Supporting Information for 2016 *ACS Med. Chem. Lett.* "Discovery of 8-Membered Ring Sulfonamides as Inhibitors of Oncogenic Mutant Isocitrate Dehydrogenase 1"

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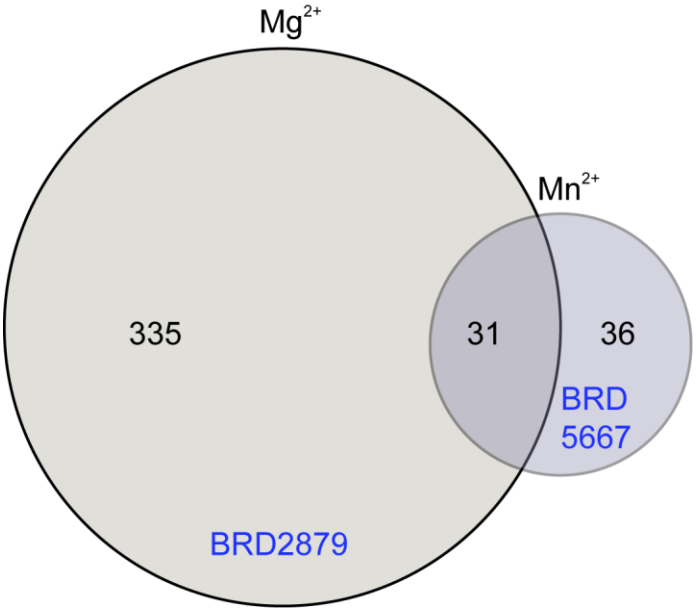


Figure S1: Number of active compounds in screens using Mg^{2+} or Mn^{2+} cofactors, out of 49,237 compounds tested in both screens. Only 31 compounds were active in both screens. The most potent hits from each screen, BRD2879 and BRD5667, were not detected in the other screen. Active compounds are those causing >60% inhibition of IDH1-R132H in both replicates of the Mg^{2+}-based assay, or causing >40% inhibition in both replicates of the Mn^{2+}-based assay.

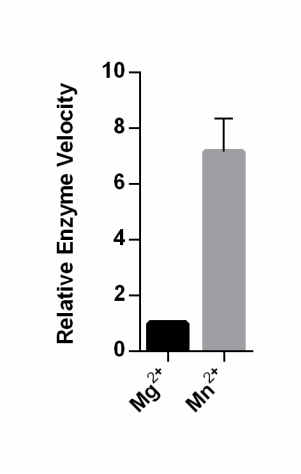
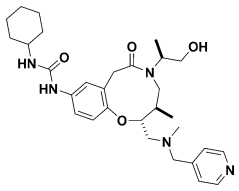
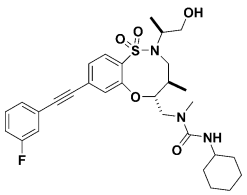


Figure S2: Relative velocity of IDH1-R132H with Mg^{2+} and Mn^{2+} cofactor. Mean ± SD of 3 technical replicates of one representative of three independent experiments.

Table S1: Comparison of Leads from Mn^{2+} and Mg^{2+} screens

Registration	**BRD5667**	**BRD2879**
Structure		
IDH1 R132H IC_{50} (μM), Mn^{2+}[a]	0.6	1.6[b]
IDH1 R132H IC_{50} (μM), Mg^{2+}	5.5[c]	0.05[d]
HA1E-M Cells EC_{50} (μM)	80[e]	0.3[f]
IDH1 wt IC_{50} (μM)[a]	>20	>20
Competition with α-KG	Noncompetitive	Competitive

[a]Geometric mean of 2 independent experiments

[b]Although BRD2879 showed detectable activity against IDH1-R132H-Mn^{2+} during retesting, the compound was not discovered in the primary screen.

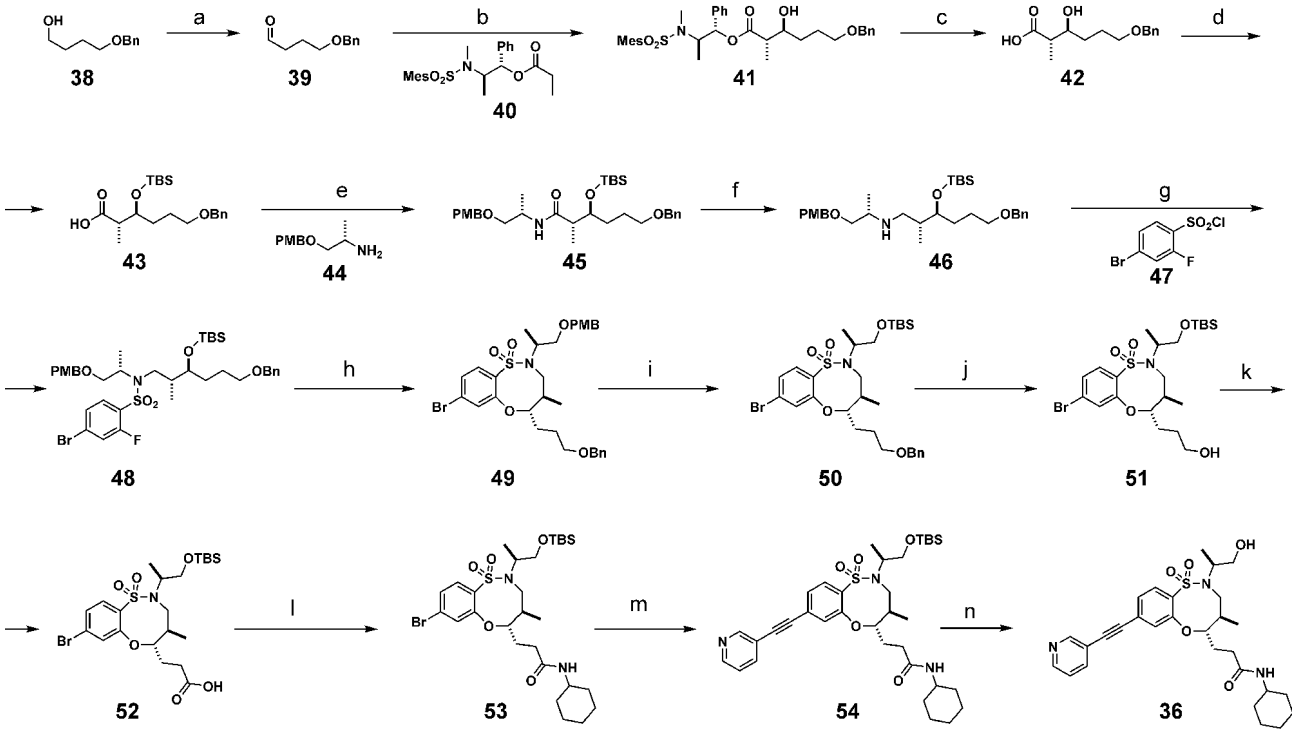
[c]Geometric mean of 8 independent experiments

[d]Geometric mean of 13 independent experiments

[e]Mean of 3 independent experiments, each in duplicate

[f]Mean of 3 independent experiments, each in triplicate

Scheme S1: Synthesis of "reverse amide" analog 36[a]



[a]Reagents and conditions: (a) $(COCl)_2$, DMSO, TEA, DCM, -78 °C, 5 h, 72 %, (b) $(c\text{-Hex})_2BOTf$, TEA, -78 °C, 6 h, 56 %, (c) H_2O_2, NaOH, MeOH/*t*-BuOH, 0 °C, 1 h, 83 %, (d) TBSOTf, 2,6-lutidine, DCM, 0 °C, 1 h, 98 %, (e) PyBOP, DIPEA, DCM, 0 °C, 2 h, (f) BH_3·DMS, THF, 50 °C, 7 h, (g) TEA, DCM, RT, 1 h, 29 % (3 steps), (h) 1) CsF, DMF, 85 °C, 20 h, 2) NaH, THF, 25 °C, 2 h, 75 %), (i) 1) DDQ, DCM, pH 7 buffer, RT, 2 h, 2) TBSOTf, 2,6-lutidine, DCM, -78 °C, 1 h, (j) DDQ, 1,2-dibromoethane, pH 7 buffer, 80 °C, 13 h, 68 % (2 steps), (k) PDC, DMF, RT, 20 h, 88 %, (l) cyclohexylamine, PyBOP, DIPEA, DCM, 0 °C, 2 h, (m) 3-ethynylpyridine, DIPEA, $Pd(PPh_3)_2Cl_2$, CuI, DMF, 50 °C, 12 h, 40 % (2 steps), (n) TBAF, THF, RT, 2 h, 68 %.

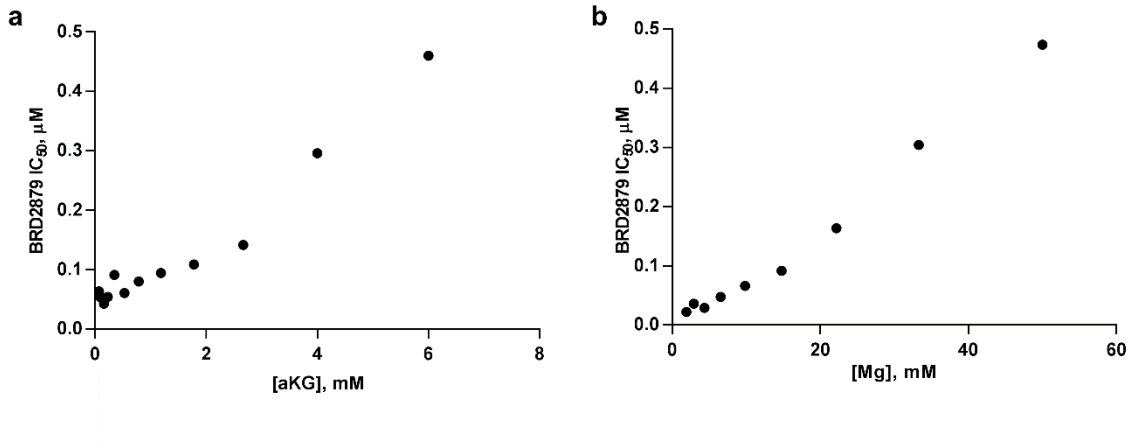


Figure S3: Mechanism of action determination for BRD2879 with respect to (a) the α-KG substrate and (b) the Mg^{2+} cofactor, using a method appropriate for tight-binding inhibitors as described by Copeland.[1] Rising IC_{50} values with increasing substrate or cofactor concentration indicate competitive inhibition. One representative experiment shown of three independent experiments.

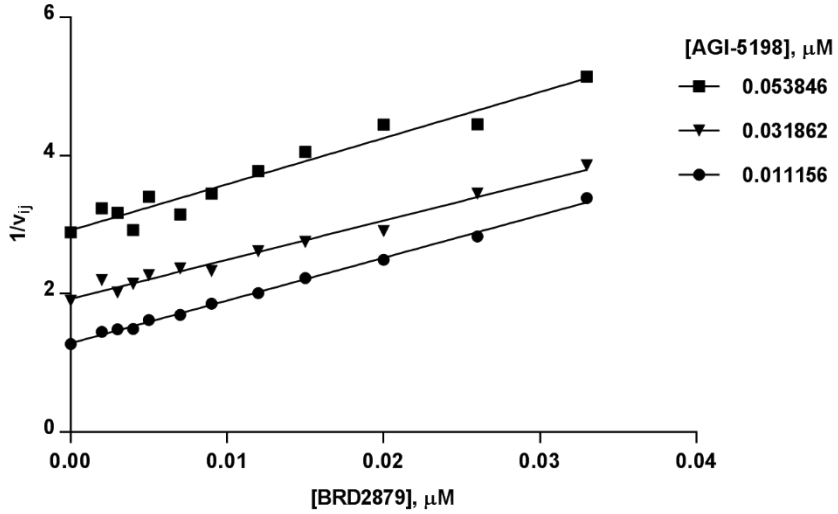


Figure S4: Evidence that binding of IDH1-R132H by BRD2879 and AGI-5198 is mutually exclusive. V_{ij} is normalized enzyme velocity in the presence of both inhibitors. The equal slopes of the [BRD2879] vs $1/v_{ij}$ trend lines at various concentrations of AGI-5198 indicate mutually exclusive binding.[1] One experiment is shown of two independent experiments.

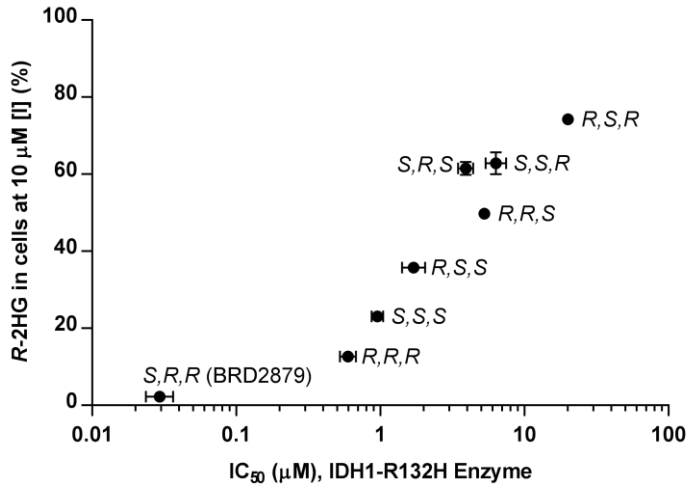


Figure S5: The potency of the stereoisomers of BRD2879 in enzymatic and cell-based assays is correlated. X-axis: IC_{50} value in the purified enzyme assay; error bars are s.e.m. of three independent experiments. Y-axis: percentage of *R*-2HG present in cells after 72 hour treatment with 10 μM compound, normalized to DMSO-treated samples; error bars are s.e.m. of three technical replicates of one representative of two experiments.

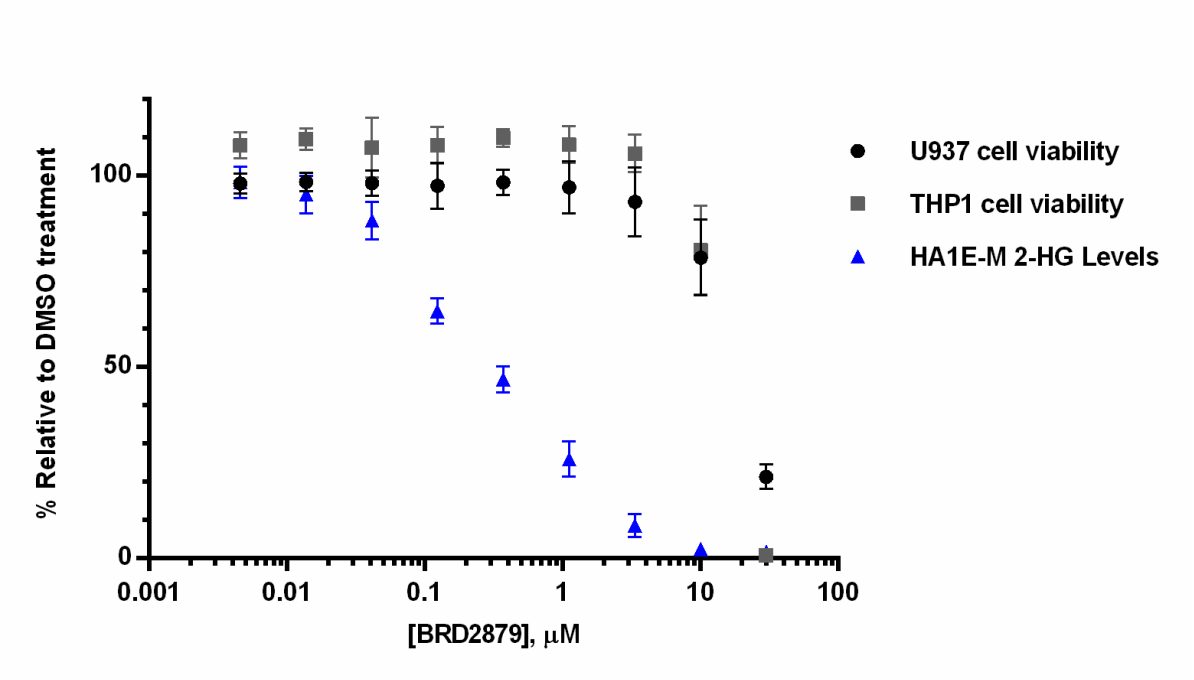


Figure S6: Effects of BRD2879 on viability of AML cell lines wild-type for IDH1 (U937, THP1), as a measure of off-target toxicity. Viability is determined by measuring ATP levels after 72 hours of treatment. The ability of BRD2879 to suppress *R*-2HG production in HA1E-M cells is shown for comparison. Values represent percentages normalized to DMSO-treated control samples, mean ± SD from three independent experiments, each run in triplicate.

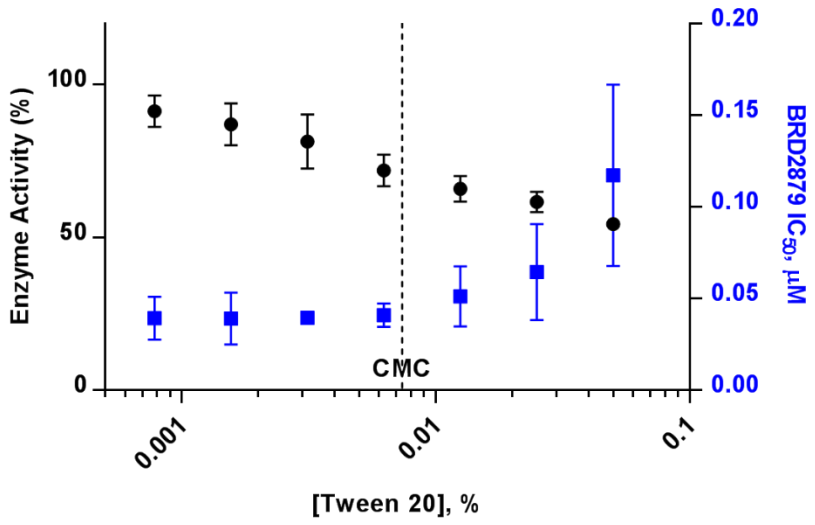


Figure S7: Effects of the detergent Tween 20 on IDH1-R132H enzyme activity and on enzyme inhibition by BRD2879. The lack of an effect of Tween 20 on the IC_{50} of BRD2879 at concentrations below its critical micelle concentration (CMC) is evidence against compound aggregation as a mechanism of enzyme inhibition.[2] Values are mean ± SD of three independent experiments.

Table S2: Statistical details for enzymatic assay IC_{50} values[a]

CMP	N	MEAN	SD	SEM	CV	GEO. MEAN	LOWER 95% CI	UPPER 95% CI
3	10	40.4	16.85	5.327	41.70%	**36.7**	25.87	52.05
4	3	32.67	8.145	4.702	24.93%	**32.04**	17.77	57.75
5	5	88.2	39.45	17.64	44.73%	**80.43**	43.44	148.9
6	3	1060	295.1	170.4	27.85%	**1035**	539	1986
7	4	615	299.4	149.7	48.69%	**552.6**	227	1346
8	3	1439	574.2	331.5	39.91%	**1371**	544.5	3451
9	3	166.7	59.23	34.2	35.54%	**160.4**	70.39	365.4
10	3	3208	737.4	425.8	22.99%	**3145**	1690	5852
11	3	11188	3128	1806	27.96%	**10868**	5119	23070
12	3	19000	1732	1000	9.12%	**18945**	15006	23918
13	5	368.2	79.61	35.6	21.62%	**361.5**	277.6	470.9
14	5	446.8	82.1	36.71	18.37%	**440.4**	346.1	560.3
15	3	157.3	19.22	11.1	12.21%	**156.6**	115.9	211.4
16	3	6099	3040	1755	49.85%	**5657**	1798	17800
17	3	8093	2521	1455	31.14%	**7856**	3802	16234
18	3	7934	3661	2114	46.14%	**7403**	2409	22752
19	3	1809	1009	582.7	55.80%	**1623**	388.9	6776
20	5	97	37.99	16.99	39.17%	**91.1**	55.48	149.6
21	5	59.8	23.06	10.31	38.56%	**56.14**	34	92.68
22	5	61.6	14.88	6.653	24.15%	**60.13**	44.18	81.83
23	5	29.6	9.476	4.238	32.01%	**28.28**	18.42	43.43
24	4	268.3	45.78	22.89	17.07%	**265.4**	203.6	346
25	13	146.5	54.75	15.19	37.38%	**136.6**	107.4	173.8
26	3	341	135.6	78.31	39.78%	**322.4**	114.5	908
27	7	98.57	38.2	14.44	38.76%	**90.53**	58.27	140.7
28	7	78.71	23.46	8.866	29.80%	**75.38**	55.61	102.2
29	5	206.4	71.21	31.84	34.50%	**194.2**	115.7	326
30	3	1594	271.5	156.7	17.03%	**1579**	1047	2381
31	3	91.67	24.79	14.31	27.04%	**89.6**	47.32	169.7
32	3	6481	1179	680.7	18.19%	**6413**	4161	9885
33	3	146	73.18	42.25	50.13%	**135.2**	42.46	430.7
34	3	7873	2041	1179	25.93%	**7676**	3785	15565
36	3	10767	8031	4637	74.60%	**9066**	1609	51093
37	3	102	44.54	25.72	43.67%	**95.62**	31.89	286.7
AGI5198	14	54.14	12.32	3.293	22.76%	**52.69**	45.59	60.9
BRD2879	13	62.15	29.89	8.291	48.10%	**56.78**	43.81	73.6
RRR	3	603.7	134	77.35	22.19%	**593.9**	343.1	1028
RRS	3	5253	29.14	16.83	0.55%	**5253**	5181	5326
RSR	3	18113	3268	1887	18.04%	**17901**	11108	28846
RSS	3	1757	562.3	324.7	32.01%	**1697**	761.2	3783
SRS	3	3966	808.5	466.8	20.39%	**3909**	2329	6562
SSR	3	6484	1728	997.9	26.66%	**6318**	3104	12857
SSS	3	960.3	144.2	83.23	15.01%	**952.6**	643.6	1410

[a]All values in nM unless otherwise indicated. The last seven entries refer to stersoisomers of BRD2879.

Biological and Biochemical Methods

Cloning, expression and purification of wild type and mutant IDH1 in E. coli.

Lysis Buffer: 20 mM Tris pH 7.4, 0.1% Triton X-100, 500 mM NaCl, 5 mM BME, 10% glycerol, protease inhibitor cocktail
Column Buffer A: 20 mM Tris pH 7.4, 500 mM NaCl, 10% glycerol
Column Buffer B: 20 mM Tris pH 7.4, 500 mM NaCl, 10% glycerol, 500 mM imidazole
Enzyme Storage Buffer: 20 mM Tris pH 7.4, 200 mM NaCl, 5 mM BME, and 10% glycerol

The human isocitrate dehydrogenase (IDH1, EC 1.1.1.42) cDNA clone was purchased from Invitrogen (Cat# IOH62682). QuickChange Lightning site-directed mutagenesis kit (Aligent Cat# 210519) was used to generate the R132H and R132C mutations; the mutation was confirmed by sequencing. Wild-type and R132H mutant IDH1 were subcloned into pET41a (EMD Biosciences) using NDEI and XHOI restriction sites to enable the *E. coli* expression of C-terminal His8-tagged proteins. Wild-type and mutant IDH1 proteins were expressed in Rosetta *E. coli* in LB media using kanamycin and chloramphenicol as selection agents and IPTG to induce protein production. After overnight protein expression at 18°C, cells were pelleted and resuspended in Lysis Buffer, then lysed using a microfluidizer at 18,000 PSI. Cell debris was removed by ultracentrifugation and the supernatant was passed through a 0.45 µm filter. Protein was purified by nickel affinity chromatography on a HisTrap 5 mL column (GE Healthcare) using a gradient of 5-100% Column Buffer B in Column Buffer A. Purity was verified by SDS-PAGE with Coomassie Blue staining. Protein concentration was determined by A280 using protein denatured in 6 M guanidinium chloride and an extinction coefficient based on the sequence of IDH1, 64080 $(M\ cm)^{-1}$, calculated with ExPASy ProtParam. Proteins were stored in 50% glycerol in Enzyme Storage Buffer at -80°C.

Cell culture and lentiviral cloning of HA1E-M cells

HA1E-M cells were described previously.[3] To generate HA1E-M-IDH1-R132H and HA1E-M-IDH1-WT cells, lentiviral expression vectors were cloned using Gateway Cloning systems (Invitrogen, Life Technologies, Grand Island, NY). Specifically, IDH1-R132H (Blue Heron, Bothell, WA) and IDH1-WT (Origene, Rockville, MD) vector templates were used to generate pDONR-223 entry clones from which pLEX-304-IDH1-R132H and pLEX-304-IDH1-WT lentiviral expression vectors were generated via enzymatic recombination as described previously.[4] HA1E-M cells were infected with lentivirus generated as described,[4] selected for 10 days in 10 µg/ml blasticidin (Invitrogen, Life Technologies, Grand Island, NY), and cultured in MEM-α medium (Gibco-BRL, Life Technologies, Grand Island, NY) supplemented with 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO) and 0.1% penicillin-streptomycin (Cellgro, Manassas, VA).

Assay development and screening for inhibitors of IDH1-R132H – Mg^{2+} conditions. ("Primary screen")

The ability of IDH1-R132H to convert α-KG to *R*-2HG using NADPH is assayed by measuring consumption of NADPH in a diaphorase-coupled assay. Briefly, resazurin is a dark blue reagent that has little intrinsic fluorescence. In the presence of NADPH, resazurin is reduced by diaphorase to resorufin, which is highly fluorescent with an excitation peak at 579 nm and an emission peak at 584 nm. The specific assay conditions are as follows: IDH1-R132H enzyme stock (10 mg/ml) and substrates (NADPH & α-KG) are diluted in assay buffer containing 25 mM Tris-HCl pH 7.5, 150 mM NaCl, 10 mM $MgCl_2$, 0.1 mM 1,10-phenanthroline, 0.1% PEG3350, and 0.01% Tween 20. To a 1536-well plate (Aurora Biotechnologies cat# 00019180BX) was added 5 nL of 10 mM compound in DMSO per well via acoustic transfer. To each well was added 2.5 µL buffer solution containing enzyme (20 µg/mL IDH1-R132H) and 44 µM NADPH, then plates were incubated for 60 min at RT. The reaction was initiated by addition of 2.5 µL buffer solution containing 1.2 mM α-KG, followed by 50 min incubation at RT, at which time the reaction is still in the linear range. Plates were read on an Envision plate reader before the addition of detection mix to identify fluorescent compounds that could be read as false positives in the assay. The reaction was then terminated by the addition of 2.5 µL of detection mix (1 µg/mL diaphorase, 60 µM resazurin) and fluorescence (excitation 535 nm, emission 595 nm) was measured again on the Envision plate reader. AGI-5198 was used as a positive control on each plate to validate the assay. We screened 89,093 compounds in duplicate, of which 551 compounds that inhibit ≥60% enzyme activity in both replicates in the primary screen were selected for further analysis.

Enzyme assay development and screening for inhibitors of IDH1-R132H – Mn^{2+} conditions.

The Mn^{2+}-based assay relied on the same resazurin-based detection technology as the Mg^{2+}-based assay, but required different buffer composition and reagent concentrations. The final IDH1-R132H assay conditions were as follows: IDH1-R132H enzyme stock (3.18 mg/ml) and substrates (NADPH & α-KG) were diluted in assay buffer containing 100 mM Tris HCl pH 7.4, 150 mM NaCl, 5 mM $MnCl_2$ and 0.03% BSA. 2.5 µL enzyme mix (16 µg/mL IDH1-R132H) and 5 nL of 10 mM compound per well are pre-incubated for 20 min. Reaction was initiated by addition of 2.5 µL of substrate mix (40 µM NADPH, 0.8 mM α-KG), followed by 50 min incubation at RT, at which time the reaction is still in the linear range. Plates were read on an Envision plate reader before the addition of detection mix to identify fluorescent compounds. IDH1-R132H enzyme reaction was then terminated by 2.5 µL of detection mix (1 µg/mL diaphorase, 50 µM resazurin) and assay plates are measured again in an Envision plate reader (excitation 535 nm, emission 595 nm). We screened 74,437 compounds in duplicate, 49,237 of which were also screened in the Mg^{2+} conditions. Compounds that inhibit ≥40% enzyme activity in the primary screen were selected as hits, corresponding to >4x the sum of the standard deviations of the negative (DMSO) and positive (no enzyme) controls.

Absorbance assay for IDH1-R132H (used to confirm screening positives), Mg^{2+} conditions

The buffer composition was identical to that of the primary screen and used Mg^{2+} as the metal cofactor. To a solution of 40 µL/well IDH1-R132H enzyme in buffer was added compound in DMSO; compound and

enzyme were incubated for 20 minutes before addition of a substrate mix containing α-KG and NADPH. Compounds were plated in 8-point dose (3x dilution starting at 16.7 μM) in clear 384-well plates (Corning #3640). The final concentration of reagents was 4.4 μg/mL IDH1-R132H, 0.6 mM α-KG, 20 μM NADPH, and 0.17% DMSO. After addition of substrates, the absorbance of NADPH at 340 nm was monitored via Spectramax M5 plate reader. The slope of the linear portion of the absorbance vs. time trace was used to determine enzyme activity.

Fluorescence assay for IDH1-R132H (used for SAR studies)

Direct fluorescence of NADPH was used to measure IDH1-R132H inhibition by tight-binding compounds in order to reduce the amount of enzyme required such that the IC_{50} determined in the assay provided a more accurate measure of compound potency. The buffer consisted of 25 mM Tris-HCl, 150 mM NaCl, 10 mM $MgCl_2$, 0.1 mM 1,10-phenanthroline, and 0.1% PEG3350, in aqueous solution with pH 7.5. No detergent was used in the assay in order to maximize enzyme activity. To a solution of 40 μL enzyme in black-walled 384-well plates (Corning #3711 or #3575) was added 100 nL of compound in DMSO by pin transfer; compound and enzyme were incubated for about 10 minutes before the reaction was started by addition of substrates NADPH and α-KG in 20 μL buffer. The final concentration of reagents was 0.5 μg/mL IDH1-R132H, 0.6 mM α-KG, 7.5 μM NADPH, and 0.17% DMSO. After addition of substrates, the progress of the reaction was monitored by the fluorescence of NADPH (ex. 340 nm, em. 465 nm) on a Spectramax M5 plate reader. The slope of the linear portion of the fluorescence vs. time trace was used to determine enzyme activity.

Fluorescence assay for other IDH alleles (IDH1-R132C and IDH2-R140Q)

Inhibition of IDH1-R132C and IDH2-R140Q by compounds was determined by a direct NADPH fluorescence assay analogous to that used for IDH1-R132H SAR studies (see above), with the following modifications. For the R132C assay, the reaction mixture contained 2 mM $MgCl_2$ and 0.34 mM α-KG due to the lower K_m for these cofactors of the R132C allele compared to the R132H allele. The IDH2-R140Q assay used the same cofactor concentrations as the IDH1-R132H assay. IDH1-R132C was tested at 2.8 μg/mL enzyme concentration and IDH2-R140Q was tested at 1.7 μg/mL enzyme concentration.

Assay for wild-type IDH1 inhibition (used to assess compound selectivity)

The wild-type IDH1 inhibition assay relied on the same resazurin-based detection technology as the primary screen, but enzyme activity was in the direction of isocitrate to α-ketoglutarate, or the "forward" direction according to the canonical citric acid cycle. Thus, we observed gain rather than loss of signal when observing resorufin fluorescence. The buffer composition was identical to that of the primary screen and used Mg^{2+} as the metal cofactor. Positives from the primary screen were plated in 8-point dose (3-

fold dilution series from 50 µM) in black 384-well plates. Compounds and enzyme were incubated together in buffer for 15 minutes, then the reaction was started by addition of substrates. The final reaction mixture contained a 20 µL volume of 0.05 µg/mL w.t. IDH1, 10 µM NADP+, 100 µM isocitrate, 1 µg/mL diaphorase, and 24 µM resazurin. Reaction progress was measured by resorufin fluorescence (excitation 535 nm, emission 595 nm) on a Spectramax M5 instrument.

Absorbance assay for IDH1-R132H, Mn^{2+} conditions

The buffer composition was as follows: 25 mM Tris-HCl, 150 mM NaCl, 0.1% PEG3350, 10 mM $MnSO_4$, pH 7.5. To a solution of 40 µL/well IDH1-R132H enzyme in buffer was added compound in DMSO; compound and enzyme were incubated for 20 minutes before addition of a substrate mix containing α-KG and NADPH. Compounds were plated in 8-point dose (3x dilution starting at 16.7 µM) in clear 384-well plates (Corning #3640). The final concentration of reagents was 3.3 µg/mL IDH1-R132H, 0.6 mM α-KG, 75 µM NADPH, and 0.17% DMSO. After addition of substrates, the absorbance of NADPH at 340 nm was monitored via Spectramax M5 plate reader. The slope of the linear portion of the absorbance vs. time trace was used to determine enzyme activity.

Use of PubChem to assess compound selectivity

The public PubChem database (pubchem.ncbi.nlm.nih.gov) was queried for CID 54619248, the record code for BRD2879. Biological test results were last observed in section 6.1 on 6/14/2016. The KDM4C (aka GASC1) assay is AID 720574. Note that while the compound is listed as inactive in AID 624101 "Development of IDH1/2 inhibitors," this *in vitro* assay was conducted by us using a Mn^{2+} cofactor and proved not to be predictive of *in vitro* activity using a Mg^{2+} cofactor nor of activity in cells.

Measurement of R-*2HG in conditioned media*

HA1E-M-IDH1-R132H cells were counted and plated in 96-well tissue-culture plates (Corning 3904) at a density of 10,000 cells/well. The plates were incubated to allow the cells to adhere and grow to confluence (about 36 hours). Media was aspirated and immediately replaced with 100 µL/well media containing compound (DMSO concentration 0.3%). Compound-treated media was aspirated and replaced after 24 hours of treatment to wash out any *R*-2HG which was secreted before compounds took effect. After 72 hours of treatment (i.e. 48 hours after last media change), 60 µL/well media was removed and added to 800 µL aqueous 80% methanol solution containing deuterated racemic 2HG (2HG-d4) as an internal standard. The methanol solution was evaporated at 40°C under reduced pressure or a stream of dry nitrogen and the resulting residue was resuspended in a solution of 9 mM ammonium hydroxide in 68% acetonitrile/22% methanol/10% water. This mixture was briefly vortexed and sonicated to encourage dissolution of *R*-2HG, then centrifuged to remove solids. Targeted LC-MS data were then acquired using a

Waters 2795 separations module and Waters 3100 mass detector. *R*-2HG was separated using a Luna NH_2 column (50 x 2 mm, 3 µm beads, cat. no. 00B-4377-B0, Phenomenex, Torrance, CA) and eluted using a 5 min linear gradient initiated with 90% mobile phase B (10 mM ammonium hydroxide in 75% acetonitrile/25% methanol) and concluding with 100% mobile phase A (aqueous solution of 20 mM ammonium hydroxide and 20 mM ammonium acetate). 2HG is measured using selected-ion monitoring in the negative ion mode at M/Z 147 (2HG) and 151 (2HG-d4). The ratio of ion counts at these M/Z is used to determine the relative amounts of *R*-2HG in various conditioned media samples.

Determination of cell viability

Cells were observed under a light microscope (100x magnification) to observe visible changes in cell morphology. At toxic compound concentrations, cells appeared shriveled up or were not present. For quantitative viability measurements, intracellular ATP levels were determined via CellTiter-Glo assay (Promega, Madison, WI).

Determination of U937, THP1 cell viability

U937 or THP1 cells were obtained from ATCC and seeded at a density of 10,000 cells/well in 96-well opaque-wall plates in 100 µL RPMI media, supplemented with 10% fetal bovine serum and penicillin/streptomycin. These conditions leave the cells ample space and nutrients to continue growth. Cells were incubated at 37 °C for 72 hours, after which viability was determined as described above.

Thermal shift assay by differential scanning fluorimetry (DSF)

In a 20 µL volume on a clear LightCycler 384-well plate (Roche cat# 05102430001), 5 µM compound was incubated with 125 µg/mL (3 µM) IDH protein of the specified allele and 1:600 dilution of SYPRO orange (Molecular Probes cat# S6650) in aqueous buffer containing 25 mM Tris-HCl, 150 mM NaCl, and 0.1% PEG3350 at pH 7.5. The plate was heated from 25 °C to 95 °C over 20 minutes while SYPRO orange fluorescence was measured on a Roche LightCycler 480 II. The protein melting point was determined using LightCycler 480 Protein Melting software.

Mechanism of action studies

BRD2879's mechanism of enzyme inhibition was investigated using steady-state kinetics using the method described by Copeland for tight binding inhibitors.[1] Assay conditions were identical to the IDH1-R132H fluorescence assay used for SAR studies (see above), except the substrate or cofactor under investigation was varied in concentration. Enzyme was treated with BRD2879 in dose in the presence of varying

concentrations of substrate/cofactor ranging from 0.2 – 10x the substrate's/cofactor's K_m. The change in the inhibitor's IC_{50} with increasing substrate concentration is diagnostic of the mechanism of action.

Calculation of dose-response curves

All dose-response curves and IC_{50}/EC_{50} values were generated/calculated with GraphPad Prism version 6 or 7 using four-parameter nonlinear regression, with curve top and bottom fixed at values associated with positive and negative control treatments. Curves were then inspected by eye for plausibility.

Purchases

IDH2-R140Q protein was purchased from BPS Bioscience (cat# 71100-1). Unless otherwise noted, assay plates were typically obtained from Corning, biological reagents were typically purchased from Life Technologies and chemicals were purchased from Sigma-Aldrich.

Analytical Chemistry/ADME Methods

General Analytical Methods

NMR spectra were recorded on a Bruker UltraShield 300 (300 MHz ^{1}H, 75 MHz ^{13}C) or Bruker Avance 400 (400 MHz ^{1}H, 100 MHz ^{13}C) NMR spectrometer. Spectra were referenced to the residual solvent peak. Data are reported as follows: chemical shift in ppm (multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, sex = sextet, m = multiplet), coupling constants (Hz), integration). Reactions were monitored by TLC and LC-MS (see below). Flash chromatography was performed on a Teledyne Isco CombiFlash Rf system using Redi*Sep* Rf columns. High-performance liquid chromatography (HPLC) was performed on a Waters HPLC system using a basic solvent system (water/acetonitrile/0.2 % NH_4OH).

Compound purity determination by LC-MS or UPLC-MS (as noted)

Compound purity and identity were determined by LC-MS (Alliance 2795, Waters, Milford, MA). Purity was measured by UV absorbance at 210 nm. Identity was determined on a SQ mass spectrometer by positive and negative electrospray ionization. Mobile phase A consisted of 0.01% formic acid in water, while mobile phase B consisted of 0.01% formic acid in acetonitrile. The gradient ran from 5% to 95% mobile phase B over 1.75 minutes at 1.75 mL/min. An Agilent Poroshell 120 EC-C18, 2.7 µm, 3.0x30 mm column was used with column temperature maintained at 40 °C. 2.1 µL of sample solution was injected.

Compound purity and identity were determined by UPLC-MS. Purity was measured by UV absorbance at 210 nm. Identity was determined on a SQ mass spectrometer by positive and/or negative electrospray ionization. Mobile phase A consisted of either 0.1% ammonium hydroxide or 0.05% trifluoroacetic acid in water, while mobile phase B consisted of either 0.1% ammonium hydroxide or 0.06% trifluoroacetic acid in acetonitrile. The gradient ran from 5% to 95% mobile phase B over 2.65 min at 0.9 mL/min. An Acquity BEH C18, 1.7 µm, 2.1x50 mm column was used with column temperature maintained at 65 °C. Compounds were dissolved in DMSO at a nominal concentration of 1 mM, and 1.0 µL of this solution was injected.

Exact mass determination

High-resolution mass-spectra were acquired on an Agilent 1290 Infinity separations module coupled to a 6230 time-of-flight (TOF) mass detector operating in ESI+ or ESI- mode. Masses were confirmed using the "Find by Formula" feature in MassHunter Qualitative Analysis vB.06.00.

Solubility (Thermodynamic)

Solubility was determined in phosphate buffered saline (PBS) pH 7.4. Each compound was prepared in triplicate at 100 µM in both 100% DMSO and PBS. Compounds were allowed to equilibrate at room temperature with a 750 rpm vortex shake for 18 hours. After equilibration, samples were analyzed by UPLC-MS (Waters, Milford, MA) with compounds detected by SIR detection on a single quadrupole mass spectrometer. The DMSO samples were used to create a two-point calibration curve to which the response in PBS was fit.

Plasma Protein Binding

Plasma protein binding was determined by equilibrium dialysis using the Rapid Equilibrium Dialysis (RED) device (Pierce Biotechnology, Rockford, IL) for both human and mouse plasma. Each compound was prepared in duplicate at 5 µM in plasma (0.95% acetonitrile, 0.05% DMSO) and added to one side of the membrane (200 µL) with PBS pH 7.4 added to the other side (350 µL). Compounds were incubated at 37ºC for 5 hours with a 350-rpm orbital shaker. After incubation, samples were analyzed by UPLC-MS (Waters, Milford, MA) with compounds detected by SIR detection on a single quadrupole mass spectrometer.

Microsomal Stability (1), Broad Institute

Microsomal stability was determined at 37°C at 60 minutes in both human and mouse microsomes. Each compound was prepared in duplicate at 1 µM with 0.3 mg/mL microsomes in PBS pH 7.4 (1% DMSO). Compounds were incubated at 37°C for 60 minutes with a 350-rpm orbital shake with time points taken at 0 minutes and 60 minutes. Samples were analyzed by UPLC-MS (Waters, Milford, MA) with compounds detected by SIR detection on a single quadrupole mass spectrometer.

Microsomal Stability (2), Wuxi AppTec

Microsomal stability measurements were performed by Wuxi AppTec. Each compound was prepared at 1 µM in 20 mM potassium phosphate buffer at pH 7.4 with 0.1% DMSO, 0.5% methanol and 0.5 mg/mL human or mouse liver microsomes. Microsomal stability was determined at 37°C at 0, 5, 10, 20, 30, and 60 minutes in both human and mouse microsomes. Remaining compound was detected by LC/MS/MS and $t_{1/2}$ was calculated by fitting measurements to a first-order kinetic decay curve.

LogD

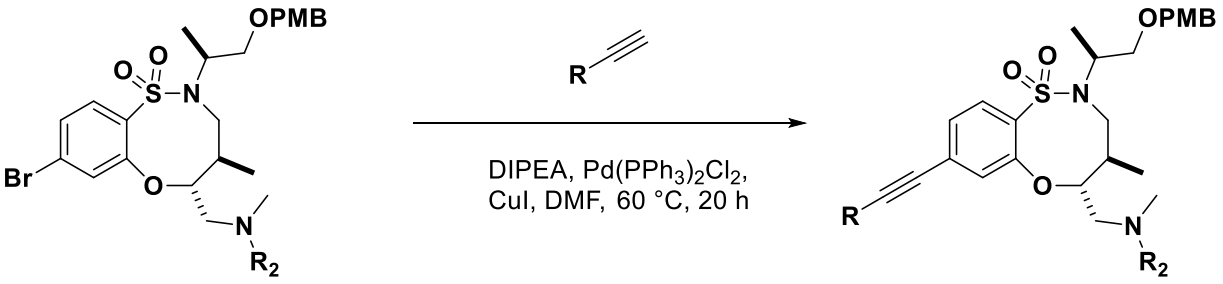
LogD was measurements were performed by Wuxi AppTec. Measurements were performed at a compound concentration of 67 µM in a two-phase system consisting of octanol and 100 mM pH 7.4 phosphate buffer containing 1% DMSO.

Stereoisomers of BRD2879

Stereoisomers of BRD2879 were used directly from the Broad Institute compound library without resynthesis. The stereoisomers were checked for purity by UPLC-MS (see above) and determined to be >94% pure.

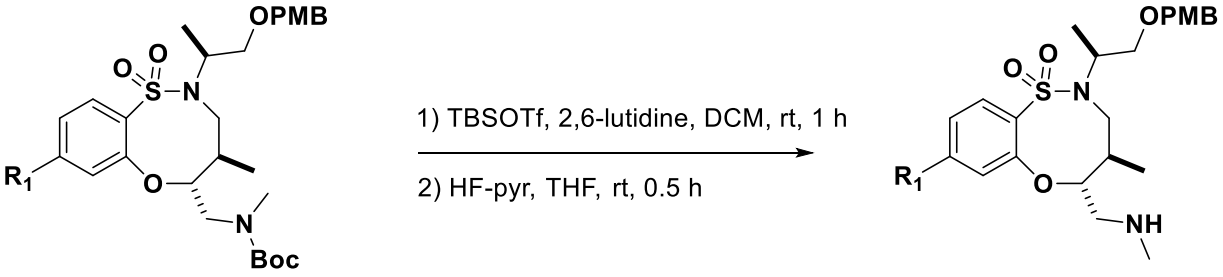
General Synthetic Protocols

General Protocol 1: addition of R_1 group



To a solution of the aryl bromide (1.0 eq) in DMF (0.05 M to 0.20 M) were added CuI (0.1 eq to 0.4 eq), $Pd(PPh_3)_2Cl_2$ (0.1 eq), DIPEA (15 eq to 25 eq), and desired alkyne (1.5 eq to 2.5 eq) under nitrogen atmosphere. The mixture was degassed with a stream of nitrogen for 15 min to 30 min before stirring at 40 °C and 60 °C until completion was observed by LC-MS (8 hours to 4 days). After cooling to room temperature the reaction was quenched with saturated aqueous sodium bicarbonate solution and extracted three times with ethyl acetate. The combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and the solvent was evaporated. The crude product was purified by flash chromatography on silica gel (gradient: 0 % to 5 % methanol in DCM).

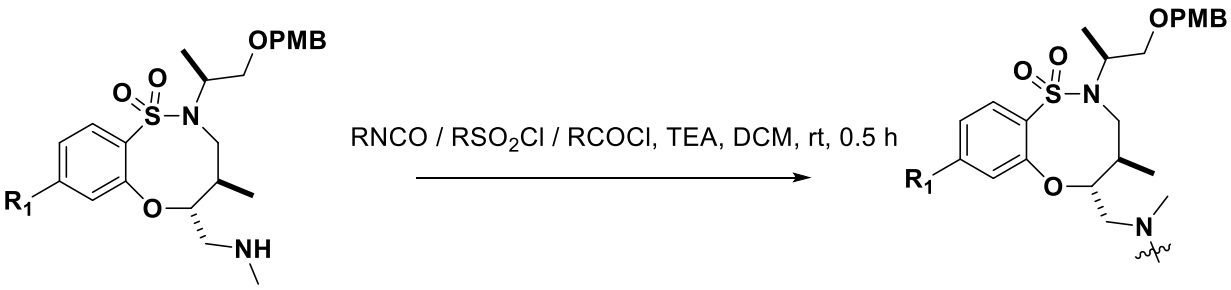
General Protocol 2: Boc deprotection



To a solution of the Boc-protected amine (1.0 eq) in DCM (0.10 M to 0.20 M) were added 2,6-lutidine (4.0 eq) and TBSOTf (3.0 eq) at room temperature. The reaction was stirred until complete consumption of starting material was observed by LC-MS (0.5 h to 1 h). The reaction was quenched with saturated aqueous ammonium chloride solution and the aqueous phase was extracted with ethyl acetate. The combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and the solvent was evaporated. The residue was redissolved in THF (0.2 M) before addition of HF·pyridine (70 % HF, 1.0 eq). Complete conversion was usually observed after 0.5 hours. The reaction was quenched with saturated aqueous

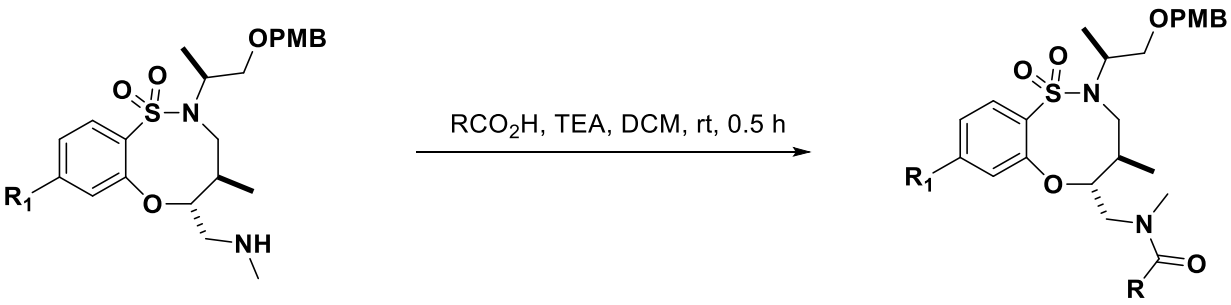
sodium bicarbonate solution and the aqueous phase was extracted with ethyl acetate. The combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and the solvent was evaporated. The resulting amine was used without further purification.

General Protocol 3: R_2 N-capping to ureas, sulfonamides, amides from isocyanates, sulfonyl chlordies, and acyl chlorides



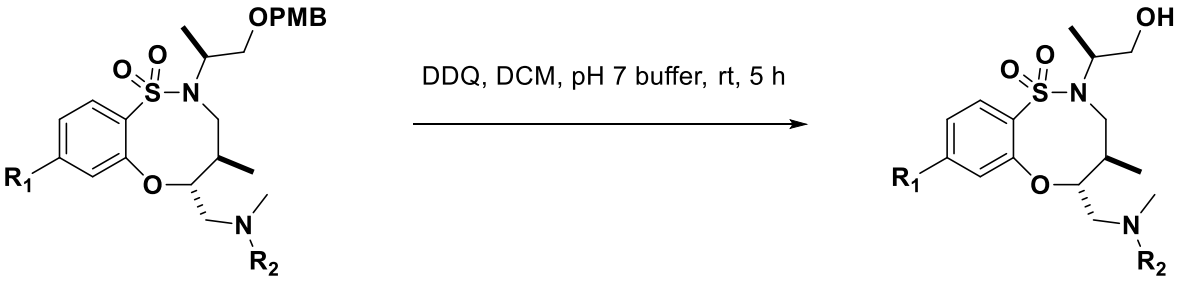
The secondary amine was dissolved in DCM (0.1 M to 0.2 M) before addition of DIPEA or TEA (1.5 eq) and the desired sulfonyl chloride, acid chloride, or isocyanate (1.5 to 3.0 eq). The mixture was stirred at room temperature until complete consumption of starting material was observed (0.5-2 h). The reaction was quenched with saturated aqueous sodium bicarbonate solution and extracted three times with ethyl acetate. The combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and the solvent was evaporated. The crude product was purified by flash chromatography on silica gel.

General Protocol 4: R_2 N-capping to amides from carboxylic acids



To a solution of carboxylic acid (1.0 eq) in DCM (80 % of total solvent, final concentration 0.1 M to 0.2 M) was added PyBOP (1.0 eq) and DIPEA (3.0 eq) under nitrogen atmosphere. The resulting solution was cooled to 0 °C before dropwise addition of the desired amine (1.5 eq) in DCM (20 % of total solvent). After complete addition the reaction was allowed to warm to room temperature and stirred for an additional 6 hours. The reaction was quenched with water and extracted with DCM three times. The combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and the solvent was evaporated. The residue was taken up in ethyl ether and the precipitated phosphoramide byproduct was removed by filtration. Evaporation of solvent yielded the crude product, which was further purified by flash chromatography on silica gel.

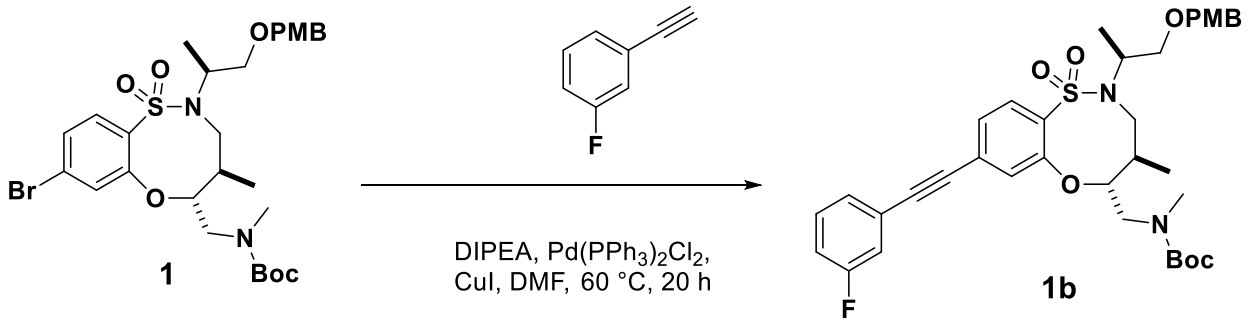
General Protocol 5: PMB deprotection



DDQ (1.2 eq to 2.0 eq) was added to a solution of the PMB-protected alcohol (1.0 eq) in a 5:1 mixture of DCM and aqueous pH 7 phosphate buffer solution (0.05 M to 0.15 M). The mixture was stirred vigorously at room temperature until completion was observed by LC-MS (1-4h). The reaction was quenched with saturated aqueous sodium bicarbonate solution and extracted three times with DCM. The combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and the solvent was evaporated. The crude product was purified by flash chromatography on silica gel (gradient: 0 % to 5 % methanol in DCM).

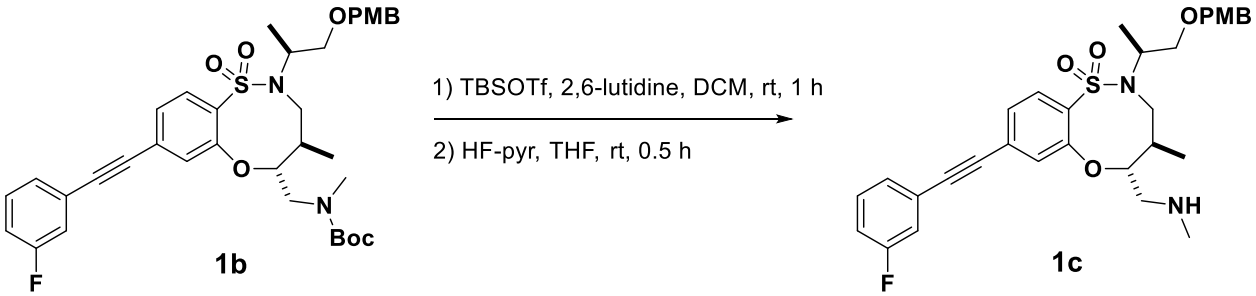
Synthetic details and characterization of compounds

A. Synthesis of BRD2879

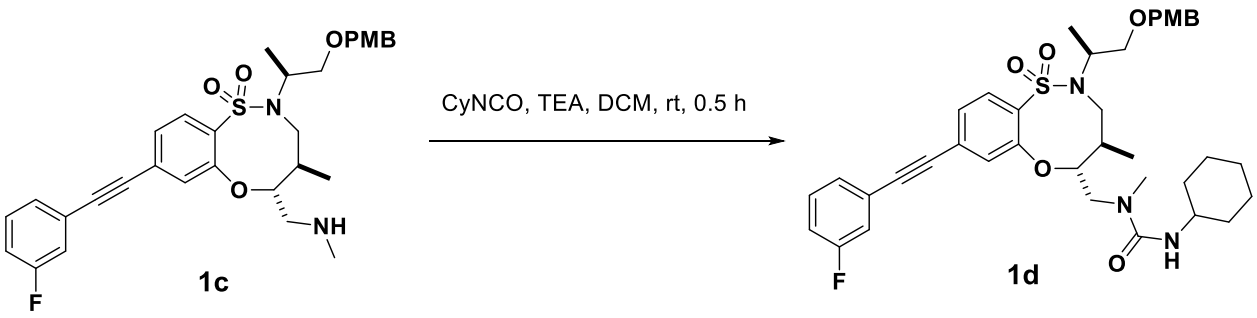


Following **General Protocol 1** aryl bromide core **1** (60 mg, 0.10 mmol, 1.0 eq) was reacted with 1-ethynyl-3-fluorobenzene (26 mg, 0.22 mmol, 2.2 eq), DIPEA (0.18 mL, 0.13 g, 1.0 mmol, 10 eq), $Pd(PPh_3)_2Cl_2$ (14 mg, 0.02 mmol, 0.2 eq), and CuI (4 mg, 0.02 mmol, 0.2 eq) in DMF (2 mL, 0.05 M) for 16 hours. Flash chromatography on silica gel (gradient: 0 % to 50 % EtOAc in hexanes) afforded **1b** (61 mg, 0.09 mmol, 91 %).

^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.85 (d, *J* = 8.2 Hz, 1H), 7.42-7.28 (m, 3H), 7.25-7.19 (m, 1H), 7.18-7.00 (m, 4H), 6.84 (d, *J* = 8.2 Hz, 2H), 4.48-4.39 (m, 1H), 4.32-4.14 (m, 2H), 3.77 (s, 3H), 4.07-3.20 (m, 6H), 3.15-2.61 (m, 4H) 2.17 (br s, 1H), 1.64-1.36 (m, 9H), 1.32 (d, *J* = 5.9 Hz, 3H), 0.93 (d, *J* = 7.0 Hz, 3H).

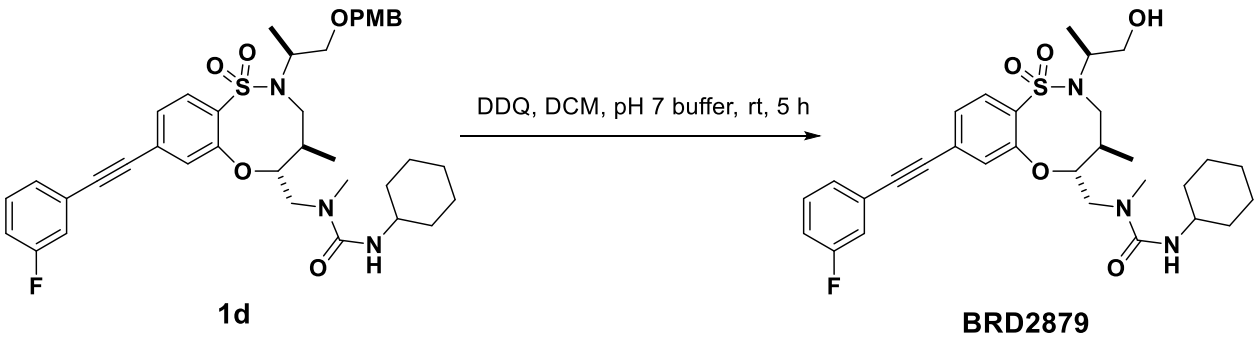


Following **General Protocol 2** Boc protected amine **1b** (44 mg, 0.066 mmol, 1.0 eq) was reacted with 2,6-lutidine (30 µL, 28 mg, 0.26 mmol, 3.9 eq) and TBSOTf (50 µL, 58 mg, 0.22 mmol, 3.3 eq) in DCM (0.7 mL, 0.09 M). The intermediate was treated with HF·pyridine (1.8 µL, 0.069 mmol, 1.1 eq) in THF (0.55 mL, 0.12 M). The crude free amine **1c** (43 mg) was identified by mass spectrometry and used without further purification.



Following **General Protocol 3** crude amine **1c** (25.0 mg, 0.044 mmol, 1.0 eq) was reacted with TEA (15 µL, 10.9 mg, 0.11 mmol, 1.6 eq) and cyclohexyl isocyanate (10 µL, 9.8 mg, 0.078 mmol, 1.8 eq) in DCM (0.45 mL, 0.10 M). Flash chromatography on silica gel (gradient: 0 % to 5 % MeOH in DCM) afforded urea **1d** (19.1 mg, 63 % over 2 steps).

^{1}H NMR (300 MHz, $CDCl_3$, 27 °C) δ 7.85 (d, *J* = 8.2 Hz, 1H), 7.36-7.28 (m, 3H), 7.25-7.19 (m, 1H), 7.14-7.00 (m, 4H), 6.87-6.79 (m, 2H), 4.38 (d, *J* = 7.7 Hz, 1H), 4.34-4.22 (m, 2H), 4.15-4.05 (m, 2H), 3.91 (dd, *J* = 15.6, 5.2 Hz, 1H), 3.77 (s, 3H), 3.81-3.61 (m, 3H), 3.56-3.43 (m, 2H), 3.04 (dd, *J* = 14.7, 2.4 Hz, 1H), 2.72 (s, 3H), 2.26-2.10 (m, 1H), 2.10-1.90 (m, 2H), 1.71-1.49 (m, 3H), 1.33 (d, *J* = 6.6 Hz, 3H), 1.41-1.21 (m, 2H), 1.21-0.87 (m, 3H), 0.96 (d, *J* = 7.1 Hz, 3H). **^{13}C NMR** (75 MHz, $CDCl_3$, 27 °C) δ 164.23, 160.96, 159.40, 158.01, 156.25, 136.15, 130.28, 130.16, 130.14, 129.38, 128.84, 128.16, 127.80, 127.76, 127.02, 126.68, 118.81, 118.51, 116.56, 116.29, 113.90, 90.98, 88.71, 86.58, 72.96, 72.53, 56.99, 55.41, 51.50, 51.34, 49.85, 36.73, 34.81, 34.31, 25.76, 25.30, 25.24, 16.86, 16.05.

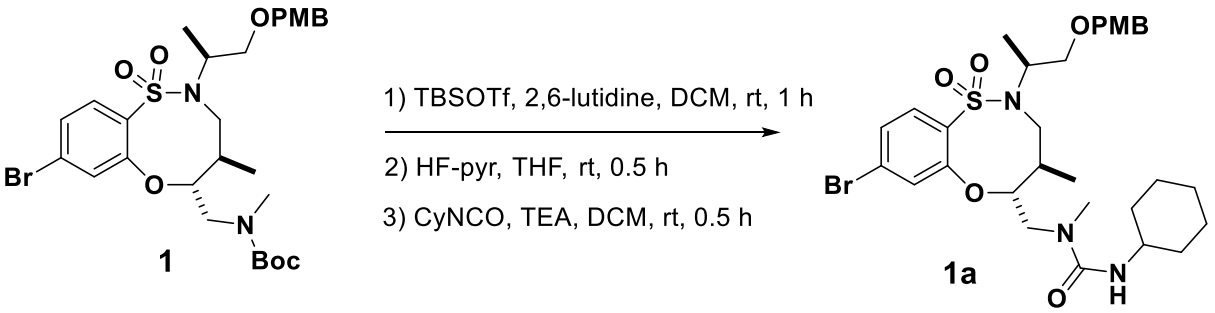


Following **General Protocol 5** PMB-protected alcohol **1d** (13.4 mg, 0.019 mmol, 1.0 eq) was reacted with DDQ (9.4 mg, 0.041 mmol, 2.2 eq) in DCM (0.4 mL, 0.05 M) and pH 7 buffer (10 drops). Flash chromatography on silica gel (gradient: 0 % to 5 % MeOH in DCM) afforded **BRD2879** (6.7 mg, 60 %). **BRD2879** was purified by HPLC prior to biological testing.

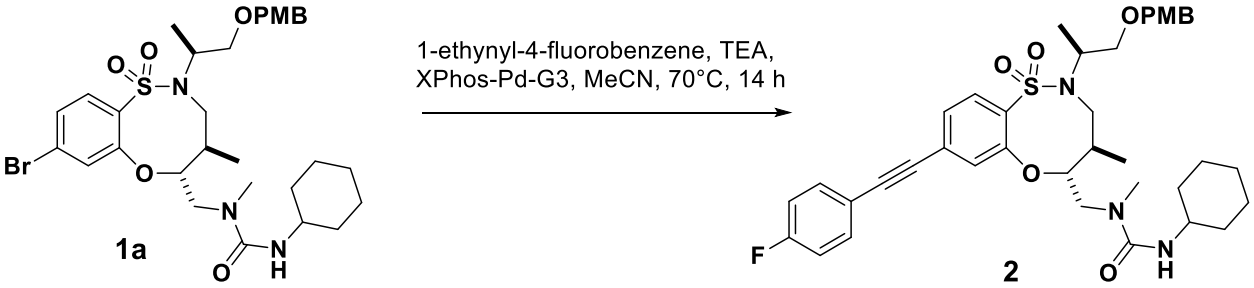
^{1}H NMR (300 MHz, $CDCl_3$, 27 °C) δ 7.88 (d, *J* = 8.3 Hz, 1H), 7.39-7.27 (m, 3H), 7.22-7.14 (m, 2H), 7.12-7.03 (m, 1H), 4.57 (td, *J* = 9.5, 2.5 Hz, 1H), 4.35-4.23 (m, 2H), 3.97-3.78 (m, 2H), 3.74-3.60 (m, 2H), 3.51 (ddd, *J* = 12.3, 9.9, 4.0 Hz, 1H), 3.40 (dd, *J* = 15.8, 5.1 Hz, 1H), 3.18 (dd, *J* = 9.9, 2.9 Hz, 1H), 3.12 (dd, *J* = 14.4, 2.6 Hz, 1H), 2.66 (s, 3H), 2.30-2.16 (m, 1H), 2.06 (d, *J* = 12.2 Hz, 1H), 1.96 (d, *J* = 12.1 Hz, 1H), 1.69-1.58 (m,

1H), 1.58-1.45 (m, 2H), 1.23 (d, *J* = 6.8 Hz, 3H), 1.40-0.97 (m, 5H), 0.94 (d, *J* = 7.0 Hz, 3H). **^{13}C NMR** (75 MHz, $CDCl_3$, 27 °C) δ 164.20, 160.92, 157.74, 154.80, 134.60, 130.26, 130.15, 129.56, 128.62, 127.82, 127.77, 127.68, 126.90, 124.27, 118.81, 118.50, 116.63, 116.35, 91.32, 88.40, 85.60, 64.86, 58.03, 51.64, 49.88, 48.51, 36.73, 34.51, 34.35, 34.31, 25.72, 25.28, 25.23, 15.76, 15.05. **HRMS** (ESI) calc'd for $C_{30}H_{38}FN_3O_5S$ $[M+H]^+$: 572.2589. Found: 572.2588.

B. Synthesis of 4 (Scheme 1)



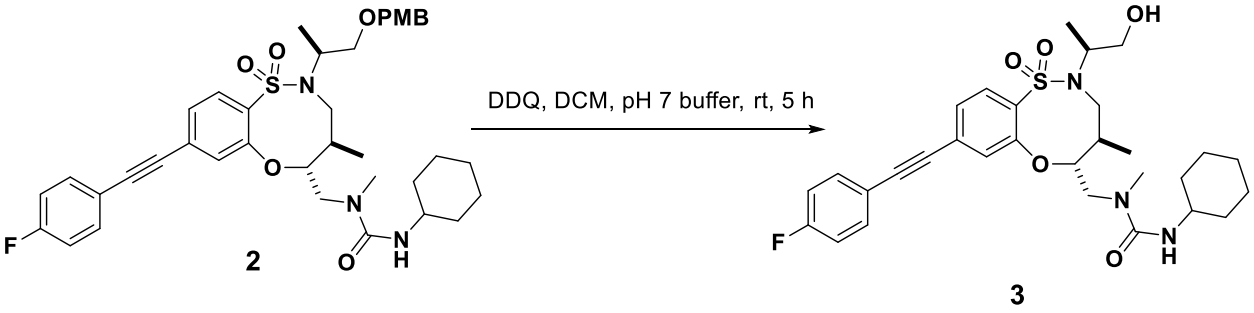
To an oven-dried, argon-filled flask containing **1** (715 mg) in dry DCM (11.4 mL, 0.1 M) was added 2,6-lutidine (537 mg, 584 µL, 4.4 eq) and *tert*-butyldimethylsilyl trifluoromethanesulfonate (843 mg, 733 µL, 2.8 eq). The mixture was stirred for 1 hour at room temperature, then quenched with saturated aqueous ammonium chloride. The organic and aqueous phases were separated and the aqueous phase was extracted 3x with ethyl acetate. The combined organic extracts were dried over magnesium sulfate, filtered, and concentrated to a yellow oil. This oil was dissolved in THF (5.7 mL, 0.2 M) and transferred to a polypropylene tube, and hydrogen fluoride-pyridine (23 mg, 1.02 eq) was added. The mixture was stirred until gas evolution ceased (5 min). The reaction was quenched with saturated aqueous sodium bicarbonate, extracted 3x with ethyl acetate, dried over magnesium sulfate, filtered, and concentrated. The residue was dissolved in DCM and transferred to a dry flask under nitrogen. Cyclohexyl isocyanate (185 mg, 189 µL, 1.3 eq) and triethylamine (184 mg, 254 µL, 1.6 eq) were added and the mixture was stirred for 25 minutes at room temperature. The reaction was quenched with saturated aqueous ammonium chloride, the aqueous and organic layers were separated, and the aqueous layer was extracted 3x with DCM. The combined organic layers were dried over magnesium sulfate. The crude material was purified by silica flash column chromatography using a solvent gradient of 0-5% methanol in DCM, with 1% triethylamine additive. 640 mg of **1a** was isolated in 86% yield and identified by LC-MS.



To a vial containing **1a** (158 mg) in acetonitrile (2.4 mL, 0.1 M, degassed by bubbling argon) was added 1-ethynyl-4-fluorobenzene (100 mg, 3.4 eq). This mixture was transferred via syringe to a vial containing XPhos Pd G3 mesylate (20.4 mg, 0.1 eq, Sigma-Aldrich, cat# 7633814) under nitrogen and the reaction was started by addition of triethylamine (489 mg, 674 µL, 20 eq., degassed by bubbling argon). The reaction was stirred overnight at 70 °C, then quenched with pH 7 aqueous phosphate buffer. Organic and

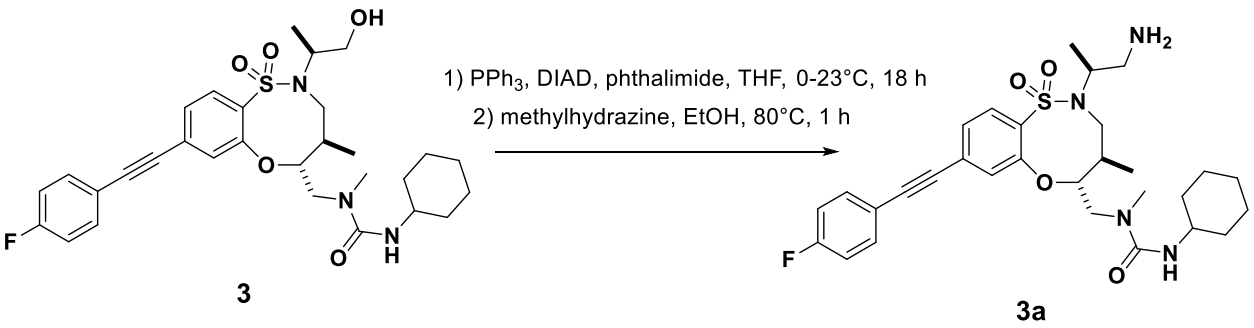
aqueous phases were separated and the aqueous phase was extracted 3x with ethyl acetate. The combined organic extracts were dried over anhydrous sodium sulfate, decanted, and concentrated. The material was purified by flash silica chromatography using a solvent gradient of 0-5% methanol in DCM. 157 mg **2** was recovered in 94% yield.

^{1}H NMR (300 MHz, $CDCl_3$, 27 °C) δ 7.84 (d, *J* = 8.2 Hz, 1H), 7.56-7.47 (m, 2H), 7.29 (dd, *J* = 8.2, 1.6 Hz, 1H), 7.12-7.00 (m, 5H), 6.87-6.79 (m, 2H), 4.39 (d, *J* = 7.7 Hz, 1H), 4.33-4.22 (m, 2H), 4.15-4.03 (m, 2H), 3.91 (dd, *J* = 15.6, 5.2 Hz, 1H), 3.77 (s, 3H), 3.82-3.59 (m, 3H), 3.56-3.43 (m, 2H), 3.03 (dd, *J* = 14.7, 2.5 Hz, 1H), 2.72 (s, 3H), 2.26-2.10 (m, 1H), 2.09- 1.90 (m, 2H), 1.70-1.49 (m, 3H), 1.33 (d, *J* = 6.6 Hz, 3H), 1.39-1.20 (m, 2H), 1.20-0.98 (m, 3H), 0.96 (d, *J* = 7.1 Hz, 3H). **^{13}C NMR** (75 MHz, $CDCl_3$, 27 °C) δ 164.71, 161.39, 159.39, 158.02, 156.24, 135.87, 133.89, 133.78, 130.14, 129.39, 128.79, 128.53, 126.91, 126.53, 116.11, 115.82, 113.89, 91.36, 87.66, 86.57, 72.94, 72.53, 56.99, 55.40, 51.50, 51.34, 49.82, 36.72, 34.80, 34.28, 25.77, 25.31, 25.24, 16.86, 16.06.



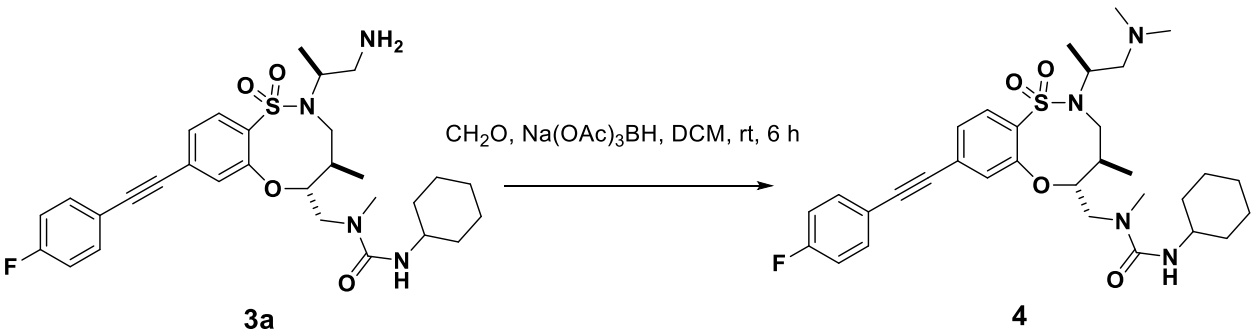
To a flask containing **2** (157 mg) in DCM (4.2 mL, 0.045 M) and phosphate-buffered water (0.84 mL, pH 7) was added DDQ (82 mg, 1.6 eq). The reaction was stirred vigorously under nitrogen at room temperature for 1 hour. The reaction was quenched with saturated aqueous sodium bicarbonate and the mixture was filtered through Celite. The mixture was partitioned between water and DCM, the phases were separated, and the aqueous layer was extracted 3x with DCM. The combined organic layers were dried over anhydrous magnesium sulfate, filtered, and evaporated. The material was purified by flash silica chromatography using a solvent gradient of 0-5% methanol in DCM. 86 mg of **3** was isolated in 66% yield.

^{1}H NMR (300 MHz, $CDCl_3$, 27 °C) δ 7.87 (d, *J* = 8.2 Hz, 1H), 7.53-7.43 (m, 2H), 7.31 (dd, *J* = 8.3, 1.6 Hz, 1H), 7.17 (d, *J* = 1.6 Hz, 1H), 7.11-7.00 (m, 2H), 4.57 (td, *J* = 9.6, 2.5 Hz, 1H), 4.36-4.22 (m, 2H), 3.97-3.78 (m, 2H), 3.74-3.59 (m, 2H), 3.51 (ddd, *J* = 12.2, 9.9, 4.0 Hz, 1H), 3.40 (dd, *J* = 15.8, 5.1 Hz, 1H), 3.19 (dd, *J* = 10.0, 3.0 Hz, 1H), 3.11 (dd, *J* = 14.4, 2.6 Hz, 1H), 2.66 (s, 3H), 2.30-2.15 (m, 1H), 2.11-2.01 (m, 1H), 2.01-1.90 (m, 1H), 1.70-1.58 (m, 1H), 1.58-1.43 (m, 2H), 1.22 (d, *J* = 6.7 Hz, 3H), 1.40-0.96 (m, 5H), 0.94 (d, *J* = 6.9 Hz, 3H). **^{13}C NMR** (75 MHz, $CDCl_3$, 27 °C) δ 157.75, 154.78, 134.30, 133.91, 133.79, 129.51, 129.00, 127.59, 126.74, 116.10, 115.81, 91.73, 87.38, 85.57, 64.86, 58.01, 51.63, 49.86, 48.48, 36.72, 34.50, 34.31, 25.74, 25.30, 25.23, 15.77, 15.05. **HRMS** (ESI) calc'd for $C_{30}H_{38}FN_3O_5S$ $[M+H]^+$: 572.2589. Found: 572.2588.



3 **3a**

To a solution of triphenylphosphine (42 mg, 3.5 eq) in THF (0.5 mL) at 0 °C was added dropwise diisopropyl azodicarboxylate (32 mg, 31 µL, 3.5 eq) and a solution of **3** (26 mg) in THF (0.5 mL, 0.05 M final). The reaction was allowed stirred overnight and allowed to warm to room temperature. The solvent was removed by evaporation and the mixture was dissolved in DCM and partially purified by silica flash chromatography using a solvent gradient of 0-5% methanol in DCM. The resulting material was dissolved in ethanol (0.57 mL, 0.1 M), methylhydrazine (44 mg, 50 µL, 17 eq) was added, and the mixture was stirred at 80 °C for 1 hr. Solvent was removed by evaporation under reduced pressure and the material was purified by silica flash chromatography using a solvent gradient of 0-10% methanol in DCM. 33 mg of **3a** was isolated in 36% yield and identified by LC-MS.



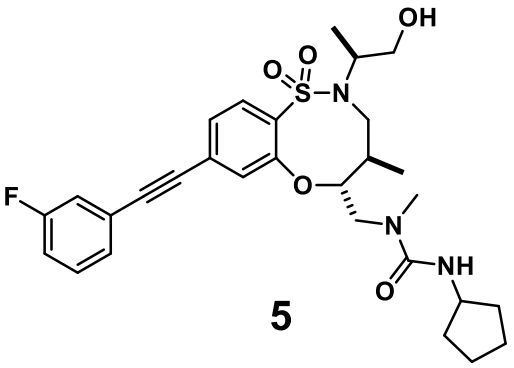
3a **4**

To a solution of **3a** (12 mg) in DCM (0.45 mL, 0.045 M) was added anhydrous magnesium sulfate (25 mg, 10 eq) and formaldehyde 30% aqueous solution (3.7 mg formaldehyde, 11.4 µL solution, 6 eq). The mixture was stirred for 1 hour at room temperature, followed by addition of sodium triacetoxyborohydride (53 mg, 12 eq) and an additional 6 hours of stirring. The solvent was evaporated and the material was purified by silica flash chromatography using a solvent gradient of 0-10% methanol in DCM. The material was further purified by HPLC to yield 6.35 mg of **4** (51% after HPLC).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C) δ 7.84 (d, *J* = 8.2 Hz, 1H), 7.55 – 7.44 (m, 2H), 7.28 (d, *J* = 1.6 Hz, 1H), 7.15 (d, *J* = 1.6 Hz, 1H), 7.11 – 7.01 (m, 2H), 4.43 (td, *J* = 9.2, 2.6 Hz, 1H), 4.35 (d, *J* = 7.6 Hz, 1H), 4.20 (dd, *J* = 14.5, 9.6 Hz, 1H), 3.76 – 3.52 (m, 3H), 3.16 (dd, *J* = 14.5, 2.6 Hz, 1H), 2.73 (s, 2H), 2.53 – 2.47 (m, 1H), 2.42 – 2.35 (m, 1H), 2.27 – 2.17 (m, 1H), 2.09 (s, 6H), 2.10 – 1.93 (m, 1H), 1.75 — 1.50 (m, 5H), 1.40 – 0.63 (m, 14H). **^{13}C NMR** (101 MHz, $CDCl_3$, 25°C) δ 157.73, 155.78, 135.06, 133.69, 133.61, 129.02, 128.34, 126.68, 126.22, 115.91, 115.68, 91.17, 85.54, 77.32, 77.21, 77.01, 76.69, 64.16, 54.81, 51.52, 50.48, 49.68, 45.82,

36.61, 34.40, 34.17, 34.14, 25.57, 25.17, 25.10, 17.53, 15.50. **HRMS** (ESI) calc'd for $C_{32}H_{43}FN_4O_4S$ $[M+H]^+$: 599.3062. Found: 599.3056.

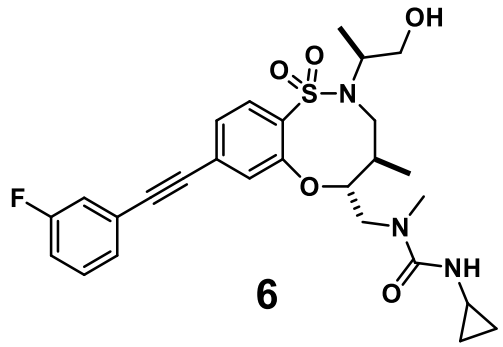
C. Selected final analogs (Table 1)



5

Following **General Protocol 3** crude amine **1c** (20 mg crude, max. 0.030 mmol, 1.0 eq) was reacted with DIPEA (10 µL, 7.2 mg, 0.058 mmol, 1.9 eq) and cyclopentyl isocyanate (6.0 µL, 5.9 mg, 0.053 mmol, 1.8 eq) in DCM (0.35 mL, 0.10 M). Crude PMB-protected alcohol (19 mg) was identified by mass spectrometry and directly used without further purification. Following **General Protocol 5** crude PMB-protected alcohol (19.0 mg, 0.028 mmol, 1.0 eq) was reacted with DDQ (8.0 mg, 0.035 mmol, 1.3 eq) in DCM (0.3 mL, 0.09 M) and pH 7 phosphate buffer (50 µL). Flash chromatography (gradient: 0 % to 10 % MeOH in DCM) afforded final compound **5** (10.0 mg, 0.018 mmol, 60 % over 3 steps). **5** was purified by HPLC prior to biological testing.

^{1}H NMR (300 MHz, $CDCl_3$, 27 °C) δ 7.88 (d, *J* = 8.2 Hz, 1H), 7.39-7.27 (m, 3H), 7.23-7.15 (m, 2H), 7.08 (tdd, *J* = 8.1, 2.7, 1.5 Hz, 1H), 4.59 (td, *J* = 9.5, 2.5 Hz, 1H), 4.39-4.25 (m, 2H), 4.14 (sex, *J* = 6.8 Hz, 1H), 3.96- 3.78 (m, 2H), 3.66 (dd, *J* = 12.3, 9.1 Hz, 1H), 3.51 (dd, *J* = 12.3, 4.0 Hz, 1H), 3.40 (dd, *J* = 15.8, 5.1 Hz, 1H), 3.12 (dd, *J* = 14.4, 2.5 Hz, 1H), 2.67 (s, 3H), 2.31-2.15 (m, 1H), 2.10-1.94 (m, 2H), 1.23 (d, *J* = 6.8 Hz, 3H), 1.63-1.16 (m, 6H), 0.94 (d, *J* = 6.9 Hz, 3H), 0.91-0.78 (m, 1H). **^{13}C NMR** (75 MHz, $CDCl_3$, 27 °C) δ 158.23, 154.80, 134.56, 130.27, 130.16, 129.58, 128.63, 127.82, 127.77, 127.64, 126.87, 118.81, 118.50, 116.64, 116.36, 88.41, 85.58, 64.86, 58.03, 53.03, 51.70, 48.50, 36.70, 34.56, 33.97, 33.88, 23.73, 23.65, 15.78, 15.06.

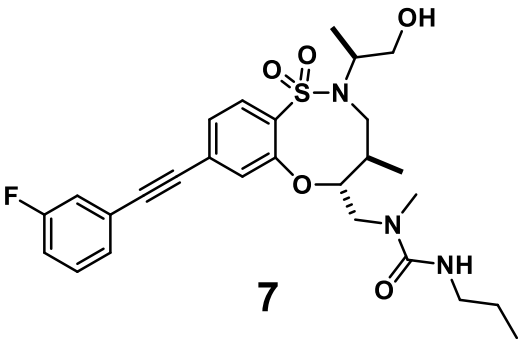


6

Following **General Protocol 3** crude amine **1c** (23.2 mg, 0.041 mmol, 1.0 eq) was reacted with DIPEA (10 µL, 7.3 mg, 0.057 mmol, 1.4 eq) and cyclopropyl isocyanate (5.0 mg, 0.060 mmol, 1.5 eq) in DCM (0.2 mL, 0.21 M). Crude PMB-protected alcohol (27.5 mg) was identified by mass spectrometry and directly used without further purification. Following **General Protocol 5** crude PMB-protected alcohol (27.5 mg crude,

max. 0.041 mmol, 1.0 eq) was reacted with DDQ (10.3 mg, 0.045 mmol, 1.1 eq) in DCM (0.3 mL, 0.14 M) and pH 5 NH_4Cl solution (3 drops). Flash chromatography (gradient: 0 % to 10 % MeOH in DCM) afforded final compound **6** (9.5 mg, 0.018 mmol, 44 % over 3 steps). **6** was purified by HPLC prior to biological testing.

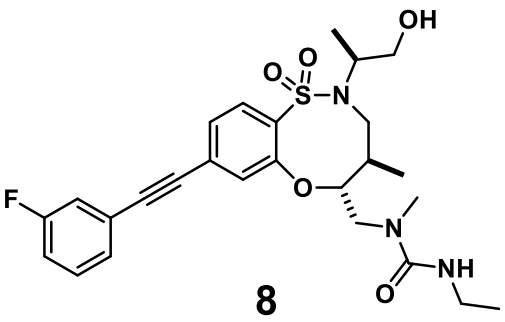
^{1}H NMR (300 MHz, $CDCl_3$, 27 °C) δ 7.87 (d, *J* = 8.2 Hz, 1H), 7.38-7.27 (m, 3H), 7.25-7.18 (m, 1H), 7.17 (d, *J* = 1.4 Hz, 1H), 7.13-7.04 (m, 1H), 4.71 (s, 1H), 4.57 (td, *J* = 9.5, 2.4 Hz, 1H), 4.33 (dd, *J* = 14.4, 9.9 Hz, 1H), 3.95-3.77 (m, 2H), 3.66 (dd, *J* = 12.2, 9.1 Hz, 1H), 3.59-3.46 (m, 1H), 3.41 (dd, *J* = 15.8, 5.1 Hz, 1H), 3.18-3.07 (m, 2H), 2.79-2.68 (m, 1H), 2.63 (s, 3H), 2.32-2.15 (m, 1H), 1.23 (d, *J* = 6.8 Hz, 3H), 0.94 (d, *J* = 6.8 Hz, 3H), 0.75-0.64 (m, 2H), 0.63-0.54 (m, 1H), 0.53-0.44 (m, 1H). **^{13}C NMR** (75 MHz, $CDCl_3$, 27 °C) δ 159.31, 154.76, 134.61, 130.29, 130.17, 129.57, 128.57, 127.80, 127.77, 127.61, 126.84, 124.38, 124.26, 118.82, 118.51, 116.68, 116.40, 91.44, 88.29, 85.52, 64.86, 58.05, 51.67, 48.55, 36.63, 34.52, 23.94, 15.78, 15.06, 7.24, 7.13.



7

Following **General Protocol 3** crude amine **1c** (30 mg crude, max. 0.041 mmol, 1.0 eq) was reacted with DIPEA (10 µL, 7.4 mg, 0.057 mmol, 1.4 eq) and propyl isocyanate (5.5 µL, 5.0 mg, 0.058 mmol, 1.4 eq) in DCM (0.2 mL, 0.21 M). Crude PMB-protected alcohol (24.7 mg) was identified by mass spectrometry and directly used without further purification. Following **General Protocol 5** crude PMB-protected alcohol (24.7 mg, 0.041 mmol, 1.0 eq) was reacted with DDQ (12.0 mg, 0.053 mmol, 1.4 eq) in DCM (0.3 mL, 0.13 M) and pH 5 NH_4Cl solution (3 drops). Flash chromatography (gradient: 0 % to 5 % MeOH in DCM) afforded final compound **7** (8.5 mg, 0.016 mmol, 39 % over 3 steps). **7** was purified by HPLC prior to biological testing.

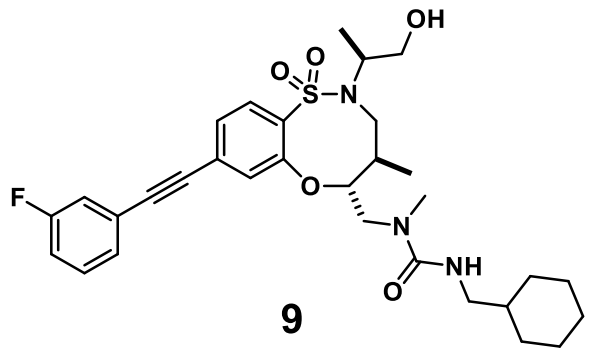
^{1}H NMR (300 MHz, $CDCl_3$, 27 °C) δ 7.88 (d, *J* = 8.2 Hz, 1H), 7.39-7.28 (m, 3H), 7.23-7.15 (m, 2H), 7.08 (dddd, *J* = 8.5, 7.8, 2.6, 1.4 Hz, 1H), 4.58 (td, *J* = 9.5, 2.5 Hz, 1H), 4.45 (t, *J* = 5.6 Hz, 1H), 4.32 (dd, *J* = 14.4, 9.9 Hz, 1H), 3.97-3.79 (m, 2H), 3.66 (dd, *J* = 12.3, 9.1 Hz, 1H), 3.51 (dd, *J* = 12.4, 4.0 Hz, 1H), 3.41 (dd, *J* = 15.8, 5.1 Hz, 1H), 3.35-3.10 (m, 3H), 3.13 (dd, *J* = 14.5, 2.4 Hz, 1H), 2.68 (s, 3H), 2.32-2.15 (m, 1H), 1.61-1.44 (m, 2H), 1.23 (d, *J* = 6.8 Hz, 3H), 0.94 (d, *J* = 6.8 Hz, 3H), 0.84 (t, *J* = 7.4 Hz, 3H). **^{13}C NMR** (75 MHz, $CDCl_3$, 27 °C) δ 158.54, 154.74, 134.62, 130.30, 130.19, 129.55, 128.56, 127.80, 127.76, 127.54, 126.99, 118.80, 118.49, 116.67, 116.38, 100.18, 91.35, 88.36, 85.72, 64.86, 58.07, 51.73, 48.55, 43.12, 36.63, 34.53, 23.79, 15.78, 15.05, 11.46.



8

Following **General Protocol 3** crude amine **1c** (23 mg crude, max. 0.035 mmol, 1.0 eq) was reacted with DIPEA (10 µL, 7.4 mg, 0.057 mmol, 1.6 eq) and ethyl isocyanate (6.5 µL, 5.8 mg, 0.082 mmol, 2.3 eq) in DCM (0.4 mL, 0.09 M). Crude PMB-protected alcohol (20 mg) was identified by mass spectrometry and directly used without further purification. Following **General Protocol 5** crude PMB-protected alcohol (20 mg, 0.031 mmol, 1.0 eq) was reacted with DDQ (9.5 mg, 0.042 mmol, 1.4 eq) in DCM (0.3 mL, 0.09 M) and water (50 µL). Flash chromatography (gradient: 0 % to 5 % MeOH in DCM) afforded final compound **8** (7 mg, 0.014 mmol, 39 % over 3 steps). **8** was purified by HPLC prior to biological testing.

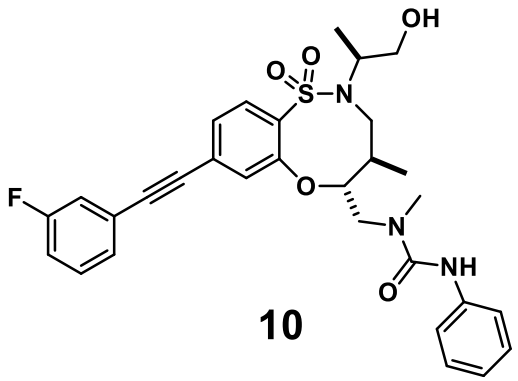
^{1}H NMR (300 MHz, $CDCl_3$, 27 °C) δ 7.88 (d, *J* = 8.2 Hz, 1H), 7.38-7.27 (m, 3H), 7.21-7.15 (m, 2H), 7.08 (tdd, *J* = 8.1, 2.6, 1.4 Hz, 1H), 4.59 (td, *J* = 9.6, 2.5 Hz, 1H), 4.43-4.28 (m, 2H), 3.97-3.79 (m, 2H), 3.66 (dd, *J* = 12.3, 9.0 Hz, 1H), 3.51 (dd, *J* = 12.3, 4.0 Hz, 1H), 3.46-3.29 (m, 3H), 3.12 (dd, *J* = 14.5, 2.5 Hz, 1H), 2.67 (s, 3H), 2.31-2.16 (m, 1H), 1.23 (d, *J* = 6.7 Hz, 3H), 1.16 (t, *J* = 7.2 Hz, 3H), 0.94 (d, *J* = 6.8 Hz, 3H). **^{13}C NMR** (75 MHz, $CDCl_3$, 27 °C) δ 158.47, 154.74, 134.60, 130.32, 130.20, 129.56, 128.55, 127.79, 127.75, 127.55, 126.94, 118.78, 118.47, 116.67, 116.39, 88.37, 85.58, 64.86, 58.06, 51.65, 48.54, 36.63, 36.15, 34.46, 15.85, 15.78, 15.03.



9

Following **General Protocol 3** crude amine **1c** (21.0 mg crude, max. 0.028 mmol, 1.0 eq) was reacted with DIPEA (15 µL, 11.1 mg, 0.086 mmol, 3.1 eq) and cyclohexanemethyl isocyanate (10 µL, 9.7 mg, 0.070 mmol, 2.5 eq) in DCM (0.4 mL, 0.07 M). Flash chromatography on silica gel (gradient: 0 % to 5 % MeOH in DCM) afforded PMB-protected alcohol (21 mg, impure). 1.26-1.02 (m, 3H), 0.99 (d, *J* = 7.1 Hz, 3H), 0.95-0.79 (m, 2H). Following **General Protocol 5** impure PMB-protected alcohol (21 mg, max. 0.028 mmol, 1.0 eq) was reacted with DDQ (9.0 mg, 0.040 mmol, 1.4 eq) in DCM (0.3 mL, 0.09 M) and pH 7 phosphate buffer (5 drops). Flash chromatography (gradient: 0 % to 3 % MeOH in DCM) afforded final compound **9** (6.5 mg, 0.011 mmol, 40 % over 3 steps).

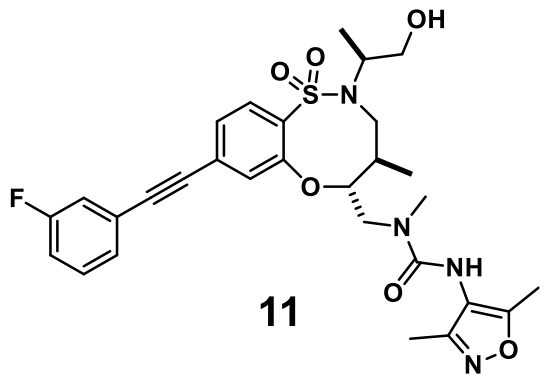
^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.87 (d, *J* = 8.1 Hz, 1H), 7.39-7.27 (m, 3H), 7.24-7.16 (m, 2H), 7.13-7.04 (m, 1H), 4.57 (t, *J* = 9.5 Hz, 1H), 4.48 (br s, 1H), 4.24 (dd, *J* = 14.5, 9.6 Hz, 1H), 3.99-3.77 (m, 2H), 3.66 (t, *J* = 10.8 Hz, 1H), 3.51 (dd, *J* = 12.3, 4.1 Hz, 1H), 3.42 (dd, *J* = 15.7, 5.0 Hz, 1H), 3.32 (dd, *J* = 13.1, 6.7 Hz, 1H), 3.20 (dd, *J* = 14.8, 2.8 Hz, 1H), 2.98 (dd, *J* = 13.1, 6.9 Hz, 1H), 2.73 (s, 3H), 2.55 (br s, 1H), 2.30-2.15 (m, 1H), 1.74-1.53 (m, 5H), 1.50-1.36 (m, 1H), 1.23 (d, *J* = 6.6 Hz, 3H), 1.19-1.00 (m, 3H), 0.94 (d, *J* = 6.6 Hz, 3H), 0.91-0.77 (m, 2H).



10

Following **General Protocol 3** crude amine **1c** (30 mg crude, max. 0.041 mmol, 1.0 eq) was reacted with DIPEA (10 µL, 7.4 mg, 0.057 mmol, 1.4 eq) and phenyl isocyanate (6.0 µL, 6.5 mg, 0.055 mmol, 1.3 eq) in DCM (0.2 mL, 0.21 M). Crude PMB-protected alcohol (29.5 mg) was identified by mass spectrometry and directly used without further purification. Following **General Protocol 5** crude PMB-protected alcohol (29.5 mg, max. 0.041 mmol, 1.0 eq) was reacted with DDQ (11.5 mg, 0.051 mmol, 1.2 eq) in DCM (0.3 mL, 0.14 M) and pH 5 NH_4Cl solution (3 drops). Flash chromatography (gradient: 0 % to 10 % MeOH in DCM) afforded final compound **10** (9.0 mg, 0.016 mmol, 39 % over 3 steps). **10** was purified by HPLC prior to biological testing.

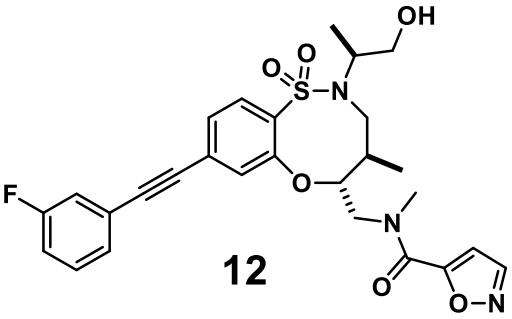
^{1}H NMR (300 MHz, $CDCl_3$, 27 °C) δ 7.89 (d, *J* = 8.2 Hz, 1H), 7.47-7.40 (m, 2H), 7.33 (dd, *J* = 8.3, 1.6 Hz, 1H), 7.26-7.15 (m, 4H), 7.06 (ddd, *J* = 8.5, 2.7, 1.1 Hz, 1H), 7.04-6.96 (m, 2H), 6.91 (ddd, *J* = 9.4, 2.7, 1.4 Hz, 1H), 6.44 (s, 1H), 4.64 (td, *J* = 9.3, 2.4 Hz, 1H), 4.45 (dd, *J* = 14.4, 10.1 Hz, 1H), 3.97-3.80 (m, 2H), 3.73-3.61 (m, 1H), 3.59-3.50 (m, 1H), 3.45 (dd, *J* = 15.8, 5.1 Hz, 1H), 3.18 (dd, *J* = 14.4, 2.5 Hz, 1H), 3.07 (d, *J* = 9.3 Hz, 1H), 2.85 (s, 3H), 2.37-2.16 (m, 1H), 1.24 (d, *J* = 6.9 Hz, 3H), 0.97 (d, *J* = 6.9 Hz, 3H). **^{13}C NMR** (75 MHz, $CDCl_3$, 27 °C) δ 155.89, 154.71, 138.93, 130.09, 129.98, 129.64, 129.11, 128.83, 127.96, 127.93, 127.61, 126.81, 123.56, 120.30, 118.91, 118.61, 116.57, 116.29, 88.03, 85.42, 64.90, 58.10, 51.79, 48.58, 36.70, 34.86, 15.78, 15.10.



11

Following **General Protocol 3** crude amine **1c** (20 mg, 0.035 mmol, 1.0 eq) was reacted with TEA (10 µL, 7.3 mg, 0.070 mmol, 2.0 eq) and 3,5- dimethylisoxazole-4-yl isocyanate (9 mg, 0.07 mmol, 2 eq) in DCM (0.35 mL, 0.10 M). Flash chromatography on silica gel (gradient: 0 % to 4 % MeOH in DCM) afforded PMB-protected alcohol (12.6 mg, 0.018 mmol, 51 % over 2 steps). Following **General Protocol 5** PMB-protected alcohol (12.6 mg, 0.018 mmol, 1.0 eq) was reacted with DDQ (6.3 mg, 0.027 mmol, 1.5 eq) in DCM (0.4 mL, 0.05 M) and pH 5 NH_4Cl solution (4 drops). Flash chromatography on silica gel (gradient: 0 % to 5 % MeOH in DCM) afforded final compound **11** (7.2 mg, 0.012 mmol, 69 %).

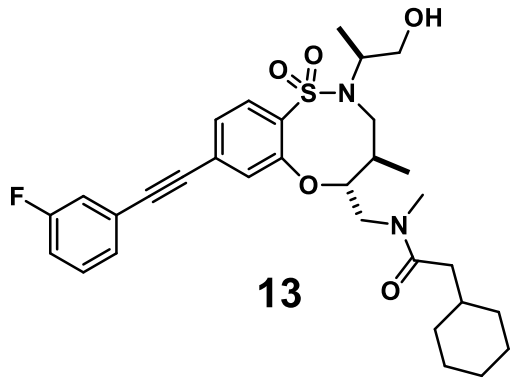
^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.92 (d, *J* = 8.2 Hz, 1H), 7.41-7.29 (m, 2H), 7.29-7.23 (m, 1H), 7.21 (d, *J* = 7.6 Hz, 1H), 7.15 (d, *J* = 9.1 Hz, 1H), 7.09 (td, *J* = 8.6, 2.7 Hz, 1H), 5.71 (s, 1H), 4.64 (td, *J* = 8.8, 3.0 Hz, 1H), 4.16-4.04 (m, 1H), 3.97-3.87 (m, 1H), 3.82 (dd, *J* = 15.7, 10.7 Hz, 1H), 3.74-3.63 (m, 1H), 3.60-3.47 (m, 2H), 3.41 (dd, *J* = 14.9, 2.8 Hz, 1H), 2.98 (s, 3H), 3.03-2.90 (m, 1H), 2.30 (s, 3H), 2.32-2.12 (m, 1H), 2.17 (s, 3H), 1.25 (d, *J* = 6.3 Hz, 3H), 0.98 (d, *J* = 6.7 Hz, 3H).



12

Following **General Protocol 3** crude amine **1c** (20.0 mg crude, max. 0.027 mmol, 1.0 eq) was reacted with DIPEA (30 µL, 22.3 mg, 0.17 mmol, 6.3 eq) and isoxazol-5-carbonyl chloride (7.5 µL, 10.2 mg, 0.078 mmol, 2.9 eq) in DCM (0.3 mL, 0.09 M). Flash chromatography on silica gel (gradient: 0 % to 5 % MeOH in DCM) afforded PMB-protected alcohol (12.0 mg, 0.018 mmol, 67 % over 2 steps). It was identified by mass spectrometry. Following **General Protocol 5** PMB-protected alcohol (12.0 mg, 0.018 mmol, 1.0 eq) was reacted with DDQ (9.8 mg, 0.043 mmol, 2.4 eq) in DCM (0.35 mL, 0.05 M) and pH 7 phosphate buffer (7 drops). Flash chromatography on silica gel (gradient: 0 % to 5 % MeOH in DCM) afforded final compound **12** (3.0 mg, 0.013 mmol, 31 %).

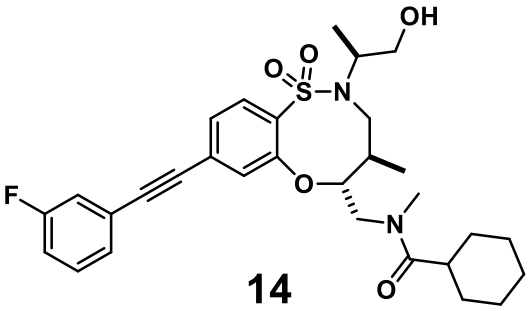
^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 8.19 (s, 1H), 7.89 (d, *J* = 8.1 Hz, 1H), 7.41-7.29 (m, 3H), 7.22-7.13 (m, 2H), 7.09 (td, *J* = 8.7, 3.3 Hz, 1H), 6.82 (s, 1H), 4.90-4.81 (m, 1H), 4.56 (dd, *J* = 14.0, 10.2 Hz, 1H), 3.99-3.81 (m, 2H), 3.66 (q, *J* = 11.9 Hz, 1H), 3.53 (dd, *J* = 12.9, 4.4 Hz, 1H), 3.45 (dd, *J* = 16.1, 5.2 Hz, 1H), 3.34 (d, *J* = 13.9 Hz, 1H), 3.12 (s, 3H), 2.95 (s, 1H), 2.39-2.18 (m, 1H), 1.24 (d, *J* = 6.7 Hz, 3H), 1.00 (d, *J* = 6.4 Hz, 3H).



13

Following **General Protocol 3** crude amine **1c** (25 mg, 0.044 mmol, 1.0 eq) was reacted with TEA (10 µL, 7.3 mg, 0.070 mmol, 1.7 eq) and cyclohexylacetyl chloride (10 µL, 10.5 mg, 0.065 mmol, 1.5 eq) in DCM (0.5 mL, 0.09 M). Flash chromatography on silica gel (gradient: 0 % to 5 % MeOH in DCM) afforded PMB-protected alcohol (14.5 mg, 0.021 mmol, 47 % over 2 steps). Following **General Protocol 5** PMB-protected alcohol (14.5 mg, 0.021 mmol, 1.0 eq) was reacted with DDQ (7.8 mg, 0.034 mmol, 1.6 eq) in DCM (0.2 mL, 0.10 M) and pH 7 phosphate buffer (5 drops). Flash chromatography on silica gel (gradient: 0 % to 5 % MeOH in DCM) afforded final compound **13** (4.6 mg, 0.012 mmol, 38 %).

^{1}H NMR (300 MHz, $CDCl_3$, 27 °C) δ 7.88 (d, *J* = 8.2 Hz, 1H), 7.39-7.27 (m, 3H), 7.20 (ddd, *J* = 9.4, 2.5, 1.3 Hz, 1H), 7.14-7.03 (m, 2H), 4.69 (td, *J* = 9.3, 2.7 Hz, 1H), 4.34 (dd, *J* = 14.1, 9.7 Hz, 1H), 3.96-3.79 (m, 2H), 3.66 (ddd, *J* = 12.1, 9.1, 2.9 Hz, 1H), 3.56-3.36 (m, 2H), 3.22 (dd, *J* = 14.0, 2.6 Hz, 1H), 3.13 (dd, *J* = 10.0, 3.1 Hz, 1H), 2.88 (s, 3H), 2.29-2.19 (m, 1H), 2.16 (dd, *J* = 9.1, 6.7 Hz, 2H), 1.95-1.82 (m, 1H), 1.82-1.52 (m, 4H), 1.23 (d, *J* = 6.7 Hz, 3H), 0.95 (d, *J* = 6.8 Hz, 3H), 1.36-0.78 (m, 6H). **^{13}C NMR** (75 MHz, $CDCl_3$, 27 °C) δ 173.44, 154.69, 134.66, 130.29, 130.17, 129.71, 128.55, 127.83, 127.66, 126.64, 118.84, 118.54, 116.70, 116.42, 91.55, 88.16, 85.08, 64.86, 58.09, 50.75, 48.51, 41.57, 36.84, 36.23, 34.59, 33.76, 33.70, 26.39, 26.31, 15.78, 15.08.

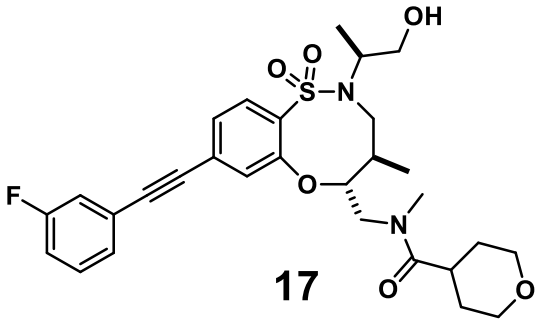


14

Following **General Protocol 3** crude amine **1c** (25.0 mg, 0.044 mmol, 1.0 eq) was reacted with TEA (10 µL, 7.3 mg, 0.071 mmol, 1.7 eq) and cyclohexylcarbonyl chloride (15 µL, 16.4 mg, 0.11 mmol, 2.5 eq) in DCM

(0.45 mL, 0.10 M). Flash chromatography on silica gel (gradient: 0 % to 4 % MeOH in DCM) afforded PMB-protected alcohol (11.5 mg, 0.017 mmol, 38 % over 2 steps). Following **General Protocol 5** PMB-protected alcohol (11.5 mg, 0.017 mmol, 1.0 eq) was reacted with DDQ (10.7 mg, 0.047 mmol, 2.8 eq) in DCM (0.2 mL, 0.09 M) and pH 7 phosphate buffer (4 drops). Flash chromatography on silica gel (gradient: 0 % to 5 % MeOH in DCM) afforded final compound **14** (6.9 mg, 0.012 mmol, 73 %).

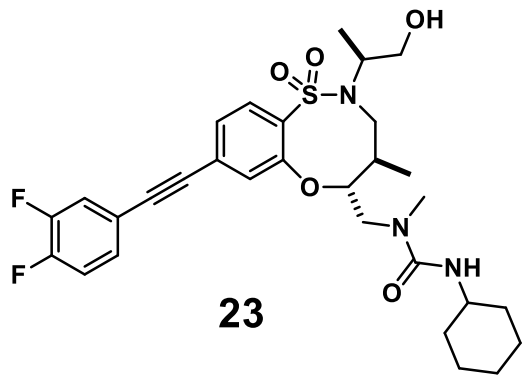
^{1}H NMR (300 MHz, $CDCl_3$, 27 °C) δ 7.88 (d, *J* = 8.2 Hz, 1H), 7.38-7.27 (m, 3H), 7.19 (ddd, *J* = 9.3, 2.6, 1.4 Hz, 1H), 7.15 (d, *J* = 1.6 Hz, 1H), 7.13- 7.04 (m, 1H), 4.70 (td, *J* = 9.5, 2.4 Hz, 1H), 4.38 (dd, *J* = 14.0, 9.8 Hz, 1H), 3.96-3.79 (m, 2H), 3.66 (ddd, *J* = 12.0, 9.3, 2.6 Hz, 1H), 3.51 (td, *J* = 11.4, 4.1 Hz, 1H), 3.41 (dd, *J* = 15.8, 5.0 Hz, 1H), 3.18 (dd, *J* = 14.0, 2.4 Hz, 1H), 3.11 (d, *J* = 10.0 Hz, 1H), 2.93 (s, 3H), 2.41 (tt, *J* = 11.5, 3.1 Hz, 1H), 2.28- 2.16 (m, 1H), 1.23 (d, *J* = 6.8 Hz, 3H), 1.90-1.09 (m, 10H), 0.94 (d, *J* = 6.8 Hz, 3H). **^{13}C NMR** (75 MHz, $CDCl_3$, 27 °C) δ 177.08, 164.20, 160.93, 154.72, 134.44, 130.28, 130.16, 129.66, 128.58, 127.81, 127.60, 126.87, 118.81, 118.49, 116.68, 116.39, 90.67, 88.08, 84.88, 64.87, 58.01, 51.16, 48.47, 41.42, 36.89, 35.85, 29.49, 29.30, 26.07, 25.97, 15.77, 15.19.



17

Following **General Protocol 3** crude amine **1c** (21.0 mg crude, max. 0.028 mmol, 1.0 eq) was reacted with DIPEA (30 µL, 22.3 mg, 0.17 mmol, 6.1 eq) and tetrahydro-2H-pyran-4-carbonyl chloride (16.5 mg, 0.11 mmol, 4.0 eq) in DCM (0.35 mL, 0.08 M). Flash chromatography on silica gel (gradient: 0 % to 5 % MeOH in DCM) afforded PMB-protected alcohol (17.0 mg, 0.025 mmol, 88 % over 2 steps). It was identified by mass spectrometry. Following **General Protocol 5** PMB-protected alcohol (17.0 mg, 0.025 mmol, 1.0 eq) was reacted with DDQ (12.4 mg, 0.055 mmol, 2.2 eq) in DCM (0.3 mL, 0.08 M) and pH 7 phosphate buffer (7 drops). Flash chromatography on silica gel (gradient: 0 % to 5 % MeOH in DCM) afforded final compound **17** (7.1 mg, 0.013 mmol, 51 %).

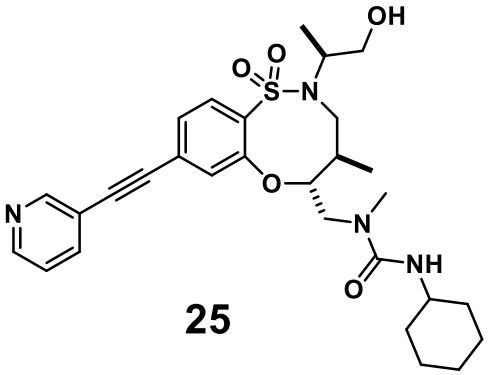
^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.89 (d, *J* = 8.2 Hz, 1H), 7.41-7.26 (m, 3H), 7.17 (d, *J* = 8.6 Hz, 1H), 7.15-7.04 (m, 2H), 4.74 (t, *J* = 10.1 Hz, 1H), 4.38 (dd, *J* = 13.9, 9.8 Hz, 1H), 4.12-3.80 (m, 4H), 3.66 (dd, *J* = 12.3, 9.0 Hz, 1H), 3.60-3.26 (m, 4H), 3.20 (d, *J* = 14.5 Hz, 1H), 2.95 (s, 3H), 2.67 (tt, *J* = 11.6, 3.8 Hz, 1H), 2.30-2.15 (m, 1H), 2.06 (qd, *J* = 12.2, 4.4 Hz, 1H), 1.91-1.67 (m, 3H), 1.66-1.50 (m, 1H), 1.23 (d, *J* = 6.6 Hz, 3H), 0.95 (d, *J* = 6.4 Hz, 3H).



23

Following **General Protocol 1** aryl bromide **1a** (23.0 mg, 0.035 mmol, 1.0 eq) was reacted with 1-ethynyl-3,4-difluorobenzene (10 µL, 11.4 mg, 0.083 mmol, 2.4 eq), DIPEA (100 µL, 74 mg, 0.57 mmol, 16 eq), $Pd(PPh_3)_2Cl_2$ (2.4 mg, 3.4 µmol, 0.10 eq), and CuI (0.9 mg, 4.7 µmol, 0.14 eq) in DMF (0.3 mL, 0.12 M) for 21 hours. Flash chromatography on silica gel (gradient: 0 % to 5 % MeOH in DCM) afforded PMB-protected alcohol (21 mg, 0.029 mmol, 84 %). Following **General Protocol 5** PMB-protected alcohol (21 mg, 0.029 mmol, 1.0 eq) was reacted with DDQ (10 mg, 0.044 mmol, 1.6 eq) in DCM (0.4 mL, 0.07 M) and pH 5 NH_4Cl solution (7 drops). Flash chromatography on silica gel (gradient: 0 % to 5 % MeOH in DCM) afforded final compound **23** (11 mg, 0.019 mmol, 66 %).

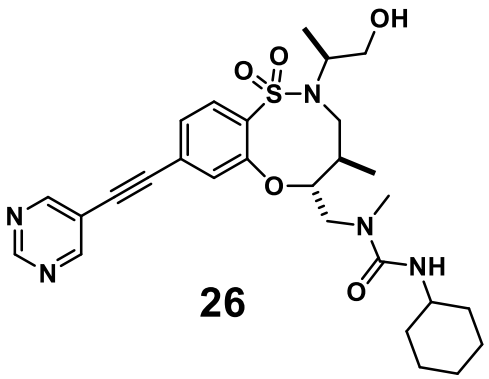
^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.88 (d, *J* = 8.3 Hz, 1H), 7.39-7.21 (m, 3H), 7.21-7.11 (m, 2H), 4.57 (td, *J* = 9.6, 2.6 Hz, 1H), 4.35-4.22 (m, 2H), 3.97-3.79 (m, 2H), 3.75-3.61 (m, 2H), 3.56-3.47 (m, 1H), 3.41 (dd, *J* = 15.8, 5.0 Hz, 1H), 3.20-3.06 (m, 2H), 2.67 (s, 3H), 2.30-2.16 (m, 1H), 2.10-1.92 (m, 2H), 1.71-1.47 (m, 3H), 1.23 (d, *J* = 6.7 Hz, 3H), 1.40-1.20 (m, 2H), 1.20-0.96 (m, 3H), 0.94 (d, *J* = 6.7 Hz, 3H).



25

Following **General Protocol 1** aryl bromide **1a** (30 mg, 0.046 mmol, 1.0 eq) was reacted with 3-ethynylpyridine (7.5 mg, 0.072 mmol, 1.6 eq), DIPEA (150 µL, 111 mg, 0.86 mmol, 19 eq), $Pd(PPh_3)_2Cl_2$ (1.7 mg, 2.4 µmol, 0.05 eq), and CuI (0.8 mg, 4.2 µmol, 0.09 eq) in DMF (0.5 mL, 0.09 M) for 24 hours. Flash chromatography on silica gel (gradient: 0 % to 5 % MeOH in DCM) afforded PMB-protected alcohol (21 mg, 0.031 mmol, 68 %). Following **General Protocol 5** PMB-protected alcohol (20 mg, 0.030 mmol, 1.0 eq) was reacted with DDQ (12.5 mg, 0.055 mmol, 1.8 eq) in DCM (0.3 mL, 0.10 M) and pH 5 NH_4Cl solution (8 drops). Flash chromatography on silica gel (gradient: 0 % to 5 % MeOH in DCM) afforded final compound **25** (9.5 mg, 0.017 mmol, 58 %). **25** was purified by HPLC prior to biological testing.

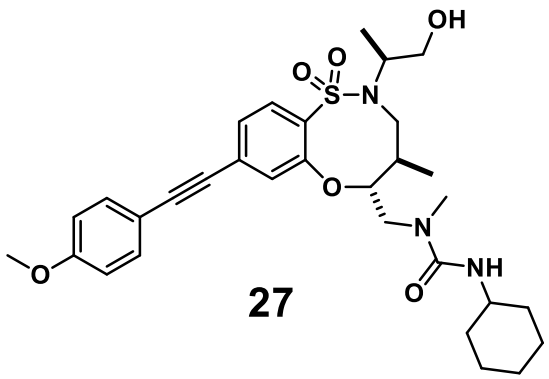
^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 8.88 (s, 1H), 8.68 (s, 1H), 8.11 (d, *J* = 7.8 Hz, 1H), 7.93 (d, *J* = 8.1 Hz, 1H), 7.62 (t, *J* = 6.7 Hz, 1H), 7.37 (d, *J* = 8.1 Hz, 1H), 7.21 (s, 1H), 4.61 (td, *J* = 9.5, 2.9 Hz, 1H), 4.43 (br s, 1H), 4.25 (dd, *J* = 14.5, 9.9 Hz, 1H), 3.97-3.77 (m, 2H), 3.75-3.60 (m, 2H), 3.53 (dd, *J* = 12.3, 4.1 Hz, 1H), 3.44 (dd, *J* = 15.8, 5.0 Hz, 1H), 3.17 (d, *J* = 14.9 Hz, 1H), 2.72 (s, 3H), 2.32-2.18 (m, 1H), 2.06-1.88 (m, 2H), 1.69-1.58 (m, 1H), 1.58-1.42 (m, 2H), 1.23 (d, *J* = 6.8 Hz, 3H), 1.40-0.96 (m, 5H), 0.95 (d, *J* = 6.6 Hz, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$, 25 °C) δ 158.09, 154.68, 148.47, 145.06, 142.59, 135.42, 129.90, 127.83, 127.22, 126.95, 93.03, 86.76, 85.33, 64.73, 58.05, 51.85, 50.05, 48.29, 36.71, 34.55, 34.26, 34.20, 25.54, 25.24, 25.08, 15.76, 14.99. **HRMS** (ESI) calc'd for $C_{29}H_{38}N_4O_5S$ $[M+H]^+$: 555.2636. Found: 555.2633.



26

Following **General Protocol 1** aryl bromide **1a** (30 mg, 0.046 mmol, 1.0 eq) was reacted with 5-ethynylpyrimidine (7.5 mg, 0.072 mmol, 1.6 eq), DIPEA (160 µL, 119 mg, 0.92 mmol, 20 eq), $Pd(PPh_3)_2Cl_2$ (1.8 mg, 2.6 µmol, 0.06 eq), and CuI (2 mg, 10.5 µmol, 0.23 eq) in DMF (0.45 mL, 0.10 M) for 4 days. Flash chromatography on silica gel (gradient: 0 % to 10 % MeOH in DCM) afforded PMB-protected alcohol (18.2 mg, 0.027 mmol, 59 %). Following **General Protocol 5** PMB-protected alcohol (18 mg, 0.027 mmol, 1.0 eq) was reacted with DDQ (9.3 mg, 0.041 mmol, 1.5 eq) in DCM (0.3 mL, 0.09 M) and pH 5 NH_4Cl solution (10 drops). Flash chromatography on silica gel (gradient: 0 % to 5 % MeOH in DCM) afforded final compound **26** (12.2 mg, 0.022 mmol, 82 %). **26** was purified by HPLC prior to biological testing.

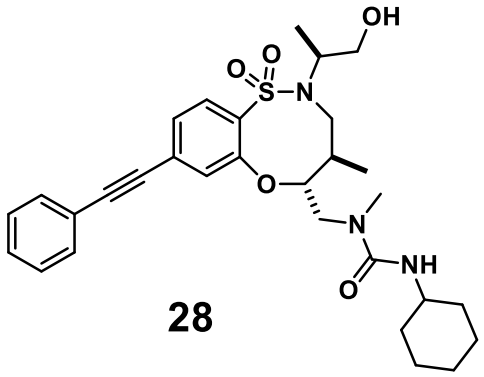
^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 9.18 (s, 1H), 8.83 (s, 2H), 7.91 (d, *J* = 8.1 Hz, 1H), 7.36 (d, *J* = 7.8 Hz, 1H), 7.23 (s, 1H), 4.58 (dt, *J* = 9.4, 4.8 Hz, 1H), 4.34-4.20 (m, 2H), 3.96-3.78 (m, 2H), 3.76-3.61 (m, 2H), 3.57-3.47 (m, 1H), 3.42 (dd, *J* = 15.7, 5.0 Hz, 1H), 3.19-3.05 (m, 2H), 2.68 (s, 3H), 2.31-2.16 (m, 1H), 2.08-1.90 (m, 2H), 1.71-1-46 (m, 3H), 1.23 (d, *J* = 6.7 Hz, 3H), 1.40-1.19 (m, 2H), 1.18-0.97 (m, 3H), 0.94 (d, *J* = 6.6 Hz, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$, 25 °C) δ 158.87, 157.64, 157.41, 154.76, 135.30, 129.76, 127.74, 127.35, 127.06, 119.19, 94.26, 85.53, 85.27, 64.77, 58.07, 51.59, 49.78, 48.29, 36.70, 34.43, 34.40, 34.32, 25.69, 25.32, 25.16, 15.78, 15.03.



27

Following **General Protocol 1** aryl bromide **1a** (21.4 mg, 0.033 mmol, 1.0 eq) was reacted with 4-ethynylanisole (6.9 µL, 7.0 mg, 0.053 mmol, 1.6 eq), DIPEA (150 µL, 111 mg, 0.86 mmol, 26 eq), $Pd(PPh_3)_2Cl_2$ (1.0 mg, 1.4 µmol, 0.05 eq), and CuI (1.0 mg, 5.3 µmol, 0.19 eq) in DMF (1 mL, 0.033 M) for 24 hours. Flash chromatography on silica gel (gradient: 0 % to 10 % MeOH in DCM) afforded PMB-protected alcohol (17.5 mg, 0.025 mmol, 76 %). Following **General Protocol 5** PMB-protected alcohol (7.5 mg, 0.011 mmol, 1.0 eq) was reacted with DDQ (6 mg, 0.026 mmol, 2.4 eq) in DCM (0.15 mL, 0.07 M) and pH 7 phosphate buffer (4 drops). Flash chromatography on silica gel (gradient: 0 % to 5 % MeOH in DCM) afforded final compound **27** (4 mg, 0.007 mmol, 65 %). **27** was purified by HPLC prior to biological testing.

^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.85 (d, *J* = 8.5 Hz, 1H), 7.43 (dt, *J* = 8.8, 2.4 Hz, 2H), 7.30 (dd, *J* = 8.3, 1.6 Hz, 1H), 7.15 (d, *J* = 1.6 Hz, 1H), 6.88 (td, *J* = 9.5, 2.6 Hz, 2H), 4.56 (td, *J* = 9.5, 2.6 Hz, 1H), 4.33-4.23 (m, 2H), 3.84 (s, 3H), 3.96-3.78 (m, 2H), 3.74-3.61 (m, 2H), 3.55-3.46 (m, 1H), 3.40 (dd, *J* = 15.8, 5.1 Hz, 1H), 3.20-3.10 (m, 2H), 2.67 (s, 3H), 2.29-2.16 (m, 1H), 2.12-2.04 (m, 1H), 2.00-1.92 (m, 1H), 1.69-1.61 (m, 1H), 1.60- 1.49 (m, 2H), 1.23 (d, *J* = 6.8 Hz, 3H), 1.39-1.20 (m, 2H), 1.20-0.97 (m, 3H), 0.94 (d, *J* = 6.8 Hz, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$, 25 °C) δ 160.32, 157.72, 154.63, 133.65, 133.43, 129.62, 129.44, 127.54, 126.48, 114.51, 114.23, 93.16, 86.51, 85.39, 64.79, 57.92, 55.51, 51.44, 49.86, 48.23, 36.69, 34.43, 34.28, 29.86, 25.72, 25.32, 25.25, 15.80, 15.01.

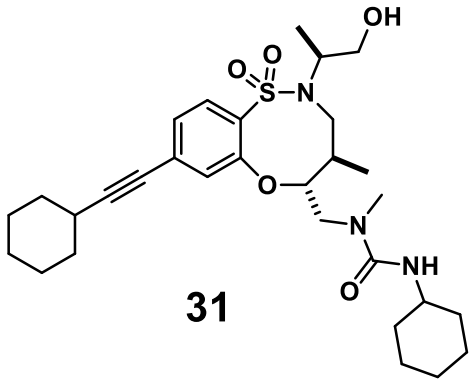


28

Following **General Protocol 1** aryl bromide **1a** (17.5 mg, 0.027 mmol, 1.0 eq) was reacted with phenylacetylene (6.0 µL, 5.6 mg, 0.055 mmol, 2.0 eq), DIPEA (150 µL, 111 mg, 0.86 mmol, 32 eq), $Pd(PPh_3)_2Cl_2$ (1 mg, 1.4 µmol, 0.05 eq), and CuI (1 mg, 5.3 µmol, 0.19 eq) in DMF (1 mL, 0.027 M) for 35 hours. Flash chromatography on silica gel (gradient: 30 % to 80 % EtOAc in hexanes) afforded PMB-

protected alcohol (20 mg, impure). A second column (gradient: 0 % to 5 % MeOH in DCM) provided pure **79** (14 mg, 0.021 mmol, 78 %). Following **General Protocol 5** PMB-protected alcohol (14 mg, 0.021 mmol, 1.0 eq) was reacted with DDQ (7.1 mg, 0.031 mmol, 1.5 eq) in DCM (0.4 mL, 0.05 M) and pH 5 NH_4Cl solution (6 drops). Flash chromatography on silica gel (gradient: 0 % to 5 % MeOH in DCM) afforded final compound **28** (4.0 mg, 0.007 mmol, 35 %). **28** was purified by HPLC prior to biological testing.

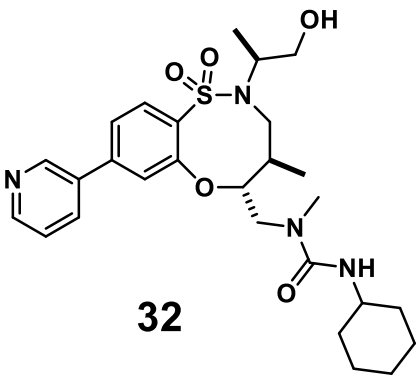
^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.87 (d, *J* = 8.0 Hz, 1H), 7.53-7.46 (m, 2H), 7.40-7.30 (m, 4H), 7.19 (s, 1H), 4.57 (td, *J* = 9.4, 2.9 Hz, 1H), 4.34-4.23 (m, 2H), 3.97-3.79 (m, 2H), 3.74-3.61 (m, 2H), 3.57-3.47 (m, 1H), 3.41 (dd, *J* = 15.9 Hz, 4.9 Hz, 1H), 3.18-3.09 (m, 2H), 2.67 (s, 3H), 2.30-2.16 (m, 1H), 2.13-2.02 (m, 1H), 2.01-1.90 (m, 1H), 1.68-1.45 (m, 3H), 1.23 (d, *J* = 6.6 Hz, 3H), 1.39-0.96 (m, 5H), 0.94 (d, *J* = 6.5 Hz, 3H).



31

Following **General Protocol 1** aryl bromide **1a** (24.9 mg, 0.038 mmol, 1.0 eq) was reacted with cyclohexylacetylene (10 µL, 8.3 mg, 0.076 mmol, 2.0 eq), DIPEA (130 µL, 96 mg, 0.75 mmol, 20 eq), $Pd(PPh_3)_2Cl_2$ (2.0 mg, 2.8 µmol, 0.07 eq), and CuI (1 mg, 5.3 µmol, 0.14 eq) in DMF (0.25 mL, 0.15 M) for 12 hours. Flash chromatography on silica gel (gradient: 0 % to 10 % MeOH in DCM) afforded PMB-protected alcohol (21 mg, 0.031 mmol, 81 %). Following **General Protocol 5** PMB-protected alcohol (21 mg, 0.031 mmol, 1.0 eq) was reacted with DDQ (10.1 mg, 0.044 mmol, 1.4 eq) in DCM (0.3 mL, 0.10 M) and pH 5 NH_4Cl solution (8 drops). Flash chromatography on silica gel (gradient: 0 % to 100 % EtOAc in hexanes) afforded final compound **31** (10.3 mg, 0.018 mmol, 60 %). **31** was purified by HPLC prior to biological testing.

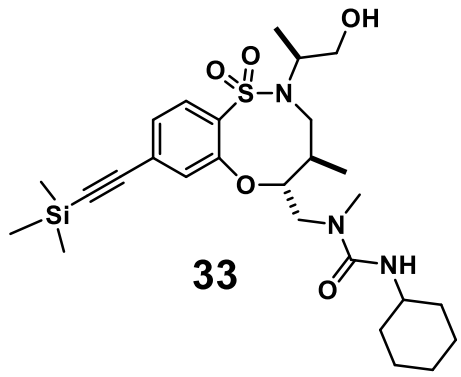
^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.79 (d, *J* = 8.2 Hz, 1H), 7.19 (dd, *J* = 8.3, 1.6 Hz, 1H), 7.02 (d, *J* = 1.6 Hz, 1H), 4.53 (td, *J* = 9.4, 2.7 Hz, 1H), 4.29 (d, *J* = 7.5 Hz, 1H), 4.20 (dd, *J* = 14.5, 9.8 Hz, 1H), 3.93-3.85 (m, 1H), 3.81 (dd, *J* = 15.8, 11.3 Hz, 1H), 3.74-3.60 (m, 2H), 3.49 (dd, *J* = 11.8, 4.2 Hz, 1H), 3.39 (dd, *J* = 15.8, 5.0 Hz, 1H), 3.18 (br s, 1H), 3.15 (dd, *J* = 14.5, 2.8 Hz, 1H), 2.68 (s, 3H), 2.58-2.49 (m, 1H), 2.26-2.14 (m, 1H), 2.14-2.05 (m, 1H), 2.02-1.93 (m, 1H), 1.91-1.81 (m, 2H), 1.79-1.29 (m, 12H), 1.21 (d, *J* = 6.8 Hz, 3H), 1.29-1.03 (m, 4H), 0.92 (d, *J* = 6.8 Hz, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$, 25 °C) δ 157.74, 154.53, 133.26, 130.27, 129.26, 127.93, 126.52, 98.58, 85.43, 79.19, 64.77, 57.86, 51.52, 49.84, 48.16, 36.70, 34.53, 34.28, 32.59, 29.96, 25.94, 25.84, 25.42, 25.30, 25.08, 15.78, 15.02.



32

Aryl bromide **1a** (25 mg, 0.038 mmol, 1.0 eq) was dissolved in DMF/EtOH (1:1, 0.5 mL, 0.08 M) under N_2 atmosphere. 3-Pyridinylboronic acid (9.5 mg, 0.077 mmol, 2.0 eq), TEA (100 µL, 73 mg, 0.72 mmol, 19 eq), and $Pd(PPh_3)_2Cl_2$ (3.0 mg, 4.3 µmol, 0.11 eq) were added before degassing the mixture for 15 minutes with a stream of N_2. The reaction was heated to 50 °C and stirred for 12 hours. The reaction was cooled to room temperature, quenched with saturated aqueous sodium bicarbonate solution, and extracted four times with EtOAc. Combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and the solvent was evaporated. Flash chromatography on silica gel (gradient: 0 % to 5 % MeOH in DCM) afforded PMB-protected alcohol (18.6 mg, 0.029 mmol, 75 %). Following **General Protocol 5** PMB-protected alcohol (18.6 mg, 0.029 mmol, 1.0 eq) was reacted with DDQ (10.2 mg, 0.045 mmol, 1.5 eq) in DCM (0.5 mL, 0.06 M) and pH 5 NH_4Cl solution (0.1 mL). Flash chromatography on silica gel (gradient: 0 % to 5 % MeOH in DCM) afforded final compound **32** (7 mg, 0.013 mmol, 46 %). **32** was purified by HPLC prior to biological testing.

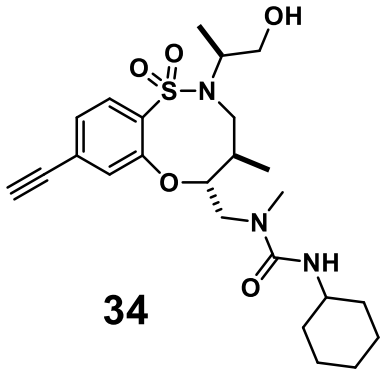
^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 8.82 (d, *J* = 2.3 Hz, 1H), 8.63 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.00 (d, *J* = 8.3 Hz, 1H), 7.89 (dt, *J* = 8.0, 2.0 Hz, 1H), 7.41 (dd, *J* = 8.2, 1.8 Hz, 1H), 7.38 (ddd, *J* = 7.9, 4.9, 0.9 Hz, 1H), 7.30 (d, *J* = 1.8 Hz, 1H), 4.65 (td, *J* = 9.6, 2.5 Hz, 1H), 4.21-4.10 (m, 2H), 4.01-3.82 (m, 2H), 3.68 (t, *J* = 10.8 Hz, 1H), 3.58-3.46 (m, 2H), 3.42 (dd, *J* = 15.8, 5.1 Hz, 1H), 3.31 (d, *J* = 10.0 Hz, 1H), 3.20 (dd, *J* = 14.6, 2.6 Hz, 1H), 2.69 (s, 3H), 2.32-2.18 (m, 1H), 1.89-1.80 (m, 1H), 1.64-1.42 (m, 3H), 1.25 (d, *J* = 6.6 Hz, 3H), 1.35-1.12 (m, 3H), 1.04 (tt, *J* = 12.4, 3.8 Hz, 1H), 0.96 (d, *J* = 6.9 Hz, 3H), 0.96-0.70 (m, 2H). **^{13}C NMR** (100 MHz, $CDCl_3$, 25 °C) δ 157.72, 155.16, 149.77, 148.36, 143.69, 134.80, 134.62, 133.72, 130.24, 123.81, 123.16, 122.73, 85.33, 64.79, 57.90, 51.99, 49.74, 48.04, 36.80, 34.51, 34.15, 33.92, 25.67, 25.21, 25.06, 15.82, 15.05.



33

Following **General Protocol 1** aryl bromide **1a** (39 mg, 0.060 mmol, 1.0 eq) was reacted with trimethylsilylacetylene (20 µL, 13.8 mg, 0.14 mmol, 2.3 eq), DIPEA (200 µL, 148 mg, 1.1 mmol, 19 eq), $Pd(PPh_3)_2Cl_2$ (4.0 mg, 5.7 µmol, 0.09 eq), and CuI (1.4 mg, 7.4 µmol, 0.12 eq) in DMF (0.6 mL, 0.10 M) for 21 hours. Flash chromatography on silica gel (gradient: 0 % to 5 % MeOH in DCM) afforded PMB-protected alcohol **33a** (39 mg, 0.058 mmol, 98 %). Following **General Protocol 5** PMB-protected alcohol (15 mg, 0.022 mmol, 1.0 eq) was reacted with DDQ (8 mg, 0.035 mmol, 1.6 eq) in DCM (0.3 mL, 0.07 M) and pH 5 NH_4Cl solution (7 drops). Flash chromatography on silica gel (gradient: 0 % to 100 % EtOAc in hexanes) afforded final compound **33** (9.5 mg, 0.021 mmol, 77 %).

^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.82 (d, *J* = 8.2 Hz, 1H), 7.32-7.22 (m, 1H), 7.08 (s, 1H), 4.52 (td, *J* = 9.0, 2.8 Hz, 1H), 4.30 (d, *J* = 7.6 Hz, 1H), 4.18 (dd, *J* = 14.5, 9.6 Hz, 1H), 3.95-3.75 (m, 2H), 3.75-3.59 (m, 2H), 3.56-3.45 (m, 1H), 3.40 (dd, *J* = 15.8, 5.0 Hz, 1H), 3.18 (dd, *J* = 14.3, 2.8 Hz, 1H), 3.09 (d, *J* = 8.5 Hz, 1H), 2.69 (s, 3H), 2.29-2.14 (m, 1H), 2.11- 1.90 (m, 2H), 1.79-1.57 (m, 3H), 1.21 (d, *J* = 6.7 Hz, 3H), 1.47-1.03 (m, 5H), 0.93 (d, *J* = 6.7 Hz, 3H), 0.23 (s, 9H).

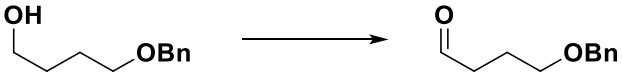


34

TMS-acetylene **33a** (25 mg, 0.037 mmol, 1.0 eq) was dissolved in THF (0.2 mL, 0.19 M) under N_2 atmosphere. 1 M TBAF solution in THF (40 µL, 0.040 mmol, 1.1 eq) was added at room temperature. After 30 min complete conversion was observed by LCMS. The reaction was quenched with sat. NH_4Cl solution and extracted with EtOAc three times. Combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and the solvent was evaporated. Terminal alkyne (20 mg, 0.033 mmol, 90 %) was isolated. Following **General Protocol 5** PMB-protected alcohol (20 mg, 0.033 mmol, 1.0 eq) was reacted with DDQ (11.5 mg, 0.051 mmol, 1.5 eq) in DCM (0.4 mL, 0.07 M) and pH 5 NH_4Cl solution (5 drops). Flash chromatography on silica gel (gradient: 0 % to 5 % MeOH in DCM) afforded final compound **34** (8.8 mg, 0.018 mmol, 55 %).

^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.85 (d, *J* = 8.2 Hz, 1H), 7.33-7.26 (m, 1H), 7.17 (s, 1H), 4.53 (dt, *J* = 9.4, 5.9 Hz, 1H), 4.33-4.16 (m, 2H), 3.96-3.77 (m, 2H), 3.75-3.60 (m, 2H), 3.56-3.46 (m, 1H), 3.41 (dd, *J* = 15.8, 5.0 Hz, 1H), 3.23-3.05 (m, 3H), 2.68 (s, 3H), 2.29-2.15 (m, 1H), 2.09 (d, *J* = 12.2 Hz, 1H), 1.97 (d, *J* = 10.9 Hz, 1H), 1.77-1.56 (m, 3H), 1.46-1.30 (m, 2H), 1.22 (d, *J* = 6.7 Hz, 3H), 1.28.1.04 (m, 3H), 0.93 (d, *J* = 6.7 Hz, 3H).

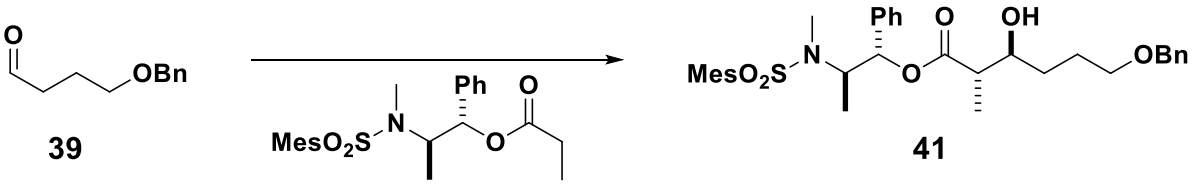
D. Synthesis of 36 (Scheme S1)



38 **39**

Oxalyl chloride (1.4 mL, 2.1 g, 16.3 mmol, 1.5 eq) was dissolved in DCM (24 mL) under nitrogen atmosphere and the solution was cooled to -78 °C. DMSO (2.4 mL, 2.6 g, 33.8 mmol, 3.0 eq) was added dropwise *via* syringe. The mixture was stirred for 30 minutes before addition of 1,4-butanediol benzyl ether **38** (2.0 mL, 2.0 g, 11.1 mmol, 1.0 eq) in DCM (20 mL, final concentration 0.25 M). Over the course of 1 hour TEA (7.0 mL, 5.1 g, 50 mmol, 4.5 eq) was added. The mixture was allowed to stir at -78 °C for 3 hours. The reaction was quenched at -78 °C with sat. $NaHCO_3$ solution and allowed to warm to room temperature. The layers were separated and the aqueous phase was extracted with DCM three times. The combined organic extracts were washed with water, dried over $MgSO_4$, filtered, and the solvent was evaporated. Flash chromatography on silica gel (gradient: 0 % to 40 % EtOAc in hexanes) afforded aldehyde **39** (1.42 g, 8.0 mmol, 72 %).

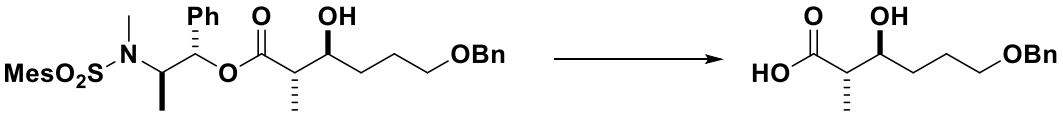
^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 9.79 (s, 1H), 7.39-7.27 (m, 5H), 4.49 (s, 2H), 3.51 (t, *J* = 6.1 Hz, 2H), 2.55 (t, *J* = 7.1 Hz, 2H), 1.95 (quin, *J* = 6.6 Hz, 2H).



39 **40** **41**

Ester **40** (3.25 g, 8.1 mmol, 1.0 eq) was dissolved in DCM (60 mL, 0.14 M) under nitrogen atmosphere and cooled to -78 °C before addition of TEA (2.7 mL, 1.96 g, 19.3 mmol, 2.4 eq). Dicyclohexylboron triflate (5.0 g, 15.3 mmol, 1.9 eq) in DCM (10 mL) was added dropwise over 30 minutes. After 2 hours or stirring aldehyde **39** (1.42 g, 8.0 mmol, 1.0 eq) was added slowly. The reaction was stirred for 2 hours at -78 °C for and then allowed to warm to room temperature. LC-MS showed complete consumption of aldehyde **39** while there was still ester **40** in the solution. The reaction was quenched with MeOH (35 mL) and pH 7 buffer (5 mL). H_2O_2 (35 % in H_2O, 4 mL) was added and the mixture stirred for 15 minutes. Volatiles were removed and the resulting slurry partitioned between DCM and water. Phases were separated and the aqueous phase extracted with DCM two more times. The combined organic extracts were dried over $MgSO_4$, filtered, and the solvent was evaporated. 1H NMR of the crude product showed formation of only one stereoisomer. Purification by flash chromatography on silica gel (gradient: 0 % to 5 % MeOH in DCM) afforded aldol product **41** (2.61 g, 4.5 mmol, 56 %). 1.15 g of ester **40** (2.9 mmol, 35 %) were recovered. The aldehyde **39** had partly decomposed and limited the reaction.

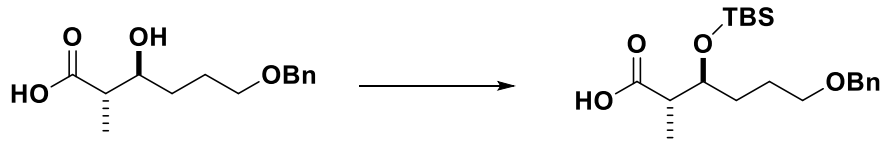
^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.37-7.27 (m, 5H), 7.25-7.13 (m, 3H), 7.04-6.99 (m, 2H), 6.87 (s, 2H), 5.76 (d, *J* = 5.4 Hz, 1H), 4.50 (s, 2H), 4.01 (quin, 6.5 Hz, 1H), 3.70 (m, 1H), 3.50 (t, *J* = 5.8 Hz, 2H), 3.03 (d, *J* = 5.6 Hz, 1H), 2.77 (s, 3H), 2.57 (quin, 7.2 Hz, 1H), 2.42 (s, 6H), 2.29 (s, 3H), 1.85-1.57 (m, 3H), 1.52-1.38 (m, 1H), 1.29 (d, *J* = 6.8 Hz, 3H), 1.13 (d, J = 7.1 Hz, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$, 25 °C) δ 174.73, 142.49, 140.55, 138.31, 138.17, 132.44, 132.07, 128.54, 128.11, 127.83, 127.79, 126.18, 78.05, 73.17, 73.07, 70.37, 55.71, 46.01, 31.66, 28.45, 26.10, 22.79, 21.06, 14.09, 12.36.



41 **42**

Aldol product **41** (2.53 g, 4.3 mmol, 1.0 eq) was dissolved in a 1:1 mixture of MeOH and *t*-BuOH (30 mL, 0.14 M). After cooling to 0 °C H_2O_2 (35 % in H_2O, 3 mL, 30 mmol, 7 eq) and 1 M NaOH (13 mL, 13 mmol, 3 eq) were added slowly over 30 minutes each. The reaction was allowed to warm to room temperature. The volatiles were removed under reduced pressure and the resulting slurry partitioned between water and EtOAc. The aqueous phase was extracted with EtOAc three times. The aqueous phase was acidified with 6 M HCl and extracted with EtOAc. The extracts of the acidic aqueous phase were combined, dried over $MgSO_4$, filtered, and the solvent was evaporated to afford carboxylic acid **42** (0.90 g, 3.6 mmol, 83 %).

^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.38-7.28 (m, 5H), 4.54 (s, 2H), 3.71 (ddd, *J* = 9.1, 6.4, 2.2 Hz, 1H), 3.59-3.49 (m, 2H), 2.53 (quin, *J* = 7.0 Hz, 1H), 1.84-1.74 (m, 3H), 1.61-1.48 (m, 1H), 1.24 (d, *J* = 7.2 Hz, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$, 25 °C) δ 178.93, 137.68, 128.67, 128.07, 128.00, 73.41, 73.08, 70.47, 45.34, 32.72, 26.29, 14.35.

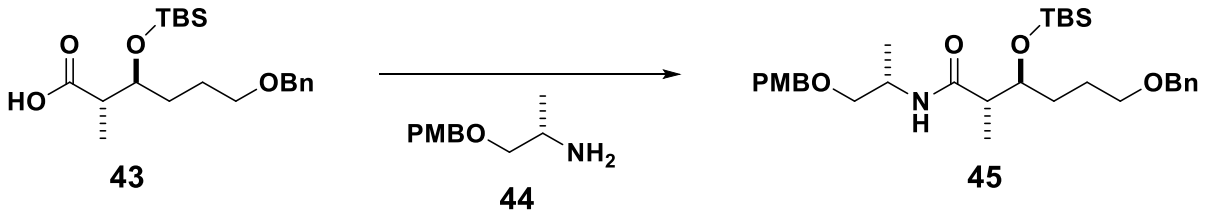


42 **43**

Carboxylic acid **42** (0.90 g, 3.6 mmol, 1.0 eq) was dissolved in DCM (40 mL, 0.09 M) under nitrogen atmosphere. After cooling to 0 °C and addition of 2,6-lutidine (1.9 mL, 1.76 g, 16.4 mmol, 4.6 eq) TBSOTf (2.3 mL, 2.6 g, 10.0 mmol, 2.8 eq) was added dropwise. The reaction was allowed to warm to room temperature and stirred for 1 hour. The reaction was quenched with sat. $NaHCO_3$ solution and the layers separated. The aqueous phase was extracted with Et_2O twice. The combined organic extracts were washed with sat. NH_4Cl solution and brine, dried over $MgSO_4$, filtered, and the solvent was evaporated. The residue was redissolved in a 1:1 mixture of MeOH and THF (40 mL, 0.09 M). An aqueous solution of K_2CO_3 (1.6 M, 5 mL, 8.0 mmol, 2.2 eq) was added and the mixture stirred at 0 °C for one hour. Volatiles were removed under reduced pressure, water added, and extracted with EtOAc three times. The combined organic extracts were washed with 1 M HCl, dried over $MgSO_4$, filtered, and the solvent was

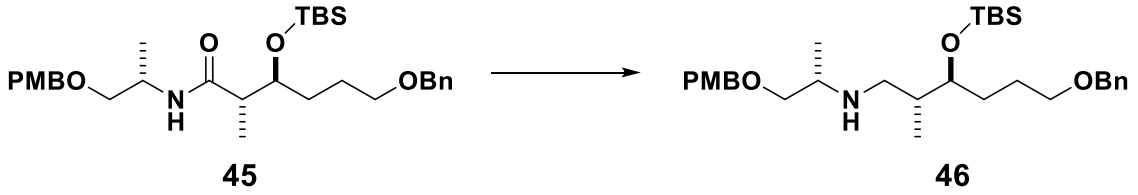
evaporated. The resulting oil was coevaporated with toluene twice yielding carboxylic acid **43** (1.3 g, 3.5 mmol, 98 %).

^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.37-7.27 (m, 5H), 4.49 (s, 2H), 3.90 (q, *J* = 4.5 Hz, 1H), 3.53-3.38 (m, 2H), 2.68 (qd, *J* = 7.3, 4.3 Hz, 1H), 1.75- 1.52 (m, 4H), 1.21 (d, *J* = 7.2 Hz, 3H), 0.91 (s, 9H), 0.11 (s, 3H), 0.10 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$, 25 °C) δ 176.93, 138.60, 128.51, 127.75, 127.70, 74.28, 73.04, 70.20, 44.55, 31.67, 25.90, 25.30, 18.12, 14.29, -4.18, -4.76.

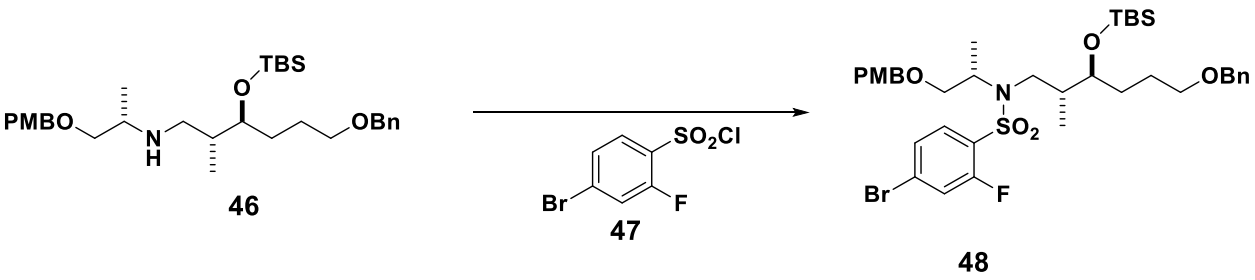


Following a procedure analogous to **General Protocol 4,** carboxylic acid **43** (1.30 g, 3.55 mmol, 1.0 eq) was reacted with PyBOP (1.86 g, 3.57 mmol, 1.0 eq), DIPEA (1.9 mL, 1.4 g, 10.9 mmol, 3.1 eq), and PMB-protected (*S*)-alaninol **44** (0.82 g, 4.2 mmol, 1.2 eq) in DCM (20 mL, 0.18 M). The crude linear amide **45** (2.23 g, impure) was identified by mass spectrometry and used without further purification. A sample was purified by flash chromatography (gradient: 0 % to 5 % MeOH in DCM) for characterization.

^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.37-7.27 (m, 5H), 7.25-7.19 (m, 2H), 6.90-6.84 (m, 2H), 6.65 (d, *J* = 8.5 Hz, 1H), 4.51-4.36 (m, 4H), 4.23-4.13 (m, 1H), 3.83-3.72 (m, 4H), 3.52-3.38 (m, 2H), 3.36 (d, *J* = 4.4 Hz, 2H), 2.42 (qd, J = 7.2, 3.7 Hz, 1H), 1.74-1.53 (m, 4H), 1.20-1.13 (m, 6H), 0.90 (s, 9H), 0.08 (s, 3H), 0.06 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$, 25 °C) δ 174.30, 159.30, 138.74, 130.53, 129.34, 128.45, 127.69, 127.59, 113.88, 74.41, 72.93, 72.88, 72.81, 70.32, 55.42, 46.11, 44.36, 32.25, 26.02, 25.67, 18.14, 18.09, 16.36, -4.22, -4.66.

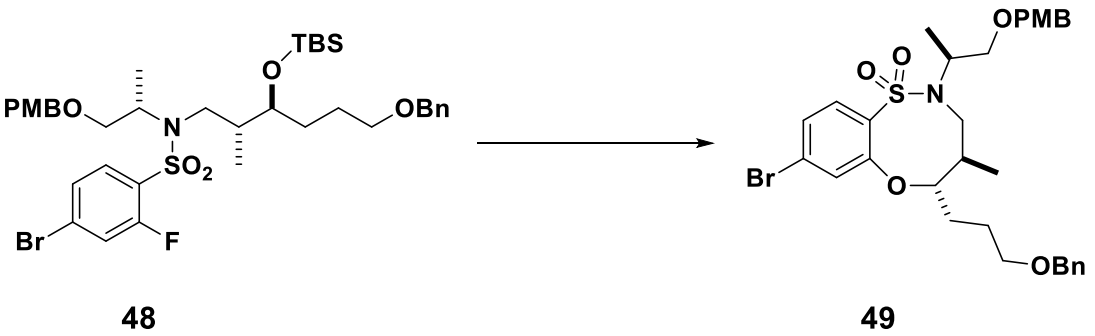


To a solution of amide **45** (2.17 g crude, max. 3.44 mmol, 1.0 eq) in anhydrous THF (35mL, 0.10 M) under nitrogen atmosphere was added BH_3·DMS (1.7 mL, 2.7 g, 17.9 mmol, 5.2 eq) via syringe. The mixture was stirred at 50 °C for 7 hours. The mixture was allowed to cool to room temperature and quenched with MeOH (20 mL), then the solvent was removed under reduced pressure. The residue was redissoved in methanol and Rochelle salt solution (0.5 M, 30 mL) was added. The resulting mixture was stirred at reflux for 13 hours. Volatile were evaporated, and the remaining aqueous phase was extracted 3x with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, filtered, and the solvent was evaporated, yielding secondary amine **46** (1.35 g, impure). The product was identified by mass spectrometry and used without further purification.



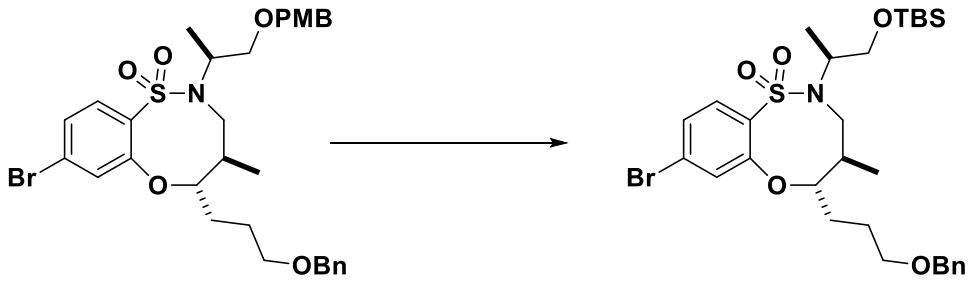
Following a procedure analogous to **General Protocol 3**, crude amine **46** (1.35 g, 2.55 mmol, 1.0 eq) was reacted with TEA (0.56 mL, 0.41 g, 4.0 mmol, 1.6 eq) and 4-bromo-2- fluorobenzene sulfonyl chloride **47** (0.88 g, 3.2 mmol, 1.3 eq) in DCM (25 mL, 0.10 M). Flash chromatography (gradient: 0 % to 30 % EtOAc in hexanes) afforded sulfonamide **48** (0.8 g, 1.0 mmol, 29 % over 3 steps).

^{1}H NMR (300 MHz, $CDCl_3$, 27 °C) δ 7.71 (dd, *J* = 8.4, 7.4 Hz, 1H), 7.37- 7.23 (m, 6H), 7.06-6.97 (m, 3H), 6.90-6.80 (m, 2H), 4.51 (s, 2H), 4.23-4.11 (m, 2H), 3.95-3.85 (m, 1H), 3.82 (s, 3H), 3.59-3.37 (m, 4H), 3.36-3.29 (m, 2H), 3.25 (dd, *J* = 10.1, 5.0 Hz, 1H), 2.00-1.84 (m, 1H), 1.72-1.59 (m, 2H), 1.59-1.41 (m, 2H), 1.18 (d, *J* = 7.0 Hz, 3H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.90 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H). **^{13}C NMR** (75 MHz, $CDCl_3$, 27 °C) δ 159.47, 138.84, 131.99, 129.85, 129.48, 128.49, 127.74, 127.64, 127.34, 127.28, 127.23, 120.78, 120.45, 113.94, 75.22, 73.04, 72.76, 70.78, 70.73, 55.43, 53.83, 47.63, 47.58, 38.29, 30.45, 26.11, 26.06, 25.44, 18.27, 17.94, 13.88, -4.10, -4.36.



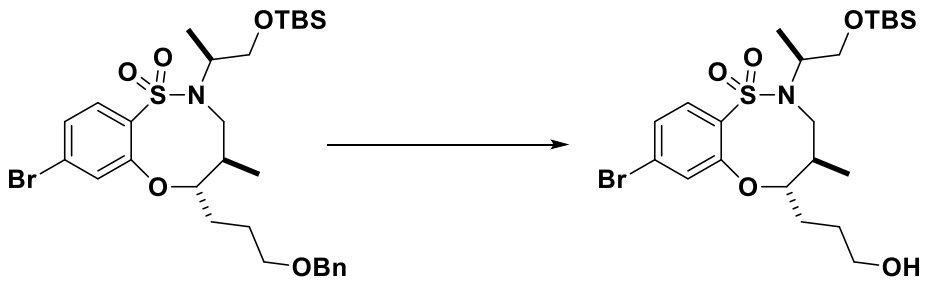
Linear sulfonamide **48** (0.68 g, 0.89 mmol, 1.0 eq) and CsF (7 g, 4.6 mmol, 5.2 eq) were tranferred to a flame-dried flask, which was flushed with nitrogen before addition of anhydrous DMF (12 mL, 0.07 M). The mixture was stirred at 85°C for 20 hours. The reaction was cooled to room temperature, quenched with saturated aqueous ammonium chloride solution, the phases were separated and the aqueous phase was extracted 3x with ethyl acetate. The combined organic phases were dried over anhydrous magnesium sulfate, filtered, and the solvent was evaporated. The residue was dissolved in THF (10 mL, 0.09 M) under nitrogen atmosphere and cooled to 0 °C, followed by treatment with NaH (60 % dispersion in mineral oil, 60 mg, 1.5 mmol, 1.7 eq). The reaction was allowed to warm to room temperature and stirred for 3 horus. The reaction was quenched with sat. NH4Cl solution and extracted three times with ethyl acetate. Combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and the solvent was evaporated. The crude product was purified by flash chromatography on silica gel (gradient: 0 % to 5 % MeOH in DCM) to afford sultam **49** (0.42 g, 0.67 mmol, 75 %).

^{1}H NMR (300 MHz, $CDCl_3$, 27 °C) δ 7.74 (d, *J* = 8.4 Hz, 1H), 7.39-7.33 (m, 4H), 7.29 (dd, *J* = 8.8, 2.3 Hz, 2H), 7.19 (d, *J* = 1.9 Hz, 1H), 7.06-7.00 (m, 2H), 6.87-6.80 (m, 2H), 4.54 (s, 2H), 4.33-4.14 (m, 3H), 3.78 (s, 3H), 3.83-3.50 (m, 6H), 3.47 (dd, *J* = 9.7, 5.0 Hz, 1H), 2.22-2.06 (m, 1H), 1.89- 1.59 (m, 4H), 1.29 (d, *J* = 6.8 Hz, 3H), 0.85 (d, *J* = 7.0 Hz, 3H). **^{13}C NMR** (75 MHz, $CDCl_3$, 27 °C) δ 159.42, 156.08, 138.71, 135.96, 130.11, 130.07, 129.32, 128.57, 127.93, 127.74, 127.71, 127.08, 126.66, 113.92, 88.47, 73.15, 73.02, 72.77, 70.28, 56.39, 55.43, 51.77, 36.06, 28.86, 24.13, 16.54, 16.10.



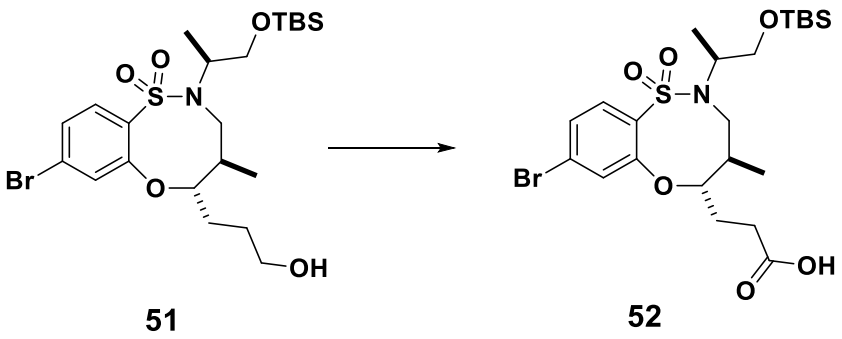
49 **50**

Following **General Protocol 5** PMB-protected alcohol **49** (160 mg, 0.25 mmol, 1.0 eq) was reacted with DDQ (90 mg, 0.40 mmol, 1.6 eq) in DCM (2.0 mL, 0.13 M) and pH 7 buffer (0.5 mL). After the aqueous work-up the residue was redissolved in DCM (2.5 mL, 0.10 M) under nitrogen atmosphere. At -78 °C 2,6-lutidine (120 µL, 111 mg, 1.0 mmol, 4.0 eq) and TBSOTf (150 µL, 172 mg, 0.65 mmol, 2.6 eq) were added. The mixture was stirred for 20 min at -78 °C before being allowed to warm to room temperature. The reaction was quenched with sat. $NaHCO_3$ solution and extracted three times with DCM. Combined organic extracts were dried over $MgSO_4$, filtered, and the solvent was evaporated. The crude TBS protected alcohol **50** (212 mg, impure, max. 0.25 mmol) was identified by mass spectrometry and used without further purification.



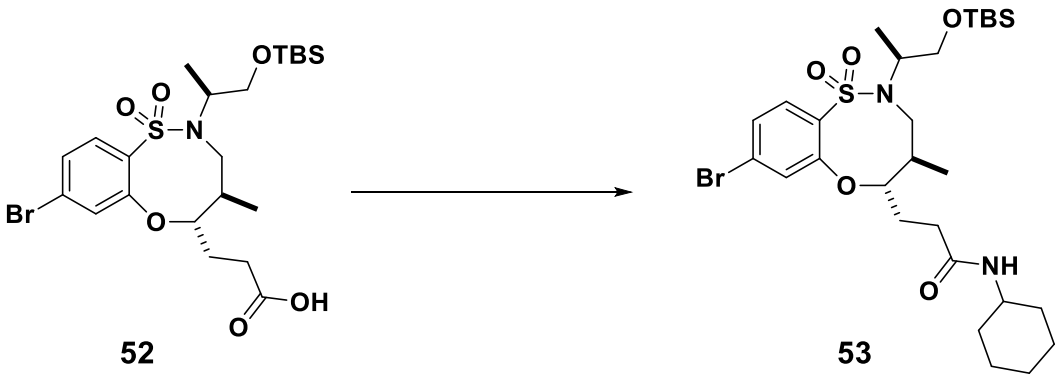
50 **51**

Benzyl ether **50** (164 mg crude, max. 0.19 mmol, 1.0 eq) was dissolved in 1,2-dibromoethane (2 mL, 0.10 M); pH 7 buffer (0.8 mL) and DDQ (132 mg, 0.58 mmol, 3.1 eq) were added. The reaction was stirred vigorously at 80 °C for 13 hours. After cooling to room temperature the reaction was quenched with sat. $NaHCO_3$ solution and extracted four times with DCM. Combined organic extracts were dried over $MgSO_4$, filtered, and the solvent was evaporated. Flash chromatography on silica gel (gradient: 0 % to 5 % MeOH in DCM) afforded free primary alcohol **51** (69 mg, 0.13 mmol, 68 % over 2 steps).



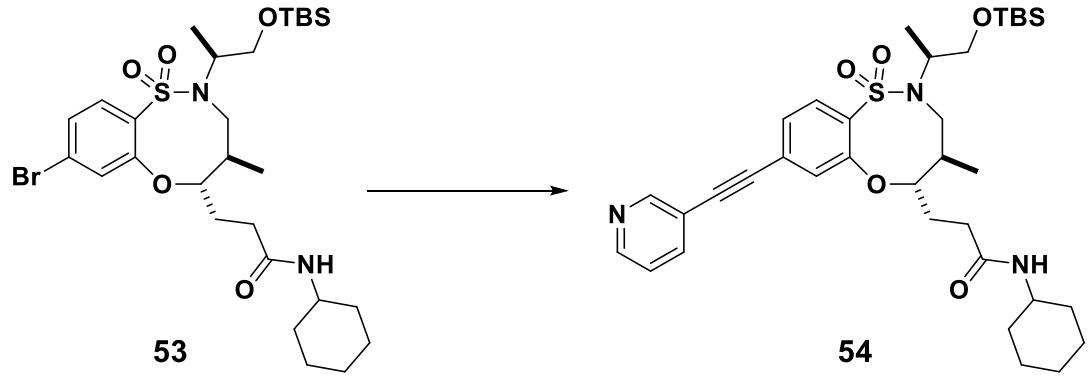
51 **52**

Primary alcohol **51** (69 mg, 0.13 mmol, 1.0 eq) was dissolved in DMF (1.5 mL) under nitrogen atmosphere before addition of PDC (96 mg, 0.26 mmol, 2.0 eq). The mixture was stirred at room temperature for 20 hours. The reaction was quenched with water and extracted with DCM four times. The combined organic extracts were dried over $MgSO_4$, filtered through a silica/Celite plug, and the solvent was evaporated. Carboxylic acid **52** (63 mg, 0.11 mmol, 88 %) was afforded as a foaming solid. The product was identified by mass spectrometry and used without further purification.



52 **53**

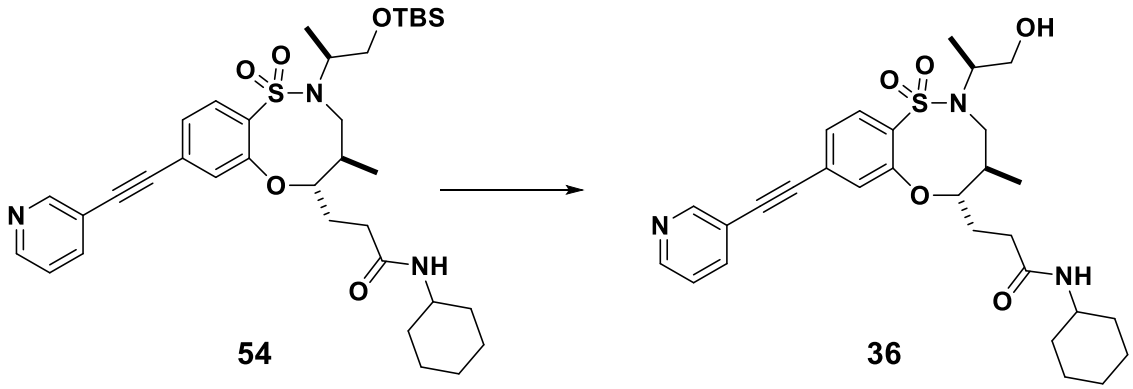
Following **General Protocol 4** carboxylic acid **52** (63 mg, 0.11 mmol, 1.0 eq) was reacted with PyBOP (65 g, 0.12 mmol, 1.1 eq), DIPEA (60 µL, 44.5 mg, 0.34 mmol, 3.1 eq), and cyclohexylamine (20 µL, 17.3 mg, 0.17 mmol, 1.6 eq) in DCM (0.8 mL, 0.14 M). Flash chromatography on silica gel (gradient: 0 % to 5 % MeOH in DCM) afforded amide **53** (64 mg, impure). The product was identified by mass spectrometry and used without further purification.



53 **54**

Following **General Protocol 1** aryl bromide **53** (30 mg, 0.047 mmol, 1.0 eq) was reacted with 3-ethynylpyridine (10 mg, 0.076 mmol, 1.6 eq), DIPEA (175 µL, 130 mg, 1.0 mmol, 21 eq), $Pd(PPh_3)_2Cl_2$ (3.7 mg, 5.3 µmol, 0.11 eq), and CuI (2.4 mg, 12.6 µmol, 0.27 eq) in DMF (0.5 mL, 0.09 M) for 12 hours. Flash chromatography on silica gel (gradient: 0 % to 5 % MeOH in DCM) afforded **54** (13.5 mg, 0.021 mmol, 40 % over 2 steps).

^{1}H NMR (300 MHz, $CDCl_3$, 27 °C) δ 8.78 (d, *J* = 1.9 Hz, 1H), 8.59 (dd, *J* = 4.9, 1.7 Hz, 1H), 7.91-7.79 (m, 2H), 7.36-7.28 (m, 2H), 7.24 (d, *J* = 1.6 Hz, 1H), 5.32 (br s, 1H), 4.33 (ddd, *J* = 9.4, 5.8, 3.6 Hz, 1H), 3.83- 3.58 (m, 6H), 2.45-2.36 (m, 2H), 2.29-2.13 (m, 2H), 2.05-1.96 (m, 1H), 1.96-1.86 (m, 2H), 1.75-1.52 (m, 3H), 1.25 (d, *J* = 6.5 Hz, 3H), 1.44-1.20 (m, 2H), 1.20-1.00 (m, 3H), 0.90 (d, *J* = 7.2 Hz, 3H), 0.83 (s, 9H), -0.01 (s, 3H), -0.05 (s, 3H).



TBS-protected alcohol **54** (13.5 mg, 0.021 mmol, 1.0 eq) was dissolved in THF (0.3 mL, 0.07 M) and cooled to 0 °C. 1 M TBAF solution in THF (25 µL, 0.025 mmol, 1.2 eq) was added and the mixture was stirred at room temperature for 2 hours. The reaction was quenched with sat. $NaHCO_3$ solution and extracted four times with EtOAc. Combined organic extracts were dried over $MgSO_4$, filtered, and the solvent was evaporated. Flash chromatography on silica gel (gradient: 0 % to 4 % MeOH in DCM) afforded final analog **36** (7.6 mg, 0.014 mmol, 68 %). **36** was purified by HPLC prior to biological testing.

^{1}H NMR (300 MHz, $CDCl_3$, 27 °C) δ 8.78 (s, 1H), 8.59 (d, *J* = 4.9 Hz, 1H), 7.91 (d, *J* = 8.2 Hz, 1H), 7.83 (dt, *J* = 7.9, 1.9 Hz, 1H), 7.39 (dd, *J* = 8.2, 1.2 Hz, 1H), 7.32 (dd, *J* = 7.9, 4.9 Hz, 1H), 7.28 (d, *J* = 1.6 Hz, 1H), 5.32 (d, *J* = 7.9 Hz, 1H), 4.46 (ddd, *J* = 9.0, 4.9, 3.4 Hz, 1H), 4.02-3.83 (m, 2H), 3.83-3.68 (m, 1H), 3.68-3.43 (m, 3H), 3.38 (dd, *J* = 15.9, 5.0 Hz, 1H), 2.45- 2.20 (m, 3H), 2.20-2.09 (m, 1H), 2.08-1.96 (m, 1H), 1.95-1.83 (m, 2H), 1.75-1.53 (m, 3H), 1.43-1.26 (m, 2H), 1.22 (d, *J* = 6.8 Hz, 3H), 1.20-0.98 (m, 3H), 0.92 (d, *J* = 6.8 Hz, 3H). **^{13}C NMR** (75 MHz, $CDCl_3$, 27 °C) δ 171.01, 153.78, 152.56, 149.42, 138.79, 135.64, 129.80, 128.28, 128.05, 127.46, 123.26, 90.56, 89.49, 87.95, 64.77, 58.10, 48.56, 48.11, 36.11, 33.37, 33.35, 30.92, 28.57, 25.68, 25.02, 15.83, 15.33. **HRMS** (ESI) calc'd for $C_{29}H_{37}N_3O_5S$ $[M+H]^+$: 540.2527. Found: 540.2528.

E. Synthesis of 2-hydroxyglutarate-D4

Deuterated α-ketoglutarate (α-KG-d6) was purchased from Sigma. To α-KG-d6 in methanol was added sodium borohydride. The mixture was heated to 60°C and stirred for 30 min. The mixture was cooled and concentrated under a stream of dry nitrogen. The residue was dissolved in water and stored as a 1 mg/mL (6.58 mM) aqueous solution.

Abbreviations

ADME: absorption, distribution, metabolism, and excretion

α-KG: α-ketoglutarate

AML: acute myeloid leukemia

BME: beta-mercaptoethanol

CMC: critical micelle concentration

DCM: dichloromethane

DDQ: 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone

DFCI: Dana-Farber Cancer Institute

DIPEA: diisopropylethylamine

DMF: dimethylformamide

DMSO: dimethyl sulfoxide

DOS: diversity-oriented synthesis

HPLC: high pressure liquid chromatography

IDH: isocitrate dehydrogenase

IPTG: Isopropyl β-D-1-thiogalactopyranoside

LC-MS: liquid chromatography-mass spectrometry

NADP/NADPH: β-nicotinamide adenine dinucleotide 2'-phosphate (oxidized/reduced)

PBS: phosphate buffered saline

PDGFRA: platelet-derived growth factor receptor alpha

PMB: *para*-methoxybenzyl

PyBOP: (Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate

R-2HG: (*R*)-2-hydroxyglutarate

SAR: structure-activity relationship

SDS-PAGE: sodium dodecyl sulfate-polyacrylamide gel electrophoresis

SIR: single ion recording

TBAF: tetra-*n*-butylammonium fluoride

TBSOTf: *tert*-butyldimethylsilyl trifluoromethanesulfonate

TEA: triethylamine

THF: tetrahydrofuran

UPLC: ultra-high pressure liquid chromatography

XPhos-Pd-G3: (2-Dicyclohexylphosphino-2′,4′,6′-triisopropyl-1,1′-biphenyl)[2-(2′-amino-1,1′-biphenyl)]palladium(II) methanesulfonate

Methods References

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(2) Ryan, A. J.; Gray, N. M.; Lowe, P. N.; Chung, C.-w. Effect of Detergent on "Promiscuous" Inhibitors. *Journal of Medicinal Chemistry* **2003**, *46*, 2003.
(3) Boehm, J. S.; Zhao, J. J.; Yao, J.; Kim, S. Y.; Firestein, R.; Dunn, I. F.; Sjostrom, S. K.; Garraway, L. A.; Weremowicz, S.; Richardson, A. L.; Greulich, H.; Stewart, C. J.; Mulvey, L. A.; Shen, R. R.; Ambrogio, L.; Hirozane-Kishikawa, T.; Hill, D. E.; Vidal, M.; Meyerson, M.; Grenier, J. K.; Hinkle, G.; Root, D. E.; Roberts, T. M.; Lander, E. S.; Polyak, K.; Hahn, W. C. Integrative genomic approaches identify IKBKE as a breast cancer oncogene. *Cell* **2007**, *129*, 2007.
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