Supplementary Data Tables and Figures

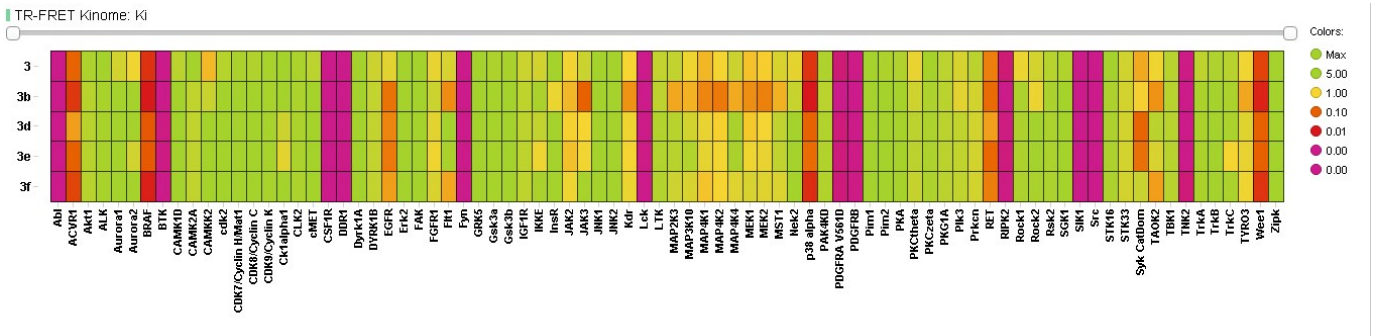
Table S1 Extended competitive TR-FRET ligand displacement data for dasatinib **3** and dasatinib analogs for tested targets for which dasatinib showed <10 nM K_i.

	TR-FRET K_i (nM)[a]											
Cmpd	BTK	ratio[b]	CSF1R	ratio[b]	DDR1	ratio[b]	Fyn	ratio[b]	Lck	ratio[b]	PDGFRA V561D	ratio[b]
3	0.51	1	6.76	1	0.28	1	0.31	1	0.85	1	2.81	1
3f	1.10	2.2	0.197	0.29	0.55	1.93	0.25	0.81	0.74	0.87	2.38	0.85
3a	2.20	4.3	0.551	0.82	1.71	6.04	0.80	2.60	2.88	3.39	5.46	1.94
3b	0.47	0.9	0.124	0.18	0.32	1.12	0.17	0.56	0.38	0.45	1.07	0.38
3c	4.00	7.8	1.7	2.51	10.0	35.3[c]	2.10	6.80	0.44	0.52	2.80	1.0
3d	1.68	3.3	0.171	0.25	0.95	3.36	0.38	1.23	1.34	1.58	2.30	0.82
3e	1.68	3.3	0.332	0.49	1.33	4.70	0.39	1.26	1.50	1.76	2.20	0.78

	TR-FRET K_i (nM)[a]							
Cmpd	PDGFRB	ratio[b]	RIPK2	ratio[b]	SIK1	ratio[b]	TNK2	ratio[b]
3	0.93	1	2.80	1	0.57	1	1.28	1
3f	1.60	1.72	1.67	0.60	1.17	2.07	2.42	1.89
3a	6.76	7.28	5.33	1.90	1.09	1.93	3.47	2.71
3b	1.03	1.11	0.792	0.28	0.32	0.56	7.61	5.95
3c	1.60	1.72	41.0	14.6[c]	7.10	12.6[c]	22.0	17.2[c]
3d	1.21	1.30	1.77	0.63	0.41	0.72	0.86	0.67
3e	1.40	1.51	2.10	0.75	0.33	0.59	0.61	0.48

[a]TR-FRET K_i values reported as the geometric mean from at least two duplicate runs. [b]Ratio indicates ratio of TR-FRET Ki values relative to compound **3** for the preceding kinase. [c]Assays for which the ratio >10-fold different from compound **3**

Figure S1: Extended TR-FRET kinase binding assay data for dasatinib derivatives across tested kinome.



Intracellular Compound Binding and Accumulation[1] – Detailed Analytical Methods

Dialysis experiments for HEK293 (obtained from ATCC) binding measurements were performed at 1 µM. Matrix receiver and donor samples were quenched with a minimum of three volume equivalents of 95:5 acetonitrile/ methanol containing 50 nM carbutamide. Samples were vortex, mixed and centrifuged, and supernatant was injected for LC-MS/MS analysis. Samples were generated in triplicate resulting in CVs of 7±4%.

Intracellular accumulation experiments were run at a concentration of 0.5 µM. Accurate cell counts were determined following two rounds of cell washing in cold PBS. For quantification, compounds were serially diluted in DSMO to produce an 11 point calibration curve ranging from 1-10,000 nM. Media (10 µL), cell (20,000 cells/µL) or standard (10 µL) samples were all matrix matched and compound was quantified in each sample from the same calibration curve. Samples were quenched with 275 µL of 80:20 acetonitrile:water containing 40 nM carbutamide, vortex mixed, and centrifuged then supernatant was injected for LC-MS/MS analysis. Samples were analyzed in triplicate resulting in CVs of 14±11%.

Compounds were quantified by either of two methods. SCD compounds as well as all of the H_3 compounds were analyzed by method **2** due to poor retention or ionization in method **1**. Method **1** consisted of an AB Sciex API-5500 mass spectrometer with electrospray ionization coupled to a CTC PAL autosampler and an Agilent 1290 HPLC binary pump. Ten microliters of sample was injected onto a Waters Xbridge C8 column, 2.1 × 50 mm (5 µm), at 25 °C with a flow rate of 0.8 mL/min. The sample was initially held at 95% of 0.1% formic acid (mobile phase A) and 5% of 0.1% formic acid in acetonitrile (mobile phase B) for 0.3 minutes. The gradient was ramped to 5% mobile phase A over the following 0.3 minutes, where it was held for an additional 0.4 minutes. The gradient was then brought back to initial conditions for 0.15 minutes. Method **2** consisted of an AB Sciex API-6500Q mass spectrometer with electrospray ionization coupled to Shimadzu Nexera X2 UHPLC system. Five microliters of sample was injected onto a Waters CORTECS T3 column, 2.1 × 100 mm (1.6 µm), at 40 °C with a flow rate of 0.8 mL/min. The sample was initially held at 95% of 10 mM ammonium formate with 0.5% formic acid (mobile phase A) and 5% of 0.1% formic acid in acetonitrile (mobile phase B) for 0.8 minutes. The gradient was ramped to 5% mobile phase A over the following 0.8 minutes, where it was held for an additional 0.4 minutes. The gradient was then brought back to initial conditions for 1 minute. Samples were kept at 10 °C until injection.

(1) A. Mateus, P. Matsson, P, Artursson, *Molecular Pharmaceutics*, 2013, **10**, 2467-2478.

Table S2 Measured unbound drug fraction in cell ($f_{u,cell}$), ratio of the total compound concentration in cells relative to media (Kp) and the unbound drug accumulation ratio (Kp_{uu}) in HEK293 cells.

	Parent (1)			**Parent (2)**			**Parent (3)**		
Cmpd	$f_{u, cell}$	Kp	Kp_{uu}	$f_{u, cell}$	Kp	Kp_{uu}	$f_{u, cell}$	Kp	Kp_{uu}
parent	0.0095	20.2	0.138	0.0931	7.74	0.721	0.00823	89.5	0.712
#a	0.0859	1.95	0.168	0.152	ND	ND	0.00153	428	0.654
#b	0.130	0.535	0.0695	0.168	ND	ND	0.00361	4.89	0.0177
#c	0.0307	0.209	0.0064	0.037	ND	ND	0.0124	0.0445	0.0000553
#d	0.162	0.135	0.0219	0.171	ND	ND	0.0375	0.162	0.00606
#e	0.0726	0.494	0.0359	0.0539	ND	ND	0.0418	0.469	0.0196
#f	---	---	---	1.00	ND	ND	0.0036	110	0.397

	Parent (4)			**Parent (5)**			**Parent (6.1)**		
Cmpd	$f_{u, cell}$	Kp	Kp_{uu}	$f_{u, cell}$	Kp	Kp_{uu}	$f_{u, cell}$	Kp	Kp_{uu}
parent	1.00	1.80	1.798	0.085	23.64	0.138	0.0720	79.3	5.712
#a	0.279	ND	ND	0.0533	9.8	0.168	0.0691	ND	ND
#b	1.00	ND	ND	0.0812	1.77	0.0695	0.129	ND	ND
#c	0.0352	ND	ND	0.0263	0.351	0064	0.0348	ND	ND
#d	0.0804	ND	ND	1.00	0.43	0219	0.0812	ND	ND
#e	0.031	ND	ND	NV[c]	0.265	NV[c]	0.0613	ND	ND
#f	---	---	---	---	---	---	NV[c]	ND	ND

[a]--- indicates the compound was not prepared. [b]ND indicates values not determined. [c]NV indicates value could not be determined.

Chemical Synthesis

Reagents: Unless otherwise specified all the reagents and solvents were obtained from Sigma-Aldrich (Milwaukee, WI). **(+)-JQ1 (1)** was obtained from Medchemexpress (Monmouth, NJ). 2-((1E,3E)-5-((E)-3,3-dimethyl-5-sulfonato-1-(3-sulfopropyl)indolin-2-ylidene)penta-1,3-dien-1-yl)-3-(6-((2,5-dioxocyclopentyl)oxy)-6-oxohexyl)-3-methyl-1-(3-sulfonatopropyl)-3H-indol-1-ium-5-sulfonate bis triethylammonium salt (**SCD-NHS**) was synthesized as described.[2] Olaparib, 2-((6-chloro-2-methylpyrimidin-4-yl)amino)-N-(2-chloro-6-methylphenyl)thiazole-5-carboxamide, *tert*-butyl (14-amino-3,6,9,12-tetraoxatetradecyl)carbamate and alprenolol hydrochloride were purchased from Ark Pharm Inc. (Arlington Heights, IL). Truncated olaparib, **2f**, 4-(4-fluoro-3-(piperazine-1-carbonyl)benzyl)phthalazin-1(2H)-one was purchased from Chemscene (Monmouth Junction, NJ). Dasatinib was obtained from Biotang (Lexington, MA).

(2) Wai-Yee Leung, Shing-Ying Cheung, Stephen, Yue, PCT Int. Appl., **2002**, WO 2002026891 A1.

Abbreviations:

DIEA	diisopropylethylamine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
HATU	1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate
HOBt	1-hydroxybenzotriazole hydrate
MeCN	acetonitrile
NHS	N-hydroxysuccinimide
NMM	N-methylmorpholine
NMP	N-methylpyrrolidone
PEG	polyethyleneglycol
PyAOP	(7-Azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
SCD-NHS	2-((1E,3E)-5-((E)-3,3-dimethyl-5-sulfonato-1-(3-sulfopropyl)indolin-2-ylidene)penta-1,3-dien-1-yl)-3-(6-((2,5-dioxocyclopentyl)oxy)-6-oxohexyl)-3-methyl-1-(3-sulfonatopropyl)-3H-indol-1-ium-5-sulfonate bis triethylammonium salt
TFA	trifluoroacetic acid

Methods

Analytical LC-MS. Analytical LC-MS was performed on a Thermo MSQ-Plus mass spectrometer and Agilent 1100/1200 HPLC system running Xcalibur 2.0.7, Open-Access 1.4, and custom login software. The mass spectrometer was operated under positive APCI or ESI ionization conditions dependent on the system used, as noted in the text. The HPLC system comprised an Agilent Binary pump, degasser, column compartment, autosampler and diode-array detector, with a Polymer Labs ELS-2100 evaporative light-scattering detector. The column used was a Phenomenex Kinetex C8, 2.6 µm 100Å (2.1mm × 30mm), at a temperature of 65°C.

"TFA method": A gradient of 5-100% acetonitrile (A) and 0.1% trifluoroacetic acid in water (B) was used, at a flow rate of 1.5 mL/min (0-0.05 min 5% A, 0.05-1.2 min 5-100% A, 1.2-1.4 min 100% A, 1.4-1.5 min 100-5% A. 0.25 min post-run delay).

"Ammonium acetate method": A gradient of 5-100% acetonitrile (A) and 10 mM ammonium acetate in water (B) was used, at a flow rate of 1.5 mL/min (0-0.05 min 5% A, 0.05-1.2 min 5-100% A, 1.2-1.4 min 100% A, 1.4-1.5 min 100-5% A. 0.25 min post-run delay).

"TFA long with integration method": A gradient of 5-100% acetonitrile (A) and 0.1% trifluoroacetic acid in water (B) was used, at a flow rate of 1.5 mL/min (0-0.1 min 5% A, 0.1-5.2 min 5-100% A, 5.2-5.7 min 100% A, 5.7-6.0 min 100-5% A. 0.25 min post-run delay).

Chiral Analytical SFC. Analytical SFC was performed on an Aurora A5 SFC Fusion and Agilent 1100 system running under Agilent Chemstation software control. The SFC system included a 10-way column switcher, CO_2 pump, modifier pump, oven, and backpressure regulator. The mobile phase comprised of supercritical CO_2 supplied by a beverage-grade CO_2 cylinder with a modifier mixture of methanol with 0.1% diethylamine at a flow rate of 3 mL/min. Oven temperature was at 35 ºC and the outlet pressure at 150 bar. The mobile phase gradient started with 5% modifier and held it for 0.1 minutes at a flow rate of 3 mL/min, then the flow rate was ramped up to 3 mL/min and held for 0.4 min. The modifier was ramped from 5% to 50% over the next 8 minutes at 3 mL/min then held for 1 minute at 50% modifier (3 mL/min). The gradient was ramped down from 50% to 5% modifier over 0.5 min (3 mL/min). The instrument was fitted with a Whelk-O1 (S,S) column with dimensions of 4.6 mm i.d. x 150 mm length with 5 µm particles.

Analytical MS (ESI). Mass spectral screening was performed on a Thermo LCQ Deca mass spectrometer with Thermo Surveyor HPLC system running Xcalibur 2.0.7 and sampled via a custom Bohdan automated sample prep system using Sirius Slate PDA Lab Automation Suite Version 2.4. The mass spectrometer was operated under positive & negative ESI ionization conditions. The HPLC system consisted of Thermo Surveyor low pressure quaternary mixing pump with built-in solvent degassing and pulse dampening systems. Method was flow injection at ambient conditions using a 70:30 methanol: 0.1M NH4OH solvent system at 250uL/min.

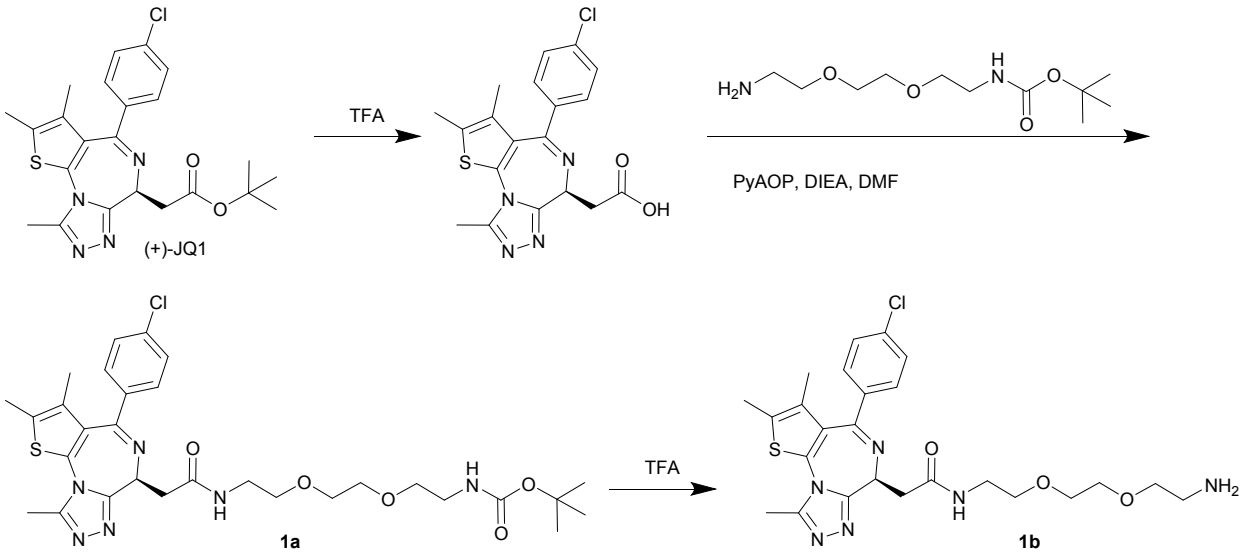
Analytical MS (DCI). Mass spectral screening was performed on a Thermo DSQ II mass spectrometer with SIS AP2000 autoprobe and LEAP HTS PAL auto sampler running Xcalibur 2.0.7 software. The mass spectrometer was operated under positive Desorption Chemical Ionization (DCI) conditions using ammonia gas as the ionization reagent. Sample was solvated in 1:1 methanol: ethyl acetate before being dried and then delivered to the mass spectrometer via the autoprobe.

Preparatory HPLC. HPLC was performed on a Gilson HPLC system equipped with liquid handler (Gison-215) and UV/Vis detector. Separations were performed on a Waters PrepPak DeltaPak C18 15 µm, 100 Å radial compression column (200 x 25 mm). The eluents were A: 0.1% TFA-water; B: acetonitrile (TFA method) or A: 10 mM ammonium acetate; B: acetonitrile (AA method). The gradient used was 0-5 min isocratic (95:5 A:B) followed by a 1%/min acetonitrile gradient until products eluted (typically up to 75% acetonitrile).

NMR Spectra were collected in DMSO-d_6 at room temperature (27 °C) on a Bruker Avance III HD spectrometer equipped with a TCI cryoprobe. ^{1}H spectra were acquired with SW= 16 ppm, 4.1 s acquisition time & 24 scans.

Elemental Analyses was performed by Intertek/QTI (Whitehouse, NJ)

***Tert*-butyl (S)-(2-(2-(2-(2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamido)ethoxy)ethoxy)ethyl)carbamate (1a) and (S)-N-(2-(2-(2-aminoethoxy)ethoxy)ethyl)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamide (JQ1-xPEG2-NH_2, 1b):**



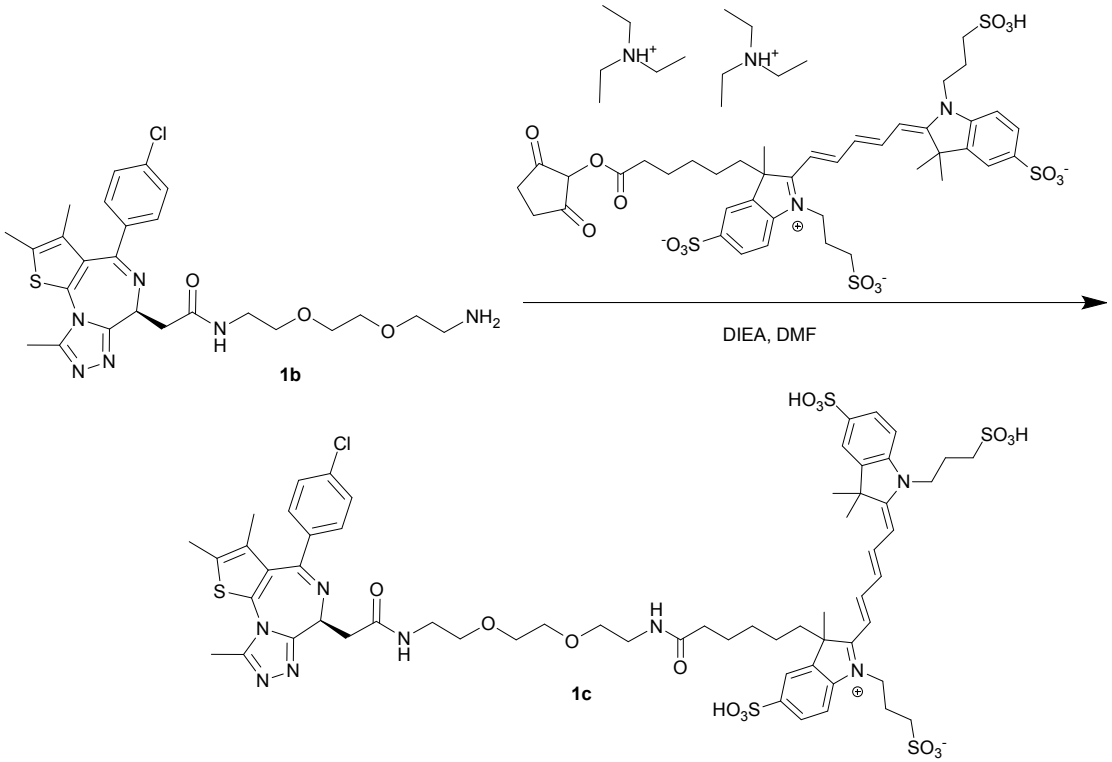
(+)-JQ1 (1 g, 2.188 mmol) was dissolved in 10 mL TFA and stirred at ambient for 30 min. The solvent was removed under a flow of dry nitrogen gas, then dissolved in 3 mL 1:1 water:MeCN and lyophilized to give the free acid [(S)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetic acid] as a yellow powder (850 mg, 97%) which was used without further purification. ^{1}H-NMR δ_H (501 MHz, DMSO-d_6) 7.50 (2 H, d, *J* 8.7), 7.45 (2 H, d), 4.47 (1 H, t, *J* 7.1), 3.48 – 3.27 (2 H, m), 2.62 (3 H, s), 2.42 (3 H, s), 1.63 (3 H, s); ESI-MS *m/z* 401.0 $[M+H]^+$; LC-MS TFA method R_t = 0.82 min, ESI-MS *m/z* 400.79 $[M+H]^+$.

(S)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetic acid (520 mg, 1.297 mmol) and tert-butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (354 mg, 1.427 mmol; Ark Pharm) were combined in 3 mL anhydrous DMF. PyAOP (676 mg, 1.297 mmol) was added and the reaction shaken at ambient for 2 h. The crude reaction mixture was diluted to 9 mL with 90% DMSO/water and purified 3 mL/injection by preparative HPLC using the TFA method. Fractions containing the desired product were combined and lyophilized to give **1a** as a light yellow powder (614 mg, 0.973 mmol, 75 % yield). ^{1}H-NMR δ_H (501 MHz, DMSO-d_6) 8.27 (1 H, t, *J* 5.7), 7.49 (2 H, d), 7.43 (2 H, d), 6.75 (1 H, d, *J* 6.0), 4.55 – 4.49 (1 H, m), 3.57 – 3.48 (4 H, m), 3.48 – 3.43 (2 H, m), 3.42 – 3.35 (2 H, m), 3.35 – 3.18 (4 H, m), 3.10 – 3.03 (2 H, m), 2.61 (3 H, s), 2.41 (3 H, s), 1.63 (3 H, s), 1.37 (9 H, s). ESI-MS *m/z* 630.9 $[M+H]^+$; LC-MS TFA w/Integration Method; R_t = 0.52 min, APCI(+) *m/z* 531.50 $[M-Boc]^+$.

Tert-butyl (S)-(2-(2-(2-(2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamido)ethoxy)ethoxy)ethyl)carbamate **1a** (500 mg, 0.792 mmol) was dissolved in 3 mL TFA and stirred at ambient for 2 min. The solvent was removed under a flow of dry nitrogen gas, then dissolved in 3 mL 1:1

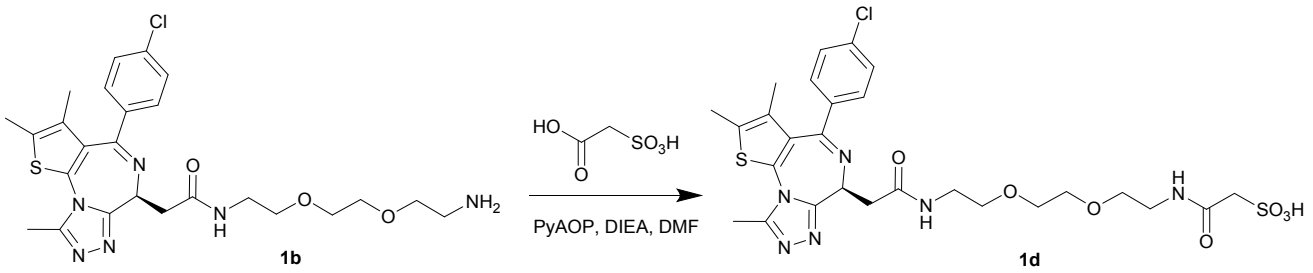
water:MeCN and lyophilized. The crude reaction mixture was diluted to 9 mL with 90% DMSO/water and purified 3 mL/injection by preparative HPLC using the TFA method. Fractions containing the desired product were combined and lyophilized to give **1b** as a light yellow powder (400 mg, 78%) ^{1}H-NMR δ_H (500 MHz, DMSO-d_6) 8.29 (1 H, t, *J* 5.7), 7.84 (1 H, s), 7.54 – 7.46 (2 H, m), 7.46 – 7.40 (2 H, m), 4.52 (1 H, t, *J* 7.5, 6.7), 3.64 – 3.54 (6 H, m), 3.47 (2 H, t, *J* 5.9), 3.34 – 3.20 (4 H, m), 3.05 – 2.95 (2 H, m), 2.60 (3 H, s), 2.42 (3 H, d, *J* 0.9), 1.63 (3 H, s). ESI-MS *m/z* 531.2 $[M+H]^+$; LC-MS TFA Long w/Integration, R_t = 2.49 min, ESI(+) *m/z* 531.02 $[M+H]^+$.

3-(3-(1-((S)-4-(4-Chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-2,13-dioxo-6,9-dioxa-3,12-diazaoctadecan-18-yl)-2-((1E,3E)-5-((E)-3,3-dimethyl-5-sulfo-1-(3-sulfopropyl)indolin-2-ylidene)penta-1,3-dien-1-yl)-3-methyl-5-sulfo-3H-indol-1-ium-1-yl)propane-1-sulfonate (JQ1-xPEG2-SCD, 1c):



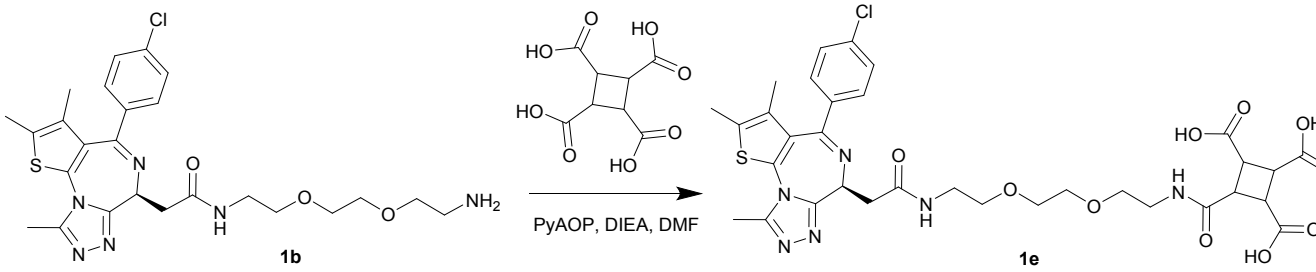
(S)-N-(2-(2-(2-aminoethoxy)ethoxy)ethyl)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamide **1b** (15.8 mg, 0.024 mmol) was combined with SCD-NHS (43.4 mg, 0.037 mmol) in 1 mL anhydrous DMF containing DIEA (9.5 mg, 0.073 mmol) and shaken at ambient for 16 h. The crude reaction mixture was diluted to 3 mL with 90% DMSO/water and purified by preparative HPLC using the TFA method. Fractions containing the desired product were combined and lyophilized to give **1c** as a dark blue solid (17.1 mg, 50.9%). ^{1}H-NMR δ_H (400 MHz, DMSO-d_6) 8.42 – 8.28 (4 H, m), 7.81 – 7.70 (4 H, m), 7.65 – 7.56 (3 H, m), 7.54 – 7.34 (7 H, m), 6.49 (3 H, dd, *J* 38.5, 12.5), 4.58 (1 H, t, *J* 7.1), 4.32 – 4.20 (5 H, m), 3.34 (2 H, t, *J* 6.2), 3.25 (3 H, q, *J* 6.5, 5.2), 3.11 (3 H, q, *J* 6.1), 2.63 – 2.57 (5 H, m), 2.04 – 1.94 (5 H, m), 1.91 – 1.82 (3 H, m), 1.71 – 1.64 (12 H, m), 1.62 (3 H, s), 1.36 – 1.20 (6 H, m), 1.12 – 1.04 (3 H, m). ESI-MS *m/z* 1371.2 $[M+H]^+$; LC-MS TFA High Mass Method, R_t = 0.62 min, ESI(+) *m/z* 1372.72 $[M+H]^+$.

(S)-14-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-2,13-dioxo-6,9-dioxa-3,12-diazatetradecane-1-sulfonic acid (JQ1-xPEG2-SAA, 1d):



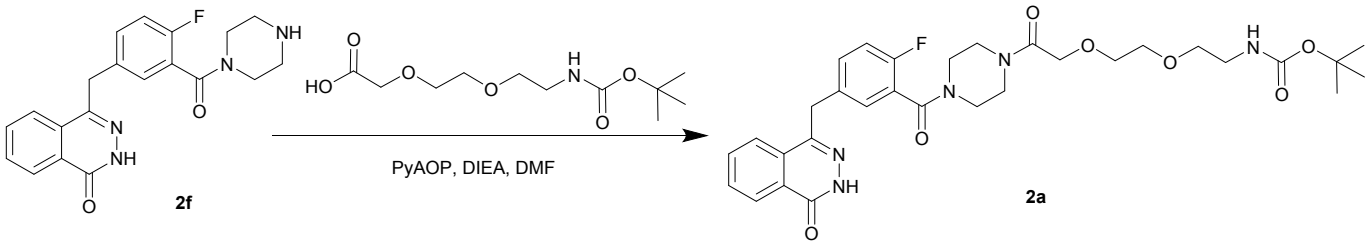
(S)-N-(2-(2-(2-aminoethoxy)ethoxy)ethyl)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamide **1b** (17.5 mg, 0.027 mmol) was combined with 2-sulfoacetic acid (19.0 mg, 0.136 mmol) in 1 mL anhydrous DMF. PyAOP (70.7 mg, 0.136 mmol) and DIEA (35.1 mg, 0.271 mmol) were added and the reaction shaken at ambient for 16 h. The crude reaction mixture was diluted to 3 mL with 90% DMSO/water and purified by preparative HPLC using the TFA method. Fractions containing the desired product were combined and lyophilized to give **1d** as a light yellow solid (12.9 mg, 62.0%) ^{1}H-NMR δ_H (400 MHz, DMSO-d_6) 8.30 (1 H, t, *J* 5.7), 7.93 (1 H, t, *J* 5.5), 7.54 – 7.47 (2 H, m), 7.47 – 7.40 (2 H, m), 4.57 (1 H, t, *J* 7.7, 6.5), 3.54 (2 H, s), 3.50 – 3.39 (4 H, m), 3.36 – 3.20 (8 H, m), 3.17 (2 H, s), 2.63 (3 H, s), 2.42 (3 H, s), 1.63 (3 H, s). ESI-MS *m/z* 653.0 $[M+H]^+$, 675 $[M+Na]^+$; LC-MS TFA Method, R_t = 0.71 min, APCI(+) *m/z* 653.30 $[M+H]^+$.

4-((2-(2-(2-(2-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamido)ethoxy)ethoxy)ethyl)carbamoyl)cyclobutane-1,2,3-tricarboxylic acid (JQ1-xPEG2-CCT, 1e):



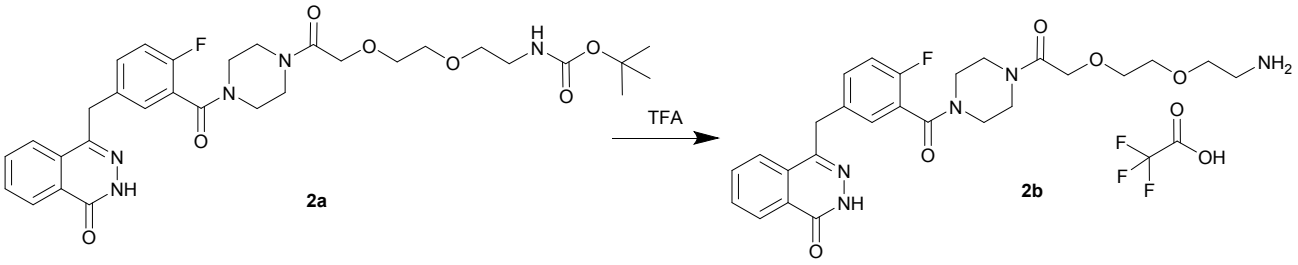
(S)-N-(2-(2-(2-aminoethoxy)ethoxy)ethyl)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamide **1b** (18.5 mg, 0.029 mmol) was combined with cyclobutane-1,2,3,4-tetracarboxylic acid (33.3 mg, 0.143 mmol) in 1 mL anhydrous DMF. PyAOP (74.8 mg, 0.143 mmol) and DIEA (37.1 mg, 0.287 mmol) were added and the reaction shaken at ambient for 16 h. The crude reaction mixture was diluted to 3 mL with 90% DMSO/water and purified by preparative HPLC using the TFA method. Fractions containing the desired product were combined and lyophilized to give **1e** as a light yellow solid (24.4 mg, 99.0%). ^{1}H-NMR δ_H (400 MHz, DMSO-d_6) 8.60 (1 H, s), 8.28 (1 H, t, *J* 5.7), 8.11 (1 H, t, *J* 5.7), 7.49 (2 H, d, *J* 8.8), 7.43 (2 H, d, *J* 8.7), 4.52 (1 H, t, *J* 7.7, 6.4), 3.54 (6 H, pq, *J* 5.7, 3.4, 2.7), 3.48 – 3.37 (5 H, m), 3.31 – 3.16 (5 H, m), 2.60 (3 H, s), 2.41 (3 H, s), 1.63 (3 H, s). ESI-MS *m/z* 653.0 $[M+H]^+$, 675.1 $[M+Na]^+$; LC-MS TFA Method, Rt = 0.64 min, APCI(+) *m/z* 745.32 $[M+H]^+$.

***Tert*-butyl (2-(2-(2-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperazin-1-yl)-2-oxoethoxy)ethoxy)ethyl)carbamate (olaparib-xPEG2-NHBoc, 2a):**



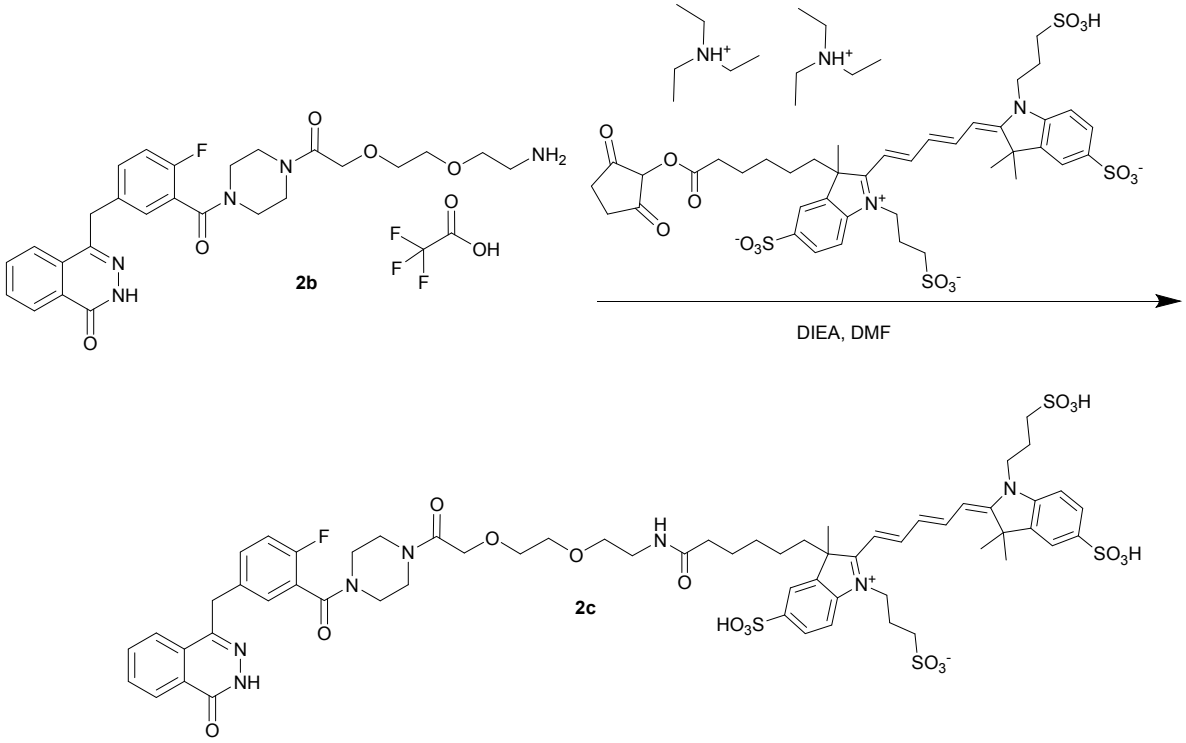
2,2-dimethyl-4-oxo-3,8,11-trioxa-5-azatridecan-13-oic acid DCHA salt was converted to the free acid by partitioning between DCM and ice-cold 1 M HCl (aq.). The DCM layer was dried over $MgSO_4$ and evaporated to give a the free acid as a light-yellow oil. 4-(4-fluoro-3-(piperazine-1-carbonyl)benzyl)phthalazin-1(2H)-one **2f** (100 mg, 0.273 mmol) was combined with 2,2-dimethyl-4-oxo-3,8,11-trioxa-5-azatridecan-13-oic acid (86 mg, 0.328 mmol) in 3 mL anhydrous DMF. PyAOP (212 mg, 0.407 mmol) and DIEA (212 mg, 1.638 mmol) were added and the reaction shaken at ambient for 2 h. The crude reaction mixture was diluted to 9 mL with 90% DMSO/water and purified 3 mL/injection by preparative HPLC using the TFA method. Fractions containing the desired product were combined and lyophilized to give **2a** as a colorless oil (128 mg, 77.0%) ^{1}H-NMR δ_H (400 MHz, DMSO-d_6) 12.18 (1 H, s), 8.31 (1 H, dd, *J* 7.6, 1.7), 7.97 – 7.92 (1 H, m), 7.91 – 7.79 (2 H, m), 7.47 – 7.40 (1 H, m), 7.36 – 7.32 (1 H, m), 7.22 – 7.15 (1 H, m), 4.34 (2 H, s), 4.17 (1 H, s), 3.64 – 3.55 (2 H, m), 3.53 – 3.42 (5 H, m), 1.81 – 1.75 (10 H, m), 1.41 (9 H, s). ESI-MS *m/z* 610.3 $[M-H]^-$; LC-MS TFA Method, R_t = 0.77 min, APCI(+) *m/z* 612.36 $[M+H]^+$.

4-(3-(4-(2-(2-(2-aminoethoxy)ethoxy)acetyl)piperazine-1-carbonyl)-4-fluorobenzyl)phthalazin-1(2H)-one 2,2,2-trifluoroacetate (olaparib-xPEG2-NH_2, 2b):



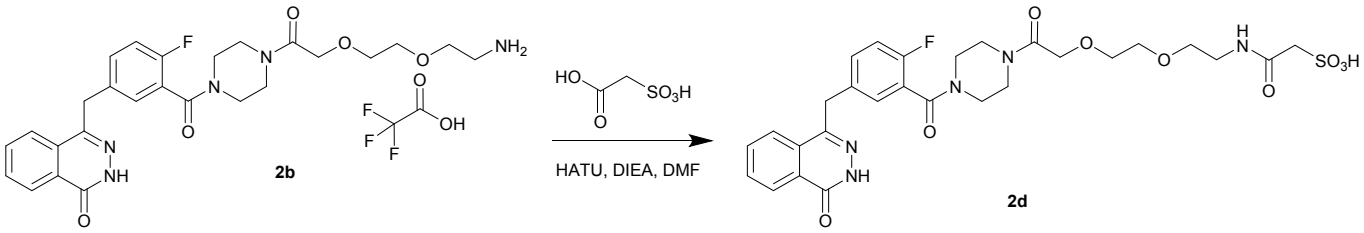
Tert-butyl (2-(2-(2-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperazin-1-yl)-2-oxoethoxy)ethoxy)ethyl)carbamate **2a** (100 mg, 0.163 mmol) was dissolved in 1 mL TFA and immediately evaporated to dryness under a stream of dry nitrogen gas. The residue was dissolved in 1 mL 1:1 water:MeCN and lyophilized to give the product **2b** a colorless solid (98 mg, 96.0%) ^{1}H-NMR δ_H (400 MHz, DMSO-d_6) 8.88 (1 H, d, *J* 137.2), 8.07 – 7.81 (2 H, m), 7.61 – 7.27 (1 H, m), 4.45 – 3.97 (3 H, m), 3.84 – 3.34 (10 H, m), 3.31 – 2.87 (8 H, m), 2.19 – 1.71 (5 H, m). ESI-MS *m/z* 510.3 $[M-H]^-$, 512.1 $[M+H]^+$; LC-MS TFA Method, R_t = 0.71 min, APCI(+) *m/z* 512.31 $[M+H]^+$.

3-(2-((1E,3E)-5-((E)-3,3-dimethyl-5-sulfo-1-(3-sulfopropyl)indolin-2-ylidene)penta-1,3-dien-1-yl)-3-(6-((2-(2-(2-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperazin-1-yl)-2-oxoethoxy)ethoxy)ethyl)amino)-6-oxohexyl)-3-methyl-5-sulfo-3H-indol-1-ium-1-yl)propane-1-sulfonate (olaparib-xPEG2-SCD, 2c):



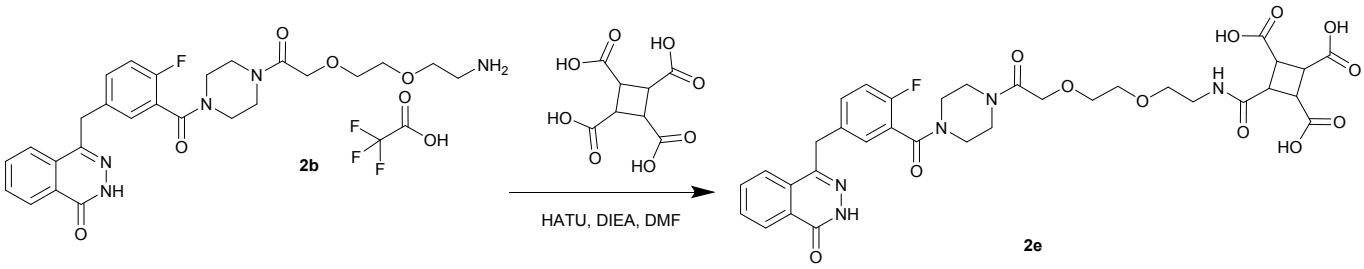
4-(3-(4-(2-(2-(2-Aminoethoxy)ethoxy)acetyl)piperazine-1-carbonyl)-4-fluorobenzyl)phthalazin-1(2H)-one 2,2,2-trifluoroacetate **2b** (10.53 mg, 0.017 mmol) was combined with SCD-NHS (21.45 mg, 0.019 mmol) in 1 mL anhydrous DMF containing DIEA (6.53 mg, 0.050 mmol) and shaken at ambient for 16 h. The crude reaction mixture was diluted to 3 mL with 90% DMSO/water and purified by preparative HPLC using the TFA method. Fractions containing the desired product were combined and lyophilized to give **2c** as a dark blue solid (14 mg, 61.4%) ^{1}H-NMR δ_H (400 MHz, DMSO-d_6) 12.58 (1 H, s), 8.38 – 8.34 (2 H, m), 8.26 (1 H, d, *J* 7.6), 7.97 (1 H, d, *J* 10.4), 7.91 – 7.83 (1 H, m), 7.81 (2 H, d, *J* 14.7), 7.75 (1 H, s), 7.64 (2 H, d, *J* 8.3), 7.43 – 7.36 (4 H, m), 7.23 (1 H, t, *J* 9.6), 6.58 – 6.54 (0 H, m), 6.48 – 6.44 (3 H, m), 4.33 (2 H, s), 4.29 – 4.22 (5 H, m), 4.17 (1 H, s), 4.12 (1 H, s), 3.33 (4 H, s), 3.17 (2 H, s), 3.11 (3 H, s), 2.90 (2 H, s), 2.74 (2 H, s), 2.63 – 2.52 (5 H, m), 2.18 – 2.13 (2 H, m), 2.02 – 1.98 (4 H, m), 1.89 – 1.84 (2 H, m), 1.68 (8 H, s), 1.31 (4 H, s), 1.24 (2 H, s), 1.09 (3 H, s), 0.86 (1 H, s), 0.78 (2 H, s), 0.44 (1 H, s). ESI-MS *m/z* 675.1 $[M-2H]^{2-}$; LC-MS TFA Method, R_t = 0.63 min, APCI(+) *m/z* 677.33 $[M+2H]^{2+}$.

2-((2-(2-(2-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperazin-1-yl)-2-oxoethoxy)ethoxy)ethyl)amino)-2-oxoethane-1-sulfonic acid (olaparib-xPEG2-SAA, 2d):



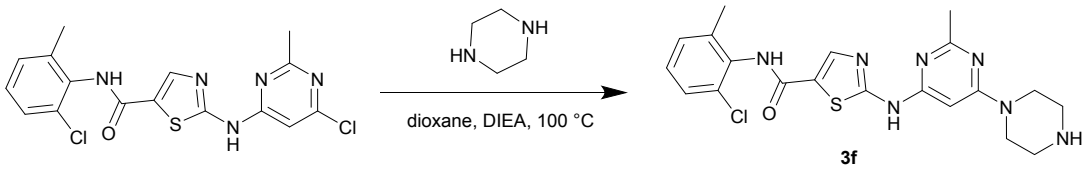
4-(3-(4-(2-(2-(2-Aminoethoxy)ethoxy)acetyl)piperazine-1-carbonyl)-4-fluorobenzyl)phthalazin-1(2H)-one 2,2,2-trifluoroacetate **2b** (20 mg, 0.032 mmol) was combined with 2-sulfoacetic acid (8.8 mg, 0.063 mmol) in 1 mL anhydrous DMF. HATU (21.9 mg, 0.058 mmol) and DIEA (21 mg, 0.160 mmol) were added and the reaction shaken at ambient for 16 h. The crude reaction mixture was diluted to 3 mL with 90% DMSO/water and purified by preparative HPLC using the TFA method. Fractions containing the desired product were combined and lyophilized to give **2d** as a colorless solid (1.6 mg, 7.9%) ^{1}H-NMR δ_H (400 MHz, DMSO-d_6) 12.58 (1 H, s), 8.27 (1 H, dd, *J* 7.9, 1.5), 8.01 – 7.80 (4 H, m), 7.49 – 7.31 (2 H, m), 7.23 (1 H, t, *J* 9.0), 4.34 (2 H, s), 4.18 (2 H, d, *J* 21.6), 3.74 – 3.54 (8 H, m), 3.44 – 3.13 (8 H, m), 1.39 (0 H, d, *J* 6.6), 1.32 – 1.22 (2 H, m). ESI-MS *m/z* 632.1 $[M-H]^-$, 654.2 $[M-2H+Na]^-$, 1265.1 $[2M-H]^-$; LC-MS TFA Method R_t = 0.77 min, ESI(+) *m/z* 633.64 $[M+H]^+$.

4-((2-(2-(2-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperazin-1-yl)-2-oxoethoxy)ethoxy)ethyl)carbamoyl)cyclobutane-1,2,3-tricarboxylic acid (olaparib-xPEG2-CCT, 2e):



4-(3-(4-(2-(2-(2-Aminoethoxy)ethoxy)acetyl)piperazine-1-carbonyl)-4-fluorobenzyl)phthalazin-1(2H)-one 2,2,2-trifluoroacetate **2b** (16 mg, 0.026 mmol) was combined with cyclobutane-1,2,3,4-tetracarboxylic acid (17.8 mg, 0.077 mmol) in 1 mL anhydrous DMF. HATU (9.7 mg, 0.026 mmol) and DIEA (20 mg, 0.153 mmol) were added and the reaction shaken at ambient for 16 h. The crude reaction mixture was diluted to 3 mL with 90% DMSO/water and purified by preparative HPLC using the TFA method. Fractions containing the desired product were combined and lyophilized to give **2e** as a colorless solid (2.5 mg, 13.5%) 1H-NMR δ_H (400 MHz, DMSO-d_6) 12.59 (3 H, s), 8.34 – 8.20 (1 H, m), 8.10 (1 H, s), 7.97 (1 H, d, *J* 7.9), 7.93 – 7.79 (2 H, m), 7.44 (1 H, t, *J* 4.4), 7.38 (1 H, d, *J* 6.2), 7.24 (1 H, t, *J* 9.0), 4.34 (2 H, s), 4.17 (2 H, d, *J* 20.6), 3.79 – 3.60 (4 H, m), 3.60 – 3.40 (9 H, m), 3.23 – 3.05 (5 H, m), 1.44 – 1.07 (1 H, m). ESI-MS *m/z* 724.1 $[M-H]^-$, 746.1 $[M-2H+Na]^-$; LC-MS TFA Method R_t = 0.63 min, ESI(+) *m/z* 726.11 $[M+H]^+$.

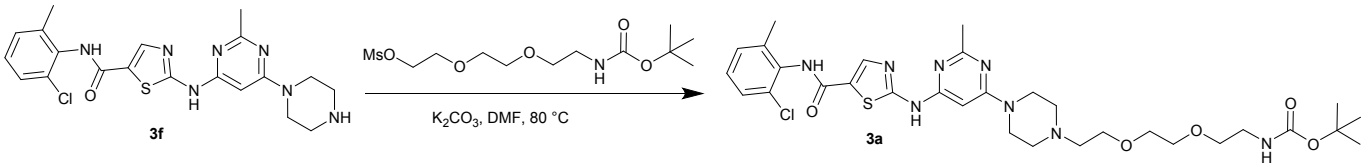
N-(2-Chloro-6-methylphenyl)-2-((2-methyl-6-(piperazin-1-yl)pyrimidin-4-yl)amino)thiazole-5-carboxamide (des-ethoxy dasatinib, 3f):



3f

2-((6-Chloro-2-methylpyrimidin-4-yl)amino)-N-(2-chloro-6-methylphenyl)thiazole-5-carboxamide (1 g, 2.54 mmol) , piperazine (2.185 g, 25.4 mmol) and DIEA (656 mg, 5.07 mmol) were combined in 30 mL 1,4-dioxane and refluxed for 4 h. The resulting white solid was filtered, washed with ether and dried in vacuo to give the product **3f** as a white solid (1.1 g, 98.0%) ^{1}H-NMR δ_H (400 MHz, DMSO-d_6) 9.92 (1 H, s), 8.25 (1 H, s), 7.40 (1 H, dd, *J* 7.5, 2.0), 7.32 – 7.21 (2 H, m), 6.06 (1 H, s), 3.51 – 3.44 (4 H, m), 2.82 – 2.75 (4 H, m), 2.40 (3 H, s), 2.24 (3 H, s). ESI-MS *m/z* 444.2 $[M+H]^+$, 442.3 $[M-H]^-$; LC-MS TFA Method R_t = 0.68 min, ESI(+) *m/z* 443.80 $[M+H]^+$

***Tert*-butyl (2-(2-(2-(4-(6-((5-((2-chloro-6-methylphenyl)carbamoyl)thiazol-2-yl)amino)-2-methylpyrimidin-4-yl)piperazin-1-yl)ethoxy)ethoxy)ethyl)carbamate (dasatinib-xPEG1-NHBoc, 3a):**

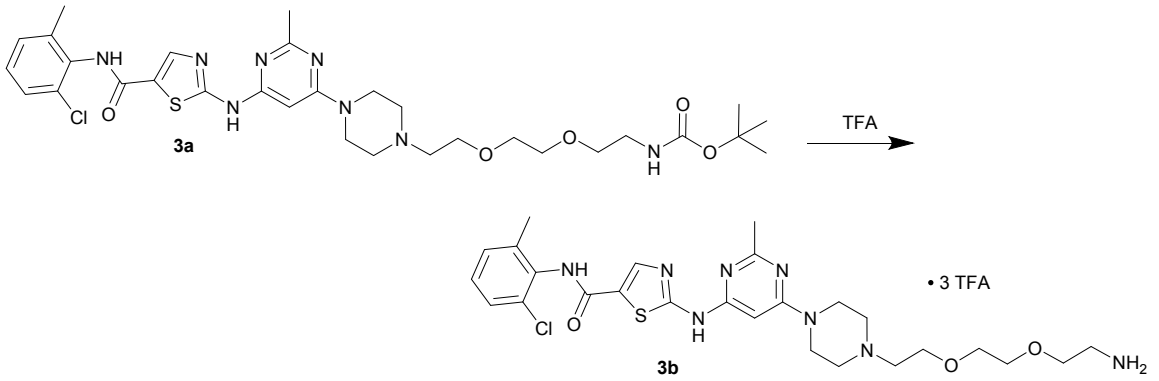


3f 3a

N-(2-Chloro-6-methylphenyl)-2-((2-methyl-6-(piperazin-1-yl)pyrimidin-4-yl)amino)thiazole-5-carboxamide **3f** (1017 mg, 2.291 mmol) was combined with 2,2-dimethyl-4-oxo-3,8,11-trioxa-5-azatridecan-13-yl methanesulfonate (900 mg, 2.75 mmol)[3] and potassium carbonate (950 mg, 6.87 mmol) in 15 mL anhydrous DMF and stirred at 80 °C for 16 h. The reaction was diluted with 50 mL water and extracted with 3 × 50 mL ethyl acetate. The ethyl acetate layers were combined, dried over $MgSO_4$ and evaporated to a brown oil. The oil was diluted with 90% DMSO/water 3 mL/injection by preparative HPLC using the TFA method. Fractions containing the desired product were combined and lyophilized to give **3a** as a colorless oil (146 mg, 10.9%); ^{1}H-NMR δ_H (500 MHz, DMSO-d_6) 11.64 (1 H, s), 9.91 (1 H, s), 8.24 (1 H, s), 7.43 – 7.38 (1 H, m), 7.30 – 7.23 (1 H, m), 6.80 (1 H, t, *J* 5.8), 6.15 (1 H, s), 4.35 (2 H, d, *J* 13.9), 3.78 (1 H, t, *J* 4.9), 3.74 – 3.66 (2 H, m), 3.66 – 3.58 (3 H, m), 3.58 – 3.52 (7 H, m), 3.38 (4 H, dt, *J* 14.9, 6.4), 3.29 (2 H, t, *J* 13.0), 3.12 – 3.02 (2 H, m), 2.45 (2 H, s), 2.24 (2 H, s), 1.37 (9 H, s). ESI-MS *m/z* 444.2 $[M+H]^+$, 442.3 $[M-H]^-$; LC-MS TFA Method, R_t = 0.68 min, ESI(+) *m/z* 443.80 $[M+H]^+$.

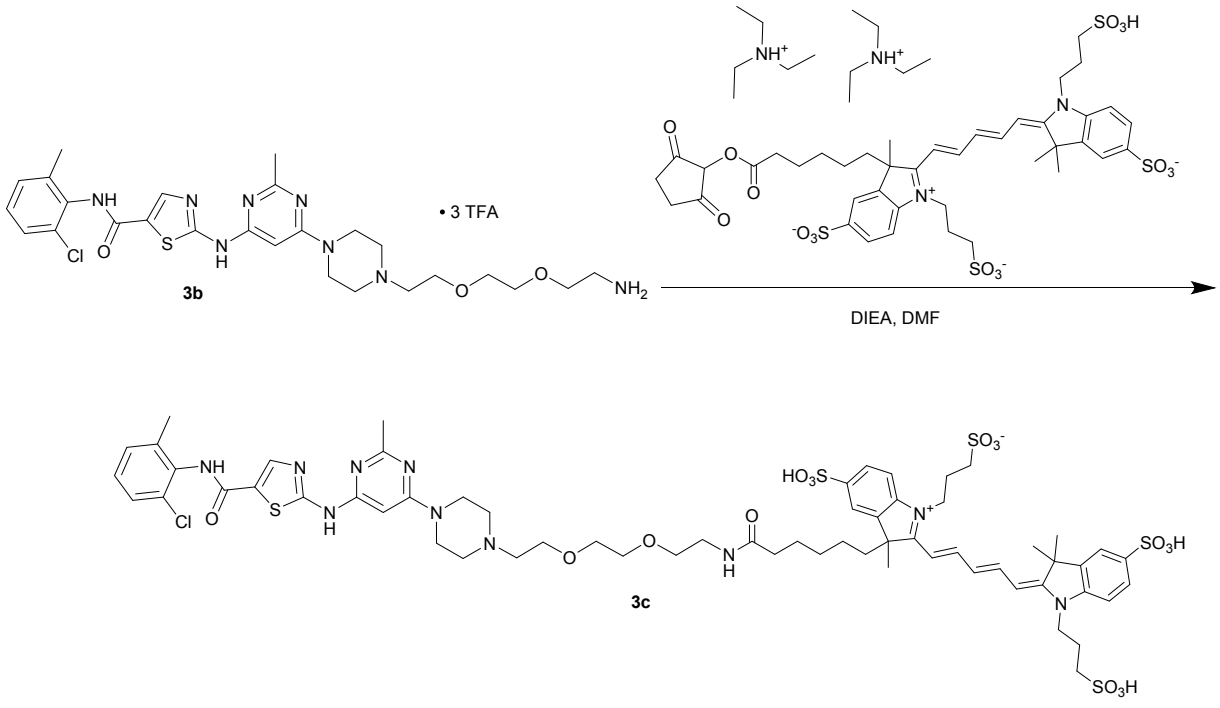
(3) Julie Moreau and Jacqueline Marchand-Brynaert, *Eur. J. Org. Chem.,* **2011**, 1641-1644.

2-((6-(4-(2-(2-(2-Aminoethoxy)ethoxy)ethyl)piperazin-1-yl)-2-methylpyrimidin-4-yl)amino)-N-(2-chloro-6-methylphenyl)thiazole-5-carboxamide tris-2,2,2-trifluoroacetate (dasatinib-xPEG1-NH_2, 3b):



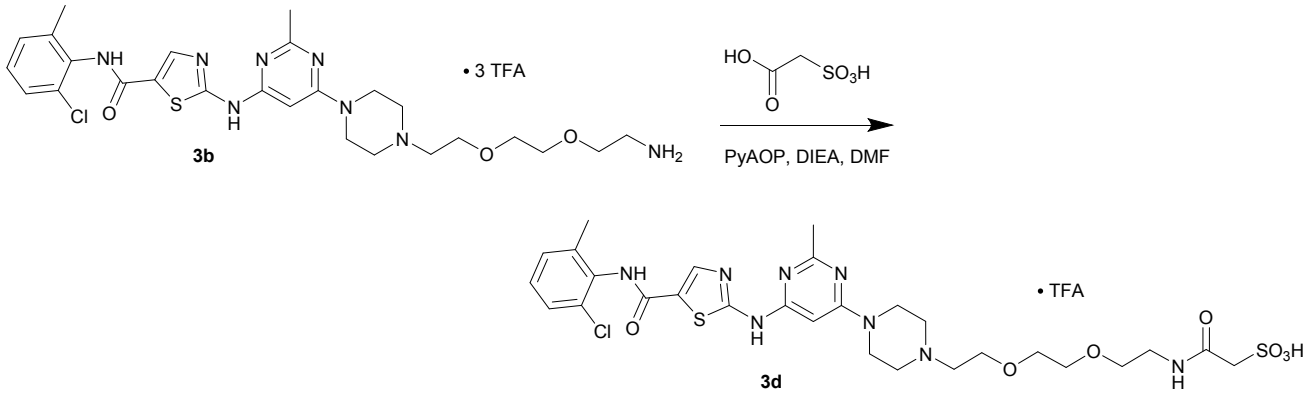
Tert-butyl (2-(2-(2-(4-(6-((5-((2-chloro-6-methylphenyl)carbamoyl)thiazol-2-yl)amino)-2-methylpyrimidin-4-yl)piperazin-1-yl)ethoxy)ethoxy)ethyl)carbamate **3a** (140 mg, 0.212 mmol) was dissolved in 1 mL TFA and immediately evaporated to dryness under a stream of dry nitrogen gas. The residue was dissolved in 1 mL 1:1 water:MeCN and lyophilized to give **3b** as a colorless solid (130 mg, 66.9%) ^{1}H-NMR δ_H (400 MHz, DMSO-d_6) 11.66 (1 H, s), 10.19 (1 H, s), 9.91 (1 H, s), 8.25 (1 H, s), 7.87 (3 H, s), 7.40 (1 H, dd, *J* 7.5, 2.0), 7.31 – 7.19 (2 H, m), 6.18 (1 H, s), 3.79 (2 H, dd, *J* 5.8, 3.9), 3.71 – 3.50 (7 H, m), 3.40 – 3.06 (6 H, m), 3.00 (2 H, h, *J* 5.6), 2.45 (3 H, s), 2.24 (3 H, s). ESI-MS *m/z* 288.3 $[M+2H]^{2+}$, 575.1 $[M+H]^+$, 597.1 $[M+Na]^+$, 633.0 $[M+CH_3CN+NH_4]^+$; LC-MS TFA Method R_t = 0.65 min, APCI(+) *m/z* 575.2$[M+H]^+$; LC-MS AA Method R_t = 0.93 min, APCI(+) *m/z* 575.33 $[M+H]^+$.

3-(3-(6-((2-(2-(2-(4-(6-((5-((2-chloro-6-methylphenyl)carbamoyl)thiazol-2-yl)amino)-2-methylpyrimidin-4-yl)piperazin-1-yl)ethoxy)ethoxy)ethyl)amino)-6-oxohexyl)-2-((1E,3E)-5-((E)-3,3-dimethyl-5-sulfo-1-(3-sulfopropyl)indolin-2-ylidene)penta-1,3-dien-1-yl)-3-methyl-5-sulfo-3H-indol-1-ium-1-yl)propane-1-sulfonate (dasatinib-xPEG1-SCD, 3c):



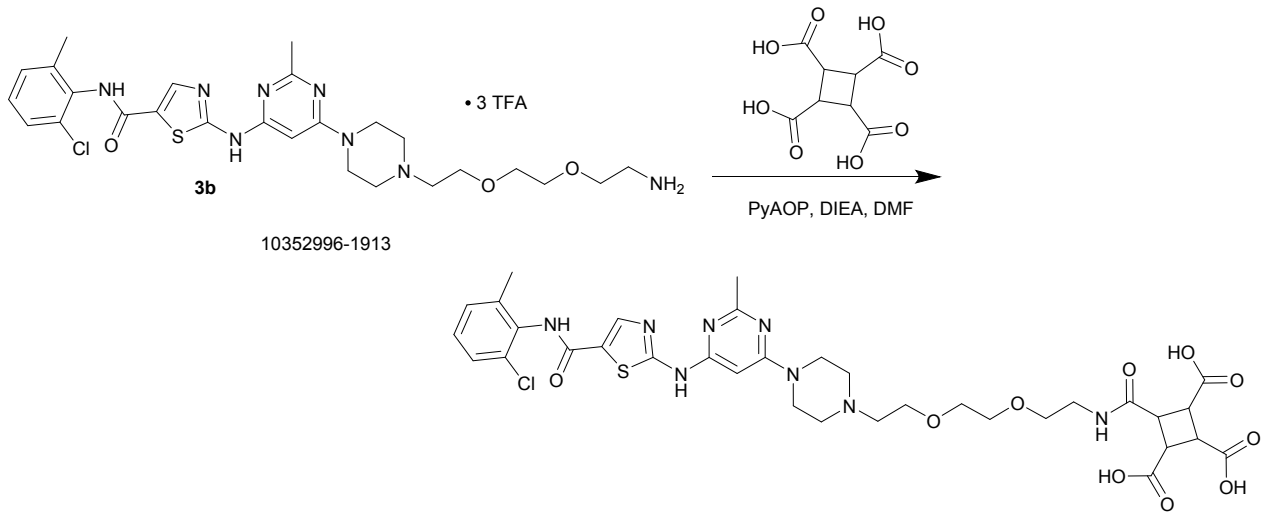
2-((6-(4-(2-(2-(2-Aminoethoxy)ethoxy)ethyl)piperazin-1-yl)-2-methylpyrimidin-4-yl)amino)-N-(2-chloro-6-methylphenyl)thiazole-5-carboxamide tris-2,2,2-trifluoroacetate **3b** (13.2 mg, 0.014 mmol) was combined with SCD-NHS (20 mg, 0.017 mmol) in 1 mL anhydrous DMF containing DIEA (5.6 mg, 0.043 mmol) and shaken at ambient for 16 h. The crude reaction mixture was diluted to 3 mL with 90% DMSO/water and purified by preparative HPLC using the TFA method. Fractions containing the desired product were combined and lyophilized to give **3c** as a dark blue solid (3.0 mg, 14.7%) ^{1}H-NMR δ_H (400 MHz, DMSO-d_6) 11.55 (1 H, s), 9.90 (1 H, s), 8.42 – 8.30 (2 H, m), 8.23 (1 H, s), 7.83 – 7.70 (3 H, m), 7.69 – 7.60 (2 H, m), 7.39 (3 H, dd, *J* 8.3, 4.0), 7.33 – 7.21 (2 H, m), 6.62 – 6.36 (3 H, m), 4.26 (5 H, s), 3.77 – 3.41 (9 H, m), 3.10 (7 H, q, *J* 7.1), 2.67 – 2.56 (4 H, m), 2.44 (3 H, s), 2.25 (3 H, s), 2.05 – 1.95 (4 H, m), 1.86 (2 H, t, *J* 7.6), 1.73 – 1.61 (9 H, m), 1.29 – 1.12 (12 H, m), 0.81 (3 H, d, *J* 25.0). ESI-MS *m/z* 708.2 $[M+2H]^{2+}$, 1415.2 $[M+H]^{+}$, 1437.4 $[M+Na]^{+}$; LC-MS TFA Method, R_t = 0.68 min; APCI(+) *m/z* 708.2 $[M+2H]^{2+}$.

2-((2-(2-(2-(4-(6-((5-((2-chloro-6-methylphenyl)carbamoyl)thiazol-2-yl)amino)-2-methylpyrimidin-4-yl)piperazin-1-yl)ethoxy)ethoxy)ethyl)amino)-2-oxoethane-1-sulfonic acid 2,2,2,-trifluoroacetate (dasatinib-xPEG1-SAA, 3d):



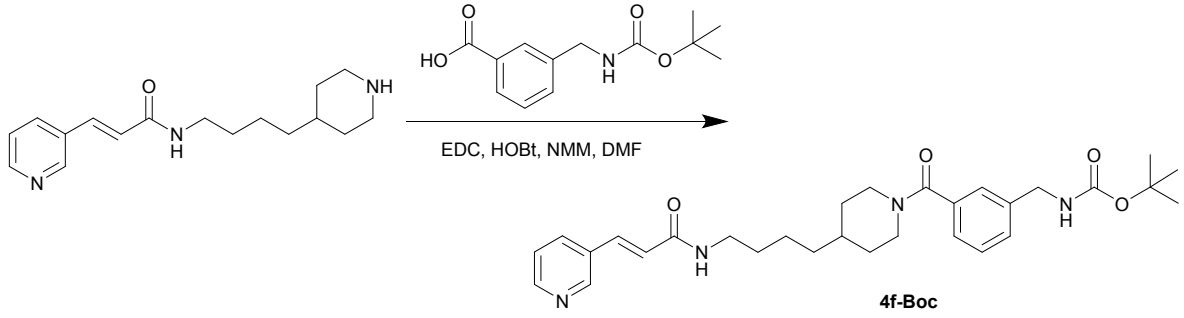
2-((6-(4-(2-(2-(2-Aminoethoxy)ethoxy)ethyl)piperazin-1-yl)-2-methylpyrimidin-4-yl)amino)-N-(2-chloro-6-methylphenyl)thiazole-5-carboxamide tris-2,2,2-trifluoroacetate **3b** (16 mg, 0.017 mmol) was combined with 2-sulfoacetic acid (12.2 mg, 0.087 mmol) in 1 mL anhydrous DMF. PyAOP (45.5 mg, 0.087 mmol) and DIEA (22.5 mg, 0.174 mmol) were added and the reaction shaken at ambient for 16 h. The crude reaction mixture was diluted to 3 mL with 90% DMSO/water and purified by preparative HPLC using the TFA method. Fractions containing the desired product were combined and lyophilized to give **3d** as a colorless solid (7.1 mg, 50.2%) ^{1}H-NMR δ_H (501 MHz, DMSO-d_6) 11.54 (1 H, s), 9.90 (1 H, s), 9.69 (1 H, s), 8.23 (1 H, s), 8.05 – 7.92 (1 H, m), 7.42 – 7.38 (1 H, m), 7.32 – 7.23 (2 H, m), 6.24 (1 H, s), 4.46 – 4.22 (2 H, m), 3.84 – 3.80 (2 H, m), 3.65 – 3.57 (6 H, m), 3.50 – 3.45 (2 H, m), 3.41 (4 H, s), 3.31 – 3.26 (2 H, m), 3.12 (2 H, d, *J* 11.4), 2.45 (3 H, s), 2.24 (3 H, s). ESI-MS *m/z* 695.2 $[M-H]^{-}$, 717.1 $[M-2H+Na]^{-}$; LC-MS TFA Method, R_t = 0.69 min; APCI(+) *m/z* 698.28 $[M+H]^{+}$.

4-((2-(2-(2-(4-(6-((5-((2-chloro-6-methylphenyl)carbamoyl)thiazol-2-yl)amino)-2-methylpyrimidin-4-yl)piperazin-1-yl)ethoxy)ethoxy)ethyl)carbamoyl)cyclobutane-1,2,3-tricarboxylic acid (dasatinib-xPEG1-CCT, 3e):



2-((6-(4-(2-(2-(2-Aminoethoxy)ethoxy)ethyl)piperazin-1-yl)-2-methylpyrimidin-4-yl)amino)-N-(2-chloro-6-methylphenyl)thiazole-5-carboxamide tris-2,2,2-trifluoroacetate **3b** (16 mg, 0.017 mmol) was combined with cyclobutane-1,2,3,4-tetracarboxylic acid (26.2 mg, 0.113 mmol) in 1 mL anhydrous DMF. PyAOP (59 mg, 0.113 mmol) and DIEA (29.2 mg, 0.226 mmol) were added and the reaction shaken at ambient for 16 h. The crude reaction mixture was diluted to 3 mL with 90% DMSO/water and purified by preparative HPLC using the TFA method. Fractions containing the desired product were combined and lyophilized to give **2e** as a colorless solid (5.4 mg, 30.3%). ^{1}H-NMR δ_H (501 MHz, DMSO-d_6) 9.86 (1 H, s), 8.36 (1 H, t, *J* 5.7), 8.21 (1 H, s), 7.40 (1 H, dd, *J* 7.7, 1.8), 7.31 – 7.22 (1 H, m), 6.09 (1 H, s), 3.55 (1 H, t, *J* 5.8), 3.51 – 3.49 (22 H, m), 3.47 (1 H, t, *J* 9.2), 3.44 – 3.39 (1 H, m), 3.33 (1 H, t, *J* 9.1), 3.24 – 3.16 (2 H, m), 2.40 (2 H, s), 2.24 (2 H, s), 1.91 (3 H, s). ESI-MS *m/z* 789.2 $[M+H]^+$, 811.1 $[M+Na]^+$; LC-MS TFA Method R_t = 0.66 min, APCI(+) *m/z* 789.36 $[M+H]^+$.

***Tert*-butyl (E)-(3-(4-(4-(3-(pyridin-3-yl)acrylamido)butyl)piperidine-1-carbonyl)benzyl)carbamate (FK866-CH_2NHBoc, 4f-Boc):**

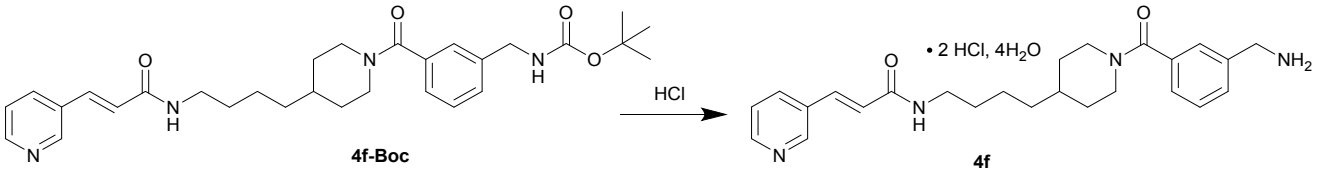


(*E*)-N-(4-(Piperidin-4-yl)butyl)-3-(pyridin-3-yl)acrylamide bis-hydrochloride[4] (100 mg, 0.278 mmol), 3-(((*tert*-butoxycarbonyl)amino)methyl)benzoic acid (91 mg, 0.361 mmol), HOBt (46.8 mg, 0.305 mmol) and NMM (140 mg, 1.388 mmol) were combined in 1.4 mL anhydrous DMF. EDC (69.2 mg, 0.361 mmol) was added and the reaction stirred at ambient for 16 h. The DMF was evaporated under a stream of dry nitrogen gas and the residue

dissolved in 20 mL ethyl acetate/20 mL brine. The separated aqueous layer was extracted with 20 mL ethyl acetate and the combined organic layers washed with 20 mL brine, dried over $MgSO_4$, stripped, and chromatographed on silica eluted with 7% methanol /methylene chloride containing 0.1% aq. ammonia to give the product **4f-Boc** as a colorless oil (129 mg, 89.0%). ^{1}H-NMR δ_H (300 MHz, DMSO-d_6) 8.74 (1 H, d, *J* 2.2), 8.54 (1 H, dd, *J* 4.7, 1.6), 8.13 (1 H, t, *J* 5.6), 8.01 – 7.93 (3 H, m), 7.72 (1 H, dt, *J* 8.3, 1.0), 7.54 (1 H, ddd, *J* 8.2, 6.8, 1.0), 7.44 – 7.26 (7 H, m), 6.71 (1 H, d, *J* 15.9), 4.14 (3 H, d, *J* 6.2), 3.24 – 3.12 (2 H, m), 2.89 (3 H, s), 2.73 (2 H, d, *J* 0.6), 1.45 (2 H, t, *J* 7.0), 1.39 (9 H, s), 1.32 – 1.20 (1 H, m), 1.03 (1 H, s). MS-ESI *m/z* 521 $[M+H]^+$, 543 $[M+Na]^+$.

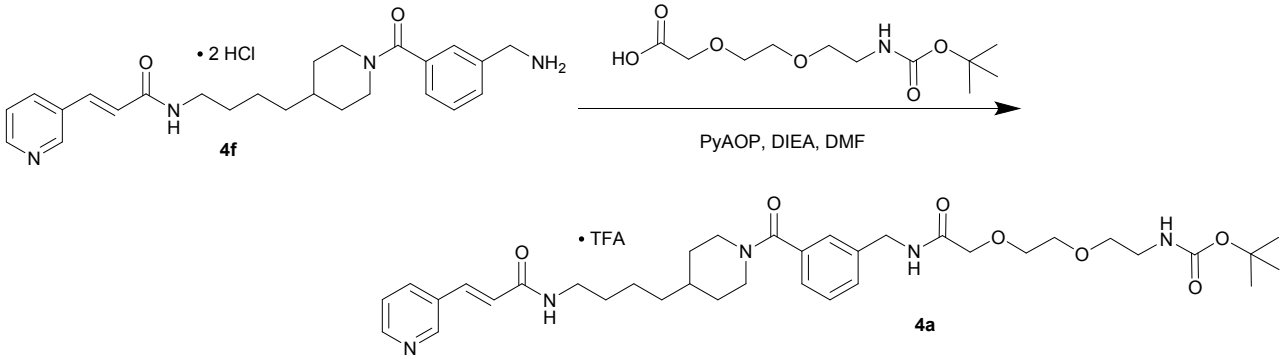
(4) Elf Biedermann, Max Hasmann, Roland Loser, Benno Rattel, Friedemann Reiter, Barbara Schein, Klaus Seibel, Klaus Vogt, PCT Int. Appl., **1997**, WO 9748696 A1 19971224.

(*E*)-N-(4-(1-(3-(aminomethyl)benzoyl)piperidin-4-yl)butyl)-3-(pyridin-3-yl)acrylamide bis-hydrochloride tetrahydrate (FK866-CH_2NH_2 , 4f):



Tert-butyl (E)-(3-(4-(4-(3-(pyridin-3-yl)acrylamido)butyl)piperidine-1-carbonyl)benzyl)carbamate **4f-Boc** (129 mg, 0.248 mmol) was treated with 12 M HCl (0.25 mL, 3 mmol) at 100 °C for 3 h. The solvent was removed by evaporation under a stream of dry nitrogen gas to give the product **4f** as a colorless oil (131 mg, 94.2 %). ^{1}H-NMR δ_H (300 MHz, DMSO-d_6) 8.85 (2 H, d, *J* 66.6), 8.52 – 8.23 (5 H, m), 7.85 – 7.76 (1 H, m), 7.58 – 7.43 (4 H, m), 7.36 (1 H, dt, *J* 7.6, 1.5), 6.86 (1 H, d, *J* 15.9), 4.06 (2 H, q, *J* 5.8), 3.25 – 3.12 (3 H, m), 1.81 – 0.97 (7 H, m). MS-ESI *m/z* 211 $[M+2H]^{2+}$, 421 $[M+H]^+$, 842 $[2M+H]^+$, 864 $[2M+Na]^+$. Elemental Analysis 2·HCl, 4·H_2O Calcd: C, 53.10; H, 7.49; N, 9.91. Found: C, 52.78; H, 7.24; N, 9.87.

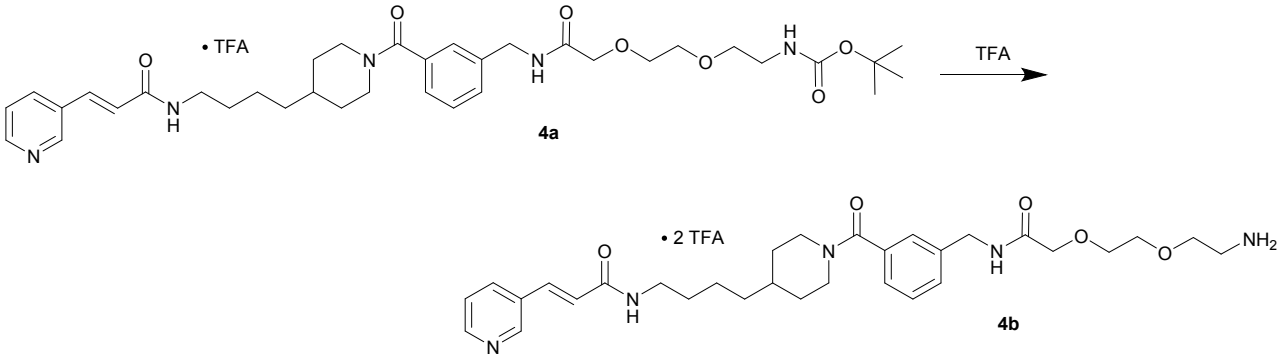
***Tert*-butyl (E)-(2-(2-(2-oxo-2-((3-(4-(4-(3-(pyridin-3-yl)acrylamido)butyl)piperidine-1-carbonyl)benzyl)amino)ethoxy)ethoxy)ethyl)carbamate 2,2,2,-trifluoroacetate (FK866-CH_2NHCO-xPEG2-NHBoc , 4a):**



(*E*)-N-(4-(1-(3-(aminomethyl)benzoyl)piperidin-4-yl)butyl)-3-(pyridin-3-yl)acrylamide bis-hydrochloride **4f** (101 mg, 0.205 mmol), 2,2-dimethyl-4-oxo-3,8,11-trioxa-5-azatridecan-13-oic acid (56.6 mg, 0.215 mmol) were combined in 3 mL anhydrous DMF. PyAOP (128 mg, 0.246 mmol) and DIEA (106 mg, 0.819 mmol) were added and the reaction shaken at ambient for 2 h. The crude reaction mixture was diluted to 9 mL with 90% DMSO/water and purified by

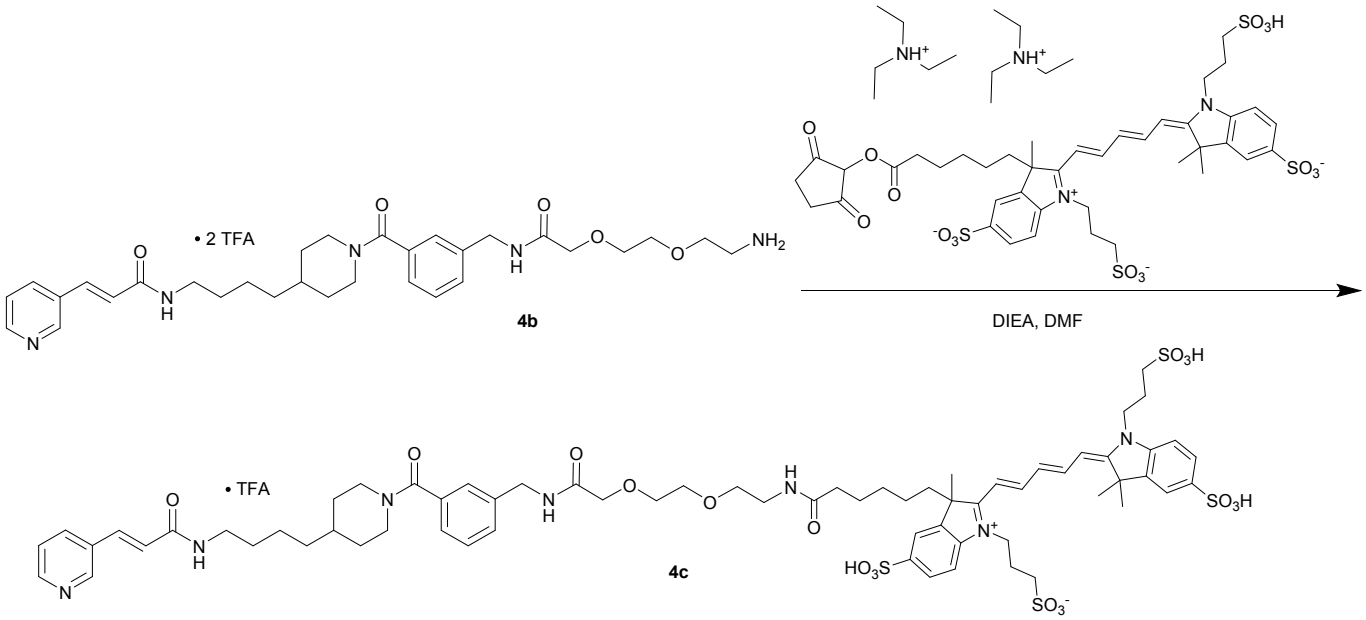
preparative HPLC 3 mL/injection using the TFA method. Fractions containing the desired product were combined and lyophilized to give **4a** as a colorless solid (68 mg, 42.6%) ^{1}H-NMR δ_H (501 MHz, DMSO-d_6) 8.84 (1 H, s), 8.63 (1 H, d, *J* 5.0), 8.39 (1 H, d, *J* 6.5), 8.29 (1 H, t, *J* 6.3), 8.23 – 8.13 (3 H, m), 7.60 (1 H, dd, *J* 8.0, 4.9), 7.47 (1 H, d, *J* 15.9), 7.40 – 7.29 (3 H, m), 7.28 – 7.19 (3 H, m), 6.82 – 6.74 (2 H, m), 4.45 (1 H, s), 4.34 (3 H, d, *J* 6.1), 4.00 – 3.91 (2 H, m), 3.41 – 3.32 (2 H, m), 3.25 – 3.10 (3 H, m), 3.11 – 2.99 (2 H, m), 1.53 – 1.42 (4 H, m), 1.36 (9 H, s), 1.34 – 1.28 (2 H, m), 1.28 – 1.22 (3 H, m), 1.08 (3 H, d, *J* 30.3). ESI-MS *m/z* 664.2 $[M-H]^-$, 686.3 $[M-2H+Na]^-$, 710.1 $[M+COOH^-]^-$; LC-MS TFA Method, R_t = 0.79 min; APCI(+) *m/z* 666.51 $[M+H]^+$.

(E)-N-(4-(1-(3-((2-(2-(2-Aminoethoxy)ethoxy)acetamido)methyl)benzoyl)piperidin-4-yl)butyl)-3-(pyridin-3-yl)acrylamide bis-2,2,2,-trifluoroacetate (FK866-CH_2NHCO-xPEG2-NH_2 , 4b):



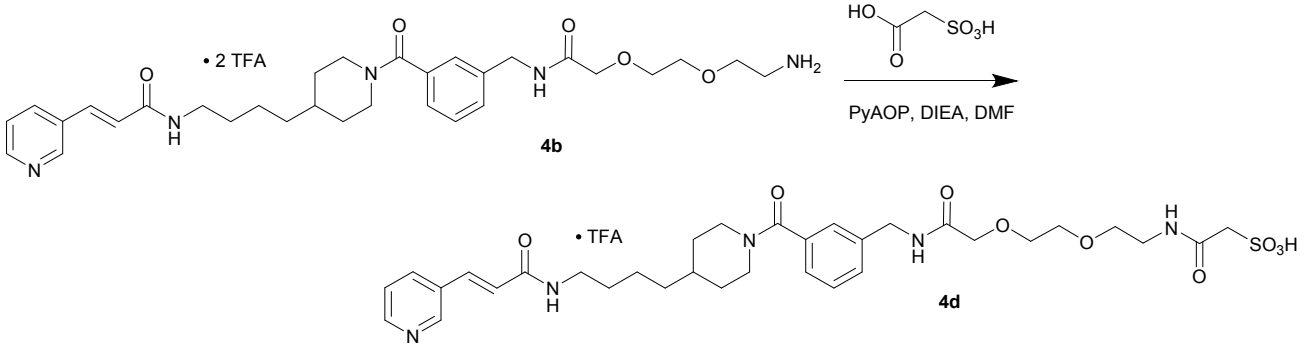
Tert-butyl (E)-(2-(2-(2-oxo-2-((3-(4-(4-(3-(pyridin-3-yl)acrylamido)butyl)piperidine-1-carbonyl)benzyl)amino)ethoxy)ethoxy)ethyl)carbamate 2,2,2,-trifluoroacetate **4a** (100 mg, 0.0.13 mmol) was dissolved in 1 mL TFA and immediately evaporated to dryness under a stream of dry nitrogen gas. The residue was dissolved in 1 mL 1:1 water:MeCN and lyophilized to give the product **4b** as a colorless solid (49.0 mg, 44.8%) ^{1}H-NMR δ_H (400 MHz, DMSO-d_6) 8.91 (1 H, s), 8.69 (1 H, d, *J* 5.1), 8.35 (1 H, t, *J* 6.2), 8.31 – 8.20 (2 H, m), 7.80 (3 H, s), 7.73 – 7.68 (1 H, m), 7.50 (1 H, d, *J* 15.9), 7.42 – 7.28 (2 H, m), 7.27 – 7.19 (2 H, m), 6.81 (1 H, d, *J* 15.9), 4.40 – 4.29 (2 H, m), 3.98 (2 H, s), 3.67 – 3.51 (7 H, m), 3.19 (2 H, q, *J* 6.6), 3.01 – 2.93 (2 H, m), 2.80 – 2.66 (1 H, m), 1.54 – 1.40 (3 H, m), 1.36 – 1.20 (4 H, m), 1.12 – 0.98 (3 H, m). ESI-MS *m/z* 566.0 $[M+H]^+$, 588.0 $[M+Na]^+$; LC-MC, TFA Method R_t = 0.69 min, APCI(+) *m/z* 566 $[M+H]^+$.

3-(2-((1E,3E)-5-((E)-3,3-dimethyl-5-sulfo-1-(3-sulfopropyl)indolin-2-ylidene)penta-1,3-dien-1-yl)-3-(3,12-dioxo-1-(3-(4-(4-((E)-3-(pyridin-3-yl)acrylamido)butyl)piperidine-1-carbonyl)phenyl)-5,8-dioxa-2,11-diazaheptadecan-17-yl)-3-methyl-5-sulfo-3H-indol-1-ium-1-yl)propane-1-sulfonate 2,2,2,-trifluoroacetate (FK866-CH_2NHCO-xPEG2-SCD , 4c):



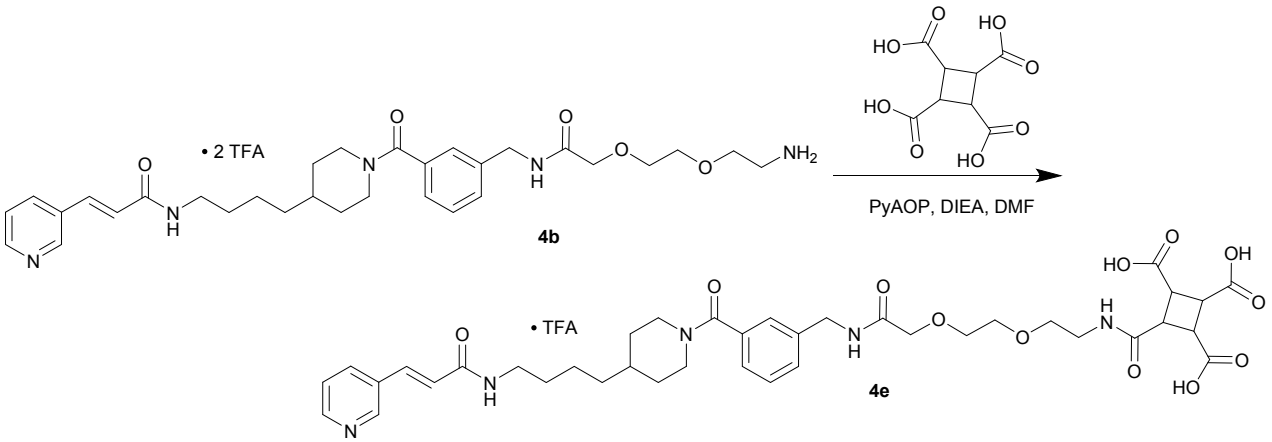
(E)-N-(4-(1-(3-((2-(2-(2-Aminoethoxy)ethoxy)acetamido)methyl)benzoyl)piperidin-4-yl)butyl)-3-(pyridin-3-yl)acrylamide bis-2,2,2,-trifluoroacetate **4b** (12.3 mg, 0.018 mmol) was combined with SCD-NHS (21 mg, 0.018 mmol) in 1 mL anhydrous DMF containing DIEA (7.0 mg, 0.054 mmol) and shaken at ambient for 16 h. The crude reaction mixture was diluted to 3 mL with 90% DMSO/water and purified by preparative HPLC using the TFA method. Fractions containing the desired product were combined and lyophilized to give **4c** as a dark blue solid (3.5 mg, 13.8%) ^{1}H-NMR δ_H (400 MHz, DMSO-d_6) 8.82 (1 H, s), 8.59 (1 H, s), 8.43 – 8.22 (3 H, m), 8.19 – 8.06 (1 H, m), 7.84 – 7.72 (1 H, m), 7.63 (2 H, d, *J* 7.9), 7.60 – 7.13 (11 H, m), 6.87 (1 H, d, *J* 15.7), 6.49 (3 H, d, *J* 35.3), 4.35 – 4.23 (6 H, m), 3.92 (2 H, s), 3.20 (4 H, d, *J* 21.2), 3.04 – 2.91 (13 H, m), 2.50 (10 H, s), 1.99 (3 H, s), 1.70 – 1.57 (12 H, m), 0.91 – 0.81 (13 H, m). ESI-MS *m/z* 467.0 $[M-3H]^{3-}$, 702.1 $[M-2H]^{2-}$, 1426.0 $[M-2H+Na]^-$; LC-MS AA Method R_t = 0.61 min, APCI(+) *m/z* 467.48 $[M-3H]^{3-}$.

(E)-3,12-Dioxo-1-(3-(4-(4-(3-(pyridin-3-yl)acrylamido)butyl)piperidine-1-carbonyl)phenyl)-5,8-dioxa-2,11-diazatridecane-13-sulfonic acid 2,2,2,-trifluoroacetate (FK866-CH_2NHCO-xPEG2-SAA, 4d):



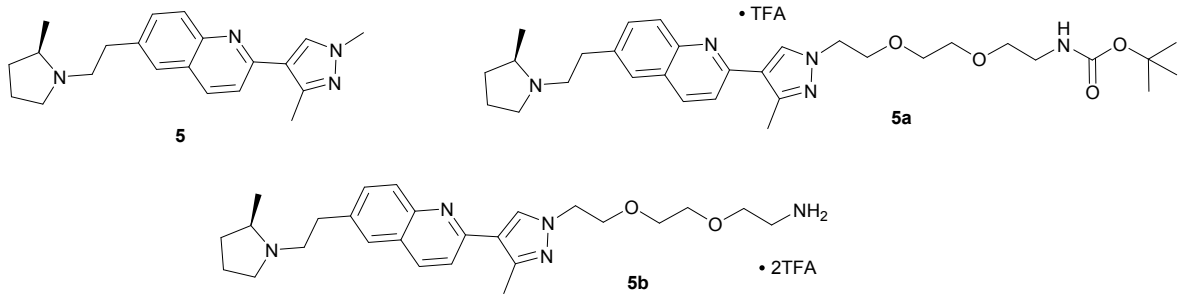
(E)-N-(4-(1-(3-((2-(2-(2-Aminoethoxy)ethoxy)acetamido)methyl)benzoyl)piperidin-4-yl)butyl)-3-(pyridin-3-yl)acrylamide bis-2,2,2,-trifluoroacetate **4b** (13.2 mg, 0.019 mmol) was combined with 2-sulfoacetic acid (13.6 mg, 0.097 mmol) in 1 mL anhydrous DMF. PyAOP (50.6 mg, 0.097 mmol) and DIEA (25.1 mg, 0.194 mmol) were added and the reaction shaken at ambient for 16 h. The crude reaction mixture was diluted to 3 mL with 90% DMSO/water and purified by preparative HPLC using the TFA method. Fractions containing the desired product were combined and lyophilized to give **4d** as a colorless solid (2.7 mg, 20.2%) ^{1}H-NMR δ_H (400 MHz, DMSO-*d6*) 8.89 (2 H, s), 8.66 (2 H, d, *J* 5.1), 8.34 (2 H, dt, *J* 12.3, 6.3), 8.29 – 8.18 (4 H, m), 7.66 (2 H, dd, *J* 8.1, 5.1), 7.49 (2 H, d, *J* 15.9), 7.41 – 7.31 (4 H, m), 7.27 – 7.16 (4 H, m), 6.82 (2 H, d, *J* 16.0), 4.34 (4 H, t, *J* 6.1), 3.92 (1 H, s), 3.29 – 3.15 (7 H, m), 1.46 (5 H, p, *J* 7.3), 1.32 (3 H, s), 1.26 (5 H, dd, *J* 10.8, 4.7). ESI-MS *m/z* 688.1 $[M+H]^+$, 710.0 $[M+Na]^+$; LC-MS TFA Method, R_t = 0.65 min, APCI(+) *m/z* 688.38 $[M+H]^+$.

(E)-4-((2-(2-(2-oxo-2-((3-(4-(4-(3-(pyridin-3-yl)acrylamido)butyl)piperidine-1-carbonyl)benzyl)amino)ethoxy)ethoxy)ethyl)carbamoyl)cyclobutane-1,2,3-tricarboxylic acid 2,2,2,-trifluoroacetate (FK866-CH$_2$NHCO-xPEG2-CCT, 4e):



(E)-N-(4-(1-(3-((2-(2-(2-Aminoethoxy)ethoxy)acetamido)methyl)benzoyl)piperidin-4-yl)butyl)-3-(pyridin-3-yl)acrylamide bis-2,2,2,-trifluoroacetate **4b** (12.5 mg, 0.018 mmol) was combined with cyclobutane-1,2,3,4-tetracarboxylic acid (21.4 mg, 0.92 mmol) in 1 mL anhydrous DMF. PyAOP (48 mg, 0.92 mmol) and DIEA (24 mg, 0.184 mmol) were added and the reaction shaken at ambient for 16 h. The crude reaction mixture was diluted to 3 mL with 90% DMSO/water and purified by preparative HPLC using the TFA method. Fractions containing the desired product were combined and lyophilized to give **4e** as a colorless solid (4.96 mg, 34.6%) ^{1}H-NMR δ_H (400 MHz, DMSO-d_6) 8.89 (2 H, s), 8.66 (2 H, d, *J* 5.1), 8.34 (2 H, dt, *J* 12.3, 6.3), 8.29 – 8.18 (4 H, m), 7.66 (2 H, dd, *J* 8.1, 5.1), 7.49 (2 H, d, *J* 15.9), 7.41 – 7.31 (4 H, m), 7.27 – 7.16 (4 H, m), 6.82 (2 H, d, *J* 16.0), 4.34 (4 H, t, *J* 6.1), 3.92 (1 H, s), 3.29 – 3.15 (7 H, m), 1.46 (5 H, p, *J* 7.3), 1.32 (3 H, s), 1.26 (5 H, dd, *J* 10.8, 4.7). ESI-MS *m/z* 762.0 $[M-H_2O]^+$, 780.0 $[M+H]^+$, 802.0 $[M+Na]^+$; LC-MS TFA Method, R_t = 0.67 min, APCI(+) *m/z* 780.55 $[M+H]^+$.

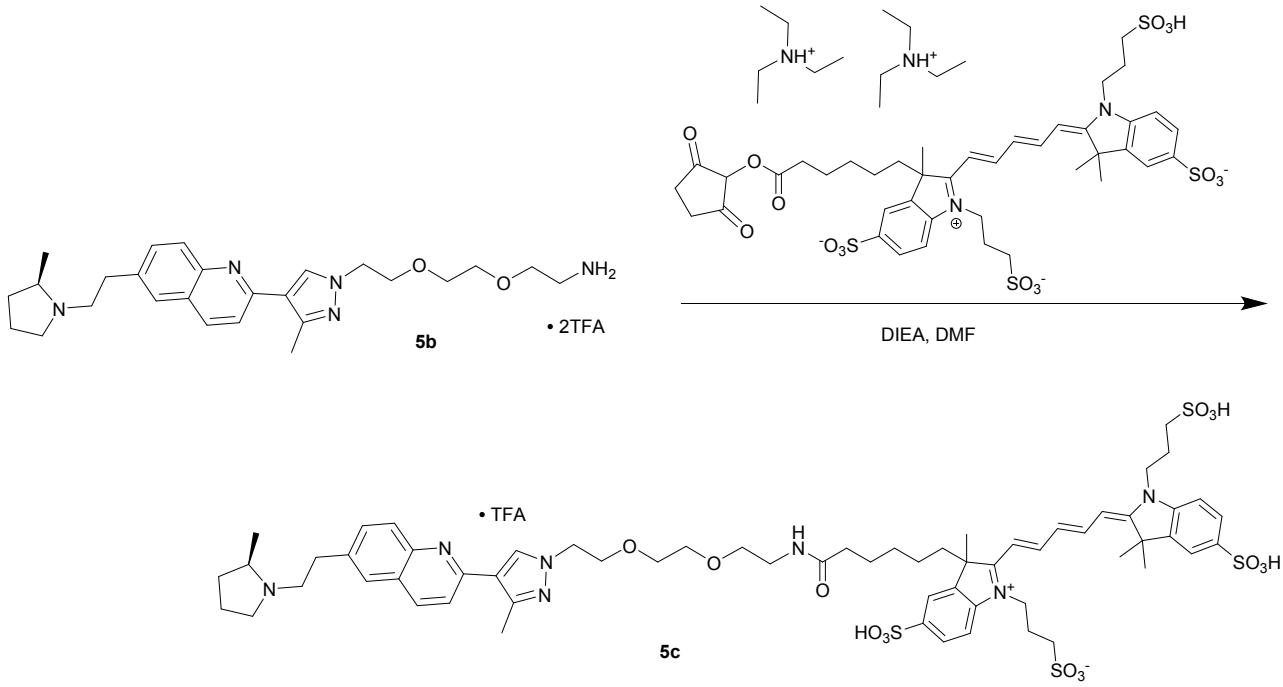
(R)-2-(1,3-dimethyl-1H-pyrazol-4-yl)-6-(2-(2-methylpyrrolidin-1-yl)ethyl)quinoline (5), tert-butyl (R)-(2-(2-(2-(3-methyl-4-(6-(2-(2-methylpyrrolidin-1-yl)ethyl)quinolin-2-yl)-1H-pyrazol-1-yl)ethoxy)ethoxy)ethyl)carbamate 2,2,2,-trifluoroacetate (5a), and (R)-2-(2-(2-(3-methyl-4-(6-(2-(2-methylpyrrolidin-1-yl)ethyl)quinolin-2-yl)-1H-pyrazol-1-yl)ethoxy)ethoxy)ethan-1-amine bis 2,2,2,-trifluoroacetate (5b): Were obtained as previously described.[5,6]



(5) Huaqing, Liu, Robert J. Altenbach, Gilbert J. Diaz, Arlene M. Manelli, Ruth L. Martin, Thomas R. Miller, Timothy A. Esbenshade, Jorge D. Brioni, Marlon D. Cowart, *Bioorg. Med. Chem. Lett.*, **2010**, *20*, 3295-3300.

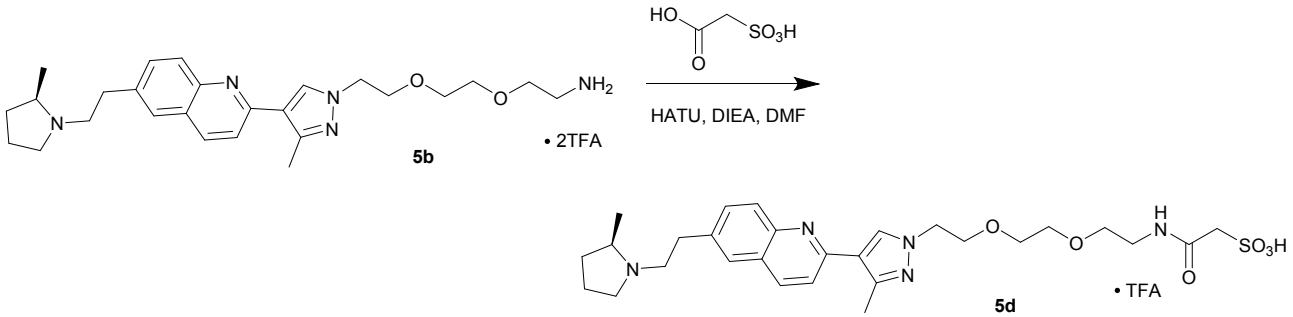
(6) Henning Stockmann, Viktor Todorovic, Paul L. Richardson, Violeta Marin, Victoria Scott, Clare Gerstein, Marc Lake, Leyu Wang, Ramkrisha Sadhukhan, Anil Vasudevan, *J. Am. Chem. Soc.,* **2017**, *139*, 16822-16829.

3-(2-((1E,3E)-5-((E)-3,3-Dimethyl-5-sulfo-1-(3-sulfopropyl)indolin-2-ylidene)penta-1,3-dien-1-yl)-3-methyl-3-(6-((2-(2-(2-(3-methyl-4-(6-(2-((R)-2-methylpyrrolidin-1-yl)ethyl)quinolin-2-yl)-1H-pyrazol-1-yl)ethoxy)ethoxy)ethyl)amino)-6-oxohexyl)-5-sulfo-3H-indol-1-ium-1-yl)propane-1-sulfonate 2,2,2-trifluoroacetate (H3-SCD, 5c):



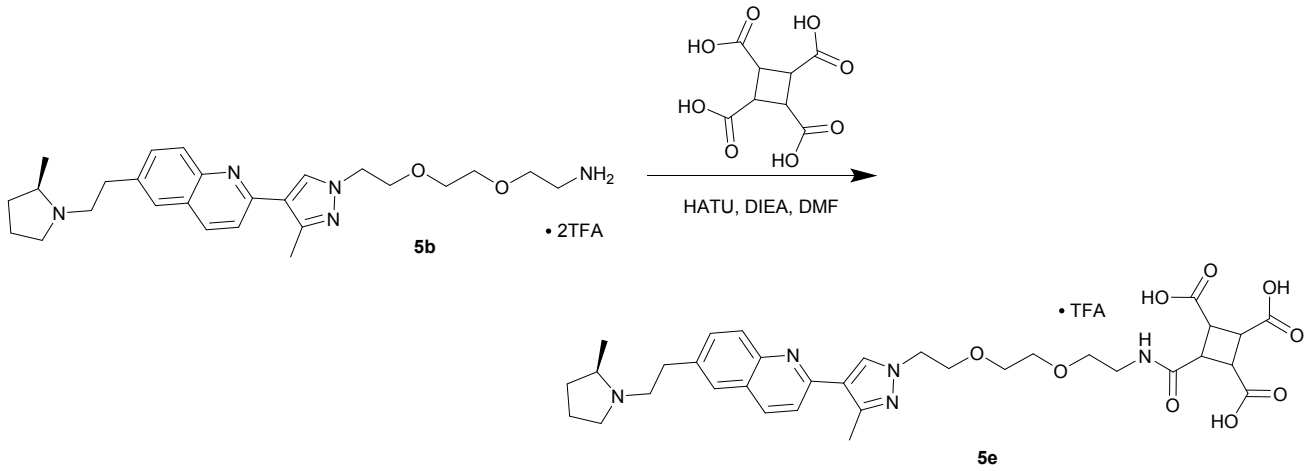
(R)-2-(2-(2-(3-methyl-4-(6-(2-(2-methylpyrrolidin-1-yl)ethyl)quinolin-2-yl)-1H-pyrazol-1-yl)ethoxy)ethoxy)ethan-1-amine bis-2,2,2,-trifluoroacetate **5b** (15.6 mg, 0.023 mmol) was combined with SCD-NHS (22 mg, 0.023 mmol) in 1 mL anhydrous DMF containing 2% DIEA (v/v) and shaken at ambient for 16 h. The crude reaction mixture was diluted to 3 mL with 90% DMSO/water and purified by preparative HPLC using the TFA method. Fractions containing the desired product were combined and lyophilized to give **5c** as a dark blue solid (16.3 mg, 50.3%) ^{1}H-NMR δ_H (500 MHz, DMSO-d_6) 9.42 (1 H, s), 8.68 (2 H, s), 8.32 (2 H, d, *J* 13.4), 8.12 – 7.92 (3 H, m), 7.80 – 7.73 (3 H, m), 7.70 – 7.56 (3 H, m), 7.48 – 7.35 (2 H, m), 6.53 – 6.36 (3 H, m), 4.32 – 4.17 (7 H, m), 3.86 – 3.76 (3 H, m), 3.45 – 3.38 (8 H, m), 3.23 (2 H, t, *J* 6.1), 2.96 (2 H, m), 2.72 – 2.59 (4 H, m), 2.58 (1 H, s), 2.53 (3 H, d, *J* 1.1), 2.28 – 2.19 (1 H, m), 2.15 – 1.88 (9 H, m), 1.72 – 1.55 (14 H, m), 1.44 – 1.37 (3 H, m), 1.27 – 1.19 (1 H, m), 1.17 – 1.01 (1 H, m), 0.95 (2 H, s), 0.69 (1 H, s), 0.34 (1 H, s). ESI-MS *m/z* 647 $[M+2H]^{2+}$, 1292 $[M+H]^+$, 1314 $[M+Na]^+$ LC-MS TFA Method, R_t = 0.57 min, ESI(+) *m/z* 1292.68 $[M+H]^+$.

(R)-2-((2-(2-(2-(3-methyl-4-(6-(2-(2-methylpyrrolidin-1-yl)ethyl)quinolin-2-yl)-1H-pyrazol-1-yl)ethoxy)ethoxy)ethyl)amino)-2-oxoethane-1-sulfonic acid 2,2,2-trifluoroacetate (H3-SAA, 5d):



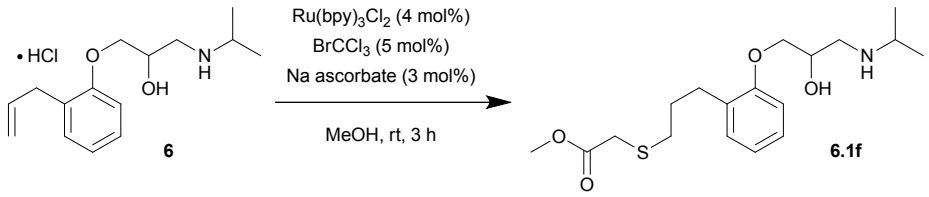
(R)-2-(2-(2-(3-methyl-4-(6-(2-(2-methylpyrrolidin-1-yl)ethyl)quinolin-2-yl)-1H-pyrazol-1-yl)ethoxy)ethoxy)ethan-1-amine bis-2,2,2,-trifluoroacetate **5b** (32 mg, 0.040 mmol) was combined with 2-sulfoacetic acid (17.0 mg, 0.121 mmol) in 1 mL anhydrous DMF. HATU (23 mg, 0.060 mmol) and DIEA (31.3 mg, 0.242 mmol) were added and the reaction shaken at ambient for 16 h. The crude reaction mixture was diluted to 3 mL with 90% DMSO/water and purified by preparative HPLC using the TFA method. Fractions containing the desired product were combined and lyophilized to give **5d** as a colorless solid (10.2 mg, 31.6%); ^{1}H-NMR δ_H (500 MHz, DMSO-d_6) 9.29 (1 H, s), 8.53 (1 H, s), 8.46 (1 H, d, *J* 8.6), 8.04 (1 H, d, *J* 8.7), 7.97 – 7.86 (3 H, m), 7.79 (1 H, d, *J* 8.8), 4.28 (2 H, t, *J* 5.1), 3.81 (4 H, t, *J* 5.2), 3.72 – 3.61 (4 H, m), 3.42 – 3.33 (5 H, m), 3.28 – 3.11 (5 H, m), 2.59 (3 H, s), 2.31 – 2.15 (1 H, m), 2.10 – 1.86 (2 H, m), 1.64 – 1.52 (1 H, m), 1.39 (3 H, d, *J* 6.5).). ESI-MS *m/z* 574.2 $[M+H]^+$, LC-MS TFA Method, R_t = 0.52 min, ESI(+) *m/z* 574.04 $[M+H]^+$.

4-((2-(2-(2-(3-methyl-4-(6-(2-((R)-2-methylpyrrolidin-1-yl)ethyl)quinolin-2-yl)-1H-pyrazol-1-yl)ethoxy)ethoxy)ethyl)carbamoyl)cyclobutane-1,2,3-tricarboxylic acid 2,2,2-trifluoroacetate (H3-CCT, 5e):



(R)-2-(2-(2-(3-methyl-4-(6-(2-(2-methylpyrrolidin-1-yl)ethyl)quinolin-2-yl)-1H-pyrazol-1-yl)ethoxy)ethoxy)ethan-1-amine bis-2,2,2,-trifluoroacetate **5b** (20 mg, 0.025 mmol) was combined with cyclobutane-1,2,3,4-tetracarboxylic acid (29.2 mg, 0.126 mmol) in 1 mL anhydrous DMF. HATU (14.4 mg, 0.038 mmol) and DIEA (32.6 mg, 0.252 mmol) were added and the reaction shaken at ambient for 16 h. The crude reaction mixture was diluted to 3 mL with 90% DMSO/water and purified by preparative HPLC using the TFA method. Fractions containing the desired product were combined and lyophilized to give **5e** as a colorless solid (10.9 mg, 48.4%) ^{1}H-NMR δ_H (400 MHz, DMSO-d_6) 12.51 (3 H, s), 9.38 (1 H, s), 8.39 (1 H, s), 8.28 (1 H, d, *J* 8.7), 8.09 (1 H, t, *J* 5.7), 7.94 (1 H, d, *J* 8.6), 7.83 (1 H, d, *J* 2.0), 7.78 (1 H, d, *J* 8.7), 7.71 (1 H, dd, *J* 8.6, 2.0), 4.25 (2 H, t, *J* 5.3), 3.82 (2 H, t, *J* 5.4), 3.57 – 3.07 (14 H, m), 2.60 (3 H, s), 2.28 – 2.17 (1 H, m), 2.08 – 1.86 (1 H, m), 1.68 – 1.53 (1 H, m), 1.39 (3 H, d, *J* 6.5), 1.33 – 1.14 (1 H, m). ESI-MS *m/z* 664.3 $[M-H]^-$, LC-MS TFA Method, R_t = 0.51 min, ESI(+) *m/z* 666.18 $[M+H]^+$.

Methyl 2-((3-(2-(2-hydroxy-3-(isopropylamino)propoxy)phenyl)propyl)thio)acetate (6.1f):

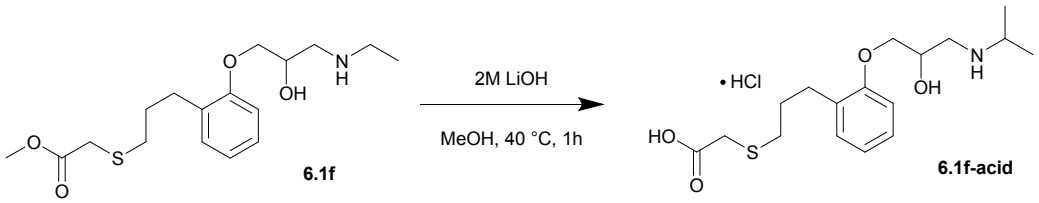


In a dry conical microwave vial equipped with a stir bar was added the solid components of the reaction: Alprenolol –HCl (1500 mg, 5.25 mmol), Tris(2,2'-bipyridyl)ruthenium (II) chloride hexahydrate (157 mg, 0.21 mmol) and sodium ascorbate (31.2 mg, 0.157 mmol). In a separate dry conical microwave vial was charged the liquid components: anhydrous methanol (15 mL), methyl thioglycolate (2.228 g, 21.0 mmol) and bromotrichloromethane (52 mg, 0.262 mmol). Each vial was sparged with nitrogen for 10 min. The mixed liquid components were transferred to the solid components via dry syringe and the reaction mixed at ambient until solids were dissolved. The reaction mixture was irradiated with a residence time of ~10 min with a 36W 450 nm blue LED lamp in the LOPHTOR flow reactor[7]. The crude eluent was partitioned between saturated aqueous sodium bicarbonate and chloroform (50 mL). The aqueous layer was extracted with 3 x 20 mL chloroform, the combined organic layers dried over MgSO4 and stripped to give **6.1f** (1.62 g, 87%) used in the next reaction without further purification. ^{1}H-NMR δ_H (400 MHz, Chloroform-*d*) 7.19 – 7.09 (2 H, m), 6.92 – 6.80 (2 H, m), 4.10 –

3.90 (3 H, m), 3.72 (3 H, s), 3.23 (2 H, s), 2.94 – 2.60 (6 H, m), 1.96 – 1.83 (2 H, m), 1.09 (6 H, d, *J* 6.2). MS (ESI+) 356 (M+H)+, LC-MS TFA Method, R_t = 0.69 min, APCI(+) *m/z* 356.10 $[M+H]^+$.

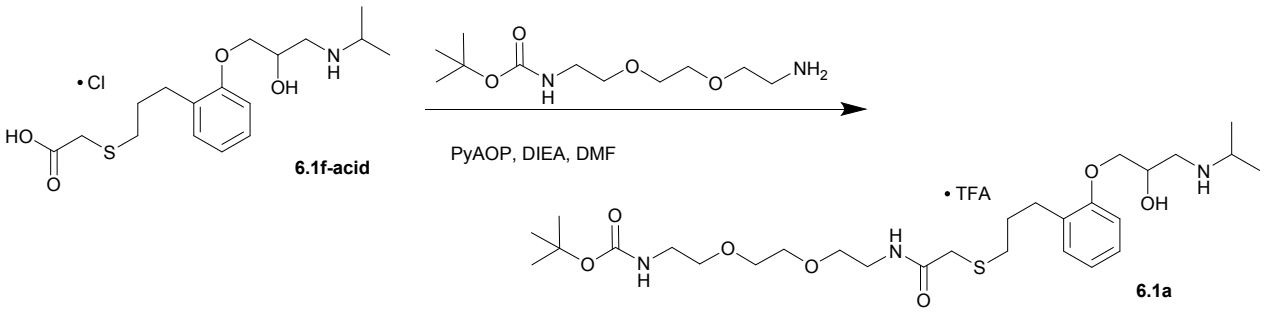
(7) Anil Vasudevan, Clara Villamil, Jonathan Trumbull, Jeff Olson, David Sutherland, Jeff Pan, Stevan Djuric , *Tet. Lett.,* **2010**, *51*, 4007-4009.

2-((3-(2-(2-Hydroxy-3-(isopropylamino)propoxy)phenyl)propyl)thio)acetic acid hydrochloride (6.1f-acid):



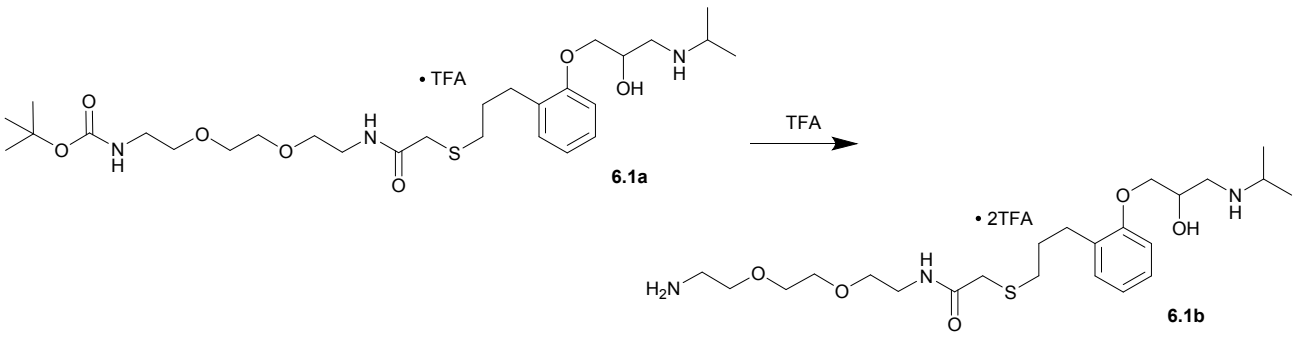
Methyl 2-((3-(2-(2-hydroxy-3-(isopropylamino)propoxy)phenyl)propyl)thio)acetate **6.1f** (1.4 g, 3.94 mmol) was dissolved in 6 mL methanol. Lithium hydroxide (2M, 7.88 mL, 15.75 mmol) was added and the reaction stirred at 40 °C for 1h. Hydrochloric acid (6M) was added dropwise until the pH ~6 and the product precipitated. The precipitate was recovered by filtration and dried to give the desired product 7.1f-acid (1.3 g, 97%) as a white solid ^{1}H-NMR δ_H (501 MHz, Chloroform-*d*) 7.21 – 7.07 (2 H, m), 6.94 – 6.87 (1 H, m), 6.79 (1 H, d, *J* 8.1), 4.11 (1 H, dd, *J* 9.2, 4.0), 3.91 (1 H, t, *J* 8.2), 3.51 – 3.26 (6 H, m), 3.15 (2 H, h, *J* 3.5), 2.68 (2 H, s), 2.64 – 2.54 (2 H, m), 1.85 – 1.79 (3 H, m), 1.48 – 1.42 (6 H, m); MS (ESI+) *m/z* 356.1 $[M+H]^+$; LC-MS TFA Method, R_t = 0.66 min, APCI(+) *m/z* 342.09 $[M+H]^+$.

Tert-butyl (15-(2-(2-hydroxy-3-(isopropylamino)propoxy)phenyl)-10-oxo-3,6-dioxa-12-thia-9-azapentadecyl)carbamate 2,2,2-trifluoroacetate (6.1a):



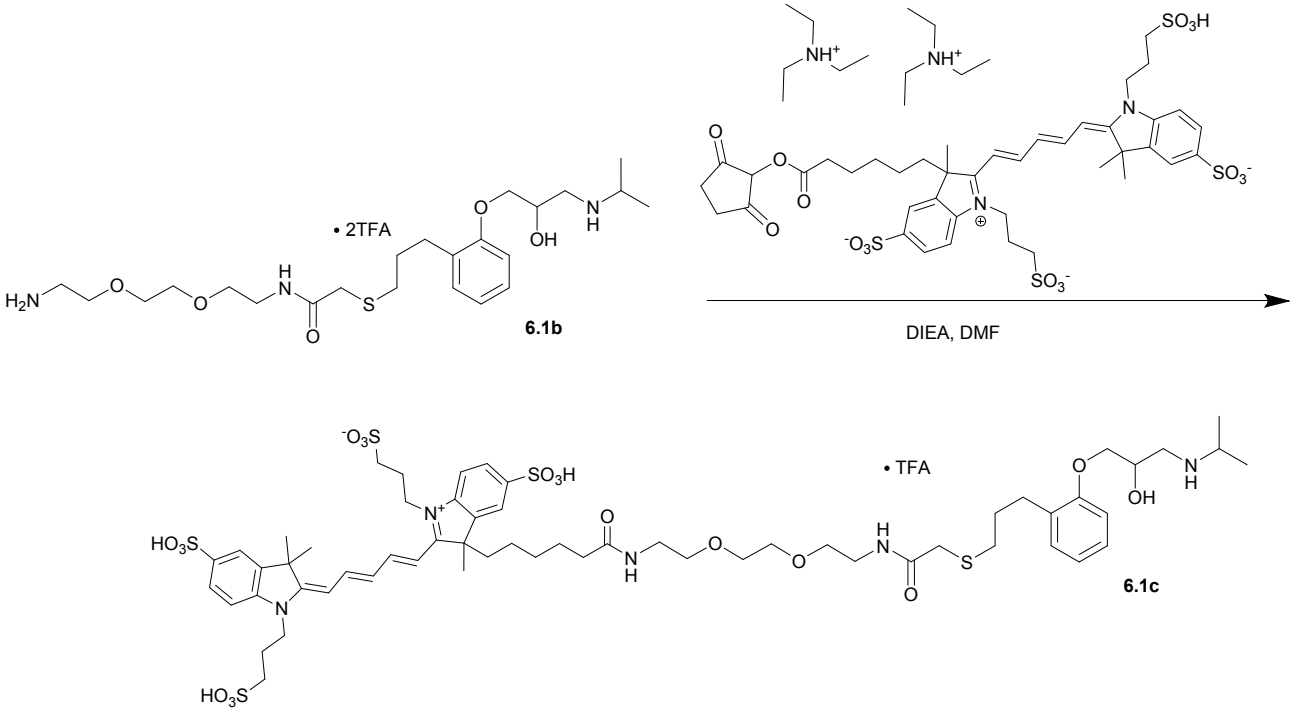
2-((3-(2-(2-Hydroxy-3-(isopropylamino)propoxy)phenyl)propyl)thio)acetic acid hydrochloride **6.1f-acid** (350 mg, 0.93 mmol) and tert-butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (345 mg, 1.39 mmol) were combined in 3 mL anhydrous NMP. PyAOP (676 mg, 1.297 mmol) and DIEA (600 mg, 4.63 mmol) were added and the reaction shaken at ambient for 3 h. The crude reaction mixture was diluted to 9 mL with 90% DMSO/water and purified 3 mL/injection by preparative HPLC using the TFA method. Fractions containing the desired product were combined and lyophilized to give **6.1a** as a white solid (372 mg, 58.6%) ^{1}H-NMR δ_H (400 MHz, DMSO-d_6) 8.45 (2 H, s), 8.04 (1 H, t, *J* 5.6), 7.21 – 7.10 (2 H, m), 6.97 – 6.84 (2 H, m), 6.75 (1 H, t, *J* 5.6), 3.48 (4 H, s), 3.38 (5 H, dt, *J* 12.5, 6.0), 3.21 (2 H, q, *J* 5.8), 2.69 – 2.61 (2 H, m), 2.57 (2 H, t, *J* 7.2), 1.84 – 1.68 (11 H, m), 1.37 (9 H, s), 1.25 (6 H, dd, *J* 6.5, 4.3). ESI-MS *m/z* 516.0 $[M\text{-tert-butyl}]^+$, 572 $[M+H]^+$, 594.1 $[M+Na]^+$; LC-MS TFA Method R_t = 0.79 min, APCI(+) *m/z* 572.39 $[M+H]^+$.

N-(2-(2-(2-Aminoethoxy)ethoxy)ethyl)-2-((3-(2-(2-hydroxy-3-(isopropylamino)propoxy)phenyl)propyl)-thio)acetamide bis-2,2,2-trifluoroacetate (6.1b):



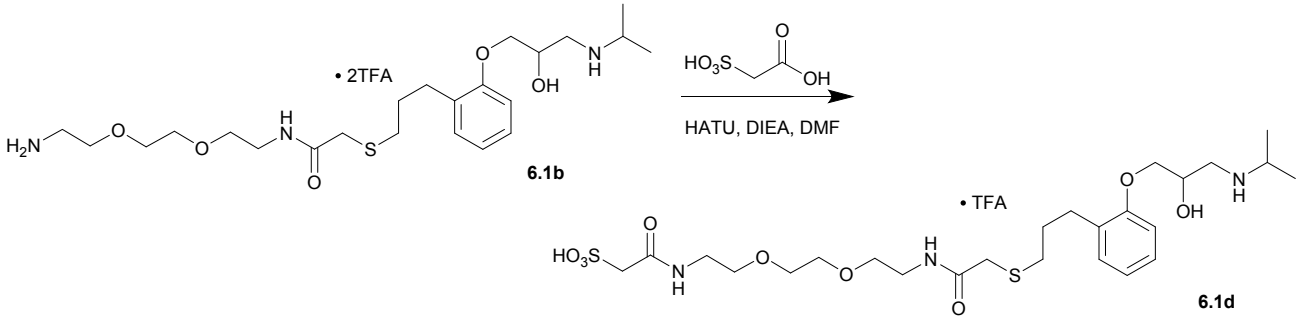
Tert-butyl (15-(2-(2-hydroxy-3-(isopropylamino)propoxy)phenyl)-10-oxo-3,6-dioxa-12-thia-9-azapentadecyl)carbamate 2,2,2-trifluoroacetate **6.1a** (400 mg, 0.70 mmol) was dissolved in 1 mL TFA and immediately evaporated to dryness under a stream of dry nitrogen gas. The residue was dissolved in 1 mL 1:1 water:MeCN and lyophilized to give the product **6.1b** as a colorless solid (497 mg, 102%). ^{1}H-NMR δ_H (501 MHz, DMSO-d_6) 8.56 (1 H, s), 8.46 (1 H, s), 8.07 (1 H, t, *J* 5.7), 7.87 (3 H, s), 7.21 – 7.10 (2 H, m), 6.93 (1 H, d, *J* 8.1), 6.88 (1 H, t, *J* 7.4), 3.62 – 3.50 (7 H, m), 3.40 (2 H, t, *J* 5.9), 3.23 – 3.16 (2 H, m), 2.95 (1 H, d, *J* 5.6), 2.64 (2 H, dd, *J* 8.6, 6.5), 2.56 (2 H, t, *J* 7.3), 1.82 – 1.71 (11 H, m), 1.28 – 1.22 (6 H, m). ESI-MS *m/z* 472.3 $[M+H]^+$; LC-MS TFA w/Integration Method R_t = 1.77 min, APCI(+) *m/z* 472.56 $[M+H]^+$.

3-(2-((1E,3E)-5-((E)-3,3-Dimethyl-5-sulfo-1-(3-sulfopropyl)indolin-2-ylidene)penta-1,3-dien-1-yl)-3-(1-(2-(2-hydroxy-3-(isopropylamino)propoxy)phenyl)-6,17-dioxo-10,13-dioxa-4-thia-7,16-diazadocosan-22-yl)-3-methyl-5-sulfo-3H-indol-1-ium-1-yl)propane-1-sulfonate 2,2,2-trifluoroacetate (6.1-SCD, 6.1c):



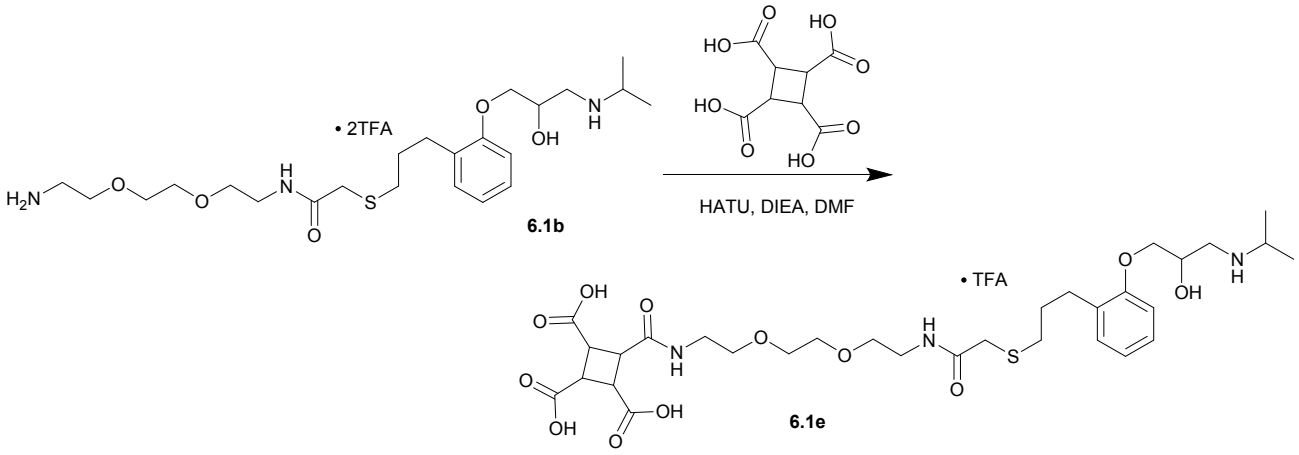
N-(2-(2-(2-Aminoethoxy)ethoxy)ethyl)-2-((3-(2-(2-hydroxy-3-(isopropylamino)propoxy)phenyl)propyl)-thio)acetamide bis-2,2,2-trifluoroacetate **6.1b** (12.1 mg, 0.17 mmol) was combined with SCD-NHS (20 mg, 0.017 mmol) in 1 mL anhydrous DMF containing 2% DIEA (v/v) and shaken at ambient for 16 h. The crude reaction mixture was diluted to 3 mL with 90% DMSO/water and purified by preparative HPLC using the TFA method. Fractions containing the desired product were combined and lyophilized to give **6.1c** as a dark blue solid (5.5 mg, 22.3%). ^{1}H-NMR δ_H (400 MHz, DMSO-d_6) 8.46 – 8.27 (3 H, m), 8.06 (1 H, t, *J* 5.6), 7.95 (1 H, s), 7.80 – 7.71 (2 H, m), 7.63 (2 H, d, *J* 8.1), 7.42 – 7.32 (2 H, m), 7.19 – 7.07 (2 H, m), 6.99 – 6.82 (2 H, m), 6.55 (1 H, t, *J* 12.3), 6.50 – 6.38 (2 H, m), 4.33 – 4.15 (4 H, m), 4.03 – 3.89 (2 H, m), 3.33 (3 H, dd, *J* 17.3, 5.9), 3.23 – 2.98 (7 H, m), 2.89 (3 H, s), 2.73 (3 H, s), 2.70 – 2.45 (24 H, m), 2.08 – 1.93 (3 H, m), 1.85 (1 H, t, *J* 7.5), 1.77 (1 H, q, *J* 7.4), 1.72 – 1.64 (7 H, m), 1.25 (6 H, dd, *J* 6.4, 4.4), 1.07 (1 H, q, *J* 7.4), 0.92 – 0.64 (2 H, m). ESI-MS *m/z* 657.1 $[M+2H]^{2+}$, 1312.2 $[M+H]^{+}$, 1334.0 $[M+Na]^{+}$; LC-MS TFA High Mass Method R_t = 0.60 min, ESI(+) *m/z* 1312.54

18-(2-(2-hydroxy-3-(isopropylamino)propoxy)phenyl)-2,13-dioxo-6,9-dioxa-15-thia-3,12-diazaoctadecane-1-sulfonic acid 2,2,2-trifluoroacetate (6.1-SAA, 6.1d):



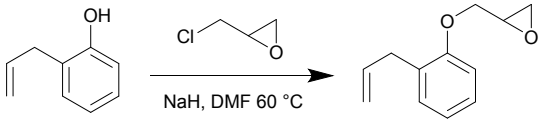
N-(2-(2-(2-Aminoethoxy)ethoxy)ethyl)-2-((3-(2-(2-hydroxy-3-(isopropylamino)propoxy)phenyl)propyl)-thio)acetamide bis-2,2,2-trifluoroacetate **6.1b** (29.5 mg, 0.042 mmol) was combined with 2-sulfoacetic acid (5 mg, 0.846 mmol) in 1 mL anhydrous DMF. HATU (12.8 mg, 0.0034 mmol) and DIEA (16.4 mg, 0.126 mmol) were added and the reaction shaken at ambient for 16 h. The crude reaction mixture was diluted to 3 mL with 90% DMSO/water and purified by preparative HPLC using the TFA method. Fractions containing the desired product were combined and lyophilized to give **6.1d** as a colorless solid (4.3 mg, 17.2%). ^{1}H-NMR δ_H (500 MHz, DMSO-d_6) 8.35 (2 H, s), 8.06 (1 H, t, *J* 5.6), 7.93 (1 H, q, *J* 5.4), 7.20 – 7.12 (2 H, m), 6.94 (1 H, d, *J* 8.2, 1.1), 6.89 (1 H, t, *J* 7.4, 1.1), 5.82 (1 H, d, *J* 5.1), 4.22 – 4.12 (1 H, m), 4.03 – 3.98 (1 H, m), 3.98 – 3.92 (1 H, m), 3.67 – 3.46 (4 H, m), 3.35 (7 H, d, *J* 12.4), 3.28 – 3.00 (8 H, m), 2.68 – 2.61 (2 H, m), 2.57 (2 H, t, *J* 7.2), 1.83 – 1.73 (2 H, m), 1.26 (6 H, t, *J* 6.2). ESI-MS *m/z* 594.2 $[M+H]^{+}$; LC-MS TFA Method R_t = 0.64 min, APCI(+) *m/z* 594.26 $[M+H]^{+}$.

4-((15-(2-(2-Hydroxy-3-(isopropylamino)propoxy)phenyl)-10-oxo-3,6-dioxa-12-thia-9-azapentadecyl)carbamoyl)-cyclobutane-1,2,3-tricarboxylic acid 2,2,2-trifluoroacetate (6.1-CCT, 6.1e):



N-(2-(2-(2-Aminoethoxy)ethoxy)ethyl)-2-((3-(2-(2-hydroxy-3-(isopropylamino)propoxy)phenyl)propyl)-thio)acetamide bis-2,2,2-trifluoroacetate **6.1b** (30 mg, 0.043 mmol) was combined with cyclobutane-1,2,3,4-tetracarboxylic acid (29.9 mg, 0.129 mmol) in 1 mL anhydrous DMF. HATU (16.3 mg, 0.043 mmol) and DIEA (33.2 mg, 0.257 mmol) were added and the reaction shaken at ambient for 16 h. The crude reaction mixture was diluted to 3 mL with 90% DMSO/water and purified by preparative HPLC using the TFA method. Fractions containing the desired product were combined and lyophilized to give **6.1e** as a colorless solid (6.2 mg, 21.1%). ^{1}H-NMR δ_H (500 MHz, DMSO-d_6) 8.13 (1 H, t, *J* 5.7), 8.06 (1 H, t, *J* 5.7), 7.20 – 7.11 (2 H, m), 6.94 (1 H, d, *J* 8.2), 6.89 (1 H, t, *J* 7.4), 4.18 – 4.12 (1 H, m), 4.02 – 3.92 (2 H, m), 3.66 – 3.46 (14 H, m), 3.30 – 3.10 (8 H, m), 3.09 – 2.98 (2 H, m), 2.89 (1 H, s), 2.73 (1 H, s), 2.69 – 2.60 (2 H, m), 2.56 (2 H, q, *J* 6.5, 5.8), 1.83 – 1.73 (2 H, m), 1.25 (6 H, t, *J* 6.0). ESI-MS *m/z* 686.2 $[M+H]^+$; LC-MS TFA Method R_t = 0.64 min, APCI(+) *m/z* 686.45 $[M+H]^+$.

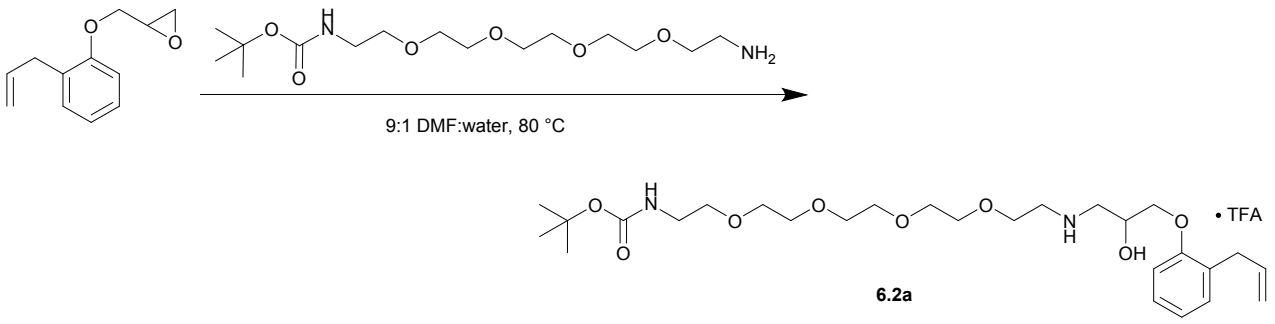
2-((2-Allylphenoxy)methyl)oxirane:



Sodium hydride (60% dispersion in mineral oil, 500 mg, 12.5 mmol) was suspended in 12.5 mL anhydrous DMF in a dry flask under nitrogen atmosphere at ambient and stirred for 10 min until gas evolution ceased. 2-Allylphenol (1.68 g, 1.641 mL, 12.5 mmol) was added dropwise with vigorous stirring followed by stirring for 30 min at ambient. Epichlorohydrin (2.317 g, 1.96 mL, 25.04 mmol) was added dropwise at ambient. After complete addition the reaction was stirred at 60 °C for 16h. The reaction was cooled to ambient and partitioned between water and diethyl ether. The water layer was extracted with 2 × ether, the combined organic layers dried over $MgSO_4$ and stripped to give the crude product as a pale yellow oil. The crude material was dissolved in 1:1 MeOH:DMSO and purified on a 30 x 100 mm Sunfire C8 column column eluted with a flowrate of 40 mL/min and a gradient of (0-2.5 min isocratic 60:40 A:B, 2.5-3.25 min to 55:45 A:B, 3.25-9.5 min 10:90 A:B; A: 10 mM ammonium acetate; B: acetonitrile) with uv-vis detection at 202 nm. Fractions containing the desired product were pooled

and lyophilized to give pure 2-((2-Allylphenoxy)methyl)oxirane (2.3 g, 97%) ^{1}H-NMR δ_H (501 MHz, DMSO-d_6) 7.18 (1 H, ddd, *J* 8.1, 7.4, 1.8), 7.12 (1 H, dd, *J* 7.5, 1.8), 6.96 (1 H, dd, *J* 8.2, 1.1), 6.90 (1 H, td, *J* 7.4, 1.1), 6.01 – 5.90 (1 H, m), 5.08 – 4.98 (2 H, m), 4.31 (1 H, dd, *J* 11.4, 2.6), 3.88 (1 H, dd, *J* 11.4, 6.1), 3.36 – 3.34 (1 H, m), 3.34 – 3.31 (2 H, m), 2.84 (1 H, dd, *J* 5.2, 4.2), 2.73 (1 H, dd, *J* 5.2, 2.6). DCI-MS *m/z* 190.2 $[M+H]^+$, 208.4 $[M+NH_4]^+$, 398.2 $[2M+NH_4)^{2+}$; LC-MS TFA Method R_t = 0.85 min, APCI(+) *m/z* not observed by this ionization method.

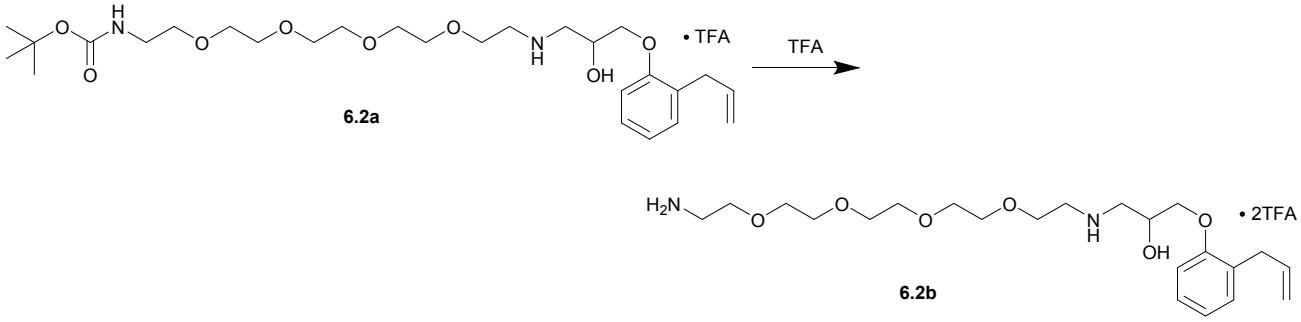
***Tert*-butyl (18-(2-allylphenoxy)-17-hydroxy-3,6,9,12-tetraoxa-15-azaoctadecyl)carbamate 2,2,2-trifluoracetate (6.2a):**



The procedure was adapted from published methods[8]. 2-((2-Allylphenoxy)methyl)oxirane(450 mg, 2.365 mmol) was combined with *tert*-butyl (14-amino-3,6,9,12-tetraoxatetradecyl)carbamate (1.00 g, 2.97 mmol) in 4 mL 9:1 DMF:water and heated at 80 °C for 16 h. The crude reaction mixture was diluted to 15 mL with 90% DMSO/water and purified 3 mL/injection by preparative HPLC using the TFA method. Fractions containing the desired product were combined and lyophilized to give **6.2a** as a light yellow oil (1.119 g, 73.8%) ^{1}H-NMR δ_H (400 MHz, DMSO-d_6) 8.70 (1 H, s), 8.61 (1 H, s), 7.19 (1 H, td, *J* 7.7, 1.8), 7.13 (1 H, dd, *J* 7.4, 1.7), 6.99 – 6.86 (2 H, m), 6.73 (1 H, d, *J* 6.0), 6.03 – 5.88 (1 H, m), 5.11 – 4.98 (2 H, m), 4.26 – 4.16 (1 H, m), 4.04 – 3.91 (2 H, m), 3.75 – 3.68 (2 H, m), 3.61 – 3.54 (4 H, m), 3.50 (8 H, d, *J* 10.7), 3.40 – 3.33 (4 H, m), 3.28 – 3.15 (3 H, m), 3.13 – 3.02 (3 H, m), 1.37 (9 H, s). ESI-MS *m/z* 527.2 $[M+H]^+$, 525 $[M-H]^-$; LC-MS TFA Method R_t = 0.82 min, APCI(+) *m/z* 527.4 $[M+H]^+$.

(8) Jillian G. Baker, Luke A. Adams, Karolina Salchow, Shailesh N. Mistry, Richard J. Middleton, Stephen J. Hill, Barrie Kellam, *J. Med. Chem.* **2011**, *54*, 6874-6887.

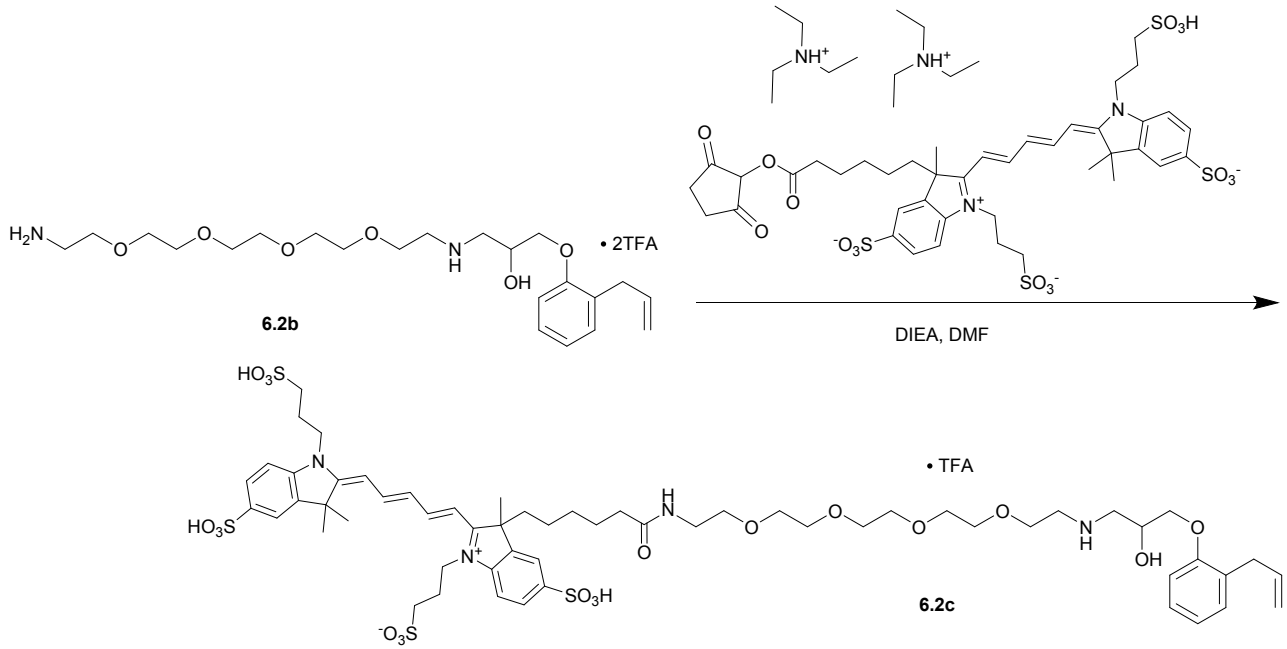
18-(2-allylphenoxy)-1-amino-3,6,9,12-tetraoxa-15-azaoctadecan-17-ol bis-2,2,2-trifluoracetate(6.2b):



Tert-butyl (18-(2-allylphenoxy)-17-hydroxy-3,6,9,12-tetraoxa-15-azaoctadecyl)carbamate 2,2,2-trifluoracetate **6.2a** (1.0 g, 1.899 mmol) was dissolved in 5 mL TFA and stirred at ambient for 5 min. The reaction was evaporated to dryness under a stream of dry nitrogen gas. The residue was dissolved in 5 mL 1:1 water:MeCN and lyophilized to

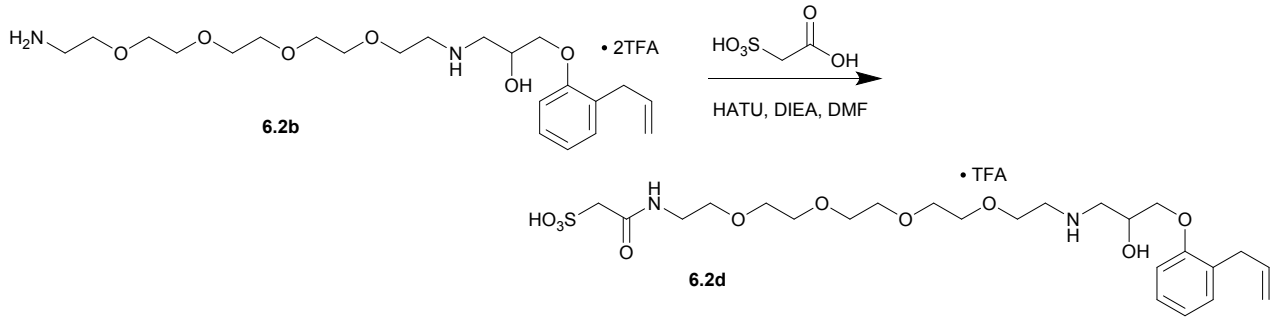
give the product **6.2b** as a colorless solid (1.20 g mg, 97%). ^{1}H-NMR δ_H (500 MHz, DMSO-d_6) 8.81 (1 H, s), 8.70 (1 H, s), 7.96 (3 H, s), 7.30 – 7.25 (1 H, m), 7.21 (1 H, dd, *J* 7.4, 1.7), 7.06 – 6.96 (1 H, m), 6.10 – 5.97 (1 H, m), 5.18 – 5.07 (2 H, m), 4.33 – 4.26 (1 H, m), 4.23 – 4.06 (6 H, m), 3.83 – 3.74 (2 H, m), 3.72 – 3.56 (12 H, m), 3.44 (1 H, d, *J* 6.7), 3.36 – 3.24 (3 H, m), 3.22 – 3.12 (1 H, m), 3.11 – 3.02 (2 H, m), 2.62 – 2.56 (1 H, m), 2.15 (0 H, d, *J* 0.5). ESI-MS *m/z* 427.3 $[M+H]^+$; LC-MS TFA w/Integration Method R_t = 1.89 min, APCI(+) *m/z* 427.38 $M+H]^+$.

3-(3-(1-(2-Allylphenoxy)-2-hydroxy-20-oxo-7,10,13,16-tetraoxa-4,19-diazapentacosan-25-yl)-2-((1E,3E)-5-((E)-3,3-dimethyl-5-sulfo-1-(3-sulfopropyl)indolin-2-ylidene)penta-1,3-dien-1-yl)-3-methyl-5-sulfo-3H-indol-1-ium-1-yl)propane-1-sulfonate 2,2,2-trifluoracetate (6.2-SCD, 6.2c):



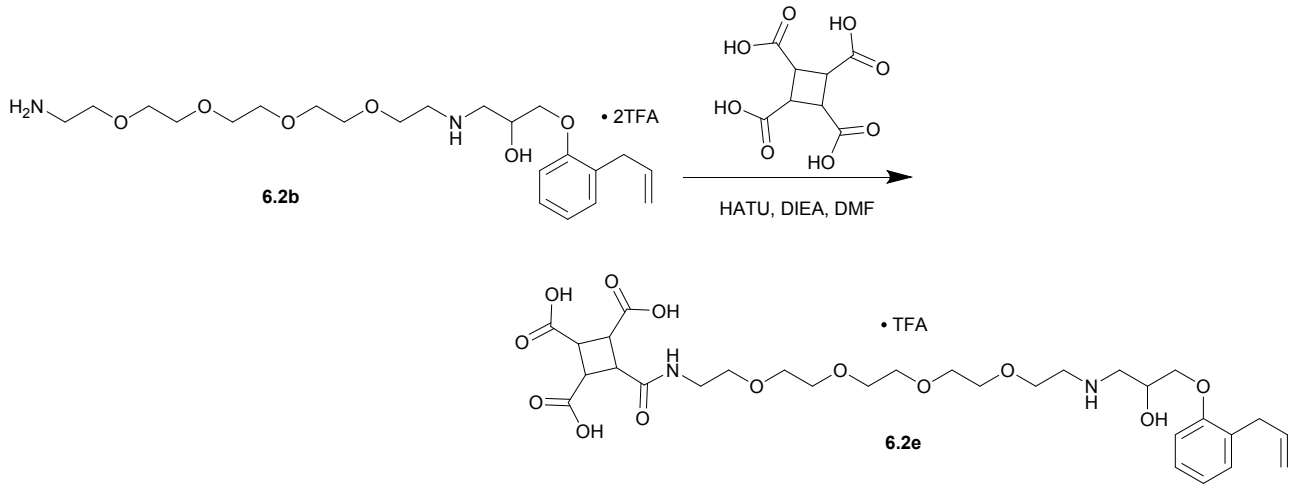
18-(2-Allylphenoxy)-1-amino-3,6,9,12-tetraoxa-15-azaoctadecan-17-ol bis-2,2,2-trifluoracetic acetate **6.2b** (7.4 mg, 0.017 mmol), SCD-NHS (20 mg, 0.017 mmol) and DIEA (6.7 mg, 0.052 mmol) were combined in 1 mL anhydrous DMF and shaken at ambient for 16 h. The crude reaction mixture was diluted to 3 mL with 90% DMSO/water and purified by preparative HPLC using the TFA method. Fractions containing the desired product were combined and lyophilized to give **6.2c** as a dark blue solid (9.6 mg, 43.9%). ^{1}H-NMR δ_H (400 MHz, DMSO-d_6) 8.53 (2 H, s), 8.43 – 8.27 (2 H, m), 7.82 – 7.69 (3 H, m), 7.66 – 7.57 (2 H, m), 7.38 (2 H, dd, *J* 8.3, 5.3), 7.22 – 7.05 (2 H, m), 6.98 – 6.85 (2 H, m), 6.51 (3 H, dt, *J* 38.6, 12.2), 6.01 – 5.88 (1 H, m), 5.09 – 4.97 (2 H, m), 4.33 – 4.15 (5 H, m), 4.02 – 3.87 (2 H, m), 3.77 – 3.43 (24 H, m), 3.25 – 2.98 (3 H, m), 2.70 – 2.53 (4 H, m), 2.22 – 2.05 (1 H, m), 2.00 (4 H, d, *J* 8.2), 1.86 (2 H, t, *J* 7.5), 1.73 – 1.59 (9 H, m), 1.38 – 1.18 (3 H, m), 1.18 – 1.01 (2 H, m), 0.76 (1 H, s), 0.43 (1 H, s). ESI-MS *m/z* 632.1 $[M-2H]^{2-}$; LC-MS TFA Method APCI(+) R_t = 0.63 min, APCI(+) *m/z* not observed by this method.

21-(2-allylphenoxy)-20-hydroxy-2-oxo-6,9,12,15-tetraoxa-3,18-diazahenicosane-1-sulfonic acid 2,2,2-trifluoracetate (6.2-SAA, 6.2d):



18-(2-Allylphenoxy)-1-amino-3,6,9,12-tetraoxa-15-azaoctadecan-17-ol bis-2,2,2-trifluoracetic acetate **6.2b** (30 mg, 0.070 mmol) was combined with 2-sulfoacetic acid (8.87 mg, 0.063 mmol) in 1 mL anhydrous DMF. HATU (21.4 mg, 0.056 mmol) and DIEA (27.3 mg, 0.211 mmol) were added and the reaction shaken at ambient for 16 h. The crude reaction mixture was diluted to 3 mL with 90% DMSO/water and purified by preparative HPLC using the TFA method. Fractions containing the desired product were combined and lyophilized to give **6.2d** as a colorless solid (14.1 mg, 36.5%). ^{1}H-NMR δ_H (501 MHz, DMSO-d_6) 8.47 (2 H, s), 7.90 (1 H, t, *J* 5.5), 7.20 – 7.13 (1 H, m), 7.10 (1 H, dd, *J* 7.4, 1.7), 6.96 – 6.83 (2 H, m), 6.00 – 5.87 (1 H, m), 5.75 (1 H, d, *J* 5.2), 5.08 – 4.96 (2 H, m), 4.24 – 4.14 (1 H, m), 4.00 – 3.86 (2 H, m), 3.75 – 3.63 (2 H, m), 3.59 – 3.53 (4 H, m), 3.52 – 3.47 (9 H, m), 3.32 (5 H, d, *J* 13.9), 3.25 – 3.14 (5 H, m), 3.04 (1 H, dd, *J* 12.8, 9.7). ESI-MS *m/z* 549.1 $[M+H]^+$; LC-MS TFA Method R_t = 0.70 min, APCI(+) *m/z* 549.30 $[M+H]^+$.

4-((18-(2-allylphenoxy)-17-hydroxy-3,6,9,12-tetraoxa-15-azaoctadecyl)carbamoyl)cyclobutane-1,2,3-tricarboxylic acid 2,2,2-trifluoracetate (6.2-CCT, 6.2e):



18-(2-Allylphenoxy)-1-amino-3,6,9,12-tetraoxa-15-azaoctadecan-17-ol bis-2,2,2-trifluoracetic acetate **6.2b** (30 mg, 0.070 mmol) was combined with cyclobutane-1,2,3,4-tetracarboxylic acid (49.0 mg, 0.211 mmol) in 1 mL anhydrous DMF. HATU (26.7 mg, 0.070 mmol) and DIEA (54.5 mg, 0.422 mmol) were added and the reaction shaken at ambient for 16 h. The crude reaction mixture was diluted to 3 mL with 90% DMSO/water and purified by preparative HPLC using the TFA method. Fractions containing the desired product were combined and lyophilized to give **6.2e** as a colorless solid (12.7 mg, 28.2%). ^{1}H-NMR δ_H (500 MHz, DMSO-d_6) 8.12 (1 H, t, *J* 5.7), 7.23 – 7.15 (1 H, m), 7.13 (1 H, dd, *J* 7.4, 1.7), 6.99 – 6.85 (2 H, m), 5.96 (1 H, ddt, *J* 16.8, 10.0, 6.7), 5.10 – 4.98 (2 H,

m), 4.24 – 4.15 (1 H, m), 4.01 – 3.89 (2 H, m), 3.75 – 3.64 (2 H, m), 3.61 – 3.37 (24 H, m), 3.29 – 3.10 (6 H, m), 3.05 (1 H, dd, *J* 12.7, 9.7). ESI-MS *m/z* 641.2 $[M+H]^+$; LC-MS TFA Method R_t = 0.69 min, APCI(+) *m/z* 641.43 $[M+H]^+$.