HHS Public Access

Author manuscript

J Med Chem. Author manuscript; available in PMC 2023 July 14.

Published in final edited form as:

J Med Chem. 2022 July 14; 65(13): 9350–9375. doi:10.1021/acs.jmedchem.2c00611.

Design, synthesis, and optimization of macrocyclic peptides as species-selective antimalaria proteasome inhibitors

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Abstract

With over 200 million cases and close to half million deaths each year, malaria is a threat to global health, particularly in developing countries. *Plasmodium falciparum*, the parasite that causes the most severe form of the disease, has developed resistance to all antimalarial drugs. Resistance to the first-line antimalarial artemisinin and to artemisinin combination therapies is widespread in Southeast Asia and is emerging in sub-Saharan Africa. The *P. falciparum* proteasome is an attractive antimalarial target because its inhibition kills the parasite at multiple stages of its life cycle and restores artemisinin sensitivity in parasites that have become resistant through mutation in Kelch K13. Here we detail our efforts to develop noncovalent, macrocyclic peptide malaria proteasome inhibitors, guided by structural analysis and pharmacokinetic properties, leading to a potent, species-selective, metabolically stable inhibitor.

Graphical Abstract

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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. / ‡These authors contributed equally. (match statement to author names with a symbol)

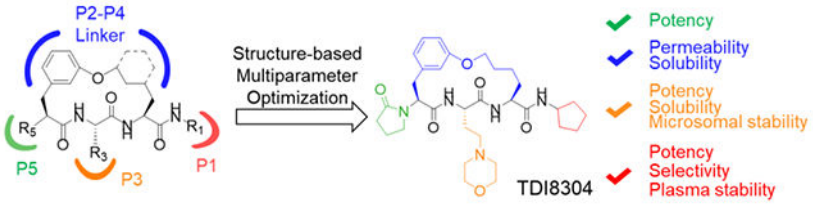
Supporting Information.

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)

The authors declare no competing financial interest.



Keywords

Plasmodium; parasite; proteasome inhibitor; antimalarials; pharmacokinetics; bioavailability

Introduction

Malaria has long been an scourge of humankind.[1] Forty percent of the world's population is at risk of contracting the disease and it is the top cause of mortality and morbidity in many underdeveloped countries.[2] There are approximately 200 million cases of malaria infections resulting in a half million deaths each year.[3] Since the discovery of quinine, the first small molecule to treat malaria, dozens of antimalarial drugs and drug combinations have been developed.[4] These agents target diverse pathways, ranging from the parasite's ability to detoxify the by-product of hemoglobin digestion to tetrahydrofolate synthesis, and artemisinins have polypharmacological effects. However, some isolates of *Plasmodium falciparum* (*Pf*), the parasite responsible for the most severe form of malaria, have developed resistance to all current treatments, even to the mainstay treatments, artemisinin (ART) drugs and their combinations.[5–6] Global spread of ART resistant strains would leave vulnerable populations with no effective therapeutics to treat malaria. It is urgent to identify novel drugs active against *Pf* parasites that are resistant to current drugs and that can synergize with them, particularly with artemisinins.

The proteasome has been validated as an antimalarial target whose inhibition can suppress resistance to artemisinins.[7–10] The *Pf* proteasome (Pf20S) is essential for parasite viability.[11] Proteasome inhibitors not only synergize with artemisinins but are active against *Pf* at multiple stages of the life cycle, offering the potential to be prophylactic, therapeutic and transmission blocking.[7, 11]

Mutations in parasite proteasomes that confer resistance to proteasome inhibitors show a fitness cost to some degree, with increased susceptibility to artemisinins.[7–9, 12–15] However, developing parasite proteasome selective inhibitors is challenging.[16] Proteasomes are highly conserved in eukaryotes, with 14 α and 14 β subunits arranged in a stack of 4 rings, α subunits forming the outer rings and β subunits the inner rings in $\alpha_{1\text{-}7}\beta_{1\text{-}7}\beta_{1\text{-}7}\alpha_{1\text{-}7}$ fashion. In addition to interacting with multiple regulatory particles, such as 19S, PA28, PA200, the outer α rings guard the entrance of substrate to the proteolytic chamber formed by the two inner β rings. Out of 7 β subunits, only β1, β2 and β5 are proteolytically active. Inhibition of β5 alone impairs viability of the *Plasmodium* parasites.

Humans have two major proteasome isoforms – constitutive proteasomes (c-20S) and immunoproteasomes (i-20S), and two minor proteasome isoforms – spermatoproteasomes

(s-20S) and thymoproteasomes (t-20S). In total, there are 7 active subunits in human proteasomes: β1cβ2cβ5c in c-20S, β1iβ2iβ5i in i-20S, β1iβ2i β5t in the t-20S, and β1cβ2cβ5c in s-20S. These proteasomes play vital roles in many cellular functions and in immune surveillance.[17] Three FDA-approved proteasome inhibitor drugs show marked anti-malarial activity, however, their toxic effects preclude their use in treating a curable infectious disease.

To serve as an antimalarial drug, a Pf20S inhibitor must be highly selective for the parasite proteasome over human proteasomes. Gantt et al was first to show that proteasome inhibitor lactacystin inhibited the development of *Plasmodium spp*.[18] However, lactacystin is unstable and shows no in vivo efficacy in mouse model of malaria. Since then, Bogyo and co-workers reported several classes of *Pf*20S selective inhibitors with reversible or irreversible modalities (Figure 1), demonstrating potent in vitro and in vivo activity.[9, 14, 19–21] Gerwick and co-workers reported an incorporation of a (*D*)-amino acid at the P3 of an epoxyketone proteasome inhibitor class that improved species selectivity.[22]

We reported the development of two classes of highly selective noncovalent *Pf*20S inhibitors, one of which was shown to have in vivo efficacy in a humanized mouse model of *Plasmodium falciparum* infection.[7, 12, 23]

Here we describe structure-activity relationship studies of a class of noncovalent, macrocyclic, peptide-based *Pf*20S inhibitors with the goal to improve potency, selectivity, and druglike pharmacokinetic properties.

RESULTS AND DISCUSSION

We were inspired by the reported *cyclic peptide 1 (CP1)* that showed potent anti-malarial activity and high species selectivity.[19] However, pharmacokinetic (PK) properties of this compound are poor (Table 1): solubility is lower than 1.6 μg/mL at pH 6.8; permeability by parallel artificial membrane permeability assay (PAMPA) is undetectable; intrinsic clearance is fast by both mouse and human liver microsomes (90.6 and 179.4 μL/min/mg, respectively). These poor physical properties are consistent with the high lipophilicity of this compound (cLogP = 6.3). Examination of the structure suggested several areas for exploration to improve physical-chemical properties as outlined in Figure 2; 1) *N*-terminal free amino group; 2) P3 homophenylalanine moiety; 3) the *C*-cap benzyl group; 4) biphenyl ether tether.

To systematically investigate moieties at the *N*-cap, *C*-cap, P3 and tether, a docking model was developed for the macrocyclic series into the β-5 subunit based on the cryo-EM structure of the *P. falciparum* proteasome (PDB entry: 5FMG).[14] Briefly, the binding pose of **CP1** in the *P. falciparum* proteasome *Pf*20S was obtained by generating a ligand conformation similar to the pose of its acyclic analog bound in the yeast proteasome crystal structure (PDB entry: 3MG4).[24] This was followed by refining the complex structure of *Pf*20S bound to this conformation of **CP1** using the Schrödinger suite Prime program.[25–26] The peptide backbone of the macrocycle **CP1** forms four hydrogen bonds with residues Ser-21, Gly-47, Ala-49, and Asp-153 of the adjacent β6 subunit (Figure 2).

Acetylating the N-terminal amino group of the compound CP1 enhanced the anti-parasitic potency by 39-fold (Table 1, compound **2**), and improved Pf20S inhibition activity by 7.6-fold, suggesting that an *N*-cap shielding the protonated primary amine group favors binding. However, the calculated lipophilicity (cLogP) of *2* is high (6.2), which likely accounts for fast clearance (171.3 μL/min/mg) by mouse liver microsomes (MLM). We thus first focused on reducing the cLogP of analogs to improve metabolic stability and permeability. Replacing the P1 *p*-methylphenyl group of **2** with trifluoromethyl yielded **3**, which maintained potency and improved permeability along with significant lowered lipophilicity.

Compound **3** showed much improved human microsomal stability, but its stability with mouse microsomes was still poor. Replacing the *N*-cap acetyl group with γ-lactam (**4**) markedly improved the proteasome inhibitory activity and potency against parasites. However, its metabolic stability was reduced. Opportunities to lower overall compound lipophilicity were limited by the presence of the biphenyl ether linker between P1 and P4. Docking compound **5** into *Pf*20S with mono-phenyl as a tether suggested that the P2 group is partially exposed to solvent and forms hydrophobic interactions with the S2 pocket (Figure 3). Changing the biphenyl ether tether of **4** to the mono-phenyl tether of **5** while maintaining a 16-membered ring motif reduced cLogP to 4.2, which translated into improved solubility (76 μg/mL at pH 6.8), without impairing anti-parasite activity. The docking pose of compound **5** showed that the P5 γ-lactam forms a critical hydrogen bond with Ser154 of the β6 subunit, which might account for the improved potency of **5** over **CP 1** (Figure 3). To further decrease lipophilicity, an oxygen atom was installed into mono-phenyl tether of **5** resulting in **6**, which showed a further decrease in cLogP and improved aqueous solubility, but suffered a 9-fold loss in potency against cultured parasites.

Compound **5**, with a monophenyl ether tether, showed good potency against cultured parasites (EC_{50} 0.0021 μM) and *Pf*20S (β5 IC_{50} 0.019 μM), good selectivity over human c-20S (hu-β5c IC_{50} 2.4 μM), excellent selectivity over human i-20S (hu-β5i IC_{50} >100 μM), good passive permeability, and improved solubility. Thus we used **5** rather than **CP1** as a scaffold for an iterative SAR study focused on modification of the P1 amide moiety (Table 2). Replacing trifluoromethyl with difluoromethyl (**7**), cyclopropyl (**8**), and 4-N-methylpyrazole (**9**) led to little loss in activity against *Pf* 3D7, suggesting that the S1 pocket can tolerate a wide range of *C*-caps. A preliminary pharmacokinetic profile of compound **5** obtained by cassette-dosing mice at 0.25 mg/kg, i.v. (Table S1) indicated that **5** had low exposure (AUC_{iv} = 0.6 ng·h/ml) and rapid clearance (Cl_{total} = 158,730 mL/h/kg), which is faster than liver blood flow (5600 mL/h/kg).[27] In agreement, **5** had rapid intrinsic clearance by mouse microsomes and was unstable in mouse but not human plasma (Figures 4a–b). After 60 min of incubation with mouse plasma, analysis by LC/MS demonstrated that the P1 amide bond of **5** was susceptible to hydrolysis (Figure 4c). We then sought to improve the mouse plasma stability. Installing a deoxy form of the P1 amide (**10**) or replacing the P1 amide with bioisosteres such as oxadiazole (**11**) and triazole (**12**) led to a complete loss of activity against cultured parasites. This was consistent with the docking model, which suggested that P1 amide forms two critical hydrogen bonds with Gly47 and Ser21 of Pf20S β5 subunit (Figure 3). Introduction of a cyclopropyl group (compound

13) to the ortho position of the P1 amide of compound **5** resulted in the excellent mouse plasma stability but decreased potency against cultured parasites by 193-fold. Presumably, the adjacent quaternary carbon center sterically hindered and retarded the hydrolysis of P1 amide. In order to balance the excellent plasma stability demonstrated by introduction of an ortho quaternary carbon center (**13**) and the high potency demonstrated by ortho primary carbon substitution (**5**), we replaced the trifluoroethyl group of **5** with the cyclopentyl group of **14**, which has a secondary carbon directly bonding to the P1 amide. Indeed, **14** maintained high potency against both cultured parasites and Pf20S, and showed improved stability in mouse plasma as compared to **5**. These results supported the hypothesis that the P1 amide is a soft spot for mouse plasma instability for this scaffold. All four test compounds demonstrated good stability in human plasma (Figure 4b).

Although improved in mouse plasma stability, **14** still suffered from high lipophilicity and rapid microsomal clearance. Examination of the docking model suggested that the P3 homophenyl substituent would tolerate modification. Substituting the P3 homophenyl group of **14** for the phenoxy group of **15** reduced lipophilicity (cLogP = 4.5) with a 72-fold loss of antiparasitic activity (Table 3). In order to block the potential microsomal metabolic soft spot, the para fluoro group was introduced in compound **16**, but this failed to improve the microsomal stability. Replacement of the phenyl group of **14** with 4-difluoropiperidine of **17** decreased the cLogP value by more than two log units, improved both mouse and human liver microsomal stability, increased solubility, and showed equipotency with **14,** suggesting that both saturated and unsaturated substituents are tolerated in the P3 pocket In order to further improve the microsomal stability, we soughtto adjust the pKa of the amine of P3 of **17**. Replacing the P3 4-difluoropiperidine of **17** with the morpholine of **TDI8304** improved microsomal stability, increased selectivity against Pf20S over human c20S, and further decreased lipophilicity (cLogP = 2.4) but demonstrated modest PAMPA permeability.

In sum, the optimization campaigns for the P1 side chain, P3 side chain and P2-P4 linker led us to **TDI8304**, which showed a balance of high potency, marked species selectivity, aqueous solubility, and high metabolic stability, but suffered from modest PAMPA permeability and a high efflux ratio. High permeability and reduced efflux ratio generally correlate with low polar surface area (PSA) and high lipophilicity (cLogP).[28–29] The P5 lactam provided an opportunity to further reduce PSA by replacing it with an alkyl or alkylamino group (Table 4). Compound **18** with a *gem*-dimethyl group showed high lipophilicity (cLogP = 3.7) and improved permeability, but unfortunately showed a significant loss in microsomal stability. Connection of the *gem*-dimethyl group of **18** with ether in **19** reduced the cLogP by 1.3 log units, restored microsomal stability and maintained the high passive permeability of **18**, but impaired permeability in an MDR1 expressing cell line.

Replacement the P5 lactam of **TDI8304** with alkylamino groups (compounds **20, 21**, and **22**) led to reduction of PSA and maintained the microsomal stability of *TDI8304*. Notably, compound **21** with a dimethylamino group reduced the PSA below the threshold of 100 and dramatically decreased efflux; however, **21** showed a 43-fold loss in potency compared to **TDI8304**. Peptidomimetic drugs have some intrinsic drawbacks, such as poor solubility,

non-selectivity, rapid proteolysis and metabolic clearance, which were addressed in our optimization campaign. However, it proved difficult to balance all the drug like properties of these macrocyclic peptidomimetics. Peptide backbone modifications, such as D-amino acid modification, α-methylation, N-methylation, and amide deoxylation, compromised the biological activity, which limited the SAR study to the side chain optimization.

The inhibitory activity of compounds **1 - 22** and **TDI8304** against α1c, β2c, β1i, and β2i subunits of human c-20S and i-20S was further determined (Table S2). Compound **9** showed moderate inhibition against hu-β2c (IC_{50} = 97.4 μM). Compounds **17** and **22** exhibited moderate inhibition against β1c and β1i. All other compounds showed <50% inhibition against all the four subunits even at 100 μM.

Correlation of Pf20S β5 inhibition with parasite growth inhibition.

Among the three active subunits of eukaryotic proteasomes, inhibition of β5 affords the strongest phenotype when solo inhibition is considered. Our compounds are primarily β5 inhibitors that yielded a high correlation with their EC_{50} against parasite growth (R^2 0.85) (Figure 5). The trend held when we combine the macrocyclic peptides and AsnEDAs (Figure 5).

Chemistry

As outlined in Scheme 1, a variety of macrocyclic proteasome inhibitors were synthesized via a convergent synthetic approach by the assembly of fragments A, B, and C following four cyclization strategies. Biphenyl ether tethered macrocycles were prepared using Suzuki coupling strategy (Scheme 2). Mono-phenyl ether linkered macrocycles were synthesized utilizing macrolactamization strategy (Scheme 3), or intramolecular etherification strategy (Scheme 4 and 5), or ring closing olefin metathesis strategy. (Scheme 6). The synthesis of intermediates fragments A, B, and C is described in Scheme S1–3.

Synthesis of compounds cyclic peptide 1, **2**, **3**, and **4** using Suzuki coupling reaction as macrocyclization strategy (Scheme 2). The commercially available N-Boc-3-bromophenylalanine **23** was subjected to benzylation and Miyaura borylation reaction to generate boronate **25**. Boc-deprotection in **25** followed by coupling with Boc-L-homophenylalanine afforded the dipeptide intermediate **27**, which subsequently underwent Boc-deprotection and condensation with carboxylic acid **28** (Scheme S1) to yield compound **29**. Oxidative hydrolysis of boronic ester to boronic acid in **29** by $NaIO_4$, followed by intramolecular Chan-Lam coupling reaction afforded macrocycle **30**. Removal of benzyl-protecting group by catalytic hydrogenation followed by amide coupling reaction provided **32a-b**, which were subjected to Boc-deprotection yielding amine **33** and **CP1**. Acetylation of free amines **33** and **CP1** afforded macrocycles **2** and **3**. Macrocycle **4** was prepared from amine *33* via subsequent alkylation with *tert-butyl* 4-bromobutanoate, Boc-deprotection, and lactamization using EDCI and HOBt.

Synthesis of compound **6** using intramolecular amidation as macrocyclization strategy (Scheme 3). Deprotection trityl group in commercially available aziridine **34** followed by reprotection with Boc group provided Boc-aziridine **35**. $BF_3{\cdot}Et_2O$-mediated SN_2-

type ring opening of aziridine **35** with 2-benzyloxyethanol afforded compound **36**. Boc-deprotection in compound **36** followed by coupling reaction with Boc-L-homophenylalanine provided dipeptide **37**, which was subjected benzyl deprotection and Mitsunobu reaction with fragment **38** (Scheme S1) affording macrocyclization precursor **39**. Subsequent debenzylation and Boc-deprotection of compound **39** followed by intramolecular amidation yielded macrocycle **41**. Macrocyle **6** was prepared from **41** via hydrolysis of methyl ester and coupling reaction with trifluoroethylamine Synthesis of compounds **5**, **7**, **8**, **9**, **10**, **11**, **12**, **13**, and **14** using intramolecular alkylation as macrocyclization strategy (Scheme 4). Dipeptide **44** was prepared from commercially available amino acid **43** via methyl esterification and HATU mediated coupling reaction with Boc-L-homophenylalanine. Boc-deprotection in dipeptide **44** and coupling reaction with fragment **45** provided tripeptide **46**, which underwent subsequent removal of two benzyl groups, chemoselective protection of primary alcohol with Tos group, and Cs_2CO_3-mediated intramolecular alkylation yielding macrocycle **47**. After hydrolysis of the methyl ester with sodium hydroxide, the free acid was coupled with various primary amines affording macrocycles **5**, **7**, **8**, **9**, **13**, and **14**. The reduction of the methyl ester **47** with lithium borohydride provided alcohol **48**, which was subjected to Dess-Martin oxidation, followed by reductive amination with trifluoroethylamine affording macrocycle **10**. Condensation of methyl ester **47** with hydrazine followed by coupling reaction with methyl trifluoroacetimidate gave hydrazide **49**, which underwent thermal cyclization affording 1,2,4-triazole **12**. Subsequent hydrolysis and dehydration of hydrazide **49** in present of Burgess reagent provided 1,3,4-oxadiazole **11**.

Synthesis of compounds **15**, **16**, **18**, **21**, and **TDI8304** using intramolecular alkylation as macrocyclization strategy (Scheme 5). The fragments **50a-c** underwent amide coupling reaction with fragments **51a-b** and subsequent deprotection affording dipeptides **52a-d**, which was coupled with fragments **53a-b** and **45** yielding tripeptides **54a-e**. The tripeptides **54a-e** were subjected to sulfonation of hydroxl group, debenzylation of aryl benzyl ether, and then intramolecular substitution between phenol and sulfonate ester group affording macrocycles **55a-b**, **15**, **18**, and **21**. Removal *tert*-butyl of **55a-b** with TFA followed by amide coupling rection with primary amine provided macrocycles **16** and **TDI8304**.[12]

Synthesis of compounds **17**, **19**, **20** and **22** using RCM reaction as macrocyclization strategy (Scheme 6). Fragments **50a** and **57** underwent subsequent amide coupling reaction with fragment **58**, Boc-deprotection, and amide coupling reaction with acids **61a-e** afforded diolefins **62a-d**, which were subjected to (RCM) in the present of the Grubbs second-generation catalyst and reducing of the C-C double bond yielding macrocycles **17**, **19**, **22**, and **63**. Removal Boc-protection of **63** provided macrocycle **20**.

Conclusion

Proteasome inhibitor drugs revolutionized the treatment of multiple myeloma, which once was unbeatable before the approval of bortezomib in 2003.[30] Since then, this barrel-shaped, ATP-dependent, compartmentalized and highly regulated N-terminal threonine hydrolase has emerged as viable targets for drug development against tuberculosis,[31–35] malaria, leishmaniasis and Chagas' diseases.[36–38] The very active field of studies offers hopes that selective pathogens' proteasome inhibitors will one day be used clinically for the treatment

of infectious diseases. Its potential as antimalarial drug target is particularly attractive as the pharmacological inhibition of its function is detrimental to the viability of the parasites at multiple stages of its life cycle, and its synergy with artemisinin, which is the core-component of artemisinin-combination therapy of malaria. Therefore, a malarial proteasome inhibitor drug could potentially be therapeutic, transmission blocking and prophylactic.

To be viable as an antiparasitic drug, proteasome inhibitors for malaria must spare the inhibition of the human proteasomes to mitigate the potential toxicity and immunosuppression from the inhibition of the human immunoproteasome. Several groups have reported the development of Pf20S selective inhibitors, for example, peptidyl vinyl sulfones guided by substrate profiling and cryo-EM structure of Pf20S,[39] peptide epoxyketone with replacement of P3 L-amino acid with a D-amino acid.[22] In addition, selectivity Here we describe our extensive rationale SAR studies to optimize the potency, selectivity and pharmacokinetic properties of a class of macrocyclic peptide proteasome inhibitors.

In this study, we systemically investigated the P1, P3, P5 and P1-P4 linker for their contribution to potency and drug like properties of macrocyclic peptides (Figure 7).[12] Moieties of the compounds at P1, P3 and P5 occupy the S1, S3 and S5 pockets of the enzyme active sites, respectively. We replaced P3 homophenyl to 4,4-diF piperidinyl and last to morpholino, which resulted in marked improvement in solubility, metabolic stability. The diF at 4-position of the piperidine and oxygen atom at the 4-position of the morpholine at the P3 also strongly improved the permeability by preventing the protonation of the tertiary amine. We further found that the linker markedly influenced the metabolic stability and permeability.

The strong correlation between inhibition of Pf20S β5 and of parasite proliferation suggests that inhibition of β5 alone is sufficient to kill *Plasmodium* parasites. we have demonstrated that co-inhibition of β5 and β2 is synergistic, similar to the observation of synergistic effect of simultaneous inhibition of β5 and β2 in tumors.[40–41] But because of the drastically different in S1 pockets at both subunits, it will be highly challenging in developing such an inhibitor while maintaining species selectivity.

Drug-target residence time influence in vivo pharmacological activity through duration of the drug-target complex. Long residence time on the target is predicted to produce durable pharamacodynamics.[42–43] Under the same experimental conditions, the shift of IC_{50} over EC_{50} were greater for time-dependent MCP inhibitors than fast equilibrium AsnEDA inhibitors, suggesting that compounds with residence time on Pf20S would have greater antimalarial activity than the compounds otherwise. This also agrees with the parasite killing rate improved from moderate for AsnEDAs to fast for MCPs, further reinforce the benefit of prolonging the residence time in drug development.

It is interesting to observe that proteasome function appears to be critical for parasites to survive at early ring stage when nutrient is short supplied due to reduced uptake of hemoglobin caused by K13 mutation. This further supports the validity of targeting Pf20S for drug development.

All in all, we have conducted an extensive lead optimization campaign guided by structure analysis and in vitro / in vivo PK studies. This strategy resulted in the identification of several compounds with potent activity against Pf20S and desirable drug like properties to include aqueous solubility, passive membrane permeability, metabolic stability, and a clean off target profile against CYP450s and the hERG channel. Further work is needed to improve oral bioavailability either by improving upon the current lead compound or by developing a pro-drug of the lead compound **TDI8304.**

Experimental Section

All commercially available reagents were used without further purification unless otherwise noted. All non-aqueous reactions were performed under argon in oven-dried glass-ware. Routine monitoring of reactions was performed using Waters Acquity Ultra Performance Liquid Chromatography (UPLC/MS). Column chromatography was generally performed on silica gel (200-300 mesh). All HPLC purifications were done by Waters prep-HPLC (mass directed purification system) using Prep C18 column. Analytical HPLC-MS was carried out using an Acquity™ Ultra Performance LC system, comprising a PDA detector, Binary Solvent Manager and SQ detector, tandem linked to a mass spectrometry system employing vendor software. Parallel evaporative light-scattering detection was incorporated into the system via a flow splitter. Column (C18 Column, 130Å, 1.7 μm, 2.1 mm X 100 mm) chromatography was carried out using a linear increasing gradient from 5-95% of solvent A (0.1% formic acid in water) to solvent B (0.1% formic acid in acetonitrile) at 0.5 ml/min over 4.0 min, or the same gradient applied over 8 min. ^{1}H- and ^{19}F- NMR spectra were acquired on Bruker 400/500 MHz system. Chemical shifts δ are expressed in parts per million, with the solvent resonance as an internal standard (chloroform-d, 1H: 7.26 ppm; Methanol-d4, 1H: 3.31 ppm; DMSO-d6, 1H: 2.50 ppm). NMR data are reported as following: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant, and integration. Purity of all final compounds were determined on a Waters UPLC/MS and all were >95%.

***Benzyl (S)-3-(3-bromophenyl)-2-((tert-butoxycarbonyl)amino)propanoate* (24).—**To a mixture of compound **23** (8.0 g, 23.24 mmol, 1.0 eq) and potassium carbonate (3.37 g, 24.40 mmol, 1.05 eq) in acetone (800 mL) was added benzyl bromide (4.37 g, 25.56 mmol, 1.1 eq) at 25°C and then the reaction mixture was stirred for 12 hours at 25°C. LCMS showed that the reaction was completed. The reaction mixture was filtered and the filtrate was concentrated in vacuum. The residue was purified by column chromatography (petroleum ether: ethyl acetate= 100:1 to 10:1) to give the desired product **24** (10.0 g, 22.74 mmol, 97.83% yield, 98.74% purity) as a white solid. LCMS: RT = 0.964 min, purity:98.75%, *m/z*= 334.0 $[M\text{-}t\text{Boc}+H]^{+}$. NMR ($CDCl_3$, 400 MHz): δ 7.39-7.36 (m, 4H), 7.33-7.30 (m, 2H), 7.26-7.25 (m, 1H), 7.10 (t, J = 7.6 Hz, 1H), 6.97 (d, J = 7.2 Hz, 1H), 5.15 (q, J = 12.4 Hz, 2H), 5.05-5.02 (m, 1H), 4.64-4.59 (m, 1H), 3.14-2.99 (m, 2H), 1.44 (s, 9H).

***Benzyl (S)-2-((tert-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoate* (25).—**To a solution of compound *24* (10.0 g, 23.02 mmol, 1.0 eq), bis(pinacolato)diboron (9.35 g, 36.83 mmol, 1.6 eq) and potassium acetate (6.78 g, 69.06 mmol, 3.0 eq) in dioxane (250 mL) was added $Pd(dppf)Cl_2$ (1.68 g,

2.30 mmol, 0.1 eq) at 25°C and then the reaction mixture was stirred at 80°C for 12 hours under nitrogen. LCMS showed that the reaction was completed. The reaction mixture was filtered and the filtrate was concentrated in vacuum. The residue was purified by column chromatography (SiO_2, petroleum ether: ethyl acetate= 100:1 to 10:1) to give the product **25** (10.0 g, 19.72 mmol, 85.66% yield, 94.92% purity) as yellow oil. LCMS: RT = 0.968 min, purity:94.92%, *m/z*= 382.1 $[M\text{-}Boc+H]^+$.

Benzyl (S)-2-((S)-2-((tert-butoxycarbonyl) amino)-4-phenylbutanamido)-3-(3-(4, 4, 5, 5-tetramethyl-1, 3, 2-dioxaborolan-2-yl) phenyl) propanoate (**27**). To a solution of compound **25** (35.0 g, 72.71 mmol, 1.0 eq) in dioxane (50 mL) was added a solution of hydrogen chloride in dioxane (4 M, 300 mL) at 25°C and then the reaction mixture was stirred at 25°C for 0.5 hour. LCMS showed that the reaction was completed. Then the reaction mixture was concentrated in vacuum to give the product **26** (30.0 g, crude, HCl salt) as yellow oil, which was used directly in the next step. To a solution of Boc-L-homophenylalanine (18.39 g, 65.84 mmol, 1.1 eq), 1-hydroxybenzotriazole (8.9 g, 65.84 mmol, 1.1 eq) in dichloromethane (250 mL) was added 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride (12.62 g, 65.84 mmol, 1.1 eq) at 0°C and then the reaction mixture was stirred for 30 mins. The compound **26** (25 g, 59.85 mmol, 1.0 eq, HCl salt) and diisopropylethylamine (23.2 g, 179.55 mmol, 3.0 eq) in dichloromethane (120 mL) was added to above reaction mixture at 0°C and then the resulting mixture was stirred at 25°C for 11.5 hours. LCMS showed that the reaction was completed. The reaction mixture was quenched with hydrochloric acid solution (1 M, 400 mL) and extracted with dichloromethane (200 mL*2). The combined organic phases were washed with brine (400 mL*2), dried over sodium sulfate and concentrated in vacuum. The residue was purified by column chromatography (SiO_2, petroleum ether: ethyl acetate= 50:1 to 2:1) to give the product **27** (29.00 g, 38.45 mmol, 64.25% yield, 85.20% purity) as yellow oil. LCMS: RT = 1.052 min, purity:85.21%, m/z= 543.3 $[M\text{-}Boc+H]^+$. ^1H NMR ($CDCl_3$, 400 MHz): δ 7.67 (d, J = 7.2 Hz, 1H), 7.54 (s, 1H), 7.40-7.29 (m, 6H), 7.28-7.20 (m, 6H), 7.17 (d, J = 7.2 Hz, 1H), 6.64 (br.s, 1H), 5.26-5.24 (s, 1H), 4.89-4.84 (m, 1H), 4.41-4.36 (m, 1H), 4.16-4.10 (m, 1H), 3.20-3.17 (m, 1H), 3.09-3.07 (m, 1H), 2.73-2.65 (m, 2H), 2.28-2.21 (m, 2H), 1.40 (s, 9H), 1.34 (s, 12H).

Benzyl (6S,9S,12S)-6-(3-hydroxybenzyl)-2,2-dimethyl-4,7,10-trioxo-9-phenethyl-12-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)-3-oxa-5,8,11-triazatridecan-13-oate (**29**). To a solution of compound **27** (31.0 g, 48.24 mmol, 1.0 eq) in dioxane (50 mL) was added a solution of hydrogen chloride in dioxane (4 M, 250 mL, 20.7 eq) at 25°C and then the reaction mixture was stirred at 25°C for 30 min. LCMS showed that the reaction was completed. The reaction mixture was concentrated in vacuum to give the product deBoc-40 (26.00 g, 38.01 mmol, 78.79% yield, 84.63% purity, HCl salt) as yellow oil. LCMS: RT = 0.784 min, purity:84.63%, m/z= 543.2 $[MS+H]^+$. ^1H NMR (CD_3OD, 400 MHz): δ 7.65-7.61 (m, 2H), 7.35-7.16 (m, 12H), 5.48 (s, 2H), 4.86-4.76 (m, 1H), 3.95-3.93 (m, 1H), 3.21-3.19 (m, 1H), 3.11-3.09 (m, 1H), 2.68-2.66 (m, 2H), 2.10-1.95 (m, 2H), 1.33 (s, 12H). To a solution of compound **deBoc-27** (5.0 g, 9.22 mmol, 1.0 eq) and compound **28** (2.59 g, 9.22 mmol, 1.0 eq) in N,N-dimethylformamide (150 mL) was added diisopropylethylamine

(5.96 g, 46.10 mmol, 5.0 eq), 1-hydroxybenzotriazole (1.37 g, 10.14 mmol, 1.1 eq) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (1.94 g, 10.14 mmol, 1.1 eq) at 0°C and the reaction was stirred at 25°C for 12 hours. LCMS showed that the reaction was completed. The reaction mixture was acidified to pH=4 with hydrochloric acid (0.5 M) and extracted with dichloromethane (150 mL*4). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated in vacuum to give the product *29* (13.60 g, 4.35 mmol, 47.16% yield, 25.760% purity) as red oil, which was used directly in the next step.

Benzyl (5S,8S,11S)-11-((tert-butoxycarbonyl)amino)-7,10-dioxo-8-phenethyl-2-oxa-6,9-diaza-1,3(1,3)-dibenzena-cyclododecaphane-5-carboxylate (**30**). To a solution of compound **29** (30.0 g, 37.23 mmol, 1.0 eq) in acetone (300 mL) was added sodium periodate (23.89 g, 111.69 mmol, 3.0 eq) and ammonium acetate (8.61 g, 111.69 mmol, 3.0 eq) in water (240 mL) at 25°C and then the reaction mixture was stirred at 25°C for 12 hours. LCMS showed that the reaction was completed. The reaction mixture was acidified to pH=4 with hydrochloric acid (0.5 M) and extracted with dichloromethane (300 mL*3). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated in vacuum. The residue was purified by reverse phase flash (HCl condition) to give the product **boronic acid-29** (9.30 g, 12.85 mmol, 25.14% yield, 100% purity) as a brown solid. LCMS: RT = 0.915 min, purity:100%, m/z= 724.1 $[MS+H]^+$. To a solution of compound **boronic acid-29** (6.0 g, 8.29 mmol, 1.0 eq) in dichloromethane (600 mL) was added copper acetate (1.51 g, 8.29 mmol, 1.0 eq), triethylamine (8.39 g, 82.90 mmol, 10.0 eq), methanol (2.66 g, 82.90 mmol, 10.0 eq) and 4Å molecular sieves (6.0 g) at 25°C and then the reaction mixture was stirred at 25°C for 12 hours under oxygen. LCMS showed that the reaction was completed. The reaction mixture was filtered and concentrated in vacuum. The residue was purified by re-crystallization from ethyl acetate (100 mL) and then re-crystallized from acetonitrile (100 mL) to give **30** (2.60 g, 3.66 mmol, 44.15% yield, 95.31% purity) as a yellow solid. LCMS: RT = 3.232 min, purity:95.31%, m/z= 678.2 $[MS+H]^+$. ^1H NMR (DMSO-d6, 400 MHz): δ 8.60 (d, J = 8.4 Hz, 1H), 8.10 (d, J = 8.8 Hz, 1H), 7.41-7.28 (m, 9H), 7.24-7.20 (m, 1H), 7.08-6.97 (m, 4H), 6.89-6.79 (m, 3H), 6.15-6.10 (m, 2H), 5.17 (s, 2H), 4.75-4.70 (m, 1H), 4.37-4.33 (m, 2H), 3.27-3.17 (m, 2H), 2.91-2.71 (m, 2H), 2.45-2.40 (m, 2H), 1.75-1.63 (m, 2H), 1.38 (s, 9H).

(5S, 8S, 11S)-11-((tert-butoxycarbonyl) amino)-7, 10-dioxo-8-phenethyl-2-oxa-6, 9-diaza-1, 3(1, 3)-dibenzenacyclododecaphane-5-carboxylic acid (**31**). To a solution of **30** (250 mg, 368.85 umol, 1.0 eq) in dichloromethane (10 mL) and isopropanol (20 mL) was added Pd/C (100 mg, 5% purity) under nitrogen. The suspension was degassed under vacuum and purged with hydrogen for several times. The mixture was stirred under hydrogen (15 psi) at 25°C for 4 hours. LCMS showed that the starting material was completed. The reaction mixture was filtered and the filtrate was concentrated in vacuum to give compound **31** (150 mg, 183.86 umol, 49.85% yield, 72.03% purity) as a brown solid. LCMS: RT = 0.900 min, purity:72.03%, m/z=588.1 $[MS+H]^+$

Tert-butyl ((5S,8S,11S)-5-((4-methylbenzyl)carbamoyl)-7,10-dioxo-8-phenethyl-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-11-yl)carbamate (**32a**). To a solution of compound **31** (500 mg, 850.83 umol, 1.0 eq), diisopropylethylamine (121 mg, 935.91 umol, 1.1 eq) and 1-hydroxy benzotriazole (126 mg, 935.91 umol, 1.1 eq) in tetrahydrofuran (10

mL) and N,N-dimethylformamide (10 mL) was added 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (179 mg, 935.91 umol, 1.1 eq) at −10°C and then the reaction mixture was stirred for 0.5 hour at −10°C. Then p-tolylmethanamine (113 mg, 935.91 umol, 1.1 eq) was added to above reaction mixture and the reaction mixture was stirred for 11.5 hours at 25°C. LCMS showed that the reaction was completed. Water (20 mL) was added to the reaction mixture and the solid was precipitate out. Then the solid was collected and purified by prep-HPLC (TFA condition, column: Agela ASB 150mm*25mm*5um, mobile phase: [water (0.1%TFA)-ACN]; B%: 60%-85%, 11 min) to give **32a** (23.00 mg, 31.20 umol, 3.67% yield, 93.72% purity) as a white solid. LCMS: RT = 1.043 min, purity:93.72%, m/z= 691.3 $[MS+H]^+$. ^1H NMR (DMSO-d6, 400 MHz): δ 8.57 (t, J = 5.6 Hz, 1H), 8.41 (d, J = 8.8 Hz, 1H), 8.01 (d, J = 8.8 Hz, 1H), 7.32-6.95 (m, 13H), 6.86-6.80 (m, 3H), 6.13 (br.s, 1H), 6.00 (d, J = 6.8 Hz, 1H), 4.64-4.63 (m, 1H), 4.42-4.23 (m, 4H), 3.05-3.01 (m, 1H), 2.92-2.66 (m, 3H), 2.47-2.45 (m, 2H), 2.22 (s, 3H), 1.79-1.64 (m, 2H), 1.39 (s, 9H).

***(5S,8S,11S)-11-amino-N-(4-methylbenzyl)-7,10-dioxo-8-phenethyl-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide* (CP1).—**To a solution of **32a** (150 mg, 217.13 umol, 1.0 eq) in dichloromethane (10 mL) and tetrahydrofuran (10 mL) was added trifluoroacetic acid (7.7 g, 67.53 mmol, 5 mL, 311.0 eq) at 25°C and then the reaction mixture was stirred for 12 hours at 25°C. LCMS showed that the reaction was completed. The reaction mixture was concentrated in vacuum. The residue was purified by prep-HPLC (TFA condition; column: Phenomenex Synergi C18 150mm*25mm*10um, mobile phase: [water (0.1%TFA)-ACN]; B%: 25%-55%, 12 min) to give the product with as TFA salt. The product was redissolved in water (5 mL) and the mixture was adjusted to pH=9 with aqueous sodium bicarbonate. The solid was precipitated out and collected to give **CP1** (32.00 mg, 54.17 umol, 24.95% yield, 100% purity) as a white solid. LCMS: RT = 0.846 min, purity:100%, *m/z*= 591.2 $[MS+H]^+$. ^1H NMR (DMSO-d6, 400 MHz): *δ* 8.55 (t, *J* = 5.6 Hz, 1H), 8.42 (d, *J* = 8.4 Hz, 1H), 7.80 (d, *J* = 8.8 Hz, 1H), 7.32-7.22 (m, 4H), 7.20-7.1 (m, 8H), 6.97-6.92 (m, 2H), 6.86-6.82 (m, 1H), 6.75 (br.s, 1H), 6.19 (br.s, 1H), 4.62 (t, *J* = 8.8 Hz, 1H), 4.44-4.41 (m, 1H), 4.32-4.19 (m, 2H), 3.58-3.57 (m, 1H), 3.02-2.98 (m, 1H), 2.86-2.84 (m, 1H), 2.68-2.66 (m, 1H), 2.47-2.44 (m, 1H), 2.22 (s, 3H), 1.77-1.57 (m, 4H).

(5S, 8S, 11S)-11-acetamido-N-(4-methylbenzyl)-7, 10-dioxo-8-phenethyl-2-oxa-6, 9-diaza-1, 3(1, 3)-dibenzenacyclododecaphane-5-carboxamide (**2**). To a solution of **CP1** (80 mg, 113.52 umol, 1.0 eq, TFA salt) and triethylamine (34 mg, 340.56 umol, 3.0 eq) in N,N-dimethylformamide (3 mL) was added a solution of acetyl chloride (11 mg, 227.04 umol, 2.0 eq) in dichloromethane (1 mL) at 0°C and then the reaction mixture was stirred for 2 hours at 25°C. LCMS showed that the reaction was completed. The reaction mixture was quenched with water (3 mL) and the solid was precipitate out. The solid was collected and washed with acetonitrile (3 mL*3) and a mixture of dichloromethane/methanol (V:V= 1:1, 2 mL*2) to give **2** (8.50 mg, 12.95 umol, 11.40% yield, 96.377% purity) as a yellow solid. LCMS: RT = 2.270 min, purity:96.38%, m/z= 633.2 $[MS+H]^+$. ^1H NMR (DMSO-d6, 400 MHz): δ 8.56 (t, J = 5.6 Hz, 1H), 8.40 (d, J = 7.2 Hz, 1H), 8.03 (d, J = 8.8 Hz, 1H), 7.61 (d, J = 7.2 Hz, 1H), 7.35-7.22 (m, 4H), 7.17-6.96 (m, 9H), 6.87-6.81 (m, 3H), 6.16 (br.s, 1H),

4.65-4.61 (m, 2H), 4.42-4.38 (m, 1H), 4.22-4.13 (m, 2H), 3.03-3.01 (m, 1H), 2.89-2.66 (m, 3H), 2.45-2.42 (m, 2H), 2.22 (s, 3H), 1.88 (s, 3H), 1.75-1.63 (m, 2H).

Tert-butyl ((5S,8S,11S)-7,10-dioxo-8-phenethyl-5-((2,2,2-trifluoroethyl)carbamoyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-11-yl)carbamate (**32b**). To a solution of compound **31** (800 mg, 1.36 mmol, 1.0 eq) and 1-hydroxybenzotria zole (202 mg, 1.50 mmol, 1.1 eq) in tetrahydrofuran (15 mL) and N, N-dimethylformamide (15 mL) was added EDCI (287 mg, 1.50 mmol, 1.1 eq) and diisopropylethylamine (352 mg, 2.72 mmol, 2.0 eq) at −10°C and then the reaction mixture was stirred for 0.5 hour at −10°C. Then 2,2,2-trifluoroethylamine (203 mg, 1.50 mmol, 1.1 eq, HCl salt) was added to above reaction mixture and the reaction mixture was stirred for 11.5 hours at 25°C. LCMS showed that the reaction was completed. Water (30mL) was added to the reaction mixture and the solid was precipitate out. The solid was collected and purified by prep-HPLC (TFA condition; column: Agela ASB 150mm*25mm*5um, mobile phase: [water (0.1%TFA)-ACN]; B%: 55%-85%, 11 min) and then purified by re-crystallization from a mixture of isopropyl ether and methanol and dichloromethane (V:V:V= 3:1:1, 5 mL *3) to give **32b** (19.00 mg, 28.41 umol, 6.33% yield, 100% purity) as a white solid. LCMS: RT = 2.379 min, purity:100%, m/z= 669.2 $[MS+H]^+$. ^{1}H NMR (DMSO-d6, 400 MHz): δ 8.90 (t, J = 5.6 Hz, 1H), 8.52 (d, J = 8.4 Hz, 1H), 8.03 (d, J = 8.0 Hz, 1H), 7.32-7.27 (m, 4H), 7.23-7.14 (m, 4H), 6.99-6.97 (m, 1H), 6.85-6.82 (m, 3H), 6.12 (br.s, 1H), 6.00 (d, J = 7.2 Hz, 1H), 4.70-4.67 (m, 1H), 4.41-4.33 (m, 2H), 4.00-3.96 (m, 2H), 3.03-2.99 (m, 1H), 2.91-2.87 (m, 1H), 2.80-2.77 (m, 2H), 2.43-2.40 (m, 2H), 1.74-1.67 (m, 2H), 1.39 (s, 9H).

(5S, 8S, 11S)-11-amino-7,10-dioxo-8-phenethyl-N-(2,2,2-trifluoroethyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclodo-decaphane-5-carboxamide (**33**). To a solution of **32b** (300 mg, 448.63 umol, 1.0 eq) in dichloromethane (10 mL) and N, N-dimethylformamide (10 mL) was added trifluoroacetic acid (7.7 g, 67.53 mmol, 5 mL, 150.5 eq) at 25°C and then the reaction mixture was stirred for 12 hours at 25°C. LCMS showed that the reaction was completed. The reaction mixture was concentrated in vacuum at 30°C. The residue was purified by prep-HPLC (TFA condition; column: Agela ASB 150mm*25mm*5um, mobile phase: [water (0.1%TFA)-ACN]; B%: 25%-55%, 11 min). The product was redissolved in water (5 mL) and the mixture was adjusted to pH=9 with aqueous sodium bicarbonate solution. The solid was collected and triturated with isopropyl ether (10 mL*2) to give **33** (38.00 mg, 66.40 umol, 14.80% yield, 99.35% purity) as a white solid. LCMS: RT = 1.906 min, purity:99.35%, m/z= 569.3 [MS+H]+. ^{1}H NMR (DMSO-d6, 400 MHz): δ 8.89 (t, J = 5.6 Hz, 1H), 8.51 (d, J = 8.8 Hz, 1H), 7.79 (d, J = 8.8 Hz, 1H), 7.31-7.09 (m, 8H), 6.95-6.85 (m, 3H), 6.75 (br.s, 1H), 6.18 (br.s, 1H), 4.66 (t, J = 9.2 Hz, 1H), 4.44-4.34 (m, 1H), 4.03-3.89 (m, 2H), 3.58-3.55 (m, 1H), 3.01-2.97 (m, 1H), 2.86-2.72 (m, 1H), 2.68-2.62 (m, 1H), 2.47-2.45 (m, 2H), 1.73-1.54 (m, 3H).

(5S, 8S, 11S)-11-acetamido-7,10-dioxo-8-phenethyl-N-(2,2,2-trifluoroethyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide (**3**). To a solution of **33** (100 mg, 175.87 umol, 1.0 eq) and triethylamine (53 mg, 527.61 umol, 3.0 eq) in N,N-dimethylformamide (4 mL) was added acetyl chloride (10 mg, 193.46 umol, 1.1 eq) at 0°C and then the reaction mixture was stirred for 3 hours at 25°C. LCMS showed that the reaction was completed. The reaction was quenched with water (5 mL) and the solid

was precipitated out. The solid was collected and purified by prep-HPLC (TFA condition; column: PhenomenexSynergi C18 150mm*25mm*10um, mobile phase: [water (0.1%TFA)-ACN]; B%: 38%-68%, 11 min) to give **3** (9.50 mg, 15.40 umol, 8.76% yield, 98.99% purity) as a white solid. LCMS: RT = 2.177 min, purity:98.99%, m/z= 611.2 [MS+H]+. ^{1}H NMR (DMSO-d6, 400 MHz): δ 8.85 (t, J = 6.4 Hz, 1H), 8.44 (d, J = 9.2 Hz, 1H), 8.01 (d, J = 8.8 Hz, 1H), 7.61 (d, J = 7.2 Hz, 1H), 7.33-7.27 (m, 4H), 7.16-7.08 (m, 4H), 6.97 (dd, J1 = 2.0 Hz, J_1 = 8.0 Hz, 1H), 6.88-6.83 (m, 3H), 6.16 (br.s, 1H), 4.71-4.66 (m, 1H), 4.63-4.58 (m, 1H), 4.43-4.38 (m, 1H), 3.99-3.93 (m, 2H), 3.03-2.99 (m, 2H), 2.89-2.74 (m, 3H), 2.45-2.43 (m, 1H), 1.88 (s, 3H), 1.76-1.65 (m, 2H).

(5S, 8S, 11S)-7,10-dioxo-11-(2-oxopyrrolidin-1-yl)-8-phenethyl-N-(2,2,2-trifluoroethyl)-2-oxa-6,9-diaza-1,3(1,3)-dibenzenacyclododecaphane-5-carboxamide (**4**). To a mixture of **33** (100 mg, 175.87 umol, 1.0 eq), sodium iodide (3 mg, 17.59 umol, 0.1 eq) and sodium carbonate (93 mg, 879.35 umol, 5.0 eq) in N,N-dimethylformamide (3 mL) was added tert-butyl 4-bromobutanoate (51 mg, 228.63 umol, 1.3 eq) at 25°C. The mixture was stirred for 12 hours at 50°C. LCMS showed that the desired compound was detected. Water (20 mL) was added to the reaction mixture and the product was precipitated out. The solid was collected and dried under vacuum to give compound **tert-butyl ester-33** (110 mg, 154.76 umol, 88.00% yield) as a yellow solid. LCMS: RT = 0.897 min, purity:54.55%, m/z= 711.5 $[MS+H]^+$. 1H NMR (DMSO-d6, 400 MHz): δ 8.78-8.72 (m, 1H), 8.39 (d, J = 8.4 Hz, 1H), 7.94 (d, J = 9.2 Hz, 1H), 7.30–7.21 (m, 4H), 7.17–7.08 (m, 4H), 7.00 (d, J = 7.6 Hz, 2H), 6.93-6.92 (m, 2H), 6.60 (br. s, 1H), 6.31 (br. s, 1H), 4.67–4.62 (m, 1H), 4.50–4.41 (m, 1H), 4.05–3.99 (m, 1H), 3.97-3.91 (m, 2H), 3.05–2.96 (m, 2H), 2.89–2.80 (m, 2H), 2.73–2.70 (m, 2H), 2.40–2.36 (m, 2H), 2.17 (t, J = 7.2 Hz, 2H), 1.74–1.72 (m, 2H), 1.62–1.57 (m, 2H), 1.34 (s, 9H). To a solution of compound **tert-butyl ester-33** (50 mg, 70.35 umol, 1.0 eq) in dichloromethane (2 mL) was added trifluoroacetic acid (1 mL). The mixture was stirred for 2 hours at 25°C. LCMS showed that the desired compound was detected. The mixture was concentrated in vacuum to give compound **acid-33** (50 mg, crude) as a yellow soild, which was used in the next step without further purification. LCMS: RT = 0.691 min, purity:63.59%, m/z= 655.3 $[MS+H]^+$. To a solution of compound **acid-33** (40 mg, 61.10 umol, 1.0 eq), 1-hydroxybenzotriazole (12 mg, 91.65 umol, 1.5 eq) and diisopropylethylamine (24 mg, 183.30 umol, 3.0 eq) in N, N-dimethylformamide (2 mL) was added EDCI (18 mg, 91.65 umol, 1.5 eq) at 25°C. The mixture was stirred for 12 hours at 25°C. LCMS showed that the starting material was consumed completely and the desired compound was detected. The mixture was added into water (10 mL) and the solid was precipitated out. The solid was filtered and purified by prep-HPLC (column: PhenomenexSynergi C18 150mm*25mm*10um; mobile phase: [water (0.1% TFA) - ACN]; B%: 44%-74%, 12min) to give **4** (15.70 mg, 23.69 umol, 38.77% yield, 96.05% purity) as a white solid. LCMS: RT = 2.791 min, purity:96.05%, m/z= 637.2 $[MS+H]^+$. 1H NMR (DMSO-d6, 400 MHz): δ 8.67 (t, J = 6.4 Hz, 1H), 8.25 (d, J = 8.0 Hz, 1H), 7.95 (d, J = 8.4 Hz, 1H), 7.34–7.28 (m, 2H), 7.24 (t, J = 7.6 Hz, 2H), 7.17–7.12 (m, 1H), 7.08 (d, J = 7.2 Hz, 2H), 7.02–6.94 (m, 4H), 8.60 (br.s, 1H), 6.51 (br.s, 1H), 4.76–4.71 (m,1H), 4.65–4.62 (m, 1H), 4.29–4.27 (m, 1H), 3.97–3.84 (m, 2H), 3.18–3.13 (m, 2H), 2.97–2.91 (m, 2H), 2.63–2.60 (m, 2H), 2.44–2.39 (m, 2H), 2.28–2.16 (m, 2H), 1.92–1.86 (m, 2H), 1.76–1.65 (m, 2H).

***1-(tert-butyl) 2-methyl (S)-aziridine-1, 2-dicarboxylate* (35).—**To a mixture of compound **34** (20 g, 58.24 mmol) in dichloromethane (60 mL) and methanol (60 mL) was added trifluoroacetic acid (199.21 g, 1.75 mol, 129.36 mL) drop wise at −30°C. The mixture was stirred at 0°C for 4 hours and 10°C for another 12 hours. TLC (petroleum ether: ethyl acetate = 10:1) and LCMS indicated the reaction was completed. The reaction mixture was concentrated in *vacuo* to afford a residue (12.5 g, crude, TFA salt) as a green solid. To a mixture of previous residue (12.5 g, 58.10 mmol, TFA salt) and triethylamine (29.4 g, 290.50 mmol) in acetonitrile (120 mL) was added di-tert-butyl dicarbonate (76.09 g, 348.60 mmol) drop wise at 0°C. The mixture was stirred at 25°C for 12 hours. New spots were formed on TLC (petroleum ether: ethyl acetate = 10:1). The mixture was concentrated in vacuum to remove the solvent. The residue solution was poured into water (200 mL), extracted with ethyl acetate (200 mL*3). The combined organic phase was washed with brine (200 mL*2), dried over anhydrous sodium sulfate, filtered and concentrated in vacuum. The residue was purified by chromatography column (petroleum ether: ethyl acetate = 100:1 - 10:1) to afford compound **35** (5 g, 24.85 mmol, 42.77% yield) as colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.79 (s, 3H), 3.06 - 3.04 (m, 1H), 2.54 - 2.53 (m, 1H), 2.43 - 2.42 (m, 1H), 1.47 (s, 9H).

***Methyl O-(2-(benzyloxy)ethyl)-N-(tert-butoxycarbonyl)-L-serinate* (36).—**To a mixture of compound **35** (5 g, 24.85 mmol) and 2-(benzyloxy)ethanol (4.92 g, 32.30 mmol) in dichloromethane (70 mL) was added a solution of boron tri-fluoride diethyl ether complex (375.19 mg, 1.24 mmol, 47% purity) in dichloromethane (10 mL) drop wise at 0°C. The mixture was stirred at 0°C for 10 minutes. LCMS indicated most of the starting material was consumed and desired mass was observed. A new spot was formed on TLC (petroleum ether: ethyl acetate = 5:1). The reaction mixture was poured into saturated sodium carbonate aqueous (200 mL), extracted with dichloromethane (200 mL*3). The combined organic phase was washed with brine (200 mL*2), dried over anhydrous sodium sulfate, filtered and concentrated in vacuum. The residue was purified chromatography column (petroleum ether: ethyl acetate = 30:1 - 10:1) to afford compound **36** (3.8 g, 10.60 mmol, 42.67% yield, 98.620% purity) as a colorless oil. LCMS: RT = 0.862 min, *m/z* 376.1 $[M+Na]^+$. Purity: 98.62%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.37 - 7.33 (m, 4H), 7.31 - 7.29 (m, 1H), 5.51 (d, *J* = 8.0 Hz, 1H), 4.56 (s, 2H), 3.96 (dd, *J* = 9.6 Hz, 3.2 Hz, 1H), 3.75 (s, 3H), 3.72 (dd, *J* = 9.6 Hz, 3.2 Hz, 1H), 3.66 - 3.64 (m, 2H), 3.61 - 3.59 (m, 2H), 1.46 (s, 9H).

***Methyl O-(2-(benzyloxy)ethyl)-N-((S)-2-((tert-butoxycarbonyl)amino)-4-phenylbutanoyl)-L-serinate* (37).—**A mixture of compound **36** (3.8 g, 10.75 mmol) in HCl/methanol (4 M, 95 mL) was stirred at 25°C for 1 hour. LCMS indicated the reaction was completed. The mixture was concentrated in *vacuo* directly to afford compound **amine-36** (3.2 g, crude, HCl salt) as a white solid. LCMS: RT = 0.586 min, *m/z* 254.2 $[M+H]^+$Purity: 98.23%. *1*H NMR (DMSO-d_6, 400 MHz): δ 8.71 (s, 3H), 7.35 −7.28 (m, 5H), 4.48 (s, 2H), 4.30 (s, 1H), 3.93 - 3.85 (m, 2H), 3.73 (s, 3H), 3.66 - 3.60 (m, 2H), 3.57 - 3.55 (m, 2H). To a mixture of compound **amine-36** (3.2 g, 11.04 mmol, HCl salt), Boc-L-homophenylalanine (3.08 g, 11.04 mmol) and HBTU (4.19 g, 11.04 mmol) in dimethyl formamide (60 mL) was added N, N-diisopropylethylamine (4.28 g, 33.13 mmol) at 25°C. The mixture was stirred at 25°C for 20 minutes. LCMS indicated the reaction was

completed. New spots were formed on TLC (petroleum ether: ethyl acetate = 1:1). The mixture was poured into water (200 mL), extracted with dichloromethane (200 mL*3). The combined organic phase was washed with saturated sodium hydrosulfate aqueous (200 mL), brine (200 mL), saturated sodium carbonate aqueous (200 mL), brine (200 mL), dried over anhydrous sodium sulfate, filtered and concentrated in vacuum. The residue was purified by column chromatography (petroleum ether: ethyl acetate = 20:1 - 5:1) to afford compound **37** (6 g, 6.53 mmol, 59.12% yield, 55% purity) as a white solid. LCMS: RT = 0.935 min, m/z 537.2 $[M+Na]^+$. Purity: 55.39%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.37 - 7.32 (m, 4H), 7.29 - 7.26 (m, 3H), 7.21 - 7.17 (m, 3H), 6.85 (d, *J* = 8.0 Hz, 1H), 5.13 (d, *J* = 6.0 Hz, 1H), 4.75 - 4.71 (m, 1H), 4.54 (s, 2H), 4.18 - 4.17 (m, 1H), 4.00 (dd, *J* = 9.6 Hz, 3.2 Hz, 1H), 3.75 - 3.70 (m, 4H), 3.67 - 3.64 (m, 2H), 3.61 - 3.58 (m, 2H), 2.70 (t, *J* = 8.0 Hz, 2H), 2.21 - 2.12 (m, 1H), 1.94 - 1.89 (m, 1H), 1.45 (s, 9H).

Benzyl (S)-3-(3-(((6S,9S)-9-(methoxycarbonyl)-2,2-dimethyl-4,7-dioxo-6-phenethyl-3,11-dioxa-5,8-diazatridecan-13-yl)oxy)phenyl)-2-(2-oxopyrrolidin-1-yl)propanoate (**39**). To a solution of compound **37** (6 g, 11.66 mmol) in methanol (60 mL) was added $Pd(OH)_2$/C (600 mg, 10% purity on carbon) 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 12 hours. LCMS indicated the reaction was completed. New spots were formed on TLC (petroleum ether: ethyl acetate = 1:2). The mixture was filtered and concentrated in vacuo. The residue was purified by prep-HPLC (TFA, MeCN/water) and followed by chromatography column (petroleum ether: ethyl acetate = 10:1 - 1:1) to afford compound **alcohol-37** (3.4 g, 7.72 mmol, 66.19% yield, 96.36% purity) as a colorless oil. LCMS: RT = 0.744 min, m/z 425.2 $[M+H]^+$. Purity: 96.36%. ^{1}H NMR (CD3OD, 400 MHz): δ 7.28 - 7.15 (m, 5H), 4.66 - 4.61 (m, 1H), 4.15 - 4.11 (m, 1H), 3.92 (dd, J = 10.0 Hz, 4.0 Hz, 1H), 3.75 - 3.72 (m, 4H), 3.67 - 3.64 (m, 2H), 3.55 - 3.53 (m, 2H), 2.74 - 2.68 (m, 2H), 2.09 - 2.03 (m, 1H), 1.95 - 1.87 (m ,1H), 1.46 (s, 9H). To a 50 mL round bottom flask was added tri-phenylphosphine (371 mg, 1.41 mmol), a solution of compound **alcohol-37** (200 mg, 471.16 umol) and compound **38** (160 mg, 471.16 umol in tetrahydrofuran (2 mL) under nitrogen atmosphere, then DEAD (246 mg, 1.41 mmol, 256.96 uL) in tetrahydrofuran (2 mL) was added drop wise. The reaction mixture was stirred at 20°C for 1 hour under nitrogen atmosphere. LCMS showed the starting material still remained and desired MS was detected. The mixture was concentrated in vacuum and the residue was purified by reverse column (water (0.05% HCl)-MeCN) to give compound **39** (280 mg) as colorless gum. LCMS: RT = 1.040 min, m/z 746.3 $[M+H]^+$. Purity: 98.13%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.36 - 7.31 (m, 5H), 7.27 - 7.24 (m, 2H), 7.20 - 7.17 (m, 4H), 6.90 (d, J = 8.4 Hz, 1H), 6.78 - 6.76 (m, 3H), 5.22 - 5.19 (m, 2H), 5.15 (d, J = 4.8 Hz, 1H), 4.76 - 4.74 (m, 1H), 4.04 - 4.01 (m, 3H), 3.79 - 3.77 (m, 3H), 3.72 (s, 3H), 3.38 - 3.36 (m, 1H), 3.29 - 3.27 (m, 1H), 2.97 (dd, J = 14.8 Hz, 10.8 Hz, 1H), 2.69 (t, J = 8.0 Hz, 2H), 2.34 - 2.27 (m, 1H), 2.20 - 2.15 (m, 2H), 1.93 - 1.91 (m , 2H), 1.84 - 1.77 (m , 2H), 1.45 (s, 9H).

(S)-3-(3-(2-((S)-2-((S)-2-amino-4-phenylbutanamido)-3-methoxy-3-oxopropoxy) ethoxy) phenyl)-2-(2-oxopyrrolidin-1-yl) propanoic acid (**40**). To a solution of compound **39** (230 mg, 308.37 umol, 1 *eq*) in methanol (6 mL) was purged with nitrogen, then $Pd(OH)_2$/C

(20 mg, 10% purity on carbon) and Pd/C (20 mg, 10% purity on carbon) was added in one portion under nitrogen atmosphere. The mixture was stirred under hydrogen atmosphere (20 psi) at 20°C for 1 hour. TLC (petroleum ether: ethyl acetate = 1:1) showed the starting material was consumed. The mixture was filtered through the celite pad and the pad washed with methanol (10 mL*3). The combined filtrate were concentrated in vacuum to give compound **acid-39** (220 mg, crude) as a white solid. LCMS: RT = 0.885 min, m/z 678.3 $[M+Na]^+$. Purity: 97.37%. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.27 - 7.15 (m, 6H), 6.83 - 6.81 (m, 2H), 6.78 (d, J = 8.0 Hz, 1H), 4.96 (dd, J = 11.6 Hz, 4.8 Hz, 1H), 4.66 (t, J = 4.0 Hz, 1H), 4.10 - 4.09 (m, 3H), 3.98 (dd, J = 11.6 Hz, 4.8 Hz, 1H), 3.84 - 3.81 (m, 3H), 3.70 (s, 3H), 3.48 - 3.40 (m, 2H), 3.35 - 3.32 (m, 1H), 3.02 (dd, J = 14.8 Hz, 11.6 Hz, 1H), 2.70 - 2.68 (m, 2H), 2.34 - 2.26 (m, 1H), 2.17 - 2.16 (m, 1H), 1.99 - 1.87 (m , 4H), 1.45 (s, 9H). To a solution of compound **acid-39** (220 mg, 335.50 umol) in dioxane (3 mL) was added HCl/dioxane (4M, 3 mL). The mixture was stirred at 20°C for 1 hour. LCMS showed the starting material was consumed and desired mass was detected. The mixture was concentrated in vacuum to give compound **40** (220 mg, crude, HCl salt) as a white solid. LCMS: RT = 0.712 min, m/z 556.3 $[M+H]^+$. Purity: 83.71%. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.30 - 7.19 (m, 6H), 6.84 - 6.78 (m, 3H), 4.99 (dd, J = 11.6 Hz, 4.8 Hz, 1H), 4.77 - 4.75 (m, 1H), 4.14 - 4.11 (m, 2H), 4.05 - 4.02 (m, 2H), 3.86 - 3.83 (m, 3H), 3.73 (s, 3H), 3.47 - 3.42 (m, 2H), 3.37 - 3.31 (m, 1H), 3.03 (dd, J = 14.8 Hz, 11.6 Hz, 1H), 2.78 - 2.76 (m, 2H), 2.35 - 2.26 (m, 1H), 2.19 - 2.15 (m, 3H), 1.98 - 1.92 (m , 1H), 1.88 - 1.79 (m, 1H).

Methyl (7S, 10S, 13S)-9, 12-dioxo-13-(2-oxopyrrolidin-1-yl)-10-phenethyl-2, 5-dioxa-8, 11-diaza-1(1, 3)-benzenacyclotetradecaphane-7-carboxylate (**41**). To a solution of compound **40** (190 mg, 260.77 umol, HCl salt) and N, N-diisopropylethylamine (152 mg, 1.17 mmol, 204.39 uL) in N, N-dimethyl formamide (19 mL) was added HOBt (53 mg, 391.15 umol) at 0°C. The mixture was stirred at 0°C for 10 minutes. EDCI (75 mg, 391.15 umol) was added at 0°C. The reaction mixture was stirred at 0°C for 20 minutes and then stirred at 20°C for another 5 hours. LCMS showed a part of starting material still remained. The mixture was stirred at 20°C for another 18 hours. LCMS showed the starting material was consumed. The mixture was quenched with water (30 mL) and then adjusted to pH = 7 with 1 N hydrochloric acid aqueous solution. The mixture was extracted with ethyl acetate (40 mL*3). The combined organic layers were washed with brine (50 mL*3), dried over anhydrous sodium sulfate, filtered, concentrated in vacuum. The residue was purified by column chromatography (SiO2, petroleum ether: ethyl acetate = 2:1 - 2:3) to give compound **41** (40 mg, 74.40 umol, 28.53% yield, 100% purity) as a light yellow solid. LCMS: RT = 0.888 min, m/z 538.3 $[M+Na]^+$. Purity: 97.31%. ^{1}H NMR (CD_3OD, 400 MHz): δ 8.12 (t, J = 8.4 Hz, 1H), 7.24 - 7.13 (m, 6H), 6.96 (d, J = 8.0 Hz, 1H), 6.81- 6.77 (m, 2H), 4.74 (dd, J = 12.0 Hz, 3.2 Hz, 1H), 4.61 - 4.57 (m, 1H), 4.51 - 4.49 (m, 1H), 4.26 - 4.24 (m, 2H), 3.78 - 3.71 (m, 9H), 3.28 (d, J = 12.4 Hz, 1H), 2.78 (dd, J = 12.8 Hz, 3.6 Hz, 1H), 2.57 - 2.53 (m, 2H), 2.45 - 2.41 (m, 2H), 2.08 - 2.07 (m, 2H), 2.02 - 1.93 (m , 1H), 1.82 - 1.81 (m , 1H).

(7S, 10S, 13S)-9, 12-dioxo-13-(2-oxopyrrolidin-1-yl)-10-phenethyl-2, 5-dioxa-8, 11-diaza-1(1, 3)-benzenacyclotetradecaphane-7-carboxylic acid (**42**). To a solution of compound **41** (40 mg, 74.40 umol) in tetrahydrofuran (0.4 mL) and water (0.2 mL) was added sodium hydroxide (21 mg, 520.83 umol) in water (0.2 mL) drop wise at 0°C. The

mixture was stirred at 0°C for 4 hours. TLC (petroleum ether: ethyl acetate = 0:1) showed the starting material was consumed completely. The mixture was adjusted pH = 3 with 1 N hydrochloric acid aqueous solution, lots of white precipitate were formed. The mixture was concentrated in vacuo to remove tetrahydrofuran. The mixture was filtered, washed with water (2 mL*3) and then dried in vacuum to give compound **42** (36 mg, 66.72 umol, 89.67% yield, 97.03% purity) as a white solid. LCMS: RT = 0.722 min, m/z 524.2 $[M+H]^+$. Purity: 97.03%. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.24 - 7.20 (m, 3H), 7.16 - 7.13 (m, 3H), 6.86 (d, J = 7.6 Hz, 1H), 6.82 - 6.79 (m, 1H), 6.77 (s, 1H), 4.74 (dd, J = 12.4 Hz, 3.6 Hz, 2H), 4.54 - 4.52 (m, 1H), 4.26 - 4.24 (m, 2H), 3.79 - 3.74 (m, 6H), 3.32 - 3.31 (m, 1H), 2.78 (dd, J = 12.4 Hz, 3.2 Hz, 1H), 2.55 (t, J = 8.0 Hz, 2H), 2.44 - 2.40 (m, 2H), 2.08 - 2.07 (m, 2H), 1.99 - 1.96 (m , 1H), 1.82 - 1.80 (m , 1H).

(7S, 10S, 13S)-9, 12-dioxo-13-(2-oxopyrrolidin-1-yl)-10-phenethyl-N-(2, 2, 2-trifluoroethyl)-2, 5-dioxa-8, 11-diaza-1(1, 3)-benzenacyclotetradecaphane-7-carboxamide (**6**). To a solution of compound **42** (31 mg, 59.21 umol) and 2,2,2-trifluoroethylamine (15 mg, 148.02 umol, 11.64 uL) in pyridine (0.3 mL) was added EDCI (28 mg, 148.02 umol) and HOBt (8 mg, 59.21 umol) at 0°C. The mixture was stirred at 20°C for 2 hours. LCMS showed the starting material was consumed and desired mass was detected. The mixture was quenched with water (15 mL) and then adjusted pH = 5 with 1N hydrochloric acid aqueous solution. The mixture was extracted with ethyl acetate (30 mL*3). The combined organic layers were washed with brine (20 mL), dried over anhydrous sodium sulfate, filtered, concentrated in vacuum to give a residue. The residue was purified by prep-HPLC (column: Phenomenex Synergi C18 150*25*10um; mobile phase: [water (0.1%TFA)-ACN]; B%: 40%-66%, 12 mins) to give **6** (32.3 mg, 77.65% yield, 99.89% purity) as a light yellow solid. LCMS: RT = 2.560 min, m/z 605.2 $[M+H]^+$. Purity: 99.89%. ^{1}H NMR (CD_3OD, 400 MHz): δ 8.35 (t, J = 6.0 Hz, 1H), 8.18 (d, J = 7.6 Hz, 1H), 7.76 (d, J = 7.6 Hz, 1H), 7.26 - 7.12 (m, 6H), 6.88 (d, J = 7.6 Hz, 1H), 6.81 - 6.79 (m, 2H), 4.82 (dd, J = 12.8 Hz, 3.6 Hz, 1H), 4.50 - 4.46 (m, 1H), 4.42 - 4.36 (m, 1H), 4.28 - 4.26 (m, 2H), 3.80 - 3.67 (m, 8H), 3.31 - 3.30 (m, 1H), 2.80 (dd, J = 12.8 Hz, 3.2 Hz, 1H), 2.55 - 2.42 (m, 4H), 2.10 - 1.97 (m, 3H), 1.83 - 1.83 (m , 1H).

***Methyl (S)-6-(benzyloxy)-2-((S)-2-((tert-butoxycarbonyl) amino)-4-phenylbutanamido) hexanoate* (44).—**To a solution of compound **43** (5 g, 21.07 mmol) in methanol (20 mL) was added HCl/methanol (4 M, 50 mL). The mixture was stirred for at 20°C 12 hours. LCMS showed the starting material was consumed. The reaction mixture was concentrated in *vacuo* to afford compound **methyl ester-43** (6.1 g, crude, HCl salt) as a yellow solid. LCMS: RT = 0.715 min, *m/z* 252.2 $[M+H]^+$. Purity: 100%. ^{1}H NMR (CD_3OD, 400 MHz): *δ* 7.36 - 7.26 (m, 5H), 4.50 (s, 2H), 4.05 (t, *J* = 6.4 Hz, 1H), 3.83 (s, 3H), 3.53 (t, *J* = 6.0 Hz, 2H), 1.98 - 1.93 (m, 2H), 1.68 - 1.54 (m, 4H). To a solution of boc-L-homophenylalanine (7 g, 25.06 mmol*)* and diisopropylethylamine (9.9 g, 76.59 mmol, 13.38 mL) in dimethyl formamide (60 mL) was added HATU (9.67 g, 25.44 mmol) at 0°C. The mixture was stirred for 10 min at 0°C. A solution of compound **methyl ester-43** (6.1 g, 21.20 mmol, HCl salt) in dimethyl formamide (20 mL) was added to the mixture at 0°C. The mixture was stirred for 20 min at 20°C. LCMS showed desired mass was detected. The reaction mixture was poured into water (300 mL), extracted with

ethyl acetate (100 mL*2). The combined organic phase was washed with 0.5 N hydrochloric acid (20 mL) and brine (200 mL*3), dried over anhydrous sodium sulfate, filtered and concentrated in *vacuo*. The residue was purified by column chromatography (petroleum ether: ethyl acetate = 10:1 - 2:1) to afford compound **44** (10.5 g, 19.16 mmol, 90.40% yield, 93.54% purity) as yellow gum. LCMS: RT = 0.954 min, *m/z* 513.2 $[M+H]^+$. Purity: 93.54%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.37 - 7.26 (m, 7H), 7.21 - 7.18 (m, 3H), 6.48 (d, J = 8.0 Hz, 1H), 5.03 (d, J = 6.8 Hz, 1H), 4.61 - 4.55 (m, 1H), 4.48 (s, 2H), 4.12 - 4.08 (m, 1H), 3.74 (s, 3H), 3.45 (t, J = 6.4 Hz, 2H), 2.69 (t, J = 8.0 Hz, 2H), 2.21 - 2.12 (m, 1H), 1.95 - 1.83 (m, 2H), 1.74 - 1.59 (m, 4H), 1.46 - 1.37 (m, 10H).

Methyl (S)-6-(benzyloxy)-2-((S)-2-((S)-3-(3-(benzyloxy) phenyl)-2-(2-oxopyrrolidin-1-yl) propanamido)-4-phenylbutanamido) hexanoate (**46**). To a solution of compound **44** (10.5 g, 20.48 mmol) in dioxane (50 mL) was added HCl/dioxane (4 M, 80 mL) at 0°C. The mixture was stirred at 20°C for 3 hours. LCMS showed desired mass was detected. The reaction mixture was concentrated in vacuo to afford compound **amine-44** (9.8 g, crude, HCl salt) as colorless gum. LCMS: RT = 0.825 min, m/z 413.2 $[M+H]^+$. Purity: 98.53%. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.34 - 7.24 (m, 10H), 4.49 - 4.42 (m, 3H), 3.99 (t, J = 6.0 Hz, 1H), 3.72 (s, 3H), 3.51 (t, J = 6.4 Hz, 2H), 2.80 - 2.70 (m, 2H), 2.19 - 2.12 (m, 2H), 1.96 - 1.88 (m, 1H), 1.82 - 1.73 (m, 1H), 1.69 - 1.62 (m, 2H), 1.56 - 1.47 (m, 2H). To a solution of compound **45** (5 g, 14.73 mmol) in dimethyl formamide (50 mL) was added diisopropylethylamine (7.62 g, 58.93 mmol, 10.29 mL, 4 eq) and HATU (6.72 g, 17.68 mmol). The mixture was stirred for 10 min at 0°C, then compound **amine-44** (6.67 g, 14.85 mmol, and HCl salt) in dimethyl formamide (30 mL) was added. The mixture was stirred at 0°C for 20 min. TLC (petroleum ether: ethyl acetate = 1:1) showed the desired mass was detected. The reaction mixture was poured into water (200 mL), extracted with ethyl acetate (200 mL*2). The combined organic phase was washed with brine (200 mL*3), dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by column chromatography (petroleum ether: ethyl acetate = 10:1 - 1:1) to afford compound **46** (7.5 g, 9.67 mmol, 65.60% yield, 94.58% purity) as colorless gum. LCMS: RT = 1.084 min, m/z 734.4 $[M+H]^+$. Purity: 94.58%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.42 - 7.37 (m, 4H), 7.35 - 7.28 (m, 7H), 7.26 - 7.25 (m, 1H), 7.22 - 7.16 (m, 4H), 6.92 (d, J = 7.6 Hz, 1H), 6.85 - 6.80 (m, 3H), 6.46 (d, J = 7.6 Hz, 1H), 5.04 (s, 2H), 4.78 (dd, J = 9.6, 6.4 Hz, 1H), 4.54 - 4.50 (m, 3H), 4.36 - 4.30 (m, 1H), 3.72 (s, 3H), 3.45 (d, J = 6.4 Hz, 2H), 3.41 - 3.35 (m, 1H), 3.29 - 3.21 (m, 2H), 3.08 (dd, J = 14.4, 10.0 Hz, 1H), 2.61 (t, J = 7.6 Hz, 2H), 2.31 (t, J = 7.6 Hz, 2H), 2.21- 2.16 (m, 1H), 2.00 - 1.89 (m, 4H), 1.65 - 1.58 (m, 3H), 1.43 - 1.33 (m, 2H).

Methyl (7S, 10S, 13S)-9, 12-dioxo-13-(2-oxopyrrolidin-1-yl)-10-phenethyl-2-oxa-8, 11-diaza-1(1, 3)-benzenacyclotetradecaphane-7-carboxylate (**47**). To a solution of compound **46** (7.5 g, 10.22 mmol) in methanol (80 mL) was added Pd/C (500 mg, 5% purity on carbon) and $Pd(OH)_2$/C (500 mg, 10% purity on carbon) under nitrogen atmosphere. The mixture was degassed with argon three times and stirred for 40 hours at 25°C under hydrogen atmosphere (50 psi). LCMS showed the starting material was consumed. The reaction mixture was filtered and concentrated in vacuo to afford compound **alcohol-46** (5.15 g, 9.30 mmol, 91.02% yield) as a white solid. LCMS: RT = 0.739 min, m/z 554.3 $[M+H]^+$. Purity: 67.88%. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.28 - 7.15 (m, 5H), 7.09 (t, J = 7.6 Hz, 1H),

6.73 - 6.63 (m, 3H), 4.95 - 4.91 (m, 1H), 4.41 - 4.34 (m, 2H), 3.71 (s, 3H), 3.58 - 3.47 (m, 4H), 3.20 (dd, J = 10.4, 5.2 Hz, 1H), 2.98 (dd, J = 14.8, 10.8 Hz, 1H), 2.73 - 2.63 (m, 2H), 2.37 - 2.23 (m, 2H), 2.13 - 2.05 (m, 1H), 2.01 - 1.82 (m, 4H), 1.77 - 1.69 (m, 1H), 1.60 - 1.40 (m, 4H). To a solution of compound **alcohol-46** (0.5 g, 903.11 umol) in pyridine (6 mL) was added p-toluenesulfonyl chloride (190 mg, 993.42 umol) at 0°C. The mixture was stirred for 12 hours at 10°C. LCMS showed desired mass was detected. The reaction mixture was concentrated in vacuo. The residue was purified by column chromatography (petroleum ether: ethyl acetate = 5:1 - 0:1) to afford compound **phenol-46** (0.2 g, 257.12 umol, 28.47% yield, 91% purity) as colorless gum. LCMS: RT = 0.942 min, m/z 708.3 $[M+H]^+$. Purity: 91.00%. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.76 (d, J = 8.4 Hz, 2H), 7.41 (d, J = 8.0 Hz, 2H), 7.28 - 7.25 (m, 2H), 7.20 - 7.15 (m, 3H), 7.09 (t, J = 8.0 Hz, 1H), 6.73 - 6.62 (m, 3H), 4.94 - 4.89 (m, 1H), 4.37 - 4.31 (m, 2H), 4.04 (t, J = 6.4 Hz, 2H), 3.70 (s, 3H), 3.54 - 3.49 (m, 2H), 3.19 (dd, J = 14.4, 6.4 Hz, 1H), 3.04 - 2.95 (m, 1H), 2.69 - 2.64 (m, 2H), 2.44 (s, 3H), 2.32 - 2.23 (m, 2H), 2.10 - 2.06 (m, 1H), 1.98 - 1.76 (m, 4H), 1.67 - 1.62 (m, 3H), 1.42 - 1.37 (m, 2H). To a solution of compound **phenol-46** (0.6 g, 847.66 umol) in dimethyl formamide (30 mL) was added potassium carbonate (468 mg, 3.39 mmol). The mixture was stirred at 50°C for 2 hours. LCMS showed desired mass was detected. The reaction mixture was poured in water (20 mL), extracted with ethyl acetate (30 mL*2). The combined organic phase was washed with brine (20 mL*3), dried over anhydrous sodium sulfate filtered and concentrated in vacuo. The residue was purified by reverse column (TFA condition) to afford compound **47** (0.3 g, 560.09 umol, 66.07% yield, 100% purity) as a white solid. LCMS: RT = 0.823 min, m/z 536.2 $[M+H]^+$. Purity:100%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.29 - 7.28 (m, 1H), 7.26 - 7.25 (m, 1H), 7.23 - 7.17 (m, 2H), 7.14 - 7.12 (m, 2H), 6.81 (d, J = 7.6 Hz, 1H), 6.74 (dd, J = 8.0, 2.4 Hz, 1H), 6.71 (t, J = 1.2 Hz, 1H), 6.49 (d, J = 8.4 Hz, 1H), 6.34 (d, J = 7.6 Hz, 1H), 4.65 (dd, J = 12.4, 2.8 Hz, 1H), 4.53 - 4.48 (m, 1H), 4.44 - 4.40 (m, 1H), 4.14 - 4.07 (m, 2H), 3.78 - 3.72 (m, 4H), 3.70 - 3.67 (m, 1H), 3.37 (t, J = 12.4 Hz, 1H), 2.71 (dd, J = 12.4, 2.8 Hz, 1H), 2.58 - 2.47 (m, 4H), 2.15 - 2.06 (m, 2H), 2.01 - 1.83 (m, 3H), 1.76 - 1.62 (m, 4H), 1.53 - 1.47 (m, 1H).

(7S, 10S, 13S)-9, 12-dioxo-13-(2-oxopyrrolidin-1-yl)-10-phenethyl-N-(2, 2, 2-trifluoroethyl)-2-oxa-8, 11-diaza-1(1, 3)-benzenacyclotetradecaphane-7-carboxamide (**5**). To a solution of compound **47** (80 mg, 149.36 umol) in tetrahydrofuran (2 mL) was added sodium hydroxide (30 mg, 746.78 umol) in water (0.6 mL) at 0°C. The mixture was stirred for 10 min at 0°C. TLC (petroleum ether: ethyl acetate = 1: 5) showed most of the starting material was consumed. The reaction mixture was poured into water (10 mL) and ethyl acetate (10 mL). The aqueous phase was adjusted to pH = 4 with 1 N hydrochloric acid, extracted with ethyl acetate (20 mL*2). The combined organic phase was washed with brine (20 mL), dried over anhydrous sodium sulfate, filtered and concentrated in vacuo to afford compound **acid-27** (50 mg, 95.86 umol, 64.18% yield, 100% purity) as a white solid. LCMS: RT = 0.764min, m/z 522.2 $[M+H]^+$. Purity: 100% (in DMSO for LCMS). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.28 (br. s, 1H), 8.27 (d, J = 8.4 Hz, 1H), 7.97 (d, J = 7.2 Hz, 1H), 7.27 - 7.24 (m, 2H), 7.18 - 7.11 (m, 4H), 6.77 - 6.74 (m, 2H), 6.67 (s, 1H), 4.61 (dd, J = 11.2, 2.4 Hz, 1H), 4.44 - 4.38 (m, 1H), 4.21 - 4.15 (m, 1H), 4.01 - 4.04 (m, 1H), 4.01 - 3.95 (m, 1H), 3.46 - 3.42 (m, 2H), 3.40 - 3.34 (m, 2H), 3.19 (t, J = 12.0 Hz, 1H), 2.58 (dd, J = 12.4, 2.4 Hz, 1H), 2.30 - 2.17 (m, 2H), 1.95 - 1.92 (m, 1H), 1.89 - 1.85

(m, 1H), 1.76 - 1.62 (m, 3H), 1.63 - 1.54 (m, 2H), 1.49 - 1.32 (m, 3H). To a solution of compound **acid-47** (60 mg, 115.03 umol) and diisopropylethylamine (60 mg, 464.24 umol, 80.86 uL) in dimethyl formamide (1 mL) was added HATU (60 mg, 157.80 umol) at 0°C. The mixture was stirred at 0°C for 5 min, then added 2, 2, 2-trifluoroethanamine (24 mg, 242.29 umol, and 19.05 uL) in dimethyl formamide (0.5 mL) at 0°C. The mixture was stirred for 25 min at 0°C. LCMS showed the starting material was consumed and desired mass was observed. The reaction mixture was poured into water (10 mL), extracted with ethyl acetate (20 mL*2). The combined organic phase was washed with brine (20 mL*3), dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by prep-HPLC (column: Phenomenex Synergi C_{18} 150*25*10um; mobile phase: [water(0.1%TFA) - ACN]; B%: 32% - 62%, 12min) to afford **5** (9 mg, 14.93 umol, 12.98% yield) (Rt=2.201 on LCMS) as a white solid. LCMS: RT = 2.202min, m/z 603.5 $[M+H]^+$. Purity: 100%. ^{1}H NMR (CD_3OD, 400 MHz): δ 8.63 (t, J = 6.0 Hz, 1H), 8.04 (d, J = 8.4 Hz, 1H), 7.27 - 7.21 (m, 7H), 6.87 - 6.84 (m, 2H), 6.81 (t, J = 2.4 Hz, 1H), 4.82 (dd, J = 12.4, 2.4 Hz, 1H), 4.38 - 4.33 (m, 1H), 4.27 - 4.15 (m, 3H), 4.02 - 3.97 (m, 1H), 3.79 - 3.63 (m, 2H), 3.55 - 3.43 (m, 2H), 2.68 (dd, J = 12.8, 2.4 Hz, 1H), 2.61 - 2.49 (m, 3H), 2.41 - 2.33 (m, 1H), 2.14 - 2.01 (m, 3H), 1.85 - 1.61 (m, 4H), 1.58 - 1.45 (m, 2H), 1.38 - 1.28 (m, 1H).

(7S, 10S, 13S)-N-(cyclopropylmethyl)-9, 12-dioxo-13-(2-oxopyrrolidin-1-yl)-10-phenethyl-2-oxa-8, 11-diaza-1(1, 3)-benzenacyclotetradecaphane-7-carboxamide (**8**). To a solution of compound **acid-47** (40 mg, 76.69 umol) and cyclopropylmethanamine (16 mg, 230.06 umol) in pyridine (0.1 mL) was added a mixture of EDCI (88 mg, 460.12 umol) and HOBt (6 mg, 46.01 umol) at 0°C. The mixture was stirred at 0°C for 1 hour, then warmed to 20°C slowly and stirred for 12 hours. LCMS showed the starting material remained, then 3.eq of EDCI was added. The mixture was stirred at 20°C for 12 hours. LCMS showed desired mass was detected. The reaction mixture was poured into water (10 mL), extracted with ethyl acetate (20 mL*2). The combined organic phase was washed with brine (20 mL), dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was triturated with ethyl acetate (10 mL) to give **8** (28.2 mg, 49.07 umol, 63.99% yield, 100% purity) as a yellow solid. LCMS: RT = 2.014 min, m/z 597.2 $[M+Na]^+$. Purity: 100%. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.25 - 7.18 (m, 3H), 7.17 - 7.11 (m, 3H), 6.85 (d, J = 8.0 Hz, 1H), 6.77 (dd, J = 8.0, 2.4 Hz, 1H), 6.70 (t, J = 2.0 Hz, 1H), 4.71 (dd, J = 12.0, 3.2 Hz, 1H), 4.66 (dd, J = 8.8, 5.6 Hz, 1H), 4.28 (dd, J = 8.8, 4.8 Hz, 1H), 4.22 - 4.17 (m, 1H), 4.11 - 4.06 (m, 1H), 3.67 (td, J = 9.2, 2.0 Hz, 2H), 3.31 - 3.25 (m, 1H), 3.07 - 2.96 (m, 2H), 2.74 (dd, J = 12.4, 2.8 Hz, 1H), 2.56 - 2.35 (m, 4H), 2.12 - 2.05 (m, 2H), 1.96 - 1.87 (m, 1H), 1.82 - 1.70 (m, 4H), 1.57 - 1.56 (m, 2H), 1.30 - 1.29 (m, 1H), 0.95 - 0.88 (m, 1H), 0.46 - 0.41 (m, 2H), 0.18 - 0.14 (m, 2H).

(7S, 10S, 13S)-N-cyclopentyl-9,12-dioxo-13-(2-oxopyrrolidin-1-yl)-10-phenethyl-2-oxa-8,11-diaza-1(1,3)-benzenacyclotetradecaphane-7-carboxamide (**14**). To a solution of compound **acid-47** (40 mg, 76.69 umol) and cyclopentanamine (13 mg, 153.37 umol, 15.13 uL) in pyridine (1 mL) was added a mixture of EDCI (147 mg, 766.87 umol, 10 eq) and HOBt (6 mg, 46.01 umol, 0.6 eq) at 0°C. The mixture was stirred for 1 hour at 0°C, then slowly warmed to 20°C and stirred for 12 hours. LCMS showed most of the starting material remained, then 5 equivalent of EDCI was added. The mixture was stirred at 20°C

for 13 hours. LCMS showed the starting material was consumed. The reaction mixture was poured into water (20 mL), extracted with ethyl acetate (20 mL*2). The combined organic phase was washed with brine (20 mL*2), dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was triturated with ethyl acetate (5 mL) to afford **14** (27.5 mg, 42.64 umol, 55.60% yield, 91.28% purity) as a yellow solid. LCMS: RT = 2.131 min, m/z 589.2 $[M+H]^+$ Purity: 91.28%. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.25 - 7.11 (m, 6H), 6.85 (d, J = 7.2 Hz, 1H), 6.77 (dd, J = 8.4, 2.4 Hz, 1H), 6.70 (t, J = 1.6 Hz, 1H), 4.71 (dd, J = 12.4, 3.6 Hz, 1H), 4.4 6 (dd, J = 8.8, 6.0 Hz, 1H), 4.24 (t, J = 7.2 Hz, 1H), 4.21 - 4.16 (m, 1H), 4.11 - 4.01 (m, 2H), 3.67 (td, J = 7.2, 1.2 Hz, 2H), 3.29 - 3.25 (m, 1H), 2.74 (dd, J = 12.4, 3.2 Hz, 1H), 2.58 - 2.37 (m, 4H), 2.12 - 2.03 (m, 2H), 1.93 - 1.84 (m, 3H), 1.82 - 1.74 (m, 2H), 1.72 - 1.66 (m, 4H), 1.62 - 1.52 (m, 4H), 1.46 - 1.36 (m, 3H).

(7S, 10S, 13S)-N-(2, 2-difluoroethyl)-9, 12-dioxo-13-(2-oxopyrrolidin-1-yl)-10-phenethyl-2-oxa-8, 11-diaza-1(1, 3)-benzenacyclotetradecaphane-7-carboxamide (**7**). To a solution of compound **acid-47** (40 mg, 76.69 umol) and 2, 2-difluoroethylamine (19 mg, 230.06 umol) in pyridine (1 mL) was added a mixture of EDCI (88 mg, 460.12 umol) and HOBt (6 mg, 46.01 umol) at 0°C. The mixture was stirred at 0°C for 1 hour, then slowly warmed to 20°C and stirred for 12 hours. LCMS showed the reaction was completed. The mixture was quenched with water (10 mL), extracted with ethyl acetate (30 mL*2). The organic layer was washed with 1N hydrochloric acid (10 mL*2), brine (10 mL*2), dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The crude was triturated with ethyl acetate (2 mL), filtered to give compound **7** (23.40 mg, 39.14 umol, 51.03% yield) as a white solid. LCMS: RT = 2.591 min, m/z 585.3 $[M+H]^+$. Purity: 97.79%. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.25 - 7.17 (m, 3H), 7.14 - 7.11 (m, 3H), 6.85 (d, J = 8.0 Hz, 1H), 6.77 (dd, J = 8.0 Hz, 2.0 Hz, 1H), 6.69 (t, J = 2.0 Hz, 1H), 5.84 (tt, J = 56.0 Hz, 4.0 Hz, 1H), 4.71 (dd, J = 12.4 Hz, 3.2 Hz, 1H), 4.47 (dd, J = 8.8 Hz, 6.4 Hz, 1H), 4.31 (dd, J = 9.6 Hz, 4.8 Hz, 1H), 4.21 - 4.05 (m, 2H), 3.69 - 3.63 (m, 2H), 3.56 - 3.44 (m, 2H), 3.29 - 3.25 (m, 1H), 2.73 (dd, J = 12.4 Hz, 3.2 Hz, 1H), 2.55 - 2.37 (m, 4H), 2.11 - 2.03 (m, 2H), 1.93 - 1.87 (m, 1H), 1.83 - 1.70 (m, 4H), 1.61 - 1.51 (m, 2H), 1.43 - 1.41 (m, 1H).

(7S, 10S, 13S)-9, 12-dioxo-13-(2-oxopyrrolidin-1-yl)-10-phenethyl-N-(1-(trifluoromethyl) cyclopropyl)-2-oxa-8, 11-diaza-1(1, 3)-benzenacyclotetradecaphane-7-carboxamide (**13**). To a solution of compound **acid-47** (50 mg, 95.86 umol) and 1-(Trifluoromethyl)cyclopropanamine (36 mg, 287.57 umol) in pyridine (1 mL) was added a mixture of EDCI (110 mg, 575.15 umol) and HOBt (8 mg, 57.51 umol) at 0°C. The mixture was stirred at 0°C for 1 hour, then slowly warmed to 20°C and stirred for 12 hours. LCMS showed compound **acid-47** still remained. EDCI (55 mg, 287.57 umol) was added at 0°C. The mixture was stirred at 0-20°C for 3 hours. LCMS showed the reaction was completed and desired mass was detected. The mixture was quenched with water (10 mL), extracted with ethyl acetate (20 mL*2). The organic layer was washed with brine (20 mL*2), dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The crude was purified by prep-HPLC (column: Phenomenex Synergi C_{18} 150*25*10um; mobile phase: [water (0.1%TFA)-ACN]; B%: 40%-70%,13min) to give compound **13** (11.8 mg, 18.20 umol, 18.99% yield, 96.99% purity) as a white solid. LCMS: RT = 2.681 min, m/z 629.2 $[M+H]^+$. Purity: 96.99%. ^{1}H NMR (CD_3OD, 400 MHz): δ 8.78 (s, 0.4H), 8.23 (d, J = 7.6 Hz, 0.5H),

8.00 (d, J = 8.8 Hz, 0.5H), 7.25 - 7.11 (m, 6H), 6.84 (t, J = 7.6 Hz, 1H), 6.76 (dd, J = 8.4 Hz, 2.4 Hz, 1H), 6.69 - 6.68 (m, 1H), 4.70 (dd, J = 12.4 Hz, 3.2 Hz, 1H), 4.48 - 4.46 (m, 1H), 4.24 - 4.17 (m, 2H), 4.09 - 4.07 (m, 1H), 3.67 (t, J = 7.6 Hz, 2H), 3.27 - 3.24 (m, 1H), 2.73 (dd, J = 12.4 Hz, 3.2 Hz, 1H), 2.53 - 2.39 (m, 4H), 2.10 - 2.08 (m, 2H), 1.91 - 1.70 (m, 5H), 1.58 - 1.56 (m, 2H), 1.45 - 1.39 (m, 1H), 1.31 - 1.19 (m, 2H), 1.09 - 0.97 (m, 2H).

(7S, 10S, 13S)-N-((1-methyl-1H-pyrazol-4-yl) methyl)-9, 12-dioxo-13-(2-oxopyrrolidin-1-yl)-10-phenethyl-2-oxa-8, 11-diaza-1(1, 3)-benzenacyclotetradecaphane-7-carboxamide (**9**). To a solution of compound **acid-47** (40 mg, 76.69 umol) and (1-methylpyrazol-4-yl) methanamine (26 mg, 230.06 umol) in pyridine (1 mL) was added a mixture of EDCI (88 mg, 460.12 umol) and HOBt (6 mg, 46.01 umol) at 0°C. The mixture was stirred at 0°C for 1 hour, then slowly warmed to 20°C and stirred for 4.5 hours. LCMS showed the reaction was completed and desired mass was detected. The mixture was quenched with water (10 mL), extracted with ethyl acetate (20 mL*2). The organic layer was washed with brine (20 mL*2), dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The crude was triturated in acetonitrile (2mL) and then by prep-HPLC (column: Boston pH-lex 150*25 10um; mobile phase: [water (0.1%TFA)-ACN]; B%: 33%-57%, 8min) to afford compound **9** (20 mg, 31.63 umol, 41.25% yield, 97.22% purity) as a white solid. LCMS: RT = 2.385 min, m/z 615.3 $[M+H]^+$. Purity: 97.22%. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.44 (s, 1H), 7.36 (s, 1H), 7.25 - 7.09 (m, 6H), 6.85 (d, J = 7.2 Hz, 1H), 6.76 (dd, J = 8.0 Hz, 2.0 Hz, 1H), 6.69 (t, J = 1.6 Hz, 1H), 5.19 (br. s, 0.5H), 4.71 (dd, J = 12.4 Hz, 3.2 Hz, 1H), 4.45 (dd, J = 8.4 Hz, 6.0 Hz, 1H), 4.28 - 4.18 (m, 4H), 4.09 - 4.08 (m, 1H), 3.73 (s, 3H), 3.66 - 3.65 (m, 2H), 3.27 - 3.24 (m, 1H), 2.74 (dd, J = 12.4 Hz, 3.6 Hz, 1H), 2.53 - 2.39 (m, 4H), 2.16 - 2.05 (m, 2H), 1.89 - 1.69 (m, 5H), 1.57 - 1.52 (m, 2H), 1.43 - 1.42 (m, 1H).

(7S, 10S, 13S)-7-(hydroxymethyl)-13-(2-oxopyrrolidin-1-yl)-10-phenethyl-2-oxa-8, 11-diaza-1(1, 3)-benzenacyclo-tetradecaphane-9, 12-Dione (**48**). To a solution of compound **47** (80 mg, 149.36 umol) in methanol (1 mL) was added lithium borohydride (13 mg, 597.43 umol) at 0°C. The mixture was stirred at 0°C for 1 hour. LCMS showed desired mass was detected. The reaction mixture was poured into water (15 ml) and 1 N hydrochloric acid (10 mL), extracted with ethyl acetate (10 mL*2). The combined organic phase was washed with brine (20 ml), dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by prep-HPLC (column: Phenomenex Synergi C_{18} 150*25*10um; mobile phase: [water (0.1%TFA)-ACN]; B%: 28%-55%, 12min) to afford **48** (51 mg, 100.43 umol, 67.24% yield, 99.96% purity) as a white solid. LCMS: RT = 2.329 min, m/z 508.3 $[M+H]^+$. Purity: 99.96%. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.95 (d, J = 8.8 Hz, 0.6H), 7.88 (d, J = 8.8 Hz, 0.5H), 7.25 - 7.11 (m, 6H), 6.84 (d, J = 7.2 Hz, 1H), 6.76 (dd, J = 8.0 Hz, 2.0 Hz, 1H), 6.70 (t, J = 1.2 Hz, 1H), 4.73 (dd, J = 12.4 Hz, 3.2 Hz, 1H), 4.42 - 4.37 (m, 1H), 4.18 - 4.07 (m, 2H), 3.87 - 3.83 (m, 1H), 3.67 (td, J = 7.6, 2.0 Hz, 2H), 3.41 (d, J = 5.6 Hz, 2H), 3.29 - 3.25 (m, 1H), 2.73 (dd, J = 12.0, 3.2 Hz, 1H), 2.57 - 2.36 (m, 4H), 2.12 - 2.01 (m, 2H), 1.93 - 1.75 (m, 3H), 1.61 - 1.39 (m, 5H).

(7S, 10S, 13S)-13-(2-oxopyrrolidin-1-yl)-10-phenethyl-7-(((2,2,2-trifluoroethyl)amino)methyl)-2-oxa-8,11-diaza-1(1,3)-benzenacyclotetradecaphane-9,12-dione (**10**). To a solution of compound **48** (80 mg, 157.60 umol) in tetrahydrofuran (1 mL) was added Dess-Martin reagent (134 mg, 315.20 umol, 97.58 uL) at 0°C. The

mixture was stirred at 20°C for 4 hours. LCMS showed the starting material was consumed. The reaction mixture was poured into water (20 mL) and ethyl acetate (20 mL). The organic phase was washed with brine (20 ml), dried over anhydrous sodium sulfate, filtrated and concentrated in vacuo. The residue was purified by prep-TLC (petroleum ether: ethyl acetate = 1:2) to afford compound **aldehyde-48** (20 mg, 36.39 umol, 23.09% yield, 92% purity) as a white solid. LCMS: RT = 0.739 min, m/z 506.3 $[M+H]^+$ Purity: 92.67%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.50 (s, 1H), 7.30 - 7.28 (m, 1H), 7.25 - 7.19 (m, 3H), 7.14 - 7.12 (m, 2H), 6.85 - 6.82 (m, 1H), 6.77 - 6.74 (m, 2H), 6.53 (d, J = 8.4 Hz, 1H), 6.40 (d, J = 6.8 Hz, 1H), 4.69 (dd, J = 12.4 Hz, 2.8 Hz, 1H), 4.45 - 4.37 (m, 2H), 4.18 - 4.07 (m, 2H), 3.66 - 3.55 (m, 2H), 3.42 (t, J = 12.4 Hz, 1H), 2.67 (dd, J = 12.4 Hz, 1.6 Hz, 1H), 2.57 - 2.34 (m, 4H), 2.12 - 2.02 (m, 3H), 1.93 - 1.86 (m, 4H), 1.66 - 1.62 (m, 1H), 1.54 - 1.47 (m, 2H). To a solution of compound **aldehyde-48** (20 mg, 39.56 umol) and 2, 2, 2-trifluoroethanamine (12 mg, 118.67 umol, 9.33 uL) in methanol (0.5 mL) was added 4A MS (100 mg). The mixture was stirred at 0°C for 1 hour, then sodium triacetoxyborohydride (17 mg, 79.11 umol) was added at 0°C. The mixture was stirred at 20°C for 24 hours. LCMS showed desired mass was detected. The reaction mixture was filtered and washed with ethyl acetate (30 mL). The organic phase was washed with brine (30 mL*2), dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by prep-HPLC (column: Phenomenex Gemini 150*25mm*10um; mobile phase: [water (0.05% ammonia hydroxide v/v)-ACN]; B%: 35%-65%, 12min) to afford compound **10** (4.6 mg, 7.06 umol, 17.85% yield, 90.372% purity) as a white solid. LCMS: RT = 1.833 min, m/z 589.2 $[M+H]^+$. Purity: 90.37%. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.25 - 7.19 (m, 3H), 7.14 - 7.11 (m, 3H), 6.84 (d, J = 7.2 Hz, 1H), 6.76 (dd, J = 8.4 Hz, 2.0 Hz, 1H), 6.69 (t, J = 1.6 Hz, 1H), 4.72 (dd, J = 12.0 Hz, 3.2 Hz, 1H), 4.38 (dd, J = 8.8 Hz, 5.6 Hz, 1H), 4.19 - 4.06 (m, 2H), 3.85 - 3.78 (m, 1H), 3.67 (td, J = 6.8 Hz, 4.6 Hz, 2H), 3.29 - 3.25 (m, 1H), 3.22 - 3.11 (m, 2H), 2.75 - 2.35 (m, 7H), 2.12 - 2.03 (m, 2H), 1.91 - 1.71 (m, 3H), 1.62 - 1.51 (m, 2H), 1.45 - 1.29 (m, 3H).

(7S, 10S, 13S)-9,12-dioxo-13-(2-oxopyrrolidin-1-yl)-10-phenethyl-N′-(2,2,2-trifluoro-1-iminoethyl)-2-oxa-8,11-diaza-1(1,3)-benzenacyclotetradecaphane-7-carbohydrazide (**49**). To a mixture of compound **47** (100 mg, 186.70 umol) in methanol (1 mL) was added hydrazine hydrate (1.03 g, 20.58 mmol, 1 mL). The result mixture was stirred at 20°C for 6 hours. LCMS showed the starting material was consumed and the desired mass was detected. The reaction mixture was quenched with 10 mL of water, the solid was collected by filtration and dried in vacuum to give compound **carbohydrazide-47** (60 mg, 97.54 umol, 52.24% yield, and 87.07% purity) as a white solid. LCMS: RT = 0.683 min, m/z 536.3 $[M+H]^+$. Purity: 87.07%. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.25 - 7.19 (m, 3H), 7.18 - 7.11 (m, 3H), 6.85 (d, J = 7.6 Hz, 1H), 6.77 (dd, J = 7.6 Hz, 2.0 Hz, 1H), 6.70 (t, J = 1.6 Hz, 1H), 4.71 (dd, J = 12.4 Hz, 2.0 Hz, 1H), 4.45 (dd, J = 8.8 Hz, 1.6 Hz, 1H), 4.23 (dd, J = 8.8 Hz, 1.2 Hz, 1H), 4.21 - 4.16 (m, 1H), 4.11 - 4.06 (m, 1H), 3.67 (dd, J = 6.4 Hz, 1.6 Hz, 2H), 3.30 - 3.25 (m, 1H), 2.74 (dd, J = 12.8 Hz, 3.6 Hz, 1H), 2.56 - 2.39 (m, 4H), 2.10 - 2.05 (m, 2H), 1.92 - 1.89 (m, 1H), 1.82 - 1.70 (m, 4H), 1.59 - 1.50 (m, 2H), 1.46 - 1.38 (m, 1H). Sodium (710 mg, 30.96 mmol, 733.85 uL) was added to methanol (150 mL) portion wise at 20°C. The mixture was stirred at 20°C for 30 min until the sodium was dissolved under nitrogen flow. To a solution of 2,2,2-trifluoroacetamide (35 g, 309.63 mmol) in pyridine (200 mL) was added trifluoroacetic anhydride (65.03 g, 309.63 mmol, 43.07 mL) in pyridine (60 mL)

drop wise at 0°C within 6 hours. The gas (BP: −60°C) was bubbled to the previous solution at −70°C. The mixture was stirred at 20°C for 1 hour until no gass released. The mixture was transferred to round bottom flask (250 mL). The mixture was distilled directly at 45°C of oil bath to afford methyl trifluoroacetimidate (5 g, 9.84 mmol, 3.18% yield, and 25% purity on NMR) as colorless oil. To a mixture of compound **carbohydrazide-47** (50 mg, 93.35 umol) in dichloromethane (2 mL) was added methyl trifluoroacetimidate (474 mg, 933.47 umol). The mixture was stirred at 20°C for 3 hours. LCMS showed the starting material was consumed and desired mass was detected. The mixture was concentrated in vacuum to give compound **49** (60 mg, crude) as a white solid. LCMS: RT = 0.765 min, m/z 631.3 $[M+H]^+$. Purity: 23.30%. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.24 - 7.18 (m, 3H), 7.13 (t, J = 8.0 Hz, 3H), 6.86 (d, J = 7.2 Hz, 1H), 6.77 (dd, J = 8.4 Hz, 2.8 Hz, 1H), 6.71 - 6.67 (m, 1H), 4.73 - 4.69 (m, 1H), 4.52 - 4.46 (m, 1H), 4.34 - 4.29 (m, 1H), 4.27 - 4.19 (m, 1H), 4.09 - 4.03 (m, 1H), 3.69 - 3.65 (m, 2H), 3.25 - 3.21 (m, 1H), 2.78 - 2.69 (m, 1H), 2.57 - 2.33 (m, 4H), 2.09 - 2.08 (m, 2H), 1.93 - 1.89 (m, 1H), 1.87 - 1.74 (m, 4H), 1.64 - 1.58 (m, 2H), 1.58 - 1.52 (m, 1H).

(7S, 10S, 13S)-13-(2-oxopyrrolidin-1-yl)-10-phenethyl-7-(5-(trifluoromethyl)-4H-1,2,4-triazol-3-yl)-2-oxa-8,11-diaza-1(1,3)-benzenacyclotetradecaphane-9,12-dione (**12**). A mixture of compound **49** (55 mg, 87.21 umol) in 1, 2-dichlorobenzene (1 mL) was stirred at 180°C for 3 hours. LCMS showed the reaction was completed. The mixture was concentrated in vacuum to give a residue. The residue was triturated with acetonitrile (10 mL) to give **12** (15 mg, 95.35% purity) as an off-white solid. LCMS: RT = 2.207 min, m/z 613.3 $[M+H]^+$. Purity: 95.35%. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.21 (t, J = 7.6 Hz, 3H), 7.15 - 7.07 (m, 3H), 6.86 (d, J = 7.6 Hz, 1H), 6.79 (dd, J = 8.0 Hz, 2.0 Hz, 1H), 6.73 (s, 1H), 5.09 (dd, J = 10.0 Hz, 4.8 Hz, 1H), 4.75 (dd, J = 12.0 Hz, 3.2 Hz, 1H), 4.44 (dd, J = 8.4 Hz, 6.0 Hz, 1H), 4.24 - 4.20 (m, 1H), 4.15 - 4.11 (m, 1H), 3.64 (td, J = 6.8 Hz, 2.8 Hz, 2H), 3.30 - 3.26 (m, 1H), 2.75 (dd, J = 12.0 Hz, 3.2 Hz, 1H), 2.53 - 2.39 (m, 4H), 2.12 - 2.02 (m, 2H), 2.00 - 1.78 (m, 5H), 1.62 - 1.57 (m, 2H), 1.51 - 1.49 (m, 1H).

(7S, 10S, 13S)-13-(2-oxopyrrolidin-1-yl)-10-phenethyl-7-(5-(trifluoromethyl)-1,3,4-oxadiazol-2-yl)-2-oxa-8,11-diaza-1(1,3)-benzenacyclotetradecaphane-9,12-dione (**11**). To a solution of compound **49** (45 mg, 71.35 umol) in dichloromethane (1 mL) was added trifluoroacetic acid (308 mg, 2.70 mmol, 0.2 mL). The mixture was stirred at 20°C for 3 hours. LCMS showed most of the starting material was consumed. The reaction was concentrated in vacuo. The residue was triturated with water (5 mL), filtered and dried under vacuo to afford compound **oxo-49** (45 mg, crude) as a white solid. LCMS: RT = 0.767 min, m/z 632.3 $[M+H]^+$. Purity: 81.71%. ^{1}H NMR (CD_3OD, 400 MHz): δ 8.39 (d, J = 8.4 Hz, 1H), 8.02 (d, J = 8.4 Hz, 1H), 7.27 - 7.20 (m, 3H), 7.15 - 7.11 (m, 3H), 6.85 (d, J = 7.2 Hz, 1H), 6.77 (dd, J = 8.0, 2.0 Hz, 1H), 6.70 (t, J = 1.6 Hz, 1H), 4.71 (dd, J = 12.4 Hz, 3.2 Hz, 1H), 4.50 - 4.37 (m, 2H), 4.22 - 4.17 (m, 1H), 4.11 - 4.05 (m, 1H), 3.67 (td, J = 6.8 Hz, 1.2 Hz, 2H), 3.29 - 3.25 (m, 1H), 2.74 (dd, J = 12.4 Hz, 2.8 Hz, 1H), 2.58 - 2.48 (m, 2H), 2.46 - 2.35 (m, 2H), 2.13 - 2.04 (m, 2H), 1.96 - 1.72 (m, 5H), 1.64 - 1.53 (m, 2H), 1.51 - 1.44 (m, 1H). ^{19}F NMR (CD_3OD, 400 MHz): δ −76.66. To a solution of compound **oxo-49** (45 mg, 71.24 umol) in tetrahydrofuran (1 mL) was added burgess reagent (51 mg, 213.73 umol). The mixture was stirred at 20°C for 12 hours. LCMS showed 40% of starting

material still remained, then another 2 equivalent of burgess reagent was added and stirred at 20°C for 24 hours. LCMS showed the starting material was consumed. The reaction mixture was poured into water (30 mL), extracted with ethyl acetate (20 mL* 2). The combined organic phase was washed with brine (20 mL), dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was triturated with ethyl acetate (5 mL), filtered. The filtrate was concentrated in vacuo, purified by prep-HPLC (column: Phenomenex Synergi C_{18} 150*25*10um; mobile phase: [water(0.1%TFA)-ACN];B%: 40% - 70%, 13min) to give compound **11** (11.5 mg, 18.23 umol, 25.59% yield, 97.281% purity) as a white solid. LCMS: RT = 2.500 min, m/z 614.1 $[M+H]^+$. Purity: 97.28%. ^{1}H NMR (CD_3OD, 400 MHz): δ 8.86 (d, J = 8.8 Hz, 0.4H), 8.12 (d, J = 8.8 Hz, 0.5H), 7.25 - 7.09 (m, 6H), 6.87 (d, J = 7.6 Hz, 1H), 6.79 (dd, J = 8.0 Hz, 2.0 Hz, 1H), 6.72 (t, J = 1.6 Hz, 1H), 5.29 - 5.24 (m, 1H), 4.74 (dd, J = 12.0 Hz, 3.2 Hz, 1H), 4.48 - 4.42 (m, 1H), 4.28 - 4.22 (m, 1H), 4.15 - 4.10 (m, 1H), 3.68 (td, J = 8.0 Hz, 1.6 Hz, 2H), 3.30 - 3.24 (m, 1H), 2.76 (dd, J = 12.4 Hz, 2.8 Hz, 1H), 2.58 - 2.36 (m, 4H), 2.14 - 2.01 (m, 4H), 1.94 - 1.79 (m, 3H), 1.67 - 1.57 (m, 3H).

***Tert-butyl (S)-2-((S)-2-amino-4-(4-fluorophenyl)butanamido)-6-hydroxyhexanoate* (52b).—**To a solution of compound **51a** (1.3 g, 5.90 mmol, 1 *eq*), compound **50c** (1.92 g, 5.78 mmol, 0.98 *eq*), T_3P (5.63 g, 8.85 mmol, 5.27 mL, 50% purity, 1.5 *eq*) in dichloromethane (30 mL) was added N, N-diisopropylethylamine (1.53 g, 11.81 mmol, 2.06 mL, 2 *eq*) at −5°C. The mixture was stirred at −5°C for 1 hour under nitrogen atmosphere. LCMS and TLC (dichloromethane: methanol = 10:1) and (petroleum ether: ethyl acetate = 0:1) showed the starting material was consumed completely and desired product mass detected. The mixture was quenched with water (15 mL) and then extracted with ethyl acetate (25 mL*3). The combined organic layers were washed with 1N hydrochloric acid (20 mL), saturated sodium bicarbonate aqueous (20 mL), and brine (20 mL), dried over anhydrous sodium sulfate, filtered and the filtrate was concentrated in vacuum. The residue was purified by column chromatography (SiO2, Petroleum ether: Ethyl acetate = 3:1 ~ 1:1) to give product **Cbz-52b** (1.07 g, 1.89 mmol, 32.09% yield, 91.449% purity) as light yellow gum. LCMS: RT = 0.881 min, Purity: 91.45%, m/z 630.4 $[M+H]^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.37 - 7.34 (m, 5H), 7.13 - 7.11 (m, 2H), 6.95 (d, J = 8.4 Hz, 2H), 6.52 (d, J = 8.0 Hz, 1H), 5.50 (d, J = 8.0 Hz, 1H), 5.15 - 5.11 (m, 2H), 4.47 - 4.44 (m, 1H), 4.19 - 4.14 (m, 1H), 3.62 - 3.58 (m, 2H), 2.66 (t, J = 7.6 Hz, 2H), 2.18 - 2.11 (m, 1H), 2.08 (s, 1H), 1.97 - 1.81 (m, 2H), 1.69 - 1.63 (m, 1H), 1.59 - 1.52 (m, 2H), 1.47 (s, 9H), 1.42 - 1.32 (m, 2H). A solution of compound **Cbz-52b** (1 g, 1.94 mmol, 1 *eq*) in tetrahydrofuran (10 mL) and trifluoroacetic acid (331.08 mg, 2.90 mmol, 214.99 uL, 1.5 *eq*) was degassed and purged with nitrogen three times, Pd/C (0.2 g, 10% purity) was added, then degassed and purged with hydrogen three times. The reaction mixture was stirred at 20°C for 1 hour under hydrogen (15 psi). LCMS indicated the starting material was consumed and desired product mass was detected. The reaction mixture was filtrated to give a solution of compound **52b** (crude, 0.74 g) in tetrahydrofuran (10 mL), which was used for the next step directly without further purification.

Tert-butyl (S)-2-((S)-2-((S)-3-(3-(benzyloxy) phenyl)-2-(2-oxopyrrolidin-1-yl) propanamido)-4-(4-fluorophenyl) bu-tanamido)-6-hydroxyhexanoate (**54b**). To a solution of compound **52b** (0.74 g, 1.93 mmol, 1 eq) in tetrahydrofuran (5 mL) was added N,

N-diisopropylethylamine (1.00 g, 7.74 mmol, 1.35 mL, 4 eq), compound **45** (657 mg, 1.93 mmol, 1 eq) and T_3P (923 mg, 1.45 mmol, 863.01 uL, 50% purity 0.75 *eq*) at −5°C and the reaction was stirred at −5°C for 1 hour under nitrogen. LCMS indicated the starting material was consumed and desired product mass was detected. The reaction was quenched with 20 ml of water, extracted with ethyl acetate (50 mL*3), washed with brine (10 mL*2), dried over anhydrous sodium sulfate, filtrated and the filtrate was concentrated in vacuum. The residue was purified combined with another batch (0.67 g scale) by column (SiO_2, petroleum ether: ethyl acetate = 2:1 ~ 1:2) to give compound **54b** (1.16 g, 1.60 mmol, 51.83% yield, 97.36% purity) as a white solid. LCMS: RT = 1.009 min, Purity: 97.36%, m/z 704.5 $[M+H]^+$. ^1H NMR (Methanol-d4, 400 MHz): δ 7.44 - 7.32 (m, 5H), 7.23 - 7.18 (m, 2H), 7.14 - 7.10 (m, 2H), 6.98 - 6.92 (m, 2H), 6.85 - 6.81 (m, 3H), 6.59 (d, J = 8.0 Hz, 1H), 5.05 (s, 2H), 4.83 - 4.79 (m, 1H), 4.47 - 4.42 (m, 1H), 4.34 - 4.30 (m, 1H), 3.63 - 3.59 (m, 2H), 3.41 - 3.37 (m, 1H), 3.31 - 3.25 (m, 2H), 3.12 - 3.06 (m, 1H), 2.58 (t, J = 8.0 Hz, 2H), 2.30 (t, J = 8.0 Hz, 2H), 2.17 - 2.11 (m, 1H), 1.96 - 1.81 (m, 4H), 1.65 - 1.60 (m, 1H), 1.56 - 1.53 (m, 2H), 1.48 - 1.43 (m, 10H), 1.40 - 1.25 (m, 2H).

Tert-butyl (7S, 10S, 13S)-10-(4-fluorophenethyl)-9, 12-dioxo-13-(2-oxopyrrolidin-1-yl)-2-oxa-8, 11-diaza-1(1, 3)-benzenacyclotetradecaphane-7-carboxylate (**55b**). To a solution of compound **54b** (1.16 g, 1.65 mmol, 1 eq) in pyridine (12 mL) was added p-toluenesulfonyl chloride (1.57 g, 8.24 mmol, 5 eq) at 20°C and then the reaction mixture was stirred at 20°C for 3 hours under nitrogen atmosphere. LCMS indicated most of the starting material was consumed and desired product mass was detected. The reaction was quenched with 20 mL of water, extracted with ethyl acetate (100 mL*2), washed with 1N hydrochloric acid (20 mL*2), saturated sodium bicarbonate aqueous (10 mL*2), brine (10 mL), dried over anhydrous sodium sulfate, filtrated and the filtrate was concentrated in vacuum. The residue was purified by column (SiO_2, petroleum ether: ethyl acetate = 3:1 ~ 1:1, monitored by TLC, petroleum ether: ethyl acetate = 1:1) to give compound **Tos-54b** (0.9 g, 903.12 umol, 54.80% yield, 86.1% purity) as a white solid. LCMS: RT = 1.124 min, Purity: 86.10%, m/z 858.4 $[M+H]^+$. ^1H NMR ($CDCl_3$, 400 MHz): δ 7.77 (d, J = 8.4 Hz, 2H), 7.53 - 7.38 (m, 5H), 7.34 - 7.32 (m, 2H), 7.23 - 7.18 (m, 1H), 7.14 - 7.09 (m, 3H), 6.99 - 6.94 (m, 2H), 6.86 - 6.82 (m, 3H), 6.50 (d, J = 7.6 Hz, 1H), 5.05 (s, 2H), 4.79 - 4.73 (m, 1H), 4.42 - 4.31 (m, 2H), 4.04 - 3.96 (m, 2H), 3.63 - 3.59 (m, 2H), 3.43 - 3.37 (m, 1H), 3.31 - 3.24 (m, 2H), 3.16 - 3.10 (m, 1H), 2.60 (t, J = 8.0 Hz, 2H), 2.43 (s, 3H), 2.31 (t, J = 8.0 Hz, 2H), 2.20 - 2.14 (m, 1H), 1.96 - 1.88 (m, 3H), 1.78 - 1.76 (m, 2H), 1.68 - 1.62 (m, 2H), 1.45 (s, 9H), 1.37 - 1.30 (m, 2H). To a solution of compound **Tos-54b** (0.9 g, 1.05 mmol, 1 *eq*) in methanol (20 mL) was added $Pd(OH)_2/C$ (90 mg, 10% purity) under nitrogen . The suspension was degassed under vacuum and purged with hydrogen several times. The mixture was stirred under hydrogen atmosphere (15 psi) at 20°C for 3 hours. TLC (petroleum ether: ethyl acetate = 0:1) showed the starting material was consumed. The mixture was filtered; the solid was washed with methanol (5 mL*2). The filtrate was concentrated in vacuum to give compound *phenol-54b* (crude, 780 mg, 818.26 umol, 78.01% yield, 80.557% purity) as white solid, which was used into the next step without further purification. LCMS: RT = 1.014 min, Purity: 80.56%, m/z 768.4 $[M+H]^+$. ^1H NMR (Methanol-d_4, 400 MHz): δ 7.77 (d, J = 8.4 Hz, 2H), 7.42 (d, J = 7.6 Hz, 2H), 7.22 - 7.18 (m, 2H), 7.11 - 7.07 (m, 1H), 6.98 (d, J = 8.8 Hz, 2H), 6.73 - 6.69 (m, 2H), 6.65 - 6.62 (m, 1H), 4.96 - 4.91 (m, 1H), 4.36 - 4.20

(m, 2H), 4.09 - 4.02 (m, 2H), 3.59 - 3.47 (m, 2H), 3.23 - 3.18 (m, 1H), 3.02 - 2.96 (m, 1H), 2.68 - 2.63 (m, 2H), 2.43 (s, 3H), 2.36 - 2.23 (m, 2H), 2.09 - 2.07 (m, 1H), 1.98 - 1.62 (m, 7H), 1.45 - 1.35 (m, 11H). To a solution of compound **phenol-54b** (780 mg, 1.02 mmol, 1 eq) in N, N-dimethylformamide (40 mL) was added cesium carbonate (993 mg, 3.05 mmol, 3 eq). The mixture was stirred at 30°C for 16 hours. LCMS showed the starting material was consumed and desired product mass was detected. The mixture was poured into ice 1N hydrochloric acid aqueous (80 mL). The mixture was extracted with ethyl acetate (50 mL*3), the combined organic layers were washed with saturated sodium bicarbonate solution (50 mL) and brine (50 mL*3), dried over anhydrous sodium sulfate, filtered and the filtrate was concentrated in vacuum. The residue was purified by column chromatography (SiO_2, Petroleum ether: Ethyl acetate = 2:1 ~ 1:1) to give compound **55b** (330 mg, 478.56 umol, 47.11% yield, 86.388% purity) as a white solid. LCMS: RT = 0.916 min, Purity: 86.39%, m/z 596.1 $[M+H]^+$.^{1}H NMR (Methanol-d_4, 400 MHz): δ 7.22 - 7.09 (m, 3H), 6.96 (d, J = 8.8 Hz, 2H), 6.85 (d, J = 7.6 Hz, 1H), 6.77 (dd, J_1 = 2.0 Hz, J_2 = 8.0 Hz, 1H), 6.73 - 6.70 (m, 1H), 6.67 - 6.66 (m, 1H), 4.69 (dd, J_1 = 3.2 Hz, J_2 = 12.0 Hz, 1H), 4.50 - 4.43 (m, 1H), 4.28 - 4.17 (m, 2H), 4.08 - 4.02 (m, 1H), 3.68 (t, J = 7.2 Hz, 2H), 3.25 (t, J = 12.4 Hz, 1H), 2.75 (dd, J_1 = 3.6 Hz, J_2 = 12.4 Hz, 1H), 2.55 (t, J = 8.0 Hz, 2H), 2.43 - 2.40 (m, 2H), 2.11 - 2.03 (m, 2H), 1.95 - 1.51 (m, 8H), 1.42 (s, 9H).

(7S, 10S, 13S)-10-(4-fluorophenethyl)-9, 12-dioxo-13-(2-oxopyrrolidin-1-yl)-2-oxa-8, 11-diaza-1(1, 3)-benzena-cyclotetradecaphane-7-carboxylic acid (**56b**). To a solution of compound **55b** in dichloromethane (4 mL) was added trifluoroacetic acid (1.93 g, 16.88 mmol, 1.25 mL,116.42 eq).The mixture was stirred at 20°C for 3 hours. LCMS showed most of the starting material was consumed and desired product mass was detected. The mixture was concentrated in vacuum to give compound **56b** (130 mg, crude) as brown gum, which was used for the next step without further purification.

(7S, 10S, 13S)-N-cyclopentyl-10-(4-fluorophenethyl)-9, 12-dioxo-13-(2-oxopyrrolidin-1-yl)-2-oxa-8, 11-diaza-1(1, 3)-benzenacyclotetradecaphane-7-carboxamide (**16**). To a solution of compound **56b** (40 mg, 74.13 umol, 1 eq), N, N-diisopropylethylamine (28.74 mg, 222.39 umol, 38.74 uL, 3 eq) and cyclopentanamine (18.94 mg, 222.39 umol, 21.94 uL, 3 eq) in dichloromethane (1 mL) was added T_3P (94.35 mg, 148.26 umol, 88.17 uL, 50% purity, 2 eq) at 0°C. The mixture was stirred at 0°C for 1 hour. LCMS showed the starting material was consumed and desired product mass was detected. The mixture was poured into water (10 mL) and then extracted with ethyl acetate (15 mL*3). The combined organic layers were washed with 1N hydrochloric acid solution (15 mL), saturated sodium bicarbonate aqueous (10 mL) and brine (10 mL), dried over anhydrous sodium sulfate, filtered and the filtrate was concentrated in vacuum. The residue was purified by prep-HPLC (column: Phenomenex Gemini 150*25mm*10um; mobile phase: [water (10mM NH_4HCO_3)-ACN]; B%: 50%-80%, 10min) to give **16** (12.1 mg, 19.56 umol, 26.38% yield, 98.073% purity) as a white solid. LCMS: RT = 2.738 min, Purity: 98.07%, m/z 607.3 $[M+H]^+$. ^{1}H NMR (Methanol-d4, 400 MHz): δ 7.19 (t, J = 8.0 Hz, 1H), 7.15 - 7.11 (m, 2H), 6.96 (t, J = 8.8 Hz, 2H), 6.85 (d, J = 8.0 Hz, 1H), 6.76 (dd, J_1 = 2.0 Hz, J_2 = 8.0 Hz, 1H), 6.70 - 6.69 (m, 1H), 4.70 (dd, J_1 = 3.6 Hz, J_2 = 12.4 Hz, 1H), 4.45 (dd, J_1 = 6.0 Hz, J_2 = 8.4 Hz, 1H), 4.26 - 4.16 (m, 2H), 4.10 - 4.02 (m, 2H), 3.69 - 3.64 (m, 2H), 3.27 (d, J = 12.4 Hz, 1H), 2.74 (dd, J_1

= 3.2 Hz, J_2 = 12.4 Hz, 1H), 2.54 - 2.38 (m, 4H), 2.11 - 2.06 (m, 2H), 1.90 - 1.67 (m, 9H), 1.57 - 1.56 (m, 4H), 1.43 - 1.40 (m, 3H).

***(S)-2-((S)-2-amino-4-morpholinobutanamido)-N-cyclopentyl-6-hydroxyhexanamide* (52c).—**To a solution of compound **51b** (1.2 g, 5.60 mmol, 1 *eq*), compound **50a** (1.61 g, 5.60 mmol, 1 *eq*) and diisopropylethylamine (2.17 g, 16.80 mmol, 2.93 mL, 3 *eq*) in dichloromethane (15 mL) was added T_3P (4.28 g, 6.72 mmol, 4.00 mL, 50% purity in ethyl acetate solution, 1.2 *eq*) drop wise at 0°C. The mixture was stirred for 1 hour at 0°C. LCMS showed the reaction was completed. The reaction mixture was poured into water (100 ml), extracted with ethyl acetate (100 mL*5). The combined organic phase was washed with brine (50 mL), dried over anhydrous sodium sulfate, filtered and concentrated in vacuum. The residue was purified by prep-HPLC (column: Phenomenex luna C18 250*50mm*10 um; mobile phase: [water (0.1%TFA)-ACN]; B%: 12%-42%, 27min, 50%min) to afford compound **Boc-52c** (1 g, 1.90 mmol, 33.85% yield, 91.868% purity) as colorless gum. LCMS: RT = 0.706 min, purity: 91.87%, *m/z*: 485.6 $[M+H]^+$. ^1H NMR ($CDCl_3$, 400 MHz): δ 7.35 (d, J = 7.2 Hz, 1H), 6.65 (br. s, 1H), 6.47 (d, J = 7.2 Hz, 1H), 4.35 - 4.29 (m, 1H), 4.19 - 4.12 (m, 2H), 3.75 (t, J = 4.4 Hz, 4H), 3.65 (t, J = 6.0 Hz, 2H), 2.55 - 2.45 (m, 6H), 2.01 - 1.85 (m, 5H), 1.69 - 1.57 (m, 7H), 1.45 - 1.23 (m, 13H). To a solution of compound **Boc-52c** (1 g, 2.06 mmol, 1 *eq*) in dioxane (10 mL) was added HCl/dioxane (4 M, 5 mL) drop wise. The mixture was stirred for 3 hours at 20°C. LCMS showed the reaction was completely. The reaction mixture was concentrated in vacuum to afford compound **52c** (1.1 g, crude, 2 HCl salt) as colorless gum. LCMS: RT = 0.526 min, purity: 85.92%, *m/z*: 385.2 $[M+H]^+$. ^1H NMR (CD_3OD, 400 MHz): δ 4.29 - 4.25 (m, 1H), 4.20 - 4.11 (m, 1H), 4.13 - 4.06 (m, 3H), 3.94 - 3.87 (m, 2H), 3.59 - 3.55 (m, 4H), 3.51 - 3.73 (m, 2H), 3.26 - 3.15 (m, 2H), 2.46 - 2.29 (m, 2H),1.93 - 1.47 (m, 14H).

(S)-2-((S)-2-((S)-3-(3-(benzyloxy) phenyl)-2-(dimethylamino) propanamido)-4-morpholinobutanamido)-N-cyclopentyl-6-hydroxyhexanamide (**54d**). To a solution of compound **53b** (0.4 g, 967.62 umol, 1 eq, TFA salt) and compound **52c** (486.88 mg, 1.06 mmol, 1.1 eq, 2HCl salt) in dichloromethane (5 mL) was added T_3P (1.23 g, 1.94 mmol, 1.15 mL, 50% in ethyl acetate solution, 2 eq) and diisopropylethylamine (625.29 mg, 4.84 mmol, 842.71 uL, 5 eq). The mixture was stirred at 0 °C for 1 hr. LCMS showed the reaction was completed. The mixture was diluted with water (2 mL) and evaporated to give a residue. The residue was purified by reversed-phase HPLC (0.1% $NH_3 \cdot H_2O$ aqueous-ACN) to obtained compound **54d** (0.25 g, 315.38 umol, 32.59% yield, 84% purity) as a white solid. LCMS: RT = 0.756 min, m/z: 666.3 $[M+H]^+$. ^1H NMR (CD_3OD, 400 MHz): δ 7.44-4.42 (m, , 2H), 7.38 - 7.35 (m, 2H), 7.32-7.28 (m, , 1H), 6.87 - 6.86 (m, 1H), 6.83 - 6.80 (m, 2H), 5.05 (s, 2H), 4.33 (t, J = 6.8 Hz, 1H), 4.19 (dd, J =8.4, 6 Hz 1H), 4.09 - 4.02 (m, 1H), 3.65 (t, J = 4.4 Hz, 3H), 3.52 (t, J = 6.8 Hz,2H), 3.28-3.26 (m, 1H), 3.03 (dd, J = 13.6, 8.4 Hz,1H), 2.93 (dd, J = 13.6, 5.6 Hz,1H),2.40 - 2.37 (m, 4H), 2.35 (s, 6H), 1.93 - 1.69 (m, 7H), 1.60 - 1.34 (m, 10H).

(7S,10S,13S)-N-cyclopentyl-13-(dimethylamino)-10-(2-morpholinoethyl)-9,12-dioxo-2-oxa-8,11-diaza-1(1,3)-benzenacyclotetradecaphane-7-carboxamide (**21**). To a solution of compound **54d** (0.17 g, 255.31 umol, 1 eq) and triethylamine (258.35 mg, 2.55 mmol, 355.36 uL, 10 eq) in dichloromethane (0.5 mL) was added TosCl (292.04

mg, 1.53 mmol, 6 eq) at 0 °C. The mixture was stirred at 0°C for 1 hr. LCMS showed the reaction was completed. The mixture was quenched with water (2 mL) at 0°C, evaporated to give a residue. The residue was purified by reversed-phase HPLC (0.1% TFA aqueous-ACN) to afford compound **Tos-54d** (150 mg, 150.17 umol, 58.82% yield, 82.1% purity) as a white solid. LCMS: RT = 0.767 min, m/z: 820.5 $[M+H]^+$,410.8 $[M/2+H]^+$. ^{1}H NMR (CD^3OD, 400 MHz): δ 7.77 - 7.75 (m, 2H), 7.44 - 7.40 (m, 4H), 7.36 - 7.30 (m, 3H), 7.21 (t, J = 7.6 Hz, 1H), 6.93 - 6.88 (m, 2H), 6.82 (d, J = 7.6 Hz, 1H), 5.08 (s, 2H), 4.39 (t, J = 6 Hz, 1H), 4.20 (dd, J = 10, 5.6 Hz, 1H), 4.08 - 4.00 (m, 5H), 3.5 - 3.4 (m, 2H), 3.14 - 3.11 (m, 5H), 3.02 (s, 1H), 2.98 (s, 6H), 2.43 (s, 3H), 2.18 - 2.11 (m, 2H), 1.93 - 1.87 (m, 2H), 1.69 - 1.41 (m, 15H). To a solution of compound **Tos-54d**(120 mg, 146.33 umol, 1 eq) in methanol (1 mL) was added $Pd(OH)_2$/C (15 mg, 10% purity) under nitrogen. The suspension was degassed under vacuum and purged with hydrogen several times. The mixture was stirred under H2 (15psi) at 20-30°C for 1 hour. LCMS showed the reaction was completed. The mixture was filtered and the filtrate was evaporated to give the crude compound **phenyl-54d** (100 mg, 137.00 umol, 93.62% yield) as a colorless oil. LCMS: RT = 0.755 min, m/z: 730.4 $[M+H]^+$, 365.8 $[M/2+H]^+$. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.79 - 7.76 (m, 2H), 7.72 (d, J = 8.0 Hz, 1H), 7.43 (d, J = 8.0 Hz, 2H), 7.25 (d, J = 8.0 Hz, 1H), 7.10 (t, J = 8.0 Hz, 1H), 6.70 - 6.66 (m, 3H), 4.40 (t, J = 6.4 Hz,1H), 4.10 - 4.04 (m, 5H), 3.29 - 3.05 (m, 7H), 2.97 (s, 6H), 2.44 (s, 3H), 2.37 (s, 1H), 2.21- 2.08 (m, 2H), 1.92 - 1.86 (m, 2H), 1.72 - 1.45 (m, 14H). To a solution of compound **phenol-54d** (90 mg, 123.30 umol, 1 eq) in dimethyl formamide (3 mL) was added cesium carbonate (120.52 mg, 369.90 umol, 3 eq). The mixture was stirred at 20-30°C for 3 hour. LCMS showed the reaction was completed. The mixture was filtered and the filtrate was collected. The filtrate was purified by prep-HPLC (column: Xtimate C18 150*25mm*5um; mobile phase: [water (0.05% ammonia hydroxide v/v)-ACN]; B%: 21%-51%, 10min) and lyophilization to afford **21** (15.41 mg, 27.15 umol, 22.02% yield, 98.25% purity) as a white solid. LCMS: RT = 1.262 min, purity: 98.25%, m/z: 558.3 $[M+H]^+$, 279.8 $[M/2+H]^+$. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.17 (t, J = 7.6 Hz, 1H), 6.80 - 6.73 (m, 2H), 6.63 - 6.62 (m, 1H), 4.53 (t, J =7.2 Hz,1H), 4.23 (dd, J =8.8 Hz ,4.8 Hz, 1H), 4.08 - 4.06 (m, 2H), 3.71 - 3.69 (m, 4H), 3.32 (dd, J = 11.6, 2.8 Hz 1H), 3.04 (t, J = 12 Hz, 1H), 2.56 (dd, J = 12.4, 2.8 Hz 1H), 2.45 - 2.44 (m, 3H), 2.43 (s, 6H), 2.42 - 2.39 (m, 3H), 2.00 - 1.74 (m, 3H), 1.74 - 1.69 (m, 6H), 1.61- 1.60 (m, 4H), 1.59 - 1.43 (m, 3H).

***(S)-2-((S)-2-amino-3-phenoxypropanamido)-N-cyclopentyl-6-hydroxyhexanamide* (52d).—**To a solution of compound **50b** (700 mg, 1.57 mmol, 1 *eq*) and compound **51b** (370.41 mg, 1.73 mmol, 1.1 *eq*) in dimethyl formamide (8 mL) was added diisopropylethylamine (406.17 mg, 3.14 mmol, 547.39 uL, 2 *eq*) and T_3P (2.00 g, 3.14 mmol, 1.87 mL, 50% purity, 2 *eq*) at 0°C. The mixture was stirred at 0°C for 1 hr. LCMS showed the reaction was completed. The mixture was diluted with water (10 mL), extracted with ethyl acetate (10 mL*2). The combined organic layer was washed with brine (10 mL), dried over anhydrous sodium sulfate, filtered and the filtrate was evaporated to give a residue. The residue was purified by reversed-phase HPLC (0.1% $NH_3•H_2O$ aqueous-ACN) to afford compound **Trt-52d** (0.4 g, 645.38 umol, 41.07% yield) as a white solid. LCMS: RT = 1.015 min, *m/z*: 620.0 $[M+H]^+$. ^{1}H NMR (DMSO, 400 MHz): *δ* 7.98 - 7.95 (m, 2H), 7.60 (d, *J* = 3.2 Hz, 1H), 7.47 - 7.45 (m, 6H), 7.28 - 7.19 (m, 11H), 6.90 (t, *J* = 6.0 Hz,

1H), 6.76(d, *J* = 8.0Hz, 2H), 4.34 (t, *J* = 5.2 Hz ,2H), 3.95 - 3.89 (m, 1H), 3.87 - 3.88 (m, 2H), 3.52 - 3.47 (m, 1H), 3.36 - 3.34 (m, 1H), 3.28 - 3.26 (m, 1H), 3.19 (d, *J* = 8.8 Hz,1H), 1.72 - 1.50 (m, 2H), 1.44 - 1.17 (m, 13H). To a solution of compound **Trt-52d** (0.4 g, 645.38 umol, 1 *eq*) in dichloromethane (5 mL) was added HCl/dioxane (4 M, 161.34 uL, 1.00 *eq*). The mixture was stirred at 20-25°C for 1 hour. LCMS showed the reaction was completed. The mixture was evaporated to give a residue. The residue was triturated in ethyl acetate (10 mL) for 0.5 hr then filtered. The filter cake was collected as the desired product to afford compound **52d** (260 mg, 566.37 umol, 87.76% yield, 90.17% purity, HCl salt) as a white solid. LCMS: RT = 0.781 min, *m/z*: 378.1 $[M+H]^+$. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.33 - 7.29 (m, 2H),7.03 - 7.01 (m, 3H), 7.45 (dd, *J* = 10.0 , 3.2 Hz, 1H), 4.38 - 4.35 (m, 2H), 4.31 - 4.27 (m, 1H), 4.08 - 4.01 (m, 1H), 3.56 (t, *J* = 7.2 Hz, 2H), 1.86 - 1.41 (m, , 14H).

(S)-2-((S)-2-((S)-3-(3-(benzyloxy) phenyl)-2-(2-oxopyrrolidin-1-yl) propanamido)-3-phenoxypropanamido)-N-cyclopentyl-6-hydroxyhexanamide (**54e**). To a solution of compound **52d** (260 mg, 628.11 umol, 1 eq, HCl salt) and compound **45** (214 mg, 628.11 umol, 1 eq) in dimethyl formamide (5 mL) was added diisopropylethylamine (406 mg, 3.14 mmol, 547.03 uL, 5 eq) and T_3P (799.42 mg, 1.26 mmol, 747.12 uL, 50% purity, 2 eq). The mixture was stirred at 0°C for 2 hr. LCMS showed the reaction was completed. The mixture was diluted with water (10 mL), extracted with ethyl acetate (10 mL*2). The combined organic layer was washed with brine (10 mL), dried over anhydrous sodium sulfate, filtered and the filtrate was evaporated to give the crude product. The residue was purified by prep-HPLC (column: Phenomenex Synergi C18 150mm*25mm*10um; mobile phase: [water (0.1%TFA)-ACN]; B%: 40%-70%, 10min) and lyophilisation to afford compound **54e** (270 mg, 366.65 umol, 58.37% yield, 94.9% purity) as a white solid. LCMS: RT = 0.917 min, m/z: 699.4 $[M+H]^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.43 - 7.28 (m, , 8H), 7.20 - 7.18 (m, 2H), 6.99 (t, J = 7.2 Hz, 1H), 6.92 - 6.84 (m, 5H), 6.39 (d, J = 7.2 Hz, 1H), 5.04 (s, 2H), 4.68 - 4.66 (m, 2H), 4.48 - 4.45 (m, 1H), 4.36 - 4.30 (m, 1H), 4.17 - 4.11 (m,2H), 3.64 (t, J = 6.0 Hz, 2H), 3.43 - 3.37 (m, 1H), 3.29 - 3.28 (m, 1H), 3.15 - 3.12 (m, 2H), 2.27 (t, J = 8.0 Hz, 2H), 1.95 - 1.90 (m, 3H), 1.66 - 1.26 (m, 13H).

(7S, 10S, 13S)-N-cyclopentyl-9, 12-dioxo-13-(2-oxopyrrolidin-1-yl)-10-(phenoxymethyl)-2-oxa-8, 11-diaza-1(1, 3)-benzenacyclotetradecaphane-7-carboxamide (**15**). To a solution of compound **54e** (170 mg, 243.26 umol, 1 eq) in pyridine (1.5 mL) was added TosCl (1.16 g, 6.08 mmol, 25 eq) at 0°C. The mixture was stirred at 0°C for 2 hours. LCMS showed the reaction was completed. The pyridine was removed by nitrogen gas flowed. The crude product was purified by reversed-phase HPLC (0.1% TFA aqueous-ACN condition) to give compound **Tos-54e** (160 mg, 182.31 umol, 74.95% yield, 97.2% purity) as a white solid. LCMS: RT = 1.131 min, m/z: 853.3 $[M+H]^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.74 (d, J = 7.6 Hz, 2H), 7.41 - 7.25 (m, 9H), 7.18 (t, J = 8.0 Hz, 2H), 6.98 (t, J = 8.0 Hz, 1H), 6.90 - 6.84(m, 4H), 6.79 (d, J = 7.6 Hz, 1H), 6.55 (d, J = 7.6 Hz, 1H), 5.02 (s, 2H), 4.75 (dd, J = 10, 6.4 Hz, 1H), 4.67 (dd, J = 10.8, 4.4 Hz, 1H), 4.47 (br s, 1H), 4.42 (dd, J = 9.6, 4.4 Hz,1H), 4.35 (td, J = 8.8, 4.8 Hz, 1H), 4.17 - 4.08 (m, 2H), 4.03 - 3.91(m, 2H), 3.38 (dd, J =16.4, 7.2 Hz,1H), 3.29 - 3.09 (m, 3H), 3.04 (br s, 4H), 2.42 (s, 3H), 2.26 (td, J = 8.0, 3.2 Hz, 2H),1.92 - 1.82 (m, 5H), 1.66 - 1.35 (m, 11H). To a solution of compound **Tos-54e** (130 mg, 152.40 umol, 1 eq) in methanol (5 mL) was added Pd/C (10 mg, 10% purity) under

nitrogen. 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 2 hours. LCMS showed the reaction was completed. The mixture was filtered and the filtrate was evaporated to give a residue. The residue was purified by reversed-phase HPLC (0.1% TFA aqueous-ACN) and lyophilization to obtained compound **phenol-54e** (110 mg, 141.16 umol, 92.62% yield, 97.9% purity) as a colorless oil. LCMS: RT = 1.006 min, m/z: 763.2 $[M+H]^+$. ^1H NMR (CD_3OD, 400 MHz): δ 7.75 (d, J = 8.0 Hz 2H), 7.41 (d, J = 8.0 Hz 2H), 7.27 (t, J = 8.0 Hz, 2H), 7.08 (t, J = 8.0 Hz, 1H), 6.97 - 6.93 (m, 3H), 6.72 - 6.69 (m, 2H), 6.63 (dd, J = 7.6, 1.6 Hz, 1H), 4.95 (dd, J = 10.8, 5.6 Hz, 1H), 4.68 (t, J = 5.2 Hz, 1H), 4.30 - 4.23 (m, 3H), 4.07 - 4.02 (m, 1H), 3.99 (t, J = 6 Hz, 2H), 3.50 - 3.40 (m, 2H), 3.23 (dd, J = 14.8, 5.6 Hz, 1H), 3.00 (dd, J = 14.4, 10.4 Hz, 1H), 2.44 (s, 3H), 2.31 - 2.14 (m, 2H), 2.43 - 1.35 (m, 17H). To a solution of compound **phenol-54e** in dimethyl formamide (5 mL) was added cesium carbonate (140.93 mg, 432.55 umol, 3 eq). The mixture was stirred at 20-30°C for 2 hr. LCMS showed the reaction was completed. The mixture was diluted with water (5 mL), extracted with ethyl acetate (5 mL*2). The combine organic layer was washed with brine (5 mL), dried over anhydrous sodium sulfate, filtered and the filtrate was evaporated to give a residue. The residue was purified by prep- HPLC (column: Luna C18 150mm*25mm*5um; mobile phase: [water (0.1%TFA)-ACN]; B%: 37%-67%,10min) and lyophilization to obtain compound **15** (13.36 mg, 21.83 umol, 15.14% yield, 96.5% purity) as a white solid. LCMS: RT = 2.114 min, m/z: 591.2 $[M+H]^+$. ^1H NMR (CD_3OD, 400 MHz): δ 8.32 (br, 0.2H NH), 8.13 (br, 0.1H NH),7.62 (d, J = 6.8 Hz, 0.6H NH), 7.28 - 7.23 (m, 3H), 6.95 - 6.86 (m, 4H), 6.81 (br d, J = 8.0 Hz, 1H), 6.73 (br s, 1H), 4.92 - 4.85 (m, 1H), 4.75 (dd, J =12.4, 2 Hz, 1H), 4.30 - 4.22 (m, 2H), 4.10 - 4.03 (m, 4H), 3.66 (t, J = 7.2 Hz, 1H), 3.33 - 3.28 (m, 1H), 2.75 (br d, J = 10.8 Hz, 1H), 2.46 - 2.31 (m, 2H), 2.12 - 2.00 (m, 2H), 1.92 - 1.68 (m, 5H), 1.59 - 1.33 (m, 7H), 1.59 - 1.32 (m, 2H). Note: The NHs at 8.32 ppm, 8.13 ppm and 7.62 ppm were not throughly exchanged with deuterated CD_3OD.

***(S)-2-((S)-2-(3-(3-(benzyloxy) phenyl)-2, 2-dimethylpropanamido)-4-morpholinobutanamido)-N-cyclopentyl-6-hydroxyhexanamide* (54c).—**To a solution of compound **53a** (130 mg, 457.18 umol, 1 *eq*) compound **52c** (293 mg, 640.06 umol, 1.4 *eq*, 2 hydrochloride acid) in dichloromethane (2 mL) was added diisopropylethylamine (148 mg, 1.14 mmol, 199.08 uL, 2.5 *eq*) at 0°C. Then, T_3P (436 mg, 685.78 umol, 407.85 uL, 50% purity in ethyl acetate, 1.5 *eq*) was added drop-wise to the mixture and stirred at 0°C for 1 hour. LCMS showed the starting material was consumed and the desired mass was detected. The mixture was diluted with 50 mL of ethyl acetate, washed with brine (20 mL*3), dried over anhydrous sodium sulfate, filtered and concentrated in vacuum to give a residue and purified by prep-HPLC (column: Phenomenex luna C18 250*50mm*10 um; mobile phase: [water (0.1% TFA) - ACN]; B%: 29%-49%, 8min) to give compound **54c** (200 mg, 307.29 umol, 67.21% yield) as colorless oil. LCMS: RT = 0.776 min, *m/z* 651.3 $[M+H]^+$, purity: 65.42%. ^1H NMR ($CDCl_3$, 400MHz): δ 8.11 (d, J = 5.6 Hz, 1H), 7.45 - 7.36 (m, 5H), 7.19 (t, J = 8.0 Hz, 1H), 7.06 (d, J = 4.8 Hz, 1H), 6.84 (dd, J = 2.4 Hz, 8.0 Hz, 1H), 6.76 - 6.73 (m, 2H), 6.35 (d, J = 6.8 Hz, 1H), 5.03 (s, 2H), 4.53 - 4.46 (m, 1H), 4.24 - 4.18 (m, 1H), 3.96 - 3.88 (m, 3H), 3.66 (t, J = 5.2 Hz, 2H), 3.56 (d, J = 12.4 Hz, 1H), 3.43 - 3.35 (m, 1H), 3.02 - 2.96 (m, 1H), 2.91 - 2.75 (m, 8H), 2.71 - 2.61 (m, 3H), 2.16

- 2.12 (m, 1H), 1.98 - 1.91 (m, 2H), 1.79 - 1.74 (m, 2H), 1.71 - 1.65 (m, 2H), 1.60 - 1.54 (m, 3H), 1.45 - 1.38 (m, 3H), 1.25 - 1.23 (m, 6H).

(7S, 10S)-N-cyclopentyl-13, 13-dimethyl-10-(2-morpholinoethyl)-9, 12-dioxo-2-oxa-8, 11-diaza-1(1, 3)-benzena-cyclotetradecaphane-7-carboxamide (**18**). To a mixture of compound **54c** (120 mg, 184.38 umol, 1 eq) in dichloromethane (2 mL) was added TosCl (105 mg, 553.13 umol, 3 eq) and triethyl amine (56 mg, 553.13 umol, 76.99 uL, 3 eq) at 0°C, the mixture was stirred at 0-20°C for 12 hours. LCMS showed 22% of starting material remained, the mixture was stirred at 25°C for another 12 hours. LCMS showed trace of starting material remained and desired mass was detected. The mixture was diluted with 30 mL of water, extracted with ethyl acetate (30 mL*3). The combined organic layer was dried over anhydrous sodium sulfate, filtered and concentrated in vacuum to give a residue and purified by prep-HPLC (column: Phenomenex Gemini 150*25mm*10um; mobile phase: [water (0.04% NH_3H_2O +10mM NH_4HCO_3)-ACN]; B%: 66%-93%,min) to give compound **Tos-54c** (40 mg, 49.69 umol, 26.95% yield) as colorless oil. LCMS: RT = 1.124 min, m/z 805.4 $[M+H]^+$, purity: 47.57%. ^1H NMR ($CDCl_3$, 400MHz): δ 7.74 (d, J = 8.0 Hz, 2H), 7.69 (br. s, 1H), 7.43 - 7.31 (m, 7H), 7.15 (t, J = 8.0 Hz, 2H), 6.82 (dd, J = 2.4 Hz, 8.0 Hz, 1H), 6.74 - 6.69 (m, 2H), 6.59 (d, J = 7.2 Hz, 1H), 5.01 (s, 2H), 4.31 - 4.23 (m, 2H), 4.17 - 4.12 (m, 1H), 4.05 - 3.94 (m, 2H), 3.61 - 3.51 (m, 4H), 3.04 (d, J = 12.8 Hz, 1H), 2.58 (d, J = 12.8 Hz, 1H), 2.45 (s, 3H), 2.38 - 2.35 (m, 2H), 2.25 - 2.23 (m, 2H), 2.03 - 1.84 (m, 5H), 1.75 - 1.39 (m, 13H), 1.26 (s, 3H), 1.19 (s, 3H). A solution of compound **Tos-54c** (40 mg, 49.69 umol, 1 eq) in methanol (2 mL) was degassed with nitrogen for three times, Pd/C (5 mg, 5% purity) and $Pd(OH)_2/C$ (5 mg, 5% purity) was added to the mixture and degassed with hydrogen for three times. The mxiture was stirred at 25°C under nitrogen (15 psi) for 1 hour. TLC (dichloromethane/methanol=10/1) showed the starting material was consumed and a new spot with larger polarity was detected. The mixture was filtered and concentrated in vacuum to give compound **phenol-54c** (33 mg, 42.53 umol, 85.59% yield, 92.132% purity) as white solid and used directly. LCMS: RT = 0.841 min, m/z 715.4 $[M+H]^+$, purity: 92.13%. ^1H NMR ($CDCl_3$, 400MHz): δ 10.79 (br. s, 1H), 8.56 (br. s, 1H), 7.77 (d, J = 7.6 Hz, 2H), 7.34 (d, J = 7.6 Hz, 2H), 7.11 (t, J = 8.0 Hz, 1H), 6.82 - 6.76 (m, 2H), 6.63 (dd, J = 7.6 Hz, 19.6 Hz, 2H), 6.33 (br. s, 1H), 4.91 (br. s, 1H), 4.21 - 4.02 (m, 8H), 3.79 - 3.69 (m, 2H), 3.33 -3.23 (m, 1H), 3.03 - 2.82 (m, 5H), 2.64 - 2.51 (m, 2H), 2.45 (s, 3H), 2.37 - 2.24 (m, 2H), 2.08 - 1.79 (m, 5H), 1.68 - 1.42 (m, 7H), 1.26 - 1.22 (m, 6H). To a solution of compound **phenol-54c** (32 mg, 44.76 umol, 1 eq) in dimethyl formamide (1 mL) was added cesium carbonate (29 mg, 89.52 umol, 2 eq) at 0°C. The mixture was stirred at 0-25°C for 12 hours and then stirred at 40°C for another 12 hours. LCMS showed the starting material was consumed. The mixture was quenched with 30 mL of water, extracted with ethyl acetate (20 mL*3). The combined organic layers were washed with brine (20 mL*2), dried over anhydrous sodium sulfate, filtered and concentrated in vacuum to give a residue and purified by prep-HPLC (column: Luna C18 150mm*25mm*5um; mobile phase: [water (0.1% TFA)-ACN]; B%: 18%-48%, 10min) and lyophilization to give **18** (2.98 mg, 4.51 umol, 10.07% yield, 99.352% purity, trifluoroacetic acid) as a white solid. LCMS: RT = 1.654 min, m/z 543.3 $[M+H]^+$, purity: 99.35%. ^1H NMR ($CDCl_3$, 400MHz): δ 7.96 (d, J = 7.6 Hz, 1H), 7.15 (t, J = 8.0 Hz, 1H), 6.75 (d, J = 6.8 Hz, 1H), 6.69 - 6.63 (m, 2H), 6.50 - 6.45 (m, 2H), 4.82 - 4.75 (m, 1H), 4.42 - 4.36 (m, 1H), 4.21 - 4.14 (m, 1H), 4.08 - 3.92 (m,

6H), 3.78 - 3.48 (m, 2H), 3.14 - 2.87 (m, 4H), 2.49 - 2.37 (m, 2H), 1.97 - 1.89 (m, 4H), 1.72 -1.63 (m, 6H), 1.60 - 1.54 (m, 3H), 1.49 - 1.45 (m, 1H), 1.42 - 1.35 (m, 2H), 1.28 (s, 3H), 1.14 (s, 3H).

***Tert-butyl ((S)-1-(((S)-1-(cyclopentylamino)-1-oxopent-4-en-2-yl)amino)-4-morpholino-1-oxobutan-2-yl)carbamate* (59a).—**To a solution of compound **58** (0.800 g, 3.66 mmol) as the hydrochloric salt and compound **50a** (0.949 g, 3.29 mmol) in N, N-dimethylformamide (10 mL) at 0°C was added N, N-diisopropylethylamine (1.91 mL, 11.0 mmol) and T_3P (3.26 mL, 5.49 mmol, 50% purity in ethyl acetate). The mixture was stirred at 0-25°C for 2 hours. The reaction mixture was diluted with water (100 mL), extracted with ethyl acetate (50 mL*2). The combined organic layers were washed with brine (100 mL), then dried over anhydrous sodium sulfate, filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 1/1 to ethyl acetate / petroleum ether = 2/1) to afford compound **59a** (0.900 g, 1.81 mmol, 49.6% yield) as yellow oil. LCMS: RT = 0.686 min, *m/z* 453.2 $[M+H]^+$. ^1H NMR ($CDCl_3$, 400 MHz): δ 7.23 - 6.95 (m, 1H), 6.48 (s, 1H), 5.86 - 5.64 (m, 1H), 5.25 - 5.06 (m, 2H), 4.48 - 4.34 (m, 1H), 4.27 - 4.06 (m, 2H), 3.76 - 3.65 (m, 4H), 2.61 - 2.52 (m, 6H), 2.31 (s, 2H), 2.02 - 1.85 (m, 4H), 1.75 - 1.52 (m, 4H), 1.46 (s, 9H), 1.42 - 1.35 (m, 2H).

***(S)-2-((S)-2-amino-4-morpholinobutanamido)-N-cyclopentylpent-4-enamide* (60a).—**To a solution of compound **59a** (0.900 g, 1.99 mmol) in dioxane (4 mL) at 0°C was added a solution of HCl in dioxane (4 M, 4.5 mL, 18 mmol). The mixture was stirred at 0°C for 2 hours. The reaction mixture was concentrated under reduced pressure to afford compound **60a** as the dihydrochloric salt (0.800 g, 1.72 mmol, 86.6% yield) as yellow oil. LCMS: RT = 0.553 min, *m/z* 353.2 $[M+H]^+$. ^1H NMR ($CDCl_3$, 400 MHz): δ 10.67 (br. s, 1H), 9.02 (br. s, 1H), 8.50 (br. s, 2H), 8.10 (br. s, 1H), 6.04 - 5.75 (m, 1H), 5.31 - 5.00 (m, 2H), 4.70 - 4.68 (m, 1H), 4.16 - 4.07 (m, 2H), 3.76 - 3.72 (m, 4H), 2.55 (s, 8H), 1.97 - 1.67 (m, 4H), 1.34 - 1.21 (m, 6H).

Tert-butyl ((S)-3-(3-(allyloxy)phenyl)-1-(((S)-1-(((S)-1-(cyclopentylamino)-1-oxopent-4-en-2-yl)amino)-4-morpholino-1-oxobutan-2-yl)amino)-1-oxopropan-2-yl)(methyl)carbamate (**62a**). To a solution of diisopropylethylamine (0.296 mL, 1.70 mmol), compound **60a** (0.181 g, 0.425 mmol) and compound **61ab** (0.150 g, 0.425 mmol) in dimethyl formamide (2 mL) was added T_3P (0.379 mL, 0.637 mmol, 50% purity in ethyl acetate) at 0°C. The mixture was stirred at 0°C for 0.5 hour and poured into water (10 mL), extracted with ethyl acetate (10 mL*3). The combined organic layers were washed with brine (20 mL), dried over anhydrous sodium sulfate, filtered and the filtrate was concentrated under reduced pressure. The residue was purified by prep-HPLC (column: Phenomenex luna C_{18} 150*40 mm* 15 um; mobile phase: [water (0.05% HCl)-ACN]; B%: 30%-60%, 11 min) to afford compound **62a** (0.200 g, 0.266 mmol, 62.6% yield) as a yellow solid. LCMS: RT = 0.903 min, m/z 670.6 $[M+H]^+$. ^1H NMR (DMSO-d_6, 400 MHz) δ7.23 - 7.13 (m, 1H), 6.87 - 6.74 (m, 3H), 6.12 - 5.96 (m, 1H), 5.80 - 5.64 (m, 1H), 5.42 - 5.34 (m, 1H), 5.29 - 5.20 (m, 1H), 5.13 - 4.97 (m, 2H), 4.57 - 4.50 (m, 2H), 4.48 - 4.39 (m, 1H), 4.26 (s, 1H), 4.23 - 4.05 (m, 1H), 4.04 - 3.83 (m, 4H), 3.77 - 3.70 (m, 1H), 3.17 - 3.14 (m, 1H), 3.07 - 2.76 (m, 6H), 2.74 -

2.58 (m, 6H), 2.36 - 2.26 (m, 2H), 2.13 - 2.03 (m, 2H), 1.75 - 1.38 (m, 8H), 1.36 and 1.17 (s, 9H).

Tert-butyl ((7S, 10S, 13S)-7-(cyclopentylcarbamoyl)-10-(2-morpholinoethyl)-9, 12-dioxo-2-oxa-8, 11-diaza-1(1, 3)-benzenacyclotetradecaphane-13-yl) (methyl) carbamate (**63**). To a solution of compound **62a** (0.130 g, 0.171 mmol) in 1, 2-dichloroethane (40 mL) was added Grubbs catalyst 2nd generation (0.058 g, 0.068 mmol). The mixture was degassed with nitrogen three times and stirred for 12 hours at 65°C under nitrogen. After being cooled to room temperature, the mixture was concentrated under reduced pressure. The residue was purified by reversed-phase HPLC (0.1% trifluoroacetic acid in water, MeCN). The fractions were adjusted pH ~ 8 with a saturated aqueous solution of sodium bicarbonate, extracted with ethyl acetate (10 mL*3). The combined organic layers were washed with brine (20 mL), dried over anhydrous sodium sulfate, filtered, and the filtrate was concentrated under reduced pressure to afford compound **RCM-62a** (0.070 g, 0.088 mmol, 51.4% yield) as an orange solid. LCMS: RT = 0.768 min, m/z 642.2 $[M+H]^+$. ^{1}H NMR: ($CDCl_3$, 400 MHz) δ7.24 (t, J = 7.6 Hz, 1H), 6.90 (d, J = 7.6 Hz, 1H), 6.79 (d, J = 5.6 Hz, 1H), 6.60 (br. s, 1H), 6.45 - 6.33 (m, 1H), 5.69 - 5.58 (m, 1H), 5.51 - 5.50 (m, 1H), 5.04 - 5.01 (m, 1H), 4.70 - 4.61 (m, 1H), 4.49 - 4.38 (m, 1H), 4.26 - 3.96 (m, 2H), 3.83 - 3.61 (m, 4H), 3.50 (s, 1H), 3.33 - 3.27 (m, 1H), 3.03 - 2.88 (m, 3H), 2.87 - 2.77 (m, 1H), 2.54 - 2.30 (m, 6H), 2.26 - 1.84 (m, 5H), 1.83 - 1.61 (m, 8H), 1.44 (s, 9H). A solution of compound **RCM-62a** (0.103 g, 0.160 mmol) in methanol (1 mL) was degassed with nitrogen for three times, Pd/C (0.010 g, 10% purity on charcoal, wet) was added and degassed with hydrogen for three times. The mixture was stirred at 25°C under hydrogen (15 psi) for 1.5 hours. The mixture was diluted with methanol (40 mL), filtered, and the filtrate was concentrated under reduced pressure to afford compound **63** (0.100 g, 0.129 mmol, 80.6% yield) as a yellow solid. LCMS: RT = 0.864 min, m/z 644.4 $[M+H]^+$. ^{1}H NMR: ($CDCl_3$, 400 MHz) δ7.27 - 7.22 (m, 1H), 7.21 - 7.04 (m, 1H), 6.93 - 6.79 (m, 1H), 6.78 - 6.64 (m, 2H), 6.61 - 6.23 (m, 1H), 6.11 - 5.66 (m, 1H), 4.75 - 4.54 (m, 1H), 4.49 - 4.20 (m, 2H), 4.19 - 4.01 (m, 3H), 3.82 - 3.64 (m, 4H), 3.50 - 3.35 (m, 1H), 3.32 - 3.11 (m, 1H), 3.01 (s, 3H), 2.82 - 2.72 (m, 1H), 2.56 - 2.31 (m, 6H), 2.03 - 1.83 (m, 4H), 1.80 - 1.57 (m, 10H), 1.48 (s, 9H).

(7S, 10S, 13S)-N-cyclopentyl-13-(methylamino)-10-(2-morpholinoethyl)-9, 12-dioxo-2-oxa-8, 11-diaza-1(1, 3)-benzenacyclotetradecaphane-7-carboxamide (**20**). To a solution of compound **63** (0.100 g, 0.134 mmol) in dioxane (1 mL) was added a solution of HCl in dioxane (4 M, 1.20 mmol, 0.300 mL) drop wise at 0°C. The mixture was stirred at 0°C for 2 hours. The mixture was concentrated under reduced pressure. The residue was purified by prep-HPLC (column: Waters Xbridge 150*25 mm*5 um; mobile phase: [water (10 mM NH_4HCO_3) - ACN]; B%: 15%-45%, 9 min) to afford compound **20** (0.025 g, 0.045 mmol, 34.0% yield) as a white solid. LCMS: RT = 1.886 min, m/z 544.5 $[M+H]^+$. ^{1}H NMR (CD_3OD, 400 MHz) δ7.19 (t, J = 8.0 Hz, 1H), 6.85 - 6.72 (m, 2H), 6.58 - 6.51 (m, 1H), 4.60 (t, J = 6.8 Hz, 1H), 4.33 (dd, J = 9.2, 4.4 Hz, 1H), 4.23 - 4.17 (m, 1H), 4.09 - 4.02 (m, 1H), 4.02 - 3.94 (m, 1H), 3.69 (t, J = 4.4 Hz, 4H), 3.47 (dd, J = 3.6, 2.0 Hz, 1H), 2.99 (dd, J = 12.8, 3.6 Hz, 1H), 2.82 - 2.72 (m, 1H), 2.55 - 2.45 (m, 4H), 2.42 (s, 3H), 2.40 - 2.30 (m, 2H), 1.96 - 1.77 (m, 5H), 1.76 - 1.67 (m, 4H), 1.66 - 1.37 (m, 7H).

***(S)-2-((S)-2-((S)-3-(3-(allyloxy) phenyl)-2-(azetidin-1-yl) propanamido)-4-morpholinobutanamido)-N-cyclopentylpent-4-enamide* (62b).—**To a solution of compound **61b** (0.400 g, 1.34 mmol), diisopropylethylamine (1.17 mL, 6.72 mmol) and compound **60a** (0.457 g, 1.07 mmol) in dimethyl formamide (10 mL) was added T_3P (1.20 mL, 2.01 mmol, 50% purity in ethyl acetate) drop-wise at 0°C. The mixture was stirred for 1 hour at 0°C. The mixture was poured into water (100 mL), extracted with ethyl acetate (50 mL*4). The combined organic phase was washed with brine (100 mL*2), dried over anhydrous sodium sulfate, filtered and the filtrate was concentrated under reduced pressure. The residue was purified by reversed phase Flash (0.1% TFA condition) to afford compound **62b** as di-trifluoroacetic acid salt (0.250 g, 0.296 mmol, 22.1% yield) as a white solid. LCMS: RT = 0.0.910 min, *m/z* 596.3 $[M+H]^+$. ^1H NMR (methanol-d_4, 400 MHz): δ= 8.15(d, *J* = 6.8 Hz, 1H), 7.28 - 7.20 (m, 1H), 6.92 - 6.80 (m, 3H), 6.12 - 5.99 (m, 1H), 5.89 - 5.71 (m, 1H), 5.48 - 5.37 (m, 1H), 5.32 - 5.24 (m, 1H), 5.20 - 5.07 (m, 2H), 4.60 - 4.54 (m, 2H), 4.51 (t, *J* = 6.4 Hz, 1H), 4.4 (t, *J* = 6.4 Hz, 1H), 4.30 - 4.24 (m, 1H), 4.17 - 3.59 (m, 10H), 3.58 - 3.38 (m, 2H), 3.30 - 3.01 (m, 7H), 2.58 - 2.30 (m, 4H), 2.26 - 2.10 (m, 2H), 1.95 - 1.84 (m, 2H), 1.78 - 1.67 (m, 2H), 1.65 - 1.54 (m, 2H), 1.53 - 1.39 (m, 2H).

(7S, 10S, 13S)-13-(azetidin-1-yl)-N-cyclopentyl-10-(2-morpholinoethyl)-9, 12-dioxo-2-oxa-8, 11-diaza-1(1, 3)-benzenacyclotetradecaphane-7-carboxamide (**22**). Compound **62b** as di-trifluoroacetic acid salt (0.200 g, 0.243 mmol) was dissolved in a solution of HCl in dioxane (4 M, 20.0 mmol 5 mL) and the mixture was concentrated under reduced pressure. The residue was dissolved in dichloromethane (15 mL) and dichloroethane (30 mL), Grubbs catalyst 2nd generation (0.042 g, 0.049 mmol) was added. The mixture was degassed and purged with nitrogen three times and stirred for 16 hours at 65°C under nitrogen atmosphere. The reaction mixture was concentrated under reduced pressure. The residue was purified by reverse phase Flash (0.1% TFA condition). The fractions were acidified pH to 8 with a saturated aqueous solution of sodium bicarbonate, extracted with ethyl acetate (50 mL*3). The combined organic phase was washed with brine (50 mL), dried over anhydrous sodium sulfate, filtered and the filtrate was concentrated under reduced pressure. The residue was dissolved in methanol (5 mL), and then Pd/C (0.020 g, 10% purity on charcoal) was added. The mixture was degassed and purged with hydrogen three times and the reaction mixture was stirred for 50 min at 20°C under hydrogen atmosphere (15 psi). The mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was purified by prep-HPLC (column: Waters Xbridge C_{18} 150*50 mm* 10 um; mobile phase: [water (10 mM NH_4HCO_3)-ACN];B%: 22%-52%,10 min), followed by chiral SFC (column: DAICEL CHIRALPAK IG (250 mm*30 mm,10 um);mobile phase: [0.1% NH_3H_2O MeOH]; B%: 60%-60%, 4.8 min; 67 min) to afford compound **22** as a white solid. LCMS: RT = 0.555 min, m/z 570.2 $[M+H]^+$. ^1H NMR (methanol-d_4, 400 MHz): δ = 7.21 - 7.12 (m, 1H), 6.81 - 6.70 (m, 2H), 6.59 - 6.54 (m, 1H), 4.61 - 4.54 (m, 1H), 4.31 - 4.22 (m, 1H), 4.20 - 4.11 (m, 1H), 4.09 - 3.97 (m, 2H), 3.72 - 3.64 (m, 4H), 3.49 - 3.39 (m, 2H), 3.36 - 3.32 (m, 1H), 3.30 - 3.28 (m, 1H), 3.13 - 3.03 (m, 1H), 2.78 - 2.65 (m, 2H), 2.51 - 2.30 (m, 6H), 2.16 - 2.06 (m, 2H), 1.95 - 1.80 (m, 3H), 1.78 - 1.65 (m, 6H), 1.63 - 1.54 (m, 4H), 1.46 - 1.38 (m, 3H).

3-(3-(allyloxy)benzyl)-N-((S)-1-(((S)-1-(cyclopentylamino)-1-oxopent-4-en-2-yl)amino)-4-morpholino-1-oxobutan-2-yl)oxetane-3-carboxamide (**62c**). To a solution of compound **60a**

as dihydrochloric acid salt (0.430 g, 1.01 mmol), compound **61d** (0.250 g, 1.01 mmol) and diisopropylethylamine (5.74 mmol, 1.00 mL) in dimethyl formamide (4 mL) was added T_3P (1.68 mmol, 1.00 mL, 50% purity in ethyl acetate) drop-wise at 0°C. The mixture was stirred for 1 hour at 0°C. The mixture was diluted with ethyl acetate (50 mL), washed with brine (50 mL*3), dried over anhydrous sodium sulfate, filtered and the filtrate was concentrated under reduced pressure. The residue was purified by prep-HPLC (column: Waters Xbridge C_{18} 150*50 mm* 10 um; mobile phase: [water (10 mM NH_4HCO_3)-ACN]; B%: 30%-60%,11.5 min) to afford compound **62c** (0.360 g, 0.547 mmol, 54.4% yield) as a white solid. LCMS: RT = 0.732 min, m/z 583.3 $[M+H]^+$. ^1H NMR ($CDCl_3$, 400 MHz): δ = 8.13 - 8.01 (m, 1H), 7.22 - 7.13 (m, 1H), 7.04 - 6.95 (m, 1H), 6.82 - 6.76 (m, 1H), 6.72 - 6.66 (m, 2H), 6.31 - 6.20 (m, 1H), 6.12 - 5.96 (m, 1H), 5.82 - 5.65 (m, 1H), 5.47 - 5.36 (m, 1H), 5.34 - 5.26 (m, 1H), 5.22 - 5.05 (m, 2H), 4.88 - 4.79 (m, 2H), 4.64 - 4.60 (m, 1H), 4.59 - 4.57 (m, 1H), 4.53 - 4.46 (m, 2H), 4.41 - 4.27 (m, 2H), 4.21 - 4.11 (m, 1H), 3.68 - 3.47 (m, 4H), 3.43 - 3.24 (m, 2H), 2.58 - 2.47 (m, 2H), 2.42 - 2.30 (m, 3H), 2.28 - 2.22 (m, 1H), 2.03 - 1.89 (m, 2H), 1.72 - 1.68 (m, 1H), 1.59 - 1.58 (m, 1H), 1.50 - 1.35 (m, 2H).

(7’S, 10’S)-N-cyclopentyl-10’-(2-morpholinoethyl)-9’, 12’-dioxospiro [oxetane-3, 13’-2-oxa-8, 11-diaza-1(1, 3)-benzenacyclotetradecaphane]-7’-carboxamide (**19**). To a solution of compound **62c** (0.200 g, 0.343 mmol) in dichloroethane (45 mL) was added Grubbs catalyst 2^{nd} generation (0.058 g, 0.069 mmol). The mixture was stirred for 52 hours at 65°C, and then concentrated under reduced pressure. The residue was purified by reversed phased Flash (0.01% TFA in water, acetonitrile). The purified residue was dissolved in methanol (5 mL) was added Pd/C (0.010 g, 5% purity on charcoal) under nitrogen. The mixture was stirred for 1 hour at 20°C under hydrogen (15 psi). The mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was purified by prep-HPLC (column: Phenomenex Gemini NX-C_{18} (75*30 mm*3 um); mobile phase: [water (10 mM NH_4HCO_3)-ACN]; B%: 20%-40%, 8 min) to afford compound **19** (0.028 g, 0.051 mmol, 90.1% yield) as a white solid. LCMS: RT = 1.664 min, m/z 557.4 $[M+H]^+$. ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.67 - 7.59 (m, 1H), 7.25 - 7.19 (m, 1H), 6.85 - 6.79 (m, 1H), 6.75 - 6.69 (m, 1H), 6.62 - 6.53 (m, 1H), 6.53 - 6.49 (m, 1H), 5.91 - 5.74 (m, 1H), 5.01 - 4.93 (m, 1H), 4.80 - 4.66 (m, 2H), 4.62 - 4.51 (m, 2H), 4.39 - 4.31 (m, 1H), 4.21 - 4.03 (m, 2H), 4.01 - 3.93 (m, 1H), 3.84 - 3.69 (m, 4H), 3.36 - 3.22 (m, 2H), 2.74 - 2.57 (m, 2H), 2.55 - 2.44 (m, 3H), 2.43 - 2.35 (m, 1H), 2.22 - 2.09 (m, 1H), 2.00 - 1.83 (m, 3H), 1.79 - 1.65 (m, 4H), 1.63 - 1.54 (m, 5H), 1.53 - 1.43 (m, 1H), 1.41 - 1.27 (m, 2H).

Tert-butyl ((S)-1-(((S)-1-(cyclopentylamino)-1-oxopent-4-en-2-yl)amino)-4-(4,4-difluoropiperidin-1-yl)-1-oxobutan-2-yl)carbamate (**59b**). To a solution of compound **57** (500 mg, 1.55 mmol, 1 eq) and compound **58** (509 mg, 2.33 mmol, 1.5 eq, HCl) in N,N-dimethylformamide(10 mL) was added diisopropylethylamine(601 mg, 4.65 mmol, 3 eq) at 0°C and T_3P (1.48 g, 2.33 mmol, 50% purity in ethyl acetate, 1.5 eq) was added the mixture at 0°C. Then the mixture was stirred at 0°C for 1 hour. TLC (Petroleum ether/Ethyl acetate = 2/1, Rf = 0.7) and LCMS showed the mixture was completed. The mixture was added water (20 mL) and ethyl acetate (50 mL), washed with aqueous sodium bicarbonate solution (50 mL), brine (50 mL*3), dried over anhydrous sodium sulfate, filtered and concentrated in vacuum. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl

acetate = 5/1 to 1/1) to give compound **59b** (550 mg, 1.13 mmol, 72.87% yield, 100% purity) as yellow oil. LCMS: RT =0.707 min, purity: 100%, m/z 487.2$[M+H]^+$. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.05 (d, J = 6.4 Hz, 1H), 6.92 (br.s, 1H), 6.47 (d, J = 2.0 Hz, 1H), 5.79 - 5.68 (m, 1H), 5.17 - 5.12 (m, 2H), 4.42 - 4.37 (m, 1H), 4.20 - 4.13 (m, 2H), 2.65 - 2.45 (m, 8H), 2.03 - 1.82 (m, 8H), 1.75 - 1.64 (m, 2H), 1.63 - 1.53 (m, 2H), 1.45(s, 9H), 1.44 - 1.34 (m, 2H).

***(S)-2-((S)-2-amino-4-(4,4-difluoropiperidin-1-yl)butanamido)-N-cyclopentylpent-4-enamide* (60b).—**To a solution of compound **59b** (550 mg, 1.13 mmol, 1 eq) in dioxane (5 mL) was added hydrochloric acid/dioxane (4 M, 10 mL, 35 eq) at 25°C and the mixture was stirried at 25°C for 1 hour. LCMS showed the mixture was completed. The mixture was concentrated in vacuum to give compound **60b** (600 mg, crude, 2HCl) as yellow oil and the residue was uesd next step directly. LCMS: RT =0.188 min, purity: 83.849%, *m/z* 387.1 $[M+H]^+$

(S)-2-((S)-2-((S)-3-(3-(allyloxy)phenyl)-2-(2-oxopyrrolidin-1-yl)propanamido)-4-(4,4-difluoropiperidin-1-yl)butanamido)-N-cyclopentylpent-4-enamide (**62d**). To a solution of compound **60b** (588 mg, 1.28 mmol, 1 eq, 2HCl) and compound **61c** (370 mg, 1.28 mmol, 1 eq) in N,N-dimethylformamide(10 mL) was added diisopropylethylamine(826 mg, 6.39 mmol, 5 eq) at 0°C and T_3P (1.22 g, 1.92 mmol, 1.14 mL, 50% purity in ethyl acetate, 1.5 eq) was added the mixture at 0°C. Then the mixture was stirred at 0°C for 0.5 hour. LCMS showed the mixture was completed. The mixture was added water (20 mL) and ethyl acetate (50 mL), washed with aqueous sodium bicarbonate solution (50 mL), brine (50 mL*3), dried over anhydrous sodium sulfate, filtered and concentrated in vacuum. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 2/1 to 0/1) to give compound **62d** (450 mg, 649.71 umol, 50.81% yield, 94.972% purity) as a white solid. LCMS: RT =0.700 min, purity: 94.972%, m/z 658.3$[M+H]^+$. ^{1}H NMR: ($CDCl_3$, 400 MHz) δ 8.49 (d, J = 6.4 Hz, 1H), 7.26 - 7.17 (m, 2H), 6.81 - 6.72 (m, 3H), 6.64 (d, J = 7.6 Hz, 1H), 6.09 - 5.99 (m, 1H), 5.78 - 5.68 (m, 1H), 5.45 - 5.37 (m, 1H), 5.31 - 5.27 (m, 1H), 5.15 - 5.04 (m, 2H), 4.62 (dd, J = 6.4, 9.6 Hz, 1H), 4.52 - 4.48 (m, 2H), 4.47 - 4.31 (m, 2H), 4.19 - 4.13 (m, 1H), 3.45 - 3.37 (m, 1H), 3.34 - 3.22 (m, 1H), 3.20 - 3.05 (m, 2H), 2.71 - 2.57 (m, 6H), 2.49 - 2.44 (m, 1H), 2.39 - 2.26 (m, 3H), 2.13 - 2.05 (m, 2H), 2.04 - 1.97 (m, 3H), 1.96 - 1.81 (m, 5H), 1.71 - 1.64 (m, 2H), 1.61 - 1.53 (m, 2H), 1.49 - 1.38 (m, 2H).

(7S,10S,13S)-N-cyclopentyl-10-(2-(4,4-difluoropiperidin-1-yl)ethyl)-9,12-dioxo-13-(2-oxopyrrolidin-1-yl)-2-oxa-8,11-diaza-1(1,3)-benzenacyclotetradecaphane-7-carboxamide (**17**). To a solution of compound **62d** (200 mg, 304.05 umol, 1 eq) in dichloroethane (40 mL) and dichloromethane (20 mL) was added Grubbs catalyst 2nd Generation (155 mg, 182.43 umol, 0.6 eq) at 25°C under nitrogen and the mixture was stirred at 60°C for 12 hours under nitrogen. LCMS showed the mixture was completed. The mixture was filtered and concentrated in vacuum. The residue was purified by prep-HPLC (TFA condition; column: Luna C18 150 mm * 25 mm * 5 um; mobile phase: [water (0.1%TFA)-ACN]; B%: 15% - 45%, 10min) to give compound **RCM-62d** (120 mg, 187.20 umol, 61.57% yield, 98.24% purity) as a black brown solid. LCMS: RT =0.649 min, purity: 98.240%, m/z 630.3 $[M+H]^+$. ^{1}H NMR (CD_3OD, 400 MHz) δ 7.19 (t, J = 8.0 Hz, 1H), 6.87 (d, J = 7.6 Hz, 1H), 6.76 (dd, J = 2.0, 8.0 Hz, 1H), 6.65 (s, 1H), 5.72 - 5.60 (m, 2H), 4.73 - 4.58 (m,

3H), 4.40 (t, J = 6.8 Hz, 1H), 4.13 (dd, J = 2.8, 11.2 Hz, 1H), 4.06 - 4.00 (m, 1H), 3.77 - 3.67 (m, 2H), 3.60 - 3.36 (m, 2H), 3.19 (t, J = 7.2 Hz, 2H), 3.07 (t, J = 12.8 Hz, 1H), 2.97 (dd, J = 4.0, 12.4 Hz, 1H), 2.48 - 2.24 (m, 9H), 2.14 - 1.83 (m, 7H), 1.75 - 1.65 (m, 2H), 1.61 - 1.52 (m, 2H), 1.46 - 1.37 (m, 2H). To a solution of compound **RCM-62d** (100 mg, 158.80 umol, 1 eq) in methanol(10 mL) was added Pd/C (20 mg, 10% purity) under nitrogen. The suspension was degassed under vacuum and purged with hydrogen several times. The mixture was stirred under hydrogen (15 psi) at 25°C for 1 hour. LCMS showed the mixture was completed. The mixture was filtered and concentrated in vacuum. The residue was purified by prep-HPLC (basic condition; column: Xtimate C18 150 mm * 25 mm * 5um; mobile phase: [water (0.05% ammonia hydroxide v/v)-ACN]; B%: 30% - 60%, 1min) to give compound **17** (44.7 mg, 69.84 umol, 43.98% yield, 98.7% purity) as a white solid. The mixture was worked up with another batch (20 mg scale) and got 53.7 mg of desired product together. LCMS: RT = 2.215 min, purity: 98.700%, m/z 632.4 $[M+H]^+$. ^{1}H NMR (CD_3OD, 400 MHz) δ 7.19 (t, J = 8.0 Hz, 1H), 6.83 (d, J = 8.0 Hz, 1H), 6.76 (dd, J = 2.0, 8.0 Hz, 1H), 6.70 - 6.65 (m, 1H), 4.65 (dd, J = 3.2, 12.4 Hz, 1H), 4.53 (t, J = 7.2 Hz, 1H), 4.26 (dd, J = 4.8, 8.8 Hz, 1H), 4.21 - 4.13 (m, 1H), 4.10 - 4.03 (m, 2H), 3.66 (t, J = 6.8 Hz, 2H), 3.26 (t, J = 12.4 Hz, 1H), 2.73 (dd, J = 3.6, 12.4 Hz, 1H), 2.57 - 2.47 (m, 4H), 2.45 - 2.33 (m, 4H), 2.13 - 1.86 (m, 8H), 1.84 - 1.65 (m, 7H), 1.64 - 1.51 (m, 4H), 1.49 - 1.36 (m, 3H).

Parallel artificial membrane permeability assay (PAMPA).—200 μL of PRISMA HT buffer in pH 7.4 (pION inc.) containing 10 μM test compounds were added to the donor wells of STIRWELL PAMPA sandwich filter plate. The PAMPA membrane was coated by pipetting 4 uL of GIT-lipid solution in dodecane (pION inc.). The acceptor wells were filled with 200 μL of acceptor sink buffer in pH 7.4 (pION inc.). The acceptor plate was put on the donor plate. After 3 hours incubation at room temperature, the amount of compound in both the acceptor and the donor wells was quantified by LC/MS/MS. Propranolol was used as positive control compound. Methyclothiazide was used as 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. 3.5*10^4 hMDR1/LLC-PK1 cells were seeded and grown on 96-well transwell (polycarbonate membrane, 0.4 μM pore size, 0.143 cm^2 surface area) for 4 days. The plated cells were preincubated with M199 media at 37 °C for 30 min. Bidirectional transport of each compound was evaluated and initiated by the addition of M199 media containing 10 μM of test compound to apical compartments or to basolateral compartments. Lucifer yellow was co-incubated with test compound as a membrane integrity marker. After 60 min incubation, aliquots from both compartments were diluted with acetonitrile and centrifuged. The amount of compound in the supernatant were quantified by LC/MS/MS. The apparent permeability (P_{app}) of text compound in both direction (A to B, and B to A) was determined. The efflux ratio (ER) of test compound was calculated using the following equation: $ER = P_{app,\ B\ to\ A}/P_{app,\ A\ to\ B}$. Where $P_{app,\ A\ to\ B}$ represent the apparent permeability in the apical-to-basal direction, $P_{app,\ B\ to\ A}$ represent the apparent permeability in the basal-to-apical direction.

Kinetic Solubility.—The kinetic solubility was determined as previously reported.[44]

Live Microsomal Stability.—The liver microsomal stability was determined in a NADPH dependent assay as previously reported.[44]

Plasma Stability.—The plasma stability of compounds was determined as previously reported.[44] The human plasma was ordered from Sigma-Aldrich (H3667). Freshly collected mouse plasma was used for the mouse plasma stability assay. Procaine was included as the positive control in each human plasma stability assay experiment. Enalapril was included as the positive control in each mouse plasma stability assay experiment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENT

We thank Drs. Leigh Baxt and Stacia Kargman at TDI for their fruitful suggestions. The Department of Microbiology and Immunology is supported by the William Randolph Hearst Foundation.

Funding Sources

The study was supported by NIH grants R01AI143714 (G.L.), R21AI123794 (G.L. and L.A.K.); The Brockman Foundation (L.A.K.); Weill Cornell Medicine Matching Fund (G.L.); Department of Medicine, Weill Cornell Medicine Seed fund (L.A.K.); Milstein Program in Chemical Biology and Translational Medicine. We gratefully acknowledge in-kind support of the Tri-Institutional Therapeutics Discovery Institute (TDI), a 501(c)(3) organization. TDI receives financial support from TDI's owners (Weill Cornell Medicine, Memorial Sloan Kettering Cancer Center and The Rockefeller University and), Takeda Pharmaceutical Company, and generous contributions from Mr. Lewis Sanders, Mr. Howard Milstein and other philanthropic sources.

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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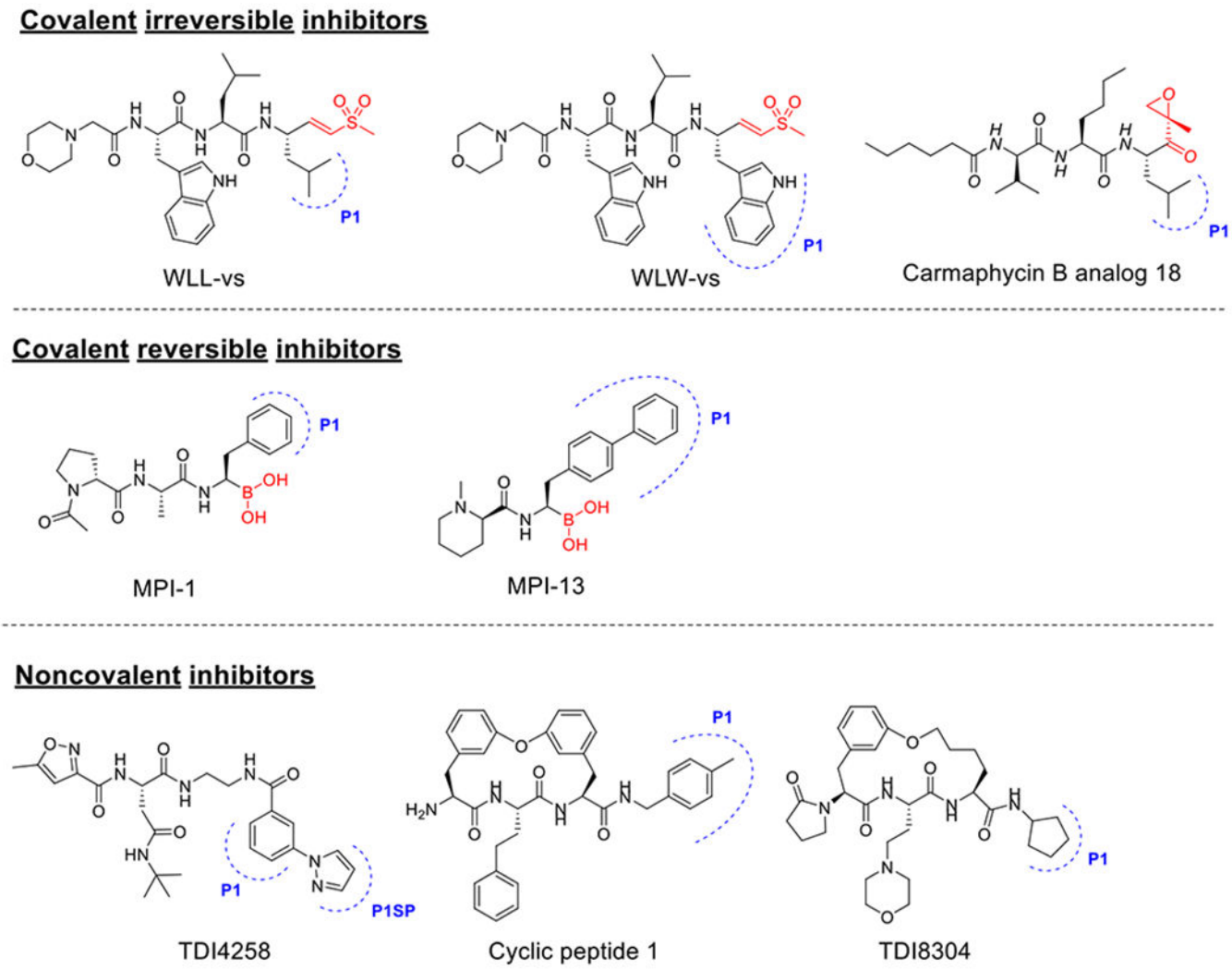


Figure 1.

Representative Pf20S inhibitors reported recently with various inhibition mechanisms and species selectivity

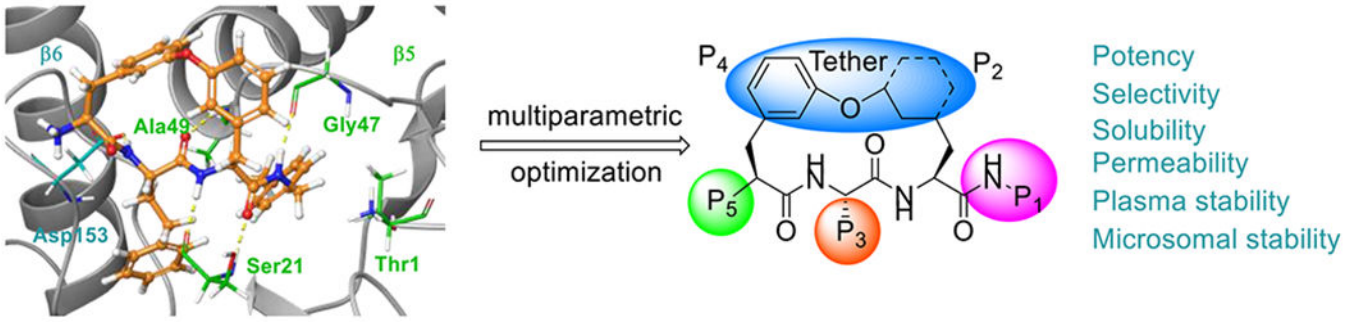


Figure 2.

Docking model of **cyclic peptide 1** into Pf20S (PDB: 5FMG) and design strategy of multiparametric optimization to improve drug-like properties.

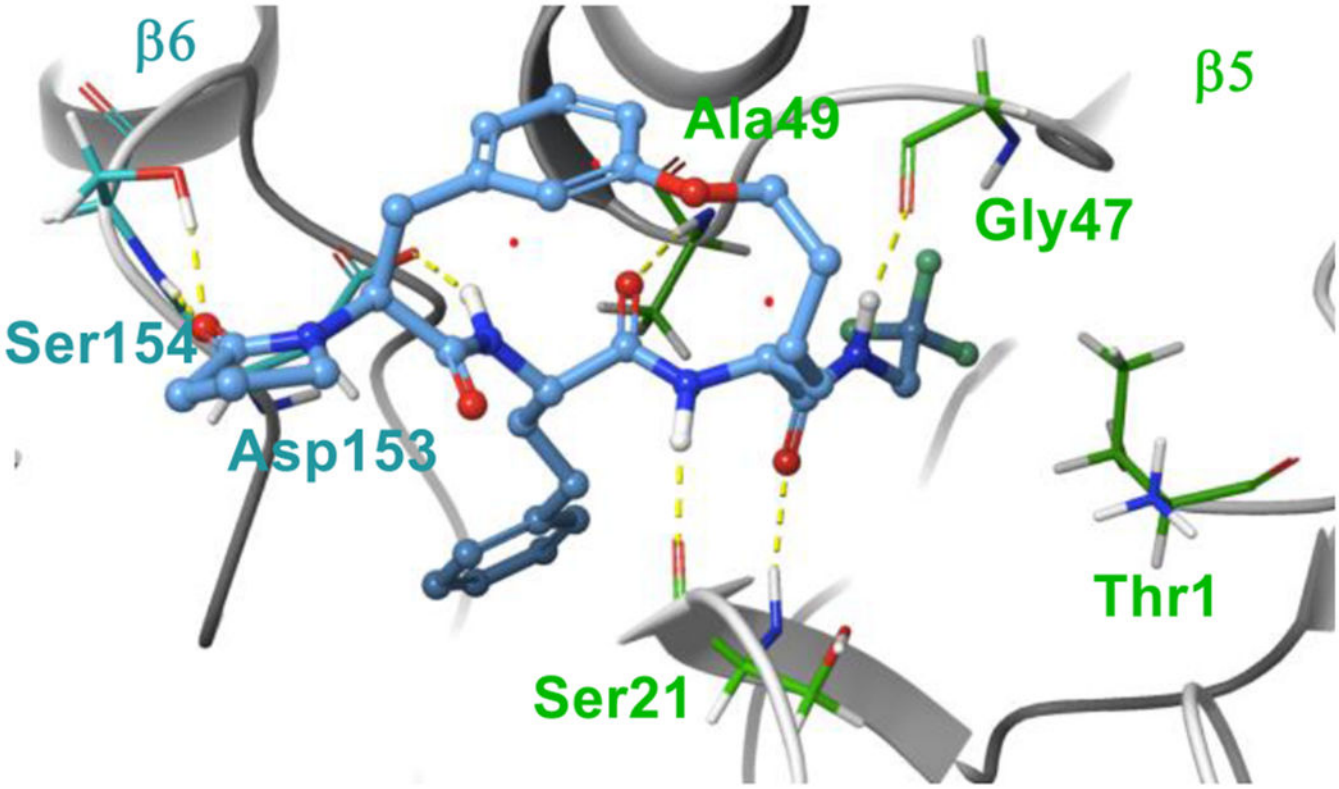


Figure 3.
Docking pose of **5** into Pf20S (PDB:5FMG).

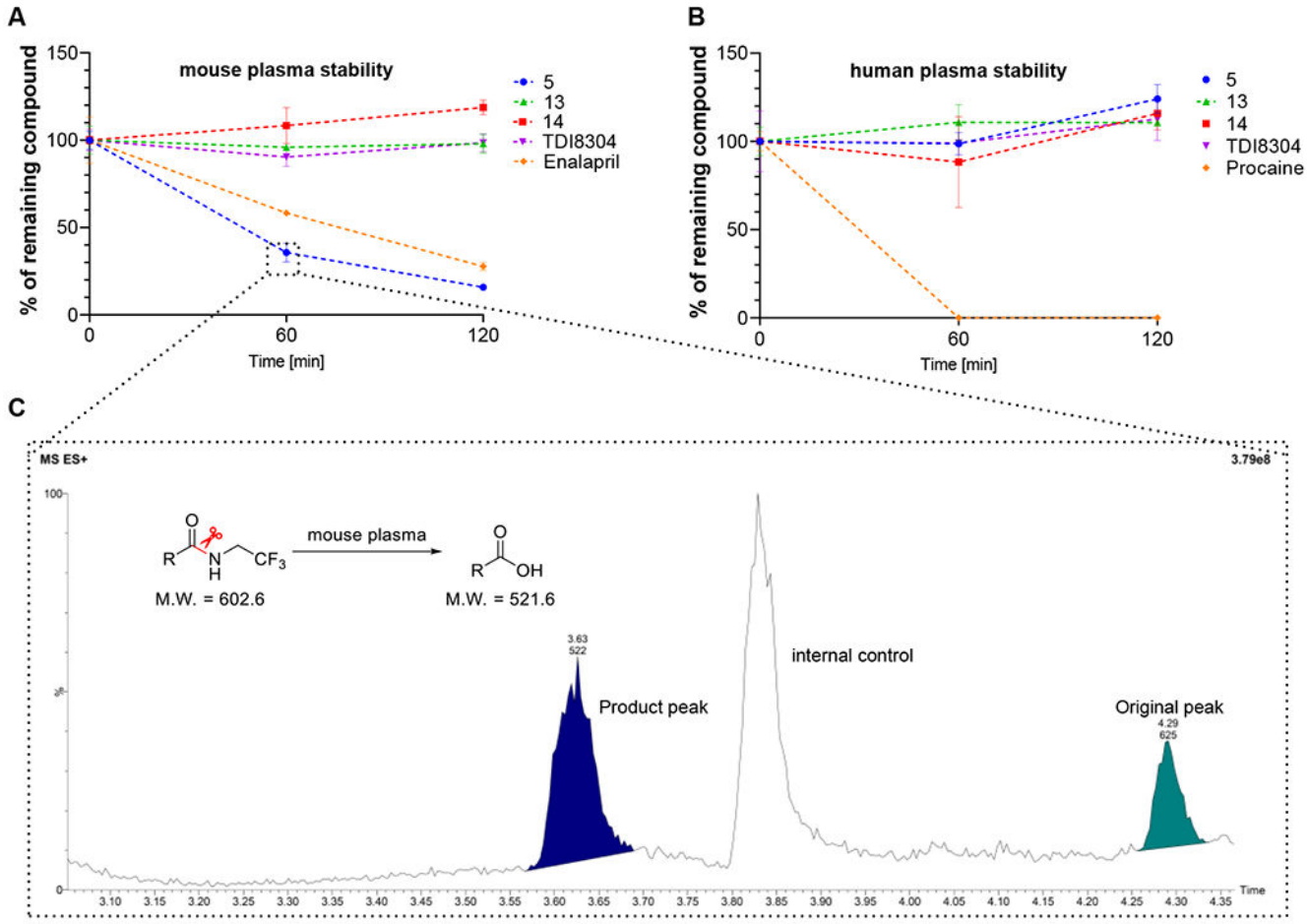


Figure 4.

A) The stability of selected compounds in mouse plasma. B) The stability of selected compounds in human plasma. C) LC/MS showed the decrease of compound **5** (retention time = 4.29 min) and the appearance of product peak (retention time = 5.41 min) after 60 min of incubation with mouse plasma at 37 °C.

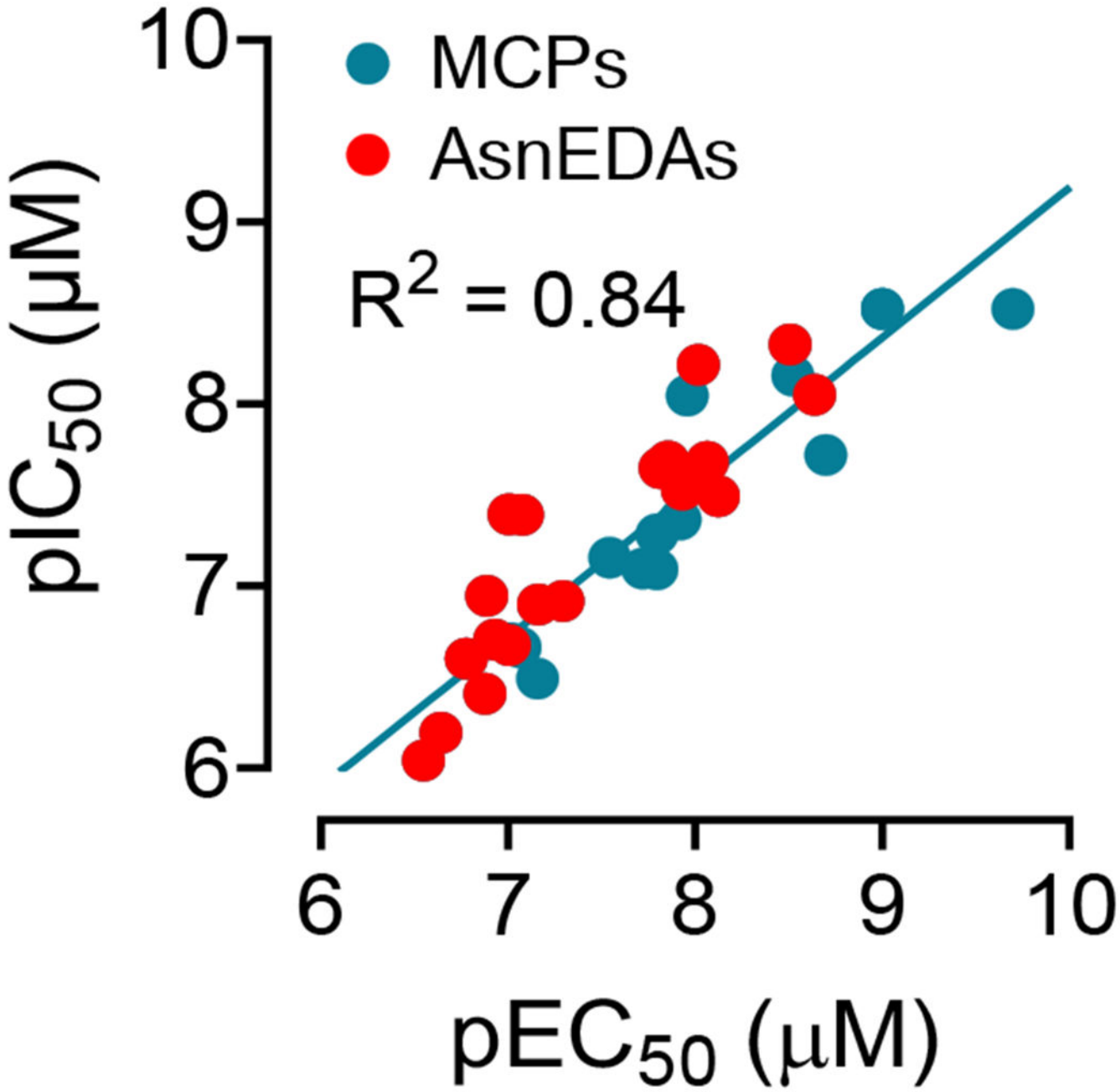


Figure 5.
Correlations of IC_{50} and EC_{50} of the compounds reported in this paper and together with compounds reported in Zhan et al JMC 2019.

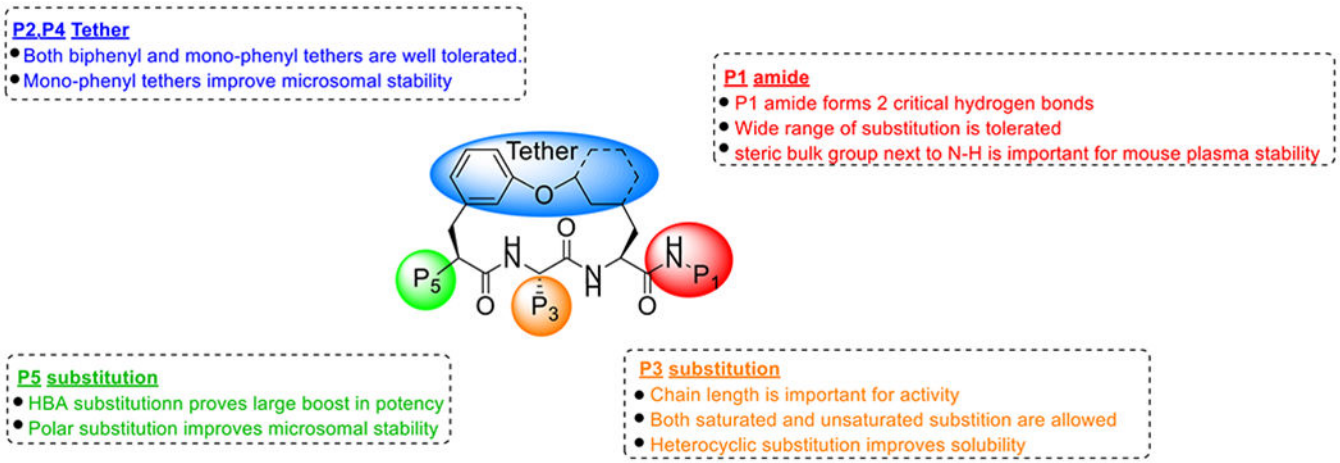
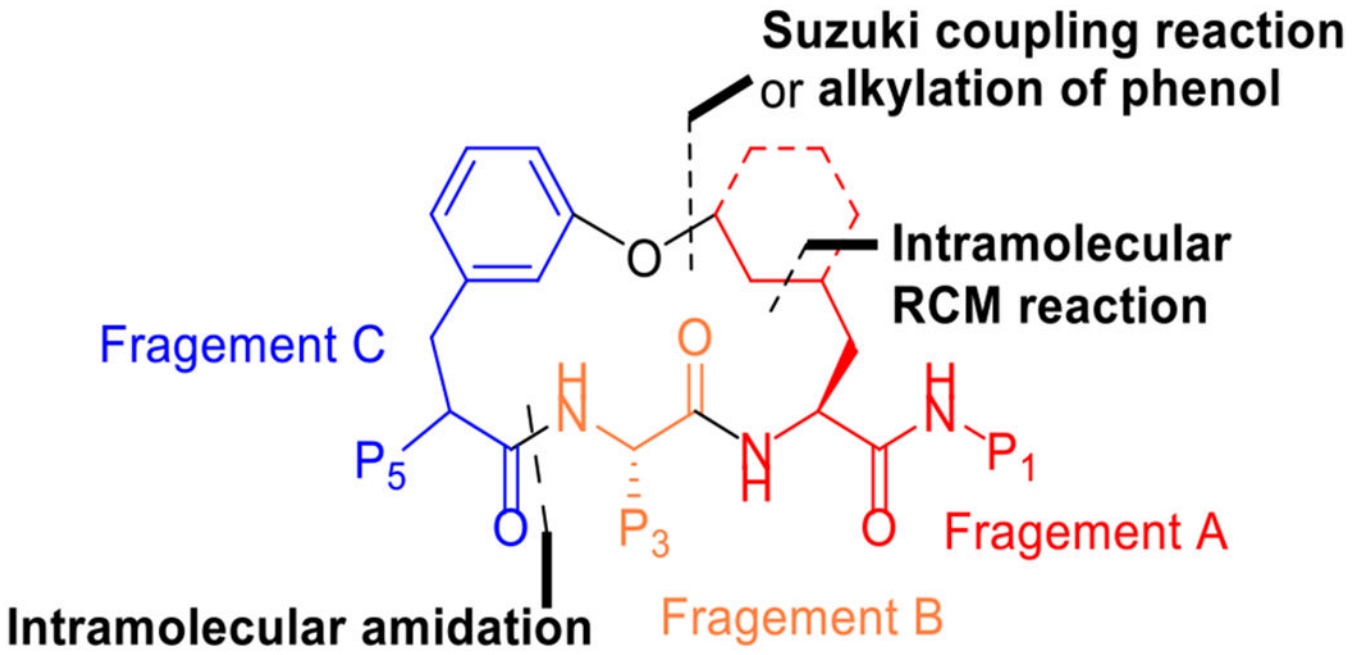
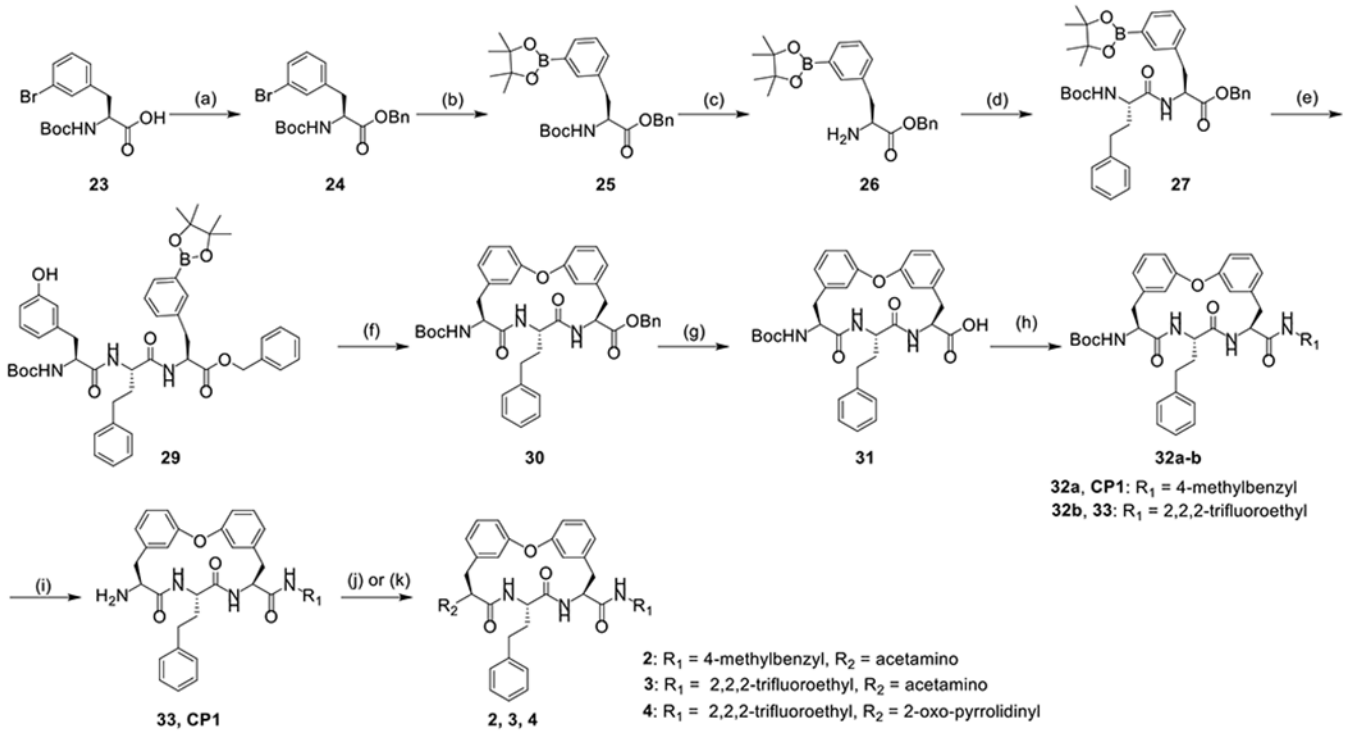


Figure 7.
Overall summary of the macrocyclic peptide series.



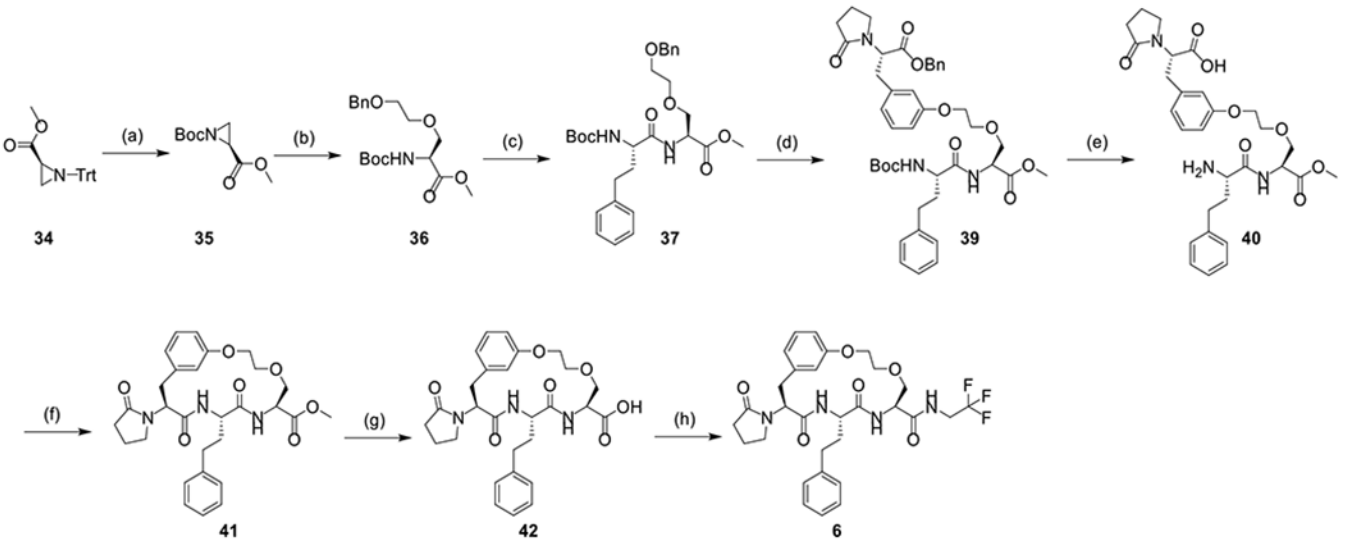
Scheme 1.
Cyclization strategies for the synthesis of macrocyclic proteasome inhibitors



Scheme 2.

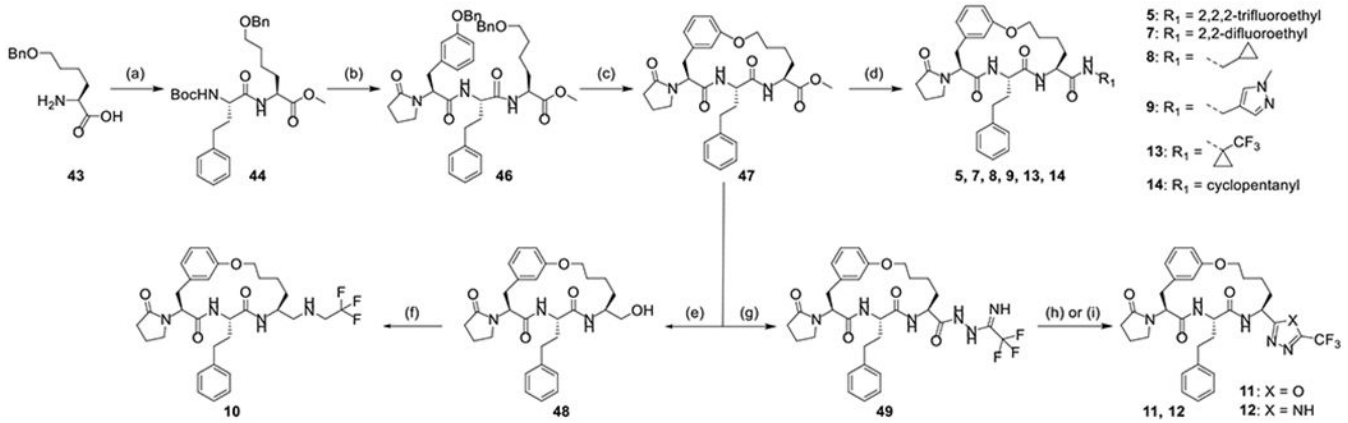
Synthesis of compounds **cyclic peptide 1**, **2**, **3**, and **4** using Suzuki coupling reaction as macrocyclization strategy

Reagents and conditions: (a) BnBr, acetaminoK_2CO_3, acetone, r.t.; (b) Bis(pinacolato)diboron, KOAc, $Pd(dppf)Cl_2$, dioxane, 80 °C; (c) 4 N HCl/dioxane; (d) Boc-L-homophenylalanine, HOBT, EDCI, DCM; (e) (1) 4 N HCl/dioxane, (2) compound **28**, HOBT, EDCI, DCM; (f) (1) $NaIO_4$, NH_4OAc, acetone, H_2O, (2) $Cu(OAc)_2$, TEA, 4Å molecular sieves, MeOH, DCM, r.t., 12 h; (g) Pd/C, H_2; (h) R_1NH_2, HOBT, EDCI, DMF; (i) TFA, DCM; (j) Acetyl chloride, TEA, DMF; (k) (1) *tert*-butyl 4-bromobutanoate, K_2CO_3, DMF, 50 °C, (2) TFA, DCM, (3) EDCI, HOBt.



Scheme 3.

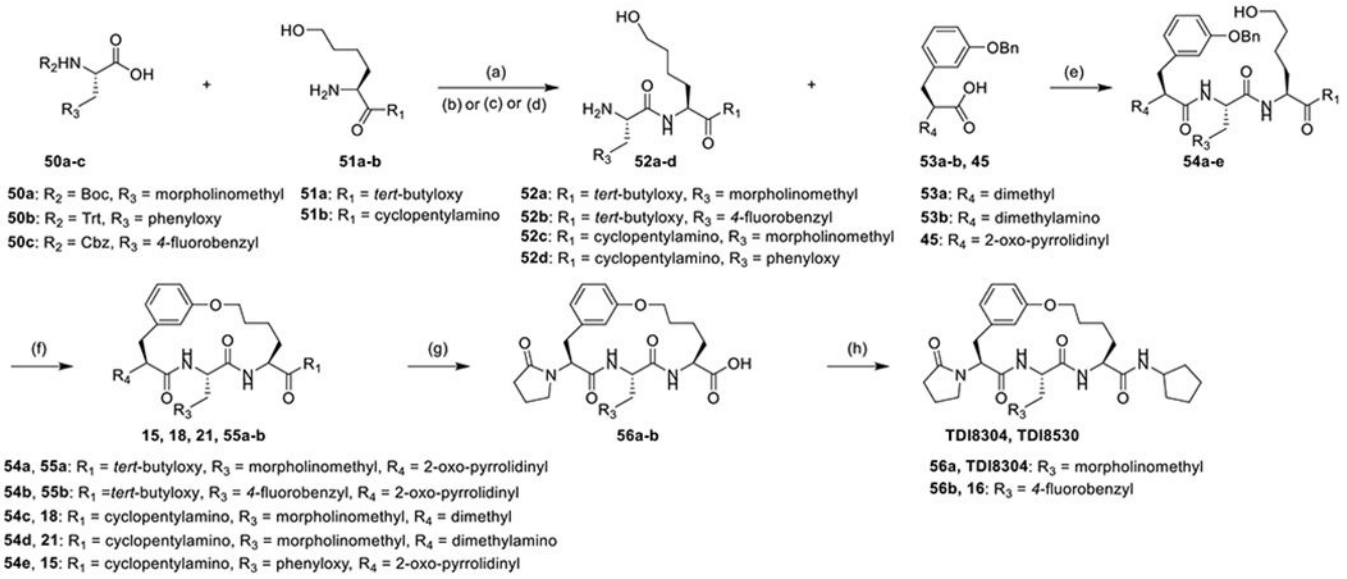
Synthesis of compound **6** using intramolecular amidation as macrocyclization strategy Reagents and conditions: (a) (1) TFA, DCM, MeOH, (2) BoC_2O, TEA, MeCN; (b) $BF_3{\cdot}Et_2O$, DCM, 2-(benzyloxy)ethanol; (c) (1) HCl, MeOH, (2) HBTU, DIPEA, DMF, boc-L-homophenylalanine; (d) (1) H_2, Pd/C, MeOH, (2) DEAD, PPh_3, THF, compound **38**; (e) (1) Pd/C, H_2, MeOH, (2) HCl/dioxane; (f) EDCI, HOBt, DMF; (g) NaOH, THF/H2O; (h) 2,2,2-Trifluoroethylamine, EDCI, HOBt, pyridine.



Scheme 4.

Synthesis of compounds **5**, **7**, **8**, **9**, **10**, **11**, **12**, **13**, and **14** using intramolecular alkylation as macrocyclization strategy.

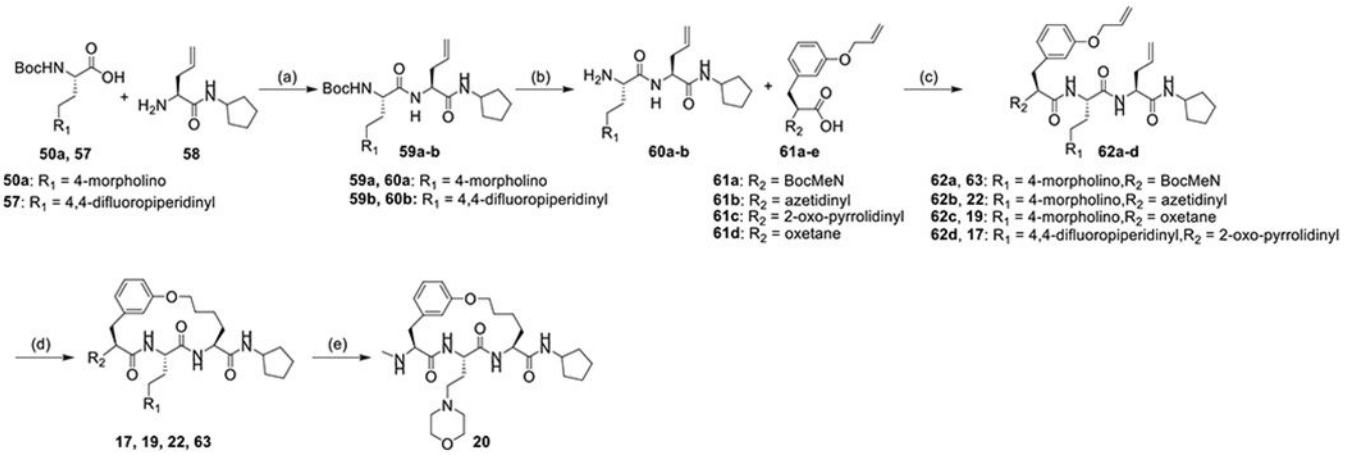
Reagent and conditions: (a) (1) HCl, MeOH, (2) boc-L-homophenylalanine, HATU, DIEA, DMF; (b) (1) HCl (4 M), dioxane, (2) compound **45**, HATU, DIEA, DMF; (c) (1) H_2, Pd/C, $Pd(OH)_2$, MeOH, (2) TosCl, pyridine, (3) Cs_2CO_3, DMF; (d) (1) NaOH, THF, H_2O, (2) HATU, DIEA, R_1NH_2; (e) $LiBH_4$, THF; (f) (1) Dess-martin, THF, (2) 4Å MS, $NaBH(OAc)_3$, MeOH, 2,2,2-trifluoroethylamine; (g) (1) $N_2H_4.H_2O$, MeOH, (2) Methyl trifluoroacetimidate, DCM; (h) 1,2-dichlorobenzene; (i) (1) TFA, DCM, (2) Burgess reagent, THF.



Scheme 5.

Synthesis of compounds **15, 16, 18, 21**, and **TDI8304** using intramolecular alkylation as macrocyclization strategy

Reagents and conditions: (a) T_3P, DIEA, DMF 0-20 °C, 1 h; (b) HCl (4M, 4eq.), dioxane, MeOH; (c) HCl, dioxane, DCM, 20 °C, 3 h; (d) H_2, Pd/C, TFA, THF; (e) T_3P, DIEA, DMF; (f) (i) TosCl, pyridine, (ii) H_2, $Pd(OH)_2/C$, MeOH, (iii) Cs_2CO_3, DMF; (g) TFA, DCM; (h) cyclopentanamine, T_3P, DIEA, DMF 0 °C, 1 h.



Scheme 6.

Synthesis of compounds **17, 19, 20** and **22** using RCM reaction as macrocyclization strategy Reagent and conditions: (a) T_3P, DMF, DIEA; (b) HCl/dioxane, MeOH; (c) T_3P, DIEA, DMF; (d) (1) Grubbs 2nd, DCE, (2) H_2, Pd/C, MeOH; (e) HCl, dioxane.

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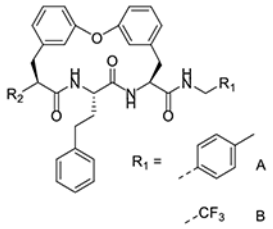
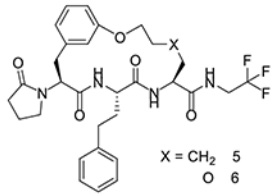
Table 1.

Optimization of N-terminal cap and linker





			EC50 (μM)	β5 IC50 (μM)			Permeability		Liver micro-somal stabil-ity	Kinetic Solubility	Cytotoxi-city HepG2	Lipo-philicity
ID	R1	R2	3D7	Pf20S	c20S	i20S	PAMPA	MDR	m/hLM	pH 6.8	Viability %	cLogP
cyclic peptide 1	A	H_2N	0.078	0.13	>100	> 100	unde-tected	>2.9/<1	90.6 179.4	<1.59	71	6.3
2	A	AcHN	0.002	0.017	>100	>100	22	>34/<1	171.3 N.D.	-	93	6.2
3	B	AcHN	0.003	0.014	4.0	> 100	151	>0.77/<4	269.7 11.2	<1.43	93	4.7
4	B		0.0002	0.003	26.3	>100	214	31/5	>768 644.3	3.8	77	5.3
5	-	-	0.002	0.019	2.4	>100	187	53/2	566.6 349.8	76	97	4.2
6	-	-	0.019	0.080	6.4	>100	120	89/1	397.4 275.7	>130	109	3.0

PAMPA unit: nm/sec; MDR unit: A→B, nm/sec; ratio (B→A/ A→B); Metabolic stability Cl_{int} unit: μL/min/mg; Kinetic solubility (μg/mL); Cytotoxicity: % viability at 30 μM. "-": not tested.

Table 2.

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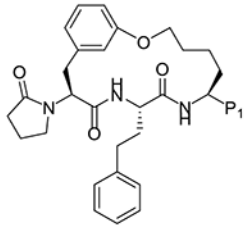
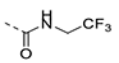
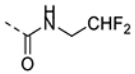
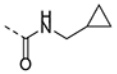
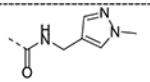
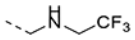
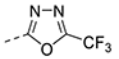
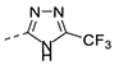
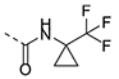
Optimization of P1 group

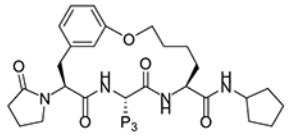


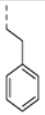
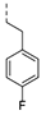
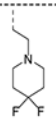
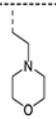
		EC_{50} (μM)	β5 IC_{50} (μM)			Permeability		Metabolic stability	Kinetic Solubility	Cytotoxicity HepG2	Lipophilicity
ID	P1	3D7	Pf20S	c20S	i20S	PAMPA	MDR	m/hLM	pH 6.8	Viability %	cLogP
5		0.002	0.019	2.4	>100	187	53/2	566.6 349.8	76	97	4.2
7		0.008	0.022	8.9	>100	72	>11/<5	288 187.7	35	101	4.0
8		0.003	0.007	3.0	>100	103	68/2	477.6 340.6	49	101	4.4
9		0.008	0.054	9.8	>100	11	>6/<1	261 187.7	>120	107	3.4
10		>2.77	23.8	>100	>100	-	-	-	-	-	4.8
11		>2.77	9.0	>100	>100	160	11/17	>768 672.1	6.2	109	3.3
12		>2.77	64.0	>100	>100	82	>21/<1	59.6 28.6	2	103	3.7
13		0.398	2.5	>100	>100	145	44/2	703.4 288	31	111	4.5
14		0.001	0.003	2.5	>100	151	57/3	657.8 596.5	100	96	4.9

PAMPA unit: nm/sec; MDR unit: A→B, nm/sec; ratio (B→A/ A→B); Metabolic stability Cl_{int} unit: μL/min/mg; Kinetic solubility (μg/mL); Cytotoxicity: % viability at 30 μM. "-": not tested.

Table 3.

Optimization of P3 group

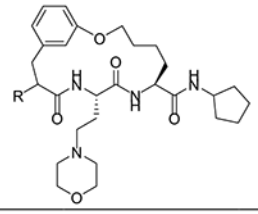


		EC50 (μM)		β5 IC50 (μM)		Permeability		Metabolic stability	Kinetic solubility	Cytotoxicity HepG2	Lipo-philicity
ID	P3	3D7	Pf20S	c-20S	i-20S	PAMPA	MDR	m/hLM	pH 6.8	Viability %	cLogP
14		0.001	0.003	2.5	>100	151	57/3	657.8 596.5	100	96	4.9
15		0.072	0.91	13.1	>100	208		529 405	20	104	4.5
16		0.011	0.009	2.7	51.0	212	45/3	>768 618	17	100	5.0
17		0.012	0.043	3.2	46.5	133	40/1	128 113	>130	102	2.8
TDI8304		0.016	0.081	>100	>100	15	>2/<1	6 19	>130	103	2.4

PAMPA pH7.4 unit: nm/sec; MDR unit: A→B, nm/sec; ratio (B→A/ A→B); Metabolic stability Cl_{int} unit: μL/min/mg; Kinetic solubility (μg/mL); Cytotoxicity: % viability at 30 μ;M

Table 4.

Optimization of P5 group



		EC_{50} (μM)	β5 IC_{50} (μM)			Permeability		Metabolic stability	Kinetic solubility	Cytotoxicity HepG2	Lipo-philicity	TPSA
ID	R	3D7	Pf20S	c-20S	i-20S	PAMPA	MDR	m/hLM	pH 6.8	Viability %	cLogP	
TDI8304	-	0.016	0.081	>100	>100	15	>2/<1	6 19	>130	103	2.4	129.3
18	gem-di-methyl	0.096	0.21	45.2	>100	269	35/11	690 167	90	97	3.7	109
19		0.034	0.56	31.1	>100	89	>28/<1	5 4	68	102	2.4	118
20		0.32	4.9	>100	>100	0	>2/<1	-14 -26	>140	100	2.2	121
21		0.7	3.9	>100	>100	20	1.2/5	-1 15	>130	104	2.8	99.8
22		0.32	2.0	13.1	17.6	0	>2/<1	-27 6	>140	100	2.9	112.2

PAMPA pH7.4 unit: nm/sec; MDR unit: A→B, nm/sec; ratio (B→A/ A→B); Metabolic stability Cl_{int} unit: μL/min/mg; Kinetic solubility (μg/mL); Cytotoxicity: % viability at 30 μM