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Structure-activity relationship studies of antimalarial *Plasmodium* proteasome inhibitors – Part II

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Abstract

With increasing reports of resistance to artemisinins and artemisinin-combination therapies, targeting the *Plasmodium* proteasome is a promising strategy for antimalarial development. We recently reported a highly selective *Plasmodium falciparum* proteasome inhibitor with anti-malarial activity in the humanized mouse model. To balance the permeability of the series macrocycles with other drug-like properties, we conducted further structure-activity relationship studies on the biphenyl ether tethered macrocyclic scaffold. Extensive SAR studies around the P1, P3, and P5 groups and peptide backbone identified compound TDI-8414. TDI-8414

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Author Contributions

The manuscript was written through contributions of all authors. / All authors have given approval to the final version of the manuscript.

ASSOCIATED CONTENT

Supporting Information.

The Supporting Information is available free of charge at http://pubs.acs.org.

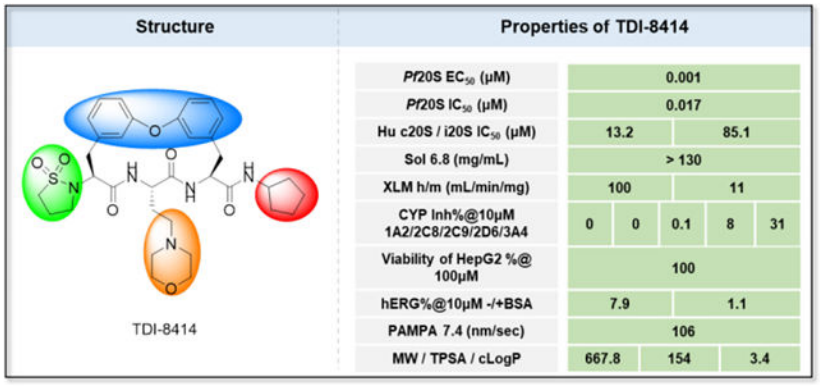
Synthetic procedures for key intermediates, NMR and HPLC spectra of final compounds, and experimental procedures for the biological assays (PDF)

Molecular formula strings (CSV)

Any additional relevant notes should be placed here.

showed nanomolar antiparasitic activity, no toxicity to HepG2 cells, high selectivity against the *Plasmodium* proteasome over the human constitutive proteasome and immunoproteasome, improved solubility and PAMPA permeability, and enhanced metabolic stability in microsomes and plasma of both humans and mice.

Graphical Abstract



Keywords

Malaria; antimalaria; Plasmodium proteasome; species-selective parasite proteasome inhibitors; pharmacokinetics

Introduction

Malaria is one of the most prevalent infectious diseases in the world. Africa carries the heaviest malaria burden, accounting for ~95% of malaria cases and deaths, primarily in young children.[1] Among the *Plasmodium* species causing human malaria, *P. falciparum* (*Pf*)is the most virulent, and infections are commonly life-threating. During symptomatic disease, parasites replicate rapidly and can reach > 10^{12} parasites in an infected individual,[2] setting the stage for selection of resistance to antimalarials. In fact, resistance to most antimalarials in clinical use is reported globally, and resistance has even been detected to some drugs at the clinical trials stage. It is alarming that resistance to artemisinins and artemisinin-combination therapies is widespread in the Greater Mekong Region of southeast Asia and now emerging independently in sub-Saharan Africa.[3–5] The loss of effectiveness of artemisinins would be disastrous for global malaria control. It is an urgent priority to develop antimalarials that target novel parasite proteins and/or demonstrate synergy with artemisinins so as to prolong their clinical effectiveness, overcome existing resistance and minimize the emergence of resistance.

Proteasomes of pathogenic microbes are novel targets for discovery and development of antimicrobials,[6] starting with the development of species selective proteasome inhibitors of *Mycobacterium tuberculosis* (Mtb)[7], followed by those of *Plasmodium*[8], *Trypanosoma,* and *Leishmania*.[9] Genetic studies validated the essentiality of the *Plasmodium* proteasome[10] and its pharmacological inhibition by small molecule inhibitors, six represents shown in Figure 1a[8, 11–16]. In addition to action against the erythrocytic stage, which is responsible for

human disease, several studies also established that proteasome inhibitors are active against the liver stage, gametocytes, and gametes (Figure 1b).[13, 16, 17] Compared to wild type strains, artemisinin resistant parasites (with K13 mutations) were slightly more sensitive to proteasome inhibitors, and proteasome inhibitor resistant mutants were slightly more sensitive to artemisinins.[16, 18] Synergy between proteasome inhibitors and artemisinins has been demonstrated by multiple groups [12, 16, 19–21].

We recently developed a proteasome inhibitor TDI8304 that is highly selective for the *P. falciparum* proteasome over both the human constitutive proteasome (c20S) and immunoproteasome (i20S) (Figure 1a), with metabolic stability and in vivo efficacy in a humanized mouse model of *P. falciparum* infection.[11] Starting from this reported cyclic peptide 1 (CP1),[14] the monophenyl linked macrocycle **TDI8304** was developed as a lead compound with a good balance of potency,[11] selectivity, solubility, plasma stability, and microsome stability, but it suffered from modest PAMPA permeability. We hypothesized that macrocyclic peptides with a biphenyl tether, which has a high lipophilicity, might balance the PAMPA permeability with other pharmacokinetic properties (Figure 2).[22] In this paper, we present our second structure-activity relationship study of biphenyl ether tethered Pf20S selective macrocycles in an attempt to improve the permeability and other pharmacokinetic properties of this class of antimalarials.

RESULTS AND DISCUSSION

Macrocycle **1** showed remarkable anti-parasitic activity and PAMPA permeability; however, it was rapidly metabolized by mouse microsomes (Table 1).[11, 14] We started by replacing the P3 homophenylalanine (homo-Phe) of **1** with hydrogen (**2**), methoxy methyl (**3**) or trifluoropropyl (**4**) groups to reduce lipophilicity (cLogP = 2.2 to 3.1), all of which resulted in much improved liver microsomal stability, but also complete loss of potency. Replacing the P3 homo-Phe of **1** with propyl (**5**) or isobutyl (**6**) groups significantly decreased potency against parasites. Replacing the P3 homo-Phe of **7** with a phenyl group (**8**) also resulted in marked potency loss, yet replacement with a piperidine (**9**) maintained the anti-parasitic activity and dramatically improved the solubility with decreased cLogP. This modification paved the way for further optimization of **9**.

Compound **9** was rapidly cleared within 30 min post cassette-dosing at 0.3 mg/kg, *i.v.* (Table S1).[23] Compound **9** showed high stability after incubation with human plasma and microsomes, but fast clearance in mouse plasma and microsomes. **9** showed good potency, solubility, and passive permeability, and was selected as the preferred compound for further structural modifications to improve metabolic and plasma stability. The piperidine was considered to be a potential metabolic liability. We introduced a metabolically stable diF at the 4-position (**10**) and a fluorine atom at the 3-position (**11**) of the piperidine of **9** to lower the electron density as well as block potential oxidative metabolism at this site. These two macrocycles showed improved anti-parasitic activity over **9;** however, both suffered significantly reduced microsome stability.

To understand the rapid microsomal clearance of **9**, we investigated the metabolites generated in the presence of mouse liver microsomes. After incubating **9** with mouse liver

microsomes for 60 min in the presence of NADPH and uridine diphosphoglucuronic acid, LC-MS/MS indicated that **9** was extensively metabolized, mostly via hydroxylation (Figure 3a, **b**). **M659a** is proposed to be formed via hydroxylation of the biphenyl linker, while **M659b** and **M659c** could be formed through oxidative metabolism of the P5 lactam by hepatic cytochrome P450 in a NADPH dependent manner. The P5 lactam appears to be the major site for oxidative metabolism. No glucuronide conjugates of **9** or its primary hydroxylated metabolites were detected, suggesting that **9** is not the substrate for UDP-glucuronosyltransferases (UGTs).

Based on the metabolite profiling of **9**, the next optimization process was focused on improving mouse microsomal stability through structural modification of the P5 group while maintaining potency and favorable biochemical properties. Blocking the metabolic soft spot and reducing the electron density of the P5 group via increased polarity are two common strategies to prevent oxidative metabolism and improve microsomal stability. We first replaced the adjacent methylene of the lactam group in macrocycle **9** with an oxygen atom in **12** to block the potential metabolic site (Table 2). This modification was tolerated for potency but failed to improve the metabolic stability, likely due to the oxazolidinone group of **12** that has higher electron density and is more prone to oxidative metabolism than the lactam group. We next directed our efforts to introducing polar substituents and decreasing electron density of the P5 group. Replacing the lactam group of **13** with a polar methyl sulfonamide group afforded macrocycle **14**, which showed an improved metabolic stability across species (m/hLM, 19/6 μL/min/mg), but a marked loss of antiparasitic activity (IC_{50} >2.7 μM). Cyclizing the methyl sulfonamide group of **14** provided compound **15** with a sultam as P5, which recovered anti-parasitic activity and maintained metabolic stability across species, but still suffered from poor permeability (Table 2). As shown in Figure 4, the improved mouse microsomal stability of **15** translated into low turnover in mouse hepatocytes (Cl_{int} = 2.8 μL/min/10^6 cells).

After incubation with human and mouse plasma for 120 min, **9** exhibited remarkable stability in human plasma, but was rapidly degraded in mouse plasma (Figure 5a, b). Mouse plasma metabolite characterization of macrocycle **9** was performed using LC/MS. A macrocyclic carboxylic acid was identified as a major metabolite, suggesting that hydrolysis of the P1 amide is a major clearance mechanism (Figure 3c). Although N-methylation of a susceptible amide can often induce resistance to hydrolysis by proteases, our docking model of compound **9** suggested that the P1 amide forms two critical hydrogen bonds with Gly47 and Ser21 residues of the β5 subunit (Figure 6). To improve the plasma stability via altering the electronic character of the P1 amide bond, the electron-withdrawing trifluoromethyl group of **9** was replaced with an electron-donating cyclopropyl group in **16** and **22** (Table 3), which maintained the high antiparasitic activity but failed to improve mouse plasma stability.

We next explored introducing an extra methyl group at the alpha position (**18** and **19**) or beta position (**17**) of the amino group of **16** to improve the plasma stability by sterically hindering the approach of proteases in plasma to the susceptible amide bond. All three compounds with the methyl substitution showed improved mouse plasma stability and better PAMPA permeability than **16**. Compounds **18** and **19** with alpha-substituted methyl

groups are slightly more stable than **17** with a beta-substituted methyl group suggesting that steric hindrance around the amide bond is a stabilizing factor for mouse plasma stability, which confirmed P1 amide as a metabolic soft spot. Compound **19**, with an (S)-methyl substitution, was 7.4-fold more potent against the parasite than the R analog, **18**. Macrocycle **20,** with cyclopentyl as the P1 group, showed high mouse plasma stability and PAMPA permeability as alpha methyl substituted compounds while maintaining high antiparasitic activity, however, **20** showed fast mouse liver microsomal clearance (195 μL/min/mg). Replacing the P1 group with a more bulky 1-bicyclo[1.1.1]pentyl group (**21**) was detrimental for antiparasitic activity. Replacing the combination of the high mouse microsomal stability of **15** and the high mouse plasma stability and PAMPA permeability of **20** might balance the drug-like properties. Consistent with our hypothesis, macrocycle **TDI-8414** achieved a balance of high potency, selectivity, solubility, PAMPA permeability, metabolic stability and plasma stability. Additionally, TDI-8414 showed synergistic effect with dihydroartemisinin (DHA) in a ring-stage survival assay (RSA)[16](Figure 7a).

MDR results suggested that this class of compounds suffered poor permeability and a high efflux ratio. The N-H bond of peptides and peptidomimetics were reported as elements that were recognized by efflux transporter.[24] We therefore used N-methylation to reduce the number of hydrogen bond donors to 2 (Table 4). We chose **7** as the starting point, and N-methylated the P2-amide (**23**) and P3-amide (**24**). Although the N-methylated products showed improved MDR properties, they suffered a 1000-fold loss of potency to 0.21 μM for **23** and a complete loss for **24**. Additionally, there was no improvement in metabolic stability over **7**. The low efflux ratio and high A to B permeability of compound **23** and **24** provided a clue that reducing the number of hydrogen bond donor to two via N-H methylation would improve the MDR properties of the macrocyclic peptides, especially the N-H methylation of P2 amide that resulted in good MDR properties and moderate antiparasitic activity. Compound **25** was developed as a species-selective inhibitor for the Mtb proteasome (Mtb20S) over human proteasomes and showed cross antimicrobial activity, but still suffered from fast clearance (Table 4).

The inhibitory activity of compounds **2**-**25** and **TDI-8414** against the other 4 active subunits of hu-c20S and hu-i20S were determined. All the compounds showed insignificant inhibition against human β1c, β2c, β1i, and β2i (Table S2).

Compounds **9**, **23** and **25** were selected for further testing for ex vivo antiparasitic activity against freshly isolated *P. falciparum* isolates from 38, 35 and 28 malaria patients, respectively in Uganda (Figure 7b–d). The EC_{50} values of **9** ranged from 6.3–30 nM, with a geometric mean of 15.2 nM, in agreement with the results for *P. falciparum* laboratory strains (Table 2). Compounds **23** and **25** showed EC_{50} ranges of 283–1203 nM and 264–860 nM, with geometric means of 732 nM and 505 nM, respectively, representing a 3-fold and 2-fold decrease in potency, respectively, over the results for laboratory strains.

Chemistry

As shown in Scheme 1, a series of biphenyl ether tethered macrocycles with various P3 groups were synthesized.[22] The synthesis of advanced intermediates is shown in

Scheme S1–3. The fragment **26** and **27a-b** underwent the Chan-Lam coupling reaction, affording biphenyl ether **28a-b**, which were then subjected to Boc-deprotection and coupling reactions with amino acid **29a-h,** yielding dipeptides **30a-h**. Subsequent acid-mediated Boc-deprotection and Pd/C mediated benzyl deprotection of compounds **30a-h** followed by intramolecular HATU mediated amide coupling reactions yield macrocycles **2, 3, 4, 5, 6, 8, 9,** and **10**.

As shown in Scheme 2–3, several biphenyl ether tethered macrocycles with various P1 groups were synthesized. The $Cu(OAc)_2$ mediated Chan-Lam coupling reaction between phenylboronic acid **32** and phenols **27b**, **33** afforded compounds **34a-b**. TFA-mediated Boc-deprotection of compounds **34a-b** followed by amide coupling reactions of resulting amines and amino acids **35a-b** yielded dipeptides **36a-c**, which were then subjected to sequential removal of benzyl and Cbz groups, and intramolecular amidation affording macrocycles **37a-c**. Macrocycles **17**, **18**, **19**, and **21** with various P1 groups were prepared via a sequence of hydrolysis of tert-butyl ester **37a** and amide coupling reactions with amines. Removal of the tert-butyl group in macrocycle **37b** and a subsequent coupling reaction with amines provided macrocycles **38a-d**, which were subjected to ceric ammonium nitrate (CAN) mediated hydrolysis of cyclic acetal[11] and reductive amination with derivatives of piperidine, yielding macrocycles **11**, **13**, **16**, **20**, and **22.**

As shown in Scheme 4, several biphenyl ether tethered macrocycles with various P5 groups were synthesized. starting from intermediate **37c.** Hydrazinolysis of phthalimide **37c** provided amine **39**, which underwent amidation with 2-chloroethyl chloroformate and subsequent alkylation yielded oxazolidone **40a**. Compound **40b** was prepared from amine **39** via sulfonamidation with MsCl. Sulfonamidation of **39** with 3-chloropropanesulfonyl chloride and subsequent base-mediated cyclization afforded sultam **40c**.[25] Compounds **40a-c** underwent TFA-mediated removal of the tert-butyl groups and followed by an amide coupling reaction, affording compounds **41a-c**, which were subjected to CAN mediated hydrolysis of cyclic acetal and reductive amination with piperidine, yielding macrocycles **12**, **14**, and **15.**

The synthesis of macrocycle **TDI-8414** using the Chan-Lam coupling reaction as a macrocyclization strategy is described in Scheme 5. Dipeptide **44** was synthesized via an amide coupling reaction of amine **42** and acid **43**. Both the Boc and pinacol groups of **44** were removed under Lewis acid $ZnBr_2$, affording compound **45**, which was coupled with acid **46** to give tripeptide **47**. Reductive debenzylation and intramolecular Chan-Lam coupling of tripeptide **47** afforded macrocycle **48**. The tert-butyl group of **48** was removed and the P5 sultam group meanwhile was hydrolyzed with TFA, affording compound **49**, which underwent a subsequent amide coupling reaction and $POCl_3$ mediated intramolecular sulfonamidation, yielding macrocycle **TDI-8414**. The synthesis of macrocycles **23-25** is shown in Scheme S4–6.

Conclusion

In summary, a series of macrocycles containing a biphenyl tether were designed, synthesized, and evaluated as highly potent Pf20S-selective inhibitors. Extensive SAR

studies around the P1, P3, and P5 groups of the macrocycles were conducted to balance potency with other PK properties (Figure 8). Both saturated and unsaturated P3 substituents were allowed. Chain length of the P3 group was important for antiparasitic activity. Replacing the phenyl group with heterocycle groups improved solubility and metabolic stability. The oxidative hydroxylation of the P5 lactam was demonstrated as the major metabolic pathway by mouse microsomes, and replacing the P5 lactam group with a polar sultam substituent improved mouse microsomal stability via decreasing the electron density of the P5 group. The improved mouse microsomal stability of **15** resulted in low liver hepatocyte intrinsic clearance. Wide ranges of primary and secondary amino P1 groups were tolerated. The metabolic profile suggested that the P1 amide was a soft spot in mouse plasma. Increasing the steric bulk of the P1 group prevented the hydrolysis of the P1 amide in mouse plasma and improved mouse plasma stability. Within this series, macrocycle **TDI-8414** demonstrated potent antiparasitic activity, high selectivity over human proteasomes, high PAMPA permeability, high solubility, high plasma stability, high mouse microsomal stability, and low CYP inhibition. Both macrocyclic peptide **TDI-8304** and **TDI-8414** have high efflux ratios, which impose a challenge for improving oral bioavailability. Reducing the number of hydrogen bond donors via N-H methylation as shown in **23** significantly improves the efflux properties providing a clue that shielding a hydrogen bond donor via noncovalent approaches may improve the oral exposure and maintain the potency and selectivity at the same time. Further optimization is under way.

Experimental Section

All purchased reagents and starting materials were used as received unless otherwise noted. All non-aqueous reactions were performed under argon in oven- or flamed-dried glassware. ^{1}H- and ^{19}F- NMR spectra were obtained on a Bruker 400/500 MHz system. Chemical shifts δ values are expressed in parts per million, with the solvent resonance as an internal standard (chloroform-d, ^{1}H: 7.26 ppm; Methanol-d_4, ^{1}H: 3.31 ppm; DMSO-d_6, ^{1}H: 2.50 ppm). NMR data are reported in an order: chemical shift, multiplicity (s: singlet; d: doublet; t: triplet; q: quartet; m: multiplet; br: broad), coupling constant, and integration. Purities of all final compounds were determined on a Waters UPLC/MS and all were > 95%.

Benzyl (S)-2-acetamido-3-(3-(3-((S)-2-((tert-butoxycarbonyl)amino)-3-oxo-3-((2,2,2-trifluoroethyl)amino)propyl)phenoxy)phenyl)propanoate (28a).

To a solution of compound **26** (477.6 mg, 1.2 mmol, 1.2 *eq*) in dichloromethane (6.0 mL) was added 4A molecular sieve (1.5 g), copper acetate (277.9 mg, 1.5 mmol, 1.5 *eq*), triethylamine (1.0 g, 10.2 mmol, 1.4 mL, 10.0 *eq*) and compound **27a** (320.0 mg, 1.0 mmol, 1.0 *eq*). The mixture was stirred at 25°C for 16 hours and then another batch of compound **26** (39.8 mg, 102.0 μmol, 0.1 *eq*) was added. The mixture was stirred at 25°C for 2 hours. LCMS showed desired compound was detected. The mixture was filtered and then filter liquor was concentrated to give crude product. The mixture was purified by reverse phase flash chromatograph (TFA) to afford compound **28a** (360.0 mg, 522.9 μmol, 51.3% yield, 95.5% purity) as a yellow brown solid. LCMS: RT = 0.97 min, m/z = 680.1 $[M+Na]^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.39 – 7.33 (m, 4H), 7.25 – 7.21 (m, 4H), 6.92 (br.s, 1H), 6.79 – 6.76 (m, 3H), 6.78 – 6.26 (m, 3H), 6.26 (d, *J* = 8.0 Hz, 1H), 5.15 (d, *J* = 8.4 Hz, 1H),

5.08 (s, 2H), 5.02–5.01 (m, 1H), 4.52–4.51 (m, 1H), 3.83 – 3.81 (m, 1H), 3.07 – 3.04 (m, 4H), 1.97 (s, 3H), 1.35 (s, 9H).

Benzyl (S)-2-acetamido-3-(3-(3-((S)-2-(2-(((benzyloxy)carbonyl)amino)acetamido)-3-oxo-3-((2,2,2-trifluoroethyl)amino)propyl)phenoxy)phenyl)propanoate (30a).

To a solution of compound **28a** (800.0 mg, 1.2 mmol, 1.0 *eq*) in dioxane (5.0 mL) was added hydrochloric acid/dioxane (4 M, 5.0 mL). The mixture was stirred at 25°C for 4 hours. LCMS showed starting material was consumed completely and desired MS was detected. The mixture was concentrated to give compound 28a-amine (770.0 mg, crude, HCl salt) as a yellow brown solid. LCMS: RT = 0.82 min, m/z = 558.2 $[M+H]^+$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 9.27 (t, *J* = 6.0 Hz, 1H), 8.45 (d, *J* = 8.0 Hz, 1H), 8.33 (br.s, 3H), 7.35 – 7.28 (m, 7H), 7.03 – 6.92 (m, 4H), 6.87 – 6.82 (m, 2H), 5.08 (dd, J1 = 12.8 Hz, J2 =18.4 Hz, 2H), 4.54 – 4.48 (m, 1H), 4.10 – 4.09 (m, 1H), 4.03 – 3.89 (m, 2H), 3.11 – 2.96 (m, 3H), 2.92 – 2.86 (m, 1H), 1.78 (s, 3H). To a solution of Z-glycine (95.4 mg, 456.0 μmol, 0.9 eq) in dimethyl formamide (DMF) (4.0 mL) was added HATU (288.0 mg, 757.6 μmol, 1.5 *eq*) and diisopropylethylamine (DIPEA) (391.6 mg, 3.0 mmol, 529.2 μL, 6.0 eq). The mixture was stirred at 25°C for 0.25 hourr and then 28a-amine (300.0 mg, 505.0 μmol, 1.0 *eq*, HCl) was added. The mixture was stirred at 25°C for 0.25 hour. LCMS showed starting material was consumed completely and desired MS was detected. The mixture was poured into water (10 mL) and then extracted by ethyl acetate (3 × 20 mL). The combined organic phase was washed by saturate sodium carbonate (3 × 20 mL), brine (20 mL) and dried over sodium sulfate. After filtration and concentration, the crude product was purified by reverse phase flash (TFA condition) to afford compound **30a** (160.0 mg, 213.7 μmol, 42.3% yield) as a white solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.71 (t, *J* = 6.4 Hz, 1H), 8.37 (d, *J* = 7.6 Hz, 1H), 8.18 (d, *J* = 7.6 Hz, 1H), 7.37 – 7.26 (m, 13H), 7.00 – 6.96 (m, 2H), 6.92 (d, *J* = 11.6 Hz, 2H), 6.84 (d, *J* = 8.0 Hz, 1H), 6.77 (d, *J* = 8.0 Hz, 1H), 5.10 (dd, J_1 = 12.8 Hz, J_2 = 19.2 Hz, 2H), 5.01 (s, 2H), 4.53 – 4.51 (m 2H), 3.89 – 3.62 (m, 2H), 3.60 – 3.53 (m, 2H), 3.05 – 2.86 (m, 3H), 2.79 – 2.74 (m, 1H), 1.78 (s, 3H).

(S)-2-acetamido-3-(3-(3-((S)-2-(2-aminoacetamido)-3-oxo-3-((2,2,2-trifluoroethyl)amino)propyl)phenoxy)phenyl)propanoic acid (31a).

To a solution of compound **30a** (160.0 mg, 213.7 μmol, 1.0 *eq*) in tetrahydrofuran (THF) (5.0 mL) was added $Pd(OH)_2/C$ (30.1 mg). The mixture was degassed and purged with hydrogen for 3 times, then the mixture was stirred at 25°C for 20 hours under hydrogen atmosphere. LCMS showed starting material and intermediate was consumed completely and desired MS was detected. The mixture was filtered and then filter cake was washed by dichloromethane (10 mL) and methanol (5 mL). The filtrate liquid was concentrated to afford compound **31a** (140.0 mg, crude) as a white solid. LCMS: RT = 0.61 min, purity:27.9%, m/z = 525.1 $[M+H]^+$.

(5S,11S)-11-acetamido-7,10-dioxo-N-(2,2,2-trifluoroethyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide (2).

To a solution of compound **31a** (140.0 mg, 266.9 μmol, 1.0 *eq*) in DMF (6.0 mL) was added DIPEA (69 mg, 533.9 μmol, 93.2 μL, 2.0 *eq*) and HATU (152.2 mg, 400.4 μmol, 1.5 *eq*)

at 0°C under nitrogen. The mixture was stirred at 0°C for 1 hour. LCMS showed starting material was consumed completely. The mixture was poured into water (10 mL) and then extracted by ethyl acetate (3 × 20 mL). The combined organic phase was washed by brine (20 mL) and dried over sodium sulfate. After filtration and concentration, the crude product was recrystallization by methanol (2 × 2 mL) to afford **2** (10.1 mg, 19.6 μmol, 7.4% yield, 98.5% purity) as a white solid. LCMS: RT = 2.67 min, m/z = 507.1 $[M+H]^+$. ^{1}H NMR (DMSO-d6, 400 MHz): δ = 8.84 (t, *J* = 6.4 Hz, 1H), 8.13 (d, *J* = 8.8 Hz, 1H), 7.99 – 7.98 (m, 1H), 7.64 (d, *J* = 6.4 Hz, 1H), 7.29 – 7.25 (m, 2H), 7.11 (d, *J* = 7.6 Hz, 1H), 6.99 – 6.98 (m, 1H), 6.87 (d, *J* = 8.0 Hz, 1H), 6.83 (d, *J* = 8.0 Hz, 1H), 6.76 (s, 1H), 3.65 (s, 1H), 4.70 – 4.68 (m, 1H), 4.65 – 4.61 (m, 1H), 3.97 – 3.89 (m, 3H), 3.45 – 3.42 (m, 1H), 3.07 (d, *J* = 11.2 Hz, 1H), 2.96 (d, *J* = 12.4 Hz, 1H), 2.81 (dd, J_1 = 6.4 Hz, J_2 = 12.8 Hz, 1H), 2.69 – 2.63 (m, 1H), 1.89 (s, 3H).

Benzyl (S)-2-acetamido-3-(3-(3-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methoxypropanamido)-3-oxo-3-((2,2,2-trifluoroethyl)amino)propyl)phenoxy)phenyl)propanoate (30b).

To a solution of Boc-O-methyl-L-serine (150.0 mg, 228.1 μmol, 1.0 *eq*) in DMF (3.0 mL) was added DIPEA (88.4 mg, 684.2 μmol, 3.0 *eq*) and HATU (130.1 mg, 342.1 μmol, 1.5 *eq*) in turn at 0°C. Then compound **28a-amine** (150.0 mg, 228.1 μmol, 1.0 *eq*) was added into the mixture and the reaction was stirred at 0°C for 4 hours. LCMS showed starting material was consumed completely and desired compound MS was detected. The reaction mixture was added water (10 mL), and extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine (3 × 20 mL), dried with anhydrous sodium sulfate. After filtration and concentration, 300.0 mg of crude compound **30b** was obtained as yellow oil. LCMS: RT = 0.99 min, purity: 54.7%, *m/z* = 759.3 $[MS+H]^+$.

(S)-2-acetamido-3-(3-(3-((S)-2-((S)-2-amino-3-methoxypropanamido)-3-oxo-3-((2,2,2-trifluoroethyl)amino)propyl)phenoxy)phenyl)propanoic acid (31b).

To a solution of compound **30b** (300.0 mg, 395.4 μmol, 1.0 *eq*) in dioxane (3 mL) was added HCl/dioxane (4 M, 5.0 mL, 50.6 *eq*). The reaction mixture was stirred at 26°C for 2.5 hours. LCMS showed starting material was consumed completely and desired compound MS was detected. The mixture was concentrated in vacuum to afford compound **30b-amine** (140.0 mg, crude, HCl salt) as yellow oil. LCMS: RT = 0.72 min, purity: 82.1%, *m/z* = 659.2 $[MS+H]^+$. To a solution of **30b-amine** (140.0 mg, 212.6 μmol, 1.0 *eq*) in THF (3 mL) was added into Palladium hydroxide (14.9 mg, 0.5 *eq*). Then the mixture was degassed under vacuum and purged hydrogen for 3 times and the reaction was stirred at 26°C for 3 hours. LCMS showed starting material was consumed completely and desired compound MS was detected, the reaction mixture was filtered, and the filtrate was concentrated in vacuum to afford compound **31b** (110.0 mg, crude) as yellow oil. LCMS: RT = 0.72 min, purity: 82.1%, *m/z* = 569.2 $[MS+H]^+$.

(5S,8S,11S)-11-acetamido-8-(methoxymethyl)-7,10-dioxo-N-(2,2,2-trifluoroethyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide (3).

To a solution of compound **31b** (110.0 mg, crude) in DMF (3.0 mL) was added DIPEA (75.0 mg, 580.4 μmol, 3.0 *eq*) and HATU (73.6 mg, 193.5 μmol, 1.0 *eq*) at 0°C. The reaction was stirred at 0°C for 2.5 hours, then HATU (36.8 mg, 96.7 μmol, 0.5 *eq*) was added into the mixture and the reaction was stirred at 0°C for 4 hours. LCMS showed starting material was consumed completely and desired compound MS was detected. The reaction mixture was added water (10 mL) and extracted with ethyl acetate (3 × 15 mL). The combined organic layers were washed with brine (3 × 20 mL) and dried with anhydrous sodium sulfate. After filtration and concentration, the residue was triturated with methanol (1 mL) to afford **3** (31.0 mg, 27.7% yield) as an off-white solid. LCMS: RT = 2.92 min, purity: 95%, *m/z* = 551.2 $[MS+H]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ = 8.54 – 8.50 (m, 2H), 8.07 (d, *J* = 8.4 Hz, 1H), 7.53 (d, *J* = 7.2 Hz, 1H), 7.33 – 7.24 (m, 2H), 7.14 (d, *J* = 8.0 Hz, 1H), 6.97 (dd, J_1 = 1.6 Hz, J_2 = 6.4 Hz, 1H), 6.85 (d, *J* = 7.2 Hz, 2H), 6.80 (d, *J* = 7.2 Hz, 1H), 6.09 (s, 1H), 4.67 – 4.61 (m, 2H), 4.56 – 4.51 (m, 1H), 4.03 – 3.95 (m, 2H), 3.20 (s, 3H), 3.12 (d, *J* = 12.0 Hz, 1H), 2.85 – 2.80 (m, 1H), 2.74 – 2.66 (m, 2H), 2.52 (d, *J* = 1.6 Hz, 2H), 1.88 (s, 3H).

Benzyl (S)-2-acetamido-3-(3-(3-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-5,5,5-trifluoropentanamido)-3-oxo-3-((2,2,2-trifluoroethyl)amino)propyl)phenoxy)phenyl)propanoate (30c).

To a solution of Boc-L-trifluoronorvaline in DMF (5.0 mL) was added HATU (211.2 mg, 555.5 μmol, 1.5 *eq*) and DIPEA (239.3 mg, 1.9 mmol, 323.4 μL, 5.0 *eq*) at 0°C. The mixture was stirred at 20°C for 30 minutes and then compound **28a-amine** (220.0 mg, 370.4 μmol, 1.0 *eq*, HCl) was added into the mixture and stirred at 0°C for 1.5 hours. LCMS showed the starting material was consumed completely and desired product Ms was detected. The mixture was poured into water (20 mL) and extracted with ethyl acetate (3 × 20 mL). The combined organic phase was washed with brine (3 × 30 mL) and dried over sodium sulfate. After filtration and concentration, the crude product was purified with silica gel column (petroleum ether: ethyl acetate=10: 1~2: 1) to provide compound **30c** (250.0 mg, 296.8 μmol, 80.1% yield, 96.3% purity) as a yellow solid. LCMS: RT = 0.98 min, *m/z* = 811.1 $[MS+H]^+$. ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.34 – 7.32 (m, 3H), 7.27 – 7.23 (m, 5H), 6.93 (td, J_1 = 2.0 Hz, J_2 = 9.6 Hz, 2H), 6.87 – 6.78 (m, 3H), 6.70 (m, 2H), 6.20 (br. s, 1H), 5.10 (s, 2H), 5.03 – 5.01 (m, 2H), 4.84 – 4.81 (m, 1H), 3.99 – 3.97 (m, 1H), 3.87 – 3.82 (m, 2H), 3.18 (dd, J_1 = 5.6 Hz, J_2 = 13.6 Hz, 1H), 3.07 (dd, J_1 = 5.2 Hz, J_2 = 14.4 Hz, 1H), 3.00 – 3.29 (m, 2H), 2.05 – 2.02 (m, 2H), 1.93 (s, 3H), 1.94 – 1.85 (m, 1H), 1.76 – 1.68 (m, 1H), 1.42 (s, 9H).

(S)-2-acetamido-3-(3-(3-((S)-2-((S)-2-amino-5,5,5-trifluoropentanamido)-3-oxo-3-((2,2,2-trifluoroethyl)amino)propyl)phenoxy)phenyl)propanoic acid (31c).

To a solution of compound **30c** (250.0 mg, 308.4 μmol, 1.0 *eq*) in dioxane (10 mL) was added hydrochloric acid/dioxane (4 M, 10.0 mL, 129.7 *eq*). The mixture was stirred at 20°C for 1 hour. LCMS showed the starting material was consumed completely. The mixture was concentrated under vacuum to provide compound **30c-amine** (220.0 mg, crude) as a white solid. LCMS: RT = 1.45 min, *m/z* = 711.3 $[MS+H]^+$. To a solution of compound **30c-amine**

(230.0 mg, 323.6 μmol, 1.0 *eq*) in THF (10.0 mL) was added $Pd(OH)_2/C$ (50.0 mg). The mixture was degassed under vacuum and purged hydrogen for 3 times. The mixture was stirred at 20°C for 2 hours under hydrogen balloon. LCMS showed the starting material was consumed completely. The mixture was filtrated out. The filtrate liquid was concentrated under vacuum to provide compound **31c** (180.0 mg, 290.1 μmol, 89.6% yield) as a white solid. ^{1}H NMR (MeOD, 400 MHz): δ = 7.35 – 7.26 (m, 2H), 7.03 – 6.87 (m, 6H), 4.74 – 4.70 (m, 1H), 4.65 – 4.62 (m, 1H), 3.95 – 3.83 (m, 3H), 3.69 – 3.69 (m, 1H), 3.58 – 3.56 (m, 1H), 3.20 – 3.09 (m, 2H), 2.95 – 2.86 (m, 2H), 2.31 – 2.21 (m, 1H), 2.11 – 2.06 (m, 1H), 1.90 (s, 3H).

(5S,8S,11S)-11-acetamido-7,10-dioxo-N-(2,2,2-trifluoroethyl)-8-(3,3,3-trifluoropropyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide (4).

To a solution of compound **31c** (120.0 mg, 193.4 μmol, 1.0 *eq*) in DMF (2.0 mL) was added DIPEA (75.0 mg, 580.1 μmol, 101.3 μL, 3.0 *eq*) and HATU (110.3 mg, 290.1 μmol, 1.5 *eq*) in turn at 0 °C. The reaction was stirred at 0°C for 1 hour. LCMS showed starting material was consumed completely and desired compound MS was detected. The reaction mixture was quenched by water (10 mL) and extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine (3 × 20 mL), dried with anhydrous sodium sulfate. After filtration and concentration, the residue was triturated with methanol (1 mL) to provide **4** (10.0 mg, 8.5% yield) as a white solid. LCMS: RT = 3.26 min, purity: 98.6%, *m/z* = 603.2 $[MS+H]^+$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.85 (t, *J* = 6.4 Hz, 1H), 8.50 (d, *J* = 8.8 Hz, 1H), 8.05 (d, *J* = 8.8 Hz, 1H), 7.66 (d, *J* = 6.8 Hz, 1H), 7.34 – 7.26 (m, 2H), 7.12 (d, *J* = 7.6 Hz, 1H), 6.98 (dd, J_1 = 1.6 Hz, J_2 = 8.0 Hz, 1H), 6.88 – 6.86 (m, 2H), 6.75 (s, 1H), 6.16 (s, 1H), 4.66 – 4.56 (m, 2H), 4.45 – 4.39 (m, 1H), 3.99 – 3.94 (m, 2H), 3.05 (d, *J* = 12.4 Hz, 1H), 2.83 – 2.71 (m, 3H), 2.17 – 2.09 (m, 2H), 1.87 (s, 3H), 1.71 – 1.54 (m, 2H).

Benzyl (S)-2-acetamido-3-(3-(3-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)pentanamido)-3-oxo-3-((2,2,2-trifluoroethyl)amino)propyl)phenoxy)phenyl)propanoate (30d).

To a solution of Boc-L-norvaline (73.2 mg, 336.7 μmol, 1.0 *eq*) in DMF (3.0 mL) was added N,N-DIPEA (130.5 mg, 1.0 mmol, 176.4 μL, 3.0 *eq*), EDCI (96.8 mg, 505.0 μmol, 1.5 *eq*) and HOBT (68.2 mg, 505.0 μmol, 1.5 *eq*) at 0°C, then compound **28a-amine** (200.0 mg, 336.7 μmol, 1.0 *eq*, HCl) was added into the mixture and the reaction was stirred at 26 °C for 6 hours. LCMS showed starting material was consumed completely and desired compound MS was detected. To the reaction mixture was added water (5 mL). The mixture was acidified by HCl (1N) until pH= 4 and extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine (3 × 20 mL), dried over anhydrous sodium sulfate. After filtration and concentration, compound **30d** (240.0 mg, crude) was obtained as yellow oil. LCMS: RT = 1.05 min, purity: 86.5%, m/z = 757.3 $[MS+H]^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.84 (dd, J_1 = 8.4 Hz, J_2 = 28.0 Hz, 1H), 7.47 – 7.43 (m, 1H), 7.34 – 7.30 (m, 3H), 7.25 – 7.20 (m, 4H), 6.91 – 6.87 (m, 3H), 6.79 (d, *J* = 7.6 Hz, 1H), 6.75 (br.s, 1H), 6.70 (br.s, 1H), 6.60 (d, *J* = 8.0 Hz, 1H), 6.27 (br.s, 1H), 5.09 (s, 2H), 4.98 (dd, J_1 = 7.2 Hz, J_2 = 23.2 Hz, 2H), 4.81 – 4.75 (m, 1H), 3.90 – 3.81 (m, 2H), 3.18 – 3.01 (m, 4H), 2.05 (d, *J* = 2.0 Hz, 3H), 1.66 – 1.61 (m, 2H), 1.39 (s, 9H), 1.26 (d, *J* = 1.2 Hz, 2H), 0.97 (t, *J* = 7.2 Hz, 3H).

(S)-2-acetamido-3-(3-(3-((S)-2-((S)-2-aminopentanamido)-3-oxo-3-((2,2,2-trifluoroethyl)amino)propyl)phenoxy)phenyl)propanoic acid (31d).

To a solution of **30d** (240.0 mg, 317.1 μmol, 1.0 *eq*) in dioxane (3.0 mL) was added HCl/dioxane (4 M, 4.0 mL, 50.5 *eq*), the reaction was stirred at 26°C for 1 hour. LCMS showed starting material was consumed completely and desired compound MS was detected. The mixture was concentrated in vacuum to give the compound **30d-amine** (270.0 mg, 270.9 μmol, 85.4% yield, 69.5% purity, HCl) as yellow oil. LCMS: RT = 0.78 min, *m/z* = 657.2 $[MS+H]^+$. To a solution of **30d-amine** (270.0 mg, 411.2 μmol, 1.0 *eq*) in THF (3.0 mL) was added $Pd(OH)_2/C$ (28.9 mg, 205.6 μmol, 0.5 *eq*) under hydrogen balloon (15 psi). Then the mixture was degassed under vacuum and purged hydrogen for 3 times and the reaction was stirred at 26°C for 1.5 hours. LCMS showed starting material was consumed completely and desired compound MS was detected. The reaction mixture was filtered and the filtrate was concentrated in vacuum to give the compound **31d** (110.0 mg, crude) as yellow oil. LCMS: RT = 0.77 min, purity: 82.5%, *m/z* = 567.2 $[MS+H]^+$.

(5S,8S,11S)-11-acetamido-7,10-dioxo-8-propyl-N-(2,2,2-trifluoroethyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide (5).

To a solution of **31d** (110.0 mg, 194.2 μmol, 1.0 *eq*) in DMF (2.0 mL) was added DIPEA (75.3 mg, 582.5 μmol, 101.7 μL, 3.0 *eq*) and HATU (110.7 mg, 291.2 μmol, 1.5 *eq*) at 0°C, the reaction was stirred at 0°C for 2.5 hours. LCMS showed starting material was consumed completely and desired compound MS was detected. The reaction mixture was added water (10 mL) and extracted with ethyl acetate (3 × 15 mL). The combined organic layers were washed with brine (3 × 20 mL), dried with anhydrous sodium sulfate, filtered and concentrated in vacuum. The residue was triturated with methanol (1 mL), filtered and the cake was collectedto give **5** (21.0 mg, 18.7% yield) as an off-white solid. LCMS: RT = 1.95 min, purity: 95.0%, *m/z* = 549.2 $[MS+H]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ = 8.81 (t, *J* = 6.0 Hz, 1H), 8.39 (d, *J* = 8.8 Hz, 1H), 7.94 (d, *J* = 8.8 Hz, 1H), 7.55 (d, *J* = 7.2 Hz, 1H), 7.33 – 7.21 (m, 2H), 7.14 (d, *J* = 7.2 Hz, 1H), 6.97 (d, *J* = 9.2 Hz, 1H), 6.86 – 6.75 (m, 3H), 6.10 (s, 1H), 4.67 – 4.58 (m, 2H), 4.37 – 4.31 (m, 1H), 3.97 – 3.90 (m, 2H), 3.00 (d, *J* = 13.2 Hz, 1H), 2.86 – 2.67 (m, 2H), 1.88 (s, 2H), 1.45 – 1.41 (m, 1H), 1.36 – 1.30 (m, 1H), 1.21 – 1.14 (m, 3H), 0.80 (t, *J* = 7.2 Hz, 4H).

Benzyl (S)-2-acetamido-3-(3-(3-((S)-2-((S)-2-(((benzyloxy)carbonyl)amino)-4-methylpentanamido)-3-oxo-3-((2,2,2-trifluoroethyl)amino)propyl)phenoxy)phenyl)propanoate (30e).

To a solution of Z-L-leucine (45.2 mg, 170.3 μmol, 1.1 eq) in DMF (3.0 mL) was added DIPEA (50.0 mg, 387.1 μmol, 67.6 μL, 2.5 eq), HOBt (27.2 mg, 201.3 μmol, 1.3 eq) and EDCI (38.6 mg, 201.3 μmol, 1.3 eq) at 0°C under nitrogen and then compound **28a-amine** (100.0 mg, 154.8 μmol, 1.0 eq) was added. The mixture was stirred at 25°C for 16 hours. LCMS showed starting material was consumed completely and desired MS was detected. TLC (dichloromethane: methanol = 10:1) indicated starting material was consumed completely and one new spot formed. The mixture was poured into water (10 mL) and then extracted by ethyl acetate (3 × 10 mL). The combined organic phase was washed by brine (10 mL) and dried over sodium sulfate. After filtration and concentration, the crude

product was purified by reverse phase flash (TFA condition) to give compound **30e** (70.0 mg, 78.6 μmol, 50.8% yield, 90.4% purity) as a yellow solid. LCMS: RT = 0.96 min, m/z = 805.2 $[M+H]^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.34 – 7.32 (m, 9H), 7.24 – 7.19 (m, 4H), 6.88– 6.86 (m, 3H), 6.77 – 6.74 (m, 2H), 6.67 – 6.62 (m, 2H), 6.24 (d, *J* = 6.4 Hz, 1H), 5.32 (d, *J* = 6.0 Hz, 1H), 5.10 (m, 3H), 4.99 (d, *J* = 11.6 Hz, 2H), 4.79 – 4.76 (m, 1H), 4.04 – 4.02 (m, 1H), 3.79 – 3.77 (m, 2H), 3.16– 3.11 (m, 2H), 3.02 – 2.97 (m, 2H), 1.91 (s, 3H), 1.48– 1.34 (m, 3H), 0.85 – 0.71 (m, 6H).

(S)-2-acetamido-3-(3-(3-((S)-2-((S)-2-amino-4-methylpentanamido)-3-oxo-3-((2,2,2-trifluoroethyl)amino)propyl)phenoxy)phenyl)propanoic acid (31e).

To a solution of compound **30e** (300.0 mg, 372.7 μmol, 1.0 *eq*) in THF (4.0 mL) was added Pd/C (90.0 mg, 10% purity). The mixture was degassed and purged with hydrogen for 3 times, and then the mixture was stirred at 25°C for 23 hours under hydrogen balloon. LCMS showed starting material was consumed completely and desired MS was detected. To the mixture was added dichloromethane (10 mL) and methanol (5 mL). The mixture was filtered and then the filter liquor was concentrated to give crude product. The crude product was triturated by acetonitrile (5 mL) to give compound **31e** (100.0 mg, 172.2 μmol, 46.2% yield, 100% purity) as an off-white solid. LCMS: RT = 0.69 min, m/z = 581.3 $[M+H]^+$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 9.78 (br.s, 1H), 8.91 (t, *J* = 5.6 Hz, 1H), 7.29 (t, *J* = 8.0 Hz, 1H), 7.17 (d, *J* = 6.4 Hz, 1H), 7.03 (d, *J* = 7.6 Hz, 1H), 6.99 – 6.96 (m, 1H), 6.90 – 6.86 (m, 4H), 6.80 (d, *J* = 7.6 Hz, 1H), 4.73 – 4.66 (m, 1H), 4.18 – 4.15 (m, 1H), 4.04 – 3.82 (m, 4H), 3.70 (t, *J* = 7.2 Hz, 1H), 3.02 – 3.01 (m, 2H), 2.93 – 2.87 (m, 1H), 1.82 (s, 3H), 1.51 – 1.45 (m, 1H), 1.33 (t, *J* = 7.2 Hz, 2H), 0.81 (t, *J* = 7.6 Hz, 6H).

(5S,8S,11S)-11-acetamido-8-isobutyl-7,10-dioxo-N-(2,2,2-trifluoroethyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide (6).

To a solution of compound **31e** (97.0 mg, 167.1 μmol, 1.0 *eq*) in DMF (7.0 mL) was added DIPEA (43.2 mg, 334.1 μmol, 58.4 μL, 2.0 *eq*) and HATU (95.3 mg, 250.6 μmol, 1.5 *eq*) at 0°C under nitrogen. The mixture was stirred at 0°C for 5 hours. LCMS showed starting material was consumed completely. The mixture was poured into water (10 mL) and then extracted by ethyl acetate (3 × 10 mL). The combined organic phase was washed with brine (20 mL), dried over sodium sulfate. After filtration and concentration, the crude product was recrystallized by acetonitrile (4 mL) to afford **6** (20.1 mg, 35.6 μmol, 21.3% yield, 99.7% purity) as a white solid. LCMS: RT = 3.04 min, m/z = 563.2 $[M+H]^+$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.78 (t, *J* = 6.0 Hz, 1H), 8.41 (d, *J* = 9.2 Hz, 1H), 7.93 (d, *J* = 8.8 Hz, 1H), 7.59 (d, *J* = 7.2 Hz, 1H), 7.32 – 7.26 (m, 2H), 7.14 (d, *J* = 7.6 Hz, 1H), 6.98 (d, *J* = 8.4 Hz, 1H), 6.87 (d, *J* = 8.0 Hz, 1H), 6.83 – 6.81 (m, 2H), 6.15 (s, 1H), 4.65 – 4.63 (m, 1H), 4.57 – 4.54 (m, 1H), 4.41 – 4.39 (m, 1H), 3.99 – 3.92 (m, 2H), 2.99 (d, *J* = 12.4 Hz, 1H), 2.87 – 2.80 (m, 2H), 2.70 – 2.67 (m, 1H), 1.87 (s, 3H), 1.46 – 1.42 (m, 1H), 1.32 – 1.25 (m, 2H), 0.83 (t, *J* = 6.8 Hz, 6H).

benzyl (S)-3-(3-(3-((S)-2-((tert-butoxycarbonyl)amino)-3-oxo-3-((2,2,2-trifluoroethyl)amino)propyl)phenoxy)phenyl)-2-(2-oxopyrrolidin-1-yl)propanoate (28b).

To a solution of compound **27b** (310.0 mg, 785.5 μmol, 1.0 *eq*) in dichloromethane (5.0 mL) was added compound **1** (674.3 mg, 1.7 mmol, 2.2 *eq*), copper acetate (214.0 mg, 1.2 mmol, 1.5 *eq*), triethylamine (794.9 mg, 7.9 mmol, 1.1 mL, 10.0 *eq*) and 4A molecular sieve (400.0 mg). The mixture was stirred at 25°C in the air for 3 hours. LCMS showed starting material was consumed completely and desired MS was detected. The mixture was filtered and then the filter was concentrated to give crude product. The crude product was purified by silica gel column (petroleum ether: ethyl acetate=3:1 to 3:1) to afford compound **28b** (510.0 mg, 677.8 μmol, 86.3% yield, 90.9% purity) as a yellow solid. LCMS: RT = 0.96 min, m/z = 684.2 $[M+H]^+$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.59 – 8.56 (m, 1H), 7.38 – 7.32 (m, 4H), 7.29 – 7.24 (m, 2H), 7.05 – 6.98 (m, 5H), 6.89 (s, 1H), 6.85 – 6.83 (m, 1H), 6.78 – 6.75 (m, 1H), 5.18 (dd, J_1 = 12.8 Hz, J_2 = 16.0 Hz, 2H), 4.97 (dd, J_1 = 5.2 Hz, J_2 = 10.8 Hz, 1H), 3.39 – 3.35 (m, 2H), 3.24 – 3.15 (m, 2H), 3.06 (dd, J_1 = 11.2, J_2 = 14.4 Hz, 1H), 2.93 – 2.89 (m, 2H), 2.80 – 2.71 (m, 2H), 2.20 – 2.02 (m, 2H), 1.85 – 1.69 (m, 2H), 1.31 (d, J = 6.8 Hz, 9H).

Benzyl (S)-3-(3-(3-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-phenylpropanamido)-3-oxo-3-((2,2,2-trifluoroethyl)amino)propyl)phenoxy)phenyl)-2-(2-oxopyrrolidin-1-yl)propanoate (30f).

To a solution of compound **28b** (320.0 mg, 468.0 μmol, 1.0 *eq*) in dioxane (5.0 mL) was added hydrochloric acid/dioxane (4 M, 10.0 mL). The mixture was stirred at 25°C for 1.5 hours. TLC (petroleum ether: ethyl acetate=1:1) indicated starting material was consumed completely and one new spot formed. The mixture was concentrated to afford compound **28b-amine** (305.0 mg, 404.8 μmol, 86.5% yield, 82.3% purity, HCl salt) as a yellow solid. LCMS: RT = 0.78 min, purity:82.3%, m/z 584.3$[M+H]^+$. ^{1}H NMR: (DMSO-d_6, 400 MHz) δ = 9.22 – 9.19 (m, 1H), 7.40 – 7.26 (m, 8H), 7.02 (d, J = 6.4 Hz, 3H), 6.93 – 6.82 (m, 4H), 5.18 (dd, J_1 = 12.8 Hz, J_2 = 16.0 Hz, 2H), 4.97 (dd, J_1 = 5.2 Hz, J_2 = 10.8 Hz, 1H), 4.47 (br.s, 1H), 4.11 – 4.07 (m, 1H), 3.98 – 3.96 (m, 1H), 3.24 – 2.94 (m, 6H), 2.19 – 2.09 (m, 1H), 2.05 – 2.00 (m, 1H), 1.88 – 1.73 (m, 2H). To a solution of Boc-L-phenylalanine (51.8 mg, 195.1 μmol, 1.1 *eq*) in DMF (2.0 mL) was added DIPEA (68.8 mg, 532.2 μmol, 93.0 μL, 3.0 *eq*), HATU (101.2 mg, 266.1 μmol, 1.5 *eq*) at 0°C, then compound **28b-amine** (110.0 mg, 177.4 μmol, 1.0 *eq*, HCl) was added into the mixture and the reaction was stirred at 26°C for 1.5 hours. LCMS showed starting material was consumed completely and desired compound MS was detected. The reaction mixture was quenched by water (3 mL), acidified by HCl (1N, 4 mL) and extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine (3 × 20 mL) and dried with anhydrous sodium sulfate. After filtration and concentration, the residue was purified by silica gel column chromatography (Petroleum ether: Ethyl acetate=20:1 to 3:1) to provide compound **30f** (140.0 mg, 128.3 μmol, 72.3% yield, 76.2% purity) as yellow oil. LCMS: RT = 1.03 min, m/z = 831.1$[M+H]^+$.

(S)-3-(3-(3-((S)-2-((S)-2-amino-3-phenylpropanamido)-3-oxo-3-((2,2,2-trifluoroethyl)amino)propyl)phenoxy)phenyl)-2-(2-oxopyrrolidin-1-yl)propanoic acid (31f).

To a solution of compound **30f** (140.0 mg, 168.5 μmol, 1.0 *eq*) in dioxane (2.0 mL) was added HCl/dioxane (4M, 5.0 mL, 118.7 *eq*). The reaction was stirred at 26°C for 2 hours. LCMS showed starting material was consumed completely and desired compound MS was detected, the residue was concentrated in vacuum to give compound **30f-amine** (128.0 mg, crude, HCl) as yellow oil. LCMS: RT = 0.86 min, purity: 82.2%, *m/z* 731.2 $[MS+H]^+$. To a solution of compound **30f-amine** (60.0 mg, 78.2 μmol, 1.0 eq, HCl salt) in THF (5.0 mL) was added $Pd(OH)_2$ (15.0 mg, 10% purity) under hydrogen (15 psi, balloon). The mixture was degassed under vacuum and purged hydrogen for 3 times and the suspension was stirred at 26°C for 1 hour. LCMS showed starting material was consumed completely and desired compound MS was detected. The reaction mixture was filtered and the filtrate was concentrated in vacuum to give crude compound **31f** (50.0 mg, 77.9% yield) as yellow oil. LCMS: RT = 0.73 min, purity: 78.0%, *m/z* = 641.2 $[MS+H]^+$.

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

To a solution of compound **31f** (50.0 mg, 73.8 μmol, 1.0 eq, HCl salt) in DMF (3.0 mL) was added DIPEA (30.3 mg, 234.1 μmol, 40.9 μL, 3.2 eq) and HATU (44.5 mg, 117.1 μmol, 1.6 eq) in turn. The reaction was stirred at 0°C for 0.5 hour. LCMS showed starting material was consumed completely and desired MS was detected. The reaction mixture was quenched by water (10 mL), acidified by HCl (1 N, 4 mL) and extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine (3 × 20 mL), dried with anhydrous sodium sulfate. After filtration and concentration, the residue was purified by prep-HPLC (column: Phenomenex Synergi C18 150 × 25 × 10 μm; mobile phase: [water(0.1%TFA)-ACN];B%: 42%–69%,12min) to give **8** (6.0 mg, 11.9% yield) as a white solid. LCMS: RT = 3.01 min, *m/z* = 623.2 $[MS+H]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ = 8.66 −8.63 (m, 1H), 8.36 (d, *J* = 8.0 Hz, 1H), 7.97 (d, *J* = 9.2 Hz, 1H), 7.31 (t, *J* = 8.0 Hz, 2H), 7.21 – 7.14 (m, 5H), 6.98 – 6.92 (m, 4H), 6.53 (s, 1H), 6.44 (s, 1H), 4.79 – 4.75 (m, 1H), 4.64 – 4.58 (m, 1H), 4.44 (dd, J_1 = 2.0 Hz, J_2 = 11.6 Hz, 1H), 4.02 – 3.89 (m, 2H), 3.04 – 3.02 (m, 2H), 2.94 – 2.86 (m, 1H), 2.79 – 2.75 (m, 1H), 2.63 – 2.57 (m, 2H), 2.41 (d, *J* = 10.4 Hz, 1H), 2.28 – 2.25 (m, 1H), 2.17 – 2.06 (m, 2H), 1.72 – 1.69 (m, 1H), 1.59 – 1.56 (m, 1H).

benzyl (S)-3-(3-(3-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-4-(piperidin-1-yl)butanamido)-3-oxo-3-((2,2,2-trifluoroethyl)amino)propyl)phenoxy)phenyl)-2-(2-oxopyrrolidin-1-yl)propanoate (30g).

To a solution of compound **29g** (95.0 mg, 331.7 μmol, 1.0 *eq*) in DMF (3.0 mL) was added HOBT (58.3 mg, 431.3 μmol, 1.3 *eq*), EDCI(82.7 mg, 431.3 μmol, 1.3 *eq*) and DIPEA (107.2 mg, 829.4 μmol, 144.5 μL, 2.5 *eq*) at 0°C under nitrogen and then compound **28b-amine** (300.0 mg, 483.8 μmol, 1.5 *eq*, HCl salt) was added. The mixture was stirred at 20°C for 16 hours. LCMS showed starting material was consumed completely and desired MS was detected. The mixture was poured into water (20 mL) and then added 1 N HCl (4 mL). The mixture was extracted by ethyl acetate (3 × 20 mL). The combined organic

phase was washed by saturate sodium carbonate (3 × 20 mL), brine (20 mL), and dried over sodium sulfate. After filtration and concentration, the crude product was purified by prep-HPLC (column: Phenomenex Synergi 10 μm C18 150 × 25 mm; mobile phase: [water(0.1%TFA)-ACN]; B%: 35%−65%,13min) to afford compound **30g** (120.0 mg, 124.8 μmol, 37.6% yield, 88.6% purity) as yellow oil. LCMS: RT = 0.833 min, m/z = 852.4 $[M+H]^+$. ^{1}H NMR (MeOD, 400 MHz): δ = 7.35 – 7.26 (m, 7H), 7.00 – 6.84 (m, 6H), 5.17 (d, *J* = 2.8 Hz, 2H), 5.08 – 5.06 (m, 1H), 4.75 – 4.73 (m, 1H), 4.15 – 4.05 (m, 1H), 3.89 – 3.87 (m, 2H), 3.48 – 3.45 (m, 3H), 3.36 – 3.34 (m, 1H), 3.09 – 2.85 (m, 7H), 2.26 – 2.16 (m, 2H), 2.01 – 1.69 (m ,10H), 1.43 (m, 10H).

(S)-3-(3-(3-((S)-2-((S)-2-amino-4-(piperidin-1-yl)butanamido)-3-oxo-3-((2,2,2-trifluoroethyl)amino)propyl)phenoxy)phenyl)-2-(2-oxopyrrolidin-1-yl)propanoic acid (31g).

A solution of compound **30g** in THF (4.0 mL) was added $Pd(OH)_2$ (40.0 mg). The suspension was degassed under vacuum and purged hydrogen for 3 times. The resulting mixture was stirred at 20°C for 1 hour under hrdrogen balloon. LCMS showed starting material was consumed completely and desired MS was detected. The mixture was filtered and the filtrated liquid was concentrated to afford compound **30g-acid** (95.0 mg, crude) as a white solid. LCMS: RT = 0.77 min, purity: 96.3%, m/z = 762.2 $[M+H]^+$. ^{1}H NMR (MeOD, 400 MHz): δ = 7.30 – 7.24 (m, 2H), 7.99 – 6.85 (m, 6H), 4.87 – 4.76 (m ,2H), 4.07 – 3.90 (m, 3H), 3.44 – 3.31 (m, 3H), 3.13 – 2.91 (m, 8H), 2.28 – 2.21 (m, 2H), 2.03 – 1.83 (m, 8H), 1.69 – 1.55 (m, 2H), 1.42 – 1.29 (m, 10H). Compound **30g-acid** (130.0 mg, 170.6 μmol, 1.0 *eq*) in dioxane (5.0 mL) was added HCl/dioxane (4 M, 10.0 mL). The mixture was stirred at 20°C for 40 minutes. LCMS showed starting material was consumed completely. The mixture was concentrated under vacuum to afford compound **31g** (115.0 mg, crude, HCl) as a light yellow solid.

(5S,8S,11S)-7,10-dioxo-11-(2-oxopyrrolidin-1-yl)-8-(2-(piperidin-1-yl)ethyl)-N-(2,2,2-trifluoroethyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide (9).

To a solution of compound **31g** (110.0 mg, 157.6 μmol, 1.0 *eq*, HCl) in DMF (10.0 mL) was added DIPEA (50.9 mg, 393.9 μmol, 68.6 μL, 2.5 *eq*), HOBt (29.8 mg, 220.6 μmol, 1.4 *eq*) and EDCI (42.3 mg, 220.6 μmol, 1.4 *eq*) at 0°C under nitrogen and the result mixture was stirred at 20°C for 16 hours. LCMS showed starting material was consumed completely and desired MS was detected. The mixture was poured into water (10 mL) and then extracted by ethyl acetate (3 × 20 mL). The combined organic phase was washed by brine (20 mL) and dried over sodium sulfate. After filtration and concentration, the crude product was purified by prep-HPLC (column: Phenomenex Gemini 10 μm C18 150 × 25mm;mobile phase: [water (0.05% ammonia hydroxide v/v)-ACN]; B%: 35%−65%,12min) to afford **9** (25.8 mg, 38.1 μmol, 24.2% yield, 95.1% purity) as a white solid. LCMS: RT = 2.62 min, m/z = 644.3$[M+H]^+$. ^{1}H NMR (MeOD, 400 MHz): δ = 7.34 – 7.26 (m, 2H), 7.09 (d, *J* = 8.4 Hz, 1H), 6.98 – 6.91 (m, 3H), 6.65 (s, 1H), 6.50 (s, 1H), 4.77 – 4.68 (m, 2H), 4.34 (t, *J* = 7.2 Hz, 1H), 4.04 – 3.82 (m, 2H), 3.64 – 3.52 (m, 2H), 3.29 – 3.22 (m, 1H), 3.14 – 3.09 (m, 1H), 3.01 (dd, J_1 = 9.2 Hz, J_2 = 15.2 Hz, 1H), 2.77 (dd, J_1 = 3.2 Hz, J_2 =12.4 Hz, 1H), 2.48 – 2.31 (m, 6H), 2.29 (t, *J* = 7.2 Hz, 2H), 2.09 – 2.01 (m, 2H), 1.85 – 1.68 (m, 2H), 1.57 – 1.55 (m, 4H), 1.49 – 1.38 (m, 2H).

Benzyl (S)-3-(3-(3-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-4-(4,4-difluoropiperidin-1-yl)butanamido)-3-oxo-3-((2,2,2-trifluoroethyl)amino)propyl)phenoxy)phenyl)-2-(2-oxopyrrolidin-1-yl)propanoate (30h).

To a solution of compound **29h** (155.7 mg, 483.1 μmol, 1.2 *eq*) in DMF (5.0 mL) was added HOBt (81.6 mg, 603.8 μmol, 1.5 *eq*), EDCI (115.8 mg, 603.8 μmol, 1.5 *eq*) and DIPEA (260.1 mg, 2.0 mmol, 350.6 μL, 5.0 *eq*) at 0°C. Then compound **28b-amine** (250.0 mg, 402.6 μmol, 1.0 *eq*, HCl) was added into the mixture and the mixture was stirred for 16 hours at 20°C. LCMS showed the starting material was consumed completely and desired product was detected. The mixture was poured into water (20 mL) and extracted with ethyl acetate (20 mL × 3). The combined organic phase was washed with brine (20 mL) and dried over sodium sulfate. After filtration and concentration, the crude product was purified with prep-HPLC(column: Phenomenex Synergi 10 μm C18 150 × 25 mm; mobile phase: [water(0.1%TFA)-ACN];B%: 35%−65%,13min) to provide compound **30h** (180.0 mg, 202.7 μmol, 50.4% yield) as colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.95 (br.s, 1H), 7.55 (d, *J* = 8.4 Hz, 1H), 7.35 – 7.33 (m, 4H), 7.27 – 7.24 (m ,4H), 6.99 – 6.91 (m, 5H), 6.85 (d, *J* = 7.6 Hz, 1H), 5.53 (br.s, 1H), 5.31 – 5.29 (m, 1H), 5.16 (dd, J_1 = 12.0 Hz, J_2 = 17.6 Hz, 2H), 5.00 – 4.93 (m, 1H), 3.86 – 3.72 (m, 2H), 3.46 – 3.31 (m, 4H), 3.11 (dd, J_1 = 4.0 Hz, J_1 = 9.6 Hz, 1H), 2.95 – 2.64 (m, 5H), 2.33 – 2.20 (m, 4H), 2.00 – 1.75 (m, 8H), 1.45 (s, 9H).

(S)-3-(3-(3-((S)-2-((S)-2-amino-4-(4,4-difluoropiperidin-1-yl)butanamido)-3-oxo-3-((2,2,2-trifluoroethyl)amino)propyl)phenoxy)phenyl)-2-(2-oxopyrrolidin-1-yl)propanoic acid (31h).

To a solution of compound **30h** (150.0 mg, 168.9 μmol, 1.0 *eq*) in methanol (2.0 mL) was added Palladium hydroxide (37.5 mg, 26.7 μmol, 10% purity). The mixture was degassed under vacuum and purged hydrogen for 3 times. The mixture was stirred at 20°C under hydrogen balloon for 2 hours. LCMS showed the starting material was consumed completely. The mixture was filtrated and the filter liquid was concentrated under vacuum to provide compound **30h-acid** (130.0 mg, 163.0 μmol, 96.5% yield) as colorless oil. LCMS: RT = 0.75 min, purity: 89.6%, m/z = 798.4$[M+H]^+$. To a solution of compound **30h-acid** (120.0 mg, 150.4 μmol, 1.0 *eq*) in dioxane (5.0 mL) was added HCl/dioxane (4 M, 5.0 mL, 133.0 *eq*). The mixture was stirred at 20°C for 0.5 hour. LCMS showed the starting material was consumed completely and desired product was detected. The mixture was filtrated and the filtrate liquid was concentrated under vacuum to provide compound **31h** (120.0 mg, crude, HCl) was obtained as a white solid. LCMS: RT = 0.68 min, purity: 89.1%, m/z = 698.3$[M+H]^+$.

(5S,8S,11S)-8-(2-(4,4-difluoropiperidin-1-yl)ethyl)-7,10-dioxo-11-(2-oxopyrrolidin-1-yl)-N-(2,2,2-trifluoroethyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide (10).

To a solution of compound **31h** (100.0 mg, 136.6 μmol, 1.0 *eq*, HCl salt) in DMF (3.0 mL) was added DIPEA (44.1 mg, 341.5 μmol, 59.5 μL, 2.5 *eq*), HOBt (24.0 mg, 177.6 μmol, 1.3 *eq*) and EDCI (34.0 mg, 177.6 μmol, 1.3 *eq*) at 0°C under nitrogen and then the mixture was stirred at 25°C for 16 hours. After 16 hours, LCMS showed starting material was consumed completely and desired MS was detected. The mixture was poured into water

(10 mL) and then extracted by ethyl acetate (3 × 20 mL). The combined organic phase was dried over sodium sulfate. After filtration and concentration, the crude product was purified by prep-HPLC(column: Phenomenex Gemini 5 μm C18 250 × 21.2 mm; mobile phase: [water (0.05% ammonia hydroxide v/v)-ACN];B%: 35%−65%,12min) to afford **10** (13.5 mg, 19.9 μmol, 14.5% yield, 100.0% purity) as a white solid. LCMS: RT = 2.65 min, m/z = 680.2[M+H]$^+$. ^{1}H NMR (MeOD, 400 MHz): δ = 7.33 (t, *J* = 7.6 Hz, 1H), 7.27 (t, *J* = 7.6 Hz, 1H), 7.10 (d, *J* = 7.6 Hz, 1H), 6.98 – 6.89 (m, 3H), 6.66 (s, 1H), 6.47 (s, 1H), 4.76 (dd, J_1 = 4.0 Hz, J_2 = 8.8 Hz, 1H), 4.71 (dd, J_1 = 3.2 Hz, J_2 = 12.0 Hz, 1H), 4.38 (t, *J* = 7.2 Hz, 1H), 4.04 – 3.82 (m, 2H), 3.64 – 3.52 (m, 2H), 3.23 (d, *J* = 12.8 Hz, 1H), 3.13 (dd, J_1 = 3.2 Hz, J_2 =14.8 Hz, 1H), 2.98 (dd, J_1 = 9.2 Hz, J_2 = 15.2 Hz, 1H), 2.77 (dd, J_1 = 3.2 Hz, J_2 = 12.4 Hz, 1H), 2.52 – 2.45 (m, 4H), 2.43 – 2.35.

benzyl (S)-3-(3-(3-((S)-3-(tert-butoxy)-2-((tert-butoxycarbonyl)amino)-3-oxopropyl)phenoxy)phenyl)-2-(2-oxopyrrolidin-1-yl)propanoate (34a).

To a solution of compound **32** (8.1 g, 22.1 mmol, 1.5 *eq*), compound **27b** (5.0 g, 14.7 mmol, 1.0 *eq*), 4A molecular sieve (5.0 g) and triethylamine (7.5 g, 73.7 mmol, 10.3 mL, 5.0 *eq*) in dichloromethane (100 mL) was added copper acetate (4.0 g, 22.1 mmol, 1.5 *eq*). The mixture was stirred at 25°C for 18 hours under oxygen (15 psi). LCMS showed 20% of material **27b** remianed. The mixture was filtered through a celite pad; the solid was washed with ethyl acetate (4 × 30 mL). 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 compound **34a** (5.7 g, 8.6 mmol, 58.5% yield, 100.0% purity) as light-yellow gum. LCMS: RT = 1.05 min, m/z = 681.1 [M+Na]$^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): 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).

Benzyl (S)-3-(3-(3-((S)-2-((S)-2-(((benzyloxy)carbonyl)amino)-4-(piperidin-1-yl)butanamido)-3-(tert-butoxy)-3-oxopropyl)phenoxy)phenyl)-2-(2-oxopyrrolidin-1-yl)propanoate (36a).

To a solution of compound **34a** (5.7 g, 8.6 mmol, 1.0 *eq*) in dichloromethane (120.0 mL) was added trifluoroacetic acid (37.0 g, 324.2 mmol, 24.0 mL, 37.6 *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 material was consumed. The mixture was poured into saturated sodium bicarbonate solution (300.0 mL, pH = 7). The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 × 100 mL). The combined organic layers were dried over anhydrous sodium sulfate, filtered, concentrated in vacuum to give compound **34a-amine** (4.2 g, 6.8 mmol, 79.4 % yield, 91.4% purity) as yellow gum, which was used for the next step without further purification. LCMS: RT = 0.89 min, m/z 559.1 [M+H]$^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): 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). To a solution of compound **35a** (231.0 mg, 531.8 μmol, 1.2 *eq*, trifluoroacetic acid salt), DIPEA (401.0 mg, 3.1 mmol, 540.3 μL, 7.0 *eq*) in DMF (3.0 mL) was added HOBt (78.0 mg, 576.1 μmol, 1.3 *eq*) at 0°C,

the mixture was stirred at 0°C for 10 minutes. EDCI (340.0 mg, 1.8 mmol, 4.0 *eq*) was added and then compound **34a-amine** (300.0 mg, 443.1 μmol, 1.0 *eq*) in DMF (1.0 mL) was drop-wise added at 0°C. The reaction mixture was stirred at 0°C for 20 minutes and then stirred at 25°C for another 16 hours under nitrogen atmosphere. LCMS showed the starting material was consumed completely and desired product mass was detected. The mixture was quenched with water (10 mL) and then combined with batch EW1319–1700. The mixture was extracted with ethyl acetate (3 × 15 mL), washed with brine (3 xx 15 mL), dried over anhydrous sodium sulfate, concentrated in vacuum. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 2/1 ~ 0/1) to give compound **36a** (450.0 mg, purity 87.5%) as yellow gum. LCMS: RT = 0.95 min, m/z = 861.4 $[M+H]^+$. ^1H NMR (Methanol-d4, 400 MHz): δ = 7.35 – 7.23 (m, 12H), 6.98 – 6.96 (m, 2H), 6.88 – 6.77 (m, 4H), 5.17 – 4.99 (m, 5H), 4.58 – 4.57 (m, 1H), 4.21 – 4.20 (m, 1H), 3.44 – 3.42 (m, 1H), 3.31 – 3.30 (m, 1H), 3.27 – 3.24 (m, 1H), 3.08 – 3.97 (m, 9H), 2.28 – 2.17 (m, 3H), 1.96 – 1.84 (m, 2H), 1.76 – 1.75 (m, 4H), 1.59 – 1.48 (m, 3H), 1.41 – 1.39 (m, 9H).

Tert-butyl (5S,8S,11S)-7,10-dioxo-11-(2-oxopyrrolidin-1-yl)-8-(2-(piperidin-1-yl)ethyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxylate (37a).

To a solution of compound **36a** (300.0 mg, 304.7 μmol, 1.0 *eq*) in isopropyl alcohol (6.0 mL) was added Pd/C (50.0 mg, 10% purity) and $Pd(OH)_2$/C (50.0 mg, 10% purity) under nitrogen atmosphere. The suspension was degassed under vacuum and purged with hydrogen several times. The mixture was stirred under hydrogen (15 psi) at 25°C for 8 hours. LCMS showed the starting material was consumed and 51% of the intermediate imine remained. The mixture was filtered. The solid was washed with isopropyl alcohol (3 × 2 mL). Pd/C (50 mg, 10% purity) and $Pd(OH)_2$/C (50 mg, 10% purity) was added into the combined filtrate under nitrogen atmosphere. The suspension was degassed under vacuum and purged with hydrogen several times. The mixture was stirred under hydrogen (15 psi) at 25°C for 14 hours. LCMS showed the starting material was consumed and 11.65% of the intermediate imine remained, the desired compound was detected. The residue was purified by reverse flash (trifluoroacetic acid condition). The fraction was adjusted to pH = 7 with saturated sodium bicarbonate solution. The mixture was lyophilized to give crude compound **36a-amino acid** (280.0 mg, crude, Na salt) as a white solid, which was used into the next step without further purification. LCMS: RT = 0.77 min, m/z 637.5 $[M+H]^+$, purity: 98.5%. To a solution of compound **36a-amino acid** (280.0 mg, 315.6 μmol, 1.0 *eq*) in DMF (280 mL) was added DIPEA (286.0 mg, 2.2 mmol, 384.8 μL, 7.0 *eq*) and HOBt (64.0 mg, 473.4 μmol, 1.5 *eq*) at 0°C. The mixture was stirred at 0°C for 10 minute. EDCI (303.0 mg, 1.6 mmol, 5.0 *eq*) was added. The reaction mixture was stirred at 0°C for 20 minutes and then stirred at 25°C for another 16 hours. LCMS showed a part of starting material remained. DIPEA (82.0 mg, 631.3 μmol, 110.0 μL, 2.0 *eq*) and EDCI (121.0 mg, 631.3 μmol, 2.0 *eq*) was added at 0°C. The mixture was stirred at 25°C for another 24 hours. LCMS showed the starting material was consumed. The mixture was poured into ice water (200 mL) and then extracted with ethyl acetate (3 × 120 mL). The combined organic layers were washed with brine (4 × 100 mL), dried over anhydrous sodium sulfate, filtered, concentrated in vacuum. The residue was purified by reverse flash column (trifluoroacetic acid condition) and then re-purified by prep-HPLC (column: Boston pH-lex 10 μm C18 150 × 25 mm; mobile phase: [water (0.1%trifluoroacetic acid)-acetonitrile]; B%: 26%−56%, 10min) to give compound

37a (40.0 mg, 63.8 μmol, 20.2% yield, 98.7% purity) as a light yellow solid. Meanwhile 20 mg of the diastereoisomer was obtained. LCMS: RT = 0.88 min, m/z 619.3 $[M+H]^+$. ^{1}H NMR (Methanol-d4, 400 MHz): δ = 7.34 (t, J = 8.0 Hz, 1H), 7.28 (t, J = 8.0 Hz, 1H), 7.12 (d, J = 7.6 Hz, 1H), 6.95 – 6.90 (m, 3H), 6.69 – 6.68 (m, 1H), 6.35 (s, 1H), 4.71 (dd, J = 12.0, 4.0 Hz, 1H), 4.62 (dd, J = 8.4, 4.0 Hz, 1H), 4.39 (t, J = 7.2 Hz, 1H), 3.63 – 3.61 (m, 2H), 3.27 – 3.21 (m, 2H), 2.99 (dd, J = 15.6, 8.8 Hz, 1H), 2.81 (dd, J = 12.4, 3.6 Hz, 1H), 2.60 – 2.32 (m, 8H), 2.08 – 2.01 (m, 2H), 1.93 – 1.79 (m, 4H), 1.66 – 1.62 (m, 4H), 1.50 (m, 9H).

(5S,8S,11S)-N-((1-methylcyclopropyl)methyl)-7,10-dioxo-11-(2-oxopyrrolidin-1-yl)-8-(2-(piperidin-1-yl)ethyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide (17).

To a solution of compound **37a** (35.0 mg, 56.6 μmol, 1.0 *eq*) in dichloromethane (1.0 mL) was added trifluoroacetic acid (0.4 mL) at 0°C. The mixture was stirred at 25°C for 3 hours. TLC showed the starting material was consumed completely. The mixture was concentrated in vacuum to give crude compound **37a-acid** (40.0 mg, crude, trifluoroacetic acid) as light-yellow gum, which was used for the next step without further purification. To a solution of **37a-acid** (35.0 mg, 51.7 μmol, 1.0 *eq*, TFA), (1-methylcyclopropyl)methanamine (19.0 mg, 155.2 μmol, 3.0 *eq*, HCl) and DIPEA (40.1 mg, 310.3 μmol, 54.1 μL, 6.0 *eq*) in THF (0.3 mL) was added HATU (39.0 mg, 103.5 μmol, 2.0 *eq*) at 0°C. The mixture was stirred at 20°C for 16 hours under nitrogen atmosphere. LCMS showed the starting material was consumed; the desired compound was detected. The mixture was quenched with water (10 mL) and then adjusted to pH=7 with HCl (1M). The mixture was extracted with ethyl acetate (3 × 15 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous sodium sulfate, filtered, concentrated in vacuum. The residue was purified by prep-HPLC (column: Phenomenex Gemini 10 μm C18 150 × 25 mm; mobile phase: [water (10mM NH4HCO3)-ACN]; B%: 50%−80%, 8 min) to give **17** (16.0 mg, 25.2 μmol, 48.8% yield, 99.3% purity) as a light yellow solid. LCMS: RT = 2.68 min, m/z = 630.3 $[M+H]^+$. ^{1}H NMR (MeOD, 400 MHz): δ = 7.33 – 7.26 (m, 2H), 7.08 (d, J = 7.6 Hz, 1H), 6.96 – 6.93 (m, 3H), 6.64 (s, 1H), 6.53 (s, 1H), 4.75 – 4.69 (m, 2H), 4.34 (t, J = 7.2 Hz, 1H), 3.59 – 3.54 (m, 2H), 3.26 (t, J = 12.4 Hz, 1H), 3.13 – 3.08 (m, 3H), 3.05 – 2.97 (m, 1H), 2.75 (dd, J = 12.8, 3.2 Hz, 1H), 2.41 – 2.27 (m, 6H), 2.23 (t, J = 7.6 Hz, 2H), 2.07 – 2.01 (m, 2H), 1.81 – 1.71 (m, 2H), 1.57 – 1.53 (m, 4H), 1.44 – 1.43 (m, 2H), 1.07 (s, 3H), 0.46 – 0.44 (m, 2H), 0.32 – 0.30 (m, 2H).

(5S,8S,11S)-N-((R)-1-cyclopropylethyl)-7,10-dioxo-11-(2-oxopyrrolidin-1-yl)-8-(2-(piperidin-1-yl)ethyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide (18).

To a solution of **37a-acid** (30.0 mg, 44.3 μmol, 1.0 eq, TFA) in pyridine (0.6 mL) was added HOBt (6.0 mg, 44.3 μmol, 1 eq) at 0°C, the mixture was stirred at 0°C for 15 minutes. (R)-1-Cyclopropylethylamine (11.0 mg, 133.0 μmol, 3.0 eq) and EDCI (26.0 mg, 133.0 μmol, 3.0 eq) were added into the mixture at 0°C and then the mixture was stirred at 25°C for another 2 hours. LCMS showed the starting material was remained. Another EDCI (26.0 mg, 133.0 μmol, 3.0 eq) and (R)-1-cyclopropylethylamine (12.0 mg, 133.0 μmol, 3.0 eq) were added into the mixture at 0°C. The mixture was stirred at 25°C for another 18 hours. LCMS showed the most starting material remained. Another (R)-1-cyclopropylethylamine

(38.0 mg, 443.3 μmol, 10.0 eq) and EDCI (51.0 mg, 266.0 μmol, 6.0 eq) was added into the mixture at 0°C. The reaction mixture was stirred at 25°C for another 18 hours. Pyridine (0.6 mL) and EDCI (51.0 mg, 266.0 μmol, 6.0 eq) were added into the mixture. The reaction mixture was stirred at 25°C for another 20 hours. LCMS showed the starting material remained. HATU (67.0 mg, 177.3 μmol, 4.0 eq) was added into the mixture. The reaction mixture was stirred at 20°C for another 24 hours. LCMS showed the most of starting material was consumed, the desired compound was detected. The mixture was poured into water (20 mL) and then extracted with ethyl acetate (3 × 25 mL). The combined organic layers were washed with brine (3 × 20 mL), dried over anhydrous sodium sulfate, filtered, concentrated in vacuum. The residue was purified by prep-HPLC (column: Boston pH-lex 10 μm C18 150 × 25 mm; mobile phase: [water(0.1%TFA)-ACN];B%: 35%−59%,8min) and then further purified by prep-HPLC (column: Waters Xbridge 5 μm C18 150 × 25 mm; mobile phase: [water (0.05% ammonia hydroxide v/v)-ACN];B%: 50%−68%,10min) to give **18** (8.7 mg, 13.7 μmol, 30.9% yield, 99.3% purity) as a white solid. LCMS: RT = 2.86 min, m/z = 630.4 $[M+H]^+$. ^{1}H NMR (MeOD, 400 MHz): δ = 7.33 (d, *J* = 8.0 Hz, 1H), 7.28 (d, *J* = 8.0 Hz, 1H), 7.09 (d, *J* = 7.6 Hz, 1H), 6.96 – 6.91 (m, 3H), 6.66 (s, 1H), 6.50 (s, 1H), 4.72 – 4.65 (m, 2H), 4.34 (t, *J* = 7.2 Hz, 1H), 3.60 – 3.56 (m, 2H), 3.35 – 3.33 (m, 1H), 3.27 – 3.24 (m, 1H), 3.09 – 2.95 (m, 2H), 2.76 (dd, *J* = 12.4, 2.8 Hz, 1H), 2.42 – 2.25 (m, 8H), 2.07 – 2.01 (m, 2H), 1.81 – 1.72 (m, 2H), 1.56 – 1.55 (m, 4H), 1.45 – 1.44 (m, 2H), 1.23 (d, *J* = 6.8 Hz, 3H), 0.88 – 0.85 (m, 1H), 0.53 – 0.38 (m, 2H), 0.28 – 0.20 (m, 2H).

(5S,8S,11S)-N-((S)-1-cyclopropylethyl)-7,10-dioxo-11-(2-oxopyrrolidin-1-yl)-8-(2-(piperidin-1-yl)ethyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide (19).

To a solution of **37a-acid** (30.0 mg, 44.3 μmol, 1.0 *eq*, TFA), (S)-1-cyclopropylethylamine (11.0 mg, 133.0 μmol, 3.0 *eq*) and DIPEA (17.0 mg, 133.0 μmol, 23.2 μL, 3.0 *eq*) in THF (0.4 mL) was added HATU (33.7 mg, 88.7 μmol, 2.0 *eq*) at 0°C. The mixture was stirred at 20°C for 16 hours under nitrogen atmosphere. LCMS showed the starting material was consumed; the desired mass was detected. The mixture was quenched with water (10 mL). The mixture was adjusted to pH=7 with HCl (1M) and then extracted with ethyl acetate (3 × 15 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous sodium sulfate, filtered, concentrated in vacuum. The residue was purified by prep-HPLC (column: Phenomenex Gemini 10 μm C18 150 × 25 mm; mobile phase: [water (10mM NH4HCO3)-ACN]; B%: 50%−80%, 8min) to give **19** (6.9 mg, purity: 99.3%) as a light-yellow solid. LCMS: RT = 2.68 min, *m*/*z* = 630.3 $[M+H]^+$. ^{1}H NMR (Methanol-d4, 400 MHz): *δ* = 7.33 (d, *J* = 8.0 Hz, 1H), 7.28 (d, *J* = 8.0 Hz, 1H), 7.09 (d, *J* = 7.6 Hz, 1H), 6.96 – 6.95 (m, 3H), 6.66 (s, 1H), 6.47 (s, 1H), 4.71 – 4.64 (m, 2H), 4.35 (t, *J* = 7.2 Hz, 1H), 3.64 – 3.53 (m, 2H), 3.31 – 3.27 (m, 2H), 3.08 – 3.00 (m, 2H), 2.75 (dd, *J* = 12.8, 3.2 Hz, 1H), 2.48 – 2.34 (m, 6H), 2.25 (t, *J* = 7.6 Hz, 1H), 2.09 – 1.99 (m, 2H), 1.82 – 1.68 (m, 2H), 1.57 – 1.54 (m, 4H), 1.44 – 1.43 (m, 2H), 1.19 (d, *J* = 6.8 Hz, 3H), 0.93 – 0.87 (m, 1H), 0.55 – 0.45 (m, 2H), 0.36 – 0.30 (m, 1H), 0.25 – 0.19 (m, 1H).

(5S,8S,11S)-N-(bicyclo[1.1.1]pentan-1-yl)-7,10-dioxo-11-(2-oxopyrrolidin-1-yl)-8-(2-(piperidin-1-yl)ethyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide (21).

To a solution of compound **37a-acid** (40.0 mg, 59.1 μmol, 1.0 *eq*, trifluoroacetic acid salt) in pyridine (0.5 mL) was added propellamine (21.0 mg, 177.3 μmol, 3.0 *eq*, HCl salt). The mixture was cooled to 0°C and then added HOBt (8.0 mg, 59.1 μmol, 1.0 *eq*). The mixture was stirred at 0°C for 10 minutes. EDCI (34.0 mg, 177.3 μmol, 3.0 *eq*) was added at 0°C. The reaction mixture was stirred at 0°C for 20 minutes and then stirred at 25°C for anothers 1.5 hours under nitrogen atmosphere. LCMS showed the starting material was consumed and desired product mass was detected. The mixture was quenched with water (5 mL). The mixture was concentrated in vacuum; the residue was diluted with water (20 mL) and then extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine (15 mL), dried over anhydrous sodium sulfate, filtered, concentrated in vacuum. The residue was purified by prep-HPLC (column: Boston pH-lex 10μm C18 150 × 25 mm; mobile phase: [water (0.1%trifluoroacetic acid)-acetonitrile]; B%: 23%−53%, 10min) and then re-purified by prep-HPLC (column: Phenomenex Synergi C18 10μm 150 × 25 mm; mobile phase: [water (0.1%trifluoroacetic acid)-acetonitrile]; B%: 23%−53%, 12min). The fraction was adjusted to pH = 7 with saturated sodium bicarbonate solution. The mixture was concentrated in vacuum to removed acetonitrile, extracted with dichloromethane (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous sodium sulfate, filtered, concentrated in vacuum. The residue was lyophilized to give **21** (15.0 mg, purity 98.4%) as a white solid. LCMS: RT = 2.42 min, m/z 628.3 $[M+H]^+$, purity: 98.4%. ^{1}H NMR (Methanol-d4, 400 MHz): δ = 7.34 (t, J = 8.0 Hz, 1H), 7.27 (t, J = 8.0 Hz, 1H), 7.11 (d, J = 7.6 Hz, 1H), 6.95 – 6.89 (m, 3H), 6.66 (s, 1H), 6.42 (s, 1H), 4.69 (dd, J = 12.4, 3.6 Hz, 1H), 4.58 – 4.55 (m, 1H), 4.37 (t, J = 6.8 Hz, 1H), 3.62 – 3.59 (m, 2H), 3.33 – 3.31 (m, 1H), 3.24 (t, J = 12.8 Hz, 1H), 3.06 (dd, J = 15.6, 8.0 Hz, 1H), 2.94 (dd, J = 15.2, 8.5 Hz, 1H), 2.82 (dd, J = 12.4, 3.6 Hz, 1H), 2.65 – 2.60 (m, 5H), 2.44 – 2.35 (m, 3H), 2.10 – 2.06 (m, 8H), 1.90 – 1.76 (m, 2H), 1.67 – 1.64 (m, 4H), 1.52 – 1.51 (m, 2H).

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 (36b).

To a solution of compound **35b** (2.6 g, 8.3 mmol, 1.2 *eq*) and DIPEA (3.5 g, 27.4 mmol, 4.8 mL, 4.0 *eq*) in DMF (45.0 mL) was added HOBt (1.2 g, 8.9 mmol, 1.3 *eq*) at 0°C. The mixture was stirred at 0°C for 10 minutes. EDCI (2.6 g, 13.7 mmol, 2.0 *eq*) was added and then compound **34a-amine** (4.2 g, 6.8 mmol, 1.0 *eq*) in DMF (15.0 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 (3 × 60 mL). The combined organic layers were washed with brine (3 × 50 mL), 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 compound **36b** (4.1 g, 4.7 mmol, 68.0% yield, 96.9% purity) as colorless gum. LCMS: RT = 1.01 min, m/z 850.4 $[M+H]^+$. ^{1}H

NMR ($CDCl_3$, 400 MHz) 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).

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 (37b).

To a solution of compound **36b** (4.1 g, 4.8 mmol, 1.0 *eq*) in THF (60.0 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 THF (4 × 10 mL) and ethyl acetate (3 × 10 mL). The combined filtrate was concentrated in vacuum to give compound **36b-amino acid** (3.1 g, crude) as a white solid, which was used into the next step without further purification. LCMS: RT = 0.81 min, m/z 626.3 $[M+H]^+$, purity: 95.1%. ^{1}H NMR (Methanol-d4, 400 MHz): 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). To a solution of compound **36b-amino acid** (1.0 g, 1.6 mmol, 1.0 *eq*) and DIPEA (1.0 g, 8.0 mmol, 1.4 mL, 5.0 *eq*) in DMF (80.0 mL) was added HOBt (324.0 mg, 2.4 mmol, 1.5 *eq*) and EDCI (613.0 mg, 3.2 mmol, 2.0 *eq*) at 0°C. The mixture was stirred at 25°C for 15 hours. LCMS showed the starting material remained. Another batch of DIPEA (516.0 mg, 4.0 mmol, 695.9 µL, 2.5 *eq*) and EDCI (613.0 mg, 3.2 mmol, 2.0 *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 (3 × 100 mL). The combined organic layers were washed with brine (3 × 50 mL), 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 **37b** (410.0 mg, 564.7 µmol, 35.3% yield, 83.7% purity) as a white solid. LCMS: RT = 0.88 min, m/z = 608.1 $[M+H]^+$. ^{1}H NMR (Methanol-d4, 400 MHz): 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).

(5S,8S,11S)-8-((1,3-dioxan-2-yl)methyl)-7,10-dioxo-11-(2-oxopyrrolidin-1-yl)-N-(2,2,2-trifluoroethyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide (38a).

To a solution of **37b** (410.0 mg, 564.7 µmol, 1.0 *eq*) in dichloromethane (5.0 mL) was added trifluoroacetic acid (2.4 g, 21.0 mmol, 1.6 mL, 37.2 *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 (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous sodium sulfate, filtered, concentrated in vacuum to give compound **37b-acid** (440.0 mg, crude) as a white solid, which was used into the next step without further purification. To a solution of compound **37b-acid** (120.0 mg, 217.6 μmol, 1.0 *eq*) in pyridine (1.5 mL) was added HOBt (29.0 mg, 217.6 μmol, 1.0 *eq*). The mixture was stirred at 0°C for 10 minutes. 2,2,2-Trifluoroethylamine (43.0 mg, 435.1 μmol, 34.2 μL, 2.0 *eq*) and EDCI (104.0 mg, 543.9 μmol, 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 6 hours. LCMS showed the starting material was consumed completely and desired product mass was detected. The mixture was quenched with water (10 mL) and then combined with another batch. The mixture was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with 1N HCl solution (30 mL), saturated sodium bicarbonate solution (30 mL) and brine (30 mL), dried over anhydrous sodium sulfate, filtered, concentrated in vacuum. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate/Ethanol = 16/3/1 ~ 12/3/1) to give compound **38a** (130.0 mg, purity 93.5%) as a light-yellow solid. LCMS: RT = 0.86 min, m/z 633.2 $[M+H]^+$. ^1H NMR (Methanol-d4, 400 MHz): δ = 7.35 – 7.26 (m, 2H), 7.07 (d, *J* = 7.6 Hz, 1H), 6.98 – 6.94 (m, 3H), 6.64 – 6.63 (s, 1H), 6.56 (s, 1H), 4.76 (dd, *J* = 9.6, 4.0 Hz, 1H), 4.76 (dd, *J* = 12.0, 3.2 Hz, 1H), 4.49 (t, *J* = 5.2 Hz, 1H), 4.44 (t, *J* = 7.2 Hz, 1H), 4.00 – 3.95 (m, 4H), 3.77 – 3.50 (m, 4H), 3.26 (t, *J* = 12.4 Hz, 1H), 3.13 (dd, *J* = 15.2, 3.6 Hz, 1H), 3.00 (dd, *J* = 15.2, 9.6 Hz, 1H), 2.74 (dd, *J* = 12.8, 2.8 Hz, 1H), 2.42 – 2.38 (m, 2H), 2.06 – 2.02 (m, 2H), 1.85 – 1.77 (m, 3H), 1.32 – 1.29 (m, 1H).

(5S,8S,11S)-8-(2-((R)-3-fluoropiperidin-1-yl)ethyl)-7,10-dioxo-11-(2-oxopyrrolidin-1-yl)-N-(2,2,2-trifluoroethyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide (11).

To a solution of compound **38a** (110.0 mg, 173.9 μmol, 1.0 *eq*) in acetonitrile (1.1 mL) was added CAN (238.0 mg, 434.7 μmol, 216.7 μL, 2.5 *eq*) in water (1.1 mL). The mixture was stirred at 70°C for 3 hours. TLC (dichloromethane: methanol =10:1) showed the starting material was consumed and a new spot was detected. The mixture was poured into water (10 mL) and then extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with saturated sodium sulfite solution (2 × 30 mL) and brine (30 mL), dried over anhydrous sodium sulfate, filtered, concentrated in vacuum to give crude product compound **38a-aldehyde** (85.0 mg, 146.4 μmol, 84.2% yield, 98.98% purity) as a light-yellow solid, which was used into the next step without further purification. To a solution of compound **38a-aldehyde** (85.0 mg, 147.9 μmol, 1.0 *eq*) and triethylamine (75.0 mg, 739.7 μmol, 103.0 μL, 5.0 *eq*) in methanol (0.5 mL) was added (R)-3-fluoropiperidine (62.0 mg, 443.8 μmol, 3.0 *eq*, HCl), acetic acid (18.0 mg, 295.9 μmol, 16.9 μL, 2.0 *eq*) and Pd/C (50.0 mg, 10% purity) under nitrogen atmosphere. The suspension was degassed under vacuum and purged with hydrogen several times. The mixture was stirred under hydrogen atmosphere (15 psi) at 25°C for 16 hours. LCMS showed the starting material and intermedaite were consumed completely and desired product mass was detected. The mixture was filtered. The solid was washed with methanol (4 × 5 mL). The combined filtrate was concentrated in vacuum. The residue was diluted with ethyl acetate (50 mL) and then washed with brine (20 mL), dried over anhydrous sodium sulfate, filtered, concentrated in vacuum. The residue was

purified by prep-HPLC (column: Boston pH-lex 10μm C18 150 × 25 mm; mobile phase: [water (0.1%trifluoroacetic acid)-acetonitrile]; B%: 21%−51%, 10 minutes). The fraction was adjusted to pH = 7 with saturated sodium bicarbonate solution. The mixture was concentrated in vacuum to removed acetonitrile, extracted with dichloromethane (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous sodium sulfate, filtered, concentrated in vacuum. The residue was lyophilized to give **11** (42.1 mg, purity 96.7%) as a white solid. LCMS: RT = 2.30 min, m/z = 662.2 $[M+H]^+$. ^1H NMR (Methanol-d4, 400 MHz): δ = 7.33 (t, J = 8.0 Hz, 1H), 7.28 (t, J = 8.0 Hz, 1H), 7.08 (d, J = 7.6 Hz, 1H), 6.96 – 6.91 (m, 3H), 6.64 (s, 1H), 6.53 (s, 1H), 4.77 (dd, J = 10.0, 4.0 Hz, 1H), 4.70 (dd, J = 12.0, 3.2 Hz, 1H), 4.66 – 4.63 (m, 0.5H), 4.53 – 4.52 (m, 0.5H), 4.37 (t, J = 7.2 Hz, 1H), 4.01 – 3.85 (m, 2H), 3.64 – 3.53 (m, 2H), 3.25 (t, J = 12.4 Hz, 1H), 3.12 (dd, J = 15.2, 3.6 Hz, 1H), 2.99 (dd, J = 15.2, 9.6 Hz, 1H), 2.77 (dd, J = 12.8, 3.2 Hz, 1H), 2.66 – 2.63 (m, 1H), 2.41 – 2.32 (m, 7H), 2.09 – 2.04 (m, 2H), 1.78 – 1.70 (m, 4H), 1.54 – 1.52 (m, 2H).

(5S,8S,11S)-8-((1,3-dioxan-2-yl)methyl)-N-((1-methyl-1H-pyrazol-4-yl)methyl)-7,10-dioxo-11-(2-oxopyrrolidin-1-yl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide (38b).

To a solution of compound **37b-acid** (200.0 mg, 362.6 μmol, 1.0 *eq*) and (1-methyl-1H-pyrazol-4-yl)methanamine (48.0 mg, 435.1 μmol, 1.2 *eq*) in pyridine (0.2 mL) was added HOBt (49.0 mg, 362.6 μmol, 1.0 *eq*) at 0°C. The mixture was stirred at 0°C for 10 minutes. EDCI (174.0 mg, 906.5 μmol, 2.5 *eq*) was added. The reaction mixture was stirred at 25°C for 16 hours under nitrogen atmosphere. LCMS showed the starting material was consumed and desired product mass was detected. The mixture was diluted with water **(**20 mL). The mixture was adjusted pH = 6 with 1N HCl solution and extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine (3 × 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/Ethanol = 1/1/0 ~ 8/9/3) to give compound **38b** (100.0 mg, 155.1 μmol, 42.8% yield, 100.0% purity) as a light-yellow solid. LCMS: RT = 0.764 min, m/z = 645.1 $[M+H]^+$. ^1H NMR (Methanol-d4, 400 MHz): δ = 7.51 (s, 1H), 7.41 (s, 1H), 7.32 (t, J = 8.0 Hz, 1H), 7.26 (t, J = 8.0 Hz, 1H), 7.07 (d, J = 7.6 Hz, 1H), 6.97 – 6.95 (m, 2H), 6.87 (d, J = 8.8 Hz, 1H), 6.63 (s, 1H), 6.54 (s, 1H), 4.69 – 4.61 (m, 2H), 4.48 (t, J = 5.2 Hz, 1H), 4.42 (t, J = 7.2 Hz, 1H), 4.24 (s, 2H), 3.95 – 3.93 (m, 2H), 3.84 (s, 3H), 3.72 – 3.50 (m, 4H), 3.24 (t, J = 12.4 Hz, 1H), 3.11 (dd, J = 15.2, 3.6 Hz, 1H), 2.94 (dd, J = 15.2, 9.6 Hz, 1H), 2.74 (dd, J = 12.4, 2.8 Hz, 1H), 2.42 – 2.37 (m, 2H), 2.05 – 2.01 (m, 2H), 1.86 – 1.84 (m, 3H), 1.30 – 1.26 (m, 1H).

(5S,8S,11S)-N-((1-methyl-1H-pyrazol-4-yl)methyl)-7,10-dioxo-11-(2-oxopyrrolidin-1-yl)-8-(2-(piperidin-1-yl)ethyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide (13).

To a solution of compound **38b** (90.0 mg, 139.6 μmol, 1.0 *eq*) in acetonitrile (0.9 mL) was added CAN (191.0 mg, 349.0 μmol, 173.9 μL, 2.5 *eq*) in water (0.9 mL). The mixture was stirred at 70°C for 3 hours. TLC (dichloromethane: methanol = 10:1) showed the starting material was consumed completely and desired product mass was detected on LCMS. The

mixture was diluted with water (10 mL) and then extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with saturated sodium sulfite solution (20 mL), brine (20 mL), dried over anhydrous sodium sulfate, filtered, concentrated in vacuum to give the crude compound **38b-aldehyde** (60.0 mg, 102.3 μmol, 73.3% yield) as a light-yellow solid, which was used into the next step without further purification. To a solution of compound **38b-aldehyde** (60.0 mg, 102.3 μmol, 1.0 *eq*) in methanol (5.0 mL) was added piperidine (44.0 mg, 511.4 μmol, 50.5 μL, 5.0 *eq*) and acetic acid (12.0 mg, 204.6 μmol, 11.7 μL, 2.0 *eq*). Pd/C (20.0 mg, 10% purity) was added under nitrogen atmosphere. The suspension was degassed under vacuum and purged with hydrogen several times. The mixture was stirred under hydrogen (15 psi) at 25°C for 14 hours. LCMS showed the intermediate remained. Pd/C (50.0 mg, 10% purity) was added. The suspension was degassed under vacuum and purged with hydrogen several times. The mixture was stirred under hydrogen (15 psi) at 25°C for another 3 hours. LCMS showed the intermediate was consumedand desired product mass was detected. The mixture was filtered through celit pad, the solid was washed with methanol (4 × 4 mL). The combined filtrates were concentrated in vacuum. The residue was diluted with ethyl acetate (40 mL) and then washed with brine (10 mL), dried over anhydrous sodium sulfate, concentrated in vacuum. The residue was purified by prep-HPLC (column: UniSil 10 μm C18 120 × 30 mm; mobile phase: [water (0.1%trifluoroacetic acid)-acetonitrile]; B%: 21%−51%, 10 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 (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous sodium sulfate, filtated and concentrated in vacuum. The residue was lyophilized to give **13** (12.6 mg) as a light-yellow solid. LCMS: RT = 2.15 min, m/z 656.3 $[M+H]^+$. ^{1}H NMR (Methanol-d4, 400 MHz): δ = 7.52 (s, 1H), 7.41 (s, 1H), 7.33 (t, J = 8.0 Hz, 1H), 7.25 (t, J = 8.0 Hz, 1H), 7.10 (d, J = 7.6 Hz, 1H), 6.98 – 6.93 (m, 2H), 6.84 (d, J = 7.6 Hz, 1H), 6.66 (s, 1H), 6.47 (s, 1H), 4.73 – 4.65 (m, 2H), 4.35 (t, J = 7.2 Hz, 1H), 4.24 (s, 2H), 3.85 (s, 3H), 3.63 – 3.55 (m, 2H), 3.24 (t, J = 12.4 Hz, 1H), 3.08 (dd, J = 15.2, 3.6 Hz, 1H), 2.97 (dd, J = 15.2, 9.2 Hz, 1H), 2.79 (dd, J = 12.8, 3.2 Hz, 1H), 2.50 – 2.36 (m, 8H), 2.07 – 2.04 (m, 2H), 1.62 – 1.58 (m, 4H), 1.49 – 1.48 (m, 2H).

(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 (38c).

To a solution of compound **37b-acid** (240.0 mg, 435.1 μmol, 1.0 *eq*) in pyridine (2.5 mL) was added HOBt (59.0 mg, 435.1 μmol, 1.0 *eq*) at 0°C. The mixture was stirred at 0°C for 10 minutes. Cyclopropylmethanamine (62.0 mg, 870.2 μmol, 2.0 *eq*) and EDCI (209.0 mg, 1.1 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 (209.0 mg, 1.1 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 HCl solution. The mixture was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with 1 N HCl 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 compound **38c** (179.0 mg, 282.9 μmol, 65.0% yield, 95.6% purity) as a light yellow solid. LCMS: RT = 0.88 min, m/z = 605.3 $[M+H]^+$, purity: 95.6%. ^{1}H NMR (Methanol-d4, 400 MHz): 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).

(5S,8S,11S)-N-(cyclopropylmethyl)-7,10-dioxo-11-(2-oxopyrrolidin-1-yl)-8-(2-(piperidin-1-yl)ethyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide (16).

To a solution of compound **38c** (194.0 mg, 320.8 μmol, 1.0 *eq*) in acetonitrile (2.0 mL) was added CAN (440.0 mg, 802.1 μmol, 399.7 μL, 2.5 *eq*) in water (2.0 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 desired product was detected. The mixture was quenched with saturated sodium bicarbonate solution (20 mL) and then extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous sodium sulfate, concentrated in vacuu to give compound **38c-aldehyde** (140.0 mg, crude) as a light yellow solid, which was used into the next step without further purification. To a solution of compound **38c-aldehyde** (140.0 mg, 256.1 μmol, 1.0 *eq*) and acetic acid (30.8 mg, 512.3 μmol, 29.3 μL, 2.0 *eq*) in methanol (5.0 mL) was added piperidine (109.0 mg, 1.3 mmol, 126.5 μL, 5.0 *eq*) and Pd/C (0.02 g, 10% purity) under nitrogen atmosphere. The mixture was degassed and purged with hydrogen several times. The reaction mixture was stirred at 25°C for 14 hours under hydrogen (15 psi). LCMS showed the starting material was consumed but the intermediate remained. The mixture was filtered and Pd/C (0.02 g, 10% purity) was added into the filtrate under nitrogen atmosphere. The mixture was degassed and purged with hydrogen several times. The reaction mixture was stirred at 25°C for another 2 hours under hydrogen (15 psi). The mixture was filtered through cilte pad and then the solid was washed with methanol (4 × 8 mL). The filtrate was concentrated in vacuum. The residue was purified by prep-HPLC (column: Boston pH-lex 10 μm C18 150 × 25 mm; mobile phase: [water (0.1%trifluoroacetic acid)-acetonitrile]; B%: 25%−55%, 10min). The fraction was adjusted to pH = 7 with saturated sodium bicarbonate solution. The mixture was concentrated in vacuum to removed acetonitrile, extracted with dichloromethane (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous sodium sulfate, filtated and concentrated in vacuum. The residue was lyophilization to give **16** (22.5 mg, 35.1 μmol, 13.7% yield, 96.0% purity) as a white solid. LCMS: RT = 2.28 min, m/z = 616.3 $[M+H]^+$. ^{1}H NMR (Methanol-d4, 400 MHz): δ = 7.34 (t, J = 8.0 Hz, 1H), 7.28 (t, J = 8.0 Hz, 1H), 7.11 (d, J = 8.0 Hz, 1H), 6.97 – 6.94 (m, 3H), 6.67 (t, J = 2.0 Hz, 1H), 6.48 (s, 1H), 4.72 – 4.67 (m, 2H), 4.38 (t, J = 7.2 Hz, 1H), 3.62 – 3.60 (m, 2H), 3.25 (t, J = 12.4 Hz, 1H), 3.13 – 3.04 (m, 4H), 2.81 (dd, J = 12.4, 2.8 Hz, 1H), 2.59 – 2.55 (m, 3H), 2.47 – 2.43 (m,

3H), 2.09 – 2.07 (m, 2H), 1.88 – 1.78 (m, 2H), 1.65 – 1.62 (m, 4H), 1.51 – 1.50 (m, 2H), 1.32 – 1.29 (m, 1H), 1.01 – 0.91 (m, 2H), 0.52 – 0.50 (m, 2H), 0.23 – 0.22 (m, 2H).

(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 (22).

To a solution of compound **38c-aldehyde** (90.0 mg, 164.7 μmol, 1.0 *eq*) in methanol (5.0 mL) was added morpholine (72.0 mg, 823.3 μmol, 72. 5 μL, 5.0 *eq*) and acetic acid (20.0 mg, 329.3 μmol, 18.8 μL, 2.0 *eq*). Pd/C (20.0 mg, 10% purity) 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, Pd/C (50 mg, 10% purity) 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 then filtered throught celite pad. The soild was washed with methanol (3 × 5 mL). The combined filtrate was concentrated in vacuum. The residue was diluted with ethyl acetate (30 mL) and then washed with brine (2 × 10 mL). The organic layers were dried over anhydrous sodium sulfate, filtered, concentrated in vacuum. The residue was purified by prep-HPLC (column: Phenomenex Synergi 10 μm C18 150 × 25 mm; 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 (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous sodium sulfate, concentrated in vacuum. The residue was lyophilized to give **22** (14.3 mg purity 98.9%) as a white solid. LCMS: RT = 2.25 min, m/z 618.3 $[M+H]^+$. ^{1}H NMR (Methanol-d4, 400 MHz): 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).

(5S,8S,11S)-8-((1,3-dioxan-2-yl)methyl)-N-cyclopentyl-7,10-dioxo-11-(2-oxopyrrolidin-1-yl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide (38d).

To a solution of compound **37b-acid** (200.0 mg, 362.6 μmol, 1.0 *eq*) and cyclopentanamine (62.0 mg, 725.2 μmol, 71.6 μL, 2.0 *eq*) in pyridine (2.5 mL) was added HOBt (49.0 mg, 362.6 μmol, 1.0 *eq*) at 0°C. The mixture was stirred at 0°C for 10 minutes. EDCI (174.0 mg, 906.5 μmol, 2.5 *eq*) was added. The reaction mixture was stirred at 25°C for 19 hours. LCMS showed the starting material was consumed and desired product mass was detected. The mixture was poured into water (15 mL) and then adjusted to pH = 6. The mixture was extracted with ethyl acetate (3 × 25 mL). The combined organic layers were washed with 1N HCl (10 mL), saturated sodium bicarbonate solution (20 mL) and brine (20 mL). 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 = 1/1~ 0/1) to give compound **38d** (130.0 mg, 210.1 μmol, 58.0% yield, 100.0% purity) as a white solid. LCMS: RT = 0.83 min, m/z = 619.2 $[M+H]^+$. ^{1}H NMR (Methanol-d_4, 400

MHz): δ = 7.95 (d, J = 7.2 Hz, 1H),7.33 (t, J = 8.0 Hz, 1H), 7.28 (t, J = 8.0 Hz, 1H), 7.07 (d, J = 7.6 Hz, 1H), 6.98 – 6.93 (m, 3H), 6.65 (s, 1H), 6.56 (s, 1H), 4.69 – 4.63 (m, 2H), 4.49 (t, J = 5.2 Hz, 1H), 4.42 (t, J = 7.2 Hz, 1H), 4.10 – 4.08 (m, 1H), 3.99 – 3.96 (m, 2H), 3.77 – 3.51 (m, 4H), 3.26 (t, J = 12.4 Hz, 1H), 3.10 – 2.96 (m, 2H), 2.76 (dd, J = 12.8, 2.8 Hz, 1H), 2.41 – 2.39 (m, 1H), 2.10 – 2.05 (m, 3H), 1.95 – 1.73 (m, 7H), 1.62 – 1.59 (m, 2H), 1.50 – 1.48 (m, 1H), 1.33 – 1.24 (m, 1H).

(5S,8S,11S)-N-cyclopentyl-7,10-dioxo-11-(2-oxopyrrolidin-1-yl)-8-(2-(piperidin-1-yl)ethyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide (20).

To a solution of compound **38d** (130.0 mg, 210.1 μmol, 1.0 *eq*) in acetonitrile (1.5 mL) was added CAN (288.0 mg, 525.3 μmol, 261.8 μL, 2.5 *eq*) in water (1.5 mL). The mixture was stirred at 60~70°C for 3 hours. TLC (dichloromethane: methanol = 10:1) showed the starting material was consumed completely. The mixture was poured into water (10 mL) and then extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with saturated sodium sulfite solution (2 × 20 mL), brine (20 mL), dried over anhydrous sodium sulfate, filtered, concentrated in vacuum to give crude compound **38d-aldehyde** (80.0 mg, 142.7 μmol, 67.9% yield, 100.0% purity) as a light yellow solid, which was used into the next step without further purification. To a solution of compound **38d-aldehyde** (80.0 mg, 142.7 μmol, 1.0 *eq*) in methanol (2.0 mL) was added piperidine (61.0 mg, 713.5 μmol, 70.5 μL, 5.0 *eq*) and acetic acid (17.0 mg, 285.4 μmol, 16.3 μL, 2.0 *eq*). Pd/C (20.0 mg, 10% purity) was added under nitrogen atmosphere. The suspension was degassed under vacuum and purged with hydrogen several times. The mixture was stirred under hydrogen (15 psi) at 30°C for 16 hours. LCMS showed the starting material was consumed completely. The mixture was filtered through celit pad, the solid was washed with methanol (4 × 4 mL). The combined filtrate was concentrated in vacuum. The residue was diluted with ethyl acetate (40 mL) and then washed with brine (10 mL), dried over anhydrous sodium sulfate, concentrated in vacuum. The residue was purified by prep-HPLC (column: Phenomenex Synergi 10 μm C18 150 × 25 mm; mobile phase: [water (0.1%trifluoroacetic acid)-acetonitrile]; B%: 28%−58%, 6min). The fraction was adjusted to pH = 7 with saturated sodium bicarbonate solution. The mixture was concentrated in vacuum to removed acetonitrile, extracted with dichloromethane (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous sodium sulfate, concentrated in vacuum. The residue was lyophilized to give **20** (30.0 mg, 47.4 μmol, 33.2% yield, 99.6% purity) as a white solid. LCMS: RT = 2.38 min, m/z 630.3 $[M+H]^+$. ^{1}H NMR (Methanol-d4, 400 MHz): δ = 7.33 (t, J = 8.0 Hz, 1H), 7.28 (t, J = 8.0 Hz, 1H), 7.09 (d, J = 7.6 Hz, 1H), 6.97 – 6.90 (m, 3H), 6.66 (s, 1H), 6.49 (s, 1H), 4.72 – 4.65 (m, 2H),4.34 (t, J = 7.2 Hz, 1H), 4.10 – 4.07 (m, 1H), 3.60 – 3.58 (m, 2H), 3.26 (t, J = 12.4 Hz, 1H), 3.09 – 2.94 (m, 2H), 2.77 (dd, J = 12.8, 3.0 Hz, 1H), 2.41 – 2.29 (m, 8H), 2.07 – 1.89 (m, 4H), 1.74 – 1.44 (m, 14H).

Benzyl (S)-3-(3-(3-((S)-3-(tert-butoxy)-2-((tert-butoxycarbonyl)amino)-3-oxopropyl)phenoxy)phenyl)-2-(1,3-dioxoisoindolin-2-yl)propanoate (34b).

To a mixture of compound **33** (2.0 g, 4.3 mmol, 1.0 *eq*), compound **32** (2.4 g, 6.5 mmol, 1.5 *eq*), copper acetate (1.2 g, 6.5 mmol, 1.5 *eq*) and triethylamine (2.2 g, 21. 7 mmol, 3.0 mL, 5.0 *eq*) in dichloromethane (25.0 mL) was added 4A molecular sieves (5.0 g). The mixture was stirred at 20°C for 16 hours under oxygen atmosphere (15 psi). The reaction mixture

was concentrated in *vacuo.* The residue was purified by column chromatography (petroleum ether: ethyl acetate = 20:1 ~ 3:1) to afford compound **34b** (2.3 g, 3.2 mmol, 72.8% yield, 98.9% purity) as yellow gum. LCMS: RT = 1.10 min, m/z = 743.3 $[M+Na]^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.82 – 7.78 (m, 2H), 7.74 – 7.70 (m, 2H), 7.35 – 7.28 (m, 5H), 7.13 (d, J = 8.0 Hz, 2H), 6.88 (d, J = 6.4 Hz, 2H), 6.84 – 6.83 (m, 1H), 6.78 – 6.75 (m, 2H), 6.67 (dd, J = 1.6 Hz, 8.0 Hz, 1H), 5.26 – 5.17 (m, 3H), 5.07 (d, J = 8.0 Hz, 1H), 4.45 – 4.41 (m, 1H), 3.61 – 3.48 (m, 2H), 3.06 – 2.91 (m, 2H), 1.39 – 1.38 (m, 18H).

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-(1,3-dioxoisoindolin-2-yl)propanoate (36c).

To a solution of compound **34b** (2.3 g, 3.2 mmol, 1.0 *eq*) in dichloromethane (40 mL) was added trifluoroacetic acid (12.3 g, 108.1 mmol, 8.0 mL, 33.9 *eq*) drop wise at 0°C. The mixture was stirred for 4 hours at 0°C. TLC (petroleum ether: ethyl acetate = 3:1) and LCMS showed the starting material was consumed. The reaction mixture was slowly added to saturated sodium bicarbonate (200 mL), extracted with ethyl acetate (3 × 200 mL). The combined organic phase was washed with brine (100 mL), dried over anhydrous sodium sulfate and concentrated in *vacuo to a*fford compound **34b-amine** (2.0 g, crude) as yellow gum. LCMS: RT = 0.84 min, m/z = 621.3 $[M+H]^+$. ^{1}H NMR (CD_3OD, 400 MHz): δ = 7.82 (s, 4H), 7.30 (s, 5H), 7.22 – 7.13 (m, 2H), 6.97 (d, J = 8.0 Hz, 1H), 6.90 – 6.78 (m, 4H), 6.68 (dd, J = 2.0 Hz, 8.4 Hz, 1H), 5.24 – 5.20 (m, 3H), 4.08 – 4.04 (m, 1H), 3.58 – 3.40 (m, 2H), 3.09 (d, J = 7.2 Hz, 2H), 1.44 – 1.39 (m, 9H). To a solution of compound **35b** (1.1 g, 3.2 mmol, 1.0 *eq*) and DIPEA (1.3 g, 9.7 mmol, 1.7 mL, 3.0 *eq*) in DMF (13.0 mL) was added HBTU (1.6 g, 4.2 mmol, 1.3 *eq*) wise-portion at 0°C. The mixture was stirred for 10 minutes at 0°C. A solution of compound **34b-amine** (2.0 g, 3.2 mmol, 1.0 *eq*) in DMF (8.0 mL) was added drop wise at 0°C. The mixture was stirred for 20 minutes at 20°C. LCMS and TLC (petroleum ether: ethyl acetate = 2:1) showed desired product was detected. The reaction mixture was poured into water (100 mL), extracted with ethyl acetate (3 × 100 mL). The combined organic phase was washed with brine (3 × 100 mL), dried over anhydrous sodium sulfate, filtered and concentrated in *vacuo.* The residue was purified by column (petroleum ether: ethyl acetate = 10:1 ~ 1:1), followed by reverse phase column (0.1% trifluoroacetic acid in water/acetonitrile) to afford compound **36c** (1.9 g, 2.1 mmol, 64.7% yield, 100.0% purity) as yellow gum. LCMS: RT = 1.13 min, m/z = 912.4 $[M+H]^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.81 – 7.77 (m, 2H), 7.72 – 7.69 (m, 2H), 7.34 – 7.26 (m, 10H), 7.13 – 7.04 (m, 3H), 6.87 (t, J = 5.2 Hz, 2H), 6.77 – 6.76 (m, 2H), 6.63 (dd, J = 2.0 Hz, 8.0 Hz, 1H), 6.02 – 6.00 (m, 1H), 5.25 – 5.17 (m, 3H), 5.13 – 5.09 (m, 2H), 4.73 – 4.66 (m, 1H), 4.65 (br. s, 1H), 4.38 (br. s, 1H), 4.01 – 3.97 (m, 2H), 3.69 – 3.56 (m, 4H), 3.06 – 3.05 (m, 2H), 2.04 – 1.96 (m, 2H), 1.38 – 1.37 (m, 9H), 1.27(s, 2H).

tert-butyl (5S,8S,11S)-8-((1,3-dioxan-2-yl)methyl)-11-(1,3-dioxoisoindolin-2-yl)-7,10-dioxo-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxylate (37c).

A solution of compound **36c** (1.9 g, 2.1 mmol, 1.0 *eq*) in THF (30.0 mL) was purged with nitrogen 10 minutes, $Pd(OH)_2$/C (0.2 g, 10% purity on carbon) and Pd/C (0.2 g, 10% purity on carbon) was added in one-portion. The mixture was degassed with hydrogen three times, then stirred for 3 hours at 20°C under hydrogen atmosphere (15 psi). LCMS showed about

20% of intermediate mass was observed. The mixture was stirred for 2 hours at 20°C under hydrogen (15 psi). LCMS showed one main peak with desired mass was detected. The reaction mixture was filtered and washed with methanol (2 × 30 mL) to afford compound **36c-amino acid** (1.2 g, 1.7 mmol, 84.0% yield) as a white solid. LCMS: RT = 0.85 min, *m*/*z* = 688.2 $[M+H]^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.80 (s, 4H), 7.17 – 7.11 (m, 2H), 6.97 – 6.92 (m, 1H), 6.87 – 6.85 (m, 2H), 6.78 – 6.75 (m, 2H), 6.64 – 6.61 (m, 1H), 5.12 (dd, *J* = 4.8 Hz, 11.6 Hz, 1H), 4.83 – 4.82 (m, 1H), 4.67 (dd, *J* = 5.6 Hz, 8.8 Hz, 1H), 4.11 – 4.05 (m, 3H), 3.85 – 3.76 (m, 2H), 3.54 – 3.41 (m, 2H), 3.17 – 3.12 (m, 1H), 3.00 – 2.94 (m, 1H), 2.22 – 2.17 (m, 1H), 2.09 – 2.03 (m, 1H), 1.46 – 1.36 (m, 11H). To a solution of compound **36c-amino acid** (500.0 mg, 727.0 μmol, 1.0 *eq*) and DIPEA (371.0 mg, 2.9 mmol, 500.0 μL, 4.0 *eq*) in DMF (50.0 mL) was added a mixture of EDCI (500 .0mg, 2.6 mmol, 3.6 *eq*) and HOBt (58.9 mg, 436.2 μmol, 0.6 *eq*) at 0°C. The mixture was stirred for 12 hours at 20°C. LCMS showed desired mass was detected. The reaction mixture was poured into ice-water (50 mL) and HCl (1N, 10 mL), a lot of white precipitate was formed and collected by filter. The residue was dried under in *vacuo* and purified by column chromatography (SiO_2, petroleum ether : ethyl acetate = 10:1 ~ 1:1) to afford compound **37c** (0.1 g, 179.2 μmol, 24.7% yield, 100.0% purity) as a white solid. Notes: This reaction worked well at low concentration (1.5 mol/L) and the Pht group is unstable at base condition. If the reaction is used for the next step directly after work-up, the yield can be improved. LCMS: RT = 1.05 min, *m*/*z* = 614.3 $[M-t\text{-}Bu+H]^+$; 670.4 $[M+H]^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.87 – 7.84 (m, 2H), 7.76 – 7.73 (m, 2H), 7.41 (t, *J* = 8.0 Hz, 1H), 7.33 (t, *J* = 8.0 Hz, 1H), 7.12 (d, *J* = 7.6 Hz, 1H), 7.08 – 7.05 (m, 3H), 7.01 – 7.00 (m, 1H), 6.96 (d, *J* = 7.6 Hz, 1H), 6.80 – 6.79 (m, 1H), 6.53 (d, *J* = 7.6 Hz, 1H), 5.14 (dd, *J* = 2.0 Hz, 11.6 Hz, 1H), 4.73 – 4.68 (m, 2H), 4.57 (dd, *J* = 4.0 Hz, 6.4 Hz, 1H), 3.96 – 3.83 (m, 3H), 3.64 – 3.49 (m, 2H), 3.25 – 3.20 (m, 2H), 3.05 (dd, *J* = 6.0 Hz, 14.0 Hz, 1H), 2.29 – 2.23 (m, 1H), 1.87 – 1.81 (m, 1H), 1.44 – 1.42 (m, 9H), 1.29 – 1.22 (m, 2H).

Tert-butyl (5S,8S,11S)-8-((1,3-dioxan-2-yl)methyl)-11-amino-7,10-dioxo-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxylate (39).

To a solution of compound **37c** (180.0 mg, 268.8 μmol, 1.0 *eq*) in THF (2.0 mL) was added hydrazine hydrate (26.9 mg, 537.5 μmol, 26.1 μL, 2.0 *eq*). The mixture was stirred for 1.5 hours at 25°C, then heated to 60°C and stirred for 12 hours. LCMS showed desired mass was detected. The reaction mixture was concentrated in *vacuo.* The residue was triturated with ethyl acetate (5 mL). The organic phase was concentrated in *vacuo* and then purified by reverse column (0.1% of trifluoroacetic acid in water/acetonitrile). The fraction was basified to pH=8 with saturated aqueous sodium bicarbonate, extracted with dichloromethane (3 × 50 mL). The combined organic phase was washed with brine (50 mL), dried over anhydrous sodium sulfate, filtered and concentrated in *vacuo to a*fford **39** (110.0 mg, 203.9 μmol, 75.8% yield, 100.0% purity) as a white solid. LCMS: RT = 0.95 min, *m*/*z* = 540.3 $[M+Na]^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.33 (d, *J* = 8.4 Hz, 1H), 7.37 (t, *J* = 8.0 Hz, 1H), 7.31 (t, *J* = 8.0 Hz, 1H), 7.09 – 7.05 (m, 2H), 6.97 (d, *J* = 7.6 Hz, 1H), 6.87 – 6.85 (m, 1H), 6.80 – 6.79 (m, 1H), 6.31 (t, *J* = 2.0 Hz, 1H), 4.69 – 4.65 (m, 1H), 4.61 (t, *J* = 5.2 Hz, 1H), 4.55 (t, *J* = 6.8 Hz, 1H), 4.15 – 4.11 (m, 1H), 4.04 – 4.00 (m, 2H), 3.82 (td, *J* = 2.4 Hz, 12.0 Hz, 1H), 3.72 (td, *J* = 2.4 Hz, 12.0 Hz, 1H), 3.26 (d, *J* = 3.2 Hz, 1H), 3.16 – 3.11(m, 1H), 3.00 (dd, *J* =

7.6 Hz, 14.4 Hz, 1H), 2.85 (dd, *J* = 10.4 Hz, 14.0 Hz, 1H), 1.97 – 1.87 (m, 2H), 1.53 (s, 9H), 1.36 – 1.26 (m, 2H).

Tert-butyl (5S,8S,11S)-8-((1,3-dioxan-2-yl)methyl)-7,10-dioxo-11-(2-oxooxazolidin-3-yl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxylate (40a).

To a solution of **39** (280.0 mg, 518.9 μmol, 1.0 *eq*) and triethylamine (157.5 mg, 1.6 mmol, 216.7 μL, 3.0 *eq*) in THF (3.0 mL) was added 2-chloroethyl carbonochloridate (112.0 mg, 783.4 μmol, 80.6 μL, 1.5 *eq*) at 0°C. The mixture was stirred for 30 minutes at 0°C. LCMS showed most of the starting material was consumed and one main peak with desired mass was observed. The reaction mixture was poured into water (10 mL), extracted with ethyl acetate (2 × 10 mL). The combined organic phase was washed with brine (2 × 10 mL), dried over anhydrous sodium sulfate, filtered and concentrated in *vacuo.* The residue was purified by reverse column (0.1% trifluoroacetic acid in water/acetonitrile) to afford compound **39-chloride** (230.0 mg, 356.0 μmol, 68.6% yield) as a white solid. LCMS: RT = 1.01 min, *m*/*z* 646.4 $[M+H]^+$. ^1H NMR (CD_3OD, 400 MHz): δ = 7.30 – 7.26 (m, 2H), 6.99 – 6.97 (m, 4H), 6.89 – 6.86 (m, 1H), 6.63 (s, 1H), 6.38 (s, 1H), 4.62 – 4.61 (m, 1H), 4.55 – 4.49 (m, 1H), 4.37 – 4.30 (m, 2H), 4.00 – 3.96 (m, 2H), 3.80 – 3.67 (m, 4H), 3.35 – 3.34 (m, 2H), 3.25 – 3.21 (m, 1H), 2.99 – 2.85 (m, 3H), 1.87 – 1.80 (m, 2H), 1.52 – 1.49 (m, 9H), 1.32 – 1.27 (m, 2H). To a solution of compound **39-chloride** (120.0 mg, 185.7 μmol, 1.0 *eq*) and sodium iodide (41.8 mg, 278.6 μmol, 1.5 *eq*) in DMF (0.5 mL) was added cesium carbonate (151.3 mg, 464.3 μmol, 2.5 *eq*) at 0°C. The mixture was stirred for 2 hours at 25°C. LCMS showed one main peak with desired mass was observed. The reaction mixture was poured into water (20 mL), extracted with ethyl acetate (3 × 20 mL). The combined organic phase was washed with brine (30 mL), dried over anhydrous sodium sulfate, filtered and concentrated in *vacuo.* The residue was purified by column (petroleum ether: ethyl acetate = 10:1 ~ 1:2) to afford compound **40a** (70.0 mg, 105.0 μmol, 56.5% yield, 91.4% purity) as a white solid. LCMS: RT = 0.86 min, *m*/*z* = 554.3 $[M-t\text{-}Bu+H]^+$; 632.3$[M+Na]^+$. ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.38 (t, *J* = 8.0 Hz, 1H), 7.24 (d, *J* = 8.0 Hz, 1H), 7.17 (d, *J* = 7.6 Hz, 1H), 7.03 (dd, *J* = 2.0 Hz, 8.4 Hz, 1H), 6.95 (dd, *J* = 1.6 Hz, 8.4 Hz, 1H), 6.88 (s, 1H), 6.76 (d, *J* = 7.2 Hz, 1H), 6.62 (d, *J* = 6.0 Hz, 1H), 6.44 (d, *J* = 7.6 Hz, 1H), 6.23 (s, 1H), 4.61 – 4.57 (m, 2H), 4.50 – 4.45 (m, 2H), 4.38 (t, *J* = 8.0 Hz, 2H), 4.03 – 3.97 (m, 3H), 3.75 – 3.61 (m, 3H), 3.29 (t, *J* = 12.4 Hz, 1H), 3.18 (dd, *J* = 5.2 Hz, 14.0 Hz, 1H), 3.04 (dd, *J* = 4.0 Hz, 14.0 Hz, 1H), 2.91 – 2.88 (m, 1H), 2.09 – 1.98 (m, 2H), 1.49 (s, 9H), 1.28 – 1.25 (m, 2H).

(5S,8S,11S)-8-((1,3-dioxan-2-yl)methyl)-7,10-dioxo-11-(2-oxooxazolidin-3-yl)-N-(2,2,2-trifluoroethyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide (41a).

To a solution of compound **40a** (55.0 mg, 90.2 μmol, 1.0 *eq*) in dichloromethane (2.0 mL) was added trifluoroacetic acid (847.0 mg, 7.4 mmol, 550.0 μL, 82.3 *eq*) drop wise at 0°C. The mixture was stirred for 2 hours at 25°C. LCMS showed most of the starting material was consumed and the desired mass was observed. The reaction mixture was poured into water (20 mL), adjusted to pH=5 with saturated sodium bicarbonate, extracted with ethyl acetate (2 × 15 mL). The combined organic phase was washed with brine (3 × 20 mL), dried over anhydrous sodium sulfate and concentrated in *vacuo to* afford compound **40a-acid** (50.0 mg, crude) as a white solid. LCMS: RT = 0.82 min, *m*/*z* = 554.2 $[M+H]^+$, purity:

50.4%. To a solution of compound **40a-acid** (50.0 mg, 90.3 μmol, 1 *eq*), DIPEA (35.0 mg, 271.0 μmol, 47.2 μL, 3.0 *eq*) and 2,2,2-trifluoroethanamine (9.0 mg, 90.3 μmol, 7.1 μL, 1.0 *eq*) in DMF (1.0 mL) was added HOBt (7.3 mg, 54.2 μmol, 0.6 *eq*) and EDCI (26.0 mg, 135.5 μmol, 1.5 *eq*) at 0°C. The mixture was stirred for 12 hours at 25°C. LCMS showed the desired mass was observed. The reaction mixture was poured into water (10 mL), extracted with ethyl acetate (3 × 10 mL). The combined organic phase was washed with brine (2 × 20 mL), dried over anhydrous sodium sulfate and concentrated in *vacuo* to afford compound **41a** (50.0 mg, crude) as yellow gum and used directly without further purification. LCMS: RT = 0.78 min, m/z = 635.1 $[M+H]^+$, purity: 83.9%.

(5S,8S,11S)-7,10-dioxo-11-(2-oxooxazolidin-3-yl)-8-(2-(piperidin-1-yl)ethyl)-N-(2,2,2-trifluoroethyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide (12).

To a solution of compound **41a** (50.0 mg, 78.8 μmol, 1.0 *eq*) in acetonitrile (0.8 mL) was added CAN (108.0 mg, 197.0 μmol, 98.2 μL, 2.5 *eq*) in water (0.8 mL). The mixture was stirred for 2 hours at 70°C. LCMS showed desired mass was detected. The reaction mixture was poured into water (10 mL), extracted with ethyl acetate (2 × 20 mL). The combined organic phase was washed with saturated sodium sulfite (20 mL), brine (20 mL), dried over anhydrous sodium sulfate and concentrated in *vacuo* to afford compound **41a-aldehyde** (50 mg, crude) as a white solid and used directly without further purification. LCMS: RT = 0.88 min, m/z = 599.3 $[M+Na]^+$. To a solution of compound **41a-aldehyde** (50.0 mg, 86.7 μmol, 1.0 *eq*) and piperidine (14.8 mg, 173.5 μmol, 17.1 μL, 2.0 *eq*) in methanol (1.0 mL) was added acetic acid (1.0 mg, 17.4 μmol, 1.0 μL, 0.2 *eq*). The mixture was purged with nitrogen atmosphere 10 minutes, then Pd/C (20.0 mg, 10% purity) was added in one portion. The mixture was degassed with hydrogen three times and stirred for 12 hours at 25°C under hydrogen atmosphere (15 psi). LCMS showed the desired mass was observed. The reaction mixture was filtered and the filtrate was concentrated in *vacuo.* The residue was purified by prep-TLC (petroleum ether: ethyl acetate: ethanol = 1:6:2), followed by prep-HPLC (column: Phenomenex Synergi 10 μm C18 150 × 25 mm; mobile phase: [water (0.04%NH_3H_2O+10mM NH_4HCO_3)-ACN]; B%: 40%−67%, 10min) to afford **12** (3.5 mg, 5.2 μmol, 6.0% yield, 96.2% purity) as a white solid. LCMS: RT = 1.70 min, m/z = 646.3 $[M+H]^+$. ^1H NMR ($CDCl_3$, 400 MHz) δ = 8.06 (s, 1H), 7.46 (s, 1H), 7.38 (d, J = 8.0 Hz, 1H), 7.30 (t, J = 8.0 Hz, 1H), 7.30 – 7.25 (m, 1H), 7.11 (d, J = 8.0 Hz, 1H), 7.09 – 7.05 (m, 2H), 6.98 – 6.97 (m, 1H), 6.54 (s, 1H), 6.47 (s, 1H), 4.89 (br. s, 1H), 4.45 (dd, J = 1.6 Hz, 8.0 Hz, 1H), 4.38 (t, J = 8.0 Hz, 2H), 4.09 – 4.05 (m, 3H), 3.73 – 3.71 (m, 2H), 3.35 – 3.25 (m, 1H), 3.10 – 3.06 (m, 2H), 2.95 – 2.80 (m, 1H), 2.38 – 2.32 (m, 5H), 1.95 – 1.80 (m, 1H), 1.48 – 1.42 (m, 9H).

Tert-butyl (5S,8S,11S)-8-((1,3-dioxan-2-yl)methyl)-11-(methylsulfonamido)-7,10-dioxo-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxylate (40b).

To a solution of **39** (230.0 mg, 426.2 μmol, 1.0 *eq*) in dichloromethane (3.0 mL) was added DIPEA (148.4 mg, 1.2 mmol, 0.2 mL, 2.7 *eq*), and then methylsufonyl chloride (0.6 g, 5.1 mmol, 391.9 μL, 11.9 *eq*) was added at 0°C drop wise. The mixture was stirred at 25°C for 1 hour. TLC (petroleum ether: ethyl acetate = 1:1) showed the starting material was consumed and desired mass was observed on LCMS. The reaction mixture was poured into water (20 mL), extracted with ethyl acetate (3 × 30 mL). The combined organic phase

was washed with brine (20 mL), dried over anhydrous sodium sulfate and concentrated in *vacuo.* The residue was purified by prep-TLC (SiO_2, petroleum ether: ethyl acetate = 1:1) to afford compound **40b** (120.0 mg, 194.3 μmol, 45.6% yield, 100.0% purity) as a white solid. LCMS: RT = 0.93 min, *m*/*z* 618.3 $[M+H]^+$. ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.38 (t, *J* = 8.0 Hz, 1H), 7.30 – 7.29 (m, 1H), 7.16 (d, *J* = 8.0 Hz, 1H), 7.03 – 6.96 (m, 2H), 6.97 (d, *J* = 7.6 Hz, 1H), 6.58 (s, 1H), 6.53 – 6.50 (m, 2H), 5.55 (br .s, 1H), 4.66 – 4.64 (m, 2H), 4.54 – 4.52 (m, 1H), 4.20 – 4.15 (m, 1H), 4.00 (dd, *J* = 4.4 Hz, 11.2 Hz, 2H), 3.74 – 3.63 (m, 2H), 3.28 (dd, *J* = 4.0 Hz, 10.0 Hz, 1H), 3.16 – 3.10 (m, 1H), 2.97 – 2.87 (m, 5H), 2.11 – 2.05 (m, 1H), 1.95 – 1.87 (m, 1H), 1.51 – 1.44 (m, 9H), 1.33 – 1.25 (m, 2H).

(5S,8S,11S)-8-((1,3-dioxan-2-yl)methyl)-N-((1-methyl-1H-pyrazol-4-yl)methyl)-11-(methylsulfonamido)-7,10-dioxo-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide (41b).

To a solution of compound **40b** (140.0 mg, 226.6 μmol, 1.0 *eq*) in dichloromethane (1.5 mL) was added trifluoroacetic acid (770.0 mg, 6.8 mmol, 0.5 mL, 29.8 *eq*) drop wise at 0°C. The mixture was stirred for 2 hours at 20°C. LCMS showed one main peak with desired mass was detected. The reaction mixture was poured into water (10 mL), adjusted to pH = 5 with saturated sodium bicarbonate, extracted with ethyl acetate (3 × 20 mL). The combined organic phase was washed with brine (20 mL), dried over anhydrous sodium sulfate and concentrated in *vacuo* to afford compound **40b-acid** (120.0 mg, crude) as a white solid. LCMS: RT = 0.80 min, *m*/*z* 562.2 $[M+Na]^+$. To a solution of compound **40b-acid** (120.0 mg, 213.7 μmol, 1.0 *eq*) and (1-methylpyrazol-4-yl) methanamine (47.5 mg, 427.4 μmol, 2.0 *eq*) in DMF (3.0 mL) was added a mixture of EDCI (90.0 mg, 469.5 μmol, 2.2 *eq*) and HOBt (20.2 mg, 149.6 μmol, 0.7 *eq*) at 0°C. The mixture was stirred for 5 hours at 25°C. LCMS showed one main peak with desired mass was detected. The reaction mixture was concentrated in *vacuo.* The residue was dissolved in ethyl acetate (20 mL), washed with HCl (0.01N, 2 × 10 mL), brine (3 × 20 mL), dried over anhydrous sodium sulfate and concentrated in *vacuo.* The residue was triturated with ethyl acetate: petroleum ether = 1:100 (50 mL) to afford compound **41b** (75.0 mg, 103.1 μmol, 48.3% yield, 90.0% purity) as a white solid. LCMS: RT = 0.79 min, *m*/*z* = 655.3 $[M+H]^+$, purity: 62.9%. ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.88 (d, *J* = 8.4 Hz, 1H), 7.74 (d, *J* = 8.0 Hz, 1H), 7.57 – 7.53 (m, 1H), 7.51 – 7.43 (m, 1H), 7.34 – 7.26 (m, 2H), 7.08 – 7.05 (m, 1H), 7.00 – 6.97 (m, 2H), 6.99 – 6.89 (m, 1H), 6.67 – 6.57 (m, 1H), 6.42 – 6.35 (m, 1H), 4.97 – 4.94 (m, 2H), 4.77 – 4.70 (m, 4H), 4.67 – 4.51 (m, 2H), 4.27 – 4.23 (m, 1H), 3.99 – 3.64 (m, 4H), 3.27 – 3.13 (m, 2H), 3.05 – 2.90 (m, 5H), 1.99 – 1.81 (m, 2H), 1.33 – 1.37 (m, 2H).

(5S,8S,11S)-N-((1-methyl-1H-pyrazol-4-yl)methyl)-11-(methylsulfonamido)-7,10-dioxo-8-(2-(piperidin-1-yl)ethyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide (14).

To a solution of compound **41b** (75.0 mg, 114.6 μmol, 1.0 *eq*) in acetonitrile (1.0 mL) was added a solution of CAN (157.0 mg, 286.4 μmol, 142.7 μL, 2.5 *eq*) in water (1.0 mL). The mixture was stirred for 2 hours at 70°C. LCMS showed the starting material was consumed. The reaction mixture was poured into ethyl acetate (30 mL), washed with saturated sodium sulfite (2 × 20 mL), brine (2 × 20 mL), dried over anhydrous sodium sulfate, filtered and concentrated in *vacuo to a*fford compound **41b-aldehyde** (70.0 mg, crude) as a white

solid. A solution of compound **41b-aldehyde** (70.0 mg, 117.3 μmol, 1.0 *eq*), piperidine (1.4 mg, 16.8 μmol, 1.7 μL, 1.0 *eq*) and acetic acid (7.1 mg, 117.3 μmol, 6.7 μL, 1.0 *eq*) in methanol (2.0 mL) was degassed with nitrogen three times and then Pd/C (20.0 mg, 10% purity on carbon) was added in one portion. The mixture was degassed with hydrogen and stirred for 12 hours at 25°C under hydrogen atmosphere (15 psi). LCMS showed one main peak with desired mass was detected. The reaction mixture was filtered, and the filtrate was concentrated in *vacuo.* The residue was purified by prep-HPLC (Column: Boston pH-lex 10um C18 150 × 25 mm, mobile phase: [water (0.1%TFA)-ACN]; B%: 19%−49 %, 8min). The fraction was basified to pH = 8 with saturated aqueous sodium bicarbonate and extracted with dichloromethane (3 × 30 mL). The combined organic phase was washed with brine (2 × 20 mL), dried over anhydrous sodium sulfate and concentrated in *vacuo.* The residue was purified by prep-HPLC (column: Gemini 5 μm C18 150 × 25 mm; mobile phase: [water (0.05% ammonia hydroxide v/v)-ACN]; B%: 25%−55%, 12min) to afford compound **14** (2.3 mg, 3.2 μmol, 2.8% yield, 95.1% purity) as a white solid. LCMS: RT = 3.41 min, m/z = 666.3 $[M+H]^+$. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.53 (s, 1H), 7.42 (s, 1H), 7.32 – 7.25 (m, 2H), 7.06 (d, J = 7.2 Hz, 1H), 6.96 (d, J = 7.2 Hz, 2H), 6.88 (dd, J = 2.0 Hz, 8.0 Hz, 1H), 6.65 – 6.64 (m, 1H), 6.36 (s, 1H), 4.64 (dd, J = 3.2 Hz, 10.0 Hz, 1H), 4.38 (t, J = 7.2 Hz, 1H), 4.27 – 4.24 (m, 3H), 3.85 (s, 3H), 3.13 – 3.08 (m, 1H), 2.96 – 2.86 (m, 6H), 2.38 – 2.25 (m, 6H), 1.81 – 1.74 (m, 2H), 1.57 – 1.55 (m, 4H), 1.46 – 1.42 (m, 2H).

Tert-butyl (5S,8S,11S)-8-((1,3-dioxan-2-yl)methyl)-11-(1,1-dioxidoisothiazolidin-2-yl)-7,10-dioxo-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxylate (40c).

To a solution of **39** (250.0 mg, 463.3 μmol, 1.0 *eq*) and triethylamine (140.6 mg, 1.4 mmol, 193.5 μL, 3.0 *eq*) in dichloromethane (1.0 mL) was added 3-chloropropane-1-sulfonyl chloride (123.0 mg, 694.9 μmol, 84.3 μL, 1.5 *eq*) at 0°C. The mixture was stirred for 30 minutes at 0°C. LCMS showed most of the starting material was consumed and one main peak with desired mass was observed. The reaction mixture was poured into water (20 mL), extracted with ethyl acetate (2 × 20 mL). The combined organic phase was washed with brine (2 × 20 mL), dried over anhydrous sodium sulfate, filtered and concentrated in *vacuo to a*fford compound **39-sulfonamide** (250.0 mg, crude) as a white solid. LCMS: RT = 1.00 min, m/z = 680.4 $[M+H]^+$, purity: 58.3%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.34 – 7.25 (m, 2H), 7.10 – 7.06 (m, 2H), 6.99 – 6.95 (m, 3H), 6.87 – 6.85 (m, 1H), 6.62 – 6.56 (m, 1H), 6.42 – 6.41 (m, 1H), 4.62 – 4.57 (m, 3H), 4.52 – 4.49 (m, 1H), 4.12 – 4.07 (m, 2H), 3.77 – 3.66 (m, 4H), 3.27 – 3.18 (m, 3H), 2.98 – 2.93 (m, 2H), 2.88 – 2.85 (m, 1H), 2.27 – 2.22 (m, 2H), 1.95 – 1.94 (m, 1H), 1.86 – 1.80 (m, 1H), 1.53 – 1.51 (m, 9H). To a solution of compound **39-sulfonamide** (260.0 mg, 382.2 μmol, 1.0 *eq*) and sodium iodide (85.9 mg, 573.4 μmol, 1.5 *eq*) in DMF (3.0 mL) was added potassium carbonate (105.7 mg, 764.5 μmol, 2.0 *eq*) at 25°C. The mixture was stirred for 12 hours at 25°C. LCMS showed one main peak with desired mass was observed. The reaction mixture was poured into water (10 mL), extracted with ethyl acetate (3 × 20 mL). The combined organic phase was washed with brine (30 mL), dried over anhydrous sodium sulfate, filtered and concentrated in *vacuo.* The residue was purified by column chromatography (SiO_2, petroleum ether: ethyl acetate = 5:1 ~ 1:2) to afford compound **40c** (160.0 mg, 235.5 μmol, 61.6% yield, 94.8% purity) as a white solid. LCMS: RT = 0.96 min, m/z = 644.3 $[M+H]^+$. ^{1}H NMR (CD_3OD, 400 MHz): δ = 7.32 (t, J = 8.0 Hz, 1H), 7.26 (t, J = 8.0 Hz, 1H), 7.09 (d, J = 8.0 Hz, 1H), 6.96 – 6.89 (m,

3H), 6.59 (t, *J* = 1.6 Hz, 1H), 6.43 (s, 1H), 4.64 – 4.59 (m, 2H), 4.46 (t, *J* = 8.0 Hz, 1H), 4.25 (dd, *J* = 3.2 Hz, 13.0 Hz, 1H), 3.98 (dd, *J* = 4.0 Hz, 11.6 Hz, 2H), 3.78 – 3.71 (m, 2H), 3.51 (t, *J* = 6.8 Hz, 2H), 3.24 – 3.21 (m, 2H), 3.19 – 3.16 (m, 2H), 3.04 – 2.99 (m, 1H), 2.89 (dd, *J* = 3.2 Hz, 12.8 Hz, 1H), 2.37 – 2.32 (m, 2H), 1.85 – 1.80 (m, 2H), 1.49 (s, 9H), 1.32 – 1.29 (m, 2H), 0.94 – 0.85 (m, 2H).

(5S,8S,11S)-8-((1,3-dioxan-2-yl)methyl)-11-(1,1-dioxidoisothiazolidin-2-yl)-N-((1-methyl-1H-pyrazol-4-yl)methyl)-7,10-dioxo-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide (41c).

To a solution of compound **40c** (160.0 mg, 248.6 μmol, 1.0 *eq*) in dichloromethane (4.0 mL) was added trifluoroacetic acid (1.5 g, 13.5 mmol, 1.0 mL, 54.3 *eq*) at 0°C. The mixture was stirred for 2 hours at 25°C. LCMS showed desired mass was observed and part of the starting material remained, then the mixture was stirred for 1 hour at 25°C. The reaction mixture was poured into water (20 mL), extracted with ethyl acetate (3 × 30 mL). The combined organic phase was washed with brine (20 mL), dried over anhydrous sodium sulfate, filtered and concentrated in *vacuo* to afford compound **40c-acid** (150.0 mg, crude) as a white solid, which was used for the next step directly without further purification. LCMS: RT = 0.74 min, *m*/*z* = 588.3 $[M+H]^+$, purity: 35.4%. To a solution of compound **40c-acid** (150.0 mg, 255.3 μmol, 1.0 *eq*), (1-methylpyrazol-4-yl) methanamine (75.4 mg, 510.5 μmol, 2.0 *eq*, HCl salt) and DIPEA (99.0 mg, 765.8 μmol, 133.4 μL, 3.0 *eq*) in DMF (2.0 mL) was added EDCI (73.4 mg, 382.9 μmol, 1.5 *eq*) and HOBt (20.7 mg, 153.2 μmol, 0.6 *eq*) at 0°C. The mixture was stirred for 12 hours at 25°C. LCMS showed desired mass was detected. The reaction mixture was poured into water (20 mL), extracted with ethyl acetate (2 × 20 mL). The combined organic phase was washed with brine (20 mL), dried over anhydrous sodium sulfate and concentrated in *vacuo to a*fford compound **41c** (100.0 mg, crude) as a white solid, which was used for the next step directly without further purification. LCMS: RT = 0.83 min, *m*/*z* = 681.3 $[M+H]^+$, purity: 45.0%.

(5S,8S,11S)-11-(1,1-dioxidoisothiazolidin-2-yl)-N-((1-methyl-1H-pyrazol-4-yl)methyl)-7,10-dioxo-8-(2-(piperidin-1-yl)ethyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide (15).

To a solution of compound **41c** (100.0 mg, 146.9 μmol, 1.0 *eq*) in acetonitrile (1.0 mL) was added a solution of CAN (201.3 mg, 367.2 μmol, 183.0 μL, 2.5 *eq*) in water (1.0 mL). The mixture was stirred for 2 hours at 70°C. LCMS showed desired mass was detected. The reaction mixture was poured into water (10 mL), extracted with ethyl acetate (2 × 20 mL). The combined organic phase was washed with saturated sodium sulfite (20 mL), brine (20 mL), dried over anhydrous sodium sulfate and concentrated in *vacuo to a*fford compound **41c-aldehyde** (100.0 mg, crude) as a white solid, which was used for the next step directly without further purification. LCMS: RT = 0.80 min, *m*/*z* = 623.3 $[M+H]^+$, purity: 47.7%. To a solution of compound **41c-aldehyde** (100.0 mg, 160.6 μmol, 1.0 *eq*) and piperidine (27.4 mg, 321.2 μmol, 31.7 μL, 2.0 *eq*) in methanol (1.5 mL) was added acetic acid (4.8 mg, 80.3 μmol, 4.6 μL, 0.5 *eq*). The mixture was degassed with nitrogen 10 minutes, then Pd/C (0.1 g, 10% purity) was added in one-portion. The mixture was degassed with hydrogen three times and stirred for 17 hours at 25°C under hydrogen atmosphere (15 psi). LCMS showed one main peak with desired mass was observed. The reaction

mixture was filtered, and the filtrate was concentrated in v*acuo.* The residue was purified by prep-HPLC (column: Phenomenex Synergi 10 μm C18 150 × 25 mm; mobile phase: [water (0.1%TFA)-ACN]; B%: 16%−46%, 13min). The fraction was basified to pH = 8 with saturated sodium bicarbonate aqueous, extracted with dichloromethane (3 × 30 mL). The combined organic phase was washed with brine (2 × 20 mL), dried over anhydrous sodium sulfate, filtered and concentrated in *vacuo* to afford compound **15** (2.3 mg, 3.0 μmol, 1.9% yield) as a white solid. LCMS: RT = 1.71 min, *m*/*z* 692.3 $[M+H]^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.55 (t, *J* = 5.0 Hz, 1H), 8.39 (d, *J* = 8.4 Hz, 0.6H), 8.30 (d, *J* = 8.4 Hz, 0.3H), 7.53 (s, 1H), 7.41 (s, 1H), 7.36 (t, *J* = 8.0 Hz, 1H), 7.24 (t, *J* = 8.0 Hz, 1H), 7.16 (d, *J* = 7.2 Hz, 1H), 6.98 – 6.93 (m, 2H), 6.80 (d, *J* = 7.6 Hz, 1H), 6.65 (s, 1H), 6.36 (s, 1H), 4.61 – 4.58 (m, 1H), 4.46 – 4.43 (m, 1H), 4.36 (dd, *J* = 3.6 Hz, 12.0 Hz, 1H), 4.25 – 4.24 (m, 2H), 3.85 (s, 3H), 3.71 – 3.67 (m, 1H), 3.63 – 3.59 (m, 1H), 3.52 – 3.44 (m, 2H), 3.25 – 3.21 (m, 2H), 3.19 – 3.15 (m, 2H), 3.12 – 3.06 (m, 2H), 2.99 – 2.94 (m, 2H), 2.86 – 2.82 (m, 2H), 2.42 – 2.31 (m, 2H), 2.11 – 2.03 (m, 1H), 1.94 – 1.87 (m, 3H), 1.85 – 1.79 (m, 1H), 1.75 – 1.68 (m, 2H), 1.50 – 1.46 (m, 1H).

Tert-butyl (S)-2-((S)-2-((tert-butoxycarbonyl)amino)-4-morpholinobutanamido)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoate (44).

To a solution of compound **43** (1.1 g, 3.8 mmol, 1.0 *eq*) in THF (10.0 mL) was added HATU (1.7 g, 4.6 mmol, 1.2 *eq*) and DIPEA (740.0 mg, 5.7 mmol, 996.7 μL, 1.5 *eq*) at 0°C and the solution was stirred at 0°C for 30 minutes. Compound **42** (1.4 g, 4.0 mmol, 1.0 *eq*) was added to the solution at 0°C and the solution was stirred at 0°C for 2 hours. LCMS showed the starting material was consumed completely and desired mass was observed. The reaction was quenched with saturated ammonium chloride (20 mL) and the solution was extracted with ethyl acetate (3 × 60 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give a residue. The residue was purified by reverse phase flash (TFA) to give compound **44** (2.2 g, 3.6 mmol, 93.4% yield) as a light yellow gum. ^{1}H NMR (CD_3OD, 400 MHz): δ = 7.63 – 7.49 (m, 2H), 7.40 – 7.29 (m, 2H), 4.62 – 4.55 (m, 1H), 4.22 – 3.99 (m, 3H), 3.83 – 3.64 (m, 2H), 3.52 – 3.40 (m, 2H), 3.24 – 2.97 (m, 6H), 2.19 – 1.86 (m, 2H), 1.46 – 1.34 (m, 26H).

(3-((S)-2-((S)-2-amino-4-morpholinobutanamido)-3-(tert-butoxy)-3-oxopropyl)phenyl)boronic acid (45).

To a solution of compound **44** (2.2 g, 3.6 mmol, 1.0 *eq*) in dichloromethane (10.0 mL) and dioxane (1.0 mL) was added zinc bromide (4.0 g, 17.8 mmol, 5.0 *eq*) at 15°C and the solution was stirred at 15°C for 8 hours. LCMS showed trace starting material still remained and desired mass was observed. The reaction was quenched with DIPEA (3.0 mL), and the mixture was concentrated to remove the solvent. The residue was purified by reverse phase flash (TFA) to give compound **45** (1.1 g, 1.9 mmol, 53.1% yield, 94.4% purity, TFA) as an off-white solid. LCMS: RT = 0.67 min, *m*/*z* = 436.2 $[M+H]^+$, purity: 94.4%. ^{1}H NMR (CD_3OD, 400 MHz): δ = 7.66 – 7.53 (m, 3H), 7.39 – 7.31 (m, 3H), 4.63 (dd, *J* = 8.4 Hz, 6.4 Hz, 1H), 4.04 (dd, *J* = 7.2 Hz, 6.0 Hz, 1H), 3.96 – 3.85 (m, 4H), 3.46 – 3.40 (m, 2H), 3.27 – 3.26 (m, 1H), 3.16 – 3.05 (m, 4H), 2.78 – 2.74 (m, 1H), 2.45 – 2.27 (m, 2H), 2.19 – 1.86 (m, 2H), 1.41 (s, 9H).

(3-((S)-2-((S)-2-((S)-3-(3-(benzyloxy)phenyl)-2-(1,1-dioxidoisothiazolidin-2-yl)propanamido)-4-morpholinobutanamido)-3-(tert-butoxy)-3-oxopropyl)phenyl)boronic acid (47).

To a solution of compound **46** (752.0 mg, 2.0 mmol, 1.0 *eq*) in DMF (10.0 mL) was added T_3P (2.6 g, 4.0 mmol, 2.4 mL, 50% purity in ethyl acetate, 2.0 *eq*) and DIPEA (1.3 g, 10.0 mmol, 1.7 mL, 5.0 *eq*) at 0°C and the solution was stirred at 0°C for 30 minutes. Then compound **45** (1.1 g, 2.0 mmol, 1.0 *eq*, TFA) was added to the solution at 0°C and the solution was stirred at 0°C for additional 1.5 hours. LCMS showed the starting material was consumed and desired MS was detected. The reaction mixture was filtered to give a crude product. The crude product was purified by reverse phase flash (TFA) to give compound **47** (0.4 g, 441.5 μmol, 22.1% yield) as a white solid. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.46 – 7.25 (m, 10H), 6.95 – 6.80 (m, 3H), 5.10 (s, 2H), 4.59 – 4.35 (m, 3H), 4.10 – 3.90 (m, 2H), 3.74 – 3.42 (m, 5H), 3.25 – 2.85 (m, 11H), 2.85 – 2.48 (m, 2H), 2.31 – 2.21 (m, 3H), 2.05 – 1.99 (m, 2H), 1.41 (s, 9H).

tert-butyl (5S,8S,11S)-11-(1,1-dioxidoisothiazolidin-2-yl)-8-(2-morpholinoethyl)-7,10-dioxo-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxylate (48).

To a solution of compound **47** (350.0 mg, 441.5 μmol, 1.0 *eq*) in methanol (3.0 mL) was added Pd/C (40.0 mg, 10% purity) and $Pd(OH)_2$/C (40.0 mg, 10% purity) at 15°C under nitrogen atmosphere and the solution was stirred at 15°C for 12 hours under hydrogen atmosphere (15 psi). LCMS showed the starting material was consumed and desired mass was observed. The reaction was filtered by celite, and the filter cake was washed with methanol (10 mL), then the solution was concentrated under reduced pressuer to give compound **47-phenol** (300.0 mg, crude) as a light yellow gum. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.28 – 7.01 (m, 4H), 6.78 – 6.65 (m, 4H), 4.58 – 4.50 (m, 1H), 4.38 – 4.30 (m, 2H), 3.76 – 3.43 (m, 7H), 3.20 – 2.93 (m, 11H), 2.81 – 2.21 (m, 5H), 2.12 – 1.94 (m, 1H), 1.74 – 1.62 (m, 1H), 1.40 (s, 9H). A mixture of compound **47-phenol** (200.0 mg, 284.7 μmol, 1.0 *eq*), copper acetate (103.0 mg, 569.3 μmol, 2.0 *eq*), 4A molecular sieve (500.0 mg) and triethylamine (144.0 mg, 1.4 mmol, 198.1 μL, 5.0 *eq*) in dichloromethane (15.0 mL) was stirred at 20°C under air balloon (15 psi) for 13 hours. LCMS showed the starting material was consumed completely and desired mass was observed. The mixture was filtered by celite, and the filter cake was washed with dichloromethane (10 mL), the solution was concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (column: Phenomenex Synergi 10 μm C18 150 × 25 mm; mobile phase: [water (0.1%TFA)-ACN]; B%: 22%−52%, 10 minutes) to give compound **48** (30.0 mg, 38.9 μmol, 13.7% yield, 100.0% purity, TFA) as an off-white solid. LCMS: RT = 0.82 min, *m/z* = 657.3 $[M+H]^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.38 (t, *J* = 8.0 Hz, 1H), 7.24 (t, *J* = 8.0 Hz, 1H), 7.15 (d, *J* = 7.6 Hz, 1H), 7.02 (dd, *J* = 8.4 Hz, *J* = 1.6 Hz, 1H), 6.96 (dd, *J* = 8.0 Hz, *J* = 1.6 Hz, 1H), 6.80 – 6.74 (m, 3H), 6.44 (d, *J* = 7.2 Hz, 1H), 6.08 (br. s, 1H), 4.58 – 4.57 (m, 1H), 4.47 – 4.42 (m, 1H), 4.08 (dd, *J* = 12.0 Hz, 3.2 Hz, 1H), 3.96 – 3.92 (m, 4H), 3.89 – 3.83 (m, 1H), 3.59 – 3.54 (m, 1H), 3.26 – 3.16 (m, 6H), 3.06 – 2.93 (m, 2H), 2.88 – 2.80 (m, 2H), 2.45 – 2.38 (m, 2H), 1.50 (s, 9H).

(5S,8S,11S)-8-(2-morpholinoethyl)-7,10-dioxo-11-((3-sulfopropyl)amino)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxylic acid (49).

To a solution of compound **48** (15.0 mg, 22.8 μmol, 1.0 eq) in dichloromethane (0.4 mL) was added trifluoroacetic acid (154.0 mg, 1.4 mmol, 100.0 μL, 59.1 eq) at 0°C. The mixture was stirred for 12 hours at 20°C. LCMS showed desired mass was observed. The reaction mixture was concentrated in vacuo to afford compound **49** (20.0 mg, crude, TFA salt) as yellow gum, which was used for the nest step without further purification.

(5S,8S,11S)-N-cyclopentyl-11-(1,1-dioxidoisothiazolidin-2-yl)-8-(2-morpholinoethyl)-7,10-dioxo-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide (TDI-8414).

To a solution of compound **49** (20.0 mg, 32.3 μmol, 1.0 *eq*), DIPEA (17.0 mg, 129.3 μmol, 22.5 μL, 4.0 *eq*) and cyclopentanamine (6.0 mg, 64.7 μmol, 6.4 μL, 2.0 *eq*) in dichloromethane (0.5 mL) was added T_3P (41.0 mg, 64.7 μmol, 38.5 μL, 50% purity in ethyl acetate, 2.0 *eq*) drop wise at 0°C. The mixture was stirred for 1 hour at 0°C. LCMS showed one main peak with desired mass was observed. The reaction mixture was concentrated in *vacuo* to afford compound **49- sulfonic acid** (25.0 mg, crude) as yellow gum, which was used for the next step without further purification. A solution of compound **49-sulfonic acid** (22.0 mg, 32.1 μmol, 1.0 *eq,* two batches) in phosphorus oxychloride (3.8 g, 24.6 mmol, 2.3 mL, 765.8 *eq*) was stirred at 0°C for 2 hours. LCMS showed the starting material was consumed and desired mass was observed. The reaction mixture was added to ice-water (20 mL), adjusted to pH~8 with 1N sodium hydroxide, extracted with ethyl acetate (4 × 30 mL). The combined organic phase was washed with brine (2 × 30 mL), dried over anhydrous sodium sulfate, filtered and concentrated in *vacuo.* The residue (two batches) was purified by prep-HPLC (column: Luna 5μM C18 150 × 25 mm; mobile phase: [water (0.225%FA)-ACN]; B%: 23%−43%,7.8min & column: Phenomenex Gemini 10 μm C18 150 × 25mm; mobile phase: [water (10mM NH_4HCO_3)-ACN]; B%: 40%−70%, 10min) to afford **TDI-8414** (4.9 mg, 7.3 μmol, 5.4% yield, 100.0% purity) as a white solid. LCMS: RT = 1.67 min, purity: 100.0%, *m/z*: = 668.3 $[M+H]^+$. ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.41 (t, J = 8.0 Hz, 1H), 7.37 – 7.34 (m, 1H), 7.30 (t, J = 8.0 Hz, 1H), 7.13 (d, J = 7.2 Hz, 1H), 7.07 – 7.01 (m, 3H), 6.91 (d, J =7.2 Hz, 1H), 6.58 (s, 1H), 6.38 (s, 1H), 6.19 – 6.16 (m, 1H), 4.73 – 4.69 (m, 1H), 4.19 – 4.06 (m, 3H), 3.87 – 3.82 (m, 1H), 3.69 – 3.95 (m, 2H), 3.59 – 3.48 (m, 3H), 3.22 – 2.98 (m, 6H), 2.49 – 2.31 (m, 6H), 2.99 – 1.89 (m, 3H), 1.72 – 1.72 (m, 4H), 1.58 – 1.42 (m, 4H), 1.32 – 1.22 (m, 1H).

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.[22]

Antimalarial activity in the erythrocytic stage.—Parasite growth inhibition assays were performed as reported.[22]

Ex vivo EC_{50} values against *P. falciparum* field isolates in Uganda.—The activity of selected compounds was tested against *P. falciparum* isolates using a 72-h growth inhibition assay with parasite DNA readout by Sybr Green detection as described.[26] These isolates were collected from patients living in the Tororo and Busia Districts, Uganda, who were newly diagnosed with *P. falciparum* malaria before antimalarial treatment was

administered. These studies 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.

Parallel artificial membrane permeability assay (PAMPA).—All PAMPA assays were performed as reported.[22] Propranolol was used as positive control compound. Methyclothiazide was used as a negative control compound.

LLC-PK1-MDR1 assay.—Human MDR1 expressing LLC-PK1 cells (hMDR1/LLC-PK1) were used to investigate if compounds are MDR1 substrates. All the MDR1 assays were performed as reported.[22] Lucifer Yellow was co-incubated with test compound as a membrane integrity marker. Digoxin was used as positive control compound.

Kinetic solubility.—The kinetic solubility of the compounds was determined as reported.[22] Diazepam was included as the positive control in each experiment.

Live microsomal stability.—The metabolic stability of the compounds by human or mouse liver microsomes was determined as reported.[22] Flutamide was included as the positive control compound in each experiment.

Plasma stability.—To 100 μL of human/mouse plasma was added 1μL of 10 mM test compound. The mixtures were incubated at 37 °C for 0, 60, 120 min. The reactions were stopped at each time point by adding 200 μL ice-cold methanol containing 50 μM internal standard. For the T0 min samples, 200 μL ice-cold methanol containing internal standard was added to human plasma prior to addition of test compound. Precipitated proteins were pelleted by centrifuging at 13,500 rpm for 20 min at 4 °C. The supernatants were collected and analyzed on an Acquity™ UPLC / MS system, coupled with a PDA detector. Column chromatography was carried out on a C18 Column, 130Å, 1.7 μm, 2.1 mm X 100 mm. The percentage of compounds remaining at each time points relative to starting concentration were calculated using integrated UV peak areas normalized to the internal control. Procaine was included as the positive control compound in each human plasma stability assay experiment. Enalapril was included as the positive control in each mouse plasma stability assay experiment.

Hepatocytes stability.—50 μL diluted compound was added to 50 μL hepatocyte cells (2×10^6 cells/mL in HT medium) was dispensed into 96-well plated. After incubating at 37 °C in 5% CO_2, 100 μL cold acetonitrile containing internal standard was added to the mixture to stop the reaction. For the T0, 100 μl cold acetonitrile containing internal standard was added to hepatocyte cell prior to addition of diluted compound. After centrifugation at 3000 rpm for 10 min at 4 °C, the supernatant was collected and analyzed by LC/MS-MS. Diazepam was included as the positive control in each experiment. The intrinsic clearance (in μL/min/10^6 cells) was calculated by dividing incubation volume (μL) by number of cells in incubation ($\times10^6$ cells) and multiplying by elimination rate constant (min^{-1}). The elimination rate constant is the negative gradient, which was calculated from the remaining ratio up to 2 h.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

PAMPA	parallel artificial membrane permeability assay
MDR	MDR1-MDCK permeability
ER	efflux ratio
m/hLM	mouse/human liver microsome stability
cLogP	calculated partition coefficient
TPSA	topological polar surface area

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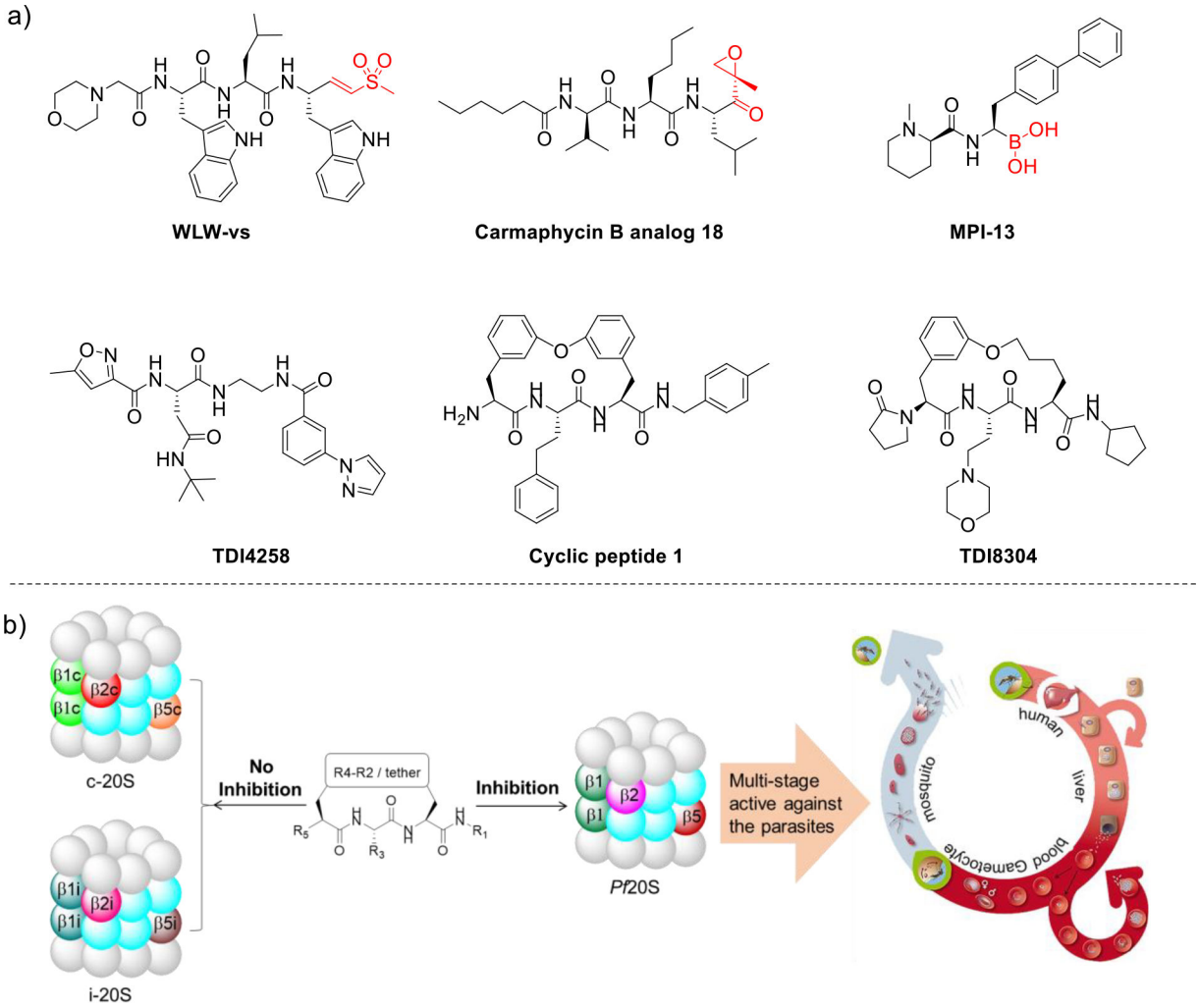


Figure 1.

a) Representative Pf20S inhibitors. b) Macrocyclic peptides selectively inhibit Pf20S over human c20S and i20S. Proteasome inhibitors are active against the *Plasmodium* parasites at multiple stages of their life cycle and are transmission blocking.

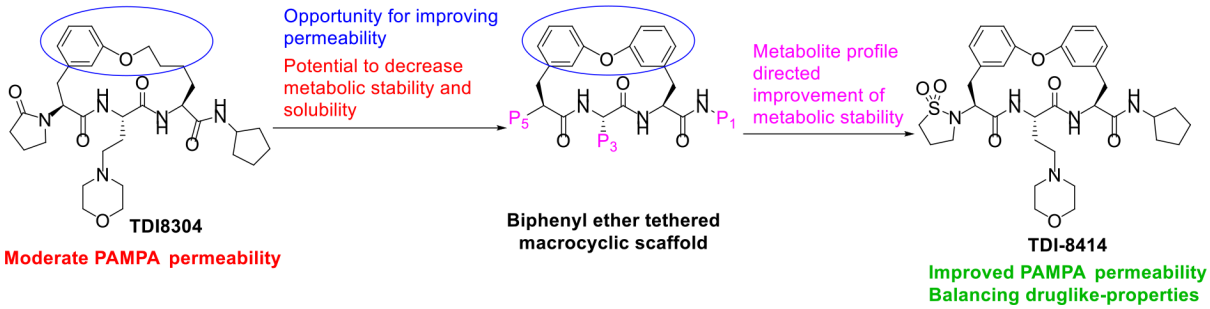


Figure 2.

Optimization strategy for biphenyl ether tethered macrocycles as antimalarial proteasome inhibitors. Note: TDI-8414 is in Table 3.

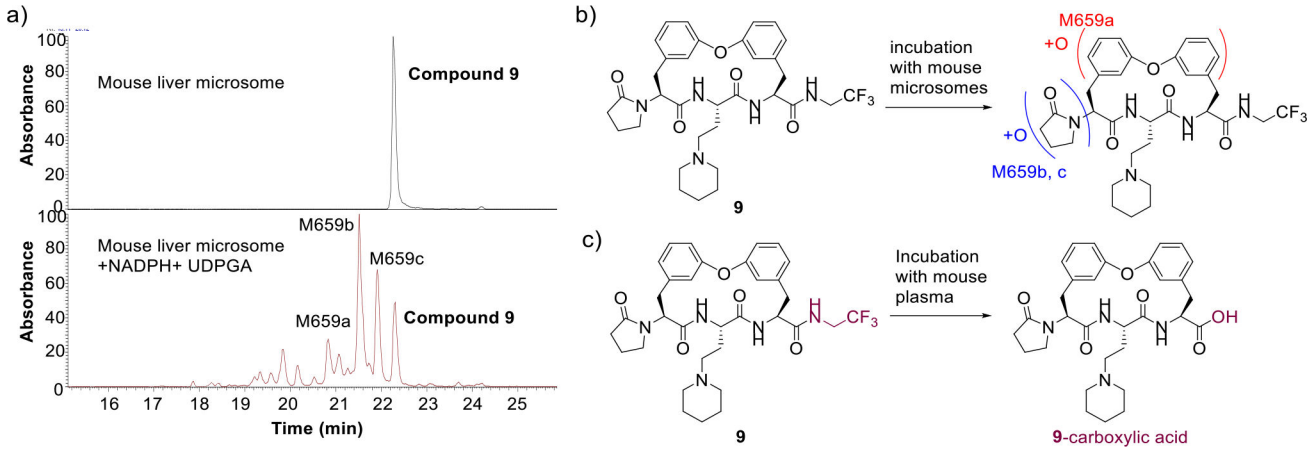


Figure 3.

Metabolite Profile of **9** in mouse liver microsome (MLM) and mouse plasma. a) LC/MS profile of **9** after incubation with MLM or MLM + NADPH + UDPGA; b) Chemical structures of metabolites of **9** in mouse liver microsome; c) Chemical structures of metabolites of **9** in mouse plasma.

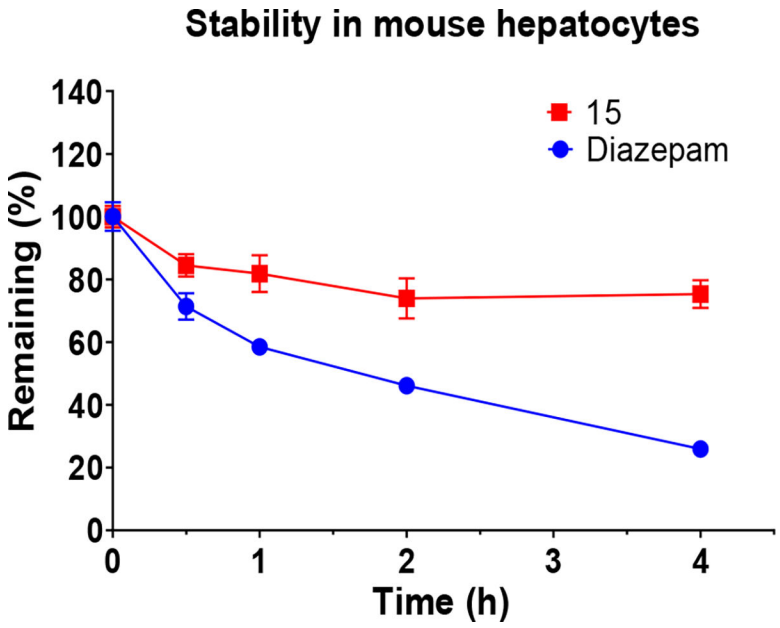


Figure 4.

In vitro metabolic stability of macrocycle **15** in mouse hepatocytes. Diazepam was used as a positive control compound.

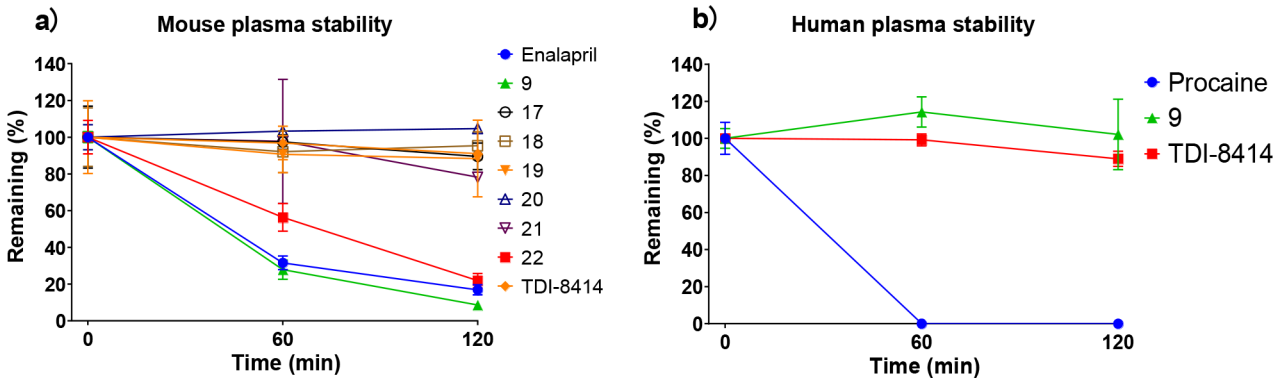


Figure 5.

a) Mouse plasma stability of selected macrocycles; b) Human plasma stability of selected macrocycles. Positive control compounds were enalapril (mouse plasma) and procaine (human plasma).

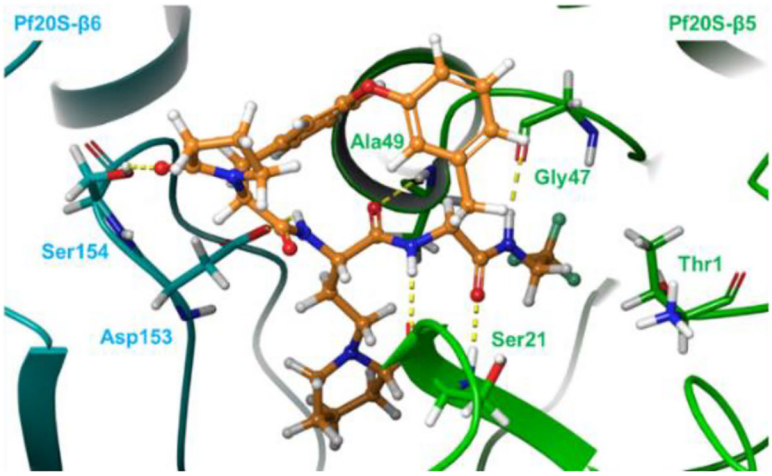


Figure 6.

Docking pose of **9** into Pf20S (PDB: 5FMG). **9** is shown in orange. The β5 subunit is in green. The β6 subunit is in cyan. Hydrogen bonds are indicated by dashed yellow lines.

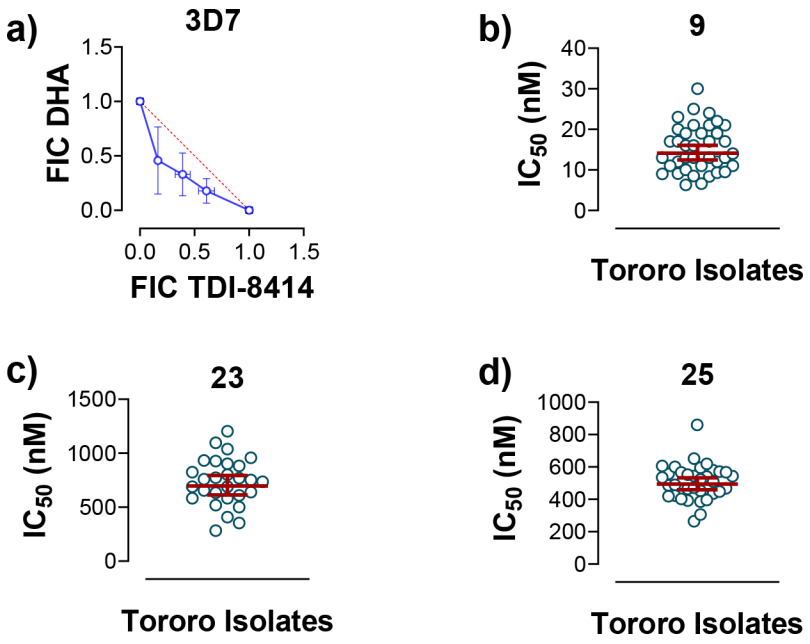


Figure 7.

Synergy and ex vivo data. a) In vitro synergy of TDI-8414 and DHA against *P. falciparum* 3D7. Date was average of two independent experiments and each in duplicates. FIC = fractional inhibitory concentration. b-d) Ex vivo data of compounds **9** (b), **23** (c) and **25** (d) against *Plasmodium* clinical isolates from patients in Tororo, Uganda. Each circle was an IC_{50} obtained from a clinical isolate. Data were shown with geometric means with 95% confidence intervals.

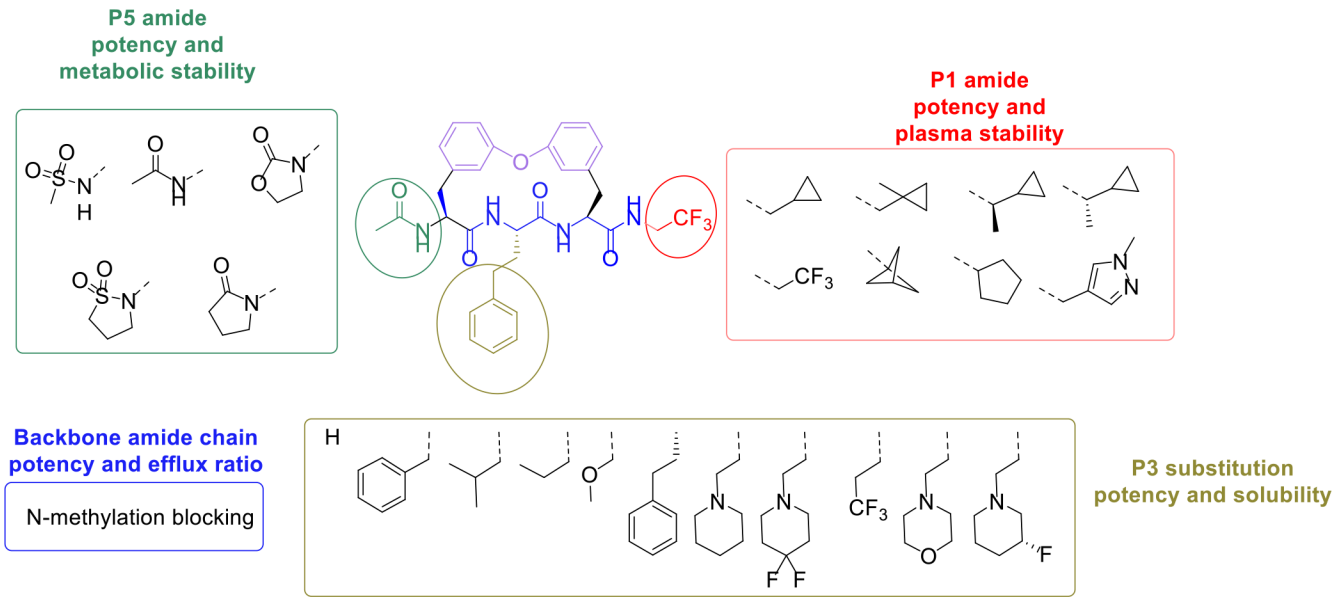
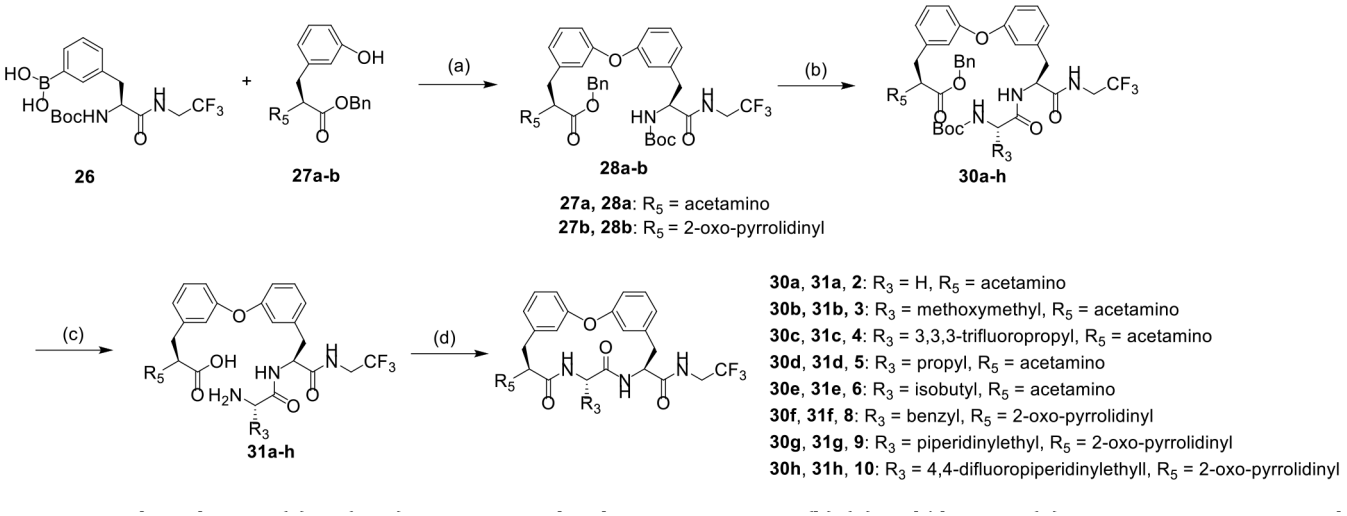


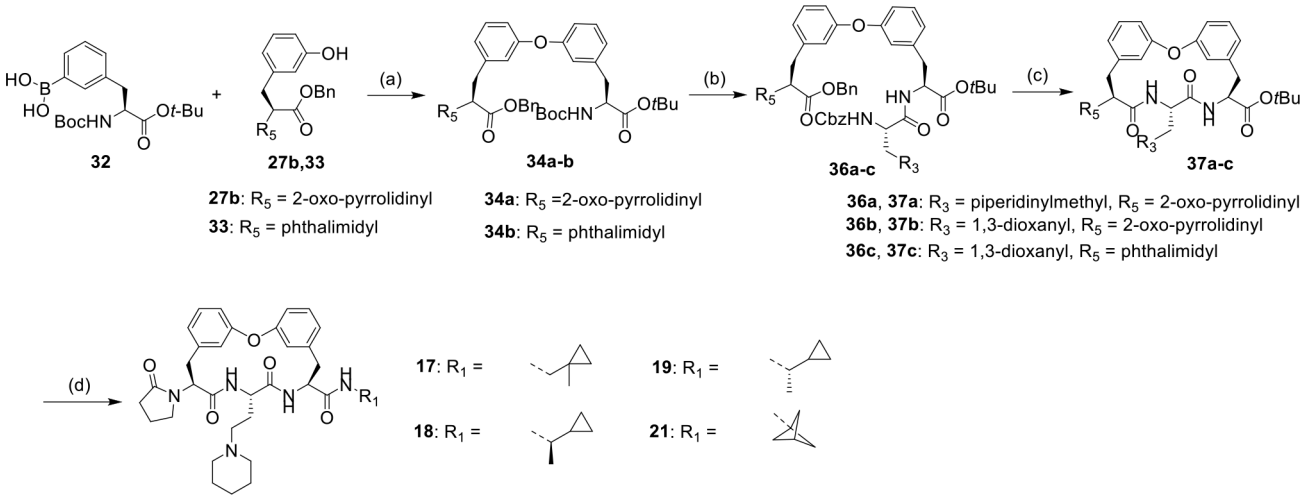
Figure 8.
Overall SAR summary of macrocyclic *Pf*20S inhibitors.



Scheme 1.

Synthesis of compound **2**, **3**, **4**, **5**, **6**, **8**, **9**, and **10** with various P3 group.

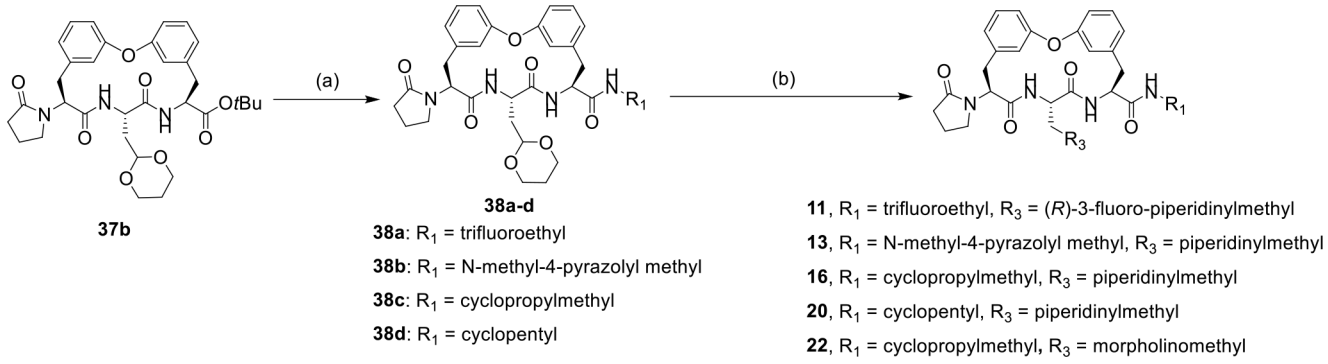
Reagents and conditions: (a) $Cu(OAc)_2$, Et_3N, 4A molecular sieve, O_2, DCM; (b) (1) HCl/dioxane, (2) HATU, DIPEA, amino acid **29a-h**, DMF; (c) (1) HCl/dioxane, (2) Pd/C, H_2, THF; (d) HATU, DIPEA, DMF.



Scheme 2.

Synthesis of compound **17**, **18**, **19**, and **21** with various P1 group.

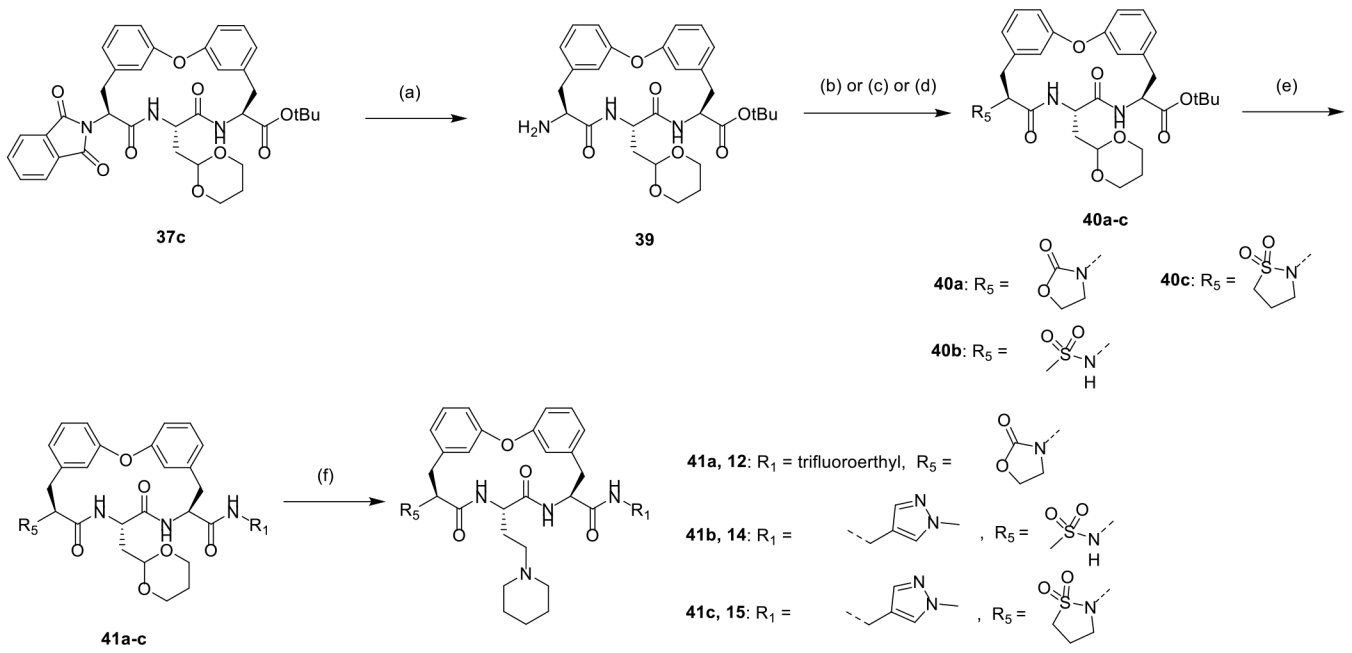
Reagents and conditions: (a) $Cu(OAc)_2$, Et_3N, 4A molecular sieve, O_2, DCM; (b) (1) TFA, DCM, 0°C, (2) EDCI, HOBt, DIPEA, DMF, **35a-b**; (c) (1) Pd/C, H_2, THF, (2) EDCI, HOBt, DIPEA, DMF; (d) (1) TFA, DCM, (2) EDCI, HOBt, Pyridine, R_1NH_2.



Scheme 3.

Synthesis of compounds **11**, **13**, **16**, **20**, and **22** with various P1 and P3 group.

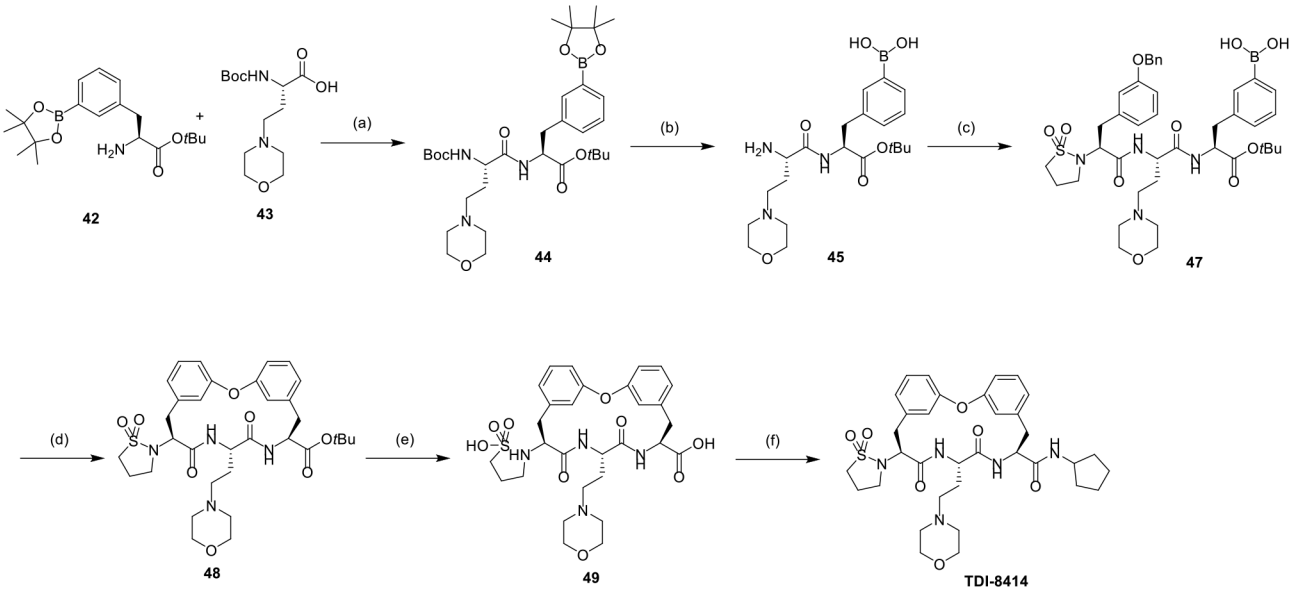
Reagents and conditions: (a) (1) TFA, DCM, (2) EDCI, HOBt, pyridine, R_1NH_2; (b) (1) ceric ammonium nitrate (CAN), $MeCN/H_2O$, (2) amines, AcOH, Pd/C, H_2, MeOH.



Scheme 4.

Synthesis of compounds **12**, **14**, and **15** with various P5 group.

Reagents and conditions: (a) $N_2H_4 \cdot H_2O$, THF; (b) (1) 2-chloroethyl chloroformate, Et_3N, THF, (2) Cs_2CO_3, NaI, DMF; (c) MsCl, DCM, DIPEA; (d) (1) 3-chloropropanesulfonyl chloride, Et_3N, DCM, (2) K_2CO_3, NaI, DMF; (e) (1) TFA, DCM, (2) R_1NH_2, EDCI, HOBt, DMF, DIPEA; (f) (1) ceric ammonium nitrate (CAN), MeCN, water, (2) piperidine, H_2, Pd/C, AcOH, MeOH.



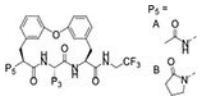
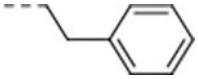
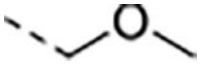
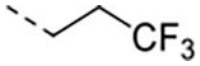
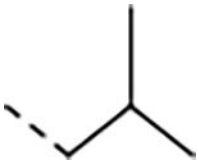
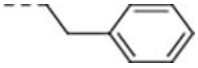
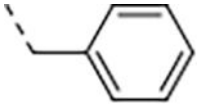
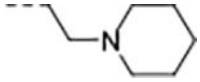
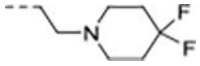
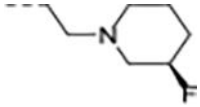
Scheme 5.

Synthesis of compound **TDI8–414** using Chan-Lam coupling reaction as macrocyclization strategy.

Reagents and conditions: (a) HATU, DIPEA, THF/DMF, 93% yield; (b) $ZnBr_2$, DCM, 53% yield; (c) compound **46**, T_3P, DIPEA, DMF, 22% yield; (d) (1) Pd/C, H_2, MeOH, (2) $Cu(OAc)_2$, Et_3N, 4A molecular sieve, O_2, DCM, 14% yield over two steps; (e) TFA, DCM, (f) (1) T_3P, DIPEA, DMF, cyclopentylamine, (2) $POCl_3$, 5.4% yield over 3 steps.

Table 1.

Optimization of P3

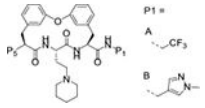
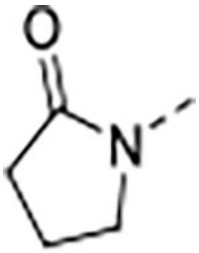
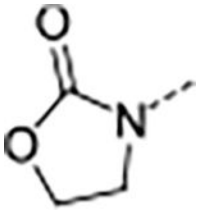
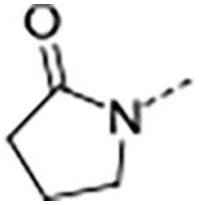
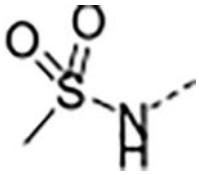
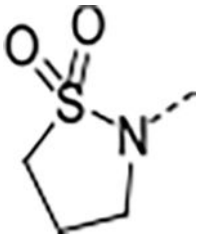
			EC_{50} (μM)	β5 IC_{50} (μM)			Permeability		Metabolic stabilityc m/hLM Cl_{int}	Kinet. Solu.	Cytotox.	cLogP
ID	P_5	P_3	3D7	Pf20S	c-20S	i-20S	PAMPA	Ratio / MDR1				
1*	A		0.003	0.014	4.0	>100	151	>0.77/ <4	270/11.2	<1.4	93	4.7
2	A	H	>2.8	>100	3.7	2.7	<18	4.1/0	−4/−17.4	4.1	104	2.2
3	A		>2.8	14.6	>100	>100	44	>8.7/< 1	−11.7/−12.7	14	98	2.4
4	A		>2.8	>100	>100	>100	73	14/0	−11.7/−2	<1.2	98	3.1
5	A		0.59	0.9	>100	>100	81	>2.5/< 2	37.7/37.7	<1.9	91	3.8
6	A		0.62	3.8	>100	>100	95	>39/< 1	25.2/−4	<1.4	94	4.2
7**	B		0.0002	0.003	0.38	33.9	214	31/5	>768/644	3.8	77	5.3
8	B		1.7	5.9	2.9	7.2	237	47/2	>768/522	22	105	4.8
9	B		0.010	0.095	1.5	9.6	30	26/1	73.9/15.5	>140	97	4.0
10	B		0.001	0.014	0.14	1.4	264	46/3	>768/687	>140	110	3.4
11	B		0.003	0.013	17	54	171	100/1	>768/238	>130	-	3.7

*: compound reported in REF 22

** : compound reported in REF 22; PAMPA unit: nm/sec at pH 7.4; MDR unit: ratio (B→A/A→B); A→B, nm/sec; Metabolic stability m/hLM Cl_{int}: mouse/human liver microsomes and unit- μL/min/mg; Kinet. Solu.: Kinetic solubility at pH 6.8 (μg/mL); Cytotox.: cytotoxicity, % viability of HepG2 cells at 30 μM. "-": not tested.

Table 2.

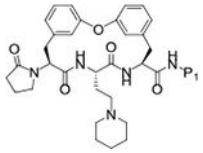
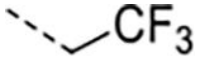
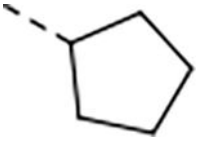
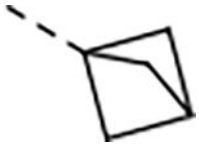
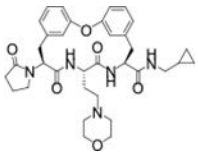
Optimization of P5

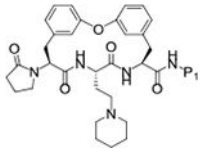
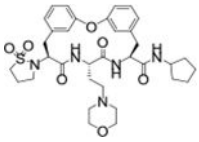
			EC_{50} (μM)	β5 IC_{50} (μM)			Permeability		Metabolic stability m/hLM Cl_{int}	Kinet. Solu.	Cyto-tox.	cLogP
ID	P1	P2	3D7	*Pf*20S	c-20S	i-20S	PAMPA	Ratio / MDR1				
9	A		0.010	0.095	1.5	9.6	30	26/1	73.9/15.5	>140	97	4.0
12	A		0.013	0.034	23.0	>100	10	>8/<1	112/16	65		4.1
13	B		0.073	0.21	33.5	88.0	<1	>1/<1	203/54	>150		3.2
14	B		>2.8	2.0	>100	>100	<1	N.D. /<1	19/6	>140		2.7
15	B		0.085	0.46	>100	59.8	<2	>0.5/<2	8/34	>140		3.1

PAMPA unit: nm/sec at pH7.4; MDR unit: ratio (B→A/A→B); A→B, nm/sec; Metabolic stability m/hLM Cl_{int}: mouse / human liver microsomes and unit- μL/min/mg; Kinet. Solu.: Kinetic solubility at pH6.8 (μg/mL); Cytotox.: cytotoxicity, % viability of HepG2 cells at 30 μM. "-": not tested.

Table 3.

Optimization of P1

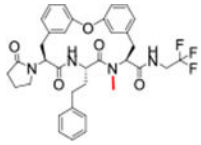
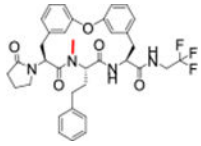
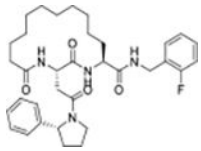
		EC$_{50}$ (μM)	β5 IC$_{50}$ (μM)			Permeability		Metabolic stability m/hLM Cl$_{int}$	Kinet. Solu.	Cytotox.	cLogP
ID	**P1**	**3D7**	**Pf20S**	**c-20S**	**i-20S**	**PAMPA**	**Ratio / MDR1**				
9		0.010	0.095	1.5	9.6	30	26/1	73.9/15.5	>140	97	4.0
16		0.031	0.083	30.0	33.3	19	>18/<1	110/46	>130	102	4.2
17		0.008	0.059	11.8	22.3	97	>30/<1	86/43	>130	-	4.7
18		0.57	4.3	>100	>100	137	>27/<1	230/58	>140	103	4.5
19		0.078	0.8	11.6	30.9	81	>20/<1	104/51	>120	-	4.5
20		0.030	0.077	28.6	17.9	49	66/1	195/75	>140	-	4.7
21		0.61	4.5	31.3	32.4	46	>55/<1	109/38	110	-	3.9
22		0.003	0.008	21.9	>100	101	>22/<1	89/41	>130	-	3.0

		EC_{50} (μM)	β5 IC_{50} (μM)			Permeability		Metabolic stability m/hLM Cl_{int}	Kinet. Solu.	Cytotox.	cLogP
ID	**P1**	**3D7**	**Pf20S**	**c-20S**	**i-20S**	**PAMPA**	**Ratio / MDR1**				
9		0.010	0.095	1.5	9.6	30	26/1	73.9/15.5	>140	97	4.0
TDI-8414		0.001	0.017	13.2	85.1	106	>39/<1	11/100	>130	105	3.4

PAMPA unit: nm/sec at pH7.4; MDR unit: ratio (B→A/B→B); A→B, nm/sec; Metabolic stability m/hLM Cl_{int}: mouse / human liver microsomes and unit- μL/min/mg; Kinet. Solu.: Kinetic solubility at pH6.8 (μg/mL); Cytotox.: cytotoxicity, % viability of HepG2 cells at 30 μM. "-": not tested.

Table 4.

N-methylated macrocyclic peptides

ID		EC_{50} (μM)	β5 IC_{50} (μM)			Permeability		Metabolic stability m/hLM Cl_{int}	Kinet. Solu.	Cytotox.	cLogP
		3D7	Pf20S	c-20S	i-20S	PAMPA	Ratio / MDR1				
23		0.21	3.2	>100	>100	186	5.5/37	>768/471	4.2	106	6.0
24		>2.8	>100	>100	>100	283	6.3/23	>768/>768	4	100	6.0
25		0.25	3.0	>100	>100	339	11/16	>768/>768	<0.85	100	6.3

PAMPA unit: nm/sec at pH7.4; MDR unit: ratio (B→A/A→B); A→B, nm/sec; Metabolic stability Cl_{int} unit: μL/min/mg; Kinet. Solu.: Kinetic solubility at pH6.8 (μg/mL); Cytotox.: cytotoxicity, % viability of HepG2 cells at 30 μM. "-": not tested.