PTK2 PROTAC | BI-3663

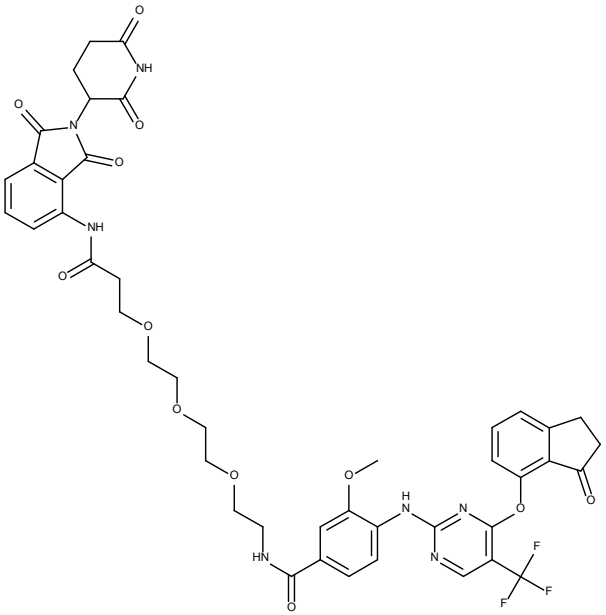
Synthesis of BI-3663 (J. Med. Chem. 2019, 62, 5, 2508-2520)

PTK2 PROTAC, BI-3663 (Compound 1)[1]
PTK2 PROTAC, BI-0319 (Compound 11)[1]
PTK2 PROTAC Negative Control, BI-4206 (Compound 12)[1]

The compound numbers mentioned herein are a reference to the numbering system employed in: Gollner A., Heine C., Hofbauer K. S. Kinase Degraders, Activators, and Inhibitors: Highlights and Synthesis Routes to the Chemical Probes on opnMe.com, Part 1. *ChemMedChem* **2023**, 18, e202300031. DOI: 10.1002/cmdc.202300031, PubMed.

[1] Popow J., Arnhof H., Bader G., Berger H., Ciulli A., Covini D., Dank C., Gmaschitz T., Greb P., Karolyi-Özguer J., Koegl M., McConnell D. B., Pearson M., Rieger M., Rinnenthal J., Roessler V., Schrenk A., Spina M., Steurer S., Trainor N., Traxler E., Wieshofer C., Zoephel A., Ettmayer P. Highly Selective PTK2 Proteolysis Targeting Chimeras to Probe Focal Adhesion Kinase Scaffolding Functions. J Med Chem **2019**, 62(5) 2508–2520. DOI: 10.1021/acs.jmedchem.8b01826, Pubmed.

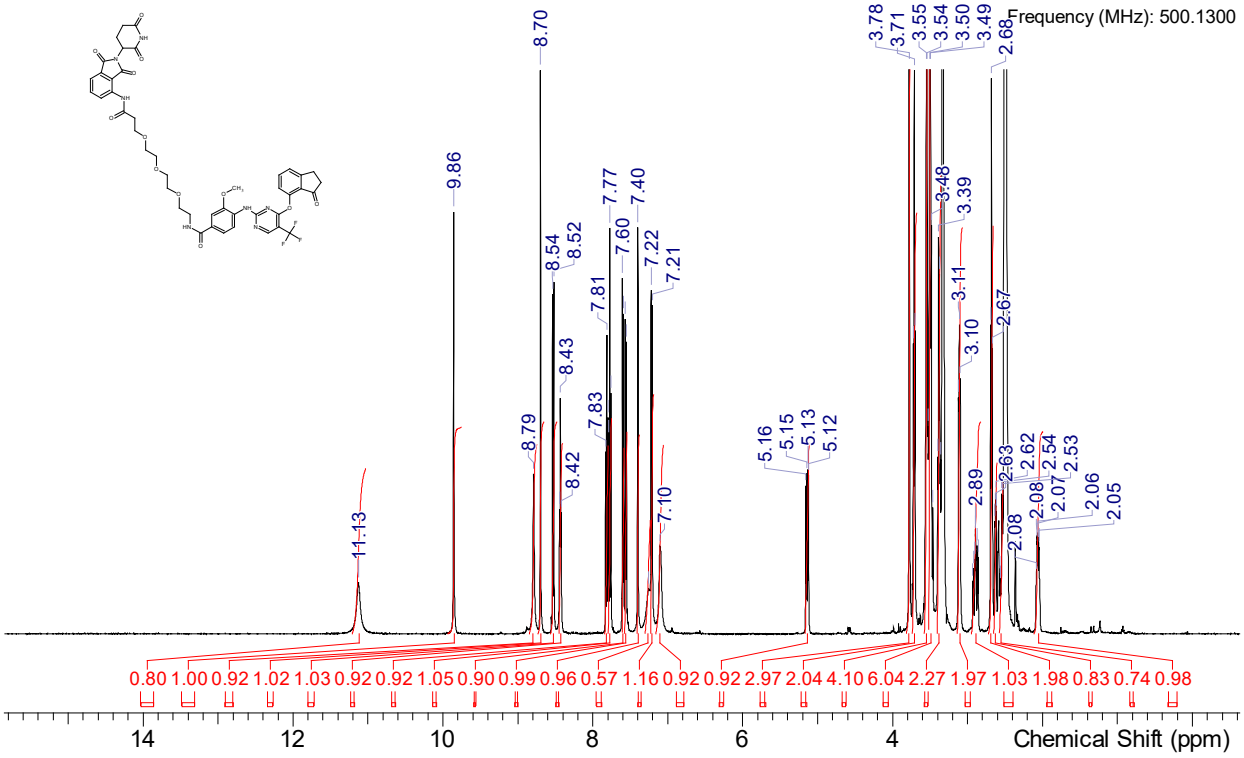
PTK2 PROTAC, BI-3663 (Compound 1)

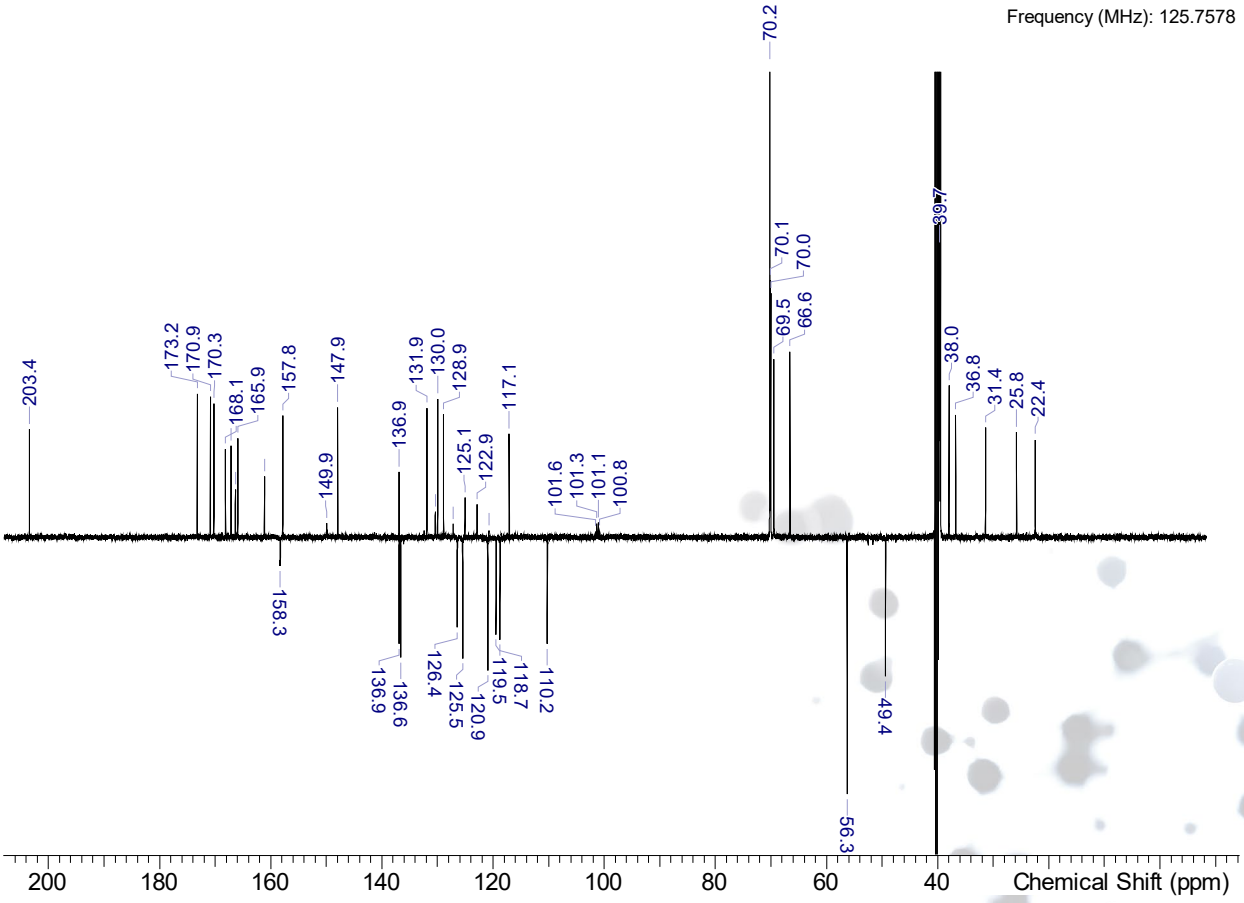


3-methoxy-4-({4-[(3-oxo-2,3-dihydro-1*H*-inden-4-yl)oxy]-5-(trifluoromethyl)pyrimidin-2-yl}amino)benzoic acid (170 mg, 1 equiv) was dissolved in DMF (2 mL). DIPEA (150 µL, 3 equiv) and HATU (155 mg, 1.2 equiv) were added. The reaction mixture was stirred at rt for 5 min, then 3-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}-*N*-[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]propanamide (162 mg, 1 equiv) was added. The mixture was stirred at rt overnight. The reaction mixture was diluted with MeCN and H_2O and filtered through a syringe filter prior to purification via RP-HPLC under acidic conditions using MeCN/H_2O as eluents in a gradient from 30:70 to 98:2 over 8 min (column: YMC Actus-Triart Prep C18, 5 µm, 30 × 50 mm; flow: 50 mL/min). The product-containing fractions were freeze-dried to give *N*-[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]-3-{2-[2-(2-{[3-methoxy-4-({4-[(3-oxo-2,3-dihydro-1*H*-inden-4-yl)oxy]-5-(trifluoromethyl)pyrimidin-2-yl}amino)phenyl]formamido}ethoxy)ethoxy]ethoxy}propanamide [(BI-3663), 32 mg, 10% yield] as an off-white lyophilizate.

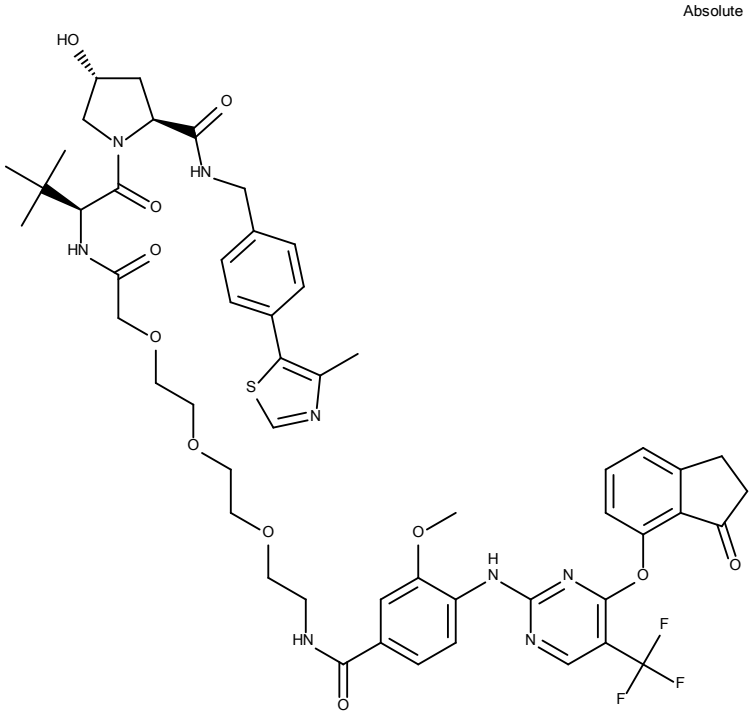
^{1}H NMR (500 MHz, DMSO-d_6): δ 11.13 (br s, 1H), 9.86 (s, 1H), 8.79 (br s, 1H), 8.70 (s, 1H), 8.53 (d, *J* = 8.51 Hz, 1H), 8.43 (t, *J* = 5.52 Hz, 1H), 7.81 (dd, *J* = 7.25, 8.51 Hz, 1H), 7.77 (t, *J* = 7.88 Hz, 1H), 7.60 (d, *J* = 7.25 Hz, 1H), 7.56 (d, *J* = 7.88 Hz, 1H), 7.40 (s, 1H), 7.21 (d, *J* = 7.88 Hz, 1H), 7.25 (br s, 1H), 7.10 (br s, 1H), 5.14 (dd, *J* = 5.52, 12.77 Hz, 1H), 3.78 (s, 3H), 3.71 (t, *J* = 5.99 Hz, 2H), 3.43–3.61 (m, 10H), 3.36–3.41 (m, 2H), 3.07–3.15 (m, 2H), 2.84–2.95 (m, 1H), 2.68 (t, *J* = 5.99 Hz, 2H), 2.52–2.65 (m, 2H), 2.02–2.11 (m, 1H), and two protons under DMSO.
^{13}C NMR (125 MHz, DMSO-d_6): δ 203.4, 173.2, 170.9, 170.3, 168.1, 167.1, 166.4, 165.9, 161.1, 158.3, 157.8, 149.9, 147.9, 136.9, 136.9, 136.6, 131.9, 130.4, 130.0, 128.9, 126.4, 125.5, 120.9, 124.0, 119.5, 118.7, 117.1, 110.2, 101.2, 70.2, 70.2, 70.1, 70.0, 69.5, 66.6, 56.3, 49.4, 39.6, 38.0, 36.8, 31.4, 25.8, 22.4, and one carbon not detected.

HRMS (*m*/*z*): $[M + H]^+$ calcd for $C_{44}H_{42}F_3N_7O_{12}$, 917.28435; found, 917.28471.
HPLC–MS ^tR = 1.35 min.


Frequency (MHz): 500.1300
11.13
9.86
8.70
8.79
8.54
8.52
8.43
8.42
7.83
7.81
7.77
7.60
7.40
7.22
7.21
7.10
5.16
5.15
5.13
5.12
3.78
3.71
3.55
3.54
3.50
3.49
3.48
3.39
3.11
3.10
2.68
2.67
2.89
2.63
2.62
2.54
2.53
2.08
2.07
2.06
2.05
0.80 1.00 0.92 1.02 1.03 0.92 0.92 1.05 0.90 0.99 0.96 0.57 1.16 0.92 0.92 2.97 2.04 4.10 6.04 2.27 1.97 1.03 1.98 0.83 0.74 0.98
14
12
10
8
6
4
Chemical Shift (ppm)


Frequency (MHz): 125.7578
203.4
173.2
170.9
170.3
168.1
165.9
157.8
147.9
149.9
136.9
131.9
130.0
128.9
125.1
122.9
117.1
101.6
101.3
101.1
100.8
70.2
70.1
70.0
69.5
66.6
39.7
38.0
36.8
31.4
25.8
22.4
158.3
136.9
136.6
126.4
125.5
120.9
119.5
118.7
110.2
56.3
49.4
200
180
160
140
120
100
80
60
40
Chemical Shift (ppm)

PTK PROTAC, BI-0319 (Compound 11)

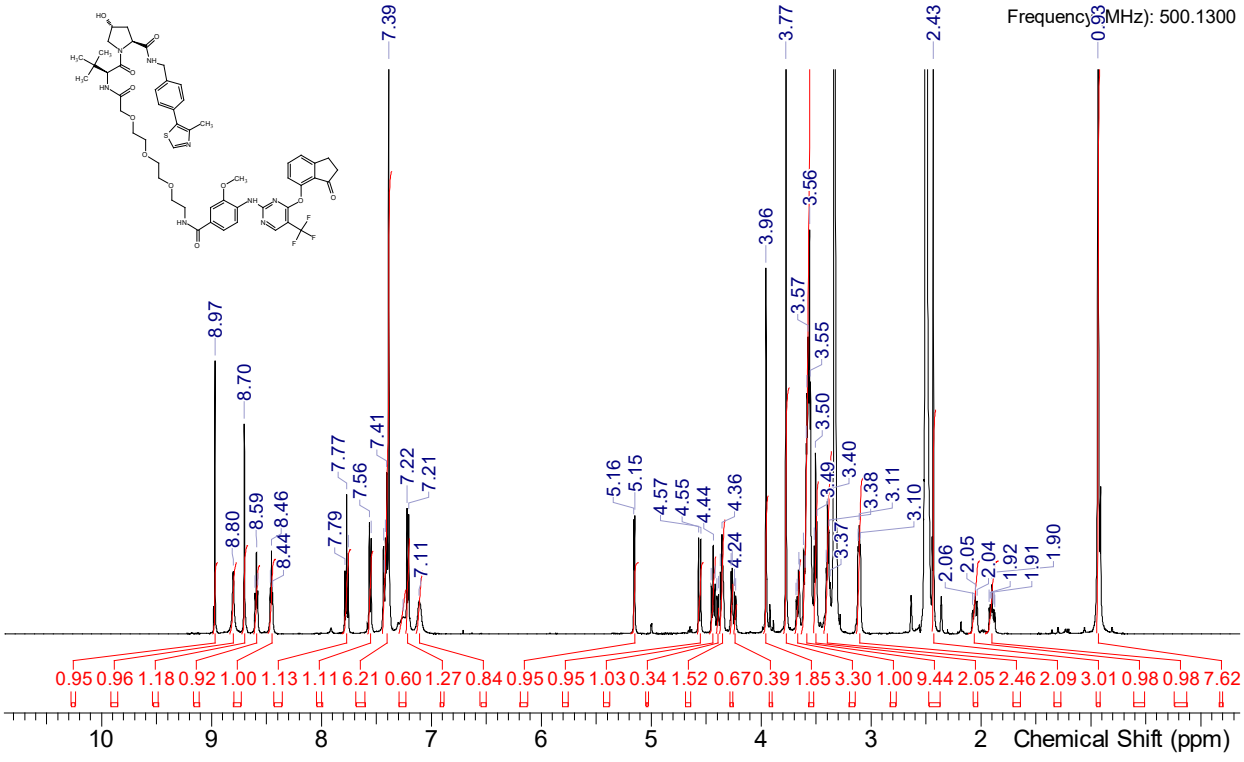


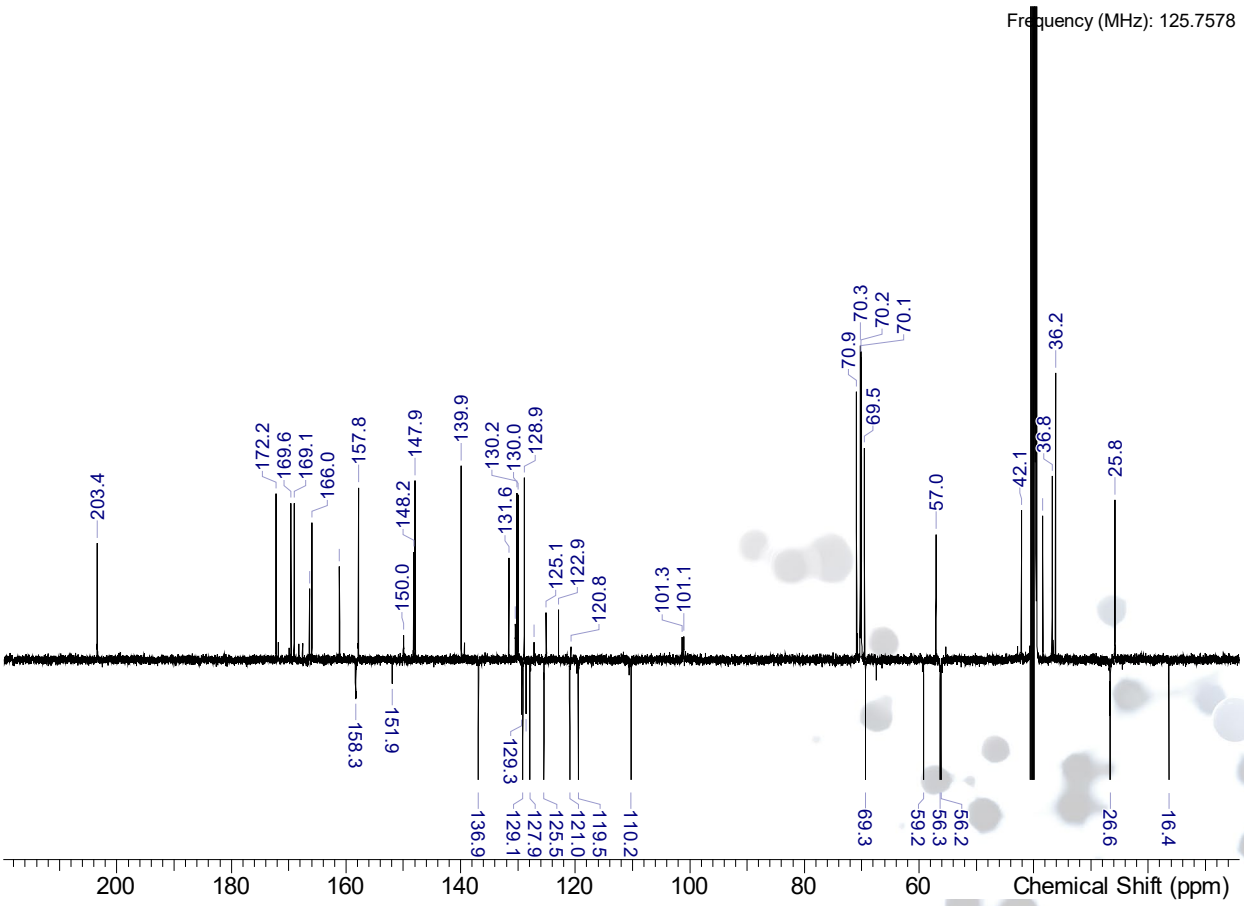
3-methoxy-4-({4-[(3-oxo-2,3-dihydro-1*H*-inden-4-yl)oxy]-5-(trifluoromethyl)pyrimidin-2-yl}amino)benzoic acid (111 mg, 1 equiv) was dissolved in DMF (3 mL). DIPEA (96 µL, 3 equiv) and HATU (101 mg; 266 µmol; 1.2 equiv) were added. The reaction mixture was stirred at rt for 5 min, and then (2*S*,4*R*)-1-[(2*S*)-2-[[2-[2-[2-(2-aminoethoxy)ethoxy] ethoxy]acetyl]amino]-3,3-dimethyl-butanoyl]-4-hydroxy-*N*-[[4-(4-methylthiazol-5-yl)phenyl]methyl]pyrrolidine-2-carboxamide (150 mg, 1 equiv) was added. The mixture was stirred at rt for 24 h. The reaction mixture was diluted with MeCN and H_2O and filtered through a syringe filter prior to purification via RP-HPLC under basic conditions using MeCN/H_2O as eluents in a gradient from 25:75 to 90:10 over 9 min (column: XBridge Prep C18, OBD 10 µm, 50 × 150 mm; flow: 150 mL/min). The product-containing fractions were pooled and freeze-dried to give (2*S*,4*R*)-4-hydroxy-1-[(2*S*)-2-(2-{2-[2-(2-{[3-methoxy-4-({4-[(3-oxo-2,3-dihydro-1*H*-inden-4-yl)oxy]-5-(trifluoromethyl)pyrimidin-2-yl}amino)phenyl]formamido}ethoxy)ethoxy]ethoxy}acetamido)-3,3-dimethylbutanoyl]-*N*-{[4-(4-methyl-1,3-thiazol-5-yl)phenyl]methyl}pyrrolidine-2-carboxamide [(BI-0319), 81 mg, 34% yield] as a colorless lyophilizate.

^{1}H NMR (500 MHz, DMSO-d_6): δ 8.97 (s, 1H), 8.80 (br s, 1H), 8.70 (s, 1H), 8.59 (t, *J* = 5.99 Hz, 1H), 8.46 (t, *J* = 5.67 Hz, 1H), 7.77 (dd, *J* = 7.57, 7.88 Hz, 1H), 7.56 (d, *J* = 7.57 Hz, 1H), 7.35–7.46 (m, 6H), 7.21 (d, *J* = 7.88 Hz, 1H), 7.23 (br s, 1H), 7.11 (br s, 1H), 5.15 (d, *J* = 3.47 Hz, 1H), 4.56 (d, *J* = 9.77 Hz, 1H), 4.20–4.48 (m, 4H), 3.96 (s, 2H), 3.77 (s, 3H), 3.36–3.71 (m, 16H), 3.06–3.15 (m, 2H), 2.43 (s, 3H), 2.00–2.10 (m, 1H), 1.85–1.94 (m, 1H), and 0.93 (s, 9H).

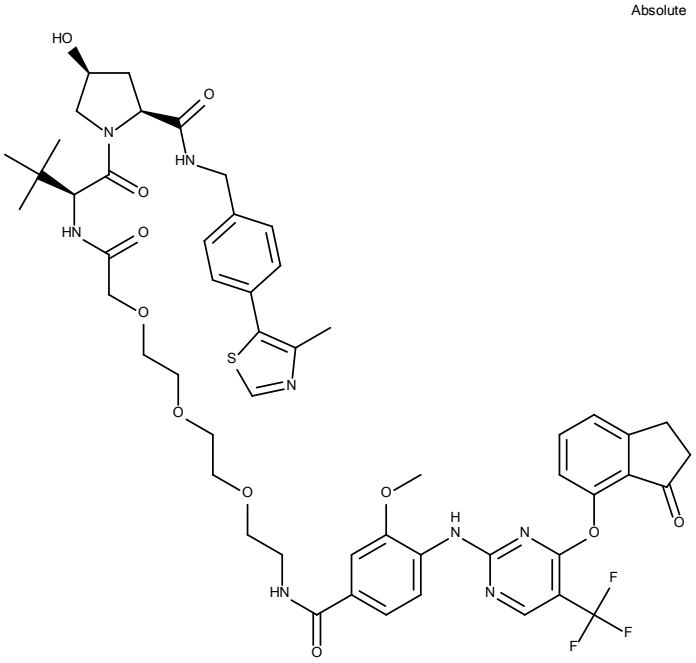
^{13}C NMR (125 MHz, DMSO-d_6): δ 203.4, 172.2, 169.6, 169.1, 166.4, 166.0, 161.1, 158.3, 157.8, 151.9, 150.0, 148.2, 147.9, 139.9, 136.9, 131.6, 130.4, 130.2, 130.0, 129.1, 128.9, 127.9, 125.5, 121.0, 124.0, 119.5, 110.2, 101.3, 101.1, 70.9, 70.3, 70.2, 70.1, 70.0, 69.5, 69.3, 59.2, 57.0, 56.3, 56.2, 42.1, 38.4, 36.8, 36.2, 26.6, 25.8, and 16.4.

HRMS (*m*/*z*): $[M + H]^+$ calcd for $C_{52}H_{59}F_3N_8O_{11}S$, 1060.39761; found, 1060.39604.
HPLC–MS ^tR = 1.35 min.


Frequency (MHz): 500.1300
Chemical Shift (ppm)


Frequency (MHz): 125.7578
Chemical Shift (ppm)

PTK PROTAC, negative control BI-4206 (Compound 12)



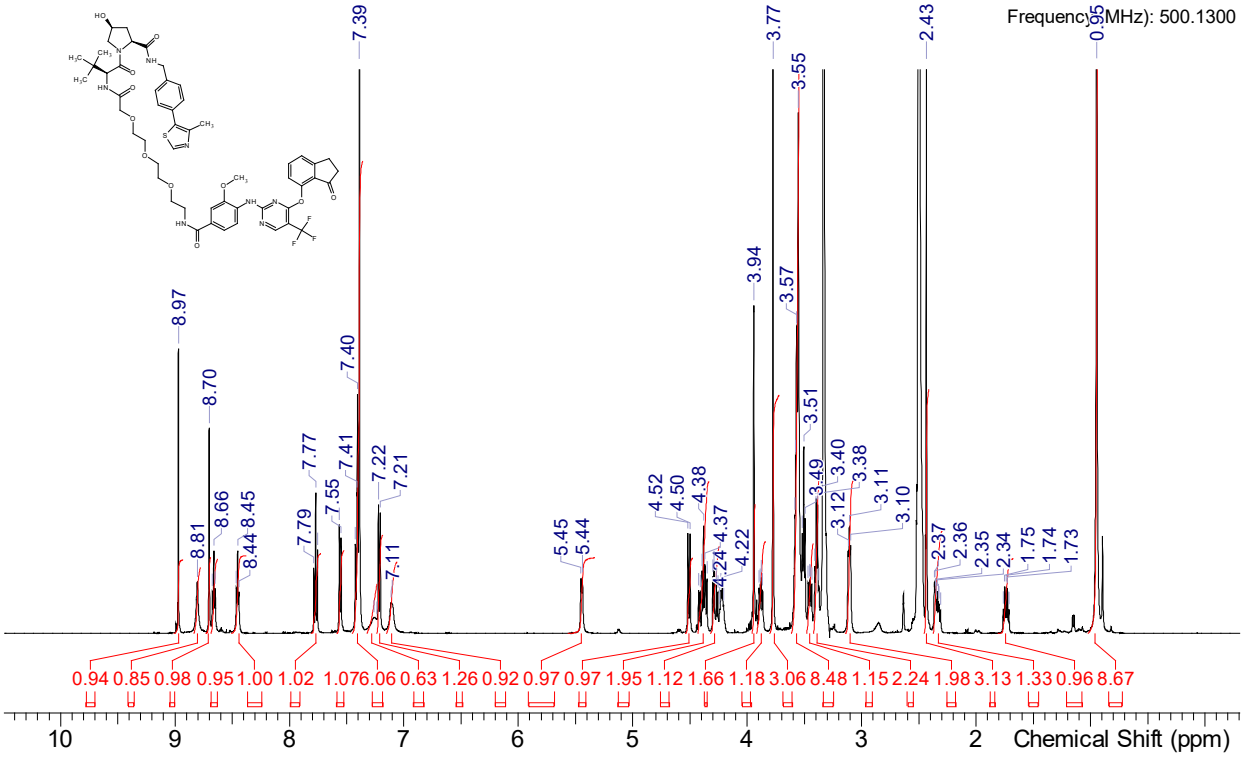
In a glass vial, 3-methoxy-4-({4-[(3-oxo-2,3-dihydro-1*H*-inden-4-yl)oxy]-5-(trifluoromethyl) pyrimidin-2-yl}amino)benzoic acid (**2**, 116 mg, 1 equiv) was dissolved in DMF (2 mL). DIPEA (100 µL, 3 equiv) and HATU (106 mg, 1.2 equiv) were added. The reaction mixture was stirred at rt for 5 min, and then (2*S*,4*S*)-1-[(2*S*)-2-(2-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}acetamido)-3,3-dimethyl butanoyl]-4-hydroxy-*N*-{[4-(4-methyl-1,3-thiazol-5-yl)phenyl]methyl}pyrrolidine-2-carboxamide (**10b**, 144 mg, 1 equiv) was added. The mixture was stirred at rt for 16 h. The reaction mixture was diluted with MeCN and H_2O and filtered through a syringe filter prior to purification via RP-HPLC under basic conditions using MeCN/H_2O as the eluents in a gradient from 30:70 to 98:2 over 8 min (column: YMC Actus-Triart Prep C18, 5 µm, 30 × 50 mm; flow: 50 mL/min). The product-containing fractions were freeze-dried to give (2*S*,4*S*)-4-hydroxy-1-[(2*S*)-2-(2-{2-[2-(2-{[3-methoxy-4-({4-[(3-oxo-2,3-dihydro-1*H*-inden-4-yl)oxy]-5-(trifluoromethyl)pyrimidin-2-yl}amino)phenyl]formamido}ethoxy) ethoxy]ethoxy}acetamido)-3,3-dimethylbutanoyl]-*N*-{[4-(4-methyl-1,3-thiazol-5-yl)phenyl]methyl}pyrrolidine-2-carboxamide [(BI-4206), 169 mg, 69% yield] as an off-white lyophilizate.

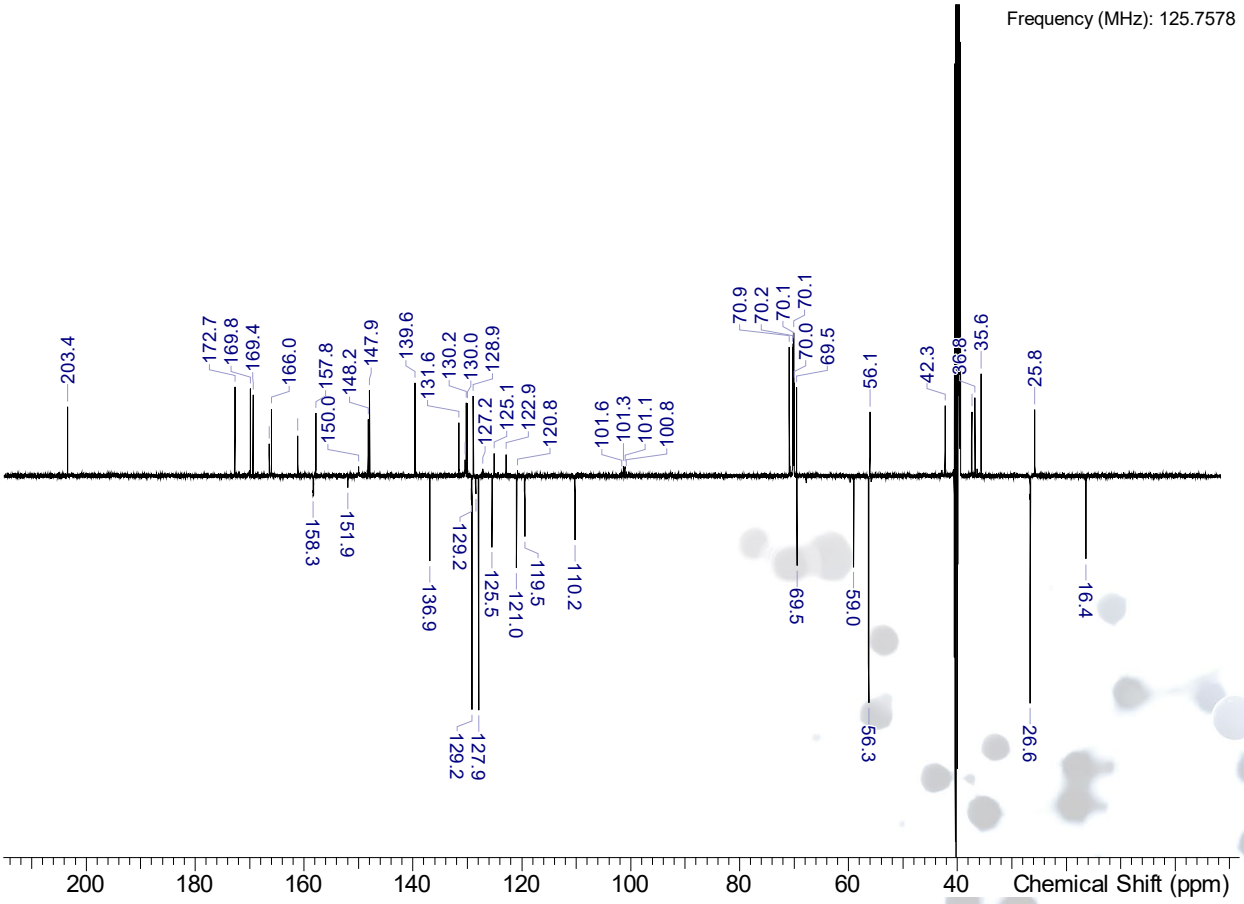
^{1}H NMR (500 MHz, DMSO-d_6): δ 8.97 (s, 1H), 8.81 (br s, 1H), 8.70 (s, 1H), 8.66 (t, *J* = 5.99 Hz, 1H), 8.45 (t, *J* = 5.52 Hz, 1H), 7.77 (dd, *J* = 7.57, 7.88 Hz, 1H), 7.56 (d, *J* = 7.57 Hz, 1H), 7.35–7.45 (m, 6H), 7.21 (d, *J* = 7.88 Hz, 1H), 7.25 (br s, 1H), 7.11 (br s, 1H), 5.45 (br d, *J* = 6.62 Hz, 1H), 4.51 (d, *J* = 9.14 Hz, 1H), 4.17–4.45 (m, 4H), 3.94 (s, 2H), 3.84–3.91 (m, 1H), 3.77 (s, 3H), 3.36–3.62 (m, 15H), 3.07–3.15 (m, 2H), 2.43 (s, 3H), 2.29–2.36 (m, 1H), 1.69–1.79 (m, 1H), and 0.95 (s, 9H).

^{13}C NMR (125 MHz, DMSO-d_6): δ 203.4, 172.7, 169.8, 169.4, 166.4, 166.0, 161.1, 158.3, 157.8, 151.9, 150.0, 148.2, 147.9, 139.6, 136.9, 131.6, 130.4, 130.2, 130.0, 129.2, 128.9, 127.9, 125.5, 121.0,

124.0, 119.5, 110.2, 101.3, 101.1, 70.9, 70.2, 70.1, 70.1, 70.0, 69.5, 69.5, 59.0, 56.3, 56.1, 42.3, 37.4, 36.8, 35.6, 26.6, 25.8, 16.4, and two carbons not detected.

HRMS (*m/z*): $[M + H]^+$ calculated for $C_{52}H_{59}F_3N_8O_{11}S$, 1060.39761; found, 1060.39665.
HPLC–MS ^tR = 1.71 min.


Frequency (MHz): 500.1300
Chemical Shift (ppm)


Frequency (MHz): 125.7578
Chemical Shift (ppm)