Supporting Information

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Table S1. Pharmacokinetic parameters of PKS21224, TDI4258 and compound1.

Cmpd ID			PKS21224	TDI4258	compound1
Solubility	(μg/mL)	pH1.2	>96		
		pH7.4	72	>110	<1.59
		GCDC	>95	>110	11
LogD		pH7.4	2.68	1.85	3.55
PAMPA	(nm/sec)	pH7.4	266	64	undetected
		pH5.0	220	56	undetected
MDR1@10 μM	B-A/A-B	ER	58	30	>2.9
	A to B	(nm/s)	5	1	<1
Metabolic rate	(μL/min/mg)	human	152	60	179.4
		mouse	126	78	90.6
		rat	74	32	136.2
mouse PK, iv (0.1mg/kg, mean, n=3)	C_{5min}	(ng/mL)	60.4	39.7	
	AUC_{iv}	(ng·h/mL)	14.8	14.1	
	MRT_{iv}	(h)	0.27	0.39	
	$V_{d(SS)}$	(mL/kg)	1958	2867	
	CL_{total}	(mL/h/kg)	7158	8157	
Comp conc determined in plasma	CL_{total}	(mL/min/kg)	119.3	135.95	
mouse PK, po (1mg/kg, mean, n=3)	C_{max}	(ng/mL)		3.4	
	T_{max}	(h)		0.25	
	AUC_{po}	(ng·h/mL)		3.3	
	MRT_{po}	(h)		0.89	
	% F	(%)	0	2.3	

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Table S2. Blood levels and PK parameters of TDI-8304 from *in vivo* therapeutic efficacy assay against *Plasmodium falciparum* (Pf3D70087/N9) using NODscidIL2rγ-/- mice.

TDI-8304; 100 mg/kg s.c (b.i.d.)						
Animal ID	C_{max} (ng/mL)	T_{max} (h)	AUC(0-8) (h*ng/mL)	C_{24h} (ng/mL)	C_{72h} (ng/mL)	C_{96h} (ng/mL)
M1	10400	0.50	14947	170	146	307
M2	10800	0.50	13165	5.23	29.6	189

Figure S1. *In vivo* pharmacokinetics of TDI-8304.

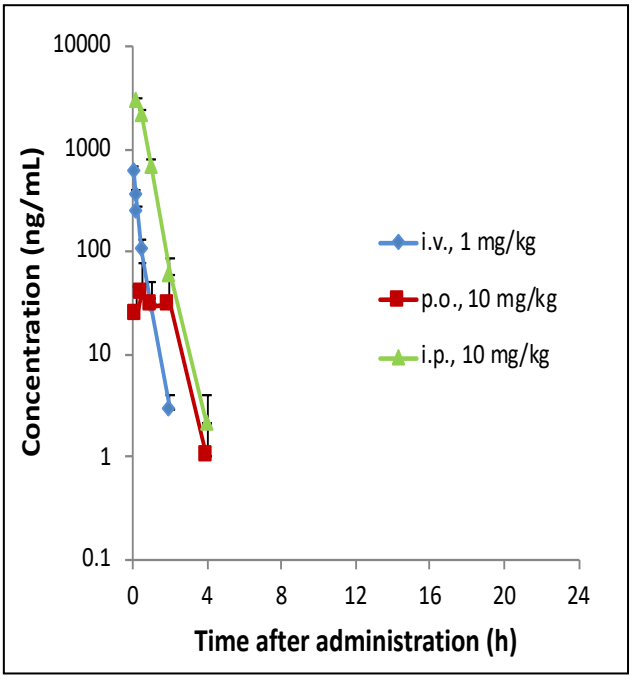
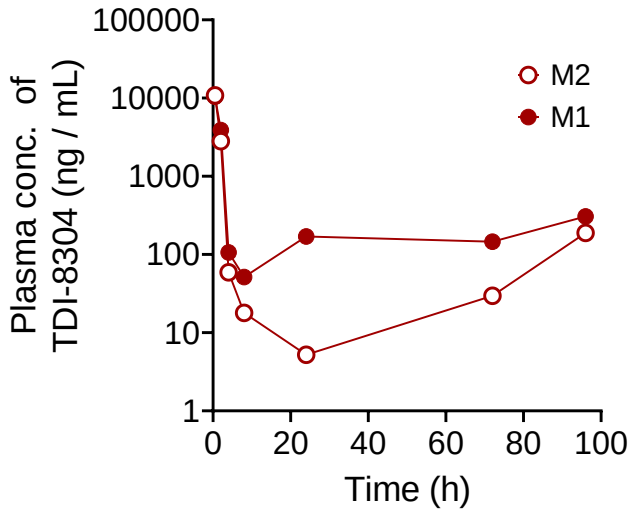


Figure S2. Plasma concentrations of TDI-8304 during the course of in vivo efficacy study.



Methods

Materials

The human 20S Immunoproteasome (i-20S, Catalog No.: E-370), human constitutive proteasome (c-20S, Catalog No: E-360) and Recombinant Human PA28 Activator alpha subunit (Catalog N0.: E-381) were purchased from Boston Biochem. The *P. falciparum* 20S proteasome (Pf20S) was enriched as previously reported[1]. Proteasome β5 substrate suc-LLVY-AMC and β5i substrate Ac-ANW-AMC were purchased from Boston Biochem. MV151 was prepared following the reported method.[1] WLW-VS was synthesized as reported.[2]

In vitro cultivation. *P. falciparum* laboratory lines were grown under standard conditions at 5% hematocrit in RPMI 1640 medium, 0.5% Albumax II (Invitrogen), 0.25% sodium bicarbonate and 0.1 mg/ml gentamicin. Parasites were placed in an incubator under 5% carbon dioxide, 5% oxygen and 90% nitrogen at 37 °C as previously reported.[3] Two Dd2-derived resistant strains (Dd2β5A49S and Dd2β6A117D) were developed in house and identified as previously described.[3-4]

IC_{50} determination. IC_{50} values of all compounds against Pf20S β5, human c-20S β5c and i-20S β5i were determined in 96-well plates as reported.[3-5] Briefly, 1μL of compound in a 3-fold series dilution in DMSO at final concentrations from 100 μM to 0.0017 μM were spotted to the bottom of a black 96-well plate. 100 μL of reaction buffer (50 mM Tris, 5 mM $MgCl_2$, 1 mM DTT, pH 7.4 for Pf20S β5; 20 mM HEPES, 0.5 mM EDTA and 0.1 mg/mL BSA, pH7.5 for human β5c and β5i) containing proteasome, substrate and activator was added to each well and the plate was spun on a desktop plate centrifuge and then placed on an orbital shaker at room temperature for 1 minutes. The progress of reactions in each well was followed by the fluorescence of the hydrolyzed AMC at Ex 360nm and Em 460 nm for 1 - 2 hours. Linear ranges of the time course are used to calculate the velocities in each well. The initial reaction velocity of each well was fit to a dose-dependent inhibition equation using PRISM to determine the IC_{50}. Final concentrations of Pf20S, c-20S and i-20S were 1 nM, 0.2 nM and 0.4 nM, respectively. Substrate

suc-LLVY-AMC was used for Pf20S and c-20S at final concentration 25μM, and Ac-ANW-AMC was used as substrate of i-20S at final concentration 15 μM. Activator PA28 at final concentration of 12 nM was used for Pf20S assay in the presence of 0.5 μM of WLW-VS, whereas 0.02% SDS was used in the assays for c-20S and i-20S.

Kinetic assay of TDI-8304 vs Pf20S. The experiments were conducted on a SpectraMax Gemini plate-reader from Molecular Devices (Sunnyvale, CA). In a black wall/clear bottom 96-well plate, 100 μL of pre-warmed assay mixture containing 1 nM Pf20S, 25 μM suc-LLVY-AMC, 12 nM PA28 and 0.5 μM WLW in buffer (50 mM Tris, 5 mM $MgCl_2$, 1 mM DTT, pH 7.4) was added to the wells that contained 1 μL inhibitor at 100x indicated concentrations in DMSO. The reaction progress of each well was immediately recorded by monitoring fluorescence at Ex 360nm and Em 460 nm for 1.2 h at 37˚C. Values of k_{obs} were derived by fitting data to equation (I): $[P] = v_s*t + [(v_i-v_s)/k_{obs}]*[1-\exp(-k_{obs}*t)]$ (v_i is initial velocity, v_s is steady state velocity) in Prism (GraphPad Software, Inc. La Jolla, CA), and then were plotted against inhibitor concentrations to obtain K_I^{app}, k_6 and k_5 by fitting to equation $k_{obs} = k_6 + (k_5 / (1 + (K_I^{app} / [I])$. The values of K_I^{*app} and $t_{1/2}$ for TDI-8304 were calculated by equation $K_I^{*app} = K_I^{app} / (1 + k_5 / k_6)$.

Anti-malarial activity in erythrocytic stage. Parasite growth inhibition assays were performed on parasites cultured in sterile 96-well plates at a total 200 μL volume per well and a 0.5% initial parasitemia and 2% hematocrit. Plates were placed in an airtight chamber flushed with 5% O_2, 5% CO_2 and 90% N_2 for 72 hours. Plates were then placed in the −80°C freezer for lysis upon thawing.[6] SYBR Green solution (100 μL of 0.2 μL SYBR Green per mL lysis buffer) was then added to each well and the plates were shaken in the dark at room temperature for 1 hour. Fluorescence was then recorded in a SpectraMax Gemini plate reader with excitation 490 nm and emission 530 nm. Fluorescent counts were normalized and plotted by non-linear least square regression to yield EC_{50} values (PRISM).

HepG2 cell viability assay. HepG2cells were plated in 96-well plate (5000 cells per well) format and treated with various concentrations of test compounds or DMSO for 72 h. Cell viability was measured using CellTiter-Glo® Assay (Promega) as per the manufacturer's instructions.

Ex vivo EC_{50} values against *P. falciparum* field isolates in Uganda. The activity of TDI-8304 was tested, as previously described,[1, 4] against *P. falciparum* isolates using a 72-h growth inhibition assay with parasite DNA readout by Sybr Green detection.[6-7] These isolates were collected in June-August, both in year 2018 and 2019, from patients living in the Tororo and Busia Districts, Uganda, who were newly diagnosed with *P. falciparum* malaria and before antimalarial treatment was administered. The relevant clinical trials and analyses of cultured parasites were approved by the Uganda National Council of Science and Technology, the Makerere University Research and Ethics Committee, and the University of California, San Francisco Committee on Human Research.

Pf20S active subunits labeling by MV151. 15 nM of Pf20S in 20 mM HEPES, 0.5 mM EDTA, PH 7.5 was treated with TDI-8304 at the designed concentrations at 37 °C for 1 h, followed by addition of activity-based probe MV151 at final concentration of 2 μM and a further incubation for 1 h at 37 °C avoiding light. The labelling assays were conducted in the absence of and in the presence of WLW-VS (0.5 μM) The samples were then heated with 4X SDS loading buffer at 95 °C for 10 min and run on a 12% Novex™ Bis-Tris Protein Gel with MOPS SDS running buffer. The gel was fixed with methanol, rinsed with distilled water and scanned at the TAMRA channel on a Typhoon Scanner (GE Healthcare).

Dual drug assays. Dual drug assays were performed as described.[3, 8] We plated 5 volume-to-volume compound combinations (4:0, 3:1, 2:2, 1:3 and 0:4) which were then diluted 3-fold in a 96 well plate. Compound combinations were tested in duplicate. Aliquots of parasites were added to compounds and incubated for 3 hours, then compounds were washed off. Parasites were then incubated with TDI-8304 at the specified concentrations from the combinations for 69 hours in an airtight chamber. Parasitemia

was then detemined by flow cytometry using (Hoechst 33342 and Thiazole Orange). FIC values were calculated as described.[8]

Ubiquitin labeling of TDI-8304 treated *P. falciparum.* *P. falciparum* parasites at schizont stage were treated with TDI-8304 at final concentration of 1 μM for 6 h, and DMSO was used as a control. The treated cultures were spun down at 4000 rpm and lysed with saponin to remove red blood cells. The parasite pellet was washed with PBS, and lysed in 50 mM Tris, 5 mM $MgCl_2$, 1 mM DTT, pH 7.4, 50 μM pan DUB inhibitor PR619, and 1× Roche protease inhibitor cocktail. Lysates were then boiled with 4X SDS loading buffer for 10 min and run on a 12% Bis-Tris gel and transferred to an Amersham Protran 0.45 NC nitrocellulose membrane (GE Healthcare) using semi-dry transfer. The membrane was pretreated with 0.5% glutaraldehyde / PBS pH 7.0 for 20 min, washed with 3× PBS, and then blocked with Odyssey® Blocking Buffer (PBS) Odessey and blotted with 1:500 dilution of primary anti-Ubiquitin antibody (Mouse monoclonal, VU-1 from LifeSensors) and 1:1000 anti-α-tubulin antibody (Rabbit monoclonal, 2144 from CST), followed by incubation with 1:10000 secondary anti-Rabbit IRDye® 800CW (LI-COR) and 1:10000 anti-Mouse IRDye® 680RD (LI-COR). The blot was further rinsed with PBS and scanned on the Licor Odyssey.

Modeling. Since the ligand bound to the β5-6 subunits in the yeast proteasome complex structure (PDB: 3MG4) is an acyclic analog of our macrocyclic series, it was used as a template to place our macrocyclic compound 2 in the Pf20S β5-6 structure. The X-ray structure of the β5-6 subunits of the yeast 20S (PDB: 3MG4, chains L and K) was superimposed on the cryo-EM structure of the β5-6 subunits of Pf20S (PDB: 5FMG, chains L and M), and the coordinates of the yeast proteasome ligand was transferred into the Pf20S β5-6 structure. The macrocyclic compound 2 was then generated by cyclization and modification of the acyclic compound. This initial pose of compound 2 was then refined using Prime minimization on the ligands and residues within 6A of the ligand.

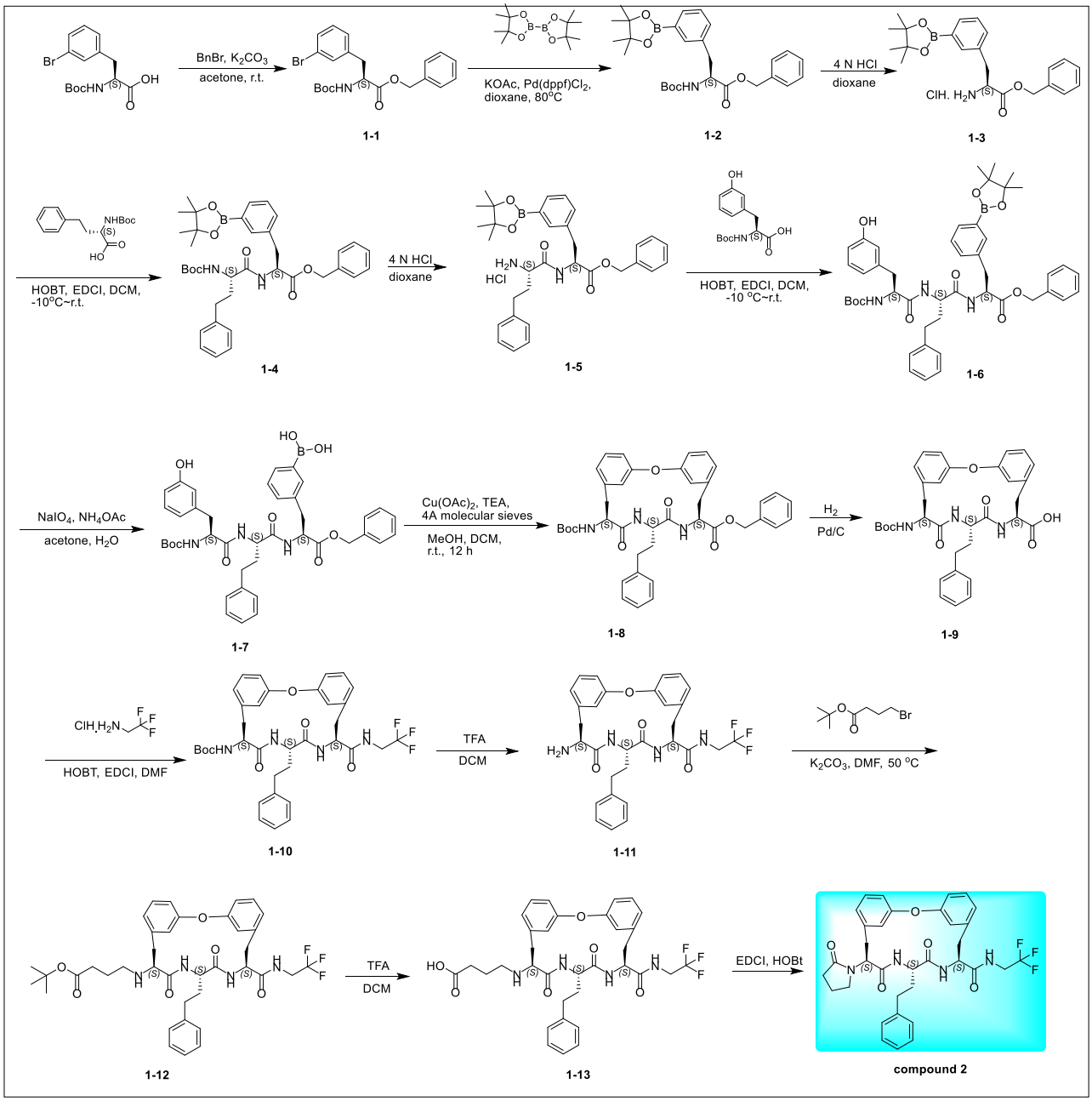
SCID mouse efficacy studies. *In vivo* efficacy of TDI-8304 was measured against *Plasmodium falciparum Pf*3D7$^{0087/N9}$ growing in peripheral blood of NODscidIL2Rγ^{null} mice (Jackson Laboratory, USA) (23-36 g) engrafted with human erythrocytes as described.[9] The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents under an IRB/EC approved protocol. Parasites (20 x 10^6) were inoculated by intravenous injection and antimalarial efficacy was assessed using a "4-day-test". Blood parasitemia was measured by FACS analysis. Compound TDI-8304 was administered twice-daily (SC) for four consecutive days starting on day 3 post infection in vehicle (5% DMSO, 45% PEG400, 50% water). All animal studies were ethically reviewed and carried out in accordance with European Directive 2010/63/EEC and the GSK Policy on the Care, Welfare and Treatment of Animals.

In the same animals, blood samples (15 μL) were collected 30 min, 2, 4, 8, 24, 72 and 96 h after first administration following first dose administration of TDI-8304, diluted with 30 μL of sterile water and stored at approximately -20º C until analyzed. The concentration in the dose suspension was confirmed and quantification of blood samples for parent compound was performed by means of LC-MS/MS with a lower limit of quantification of 1 ng/mL.

Non-compartmental methods were used to obtain estimates of pharmacokinetic parameters using Phoenix 64 (Pharsight, Certara).

Chemistry: All commercially available reagents were used without further purification unless otherwise noted. All non-aqueous reactions were performed under argon in oven-dried glasswares. Routine monitoring of reactions were performed using Waters Acquity Ultra Performance Liquid Chromatography (UPLC/MS) and TLC. Column chromatography was generally performed on silica gel (200-300 mesh). Analytical HPLC-MS was carried out using an Acquity™ Ultra Performance LC system, comprising a PDA detector, Binary Solvent Manager and SQ detector, tandem linked to a mass spectrometry system employing vendor software. Parallel evaporative light-scattering detection was

incorporated into the system via a flow splitter. Column (C18 Column, 130Å, 1.7 μm, 2.1 mm X 100 mm) chromatography was carried out using a linear increasing gradient from 5-95% of solvent A (0.1% formic acid in water) to solvent B (0.1% formic acid in acetonitrile) at 0.5 ml/min over 4.0 min, or the same gradient applied over 8 min. All HPLC purifications were done by Waters prep-HPLC (mass directed purification system) using Prep C18 column. Chiral SFC was in general performed with Chiralcel Column (OD-3 50×4.6mm I.D., 3um) using a linear increasing gradient MeOH (0.05% DEA) in CO2 from 5% to 40% at 3mL/min, detection by UV absorbance. ^{1}H NMR spectra was acquired on a Bruker DRX-400 spectrometer. Chemical shifts δ are expressed in parts per million, with the solvent resonance as an internal standard ($CDCl_3$, ^{1}H: 7.26; DMSO-*d6*, ^{1}H: 2.50 ppm; CD_3OD, ^{1}H: 3.31 ppm). NMR data are reported as following: chemical shift, multiplicity (br = broad, d = doublet, m = multiplet, q = quartet, s = singlet, t = triplet), coupling constant, and integration.



Scheme S1 Synthetic route of compound 2

Preparation of benzyl (S)-3-(3-bromophenyl)-2-((tert-butoxycarbonyl)amino)propanoate 1-1

To a mixture of (S)-3-(3-bromophenyl)-2-((tert-butoxycarbonyl)amino)propanoic acid (8.0 g, 23.24 mmol, 1.0 eq) and potassium carbonate (3.37 g, 24.40 mmol, 1.05 eq) in acetone (800 mL) was added benzyl bromide (4.37 g, 25.56 mmol, 1.1 eq) at 25°C and then the reaction mixture was stirred overnight at 25°C. LCMS showed that the reaction was completed. The reaction mixture was filtered and the filtrate was concentrated in vacuum. The residue

was purified by column chromatography (petroleum ether: ethyl acetate= 100:1 to 10:1) to give the desired product **1-1** (10.0 g, 22.74 mmol, 97.8% yield, 98.7% purity) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39-7.36 (m, 4H), 7.33-7.30 (m, 2H), 7.26-7.25 (m, 1H), 7.10 (t, *J* = 7.6 Hz, 1H), 6.97 (d, *J* = 7.2 Hz, 1H), 5.15 (q, *J* = 12.4 Hz, 2H), 5.05-5.02 (m, 1H), 4.64-4.59 (m, 1H), 3.14-2.99 (m, 2H), 1.44 (s, 9H).

LCMS for **1-1**: RT = 0.964 min, purity: 98.8%, *m*/*z*= 334.0 $[M\text{-}t\text{Boc}+H]^+$.

SFC for **1-1**: RT = 1.664 min; ee% = 99.6%.

Preparation of benzyl (S)-2-((tert-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoate 1-2

To a solution of **1-1** (10.0 g, 23.02 mmol, 1.0 eq), bis(pinacolato)diboron (9.35 g, 36.83 mmol, 1.6 eq) and potassium acetate (6.78 g, 69.06 mmol, 3.0 eq) in dioxane (250 mL) was added $Pd(dppf)Cl_2$ (1.68 g, 2.30 mmol, 0.1 eq) at 25°C and then the reaction mixture was stirred at 80°C for 12 h under nitrogen. LCMS showed that the reaction was completed. The reaction mixture was filtered and the filtrate was concentrated in vacuum. The residue was purified by column chromatography (SiO_2, petroleum ether: ethyl acetate= 100:1 to 10:1) to give the product **1-2** (10.0 g, 19.72 mmol, 85.7% yield, 94.9% purity) as yellow oil.

LCMS for **1-2**: RT = 0.968 min, purity: 94.9%, *m*/*z*= 382.1 $[M\text{-Boc}+H]^+$.

Preparation of benzyl (S)-2-((S)-2-((tert-butoxycarbonyl)amino)-4-phenylbutanamido)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoate 1-4

To a solution of **1-2** (35.0 g, 72.71 mmol, 1.0 eq) in dioxane (50 mL) was added a solution of hydrogen chloride in dioxane (4 M, 300 mL) at 25°C and then the reaction mixture was stirred at 25°C for 0.5 hour. LCMS showed that the reaction was completed. Then the reaction mixture was concentrated in vacuum to give the benzyl (S)-2-amino-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoate **1-3** (30.0 g, crude, HCl salt) as yellow oil, which was used directly in the next step.

To a solution of (S)-2-((tert-butoxycarbonyl)amino)-4-phenylbutanoic acid (18.39 g, 65.84 mmol, 1.1 eq), 1-hydroxybenzotriazole (8.9 g, 65.84 mmol, 1.1 eq) in dichloromethane (250 mL) was added 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride (12.62 g, 65.84 mmol, 1.1 eq) at 0°C and then the reaction mixture was

stirred for 30 mins. Then **1-3** (25 g, 59.85 mmol, 1.0 eq, HCl salt) and diisopropylethylamine (23.2 g, 179.55 mmol, 3.0 eq) in dichloromethane (120 mL) was added to above reaction mixture at 0°C and then the resulting mixture was stirred at 25°C for 11.5 hours. LCMS showed that the reaction was completed. The reaction mixture was quenched with hydrochloric acid solution (1 M, 400 mL) and extracted with dichloromethane (200 mL*2). The combined organic phases were washed with brine (400 mL*2), dried over sodium sulfate and concentrated in vacuum. The residue was purified by column chromatography (SiO_2, petroleum ether: ethyl acetate= 50:1 to 2:1) to give the product **1-4** (29.0 g, 38.45 mmol, 64.2% yield, 85.2% purity) as yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, J = 7.2 Hz, 1H), 7.54 (s, 1H), 7.40-7.29 (m, 6H), 7.28-7.20 (m, 6H), 7.17 (d, J = 7.2 Hz, 1H), 6.64 (br.s, 1H), 5.26-5.24 (s, 1H), 4.89-4.84 (m, 1H), 4.41-4.36 (m, 1H), 4.16-4.10 (m, 1H), 3.20-3.17 (m, 1H), 3.09-3.07 (m, 1H), 2.73-2.65 (m, 2H), 2.28-2.21 (m, 2H), 1.40 (s, 9H), 1.34 (s, 12H).

LCMS for **1-4**: RT = 1.052 min, purity: 85.2%, m/z= 543.3 $[M\text{-Boc}+H]^+$.

Preparation of benzyl (S)-2-((S)-2-amino-4-phenylbutanamido)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoate hydrochloride 1-5

To a solution of **1-4** (31.0 g, 48.24 mmol, 1.0 eq) in dioxane (50 mL) was added a solution of hydrogen chloride in dioxane (4 M, 250 mL, 20.7 eq) at 25°C and then the reaction mixture was stirred at 25°C for 30 min. LCMS showed that the reaction was completed. The reaction mixture was concentrated in vacuum to give the product **1-5** (26.00 g, 38.01 mmol, 78.8% yield, 84.6% purity, HCl salt) as yellow oil. ^{1}H NMR (400 MHz, CD_3OD) δ 7.65-7.61 (m, 2H), 7.35-7.16 (m, 12H), 5.48 (s, 2H), 4.86-4.76 (m, 1H), 3.95-3.93 (m, 1H), 3.21-3.19 (m, 1H), 3.11-3.09 (m, 1H), 2.68-2.66 (m, 2H), 2.10-1.95 (m, 2H), 1.33 (s, 12H).

LCMS for **1-5**: RT = 0.784 min, purity: 84.6%, m/z= 543.2 $[MS+H]^+$.

Preparation of (3-((6S,9S,12S)-12-((benzyloxy)carbonyl)-6-(3-hydroxybenzyl)-2,2-dimethyl-4,7,10-trioxo-9-phenethyl-3-oxa-5,8,11-triazatridecan-13-yl)phenyl)boronic acid 1-7

To a solution of **1-5** (5.0 g, 9.22 mmol, 1.0 eq) and (S)-2-((tert-butoxycarbonyl)amino)-3-(3-hydroxyphenyl)propanoic acid (2.59 g, 9.22 mmol, 1.0 eq) in N,N-dimethylformamide (150 mL) was added diisopropylethylamine (5.96 g, 46.10 mmol, 5.0 eq), 1-hydroxybenzotriazole (1.37 g, 10.14 mmol, 1.1 eq) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (1.94 g, 10.14 mmol, 1.1 eq) at 0°C and the reaction

was stirred at 25°C for 12 hours. LCMS showed that the reaction was completed. The reaction mixture was acidified to pH=4 with hydrochloric acid (0.5 M) and extracted with dichloromethane (150 mL*4). The organic layers were dried over anhydrous sodium sulfate, filtered and concentrated in vacuum to give the benzyl (6S,9S,12S)-6-(3-hydroxybenzyl)-2,2-dimethyl-4,7,10-trioxo-9-phenethyl-12-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)-3-oxa-5,8,11-triazatridecan-13-oate **1-6** (13.60 g, 4.35 mmol, 47.2% yield, 25.8% purity) as red oil, which was used directly in the next step.

To a solution of **1-6** (30.0 g, 37.23 mmol, 1.0 eq) in acetone (300 mL) was added sodium periodate (23.89 g, 111.69 mmol, 3.0 eq) and ammonium acetate (8.61 g, 111.69 mmol, 3.0 eq) in water (240 mL) at 25°C and then the reaction mixture was stirred at 25°C for 12 hours. LCMS showed that the reaction was completed. The reaction mixture was acidified to pH=4 with hydrochloric acid (0.5 M) and extracted with dichloromethane (300 mL*3). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated in vacuum. The residue was purified by reverse phase flash (HCl condition) to give the product **1-7** (9.30 g, 12.85 mmol, 25.1% yield, 100% purity) as a brown solid.

LCMS for **1-7**: RT = 0.915 min, purity: 100%, *m*/*z*= 724.1 $[MS+H]^+$.

Preparation of benzyl (5S,8S,11S)-11-((tert-butoxycarbonyl)amino)-7,10-dioxo-8-phenethyl-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxylate 1-8

To a solution of **1-7** (6.0 g, 8.29 mmol, 1.0 eq) in dichloromethane (600 mL) was added copper acetate (1.51 g, 8.29 mmol, 1.0 eq), triethylamine (8.39 g, 82.90 mmol, 10.0 eq), methanol (2.66 g, 82.90 mmol, 10.0 eq) and 4Å molecular sieves (6.0 g) at 25°C and then the reaction mixture was stirred at 25°C for 12 hours under oxygen. LCMS showed that the reaction was completed. The reaction mixture was filtered and concentrated in vacuum. The residue was purified by re-crystallization from ethyl acetate (100 mL) and then re-crystallized from acetonitrile (100 mL) to give **1-8** (2.60 g, 3.66 mmol, 44.2% yield, 95.3% purity) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.60 (d, J = 8.4 Hz, 1H), 8.10 (d, J = 8.8 Hz, 1H), 7.41-7.28 (m, 9H), 7.24-7.20 (m, 1H), 7.08-6.97 (m, 4H), 6.89-6.79 (m, 3H), 6.15-6.10 (m, 2H), 5.17 (s, 2H), 4.75-4.70 (m, 1H), 4.37-4.33 (m, 2H), 3.27-3.17 (m, 2H), 2.91-2.71 (m, 2H), 2.45-2.40 (m, 2H), 1.75-1.63 (m, 2H), 1.38 (s, 9H).

LCMS for **1-8**: RT = 3.232 min, purity: 95.3%, *m*/*z*= 678.2 $[MS+H]^+$.

SFC for **1-8**: RT = 2.443 min; de% = 100.00%.

Preparation of (5S,8S,11S)-11-((tert-butoxycarbonyl)amino)-7,10-dioxo-8-phenethyl-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxylic acid 1-9

To a solution of **1-8** (250 mg, 368.85 umol, 1.0 eq) in dichloromethane (10 mL) and isopropanol (20 mL) was added Pd/C (100 mg, 5% purity) under nitrogen. The suspension was degassed under vacuum and purged with hydrogen for several times. The mixture was stirred under hydrogen (15 psi) at 25°C for 4 hours. LCMS showed that the starting material was completed. The reaction mixture was filtered and the filtrate was concentrated in vacuum to give compound **1-9** (150 mg, 183.86 umol, 49.8% yield, 72.0% purity) as a brown solid.

LCMS for **1-9**: RT = 0.900 min, purity: 72.0%, *m*/*z*= 588.1 $[MS+H]^+$.

Preparation of tert-butyl ((5S,8S,11S)-7,10-dioxo-8-phenethyl-5-((2,2,2-trifluoroethyl)carbamoyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-11-yl)carbamate 1-10

To a solution of **1-9** (800 mg, 1.36 mmol, 1.0 eq) and 1-hydroxybenzotriazole (202 mg, 1.50 mmol, 1.1 eq) in tetrahydrofuran (15 mL) and N,N-dimethylformamide (15 mL) was added EDCI (287 mg, 1.50 mmol, 1.1 eq) and diisopropylethylamine (352 mg, 2.72 mmol, 2.0 eq) at -10°C and then the reaction mixture was stirred for 0.5 hour at -10°C. Then 2,2,2-trifluoroethan-1-amine hydrochloride (203 mg, 1.50 mmol, 1.1 eq, HCl salt) was added to above reaction mixture and the reaction mixture was stirred for 11.5 hours at 25°C. LCMS showed that the reaction was completed. Water (30mL) was added to the reaction mixture and the solid was precipitate out. The solid was collected and purified by prep-HPLC (TFA condition; column: Agela ASB 150mm*25mm*5um, mobile phase: [water (0.1%TFA)-ACN]; B%: 55%-85%, 11 min) and then purified by re-crystallization from a mixture of isopropyl ether and methanol and dichloromethane (V:V:V= 3:1:1, 5 mL *3) to give **1-10** (19.00 mg, 28.41 umol, 6.3% yield, 100% purity) as a white solid. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.90 (t, J = 5.6 Hz, 1H), 8.52 (d, J = 8.4 Hz, 1H), 8.03 (d, J = 8.0 Hz, 1H), 7.32-7.27 (m, 4H), 7.23-7.14 (m, 4H), 6.99-6.97 (m, 1H), 6.85-6.82 (m, 3H), 6.12 (br.s, 1H), 6.00 (d, J = 7.2 Hz, 1H), 4.70-4.67 (m, 1H), 4.41-4.33 (m, 2H), 4.00-3.96 (m, 2H), 3.03-2.99 (m, 1H), 2.91-2.87 (m, 1H), 2.80-2.77 (m, 2H), 2.43-2.40 (m, 2H), 1.74-1.67 (m, 2H), 1.39 (s, 9H).

LCMS for **1-10**: RT = 2.379 min, purity: 100%, *m*/*z*= 669.2 $[MS+H]^+$.

SFC for **1-10**: RT_1 = 1.674 min; RT_2 = 1.861 min; de% = 92.7%.

Preparation of (5S,8S,11S)-11-amino-7,10-dioxo-8-phenethyl-N-(2,2,2-trifluoroethyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide 1-11

To a solution of **1-10** (300 mg, 448.63 umol, 1.0 eq) in dichloromethane (10 mL) and N,N-dimethylformamide (10 mL) was added trifluoroacetic acid (7.7 g, 67.53 mmol, 5 mL, 150.5 eq) at 25°C and then the reaction mixture was stirred for 12 hours at 25°C. LCMS showed that the reaction was completed. The reaction mixture was concentrated in vacuum at 30°C. The residue was purified by prep-HPLC (TFA condition; column: Agela ASB 150mm*25mm*5um, mobile phase: [water (0.1%TFA)-ACN]; B%: 25%-55%, 11 min). The product was redissolved in water (5 mL) and the mixture was adjusted to pH=9 with aqueous sodium bicarbonate solution. The solid was collected and triturated with isopropyl ether (10 mL*2) to give **1-11** (38.00 mg, 66.40 umol, 14.8% yield, 99.4% purity) as a white solid. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.89 (t, J = 5.6 Hz, 1H), 8.51 (d, J = 8.8 Hz, 1H), 7.79 (d, J = 8.8 Hz, 1H), 7.31-7.09 (m, 8H), 6.95-6.85 (m, 3H), 6.75 (br.s, 1H), 6.18 (br.s, 1H), 4.66 (t, J = 9.2 Hz, 1H), 4.44-4.34 (m, 1H), 4.03-3.89 (m, 2H), 3.58-3.55 (m, 1H), 3.01-2.97 (m, 1H), 2.86-2.72 (m, 1H), 2.68-2.62 (m, 1H), 2.47-2.45 (m, 2H), 1.73-1.54 (m, 3H).

LCMS for **1-11**: RT = 1.906 min, purity: 99.4%, m/z= 569.3 $[MS+H]^+$.

SFC for **1-11:** RT_1 = 1.993 min; RT_2 = 2.239 min; de% = 87.1%.

Preparation of tert-butyl 4-(((5S,8S,11S)-7,10-dioxo-8-phenethyl-5-((2,2,2-trifluoroethyl)carbamoyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-11-yl)amino)butanoate 1-12

To a mixture of **1-11**(100 mg, 175.87 umol, 1.0 eq), sodium iodide (3 mg, 17.59 umol, 0.1 eq) and sodium carbonate (93 mg, 879.35 umol, 5.0 eq) in N,N-dimethylformamide (3 mL) was added tert-butyl 4-bromobutanoate (51 mg, 228.63 umol, 1.3 eq) at 25°C. The mixture was stirred for 12 hours at 50°C. LCMS showed that the desired compound was detected. Water (20 mL) was added to the reaction mixture and the product was precipitated out. The solid was collected and dried under vacuum to give **1-12** (110 mg, 154.76 umol, 88.0% yield) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.78-8.72 (m，1H), 8.39 (d, J = 8.4 Hz, 1H), 7.94 (d, J = 9.2 Hz, 1H), 7.30–7.21 (m, 4H), 7.17–7.08 (m, 4H), 7.00 (d, J = 7.6 Hz, 2H), 6.93-6.92 (m, 2H), 6.60 (br. s, 1H), 6.31 (br. s, 1H),

4.67–4.62 (m, 1H), 4.50–4.41 (m, 1H), 4.05–3.99 (m, 1H), 3.97-3.91 (m, 2H), 3.05–2.96 (m, 2H), 2.89–2.80 (m, 2H), 2.73–2.70 (m, 2H), 2.40–2.36 (m, 2H), 2.17 (t, *J* = 7.2 Hz, 2H), 1.74–1.72 (m, 2H), 1.62–1.57 (m, 2H), 1.34 (s, 9H).

LCMS for **1-12**: RT = 0.897 min, purity: 54.6%, *m*/*z*= 711.5 $[MS+H]^+$.

Preparation of 4-(((5S,8S,11S)-7,10-dioxo-8-phenethyl-5-((2,2,2-trifluoroethyl)carbamoyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-11-yl)amino)butanoic acid 1-13

To a solution of **1-12** (50 mg, 70.35 umol, 1.0 eq) in dichloromethane (2 mL) was added trifluoroacetic acid (1 mL). The mixture was stirred for 2 hours at 25°C. LCMS showed that the desired compound was detected. The mixture was concentrated in vacuum to give compound **17** (50 mg, crude) as a yellow solid, which was used in the next step without further purification.

LCMS for **1-13**: RT = 0.691 min, purity: 63.6%, *m*/*z*= 655.3 $[MS+H]^+$.

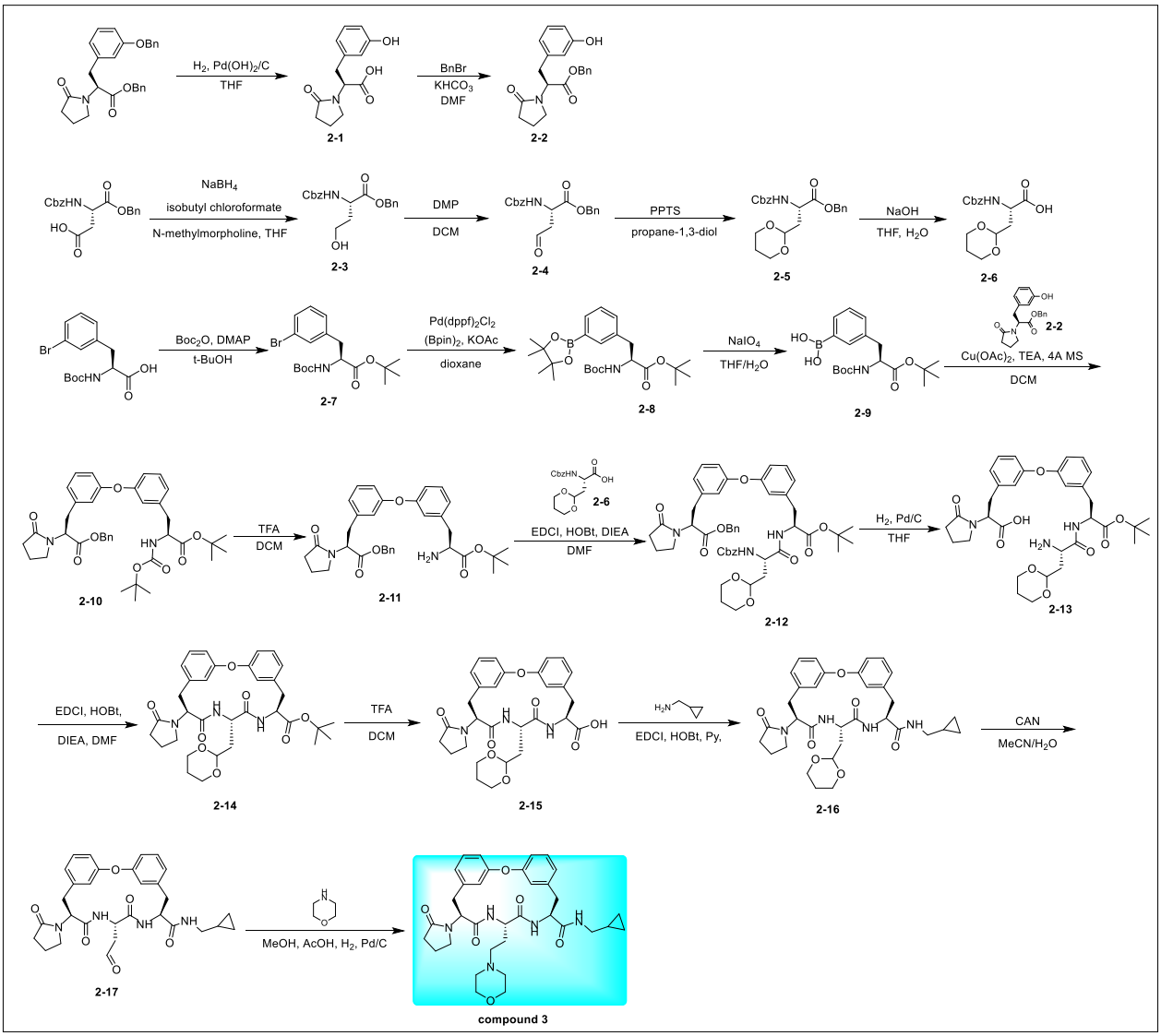
Preparation of (5S,8S,11S)-7,10-dioxo-11-(2-oxopyrrolidin-1-yl)-8-phenethyl-N-(2,2,2-trifluoroethyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide (compound 2)

To a solution of crude **1-13** (40 mg, 61.10 umol, 1.0 eq), 1-hydroxybenzotriazole (12 mg, 91.65 umol, 1.5 eq) and diisopropylethylamine (24 mg, 183.30 umol, 3.0 eq) in N,N-dimethylformamide (2 mL) was added EDCI (18 mg, 91.65 umol, 1.5 eq) at 25°C. The mixture was stirred for 12 hours at 25°C. LCMS showed that the starting material was consumed completely and the desired compound was detected. The mixture was added into water (10 mL) and the solid was precipitated out. The solid was filtered and purified by prep-HPLC (column: PhenomenexSynergi C18 150mm*25mm*10um; mobile phase: [water (0.1% TFA) - ACN]; B%: 44%-74%, 12min) to give **compound 2** (15.70 mg, 23.69 umol, 38.8% yield, 96.0% purity) as a white solid. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.67 (t, *J* = 6.4 Hz, 1H), 8.25 (d, *J* = 8.0 Hz, 1H), 7.95 (d, *J* = 8.4 Hz, 1H), 7.34–7.28 (m, 2H), 7.24 (t, *J* = 7.6 Hz, 2H), 7.17–7.12 (m, 1H), 7.08 (d, *J* = 7.2 Hz, 2H), 7.02–6.94 (m, 4H), 8.60 (br.s, 1H), 6.51 (br.s, 1H), 4.76–4.71 (m,1H), 4.65–4.62 (m, 1H), 4.29–4.27 (m, 1H), 3.97–3.84 (m, 2H), 3.18-3.13 (m, 2H), 2.97–2.91 (m, 2H), 2.63–2.60 (m, 2H), 2.44–2.39 (m, 2H), 2.28–2.16 (m, 2H), 1.92–1.86 (m, 2H), 1.76–1.65 (m, 2H).

LCMS for **compound 2**: RT = 2.791 min, purity: 98.0%, *m*/*z*= 637.2 $[MS+H]^+$.

SFC for **compound 2**: RT_1 = 2.139 min; RT_2 = 2.255 min; de% = 98.1%.

HRMS for **compound 2**: calc. for $C_{34}H_{36}F_3N_4O$ $[M+H]^+$: 637.2632. Found: 637.2645.



Scheme S2 Synthetic route of compound 3

Preparation of (S)-3-(3-hydroxyphenyl)-2-(2-oxopyrrolidin-1-yl)propanoic acid 2-1

A solution of benzyl (S)-3-(3-(benzyloxy)phenyl)-2-(2-oxopyrrolidin-1-yl)propanoate (15 g, 34.92 mmol, 1 *eq*) in tetrahydrofuran (200 mL) was degassed with nitrogen three times, then $Pd(OH)_2/C$ (1 g, 10% purity on carbon) was added in one portion. The mixture was stirred for 4 hours at 20°C under hydrogen atmosphere (15 psi). LCMS showed the starting material was consumed. The reaction mixture was filtered and concentrated in *vacuo* to afford **2-1** (9.5 g, 33.54 mmol, 96.0% yield, 88.3% purity) as white gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.89 (br. s, 1H),

9.30 (s, 1H), 7.06 (t, *J* = 7.2 Hz, 1H), 6.63 - 6.59 (m, 3H), 4.74 - 4.70 (m, 1H), 3.35 - 3.26 (m, 2H), 3.13 - 3.09 (m, 1H), 2.93 - 2.86 (m, 1H), 2.18 - 2.09 (m, 2H), 1.92 - 1.85 (m, 1H), 1.77 - 1.74 (m, 1H).

LCMS for **2-1**: RT = 0.423 min, *m*/*z* 250.1[M+Na]$^+$, purity: 88.3%.

Preparation of benzyl (S)-3-(3-hydroxyphenyl)-2-(2-oxopyrrolidin-1-yl)propanoate 2-2

To a solution of **2-1** (9.5 g, 33.54 mmol, 1 *eq*) in N, N-dimethyl formamide (100 mL) was added potassium bicarbonate (5.04 g, 50.31 mmol, 1.50 *eq*) wise-portion at 0°C. The mixture was stirred for at 0°C 30 minutes. Then benzyl bromide (6.02 g, 35.22 mmol, 4.18 mL, 1.05 *eq*) was added at 0°C dropwise. The mixture was stirred for at 0°C 2 hours. TLC (petroleum ether: ethyl acetate = 2:1) showed the starting material was consumed. The reaction mixture was poured into water (200 mL), extracted with ethyl acetate (200 mL*2). The combined organic phase was washed with brine (200 mL*3), dried over anhydrous sodium sulfate, filtered and concentrated in *vacuo.* The residue was purified by column (petroleum ether: ethyl acetate = 10:1 ~ 1:1) to afford **2-2** (11.1 g, 32.35 mmol, 96.4% yield, 98.9% purity) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 - 7.31 (m, 5H), 7.14 - 7.10 (m, 1H), 6.72 - 6.69 (m, 3H), 5.20 - 5.16 (m, 3H), 3.41 - 3.28 (m, 3H), 3.00 - 2.90 (m, 1H), 2.35 - 2.24 (m, 2H), 1.94 - 1.82 (m, 2H).

LCMS for **2-2**: RT = 0.870 min, *m*/*z* 340.0 [M+H]$^+$, purity: 98.9%.

SFC for **2-2**: RT = 2.519 min; de% = 94.7%.

Preparation of benzyl ((benzyloxy)carbonyl)-L-homoserinate 2-3

To a mixture of (S)-4-(benzyloxy)-3-(((benzyloxy)carbonyl)amino)-4-oxobutanoic acid (58 g, 132.11 mmol, 1 *eq*) and N-methylmorpholine (13.36 g, 132.11 mmol, 14.53 mL, 1 *eq*) in tetrahydrofuran (500 mL) was added isobutyl chloroformate (18.04 g, 132.11 mmol, 17.35 mL, 1 *eq*) dropwise at -20°C under nitrogen atmosphere. The mixture was stirred at -20 °C for 2 hours. Then sodium borohydride (7.50 g, 198.17 mmol, 1.5 *eq*) in water (80 mL) was added drop wise. The mixture was stirred at -20 °C for 1 hour. The mixture was quenched with 1N hydrochloric acid (800mL). The aqueous phase was extracted with ethyl acetate (500 mL*3). The combined organic phase was washed with brine (500 mL*2), dried with anhydrous anhydrous sodium sulfate, filtered and concentrated in vacuum. The residue was purified by silica gel chromatography (column height: 250

mm, diameter: 100 mm, 100-200 mesh silica gel, Petroleum ether/Ethyl acetate = 10/1 ~ 2/1) to afford the compound 5 (46.5 g, 97.31 mmol, 73.7% yield, 71.9% purity) as a light yellow oil.

LCMS for **2-3**: RT = 0.653 min, *m*/*z* 366.1 $[M+Na]^+$, purity: 71.9%.

Preparation of benzyl (S)-2-(((benzyloxy)carbonyl)amino)-4-oxobutanoate 2-4

To a solution of **2-3** (23.5 g, 49.25 mmol, 1 *eq*) in dichloromethane (500 mL) was added Dess-Martin (41.78 g, 98.50 mmol, 30.49 mL, 2 *eq*) at 0 °C under nitrogen atmosphere. The mixture was stirred at 20°C for 3 hours. TLC (petroleum ether：ethyl acetate = 2:1) indicated trace material remained and one new spot formed. The mixture was concentrated in vacuum. The residue was purified by silica gel chromatography (column height: 250 mm, diameter: 100 mm, 100-200 mesh silica gel, Petroleum ether/Ethyl acetate = 10/1 ~ 3/1) to afford **2-4** (11 g, 23.91 mmol, 48.6% yield, 74.2% purity) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.63 (s, 1H), 7.29 - 7.25 (m, 10H), 5.72 - 5.60 (m, 1H), 5.11 - 4.96 (m, 4H), 4.63 - 4.61 (m, 1H), 3.07 - 2.82 (m, 2H).

LCMS for **2-4**: RT = 0.660 min, *m*/*z* 342.1 $[M+H]^+$, purity: 74.2%.

Preparation of benzyl (S)-2-(((benzyloxy)carbonyl)amino)-3-(1,3-dioxan-2-yl)propanoate 2-5

To a mixture of **2-4** (2.2 g, 4.78 mmol, 1 *eq*, 5 batches) and propane-1, 3-diol (9.45 g, 124.19 mmol, 9 mL, 25.97 *eq*) was added PPTS (324 mg, 1.29 mmol, 0.27 *eq*) and 4A MS (4.4 g) in one portion at 20°C under nitrogen atmosphere. The mixture was stirred at 80°C for 12 hours. TLC (petroleum ether：ethyl acetate = 2:1) indicated the starting material was consumed completely and one new spot was formed. The mixture was cooled to 20°C and poured into water (100mL). The aqueous phase was extracted with ethyl acetate (100 mL*3). The combined organic phase was washed with brine (100 mL), dried with anhydrous anhydrous sodium sulfate, filtered and concentrated in vacuum to give **2-5** (11 g, crude) as a light yellow oil, which was used for the next step directly without further purification.

LCMS for **2-5**: RT = 1.003 min, *m*/*z* 400.1 $[M+H]^+$, purity: 32.0%.

Preparation of (S)-2-(((benzyloxy)carbonyl)amino)-3-(1,3-dioxan-2-yl)propanoic acid 2-6

To a mixture of **2-5** (5.5 g, 13.77 mmol, 1 *eq*, 2 batches) in tetrahydrofuran (50 mL) and water (50 mL) was added sodium hydroxide (3.86 g, 96.39 mmol, 7 *eq*) in one portion at 0°C under nitrogen atmosphere. The mixture was stirred at 0°C for 30 min. TLC (petroleum ether： ethyl acetate = 2:1) indicated the starting material was consumed completely and one new spot was formed. The mixture was poured into ice-water (50 mL) and the aqueous phase was extracted with ethyl acetate (200 mL*2). The combined organic phase was discarded, then the pH value of the aqueous phase was adjusted to 2 with 1 N hydrochloric acid aqueous (220 mL), extracted with ethyl acetate (200 mL*3). The combined organic phase was washed with brine (200 mL), dried with anhydrous anhydrous sodium sulfate, filtered and concentrated in vacuum to give **2-6** (4.6 g, 14.43 mmol, 52.4% yield, 97.4% purity) as a light yellow oil. ^{1}H NMR (400 MHz, DMSO-d6) δ 7.58 (d, J = 8.0 Hz, 1H), 7.39 - 7.30 (m, 5H), 5.03 (s, 2H), 4.55 - 4.54 (m, 1H), 4.05 - 3.96 (m, 3H), 3.66 - 3.61 (m, 2H), 1.88 - 1.81 (m, 3H), 1.33 - 1.30 (m, 1H).

LCMS for **2-6**: RT = 0.754 min, *m*/*z* 310.1 $[M+H]^+$, purity: 97.4%.

SFC for **2-6**: RT = 1.075 min, ee%: 95.5%.

Preparation of tert-butyl (S)-3-(3-bromophenyl)-2-((tert-butoxycarbonyl)amino)propanoate 2-7

To a solution (S)-3-(3-bromophenyl)-2-((tert-butoxycarbonyl)amino)propanoic acid (20 g, 58.11 mmol, 1 *eq*) in ter-butyl alcohol (108.50 g, 1.46 mol, 140.00 mL, 25.19 *eq*) was added DMAP (710 mg, 5.81 mmol, 0.1 *eq*) and di-tert-butyl dicarbonate (16.49 g, 75.54 mmol, 17.35 mL, 1.3 *eq*). The mixture was stirred at 20°C for 18 hours under nitrogen atmosphere. TLC (petroleum ether： ethyl acetate = 0:1) showed the starting material was consumed. The mixture was dulited with ethyl acetate (500 mL) and then washed with brine (100 mL*2). The organic layer was dried over anhydrous sodium sulfate, filtered, concentrated in vacuum. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 100/1 ~ 60/1) to give **2-7** (14.9 g, 37.22 mmol, 64.1% yield, 100% purity) as colorless gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, J = 7.6 Hz, 1H), 7.33 (s, 1H), 7.18 - 7.16 (m, 2H), 5.04 (d, J = 7.6 Hz, 1H), 4.46 - 4.41 (m, 1H), 3.08 - 3.03 (m, 2H), 1.44 (s, 9H), 1.42 (s, 9H).

LCMS for **2-7**: RT = 0.922 min, m/z 422.1, 424.1 $[M+Na]^+$, purity: 100.0%.

SFC for **2-7**: RT = 0.889 min, ee%: 100.0%.

Preparation of (S)-(3-(3-(tert-butoxy)-2-((tert-butoxycarbonyl)amino)-3-oxopropyl)phenyl)boronic acid 2-9

To a solution of **2-7** (14.9 g, 37.22 mmol, 1 *eq*), 4, 4, 5, 5-tetramethyl-2- (4, 4, 5, 5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (12.29 g, 48.39 mmol, 1.3 *eq*) and potassium acetate (9.13 g, 93.05 mmol, 2.5 *eq*) in dry dioxane (200 mL) was added Pd(dppf)Cl_2 (1.09 g, 1.49 mmol, 0.04 *eq*) under nitrogen atmosphere. The mixture was degassed and then stirred at 80°C for 7 hours under nitrogen atmosphere. LCMS showed the starting material was consumed. The mixture was concentrated in vacuum. The residue was diluted with ethyl acetate (500 mL). The mixture was filtered and the solid was washed with ethyl acetate (30 mL*3). The combined organic layers were combined and concentrated in vacuum to give tert-butyl (S)-2-((tert-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoate **2-8** (26.6 g, crude) as black gum, which was used into the next step without further purification.

To a solution of **2-8** (26.6 g, 37.22 mmol, 1 *eq*) and ammonium acetate (14.35 g, 186.11 mmol, 5 *eq*) in acetone (300 mL) and water (150 mL) was added sodium periodate (31.84 g, 148.88 mmol, 8.25 mL, 4 *eq*) over a period of 1 hour. The mixture was stirred at 20°C for 18 hours. LCMS showed a part of starting material remained. Another batch of ammonium acetate (14.35 g, 186.11 mmol, 5 *eq*) and sodium periodate (31.84 g, 148.88 mmol, 8.25 mL, 4 *eq*) were added. The reaction mixture was stirred at 25°C for another 24 hours. TLC (petroleum ether：ethyl acetate = 10:1) showed most of the starting mateiral was consumed. The mixture was diluted with ethyl acetate (200 mL) and then filtered. The filtrate was extracted with ethyl acetate (200 mL*4). The combined organic layers were washed with saturated sodium sulfite solution (200 mL) and brine (200 mL), dried over anhydrous sodium sulfate, filtered, concentrated in vacuum. The residue was purified by flash silica gel chromatography (ISCO®; 220 g SepaFlash® Silica Flash Column, Eluent of 0~100% Ethyl acetate/Petroleum ethergradient @ 80 mL/min) to give **2-9** (12.73 g, 34.70 mmol, 93.2% yield, 99.6% purity) as a red solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.62 (s, 1H), 7.47 (s, 1H), 7.31 - 7.26 (m, 2H), 4.23 - 4.21 (m, 1H), 3.04 - 3.02 (m, 1H), 2.94 - 2.90 (m, 1H), 1.40 - 1.39 (m, 18H).

LCMS for **2-9**: RT = 0.839 min, *m*/*z* 388.0 $[M+Na]^+$, purity: 99.6%.

Preparation of benzyl (S)-3-(3-(3-((S)-3-(tert-butoxy)-2-((tert-butoxycarbonyl)amino)-3-oxopropyl)phenoxy)phenyl)-2-(2-oxopyrrolidin-1-yl)propanoate 2-10

To a solution of **2-9** (8.07 g, 22.10 mmol, 1.5 *eq*), **2-2** (5 g, 14.73 mmol, 1 *eq*), 4A molecular sieve (5 g) and triethylamine (7.45 g, 73.66 mmol, 10.25 mL, 5 *eq*) in dichloromethane (100 mL) was added copper acetate (4.01 g, 22.10 mmol, 1.5 *eq*). The mixture was stirred at 25℃ for 18 hours under oxygen (15 psi). LCMS showed 20% of material **2-2** remained. The mixture was filtered through a celite pad; the solid was washed with ethyl acetate (30 mL*4). The combined filtrates were concentrated in vacuum. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 10/1 ~ 4/1) to give **2-10** (5.68 g, 8.62 mmol, 58.5% yield, 100% purity) as light yellow gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 - 7.31 (m, 5H), 7.25 - 7.21 (m, 2H), 6.94 - 6.91 (m, 2H), 6.85 - 6.83 (m, 4H), 5.19 - 5.09 (m, 4H), 4.44 - 4.41 (m, 1H), 3.39 - 3.31 (m, 3H), 3.04 - 2.98 (m, 3H), 2.32 - 2.23 (m, 2H), 1.96 - 1.77 (m, 2H), 1.41 (s, 9H), 1.40 (s, 9H).

LCMS for **2-10**: RT = 1.052 min, *m/z* 681.1 $[M+Na]^+$, purity: 100.0%.

SFC for **2-10**: RT = 2.298 min, de%: 83.1%.

Preparation of benzyl (S)-3-(3-(3-((S)-2-amino-3-(tert-butoxy)-3-oxopropyl)phenoxy)phenyl)-2-(2-oxopyrrolidin-1-yl)propanoate 2-11

To a solution of **2-10** (5.68 g, 8.62 mmol, 1 *eq*) in dichloromethane (120 mL) was added trifluoroacetic acid (36.96 g, 324.15 mmol, 24 mL, 37.60 *eq*) at 0°C. The mixture was stirred at 0°C for 4 hours. TLC (petroleum ether： ethyl acetate = 2:1) showed most of starting the material was consumed. The mixture was poured into saturated sodium bicarbonate solution (300 mL, pH = 7). The organic layer was separated. The aqueous layer was extracted with ethyl acetate (100 mL*3). The combined organic layers were dried over anhydrous sodium sulfate, filtered, concentrated in vacuum to give **2-11** (4.18 g, 6.84 mmol, 79.4% yield, 91.4% purity) as yellow gum, which was used for the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 - 7.27 (m, 7H), 6.99 - 6.97 (m, 2H), 6.86 - 6.82 (m, 4H), 5.20 - 5.16 (m, 2H), 4.94 (dd, *J* = 10.8, 5.6 Hz, 1H), 3.58 - 3.56 (m, 1H), 3.46 - 3.40 (m, 1H), 3.33 - 3.29 (m, 2H), 3.05 (dd, *J* = 14.4, 10.8 Hz, 1H), 2.91 (d, *J* = 6.8 Hz, 1H), 2.25 - 2.22 (m, 2H), 1.90 - 1.84 (m, 2H), 1.37 (s, 9H).

LCMS for **2-11**: RT = 0.885 min, *m/z* 559.1 $[M+H]^+$, purity: 91.4%.

SFC for **2-11:** RT = 1.595 min, de%: 93.0%.

Preparation of benzyl (S)-3-(3-(3-((S)-2-((S)-2-(((benzyloxy)carbonyl)amino)-3-(1,3-dioxan-2-yl)propanamido)-3-(tert-butoxy)-3-oxopropyl)phenoxy)phenyl)-2-(2-oxopyrrolidin-1-yl)propanoate 2-12

To a solution of **2-6** (2.56 g, 8.28 mmol, 1.21 *eq*) and N, N-diisopropylethylamine (3.54 g, 27.37 mmol, 4.77 mL, 4 *eq*) in N, N-dimethylformamide (45 mL) was added HOBt (1.20 g, 8.89 mmol, 1.3 *eq*) at 0°C. The mixture was stirred at 0°C for 10 minutes. EDCI (2.62 g, 13.68 mmol, 2 *eq*) was added and then **2-11** (4.18 g, 6.84 mmol, 1 *eq*) in N, N-dimethylformamide (15 mL) was added. The reaction mixture was stirred at 0°C for 20 minutes and then stirred at 25°C for another 1.5 hours under nitrogen atmosphere. LCMS showed most of the starting material was consumed and desired compound was detected. The mixture was quenched with ice water (100 mL) and then extracted with ethyl acetate (60 mL*3). The combined organic layers were washed with brine (50 mL*3), dried over anhydrous sodium sulfate, filtered, concentrated in vacuum. The residue was purified by column chromatography (SiO2, Petroleum ether/Ethyl acetate = 6:1 ~ 1:1) to give **2-12** (4.08 g, 4.65 mmol, 68.0% yield, 96.9% purity) as colorless gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 - 7.30 (m, 10H), 7.24 - 7.19 (m, 2H), 7.00 (d, J = 5.6 Hz, 1H), 6.91(d, J = 6.8 Hz, 2H), 6.83 - 6.81 (m, 4H), 5.94 (d, J = 6.4 Hz, 1H), 5.15 - 5.09 (m, 5H), 4.71 - 4.68 (m, 2H), 4.36 - 4.34 (m, 1H), 4.03 - 4.00 (m, 2H), 3.72 - 3.63 (m, 2H), 3.35 - 3.30 (m, 3H), 3.06 (d, J = 6.0 Hz, 2H), 3.30 - 2.94 (m, 1H), 2.31 - 2.21 (m, 2H), 2.05 - 1.72 (m, 6H), 1.38 (s, 9H).

LCMS for **2-12**: RT = 1.012 min, m/z 850.4 $[M+H]^+$, purity: 96.9%.

SFC for **2-12**: RT = 1.995 min, de%: 82.6%.

Preparation (S)-3-(3-(3-((S)-2-((S)-2-amino-3-(1,3-dioxan-2-yl)propanamido)-3-(tert-butoxy)-3-oxopropyl)phenoxy)phenyl)-2-(2-oxopyrrolidin-1-yl)propanoic acid 2-13

To a solution of **2-12** (4.08 g, 4.80 mmol, 1 *eq*) in tetrahydrofuran (60 mL) was added Pd/C (0.4 g, 10% purity) and $Pd(OH)_2$/C (0.4 g, 10% purity) under nitrogen atmosphere. The mixture was degassed under vacuum and purged with hydrogen several times. The mixture was stirred under hydrogen atmosphere (15 psi) at 25°C for 14 hours. LCMS showed the starting material was consumed and desired product mass was detected. The mixture was filtered, the solid was washed with tetrahydrofuran (10 mL*4) and ethyl acetate (10 mL*3). The combined filtrate was concentrated in vacuum to give **2-13** (3.1 g, crude) as a white solid, which was used into the next step without further purification. ^{1}H NMR (400 MHz, CD_3OD) δ 7.28 - 7.26 (m, 2H), 7.01- 6.98 (m, 2H), 6.86 - 6.84 (m, 4H),

4.84 - 4.82 (m, 1H), 4.75 - 4.71 (m, 1H), 4.60 (dd, *J* = 9.2, 5.2 Hz, 1H), 4.10 - 4.06 (m, 2H), 4.00 – 3.98 (m, 1H), 3.83 - 3.72 (m, 2H), 3.59 - 3.56 (m, 1H), 3.41 - 3.33 (m, 2H), 3.19 - 3.14 (m, 1H), 2.99 - 2.86 (m, 2H), 2.32 - 2.01 (m, 7H), 1.87 - 1.86 (m, 1H), 1.45 (s, 9H).

LCMS for **2-13**: RT = 0.813 min, m/z 626.3 $[M+H]^+$, purity: 95.1%.

Preparation of tert-butyl (5S,8S,11S)-8-((1,3-dioxan-2-yl)methyl)-7,10-dioxo-11-(2-oxopyrrolidin-1-yl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxylate 2-14

To a solution of **2-13** (1 g, 1.60 mmol, 1 *eq*) and N, N-diisopropylethylamine (1.03 g, 7.99 mmol, 1.39 mL, 5 *eq*) in N, N-dimethylformamide (80 mL) was added HOBt (324 mg, 2.40 mmol, 1.5 *eq*) and EDCI (613 mg, 3.20 mmol, 2 *eq*) at 0°C. The mixture was stirred at 25°C for 15 hours. LCMS showed the starting material remained. Another batch of N, N-diisopropylethylamine (516 mg, 4.00 mmol, 695.94 uL, 2.5 *eq*) and EDCI (613 mg, 3.20 mmol, 2 *eq*) were added at 0°C. The reaction mixture was stirred at 25°C for another 15 hours. LCMS showed the starting material was consumed. The mixture was quenched with ice water (100 mL) and then extracted with ethyl acetate (100 mL*3). The combined organic layers were washed with brine (50 mL*3), dried over anhydrous sodium sulfate, filtered, concentrated in vacuum. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 1/1~0/1) to give ***2-14*** (410 mg, 564.71 umol, 35.3% yield, 83.7% purity) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.11 (d, *J* = 8.0 Hz, 1H), 8.02 (d, *J* = 8.0 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.27 (d, *J* = 8.0 Hz, 1H), 7.08 (d, *J* = 7.6 Hz, 1H), 6.95 - 6.92 (m, 3H), 6.66 - 6.65 (m, 1H), 6.45 (m, 1H), 4.70 (dd, *J* = 12.0, 3.2 Hz, 1H), 4.66 - 4.61 (m, 1H), 4.52 - 4.48 (m, 2H), 4.00 - 3.97 (m, 2H), 3.75 - 3.53 (m, 5H), 3.26 (t, *J* = 12.4 Hz, 1H), 3.18 (dd, *J* = 15.2, 4.0 Hz, 1H), 3.00 (dd, *J* = 15.2, 8.8 Hz, 1H), 2.73 (dd, *J* = 12.4, 2.8 Hz, 1H), 2.43 - 2.41 (m, 2H), 2.01 - 1.84 (m, 8H), 1.50 (s, 9H).

LCMS for **2-14**: RT = 0.875 min, m/z 608.1 $[M+H]^+$, purity: 83.7%.

Preparation of (5S,8S,11S)-8-((1,3-dioxan-2-yl)methyl)-N-(cyclopropylmethyl)-7,10-dioxo-11-(2-oxopyrrolidin-1-yl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide 2-16

To a solution of **2-14** (410 mg, 564.71 umol, 1 *eq*) in dichloromethane (5 mL) was added trifluoroacetic acid (2.39 g, 21.00 mmol, 1.56 mL, 37.19 *eq*). The mixture was stirred at 25°C for 5.5 hours. TLC (petroleum ether：ethyl

acetate = 0:1) showed most of the starting material was consumed. The mixture was poured into water (50 mL) and then adjusted pH = 4~5 with saturated sodium bicarbonate aqueous. The mixture was extracted with ethyl acetate (50 mL*3). The combined organic layers were washed with brine (50 mL), dried over anhydrous sodium sulfate, filtered, concentrated in vacuum to give (5S,8S,11S)-8-((1,3-dioxan-2-yl)methyl)-7,10-dioxo-11-(2-oxopyrrolidin-1-yl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxylic acid **2-15** (440 mg, crude) as a white solid, which was used into the next step without further purification.

To a solution of **2-15** (240 mg, 435.11 umol, 1 *eq*) in pyridine (2.5 mL) was added HOBt (59 mg, 435.11 umol, 1 *eq*) at 0°C. The mixture was stirred at 0°C for 10 minutes. Cyclopropylmethanamine (62 mg, 870.22 umol, 2 *eq*) and EDCI (209 mg, 1.09 mmol, 2.5 *eq*) were added at 0°C. The reaction mixture was stirred at 0°C for 20 minutes and then stirred at 25°C for 16 hours under nitrogen atmosphere. LCMS showed most of the starting material remained. Another batch of EDCI (209 mg, 1.09 mmol, 2.5 *eq*) was added, the reaction mixture was stirred at 25°C for another 18 hours. LCMS showed the starting material was consumed and desired product mass was detected. The reaction mixture was quenched with ice water (20 mL), and then adjusted pH=6~7 with 1 N hydrochloric acid solution. The mixture was extracted with ethyl acetate (20 mL * 3). The combined organic layers were washed with 1 N hydrochloric acid solution (20 mL), saturated sodium bicarbonate solution (20 mL) and brine (20 mL), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 1/1 ~ 0/1) to give **2-16** (179 mg, 282.88 umol, 65.0% yield, 95.6% purity) as a light yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.11 (t, *J* = 5.2 Hz, 1H), 7.98 (dd, *J* = 21.2, 8.0 Hz, 1H), 7.33 (t, *J* = 8.0 Hz, 1H), 7.28 (t, *J* = 8.0 Hz, 1H), 7.08 (d, *J* = 7.6 Hz, 1H), 6.98 - 6.94 (m, 3H), 6.65 (s, 1H), 6.55 (s, 1H), 4.72 - 4.69 (m, 2H), 4.50 (t, *J* = 4.8 Hz, 1H), 4.45 - 4.41 (m, 1H), 3.99 - 3.96 (m, 2H), 3.76 - 3.48 (m, 4H), 3.26 (t, *J* = 12.4 Hz, 1H), 3.11 - 3.04 (m, 4H), 2.75 (dd, *J* = 12.8, 2.8 Hz, 1H), 2.42 - 2.38 (m, 2H), 2.06 - 1.86 (m, 5H), 1.33 - 1.29 (m, 1H), 1.01 - 0.96 (m, 1H), 0.52 - 0.50 (m, 2H), 0.23 - 0.22 (m, 2H).

LCMS for **2-16**: RT = 0.875 min, m/z 605.3 $[M+H]^+$, purity: 95.6%.

Preparation of (5S,8S,11S)-N-(cyclopropylmethyl)-8-(2-morpholinoethyl)-7,10-dioxo-11-(2-oxopyrrolidin-1-yl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide (compound 3)

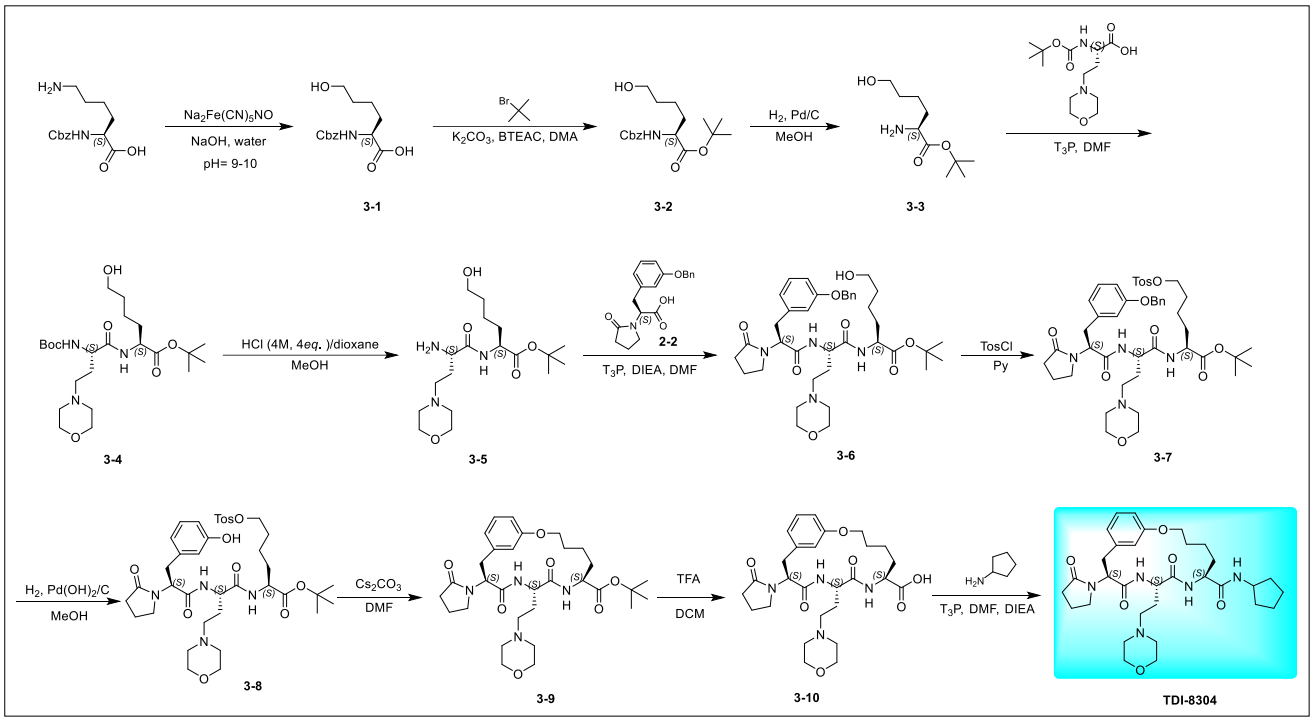
To a solution of **2-16** (194 mg, 320.82 umol, 1 *eq*) in acetonitrile (2 mL) was added CAN (440 mg, 802.06 umol, 399.73 uL, 2.5 *eq*) in water (2 mL) at 25°C. The mixture was stirred at 70°C for 2.5 hours. LCMS and TLC (petroleum ether： ethyl acetate = 0:1) showed most of the starting material was consumed and the desired product was detected. The mixture was quenched with saturated sodium bicarbonate solution (20 mL) and then extracted with ethyl acetate (20 mL*3). The combined organic layers were washed with brine (20 mL), dried over anhydrous sodium sulfate, concentrated in vacuum to give (5S,8S,11S)-N-(cyclopropylmethyl)-7,10-dioxo-8-(2-oxoethyl)-11-(2-oxopyrrolidin-1-yl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide **2-17** (140 mg, crude) as a light yellow solid, which was used into the next step without further purification.

To a solution of **2-17** (90 mg, 164.65 umol, 1 *eq*) in methanol (5 mL) was added morpholine (72 mg, 823.25 umol, 72.45 uL, 5 *eq*) and acetic acid (20 mg, 329.30 umol, 18.83 uL, 2 *eq*). Pd/C (20 mg, 10%) was added under nitrogen atmosphere. The suspension was degassed under vacuum and purged with hydrogen several times. The mixture was stirred at 25°C for 16 hours under hydrogen (15 psi). LCMS showed the intermediate remained, additional batch of Pd/C (50 mg, 10%) was added. The suspension was degassed under vacuum and purged with hydrogen several times. The mixture was stirred at 25°C for 26 hours under hydrogen (15 psi). LCMS showed the most of intermediate was consumed and desired product mass was detected. The mixture was filtered through celite pad and washed with methanol (5 mL*3). The combined filtrate was concentrated in vacuum. The residue was diluted with ethyl acetate (30 mL) and then washed with brine (10 mL*2). The organic layers were dried over anhydrous sodium sulfate, filtered, concentrated in vacuum. The residue was purified by prep-HPLC (column: Phenomenex Synergi C18 150*25*10um; mobile phase: [water (0.1%trifluoroacetic acid)-acetonitrile]; B%: 20%-50%, 13 mins). The fraction was adjusted to pH = 7 with saturated sodium bicarbonate solution. The mixture was concentrated in vacuum to removed acetonitrile, extracted with dichloromethane (20 mL*3). The combined organic layers were washed with brine (20 mL), dried over anhydrous sodium sulfate, concentrated in vacuum. The residue was lyophilized to give **compound 3** (14.3 mg purity 98.9%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.34 (t, J = 8.0 Hz, 1H), 7.27 (t, J = 8.0 Hz, 1H), 7.10 (d, J = 8.0 Hz, 1H), 6.98 - 6.91 (m, 3H), 6.67 (s, 1H), 6.47 (s, 1H), 4.72 - 4.68 (m, 2H), 4.39 (t, J = 7.2 Hz, 1H), 3.67 - 3.64 (m, 6H), 3.27 - 3.24 (m, 1H), 3.09 - 3.02 (m, 4H), 2.77 (dd, J = 12.8, 3.2 Hz, 1H), 2.44 - 2.30 (m, 8H), 2.05 - 2.03 (m, 2H), 1.79 - 1.72 (m, 2H), 1.00 - 0.96 (m, 1H), 0.53 - 0.49 (m, 2H), 0.24 - 0.20 (m, 2H).

LCMS for **compound 3**: RT = 2.249 min, m/z 618.3 $[M+H]^+$, purity: 98.9%.

SFC for **compound 3:** RT_1 = 1.402 min, RT_2 = 1.575 min, de%: 98.1%.

HRMS for **compound 3**: calc. for $C_{34}H_{44}N_5O_6$ $[M+H]^+$: 618.3286. Found: 618.3276.



Scheme S2 Synthetic route of 8304

Preparation of (S)-2-(((benzyloxy)carbonyl)amino)-6-hydroxyhexanoic acid 3-1

((Benzyloxy)carbonyl)-L-lysine (19 g, 67.78 mmol, 1 *eq*, 2 batches) was dissolved in water (250 mL) and the pH adjusted to 9-10 using sodium hydroxide (4 M). The mixture was heated to 60-65°C in an oil bath. Sodium nitroprusside dihydrate (36.35 g, 122.00 mmol, 21.13 mL, 1.8 *eq*) was added portion wise over 1 hour while maintaining the pH of the reaction mixture between 9~10 using sodium hydroxide aqueous (4 M). The resulting mixture was heated for additional 5 hours while maintaining the pH between 9~10 with occasional addition of sodium hydroxide aqueous (4 M). LCMS showed the desired mass was observed. The mixture was filtered and the filtrate was adjusted to pH 2 using 6M hydrochloric acid carefully (Caution: HCN was released during the acidification, which can be monitored by HCN detector). We suggest conducting the filtration to remove sodium nitroprusside dihydrate before acidification. The filtration can reduce HCN gas production and almost no HCN gas was released after twice filtration and extracted with ethyl acetate (250 mL*3). The combined organic layers were

washed with brine (100 mL), dried over anhydrous sodium sulfate, filtered and concentrated in *vacuo*. The residue was purified by reverse phase column (C_{18}, 0.1% TFA in water/acetonitrile) to afford **3-1** (18 g, 57.59 mmol, 42.5% yield) as pink gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 - 7.28 (m, 5H), 5.10 (s, 2H), 4.19 - 4.11 (m, 1H), 3.56 (t, *J* = 6.4 Hz, 2H), 1.75 - 1.65 (m, 1H), 1.58 - 1.56 (m, 1H), 1.47 - 1.24 (m, 4H).

LCMS for **3-1**: RT = 0.614 min, *m*/*z* 282.1$[M+H]^+$, purity: 74.2%.

Preparation of tert-butyl (S)-2-(((benzyloxy)carbonyl)amino)-6-hydroxyhexanoate 3-2

To a solution of **3-1** (7 g, 24.88 mmol, 1 *eq*) in N, N-dimethylacetamide (100 mL) was added potassium carbonate (89.42 g, 646.99 mmol, 26 *eq*), followed by addition of 2-bromo-2-methyl-propane (163.66 g, 1.19 mol, 138.69 mL, 48 *eq*). The mixture was stirred at 55°C for 24 hours. LCMS showed desired mass was observed. The reaction mixture was filtered and the filtrate was poured into water (400 mL), extracted with ethyl acetate (200 mL*3). The combined organic phase was washed with brine (300 mL*3), dried over anhydrous sodium sulfate, filtered and concentrated in *vacuo*. The residue was purified by column (SiO_2, petroleum ether: ethyl acetate = 10:1 ~ 1:1) to afford **3-2** (6.5 g, 17.34 mmol, 69.7% yield) as yellow gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 - 7.31 (m, 5H), 5.35 (br. d, *J* = 8.0 Hz, 1H), 5.12 (m, 2H), 4.30 - 4.27 (m, 1 H), 3.64 (t, *J* = 6.0 Hz, 2H), 1.83 - 1.63 (m, 1H), 1.61 - 1.56 (m, 4H), 1.48 (s, 9H), 1.43 - 1.41 (m, 1H).

LCMS for **3-2**: RT = 0.883 min, *m*/*z* 360.3$[M+Na]^+$, purity: 49.1%.

Preparation of tert-butyl (S)-2-amino-6-hydroxyhexanoate 3-3

A solution of **3-2** (6.4 g, 18.97 mmol, 1 *eq*) in methanol (60 mL) was degassed and purged with nitrogen 10 minutes, then Pd/C (0.6 g, 10% purity on carbon) was added in one portion. The mixture was degassed and purged with hydrogen three times and then stirred for 3 hours at 20°C under hydrogen atmosphere (15 psi). TLC (petroleum ether: ethyl acetate = 1:1) showed the starting material was consumed. The reaction mixture was filtered and the filtrate was concentrated in *vacuo* to afford **3-3** (3.5 g, 16.36 mmol, 86.2% yield, 95.0% purity) as colorless gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.66 (t, *J* = 6.4 Hz, 2H), 3.35 - 3.12 (m, 1H), 1.76 - 1.71 (m, 1H), 1.65 - 1.51 (m, 5H), 1.47 (s, 9H).

Preparation of tert-butyl (S)-2-((S)-2-((tert-butoxycarbonyl)amino)-4-morpholinobutanamido)-6-hydroxyhexanoate 3-4

To a solution of (S)-2-((tert-butoxycarbonyl)amino)-4-morpholinobutanoic acid (3.19 g, 11.07 mmol, 0.9 *eq*), **3-3** (2.5 g, 12.30 mmol, 1 *eq*) and diisopropylethylamine (4.77 g, 36.90 mmol, 6.43 mL, 3 *eq*) in N, N-dimethyl formamide (20 mL) was added T_3P (11.74 g, 18.45 mmol, 10.97 mL, 50% in ethyl acetate, 1.5 *eq*) drop wise at 0°C. The mixture was stirred for 1 hour at 0°C. LCMS showed the starting material was consumed and desired mass was observed. The reaction mixture was poured into water (100 mL), extracted with ethyl acetate (100 mL*3). The combined organic phase was washed with brine (200 mL*3), dried over anhydrous sodium sulfate, filtered and concentrated in *vacuo*. The residue was purified by column chromatography (SiO_2, petroleum ether: ethyl acetate: ethanol = 1:1:0 ~ 4:3:1) to afford **3-4** (4.0 g, 8.45 mmol, 68.7% yield) as colorless gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (d, J = 4.0 Hz, 1H), 6.26 (d, J = 6.8 Hz, 1H), 4.49 - 4.44 (m, 1H), 4.25 - 4.23 (m, 1H), 3.75 - 3.69 (m, 4H), 3.65 - 3.49 (m, 2H), 2.61 - 2.43 (m, 6H), 2.02 - 1.97 (m, 1H), 1.91 - 1.82 (m, 1H), 1.71 - 1.65 (m, 4H), 1.48 - 1.41 (m, 20H).

LCMS for **3-4**: RT = 0.654 min, *m*/*z* 474.3$[M+H]^+$, purity: 70.4%.

Preparation of tert-butyl (S)-2-((S)-2-amino-4-morpholinobutanamido)-6-hydroxyhexanoate 3-5

To a solution of **3-4** (4 g, 8.45 mmol, 1 *eq*) in methanol (40 mL) was added HCl/dioxane (4 M, 9.33 mL, 4.42 *eq*) at 0°C. The mixture was stirred for 12 hours at 20°C. LCMS showed the starting material was consumed and desired mass was observed. The reaction mixture was concentrated in *vacuo* to afford **3-5** (3.8 g, crude, 2HCl salt) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 4.36 - 4.33 (m, 1H), 4.28 - 4.24 (m, 1H), 4.11 - 4.07 (m, 2H), 3.94 - 3.92 (m, 2H), 3.62 - 3.58 (m, 4H), 3.51 - 3.49 (m, 2H), 3.36 - 3.33 (m, 2H), 2.51 - 2.34 (m, 2H), 1.91 - 1.62 (m, 2H), 1.59 - 1.53 (m, 2H), 1.51 (s, 9H).

LCMS for **3-5**: RT = 0.553 min, *m*/*z* 374.2$[M+H]^+$, purity: 58.0%.

Preparation of tert-butyl (S)-2-((S)-2-((S)-3-(3-(benzyloxy)phenyl)-2-(2-oxopyrrolidin-1-yl)propanamido)-4-morpholinobutanamido)-6-hydroxyhexanoate 3-6

To a solution of **3-5** (3.6 g, 8.06 mmol, 1 *eq*, 2HCl salt), (S)-3-(3-(benzyloxy)phenyl)-2-(2-oxopyrrolidin-1-yl)propanoic acid **2-2** (2.46 g, 7.26 mmol, 0.9 *eq*) and diisopropylethylamine (4.17 g, 32.26 mmol, 5.62 mL, 4 *eq*)

in N, N-dimethyl formamide (30 mL) was added T_3P (6.16 g, 9.68 mmol, 5.76 mL, 50% in ethyl acetate, 1.2 *eq*) at 0°C. The mixture was stirred for 1 hour at 0°C. LCMS showed **3-5** was consumed and desired mass was observed. The reaction mixture was poured into water (100 mL), extracted with ethyl acetate (50 mL*3). The combined organic phase was washed with saturated sodium bicarbonate aqueous (50 mL), brine (100 mL*3), dried over anhydrous sodium sulfate, filtered and concentrated in *vacuo*. The residue was purified by column chromatography (SiO_2, petroleum ether: ethyl acetate: ethanol = 10:1:1 ~ 3:3:1) to afford **3-6** (2.6 g, 3.74 mmol, 46.4% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, J = 6.4 Hz, 1H), 7.44 - 7.33 (m, 5H), 7.23 - 7.18 (m, 1H), 6.85 - 6.80 (m, 3H), 5.05 (s, 2H), 4.79 - 4.76 (m, 1H), 4.44 - 4.41 (m, 2H), 7.78 - 3.63 (m, 6H), 3.38 - 3.34 (m, 2H), 3.29 - 3.24 (m, 1H), 3.12 - 3.06 (m, 1H), 2.68 - 2.38 (m, 6H), 2.32 - 2.25 (m, 2H), 2.11 - 2.05 (m, 1H), 1.91 - 1.81 (m, 4H), 1.68 - 1.56 (m, 3H), 1.48 - 1.41 (m, 11H).

LCMS for **3-6**: RT = 0.735 min, *m*/*z* 695.4$[M+H]^+$, purity: 94.0%.

Preparation of tert-butyl (S)-2-((S)-2-((S)-3-(3-(benzyloxy)phenyl)-2-(2-oxopyrrolidin-1-yl)propanamido)-4-morpholinobutanamido)-6-(tosyloxy)hexanoate 3-7

To a solution of p-toluenesulfonyl chloride (856 mg, 4.49 mmol, 1.2 *eq*) in pyridine (30 mL) was added **3-6** (2.6 g, 3.74 mmol, 1 *eq*). The mixture was stirred for 2 hours at 15°C. LCMS showed the starting material was consumed and desired mass was observed. The mixture was poured into water (50 mL), adjusted to pH = 7 with 1 N hydrochloric acid aqueous and extracted with ethyl acetate (50 mL*2). The combined organic phase was washed with brine (30 mL*3), dried over anhydrous sodium sulfate, filtered and concentrated in *vacuo* to afford **3-7** (3.3 g, crude) as yellow gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (d, J = 4.4 Hz, 1H), 7.77 (d, J = 8.0 Hz, 2H), 7.44 - 7.35 (m, 5H), 7.34 (d, J = 8.0 Hz, 2H), 7.19 (t, J = 7.2 Hz, 1H), 6.84 - 6.81 (m, 3H), 5.04 (s, 2H), 4.84 - 4.80 (m, 1H), 4.48 - 4.37 (m, 2H), 4.01 (t, J = 5.6 Hz, 2H), 3.78 - 3.69 (m, 4H), 3.36 (t, J = 6.8 Hz, 2H), 3.29 - 3.23 (m, 1H), 3.11 - 3.04 (m, 1H), 2.65 - 2.51 (m, 4H), 2.44 (s, 3H), 2.38 - 2.24 (m, 4H), 2.11 - 2.05 (m, 1H), 1.93 - 1.85 (m, 2H), 1.74 - 1.56 (m, 5H), 1.45 (s, 9H) 1.40 - 1.37 (m, 2H).

LCMS for **3-7**: RT = 0.881 min, *m*/*z* 849.6$[M+H]^+$, purity: 86.7%.

Preparation of tert-butyl (S)-2-((S)-2-((S)-3-(3-hydroxyphenyl)-2-(2-oxopyrrolidin-1-yl)propanamido)-4-morpholinobutanamido)-6-(tosyloxy)hexanoate 3-8

A solution of **3-7** (3.3 g, 3.89 mmol, 1 *eq*) in methanol (3 mL) was degassed and purged with nitrogen 10 minutes and $Pd(OH)_2/C$ (0.1 g, 10% purity on carbon) was added in one portion. The mixture was degassed purged with hydrogen three times and stirred at 20°C for 12 hours under hydrogen atmosphere (15 psi). LCMS showed the starting material was consumed and desired mass was observed. The mixture was filtered and the filtrate was concentrated in *vacuo* to afford **3-8** (2.7 g, crude) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, J = 6.0 Hz, 1H), 7.77 (d, J = 8.0 Hz, 2H), 7.34 (d, J = 8.0 Hz, 2H), 7.15 - 7.11 (m, 1H), 6.73 - 6.72 (m, 3H), 4.73 - 4.69 (m, 1H), 4.42 - 4.38 (m, 2H), 4.01 (t, J = 6.2 Hz, 2H), 3.74 - 3.66 (m, 4H), 3.64 - 3.61 (m, 3H), 3.51 - 3.47 (m, 1H), 3.34 - 3.28 (m, 1H), 3.11 - 3.04 (m, 2H), 2.89 - 2.86 (m, 2H), 2.56 - 2.46 (m, 4H), 2.45 (s, 3H), 2.39 - 2.31 (m, 4H), 1.98 - 1.87 (m, 4H), 1.82 - 1.60 (m, 8H), 1.46 - 1.42 (m, 11H).

LCMS for **3-8**: RT = 0.774 min, *m/z* 759.3$[M+H]^+$, purity: 76.2%.

Preparation of tert-butyl (7S,10S,13S)-10-(2-morpholinoethyl)-9,12-dioxo-13-(2-oxopyrrolidin-1-yl)-2-oxa-8,11-diaza-1(1,3)-benzenacyclotetradecaphane-7-carboxylate 3-9

To a solution of **3-8** (1.5 g, 1.98 mmol, 1 *eq*) in N, N-dimethyl formamide (15 mL) was added cesium carbonate (1.5 g, 4.60 mmol, 2.33 *eq*) at 20°C. The mixture was stirred at 30°C for 3 hours. LCMS showed desired mass was observed. The reaction mixture was poured into water (20 mL), extracted with ethyl acetate (30 mL*3). The combined organic phase was washed with brine (30 mL *3), dried over anhydrous sodium sulfate, filtered and concentrated in *vacuo*. The residue was purified by column (SiO_2, petroleum ether: ethyl acetate: ethanol = 20:20:1 ~ 5:5:1), followed by prep-HPLC (column: Phenomenex Synergi Max-RP 250*50mm*10 um; mobile phase: [water(0.1%TFA)-ACN]; B%: 28ACN%-58ACN%,28min,50%min) to afford **3-9** (480 mg, 736.30 umol, 18.6% yield) as colorless gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (d, J = 6.4 Hz, 1H), 7.18 (t, J = 8.0 Hz, 1H), 7.76 (d, J = 7.2 Hz, 1H), 6.67 - 6.64 (m, 1H), 6.56 (s, 1H), 6.35 (d, J = 6.8 Hz, 1H), 4.53 - 4.49 (m, 1H), 4.39 - 4.34 (m, 2H), 4.08 - 4.05 (m, 1H), 3.97 - 3.94 (m, 1H), 3.81 - 3.79 (m, 1H), 3.73 - 3.65 (m, 4H), 3.55 - 3.49 (m, 1H), 3.16 (t, J = 12.4 Hz, 1H), 2.67 - 2.63 (m, 1H), 2.48 -2.44 (m, 2H), 2.39 - 2.35 (m, 5H), 2.21 - 2.18 (m, 1H), 2.13 - 1.96 (m, 2H), 1.92 - 1.82 (m, 2H), 1.73 - 1.60 (m, 3H), 1.56 - 1.48 (m, 3H), 1.37 (s, 9H).

LCMS for **3-9**: RT = 0.689 min, *m/z* 587.3 $[M+H]^+$, purity: 88.8%.

Preparation of (7S,10S,13S)-10-(2-morpholinoethyl)-9,12-dioxo-13-(2-oxopyrrolidin-1-yl)-2-oxa-8,11-diaza-1(1,3)-benzenacyclotetradecaphane-7-carboxylic acid 3-10

To a solution of **3-9** (40 mg, 68.18 umol, 1 *eq*) in dichloromethane (0.6 mL) was added TFA (462 mg, 4.05 mmol, 0.3 mL, 59.43 *eq*). The mixture was stirred for 1 hour at 20°C. LCMS showed the starting material was consumed. The reaction mixture was concentrated in *vacuo.* The residue was purified by prep-HPLC (column: Phenomenex luna C18 150*25 10u; mobile phase: [water(0.1%TFA)-ACN]; B%: 9%-39%, 10min) to afford **3-10** (22.35 mg, 33.53 umol, 49.2% yield, 96.7% purity, TFA salt) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 - 7.71 (m, 1H), 7.28 - 7.25 (m, 1H), 6.81 (d, *J* = 7.2 Hz, 1H), 6.75 (d, *J* = 8.0 Hz, 1H), 6.59 (s, 1H), 6.48 - 6.46 (m, 1H), 4.69 - 4.64 (m, 1H), 4.67 - 4.64 (m, 2H), 4.22 - 4.21 (m, 1H), 4.03 - 3.94 (m, 3H), 3.88 - 3.81 (m, 3H), 3.68 - 3.63 (m, 1H), 3.56 - 3.46 (m, 2H), 3.13 - 3.08 (m, 3H), 2.94 - 2.84 (m, 3H), 2.51 - 2.26 (m, 3H), 2.16 - 2.08 (m, 2H), 1.97 - 1.95 (m, 1H), 1.88 - 1.72 (m, 3H), 1.66 - 1.56 (m, 2H), 1.47 - 1.38 (m, 1H).

LCMS for **3-10**: RT =1.522 min, *m/z* 531.4$[M+H]^+$, purity: 96.7%.

Preparation of (7S,10S,13S)-N-cyclopentyl-10-(2-morpholinoethyl)-9,12-dioxo-13-(2-oxopyrrolidin-1-yl)-2-oxa-8,11-diaza-1(1,3)-benzenacyclotetradecaphane-7-carboxamide (TDI-8304)

To a solution of **3-10** (140 mg, 217.18 umol, 1 *eq*, TFA salt), cyclopentanamine (37 mg, 434.35 umol, 42.86 uL, 2 *eq*) and diisopropylethylamine (113 mg, 868.71 umol, 151.31 uL, 4 *eq*) in dimethyl formamide (2 mL) was added T_3P (208 mg, 325.77 umol, 193.74 uL, 50% in ethyl acetate, 1.5 *eq*) at 0°C. The mixture was stirred for 1 hour at 0°C. LCMS showed desired mass was observed. The reaction mixture was poured into water (20 mL), extracted with ethyl acetate (20 mL*3). The combined organic phase was washed with brine (20 mL), dried over anhydrous sodium sulfate filtered and concentrated in *vacuo.* The residue was purified by prep-HPLC (column: Phenomenex luna C18 150*25 10u; mobile phase: [water (0.1%TFA)-ACN]; B%: 15%-45%, 4min). The residue was dissolved in water (10 mL), adjusted to pH = 9 with 1 N sodium hydroxide aqueous, extracted with dichloromethane: methanol (v/v=10:1, 20 mL*4). The combined organic phase was dried over anhydrous sodium sulfate filtered and concentrated in *vacuo* to afford **TDI-8304** (60.01 mg, 100.19 umol, 46.1% yield, 100% purity) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 - 7.23 (m, 1H), 6.91 (d, *J* = 6.0 Hz, 1H), 6.86 (d, *J* = 7.2 Hz, 1H), 6.72 (d, *J* = 8.0 Hz, 1H), 6.66 (s, 1H), 6.10 (d, *J* = 7.2 Hz, 1H), 4.69 - 4.65 (m, 1H), 4.39 - 4.28 (m, 2H), 4.17 - 4.05 (m, 3H), 3.88

- 3.84 (m, 1H), 3.79 - 3.71 (m, 4H), 3.64 - 3.58 (m, 1H), 3.21 (t, *J* = 12.4 Hz, 1H), 2.80 - 2.76 (m, 1H), 2.52 - 2.38 (m, 8H), 2.12 - 2.05 (m, 2H), 1.98 - 1.91 (m, 3H), 1.81 - 1.72 (m, 3H), 1.69 - 1.59 (m, 7H), 1.42 - 1.26 (m, 3H). ^{13}C NMR (126 MHz, DMSO) δ 173.52, 170.81, 170.43, 167.91, 158.00, 138.99, 129.26, 121.50, 116.24, 112.65, 66.79, 66.16, 56.16, 54.35, 53.26, 51.22, 50.30, 50.16, 44.09, 35.08, 32.35, 32.10, 31.70, 30.52, 29.07, 27.04, 23.40, 23.36, 21.12, 17.91.

LCMS for **TDI-8304**: RT =2.104 min, *m/z* 598.3$[M+H]^+$, purity: 100%.

HRMS for **TDI-8304**: calc. for $C_{32}H_{48}N_5O_6$ $[M+H]^+$: 598.3599. Found: 598.3608.

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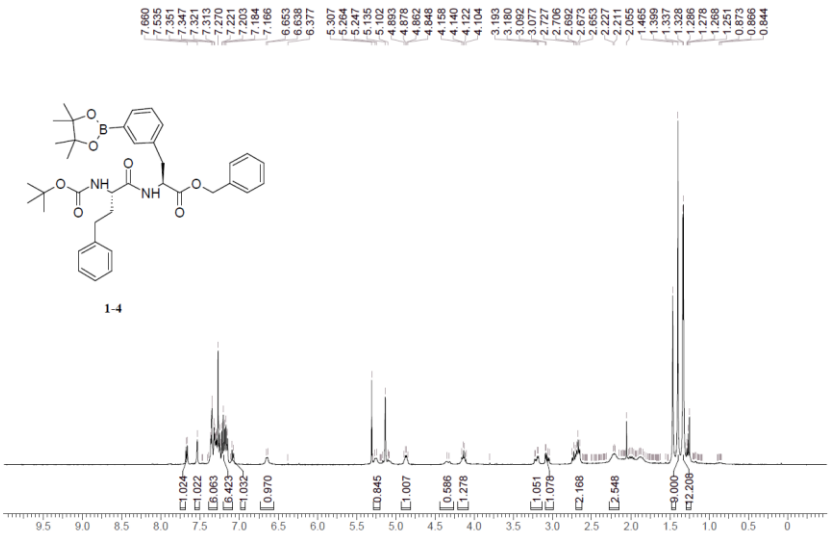
NMR Spectra



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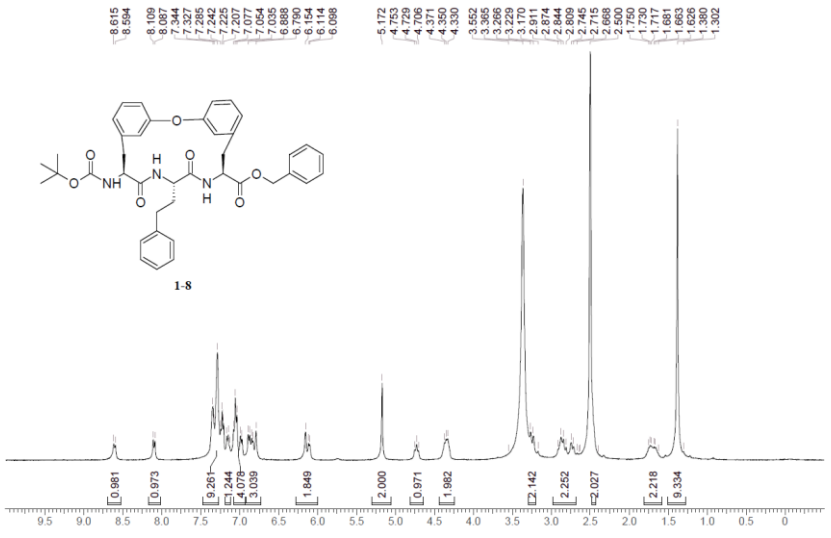

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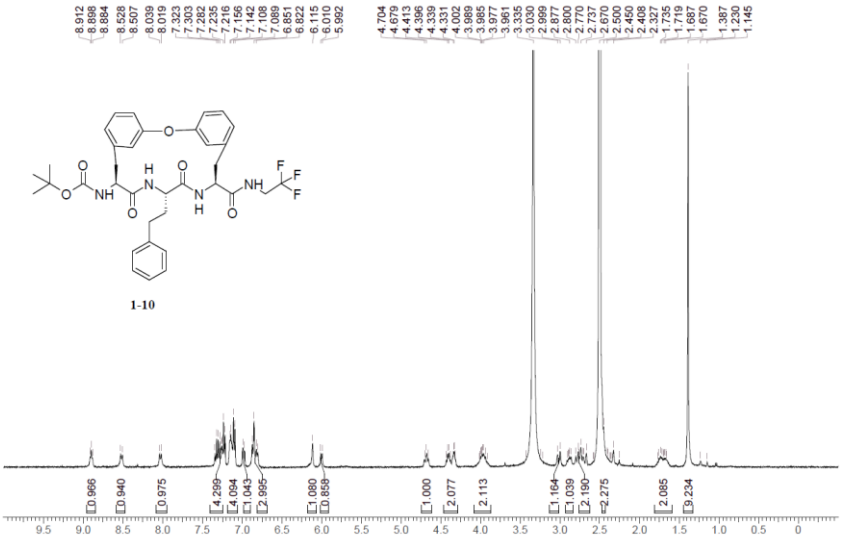

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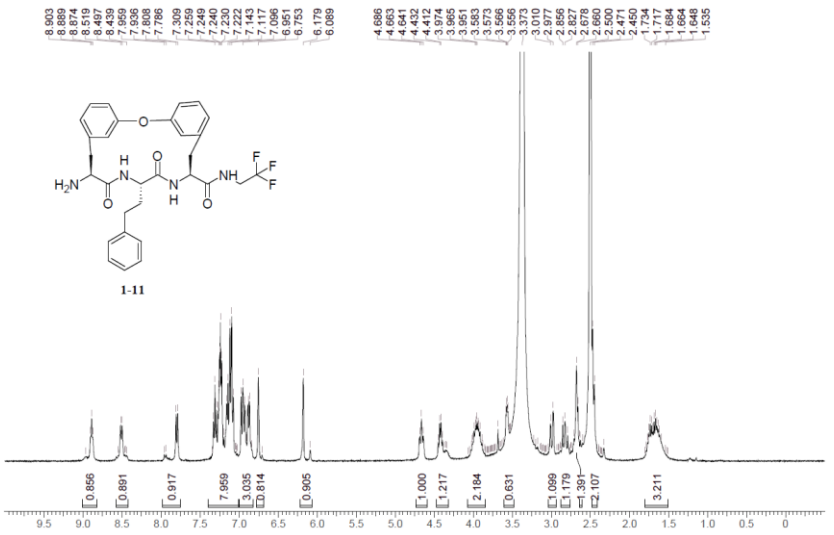

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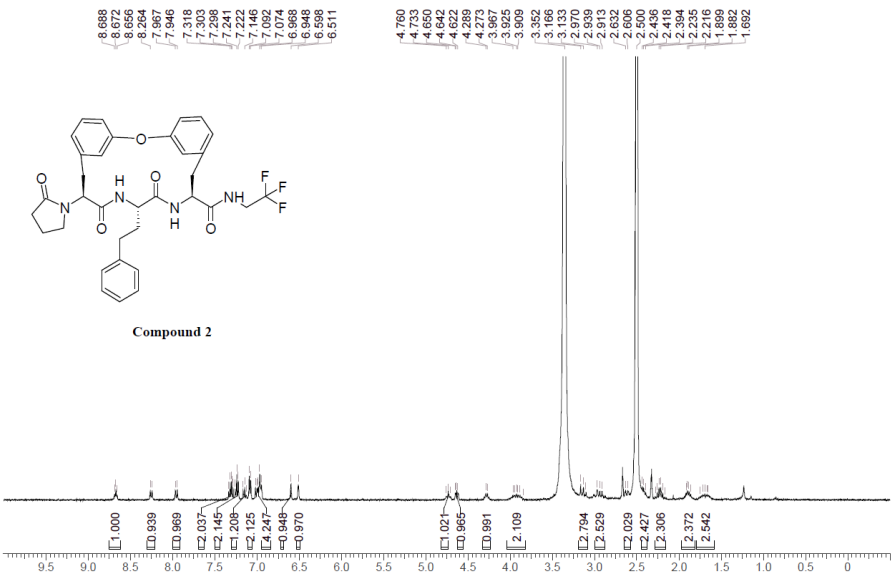

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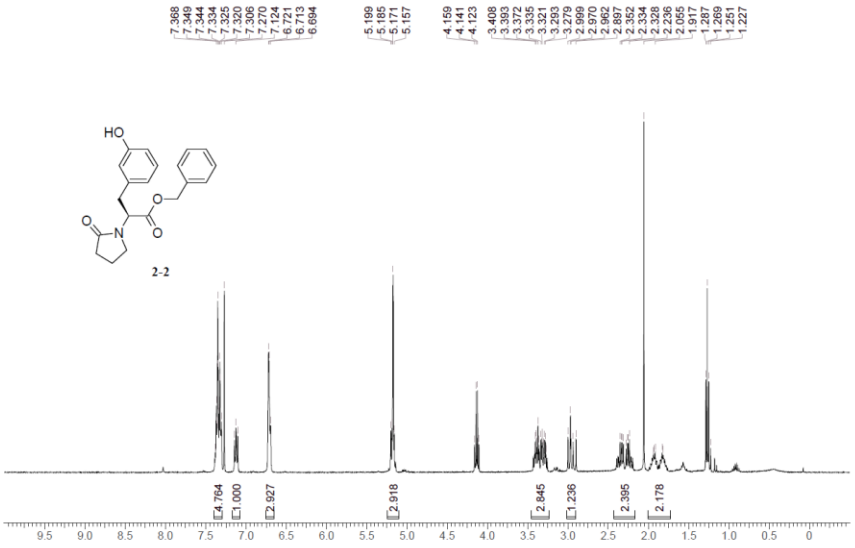

2-2

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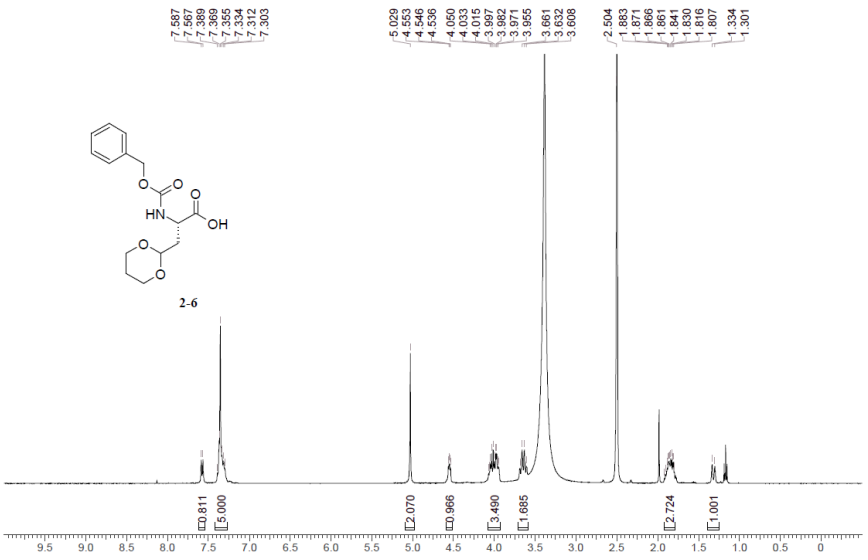

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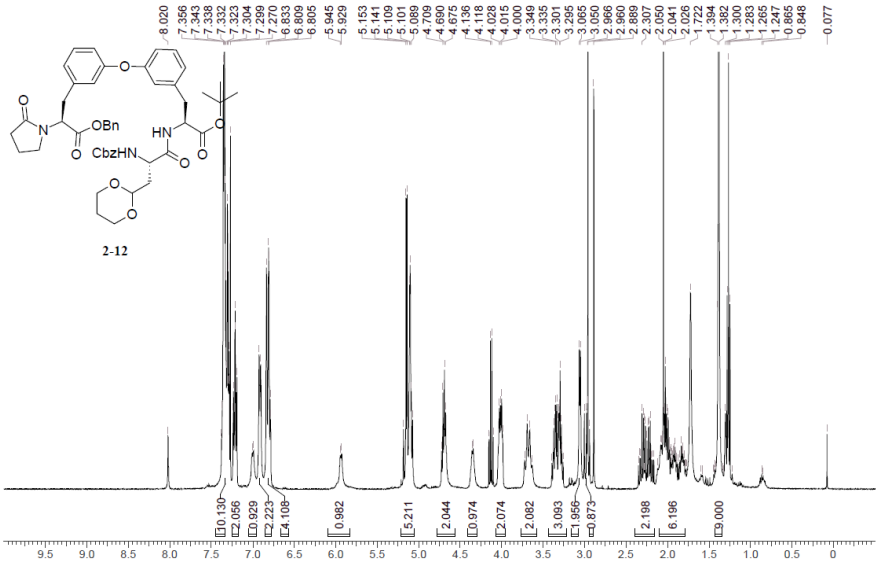

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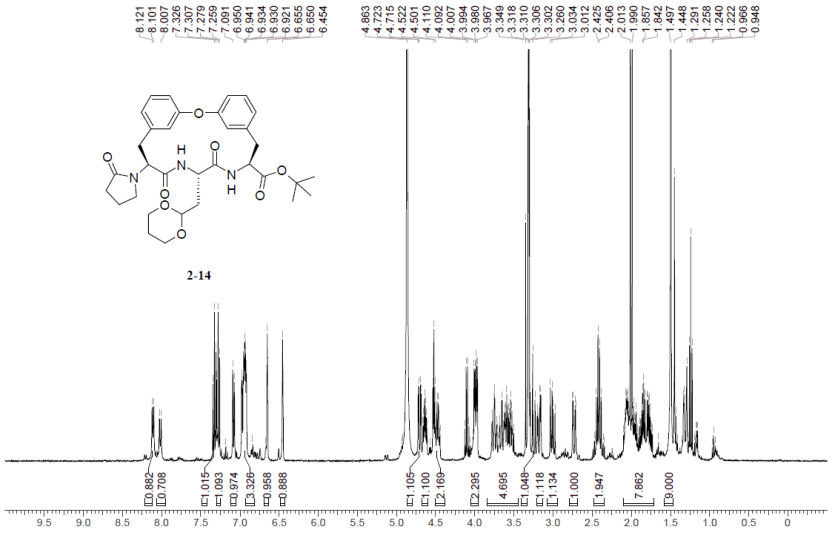

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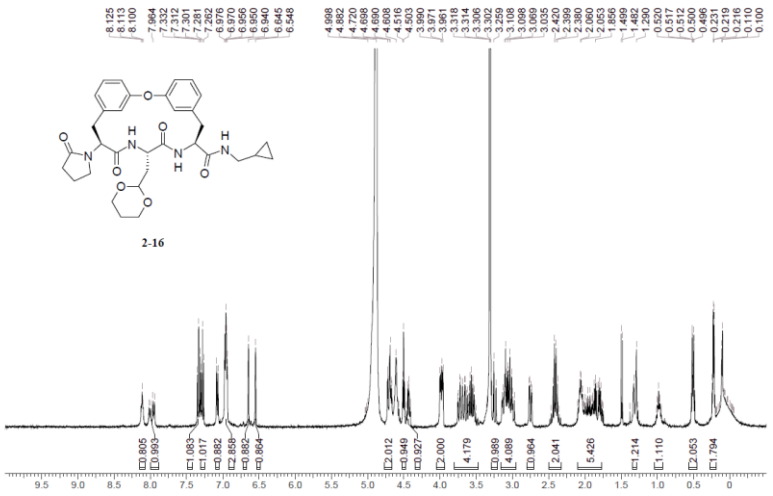

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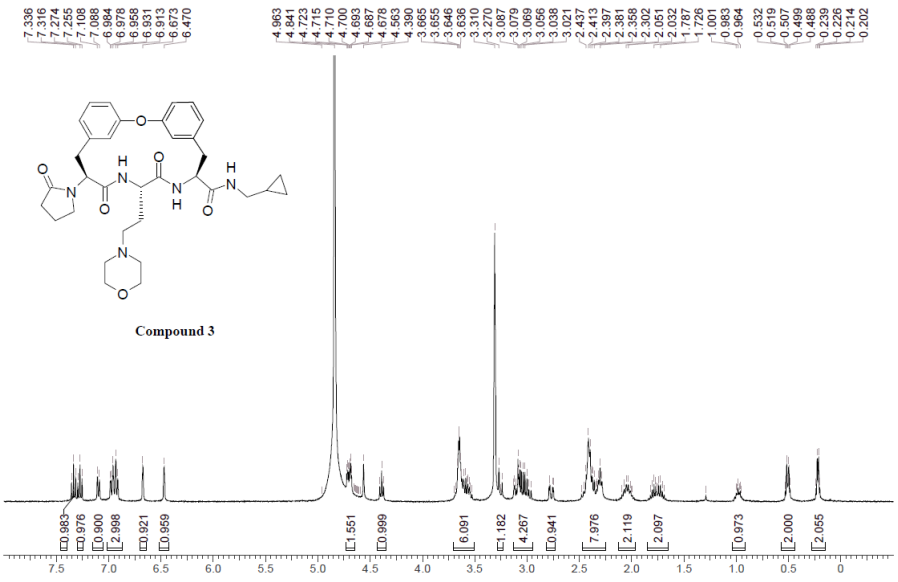

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Points Count	65536
Pulse Sequence	zg30
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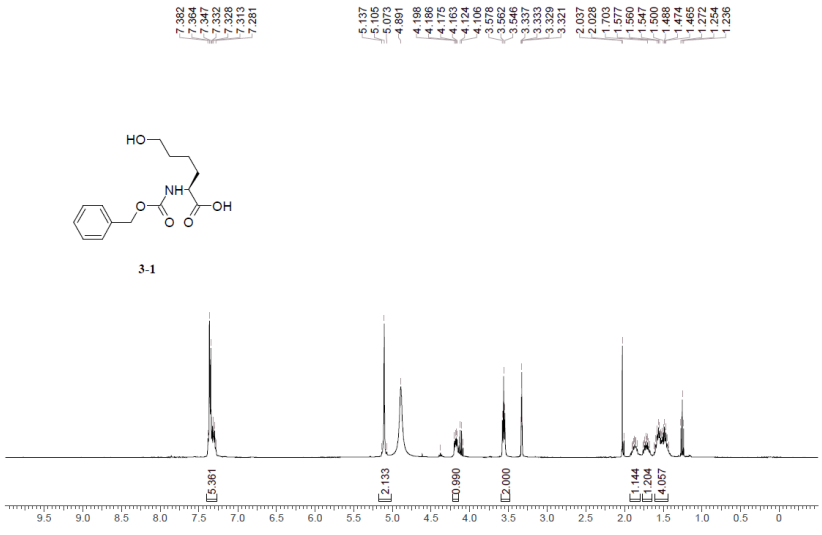

3-1

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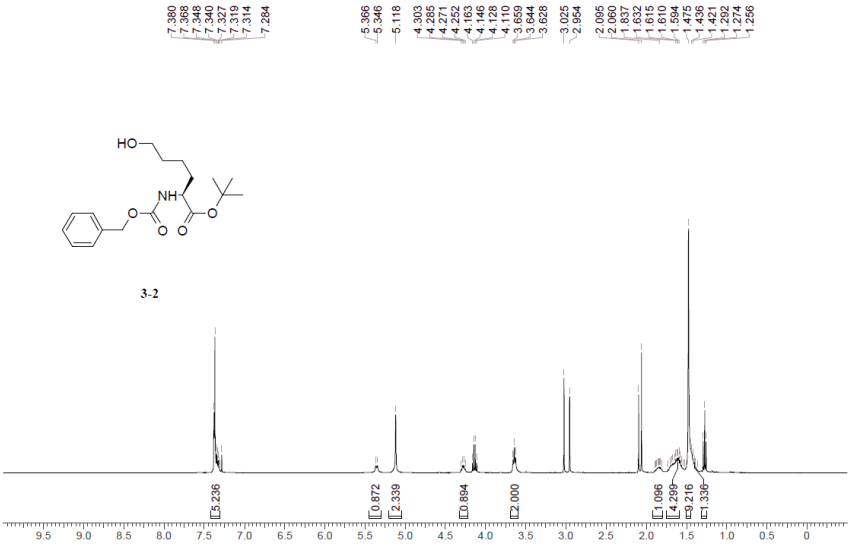

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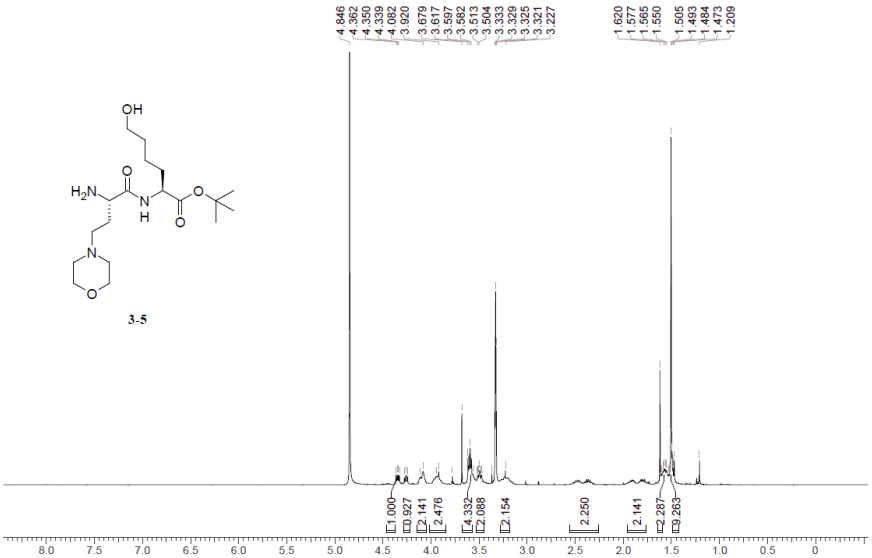
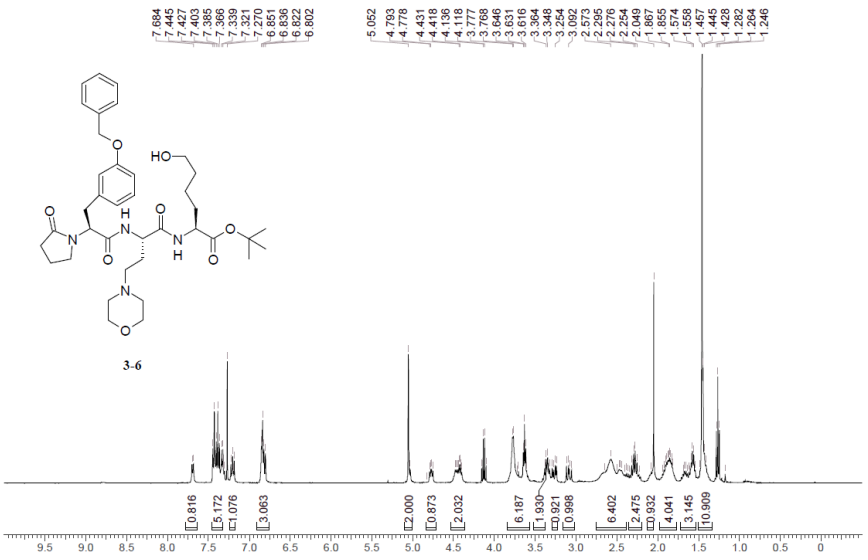
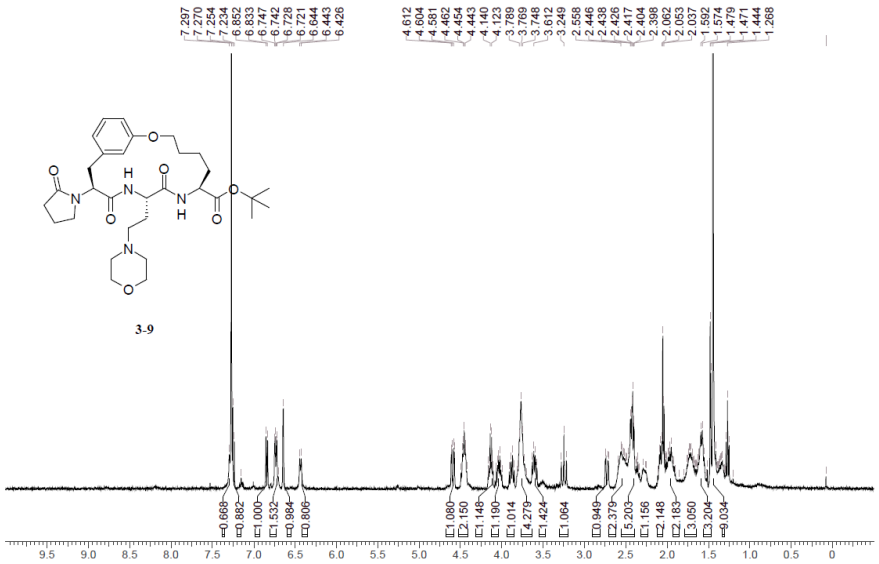
SW(cyclical) (Hz)	8223.68
Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	2470.9683
Spectrum Type	standard
Sweep Width (Hz)	8223.56
Temperature (degree C)	24.945



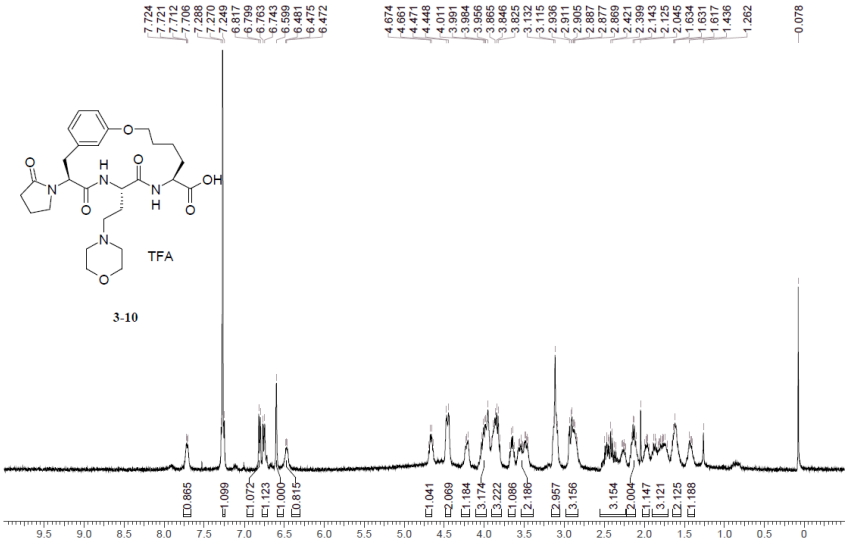
Acquisition Time (sec)	3.0671
Comment	EW1632-2 034-P1A MeOD Bruker_D_ 400MHz
Date	12 Jan 2018 11:44:20
Frequency (MHz)	400.1300
Nucleus	1H
Number of Transients	8
Origin	spect
Original Points Count	24576
Owner	nmrsu
Points Count	65536
Pulse Sequence	zg30
Receiver Gain	71.54
SW(cyclical) (Hz)	8012.82
Solvent	METHANOL-d4
Spectrum Offset (Hz)	2400.7795
Spectrum Type	standard
Sweep Width (Hz)	8012.70
Temperature (degree C)	28.142



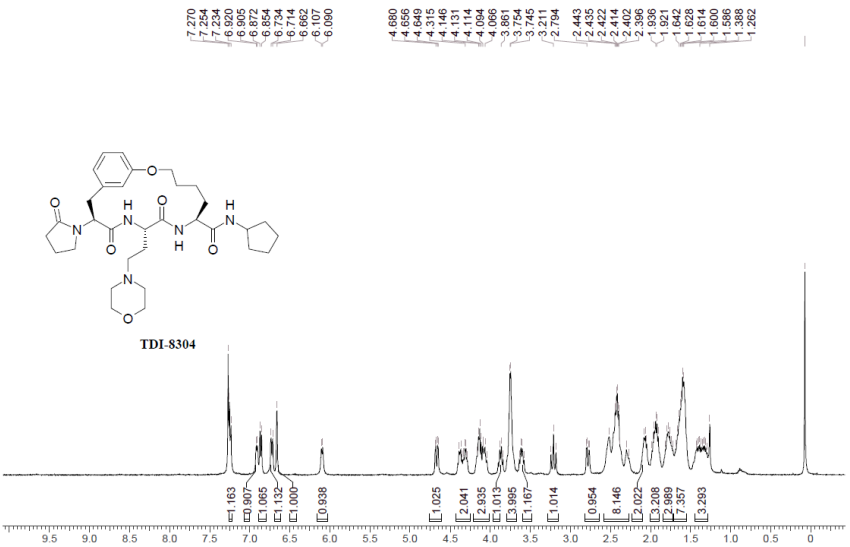
Acquisition Time (sec)	3.0671
Comment	EW1632-2 048-P1A CDCl3 Bruker_D_ 400MHz
Date	19 Jan 2018 19:07:06
Frequency (MHz)	400.1300
Nucleus	1H
Number of Transients	8
Origin	spect
Original Points Count	24576
Owner	nmrsu
Points Count	65536
Pulse Sequence	zg30
Receiver Gain	71.54
SW(cyclical) (Hz)	8012.82
Solvent	CHLORO FORM-d
Spectrum Offset (Hz)	2395.2576
Spectrum Type	standard
Sweep Width (Hz)	8012.70
Temperature (degree C)	27.161



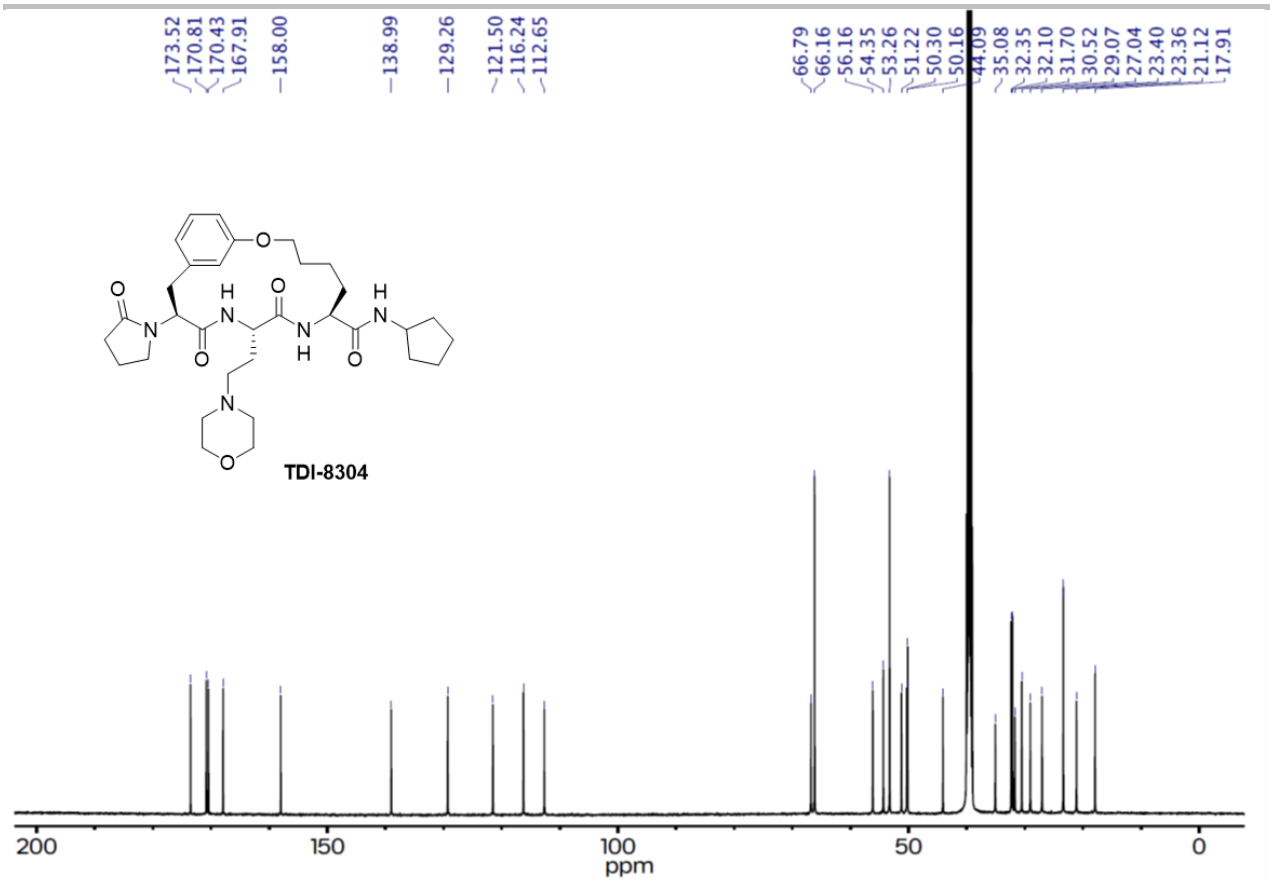
Acquisition Time (sec)	3.0671
Comment	EW1632-2 055-P1A CDCl3 Bruker_A_ 400MHz
Date	30 Jan 2018 16:32:57
Frequency (MHz)	400.1300
Nucleus	1H
Number of Transients	8
Origin	spect
Original Points Count	24576
Owner	nmrsu
Points Count	65536
Pulse Sequence	zg30
Receiver Gain	175.36
SW(cyclical) (Hz)	8012.82
Solvent	CHLORO FORM-d
Spectrum Offset (Hz)	2395.3501
Spectrum Type	standard
Sweep Width (Hz)	8012.70
Temperature (degree C)	28.147



Acquisition Time (sec)	3.0671
Comment	EW1632-2 072-P1A1 CDCl3 Bruker_F_ 400MHz
Date	09 Feb 2018 10:28:47
Frequency (MHz)	400.1700
Nucleus	1H
Number of Transients	8
Origin	spect
Original Points Count	24576
Owner	nmrsu
Points Count	65536
Pulse Sequence	zg30
Receiver Gain	128.11
SW(cyclical) (Hz)	8012.82
Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	2395.3062
Spectrum Type	standard
Sweep Width (Hz)	8012.70
Temperature (degree C)	24.159



Acquisition Time (sec)	3.0671
Comment	EW1632-2 063-P1C CDCl3 Bruker_F_ 400MHz
Date	07 Feb 2018 11:43:24
Frequency (MHz)	400.1700
Nucleus	1H
Number of Transients	8
Origin	spect
Original Points Count	24576
Owner	nmrsu
Points Count	65536
Pulse Sequence	zg30
Receiver Gain	80.69
SW(cyclical) (Hz)	8012.82
Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	2395.4063
Spectrum Type	standard
Sweep Width (Hz)	8012.70
Temperature (degree C)	28.162


173.52
170.81
170.43
167.91
158.00
138.99
129.26
121.50
116.24
112.65
66.79
66.16
56.16
54.35
53.26
51.22
50.30
50.16
44.09
35.08
32.35
32.10
31.70
30.52
29.07
27.04
23.40
23.36
21.12
17.91
TDI-8304
200
150
100
50
0
ppm

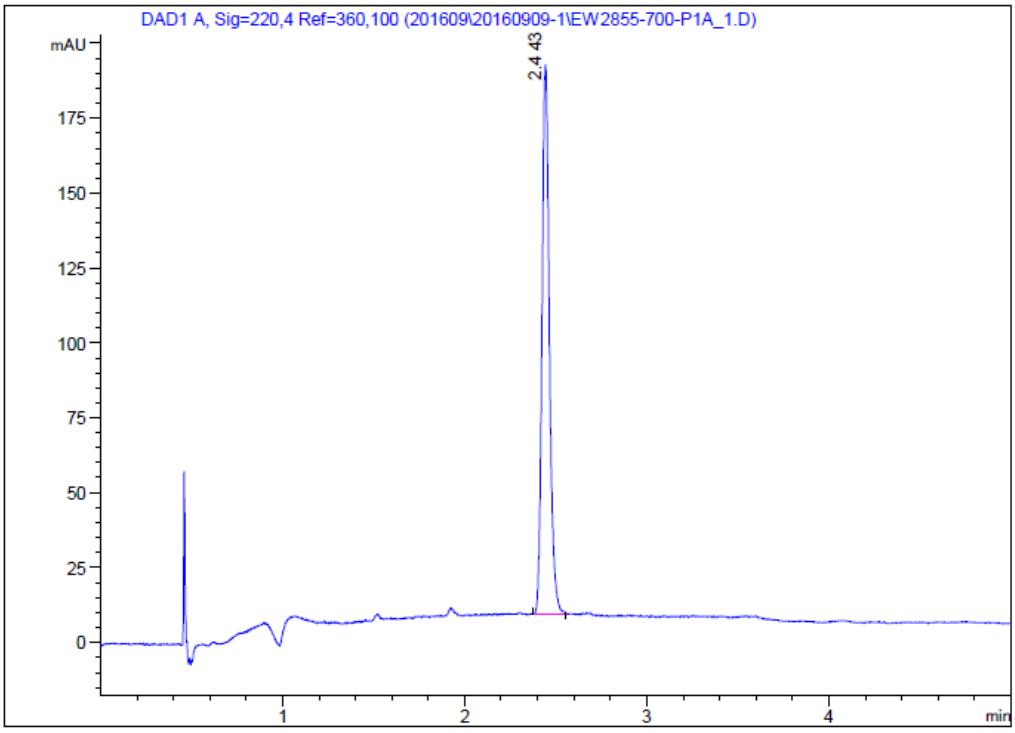
SFC

COMPOUND ID : 1-8

Sample ID : EW2855-700-P1A_1
Injection Date: 9/9/2016

Acq Method : D:\DATA\201609\20160909-1\OD-3S_3_5_40_3ML_T35.M

Data Filename : D:\DATA\201609\20160909-1\EW2855-700-P1A_1.D

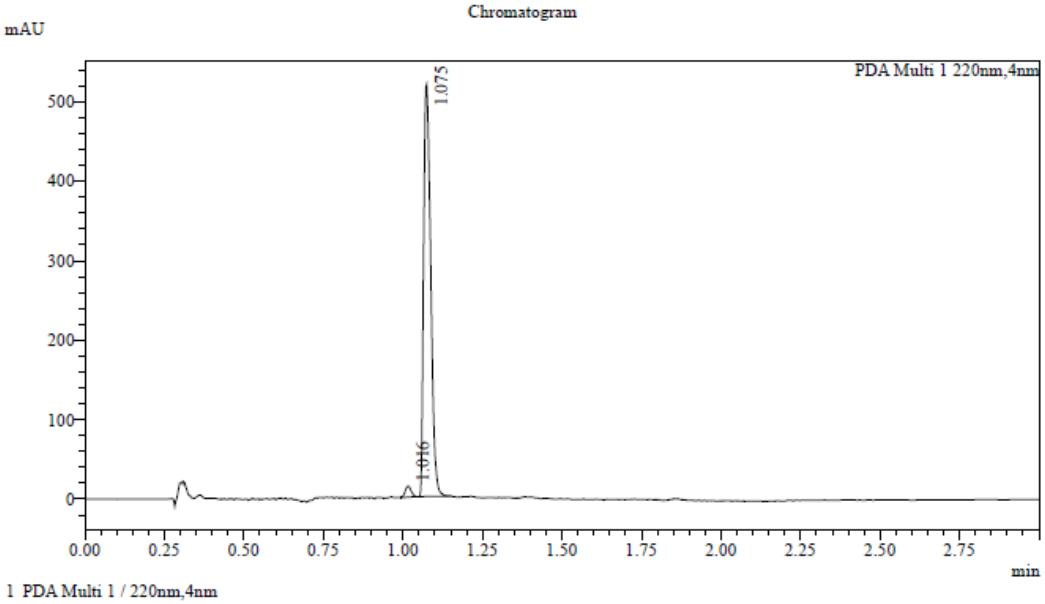


==========

DAD1 A, Sig=220,4 Ref=360,100

#	Meas. Ret. Time	Height	Width	Symmetry	Area	Area %
1	2.443	184.053	0.047	0.789	514.919	100.000

Compound ID : 2-6
Sample ID : EW7163-136-P1A_2
Injection Vol : 3ul
Location : vial43
Acq Method : D:\METHODS\Cellucoat-MeOH(DEA)-5-40-3mL-35
Org DateFile : D:\DATA\1707\20170707\EW7163-136-P1A_2.lcd
Injection Date : 7/7/2017 17:13:04
Instrument : SFC-C



1 PDA Multi 1 / 220nm,4nm

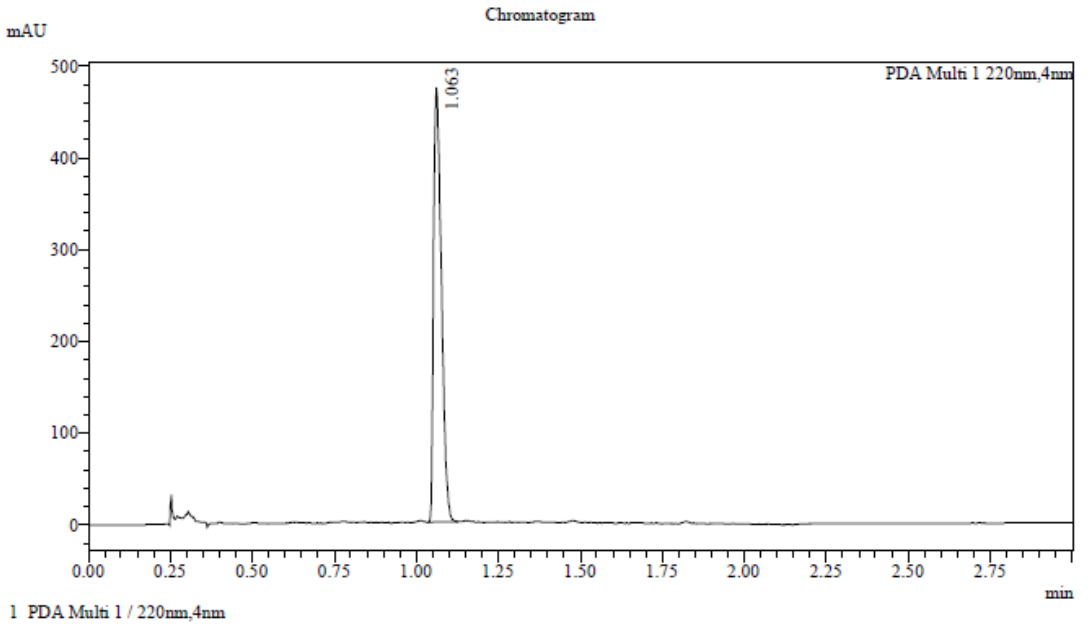
Integration Result

Peak Table

PDA Ch1 220nm

Peak#	Ret. Time	Height	Height%	USP Width	Area	Area%
1	1.016	12874	2.562	0.040	18466	2.252
2	1.075	489576	97.438	0.044	801489	97.748

Compound ID : 3-2
Sample ID : EW1632-1934-P1A_2
Injection Vol : 4ul
Location : vial70
Acq Method : D:\METHODS\Cellucoat-MeOH(DEA)-5-40-3mL-35
Org DateFile : D:\DATA\1711\20171121\EW1632-1934-P1A_2.lcd
Injection Date : 21/11/2017 PM 7:46:44
Instrument : SFC-C



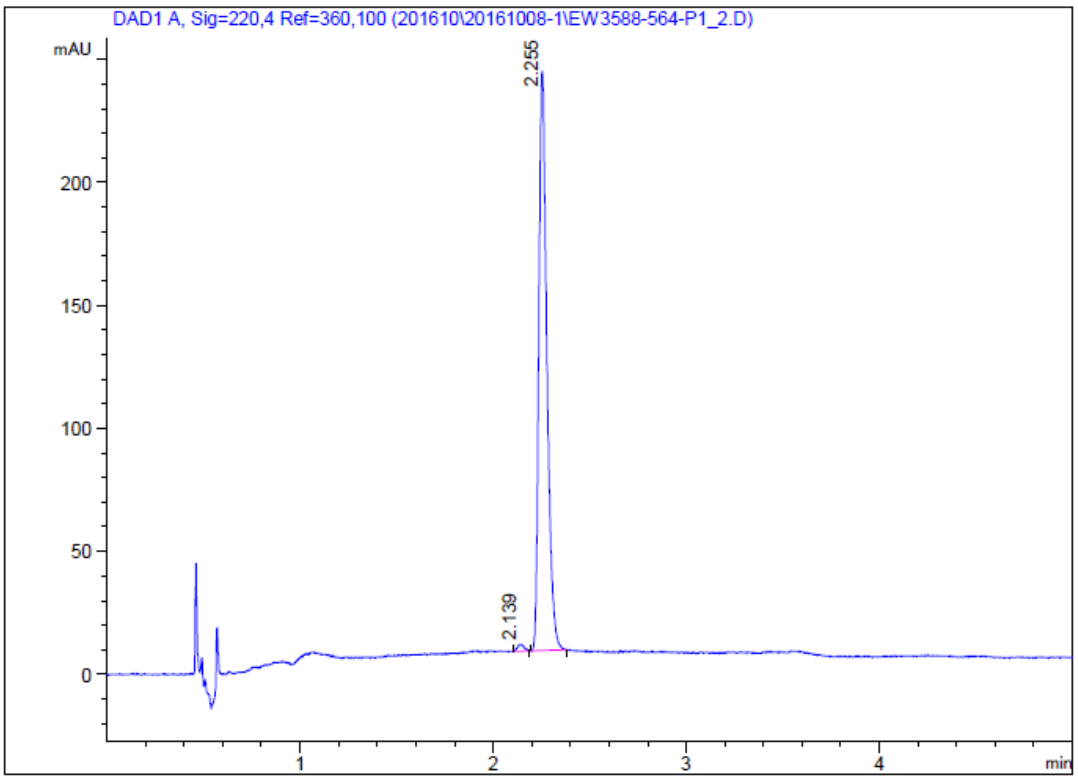
Integration Result

Peak Table

PDA Ch1 220nm

Peak#	Ret. Time	Height	Height%	USP Width	Area	Area%
1	1.063	464415	100.000	0.049	782691	100.000

```
COMPOUND ID    : compound 2
Sample ID      : EW3588-564-P1_2
Injection Date: 10/8/2016
Acq Method     : D:\DATA\201610\20161008-1\OD-3S_3_5_40_3ML_T35.M
Data Filename : D:\DATA\201610\20161008-1\EW3588-564-P1_2.D
Instrument     : SFC-A
================================================================================
```

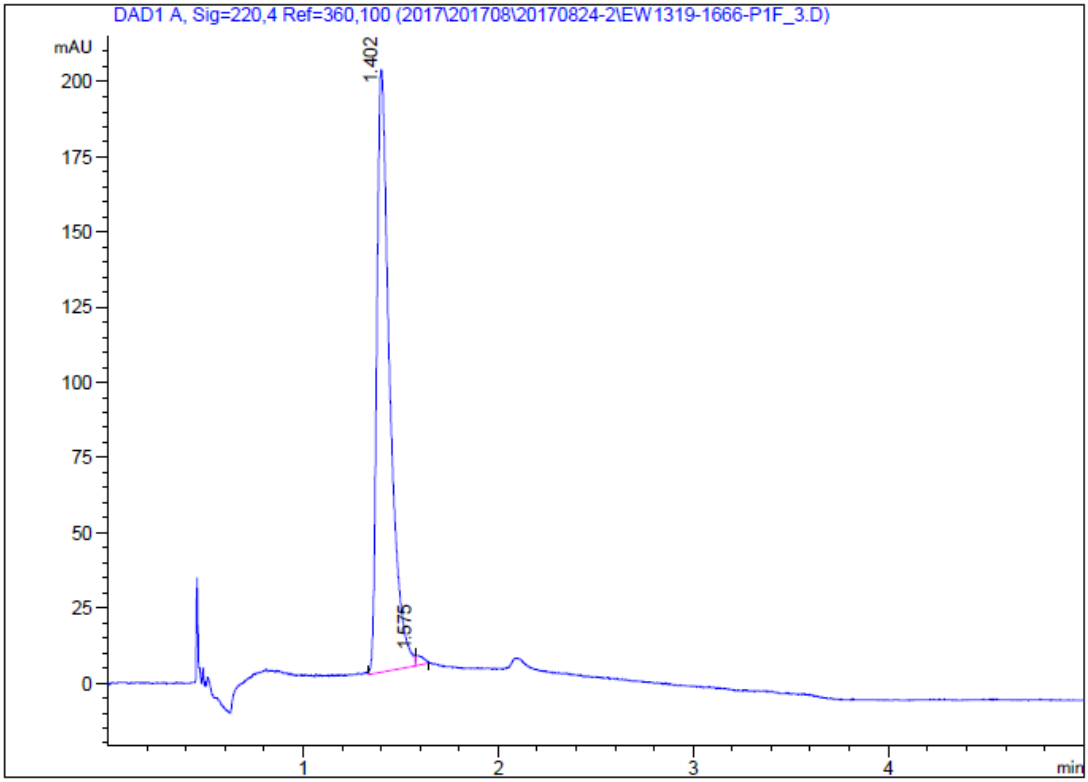



```
================================================================================
```

DAD1 A, Sig=220,4 Ref=360,100

#	Meas. Ret. Time	Height	Width	Symmetry	Area	Area %
1	2.139	2.827	0.039	0.478	6.660	0.962
2	2.255	237.247	0.048	0.558	685.371	99.038

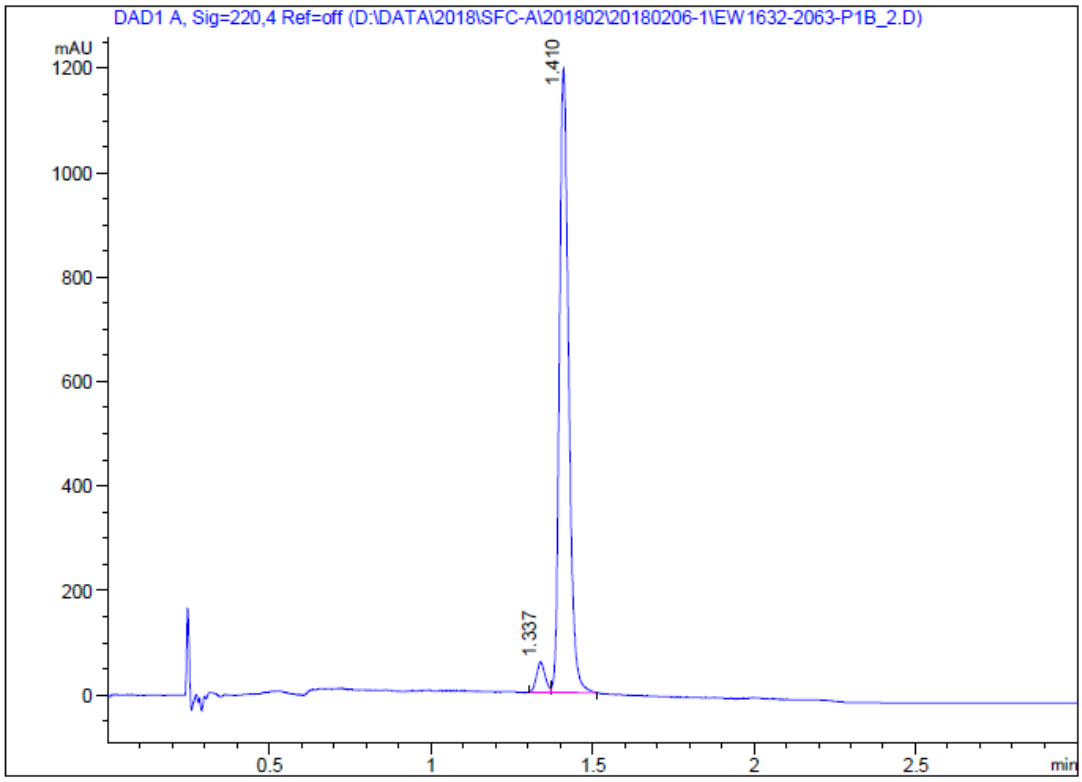
```
COMPOUND ID    : compound 3
Sample ID      : EW1319-1666-P1F_3
Injection Date: 8/24/2017              11:45:58 PM
Acq Method     : D:\DATA\2017\201708\20170824-2\OJ-3S_3_5_40_3ML_T35.M
Location       : Vial 51
Data Filename  : D:\DATA\2017\201708\20170824-2\EW1319-1666-P1F_3.D
Instrument     : SFC-A
================================================================================
```




```
================================================================================

DAD1 A, Sig=220,4 Ref=360,100
  #   Meas. Ret. Time    Height      Width     Symmetry      Area      Area %
--------------------------------------------------------------------------------
    1           1.402   201.027      0.077       0.576   925.518      99.038
    2           1.575     3.498      0.043       0.000     8.991       0.962
--------------------------------------------------------------------------------
```

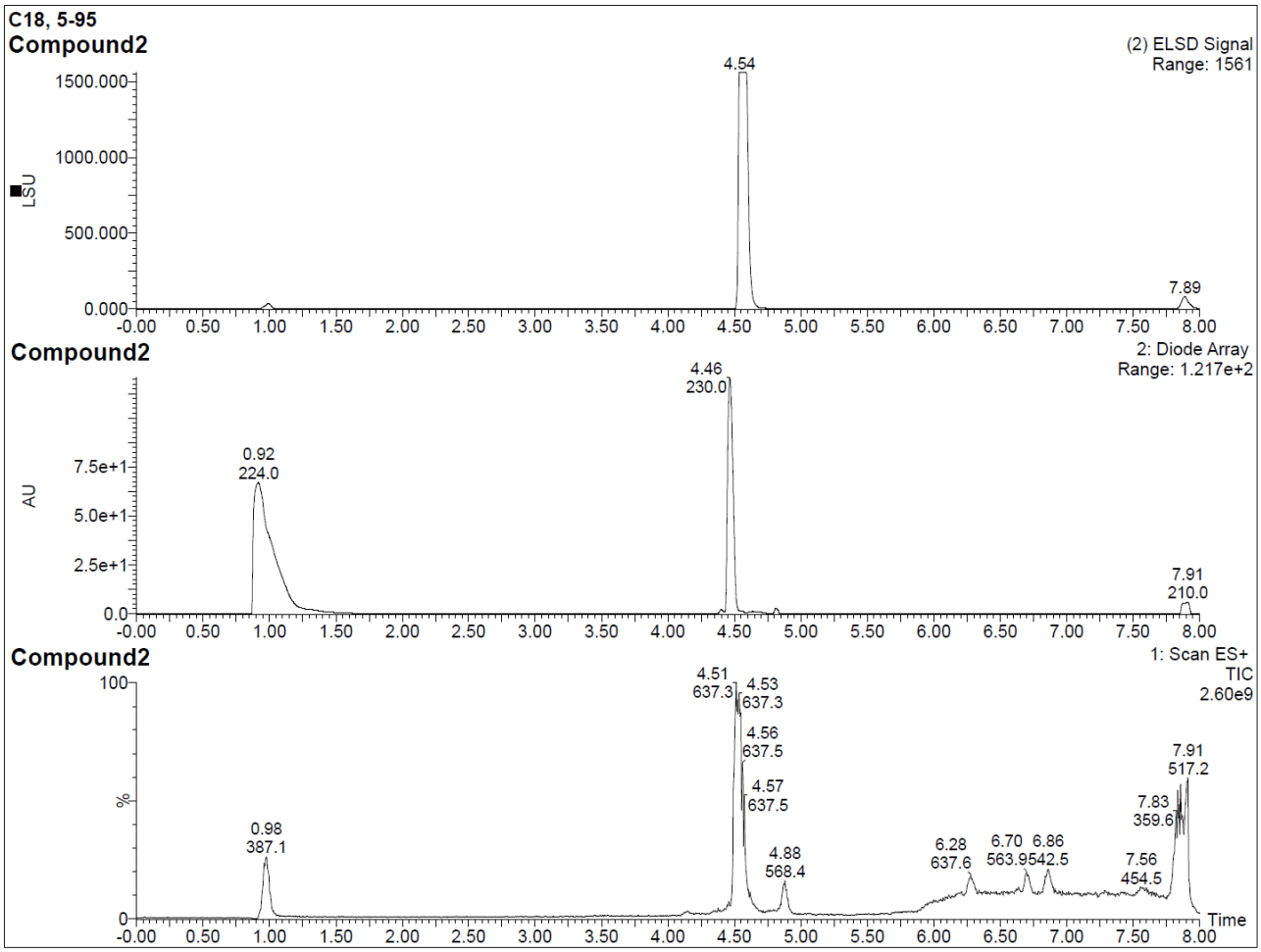
```
COMPOUND ID    : TDI-8304
Sample ID      : EW1632-2063-P1B_2
Injection Date: 2/6/2018                  9:25:06 PM
Acq Method     : D:\DATA\2018\201802\20180206-1\OD-3_5CM_MEOH(DEA)_5_40_3ML_T35.M
Location       : Vial 14
Data Filename : D:\DATA\2018\SFC-A\201802\20180206-1\EW1632-2063-P1B_2.D
Instrument     : SFC-A
================================================================================
```

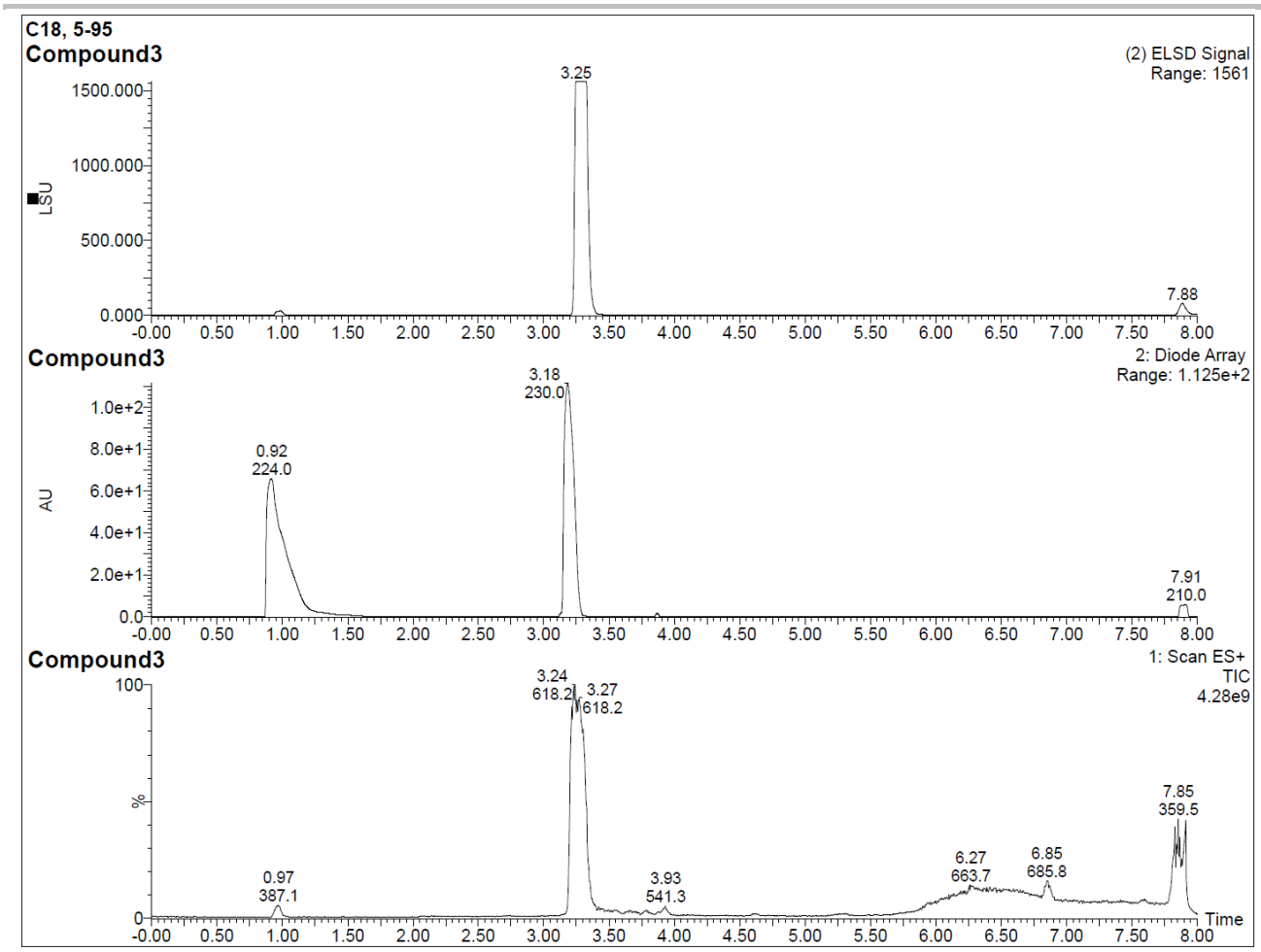


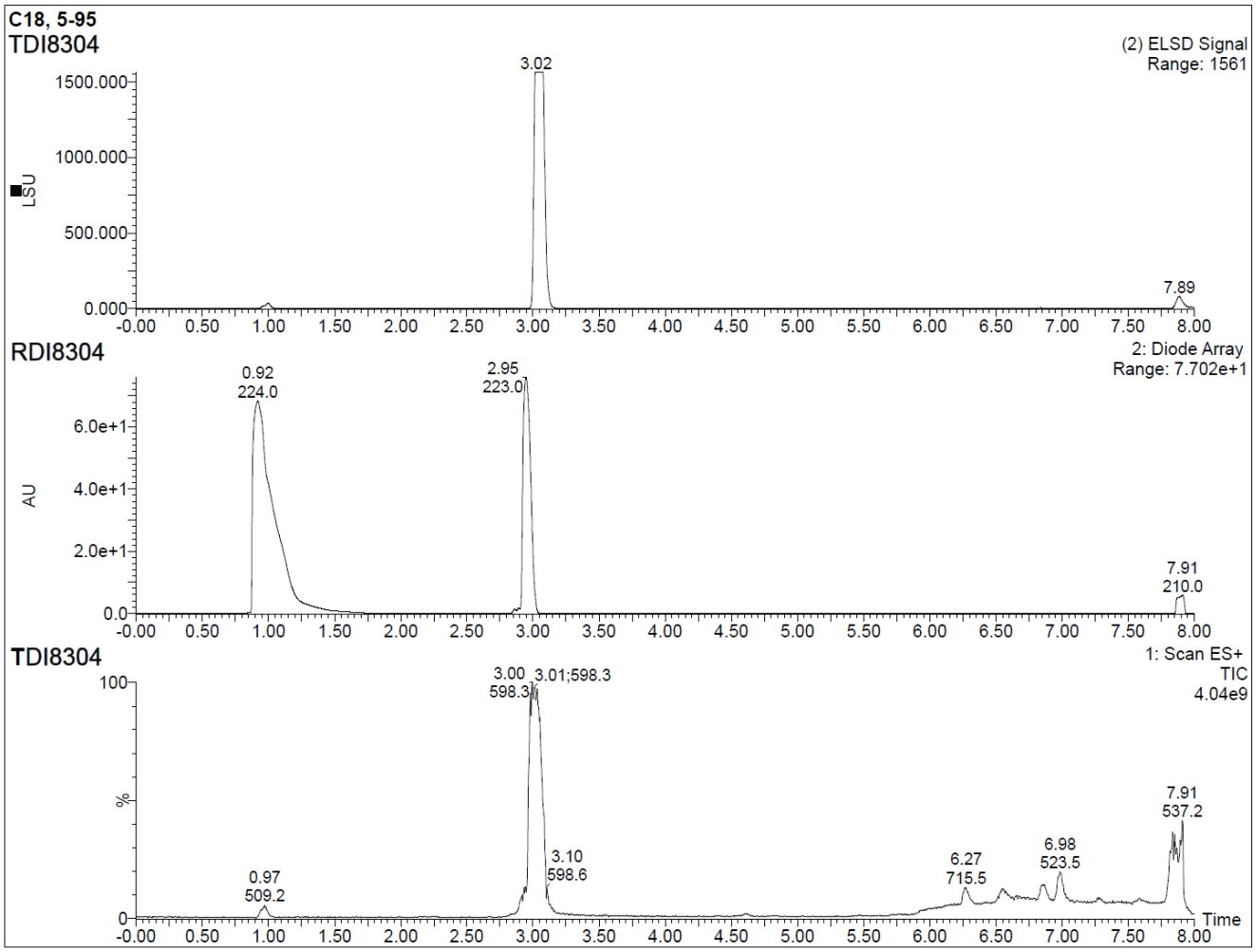

```
================================================================================

DAD1 A, Sig=220,4 Ref=off
  #   Meas. Ret. Time    Height      Width     Symmetry      Area      Area %
--------------------------------------------------------------------------------
    1           1.337     58.187      0.031       0.708   106.510       4.283
    2           1.410   1202.284      0.033       0.816  2380.530      95.717
--------------------------------------------------------------------------------
```

HPLC


C18, 5-95
Compound2
(2) ELSD Signal
Range: 1561
4.54
7.89
LSU
Compound2
2: Diode Array
Range: 1.217e+2
4.46
230.0
0.92
224.0
7.91
210.0
AU
Compound2
1: Scan ES+
TIC
2.60e9
4.51
637.3
4.53
637.3
4.56
637.5
4.57
637.5
7.91
517.2
7.83
359.6
0.98
387.1
4.88
568.4
6.28
637.6
6.70
563.9
6.86
542.5
7.56
454.5
%
Time


C18, 5-95
Compound3
(2) ELSD Signal
Range: 1561
3.25
7.88
LSU
Compound3
2: Diode Array
Range: 1.125e+2
3.18
230.0
0.92
224.0
7.91
210.0
AU
Compound3
1: Scan ES+
TIC
4.28e9
3.24
618.2
3.27
618.2
7.85
359.5
6.27
663.7
6.85
685.8
0.97
387.1
3.93
541.3
%
Time


C18, 5-95
TDI8304
(2) ELSD Signal
Range: 1561
3.02
7.89
LSU
RDI8304
2: Diode Array
Range: 7.702e+1
0.92
224.0
2.95
223.0
7.91
210.0
AU
TDI8304
1: Scan ES+
TIC
4.04e9
3.00
598.3
3.01;598.3
3.10
598.6
0.97
509.2
6.27
715.5
6.98
523.5
7.91
537.2
%
Time

HRMS

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

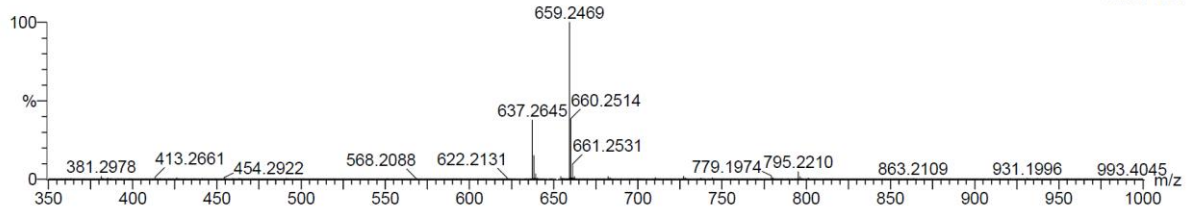
Monoisotopic Mass, Even Electron Ions
92 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-36 H: 0-36 N: 0-4 O: 0-5 F: 0-3

GANG
GANG
C34H35F3N4O5
compound2 64 (1.436) Cm (62:64)

NMR Analytical Core Facility
LCT Premier XE

15-Jul-2020
11:56:14

1: TOF MS ES+
7.05e+004



Minimum:				-1.5					
Maximum:		5.0	5.0	50.0					
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula		
637.2645	637.2638	0.7	1.1	17.5	169.9	0.0	C34	H36	N4 O5 F3

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

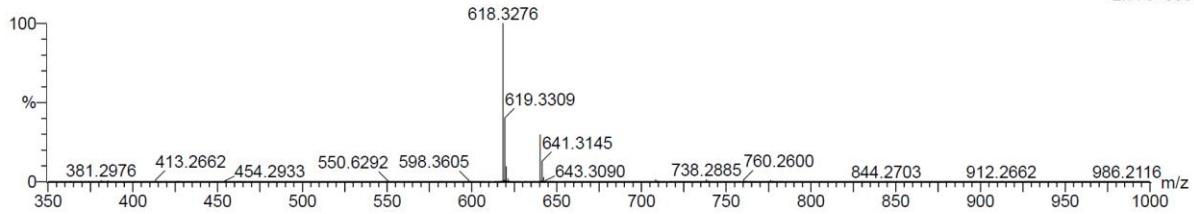
Monoisotopic Mass, Even Electron Ions
35 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-36 H: 0-48 N: 0-5 O: 0-6

GANG
GANG
C34H43N5O6
Compound3 19 (0.425) Cm (18:23)

NMR Analytical Core Facility
LCT Premier XE

15-Jul-2020
12:05:26

1: TOF MS ES+
2.77e+005



Minimum:				-1.5					
Maximum:		5.0	5.0	50.0					
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula		
618.3276	618.3292	-1.6	-2.6	15.5	288.9	0.0	C34	H44	N5 O6

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

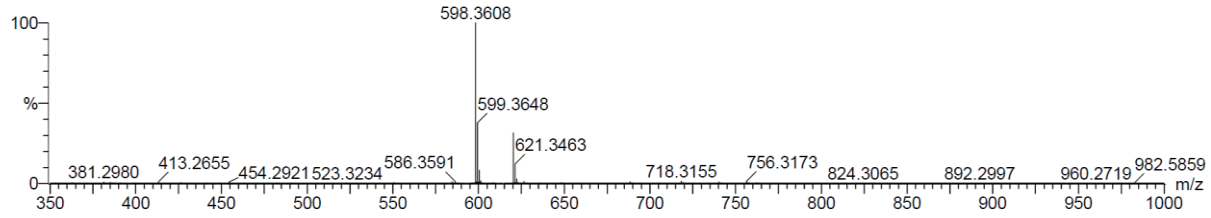
Monoisotopic Mass, Even Electron Ions
42 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-36 H: 0-48 N: 0-5 O: 0-6

GANG
GANG
C32H47N5O6
TDI8304 30 (0.679) Cm (27:32)

NMR Analytical Core Facility
LCT Premier XE

15-Jul-2020
12:01:36

1: TOF MS ES+
2.55e+005



Minimum:				-1.5						
Maximum:		5.0	5.0	50.0						
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula			
598.3608	598.3605	0.3	0.5	11.5	279.8	0.0	C32	H48	N5	O6