Supporting Information

Solid-Phase Photochemical Decarboxylative Hydroalkylation of Peptides

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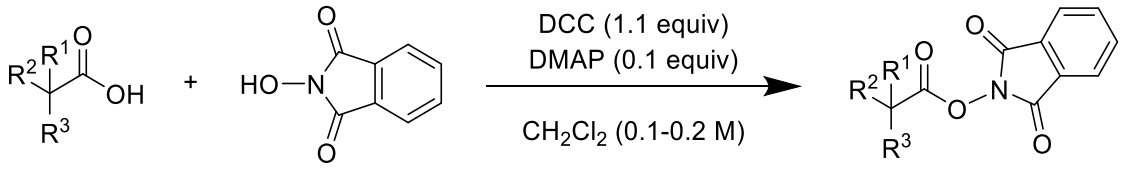
General Considerations

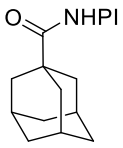
Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous material, unless otherwise stated. Reactions were monitored by LC/MS and thin layer chromatography (TLC). TLC was performed using 0.25 mm E. Merck silica plates (60F-254), using short-wave UV light as the visualizing agent, and bromocresol green and heat as developing agents. NMR spectra were recorded on Bruker DRX-600, DRX-500, and AMX-400 instruments and are calibrated using residual non-deuterated solvent ($CHCl_3$ at 7.26 ppm ^{1}H NMR, 77.16 ppm ^{13}C NMR; CH_3OH at 3.31 ppm ^{1}H NMR, 49.00 ppm ^{13}C NMR; $(CH_3)_2SO$ at 2.05 ppm ^{1}H NMR, 39.52 ppm ^{13}C NMR). The following abbreviations were used to explain multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Column chromatography was performed using E. Merck silica gel (60, particle size 0.043–0.063 mm). High-resolution mass spectra (HRMS) were recorded on an Agilent LC/MSD TOF mass spectrometer by electrospray ionization time of flight reflectron experiments. HPLC was performed using an Agilent Infinity II Analytical HPLC equipped with a Phenomenex Aeris C18 column (5 μM particle size, 100 A pore size, 250 x 4.6 mm for analytical (1 mL/min)) in an Agilent G7116A column heater; or Agilent 1260 II prep HPLC equipped with a Phenomenex Luna Omega C18 column (5 μM particle size, 250 x 21.2 mm (20 mL/min)).

General Procedure for the Synthesis of Redox Active Esters

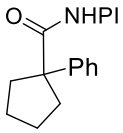


A round-bottom flask was charged with (if solid) the carboxylic acid (5.0 mmol, 1.0 equiv), *N*-hydroxyphthalimide (5.0 mmol, 1.0 equiv), DCC (5.0 mmol, 1.0 equiv) and DMAP (0.5 mmol, 0.1 equiv). The mixture was stirred vigorously in CH_2Cl_2 (0.1-0.2 M). The carboxylic acid (5.0 mmol, 1.0 equiv) was added dropwise (if liquid), and the mixture was stirred until the acid was consumed (determined by TLC). Total starting material consumption required 0.5 h to 12 h. The reaction mixture was filtered through a fritted funnel and rinsed with CH_2Cl_2/Et_2O. The solvent was removed in vacuo, and the corresponding redox-active ester was purified via reverse-phase chromatography.

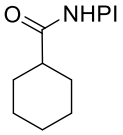
The following redox active esters are known compounds and were prepared from their corresponding carboxylic acids following previously reported procedures (NHPI = *N*-hydroxyphthalimide).[1-12]



Org. Lett. 2018, 20, 1338



Angew. Chem. Int. 2020, 59, 8907



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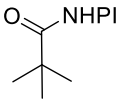
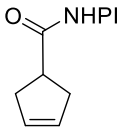


Org. Lett. 2019, 21, 8037



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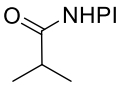

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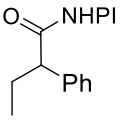

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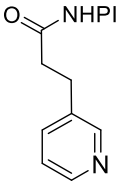
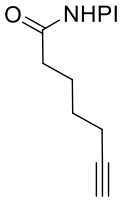
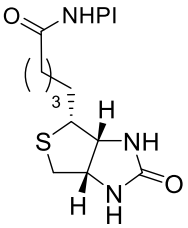


J. Am. Chem. Soc. 2020, 142, 20143



Chem. 2020, 11, 488



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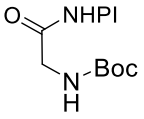
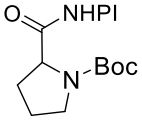
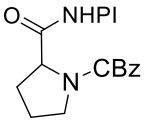


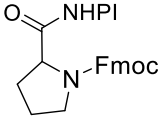
Nat. Catal. 2020, 3, 393



ACS Catal. 2019, 9, 9103



Chem. Sci., 2020, 11, 4939



PNAS. 2018, 115, E6404

General Procedure for Loading Resin

Resin loading was conducted using the manufacturer's loading on label in a fritted plastic syringe with a stir-bar. RA Chem-Matrix resin (0.47 mmol/g) was swelled in NMP for 20 min, while RA polystyrene LL resin (0.34 mmol/g) was swelled in DMF for 20 min, Fmoc-deprotected, washed 3 times with DMF, then allowed to equilibrate in NMP.

Amino acid Fmoc-(4-acrylamide)Phe-COOH was coupled at 3 equivalents to resin loading. The Fmoc-amino acid was combined with COMU (3 equivalents to resin loading) and dissolved in NMP (0.2 M). To the solution was added TMP (4.5 equivalents to resin loading); the solution was vortexed and allowed to sit for 5 min. The reaction solution was then added to the swelled drained resin and allowed to react for 60 min. The resin was then wash 3 times with DMF

Amino acids Fmoc-Gly-COOH was coupled at 4 equivalents to resin loading. The Fmoc-amino acid was combined with COMU (4 equivalents to resin loading) and dissolved in NMP (0.2 M). To the solution was added TMP (6 equivalents to resin loading); the solution was vortexed and allowed to sit for 5 min. The reaction solution was then added to the swelled drained resin and allowed to react for 60 min. The resin was then wash 3 times with DMF.

Peptide Elongation (0.1 mmol resin scale)

Commercially available Fmoc-Xaa-OH (0.5 mmol, 5 equiv) were combined with 1-[(1-(Cyano-2-ethoxy-2-oxoethylideneaminooxy) dimethylaminomorpholino)] uronium hexafluorophosphate (COMU) (0.5 mmol, 5 equiv) in a glass vial and dissolved in NMP (2 mL). TMP (0.75 mmol, 7.5 equiv) was added to the solution and allowed to react for 5 min, then added to the resin and allowed to react for >30 min at rt.

Synthesized Fmoc-Xaa-OH (0.03 mmol, 3 equiv) were combined with COMU (0.03 mmol, 3 equiv) in a glass vial and dissolved in NMP (2 mL). TMP (0.45 mmol, 4.5 equiv) was added to the solution and allowed to react for 5 min then added to the resin and allowed to react for >30 min at rt.

Collagen model peptide was assembled using Fmoc-Pro-Hyp(OtBu)-Gly-OH building blocks as previously described.[13]

Fmoc-Deprotection

The resin was then washed with DMF (3x) and deprotected (3 x 2 mL) with a solution of 1% HOBT (w/v), 2% DBU (v/v), in DMF at rt for 2 min. The resin was then washed with DMF (3x).

N-Terminal Acylation

To resin swelled in DMF and drained was added a 3 mL solution of 5% acetic anhydride in pyridine and mixed for 5 min. The resin was drained, then acylation was repeated two additional times.

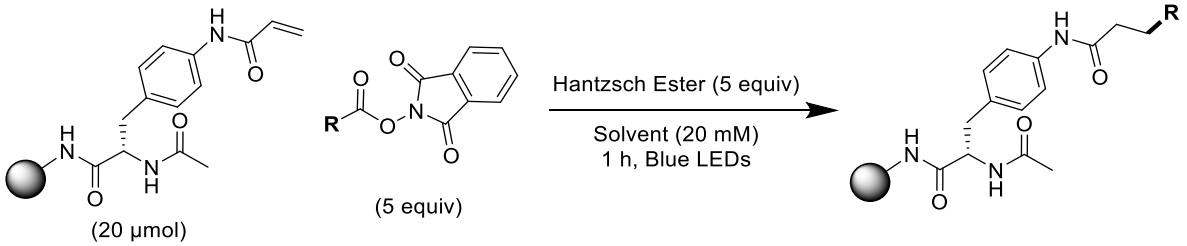
Trityl Deprotection

Resin containing trityl-protected peptide was swelled in CH_2Cl_2 for 30 min or washed in CH_2Cl_2 for 5 min, then drained. The resin was then washed with a 2.5 mL-solution of 3% TFA, 5% TIPS in CH_2Cl_2 for 45 sec and drained (solution turns yellow, then clear). This was repeated 3 times. After the third treatment, the resin was washed with CH_2Cl_2, then treated a fourth time to ensure no yellow color was observed.[14]

General Procedure for Determination of Resin Loading

After loading the resin following the **General Procedure for Loading Resin**, a 10-mg portion resin was cleaved following the **General Procedure for Peptide Cleavage and Work-up**. To the cleaved residue was added 200 µL of a stock solution of 2,3,5,6-tetrachloronitrobenzene (0.02 M in MeCN). The solvent was removed via genevac then dissolved in the appropriate deuterated solvent for NMR analysis. The moles of starting material were calculated based on relative integrations of the aromatic peak of the 2,3,5,6-tetrachloronitrobenzene and the baseline-resolution peaks in the starting material. The loading (mmol/mg) was then calculated by dividing the moles of starting material by the mass of cleaved resin.

General Procedure for Solid Phase Hydroalkylation



To a 1-mL glass shell vial equipped with a tumble stirrer bar was added resin (20 µmol, 1 equiv), diethyl 1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate (100 µmol, 5 equiv) and the appropriate redox active ester (100 µmol, 5 equiv) in DMF (20 mM), unless otherwise stated. The mixture was then stirred for 1 h under blue LED irradiation (470 nm). A 24-well photoredox Para-dox® aluminum reactor block assembly was used to suspend the reaction vessel ~2 mm above the Lumidox® 24-well blue LED array. Upon completion, the resin was rinsed with DMF (5 x 2) then CH_2Cl_2 (5 x 2 mL) and dried under vacuum. The substrate was cleaved following the **General Procedure for Peptide Cleavage and Work-up**. To the cleaved residue was added 200 µL of a stock solution of 2,3,5,6-tetrachloronitrobenzene (0.02 M in MeCN). NMR yields were calculated based on relative integrations of the aromatic peak of the 2,3,5,6-tetrachloronitrobenzene and the aromatic phenylalanine peaks in the starting materials and products. The product was then isolated via analytical HPLC and characterized via ^{1}H NMR and HRMS.

General Procedure for Peptide Cleavage and Work-up

Cleavage: The resin was stirred in a fritted syringe with 1 mL mixture of TFA, triisopropylsilane (TIPS), and H_2O (85:5:10 v/v) for 1 h. The TFA solution was drained and collected. The resin was then washed with 1:1 CH_2Cl_2/MeOH (3 x 1 mL), the rinsate was collected.

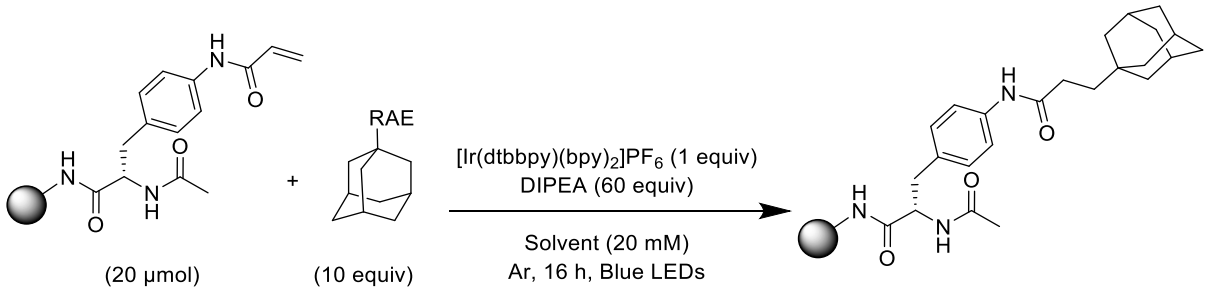
Work-up: The combined cleavage solution and CH_2Cl_2/MeOH washes were concentrated under a stream of argon. The residue was used to evaluate reaction progress via LC-MS or generate crude yields by ^1H NMR. The residue was also dissolved in H_2O/MeCN containing 0.1% TFA, filtered, and purified by reverse-phase HPLC to generate NMR spectra for the scope table.

Optimization and Control Studies

General Optimization Procedure A: To a 1-mL glass shell vial equipped with a tumble stirrer bar was added resin (20 µmol, 1 equiv), the appropriate photocatalyst (20 µmol, 1 equiv), an amine base (120 µmol, 60 equiv), and the appropriate redox active ester (100 µmol, 5 equiv) in DMF (20 mM). The mixture was stirred for 16 h under argon and blue LED irradiation, unless otherwise stated. All reactions were carried out using 24 well reactor blocks. Upon completion, the resin was rinsed with DMF (5 x 2 mL during optimization and scope; 10 x 3 mL during scale-up), then CH_2Cl_2 (5 x 2 mL). The resin was then dried under vacuum, and the peptide was cleaved. To the cleaved residue was added 200 µL of a stock solution of internal standard (methyl 5-bromopicolinate, 0.02 M in MeCN). Reaction yields and conversions were evaluated based on product-to-internal standard ratio (P/IS). These ratios were determined by comparison of the corrected peak areas of the desired product against methyl 5-bromopicolinate.

General Optimization Procedure B: To a 1-mL glass shell vial equipped with a tumble stirrer bar was added resin (20 µmol, 1 equiv), diethyl 1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate (100 µmol, 5 equiv), and the appropriate redox active ester (100 µmol, 5 equiv) in DMF (20 mM). The mixture was then stirred for 1 h under blue LED irradiation, unless otherwise stated. All reactions were carried out using 24 well reactor blocks. Upon completion, the resin was rinsed with DMF (5 x 2 mL during optimization and scope; 10 x 3 mL during scale-up), then CH_2Cl_2 (5 x 2 mL during optimization and scope; 10 x 3 mL during scale-up). The resin was then dried under vacuum, and the peptide was cleaved. To the cleaved residue was added 200 µL of a stock solution of 2,3,5,6-tetrachloronitrobenzene (0.02 M in MeCN). NMR yields were calculated based on relative integrations of the aromatic peak of the 2,3,5,6-tetrachloronitrobenzene and the baseline-resolution peaks in the starting materials and products.

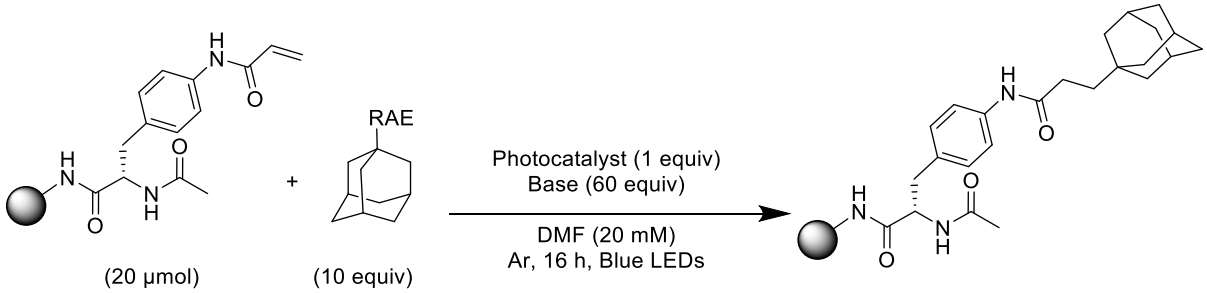
Table S1: Solvent Screen for Solid-Phase Hydroalkylation[a]



Solvent	P/IS	P/SM (%)
DMF	0.40	94
DMSO	0.32	74
DMA	0.38	91
MeCN	0.36	84
DME	0.25	84
THF	0.24	89

[a]Performed according to **General Optimization Procedure A**

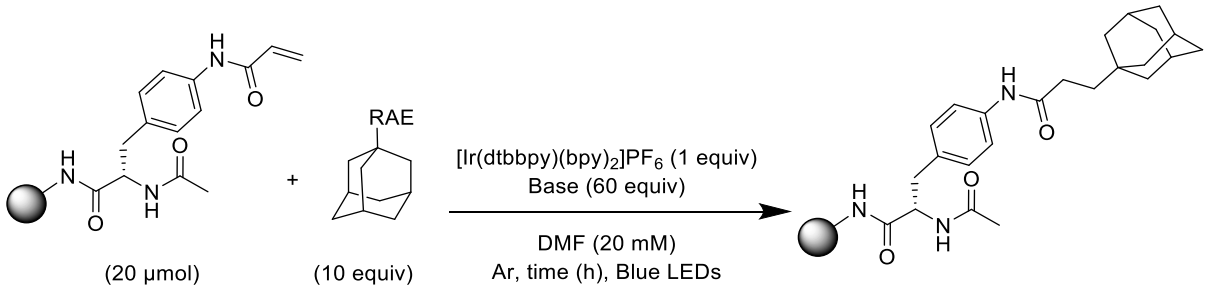
Table S2: Photocatalyst and Base Screen for Solid-Phase Hydroalkylation[a]



Photocatalyst	Base	P/IS	P/SM (%)
$[Ir(dtbbpy)(bpy)_2]PF_6$	DIPEA	0.38	93
$[Ir(dtbbpy)(dtbpy)_2]PF_6$	DIPEA	0.31	88
1,2,3,5-Tetrakis(carbazol-9-yl)-4,6-dicyanobenzene	DIPEA	0.14	59
2,4,5,6-tetrakis(3,6-dichloro-9H-carbazol-9-yl)isophthalonitrile	DIPEA	0.10	31
EosinY	DIPEA	0.19	92
$Ru(bpy)_3(PF_6)_2$	DIPEA	0.26	30
$[Ir(dtbbpy)(bpy)_2]PF_6$	TEA	0.33	79
$[Ir(dtbbpy)(bpy)_2]PF_6$	DIPA	0.31	92
$[Ir(dtbbpy)(bpy)_2]PF_6$	TMEDA	0.36	80

[a]Performed according to **General Optimization Procedure A**

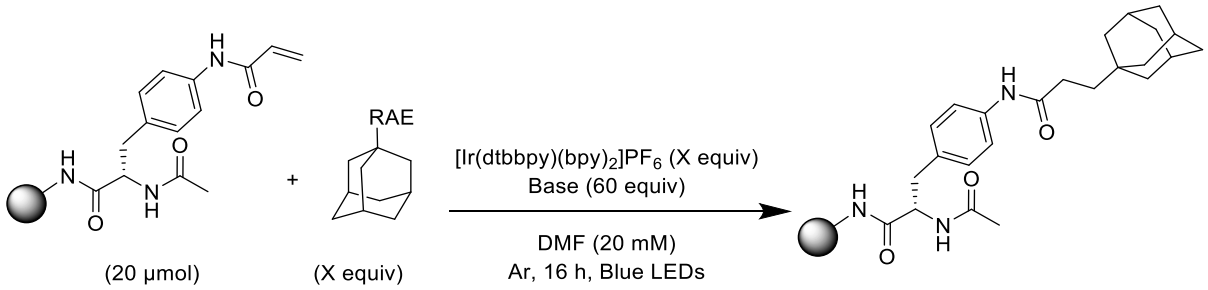
Table S3: Reaction Time Variation for Solid-Phase Hydroalkylation[a]



Reaction Time (h)	P/IS	P/SM (%)
16	0.38	94
8	0.17	37
4	0.14	24
2	0.07	10
1	0.04	5

[a]Performed according to **General Optimization Procedure A**

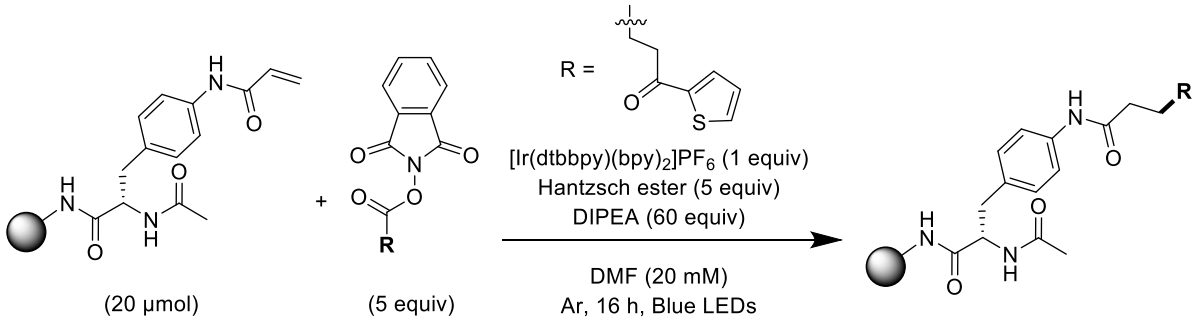
Table S4: Stoichiometry Variation for Solid-Phase Hydroalkylation[a]



Photocatalyst Equiv	Radical Precursor Equiv	P/IS	P/SM (%)
1	15	0.32	93
0.5	15	0.26	59
0.25	15	0.20	49
1	10	0.36	95
1	5	0.40	100

[a]Performed according to **General Optimization Procedure A**

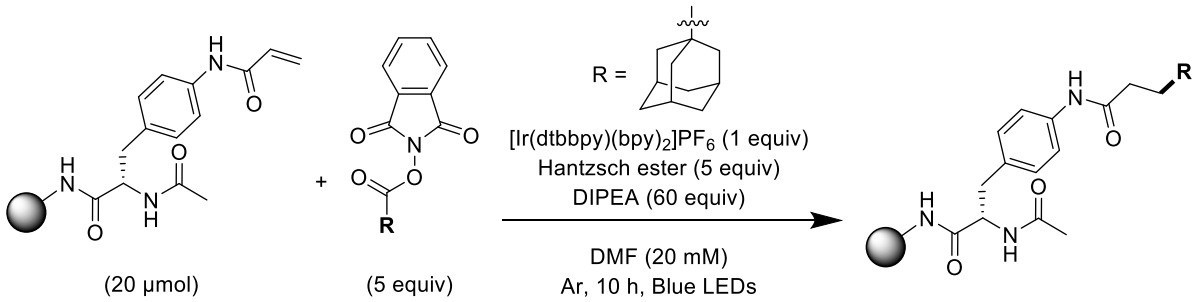
Table S5: Effects of Hantzsch Ester on Primary Redox-Active Esters[a]



Condition	P/IS
PC (1 equiv) + Hantzsch ester (0 equiv)	0.32
PC (1 equiv) + Hantzsch ester (5 equiv)	0.76
PC (1 equiv) + Hantzsch ester (20 equiv)	0.70

[a]Performed according to **General Optimization Procedure A.**

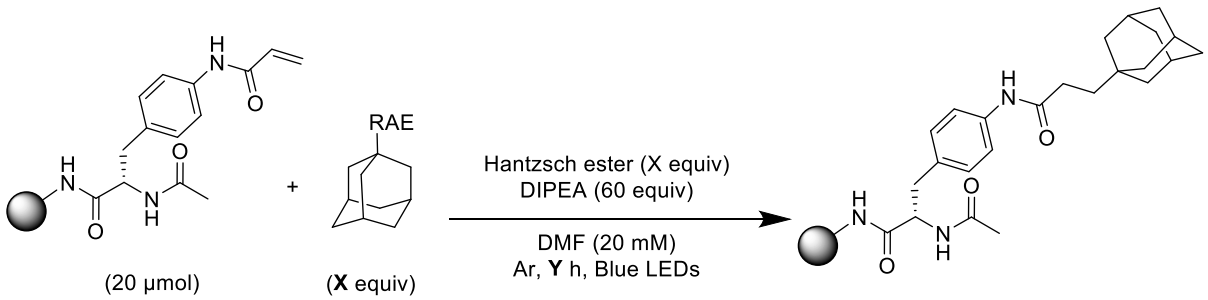
Table S6: Performance of Hantzsch Ester without Photocatalyst[a]



Condition	NMR Conversion (%)	NMR Yield (%)
Hantzsch ester only (5 equiv)	100	94
Photocatalyst only (1 equiv)	62	52
Hantzsch ester (5 equiv) + Photocatalyst (1 equiv)	87	80

[a]Performed according to **General Optimization Procedure B.**

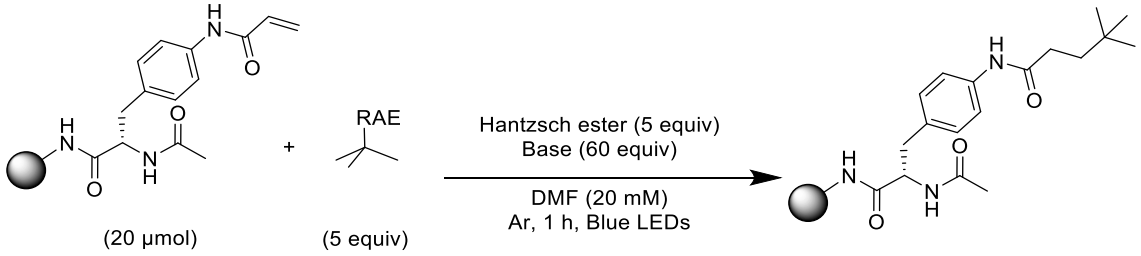
Table S7: Stoichiometry and Reaction Time Variation for Solid-Phase Hydroalkylation[a]



RAE and HE equiv	Reaction Time (h)	NMR Conversion (%)	NMR Yield (%)
5	16	100	90
10	16	100	72
20	16	100	70
5	8	100	94
5	4	100	93
5	2	100	94
5	1	100	95
5	0.5	100	95
5	0.25	72	59

[a]Performed according to **General Optimization Procedure B**

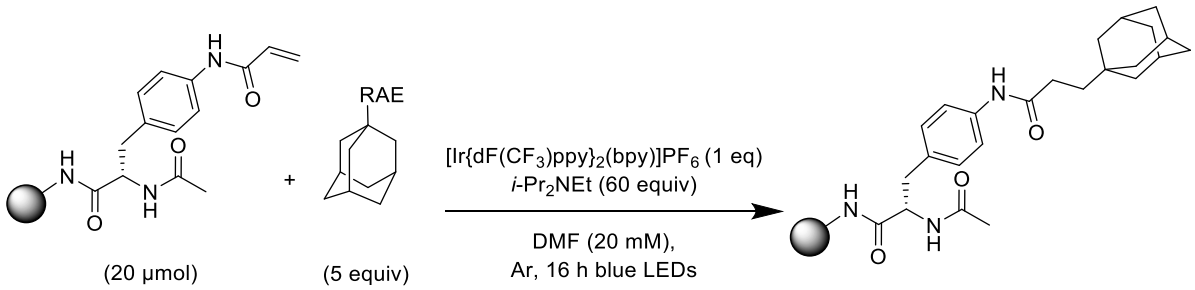
Table S8: Control Studies for Solid-Phase Hydroalkylation[a]



Deviation from initial conditions	NMR Conversion (%)	NMR Yield (%)
none	100	90
Excluding Hantzsch ester	0	0
Excluding base	100	88
Under air	100	89
Without irradiation	0	0
Excluding base, under air	100	90
10 equiv HE and RAE	100	88
2.5 equiv HE and RAE	35	-
2.5 equiv HE and RAE	59	-
2.5 equiv HE and RAE	43	-

[a]Performed according to **General Optimization Procedure B**

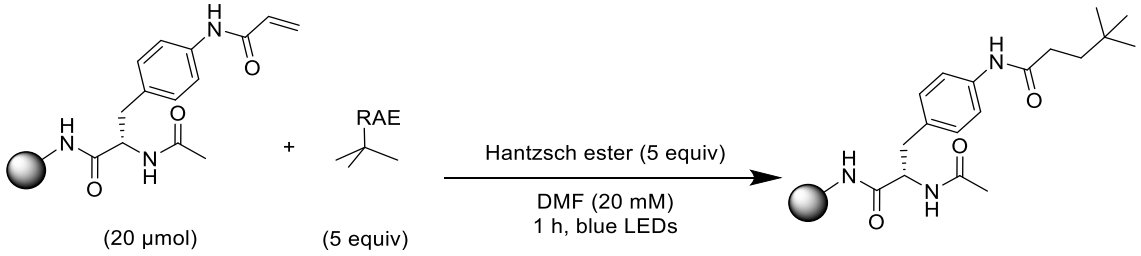
Table S9: Rink Amide Chem-Matrix vs Rink Amide Polystyrene Resin[a]



Resin (rink amide)	LC-MS Conversion (%)	P/IS
Chem-Matrix	100	0.40
Polystyrene	100	0.40

[a]Performed according to **General Optimization Procedure A**

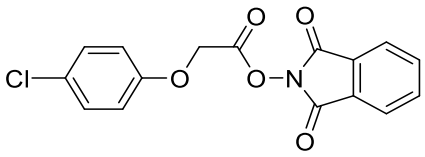
Table S10: Rink Amide Chem-Matrix vs Rink Amide Polystyrene Resin[a]



Resin (rink amide)	LC-MS Conversion (%)	P/IS
Chem-Matrix	100	0.40
Polystyrene	100	0.40

[a]Performed according to **General Optimization Procedure A**

Redox-Active Esters: Experimental Procedures and Characterization Data



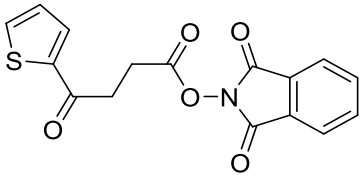
1,3-Dioxoisoindolin-2-yl 2-(4-chlorophenoxy)acetate (S1)

Following the general procedure with 2-(4-chlorophenoxy)acetic acid (560 mg, 3.00 mmol, 1.0 equiv) and purification by silica plug afforded 1,3-dioxoisoindolin-2-yl 2-(4-chlorophenoxy)acetate as a yellow solid (615 mg, 62%). mp = 105.5-106.5 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 (m, 2H), 7.81 (m, 2H), 7.30 (d, *J* = 9.0 Hz, 2H), 6.93 (d, *J* = 8.9 Hz, 2H), 5.02 (s, 2H)

^{13}C NMR (101 MHz, $CDCl_3$): δ 165.3, 161.5, 155.9, 135.0, 129.7, 128.8, 127.6, 124.2, 116.2, 63.7.

HRMS (ESI+, *m/z*): calc'd for $C_{16}H_{10}ClNO_5$ $[M]^+$ 331.0248; found 331.0241.



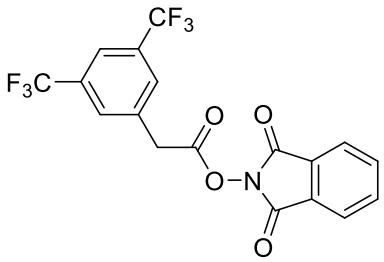
1,3-Dioxoisoindolin-2-yl 4-oxo-4-(thiophen-2-yl)butanoate (S2)

Following the general procedure with 4-oxo-4-(thiophen-2-yl)butanoic acid (300 mg, 1.63 mmol, 1.0 equiv) and purification by flash chromatography (C18, 30-50% MeCN/H_2O) afforded 1,3-dioxoisoindolin-2-yl 4-oxo-4-(thiophen-2-yl)butanoate as a white solid (381 mg, 71.0%). mp = 121.0-122.0 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.91 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.81 (br, 3H), 7.69 (d, *J* = 4.9 Hz, 1H), 7.17 (t, *J* = 4.4 Hz, 1H), 3.43 (t, *J* = 7.0 Hz, 2H), 3.18 (t, *J* = 7.0 Hz, 2H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 189.3, 169.2, 161.8, 143.1, 134.8, 134.1, 132.3, 128.9, 128.2, 124.0, 33.6, 25.4.

HRMS (ESI+, *m/z*): calc'd for $C_{16}H_{11}NO_5NaS$ $[M+Na]^+$ 352.0256; found 352.0248.



1,3-Dioxoisoindolin-2-yl 2-(3,5-bis(trifluoromethyl)phenyl)acetate (S3)

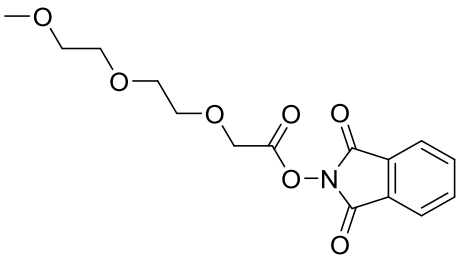
Following the general procedure with 2-(3,5-bis(trifluoromethyl)phenyl)acetic acid (1.361 g, 5.00 mmol, 1.0 equiv) and purification by flash chromatography (C18, 50-80% MeCN/H_2O) afforded 1,3-dioxoisoindolin-2-yl 2-(3,5-bis(trifluoromethyl)phenyl)acetate as a white solid (1.544 g, 61%). mp = 143.0-146.0 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.90 (br, 3H), 7.84 (dd, *J* = 5.5, 3.1 Hz, 2H), 4.17 (s, 2H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 166.5, 161.6, 135.0, 133.9, 132.4, 132.1, 129.7, 128.8, 124.2, 122.1, 37.1.

^{19}F NMR (376 MHz, $CDCl_3$): δ -63.89, (calibrated using TFA at -76.55 ppm).

HRMS (ESI+, *m/z*): calc'd for $C_{18}H_9F_6NO_4$ $[M]^+$ 417.0436; found 417.0434.



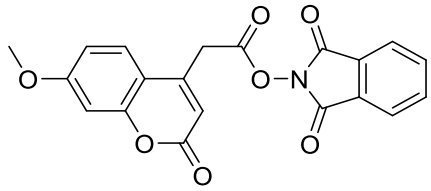
1,3-Dioxoisoindolin-2-yl 2-(2-(2-methoxyethoxy)ethoxy)acetate (S4)

Following the general procedure with 2-(2-(2-methoxyethoxy)ethoxy)acetic acid (5.000 g, 28.1 mmol, 1.0 equiv) and purification by flash chromatography (silica gel, linear gradient of 30-70% EtOAc/Hexane) afforded 1,3-dioxoisoindolin-2-yl 2,5,8,11-tetraoxatridecan-13-oate as a clear oil (371 mg, 4.1%).

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.91 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.82 (m, 2H), 4.61 (s, 2H), 3.87 (m, 2H), 3.75 (m, 2H), 3.69 (m, 2H), 3.59 (m, 2H), 3.41 (s, 3H).

^{13}C NMR (151 MHz, $CDCl_3$): δ 166.9, 161.6, 134.9, 128.9, 124.1, 71.9, 71.4, 70.6, 70.6, 66.6, 59.1.

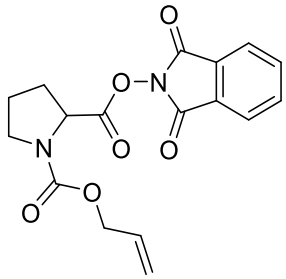
HRMS (ESI+, *m/z*): calc'd for $C_{15}H_{18}NO_7$ $[M+H]^+$ 346.0903; found 346.0907.



1,3-Dioxoisoindolin-2-yl 2-(7-methoxy-2-oxo-2H-chromen-4-yl)acetate (S5)

Following the general procedure with 2-(7-methoxy-2-oxo-2H-chromen-4-yl)acetic acid (1.171 g, 5.00 mmol, 1.0 equiv) and purification by silica plug afforded 1,3-dioxoisoindolin-2-yl 2-(7-methoxy-2-oxo-2H-chromen-4-yl)acetate as a crude tan oil (1.214 g, 64%). Reagent identity confirmed by crude ^{1}H NMR, LC-MS and HRMS.

HRMS (ESI+, *m/z*): calc'd for $C_{20}H_{13}NO_7$ $[M+H]^+$ 380.0770; found 380.0761.



1-Allyl 2-(1,3-dioxoisoindolin-2-yl) pyrrolidine-1,2-dicarboxylate (S6)

Following the general procedure with ((allyloxy)carbonyl)proline (996 mg, 5.00 mmol, 1.0 equiv) and purification by flash chromatography (C18, 40-80% MeCN/H_2O) afforded 1-allyl 2-(1,3-dioxoisoindolin-2-yl) pyrrolidine-1,2-dicarboxylate as an orange oil (1.308 g, 76%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 – 7.74 (m, 4H), 5.97 (tdt, *J* = 16.3, 10.7, 5.5 Hz, 1H), 5.34 – 5.22 (m, 2H), 4.79 – 4.55 (m, 3H), 3.72 – 3.47 (m, 2H), 2.61 – 2.27 (m, 2H), 2.14 – 1.93 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 169.3, 161.6, 134.9, 132.6, 128.9, 124.1, 117.9, 66.6, 57.0, 46.8, 35.0, 31.4, 23.6.

HRMS (ESI+, *m/z*): calc'd for $C_{17}H_{16}N_2O_6$ $[M+Na]^+$ 367.0906; found 367.0900.

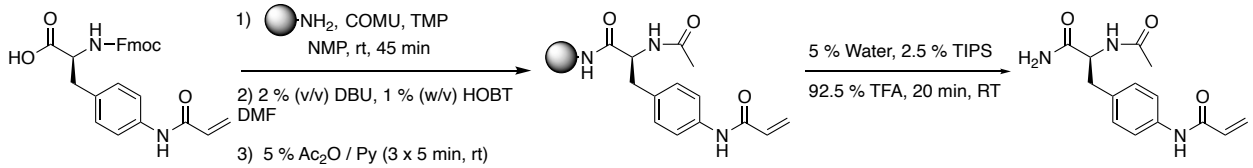
Starting Material Assembly on Resin

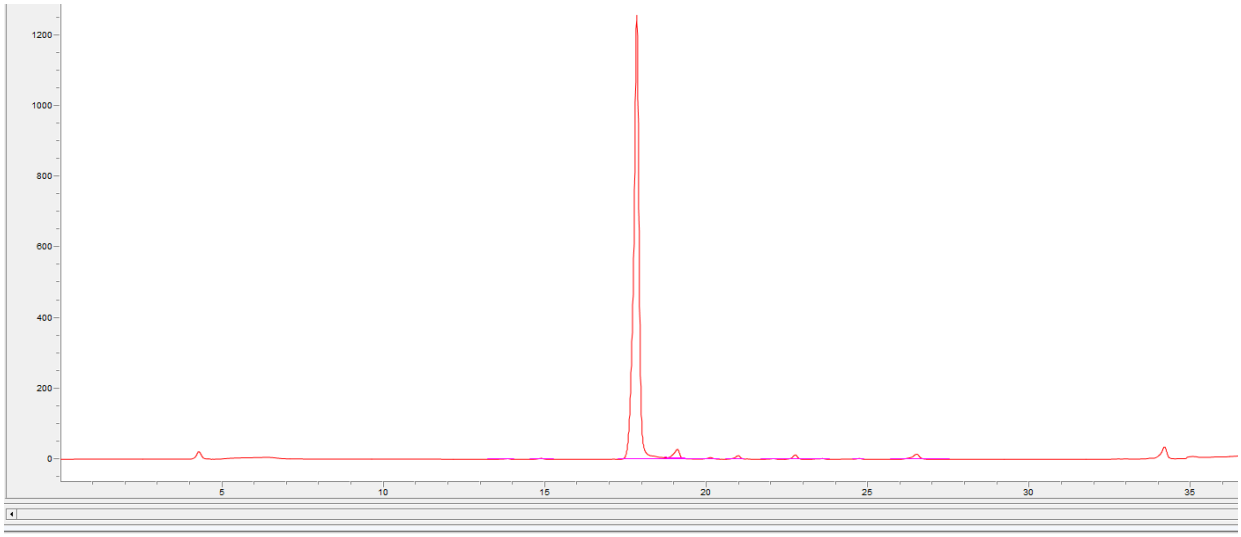
4-Amino-Phe Acrylation

Resin containing 4-amino-Phe was swelled for 30 min or washed for 10 min in NMP. COMU (5 equiv) was dissolved in NMP (0.052 M (resin loading)) followed by acrylic acid (5 equiv), then TMP (7.5 equiv). The mixture was allowed to react for 5 min, then was added to the filtered resin and allowed to react for 45 min at rt. The resin was then drained and washed with DMF (3x).

Characterization of Giese Acceptor

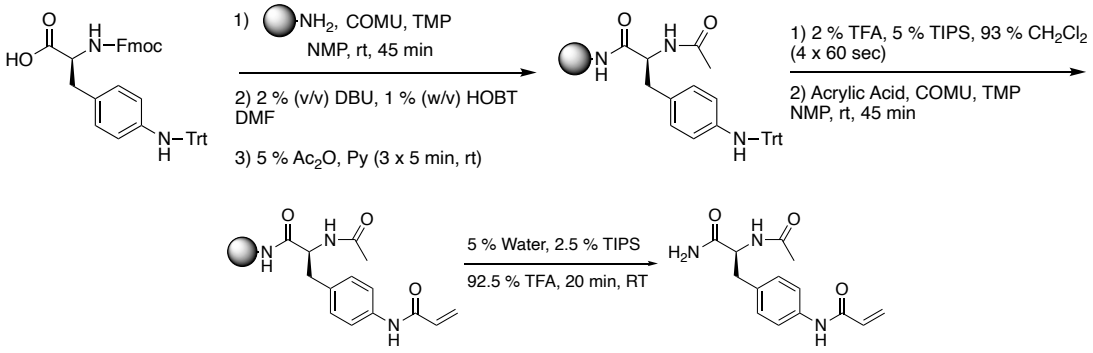
Method A

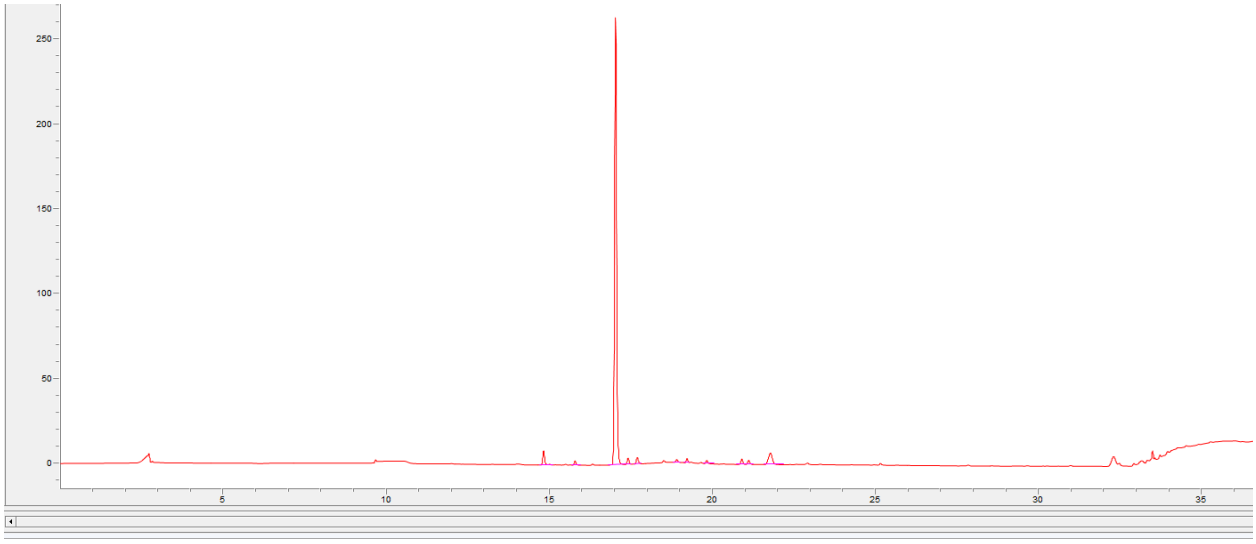




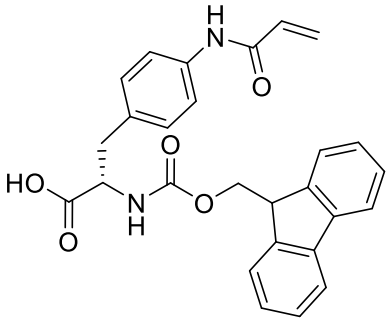
Reinjection of above crude product using a gradient of 2-40% MeCN/H_2O over 30 min, data collected at 254 nm.

Method B

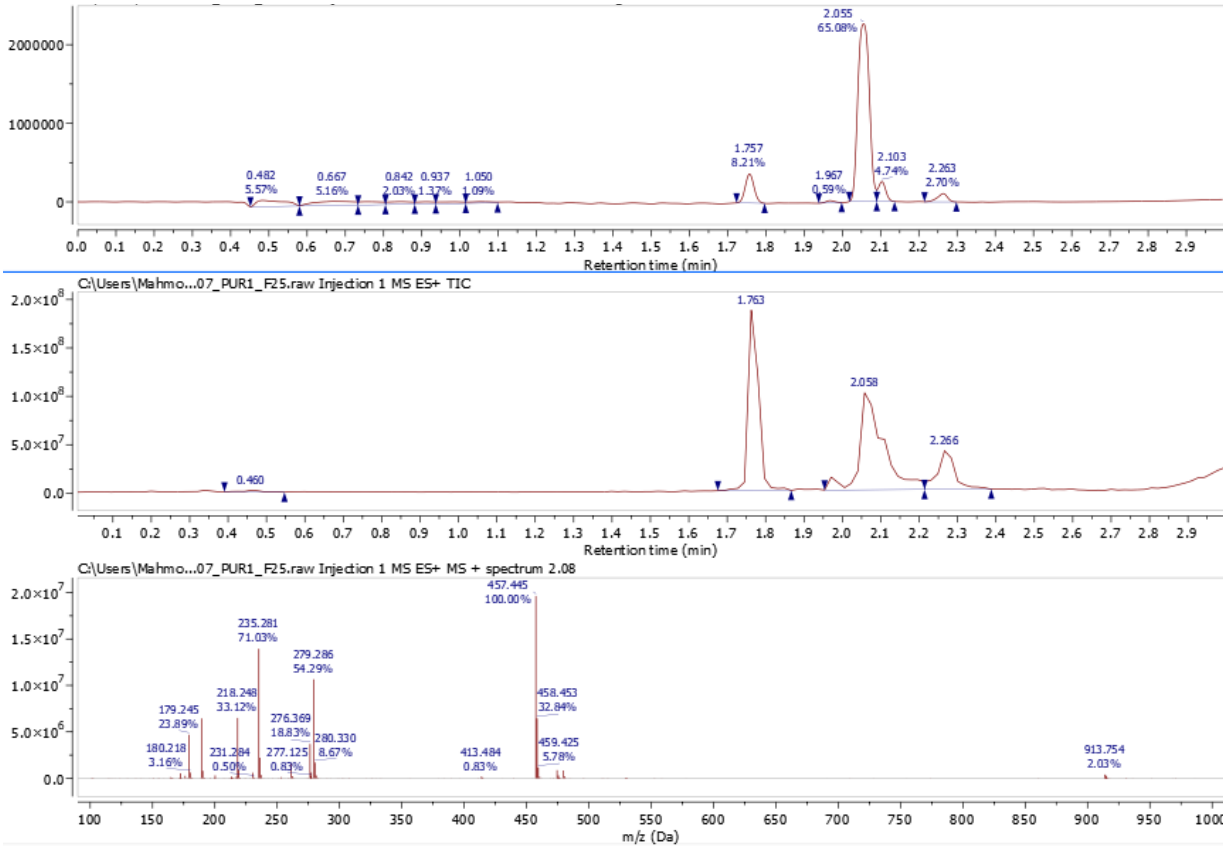




Reinjection of above crude product using a gradient of 2-40% MeCN/H_2O over 30 min, data collected at 254 nm.

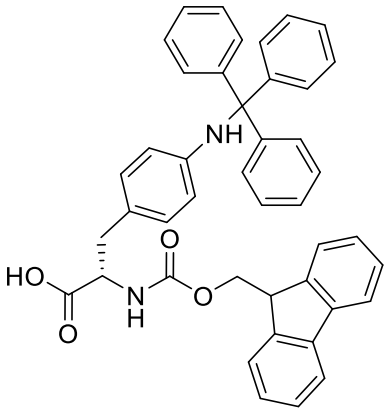


(*R*)-2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-acrylamidophenyl)propanoic acid (S7)

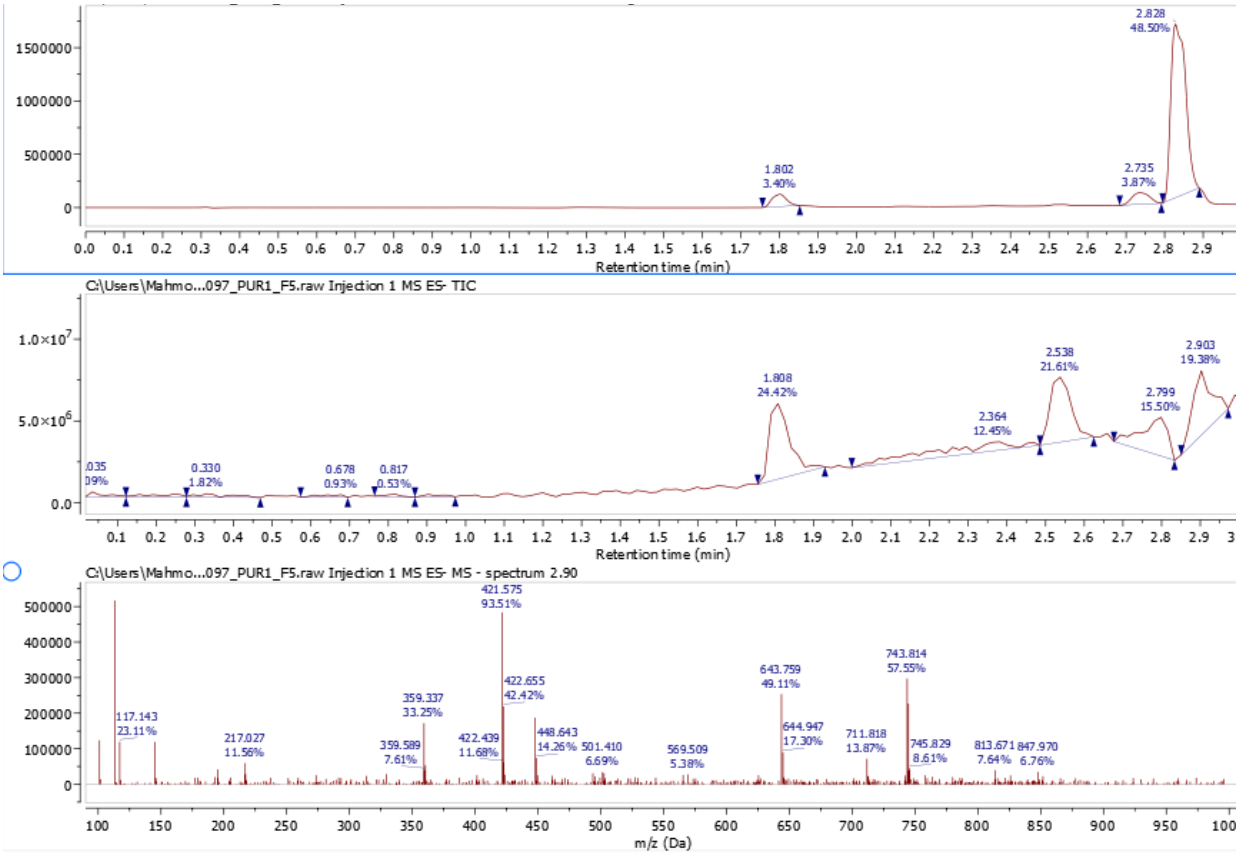


LC-MS of crude material using a gradient of 5-95% MeCN/H_2O over 2 min, data collected at 254 nm. The crude material was loaded onto resin.

HRMS (ESI+, *m/z*): calc'd for $C_{27}H_{24}N_2NaO_5$ $[M+Na]^+$ 479.1583; found 479.1588.



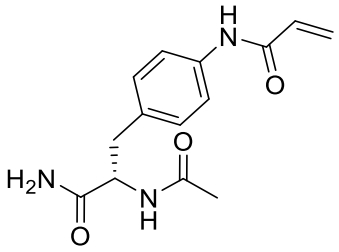
(R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-(tritylamino)phenyl)propanoic acid (S8)



LC-MS of crude material using a gradient of 5-95% MeCN/H_2O over 2 min, data collected at 254 nm. The crude material was loaded onto resin.

HRMS (ESI+, *m/z*): calc'd for $C_{43}H_{36}N_2NaO_4$ $[M+Na]^+$ 667.2573; found 667.2566.

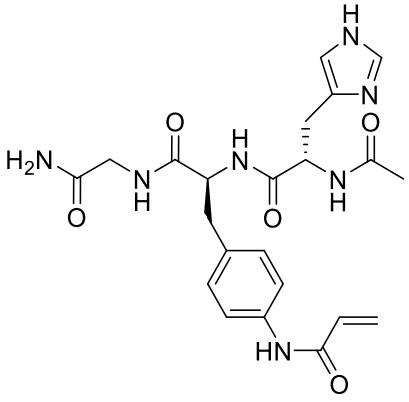
Starting materials S9-S17 were assembled on-resin and characterized crude by ^{1}H-NMR and MS



N-(4-(2-acetamido-3-amino-3-oxopropyl)phenyl)acrylamide (S9)

^{1}H NMR (400 MHz, CD_3OD) δ 7.57 (d, *J* = 8.5 Hz, 2H), 7.24 (d, *J* = 8.5 Hz, 2H), 6.49 – 6.29 (m, 2H), 5.77 (dd, *J* = 9.7, 2.2 Hz, 1H), 4.61 (dd, *J* = 9.1, 5.6 Hz, 1H), 3.13 (dd, *J* = 13.9, 5.6 Hz, 1H), 2.92 – 2.83 (m, 2H), 1.92 (s, 3H).

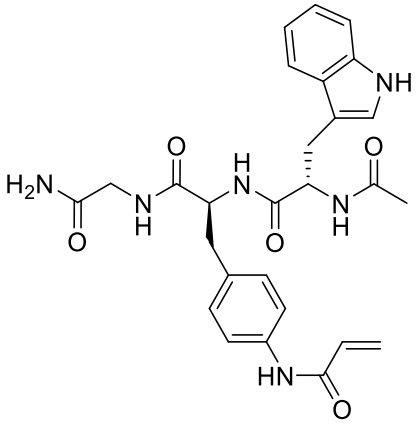
HRMS (ESI+, *m/z*): calc'd for $C_{14}H_{18}N_3O_3$ $[M+H]^+$ 276.1348; found 276.1339.



***tert*-Butyl 4-((*S*)-2-acetamido-3-(((*S*)-1-((2-amino-2-oxoethyl)amino)-1-oxo-3-(4-(*N*-tritylacrylamido)phenyl)propan-2-yl)amino)-3-oxopropyl)-1H-imidazole-1-carboxylate (S10)**

^{1}H NMR (400 MHz, CD_3OD) δ 8.78 (d, *J* = 1.4 Hz, 1H), 7.58 (d, *J* = 8.2 Hz, 2H), 7.29 (s, 1H), 7.24 (d, *J* = 8.4 Hz, 2H), 6.50 – 6.36 (m, 2H), 5.79 (dd, *J* = 9.5, 2.4 Hz, 1H), 4.68 (t, *J* = 6.7 Hz, 1H), 4.56 (dd, *J* = 8.6, 6.3 Hz, 1H), 3.94 (s, 1H), 3.72 (s, 1H), 3.16 – 2.96 (m, 4H), 1.96 (s, 3H).

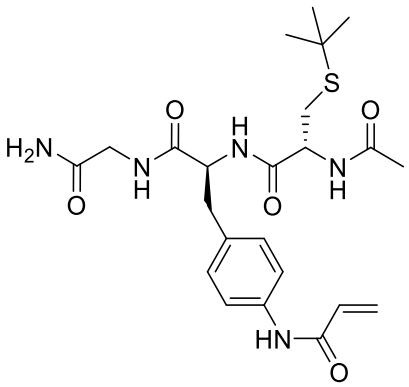
MS (ESI+, *m/z*): calc'd for $C_{22}H_{28}N_7O_5$ $[M+H]^+$ 470.502; found 470.491.



***N*-(4-((*S*)-2-((*S*)-2-Acetamido-3-(1H-indol-3-yl)propanamido)-3-((2-amino-2-oxoethyl)amino)-3-oxopropyl)phenyl)acrylamide (S11)**

^{1}H NMR (400 MHz, CD_3OD) δ 7.67 – 7.48 (m, 3H), 7.40 – 6.85 (m, 6H), 6.53 – 6.30 (m, 2H), 5.78 (dd, *J* = 9.6, 2.2 Hz, 1H), 4.71 – 4.58 (m, 1H), 4.55 – 4.44 (m, 1H), 3.92 – 3.79 (m, 1H), 3.62 (d, *J* = 17.1 Hz, 1H), 3.22 – 2.89 (m, 4H), 1.92 (s, 3H).

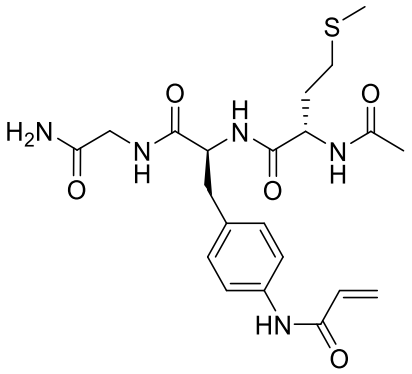
MS (ESI+, *m/z*): calc'd for $C_{27}H_{31}N_6O_5$ $[M+H]^+$ 519.574; found 519.695.



***N*-(4-((*S*)-2-((*R*)-2-Acetamido-3-(*tert*-butylthio)propanamido)-3-((2-amino-2-oxoethyl)amino)-3-oxopropyl)phenyl)-*N*-tritylacrylamide (S12)**

^{1}H NMR (400 MHz, CD_3OD) δ 7.59 (d, *J* = 8.5 Hz, 2H), 7.23 (d, *J* = 8.5 Hz, 2H), 6.50 – 6.35 (m, 2H), 5.78 (dd, *J* = 9.6, 2.3 Hz, 1H), 4.55 (dd, *J* = 9.2, 5.6 Hz, 1H), 4.40 (dd, *J* = 8.5, 5.9 Hz, 1H), 3.95 (d, *J* = 17.0 Hz, 1H), 3.74 (d, *J* = 17.1 Hz, 1H), 3.24 (dd, *J* = 14.0, 5.6 Hz, 1H), 3.01 – 2.94 (m, 1H), 2.89 – 2.83 (m, 1H), 2.71 (dd, *J* = 12.9, 8.5 Hz, 1H), 1.98 (s, 3H).

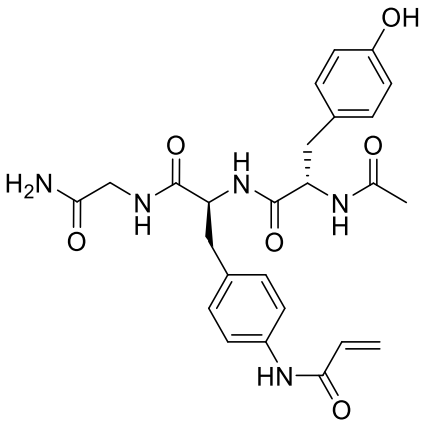
MS (ESI+, *m/z*): calc'd for $C_{23}H_{34}N_5O_5S$ $[M+H+t\text{-butyl}]^+$ 492.607; found 492.556.



***N*-(4-((*S*)-2-((*R*)-2-Acetamido-3-(methylthio)propanamido)-3-((2-amino-2-oxoethyl)amino)-3-oxopropyl)phenyl)-*N*-tritylacrylamide (S13)**

^{1}H NMR (400 MHz, CD_3OD) δ 7.60 (d, *J* = 8.5 Hz, 2H), 7.23 (d, *J* = 8.5 Hz, 2H), 6.49 – 6.36 (m, 2H), 5.78 (dd, *J* = 9.7, 2.2 Hz, 1H), 4.55 (dd, *J* = 9.1, 5.9 Hz, 1H), 4.34 (dd, *J* = 8.3, 5.6 Hz, 1H), 3.95 (d, *J* = 17.0 Hz, 1H), 3.70 (d, *J* = 17.0 Hz, 1H), 3.23 (dd, *J* = 13.9, 6.0 Hz, 1H), 3.03 – 2.95 (m, 2H), 2.05 (s, 3H), 1.99 (s, 3H).

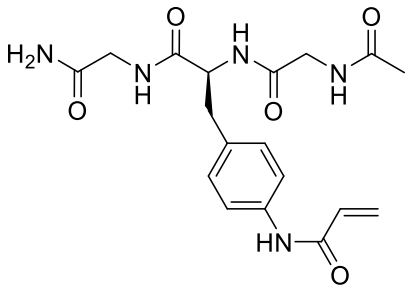
MS (ESI+, *m/z*): calc'd for $C_{21}H_{30}N_5O_5S$ $[M+H]^+$ 464.553; found 464.517.



***N*-(4-((S)-2-((S)-2-Acetamido-3-(4-(*tert*-butoxy)phenyl)propanamido)-3-((2-amino-2-oxoethyl)amino)-3-oxopropyl)phenyl)-*N*-tritylacrylamide (S14)**

^{1}H NMR (400 MHz, CD_3OD): δ 7.58 (d, *J* = 8.5 Hz, 2H), 7.21 (d, *J* = 8.5 Hz, 2H), 7.02 (d, *J* = 8.5 Hz, 2H), 6.70 (d, *J* = 8.5 Hz, 2H), 6.47 – 6.35 (m, 2H), 5.78 (dd, *J* = 9.7, 2.3 Hz, 1H), 4.50 (s, 2H), 3.89 (d, *J* = 17.0 Hz, 1H), 3.70 (d, *J* = 17.0 Hz, 1H), 3.19 (dd, *J* = 13.9, 5.9 Hz, 1H), 2.93 (s, 2H), 2.74 (dd, *J* = 14.0, 8.7 Hz, 1H), 1.90 (s, 3H).

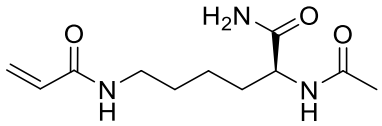
MS (ESI+, *m/z*): calc'd for $C_{25}H_{30}N_5O_6$ $[M+H]^+$ 496.536; found 496.584.



(*S*)-*N*-(4-(2-(2-Acetamidoacetamido)-3-((2-amino-2-oxoethyl)amino)-3-oxopropyl)phenyl)acrylamide (S15)

^{1}H NMR (400 MHz, CD_3OD) δ 7.58 (d, *J* = 8.5 Hz, 2H), 7.21 (d, *J* = 8.5 Hz, 2H), 7.02 (d, *J* = 8.5 Hz, 2H), 6.70 (d, *J* = 8.5 Hz, 2H), 6.47 – 6.35 (m, 2H), 5.78 (dd, *J* = 9.7, 2.3 Hz, 1H), 4.54 – 4.45 (m, 2H), 3.89 (d, *J* = 17.0 Hz, 1H), 3.70 (d, *J* = 17.0 Hz, 1H), 3.22 – 3.16 (m, 1H), 2.93 (s, 2H), 2.74 (dd, *J* = 14.0, 8.7 Hz, 1H), 1.90 (s, 3H).

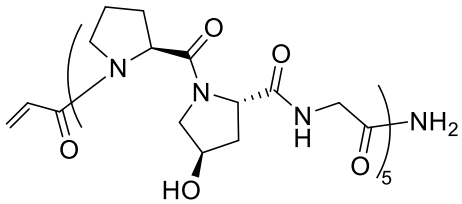
MS (ESI+, *m/z*): calc'd for $C_{18}H_{24}N_5O_5$ $[M+H]^+$ 390.412; found 390.547.



(*S*)-2-Acetamido-6-acrylamidohexanamide (S16)

^{1}H NMR (600 MHz, DMSO-*d*$_6$) δ 6.19 (dd, *J* = 17.1, 10.2 Hz, 1H), 6.10 – 6.02 (m, 1H), 5.61 – 5.54 (m, 1H), 4.11 (dd, *J* = 9.0, 5.0 Hz, 1H), 3.09 (t, *J* = 7.0 Hz, 2H), 1.84 (s, 3H), 1.62 (s, 1H), 1.52 – 1.37 (m, 3H), 1.25 (s, 2H).

MS (ESI+, *m/z*): calc'd for $C_{11}H_{19}N_3NaO_3$ $[M+Na]^+$ 264.1310; found 264.1310.



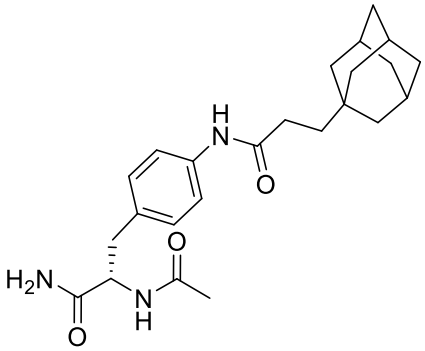
(S17)

HRMS (ESI+, *m/z*): calc'd for $C_{63}H_{92}N_{16}O_{21}$ $[M+2H]^{2+}$ 704.3312; found 704.3299.

Analytical HPLC: Linear gradient of 2-25% MeCN/H_2O (0.1% TFA) over 30 min, R_t = 18.72 min.

Conjugate Addition Products: Experimental Procedures and Characterization Data

The following compounds were assembled on-resin and were evaluated by crude quantitative ^{1}H NMR, then characterized by pure ^{1}H NMR, HRMS, and Analytical HPLC.

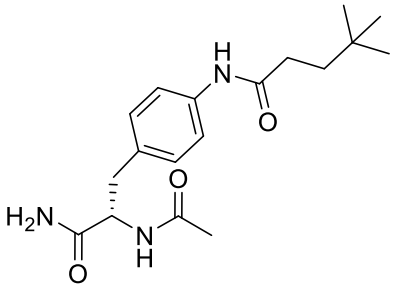


(2*R*)-2-Acetamido-3-(4-(3-((1*S*,3*S*)-adamantan-1-yl)propanamido)phenyl)propenamide (S18) (18.9 µmol, 94% yield, 100% conversion) was prepared according to the general hydroalkylation procedure with 1,3-dioxoisoindolin-2-yl (1*R*,3*S*)-adamantane-1-carboxylate.

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 7.45 (d, *J* = 8.4 Hz, 2H), 7.13 (d, *J* = 8.4 Hz, 2H), 4.36 (dd, *J* = 5.1, 4.7 Hz, 1H), 2.92 (dd, *J* = 13.6, 4.6 Hz, 1H), 2.66 (dd, *J* = 13.6, 9.9 Hz, 1H), 2.22 (t, *J* = 7.1 Hz, 2H), 1.76 (s, 3H), 1.92 (m, 3H), 1.67 (m, 3H), 1.59 (m, 3H), 1.46 (m, 6H), 1.36 (t, *J* = 7.4 Hz, 2H)

HRMS (ESI+, *m/z*): calc'd for $C_{24}H_{34}N_3O_3$ $[M+H]^+$ 412.2600; found 412.2608.

Analytical HPLC: Linear gradient of 10-60% MeCN/H_2O (0.1% TFA) over 30 min, R_t = 24.8 min.

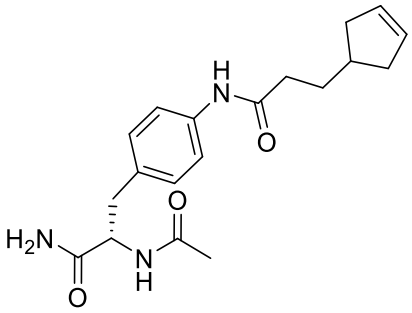


(*R*)-*N*-(4-(2-Acetamido-3-amino-3-oxopropyl)phenyl)-4,4-dimethylpentanamide (S19) (19.7 µmol, 99% yield, 100% conversion) was prepared according to the general hydroalkylation procedure with 1,3-dioxoisoindolin-2-yl pivalate.

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 7.45 (d, *J* = 8.4 Hz, 2H), 7.13 (d, *J* = 8.4 Hz, 2H), 4.36 (dd, *J* = 5.1, 4.7 Hz, 1H), 2.92 (dd, *J* = 13.6, 4.6 Hz, 1H), 2.66 (dd, *J* = 13.6, 9.9 Hz, 1H), 2.24 (t, *J* = 8.1 Hz, 2H), 1.76 (s, 3H), 1.49 (t, *J* = 8.5 Hz, 2H), 0.89 (s, 9H).

HRMS (ESI+, *m/z*): calc'd for $C_{18}H_{28}N_3O_3$ $[M+H]^+$ 334.2131; found 334.2119.

Analytical HPLC: Linear gradient of 5-40% MeCN/H_2O (0.1% TFA) over 30 min, R_t = 24.3 min.

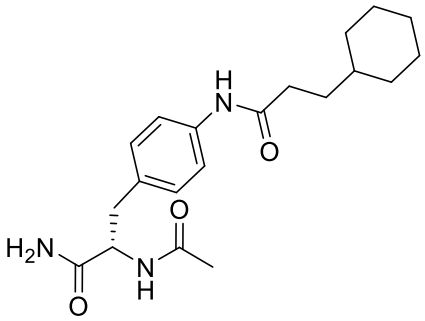


(*R*)-2-Acetamido-3-(4-(3-(cyclopent-3-en-1-yl)propanamido)phenyl)propanamide (S20) (15.4 µmol, 77% yield, 77% conversion) was prepared according to the general hydroalkylation procedure with 1,3-dioxoisoindolin-2-yl cyclopent-3-ene-1-carboxylate

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.45 (d, *J* = 8.4 Hz, 2H), 7.13 (d, *J* = 8.4 Hz, 2H), 5.67 (s, 2H), 4.36 (dd, *J* = 5.1, 4.7 Hz, 1H), 2.92 (dd, *J* = 13.6, 4.6 Hz, 1H), 2.66 (dd, *J* = 13.6, 9.9 Hz, 1H), 2.44 (m, 2H), 2.29 (t, *J* = 7.6 Hz, 2H), 2.19 (m, 1H), 1.96 (m, 2H), 1.76 (s, 3H), 1.66 (q, *J* = 7.4 Hz, 2H).

HRMS (ESI+, *m/z*): calc'd for $C_{19}H_{25}N_3NaO_3$ $[M+Na]^+$ 366.1794; found 366.1815.

Analytical HPLC: Linear gradient of 5-60% MeCN/H_2O (0.1% TFA) over 30 min, R_t = 18.0 min.

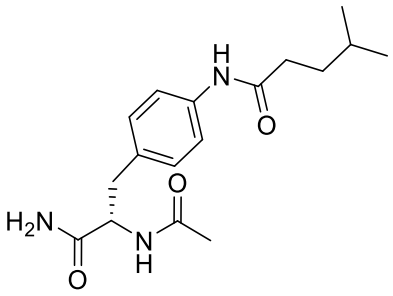


(*R*)-2-Acetamido-3-(4-(3-cyclohexylpropanamido)phenyl)propanamide (S21) (18.0 µmol, 90% yield, 100% conversion) was prepared according to the general hydroalkylation procedure with 1,3-dioxoisoindolin-2-yl cyclohexanecarboxylate.

^{1}H NMR (400 MHz, CD_3OD): δ 7.46 (d, *J* = 9.5 Hz, 2H), 7.20 (d, *J* = 7.8 Hz, 2H), 4.58 (dd, J = 9.1, 5.7 Hz, 1H), 3.10 (dd, *J* = 14.3, 5.7 Hz, 1H), 2.84 (dd, *J* = 13.9, 9.1 Hz, 1H), 2.36 (t, *J* = 7.7 Hz, 2H), 1.90 (s, 3H), 1.73 (m, 7H), 1.58 (q, *J* = 7.2 Hz, 2H), 1.29 (m, 4H).

HRMS (ESI+, *m/z*): calc'd for $C_{20}H_{30}N_3O_3$ $[M+H]^+$ 360.2287; found 360.2316.

Analytical HPLC: Linear gradient of 5-50% MeCN/H_2O (0.1% TFA) over 30 min, R_t = 24.6 min.

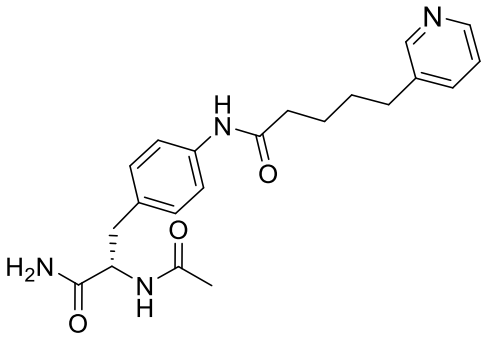


(*R*)-*N*-(4-(2-Acetamido-3-amino-3-oxopropyl)phenyl)-4-methylpentanamide (S22) (19.8 µmol, 99% yield, 100% conversion) was prepared according to the general hydroalkylation procedure with 1,3-dioxoisoindolin-2-yl isobutyrate.

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.45 (d, *J* = 8.4 Hz, 2H), 7.13 (d, *J* = 8.4 Hz, 2H), 4.36 (dd, *J* = 5.1, 4.7 Hz, 1 H), 2.92 (dd, *J* = 13.6, 4.6 Hz, 1H), 2.66 (dd, *J* = 13.6, 9.9 Hz, 1H), 2.27 (t, *J* = 7.9 Hz, 2H), 1.76 (s, 3H), 1.54 (sept, *J* = 6.4 Hz, 1H), 1.47 (m, 2H), 0.88 (s, 6H).

HRMS (ESI+, *m/z*): calc'd for $C_{17}H_{25}N_3NaO_3$ $[M+Na]^+$ 342.1794; found 342.1819.

Analytical HPLC: Linear gradient of 5-40% MeCN/H_2O (0.1% TFA) over 30 min, R_t = 21.2 min.

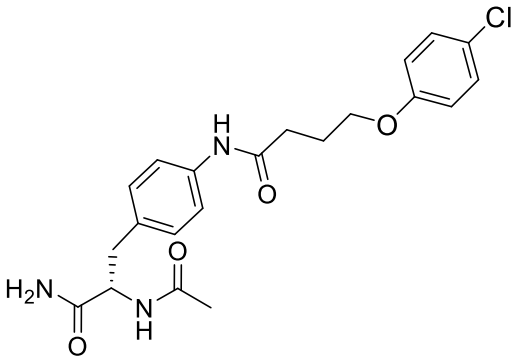


(*R*)-*N*-(4-(2-Acetamido-3-amino-3-oxopropyl)phenyl)-5-(pyridin-3-yl)pentanamide (S23) (15.9 µmol, 80% yield, 87% conversion) was prepared according to the general hydroalkylation procedure with 1,3-dioxoisoindolin-2-yl 3-(pyridin-3-yl)propanoate.

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.57 (s, 1H), 8.53 (d, *J* = 4.9 Hz, 1H), 7.97 (d, *J* = 7.2 Hz, 1H), 7.58 (t, *J* = 6.2 Hz, 1H), 7.45 (d, *J* = 8.4 Hz, 2H), 7.13 (d, *J* = 8.4 Hz, 2H), 4.36 (dd, *J* = 5.1, 4.7 Hz, 1 H), 2.92 (dd, *J* = 13.6, 4.6 Hz, 1H), 2.70 (t, *J* = 6.9 Hz, 2H), 2.31 (t, *J* = 7.9 Hz, 2H), 1.61 (m, 4H), 2.66 (dd, *J* = 13.6, 9.9 Hz, 1H), 1.76 (s, 3H).

HRMS (ESI+, *m/z*): calc'd for $C_{21}H_{27}N_4O_3$ $[M+H]^+$ 383.2083; found 383.2078.

Analytical HPLC: Linear gradient of 2-20% MeCN/H_2O (0.1% TFA) over 30 min, R_t = 19.8 min.

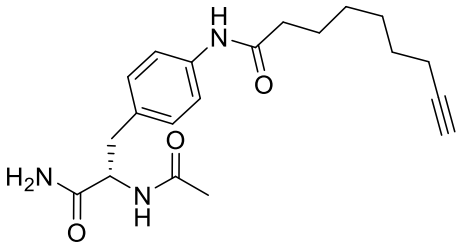


(*R*)-*N*-(4-(2-Acetamido-3-amino-3-oxopropyl)phenyl)-4-(4-chlorophenoxy)butanamide (S24) (19.8 µmol, 99% yield, 100% conversion) was prepared according to the general hydroalkylation procedure with 1,3-dioxoisoindolin-2-yl 2-(4-chlorophenoxy)acetate.

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.45 (d, *J* = 8.4 Hz, 2H), 7.31 (d, *J* = 9.0 Hz, 2H), 7.13 (d, *J* = 8.4 Hz, 2H), 6.93 (d, *J* = 9.4 Hz, 2H), 4.36 (dd, *J* = 5.1, 4.7 Hz, 1 H), 4.00 (t, *J* = 6.4 Hz, 2H), 2.92 (dd, *J* = 13.6, 4.6 Hz, 1H), 2.66 (dd, *J* = 13.6, 9.9 Hz, 1H), 2.45 (t, *J* = 7.5 Hz, 2H), 2.00 (p, *J* = 7.0 Hz, 2H), 1.76 (s, 3H).

HRMS (ESI+, *m/z*): calc'd for $C_{21}H_{24}ClN_3NaO_4$ $[M+Na]^+$ 440.1353; found 440.1353.

Analytical HPLC: Linear gradient of 10-60% MeCN/H_2O (0.1% TFA) over 30 min, R_t = 20.1 min.

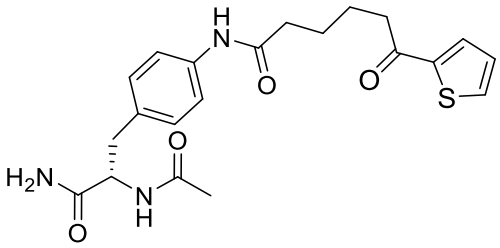


(*R*)-*N*-(4-(2-Acetamido-3-amino-3-oxopropyl)phenyl)non-8-ynamide (S25) (13.2 µmol, 66% yield, 67% conversion) was prepared according to the general hydroalkylation procedure with 1,3-dioxoisoindolin-2-yl hept-6-ynoate.

^{1}H NMR (400 MHz, CD_3OD): δ 7.46 (d, *J* = 9.5 Hz, 2H), 7.20 (d, *J* = 7.8 Hz, 2H), 4.58 (dd, *J* = 9.1, 5.7 Hz, 1H), 1.90 (s, 3H), 3.10 (dd, *J* = 14.3, 5.7 Hz, 1H), 2.84 (dd, *J* = 13.9, 9.1 Hz, 1H), 2.36 (t, *J* = 7.4 Hz, 2H), 2.16 (d, *J* = 5.4 Hz, 2H), 2.03 (s, 1H), 1.50 (m, 6H), 1.40 (m, 2H).

HRMS (ESI+, *m/z*): calc'd for $C_{20}H_{28}N_3O_3$ $[M+H]^+$ 358.2131; found 358.2111.

Analytical HPLC: Linear gradient of 5-60% MeCN/H_2O (0.1% TFA) over 30 min, R_t = 19.1 min.

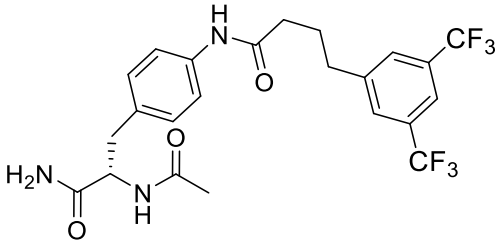


(*R*)-*N*-(4-(2-Acetamido-3-amino-3-oxopropyl)phenyl)-6-oxo-6-(thiophen-2-yl)hexanamide (S26) (11.7 µmol, 59% yield, 65% conversion) was prepared according to the general hydroalkylation procedure with 1,3-dioxoisoindolin-2-yl 4-oxo-4-(thiophen-2-yl)butanoate.

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 7.96 (dd, *J* = 4.9, 1.2 Hz, 2H), 7.94 (dd, *J* = 3.8, 1.1, 1H), 7.45 (d, *J* = 8.6 Hz, 2H), 7.13 (d, *J* = 8.4 Hz, 2H), 4.36 (dd, *J* = 9.8, 4.7 Hz, 1 H), 2.98 (t, *J* = 6.7 Hz, 2H), 2.92 (dd, *J* = 13.6, 4.6 Hz, 1H), 2.66 (dd, *J* = 13.6, 9.9 Hz, 1H), 2.31 (t, *J* = 6.6 Hz, 2H), 1.76 (s, 3H), 1.64 (m, 4H).

HRMS (ESI+, *m/z*): calc'd for $C_{21}H_{26}N_3O_4S$ $[M+H]^+$ 416.1644; found 416.1631.

Analytical HPLC: Linear gradient of 5-40% MeCN/H_2O (0.1% TFA) over 30 min, R_t = 22.3 min.



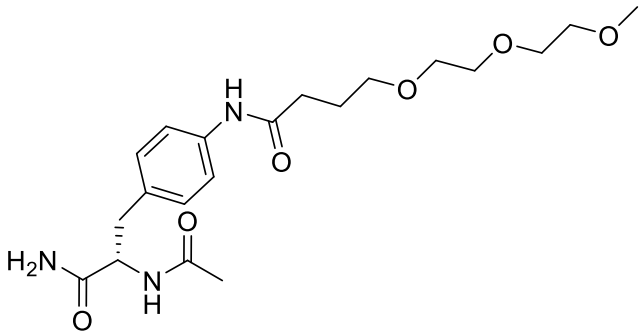
(*R*)-*N*-(4-(2-Acetamido-3-amino-3-oxopropyl)phenyl)-4-(3,5-bis(trifluoromethyl)phenyl)butanamide (S27) (10.7 µmol, 54% yield, 55% conversion) was prepared according to the general hydroalkylation procedure with 1,3-dioxoisoindolin-2-yl 2-(3,5-bis(trifluoromethyl)phenyl)acetate.

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 7.94 (s, 2H), 7.90 (s, 1H), 7.45 (d, *J* = 8.4 Hz, 2H), 7.13 (d, *J* = 8.4 Hz, 2H), 4.36 (dd, *J* = 9.8, 4.7 Hz, 1H), 2.92 (dd, *J* = 13.6, 4.6 Hz, 1H), 2.84 (t, *J* = 7.2 Hz, 2H), 2.66 (dd, *J* = 13.6, 9.9 Hz, 1H), 2.31 (t, *J* = 7.2 Hz, 2H), 1.95 (m, 2H), 1.76 (s, 3H).

^{19}F NMR (376 MHz, CDCl$_3$): δ -64.21, (calibrated using TFA at -76.55 ppm).

HRMS (ESI+, *m/z*): calc'd for $C_{23}H_{24}F_6N_3O_3$ $[M+H]^+$ 504.1722; found 504.1714.

Analytical HPLC: Linear gradient of 5-60% MeCN/H_2O (0.1% TFA) over 30 min, R_t = 25.8 min.

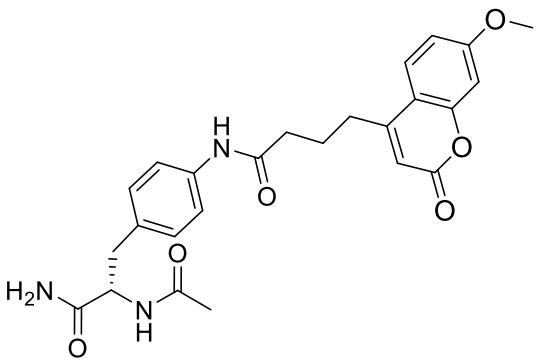


(*R*)-*N*-(4-(2-Acetamido-3-amino-3-oxopropyl)phenyl)-4-(2-(2-methoxyethoxy)ethoxy)butanamide (S28) (18.4 µmol, 92% yield, 100% conversion) was prepared according to the general hydroalkylation procedure with 1,3-dioxoisoindolin-2-yl 2-(2-(2-methoxyethoxy)ethoxy)acetate.

^{1}H NMR (400 MHz, CD_3OD): δ 7.46 (d, *J* = 9.5 Hz, 2H), 7.20 (d, *J* = 7.8 Hz, 2H), 4.58 (dd, *J* = 9.1, 5.7 Hz, 1H), 3.60 (m, 6H), 3.53 (m, 4H), 3.34 (s, 3H), 3.10 (dd, *J* = 14.3, 5.7 Hz, 1H), 1.90 (s, 3H), 2.84 (dd, *J* = 13.9, 9.1 Hz, 1H), 2.45 (t, *J* = 7.2 Hz, 2H), 1.94 (m, 2H).

HRMS (ESI+, *m/z*): calc'd for $C_{20}H_{32}N_3O_6$ $[M+H]^+$ 410.2291; found 410.2291.

Analytical HPLC: Linear gradient of 5-40% MeCN/H_2O (0.1%TFA) over 30 min, R_t = 14.8 min.

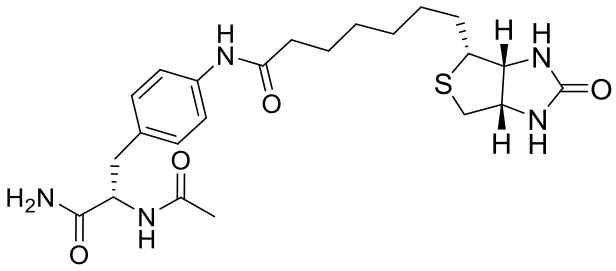


(*R*)-*N*-(4-(2-Acetamido-3-amino-3-oxopropyl)phenyl)-4-(7-methoxy-2-oxo-2H-chromen-4-yl)butanamide (S29) (5.02 µmol, 25% yield, 27% conversion) was prepared according to the general hydroalkylation procedure with 1,3-dioxoisoindolin-2-yl 2-(7-methoxy-2-oxo-2H-chromen-4-yl)acetate.

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.45 (d, *J* = 8.4 Hz, 2H), 7.13 (d, *J* = 8.4 Hz, 2H), 4.36 (dd, *J* = 9.8, 4.7 Hz, 1H), 2.92 (dd, *J* = 13.6, 4.6 Hz, 1H), 2.66 (dd, *J* = 13.6, 9.9 Hz, 1H), 1.76 (s, 3H), 7.80 (d, *J* = 8.5 Hz, 1H), 6.99 (m, 2H), 6.19 (s, 1H), 3.85 (s, 3H), 2.81 (t, *J* = 7.4 Hz, 2H), 2.41 (t, *J* = 7.1 Hz, 2H), 1.93 (m, 2H).

HRMS (ESI+, *m/z*): calc'd for $C_{25}H_{27}N_3NaO_6$ $[M+Na]^+$ 488.1798; found 488.1803.

Analytical HPLC: Linear gradient of 10-60% MeCN/H_2O (0.1% TFA) over 30 min, R_t = 23.3 min.

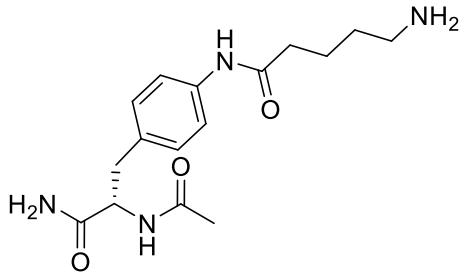


***N*-(4-((*R*)-2-Acetamido-3-amino-3-oxopropyl)phenyl)-7-((3a*R*,4*R*,6a*S*)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)heptanamide (S30)** (16.6 µmol, 83% yield, 86% conversion) was prepared according to the general hydroalkylation procedure with 1,3-dioxoisoindolin-2-yl 5-((3a*R*,4*R*,6a*S*)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanoate.

^{1}H NMR (400 MHz, CD_3OD): δ 7.46 (d, *J* = 9.5 Hz, 2H), 7.20 (d, *J* = 7.8 Hz, 2H), 4.58 (dd, *J* = 9.1, 5.7 Hz, 1H), 1.90 (s, 3H), 3.10 (dd, *J* = 14.3, 5.7 Hz, 1H), 2.84 (dd, *J* = 13.9, 9.1 Hz, 1H), 4.48 (dd, *J* = 8.0, 4.7 Hz, 1H), 4.29 (dd, *J* = 8.0, 4.4 Hz, 1H), 2.92 (d, *J* = 4.9 Hz, 1H), 2.90 (d, *J* = 5.1 Hz, 1H), 2.36 (t, *J* = 7.4 Hz, 2H), 1.70 (m, 4H), 1.58 (m, 2H), 1.43 (m, 5H).

HRMS (ESI+, *m/z*): calc'd for $C_{23}H_{34}N_5O_4S$ $[M+H]^+$ 476.2332; found 476.2332.

Analytical HPLC: Linear gradient of 5-25% MeCN/H_2O (0.1% TFA) over 30 min, R_t = 28.1 min.

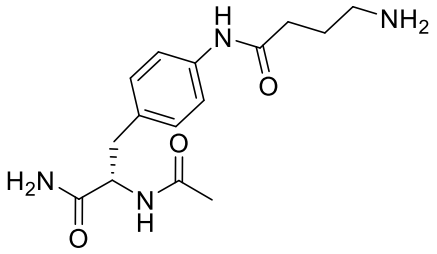


(*R*)-*N*-(4-(2-Acetamido-3-amino-3-oxopropyl)phenyl)-5-aminopentanamide (S31)

^{1}H NMR (600 MHz, CD_3OD): δ 7.47 (d, *J* = 8.5 Hz, 2H), 7.21 (d, *J* = 8.4 Hz, 2H), 4.58 (dd, *J* = 9.2, 5.6 Hz, 1H), 3.23 (m, 2H), 3.12 (dd, *J* = 13.9, 5.5 Hz, 1H), 2.96 (t, *J* = 7.2 Hz, 2H), 2.82 (dd, *J* = 14.0, 9.4 Hz, 1H), 2.44 (s, 2H), 1.90 (s, 3H), 1.80 – 1.67 (m, 4H).

HRMS (ESI+, *m/z*): calc'd for $C_{16}H_{24}N_4O_3$ $[M+H]^+$ 321.1927 found 321.1946.

Analytical HPLC: Linear gradient of 2-10% MeCN/H_2O (0.1% TFA) over 30 min, R_t = 19.2 min.

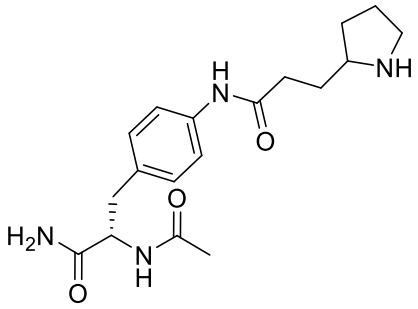


(*R*)-*N*-(4-(2-Acetamido-3-amino-3-oxopropyl)phenyl)-4-aminobutanamide (S32) (19.8 µmol, 99% yield, 100% conversion) was prepared according to the general hydroalkylation procedure with 1,3-dioxoisoindolin-2-yl (*tert*-butoxycarbonyl)glycinate.

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.45 (d, *J* = 8.4 Hz, 2H), 7.13 (d, *J* = 8.4 Hz, 2H), 4.36 (dd, *J* = 9.8, 4.7 Hz, 1 H), 2.92 (dd, *J* = 13.6, 4.6 Hz, 1H), 2.83 (t, *J* = 7.6 Hz, 2H), 2.66 (dd, *J* = 13.6, 9.9 Hz, 1H), 2.39 (t, *J* = 7.4 Hz, 2H), 1.84 (m, 2H), 1.76 (s, 3H).

HRMS (ESI+, *m/z*): calc'd for $C_{15}H_{23}N_4O_3$ $[M+H]^+$ 307.1770; found 307.1761.

Analytical HPLC: Linear gradient of 2-20% MeCN/H_2O (0.1% TFA) over 30 min, R_t = 12.8 min.

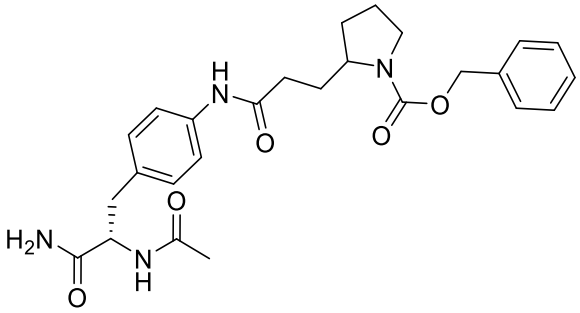


(*2R*)-2-Acetamido-3-(4-(3-(pyrrolidin-2-yl)propanamido)phenyl)propanamide (S33) (19.5 µmol, 98% yield, 100% conversion) was prepared according to the general hydroalkylation procedure with 1-(*tert*-butyl) 2-(1,3-dioxoisoindolin-2-yl) pyrrolidine-1,2-dicarboxylate.

^{1}H NMR (400 MHz, CD_3OD): δ 7.46 (d, *J* = 9.5 Hz, 2H), 7.20 (d, *J* = 7.8 Hz, 2H), 4.58 (dd, *J* = 9.1, 5.7 Hz, 1H), 3.60 (m, 1H), 3.10 (dd, *J* = 14.3, 5.7 Hz, 1H), 2.84 (dd, *J* = 13.9, 9.1 Hz, 1H), 2.58 (m, 2H), 2.25 (m, 1H), 2.12 (m, 2H), 2.02 (m, 2H), 1.90 (s, 3H), 1.72 (m, 1H).

HRMS (ESI+, *m/z*): calc'd for $C_{18}H_{27}N_4O_3$ $[M+H]^+$ 347.2083; found 347.2075.

Analytical HPLC: Linear gradient of 2-20% MeCN/H_2O (0.1% TFA) over 30 min, R_t = 15.4 min.

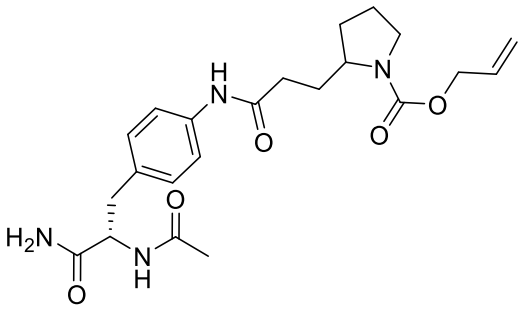


Benzyl 2-(3-((4-((*R*)-2-Acetamido-3-amino-3-oxopropyl)phenyl)amino)-3-oxopropyl)pyrrolidine-1-carboxylate (S34) (19.8 µmol, 99% yield, 100% conversion) was prepared according to the general hydroalkylation procedure with 1-benzyl 2-(1,3-dioxoisoindolin-2-yl) pyrrolidine-1,2-dicarboxylate.

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.45 (d, *J* = 8.4 Hz, 2H), 7.34 (m, 5H), 7.13 (d, *J* = 8.4 Hz, 2H), 5.06 (s, 2H), 4.36 (dd, *J* = 5.1, 4.7 Hz, 1H), 3.35 (m, 1H), 2.92 (dd, *J* = 13.6, 4.6 Hz, 1H), 2.66 (dd, *J* = 13.6, 9.9 Hz, 1H), 2.27 (m, 2H), 1.89 (m, 5H), 1.76 (s, 3H), 1.66 (m, 3H).

HRMS (ESI+, *m/z*): calc'd for $C_{26}H_{32}N_4NaO_5$ $[M+Na]^+$ 503.2270; found 503.2271.

Analytical HPLC: Linear gradient of 5-60% MeCN/H_2O (0.1% TFA) over 30 min, R_t = 20.5 min.

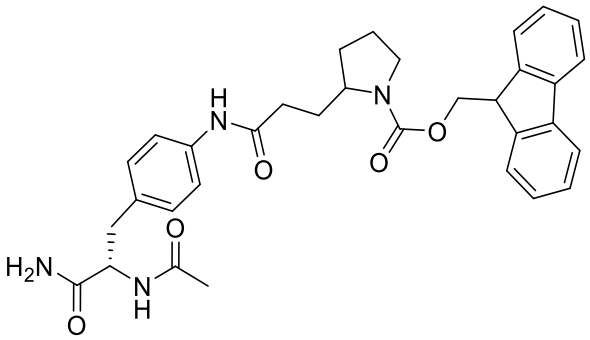


Allyl 2-(3-((4-((*R*)-2-Acetamido-3-amino-3-oxopropyl)phenyl)amino)-3-oxopropyl)pyrrolidine-1-carboxylate (S35) (19.7 µmol, 98% yield, 100% conversion) was prepared according to the general hydroalkylation procedure with 1-allyl 2-(1,3-dioxoisoindolin-2-yl) pyrrolidine-1,2-dicarboxylate.

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.45 (d, *J* = 8.5 Hz, 2H), 7.14 (d, *J* = 8.5 Hz, 2H), 5.90 (d, *J* = 5.5 Hz, 2H), 5.26 (d, *J* = 17.4 Hz, 1H), 5.18 – 5.11 (m, 1H), 4.49 (s, 2H), 4.36 (d, *J* = 4.9 Hz, 1H), 3.78 (s, 2H), 3.33 (d, *J* = 8.2 Hz, 2H), 2.91 (d, *J* = 4.8 Hz, 1H), 2.67 (d, *J* = 9.7 Hz, 1H), 2.26 (m, 2H), 1.89 (m, 2H), 1.76 (s, 3H), 1.65 (m, 2H).

HRMS (ESI+, *m/z*): calc'd for $C_{22}H_{31}N_4O_5$ $[M+H]^+$ 431.2294; found 431.2285.

Analytical HPLC: Linear gradient of 5-40% MeCN/H_2O (0.1% TFA) over 30 min, R_t = 20.8 min.

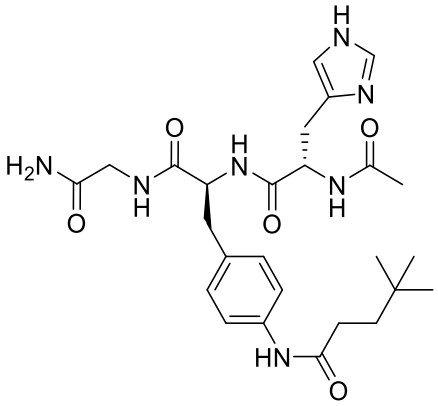


(9H-Fluoren-9-yl)methyl 2-(3-((4-((*R*)-2-Acetamido-3-amino-3-oxopropyl)phenyl)amino)-3-oxopropyl)pyrrolidine-1-carboxylate (S36) (12.9 mmol, 65%) was prepared according to the general hydroalkylation procedure with 1-((9H-fluoren-9-yl)methyl) 2-(1,3-dioxoisoindolin-2-yl) pyrrolidine-1,2-dicarboxylate.

^{1}H NMR (600 MHz, 5% D_2O/DMSO-d_6): δ 7.91 – 7.80 (m, 2H), 7.62 (d, *J* = 7.6 Hz, 2H), 7.52 – 7.34 (m, 4H), 7.34 – 7.25 (m, 2H), 7.12 (d, *J* = 8.2 Hz, 2H), 4.46 – 4.32 (m, 2H), 4.29 (d, *J* = 6.8 Hz, 1H), 4.24 (t, *J* = 6.4 Hz, 1H), 3.26 – 3.17 (m, 2H), 2.91 (d, *J* = 13.7 Hz, 1H), 2.65 (dd, *J* = 13.8, 9.7 Hz, 1H), 2.24 (s, 1H), 2.05 (s, 1H), 1.93 (s, 1H), 1.85 (s, 2H), 1.74 (s, 3H), 1.60 (s, 2H), 1.46 (s, 1H).

HRMS (ESI+, *m/z*): calc'd for $C_{33}H_{37}N_4O_5$ $[M+H]^+$ 569.2764; found 569.2764.

Analytical HPLC: Linear gradient of 5-60% MeCN/H_2O (0.1% TFA) over 30 min, R_t = 25.2 min.

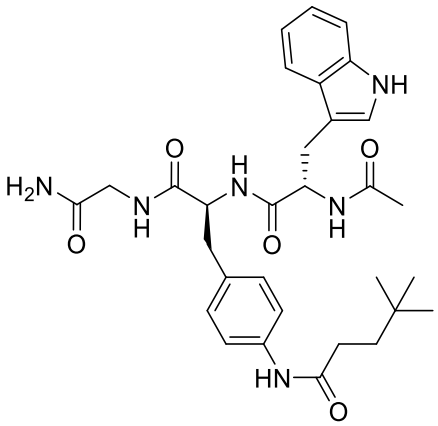


***tert*-Butyl 4-((*S*)-2-Acetamido-3-(((*S*)-1-((2-amino-2-oxoethyl)amino)-1-oxo-3-(4-(*N*-tritylacrylamido)phenyl)propan-2-yl)amino)-3-oxopropyl)-1H-imidazole-1-carboxylate (S37)**

^{1}H NMR (600 MHz, CD_3OD) δ 8.78 (d, *J* = 1.4 Hz, 1H), 7.49 (d, *J* = 8.5 Hz, 2H), 7.28 (d, *J* = 1.3 Hz, 1H), 7.21 (d, *J* = 8.5 Hz, 2H), 4.67 (t, *J* = 6.7 Hz, 1H), 4.54 (d, *J* = 2.2 Hz, 1H), 3.94 (s, 1H), 3.67 (s, 1H), 3.15 (s, 3H), 3.06 (dd, *J* = 8.8, 0.8 Hz, 1H), 2.95 (d, *J* = 5.3 Hz, 1H), 2.37 (d, *J* = 8.3 Hz, 2H), 1.96 (s, 3H), 1.62 (s, 2H), 0.97 (s, 9H).

HRMS (ESI+, *m/z*): calc'd for $C_{26}H_{38}N_7O_5$ $[M+H]^+$ 528.2934; found 528.2939.

Analytical HPLC: Linear gradient of 5-40% MeCN/H_2O (0.1% TFA) over 30 min, R_t = 21.3 min.

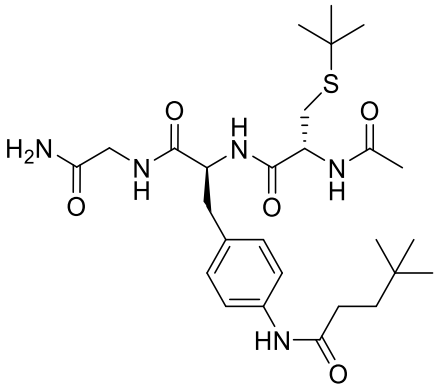


***N*-(4-((*S*)-2-((*S*)-2-Acetamido-3-(1H-indol-3-yl)propanamido)-3-((2-amino-2-oxoethyl)amino)-3-oxopropyl)phenyl)-4,4-dimethylpentanamide (S38)**

^{1}H NMR (600 MHz, CD_3OD) δ 7.57 (d, *J* = 7.9 Hz, 1H), 7.47 (d, *J* = 8.2 Hz, 2H), 7.35 (d, *J* = 8.1 Hz, 1H), 7.11 (dd, *J* = 8.1, 5.8 Hz, 3H), 7.06 (s, 1H), 7.03 (t, *J* = 7.4 Hz, 1H), 4.61 (t, *J* = 7.1 Hz, 1H), 4.46 (dd, *J* = 8.6, 5.9 Hz, 1H), 3.79 (d, *J* = 17.0 Hz, 1H), 3.61 (d, *J* = 17.1 Hz, 1H), 3.19 – 3.16 (m, 1H), 3.12 – 3.00 (m, 3H), 2.89 – 2.86 (m, 1H), 2.37 – 2.34 (m, 2H), 1.91 (s, 3H), 1.64 – 1.60 (m, 2H), 0.97 (s, 9H).

HRMS (ESI+, *m/z*): calc'd for $C_{31}H_{41}N_6O_5$ $[M+H]^+$ 577.3138; found 577.3140.

Analytical HPLC: Linear gradient of 10-50% MeCN/H_2O (0.1% TFA) over 30 min, R_t = 24.0 min.

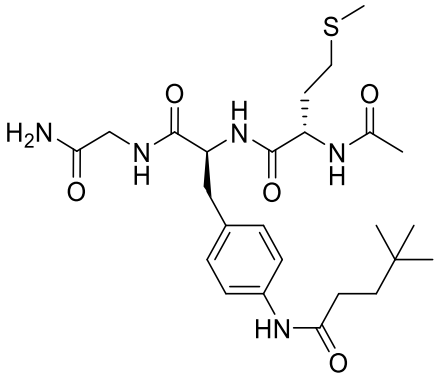


***N*-(4-((*S*)-2-((*R*)-2-Acetamido-3-(*tert*-butylthio)propanamido)-3-((2-amino-2-oxoethyl)amino)-3-oxopropyl)phenyl)-4,4-dimethylpentanamide (S39)**

^{1}H NMR (400 MHz, CD_3OD) δ 7.48 (d, *J* = 8.5 Hz, 2H), 7.17 (d, *J* = 8.6 Hz, 2H), 4.55 – 4.47 (m, 1H), 4.38 (dd, *J* = 8.5, 5.9 Hz, 1H), 3.91 (d, *J* = 17.0 Hz, 1H), 3.71 (d, *J* = 17.0 Hz, 1H), 3.20 (dd, *J* = 14.0, 5.6 Hz, 1H), 2.94 (dd, *J* = 14.0, 9.2 Hz, 1H), 2.83 (dd, *J* = 12.8, 5.9 Hz, 1H), 2.68 (dd, *J* = 12.8, 8.6 Hz, 1H), 2.37 – 2.30 (m, 2H), 1.95 (s, 3H), 1.62 – 1.56 (m, 2H), 1.29 (s, 9H), 0.95 (s, 9H).

HRMS (ESI+, *m/z*): calc'd for $C_{27}H_{44}N_5O_5S$ $[M+H]^+$ 550.3063; found 550.3046.

Analytical HPLC: Linear gradient of 10-50% MeCN/H_2O (0.1% TFA) over 30 min, R_t = 24.0 min.

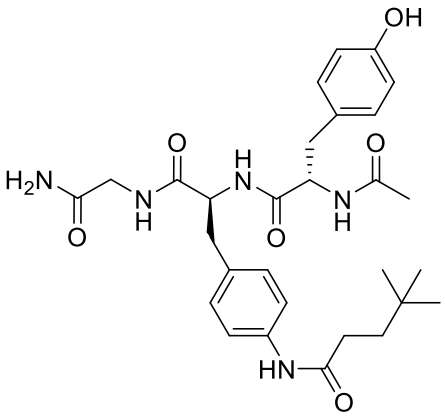


***N*-(4-((*S*)-2-((*R*)-2-Acetamido-3-(methylthio)propanamido)-3-((2-amino-2-oxoethyl)amino)-3-oxopropyl)phenyl)-4,4-dimethylpentanamide (S40)**

^{1}H NMR (400 MHz, CD_3OD) δ 7.48 (d, *J* = 8.5 Hz, 2H), 7.17 (d, *J* = 8.6 Hz, 2H), 4.51 (dd, *J* = 9.1, 5.8 Hz, 1H), 4.31 (dd, *J* = 8.3, 5.7 Hz, 1H), 3.92 (d, *J* = 17.0 Hz, 1H), 3.67 (d, *J* = 17.1 Hz, 1H), 3.19 (dd, *J* = 14.0, 6.0 Hz, 1H), 2.95 (dd, *J* = 13.9, 9.2 Hz, 1H), 2.40 – 2.31 (m, 3H), 2.03 (s, 3H), 1.96 (s, 3H), 1.94 – 1.71 (m, 3H), 1.63 – 1.56 (m, 2H), 0.95 (s, 9H).

HRMS (ESI+, *m/z*): calc'd for $C_{25}H_{40}N_5O_5S$ $[M+H]^+$ 522.2750; found 522.2722.

Analytical HPLC: Linear gradient of 10-50% MeCN/H_2O (0.1% TFA) over 30 min, R_t = 20.8 min.

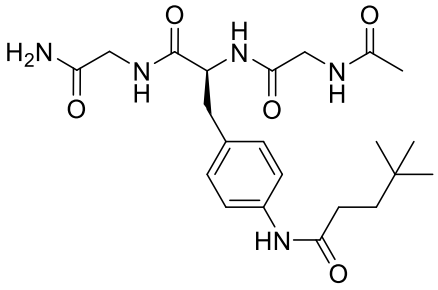


***N*-(4-((*S*)-2-((*S*)-2-Acetamido-3-(4-hydroxyphenyl)propanamido)-3-((2-amino-2-oxoethyl)amino)-3-oxopropyl)phenyl)-4,4-dimethylpentanamide (S41)**

^{1}H NMR (600 MHz, CD_3OD) δ 7.49 (d, *J* = 7.9 Hz, 2H), 7.18 (d, *J* = 7.8 Hz, 2H), 7.01 (d, *J* = 7.3 Hz, 2H), 6.69 (d, *J* = 6.6 Hz, 2H), 4.49 (dt, *J* = 9.7, 5.2 Hz, 2H), 3.88 (d, *J* = 17.1 Hz, 1H), 3.69 (d, *J* = 17.2 Hz, 1H), 3.19 – 3.15 (m, 1H), 2.94 – 2.90 (m, 2H), 2.73 (d, *J* = 5.2 Hz, 1H), 2.37 – 2.34 (m, 2H), 1.90 (s, 3H), 1.64 – 1.60 (m, 2H), 0.97 (d, *J* = 1.7 Hz, 9H).

HRMS (ESI+, *m/z*): calc'd for $C_{29}H_{40}N_5O_6$ $[M+H]^+$ 554.2979; found 554.2972.

Analytical HPLC: Linear gradient of 5-40% MeCN/H_2O (0.1% TFA) over 30 min, R_t = 25.4 min.

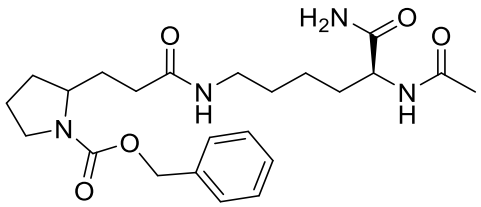


(*S*)-*N*-(4-(2-(2-Acetamidoacetamido)-3-((2-amino-2-oxoethyl)amino)-3-oxopropyl)phenyl)-4,4-dimethylpentanamide (S42)

^{1}H NMR (400 MHz, CD_3OD) δ 7.47 (d, *J* = 8.5 Hz, 2H), 7.18 (d, *J* = 8.5 Hz, 2H), 4.51 (dd, *J* = 8.8, 5.8 Hz, 1H), 3.80 (s, 4H), 3.18 – 3.12 (m, 1H), 2.92 (dd, *J* = 14.0, 8.8 Hz, 1H), 2.37 – 2.30 (m, 2H), 1.97 (s, 3H), 1.63 – 1.57 (m, 2H), 0.95 (s, 9H).

HRMS (ESI+, *m/z*): calc'd for $C_{22}H_{34}N_5O_5$ $[M+H]^+$ 448.2560; found 448.2505.

Analytical HPLC: Linear gradient of 10-50% MeCN/H_2O (0.1% TFA) over 30 min, R_t = 17.3 min.

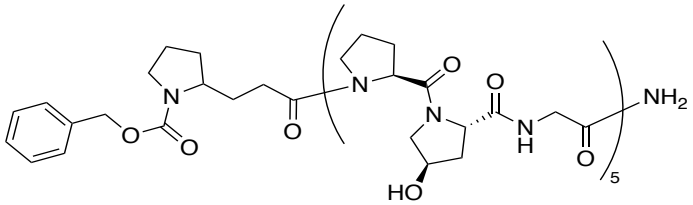


Benzyl 2-(3-(((*S*)-5-Acetamido-6-amino-6-oxohexyl)amino)-3-oxopropyl)pyrrolidine-1-carboxylate (S43)

^{1}H NMR (600 MHz, DMSO-d_6) δ 7.35 (m, 5H), 5.05 (br, 2H), 4.10 (dd, *J* = 9.0, 5.0 Hz, 1H), 3.71 (br, 1H), 3.32 (br, 2H), 3.01-2.94 (m, 2H), 2.03 (br, 2H), 1.84 (br, 4H), 1.76 (br, 1H), 1.64 – 1.42 (m, 5H), 1.39 – 1.17 (m, 5H).

HRMS (ESI+, *m/z*): calc'd for $C_{23}H_{35}N_4O_5$ $[M+H]^+$ 447.2607; found 447.2607.

Analytical HPLC: Linear gradient of 10-50% MeCN/H_2O (0.1% TFA) over 30 min, R_t = 18.6 min.

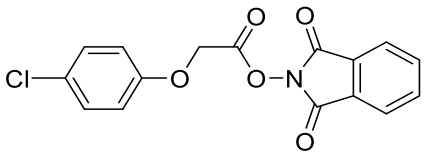


(S44)

HRMS (ESI+, *m/z*): calc'd for $C_{75}H_{107}N_{17}O_{23}$ $[M+2H]^{2+}$ 806.8863; found 806.8851.

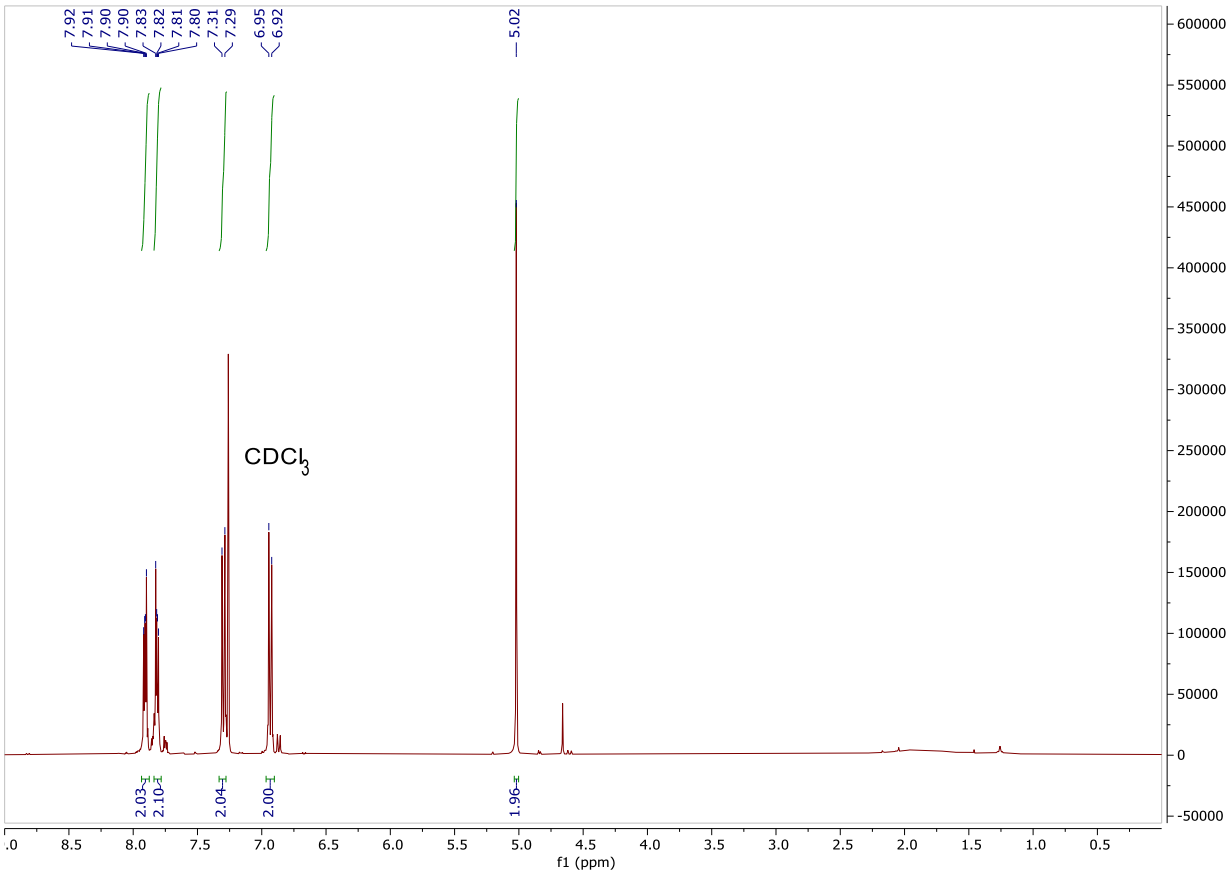
Analytical HPLC: Linear gradient of 5-40% MeCN/ H_2O (0.1% TFA) over 30 min, R_t = 22.0 min.

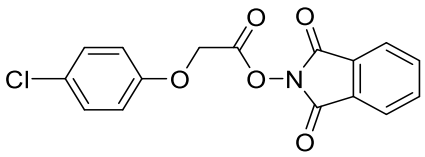
Spectra for Synthesized Compounds



1,3-Dioxoisoindolin-2-yl 2-(4-chlorophenoxy)acetate (S1)

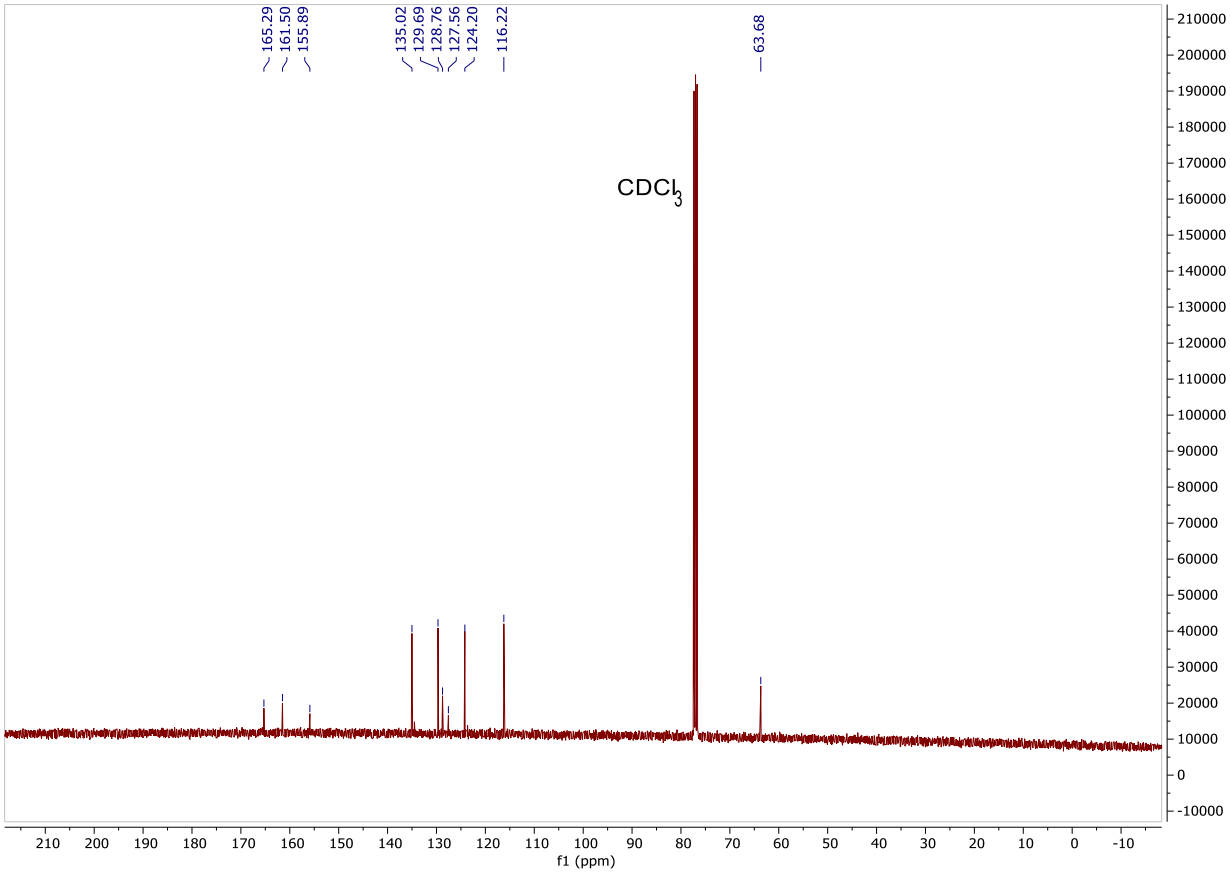
^{1}H NMR (400 MHz, $CDCl_3$)

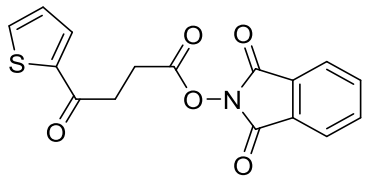




1,3-Dioxoisoindolin-2-yl 2-(4-chlorophenoxy)acetate (S1)

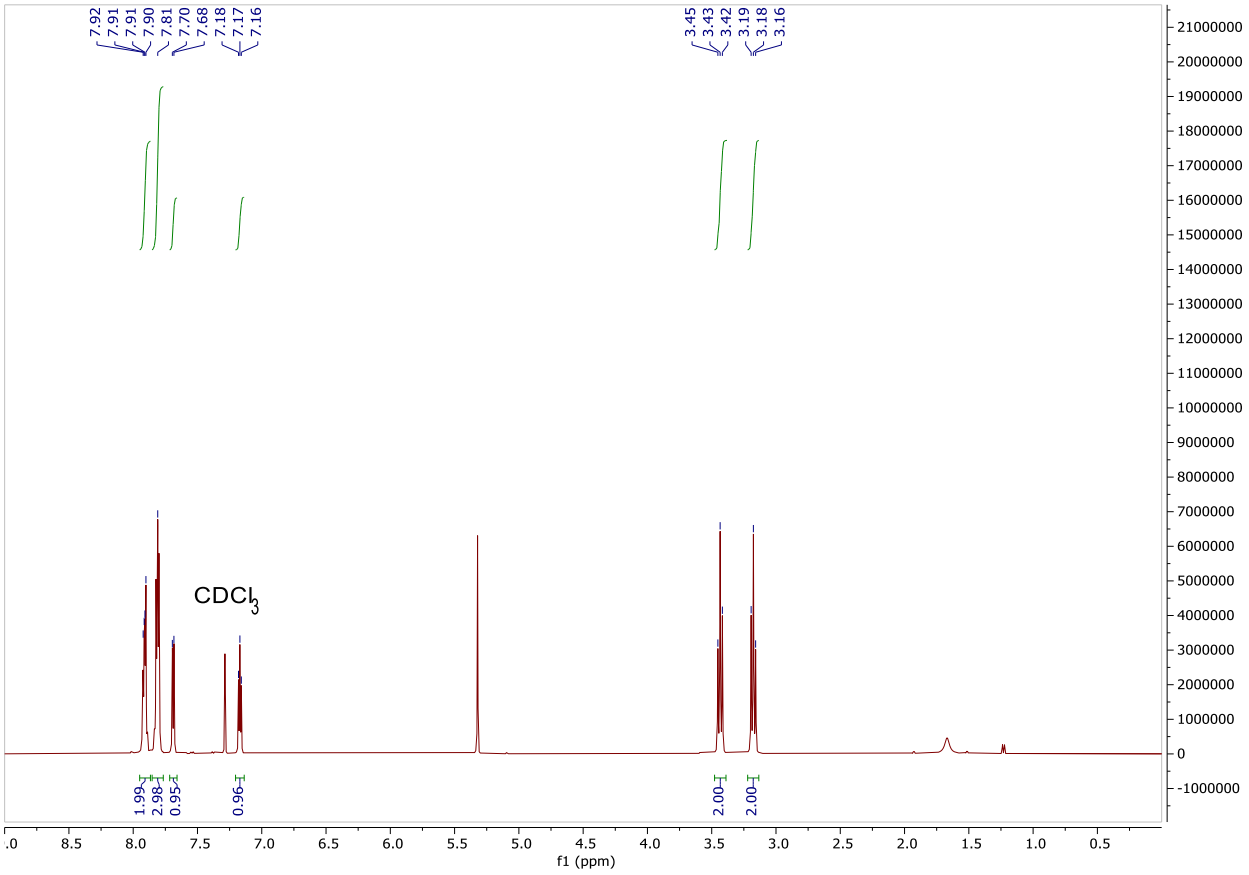
^{13}C NMR (101 MHz, $CDCl_3$)

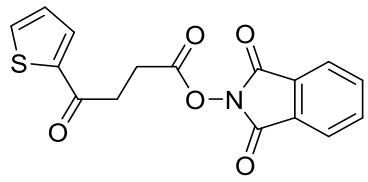




1,3-Dioxoisoindolin-2-yl 4-oxo-4-(thiophen-2-yl)butanoate (S2)

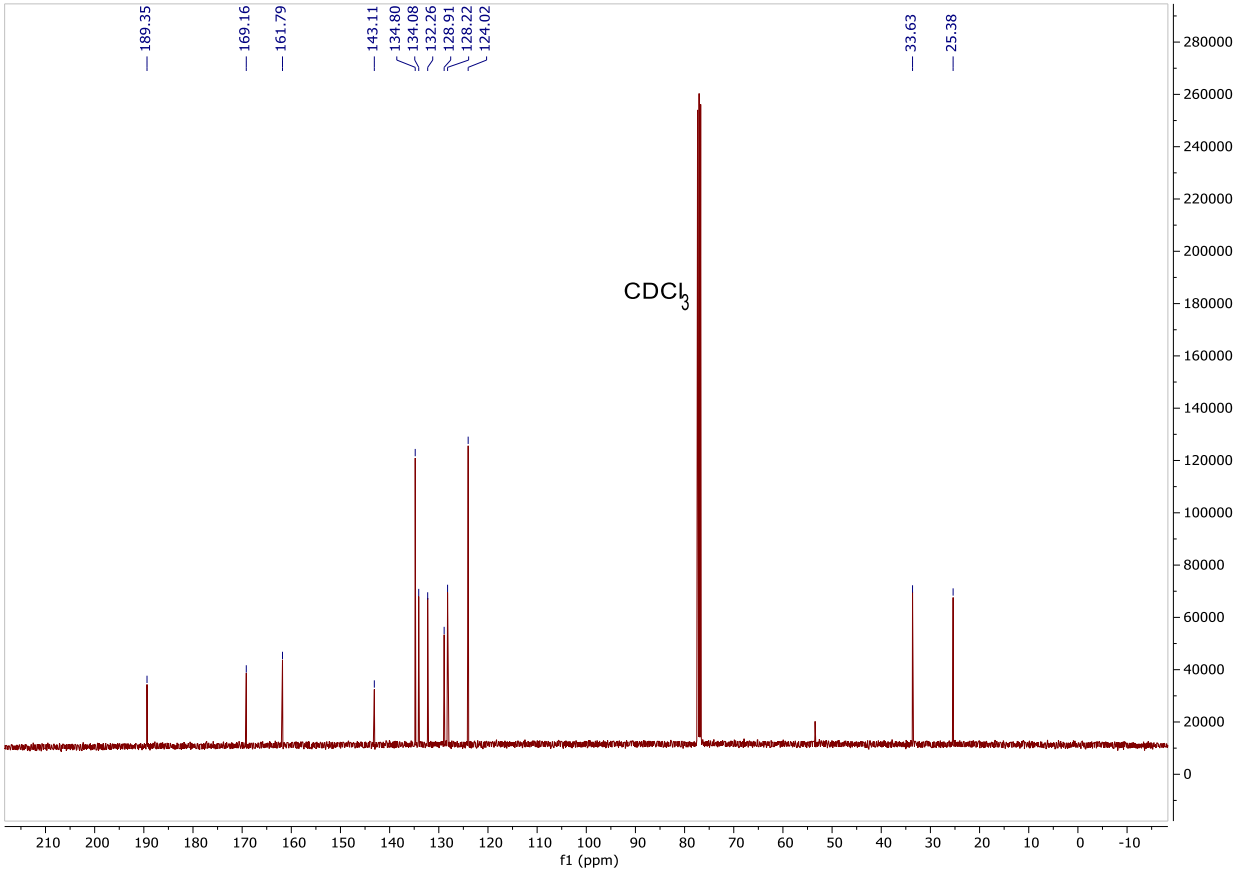
^{1}H NMR (400 MHz, $CDCl_3$)

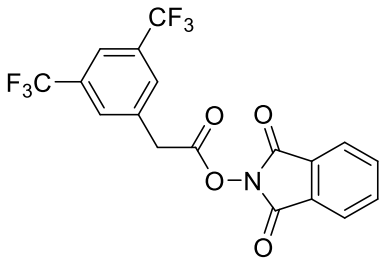




1,3-Dioxoisoindolin-2-yl 4-oxo-4-(thiophen-2-yl)butanoate (S2)

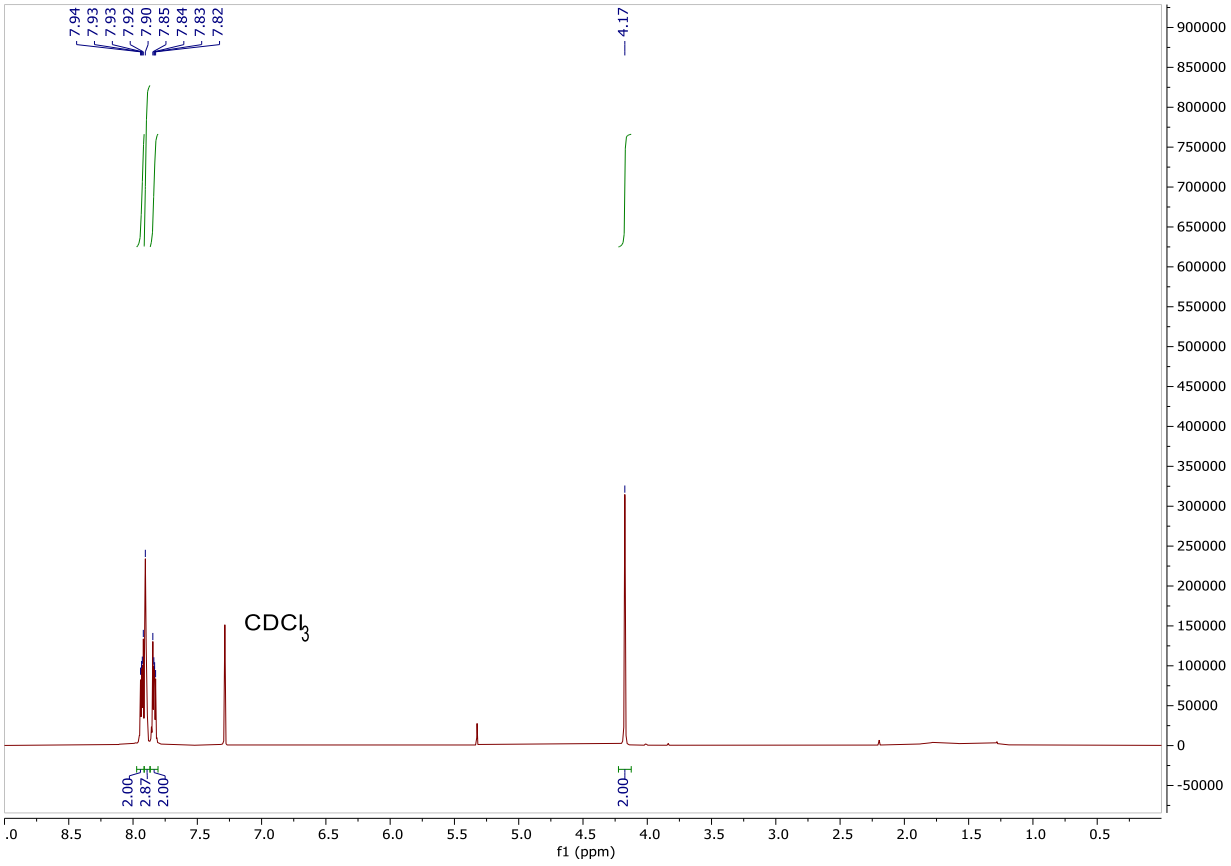
^{13}C NMR (101 MHz, $CDCl_3$)

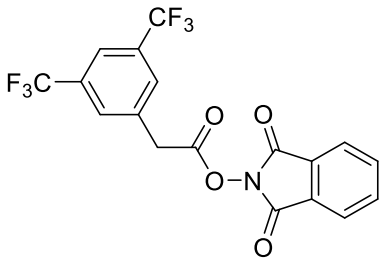




1,3-Dioxoisoindolin-2-yl 2-(3,5-bis(trifluoromethyl)phenyl)acetate (S3)

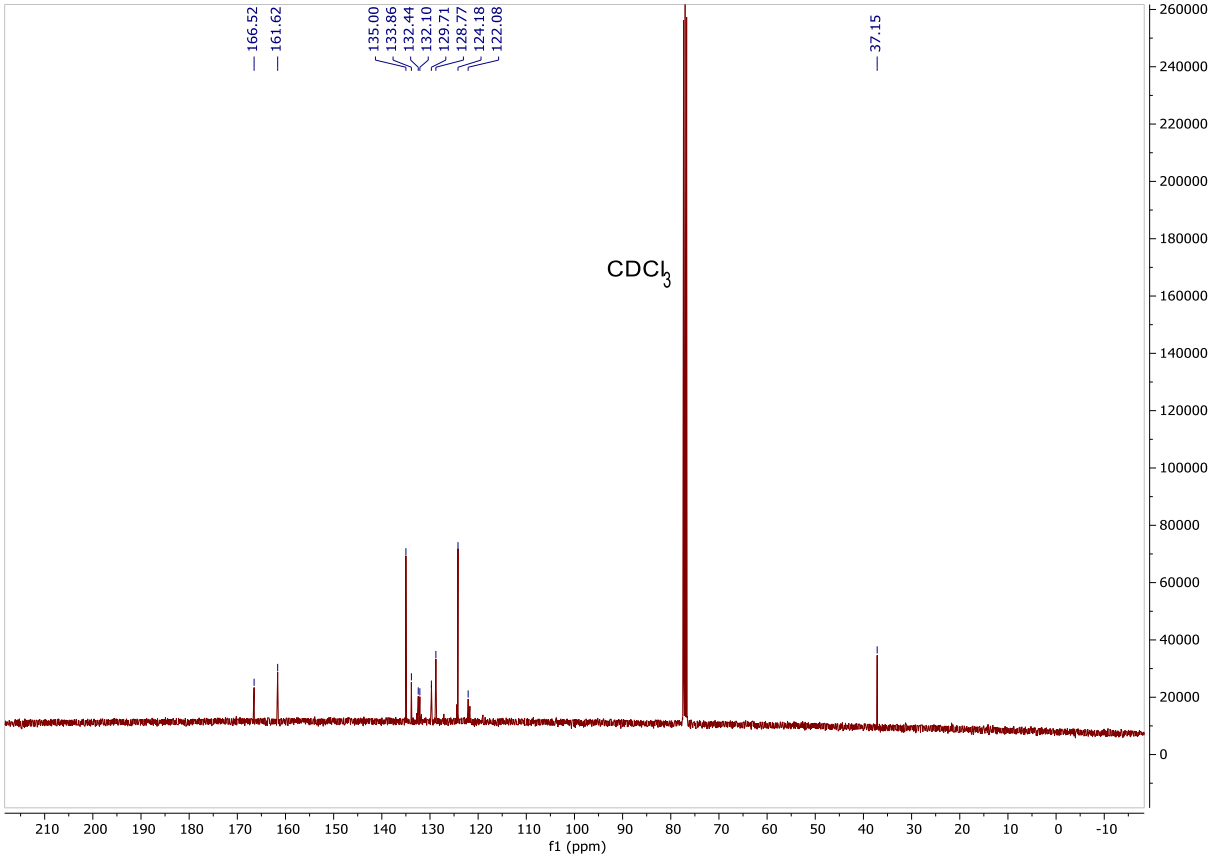
^{1}H NMR (400 MHz, $CDCl_3$)

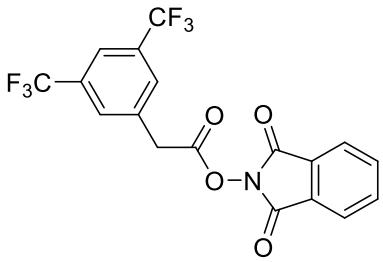




1,3-Dioxoisoindolin-2-yl 2-(3,5-bis(trifluoromethyl)phenyl)acetate (S3)

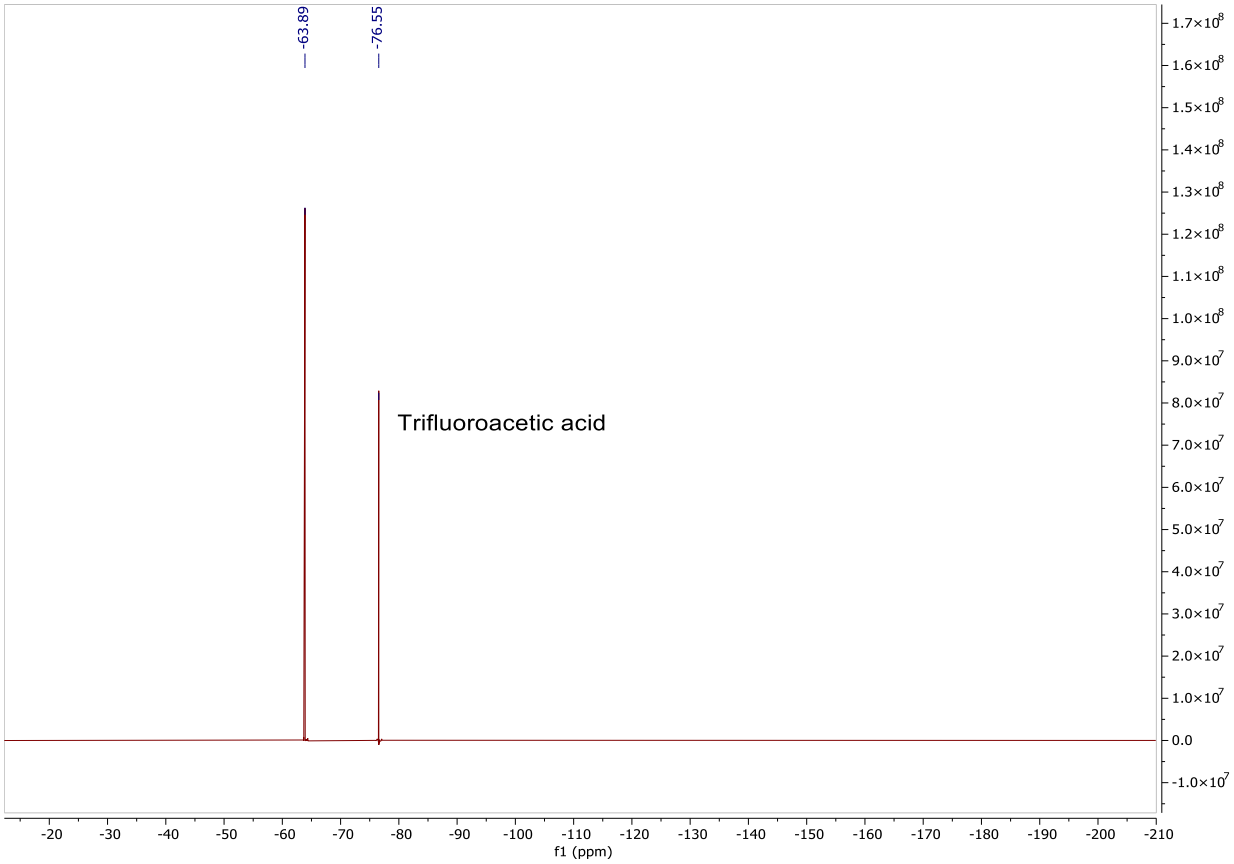
^{13}C NMR (101 MHz, $CDCl_3$)



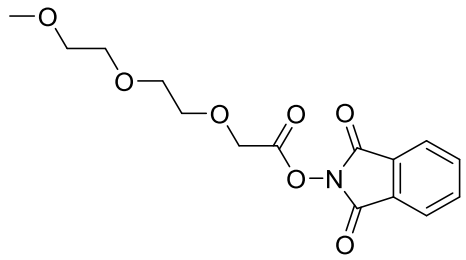


1,3-Dioxoisoindolin-2-yl 2-(3,5-bis(trifluoromethyl)phenyl)acetate (S3)

^{19}F NMR (376 MHz, $CDCl_3$)

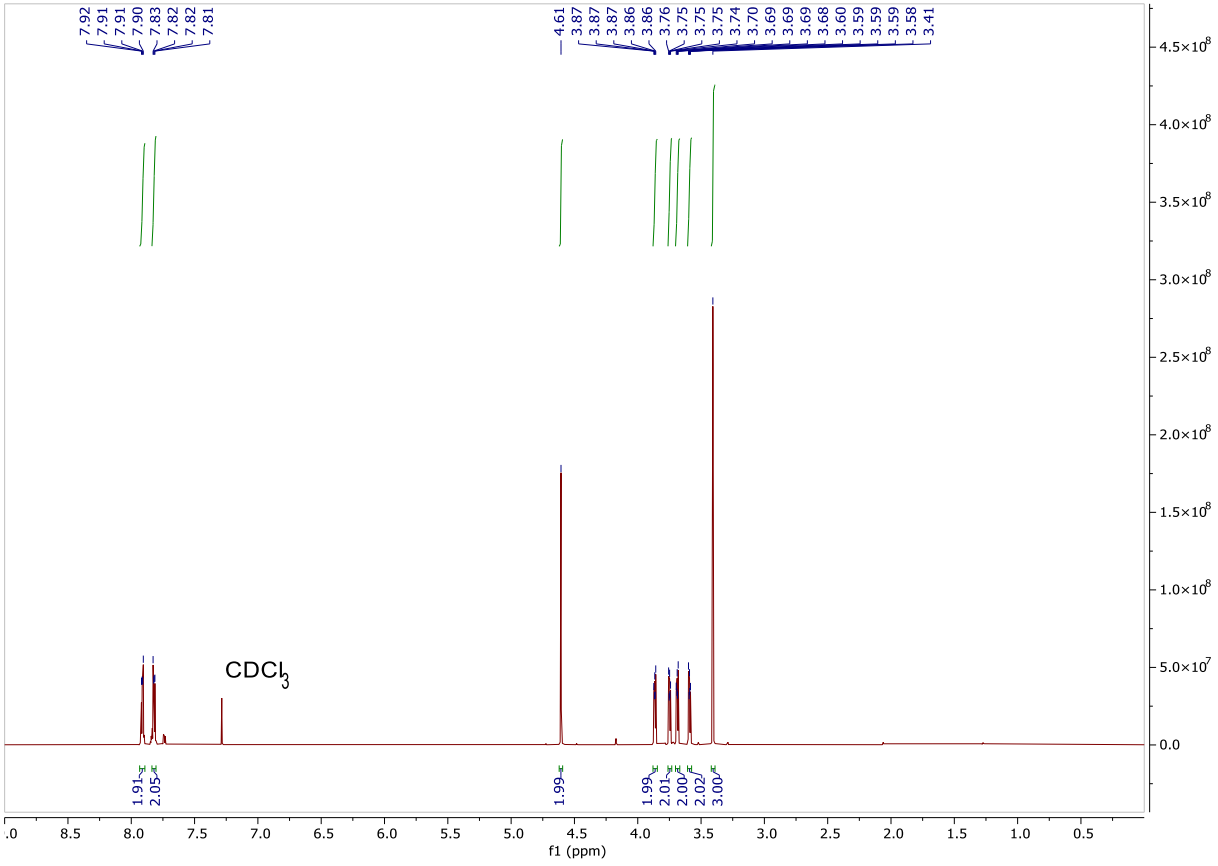


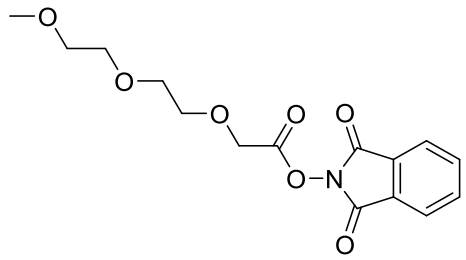
^{19}F NMR. Trifluoroacetic acid used as internal standard.



1,3-Dioxoisoindolin-2-yl 2-(2-(2-methoxyethoxy)ethoxy)acetate (S4)

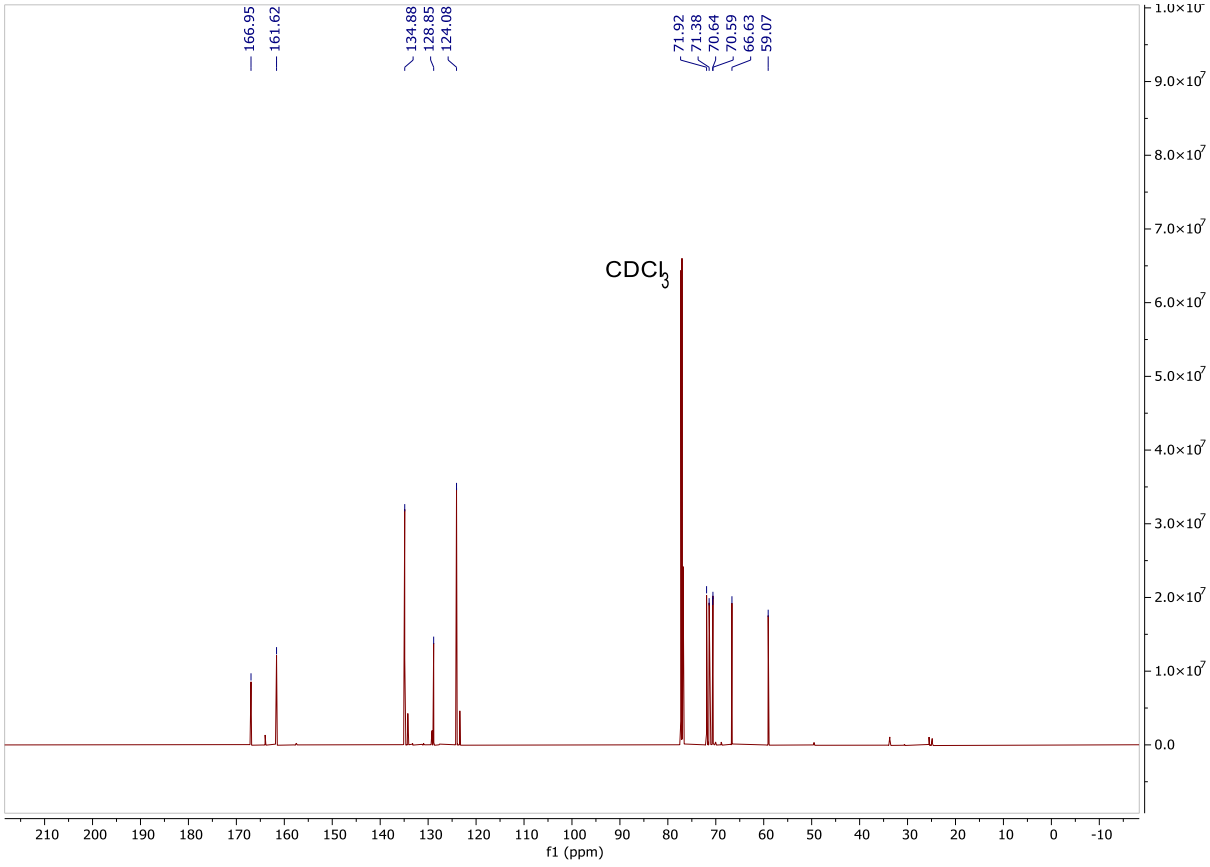
^{1}H NMR (600 MHz, $CDCl_3$)

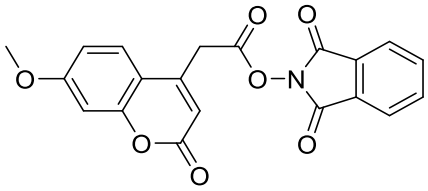




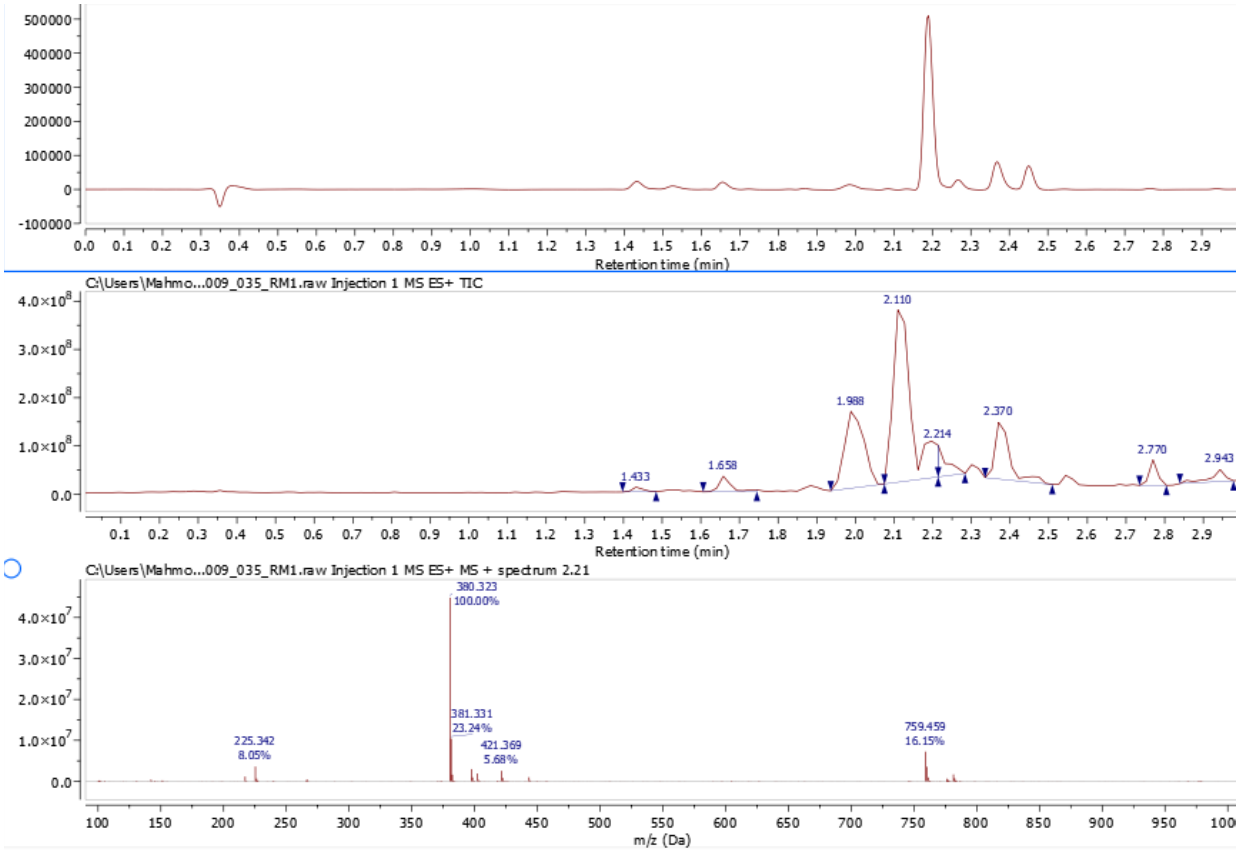
1,3-Dioxoisoindolin-2-yl 2-(2-(2-methoxyethoxy)ethoxy)acetate (S4)

^{13}C NMR (151 MHz, $CDCl_3$)

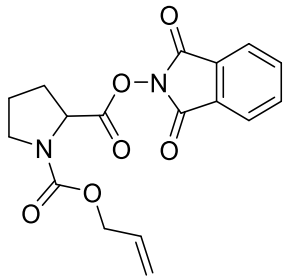




1,3-Dioxoisoindolin-2-yl 2-(7-methoxy-2-oxo-2H-chromen-4-yl)acetate (S5)

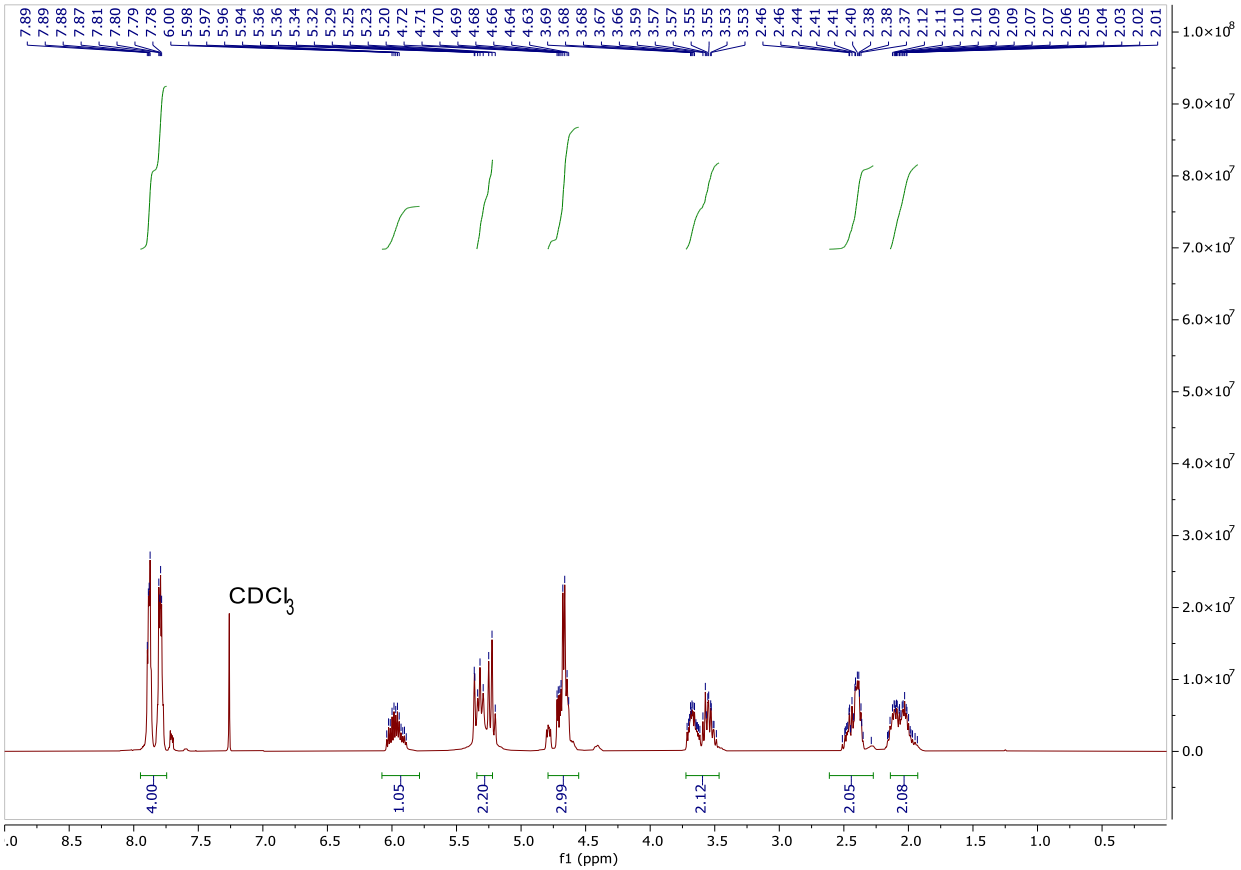


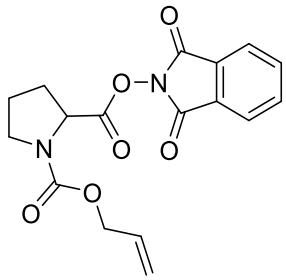
LC-MS of crude material after silica plug using a gradient of 5-95% MeCN/H_2O over 2 min, data collected at 254 nm. The crude material was used for scope table.



1-Allyl 2-(1,3-dioxoisoindolin-2-yl) pyrrolidine-1,2-dicarboxylate (S6)

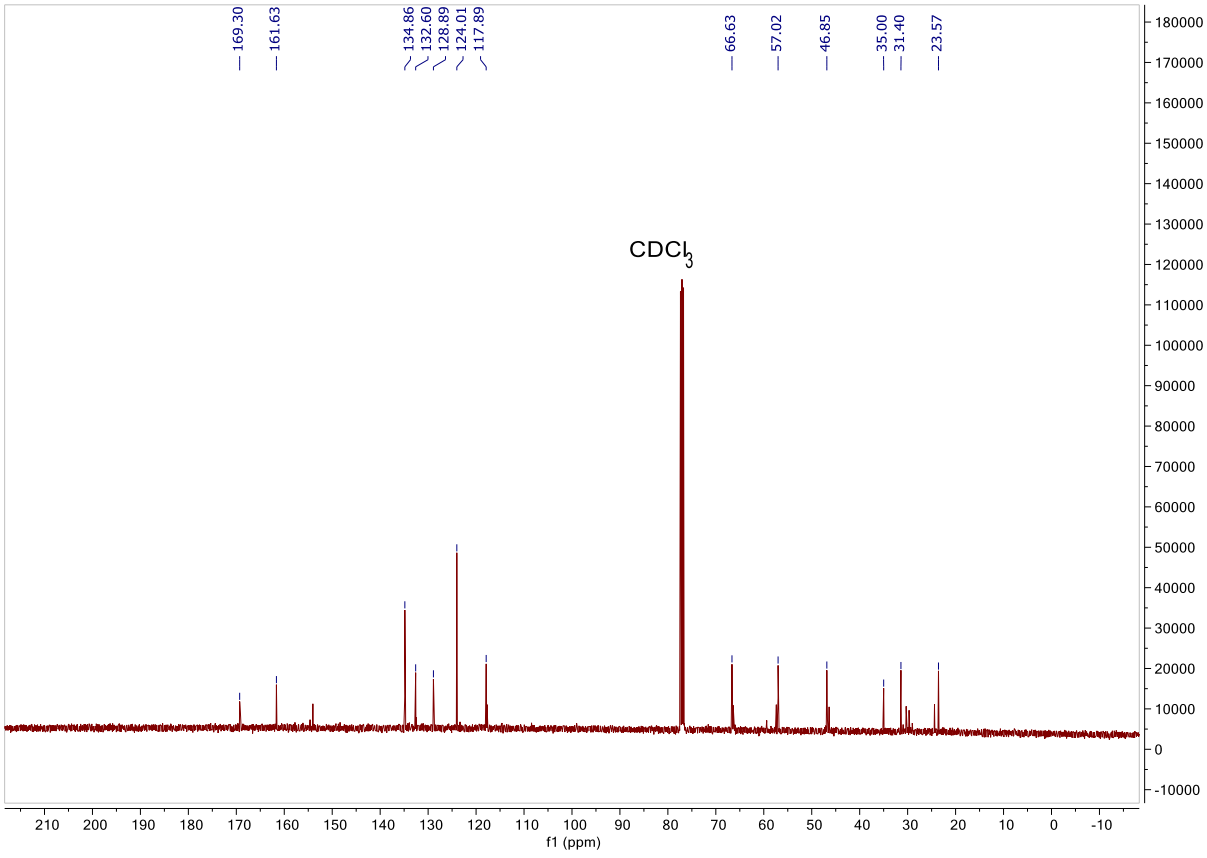
^{1}H NMR (400 MHz, $CDCl_3$)



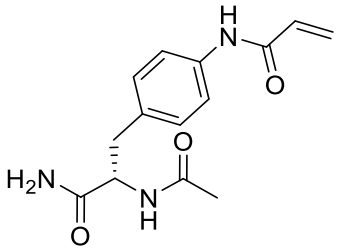


1-Allyl 2-(1,3-dioxoisoindolin-2-yl) pyrrolidine-1,2-dicarboxylate (S6)

^{13}C NMR (101 MHz, $CDCl_3$)

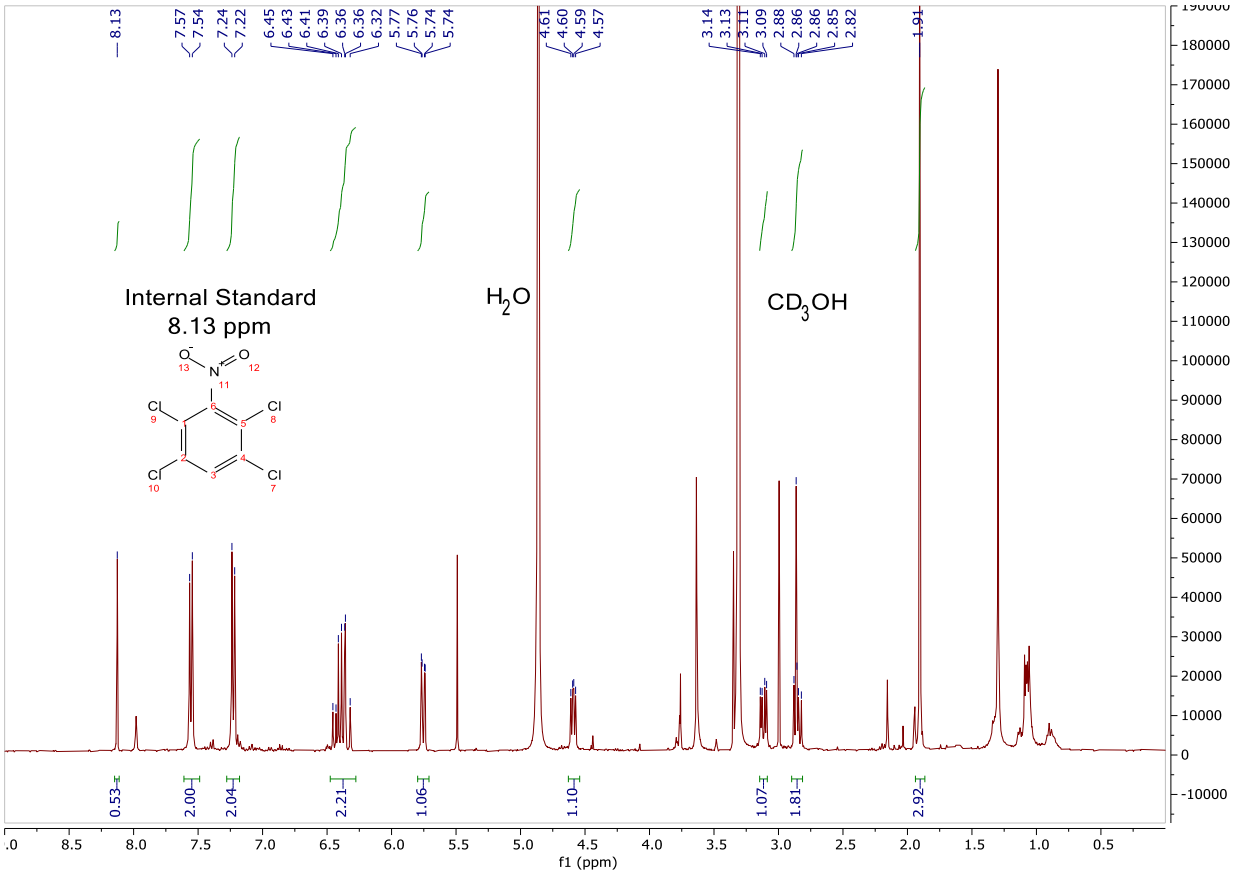


Compounds S9-S17 were assembled on-resin and used crude without further purification:

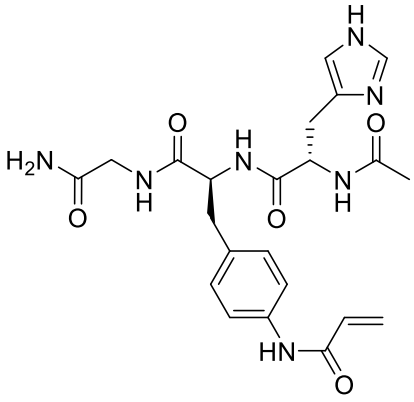


N-(4-(2-acetamido-3-amino-3-oxopropyl)phenyl)acrylamide (S9)

^{1}H NMR (400 MHz, CD_3OD)

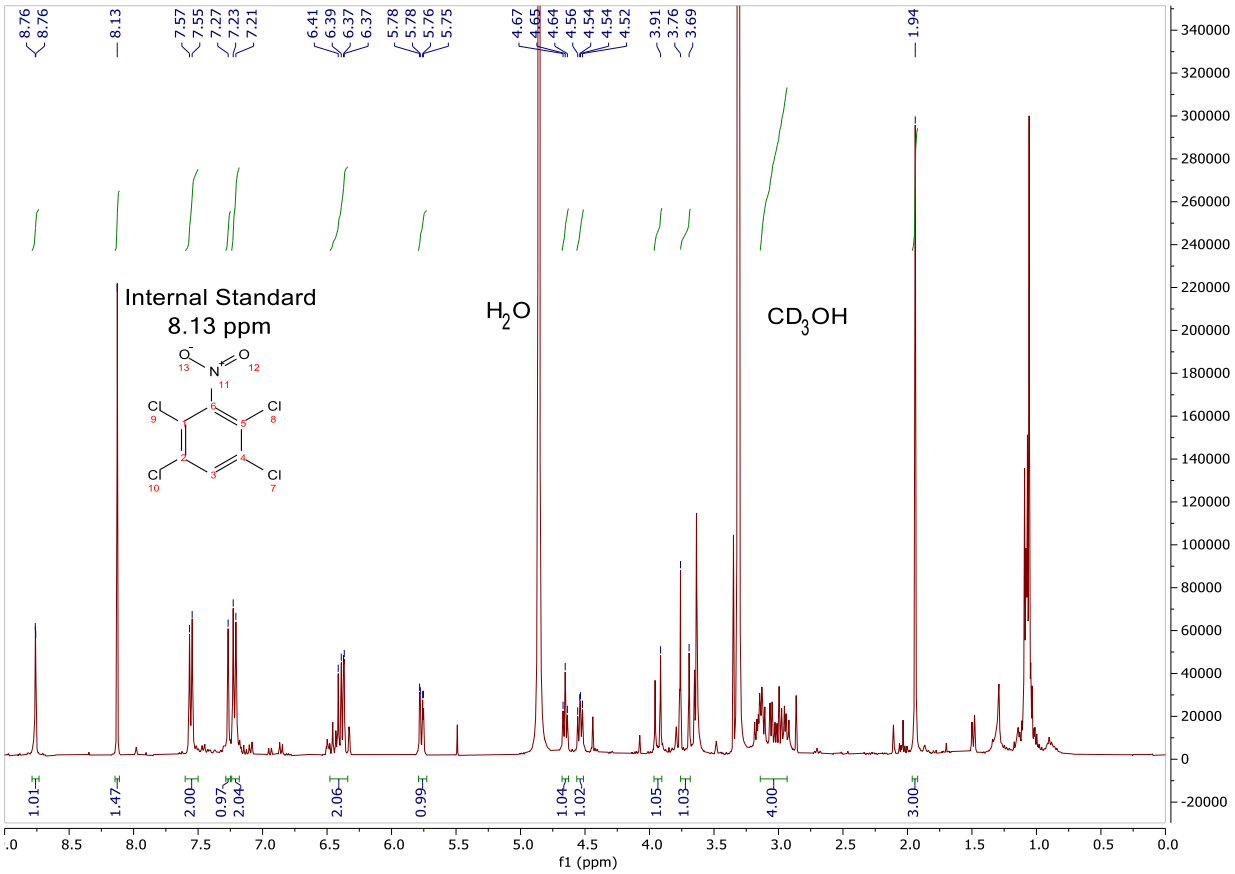


Crude ^{1}H NMR of cleaved starting material. Internal standard used to calculate resin loading.

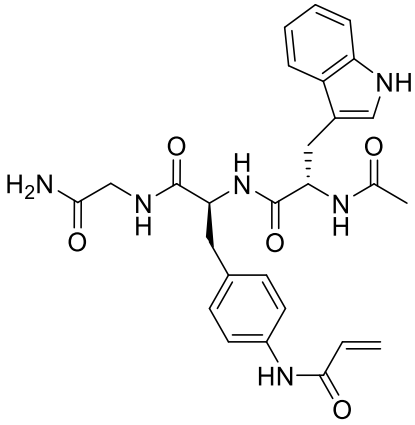


_N_-(4-((S)-2-((_S_)-2-Acetamido-3-(1H-imidazol-4-yl)propanamido)-3-((2-amino-2-oxoethyl)amino)-3-oxopropyl)phenyl)acrylamide (S10)

^{1}H NMR (400 MHz, CD_3OD)

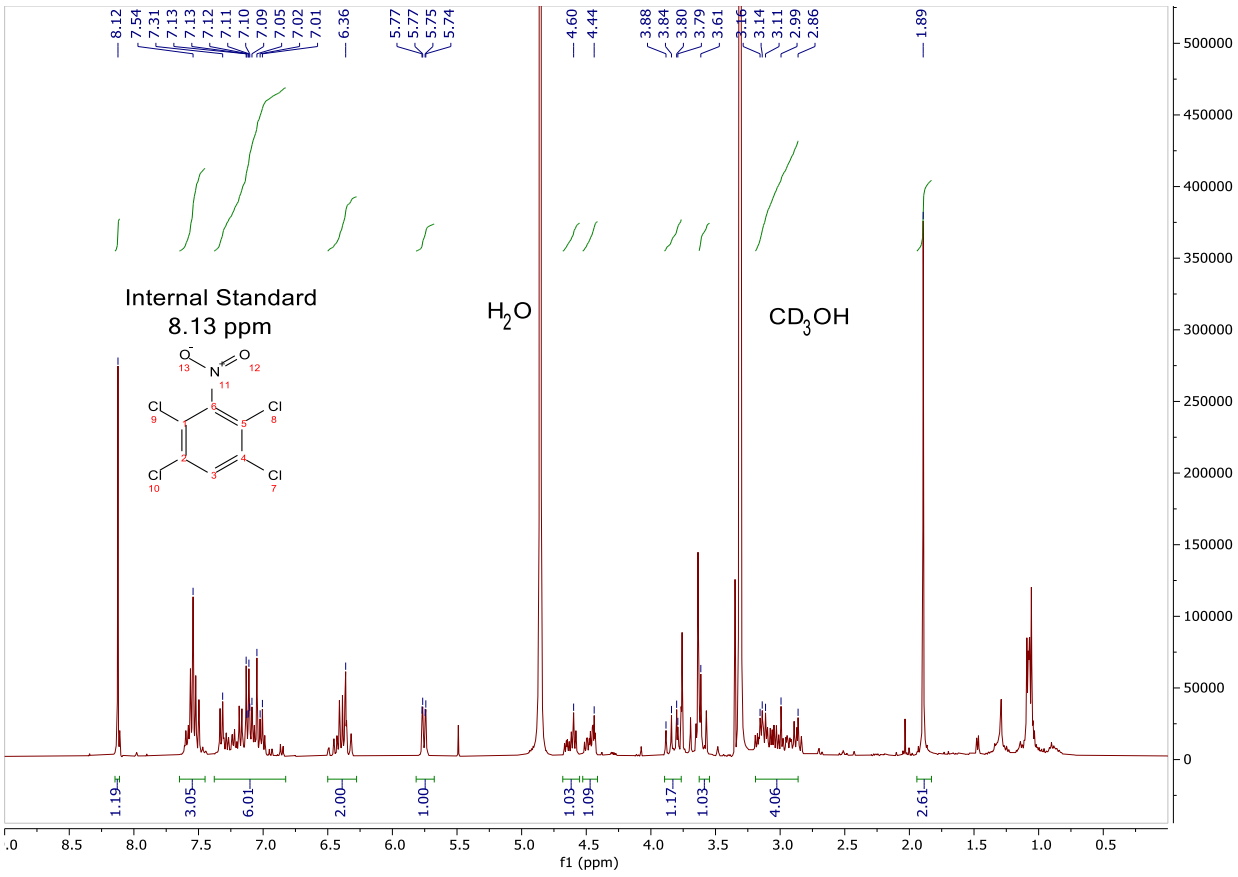


Crude ^{1}H NMR of cleaved starting material. Internal standard used to calculate resin loading.

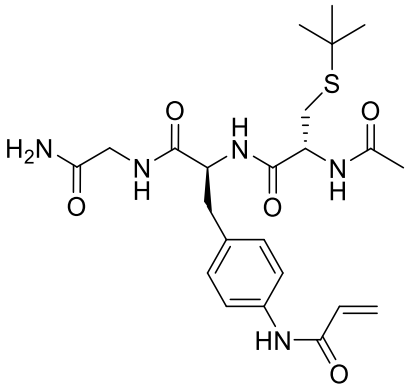


***N*-(4-((*S*)-2-((*S*)-2-Acetamido-3-(1H-indol-3-yl)propanamido)-3-((2-amino-2-oxoethyl)amino)-3-oxopropyl)phenyl)acrylamide (S11)**

^{1}H NMR (400 MHz, CD_3OD)

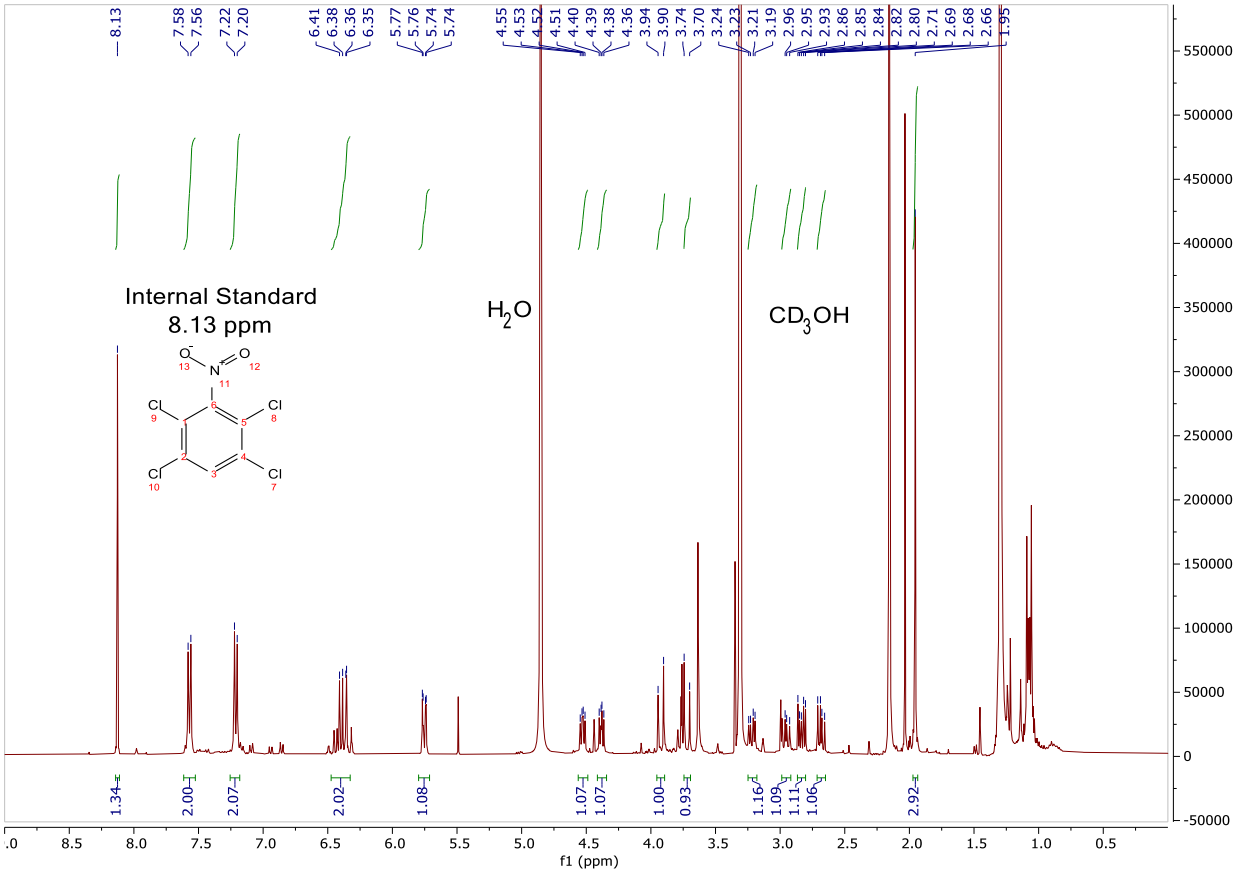


Crude ^{1}H NMR of cleaved starting material. Internal standard used to calculate resin loading.

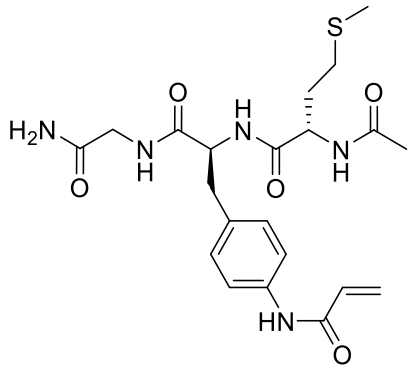


***N*-(4-((*S*)-2-((*R*)-2-Acetamido-3-(*tert*-butylthio)propanamido)-3-((2-amino-2-oxoethyl)amino)-3-oxopropyl)phenyl)-*N*-tritylacrylamide (S12)**

^{1}H NMR (400 MHz, CD_3OD)

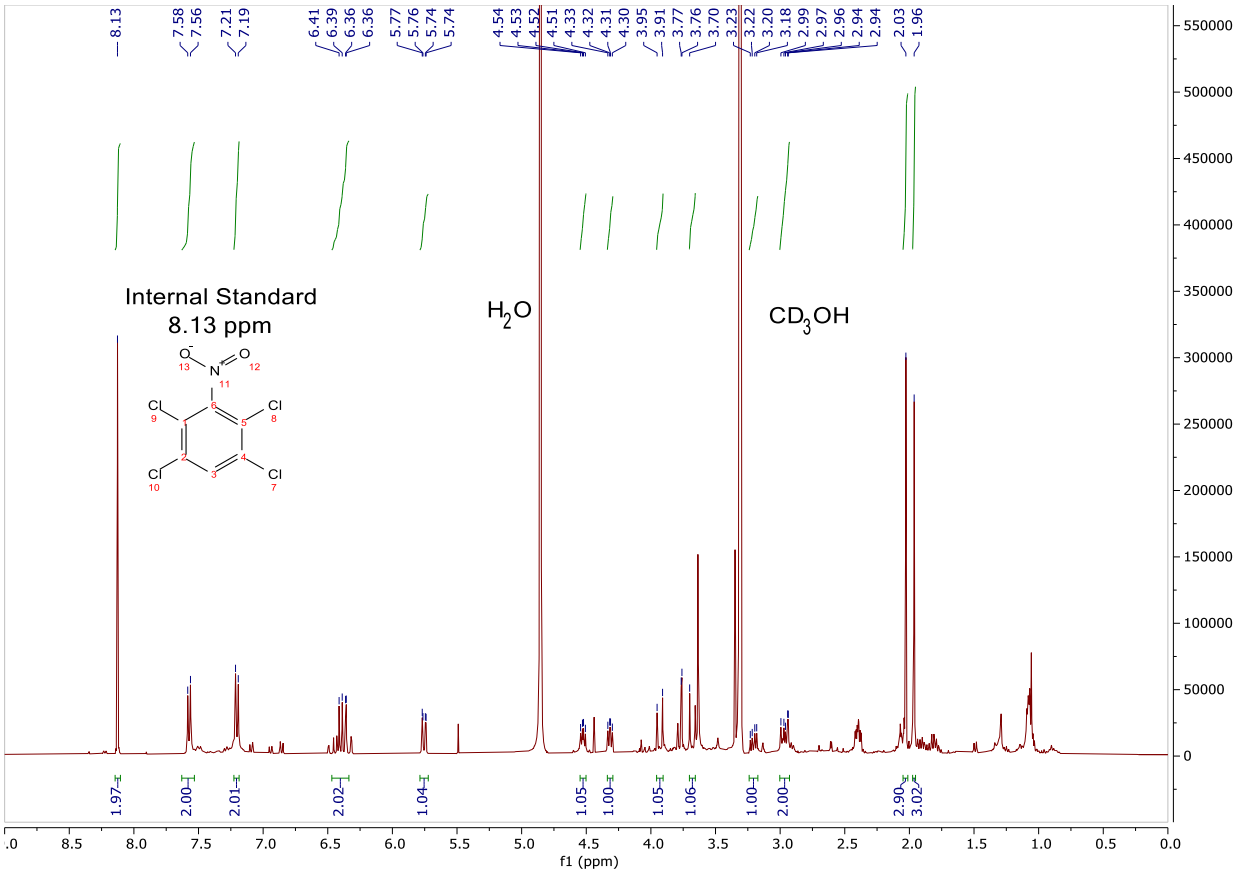


Crude ^{1}H NMR of cleaved starting material. Internal standard used to calculate resin loading.

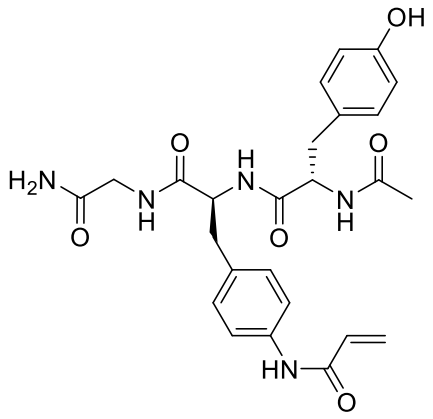


***N*-(4-((*S*)-2-((*R*)-2-Acetamido-3-(methylthio)propanamido)-3-((2-amino-2-oxoethyl)amino)-3-oxopropyl)phenyl)-*N*-tritylacrylamide (S13)**

^{1}H NMR (400 MHz, CD_3OD)

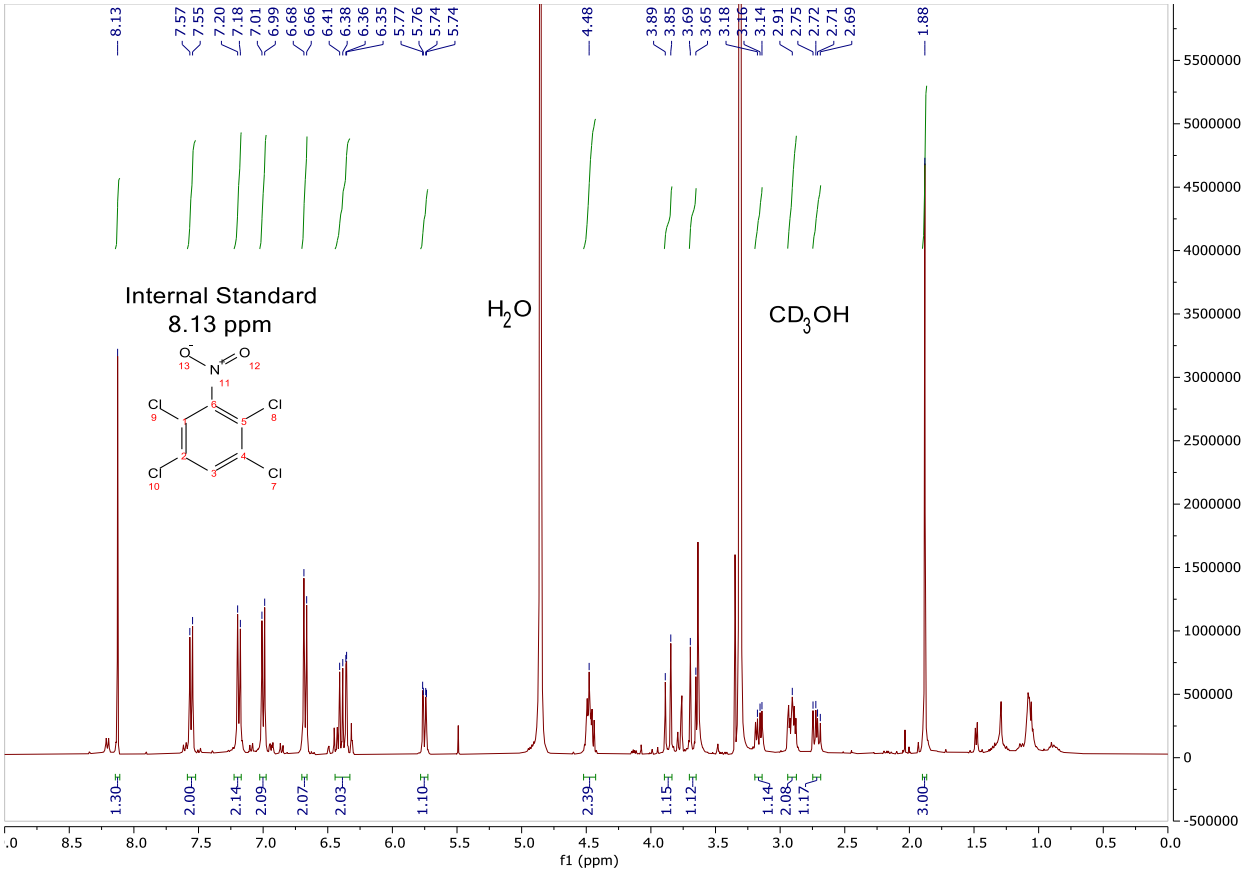


Crude ^{1}H NMR of cleaved starting material. Internal standard used to calculate resin loading.

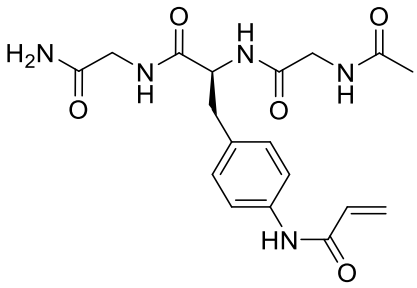


_N_-(4-((_S_)-2-((_S_)-2-Acetamido-3-(4-hydroxyphenyl)propanamido)-3-((2-amino-2-oxoethyl)amino)-3-oxopropyl)phenyl)acrylamide (S14)

^{1}H NMR (400 MHz, CD_3OD)

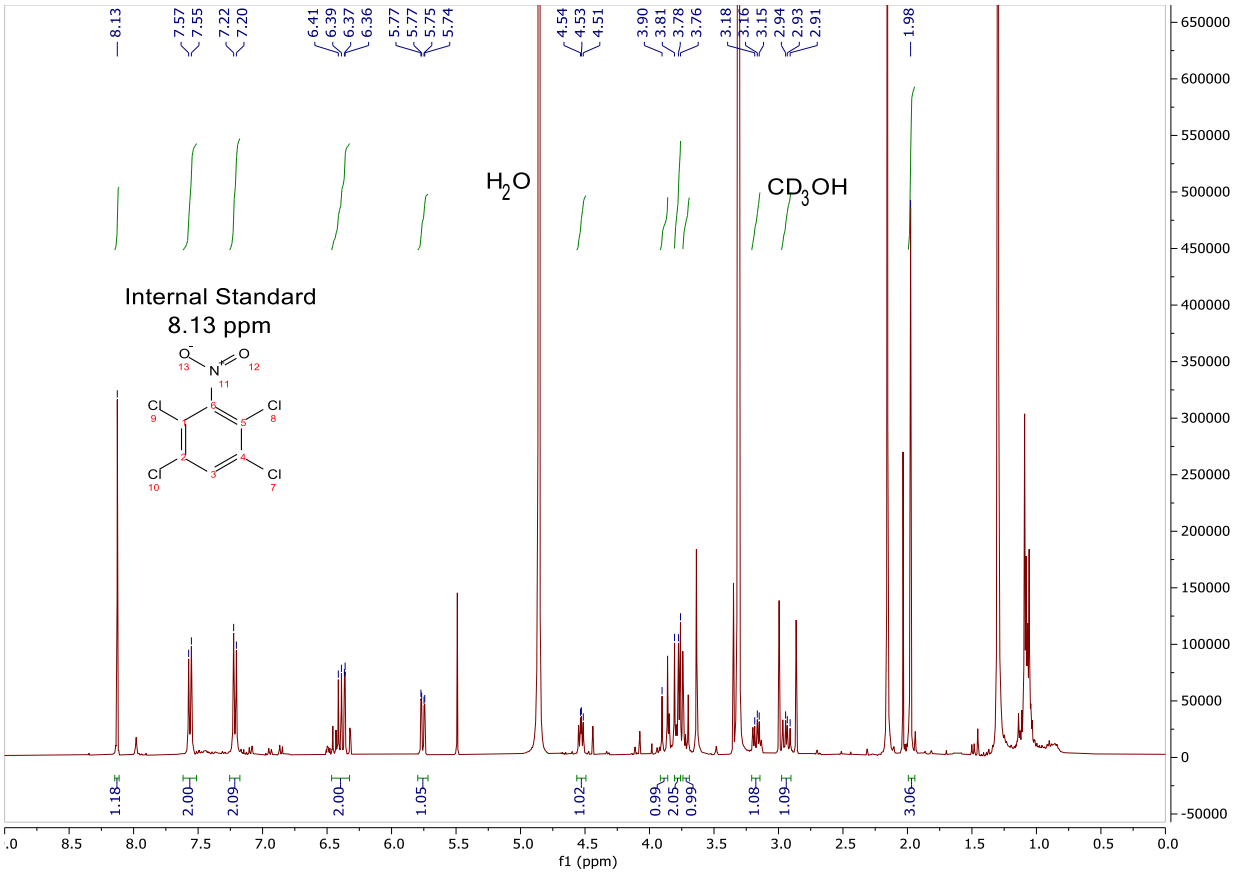


Crude ^{1}H NMR of cleaved starting material. Internal standard used to calculate resin loading.

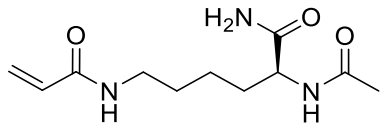


(*S*)-*N*-(4-(2-(2-Acetamidoacetamido)-3-((2-amino-2-oxoethyl)amino)-3-oxopropyl)phenyl)acrylamide (S15)

^{1}H NMR (400 MHz, CD_3OD)

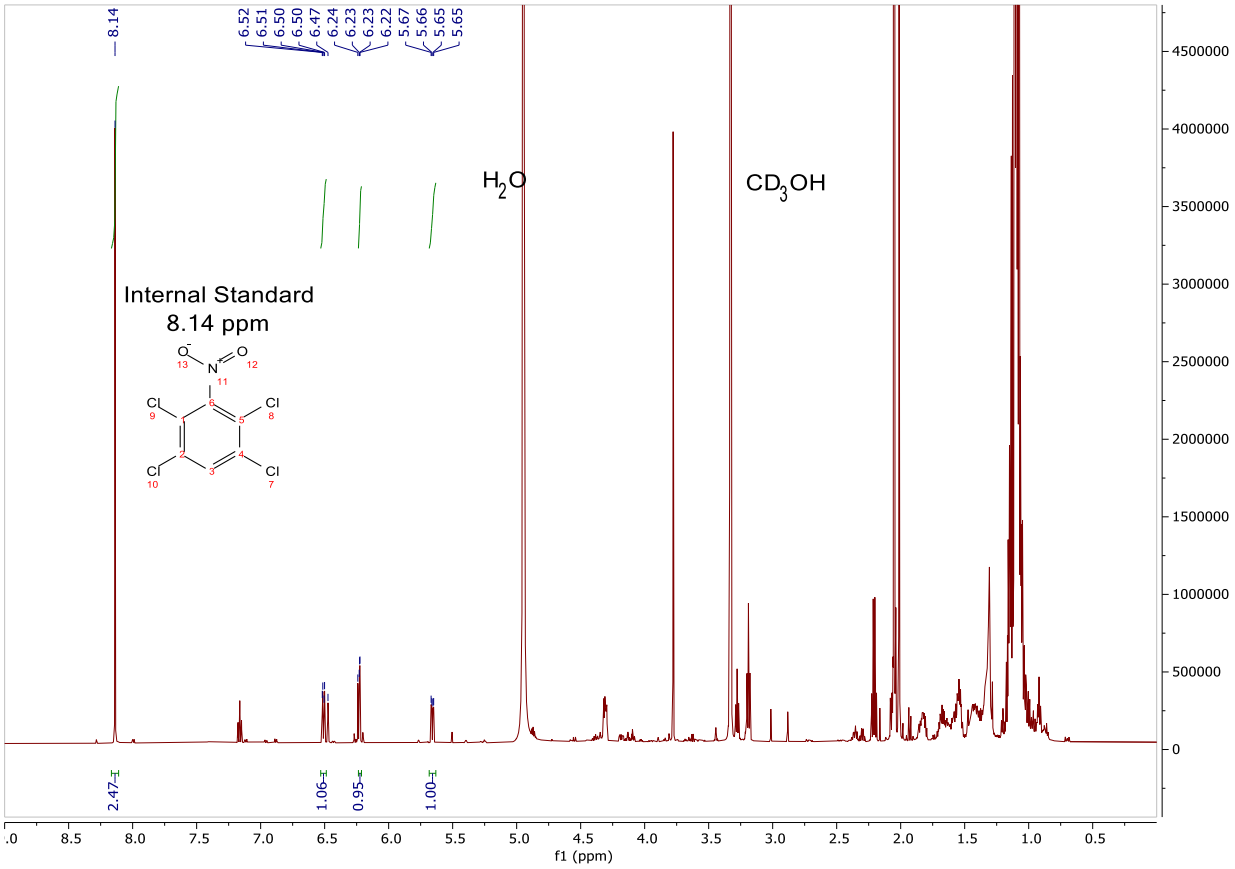


Crude ^{1}H NMR of cleaved starting material. Internal standard used to calculate resin loading.

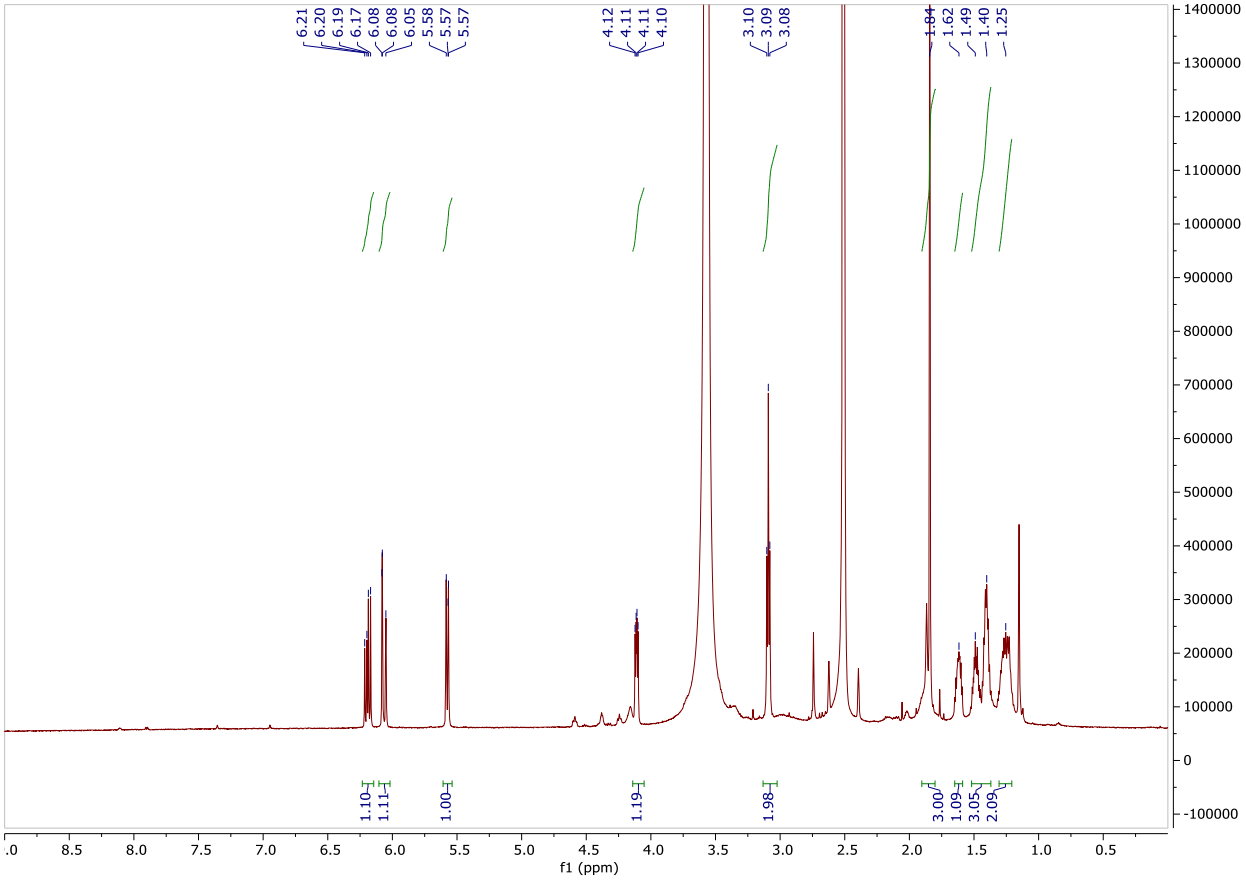


(S)-2-acetamido-6-acrylamidohexanamide (S16)

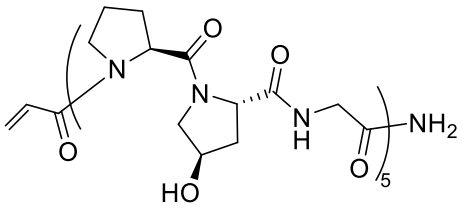
^{1}H NMR (400 MHz, CD_3OD)



Crude ^{1}H NMR of cleaved starting material. Internal standard used to calculate resin loading.

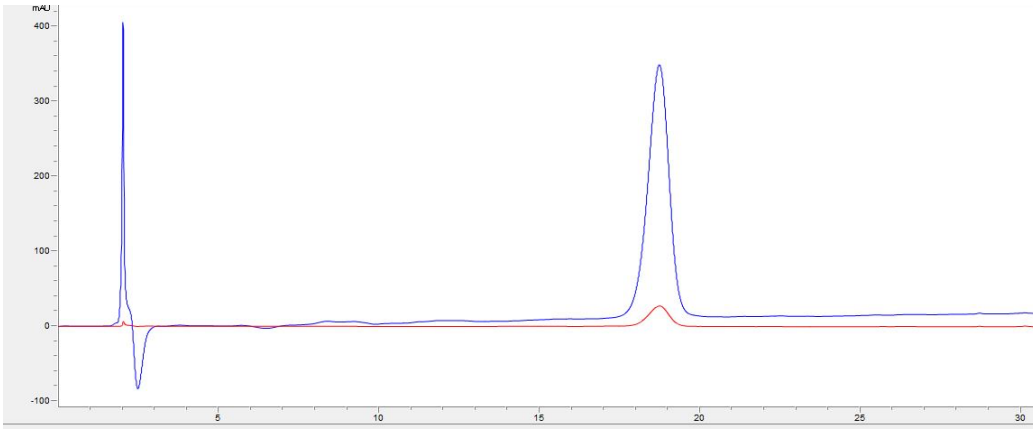


^{1}H NMR of purified starting material.



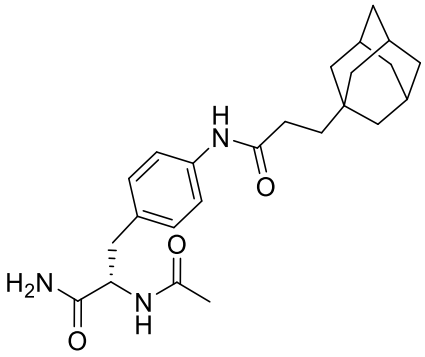
(S17)

Compound was purified on preparatory HPLC using a linear gradient of 2-25 % MeCN/H_2O (0.1 % TFA) over 30 min. (R_t = 19.6 min).



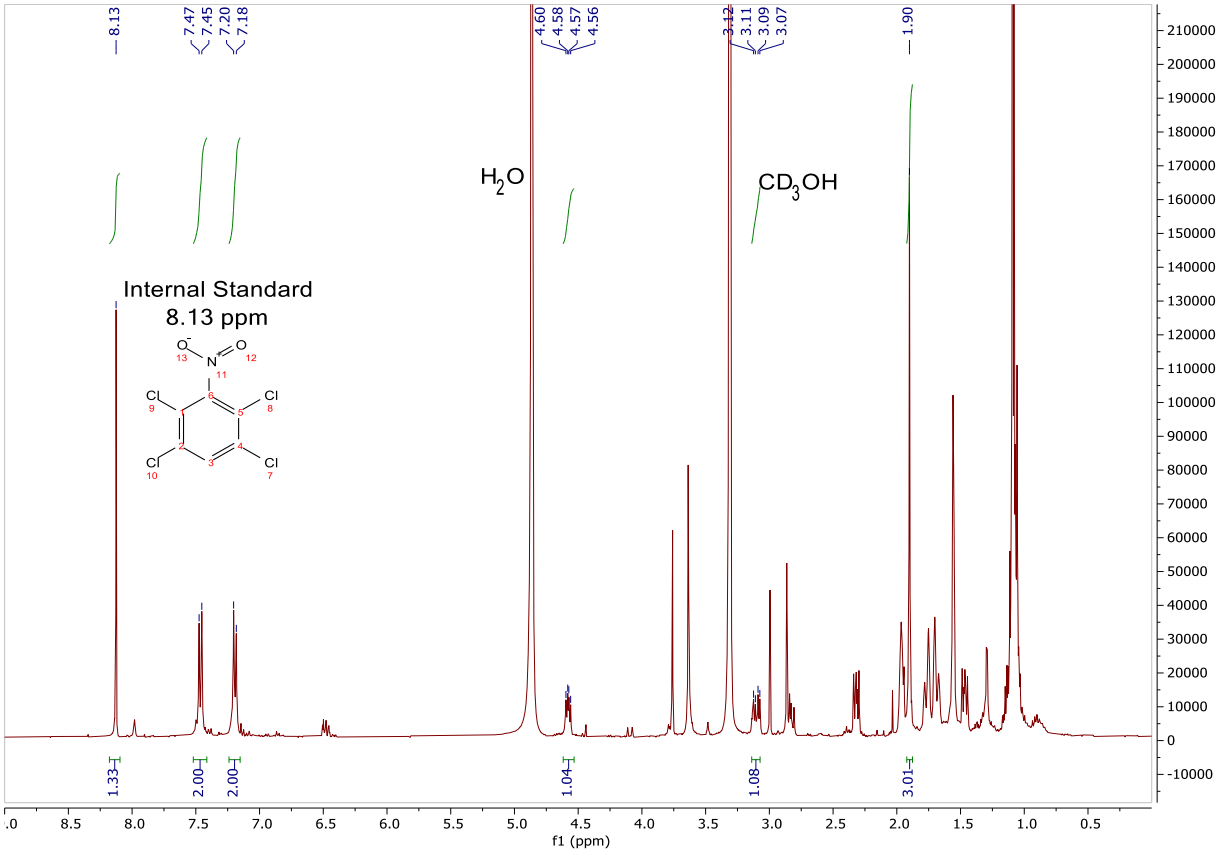
Reinjection of compound on analytical HPLC using a linear gradient of 2-25 % (0.1 % TFA) over 30 min at 254 nm (red) and 214 nm (blue). R_t = 18.72 min.

For compounds S18-S44, crude quantitative ^{1}H NMR with internal standard shown first, followed by ^{1}H NMR and HPLC reinjection of pure material:



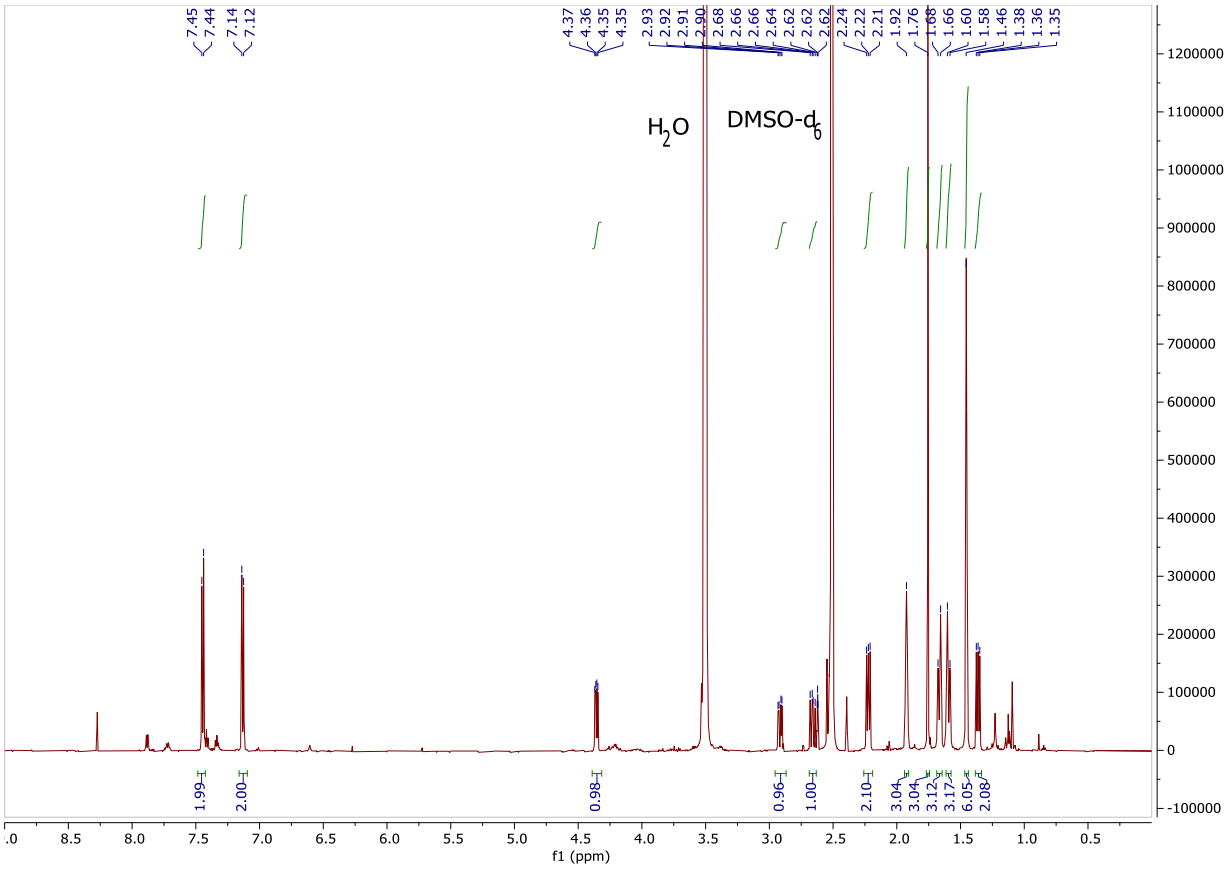
(2*R*)-2-Acetamido-3-(4-(3-((1*S*,3*S*)-adamantan-1-yl)propanamido)phenyl)propenamide (S18)

^{1}H NMR (400 MHz, CD_3OD)

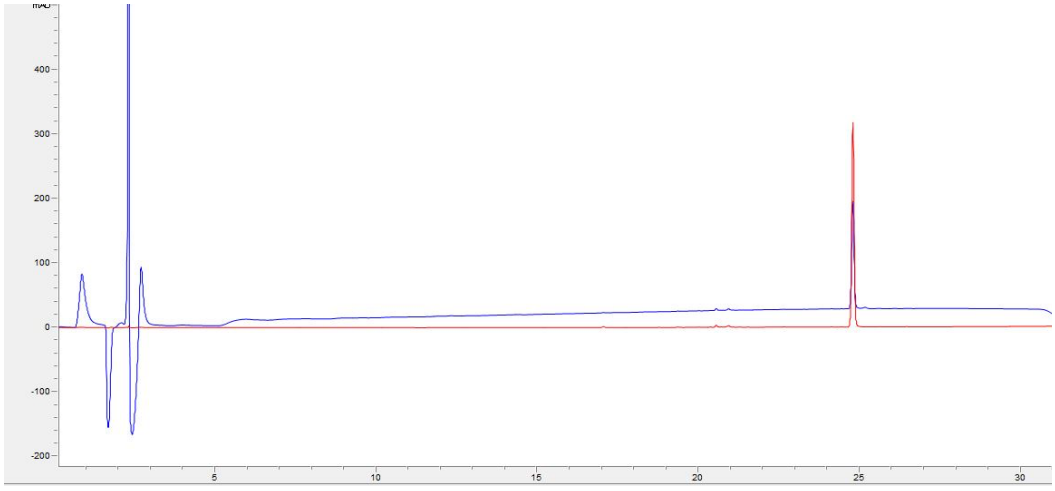


Crude ^{1}H NMR of product off-resin. Internal standard used to calculate conversion and yield.

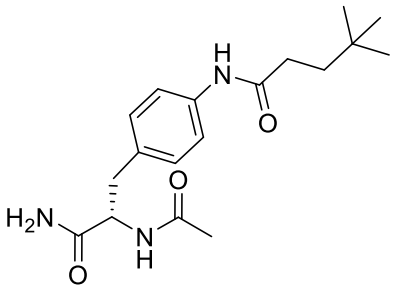
^{1}H NMR (400 MHz, CD_3OD)



^{1}H NMR of purified compound.

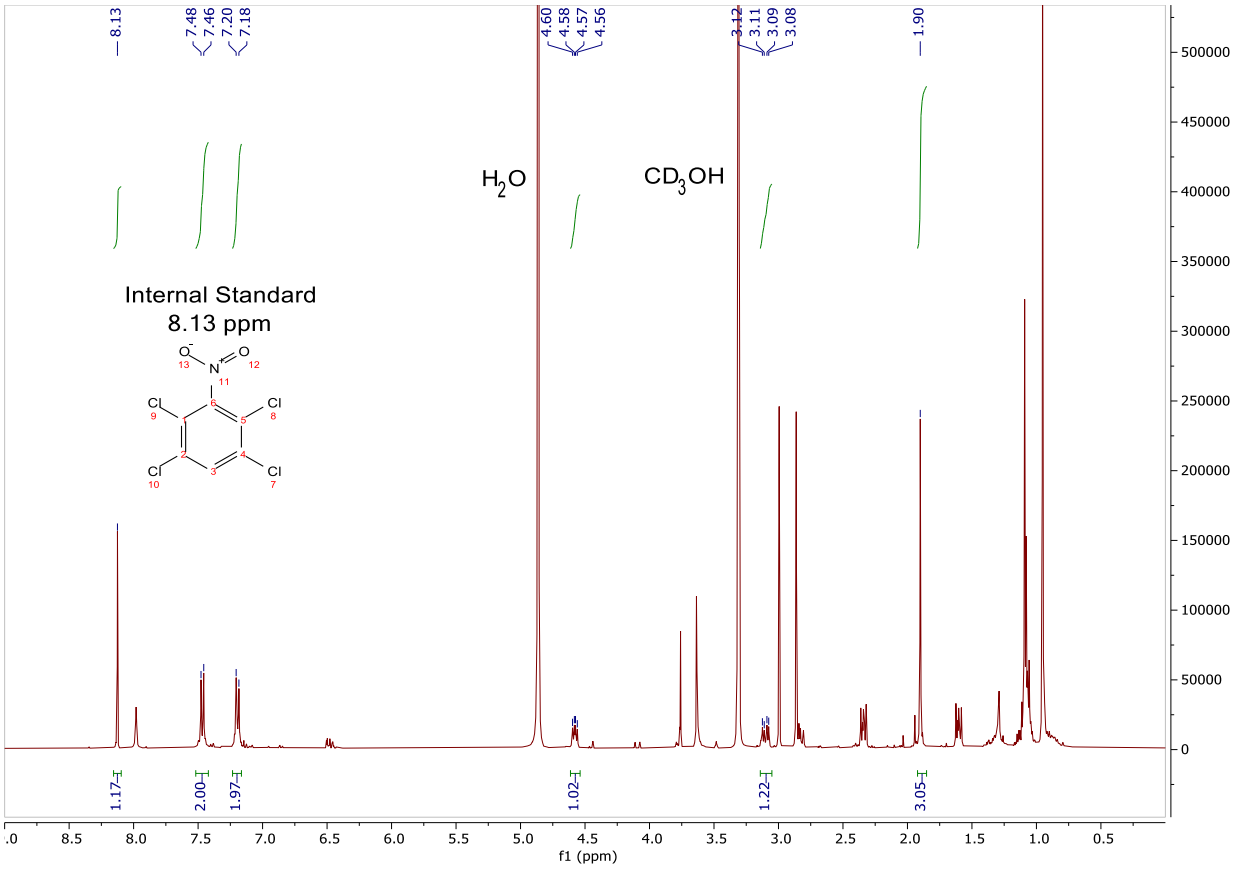


Chromatogram after HPLC purification. Reinjection of compound on analytical HPLC using a linear gradient of 10-60 % MeCN/H_2O (0.1 % TFA) over 30 min at 254 nm (red) and 214 nm (blue).



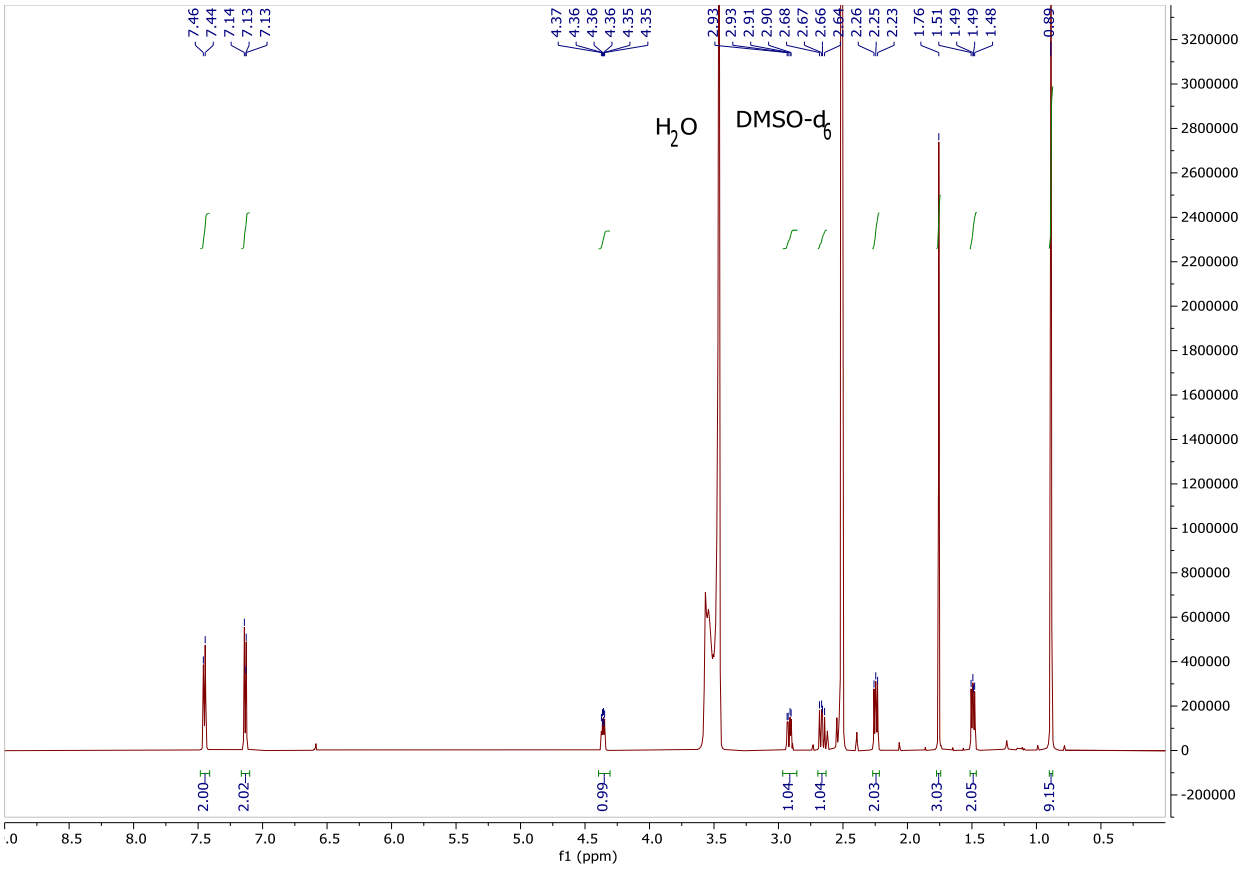
(*R*)-*N*-(4-(2-Acetamido-3-amino-3-oxopropyl)phenyl)-4,4-dimethylpentanamide (S19)

^{1}H NMR (400 MHz, CD_3OD)

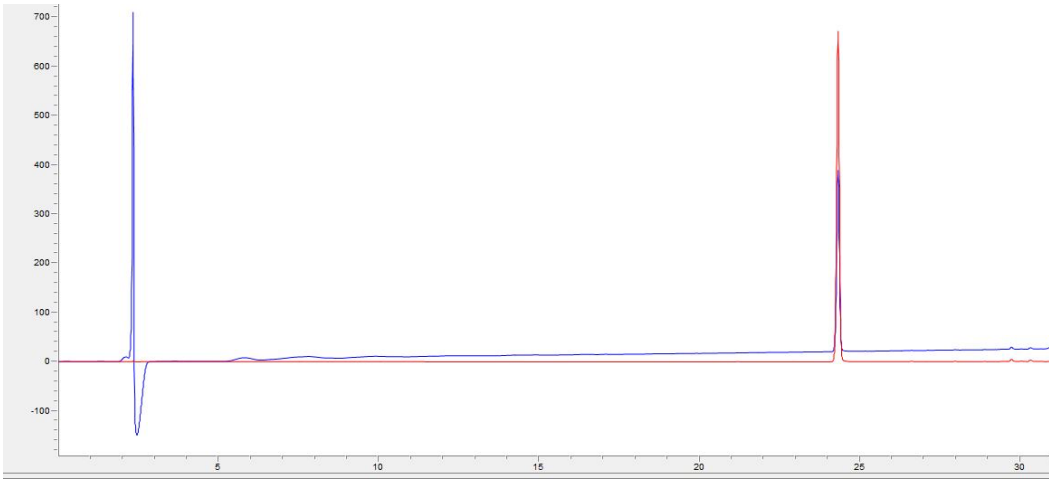


Crude ^{1}H NMR of product off-resin. Internal standard used to calculate conversion and yield.

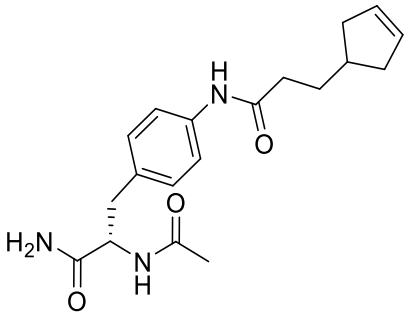
^{1}H NMR (400 MHz, DMSO-d*6*)



^{1}H NMR of purified compound.

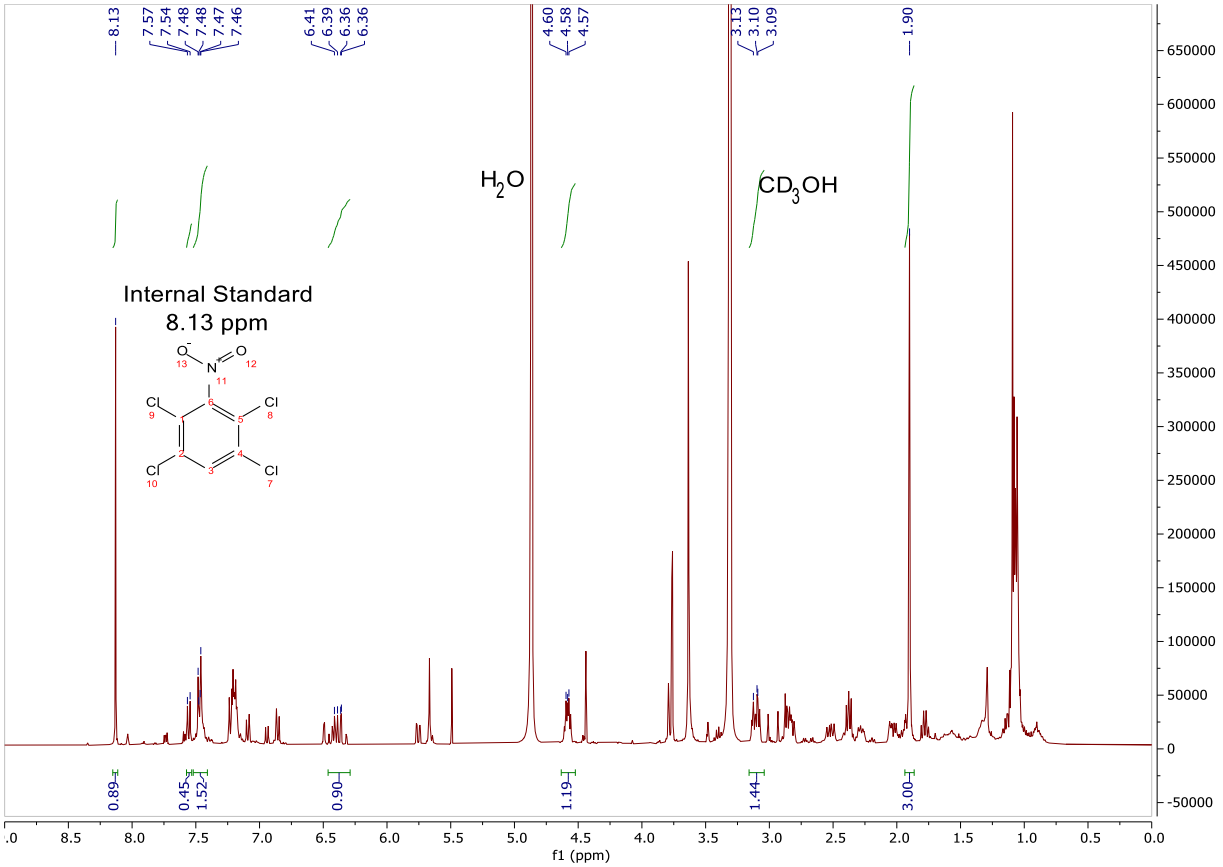


Chromatogram after HPLC purification. Reinjection of compound on analytical HPLC using a linear gradient of 5-40 % MeCN/H_2O (0.1 % TFA) over 30 min at 254 nm (red) and 214 nm (blue).



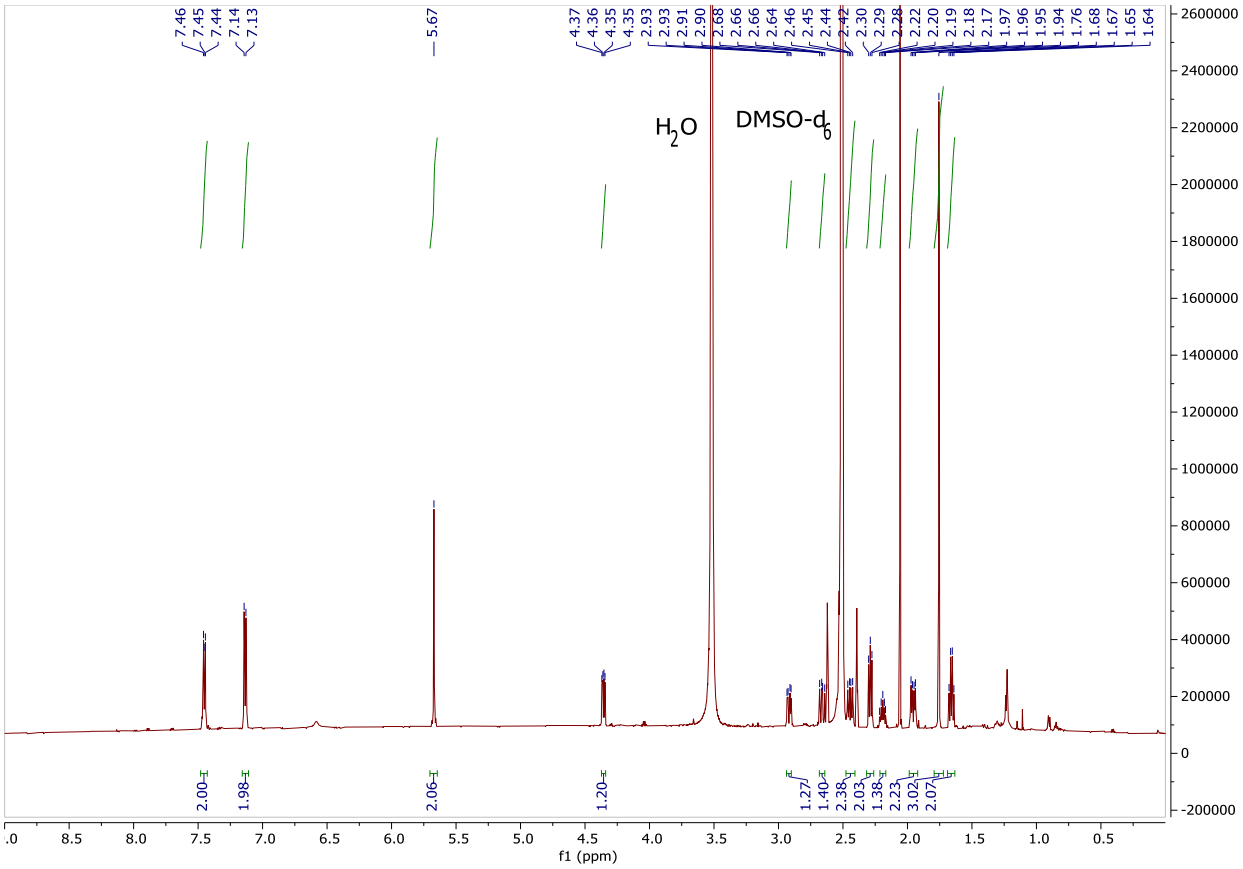
(*R*)-2-Acetamido-3-(4-(3-(cyclopent-3-en-1-yl)propanamido)phenyl)propanamide (S20)

^{1}H NMR (400 MHz, CD_3OD)

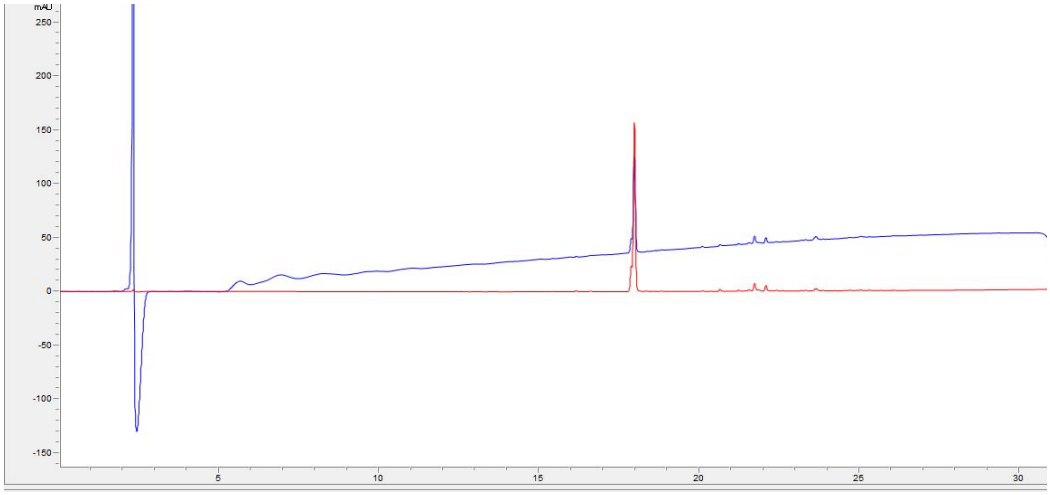


Crude ^{1}H NMR of product off-resin. Internal standard used to calculate conversion and yield.

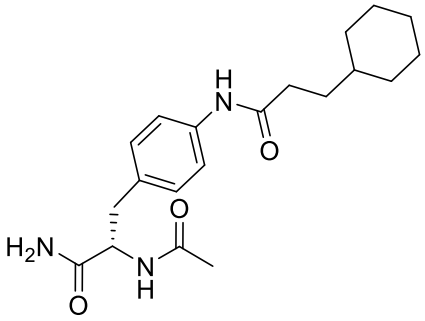
^{1}H NMR (400 MHz, DMSO-*d6*)



^{1}H NMR of purified compound.

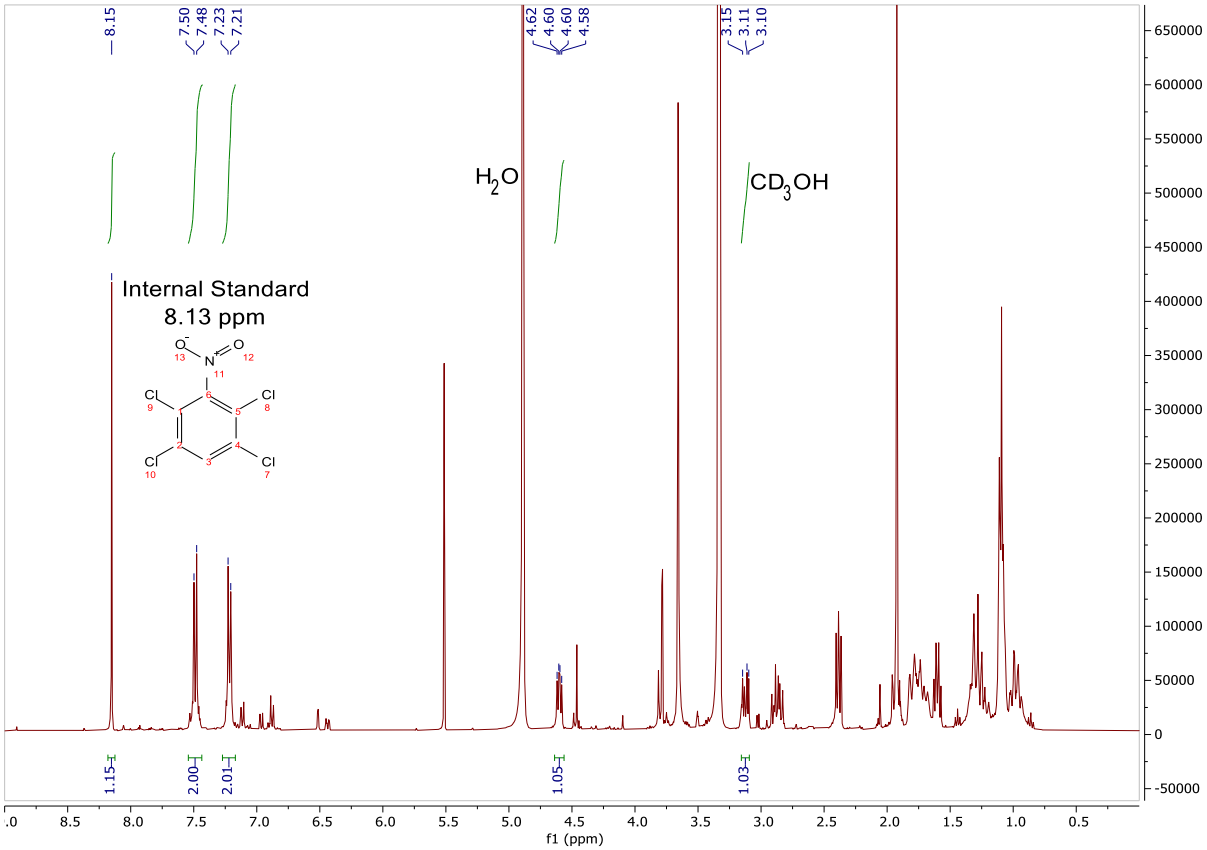


Chromatogram after HPLC purification. Reinjection of compound on analytical HPLC using a linear gradient of 5-60 % MeCN/H_2O (0.1 % TFA) over 30 min at 254 nm (red) and 214 nm (blue).



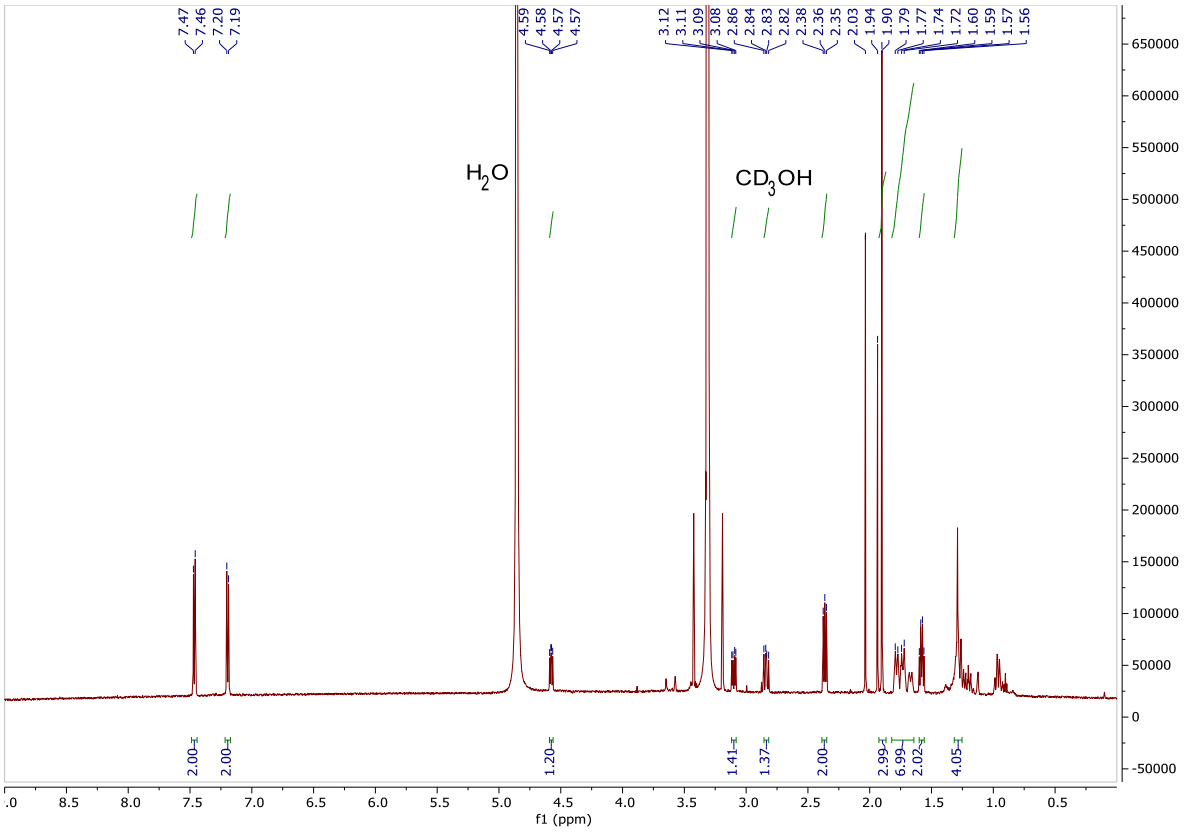
(*R*)-2-Acetamido-3-(4-(3-cyclohexylpropanamido)phenyl)propanamide (S21)

^{1}H NMR (400 MHz, CD_3OD)

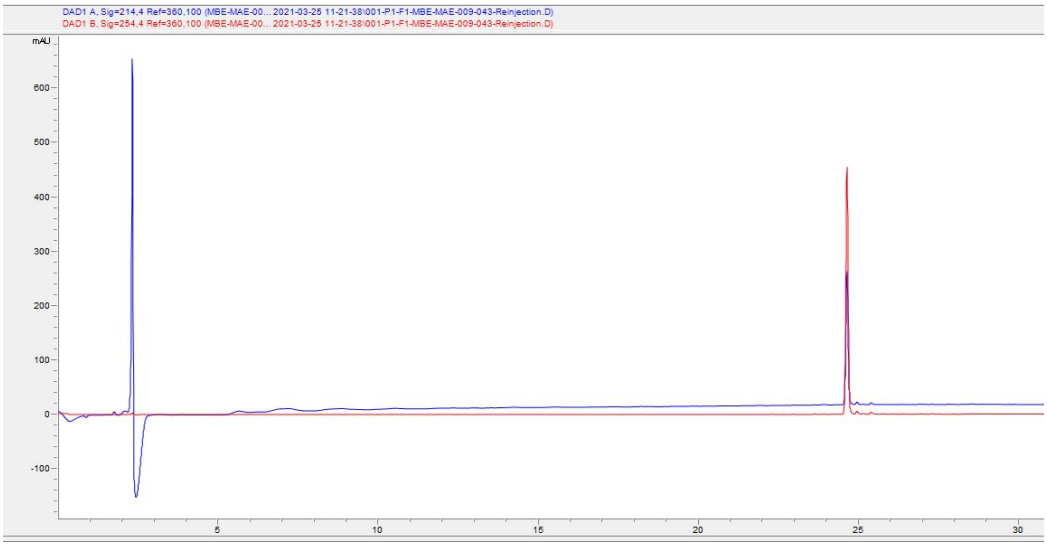


Crude ^{1}H NMR of product off-resin. Internal standard used to calculate conversion and yield.

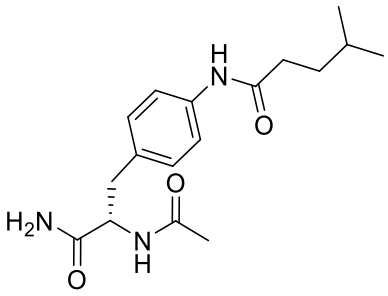
^{1}H NMR (400 MHz, CD_3OD)



^{1}H NMR of purified compound.

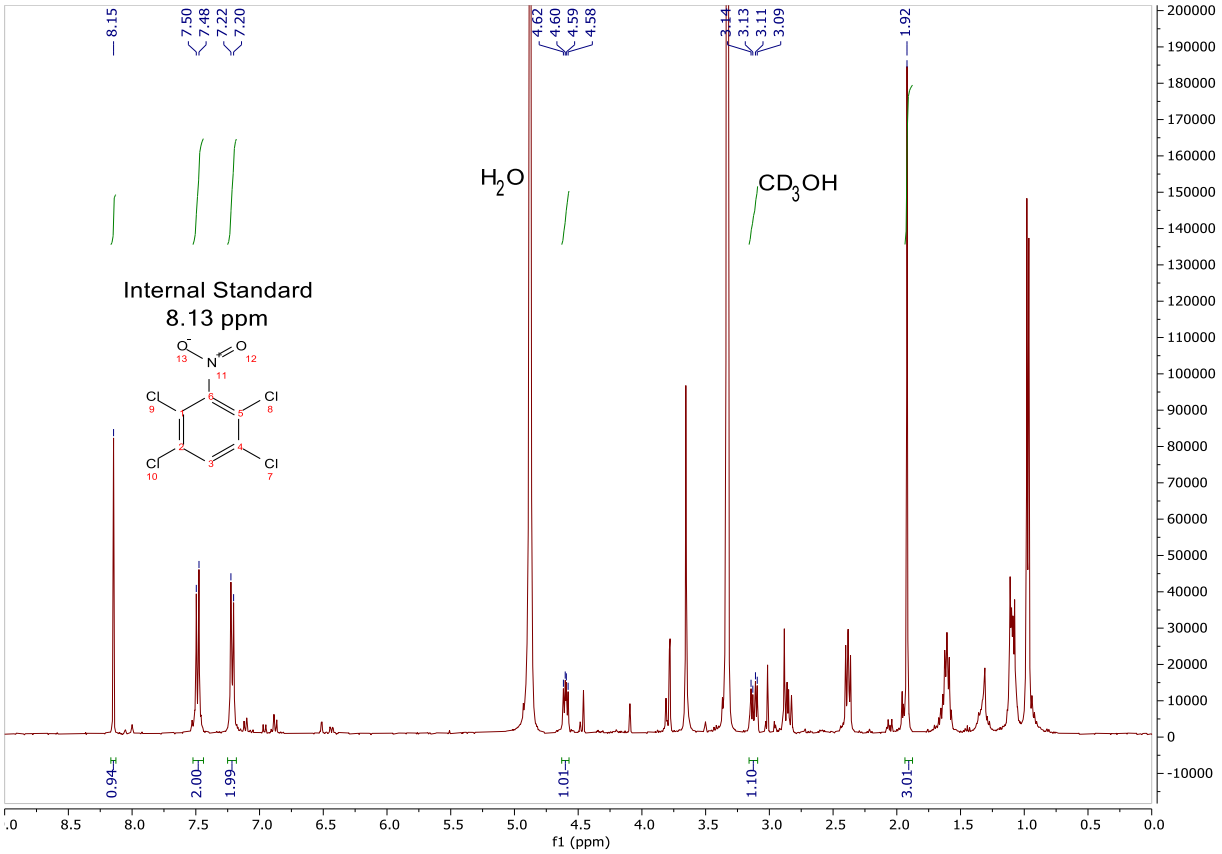


Chromatogram after HPLC purification. Reinjection of compound on analytical HPLC using a linear gradient of 5-50 % MeCN/H_2O (0.1 % TFA) over 30 min at 254 nm (red) and 214 nm (blue).



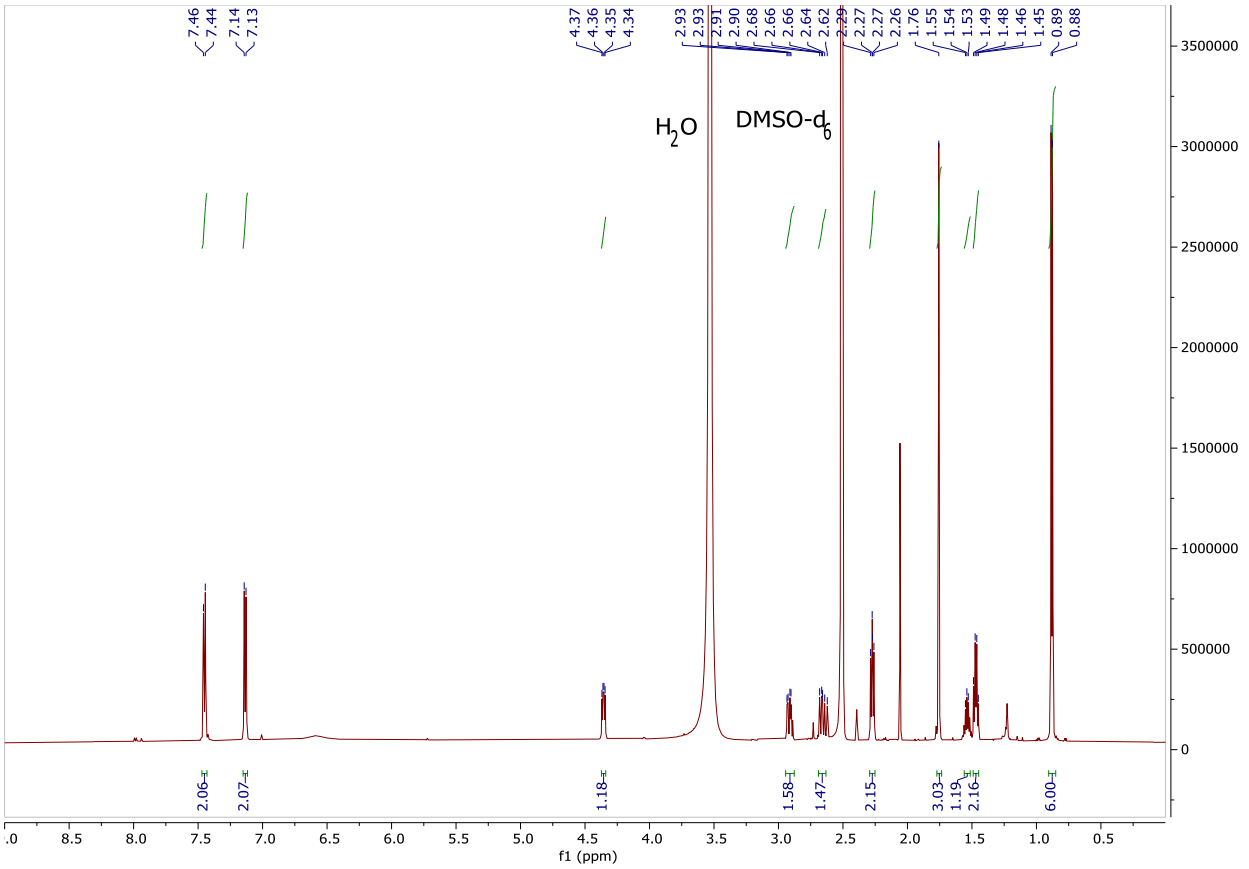
(*R*)-*N*-(4-(2-Acetamido-3-amino-3-oxopropyl)phenyl)-4-methylpentanamide (S22)

^{1}H NMR (400 MHz, CD_3OD)

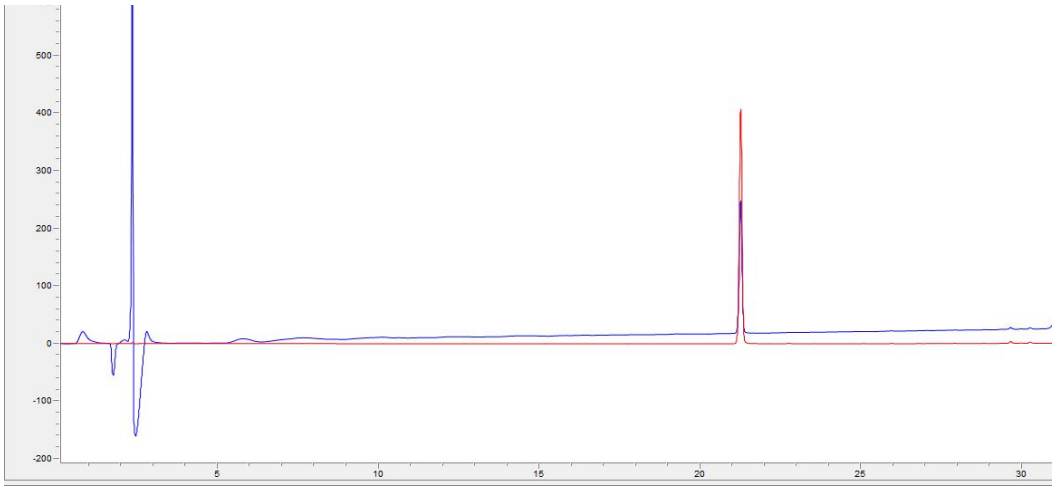


Crude ^{1}H NMR of product off-resin. Internal standard used to calculate conversion and yield.

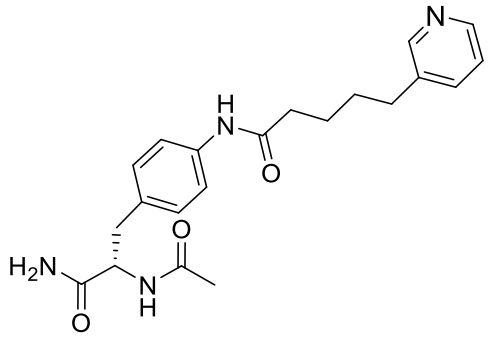
^{1}H NMR (400 MHz, DMSO-*d6*)



^{1}H NMR of purified compound.

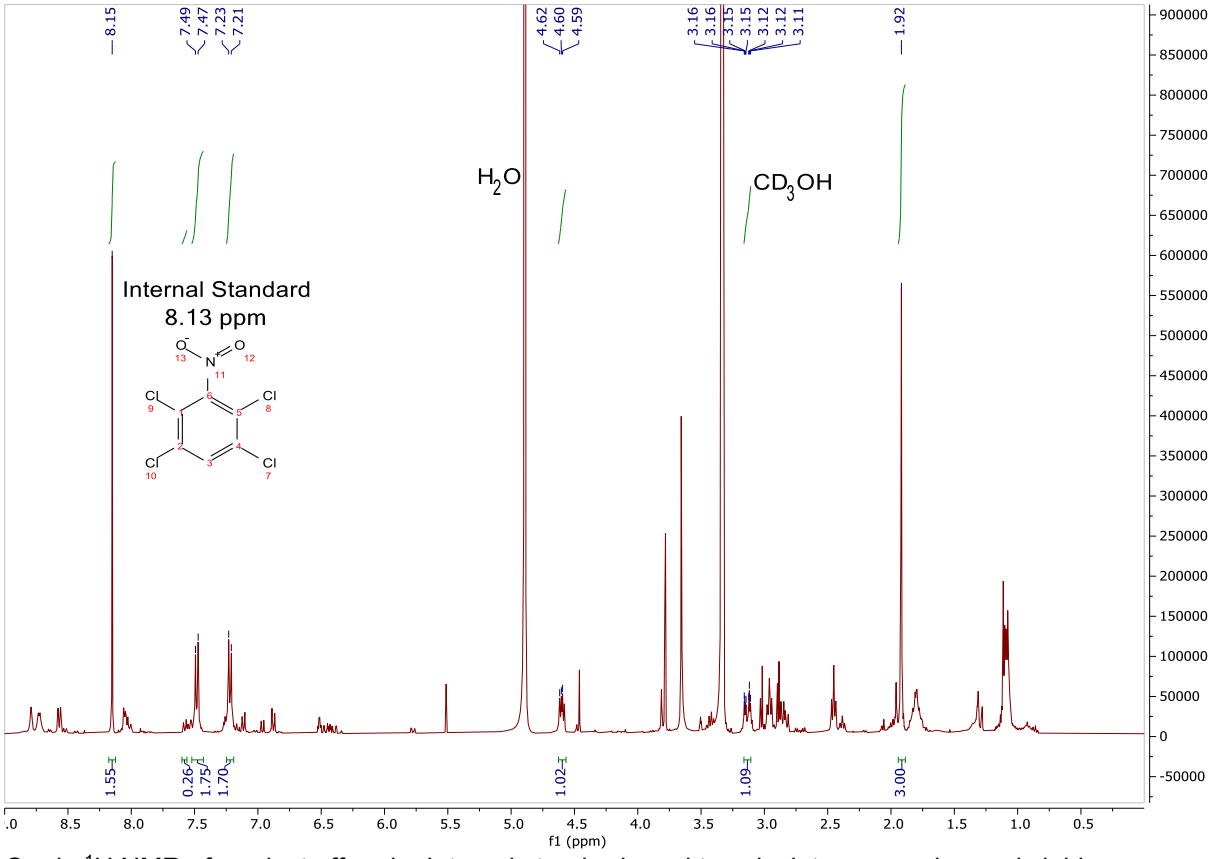


Chromatogram after HPLC purification. Reinjection of compound on analytical HPLC using a linear gradient of 5-40 % MeCN/H_2O (0.1 % TFA) over 30 min at 254 nm (red) and 214 nm (blue).



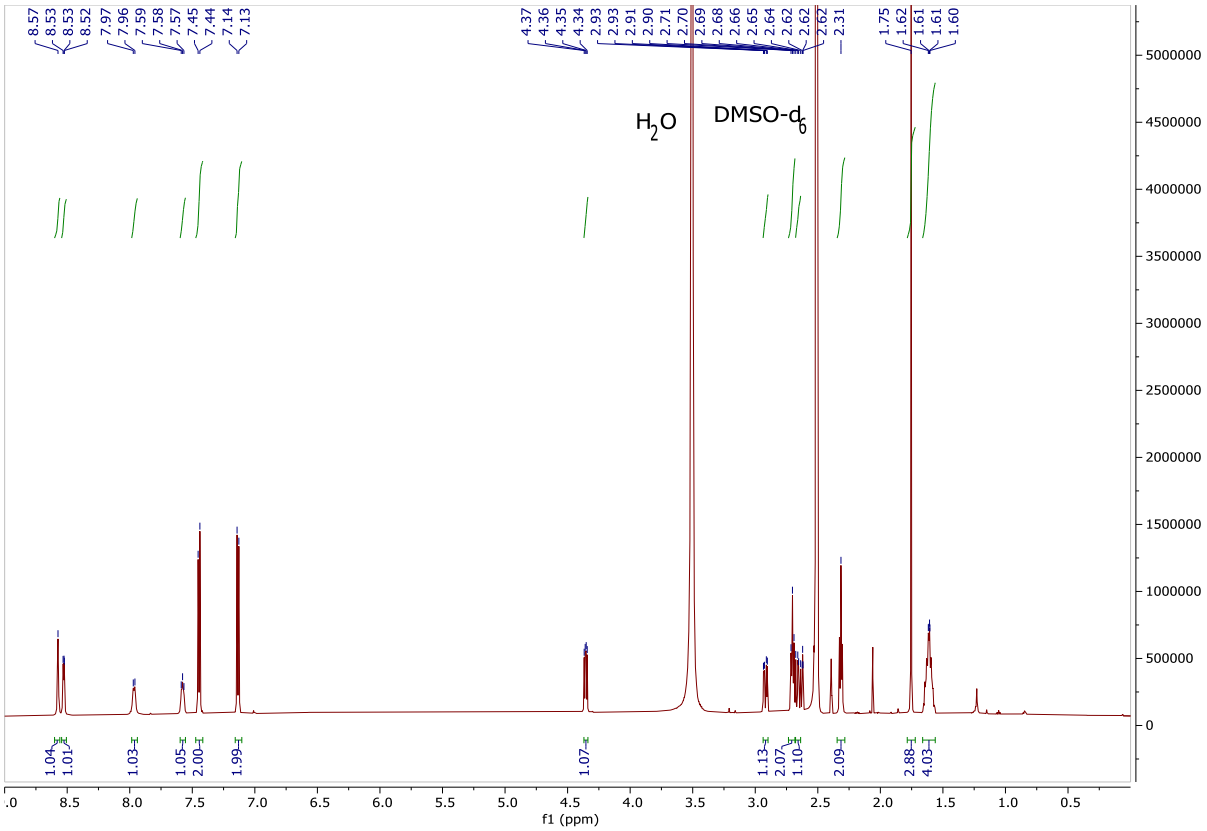
(*R*)-*N*-(4-(2-Acetamido-3-amino-3-oxopropyl)phenyl)-5-(pyridin-3-yl)pentanamide (S23)

^{1}H NMR (400 MHz, CD_3OD)

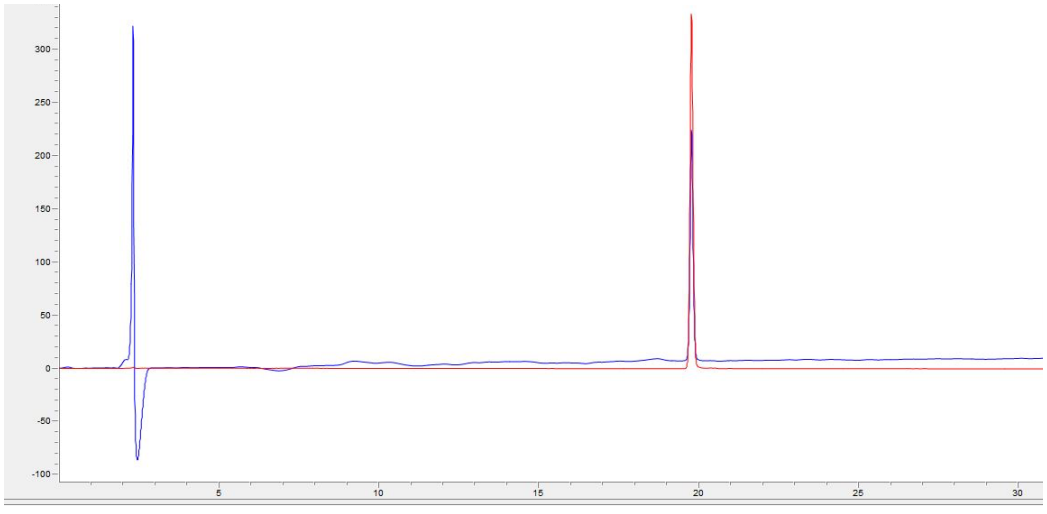


Crude ^{1}H NMR of product off-resin. Internal standard used to calculate conversion and yield.

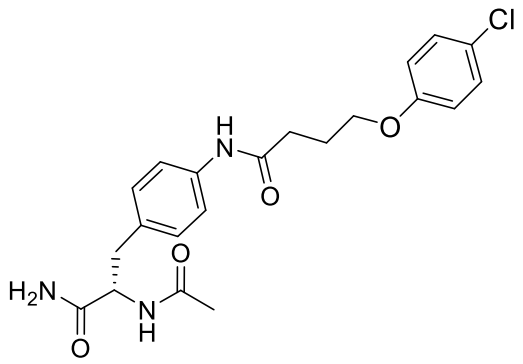
^{1}H NMR (400 MHz, DMSO-*d6*)



^{1}H NMR of purified compound.

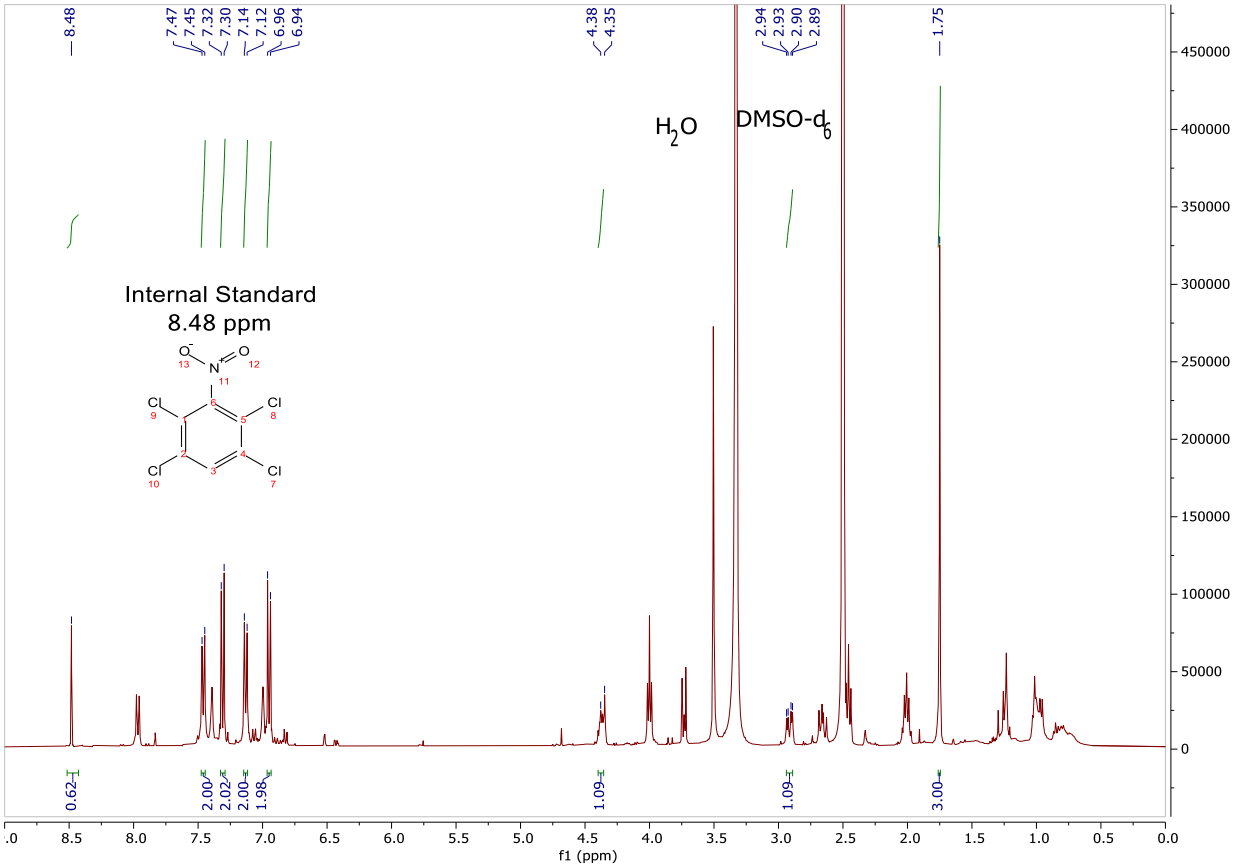


Chromatogram after HPLC purification. Reinjection of compound on analytical HPLC using a linear gradient of 2-20 % MeCN/H_2O (0.1 % TFA) over 30 min at 254 nm (red) and 214 nm (blue).



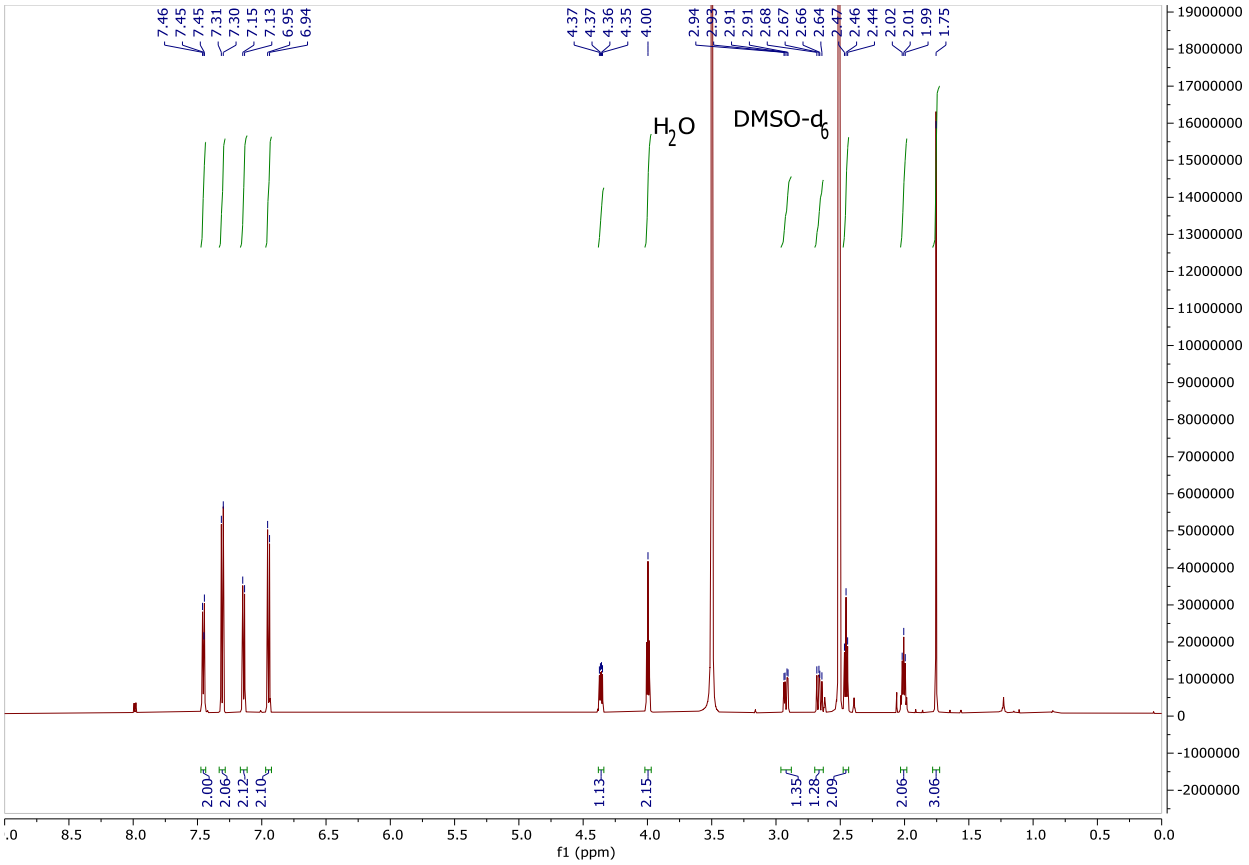
(*R*)-*N*-(4-(2-Acetamido-3-amino-3-oxopropyl)phenyl)-4-(4-chlorophenoxy)butanamide (S24)

^{1}H NMR (400 MHz, DMSO-*d6*)

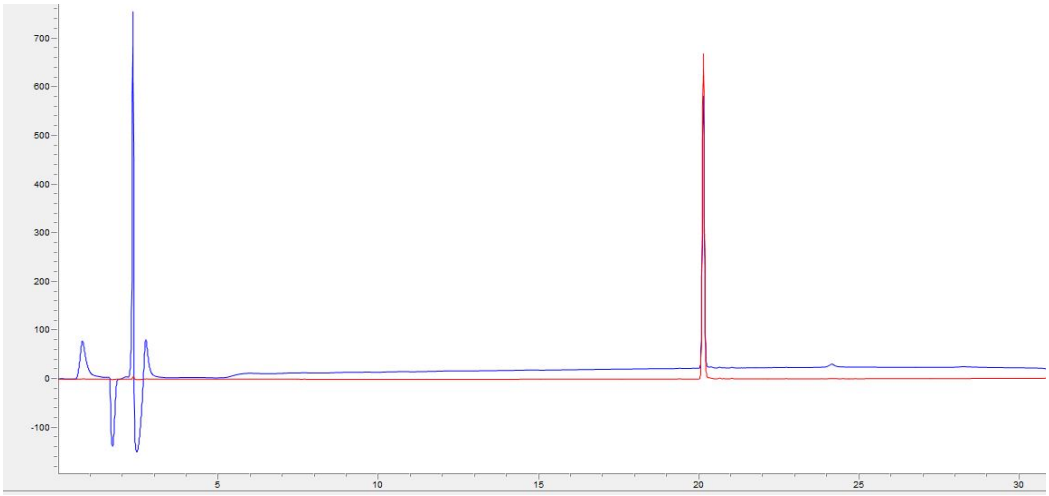


Crude ^{1}H NMR of product off-resin. Internal standard used to calculate conversion and yield.

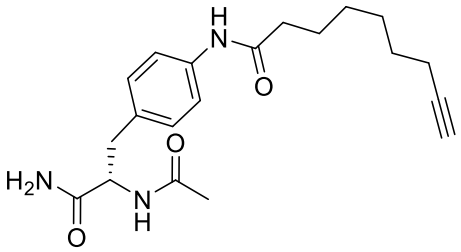
^{1}H NMR (400 MHz, DMSO-*d6*)



^{1}H NMR of purified compound.

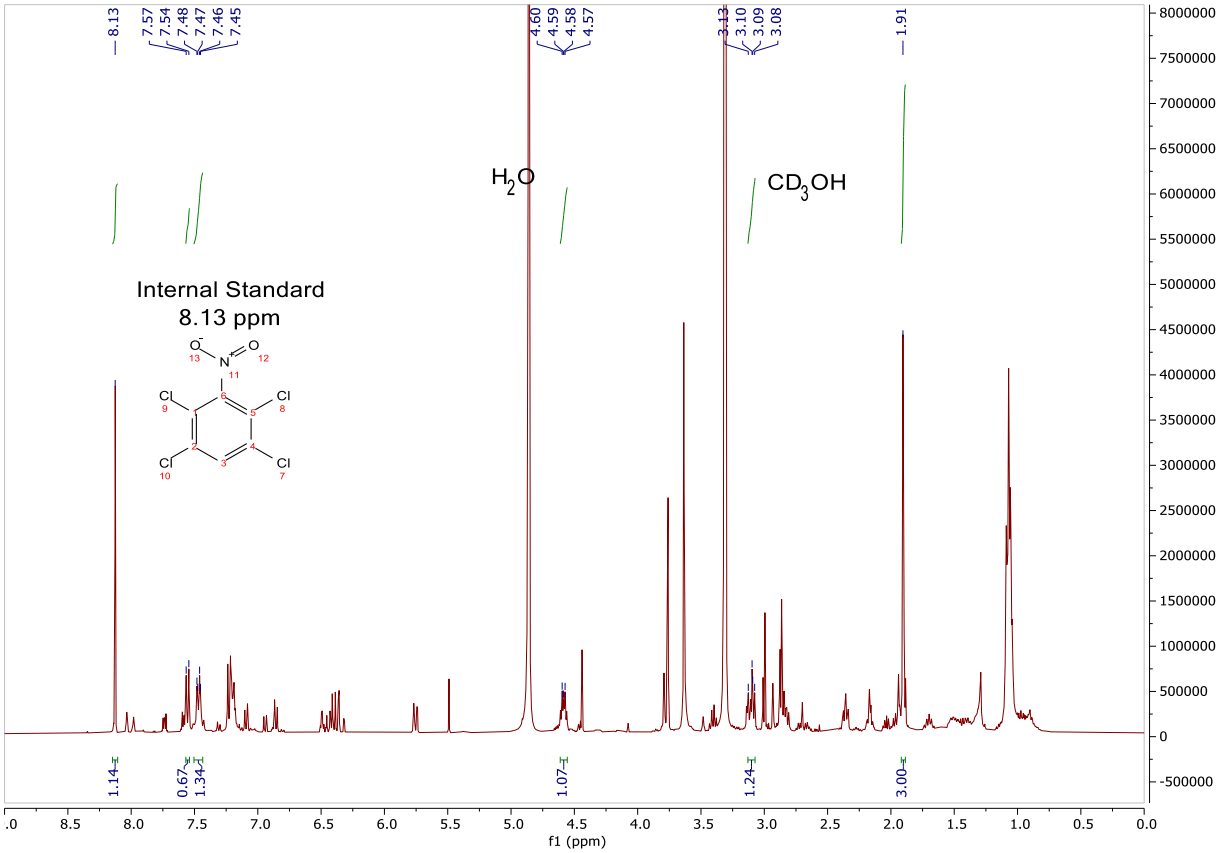


Chromatogram after HPLC purification. Reinjection of compound on analytical HPLC using a linear gradient of 10-60 % MeCN/H_2O (0.1 % TFA) over 30 min at 254 nm (red) and 214 nm (blue).



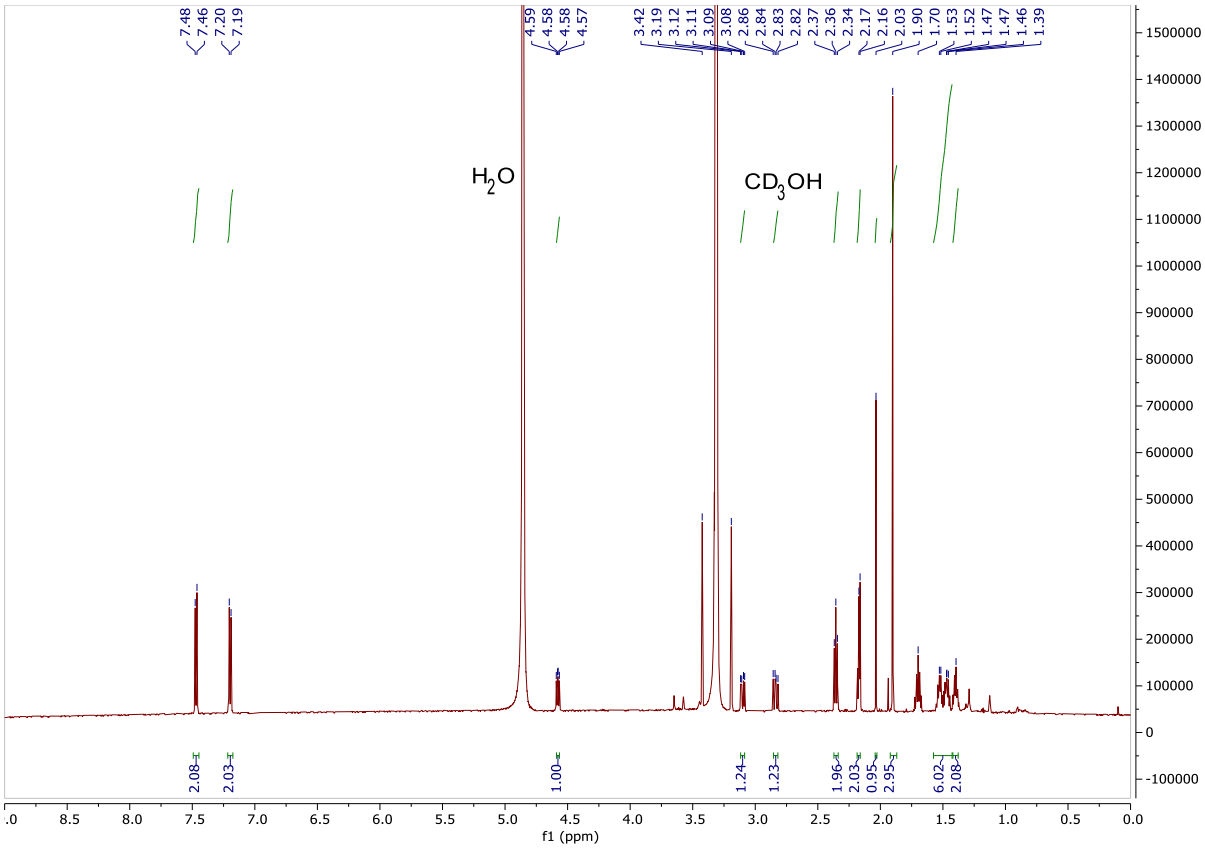
(*R*)-*N*-(4-(2-Acetamido-3-amino-3-oxopropyl)phenyl)non-8-ynamide (S25)

^{1}H NMR (400 MHz, CD_3OD)

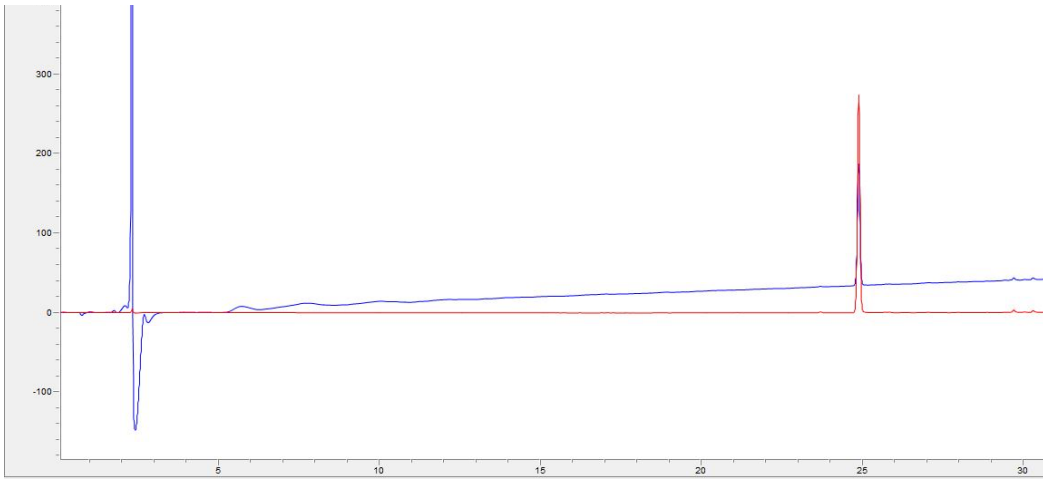


Crude ^{1}H NMR of product off-resin. Internal standard used to calculate conversion and yield.

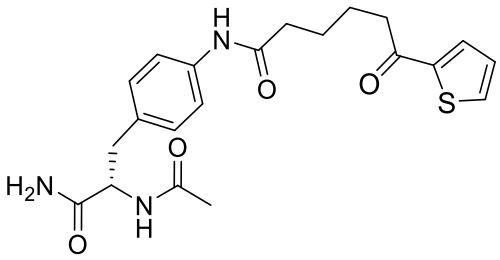
^{1}H NMR (400 MHz, CD_3OD)



^{1}H NMR of purified compound.

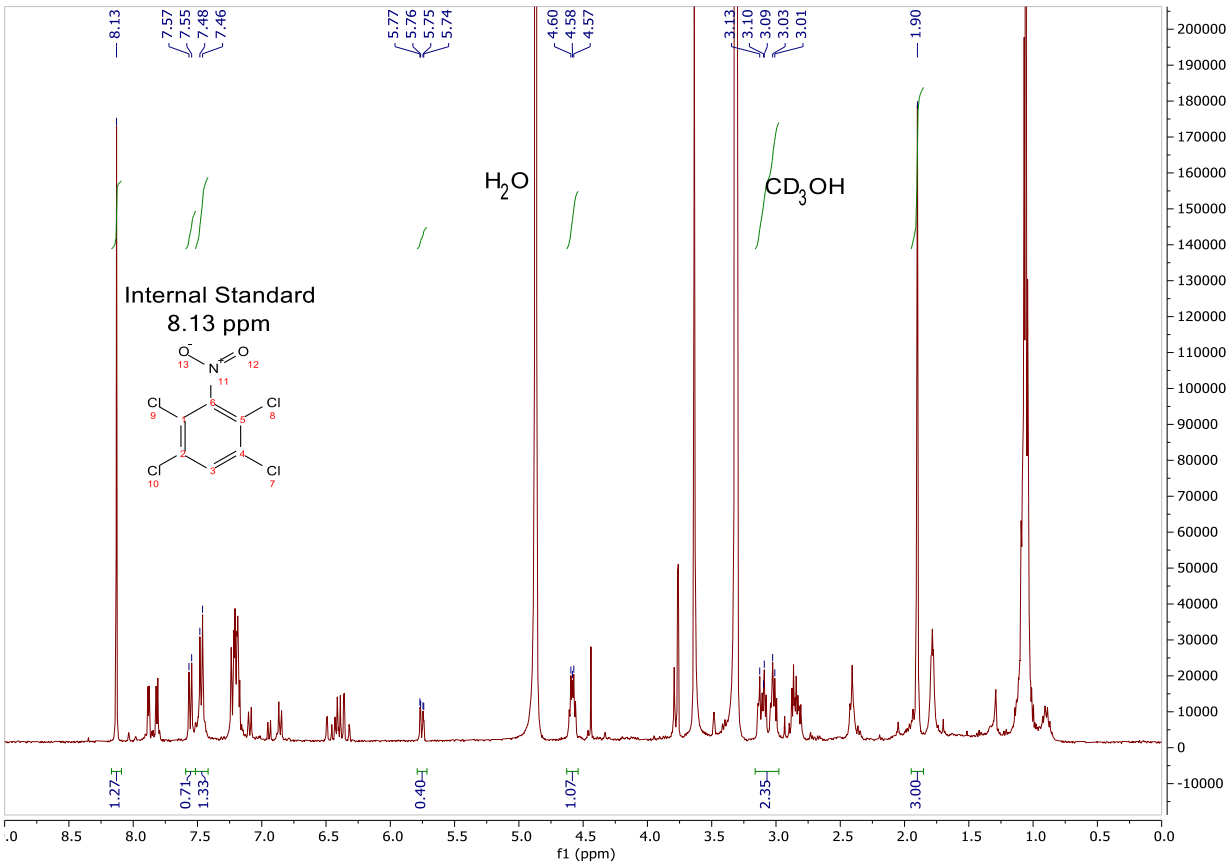


Chromatogram after HPLC purification. Reinjection of compound on analytical HPLC using a linear gradient of 5-60 % MeCN/H_2O (0.1 % TFA) over 30 min at 254 nm (red) and 214 nm (blue).



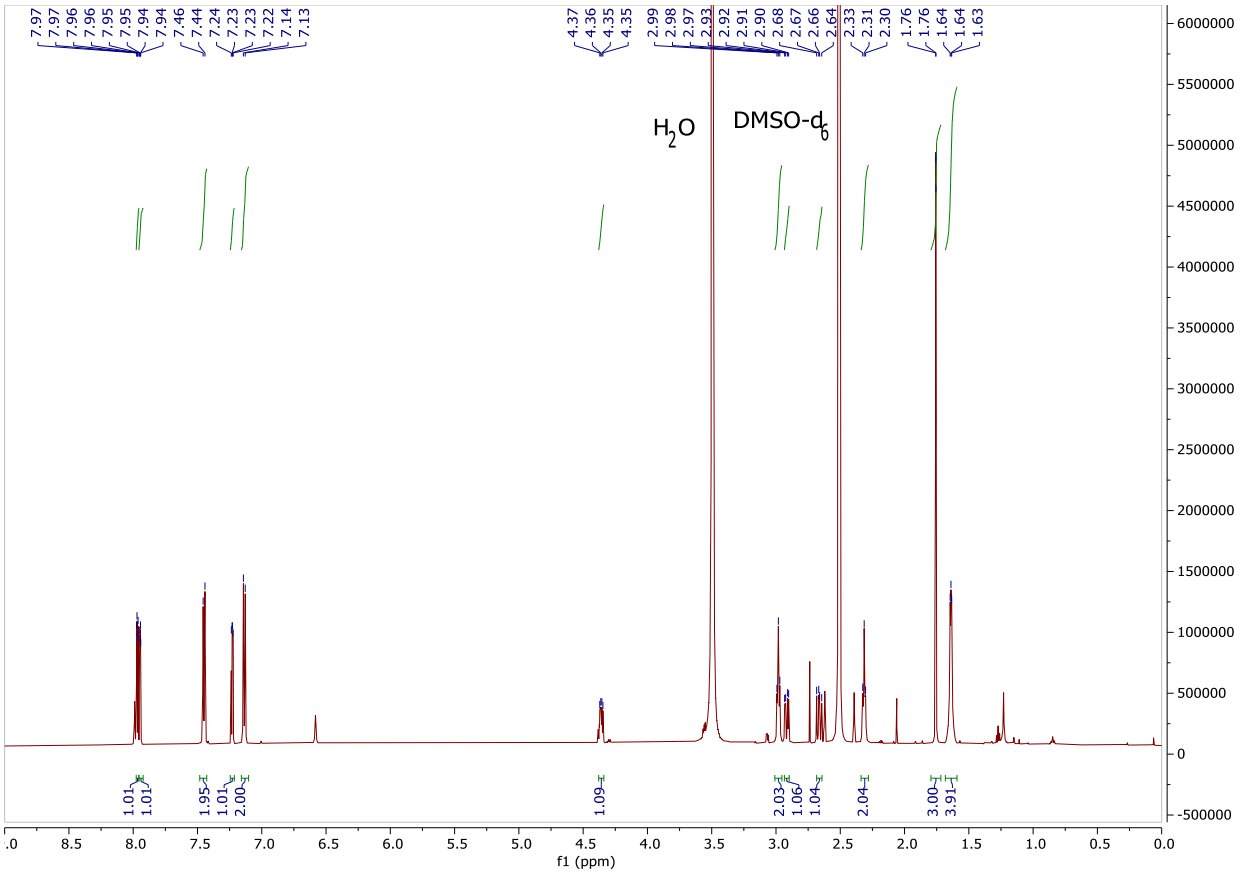
(*R*)-*N*-(4-(2-Acetamido-3-amino-3-oxopropyl)phenyl)-6-oxo-6-(thiophen-2-yl)hexanamide (S26)

^{1}H NMR (400 MHz, CD_3OD)

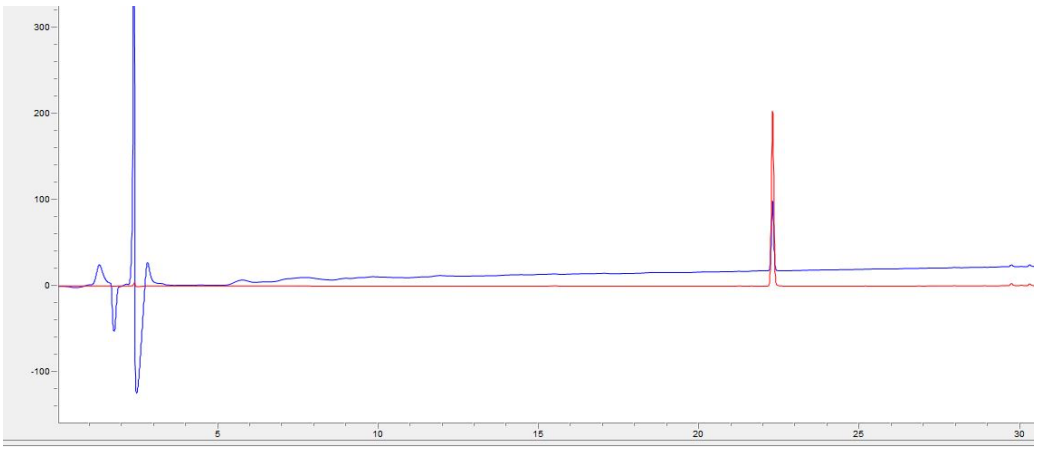


Crude ^{1}H NMR of product off-resin. Internal standard used to calculate conversion and yield.

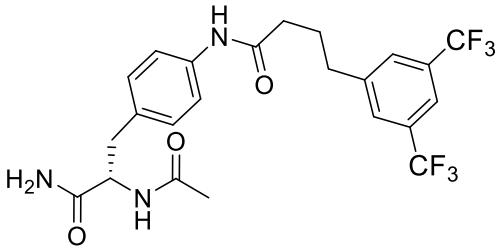
^{1}H NMR (400 MHz, DMSO-*d6*)



^{1}H NMR of purified compound.

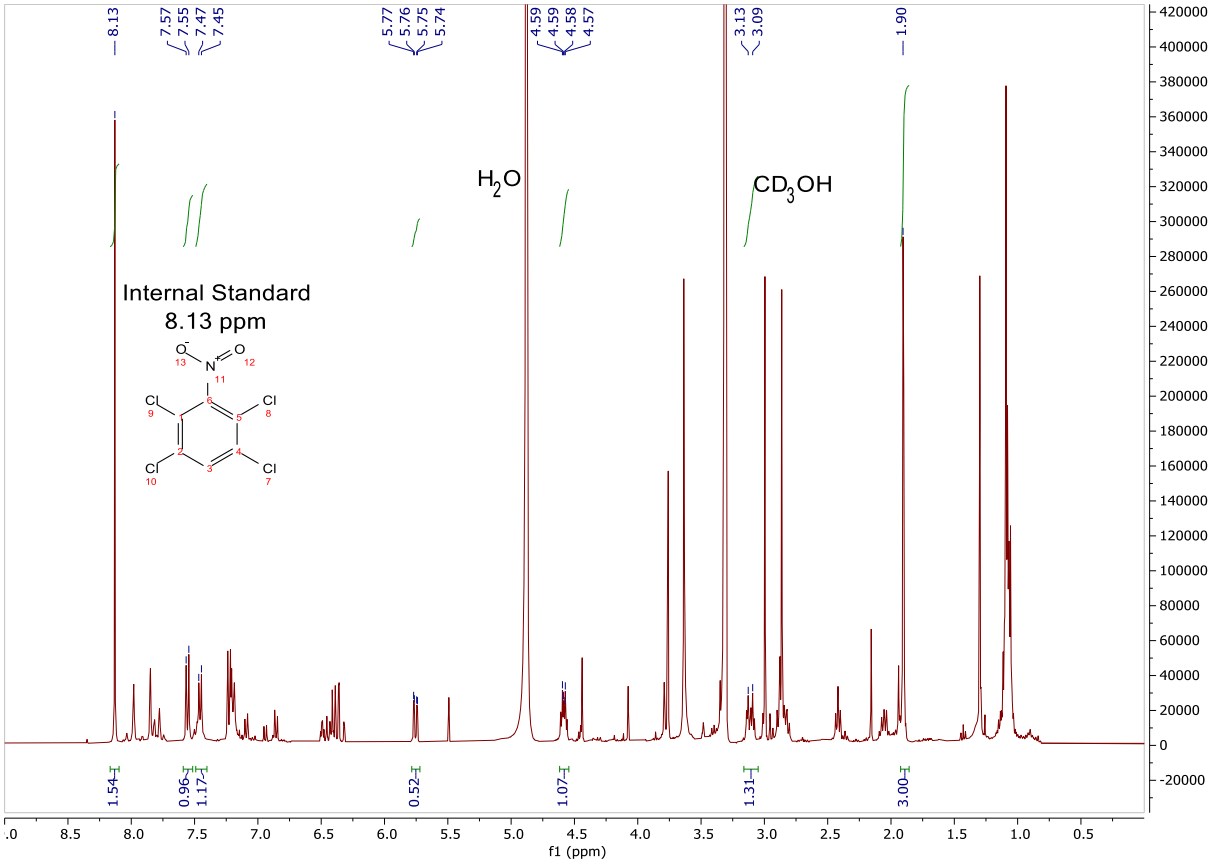


Chromatogram after HPLC purification. Reinjection of compound on analytical HPLC using a linear gradient of 5-40 % MeCN/H_2O (0.1 % TFA) over 30 min at 254 nm (red) and 214 nm (blue).



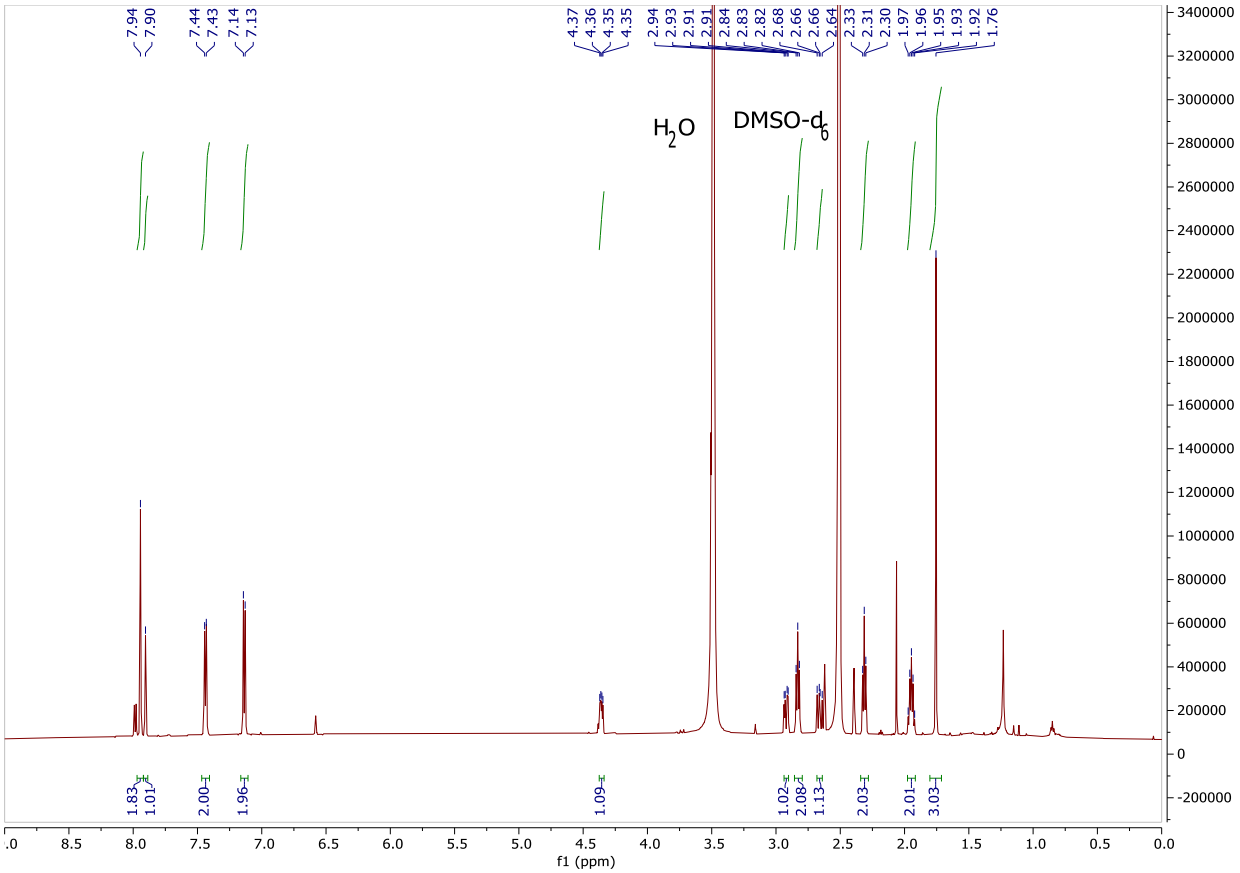
(*R*)-*N*-(4-(2-Acetamido-3-amino-3-oxopropyl)phenyl)-4-(3,5-bis(trifluoromethyl)phenyl)butanamide (S27)

^{1}H NMR (400 MHz, CD_3OD)



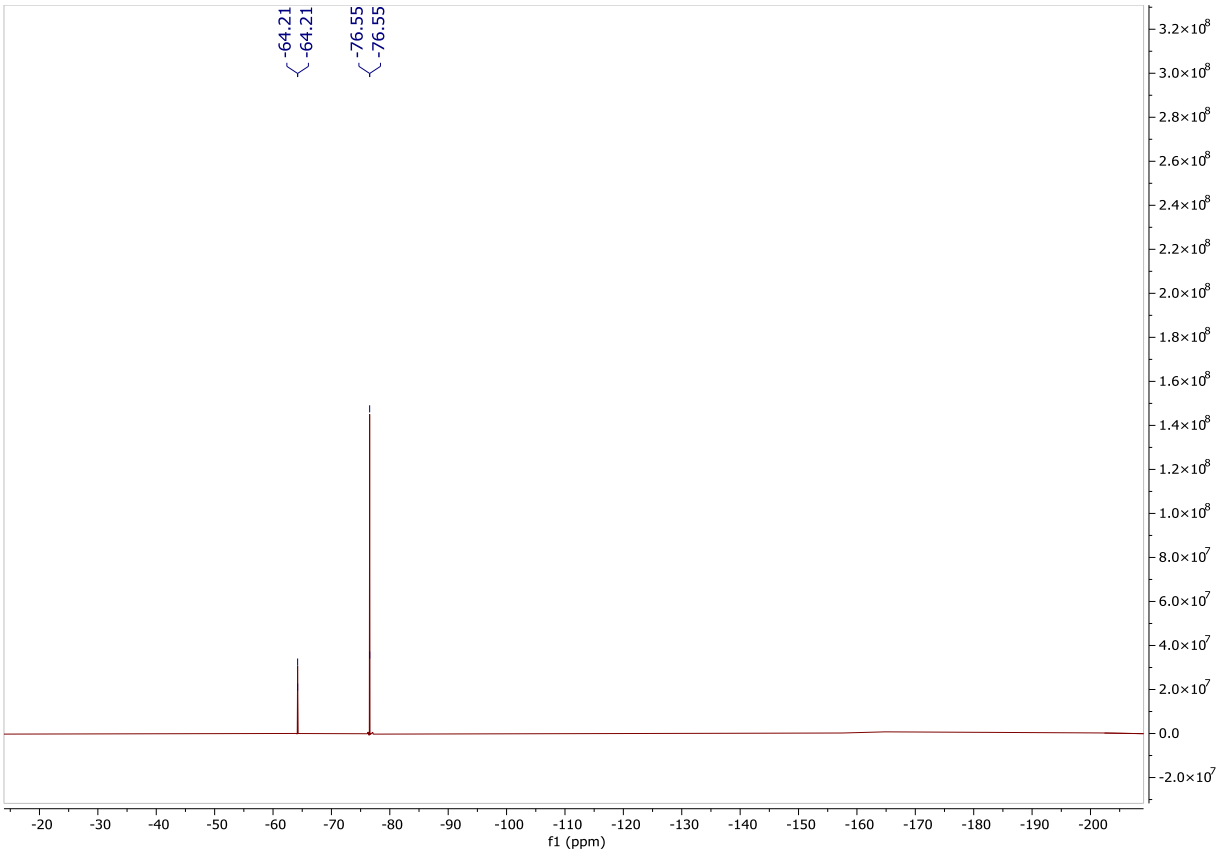
Crude ^{1}H NMR of product off-resin. Internal standard used to calculate conversion and yield.

^{1}H NMR (400 MHz, DMSO-*d6*)

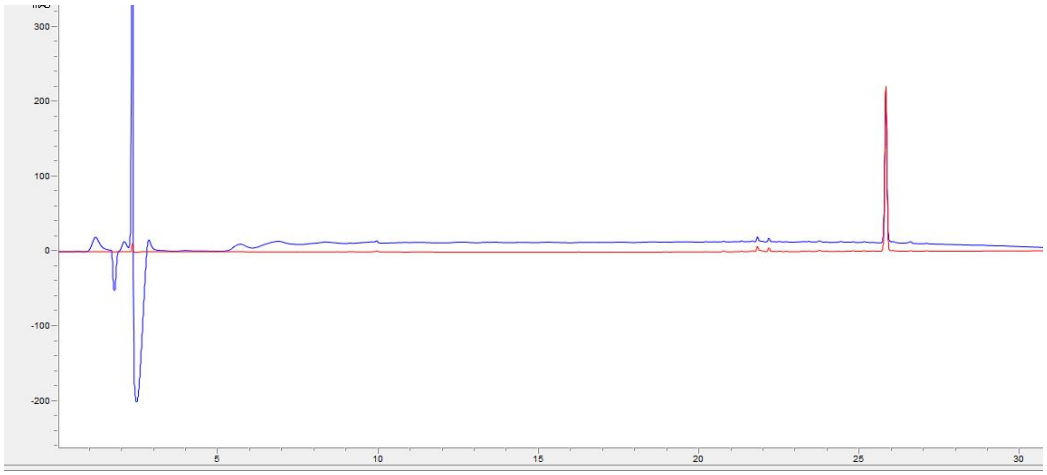


^{1}H NMR of purified compound.

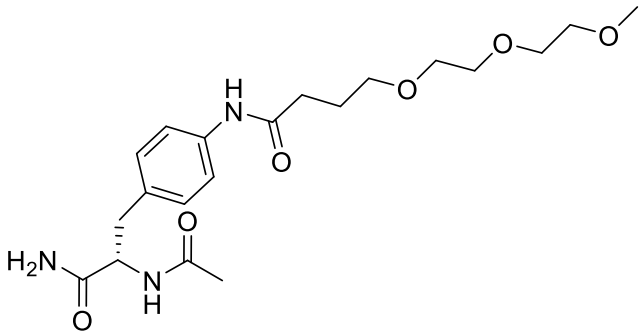
^{19}F NMR (376 MHz, $CDCl_3$)



^{19}F NMR of purified compound. Trifluoroacetic acid used as internal standard.

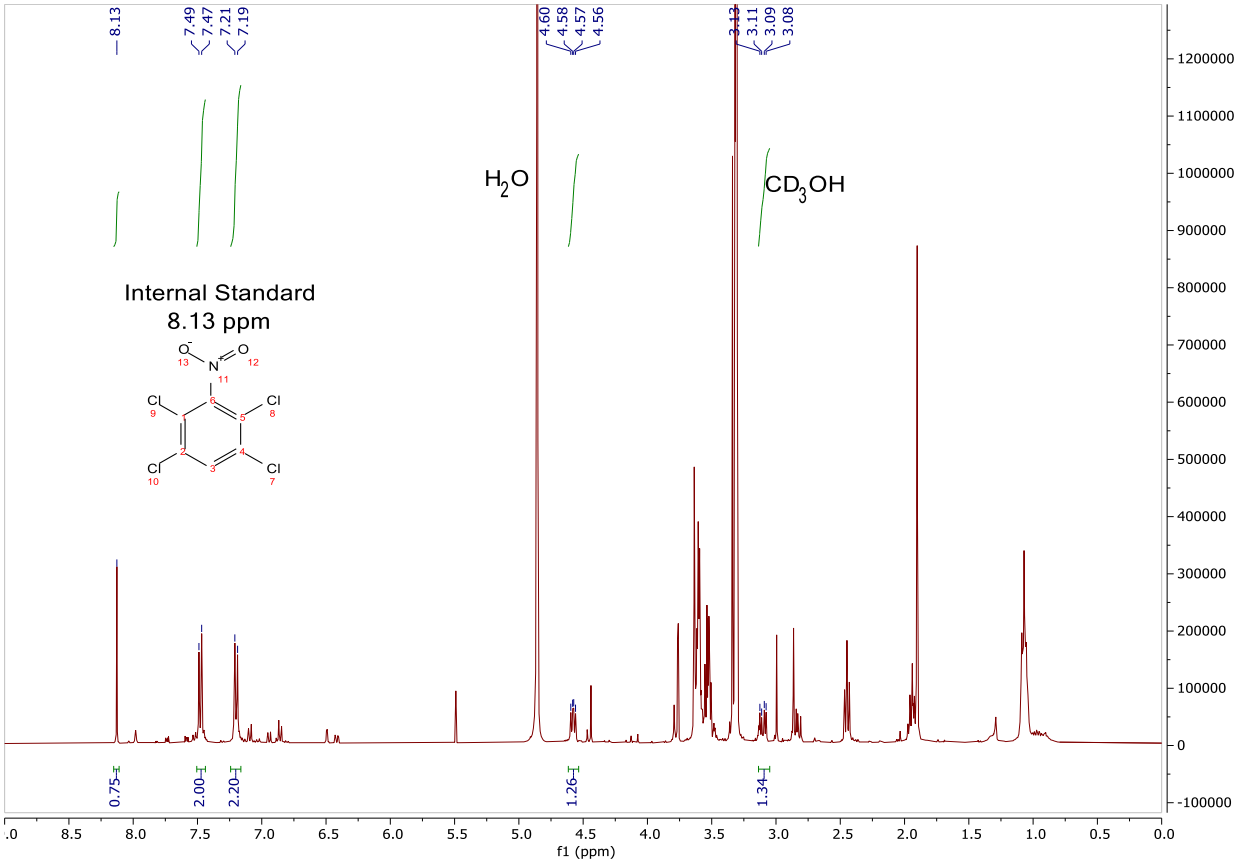


Chromatogram after HPLC purification. Reinjection of compound on analytical HPLC using a linear gradient of 5-60 % MeCN/H_2O (0.1 % TFA) over 30 min at 254 nm (red) and 214 nm (blue).



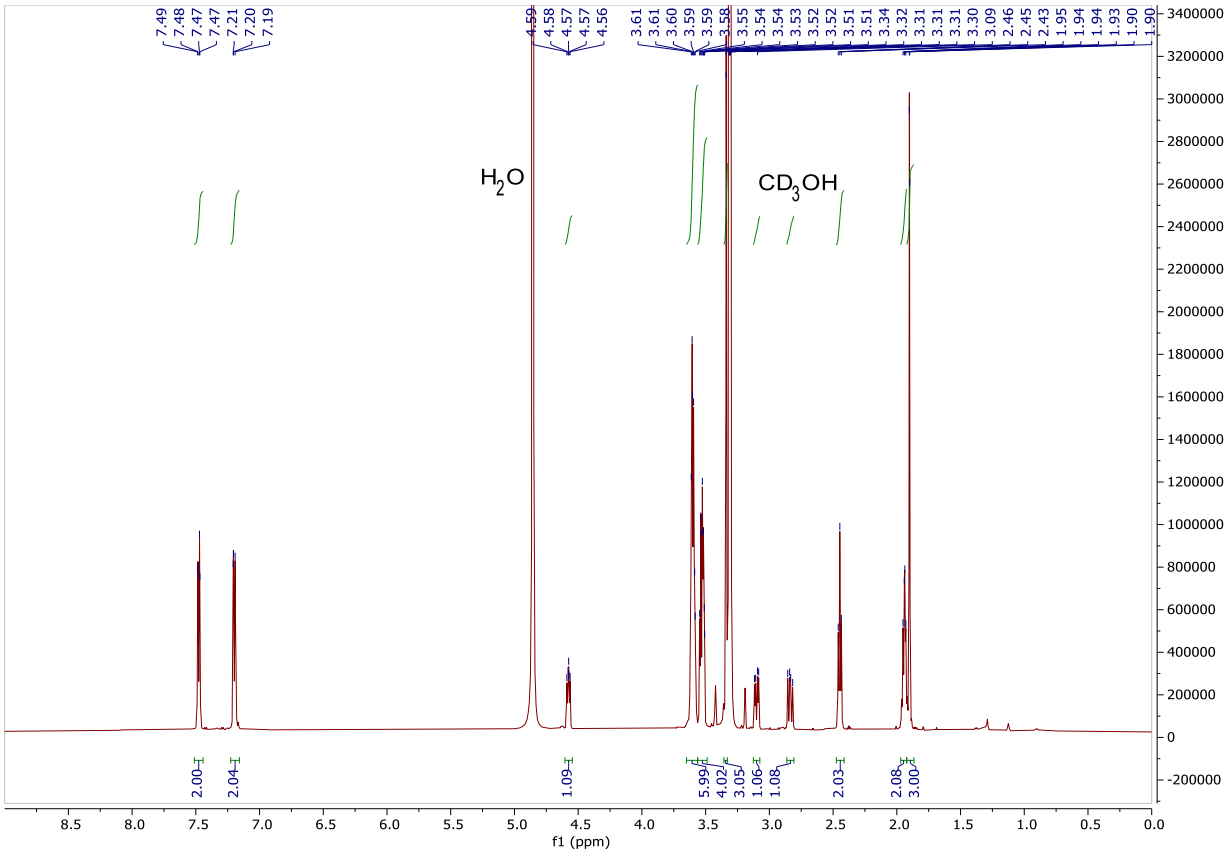
(*R*)-*N*-(4-(2-Acetamido-3-amino-3-oxopropyl)phenyl)-4-(2-(2-methoxyethoxy)ethoxy)butanamide (S28)

^{1}H NMR (400 MHz, CD_3OD)

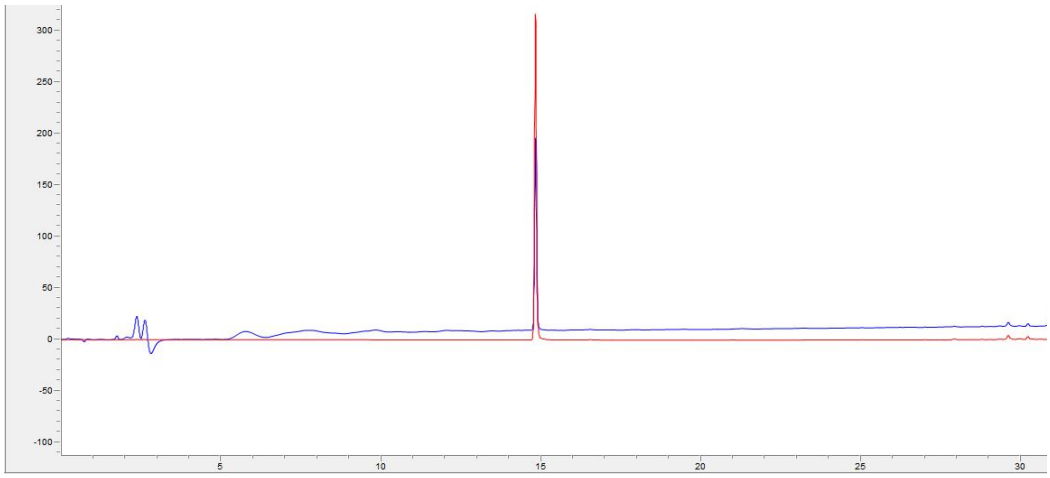


Crude ^{1}H NMR of product off-resin. Internal standard used to calculate conversion and yield.

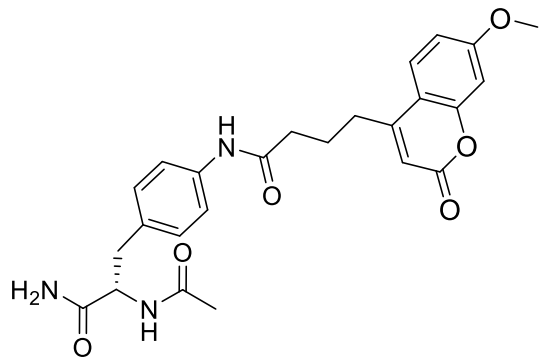
^{1}H NMR (400 MHz, CD_3OD)



^{1}H NMR of purified compound.

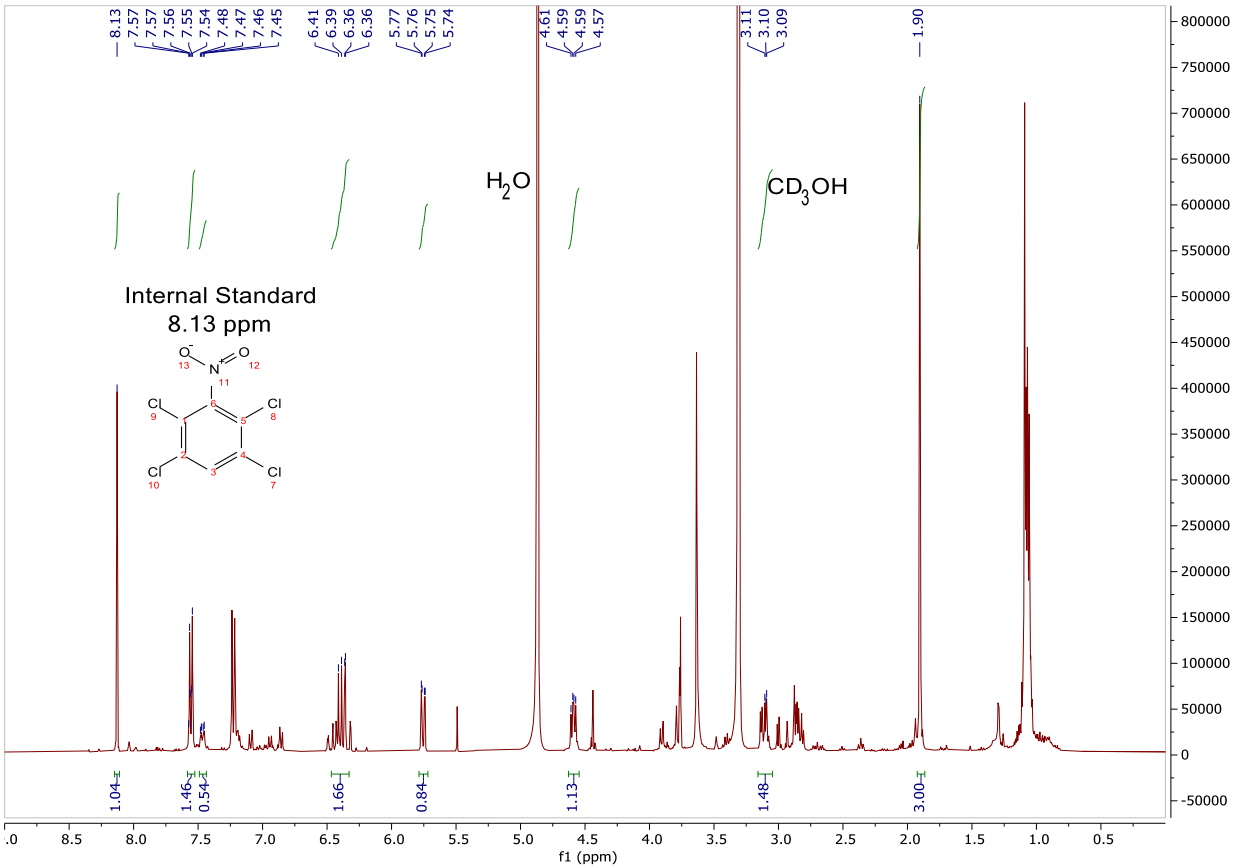


Chromatogram after HPLC purification. Reinjection of compound on analytical HPLC using a linear gradient of 5-40 % MeCN/H_2O (0.1 % TFA) over 30 min at 254 nm (red) and 214 nm (blue).



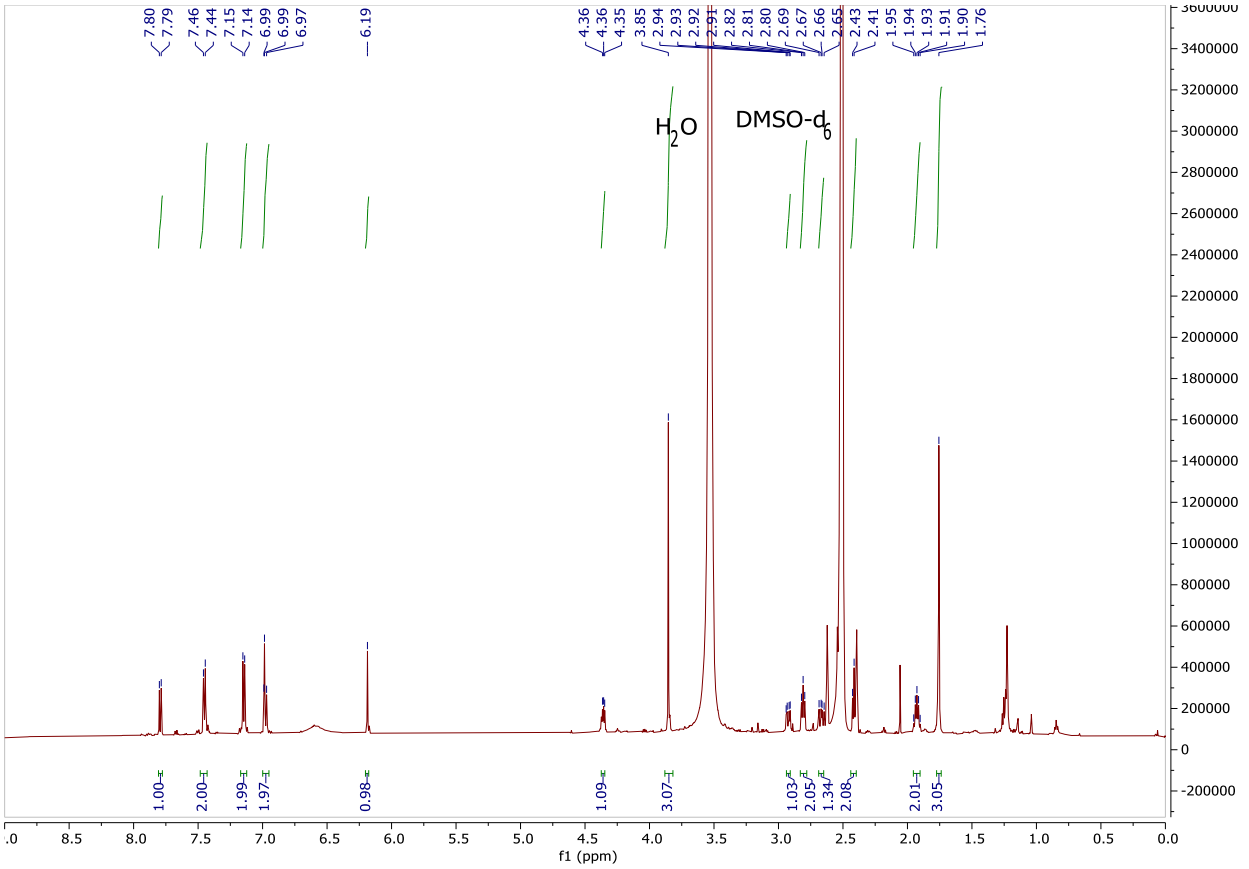
(*R*)-*N*-(4-(2-Acetamido-3-amino-3-oxopropyl)phenyl)-4-(7-methoxy-2-oxo-2H-chromen-4-yl)butanamide (S29)

^{1}H NMR (400 MHz, CD_3OD)

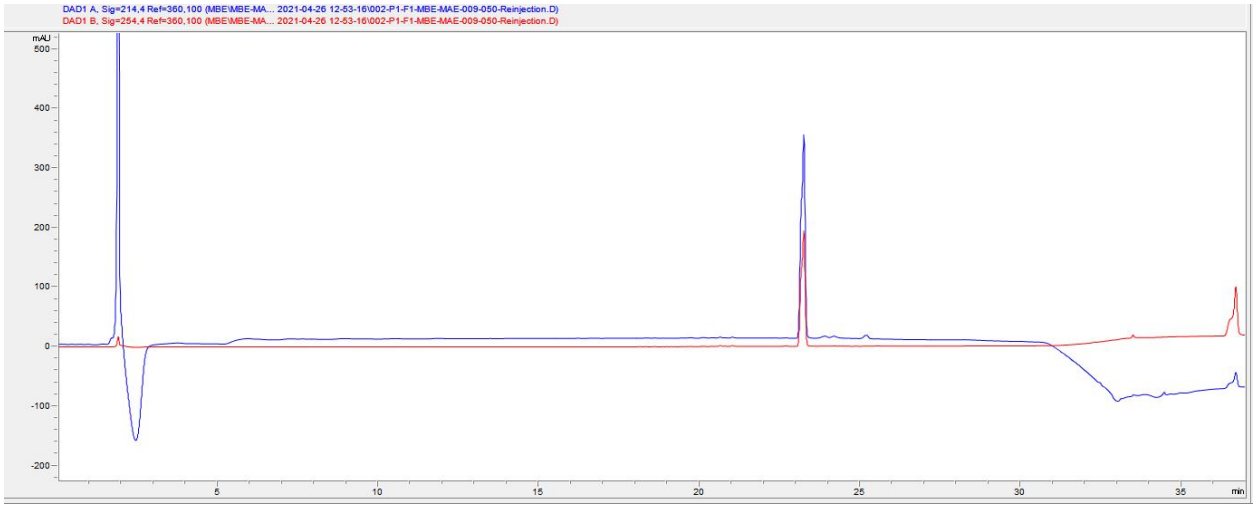


Crude ^{1}H NMR of product off-resin. Internal standard used to calculate conversion and yield.

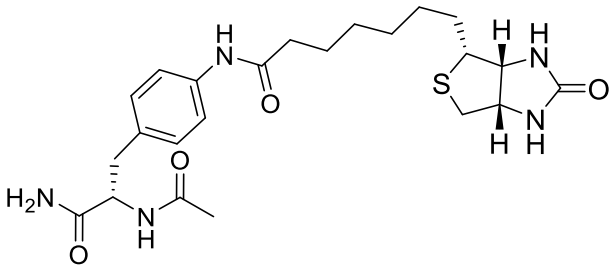
^{1}H NMR (400 MHz, DMSO-*d6*)



^{1}H NMR of purified compound.

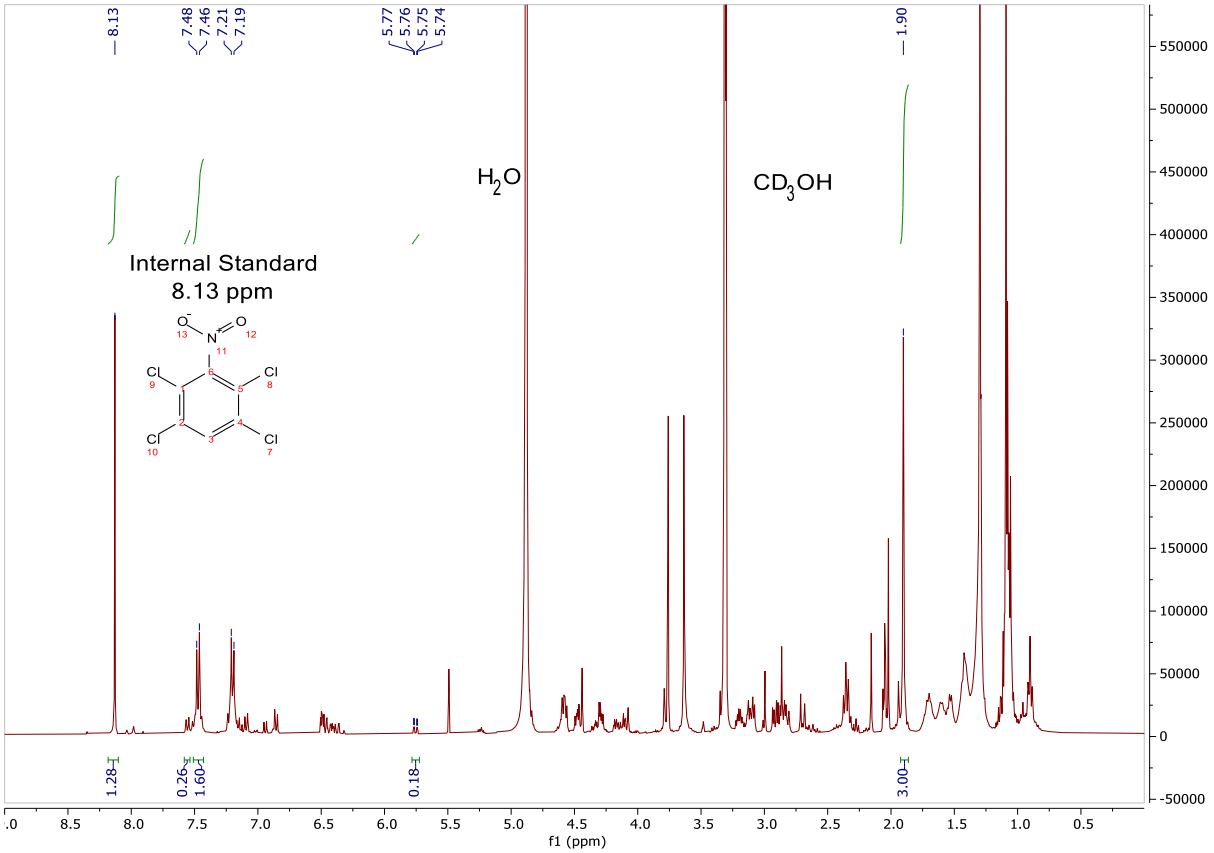


Chromatogram after HPLC purification. Reinjection of compound on analytical HPLC using a linear gradient of 10-60 % MeCN/H_2O (0.1 % TFA) over 30 min at 254 nm (red) and 214 nm (blue).



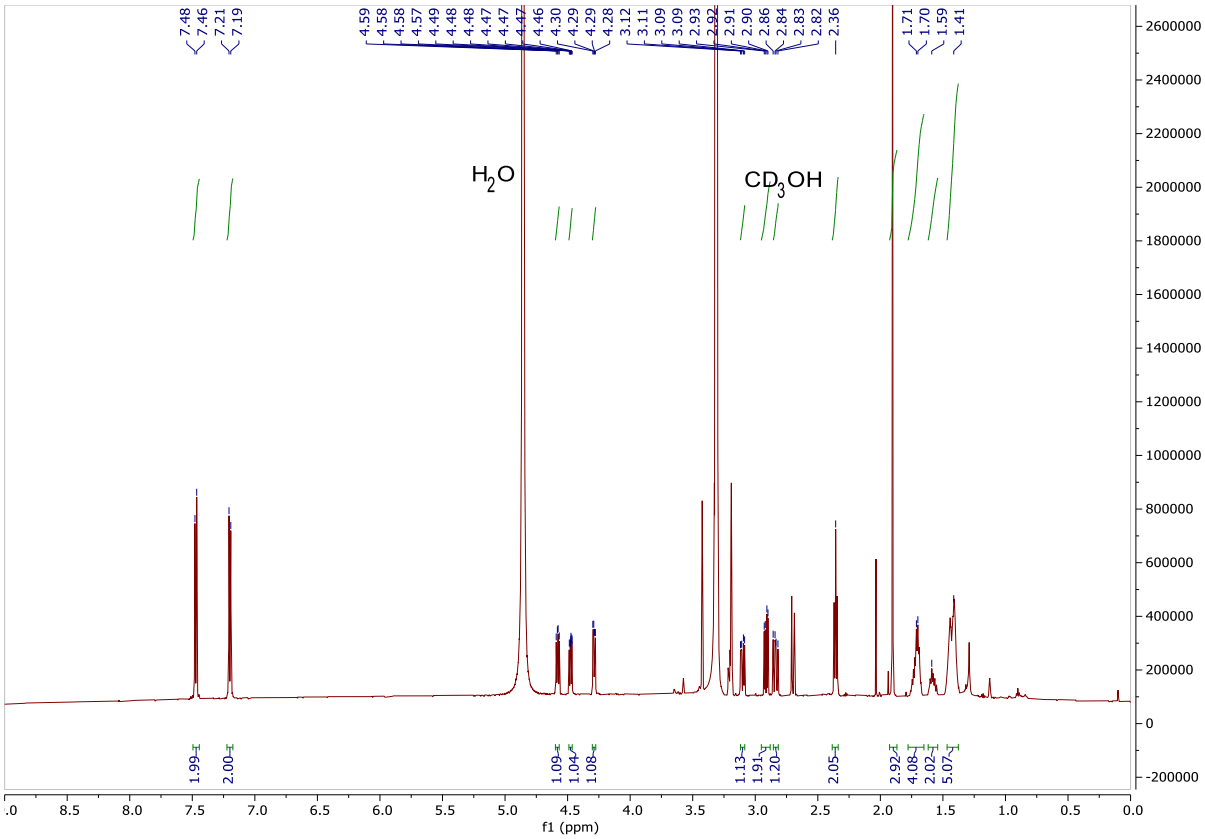
***N*-(4-((*R*)-2-Acetamido-3-amino-3-oxopropyl)phenyl)-7-((3a*R*,4*R*,6a*S*)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)heptanamide (S30)**

^{1}H NMR (400 MHz, CD_3OD)

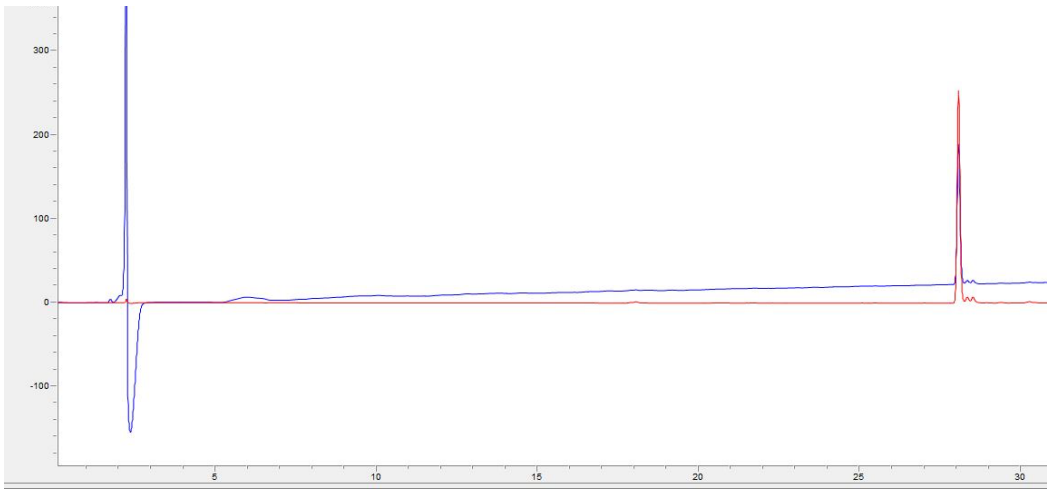


Crude ^{1}H NMR of product off-resin. Internal standard used to calculate conversion and yield.

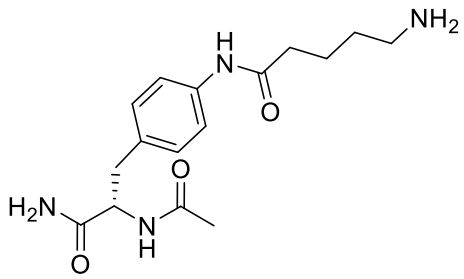
^{1}H NMR (400 MHz, CD_3OD)



^{1}H NMR of purified compound.

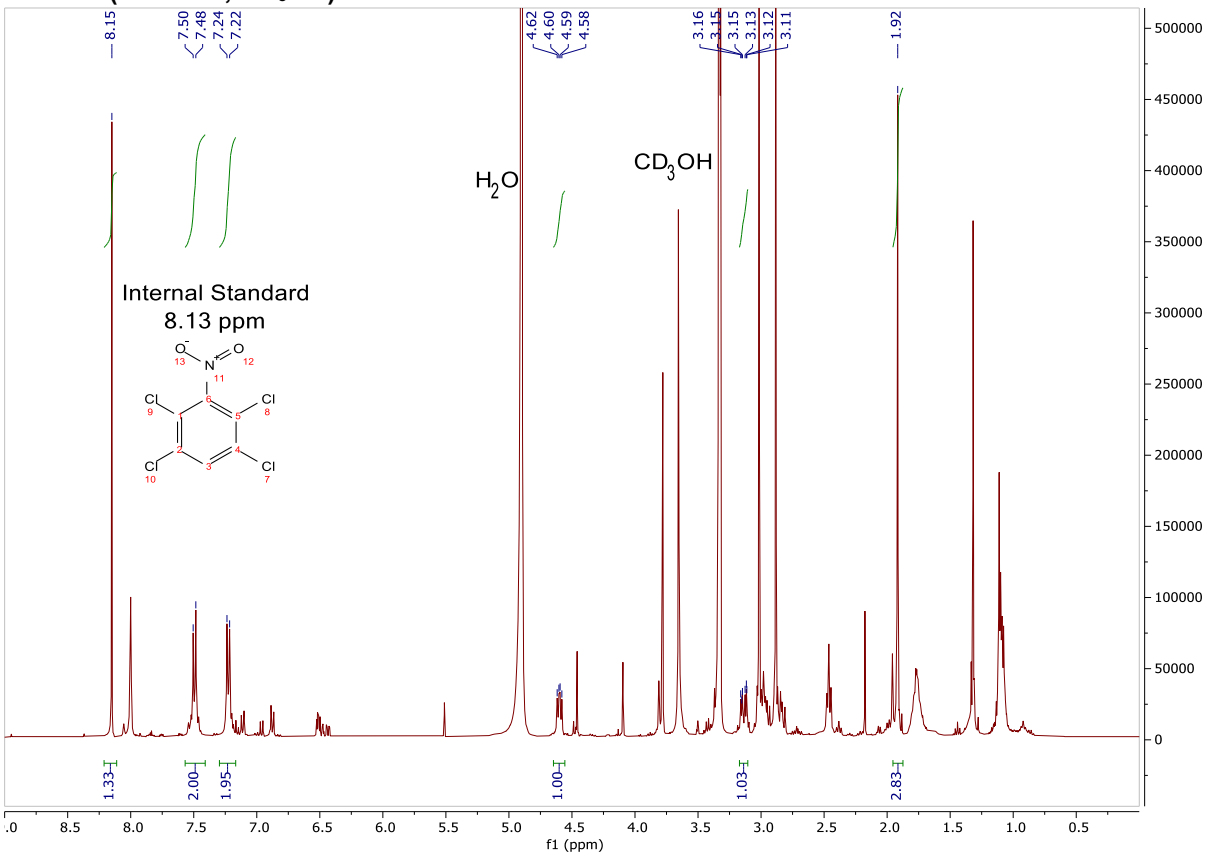


Chromatogram after HPLC purification. Reinjection of compound on analytical HPLC using a linear gradient of 5-25 % MeCN/H_2O (0.1 % TFA) over 30 min at 254 nm (red) and 214 nm (blue).



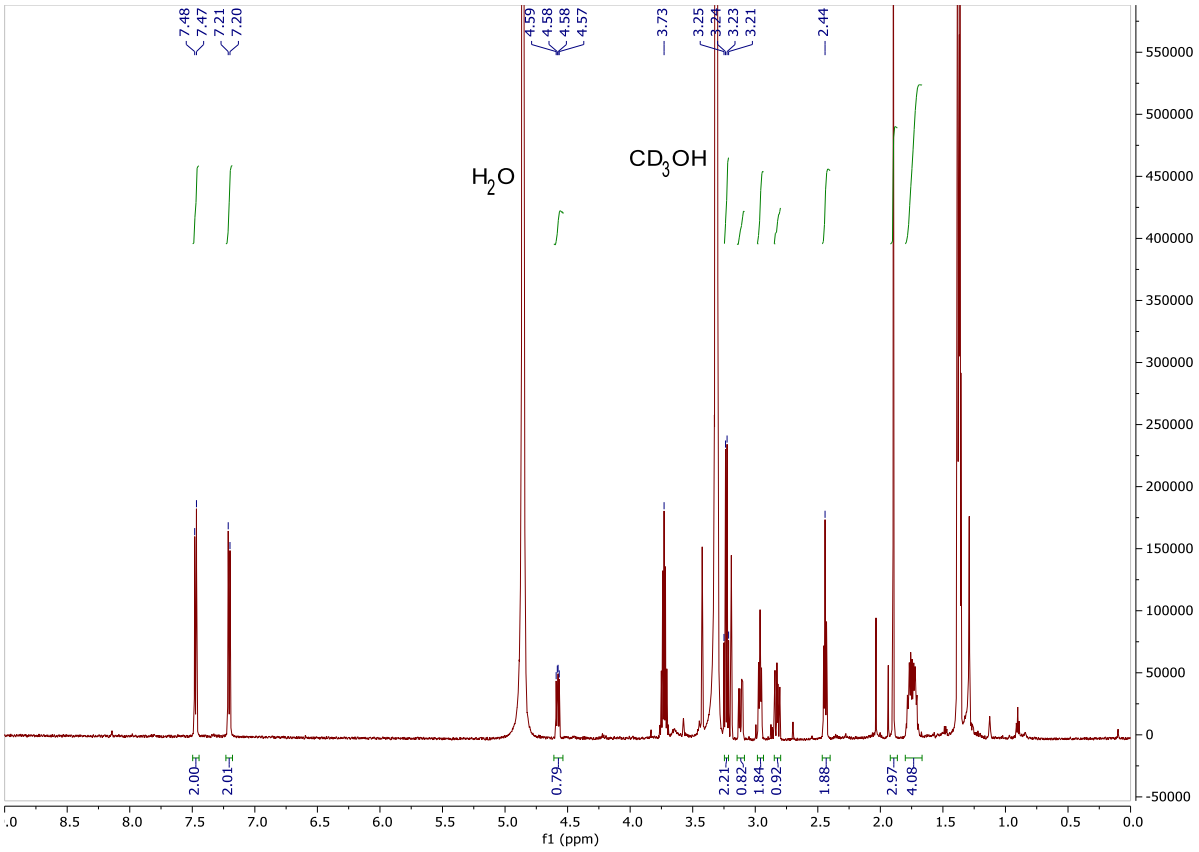
(R)-N-(4-(2-acetamido-3-amino-3-oxopropyl)phenyl)-5-aminopentanamide (S31)

^{1}H NMR (400 MHz, CD_3OD)

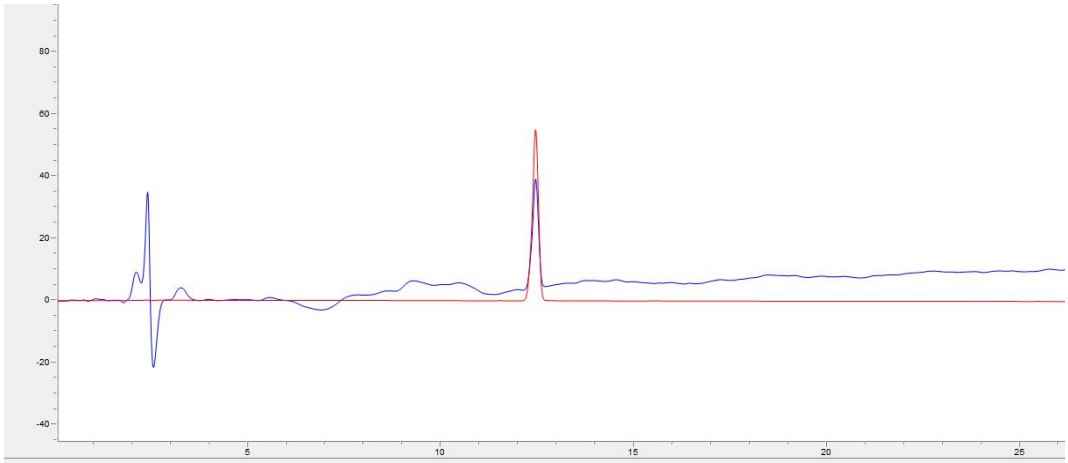


Crude ^{1}H NMR of product off-resin. Internal standard used to calculate conversion and yield.

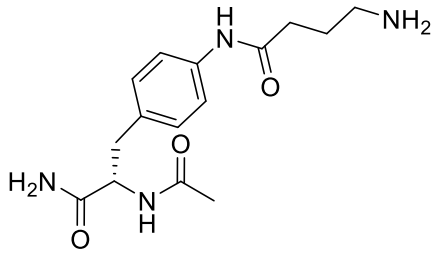
^{1}H NMR (400 MHz, CD_3OD)



^{1}H NMR of purified compound.

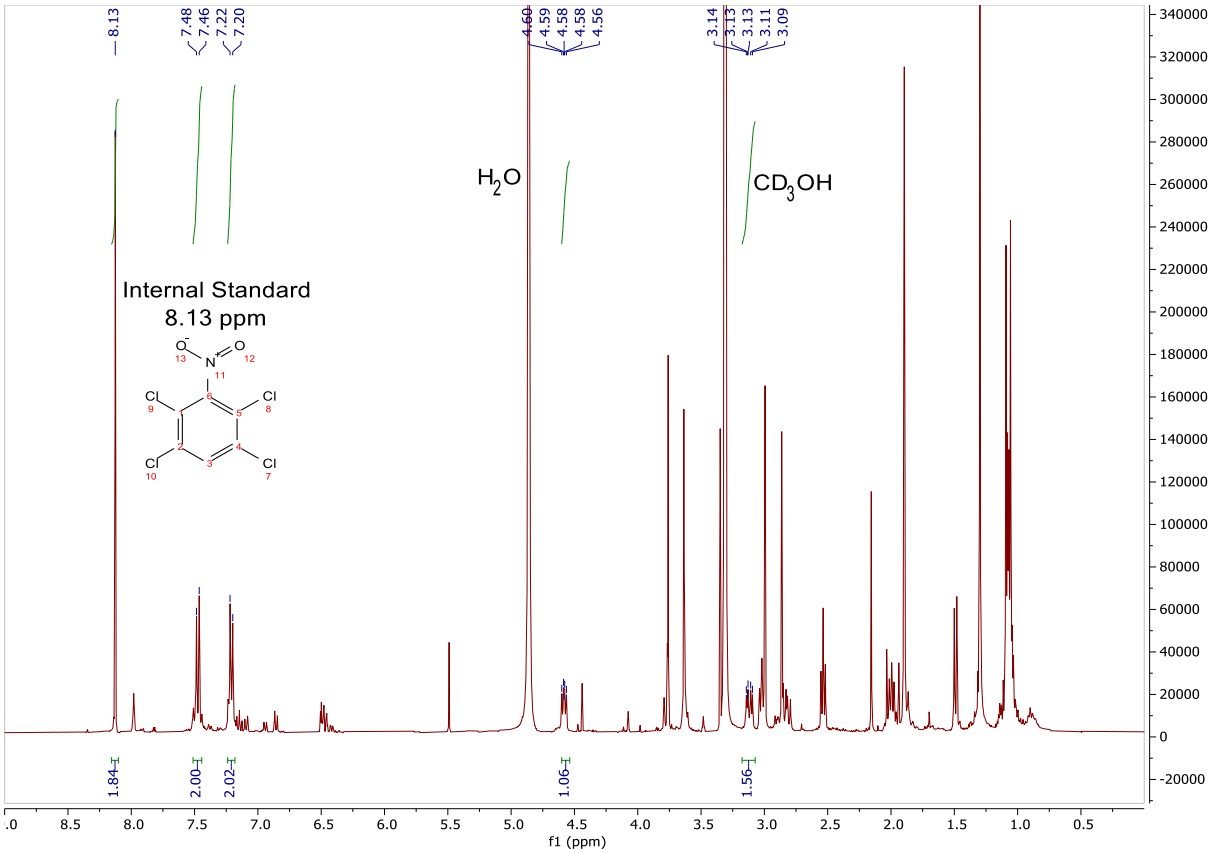


Reinjection of compound on analytical HPLC using a linear gradient of 2-20 % MeCN/H_2O (0.1 % TFA) over 30 min at 254 nm (red) and 214 nm (blue). R_t = 12.5 min



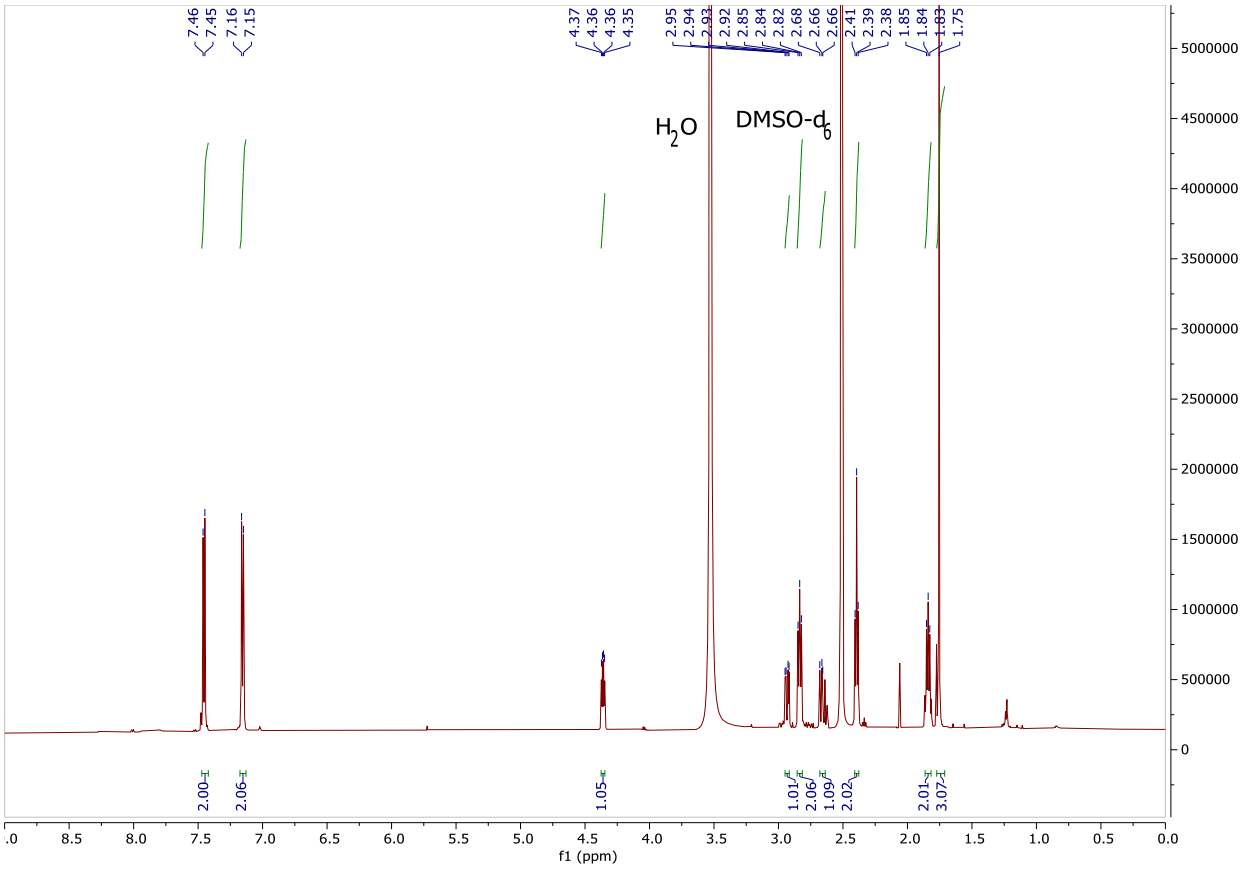
(*R*)-*N*-(4-(2-acetamido-3-amino-3-oxopropyl)phenyl)-4-aminobutanamide (S32)

^{1}H NMR (400 MHz, CD_3OD)

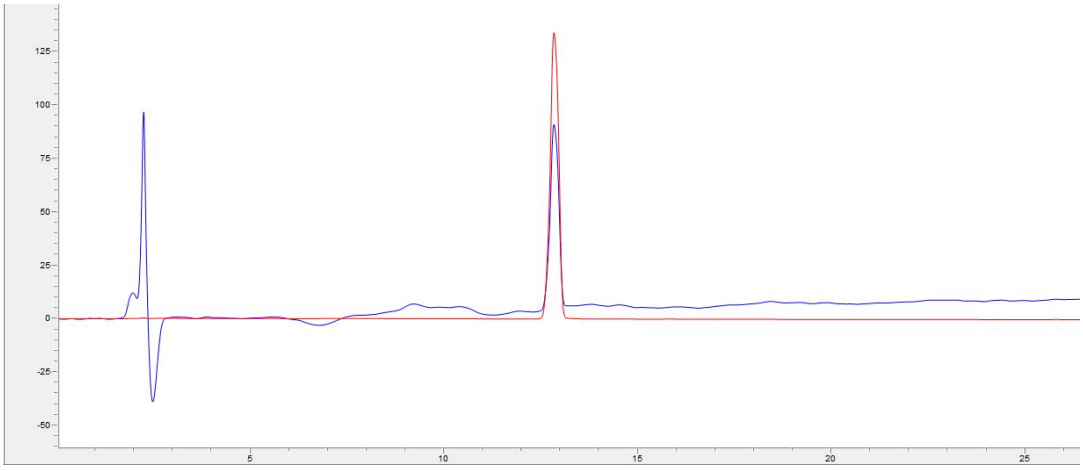


Crude ^{1}H NMR of product off-resin. Internal standard used to calculate conversion and yield.

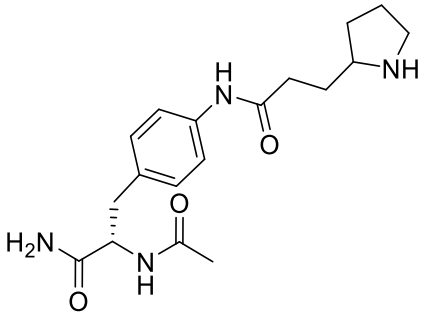
^{1}H NMR (400 MHz, DMSO-*d6*)



^{1}H NMR of purified compound.

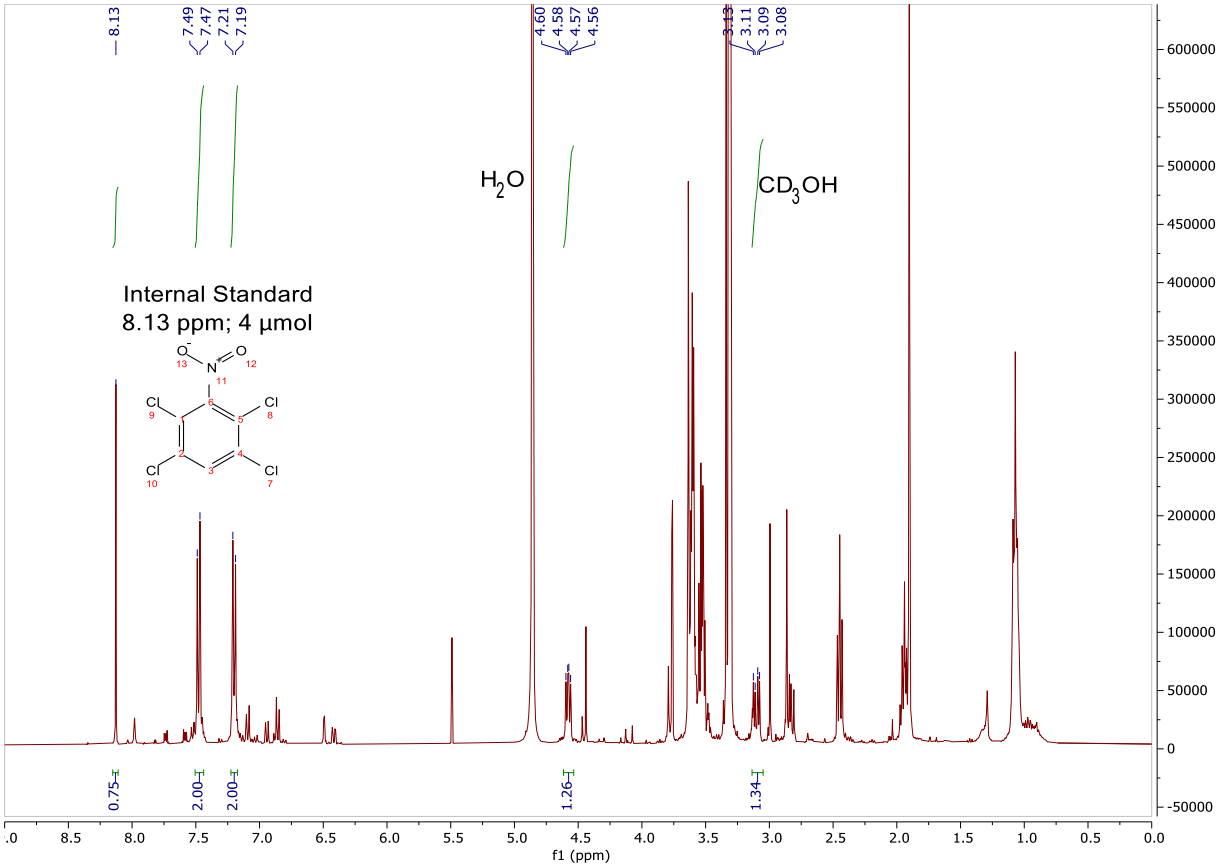


Chromatogram after HPLC purification. Reinjection of compound on analytical HPLC using a linear gradient of 2-20 % MeCN/H_2O (0.1 % TFA) over 30 min at 254 nm (red) and 214 nm (blue).



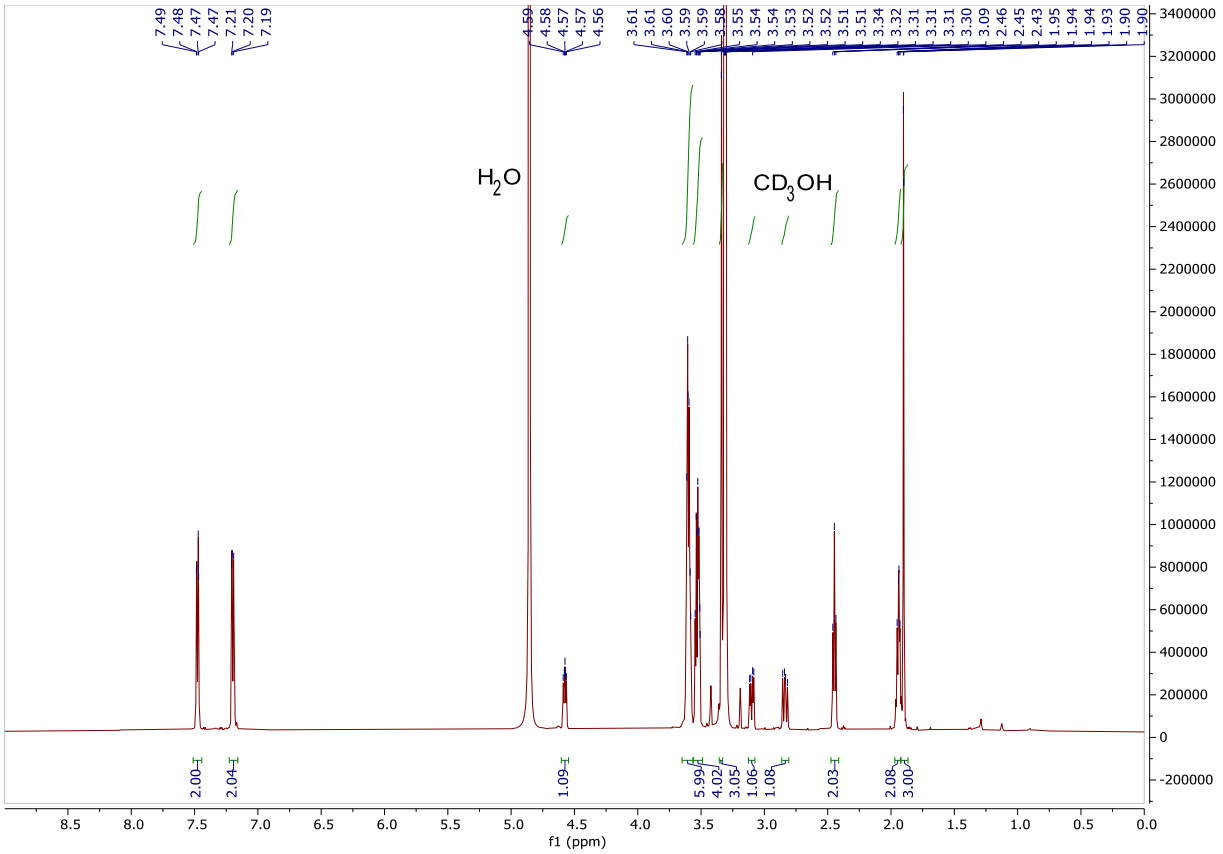
(*2R*)-2-Acetamido-3-(4-(3-(pyrrolidin-2-yl)propanamido)phenyl)propanamide (S33)

^{1}H NMR (400 MHz, CD_3OD)

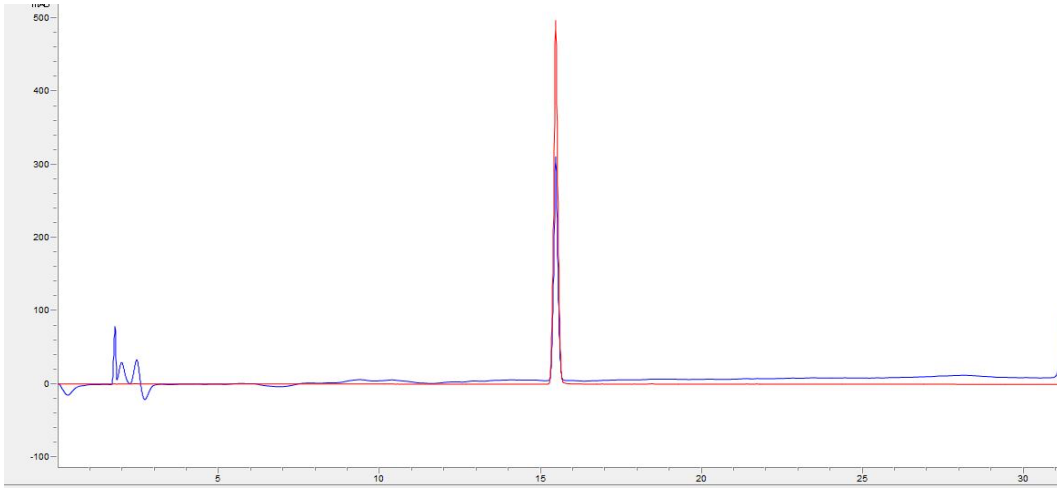


Crude ^{1}H NMR of product off-resin. Internal standard used to calculate conversion and yield.

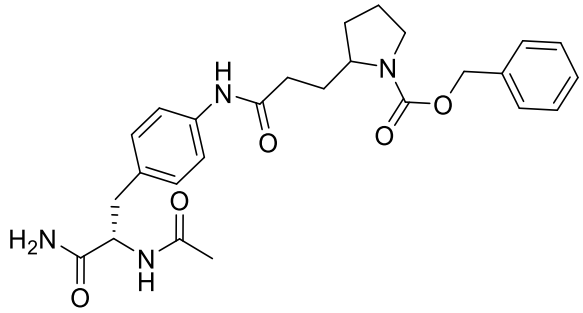
^{1}H NMR (400 MHz, CD_3OD)



^{1}H NMR of purified compound.

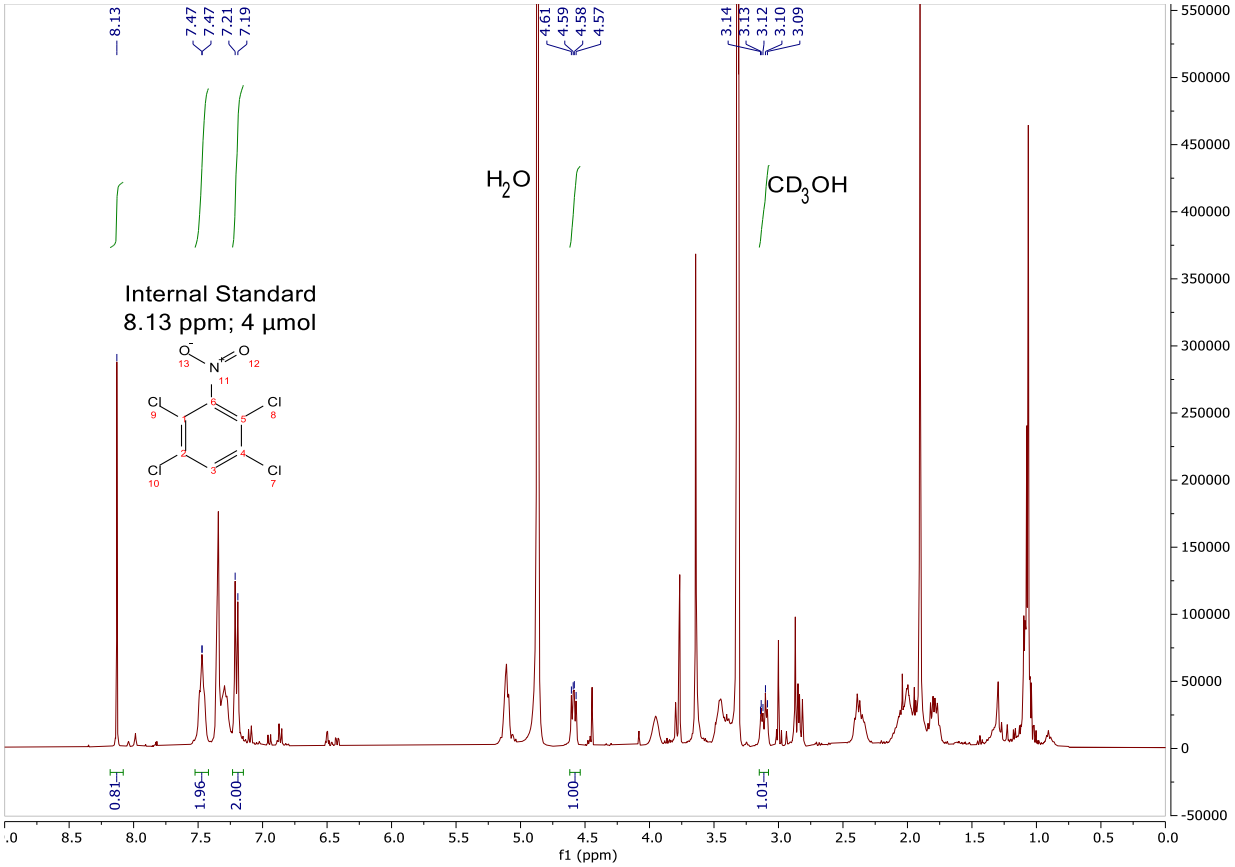


Chromatogram after HPLC purification. Reinjection of compound on analytical HPLC using a linear gradient of 2-20 % MeCN/H_2O (0.1 % TFA) over 30 min at 254 nm (red) and 214 nm (blue).



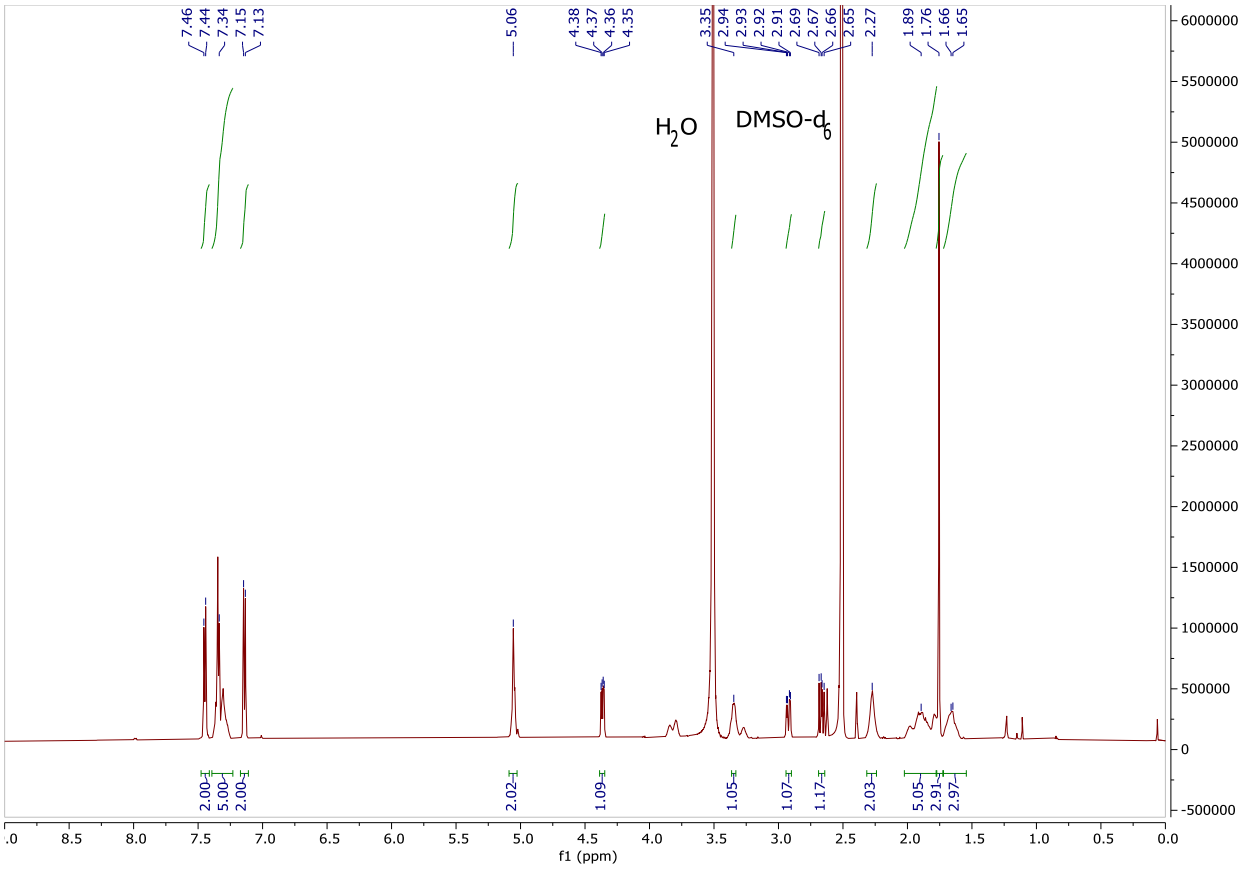
Benzyl 2-(3-((4-((*R*)-2-Acetamido-3-amino-3-oxopropyl)phenyl)amino)-3-oxopropyl)pyrrolidine-1-carboxylate (S34)

^{1}H NMR (400 MHz, CD_3OD)

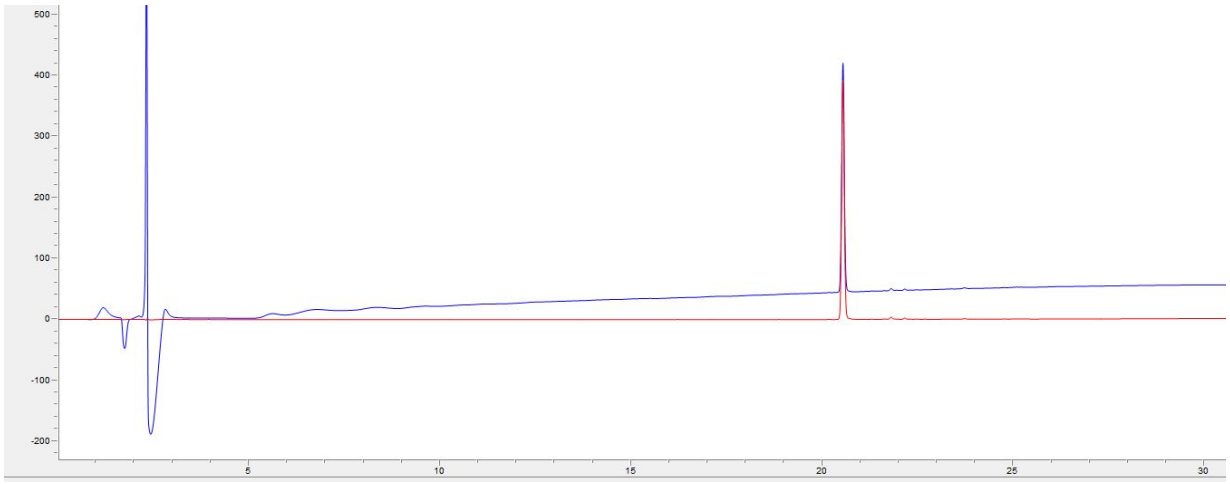


Crude ^{1}H NMR of product off-resin. Internal standard used to calculate conversion and yield.

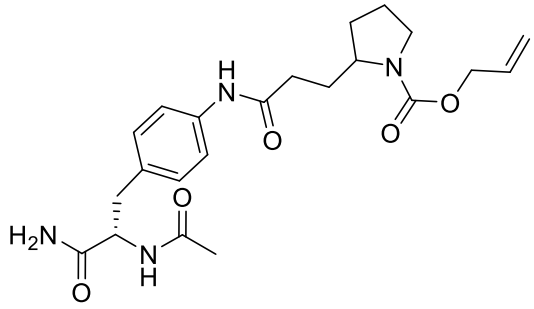
^{1}H NMR (400 MHz, DMSO-*d6*)



^{1}H NMR of purified compound.

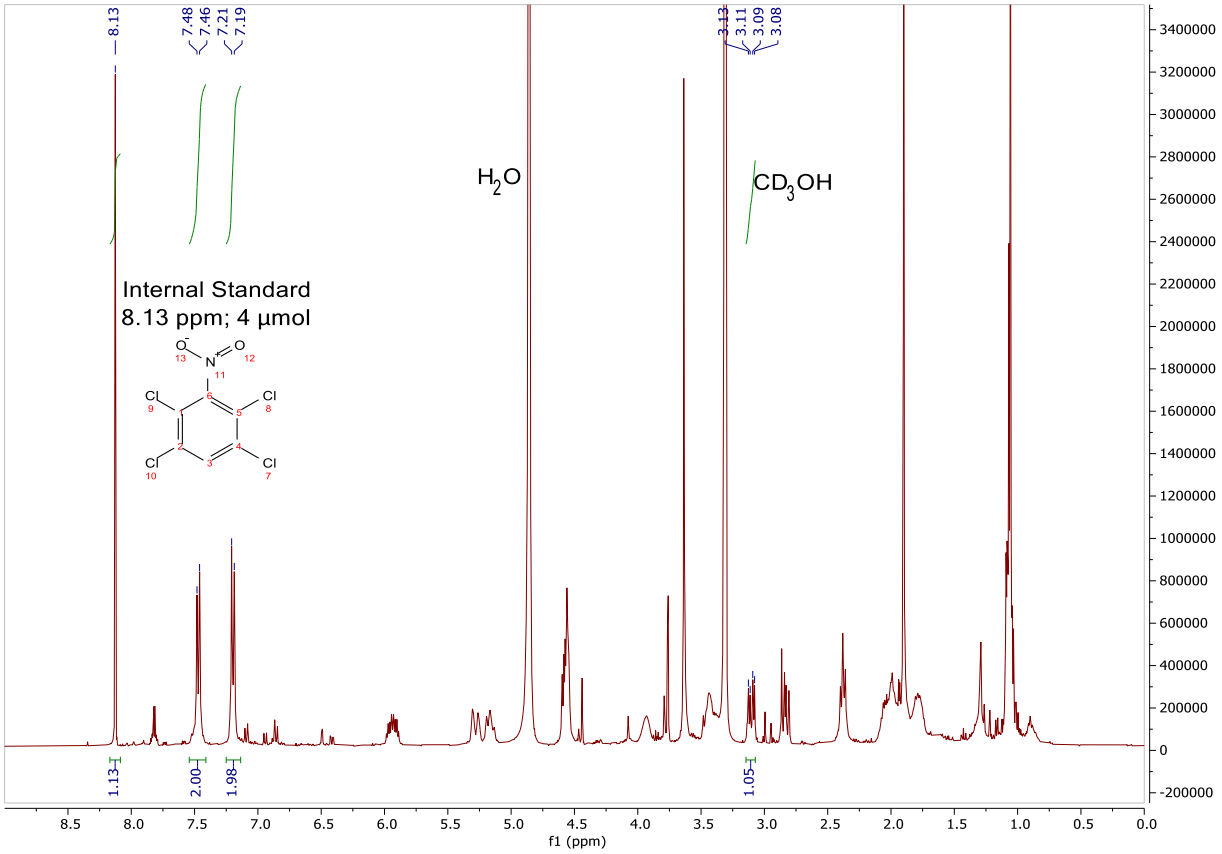


Chromatogram after HPLC purification. Reinjection of compound on analytical HPLC using a linear gradient of 5-60 % MeCN/H_2O (0.1 % TFA) over 30 min at 254 nm (red) and 214 nm (blue).



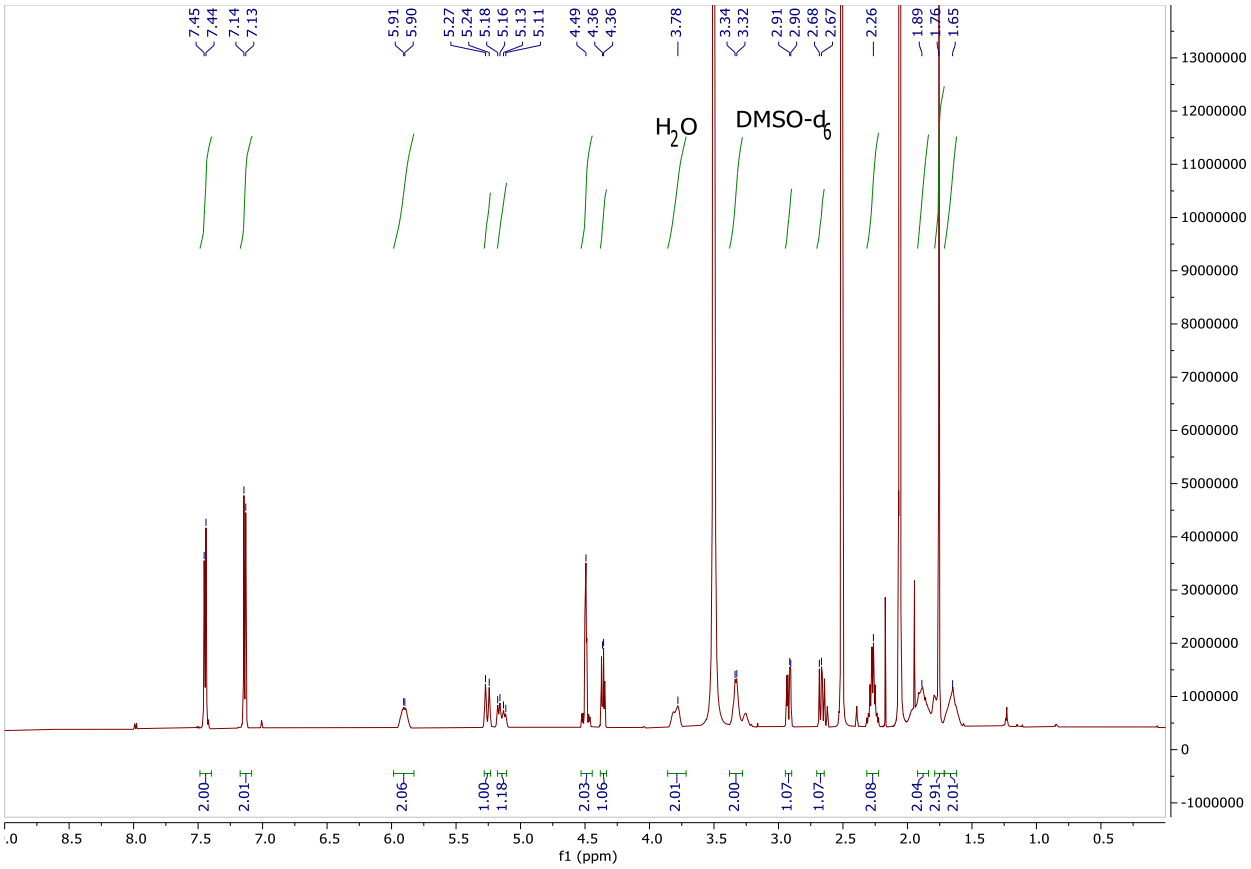
Allyl 2-(3-((4-((*R*)-2-Acetamido-3-amino-3-oxopropyl)phenyl)amino)-3-oxopropyl)pyrrolidine-1-carboxylate (S35)

^{1}H NMR (400 MHz, CD_3OD)

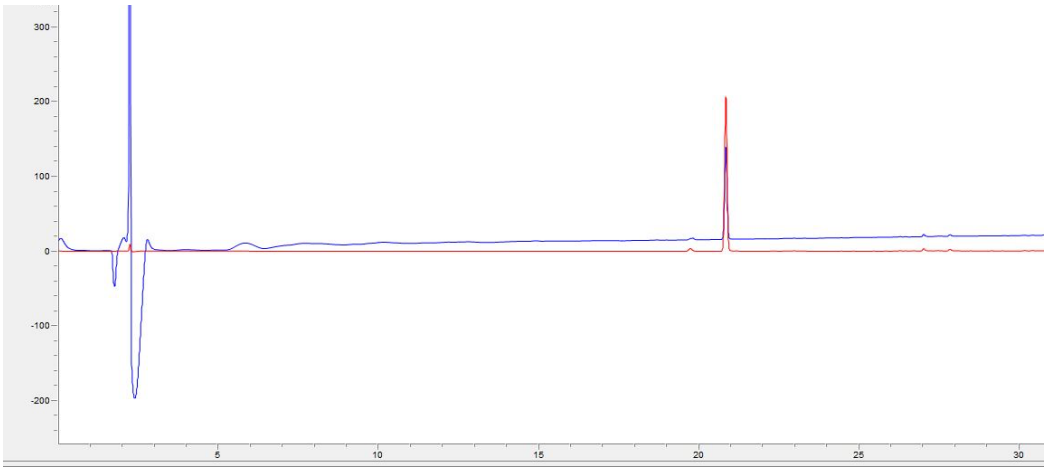


Crude ^{1}H NMR of product off-resin. Internal standard used to calculate conversion and yield.

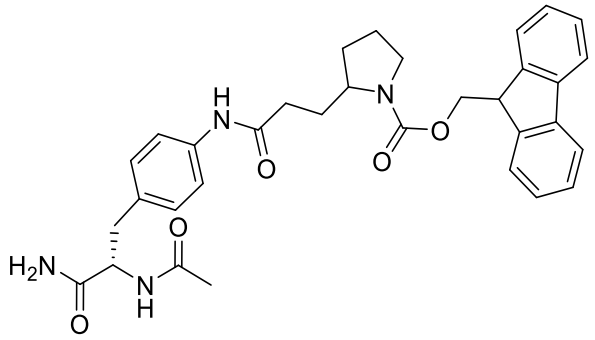
^{1}H NMR (400 MHz, DMSO-*d6*)



^{1}H NMR of purified compound.

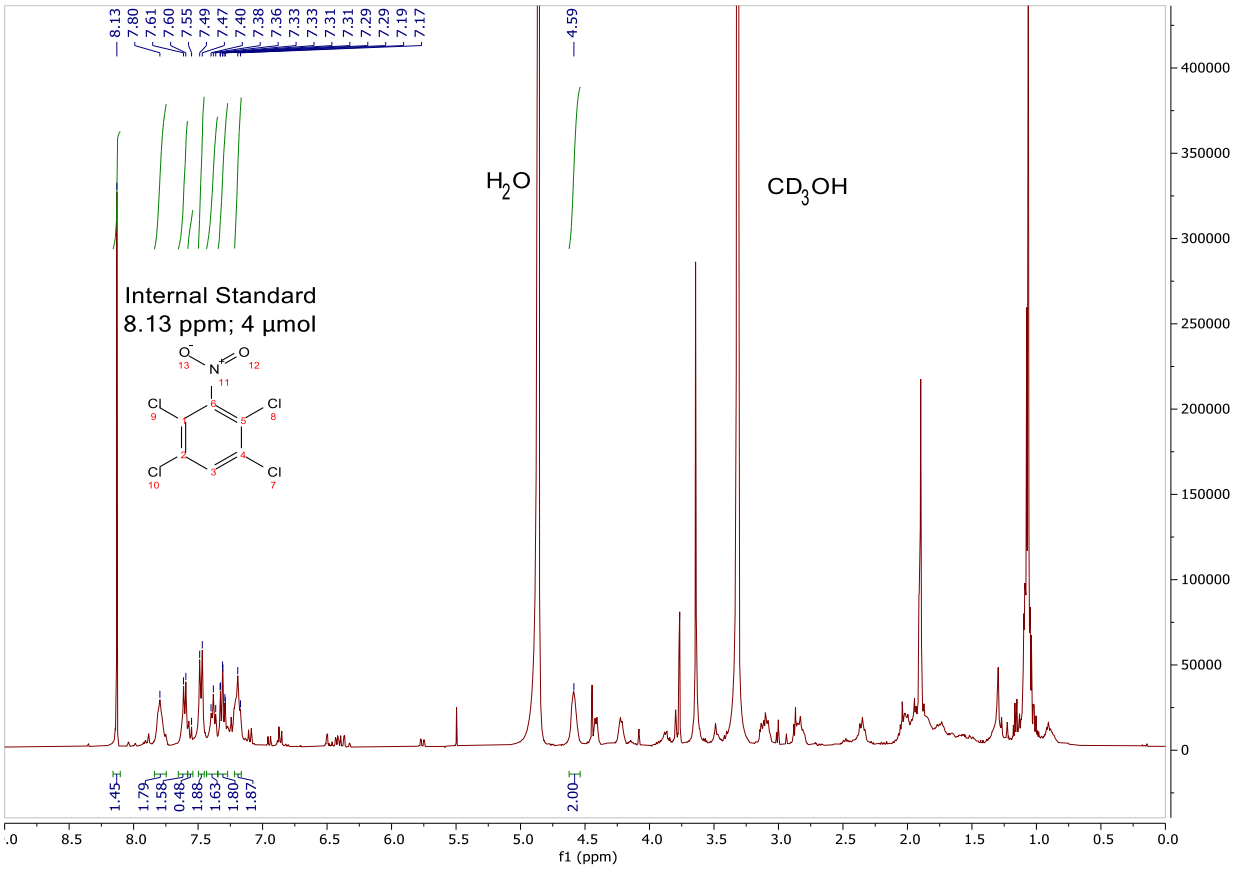


Chromatogram after HPLC purification. Reinjection of compound on analytical HPLC using a linear gradient of 5-40 % MeCN/H_2O (0.1 % TFA) over 30 min at 254 nm (red) and 214 nm (blue).



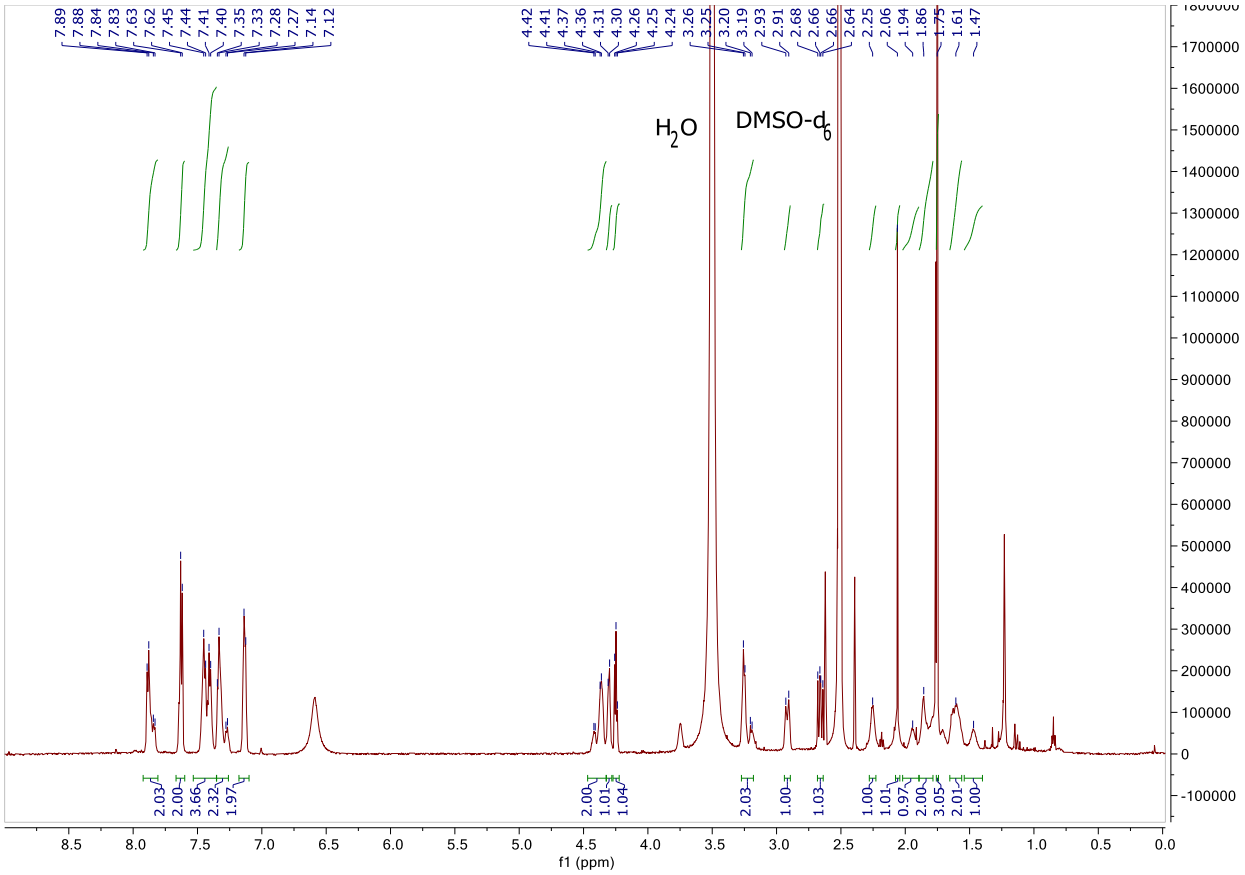
(9H-Fluoren-9-yl)methyl 2-(3-((4-((*R*)-2-Acetamido-3-amino-3-oxopropyl)phenyl)amino)-3-oxopropyl)pyrrolidine-1-carboxylate (S36)

^{1}H NMR (400 MHz, CD_3OD)

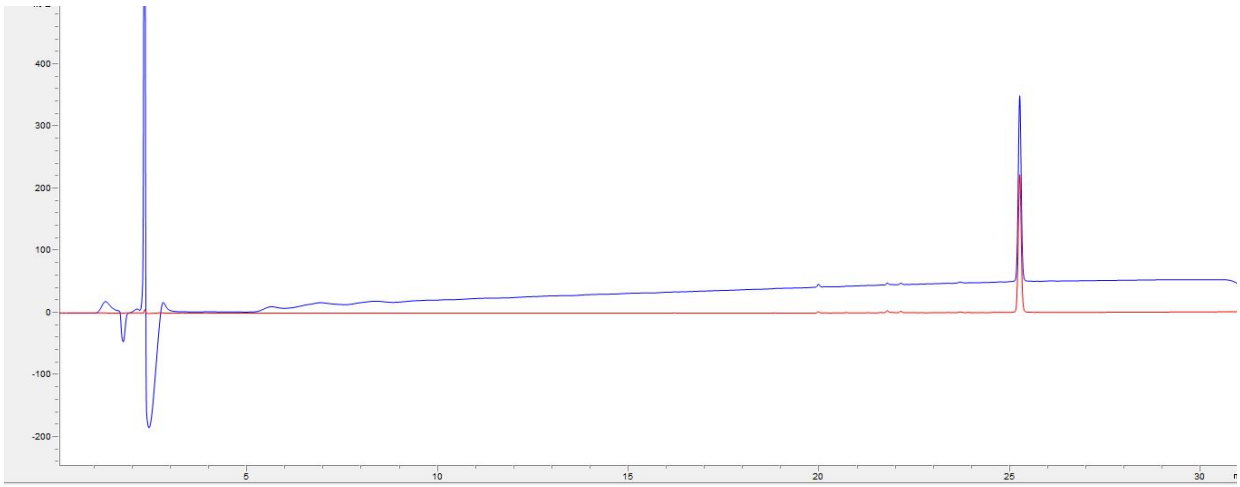


Crude ^{1}H NMR of product off-resin. Internal standard used to calculate conversion and yield.

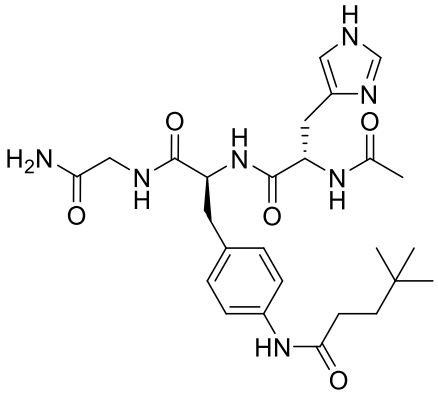
^{1}H NMR (400 MHz, DMSO-*d6*)



^{1}H NMR of purified compound.

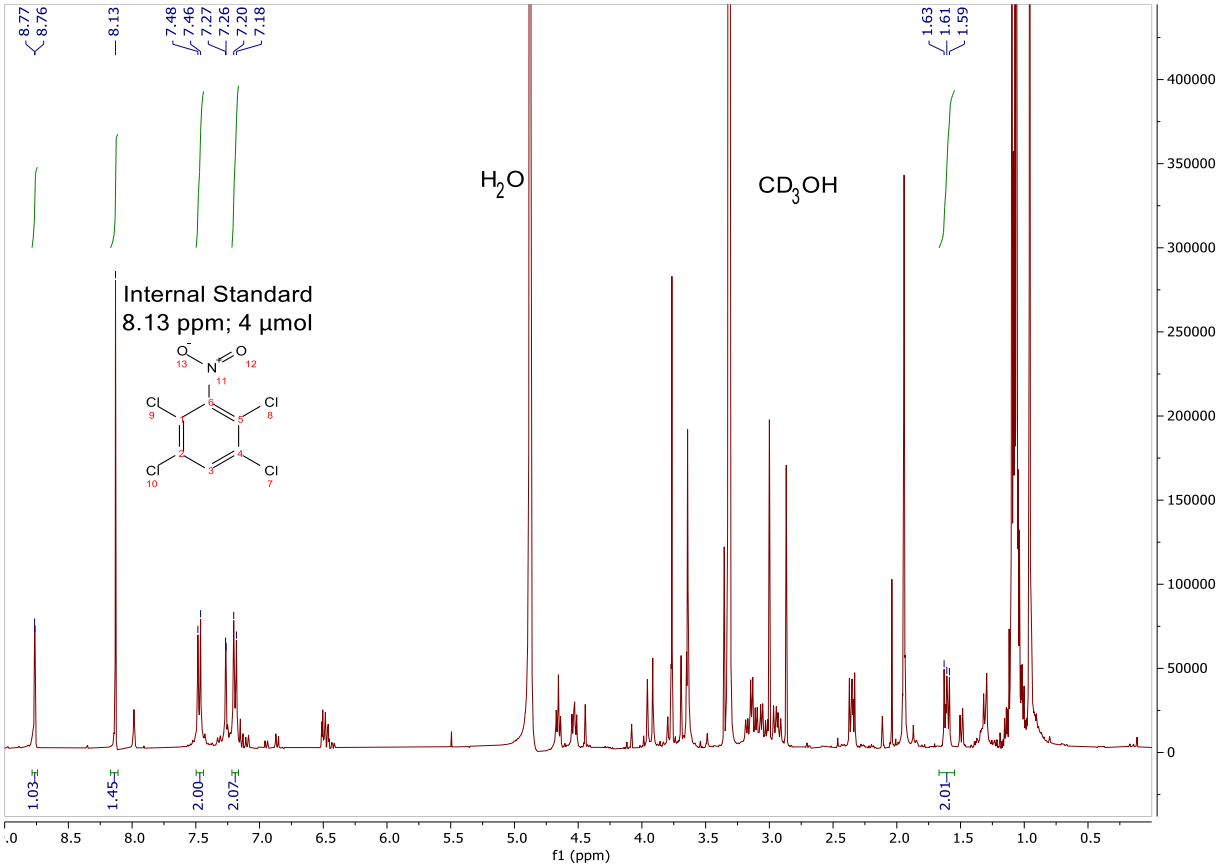


Chromatogram after HPLC purification. Reinjection of compound on analytical HPLC using a linear gradient of 5-60 % MeCN/H_2O (0.1 % TFA) over 30 min at 254 nm (red) and 214 nm (blue).



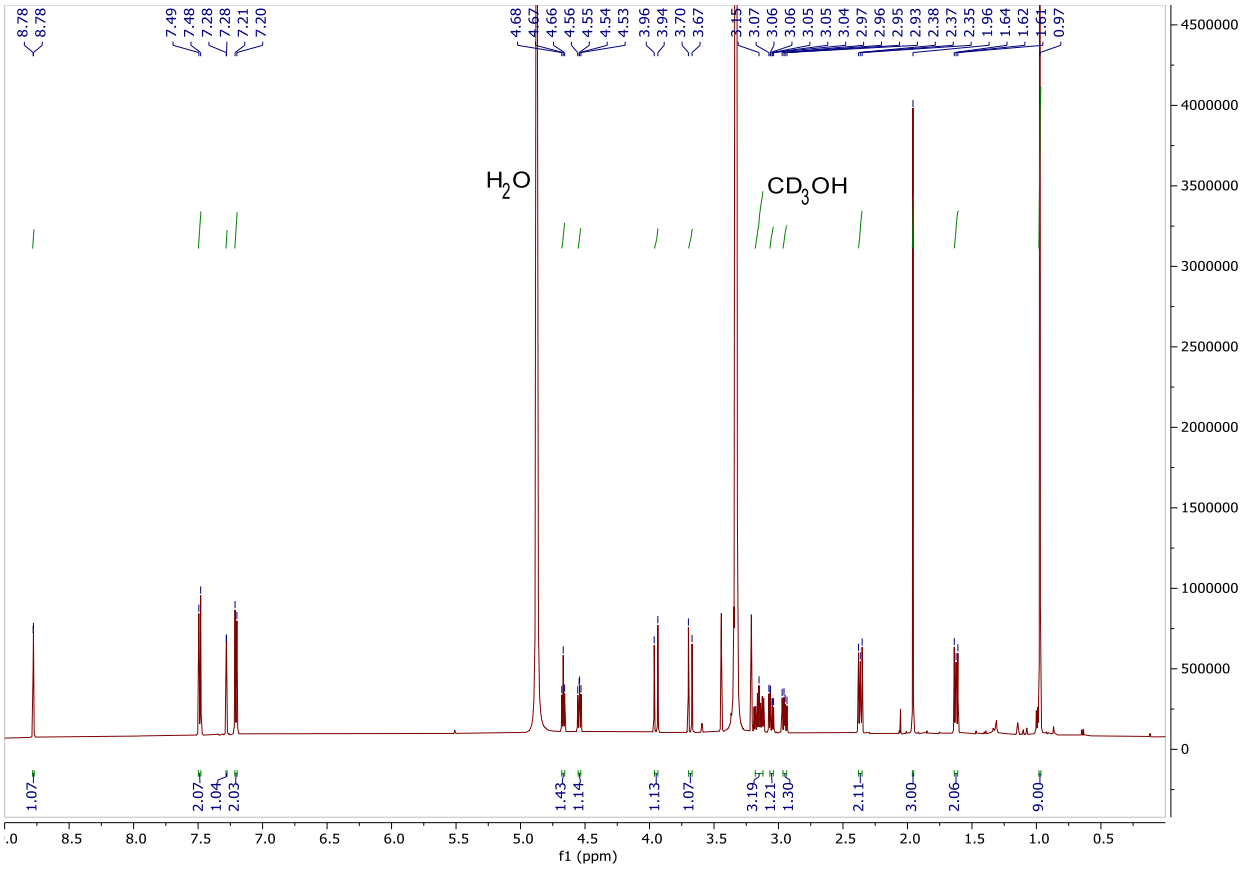
***tert*-Butyl 4-((*S*)-2-Acetamido-3-(((*S*)-1-((2-amino-2-oxoethyl)amino)-1-oxo-3-(4-(*N*-tritylacrylamido)phenyl)propan-2-yl)amino)-3-oxopropyl)-1H-imidazole-1-carboxylate (S37)**

^{1}H NMR (400 MHz, CD_3OD)

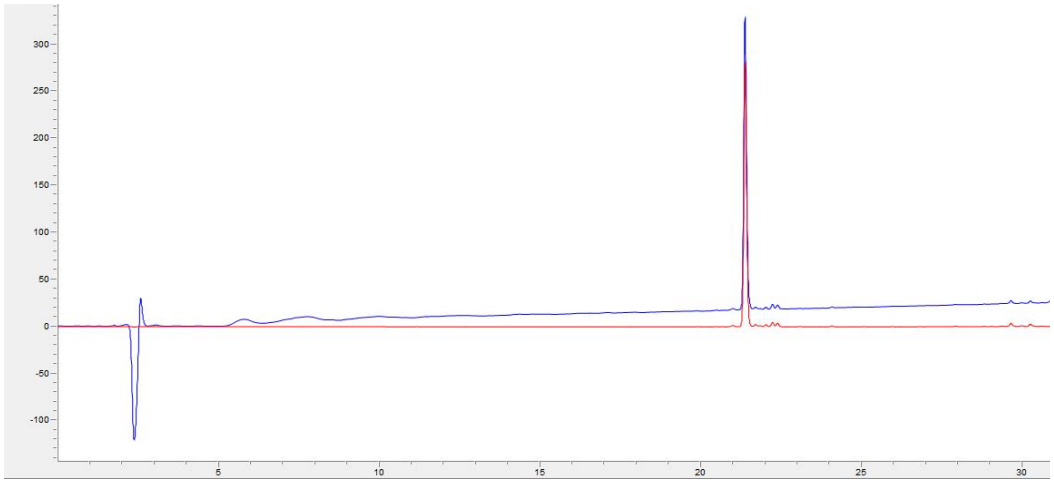


Crude ^{1}H NMR of product off-resin. Internal standard used to calculate conversion and yield.

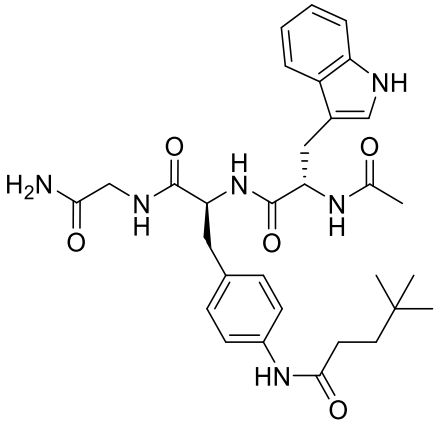
^{1}H NMR (400 MHz, CD_3OD)



^{1}H NMR of purified compound.

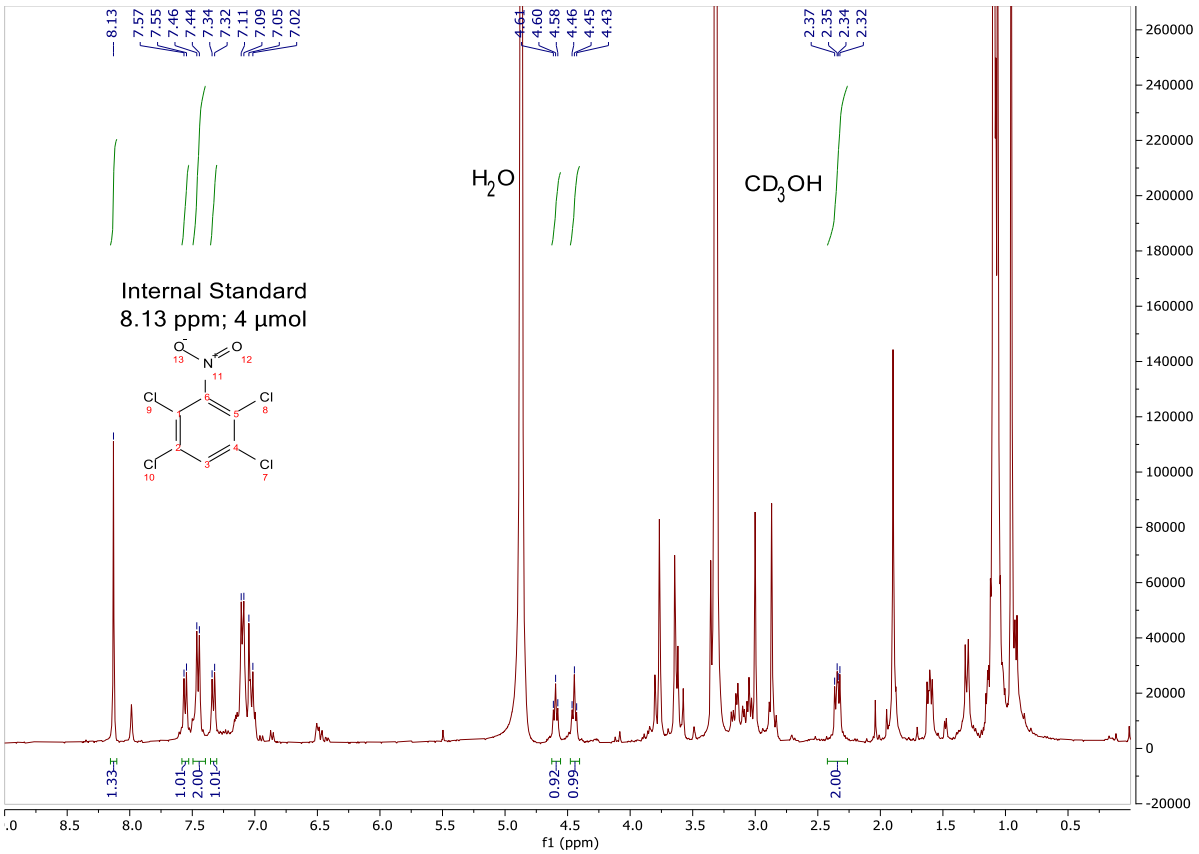


Chromatogram after HPLC purification. Reinjection of compound on analytical HPLC using a linear gradient of 10-50 % MeCN/H_2O (0.1 % TFA) over 30 min at 254 nm (red) and 214 nm (blue).



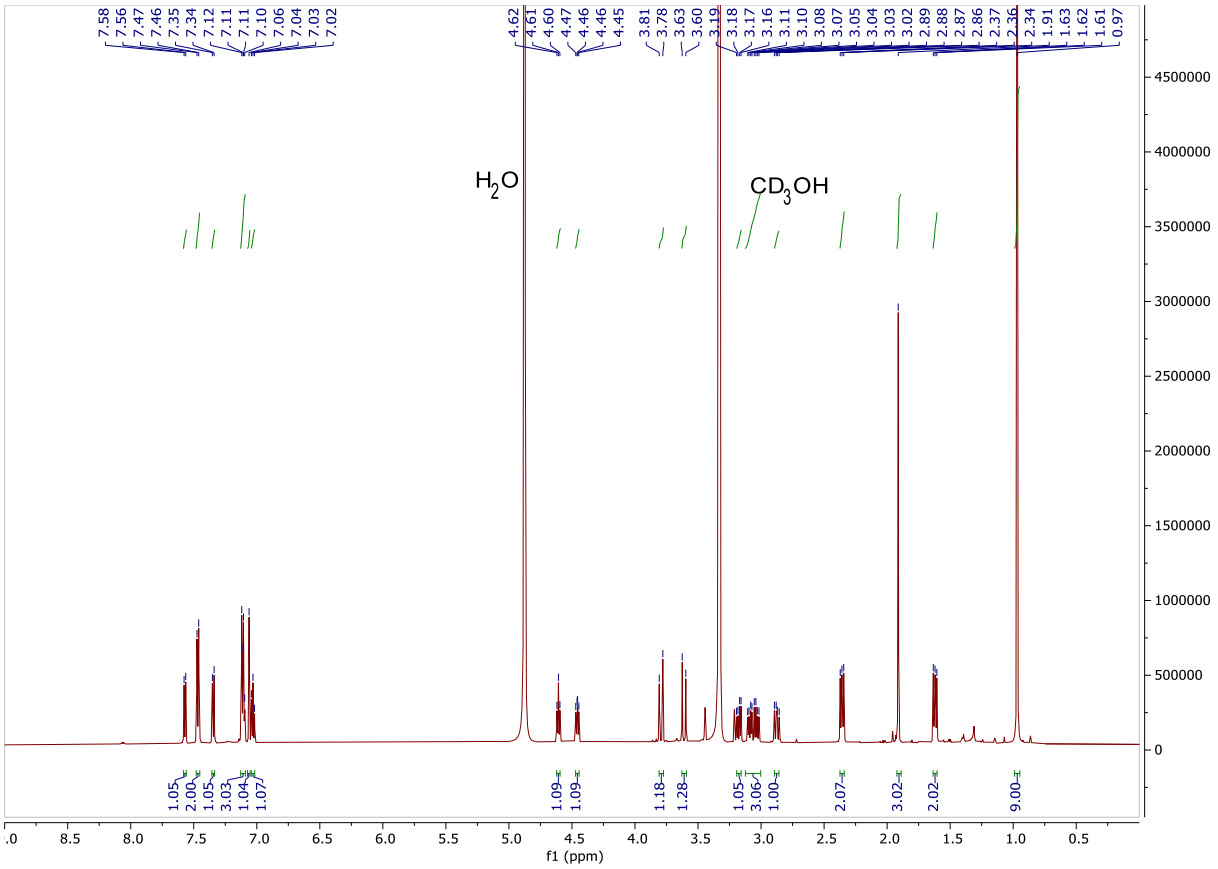
***N*-(4-((*S*)-2-((*S*)-2-Acetamido-3-(1H-indol-3-yl)propanamido)-3-((2-amino-2-oxoethyl)amino)-3-oxopropyl)phenyl)-4,4-dimethylpentanamide (S38)**

^{1}H NMR (400 MHz, CD_3OD)

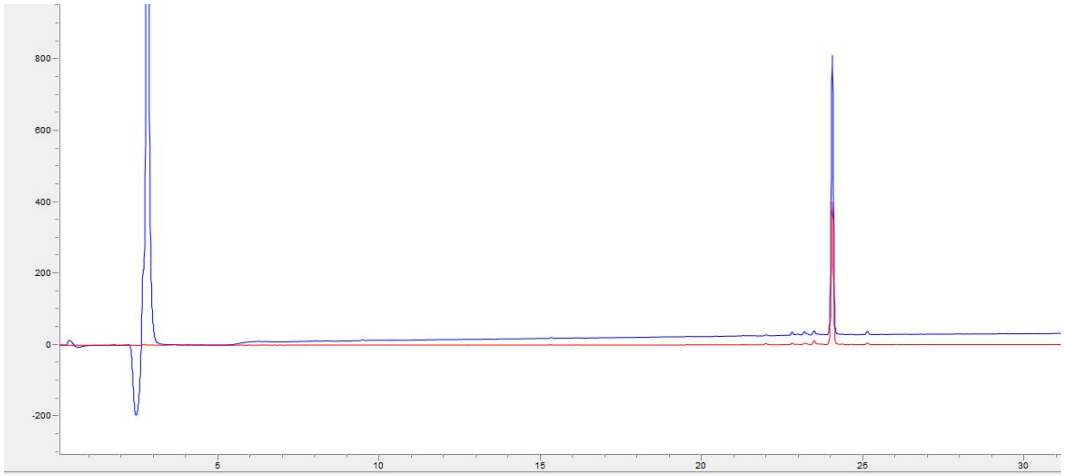


Crude ^{1}H NMR of product off-resin. Internal standard used to calculate conversion and yield.

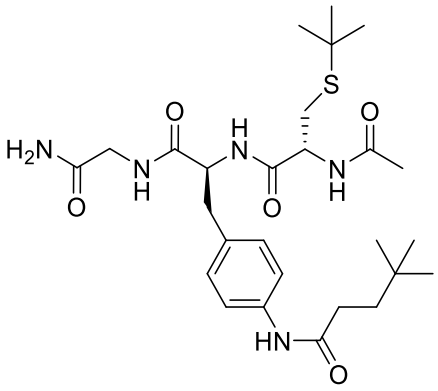
^{1}H NMR (400 MHz, CD_3OD)



^{1}H NMR of purified compound.

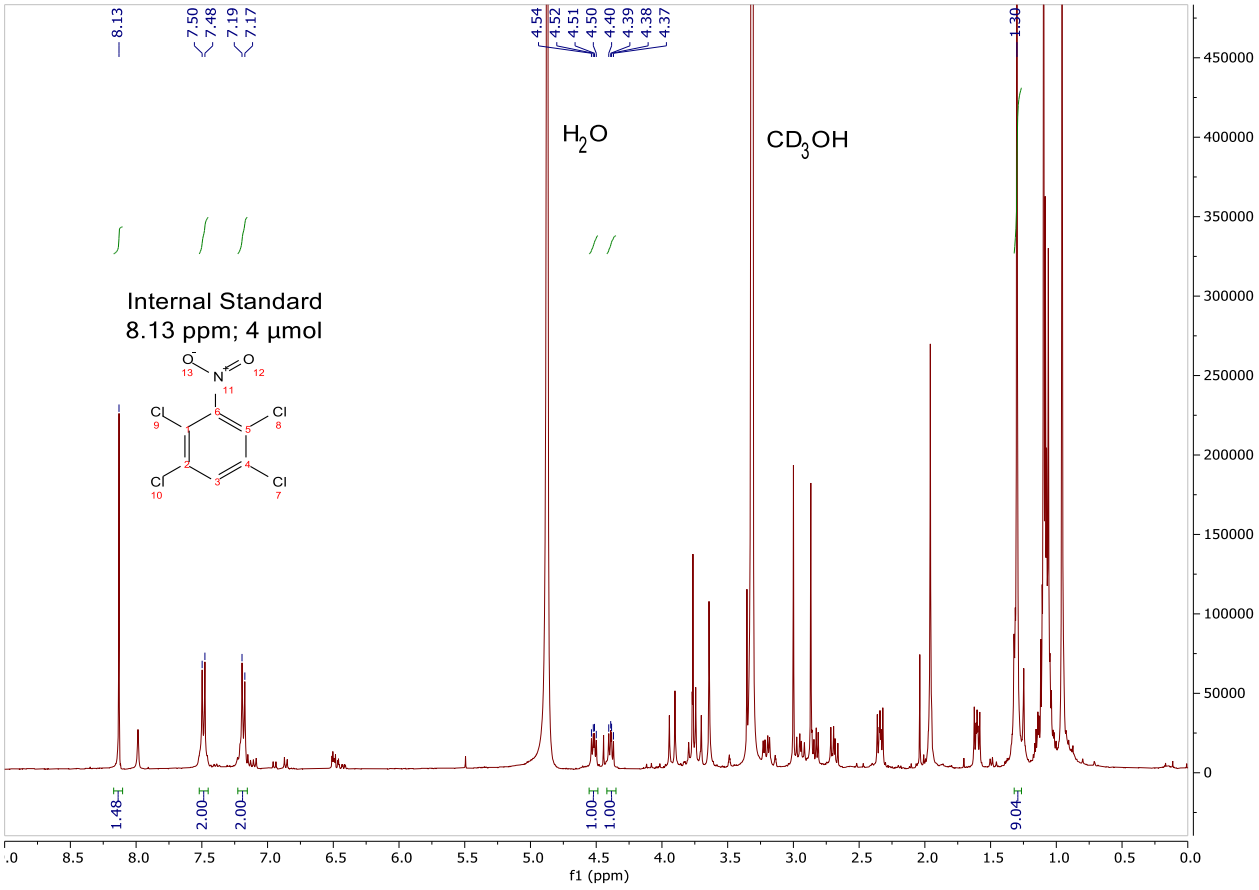


Chromatogram after HPLC purification. Reinjection of compound on analytical HPLC using a linear gradient of 10-50 % MeCN/H_2O (0.1 % TFA) over 30 min at 254 nm (red) and 214 nm (blue).



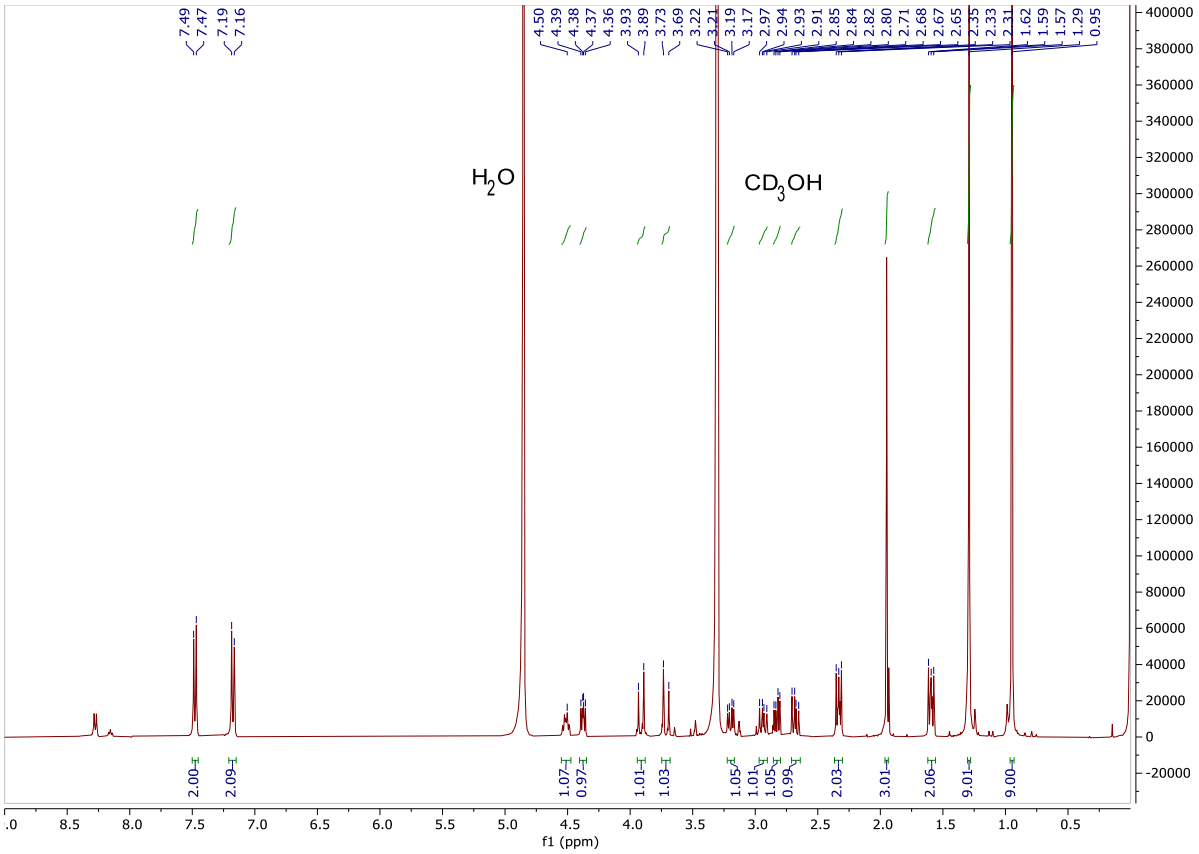
***N*-(4-((*S*)-2-((*R*)-2-Acetamido-3-(*tert*-butylthio)propanamido)-3-((2-amino-2-oxoethyl)amino)-3-oxopropyl)phenyl)-4,4-dimethylpentanamide (S39)**

^{1}H NMR (400 MHz, CD_3OD)

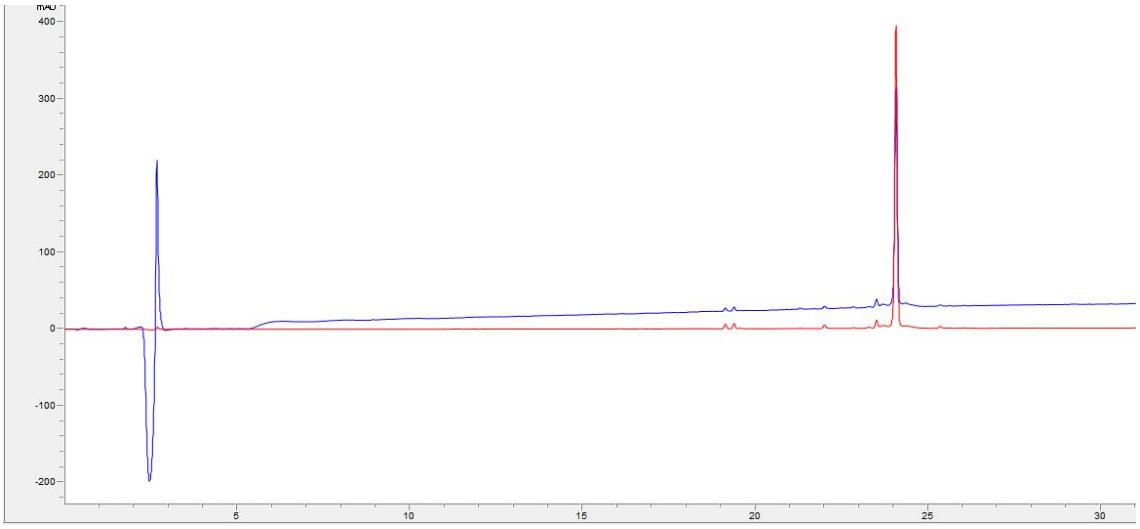


Crude ^{1}H NMR of product off-resin. Internal standard used to calculate conversion and yield.

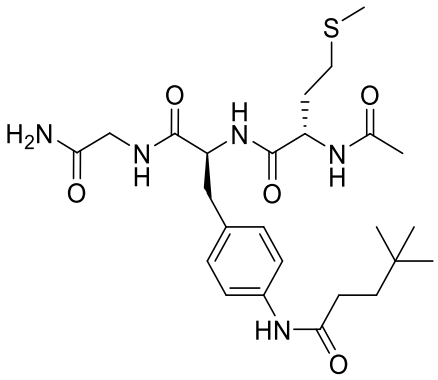
^{1}H NMR (400 MHz, CD_3OD)



^{1}H NMR of purified compound.

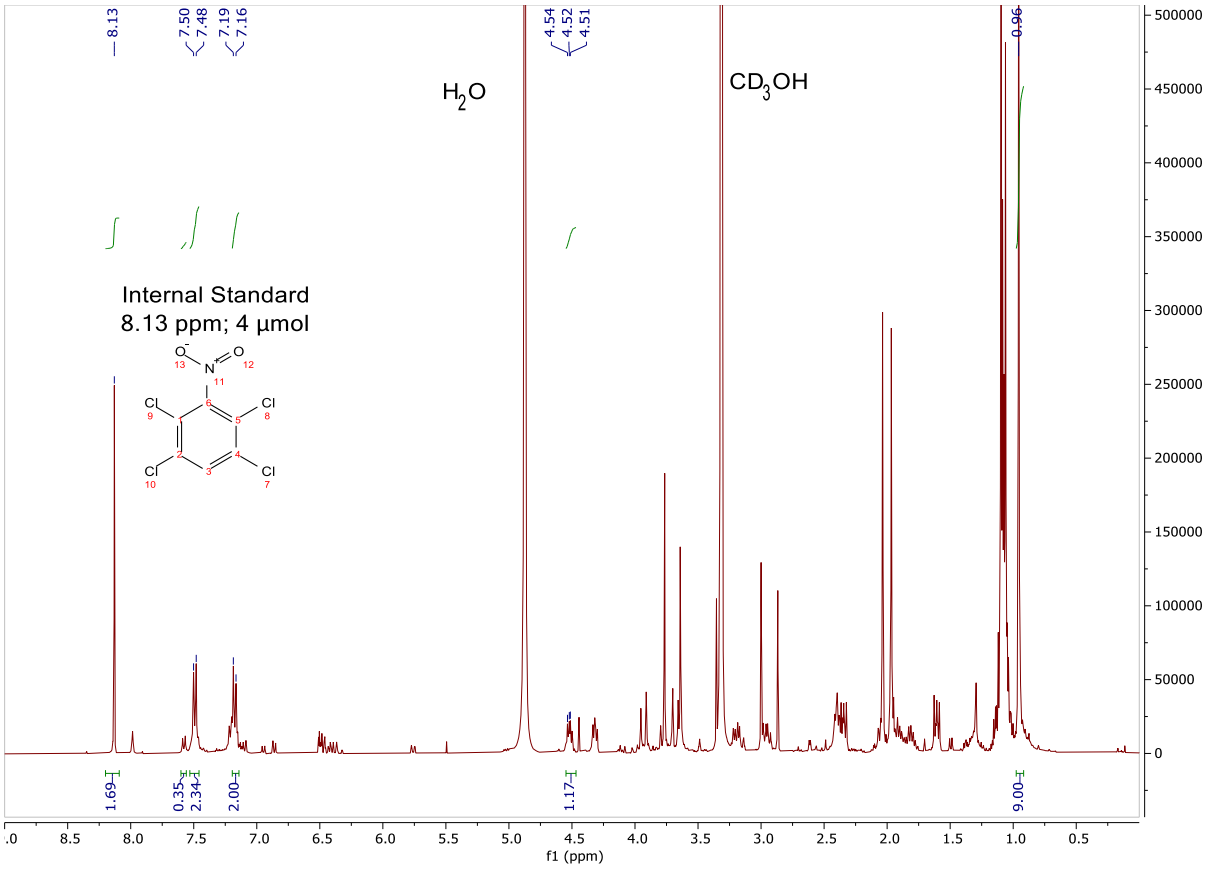


Chromatogram after HPLC purification. Reinjection of compound on analytical HPLC using a linear gradient of 5-60 % MeCN/H_2O (0.1 % TFA) over 30 min at 254 nm (red) and 214 nm (blue).



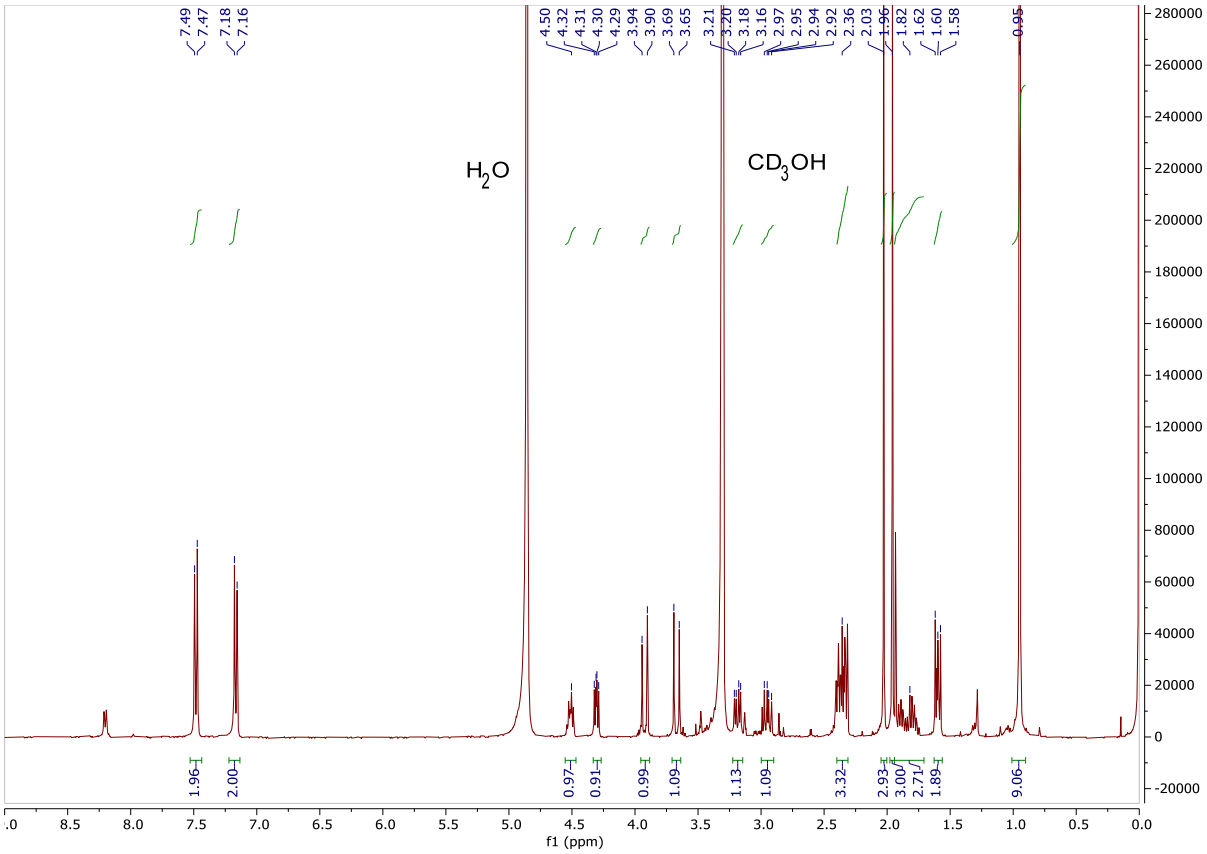
***N*-(4-((*S*)-2-((*R*)-2-Acetamido-3-(methylthio)propanamido)-3-((2-amino-2-oxoethyl)amino)-3-oxopropyl)phenyl)-4,4-dimethylpentanamide (S40)**

^{1}H NMR (400 MHz, CD_3OD)

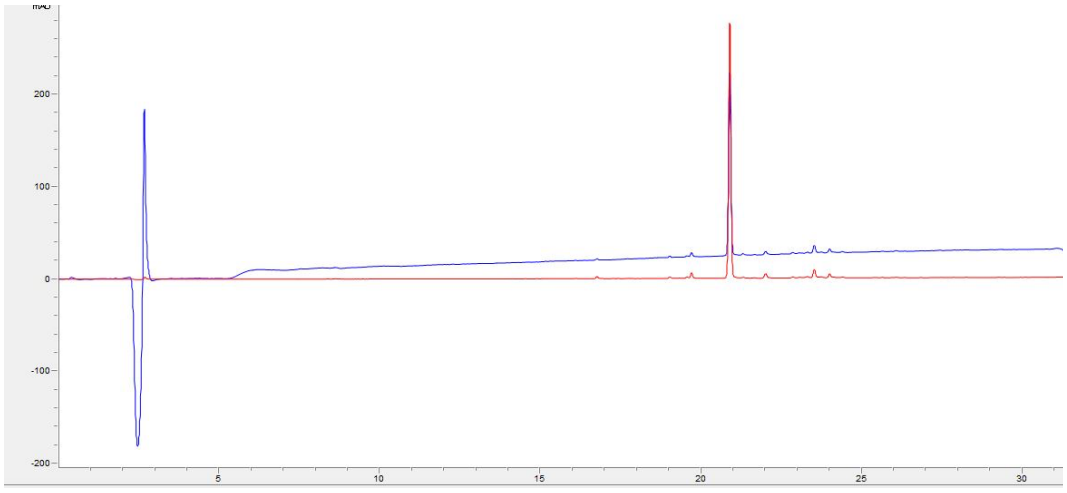


Crude ^{1}H NMR of product off-resin. Internal standard used to calculate conversion and yield.

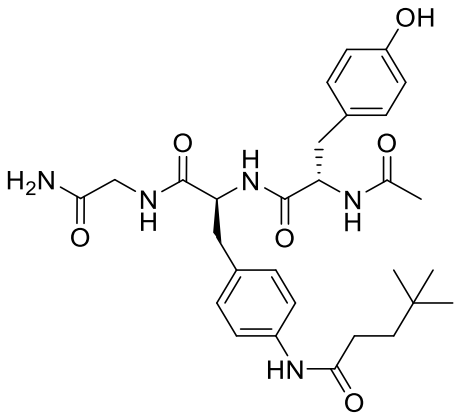
^{1}H NMR (400 MHz, CD_3OD)



^{1}H NMR of purified compound.

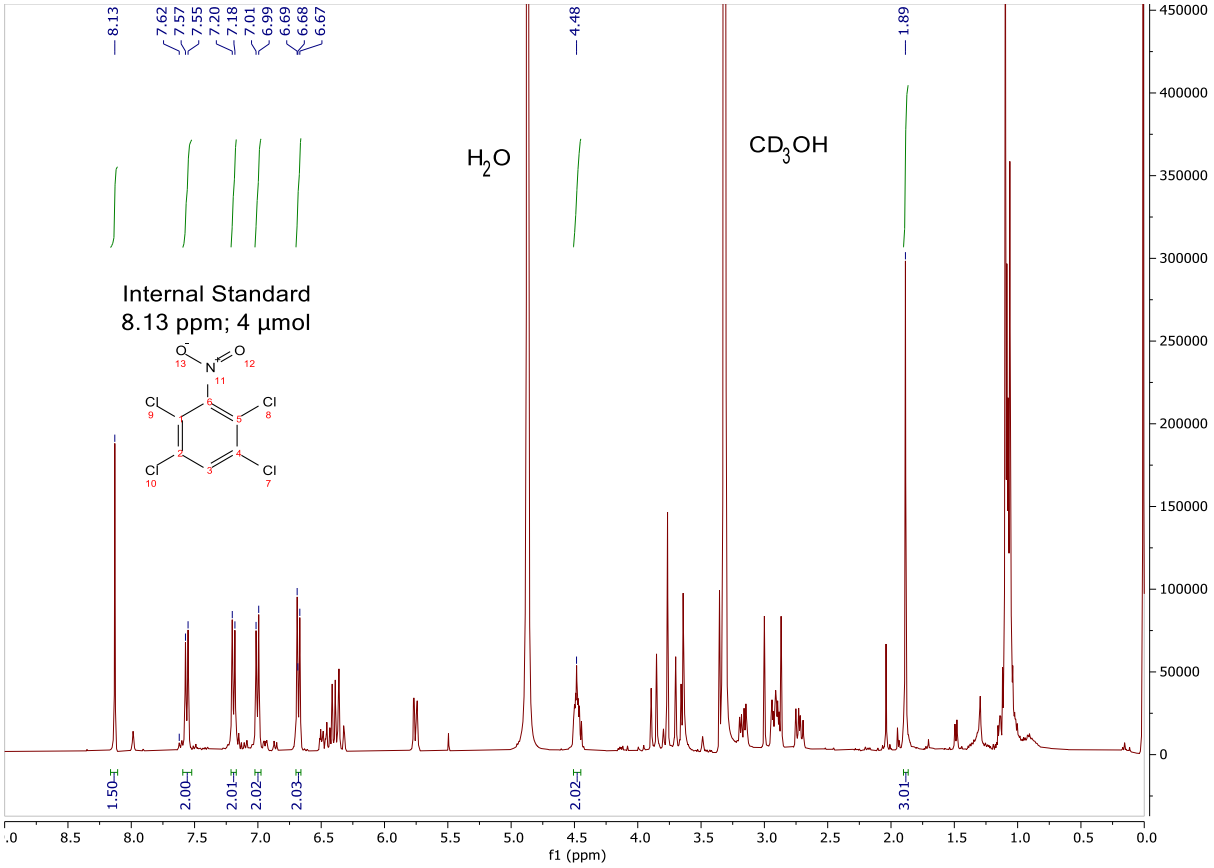


Chromatogram after HPLC purification. Reinjection of compound on analytical HPLC using a linear gradient of 10-50 % MeCN/H_2O (0.1 % TFA) over 30 min at 254 nm (red) and 214 nm (blue).



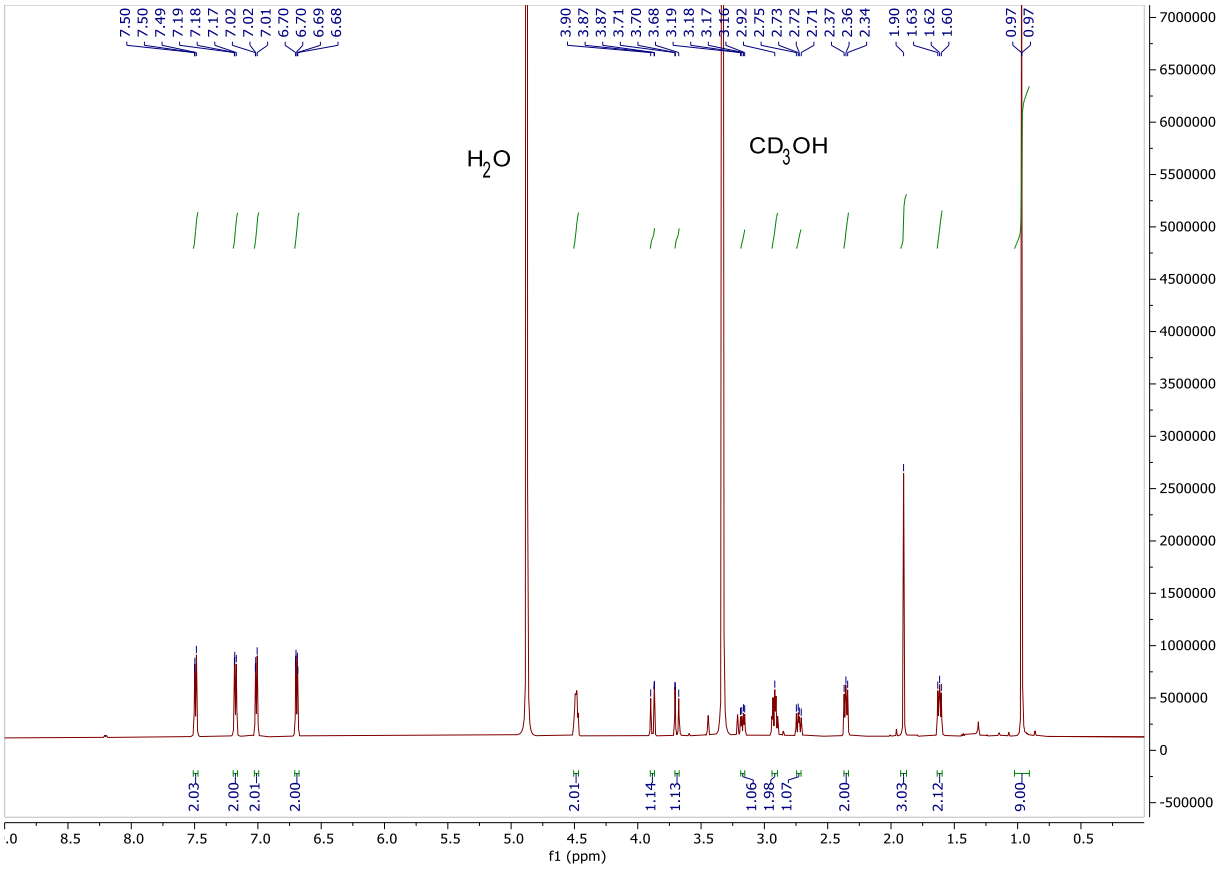
***N*-(4-((*S*)-2-((*S*)-2-Acetamido-3-(4-(*tert*-butoxy)phenyl)propanamido)-3-((2-amino-2-oxoethyl)amino)-3-oxopropyl)phenyl)-4,4-dimethylpentanamide (S41)**

^{1}H NMR (400 MHz, CD_3OD)

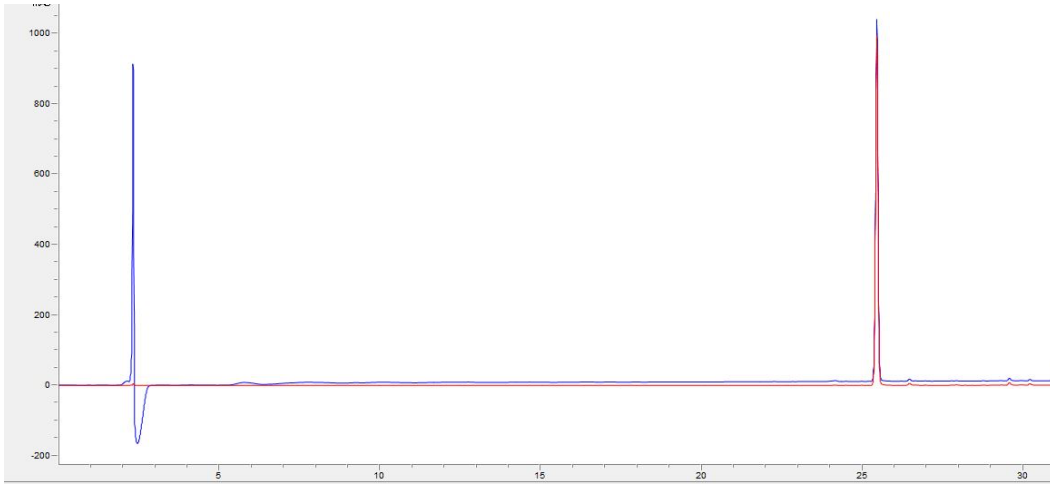


Crude ^{1}H NMR of product off-resin. Internal standard used to calculate conversion and yield.

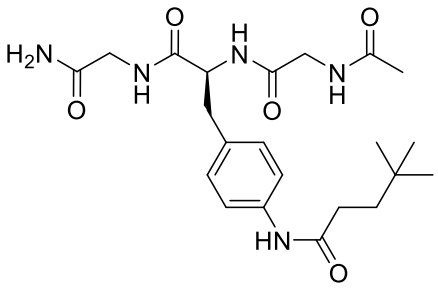
^{1}H NMR (400 MHz, CD_3OD)



^{1}H NMR of purified compound.

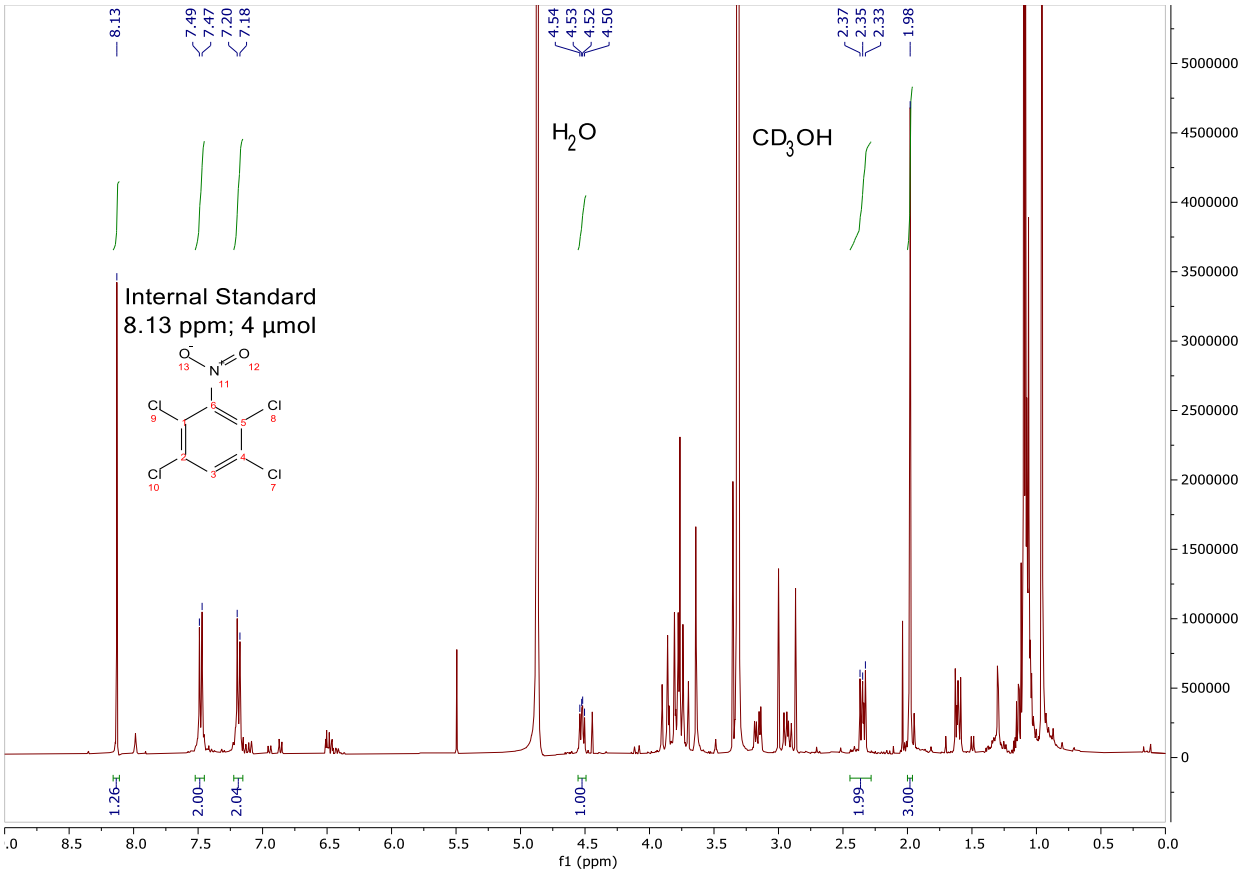


Chromatogram after HPLC purification. Reinjection of compound on analytical HPLC using a linear gradient of 5-40 % MeCN/H_2O (0.1 % TFA) over 30 min at 254 nm (red) and 214 nm (blue).



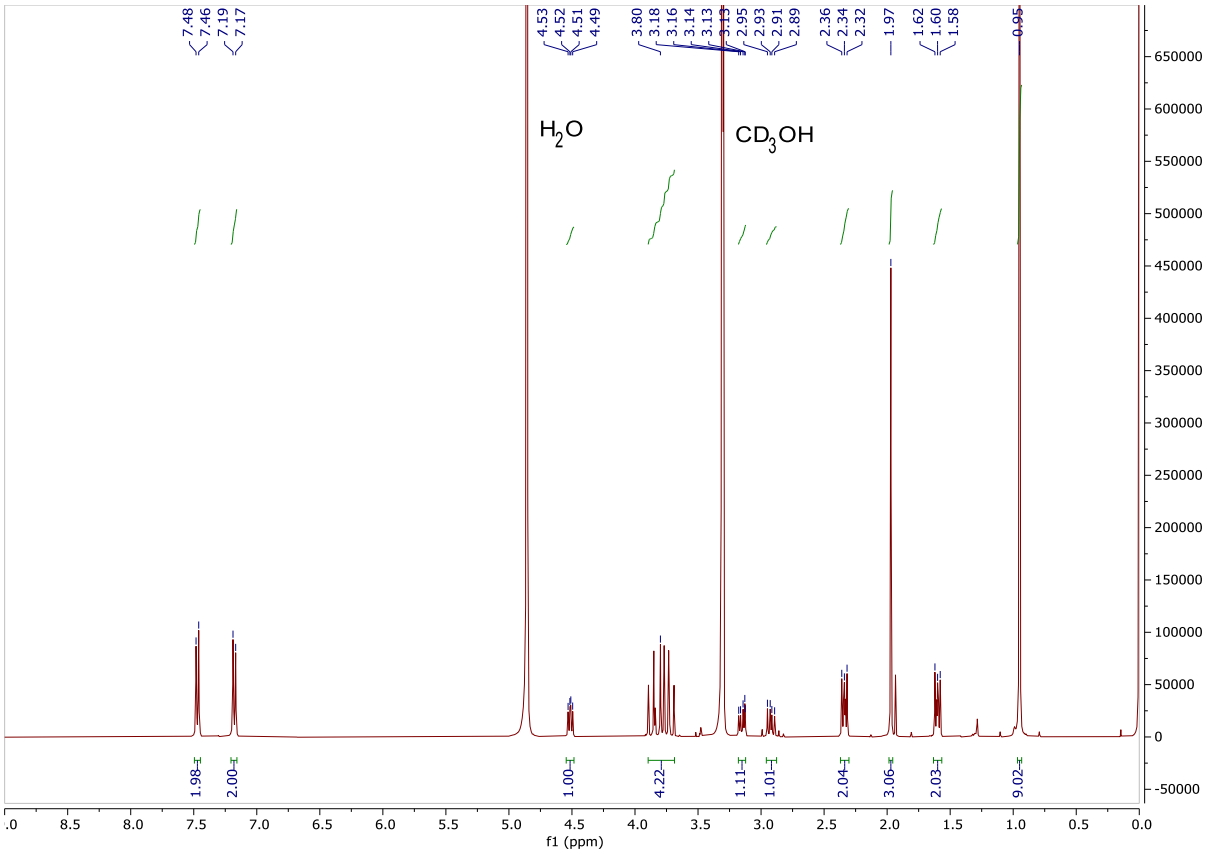
(*S*)-*N*-(4-(2-(2-Acetamidoacetamido)-3-((2-amino-2-oxoethyl)amino)-3-oxopropyl)phenyl)-4,4-dimethylpentanamide (S42)

^{1}H NMR (400 MHz, CD_3OD)

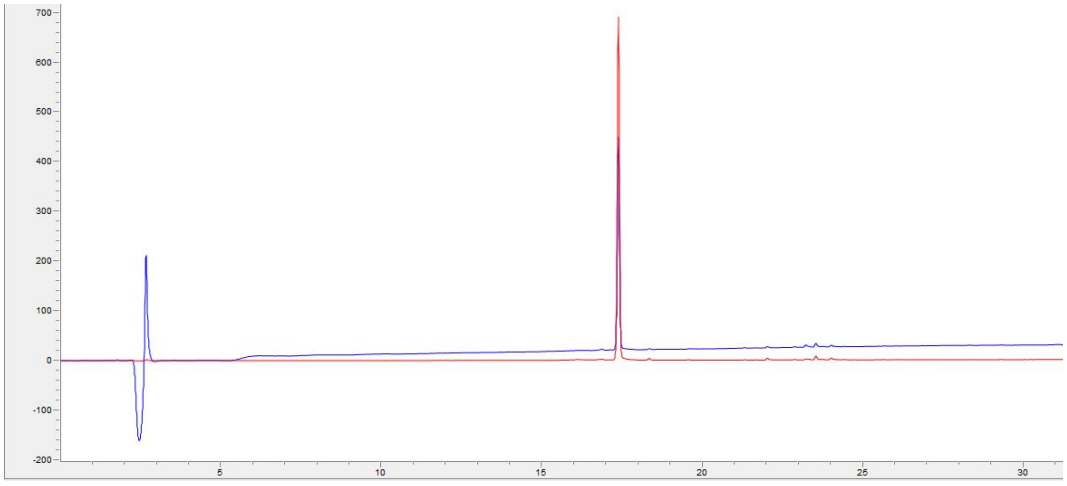


Crude ^{1}H NMR of product off-resin. Internal standard used to calculate conversion and yield.

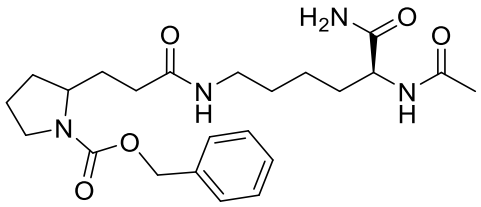
^{1}H NMR (400 MHz, CD_3OD)



^{1}H NMR of purified compound.

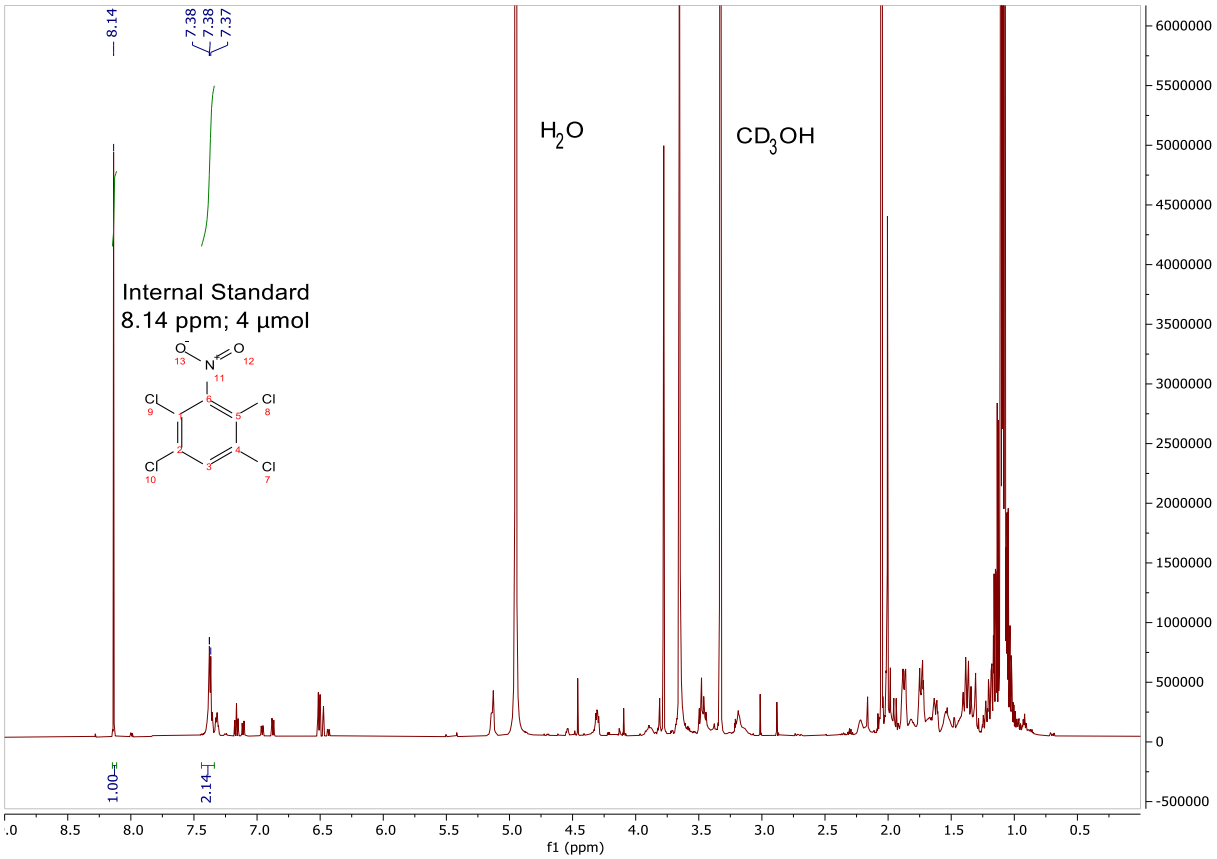


Chromatogram after HPLC purification. Reinjection of compound on analytical HPLC using a linear gradient of 10-50 % MeCN/H_2O (0.1 % TFA) over 30 min at 254 nm (red) and 214 nm (blue).



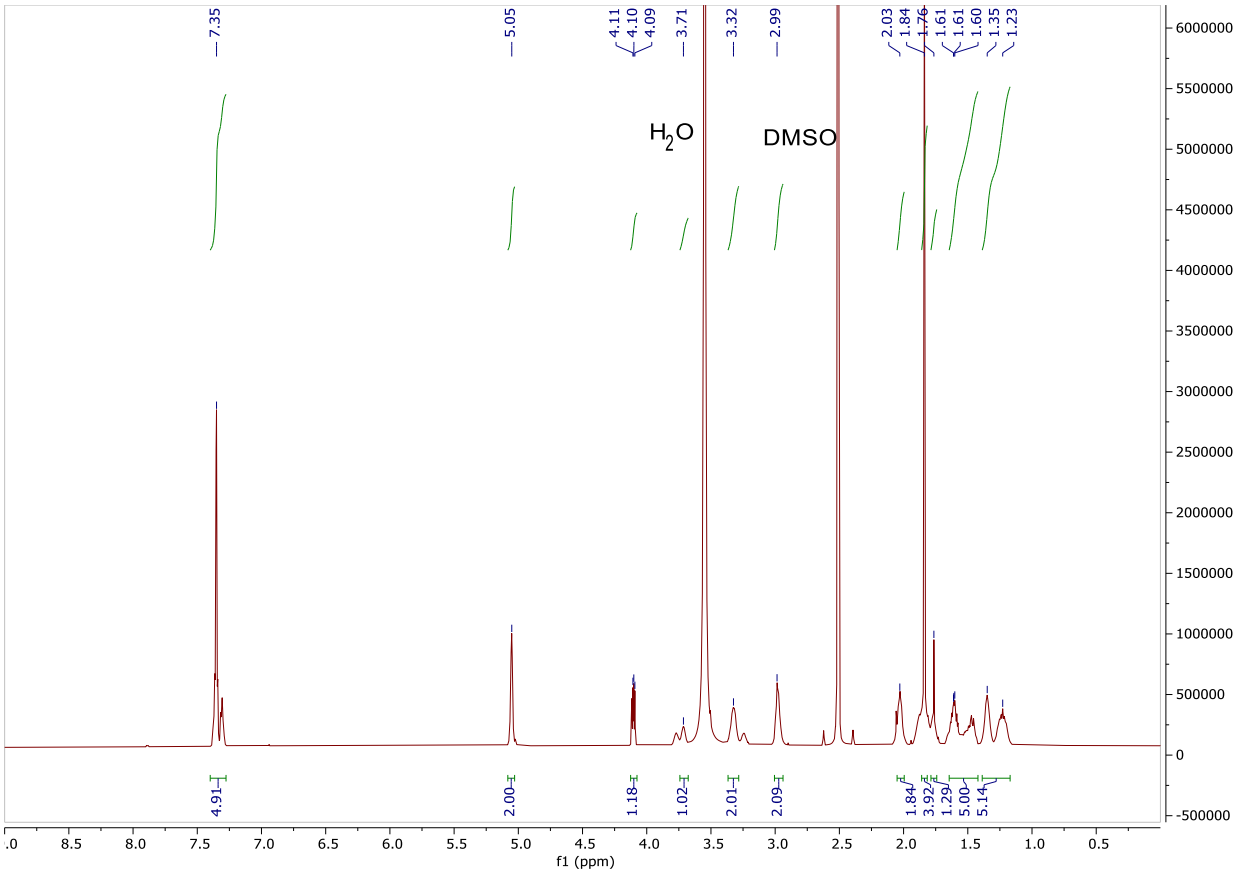
Benzyl 2-(3-(((S)-5-acetamido-6-amino-6-oxohexyl)amino)-3-oxopropyl)pyrrolidine-1-carboxylate (S43)

^{1}H NMR (600 MHz, CD_3OD)

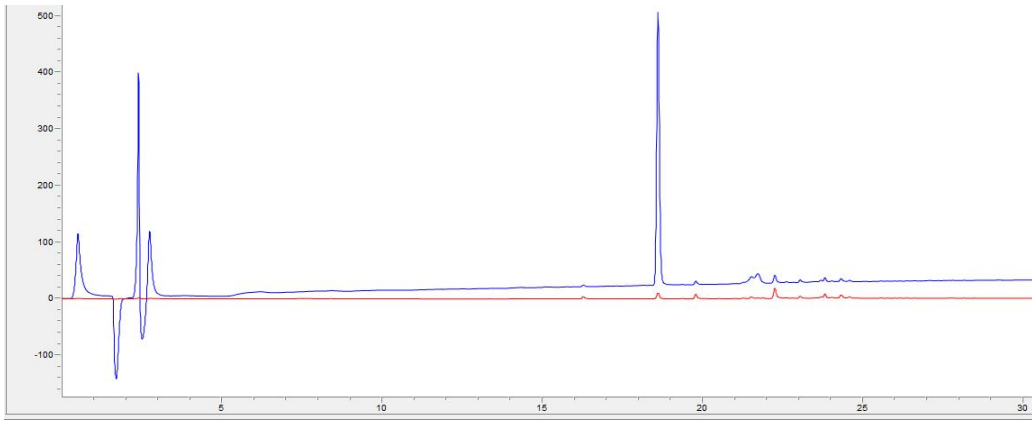


Crude ^{1}H NMR of product off-resin. Internal standard used to calculate conversion and yield.

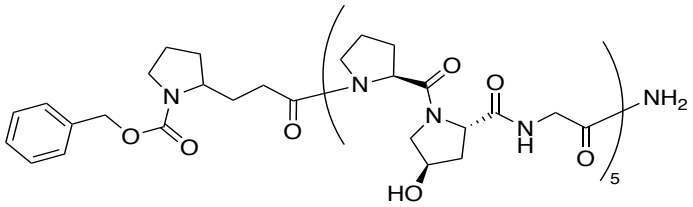
^{1}H NMR (600 MHz, DMSO-d6)



^{1}H NMR of purified compound.



Chromatogram after HPLC purification. Reinjection of compound on analytical HPLC using a linear gradient of 10-50 % (0.1 % TFA) over 30 min at 254 nm (red) and 214 nm (blue).



(S44)

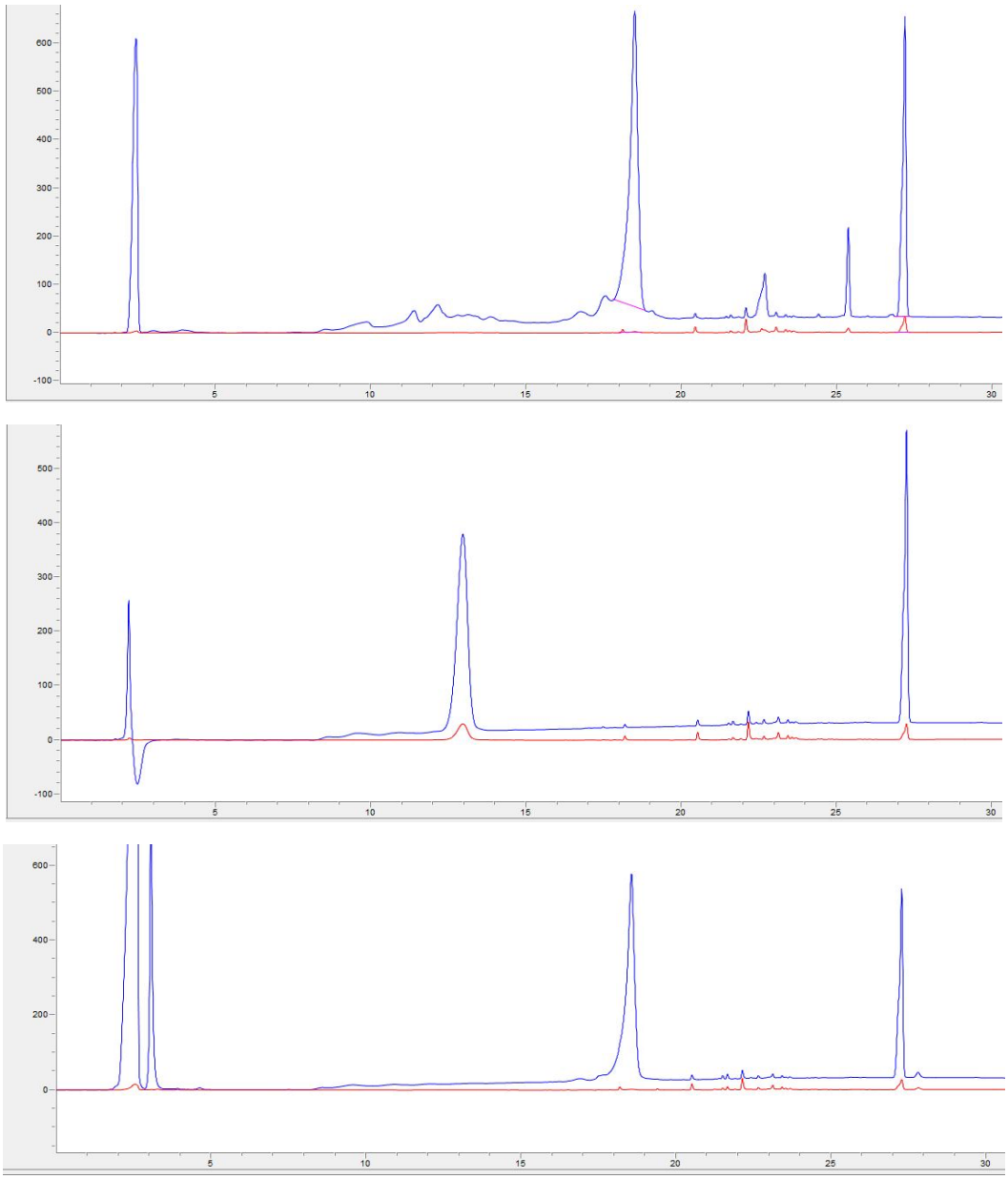
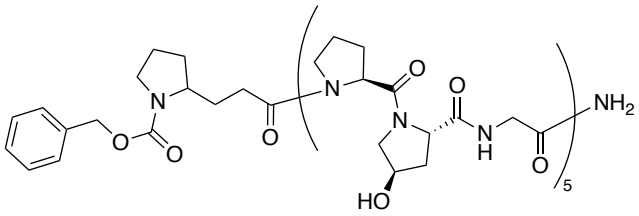
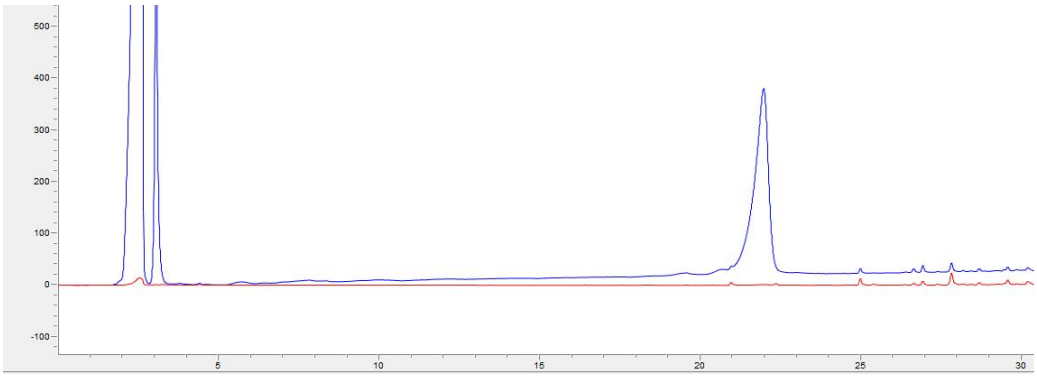


Figure 1. (Top) Crude analytical HPLC spectra of above reaction indicating product [18.5 min, area = 11530 (214 nm)], starting material not observed. Pure analytical HPLC spectra of starting material (middle) and product (bottom). Fenoprofin used as an internal standard (28.8 min). Data was collected using a gradient of 0-5 % MeCN/H_2O over 10 min then 5-60 % MeCN/H_2O over 20 min, data recorded at 254 nm (red) and 214 nm (blue).



(S44)

Compound S44 was purified on preparatory HPLC using a linear gradient of 5-40 % MeCN/H_2O (0.1 % TFA) over 30 min. (R_t = 28.5 min).



Reinjection of compound on analytical HPLC using a linear gradient of 5-40 % (0.1 % TFA) over 30 min at 254 nm (red) and 214 nm (blue). R_t = 22.0 min.

References

(1) Li, H.; Breen, C. P.; Seo, H.; Jamison, T. F.; Fang, Y. Q.; Bio, M. M. Ni-Catalyzed Electrochemical Decarboxylative C-C Couplings in Batch and Continuous Flow. *Org. Lett.* **2018**, *20*, 1338–1341. https://doi.org/10.1021/acs.orglett.8b00070.

(2) Li, Y.; Chen, S.; Wang, M.; Jiang, X. Sodium Dithionite-Mediated Decarboxylative Sulfonylation: Facile Access to Tertiary Sulfones. *Angew. Chemie - Int. Ed.* **2020**, *59*, 8907–8911. https://doi.org/10.1002/anie.202001589.

(3) Zheng, C.; Wang, Y.; Xu, Y.; Chen, Z.; Chen, G.; Liang, S. H. Ru-Photoredox-Catalyzed Decarboxylative Oxygenation of Aliphatic Carboxylic Acids through N-(Acyloxy)Phthalimide. *Org. Lett.* **2018**, *20*, 4824–4827. https://doi.org/10.1021/acs.orglett.8b01885.

(4) Chan, C. M.; Xing, Q.; Chow, Y. C.; Hung, S. F.; Yu, W. Y. Photoredox Decarboxylative C(Sp3)-N Coupling of α-Diazoacetates with Alkyl N-Hydroxyphthalimide Esters for Diversified Synthesis of Functionalized N-Alkyl Hydrazones. *Org. Lett.* **2019**, *21*, 8037–8043. https://doi.org/10.1021/acs.orglett.9b03020.

(5) Yu, L.; Tang, M. L.; Si, C. M.; Meng, Z.; Liang, Y.; Han, J.; Sun, X. Zinc-Mediated Decarboxylative Alkylation of Gem-Difluoroalkenes. *Org. Lett.* **2018**, *20*, 4579–4583. https://doi.org/10.1021/acs.orglett.8b01866.

(6) Suzuki, N.; Hofstra, J. L.; Poremba, K. E.; Reisman, S. E. Nickel-Catalyzed Enantioselective Cross-Coupling of N-Hydroxyphthalimide Esters with Vinyl Bromides. *Org. Lett.* **2017**, *19*, 2150–2153. https://doi.org/10.1021/acs.orglett.7b00793.

(7) Chowdhury, R.; Yu, Z.; Tong, M. L.; Kohlhepp, S. V.; Yin, X.; Mendoza, A. Decarboxylative Alkyl Coupling Promoted by NADH and Blue Light. *J. Am. Chem. Soc.* **2020**, *142*, 20143–20151. https://doi.org/10.1021/jacs.0c09678.

(8) Jiang, W. T.; Yang, S.; Xu, M. Y.; Xie, X. Y.; Xiao, B. Zn-Mediated Decarboxylative Carbagermatranation of Aliphatic: N -Hydroxyphthalimide Esters: Evidence for an Alkylzinc Intermediate. *Chem. Sci.* **2020**, *11*, 488–493. https://doi.org/10.1039/c9sc04288a.

(9) Huang, H. M.; Koy, M.; Serrano, E.; Pflüger, P. M.; Schwarz, J. L.; Glorius, F. Catalytic Radical Generation of π-Allylpalladium Complexes. *Nat. Catal.* **2020**, *3*, 393–400. https://doi.org/10.1038/s41929-020-0434-0.

(10) Bosque, I.; Bach, T. 3-Acetoxyquinuclidine as Catalyst in Electron Donor-Acceptor Complex-Mediated Reactions Triggered by Visible Light. *ACS Catal.* **2019**, *9* , 9103–9109. https://doi.org/10.1021/acscatal.9b01039.

(11) Mao, Y.; Zhao, W.; Lu, S.; Yu, L.; Wang, Y.; Liang, Y.; Ni, S.; Pan, Y. Copper-Catalysed Photoinduced Decarboxylative Alkynylation: A Combined Experimental and Computational Study. *Chem. Sci.* **2020**, *11*, 4939–4947. https://doi.org/10.1039/d0sc02213f.

(12) Wang, J.; Lundberg, H.; Asai, S.; Martín-Acosta, P.; Chen, J. S.; Brown, S.; Farrell, W.; Dushin, R. G.; O'Donnell, C. J.; Ratnayake, A. S.; Richardson, P.; Liu, Z.; Qin, T.; Blackmond, D. G.; Baran, P. S. Kinetically Guided Radical-Based Synthesis of C(Sp3)−C(Sp3) Linkages on DNA. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115*, E6404–E6410. https://doi.org/10.1073/pnas.1806900115.

(13) Kasznel, A. J.; Zhang, Y.; Hai, Y.; Chenoweth, D. M. Structural Basis for Aza-Glycine Stabilization of Collagen. *J. Am. Chem. Soc.* **2017**, *139*, 9427–9430. https://doi.org/10.1021/jacs.7b03398.

(14) Elbaum, M. B.; Zondlo, N. J. OGlcNAcylation and Phosphorylation Have Similar Structural Effects in α-Helices: Post-Translational Modifications as Inducible Start and Stop Signals in α-Helices, with Greater Structural Effects on Threonine Modification. *Biochemistry* **2014**, *53*, 2242–2260. https://doi.org/10.1021/bi500117c.