

## **Supporting Information**

### **Discovery of a Selective Covalent Inhibitor of Lysophospholipase-like 1 (LYPLAL1) as a Tool to Evaluate the Role of this Serine Hydrolase in Metabolism**

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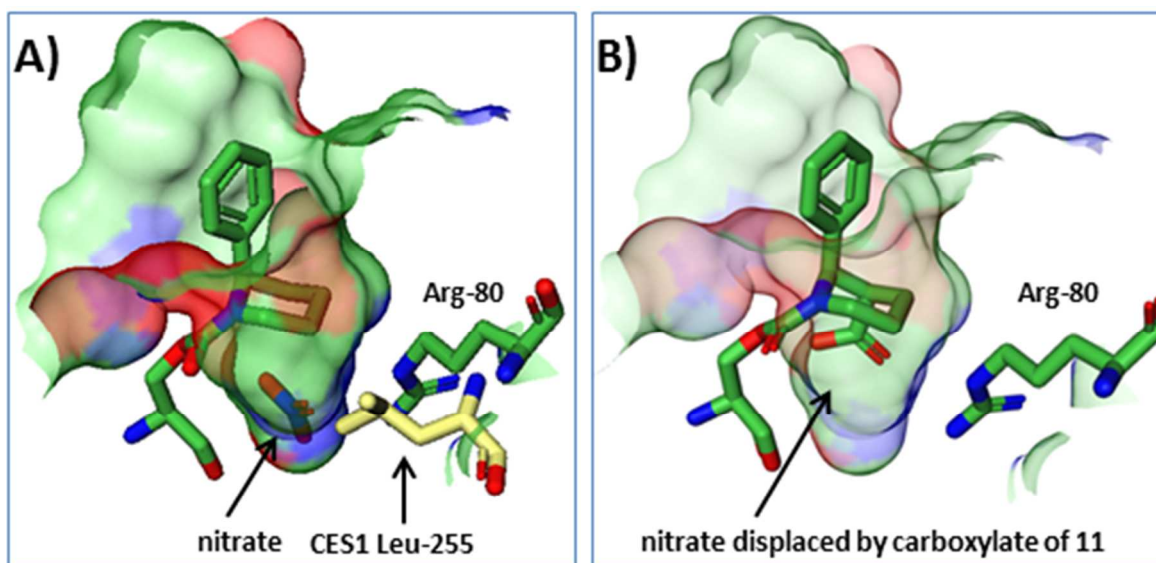
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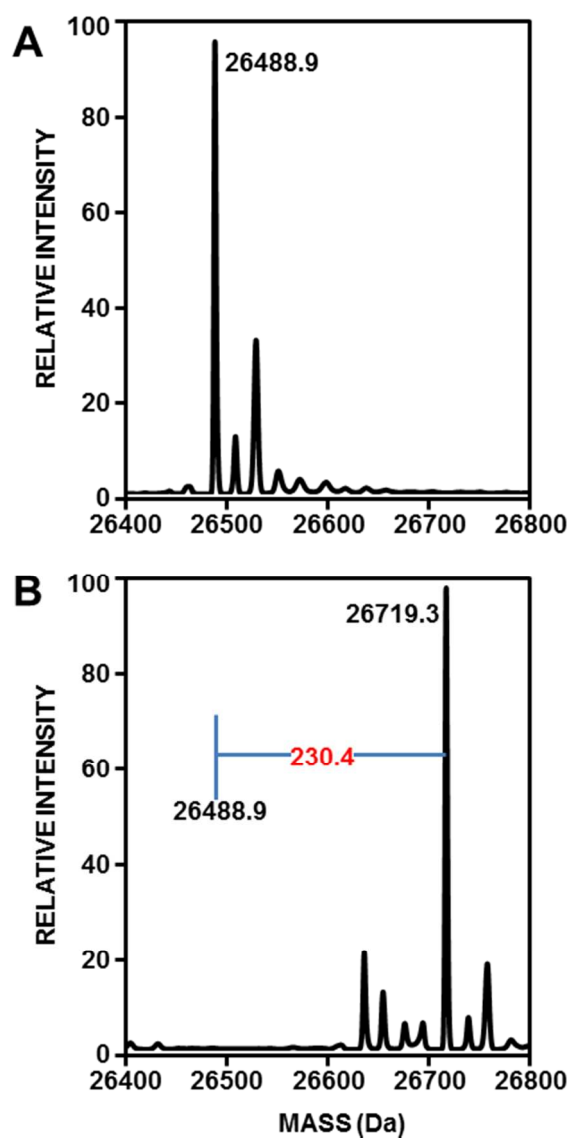
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**Comparison of the Experimental Structure of the LYPLAL1 Active Site Acylated by Compound 1 and 2 with Modeled X-Ray Structure of the Active Site Modified by Compound 11**



**Figure S1:** A) X-ray structure of LYPLAL1 active site after reaction with compounds **1** and **2**, as shown also in Figure 3B. Nitrate derived from the crystallization buffer interacts with the side chain of Arg-80, a distinctive feature of LYPLAL1. B) Modeled structure of the LYPLAL1 active site modified by reaction with compound **11**, showing how the carboxylate substituent of the modifying group would occupy the position previously taken by the nitrate.

### Mass Spectrometry of LYPLAL1 Before and After Acylation by Compound 11



**Figure S2.** Mass spectrum of A) human recombinant LYPLAL1 (mass theor. 26,488.7 Da), and B) LYPLAL1 treated with **11**. The mass shift observed in panel B indicates that the enzyme was covalently modified by the (2*R*,3*R*)-1-(carbonyl)-2-phenylpiperidine-3-carboxylic acid moiety derived from **11**. Spectra were collected using a Waters LCT Premier XE mass spectrometer as described previously.<sup>1</sup>

## X-Ray Refinement Statistics (LYPLAL1 with compound 2) <sup>2-5</sup>

### Data Collection

X-Ray diffraction data at 100 K were collected at the 17-ID (IMCA) beamline at the APS with a Pilatus 6M detector. For each crystal, a total of 180° of data were collected in 0.25° oscillations, generally yielding ~100% complete data coverage at 4-5X redundancy for accurate scaling and outlier rejection. Data processing was performed using HKL2000.

<b><u>Data Collection Statistics</u></b>	<b><u>Total (Å)</u></b> (50-2.0)	<b><u>Outer Resolution Shell (Å)</u></b> (2.03-2.00)
R <sub>sym</sub>	0.095	0.302
I/σ(I)	16.4	2.6
Completeness (%)	99.7	95.9

### Structure Solution

The LYPLAL1 co-structure with **2** was determined by using molecular replacement (Phaser, CCP4 suite) utilizing RCSB entry 3U0V as the search model. Binding of **2** to the LYPLAL1 active site was assessed by visual inspection of the resulting difference maps using the program COOT.<sup>3</sup> Final refinement was done using AUTOBUSTER.<sup>5</sup> Refinement statistics are shown below.

<b>Model Refinement</b>	<b>Compound 2</b>
Maximum resolution (Å)	2.00
R <sub>work</sub> (%) <sup>a</sup>	16.5
R <sub>free</sub> (%)	21.2
Mean B value (Å <sup>2</sup> )	27.4
Rms deviations from ideal geometry	
Bonds (Å)	0.010
Angles (°)	1.04
Water molecules	153
Ions	One nitrate

<sup>a</sup>R<sub>work</sub> =  $\sum |F_{\text{obs}}| - |F_{\text{calc}}| / \sum |F_{\text{obs}}|$ . R<sub>free</sub> is equivalent to R<sub>work</sub>, except that it is calculated for a randomly chosen 5% of reflections omitted from the refinement process.

**<sup>35</sup>S Pulse Chase Analysis of LYPLAL1 protein turnover****Table of data**

<b>Time (h)</b>	<b>Percentage</b>
0	100
0.25	88.9
0.5	85.5
1	79.4
3	71.7
6	62.3
24	19.3
48	5.2
72	2.7

## Details of Human Liver Samples used for mRNA analysis

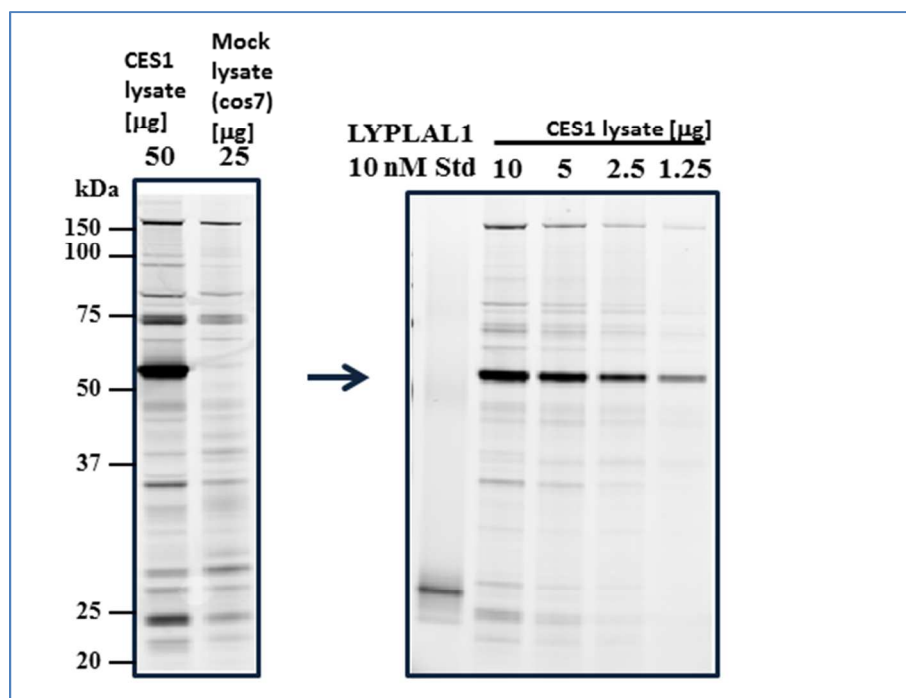
COLLECTION	SUBJECT ID	TISSUE TYPE	TISSUE SUBDETAIL	AGE	GENDER
U of MICHIGAN	09-0024	LIVER	Fibrosis	57	MALE
U of MICHIGAN	08-0414	LIVER	Fibrosis	61	MALE
U of MICHIGAN	Jul-87	LIVER	Fibrosis	71	FEMALE
U of MICHIGAN	06-0984	LIVER	Fibrosis	80	FEMALE
U of MICHIGAN	06-0429	LIVER	HCC	76	FEMALE
U of MICHIGAN	08-0015	LIVER	HCC	61	MALE
U of MICHIGAN	08-1002	LIVER	HCC	68	FEMALE
U of MICHIGAN	06-1123	LIVER	HCC	74	MALE
U of MICHIGAN	07-0821	LIVER	HCC	68	MALE
U of MICHIGAN	07-1162	LIVER	HCC	62	MALE
U of MICHIGAN	08-0305	LIVER	HCC	49	MALE
U of MICHIGAN	08-0953	LIVER	HCC	57	FEMALE
U of MICHIGAN	08-1211	LIVER	HCC	61	MALE
U of MICHIGAN	07-1295	LIVER	HCC	59	MALE
U of MICHIGAN	08-0414	LIVER	HCC	61	MALE
U of MICHIGAN	08-0937	LIVER	steatosis	49	FEMALE
U of MICHIGAN	07-1237	LIVER	steatosis	29	FEMALE
U of MICHIGAN	07-0068	LIVER	steatosis	63	MALE
U of MICHIGAN	07-0367	LIVER	steatosis	60	FEMALE
U of MICHIGAN	08-0334	LIVER	steatosis	69	MALE
U of MICHIGAN	08-1110	LIVER	steatosis	63	FEMALE
U of MICHIGAN	08-0929	LIVER	Steatosis	80	MALE
U of MICHIGAN	07-1143	LIVER	Steatosis	52	MALE
U of MICHIGAN	09-0679	LIVER	Steatosis	67	FEMALE
U of MICHIGAN	Aug-68	LIVER	Steatosis	53	MALE
U of MICHIGAN	07-1219	LIVER	Steatosis	44	FEMALE
U of MICHIGAN	08-1218	LIVER	Normal	60	FEMALE
U of MICHIGAN	08-1420	LIVER	Normal	78	MALE
U of MICHIGAN	07-1156	LIVER	Normal	33	FEMALE
CLEVELAND CLINIC	303C133Cc	LIVER	Normal	71	FEMALE
CLEVELAND CLINIC	303C002Ca	LIVER	Normal	54	MALE
PROTEOGENEX	09275A	LIVER	Normal	84	FEMALE
PROTEOGENEX	09278A	LIVER	Normal	84	MALE
PROTEOGENEX	09284A	LIVER	Normal	81	FEMALE
Advanced Tissue Services	14-0919	LIVER	Normal	66	MALE
Advanced Tissue Services	15-0021	LIVER	Normal	84	MALE
BioServe Biotechnologies, Ltd.	QZFIXNV7	LIVER	Normal	79	MALE
BioServe Biotechnologies, Ltd.	PZD4WNUY	LIVER	Normal	66	FEMALE
BioServe Biotechnologies, Ltd.	1X92PNC2	LIVER	Normal	73	MALE
BioServe Biotechnologies, Ltd.	ZPEP4Y11	LIVER	Normal	63	FEMALE



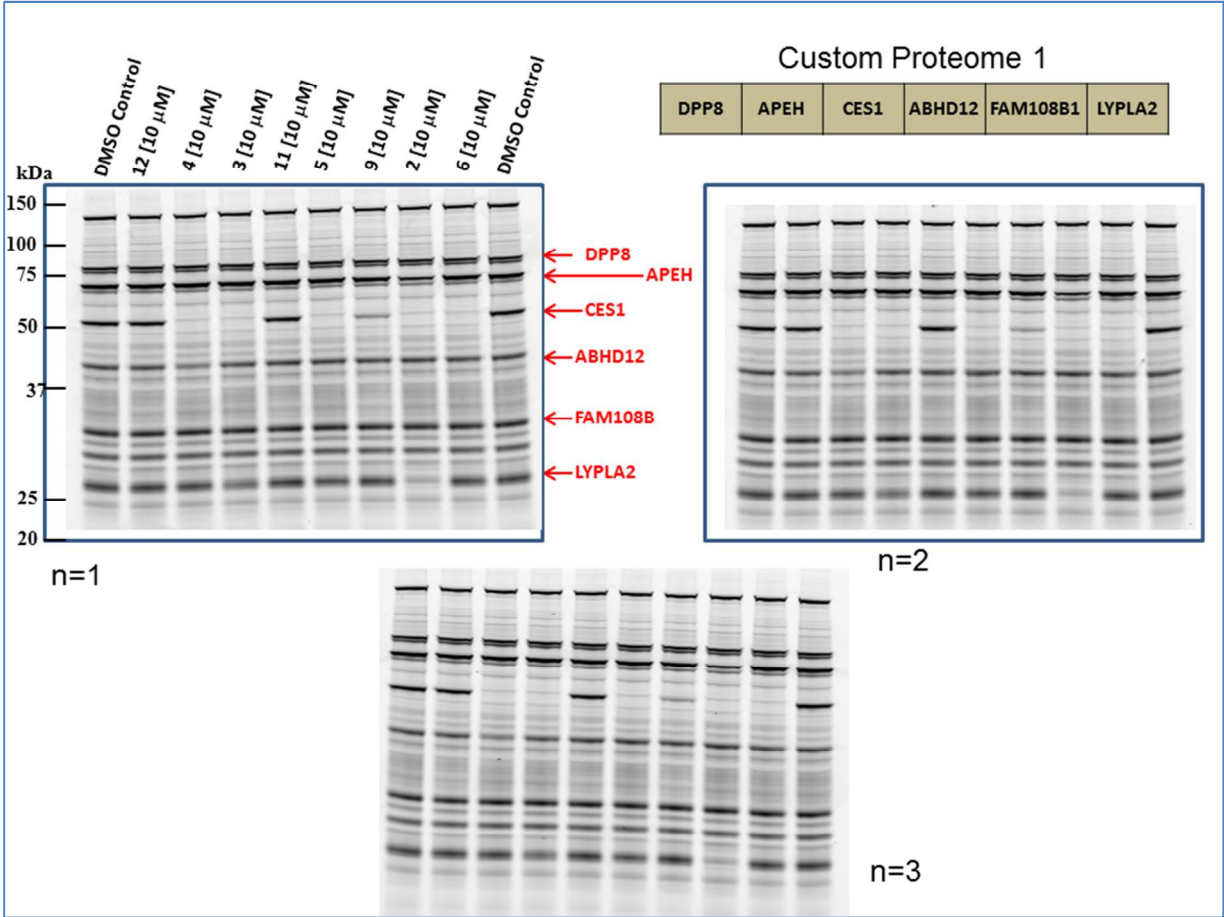
## Human Liver Samples used in assessment of LYPLAL1 levels in human liver by click chemistry

Lane in Figure 4A	Raw Counts [LYPLAL1 Band Intensity]	COLLECTION	SUBJECT ID	TISSUE TYPE	TISSUE SUBDETAIL	AGE	GENDER	ETHNICITY
1	4212197.23	Advanced Tissue Services	14-0919	LIVER	Normal	66	MALE	CAUCASIAN
2	15196966.41	Advanced Tissue Services	15-0021	LIVER	Normal	84	MALE	CAUCASIAN
3	13224542.05	BioServe Biotechnologies, Ltd.	QZFIXNV7	LIVER	Normal	79	MALE	CAUCASIAN
4	12863632.72	BioServe Biotechnologies, Ltd.	PZD4WNUY	LIVER	Normal	66	FEMALE	CAUCASIAN
5	11336187.46	BioServe Biotechnologies, Ltd.	1X92PNC2	LIVER	Normal	73	MALE	CAUCASIAN
6	14002573.92	BioServe Biotechnologies, Ltd.	ZPEP4Y11	LIVER	Normal	63	FEMALE	CAUCASIAN
7	14268674.11	BioServe Biotechnologies, Ltd.	WV4YNNFA	LIVER	Normal	51	MALE	CAUCASIAN
8	13824902.84	BioServe Biotechnologies, Ltd.	Q8HY3NWY	LIVER	Fatty	48	MALE	CAUCASIAN
9	15030379.32	BioServe Biotechnologies, Ltd.	NIHGJN16	LIVER	Fatty	57	MALE	CAUCASIAN
10	985446.87	BioServe Biotechnologies, Ltd.	8MTC7NN7	LIVER	Fatty	71	MALE	AFRICAN AMERICAN
11	7556857.14	BioServe Biotechnologies, Ltd.	6EVY7NHR	LIVER	Fatty	69	FEMALE	CAUCASIAN
12	15090617.23	Analytical Biological Services, Inc.	UH1005-22	LIVER	Fatty	64	FEMALE	CAUCASIAN
13	10866346.53	Analytical Biological Services, Inc.	UH1010-33	LIVER	Fatty	62	FEMALE	CAUCASIAN
14	9684566.98	Analytical Biological Services, Inc.	UH1012-06	LIVER	Fatty	57	FEMALE	CAUCASIAN
15	14270781.26	BioServe Biotechnologies, Ltd.	192J9ACD	LIVER	HCC	37	MALE	CAUCASIAN
16	15731045.97	BioServe Biotechnologies, Ltd.	DLEE6A5B	LIVER	HCC	78	FEMALE	CAUCASIAN
17	18551097.18	BioServe Biotechnologies, Ltd.	Q2FIXAH1	LIVER	HCC	79	MALE	CAUCASIAN
18	7726650.45	BioServe Biotechnologies, Ltd.	YE4RAAHL	LIVER	HCC	78	MALE	CAUCASIAN
19	22956961.81	BioServe Biotechnologies, Ltd.	WU25ZAWF	LIVER	HCC	54	MALE	CAUCASIAN

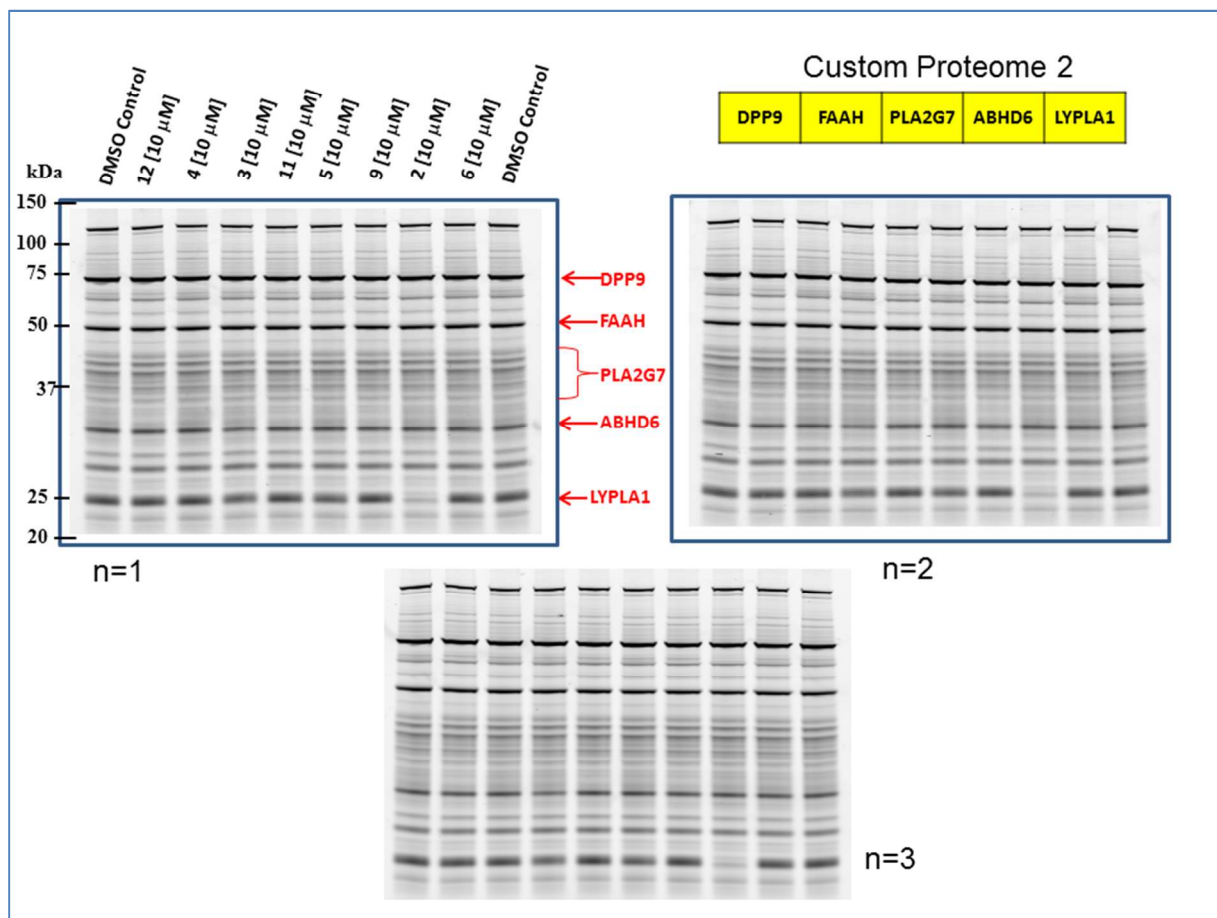
## Example of Characterization of a Serine Hydrolase (CES1) Before Pooling for Custom Proteome Generation



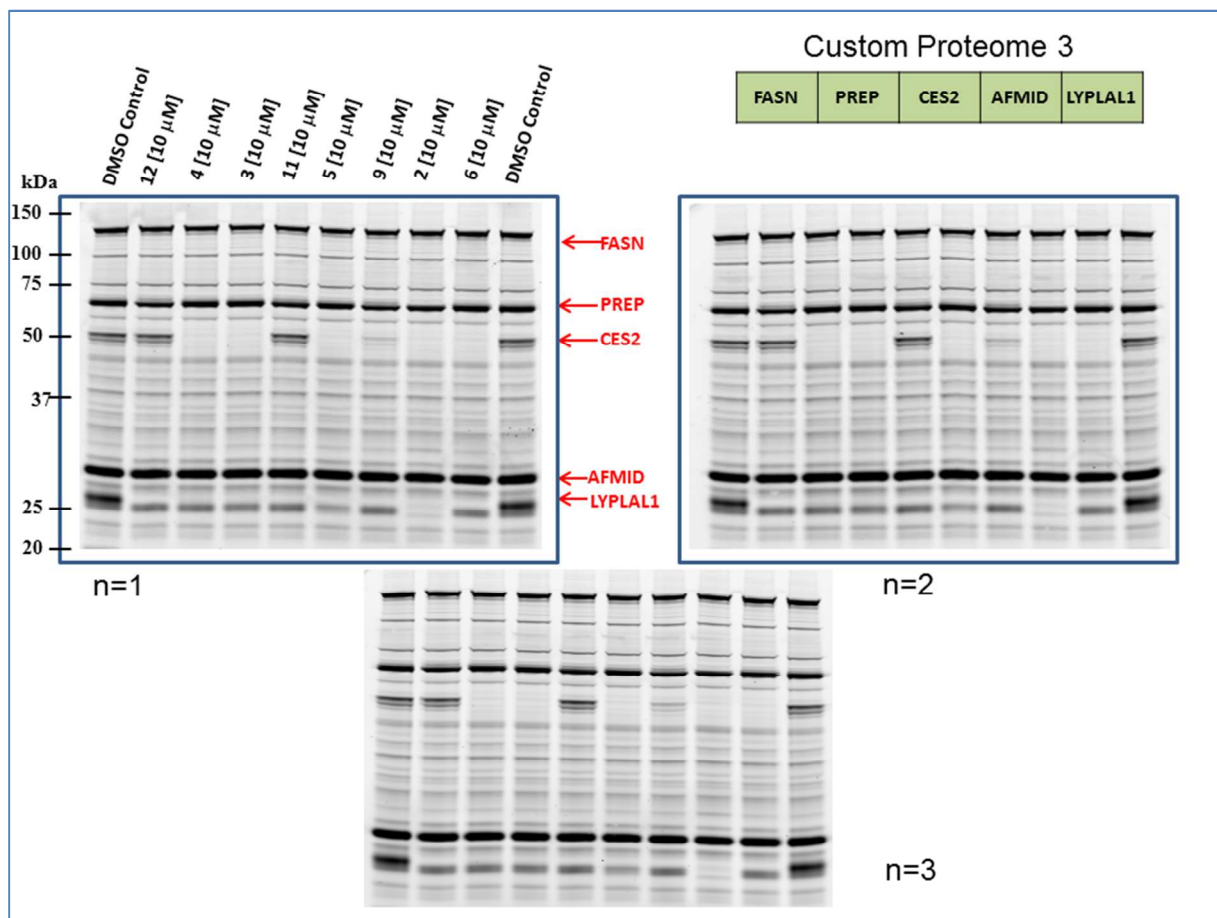
**Custom Proteome 1 ABPP Fluorescent Gels**



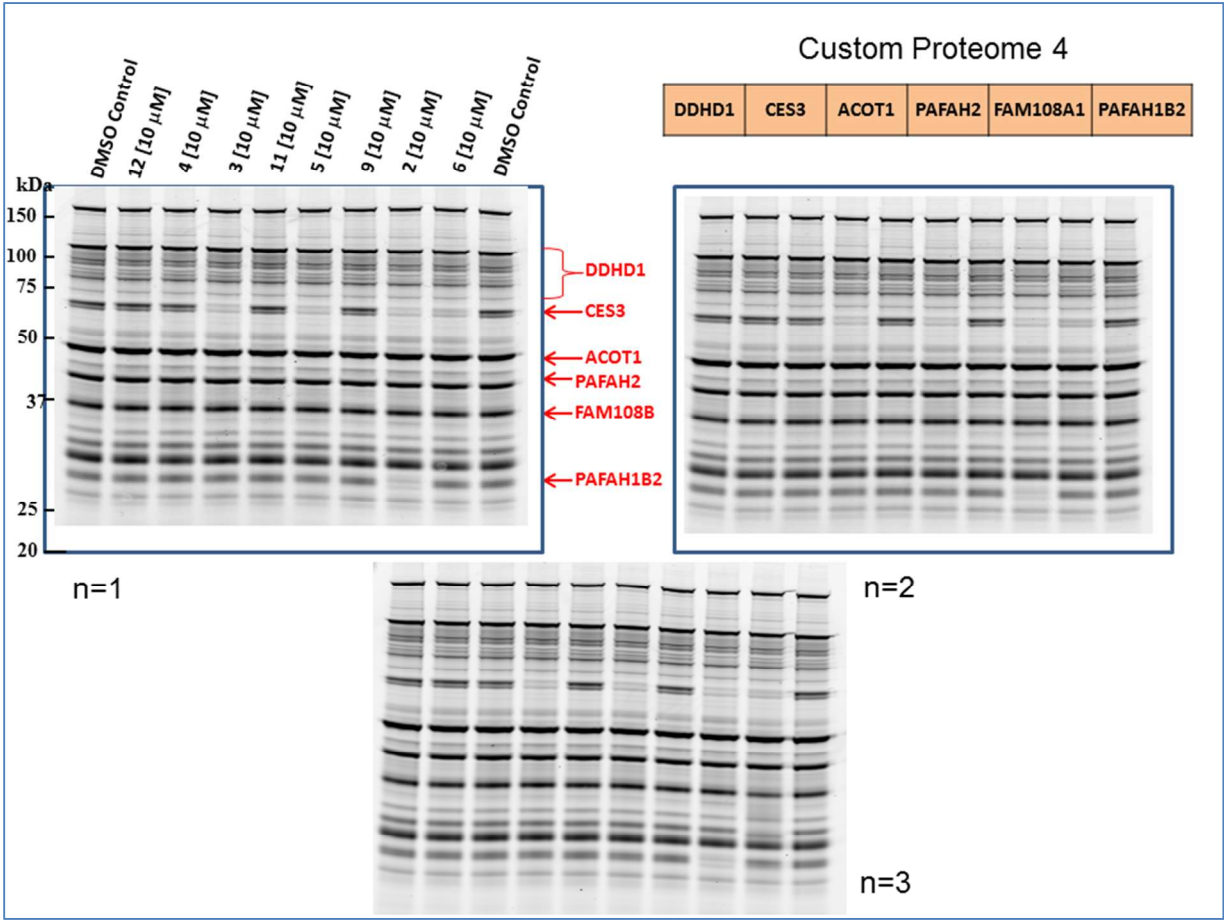
## Custom Proteome 2 ABPP Fluorescent Gels



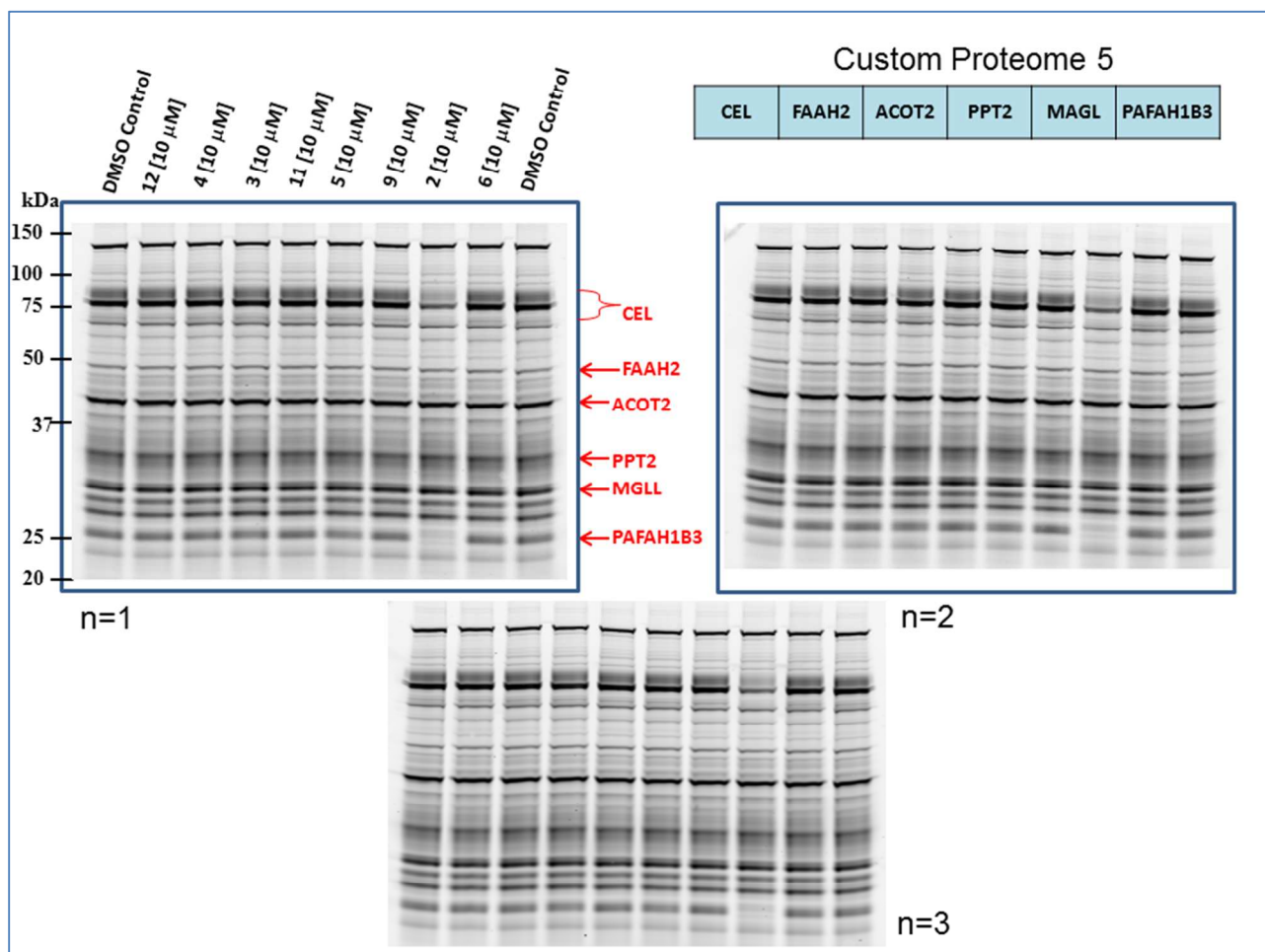
### Custom Proteome 3 ABPP Fluorescent Gels



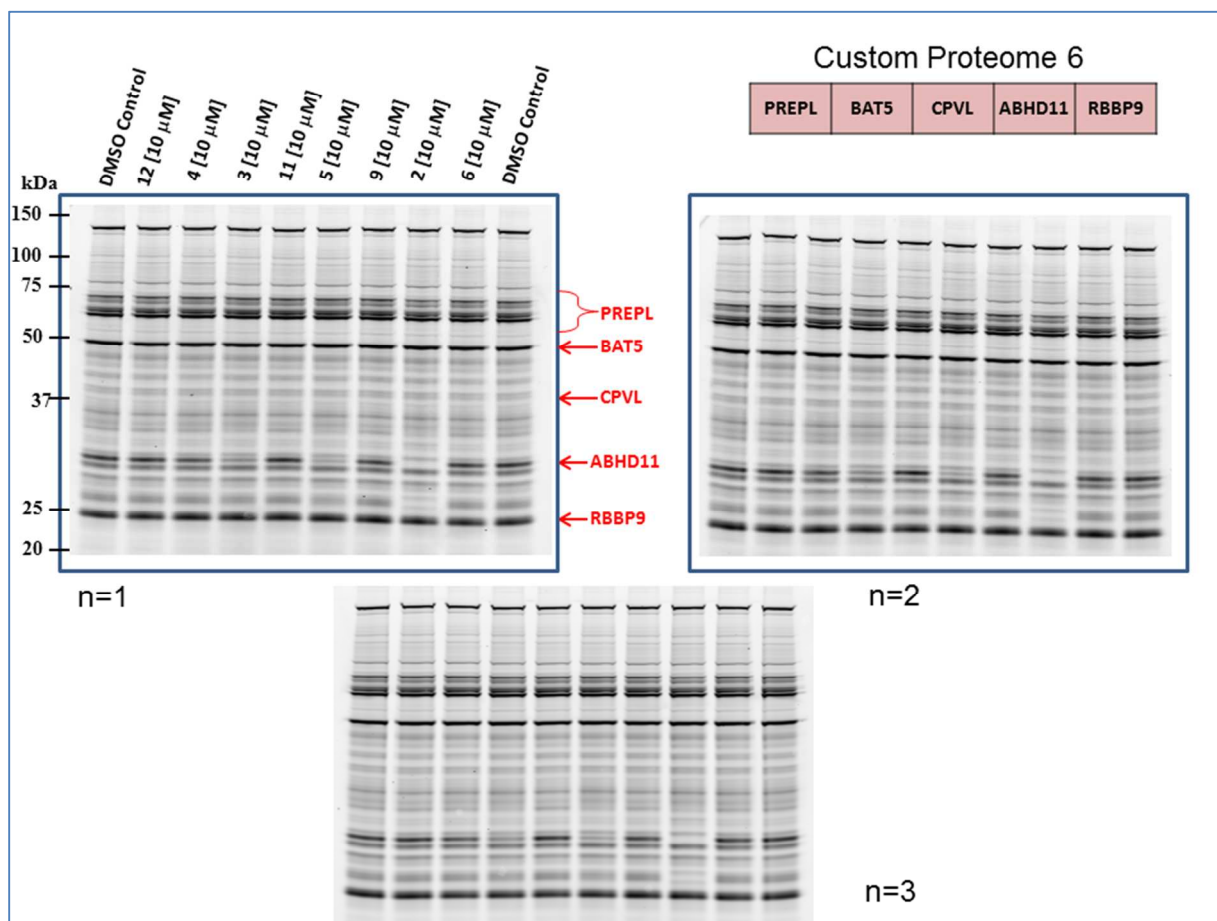
Custom Proteome 4 ABPP Fluorescent Gels



## Custom Proteome 5 ABPP Fluorescent Gels

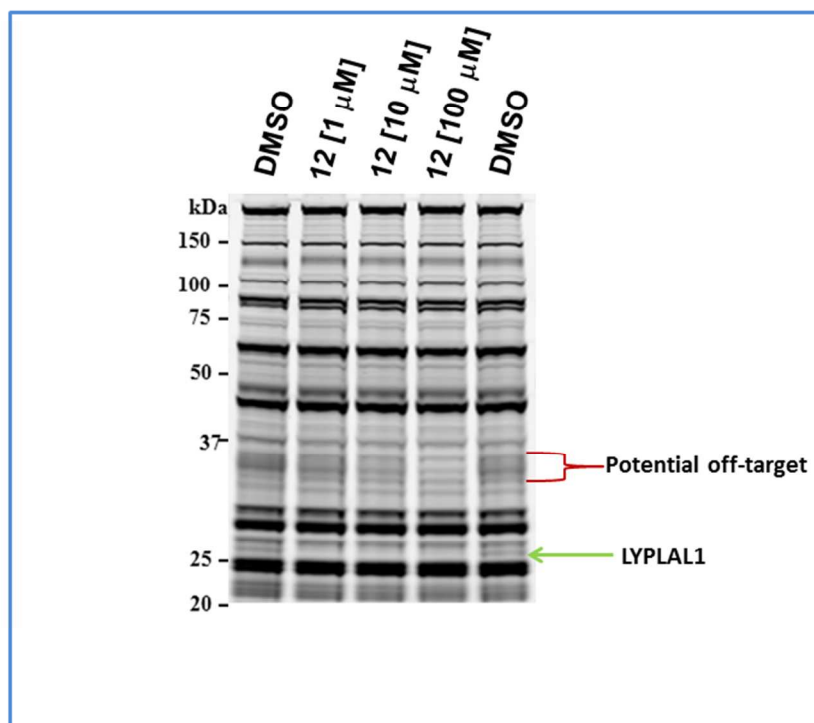


## Custom Proteome 6 ABPP Fluorescent Gels



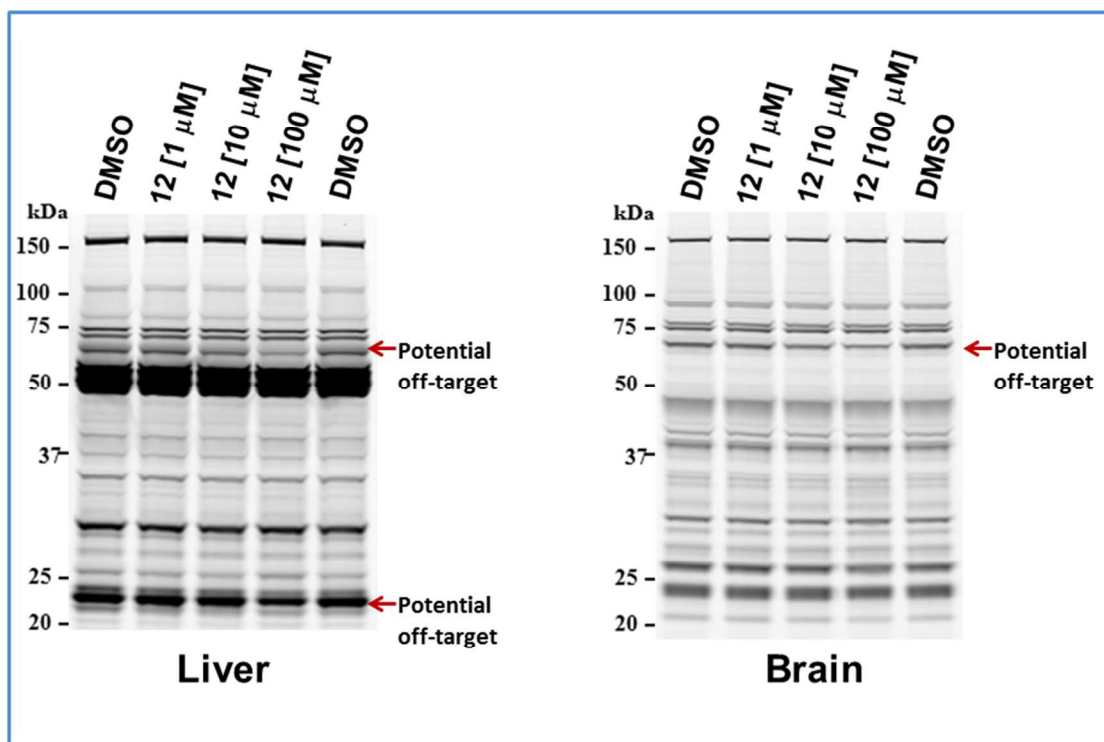


### LYPLAL1 Alkyne Probe Selectivity: HepG2 Lysate ABPP Fluorescent Gel

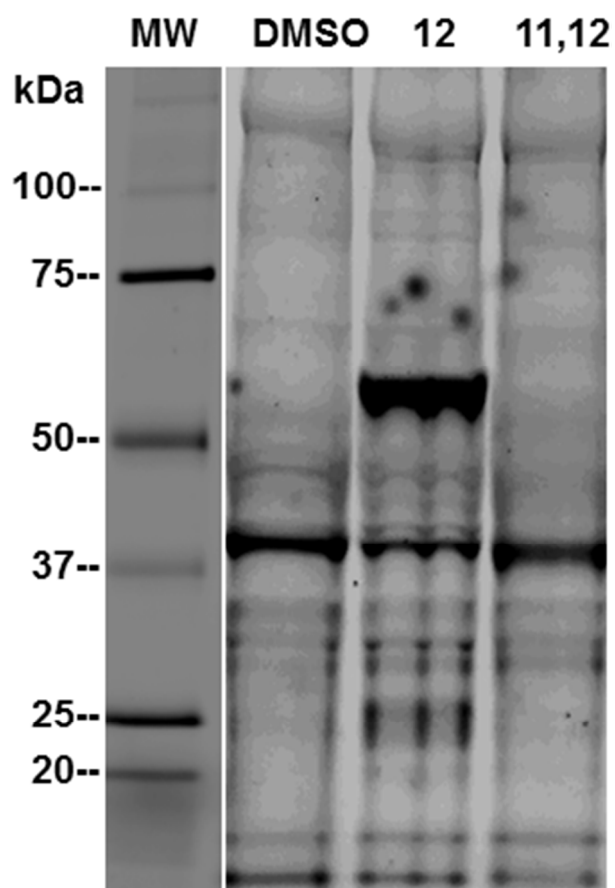


**Figure S3:** Labelling of HepG2 endogenous serine hydrolases by FP-rhodamine probe pretreated by DMSO or alkyne probe **12**.

**LYPLAL1 Alkyne Probe Selectivity: C57Bl/6J Mouse Tissue Lysate**  
**ABPP Fluorescent Gels**

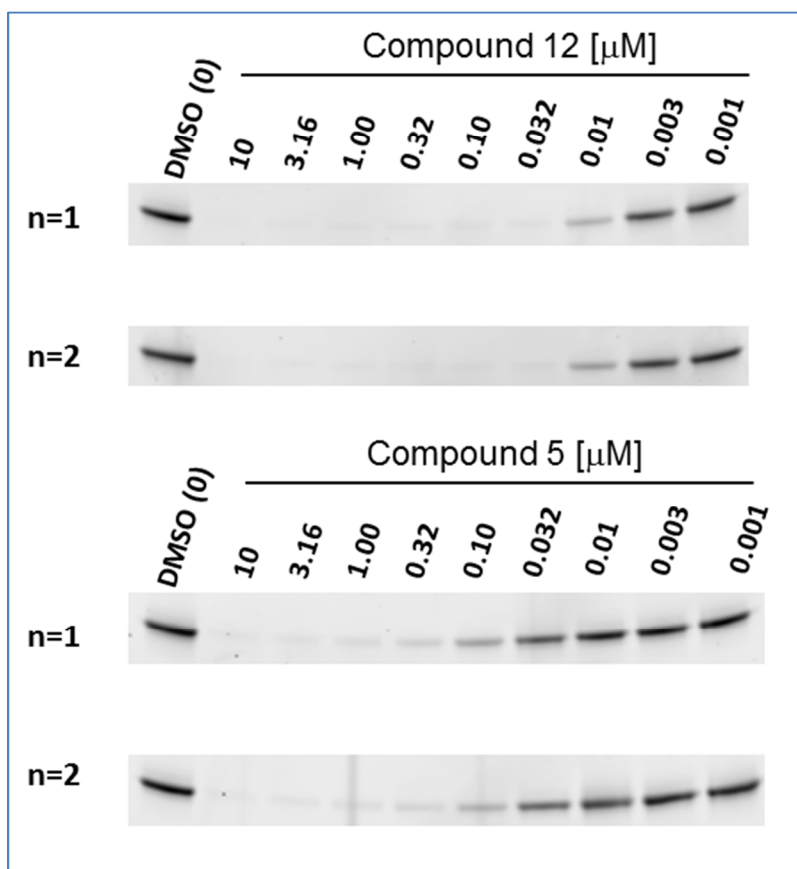


**Figure S4:** Labelling of liver and brain endogenous serine hydrolases from C57Bl/6J mouse by FP-rhodamine probe pretreated by DMSO or alkyne probe **12**.

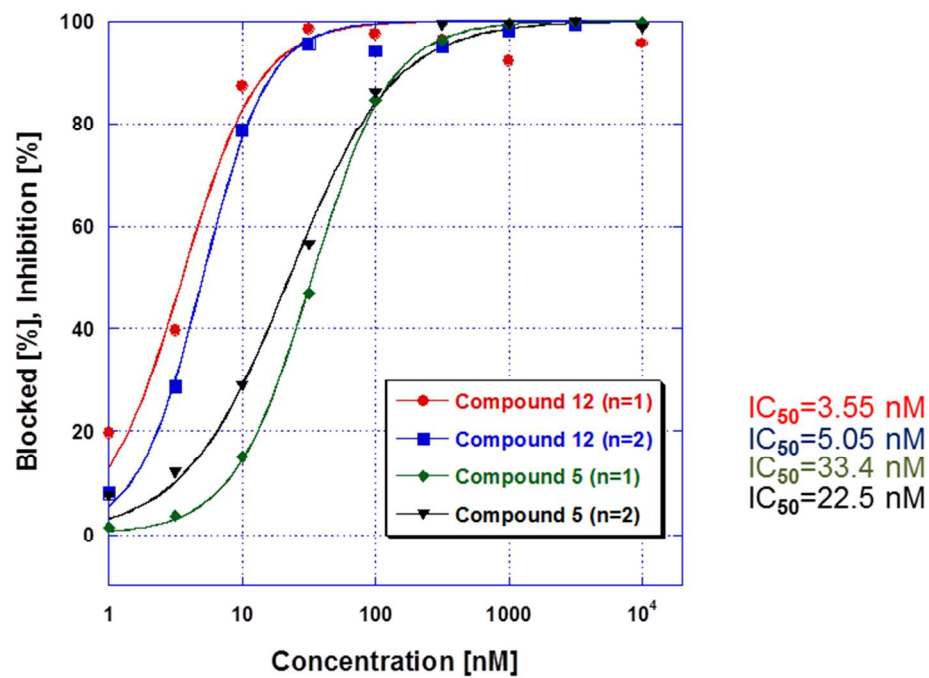


**Figure S5:** ABPP-based selectivity test of compound **12** in rat hepatocytes. See Methods for details. TAMRA was covalently linked by click chemistry to proteins labeled by alkyne probe **12**. Proteomic analysis detected the presence of LYPLAL1 in the selectively TAMRA-labeled band at ~26 kDa and of multiple carboxylesterases in the labeled band at ~56 kDa.

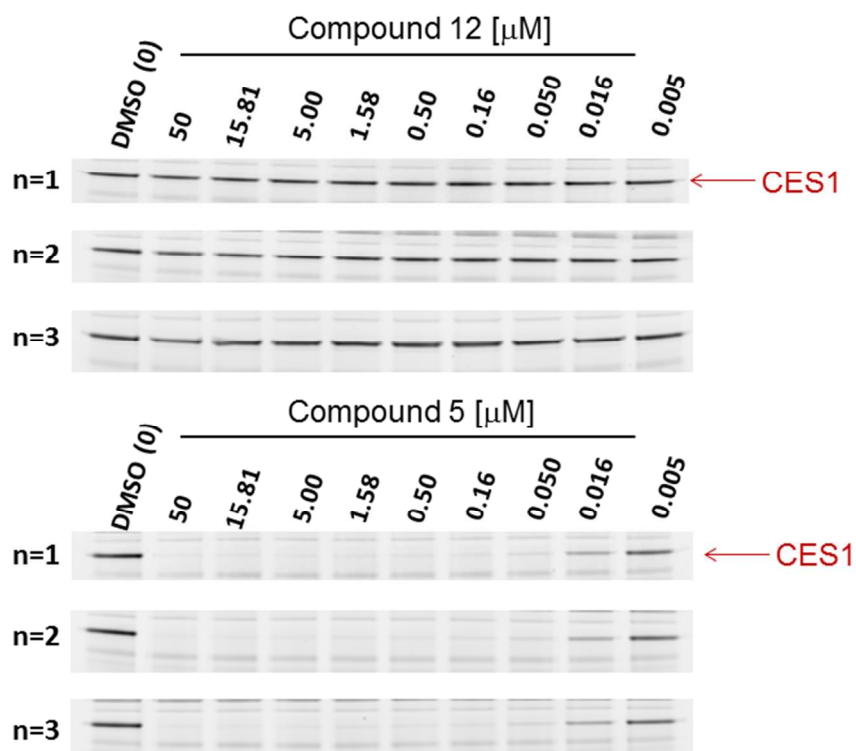
**LYPLAL1 ABPP Fluorescent IC<sub>50</sub> Gels (~25kDa region of gel)**  
**[Example from Compounds 5 and 12]**



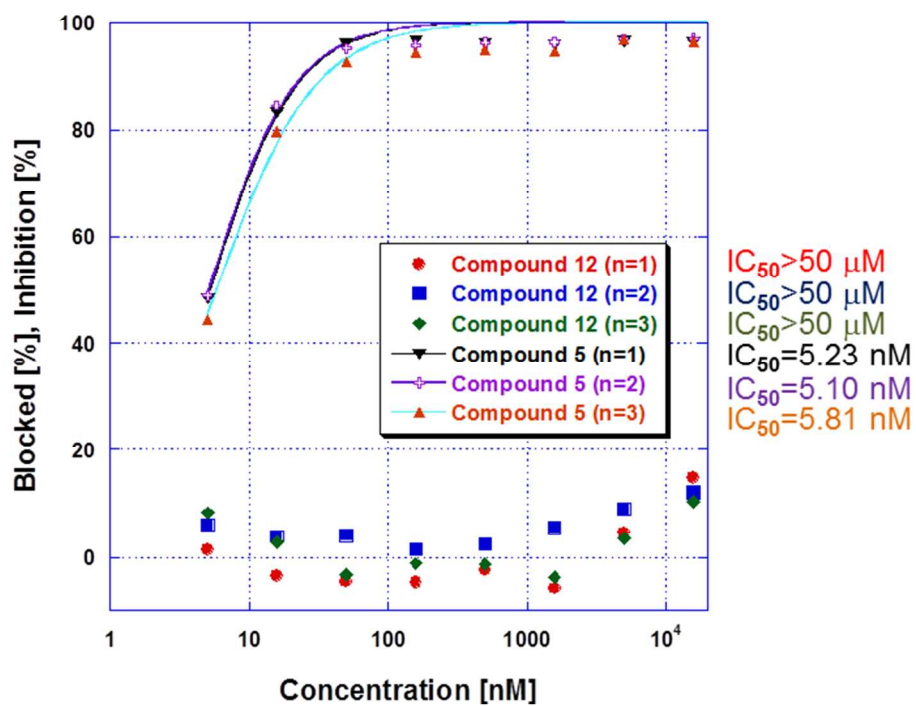
## LYPLAL1 IC<sub>50</sub> Curves Generated from In-Gel Fluorescence [Example from Compound 5 and 12]



**CES1 ABPP Fluorescent IC<sub>50</sub> Gels (~50kDa region of gel)**  
**[Example from Compound 5 and 12]**



## CES1 IC<sub>50</sub> Curves Generated from In-Gel Fluorescence [Example from Compound 5 and 12]



## **Synthesis of LYPLAL1 Inhibitors**

### **Chemical Synthesis and General Methods for Characterization**

All chemicals, reagents, and solvents were purchased from commercial sources and used without further purification.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were obtained at the indicated field as solutions in the indicated deuterated solvent. Chemical shifts for  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra are reported in parts per million (ppm,  $\delta$ ) and referenced to the indicated deuterated solvent. Coupling constants ( $J$ ) are reported in Hertz (Hz). The multiplicities are denoted as follows, s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br s, broad singlet; td, triplet of doublets; qd, quartet of doublets. Silica gel flash chromatography was performed using medium pressure Biotage or ISCO systems using pre-packaged columns by various commercial vendors including Biotage and ISCO.

NMR instrumentation: Agilent 600 MHz with an AutoX-ID probe (VNMRS console), Bruker 600 MHz Avance III with CryoProbe TCI, Bruker 500 MHz Avance HD with CryoProbe Prodigy BBO, and Bruker 400 MHz Avance III with BBFO ATM were used to acquire NMR data.

Low resolution mass spectrometry: Agilent 6120, Agilent 6110, and Waters Acquity UPLC/ Waters Acquity SQ systems (Waters SQ MS(single quad) Tune).

High resolution mass spectrometry: Accurate Mass Spectrometry analyses were conducted on an Agilent 6220 TOF mass spectrometer (Agilent Technologies, Wilmington, DE) in positive or negative electrospray mode. The system was calibrated to greater than 1 ppm accuracy across the mass range prior to analyses according to manufacturer's specifications. The samples were separated using UHPLC on an Agilent 1200 (Agilent Technologies, Wilmington, DE) system prior to mass spectrometric analysis. The resulting spectra were automatically lock mass corrected and the target mass ions and any confirming adducts ( $\text{Na}^+$ ,  $\text{NH}_4^+$ ) were extracted and combined as a chromatogram. The mass accuracy was calculated for all observed isotopes against the theoretical mass ions derived from the chemical formula using MassHunter software (Agilent Technologies, Wilmington, DE).

#### **Chiral SFC purification**

Method 1: Chiralpak AD (20 mm x 30 mm, 10  $\mu\text{m}$ ); eluting with 55% EtOH (0.1%  $\text{NH}_4\text{OH}$ ) in  $\text{CO}_2$  at a flow rate of 80 mL/min.

Method 2: Column: Chiral Tech ID (250mm x 21.2mm, 5  $\mu\text{m}$ ); eluting with 50%  $\text{CO}_2$  and 50% acetonitrile at flow rate of 80 mL/min.



## Experimental procedures for compounds 1-13

### Urea formation

The preparation of urea analogs of the 1,2,4-triazoles typically involved a two-step sequence, urea formation of 3-(4-bromophenyl)-1H-1,2,4-triazole with a substituted piperidine or other secondary amine followed by a Suzuki coupling. For the urea formation step, a separate protocol, using bis(pentafluorophenyl) carbonate, was also employed in addition to the standard triphosgene protocol described for the 1,2,3-triazole ureas.

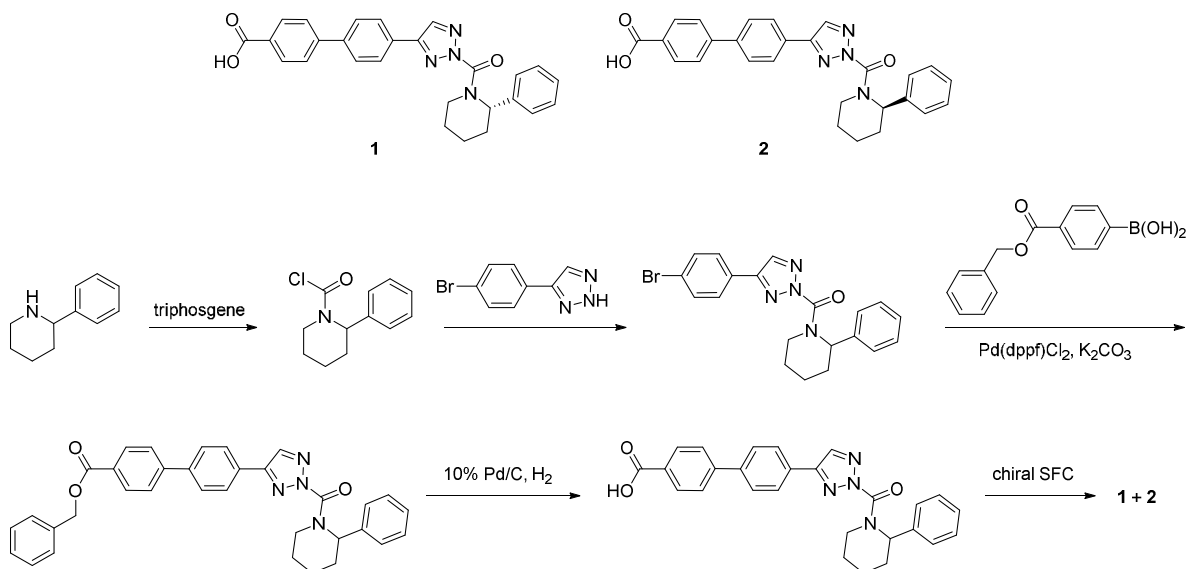
#### Urea method A: Triphosgene method

To a solution of the appropriate piperidine (1.26 mmol) in  $\text{CH}_2\text{Cl}_2$  (12 mL) was added triphosgene (261 mg, 0.88 mmol) and DIPEA (1.15 mL, 6.3 mmol) at 0 °C. The mixture was stirred at -10 °C for 15 min, then it was warmed up to rt and stirred for 2 h. LC-MS showed that the starting material was consumed completely. The solution was added into cold water (10 mL) and extracted with  $\text{CH}_2\text{Cl}_2$  (2 x 5 mL). The combined organic layers were dried ( $\text{Na}_2\text{SO}_4$ ), filtered and concentrated to give the crude carbamoyl chloride as an oil. The carbamoyl chloride was in the acylation step without further purification. To a solution of the crude carbamoyl chloride in THF (15 mL), was added 4-(5-(4-(1H-1,2,4-triazol-3-yl)phenyl)pyrimidin-2-yl)morpholine (427 mg, 1.38 mmol), DMAP (154 mg, 1.26 mmol) and DIPEA (1.15 mL, 6.3 mmol). The reaction mixture was stirred at 60 °C for 1 h. After cooling to rt, the solvent was removed under reduced pressure. The crude urea product was then purified via flash chromatography.

#### Urea method B: Bis(pentafluorophenyl) carbonate

To a 40 mL vial equipped with a magnetic stirrer was added 3-(4-bromophenyl)-1H-1,2,4-triazole (1.20 g, 5.4 mmol), bis(pentafluorophenyl) carbonate (2.40 g, 6.1 mmol) and anhydrous THF (22 mL). The mixture was cooled to 0 °C and triethylamine (3.0 mL, 22 mmol) was added slowly. The mixture was then allowed to then stirred at rt for 1.5 h. The mixture was re-cooled to 0 °C, and 2-phenylpiperidine HCl salt (950 mg, 5.9 mmol) was added as well as more trimethylamine (1.0 mL, 7.1 mmol). The mixture was allowed to warm to rt and stirred at this temperature for 1 h. The reaction mixture was diluted with  $\text{CH}_2\text{Cl}_2$  and washed with brine. The organic later was dried ( $\text{MgSO}_4$ ), filtered, and concentrated under reduced pressure to furnish the crude product which was further purified to afford the designate *N*-2 urea product typically in >70% yield.

## Synthesis of compounds 1 and 2



To a solution of 2-phenylpiperidine (620 mg, 3.85 mmol) in THF (40 mL) was added DIPEA (1.49 g, 11.5 mmol) followed by triphosgene (571 mg, 1.92 mmol) at 0 °C; the reaction mixture was stirred at 0 °C for 2 h. The suspension was then poured in cold water (10 mL) and extracted with EtOAc (3x 20 mL). The combined organic layers were washed with brine (2 x 20 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated under reduced pressure to give 920 mg (~100%) of the carbamoyl chloride as a pale yellow oil which was used in the next step without further purification. To a solution of the crude carbamoyl chloride (860 mg, 3.84 mmol) in THF (32 mL) was added 4-(4-bromophenyl)-2H-1,2,3-triazole (861mg, 3.84 mmol), DIPEA(1.49 g, 11.5 mmol) followed by DMAP (470 mg, 3.84 mmol) at rt. The reaction mixture was stirred at 60 °C for 12 h. The reaction mixture was combined with a parallel reaction [starting with 139 mg (0.621 mmol) of 4-(4-bromophenyl)-2H-1,2,3-triazole] and poured into aqueous NH<sub>4</sub>Cl (20 mL). The mixture was then extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine (2 x 20 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated to give a mixture of regioisomers (*N*-2 acylated urea and *N*-1 acylated urea). The regioisomers were separated by flash chromatography eluting with a gradient of petroleum ether:ethyl acetate (100:0 to 94:6) to deliver 900 mg (57%, combined yield) the desired *N*-2 acylated urea as a colorless gum. The regiochemistry of the product was confirmed by HMBC NMR experiment.

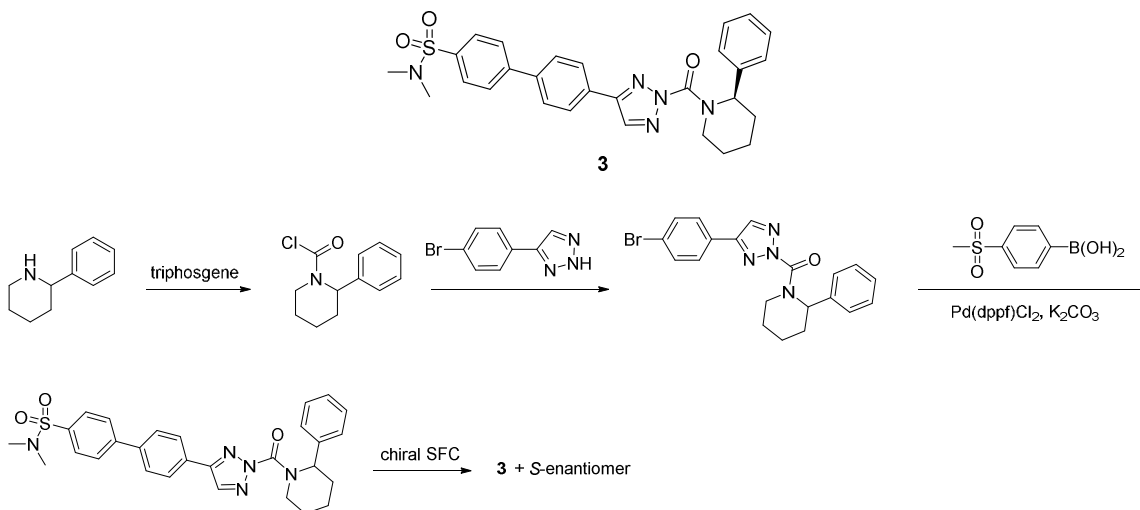
To a mixture of the desired *N*-2-acylated urea (400 mg, 0.973 mmol) in THF (12 mL) and H<sub>2</sub>O (1.2 mL) was added (4-((benzyloxy)carbonyl)phenyl)boronic acid (274 mg, 1.07 mmol), K<sub>2</sub>CO<sub>3</sub> (336 mg, 2.43 mol) and Pd(dppf)Cl<sub>2</sub> (71.2 mg, 0.0973 mmol) at rt. Then, the reaction was stirred at 80 °C under a nitrogen atmosphere for 16 h. The reaction mixture was concentrated in vacuum to give crude product. The crude of product was purified by flash chromatography eluting with a gradient of petroleum ether:ethyl acetate (100:0 to 85:15) to give deliver 362 mg (69%) of the desired product as a colorless gum.

To a solution of the benzyl ester (362 mg, 0.667 mmol) in anhydrous THF (20 mL) was added 10% Pd/C (159 mg), and the mixture was stirred under H<sub>2</sub> (50 psi) for 16 h at rt. The mixture was filtered washed with ethyl acetate (3 x 50 mL) and MeOH (3 x 20 mL). The filtrate was concentrated in vacuum to give 290 mg (96%) of the racemic carboxylic acid as a white solid. 250 mg of the racemic carboxylic acid was then separated by chiral SFC method 1 to afford 61 mg of carboxylic acid **1** and 63.1 mg of carboxylic acid **2**.

**(*S*)-4'-(2-(2-phenylpiperidine-1-carbonyl)-2H-1,2,3-triazol-4-yl)-[1,1'-biphenyl]-4-carboxylic acid (1).** <sup>1</sup>H NMR (600 MHz, DMSO-d<sub>6</sub>) δ 13.02 (br s, 1 H), 8.72 (s, 1 H), 8.04 (m, 4 H), 7.90 (d, *J*=8.2 Hz, 2 H), 7.89 (d, *J*= 8.2 Hz, 2 H), 7.47-7.40 (m, 4 H), 7.35 - 7.28 (m, 1 H), 5.60 - 5.42 (m, 1 H), 3.97-3.79 (m, 1 H), 3.07-3.03 (m, 1 H), 2.02 (m, 1 H), 1.76 - 1.61 (m, 3 H), 1.51-1.42 (m, 1 H), 1.30-1.18 (m, 1 H); <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>) δ 167.10, 149.87, 148.01, 143.24, 139.80, 138.09, 134.13, 130.14, 129.97, 128.83, 128.57, 127.64, 126.96, 126.85, 126.76, 126.39, 53.23, 27.63, 24.99, 18.93, 17.20. HRMS (ESI) *m/z* [M+Na]<sup>+</sup> calcd for C<sub>27</sub>H<sub>24</sub>N<sub>4</sub>O<sub>3</sub>Na 475.1741, found 475.1741.

**(*R*)-4'-(2-(2-phenylpiperidine-1-carbonyl)-2H-1,2,3-triazol-4-yl)-[1,1'-biphenyl]-4-carboxylic acid (2).** <sup>1</sup>H NMR (600 MHz, DMSO-d<sub>6</sub>) δ 13.02 (br s, 1 H), 8.72 (s, 1 H), 8.04 (m, 4 H), 7.90 (d, *J*=8.2 Hz, 2 H), 7.89 (d, *J*= 8.2 Hz, 2 H), 7.47-7.40 (m, 4 H), 7.35 - 7.28 (m, 1 H), 5.60 - 5.42 (m, 1 H), 3.97-3.79 (m, 1 H), 3.07-3.03 (m, 1 H), 2.02 (br. s., 1 H), 1.76 - 1.61 (m, 3 H), 1.51-1.42 (m, 1H), 1.30-1.18 (m, 1 H); <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>) δ 167.13, 149.87, 148.01, 143.20, 139.82, 138.09, 134.13, 130.23, 129.97, 128.83, 128.57, 127.64, 126.96, 126.85, 126.75, 126.39, 53.23, 27.62, 24.99, 18.92, 17.20. HRMS (ESI) *m/z* [M+Na]<sup>+</sup> calcd for C<sub>27</sub>H<sub>24</sub>N<sub>4</sub>O<sub>3</sub>Na 475.1741, found 475.1740.

### Synthesis of Compounds 3-6

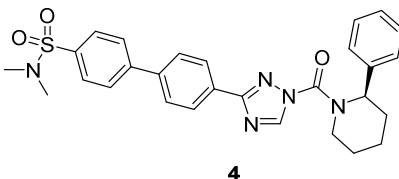


Step 1 (urea formation): In a round-bottom flask equipped with a magnetic stirrer were added 2-phenylpiperidine (6.91 g, 42.8 mmol) and THF (400 mL). The mixture was cooled to 0 °C with ice. DIPEA (22.4 mL, 129 mmol) was added followed by the addition of triphosgene (6.5 g, 21.4 mmol) portion wise. The reaction mixture was then stirred at rt for 2 h. The mixture was poured into cold water (100 mL), extracted with ethyl acetate (2x 200 mL). The combined organic layers was dried (MgSO<sub>4</sub>), filtered and concentrated to furnish 2-phenylpiperidine-1-carbonyl chloride which was immediately dissolved in THF (200 mL). To this solution were added Hunig's base (22.4 mL, 129 mmol) and DMAP (5.23 g, 42.8 mmol). A solution of 4-(4-bromophenyl)-1H-1,2,3-triazole (9.60 g, 43.0 mmol) in THF (200 mL) was added to the reaction mixture which was then heated at 60 °C for 12 h. The reaction was quenched with saturated aqueous NH<sub>4</sub>Cl solution (200 mL). The mixture was extracted with ethyl acetate (3 x 250 mL). The organic layers were combined, washed with brine (200 mL), dried (MgSO<sub>4</sub>), filtered and concentrated under reduced pressure. The crude material was purified on a silica gel column (220 g) twice, eluting with a gradient of ethyl acetate/heptane (5:95 to 25:75), to afford the separated pure regioisomers: 2.1 g (12%) of *N*-1 acylated isomer (4-(4-bromophenyl)-1H-1,2,3-triazol-1-yl)(2-phenylpiperidin-1-yl)methanone as a white solid; 3.3 g (19%) of *N*-2 acylated isomer (4-(4-bromophenyl)-2H-1,2,3-triazol-2-yl)(2-phenylpiperidin-1-yl)methanone as a white solid.

Step 2 (Suzuki coupling): To a septa capped vial were added (4-(4-bromophenyl)-2H-1,2,3-triazol-2-yl)(2-phenylpiperidin-1-yl)methanone (300 mg, 0.729 mmol), 4-(di(1-oxidanyl)boranyl)-*N,N*-dimethylbenzenesulfonamide (200 mg, 0.873 mmol) and Pd(dppf)Cl<sub>2</sub>·CH<sub>2</sub>Cl<sub>2</sub> (90 mg, 0.11 mmol). The mixture was evacuated and backfilled with nitrogen (3x). DMF (8 mL) was added followed by the addition of a 2.0 M solution of Na<sub>2</sub>CO<sub>3</sub> (0.90 mL, 1.8 mmol). The reaction mixture was evacuated and backfilled

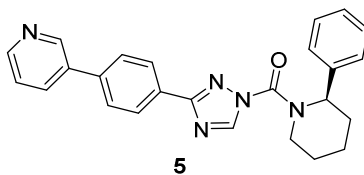
with nitrogen (3x) and then heated at 70 °C for 16 h. The reaction was then quenched with saturated aqueous solution of NH<sub>4</sub>Cl (3 mL) followed by the addition of water (10 mL). The mixture was then extracted with ethyl acetate (3x 50 mL). Organic phases were combined, washed with brine (3x 50 mL), dried (MgSO<sub>4</sub>), filtered and concentrated *in vacuo*. The residue was purified on a silica column (40 g) eluting with a gradient of ethyl acetate/heptane (10:90 to 90:10) to furnish 320 mg (82%) of *N,N*-dimethyl-4'-(2-(2-phenylpiperidine-1-carbonyl)-2H-1,2,3-triazol-4-yl)-[1,1'-biphenyl]-4-sulfonamide as an off white solid. Chiral separation was achieved using chiral SFC method 2 to afford the enantiomers.

**(*R*)-*N,N*-dimethyl-4'-(2-(2-phenylpiperidine-1-carbonyl)-2H-1,2,3-triazol-4-yl)-[1,1'-biphenyl]-4-sulfonamide (3).** <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 8.73 (s, 1H), 8.07 (d, *J* = 8.1 Hz, 2 H), 8.04 - 8.00 (m, 2H), 7.93 (d, *J* = 8.6 Hz, 2 H), 7.88 - 7.83 (m, 2 H), 7.48 - 7.40 (m, 4 H), 7.32 (s, 1 H), 5.65 - 5.44 (m, 1 H), 4.09 - 3.71 (m, 1 H), 3.17 - 2.93 (m, 1 H), 2.66 (s, 6 H), 2.50 (br. s., 1H), 2.15 - 1.90 (m, 1H), 1.69 (d, *J* = 14.4 Hz, 3 H), 1.55 - 1.36 (m, 1 H); <sup>13</sup>C NMR (126 MHz, DMSO-*d*<sub>6</sub>) δ 150.36, 148.44, 144.00, 139.60, 138.57, 134.68, 134.33, 129.43, 129.35, 128.75, 128.33, 127.99, 127.47, 127.43, 126.90, 55.90, 43.39, 38.08, 28.13, 25.50, 19.44; HRMS-ESI (*m/z*) [M+Na]<sup>+</sup> calcd for C<sub>28</sub>H<sub>29</sub>N<sub>5</sub>O<sub>3</sub>Na 538.1883, found 538.1886.



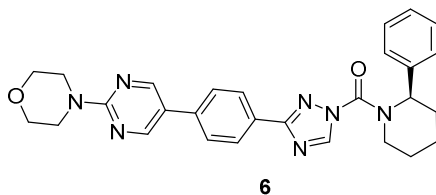
Compound **4** was synthesized in a similar manner as compounds **3** except 3-(4-bromophenyl)-1H-1,2,4-triazole was used for the urea formation and (4-(*N,N*-dimethylsulfamoyl)phenyl)boronic acid was required for the Suzuki reaction.

**(*R*)-4'-(1-(2-phenylpiperidine-1-carbonyl)-1H-1,2,4-triazol-3-yl)-[1,1'-biphenyl]-4-sulfonamide (4).** <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 9.23 (s, 1 H), 8.13 (d, *J* = 8.3 Hz, 2 H), 8.04 - 7.97 (m, 2 H), 7.94 - 7.88 (m, 2 H), 7.87 - 7.81 (m, 2 H), 7.48 - 7.42 (m, 4 H), 7.33 (br. s., 1 H), 5.73 - 5.66 (m, 1 H), 4.26 - 4.16 (m, 1 H), 3.09 - 2.97 (m, 1 H), 2.66 (s, 6 H), 2.13 - 1.98 (m, 1 H), 1.68 (d, *J* = 1.5 Hz, 3 H), 1.56 - 1.42 (m, 1H), 1.30 - 1.26 (m, 1H); <sup>13</sup>C NMR (126 MHz, DMSO-*d*<sub>6</sub>) δ 166.96, 160.84, 149.53, 148.16, 143.55, 139.70, 138.26, 133.88, 129.79, 128.77, 128.26, 127.68, 127.52, 126.97, 126.89, 126.52, 67.38, 55.65, 42.93, 31.23, 28.35, 24.94; HRMS (ESI) *m/z* [M+H]<sup>+</sup> calcd for C<sub>28</sub>H<sub>30</sub>N<sub>5</sub>O<sub>3</sub>S 516.2064, found 516.2065.



Compound **5** was synthesized in a similar manner as compound **3** except 3-(4-bromophenyl)-1H-1,2,4-triazole was used for the urea formation and pyridin-3-ylboronic acid was required for the Suzuki reaction.

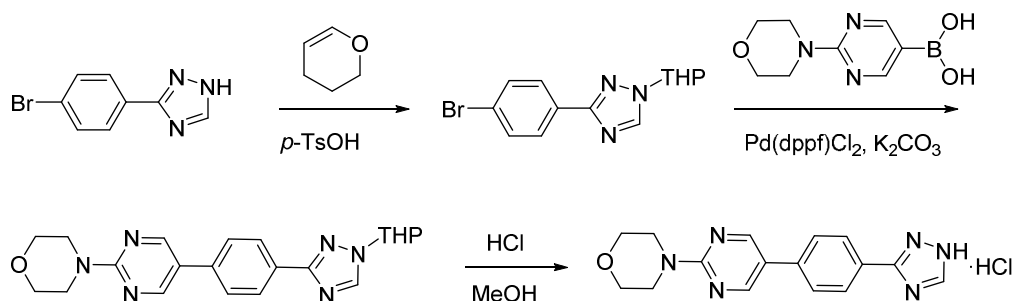
**(R)-(2-phenylpiperidin-1-yl)(3-(4-(pyridin-3-yl)phenyl)-1H-1,2,4-triazol-1-yl)methanone (5).**  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  9.22 (s, 1 H), 8.96 (d,  $J$  = 2.0 Hz, 1H), 8.60 (dd,  $J$  = 1.5, 4.9 Hz, 1 H), 8.19 - 8.04 (m, 3 H), 7.94 - 7.79 (m, 2 H), 7.55 - 7.49 (m, 1H), 7.47 - 7.40 (m, 4 H), 7.35 - 7.26 (m, 1 H), 5.69 (br. s., 1 H), 4.19 (d,  $J$  = 13.7 Hz, 1H), 3.10 - 2.92 (m, 1 H), 2.49 - 2.45 (m, 1 H), 2.10 - 1.93 (m, 1 H), 1.84 - 1.61 (m, 3 H), 1.55 - 1.38 (m, 1 H);  $^{13}\text{C}$  NMR (126 MHz, DMSO- $d_6$ )  $\delta$  161.24, 149.87, 149.20, 148.48, 147.97, 138.85, 138.59, 135.08, 134.45, 129.72, 129.10, 128.89, 127.73, 127.28, 127.23, 126.85, 124.26, 56.00, 43.25, 27.93, 25.27, 19.31; HRMS (ESI)  $m/z$   $[\text{M}+\text{Na}]^+$  calcd for  $\text{C}_{25}\text{H}_{23}\text{N}_5\text{ONa}$  432.1795, found 432.1802.



Compound **6** was synthesized in a similar manner as compound **3** except 3-(4-bromophenyl)-1H-1,2,4-triazole was used for the urea formation and (2-morpholinopyrimidin-5-yl)boronic acid was required for the Suzuki reaction.

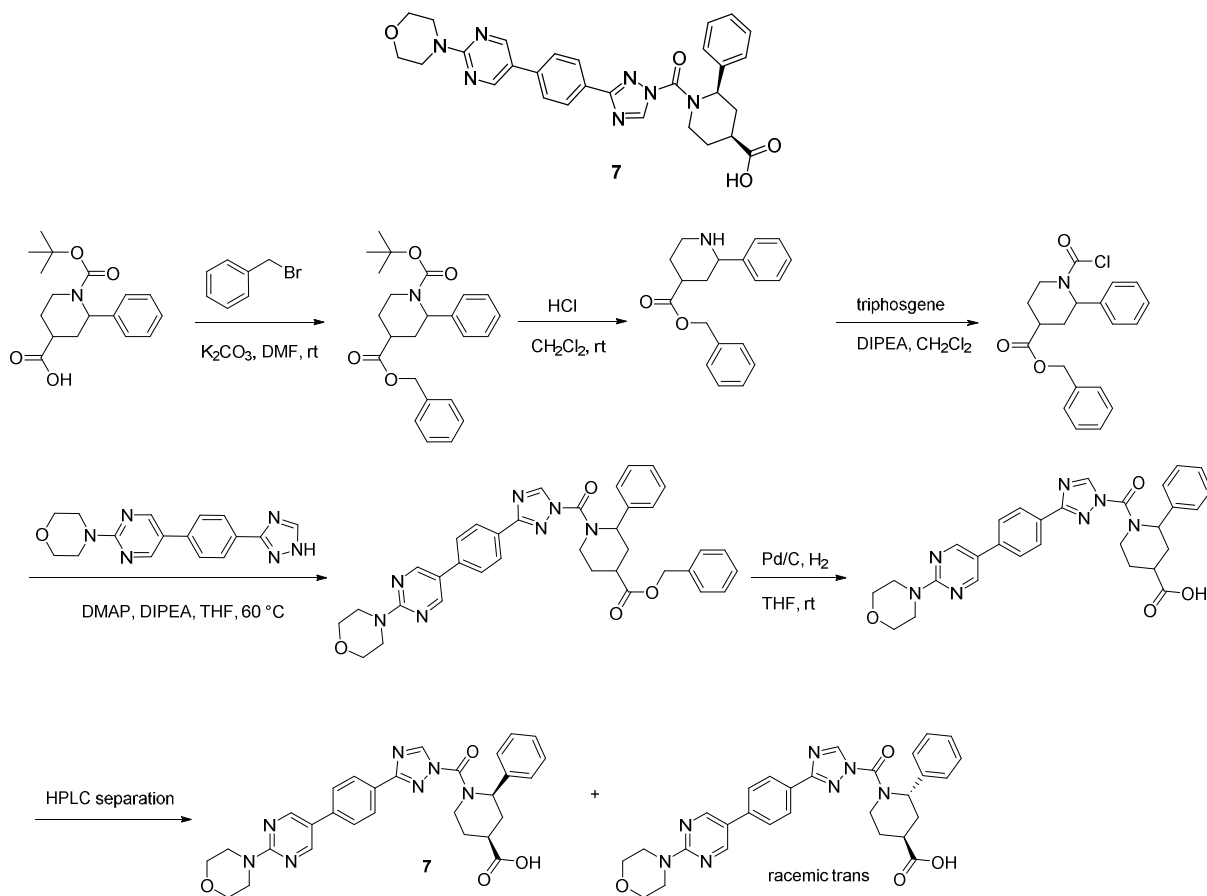
**(R)-(3-(4-(2-morpholinopyrimidin-5-yl)phenyl)-1H-1,2,4-triazol-1-yl)(2-phenylpiperidin-1-yl)methanone (6).**  $^1\text{H}$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  9.18 (s, 1 H), 8.79 (s, 2 H), 8.04 (d,  $J$  = 8.2 Hz, 2 H), 7.79 (d,  $J$  = 8.2 Hz, 2 H), 7.45 - 7.39 (m, 4 H), 7.32-7.29 (m, 1 H), 5.68 (br. s., 1H), 4.18 (d,  $J$  = 14.1 Hz, 1H), 3.78 - 3.73 (m, 4 H), 3.70 - 3.63 (m, 4 H), 2.99 (t,  $J$  = 12.3 Hz, 1 H), 2.06 - 1.97 (m, 1 H), 1.79-1.70 (m, 1H), 1.70 - 1.61 (m, 2 H), 1.50-1.42 (m, 1 H), 1.28-1.19 (m, 1 H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  161.01, 160.67, 155.81, 149.54, 148.05, 138.26, 136.40, 128.75, 128.37, 126.88, 126.52, 125.75, 121.41, 65.93, 55.66, 44.00, 42.88, 27.59, 24.92, 18.97. HRMS (ESI)  $m/z$   $[\text{M}+\text{H}]^+$  calcd for  $\text{C}_{28}\text{H}_{30}\text{N}_7\text{O}_2$  496.2455, found 496.2449.

A key intermediate for compounds **7-12**: Synthesis of 4-(5-(4-(1H-1,2,4-triazol-3-yl)phenyl)pyrimidin-2-yl)morpholine hydrochloride:



To a solution of 3-(4-bromophenyl)-1H-1,2,4-triazole (20.0 g, 89.3 mmol) in THF (300 mL) was added 3,4-dihydro-2H-pyran (15 g, 178.6 mmol) and 4-methylbenzenesulfonic acid (3.4 g, 17.8 mmol). The mixture was stirred at 70 °C for 16 h. The mixture was cooled down to rt, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (silica gel) eluting with a gradient of petroleum ether: ethyl acetate (100:0 to 70:30) to afford 21 g (76%) of 3-(4-bromophenyl)-1-(tetrahydro-2H-pyran-2-yl)-1H-1,2,4-triazole as a white solid. To a mixture of 3-(4-bromophenyl)-1-(tetrahydro-2H-pyran-2-yl)-1H-1,2,4-triazole (18.3 g, 59.6 mmol), 2-morpholino-pyrimidin-5-ylboronic acid (15 g, 71.7 mmol) in THF (280 mL) was added a solution of K<sub>2</sub>CO<sub>3</sub> (16.5 g, 119.6 mmol) in water (70 mL) and Pd(dppf)Cl<sub>2</sub> (3.66 g, 4.99 mmol) successively. The vessel was degassed and then refilled with N<sub>2</sub> (g). The mixture was stirred at 75 °C for 16 h. After cooling to rt, the mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel) eluting with a gradient of CH<sub>2</sub>Cl<sub>2</sub>:MeOH (100:0 to 95:5) to afford 21.1 g (89%) of 4-(5-(4-(1-(tetrahydro-2H-pyran-2-yl)-1H-1,2,4-triazol-3-yl)phenyl)pyrimidin-2-yl)morpholine as a brown solid. A solution of 4-(5-(4-(1-(tetrahydro-2H-pyran-2-yl)-1H-1,2,4-triazol-3-yl)phenyl)pyrimidin-2-yl)morpholine (21.1 g, 53.8 mmol) in hydrogen chloride in MeOH (1 M, 150 mL, 150 mmol) was stirred at rt for 30 min. The mixture was concentrated under reduced pressure. MeOH (100 mL) was added to the resulting residue, and the suspension was stirred at rt for 2 h. The mixture was filtered to give 17.7 g (95%) of 4-(5-(4-(1H-1,2,4-triazol-3-yl)phenyl)pyrimidin-2-yl)morpholine hydrochloride as an off-white solid. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 10.76 (br s, 2 H), 8.83 (s, 2 H), 8.72 (s, 1 H), 8.12 (d, *J* = 8.3 Hz, 2 H), 7.83 (d, *J* = 8.3 Hz, 2 H), 3.81 - 3.76 (m, 4 H), 3.73 - 3.66 (m, 4 H); LRMS (ESI) *m/z* 309.2 (M + H)<sup>+</sup>.

## Synthesis of compound 7



A mixture of 1-(tert-butoxycarbonyl)-2-phenylpiperidine-4-carboxylic acid (0.61 g, 2.0 mmol), (bromomethyl)benzene (0.51 g, 3.0 mmol),  $K_2CO_3$  (0.552 g, 4.0 mmol) and DMF (20 mL) was stirred at rt overnight. The reaction mixture was diluted with water (100 mL) and extracted with ethyl acetate (3 x 40 mL). The combined organic layers were dried ( $Na_2SO_4$ ), filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography (silica gel), eluting with a gradient of ethyl acetate/petroleum ether (0:100 to 20:80) to give 0.64 g (81%) of 4-benzyl 1-tert-butyl 2-phenylpiperidine-1,4-dicarboxylate as colorless oil. LRMS (ESI-API)  $m/z$  296.2 (M-BOC).

To a solution of 4-benzyl 1-tert-butyl 2-phenylpiperidine-1,4-dicarboxylate (1.27 g, 3.2 mmol) in  $CH_2Cl_2$  (30 mL) was bubbled HCl (gas) for 30 min, and the resulting mixture was stirred at rt for 2 h. The reaction mixture was diluted with  $CH_2Cl_2$  (20 mL) and washed with saturated aqueous  $NaHCO_3$  solution. The aqueous phase was extracted with  $CH_2Cl_2$  (50 mL). The combined organic layers was dried ( $Na_2SO_4$ ) and concentrated *in*



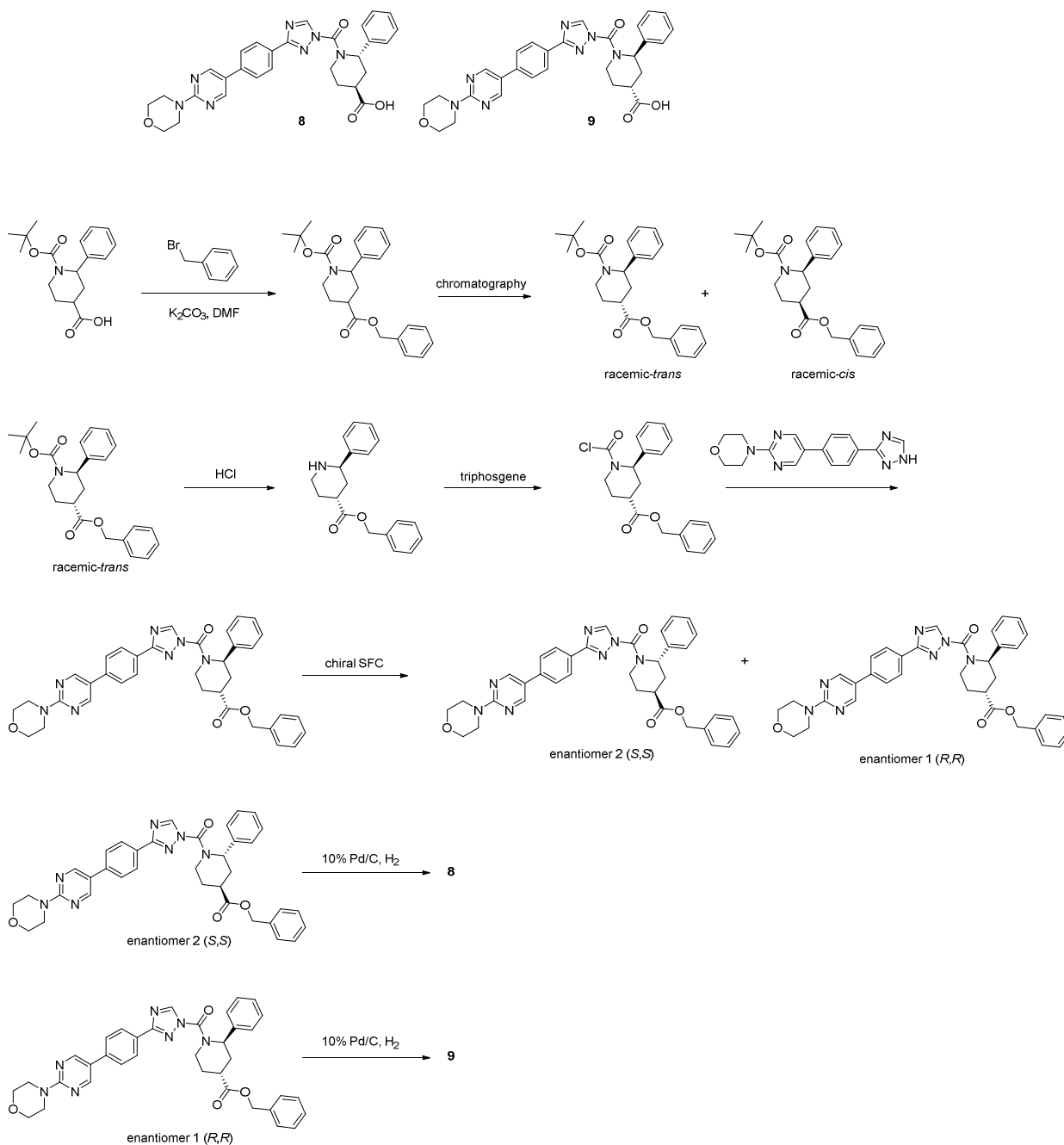
*vacuo* to give 0.86 g (85%) of benzyl 2-phenylpiperidine-4-carboxylate as light yellow oil. LRMS (ESI-API)  $m/z$  (M+H)<sup>+</sup> 296.2

The corresponding urea was then synthesized using the urea method A (triphosgene method) with the carbamoyl chloride of benzyl 2-phenylpiperidine-4-carboxylate (1.05 g, 2.73 mmol) and *bis*-HCl of 4-(5-(4-(1H-1,2,4-triazol-3-yl)phenyl)pyrimidin-2-yl)morpholine (1.04 g, 2.73 mmol) to deliver 1.3 g of a diastereomeric benzyl esters.

A mixture of the diastereomeric benzyl esters (1.3 g, 2.1 mmol), 10% Pd/C (0.26 g, 56.5% water) and THF (20 mL) was degassed with hydrogen gas (6x), and then stirred under 1 atm of hydrogen for 4 h at rt. Then, the mixture was filtered, and the filtrate was concentrated under reduced pressure to give 1.2 g crude product. 900 mg of the diastereomeric carboxylic acids were then purified by reverse phase chromatography (Gemini-C18; 150×21.2 mm; 5μm, eluting with CH<sub>3</sub>CN-H<sub>2</sub>O (0.1% TFA) from 50% to 55% at a flow rate of 20 mL/min) to deliver impure racemic-*cis* **7** and racemic-*trans*. Compound **7** was further purified via prep-HPLC (Gemini-C18; 150×21.2 mm, 5μm eluting with CH<sub>3</sub>CN-H<sub>2</sub>O (0.1% FA) from 48% to 55% at a flow rate of 20 mL/min to give racemic *cis*-1-(3-(4-(2-morpholinopyrimidin-5-yl)phenyl)-1H-1,2,4-triazole-1-carbonyl)-2-phenylpiperidine-4-carboxylic acid (**7**) as white solid (240 mg). The racemic-*trans* compound was then added to 1 N HCl (5 mL) and lyophilized to give the HCl salt of racemic *trans*-1-(3-(4-(2-morpholinopyrimidin-5-yl)phenyl)-1H-1,2,4-triazole-1-carbonyl)-2-phenylpiperidine-4-carboxylic acid as yellow solid (121 mg). See compounds **8** and **9** for the full characterization of the racemic-*trans* enantiomers.

**Racemic-*cis*-1-(3-(4-(2-morpholinopyrimidin-5-yl)phenyl)-1H-1,2,4-triazole-1-carbonyl)-2-phenylpiperidine-4-carboxylic acid (**7**).** <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 12.31 (m, 1H), 9.08 (s, 1 H), 8.81 (s, 2 H), 8.08 - 8.01 (m, 2 H), 7.85 - 7.76 (m, 2 H), 7.42 - 7.31 (m, 4 H), 7.30 - 7.22 (m, 1 H), 5.33 (dd, *J* = 6.4, 9.3 Hz, 1 H), 4.31 - 4.20 (m, 1 H), 3.81 - 3.74 (m, 4 H), 3.72 - 3.66 (m, 4 H), 3.66 - 3.57 (m, 1 H), 2.85 - 2.71 (m, 1H), 2.39 (ddd, *J* = 4.6, 6.0, 13.8 Hz, 1 H), 2.30 - 2.20 (m, 1H), 2.19 - 2.10 (m, 1H), 1.98 - 1.89 (m, 1H); <sup>13</sup>C NMR (126 MHz, DMSO-d<sub>6</sub>) δ 175.78, 161.12, 160.86, 156.01, 149.55, 147.93, 141.28, 136.57, 128.58, 128.53, 127.10, 127.03, 125.94, 125.74, 121.63, 66.12, 57.70, 44.18, 41.20, 35.56, 31.58, 25.19; HRMS (ESI)  $m/z$  [M+H]<sup>+</sup> calcd for C<sub>29</sub>H<sub>30</sub>N<sub>7</sub>O<sub>4</sub> 540.2354, found 540.2356.

## Synthesis of Compounds 8 and 9



A solution of *N*-Boc-2-phenyl-isonipecotic acid (6.00 g, 19.7 mmol) in DMF (30 mL) was treated with  $\text{K}_2\text{CO}_3$  (6.40 g, 40 mmol) followed by benzyl bromide (4.20 g, 24.6 mmol). The reaction mixture was stirred at rt for 3 h and then concentrated under reduced pressure. The crude product was purified by flash chromatography (silica gel) eluting

with a gradient of heptane:EtOAc (100:0 to 85:15) to deliver 2.1 g of the racemic *trans*-isomer and 5.5 g of a *cis:trans* mixture (5:1). racemic-*trans*:  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.40-7.31 (m, 7 H), 7.26-7.19 (m, 3 H), 5.56 (br s, 1 H), 5.14 (s, 2 H), 4.15 (br s, 1 H), 2.80 (td,  $J = 13.5, 2.9$  Hz, 1 H), 2.67-2.64 (m, 1 H), 2.58-2.52 (m, 1 H), 2.04 (td,  $J = 13.0, 5.4$  Hz, 1 H), 1.91-1.88 (m, 1 H), 1.67 (qd,  $J = 13.0, 4.9$  Hz, 1 H), 1.48 (s, 9 H). LRMS (ESI)  $m/z$  296.36 (M-BOC+H) $^+$ . 2D NMR experiments (i.e., HSQC, COSY, and ROESY) used to support relative *trans*-stereochemistry.

A solution of the racemic *trans*-isomer (2.1 g, 5.3 mmol) in  $\text{CH}_2\text{Cl}_2$  (20 mL) was treated with HCl in dioxane (4 N, 20 mL), and the reaction mixture was stirred at rt for 1 h. The mixture was concentrated under reduced pressure and dissolved in  $\text{CH}_2\text{Cl}_2$  (50 mL). The resulting solution was then washed with saturated aqueous sodium carbonate (50 mL). The aqueous layer was then extracted with  $\text{CH}_2\text{Cl}_2$  (2 x 50 mL). The combined organic layers were dried ( $\text{MgSO}_4$ ), filtered, and concentrated under reduced pressure to deliver 1.71 g piperidine hydrochloride as a colorless residue.

To the solution of the amine (1.70 g, 5.80 mmol) in  $\text{CH}_2\text{Cl}_2$  (90 mL) was added DIPEA (2.02 mL, 11.6 mmol) and triphosgene (1.21 g, 4.06 mmol) successively at 0 °C. After stirring at 0 °C for 30 min, the reaction mixture was warmed up to rt and stirred overnight. The mixture was poured into cold water (50 mL) and extracted with  $\text{CH}_2\text{Cl}_2$  (2 x 50 mL). The combined organic layers were dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated *in vacuo* to give 2.05 g of the acid chloride as a light yellow oil residue. The acid chloride was used in the next step without further purification.

To the solution of the carbamoyl chloride (1.90 g, 5.30 mmol) in THF (60 mL) was added DIPEA (2.8 mL, 16 mmol), DMAP (0.648 g, 5.3 mmol) and 4-(5-(4-(1H-1,2,4-triazol-3-yl) phenyl)pyrimidin-2-yl)morpholine (2.19 g, 6.4 mmol) successively at rt. Then, the mixture was heated to 60 °C and stirred under  $\text{N}_2$  for 5 h. the mixture was then concentrated under reduced pressure. The crude product was purified via flash chromatography (silica gel) eluting with a gradient of heptane:EtOAc (100:0 to 55:45) to afford 2.8 g (84%) of the desired racemic urea as a white solid. The racemate was purified by chiral SFC ( $\text{MeOH}/\text{CO}_2$ ) to deliver 1.01 g of enantiomer 1 [presumed to be (*R,R*), LRMS (ESI)  $m/z$  630.56 (M+H) $^+$ ] and 1.07 g of enantiomer 2 [presumed to be (*S,S*), LRMS (ESI)  $m/z$  630.58 (M+H) $^+$ ].

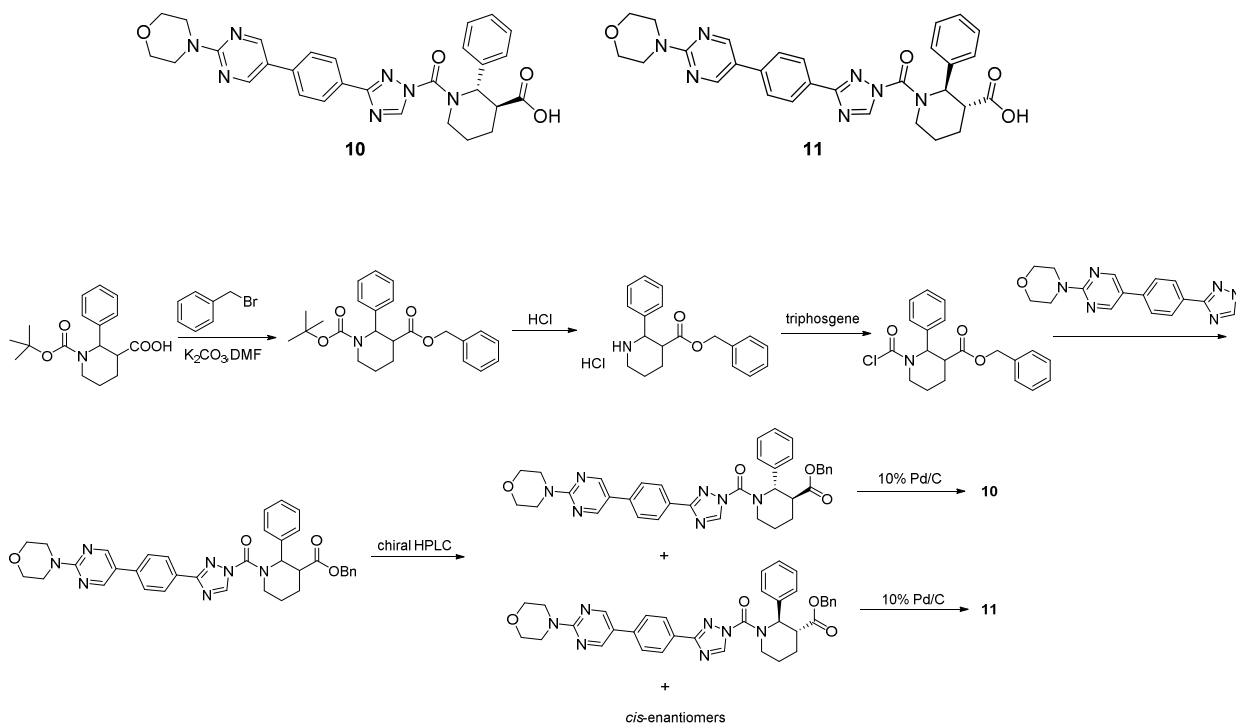
Enantiomer 2 (*S,S*) (700 mg, 1.10mmol) in THF (70 mL) was partitioned between 7 vials equipped with stir bars. Each vial was charged with 10% Pd/C (50% water, 100 mg) and hydrogenated at rt at 100 psi for 20 h. The mixture were combined and filtered through celite (~1 g) and rinsed with EtOAc (5 x 10 mL). The combined organic filtrates were concentrated to furnish white solid. The crude product was purified by flash chromatography eluting with a gradient of heptane:1% formic acid in EtOAc (100:0 to 0:100) to deliver 180 mg (30%) of **8** as a white solid.

**(2*S*,4*S*)-1-(3-(4-(2-morpholinopyrimidin-5-yl)phenyl)-1*H*-1,2,4-triazole-1-carbonyl)-2-phenylpiperidine-4-carboxylic acid (8).** <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 12.43 (br. s., 1 H), 9.21 (s, 1 H), 8.90 - 8.73 (m, 2 H), 8.22 - 7.95 (m, 2 H), 7.80 (d, *J*=7.8 Hz, 2 H), 7.58 - 7.42 (m, 4 H), 7.39 - 7.22 (m, 1 H), 5.82 (br. s., 1 H), 4.28 (d, *J*=13.7 Hz, 1 H), 3.88 - 3.73 (m, 4 H), 3.73 - 3.64 (m, 4 H), 3.03 (br. s., 1 H), 2.75 (d, *J*=13.7 Hz, 1 H), 2.49 - 2.42 (m, 1 H), 2.15 - 2.04 (m, 1 H), 1.92 (d, *J*=10.5 Hz, 1 H), 1.82 (br. s., 1 H). <sup>13</sup>C NMR (126 MHz, DMSO-*d*<sub>6</sub>) δ 175.42, 161.04, 160.69, 155.83, 149.53, 148.21, 137.61, 136.44, 128.90, 128.34, 127.11, 126.91, 126.45, 125.78, 121.42, 65.95, 55.29, 44.00, 42.82, 36.03, 30.20, 27.64. HRMS (ESI) *m/z* [M+H]<sup>+</sup> calcd for C<sub>29</sub>H<sub>30</sub>N<sub>7</sub>O<sub>4</sub> 540.2354, found: 540.2354

Enantiomer 1 (*R,R*) (1.01 g, 1.60 mmol) in THF (120 mL) was partitioned between 12 vials equipped with stir bars. Each vial was charged with 10% Pd/C (50% water, 100 mg) and hydrogenated at rt at 100 psi for 20 h. The mixture were combined and filtered through celite (~1 g) and rinsed with EtOAc (5 x 10 mL). The combined organic filtrates were concentrated to furnish 705 mg of white solid. The crude product was purified by flash chromatography eluting with a gradient of heptane:1% formic acid in EtOAc (100:0 to 0:100) to deliver 417 mg (47%) of **9** as a white solid.

**(2*R*, 4*R*)-1-(3-(4-(2-morpholinopyrimidin-5-yl)phenyl)-1*H*-1,2,4-triazole-1-carbonyl)-2-phenylpiperidine-4-carboxylic acid (9).** <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 12.43 (br. s., 1H), 9.21 (s, 1H), 8.90 - 8.73 (m, 2 H), 8.22 - 7.95 (m, 2 H), 7.80 (d, *J* = 7.8 Hz, 2 H), 7.58 - 7.42 (m, 4 H), 7.39 - 7.22 (m, 1 H), 5.82 (br. s., 1 H), 4.28 (d, *J* = 13.7 Hz, 1 H), 3.88 - 3.73 (m, 4 H), 3.73 - 3.64 (m, 4 H), 3.03 (br. s., 1 H), 2.75 (d, *J* = 13.7 Hz, 1H), 2.49 - 2.42 (m, 1 H), 2.15 - 2.04 (m, 1 H), 1.92 (d, *J* = 10.5 Hz, 1 H), 1.82 (br. s., 1 H); <sup>13</sup>C NMR (126 MHz, DMSO-*d*<sub>6</sub>) δ 175.42, 161.04, 160.69, 155.83, 149.53, 148.21, 137.61, 136.44, 128.90, 128.34, 127.11, 126.91, 126.45, 125.78, 121.42, 65.95, 55.12, 44.00, 42.67, 36.03, 30.20, 27.64; HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd for C<sub>29</sub>H<sub>30</sub>N<sub>7</sub>O<sub>4</sub> 540.2354, found 540.2356.

## Synthesis of compounds 10 and 11.



To a solution of 1-(*tert*-butoxycarbonyl)-2-phenylpiperidine-3-carboxylic acid (12 g, 39.3 mmol) and benzyl bromide (13.44 g, 78.6 mmol) in DMF (100 mL) was added potassium carbonate (8.15 g, 59 mmol), and it was stirred at rt for 2 h. The mixture was quenched with water (300 mL), and it was extracted with ethyl acetate (4 x 200 mL). The combined organic layers were washed with brine (4 x 200 mL), dried ( $Na_2SO_4$ ), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel) eluting with a gradient of petroleum ether:ethyl acetate (80:20 to 75:25) to give 15 g (96%) of the desired benzyl ester. LRMS (ES-API)  $m/z$  340.2 ( $M-(t-Bu) + H$ )<sup>+</sup>.

To a solution of 3-benzyl 1-*tert*-butyl 2-phenylpiperidine-1,3-dicarboxylate (15 g, 37.9 mmol) in  $CH_2Cl_2$  was added 2 M HCl (100 mL) which was stirred at rt for 5 h. The mixture was concentrated under reduced pressure to afford the crude product (12.57 g, 100% yield) as a white solid. LRMS (ES-API)  $m/z$ , 296.2 ( $M + H$ )<sup>+</sup>.

The corresponding urea was then synthesized using the urea method A (triphosgene method) with the carbamoyl chloride of benzyl 2-phenylpiperidine-3-carboxylate (12.57 g, 37.9 mmol) and *bis*- HCl of 4-(5-(4-(1H-1,2,4-triazol-3-yl)phenyl)pyrimidin-2-yl)morpholine (11.68 g, 37.9 mmol) to deliver 11.9 g of the diastereomeric benzyl esters.

The diastereomeric benzyl esters (2.02 g, 3.2 mmol) were separated by chiral chromatography [Column: Chiralpak ID; mobile phase: hexane/CH<sub>2</sub>Cl<sub>2</sub>/diethyl amine (20:80:0.1 by volume)] to give (2*S*,3*S*)-benzyl 1-(3-(4-(2-morpholinopyrimidin-5-yl)phenyl)-1*H*-1,2,4-triazole-1-carbonyl)-2-phenylpiperidine-3-carboxylate (4.3 g) and (2*R*,3*R*)-benzyl 1-(3-(4-(2-morpholinopyrimidin-5-yl)phenyl)-1*H*-1,2,4-triazole-1-carbonyl)-2-phenylpiperidine-3-carboxylate (5.2 g) as white solids. Note: *cis*-enantiomers were also separated.

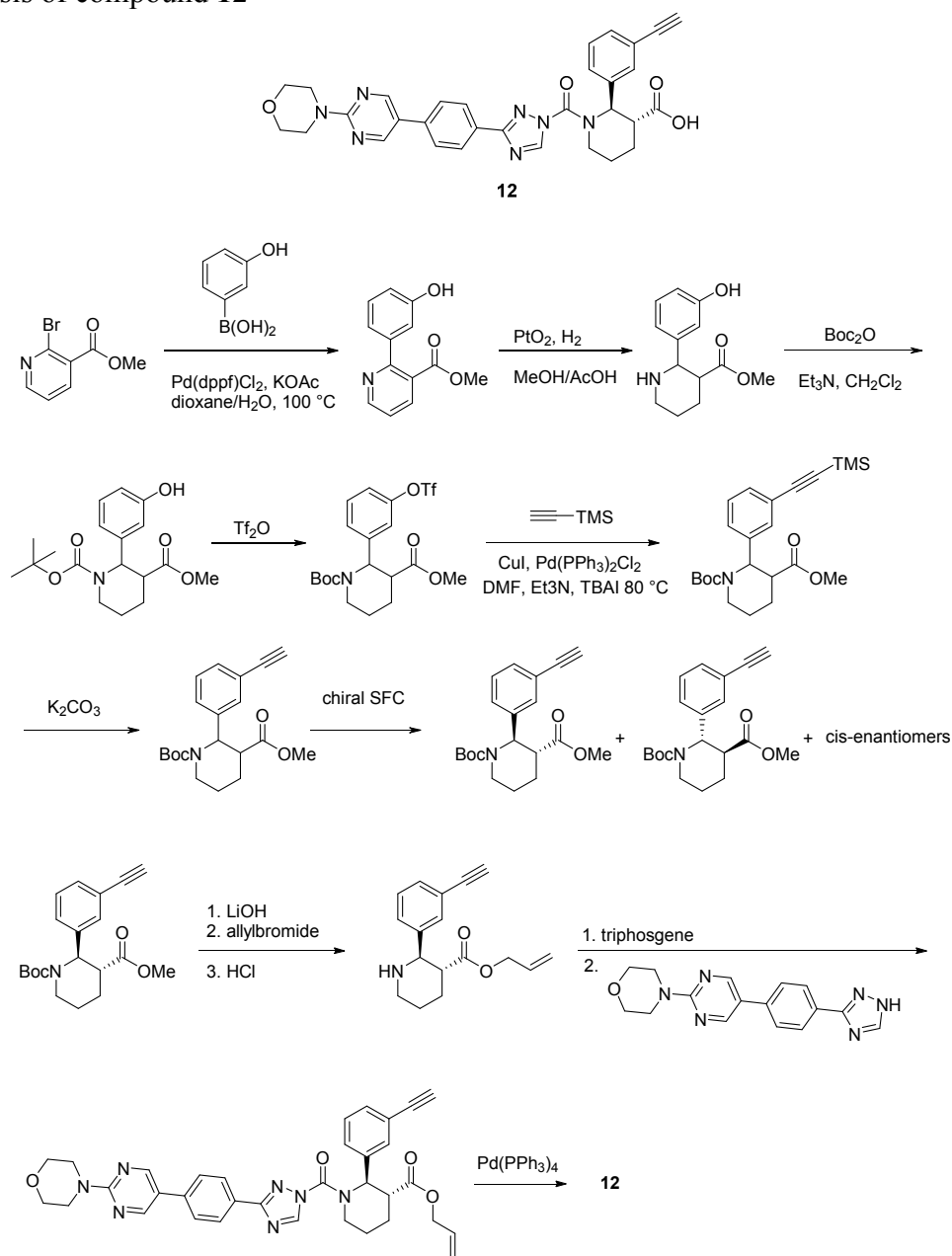
To a solution of (2*S*,3*S*)-benzyl 1-(3-(4-(2-morpholinopyrimidin-5-yl)phenyl)-1*H*-1,2,4-triazole-1-carbonyl)-2-phenylpiperidine-3-carboxylate (4.3 g, 6.83 mmol) in THF (200 mL) was added 10% Pd/C (215 mg), and it was stirred at rt for 4 h under hydrogen atmosphere. The mixture was filtered, and the filtrate concentrated under reduced pressure. The residue was triturated with petroleum ether:ethyl acetate (1:1), and crystallized from dichloromethane and hexane to give 2.8 g (76%) of compound **10** as a white solid.

**(2*S*,3*S*)-1-(3-(4-(2-morpholinopyrimidin-5-yl)phenyl)-1*H*-1,2,4-triazole-1-carbonyl)-2-phenylpiperidine-3-carboxylic acid (**10**).** <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 9.15 (s, 1 H), 8.77 (s, 2 H), 7.93 (d, *J*=7.1 Hz, 2 H), 7.75 (d, *J*=8.3 Hz, 2 H), 7.56 (d, *J*=7.8 Hz, 2 H), 7.46 (t, *J*=7.8 Hz, 2 H), 7.40 - 7.31 (m, 1 H), 6.04 (s, 1 H), 4.13 (d, *J*=13.2 Hz, 1 H), 3.79 - 3.72 (m, 4 H), 3.69 - 3.63 (m, 4 H), 3.52 (br. s., 1 H), 3.32 (br. s., 1 H), 2.98 - 2.86 (m, 1 H), 2.10 (d, *J*=10.8 Hz, 1 H), 1.80 - 1.73 (m, 1 H), 1.72 - 1.64 (m, 1 H), 1.63 - 1.52 (m, 1 H). <sup>13</sup>C NMR (126 MHz, DMSO-*d*<sub>6</sub>) δ 174.56, 160.72, 160.67, 155.81, 150.14, 147.93, 137.26, 136.34, 128.66, 128.39, 127.13, 126.75, 126.72, 125.73, 121.38, 65.95, 57.48, 44.01, 41.91, 41.55, 21.71, 21.64. HRMS (ESI) *m/z* [M+H]<sup>+</sup> calcd for C<sub>29</sub>H<sub>30</sub>N<sub>7</sub>O<sub>4</sub> 540.2354, found: 540.2355.

To a solution of (2*R*,3*R*)-benzyl 1-(3-(4-(2-morpholinopyrimidin-5-yl)phenyl)-1*H*-1,2,4-triazole-1-carbonyl)-2-phenylpiperidine-3-carboxylate (5.2 g, 8.25 mmol) in THF (200 mL) was added 10% Pd/C (260 mg), and it was stirred at rt for 4 h under hydrogen atmosphere. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was triturated with petroleum ether:ethyl acetate (1:1), and recrystallized from CH<sub>2</sub>Cl<sub>2</sub> and hexane to give 2.83 g (64%) of **11** as a white solid.

**(2*R*,3*R*)-1-(3-(4-(2-morpholinopyrimidin-5-yl)phenyl)-1*H*-1,2,4-triazole-1-carbonyl)-2-phenylpiperidine-3-carboxylic acid (**11**).** <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 9.15 (s, 1 H), 8.77 (s, 2 H), 7.93 (d, *J*=7.1 Hz, 2 H), 7.75 (d, *J*=8.3 Hz, 2 H), 7.56 (d, *J*=7.8 Hz, 2 H), 7.46 (t, *J*=7.8 Hz, 2 H), 7.40 - 7.31 (m, 1 H), 6.04 (s, 1 H), 4.13 (d, *J*=13.2 Hz, 1 H), 3.79 - 3.72 (m, 4 H), 3.69 - 3.63 (m, 4 H), 3.52 (br. s., 1 H), 3.32 (br. s., 1 H), 2.98 - 2.86 (m, 1 H), 2.10 (d, *J*=10.8 Hz, 1 H), 1.80 - 1.73 (m, 1 H), 1.72 - 1.64 (m, 1 H), 1.63 - 1.52 (m, 1 H); <sup>13</sup>C NMR (126 MHz, DMSO-*d*<sub>6</sub>) δ 174.52, 160.73, 160.66, 155.81, 150.12, 147.93, 137.18, 136.34, 128.66, 128.37, 127.13, 126.74, 125.72, 121.37, 67.00, 65.94,

57.41, 44.00, 41.99, 41.47, 25.1, 21.66, 21.62; HRMS (ESI)  $m/z$   $[M+H]^+$  calcd for  $C_{29}H_{30}N_7O_4$  540.2354, found 540.2353. Relative *trans*-stereochemistry was established by 2D NMR experiments. See 2D NMR section. The absolute stereochemistry of compound **11** was established by synthesizing compound **13** from **11** and obtaining x-ray crystallographic data of **13**.

Synthesis of compound **12**

To a mixture of methyl 2-bromonicotinate (18 g, 83.3 mmol) in 1,4-dioxane/water (120 mL/ 30 mL) was added 3-hydroxyphenylboronic acid (13.8 g, 100 mmol),  $\text{Na}_2\text{CO}_3$  (17.66 g, 166.6 mmol) and  $\text{Pd(dppf)Cl}_2$  (6 g, 8.3 mmol). The vessel was degassed and refilled with  $\text{N}_2$  (g). Then, the reaction mixture was stirred at  $100^\circ\text{C}$  for 2 h. The reaction mixture was cooled to rt and filtered through a celite pad. Water (40 mL) was added to the filtrate, and the mixture was extracted with  $\text{CH}_2\text{Cl}_2$  (3 x 80 mL). The combined organic layers were washed with brine (80 mL), dried ( $\text{Na}_2\text{SO}_4$ ), filtered and



concentrated. The residue was purified by column chromatography (silica gel) eluting with a gradient of  $\text{CH}_2\text{Cl}_2$ :MeOH (100:0 to 80:20) to deliver 14 g (74%) of methyl 2-(3-hydroxyphenyl)nicotinate (14 g, 74% yield) as an oil. LRMS (ES-API)  $m/z$  230.2 ( $\text{M}+\text{H}$ )<sup>+</sup>.

To a solution of methyl 2-(3-hydroxyphenyl) nicotinate (14 g, 61 mmol) in EtOH (150 mL) was added  $\text{PtO}_2$  (1.39 g, 6.1 mmol) and HCl (28 mL). The mixture was stirred at rt for 3 h under hydrogen (4 atm). Then, the reaction mixture was filtered through a celite pad and washed with EtOH (50 mL). The solvent was removed, and the crude product was purified by flash chromatography (silica gel) eluting with a gradient of  $\text{CH}_2\text{Cl}_2$ :MeOH (100:0 to 70:30) to afford 9.5 g (68%) of methyl 2-(3-hydroxyphenyl)piperidine-3-carboxylate as an off-white solid. LRMS (ES-API)  $m/z$  236.3 ( $\text{M}+\text{H}$ )<sup>+</sup>.

To a solution of methyl 2-(3-hydroxyphenyl)piperidine-3-carboxylate (9.5 g, 40 mmol) in  $\text{CH}_2\text{Cl}_2$  (80 mL) was added  $(\text{Boc})_2\text{O}$  (10.48 g, 48 mmol). Then, the reaction mixture was stirred at rt for 1 h. The solvent was removed by *in vacuo*. The crude product was purified by flash chromatography (silica gel) eluting with a gradient of petroleum ether:EtOAc (100:0 to 70:30) to give 12 g (89%) of 1-*tert*-butyl 3-methyl 2-(3-hydroxyphenyl)piperidine-1,3-dicarboxylate as an oil. LRMS (ES-API)  $m/z$  358.2 ( $\text{M}+\text{Na}$ )<sup>+</sup>.

To a solution of 1-*tert*-butyl 3-methyl 2-(3-hydroxyphenyl)piperidine-1,3-dicarboxylate (12 g, 35.8 mmol) in  $\text{CH}_2\text{Cl}_2$  (100 mL) was added pyridine (4.25 g, 53.7 mmol) and  $\text{Tf}_2\text{O}$  (12.1 g, 43 mmol) at 0 °C. Then, the reaction mixture was stirred at rt for 1 h. The reaction mixture was then quenched with aqueous sodium bicarbonate (70 mL), and extracted with  $\text{CH}_2\text{Cl}_2$  (2 x 50 mL). The combined organic layers were washed with brine (50 mL), dried ( $\text{Na}_2\text{SO}_4$ ), filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (silica gel) eluting with a gradient of petroleum ether:EtOAc (100:0 to 80:20) to give 10 g (60%) of 1-*tert*-butyl 3-methyl 2-(3-(trifluoromethyl-sulfonyloxy)phenyl)piperidine-1,3-dicarboxylate as an oil. LRMS (ES-API)  $m/z$  490.1 ( $\text{M}+\text{Na}$ )<sup>+</sup>.

A solution of 1-*tert*-butyl 3-methyl 2-(3-(trifluoromethylsulfonyloxy)phenyl)piperidine-1,3-dicarboxylate (10 g, 21.4 mmol) and trimethylamine (60 mL, 428 mmol) in DMF (120 mL) was purged with  $\text{N}_2$  (g). To this solution was added ethynyltrimethylsilane (21 g, 214 mmol), TBAI (15.8 g, 42.8 mmol), CuI (1.22 g, 6.42 mmol), and  $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$  (1.5 g, 2.14 mmol). Then, the reaction mixture was stirred at 90 °C for 12 h. After the reaction mixture was cooled to room temperature, it was filtered through a celite pad. Water (100 mL) was added to the filtrate, and the mixture was extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine (20 mL), dried ( $\text{Na}_2\text{SO}_4$ ), filtered and concentrated. The crude product was purified by column chromatography

(silica gel) eluting with a gradient of petroleum ether and EtOAc (100:0 to 85:15) to give 7.5 g (84%) of 1-*tert*-butyl 3-methyl 2-(3-((trimethylsilyl)-ethynyl)phenyl)piperidine-1,3-dicarboxylate as an oil. LRMS (ES-API)  $m/z$  438.2 (M+Na)<sup>+</sup>.

To a solution of 1-*tert*-butyl 3-methyl 2-(3-((trimethylsilyl)ethynyl)phenyl)piperidine-1,3-dicarboxylate (7.5 g, 18 mmol) in a mixture of MeOH:CH<sub>2</sub>Cl<sub>2</sub> (1:1, 50 mL) was added K<sub>2</sub>CO<sub>3</sub> (4.97 g, 36 mmol). Then, the reaction mixture was stirred at rt for 1.5 h. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography (silica gel) eluting with a gradient of petroleum ether:EtOAc (100:0 to 80:20) to afford 5.5 g (89%) of 1-*tert*-butyl 3-methyl 2-(3-ethynylphenyl)piperidine-1,3-dicarboxylate as an oil. LRMS (ES-API)  $m/z$  366.2 (M+Na)<sup>+</sup>.

(1-*tert*-butyl 3-methyl 2-(3-ethynylphenyl)piperidine-1,3-dicarboxylate was separated by chiral chromatography (Column: CHIRALPAK IE ODH; Mobile phase: Hexane/IPA 95:5 by volume) to give the (2*R*,3*R*)-1-*tert*-butyl 3-methyl 2-(3-ethynylphenyl)piperidine-1,3-dicarboxylate (700 mg) and (2*S*,3*S*)-1-*tert*-butyl 3-methyl 2-(3-ethynylphenyl)piperidine-1,3-dicarboxylate (900 mg). Note: *cis*-enantiomers were also isolated.

To a solution of (2*R*,3*R*)-1-*tert*-butyl 3-methyl 2-(3-ethynylphenyl)piperidine-1,3-dicarboxylate (700 mg, 2.04 mmol) in MeOH (20 mL) was added LiOH (245 mg, 10.2 mmol) in water (4 mL). The reaction mixture was stirred at rt for 16 h. The solvent was removed *in vacuo*, and a solution of 1 N HCl was added to adjust the pH to 2. The mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 x 30 mL). The combined organic layers were washed with brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated under reduced pressure to afford 660 mg (98%) of (2*R*,3*R*)-1-*tert*-butyl 2-(3-ethynylphenyl)piperidinecarboxylic acid as an oil. LRMS (ES-API)  $m/z$  352.2 (M+Na)<sup>+</sup>.

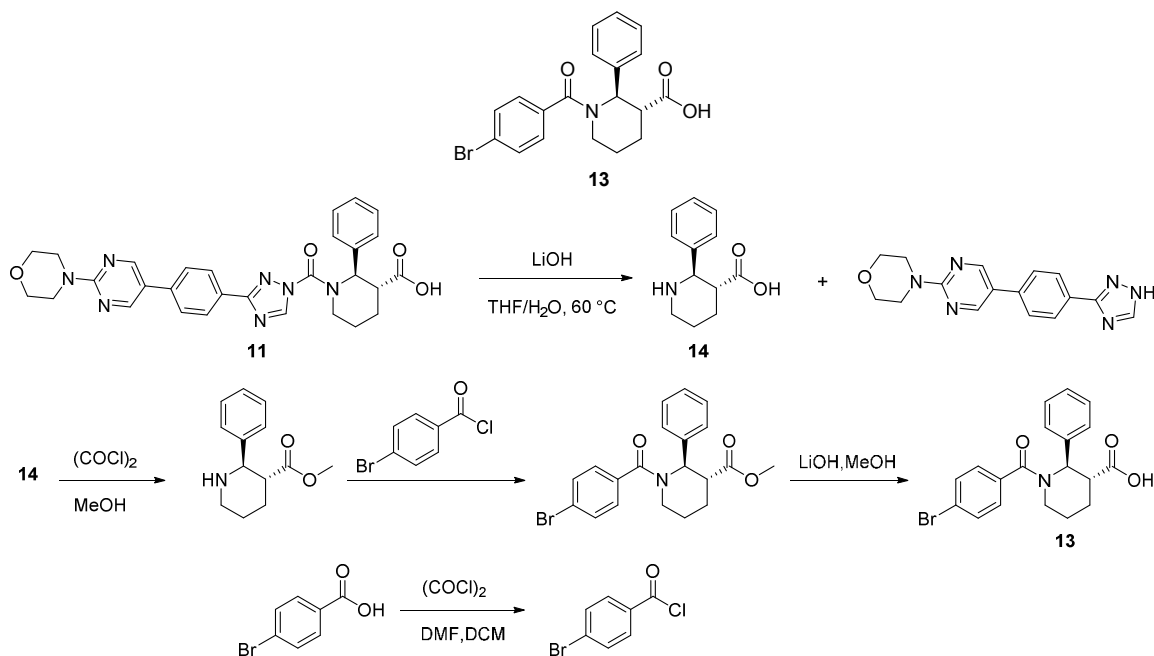
To a solution of (2*R*,3*R*)-1-*tert*-butyl 2-(3-ethynylphenyl)piperidinecarboxylic acid (660 mg, 2.0 mmol) in DMF (10 mL) was added allyl bromide (364 mg, 3.01 mmol) and K<sub>2</sub>CO<sub>3</sub> (415 mg, 3.01 mmol). The mixture was stirred at rt for 2 h. Then, the solvent was removed and water (50 mL) was added, and the mixture was extracted with EtOAc (2 x 30 mL). The combined organic layers were washed with brine (10 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel) eluting with a gradient of petroleum ether:EtOAc (100:0 to 85:15) to afford 642 mg (87%) of (2*R*,3*R*)-3-allyl 1-*tert*-butyl 2-(3-ethynylphenyl)piperidine-1,3-dicarboxylate as a yellow oil. LRMS (ES-API) 392.2 (M+Na)<sup>+</sup>.

To a solution of (2*R*,3*R*)-3-allyl 1-*tert*-butyl 2-(3-ethynylphenyl)piperidine-1,3-dicarboxylate (642 mg, 1.74 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) was added HCl (16 mL, 2 M in CH<sub>2</sub>Cl<sub>2</sub>). The mixture was stirred at rt for 4 h. The solvent was removed to deliver 580 mg of (2*R*,3*R*)-allyl 2-(3-ethynylphenyl)piperidine-3-carboxylate hydrochloride as a brown solid. LRMS (ES-API)  $m/z$  270.2 (M+H)<sup>+</sup>.

To a solution of (2*R*,3*R*)-allyl 2-(3-ethynylphenyl)piperidine-3-carboxylate hydrochloride (580 mg, 1.89 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) was added triphosgene (390 mg, 1.53 mmol) and DIPEA (732 mg, 5.67 mmol) at 0 °C. The mixture was stirred at -10 °C for 15 min, then it was warmed up to rt and stirred for 2 h. The solution was added to cold water (40 mL) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 x 20 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated to give the crude product as an oil. It was used in the next step without further purification. To a solution of (2*R*,3*R*)-allyl 1-(chlorocarbonyl)-2-(3-ethynylphenyl)piperidine-3-carboxylate in THF (30 mL), was added 4-(5-(4-(1*H*-1,2,4-triazol-3-yl)phenyl)pyrimidin-2-yl)morpholine (565 mg, 1.83 mmol), DMAP (228 mg, 1.83 mmol) and DIPEA (473 mg, 3.67 mmol). The reaction mixture was stirred at 60 °C for 2 h. After cooling to rt, the solvent was removed under reduced pressure. The residue was purified by flash chromatography (silica gel) eluting with a gradient of CH<sub>2</sub>Cl<sub>2</sub>:MeOH (100:0 to 95:5) to give 800 mg (72%) of (2*R*,3*R*)-allyl 2-(3-ethynylphenyl)-1-(3-(4-(2-morpholinopyrimidin-5-yl)phenyl)-1*H*-1,2,4-triazole-1-carbonyl)piperidine-3-carboxylate as a brown solid. To a solution of (2*R*,3*R*)-allyl 2-(3-ethynylphenyl)-1-(3-(4-(2-morpholinopyrimidin-5-yl)phenyl)-1*H*-1,2,4-triazole-1-carbonyl)piperidine-3-carboxylate (800 mg, 1.33 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (20 mL), was added TMSEt<sub>2</sub>N (193 mg, 1.33 mmol), and Pd(PPh<sub>3</sub>)<sub>4</sub> (768 mg, 0.665 mmol). The vessel was degassed and refilled with N<sub>2</sub> (g). The reaction mixture was stirred at rt for 2 h. Then, the reaction mixture was filtered through a celite pad, and the filtrate was concentrated under reduced pressure. The crude product was purified by flash chromatography (silica gel), eluting with a gradient of CH<sub>2</sub>Cl<sub>2</sub>:MeOH (100:0 to 88:12) followed by prep HPLC to afford 280 mg (38% over 3 steps) of **12** as a brown solid.

**(2*R*,3*R*)-2-(3-ethynylphenyl)-1-(3-(4-(2-morpholinopyrimidin-5-yl)phenyl)-1*H*-1,2,4-triazole-1-carbonyl)piperidine-3-carboxylic acid (**12**).** <sup>1</sup>H NMR (600 MHz, DMSO-*d*<sub>6</sub>) δ 12.78 (br s, 1 H), 9.17 (s, 1 H), 8.79 (s, 2 H), 7.93 (br. s., 2 H), 7.76 (d, *J* = 7.6 Hz, 2 H), 7.71 (s, 1 H), 7.62 (s, 1 H), 7.51 - 7.45 (m, 2 H), 5.98 (s, 1 H), 4.24 (s, 1 H), 4.16-4.11 (m, 1 H), 3.79 - 3.73 (m, 4 H), 3.71 - 3.64 (m, 4 H), 3.51 (s, 1 H), 2.94-2.87 (m, 1 H), 2.14-2.08 (m, 1 H), 1.82 - 1.72 (m, 1 H), 1.71-1.63 (m, 1 H), 1.62-1.56 (m, 1 H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 174.34, 160.50, 160.46, 155.60, 149.99, 147.76, 137.88, 136.16, 130.30, 129.94, 128.84, 128.14, 127.36, 126.49, 125.52, 121.92, 121.16, 83.35, 80.76, 65.73, 57.07, 43.79, 41.29, 21.56, 21.36. HRMS (ESI) *m/z* [M+H]<sup>+</sup> calcd for C<sub>31</sub>H<sub>30</sub>N<sub>7</sub>O<sub>4</sub> 564.2354, found 564.2353

## Synthesis of compound **13**



The absolute stereochemistry of compound **11** was established by isolating enantiopure *trans*-2-phenylpiperidine-3-carboxylic acid (**14**) via basic hydrolysis. Compound **14** was then converted to enantiopure bromobenzamide **13** in multiple steps. Single crystal X-ray diffraction of bromobenzamide **13** showed the absolute stereochemistry (2*R*,3*R*). Thus, compound **11** also had the absolute configuration of (2*R*,3*R*).

To a solution of (2*R*,3*R*)-1-(3-(4-(2-morpholinopyrimidin-5-yl)phenyl)-1*H*-1,2,4-triazole-1-carbonyl)-2-phenylpiperidine-3-carboxylic acid (**11**) (300 mg, 0.556 mmol) in THF (6 mL) and water (3 mL) was added LiOH (66.5 mg, 2.78 mmol) and the mixture was stirred at 60 °C for 3 h. The mixture was concentrated and dried under vacuum to give 360 mg (100%) of the crude (2*R*,3*R*)-2-phenylpiperidine-3-carboxylic acid as a white solid. LRMS (ES-API) *m/z* 206.2 (M+H)<sup>+</sup>.

To a suspension of crude (2*R*,3*R*)-2-phenylpiperidine-3-carboxylic acid (360 mg crude, 0.556 mmol) in MeOH (10 mL) was added oxalyl chloride (1.06 g, 8.34 mmol) at 0 °C in drops. This mixture was stirred at room temperature for 18 h. The mixture was concentrated and diluted with water (5 mL), extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 20 mL). The water phase was filtered and the filtrate was basified with saturated aqueous sodium carbonate to pH = 10. Then, the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (6 x 30 mL). The combined organic layers were washed with brine (50 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated to deliver 100 mg (74% over two steps) of the crude product as a white solid. LRMS (ES-API) *m/z* 220.2 (M+H)<sup>+</sup>.

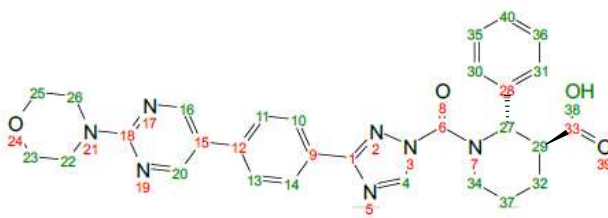
To a suspension of 4-bromobenzoic acid (201 mg, 1 mmol) in DMF (1 drop) and CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was added oxalyl chloride (635 mg, 5 mmol) dropwise at 0 °C. This mixture was stirred at room temperature for 18 h. The mixture was concentrated to give 240 mg (100%) of the crude product as a yellow solid and used directly in the next step.

To a solution of (2*R*,3*R*)-methyl 2-phenylpiperidine-3-carboxylate (55 mg crude, 0.226 mmol) and ethyldiisopropylamine (146 mg, 1.13 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (6 mL) was added a solution of 4-bromobenzoyl chloride (240 mg crude, 1 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) dropwise at 0 °C. This mixture was stirred at rt for 1 h. The mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> and washed with water (20 mL), saturated aqueous sodium carbonate solution (2 x 20 mL), brine (20 mL), dried over (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated. The residue was purified by prep-TLC eluting with a mixture of petroleum ether:EtOAc (5:1) to give 70 mg (77%) of the product as a yellow solid. LRMS (ES-API) *m/z* 402.1 (M+1 for <sup>79</sup>Br), 404.1 (M+1 for <sup>81</sup>Br).

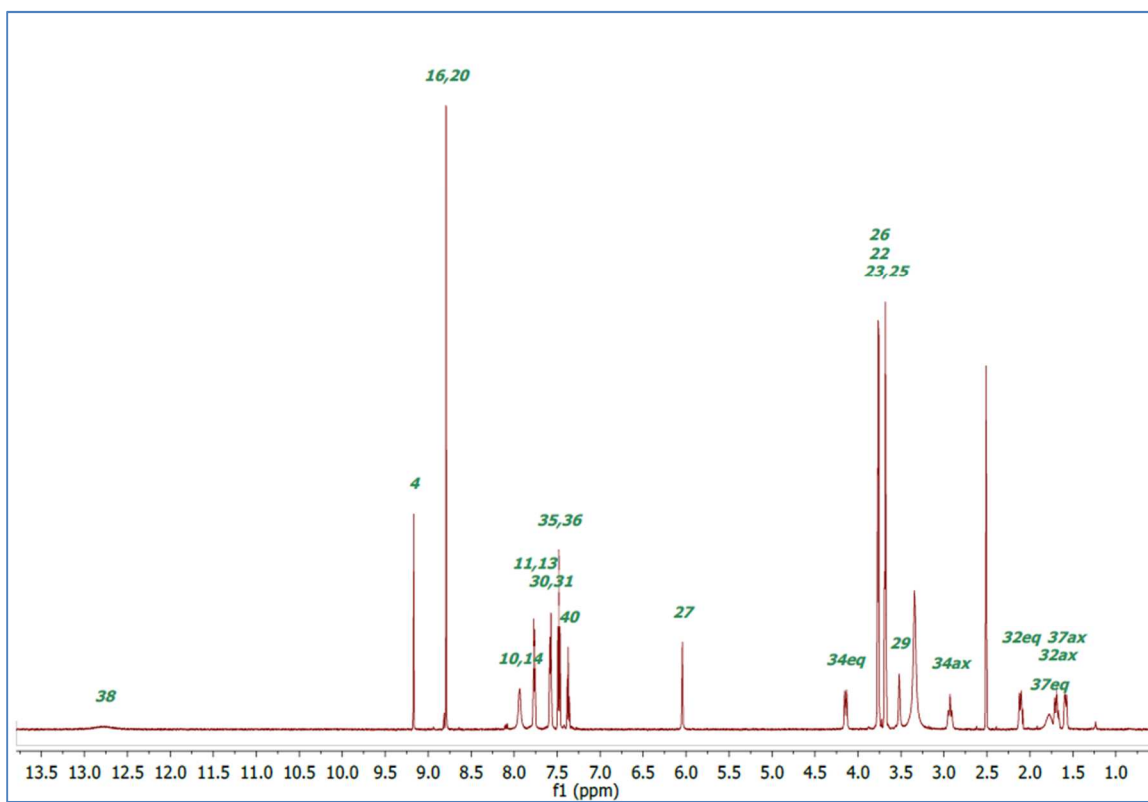
To a solution of (2*R*,3*R*)-methyl 1-(4-bromobenzoyl)-2-phenylpiperidine-3-carboxylate (70 mg, 0.174 mmol) in MeOH (3 mL) and water (1 mL) was added lithium hydroxide (25 mg, 1.044 mmol), and the mixture was stirred at room temperature for 3 h. The mixture was diluted with water (7 mL) and concentrated, acidified with 1N HCl to pH = 2. The mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 30 mL). The combined organic layers were dried over (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated. The residue was purified by prep-TLC eluting with a mixture of CH<sub>2</sub>Cl<sub>2</sub>:MeOH:formic acid (20:1:0.0125) to deliver 60 mg (89%) of **13** as a white solid.

**(2*R*,3*R*)-1-(4-bromobenzoyl)-2-phenylpiperidine-3-carboxylic acid (13).** <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>, T = 60 °C) δ 12.40 (brs, 1 H), 7.62-7.57 (m, 2 H), 7.44-7.28 (m, 7H), 5.76 (brs, 1 H), 3.92 (brs, 1 H), 3.15 (br s, 1 H), 2.97-2.94 (m, 1 H), 2.02-1.98 (m, 1 H), 1.76-1.65 (m, 1 H), 1.60-1.46 (m, 2 H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>, T = 60 °C) 173.96, 169.45, 138.17, 135.54, 130.92, 128.49, 128.17, 126.60, 125.95, 122.28, 54.97\*, 41.90, 40.4\*, 21.13, 20.94. HRMS (ESI) *m/z* [M+H]<sup>+</sup> calcd for C<sub>19</sub>H<sub>19</sub>BrNO<sub>3</sub> 388.0543 (<sup>79</sup>Br) and 390.0525 (<sup>81</sup>Br), found 388.0547 and 390.0522. See X-ray data showed (2*R*, 3*R*) absolute stereochemistry for **13** which established the absolute stereochemistry for compound **11** as (2*R*, 3*R*). See X-ray section.

\*(from HSQC NMR experiment at 60 °C)

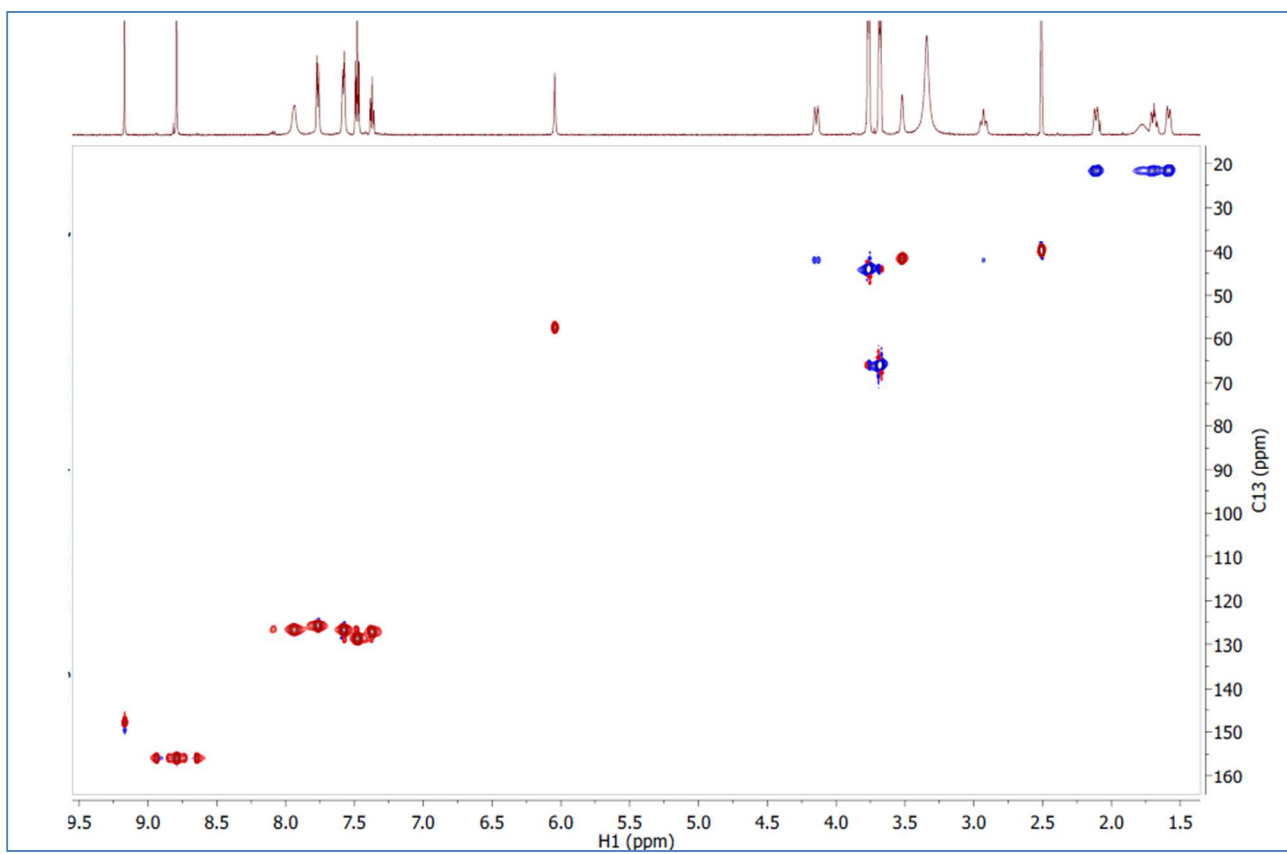
**NMR data establishing the *trans*-stereochemistry of compounds **10** and **11******10**

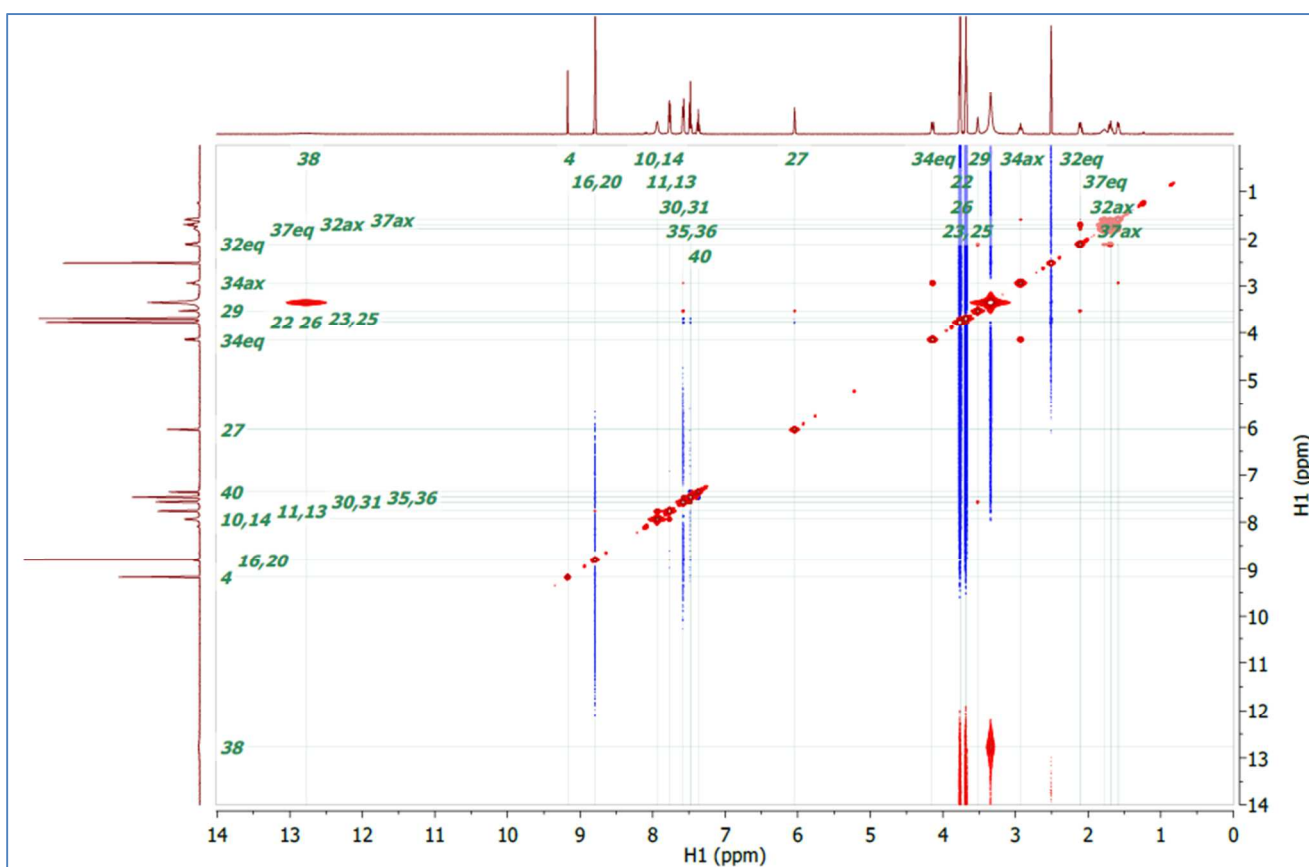
$^1\text{H}$  NMR of **10** at 298 K, 600 MHz in DMSO- $\text{d}_6$



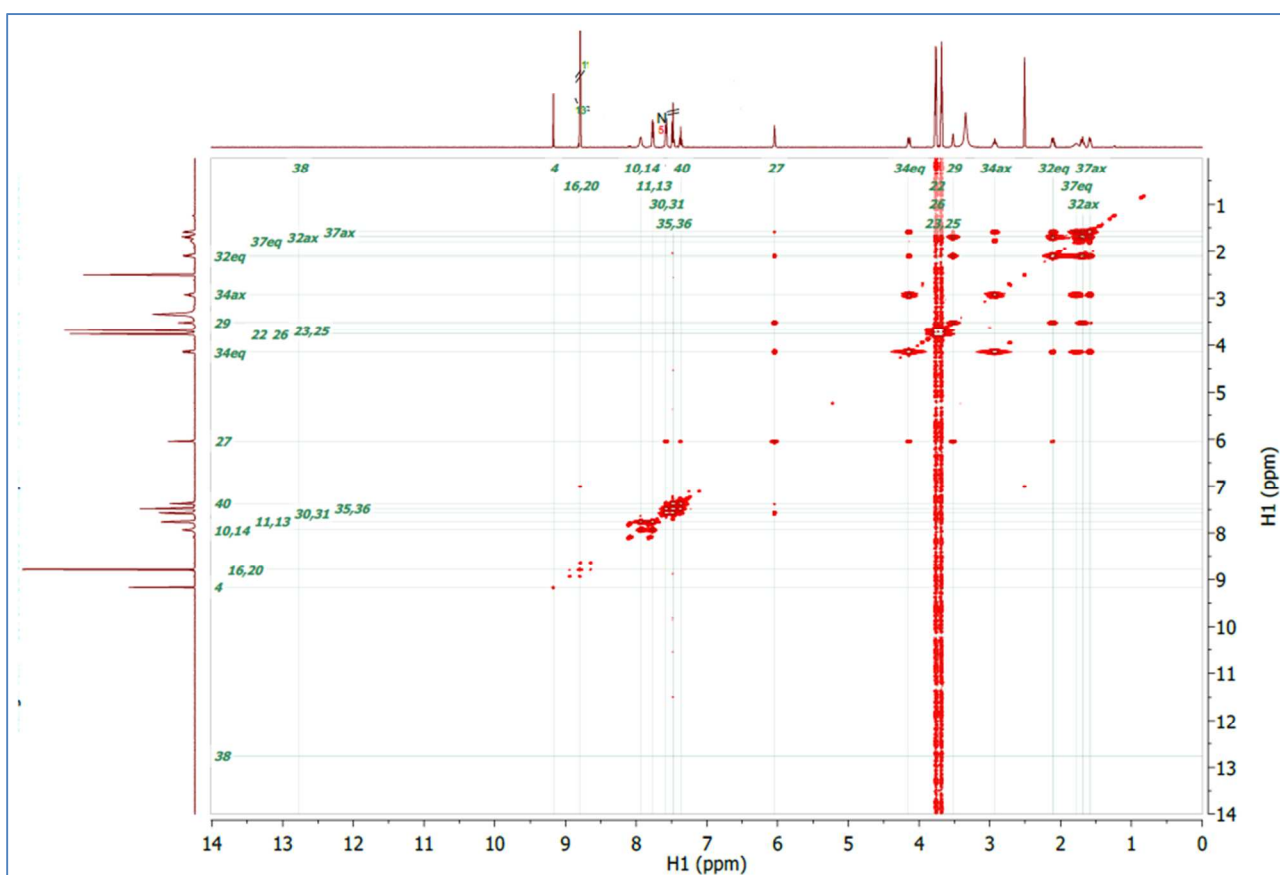
**NMR data establishing the *trans*-stereochemistry of compounds **10** and **11** (contd.)**

$^{13}\text{C}$ - $^1\text{H}$ , HSQC (DMSO- $\text{d}_6$ ) of **10** at 298 K

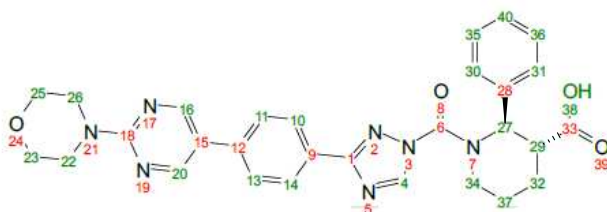


NMR data establishing the *trans*-stereochemistry of compounds **10** and **11** (contd.) $^1\text{H}$ - $^1\text{H}$  NOESY (DMSO- $d_6$ ) of **10** at 298 K

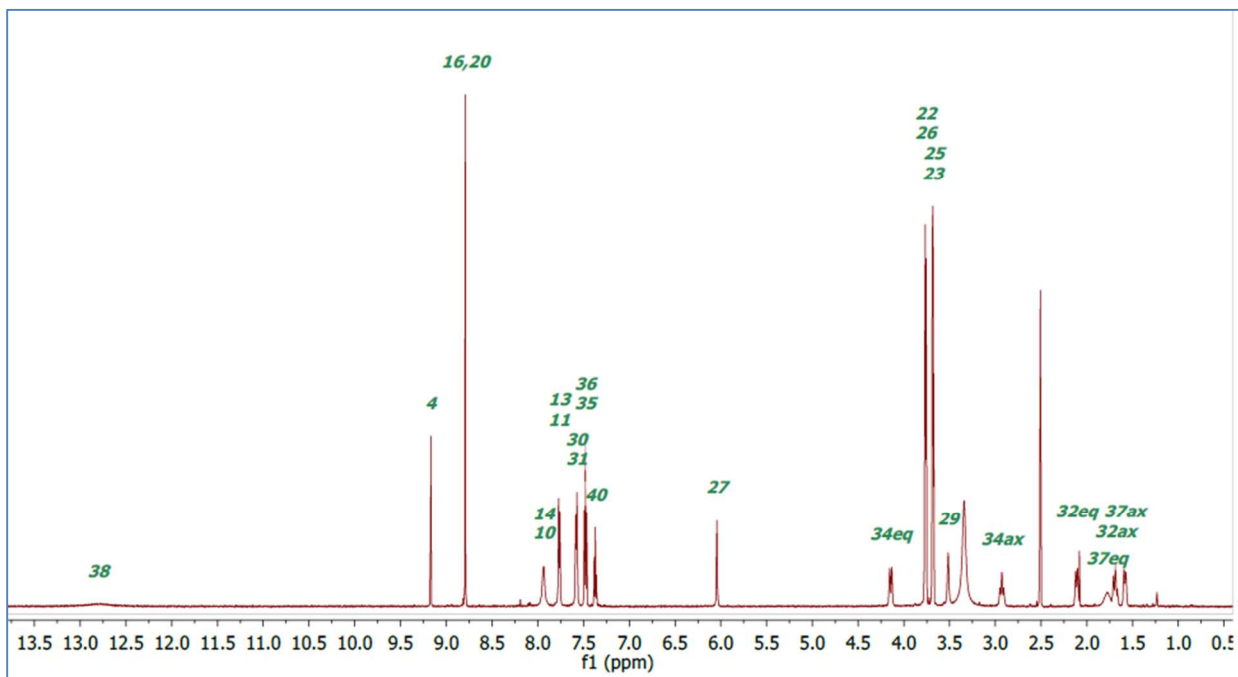


NMR data establishing the *trans*-stereochemistry of compounds **10** and **11** (contd.) $^1\text{H}$ - $^1\text{H}$  COSY (DMSO- $d_6$ ) of **10** at 298 K



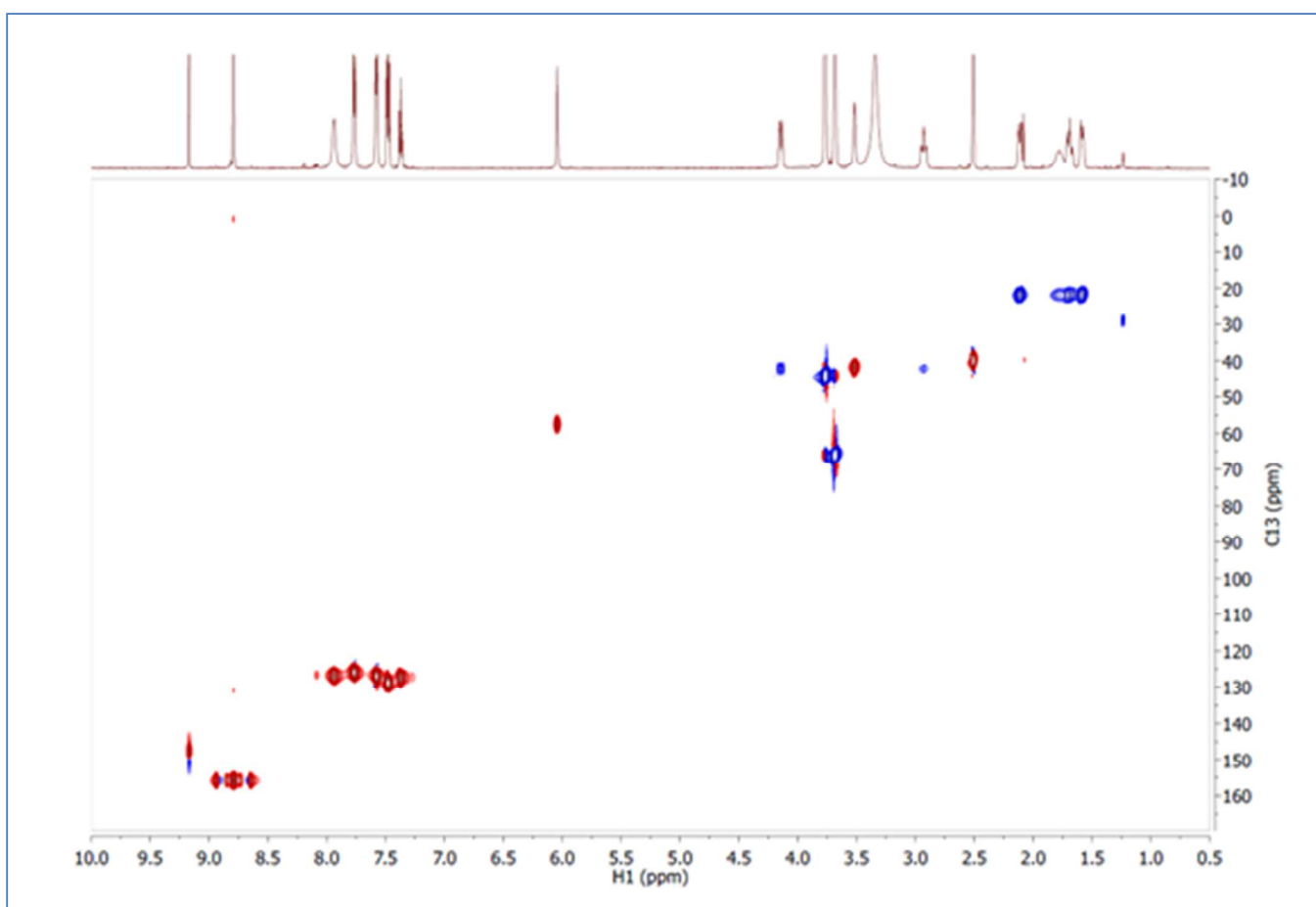
NMR data establishing the *trans*-stereochemistry of compounds **10** and **11** (contd.)**11**

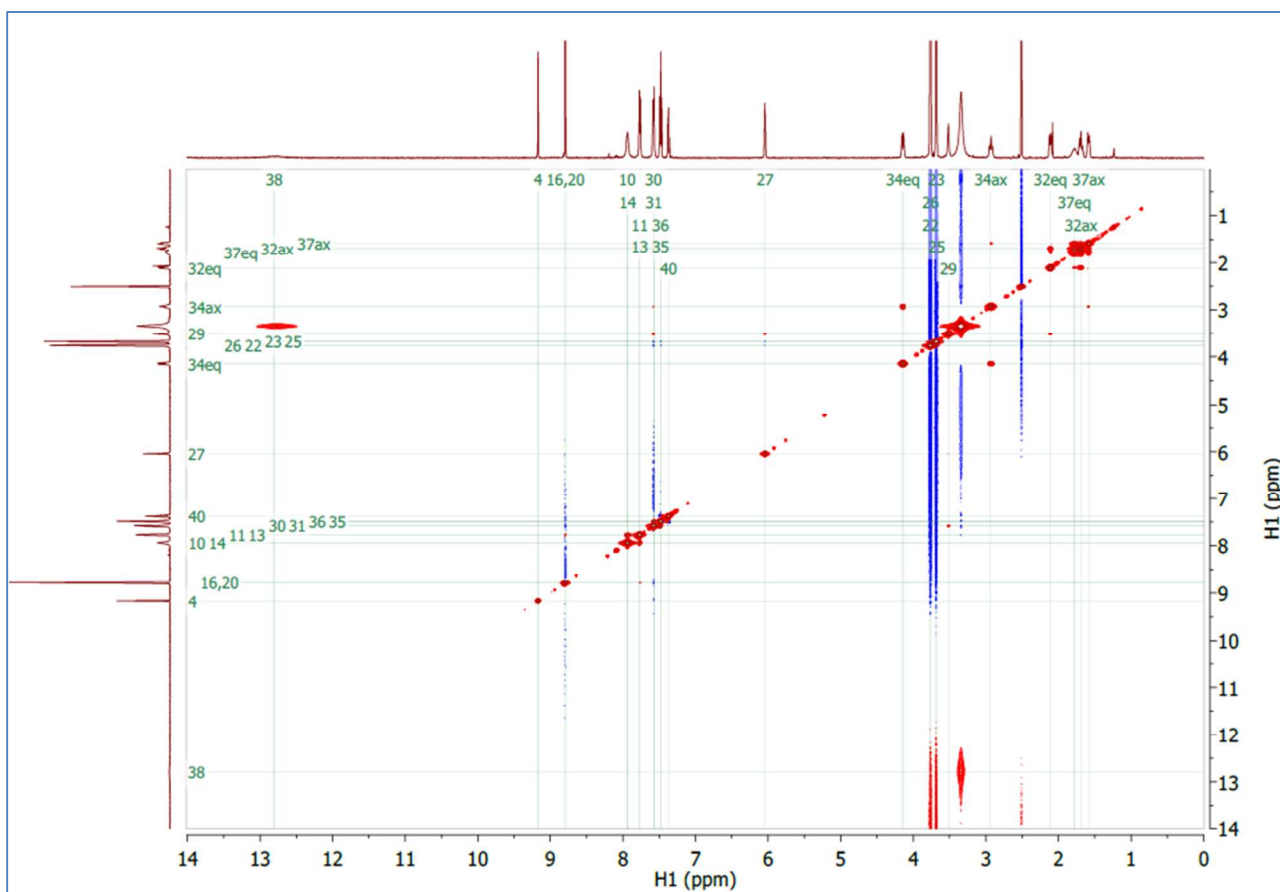
$^1\text{H}$  NMR (DMSO- $\text{d}_6$ ) of **11** at 298 K, 600 MHz in DMSO- $\text{d}_6$

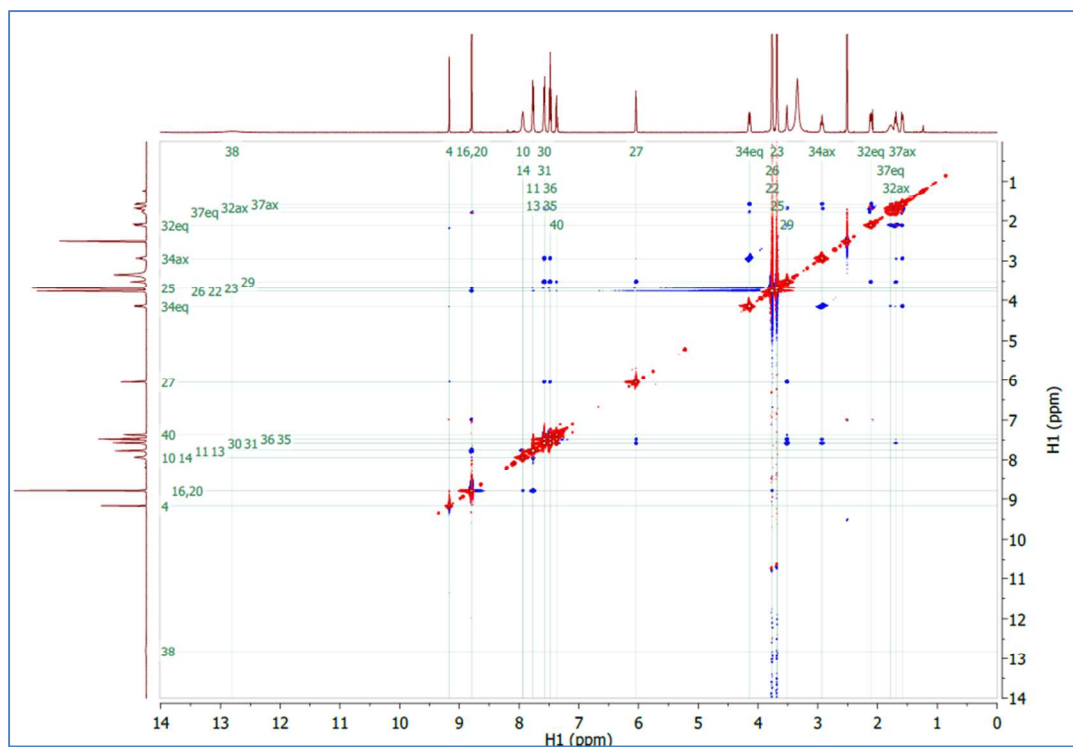
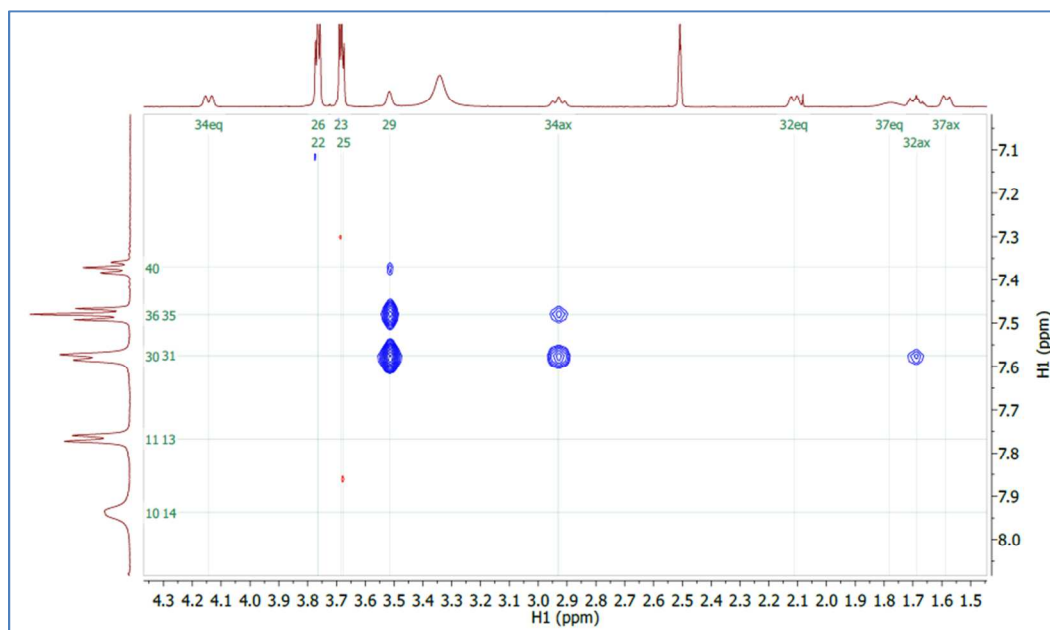


**NMR data establishing the *trans*-stereochemistry of compounds 10 and 11 (contd.)**

$^{13}\text{C}$ - $^1\text{H}$ , HSQC (DMSO- $\text{d}_6$ ) of **11** at 298 K

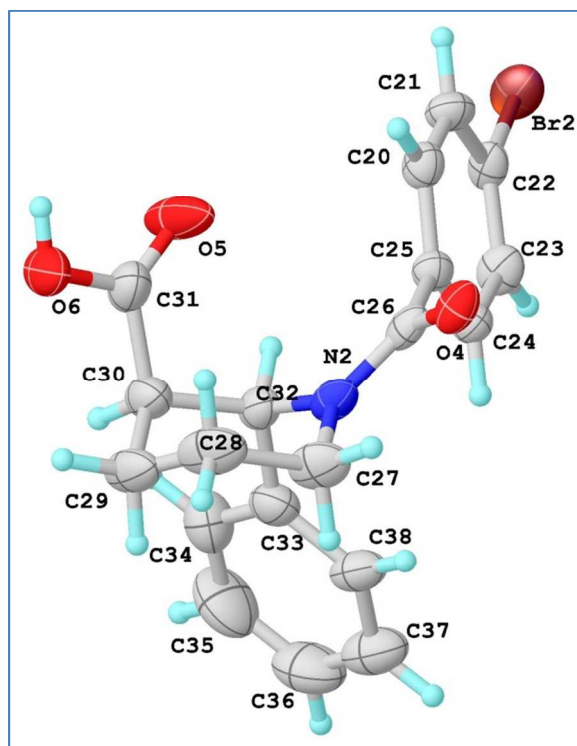


NMR data establishing the *trans*-stereochemistry of compounds **10** and **11** (contd.) $^1\text{H}$ - $^1\text{H}$ -NOESY of **11** at 298 K

**NMR data establishing the *trans*-stereochemistry of compounds 10 and 11 (contd.)** $^1\text{H}$ - $^1\text{H}$ -ROESY (DMSO- $d_6$ ) of 11 at 298 K**expanded view of key peaks**

**X-Ray Data for (2*R*,3*R*)-1-(4-bromobenzoyl)-2-phenylpiperidine-3-carboxylic acid (13)**

- The structure was solved in the  $P2_1$  space group
- The asymmetric unit is comprised of two molecules and one half occupied ethyl acetate sitting on a special position.
- R value 5.5%
- Absolute configuration



**Figure S6. Compound 13 in the asymmetric unit. Displacement parameters are drawn at 50% probability.**

### Small-molecule X-ray diffraction: experimental details:

Data collection was performed on a Bruker APEX diffractometer at -150 °C. Data collection consisted of omega and phi scans.

The structure was solved by direct methods using SHELX software suite in the space group  $P2_1$ . The structure was subsequently refined by the full-matrix least squares method. All non-hydrogen atoms were found and refined using anisotropic displacement parameters.

The asymmetric unit is comprised of two molecules and one half occupied molecule of ethyl acetate.

All hydrogen atoms (including those bonded to heteroatoms) were placed in idealized positions and were allowed to ride on their carrier atoms. The final refinement included isotropic displacement parameters for all hydrogen atoms.

Analysis of the absolute structure using likelihood methods (Hooft 2008) was performed using PLATON (Spek 2010). The results indicate that the absolute structure has been correctly assigned. The method calculates that the probability that the structure is correct is 100.0. The Hooft parameter is reported as 0.001 with an esd of 0.018.

The final R-index was 5.5%. A final difference Fourier revealed no missing or misplaced electron density.

Pertinent crystal, data collection and refinement are summarized in table 1. Atomic coordinates, bond lengths, bond angles, Torsion angles and displacement parameters are listed in tables 2 –6.

### Software and References

**SHELXTL**, Version 5.1, Bruker AXS, 1997

**PLATON**, A.L. Spek, *J. Appl. Cryst.* 2003, 36, 7-13.

**MERCURY**, C.F. Macrae, P.R. Edington, P. McCabe, E. Pidcock, G.P. Shields, R. Taylor, M. Towler and J. van de Streek, *J. Appl. Cryst.* **39**, 453-457, 2006.

**OLEX2**, Dolomanov, O.V.; Bourhis, L.J.; Gildea, R.J.; Howard, J.A.K.; Puschmann, H., (2009). *J. Appl. Cryst.*, 42, 339-341.

R.W.W. Hooft et al. *J. Appl. Cryst.* (2008). 41. 96-103.

H.D. Flack, *Acta Cryst.* 1983, **A39**, 867-881.



**Table S1. Crystal data and structure refinement for compound 13**

Identification code	z514
Crystallization	EtOAc-heptane
Empirical formula	C <sub>40</sub> H <sub>40</sub> Br <sub>2</sub> N <sub>2</sub> O <sub>7</sub>
Formula weight	820.56
Temperature	123(2) K
Wavelength	1.54178 Å
Crystal system	Monoclinic
Space group	P2(1)
Unit cell dimensions	a = 11.1307(7) Å      α = 90° b = 9.2916(7) Å      β = 103.408(4)° c = 18.7125(16) Å      γ = 90°
Volume	1882.5(2) Å <sup>3</sup>
Z	2
Density (calculated)	1.448 Mg/m <sup>3</sup>
Absorption coefficient	3.155 mm <sup>-1</sup>
F(000)	840
Crystal size	0.66 x 0.10 x 0.04 mm <sup>3</sup>
Theta range for data collection	2.43 to 68.58°
Index ranges	-13 ≤ h ≤ 13, -11 ≤ k ≤ 10, -21 ≤ l ≤ 22
Reflections collected	19712
Independent reflections	6175 [R(int) = 0.0899]
Completeness to theta = 68.58°	98.2 %
Absorption correction	None
Max. and min. transmission	0.8842 and 0.2299
Refinement method	Full-matrix least-squares on F <sup>2</sup>
Data / restraints / parameters	6175 / 1 / 499
Goodness-of-fit on F <sup>2</sup>	1.105
Final R indices [I > 2σ(I)]	R1 = 0.0550, wR2 = 0.1374
R indices (all data)	R1 = 0.0624, wR2 = 0.1420
Absolute structure parameter	0.00(2)
Largest diff. peak and hole	0.542 and -0.759 e.Å <sup>-3</sup>

**Table S2. Atomic coordinates ( $\times 10^4$ ) and equivalent isotropic displacement parameters ( $\text{\AA}^2 \times 10^3$ ).**

U(eq) is defined as one third of the trace of the orthogonalized  $U^{ij}$  tensor.

	x	y	z	U(eq)
Br(1)	13168(1)	10190(1)	3951(1)	49(1)
Br(2)	13798(1)	3189(1)	2607(1)	48(1)
C(1)	9355(5)	10411(7)	3396(3)	35(1)
C(2)	10600(5)	10653(6)	3718(3)	34(1)
C(3)	11474(5)	9802(6)	3510(3)	35(1)
C(4)	11151(5)	8722(7)	3006(4)	44(2)
C(5)	9921(5)	8509(7)	2666(4)	40(2)
C(6)	9012(5)	9366(7)	2864(3)	34(1)
C(7)	7713(5)	9254(7)	2427(4)	38(1)
C(8)	5496(5)	9248(8)	2343(4)	46(2)
C(9)	4859(5)	7789(8)	2276(5)	55(2)
C(10)	4938(5)	7141(8)	3042(5)	57(2)
C(11)	6275(5)	7057(7)	3460(4)	46(2)
C(12)	6984(6)	5919(8)	3131(5)	59(2)
C(13)	6952(5)	8517(7)	3509(4)	39(1)
C(14)	6584(5)	9547(7)	4055(4)	41(2)
C(15)	6516(5)	9061(8)	4758(4)	47(2)
C(16)	6210(6)	10006(10)	5266(4)	54(2)
C(17)	5996(5)	11433(9)	5104(4)	52(2)
C(18)	6078(5)	11934(8)	4417(4)	47(2)
C(19)	6373(5)	11005(7)	3903(4)	39(1)

C(20)	10136(5)	4027(7)	2589(3)	38(1)
C(21)	11418(5)	4067(7)	2745(3)	40(1)
C(22)	12043(4)	3054(7)	2436(3)	35(1)
C(23)	11437(5)	2001(7)	1976(3)	39(1)
C(24)	10164(5)	1976(7)	1815(3)	34(1)
C(25)	9507(4)	2993(7)	2114(3)	32(1)
C(26)	8121(5)	2933(6)	1974(3)	36(1)
C(27)	6121(5)	2789(8)	1125(4)	54(2)
C(28)	5401(6)	4128(10)	835(5)	69(3)
C(29)	5813(7)	4678(8)	174(5)	67(3)
C(30)	7222(7)	4996(8)	366(4)	57(2)
C(31)	7582(7)	6225(8)	895(4)	56(2)
C(32)	7978(6)	3648(7)	692(4)	41(2)
C(33)	8123(6)	2534(8)	112(4)	48(2)
C(34)	8601(7)	2951(10)	-476(4)	64(2)
C(35)	8845(10)	1948(14)	-976(5)	83(3)
C(36)	8616(8)	502(12)	-895(5)	78(3)
C(37)	8142(7)	78(11)	-311(5)	64(2)
C(38)	7898(6)	1070(8)	186(4)	50(2)
N(1)	6784(4)	9118(6)	2772(3)	37(1)
N(2)	7463(4)	3054(6)	1279(3)	39(1)
O(1)	7520(4)	9407(5)	1746(3)	43(1)
O(2B)	8173(18)	6101(19)	3280(30)	36(9)
O(2A)	7770(18)	6146(10)	2782(17)	70(7)
O(3)	6451(4)	4650(5)	3083(3)	49(1)
O(4)	7631(4)	2702(4)	2499(2)	40(1)

O(5)	8501(6)	6242(7)	1368(4)	98(3)
O(6)	6797(5)	7338(6)	761(3)	61(1)
C(91)	-603(18)	11480(60)	5486(17)	160(20)
C(92)	402(16)	10660(30)	5453(9)	80(6)
O(92)	125(14)	9230(20)	5322(9)	107(6)
C(94)	1011(18)	8390(30)	5302(13)	104(9)
O(91)	1444(10)	11130(20)	5529(7)	102(5)
C(95)	590(30)	7110(50)	4812(14)	150(20)

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**Table S3. Bond lengths [Å] and angles [°].**

Br(1)-C(3)	1.909(5)	C(16)-C(17)	1.369(12)
Br(2)-C(22)	1.909(5)	C(17)-C(18)	1.390(10)
C(1)-C(6)	1.379(9)	C(18)-C(19)	1.388(9)
C(1)-C(2)	1.395(7)	C(20)-C(25)	1.384(8)
C(2)-C(3)	1.377(8)	C(20)-C(21)	1.388(8)
C(3)-C(4)	1.367(9)	C(21)-C(22)	1.375(9)
C(4)-C(5)	1.383(8)	C(22)-C(23)	1.372(9)
C(5)-C(6)	1.402(8)	C(23)-C(24)	1.380(8)
C(6)-C(7)	1.491(8)	C(24)-C(25)	1.389(8)
C(7)-O(1)	1.251(8)	C(25)-C(26)	1.504(7)
C(7)-N(1)	1.346(8)	C(26)-O(4)	1.248(7)
C(8)-N(1)	1.476(7)	C(26)-N(2)	1.341(8)
C(8)-C(9)	1.522(10)	C(27)-N(2)	1.476(7)
C(9)-C(10)	1.540(11)	C(27)-C(28)	1.511(11)
C(10)-C(11)	1.513(9)	C(28)-C(29)	1.503(13)
C(11)-C(12)	1.532(9)	C(29)-C(30)	1.554(10)
C(11)-C(13)	1.544(9)	C(30)-C(31)	1.503(10)
C(12)-O(2A)	1.225(11)	C(30)-C(32)	1.553(9)
C(12)-O(2B)	1.30(2)	C(31)-O(5)	1.188(8)
C(12)-O(3)	1.314(8)	C(31)-O(6)	1.340(9)
C(13)-N(1)	1.458(8)	C(32)-N(2)	1.459(9)
C(13)-C(14)	1.525(9)	C(32)-C(33)	1.535(9)
C(14)-C(19)	1.393(9)	C(33)-C(34)	1.384(11)
C(14)-C(15)	1.409(9)	C(33)-C(38)	1.396(11)
C(15)-C(16)	1.393(11)	C(34)-C(35)	1.392(13)

C(35)-C(36)	1.383(16)	C(10)-C(11)-C(13)	113.2(6)
C(36)-C(37)	1.376(14)	C(12)-C(11)-C(13)	109.8(5)
C(37)-C(38)	1.381(11)	O(2A)-C(12)-O(2B)	43.1(9)
C(91)-C(92)	1.36(4)	O(2A)-C(12)-O(3)	119.0(9)
C(92)-O(91)	1.22(2)	O(2B)-C(12)-O(3)	123.5(10)
C(92)-O(92)	1.37(3)	O(2A)-C(12)-C(11)	126.5(7)
O(92)-C(94)	1.27(3)	O(2B)-C(12)-C(11)	115.0(13)
C(94)-C(95)	1.50(5)	O(3)-C(12)-C(11)	112.3(5)
		N(1)-C(13)-C(14)	113.4(5)
C(6)-C(1)-C(2)	120.4(5)	N(1)-C(13)-C(11)	109.1(5)
C(3)-C(2)-C(1)	118.8(6)	C(14)-C(13)-C(11)	112.8(5)
C(4)-C(3)-C(2)	121.7(5)	C(19)-C(14)-C(15)	117.4(7)
C(4)-C(3)-Br(1)	120.8(4)	C(19)-C(14)-C(13)	122.2(6)
C(2)-C(3)-Br(1)	117.4(4)	C(15)-C(14)-C(13)	120.4(6)
C(3)-C(4)-C(5)	119.8(5)	C(16)-C(15)-C(14)	120.6(7)
C(4)-C(5)-C(6)	119.7(6)	C(17)-C(16)-C(15)	121.2(7)
C(1)-C(6)-C(5)	119.6(5)	C(16)-C(17)-C(18)	118.9(7)
C(1)-C(6)-C(7)	121.0(5)	C(19)-C(18)-C(17)	120.6(7)
C(5)-C(6)-C(7)	118.9(5)	C(18)-C(19)-C(14)	121.3(7)
O(1)-C(7)-N(1)	122.0(5)	C(25)-C(20)-C(21)	119.7(6)
O(1)-C(7)-C(6)	117.8(5)	C(22)-C(21)-C(20)	119.2(6)
N(1)-C(7)-C(6)	119.9(6)	C(23)-C(22)-C(21)	121.9(5)
N(1)-C(8)-C(9)	110.3(5)	C(23)-C(22)-Br(2)	119.1(4)
C(8)-C(9)-C(10)	110.2(6)	C(21)-C(22)-Br(2)	118.8(5)
C(11)-C(10)-C(9)	109.8(5)	C(22)-C(23)-C(24)	118.6(6)
C(10)-C(11)-C(12)	111.3(6)	C(23)-C(24)-C(25)	120.8(5)

C(20)-C(25)-C(24)	119.7(5)	C(7)-N(1)-C(13)	123.0(5)
C(20)-C(25)-C(26)	118.9(5)	C(7)-N(1)-C(8)	119.2(5)
C(24)-C(25)-C(26)	121.3(5)	C(13)-N(1)-C(8)	116.2(5)
O(4)-C(26)-N(2)	122.5(5)	C(26)-N(2)-C(32)	122.7(5)
O(4)-C(26)-C(25)	119.1(5)	C(26)-N(2)-C(27)	118.4(5)
N(2)-C(26)-C(25)	118.3(5)	C(32)-N(2)-C(27)	118.2(5)
N(2)-C(27)-C(28)	111.2(6)	O(91)-C(92)-C(91)	124(3)
C(29)-C(28)-C(27)	109.8(7)	O(91)-C(92)-O(92)	122(2)
C(28)-C(29)-C(30)	111.0(6)	C(91)-C(92)-O(92)	113(2)
C(31)-C(30)-C(32)	108.4(5)	C(94)-O(92)-C(92)	117.5(19)
C(31)-C(30)-C(29)	113.4(7)	O(92)-C(94)-C(95)	112(2)
C(32)-C(30)-C(29)	111.3(6)		
O(5)-C(31)-O(6)	122.8(7)		
O(5)-C(31)-C(30)	123.6(7)		
O(6)-C(31)-C(30)	113.6(6)		
N(2)-C(32)-C(33)	113.6(5)		
N(2)-C(32)-C(30)	109.2(6)		
C(33)-C(32)-C(30)	113.7(5)		
C(34)-C(33)-C(38)	117.6(7)		
C(34)-C(33)-C(32)	119.7(7)		
C(38)-C(33)-C(32)	122.5(7)		
C(33)-C(34)-C(35)	121.2(9)		
C(36)-C(35)-C(34)	120.5(9)		
C(37)-C(36)-C(35)	118.7(9)		
C(36)-C(37)-C(38)	121.0(9)		
C(37)-C(38)-C(33)	121.0(8)		

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Symmetry transformations were used to generate equivalent atoms.

**Table S4. Anisotropic displacement parameters ( $\text{\AA}^2 \times 10^3$ ). The anisotropic displacement factor exponent takes the form:  $-2\pi^2 [h^2 a^{*2} U^{11} + \dots + 2 h k a^* b^* U^{12}]$**

	$U^{11}$	$U^{22}$	$U^{33}$	$U^{23}$	$U^{13}$	$U^{12}$
Br(1)	23(1)	44(1)	78(1)	-4(1)	8(1)	0(1)
Br(2)	24(1)	47(1)	71(1)	2(1)	7(1)	1(1)
C(1)	28(2)	32(4)	47(3)	5(3)	14(2)	9(2)
C(2)	30(3)	32(3)	39(3)	0(2)	6(2)	1(2)
C(3)	22(2)	29(4)	55(3)	3(3)	11(2)	-3(2)
C(4)	28(3)	36(4)	71(4)	-5(3)	19(3)	4(2)
C(5)	28(3)	35(4)	60(4)	-9(3)	16(3)	-6(2)
C(6)	22(2)	33(3)	49(3)	1(3)	12(2)	1(2)
C(7)	30(3)	27(3)	54(4)	3(3)	7(3)	5(2)
C(8)	24(3)	43(4)	65(4)	-9(3)	1(3)	4(3)
C(9)	24(3)	52(5)	90(5)	-26(4)	13(3)	-7(3)
C(10)	28(3)	31(4)	118(7)	-16(4)	31(4)	-5(3)
C(11)	35(3)	27(3)	85(5)	0(3)	33(3)	2(2)
C(12)	45(4)	29(4)	116(7)	8(4)	46(4)	3(3)
C(13)	24(2)	31(4)	63(4)	6(3)	16(2)	2(2)
C(14)	20(2)	39(4)	62(4)	3(3)	8(2)	-4(2)
C(15)	26(3)	46(4)	65(4)	10(4)	1(3)	-6(3)
C(16)	36(3)	69(6)	57(4)	-7(4)	9(3)	-18(3)
C(17)	24(3)	63(5)	69(5)	-16(4)	14(3)	-7(3)
C(18)	25(3)	52(4)	61(4)	-5(3)	6(3)	5(3)
C(19)	23(2)	40(4)	55(4)	-6(3)	10(2)	-2(2)
C(20)	34(3)	33(3)	45(3)	3(3)	6(2)	4(2)



C(21)	35(3)	36(4)	46(3)	-6(3)	2(2)	-8(3)
C(22)	20(2)	33(3)	49(3)	8(3)	4(2)	2(2)
C(23)	26(3)	37(4)	56(4)	1(3)	11(2)	5(2)
C(24)	35(3)	28(3)	37(3)	-1(2)	3(2)	1(2)
C(25)	29(2)	30(3)	37(3)	1(3)	4(2)	-2(2)
C(26)	28(2)	20(3)	59(4)	1(3)	8(2)	3(2)
C(27)	26(3)	53(5)	76(5)	-14(4)	-3(3)	0(3)
C(28)	30(3)	59(5)	101(7)	-26(5)	-17(4)	8(3)
C(29)	54(4)	42(4)	82(6)	-13(4)	-33(4)	12(3)
C(30)	59(4)	37(4)	57(4)	-3(3)	-20(3)	4(3)
C(31)	56(4)	35(4)	64(4)	8(3)	-10(3)	1(3)
C(32)	38(3)	30(4)	48(4)	1(3)	-6(3)	3(2)
C(33)	46(3)	46(4)	45(4)	-6(3)	-7(3)	1(3)
C(34)	72(5)	61(6)	57(4)	13(4)	8(4)	6(4)
C(35)	95(7)	108(9)	47(5)	4(5)	19(4)	16(6)
C(36)	74(5)	84(8)	68(5)	-31(5)	0(4)	11(5)
C(37)	51(4)	53(5)	81(5)	-23(5)	3(4)	1(4)
C(38)	40(3)	40(4)	63(4)	-15(3)	-1(3)	1(3)
N(1)	26(2)	27(3)	56(3)	-5(2)	7(2)	-1(2)
N(2)	24(2)	29(3)	59(3)	-3(3)	1(2)	-1(2)
O(1)	40(2)	33(3)	54(3)	-1(2)	6(2)	4(2)
O(2B)	20(8)	22(9)	70(20)	-3(9)	20(11)	0(6)
O(2A)	57(8)	49(5)	126(18)	-1(6)	67(11)	1(4)
O(3)	36(2)	33(3)	84(3)	-2(2)	24(2)	0(2)
O(4)	31(2)	29(2)	62(3)	5(2)	16(2)	5(2)
O(5)	80(4)	50(4)	124(5)	-33(4)	-60(4)	21(3)

O(6)	71(3)	37(3)	60(3)	-3(2)	-15(2)	18(2)
C(91)	26(9)	340(60)	120(30)	-130(30)	25(12)	-46(18)
C(92)	53(10)	130(20)	48(9)	-1(10)	-5(7)	-25(10)
O(92)	79(10)	137(16)	106(12)	49(12)	25(8)	-4(11)
C(94)	63(11)	120(20)	122(18)	83(17)	-2(11)	9(12)
O(91)	39(6)	177(16)	85(8)	-9(10)	5(6)	-9(8)
C(95)	140(30)	240(40)	52(15)	80(20)	-43(15)	-130(30)

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**Table S5. Hydrogen coordinates (  $\times 10^4$  ) and isotropic displacement parameters ( $\text{\AA}^2 \times 10^3$ ).**

	x	y	z	U(eq)
H(1)	8740	10970	3543	42
H(2)	10841	11390	4074	41
H(4)	11768	8121	2889	53
H(5)	9693	7787	2301	48
H(8A)	5488	9626	1848	55
H(8B)	5043	9935	2589	55
H(9A)	5258	7131	1984	66
H(9B)	3982	7900	2016	66
H(10A)	4467	7747	3316	68
H(10B)	4570	6165	2993	68
H(11)	6282	6750	3974	55
H(13)	7854	8315	3691	46
H(15)	6681	8080	4887	57
H(16)	6147	9654	5732	65
H(17)	5796	12071	5456	62
H(18)	5930	12922	4298	56
H(19)	6432	11370	3438	47
H(20)	9693	4708	2807	45
H(21)	11858	4786	3062	48
H(23)	11886	1303	1773	47
H(24)	9731	1256	1497	41

H(27A)	5892	2475	1581	65
H(27B)	5903	2006	759	65
H(28A)	4507	3904	698	82
H(28B)	5538	4877	1222	82
H(29A)	5354	5569	-6	81
H(29B)	5621	3952	-223	81
H(30)	7464	5256	-100	68
H(32)	8828	3994	928	49
H(34)	8765	3940	-539	77
H(35)	9172	2259	-1376	99
H(36)	8781	-185	-1236	93
H(37)	7981	-913	-249	77
H(38)	7571	751	585	60
H(3)	6886	4048	2923	74
H(6)	7035	7984	1076	92
H(91A)	-1338	10864	5406	241
H(91B)	-723	12225	5106	241
H(91C)	-472	11932	5971	241
H(94A)	1386	8042	5805	125
H(94B)	1653	8916	5121	125
H(95A)	1298	6479	4815	232
H(95B)	246	7446	4309	232
H(95C)	-40	6581	4991	232

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## REFERENCES

- (1) Hett, E. C., Xu, H., Geoghegan, K. F., Gopalsamy, A., Kyne, R. E., Menard, C. A., Narayanan, A., Parikh, M. D., Liu, S., Roberts, L., Robinson, R. P., Tones, M. A., et al. (2015) Rational targeting of active-site tyrosine residues using sulfonyl fluoride probes. *ACS Chem. Biol.* *10*, 1094-1098.
- (2) Bricogne, G., Blanc, E., Brandl, M., Flensburg, C., Keller, P., Paciorek, W., Roversi, P., Sharff, A., Smart, O. S., Vonnrhein, C., and Womack, T. O. (2011) BUSTER version 2.11.5. Cambridge, United Kingdom: Global Phasing Ltd.
- (3) Emsley, P., and Cowtan, K. (2004) Coot: model-building tools for molecular graphics. *Acta Cryst. D60*, 2126-2132.
- (4) McCoy, A. J., Grosse-Kunstleve, R. W., Adams, P. D., Winn, M. D., Storoni, L. C., and Read, R. J. (2007) Phaser crystallographic software. *J. Appl. Crystallogr.* *40*, 658-674.
- (5) Vonnrhein, C., Flensburg, C., Keller, P., Sharff, A., Smart, O., Paciorek, W., Womack, T., and Bricogne, G. (2011) Data processing and analysis with the autoPROC toolbox. *Acta Cryst. D67*, 293-302.