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Research paper

Exploration of novel macrocyclic dipeptide *N*-benzyl amides as proteasome inhibitors

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ABSTRACT

As proteasome inhibitors, a series of novel macrocyclic dipeptide *N*-benzyl amides were designed, synthesized and evaluated. Most of them exhibited potent proteasome inhibition and excellent anti-proliferative activity against RPMI 8226, MM1S, and MV-4-11 cell lines. As the most distinguished one among this series, compound **23h** displayed potent and selective proteasome inhibitory potency (IC_{50}: β5c = 29 nM, β5i = 35 nM, β_{1c}, β_{2c}, β_{1i}, β_{2i} > 10 μM), excellent anti-proliferative activity against RPMI 8226, MM1S, and MV-4-11 cell lines with IC_{50} values of 18 nM, 15 nM, and 21 nM, respectively, as well as favorable metabolic stability in human liver microsomes (HLMs), highlighting that it is a promising lead compound for further development of proteasome inhibitors.

1. Introduction

The eukaryotic 26S proteasome is a large (1.6–2.4 MDa) ATP-dependent proteolytic complex, composed of a cylindrical 20S core particle (CP) capped by two 19S regulatory complexes [1]. The 20S proteasome is the proteolytically active key element of the ubiquitin proteasome system (UPS) that directs the majority of intracellular protein degradation in eukaryotic cells. It is composed by four heptameric rings stacked in a $\alpha_7\beta_7\beta_7\alpha_7$ arrangement and contains three proteolytic subunits, β1, β2, and β5 [2], respectively. There are two main proteasome subtypes: the constitutive proteasome (cCP) which contains three catalytic subunits denoted as β1c, β2c, and β5c and the immunoproteasome (iCP) which contains three catalytic subunits denoted as β1i, β2i, and β5i [3]. Inhibition of the 20S proteasome leads to the accumulation of substrate proteins involved in signal transduction, antigen presentation, cell-cycle progression and apoptosis, and is preferentially cytotoxic to cancer cells [4–8]. Indeed, proteasome inhibitors are recognized as clinically effective anti-cancer agents, primarily for hematological malignancies [9]. To date, three proteasome inhibitors Bortezomib, Carfilzomib, and Ixazomib (Fig. 1) are FDA approved for the treatment of multiple myeloma (MM) and mantle cell lymphoma [10–12]. All three are covalent inhibitors with an electrophilic warhead at the C-terminal end of a peptidyl backbone for covalent attachment to the catalytic Thr1 residues of the proteasome [13]. However, the electrophilic warhead is often related to excessive reactivity, lack of specificity and instability, which is believed to be the major cause of side effects during therapy [12,14]. These inhibitors have also been unsuccessful in the treatment of solid

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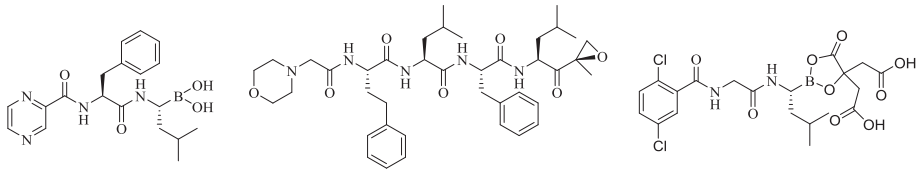


Fig. 1. FDA-approved proteasome inhibitor Bortezomib, Carfilzomib, and Ixazomib.

cancers [15,16], with the lack of efficacy most likely due to their covalent binding to the proteasome, limiting their widespread tissue distribution. In contrast, non-covalent proteasome inhibitors do not possess an electrophilic warhead with less reactivity. Non-covalent and reversible binding mode ensure them with rapid binding and dissociation kinetics. These features may allow non-covalent inhibitors to overcome drawbacks arising in therapeutics of covalent ones.

Representative peptidyl non-covalent proteasome inhibitors have been reported. TMC-95A (**4**, Fig. 2) is a macrocyclic natural product isolated from *Apiospora montagnei* Sacc TC 1093, which potently inhibits all the three proteasome catalytic activities with preference for β5c activity ($IC_{50} = 5.4$ nM) [17,18]. However, the structural complexity of TMC-95A maybe one of important obstacle to its further development. Additionally, a series of 5-methoxy-1-indanone di-peptide benzyl amides have been reported, including CVT-659 (**5**, Fig. 2) which selectively inhibits β5c site with submicromolar potency ($IC_{50} = 0.14$ μM), but this compound displayed poor cellular activity ($IC_{50} = 8$ μM) [19]. Researchers from Millennium Pharmaceuticals, Inc. identified a series of di- and tripeptides (e. g. **6** and **7**, Fig. 2) with potency and selectivity for both constitutive proteasome and immunoproteasome β5 sites [20]. Moreover, the X-ray structures of the inhibitors in complex with proteasome suggest that the occupancy of S1 and S3 pockets was crucial to inhibitor potency. Of note, the size of the hydrophobic benzyl group was well suited to S1 pocket, which may be a reason for the wide use of various substituted benzyl groups in peptidyl non-covalent proteasome inhibitors. Meanwhile, the 2-(neopentylamino)-2-oxoethyl group (as present in compound **6**) has been reported to provide a near-optimal fit for the S3 binding pocket [20–22]. Other reported peptidyl non-covalent proteasome inhibitors include 2-aminobenzylstatine derivatives [23,24] and linear TMC-95A analogues [25,26]. Overall, linear peptide-based proteasome inhibitors remain to be the mainstream of this field [13,21,27–30].

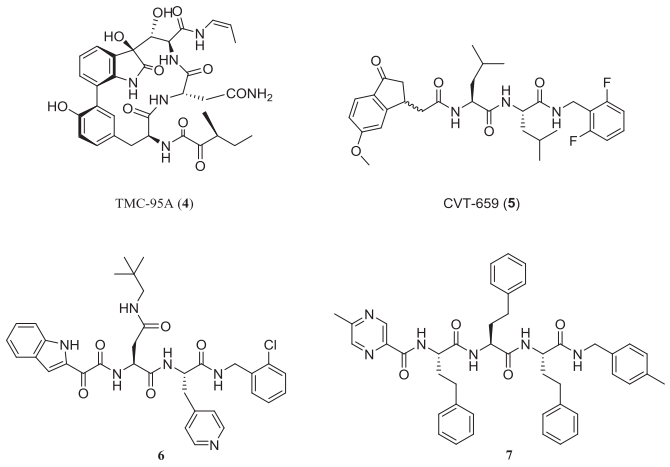


Fig. 2. Structures of representative peptidyl non-covalent proteasome inhibitors.

However, linear peptides are always thought to be unstable with unsatisfied pharmacokinetic profiles. In contrast, macrocyclic peptides often show distinct and (in comparison to linear peptides) superior pharmacokinetic and pharmacological properties [31,32]. Desirable properties introduced by the macrocycle include increased metabolic stability, cellular penetration and selectivity [33–35]. We have identified a series of oral and potent macrocyclic dipeptide epoxyketones as covalent proteasome inhibitors in our previous work [36]. Due to the disadvantages of the electrophilic warhead discussed, in this manuscript, we explored novel proteasome inhibitors based on the peptidyl macrocyclic skeleton of macrocyclic dipeptide epoxyketones, replacing the electrophilic warhead with substituted benzyl groups (Fig. 3). During further optimization, a series of macrocyclic dipeptide *N*-benzyl amides were designed and synthesized. Moreover, the anti-proliferative activity against various cancer cells of selected compounds were evaluated. In addition, we explored the differences in metabolic stability between our macrocyclic dipeptides and the corresponding linear analogues.

2. Results and discussion

2.1. Chemistry

All the target compounds were synthesized by following routes as described in Schemes 1–5. Schemes 1 and 2 show the synthetic routes of the intermediates which would be used in Schemes 3–5. Schemes 3 and 4 show the synthetic routs of macrocyclic dipeptides **23a-23j** and **31**. Finally, Scheme 5 shows the synthetic rout of linear analogue **33.**

The synthetic routes of terminal alkene-containing carboxylic intermediates **10a** - **10e** are displayed in Scheme 1. (a) Treatment of **8a** with 5-bromopent-1-ene in the presence of potassium carbonate, followed by removal of the methyl group yielded the carboxylic intermediate **10a**. (b) The preparation of **10b** was initiated from ethyl 5-bromo-1-methyl-1H-pyrazole-4-carboxylate **8b**, through nucleophilic displacement with pent-4-en-1-ol using NaHMDS to yield alkenyl esters **9b**, which was hydrolyzed to furnish **10b**. (c) The ethyl 5-bromo-1-methyl-1H-pyrazole-4-carboxylate **8b** was coupled with allyltributyltin to provide **9c**, followed by hydrolysis to obtain **10c**. (d) Electrophilic substitution of ethyl 1H-imidazole-2-carboxylate **8c** with 4-bromobut-1-ene or 6-bromohex-1-ene, afforded **9d** or **9e**, which were subsequently converted to **10d** or **10e** through hydrolysis.

The terminal alkene-containing amino acid intermediates **13a**, **13b**, and **17** were prepared through the following routes (Scheme 2). (a) Benzyl (tert-butoxycarbonyl)-*L*-serinate **11a** or benzyl (tert-butoxycarbonyl)-*L*-threoninate coupled **11b** with allylmethyl carbonate in the presence of a palladium catalyst to afford **12a** or **12b**. Following deprotection in HCl-saturated ethyl acetate, **13a** or **13b** was formed. (b) Boc-*L*-serine **14** underwent nucleophilic substitution with 6-bromohex-1-ene, followed by benzylation and removal of the Boc group to afford **17**.

The synthetic routes for target compounds **23a-23j** are outlined

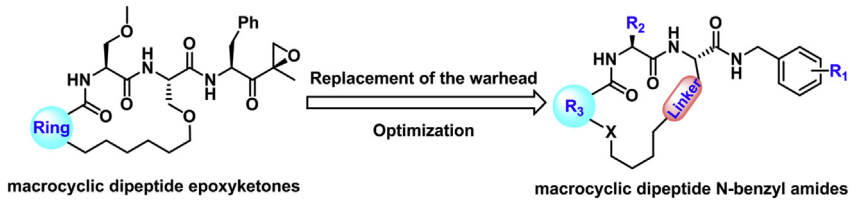
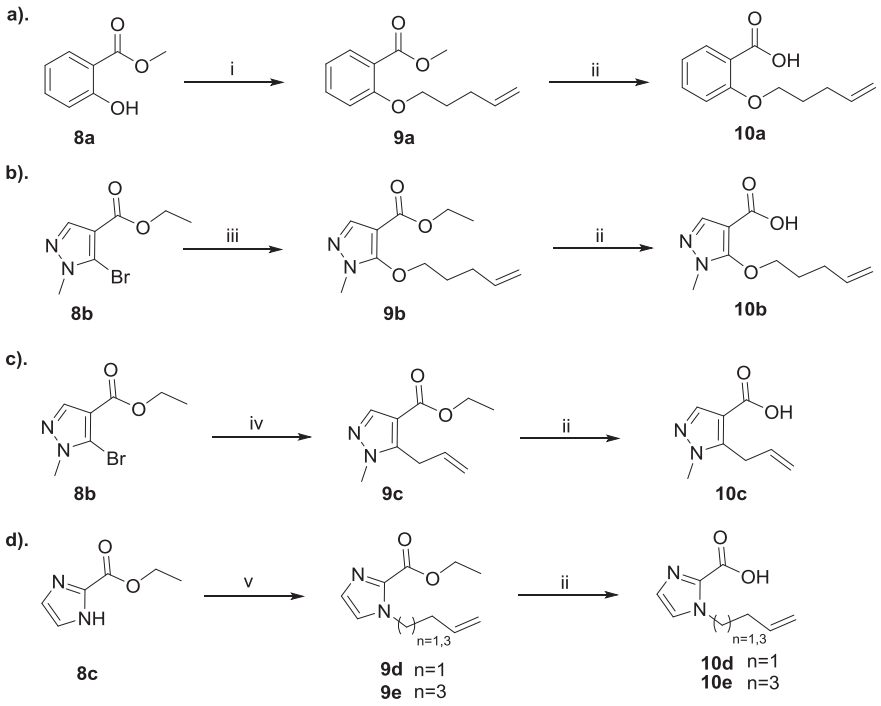
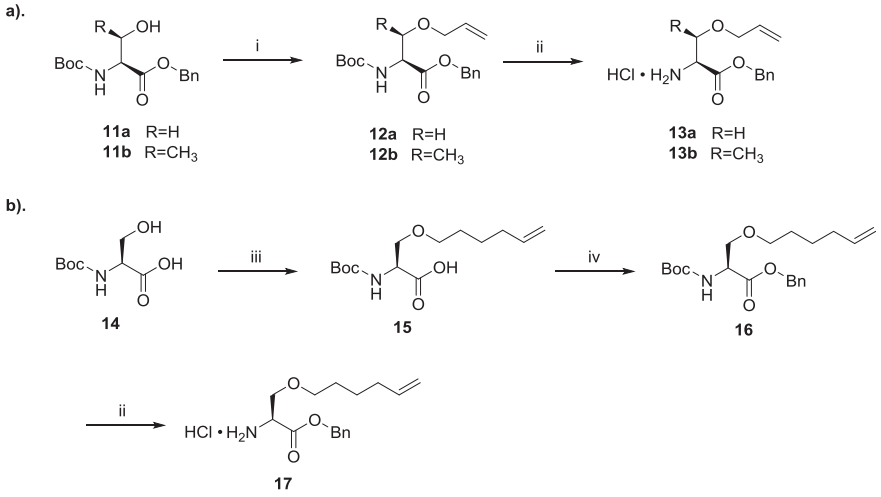


Fig. 3. Design and optimization of the macrocyclic dipeptide *N*-benzyl amides.

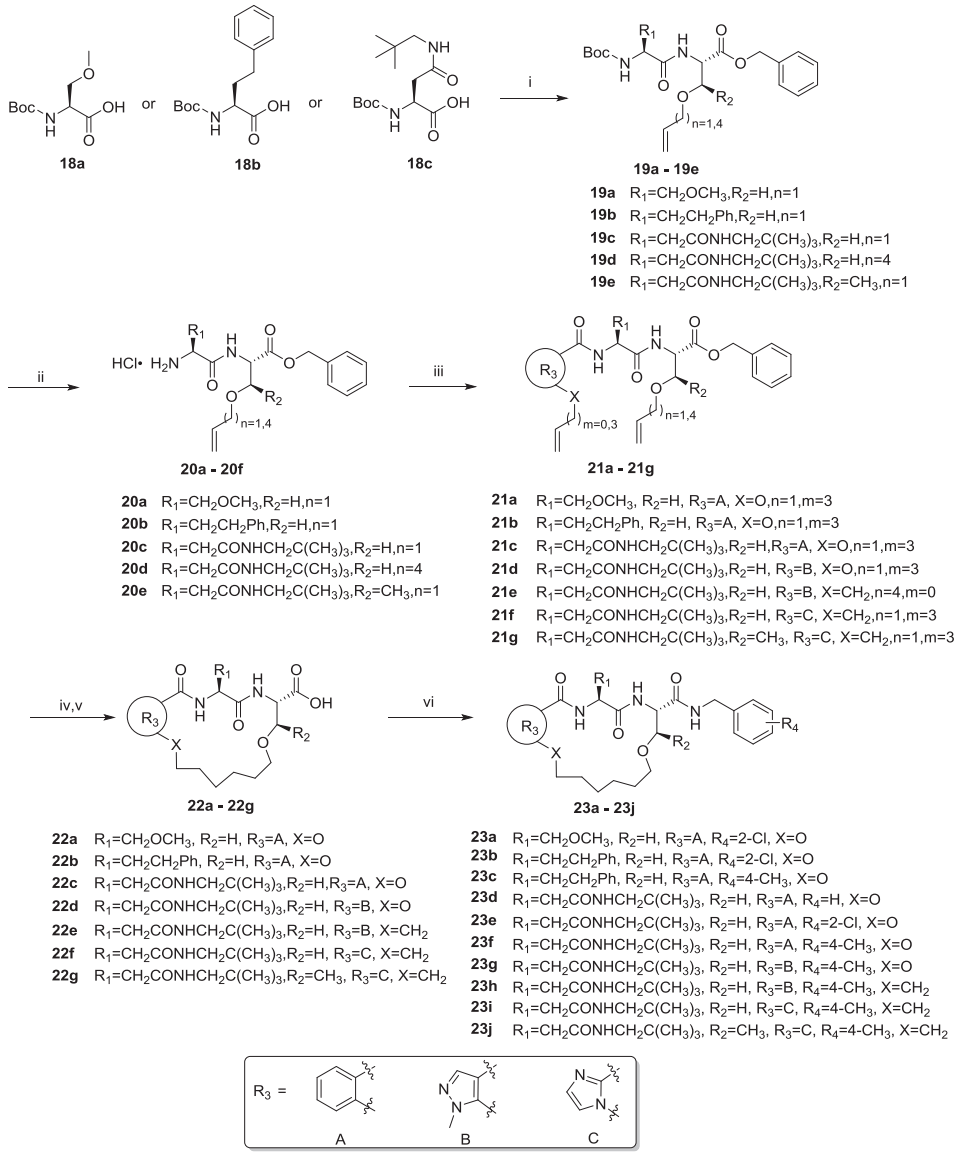


Scheme 1. Synthetic routes for the carboxylic intermediates **10a** – **10e**. Reagents and conditions: (i) 5-bromopent-1-ene, K_2CO_3, DMF, 80 °C, 2 h; (ii) 2M NaOH (aq), THF/MeOH, r.t., 3 h; (iii) pent-4-en-1-ol, NaHMDS, THF, −8 °C; (iv) allyltributyltin, $Pd_2(dba)_3$, $PtBu_3$, CsF, THF, reflux, 5 h; (v) 4-bromobut-1-ene or 6-bromohex-1-ene, K_2CO_3, DMF, 80 °C, 3 h.



Scheme 2. Synthetic routes for terminal alkene-containing amino acid intermediates **13a**, **13b**, **17**. Reagents and conditions: (i) allylmethyl carbonate, $Pd(PPh_3)_4$, THF, 60 °C, 4 h; (ii) HCl-saturated ethyl acetate, 0°C - r.t., 2 h; (iii) 6-bromohex-1-ene, NaH, DMF, rt, 18 h; (iv) BnBr, K_2CO_3, DMF, 0 °C – r.t., 20 h.

in Scheme 3. Boc protected amino acids with different side chains **18a-18c**, as the starting material, were firstly condensed with **13a**, **13b** or **17** to yield dipeptide intermediates **19a-19e,** which subsequently underwent deprotection of the Boc group and condensation with the corresponding carboxylic intermediates **10a-10c** or **10f** to afford the diolefin derivatives **21a-21g**. Following



Scheme 3. Synthetic routes for target compounds **23a-23j**. Reagents and conditions: (i) **13a**, **13b** or **17**, HOBt, EDCI, DIPEA, DCM, 0 °C - rt, 5 h; (ii) HCl-saturated ethyl acetate, 0°C - r.t., 2 h; (iii) **10a - 10c** or **10f**, HOBt, EDCI, DIPEA, DCM, 0 °C - rt, 5 h; (iv) Grubbs second generation catalyst, toluene, 100 °C; (v) 10% Pd/C, MeOH, rt, 2 h; (vi) phenylmethanamine, (2-chlorophenyl)methanamine or *p*-tolylmethanamine, HOBt, EDCI, DIPEA, DCM, 0 °C - rt, 5 h.

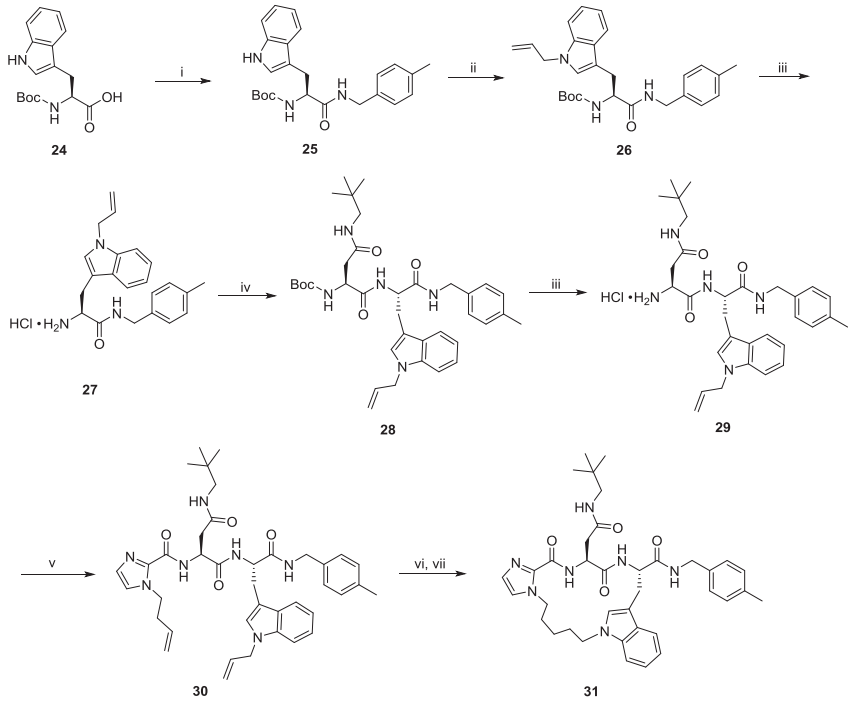
this, diolefin derivatives through ring-closing metathesis (RCM) in the presence of Grubbs second generation catalyst, as well as reduction of the C–C double bond and removal of the benzyl-protecting group on one pot, gave the macrocyclic carboxylic derivatives **22a-22g.** Finally, **22a-22g** was condensed with phenylmethanamine, (2-chlorophenyl)methanamine or *p*-tolylmethanamine to produce **23a-23j** as the target compounds.

Target compound **31** was prepared through the following route (Scheme 4). Firstly, commercially available Boc-L-tryptophan **24**, was used as the starting material, which underwent condensation with *p*-tolylmethanamine to yield **25**, which was converted to **26** through electrophilic substitution with 3-bromoprop-1-ene in the presence of NaH in THF. Subsequent removal of the Boc-protecting group and condensation with N^2-(tert-butoxycarbonyl)-N^4-neopentyl-L-asparagine **18c** provided dipeptide **28**. This was then converted into the hydrochloride **29** via unmasking the amine group, followed by condensation with the carboxylic intermediate **10e** to afford **30**. Finally, diolefin intermediate via RCM and reduction produced target compound **31.**

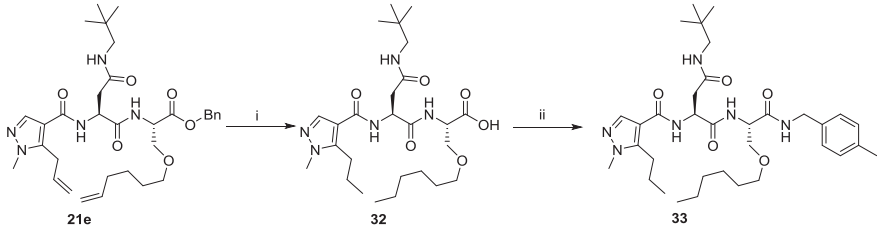
To explore the advantages of the designed macrocyclic dipeptide *N*-benzyl amides, we synthesized linear analogue **33**, corresponding to **23h**. As show in Scheme 5, the synthesis of **33** was initiated from a diolefin intermediate **21e**, which was converted into a carboxylic intermediate **32** through reduction and debenzylation in the present of 10% Pd/C under a H_2 atmosphere. Subsequently, **32** underwent condensation with *p*-tolylmethanamine to produce the linear analogue **33**.

2.2. Biological evaluation

All synthesized target compounds (**23a-23j**, **31**) were evaluated for their 20S proteasome β5c inhibitory activity. Nine compounds with promising β5 inhibition were further tested for their anti-proliferative activity against three tumor cell lines including RPMI 8226, MM1S, and MV-4-11 (all of the three are peripheral blood cell lines). The results are summarized in Table 1.



Scheme 4. Synthetic route for target compound **31**. Reagents and conditions: (i) *p*-tolylmethanamine, HOBt, EDCI, DIPEA, DCM, 0 °C - rt, 5 h; (ii) 3-bromoprop-1-ene, NaH, THF, 0°C - r.t., 3 h; (iii) HCl-saturated ethyl acetate, 0°C - r.t., 2 h; (iv) N^2-(tert-butoxycarbonyl)-N^4-neopentyl-*L*-asparagine, HOBt, EDCI, DIPEA, DCM, 0 °C - rt, 5 h; (v) **10d**, HOBt, EDCI, DIPEA, DCM, 0 °C - rt, 5 h; (vi) Grubbs second generation catalyst, toluene, 100 °C, 3 h; (vii) 10% Pd/C, MeOH, rt, 2 h.



Scheme 5. Synthetic route for the corresponding linear analogue **33**. Reagents and conditions: (i) 10% Pd/C, MeOH, rt, 2 h; (ii) *p*-tolylmethanamine, HOBt, EDCI, DIPEA, DCM, 0 °C - rt, 5 h.

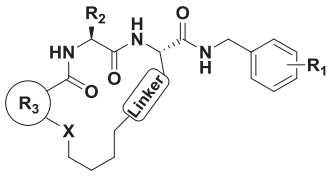
Firstly, we found that R_2 substituent groups significantly influenced proteasome inhibitory activity. 2-(Neopentylamino)-2-oxoethyl substituted compound **23e** was more potent than methoxymethyl and phenethyl substituted compounds (**23a** & **23b**). Therefore, 2-(Neopentylamino)-2-oxoethyl was employed at the R_2 group for further optimization. Secondly, in order to ensure suitable R_1 substitutions, the H, 2-Cl, and 4-CH_3 groups, which are often used in linear non-covalent proteasome inhibitors (e. g. compounds **6** and **7**), were investigated with the 4-CH_3 (as in **23f**) being 10-fold and 2-fold more potent than the H (as in **23d**) and 2-Cl (as in **23e**), respectively. Furthermore, three different rings were employed in R_3 with the methylpyrazole and imidazole rings improving inhibitory potency compared to the phenyl rings, as exemplified by compounds **23g**-**23i**, which were approximately 2-fold more potent than **23f** with IC_{50} values about 30 nM. Meanwhile, compounds **23g**-**23i** exhibited excellent anti-proliferative activity against the three cancer cell lines, particularly compound **23h** and **23i**. In addition, compared to **23g** and **23h**, we found that the oxygen and carbon atoms were both suitable for the X atom. Finally, different linkers (e. g. **23i**, **23j**, & **31**) when introduced into the skeleton displayed little impact on inhibitory potency, but the anti-proliferative activity against MM1S and MV-4-11 of compound **23j** significantly decreased compared to **23i** or **31**.

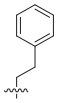
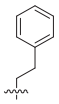
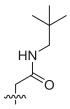
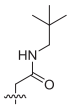
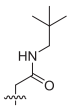
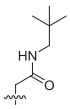
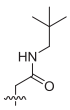
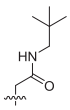
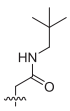
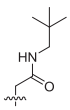
Compound **23h** and compound **23i** exhibited excellent anti-proliferative activity against RPMI 8226, MM1S, and MV-4-11 cell lines, and all the three cell lines are blood cancer cell lines. Several studies [37,38] have reported that immunoproteasome expressed more than constitutive proteasome in B-cell malignancies, suggesting the importance of the immunoproteasome in hematologic malignancies [39–41]. Therefore, we assessed compound 23h and 23i against different subunits of cCP and iCP (Table 2.). We found our compounds displayed potent and selective inhibitory activity against β_{5c} and $\beta_{5i.}$ The co-inhibition against β_{5c} and β_{5i} of **23h** and **23i** may contribute to well anti-proliferative activity against the three cancer cell lines.

To assess metabolic stability profiles of **23h** and **23i**, we tested they metabolic stability in HLMs. As shown in Fig. 4, compound **23h** showed more than 66% remaining after 30 min incubation, while **23i** showed less than 20% remaining. This result demonstrates that **23h** was more stable than **23i**.

In order to investigate the advantages of our marcocyclic dipeptides compared to the linear dipeptides, we also synthesized linear analogue **33** which correspond to **23h**. The β5c inhibitory activity, anti-proliferative activity and metabolic stability profiles in

Table 1
20S proteasome (β5c) inhibitory activity and anti-proliferative activity of the target compounds (**22a-22j**, **31**).



Compd.	R_1	R_2	R_3	X	Linker	β_{5c} IC_{50} (nM)[a]/inhibition rate[b]	Anti-proliferative activity (IC_{50}, nM)[a]		
							RPMI 8226	MM1S	MV-4-11
23a	2-Cl			O		34.01%[b]	–	–	–
23b	2-Cl			O		36.67%[b]	–	–	–
23c	4-CH_3			O		38.39%[b]	–	–	–
23d	H			O		660 ± 30	–	–	–
23e	2-Cl			O		127 ± 36	301 ± 20	343 ± 11	718 ± 58
23f	4-CH_3			O		67 ± 12	175 ± 6	37 ± 2	76 ± 5
23g	4-CH_3			O		30 ± 4	58 ± 9	23 ± 3	55 ± 2
23h	4-CH_3			C		29 ± 3	18 ± 2	15 ± 1	21 ± 9
23i	4-CH_3			C		34 ± 6	15 ± 1	6 ± 0.5	12 ± 0.3
23j	4-CH_3			C		50 ± 7	27 ± 2	220 ± 32	208 ± 41
31	4-CH_3			C		26 ± 7	59 ± 4	17 ± 1	52 ± 1
Carfilzomib	–	–	–	–	–	7 ± 0.5	2 ± 0.1	<1	–
Oprozomib	–	–	–	–	–	47 ± 2	19 ± 3	9 ± 1	28 ± 3

[a] IC_{50} values are shown as an average of three independent determinations.
[b] Inhibition rates of β5c were determined at a concentration of 10 μM.

Table 2
The inhibition of β_{1c}, β_{2c}, β_{5c}, β_{1i}, β_{2i}, and β_{5i} of compound **23h**, **23i**, and Bortezomib.

Compd.	IC_{50}(nM)[a]					
	β_{1c}	β_{2c}	β_{5c}	β_{1i}	β_{2i}	β_{5i}
23h	>10000	>10000	29 ± 3	>10000	>10000	35 ± 5
23i	>10000	>10000	34 ± 6	>10000	>10000	19 ± 2
Bortezomib	104 ± 38	2119 ± 819	11 ± 1	3 ± 1	517 ± 10	4 ± 1

[a] The IC_{50} values are shown as an average of three independent determinations.

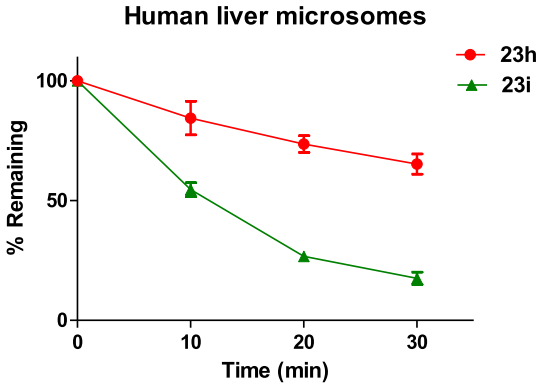


Fig. 4. Metabolic stability profiles of compound **23h** and **23i** in HLMs.

HLMs of the linear analogue **33** were evaluated. The results are summarized in Table 3.

As illustrated in Table 3, proteasome β5c inhibition of linear analogue **33** was better than that of the macrocyclic compound, but its anti-proliferative activity against all of the three cancer cell lines was lower. In comparison to **33** with **23h**, ring breaking of **23h** led to a 5-fold, 5-fold, and 3-fold loss of anti-proliferative activity against RPMI 8226, MM1S, and MV-4-11, respectively. These results indicated that macrocylic dipeptide *N*-benzyl amides were likely to display improved cellular penetration compared to linear analogues. Furthermore, **23h** demonstrated excellent metabolic stability in HLMs (Table 3. & Fig. 5), as compared to **33** and carfilzomib. This result suggested that our macrocylic dipeptide *N*-benzyl

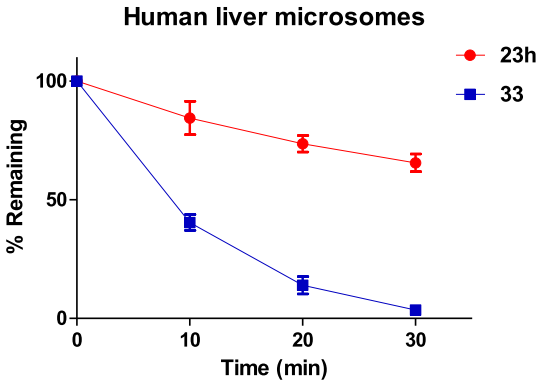


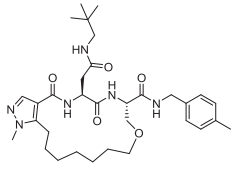
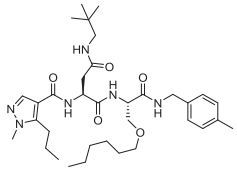
Fig. 5. Metabolic stability profiles of compound **23h** and **33** in HLMs.

amides might possess improved metabolic stability profiles than carfilzomib and their corresponding linear analogues.

2.3. Binding mode analysis

To explore the binding modes of these macrocyclic dipeptide *N*-benzyl amides within the active site of proteasome, molecular docking calculations were performed. First, the ligand in the crystal structure of 3MG6 (PDB ID) was extracted and re-docked into the binding pocket [43–46]. The root mean square deviation (RMSD) between the docked pose and the original conformation of the ligand was calculated, and a RMSD = 0.3 Å suggests that the Glide docking with the SP scoring can successfully recognize the near-native conformations. Then, the binding mode of compound **23h** within the proteasome was predicted by the Glide docking and shown in Fig. 6. The docking simulations clearly demonstrated the presence of **23h** in the proximity of the β5 site. As depicted in Fig. 6A, the C-terminal 4-methylbenzyl group and the neopentylamino group occupied the S1 and S3 pockets, respectively, while the linker was exposed to the solvent. In addition, a similar binding mode reported by Blackburn et al. [20] involving β-sheet-type H-bonding interactions was also observed (Fig. 6B). Six critical hydrogen bonds were formed between the carbonyl and amino groups of the peptide skeleton and Gly47, Thr21, Ala49, Ala50 and

Table 3
Biological evaluation of compounds **23h** & **33**.

Compd.	Structure	β_{5c} IC_{50} (nM)[a]	Anti-proliferative activity (IC_{50}, nM)[a]			HLMs Stability[b]
			RPMI 8226	MM1S	MV-4-11	
23h		29 ± 3	18 ± 2	15 ± 1	21 ± 9	66%
33		9 ± 0.5	97 ± 26	64 ± 8	73 ± 3	<5%
Carfilzomib	–	7 ± 0.5	2 ± 0.1	<1	–	<5%[c]

[a] IC_{50} values are shown as an average of three independent determinations.
[b] % remaining was determined after 30 min of incubation in HLMs. The higher of % remaining, the more stable is the compound in HLMs. % remaining was calculated as described in 4.2.3 section.
[c] Data from reported reference [42].

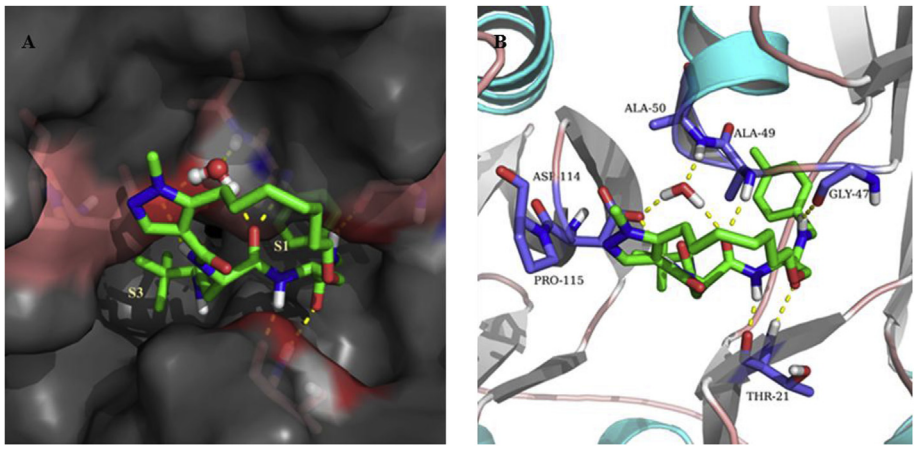


Fig. 6. Binding mode of compound **23h** within the active site of proteasome. A: Occupancy of the S1 and S3 pockets of the 20S proteasome by the 4-methylbenzyl group and the neopentylamino group of compound **23h** displayed as Connolly surface. B: Predicted H-bonds between compound **23h** and the protein represented as a ribbon model, H-bonds are shown as dashed yellow lines. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Asp114 of the proteasome active site. Notably, **23h** established H-bonds with Ala50N and ASP114O$^{\gamma}$ via a bridge water molecule. In brief, specific binding to the S1 and S3 pockets and the β-sheet-type H-bonding interactions of compound **23h** contribute to its inhibitory potency.

3. Conclusions

In this study, a series of novel macrocyclic dipeptide *N*-benzyl amides were rationally designed, synthesized, and biologically evaluated. Most of them exhibited potent proteasomal inhibition, as well as excellent anti-proliferative activity against RPMI 8226, MM1S, and MV-4-11 cell lines. Compound **23h** and **23i** displayed potent and selective inhibition against β5c and β5i. Further studies found that compound **23h** was more potent and more stable than its corresponding linear analogues, proving that the introduction of a macrocycle might increase potency and metabolic stability. Finally, docking studies were performed to analyze the binding mode of **23h** within β5 site of the proteasome. Overall, **23h** exhibited excellent metabolic stability with promising proteasome inhibitory activity and vigorous cellular activity, which merits further development as a potential anticancer agent.

4. Experimental section

^{1}H and ^{13}C NMR spectra were recorded on Brüker 500 MHz spectrometer (Brüker Bioscience, Billerica, MA, USA) with $CDCl_3$ or DMSO-d_6 as solvent. Chemical shifts (d) were reported in parts per million (ppm) relative to internal TMS, and coupling constants (J) were reported in Hertz (Hz). Splitting patterns were designated as singlet (s), broad singlet (brs), doublet (d), double doublet (dd), triplet (t), quartet (q) and multiplet (m). Melting points were determined using a Buchi B-540 capillary melting point apparatus and are uncorrected. Electrospray ionization mass spectroscopy (ESI-MS) spectra were obtained with a Shimadzu LCMS-2020 mass spectrometer with mobile phases as methanol and water containing 0.1% formic acid. HPLC analysis was performed using an Agilent 1260 Series system with a COSMOSIL 5C18-MS-II (4.6 mm I.D. × 250 mm) column and detected at 254 nm wavelength. The details of the HPLC method can be found in supplementary data. Reagents and solvents were purchased from common commercial suppliers and were used without further purification unless stated otherwise. Column chromatography was performed using silica gel (200–300 mesh). All yields are unoptimized and generally represent.

4.1. Chemistry

4.1.1. Methyl 2-(pent-4-en-1-yloxy)benzoate **9a**

A mixture of methyl 2-hydroxybenzoate (5.00 g, 33.0 mmol), potassium carbonate (9.11 g, 66.0 mmol) and 5-bromopent-1-ene (6.81 g, 46.0 mmol) in 50 mL DMF was heated to 80 °C for 2 h. TLC analysis indicated that the reaction had ceased. After cooling to room temperature, the solution was diluted with EtOAc (100 mL) and washed with water (100 mL × 3), brine (50 mL × 3). The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was then purified by SiO_2 chromatography (ethyl acetate: petroleum ether = 1:15) to give compound **9a** as colorless oil (5.8 g, 80%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (dd, J = 7.5, 1.5 Hz, 1H), 7.44 (ddd, J = 8.0, 7.5, 1.5 Hz, 1H), 6.99–6.94 (m, 2H), 5.92–5.80 (m, 1H), 5.09–5.04 (m, 1H), 5.02–4.98 (m, 1H), 4.05 (t, J = 6.5 Hz, 2H), 3.89 (s, 3H), 2.34–2.25 (m, 2H), 1.98–1.88 (m, 2H); ESI-MS: m/z = 221 $[M+H]^+$.

4.1.2. Ethyl 5-allyl-1-methyl-1H-pyrazole-4-carboxylate **9c**

Ethyl 5-bromo-1-methyl-1*H*-pyrazole-4-carboxylate **8b** (1.23 g, 5.3 mmol), allyltributyltin (1.8 mL, 5.8 mmol), $Pd_2(dba)_3$ (240 mg, 5 mol%), $PtBu_3$ (0.27 mL, 18 mol %), and CsF (1.61 g, 10.0 mmol) was charged in a three-necked flask under N_2. To this mixture was added dry THF (25 mL), and the reaction mixture was heated to reflux for 5 h. The reaction mixture was filtered off, the solvent removed in a vacuum, and the crude product purified by SiO_2 chromatography (ethyl acetate: petroleum ether = 1:8) to give **9c** as colorless oil (0.82 g, 80%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (s, 1H), 5.94–5.80 (m, 1H), 5.16–5.06 (m, 1H), 5.02–4.90 (m, 1H), 4.28 (q, J = 7.0 Hz, 2H), 3.85–3.73 (m, 5H), 1.34 (t, J = 7.0 Hz, 3H); ESI-MS: m/z = 195 $[M+H]^+$.

4.1.3. Ethyl 1-(but-3-en-1-yl)-1H-imidazole-2-carboxylate **9d**

A mixture of ethyl 1H-imidazole-2-carboxylate (700 mg, 5.0 mmol), potassium carbonate (1.38 g, 10.0 mmol) and 4-bromobut-1-ene (800 mg, 6.0 mmol) in 5 mL DMF was heated to 80 °C for 3 h. TLC analysis indicated that the reaction had ceased. After cooling to room temperature, the solution was diluted with EtOAc (15 mL) and washed with water (25 mL × 3), brine (25 mL × 3). The organic layer was dried over Na_2SO_4, filtered, and

concentrated under reduced pressure. The residue was then purified by SiO_2 chromatography (ethyl acetate: petroleum ether = 1:1) to give compound **9d** as colorless oil (0.93 g, 96%).^{1}H NMR (500 MHz, $CDCl_3$) δ 7.14 (d, J = 1.0 Hz, 1H), 7.05 (d, J = 1.0 Hz, 1H), 5.80–5.70 (m, 1H), 5.08–5.01 (m, 2H), 4.47 (t, J = 7.0 Hz, 2H), 4.41 (q, J = 7.0 Hz, 2H), 2.59–2.50 (m, 2H), 1.43 (t, J = 7.0 Hz, 3H); ESI-MS: m/z = 195 $[M+H]^+$.

4.1.4. Ethyl 1-(hex-5-en-1-yl)-1H-imidazole-2-carboxylate **9e**

This compound was prepared from **8c** (700 mg, 5.0 mmol), 6-bromohex-1-ene (972 mg, 6 mmol) and K_2CO_3 (1.38 g, 10.0 mmol) in a similar manner as described for compound **9d**. The product was obtained as colorless oil (1.04 g, 94%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.16 (s, 1H), 7.07 (s, 1H), 5.77 (ddt, J = 17.0, 10.0, 7.0 Hz, 1H), 5.00 (dd, J = 17.0, 1.0 Hz, 1H),4.96(d,J = 10, 1H) 4.43–4.39 (m, 4H), 2.09 (dt, J = 7.0, 7.0 Hz, 2H), 1.85–1.79 (m, 2H), 1.50–1.34 (m, 5H); ESI-MS: m/z = 223 $[M+H]^+$.

4.1.5. 2-(Pent-4-en-1-yloxy)benzoic acid **10a**

To a solution of compound **9a** (1.10 g, 5.0 mmol) in 10 mL MeOH/THF (V_{MeOH}:V_{THF} = 1:1) at 0°C was added 10 mL 2M NaOH (aq). The mixture was stirred at room temperature for 3 h and MeOH and THF were evaporated in vacuo. The residue was acidified to pH = 2–3 with 1 N HCl and extracted with ethyl acetate (20 mL × 3). The combined organic layers were washed with brine (20 mL × 3), dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford compound **10a** as colorless oil (0.71 g, 68%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.47 (s, 1H), 7.62 (dd, J = 7.5, 2.0 Hz, 1H), 7.50–7.45 (m, 1H), 7.10 (d, J = 8.5 Hz, 1H), 7.00–6.95 (m, 1H), 5.92–5.81 (m, 1H), 5.08–5.01 (m, 1H), 5.01–4.94 (m, 1H), 4.03 (t, J = 6.0 Hz, 2H), 2.26–2.17 (m, 2H), 1.84–1.74 (m, 2H); ESI-MS: m/z = 205 $[M-H]^-$.

4.1.6. 1-Methyl-5-(pent-4-en-1-yloxy)-1H-pyrazole-4-carboxylic acid **10b**

A solution of pent-4-en-1-ol (1.45 mL, 14.0 mmol) in THF (24.0 mL) was cooled to −8 °C and treated with 1.0 M sodium hexamethyldisilazane in THF (14.0 mL, 14.0 mmol). The reaction mixture was stirred at the reduced temperature for 5 min before the ice bath was removed. Stirring was continued for an additional 25 min before being treated with a solution of ethyl 5-bromo-1-methyl-1*H*-pyrazole-4-carboxylate (1.1 g, 4.7 mmol) in THF (24 mL). The reaction mixture was stirred for 1 hour at room temperature before being quenched with 50 mL of sat. NH_4Cl. The crude product was extracted with DCM (60 mL x 3), dried with Na_2SO_4 and concentrated under reduced pressure. This material purified by SiO_2 chromatography (ethyl acetate: petroleum ether = 1:5) to afford 0.65 g impure product **9b** mixed with pent-4-en-1-yl 1-methyl-5-(pent-4-en-1-yloxy)-1*H*-pyrazole-4-carboxylate (produced through transesterification reaction), the mixed esters could both be the raw materials for next hydrolysis, so the mixture was put into next step without further separation. To a solution of the mixed esters in 5 mL MeOH/THF (V_{MeOH}:V_{THF} = 1:1) at 0°C was added 5 mL 2M NaOH (aq). The mixture was stirred at room temperature for 3 h and MeOH and THF were evaporated in vacuo. The residue was acidified to pH = 2–3 with 1 N HCl and extracted with ethyl acetate (10 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford compound **10b** as yellow solid (0.45 g, 46%).^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 (s, 1H), 5.89–5.77 (m, 1H), 5.10–5.00 (m, 2H), 4.46 (t, J = 6.5 Hz, 2H), 3.70 (s, 4H), 2.28–2.19 (m, 2H), 1.93–1.85 (m, 2H); ESI-MS: m/z = 209 $[M-H]^-$.

4.1.7. 5-Allyl-1-methyl-1H-pyrazole-4-carboxylic acid **10c**

This compound was prepared from **9c** (970 mg, 5.0 mmol), 10 mL MeOH/THF (V_{MeOH}:V_{THF} = 1:1) and 10 mL 2M NaOH (aq) in a similar manner as described for compound **10a**. The product was obtained as colorless oil yellow solid (747 mg, 90%).^{1}H NMR (500 MHz, DMSO-d_6) δ 12.26 (s, 1H), 7.74 (s, 1H), 5.93–5.78 (m, 1H), 5.09–5.05 (m, 1H), 4.98–4.92 (m, 1H), 3.78–3.75 (m, 2H), 3.75 (s, 3H); ESI-MS: m/z = 165 $[M-H]^-$.

4.1.8. 1-(But-3-en-1-yl)-1H-imidazole-2-carboxylic acid **10d** and 1-(hex-5-en-1-yl)-1H-imidazole-2-carboxylic acid **10e**

To a solution of compound **9d** (0.93 g, 4.8 mmol) or **9e** (1.11 g, 5.0 mmol) in 5 mL MeOH/THF (V_{MeOH}:V_{THF} = 1:1) at 0°C was added 5 mL 2M NaOH (aq). The mixture was stirred at room temperature for 3 h and MeOH and THF were evaporated in vacuo. The residual water layer was washed with DCM (5 mL × 3), acidified to pH = 3–4 with 1 N HCl and extracted with *N*-butanol (5 mL × 3). The combined organic layers were evaporated in vacuo at room temperature to afford compound **10d** or **10e**, the crude product was put into next step without further purification.

4.1.9. Benzyl O-allyl-N-(tert-butoxycarbonyl)-L-serinate **12a**

A three-necked flask was charged with **11a** (4.42 g, 15.0 mmol) and $Pd(PPh_3)_4$ (0.87 g, 0.75 mmol) under N_2. The allylmethyl carbonate (2.3 mL, 21.0 mmol) in anhydrous THF (50 mL) was syringed over 10 min. The reaction mixture was heated to 60 °C for 4 h upon which TLC indicated the loss of starting material. The solvent was removed under reduced pressure and the residue was diluted with EtOAc (40 mL) and washed with $NaHCO_3$ (40 mL × 3) and brine (40 mL × 3). The organic layer was dried over $NaSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by SiO_2 chromatography (ethyl acetate: petroleum ether = 1:2) to afford compound **12a** as colorless oil (4.11 g, 82%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41–7.30 (m, 5H), 5.86–5.68 (m, 1H), 5.41 (d, J = 8.5 Hz, 1H), 5.32–5.17 (m, 2H), 5.17–5.10 (m, 2H), 4.51–4.42 (m, 1H), 3.99–3.84 (m, 3H), 3.65 (dd, J = 9.5, 3.0 Hz, 1H), 1.45 (s, 9H); ESI-MS: m/z = 336 $[M+H]^+$.

4.1.10. Benzyl O-allyl-N-(tert-butoxycarbonyl)-L-threoninate **12b**

This compound was prepared from **11b** (4.63 g, 15 mmol), $Pd(PPh_3)_4$ (0.87 g, 0.75 mmol) and allylmethyl carbonate (2.3 mL, 21.0 mmol) in a similar manner as described for compound **12a**. The product was obtained as colorless oil (4.55 g, 87%).^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40–7.30 (m, 5H), 5.68 (ddt, J = 16.5, 10.5, 5.5 Hz, 1H), 5.31–5.05 (m, 5H), 4.32 (d, J = 9.5 Hz, 1H), 4.11–4.03 (m, 1H), 3.96–3.89 (m, 1H), 3.79–3.68 (m, 1H), 1.45 (s, 9H), 1.20 (d, J = 6.0 Hz, 3H); ESI-MS: m/z = 350 $[M+H]^+$.

4.1.11. N-(tert-butoxycarbonyl)–O-(hex-5-en-1-yl)-L-serine **15**

(*tert*-Butoxycarbonyl)-L-serine **14** (2.05 g, 10.0 mmol) was dissolved in DMF (25 mL). The solution was cooled to 0 °C (ice bath) before sodium hydride (60% dispersion in mineral oil, 0.60 g, 25.0 mmol) was added slowly under stirring. After gas evolution had ceased 6-bromohex-1-ene (1.3 mL, 10.0 mmol) was added dropwise and the reaction mixture stirred for 18 h at room temperature. The reaction mixture was cooled to 0 °C and diluted with EtOAc (50 mL) and H_2O (50 mL), the organic layer was separated and washed with H_2O (50 mL × 3), and brine (50 mL × 3). Dried over Na_2SO_4, filtrated, and evaporated under reduced pressure to yield compound **15** as colorless oil (1.61 g, 56%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.84–5.72 (m, 1H), 5.42 (d, J = 8.0 Hz, 1H), 5.06–4.88 (m, 2H), 4.47–4.37 (m, 1H), 3.87 (dd, J = 11.0, 3.0 Hz, 2H), 3.65 (dd, J = 9.5, 3.5 Hz, 1H), 3.45 (t, J = 6.5 Hz, 2H), 2.08–2.02 (m, 2H), 1.60–1.52 (m, 2H), 1.49–1.38 (m, 11H); ESI-MS: m/z = 288 $[M+H]^+$.

4.1.12. Benzyl N-(tert-butoxycarbonyl)–O-(hex-5-en-1-yl)-L-serinate ***16***

Benzyl bromide (0.79 mL, 6.7 mmol) was slowly added to a mixture of **15** (1.61 g, 5.6 mmol) and K_2CO_3 (0.92 g, 6.7 mmol) in DMF (15 mL). The solution was stirred at room temperature for 20 h and then diluted with EtOAc (30 mL) and H_2O (30 mL). The organic layer was separated and washed with saturated $NaHCO_3$ (20 mL × 3) and brine (20 mL × 3), dried over Na_2SO_4. Filtrated, and evaporated under vacuum to give compound **16** as colorless oil (1.10 g, 52%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41–7.29 (m, 5H), 5.85–5.71 (m, 1H), 5.38 (d, J = 8.5 Hz, 1H), 5.28–5.09 (m, 2H), 5.05–4.89 (m, 2H), 4.52–4.39 (m, 1H), 3.84 (dd, J = 9.5, 3.0 Hz, 1H), 3.63 (dd, J = 9.5, 3.0 Hz, 1H), 3.45–3.27 (m, 2H), 2.07–1.99 (m, 2H), 1.55–1.33 (m, 13H); ESI-MS: m/z = 378 $[M+H]^+$.

4.1.13. General procedure for compounds ***13a***, ***13b***, ***17***, ***20a*** *–* ***20e***, ***27*** *and* ***29***

To a solution of **12a**, **12b**, **16**, **19a** – **19e**, **27** and **29** (3.0 mmol) in 6 mL ethyl acetate was added saturated hydrogen chloride in ethyl acetate (12 mL) at 0°C. After stirring at room temperature for 2 h, the reaction mixture was concentrated. The residue was washed with EtO_2 (15 mL × 3) and evaporated under vacuum to give crude products **13a**, **13b**, **17**, **20a** – **20e**, **27** and **29**, the crude products were put into next step without further purification.

4.1.14. Benzyl O-allyl-N-(N-(tert-butoxycarbonyl)–O-methyl-L-seryl)-L-serinate ***19a***

To a solution of *N*-(tert-butoxycarbonyl)–O-methyl-L-serine **18a** (2.19 g, 10.0 mmol) in DCM (30 mL) were added HOBt (162 g, 12.0 mmol) and EDCI (2.88 g, 15.0 mmol) at 0°C. The reaction mixture was stirred for 30 min. Then compound **13a** (2.47 mg, 10.5 mmol) and diisopropylethylamine (6.61 mL) were added. After stirring at room temperature for another 5 h, the resulting mixture was washed with aqueous $NaHCO_3$ solution (30 mL × 3), brine (30 mL × 3) and dried over Na_2SO_4. The organic layer was evaporated in vacuo and the crude product was purified on silica gel to afford the compound **19a** as a white solid (3.61 mg, 82%).^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 (d, J = 6.5 Hz, 1H), 7.36–7.29 (m, 5H), 5.80–5.70 (m, 1H), 5.43 (d, J = 5.0 Hz, 1H), 5.26–5.21 (m, 1H), 5.21–5.16 (m, 1H), 5.16–5.11 (m, 2H), 4.74 (dt, J = 8.0, 3.0 Hz, 1H), 4.31–4.24 (m, 1H), 3.94–3.87 (m, 3H), 3.77 (dd, J = 9.0, 4.0 Hz, 1H), 3.65 (dd, J = 9.5, 3.0 Hz, 1H), 3.43 (dd, J = 9.0, 7.0 Hz, 1H), 3.33 (s, 3H), 1.44 (s, 9H); ESI-MS: m/z = 437 $[M+H]^+$.

4.1.15. Benzyl O-allyl-N-((S)-2-((tert-butoxycarbonyl)amino)-4-phenylbutanoyl)-L-serinate ***19b***

This compound was prepared from (S)-2-((tert-butoxycarbonyl) amino)-4-phenylbutanoic acid **18b** (2.79 g, 10 mmol), HOBt (162 g, 12.0 mmol), EDCI (2.88 g, 15.0 mmol), compound **13a** (2.47 mg, 10.5 mmol) and diisopropylethylamine (6.61 mL) in a similar manner as described for compound **19a**. The product was obtained as a white solid (4.31 g, 87%).^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36–7.30 (m, 5H), 7.29–7.24 (m, 2H), 7.21–7.15 (m, 3H), 6.71 (d, J = 7.5 Hz, 1H), 5.82–5.69 (m, 1H), 5.29–5.06 (m, 5H), 4.81–4.71 (m, 1H), 4.23–4.11 (m, 1H), 3.98–3.82 (m, 3H), 3.63 (dd, J = 9.5, 3.0 Hz, 1H), 2.70 (t, J = 8.0 Hz, 2H), 2.19–2.08 (m, 1H), 1.99–1.88 (m, 1H), 1.44 (s, 9H); ESI-MS: m/z = 497 $[M+H]^+$.

4.1.16. Benzyl O-allyl-N-(N^2-(tert-butoxycarbonyl)-N^4-neopentyl-L-asparaginyl)-L-serinate ***19c***

This compound was prepared from N^2-(tert-butoxycarbonyl)-N^4-neopentyl-L-asparagine **18c** (3.02 g, 10 mmol), HOBt (162 g, 12.0 mmol), EDCI (2.88 g, 15.0 mmol), compound **13a** (2.47 mg, 10.5 mmol) and diisopropylethylamine (6.61 mL) in a similar manner as described for compound **19a**. The product was obtained as a white solid (5.09 g, 98%).^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64 (d, J = 7.0 Hz, 1H), 7.48–7.28 (m, 5H), 6.19 (d, J = 5.0 Hz,1H), 5.99 (s, 1H), 5.83–5.71 (m, 1H), 5.27–5.11 (m, 4H), 4.70 (dt, J = 8.5, 3.5 Hz, 1H), 4.52 (d, J = 3.5 Hz, 1H), 3.99–3.90 (m, 2H), 3.88 (dd, J = 9.5, 3.5 Hz, 1H), 3.61 (dd, J = 9.5, 3.5 Hz, 1H), 3.03 (d, J = 6.0 Hz, 2H), 2.84 (dd, J = 14.0,2.0 Hz, 1H), 2.58 (dd, J = 15.0, 6.0 Hz, 1H), 1.44 (s, 9H), 0.88 (s, 9H); ESI-MS: m/z = 520 $[M+H]^+$.

4.1.17. Benzyl N-(N^2-(tert-butoxycarbonyl)-N^4-neopentyl-L-asparaginyl)–O-(hex-5-en-1-yl)-L-serinate ***19d***

This compound was prepared from N^2-(tert-butoxycarbonyl)-N^4-neopentyl-L-asparagine **18c** (3.02 g, 10 mmol), HOBt (162 g, 12.0 mmol), EDCI (2.88 g, 15.0 mmol), compound **17** (2.91 g, 10.5 mmol) and diisopropylethylamine (6.61 mL) in a similar manner as described for compound **19a**. The product was obtained as a white solid (5.11 g, 91%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (d, J = 6.0 Hz, 1H), 7.41–7.29 (m, 5H), 6.16 (d, J = 4.5 Hz, 1H), 5.91 (s, 1H), 5.78 (ddt, J = 17.0, 11.0, 6.5 Hz, 1H), 5.22 (d, J = 12.5 Hz, 1H), 5.13 (d, J = 12.5 Hz, 1H), 4.99 (ddd, J = 17.0, 3.5, 1.5 Hz, 1H), 4.97–4.92 (m, 1H), 4.68 (dt, J = 8.0, 3.5 Hz, 1H), 4.52 (s, 1H), 3.86 (dd, J = 9.5, 3.5 Hz, 1H), 3.59 (dd, J = 9.5, 3.5 Hz, 1H), 3.45–3.29 (m, 2H), 3.07–2.97 (m, 2H), 2.84 (d, J = 13.0 Hz, 1H), 2.57 (dd, J = 15.0, 6.0 Hz, 1H), 2.05–2.00 (m, 2H), 1.55–1.47 (m, 2H), 1.44 (s, 9H), 1.41–1.33 (m, 2H), 0.88 (s, 9H); ESI-MS: m/z = 562 $[M+H]^+$.

4.1.18. Benzyl O-allyl-N-(N^2-(tert-butoxycarbonyl)-N^4-neopentyl-L-asparaginyl)-L-threoninate ***19e***

This compound was prepared from N^2-(tert-butoxycarbonyl)-N^4-neopentyl-L-asparagine **18c** (3.02 g, 10 mmol), HOBt (162 g, 12.0 mmol), EDCI (2.88 g, 15.0 mmol), compound **13b** (2.61 mg, 10.5 mmol) and diisopropylethylamine (6.61 mL) in a similar manner as described for compound **19a**. The product was obtained as a white solid (4.63 g, 87%).^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48 (d, J = 9.5 Hz, 1H), 7.39–7.28 (m, 5H), 6.21 (s, 1H), 5.93 (s, 1H), 5.74–5.59 (m, 1H), 5.26–5.02 (m, 4H), 4.59 (dd, J = 9.0, 2.5 Hz, 1H), 4.57–4.50 (m, 1H), 4.13–4.02 (m, 1H), 3.98–3.89 (m, 1H), 3.81–3.70 (m, 1H), 3.01 (d, J = 6.0 Hz, 2H), 2.87 (d, J = 13.5 Hz, 1H), 2.60 (dd, J = 15.0, 6.0 Hz, 1H), 1.44 (s, 9H), 1.13 (d, J = 6.3 Hz, 3H), 0.88 (s, 9H); ESI-MS: m/z = 534 $[M+H]^+$.

4.1.19. Benzyl O-allyl-N-(O-methyl-N-(2-(pent-4-en-1-yloxy) benzoyl)-L-seryl)-L-serinate ***21a***

This compound was prepared from **10a** (206 mg, 1.0 mmol), HOBt (162 mg, 1.2 mmol), EDCI (288 mg, 1.5 mmol), **20a** (390 mg, 1.05 mmol) and diisopropylethylamine (0.66 mL) in a similar manner as described for compound **19a**. The product was obtained as a white solid (445 mg, 85%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.85 (d, J = 6.5 Hz, 1H), 8.21 (d, J = 8.0 Hz, 1H), 7.48–7.42 (m, 1H), 7.40 (d, J = 8.0 Hz, 1H), 7.36–7.29 (m, 5H), 7.09–7.03 (m, 1H), 6.96 (d, J = 8.0 Hz, 1H), 5.89–5.79 (m, 1H), 5.79–5.69 (m, 1H), 5.27–4.98 (m, 6H), 4.88–4.82 (m, 1H), 4.81–4.77 (m, 1H), 4.16–4.09 (m, 2H), 3.97–3.87 (m, 4H), 3.70–3.65 (m, 1H), 3.55–3.49 (m, 1H), 3.38 (s, 3H), 2.30–2.22 (m, 2H), 2.11–1.97 (m, 2H); ESI-MS: m/z = 525 $[M+H]^+$.

4.1.20. Benzyl O-allyl-N-((S)-2-(2-(pent-4-en-1-yloxy)benzamido)-4-phenylbutanoyl)-L-serinate ***21b***

This compound was prepared from **10a** (206 mg, 1.0 mmol), HOBt (162 mg, 1.2 mmol), EDCI (288 mg, 1.5 mmol), **20b** (454 mg, 1.05 mmol) and diisopropylethylamine (0.66 mL) in a similar manner as described for compound **19a**. The product was obtained as a white solid (461 mg, 79%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.63 (d, J = 7.5 Hz, 1H), 8.21 (dd, J = 8.0, 2.0 Hz, 1H), 7.47–7.41 (m, 1H), 7.35–7.30 (m, 5H), 7.28–7.23 (m, 2H), 7.21–7.15 (m, 3H), 7.09–7.04 (m, 1H), 6.97 (d, J = 8.0 Hz, 1H), 6.85 (d, J = 8.5 Hz, 1H), 5.88–5.78

(m, 1H), 5.79–5.67 (m, 1H), 5.27 (d, $J = 12.0$ Hz, 1H), 5.21–5.13 (m, 2H), 5.13–5.09 (m, 1H), 5.09–5.04 (m, 1H), 5.03–4.99 (m, 1H), 4.83–4.72 (m, 2H), 4.18–4.10 (m, 2H), 3.96–3.85 (m, 3H), 3.62 (dd, $J = 9.5$, 3.0 Hz, 1H), 2.80–2.74 (m, 2H), 2.34–2.25 (m, 3H), 2.13–2.01 (m, 3H); ESI-MS: $m/z = 607$ $[M+Na]^+$.

4.1.21. Benzyl O-allyl-N-(N^4-neopentyl-N^2-(2-(pent-4-en-1-yloxy) benzoyl)-L-asparaginyl)-L-serinate **21c**

This compound was prepared from **10a** (206 mg, 1.0 mmol), HOBt (162 mg, 1.2 mmol), EDCI (288 mg, 1.5 mmol), **20c** (478 mg, 1.05 mmol) and diisopropylethylamine (0.66 mL) in a similar manner as described for compound **19a**. The product was obtained as a white solid (461 mg, 76%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.21 (d, $J = 6.5$ Hz, 1H), 8.16 (dd, $J = 8.0$, 1.5 Hz, 1H), 8.10–8.01 (m, 1H), 7.46–7.41 (m, 1H), 7.38–7.28 (m, 5H), 7.06–7.02 (m, 1H), 6.96 (d, $J = 8.5$ Hz, 1H), 6.11–6.02 (m, 1H), 5.90–5.80 (m, 1H), 5.79–5.69 (m, 1H), 5.24 (d, $J = 12.5$ Hz, 1H), 5.21–5.15 (m, 1H), 5.13 (d, $J = 12.5$ Hz, 1H), 5.10–4.96 (m, 4H), 4.78–4.70 (m, 1H), 4.15 (t, $J = 6.5$ Hz, 2H), 3.97–3.85 (m, 3H), 3.64 (dd, $J = 9.5$, 3.5 Hz, 1H), 3.05 (d, $J = 6.5$ Hz, 2H), 2.92 (dd, $J = 15.0$, 3.5 Hz, 1H), 2.67 (dd, $J = 15.0$, 7.0 Hz, 1H), 2.32–2.17 (m, 2H), 2.16–1.98 (m, 2H), 0.89 (s, 9H); ESI-MS: $m/z = 630$ $[M+Na]^+$.

4.1.22. Benzyl O-allyl-N-(N^2-(1-methyl-5-(pent-4-en-1-yloxy)-1H-pyrazole-4-carbonyl)-N^4-neopentyl-L-asparaginyl)-L-serinate **21d**

This compound was prepared from **10b** (210 mg, 1.0 mmol), HOBt (162 mg, 1.2 mmol), EDCI (288 mg, 1.5 mmol), **20c** (478 mg, 1.05 mmol) and diisopropylethylamine (0.66 mL) in a similar manner as described for compound **19a**. The product was obtained as a white solid (477 mg, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99–7.85 (m, 2H), 7.74 (s, 1H), 7.39–7.29 (m, 5H), 6.03 (s, 1H), 5.88–5.70 (m, 2H), 5.27–4.99 (m, 6H), 4.92 (s, 1H), 4.75–4.67 (m, 1H), 4.34–4.20 (m, 2H), 3.97–3.85 (m, 3H), 3.70 (s, 3H), 3.63 (dd, $J = 9.5$, 3.0 Hz, 1H), 3.05 (d, $J = 6.5$ Hz, 2H), 2.89 (dd, $J = 15.0$,3.0 Hz, 1H), 2.61 (dd, $J = 15.0$, 6.5 Hz, 1H), 2.24 (dd, $J = 14.0$, 7.5 Hz, 2H), 2.02–1.90 (m, 2H), 0.89 (s, 9H); ESI-MS: $m/z = 612$ $[M+H]^+$.

4.1.23. Benzyl N-(N^2-(5-allyl-1-methyl-1H-pyrazole-4-carbonyl)-N^4-neopentyl-L-asparaginyl)–O-(hex-5-en-1-yl)-L-serinate **21e**

This compound was prepared from **10c** (166 mg, 1.0 mmol), HOBt (162 mg, 1.2 mmol), EDCI (288 mg, 1.5 mmol), **20d** (522 mg, 1.05 mmol) and diisopropylethylamine (0.66 mL) in a similar manner as described for compound **19a**. The product was obtained as a white solid (438 mg, 72%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 (d, $J = 8.0$ Hz, 1H), 7.73 (d, $J = 5.5$ Hz, 1H), 7.68 (s, 1H), 7.27–7.23 (m, 5H), 6.12 (s, 1H), 5.85–5.77 (m, 1H), 5.75–5.61 (m, 1H), 5.15 (d, $J = 12.0$ Hz, 1H), 5.06–5.02 (m, 2H), 4.94–4.85 (m, 4H), 4.62–4.60 (m, 1H), 3.79 (dd, $J = 9.5$, 3.5 Hz, 1H), 3.77–3.64 (m, 5H), 3.53 (dd, $J = 9.5$, 3.5 Hz, 1H), 3.36–3.22 (m, 2H), 2.97 (d, $J = 6.5$ Hz, 2H), 2.82 (dd, $J = 15.0$, 3.5 Hz, 1H), 2.53 (dd, $J = 15.0$, 6.5 Hz, 1H), 1.95–1.89 (m, 2H), 1.46–1.35 (m, 2H), 1.27–1.23 (m, 2H), 0.82 (s, 9H); ESI-MS: $m/z = 610$ $[M+H]^+$.

4.1.24. Benzyl O-allyl-N-(N^2-(1-(hex-5-en-1-yl)-1H-imidazole-2-carbonyl)-N^4-neopentyl-L-asparaginyl)-L-serinate **21f**

This compound was prepared from **10e** (194 mg, 1.0 mmol), HOBt (162 mg, 1.2 mmol), EDCI (288 mg, 1.5 mmol), **20c** (478 mg, 1.05 mmol) and diisopropylethylamine (0.66 mL) in a similar manner as described for compound **19a**. The product was obtained as a white solid (410 mg, 69%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.65 (d, $J = 7.5$ Hz, 1H), 7.57 (d, $J = 8.0$ Hz, 1H), 7.26 (m, 5H),6.97 (d, $J = 1.0$ Hz, 1H), 6.92 (d, $J = 1.0$ Hz, 1H), 5.91 (s, 1H), 5.76–5.61 (m, 2H), 5.30–7.21 (d, $J = 12.0$ Hz, 1H), 5.12–5.06 (m, 1H), 5.05 (d, $J = 12.0$ Hz, 1H), 5.03–4.99 (m, 1H), 4.98–4.93 (m, 2H), 4.68–4.62 (m, 1H), 4.43–4.30 (m, 2H),4.09–4.00 (m, 1H), 3.86–3.78 (m, 3H), 3.57–3.52 (m, 1H), 3.05–3.00 (m, 2H), 2.80 (dd, $J = 15.0$, 4.5 Hz, 1H), 2.62 (dd, $J = 15.0$, 6.5 Hz, 1H), 2.06–1.95 (m, 2H), 1.78–1.68 (m, 2H), 1.21–1.17 (m, 2H), 0.80 (s, 9H); ESI-MS: $m/z = 596$ $[M+H]^+$.

4.1.25. Benzyl O-allyl-N-(N^2-(1-(hex-5-en-1-yl)-1H-imidazole-2-carbonyl)-N^4-neopentyl-L-asparaginyl)-L-threoninate **21g**

This compound was prepared from **10e** (194 mg, 1.0 mmol), HOBt (162 mg, 1.2 mmol), EDCI (288 mg, 1.5 mmol), **20e** (492 mg, 1.05 mmol) and diisopropylethylamine (0.66 mL) in a similar manner as described for compound **19a**. The product was obtained as a white solid (396 mg, 65%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.78 (d, $J = 8.0$ Hz, 1H), 7.55 (d, $J = 9.0$ Hz, 1H), 7.39–7.31 (m, 5H), 7.06 (d, $J = 1.0$ Hz, 1H), 7.01 (d, $J = 1.0$ Hz, 1H), 5.99 (s, 1H), 5.83–5.73 (m, 1H), 5.71–5.64 (m, 1H), 5.23 (d, $J = 12.5$ Hz, 1H), 5.17–5.10 (m, 2H), 5.06–4.94 (m, 4H), 4.64 (dd, $J = 9.0$, 2.5 Hz, 1H), 4.51–4.39 (m, 2H), 4.09 (qd, $J = 6.5$, 2.5 Hz, 1H), 3.95–3.91 (m, 1H), 3.76 (ddt, $J = 13.0$, 5.5, 1.5 Hz, 1H), 3.12–3.02 (m, 2H), 2.91 (dd, $J = 15.0$, 4.5 Hz, 1H), 2.72 (dd, $J = 15.0$, 6.5 Hz, 1H), 2.12–2.06 (m, 2H), 1.87–1.77 (m, 2H), 1.47–1.38 (m, 2H), 1.14 (d, $J = 6.5$ Hz, 3H), 0.89 (s, 9H); ESI-MS: $m/z = 610$ $[M+H]^+$.

4.1.26. (10S,13S)-13-(Methoxymethyl)-12,15-dioxo-2,3,4,5,6,7,10,11,12,13,14,15-dodecahydro-9H-benzo[i][1,11]dioxa[4,7]diazacycloheptadecine-10-carboxylic acid **22a**

A solution of **21a** (105 mg, 0.20 mmol) in toluene (200 mL) was degassed with dry nitrogen for 15 min. The mixture was stirred for 5 min at 100 °C. after which, a degassed solution of grubbs second-generation catalyst (17 mg, 0.02 mmol) in toluene (20 mL) was injected with a syringe for 30 min. The reaction was stirred for half an additional hour. After cooling to room temperature, the solvent was evaporated under reduced pressure, and purified by flash chromatography on silica gel. The product (a mixture of geometric isomers) was redissolved in methanol (5 mL), stirred 3 h with 10% Pd/C (10 mol %) under H_2 atmosphere at room temperature. The mixture was then filtered over Celite, concentrated to provide **22a** as white solid (62 mg, 76%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.00 (d, $J = 8.0$ Hz, 1H), 8.18 (d, $J = 7.0$ Hz, 1H), 7.47–7.38 (m, 1H), 7.23 (d, $J = 6.5$ Hz, 1H), 7.06–6.99 (m, 1H), 6.94 (d, $J = 8.5$ Hz, 1H), 4.92–4.82 (m, 1H), 4.65–4.55 (m, 1H), 4.23–4.16 (m, 1H), 4.14–4.05 (m, 2H), 3.80–3.67 (m, 2H), 3.56–3.47 (m, 2H), 3.45–3.40 (m, 1H), 3.36 (s, 3H), 1.98–1.88 (m, 1H), 1.82–1.69 (m, 2H), 1.63–1.36 (m, 6H); ESI-MS: $m/z = 407$ $[M-H]^-$.

4.1.27. (10S,13S)-12,15-Dioxo-13-phenethyl-2,3,4,5,6,7,10,11,12,13,14,15-dodecahydro-9H-benzo[i][1,11]dioxa[4,7]diazacycloheptadecine-10-carboxylic acid **22b**

This compound was prepared from **21b** (117 mg, 0.2 mmol) in a similar manner as described for compound **21a**. The product was obtained as a white solid (64 mg, 69%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.04 (brs, 1H), 8.46 (d, $J = 8.0$ Hz, 1H), 8.12 (d, $J = 8.5$ Hz, 1H), 7.90 (d, $J = 8.0$ Hz, 1H), 7.54–7.48 (m, 1H), 7.26 (t, $J = 7.5$ Hz, 2H), 7.21–7.13 (m, 4H), 7.07 (t, $J = 7.5$ Hz, 1H), 4.68–4.58 (m, 2H), 4.25–4.17 (m, 1H), 4.16–4.05 (m, 1H), 3.74–3.60 (m, 2H), 3.47–3.36 (m, 2H), 2.71–2.55 (m, 2H), 2.17–2.05 (m, 1H), 1.96 (m, 1H), 1.87–1.68 (m, 2H), 1.69–1.50 (m, 2H), 1.50–1.30 (m, 4H); ESI-MS: $m/z = 467$ $[M-H]^-$.

4.1.28. (10S,13S)-13-(2-(Neopentylamino)-2-oxoethyl)-12,15-dioxo-2,3,4,5,6,7,10,11,12,13,14,15-dodecahydro-9H-benzo[i][1,11]dioxa[4,7]diazacycloheptadecine-10-carboxylic acid **22c**

This compound was prepared from **21c** (121 mg, 0.2 mmol) in a similar manner as described for compound **21a**. The product was obtained as a white solid (71 mg, 73%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.93 (brs, 1H), 9.15 (d, $J = 8.5$ Hz, 1H), 8.03 (dd, $J = 8.0$, 2.0 Hz, 1H), 7.91 (t, $J = 6.0$ Hz, 1H), 7.56–7.44 (m, 2H), 7.16 (d,

J = 8.5 Hz, 1H), 7.06 (t, J = 7.5 Hz, 1H), 4.99–4.90 (m, 1H), 4.52–4.42 (m, 1H), 4.22–4.07 (m, 2H), 3.68 (dd, J = 10.0, 6.0 Hz, 1H), 3.57 (dd, J = 10.0, 3.0 Hz, 1H), 3.46–3.32 (m, 2H), 2.94 (dd, J = 13.0, 7.0 Hz, 1H), 2.87 (dd, J = 15.5, 5.5 Hz, 1H), 2.79 (dd, J = 13.0, 6.0 Hz, 1H), 2.54 (dd, J = 15.5, 4.5 Hz, 1H), 2.22–2.09 (m, 1H), 1.88–1.76 (m, 1H), 1.72–1.54 (m, 2H), 1.53–1.39 (m, 3H), 1.38–1.28 (m, 1H), 0.78 (s, 9H); ESI-MS: m/z = 490 $[M\text{-}H]^-$.

4.1.29. (6S,9S)-1-Methyl-6-(2-(neopentylamino)-2-oxoethyl)-4,7-dioxo-1,4,5,6,7,8,9,10,12,13,14,15,16,17-tetradecahydropyrazolo[4,3-i][1,11]dioxa[4,7]diazacycloheptadecine-9-carboxylic acid ***22d***

This compound was prepared from **21d** (112 mg, 0.2 mmol) in a similar manner as described for compound **21a**. The product was obtained as a white solid (60 mg, 61%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.87 (brs, 1H), 8.21 (d, J = 7.5 Hz, 1H), 7.91 (d, J = 8.0 Hz, 1H), 7.75 (t, J = 6.5 Hz, 1H), 7.69 (s, 1H), 4.73 (td, J = 8.5, 5.0 Hz, 1H), 4.56–4.51 (m, 1H), 4.44–4.37 (m, 1H), 4.27–4.18 (m, 1H), 3.71–3.55 (m, 5H), 3.47–3.37 (m, 2H), 2.96 (dd, J = 13.0, 6.5 Hz, 1H), 2.79 (dd, J = 13.0, 5.5 Hz, 1H), 2.65 (dd, J = 15.0, 9.0 Hz, 1H), 2.57 (dd, J = 15.0, 5.0 Hz, 1H), 1.72 (m, 2H), 1.54–1.27 (m, 6H), 0.81 (s, 9H); ESI-MS: m/z = 494 $[M\text{-}H]^-$.

4.1.30. (6S,9S)-1-Methyl-6-(2-(neopentylamino)-2-oxoethyl)-4,7-dioxo-4,5,6,7,8,9,10,12,13,14,15,16,17,18-tetradecahydro-1H-pyrazolo[4,3-i][1]oxa[4,7]diazacycloheptadecine-9-carboxylic acid ***22e***

This compound was prepared from **21e** (121 mg, 0.2 mmol) in a similar manner as described for compound **21a**. The product was obtained as a white solid (67 mg, 68%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.70 (s, 1H), 8.15 (d, J = 8.0 Hz, 1H), 7.91 (d, J = 8.0 Hz, 1H), 7.81 (s, 1H), 7.67 (t, J = 6.0 Hz, 1H), 4.80–4.72 (m, 1H), 4.43–4.35 (m, 1H), 3.74 (s, 3H), 3.66 (dd, J = 10.0, 5.0 Hz, 1H), 3.59 (dd, J = 10.0, 3.5 Hz, 1H), 3.42–3.34 (m, 1H), 3.33–3.29 (m, 1H), 3.17 (d, J = 3.5 Hz, 2H), 2.95 (dd, J = 13.0, 6.5 Hz, 1H), 2.79 (dd, J = 13.0, 5.5 Hz, 1H), 2.61 (d, J = 7.5 Hz, 2H), 1.65–1.54 (m, 1H), 1.52–1.43 (m, 1H), 1.43–1.20 (m, 8H), 0.80 (s, 9H); ESI-MS: m/z = 492 $[M\text{-}H]^-$.

4.1.31. (14S,17S)-17-(2-(Neopentylamino)-2-oxoethyl)-16,19-dioxo-6,7,8,9,10,11,14,15,16,17,18,19-dodecahydro-5H,13H-imidazo[2,1-i][1]oxa[4,7,10]triazacycloheptadecine-14-carboxylic acid ***22f***

This compound was prepared from **21f** (119 mg, 0.2 mmol) in a similar manner as described for compound **21a**. The product was obtained as a white solid (52 mg, 55%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.68 (d, J = 8.0 Hz, 1H), 7.85 (t, J = 6.5 Hz, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.39 (d, J = 1.0 Hz, 1H), 6.99 (d, J = 1.0 Hz, 1H), 4.82–4.72 (m, 2H), 4.39–4.32 (m, 1H), 4.07–3.98 (m, 1H), 3.66 (dd, J = 10.0, 5.0 Hz, 1H), 3.61 (dd, J = 10.0, 3.5 Hz, 1H), 3.36–3.28 (m, 2H), 2.91 (dd, J = 13.0, 6.5 Hz, 1H), 2.84 (dd, J = 13.0, 6.0 Hz, 1H), 2.75 (dd, J = 15.0, 8.5 Hz, 1H), 2.58 (dd, J = 15.0, 4.5 Hz, 1H), 1.81–1.70 (m, 1H), 1.70–1.61 (m, 1H), 1.36–1.21 (m, 6H), 1.20–1.06 (m, 2H), 0.81 (s, 9H); ESI-MS: m/z = 478 $[M\text{-}H]^-$.

4.1.32. (13R,14S,17S)-13-Methyl-17-(2-(neopentylamino)-2-oxoethyl)-16,19-dioxo-6,7,8,9,10,11,14,15,16,17,18,19-dodecahydro-5H,13H-imidazo[2,1-i][1]oxa[4,7,10]triazacycloheptadecine-14-carboxylic acid ***22g***

This compound was prepared from **21g** (121 mg, 0.2 mmol) in a similar manner as described for compound **21a**. The product was obtained as a white solid (66 mg, 67%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.08 (d, J = 8.0 Hz, 1H), 7.89 (t, J = 6.0 Hz, 1H), 7.42 (s, 1H), 7.20 (d, J = 9.0 Hz, 1H), 7.00 (s, 1H), 4.87–4.71 (m, 2H), 4.14 (dd, J = 9.0, 1.5 Hz, 1H), 4.08–3.97 (m, 1H), 3.98–3.87 (m, 1H), 3.43–3.33 (m, 1H), 3.19–3.10 (m, 1H), 2.91 (dd, J = 13.0, 6.5 Hz, 1H), 2.88–2.75 (m, 2H), 2.60 (dd, J = 15.5, 4.5 Hz, 1H), 1.86–1.73 (m, 1H), 1.73–1.57 (m, 1H), 1.34–1.06 (m, 8H), 1.00 (d, J = 6.5 Hz, 3H), 0.79 (s, 9H); ESI-MS: m/z = 492 $[M\text{-}H]^-$.

4.1.33. (10S,13S)-N-(2-Chlorobenzyl)-13-(methoxymethyl)-12,15-dioxo-2,3,4,5,6,7,10,11,12,13,14,15-dodecahydro-9H-benzo[i][1,11]dioxa[4,7]diazacycloheptadecine-10-carboxamide ***23a***

This compound was prepared from **22a** (40.8 mg, 0.1 mmol), HOBt (16.2 mg, 0.12 mmol), EDCI (28.8 mg, 0.15 mmol), (2-chlorophenyl)methanamine (14.8 mg, 0.105 mmol) and diisopropylethylamine (66 μL) in a similar manner as described for compound **19a**. The product was obtained as a white solid (43.5 mg, 82%). Mp: 141.8–147.7 °C. HPLC purity = 100.00%, HPLC t_R = 9.76 min; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.82 (d, J = 6.0 Hz, 1H), 7.95 (dd, J = 7.5, 2.0 Hz, 1H), 7.49–7.41 (m, 1H), 7.30–6.93 (m, 8H), 4.77–4.70 (m, 1H), 4.63–4.58 (m, 1H), 4.50 (d, J = 6.0 Hz, 2H), 4.34–4.26 (m, 3H), 4.01 (dd, J = 9.0, 2.0 Hz, 1H), 3.73–3.67 (m, 1H), 3.64 (dd, J = 9.5, 3.5 Hz, 1H), 3.42 (s, 3H), 3.38 (dd, J = 8.5, 3.5 Hz, 1H), 3.28–3.19 (m, 1H), 1.93–1.43 (m, 8H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.65, 169.60, 166.70, 157.25, 135.50, 133.57, 133.17, 132.43, 129.17, 128.89, 128.19, 126.79, 121.14, 120.09, 111.79, 71.31, 70.68, 68.94, 68.70, 59.18, 55.59, 52.66, 41.30, 29.07, 28.40, 27.04, 26.88; ESI-MS: m/z = 554 $[M+Na]^+$.

4.1.34. (10S,13S)-N-(2-Chlorobenzyl)-12,15-dioxo-13-phenethyl-2,3,4,5,6,7,10,11,12,13,14,15-dodecahydro-9H-benzo[i][1,11]dioxa[4,7]diazacycloheptadecine-10-carboxamide ***23b***

This compound was prepared from **22b** (46.8 mg, 0.1 mmol), HOBt (16.2 mg, 0.12 mmol), EDCI (28.8 mg, 0.15 mmol), (2-chlorophenyl)methanamine (14.8 mg, 0.105 mmol) and diisopropylethylamine (66 μL) in a similar manner as described for compound **19a**. The product was obtained as a white solid (47 mg, 79%). Mp: 154.3–158.4 °C. HPLC purity = 96.33%, HPLC t_R = 11.79 min; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (d, J = 5.0 Hz, 1H), 7.97 (dd, J = 7.5, 1.5 Hz, 1H), 7.51–7.44 (m, 1H), 7.32 (t, J = 6.0 Hz, 1H), 7.25–7.22 (m, 4H), 7.20–7.14 (m, 3H), 7.14–7.06 (m, 2H), 7.03 (t, J = 7.5 Hz, 1H), 6.96 (t, J = 7.5 Hz, 2H), 4.72–4.67 (m, 1H), 4.55–4.47 (m, 3H), 4.32–4.25 (m, 1H), 4.21–4.13 (m, 1H), 3.95 (dd, J = 9.5, 4.5 Hz, 1H), 3.60 (dd, J = 9.5, 4.0 Hz, 1H), 3.54–3.47 (m, 1H), 3.43–3.36 (m, 1H), 2.86–2.73 (m, 2H), 2.44–2.34 (m, 1H), 2.11–2.00 (m, 1H), 1.97–1.78 (m, 2H), 1.68–1.42 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.59, 169.60, 166.94, 156.91, 140.39, 135.51, 133.60, 133.17, 132.44, 129.18, 128.94, 128.64, 128.37, 128.21, 126.80, 126.37, 121.32, 120.15, 112.01, 70.95, 69.09, 69.03, 55.62, 52.49, 41.33, 33.62, 32.69, 29.30, 28.62, 27.49, 27.28; ESI-MS: m/z = 614 $[M+Na]^+$.

4.1.35. (10S,13S)-N-(4-Methylbenzyl)-12,15-dioxo-13-phenethyl-2,3,4,5,6,7,10,11,12,13,14,15-dodecahydro-9H-benzo[i][1,11]dioxa[4,7]diazacycloheptadecine-10-carboxamide ***23c***

This compound was prepared from **22b** (46.8 mg, 0.1 mmol), HOBt (16.2 mg, 0.12 mmol), EDCI (28.8 mg, 0.15 mmol), *p*-tolylmethanamine (12.7 mg, 0.105 mmol) and diisopropylethylamine (66 μL) in a similar manner as described for compound **19a**. The product was obtained as a white solid (48 mg, 85%). Mp: 208.1–208.7 °C. HPLC purity = 99.73%, HPLC t_R = 11.80 min; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (d, J = 5.0 Hz, 1H), 7.96 (d, J = 7.5 Hz, 1H), 7.48 (t, J = 7.5 Hz, 1H), 7.24 (d, J = 7.5 Hz, 2H), 7.21–7.14 (m, 4H), 7.10 (d, J = 7.5 Hz, 2H), 7.06–6.94 (m, 4H), 6.90 (d, J = 8.5 Hz, 1H), 4.71–4.65 (m, 1H), 4.54–4.48 (m, 1H), 4.41 (dd, J = 15.0, 6.0 Hz, 1H), 4.35–4.25 (m, 2H), 4.19–4.10 (m, 1H), 3.96 (dd, J = 9.5, 4.5 Hz, 1H), 3.58 (dd, J = 9.5, 4.0 Hz, 1H), 3.54–3.47 (m, 1H), 3.42–3.35 (m, 1H), 2.85–2.71 (m, 2H), 2.43–2.32 (m, 1H), 2.28 (s, 3H), 2.09–1.98 (m, 1H), 1.95–1.77 (m, 2H), 1.68–1.41 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.47, 169.25, 166.84, 156.94, 140.42, 136.41, 135.17, 133.52, 132.47, 129.03, 128.63, 128.37, 127.51, 126.34, 121.27, 120.12, 111.98, 70.95, 69.19, 69.06, 55.52, 52.36, 43.23, 33.61, 32.68, 29.31, 28.68, 27.48, 27.32, 21.12; ESI-MS: m/z = 594 $[M+Na]^+$.

4.1.36. (10S,13S)-N-Benzyl-13-(2-(neopentylamino)-2-oxoethyl)-12,15-dioxo-2,3,4,5,6,7,10,11,12,13,14,15-dodecahydro-9H-benzo[i][1,11]dioxa[4,7]diazacycloheptadecine-10-carboxamide ***23d***

This compound was prepared from **22c** (49.1 mg, 0.1 mmol), HOBt (16.2 mg, 0.12 mmol), EDCI (28.8 mg, 0.15 mmol), phenylmethanamine (10.7 mg, 0.105 mmol) and diisopropylethylamine (66 μL) in a similar manner as described for compound **19a**. The product was obtained as a white solid (42 mg, 73%). Mp: 171.9–173.0 °C. HPLC purity = 99.80%, HPLC t_R = 10.14 min; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.50 (d, *J* = 6.5 Hz, 1H), 7.98 (dd, *J* = 8.0, 2.0 Hz, 1H), 7.49–7.39 (m, 1H), 7.31 (t, *J* = 6.0 Hz, 1H), 7.24–7.14 (m, 5H), 7.09 (d, *J* = 8.5 Hz, 1H), 7.02–6.94 (m, 2H), 5.94 (s, 1H), 4.87–4.82 (m, 1H), 4.69–4.62 (m, 1H), 4.47 (dd, *J* = 15.0, 6.5 Hz, 1H), 4.43–4.35 (m, 2H), 4.27–4.21 (m, 1H), 3.89 (dd, *J* = 9.5, 4.5 Hz, 1H), 3.59 (dd, *J* = 9.5, 4.0 Hz, 1H), 3.54–3.46 (m, 1H), 3.43–3.35 (m, 1H), 3.08–2.93 (m, 3H), 2.65 (dd, *J* = 15.0, 5.0 Hz, 1H), 2.15–2.05 (m, 1H), 1.90–1.80 (m, 1H), 1.67–1.56 (m, 2H), 1.51–1.39 (m, 4H), 0.88 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.33, 170.09, 169.38, 167.07, 157.16, 138.26, 133.35, 132.30, 128.33, 127.43, 126.93, 120.68, 120.40, 112.03, 71.06, 69.01, 68.96, 53.03, 52.79, 50.70, 43.39, 36.88, 31.86, 28.66, 28.40, 27.37, 27.24, 27.20; ESI-MS: *m*/*z* = 603 $[M+Na]^+$

4.1.37. (10S,13S)-N-(2-Chlorobenzyl)-13-(2-(neopentylamino)-2-oxoethyl)-12,15-dioxo-2,3,4,5,6,7,10,11,12,13,14,15-dodecahydro-9H-benzo[i][1,11]dioxa[4,7]diazacycloheptadecine-10-carboxamide ***23e***

This compound was prepared from **22c** (49.1 mg, 0.1 mmol), HOBt (16.2 mg, 0.12 mmol), EDCI (28.8 mg, 0.15 mmol), (2-chlorophenyl)methanamine (14.8 mg, 0.105 mmol) and diisopropylethylamine (66 μL) in a similar manner as described for compound **19a**. The product was obtained as a white solid (44 mg, 71%). Mp: 195.7–198.1 °C. HPLC purity = 99.52%, HPLC t_R = 10.63 min; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.53 (d, *J* = 6.0 Hz, 1H), 7.96 (dd, *J* = 7.0, 1.5 Hz, 1H), 7.47–7.36 (m, 2H), 7.26–7.21 (m, 2H), 7.16 (d, *J* = 8.0 Hz, 1H), 7.13–7.04 (m, 2H), 7.00–6.94 (m, 2H), 6.23–6.08 (m, 1H), 4.89–4.81 (m, 1H), 4.69–4.61 (m, 1H), 4.57–4.44 (m, 2H), 4.44–4.38 (m, 1H), 4.29–4.22 (m, 1H), 3.87 (dd, *J* = 9.5, 4.5 Hz, 1H), 3.59 (dd, *J* = 9.5, 3.5 Hz, 1H), 3.53–3.45 (m, 1H), 3.44–3.34 (m, 1H), 3.05 (dd, *J* = 13.0, 6.5 Hz, 1H), 3.02–2.94 (m, 2H), 2.66 (dd, *J* = 14.5, 5.0 Hz, 1H), 2.17–2.05 (m, 1H), 1.91–1.79 (m 1H), 1.69–1.57 (m, 2H), 1.53–1.40 (m, 4H), 0.87 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.46, 170.11, 169.64, 167.11, 157.14, 135.52, 133.36, 133.15, 132.30, 129.18, 128.84, 128.19, 126.74, 120.66, 120.41, 112.02, 71.07, 68.98, 68.90, 53.13, 52.81, 50.69, 41.31, 36.82, 31.87, 28.63, 28.40, 27.39, 27.24, 27.18; ESI-MS: *m*/*z* = 637 $[M+Na]^+$.

4.1.38. (10S,13S)-N-(4-Methylbenzyl)-13-(2-(neopentylamino)-2-oxoethyl)-12,15-dioxo-2,3,4,5,6,7,10,11,12,13,14,15-dodecahydro-9H-benzo[i][1,11]dioxa[4,7]diazacycloheptadecine-10-carboxamide ***23f***

This compound was prepared from **22c** (49.1 mg, 0.1 mmol), HOBt (16.2 mg, 0.12 mmol), EDCI (28.8 mg, 0.15 mmol), *p*-tolylmethanamine (12.7 mg, 0.105 mmol) and diisopropylethylamine (66 μL) in a similar manner as described for compound **19a**. The product was obtained as a white solid (47 mg, 79%). Mp: 183.6–186.1 °C. HPLC purity = 97.15%, HPLC t_R = 10.65 min; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.52 (d, *J* = 6.0 Hz, 1H), 7.94 (d, *J* = 7.5 Hz, 1H), 7.43 (t, *J* = 7.5 Hz, 1H), 7.31 (t, *J* = 5.5 Hz, 1H), 7.13 (d, *J* = 8.5 Hz, 1H), 7.08 (d, *J* = 8.0 Hz, 2H), 7.01–6.90 (m, 4H), 6.44–6.32 (m, 1H), 4.86–4.74 (m, 1H), 4.65–4.57 (m, 1H), 4.46–4.36 (m, 2H), 4.29 (dd, *J* = 15.0, 5.0 Hz, 1H), 4.25–4.17 (m, 1H), 3.88 (dd, *J* = 9.0, 4.0 Hz, 1H), 3.56 (dd, *J* = 9.0, 3.5 Hz, 1H), 3.53–3.46 (m, 1H), 3.42–3.34 (m, 1H), 3.06–2.91 (m, 3H), 2.65 (dd, *J* = 15.0, 5.0 Hz, 1H), 2.26 (s, 3H), 2.17–2.06 (m, 1H), 1.88–1.78 (m, 1H), 1.67–1.51 (m, 2H), 1.53–1.39 (m, 4H), 0.86 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.40, 170.15, 169.29, 167.03, 157.19, 136.37, 135.23, 133.27, 132.28, 129.00, 127.43, 120.59, 120.42, 111.99, 71.09, 69.06, 69.03, 53.03, 52.85, 50.66, 43.15, 36.77, 31.89, 28.70, 28.43, 27.38, 27.25, 27.21, 21.08; ESI-MS: *m*/*z* = 617 $[M+Na]^+$.

4.1.39. (6S,9S)-1-Methyl-N-(4-methylbenzyl)-6-(2-(neopentylamino)-2-oxoethyl)-4,7-dioxo-1,4,5,6,7,8,9,10,12,13,14,15,16,17-tetradecahydropyrazolo[4,3-i][1,11]dioxa[4,7]diazacycloheptadecine-9-carboxamide ***23g***

This compound was prepared from **22d** (49.5 mg, 0.1 mmol), HOBt (16.2 mg, 0.12 mmol), EDCI (28.8 mg, 0.15 mmol), *p*-tolylmethanamine (12.7 mg, 0.105 mmol) and diisopropylethylamine (66 μL) in a similar manner as described for compound **19a**. The product was obtained as a white solid (46 mg, 77%). Mp: 211.6–213.6 °C. HPLC purity = 97.17%, HPLC t_R = 9.45 min; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.16 (d, *J* = 5.5 Hz, 1H), 7.73 (s, 1H), 7.69 (t, *J* = 6.0 Hz, 1H), 7.40 (d, *J* = 8.0 Hz, 1H), 7.16 (d, *J* = 8.0 Hz, 2H), 7.05 (d, *J* = 8.0 Hz, 2H), 6.93 (t, *J* = 6.0 Hz, 1H), 4.72–4.62 (m, 1H), 4.54 (dd, *J* = 14.5, 6.5 Hz, 1H), 4.50–4.41 (m, 2H), 4.26 (dd, *J* = 14.5, 5.0 Hz, 1H), 4.03–3.94 (m, 1H), 3.91 (dd, *J* = 9.0, 2.0 Hz, 1H), 3.63 (s, 3H), 3.57 (dd, *J* = 9.0, 3.0 Hz, 1H), 3.47–3.33 (m, 2H), 3.01 (dd, *J* = 13.5, 6.5 Hz, 1H), 2.88 (dd, *J* = 13.5, 6.0 Hz, 1H), 2.82–2.70 (m, 2H), 2.28 (s, 3H), 1.82–1.67 (m, 1H), 1.63–1.50 (m, 1H), 1.50–1.35 (m, 3H), 1.34–1.19 (m, 3H), 0.83 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.22, 170.82, 169.86, 163.45, 153.88, 138.64, 136.51, 135.54, 129.04, 127.68, 101.25, 74.04, 70.98, 69.31, 54.12, 51.92, 50.56, 43.21, 35.87, 34.29, 31.94, 28.08, 27.95, 27.17, 24.43, 23.66, 21.06; ESI-MS: *m*/*z* = 599 $[M+H]^+$.

4.1.40. (6S,9S)-1-Methyl-N-(4-methylbenzyl)-6-(2-(neopentylamino)-2-oxoethyl)-4,7-dioxo-4,5,6,7,8,9,10,12,13,14,15,16,17,18-tetradecahydro-1H-pyrazolo[4,3-i][1]oxa[4,7]diazacycloheptadecine-9-carboxamide ***23h***

This compound was prepared from **22e** (49.3 mg, 0.1 mmol), HOBt (16.2 mg, 0.12 mmol), EDCI (28.8 mg, 0.15 mmol), *p*-tolylmethanamine (12.7 mg, 0.105 mmol) and diisopropylethylamine (66 μL) in a similar manner as described for compound **19a**. The product was obtained as a white solid (43 mg, 72%). Mp: 227.9–229.7 °C. HPLC purity = 97.22%, HPLC t_R = 9.78 min; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.18 (d, *J* = 5.0 Hz, 1H), 7.81 (s, 1H), 7.74 (s, 1H), 7.33 (d, *J* = 7.5 Hz, 1H), 7.18 (d, *J* = 8.0 Hz, 2H), 7.08–6.95 (m, 3H), 4.64–4.58 (m, 1H), 4.50 (dd, *J* = 14.5, 6.0 Hz, 1H), 4.40 (d, *J* = 7.5 Hz, 1H), 4.29 (dd, *J* = 14.5, 5.0 Hz, 1H), 3.94–3.88 (m, 1H), 3.75 (s, 3H), 3.51 (dd, *J* = 8.5, 3.0 Hz, 1H), 3.40–3.33 (m, 1H), 3.32–3.23 (m, 2H), 3.00 (dd, *J* = 13.0, 6.5 Hz, 1H), 2.87 (dd, *J* = 13.0, 6.0 Hz, 1H), 2.82–2.67 (m, 2H), 2.49–2.39 (m, 1H), 2.26 (s, 3H), 1.58–1.11 (m, 10H), 0.81 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.13, 170.92, 170.07, 164.72, 147.11, 138.17, 136.44, 135.52, 129.01, 127.78, 112.59, 70.97, 69.24, 54.32, 52.11, 50.58, 43.38, 36.47, 35.78, 31.97, 27.81, 27.17, 26.79, 26.45, 25.91, 24.29, 23.52, 21.08; ESI-MS: *m*/*z* = 597 $[M+H]^+$.

4.1.41. (14S,17S)-N-(4-Methylbenzyl)-17-(2-(neopentylamino)-2-oxoethyl)-16,19-dioxo-6,7,8,9,10,11,14,15,16,17,18,19-dodecahydro-5H,13H-imidazo[2,1-i][1]oxa[4,7,10]triazacycloheptadecine-14-carboxamide ***23i***

This compound was prepared from **22f** (49.3 mg, 0.1 mmol), HOBt (16.2 mg, 0.12 mmol), EDCI (28.8 mg, 0.15 mmol), *p*-tolylmethanamine (12.7 mg, 0.105 mmol) and diisopropylethylamine (66 μL) in a similar manner as described for compound **19a**. The product was obtained as a white solid (33 mg, 69%). Mp: 184.4–187.5 °C. HPLC purity = 99.27%, HPLC t_R = 9.89 min; ^{1}H NMR (500 MHz, CDCl3) δ 8.99 (d, *J* = 6.5 Hz, 1H), 7.48 (t, *J* = 5.5 Hz, 1H), 7.22 (d, *J* = 8.0 Hz, 2H), 7.17 (d, *J* = 7.5 Hz, 1H), 7.09 (d, *J* = 8.0 Hz, 2H), 7.05 (s, 1H), 6.95 (s, 1H), 5.93 (s, 1H), 4.94–4.82 (m, 1H), 4.71–4.65 (m, 1H), 4.57 (dd, *J* = 14.5, 6.5 Hz, 1H), 4.49–4.43 (m, 1H), 4.32 (dd, *J* = 14.5, 5.5 Hz, 1H), 3.95 (dd, *J* = 9.0, 2.0 Hz, 1H), 3.76–3.67 (m, 1H),

3.51 (dd, $J = 9.0$, 3.5 Hz, 1H), 3.38–3.33 (m, 1H), 3.33–3.26 (m, 1H), 3.10 (dd, $J = 13.5$, 7.0 Hz, 1H), 2.94–2.86 (m, 2H), 2.82 (dd, $J = 14.5$, 6.5 Hz, 1H), 2.31 (s, 3H), 1.71–1.60 (m, 1H), 1.35–1.11 (m, 8H), 1.06–0.94 (m, 1H), 0.86 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ13C NMR (126 MHz, CDCl3) δ 170.39, 170.15, 169.92, 160.10, 137.17, 136.49, 135.50, 129.04, 129.00, 128.23, 127.82, 125.77, 70.88, 69.20, 54.26, 51.69, 50.53, 48.81, 43.38, 35.92, 31.80, 28.60, 27.60, 27.13, 26.59, 23.59, 23.49, 21.09; ESI-MS: $m/z = 583$ $[M+H]^+$.

4.1.42. (13R,14S,17S)-13-Methyl-N-(4-methylbenzyl)-17-(2-(neopentylamino)-2-oxoethyl)-16,19-dioxo-6,7,8,9,10,11,14,15,16,17,18,19-dodecahydro-5H,13H-imidazo[2,1-i][1]oxa[4,7,10]triazacycloheptadecine-14-carboxamide **23j**

This compound was prepared from **22f** (49.3 mg, 0.1 mmol), HOBt (16.2 mg, 0.12 mmol), EDCI (28.8 mg, 0.15 mmol), *p*-tolylmethanamine (12.7 mg, 0.105 mmol) and diisopropylethylamine (66 μL) in a similar manner as described for compound **19a**. The product was obtained as a white solid (47 mg, 80%). Mp: 204.4–206.4 °C. HPLC purity = 99.37%, HPLC $t_R = 10.33$ min; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.30 (d, $J = 5.5$ Hz, 1H), 7.42 (t, $J = 5.5$ Hz, 1H), 7.19 (d, $J = 8.0$ Hz, 2H), 7.11 (d, $J = 8.5$ Hz, 1H), 7.06 (d, $J = 8.0$ Hz, 2H), 7.04 (s, 1H), 6.94 (s, 1H), 6.46 (s, 1H), 4.90–4.77 (m, 1H), 4.68–4.59 (m, 1H), 4.46–4.32 (m, 2H), 4.24 (d, $J = 8.5$ Hz, 1H), 4.19–4.09 (m, 1H), 3.81–3.66 (m, 1H), 3.54–3.41 (m, 1H), 3.11–2.98 (m, 2H), 2.98–2.86 (m, 2H), 2.80 (dd, $J = 14.5$, 5.5 Hz, 1H), 2.28 (s, 3H), 1.74–1.60 (m, 1H), 1.36–0.99 (m, 12H), 0.82 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.85, 170.32, 170.16, 160.05, 137.02, 136.62, 135.29, 129.08, 127.97, 127.92, 125.76, 73.80, 68.39, 58.78, 52.17, 50.58, 48.89, 43.47, 35.73, 31.88, 28.87, 28.02, 27.18, 26.69, 23.82, 23.63, 21.09, 16.50; ESI-MS: $m/z = 597$ $[M+H]^+$.

4.1.43. tert-Butyl (S)-(3-(1H-indol-3-yl)-1-((4-methylbenzyl)amino)-1-oxopropan-2-yl)carbamate **25**

This compound was prepared from (tert-butoxycarbonyl)-L-tryptophan **24** (3.04 g, 10.0 mmol), HOBt (1.62 g, 12.0 mmol), EDCI (2.88 g, 15.0 mmol), *p*-tolylmethanamine (1.27 g, 10.5 mmol) and diisopropylethylamine (6.61 mL) in a similar manner as described for compound **19a**. The product was obtained as a white solid (3.62 g, 89%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.81 (s, 1H), 8.35 (t, $J = 6.0$ Hz, 1H), 7.60 (d, $J = 8.0$ Hz, 1H), 7.33 (d, $J = 8.0$ Hz, 1H), 7.15–7.00 (m, 6H), 6.97 (t, $J = 7.5$ Hz, 1H), 6.81 (d, $J = 8.0$ Hz, 1H), 4.28–4.17 (m, 3H), 3.08 (dd, $J = 14.5$, 5.0 Hz, 1H), 2.92 (dd, $J = 14.5$, 9.0 Hz, 1H), 2.26 (s, 3H), 1.32 (s, 9H); ESI-MS: $m/z = 408$ $[M+H]^+$.

4.1.44. tert-Butyl (S)-(3-(1-allyl-1H-indol-3-yl)-1-((4-methylbenzyl)amino)-1-oxopropan-2-yl)carbamate **26**

To a solution of **25** (500 mg, 1.2 mmol) in anhydrous THF (10 mL) were added NaH (98 mg, 12.0 mmol) and 3-bromoprop-1-ene (295 mg, 2.5 mmol) at 0°C. After stirring at room temperature for 3 h, 10 mL water was added at 0°C. Then, the reaction mixture was concentrated and dissolved with ethyl acetate (15 mL), washed with brine (10 mL × 3). The organic layer was dried over Na_2SO_4, concentrated under vacuum and purified by SiO_2 chromatography (ethyl acetate: petroleum ether = 1:2) to afford the compound **26** as a white solid (430 mg, 79%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (d, $J = 8.0$ Hz, 1H), 7.29 (d, $J = 8.0$ Hz, 1H), 7.24–7.19 (m, 1H), 7.14–7.11 (m, 1H), 7.03 (d, $J = 7.5$ Hz, 2H), 6.93–6.85 (m, 3H), 6.00–5.84 (m, 2H), 5.23–5.09 (m, 2H), 5.03 (d, $J = 17.0$ Hz, 1H), 4.61 (dd, $J = 5.5$, 1.5 Hz, 2H), 4.49–4.38 (m, 1H), 4.25 (s, 2H), 3.33 (dd, $J = 14.5$, 5.0 Hz, 1H), 3.16 (dd, $J = 14.5$, 7.5 Hz, 1H), 2.30 (s, 3H), 1.41 (s, 9H); ESI-MS: $m/z = 448$ $[M+H]^+$.

4.1.45. tert-Butyl ((S)-1-(((S)-3-(1-allyl-1H-indol-3-yl)-1-((4-methylbenzyl)amino)-1-oxopropan-2-yl)amino)-4-(neopentylamino)-1,4-dioxobutan-2-yl)carbamate **28**

This compound was prepared from **18c** (302 mg, 1.0 mmol), HOBt (162 mg, 1.2 mmol), EDCI (288 mg, 1.5 mmol), compound **27** (364 mg, 1.1 mmol) and diisopropylethylamine (0.66 mL) in a similar manner as described for compound **19a**. The product was obtained as a white solid (517 g, 82%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (d, $J = 8$ Hz, 1H), 7.28 (s, 1H), 7.24–7.08 (m, 3H), 7.03 (d, $J = 7.5$ Hz, 1H), 7.00 (d, $J = 7.5$ Hz, 2H), 6.97–6.83 (m, 4H), 5.98–5.82 (m, 2H), 5.18–5.12 (m, 1H), 5.07–4.98 (m, 1H), 4.75–4.66 (m, 1H), 4.63–4.56 (m, 2H), 4.40–4.24 (m, 2H), 4.23–4.18 (m, 1H), 3.47 (dd, $J = 10.0$, 4.0 Hz, 1H), 3.14–3.06 (m, 1H), 3.01–2.89 (m, 2H), 2.72 (dd, $J = 15.0$, 5.0 Hz, 1H), 2.58 (dd, $J = 15.0$, 6.0 Hz, 1H), 2.28 (s, 3H), 1.21 (s, 9H), 0.86 (s, 9H); ESI-MS: $m/z = 632$ $[M+H]^+$.

4.1.46. (S)-N^1-((S)-3-(1-Allyl-1H-indol-3-yl)-1-((4-methylbenzyl)amino)-1-oxopropan-2-yl)-2-(1-(but-3-en-1-yl)-1H-imidazole-2-carboxamido)-N^4-neopentylsuccinamide **30**

This compound was prepared from **10d** (166 mg, 1.0 mmol), HOBt (162 mg, 1.2 mmol), EDCI (288 mg, 1.5 mmol), **29** (531 mg, 1.05 mmol) and diisopropylethylamine (0.66 mL) in a similar manner as described for compound **19a**. The product was obtained as a white solid (421 mg, 62%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.65 (d, $J = 7.5$ Hz, 1H), 7.67 (d, $J = 8.0$ Hz, 1H), 7.22 (d, $J = 8.5$ Hz, 1H), 7.18–7.13 (m, 1H), 7.10 (d, $J = 8.0$ Hz, 1H), 7.07–7.00 (m, 4H), 6.99 (d, $J = 1.0$ Hz, 1H), 6.94 (m, 3H), 6.69 (t, $J = 5.5$ Hz, 1H), 5.94–5.65 (m, 3H), 5.13 (m, 1H), 5.05–5.01 (m, 2H), 5.01–4.97 (m, 1H), 4.79–4.70 (m, 2H), 4.57–4.48 (m, 2H), 4.37–4.30 (m, 1H), 4.28 (dd, $J = 6.0$, 2.5 Hz, 2H), 4.26–4.18 (m, 1H), 3.46 (dd, $J = 14.5$, 5.0 Hz, 1H), 3.15 (dd, $J = 14.5$, 7.0 Hz, 1H), 3.02 (dd, $J = 13.5$, 7.0 Hz, 1H), 2.90 (dd, $J = 13.5$, 6.0 Hz, 1H), 2.82 (dd, $J = 15.5$, 4.5 Hz, 1H), 2.73 (dd, $J = 15.0$, 7.0 Hz, 1H), 2.44 (q, $J = 7.5$ Hz, 2H), 2.31 (s, 3H), 0.87 (s, 9H); ESI-MS: $m/z = 680$ $[M+H]^+$.

4.1.47. (6S,9S)-N-(4-Methylbenzyl)-6-(2-(neopentylamino)-2-oxoethyl)-4,7-dioxo-4,5,6,7,8,9,17,18,19,20-decahydro-11,16-(metheno)benzo[q]imidazo[1,2-g][1,7,10,13]tetraazacyclooctadecine-9-carboxamide **31**

This compound was prepared from **30** (135 mg, 0.2 mmol) in a similar manner as described for compound **21a**. The product was obtained as a white solid (58 mg, 45%). Mp: 278.6–282.2 °C. HPLC purity = 98.31%, HPLC $t_R = 10.70$ min; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.62 (d, $J = 8.0$ Hz, 1H), 8.56 (t, $J = 6.0$ Hz, 1H), 8.26 (d, $J = 8.0$ Hz, 1H), 8.02 (t, $J = 6.5$ Hz, 1H), 7.50 (d, $J = 7.5$ Hz, 1H), 7.44 (d, $J = 1.0$ Hz, 1H), 7.37 (d, $J = 8.0$ Hz, 1H), 7.16–7.07 (m, 5H), 7.03–6.97 (m, 3H), 5.01–4.93 (m, 1H), 4.75–4.67 (m, 1H), 4.63–4.57 (m, 1H), 4.34 (dd, $J = 15.0$, 6.5 Hz, 1H), 4.24–4.11 (m, 2H), 4.08–4.01 (m, 1H), 3.88–3.79 (m, 1H), 3.23–3.17 (m, 1H), 3.05 (dd, $J = 16.0$, 11.0 Hz, 1H), 2.85 (dd, $J = 13.5$, 6.5 Hz, 1H), 2.79 (dd, $J = 13.5$, 6.0 Hz, 1H), 2.68 (d, $J = 7.0$ Hz, 2H), 2.28 (s, 3H), 1.84–1.72 (m, 2H), 1.71–1.63 (m, 1H), 1.63–1.53 (m, 1H), 1.05–0.93 (m, 2H), 0.79 (s, 9H); ^{13}C NMR (125 MHz, DMSO) δ 171.77, 171.57, 170.15, 158.86, 138.51, 136.79, 136.15, 136.07, 129.22, 128.32, 127.61, 126.69, 125.19, 121.53, 118.83, 118.79, 110.46, 109.99, 53.38, 50.39, 50.19, 46.30, 45.88, 42.47, 37.75, 32.27, 30.66, 28.85, 27.64, 27.50, 22.92, 21.15; ESI-MS: $m/z = 654$ $[M+H]^+$.

4.1.48. O-Hexyl-N-(N^2-(1-methyl-5-propyl-1H-pyrazole-4-carbonyl)-N^4-neopentyl-L-asparaginyl)-L-serine **32**

Compound **21e** (183 mg, 0.3 mmol) was dissolved in methanol (5 mL) in the presence of 10% palladium on carbon (10 mol %), and the reaction mixture was stirred under an atmosphere of H_2 for 2 h. The Pd/C was removed via filtration through celite, and the solvent was evaporated to obtain compound **32** as a white solid (150 mg,

95%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.90 (brs, 1H), 8.12 (d, J = 8.0 Hz, 1H), 7.94 (d, J = 8.0 Hz, 1H), 7.86 (s, 1H), 7.74 (t, J = 6.0 Hz, 1H), 4.84–4.75 (m, 1H), 4.38–4.30 (m, 1H), 3.75 (s, 3H), 3.69 (dd, J = 10.0, 5.0 Hz, 1H), 3.54 (dd, J = 10.0, 4.0 Hz, 1H), 3.37–3.30 (m, 2H), 2.96–2.85 (m, 3H), 2.81 (dd, J = 13.0, 6.0 Hz, 1H), 2.64 (dd, J = 14.5, 5.0 Hz, 1H), 2.56 (dd, J = 14.5, 9.5 Hz, 1H), 1.55–1.46 (m, 2H), 1.44–1.36 (m, 2H), 1.26–1.15 (m, 6H), 0.88 (t, J = 7.5 Hz, 3H), 0.84 (t, J = 7.0 Hz, 3H), 0.79 (s, 9H); ESI-MS: m/z = 524 $[M+H]^+$.

4.1.49. (S)-N^1-((S)-3-(Hexyloxy)-1-((4-methylbenzyl)amino)-1-oxopropan-2-yl)-2-(1-methyl-5-propyl-1H-pyrazole-4-carboxamido)-N^4-neopentylsuccinamide **33**

This compound was prepared from **32a** (52.3 mg, 0.1 mmol), HOBt (16.2 mg, 0.12 mmol), EDCI (28.8 mg, 0.15 mmol), *p*-tolylmethanamine (12.7 mg, 0.105 mmol) and diisopropylethylamine (66 μL) in a similar manner as described for compound **19a**. The product was obtained as a white solid (47 mg, 75%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84–7.69 (m, 2H), 7.51 (s, 1H), 7.28 (s, 1H), 7.14 (d, J = 7.5 Hz, 2H), 7.06 (d, J = 7.5 Hz, 2H), 6.29 (s, 1H), 4.74 (s, 1H), 4.56–4.42 (m, 2H), 4.39–4.26 (m, 1H), 3.98–3.88 (m, 1H), 3.78 (s, 3H), 3.47 (s, 1H), 3.42–3.30 (m, 2H), 3.06–2.66 (m, 6H), 2.29 (s, 3H), 1.62–1.48 (m, 2H), 1.46–1.33 (m, 2H), 1.28–1.10 (m, 6H), 0.91 (t, J = 7.0 Hz, 3H), 0.87–0.80 (m, 12H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.89, 169.77, 164.22, 146.36, 137.70, 136.54, 135.34, 129.08, 127.35, 116.79, 113.22, 71.63, 69.64, 53.57, 51.22, 50.66, 43.18, 37.11, 36.33, 31.88, 31.60, 29.39, 27.17, 26.35, 25.59, 22.49, 22.19, 21.08, 14.07, 13.82; ESI-MS: m/z = 627 $[M+H]^+$.

4.2. Biological evaluation

4.2.1. In vitro assays of chymotrypsin-like, trypsin-like, and caspase-like activities of the proteasome and immunoproteasome

The human constitutive proteasome was given by Dr. Jiang-ping Wu (Notre-Dame Hospital, Montreal, QC, Canada), derived from human hepatic cells; the immunoproteasome was purchased from Boston Biochem, derived from human peripheral blood mononuclear cell. We used several fluorogenic substrates, the fluorogenic substrate Suc-LLE-AMC was used to measure the Capase-Like activity of the proteasome, β1c. Suc-KQL-AMC was used to measure the Trypsin-Like activity of the proteasome, β2c. Suc-WLA-AMC was used to measure the Chymotrypsin-Like activity of the proteasome, β5c. The fluorogenic substrate Suc-PAL-AMC was used to measure the Capase-Like activity of the immunoproteasome, β1i. Suc-VGR-AMC was used to measure the Trypsin-Like activity of the immunoproteasome, β2i. Suc-ANW-AMC was used to measure the Chymotrypsin-Like activity of the immunoproteasome, β5i. All compounds were solved in DMSO, then diluted with water to be 10% DMSO containing solution. The reaction under the following buffer conditions: 100 mM Tris-HCl, pH7.5. Positive control: bortezomib. Negative control: 2% DMSO. Blank: no enzyme, replaced by reaction buffer. All the final concentration of the substrates were 100 μM. All assays were carried out in a 50 μL volume. 10 μL compound (contain 10% DMSO, the fianl concentration of DMSO is 2%), at different concentrations were added to 20 μL human proteasome (40 μg/mL) or immunoproteasome (1 μg/mL), after 15 min, the flurogenic substrates were added into the reaction plate, the plates were covered with a plate sealer and incubated at room temperature (25 °C) for 60 min. The AMC of the probe was detected by monitoring the increase in fluorescence with EnVision, at a 355 nm excitation and 460 nm emission wavelength. The calculation for Inhibition% as following formula: Inhibition% = $(OD_{DMSO} - OD_{Compound})/(OD_{DMSO} - OD_{Blank}) \times 100\%$. IC_{50} data were calculated using GraphPad Prism software, and the equation 'sigmoidal dose–response (variable slope)' was chosen for curve fitting.

4.2.2. Cancer cell proliferation assay

4.2.2.1. Cell culture. RPMI 8226, MM.1S and MV-4-11 cell lines were obtained from the American Type Culture Collection (ATCC) (Manassas, VA, USA) and maintained at 37 °C in a humidified atmosphere containing 5% CO_2. The human MM cell lines PRMI 8226 and MM.1S were cultured in RPMI-1640 media supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin from Invitrogen (Grand Island, NY, USA). The human AML cell line MV-4-11 was cultured in Iscove's modified Dulbecco's medium supplemented with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin.

4.2.2.2. Cell proliferation assays. A 90 μL aliquot of RPMI 8226 (5×10^3 cells per well), MM-1S (3×10^4 cells per well), or MV-4-11 cells (8×10^3 cells per well) was seeded into 96-well plates and then treated with 10 μL of 0.2% DMSO or varying concentrations of tested compounds for 72 h. Cell viability was measured using the CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay (MTS; Promega, Madison, WI). Briefly, 20 μL of the combined MTS/PMS solution was pipetted into each of the 96-wells plate and then incubated for 2–4 h at 37 °C in a humidified, 5% CO_2 atmosphere. The optical density was determined at 490 nm (background subtraction at 690 nm) using a SpectraMax 340 microplate reader (Molecular Devices, Sunnyvale, CA, USA). Growth inhibitory ratios were calculated as follows: Growth inhibitory ratio = $(A_{control} - A_{sample})/A_{control}$. IC_{50} values were derived from a nonlinear regression model (curvefit) based on a sigmoidal dose response curve (variable slope) and computed using GraphPad Prism version 5.02, GraphPad Software.

4.2.3. Human liver microsomal stability assays

The metabolic stability profiles of **23h** and **33** were assessed by monitoring the loss of test compounds in the presence of human liver microsomes. A typical incubation mixture (100 μL total volume) for metabolic stability studies contained 1 μM of test compounds, 1.0 mg/mL microsomal protein (pooled Balb/c mouse liver microsomes prepared in-house or BD UltraPool human liver microsomes), 0.1M phosphate buffered saline (pH 7.4), and 1 mM NADPH. After preincubation at 37 °C for 5 min, reactions were started by the addition of test compounds and further incubated for 0, 10, 20, and 30 min. Reactions were terminated by the addition of 400 μL ice-cold methanol containing internal standard (1 μM), followed by centrifugation at 15000*g* for 10 min to obtain the supernatant. Aliquots (100 μL) of the supernatant were taken, which were subsequently analyzed using a Shimadzu LCMS-2020 mass spectrometer. The peak area response ratio to internal standard (PARR) of the compounds at different time points were compared to the PARR at 0 min to determine the percentage of test compounds remaining.

4.3. Molecular modeling

Docking calculations were performed using the Glide module in Schrodinger (version 11.1) with the default option [47,48]. The X-ray crystal structure of the proteasome (PDB entry: 3MG6) was used as the docking template, and it was prepared by the protein preparation Wizard in Schrodinger by adding hydrogens and disulfide bridges, removing crystallographic waters and ions, fixing bond orders, assigning partial charges with the OPLS force field. The ligand **23h** was prepared using the *Ligprep* module in Schrodinger. The binding box with the size of 10 Å × 10 Å × 10 Å centered on the centroid of the ligand in the crystal structure of 3MG6. For the docking calculations of **23h**, the standard precision (SP) scoring function of Glide was used. All graphical images were created using Pymol.

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Appendix A. Supplementary data

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