). MPA (3 equiv relative to the initial **7**, 8 μL of 11.4 mM stock, FW: 106.14, d: 1.218) was added to the crude **3** (R¹=2-pyridyl) dissolved in 400 μL of CH₃OH. This solution was stirred at room temperature for 3 h. Mass spec analysis showed complete conversion to **3b**. (ESI-MS m/z calc'd (MH⁺): 717.24, (MNa⁺): 739.24 Found: 717.1 and 739.4, Figure S8). The crude product in CH₃OH was mixed with an equal volume of H₂O and the pH adjusted to 6 with Et₃N. About 75% of the free 2-thiopyridone was extracted from the mixture with an equal volume of chloroform. The crude **3b**, found both in the aqueous phase and interface layer, was purified by reverse-phase preparative HPLC to yield a yellowish solid. Yield: 6.1 mg, 8.5 μmol (30%). HPLC gradient: solvent A, water with 0.1% CF₃COOH; solvent B, CH₃CN with 0.075% CF₃COOH, 0-45 min, 3-40% B; 45-50 min, 40-100% B; 50-60 min, 100% B; 60-70 min, 100-3% B, 7mL/min. Peak containing the product as confirmed by mass spec was eluted between 25 and 28.3 min, 22.3 to 25% B.

(S)-2-(4-*L*-cysteinyl(mercaptoacetic acid-disulfide)-amidobenzyl)-1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid (MAASSCys-ABD) **3a** (Scheme 1). A similar disulfide exchange was carried out with mercaptoacetic acid (MAA) on the vacuum-dried, crude **3** (R=2-pyridyl). MAA (3 equiv relative to initial **7** (14 μmol), 3 μL of 14 mM stock, FW: 92.1g/mol, d: 1.32) was added to the crude **3** (R=2-pyridyl) dissolved in 200 μL of CH₃OH. This solution was stirred at room temperature for 3 h. Mass spec analysis showed complete conversion. The crude **3a** was HPLC purified. Yield: 2.5 mg, 3.5 μmol (25%). HPLC gradient: solvent A, water with 0.1% CF₃COOH; solvent B, CH₃CN with 0.075% CF₃COOH, 0-30 min, 3-45% B; 30-35 min, 45-100% B; 35-40 min, 100% B; 40-45 min, 100-3% B, 7mL/min. Peak containing the sample as

Supporting Information - New covalent capture probes

confirmed by mass spec was eluted between 19 and 20.8 min, 26.9% B. (ESI-MS m/z calc'd (MH⁺): 703.24, (MNa⁺): 725.22 Found: 703.5 and 725.4, Figure S9).

N'-Boc-aminoxyacetyl-*N*-hydroxysuccinimide Ester (*BocAOA-NHS(4)*). *N*-Boc-aminoxyacetic acid, Boc-AOA (100 mg, 520 μmol) was stirred in a mixture of ethyl acetate/dioxane (1:1, 2 mL) on an ice bath for 5 min. *N*-hydroxysuccinimide, NHS (62 mg, 540 μmol) was added to this mixture, then dicyclohexylcarbodiimide DCC (94 mg, 450 μmol, 0.8 equiv to NHS and BocAOA) was added. The reaction mixture was stirred at room temperature for 22 h under argon. Ethyl acetate (2mL) was added to the reaction mixture before filtering through a coarse-grade pyrex filter followed by a medium-grade filter. The resulting filtrate was dried under high vacuum and then re-crystallized in 1050 μL of 1:1:1 CH₂Cl₂/Et₂O/pentane to yield about 90 mg of pure Boc-AOA-NHS ester (yield was approximately 60%). IR analysis shows the presence of the ester group at 1739cm⁻¹ and the complete disappearance of the carboxylic acid stretch at 1719cm⁻¹. A portion of the resulting Boc-AOA-NHS ester was dried and stored in -80 °C under argon. ¹H NMR (300 MHz, CD₂Cl₂) δ 8.95 (s, 1H), 5.64 (s, 2H), 3.72 (s, 4H), 2.34 (d, *J*=3.4, 9H).

(*S*)-2-(4-(2-aminoxy-acetylamido)-*L*-cysteinyl(methylsulfide)-amidobenzyl)-1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid (*AOCD-SS-CH₃*) **4** (R=CH₃). The vacuum-dried crude **3** (R¹=CH₃) (approximately 20 μmol) was dissolved and stirred in 300 μL of DMF, adjusted to pH 8 with 50 μL of DIPEA for about 5 min. Boc-AOA-NHS ester (60 μmol in 500 μL CH₂Cl₂) was added and pH adjusted to 8 again using an additional 20 μL of DIPEA. The mixture was maintained at pH 8 for another 3 h by adding DIPEA, and then stirred under argon at room temperature for 21 h. The Boc group of the crude product was removed in 2 mL CF₃COOH:CH₂Cl₂:TIS (50:50:2) solution for 4 h at room temperature. The crude **4** (R=CH₃) was HPLC purified. Peak containing the sample was eluted between 17.3 and 18 min, 26.8-27.6% B as confirmed by mass spec (ESI-MS m/z calc'd (MH⁺): 732.26, (MNa⁺): 754.25 Found: 732.1 and 754.3, Figure S10).

HPLC purification of all the aminoxy adducts was done using the same solvents and gradients: solvent A, water with 0.1% CF₃COOH; solvent B, CH₃CN with 0.075% CF₃COOH, 0-10 min, 3-25% B; 10-50 min, 25-40% B; 50-55 min, 40-100% B; 55-75 min, 100% B, 2 mL/min.

(*S*)-2-(4-(2-aminoxyacetylamido)-*L*-cysteinyl(mercaptopropionic acid-disulfide)-amidobenzyl)-1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid (*AOCD-SS-MPA*) **4b** (Scheme 1). The purified **3b**, approximately 3.4 μmol, was dissolved and stirred in 150 μL of DMF with pH adjusted to 8 using 10 μL of DIPEA for about 5 min (pH check was done on 1 μL aliquot of the mixture diluted with 3 μL of water). Boc-AOA-NHS ester (20 μmol in 20 μL of CH₂Cl₂) was added and the pH adjusted to 8 again, using DIPEA. The mixture was maintained at pH 8 for another 3 h by adding DIPEA as required, and then stirred under argon at room temperature for 21 h. The reaction was quenched with excess H₂O for 1 h and then dried under high vacuum. The

Supporting Information - New covalent capture probes

Boc group of the crude product was removed in 2 mL CF₃COOH:CH₂Cl₂:TIS (50:50:2) solution for 3 h at room temperature. The reaction was dried under high vacuum and the residue containing **4b** was stored at -80 °C until needed. Yield: 1.7 mg, 2.2 μmol (64%). (ESI-MS m/z calc'd (MH⁺): 790.27, (MNa⁺): 812.26 Found: 790.3 and 812.1, Figure S11).

(S)-2-(4-(2-aminooxyacetyl-amido)-L-cysteinyl(mercaptoacetic acid-disulfide)-amidobenzyl)-1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (AOCD-SS-MAA) **4a** (Scheme 1). The purified **3a**, approximately 1.65 μmol, was dissolved and stirred in 100 μL DMF with pH adjusted to 8 using 5 μL DIPEA. Boc-AOA-NHS ester (10 μmol in 10 μL CH₂Cl₂) was added and the pH adjusted to 8 again, using DIPEA. The mixture was maintained at pH 8 for another 3 h by adding DIPEA as required, and then stirred under argon at room temperature for 21 h. The reaction was quenched with H₂O for 1 h and then dried under high vacuum. The Boc group of the crude product was removed in 1.5 mL CF₃COOH:CH₂Cl₂:TIS (50:50:2) solution for 3 h at room temperature. The reaction was dried under high vacuum and stored at -80 °C until needed. (ESI-MS m/z calc'd (MH⁺): 776.25, (MNa⁺): 798.24 Found: 776.8 and 798.5, Figure S12).

Analytical fractions of the crude **4a** and **4b** were separately HPLC purified using the same column conditions described above. The peak containing **4b** eluted between 17 and 17.4 min at 27.4-27.9% B while **4a** eluted between 16.2 and 16.5 min at 27.4% B, as confirmed by mass spec.

Glucose-AOCD-SS-MPA 5b (Scheme 1). Glucose (26 mg, 144 μmol, 20 equiv to total **4b**) was dissolved and stirred in 70 μL normal saline with 1 μL CF₃COOH for 10 min. In another vial, crude **4b** was dissolved in 200 μL of 100% ethanol with 30 μL of normal saline added to achieve complete dissolution. The acidic glucose solution was added into the **4b** ethanolic solution and incubated while stirring at 100 °C for 40 min. The solution was cooled and taken to dryness before HPLC purification using the gradient described above. Yield: 3.2 mg, 3.3 μmol (46%). (ESI-MS m/z calc'd (MH⁺): 952.32, Found: 952.4, Figure S13).

Glucose-AOCD-SS-MAA 5a (Scheme 1) was dissolved and stirred in 35 μL normal saline with 0.6 μL CF₃COOH for 10 min. In another vial, **4a** was dissolved in 100 μL of 100% ethanol with 15 μL of normal saline added to achieve complete dissolution. The acidic glucose solution was added into the **4a** ethanolic solution and incubated while stirring at 100 °C for 40 min. The solution was cooled and taken to dryness before HPLC purification using the gradient described above. Yield: 1.3 mg, 1.4 μmol (38%). (ESI-MS m/z calc'd (MH⁺): 938.30 Found: 938.4, Figure S14).

Radiochemistry. HPLC was performed on a Waters Breeze system with RA and UV (220 and 254 nm) detection, using a Luna C18 10 x 250 mm, 10 micron column (Phenomenex). Solvents 0.1% (v/v) TFA (aq) (A) and CH₃CN (B) were eluted at 3.0 mL/min using the linear gradient: 0 min, 97% A; 2 min, 97% A; 52 min, 0% A; 54 min, 0% A; 58 min, 97% A, 60 min, 97% A. The following elution times were observed: **1**,

Supporting Information - New covalent capture probes

16.96 min; **1**-⁹⁰Y, 16.43 min; **5b** (Scheme 1), 15.82 min; **5b**-⁹⁰Y (Figure 1), 15.06 and 15.39 min (two peaks); **5a**, 14.92 min; and **5a**-⁹⁰Y, 14.06 and 14.45 min (two peaks).

⁹⁰YCl₃ (0.5 to 2.6 mCi; Perkinelmer), supplied in dilute HCl, was buffered and added to DOTA constructs (80 to 270 nmol), such that the reaction solutions contained 0.7 to 2.6 mM DOTA construct in 0.1 to 0.6 M ammonium acetate, pH 6 to 7. The reactions were incubated for an hour or longer at 40 to 60 °C. DTPA was added to a final concentration of 10 mM to scavenge unchelated ⁹⁰Y³⁺, and the ⁹⁰Y-DOTA constructs were purified and evaluated for radiochemical yield (>95% of ⁹⁰Y chelated) by HPLC. The chelated ⁹⁰Y-DOTA products were well resolved from unchelated starting material, and 80 per cent or more of the latter was removed. The product HPLC peaks were dried at 110 °C under gentle helium, formulated for injection in PBS, and evaluated by HPLC for radiochemical purity (>95%) and mass content. Doses for injection contained 8 to 10 μCi of ⁹⁰Y-DOTA construct on 0.06 to 0.15 nmol total (chelated plus unchelated) DOTA construct.

Mass Spectra:

HPLC T1_C #47-56 RT: 1.08-1.30 AV: 10 NL: 2.02E5
T: - c Full ms [350.00-2000.00]

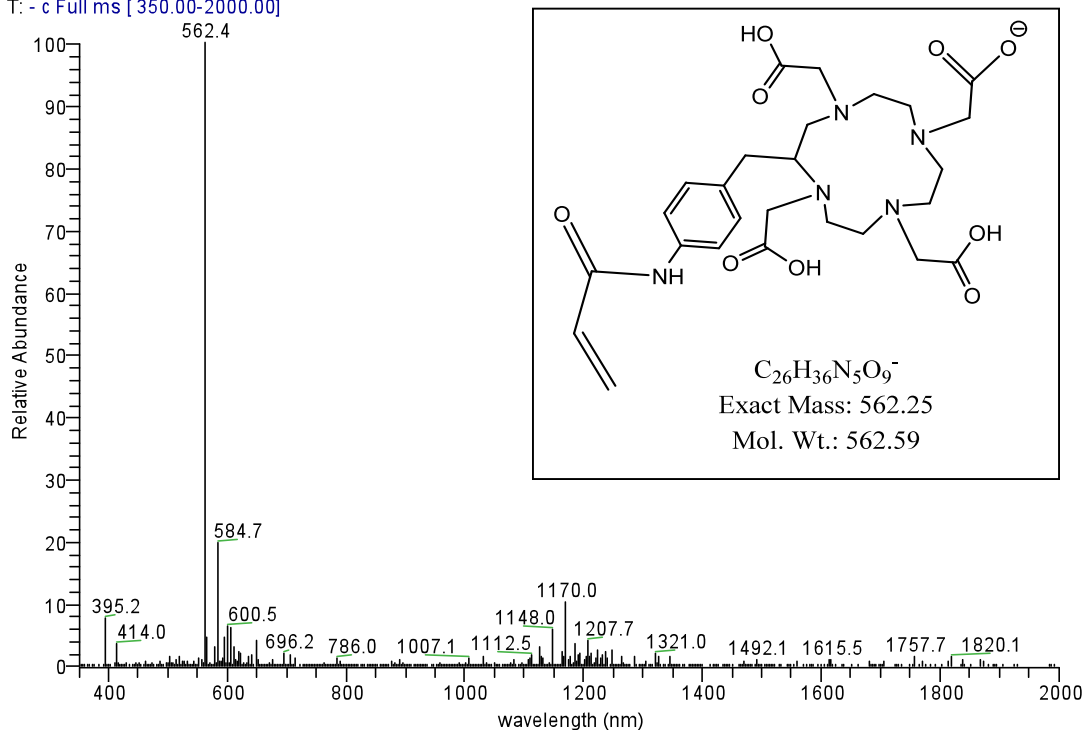


Figure s1. Mass spectrum for **1** after HPLC purification in the negative mode.

Supporting Information - New covalent capture probes

HPLC T1_C #57-73 RT: 1.34-1.76 AV: 17 NL: 4.06E5
T: + c Full ms [350.00-2000.00]

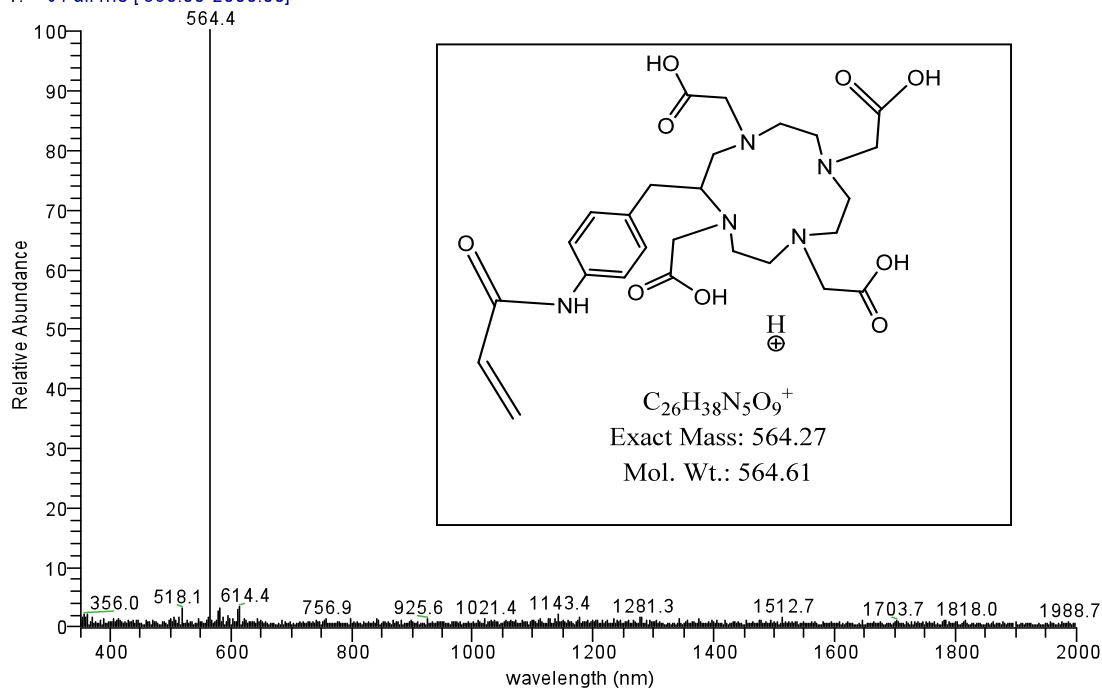


Figure s2. Mass spectrum for **1** after HPLC purification in the positive mode.

052010ABDCysTrtFmoccrude #1 RT: 0.00 AV: 1 NL: 7.01E4
T: + c Full ms [490.00-2000.00]

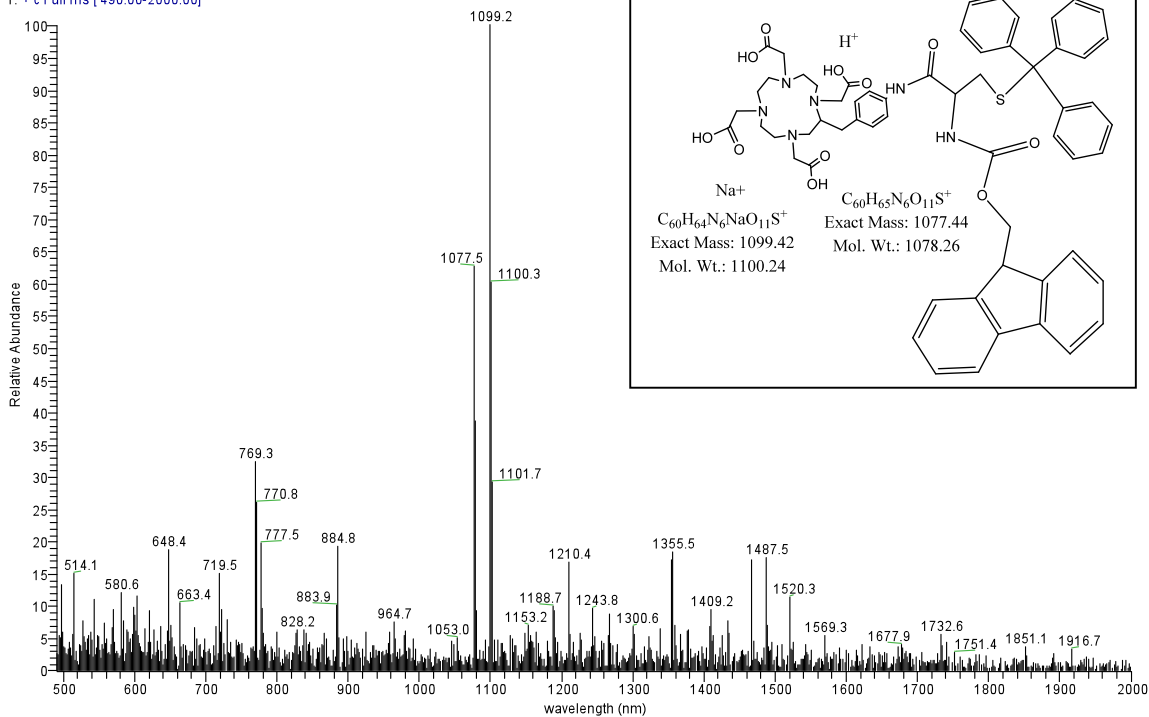


Figure s3. Mass Spec of ABD-Cys(Trt)-Fmoc after 48 h of incubation

Supporting Information - New covalent capture probes

121610-ABDcyst-tr-pure #59-69 RT: 1.57-1.83 AV: 11 NL: 5.75E7
T: + c Full ms [400.00-2000.00]

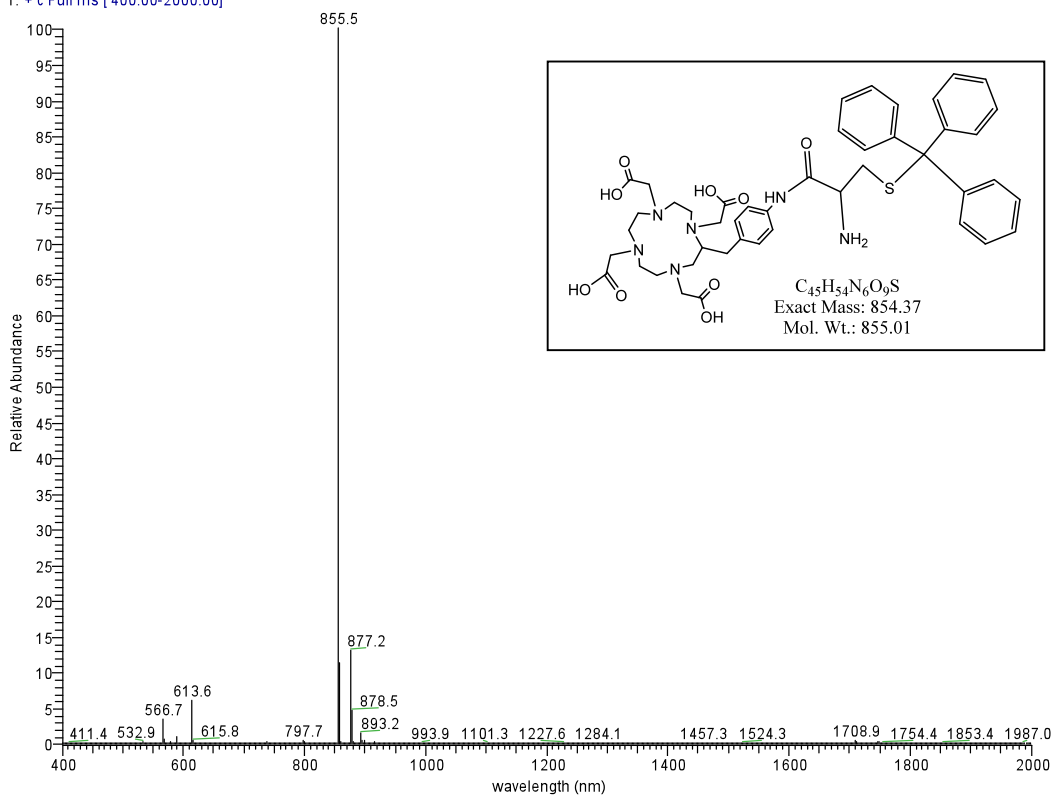


Figure s4. Mass spectrum for ABD-Cys(Trt) after HPLC purification in the positive mode.

Supporting Information - New covalent capture probes

12210-ABDcys #20-27 RT: 0.51-0.69 AV: 8 NL: 1.03E8
T: + c Full ms [400.00-2000.00]

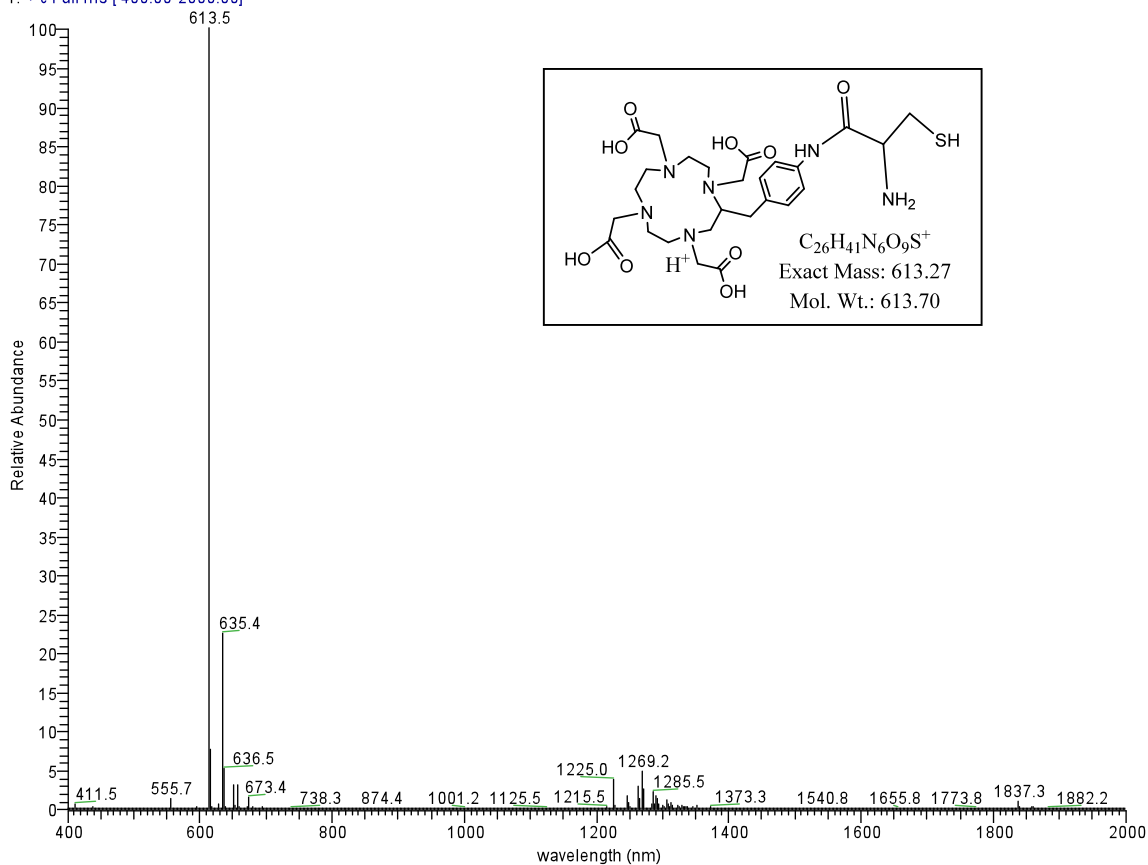


Figure s5. Mass spectrum for Cys-ABD after HPLC purification in the positive mode.

Supporting Information - New covalent capture probes

ABDCys-SH #69-74 RT: 1.69-1.81 AV: 6 NL: 7.40E4
T: + c Full ms [400.00-2000.00]

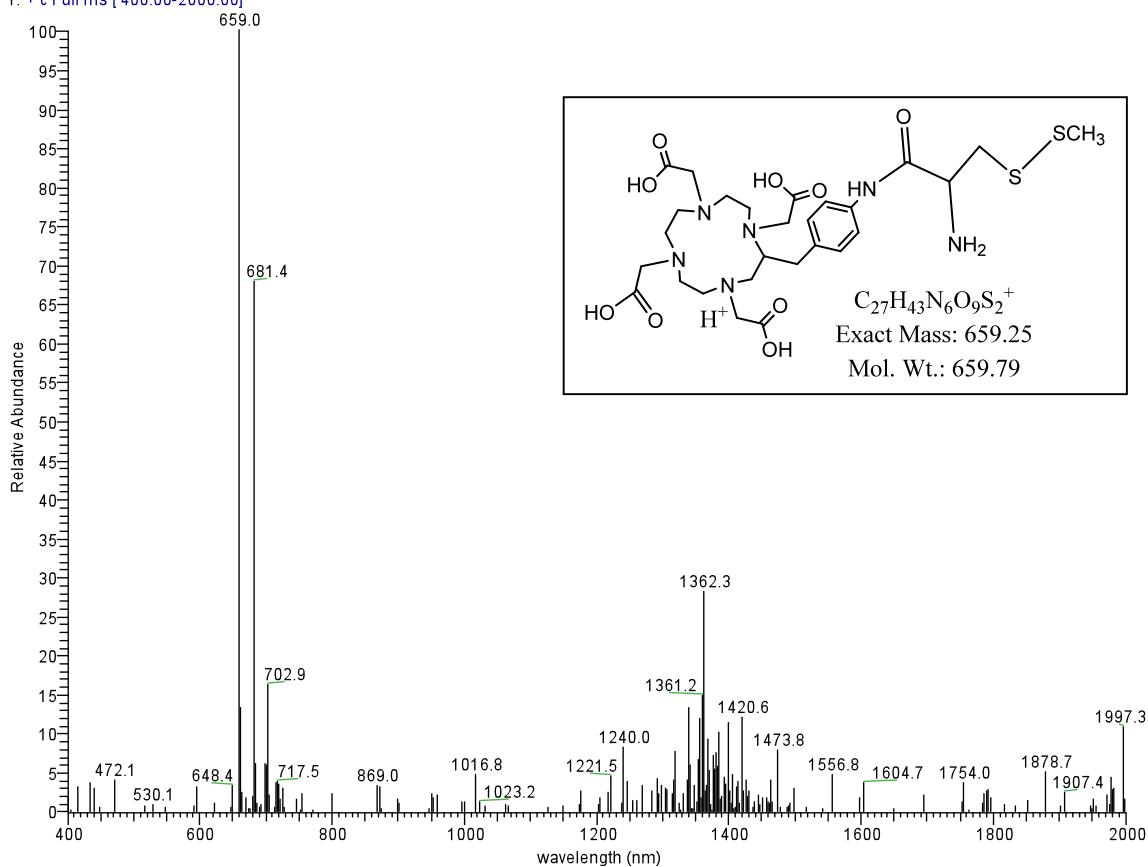


Figure s6. Mass spectrum for CH_3SSCys -ABD after HPLC purification in the positive mode.

Supporting Information - New covalent capture probes

12270-ABDcysSSDTP #32-36 RT: 0.83-0.94 AV: 5 NL: 3.06E7
T: + c Full ms [400.00-2000.00]

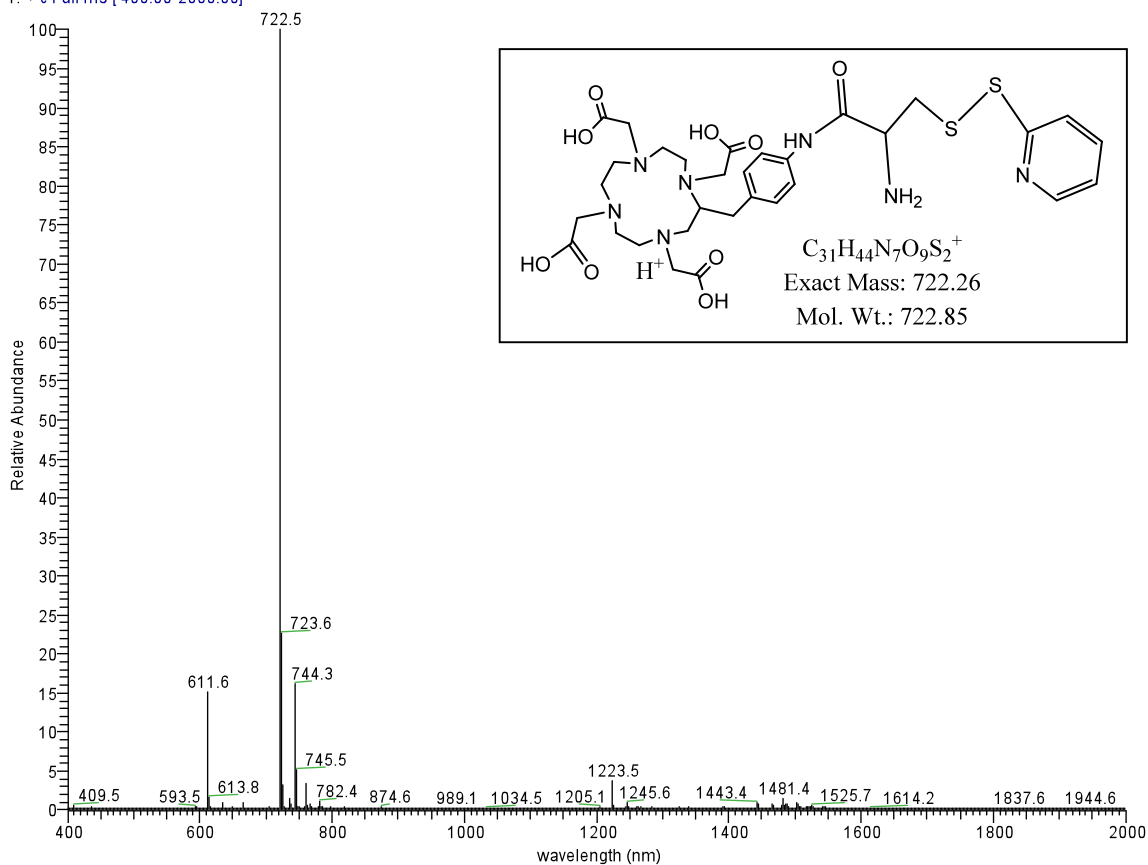


Figure s7. Mass spectrum for Pyr-SS-Cys-ABD in the positive mode.

Supporting Information - New covalent capture probes

011411-ABDcysSSMPA- HPLC @23min-BM #55-65 RT: 1.44-1.70 AV: 11 NL: 1.12E5
T: + c Full ms [400.00-2000.00]

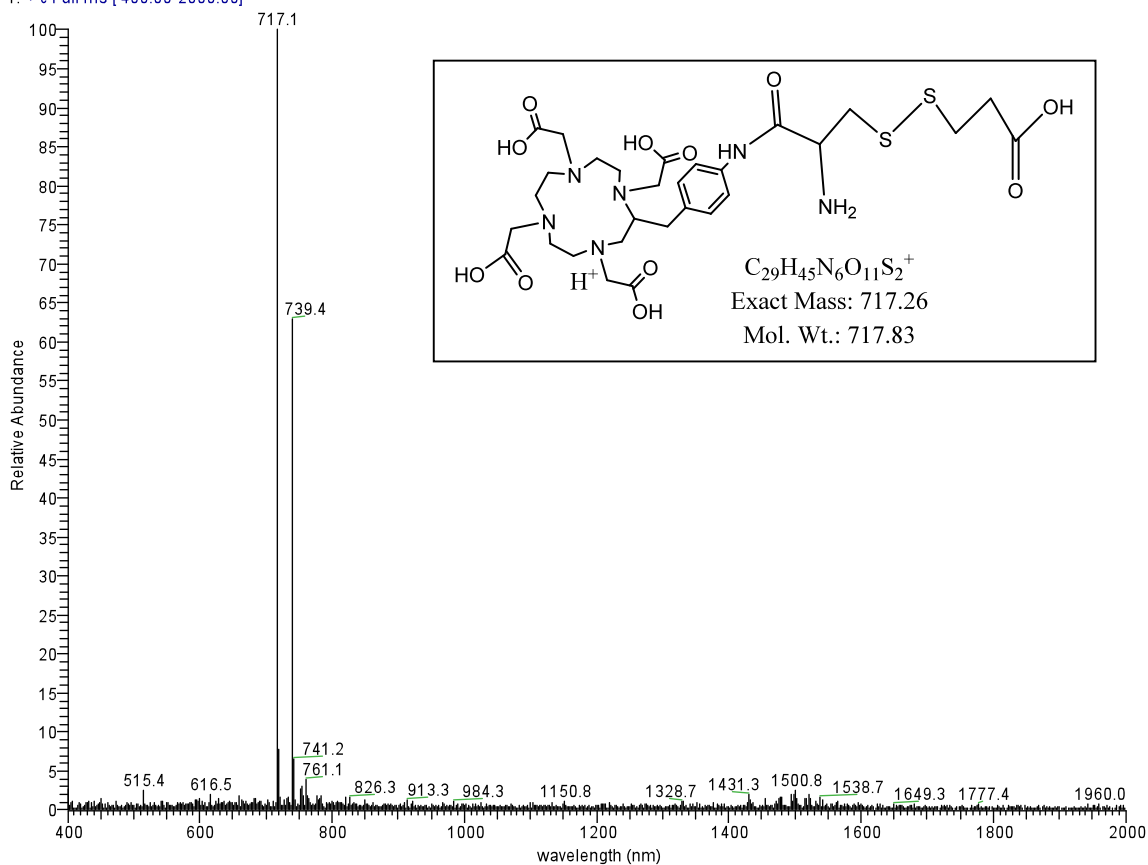


Figure s8. Mass spectrum for **3b** (Scheme 1) after HPLC purification in the positive mode.

Supporting Information - New covalent capture probes

111010ABDCys-SSMEA-mixt HPLC-fra5 #55-60 RT: 1.48-1.61 AV: 6 NL: 1.72E6
T: + c Full ms [400.00-2000.00]

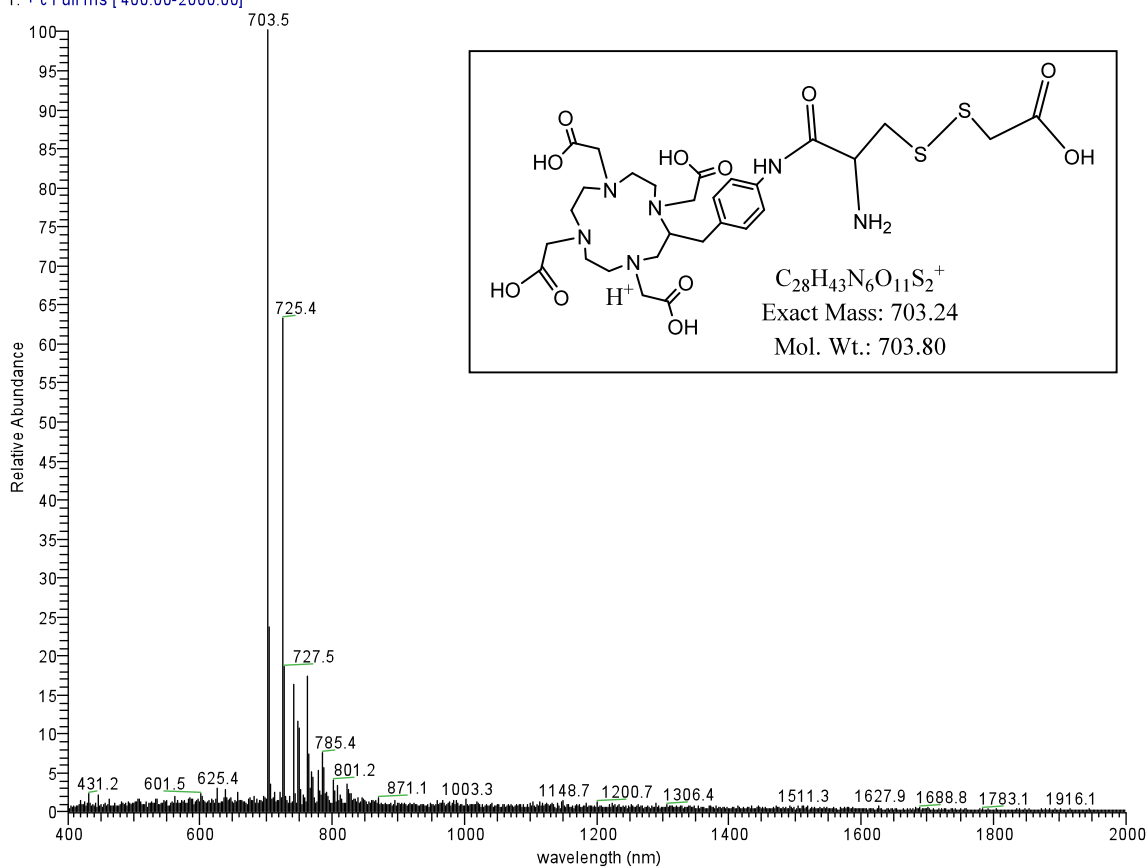


Figure s9. Mass spectrum for **3a** (Scheme 1) after HPLC purification in the positive mode.

Supporting Information - New covalent capture probes

111510-AOCD-MPA HPLC frac at 17min #48-58 RT: 1.16-1.43 AV: 11 NL: 4.29E5
T: + c Full ms [400.00-2000.00]

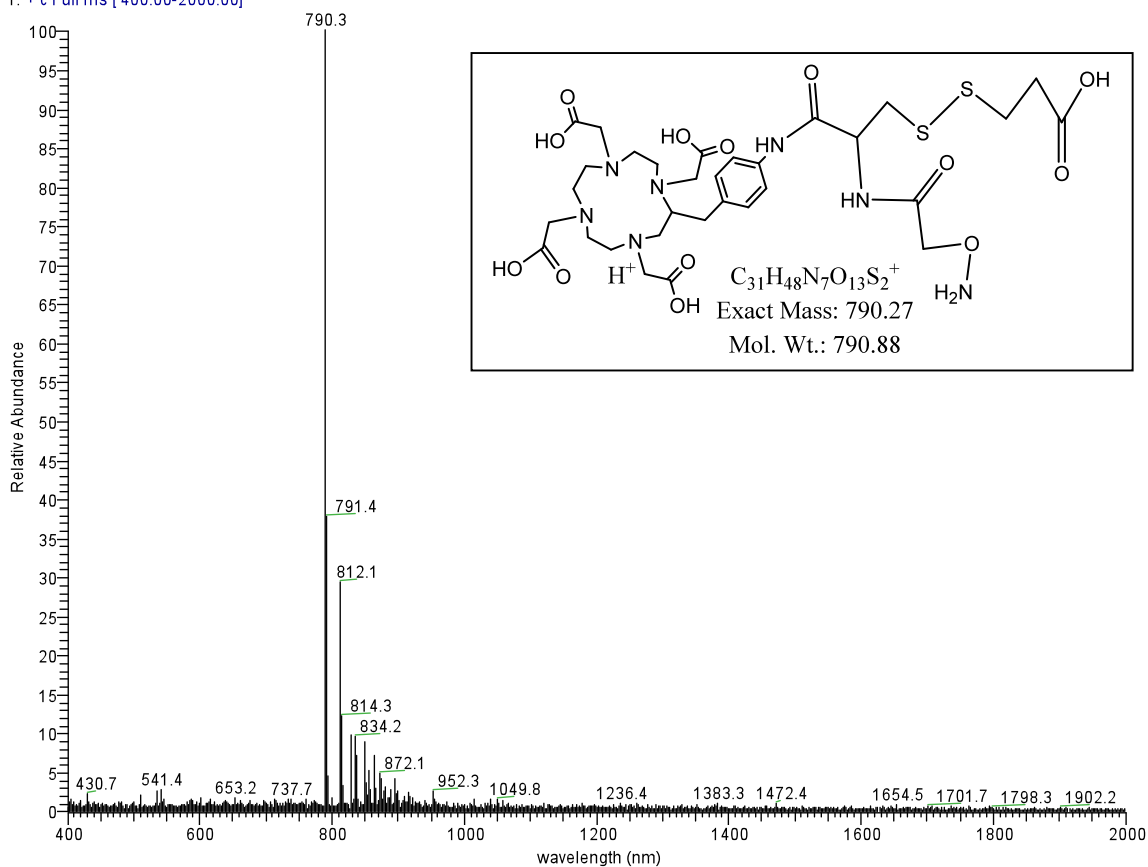


Figure s11. Mass spectrum for **4b** (Scheme 1) after HPLC purification in the positive mode.

Supporting Information - New covalent capture probes

111210-AOCD-DS-MEA#61-64 RT: 1.64-1.72 AV: 4 NL: 2.90E5
T: + c ms [400.00-2000.00]

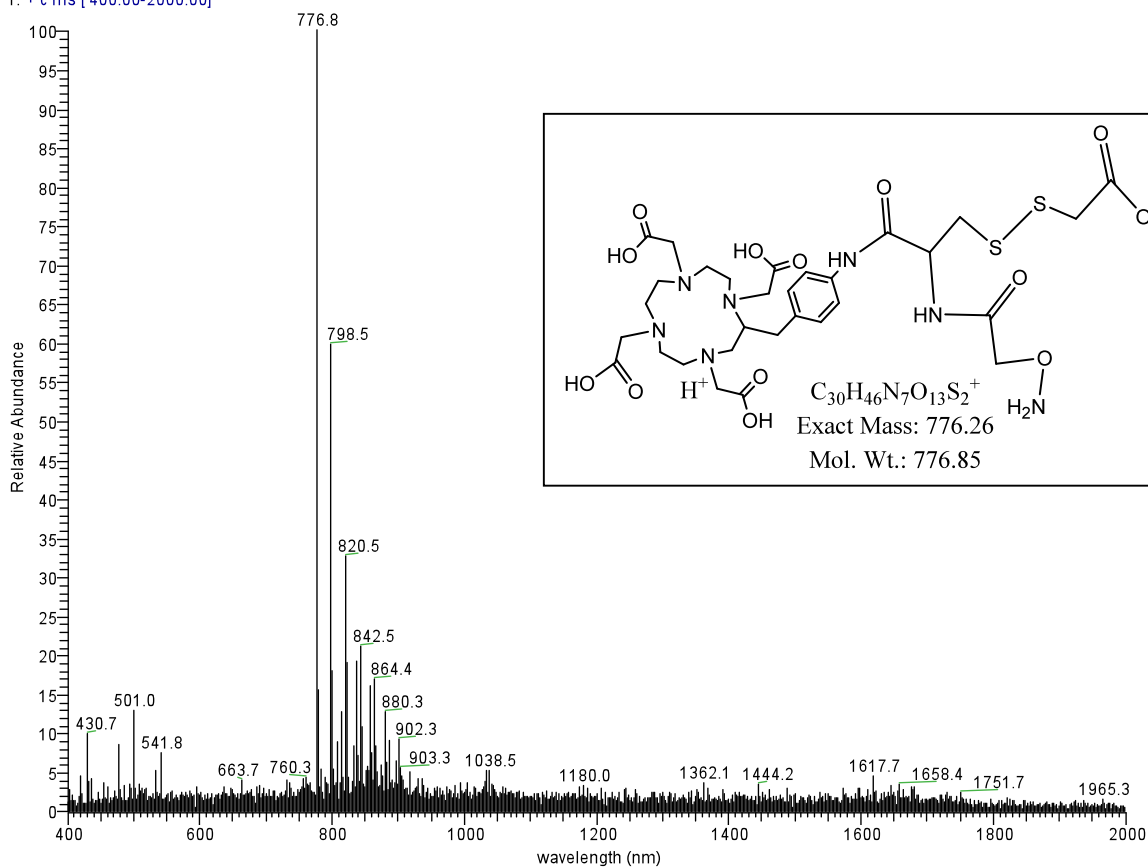


Figure s12. Mass spectrum for **4a** (Scheme 1) after HPLC purification in the positive mode.

Supporting Information - New covalent capture probes

111710-AoCD_MPA gluR5 #3-9 RT: 0.06-0.23 AV: 7 NL: 1.62E6
T: + c Full ms [400.00-2000.00]

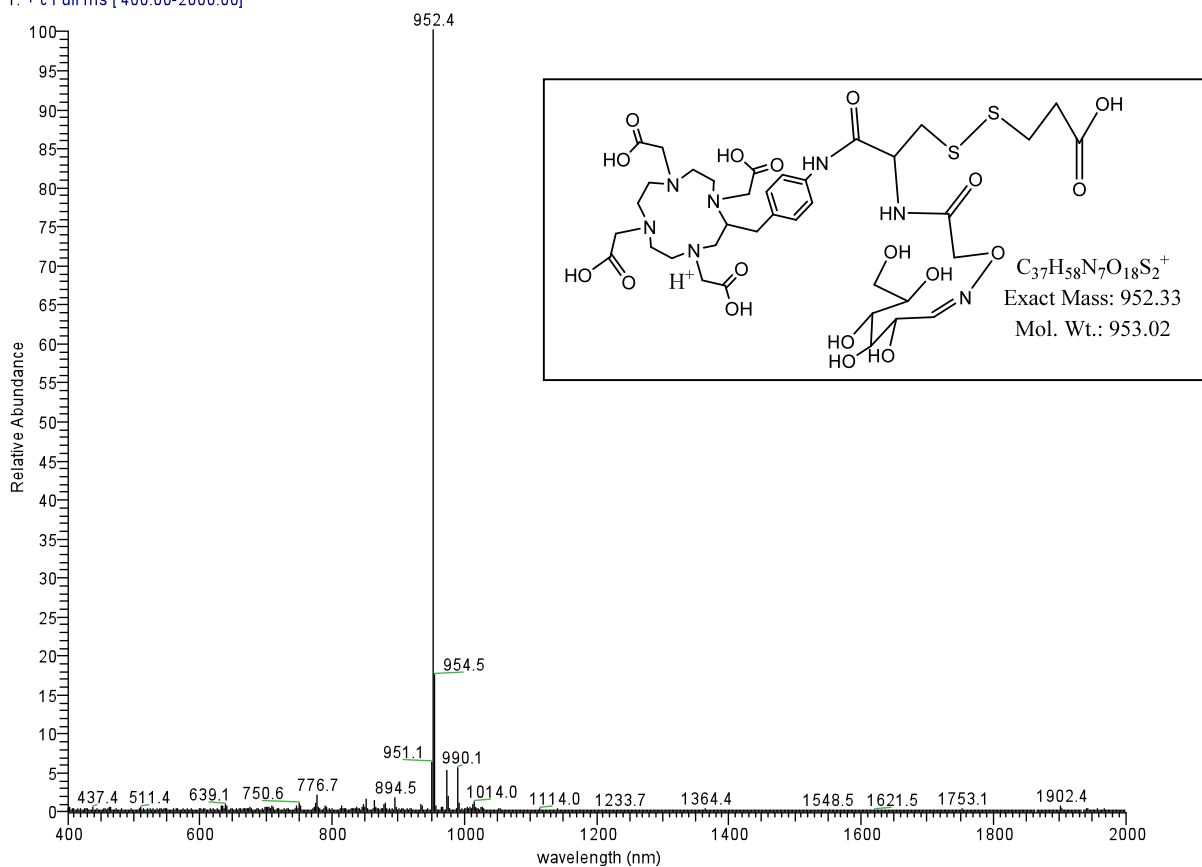


Figure s13. Mass spectrum for **5b** (Scheme 1) after HPLC purification, in the positive mode.

Supporting Information - New covalent capture probes

111510-AOCD-MEA glucos-fr@16min R3#2-8 RT: 0.04-0.20 AV: 7 NL: 5.17E5
T: + c ms [400.00-2000.00]

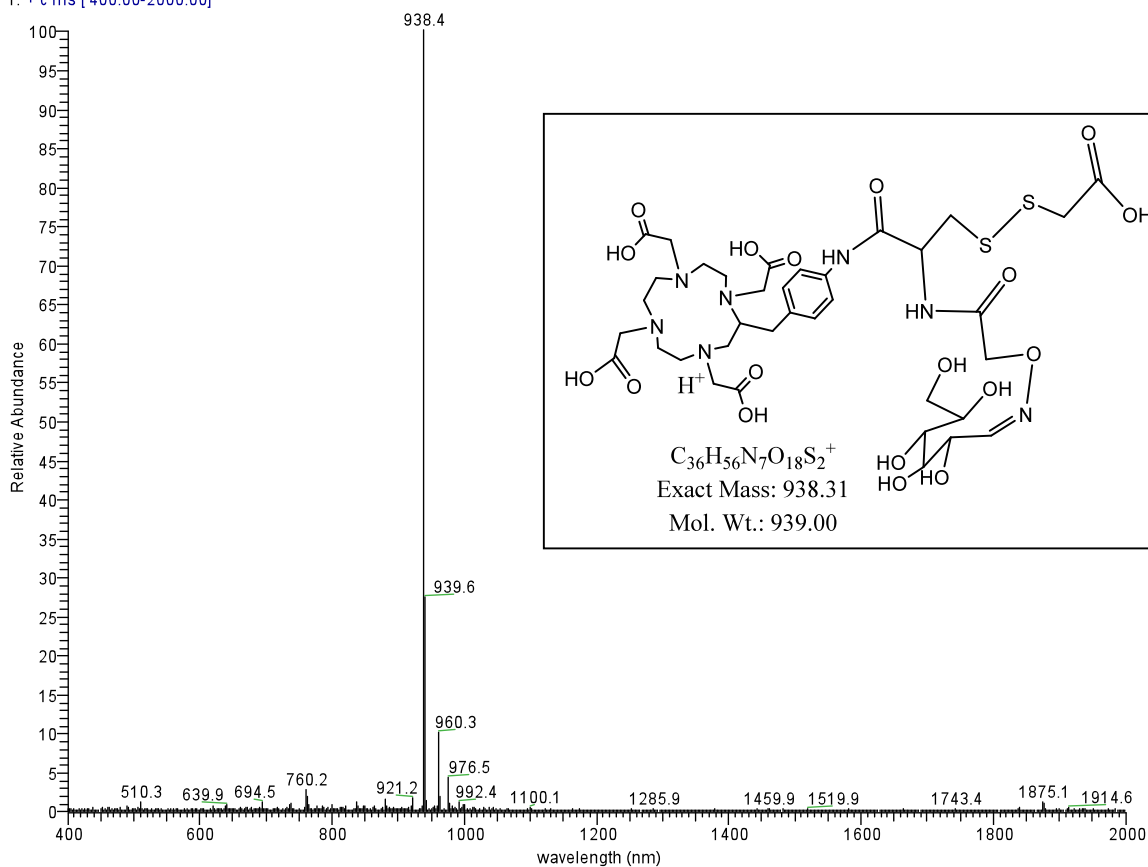


Figure s14. Mass spectrum for **5a** (Scheme 1) after HPLC purification, in the positive mode.

Supporting Information - New covalent capture probes

Construction of DAbR1-2A-mCherry multimodal reporter. The lentiviral plasmid backbone used for mammalian delivery in this study is a modified form of pLenti6/TR (Invitrogen). Briefly, the sequence containing the β -Globin Intron II and tetracycline receptor was excised and replaced with a customized multiple cloning sequence designed specifically for acceptance of a variety of similarly cloned reporter genes. Relevant to this manuscript, the MCS contains recognition sites for *CpoI*, *BamHI* and *XbaI*, in that order, immediately downstream from the cytomegalovirus (CMV) immediate early promoter.

Sequence encoding DAbR1 was PCR-amplified from pcDNA3.1/DAbR1 (a generous gift from Dr. Anna Wu, UCLA) using the following primer pair:

F: 5'-cggtcggccaccatggagacagacacactcc-3'
R: 5'-ggatccggcgtctgcgtcctgctgaac-3'

The PCR amplicon was TOPO cloned and verified for sequence accuracy. It was then digested with *CpoI* and *BamHI* (Fermentas) and ligated into the modified form of the pLenti6/CMV vector to create pLenti6/CMV/DAbR1. Sequence encoding the fluorescent protein mCherry was PCR-amplified from pmCherry-N1 (Clontech) using the following primers (with sequence encoding the 2A peptide shown in bold):

F: 5'-
ggatcc**gagggcagaggaagtcttctaacatgcggtgacgtggaggagaatccggccctatggtgagcaagggcgagg**-3'
R: 5'-tctagaccgaccgctgtacagctgcatgcc-3'

The PCR amplicon was TOPO cloned and verified for sequence accuracy. It was then digested with *BamHI* and *XbaI* (Fermentas) and ligated into the similarly digested pLenti6/CMV/DAbR1 to create pLenti6/CMV/DAbR1-2A-mCherry.

Establishment of the U-87/DC cell line. 293T cells were obtained from ATCC and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin/L-Glutamine (complete DMEM) and maintained at 37 C in the presence of 5% CO₂ in air. 293T cells were seeded to 10cm culture dishes and, at approximately 75% confluency, were co-transfected with pLenti6/CMV/DAbR1-2A-mCherry and the pLP1, pLP2 and pVSV-G series of viral packaging plasmids (Invitrogen) in a 1:1:1:1 ratio. Transfected cells were cultured 48-hours (with a single medium change at 24-hours post-transfection) and lentiviral particles were harvested and filtered through a 0.45 μ M acrodisc (PALL Corporation) to remove cell debris and residual 293T cells. Filtered supernatant containing lentiviral particles was then applied to a culture of U-87 glioma cells (ATCC). Beginning 48 h post-transduction, stably transduced U-87 cells were selected for with 10 μ g/mL blasticidin (Invitrogen) in complete DMEM for 30 days to generate the mixed U-87/DC cell line. Expression of mCherry (and indirectly DAbR1) was verified by fluorescent microscopy prior to *in vivo* studies.

Supporting Information - New covalent capture probes

Establishment of U-87 and U-87/DC tumors. Cultures of U-87 and U-87/DC were expanded in T150 flasks in complete DMEM. Expression of the DAbR1-2A-mCherry reporter was maintained in U-87/DC with 5 µg/mL blasticidin until five days prior to injection. At that time, selection was removed in order to prevent negative effects on tumor take rate. At approximately 80% confluency, cells were trypsinized, resuspended in complete DMEM and centrifuged (2000 rpm, 5 min, RT). The cell pellet was then washed with PBS, centrifuged and resuspended in fresh PBS at a concentration of 3×10^7 cells/mL. One hundred microliters of the cell slurry (3×10^6 cells) was injected bilaterally into the subscapular region of six SCID Hairless Outbred (SHO) mice (Charles River) with U-87/DC cells delivered to the right shoulder and U-87 cells delivered to the left shoulder.

In vivo fluorescent imaging. Two hours prior to injection of radiolabeled probe, mice were imaged on a Maestro 2 (CRi) to verify mCherry expression. Images were acquired using the yellow filter set (excitation filter range – 576 to 621 nm; emission filter – 635 nm longpass; acquisition settings – 630 to 800 nm in 10 nm increments), 1x1 binning and an exposure time of 8 s. The spectrally unmixed composite images were overlaid with corresponding monochrome images using ImageJ software (<http://rsweb.nih.gov>).

In vivo Cerenkov Luminescence Imaging. At denoted intervals following administration of radiolabeled probe, Cerenkov luminescence imaging (CLI) was conducted for all mice. Images were acquired on an IVIS 100 (Caliper Life Sciences) using field of view “B” (15 cm), *f*/1, medium binning (binning factor 8), and an exposure time of 180 s. Luminescence images were overlaid with corresponding white light photograph images using Living Image software.

Ex vivo biodistribution. Animals (two cohorts, *n*=3 each) were euthanized by cervical dislocation following deep anesthesia 48 h after injection. Heart, lung, liver, kidney, spleen, stomach, intestine, bladder, and two (left and right) tumor tissues were removed, weighed, and counted for radioactivity with a calibrated gamma counter (1470 Wallac Wizard, Perkin Elmer) which measured the bremsstrahlung produced by the ^{90}Y radioactivity. Uptakes of radioisotope were calculated as the percentage of the injected dose per gram of tissue (%ID/g).

Literature Cited

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Supporting Information - New covalent capture probes

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