

SUPPORTING MATERIALS

Small Molecule Inhibitors Of The PCSK9•LDLR Interaction

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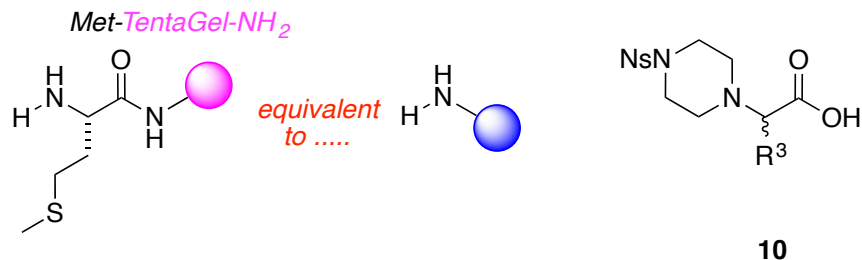
E-mail: burgess@tamu.edu

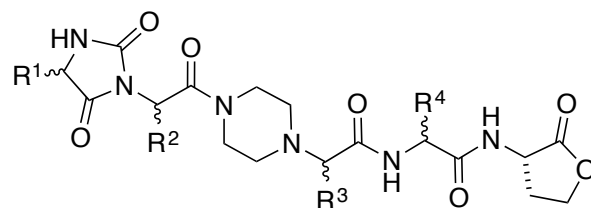
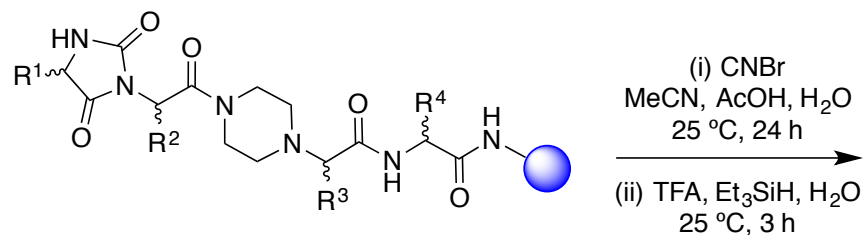
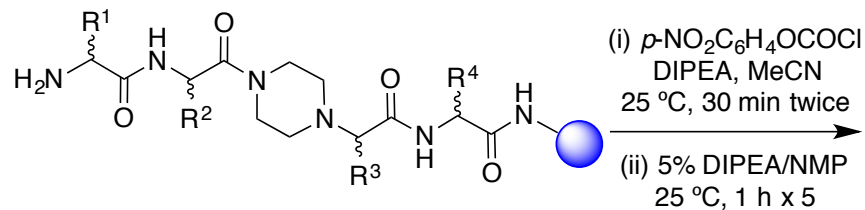
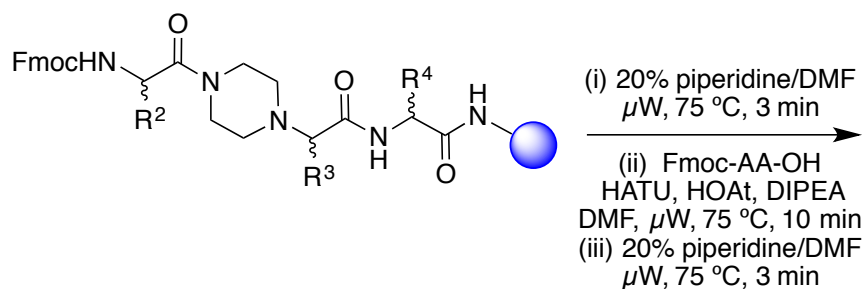
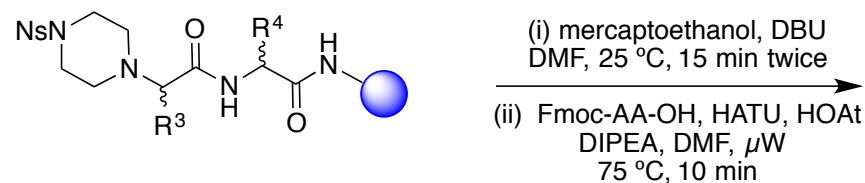
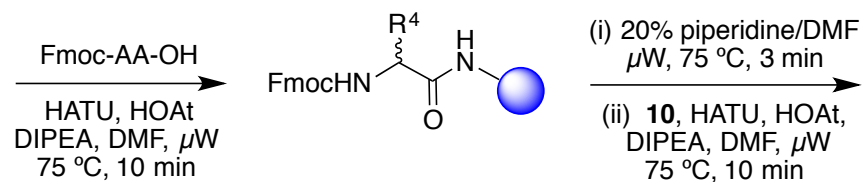
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A. General Experimental Procedures

All reactions were carried out under an inert atmosphere (nitrogen, or argon where stated) with dry solvents under anhydrous conditions. Glassware for anhydrous reactions was dried in an oven at 140 °C for minimum 6 h prior to use. Dry solvents were obtained by passing the previously degassed solvents through activated alumina columns. Reagents were purchased at a high commercial quality (typically 97 % or higher) and used without further purification, unless otherwise stated. High field NMR spectra were recorded with Bruker Avance III at 400 MHz for ^1H , and 100 MHz for ^{13}C and were calibrated using residual non-deuterated solvent as an internal reference (CDCl_3 : ^1H NMR = 7.24, ^{13}C NMR = 77.0, MeOD: ^1H NMR = 3.30, ^{13}C NMR = 49.0, DMSO- d_6 : ^1H NMR = 2.50, ^{13}C NMR = 39.5). Flash chromatography was performed using silica gel (230-600 mesh). Analytical thin layer chromatography (TLC) was carried out on Merck silica gel plates with QF 254 indicator and visualized by UV, ceric ammonium molybdate, ninhydrin, *para*-methoxybenzaldehyde and/or potassium permanganate stains. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, dd = double doublet, dt = double triplet, dq = double quartet, m = multiplet, br = broad. Electrospray ionization mass spectrometry (ESI-MS) data were collected on triple-stage quadrupole instrument in a positive mode. LC-MS analyses were collected from Agilent 1260 Infinity Quaternary LC and Agilent 6120 Quadrupole LC/MS modules using Poroshell 120 EC-C18 2.7 μm (4.6 x 50 mm) column in 5-95% MeCN/water gradient with 0.1% formic acid over 10 minutes. Microwave irradiation for solid-phase syntheses was done using CEM MARS 5® system. All statistical analyses were carried out by Graphpad Prism version 6.0 (Graphpad Software). Student's t-test was used to determine significant differences between compounds and negative control. Results are represented as means \pm SD.





1

DLLD-1ncl_k; DLLD-1ncl_r; DDDL-1vcl_r
 DDDD-1vcl_r; LDLL-1dln_r; LDLL-1dl(CN)_r
 LLLD-1qnd_w; LLLD-1qnd_r; DLLL-1qnd_r
 LLDL-1qnd_r; LLDD-1qnd_r; DLDL-1end_r
 DLDD-1end_h; LLLD-1ldn_q; LLLL-1aa_r

B. Solid-phase Syntheses and Characterizations of Compounds

1

Loading methionine linker on resin: Tentagel-amine resin (600 mg, 0.26 mequiv/g) was swollen with CH_2Cl_2 (5 mL) in a fritted syringe for 15 min, and then in DMF for at least 1 h. After removing DMF, Fmoc-Met-OH (232 mg, 0.62 mmol), $i\text{Pr}_2\text{NEt}$ (0.22 mL, 1.25 mmol) dissolved 2.5 mL of 0.25 M HATU/HOAt in DMF solution were added into the fritted syringe. The reaction was heated while stirring under microwave irradiation (100 W, 75 °C, 10 min). Solution was drained, and washed with DMF (4 mL x 5). The completeness of loading was determined by negative Kaiser test.

Fmoc deprotection: The Fmoc deprotection was performed by shaking beads in 2 mL of 20% piperidine in DMF for 1 min, drained, and beads were heated under microwave irradiation (160 W, 75 °C, 3 min) with fresh 4 mL of 20% piperidine solution. Solution was drained, and beads were washed with DMF (4 mL x 5), CH_2Cl_2 (4 mL x 3), MeOH (4 mL x 3) and DMF (4 mL x 3).

Amide coupling: Fmoc-amino acid or nosyl-containing amino acid (4 eq) and $i\text{Pr}_2\text{NEt}$ (8 eq) dissolved 2.5 mL of 0.25 M HATU/HOAt in DMF solution were added into the resin containing primary- or secondary-amine. Beads were heated under microwave irradiation (100 W, 75 °C, 10 min), then washed with 4 mL x 5 of DMF. For arginine amino acid, beads were shaken in the coupling solution for 20 min at room temperature, and then irradiated with microwave (100 W, 75 °C, 10 min). The solution was changed with the fresh Fmoc-arginine coupling solution and irradiated again with the same condition. For cysteine and histidine amino acids, the coupling reaction was performed under microwave at lower temperature to avoid epimerization (100 W, 50 °C, 15 min).

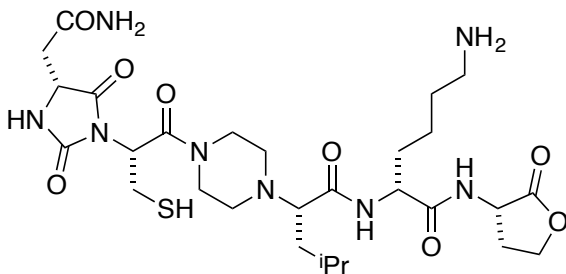
Nosyl deprotection: After coupling with nosyl-containing amino-acid derivatives, beads were shaken in the solution of mercaptoethanol (5 eq) and DBU (5 eq) in 3 mL of DMF for 15 min at room temperature. The orange solution was drained, washed thrice with 3 mL DMF, and beads were shaken again with the fresh solution of mercaptoethanol/DBU for 15 min at room temperature. Beads were washed 5 times with DMF, and a few beads were analyzed by chloranil test to confirm the presence of secondary amine.

Hydantoin cyclization: After the Fmoc deprotection at the R¹ position, beads were treated twice with the solution of 2 equiv of *p*-nitrophenyl chloroformate and 4 equiv of ⁱPr₂NEt in MeCN at ~ 0.05 M concentration. Beads were shaken at room temperature for 30 min each. After coupling step, beads were washed with MeCN (4 mL x 3), CH₂Cl₂ (4 mL x 3) and NMP (4 mL x 3). Beads were shaken 5 times with 5 mL of 5 % ⁱPr₂NEt /NMP solution for 1 h each to remove any byproducts. Beads were washed with NMP (4 mL x 5), CH₂Cl₂ (4 mL x 5) and MeOH (4 mL x 5) and dried.

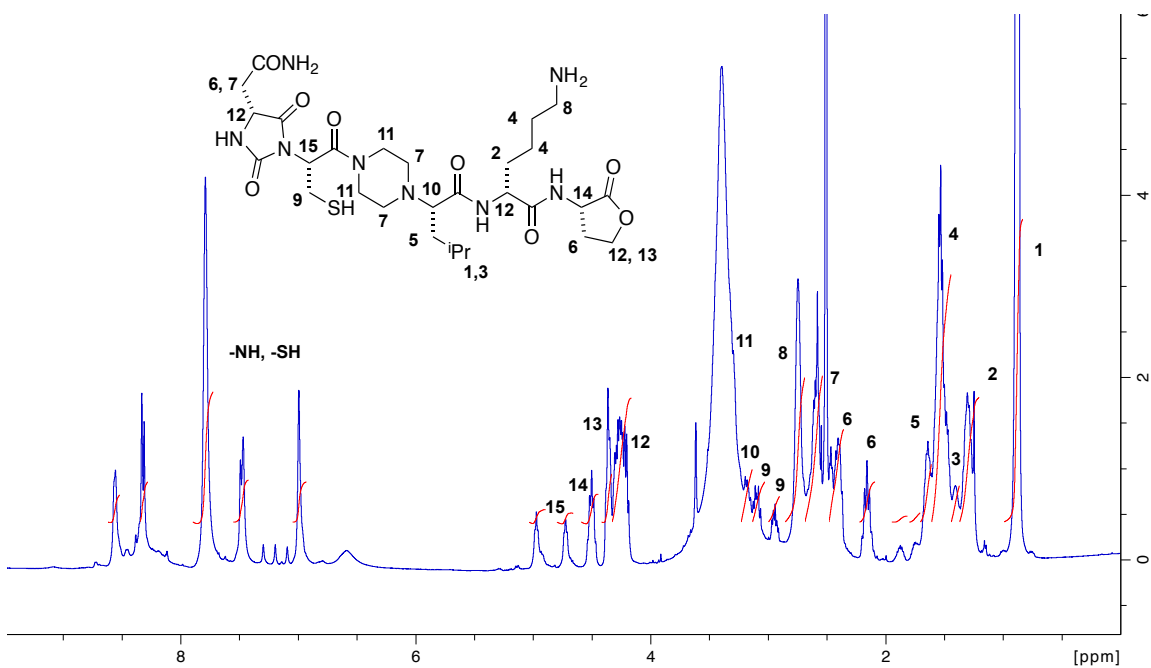
Cleavage from the resin: Compounds were cleaved off from the beads by treating with cyanogen bromide (30 mg/mL) in MeCN/acetic acid/water (5:4:1 v/v) solution. Beads were shaken at room temperature for at least 24 h. After filtration, the crude material was dried under nitrogen stream and purified by preparative reversed-phase HPLC (50% - 95% MeCN/water containing 0.05% TFA). Compounds were lyophilized to obtain white powders.

Side-chain deprotection: Purified compounds were stirred in TFA/Et₃SiH/water (95:2.5:2.5 v/v) cocktail for 4 h at room temperature. The crude materials were dried under vacuum, and then precipitated in cold *tert*-butyl methyl ether. The solid compounds were collected, washed several times with *tert*-butyl methyl ether. Compounds were dissolved in water and lyophilized to obtain products as white solids.

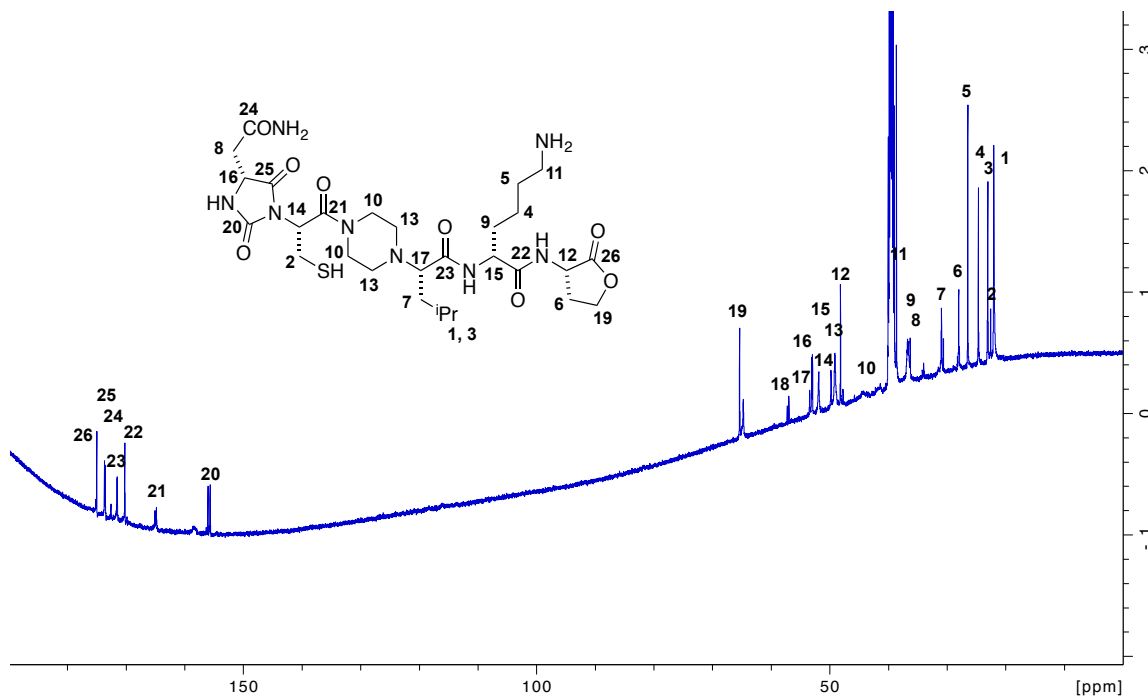
(*R*)-6-Amino-2-((*S*)-2-(4-((*R*)-2-((*R*)-4-(2-amino-2-oxoethyl)-2,5-dioxoimidazolidin-1-yl)-3-mercaptopropanoyl)piperazin-1-yl)-4-methylpentanamido)-*N*-((*S*)-2-oxotetrahydrofuran-3-yl)hexanamide (DLLD-1nclk)



Data for DLLD-1nclk. Yield 8%. $^1\text{H NMR}$ (400 MHz, DMSO-d_6) δ 8.56 (br, 1H), 8.32 (m, 1H), 7.79 (m, 4H), 7.48 (m, 1H), 6.99 (m, 1H), 4.72 (m, 1H), 4.50 (m, 1H), 4.37 (m, 1H), 4.33-4.17 (m, 3H), 3.49-3.39 (m, 4H), 3.37 (m, 1H), 3.10 (m, 1H), 2.94 (m, 1H), 2.75 (m, 2H), 2.64-2.54 (m, 5H), 2.49-2.36 (m, 2H), 2.16 (m, 1H), 1.69-1.22 (m, 9H), 0.88 (m, 6H);

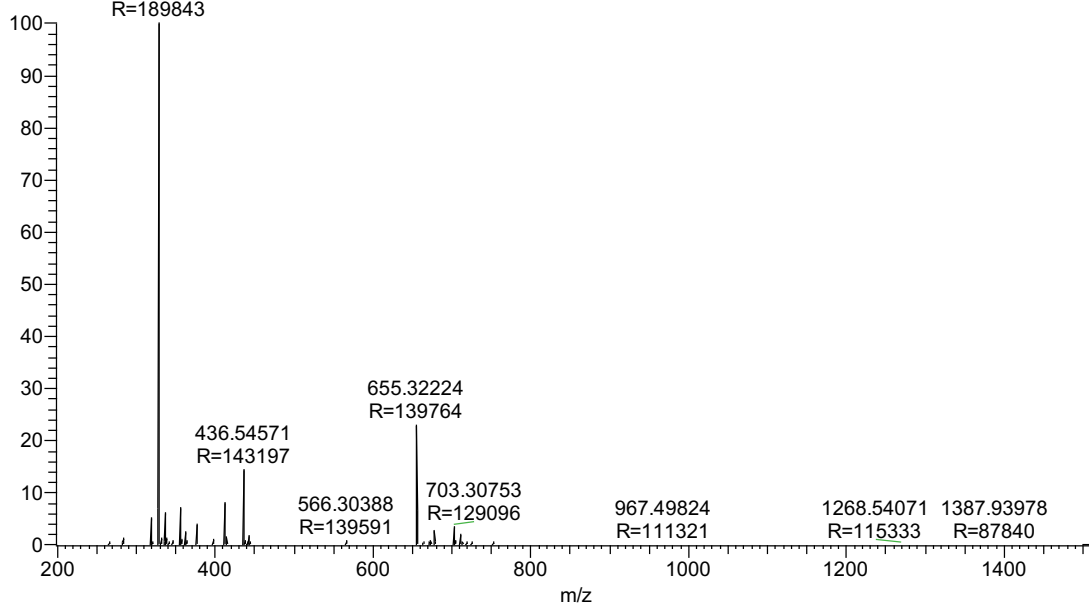


^{13}C NMR (100 MHz, DMSO-d_6) δ 175.0, 173.7, 173.6, 171.5, 170.2, 165.0, 155.7, 65.4, 57.1, 53.4, 53.1, 52.9, 51.9, 49.8, 49.1, 48.2, 44.5, 41.8, 38.6, 36.5, 36.3, 31.0, 28.0, 26.4, 24.7, 23.0, 22.6, 22.0

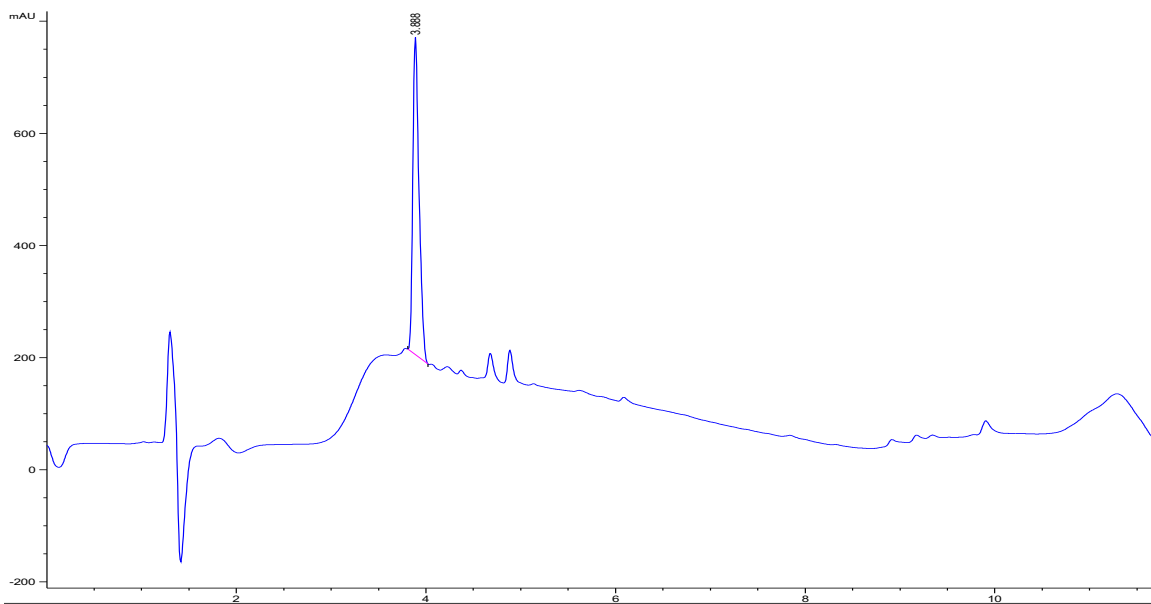


HRMS (ESI) m/z calcd for $\text{C}_{28}\text{H}_{47}\text{N}_8\text{O}_8\text{S}^+$ 655.3232; found 655.3222 ($\text{M}+\text{H}^+$)

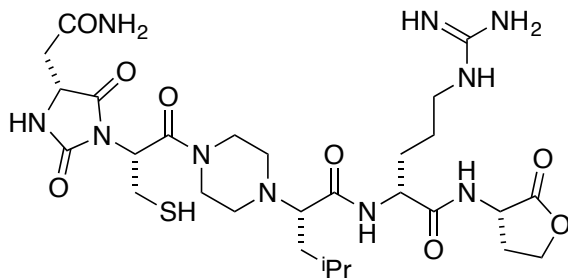
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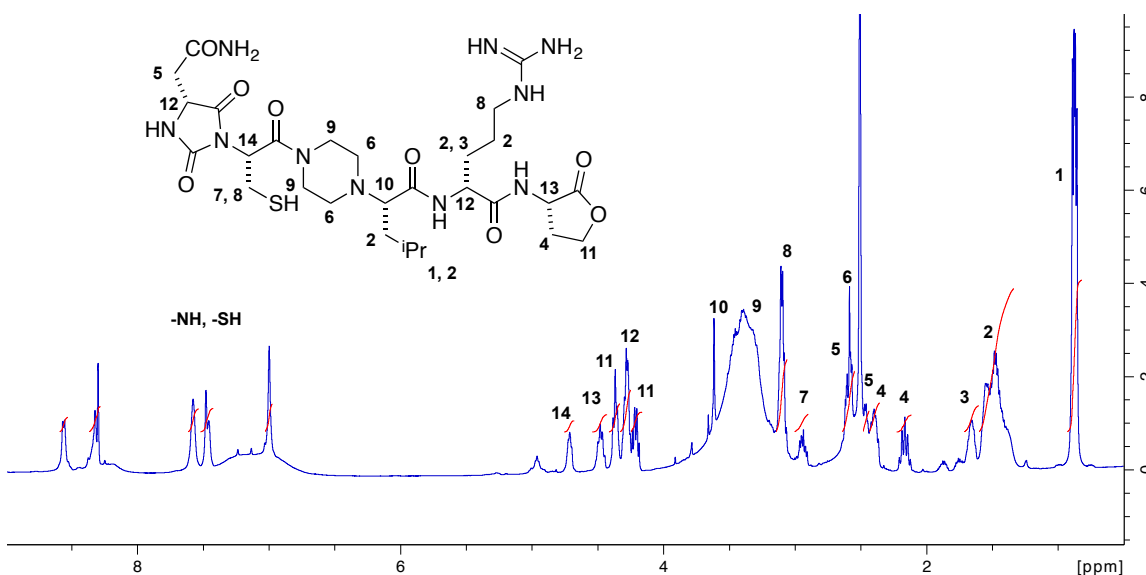
LC



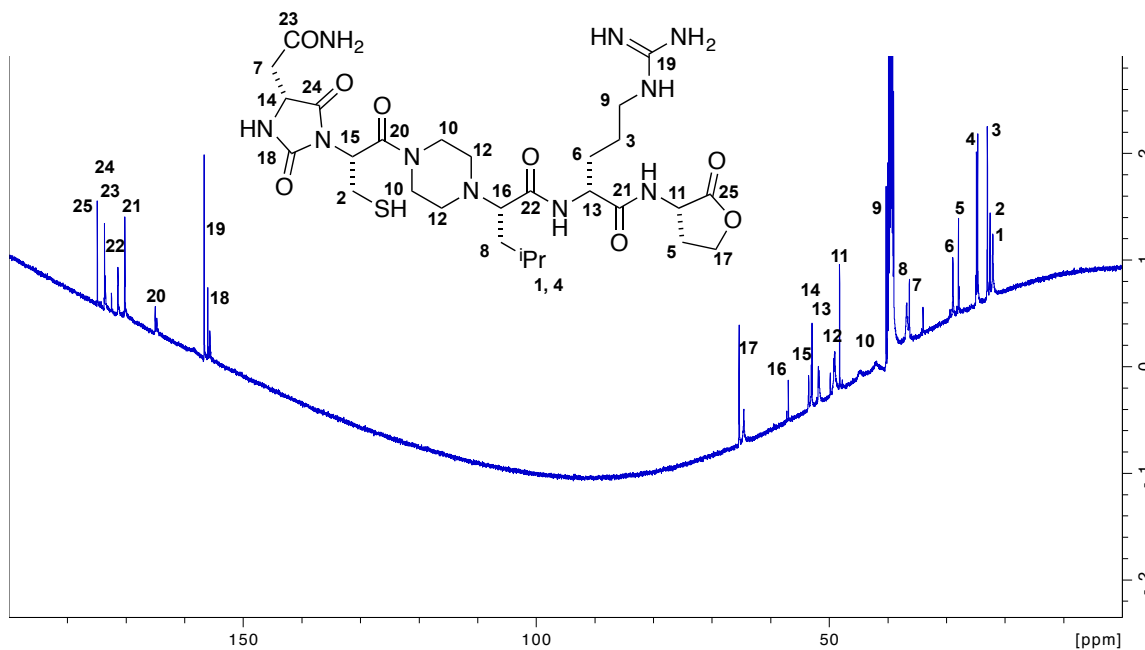
(S)-2-(4-((R)-2-((R)-4-(2-Amino-2-oxoethyl)-2,5-dioxoimidazolidin-1-yl)-3-mercaptopropanoyl)piperazin-1-yl)-N-((R)-5-guanidino-1-oxo-1-((S)-2-oxotetrahydrofuran-3-yl)amino)pentan-2-yl)-4-methylpentanamide (DLLD-1nclr)



Data for DLLD-1nclr. Yield 17%. $^1\text{H NMR}$ (400 MHz, DMSO-d_6) δ 8.57 (m, 1H), 8.32 (m, 1H), 7.58 (br, 1H), 7.47 (m, 1H), 7.00 (br, 1H), 4.72 (m, 1H), 4.48 (m, 1H), 4.37 (m, 1H), 4.28 (m, 2H), 4.21 (m, 1H), 3.62 (m, 1H), 3.40-3.23 (m, 4H), 3.10 (m, 3H), 2.94 (m, 1H), 2.63-2.55 (m, 3H), 2.53-2.48 (m, 2H), 2.48-2.44 (m, 1H), 2.44-2.36 (m, 1H), 2.17 (m, 1H), 1.66 (m, 1H), 1.61-1.32 (m, 6H), 0.88 (m, 6H);



^{13}C NMR (100 MHz, DMSO-d_6) δ 174.9, 173.7, 172.5, 171.4, 170.2, 165.0, 156.7, 156.0, 65.4, 64.6, 57.0, 53.5, 52.9, 51.8, 49.0, 48.3, 44.8, 42.1, 40.4, 36.8, 36.4, 28.9, 28.0, 24.9, 23.0, 22.5, 22.1



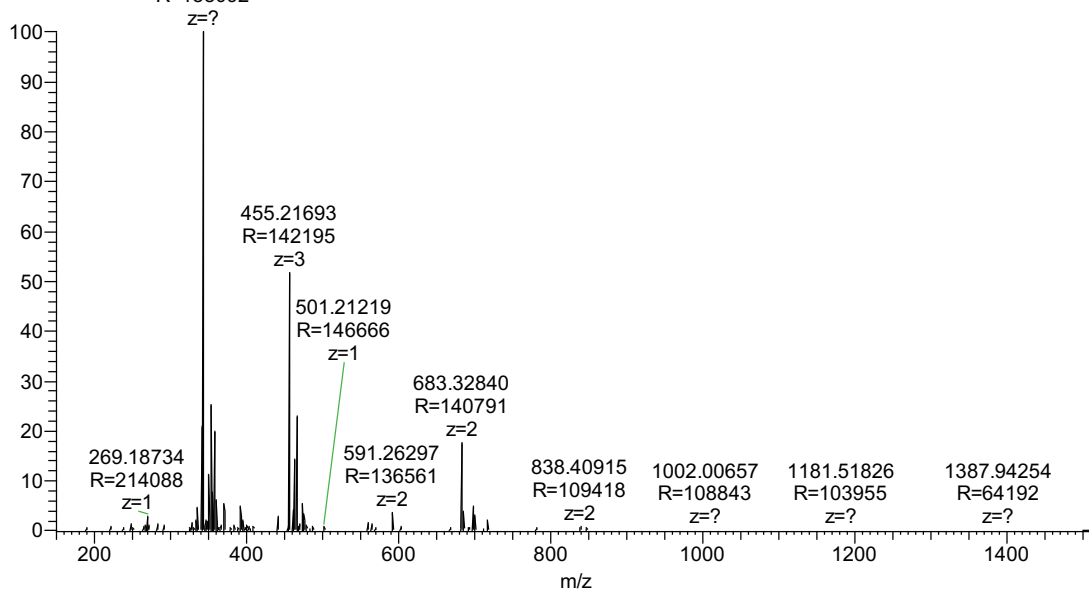
HRMS (ESI) m/z calcd for $\text{C}_{28}\text{H}_{47}\text{N}_{10}\text{O}_8\text{S}^+$ 683.3294; found 683.3284 ($\text{M}+\text{H}^+$)

06082017_DLLD-NCLR#1-140 RT: 0.01-1.34) NL: 3.72E7

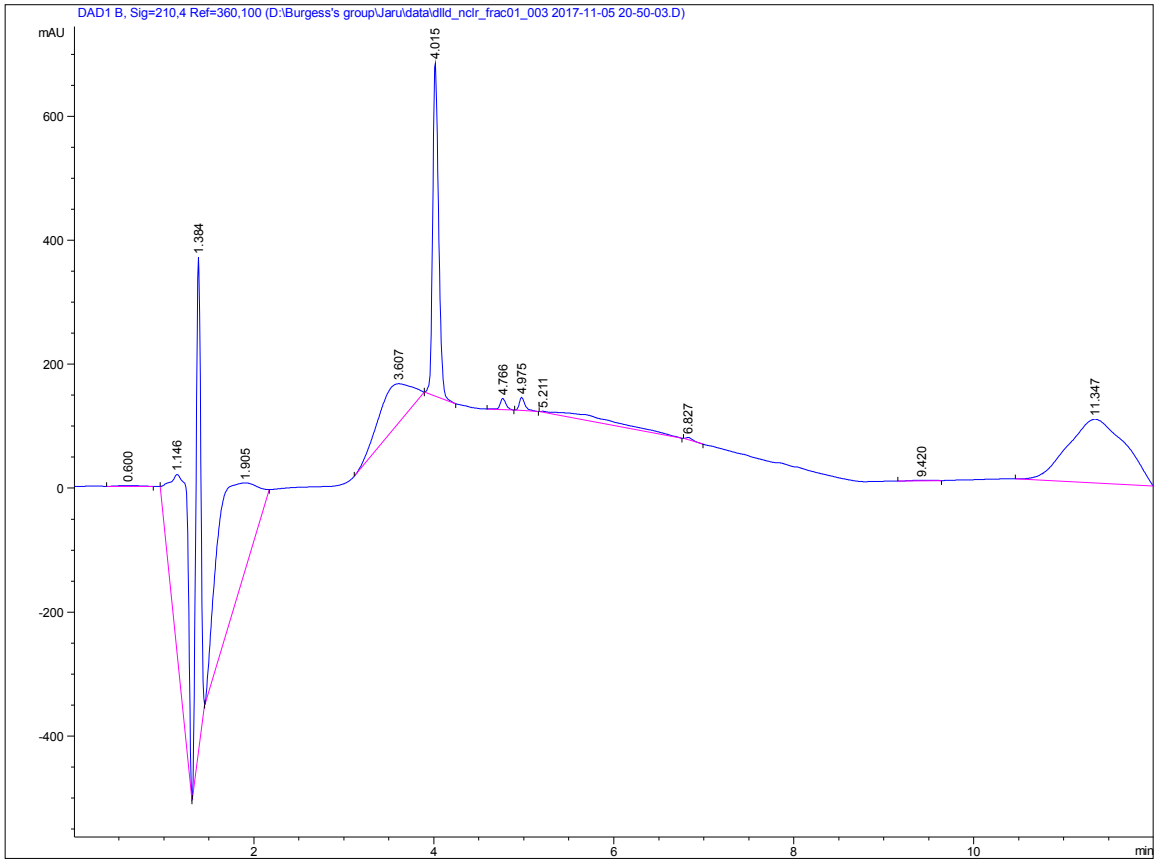
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342.16710

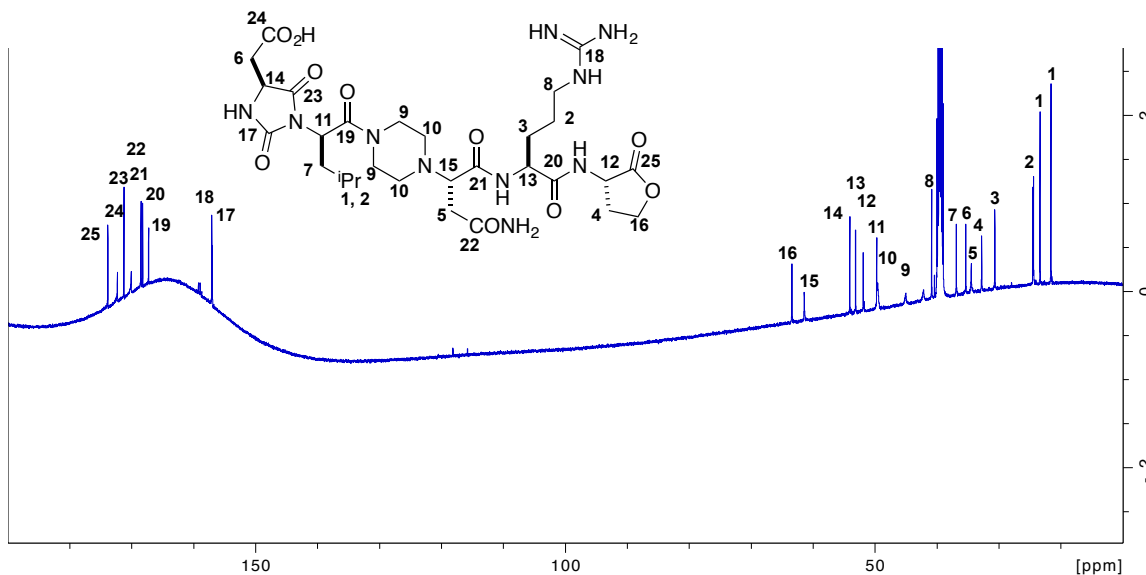
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LC

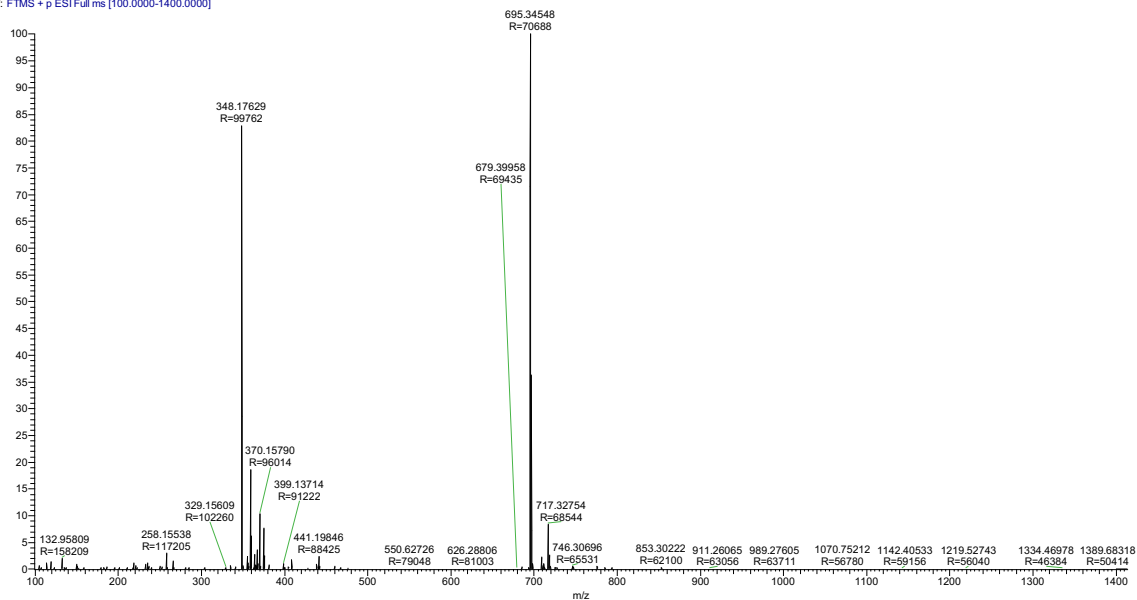


^{13}C NMR (100 MHz, DMSO-d_6) δ ; 173.9, 172.3, 171.3, 170.1, 168.5, 168.3, 167.3, 157.1, 157.0, 63.5, 61.5, 54.1, 53.2, 51.9, 49.8, 49.6, 45.1, 42.2, 40.9, 36.9, 35.4, 34.5, 32.8, 30.7, 24.6, 24.4, 23.4, 21.6

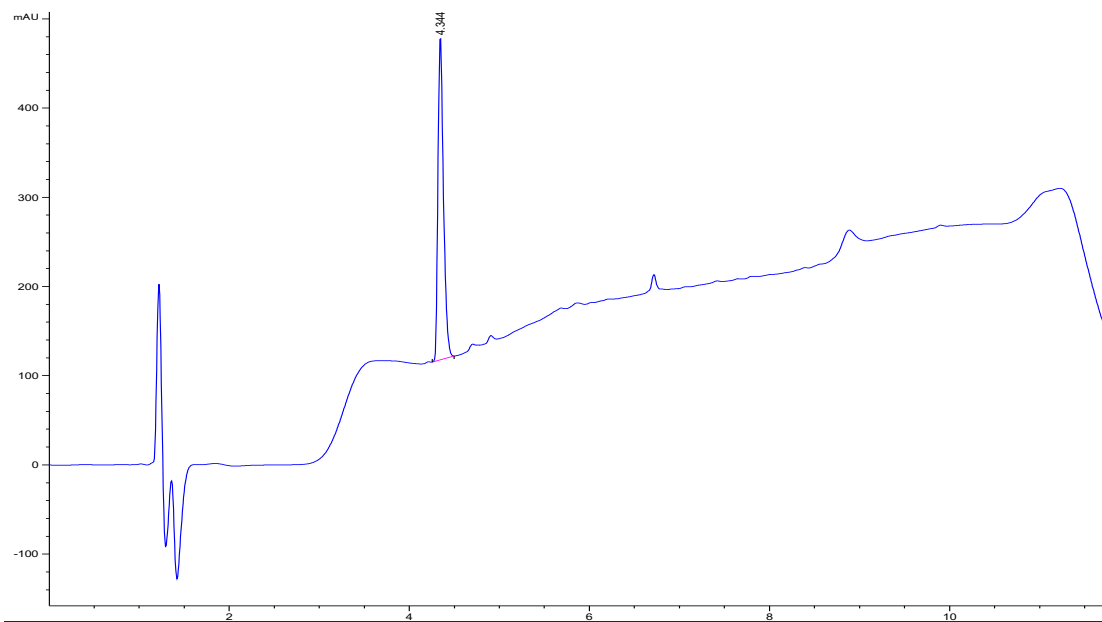


HRMS (ESI) m/z calcd for $\text{C}_{29}\text{H}_{47}\text{N}_{10}\text{O}_{10}^+$ 695.3471; found 695.3455 ($\text{M}+\text{H}^+$)

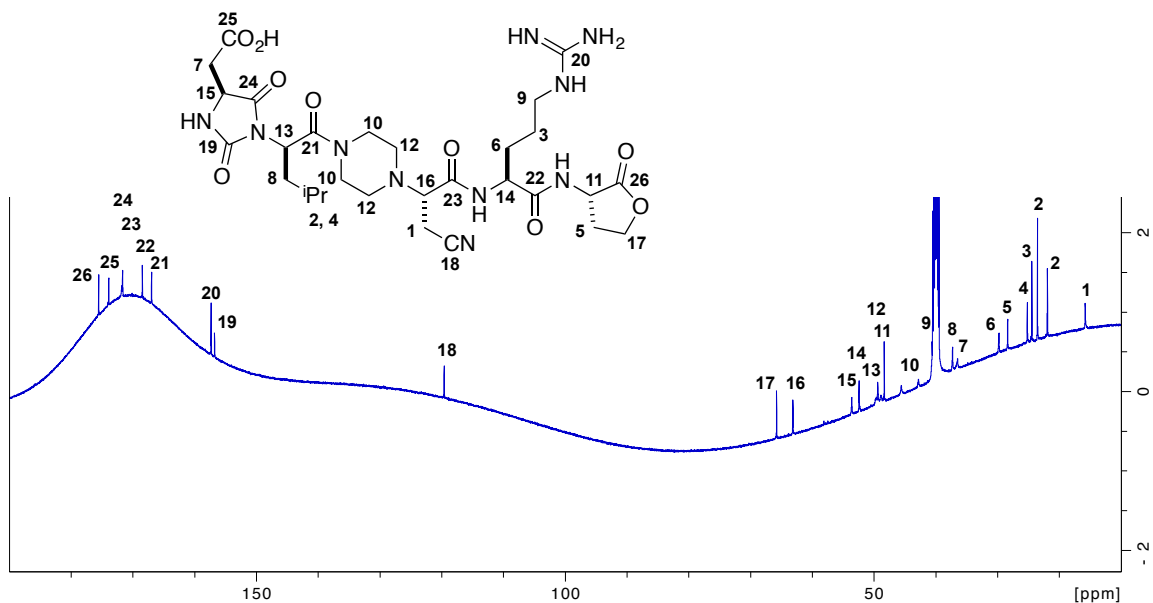
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LC

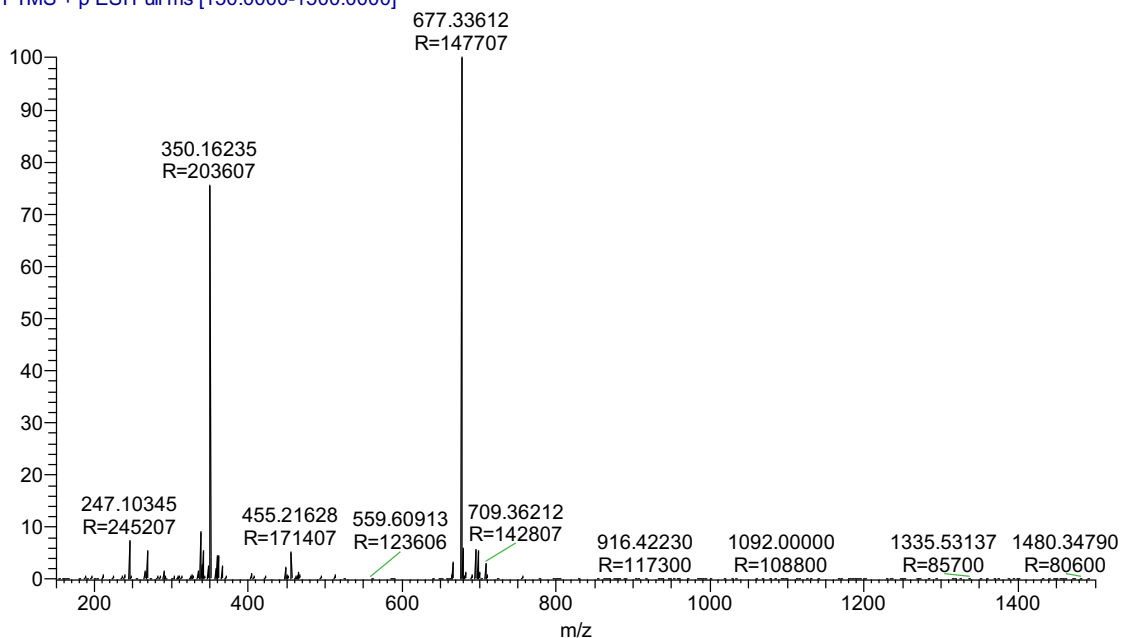


^{13}C NMR (100 MHz, DMSO-d_6) δ 175.0, 173.4, 171.3, 171.2, 168.0, 166.5, 156.8, 156.3, 119.1, 65.3, 62.7, 53.1, 51.9, 49.2, 48.9, 48.4, 47.9, 45.1, 42.3, 40.2, 36.8, 36.0, 29.2, 27.9, 24.7, 24.0, 23.0, 21.4, 15.3

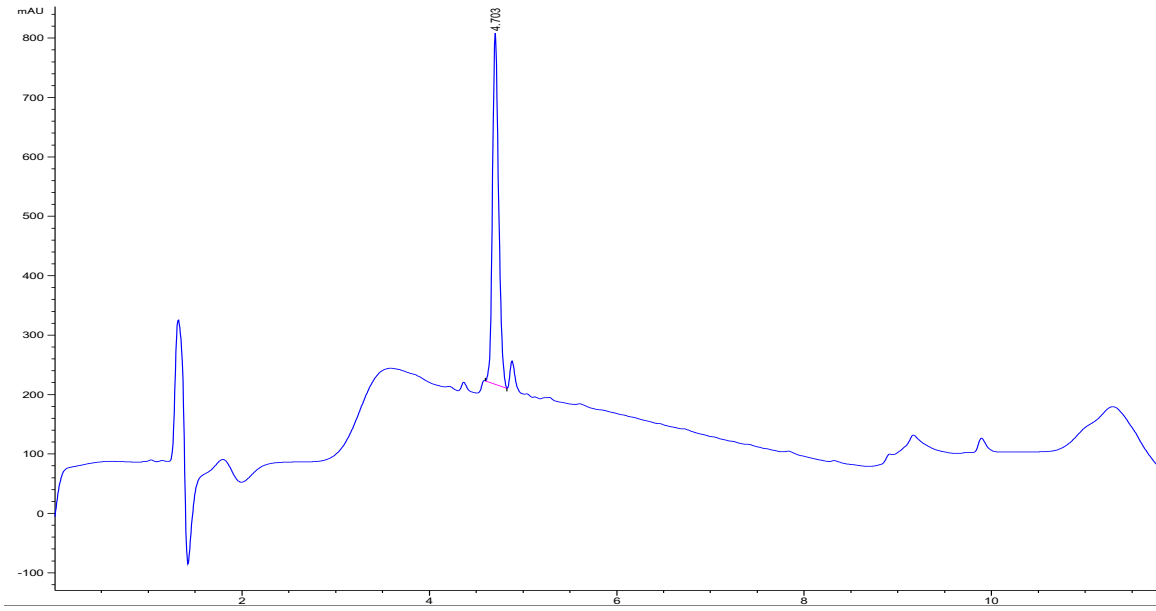


HRMS (ESI) m/z calcd for $\text{C}_{29}\text{H}_{45}\text{N}_{10}\text{O}_9^+$ 677.3365; found 677.3361 ($\text{M}+\text{H}$)⁺

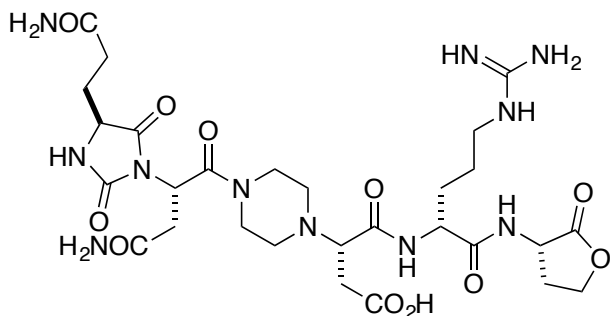
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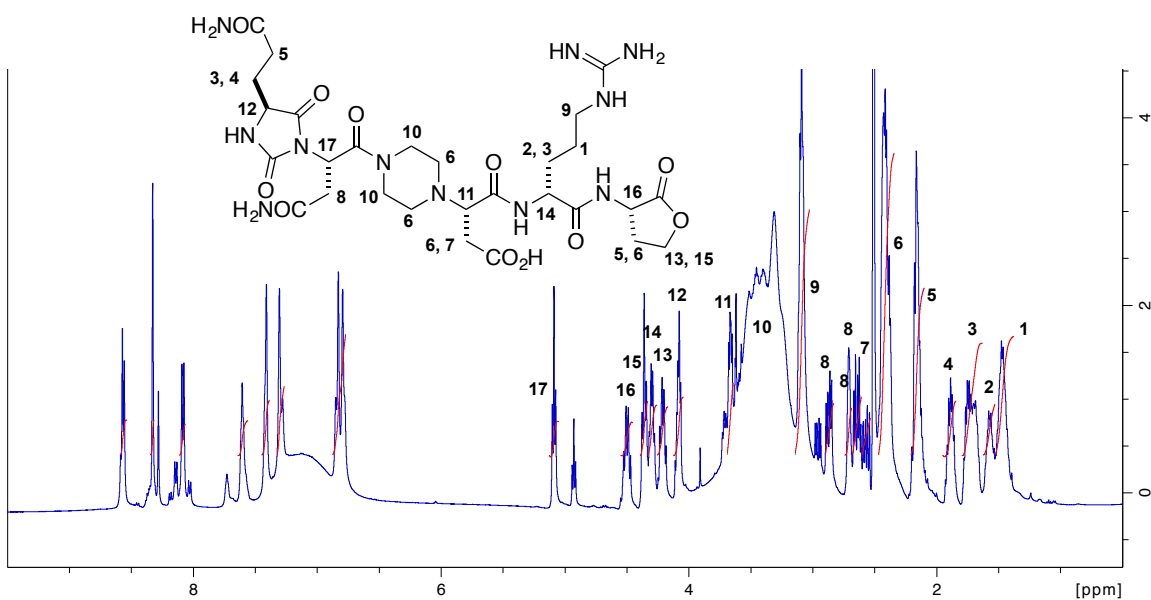
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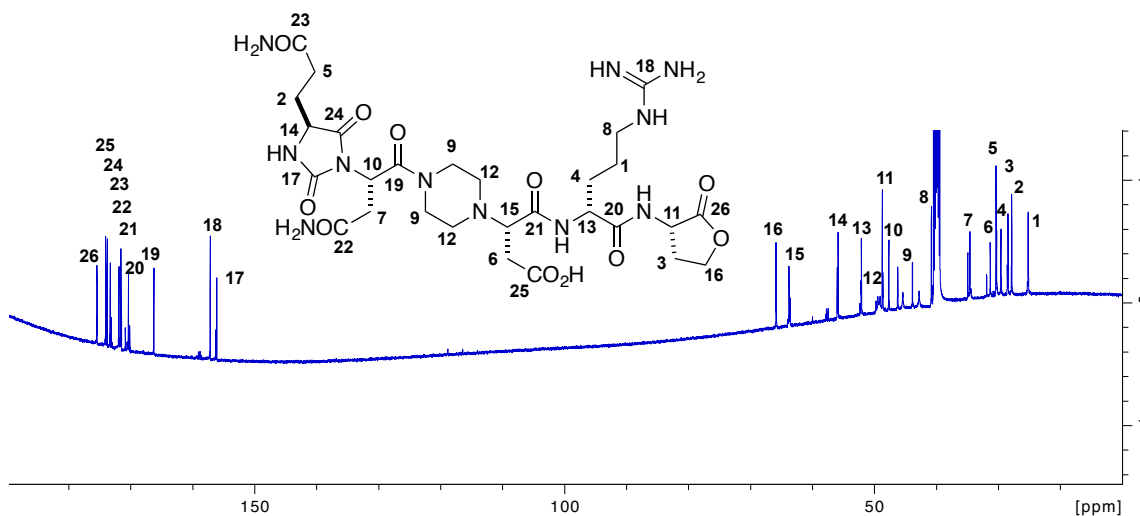
(S)-3-(4-((S)-4-Amino-2-((S)-4-(3-amino-3-oxopropyl)-2,5-dioxoimidazolidin-1-yl)-4-oxobutanoyl)piperazin-1-yl)-4-(((R)-5-guanidino-1-oxo-1-(((S)-2-oxotetrahydrofuran-3-yl)amino)pentan-2-yl)amino)-4-oxobutanoic acid (LLLD-1qndr)



Data for LLLD-1qndr. Yield 26%. $^1\text{H NMR}$ (400 MHz, DMSO-d_6) δ 8.57 (m, 1H), 8.33 (s, 1H), 8.25 (d, $J = 8.3$ Hz, 1H), 7.60 (br, 1H), 7.41 (br, 2H), 7.30 (br, 2H), 6.81 (m, 3H), 5.09 (t, $J = 7.0$ Hz, 1H), 4.50 (m, 1H), 4.36 (m, 1H), 4.30 (m, 1H), 4.21 (m, 1H), 4.09 (m, 1H), 3.67 (m, 1H), 3.42-3.17 (m, 4H), 3.09 (m, 2H), 2.87 (dd, $J = 15.5, 7.1$ Hz, 1H), 2.65 (dd, $J = 15.3, 6.8$ Hz, 1H), 2.56 (m, 1H), 2.42 (m, 6H), 2.16 (m, 3H), 1.89 (m, 1H), 1.75 (m, 1H), 1.69 (m, 1H), 1.58 (m, 1H), 1.47 (m, 2H)

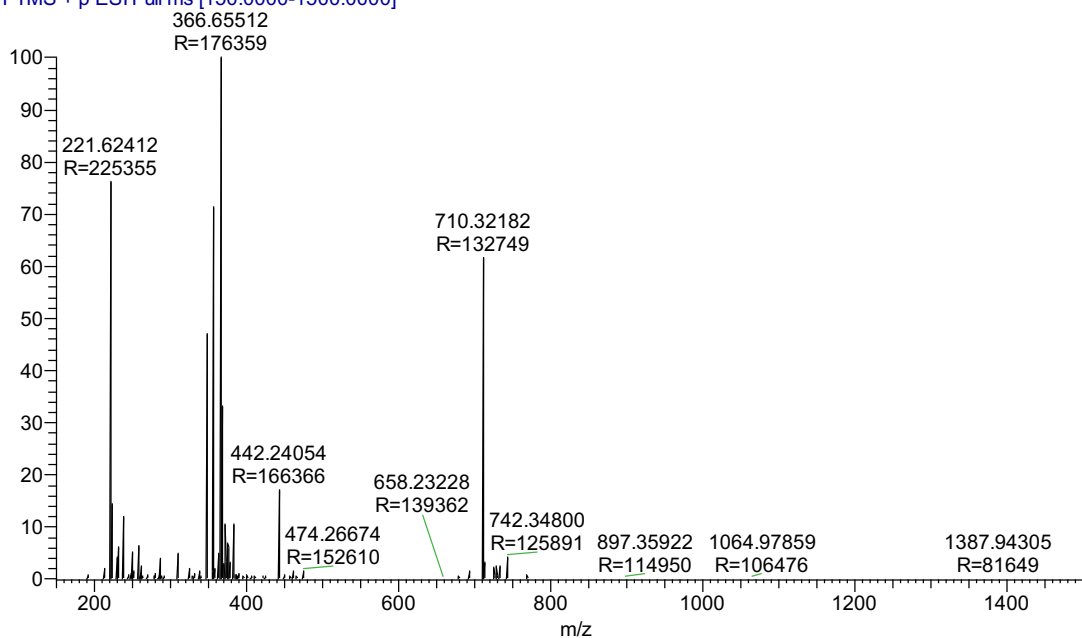


^{13}C NMR (100 MHz, DMSO-d_6) δ 175.0, 173.6, 173.3, 172.8, 171.4, 171.1, 169.9, 165.8, 156.7, 155.7, 65.4, 63.4, 55.4, 51.7, 48.9, 48.3, 47.2, 45.0, 42.3, 40.4, 34.2, 30.9, 30.0, 29.1, 28.0, 27.4, 24.7

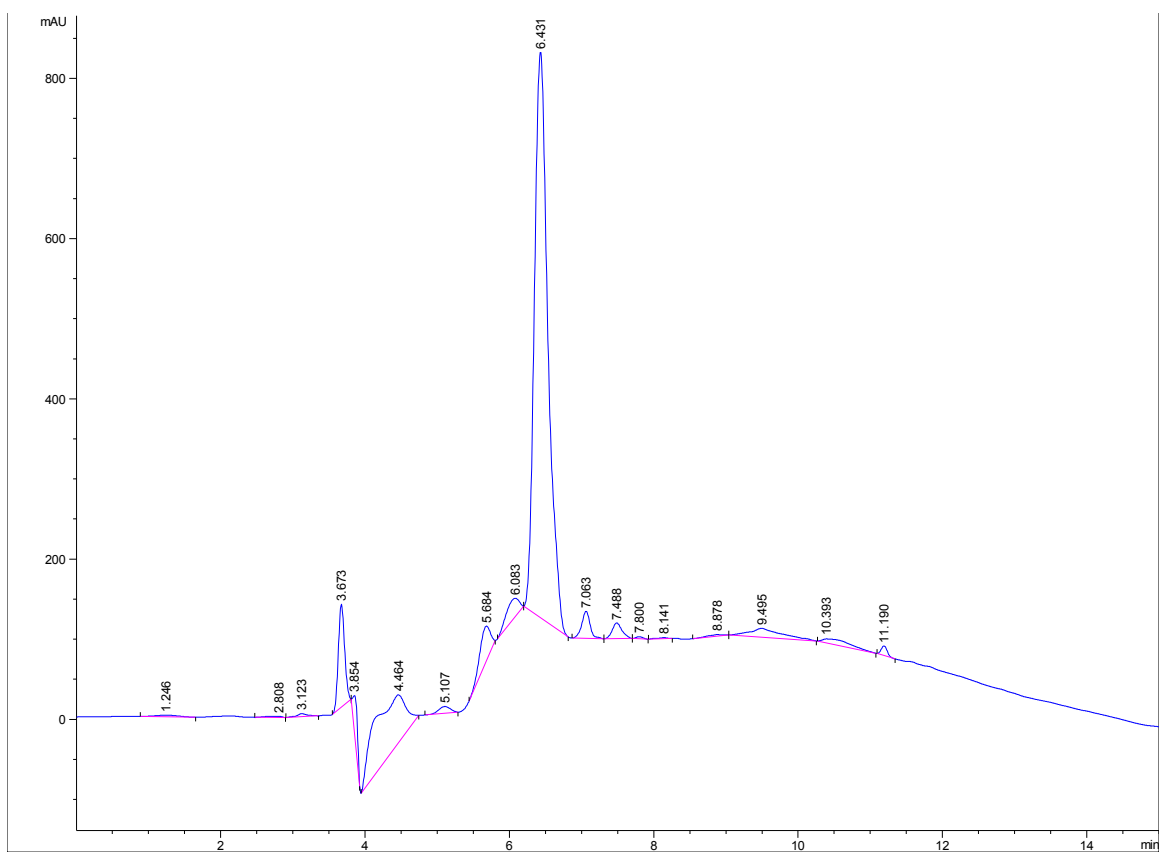


HRMS (ESI) m/z calcd for $\text{C}_{28}\text{H}_{44}\text{N}_{11}\text{O}_{11}^+$ 710.3216; found 710.3218 ($\text{M}+\text{H}^+$)

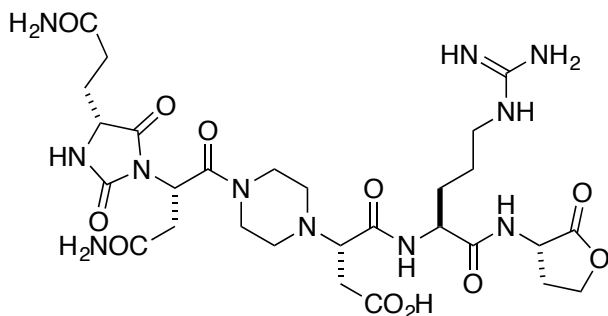
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T: FTMS + p ESI Full ms [150.0000-1500.0000]



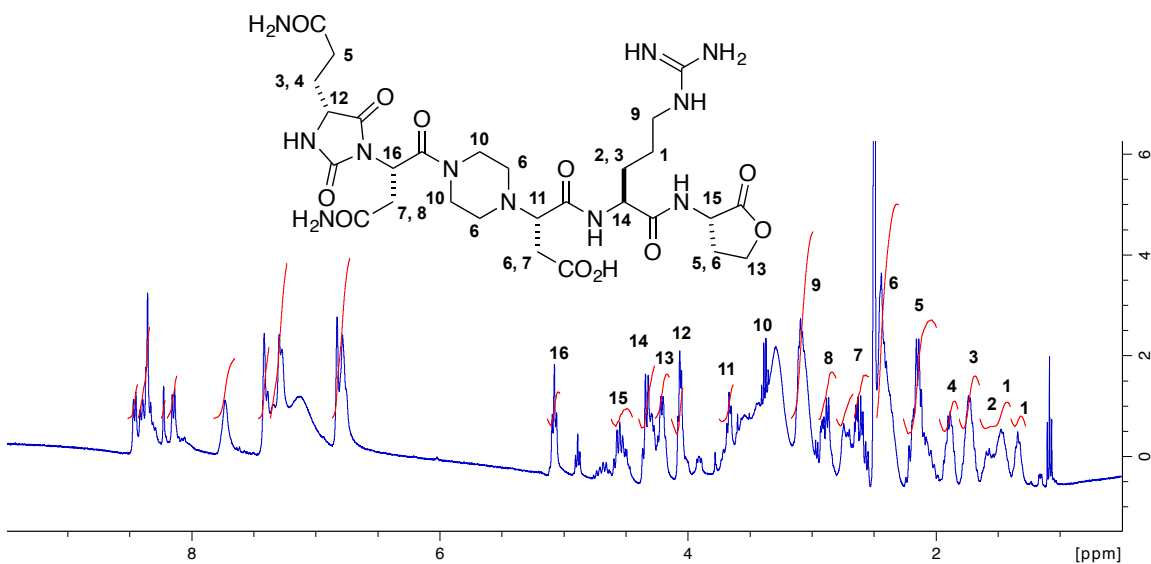
LC



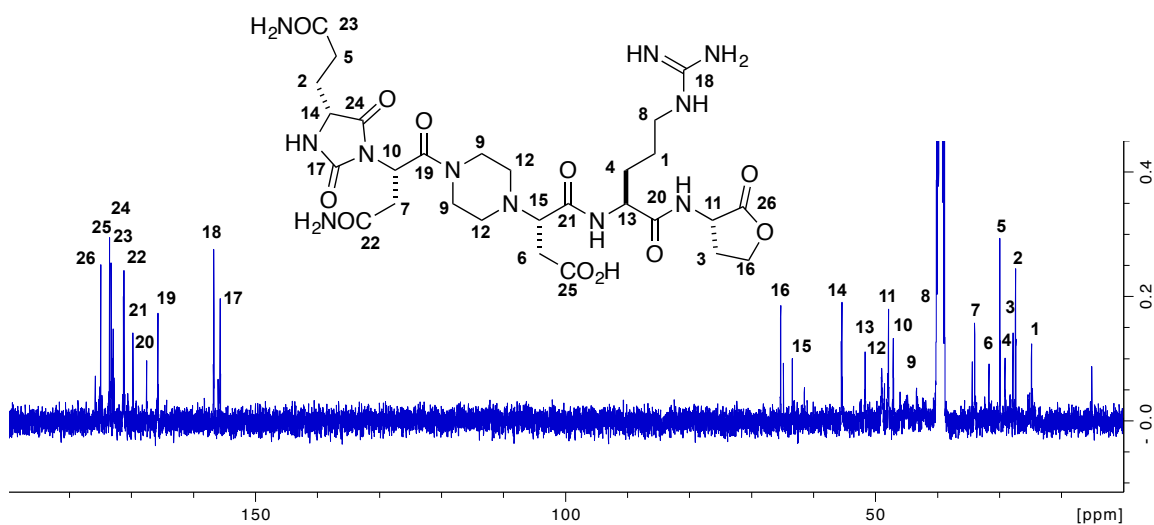
(S)-3-(4-((S)-4-Amino-2-((R)-4-(3-amino-3-oxopropyl)-2,5-dioxoimidazolidin-1-yl)-4-oxobutanoyl)piperazin-1-yl)-4-(((S)-5-guanidino-1-oxo-1-(((S)-2-oxotetrahydrofuran-3-yl)amino)pentan-2-yl)amino)-4-oxobutanoic acid (DLLL-1qndr)



Data for DLLL-1qndr. Yield 19%. ^1H NMR (400 MHz, DMSO- d_6) δ 8.46 (d, $J = 7.7$ Hz, 1H), 8.43-8.34 (m, 3H), 8.23 (br, 1H), 8.15 (d, $J = 8.2$ Hz, 1H), 7.73 (br, 1H), 7.42 (m, 1H), 7.28 (m, 2H), 6.81 (m, 2H), 5.08 (m, 1H), 4.53 (m, 1H), 4.39-4.26 (m, 2H), 4.21 (m, 1H), 4.07 (m, 1H), 3.67 (m, 1H), 3.29 (m, 4H), 3.08 (m, 2H), 2.89 (m, 1H), 2.67-2.53 (m, 2H), 2.48-2.30 (m, 6H), 2.26-1.99 (m, 3H), 1.90 (m, 1H), 1.73 (m, 2H), 1.64-1.40 (m, 2H), 1.34 (m, 1H)

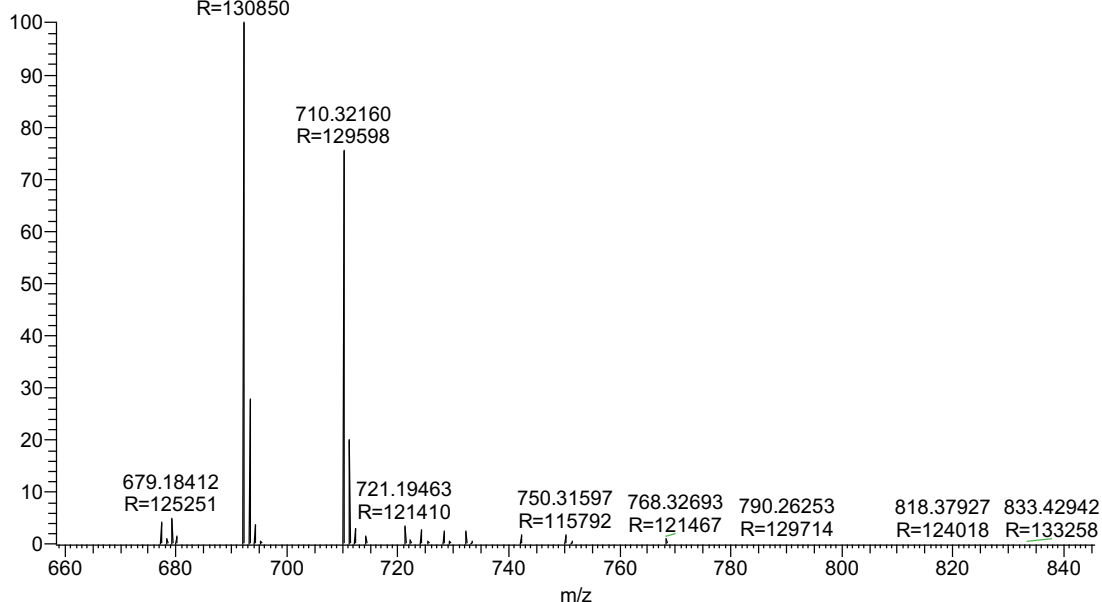


^{13}C NMR (100 MHz, DMSO-d_6) δ 175.0, 173.5, 173.3, 171.3, 171.2, 169.8, 167.5, 165.7, 156.7, 155.7, 65.3, 63.5, 55.4, 51.8, 49.0, 48.0, 47.2, 45.1, 42.2, 40.4, 34.1, 31.7, 30.0, 29.2, 27.8, 27.5, 24.9

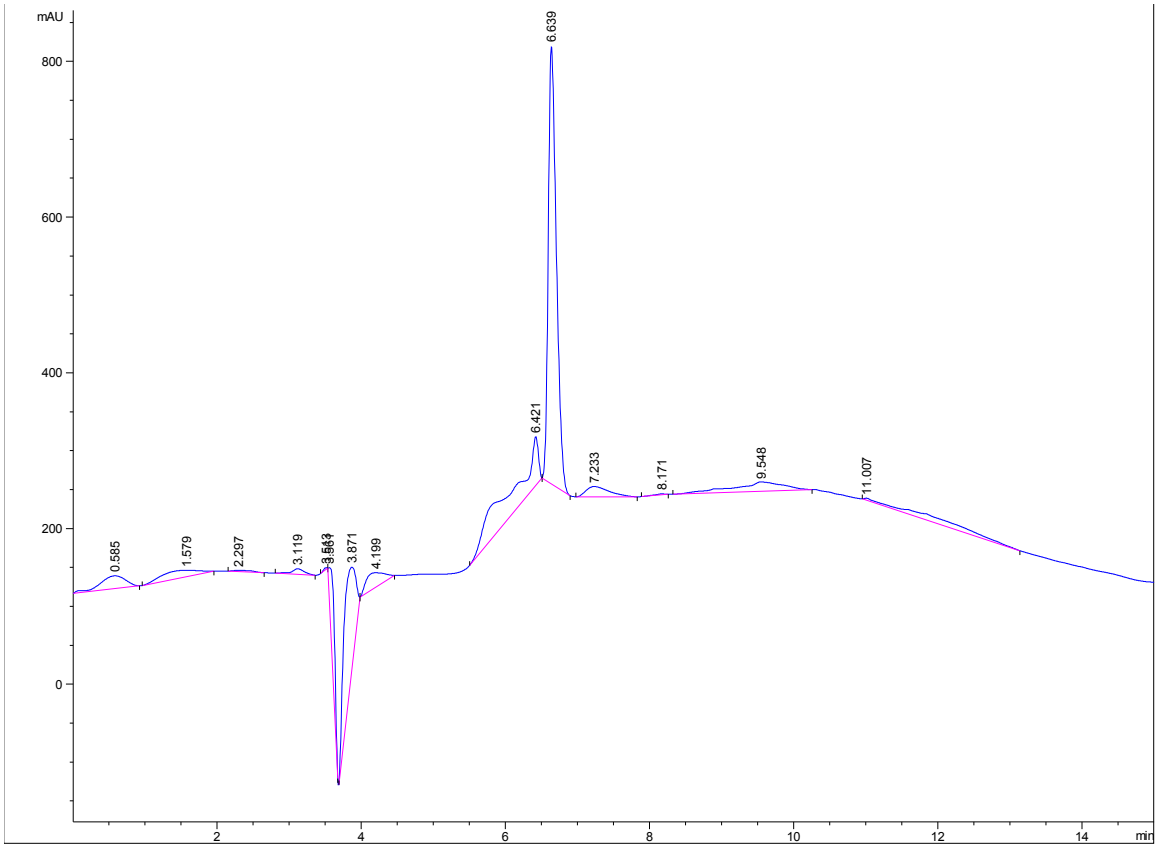


HRMS (ESI) m/z calcd for $\text{C}_{28}\text{H}_{44}\text{N}_{11}\text{O}_{11}^+$ 710.3216; found 710.3216 ($\text{M}+\text{H}^+$)

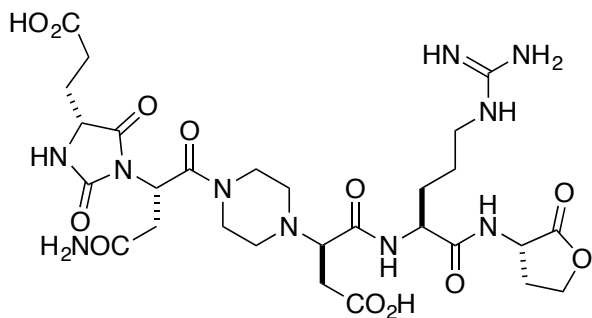
06082017_DLLL-QNDR #48-113 RT: 0.51-1.1: i6 NL: 5.05E6
 T: FTMS + p ESI Full ms [150.0000-1500.0000]
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 R=130850



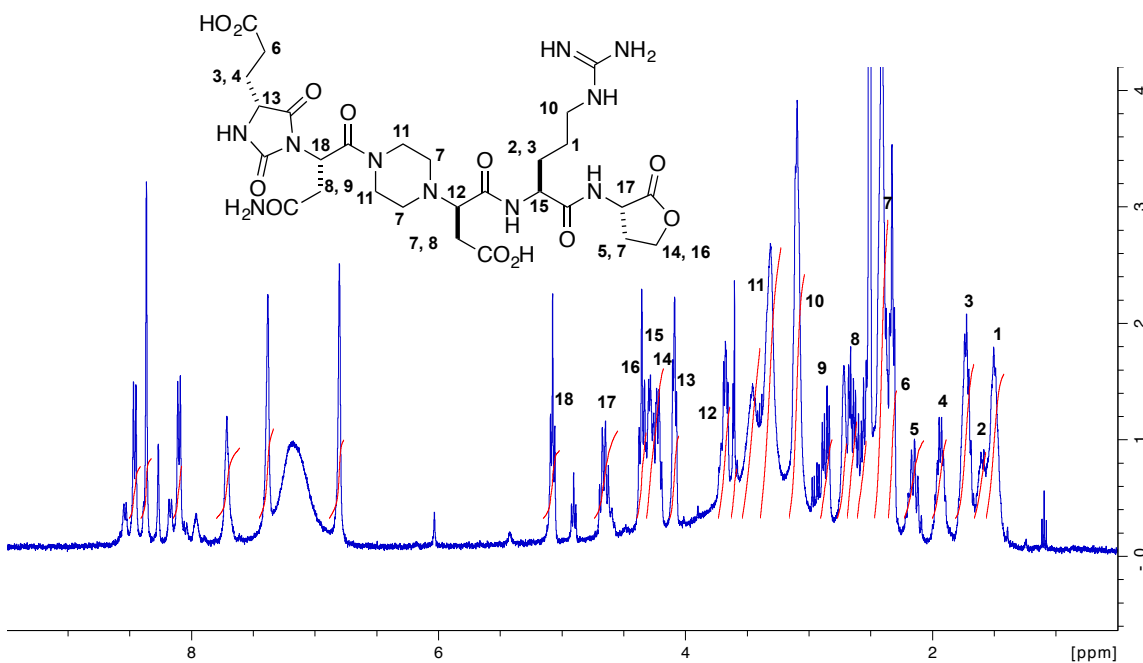
LC



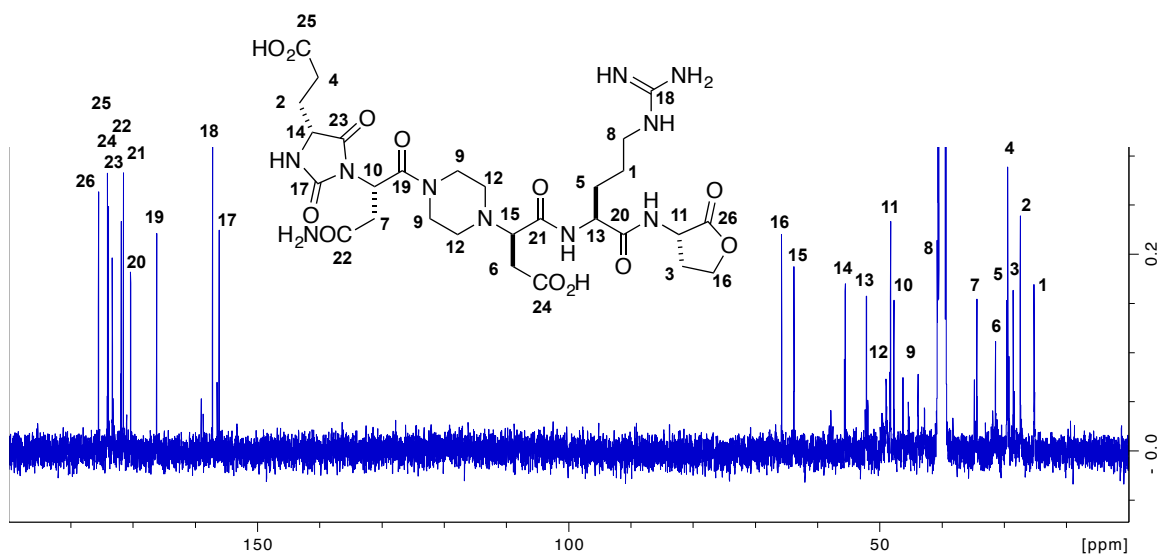
(*R*)-3-(4-((*S*)-4-Amino-2-((*R*)-4-(2-carboxyethyl)-2,5-dioximidazolidin-1-yl)-4-oxobutanoyl)piperazin-1-yl)-4-(((*S*)-5-guanidino-1-oxo-1-(((*S*)-2-oxotetrahydrofuran-3-yl)amino)pentan-2-yl)amino)-4-oxobutanoic acid (DLDL-1endr)



Data for DLDL-1endr. Yield 20%. ^1H NMR (400 MHz, DMSO- d_6) δ 8.46 (d, $J = 8.0$ Hz, 1H), 8.37 (s, 1H), 8.10 (d, $J = 8.3$ Hz, 1H), 7.71 (br, 1H), 7.38 (br, 1H), 7.31-6.97 (br, 4H), 6.80 (br, 1H), 5.08 (d, $J = 7.0$ Hz, 1H), 4.66 (m, 1H), 4.35 (m, 1H), 4.29 (m, 1H), 4.22 (m, 1H), 4.09 (m, 1H), 3.67 (m, 1H), 3.31 (m, 4H), 3.11 (m, 1H), 2.86 (dd, $J = 15.5$, 7.1 Hz, 1H), 2.65 (m, 1H), 2.61-2.52 (m, 2H), 2.46-2.36 (m, 6H), 2.33 (m, 2H), 2.15 (m, 1H), 1.94 (m, 1H), 1.73 (m, 2H), 1.61 (m, 1H), 1.50 (m, 2H)

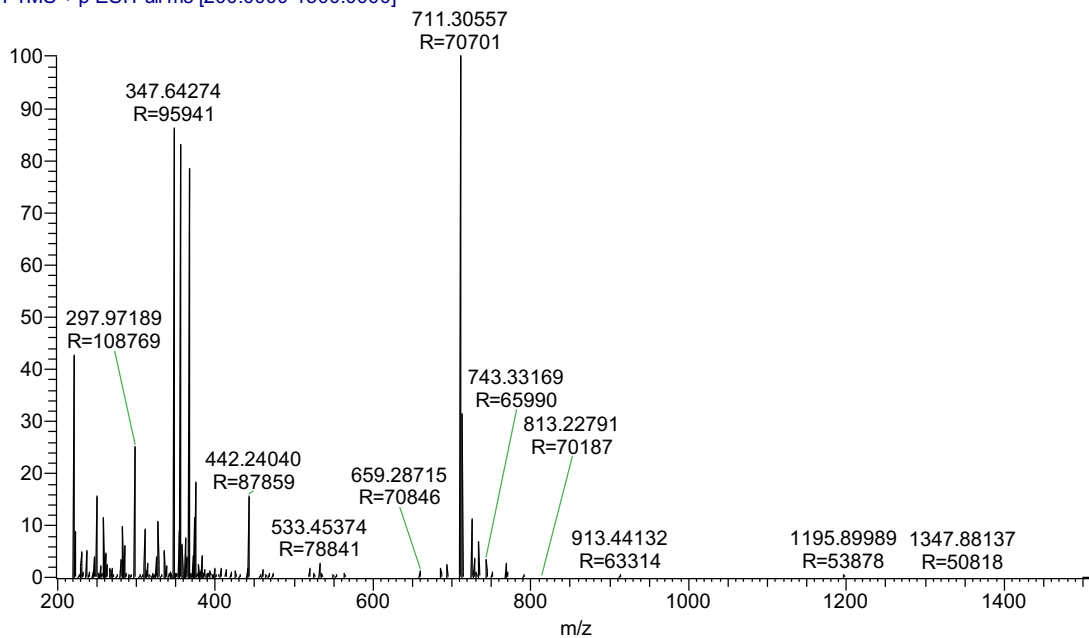


^{13}C NMR (100 MHz, DMSO-d_6) δ 175.6, 174.1, 174.0, 173.3, 171.9, 171.6, 170.4, 166.2, 156.7, 155.7, 65.3, 63.3, 55.1, 51.7, 48.5, 47.8, 47.2, 44.9, 42.3, 40.1, 33.9, 30.9, 29.1, 29.0, 28.1, 26.9, 24.7

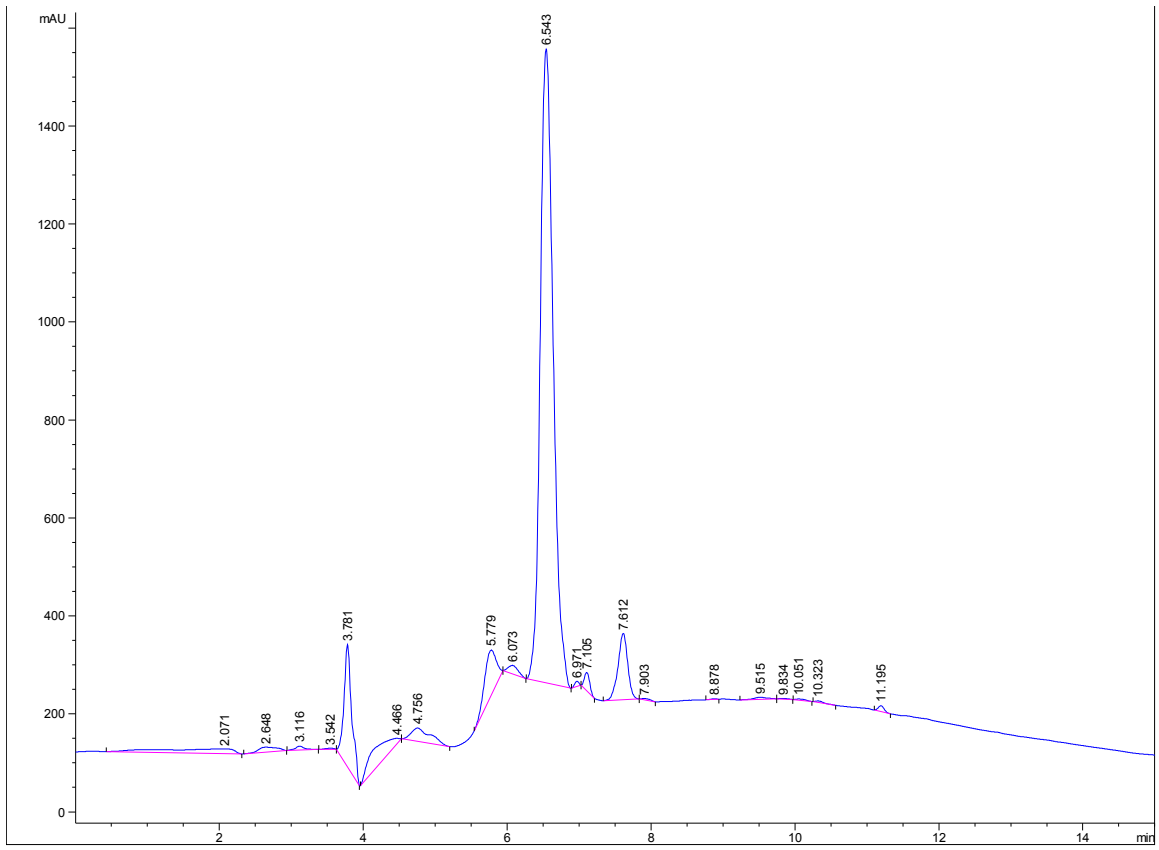


HRMS (ESI) m/z calcd for $\text{C}_{28}\text{H}_{43}\text{N}_{10}\text{O}_{12}^+$ 711.3056; found 711.3056 ($\text{M}+\text{H}^+$)

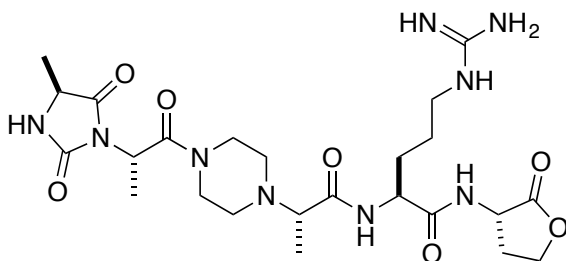
06122017_dldl-ENDR #1-176 RT: 0.00-0.94 / NL: 1.68E7
T: FTMS + p ESI Full ms [200.0000-1500.0000]



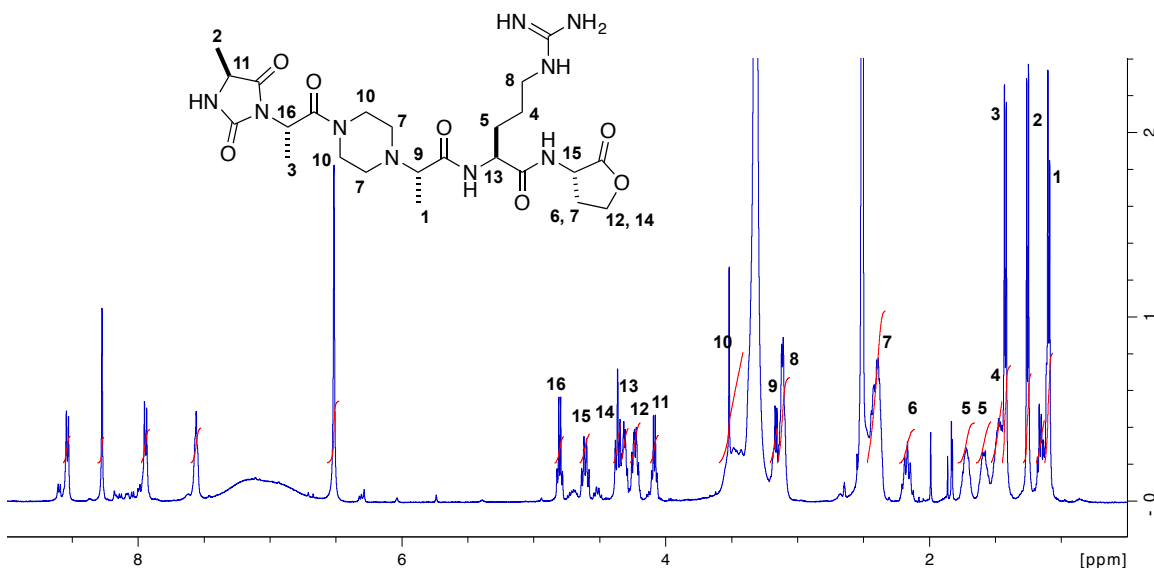
LC



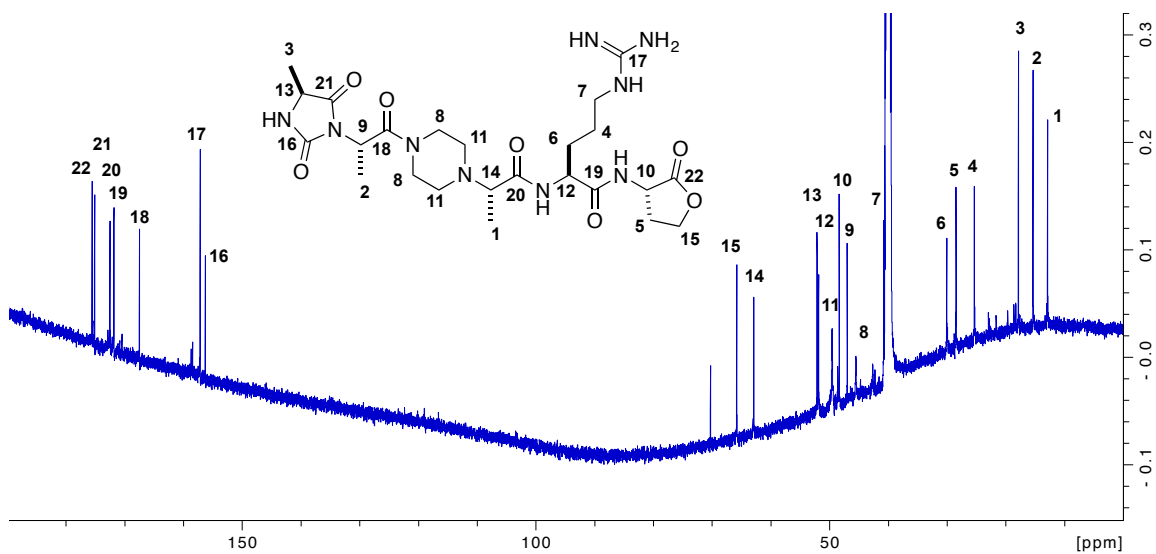
(S)-5-Guanidino-2-((S)-2-(4-((S)-2-((S)-4-methyl-2,5-dioxoimidazolidin-1-yl)propanoyl)piperazin-1-yl)propanamido)-N-((S)-2-oxotetrahydrofuran-3-yl)pentanamide (LLLL-1a_{aa}r)



Data for LLLL-1a_{aa}r. Yield 6%. ¹H NMR (400 MHz, DMSO-d₆) δ 8.54 (d, *J* = 8.0 Hz, 1H), 8.28 (s, 1H), 7.94 (d, *J* = 8.4 Hz, 1H), 7.56 (br, 1H), 6.52 (s, 2H), 4.80 (q, *J* = 7.1 Hz, 1H), 4.61 (q, *J* = 9.3 Hz, 1H), 4.36 (t, *J* = 8.7 Hz, 1H), 4.31 (m, 1H), 4.23 (m, 1H), 4.09 (q, *J* = 7.0 Hz, 1H), 3.60-3.41 (m, 4H), 3.17 (m, 1H), 3.12 (m, 2H), 2.41 (m, 5H), 2.17 (m, 1H), 1.72 (m, 1H), 1.59 (m, 1H), 1.48 (m, 2H), 1.42 (d, *J* = 7.1 Hz, 3H), 1.26 (d, *J* = 6.9 Hz, 3H), 1.09 (d, *J* = 6.9 Hz, 3H)

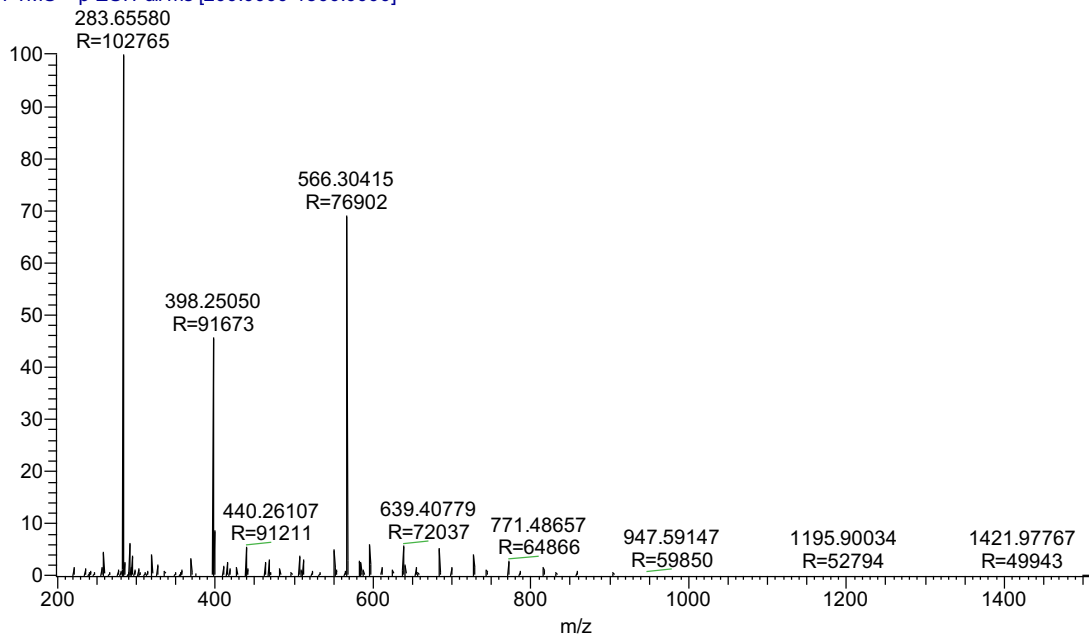


^{13}C NMR (100 MHz, DMSO-d_6) δ 175.0, 174.6, 172.0, 171.3, 167.0, 156.6, 155.7, 65.3, 62.4, 51.6, 51.3, 49.1, 47.8, 46.5, 45.0, 42.1, 40.3, 29.5, 27.9, 24.8, 17.3, 14.8, 12.3

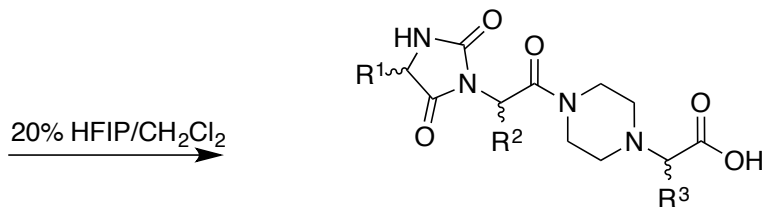
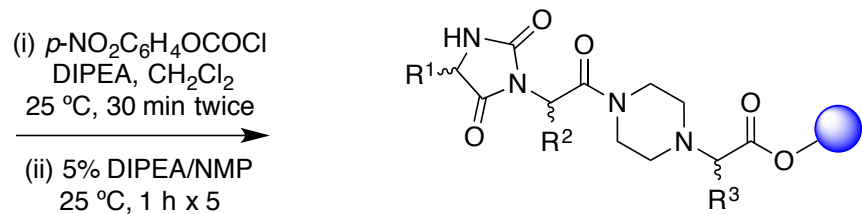
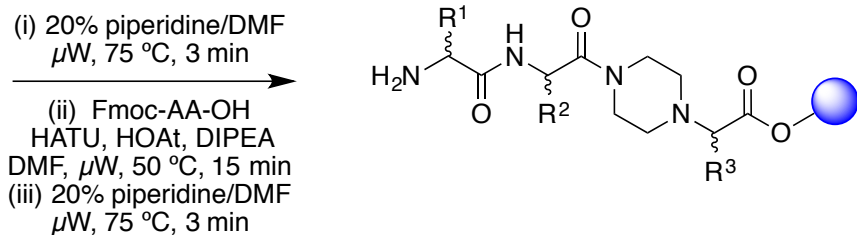
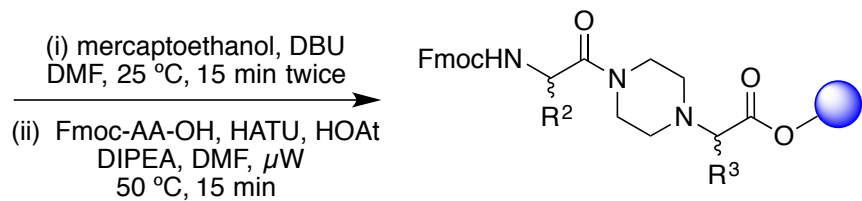
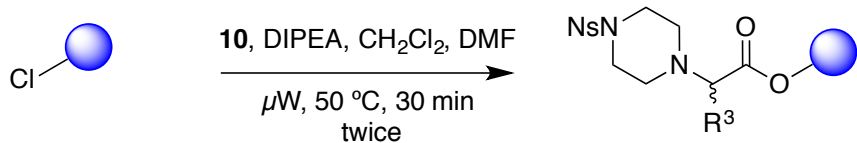


HRMS (ESI) m/z calcd for $\text{C}_{24}\text{H}_{40}\text{N}_9\text{O}_7^+$ 566.3045; found 566.3042 ($\text{M}+\text{H}$) $^+$

06122017_III-AAAR #1-152 RT: 0.00-0.81 AV NL: 7.81E7
T: FTMS + p ESI Full ms [200.0000-1500.0000]



2-Cl-Trt resin



A

DLL-**Ancl**; DDD-**Avcl**; LLL-**Aldn**
LDL-**Adln**; LLL-**Acnd**; DLL-**Acnd**
LLD-**Acnd**; DLD-**Acnd**

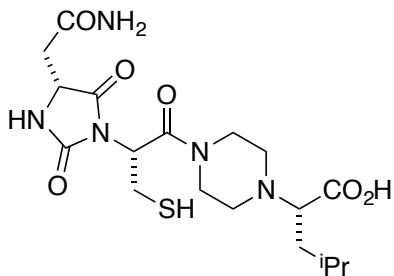
C. Solid-phase Syntheses and Characterizations of Compounds **A**

Loading, amide coupling and deprotection: 2-Cl-Trt resin (200 mg, 1.2 mequiv/g) in fritted syringe was swollen in CH₂Cl₂ for 1 h. Nosyl-protected piperazine amino acid (1.2 eq) and ⁱPr₂NEt (3 eq) in 3 mL of CH₂Cl₂/DMF (50:50) were added into the syringe, and beads were subjected to the microwave irradiation (100 W, 50 °C, 30 min). Reaction was repeated with a fresh solution. Beads were washed thrice with CH₂Cl₂ (4 mL x 3), then the remaining active beads were blocked with MeOH/ⁱPr₂NEt (9:1) for 1 h. Beads were washed thoroughly with CH₂Cl₂ (4 mL x 5 mL), MeOH (4 mL x 3), CH₂Cl₂ (4 mL x 3) and DMF (4 mL x 3). The coupling reaction was carried out similar to the procedure for preparing compound **1** described above except using microwave irradiation at lower temperature (50 °C, 15 min). Fmoc- and nosyl-deprotection were done same as described above.

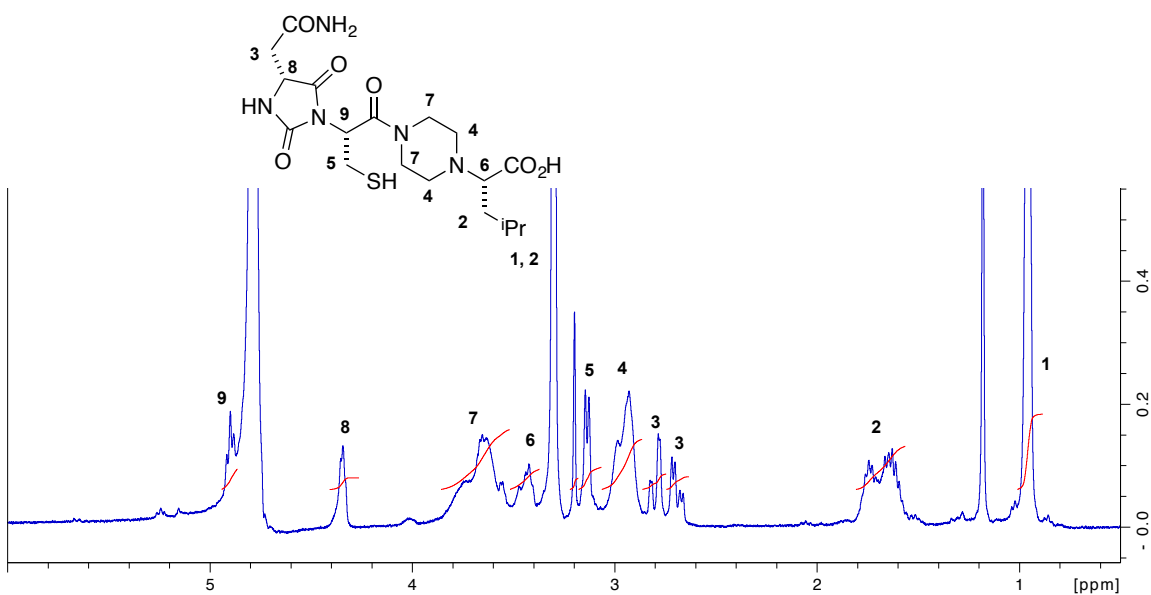
Hydantoin cyclization: The hydantoin cyclization was performed similar to the protocol described above except the coupling reaction with *p*-nitrophenyl chloroformate was performed in CH₂Cl₂ instead of MeCN.

Cleavage from solid support: Products were cleaved with 20% HFIP/CH₂Cl₂ solution for 24 h. The crude material was dried under N₂ stream and purified by preparative reverse-phase HPLC (50-95% MeCN/water containing 0.05% TFA). The purified products were lyophilized to obtain protected-form of compounds **A** as white powder. Side-chain deprotection was performed similar to compounds **1** described above.

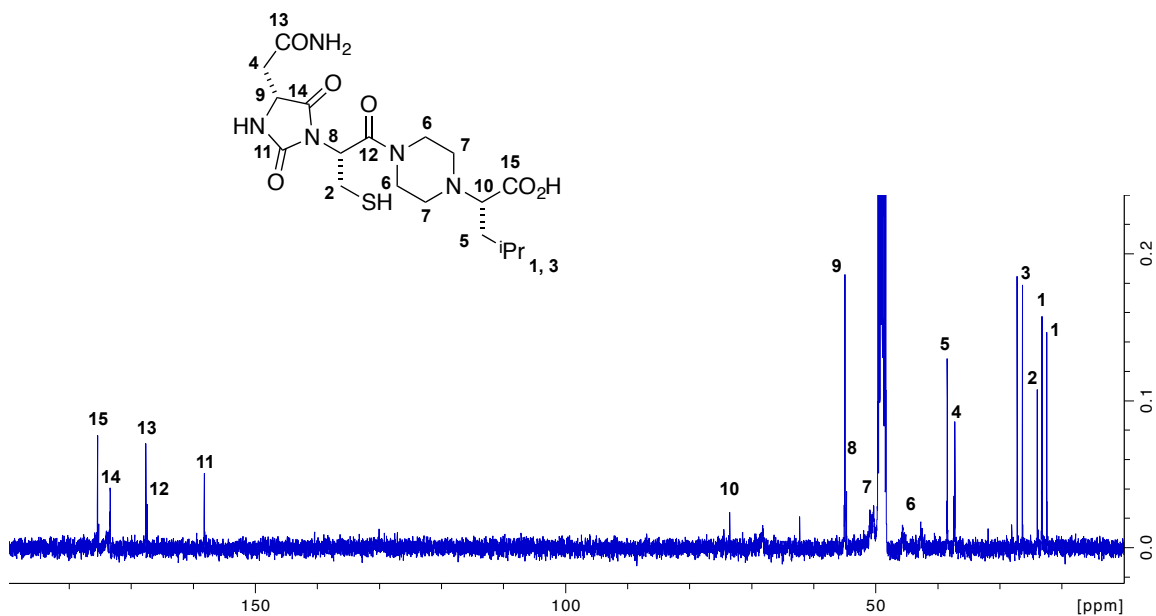
(S)-2-(4-((R)-2-((R)-4-(2-Amino-2-oxoethyl)-2,5-dioxoimidazolidin-1-yl)-3-mercaptopropanoyl)piperazin-1-yl)-4-methylpentanoic acid (DLL-Ancl)



Data for DLL-Ancl. Yield 13%. ^1H NMR (400 MHz, MeOD- d_4) δ 4.91 (m, 1H), 4.34 (m, 1H), 3.85-3.50 (m, 4H), 3.50-3.36 (m, 1H), 3.13 (d, $J = 7.5$ Hz, 2H), 3.02-2.85 (m, 4H), 2.80 (dd, $J = 16.1, 4.2$ Hz, 1H), 2.68 (dd, $J = 16.1, 6.5$ Hz, 1H), 1.81-1.49 (m, 3H), 0.96 (m, 6H);

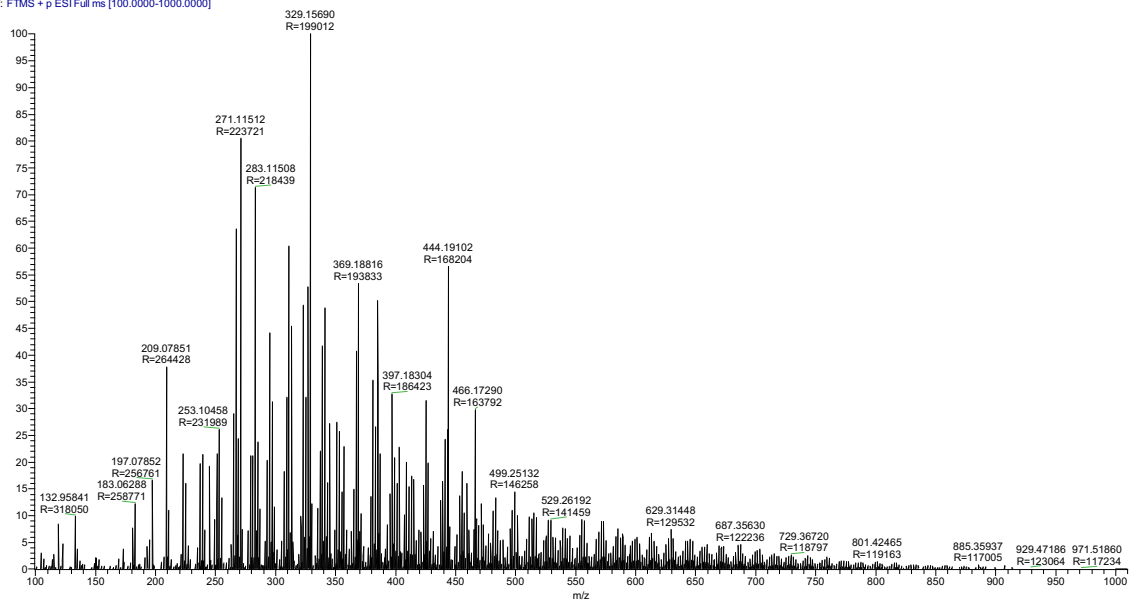


^{13}C NMR (100 MHz, MeOD-d_4) δ 175.4, 173.4, 167.6, 167.4, 158.2, 73.5, 55.0, 54.7, 50.9, 45.7, 42.7, 38.5, 37.3, 27.2, 26.4, 24.0, 23.2, 22.4

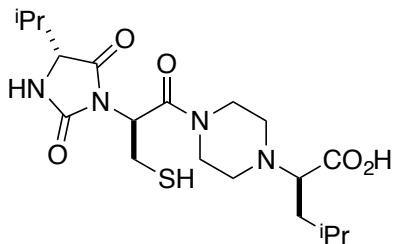


HRMS (ESI) m/z calcd for $\text{C}_{18}\text{H}_{30}\text{N}_5\text{O}_6\text{S}^+$ 444.1911; found 444.1910 ($\text{M}+\text{H}^+$)

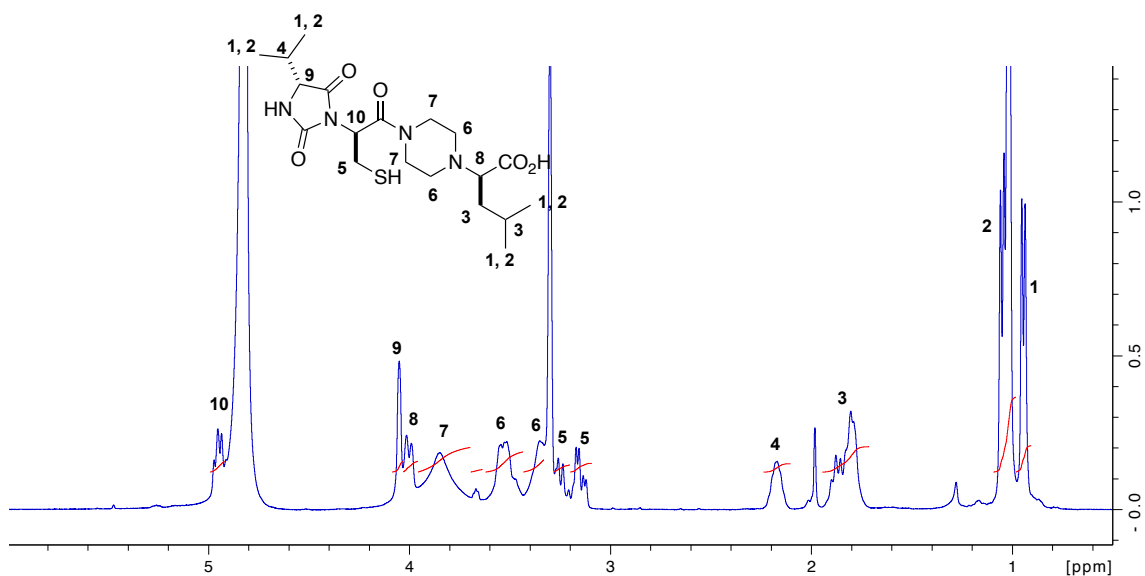
06052017_DLL-Anc+nacl #6-133 RT: 0.06-1.27 AV: 128 NL: 3.66E7
T: FTMS + p ESI Full ms [100.0000-1000.0000]



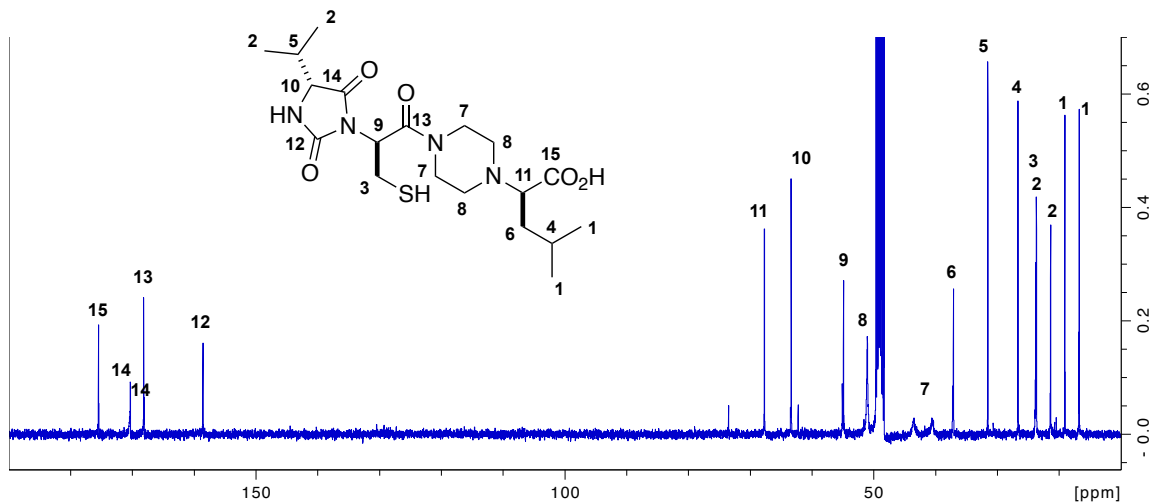
(*R*)-2-(4-((*S*)-2-((*R*)-4-isopropyl-2,5-dioxoimidazolidin-1-yl)-3-mercaptopropanoyl)piperazin-1-yl)-4-methylpentanoic acid (DDD-**Avcl**)



Data for DDD-Avcl. Yield 61%. $^1\text{H NMR}$ (400 MHz, MeOD- d_4) δ 4.95 (m, 1H), 4.05 (m, 1H), 4.00 (m, 1H), 3.96-3.64 (m, 3H), 3.62-3.43 (m, 3H), 3.43-3.32 (m, 2H), 3.28-3.20 (m, 1H), 3.20-3.09 (m, 1H), 2.18 (m, 1H), 1.95-1.70 (m, 3H), 1.10-0.98 (m, 9H), 0.94 (d, $J = 6.9$ Hz, 3H)

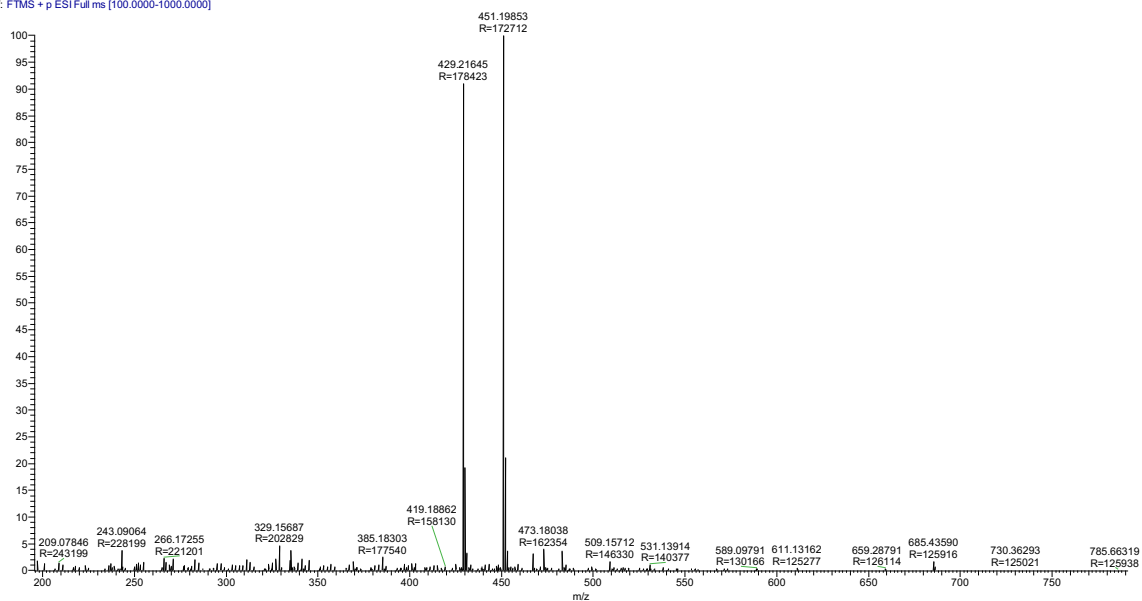


^{13}C NMR (100 MHz, MeOD- d_4) δ 175.5, 170.3, 168.2, 158.5, 67.7, 63.4, 54.9, 51.1, 43.5, 40.6, 37.1, 31.6, 26.7, 23.8, 23.7, 21.4, 19.1, 16.8

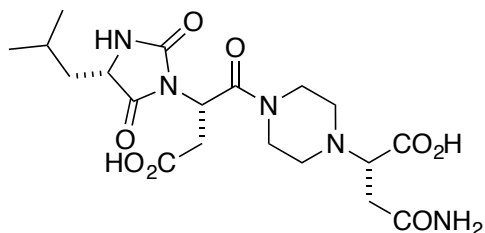


HRMS (ESI) m/z calcd for $\text{C}_{19}\text{H}_{33}\text{N}_4\text{O}_5\text{S}^+$ 429.2166; found 429.2165 ($\text{M}+\text{H}^+$)

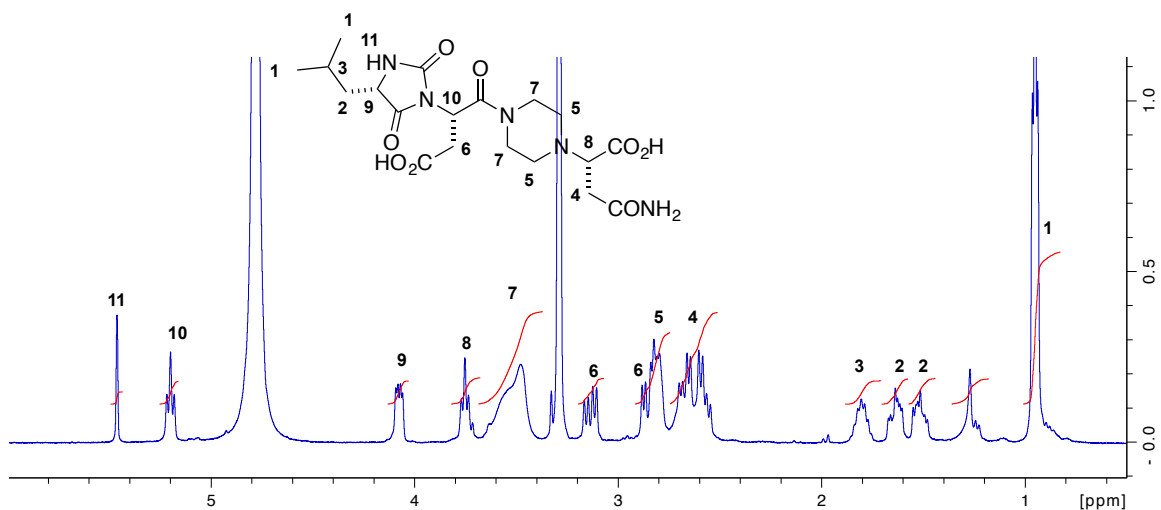
06052017_DDD-Acnd+nacl #99-205 RT: 0.95-1.96 AV: 107 NL: 1.02E8
T: FTMS + p ESI Full ms [100.0000-1000.0000]



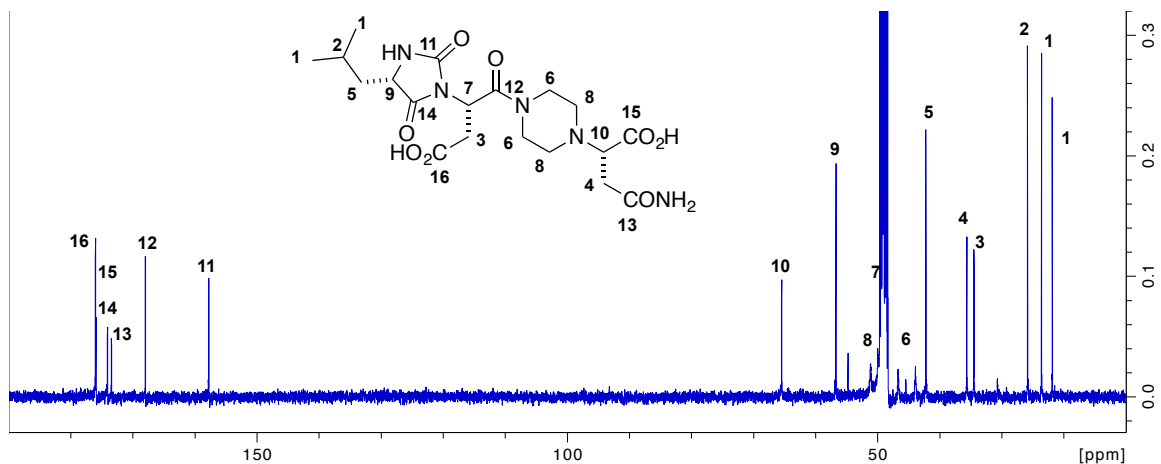
(S)-4-Amino-2-(4-((S)-3-carboxy-2-((S)-4-isobutyl-2,5-dioximidazolidin-1-yl)propanoyl)piperazin-1-yl)-4-oxobutanoic acid (LLL-Aldn)



Data for LLL-Aldn. Yield 13%. ¹H NMR (400 MHz, MeOD-d₄) δ 5.46 (s, 1H), 5.20 (t, *J* = 7.2 Hz, 1H), 4.07 (m, 1H), 3.75 (t, *J* = 6.9 Hz, 1H), 3.62-3.37 (m, 4H), 3.13 (dd, *J* = 16.4, 7.8 Hz, 1H), 2.91-2.75 (m, 3H), 2.74-2.50 (m, 4H), 1.81 (m, 1H), 1.64 (m, 1H), 1.52 (m, 1H), 0.95 (m, 6H)

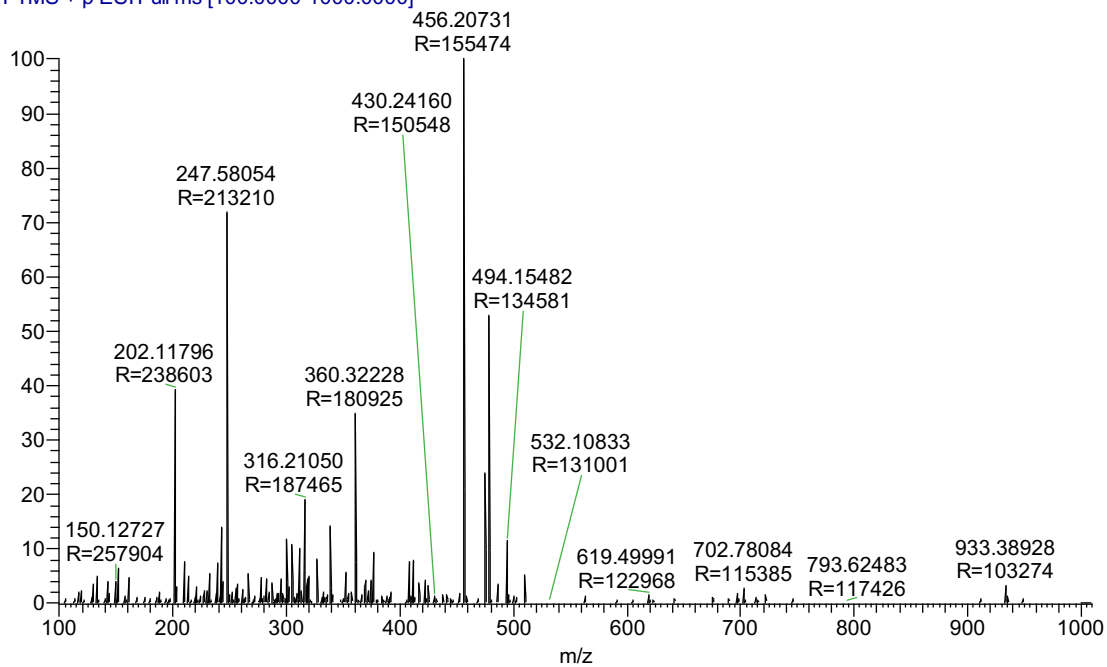


^{13}C NMR (100 MHz, MeOD- d_4) δ 176.0, 175.9, 174.1, 173.5, 168.0, 157.8, 65.5, 56.7, 51.2, 50.0, 49.7, 46.7, 43.9, 42.2, 35.6, 34.5, 25.9, 23.6, 21.9

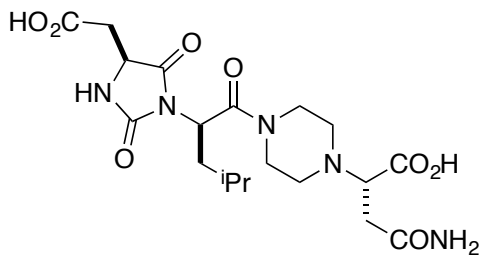


HRMS (ESI) m/z calcd for $\text{C}_{19}\text{H}_{30}\text{N}_5\text{O}_8^+$ 456.2089; found 456.2073 ($\text{M}+\text{H}$) $^+$

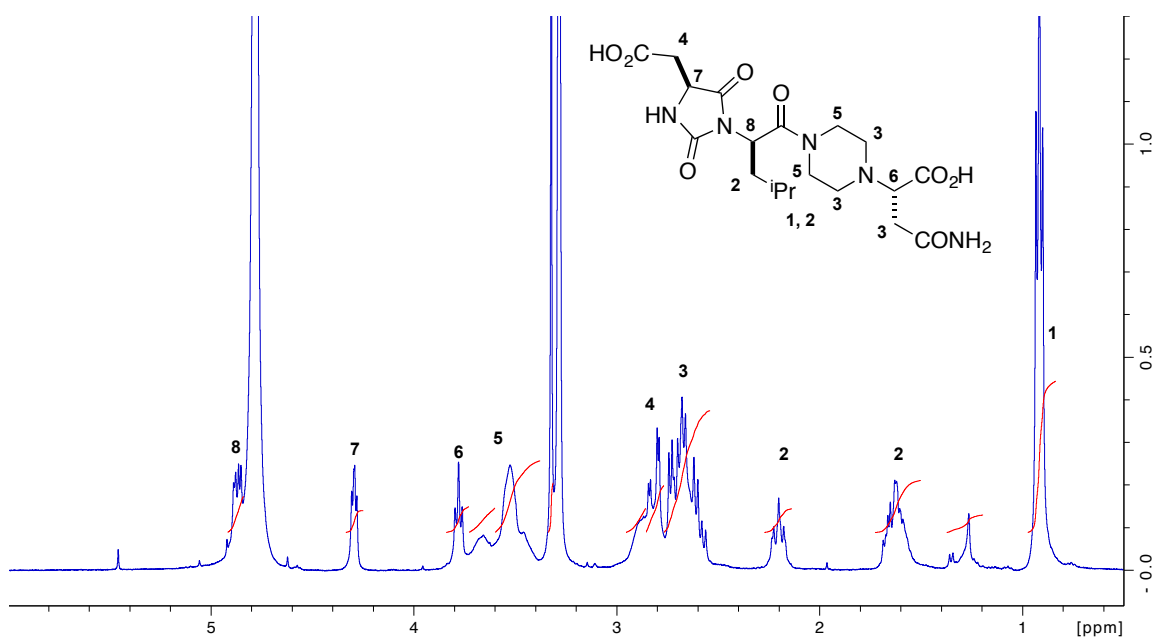
06232017_ldl-LDN #83-174 RT: 0.79-1.67 Av L: 3.04E7
T: FTMS + p ESI Full ms [100.0000-1000.0000]



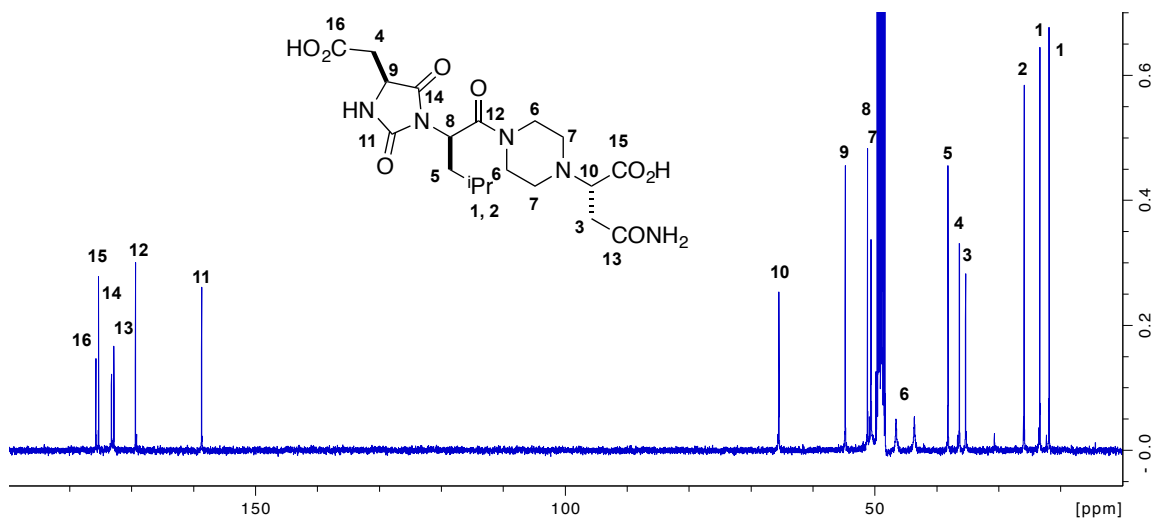
(S)-4-Amino-2-(4-((R)-2-((S)-4-(carboxymethyl)-2,5-dioximidazolidin-1-yl)-4-methylpentanoyl)piperazin-1-yl)-4-oxobutanoic acid (LDL-AdIn)



Data for LDL-AdIn. Yield 24%. $^1\text{H NMR}$ (400 MHz, MeOD- d_4) δ 4.87 (m, 1H), 4.30 (m, 1H), 3.78 (m, 1H), 3.72-3.37 (m, 4H), 2.95-2.76 (m, 3H), 2.77-2.54 (m, 5H), 2.20 (m, 1H), 1.63 (m, 2H), 0.92 (m, 6H)

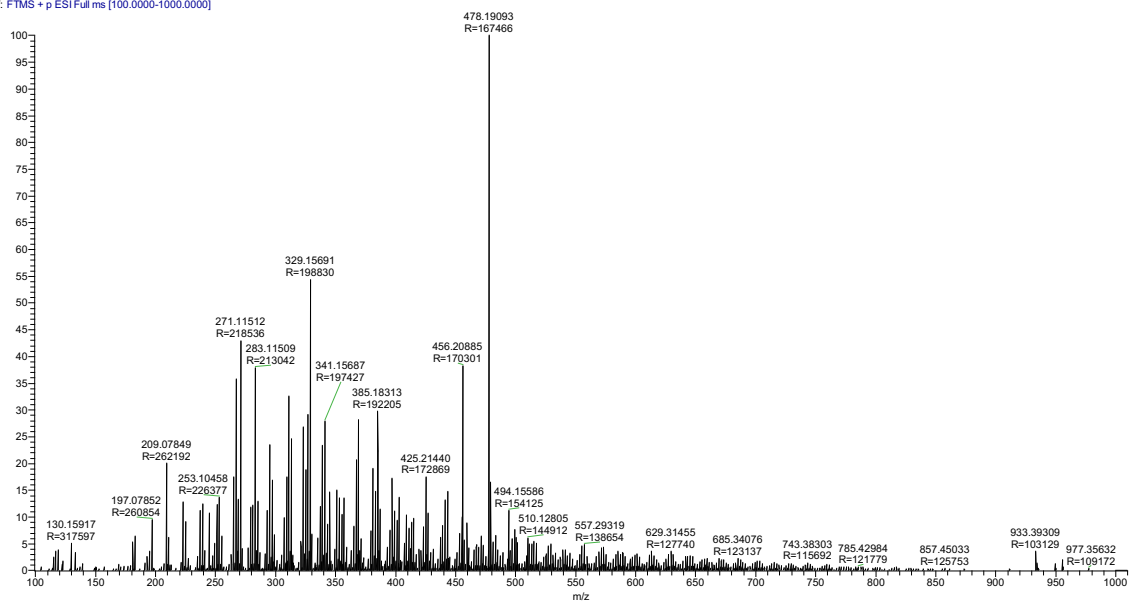


^{13}C NMR (100 MHz, MeOD- d_4) δ 175.8, 175.3, 173.3, 172.9, 169.4, 158.7, 65.5, 54.8, 51.3, 50.7, 46.6, 43.6, 38.2, 36.4, 35.3, 25.9, 23.4, 21.9

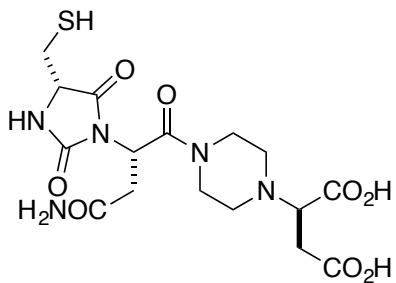


HRMS (ESI) m/z calcd for $\text{C}_{19}\text{H}_{30}\text{N}_5\text{O}_8^+$ 456.2089; found 456.2089 ($\text{M}+\text{H}$) $^+$

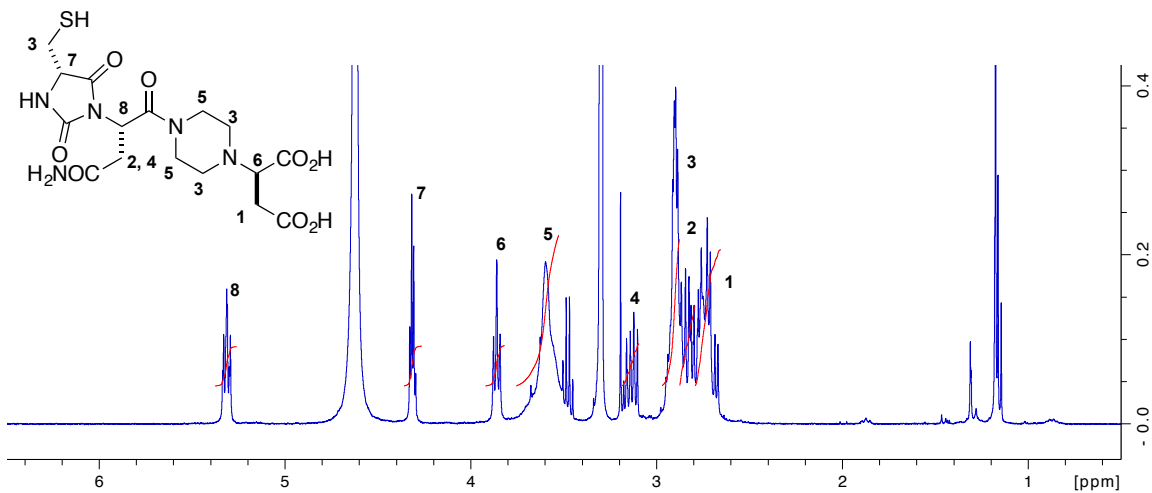
06052017_LDL-Acnd+nacl-good #1-28 RT: 0.01-0.27 AV: 28 NL: 6.46E7
T: FTMS + p ESI Full ms [100.0000-1000.0000]



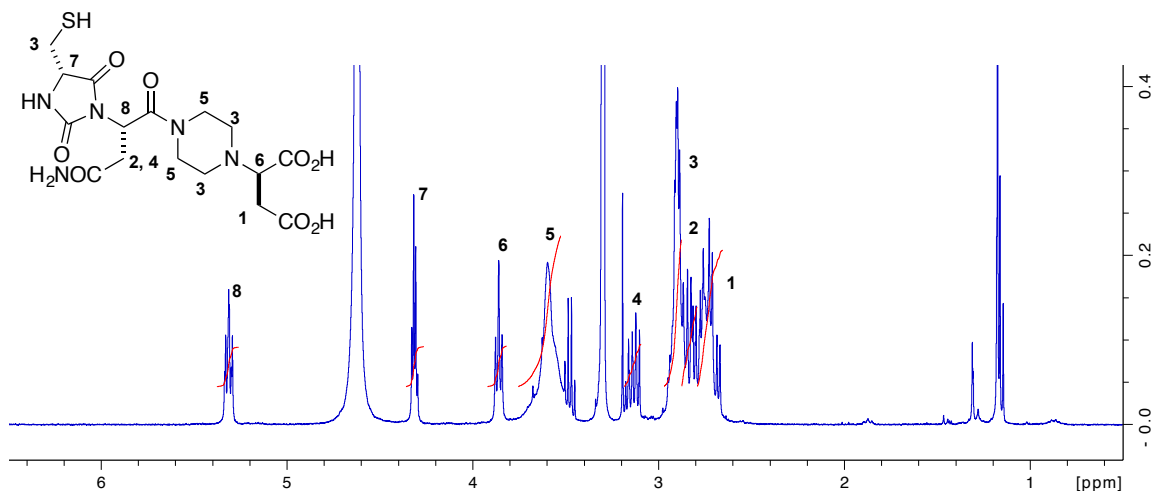
(*R*)-2-(4-((*S*)-4-Amino-2-((*S*)-4-(mercaptomethyl)-2,5-dioxoimidazolidin-1-yl)-4-oxobutanoyl)piperazin-1-yl)succinic acid (DLD-**Acnd**)



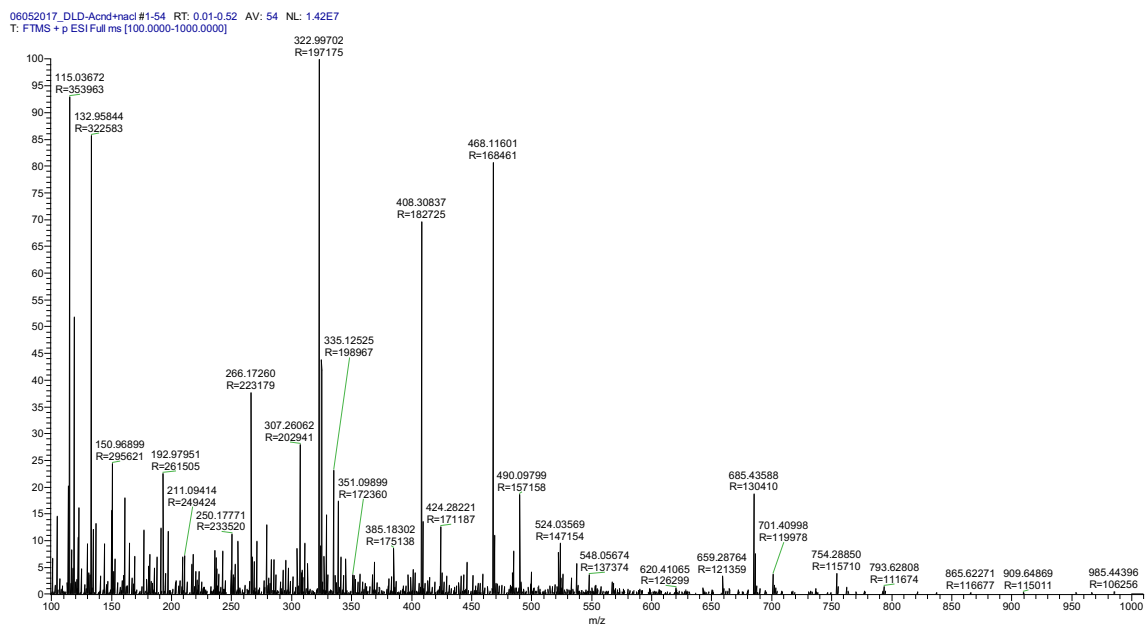
Data for DLD-Acnd. Yield 32%. $^1\text{H NMR}$ (400 MHz, MeOD- d_4) δ 5.32 (m, 1H), 4.31 (m, 1H), 3.86 (t, $J = 7.0$ Hz, 1H), 3.75-3.52 (m, 4H), 3.14 (m, 1H), 2.97-2.87 (m, 4H), 2.87-2.79 (m, 2H), 2.79-2.65 (m, 3H)



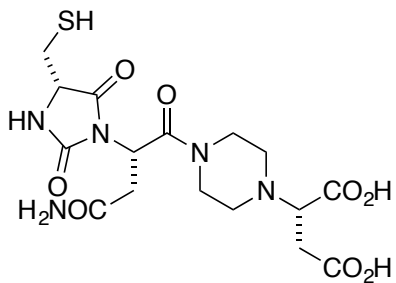
^{13}C NMR (100 MHz, MeOD- d_4) δ 174.6, 174.3, 174.0, 172.6, 168.0, 158.1, 65.1, 59.4, 50.8, 49.1, 46.4, 43.6, 35.4, 34.4, 26.1



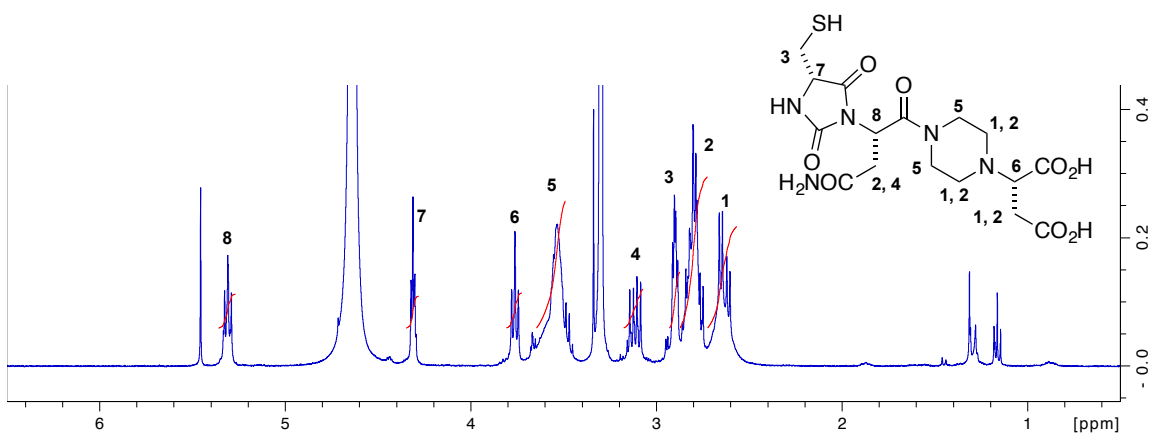
HRMS (ESI) m/z calcd for $\text{C}_{16}\text{H}_{23}\text{N}_5\text{O}_8\text{SNa}^+$ 468.1160; found 468.1160 ($\text{M}+\text{Na}$) $^+$



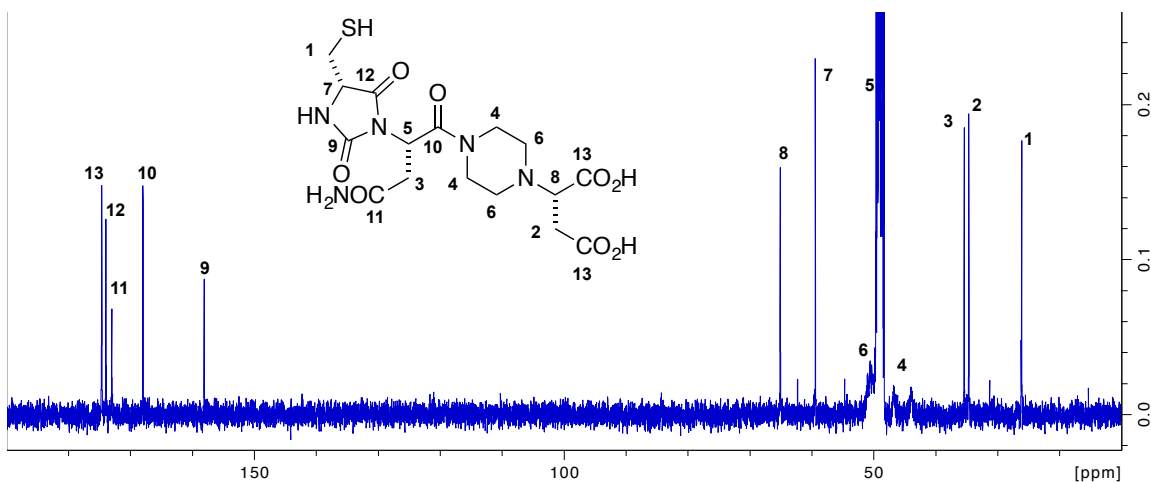
(S)-2-(4-((S)-4-Amino-2-((S)-4-(mercaptomethyl)-2,5-dioxoimidazolidin-1-yl)-4-oxobutanoyl)piperazin-1-yl)succinic acid (DLL-Acnd)



Data for DLL-Acnd. Yield 58%. ^1H NMR (400 MHz, MeOD- d_4) δ 5.31 (m, 1H), 4.31 (m, 1H), 3.76 (t, $J = 7.3$ Hz, 1H), 3.69-3.45 (m, 4H), 3.11 (dd, $J = 15.5, 7.9$ Hz, 1H), 2.90 (m, 2H), 2.87-2.73 (m, 4H), 2.72-2.56 (m, 3H)

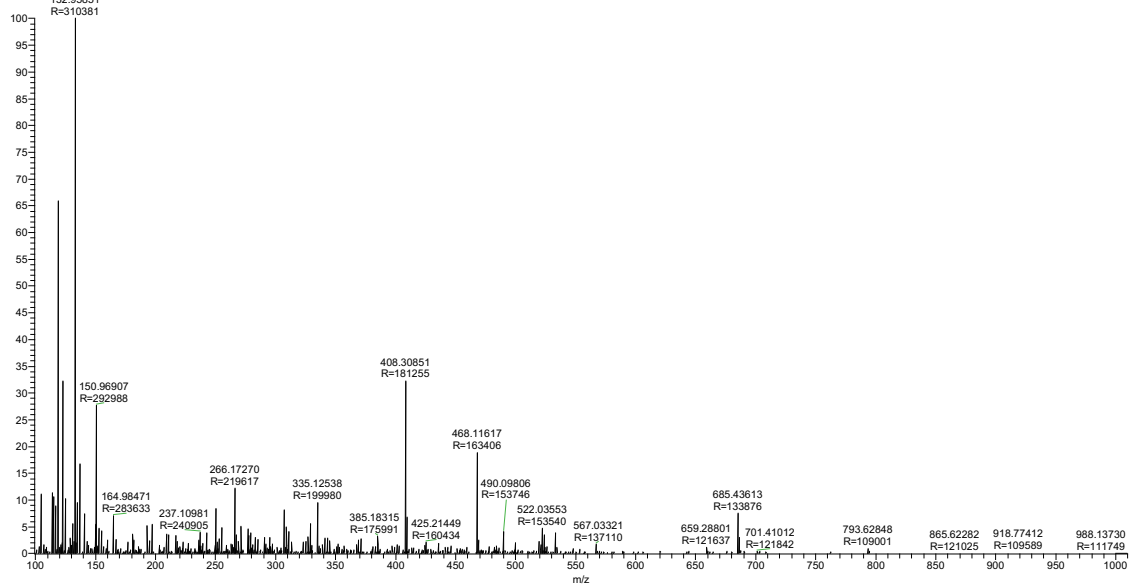


^{13}C NMR (100 MHz, MeOD- d_4) δ 174.6, 174.0, 168.0, 158.1, 65.1, 59.4, 50.6, 48.9, 46.8, 44.0, 35.4, 34.7, 26.1

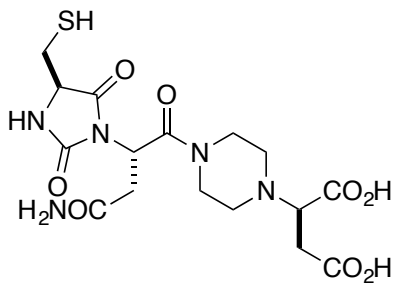


HRMS (ESI) m/z calcd for C₁₆H₂₃N₅O₈SNa⁺ 468.1160; found 468.1162 (M+Na)⁺

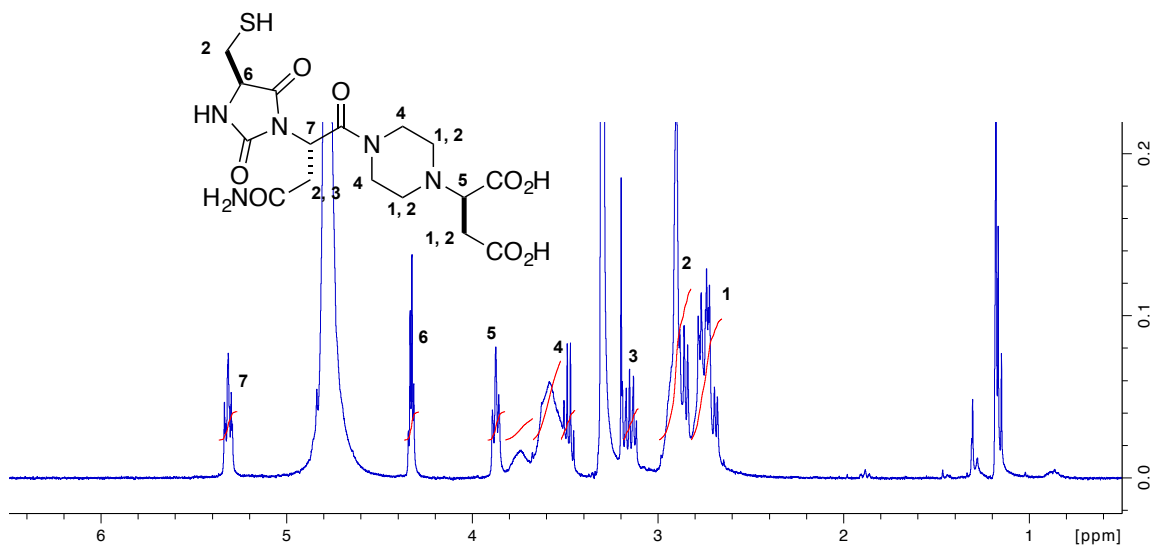
06052017_DLL-Acnd+nacl_20170605043521 #125-200 RT: 1.39-2.11 AV : 3.39E7
T: FTMS + p ESI Full ms [100.0000-1000.0000]
132.95851
R=310381



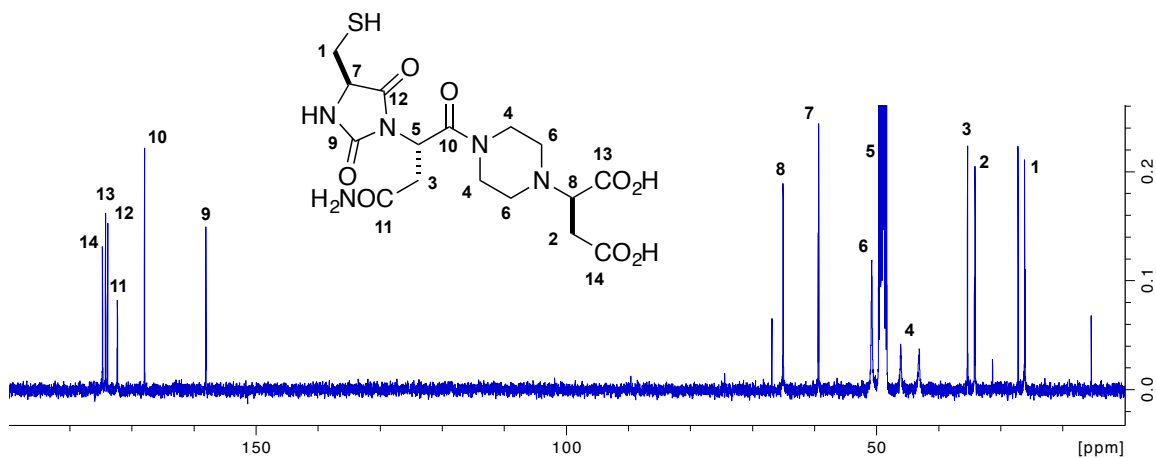
(*R*)-2-(4-((*S*)-4-Amino-2-((*R*)-4-(mercaptomethyl)-2,5-dioximidazolidin-1-yl)-4-oxobutanoyl)piperazin-1-yl)succinic acid (LLD-**Acnd**)



Data for LLD-Acnd. Yield 31%. ¹H NMR (400 MHz, MeOD-d₄) δ 5.31 (m, 1H), 4.33 (m, 1H), 3.88 (t, *J* = 6.9 Hz, 1H), 3.82-3.53 (m, 4H), 3.15 (m, 1H), 3.00-2.82 (m, 5H), 2.82-2.65 (m, 4H)

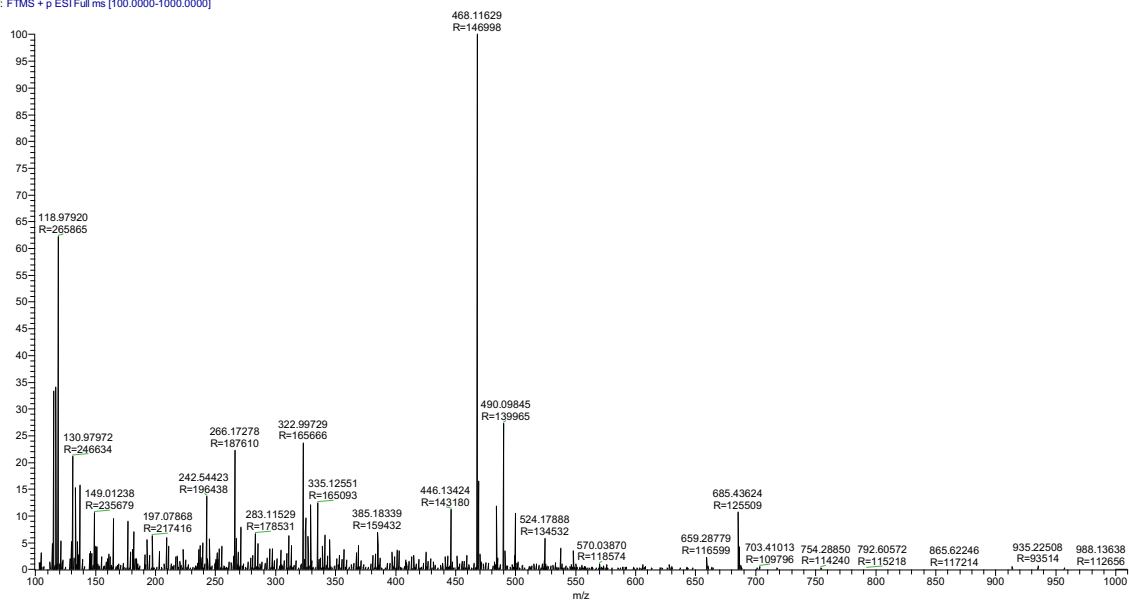


^{13}C NMR (100 MHz, MeOD- d_4) δ 174.8, 174.2, 173.9, 172.3, 168.0, 158.1, 66.9, 65.1, 59.4, 50.8, 48.8, 46.1, 43.1, 35.3, 34.1, 26.2

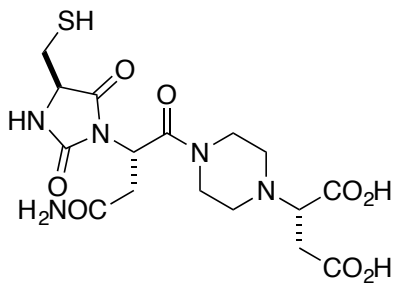


HRMS (ESI) m/z calcd for $\text{C}_{16}\text{H}_{23}\text{N}_5\text{O}_8\text{SNa}^+$ 468.1160; found 468.1163 ($\text{M}+\text{Na}$) $^+$

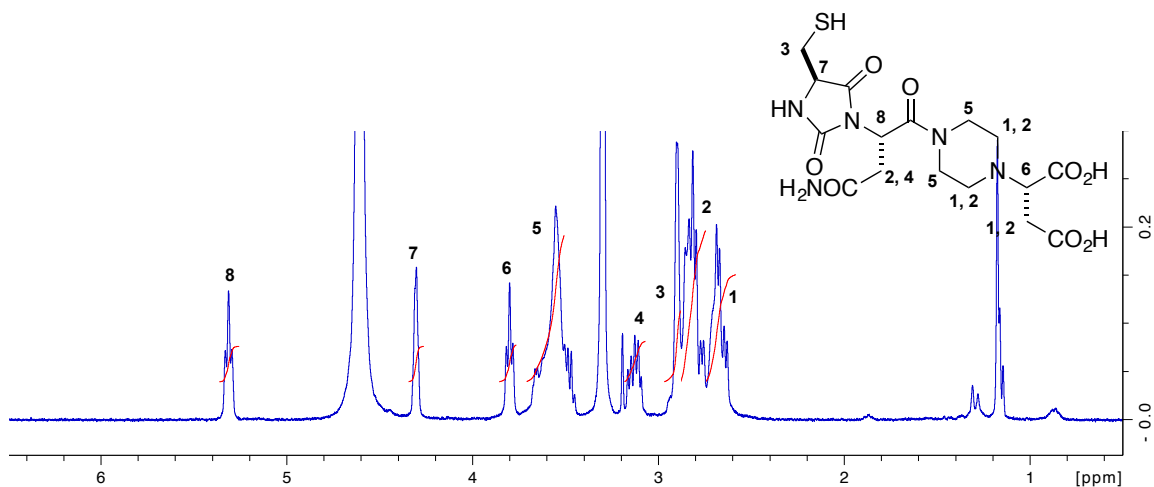
06052017_LLD-Acrid+red#15-147 RT: 0.14-1.42 AV: 133 NL: 9.47E6
T: FTMS +p ESI Full ms [100.0000-1000.0000]



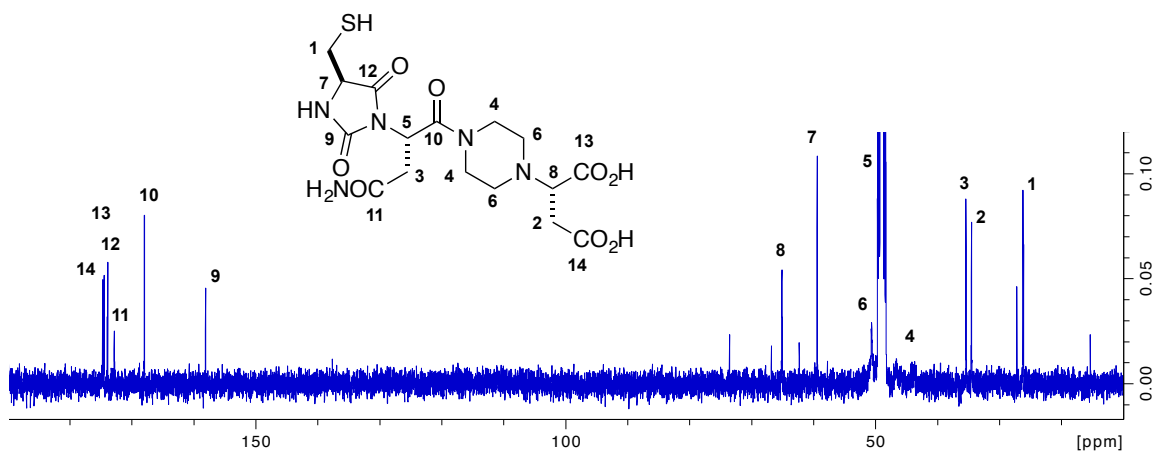
(S)-2-(4-((S)-4-Amino-2-((R)-4-(mercaptomethyl)-2,5-dioxoimidazolidin-1-yl)-4-oxobutanoyl)piperazin-1-yl)succinic acid (LLL-Acnd)



Data for LLL-Acnd. Yield 10%. ¹H NMR (400 MHz, MeOD-d₄) δ 5.31 (t, *J* = 7.1 Hz, 1H), 4.31 (m, 1H), 3.80 (t, *J* = 7.0 Hz, 1H), 3.71-3.50 (m, 4H), 3.14 (m, 1H), 2.97-2.88 (m, 2H), 2.88-2.75 (m, 4H), 2.75-2.58 (m, 3H)

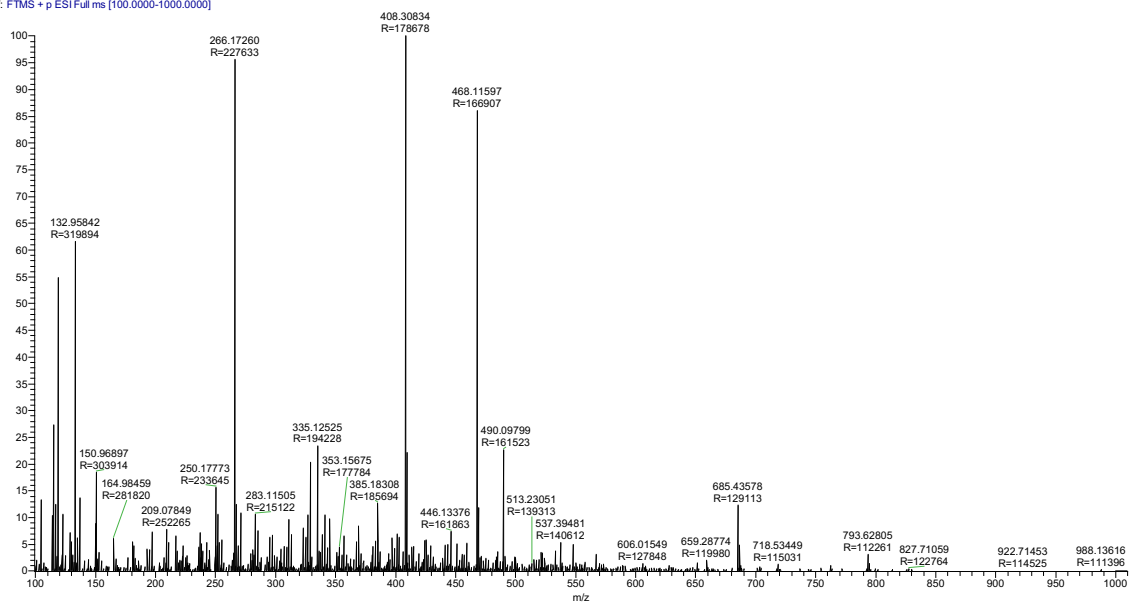


^{13}C NMR (100 MHz, MeOD- d_4) δ 174.7, 174.5, 173.9, 172.9, 168.0, 158.1, 65.1, 59.4, 50.6, 48.9, 46.6, 43.8, 35.4, 34.5, 27.2, 26.2

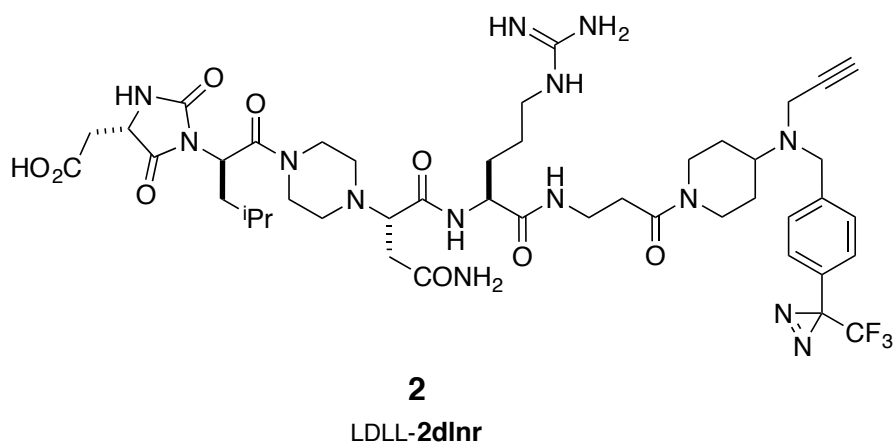
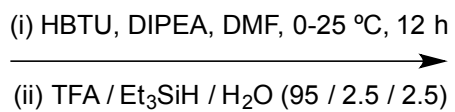
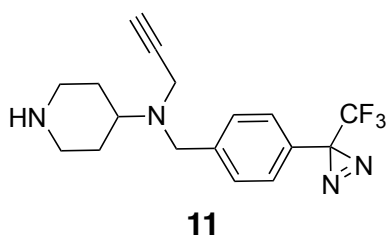
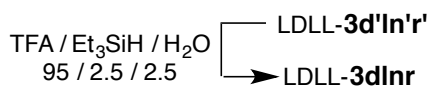
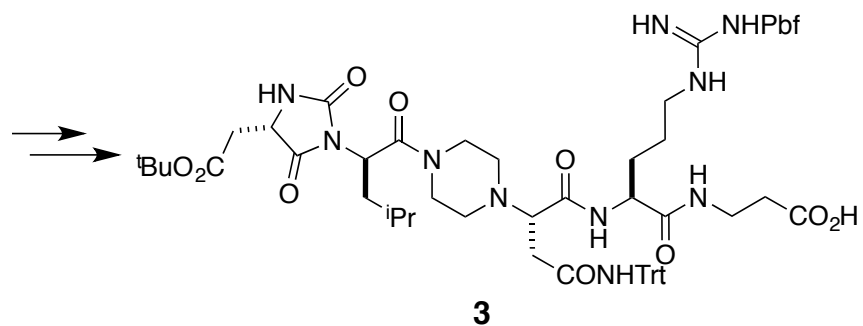
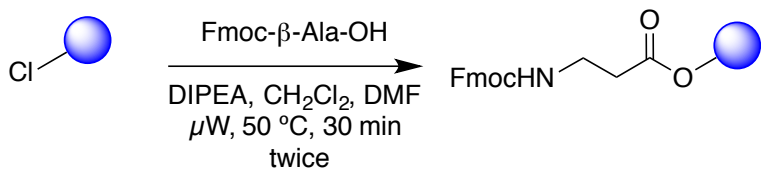


HRMS (ESI) m/z calcd for $\text{C}_{16}\text{H}_{23}\text{N}_5\text{O}_8\text{SNa}^+$ 468.1160; found 468.1160 ($\text{M}+\text{Na}^+$)

06052017_LLL_Acrid-nacrl-good #4-87 RT: 0.04-0.84 AV: 84 NL: 1.78E7
T: FTMS - p ESI Full ms [100.0000-1000.0000]



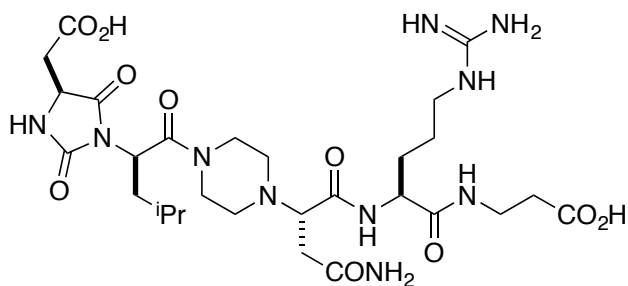
2-Cl-Trt resin



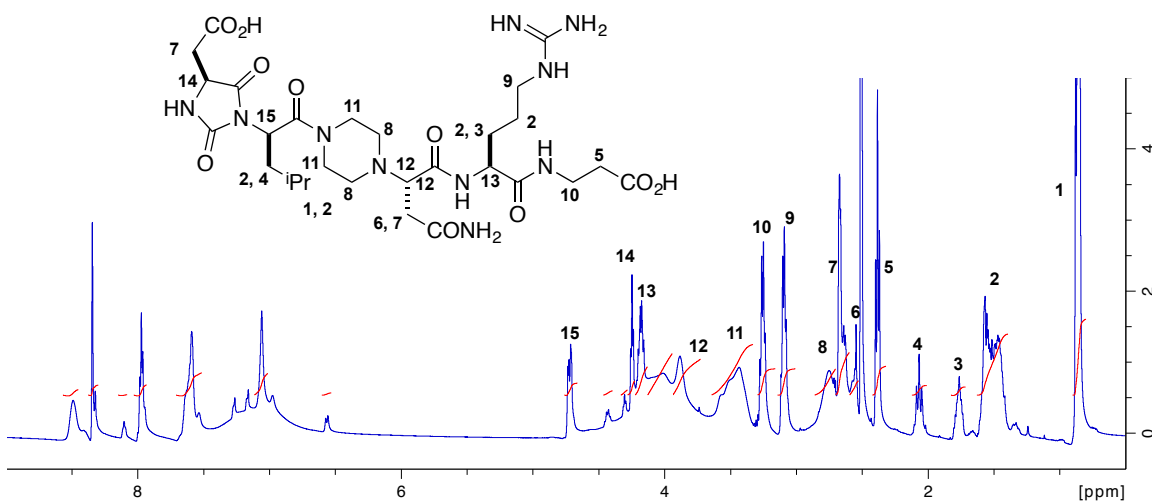
D. Solid-phase Syntheses and Characterizations of Compounds 2 and 3

The derivatives of LDLL-**1dlnr**, compounds **2** and **3**, were synthesized by solid-phase and solution-phase syntheses. To synthesize the core structure, the solid-phase synthesis was performed using 2-Cl-Trt resins loaded with Fmoc- β -Ala-OH. The protocols were the same as the syntheses of compounds **A** to obtain the protected-form of compound **3**. The crude product after cleaving with 20% HFIP/CH₂Cl₂ was reacted with the photoaffinity fragment **11** (1.1 eq), HBTU (1.1 eq) and ⁱPr₂NEt (3 eq) in DMF. The reaction was stirred at room temperature for 12 h. Solvent was completely removed, redissolved in CH₂Cl₂ and extracted with 10% citric acid, saturated NaHCO₃ and brine. The organic solution was collected, dried and removed in vacuo. The crude material was subsequently treated with TFA/Et₃SiH/water (95:2.5:2.5 v/v) for 3 h. Solution was removed with N₂ stream, and the crude material was purified by preparative reverse-phase HPLC (10% - 50% MeCN/water containing 0.05% TFA) to obtain compound **2** as white solids. For compound **3**, the crude material after cleavage from beads was treated with TFA/Et₃SiH/water cocktail and purified by preparative reverse-phase HPLC (10% - 50% MeCN/water containing 0.05% TFA).

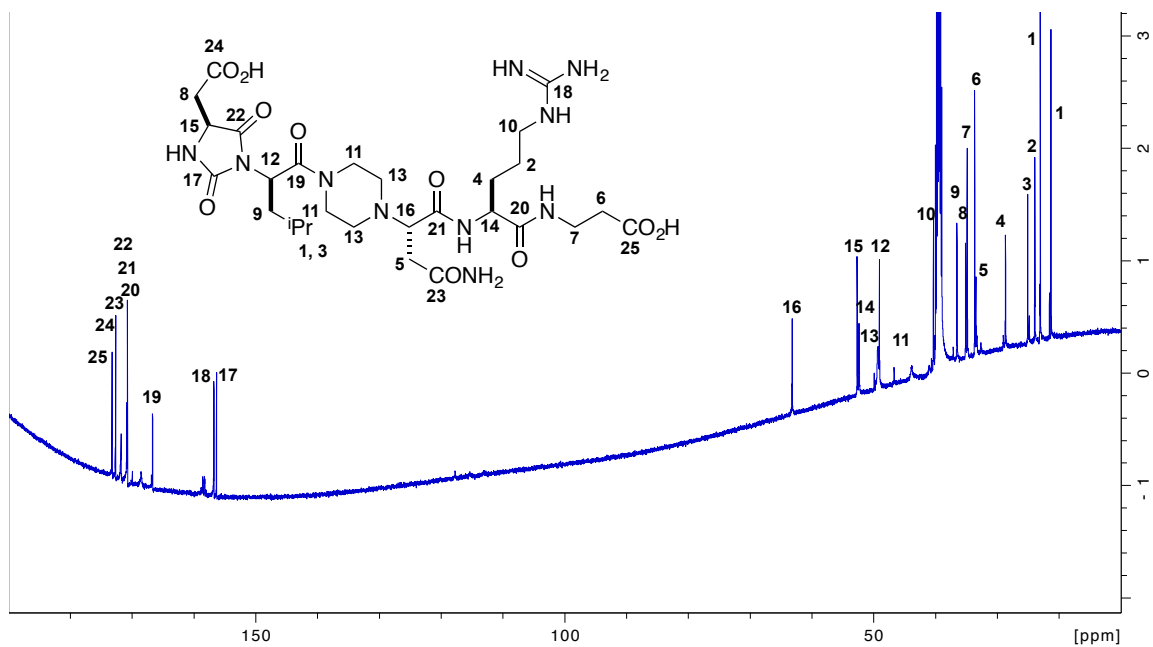
3-((*S*)-2-((*S*)-4-Amino-2-(4-((*R*)-2-((*S*)-4-(carboxymethyl)-2,5-dioximidazolidin-1-yl)-4-methylpentanoyl)piperazin-1-yl)-4-oxobutanamido)-5-guanidinopentanamido)propanoic acid (**3**)



Data for Compound 3. Yield 18%. $^1\text{H NMR}$ (400 MHz, DMSO-d_6) δ 8.49 (br, 1H), 8.34 (m, 1H), 7.98 (m, 1H), 7.62 (br, 2H), 7.06 (br, 2H), 4.72 (m, 1H), 4.25 (m, 1H), 4.18 (m, 1H), 3.89 (m, 1H), 3.65-3.33 (m, 4H), 3.26 (m, 2H), 3.10 (m, 2H), 2.86-2.70 (m, 4H), 2.70-2.60 (m, 3H), 2.60-2.53 (m, 1H), 2.38 (t, $J = 7.2$ Hz, 2H), 2.07 (m, 1H), 1.76 (m, 1H), 1.62-1.40 (m, 5H), 0.87 (m, 6H);

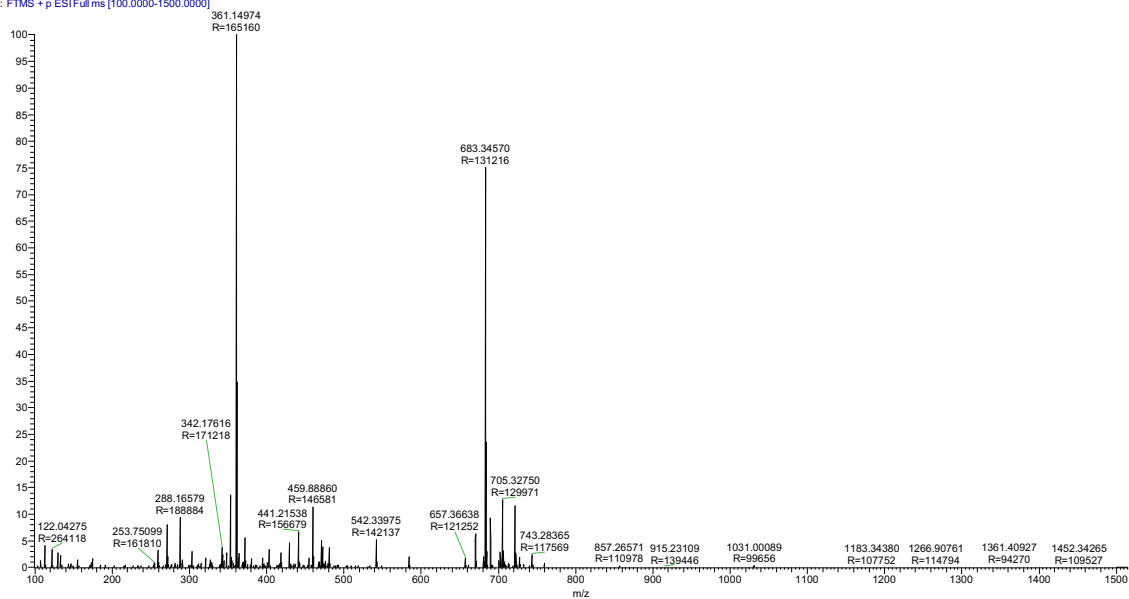


^{13}C NMR (100 MHz, DMSO-d_6) δ 173.2, 172.8, 172.7, 171.8, 170.9, 170.8, 166.7, 156.8, 156.4, 63.2, 52.7, 52.4, 49.4, 49.1, 43.9, 41.1, 40.4, 36.6, 35.2, 34.9, 33.7, 33.5, 28.7, 25.1, 24.0, 23.1, 21.3

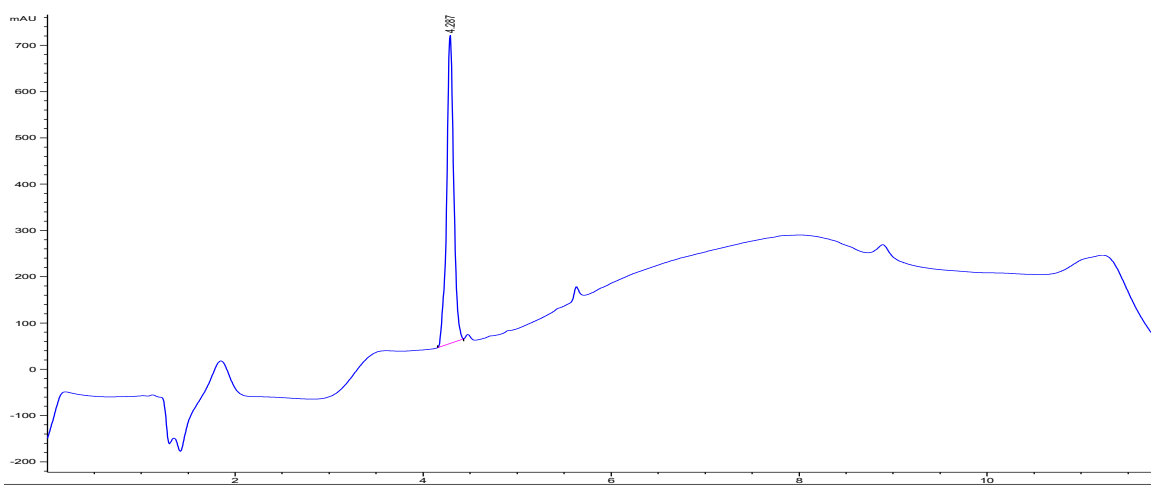


HRMS (ESI) m/z calcd for $\text{C}_{28}\text{H}_{47}\text{N}_{10}\text{O}_{10}^+$ 683.3471; found 683.3457 ($\text{M}+\text{H}^+$)

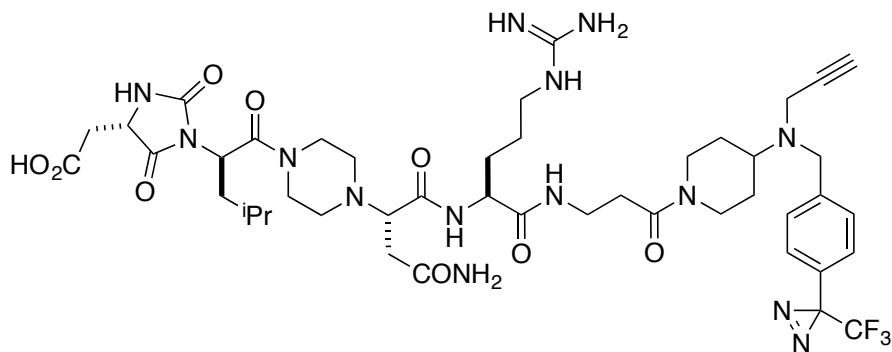
06142017-4dl-DLNR-beta #1-78 RT: 0.01-0.83 AV: 78 NL: 8.24E5
T: FTMS + p ESI Full ms [100.0000-1500.0000]



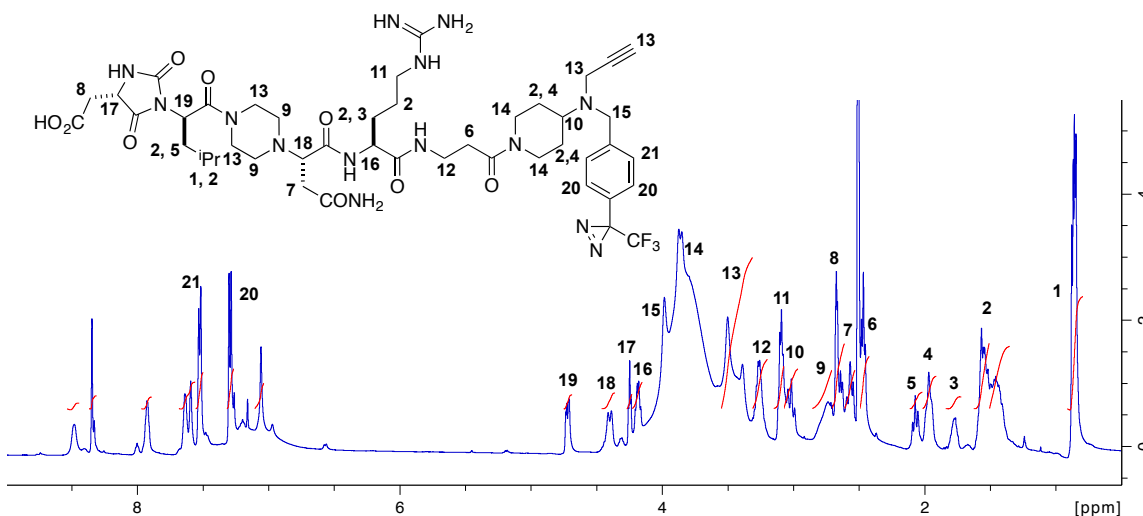
LC



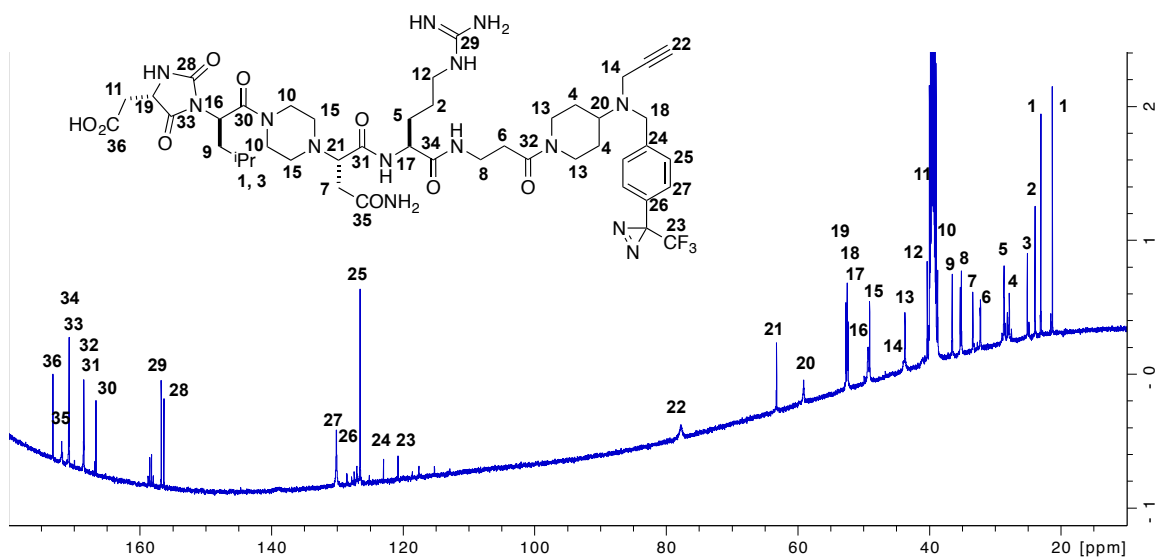
2-((*S*)-1-((*R*)-1-(4-((*S*)-4-Amino-1-(((*S*)-5-guanidino-1-oxo-1-((3-oxo-3-(4-(prop-2-yn-1-yl)(4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)phenyl)amino)piperidin-1-yl)propyl)amino)pentan-2-yl)amino)-1,4-dioxobutan-2-yl)piperazin-1-yl)-4-methyl-1-oxopentan-2-yl)-2,5-dioximidazolidin-4-yl)acetic acid (**2**)



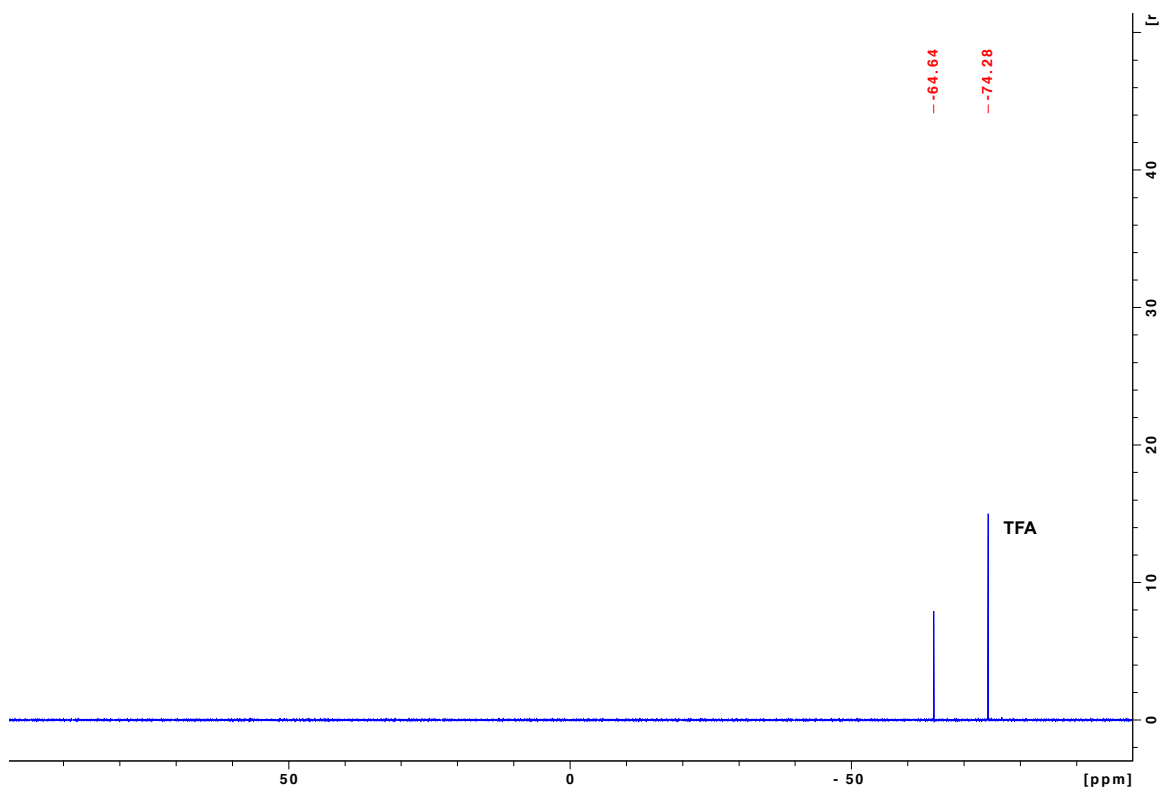
Data for Compound 2. Yield 8%. ¹H NMR (400 MHz, DMSO-d₆) δ 8.49 (br, 1H), 8.34 (m, 1H), 7.93 (br, 1H), 7.66-7.57 (br, 2H), 7.53 (d, *J* = 7.7 Hz, 2H), 7.30 (d, *J* = 7.9 Hz, 2H), 7.06 (br, 2H), 4.72 (m, 1H), 4.40 (m, 1H), 4.25 (m, 1H), 4.19 (m, 1H), 3.99 (s, 2H), 3.85 (m, 4H), 3.55-3.32 (m, 7H), 3.26 (m, 2H), 3.10 (m, 2H), 3.02 (m, 1H), 2.85-2.70 (m, 4H), 2.68 (m, 2H), 2.63 (m, 2H), 2.47 (m, 2H), 2.07 (m, 1H), 1.97 (m, 2H), 1.78 (m, 1H), 1.61-1.38 (m, 7H), 0.86 (m, 6H)



^{13}C NMR (100 MHz, DMSO-d_6) δ 173.3, 171.9, 170.9, 170.8, 168.6, 168.5, 166.7, 156.8, 156.4, 130.1, 127.0, 126.6, 123.0, 120.9, 77.8, 63.3, 59.1, 52.7, 52.5, 52.4, 49.4, 49.1, 43.9, 43.7, 41.1, 40.3, 38.7, 36.6, 35.4, 35.1, 33.4, 32.3, 28.7, 27.9, 25.1, 24.0, 23.1, 21.3

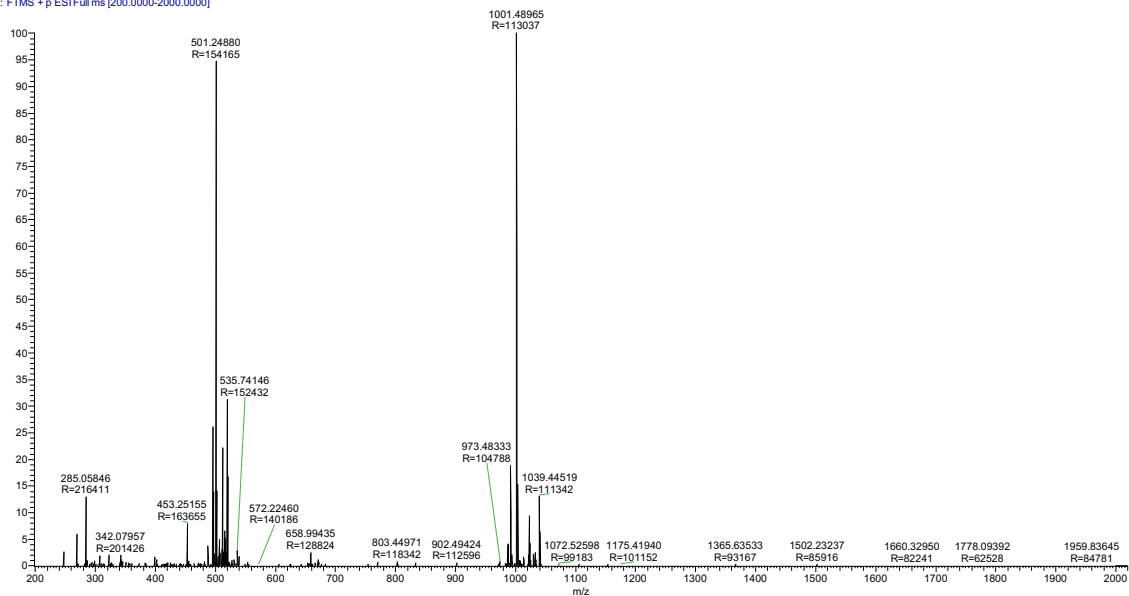


^{19}F NMR spectrum

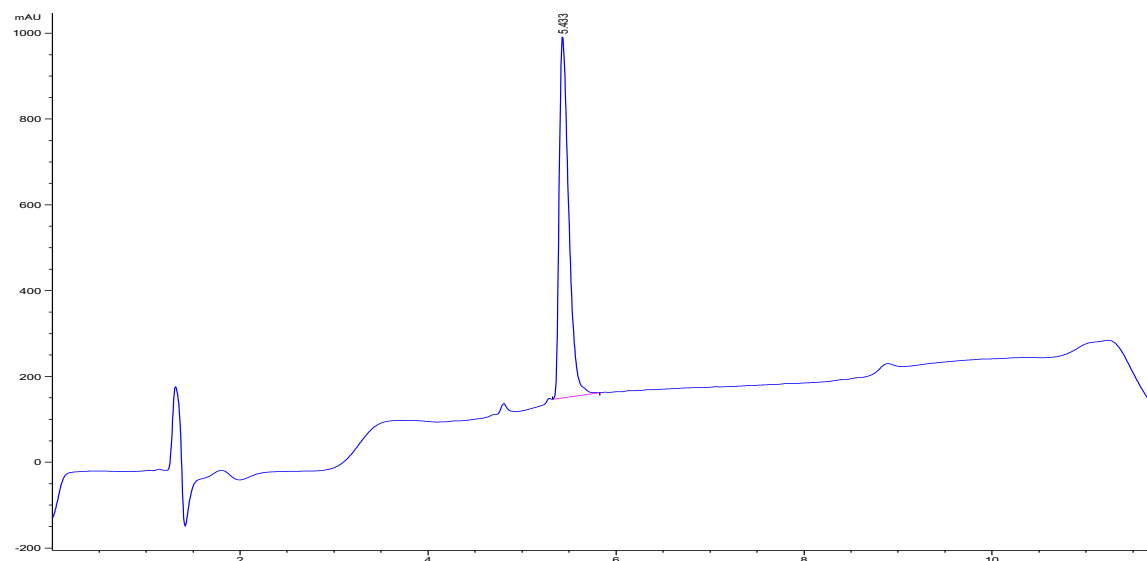


HRMS (ESI) m/z calcd for $C_{45}H_{64}F_3N_{14}O_9^+$ 1001.4927; found 1001.4897 ($M+H$)⁺

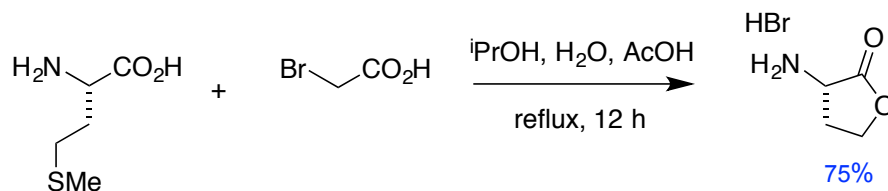
06142017-ldl-DLNR-probe #15-88 RT: 0.16-0.96 AV: 74 NL: 1.18E6
T: FTMS + p ESI Full ms [200.0000-2000.0000]



LC

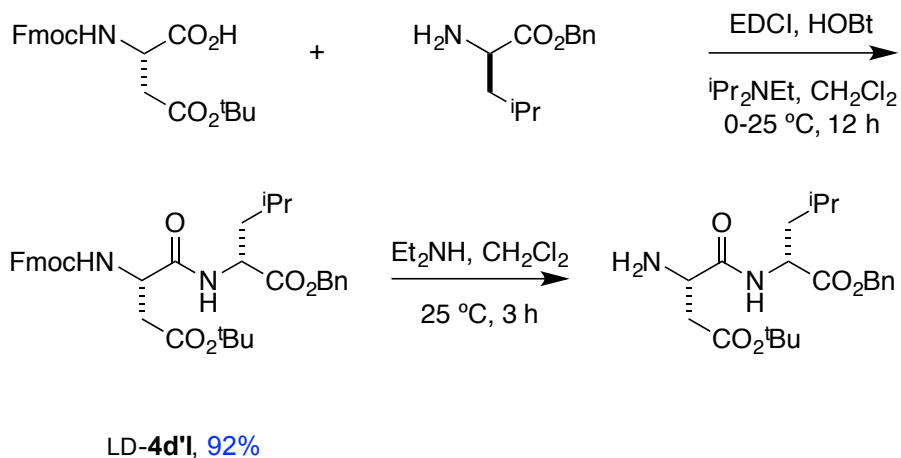


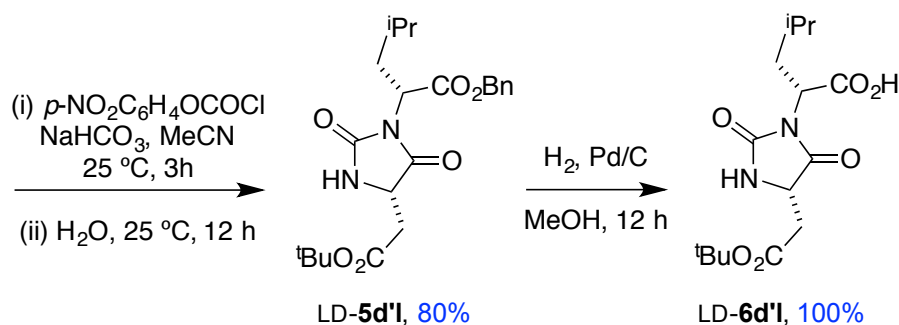
E. Solution-phase Syntheses of LDLL-1dInr



Synthesis of *L*-homoserine lactone hydrobromide salt¹

L-methionine (2.98 g, 20 mmol) and bromoacetic acid (3.06 g, 22 mmol) were dissolved in *i*PrOH, water and AcOH (5:5:2, 30 mL). The mixture was stirred in the reflux condition for 12 h. The solvent was completely removed under vacuum to obtain dark brown oil. This crude was dissolved in 4:1 *i*PrOH : 33% HBr in AcOH and let it cool in the fridge to induce white precipitate. The precipitate was filtered and washed with *i*PrOH. The supernatant was concentrated in vacuo and reprecipitated again at the same condition. The precipitation was repeated until no further solid formed. All crops of precipitate were combined to obtain the product as a hydrobromide salt (2.76 g, 75%). NMR spectra are identical to the reference.¹





Synthesis of dipeptide LD-4d'I

L-Fmoc-Asp(^tBu)-OH (1.94 g, 4.72 mmol), EDCI (1.00 g, 5.19 mmol) and HOBt (0.70 g, 5.19 mmol) were stirred in 50 mL CH_2Cl_2 at $0\text{ }^\circ\text{C}$ under N_2 atmosphere for 30 min. Pr_2NEt (2.5 mL, 14.2 mmol) was added followed by D-Leu-OBn (1.04 g, 4.72 mmol). The reaction was allowed to stir at room temperature for 12 h. The reaction was diluted with 100 mL CH_2Cl_2 and extracted three times with 10% citric acid, three times with saturated NaHCO_3 and brine. The organic solution was dried with MgSO_4 and evaporated in vacuo to obtain the product as a white solid (2.67 g, 92%), which was used in the next step without further purification.

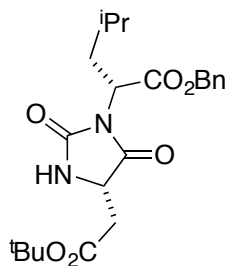
Synthesis of hydantoin LD-5d'I

The dipeptide LD-4d'I (0.62 g, 1.00 mmol) was stirred in $\text{Et}_2\text{NH}/\text{CH}_2\text{Cl}_2$ solution (4 mL, 1:1 v/v) for 3 h, then solvent was removed in vacuo, and the crude material was purified by flash column chromatography using 2-5% MeOH/ CH_2Cl_2 with 0.1% Et_3N to obtain product as a clear oil. Purified NH_2 -dipeptide (0.40 g, 1.00 mmol) and NaHCO_3 (0.25 g, 3.03 mmol) were dissolved in 20 mL MeCN. p -Nitrophenyl chloroformate (0.21 g, 1.1 mmol) was added and the reaction was stirred at room temperature for 3 h under N_2 atmosphere. Water (~ 10 mL) was added to produce bright yellow solution, which was stirred for additional 12 h at room temperature. Organic solvent was removed in vacuo, then EtOAc (100 mL) was added into the aqueous solution. Organic layer was separated, and extracted with 5% Na_2CO_3 five times to remove p -nitrophenol byproduct. The clear organic solution was further dried with brine and MgSO_4 . The mixture was reduced in vacuo to obtain crude oil which was purified by flash column chromatography using EtOAc:hexanes (1:4 v/v) to obtain product as a white solid (0.34 g, 80%)

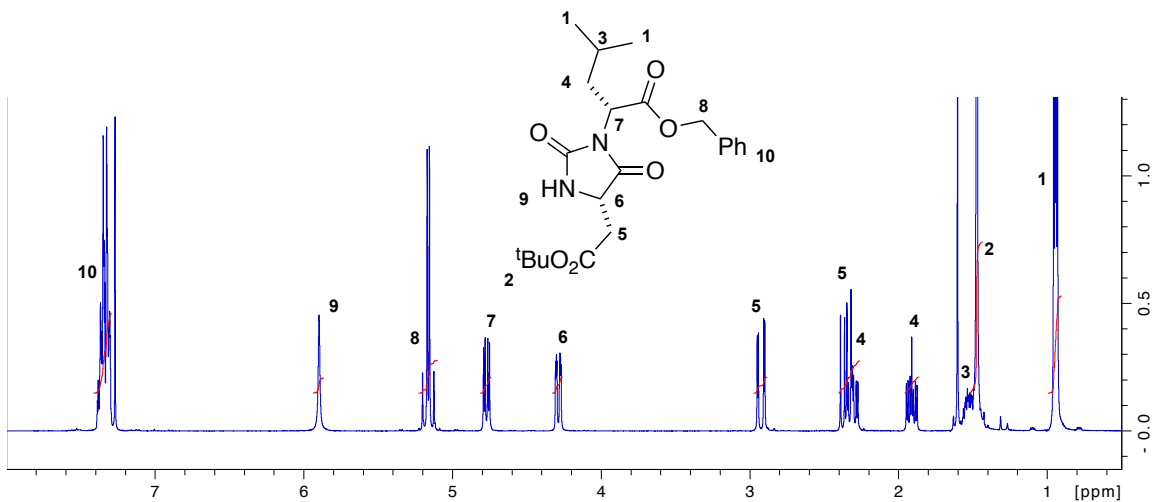
Benzyl ester deprotection (LD-6d'I)

Hydantoin LD-5d'I (2.51 g, 6 mmol) and 10% Pd/C (620 mg, 0.6 mmol) were purged under N₂ before adding 50 mL MeOH. H₂ balloon was purged for 10 min, and the reaction was stirred under H₂ atmosphere for 12 h. The mixture was filtered through celite and the clear solution was reduced in vacuo to obtain compound LD-6d'I (1.99 g, 100%) as clear oil.

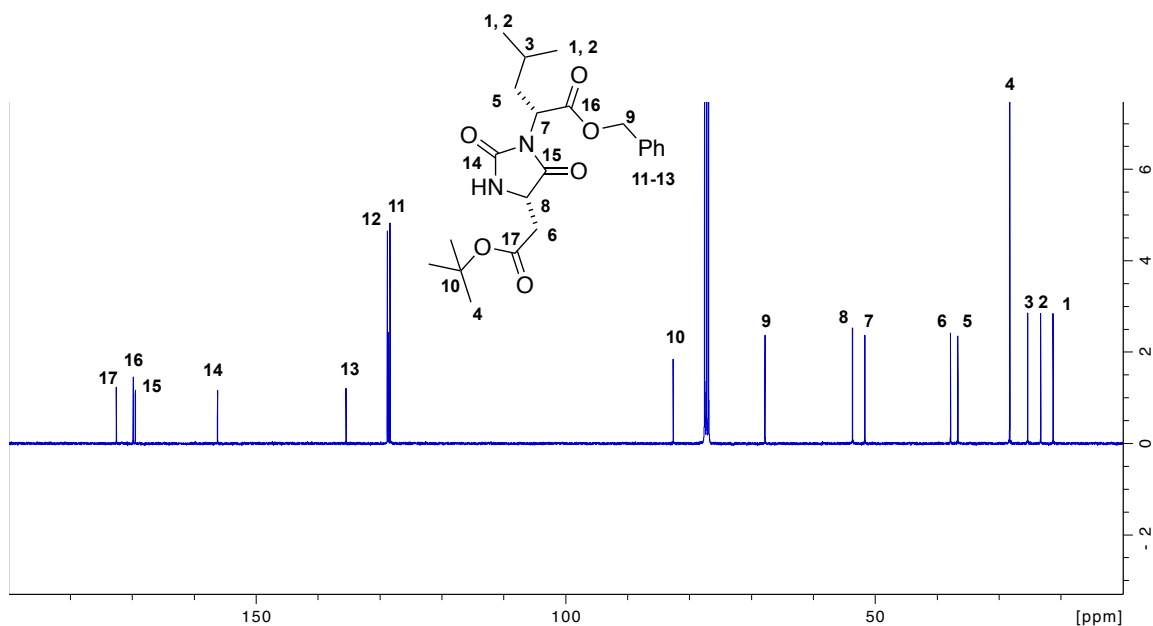
Benzyl (*R*)-2-((*S*)-4-(2-(*tert*-butoxy)-2-oxoethyl)-2,5-dioxoimidazolidin-1-yl)-4-methylpentanoate (LD-5d'I)



Data for LD-5d'I. ¹H NMR (400 MHz, CDCl₃) δ 7.39-7.30 (m, 5H), 5.90 (br, 1H), 5.17 (d, *J* = 6.0 Hz, 2H), 4.77 (dd, *J* = 11.5, 4.4 Hz, 1H), 4.29 (m, 1H), 2.93 (dd, *J* = 17.4, 2.8 Hz, 1H), 2.39-2.27 (m, 2H), 1.96-1.87 (m, 1H), 1.58-1.50 (m, 1H), 1.48 (s, 9H), 0.94 (dd, *J* = 6.6, 3.6 Hz, 6H)

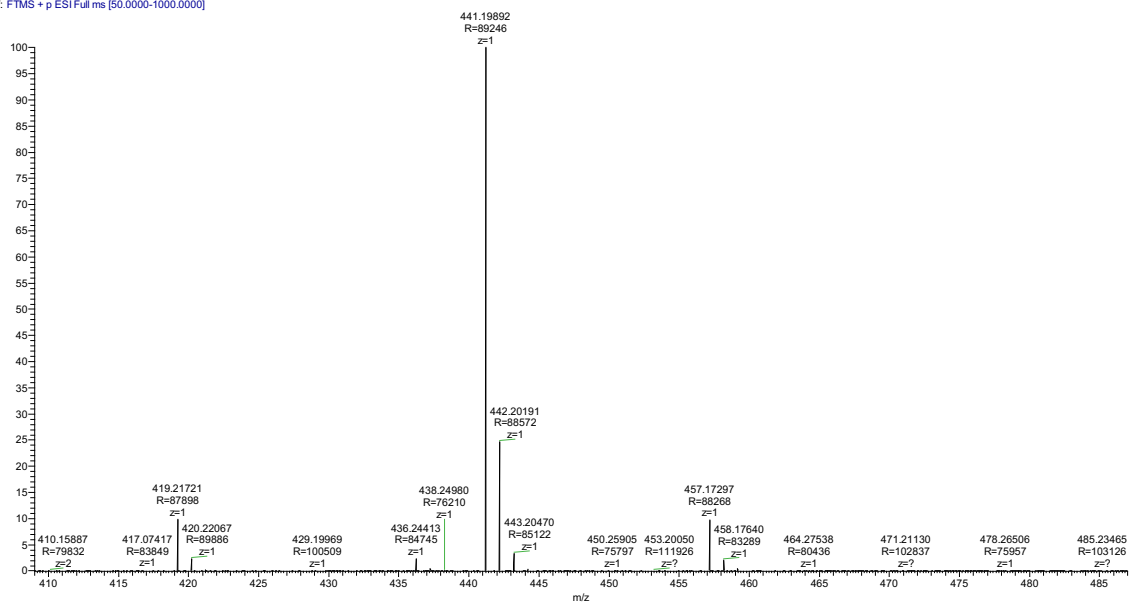


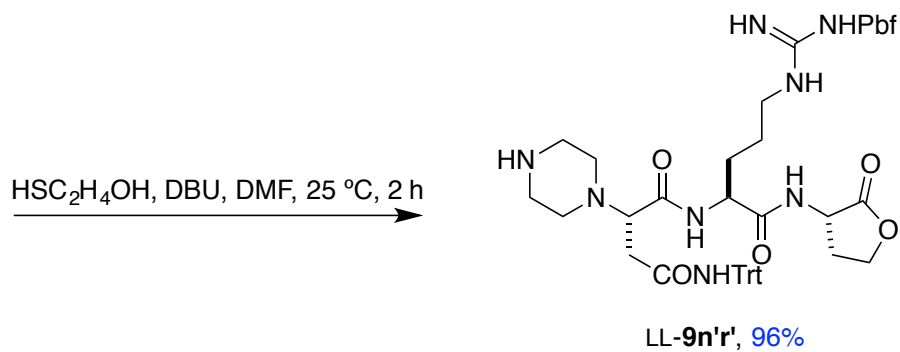
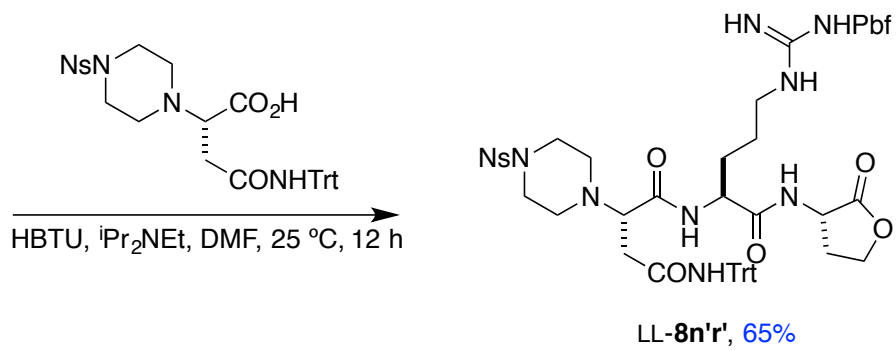
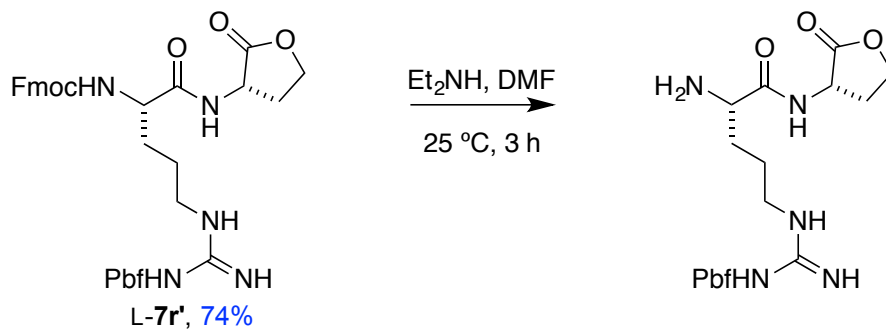
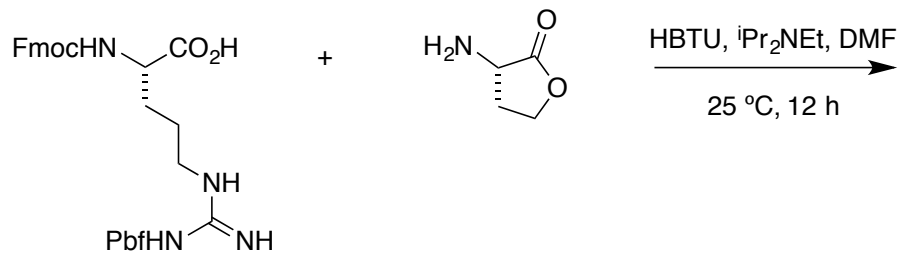
^{13}C NMR (100 MHz, CDCl_3) δ 172.6, 169.8, 169.5, 156.2, 135.5, 128.8, 128.6, 128.4, 82.6, 67.8, 53.7, 51.7, 37.8, 36.7, 28.3, 25.4, 23.3, 21.3

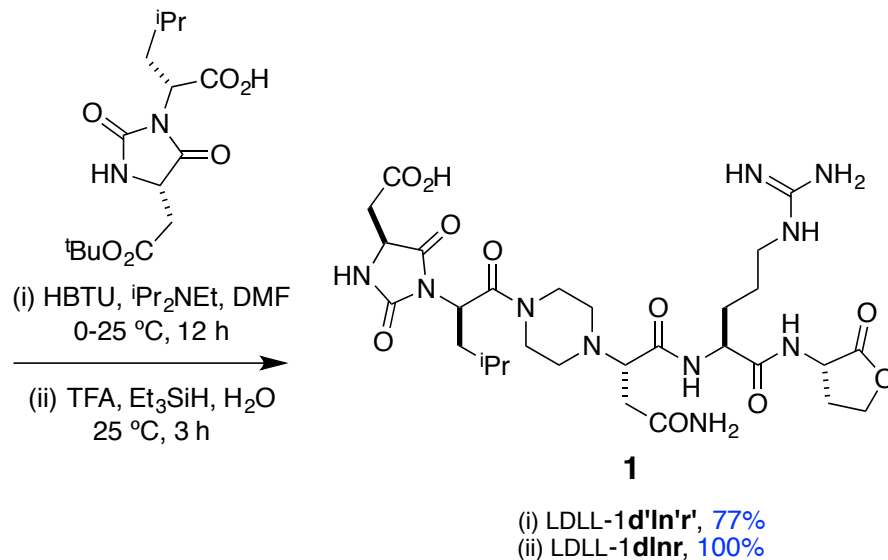


HRMS (ESI) m/z calcd for $\text{C}_{22}\text{H}_{30}\text{N}_2\text{NaO}_6\text{Na}^+$ 441.1996; found 441.1989 ($\text{M}+\text{Na}$) $^+$

05312017_hyd-d[bul]-obn#1-189 RT: 0.00-1.01 AV: 189 NL: 2.28E8
T: FTMS + p ESI Full ms [50.0000-1000.0000]







General Procedure of Amide Coupling with HBTU

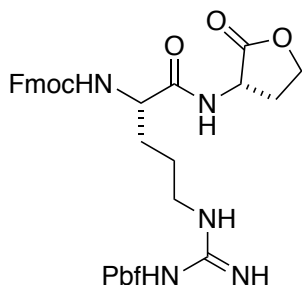
The N-protected amino acid (1 eq) was activated with HBTU (1.1 eq) and $i\text{Pr}_2\text{NEt}$ (1 eq) in DMF (0.2 M) at 0 °C for 10 min. Then, amine compound (1 eq) was added followed by $i\text{Pr}_2\text{NEt}$ (2.5 eq). The reaction was stirred at ambient temperature for 12 h under N_2 atmosphere. DMF was removed in vacuo, then crude material was redissolved in EtOAc and extracted with 10% citric acid, saturated NaHCO_3 and brine. After drying with MgSO_4 , compound was purified by flash column chromatography using 5% $\text{MeOH}/\text{CH}_2\text{Cl}_2$ to obtain product.

Nosyl deprotection

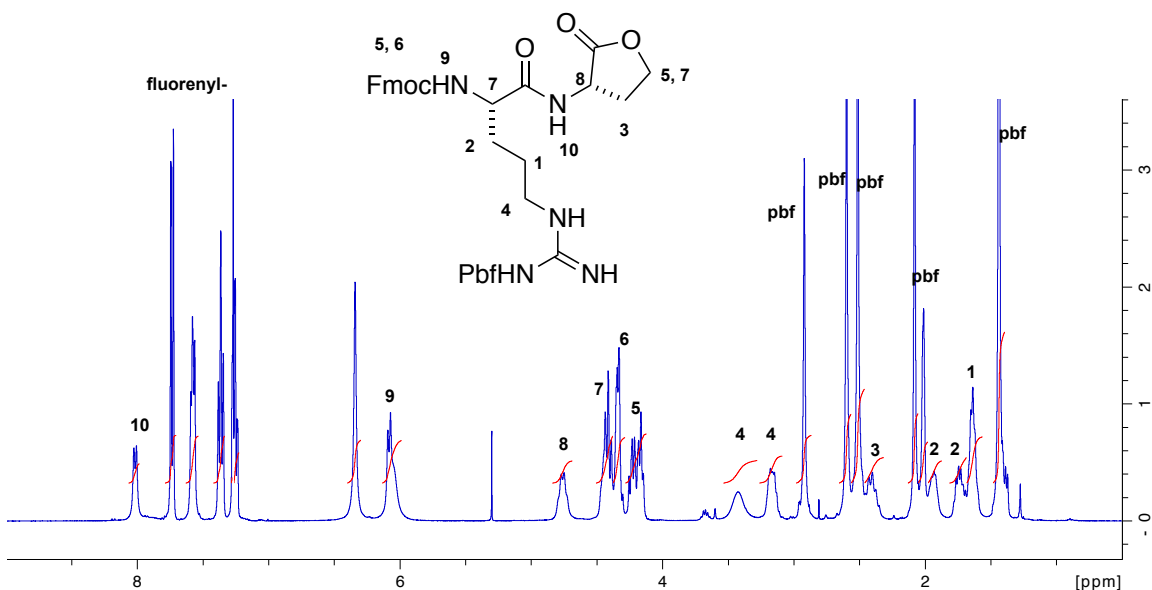
Compound LL-**8n'r'** (0.80 g, 0.71 mmol) was dissolved in 7 mL DMF, then mercaptoethanol (0.10 mL, 1.43 mmol) and DBU (0.21 mmol, 1.43 mmol) were added subsequently, and the reaction was stirred for 2 h. After the reaction completed, the mixture was diluted with EtOAc and extracted 3 times with saturated NaHCO_3 . The organic phase was dried over Na_2SO_4 and removed in vacuo to obtain crude oil. Small amount of CH_2Cl_2 was added to dissolve oil, then cold *tert*-butyl methyl ether was added to induce precipitation. The solid was filtered, washed with ether to obtain product LL-**9n'r'** as a solid (0.64 g, 96%).

Side-chain deprotection and purification were performed using TFA/Et₃SiH/water (95:2.5:2.5 v/v) cocktail same as described in solid-phase synthesis of compound **1** above.

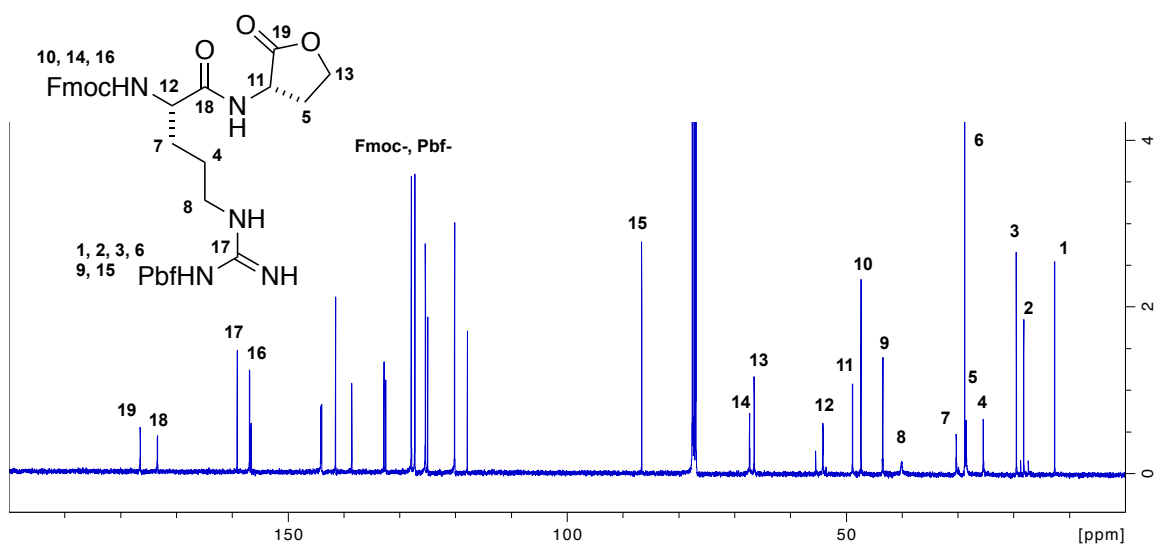
(9*H*-Fluoren-9-yl)methyl ((*S*)-1-oxo-1-(((*S*)-2-oxotetrahydrofuran-3-yl)amino)-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)carbamate (**L-7r'**)



Data for L-7r'. ¹H NMR (400 MHz, CDCl₃) δ 8.02 (br, 1H), 7.74 (d, *J* = 7.7 Hz, 2H), 7.58 (m, 2H), 7.36 (t, *J* = 7.4 Hz, 2H), 7.30-7.22 (m, 2H), 6.34 (br, 1H), 6.07 (br, 2H), 4.76 (m, 1H), 4.49-4.37 (m, 2H), 4.37-4.29 (m, 2H), 4.27-4.12 (m, 2H), 3.43 (m, 1H), 3.17 (m, 1H), 2.92 (s, 2H), 2.60 (s, 3H), 2.51 (m, 4H), 2.46-2.31 (m, 1H), 2.08 (s, 3H), 1.98-1.88 (m, 1H), 1.80-1.68 (m, 1H), 1.68-1.58 (m, 2H), 1.44 (m, 7H)

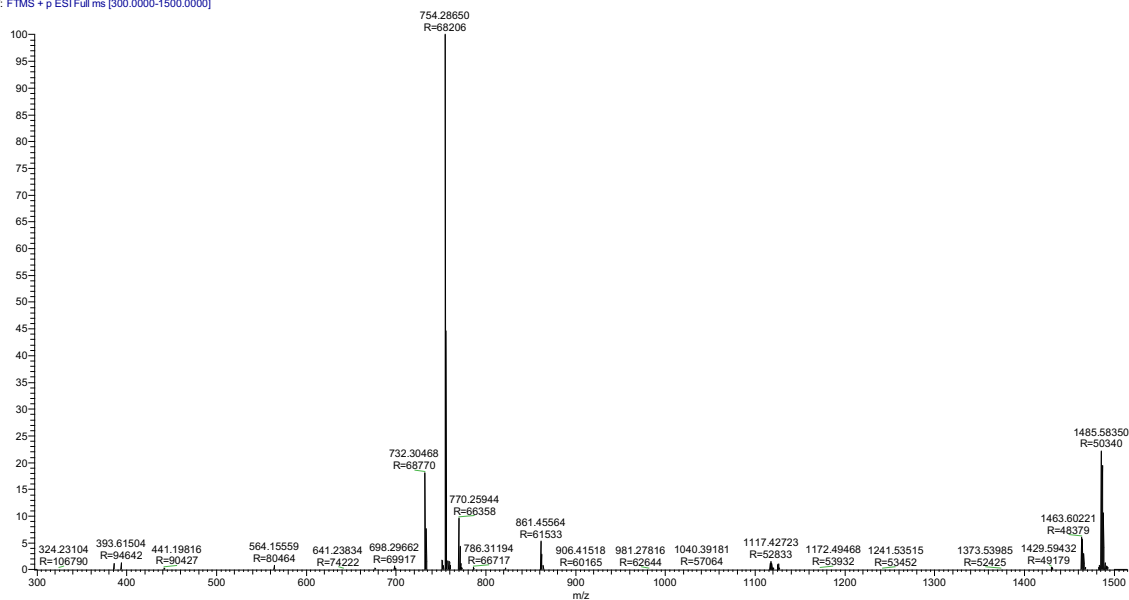


^{13}C NMR (100 MHz, CDCl_3) δ 176.5, 173.4, 159.1, 156.9, 156.6, 144.1, 144.0, 141.5, 138.6, 132.8, 132.5, 127.9, 127.3, 125.4, 124.9, 120.1, 117.8, 86.6, 67.3, 66.5, 54.2, 48.9, 47.3, 43.4, 40.1, 30.3, 28.8, 28.5, 25.4, 19.5, 18.2, 12.7, 12.6

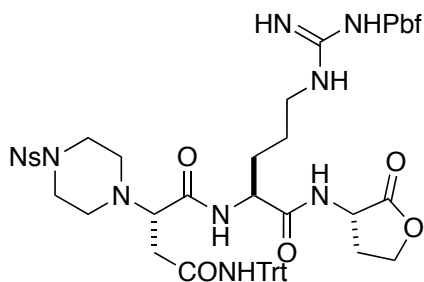


HRMS (ESI) m/z calcd for $\text{C}_{38}\text{H}_{46}\text{N}_5\text{O}_8\text{S}^+$ 732.3062; found 732.3047 ($\text{M}+\text{H}^+$)

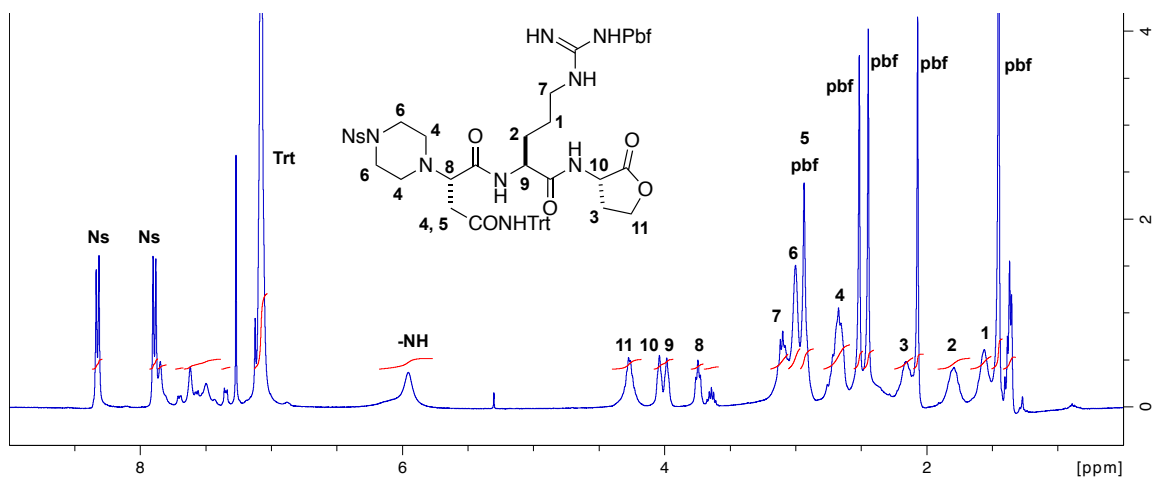
05312017_fmoc-arg(pbf)-lac #1-299 RT: 0.00-1.58 AV: 299 NL: 7.07E8
T: FTMS + p ESI Full ms [300.0000-1500.0000]



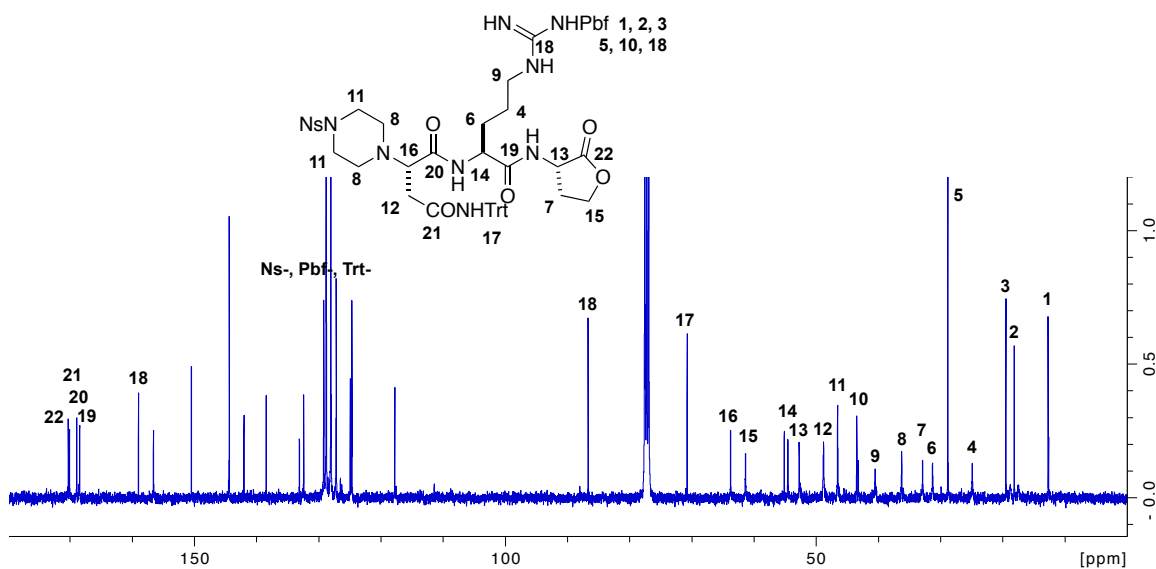
(S)-2-(4-((4-Nitrophenyl)sulfonyl)piperazin-1-yl)-N¹-((S)-1-oxo-1-(((S)-2-oxotetrahydrofuran-3-yl)amino)-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)-N⁴-tritylsuccinamide (LL-8n'r')



Data for LL-8n'r'. ¹H NMR (400 MHz, CDCl₃) δ 8.33 (d, *J* = 8.7 Hz, 2H), 7.89 (d, *J* = 8.6 Hz, 2H), 7.08 (s, 15H), 5.96 (br, 2H), 4.27 (m, 2H), 4.01 (m, 2H), 3.74 (m, 1H), 3.16-3.04 (m, 3H), 3.00 (m, 4H), 2.94 (m, 4H), 2.78-2.59 (m, 5H), 2.51 - 2.44 (m, 8H), 2.22-2.10 (m, 2H), 2.08 (s, 3H), 1.79 (m, 2H), 1.56 (m, 2H), 1.45 (s, 6H)

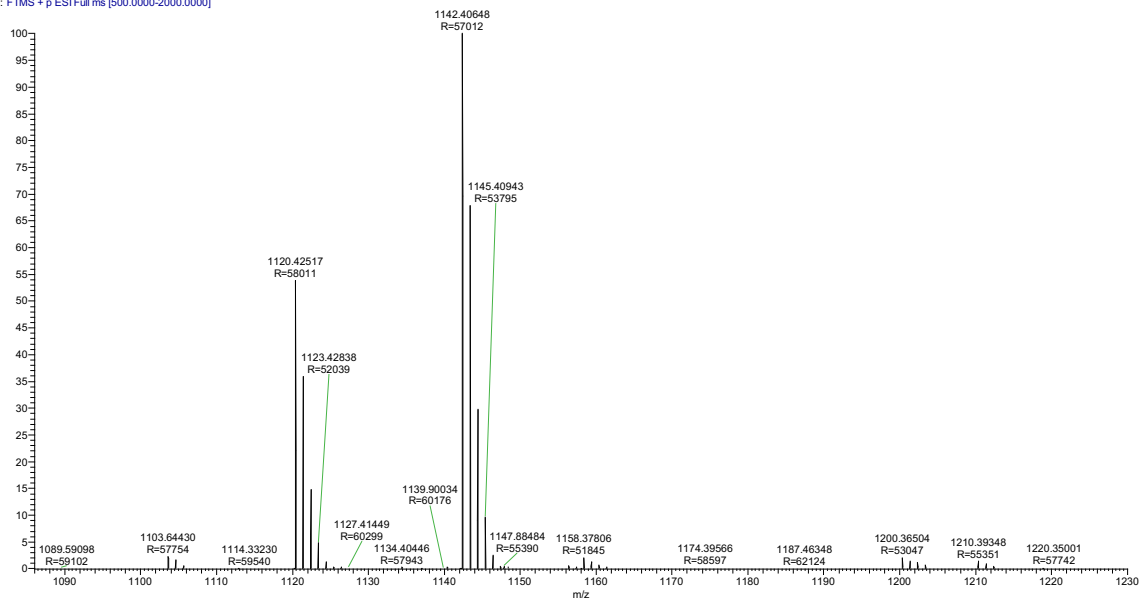


^{13}C NMR (100 MHz, CDCl_3) δ 170.3, 170.1, 168.9, 168.4, 159.0, 156.6, 150.5, 144.4, 142.0, 138.4, 133.1, 132.4, 129.2, 128.8, 128.0, 127.2, 124.9, 124.7, 117.7, 86.7, 70.7, 63.8, 61.3, 55.1, 54.5, 52.7, 48.8, 46.5, 43.5, 43.2, 40.5, 36.2, 32.9, 31.3, 28.8, 24.9, 19.5, 18.1, 12.7, 12.6

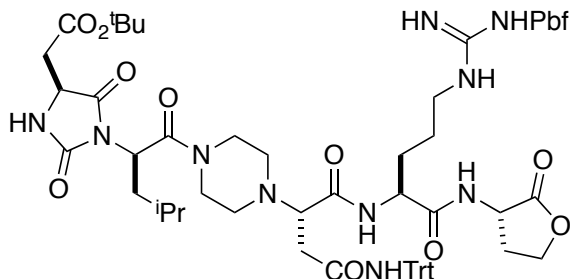


HRMS (ESI) m/z calcd for $\text{C}_{56}\text{H}_{66}\text{N}_9\text{O}_{12}\text{S}_2^+$ 1120.4267; found 1120.4252 ($\text{M}+\text{H}$) $^+$

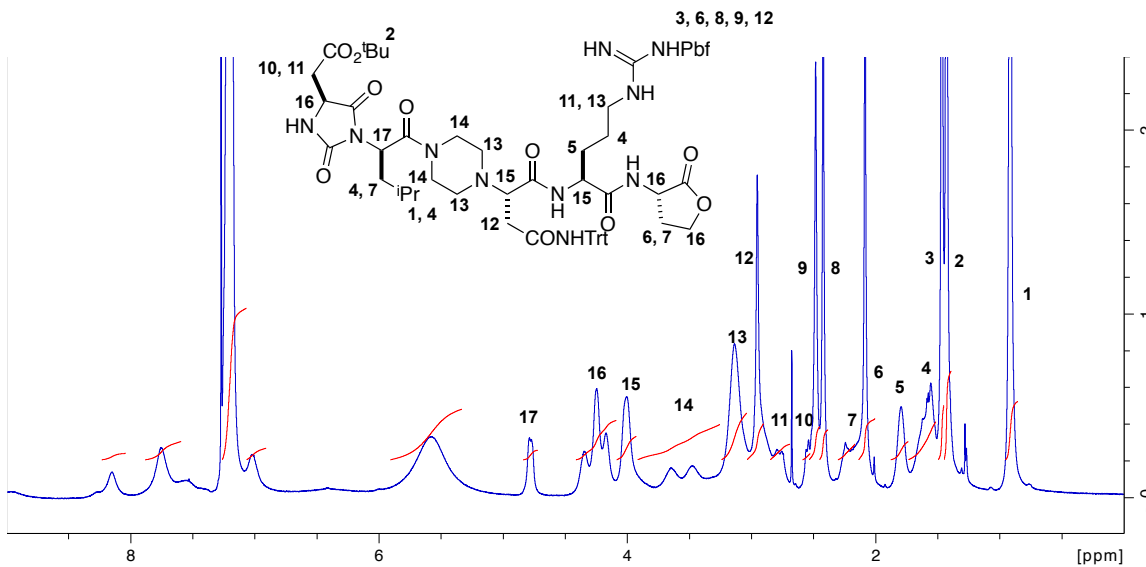
05312017_hyding-pip-asn(triang-lac #1-154 RT: 0.00-0.82 AV: 154 NL: 1.
T: FTMS -p ESI Full ms [500.0000-2000.0000]



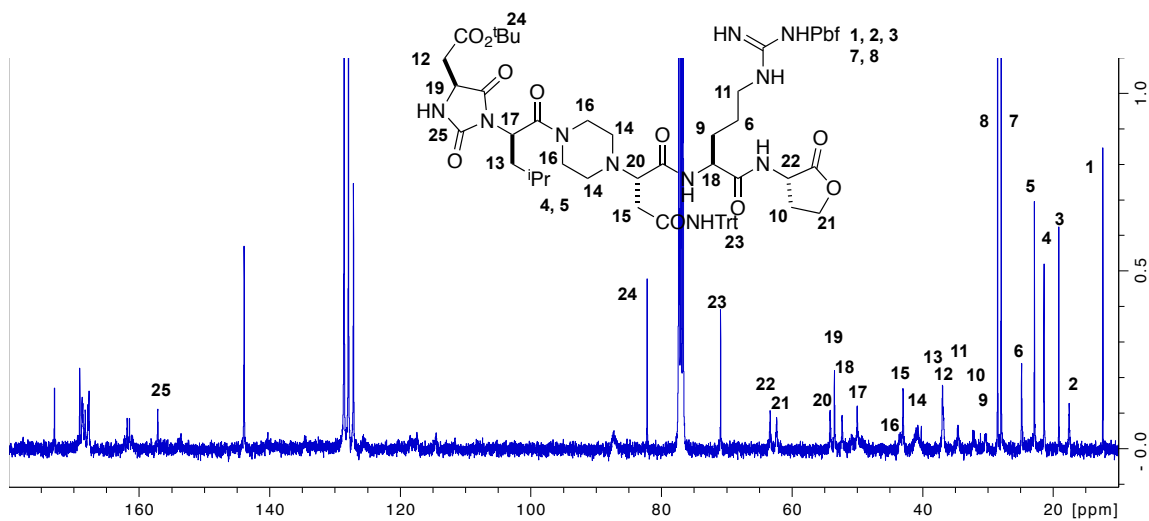
tert-Butyl 2-((*S*)-1-((*R*)-1-(4-((*S*)-1,4-dioxo-1-(((*S*)-1-oxo-1-(((*S*)-2-oxotetrahydrofuran-3-yl)amino)-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)amino)-4-(tritylamino)butan-2-yl)piperazin-1-yl)-4-methyl-1-oxopentan-2-yl)-2,5-dioximidazolidin-4-yl)acetate (LDLL-1d'In'r')



Data for LDLL-1d'In'r'. ^1H NMR (400 MHz, CDCl_3) δ 8.15 (br, 1H), 7.75 (br, 2H), 7.20 (m, 15H), 7.02 (m, 1H), 5.58 (m, 3H), 4.79 (m, 1H), 4.26 (m, 4H), 4.01 (m, 2H), 3.79-3.39 (m, 4H), 3.14 (m, 5H), 2.96 (m, 4H), 2.84-2.69 (m, 2H), 2.55 (m, 1H), 2.50 (s, 3H), 2.43 (s, 3H), 2.21 (m, 2H), 2.09 (m, 4H), 1.80 (m, 2H), 1.71-1.51 (m, 4H), 1.47 (m, 6H), 1.43 (s, 9H) 0.92 (m, 6H);

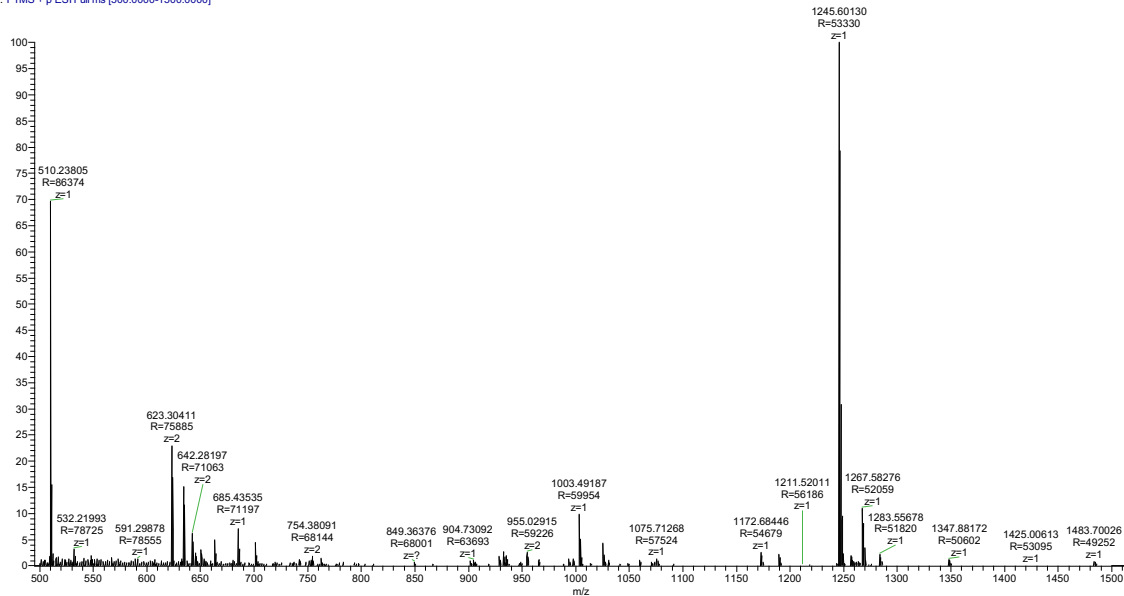


^{13}C NMR (100 MHz, CDCl_3) δ 173.0, 169.1, 168.7, 168.6, 168.3, 167.8, 167.7, 161.8, 161.5, 157.1, 143.9, 128.6, 127.9, 127.2, 82.2, 70.9, 63.4, 62.4, 54.2, 53.5, 52.3, 50.0, 43.5, 43.3, 40.7, 37.0, 36.9, 34.6, 32.3, 30.4, 28.5, 28.0, 24.8, 22.9, 21.4, 19.1, 17.6, 12.4



HRMS (ESI) m/z calcd for $\text{C}_{65}\text{H}_{85}\text{N}_{10}\text{O}_{13}\text{S}^+$ 1245.6013; found 1245.6013 ($\text{M}+\text{H}$) $^+$

05312017_hyd-dlnr-lac #269-402 RT: 1.88-2.59 AV: 134 NL: 1.85E7
T: FTMS +p ESI Full ms [500.0000-1500.0000]



F. Biological Studies

LDL-uptake Assay

LDL-uptake assay was performed according to the reference with modifications.² Briefly, HepG2 cells were seeded into 96-well plate at a density of 3×10^4 cells/well in DMEM with 10% FBS medium (Sigma-Aldrich). After 24 h, the medium was aspirated and cells were washed twice with PBS. Cells were treated with 10% lipoprotein-deficient serum (Gemini) in DMEM and incubated for another 24 h. After washing cells with PBS twice, cells were incubated 2 h with 100 μ L of Pep2-8 (30 μ M) or inhibitors at various concentrations (50, 5, 0.5 μ M), which were pre-incubated with 15 μ g/mL PCSK9 in 10% lipoprotein-deficient DMEM for 30 min prior. BODIPY-LDL (10 μ g/mL, Invitrogen) was added to each well and cells were incubated for another 3 h. Cells were washed three times with PBS, and fluorescent intensity was measured on Synergy H4 plate reader (Biotek) with excitation and emission wavelength at 488 and 520 nm, respectively.

In vitro PCSK9-LDLR Binding Assay

Compounds efficiencies to inhibit PCSK9-LDLR interaction were tested using the PCSK9-LDLR *in vitro* binding assay kit (MBL Int. Co., MA) according to the manufacture's protocol. Briefly, compounds (50 μ M), Pep2-8 (50 μ M) or vehicle (DMSO) were pre-incubated with 50 ng/mL of His-tagged PCSK9 in the reaction buffer (100 μ L) 30 min prior the experiments. The premix mixtures were added into the each well of microplate containing pre-coated LDLR-EGF-AB domain. The microplate was incubated for 2 h at room temperature while shaking at 300 rpm on an orbital microplate shaker. Each well was washed 4 times with wash buffer (350 μ L each), and the biotinylated anti-His-tag mAb was added (100 μ L). The microplate was shaken at 300 rpm for 1 h at room temperature. After washing 4 times with washing buffer, HRP-conjugated Streptavidin (100 μ L) was added into each well, incubated for 20 min at room temperature while shaking at 300 rpm. Microplate was washed 4 times with washing buffer, then the substrate reagent (tetra-methylbenzidine, 100 μ L) was added into each well and the microplate was shaken for 20 min at room temperature. Finally, the reaction was stopped with 1 N H₂SO₄ (100 μ L), and the absorbance was measured on plate reader at dual wavelengths of 450/540 nm.

TR-FRET Assay

Compounds **A** were screened using PCSK9-LDLR TR-FRET assay kit (BPS Bioscience, CA) following manufacture's protocol. LDLR-Eu (125 ng/mL), dye-labeled acceptor and assay buffer were added into each well of 384-well microplate. Compounds **A**, Pep2-8 and vehicle at various concentrations were added, and the reactions were initiated by adding biotinylated PCSK9 (2 µg/mL). Plates were shaken at room temperature for 2 h. The TR-FRET fluorescent intensities were measured using plate reader with two pairs of excitation/emission wavelengths (320/620 and 320/665 nm) with 60 µs lag time and 500 µs integration time. Results were analyzed by normalizing TR-FRET emission ratio (665/620 nm) of vehicle with biotinylated PCSK9 as 0% (negative), vehicle without biotinylated PCSK9 as 100% (positive), and compounds were scaled according to those controls.

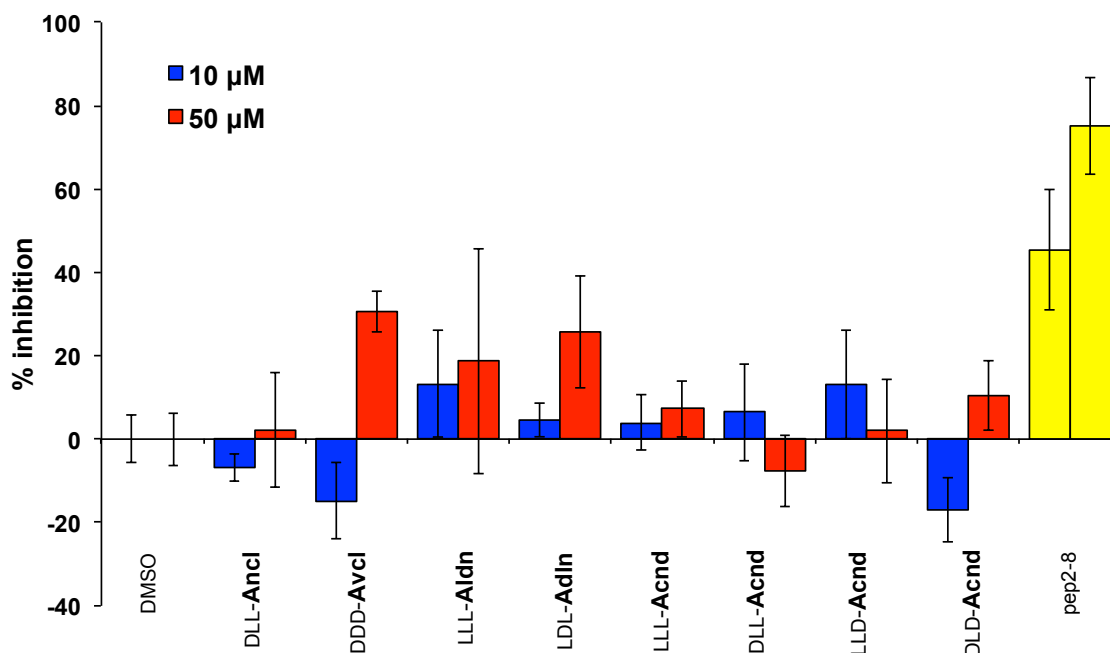


Figure S1. Compounds **A** were screened for their inhibition using a commercial PCSK9-LDLR TR-FRET kit (BPS Bioscience). Compounds and Pep2-8 were tested at 10 and 50 µM. The results, however, were inconclusive, so the subsequent experiments were discontinued.

MTT Assay

HepG2 cells were seeded at 3000 cells/well in 96-well plate and incubated in DMEM + 10% FBS medium for 24 h. Then, medium containing inhibitors was added to the final concentrations of 50 μM or 100 μM (100 μL) and cells were incubated for another 2 days. MTT substrate in HBSS buffer (5 mg/mL, 20 μL) was added and cells were incubated for additional 3 h. Medium was aspirated, then DMSO (100 μL) was added subsequently, and the microplate was shaken for 5 min. The absorbance was measured with plate reader at 570 nm wavelength.

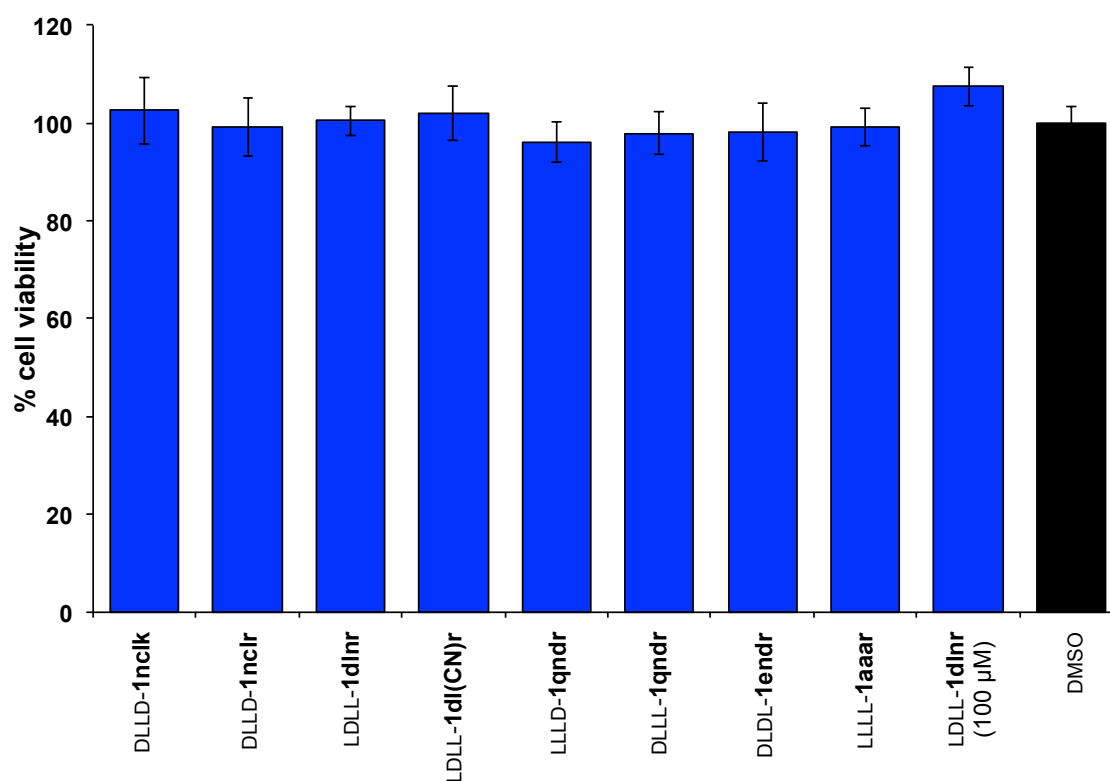


Figure S2. Seven featured compounds **1** and one partial negative control were tested for their cytotoxicity with HepG2 cells. All compounds showed no cytotoxicity compared to the control (DMSO, black).

Cell-surface LDLR Assay

Flow Cytometry

HepG2 cells were seeded at a density of 1.0×10^5 cells/well in 24-well plate with DMEM + 10% FBS medium for 18 h. After aspiration, cells were washed two times with PBS and treated with DMEM supplemented with 10% lipoprotein-deficient serum for another 18 h. Inhibitors (50 μ M) or pep2-8 (50 μ M) at 0.5 % DMSO level and 3 μ g/mL PCSK9 in 10% lipoprotein-deficient DMEM were pre-incubated for 30 minutes at room temperature before adding into each well. Cells were incubated for 4 h at 37 °C with 5% CO₂. After treatment, cells were washed twice with PBS and detached with enzyme-free cell dissociation buffer (gibco) for 15 min at 37 °C. After centrifugation (500 rcf, 5 mins, 4 °C), cells were incubated with anti-LDLR antibody (100 μ L of 10 μ g/mL in 0.5% BSA/PBS, Invitrogen) on ice for 20 minutes. Cells were washed twice with 0.5% BSA/PBS and incubated with goat anti-mouse IgG (H+L) Alexa Fluor 647 (100 μ L of 10 μ g/mL in 0.5 % BSA/PBS, Invitrogen) on ice for 10 minutes. After washing twice with 0.5% BSA/PBS, cells were resuspended in 100 μ L 0.5% BSA/PBS and analyzed by flow cytometer (BD Accuri™ C6 cytometer, BD Biosciences). The fluorescence of 30,000 events of each sample was acquired for data analysis. Forward scatter versus side scatter gates were set to exclude dead cells and debris. Mean values of the cells treated with PCSK9 were set as the minimum LDLR level (0%, negative) and without PCSK9 as the maximum LDLR level (100%, positive). Data from inhibitors were scaled as 0-100% according to the negative and positive controls. All experiments were repeated three times individually.

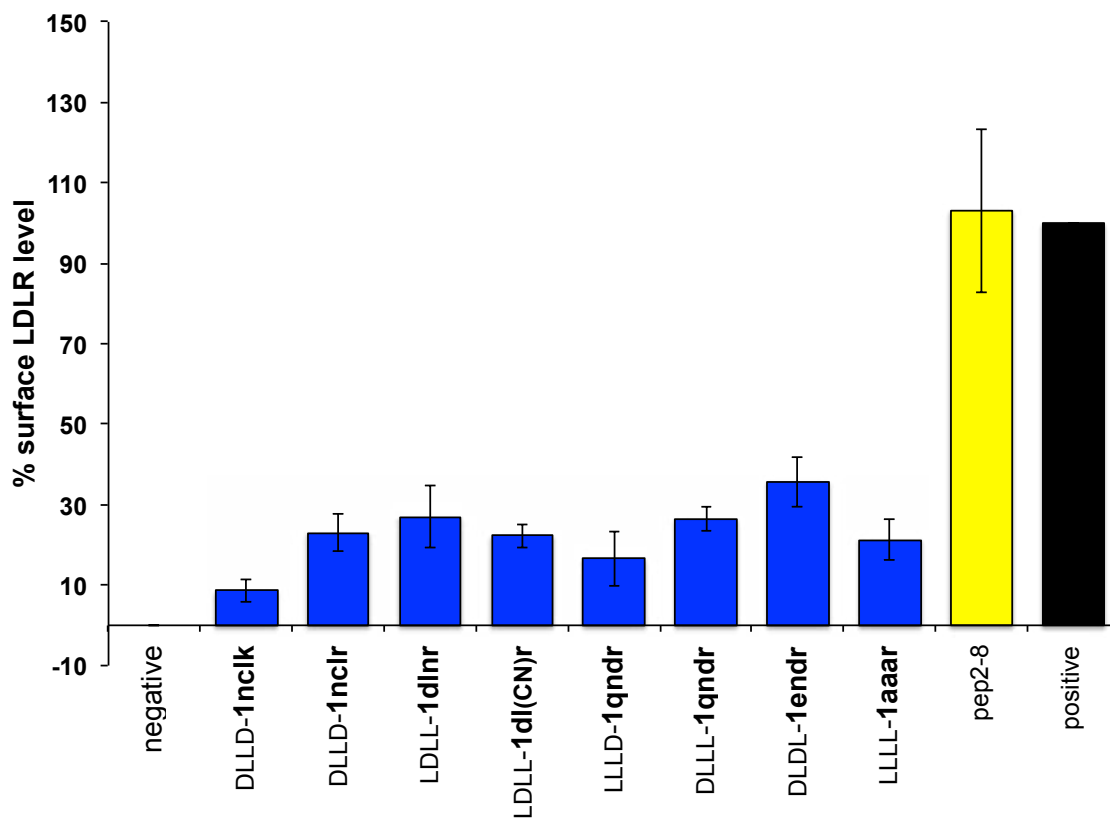
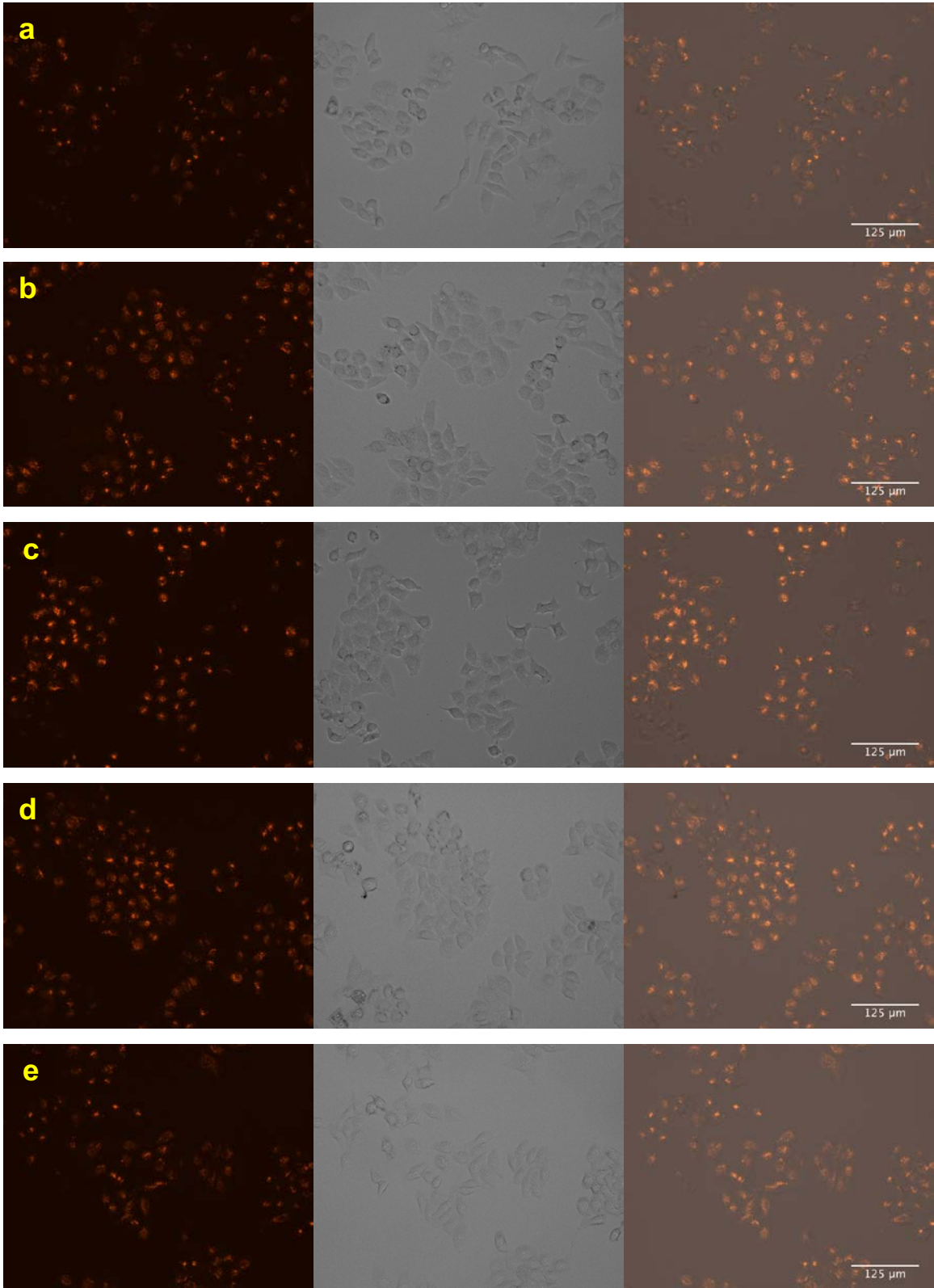


Figure S3. The percentages of cell-surface LDLR of HepG2 cells treated with compounds **1** were quantified by flow cytometry. Results showed the increase of LDLR on cell surface comparing to the cells treated with PCSK9 alone (negative). Results represent averages \pm SD of three independent experiments.

Fluorescent Imaging

HepG2 cells were seeded at the density of 1.0×10^4 cells/well in 24-well plate with DMEM + 10% FBS medium overnight, and then incubated in 10% lipoprotein-deficient DMEM for another 24 h. Inhibitors (50 μ M) or pep2-8 (50 μ M) at 0.5 % DMSO level and 3 μ g/mL PCSK9 in 10% lipoprotein-deficient DMEM were pre-incubated for 30 minutes at room temperature before adding into each well. Cells were incubated for 4 h at 37 $^{\circ}$ C with 5% CO₂. After treatment, cells were washed twice with PBS and incubated with 300 μ L anti-LDLR antibody (5 μ g/mL in delipidated DMEM, Invitrogen) at 37 $^{\circ}$ C for 30 minutes. Cells were washed twice with PBS and incubated with 300 μ L goat anti-mouse IgG (H+L) Alexa Fluor 647 (10 μ g/mL in delipidated DMEM, Invitrogen) at 37 $^{\circ}$ C for 30 minutes. Cells were washed twice with PBS, and then treated with FluoroBrite™ DMEM (gibco) before analyzing with fluorescent microscope (Evos FL Auto 2, Invitrogen). The average corrected total cell fluorescence (CTCF) was quantified by ImageJ software.

Cells treated with PCSK9 alone were calibrated as 0% (negative), untreated with PCSK9 as 100% (positive) and cells treated with compounds and PCSK9 were scaled.



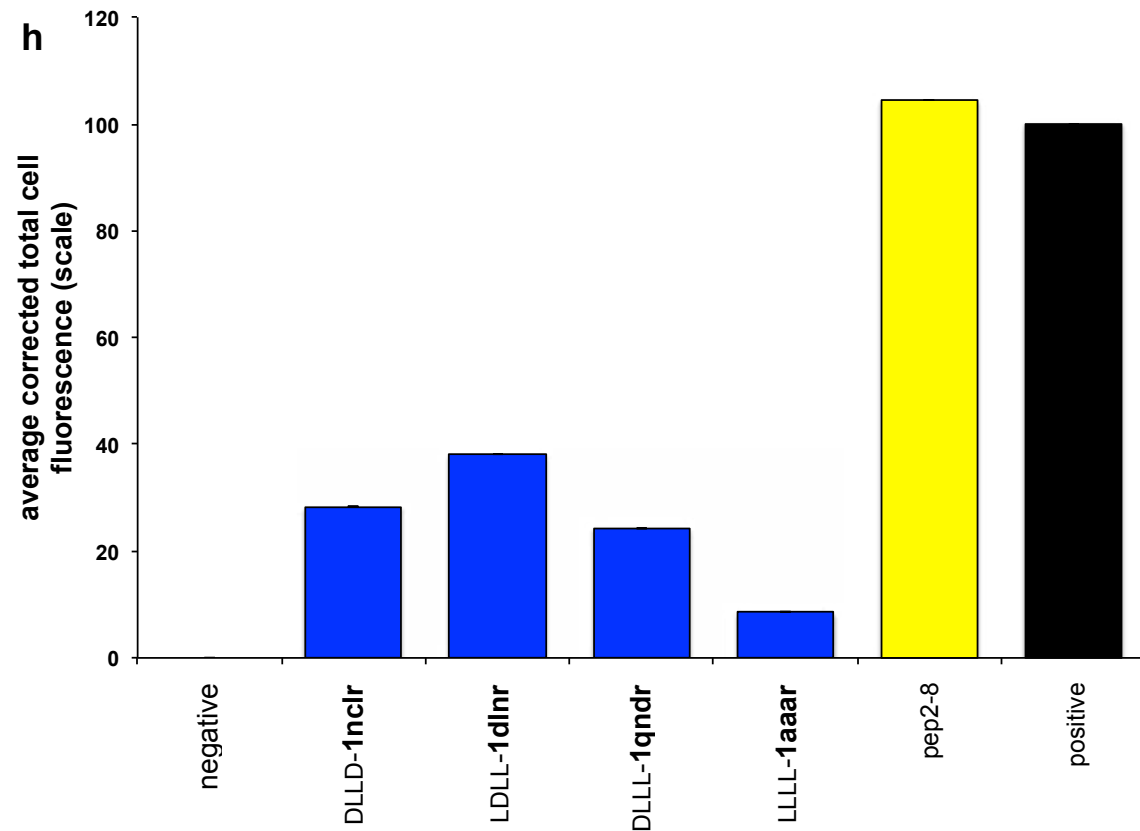
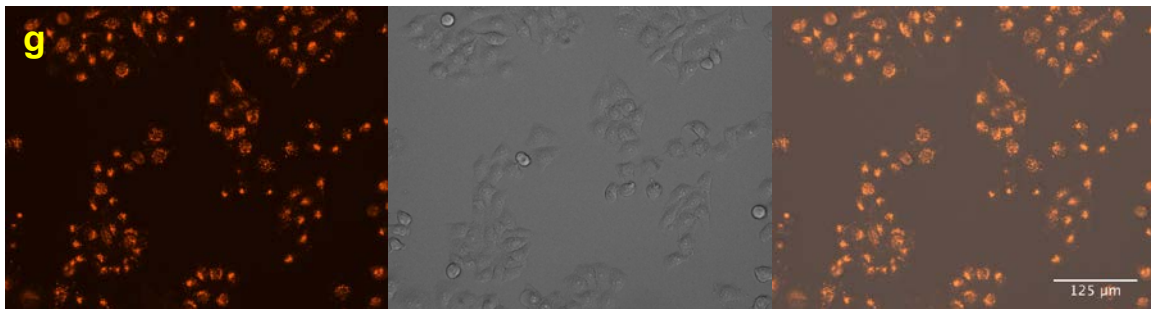
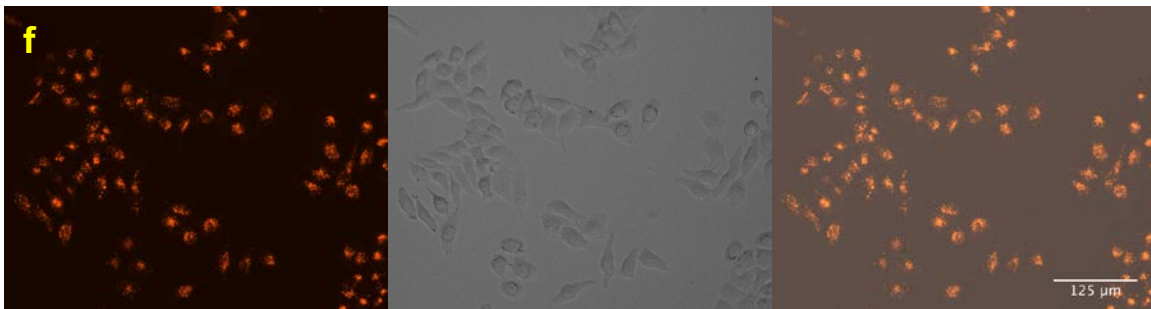
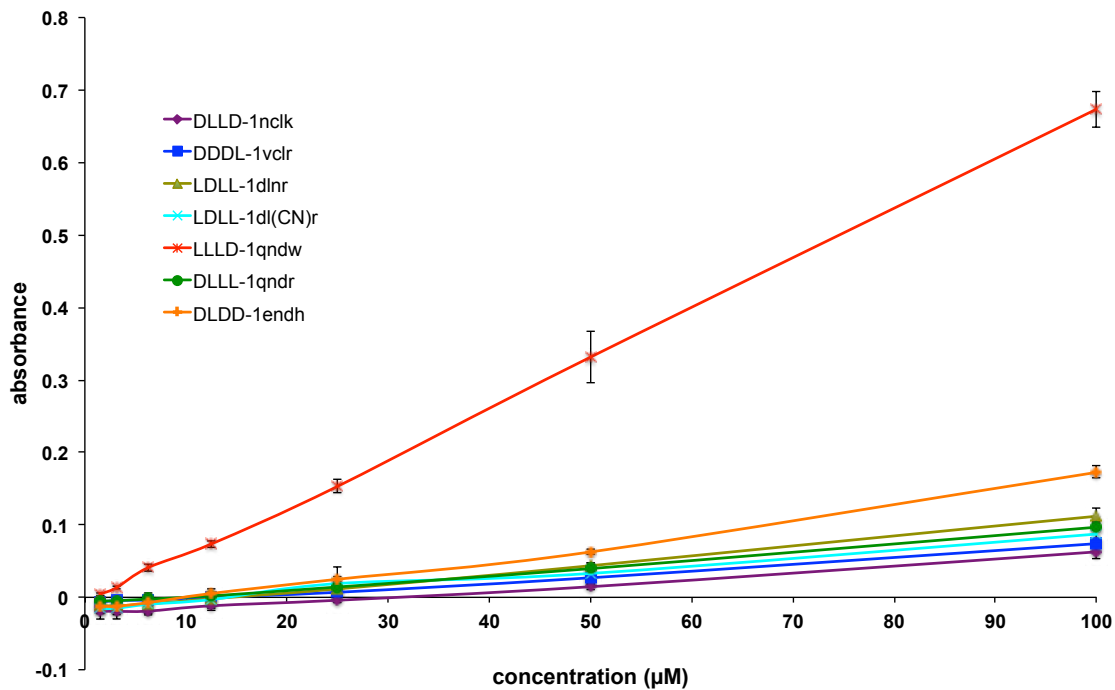


Figure S4. Fluorescent imagings of HepG2 cells treated with PCSK9 and **a** DMSO; **b** DLDD-1nc1r; **c** LDLL-1dl1r; **d** DLLL-1qndr; **e** LLLL-1aaar; **f** pep2-8; and **g** DMSO without PCSK9. **h** Fluorescent intensity was quantified by ImageJ software. All cells in one image were quantified manually, subtracted background and averaged, so that the error bars were not reported.

G. Determination of Water Solubilities

Compound solubility in water was measured by UV absorbance following the literature.³ Briefly, compounds at various concentrations were dissolved in MeOH and plated on 96-well plate. Solvent was evaporated at room temperature. Compounds were dissolved in water at various volumes to reach their designated concentrations. Microplate was shaken for 5 h, and left overnight at room temperature to reach equilibrium. Microplate was centrifuged for 20 min to remove any particulates. The clear solution was transferred to a 96-well UV-transparent plate to measure the UV absorbance at 230 nm wavelength.



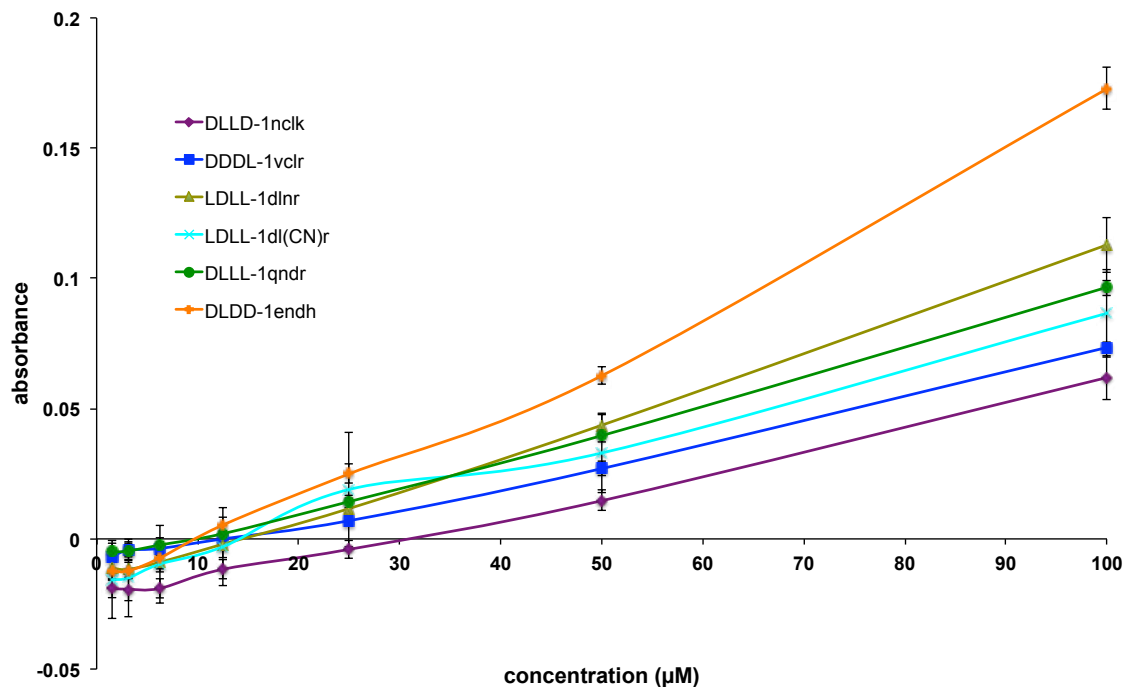
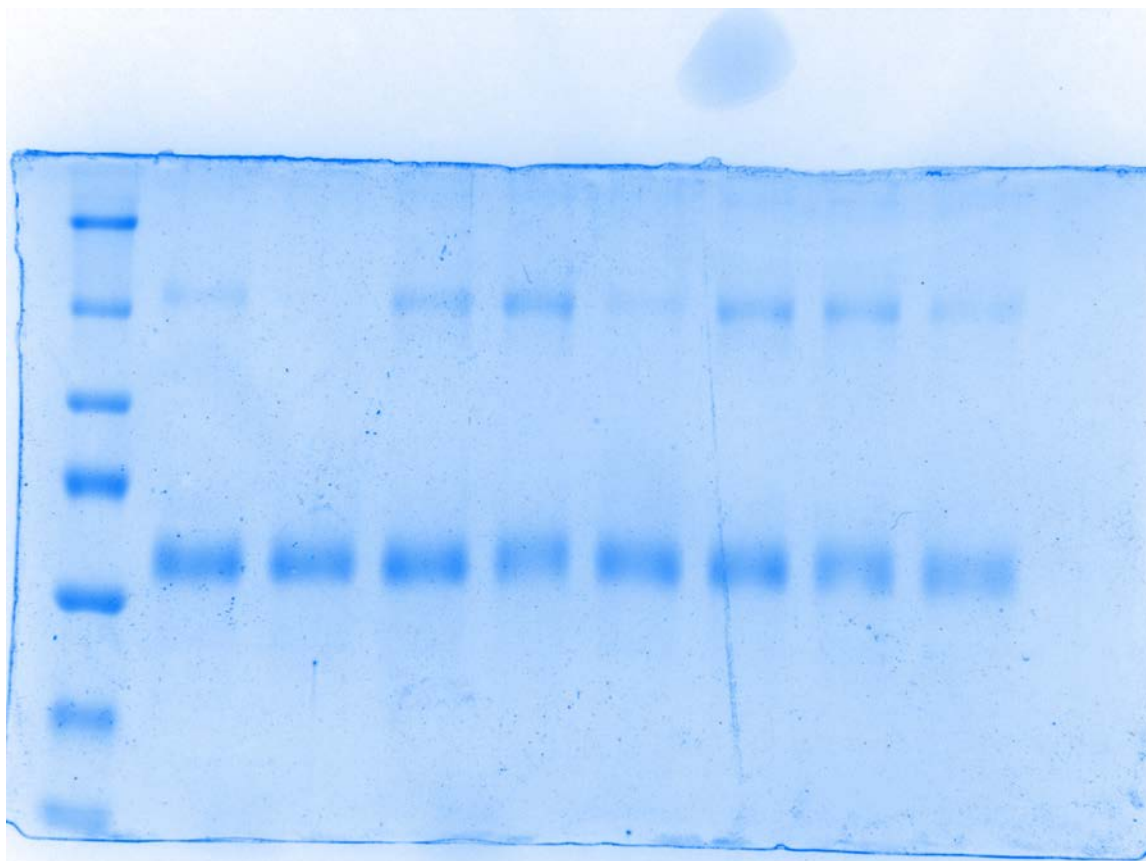


Figure S5. Selected compounds **1** were tested for their solubility in water by UV absorbance method. Compounds at seven concentrations up to 100 μM gave linear absorbance trend, which implied the solubility of compounds were at least $> 100 \mu\text{M}$.

H. Photoaffinity Labeling

Compounds **2** (100, 200 and 300 μM) were incubated with 0.25 $\mu\text{g}/\mu\text{L}$ PCSK9 in 100 mM HEPES buffer to a final volume of 20 μL at 4 $^{\circ}\text{C}$ for 1.5 h. Mixtures were transferred into 96-well plate, and then irradiated by a LED UV flashlight (365 nm) at 4 $^{\circ}\text{C}$ for 30 min. Samples (19.5 μL) were transferred into Eppendorf tubes, mixed with 2.5 μL of 10% SDS, and added 0.5 μL of 10 mM azide-fluor 488 (Sigma-Aldrich) to each tube. Huisgen cycloaddition was initiated by adding 2.5 μL of catalyst mixture, which was prepared immediately before use by mixing 3:1:1 v/v of 1.7 mM TBTA in 80% *tert*-butanol/20% DMSO, 50 mM CuSO_4 and 50 mM TCEP in H_2O . The reaction was kept at room temperature for 4 h. Finally, the reactions were quenched with 5 μL of 6x SDS buffer, and boiled at 95 $^{\circ}\text{C}$ for 10 min to denature PCSK9 protein. SDS-PAGE was performed by loading on 10% polyacrylamide gel. The in-gel fluorescence was scanned by Typhoon FLA 9500 imager (Alexa Fluor-488), and all proteins were stained with

Coomassie Brilliant Blue G250. For the competitive experiment, 50-fold concentration of compounds **3** were incubated with PCSK9 at 4 °C for 1 h before adding compounds **2**. Also, the mixture of compound **2** and PCSK9 without irradiating UV flashlight (negative control) had been tested to gauge non-specific binding.



=

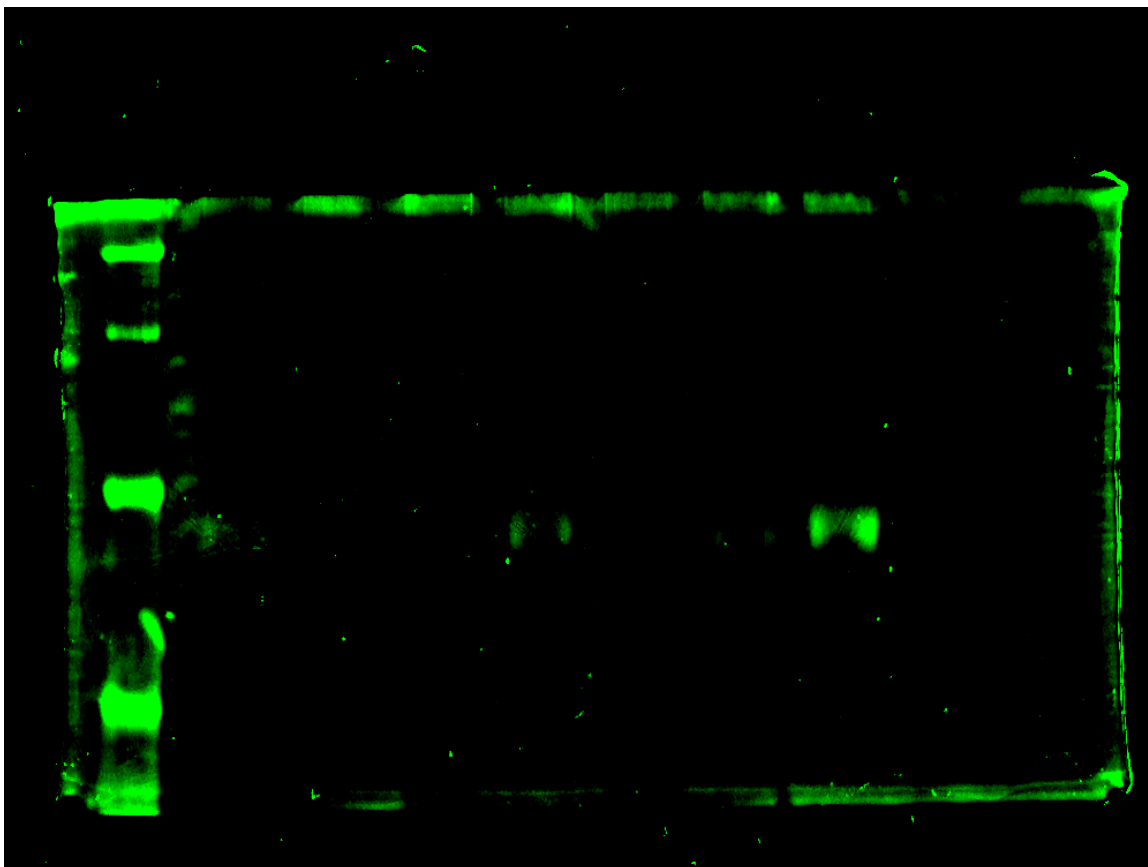


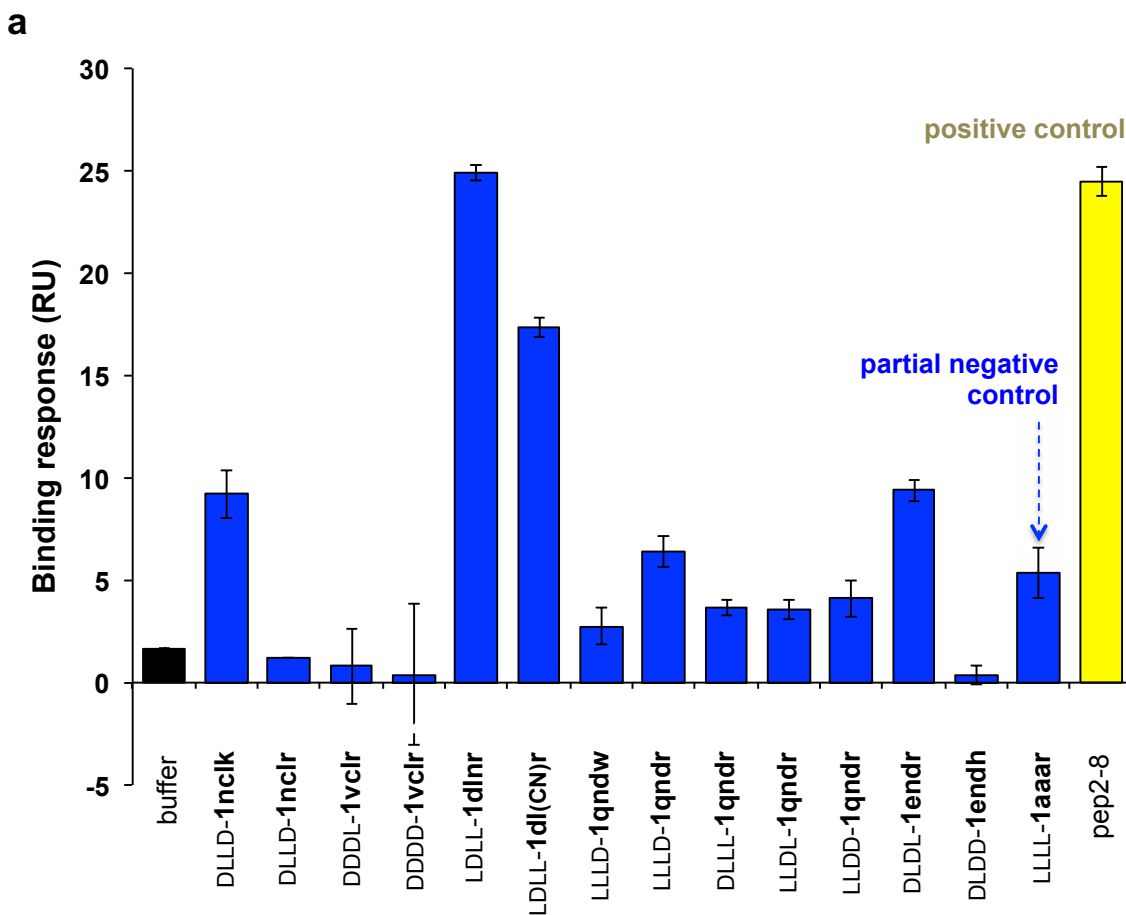
Figure S6. Full SDS-PAGE gel from photoaffinity labeling experiment. Gel was stained with CBB-G250 (top) and visualized by in-gel fluorescence (bottom). Protein ladder was on the left lane, and the sequence of samples was the same as Figure 4

I. Surface Plasmon Resonance (SPR)

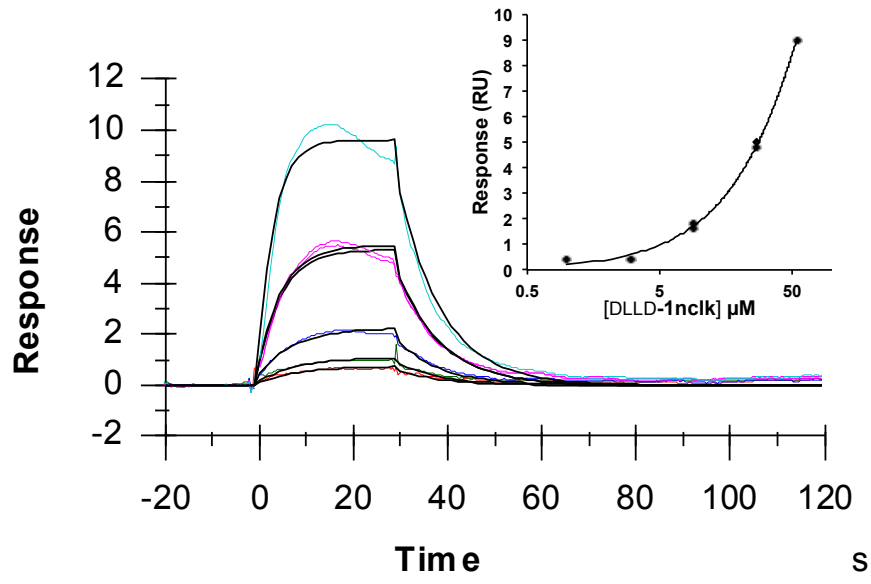
Direct binding of each compound to PCSK9 was measured by SPR using a Biacore T200 optical biosensor (GE Healthcare) at 25°C. CM5 sensor chips (GE Healthcare) were used to create PCSK9 biosensors using standard amine coupling chemistry. Briefly, after mixing equal volume mixture of 0.1 M N-hydroxysuccinimide and 0.4 M 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide, the mixture was injected for 7 min at 10 μ l/min to activate the flow cell surface. PCSK9 stock (about 1 mg/ml) was diluted to 10 μ g/ml in 50 mM sodium acetate (pH 5.5) and injected to the activated surface and immediately followed by a 5-min injection of 1 M ethanolamine (pH 9) to deactivate the surface. By varying the ligand contact times for 5, 7 or 10 minutes, different density of

PCSK9 surfaces were generated: 2600, 4300 and 5400 RU respectively. Phosphate buffered saline (PBS: 8.06 mM Na₂HPO₄ and 1.94 mM KH₂PO₄, pH 7.4, 2.7 mM KCl, 137 mM NaCl) was used as running buffer for immobilization. A reference flow cell was prepared with activation and deactivation steps but no protein coupled. All binding experiments were performed in a running buffer of 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 5% (v/v) DMSO (TBSD) using a flow rate of 50 µl/min.

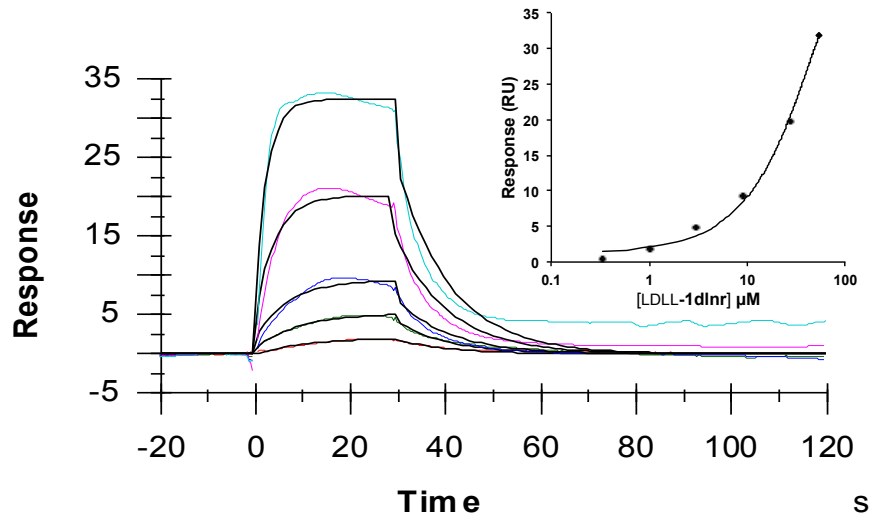
To evaluate compound binding, each compound was dissolved in 100% DMSO to 10 mM and then diluted 20-fold in TBS to 500 µM final concentration as working stock for further dilution in TBSD. Samples were injected over the PCSK9 surface for 30 s followed by 60 s of dissociation and a subsequent wash step with 50% DMSO solution. In the screening experiment, the positive control Pep2-8 was injected three times (beginning, middle and end) during the run. The signal 10 s before injection stop of the sensorgram was treated as PCSK9-binding response. The SPR sensorgrams were solvent corrected, reference and buffer subtracted, and evaluated using the Biacore T200 Evaluation Software (version 3.1).



b



c



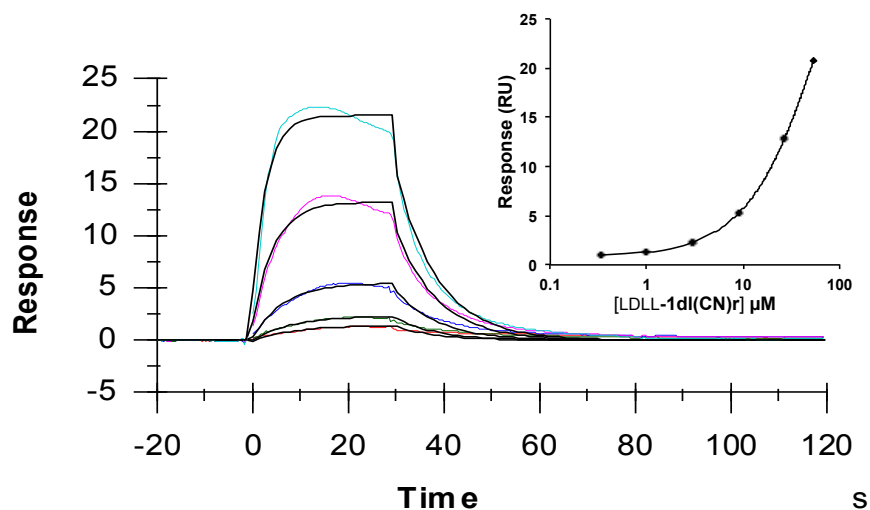
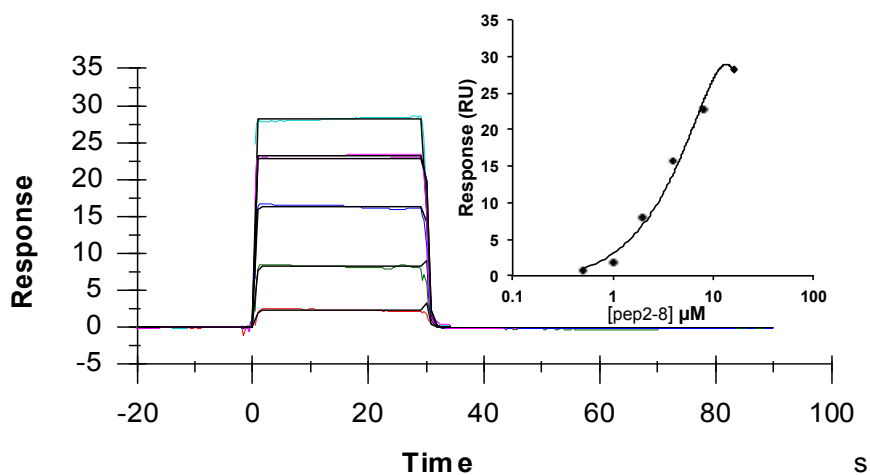
d**e**

Figure S7. SPR measurement of PCSK9 direct binding. **a.** Each compound was injected at 50 μM and Pep2-8 at 5 μM to the PCSK9 surface in triplicate to detect direct binding. **b-e.** The representative SPR profiles for the DLLD-1nclk, LDLL-1dlnr, LDLL-1dl(CN)r (1, 3, 9, 27 and 54 μM) and peptide Pep2-8 (1, 2, 4, 8 and 16 μM) binding to immobilized PCSK9 (4000 RU). SPR response curves (colored lines) for the compound binding were fitted to a 1:1 Langmuir model (black lines) to obtain the association and dissociation rate constants (k_{on} , k_{off}). The kinetic dissociation constant K_d was derived ($K_d = k_{\text{off}}/k_{\text{on}}$). Steady state analysis was performed for each tested compounds to plot dose-dependent PCSK9-binding. A binding isotherm (inset) was generated to calculate the steady state affinity K_D^{SS} . All the binding parameters are listed in Table S1.

Table S1. Confirmation of compound binding and their dissociation constants for PCSK9 as determined by SPR.

Compound	k_{on} ($\text{M}^{-1}\text{s}^{-1}$)	k_{off} (s^{-1})	K_{d} (μM)	$\%R_{\text{max}}$	K_{D}^{SS} (μM)
DLLD-1ncl \mathbf{k}	2.99 (0.89) $\times 10^3$	1.02 (0.11) $\times 10^{-1}$	41.2 (17.5)	30 (13)	303 (94)
LDLL-1dl \mathbf{n} r	4.04 (2.20) $\times 10^3$	8.74 (3.40) $\times 10^{-2}$	24.8 (9.1)	70 (24)	55.4 (13.2)
LDLL-1dl(CN) \mathbf{r}	2.50 (1.44) $\times 10^3$	8.06 (3.07) $\times 10^{-2}$	35.8 (11.4)	59 (12)	74.5 (15.6)
Pep2-8	4.35 (2.02) $\times 10^5$	1.56 (0.78)	3.56 (0.16)	32 (11)	7.63 (1.42)

Data are average of three independent experiments using different PCSK9 surface density ($R_{\text{PCSK9}} = 2600 \text{ RU}$, 4300 RU , or 5400 RU) with standard deviations shown in parentheses. The theoretical maximal binding response of each surface was calculated using equation: $R_{\text{max}} = (\text{MW}_{\text{compound}}/\text{MW}_{\text{PCSK9}}) \times R_{\text{PCSK9}}$. The experimental maximal binding was obtained from the kinetic fitting and convert to $\%R_{\text{max}}$ so that the compound MW and PCSK9 density for each experiment can be normalized and compared.

J. Computational Studies

QMD & EKO

QMD was used to generate simulated conformations of all diastereomers of **Aaaa**. The procedures had been reported previously.⁴ Approximately 1500 conformers of each diastereomer within 3.0 kcal/mol of the global minimum were matched on PCSK9-LDLR protein-protein interaction (PDBID: 3gcx)⁵ using EKO analyses.⁶ The C_{α} - C_{β} coordinates from the side chains of peptidomimetics were systematically overlaid on C_{α} - C_{β} coordinates of the LDLR side chains at protein-protein interface using in-house algorithm. The goodness of fit was reported as root mean square deviation (RMSD). Lower RMSDs mean the C_{α} - C_{β} orientations between chemotypes and protein side chains are similar, thus chemotypes can *mimic* the protein side chains. Conformers within $\text{RMSD} \leq 0.50 \text{ \AA}$ were considered as “potential hits” which were summarized in Table S2. Compounds **A** were synthesized based on the feasibility to obtain the starting materials.

Table S2. Potential LDLR mimics (**A**) from EKO analyses within RMSD ≤ 0.50 Å

Isomer	Residues	RMSD (Å)
DDD	D299-L298-N301	0.39
LDD	D299-L298-N301	0.41
LLD	C297-N301-D299	0.42*
DDL	D299-L298-N301	0.43
LLL	C297-N301-D299	0.44*
LDL	D299-L298-N301	0.45*
DLD	C297-N301-D299	0.46*
LLL	L298-D299-N301	0.46*
DLL	C297-N301-D299	0.47*
DDD	V307-C308-L318	0.47*
LLD	L298-D299-N301	0.48
DLL	N309-C308-L318	0.50*

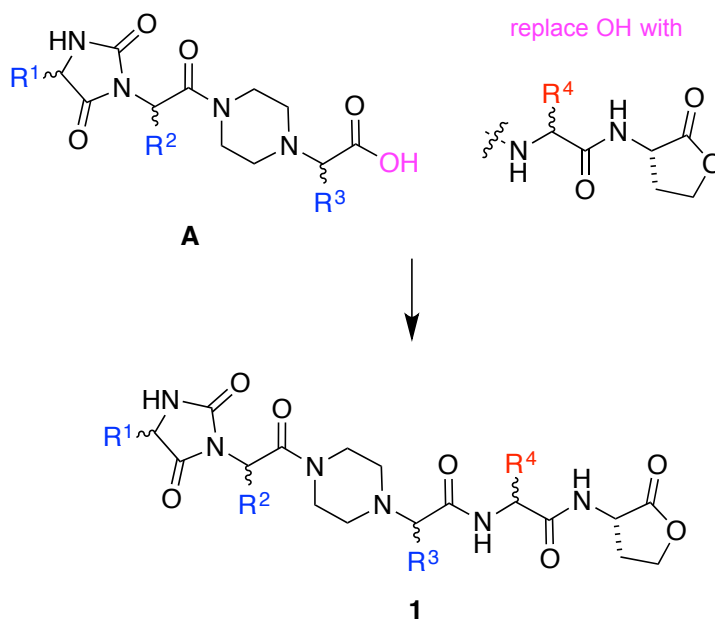
* Selected hits

Glide

Molecular dockings of the virtual libraries were performed using Glide in Schrödinger package (version 2015-4).⁷⁻¹⁰ Selected hits from EKO analyses were used as the templates for optimizations. Side-chain substitutions and C-terminus modifications were enumerated using CombiGlide to obtain virtual combinatorial libraries. PCSK9 protein was obtained by deleting the LDLR protein from the same PDB file (3gcx). The docking of virtual compounds was performed using OPLS_2005 force field within 20 Å of the grid box, and the conformations of virtual molecules were restricted within 5 Å from the parental conformers.

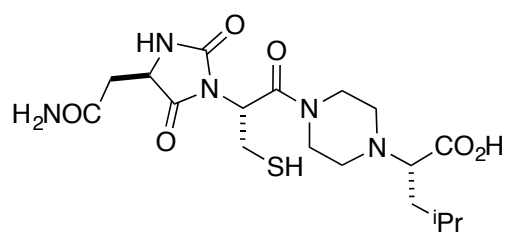
Scheme S1 showed the representative how compounds **1** were obtained from Glide. R¹-R³ side chains were replaced with the natural side chains from LDLR protein (Table S2). Due to the synthetic difficulty and poor stability of the hydantoin when R¹ was cysteine, this position was enumerated with other amino-acid side chains to obtain ones that had best docking scores, which were glutamine and glutamic derivatives. After modifying R¹-R³ side chains, the carboxylic acid was replaced with amino acid-lactone moiety, in

which R⁴ was the library of *D*-, *L*- amino-acid side chains. The top results, which were mostly positive functional groups, were listed below.

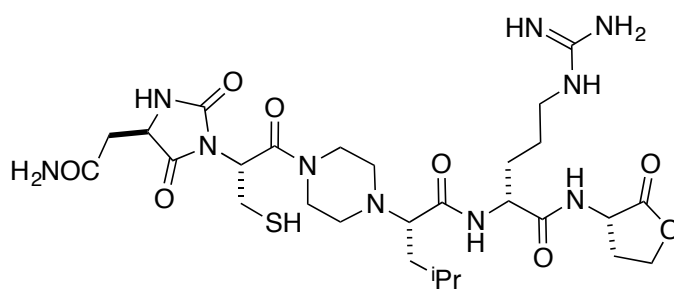


Scheme S1. Compounds **1** were conceived from their parental compounds **A** using Glide. Side chains of R¹-R³ were substituted based on the natural side chains of LDLR in which conformers **A** overlaid on, except cysteine side chain at R¹ was modified. C-terminus was replaced with *D*-, *L*- amino acid-lactone libraries.

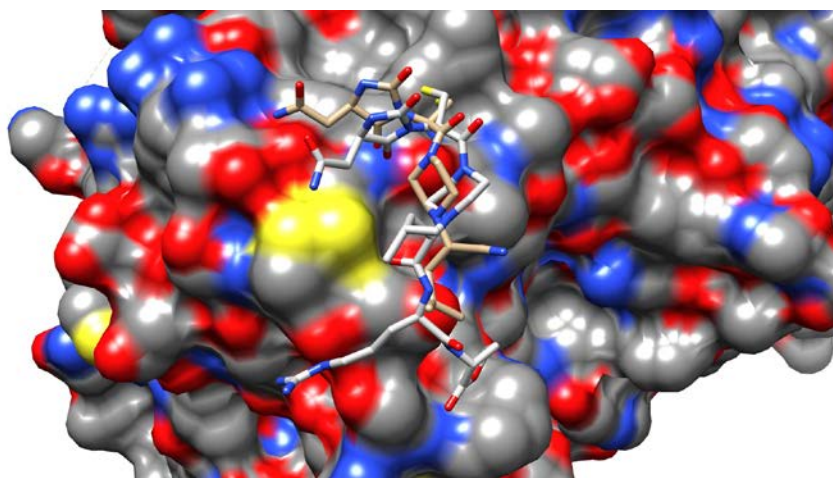
DLLD-1nclr



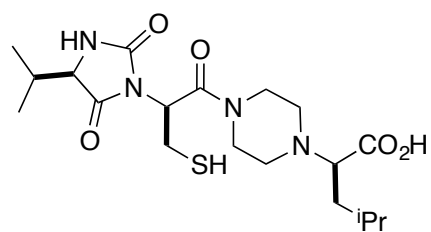
DLL-Ancl (gold), $\Delta G = -2.04$ kcal/mol



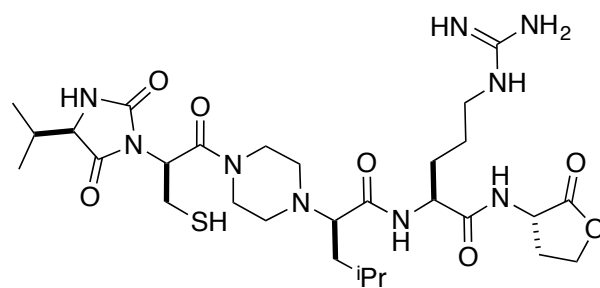
DLLD-1nclr (silver), $\Delta G = -3.41$ kcal/mol



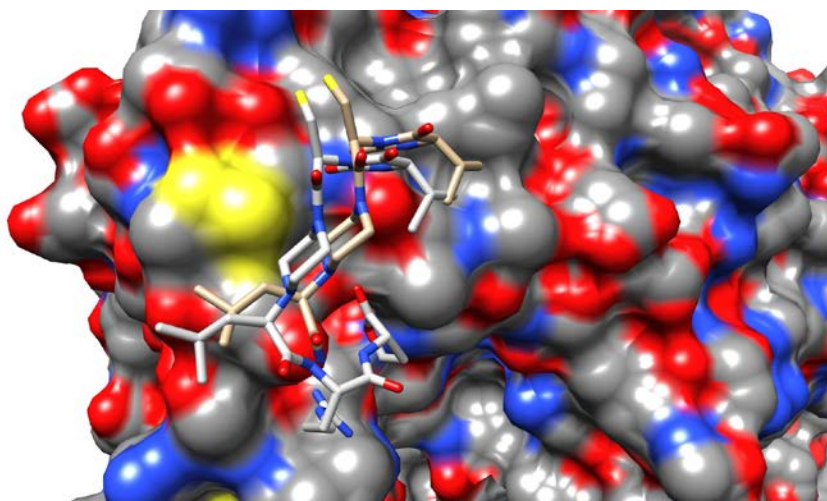
DDDL-1vclr



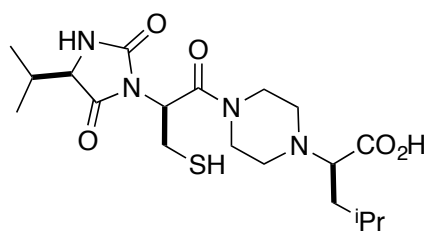
DDD-Avcl (gold), $\Delta G = -1.25$ kcal/mol



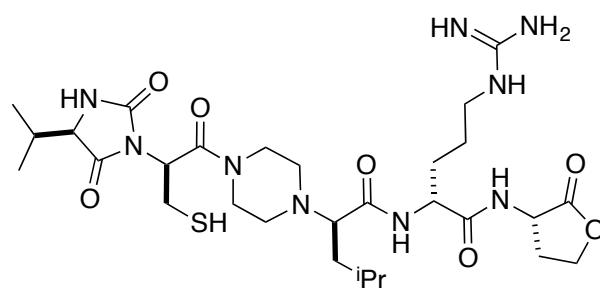
DDDL-1vclr (silver), $\Delta G = -4.88$ kcal/mol



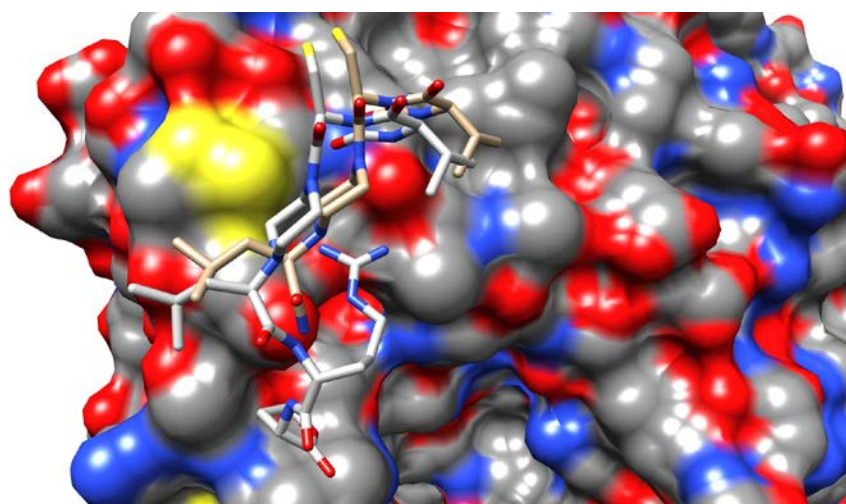
DDDD-1vclr



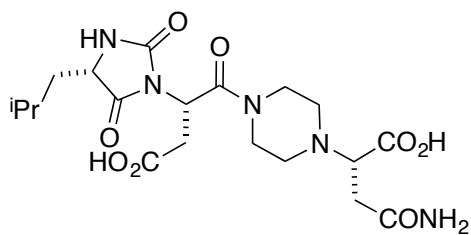
DDD-Avcl (gold), $\Delta G = -1.25$ kcal/mol



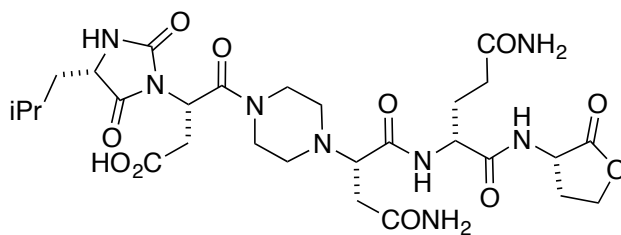
DDDD-1vclr (silver), $\Delta G = -4.46$ kcal/mol



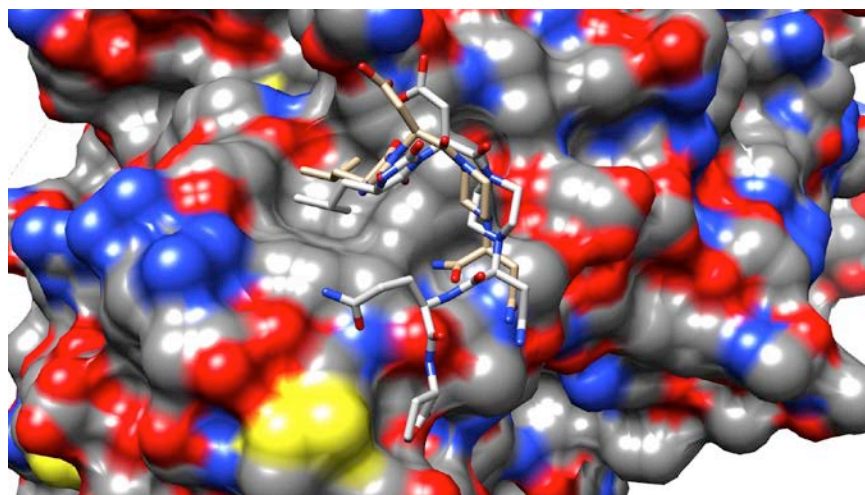
LLLD-1ldnq



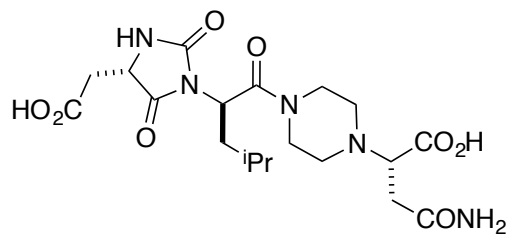
LLL-Aldn (gold), $\Delta G = -2.29$ kcal/mol



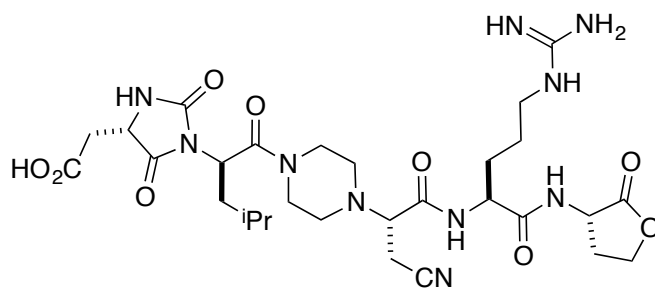
LLLD-1ldnq (silver), $\Delta G = -5.44$ kcal/mol



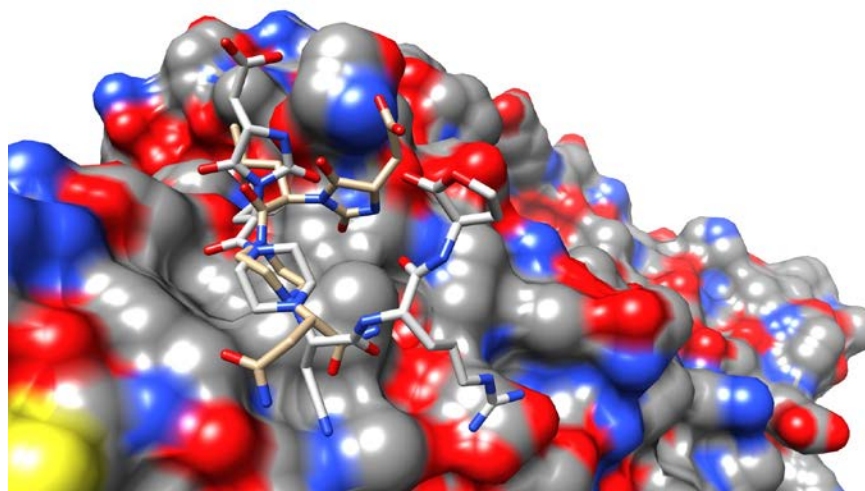
LDLL-1dl(CN)r



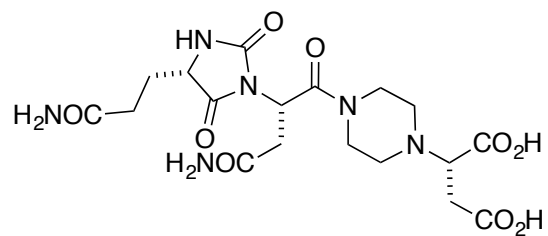
LDL-AdIn (gold), $\Delta G = -1.25$ kcal/mol



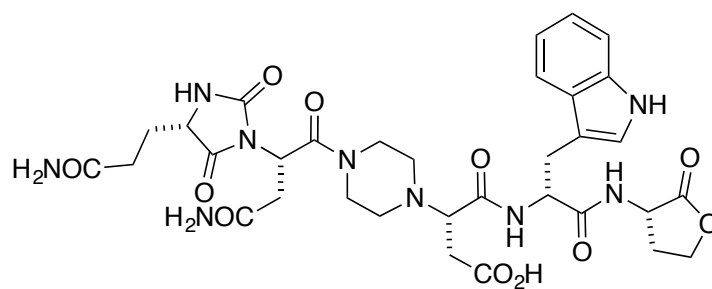
LDLL-1dl(CN)r (silver), $\Delta G = -2.67$ kcal/mol



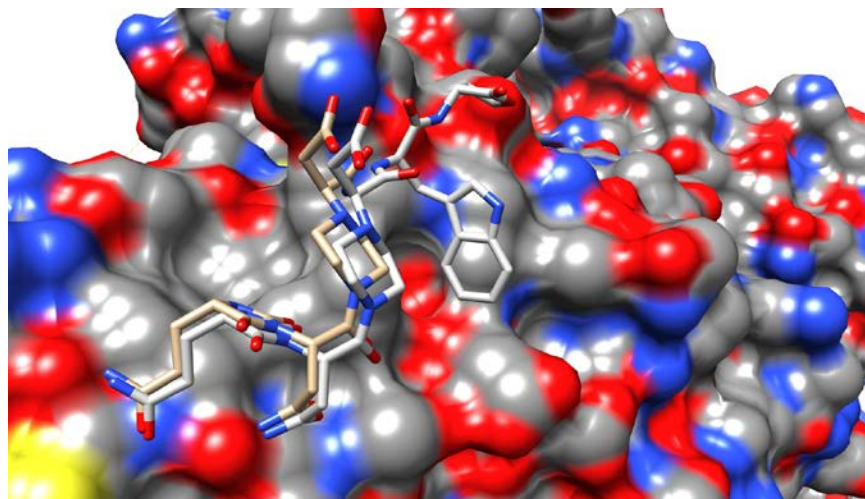
LLLD-1qndw



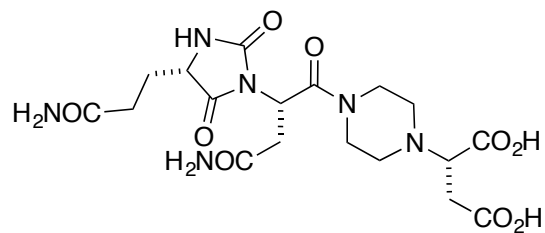
LLL-Aqnd (gold), $\Delta G = -3.17$ kcal/mol



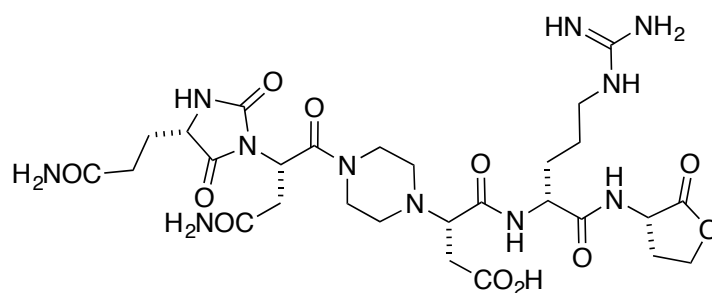
LLLD-1qndw (silver), $\Delta G = -3.63$ kcal/mol



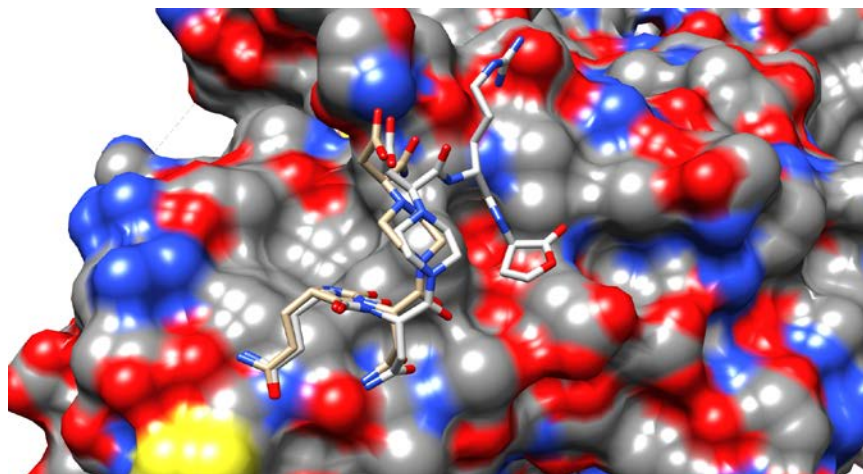
LLLD-1qndr



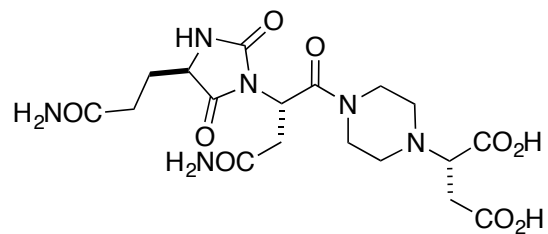
LLL-Aqnd (gold), $\Delta G = -3.71$ kcal/mol



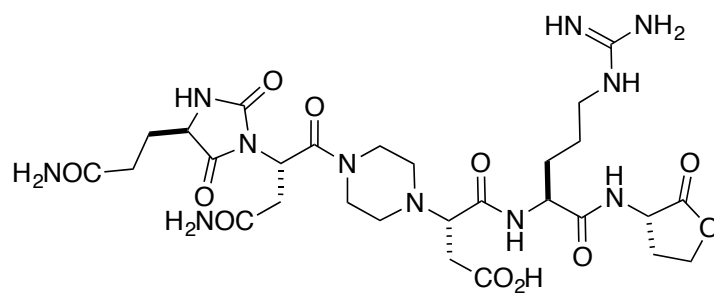
LLLD-1qndr (silver), $\Delta G = -6.15$ kcal/mol



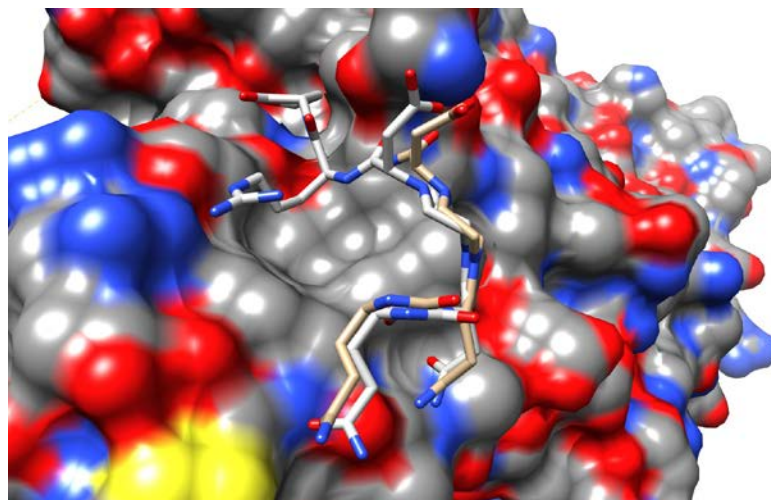
DLLL-1qndr



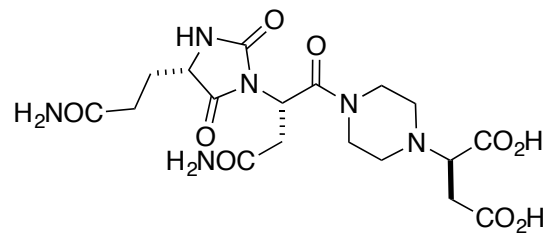
DLL-Aqnd (gold), $\Delta G = -3.15$ kcal/mol



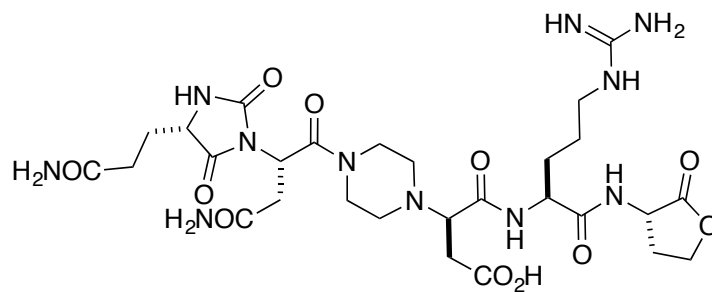
DLLL-1qndr (silver), $\Delta G = -4.77$ kcal/mol



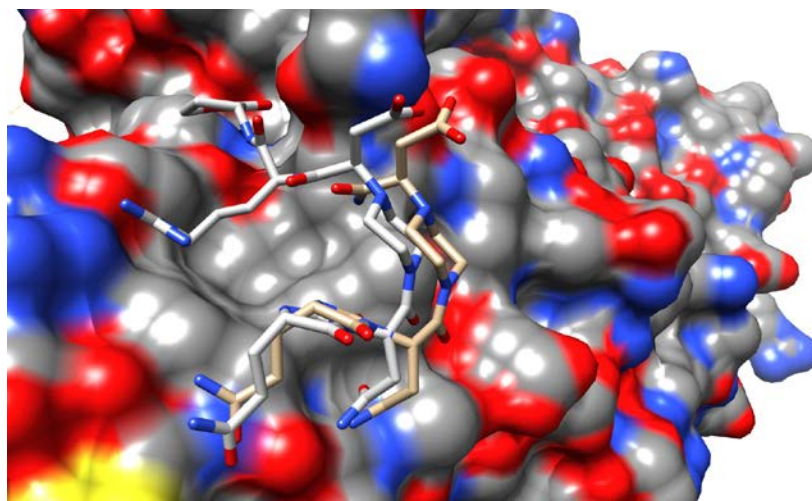
LLDL-1qndr



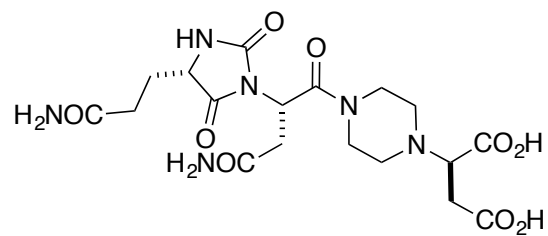
LLD-Aqnd (gold), $\Delta G = -2.39$ kcal/mol



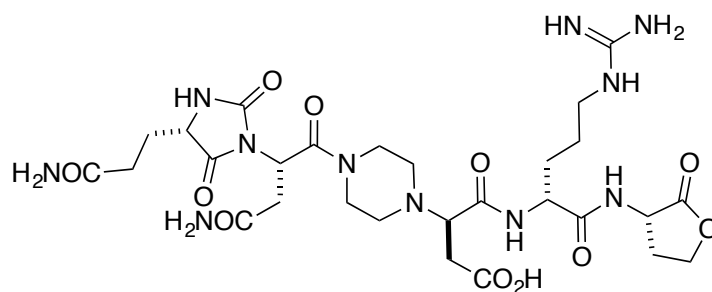
LLDL-1qndr (silver), $\Delta G = -5.21$ kcal/mol



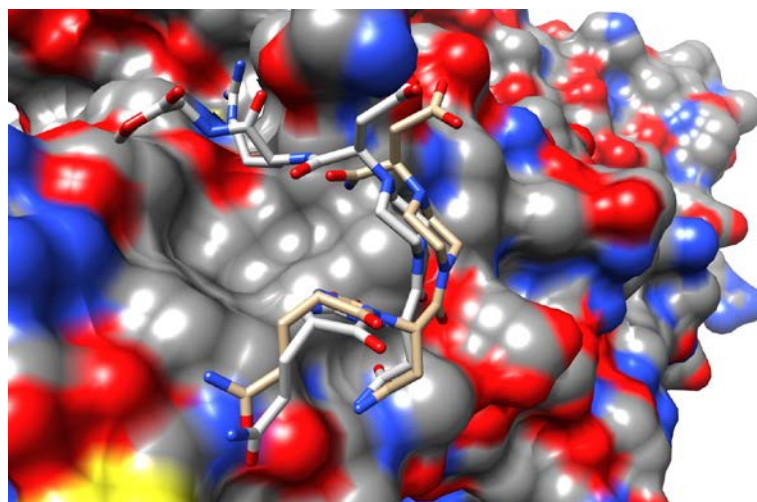
LLDD-1qndr



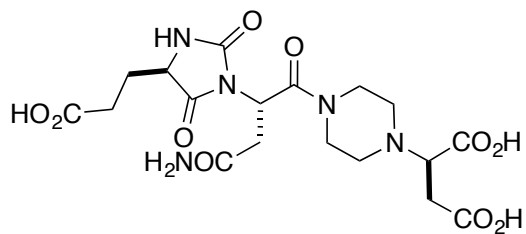
LLD-Aqnd (gold), $\Delta G = -2.39$ kcal/mol



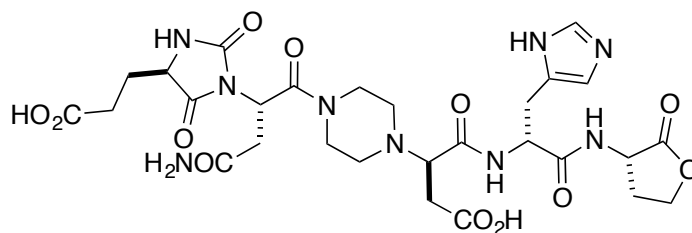
LLDD-1qndr (silver), $\Delta G = -5.37$ kcal/mol



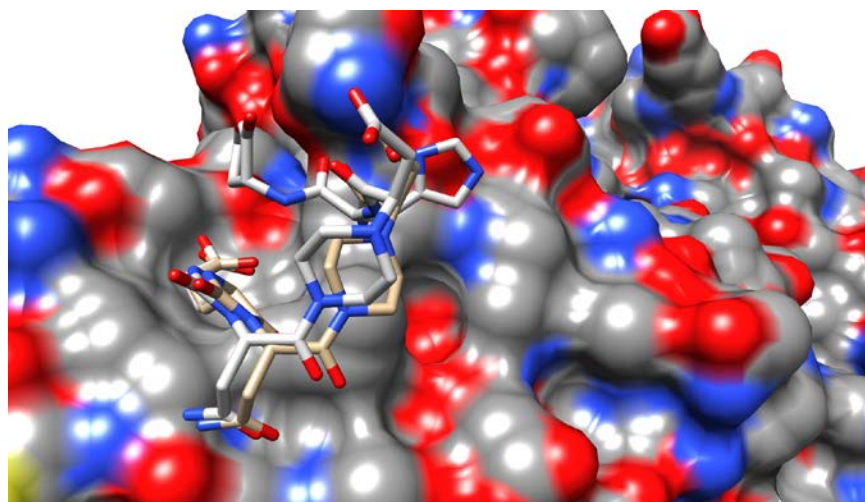
DLDD-1endh



DLD-Aend (gold), $\Delta G = -2.73$ kcal/mol



DLDD-1endh (silver), $\Delta G = -3.59$ kcal/mol



K. QikProp Calculation

QikProp^{11,12} from Schrödinger package (2015-4) was used to evaluate pharmaceutically properties of compounds **1** and **A**.

Table S3. QikProp calculations of compounds **1**

Compounds	QPlogP _{o/w}	PSA (Å ³)	QPPCaco (nm/s)	Rule of Five	Rule of Three
DLLD-1nclk	-2.01	278	0.031	3	2
DLLD-1nclr	-2.59	319	0.023	3	2
DDDL-1vclr	-0.38	257	0.62	3	2
DDDD-1vclr	-0.34	262	0.55	3	2
LDLL-1dlnr	-5.68	366	0.001	3	2
LDLL-1dl(CN)r	-4.37	338	0.006	3	2
LLLL-1qndw	-6.19	362	0.001	3	2
LLLD-1qndr	-8.54	421	0	3	2
DLLL-1qndr	-8.54	421	0	3	2
LLDL-1qndr	-8.54	421	0	3	2
LLDD-1qndr	-8.54	421	0	3	2
DLDL-1endr	-7.12	416	0	3	2
DLDD-1endh	-6.45	368	0	3	2
LLLD-1ldnq	-7.08	348	0.002	3	2
LLLL-1aaar	-2.38	270	0.27	3	2

Table S4. QikProp calculations of compounds **A**

Compounds	QPlogP _{o/w}	PSA (Å ³)	QPPCaco (nm/s)	Rule of Five	Rule of Three
DLL-Ancl	-2.30	188	0.51	1	2
DDD-Avcl	-0.04	137	10.80	0	1
LLL-Aldn	-3.93	238	0.017	1	2
LDL-Adln	-3.63	241	0.01	1	2
LLL-Acnd	-3.92	235	0.037	1	2

DLL-Acnd	-3.92	235	0.037	1	2
LLD-Acnd	-3.92	235	0.037	1	2
DLD-Acnd	-3.92	235	0.037	1	2

QlogP_{ow} – predicted octanol/water partition coefficient; PSA – Van der Waals surface area of polar nitrogen and oxygen atoms; QPPCaco – predicted Caco-2 cell permeability; Rule of Five – number of violations of Lipinski's rule of five; Rule of Three – number of violations of Jorgensen's rule of three.

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