Supporting Information

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Supplemental Figures

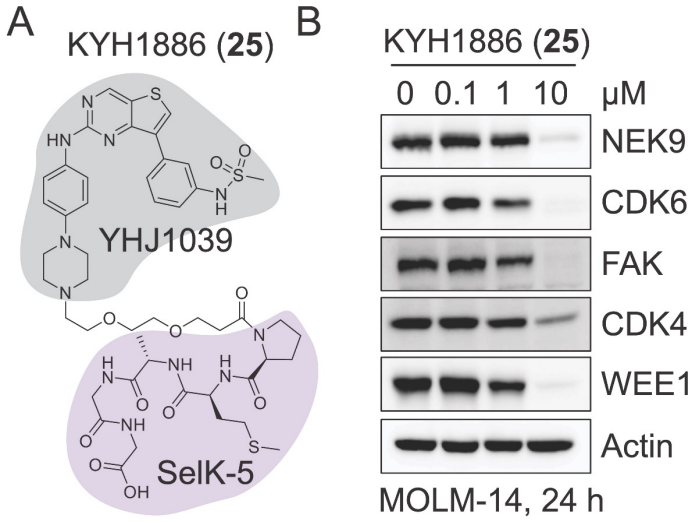


Figure S1. Structure and activity of KYH1886 (25), related to Figure 1.

A) Structure of KYH1886 (**25**).
B) Western blot result showing kinase levels after treatment of MOLM-14 cells with KYH1886 (**25**) for 24 hours at the indicated concentrations.

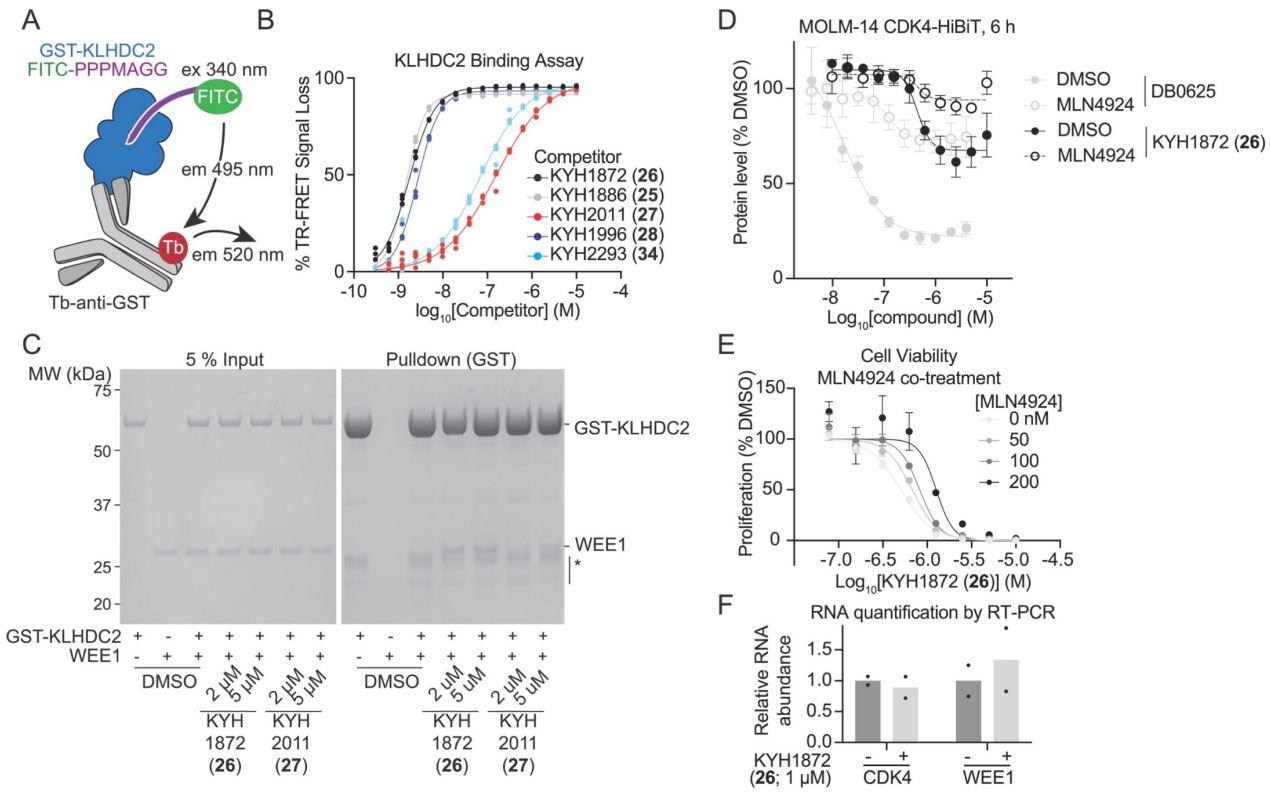


Figure S2. KLHDC2-SelK binding and compound mechanism of action, related to Figure 2.

A) Schematic showing the TR-FRET (Time-Resolved Förster Resonance Energy Transfer) assay setup. Upon incubation with compounds that engage KLHDC2, the FITC-labeled SelK peptide (purple) is displaced from recombinant GST-KLHDC2.
B) KLHDC2 biochemical binding assay showing the ability of the indicated compounds to compete with FITC-SelK for KLHDC2 binding. Error bars show +/- SD.
C) GST pulldown assay showing induced association of purified KLHDC2 and WEE1 kinase domain upon treatment with the indicated concentrations of KYH1872 or KYH2011 (D-Met-containing compound). A Coomassie stain is shown (total protein). The bands labeled by the asterisk correspond to GST-KLHDC2 truncation products; they are also visible in the pulldown sample without WEE1 (lane 2).
D) HiBiT experiment showing levels of CDK4-HiBiT protein after treatment with the indicated compounds. Cells were pretreated for two hours with either DMSO or 1 µM MLN4924.
E) Cell viability experiment showing the effects of increasing doses of MLN4924. Maximal cell viability was calculated separately for each MLN4924 dose (listed at right). Error bars show +/- SD.
F) RNA levels were determined by RT-PCR. GAPDH- and DMSO-normalized values from two biological replicates are shown with measurements from independent experiments plotted as dots. RNA levels were determined by densitometric quantification of RT-PCR band intensity.

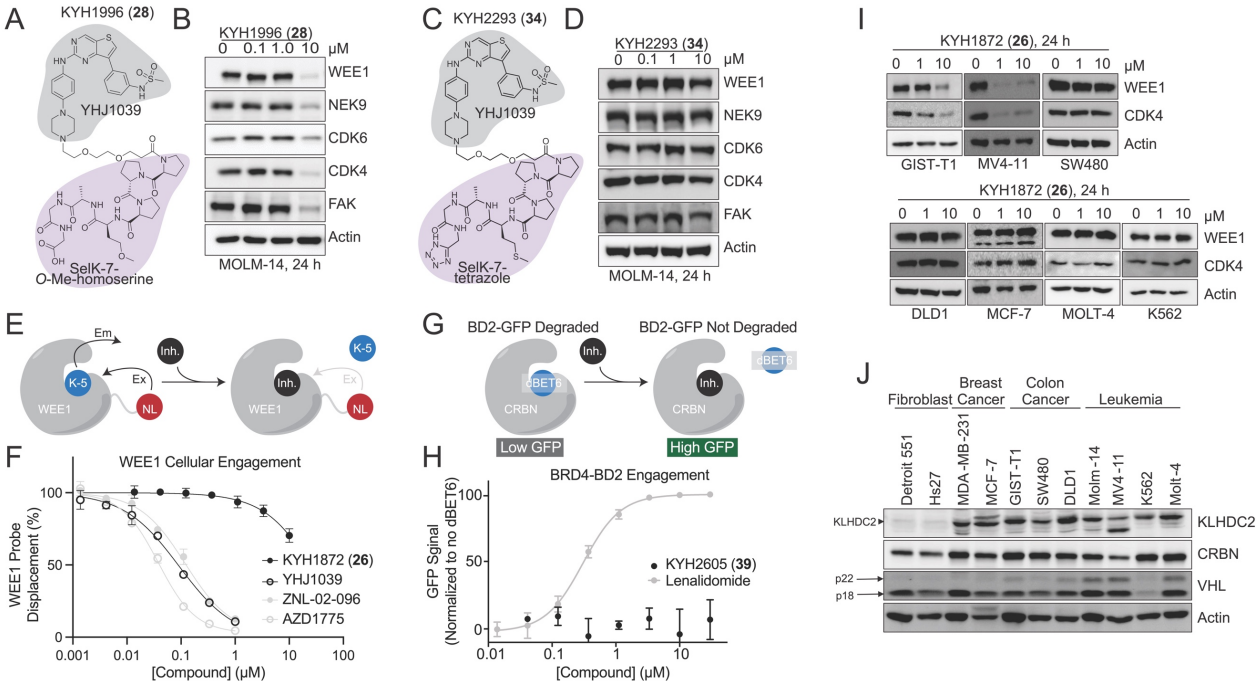


Figure S3. Further characterization of SelK-bearing compounds, related to Figure 3.

A) Structure of KYH1996 (**28**) compound.
B) Western blot showing kinase levels in MOLM-14 cells treated with the indicated concentrations of KYH1996 (**28**).
C) Structure of KYH2293 (**34**).
D) Western blot showing kinase levels in MOLM-14 cells treated with the indicated concentrations of KYH2293 (**34**).
E) Schematic showing the WEE1 cellular engagement assay. K-5 is the kinase-binding competitive inhibitor fluorescent probe used in this assay. NL indicates nanoluciferase, which is fused to WEE1. Inh. indicates the tested inhibitor compound. Em and Ex refer to the Emitted and Excitation light from the K-5 probe, respectively. The excitation is produced by nanoluciferase enzyme activity upon addition of the substrate to cells. Displacement of the K-5 probe from the WEE1 active site results in loss of energy transfer.
F) WEE1 cellular engagement data showing K-5 probe WEE1 occupancy as a percent of the signal seen in DMSO-treated cells.
G) Schematic showing the CRBN cellular engagement assay. dBET6 induces BD2-GFP (BRD4 bromodomain 2) degradation via CRBN. Test compound (Inh.) CRBN displaces dBET6, sparing BD2-GFP.
H) CRBN cellular engagement data. Curve fitting was not attempted for the test compound (KYH2605, **39**; thalidomide-SelK-7 conjugate). Lenalidomide was used as a positive control for CRBN engagement.
I) Western blots showing kinase levels (WEE1 and CDK4) in the indicated cell lines treated for 24 h with KYH1872 (**26**).
J) KLHDC2, VHL, and CRBN expression levels assessed by Western blot in the indicated cell lines. The arrow (left) points to the KLHDC2 band.

Compounds	Papp (10^{-6} cm/s)		Recovery %		Recovery % with Cell Lysate		Rank
	A to B	B to A	A to B	B to A	A to B	B to A	Papp
Atenolol	0.29	NA	89.5	NA			Low
Propranolol	22.78	NA	78.4	NA			High
Digoxin	0.43	17.87	107.0	117.6			Low
KYH1872 (**26**)	0.06	0.02	31.4	87.5	32.5	87.6	Low
SelK-7 (**35**)	0.06	0.02	16.7	95.2	16.9	95.2	Low
YHJ1039	2.40	3.72	30.6	74.7	70.9	79.4	Moderate

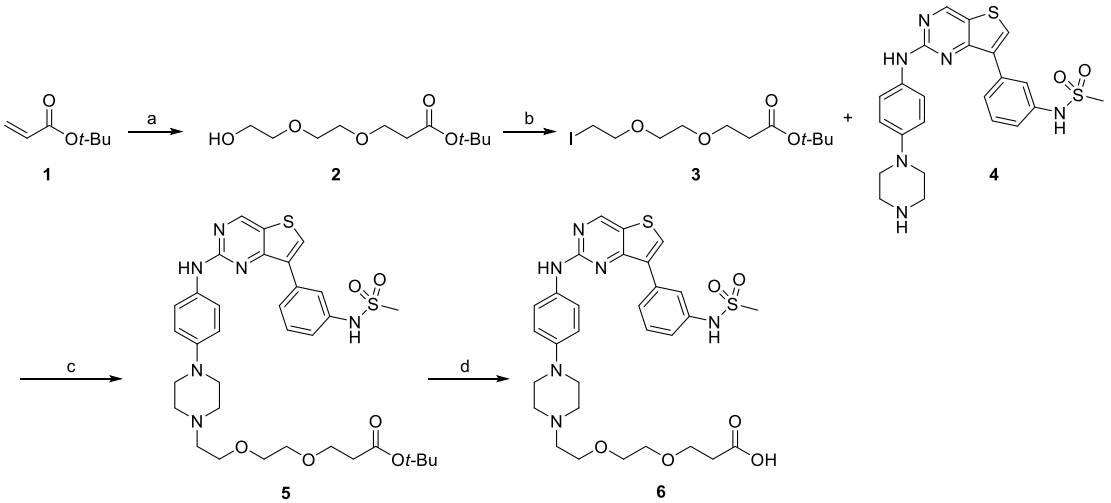
Table S1. Caco-2 permeability assay for KYH1872 (26), SelK-7 (35), and YHJ1039, related to Figure 3.
Atenolol, propranolol, and digoxin were used as control compounds. KYH1872 (**26**) and SelK-7 (**35**) have low permeability, YHJ1039 has moderate permeability (Papp(AtoB) < 2 : Low permeability; 2 < Papp(AtoB) < 10 : Moderate permeability; Papp(AtoB) > 10 : High permeability).

Data S1. Materials and methods for chemical synthesis of compounds described in the text, related STAR methods.

General information. Unless otherwise described, all commercial reagents and solvents were purchased from commercial suppliers and used without further purification. All reactions were performed under a N_2 atmosphere in flame-dried glassware. Reactions were monitored by using TLC with 0.25 mm E. Merck pre-coated silica gel plates (60 F254). Reaction progress was monitored by using TLC analysis using a UV lamp, ninhydrin, or p-anisaldehyde stain for detection purposes. All solvents were purified by using standard techniques. Purification of reaction products was carried out by using silica gel column chromatography with Kieselgel 60 Art. 9385 (230–400 mesh). Purities of all compounds were ≥ 95%. Mass spectra and purities of all compounds were assessed using LC/mass spec analysis with Waters LCMS system (Waters 2998 Photodiode Array Detector, Waters 3100 Mass Detector, Waters SFO System Fluidics Organizer, Water 2545 Binary Gradient Module, Waters Reagent Manager, and Waters 2767 Sample Manager) using SunFireTM C18 column (4.6 × 50 mm, 5 μm particle size): solvent gradient = 30% B at 0.00 min, 100% B at 7.00 min, 100% B at 8.50 min, 30% B at 8.51 min, 30% B at 10.00 min. Solvent A = 0.1% formic acid in H_2O; Solvent B = 0.1% formic acid in methanol; flow rate = 0.8 mL/min. ^1H and ^{13}C NMR spectra were obtained using Bruker 400 MHz FT-NMR (400 MHz for ^1H, and 100 MHz for ^{13}C) spectrometer and Agilent 600 MHz FT-NMR (150 MHz for ^{13}C). Standard abbreviations are used for denoting the signal multiplicities.

Pathways for synthesis of PROTAC molecules having a broad-spectrum kinase inhibitor (YHJ1039) fused with KLHDC2 substrate peptides are outlined in Schemes 1-7. The synthetic route employed to prepare the linker-attached warhead **6**, given in Scheme 1, begins with the treatment of commercially available acrylate **l** and 2-(2-hydroxyethoxy)ethanol with benzyltrimethylammonium hydroxide, followed by iodination using I_2 to yield the iodide **3** (85%). The kinase inhibitor **4** prepared as previously reported was subjected to alkylation with iodide **3** using NaI and DIPEA, followed by hydrolysis of the *tert*-butyl ester to yield the key intermediate **6** [1].

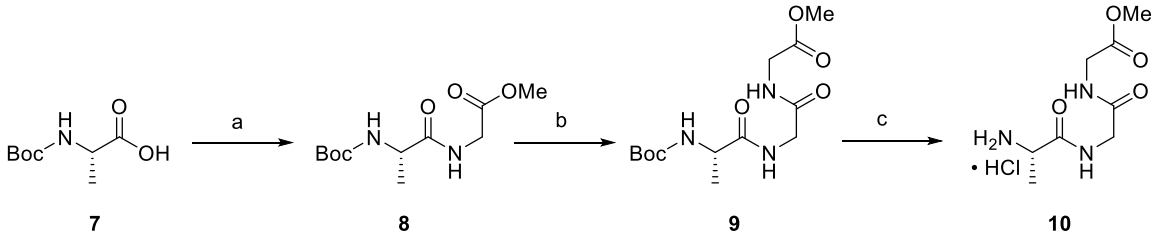
Scheme 1. Synthesis of Linker-attached Warhead **6**.



Reagent and condition: (a) 2-(2-hydroxyethoxy)ethanol, benzyltrimethylammonium hydroxide, MeCN, rt, 12 h, 44%; (b) I_2, triphenylphosphine, imidazole, DCM, rt, 12 h, 85%; (c) NaI, DIPEA, DMF, 60 °C, 3 h, 43%; (d) 4 M HCl in dioxane, DCM, 0°C to rt, 6 h, 96%.

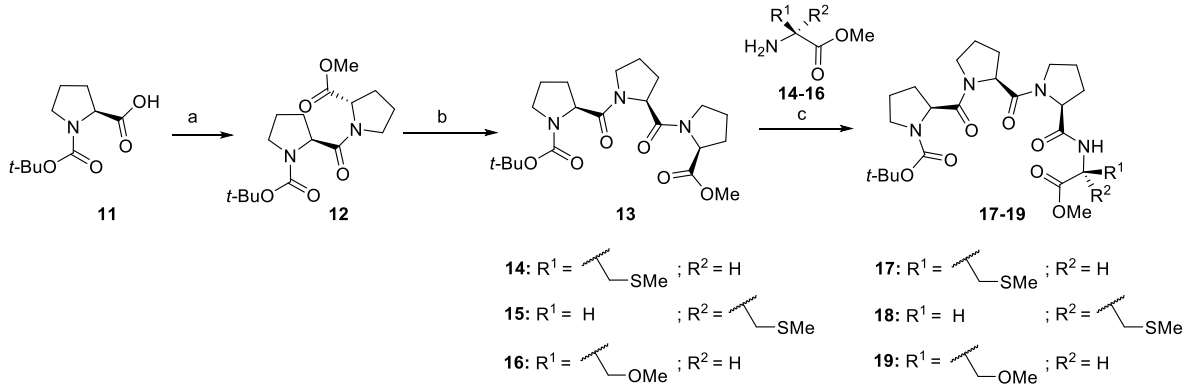
Peptide fragments **10** and **17-19** were prepared by the synthetic strategy described in Schemes 2-3. Amide coupling reactions using EDCI and 1-hydroxybenzotriazole carried out on the commercially available amino acid **7** with glycine methyl ester hydrochloride yielded the dipeptide **8** (87%). The hydrolysis of the methyl ester, followed by another amide coupling reaction formed **9** (91%), which upon removal of *tert*-butyloxycarbonyl group formed the tripeptide **10** (91%). The same synthetic route as for **9** by using the commercially available amino acid **11** and *L*-proline methyl ester hydrochloride was employed to synthesize the tripeptide **13** (79%), which upon hydrolysis of methyl ester and amide coupling with the corresponding amino acid methyl ester hydrochloride formed **17**-**19** (75-82%).

Scheme 2. Synthesis of Tripeptide Fragment **10**.



Reagent and condition: (a) glycine methyl ester hydrochloride, EDCI, 1-hydroxybenzotriazole, DIPEA, DCM, rt, 3 h, 87%; (b) i) LiOH, THF/H_2O (1:1), 0 °C to rt, 3 h; ii) glycine methyl ester hydrochloride, EDCI, 1-hydroxybenzotriazole, DIPEA, DCM, rt, 3 h, 91% over two steps; (c) 4 M HCl in dioxane, DCM, 0°C to rt, 3 h, 91%.

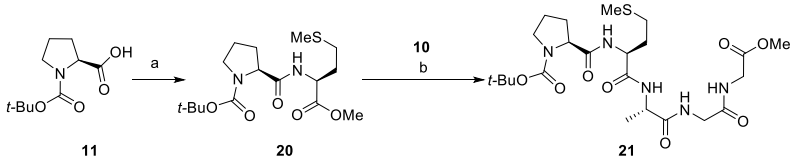
Scheme 3. Synthesis of Tetrapeptide Fragments **17-19** .



Reagent and condition: (a) amino acid methyl ester hydrochloride, EDCI, 1-hydroxybenzotriazole, DIPEA, DCM, rt, 3 h, 86%; (b) i) LiOH, THF/H_2O (1:1), 0 °C to rt, 3 h; ii) *L*-proline methyl ester hydrochloride, EDCI, 1-hydroxybenzotriazole, DIPEA, DCM, rt, 3 h, 79% over two steps; (c) i) LiOH, THF/H_2O (1:1), 0 °C to rt, 3 h; ii) corresponding amino acid methyl ester hydrochloride, EDCI, 1-hydroxybenzotriazole, DIPEA, DCM, rt, 3 h, 75-82% over two steps.

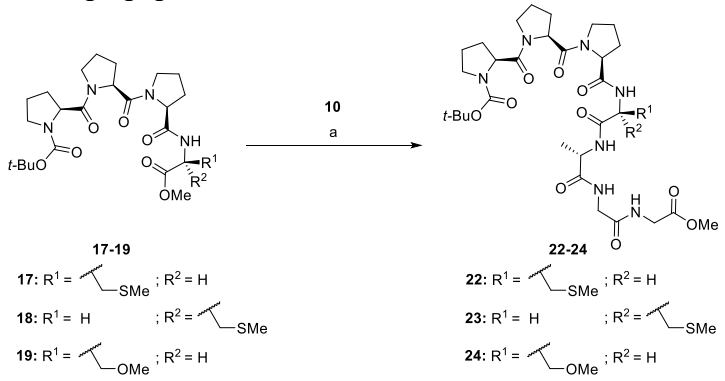
The synthesis of penta- and heptapeptide KLHDC2 binders is shown in Schemes 4-5. Amide coupling reactions using EDCI and 1-hydroxybenzotriazole carried out on the commercially available amino acid **11** with *L*-methionine methyl ester hydrochloride yielded the dipeptide **20** (65%), which upon hydrolysis of the methyl ester and amide coupling reaction with the tripeptide **10** formed the pentapeptide KLHDC2 binder **21** (32%). The hydrolysis of ester moieties of tetrapeptides **17**-**19**, followed by amide coupling reaction with the tripeptide **10** generated heptapeptide KLHDC2 binders **22**-**24** (30-45%).

Scheme 4. Synthesis of Pentapeptide KLHDC2 Binder **21**.



Reagent and condition: (a) amino acid methyl ester hydrochloride, EDCI, 1-hydroxybenzotriazole, DIPEA, DCM, rt, 3 h, 65%; (b) i) LiOH, THF/H_2O (1:1), 0 °C to rt, 3 h; ii) **10**, EDCI, 1-hydroxybenzotriazole, DIPEA, DCM, rt, 3 h, 32% over two steps.

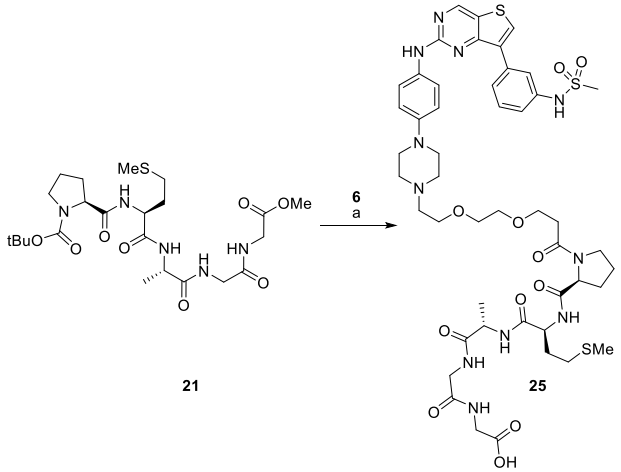
Scheme 5. Synthesis of Heptapeptide KLHDC2 Binders **22-24** .



Reagent and condition: (a) i) LiOH, THF/H_2O (1:1), 0 °C to rt, 3 h; ii) amino acid methyl ester hydrochloride, EDCI, 1-hydroxybenzotriazole, DIPEA, DCM, rt, 3 h, 30-45% over two steps.

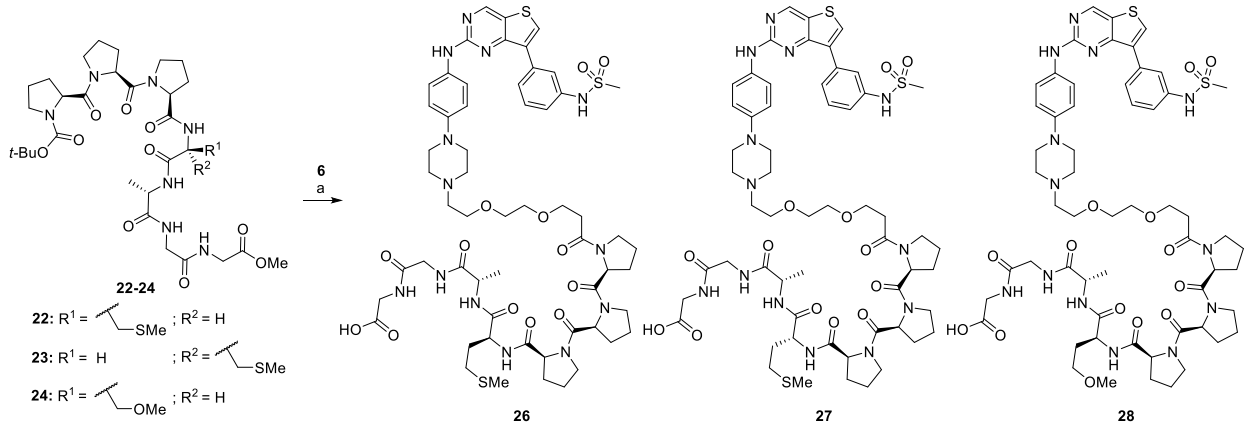
The synthetic routes towards the various KLHDC2 PROTAC molecules using penta- and heptapeptides are shown in Schemes 6-7. The peptide key intermediates **21** and **22-24** were treated with HCl (4.0 M in 1,4-dioxane) to deprotect *tert*-butyloxycarbonyl group, followed by amide coupling reactions with the linker-attached warhead **6** using 1-propanephosphonic anhydride (50% w/w in ethyl acetate). The synthesis involved, as a final step, removal of the methyl ester to yield KLHDC2 PROTAC molecules **25-28** (33-57%).

Scheme 6. Synthesis of Pentapeptide KLHDC2 PROTAC **25**.



Reagent and condition: (a) i) 4 M HCl in dioxane, DCM, 0°C to rt, 3 h ii) **6**, Et_3N, 1-propanephosphonic anhydride (50% w/w in ethyl acetate), DMF, -10 °C, 0.5 h; iii) LiOH, THF/H_2O (1:1), -10 °C, 0.5 h, 43% over two steps.

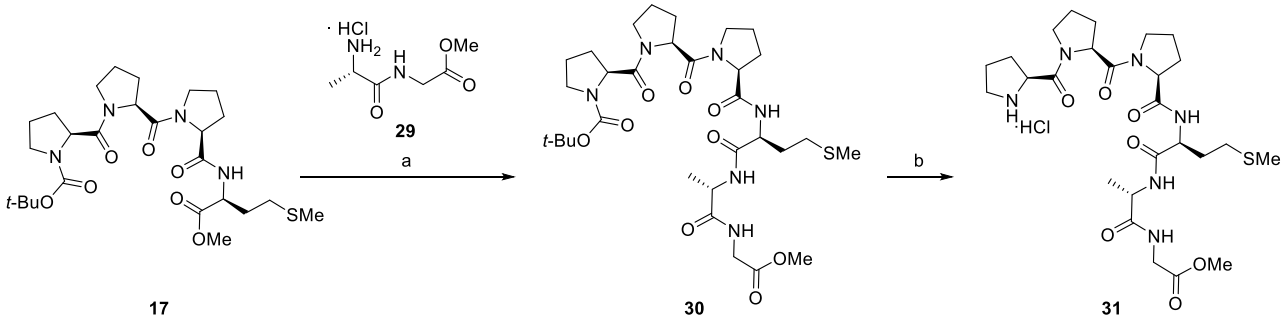
Scheme 7. Synthesis of Heptapeptide KLHDC2 PROTACs **26**-**28**.



Reagent and condition: (a) i) 4 M HCl in dioxane, DCM, 0°C to rt, 3 h ii) **6**, Et_3N, 1-propanephosphonic anhydride (50% w/w in ethyl acetate), DMF, -10 °C, 0.5 h; iii) LiOH, THF/H_2O (1:1), -10 °C, 0.5 h, 33-57% over two steps.

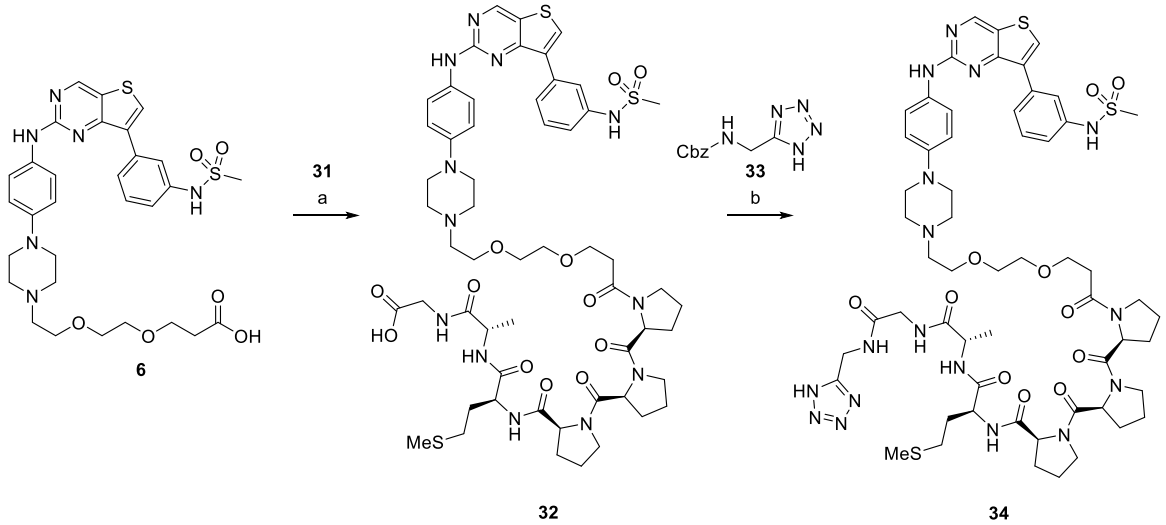
The synthesis of hexapeptide **31** and tetrazole analogue **34** is shown in Schemes 8-9. The hydrolysis of the methyl ester moiety of **17**, followed by amide coupling reaction with the dipeptide **29** and deprotection of *tert*-butyloxycarbonyl group with HCl (4.0 M in 1,4-dioxane) afforded hexapeptide **31** (93%). Subsequent coupling reaction of **31** with the intermediate **6** using 1-propanephosphonic anhydride (50% w/w in ethyl acetate) and hydrolysis of the methyl ester provided hexapeptide KLHDC2 PROTAC **32** (56%). *N*-benzyloxycarbonyl group of tetrazole **33** was removed under concentrated hydrochloric acid, which was then subjected to amide coupling reaction with **32** using *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate to provide tetrazole analogue **34** (16%).

Scheme 8. Synthesis of Hexapeptide **31**.



Reagent and condition: (a) i) LiOH, THF/H_2O (1:1), 0 °C to rt, 3 h; ii) amino acid methyl ester hydrochloride, EDCI, 1-hydroxybenzotriazole, DIPEA, DCM, rt, 3 h, 90% over two steps; (b) 4 M HCl in dioxane, DCM, 0°C to rt, 3 h, 93%.

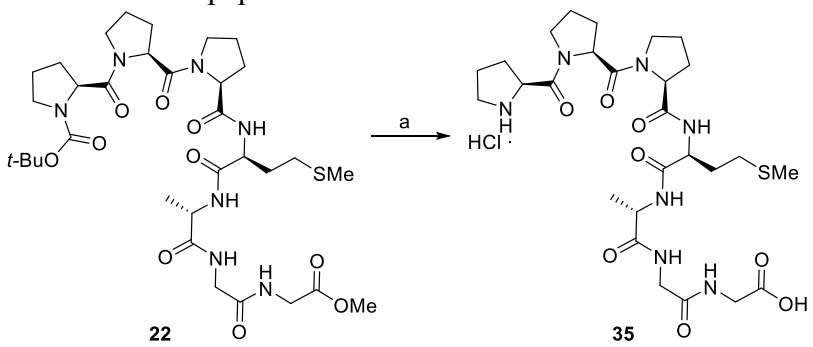
Scheme 9. Synthesis of KLHDC2 PROTAC Tetrazole Analogue **34**.



Reagent and condition: (a) i) **31**, Et_3N, 1-propanephosphonic anhydride (50% w/w in ethyl acetate), DMF, -10 °C, 0.5 h; ii) LiOH, THF/H_2O (1:1), -10 °C, 0.5 h, 56% over two steps; (b) i) **33**, concd hydrochloric acid, 100 °C, 3 h; ii) *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, DIPEA, DMF, -10°C, 3 h, 16% over two steps.

The synthesis of SelK-7 peptide (**35**) is shown in Scheme 10. The hydrolysis of the methyl ester moiety of the heptapeptide **22**, followed by deprotection of *tert*-butyloxycarbonyl group with HCl (4.0 M in 1,4-dioxane) afforded SelK-7 (**35**, 90%).

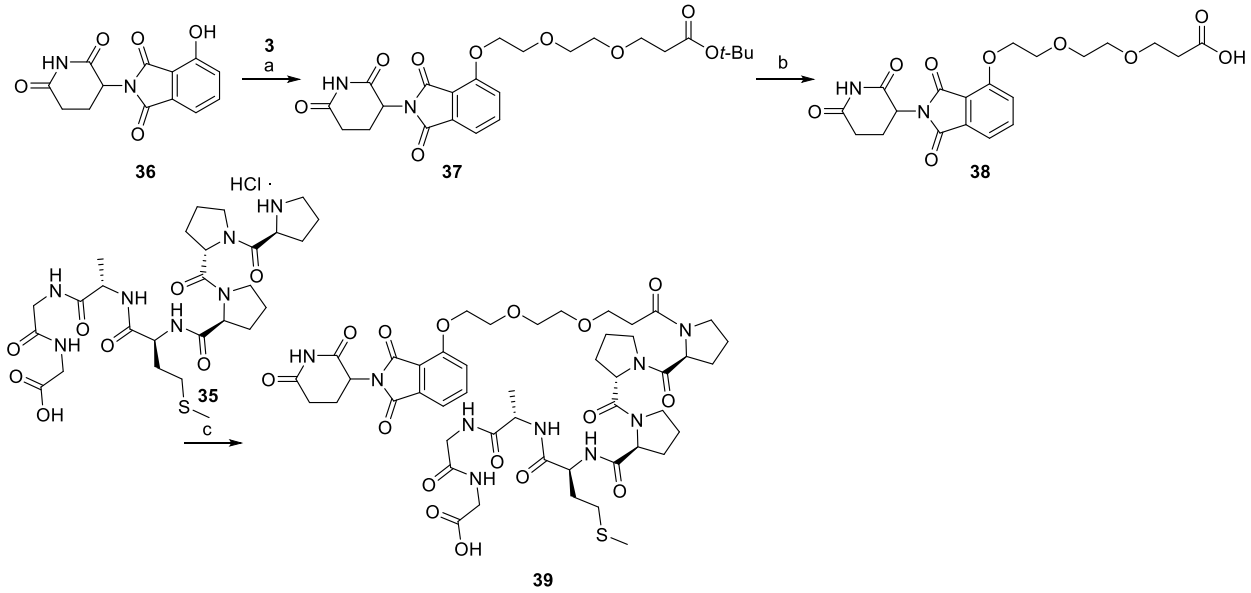
Scheme 10. Synthesis of SelK-7 peptide **35**



Reagent and condition: (a) i) LiOH, THF/H_2O (1:1), 0 °C to rt, 2 h; ii) 4 M HCl in dioxane, DCM, 0°C to rt, 3 h, 90% over two steps.

The synthesis of thalidomide-SelK-7 conjugate (**39**) is shown in Scheme 11. The CRBN ligand **36** was subjected to alkylation with iodide **3** using NaI and $KHCO_3$, followed by hydrolysis of the *tert*-butyl ester to yield the key intermediate **38**. The acid **38** was then coupled with the Selk-7 peptide (**35**) using perfluorophenyl ester generated *in-situ* to afford thalidomide-SelK-7 conjugate **39** (26%).

Scheme 11. Synthesis of Thalidomide-SelK-7 Conjugate **39**.



Reagent and condition: (a) **3**, NaI, $KHCO_3$, DMF, 70 °C, 12 h, 60%; (b) 4 M HCl in dioxane, DCM, 0°C to rt, 12 h, 95%; (c) i) EDCI, DMAP, perfluorophenyl trifluoroacetate, DCM rt, 1 h; ii) **35**, DIPEA, DCM, rt, 1 h, 26% over two steps.

tert-Butyl 3-(2-(2-hydroxyethoxy)ethoxy)propanoate (**2**).
To a solution of *tert*-butyl acrylate (3 mL, 20.7 mmol) and 2-(2-hydroxyethoxy)ethanol (2 mL, 20.7 mmol) in MeCN (30 mL) was added benzyltrimethylammonium hydroxide (0.3 mL, 0.75 mmol) and stirred at room temperature for 12 h. The reaction mixture was concentrated and diluted with ethyl acetate and water. The organic phase was washed with $NaHCO_3$ solution and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was subjected to chromatography on silica gel (30% to 50% ethyl acetate/hexane) to afford **2** (2.12 g, 44%).^{1}H NMR (400 MHz, $CDCl_3$): δ 3.75 – 3.68 (m, 4H), 3.66 – 3.56 (m, 6H), 2.57 – 2.44 (m, 3H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.07, 80.81, 72.59, 70.46, 70.44, 66.94, 61.88, 36.27, 28.19. LRMS (ESI) *m/z* calculated for $C_{11}H_{22}O_5Na^+$ $[M + Na]^+$: 257.1. Found 257.
tert-Butyl 3-(2-(2-iodoethoxy)ethoxy)propanoate (**3**).
To a solution of **2** (1.00 g, 4.27 mmol) and triphenylphosphine (1.17 g, 4.50 mmol) in DCM (30 mL), imidazole (363 mg, 5.30 mmol) was added followed by I_2 (1.19 g, 4.70 mmol). The mixture was stirred at room temperature for 12 h. The reaction mixture was quenched by addition of saturated aqueous sodium thiosulfate solution and extracted with DCM. The organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was subjected to chromatography on silica gel (0% to 20% ethyl acetate/hexane) to afford **3** (1.25 g, 85%) as yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.74 (t, J = 5.9 Hz, 2H), 3.71 (t, J = 5.5 Hz, 2H), 3.66 – 3.59 (m, 4H), 3.24 (t, J = 6.9 Hz, 2H), 2.50 (t, J = 6.5 Hz, 2H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.00, 80.68, 72.07, 70.46, 70.24, 67.06, 36.34, 28.22, 3.03. LRMS (ESI) *m/z* calculated for $C_{11}H_{21}IO_4Na^+$ $[M + Na]^+$: 367.0. Found 367.

tert-Butyl 3-(2-(2-(4-(4-((7-(3-(methylsulfonamido)phenyl)thieno[3,2-d]pyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethoxy)ethoxy)propanoate (**5**).
To a solution of **4** (335 mg, 0.70 mmol) in DMF (5.0 mL) was added **3** (288 mg, 0.84 mmol), NaI (524 mg, 3.49 mmol), DIPEA (0.49 ml, 2.79 mmol) at room temperature. The reaction

mixture was then stirred at 60 ˚C for 3 h, quenched with water and diluted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (50% to 70% THF/hexane) to afford **5** (210 mg, 43%) as a yellow solid.
^{1}H NMR (400 MHz, DMSO-d_6): δ 9.84 (s, 1H), 9.44 (s, 1H), 9.16 (s, 1H), 8.45 (s, 1H), 7.83 (t, J = 1.74, 2H), 7.82 – 7.79 (m, 1H), 7.70 (d, J = 9.1, 2H), 7.48 (t, J = 7.9, 1H), 7.27 (ddd, J = 0.9, 2.2, 8.1 1H), 6.91 (d, J = 9.1, 2H), 3.60 (t, J = 6.2, 2H), 3.54 (t, J = 5.9, 2H), 3.50 (s, 4H), 3.06 (t, J = 4.4, 4H), 3.02 (s, 3H), 2.56 (t, J = 4.4, 4H), 2.53 – 2.50 (m, 2H), 2.42 (t, J = 6.2, 2H), 1.40 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.47, 158.41, 157.95, 154.00, 146.12, 138.57, 134.60, 134.38, 133.69, 132.73, 129.34, 123.81, 122.19, 120.07, 119.97, 119.10, 115.89, 79.75, 69.69, 69.66, 68.43, 66.27, 57.31, 53.28, 49.07, 35.89, 27.79. LRMS (ESI) *m/z* calculated for $C_{34}H_{45}N_6O_6S_2^+$ $[M + H]^+$: 697.3. Found 697.

3-(2-(2-(4-(4-((7-(3-(Methylsulfonamido)phenyl)thieno[3,2-d]pyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethoxy)ethoxy)propanoic acid (**6**).
To a solution of **5** (500 mg, 0.72 mmol) in DCM (1.44 mL) was added HCl (4.0 M in 1,4-dioxane, 1.80 mL) at 0 °C. After stirring at room temperature for 6 h, the solution was concentrated under reduced pressure. The residue was solidified by swirling in DCM/diethyl ether (1:4) and separation of the formed solid by filtration followed by drying yielded **6** (443 mg, 0.69 mmol, 96%) as a yellow solid that was used for next reaction without any further purification. LRMS (ESI) *m/z* calculated for $C_{30}H_{37}N_6O_6S_2^+$ $[M + H]^+$: 641.2. Found 641.

General Procedure A for the Synthesis of Dipeptides.
To a solution of Boc-protected amino acid (1.0 equiv) in DCM (0.2 M) was added EDCI (1.5 equiv) and 1-hydroxybenzotriazole hydrate (1.5 equiv). The mixture was stirred for 5 min, treated slowly with a solution of amino acid methyl ester hydrochloride (1.2 equiv) and DIPEA (4 equiv) in DCM (0.4 M). The reaction mixture was stirred at room temperature for 2 h, quenched by addition of ice water, and extracted with isopropyl alcohol/chloroform (1:4). The organic layer was washed with NH_4Cl solution and $NaHCO_3$ solution, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residues were subjected to flash column chromatography on silica gel.

General Procedure B for the Hydrolysis of Ester Group and Amide Bond Formation.
To a solution of Boc-protected amino acid methyl ester (1.0 equiv) in THF/H_2O (1:1, 0.1 M) was added aqueous LiOH solution (3.0 equiv) at 0 °C. The reaction mixture was stirred at room temperature for 2 h and pH was adjusted to 2 by addition of 1 N aqueous HCl solution. The mixture was then extracted with isopropyl alcohol/chloroform (1:4), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. To the solution of crude acid in DCM (0.2 M) was added EDCI (1.5 equiv) and 1-hydroxybenzotriazole hydrate (1.5 equiv). The mixture was stirred for 5 min, treated slowly with a solution of corresponding amino acid methyl ester hydrochloride (1.2 equiv) and DIPEA (4 equiv) in DCM (0.4 M). The reaction mixture was stirred at room temperature for 2 h, quenched by addition of ice water and extracted with isopropyl alcohol/chloroform (1:4). The organic layer was washed with NH_4Cl solution and $NaHCO_3$ solution, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residues were subjected to flash column chromatography on silica gel.

Methyl (*tert*-butoxycarbonyl)-*L*-alanylglycinate (**8**).
(*tert*-Butoxycarbonyl)-*L*-alanine (2.00 g, 10.6 mmol) was converted to the target compound using general procedure A. The crude product was purified using flash column chromatography (0% to 5% methanol/DCM) on silica gel to afford **8** (2.39 g, 9.2 mmol, 87%) as a pale yellow oil. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.30 – 8.13 (m, 2H), 6.96 (d, J = 7.6 Hz, 1H), 4.06 – 3.94 (m, 1H), 3.83 (ddd, J = 6.56, 17.5 36.9 Hz, 2H), 3.62 (s, 3H), 1.37 (s, 9H), 1.18 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.34, 170.31, 155.04, 78.02, 51.70, 49.48, 40.54, 28.23, 18.19. LRMS (ESI) *m/z* calculated for $C_{11}H_{20}N_2O_5Na^+$ [M + Na]$^+$: 283.2. Found 283.

Methyl (*tert*-butoxycarbonyl)-*L*-alanylglycylglycinate (**9**).
Compound **8** (2.39 g, 9.2 mmol) was converted to the target compound using general procedure B. The crude product was purified using flash column chromatography (5% to 10% methanol/DCM) on silica gel to afford **9** (2.64 g, 8.33 mmol, 91%) as a pale yellow oil. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.21 (t, J = 5.57, 1H), 8.13 (t, J = 5.45 Hz, 1H), 7.05 (d, J = 6.95 Hz, 1H), 4.02 – 3.91 (m, 1H), 3.90 – 3.78 (m, 2H), 3.72 (d, J = 5.7 Hz, 2H), 3.62 (s, 3H), 1.37 (s, 9H), 1.17 (d, J = 7.12 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.05, 170.16, 169.40, 155.34, 78.25, 51.76, 49.84, 41.82, 40.53, 28.23, 17.95. LRMS (ESI) *m/z* calculated for $C_{13}H_{23}N_3O_6Na^+$ [M + Na]$^+$: 340.1. Found 340.

Methyl *L*-alanylglycylglycinate hydrochloride (**10**).
To a solution of **9** (1.40 g, 4.42 mmol) in DCM (4.4 mL) was added HCl (4.0 M in 1,4-dioxane, 11.0 mL) at 0 °C. After stirring at room temperature for 3 h, the solution was concentrated under reduced pressure. The residue was solidified by swirling in DCM/diethyl ether (1:4) and separation of the formed solid by filtration, followed by drying yielded **10** (1.02 g, 4.03 mmol, 91%) as a white solid that was used for next reaction without any further purification. LRMS (ESI) *m/z* calculated for $C_8H_{15}N_3O_4Na^+$ [M + Na]$^+$: 240.1. Found 240.

tert-Butyl (*S*)-2-((*S*)-2-(methoxycarbonyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carboxylate (**12**).
(*tert*-Butoxycarbonyl)-*L*-proline (10.0 g, 46.4 mmol) was converted to the target compound using general procedure A. The crude product was purified using flash column chromatography (0% to 50% THF/hexane) on silica gel to afford **12** (13.1 g, 40.1 mmol, 86%) as a pale yellow oil.
^{1}H NMR (400 MHz, DMSO-d_6): δ two rotamers 4.43 (dd, J = 3.1, 8.7 Hz, minor), 4.39 (dd, J = 4.3, 8.3 Hz, major, 1H), 4.35 -4.28 (m, 1H), 3.7 – 3.46 (m, 2H), 3.61 (s, major, 3H), 3.60 (s, minor), 3.35 – 3.24 (m, 2H), 2.27 – 2.06 (m, 2H), 2.00 – 1.89 (m, 2H), 1.87 – 1.68 (m, 4H), 1.36 (s, minor), 1.30 (s, major, 9H);^{13}C NMR (100 MHz, DMSO-d_6): δ two rotamers 172.34 (minor), 172.20 (major), 170.94 (major), 170.35 (minor), 153.33 (minor), 152.95 (major), 78.40 (minor), 78.30 (major), 58.33 (major), 58.32 (minor), 57.33 (minor), 57.26 (major), 51.76 (major), 51.70 (minor), 46.51 (minor), 46.37 (major), 46.23 (major), 46.16 (minor), 29.36 (major), 28.46 (minor), 28.41 (major), 28.33 (minor), 28.15 (minor), 27.92 (major), 24.65 (major), 24.63 (minor), 23.59 (minor), 23.11 (major). LRMS (ESI) *m/z* calculated for $C_{16}H_{26}N_2O_5Na^+$ [M + Na]$^+$: 349.2. Found 349.

*t*ert-Butyl (*S*)-2-((*S*)-2-((*S*)-2-(methoxycarbonyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carboxylate (**13**).

Compound **12** (6.00 g, 18.4 mmol) was converted to the target compound using general procedure B. The crude product was purified using flash column chromatography (30% to 50% THF/hexane) on silica gel to afford **13** (6.13 g, 14.5 mmol, 79%) as a colorless oil.
^{1}H NMR (400 MHz, DMSO-d_6): δ two rotamers 4.61 (dd, J = 4.0, 8.0 Hz, minor), 4.58 (dd, J = 4.0, 8.4 Hz, major, 1H), 4.45 -4.34 (m, 1H), 4.33 – 4.24 (m, 1H), 3.72 – 3.38 (m, 4H), 3.60 (s, 3H), 3.30 – 3.25 (m, 2H), 2.24 – 2.05 (m, 3H), 2.00 – 1.86 (m, 4H), 1.86 – 1.67 (m, 5H), 1.37 (s, minor), 1.31 (s, major, 9H); ^{13}C NMR (100 MHz, DMSO-d_6): δ two rotamers 172.30, 170.41(major), 169.95 (minor), 169.83 (minor), 169.79 (major), 153.31 (minor), 153.02 (major), 78.29 (minor), 78.21 (major), 58.32 (major), 58.27 (minor), 57.35, 57.29, 51.72, 46.51 (minor), 46.41 (major), 46.36, 46.25 (major), 46.20 (minor), 29.24 (major), 28.34 (minor), 28.37, 28.17 (minor), 27.96 (major), 27.60 (major), 27.41 (minor), 24.60 , 24.34 (major), 24.33 (minor), 23.56 (minor), 23.04 (major). LRMS (ESI) *m/z* calculated for $C_{21}H_{33}N_3O_6Na^+$ $[M + Na]^+$: 446.2. Found 446.

Methyl *O*-methyl-*L*-homoserinate hydrochloride (**16**).
To a solution of *N*-(*tert*-butoxycarbonyl)-*L*-homoserine (1 g, 4.3 mmol) in methanol (4.3 mL) was added trimethylsilyl chloride (2.8 mL, 21.5 mmol) slowly and stirred at room temperature for 2 h. The solution was concentrated under reduced pressure. The residue was solidified by swirling in DCM/diethyl ether (1:4) and separation of the formed solid by filtration, followed by drying yielded **16** (625 mg, 3.41 mmol, 79%) as a white solid.
^{1}H NMR (400 MHz, DMSO-d_6): δ 5.59 (brs, 3H), 4.02 (t, J = 6.1 Hz, major, 1H), 3.73 (s, 3H), 3.44 (t, J = 6. Hz, 2H), 3.22 (s, major, 3H), 2.12 – 1.98 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.85, 66.92, 58.03, 52.72, 49.61, 29.93. LRMS (ESI) *m/z* calculated for $C_6H_{14}NO_3^+$ $[M + H]^+$: 148.1. Found 148.

tert-Butyl (*S*)-2-((*S*)-2-((*S*)-2-(((*S*)-1-methoxy-4-(methylthio)-1-oxobutan-2-yl)carbamoyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carboxylate (**17**).
Compound **13** (3.80 g, 8.98 mmol) was converted to the target compound using general procedure B. The crude product was purified using flash column chromatography (30% to 50% THF/hexane) on silica gel to afford **17** (4.02 g, 7.24 mmol, 81%) as a white solid.
^{1}H NMR (400 MHz, DMSO-d_6) δ diastereomeric mixtures (85:10) 8.70 (dd, J = 7.6, 29.8 Hz, minor), 8.24 (dd, J = 4.0, 7.6 Hz, major, 1H), two rotamers 4.58 (dd, J = 4.1, 8.9 Hz, minor), 4.55 (dd, J = 3.7, 8.6 Hz, major, 1H), 4.47 – 4.24 (m, 3H), 3.63 (s, 3H), 3.62 – 3.39 (m, 4H), 3.32 – 3.27 (m, 2H), 2.58 – 2.43 (m, 2H), 2.33 – 2.05 (m, 3H), 2.04 (s, 3H), 1.98 – 1.67 (m, 11H), 1.38 (s, minor), 1.31 (s, major, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ two rotamers 172.19, 171.98 (minor), 171.97 (major), 170.39 (major), 169.82 (minor), 169.77 (minor), 169.61 (major), 153.31 (minor), 153.04 (major), 78.31 (minor), 78.22 (major), 58.89 (major), 58.84 (minor), 57.41 (major), 57.30 (minor), 57.36, 51.92, 50.85 (minor), 50.84 (major), 46.54, 46.50 (minor), 46.44 (major), 46.38, 30.82, 29.38, 29.24 (major), 28.33 (minor), 28.80, 28.19 (minor), 27.97 (major), 27.70 (major), 27.51 (minor), 24.46, 24.32 (major), 24.29 (minor), 23.60 (minor), 23.07 (major), 14.58. LRMS (ESI) *m/z* calculated for $C_{26}H_{42}N_4O_7SNa^+$ $[M + Na]^+$: 577.3. Found 577.

tert-Butyl (*S*)-2-((*S*)-2-((*S*)-2-(((*R*)-1-methoxy-4-(methylthio)-1-oxobutan-2-yl)carbamoyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carboxylate (**18**).

Compound **13** (1.50 g, 3.55 mmol) was converted to the target compound using general procedure B. The crude product was purified using flash column chromatography (30% to 50% THF/hexane) on silica gel to afford **18** (1.61 g, 2.91 mmol, 82%) as a white solid.
^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ diastereomeric mixtures (85:13) 8.26 (dd, *J* = 7.2, 18.7 Hz, minor), 8.05 (d, *J* = 8.2 Hz, major, 1H), two rotamers 4.57 (dd, *J* = 3.8, 8.6 Hz, minor), 4.53 (dd, *J* = 3.7, 8.3 Hz, major, 1H), 4.47 – 4.34 (m, 2H), 4.33 – 4.22 (m, 1H), 3.69 – 3.34 (m, 4H), 3.63 (s, 3H), 3.32 – 3.24 (m, 2H), 2.49 – 2.32 (m, 2H), 2.22 – 1.99 (m, 3H), 2.02 (s, 3H), 2.00 – 1.71 (m, 11H), 1.37 (s, minor), 1.31 (s, major, 9H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ two rotamers 172.13, 171.75 (minor), 171.71 (major), 170.54 (major), 170.17 (minor), 170.01 (major), 169.92 (major), 153.33 (minor), 153.04 (major), 78.34 (minor), 78.25 (major), 59.57 (major), 59.51 (minor), 57.70, 57.39 (minor), 57.34 (major), 51.98, 50.56, 46.66 (major), 46.63 (minor), 46.58 (minor), 46.55 (major), 46.48 (minor), 46.40 (major), 30.72, 29.42, 29.31 (major), 28.38 (minor), 29.11, 28.21 (minor), 28.00 (major), 27.81 (major), 27.66 (minor), 24.48, 24.47 (major), 24.43 (minor), 23.62 (minor), 23.12 (major), 14.59. LRMS (ESI) *m/z* calculated for $C_{26}H_{42}N_4O_7SNa^+$ $[M + Na]^+$: 577.3. Found 577.

tert-Butyl (*S*)-2-((*S*)-2-((*S*)-2-(((*S*)-1,4-dimethoxy-1-oxobutan-2-yl)carbamoyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carboxylate (**19**).
Compound **13** (1.00 g, 2.36 mmol) was converted to the target compound using general procedure B. The crude product was purified using flash column chromatography (30% to 50% THF/hexane) on silica gel to afford **19** (950 mg, 1.76 mmol, 75%) as a white solid.
^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ diastereomeric mixtures (84:12) 8.67 (dd, *J* = 7.5, 13.5 Hz, minor), 8.17 (dd, *J* = 7.5, 4.4 Hz, major, 1H), two rotamers 4.58 (dd, *J* = 3.6, 8.4 Hz, minor), 4.55 (dd, *J* = 3.7, 8.5 Hz, major, 1H), 4.51 – 4.20 (m, 3H), 3.68 – 3.42 (m, 4H), 3.63 (s, 3H), 3.39 – 3.33 (m, 2H), 3.30 – 3.24 (m, 2H), 3.20 (s, 3H), 2.22 – 1.98 (m, 3H), 1.97 – 1.68 (m, 11H), 1.37 (s, minor), 1.31 (s, major, 9H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ two rotamers 172.45, 171.85 (minor), 171.83 (major), 170.38 (major), 169.81 (minor), 169.78 (minor), 169.63 (major), 153.31 (minor), 153.03 (major), 78.30 (minor), 78.21 (major), 67.86, 58.84 (major), 58.79 (minor), 57.88, 57.40 (minor), 57.30 (major), 57.35, 51.81, 49.12 (minor), 49.11 (major), 46.53, 46.49 (minor), 46.44 (major), 46.37, 31.07, 29.24 (major), 28.32 (minor), 28.81, 28.18 (minor), 27.97 (major), 27.71 (major), 27.52 (minor), 24.41, 24.34 (major), 24.30 (minor), 23.59 (minor), 23.06 (major). LRMS (ESI) *m/z* calculated for $C_{26}H_{42}N_4O_8Na^+$ $[M + Na]^+$: 561.3. Found 561.

tert-Butyl (*S*)-2-(((*S*)-1-methoxy-4-(methylthio)-1-oxobutan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (**20**).
(*tert*-Butoxycarbonyl)-*L*-proline (2.00 g, 9.30 mmol) was converted to the target compound using general procedure A. The crude product was purified using flash column chromatography (0% to 50% THF/hexane) on silica gel to afford **20** (2.19 g, 6.08 mmol, 65%) as a pale yellow oil.
^{1}H NMR (400 MHz, DMSO-*d*$_6$): *δ* two rotamers 8.27 (d, *J* = 7.5 Hz, major, 1H), 8.23 (d, *J* = 7.3 Hz, minor), 4.45 – 4.35 (m, 1H), 4.17 – 4.07 (m, 1H), 3.63 (s, 3H), 3.41 – 3.34 (m, 1H), 3.30 – 3.22 (m, 1H), 2.60 – 2.40 (m, 2H), 2.18 – 2.07 (m, 1H), 2.03 (s, 3H), 2.00 – 1.85 (m, 2H), 1.84 – 1.72 (m, 3H), 1.38 (s, minor), 1.33 (s, major 9H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$): *δ* two rotamers 172.85 (major), 172.48 (minor), 172.28 (minor), 172.24 (major), 153.51 (minor), 153.29 (major), 78.55 (minor), 78.41 (major), 59.30 (major), 59.05 (minor), 51.95, 50.75

(minor), 50.71 (major), 46.64 (minor), 46.49 (major), 30.92 (major), 30.75 (minor), 30.25 (major), 29.75 (minor), 29.64 (major), 29.45 (minor), 28.14 (minor), 27.98 (major), 23.89 (minor), 23.02 (major), 14.65 (minor), 14.43 (major). LRMS (ESI) *m/z* calculated for $C_{16}H_{28}N_2O_5SNa^+$ $[M + Na]^+$: 383.2. Found 383.

tert-Butyl (*S*)-2-(((10*S*,13*S*)-10-methyl-3,6,9,12-tetraoxo-2-oxa-16-thia-5,8,11-triazaheptadecan-13-yl)carbamoyl)pyrrolidine-1-carboxylate (**21**).
Compound **20** (500 mg, 1.38 mmol) was converted to the target compound using general procedure B. The crude product was purified using flash column chromatography (30% to 50% THF/hexane) on silica gel to afford **21** (240 mg, 0.44 mmol, 32%) as a colorless oil.
^{1}H NMR (400 MHz, DMSO-d_6): δ two rotamers 8.27 – 8.15 (m, 2H), 8.06 (d, J = 6.9 Hz, major, 1H), 7.99 (brd, minor), 8.00 (d, J = 7.6, 1H), 4.39 – 4.29 (m, 1H), 4.28 – 4.21 (m, 1H), 4.17 – 4.09 (m, 1H), 3.85 (dd, J = 4.3, 5.73 Hz, 2H), 3.78 – 3.69 (m, 2H), 3.63 (s, 3H), 3.40 – 3.33 (m, 1H), 3.30 – 3.21 (m, 1H), 2.48 – 2.40 (m, 1H), 2.15 – 2.06 (m, 1H), 2.03 (s, 3H), 1.98 – 1.87 (m, 1H), 1.86 – 1.70 (m, 4H), 1.39 (s, minor), 1.32 (s, major, 9H), 1.22 (d, J = 7.05 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ two rotamers 172.57 (major), 172.32 (minor), 172.46, 170.97 (major), 170.90 (minor), 170.94 (minor), 170.21 (major), 169.29, 153.74 (minor), 153.31 (major), 78.72 (minor), 78.40 (major), 59.26 (minor), 59.23 (major), 51.76, 51.71 (minor), 51.61 (major), 48.41(minor), 48.34 (major), 46.72 (minor), 46.52 (major), 41.73, 40.54, 31.87 (major), 31.01 (minor), 29.74, 29.58 (major), 29.46 (minor), 28.16 (minor), 28.02 (major), 23.99 (minor), 23.06 (major), 18.02 (major), 17.90 (minor), 14.68 (minor), 14.54 (major). LRMS (ESI) *m/z* calculated for $C_{23}H_{40}N_5O_8S^+$ $[M + H]^+$: 546.3. Found 546.

tert-Butyl (*S*)-2-((*S*)-2-((*S*)-2-(((10*S*,13*S*)-10-methyl-3,6,9,12-tetraoxo-2-oxa-16-thia-5,8,11-triazaheptadecan-13-yl)carbamoyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carboxylate (**22**).
Compound **17** (3.5 g, 6.31 mmol) was converted to the target compound using general procedure B. The crude product was purified using flash column chromatography (0% to 10% methanol/chloroform) on silica gel to afford **22** (2.01 g, 2.84, 45%) as a white solid.
^{1}H NMR (400 MHz, DMSO-d_6) δ two rotamers 8.19 (dd, J = 5.4, 6.7 Hz, 2H), 8.02 – 7.95 (m, 2H), 4.58 (dd, J = 4.0, 8.7 Hz, minor), 4.55 (dd, J = 3.7, 8.5 Hz, major, 1H) 4.43 – 4.18 (m, 4H), 3.86 (dd, J = 3.2, 5.8 Hz, 2H), 3.73 (dd, J = 6.0, 8.4 Hz, 2H), 3.63 (s, 3H), 3.66 – 3.40 (m, 4H), 3.31 – 3.26 (m, 2H), 2.48 – 2.37 (m, 2H), 2.20 – 1.98 (m, 3H), 2.03 (s, 3H), 1.98 – 1.68 (m, 11H), 1.37 (s, minor), 1.31 (s, major, 9H), 1.22 (d, J = 7.2, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ two rotamers 172.45, 171.89 (minor), 171.87 (major), 170.91, 170.51 (major), 170.09 (minor), 170.20, 169.93 (major), 169.91 (minor), 169.31, 153.34 (minor), 153.06 (major), 78.37 (minor), 78.28 (major), 59.33 (major), 59.27 (minor), 57.54, 57.38 (minor), 57.31 (major), 51.88, 51.76, 48.39, 46.67 (major), 46.62 (minor), 46.57 (minor), 46.50 (major), 46.41, 41.76, 40.54, 31.86, 29.45, 29.28 (major), 28.37 (minor), 28.82, 28.22, 28.01, 27.76 (major), 27.57 (minor), 24.62, 24.40 (major), 24.36 (minor), 23.66 (minor), 23.15 (major), 17.87, 14.65. LRMS (ESI) *m/z* calculated for $C_{33}H_{54}N_7O_{10}S^+$ $[M + H]^+$: 740.4. Found 740. HRMS (ESI) *m/z* calculated for $C_{33}H_{54}N_7O_{10}S^+$ $[M + H]^+$: 740.3653. Found: 740.3612.

tert-Butyl (*S*)-2-((*S*)-2-((*S*)-2-(((10*S*,13*R*)-10-methyl-3,6,9,12-tetraoxo-2-oxa-16-thia-5,8,11-triazaheptadecan-13-yl)carbamoyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carboxylate (**23**).

Compound **18** (1.47 g, 2.65 mmol) was converted to the target compound using general procedure B. The crude product was purified using flash column chromatography (0% to 10% methanol/chloroform) on silica gel to afford **23** (750 mg, 1.01 mmol, 38%) as a white solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ two rotamers 8.31 (d, *J* = 8.3 Hz, major, 1H), 8.30 (d, *J* = 8.0 Hz, minor, 1H), 8.21 – 8.10 (m, 2H), 7.80 (d, *J* = 6.6 Hz, major, 1H), 7.77 (d, *J* = 6.8 Hz, minor, 1H), 4.59 (dd, *J* = 4.5, 8.5 Hz, minor), 4.55 (dd, *J* = 4.3, 8.4 Hz, major, 1H) 4.44 – 4.35 (m, 1H), 4.31 – 4.17 (m 3H), 3.90 – 3.80 (m, 2H), 3.77 – 3.70 (m, 2H), 3.63 (s, 3H), 3.69 – 3.37 (m, 4H), 3.45 – 3.37 (m, 1H) 3.31 – 3.25 (m, 2H), 2.48 – 2.34 (m, 2H), 2.21 – 1.97 (m, 4H), 2.01 (s, 3H), 1.95 – 1.65 (m, 10H), 1.37 (s, minor), 1.31 (s, major, 9H), 1.23 (d, *J* = 7.1, 3H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ two rotamers 172.83, 172.60 (minor), 172.56 (major), 171.58 (major), 171.55 (minor), 170.92 (major), 170.80 (minor), 170.63, 170.61 (major), 170.31 (minor), 169.79, 153.76 (minor), 153.47 (major), 78.79 (minor), 78.71 (major), 60.36 (major), 60.27 (minor), 57.96, 57.74 (minor), 57.67 (major), 52.18, 52.05, 49.13 (major), 49.09 (minor), 47.33, 46.98 (major), 46.96 (minor), 46.89 (minor), 46.83 (major), 42.18, 40.94, 31.46, 30.01, 29.67 (major), 28.77 (minor), 29.36 (minor), 29.33 (major), 28.64 (minor), 28.43 (major), 28.14 (major), 27.97 (minor), 25.32, 24.99 (major), 24.97 (minor), 24.12 (minor), 23.60 (major), 18.01 (minor), 17.98 (major), 15.05. LRMS (ESI) *m/z* calculated for $C_{33}H_{54}N_7O_{10}S^+$ $[M + H]^+$: 740.4. Found 740.

tert-Butyl (*S*)-2-((*S*)-2-((*S*)-2-(((10*S*,13*S*)-10-methyl-3,6,9,12-tetraoxo-2,16-dioxa-5,8,11-triazaheptadecan-13-yl)carbamoyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carboxylate (**24**).
Compound **19** (1.0 g, 1.86 mmol) was converted to the target compound using general procedure B. The crude product was purified using flash column chromatography (0% to 10% methanol/chloroform) on silica gel to afford **24** (405 mg, 0.56 mmol, 30%) as a white solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ two rotamers 8.17 (t, *J* = 5.9 Hz, 1H), 8.12 (t, *J* = 5.9 Hz, 1H), 7.95 (dd, *J* = 3.7, 7.6 Hz), 7.90 (d, *J* = 7.0 Hz), 4.59 (dd, *J* = 4.2, 8.8 Hz, minor), 4.55 (dd, *J* = 3.7, 8.5 Hz, major, 1H), 4.45 – 4.35 (m, 1H), 4.34 – 4.28 (m, 1H), 4.27 – 4.16 (m, 2H), 3.85 (dd, *J* = 2.8, 5.8 Hz, 2H), 3.73 (dd, *J* = 6.0, 8.4 Hz, 2H), 3.65 – 3.41 (m, 4H), 3.62 (s, 3H), 3.36 – 3.27 (m, 4H), 3.18 (s, 3H), 2.18 – 1.98 (m, 3H), 1.97 – 1.67 (m, 11H), 1.37 (s, minor), 1.31 (s, major, 9H), 1.21 (d, *J* = 7.1, 3H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ two rotamers 172.41, 171.86 (minor), 171.84 (major), 171.22, 170.54 (major), 170.20 (minor), 170.21, 170.03 (major), 169.94 (minor), 169.32, 153.36 (minor), 153.07 (major), 93.89, 78.38 (minor), 78.29 (major), 68.51, 59.42 (major), 59.35 (minor), 57.91, 57.60, 57.38 (minor), 57.32 (major), 51.76, 50.04, 48.38, 46.69 (major), 46.65 (minor), 46.58 (minor), 46.51 (major), 46.42, 41.78, 40.54, 31.60, 29.30 (major), 28.38 (minor), 28.85, 28.22 (minor), 28.01 (major), 27.77 (major), 27.59 (minor), 24.60, 24.44 (major), 24.41 minor), 23.68 (minor), 23.16 (major), 17.87. LRMS (ESI) *m/z* calculated for $C_{33}H_{54}N_7O_{11}^+$ $[M + H]^+$: 724.4. Found 724.

General Procedure C for the Synthesis of KLHDC2 PROTACs.
To a solution of corresponding peptide intermediates (1.0 equiv) in DCM (0.5 M) was added HCl (4.0 M in 1,4-dioxane, 10 equiv) at 0 °C. After stirring at room temperature for 3 h, the solution was concentrated. To the crude peptide in DMF (0.1 M) was added **6** (0.8 equiv), Et_3N (4.0 equiv) followed by 1-propanephosphonic anhydride (50% w/w in ethyl acetate, 1.6 equiv) at -10 °C. The reaction mixture was then stirred at -10 °C for 30 min, quenched with methanol and concentrated under reduced pressure. To the solution of crude ester in THF (0.1 M) was added aqueous LiOH solution (5.0 equiv) at -10 °C. The reaction mixture was then stirred at -10 °C for

30 min and diluted with DMF. The resulting mixture was subjected to ACCQPREP HP150 system (0% to 50% MeCN/H_2O (0.1% formic acid) to afford compound **25-28** as yellow solid (33-57%).

(3-(2-(2-(4-(4-((7-(3-(Methylsulfonamido)phenyl)thieno[3,2-*d*]pyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethoxy)ethoxy)propanoyl)-*L*-prolyl-*L*-methionyl-*L*-alanylglycylglycine (**25**).
Compound **21** (50 mg, 0.07 mmol) was converted to the target compound using general procedure C. The product was purified with ACCQPREP HP150 system using XBridge BEH Shield RP18 column (19 × 250 mm, 10 μm particle size; 0% to 50% MeCN/H_2O (0.1% formic acid) to afford compound **25** as a yellow solid (41.0 mg, 0.03 mmol, 43%).
^{1}H NMR (400 MHz, DMSO-d_6): δ two rotamers 9.84 (s, 1H), 9.44 (s, 1H), 9.16 (s, 1H), 8.46 (s, 1H), 8.29 (d, J = 8.2 Hz, minor), 8.19 (t, J = 5.8 Hz, minor), 8.13 – 7.99 (m, 3H), 7.86 (d, J = 7.0 Hz, 1H), 7.83 (s, 1H), 7.80 (d, J = 7.8 Hz, 1H), 7.69 (d, J = 9.0 Hz, 2H), 7.48 (t, J = 7.9 Hz, 1H), 7.27 (dd, J = 1.3, 8.1 Hz, 1H), 6.91 (d, J = 9.1 Hz, 2H), 4.45 (dd, J = 2.7, 8.5, minor), 4.41 – 4.34 (m, minor), 4.32 – 4.27 (m, 2H), 4.26 – 4.19 (m, 1H), 3.77 – 3.70 (m, 4H), 3.67 – 3.53 (m, 6H), 3.50 – 3.35 (m, 6H), 3.07 (brt, 4H), 3.02 (s, 3H), 2.64 – 2.52 (m, 6H), 2.48 – 2.37 (m, 2H), 2.23 – 2.11 (m, 1H), 2.04 (s, minor), 2.03 (s, major, 3H), 2.00 – 1.71 (m, 5H), 1.24 (d, J = 7.1 Hz, major, 3H), 1.22 (d, J = 7.0 Hz, minor); ^{13}C NMR (100 MHz, DMSO-d_6): δ two rotamers 172.45 (minor), 172.01 (major), 172.40, 172.05 (minor), 171.18 (major), 170.97 (major), 170.95 (minor), 169.43 (major), 169.41 (minor), 167.00, 158.41, 157.95, 154.01, 146.05, 138.56, 134.60, 134.40, 133.69, 132.77, 129.35, 123.82, 122.20, 120.08, 120.00, 119.13, 115.93, 69.67, 68.21, 66.48 (minor), 66.32 (major), 59.51 (minor), 59.44 (major), 57.15, 53.16, 51.79 (major), 51.74 (minor), 48.93, 48.44 (major), 48.36 (minor), 47.12 (minor), 47.02 (major), 41.85 (major), 41.79 (minor), 40.77, 34.46, 31.45, 29.67 (minor), 29.33 (major), 29.63, 24.34, 18.06 (minor), 17.87 (major), 14.68. LRMS (ESI) *m/z* calculated for 1/2 $C_{47}H_{64}N_{11}O_{11}S_3^+$ $[M/2 + H]^+$: 527.7. Found 527; $C_{47}H_{64}N_{11}O_{11}S^+$ $[M + H]^+$: 1054.4. Found 1054. HRMS (ESI) *m/z* calculated for 1/2 $C_{47}H_{64}N_{11}O_{11}S^+$ $[M/2 + H]^+$: 527.7014. Found: 527.6990; $C_{47}H_{64}N_{11}O_{11}S^+$ $[M + H]^+$: 1054.3949. Found: 1054.3889.

(3-(2-(2-(4-(4-((7-(3-(Methylsulfonamido)phenyl)thieno[3,2-*d*]pyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethoxy)ethoxy)propanoyl)-*L*-prolyl-*L*-prolyl-*L*-prolyl-*L*-methionyl-*L*-alanylglycylglycine (**26**).
Compound **22** (50 mg, 0.07 mmol) was converted to the target compound using general procedure C. The product was purified with ACCQPREP HP150 system using XBridge BEH Shield RP18 column (19 × 250 mm, 10 μm particle size; 0% to 50% MeCN/H_2O (0.1% formic acid) to afford compound **26** as a yellow solid (31.5 mg, 0.03 mmol, 43%).
^{1}H NMR (400 MHz, DMSO-d_6): δ two rotamers 9.84 (brs, 1H), 9.44 (s, 1H), 9.16 (s, 1H), 8.46 (s, 1H), 8.16 – 8.13 (m, 1H), 8.06 – 8.00 (m, 1H), 7.99 – 7.93 (m, 2H), 7.84 (s, 1H), 7.80 (d, J = 7.9 Hz 1H), 7.69 (d, J = 9.0 Hz, 2H), 7.48 (t, J = 7.9 Hz, 1H), 7.27 (dd, J = 1.3, 8.1 Hz, 1H), 6.91 (d, J = 9.0 Hz , 2H), 4.78 (dd, J = 2.7, 8.5 Hz, minor), 4.62 (dd, J = 4.5, 8.5 Hz, minor), 4.57 – 4.49 (m, 2H, major), 4.34 – 4.29 (m, 1H), 4.29 – 4.19 (m, 2H), 3.78 – 3.69 (m, 4H), 3.68 – 3.51 (m, 10 H), 3.47 – 3.33 (m, 6H), 3.05 (brt, 4H), 3.02 (s, 3H), 2.60 – 2.56 (brt, 4H), 2.55 – 2.52 (m, 2H), 2.48 – 2.38 (m, 2H), 2.24 – 2.01 (m, 3H), 2.02 (s, 3H), 1.97 – 1.71 (m, 11H), 1.22 (d, J = 7.1 Hz, 3H); 13C NMR (150 MHz, DMSO-*d6*): δ two rotamers 172.70, 171.14 (major), 172.10 (minor), 171.45 (minor), 171.12 (major), 170.45, 170.30 (minor), 170.16 (major), 169.93

(major), 169.87 (minor), 169.12 (major), 169.09 (minor), 168.54, 158.74, 158.28, 154.28, 146.44, 138.88, 134.91, 134.66, 134.00, 133.05, 129.64, 124.14, 122.52, 120.42, 120.36, 119.50, 116.21, 70.02 (minor), 69.98 (major), 68.71 (major), 68.68 (minor), 66.82 (minor), 66.63 (major), 59.59 (major), 59.52 (minor), 57.81, 57.58, 57.45, 53.55, 52.22, 49.37, 48.68, 47.19, 46.95 (minor), 46.85 (major), 46.80 (major), 46.65 (minor), 42.19, 41.42, 34.69 (major), 34.37 (minor), 32.21 (minor), 32.19 (major), 29.79, 29.12, 28.24, 28.01 (major), 27.84 (minor), 24.91 (major), 24.88 (minor), 24.80 (minor), 24.70 (major), 24.47 (minor), 24.45 (major), 18.29 (minor) 18.27 (major), 14.99. LRMS (ESI) *m/z* calculated for 1/2 $C_{57}H_{78}N_{13}O_{13}S_3^+$ $[M/2 + H]^+$: 624.8. Found 625; $C_{57}H_{78}N_{13}O_{13}S_3^+$ $[M + H]^+$: 1248.5. Found 1248. HRMS (ESI) *m/z* calculated for 1/2 $C_{57}H_{77}N_{13}O_{13}S_3^+$ $[M/2 + H]^+$: 624.7541. Found: 624.7516; $C_{57}H_{78}N_{13}O_{13}S_3^+$ $[M + H]^+$: 1248.5004. Found: 1248.4956.

(3-(2-(2-(4-(4-((7-(3-(Methylsulfonamido)phenyl)thieno[3,2-*d*]pyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethoxy)ethoxy)propanoyl)-*L*-prolyl-*L*-prolyl-*L*-prolyl-*D*-methionyl-*L*-alanylglycylglycine (**27**).
Compound **23** (50 mg, 0.07 mmol) was converted to the target compound using general procedure C. The product was purified with ACCQPREP HP150 system using XBridge BEH Shield RP18 column (19 × 250 mm, 10 μm particle size; 0% to 50% MeCN/H_2O (0.1% formic acid) to afford compound **27** as a yellow solid (42.8 mg, 0.04 mmol, 57%).
^{1}H NMR (400 MHz, DMSO-d_6): δ two rotamers 9.85 (brs, 1H), 9.45 (s, 1H), 9.17 (s, 1H), 8.47 (s, 1H), 8.35 – 8.27 (m, 1H), 8.12 (t, J = 5.9 Hz, 1H), 8.03 – 7.95 (m, 1H), 7.84 (s, 1H), 7.83 – 7.75 (m, 2H), 7.70 (d, J = 9.0 Hz, 2H), 7.49 (t, J = 7.9 Hz, 1H), 7.27 (dd, J = 1.3, 8.1 Hz, 1H), 6.92 (d, J = 9.07 Hz, 2H), 4.79 (dd, J = 2.8, 8.6 Hz, minor), 4.63 (dd, J = 5.0, 8.4 Hz, minor), 4.58 – 4.50 (m, 2H), 4.33 – 4.17 (m, 3H), 3.77 – 3.71 (m, 4H), 3.69 – 3.53 (m, 9H), 3.50 – 3.36 (m, 7H), 3.07 (brt, 4H), 3.03 (s, 3H), 2.63 – 2.53 (m, 6H), 2.48 – 2.35 (m, 2H), 2.24 – 1.96 (m, 5H), 2.02 (s, 3H), 1.94 – 1.68 (m, 9H), 1.24 (d, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ two rotamers 172.36, 172.31 (minor), 172.11 (major), 172.13 (major), 171.19 (minor), 171.07 (major), 171.03 (minor), 170.40 (minor), 169.61 (major), 169.65 (minor), 169.51 (major), 169.03, 168.22, 158.40, 157.94, 154.02, 146.07, 138.56, 134.60, 134.41, 133.68, 132.76, 129.36, 123.82, 122.20, 120.67, 120.01, 119.13, 115.92, 69.70, 69.66, 68.29, 66.49 (minor), 66.30 (major), 59.88 (major), 59.81 (minor), 57.51, 57.20, 57.08, 53.19, 51.60, 48.97, 48.65 (major), 48.56 (minor), 46.87, 46.86, 46.55, 41.82, 40.77, 34.34, 31.03, 29.59, 28.92, 28.89 (minor), 28.05 (major), 27.65 (major), 27.52 (minor), 24.88, 24.84, 24.54 (minor), 24.18 (major), 17.89 (minor), 17.62 (major), 14.64. LRMS (ESI) *m/z* calculated for 1/2 $C_{57}H_{78}N_{13}O_{13}S_3^+$ $[M/2 + H]^+$: 624.8. Found 625; $C_{57}H_{78}N_{13}O_{13}S_3^+$ $[M + H]^+$: 1248.5. Found 1248. HRMS (ESI) *m/z* calculated for 1/2 $C_{57}H_{77}N_{13}O_{13}S_3^+$ $[M/2 + H]^+$: 624.7541. Found: 624.7515; $C_{57}H_{78}N_{13}O_{13}S_3^+$ $[M + H]^+$: 1248.5004. Found: 1248.4953.

O-methyl-*N*-(3-(2-(2-(4-(4-((7-(3-(methylsulfonamido)phenyl)thieno[3,2-d]pyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethoxy)ethoxy)propanoyl)-*L*-prolyl-*L*-prolyl-*L*-prolyl-*L*-homoseryl-*L*-alanylglycylglycine (**28**).
Compound **24** (50 mg, 0.09 mmol) was converted to the target compound using general procedure C. The product was purified with ACCQPREP HP150 system using XBridge BEH Shield RP18 column (19 × 250 mm, 10 μm particle size; 0% to 50% MeCN/H_2O (0.1% formic acid) to afford compound **28** as a yellow solid (28.3 mg, 0.03 mmol, 33%).

^{1}H NMR (400 MHz, DMSO-d_6): δ two rotamers 9.84 (s, 1H), 9.44 (s, 1H), 9.16 (s, 1H), 8.46 (s, 1H), 814 – 8.08 (m, 1H), 8.03 (m, 1H), 7.97 – 7.88 (m, 2H), 7.84 (s, 1H), 7.80 (d, J = 7.81 Hz, 1H), 7.70 (d, J = 7.8 Hz, 2H), 7.48 (t, J = 7.9 Hz, 1H), 7.27 (dd, J = 1.3, 8.1 Hz, 1H), 6.91 (d, J = 1.3, 8.1 Hz, 2H), 4.78 (dd, J = 8.7, 2.6 Hz, minor), 4.62 (dd, J = 4.5, 8.6 Hz, minor), 4.58 – 4.45 (m, 2H), 4.36 – 4.26 (m, 1H), 4.26 – 4.16 (m, 2H), 3.77 – 3.69 (m, 4H), 3.68 – 3.52 (m, 7H), 3.49 – 3.27 (m, 11H), 3.19 (s, 3H), 3.06 (brt, 4H), 3.02 (s, 3H), 2.62 – 2.52 (m, 6H), 2.24 – 1.96 (m, 3H), 1.97 – 1.64 (m, 11H), 1.21 (d, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ two rotamers 172.37, 171.80 (minor), 171.16 (major), 171.74 (major), 171.63 (minor), 171.15, 170.20 (minor), 169.62 (major), 169.90 (major), 169.55 (minor), 169.00, 168.23, 158.40, 157.94, 154.01, 146.08, 138.56, 134.60, 134.40, 133.68, 132.76, 129.36, 123.81, 122.20, 120.06, 120.01, 119.13, 115.91, 69.70, 69.66, 68.49, 68.31, 66.48 (minor), 66.29 (major), 59.29 (major), 59.28 (minor), 57.90, 57.49, 57.22, 57.12, 53.21, 50.01, 48.99, 48.34, 46.68, 46.64 (major), 46.63 (minor), 46.52 (major), 46.32 (minor), 41.82, 40.74, 34.34, 31.62, 28.84, 28.76 (minor), 27.91 (major), 27.69 (major), 27.52 (minor), 24.56, 24.53 (major), 24.51 (minor), 24.39 (minor), 24.14 (major), 17.91. LRMS (ESI) *m/z* calculated for 1/2 $C_{57}H_{78}N_{13}O_{13}S_3^+$ $[M/2 + H]^+$: 616.8. Found 617; $C_{57}H_{78}N_{13}O_{13}S_3^+$ $[M + H]^+$: 1232.5. Found 1232. HRMS (ESI) *m/z* calculated for 1/2 $C_{57}H_{77}N_{13}O_{13}S_3^+$ $[M/2 + H]^+$: 616.7655. Found: 616.7630; $C_{57}H_{78}N_{13}O_{13}S_3^+$ $[M + H]^+$: 1232.5233. Found: 1232.5182.

Methyl *L*-alanylglycinate hydrochloride (**29**).
To a solution of **8** (1.0 g, 3.84 mmol) in DCM (7.0 mL) was added HCl (4.0 M in 1,4-dioxane, 5.0 mL) at 0 °C. After stirring at room temperature for 3 h, the solution was concentrated under reduced pressure. The residue was solidified by swirling in DCM/diethyl ether (1:4) and separation of the formed solid by filtration, followed by drying yielded **10** (990 mg, 5.05 mmol, 76%) as a white solid that was used for next reaction without any further purification. LRMS (ESI) *m/z* calculated for $C_6H_{12}N_2O_3Na^+$ $[M + Na]^+$: 183.1. Found 183.

tert-Butyl (*S*)-2-((*S*)-2-((*S*)-2-(((7*S*,10*S*)-7-methyl-3,6,9-trioxo-2-oxa-13-thia-5,8-diazatetradecan-10-yl)carbamoyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carboxylate (**30**).
Compound **17** (500 mg, 0.90 mmol) was converted to the target compound using general procedure B. The crude product was purified using flash column chromatography (5% to 10% methanol/chloroform) on silica gel to afford **30** (550 mg, 0.81 mmol, 90%) as a white semi-solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ two rotamers 8.27 (t, J = 5.9 Hz, 1H), 7.99 – 7.94 (dd, J = 3.5, 7.9 Hz 1H), 7.92 (d, J = 7.4 Hz, 1 H), 4.58 (dd, J = 4.0, 8.6 Hz, minor), 4.55 (dd, J = 3.5, 8.2 Hz, major, 2H), 4.35 – 4.21 (m, 3H), 3.84 (dd, J = 6.0, 13.2 Hz, 2H), 3.67 – 3.40 (m, 4H), 3.62 (s, 3H), 3.31 – 3.25 (m, 2H), 2.53 – 2.36 (m, 2H), 2.21 – 1.98 (m, 3H), 2.03 (s, 3H), 1.98 – 1.68 (m, 11H), 1.37 (s, minor), 1.31 (s, major, 9H), 1.22 (d, J = 7.1, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ two rotamers 172.64, 171.72 (minor), 171.71 (major), 170.53, 170.44 (major), 169.99 (minor), 170.14, 169.85 (minor), 169.83 (minor), 153.31 (minor), 153.02 (major), 78.31 (minor), 78.22 (major), 59.22 (major), 59.17 (minor), 57.52 (minor), 57.50 (major), 57.34 (major), 57.29 (minor), 51.81, 51.67, 47.89, 46.61 (major), 46.57 (minor), 46.53 (minor), 46.44 (major), 46.37, 40.50, 31.95, 29.46, 29.24 (major), 28.32 (minor), 28.77, 28.19, 27.97, 27.73 (major), 27.54 (minor), 24.55, 24.36 (major), 24.32 (minor), 23.61 (minor), 23.09 (major), 18.14, 14.63. LRMS (ESI) *m/z* calculated for $C_{31}H_{51}N_6O_9S^+$ $[M + H]^+$: 683.3. Found 683.

Methyl *L*-prolyl-*L*-prolyl-*L*-prolyl-*L*-methionyl-*L*-alanylglycinate hydrochloride (**31**).
To a solution of **30** (300 mg, 0.44 mmol) in DCM (1.1 mL) was added HCl (4.0 M in 1,4-dioxane, 2.9 mL) at 0 °C. After stirring at room temperature for 3 h, the solution was concentrated under reduced pressure. The residue was solidified by swirling in DCM/diethyl ether (1:4) and separation of the formed solid by filtration, followed by drying yielded **31** (250 mg, 0.41 mmol, 93%) as a white solid that was used for next reaction without any further purification. LRMS (ESI) *m/z* calculated for $C_{26}H_{43}N_6O_7S^+$ $[M + H]^+$: 583.3. Found 583.

(3-(2-(2-(4-(4-((7-(3-(Methylsulfonamido)phenyl)thieno[3,2-*d*]pyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethoxy)ethoxy)propanoyl)-*L*-prolyl-*L*-prolyl-*L*-prolyl-*L*-methionyl-*L*-alanylglycine (**32**).
To a solution of **31** (50 mg, 0.078 mmol) in DMF (0.1 M) was added **6** (0.8 equiv), Et_3N (4.0 equiv) followed by 1-propanephosphonic anhydride (50% w/w in ethyl acetate, 1.6 equiv) at -10 °C. The reaction mixture was then stirred at -10 °C for 30 min, quenched with methanol and concentrated under reduced pressure. To the solution of crude ester in THF (0.1 M) was added aqueous LiOH solution (5.0 equiv) at -10 °C. The reaction mixture was then stirred at -10 °C for 30 min and diluted with DMF. The resulting mixture was subjected to ACCQPREP HP150 system (0% to 50% MeCN/H_2O (0.1% formic acid) to afford compound **32** as a yellow solid (52 mg, 0.044 mmol, 56%).
^{1}H NMR (400 MHz, DMSO-d_6): δ two rotamers 9.82 (s, 1H), 9.44 (s, 1H), 9.16 (s, 1H), 8.46 (s, 1H), 8.10 (t, J = 5.7 Hz, 1H), 7.97 (t, J = 8.9 Hz, 1H), 7.92 (d, J = 7.4 Hz, 1H), 7.84 (s, 1H), 7.80 (d, J = 7.8 Hz, 1H), 7.70 (d, J = 9.0 Hz, 2H), 7.48 (t, J = 7.9 Hz, 1H), 7.27 (dd, J = 7.9, 1.52 Hz, 2H), 6.91 (d, J = 8.9 Hz, 2H), 4.78 (dd, J = 8.8, 2.5 Hz, minor), 4.62 (dd, J = 8.5, 4.5 Hz, minor), 4.56 – 4.49 (m, 2H, major), 4.34 – 4.22 (m, 3H), 3.73 (dd, J = 14.2, 5.8 Hz, 2H), 3.68 – 3.35 (m, 16H), 3.07 (brt, 4H), 3.02 (s, 3H), 2.65 – 2.54 (m, 6H), 2.48 – 2.37 (m, 2H), 2.13 – 1.99 (m, 3H), 2.02 (s, 3H), 1.97 – 1.69 (m, 11H), 1.21 (d, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ two rotamers 172.41, 171.76, 171.20, 170.59, 170.03 (major), 169.76 (minor), 169.61 (major), 169.55 (minor), 168.80, 168.25, 158.42, 157.96, 154.03, 146.06, 138.57, 134.61, 134.42, 133.70, 132.80, 129.38, 123.84, 122.22, 120.08, 120.03, 119.15, 115.94, 69.71(minor), 69.66 (major), 68.22, 66.49 (minor), 66.31 (major), 59.16 (major), 59.13 (minor), 57.48, 57.15, 53.17, 51.86, 48.94, 47.97, 46.88, 46.62 (major), 46.53 (minor), 46.35, 40.77, 34.35 (major), 34.03 (minor), 32.04, 29.51, 28.85, 27.93, 27.70 (major), 27.53 (minor), 24.59 (major), 24.49 (minor), 24.38, 24.14, 18.29, 14.69. LRMS (ESI) *m/z* calculated for 1/2 $C_{55}H_{75}N_{12}O_{12}S_3^+$ $[M/2 + H]^+$: 596.7. Found 596; $C_{55}H_{75}N_{12}O_{12}S_3^+$ $[M + H]^+$: 1191.5. Found 1192. HRMS (ESI) *m/z* calculated for 1/2 $C_{55}H_{75}N_{12}O_{12}S_3^+$ $[M/2 + H]^+$: 596.7473. Found: ; $C_{55}H_{75}N_{12}O_{12}S_3^+$ $[M + H]^+$: 1191.4790. Found: 1191.4785.

Benzyl ((2H-tetrazol-5-yl)methyl)carbamate (**33**).
To a solution of benzyl (cyanomethyl)carbamate (1.0 g, 5.26 mmol) in 1:2 isopropyl alcohol:H_2O (18 mL) was added NaN_3 (684 mg, 10.5 mmol) and $ZnBr_2$ (591 mg, 2.63 mmol) at room temperature. The reaction mixture was then stirred under reflux overnight, quenched with water and diluted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (30% to 50% THF/hexane) to afford **33** (1.1 g, 4.72 mmol, 90%) as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.02 – 7.96 (br, 1H), 7.40 – 7.29 (m, 5H), 5.05 (s, 2H), 4.51 (d, J = 5.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 156.77, 155.51, 137.22, 128.84, 128.38, 128.33, 66.26, 34.93. LRMS (ESI) *m/z* calculated for $C_{11}H_{11}N_5O_2^+$ $[M + H]^+$: 234.1. Found 234.

(*S*)-*N*-((*S*)-1-(((*S*)-1-((2-(((2*H*-tetrazol-5-yl)methyl)amino)-2-oxoethyl)amino)-1-oxopropan-2-yl)amino)-4-(methylthio)-1-oxobutan-2-yl)-1-((3-(2-(2-(4-(4-((7-(3-(methylsulfonamido)phenyl)thieno[3,2-*d*]pyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethoxy)ethoxy)propanoyl)-*L*-prolyl-*L*-prolyl)pyrrolidine-2-carboxamide (**34**).
To a solution of **33** (23 mg, 0.1 mmol) in DCM (0.5 M) was added concd hydrochloric acid (20 equiv) at 0 °C. After stirring at 100 °C for 3 h, the solution was concentrated. To the crude mixture in DMF (0.1 M) was added **32** (0.25 equiv), DIPEA (2.0 equiv) followed by *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (1.5 equiv) at -10 °C. The reaction mixture was then stirred at -10 °C for 30 min and diluted with DMF. The resulting mixture was subjected to ACCQPREP HP150 system (0% to 50% MeCN/H_2O (0.1% formic acid) to afford compound **34** as a yellow solid (21 mg, 0.016 mmol, 16%).
^{1}H NMR (400 MHz, DMSO-d_6): δ two rotamers 9.84 (brs, 1H), 9.46 (s, 1H), 9.17 (s, 1H), 8.46 (s, 1H), 8.43 – 8.38 (m, 1H), 8.13 – 8.10 (m, 1H), 8.02 – 7.95 (m, 2H), 7.83 (d, J = 1.6 Hz, 1H), 7.80 (d, J = 7.9 Hz, 1H), 7.71 (d, J = 8.9 Hz, 2H), 7.48 (t, J = 7.9 Hz, 1H), 7.27 (dd, J = 8.0, 1.3 Hz, 1H), 6.92 (d, J = 9.1 Hz, 2H), 4.80 – 4.75 (dd, J = 8.7, 2.2 Hz, minor), 4.61 (dd, J = 8.5, 4.5 Hz, minor), 4.49 – 4.46 (m, 2H, major), 4.34 – 4.27 (m, 1H), 4.27 – 4.20 (m, 2H), 3.78 – 3.55 (m, 10H), 3.53 – 3.33 (m, 10H), 3.15 – 3.09 (brt, 4H), 3.02 (s, 2H), 2.77 – 2.63 (m, 6H), 2.48 – 2.38 (m, 2H), 2.12 – 1.96 (m, 4H), 2.02 (s, 3H), 1.94 – 1.70 (m, 11H), 1.21 (d, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.50, 171.85 (major), 171.82 (minor), 170.87, 170.09 (major), 169.81 (minor), 169.62 (major), 169.56 (minor), 169.12, 168.80, 168.26, 158.41, 157.94, 154.06, 145.78, 138.58, 134.62, 134.47, 133.70, 132.99, 129.39, 123.84, 122.26, 120.08, 120.03, 119.14, 116.06, 69.70 (minor), 69.62 (major), 67.65 (minor), 67.56 (major), 66.46 (minor), 66.28 (major), 59.26 (major), 59.20 (minor), 57.51, 57.16, 56.80, 52.87, 51.85, 48.51, 48.35, 46.87, 46.64, 46.54, 41.90, 34.34 (major), 34.00 (minor), 33.11, 31.93, 29.46, 28.86, 27.93, 27.69 (major), 27.53 (minor), 24.60 (major), 24.49 (minor), 24.38, 24.14, 18.00 (minor), 17.97 (major), 14.68. LRMS (ESI) *m/z* calculated for 1/2 $C_{57}H_{78}N_{17}O_{11}S_3^+$ $[M/2 + H]^+$: 636.8. Found 637. HRMS (ESI) *m/z* calculated for 1/2 $C_{57}H_{78}N_{17}O_{11}S_3^+$ $[M/2 + H]^+$: 636.7654. Found: 636.7655; $C_{57}H_{78}N_{17}O_{11}S_3^+$ $[M + H]^+$: 1272.5229. Found: 1272.5227.

L-prolyl-*L*-prolyl-*L*-prolyl-*L*-methionyl-*L*-alanylglycylglycine (**35**).
To a solution of **22** (300 mg, 0.41 mmol) in methanol/H_2O (1:1, 4.0 mL)) was added aqueous LiOH solution (52 mg, 1.23 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 2 h and pH was adjusted to 2 by addition of 1 N aqueous HCl solution. The mixture was then extracted with isopropyl alcohol/chloroform (1:4), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. To the solution of crude acid in DCM (0.2 M) was added HCl (4.0 M in 1,4-dioxane, 3.00 mL) at 0 °C. After stirring at room temperature for 3 h, the solution was concentrated under reduced pressure. The residue was solidified by swirling in DCM/diethyl ether (1:4) and separation of the formed solid by filtration, followed by drying yielded **38** as a white solid (250 mg, 90%).
LRMS (ESI) *m/z* calculated for $C_{20}H_{23}N_2O_9^+$ $[M + H]^+$: 626.3. Found 626.

tert-Butyl 3-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)ethoxy)ethoxy)propanoate (**37**).
To a solution of (2,6-dioxopiperidin-3-yl)-4-hydroxyisoindoline-1,3-dione (**36**), 300 mg, 1.09 mmol) in DMF (2.00 mL) was added sodium iodide (250 mg, 1.67 mmol), compound **3** (452 mg, 1.31 mmol) and potassium bicarbonate (179 mg. 1.68 mmol) at room temperature. The reaction mixture was then stirred at 70 °C for 12 h, quenched with water and diluted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on 38 g WELUX™ spherical C18 column (20~30 μm particle size; 50% to 80% MeCN/H_2O (0.1% formic acid)) to afford compound **37** as a white solid (320 mg, 0.65 mmol, 60%).
^{1}H NMR (400 MHz, DMSO-d_6): δ 11.12 (s, 1H), 7.81 (dd, J = 7.2, 8.5 Hz, 1H), 7.53 (dd, J = 0.7, 8.6 Hz 1H), 7.46 (dd, J = 0.6, 7.3 Hz 1H), 5.09 (dd, J = 5.4, 12.8 Hz 1H), 4.37 – 4.29 (m, 2H), 3.83 – 3.76 (m, 2H), 3.64 – 3.60 (m, 2H), 3.58 (t, J = 6.3 Hz, 2H), 3.52 – 3.48 (m, 2H), 2.95 – 2.81 (m, 1H), 2.63 – 2.45 (m, 2H), 2.39 (t, J = 6.2 Hz, 2H), 2.06 – 1.95 (m, 1H), 1.37 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.86, 170.47, 169.99, 166.86, 165.30, 155.86, 137.03, 133.28, 120.03, 116.33, 115.43, 79.74, 70.12, 69.74, 68.88, 68.70, 66.26, 48.76, 35.84, 30.98, 30.75, 27.76, 22.02. LRMS (ESI) *m/z* calculated for $C_{24}H_{30}N_2O_9Na^+$ $[M + Na]^+$: 513.5. Found 513.

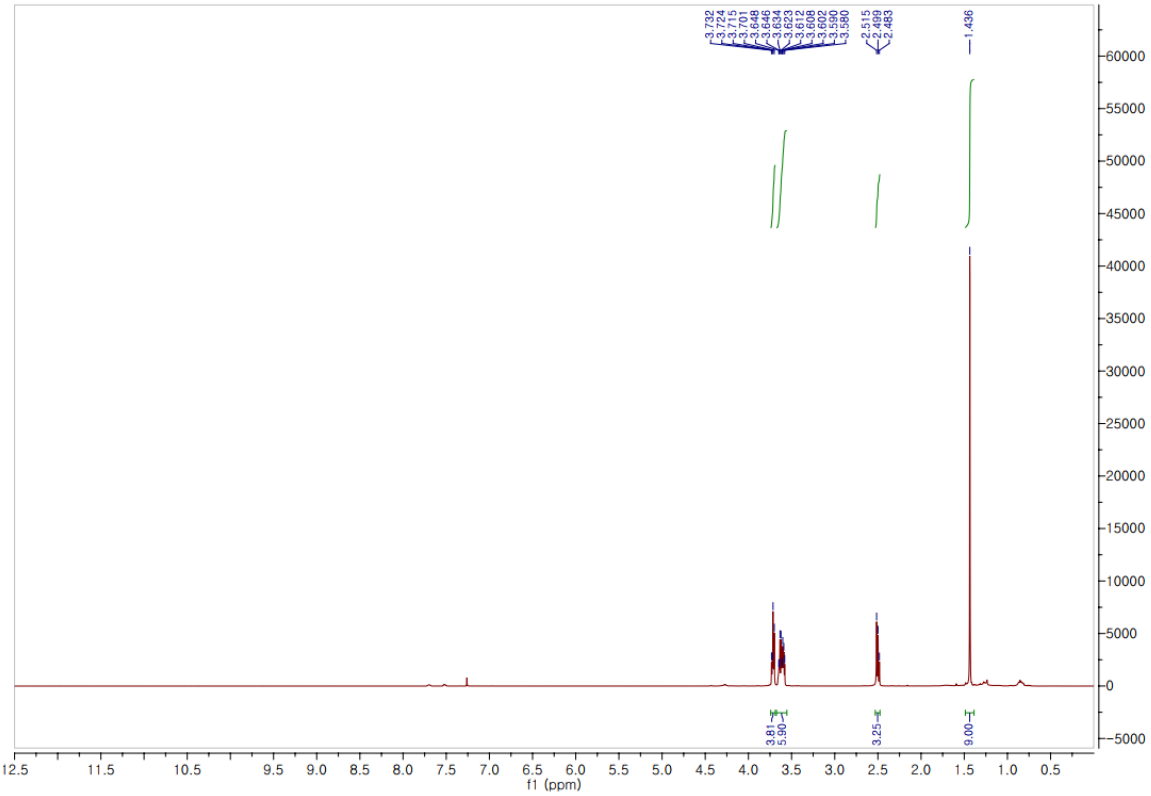
3-(2-(2-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)ethoxy)ethoxy)propanoic acid (**38**).
To a solution of **37** (300 mg, 0.61 mmol) in DCM (2.00 mL) was added HCl (4.0 M in 1,4-dioxane, 3.00 mL) at 0 °C. After stirring at room temperature for 12 h, the solution was concentrated under reduced pressure to yield compound **38** as a white semi-solid (250 mg, 0.58 mmol, 95%) that was used for next reaction without any further purification.
LRMS (ESI) *m/z* calculated for $C_{20}H_{23}N_2O_9^+$ $[M + H]^+$: 435.4. Found 435.

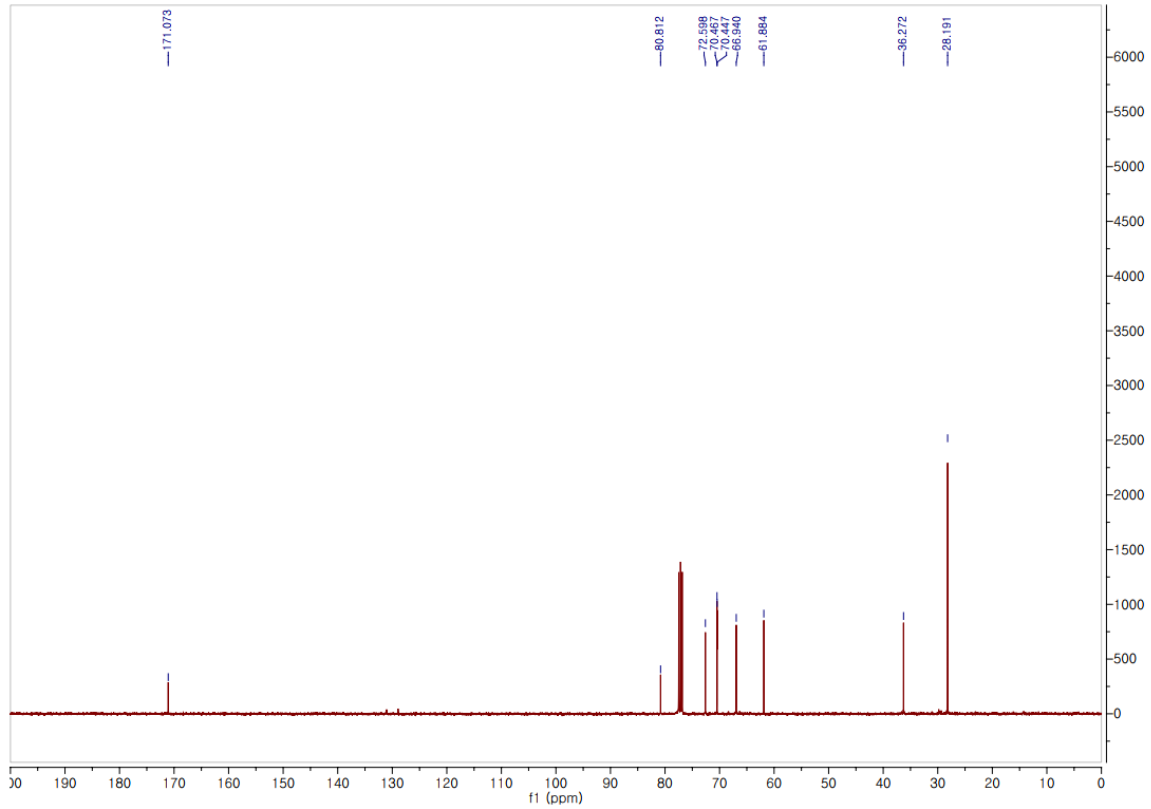
(3-(2-(2-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)ethoxy)ethoxy)propanoyl)-*L*-prolyl-*L*-prolyl-*L*-prolyl-*L*-methionyl-*L*-alanylglycylglycine (**39**).
To a solution of **38** (40 mg, 0.09 mmol) in DCM (0.40 mL) was added EDCI (22 mg, 0.14 mmol) and DMAP (22 mg, 0.18 mmol). The mixture was stirred for 5 min, treated slowly with perfluorophenyl trifluoroacetate (39 mg, 0.14 mmol). The reaction mixture was stirred at room temperature for 1 h, quenched by addition of ice water, and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. To the solution of crude ester in DCM was added **35** (73 mg, 0.11 mmol) and DIPEA (0.06 mL, 0.36 mmol). The reaction mixture was then stirred at room temperature for 1 h and diluted with DMF. The resulting mixture was subjected to ACCQPREP HP150 system (0% to 50% MeCN/H_2O (0.1% formic acid) to afford compound **39** as a white solid (21 mg, 0.02 mmol, 22%).
^{1}H NMR (400 MHz, DMSO-d_6): δ two rotamers 12.57 (brs, 1H), 11.09 (s, 1H), 8.18 – 8.13 (m, 1H), 8.10 – 8.04 (m, 1H), 7.97 (d, J = 7.4 Hz, 2H), 7.84 – 7.76 (dd, J = 8.9, 7.26 Hz 1H), 7.54 (d, J = 8.6 Hz, 1H), 7.46 (d, J = 7.4 Hz, 1H), 5.08 (dd, J = 12.8, 5.4 Hz, 1H), 4.76 (dd, J = 8.6, 2.5 Hz, minor), 4.61 (dd, J = 8.8, 4.4 Hz, minor), 4.53 (dd, J = 8.6, 3.7 Hz, 2H, major), 4.36 – 4.29 (m, 3H), 4.28 – 4.19 (m, 2H), 3.82 – 3.68 (m, 7H), 3.65 – 3.55 (m, 5H), 3.55 – 3.40 (m, 6H), 2.95 – 2.81 (m, 1H), 2.62 – 2.52 (m, 2H), 2.48 – 2.32 (m, 4H), 2.14 – 1.97 (m, 4H), 2.03 (s, 3H), 1.95 – 1.72 (m, 11H), 1.22 (d, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ . LRMS (ESI) *m/z* calculated for 1/2 $C_{55}H_{75}N_{12}O_{12}S_3^+$ $[M/2 + H]^+$: 521.7. Found 522; $C_{47}H_{64}N_9O_{16}S^+$ [M

+ H]$^+$: 1042.4. Found 1042. HRMS (ESI) *m/z* calculated for 1/2 $C_{55}H_{75}N_{12}O_{12}S_3^+$ [M/2 + H]$^+$: 521.7135. Found: 521.7130; $C_{47}H_{64}N_9O_{16}S^+$ [M + H]$^+$: 1042.4192. Found: 1042.4180.

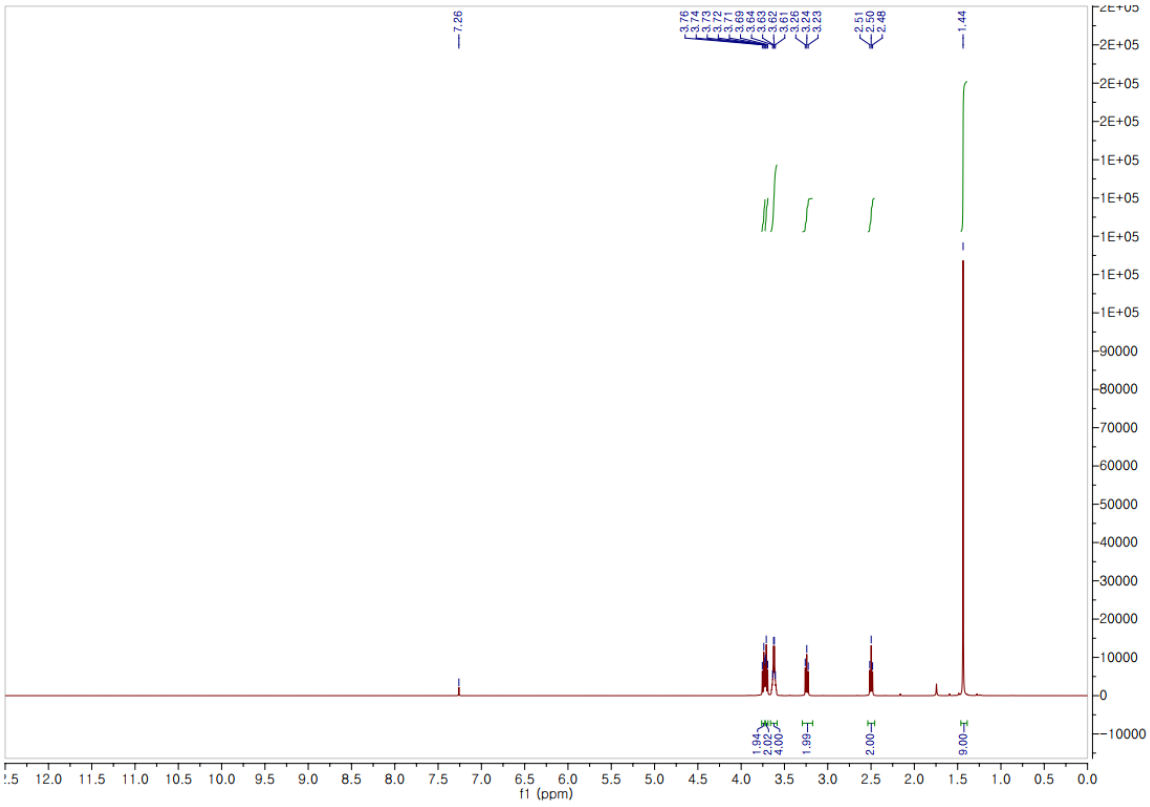
^{1}H and ^{13}C NMR spectra of intermediates

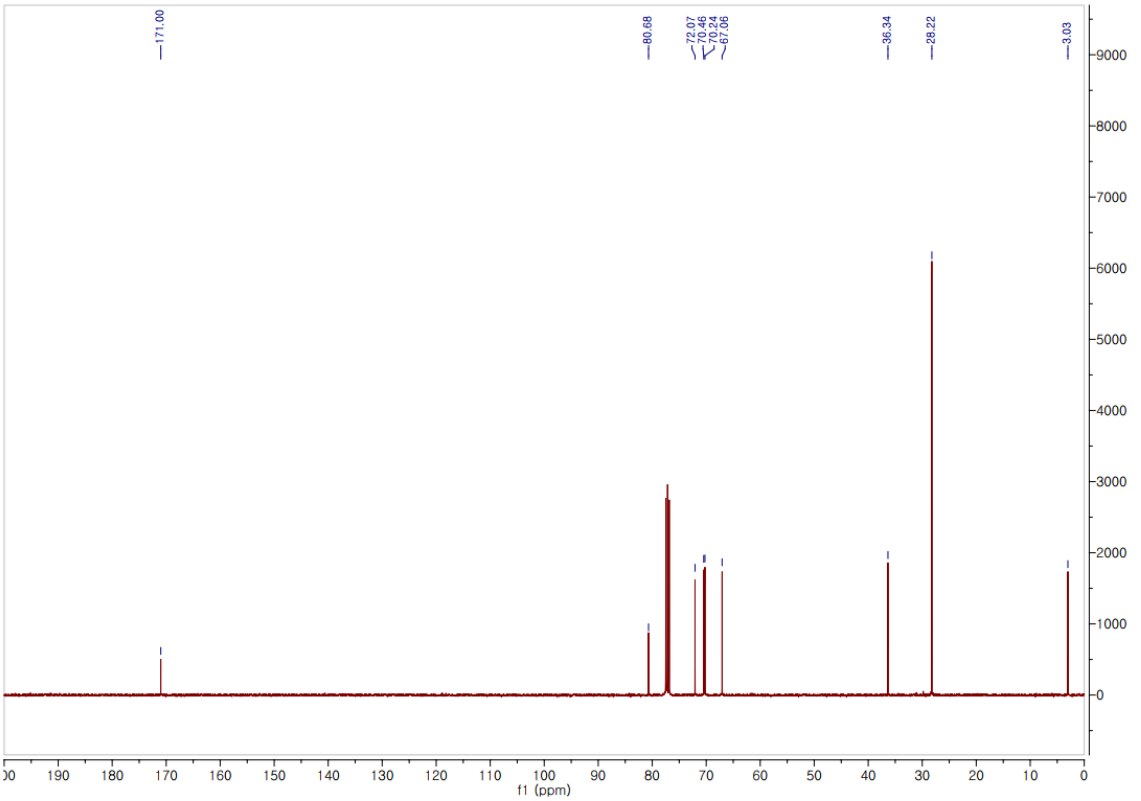
tert-butyl 3-(2-(2-hydroxyethoxy)ethoxy)propanoate **(2)**.



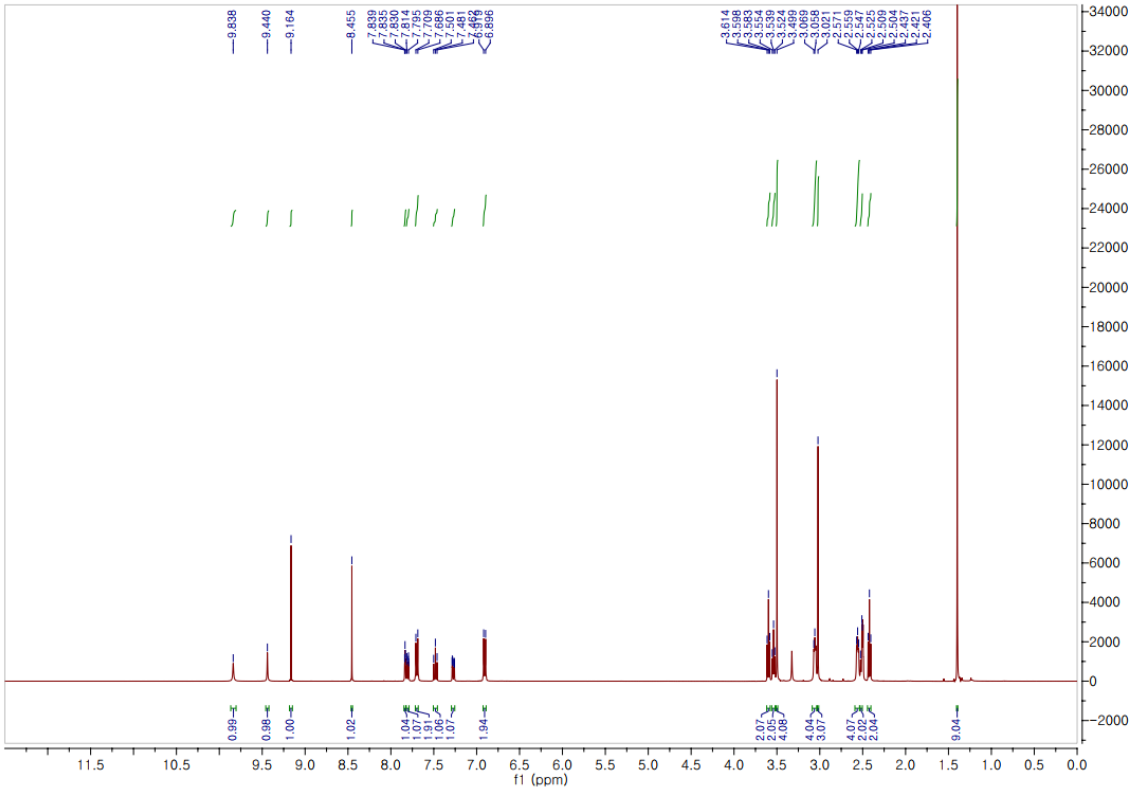


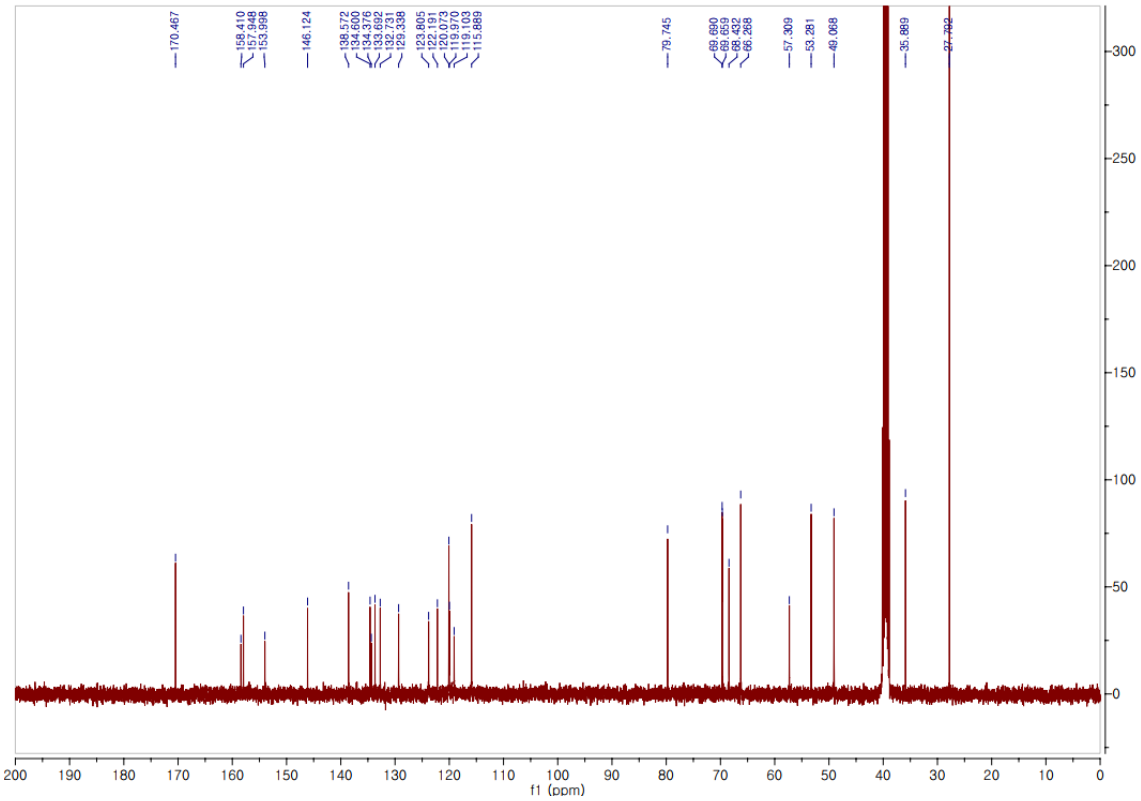
tert-butyl 3-(2-(2-iodoethoxy)ethoxy)propanoate **(3)**.



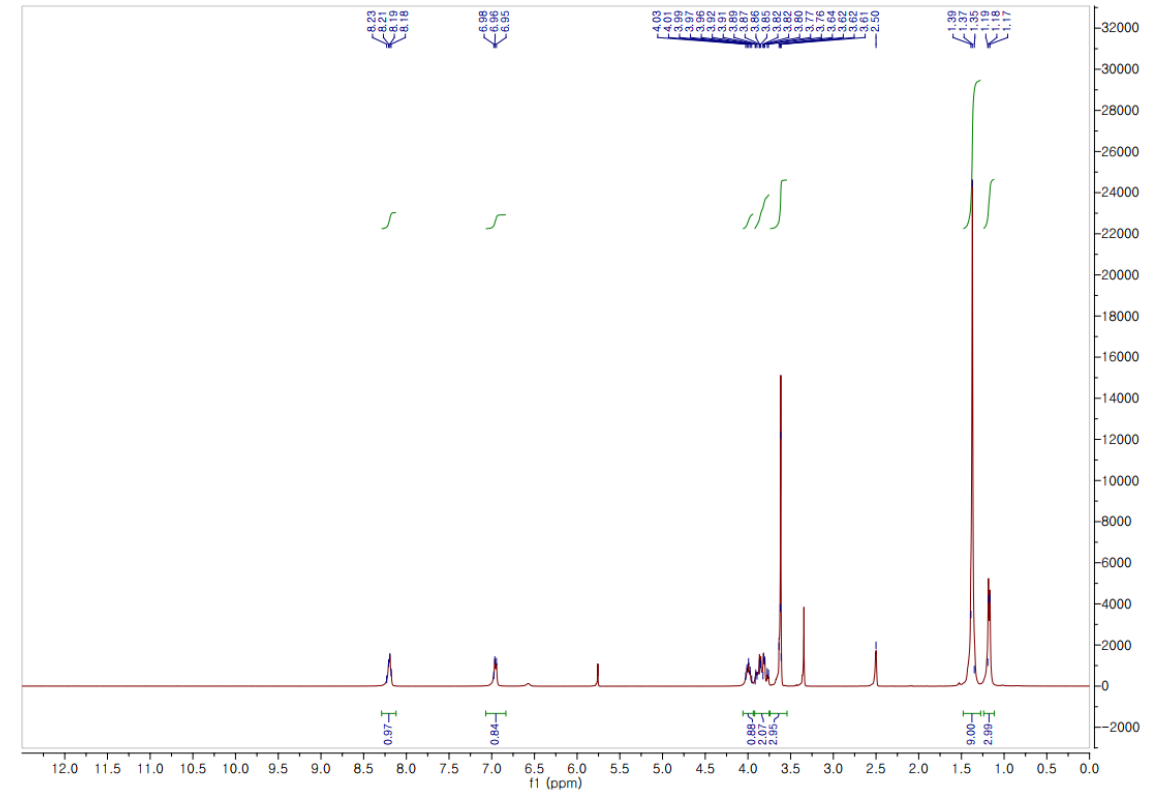


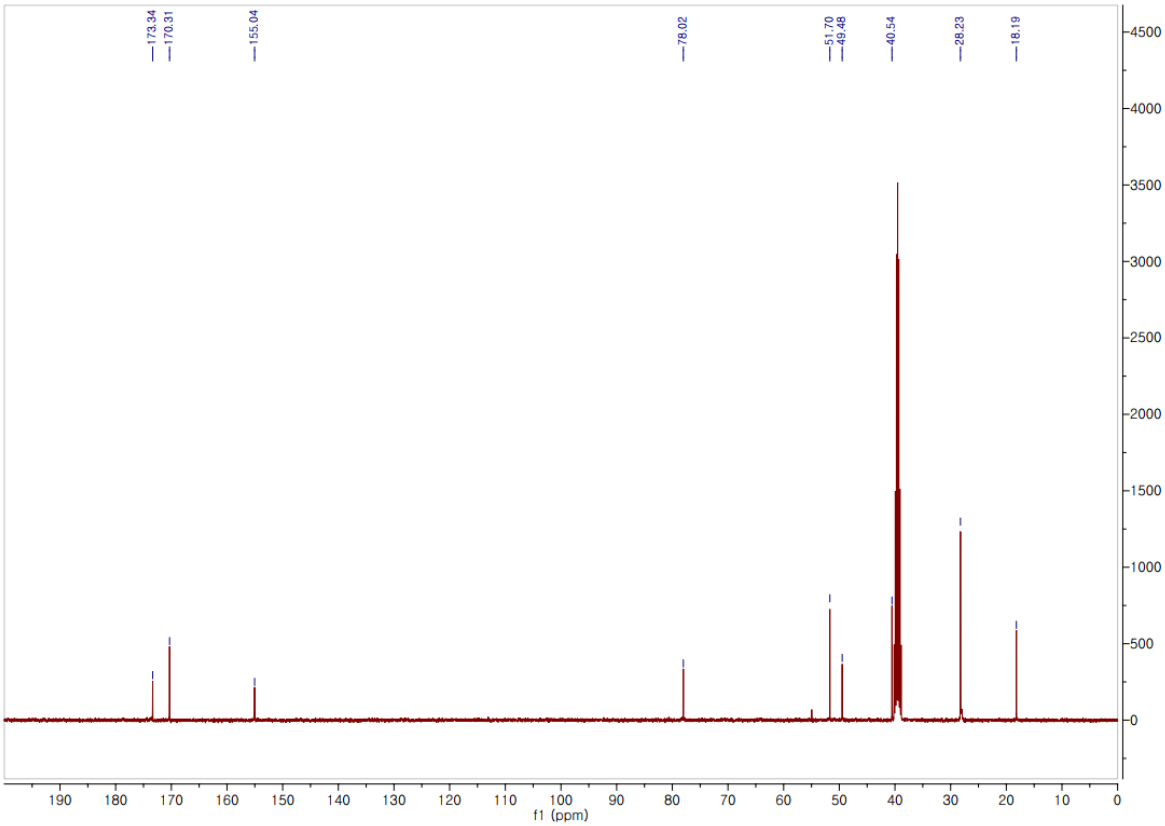
tert-butyl 3-(2-(2-(4-(4-((7-(3-(methylsulfonamido)phenyl)thieno[3,2-d]pyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethoxy)ethoxy)propanoate **(5)**.



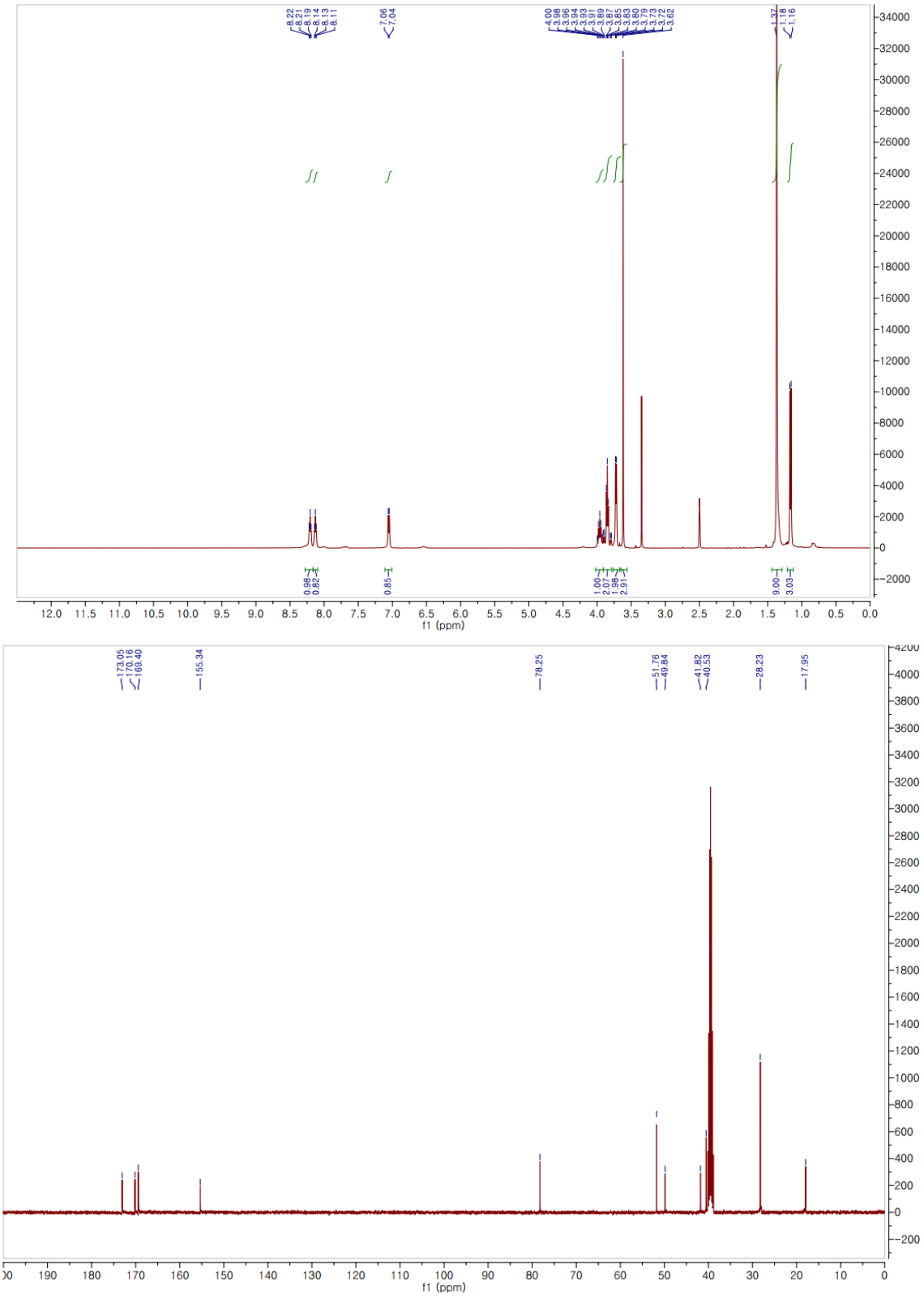


Methyl (*tert*-butoxycarbonyl)-*L*-alanylglycinate **(8)**.

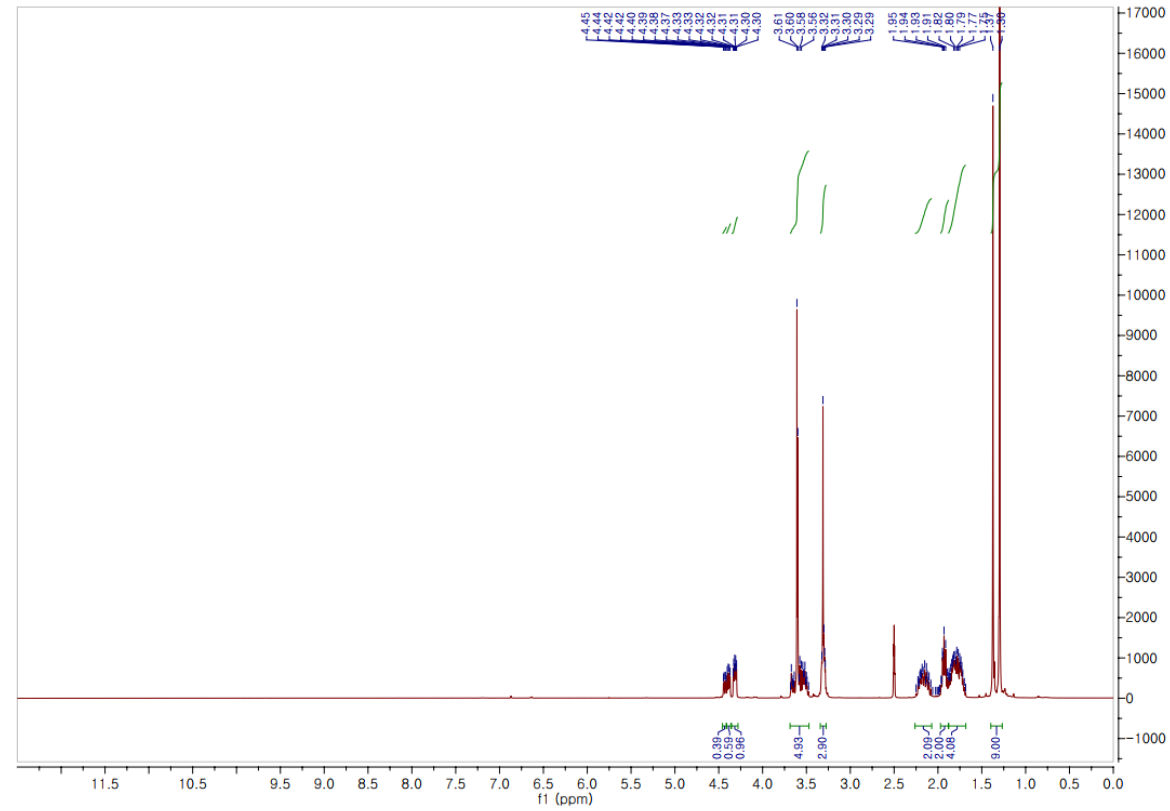


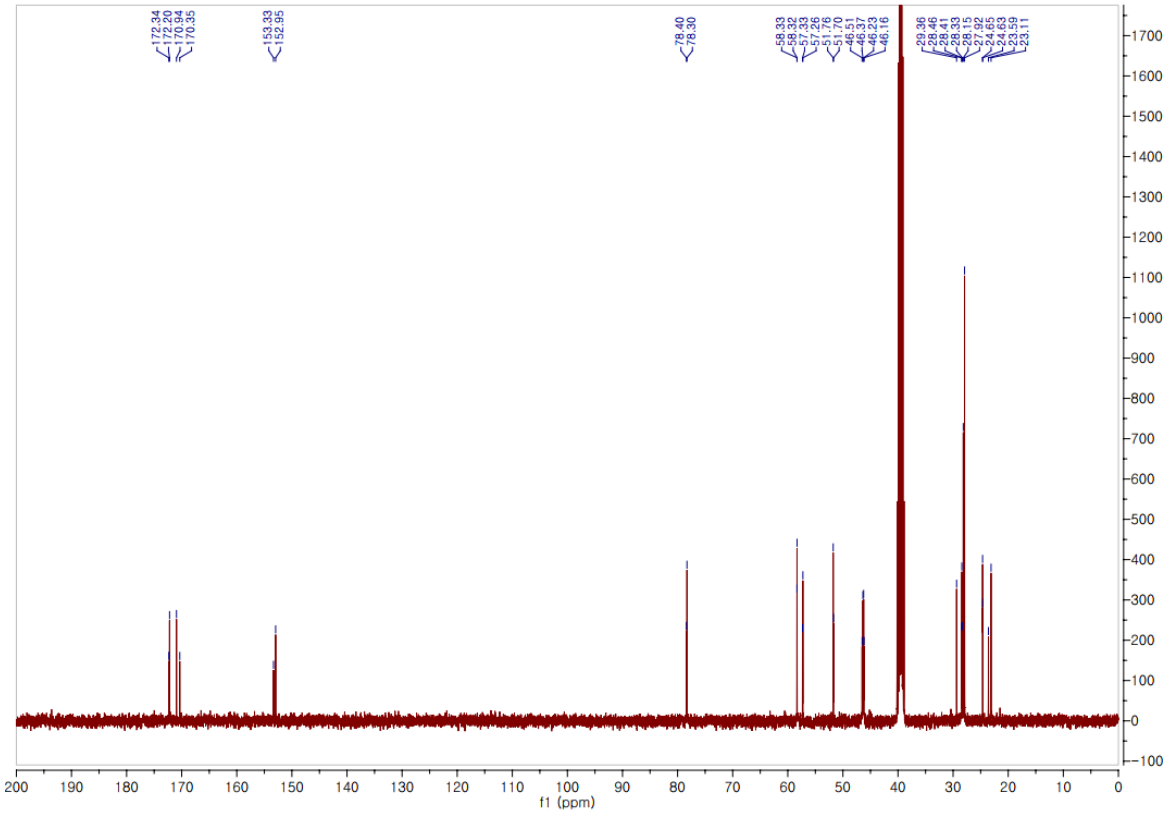


Methyl (*tert*-butoxycarbonyl)-*L*-alanylglycylglycinate **(9)**.

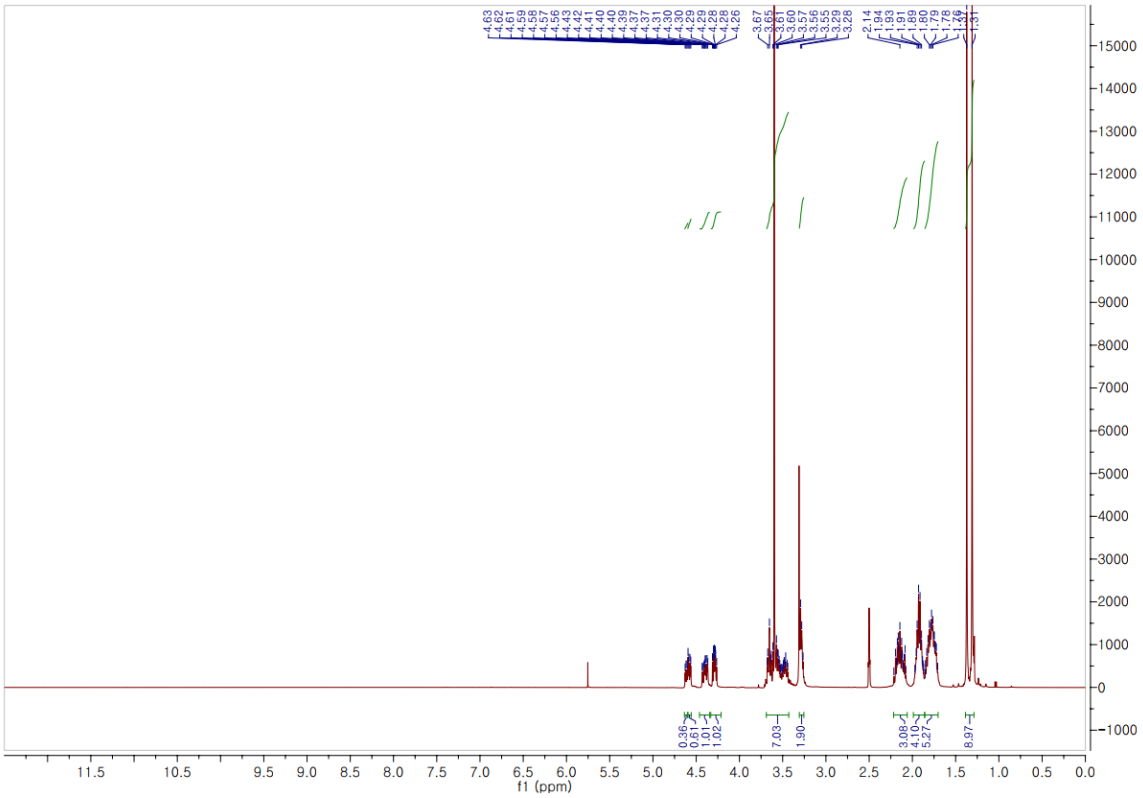


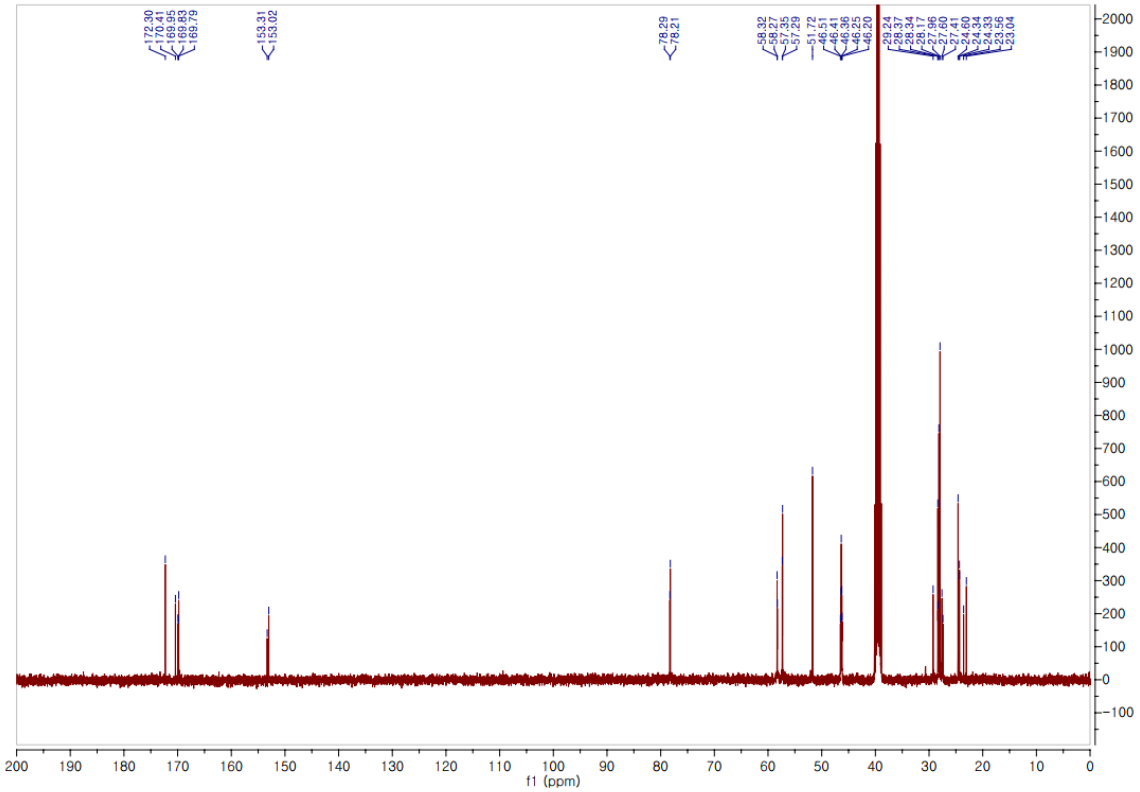
tert-Butyl (*S*)-2-((*S*)-2-(methoxycarbonyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carboxylate **(12)**.



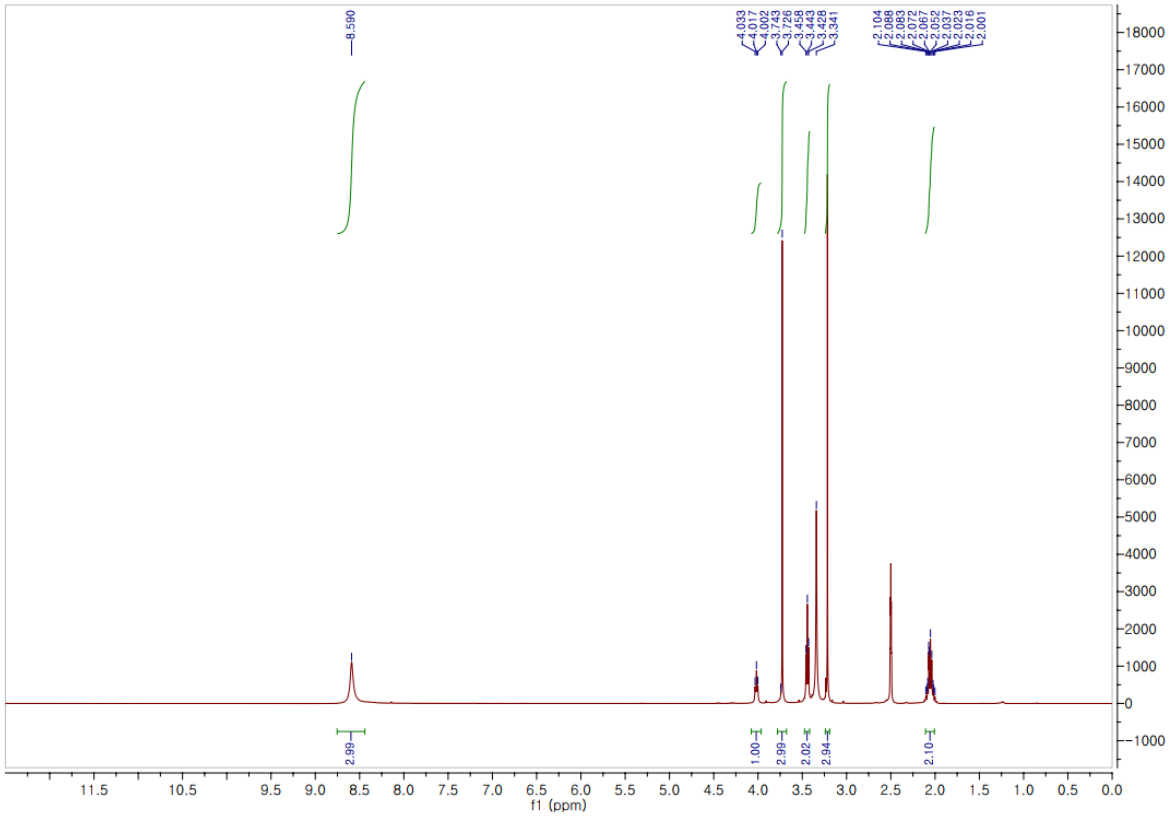


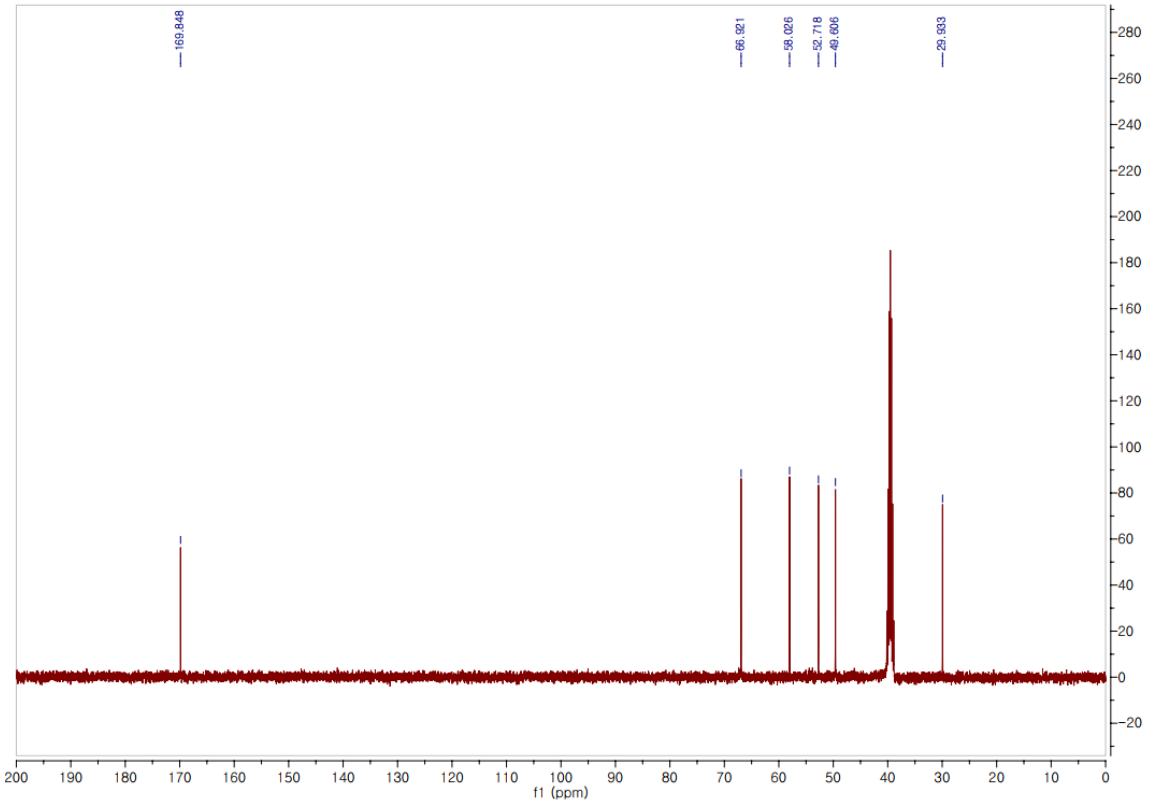
*t*ert-Butyl (*S*)-2-((*S*)-2-((*S*)-2-(methoxycarbonyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carboxylate **(13)**.



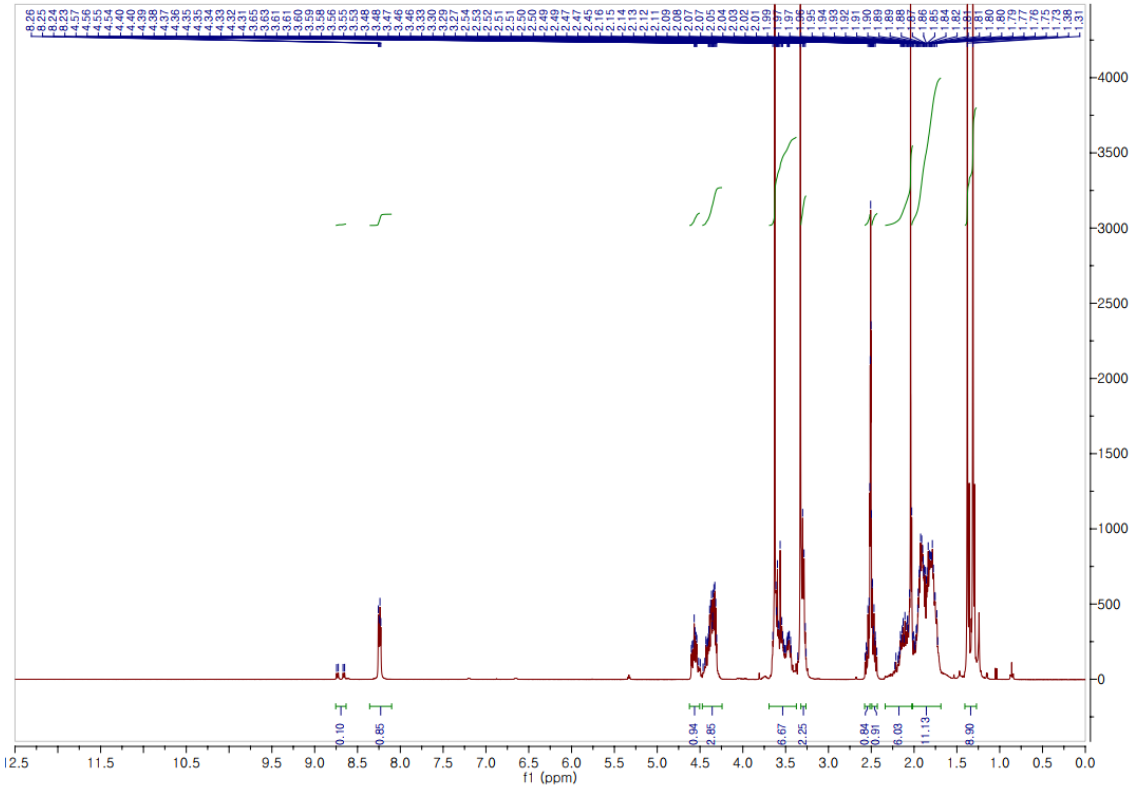


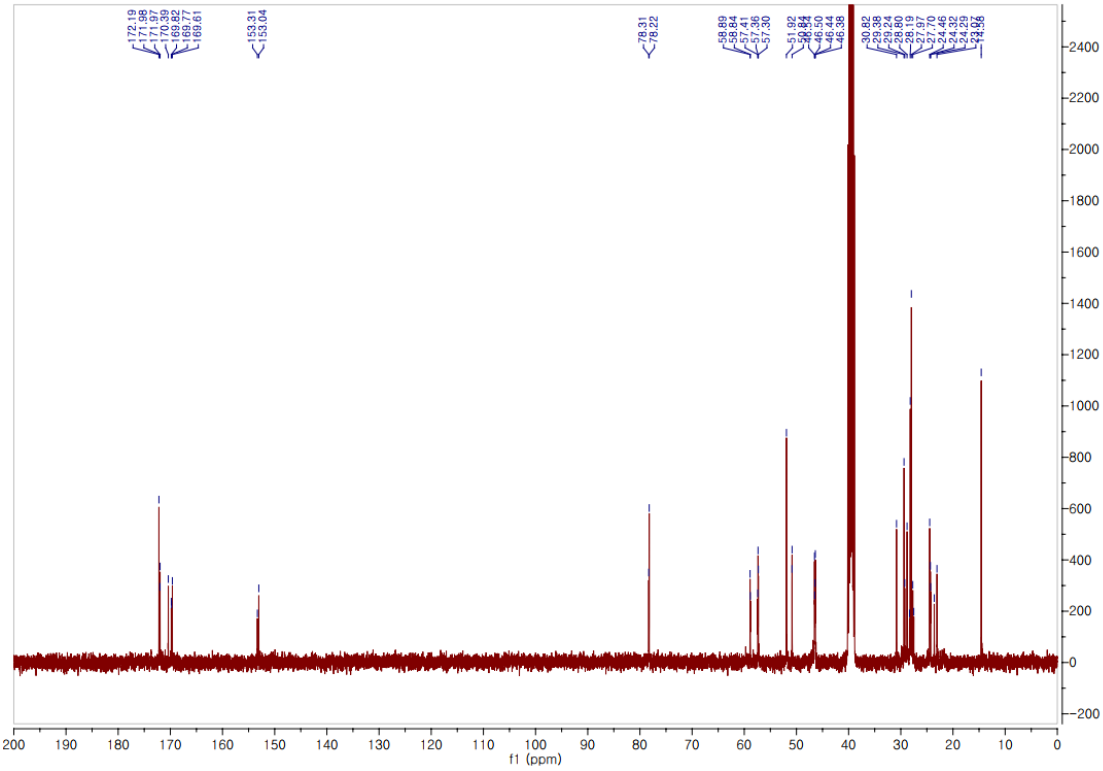
Methyl *O*-methyl-*L*-homoserinate hydrochloride **(16)**.



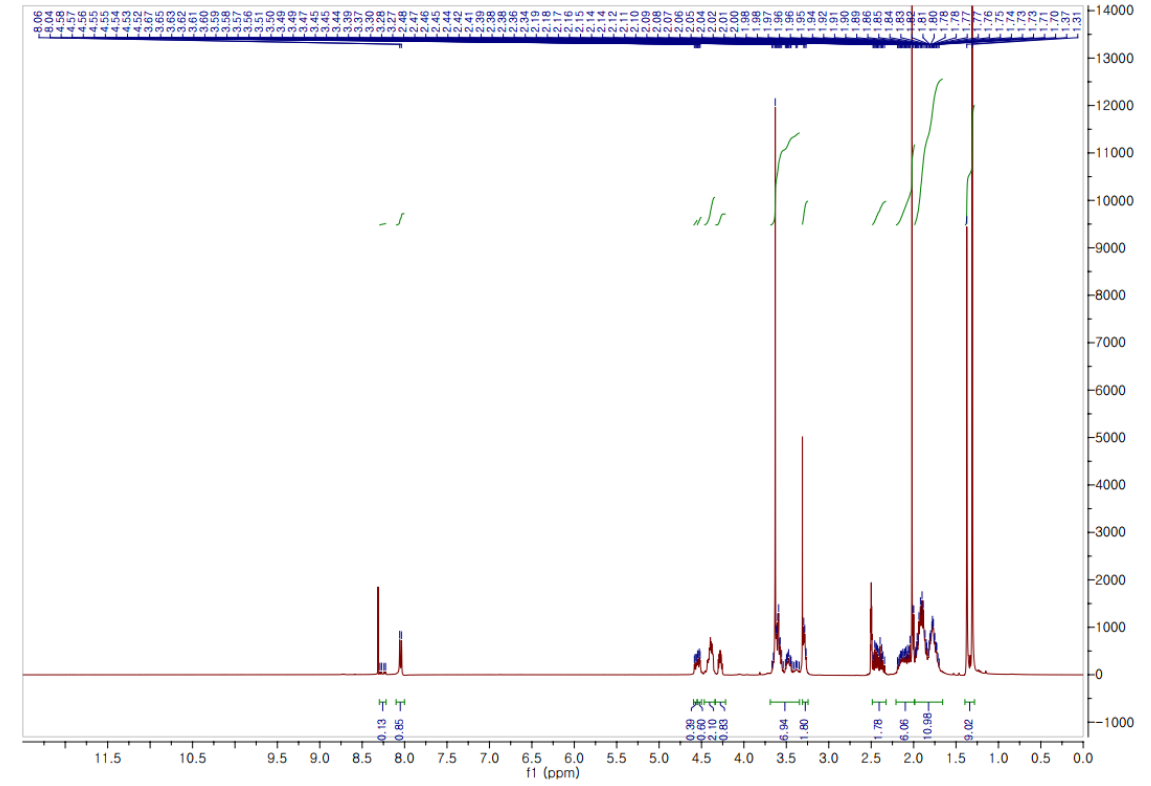


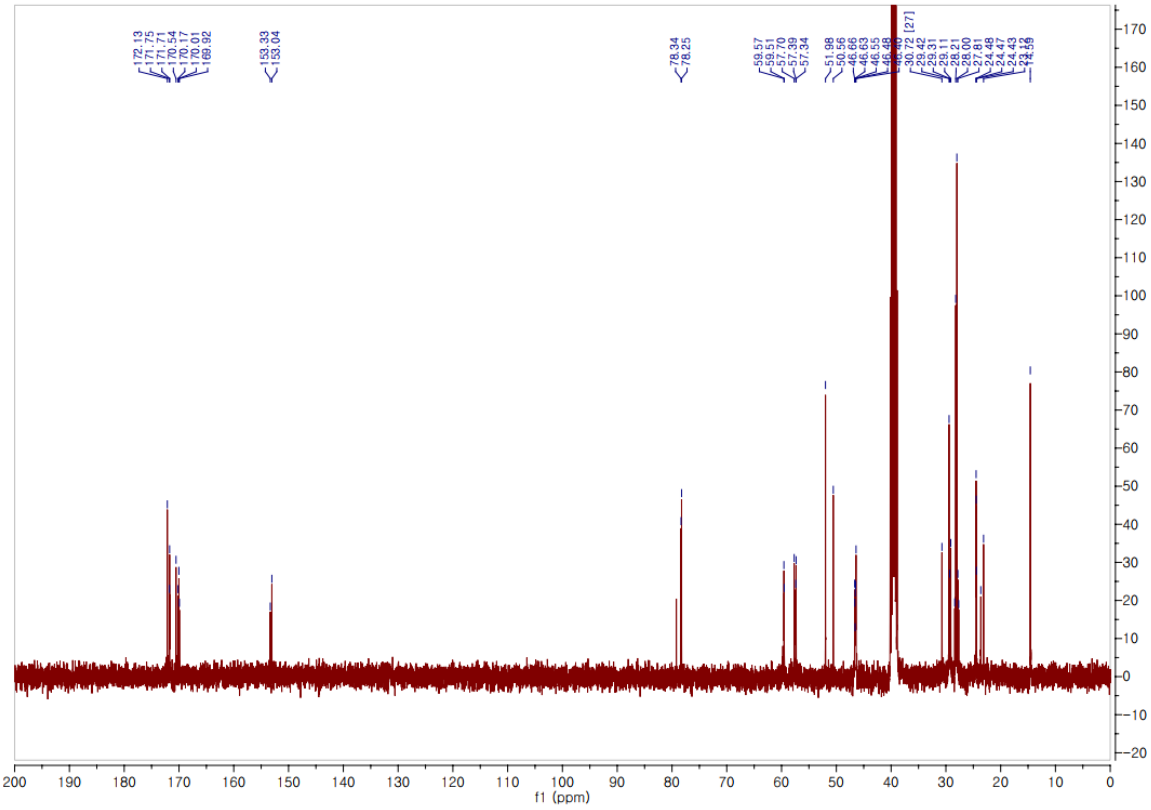
tert-Butyl (*S*)-2-((*S*)-2-((*S*)-2-(((*S*)-1-methoxy-4-(methylthio)-1-oxobutan-2-yl)carbamoyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carboxylate **(17)**.



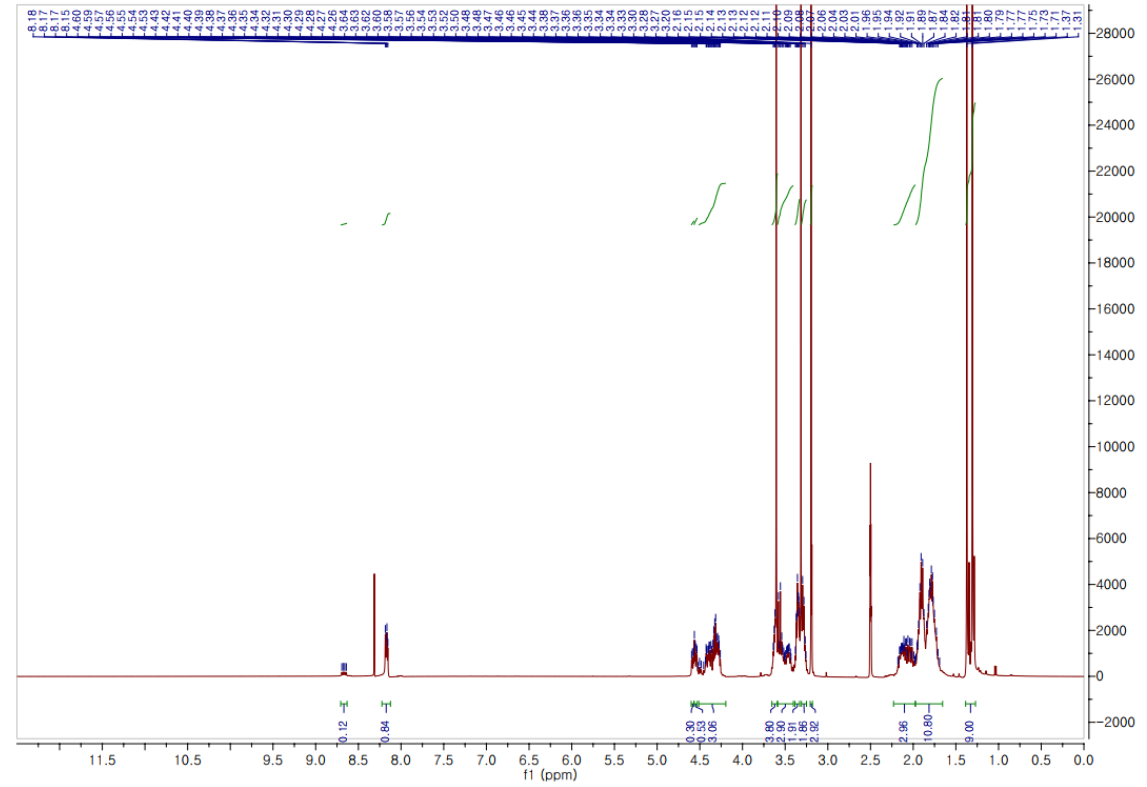


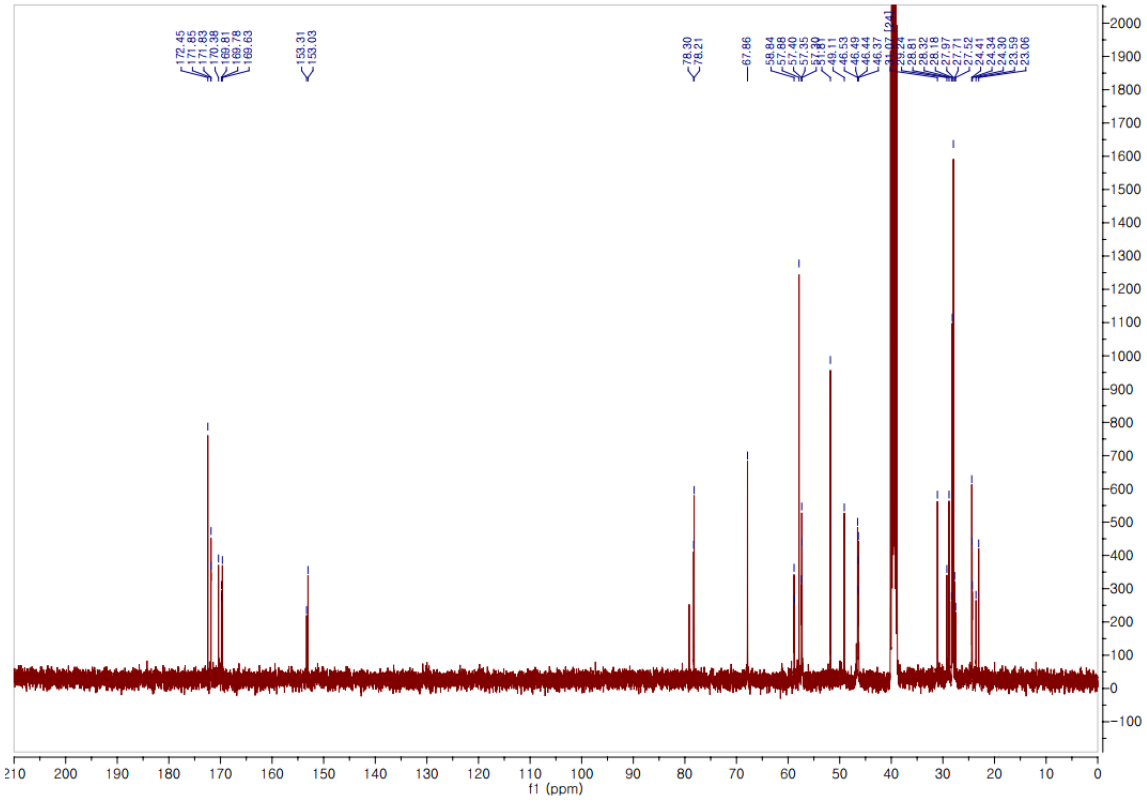
tert-Butyl (*S*)-2-((*S*)-2-((*S*)-2-(((*R*)-1-methoxy-4-(methylthio)-1-oxobutan-2-yl)carbamoyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carboxylate **(18)**.



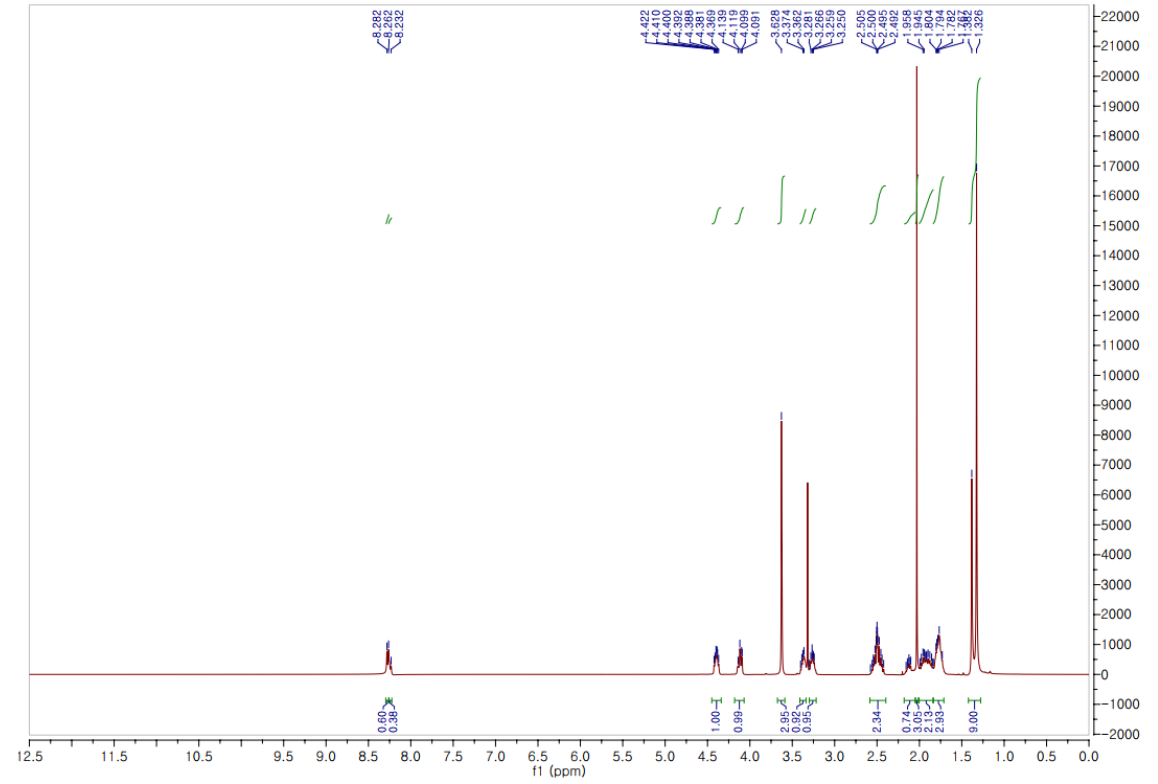


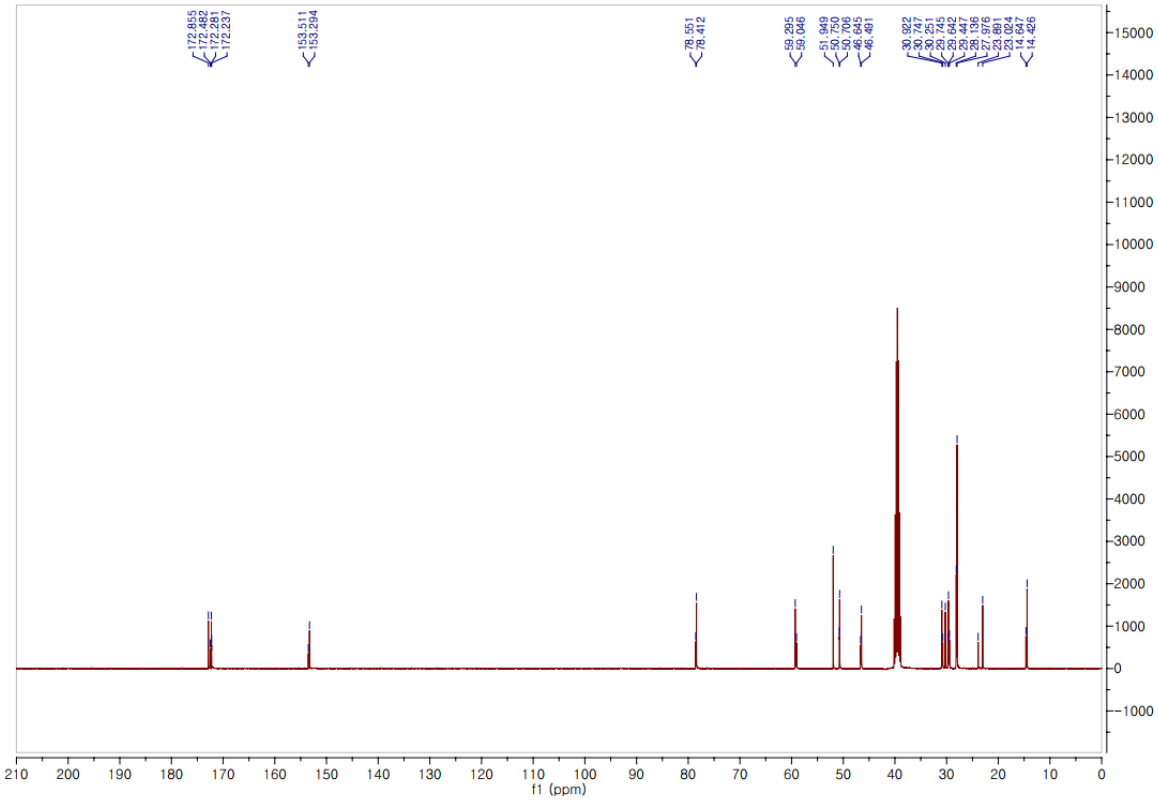
tert-Butyl (*S*)-2-((*S*)-2-((*S*)-2-(((*S*)-1,4-dimethoxy-1-oxobutan-2-yl)carbamoyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carboxylate **(19)**.



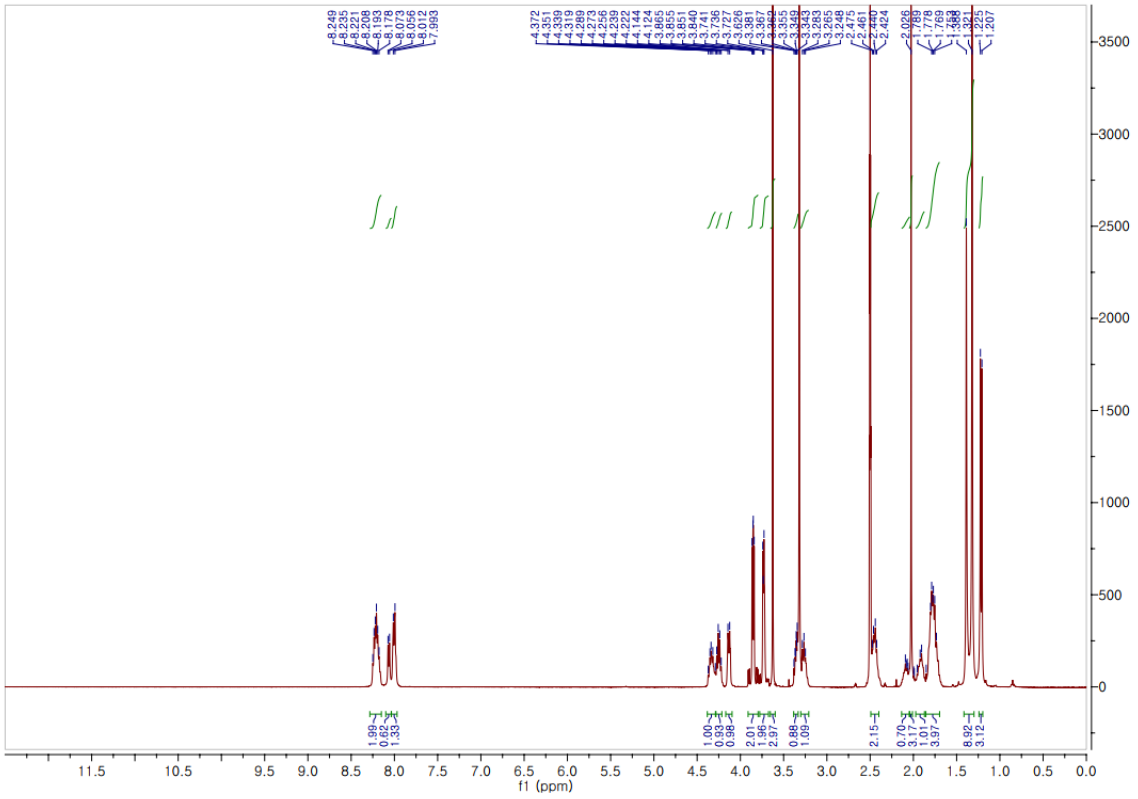


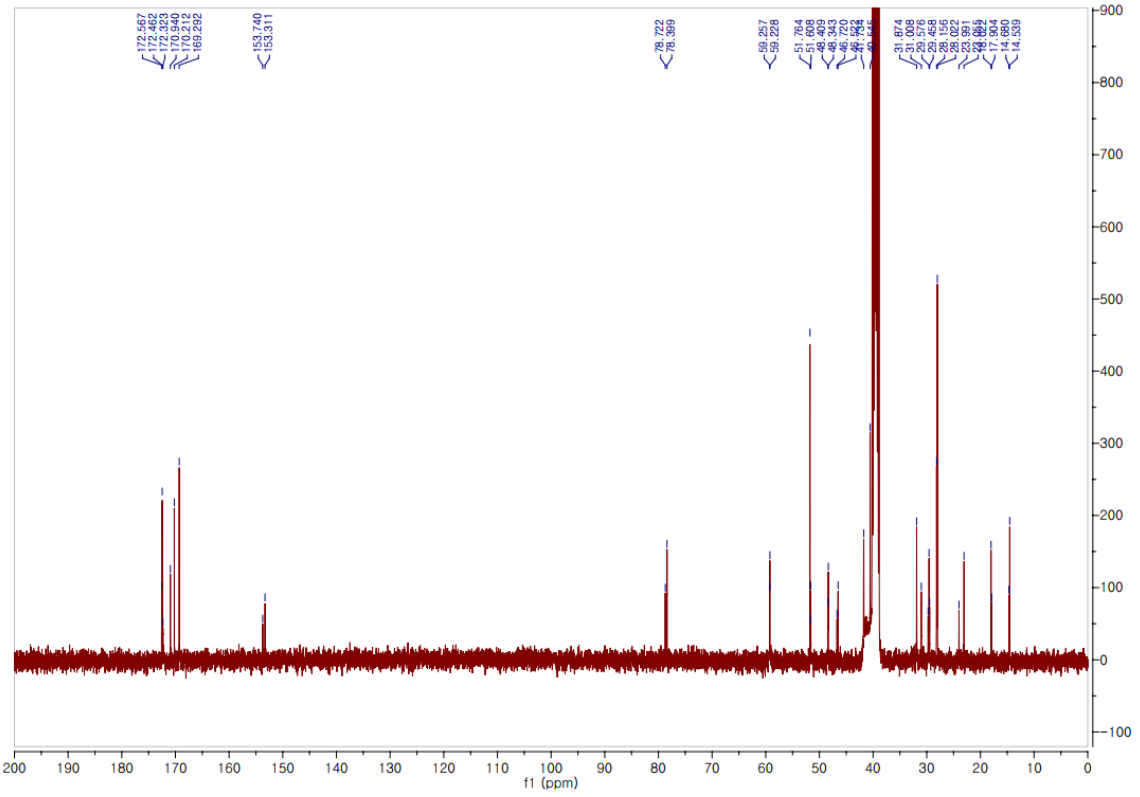
tert-Butyl (*S*)-2-(((*S*)-1-methoxy-4-(methylthio)-1-oxobutan-2-yl)carbamoyl)pyrrolidine-1-carboxylate **(20)**.



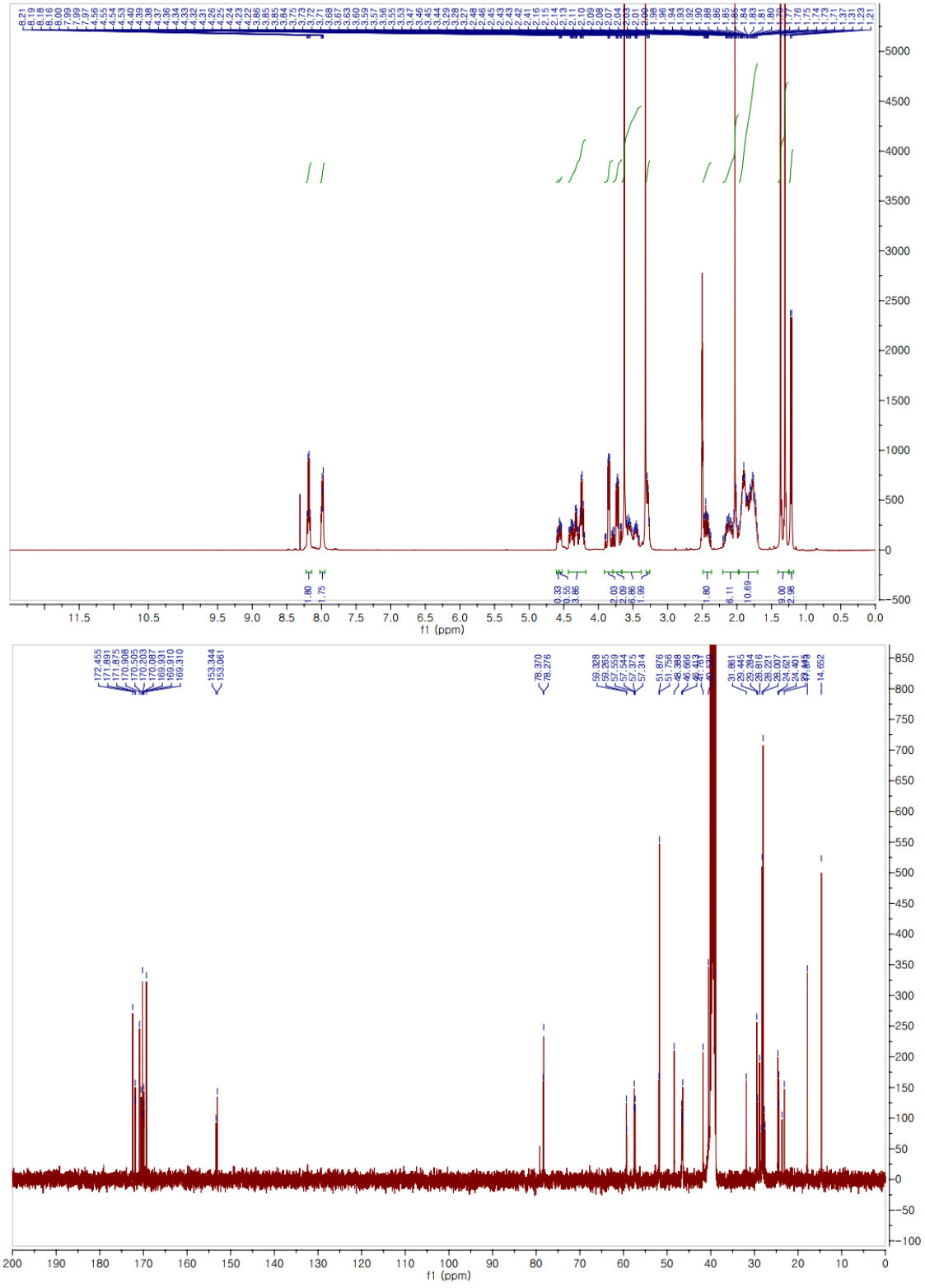


tert-Butyl (*S*)-2-(((10*S*,13*S*)-10-methyl-3,6,9,12-tetraoxo-2-oxa-16-thia-5,8,11-triazaheptadecan-13-yl)carbamoyl)pyrrolidine-1-carboxylate **(21)**.

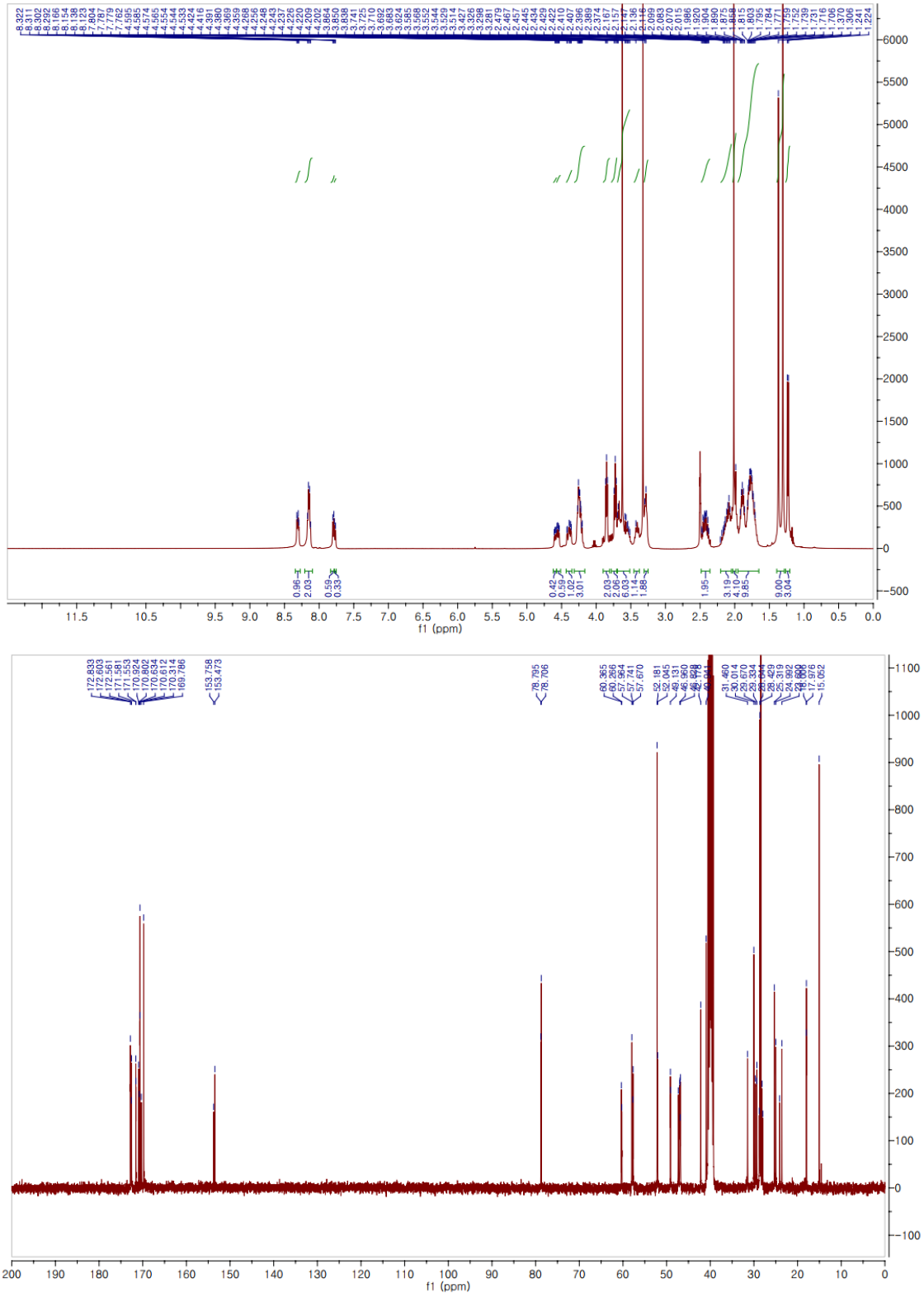




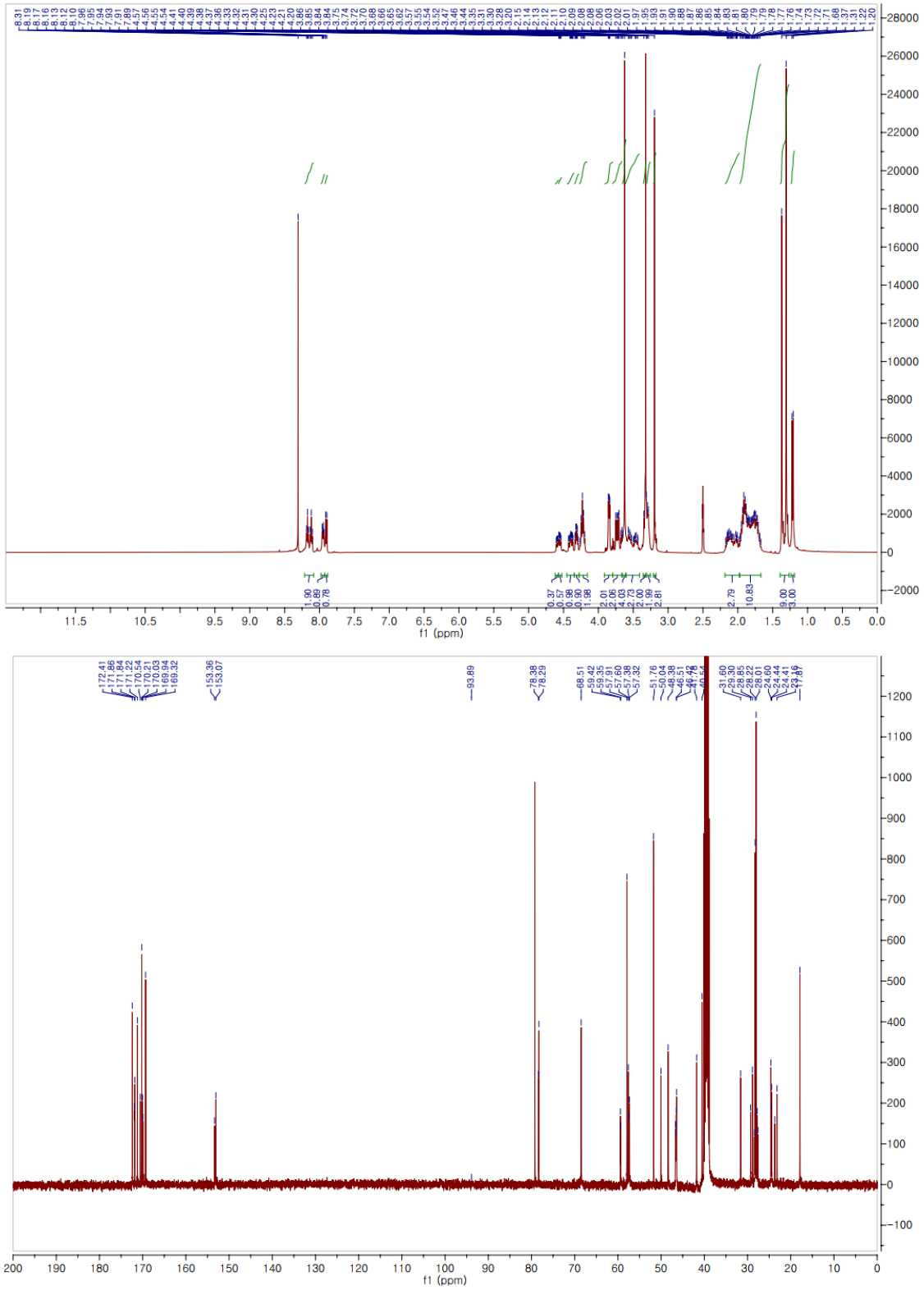
tert-Butyl (*S*)-2-((*S*)-2-((*S*)-2-(((10*S*,13*S*)-10-methyl-3,6,9,12-tetraoxo-2-oxa-16-thia-5,8,11-triazaheptadecan-13-yl)carbamoyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carboxylate **(22)**.



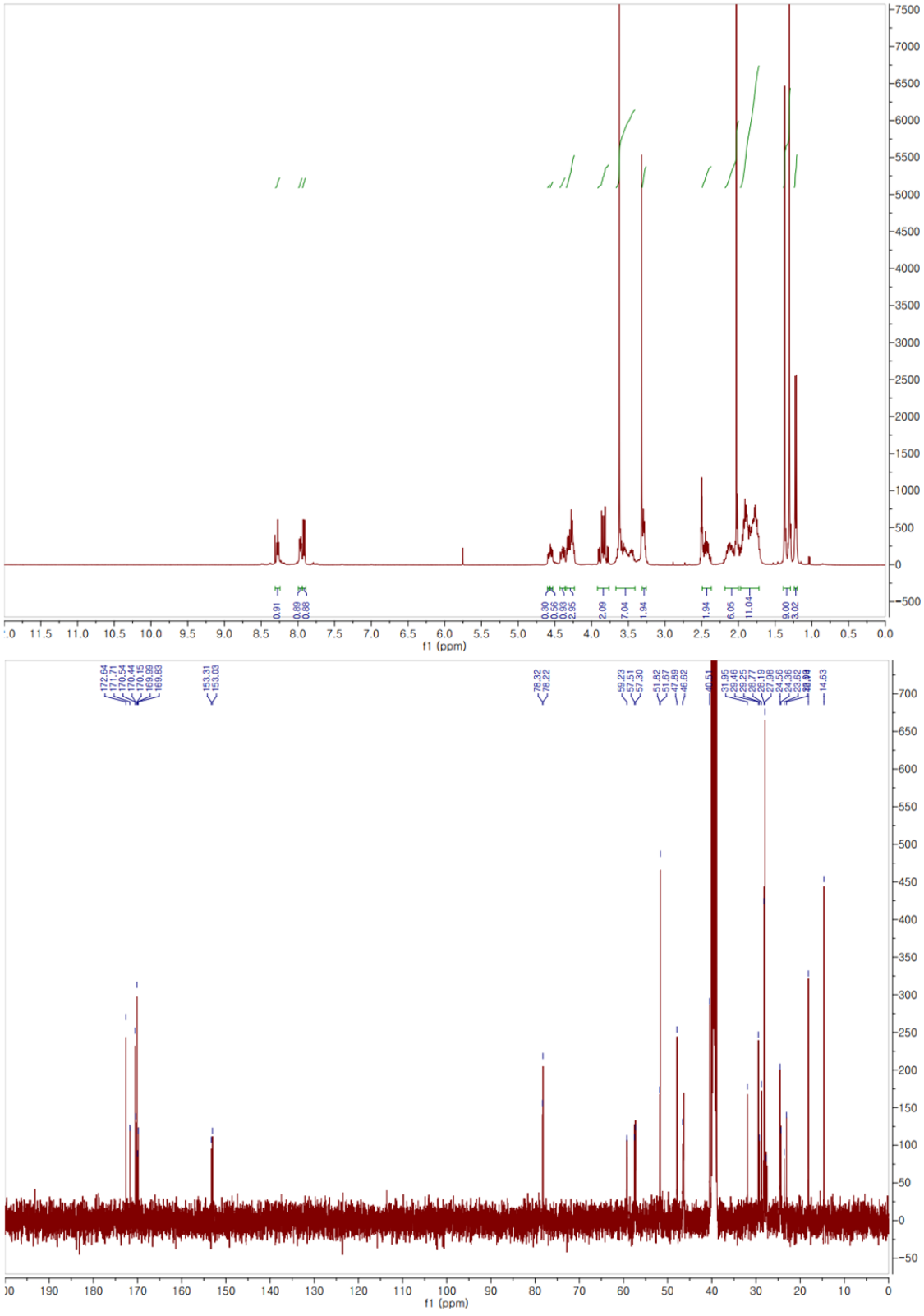
tert-Butyl (*S*)-2-((*S*)-2-((*S*)-2-(((10*S*,13*R*)-10-methyl-3,6,9,12-tetraoxo-2-oxa-16-thia-5,8,11-triazaheptadecan-13-yl)carbamoyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carboxylate **(23)**.



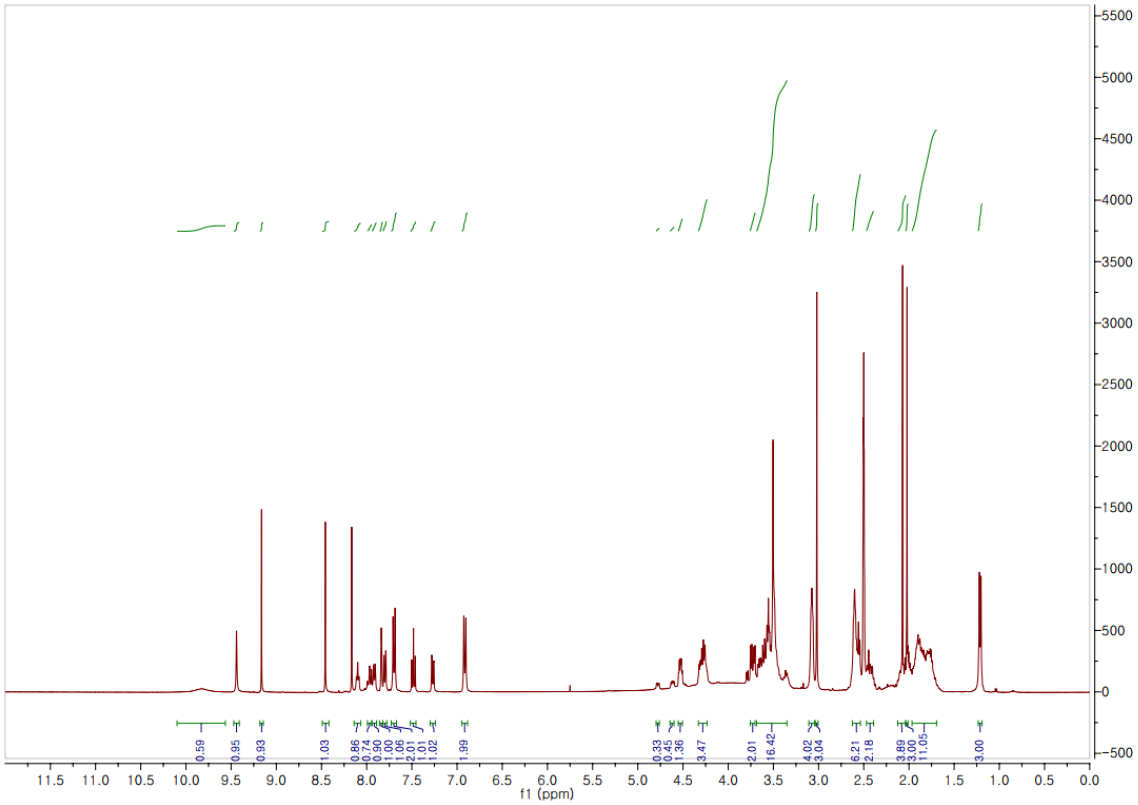
tert-Butyl (*S*)-2-((*S*)-2-((*S*)-2-(((10*S*,13*S*)-10-methyl-3,6,9,12-tetraoxo-2,16-dioxa-5,8,11-triazaheptadecan-13-yl)carbamoyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carboxylate **(24)**.

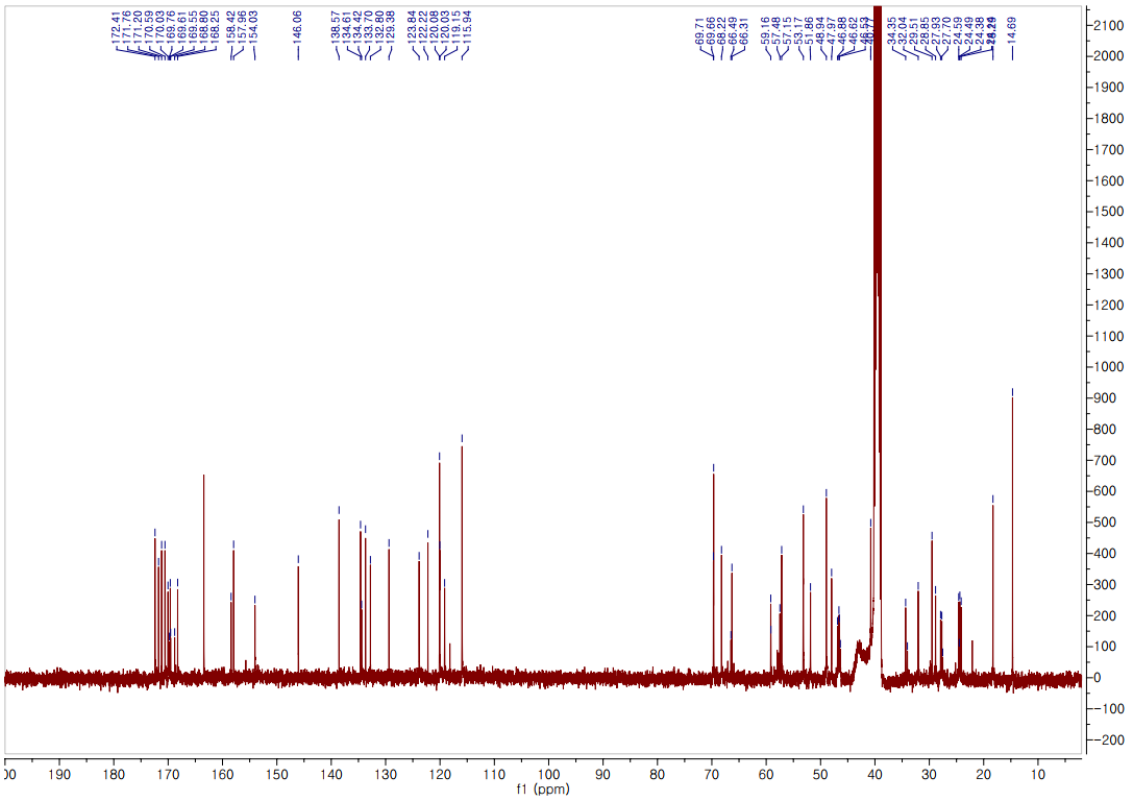


tert-Butyl (*S*)-2-((*S*)-2-((*S*)-2-(((7*S*,10*S*)-7-methyl-3,6,9-trioxo-2-oxa-13-thia-5,8-diazatetradecan-10-yl)carbamoyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carboxylate (**30**).

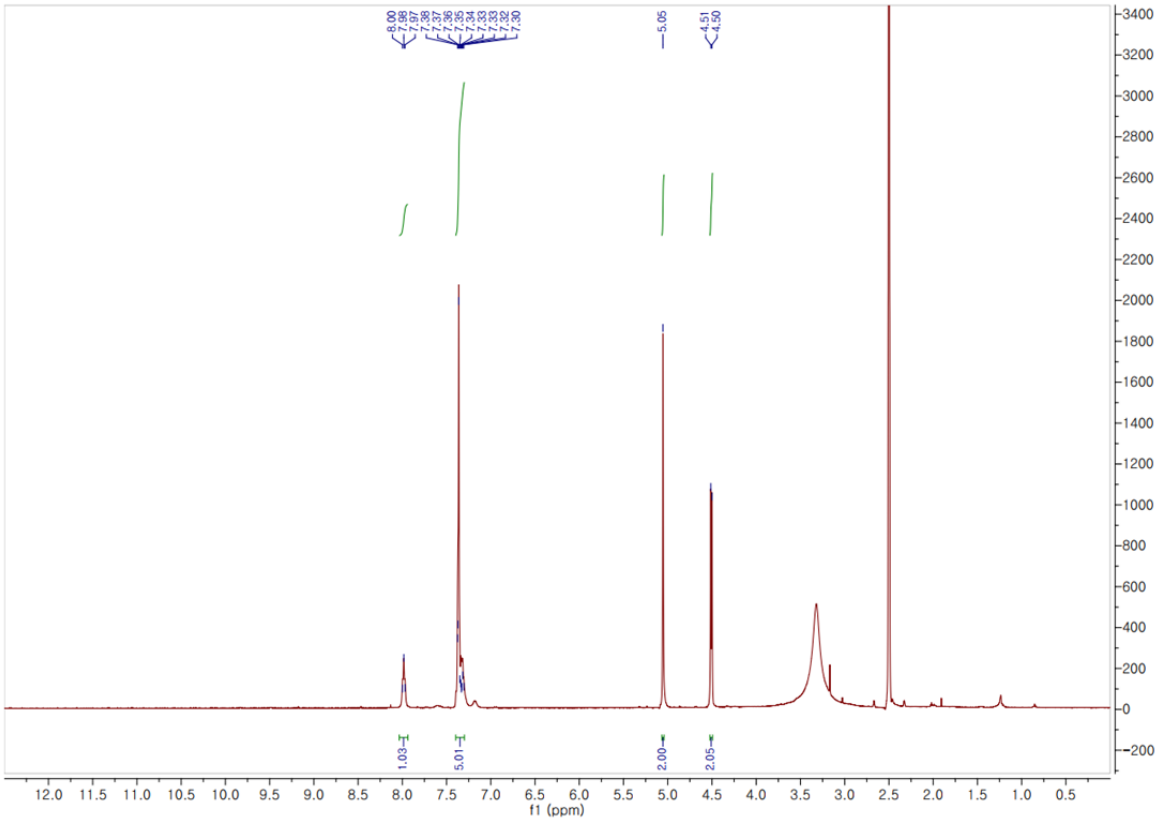


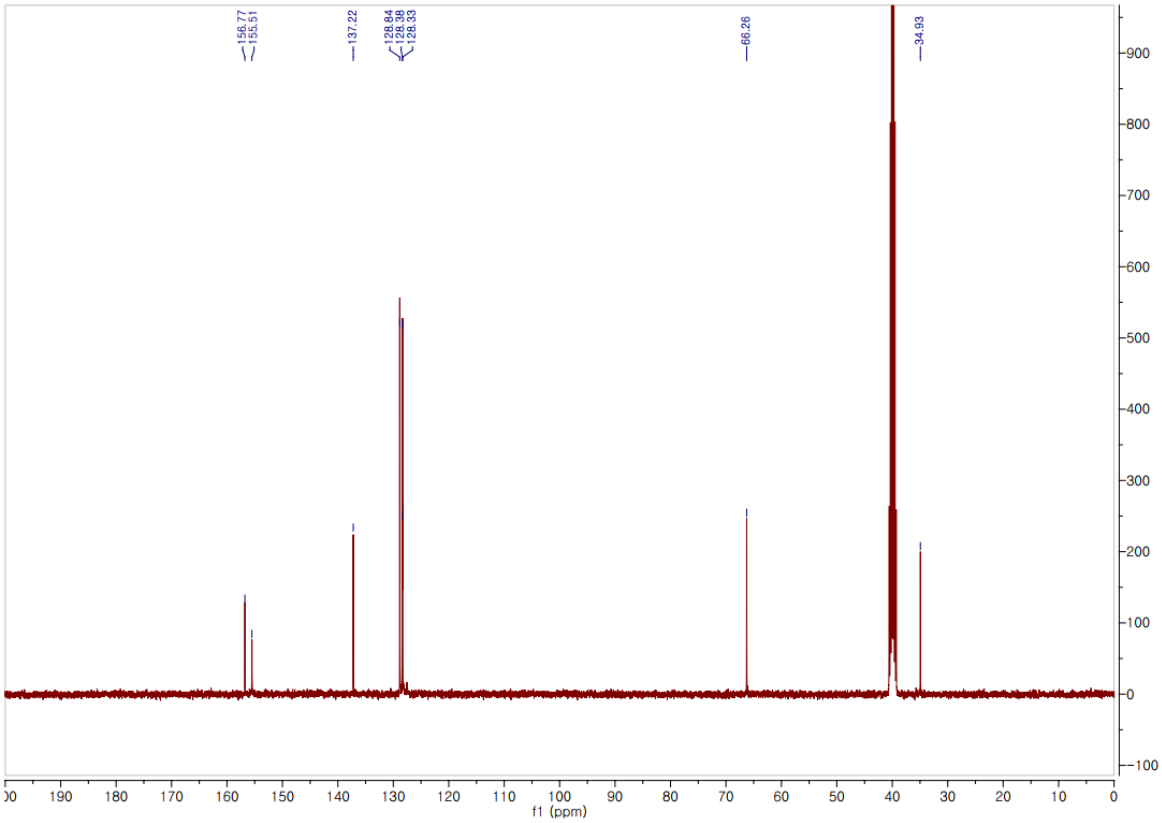
(3-(2-(2-(4-(4-((7-(3-(Methylsulfonamido)phenyl)thieno[3,2-*d*]pyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethoxy)ethoxy)propanoyl)-*L*-prolyl-*L*-prolyl-*L*-prolyl-*L*-methionyl-*L*-alanylglycine (**32**).



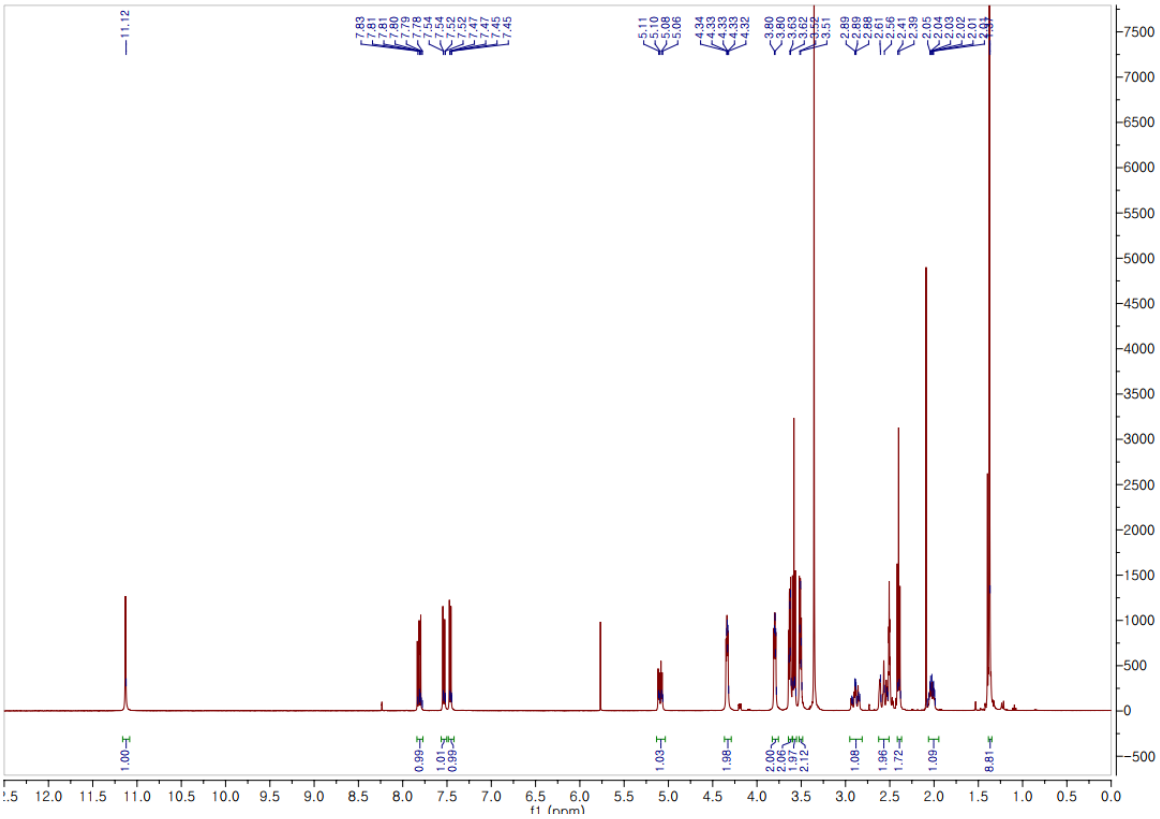


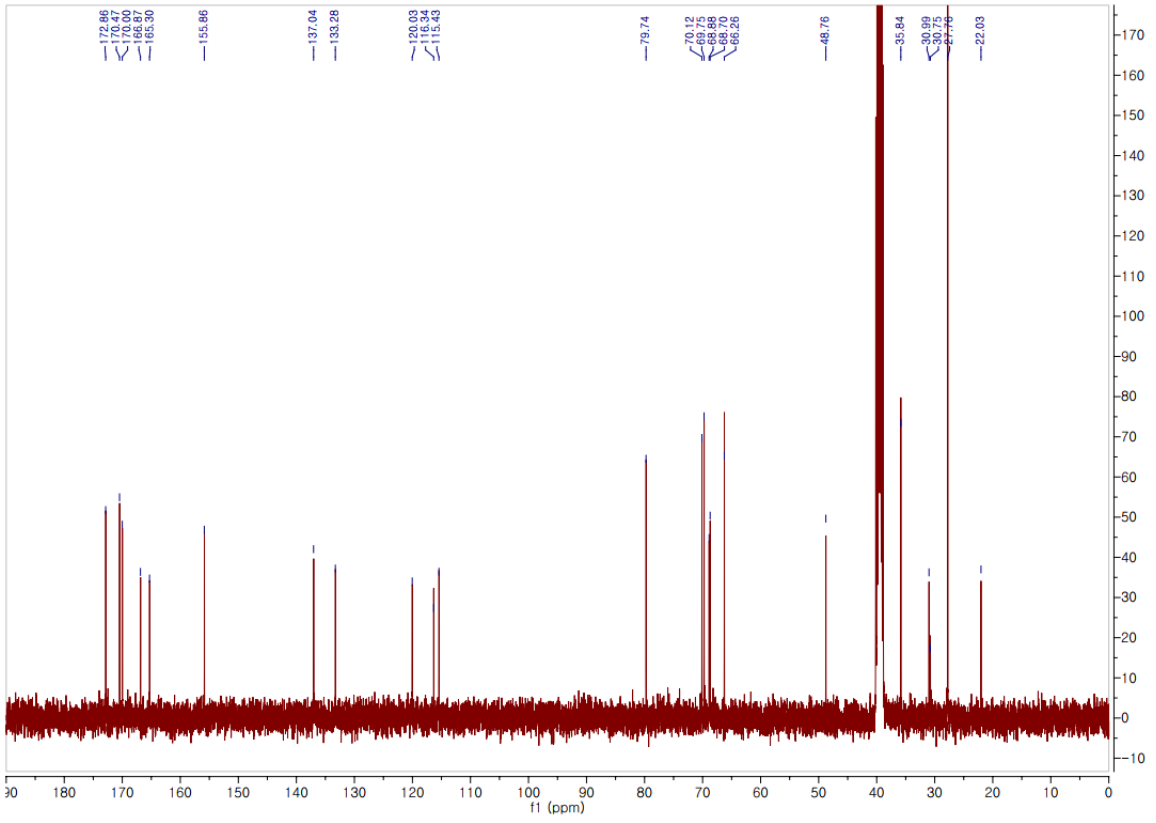
Benzyl ((2H-tetrazol-5-yl)methyl)carbamate (**33**).





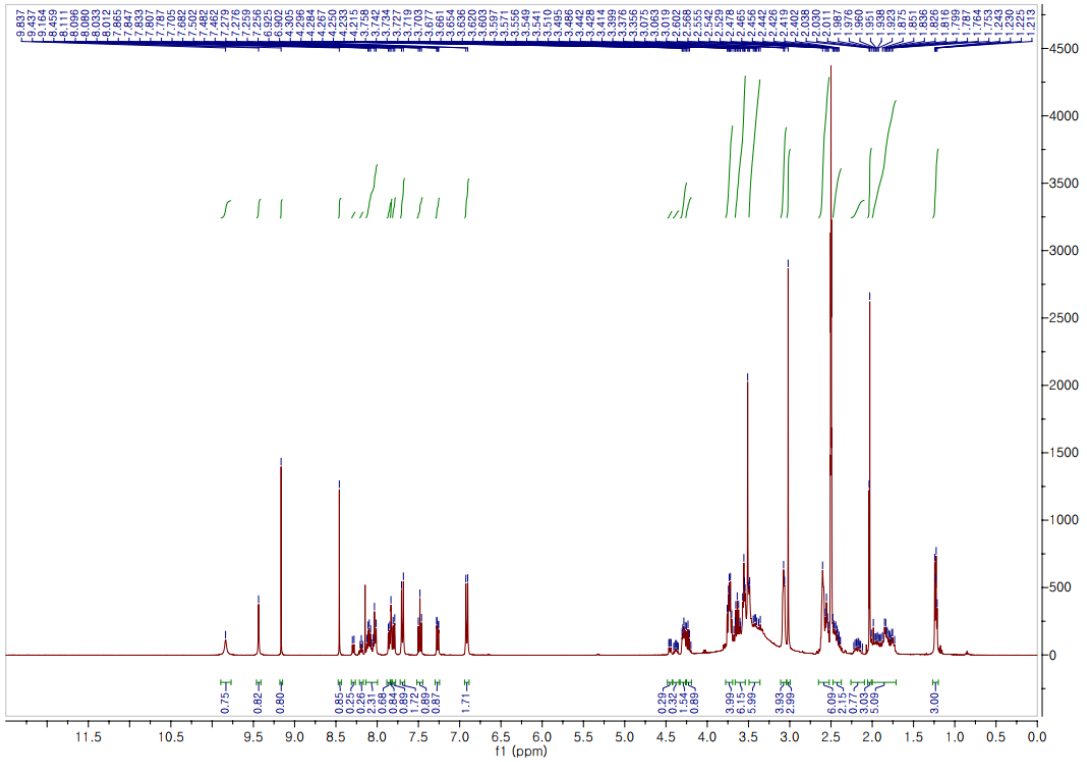
tert-Butyl 3-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)ethoxy)ethoxy)propanoate (**37**).

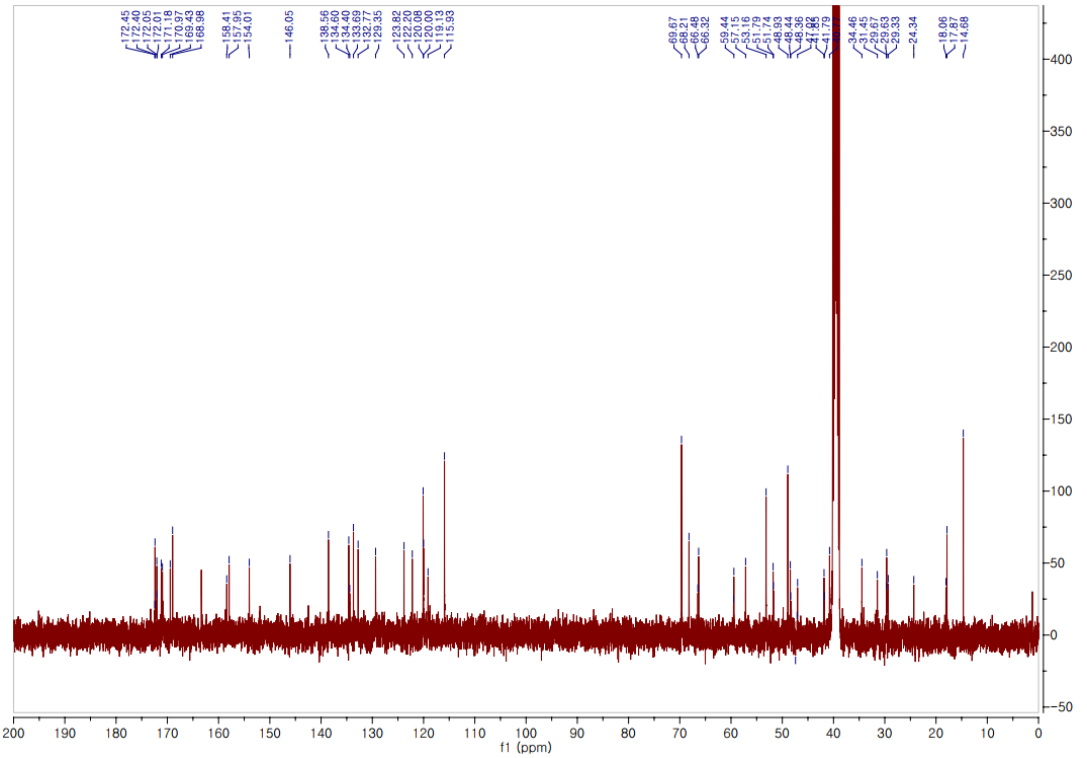


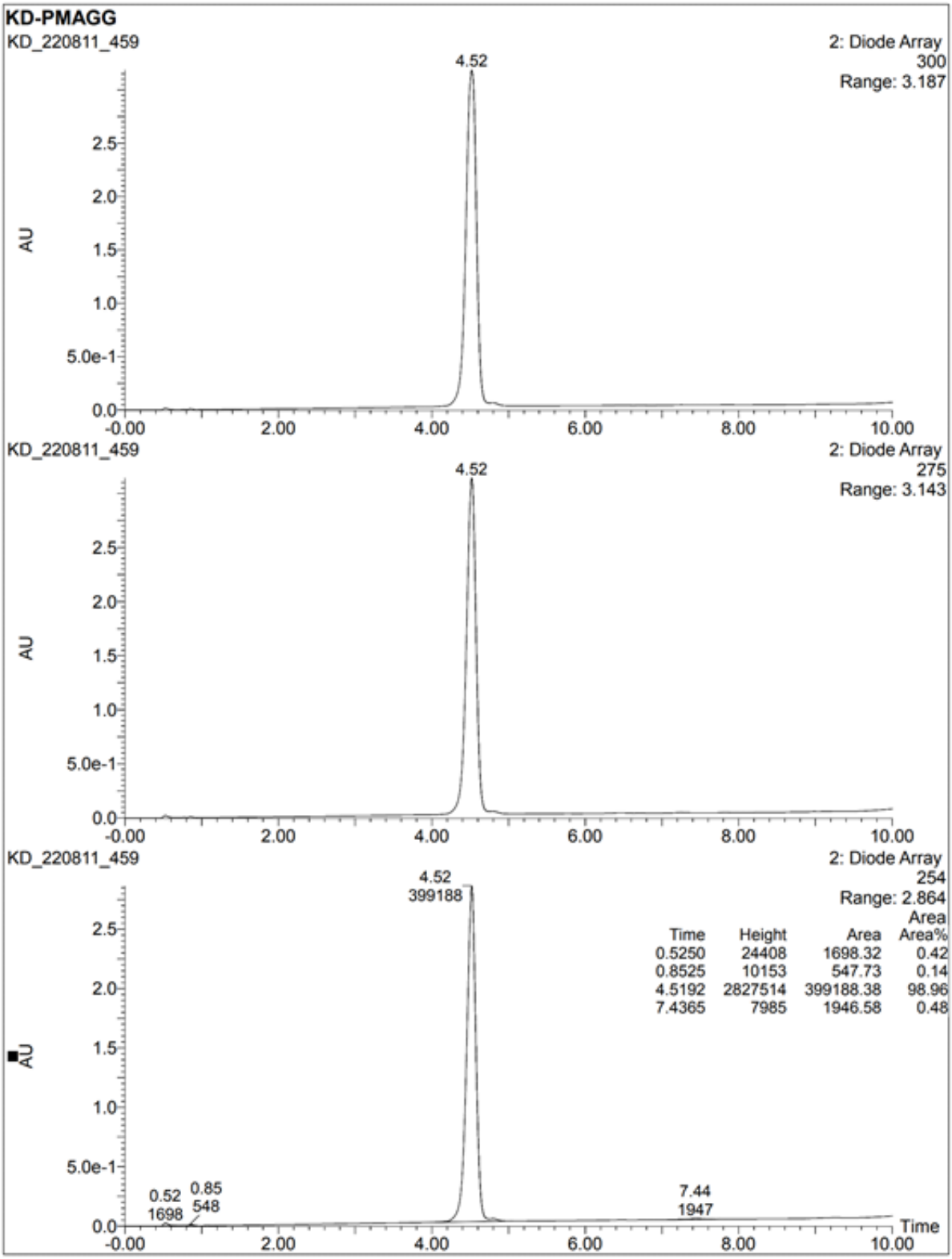


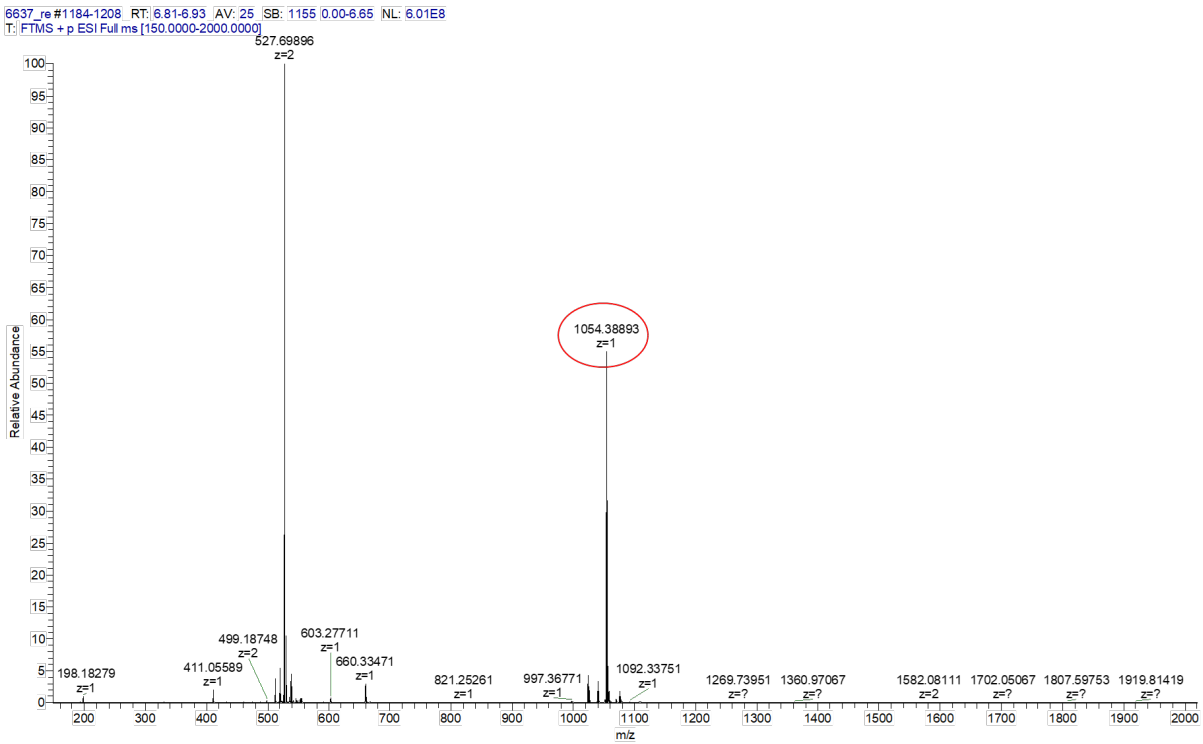
^{1}H, ^{13}C NMR, HPLC traces, and ESI-HRMS spectra of final compounds

(3-(2-(2-(4-(4-((7-(3-(methylsulfonamido)phenyl)thieno[3,2-d]pyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethoxy)ethoxy)propanoyl)-*L*-prolyl-*L*-methionyl-*L*-alanylglycylglycine **(25)**.

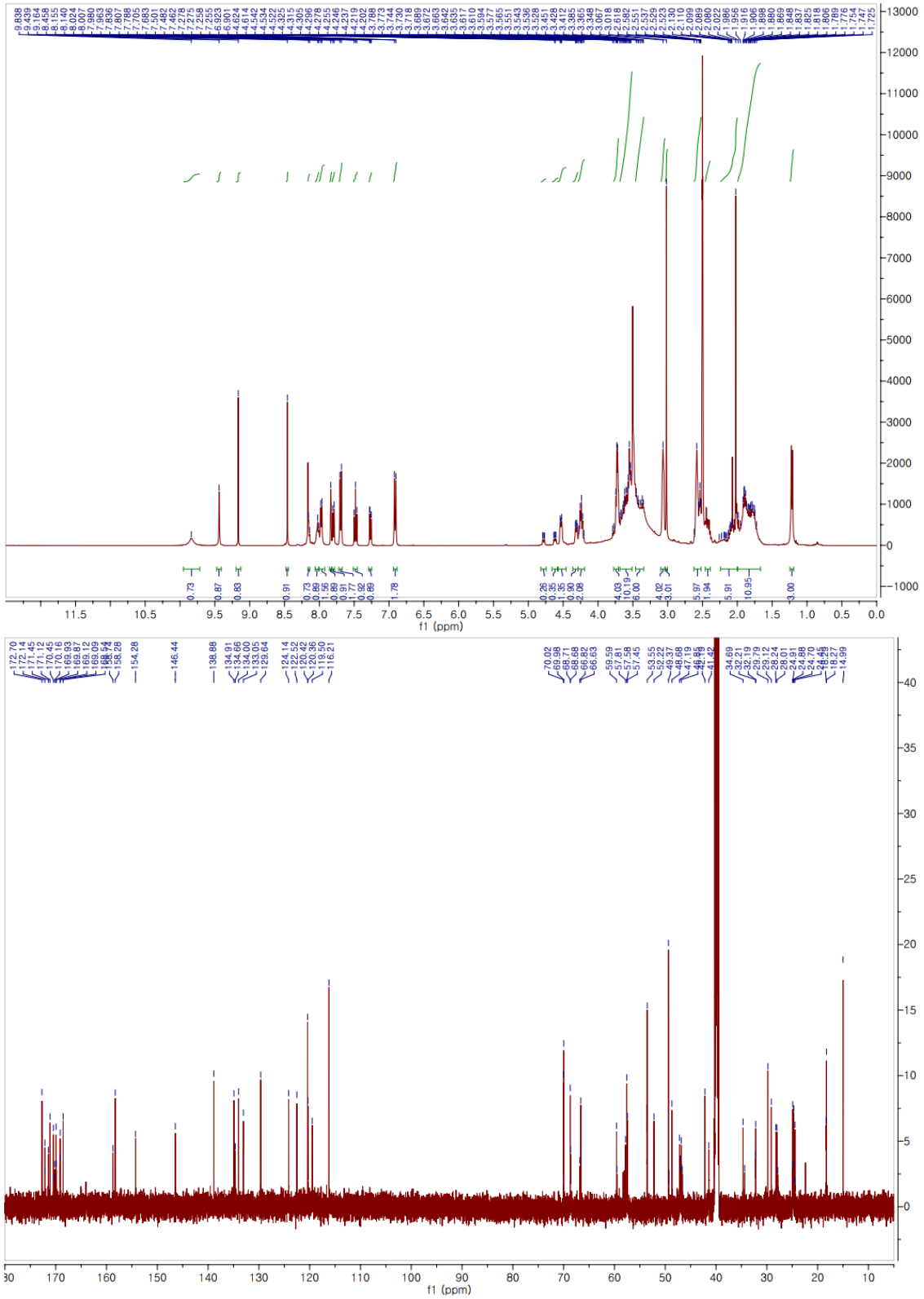


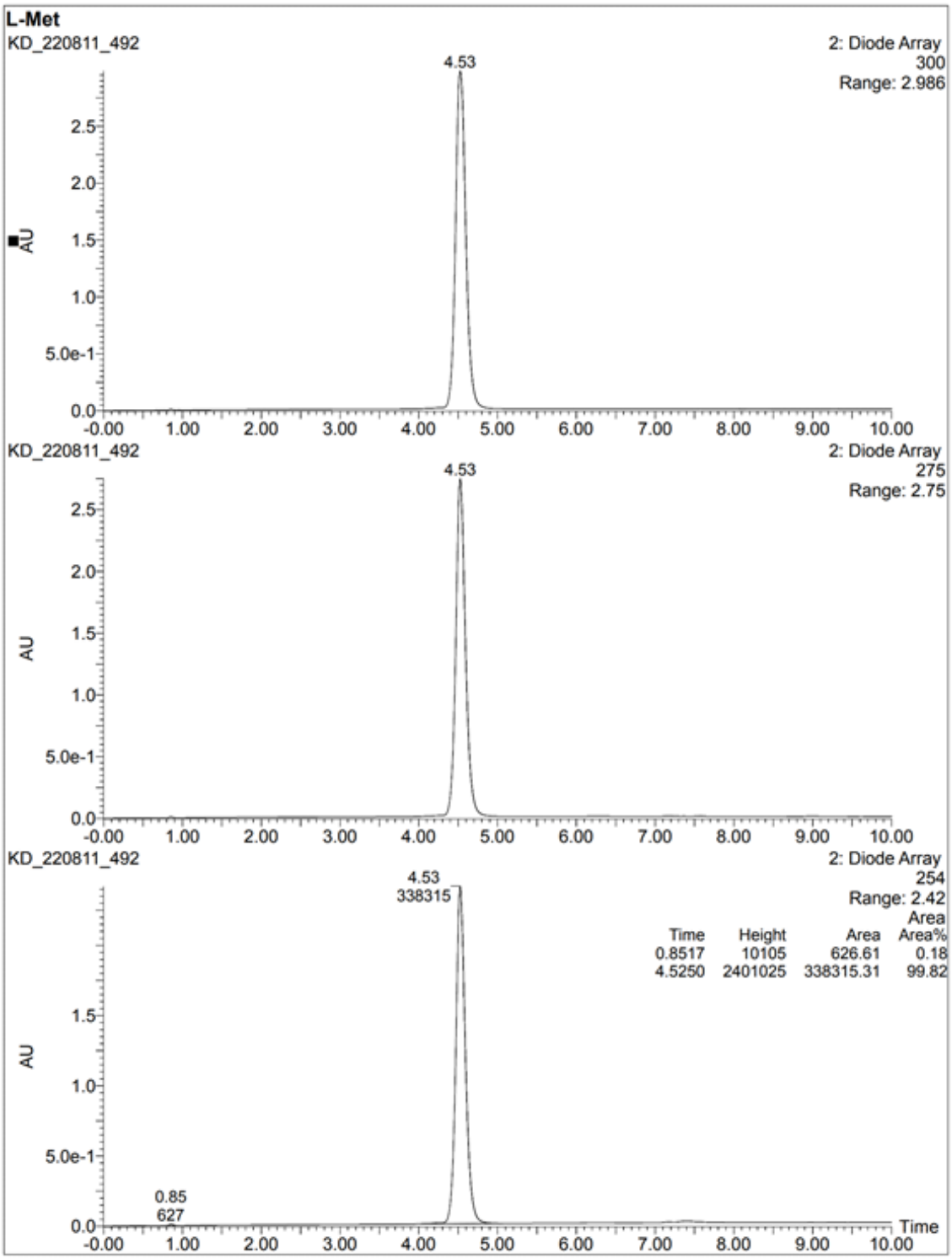


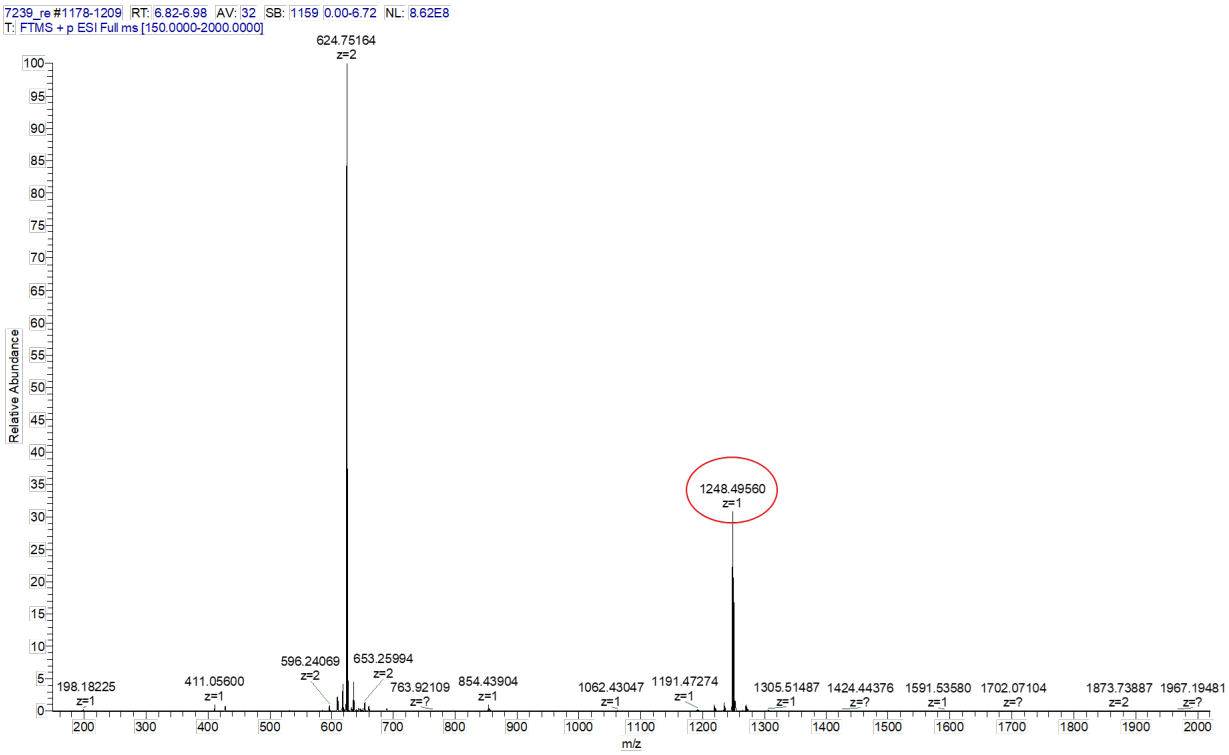

KD-PMAGG
KD_220811_459
2: Diode Array
300
Range: 3.187
4.52
AU
2.5
2.0
1.5
1.0
5.0e-1
0.0
-0.00
2.00
4.00
6.00
8.00
10.00
KD_220811_459
2: Diode Array
275
Range: 3.143
4.52
KD_220811_459
2: Diode Array
254
Range: 2.864
4.52
399188
Time Height Area Area%
0.5250 24408 1698.32 0.42
0.8525 10153 547.73 0.14
4.5192 2827514 399188.38 98.96
7.4365 7985 1946.58 0.48
0.52
1698
0.85
548
7.44
1947
Time


6637_re #1184-1208 RT: 6.81-6.93 AV: 25 SB: 1155 0.00-6.65 NL: 6.01E8
T: FTMS + p ESI Full ms [150.0000-2000.0000]
527.69896
z=2
1054.38893
z=1
499.18748
z=2
603.27711
z=1
411.05589
z=1
660.33471
z=1
198.18279
z=1
821.25261
z=1
997.36771
z=1
1092.33751
z=1
1269.73951
z=?
1360.97067
z=?
1582.08111
z=2
1702.05067
z=?
1807.59753
z=?
1919.81419
z=?
Relative Abundance
m/z

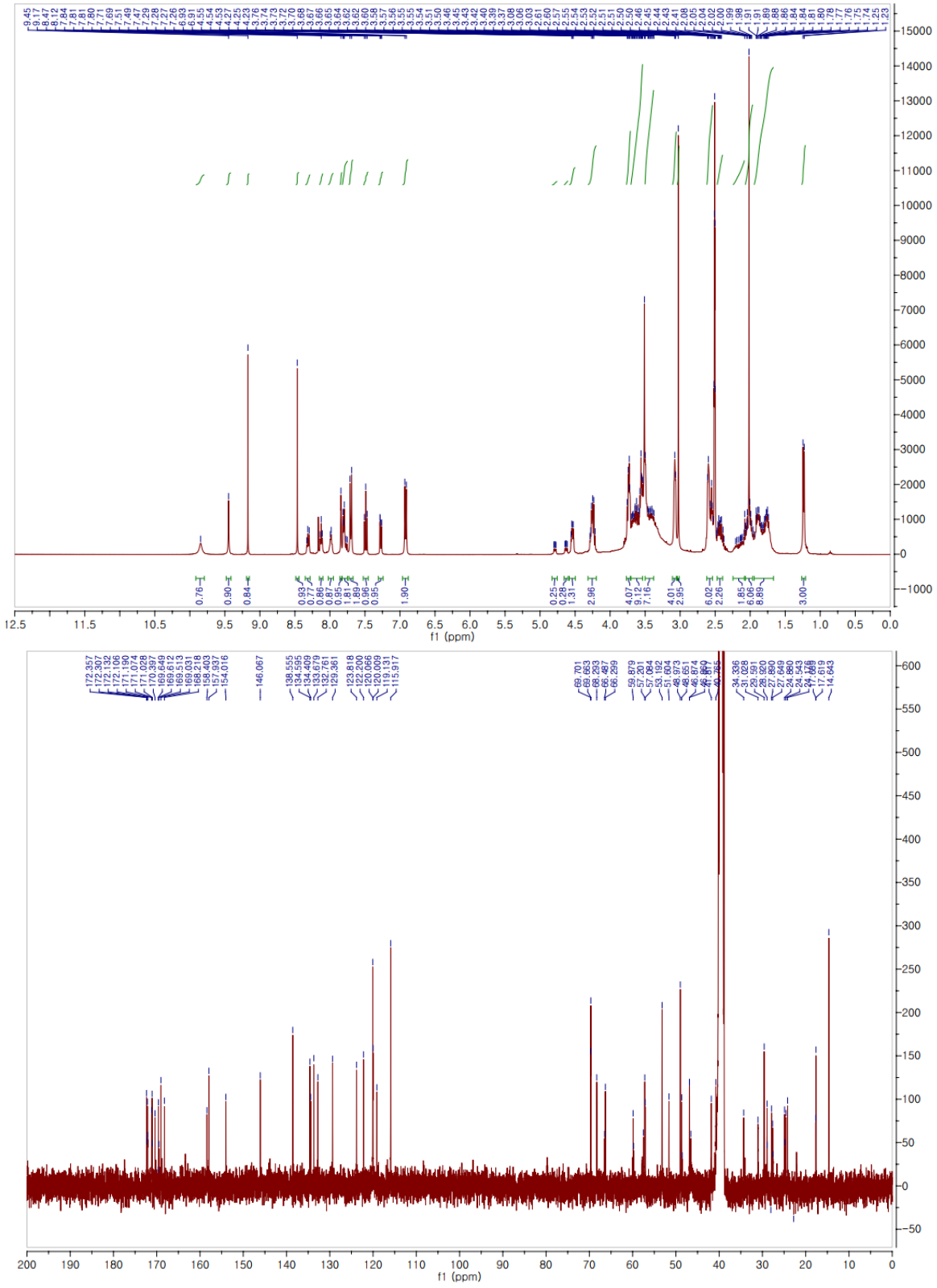
(3-(2-(2-(4-(4-((7-(3-(methylsulfonamido)phenyl)thieno[3,2-d]pyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethoxy)ethoxy)propanoyl)-*L*-prolyl-*L*-prolyl-*L*-prolyl-*L*-methionyl-*L*-alanylglycylglycine **(26)**.

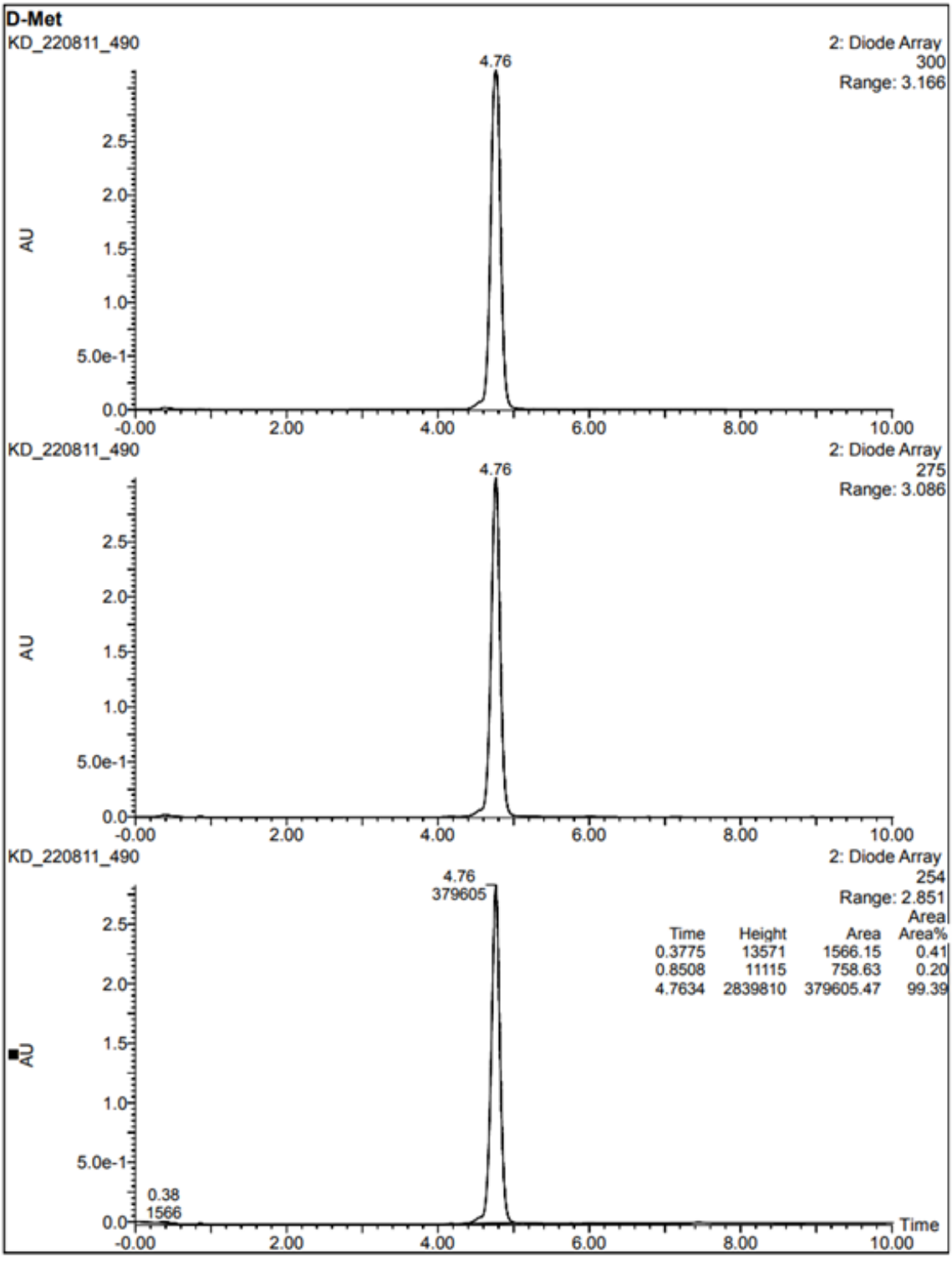


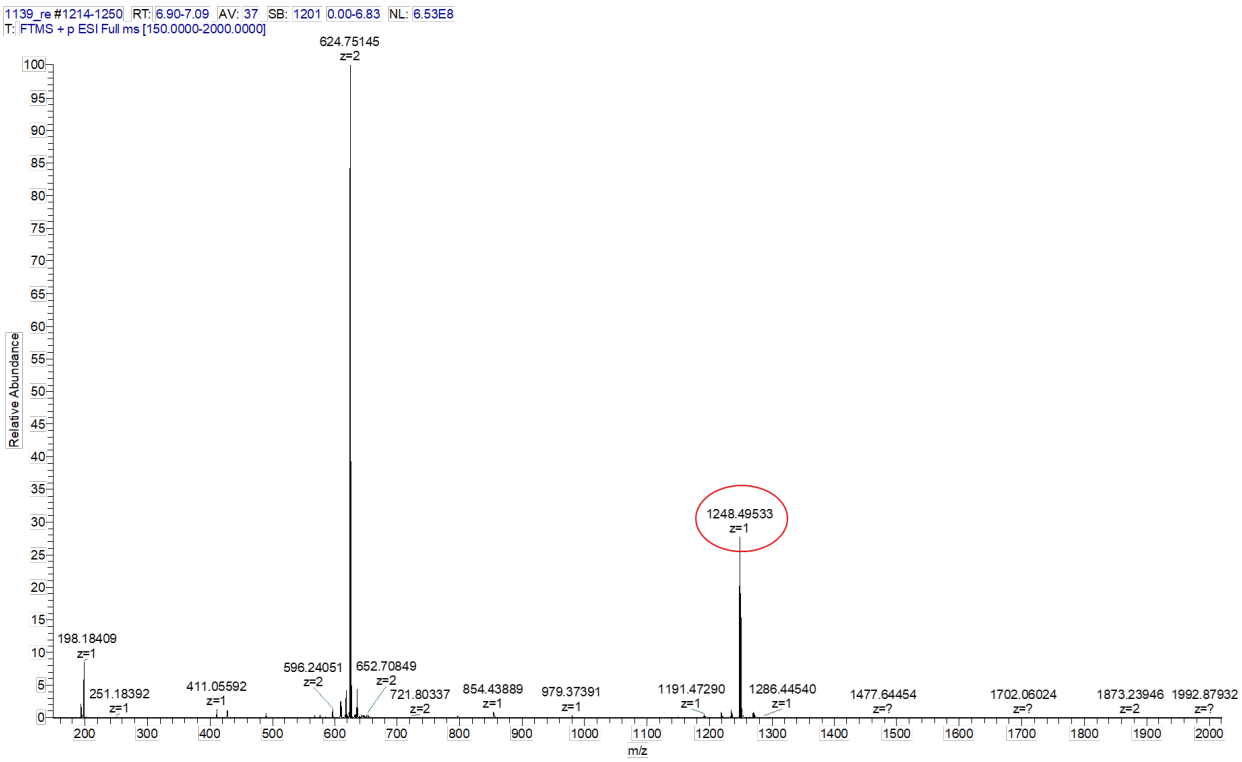

L-Met
KD_220811_492
2: Diode Array
300
Range: 2.986
4.53
AU
KD_220811_492
2: Diode Array
275
Range: 2.75
4.53
AU
KD_220811_492
2: Diode Array
254
Range: 2.42
4.53
338315
Area
Time Height Area Area%
0.8517 10105 626.61 0.18
4.5250 2401025 338315.31 99.82
AU
0.85
627
Time


7239_re #1178-1209 RT: 6.82-6.98 AV: 32 SB: 1159 0.00-6.72 NL: 8.62E8
T: FTMS + p ESI Full ms [150.0000-2000.0000]
624.75164
z=2
1248.49560
z=1
596.24069
z=2
653.25994
z=2
198.18225
z=1
411.05600
z=1
763.92109
z=?
854.43904
z=1
1062.43047
z=1
1191.47274
z=1
1305.51487
z=1
1424.44376
z=?
1591.53580
z=1
1702.07104
z=?
1873.73887
z=2
1967.19481
z=?
Relative Abundance
m/z

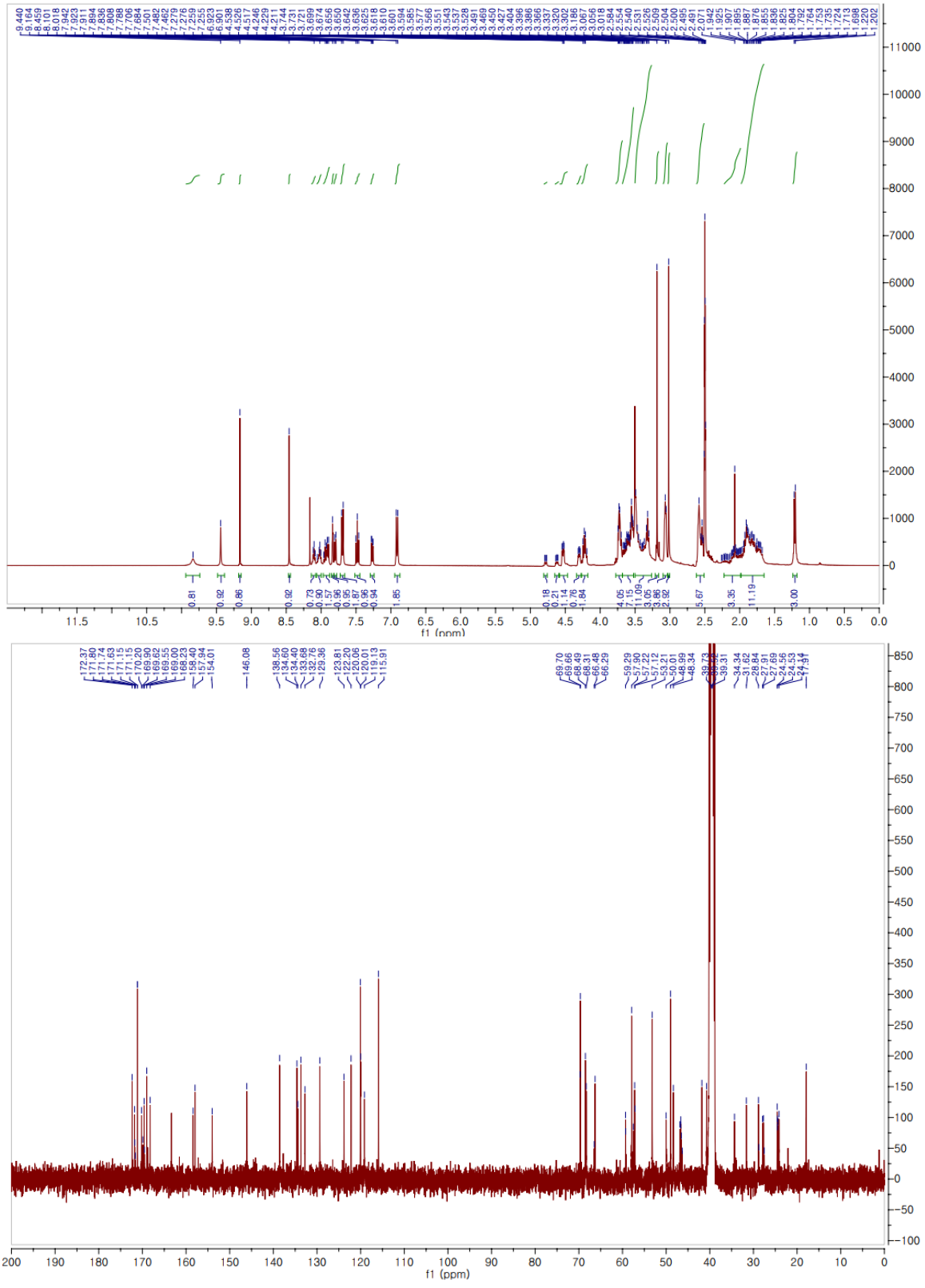
(3-(2-(2-(4-(4-((7-(3-(methylsulfonamido)phenyl)thieno[3,2-d]pyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethoxy)ethoxy)propanoyl)-*L*-prolyl-*L*-prolyl-*L*-prolyl-*D*-methionyl-*L*-alanylglycylglycine **(27)**.

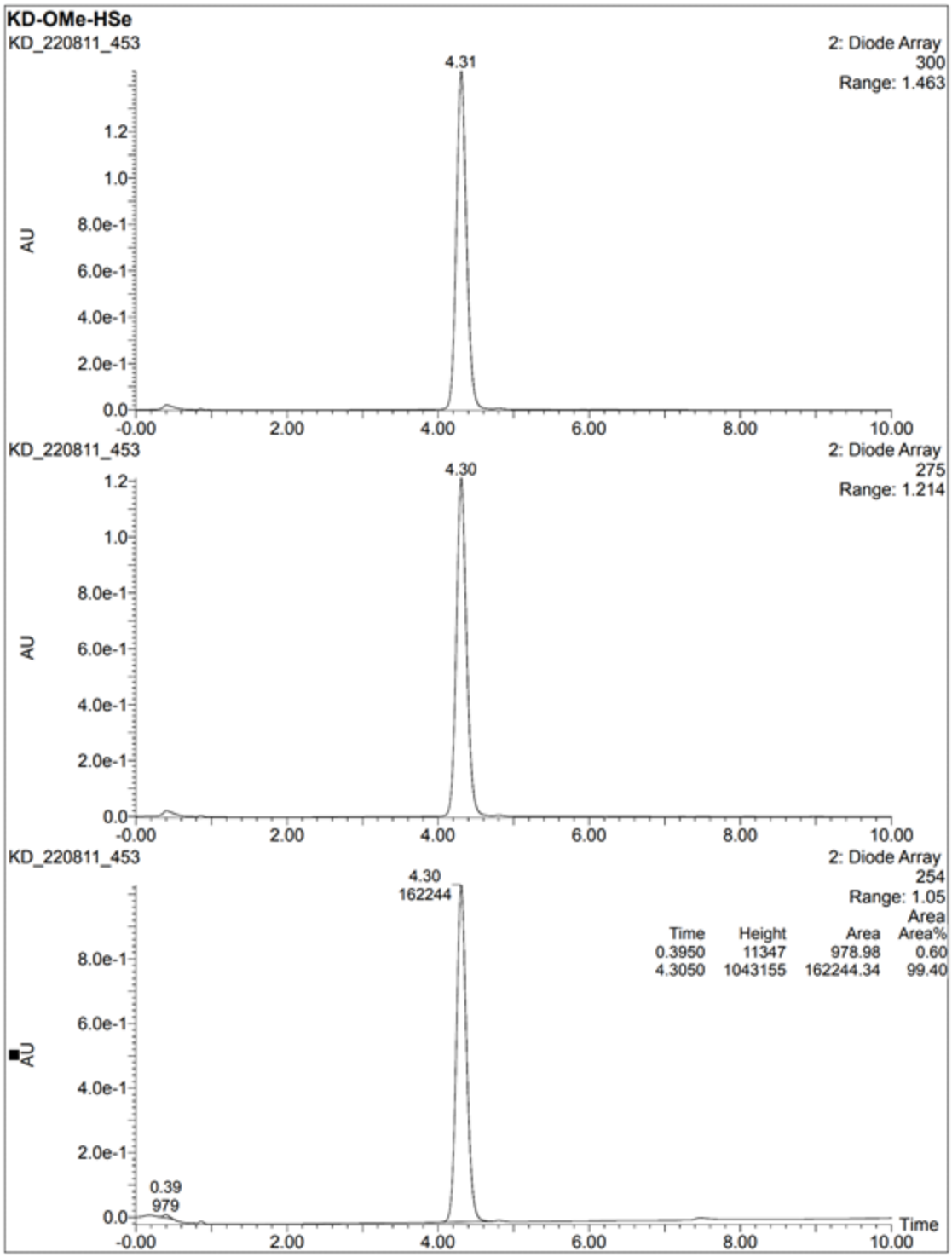


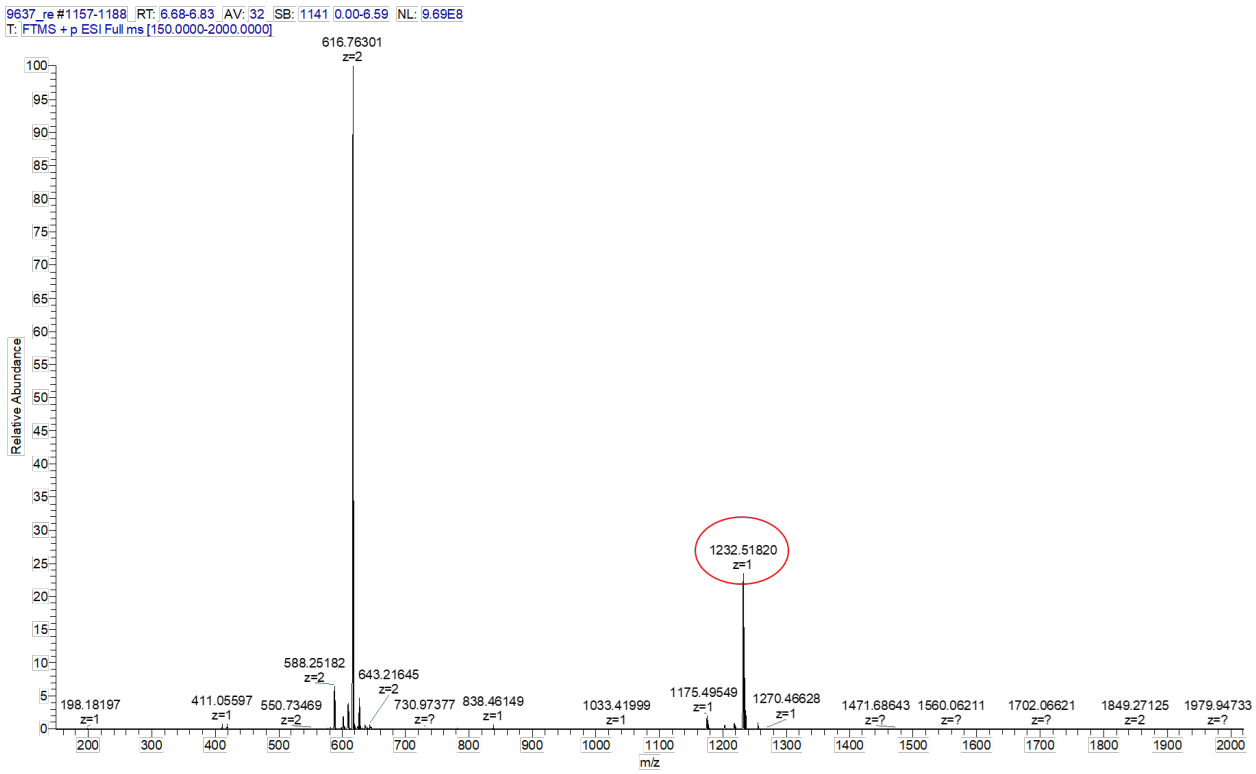

D-Met
KD_220811_490
2: Diode Array
300
Range: 3.166
4.76
AU
2.5
2.0
1.5
1.0
5.0e-1
0.0
-0.00
2.00
4.00
6.00
8.00
10.00
KD_220811_490
2: Diode Array
275
Range: 3.086
4.76
AU
KD_220811_490
2: Diode Array
254
Range: 2.851
4.76
379605
Area
Time Height Area Area%
0.3775 13571 1566.15 0.41
0.8508 11115 758.63 0.20
4.7634 2839810 379605.47 99.39
AU
0.38
1566
Time


1139_re #1214-1250 RT: 6.90-7.09 AV: 37 SB: 1201 0.00-6.83 NL: 6.53E8
T: FTMS + p ESI Full ms [150.0000-2000.0000]
624.75145
z=2
1248.49533
z=1
198.18409
z=1
251.18392
z=1
411.05592
z=1
596.24051
z=2
652.70849
z=2
721.80337
z=2
854.43889
z=1
979.37391
z=1
1191.47290
z=1
1286.44540
z=1
1477.64454
z=?
1702.06024
z=?
1873.23946
z=2
1992.87932
z=?
Relative Abundance
m/z

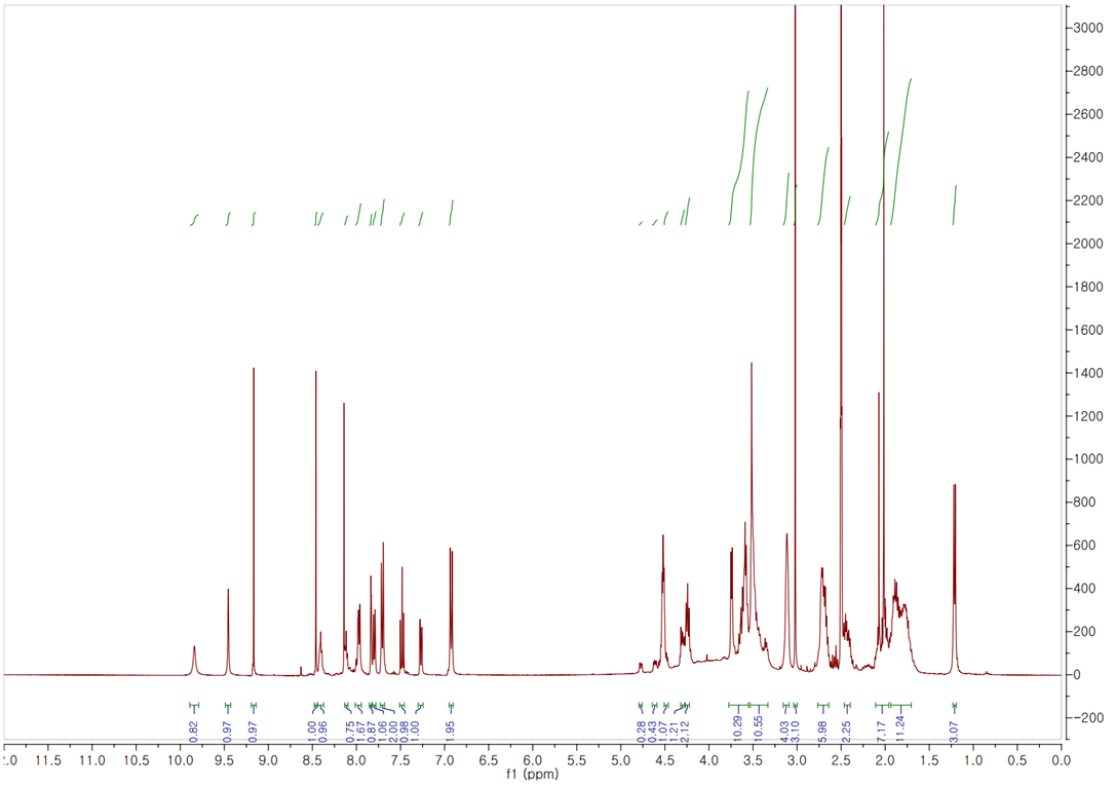
O-methyl-*N*-(3-(2-(2-(4-(4-((7-(3-(methylsulfonamido)phenyl)thieno[3,2-d]pyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethoxy)ethoxy)propanoyl)-*L*-prolyl-*L*-prolyl-*L*-prolyl-*L*-homoseryl-*L*-alanylglycylglycine **(28)**.

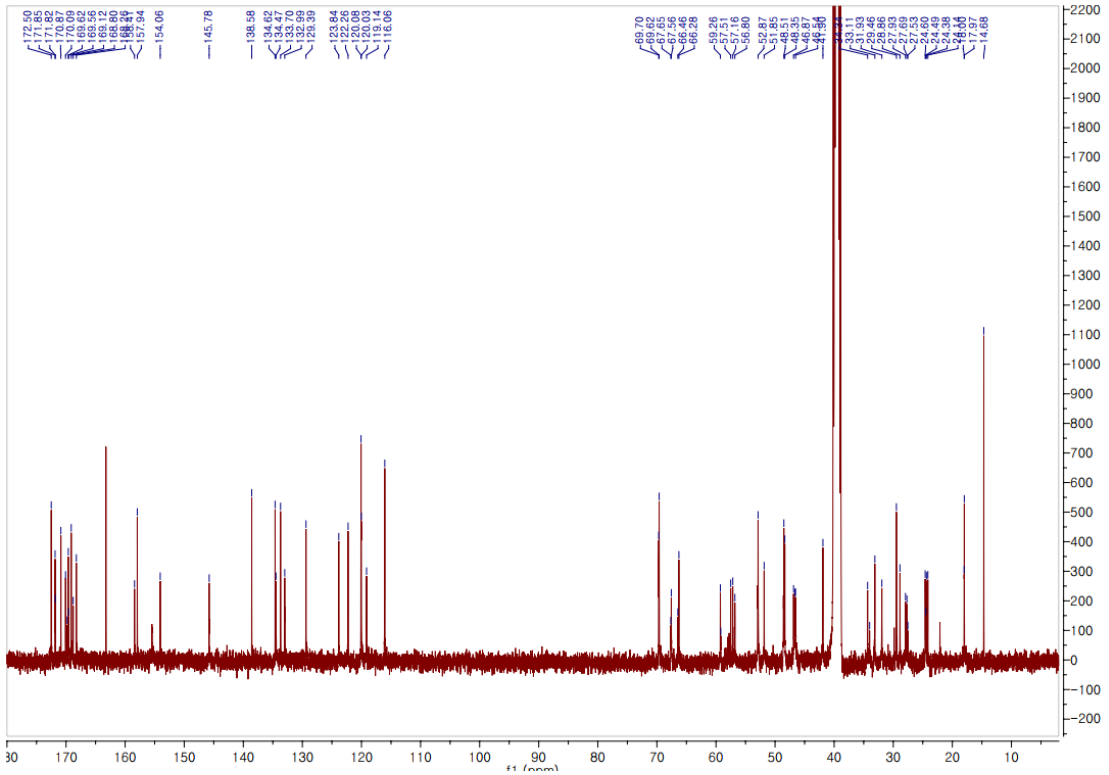


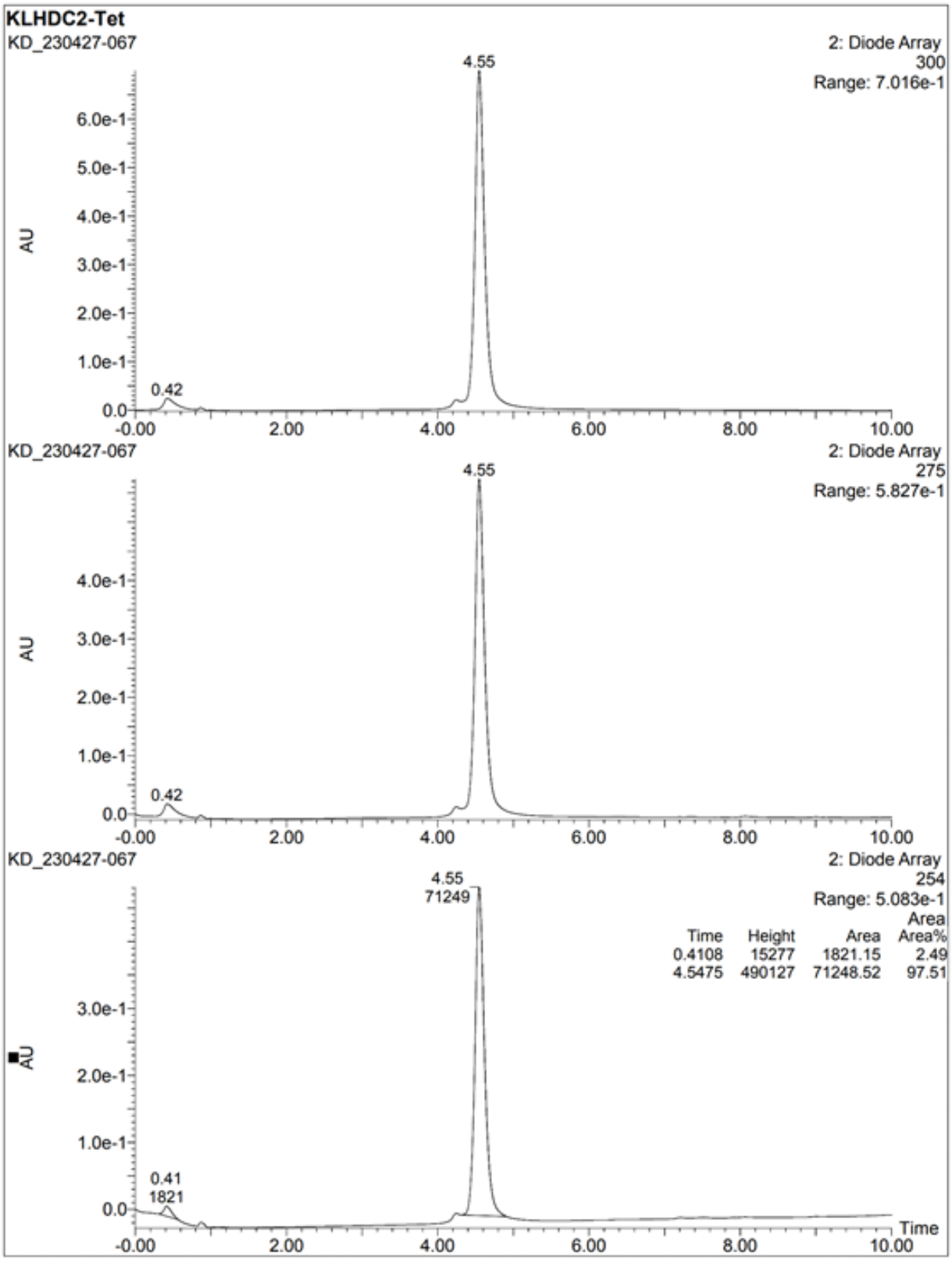

KD-OMe-HSe
KD_220811_453
2: Diode Array
300
Range: 1.463
4.31
AU
1.2
1.0
8.0e-1
6.0e-1
4.0e-1
2.0e-1
0.0
-0.00
2.00
4.00
6.00
8.00
10.00
KD_220811_453
2: Diode Array
275
Range: 1.214
4.30
AU
KD_220811_453
2: Diode Array
254
Range: 1.05
4.30
162244
Area
Time Height Area Area%
0.3950 11347 978.98 0.60
4.3050 1043155 162244.34 99.40
AU
0.39
979
Time

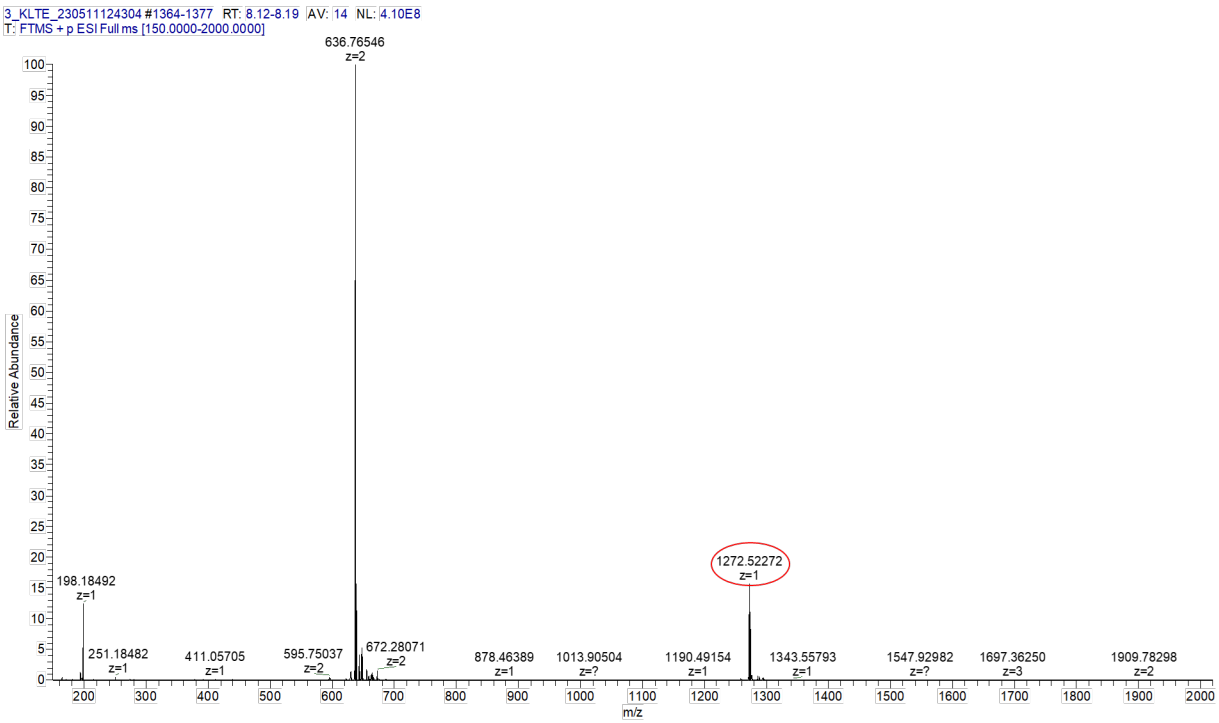

9637_re #1157-1188 RT: 6.68-6.83 AV: 32 SB: 1141 0.00-6.59 NL: 9.69E8
T: FTMS + p ESI Full ms [150.0000-2000.0000]
Relative Abundance
m/z
616.76301
z=2
1232.51820
z=1
588.25182
z=2
643.21645
z=2
198.18197
z=1
411.05597
z=1
550.73469
z=2
730.97377
z=?
838.46149
z=1
1033.41999
z=1
1175.49549
z=1
1270.46628
z=1
1471.68643
z=?
1560.06211
z=?
1702.06621
z=?
1849.27125
z=2
1979.94733
z=?

(*S*)-*N*-((*S*)-1-(((*S*)-1-((2-(((2*H*-tetrazol-5-yl)methyl)amino)-2-oxoethyl)amino)-1-oxopropan-2-yl)amino)-4-(methylthio)-1-oxobutan-2-yl)-1-((3-(2-(2-(4-(4-((7-(3-(methylsulfonamido)phenyl)thieno[3,2-*d*]pyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethoxy)ethoxy)propanoyl)-*L*-prolyl-*L*-prolyl)pyrrolidine-2-carboxamide (**34**).

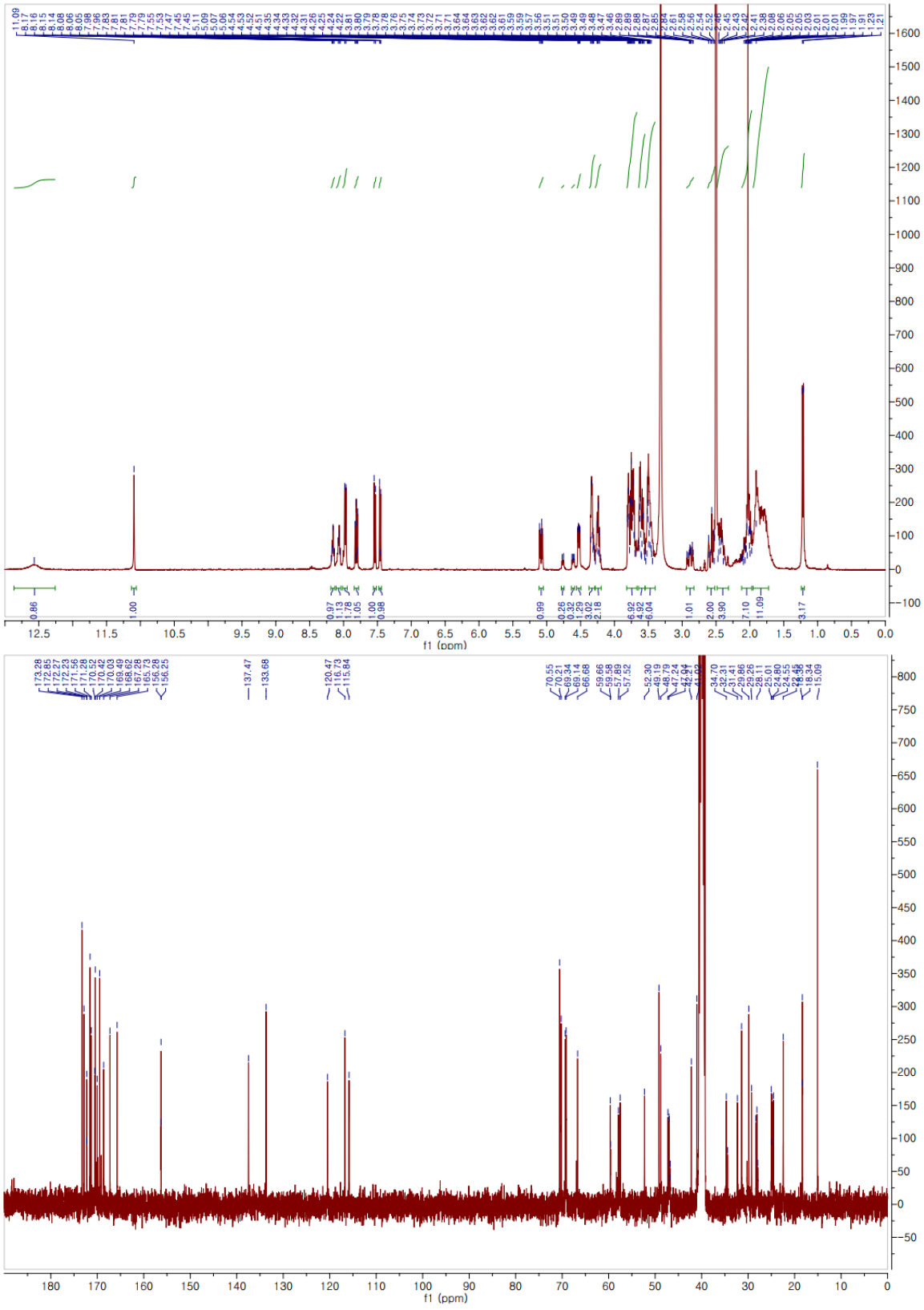


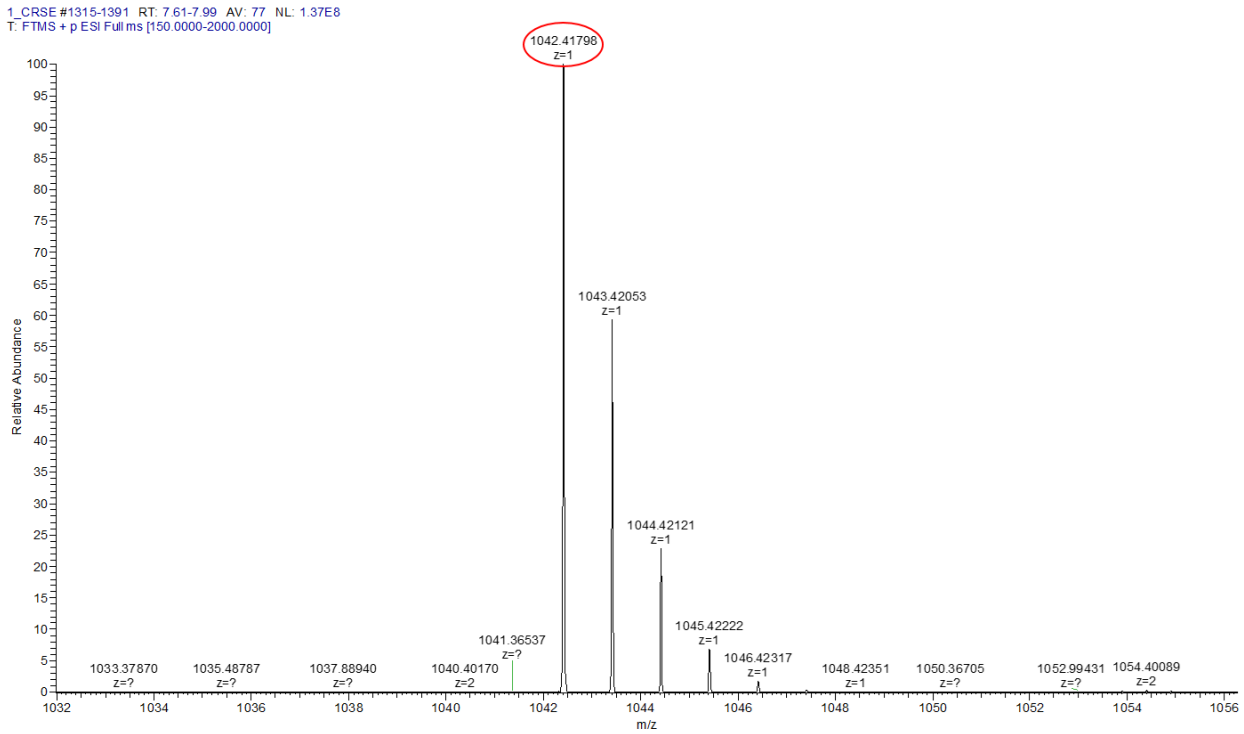


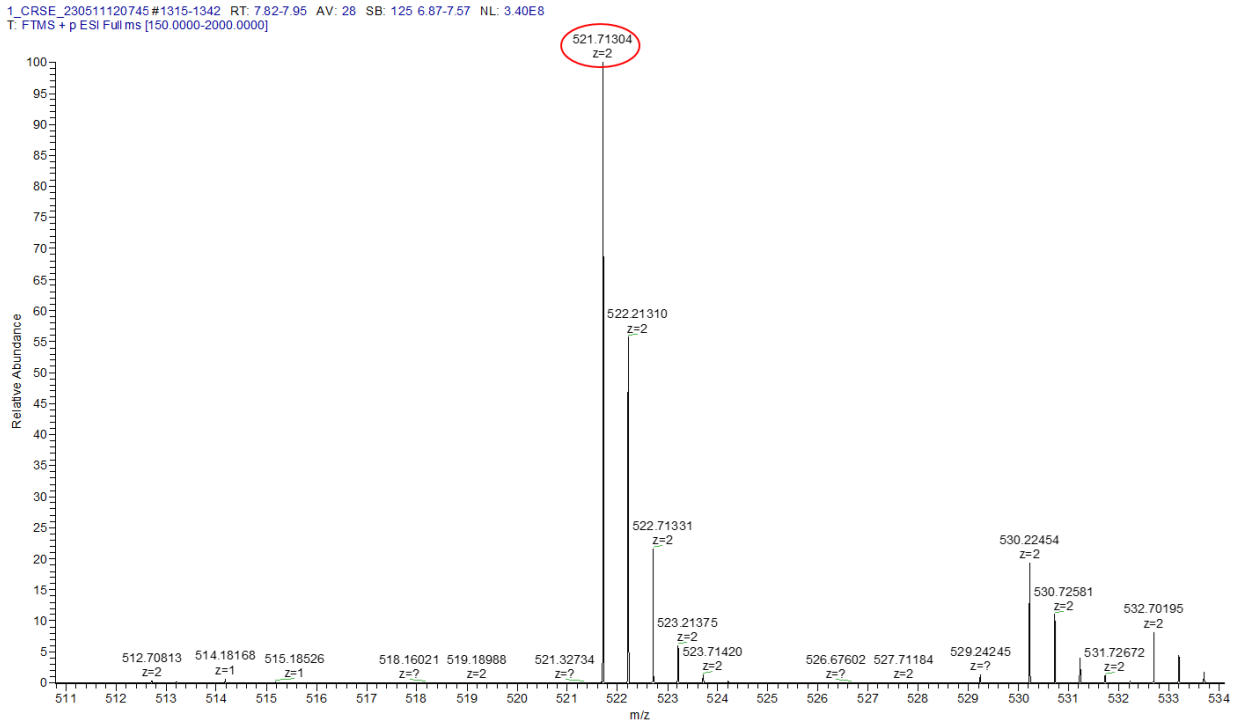

KLHDC2-Tet
KD_230427-067
2: Diode Array
300
Range: 7.016e-1
4.55
0.42
AU
6.0e-1
5.0e-1
4.0e-1
3.0e-1
2.0e-1
1.0e-1
0.0
-0.00
2.00
4.00
6.00
8.00
10.00
KD_230427-067
2: Diode Array
275
Range: 5.827e-1
4.55
0.42
AU
4.0e-1
3.0e-1
2.0e-1
1.0e-1
0.0
-0.00
2.00
4.00
6.00
8.00
10.00
KD_230427-067
2: Diode Array
254
Range: 5.083e-1
4.55
71249
Time Height Area Area%
0.4108 15277 1821.15 2.49
4.5475 490127 71248.52 97.51
0.41
1821
AU
3.0e-1
2.0e-1
1.0e-1
0.0
-0.00
2.00
4.00
6.00
8.00
10.00
Time


3_KLTE_230511124304 #1364-1377 RT: 8.12-8.19 AV: 14 NL: 4.10E8
T: FTMS + p ESI Full ms [150.0000-2000.0000]
636.76546
z=2
1272.52272
z=1
198.18492
z=1
251.18482
z=1
411.05705
z=1
595.75037
z=2
672.28071
z=2
878.46389
z=1
1013.90504
z=?
1190.49154
z=1
1343.55793
z=1
1547.92982
z=?
1697.36250
z=3
1909.78298
z=2
Relative Abundance
m/z

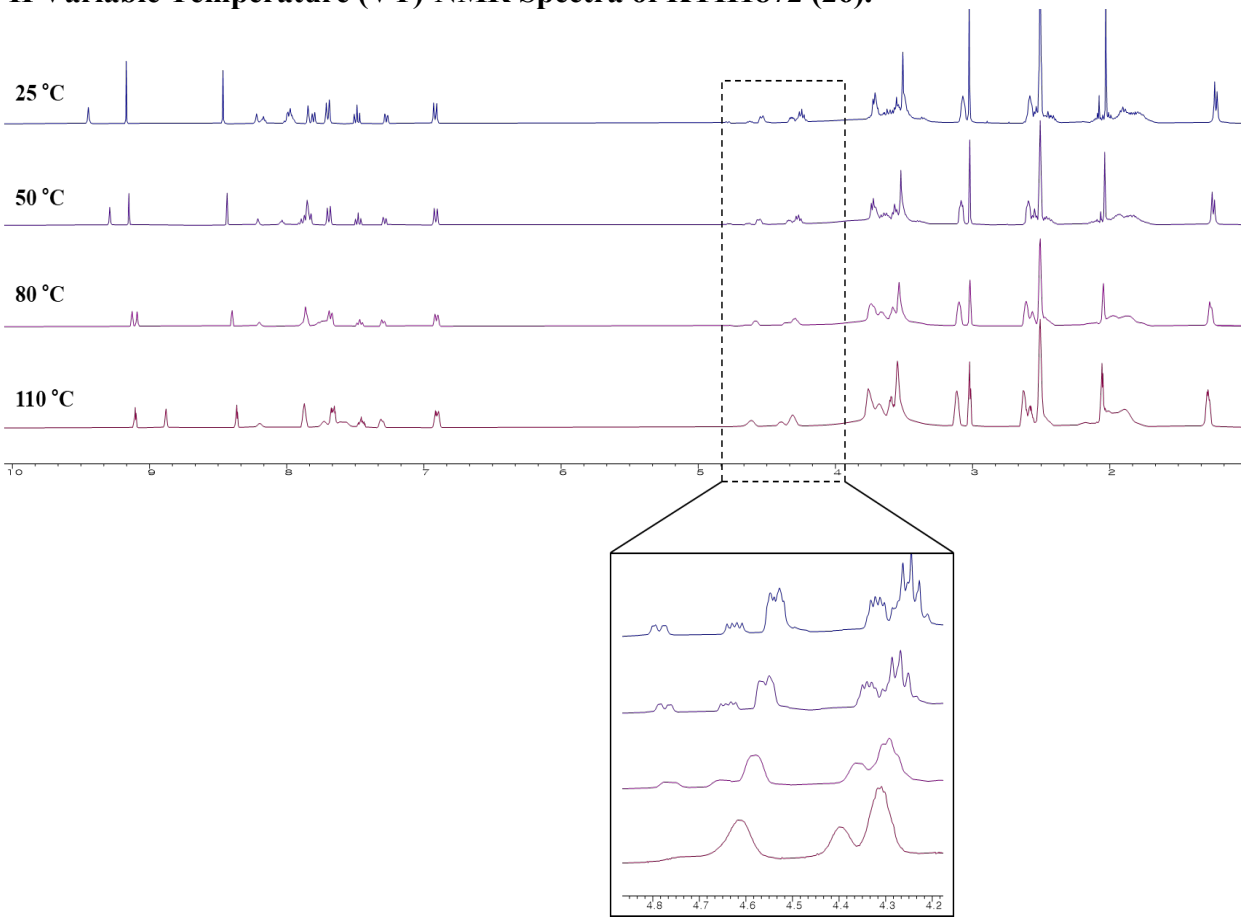
(3-(2-(2-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)ethoxy)ethoxy)propanoyl)-*L*-prolyl-*L*-prolyl-*L*-prolyl-*L*-methionyl-*L*-alanylglycylglycine (**39**).




1_CRSE #1315-1391 RT: 7.61-7.99 AV: 77 NL: 1.37E8
T: FTMS + p ESI Full ms [150.0000-2000.0000]
1042.41798 z=1
1043.42053 z=1
1044.42121 z=1
1045.42222 z=1
1041.36537 z=?
1046.42317 z=1
1033.37870 z=?
1035.48787 z=?
1037.88940 z=?
1040.40170 z=2
1048.42351 z=1
1050.36705 z=?
1052.99431 z=?
1054.40089 z=2
Relative Abundance
m/z


1_CRSE_230511120745 #1315-1342 RT: 7.82-7.95 AV: 28 SB: 125 6.87-7.57 NL: 3.40E8
T: FTMS + p ESI Full ms [150.0000-2000.0000]
521.71304 z=2
522.21310 z=2
522.71331 z=2
523.21375 z=2
523.71420 z=2
530.22454 z=2
530.72581 z=2
532.70195 z=2
531.72672 z=2
512.70813 z=2
514.18168 z=1
515.18526 z=1
518.16021 z=?
519.18988 z=2
521.32734 z=?
526.67602 z=?
527.71184 z=2
529.24245 z=?
Relative Abundance
m/z

^{1}H Variable Temperature (VT)-NMR Spectra of KYH1872 (26).



The coalescence of signals was observed at temperatures > 80 °C indicating the presence of rotamers, not diastereomers.

Supplemental References

1. Cho, H., Shin, I., Yoon, H., Jeon, E., Lee, J., Kim, Y., Ryu, S., Song, C., Kwon, N.H., and Moon, Y. (2021). Identification of Thieno [3, 2-d] pyrimidine Derivatives as Dual Inhibitors of Focal Adhesion Kinase and FMS-like Tyrosine Kinase 3. Journal of Medicinal Chemistry *64*, 11934-11957.