Supporting Information for

Original article

NAMPT-targeting PROTAC promotes antitumor immunity *via* suppressing myeloid-derived suppressor cell expansion

Ying Wu[a,†], Congying Pu[b,†], Yixian Fu[b], Guoqiang Dong[a,*], Min Huang[b,*], Chunquan Sheng[a,*]

[a]*School of Pharmacy, Second Military Medical University, Shanghai 200433, China*

[b]*State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, University of Chinese Academy of Sciences, Shanghai 201203, China*

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[†]These authors made equal contributions to this work.

*Corresponding authors: dgq-81@163.com (Guoqiang Dong); mhuang@simm.ac.cn (Min Huang); shengcq@smmu.edu.cn (Chunquan Sheng).

Supporting figures (Figure S1–S5)

Supporting schemes (Scheme S1–S4)

Supporting methods

Supporting structure identification spectra

Supporting references

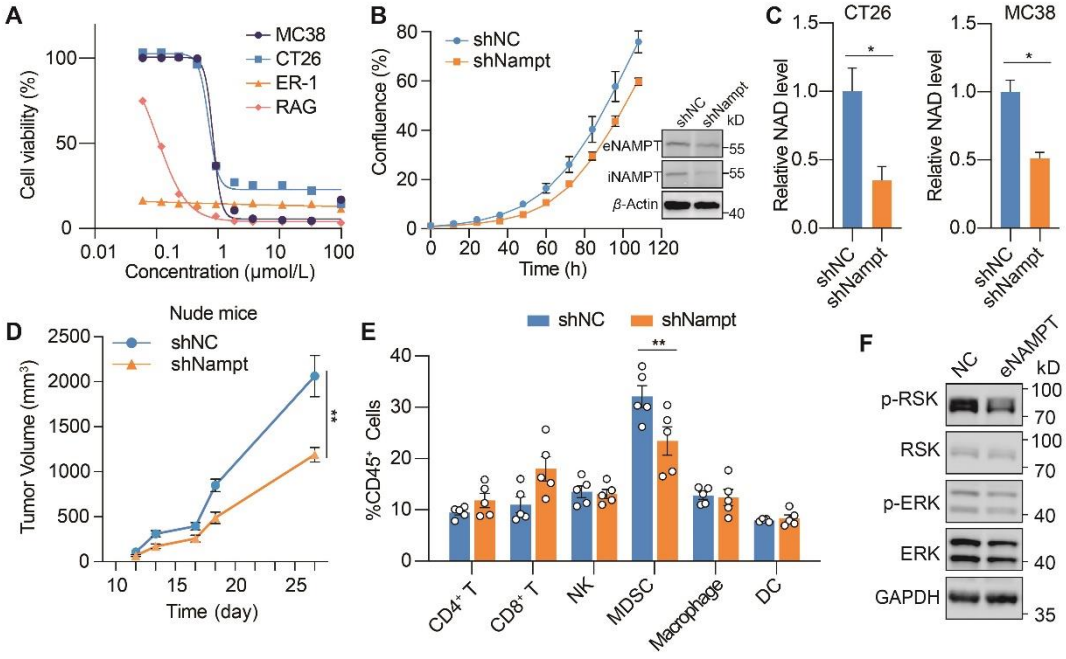


Figure S1 NAMPT facilitates the expansion of MDSCs independent of its enzymatic activity. (A) FK866 sensitivity. Cells were treated with FK866 at gradient concentrations for 72 h and cell viability was examined by (sulforhodamine B) SRB assay. (B) Growth of MC38 cells with stable knockdown of NAMPT. Left, MC38 scramble control (shNC) or shNAMPT cell confluency assessed by incuCyte proliferation assay; Right, immunoblot analysis of NAMPT knockdown efficiency. (C) Intracellular NAD^+ level change in CT26 and MC38 cells with stable knockdown of NAMPT. (D) Tumor growth curve in nude mice. CT26 scramble control or shNAMPT cells were inoculated subcutaneously in BALB/c nude mice (n = 10). (E) MC38 scramble control or shNAMPT cells were inoculated subcutaneously in C57BL/6 mice (n = 5). Tumor infiltrating immune cells were analyzed by flow cytometry. The proportion of the indicated immune cells in tumor infiltrating $CD45^+$ cells. (F) MDSCs from mouse bone marrow were treated with recombinant NAMPT (200 ng) for 2 h and immunoblot analysis was performed. All data depict the means ± SEM; *P<0.05, **P<0.01.

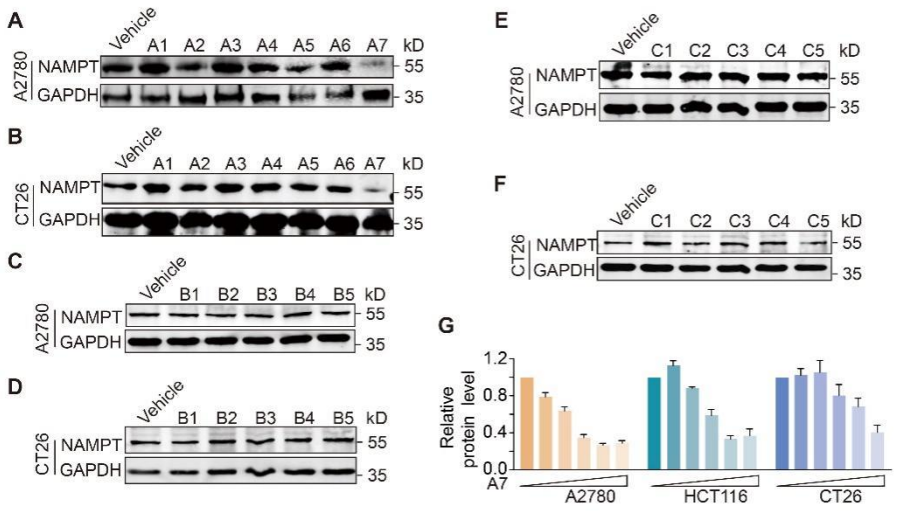


Figure S2 The rational design of NAMPT-specific PROTACs. (A–F) Immunoblot analysis of NAMPT in A2780 (A, C, E) or CT26 (B, D, F) cells. Cells were treated with Compound **A1**–**7**, **B1**–**5** or **C1**–**5** at 10 nmol/L or 100 nmol/L for 24 h. (G) Semi-quantification of NAMPT expression level versus that of GAPDH based on repeated immunoblot analysis.

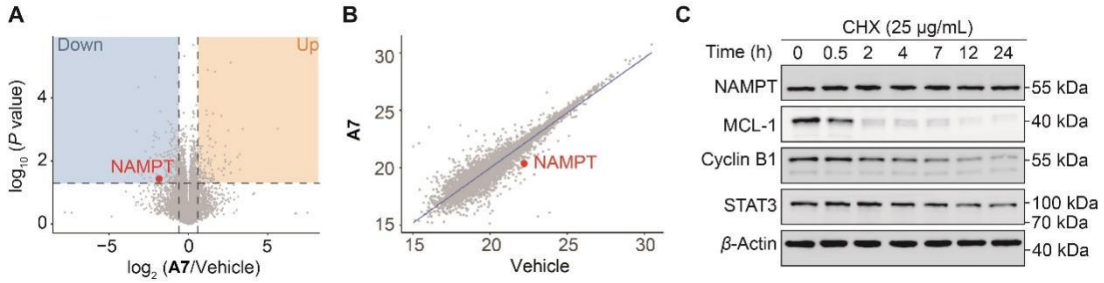


Figure S3 PROTAC **A7** is a selective degrader of NAMPT. (A, B) The proteomic analysis of **A7**-caused protein degradation in A2780 cells. Cells were treated with **A7** (10 nM, 24 h) or vehicle control. Proteomic analysis was performed to compare the protein level change between **A7** and the control group. (C) Immunoblot analysis of the indicated proteins in A2780 cells treated with cycloheximide (CHX, 25 μg/mL) at different timepoints.

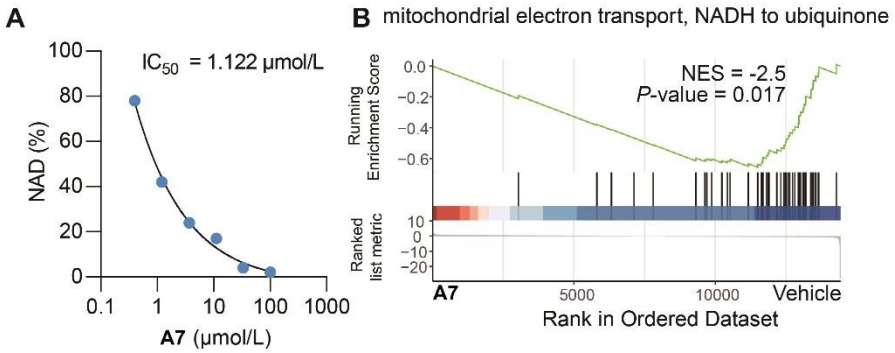


Figure S4 The impact of compound **A7** on NAD^+-relating pathway. (A) Intracellular NAD^+ levels in A2780 cells. Cells were treated with PROTAC **A7** as indicated for 24 h. (B) RNAseq analysis of CT26 cells treated with **A7** (100 nmol/L, 24 h) using Gene Set Enrichment Analysis (GSEA).

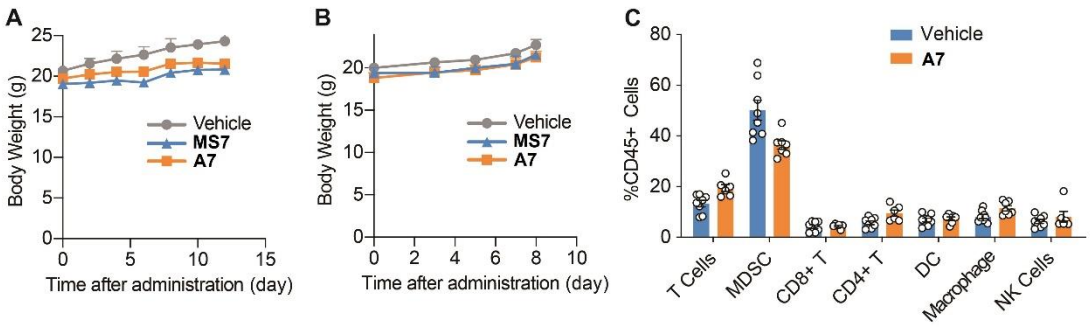
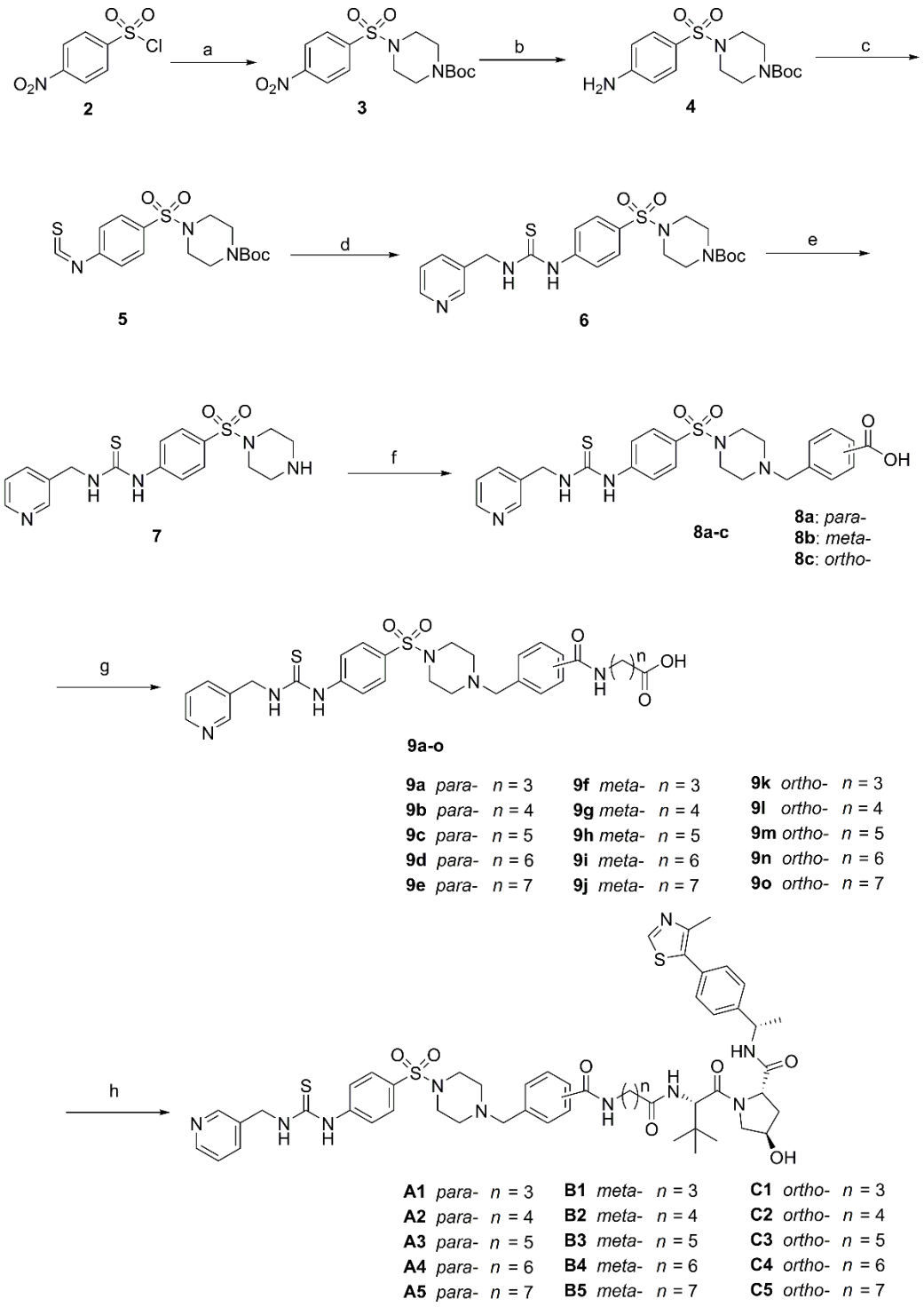
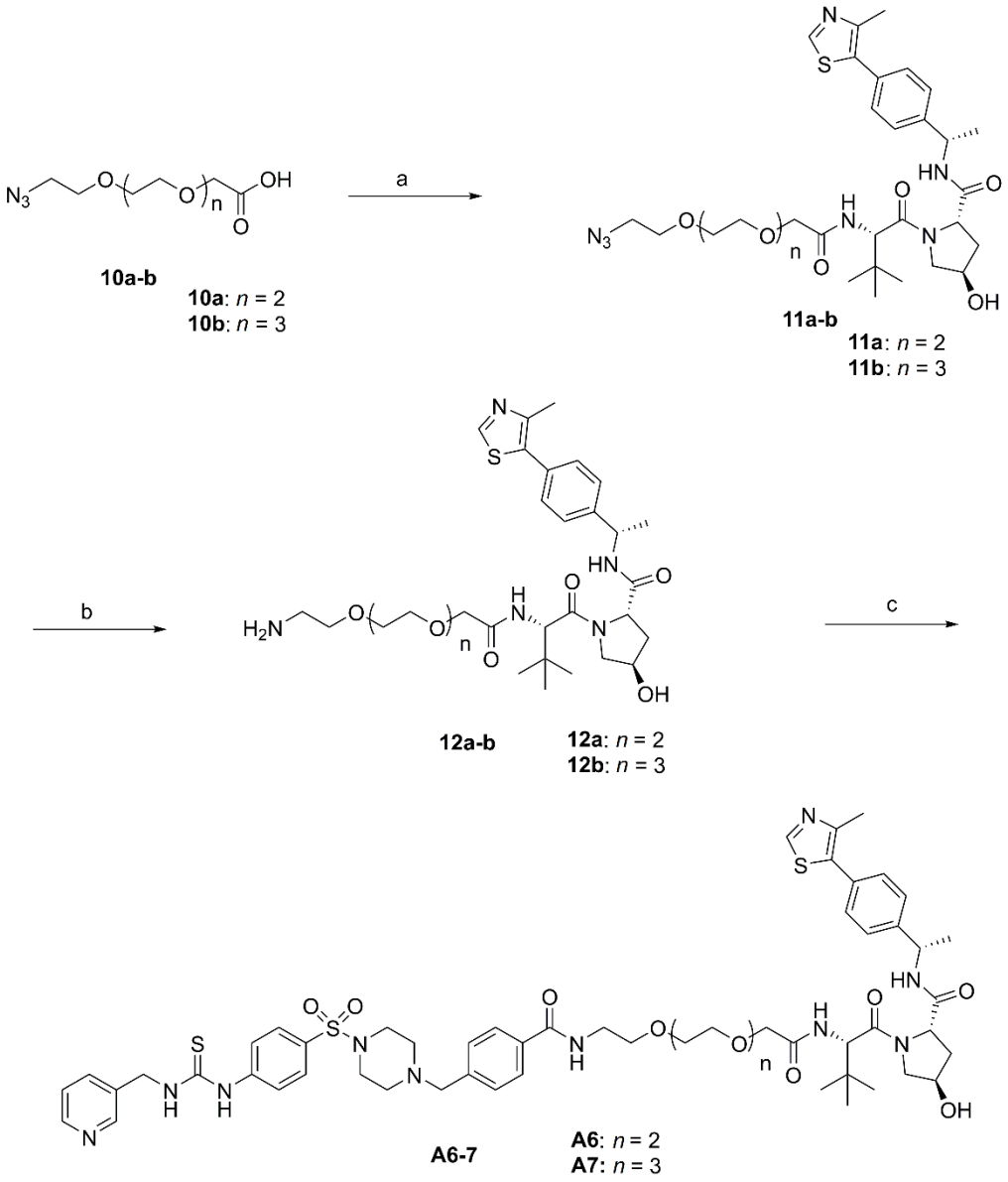


Figure S5 PROTAC **A7** inhibits MDSCs infiltration and revives antitumor immunity. CT26 tumor bearing BALB/c or nude mice were treated with PROTAC **A7** (16 mg/kg, i.p), **MS7** (16 mg/kg, i.p.) or vehicle for 12 consecutive days. (A) Body weight in BALB/c mice (n = 6). (B) Body weight in nude mice (n = 6). (C) The proportion of the indicated immune cells in tumor infiltrating $CD45^+$ cells. CT26 tumor-bearing BALB/c mice (n = 7 or 8) were treated as in (A) for 7 consecutive days. Tumor infiltrating immune cells were analyzed by flow cytometry.



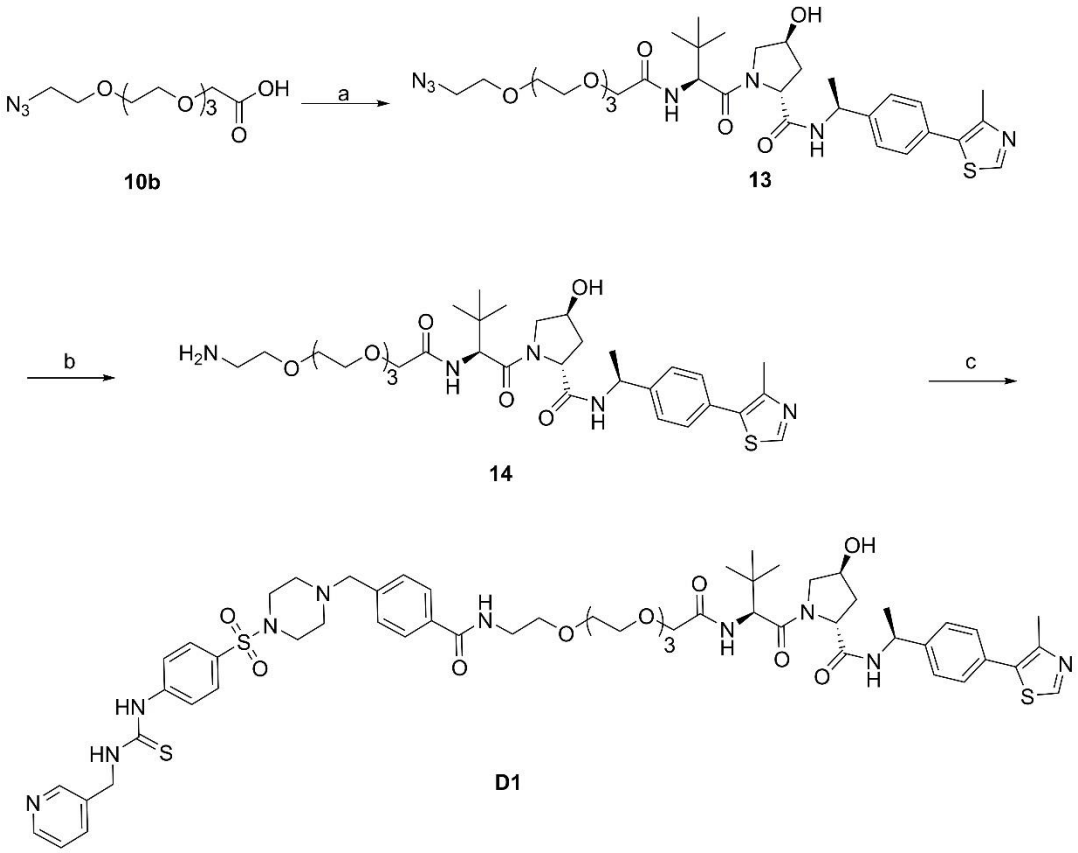
Scheme S1 Synthetic routes of target compounds **A1**–**5**, **B1**–**5** and **C1**–**5**[a].

[a]**Reagents and conditions**: (a) *tert*-butyl piperazine-1-carboxylate, Et_3N, CH_2Cl_2, rt, 2 h, 89%; (b) Pd/C, H_2, CH_2Cl_2, rt, overnight, 96%; (c) di(1*H*-imidazol-1-yl)methanethione, CH_2Cl_2, rt, overnight, 85%; (d) pyridin-3-ylmethanamine, CH_2Cl_2, rt, 6 h, 92%; (e) CF_3COOH, CH_2Cl_2, rt, overnight, 90%; (f) (i) substituted methyl (bromomethyl)benzoate, Et_3N, CH_2Cl_2, rt, 4 h; (ii) LiOH, THF/MeOH/H_2O, rt, 2 h, 40%–56%; (g) (i) amino acid esters, HATU, DIPEA, DMF, rt, 4 h; (ii) LiOH, THF/MeOH/H_2O, rt, 4 h, 15%–30%; (h) VHL ligand, HATU, DIPEA, DMF, rt, 5 h, 17%–26%.



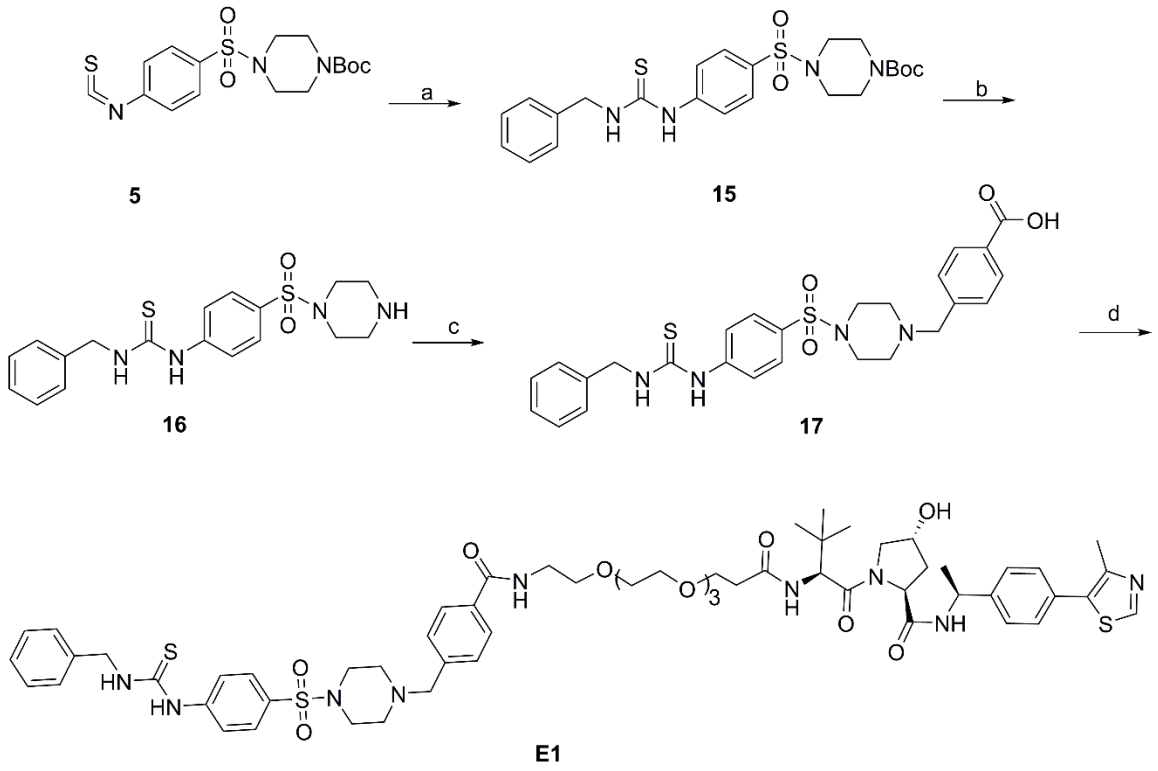
Scheme S2 Synthetic routes of target compounds **A6** and **A7**[a].

[a]**Reagents and conditions**: (a) VHL ligand, HATU, DIPEA, DMF, rt, 5 h, 21%–28%; (b) Pd/C, H_2, CH_2Cl_2, rt, overnight, 96%; (c) compound **8a**, HATU, DIPEA, DMF, rt, 5 h, 23%–29%.



Scheme S3 Synthetic route of target compound **D1**[a]

[a]**Reagents and conditions**: (a) VHL ligand analog, HATU, DIPEA, DMF, rt, 5 h, 18%; (b) Pd/C, H_2, CH_2Cl_2, rt, overnight, 95%; (c) compound **8a**, HATU, DIPEA, DMF, rt, 5 h, 31%.



Scheme S4 Synthetic Route of Target Compound **E1**[a]

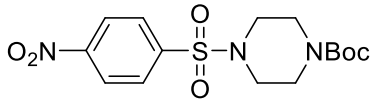
[a]**Reagents and conditions**: (a) phenylmethanamine, CH_2Cl_2, rt, 6 h, 90%; (b) CF_3COOH, CH_2Cl_2, rt, overnight, 92%; (c) (i) substituted methyl (bromomethyl)benzoate, Et_3N, CH_2Cl_2, rt, 4 h; (ii) LiOH, THF/MeOH/H_2O, rt, 2 h, 46%; (d) compound **12b**, HATU, DIPEA, DMF, rt, 5 h, 14%.

Supporting methods

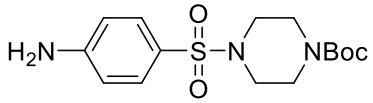
1. Chemistry

General information. The materials used in the experiments were commercially available. Column chromatography was performed on 200–300 mesh silica gel. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AVANCE300 or AVANCE600 spectrometer (Bruker Company, Germany) with DMSO-d_6, CD_3OD or $CDCl_3$ as the solvents and TMS as the internal standard. Chemical shift (δ) was given in ppm and the coupling constant (J) is reported in hertz (Hz).

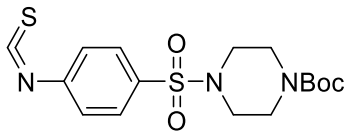
The VHL ligand analog was prepared according to the literature[14]. Intermediate **13** was obtained through amidation between the VHL ligand analog and compound **10b**. Then, intermediate **13** was reduced under a H_2 atmosphere to afford compound **14**. Target compound **D1** was obtained through amidation between compound **8a** and intermediate **14** (**Scheme S3**). Intermediate **15** was obtained through the reaction between intermediate **5** and phenylmethanamine. After the removal of the Boc protecting group with trifluoroacetic acid, intermediate **16** was obtained. Then, substituted methyl (bromomethyl)benzoate was added to give esters, which were converted to the corresponding acid intermediate **17** under basic conditions. Amidation of compound **17** with intermediate **12b** afforded the negative control compound **E1** (**Scheme S4**).



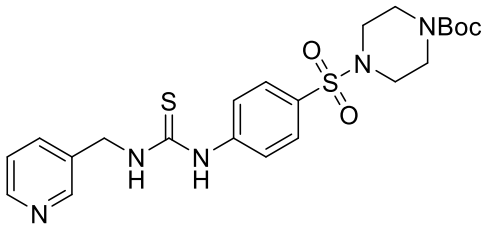
***tert*-Butyl-4-((4-nitrophenyl)sulfonyl)piperazine-1-carboxylate (3).** The mixture of compound **2** (500 mg, 2.3 mmol), *tert*-butyl piperazine-1-carboxylate (840 mg, 4.6 mmol) and Et_3N (230 mg, 2.3 mmol) was dissolved in CH_2Cl_2 (20 mL) and stirred at room temperature for 2 h. Then, the mixture was washed with 1 mol/L HCl aqueous solution (10 mL) twice. The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford the desired compound **3** (760 mg, 89%). ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 7.78 (d, J = 8.78 Hz, 2H), 7.67 (d, J = 8.78 Hz, 2H), 3.37–3.40 (m, 4H), 2.85–2.89 (m, 4H), 1.34 (s, 9H).



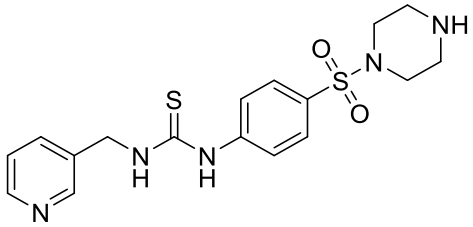
***tert*-Butyl-4-((4-aminophenyl)sulfonyl)piperazine-1-carboxylate (4).** The intermediate **3** (750 g, 2.0 mmol) was dissolved in CH_2Cl_2 (20 mL) and Pd/C (200 mg, 1.2 mmol, 45% purity) was added. The mixture was stirred at room temperature and maintained overnight under the atmosphere of hydrogen. Then the resulting mixture was filtrated through diatomite and the filtrate was concentrated under reduced pressure to afford the intermediate **4** (650 mg, 96%) as a white solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 7.33 (d, J = 8.7 Hz, 2H), 6.64 (d, J = 8.9 Hz, 2H), 6.11 (s, 2H), 3.33-3.41 (m, 4H), 2.73 (t, J = 4.9 Hz, 4H), 1.34 (s, 9H).



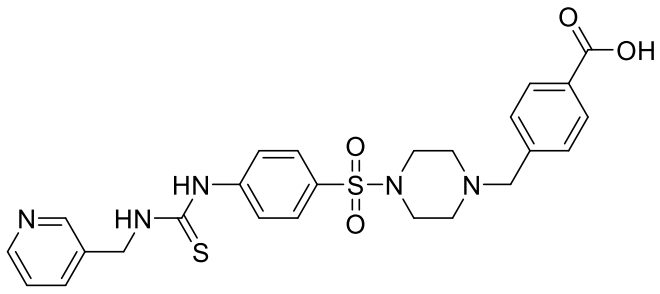
***tert*-Butyl-4-((4-(1*H*-imidazole-1-carbothioamido)phenyl)sulfonyl)piperazine-1-carboxylate (5).** The intermediate **4** (650 mg, 1.9 mmol) was previously dissolved in CH_2Cl_2 and stirred for 5 min at 0 °C. Then, TCDI (462 mg, 2.9 mmol) was added and the solution was allowed to slowly warm to room temperature and stirred overnight. The solvent was removed under reduced pressure and the crude product was purified by silica gel flash chromatography (PE:EA = 20: 1) to afford intermediate **5** (730 mg, 85%) as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 7.76–7.79 (m, 2H), 7.64–7.66 (m, 2H), 3.38 (t, J = 8.7 Hz, 4H), 2.87 (t, J = 5.1 Hz, 4H), 1.33 (s, 9H).



***tert*-Butyl-4-((4-(3-(pyridin-3-ylmethyl)thioureido)phenyl)sulfonyl)piperazine-1-carboxylate (6)** To a solution of the intermediate **5** (780 mg, 1.59 mmol) in DCM (10 mL) was added 3-aminomethylpyridine (171 mg, 1.59 mmol) and then the mixture was reacted at room temperature for 6 h. The solid is filtered to obtain the intermediate **6** (720 mg, 92%) as a white solid. ^{1}H NMR (600 MHz, CD_3OD) δ: 8.55–8.56 (m, 1H), 8.43 (m, 1H), 7.86–7.89 (m, 1H), 7.69–7.74 (m, 4H), 7.39–7.42 (m, 1H), 4.88 (s, 2H), 3.48 (s, 4H), 2.94 (t, J = 5.03 Hz, 4H), 1.39 (s, 9H).

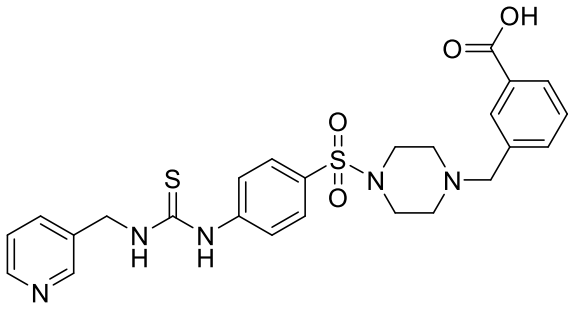


1-(4-(Piperazin-1-ylsulfonyl)phenyl)-3-(pyridin-3-ylmethyl)thiourea(7) Compound **6** (500 mg, 1.01 mmol) was dissolved in dry CH_2Cl_2 (5 mL) containing 20% TFA, and then the mixture was stirred overnight at room temperature. The reaction solution was washed with saturated brine (20 mL), and the organic The phase was concentrated to obtain intermediate **7** as a white solid (360 mg, 90%).^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.13 (s, 1H), 8.61(s, 1H), 8.56-8.58 (m, 1H), 8.46–8.49 (m, 1H), 7.82 (d, J = 8.8 Hz, 2H), 7.66 (d, J = 8.8 Hz, 2H), 7.49 (d, J = 8.3 Hz, 2H), 4.79 (d, J = 5.3 Hz, 2H), 4.30 (s, 1H), 3.74–3.37 (m, 4H), 2.86–3.03 (m, 4H).

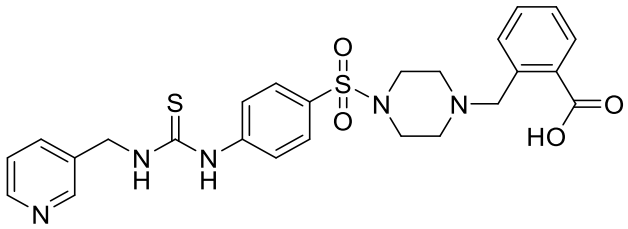


4-((4-((4-(3-(Pyridin-3-ylmethyl)thioureido)phenyl)sulfonyl)piperazin-1-yl)methyl)benzoic acid (8a) Compound **7** (75 mg, 0.14 mmol) and methyl 4-bromomethylbenzoate (70 mg, 0.31 mmol) were dissolved in dry CH_2Cl_2 (6 mL) containing TEA (100 μL), then the mixture was stirred at room temperature for 4 h. The solution was concentrated and purified by silica gel column chromatography (CH_2Cl_2/MeOH = 100/1) to obtain a white solid (110 mg). Then it was then dissolved in THF/MeOH/H_2O (*v*/*v*/*v* = 3/2/1, 15 mL) containing LiOH (20 mg, 0.84 mmol). The mixture was stirred at room temperature for 2 h, the organic phase is evaporated under vacuum. Then pH was adjusted to about 3 with 1 mol/L HCl. The precipitant was filtered and dried to obtain the intermediate **8a** as a white solid (75 mg, 56%). ^{1}H NMR (600 MHz, DMSO-d_6) δ: 12.82 (s, 1H), 10.17 (s, 1H), 8.63-8.68 (m, 1H), 8.57 (d, *J* = 1.8 Hz, 1H), 8.48 (dd, *J* = 4.7, 1.6 Hz, 1H), 7.83 (d, *J* = 8.1 Hz, 2H), 7.81 (d, *J* = 9.1 Hz, 2H), 7.77 (d, *J* = 7.7 Hz, 1H), 7.65 (d, *J* = 9.1 Hz, 2H), 7.34–7.40 (m, 3H), 4.79 (d, *J* = 5.4 Hz, 2H), 3.57 (s, 2H), 2.83–2.95 (m, 4H), 2.39–2.49 (m, 4H).

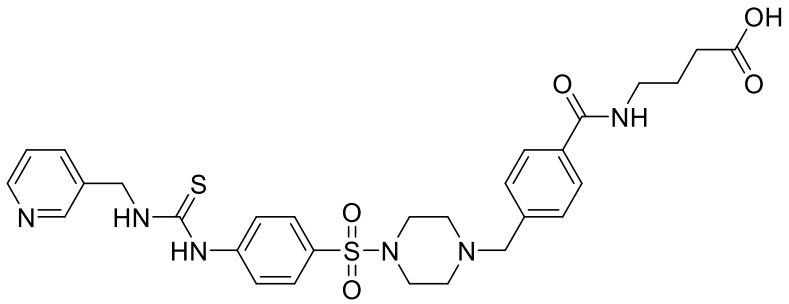
The synthetic routes of compounds **8b** and **8c** were similar to **8a**.



3-((4-((4-(3-(Pyridin-3-ylmethyl)thioureido)phenyl)sulfonyl)piperazin-1-yl)methyl)benzoic acid (8b) White solid, 40%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 12.91 (s, 1H), 10.13 (s, 1H), 8.64 (s, 1H), 8.55–8.59 (m, 1H), 8.47 (dd, *J* = 4.6, 1.0 Hz, 1H), 7.80–7.83 (m, 2H), 7.78–7.83 (m, 2H), 7.76–7.78 (m, 1H), 7.65 (d, *J* = 7.6 Hz, 2H), 7.48 (d, *J* = 7.6 Hz, 1H), 7.41 (t, *J* = 15.4, 7.9 Hz, 1H), 7.38 (dd, *J* = 7.8, 4.7 Hz, 1H), 4.79 (d, *J* = 5.4 Hz, 2H), 3.54 (s, 2H), 2.89 (s, 4H), 2.45 (s, 4H).

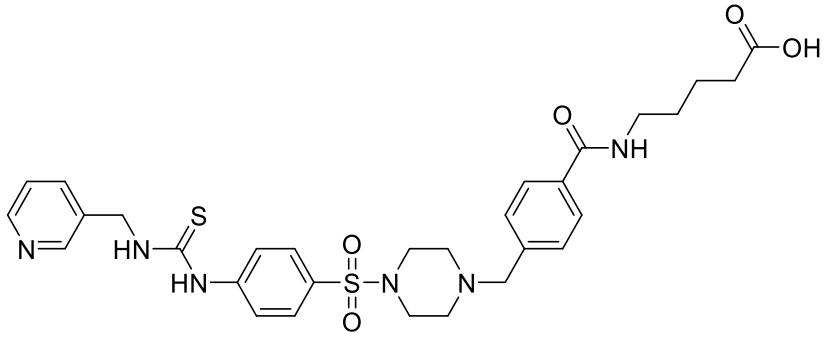


4-((4-((4-(3-(pyridin-3-ylmethyl)thioureido)phenyl)sulfonyl)piperazin-1-yl)methyl)benzoic acid (8c) White solid, 48%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.13 (s, 1H), 8.64 (s, 1H), 8.47–8.57 (m, 1H), 8.47–8.48 (m, 1H), 7.80–7.82 (m, 2H), 7.76–7.78 (m, 1H), 7.72–7.73 (m, 1H), 7.64–7.66 (m, 2H), 7.45–7.47 (m, 1H), 7.35–7.39 (m, 3H), 4.80 (d, *J* = 5.1 Hz, 2H), 3.82 (s, 2H), 2.79-2.91 (m, 4H), 2.30–2.40 (m, 4H).

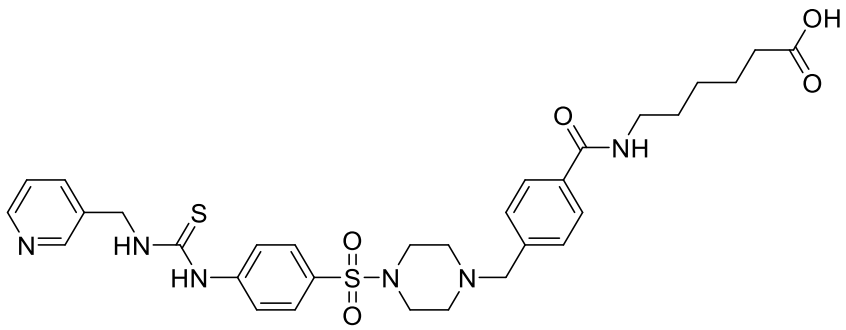


4-(4-((4-((4-(3-(Pyridin-3-ylmethyl)thioureido)phenyl)sulfonyl)piperazin-1-yl)methyl) benzamido)butanoic acid (9a). The compound **8** (75 mg, 0.14 mmol), methyl butyrate (15 mg, 0.14 mmol), HATU (81 mg, 0.22 mmol), DIPEA (28 mg, 0.22 mmol) were dissolved in dry DMF (5 mL) and then the mixture was stirred at room temperature for 4 h. The mixture was poured saturated NaCl solution (20 mL) and then extracted with ethyl acetate (10 mL × 3). The organic phases were combined and washed with of saturated NaCl solution (10 mL). After dried over sodium sulfate, it was concentrated and purified by silica gel column chromatography (dichloromethane/methanol=100/1) to obtain 80 mg of white solid. The obtained product was dissolved in THF/MeOH/H_2O (*v*/*v*/*v* = 3/2/1, 10 mL) containing LiOH (15 mg, 0.63 mmol). The mixture was stirred at room temperature for 4 h, the organic phase is evaporated under vacuum. Then pH was adjusted to about 3 with 1 mol/L HCl. The precipitant was filtered and dried to obtain the intermediate **9a** as a white solid (26 mg, 30%). ^{1}H NMR (600 MHz, DMSO-d_6) δ: 12.08 (s, 1H), 10.39 (s, 1H), 8.79 (s, 1H), 8.41–8.61 (m, 2H), 7.85 (d, *J* = 8.1 Hz, 2H), 7.77 (d, *J* = 7.5 Hz, 2H), 7.63 (d, *J* = 8.8 Hz, 2H), 7.24–7.46 (m, 5H), 4.78 (d, *J* = 5.2 Hz, 2H), 3.59 (s, 2H), 3.11 (d, *J* = 4.8 Hz, 2H), 2.76–3.01 (m, 4H), 2.27–2.49 (m, 4H), 2.22 (t, *J* = 7.1 Hz, 2H), 1.62 (t, *J* = 6.3 Hz, 2H).

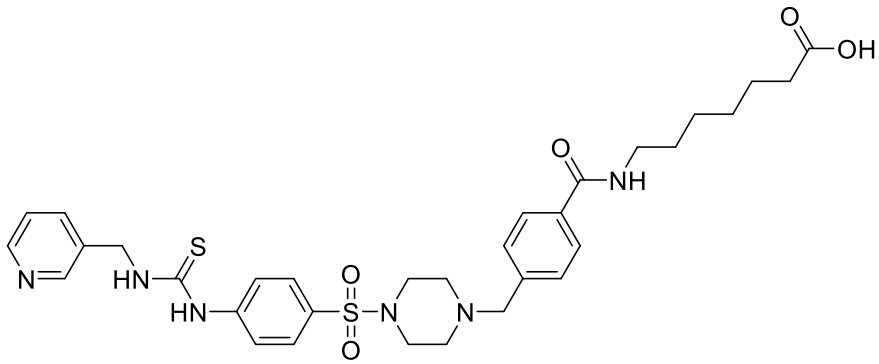
The synthetic routes of **9b–o** were similar to that of **9a**.



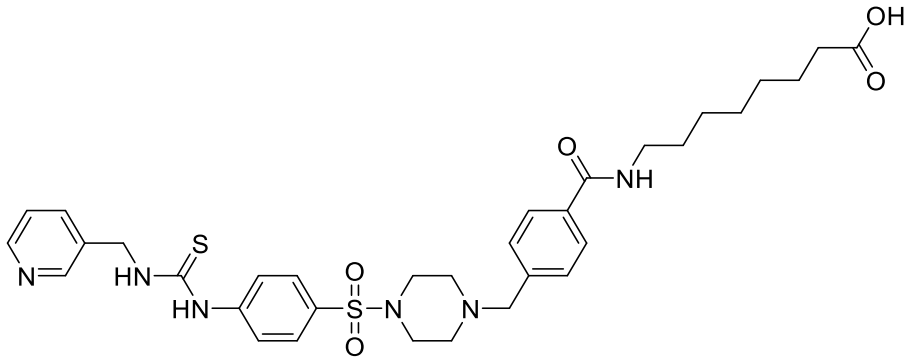
5-(4-((4-((4-(3-(Pyridin-3-ylmethyl)thioureido)phenyl)sulfonyl)piperazin-1-yl)methyl)benzamido)pentanoic acid (9b). White solid, 21%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 11.97 (s, 1H), 10.45 (s, 1H), 8.76–8.91 (m, 1H), 8.55 (d, *J* = 1.7 Hz, 1H), 8.46 (dd, *J* = 4.7, 1.3 Hz, 1H), 8.37 (t, *J* = 4.9 Hz, 1H), 7.84 (d, *J* = 7.4 Hz, 2H), 7.72–7.77 (m, 3H), 7.63 (d, *J* = 8.7 Hz, 2H), 7.36 (dd, *J* = 7.7, 4.9 Hz, 1H), 7.31 (d, *J* = 7.3 Hz, 2H), 4.78 (d, *J* = 5.6 Hz, 2H), 3.46–3.69 (m, 4H), 3.20 (q, *J* = 6.4 Hz, 2H), 2.88 (s, 4H), 2.36–2.57 (m, 4H), 1.45–1.49 (m, 4H).



6-(4-((4-((4-(3-(Pyridin-3-ylmethyl)thioureido)phenyl)sulfonyl)piperazin-1-yl)methyl)benz-amido)hexanoic acid(9c). White solid, 26%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.27 (s, 1H), 8.71 (s, 1H), 8.55 (s, 1H), 8.46 (d, J = 4.4 Hz, 1H), 8.38 (s, 1H), 7.70–7.84 (m, 6H), 7.63 (d, J = 1.6 Hz, 2H), 7.37 (dd, J = 7.7, 4.7 Hz, 1H), 7.31 (s, 1H), 4.77 (d, J = 5.4 Hz, 2H), 3.17–3.22 (m, 3H), 2.86 (s, 4H), 2.35–2.48 (m, 4H), 2.18 (t, J = 7.4 Hz, 2H), 1.34–1.52 (m, 5H), 1.25–1.30 (m, 2H).

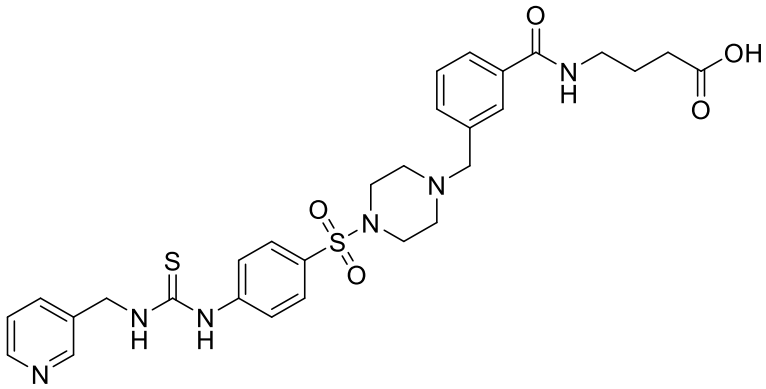


7-(4-((4-((4-(3-(Pyridin-3-ylmethyl)thioureido)phenyl)sulfonyl)piperazin-1-yl)methyl)benz-amido)heptanoic acid(9d) White solid, 27%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 11.94 (s, 1H), 10.47 (s, 1H), 8.84 (s, 1H), 8.55 (d, J = 1.6 Hz, 1H), 8.46 (dd, J = 4.9, 1.3 Hz, 1H), 8.37–8.43 (m, 1H), 7.85 (d, J = 8.4 Hz, 2H), 7.76 (d, J = 7.3 Hz, 3H), 7.63 (d, J = 8.8 Hz, 2H), 7.32-7.42 (m, 3H), 4.78 (d, J = 5.6 Hz, 2H), 3.72 (s, 2H), 3.20 (q, J = 6.6 Hz, 2H), 2.93 (s, 4H), 2.65 (s, 4H), 2.17 (t, J = 7.1 Hz, 2H), 1.43–1.51 (m, 4H), 1.23-1.31 (m, 4H).

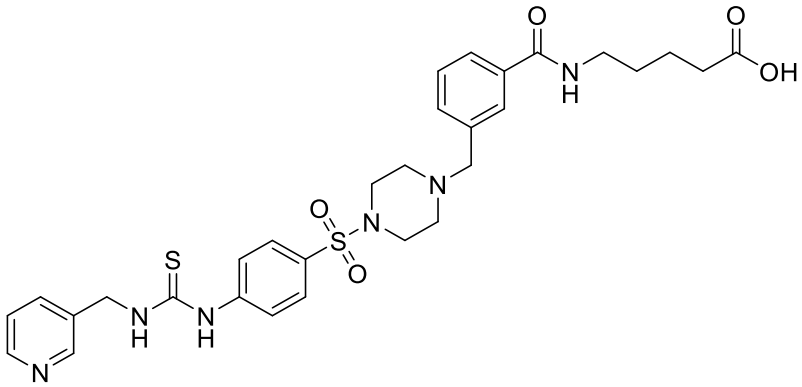


8-(4-((4-((4-(3-(Pyridin-3-ylmethyl)thioureido)phenyl)sulfonyl)piperazin-1-yl)methyl)benz-amido)octanoic acid(9e) White solid, 15%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 12.88 (s, 1H), 11.69 (s, 1H),

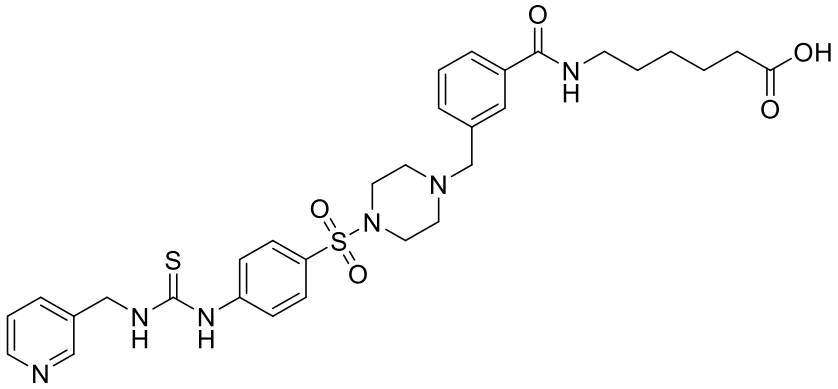
8.53 (s, 1H), 8.43 (d, *J* = 4.2 Hz, 1H), 8.35 (t, *J* = 4.8 Hz, 1H), 8.00 (d, *J* = 8.2 Hz, 2H), 7.67–7.78 (m, 3H), 7.55 (d, *J* = 8.8 Hz, 2H), 7.34 (dd, *J* = 7.8, 4.8 Hz, 1H), 7.28 (d, *J* = 7.8 Hz, 2H), 4.76 (s, 2H), 3.45 (s, 2H), 3.17–3.25 (m, 2H), 2.75 (s, 4H), 2.41 (s, 4H), 1.94 (t, *J* = 7.6 Hz, 2H), 1.30–1.53 (m, 4H), 1.18–1.31 (m, 6H).



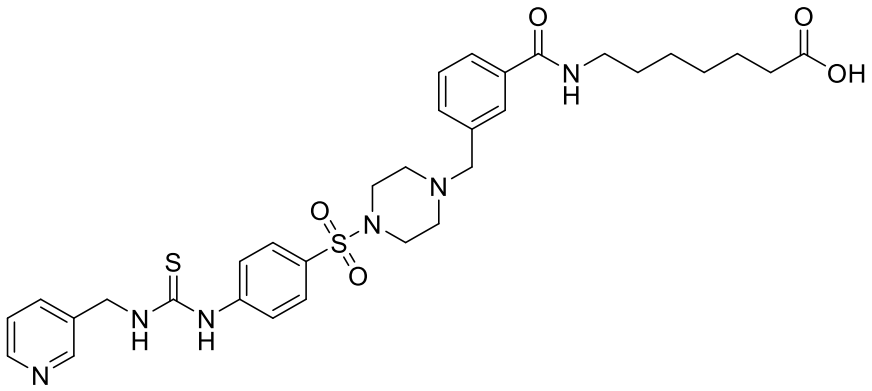
4-(3-((4-((4-(3-(Pyridin-3-ylmethyl)thioureido)phenyl)sulfonyl)piperazin-1-yl)methyl)benz-amido)butanoic acid (9f) White solid, 28%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 12.08 (s, 1H), 10.13 (s, 1H), 8.64 (s, 1H), 8.57 (d, *J* = 1.5 Hz, 1H), 8.47 (dd, *J* = 4.7, 1.4 Hz, 1H), 8.44 (t, *J* = 5.6 Hz, 1H), 7.75–7.80 (m, 3H), 7.68-7.70 (m, 2H), 7.64 (d, *J* = 8.9 Hz, 2H), 7.35–7.40 (m, 3H), 4.79 (d, *J* = 5.5 Hz, 2H), 3.51 (s, 2H), 3.24 (q, *J* = 6.6 Hz, 2H), 2.89 (s, 4H), 2.45 (s, 4H), 2.26 (t, *J* = 7.5 Hz, 2H), 1.70–1.75 (m, 2H).



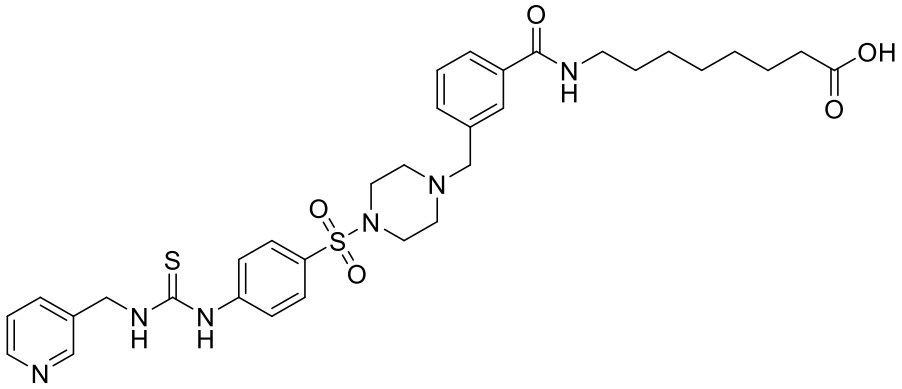
5-(3-((4-((4-(3-(Pyridin-3-ylmethyl)thioureido)phenyl)sulfonyl)piperazin-1-yl)methyl)benz-amido)pentanoic acid (9g) White solid, 27%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.28 (s, 1H), 8.77 (s, 1H), 8.54 (d, *J* = 1.7 Hz, 1H), 8.44 (dd, *J* = 4.8, 1.4 Hz, 1H), 8.40 (t, *J* = 5.6 Hz, 1H), 7.77 (d, *J* = 8.7 Hz, 2H), 7.70–7.76 (m, 1H), 7.63–7.69 (m, 2H), 7.60 (d, *J* = 8.7 Hz, 2H), 7.29–7.38 (m, 3H), 4.76 (d, *J* = 5.3 Hz, 2H), 3.41–3.55 (m, 4H), 3.21 (q, *J* = 6.8 Hz, 2H), 2.80–2.93 (m, 4H), 2.39 (s, 4H), 2.21 (t, *J* = 7.6 Hz, 2H), 1.66–1.75 (m, 2H).



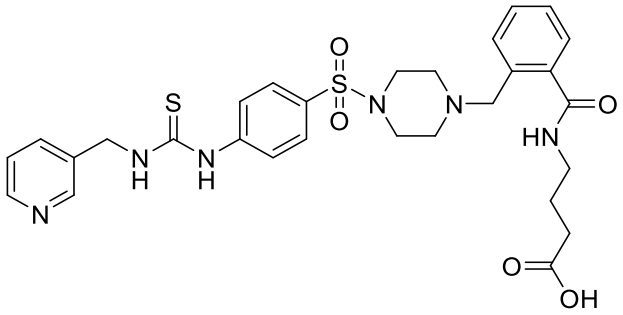
6-(3-((4-((4-(3-(Pyridin-3-ylmethyl)thioureido)phenyl)sulfonyl)piperazin-1-yl)methyl)benz-amido)hexanoic acid (9h) White solid, 22%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 11.99 (s, 1H), 10.37 (s, 1H), 8.81 (s, 1H), 8.57 (s, 1H), 8.47 (d, J = 4.1 Hz, 1H), 8.40 (t, J = 5.6 Hz, 1H), 7.80–7.85 (m, 2H), 7.77 (d, J = 8.3 Hz, 1H), 7.66–7.70 (m, 2H), 7.64 (d, J = 8.8 Hz, 2H), 7.34–7.40 (m, 3H), 4.79 (d, J = 5.1 Hz, 2H), 3.49 (s, 2H), 3.32 (q, J = 6.6 Hz, 2H), 2.88 (s, 4H), 2.43 (s, 4H), 2.19 (t, J = 7.5 Hz, 2H), 1.47–1.53 (m, 4H), 1.26–1.31 (m, 2H).



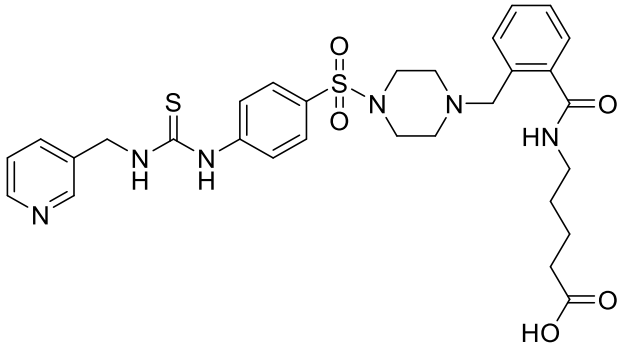
7-(3-((4-((4-(3-(pyridin-3-ylmethyl)thioureido)phenyl)sulfonyl)piperazin-1-yl)methyl)benz-amido)heptanoic acid (9i) White solid, 27%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 12.00 (s, 1H), 10.23 (s, 1H), 8.70 (s, 1H), 8.57 (d, J = 1.9 Hz, 1H), 8.47 (dd, J = 4.7, 1.3 Hz, 1H), 8.38–8.43 (m, 1H), 7.79–7.85 (m, 2H), 7.75–7.79 (m, 1H), 7.71 (s, 2H), 7.65 (d, J = 8.8 Hz, 2H), 7.33–7.43 (m, 3H), 4.79 (d, J = 5.5 Hz, 2H), 3.55 (s, 2H), 3.21 (q, J = 6.8 Hz, 2H), 2.89 (s, 4H), 2.37-2.49 (m, 4H), 2.19 (t, J = 7.4 Hz, 2H), 1.46–1.51 (m, 4H), 1.26–1.30 (m, 4H).



8-(3-((4-((4-(3-(Pyridin-3-ylmethyl)thioureido)phenyl)sulfonyl)piperazin-1-yl)methyl)benzamido)octanoic acid (9j) White solid, 21%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 11.97 (s, 1H), 10.35 (s, 1H), 8.78 (s, 1H), 8.47 (s, 1H), 8.40 (t, J = 5.2 Hz, 1H), 7.83 (d, J = 8.5 Hz, 2H), 7.77 (d, J = 7.8 Hz, 1H), 7.69–7.75 (m, 2H), 7.65 (d, J = 8.7 Hz, 2H), 7.34–7.43 (m, 2H), 7.79 (d, J = 5.4 Hz, 2H), 3.60 (s, 2H), 3.32 (q, J = 6.3 Hz, 2H), 2.91 (s, 4H), 2.50–2.68 (m, 4H), 2.18 (t, J = 7.0 Hz, 2H), 1.43–1.53 (m, 4H), 1.25–1.29 (m, 8H).

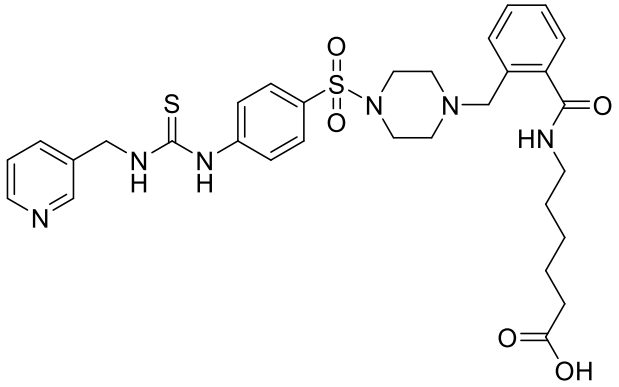


4-(2-((4-((4-(3-(Pyridin-3-ylmethyl)thioureido)phenyl)sulfonyl)piperazin-1-yl)methyl)benzamido)butanoic acid (9k) White solid, 28%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.75 (s, 1H), 9.27 (s, 1H), 8.56 (d, J = 1.6 Hz, 1H), 8.49 (t, J = 11.0, 5.7 Hz, 1H), 8.46 (dd, J = 4.6, 1.1 Hz, 1H), 7.84 (d, J = 8.6 Hz, 2H), 7.76 (d, J = 7.9 Hz, 1H), 7.71 (d, J = 8.7 Hz, 2H), 7.25–7.39 (m, 5H), 4.78 (d, J = 4.4 Hz, 2H), 3.54 (s, 2H), 3.30 (q, J = 6.5 Hz, 2H), 2.84 (s, 4H), 2.39 (s, 4H), 2.16 (t, J = 7.3 Hz, 2H), 1.56–1.63 (m, 2H).

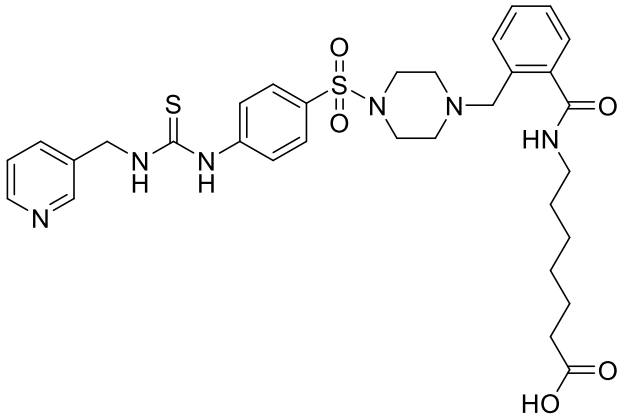


5-(2-((4-((4-(3-(Pyridin-3-ylmethyl)thioureido)phenyl)sulfonyl)piperazin-1-yl)methyl)benzamido)pentanoic acid (9l) White solid, 18%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 12.05 (s, 1H), 10.23 (s, 1H),

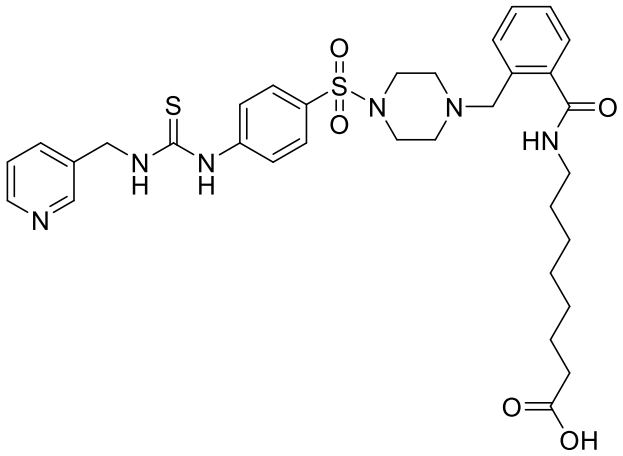
8.66 (s, 2H), 8.57 (d, *J* = 1.7 Hz, 1H), 8.44–8.50 (m, 1H), 7.85 (d, *J* = 8.6 Hz, 2H), 7.78 (d, *J* = 7.6 Hz, 1H), 7.66 (d, *J* = 8.6 Hz, 2H), 7.33–7.55 (m, 5H), 4.79 (d, *J* = 5.3 Hz, 2H), 3.6–4.15 (m, 2H), 3.16 (s, 3H), 2.60–3.10 (m, 6H), 2.22 (t, *J* = 7.0 Hz, 2H), 1.34–1.60 (m, 5H).



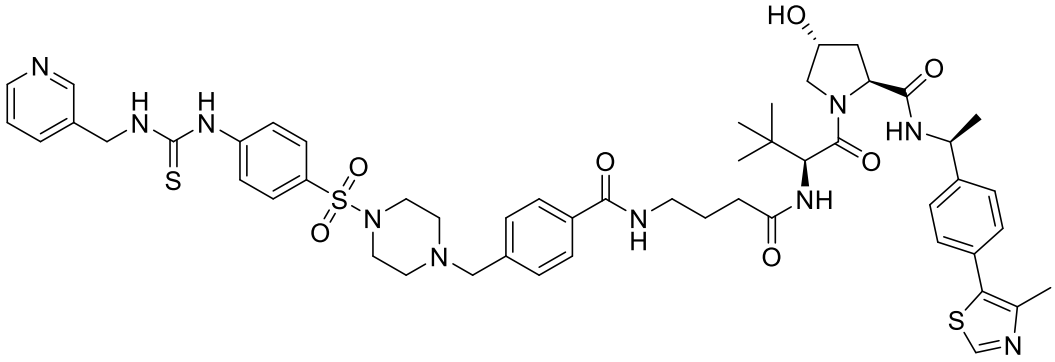
6-(2-((4-((4-(3-(Pyridin-3-ylmethyl)thioureido)phenyl)sulfonyl)piperazin-1-yl)methyl)benz-amido)hexanoic acid (9m) White solid, 29%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.20 (s, 1H), 8.67 (s, 1H), 8.54 (d, *J* = 1.5 Hz, 1H), 8.44 (dd, *J* = 4.7, 1.3 Hz, 1H), 7.79 (s, 1H), 7.78 (s, 1H), 7.69–7.76 (m, 2H), 7.62 (s, 1H), 7.61 (s, 1H), 7.43–7.48 (m, 1H), 7.33–7.38 (m, 4H), 4.75 (d, *J* = 5.5 Hz, 2H), 3.88 (s, 4H), 3.02–3.30 (m, 4H), 2.76-3.10 (m, 8H), 2.65 (s, 4H).



7-(2-((4-((4-(3-(Pyridin-3-ylmethyl)thioureido)phenyl)sulfonyl)piperazin-1-yl)methyl)benz-amido)heptanoic acid (9n) White solid, 18%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 12.00 (s, 1H), 10.34 (s, 1H), 8.73 (s, 1H), 8.52–8.60 (m, 2H), 8.47 (dd, *J* = 4.7, 1.3 Hz, 1H), 7.86 (d, *J* = 8.4 Hz, 2H), 7.75–7.78 (m, 1H), 7.63 (d, *J* = 8.7 Hz, 2H), 7.27–7.41 (m, 4H), 4.79 (d, *J* = 5.9 Hz, 2H), 3.56 (s, 2H), 3.05 (d, *J* = 4.2 Hz, 2H), 2.84 (s, 4H), 2.43 (s, 4H), 2.19 (t, *J* = 7.4 Hz, 2H), 1.43–1.49 (m, 2H), 1.29–1.38 (m, 2H), 1.17–1.23 (m, 4H).



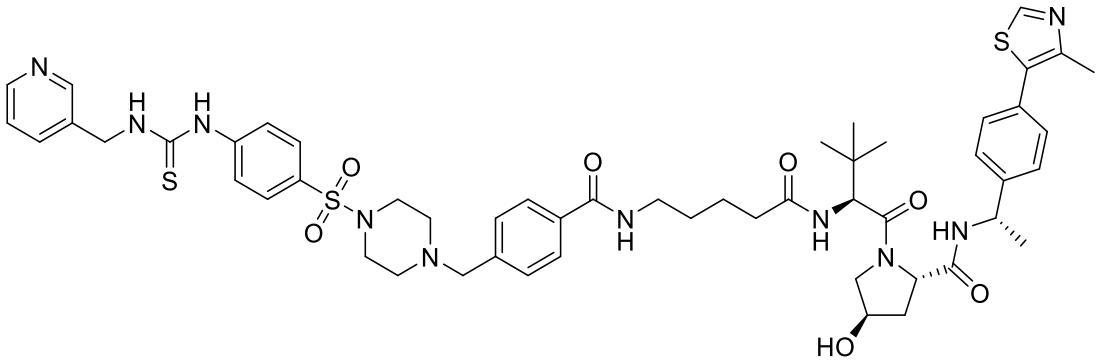
8-(2-((4-((4-(3-(Pyridin-3-ylmethyl)thioureido)phenyl)sulfonyl)piperazin-1-yl)methyl)benz-amido)octanoic acid (9o) White solid, 29%. ^{1}H NMR (DMSO-d_6, 600MHz) δ: 11.95 (s, 1H), 10.38 (s, 1H), 8.53–8.83 (m, 3H), 8.48 (dd, J = 1.2 Hz, 4.6 Hz, 1H), 7.88 (d, J = 8.0 Hz, 2H), 7.77 (d, J = 8.0 Hz, 1H), 7.64 (d, J = 8.6 Hz, 2H), 7.25–7.54 (m, 5H), 4.79 (d, J = 5.6 Hz, 2H), 3.57 (s, 2H), 2.98–3.28 (m, 4H), 2.64–2.96 (m, 4H), 2.28–2.48 (m, 2H), 2.19 (t, J = 7.6 Hz, 2H), 1.45–1.51 (m, 2H), 1.37 (s, 2H), 1.19–1.28 (m, 6H).



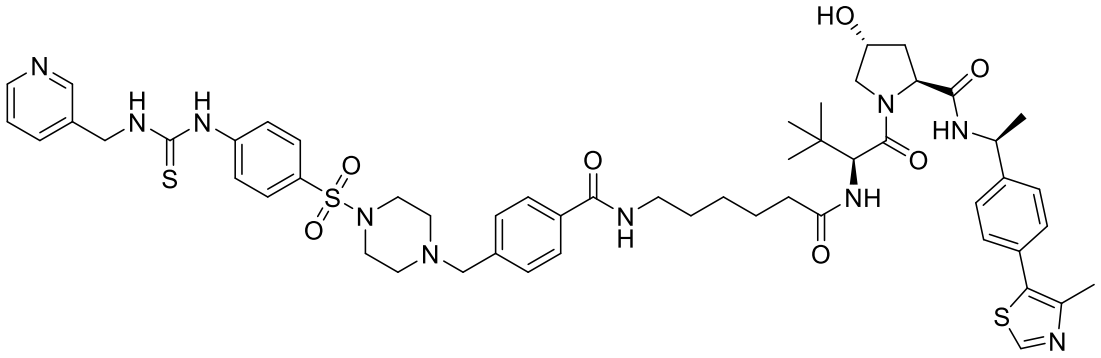
(2*S*,4*R*)-1-((*S*)-3,3-Dimethyl-2-(4-(4-((4-((4-(3-(pyridin-3-ylmethyl)thioureido)phenyl)sulfo-nyl)pi-perazin-1-yl)methyl)benzamido)butanamido)butanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxa-mide1 (A1). The compound **9a** (25 mg, 0.04 mmol), VHL ligand (18 mg, 0.04 mmol), HATU (30 mg, 0.08 mmol), DIPEA (10 mg, 0.08 mmol) were dissolved in dry DMF (5 mL) and then the mixture was stirred at room temperature for 6 h. The mixture was poured saturated NaCl solution (20 mL) and then extracted with ethyl acetate (10 mL × 3). The organic phases were combined and washed with of saturated NaCl solution (10 mL). After dried over sodium sulfate, it was concentrated and purified by silica gel column chromatography (dichloromethane/methanol=100/3) to obtain 80 mg of white solid (11 mg, yield 26%, purity 99.5%). ^{1}H NMR (600 MHz, DMSO-d_6) δ:10.13 (s, 1H), 8.96 (s, 1H), 8.62 (s, 1H), 8.56 (s, 1H), 8.46 (d, J = 4.1 Hz, 1H), 8.31–8.36 (m, 2H), 7.73–7.80 (m, 7H), 7.63 (d, J = 4.4 Hz, 2H), 7.41 (d, J = 3.8 Hz, 2H), 7.35–7.37 (m, 3H), 7.29 (d, J = 3.9 Hz, 2H), 5.05–5.10 (m, 1H), 4.88–4.93 (m, 1H), 4.78 (d, J = 5.0 Hz, 2H), 4.49 (d, J = 9.1 Hz, 1H), 4.40 (t, J = 7.9 Hz, 1H), 4.26 (s, 1H), 3.56–3.61 (m, 2H), 3.51 (s, 2H), 3.16–3.21 (m, 2H), 2.83–2.93 (m, 4H), 2.40–2.47 (m, 6H), 2.19–2.26 (m, 1H), 2.08–2.13 (m, 1H), 1.97–2.02 (m, 1H), 1.75–1.80 (m, 1H), 1.35 (d, J = 6.8 Hz, 3H), 1.22–1.27 (m, 2H), 0.90 (s, 9H);

^{13}C NMR (150 MHz, DMSO-d_6) δ: 181.22, 172.46, 171.07, 170.07, 166.29, 151.88, 149.39, 148.65, 148.21, 145.08, 144.49, 135.75, 134.61, 134.08, 131.56, 130.15, 129.26, 128.98, 128.86, 127.57, 126.83, 123.88, 121.95, 69.22, 61.31, 59.00, 56.82, 56.68, 51.89, 48.14, 46.29, 45.18, 38.16, 35.60, 35.36, 29.36, 26.89, 26.65, 25.67, 22.84, 16.42; HRMS (ESI, positive) *m*/*z* calcd for $C_{52}H_{65}N_{10}O_7S_3$ $(M+H)^+$ 1037.4194, found 1037.4211.

The synthetic routes of **A2–5**, **B1–5** and **C1–5** were similar to that of **A1**.

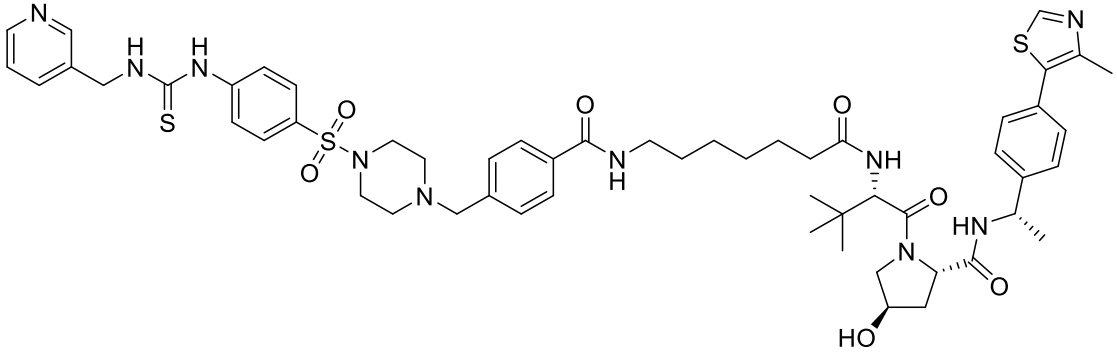


(2*S*,4*R*)-1-((*S*)-3,3-Dimethyl-2-(5-(4-((4-((4-(3-(pyridin-3-ylmethyl)thioureido)phenyl)sulfon-yl)piperazin-1-yl)methyl)benzamido)pentanamido)butanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (A2) White solid, yield 21%, purity 99.0%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.18 (s, 1H), 8.99 (s, 1H), 8.67 (s, 1H), 8.59 (s, 1H), 8.49 (d, J = 3.6 Hz, 1H), 8.40 (s, 1H), 8.37 (d, J = 7.9 Hz, 1H), 7.74-7.86 (m, 6H), 7.66 (d, J = 8.7 Hz, 2H), 7.33-7.45 (m, 7H), 5.10 (s, 1H), 4.93 (t, J = 7.6 Hz, 1H), 4.81 (d, J = 5.4 Hz, 2H), 4.51 (d, J = 9.1 Hz, 1H), 4.43 (t, J = 8.0 Hz, 1H), 4.29 (s, 1H), 3.60–3.65 (m, 2H), 3.20–3.27 (m, 3H), 2.76–3.02 (m, 4H), 2.52–2.56 (m, 2H), 2.46 (s, 3H), 2.25–2.31 (m, 1H), 2.12–2.17 (m, 1H), 1.99–2.03 (m, 1H), 1.76–1.82 (m, 1H), 1.43–1.56 (m, 5H), 1.38 (d, J = 7.0 Hz, 3H), 1.24–1.26 (m, 2H), 0.93 (s, 9H); ^{13}C NMR (150 MHz, CD_3OD) δ:181.84, 174.38, 171.82, 170.93, 168.50, 151.42, 148.11, 147.68, 147.31, 144.22, 143.91, 141.81, 140.66, 136.45, 135.17, 133.59, 131.93, 130.13, 129.09, 129.01, 128.42, 128.03, 126.98, 126.22, 125.92, 125.33, 123.77, 122.20, 117.29, 110.23, 69.57, 61.32, 59.17, 57.73, 56.55, 51.73, 48.74, 45.71, 44.81, 39.10, 37.36, 35.10, 34.76, 28.55, 25.67, 22.97, 20.99, 14.41; HRMS (ESI, positive) *m*/*z* calcd for $C_{53}H_{67}N_{10}O_7S_3$ $(M+H)^+$ 1051.4351, found 1051.4334.

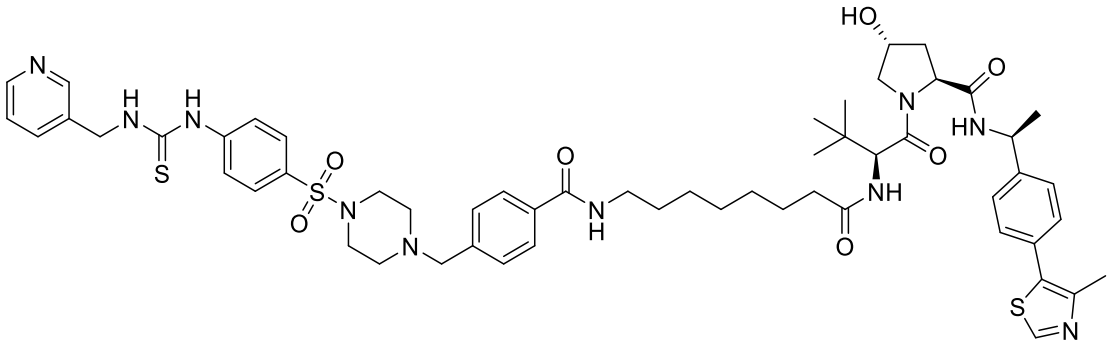


(2*S*,4*R*)-1-((*S*)-3,3-Dimethyl-2-(6-(4-((4-((4-(3-(pyridin-3-ylmethyl)thioureido)phenyl)sulfon-yl)piperazin-1-yl)methyl)benzamido)hexanamido)butanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-

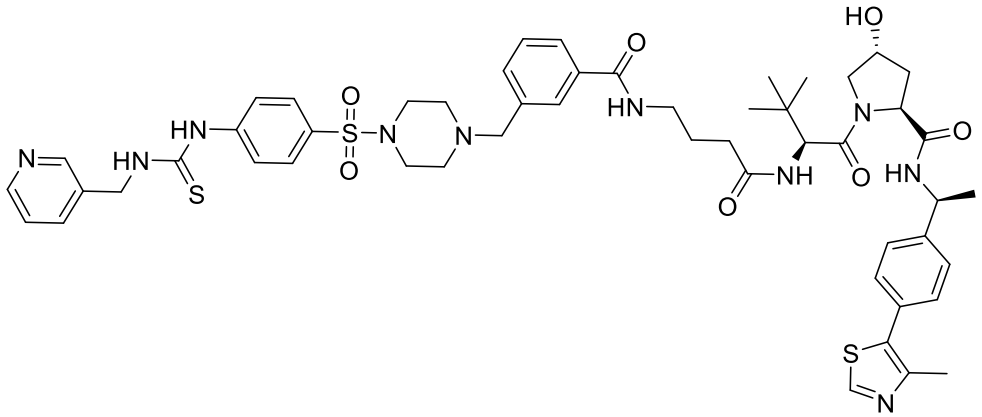
yl)phenyl)ethyl)pyrrolidine-2-carboxamide (A3) White solid, yield 26%, purity 99.0%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.09 (s, 1H), 8.97 (s, 1H), 8.60 (s, 1H), 8.56 (d, J = 1.9 Hz, 1H), 8.44–8.48 (m, 1H), 8.29–8.36 (m, 2H), 7.72–7.80 (m, 6H), 7.63 (d, J = 8.8 Hz, 2H), 7.41–7.43 (m, 2H), 7.35–7.38 (m, 3H), 7.29 (d, J = 8.1 Hz, 2H), 5.06 (d, J = 3.6 Hz, 1H), 4.88–4.92 (m, 1H), 4.78 (d, J = 5.4 Hz, 2H), 4.49 (d, J = 9.4 Hz, 1H), 4.41 (t, J = 8.1 Hz, 1H), 4.25–4.29 (m, 1H), 3.60–3.56 (m, 2H), 3.49 (s, 2H), 3.17–3.23 (m, 2H), 2.81–2.93 (m, 4H), 2.40 (s, 3H), 2.39–2.43 (m, 4H), 2.20–2.26 (m, 1H), 2.09–2.13 (m, 1H), 1.98–2.01 (m, 1H), 1.76–1.81 (m, 1H), 1.44–1.51 (m, 4H), 1.36 (d, J = 6.9 Hz, 3H), 0.91 (s, 9H), 0.85 (t, J = 7.2 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ:181.21, 172.48, 171.09, 170.08, 166.30, 151.93, 149.38, 148.66, 148.21, 145.11, 144.52, 135.77, 131.57, 130.15, 129.28, 128.89, 127.59, 126.84, 123.91, 121.93, 69.22, 61.29, 59.00, 56.82, 56.71, 51.88, 48.15, 46.29, 45.17, 38.18, 35.62, 35.36, 29.38, 26.90, 26.66, 25.69, 22.88, 16.44; HRMS (ESI, positive) m/z calcd for $C_{54}H_{69}N_{10}O_7S_3$ $(M+H)^+$ 1065.4507, found 1065.4521.



(2*S*,4*R*)-1-((*S*)-3,3-dimethyl-2-(7-(4-((4-((4-(3-(pyridin-3-ylmethyl)thioureido)phenyl)sulfon-yl)piperazin-1-yl)methyl)benzamido)heptanamido)butanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (A4) White solid, yield 17%, purity 98.9%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.46 (s, 1H), 8.99 (s, 1H), 8.85 (s, 1H), 8.59 (s, 1H), 8.49 (d, J = 4.0 Hz, 1H), 8.35–8.40 (m, 2H), 7.83–7.90 (m, 2H), 7.76–7.81 (m, 4H), 7.65 (d, J = 8.7 Hz, 2H), 7.43–7.45 (m, 2H), 7.36–7.39 (m, 3H), 7.32 (d, J = 7.6 Hz, 2H), 5.10 (d, J = 3.0 Hz, 1H), 4.92 (t, J = 7.2 Hz, 1H), 4.80 (d, J = 5.1 Hz, 2H), 4.51 (d, J = 9.4 Hz, 1H), 4.44 (t, J = 8.1 Hz, 1H), 4.26–4.31 (m, 1H), 3.60–3.62 (m, 2H), 3.52 (s, 2H), 3.18–3.25 (m, 2H), 3.11–3.15 (s, 1H), 2.74–3.00 (m, 4H), 2.40–2.49 (m, 7H), 2.22–2.27 (m, 1H), 2.07–2.15 (m, 1H), 1.98–2.04 (m, 1H), 1.74–1.83 (m, 1H), 1.45–1.52 (m, 4H), 1.38 (d, J = 3.9 Hz, 3H), 1.18 (t, J = 7.2 Hz, 1H), 0.94 (s, 9H), 0.85–0.87 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 181.26, 172.49, 171.07, 170.08, 166.13, 151.89, 149.11, 148.41, 148.21, 135.98, 134.76, 131.56, 130.14, 129.26, 128.86, 127.72, 126.83, 124.00, 121.72, 69.20, 58.99, 56.81, 56.68, 53.90, 48.14, 45.00, 38.16, 35.61, 35.31, 29.48, 28.88, 26.90, 26.70, 25.83, 18.49, 17.18, 16.42; HRMS (ESI, positive) m/z calcd for $C_{56}H_{71}N_{10}O_7S_3$ $(M+H)^+$ 1079.4664, found 1079.4675.

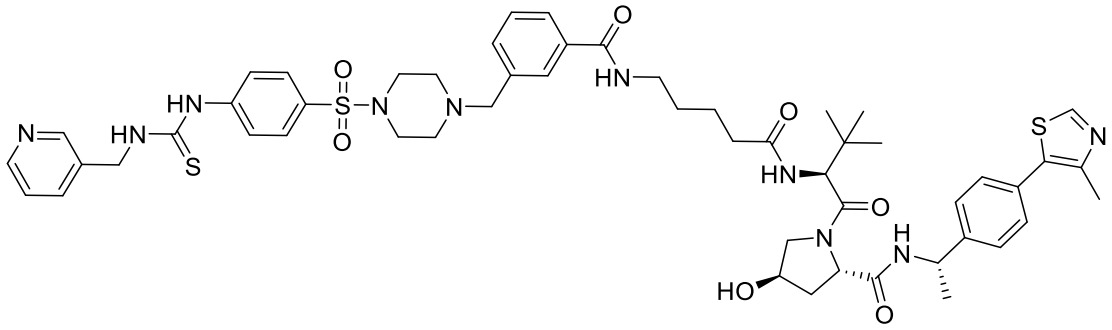


(2*S*,4*R*)-1-((*S*)-3,3-Dimethyl-2-(8-(4-((4-((4-(3-(pyridin-3-ylmethyl)thioureido)phenyl)sulfon-yl)piperazin-1-yl)methyl)benzamido)octanamido)butanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (A5) White solid, yield 23%, purity 95.2%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.17 (s, 1H), 8.98 (s, 1H), 8.68 (s, 1H), 8.57 (s, 1H), 8.47 (d, J = 3.8 Hz, 1H), 8.33-8.38 (m, 2H), 7.73-7.81 (m, 6H), 7.64 (d, J = 8.2 Hz, 2H), 7.36-7.44 (m, 5H), 7.27-7.31 (m, 2H), 5.09 (s, 1H), 4.91 (t, J = 6.8 Hz, 1H), 4.79 (s, 2H), 4.51 (d, J = 9.5 Hz, 1H), 4.42 (t, J = 7.8 Hz, 1H), 4.27 (s, 1H), 4.03–4.14 (m, 1H), 3.59 (s, 2H), 3.49 (s, 2H), 3.13–3.27 (m, 5H), 2.88 (s, 4H), 2.45 (s, 3H), 2.42 (s, 4H), 2.21–2.27 (m, 1H), 2.07–2.13 (m, 1H), 1.96–2.03 (m, 1H), 1.75–1.81 (m, 1H), 1.44–1.50 (m, 4H), 1.37 (d, J = 7.0 Hz, 1H), 1.22–1.24 (m, 2H), 0.92 (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 181.20, 172.50, 171.07, 170.07, 166.33, 151.89, 149.42, 148.66, 148.20, 145.08, 144.48, 141.30, 135.72, 134.61, 134.02, 131.55, 130.14, 129.26, 129.03, 128.89, 127.54, 126.82, 123.87, 121.93, 69.20, 61.39, 58.99, 56.79, 56.68, 51.93, 49.04, 48.13, 46.38, 45.18, 38.16, 35.61, 35.32, 29.57, 29.07, 28.95, 26.88, 25.82, 22.84, 16.42; HRMS (ESI, positive) m/z calcd for $C_{56}H_{73}N_{10}O_7S_3$ $(M+H)^+$ 1093.4820, found 1093.4848.

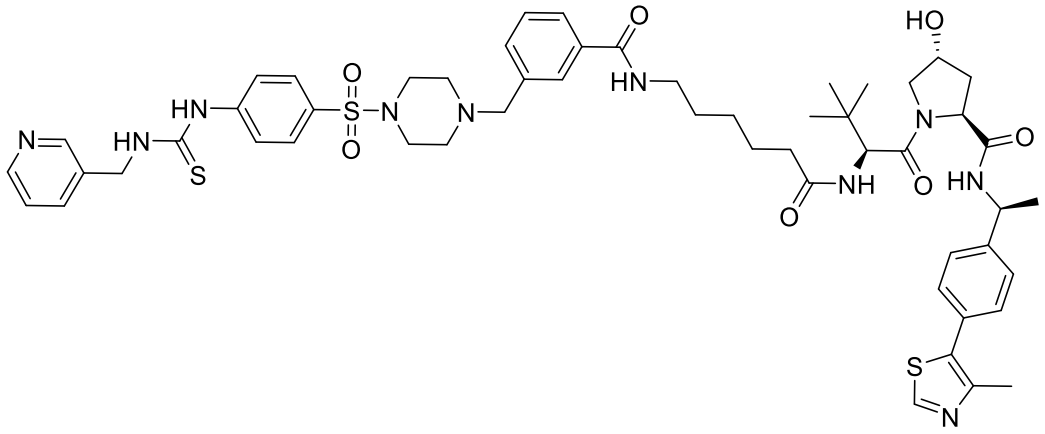


(2*S*,4*R*)-1-((*S*)-3,3-Dimethyl-2-(4-(3-((4-((4-(3-(pyridin-3-ylmethyl)thioureido)phenyl)sulfon-yl)-piperazin-1-yl)methyl)benzamido)butanamido)butanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (B1) White solid, yield 25%, purity 100%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.42 (s, 1H), 8.98 (s, 1H), 8.74–8.86 (m, 1H), 8.57 (s, 1H), 8.47 (d, J = 4.4 Hz, 1H), 8.44 (t, J = 4.5 Hz, 1H), 8.39 (d, J = 7.9 Hz, 1H), 7.89 (d, J = 9.1 Hz, 1H), 7.82-7.86 (m, 2H), 7.77 (d, J = 7.9 Hz, 1H), 7.67–7.73 (m, 2H), 7.63 (d, J = 8.3 Hz, 2H), 7.43 (d, J = 8.0 Hz, 1H), 7.35–7.40 (m, 5H), 5.12 (d, J = 2.9 Hz, 1H), 4.91 (t, J = 6.8 Hz, 1H), 4.79 (d, J = 5.2 Hz, 2H), 4.52 (d, J = 9.1 Hz, 1H), 4.42 (t, J = 8.0 Hz,

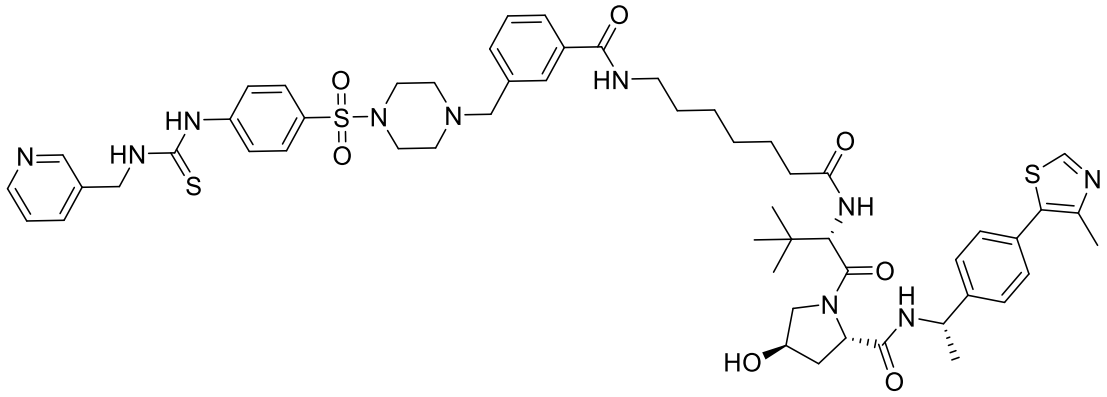
1H), 4.25–4.31 (m, 1H), 3.56–3.65 (m, 2H), 3.50 (s, 2H), 3.19–3.24 (m, 2H), 2.88 (s, 4H), 2.45 (s, 3H), 2.37–2.44 (m, 4H), 2.26–2.32 (m, 1H), 2.16–2.21 (m, 1H), 2.00–2.02 (m, 1H), 1.69–1.75 (m, 2H), 1.62 (s, 1H), 1.36 (d, *J* = 7.0 Hz, 3H), 0.93 (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6) δ:181.22, 172.26, 171.06, 170.01, 166.53, 151.92, 149.36, 148.64, 148.20, 145.10, 144.56, 135.69, 135.04, 134.62, 131.87, 131.56, 130.13, 129.26, 128.84, 128.60, 128.02, 126.82, 126.36, 123.89, 121.75, 69.21, 61.64, 58.99, 56.93, 56.69, 51.92, 48.14, 46.34, 45.05, 38.16, 35.65, 33.10, 26.90, 22.87, 16.42; HRMS (ESI, positive) *m*/*z* calcd for $C_{52}H_{65}N_{10}O_7S_3$ $(M+H)^+$ 1037.4194, found 1037.4192.



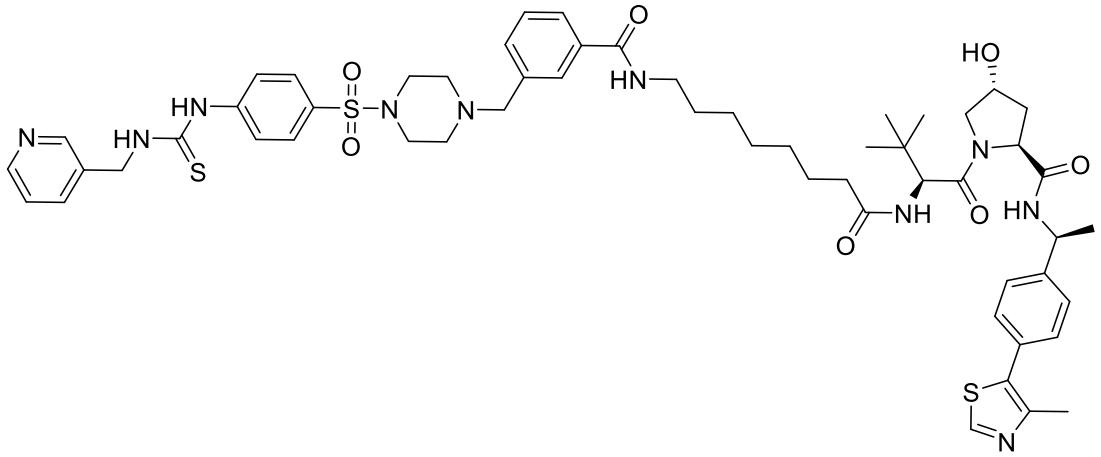
(2*S*,4*R*)-1-((*S*)-3,3-Dimethyl-2-(5-(3-((4-((4-(3-(pyridin-3-ylmethyl)thioureido)phenyl)sulfon-yl)piperazin-1-yl)methyl)benzamido)pentanamido)butanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (B2) White solid, yield 20%, purity 99.4%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.17 (s, 1H), 8.66 (s, 1H), 8.58 (s, 1H), 8.48 (d, *J* = 4.5 Hz, 1H), 8.3-8.41 (m, 2H), 7.81 (d, *J* = 8.7 Hz, 3H), 7.78 (d, *J* = 7.8 Hz, 1H), 7.75 (d, *J* = 8.2 Hz, 2H), 7.66 (s, 1H), 7.65 (s, 1H), 7.37–7.45 (m, 5H), 7.30 (d, *J* = 8.1 Hz, 2H), 5.13 (d, *J* = 3.5 Hz, 1H), 4.88–4.95 (m, 1H), 4.80 (d, *J* = 5.2 Hz, 2H), 4.51 (d, *J* = 9.3 Hz, 1H), 4.43 (t, *J* = 8.2 Hz, 1H), 4.26–4.30 (m, 1H), 3.58–3.63 (m, 1H), 3.51 (s, 2H), 3.19–3.24 (m, 2H), 2.82–2.96 (m, 4H), 2.45 (s, 3H), 2.41–2.45 (m, 4H), 2.22–2.28 (m, 1H), 2.09–2.15 (m, 1H), 1.96–2.05 (m, 2H), 1.77–1.83 (m, 1H), 1.47–1.53 (m, 4H), 1.38 (d, 3H), 0.92 (s, 9H), 0.82–0.89 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ:181.20, 172.54, 171.10, 170.08, 166.36, 151.94, 149.38, 148.67, 148.21, 145.10, 144.16, 141.33, 135.76, 134.64, 133.97, 131.58, 130.15, 129.28, 128.93, 127.56, 126.84, 126.72, 123.92, 121.97, 69.22, 61.39, 59.01, 56.84, 56.71, 51.93, 48.16, 46.40, 45.18, 40.48, 38.16, 35.61, 35.36, 26.89, 25.68, 22.87, 16.43; HRMS (ESI, positive) *m*/*z* calcd for $C_{53}H_{67}N_{10}O_7S_3$ $(M+H)^+$ 1051.4351, found 1051.4346.



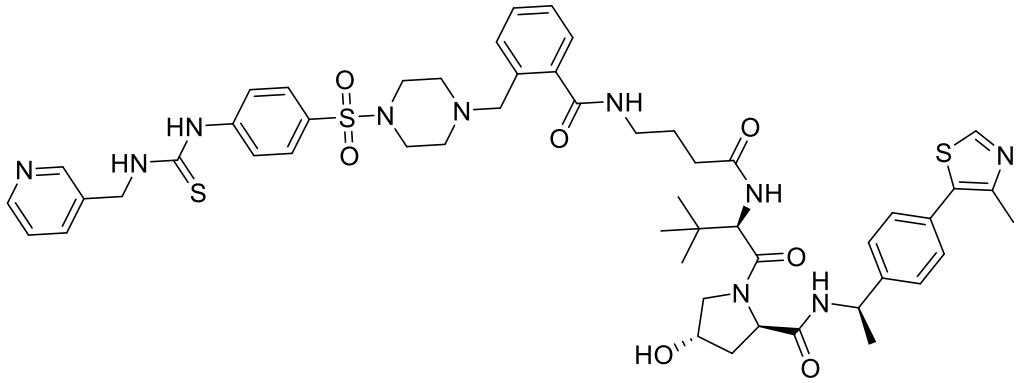
(2*S*,4*R*)-1-((*S*)-3,3-Dimethyl-2-(6-(3-((4-((4-(3-(pyridin-3-ylmethyl)thioureido)phenyl)sulfonyl) piperazin-1-yl)methyl)benzamido)hexanamido)butanoyl)-4-hydro-xy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (B3) White solid, yield 21%, purity 97.1%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.29 (s, 1H), 8.98 (s, 1H), 8.73 (s, 1H), 8.58 (s, 1H), 8.47 (d, J = 3.8 Hz, 1H), 8.36–8.40 (m, 2H), 7.71–7.89 (m, 6H), 7.62–7.66 (m, 2H), 7.42–7.44 (m, 2H), 7.35–7.40 (m, 4H), 7.30 (d, J = 8.3 Hz, 2H), 5.10 (s, 1H), 4.89–4.94 (m, 1H), 4.79 (d, J = 5.4 Hz, 2H), 4.50 (d, J = 9.2 Hz, 1H), 4.42 (t, J = 8.3 Hz, 1H), 4.27 (s, 1H), 3.56–3.63 (m, 2H), 3.50 (s, 2H), 3.20–3.24 (m, 2H), 2.79–2.94 (m, 4H), 2.45 (s, 3H), 2.40–2.44 (m, 4H), 2.24–2.30 (m, 1H), 2.11–2.16 (m, 1H), 1.95–2.03 (m, 2H), 1.76–1.82 (m, 1H), 1.45–1.55 (m, 4H), 1.37 (d, J = 7.1 Hz, 3H), 0.93 (s, 9H), 0.84–0.86 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 181.23, 172.42, 171.08, 170.07, 166.36, 151.93, 149.41, 148.68, 148.22, 145.11, 144.54, 141.37, 135.73, 134.63, 133.99, 131.57, 130.16, 129.28, 128.92, 127.56, 126.85, 124.23, 123.90, 121.85, 119.22, 110.59, 69.23, 61.40, 59.02, 56.85, 56.71, 55.37, 51.96, 48.15, 46.40, 45.14, 39.38, 38.17, 35.65, 35.13, 29.33, 26.92, 23.53, 22.88, 16.44; HRMS (ESI, positive) m/z calcd for $C_{54}H_{69}N_{10}O_7S_3$ $(M+H)^+$ 1065.4507, found 1065.4492.



(2*S*,4*R*)-1-((*S*)-3,3-Dimethyl-2-(7-(3-((4-((4-(3-(pyridin-3-ylmethyl)thioureido)phenyl)sulfon-yl)piperazin-1-yl)methyl)benzamido)heptanamido)butanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (B4) White solid, yield 26%, purity 97.4%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.19 (s, 1H), 8.99 (s, 1H), 8.68 (s, 1H), 8.58 (s, 1H), 8.48 (d, J = 4.6 Hz, 1H), 8.36–8.42 (m, 2H), 7.76–7.82 (m, 4H), 7.64–7.71 (m, 4H), 7.44 (d, J = 7.9 Hz, 2H), 7.35–7.41 (m, 5H), 5.12 (d, J = 3.2 Hz, 1H), 4.88–4.94 (m, 1H), 4.80 (d, J = 5.4 Hz, 2H), 4.52 (d, J = 9.4 Hz, 1H), 4.43 (t, J = 8.0 Hz, 1H), 4.29 (s, 1H), 3.60–3.63 (m, 2H), 3.50 (s, 3H), 3.18–3.25 (m, 2H), 2.83–2.96 (m, 4H), 2.46 (s, 3H), 2.40–2.45 (m, 4H), 2.22–2.30 (m, 1H), 2.09–2.15 (m, 1H), 1.99–2.03 (m, 1H), 1.77–1.82 (m, 1H), 1.40–1.61 (m, 6H), 1.37 (d, J = 7.1 Hz, 3H), 0.94 (s, 9H), 0.86 (s, J = 7.1 Hz, 1H); ^{13}C NMR (600 MHz, DMSO-d_6) δ:181.19, 172.52, 171.07, 170.07, 166.45, 151.92, 149.38, 148.65, 148.20, 145.10, 144.44, 138.35, 135.73, 135.12, 134.62, 131.81, 131.56, 130.13, 129.26, 128.87, 128.58, 127.99, 126.82, 126.31, 123.88, 121.98, 69.20, 61.65, 58.99, 56.81, 56.69, 51.93, 48.14, 46.38, 45.18, 38.16, 35.61, 35.30, 29.48, 28.86, 26.89, 26.71, 25.84, 22.86, 16.42; HRMS (ESI, positive) m/z calcd for $C_{55}H_{71}N_{10}O_7S_3$ $(M+H)^+$ 1079.4664, found 1079.4651.

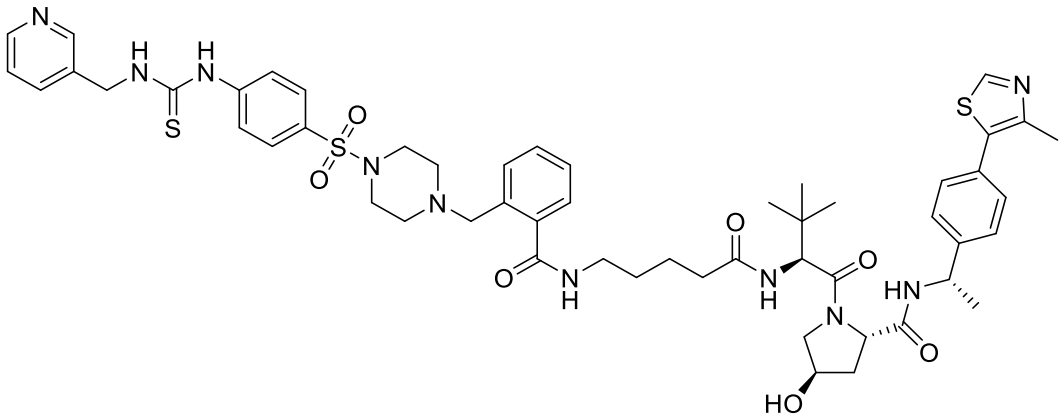


(2*S*,4*R*)-1-((*S*)-3,3-Dimethyl-2-(8-(3-((4-((4-(3-(pyridin-3-ylmethyl)thioureido)phenyl)sulfonyl)piperazin-1-yl)methyl)benzamido)octanamido)butanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (B5) White solid, yield 19%, purity 98.9%. ^{1}H NMR (600 MHz, CD_3OD) δ: 8.88 (s, 1H), 8.59 (d, J = 1.7 Hz, 1H), 8.46 (dd, J = 4.8, 1.5 Hz, 1H), 7.90–7.92 (m, 1H), 7.76–7.79 (m, 2H), 7.73 (s, 1H), 7.72 (s, 1H), 7.71 (s, 1H), 7.68–7.70 (m, 1H), 7.40–7.46 (m, 7H), 5.34–5.37 (m, 1H), 5.00 (q, J = 7.0 Hz, 2H), 4.92 (s, 2H), 4.64 (s, 1H), 4.56–4.58 (m, 2H), 4.42–4.46 (m, 1H), 3.87–3.90 (m, 1H), 3.75 (dd, J = 10.9, 4.4 Hz, 1H), 3.66 (s, 1H), 3.57 (s, 2H), 3.36 (t, J = 7.2 Hz, 2H), 3.03 (s, 4H), 2.54 (s, 4H), 2.49 (s, 3H), 2.26–2.31 (m, 2H), 2.20 (t, J = 7.4 Hz, 2H), 2.02-2.07 (m, 2H), 1.94–1.98 (m, 1H), 1.58–1.65 (m, 6H), 1.50 (d, J = 7.0 Hz, 1H), 1.04 (s, 9H), 1.02 (s, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 172.61, 171.09, 170.08, 166.52, 155.30, 149.13, 148.49, 145.35, 145.07, 138.36, 136.02, 135.47, 135.13, 131.79, 131.49, 130.10, 129.31, 129.26, 128.59, 127.92, 126.82, 126.48, 126.30, 123.93, 117.69, 69.19, 61.62, 58.99, 56.82, 56.67, 51.91, 48.15, 40.94, 38.13, 35.60, 35.55, 35.33, 31.70, 29.50, 29.44, 29.24, 29.16, 29.11, 29.05, 28.99, 28.93, 26.98, 26.87, 25.81, 25.54, 22.82, 22.52, 16.40, 14.37; HRMS (ESI, positive) *m*/*z* calcd for $C_{56}H_{73}N_{10}O_7S_3$ $(M+H)^+$ 1093.4820, found 1093.4827.

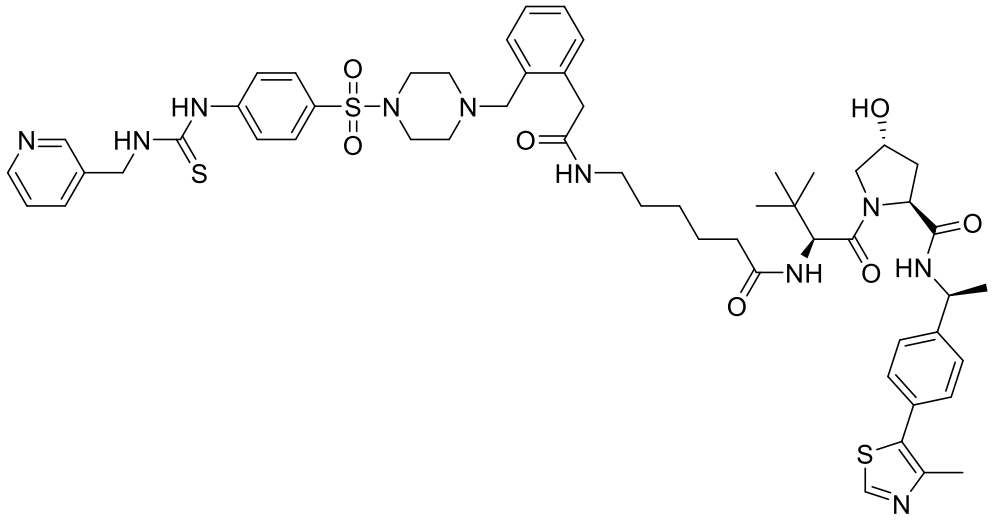


(2*R*,4*S*)-1-((*R*)-3,3-Dimethyl-2-(4-(2-((4-((4-(3-(pyridin-3-ylmethyl)thioureido)phenyl)sulfonyl)piperazin-1-yl)methyl)benzamido)butanamido)butanoyl)-4-hydroxy-*N*-((*R*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (C1) White solid, yield 21%, purity 96.7%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.15 (s, 1H), 8.97 (s, 1H), 8.64 (s, 1H), 8.57 (s, 1H), 8.47 (d, J = 4.6 Hz, 2H), 8.36 (d,

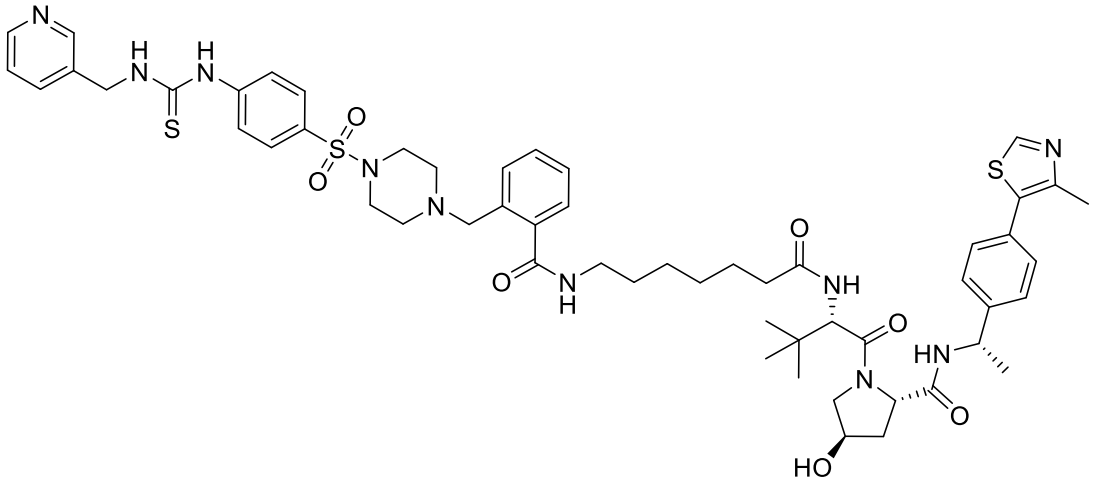
J = 7.6 Hz, 1H), 7.76–7.90 (m, 4H), 7.64 (d, *J* = 8.8 Hz, 2H), 7.41–7.44 (m, 2H), 7.30–7.39 (m, 7H), 5.09–5.15 (m, 1H), 4.92 (t, *J* = 7.1 Hz, 1H), 4.80 (d, *J* = 5.0 Hz, 2H), 4.53 (d, *J* = 9.3 Hz, 1H), 4.44 (t, *J* = 8.2 Hz, 1H), 4.29 (s, 1H), 3.55–3.65 (m, 4H), 3.07–3.02 (m, 2H), 2.86 (s, 4H), 2.45 (s, 3H), 2.41 (s, 4H), 2.24–2.29 (m, 1H), 2.09–2.15 (m, 1H), 2.00–2.05 (m, 1H), 1.78–1.83 (m, 1H), 1.58–1.64 (m, 2H), 1.37 (d, *J* = 6.9 Hz, 3H), 0.95 (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6) δ:181.19, 172.15, 171.05, 170.04, 168.99, 151.90, 149.39, 148.66, 148.21, 145.09, 144.44, 137.91, 135.73, 135.46, 134.61, 131.56, 130.47, 130.15, 129.65, 129.27, 128.83, 128.39, 127.52, 126.90, 126.83, 123.88, 121.92, 69.23, 59.33, 59.02, 56.99, 56.70, 51.72, 48.16, 46.32, 45.19, 40.89, 40.52, 39.03, 38.18, 35.68, 33.08, 26.91, 26.14, 22.85, 16.42; HRMS (ESI, positive) *m*/*z* calcd for $C_{52}H_{65}N_{10}O_7S_3$ $(M+H)^+$ 1037.4194, found 1037.4175.



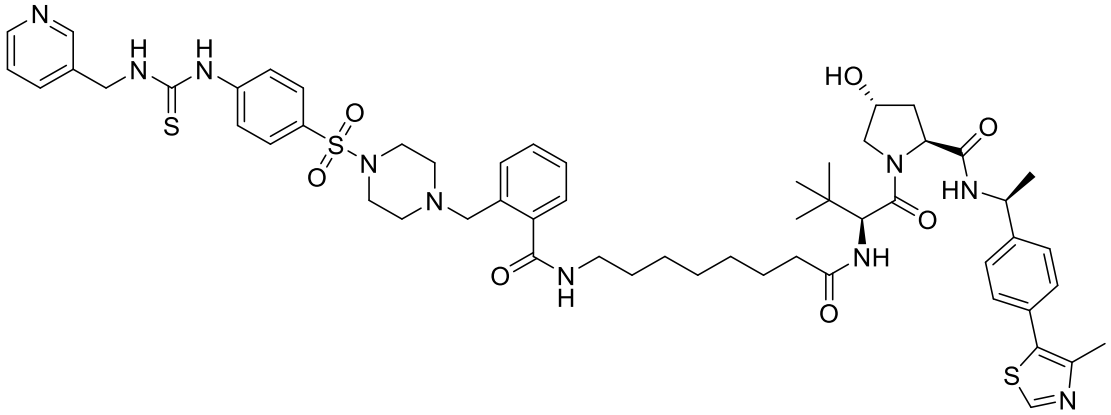
(2*S*,4*R*)-1-((*S*)-3,3-Dimethyl-2-(5-(2-((4-((4-(3-(pyridin-3-ylmethyl)thioureido)phenyl)sulfon-yl)piperazin-1-yl)methyl)benzamido)pentanamido)butanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (C2) White solid, yield 25%, purity 94.8%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.29 (s, 1H), 8.98 (s, 1H), 8.74 (s, 1H), 8.58 (s, 1H), 8.47 (d, *J* = 3.8 Hz, 1H), 8.36–8.40 (m, 2H), 7.73–7.83 (m, 6H), 7.62–7.66 (m, 2H), 7.42–7.45 (m, 2H), 7.35–7.40 (m, 4H), 7.30 (d, *J* = 8.2 Hz, 2H), 5.10 (s, 1H), 4.91 (q, *J* = 7.1 Hz, 1H), 4.79 (d, *J* = 5.5 Hz, 2H), 4.50(d, *J* = 9.3 Hz, 1H), 4.42 (t, *J* = 8.4 Hz, 1H), 4.27 (s, 1H), 3.56–3.64 (m, 2H), 3.50 (s, 2H), 3.20–3.24 (m, 2H), 2.79–2.94 (m, 4H), 2.45 (s, 3H), 2.40–2.44 (m, 4H), 2.24–2.30 (m, 1H), 2.11–2.17 (m, 1H), 1.94–2.03 (m, 2H), 1.76–1.82 (m, 1H), 1.47–1.50 (m, 2H), 1.37 (d, *J* = 7.0 Hz, 3H), 0.93 (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 181.23, 172.42, 171.08, 170.07, 166.36, 151.93, 149.41, 148.68, 148.22, 145.12, 144.54, 141.37, 135.73, 134.63, 133.99, 131.57, 130.16, 129.28, 128.92, 127.58, 126.85, 124.23, 123.90, 121.85, 119.22, 110.59, 69.23, 61.40, 59.02, 56.85, 56.71, 55.37, 51.95, 48.15, 46.40, 39.38, 38.17, 35.65, 35.13, 23.33, 26.92, 23.53, 22.88, 16.44; HRMS (ESI, positive) *m*/*z* calcd for $C_{53}H_{67}N_{10}O_7S_3$ $(M+H)^+$ 1051.4351, found 1051.4346.



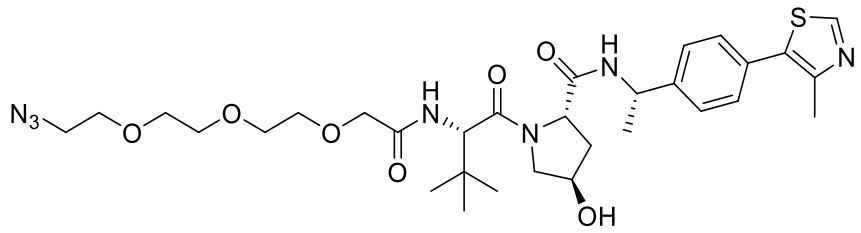
(2*S*,4*R*)-1-((*S*)-3,3-Dimethyl-2-(6-(2-(2-((4-((4-(3-(pyridin-3-ylmethyl)thioureido)phenyl)sulfo-nyl) piperazin-1-yl)methyl)phenyl)acetamido)hexanamido)butanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (C3) White solid, yield 23%, purity 97.9%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.71 (s, 1H), 8.99 (s, 1H), 8.97 (s, 1H), 8.52-8.61 (m, 2H), 8.47 (d, J = 4.7 Hz, 1H), 8.40 (d, J = 7.3 Hz, 1H), 7.93 (d, J = 8.0 Hz, 2H), 7.79 (d, J = 9.4 Hz, 1H), 7.77 (d, J = 7.3 Hz, 2H), 7.63 (d, J = 8.4 Hz, 2H), 7.42–7.44 (m, 2H), 7.36–7.39 (m, 2H), 7.26–7.33 (m, 5H), 5.14 (s, 1H), 4.88–4.96 (m, 1H), 4.80 (d, J = 4.9 Hz, 2H), 4.53 (d, J = 9.1 Hz, 1H), 4.44 (t, J = 8.0 Hz, 1H), 4.3 (s, 1H), 3.61 (s, 4H), 3.06 (s, 2H), 2.85 (s, 4H), 2.29–2.48 (m, 7H), 2.21–2.28 (m, 1H), 2.08–2.15 (m, 1H), 2.00–2.05 (m, 1H), 1.78–1.83 (m, 1H), 1.42–1.49 (m, 2H), 1.36 (d, J = 7.0 Hz, 3H),1.17–1.26 (m, 3H), 0.94 (s, 9H), 0.80–0.87 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 181.24, 172.56, 171.07, 170.08, 168.71, 151.90, 149.29, 148.60, 148.20, 145.11, 144.84, 135.70, 134.64, 131.56, 130.13, 129.26, 128.77, 128.56, 126.83, 123.90, 121.35, 69.21, 59.01, 56.88, 56.70, 55.36, 51.51, 48.16, 44.87, 39.31, 38.17, 35.64, 35.36, 29.23, 26.90, 26.63, 25.55, 22.87, 16.43; HRMS (ESI, positive) *m*/*z* calcd for $C_{54}H_{69}N_{10}O_7S_3$ $(M+H)^+$ 1065.4507, found 1065.4508.



(2*S*,4*R*)-1-((*S*)-3,3-Dimethyl-2-(7-(2-((4-((4-(3-(pyridin-3-ylmethyl)thioureido)phe-nyl)sulfonyl) piperazin-1-yl)methyl)benzamido)heptanamido)butanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (C4) White solid, yield 18%, purity 98.5%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.17 (s, 1H), 8.99 (s, 1H), 8.63 (s, 1H), 8.53-8.60 (m, 2H), 8.49 (d, J = 4.4 Hz, 1H), 8.40 (d, J = 7.7 Hz, 1H), 7.80–7.86 (m, 3H), 7.78 (d, J = 7.7 Hz, 1H), 7.64 (d, J = 8.6 Hz, 2H), 7.44 (d, J = 8.1 Hz, 2H), 7.29–7.40 (m, 7H), 5.14 (d, J = 3.5 Hz, 1H), 4.92 (q, J = 7.2 Hz, 1H), 4.80 (d, J = 5.3 Hz, 2H), 4.54 (d, J = 9.0 Hz, 1H), 4.44 (t, J = 8.1 Hz, 1H), 4.27–4.31 (m, 1H), 3.59–3.64 (m, 2H), 3.56 (s, 2H), 3.06 (q, J = 6.6 Hz, 2H), 2.85 (s, 4H), 2.46 (s, 3H), 2.43 (s, 4H), 2.22–2.30 (m, 1H), 2.10-2.15 (m, 1H), 1.98–2.05 (m, 2H), 1.76–1.83 (m, 1H), 1.41–1.52 (m, 2H), 1.38 (d, J = 7.0 Hz, 3H), 1.30–1.35 (m, 2H), 1.19–1.22 (m, 2H), 0.95 (s, 9H), 0.86 (t, J = 6.4 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ:181.15, 172.61, 171.07, 170.07, 168.74, 151.92, 149.37, 148.66, 148.20, 145.07, 144.54, 138.03, 135.73, 135.21, 134.60, 131.55, 130.67, 130.13, 129.21, 129.26, 128.81, 128.54, 127.63, 126.82, 123.89, 121.71, 69.20, 59.47, 59.00, 56.83, 56.69, 51.60, 48.14, 46.36, 45.12, 38.17, 35.64, 35.35, 29.32, 28.784, 26.89, 26.65, 25.83, 22.87, 16.42; HRMS (ESI, positive) *m*/*z* calcd for $C_{55}H_{71}N_{10}O_7S_3$ $(M+H)^+$ 1079.4664, found 1079.4657.

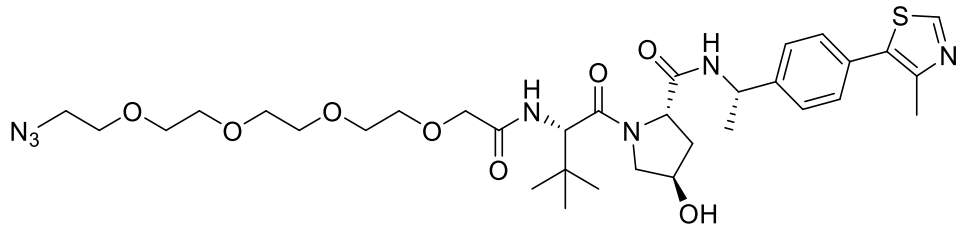


(2*S*,4*R*)-1-((*S*)-3,3-Dimethyl-2-(8-(2-((4-((4-(3-(pyridin-3-ylmethyl)thioureido)phenyl)sulfon-yl)pi-perazin-1-yl)methyl)benzamido)octanamido)butanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (C5) White solid, yield 22%, purity 95.7%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.15 (s, 1H), 8.97 (s, 1H), 8.53-8.64 (m, 3H), 8.48 (d, J = 4.5 Hz, 1H), 8.36 (d, J = 7.5 Hz, 1H), 7.83 (m, J = 8.3 Hz, 2H), 7.74–7.80 (m, 2H), 7.64 (d, J = 8.7 Hz, 2H), 7.43 (d, J = 8.3 Hz, 2H), 7.26–7.41 (m, 7H), 5.10 (s, 1H), 4.86–4.94 (m, 1H), 4.79 (d, J = 5.4 Hz, 2H), 4.52 (d, J = 9.3 Hz, 1H), 4.42 (t, J = 8.0 Hz, 1H), 4.28 (s, 1H), 3.53-3.63 (m, 3H), 3.07 (s, 2H), 2.72–2.95 (m, 4H), 2.36–2.48 (m, 7H), 2.22–2.28 (m, 1H), 2.08–2.13 (m, 1H), 1.99–2.03 (m, 1H), 1.77–1.81 (m, 1H), 1.44–1.52 (m, 2H), 1.37 (d, J = 7.0 Hz, 3H), 1.28–1.36 (m, 2H), 1.17–1.23 (m, 6H), 0.93 (s, 9H), 0.92 (s, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ:181.16, 172.57, 171.06, 170.08, 168.70, 151.92, 149.33, 148.62, 148.20, 134.62, 131.55, 130.14, 129.26, 128.82, 128.60, 126.83, 123.90, 121.71, 69.20, 59.44, 59.00, 56.81, 56.68, 51.59, 48.14, 45.11, 40.89, 38.17, 36.64, 35.38, 29.36, 29.08, 28.83, 26.89, 26.81, 25.82, 22.84, 16.42; HRMS (ESI, positive) *m*/*z* calcd for $C_{56}H_{73}N_{10}O_7S_3$ $(M+H)^+$ 1093.4820, found 1093.4809.

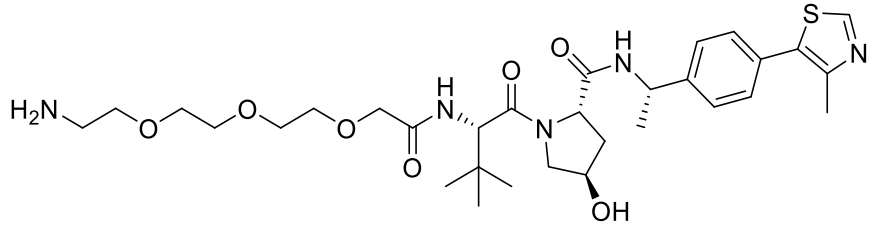


(2*S*,4*R*)-1-((*S*)-14-Azido-2-(*tert*-butyl)-4-oxo-6,9,12-trioxa-3-azatetradecanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxam-ide (11a) The compound **10a** (233 mg, 1 mmol), VHL ligand (444 mg, 1 mmol), HATU (570 mg, 1.5 mmol), DIPEA (194 mg, 1.5mmol) were dissolved in dry DMF (15 mL) and then the mixture was stirred at room temperature for 5 h. The mixture was poured saturated NaCl solution (60 mL) and then extracted with ethyl acetate (20 mL × 3). The organic phases were combined and washed with of saturated NaCl solution (20 mL). After dried over sodium sulfate, it was concentrated and purified by silica gel column chromatography (dichloromethane/methanol=100/2) to obtain the intermediate **11a** as a colorless oil (138 mg, yield 21%). ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.99 (s, 1H), 8.43 (d, J = 8.1 Hz, 1H), 8.23 (d, J = 9.4 Hz, 1H), 7.42 (dd, J = 34.0, 8.0 Hz, 4H), 5.14 (d, J = 3.4 Hz, 1H), 4.93 (t, J = 7.3 Hz, 1H), 4.53 (d, J = 9.0 Hz, 1H), 4.45 (t, J = 8.2 Hz, 1H), 4.30 (s, 1H), 4.10 (q, J = 5.1 Hz, 2H), 3.53–3.67 (m, 4H), 3.15–3.21 (m, 9H), 2.46 (s, 3H), 1.84–1.76 (m, 1H), 1.39 (d, J = 7.1 Hz, 3H), 1.23–1.29 (m, 2H), 0.96 (s, 9H).

The synthetic route of **11b** is similar to that of **11a**.



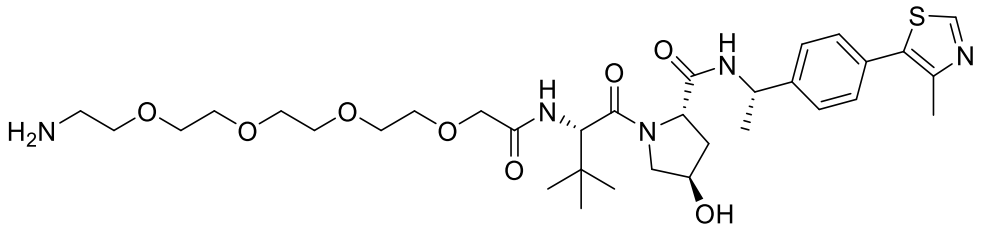
(2*S*,4*R*)-1-((*S*)-17-Azido-2-(*tert*-butyl)-4-oxo-6,9,12,15-tetraoxa-3-azahepta-0decan-oyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (11b) Colorless oil, yield 28%. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.59 (s, 1H), 7.38–7.48 (m, 4H), 7.31 (d, J = 7.9 Hz, 1H), 5.07 (t, J = 7.0 Hz, 1H), 4.77 (t, J = 7.9 Hz, 1H), 4.46–4.52 (m, 2H), 4.17 (d, J = 10.6 Hz, 1H), 3.99–4.07 (m, 2H), 3.64–3.71 (m, 16H), 3.38 (t, J = 4.8 Hz, 2H), 2.67–2.74 (m, 3H), 1.99–2.11 (m, 2H), 1.47 (d, J = 7.1 Hz, 3H), 1.07 (s, 9H).



(2*S*,4*R*)-1-((*S*)-14-amino-2-(*tert*-butyl)-4-oxo-6,9,12-trioxa-3-azatetradecanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (12a) The intermediate **11a** (100 mg, 0.15

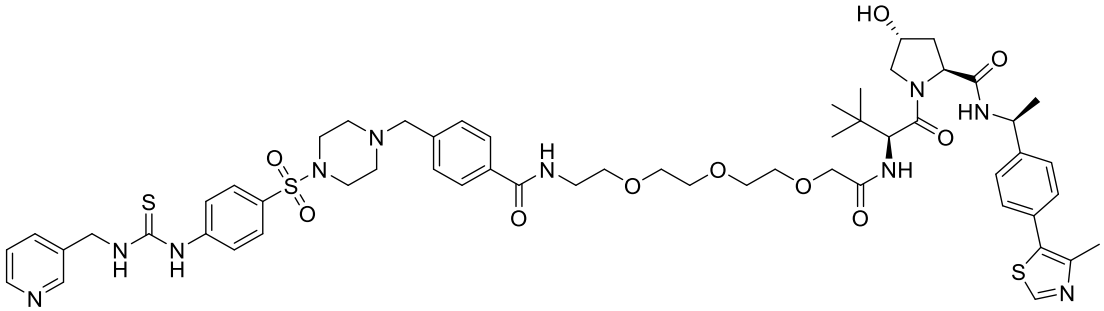
mmol) was dissolved in CH_2Cl_2 (15 mL) and Pd/C (100 mg, 0.6 mmol, 45% purity) was added. The mixture was stirred at room temperature and maintained overnight under the atmosphere of hydrogen. Then the resulting mixture was filtrated through diatomite and the filtrate was concentrated under reduced pressure to afford the intermediate **12a** (96 mg, yield 96%) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.67 (s, 1H), 8.17 (s, 1H), 7.36–7.43 (m, 5H), 5.12 (t, J = 7.5 Hz, 1H), 4.82 (t, J = 8.3 Hz, 1H), 4.66 (d, J = 10.0 Hz, 1H), 4.43 (s, 1H), 4.18 (d, J = 15.2 Hz, 1H), 3.99–4.06 (m, 2H), 3.58–3.81 (m, 14H), 3.08–3.20 (m, 2H), 2.53 (s, 3H), 2.14–2.22 (m, 2H), 1.51 (d, J = 6.7 Hz, 3H), 1.44–1.49 (m, 1H), 0.95 (s, 9H).

The synthetic route of **12b** is similar to that of **12a**.



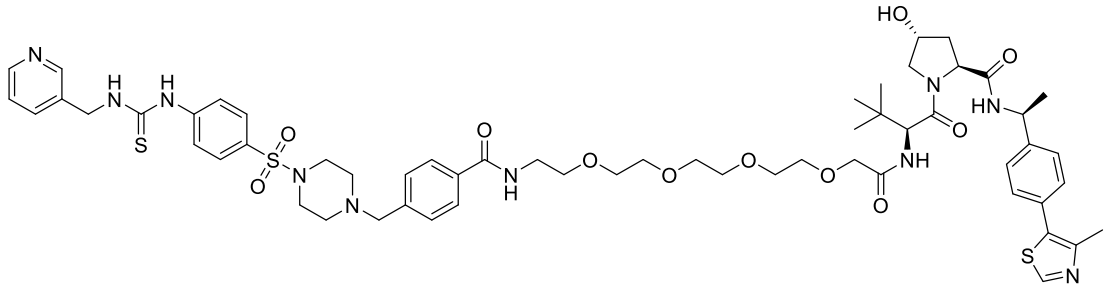
(2*S*,4*R*)-1-((*S*)-17-amino-2-(*tert*-butyl)-4-oxo-6,9,12,15-tetraoxa-3-azaheptadecan-oyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-car-boxamide (12b) Colorless oil, yield 96%. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.75 (s, 1H), 7.48 (d, J = 7.8 Hz, 1H), 7.36–7.44 (m, 6H), 5.08 (t, J = 7.3 Hz, 1H), 4.76 (t, J = 8.3 Hz, 1H), 4.50–4.54 (m, 2H), 4.17 (s, 1H), 4.13–4.17 (m, 1H), 3.75 (s, 1H), 3.66–3.72 (m, 16H), 3.59 (dd, J = 11.5, 3.5 Hz, 1H), 3.39 (q, J = 6.3 Hz, 2H), 2.57–2.63 (m, 1H), 2.56 (s, 3H), 2.05–2.09 (m, 1H), 1.47 (d, J = 7.0 Hz, 3H), 1.07 (s, 9H).

The synthetic routes of **A6** and **A7** is similar to that of **A1**.



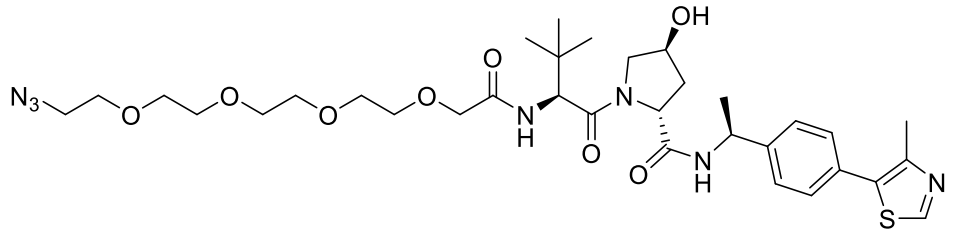
(2*S*,4*R*)-1-((*S*)-15-(*tert*-Butyl)-1,13-dioxo-1-(4-((4-((4-(3-(pyridin-3-ylmethyl)thioureido)phen-yl) sulfonyl)piperazin-1-yl)methyl)phenyl)-5, 8, 11-trioxa-2, 14-diazahexade-can-16-oyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (A6) White solid, yield 29%, purity 99.8%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.14 (s, 1H), 8.98 (s, 1H), 8.63 (s, 1H), 8.58 (s, 1H), 8.48 (d, J = 7.1 Hz, 1H), 8.41 (d, J = 8.9 Hz, 1H), 8.12 (d, J = 9.0 Hz, 1H), 7.91 (d, J = 7.6 Hz, 2H), 7.74–7.84 (m, 4H), 7.65 (d, J = 8.1 Hz, 2H), 7.37–7.44 (m, 7H), 5.12 (s, 1H), 4.67–5.00 (m, 7H), 4.53 (d, J = 9.0 Hz, 1H), 4.44 (t, J = 7.7 Hz, 1H), 4.28 (s, 1H), 3.48–3.65 (m, 6H), 2.89 (m, 4H), 2.33–2.48 (m, 10H), 2.00–2.08 (m, 1H), 1.74–1.83 (m, 1H), 1.38 (d, J = 6.4 Hz, 3H), 1.15–1.35 (m, 3H), 0.95 (s, 9H), 0.79–0.93 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ:181.17, 170.96, 169.48, 166.66, 166.53, 151.86, 149.38, 148.64, 148.17, 145.07, 144.44, 144.34, 135.70, 134.58, 131.53, 130.35, 129.78, 129.29, 128.86, 128.52, 126.79, 123.85,

121.95, 69.19, 62.97, 61.30, 59.03, 56.84, 51.98, 48.15, 46.37, 45.17, 38.14, 36.02, 26.77, 22.83, 16.40; HRMS (ESI, positive) *m*/*z* calcd for $C_{56}H_{73}N_{10}O_{10}S_3$ $(M+H)^+$ 1141.4668, found 1141.4675.



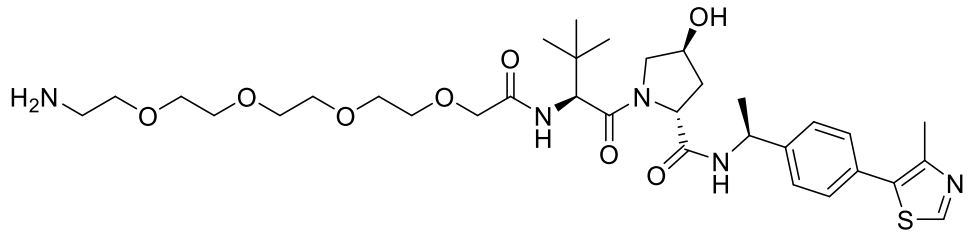
(2*S*,4*R*)-1-((*S*)-18-(*tert*-Butyl)-1,16-dioxo-1-(4-((4-((4-(3-(pyridin-3-ylmethyl)thioureido)phe-nyl) sulfonyl)piperazin-1-yl)methyl)phenyl)-5, 8, 11, 14-tetraoxa-2, 17-diazanonadecan-19-oyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)-pyroledine-2-carboxamide (A7) White solid, yield 23%, purity 99.7%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.15 (s, 1H), 8.99 (s, 1H), 8.64 (s, 1H), 8.58 (d, *J* = 1.7 Hz, 1H), 8.49 (dd, *J* = 5.0, 1.4 Hz, 1H), 8.41–8.46 (m, 2H), 7.79 (dd, *J* = 24.7, 8.7 Hz, 4H), 7.78–7.79 (m, 1H), 7.65 (d, *J* = 8.8 Hz, 2H), 7.43 (d, *J* = 8.2 Hz, 2H), 7.34–7.40 (m, 4H), 7.31 (d, *J* = 8.2 Hz, 2H), 5.13 (d, *J* = 3.5 Hz, 1H), 4.88–4.93 (m, 1H), 4.80 (d, *J* = 5.4 Hz, 2H), 4.55 (d, *J* = 9.6 Hz, 1H), 4.45 (t, *J* = 8.3 Hz, 1H), 4.27–4.31 (m, 1H), 3.96 (s, 2H), 3.51–3.61 (m, 18H), 3.39–3.42 (m, 2H), 2.89 (s, 4H), 2.46 (s, 3H), 2.43 (s, 4H), 2.03–2.08 (m, 1H), 1.75–1.82 (m, 1H), 1.38 (d, *J* = 6.9 Hz, 3H), 0.94 (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6) δ:181.20, 170.90, 169.47, 168.95, 166.51, 151.89, 149.41, 148.67, 148.20, 145.14, 144.45, 141.49, 135.72, 134.60, 133.69, 131.55, 130.14, 129.27, 129.07, 128.91, 127.60, 126.77, 123.87, 70.88, 70.29, 70.21, 70.19, 70.05, 69.35, 69.22, 61.37, 59.01, 56.96, 56.15, 51.94, 48.19, 46.38, 45.19, 38.17, 36.17, 26.67, 22.88, 16.43; HRMS (ESI, positive) *m*/*z* calcd for $C_{58}H_{76}N_{10}O_{11}S_3Na$ $(M+Na)^+$ 1207.4749, found 1207.4753.

The synthetic route of the intermediate **13** is similar to that of **11a**.



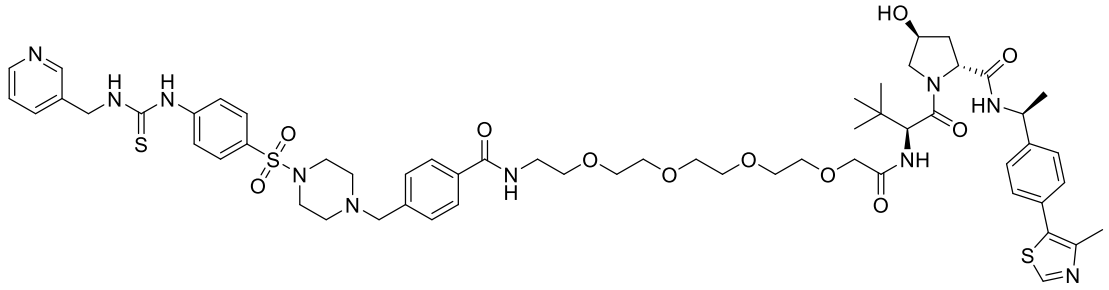
(2*R*,4*S*)-1-((*S*)-17-Azido-2-(*tert*-butyl)-4-oxo-6,9,12,15-tetraoxa-3-azahepta-decanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (13) Colorless oil, yield 18%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.98 (s, 1H), 8.14 (d, *J* = 8.1 Hz, 1H), 7.38–7.50 (m, 6H), 4.86–4.94 (m, 1H), 4.49 (d, *J* = 8.7 Hz, 1H), 4.37–4.43 (m, 1H), 4.30–4.36 (m, 1H), 3.44–3.64 (m, 18H), 3.36 (t, *J* = 5.2 Hz, 2H), 2.46 (s, 3H), 2.00–2.06 (m, 1H), 1.90–1.96 (m, 1H), 1.32 (d, *J* = 7.0 Hz, 3H), 0.99 (s, 9H).

The synthetic route of the intermediate **14** is similar to that of **12a**.



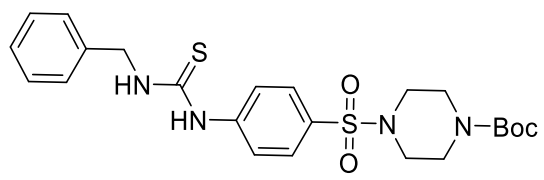
(2*R*,4*S*)-1-((*S*)-17-amino-2-(*tert*-butyl)-4-oxo-6,9,12,15-tetraoxa-3-azahepta-decanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (14) Colorless oil, yield 95%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.83–9.01 (m, 1H), 8.12–8.24 (m, 1H), 7.26–7.52 (m, 7H), 5.21 (s, 1H), 4.83 (t, J = 6.7 Hz, 1H), 4.20–4.49 (m, 4H), 3.61–3.77 (m, 1H), 3.42–3.57 (m, 18H), 2.36 (s, 3H), 1.82–2.02 (m, 2H), 1.27 (d, J = 6.8 Hz, 3H), 0.90 (s, 9H).

The synthetic route of the intermediate **D1** is similar to that of **A1**.



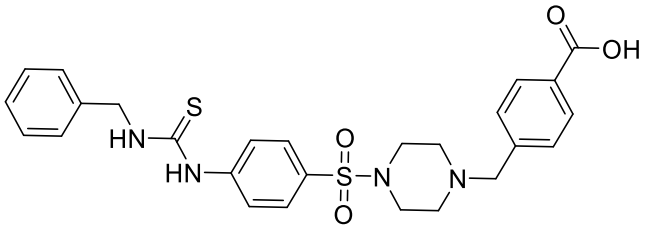
(2*R*,4*S*)-1-((*S*)-18-(*tert*-Butyl)-1,16-dioxo-1-(4-((4-((4-(3-(pyridin-3-ylmethyl)thioureido)phe-nyl) sulfonyl)piperazin-1-yl)methyl)phenyl)-5, 8, 11, 14-tetraoxa-2, 17-diazanonadecan-19-oyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)-pyrrolidine-2-carboxamide (D1) White solid, yield 31%, purity 99.3%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.12 (s, 1H), 8.95 (s, 1H), 8.61 (s, 1H), 8.56 (s, 1H), 8.46 (d, J = 4.2 Hz, 1H), 8.40 (t, J = 5.4 Hz, 1H), 8.11 (d, J = 8.0 Hz, 1H), 7.72–7.80 (m, 5H), 7.63 (d, J = 8.6 Hz, 2H), 7.35–7.44 (m, 6H), 7.28 (d, 2H), 5.11 (d, J = 3.7 Hz, 1H), 4.85-4.91 (m, 1H), 4.78 (d, J = 5.1 Hz, 2H), 4.47 (d, J = 8.7 Hz, 1H), 4.38 (t, J = 6.5 Hz, 1H), 4.28–4.35 (m, 1H), 3.91 (q, J = 15.4 Hz, 2H), 3.70–3.76 (m, 1H), 3.43–3.54 (m, 16H), 3.34–3.37 (m, 2H), 2.86 (s, 4H), 2.43 (s, 3H), 2.38–2.42 (m, 4H), 1.97–2.04 (m, 1H), 1.88–1.95 (m, 1H), 1.37 (s, 1H), 1.30 (d, J = 7.0 Hz, 3H), 0.94 (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 181.20, 170.99, 169.61, 169.48, 166.51, 151.86, 149.41, 148.67, 148.20, 144.77, 144.46, 141.49, 135.73, 134.60, 133.69, 131.57, 130.10, 129.18, 128.90, 127.59, 127.24, 127.03, 123.87, 121.94, 70.80, 70.22, 70.17, 69.93, 69.34, 68.91, 61.38, 59.17, 56.54, 55.81, 51.94, 47.90, 46.38, 45.19, 38.27, 35.27, 26.77, 22.81, 16.40; HRMS (ESI, positive) m/z calcd for $C_{58}H_{77}N_{10}O_{11}S_3$ $(M+H)^+$ 1185.4930, found 1185.4927.

The synthetic route of the intermediate **15** is similar to that of **6**.



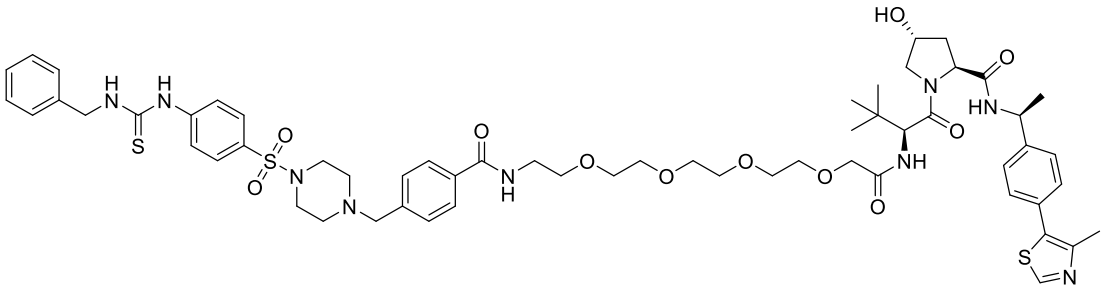
***tert*-Butyl 4-((4-(3-benzylthioureido)phenyl)sulfonyl)piperazine-1-carboxylate (15)** White solid, yield 90%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.06 (s, 1H), 8.54 (s, 1H), 7.83 (d, J = 8.7 Hz, 2H), 7.65 (d, J = 8.7 Hz, 2H), 7.35 (d, J = 4.4 Hz, 4H), 7.26–7.30 (m, 1H), 4.76 (s, 2H), 3.36–3.41 (m, 4H), 2.83 (t, J = 4.8 Hz, 4H), 1.34 (s, 9H).

The synthetic route of the intermediate **17** is similar to that of **8**.



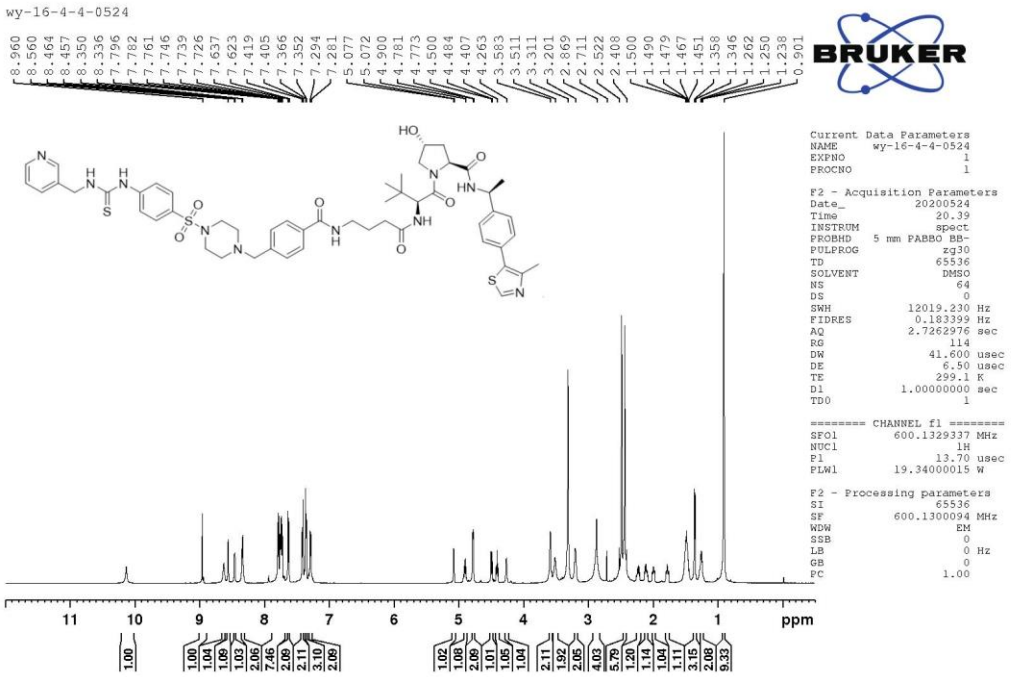
4-((4-((4-(3-Benzylthioureido)phenyl)sulfonyl)piperazin-1-yl)methyl)benzoic acid (17) White solid, yield 46%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 12.81 (s, 1H) ,10.07 (s, 1H), 8.57 (s, 1H), 7.83-7.86 (m, 4H), 7.64–7.65 (m, 2H), 7.34–7.36 (m, 6H), 7.26–7.29 (m, 1H), 4.76 (d, J = 4.7 Hz, 2H), 3.53 (s, 2H), 2.89 (s, 4H), 2.44 (s, 4H).

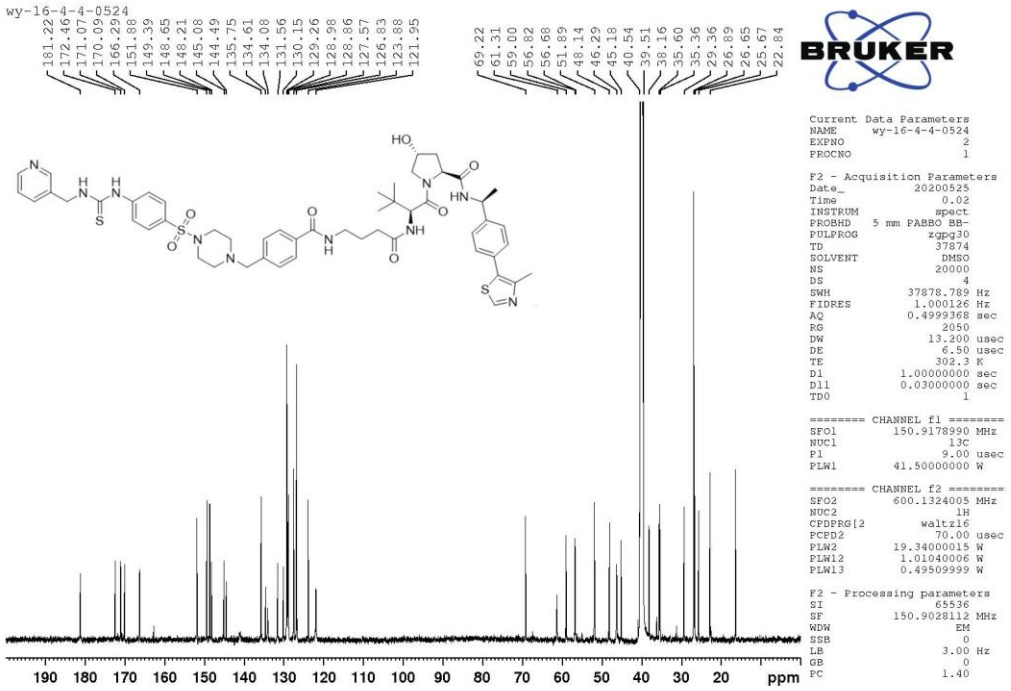
The synthetic route of compound **E1** is similar to that of **A1**.

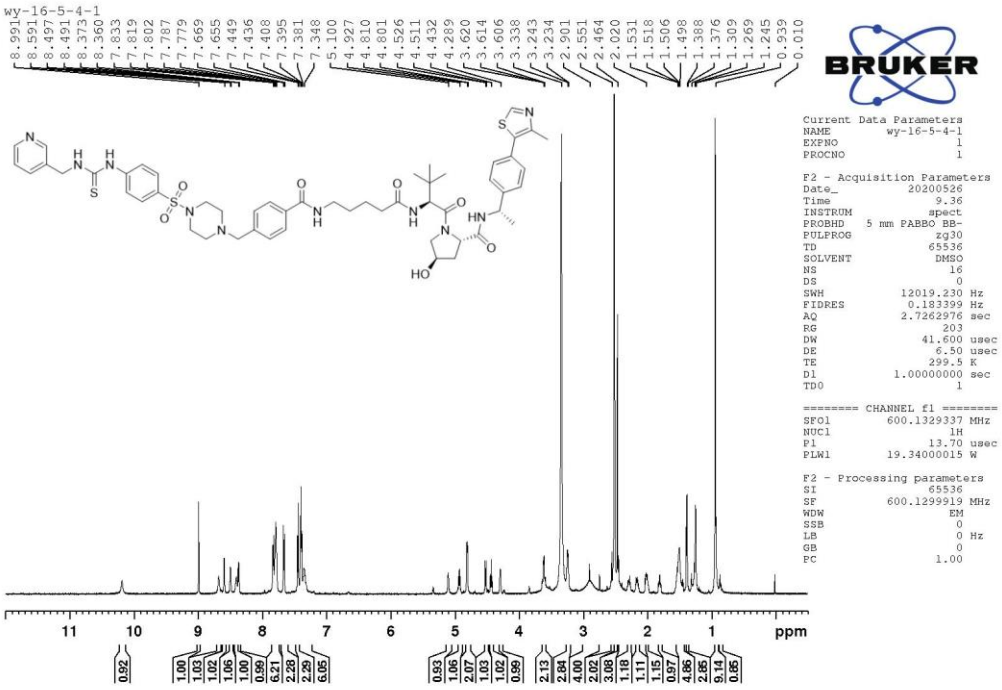


(2*S*,4*R*)-1-((*S*)-1-(4-((4-((4-(3-benzylthioureido)phenyl)sulfonyl)piperazin-1-yl)-methyl)phen-yl)-18-(*tert*-butyl)-1,16-dioxo-5, 8, 11, 14-tetraoxa-2,17-diazanonade-can-19-oyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrole-dine-2-carboxamide (E1) White solid, yield 14%, purity 95.8%. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.04 (s, 1H), 8.53 (s, 1H), 8.37–8.45 (m, 2H), 7.81 (d, J = 8.1 Hz, 2H), 7.74 (d, J = 7.8 Hz, 2H), 7.62 (d, J = 8.5 Hz, 2H), 7.40–7.43 (m, 1H), 7.23–7.38 (m, 10H), 7.06–7.21 (m, 2H), 5.07–5.14 (m, 1H), 4.80–4.93 (m, 1H), 4.75 (d, J = 4.4 Hz, 2H), 4.53 (d, J = 9.4 Hz, 1H), 4.38–4.45 (m, 1H), 4.26 (s, 1H), 3.86–3.97 (m, 2H), 3.47–3.60 (m, 18H), 3.35–3.41 (m, 2H), 2.86 (s, 4H), 2.43 (s, 1H), 2.41 (s, 4H), 1.98–2.07 (m, 1H), 1.68–1.80 (m, 1H), 1.35 (d, J = 6.6 Hz, 3H), 1.18–1.24 (m, 2H), 0.92 (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 206.47, 180.98, 170.90, 169.47, 168.96, 166.53, 151.89, 148.20, 145.13, 144.61, 143.62, 141.50, 138.88, 133.69, 133.49, 131.56, 130.15, 129.87, 129.27, 128.80, 127.99, 127.60, 127.50, 126.78, 126.17, 121.70, 70.89, 70.30, 70.22, 70.19, 70.06, 69.35, 69.22, 61.38, 60.19, 59.01, 56.96, 56.15, 51.94, 49.66, 48.20, 48.10, 47.61, 46.39, 38.17, 36.17, 29.91, 29.46, 26.76, 26.68, 23.01, 22.88, 21.20, 16.43, 14.53; HRMS (ESI, positive) m/z calcd for $C_{59}H_{78}N_9O_{11}S_3$ $(M+H)^+$ 1184.4977, found 1184.5001.

NMR spectra and HRMS reports



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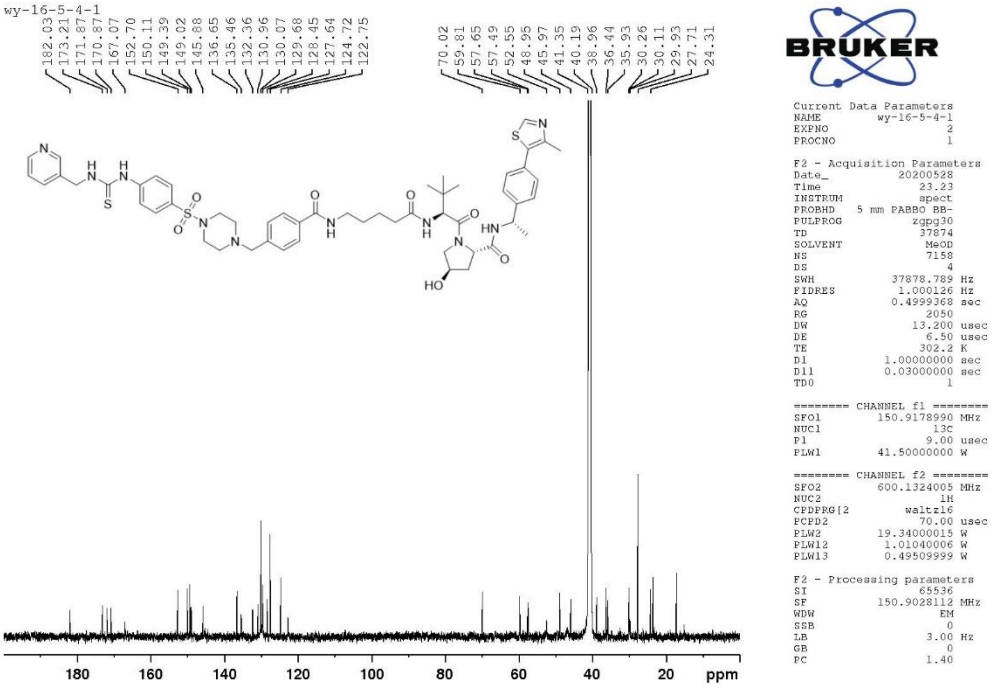
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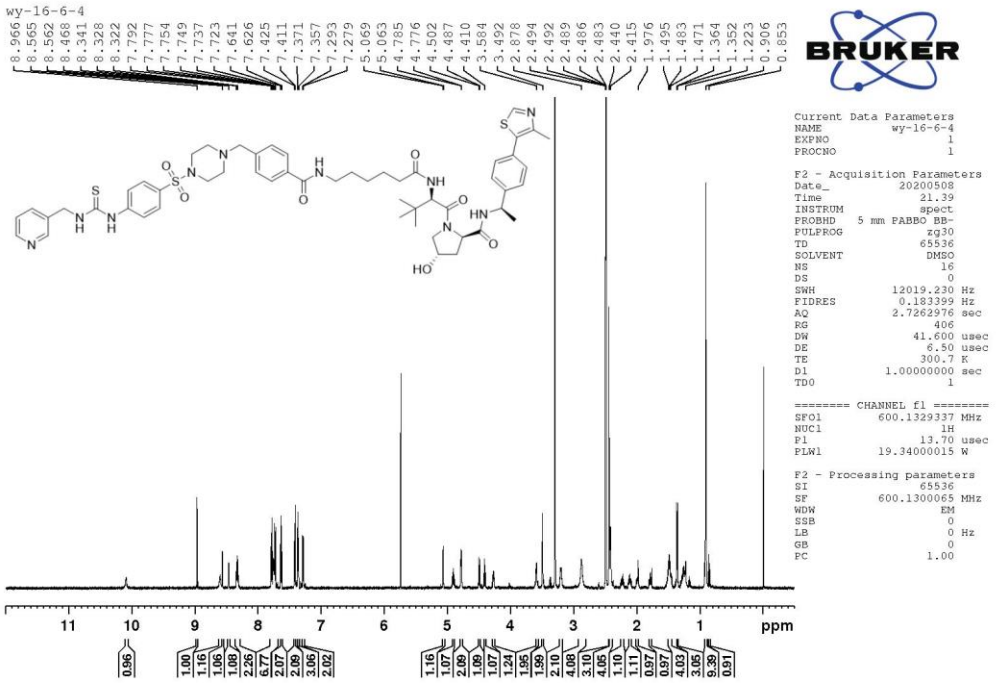
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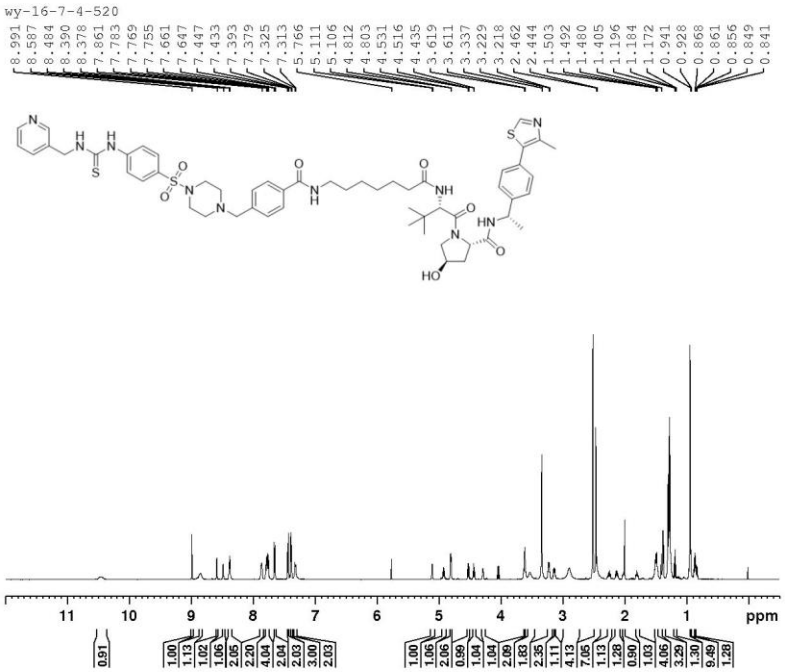

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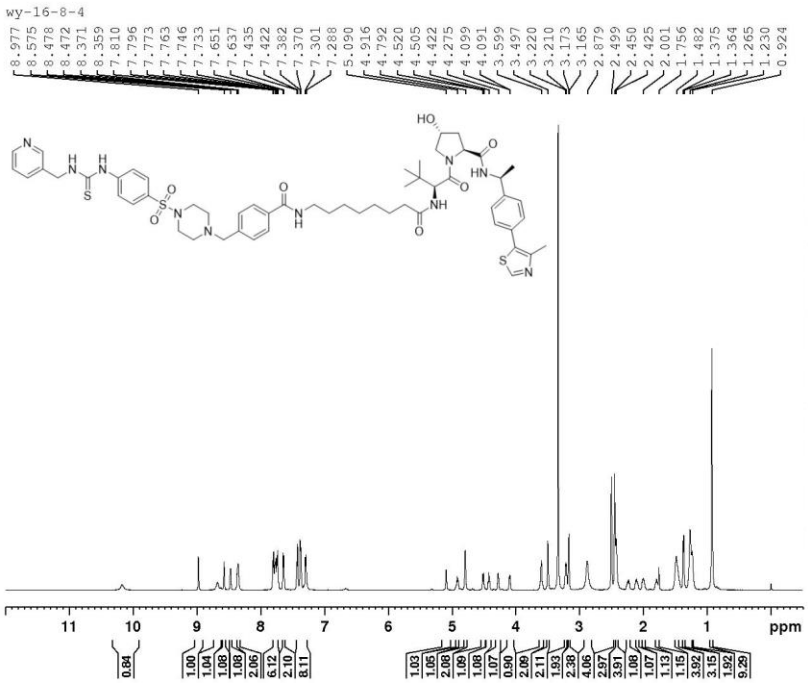

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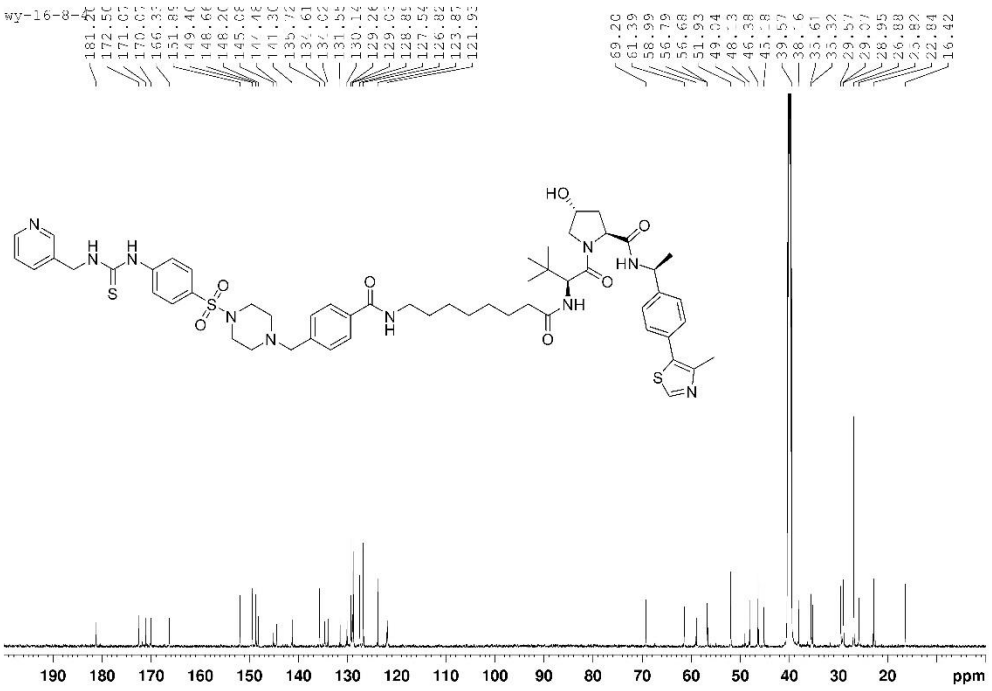

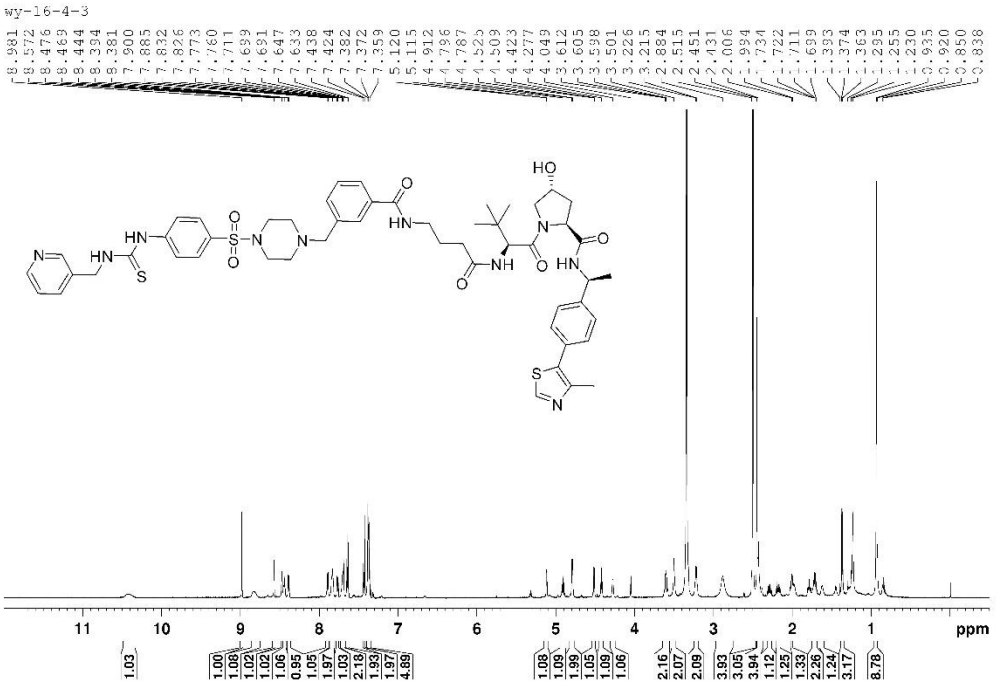

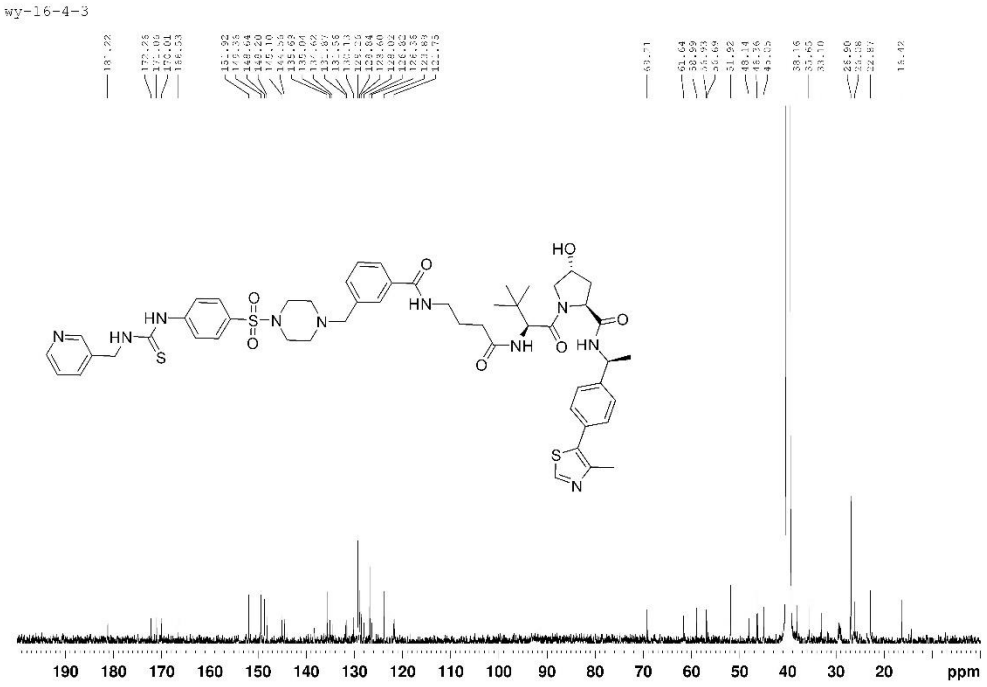
wy-16-4-3
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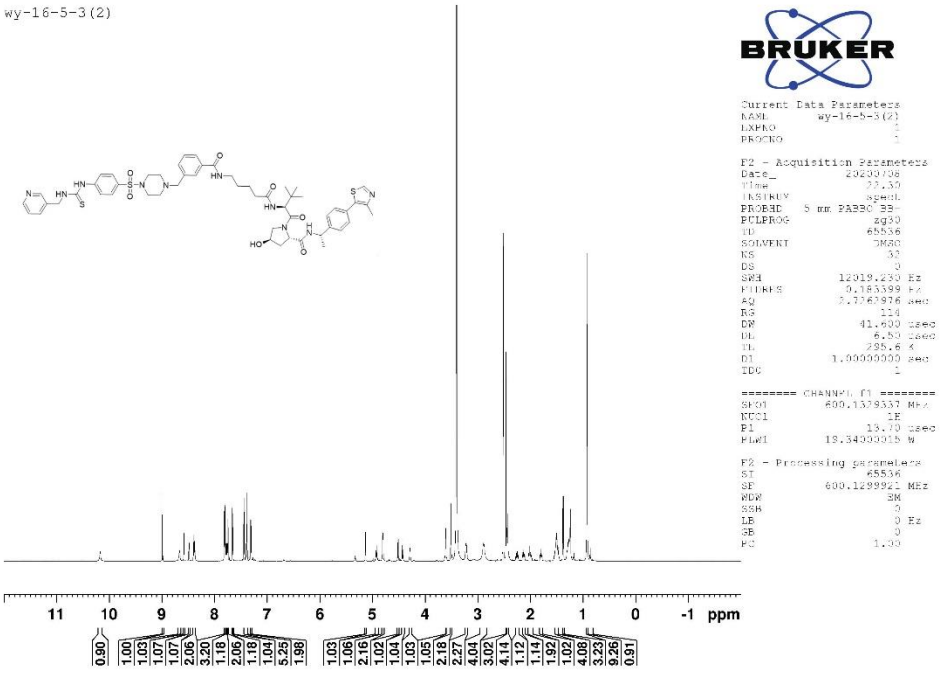
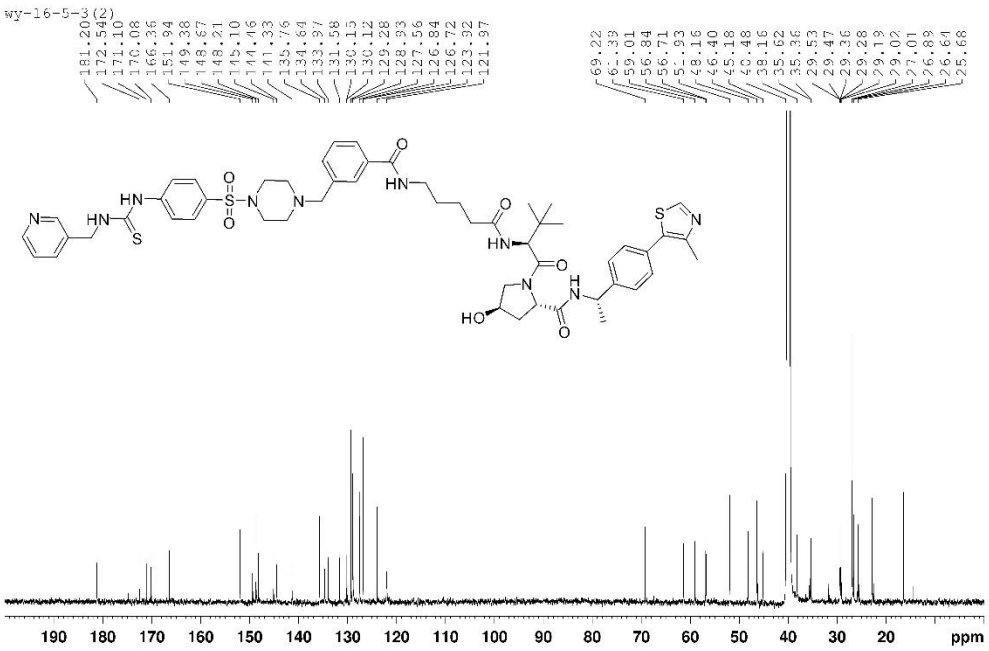

wy-16-5-3(2)
BRUKER
Current Data Parameters
NAME wy-16-5-3(2)
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200708
Time 23.30
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 32
DS 0
SWH 12019.230 Hz
FIDRES 0.183388 Hz
AQ 2.7262976 sec
RG 114
DW 41.600 usec
DE 6.50 usec
TE 295.6 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 600.1329933 MHz
NUC1 1H
P1 13.70 usec
PLW1 18.34000015 W
F2 - Processing parameters
SI 65536
SF 600.1299922 MHz
WDW EM
SSB 0
LB 0 Hz
GB 0
PC 1.00
11 10 9 8 7 6 5 4 3 2 1 0 -1 ppm
0.90 1.00 1.03 1.07 1.07 2.06 3.20 1.18 2.06 1.18 1.04 5.25 1.98 1.03 1.06 2.16 1.02 1.04 1.03 1.05 2.18 2.27 4.04 3.02 4.14 1.12 1.14 1.92 1.02 4.08 3.23 9.26 0.91


wy-16-5-3(2)
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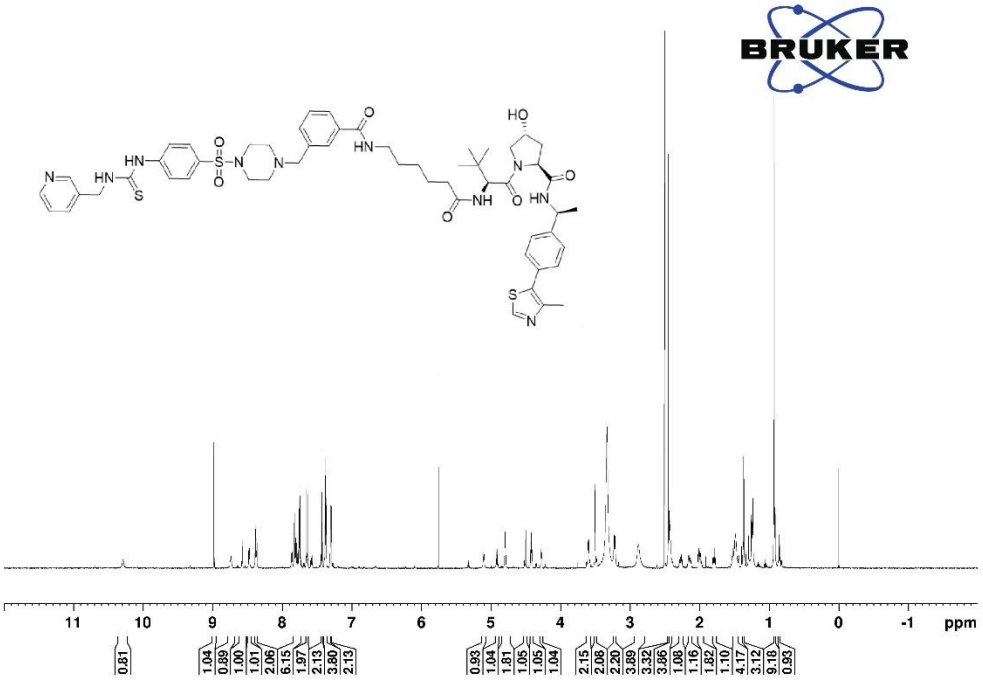
171.10
170.08
166.36
151.94
149.38
148.67
148.21
115.10
114.46
111.33
135.76
134.64
133.97
131.58
130.15
130.12
129.28
128.93
127.56
126.84
126.72
123.92
121.97
69.22
60.33
59.01
56.84
56.71
55.93
48.16
46.40
45.18
40.48
38.16
35.62
35.36
29.53
29.47
29.36
29.28
29.19
29.02
27.01
26.89
26.61
25.68
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm


BRUKER
11 10 9 8 7 6 5 4 3 2 1 0 -1 ppm
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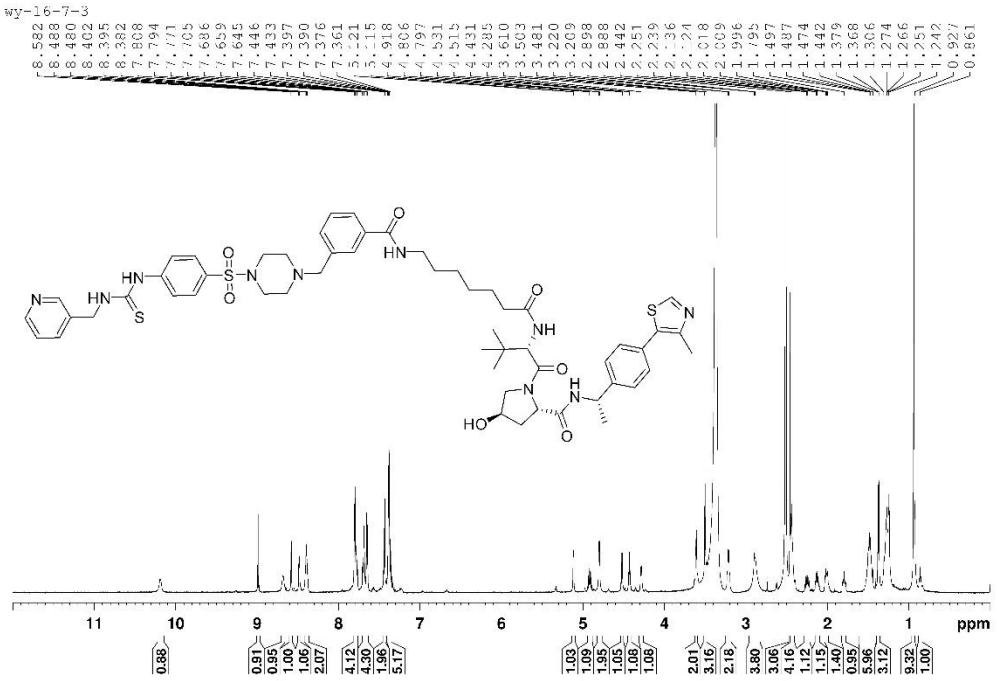
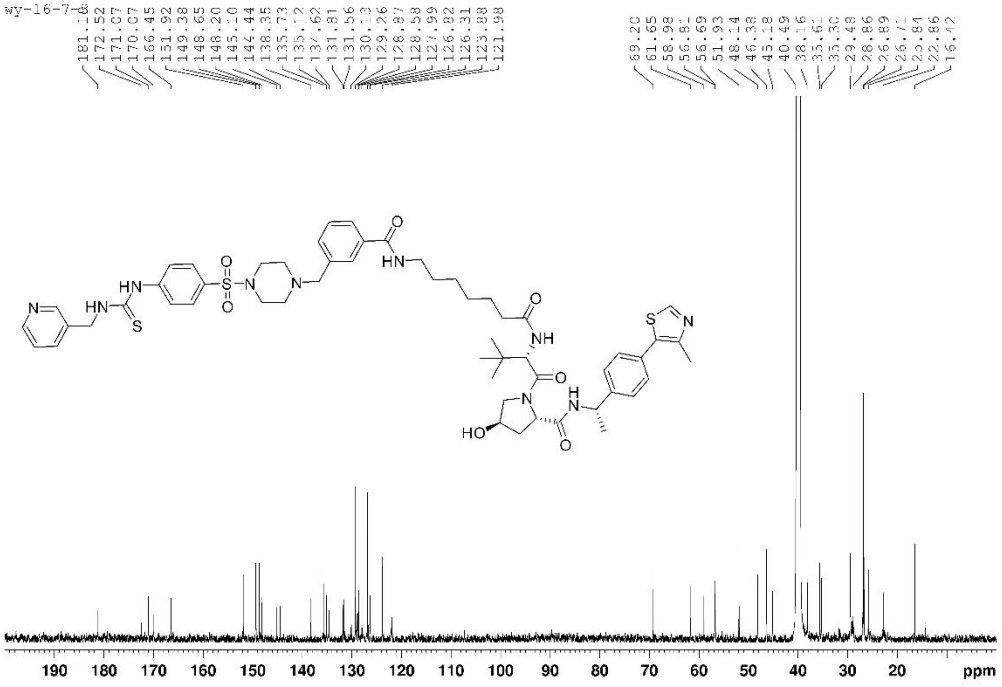
1.04
0.89
1.00
1.01
2.06
6.15
1.97
2.13
3.80
2.13
0.93
1.04
1.81
1.05
1.05
1.04
2.15
2.08
2.20
3.89
3.32
3.86
1.08
1.16
1.82
1.10
4.17
3.12
9.19
0.93


wy-16-7-3
ppm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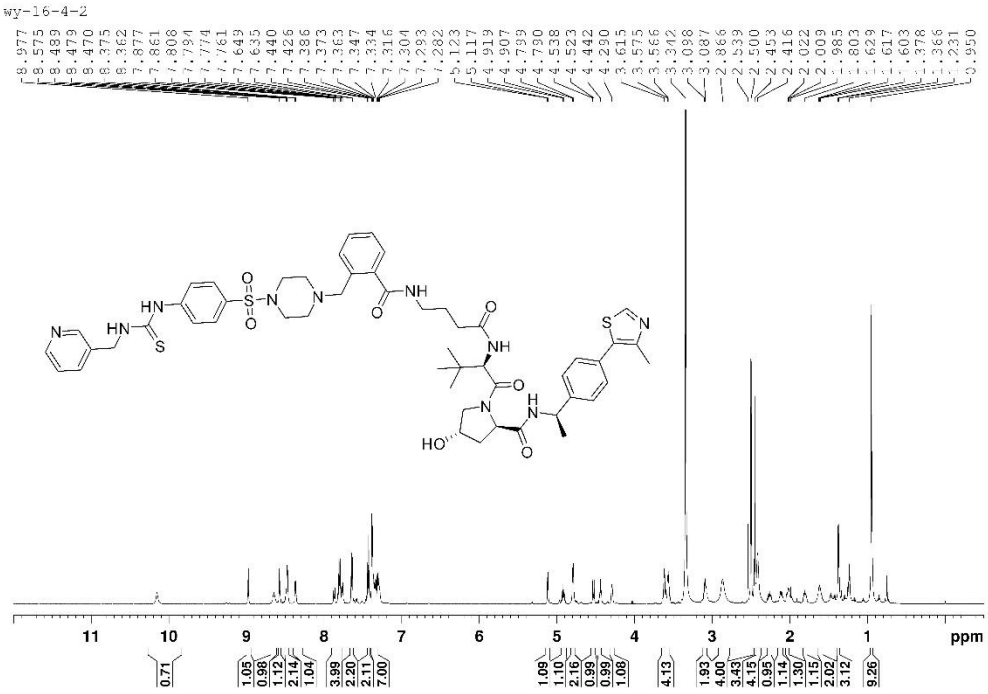
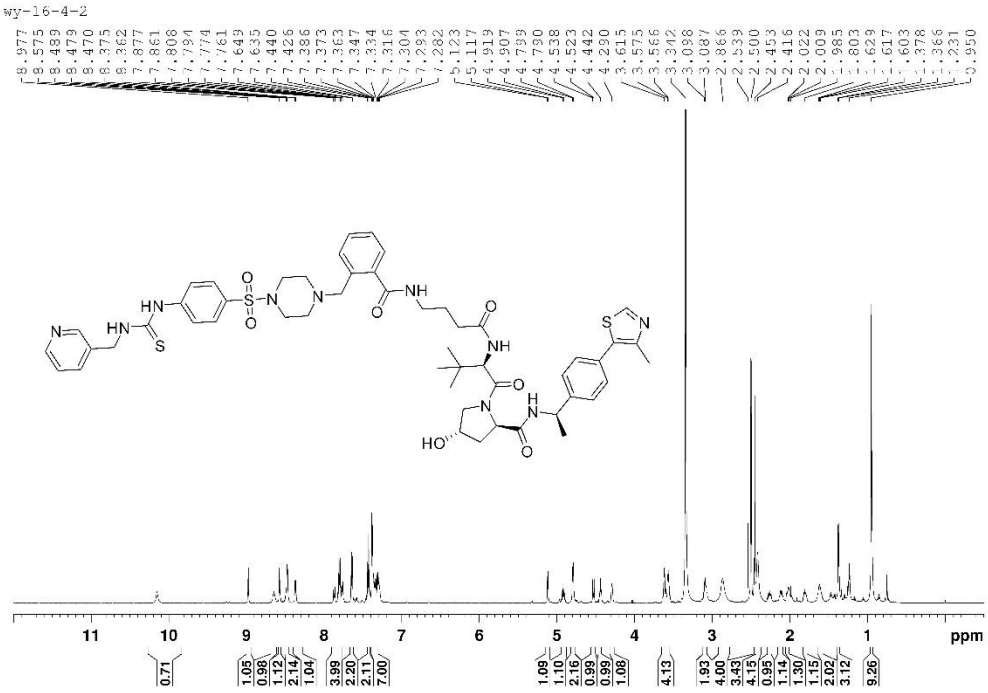
ppm


wy-16-4-2
8.977
8.575
8.489
8.479
8.470
8.375
8.362
7.877
7.861
7.808
7.794
7.774
7.761
7.649
7.635
7.440
7.426
7.386
7.373
7.363
7.347
7.334
7.316
7.304
7.293
7.282
5.123
4.919
4.907
4.799
4.790
4.538
4.523
4.442
4.290
3.615
3.575
3.566
3.342
3.098
3.087
2.866
2.539
2.500
2.453
2.416
2.022
2.009
1.985
1.803
1.629
1.617
1.603
1.378
1.366
1.231
0.950
HN
HN
S
N
O
S
O
N
N
NH
O
NH
O
N
H
N
O
HO
S
N
11
10
9
8
7
6
5
4
3
2
1
ppm
0.71
1.05
0.98
1.12
2.14
1.04
3.99
2.20
2.11
7.00
1.09
1.10
2.16
0.99
0.99
1.08
4.13
1.93
4.00
3.43
4.15
0.95
1.14
1.30
1.15
2.02
3.12
9.26


wy-16-4-2
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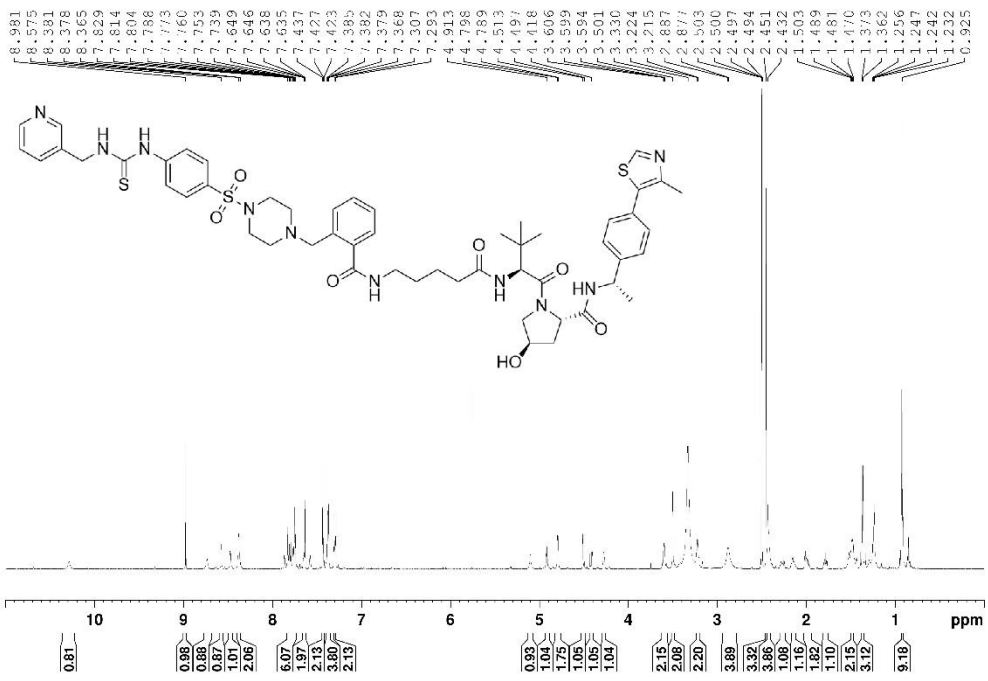
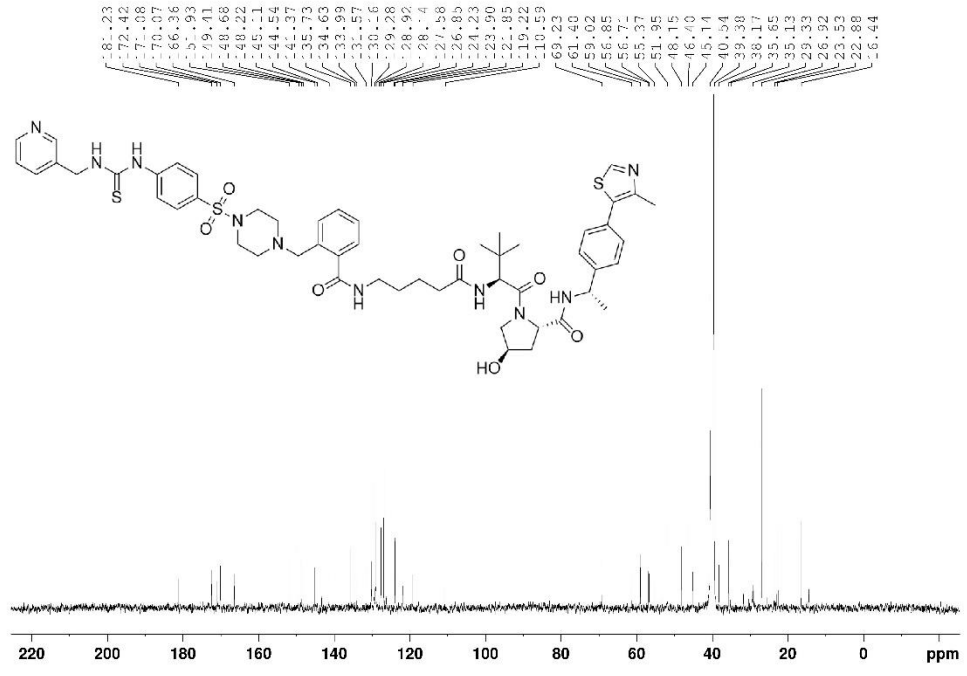
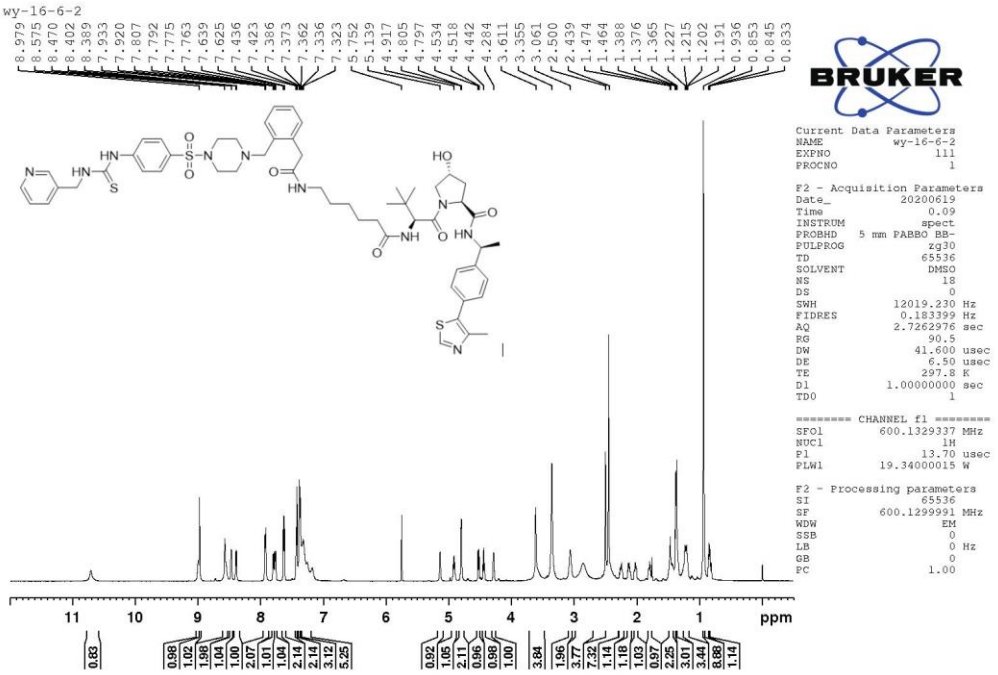
8.575
8.489
8.479
8.470
8.375
8.362
7.877
7.861
7.808
7.794
7.774
7.761
7.649
7.635
7.440
7.426
7.386
7.373
7.363
7.347
7.334
7.316
7.304
7.293
7.282
5.123
4.919
4.907
4.799
4.790
4.538
4.523
4.442
4.290
3.615
3.575
3.566
3.342
3.098
3.087
2.866
2.539
2.500
2.453
2.416
2.022
2.009
1.985
1.803
1.629
1.617
1.603
1.378
1.366
1.231
0.950
HN
HN
S
N
O
S
O
N
N
NH
O
NH
O
N
H
N
O
HO
S
N
11
10
9
8
7
6
5
4
3
2
1
ppm
0.71
1.05
0.98
1.12
2.14
1.04
3.99
2.20
2.11
7.00
1.09
1.10
2.16
0.99
0.99
1.08
4.13
1.93
4.00
3.43
4.15
0.95
1.14
1.30
1.15
2.02
3.12
9.26



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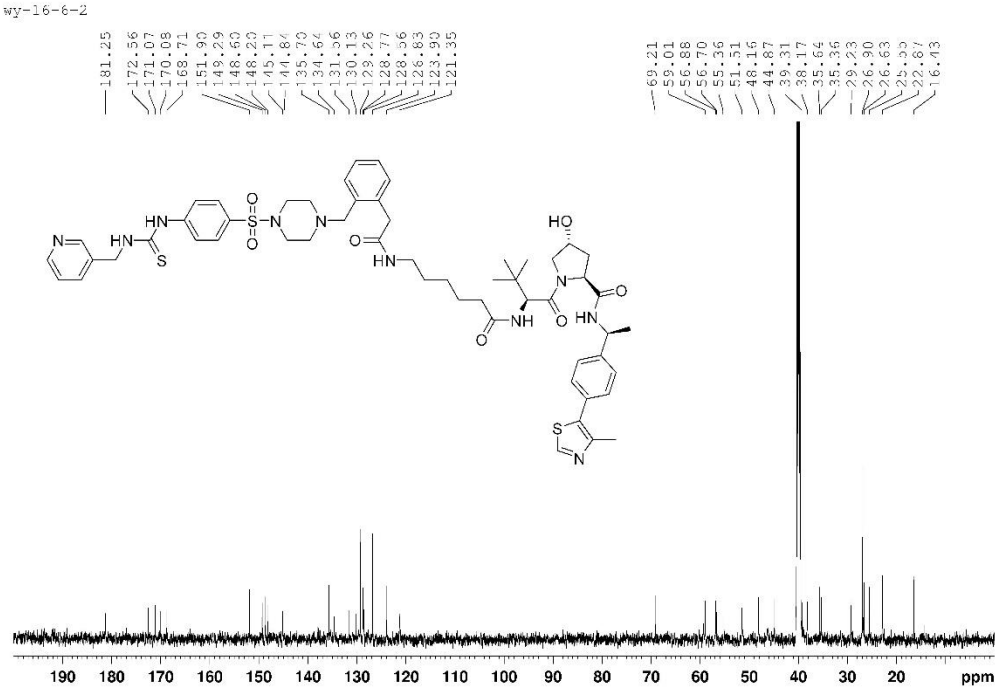
7.807
7.792
7.775
7.763
7.639
7.625
7.436
7.423
7.386
7.373
7.362
7.336
7.323
5.752
5.139
4.917
4.805
4.797
4.534
4.518
4.442
4.284
3.611
3.355
3.061
2.500
2.439
1.474
1.464
1.388
1.376
1.365
1.227
1.215
1.202
1.191
0.936
0.853
0.845
0.833
BRUKER
Current Data Parameters
NAME wy-16-6-2
EXPNO 111
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200619
Time 0.09
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 18
DS 0
SWH 12019.230 Hz
FIDRES 0.183399 Hz
AQ 2.7262976 sec
RG 90.5
DW 41.600 usec
DE 6.50 usec
TE 297.8 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 600.1329337 MHz
NUC1 1H
P1 13.70 usec
PLW1 19.34000015 W
F2 - Processing parameters
SI 65536
SF 600.1299991 MHz
WDW EM
SSB 0
LB 0 Hz
GB 0
PC 1.00
11 10 9 8 7 6 5 4 3 2 1 ppm
0.83
0.98 1.02 1.98 1.04 1.00 2.07 1.01 1.04 2.14 2.14 3.12 5.25
0.92 1.05 2.11 0.96 0.98 1.00
3.84
1.96 3.77 7.32 1.14 1.18 1.03 0.97 2.25 3.01 3.44 8.88 1.14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181.25
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170.08
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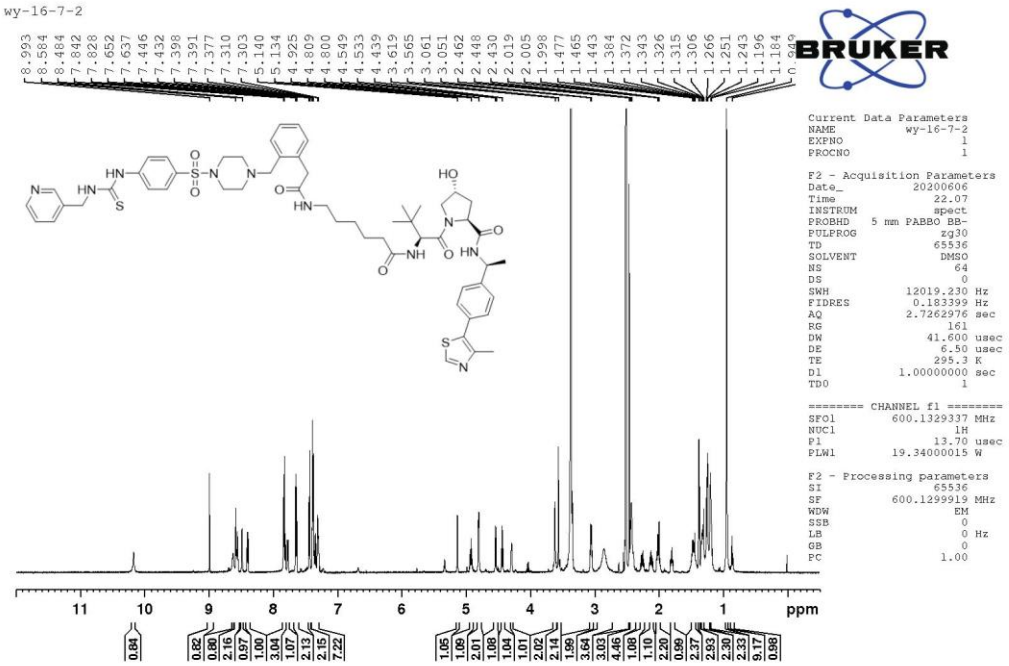
151.90
149.29
148.60
148.23
145.11
141.84
135.70
134.64
131.96
130.13
129.26
128.77
128.36
126.83
123.90
121.35
69.21
59.01
56.88
56.70
55.36
51.51
48.16
44.87
39.31
38.17
35.64
35.36
29.23
26.90
26.63
22.60
22.87
16.43
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm


wy-16-7-2
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NAME wy-16-7-2
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
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Time 22.07
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 64
DS 0
SWH 12019.230 Hz
FIDRES 0.183399 Hz
AQ 2.7262976 sec
RG 161
DW 41.600 usec
DE 6.50 usec
TE 295.3 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 600.1329337 MHz
NUC1 1H
P1 13.70 usec
PLW1 19.34000015 W
F2 - Processing parameters
SI 65536
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SSB 0
LB 0 Hz
GB 0
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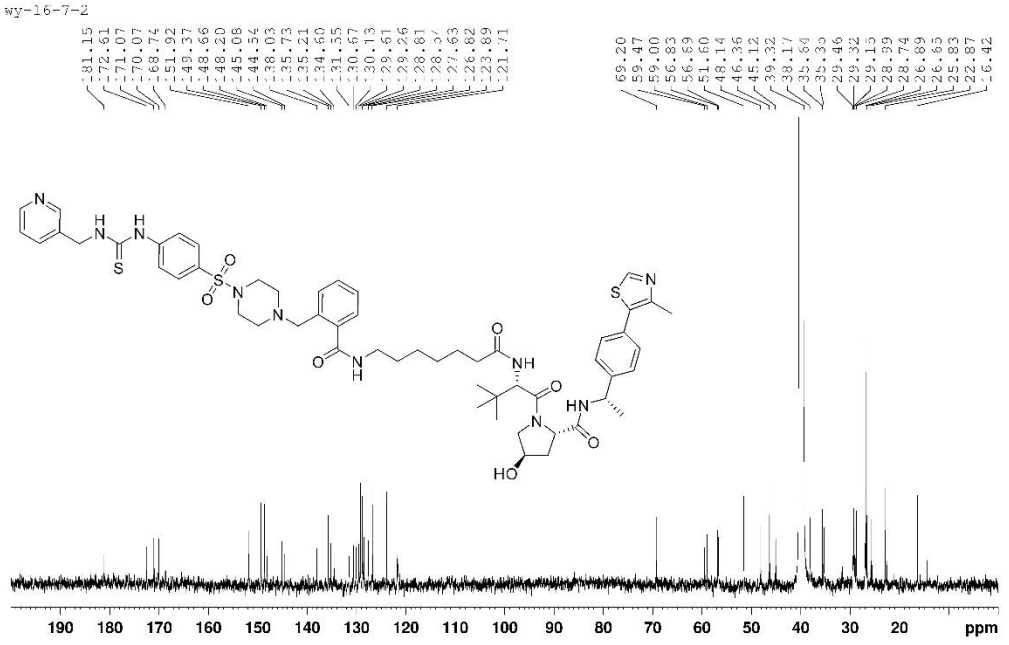
ppm


wy-16-7-2
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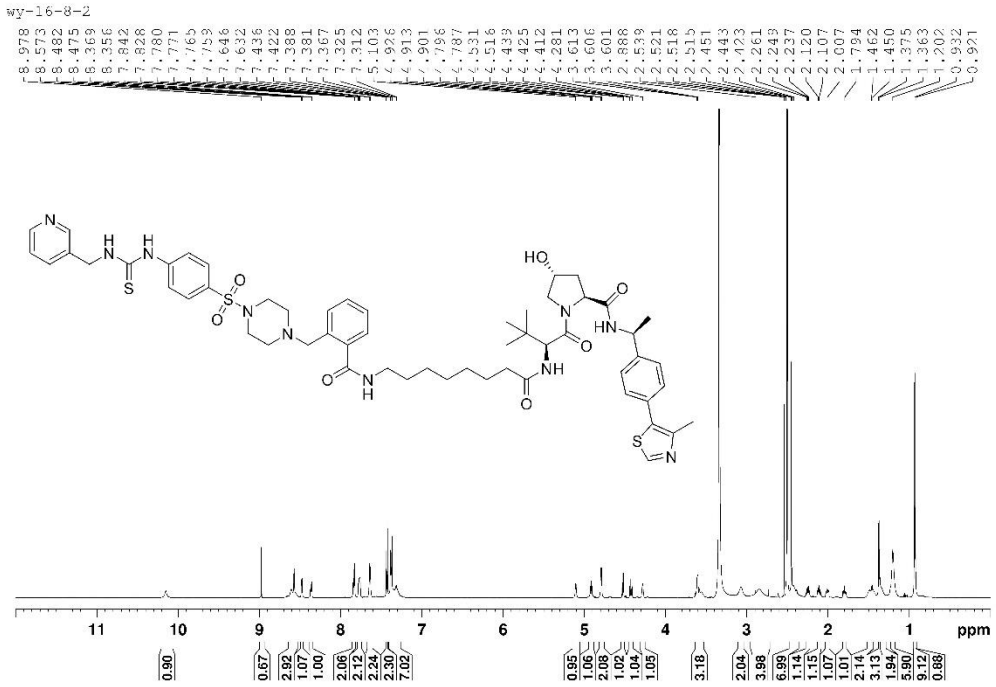
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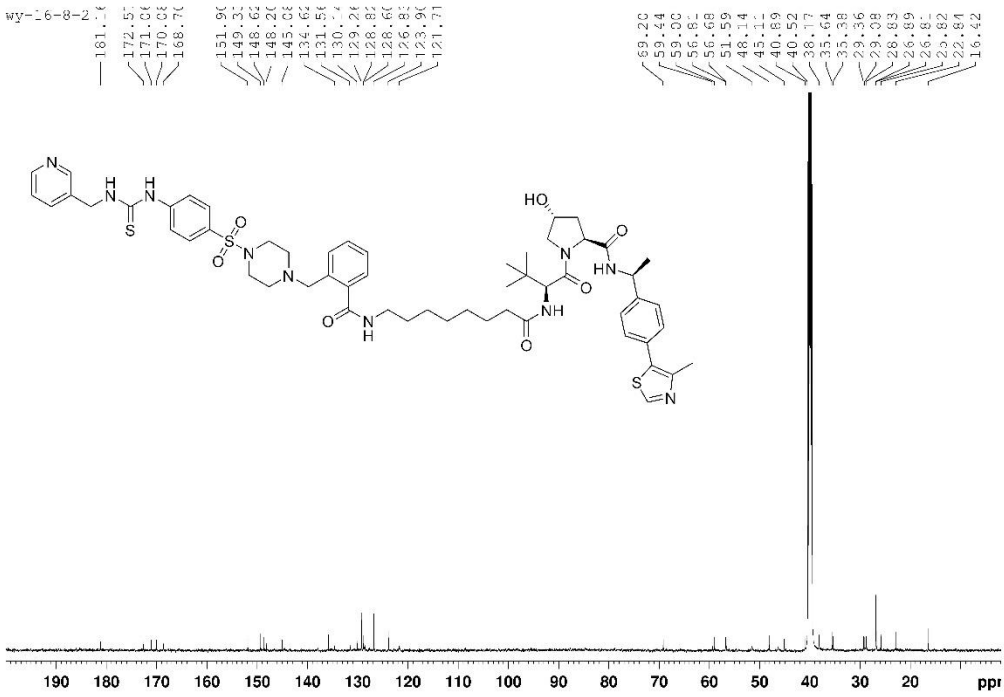
wy-16-8-2
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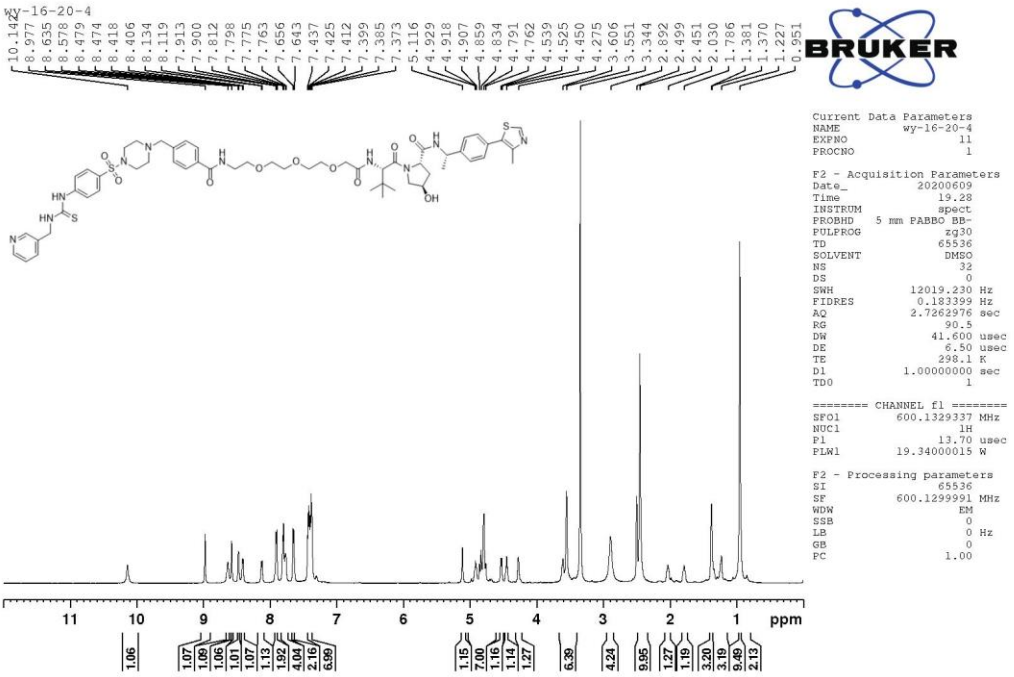
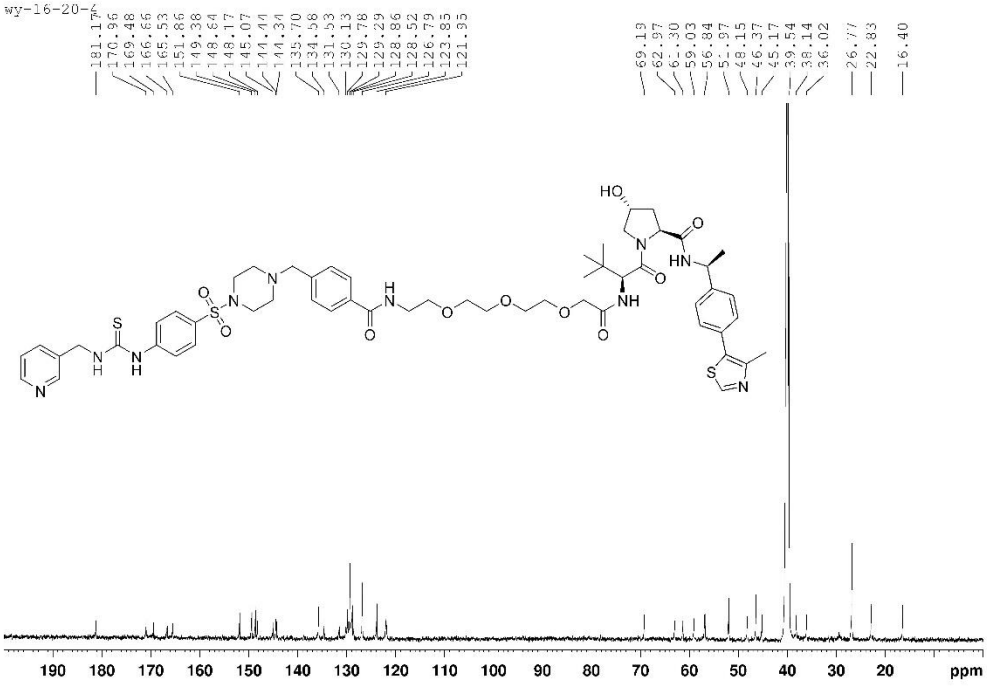

wy-16-20-4
BRUKER
Current Data Parameters
NAME wy-16-20-4
EXPNO 11
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200609
Time 19.28
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 32
DS 0
SWH 12019.230 Hz
FIDRES 0.183399 Hz
AQ 2.7262976 sec
RG 90.5
DW 41.600 usec
DE 6.50 usec
TE 298.1 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 600.1329337 MHz
NUC1 1H
P1 13.70 usec
PLW1 19.34000015 W
F2 - Processing parameters
SI 65536
SF 600.1299991 MHz
WDW EM
SSB 0
LB 0 Hz
GB 0
PC 1.00
ppm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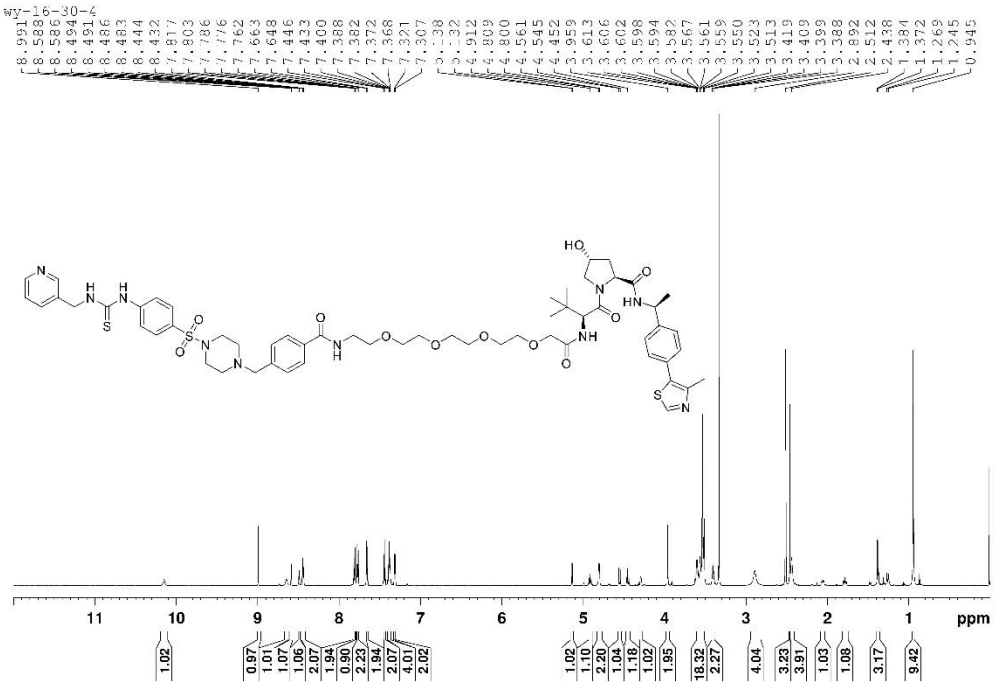
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8.483
8.444
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7.803
7.786
7.776
7.762
7.663
7.648
7.446
7.433
7.400
7.388
7.382
7.372
7.368
7.321
7.307
5.138
5.132
4.912
4.809
4.800
4.561
4.545
4.452
3.959
3.613
3.606
3.602
3.598
3.594
3.582
3.567
3.561
3.559
3.550
3.523
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3.409
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2.892
2.512
2.438
1.384
1.372
1.269
1.245
0.945
HO
HN
NH
ppm
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0.97
1.01
1.07
1.06
2.07
1.94
0.90
2.23
1.94
2.07
4.01
2.02
1.02
1.10
2.20
1.04
1.18
1.02
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18.32
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4.04
3.23
3.91
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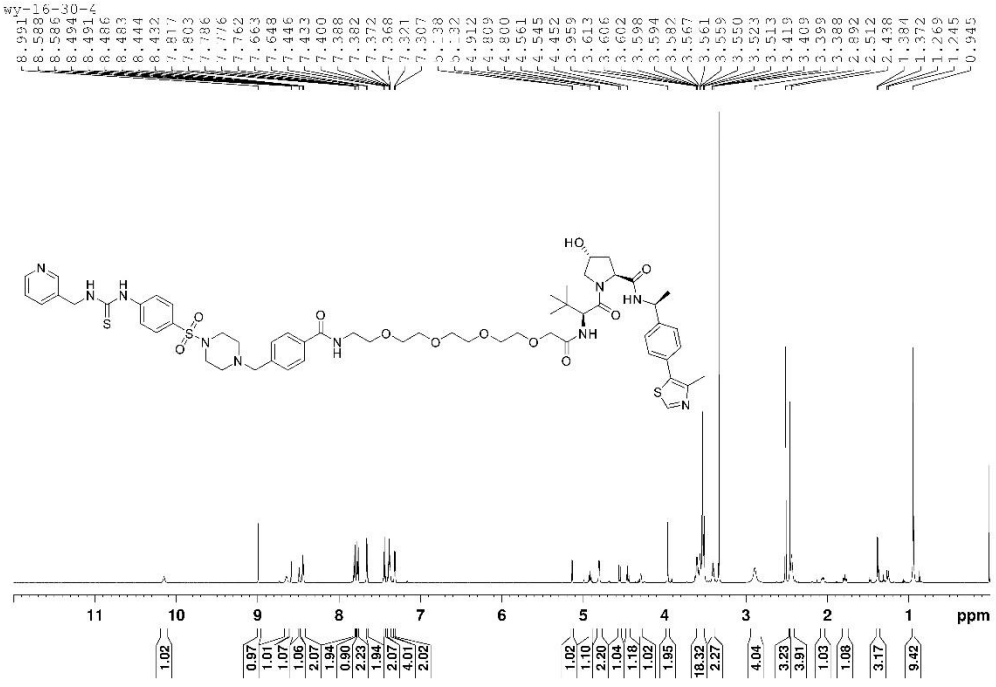
1.08
3.17
9.42


wy-16-30-4
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8.588
8.586
8.494
8.491
8.486
8.483
8.444
8.432
7.817
7.803
7.786
7.776
7.762
7.663
7.648
7.446
7.433
7.400
7.388
7.382
7.372
7.368
7.321
7.307
5.138
5.132
4.912
4.809
4.800
4.561
4.545
4.452
3.959
3.613
3.606
3.602
3.598
3.594
3.582
3.567
3.561
3.559
3.550
3.523
3.513
3.419
3.409
3.399
3.388
2.892
2.512
2.438
1.384
1.372
1.269
1.245
0.945
HO
HN
NH
ppm
1.02
0.97
1.01
1.07
1.06
2.07
1.94
0.90
2.23
1.94
2.07
4.01
2.02
1.02
1.10
2.20
1.04
1.18
1.02
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18.32
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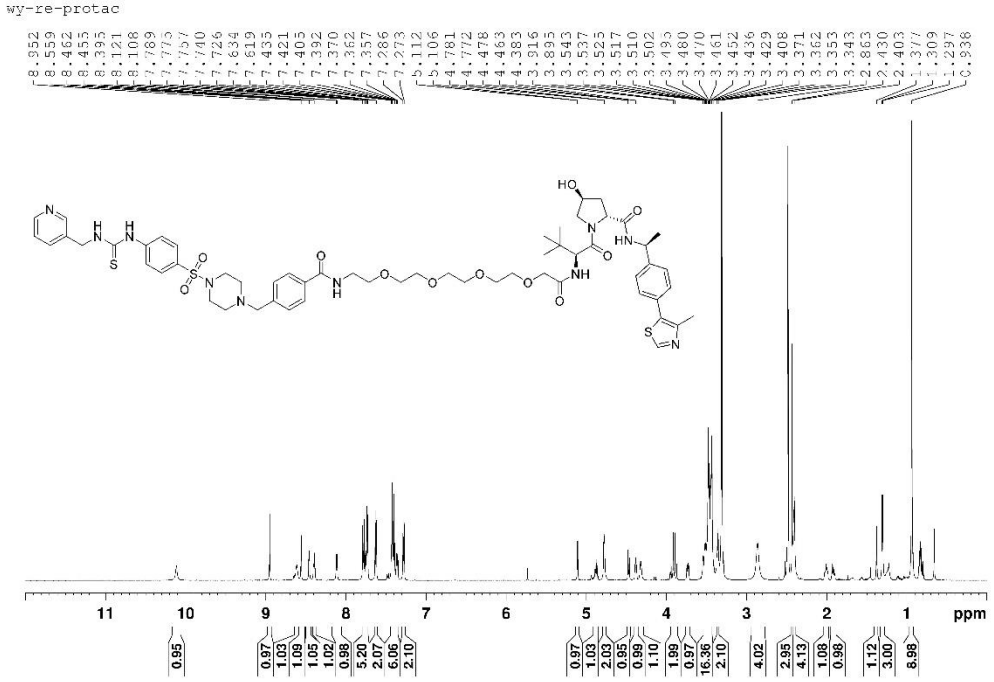
3.23
3.91
1.03
1.08
3.17
9.42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8.108
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7.775
7.757
7.740
7.726
7.634
7.619
7.435
7.421
7.405
7.392
7.370
7.362
7.357
7.286
7.273
5.112
5.106
4.781
4.772
4.478
4.463
4.383
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3.895
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3.537
3.525
3.517
3.510
3.502
3.493
3.480
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3.429
3.408
3.371
3.362
3.353
3.343
2.863
2.430
2.403
1.377
1.309
1.297
0.938
HO
HN
NH
S
N
O
11
10
9
8
7
6
5
4
3
2
1
ppm
0.95
0.97
1.03
1.08
1.05
1.02
0.98
5.20
2.07
6.06
2.10
0.97
1.03
2.03
0.85
0.99
1.10
1.99
0.97
16.36
2.10
4.02
2.95
4.13
1.08
0.98
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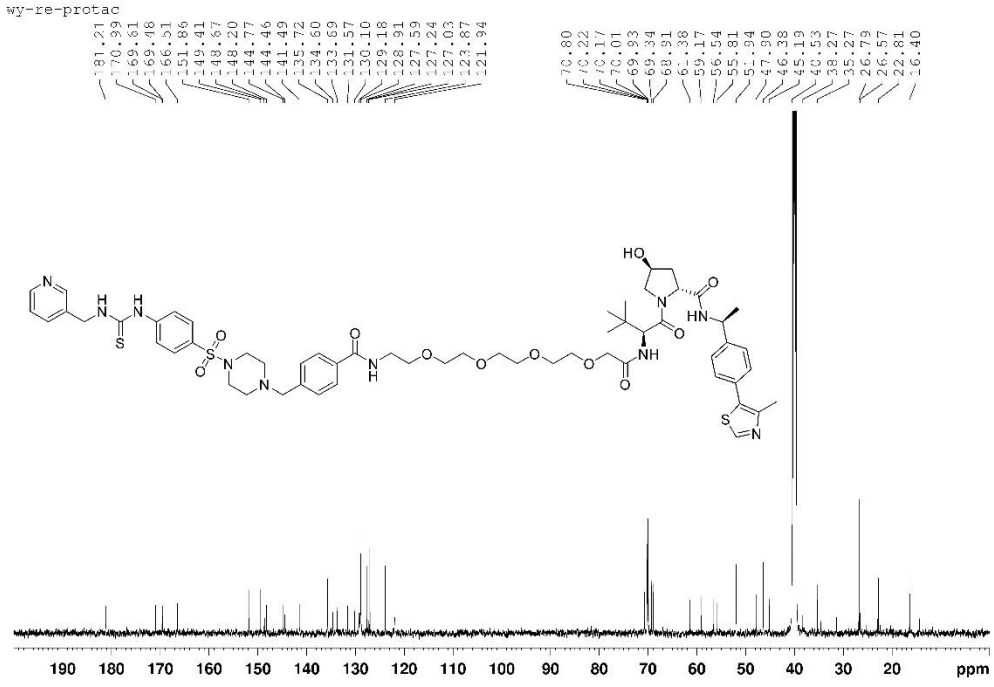
3.00
8.98


wy-re-protac
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166.31
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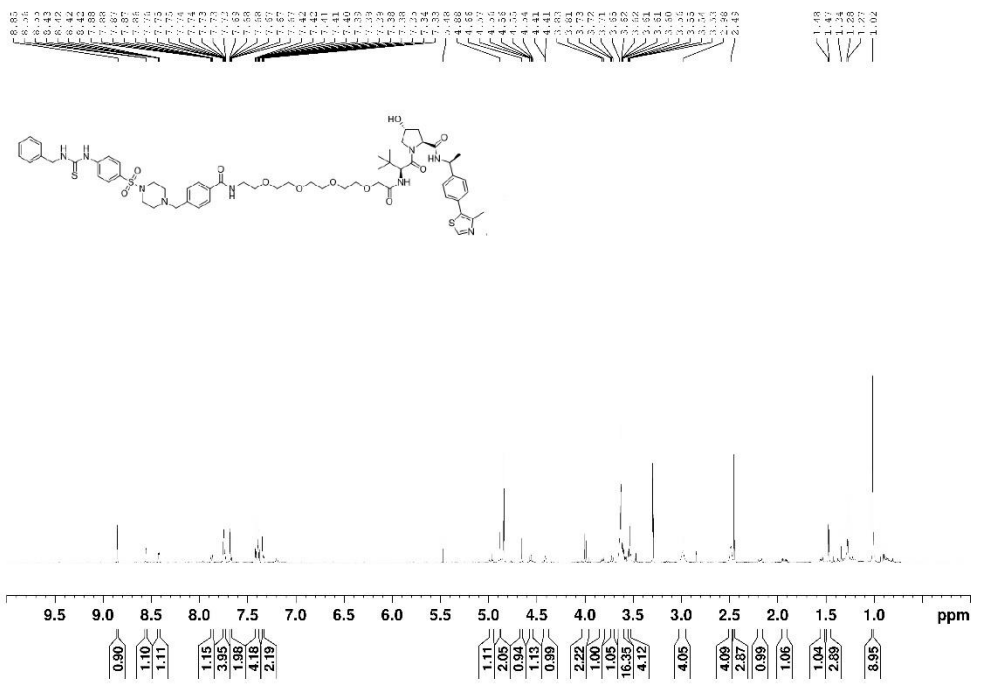
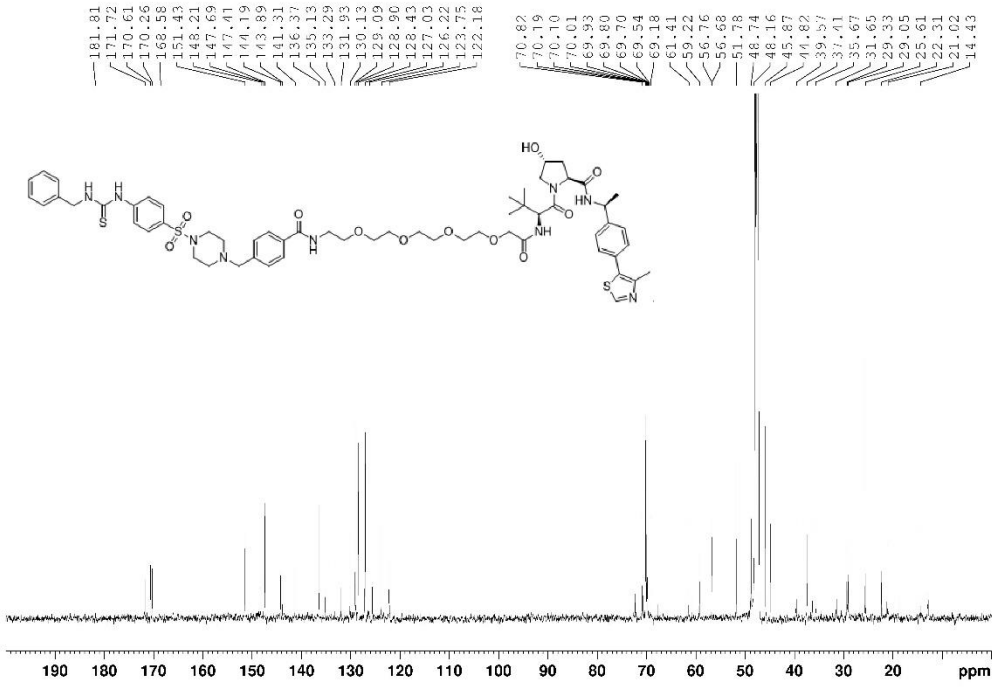
148.20
144.77
144.46
141.49
135.72
134.60
133.69
131.57
130.10
129.18
128.91
127.59
127.24
127.03
123.87
121.94
70.80
70.22
70.17
70.01
69.93
69.34
68.91
61.38
59.17
56.54
55.81
55.94
47.90
46.38
45.19
40.53
38.27
35.27
26.79
26.57
22.81
16.40
HO
HN
NH
S
N
O
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm



9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
0.90 1.10 1.11 1.15 3.95 1.98 4.18 2.19 1.11 2.05 0.94 1.13 0.99 2.22 1.00 1.05 16.35 4.12 4.05 4.09 2.87 0.99 1.06 1.04 2.89 8.95


190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

4-4

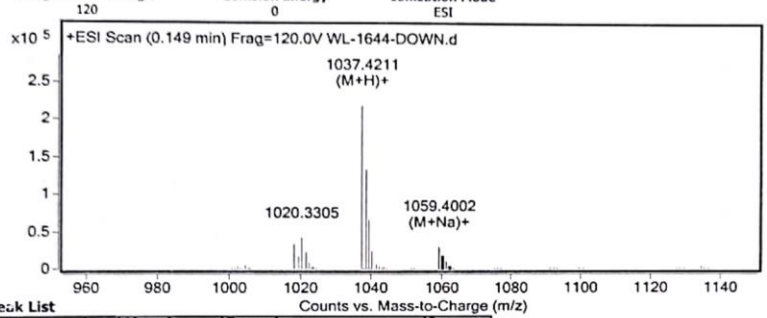
Qualitative Analysis Report

Data Filename	WL-1644-DOWN.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-B2
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	11/5/2018 11:16:11 AM
IRM Calibration Status	Success	**DA Method**	1.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage 120 **Collision Energy** 0 **Ionization Mode** ESI



Peak List

m/z	z	Abund	Formula	Ion
150.1064	1	597236.5		
152.1019	1	186839		
254.5785	2	173572.4		
332.1446	1	307275.8		
346.4826	1	1220190.6		
346.8168	1	781629.7		
347.1492		410332.7		
519.2182	2	403379.6		
519.7171	2	250899.4		
1037.4211	1	218043.3	C54 H67 N7 O8 S3	(M+H)+

Formula Calculator Element Limits

Element	Min	Max
C	0	100
H	0	200
O	0	20
N	0	10
S	3	3

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C52 H64 N10 O7 S3	TRUE	1036.4138	1036.4122	-1.57	C52 H65 N10 O7 S3	96.52
C52 H64 N10 O7 S3	TRUE	1036.4108	1036.4122	1.31	C52 H64 N10 Na O7 S3	97.32

--- End Of Report ---

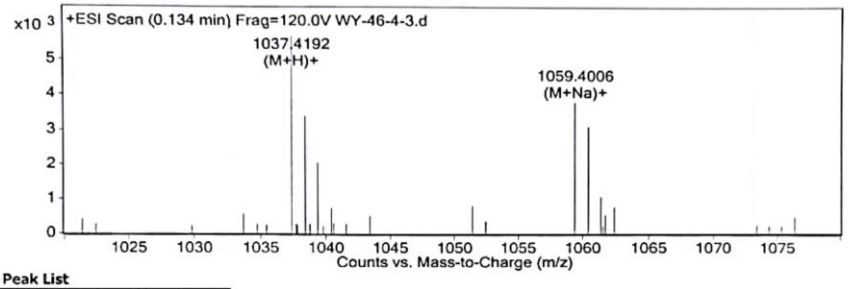
Qualitative Analysis Report

Data Filename	WY-46-4-3.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-B5
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	12/21/2018 3:20:16 PM
IRM Calibration Status	Success	**DA Method**	Default.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage 120 **Collision Energy** 0 **Ionization Mode** ESI



Peak List

m/z	z	Abund
102.1273	1	74248
130.1591	1	229474.8
239.2359	1	71746
257.2478	1	61538.4
283.2637	1	71105.9
285.2795	1	60575.3
331.285	1	55004.6
346.4795	1	58636.2
369.3524	1	61873.1
437.194	1	68196.8

Formula Calculator Element Limits

Element	Min	Max
C	3	80
H	0	120
O	0	30
N	0	30
S	1	3

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C52 H64 N10 O7 S3	TRUE	1036.412	1036.4122	0.19	C52 H65 N10 O7 S3	95.59
C64 H52 N12 O S	TRUE	1036.4114	1036.4108	-0.61	C64 H52 N12 Na O S	84
C67 H60 N2 O7 S		1036.4113	1036.4121	0.8	C67 H60 N2 Na O7 S	82.17
C49 H48 N24 O2 S		1036.4116	1036.4113	-0.31	C49 H48 N24 Na O2 S	81.15
C63 H56 N8 O5 S		1036.4114	1036.4094	-1.86	C63 H56 N8 Na O5 S	80.59
C79 H56 S		1036.4113	1036.4103	-0.96	C79 H56 Na S	80.53
C68 H56 N6 O3 S		1036.4113	1036.4135	2.05	C68 H56 N6 Na O3 S	80.18

--- End Of Report ---

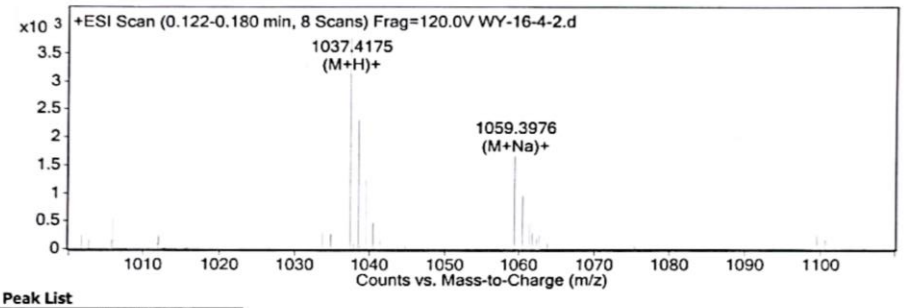
Qualitative Analysis Report

Data Filename	WY-16-4-2.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-B7
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	12/21/2018 3:23:27 PM
IRM Calibration Status	Success	**DA Method**	Default.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
120	0	ESI



Peak List

m/z	z	Abund
130.1605	1	391633.8
239.2369	1	63927.1
257.2479	1	65031.4
267.2684	1	55264.2
274.2743	1	49621
283.2639	1	79932.6
285.2797	1	61974.8
369.3523	1	66636.4
415.2121	1	59390.5
437.1942	1	84760.8

Formula Calculator Element Limits

Element	Min	Max
C	3	80
H	0	120
O	0	30
N	0	30
S	1	3

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C49 H56 N20 O S3		1036.4103	1036.4108	0.53	C49 H57 N20 O S3	99.28
C48 H60 N16 O5 S3		1036.4102	1036.4095	-0.74	C48 H61 N16 O5 S3	99.2
C51 H68 N6 O11 S3		1036.4102	1036.4108	0.64	C51 H69 N6 O11 S3	99.16
C50 H72 N2 O15 S3		1036.4101	1036.4095	-0.62	C50 H73 N2 O15 S3	98.6
C58 H68 O13 S2		1036.4101	1036.4101	0.08	C58 H69 O13 S2	98.21
C52 H64 N10 O7 S3	TRUE	1036.4102	1036.4122	1.91	C52 H65 N10 O7 S3	96.66
C56 H56 N14 O3 S2		1036.4102	1036.4101	-0.04	C56 H57 N14 O3 S2	96.39
C43 H64 N12 O14 S2		1036.4102	1036.4106	0.43	C43 H65 N12 O14 S2	96.16
C55 H60 N10 O7 S2		1036.4101	1036.4088	-1.3	C55 H61 N10 O7 S2	96.1
C47 H64 N12 O9 S3		1036.4102	1036.4081	-2.01	C47 H65 N12 O9 S3	96.07
C47 H64 N12 O9 S3	TRUE	1036.4086	1036.4081	-0.48	C47 H64 N12 Na O9 S3	96.87

Qualitative Analysis Report

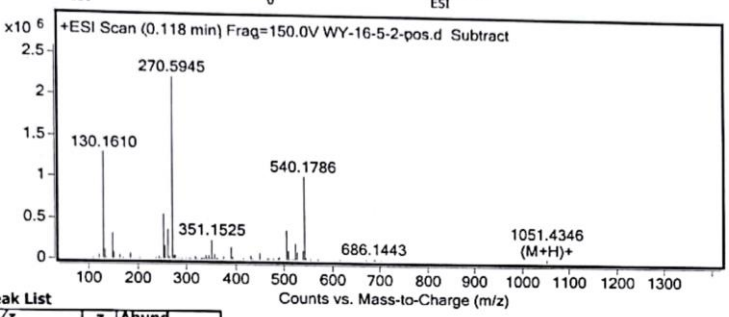
5-2

Data Filename	WY-16-5-2-pos.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-B3
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	11/27/2018 2:16:54 PM
IRM Calibration Status	Success	**DA Method**	1.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage 150 **Collision Energy** 0 **Ionization Mode** ESI



Peak List

m/z	z	Abund
130.161		1296849.4
149.0608		305581.6
253.5995	2	546311.6
262.6044	2	358224.1
270.5946	2	2228949
271.0954	2	691991.6
271.5918	2	294586.2
506.1893		356574.3
540.1787	1	1027384.4
541.1792	1	319474.6

Formula Calculator Element Limits

Element	Min	Max
C	0	60
H	0	200
O	0	10
N	0	10
S	0	3

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C53 H66 N10 O7 S3	TRUE	1050.427	1050.4278	0.72	C53 H67 N10 O7 S3	97.05

--- End Of Report ---

Qualitative Analysis Report

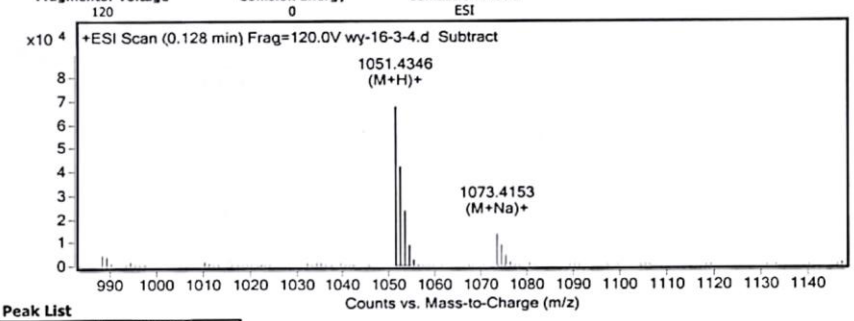
5-24

Data Filename	wy-16-3-4.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-A1
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	11/14/2018 10:42:17 AM
IRM Calibration Status	Success	**DA Method**	1.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
120	0	ESI



Peak List

m/z	z	Abund
130.1592	1	144737
163.1331	1	122827.8
274.2744	1	130406.8
280.1296	1	142290.4
338.3419	1	113377.3
351.1527	1	312334.9
351.4862	1	188228.1
526.2222	2	136549.7
574.2819	1	697019.6
575.282	1	229315.8

Formula Calculator Element Limits

Element	Min	Max
C	0	60
H	0	200
O	0	20
N	0	10
S	3	3

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C53 H66 N10 O7 S3	TRUE	1050.4273	1050.4278	0.52	C53 H67 N10 O7 S3	98.74
C53 H66 N10 O7 S3	TRUE	1050.4259	1050.4278	1.8	C53 H66 N10 Na O7 S3	94.33

--- End Of Report ---

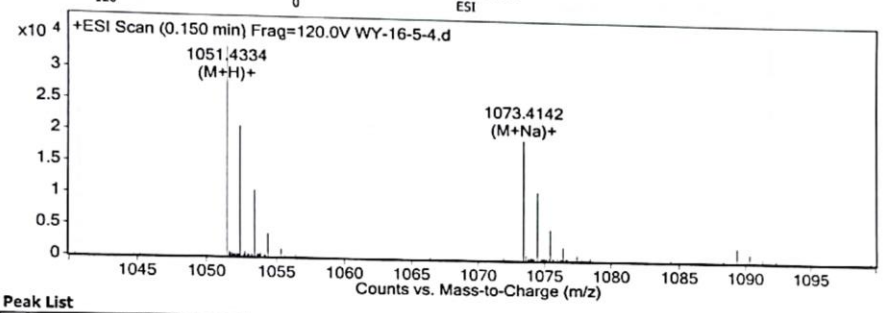
Qualitative Analysis Report

Data Filename	WY-16-5-4.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-B6
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	12/21/2018 3:21:53 PM
IRM Calibration Status	Success	**DA Method**	Default.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage 120 **Collision Energy** 0 **Ionization Mode** ESI



Peak List

m/z	z	Abund
130.1608	1	452745.9
136.0508		110095.6
274.2739	1	93721.3
283.2637	1	86906
318.3002	1	94702.5
351.1519	1	205352.2
351.4856		143253.3
351.818		79197.2
526.2216	2	113773.1
608.2881	1	162641.5

Formula Calculator Element Limits

Element	Min	Max
C	3	80
H	0	120
O	0	30
N	0	30
S	1	3

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C50 H58 N20 O S3		1050.4259	1050.4265	0.5	C50 H59 N20 O S3	98.57
C49 H62 N16 O5 S3		1050.4259	1050.4251	-0.75	C49 H63 N16 O5 S3	98.17
C59 H70 O13 S2		1050.4257	1050.4258	0.05	C59 H71 O13 S2	97.77
C52 H70 N6 O11 S3		1050.4258	1050.4265	0.61	C52 H71 N6 O11 S3	97.31
C42 H54 N26 O4 S2		1050.426	1050.4263	0.28	C42 H55 N26 O4 S2	97.26
C44 H66 N12 O14 S2		1050.4259	1050.4263	0.4	C44 H67 N12 O14 S2	97.04
C57 H58 N14 O3 S2		1050.4258	1050.4258	-0.06	C57 H59 N14 O3 S2	96.98
C56 H62 N10 O7 S2		1050.4258	1050.4244	-1.31	C56 H63 N10 O7 S2	96.46
C51 H74 N2 O15 S3		1050.4258	1050.4251	-0.63	C51 H75 N2 O15 S3	96.45
C41 H58 N22 O8 S2		1050.426	1050.4249	-0.97	C41 H59 N22 O8 S2	96.03
C50 H70 N2 O20 S	TRUE	1050.4251	1050.4243	-0.83	C50 H70 N2 Na O20 S	97.62

Qualitative Analysis Report

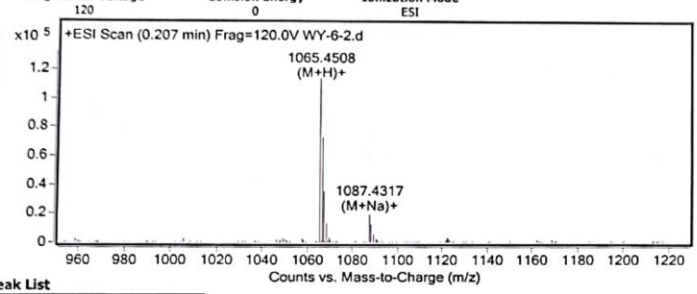
6-2

Data Filename	WY-6-2.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-A9
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	11/27/2018 12:48:08 PM
IRM Calibration Status	Success	**DA Method**	1.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage 120 **Collision Energy** 0 **Ionization Mode** ESI



Peak List

m/z	z	Abund
274.277	1	489947.3
302.3057	1	114393.4
318.3021	1	273268.3
355.8262	1	1061775.9
356.1606	1	696861.6
356.4922	1	383640
356.8243	1	139579.8
533.2331	2	357800.1
533.7327	2	229101.3
534.2308	2	124255.8

Formula Calculator Element Limits

Element	Min	Max
C	0	60
H	0	200
O	1	10
N	0	10
S	0	3

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C54 H68 N10 O7 S3	TRUE	1064.4433	1064.4435	0.12	C54 H69 N10 O7 S3	97.69
C54 H68 N10 O7 S3	TRUE	1064.4421	1064.4435	1.25	C54 H68 N10 Na O7 S3	91.62

--- End Of Report ---

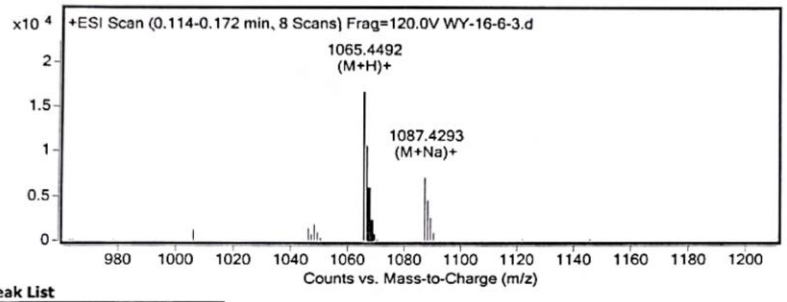
Qualitative Analysis Report

Data Filename	WY-16-6-3.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-A7
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	12/12/2018 10:29:37 AM
IRM Calibration Status	Success	**DA Method**	1.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
120	0	ESI



Peak List

m/z	z	Abund
130.1634	1	6028415.5
131.1644	1	736262.8
136.0504	1	43294.7
295.2886	1	265551.5
296.2906	1	46558.8
297.2851	1	84263.2
338.3417	1	43707.6
355.8235	1	109242.2
356.1576	1	74718.3
373.3039	1	67458

Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	120
O	0	20
N	0	10
S	3	3

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C54 H68 N10 O7 S3	TRUE	1064.4403	1064.4435	2.93	C54 H68 N10 Na O7 S3	90.59
C54 H68 N10 O7 S3	TRUE	1064.4417	1064.4435	1.64	C54 H69 N10 O7 S3	96.85

--- End Of Report ---

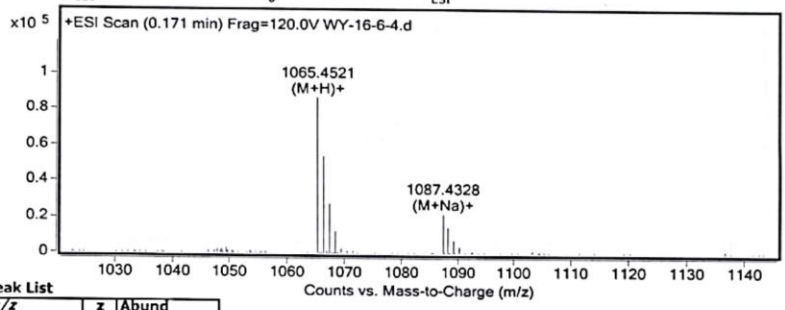
Qualitative Analysis Report

Data Filename	WY-16-6-4.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-B1
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	11/22/2018 11:11:02 AM
IRM Calibration Status	Success	**DA Method**	1.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage 120 **Collision Energy** 0 **Ionization Mode** ESI



Peak List

m/z	z	Abund
163.1333		86480.9
332.1446	1	232274.8
338.3435	1	233186.6
355.8261	1	436872.4
356.1587	1	289650.8
356.492		142558.5
367.6633	2	122868.7
533.2321	2	236132.2
533.7328	2	165129.4
534.2323	2	87010.5

Formula Calculator Element Limits

Element	Min	Max
C	0	60
H	0	200
O	0	10
N	0	10
S	3	3
F	0	1

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C54 H68 N10 O7 S3	TRUE	1064.4445	1064.4435	-0.95	C54 H69 N10 O7 S3	97.3
C54 H68 N10 O7 S3	TRUE	1064.4429	1064.4435	0.5	C54 H68 N10 Na O7 S3	95.85

--- End Of Report ---

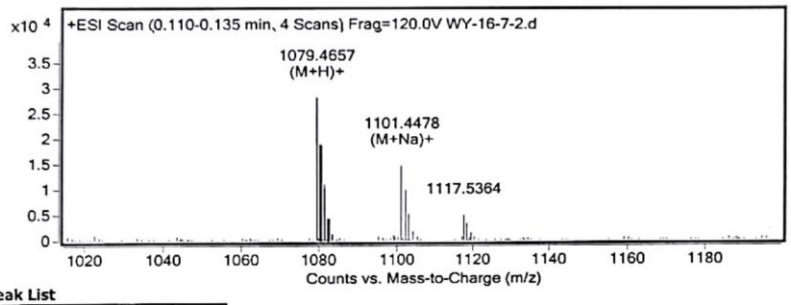
Qualitative Analysis Report

Data Filename	WY-16-7-2.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-B4
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	12/12/2018 10:39:13 AM
IRM Calibration Status	Success	**DA Method**	1.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage 120 **Collision Energy** 0 **Ionization Mode** ESI



Peak List

m/z	z	Abund
163.1331	1	205581.1
185.1148	1	69674.9
338.344	1	303360.4
339.3451	1	74379.5
360.4971	1	448783.8
360.8308	1	299467.4
361.1632	1	149886.2
437.1938	1	87962.2
540.2392	2	134560.8
540.7398	2	93174

Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	120
O	0	10
N	0	10
S	3	3

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C55 H70 N10 O7 S3	TRUE	1078.4585	1078.4591	0.55	C55 H70 N10 Na O7 S3	95.89
C55 H70 N10 O7 S3	TRUE	1078.4584	1078.4591	0.63	C55 H71 N10 O7 S3	98.63

--- End Of Report ---

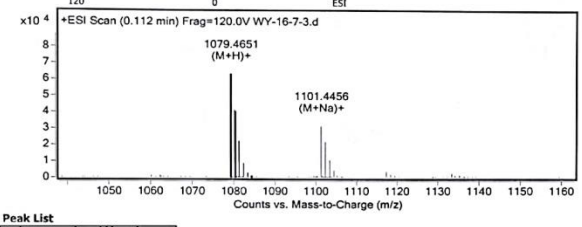
Qualitative Analysis Report

Data Filename	WY-16-7-3.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-B3
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	12/12/2018 10:37:40 AM
IRM Calibration Status	Success	**DA Method**	1.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
120	0	ESI



Peak List

m/z	z	Abund
130.1609	1	779102.6
163.1331	1	199364.8
332.1428	1	120873.9
338.3428	1	254406.2
360.4972	1	483616.3
360.8307	2	325578.9
361.1637	1	166039.6
437.1939	1	93817.5
540.2391	2	192698.8
540.7405	2	129686.3

Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	120
O	0	10
N	0	10
S	3	3

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C55 H70 N10 O7 S3	TRUE	1078.4567	1078.4591	2.26	C55 H70 N10 Na O7 S3	92.99
C55 H70 N10 O7 S3	TRUE	1078.4575	1078.4591	1.48	C55 H71 N10 O7 S3	96.15

--- End Of Report ---

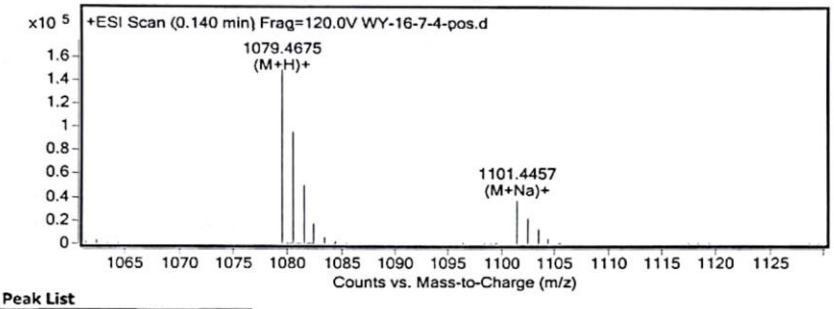
Qualitative Analysis Report

Data Filename	WY-16-7-4-pos.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-B1
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	12/12/2018 12:19:36 PM
IRM Calibration Status	Success	**DA Method**	1.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
120	0	ESI



Peak List

m/z	z	Abund
130.1611	1	647301.1
163.1355	1	1325325.3
185.117	1	399494.8
332.1437	1	219393.2
338.3454	1	661168.6
360.4978	1	598103.7
360.8317	1	390964.1
361.1645		203232.2
540.2407	2	381808.8
540.7409	2	245740.7

Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	120
O	7	7
N	0	30
S	3	3

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C55 H70 N10 O7 S3	TRUE	1078.4602	1078.4591	-0.99	C55 H71 N10 O7 S3	98.12
C55 H70 N10 O7 S3	TRUE	1078.4567	1078.4591	2.24	C55 H70 N10 Na O7 S3	94.33

--- End Of Report ---

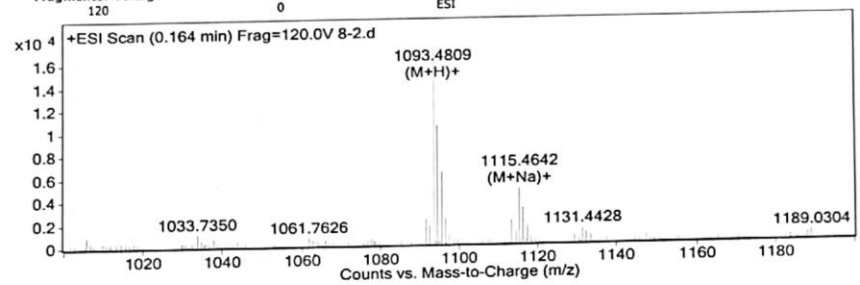
Qualitative Analysis Report

Data Filename	8-2.d	**Sample Name**	BK
Sample Type	Sample	**Position**	P1-A5
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	1/2/2019 3:47:19 PM
IRM Calibration Status	Success	**DA Method**	Default.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
120	0	ESI



Peak List

m/z	z	Abund
130.1596	1	286288.9
364.5131		37439.9
365.17	1	710847.1
365.5037	1	505738
365.8351	1	257849.8
366.1685	1	102987.9
547.2466	2	158171.7
547.748	2	103174
548.2474	2	55056.5
922.0098	1	34977.7

Formula Calculator Element Limits

Element	Min	Max
C	3	105
H	0	130
O	0	7
N	0	10
S	1	3

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C56 H72 N10 O7 S3	TRUE	1092.4742	1092.4748	0.55	C56 H72 N10 Na O7 S3	91.22
C64 H68 N8 O5 S2		1092.4741	1092.4754	1.21	C64 H68 N8 Na O5 S2	91.15
C56 H72 N10 O7 S3	TRUE	1092.4739	1092.4748	0.81	C56 H73 N10 O7 S3	96.72
C68 H68 N8 S3		1092.4738	1092.4729	-0.84	C68 H69 N8 S3	91.69
C67 H72 N4 O4 S3		1092.4738	1092.4716	-2.04	C67 H73 N4 O4 S3	90.27

--- End Of Report ---

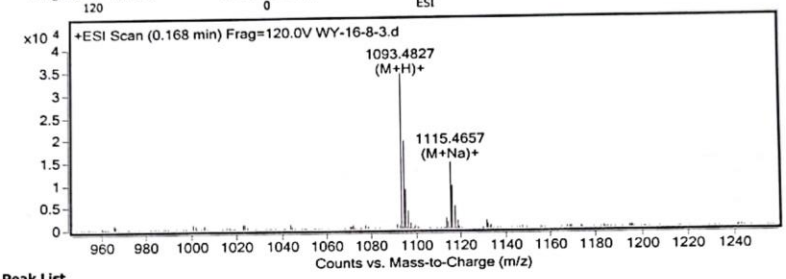
Qualitative Analysis Report

Data Filename	WY-16-8-3.d	**Sample Name**	WY-16-8-3
Sample Type	Sample	**Position**	P1-A4
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	1/8/2019 10:04:55 AM
IRM Calibration Status	Success	**DA Method**	00000.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage 120 **Collision Energy** 0 **Ionization Mode** ESI



Peak List

m/z	z	Abund
274.2761	1	160851.9
318.3028	1	136236.2
338.3439	1	146855.2
365.1713	1	469343.8
365.5042	1	331310
365.837		172551.3
366.1694	1	72154
547.2486	2	147798.1
547.7496	2	100624.4
650.3374	1	145979.4

Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	120
N	0	10
S	0	3
O	0	10

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C56 H72 N10 O7 S3	TRUE	1092.4752	1092.4748	-0.4	C56 H73 N10 O7 S3	92.25
C56 H72 N10 O7 S3	TRUE	1092.476	1092.4748	-1.14	C56 H72 N10 Na O7 S3	96.59

--- End Of Report ---

Qualitative Analysis Report

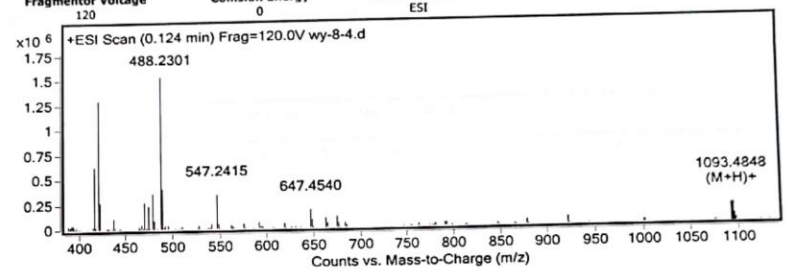
8-L

Data Filename	wy-8-4.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-A4
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	11/14/2018 12:05:57 PM
IRM Calibration Status	Success	**DA Method**	1.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage 120 **Collision Energy** 0 **Ionization Mode** ESI



Peak List

m/z	z	Abund
151.0264		897297.8
163.1267		909274.5
185.1085		525805.7
244.6147	2	899317.9
338.3364	1	534974.9
416.26	1	617868.8
421.2161	1	1293221
479.2211		357185.9
488.2301	1	1525485.6
489.2323	1	402292.5

Formula Calculator Element Limits

Element	Min	Max
C	0	60
H	0	200
O	0	20
N	0	10
S	3	3

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C56 H72 N10 O7 S3	TRUE	1092.4776	1092.4748	-2.61	C56 H73 N10 O7 S3	91.32

--- End Of Report ---

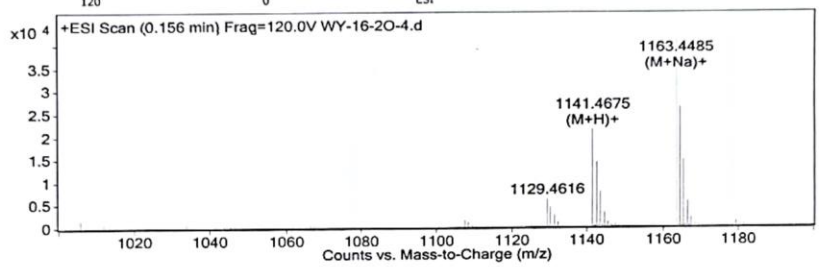
Qualitative Analysis Report

Data Filename	WY-16-2O-4.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-A8
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	12/3/2018 9:44:24 AM
IRM Calibration Status	Success	**DA Method**	1.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
120	0	ESI



Peak List

m/z	z	Abund	Formula	Ion
121.0509		37332.2		
381.1626	1	136839		
381.4967	1	96133.4		
381.8298		55613.7		
571.2387	2	53489.7		
676.3386	1	57398.8		
698.3209	1	108053		
699.3232	1	36767.5		
922.0098	1	51557.9		
1163.4485	1	41067.9	C56 H72 N10 Na O10 S3	(M+Na)+

Formula Calculator Element Limits

Element	Min	Max
C	0	100
H	0	200
O	0	20
N	0	20
S	0	10

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C56 H72 N10 O10 S3	TRUE	1140.4598	1140.4595	-0.3	C56 H73 N10 O10 S3	98.8
C55 H66 N17 O5 S3		1140.4599	1140.4595	-0.36	C55 H67 N17 O5 S3	98.79
C57 H78 N3 O15 S3		1140.4598	1140.4595	-0.24	C57 H79 N3 O15 S3	98.48
C57 H68 N14 O6 S3		1140.4599	1140.4608	0.85	C57 H69 N14 O6 S3	98.02
C57 H78 N3 O15 S3		1140.4594	1140.4595	0.13	C57 H78 N3 Na O15 S3	99.13
C56 H72 N10 O10 S3	TRUE	1140.4594	1140.4595	0.07	C56 H72 N10 Na O10 S3	99.12

--- End Of Report ---

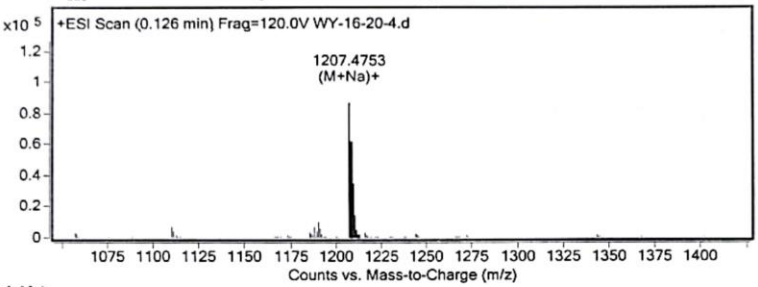
Qualitative Analysis Report

Data Filename	WY-16-20-4.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-A8
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	12/12/2018 10:31:15 AM
IRM Calibration Status	Success	**DA Method**	1.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage 120 **Collision Energy** 0 **Ionization Mode** ESI



Peak List

m/z	z	Abund	Formula	Ion
130.1607	1	512342.4		
136.0506		56825.2		
185.1167	1	469091.8		
338.3417		83111.6		
353.2664		73337.6		
381.2971		73380.1		
395.8374		110749.4		
396.1715		82508.4		
1207.4753	1	87043	C58 H76 N10 Na O11 S3	(M+Na)+
1208.4786	1	57264.1	C58 H76 N10 Na O11 S3	(M+Na)+

Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	120
O	0	20
N	0	10
S	3	3

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C58 H76 N10 O11 S3	TRUE	1184.4862	1184.4857	-0.39	C58 H76 N10 Na O11 S3	98.69

--- End Of Report ---

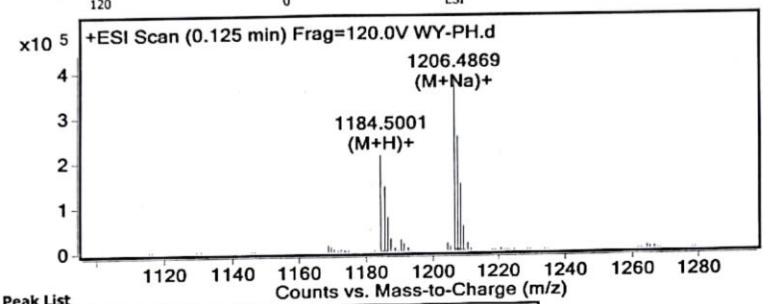
Qualitative Analysis Report

Data Filename	WY-PH.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-D5
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	3/28/2019 10:56:58 AM
IRM Calibration Status	Success	**DA Method**	Default.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage 120 **Collision Energy** 0 **Ionization Mode** ESI



Peak List

m/z	z	Abund	Formula	Ion
592.7593	2	1366359.5		
593.2605	2	977482.8		
593.7596	2	536137.5		
594.2572	2	220685.3		
603.7471	2	251307		
758.3461	1	426330.3		
759.3453	1	175976.6		
1184.5001	1	213756.5	C59 H78 N9 O11 S3	(M+H)+
1206.4869	1	378930.3	C59 H77 N9 Na O11 S3	(M+Na)+
1207.4861	1	255894.2	C59 H77 N9 Na O11 S3	(M+Na)+

Formula Calculator Element Limits

Element	Min	Max
C	0	80
H	0	120
O	11	11
N	9	9
S	3	3

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C59 H77 N9 O11 S3	TRUE	1183.4927	1183.4905	-1.9	C59 H78 N9 O11 S3	95.46
C59 H77 N9 O11 S3	TRUE	1183.4966	1183.4905	-5.18	C59 H77 N9 Na O11 S3	75.59

--- End Of Report ---

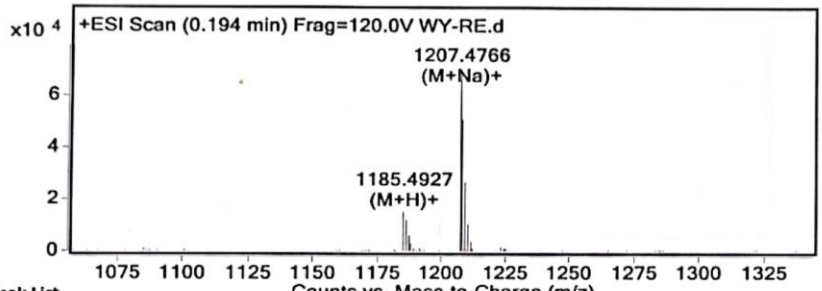
Qualitative Analysis Report

Data Filename	WY-RE.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-B8
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	10/9/2019 10:21:16 AM
IRM Calibration Status	Some Ions Missed	**DA Method**	SERUM-POS-19MIN.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
120	0	ESI



Peak List

m/z	z	Abund	Formula	Ion
395.8402		68454		
396.1741		48828.8		
396.5065		28088.4		
593.2539	2	30749.2		
593.7529	2	24517.6		
594.2543	2	15678.2		
742.3467		20105.3		
1207.4766	1	68637.1	C58 H76 N10 Na O11 S3	(M+Na)+
1208.478	1	51361.7	C58 H76 N10 Na O11 S3	(M+Na)+
1209.4765	1	26804	C58 H76 N10 Na O11 S3	(M+Na)+

Formula Calculator Element Limits

Element	Min	Max
C	0	100
H	0	150
N	10	10
O	11	11
S	3	3

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C58 H76 N10 O11 S3	TRUE	1184.4845	1184.4857	1	C58 H77 N10 O11 S3	94.67
C58 H76 N10 O11 S3	TRUE	1184.4868	1184.4857	-0.94	C58 H76 N10 Na O11 S3	96.18

--- End Of Report ---

Supporting references

1. Neuhaus JM, Sticher L, Meins F Jr, Boller T. A short C-terminal sequence is necessary and sufficient for the targeting of chitinases to the plant vacuole. *Proc Natl Acad Sci U S A* 1991;**88**:10362-6.

2. van Sebille E, Doblin M. Data from "Drift in ocean currents impacts intergenerational microbial exposure to temperature." Figshare. Available from: https://dx.doi.org/10.6084/m9.figshare.3178534.v2. Deposited 15 April 2016.

3. Hill AVS. HLA associations with malaria in Africa: Some implications for MHC evolution. In: Klein J, Klein D, Editors. *Molecular evolution of the major histocompatibility complex.* 1991; Springer. p. 403–20.