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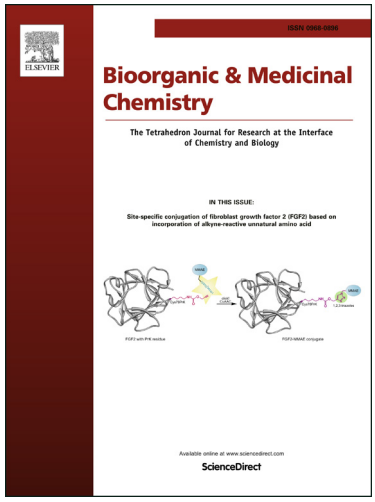

Synthesis and biological activity of peptide proline-boronic acids as proteasome inhibitors

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Graphical Abstract

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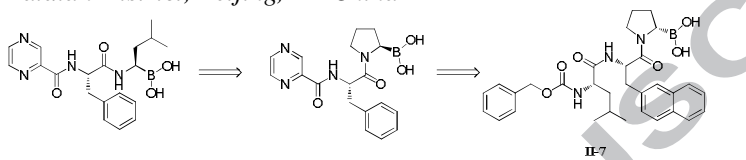

Bortezomib

IE-7

3 Cell lines inhibition: $IC_{50} < 10$ nM
HL-60 inhibition: $IC_{50} < 1$ nM
Improved subunit selectivty over proteasome

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ABSTRACT

On the basis of the application of proline-boronic acid as pharmacophore in the kinase inhibitors and our previous research results, using proline-boronic acid as warhead, two series of peptide proline-boronic acids, dipeptide proline-boronic acids (**I**) and tripeptide proline-boronic acids (**II**), were designed and synthesized. All the synthesized compounds were first evaluated for their biological activity against MGC803 cell, and then, the best compound **II-7** was selected to test its anti-tumor spectrum on six human tumor cell lines and proteasome inhibition against three subunits. The results indicated that series **II** have much better biological activities than series **I**. The compound **II-7** exhibited not only excellent biological activities with IC_{50} values of nM level in both cell and proteasome models, but also much better subunit selectivity. Thus, proline-boronic acid as warhead is reasonable in the design of proteasome inhibitors.

1. Introduction

The ubiquitin-proteasome pathway is critical to protein homeostasis.[1] It is involved in large numbers of cellular processes like cell cycle regulation, cytokine-stimulated signal transduction, immunity adjustment and cell apoptosis.[2] It has been confirmed that the proteasome is a promising target for the treatment of diseases such as inflammation, immune diseases, and cancer.[3] So far, a series of proteasome inhibitors have advanced to clinic or clinical trials. Bortezomib, Carfilzomib (PR-171)[4] and Ixazomib (MLN-9708)[5] have been approved by FDA for treatment of multiple myeloma (MM). Another three second-generation proteasome inhibitors, Marizomib (Salinosporamide A, NPI-0052),[6] Delanzomib (CEP-18770)[7] and Oprozomib (ONX-0912, PR-047)[8] are in clinical testing. Although several noncovalent proteasome inhibitors with specificity for the β5 subunits, such as benzyl amide[9] and oxdiazole[10] as pharmacophore, have been identified, the study of covalent inhibitors still are the most common and attractive due to their various advantages, including prolonged residence times, lower sensitivity against pharmacokinetic aspects, and high efficacy.[11] Most covalent inhibitors are based on peptide skeletons with a certain pharmacophore on their end by which the inhibitors can be covalently binding to the threonine residue in proteasome.

Peptide boronic acids are one of the most important chemical classes of covalent proteasome inhibitors. Unlike other kind of proteasome inhibitors with aldehydes or Michael acceptor pharmacophore, peptide boronates are poor inhibitors of cysteine proteases due to the weak interaction between sulfur and boron atoms,[12] which can reduce its side effects. However, most of peptide boronic acids have poor pharmacokinetic properties, which greatly limited their clinical applications.[13] To date, many efforts in improving the PK/PD properties of peptide boronic acid proteasome inhibitors mostly focus on the modifying the amino acid residues and the N-end substituents,[14] but neglect the changes of amide bonds and C-end substituents.

However, will the widely used leucine-boronic acid be the best choice as the warhead in the design of peptide boronic acid proteasome inhibitors? One of the strategies in drug design to make compounds more metabolically stable and cell penetrating is that of obtaining conformational constrained surrogates of peptide structures in such a way to eliminate at least a cleavable amide bond and reduce consequently the overall hydrophilic character of the molecule.[15] In our previous 3D-QSAR models of tripeptide aldehyde,[16] both CoMFA and CoMSIA contour maps had offered us that bulky and positive groups at C-end position will increase selectivity between the three subunits of proteasome. We also noticed that conformational constrained proline-boronic acids as potent inhibitors of both DPP4, DPP7 and FAP have been reported, such as a highly selective inhibitor of human dipeptidyl peptidase IV (**1**),[17] the inhibitor of bacteria IgA proteinase (**2**),[18] (IC_{50} < 10 nM), and a FAP inhibitor (**3**)[19] (**Fig. 1**). Inspired by above, we designed and synthesized both dipeptide proline-boronic acids (**I**) and tripeptide proline-

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† These authors contribute equally to this work.

boronic acids (**II**) as proteasome inhibitor based on the known effective peptide boronic acid kind of proteasome inhibitors and our previous research results.[16, 20]

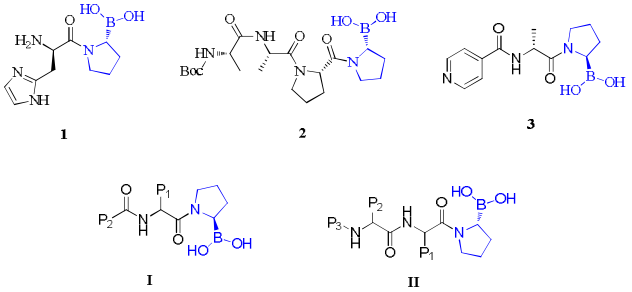


Fig.1. Design of peptide proline-boronic acids as proteasome inhibitor.

2. Results and discussion

2.1 Design, synthesis and biological activities of dipeptide proline-boronic acids (I)

In the specific design of the compounds, we refer to some practical structures reported recent years as listed in **Figure 2**.

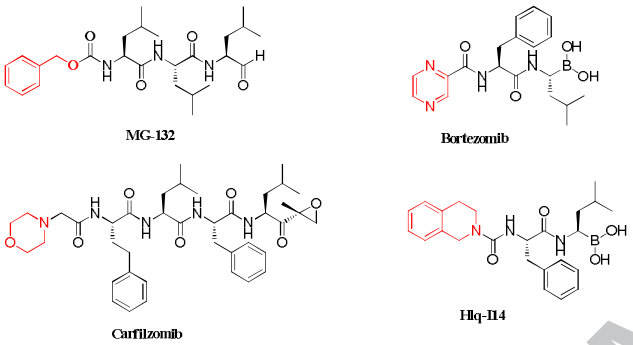
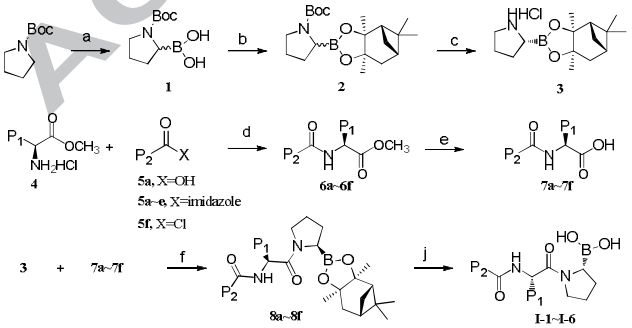


Fig.2. Some practical proteasome inhibitors with classical peptide bones.

MG-132 is a well-studied tripeptide aldehyde (Z-L-leu-L-leu-L-leu-H) proteasome inhibitor that exerts antitumor activity and enhances cytostatic/cytotoxic effects of chemo- and radiotherapy.[21] The dipeptide boronic acid Bortezomib[22] represents the first 20S proteasome inhibitor approved by FDA for the treatment of multiple myeloma in 2003 and mantle cell lymphoma in 2006. Carfilzomib is the first epoxy ketone proteasome inhibitor approved by FDA in July 2012 for treatment of multiple myeloma.[4] And in our previous work, we found that 1, 2, 3, 4-tetrahydroisoquinoline substituted urea scaffold at the N-end of peptide boronic acid (**Hlq-I14**) show significantly superior toxicity profile in rats and more metabolically stable than Bortezomib while without loss of activity in anti-tumors.[23] Based on the N-end substituent groups of above representative proteasome inhibitors, six dipeptide proline-boronic acids as proteasome inhibitors were designed and synthesized (**Table 1**).

Synthesis

The synthetic route is illustrated in **Scheme 1**. The key intermediate, α-proline-boronic acid pinanediol ester hydrochloride (**3**), was synthesized from pyrrolidine as starting material according to the literatures.[24-25] The N-Boc-tetrahydropyrrole was reacted with $B(OMe)_3$ in the presence of s-BuLi affording the N-Boc-α-proline-boronic acid (**1**). Esterification of **1** with (+)-pinanediol gave the N-Boc-α-proline-boronic acid pinanediol ester (**2**). Finally, removing the Boc protecting group in the solution of HCl-AcOEt obtained the key intermediate (**3**).



Scheme1. Synthesis of **I-1~I-6**. Reagents and conditions: (a) s-BuLi, $B(OCH_3)_3$, THF, -78°C; (b) (+)-Pinanediol, EtOAc, rt; (c) i, HCl/EtOAc, rt; ii, DCM, i-BuOH; (d) DCC, HOBt, 4-Methylmorpholine, THF, 0°C; or CDI, THF/DCM, 0°C -rt; or Sodium Carbonate,

PhMe, 0°C -rt; (e) i, LiOH (3 N, aq), THF/H_2O, 0 °C; ii, HCl (4 N, aq), H_2O, 0°C; (f) EDCI, HOBt, N,N-Diisopropylethylamine, DCM, 0°C -rt; (j) isobutaneboronic acid, n-hexane, methanol, rt.

Another key intermediates (**7**), the peptide acids containing different N-end substituted groups, was prepared according to the related literature.[26-27] For compound **I-1**, L-phenylalanine methyl ester hydrochloride **4** was coupled with pyrazine 2-carboxylic acid in the presence of DCC and HOBt to give the methyl (pyrazine-2-carbonyl)-L-phenylalaninate **6a**. For compounds **I-2~I-5**, the urea scaffold at N-end was introduced to peptide acid methyl esters **6b~6e** by using 1,1-carbonyldiimidazole (CDI) to form the urea part. Reaction of L-Naphthylalanine methyl ester hydrochloride **5** with corresponding Cbz-Cl furnished **6f**. All the methyl esters **6a~6f** were not separated and directly used for saponification to afford the intermediates **7a~7f**.

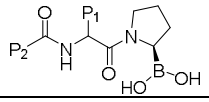
Then intermediates **3** and **7a~7f** were coupled in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI) to provide dipeptide proline-boronic acid pinanediol ester **8a~8f** in high yield after chromatographic purification. And then **8a~8f** were treated with phenylboronic acid in mixture solvent of hexane-methanol to give target peptide boronic acids **I-1~I-6**. All the key intermediates and final products were characterized by ^{1}H NMR, ^{13}C NMR and MS spectroscopic analyses.

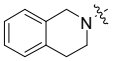
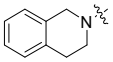
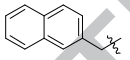
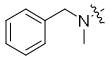
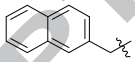
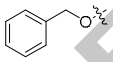
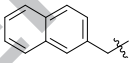
Biological activities

Refer to the results of our previous article,[23] we firstly selected MG803 cell line to evaluate the anti-tumor activity of **I-1~I-6** and the results are summarized in **Table 1**.

As shown in **Table 1**, the compounds with N-end urea scaffold (**I-4** and **I-5**) showed significant inhibitory activity against MG803 though the activity of others is weak (IC_{50} > 10 μM). Thus, proline-boronic acid as the warhead seem to be effective. Meanwhile, comparing the results of **I-2** and **I-4**, it is clear that the larger P_1 is more favorable to the activity than the smaller P_1 which is consistent with the literature description.[16]

Table 1. Structures and cell viability of dipeptide proline-boronic acids (**I**).



Compd	P_2	P_1	IC_{50}/μM(MGC803)
I-1			>10
I-2			>10
I-3			>10
I-4			5.362±0.422
I-5			7.480±0.271
I-6			>10

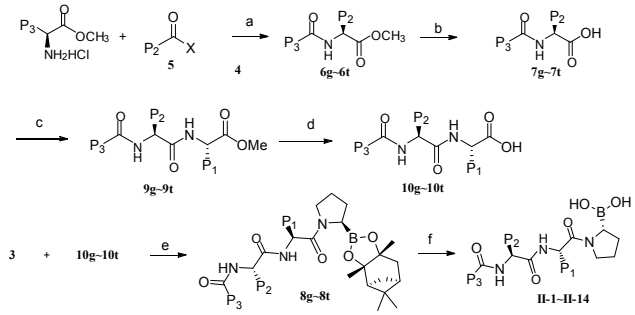
2.2 Design, synthesis and biological activities of tripeptide proline-boronic acids (II)

Design

In our previous works,[16, 23] we found that it's remarkably useful and practical to extend the length of peptide which show more linear capacity when designing the peptides proteasome inhibitors and the same phenomenon has been found in other reported papers.[28] Hence, aiming at more potent proteasome inhibitors containing proline-boronic, we designed and synthesized fourteen tripeptide proline-boronic acids (**II-1~II-14**) as the proteasome inhibitors.

Synthesis

All the designed tripeptide proline-boronic acids were synthesized by the similar methodology as **I-1** (**Scheme 2**).



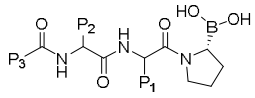
Scheme 2. Synthesis of **II-1~II-14**. Reagents and conditions: (a) DCC, HOBt, 4-Methylmorpholine, THF, 0°C; or CDI, THF/DCM, 0°C -rt; or Sodium Carbonate, PhMe, H_2O, 0°C -rt. (b) i, LiOH (3 N, aq), THF/H_2O, 0°C; ii, HCl (4 N, aq), H_2O, 0°C. (c) DCC, HOBt, 4-Methylmorpholine, THF, 0°C. (d) i, LiOH (3 N, aq), THF/H_2O, 0°C; ii, HCl (4 N, aq), H_2O, 0°C. (e) EDCI, HOBt, N,N-diisopropylethylamine, DCM, 0°C -rt. (f) isobutaneboronic acid, n-hexane, methanol, rt.

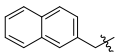
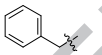
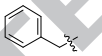
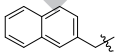
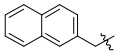
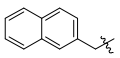
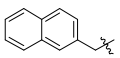
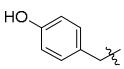
The acid intermediates **7g~7t** were prepared as synthesis of **7a~7f** in **Scheme 1**. Then, **7g~7t** were reacted with another amino acid residue in the presence of DCC to provide dipeptide ester **9g~9t,** which were saponified without purification affording the dipeptide acids **10g~10t**. Finally, intermediate **3** were respectively coupled with **10g~10t,** and then deprotected to give the target tripeptide proline-boronic acids **II-1~II-14** as the synthesis of **I**.

Biological activities

The structures and results of the anti-tumor assay are illustrated in **Table 2**. MG803 cell line is still selected to evaluate the activity.

Table 2. Structures and cell biological activity of the tripeptide proline-boronic acids (**II**)



Compd	P_3	P_2	P_1	IC_{50}/μM (MGC803)
II-1				0.02958±0.000
II-2				0.02724±0.002
II-3				0.4777±0.002
II-4				0.1103±0.000
II-5				4.426±0.055
II-6				0.1495±0.001
II-7				0.009765±0.000
II-8				0.01091±0.001
II-9				4.167±0.504
II-10				2.416±0.254
II-11				7.641±0.950

II-12	>10
II-13	1.432±0.175
II-14	1.654±0.251
Bortezomib	0.0133±0.000

As shown in **Table 2**, the activity of tripeptide proline-boronic acids was significantly better than the dipeptide proline-boronic acids. Among the three substituents at P_1, P_2 and P_3, apparently the most crucial influence factor is the N-end P_3 substituted groups. For the same N-end P_3 compounds **II-1~II-5, II-6~II-8** and **II-9~II-13**, no matter how P_1 and P_2 change, the IC_{50} level show average differences at the order of magnitude. Cbz group shows significant superiority over tetrahydroisoquinoline substituted, N-methyl benzylamine and pyrazinyl substituted group, while pyrazinyl substituted group behaves moderately (**II-6 over II-10; II-7 over II-13, II-1; II-8 over II-5**). The best choice of P_1 is still aromatic group, and more bulky naphthyl group is slightly better than benzyl group (**II-4** over **II-3**, **II-10** over **II-9**). The activity of compounds with aliphatic isopropyl group at P_1 decrease sharply (**II-5** over **II-1~2, II-12** over **II-9~10**). However, when it comes to P_2 position, isopropyl group show dramatically advantages over aromatic benzyl group (**II-2 over II-3, II-7 over 6 and II-13 over II-10**). In particular, compound **II-7,** which represents the best choices of P_1, P_2 and P_3, reaches the IC_{50} value of 0.009 μM against MGC803 cells while the IC_{50} of Bortezomib is 0.0133 μM. All the structure-activity relationship (SAR) analysis are summarized in **Figure 3**.

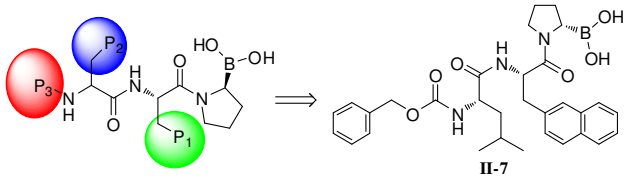


Fig.3. Potential sites suitable for modification and structure of **II-7**. P_3 plays the most crucial role between the three sites; P_2, alkyl groups are better than aromatic ones; P_1, aromatic and bulky groups are preferred.

We next further investigated SAR by docking simulation of **II-7** to the β5 subunit of the proteasome.(**Fig. 4**)

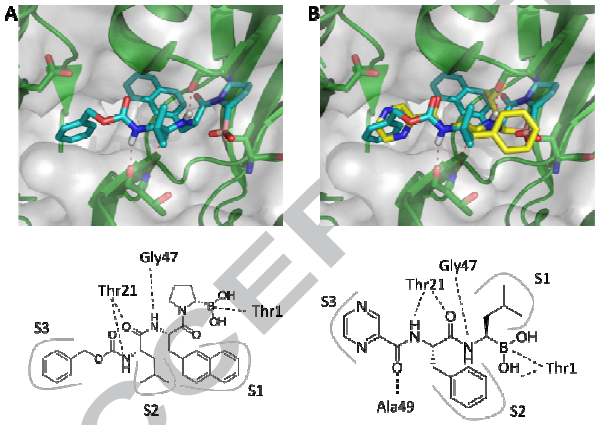


Fig.4. Predicted binding mode of **II-7**(cyan sticks, left panel) and its comparison with bortezomib(yellow sticks, from PDB 2f16). Binding mode **II-7** was handcrafted and minimized using Amber16.[29]

As seen in the predicted binding mode (**Figure 4A**), the proline ring in **II-7** extends into a new space and the boronic aicd retraces and binds to the Thr as leucine-boronic acid in bortezomib does. The bulky naphthyl group is predicted to occupy the S1 subsite located at the interface between β5 and β6 subunits and leucine residue in **II-7** stretches into the S2 subsite where the phenylalanine is supposed to occupy with a little deviation. Cbz group of **II-7** is predicted to spread straightly and deeply as the pyrazinyl substituted group of bortezomib (**Figure 4B**). Overall, **II-7** is predicted to occupy three subsites, as compared to three for bortezomib, and this predicted similarity may indicate that proline-boronic acid as warhead is reasonable in the design of proteasome inhibitors.

Subsequently, we choose **II-7** to investigate the capacity of anti-tumor spectrum on 6 human tumor cell lines and Bortezomib was used as positive control. The results are summarized in **Table 3.**

Table 3. Biological results of compound **II-7** over 6 cell lines (IC_{50}, μM)

Compd	A549	95D	HCT1	MDA	HL-60	MGC

			16	-MB-231		803
II-7	5.99	0.012	0.069	0.007	<0.001	0.010
Bortezomib	2.14	0.038	0.001	0.017	0.008	0.008

As shown in **Table 3**, comparing to positive drug Bortezomib, **II-7** exhibits approximate activities in different cells lines with Bortezomib. Accordingly, proline-boronic acids can also serve as an excellent warhead in the design of proteasome inhibitors.

Finally, the proteasome inhibitory activity of compound **II-7** was examined in an isolated 20S mouse proteasome, including CT-L, T-L, and PGPH, with Bortezomib as a control. The results are summarized in **Table 4**.

Table 4. Biological activity towards proteasome

	Against proteasome (IC_{50}, μM)				
Compd	CT-L	T-L	PGPH	T-L/ CT-L	PGPH/ CT-L
II-7	0.018	1.66	1.25	92.2	69.4
Bortezomib	0.004	0.44	0.03	110	7.5

Compound **II-7** inhibited proteasome ChT-L activity with IC_{50} values of 18 nM, which is comparable with Bortezomib (4 nM). However, compound **II-7** (IC_{50}: T-L / CT-L = 92.2; PGPH/CT-L = 69.4) showed better subunit selectivity than Bortezomib (IC_{50}: T-L / CT-L = 110; PGPH/CT-L = 7.5). These results demonstrate that proline-boronic acid as the warhead can really improve the subunit selectivity, which is consistent with our original research objectives. Thus, compound **II-7** with high subunit selectivity is expected to have lower side-effect.[30-32]

3. Conclusion

Using the proline-boronic acid as warhead, we designed and synthesized six dipeptide proline-boronic acids and fourteen tripeptide proline-boronic acids as proteasome inhibitors. All the synthesized compounds were first evaluated for their biological activity against MGC803 cell. The results show that tripeptide proline-boronic acids obviously exhibit better activity than dipeptide proline-boronic acids and **II-7** is the best one with IC_{50} value of 0.009 μM. Then, the best compound **II-7** was selected to test its anti-tumor spectrum on six human tumor cell lines and the inhibition for all the cell lines is very strong with IC_{50} value of < 70 nM except A549 cell line. Finally, the proteasome inhibition of **II-7** was examined and the results indicated that **II-7** has a comparable activity against ChT-L and higher subunit selectivity comparing with Bortezomib. Further research on the compound **II-7** as a potential proteasome inhibitor is being carried out in our laboratory.

4. Experimental section

4.1 General information

All commercial reagents were purchased from commercial suppliers and used without further purification. Dry solvents were prepared according to standard procedures. Melting points were taken with an X4 apparatus and were uncorrected. Column chromatography was carried out on silica gel 60 (300-400 mesh) supplied by Qingdao Haiyang Chemical Co. Yields refer to chromatographically and spectroscopically pure compounds unless otherwise stated. ^{1}H-NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 at ambient temperature on a Bruker Avance III 400 MHz system. ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 at ambient temperature unless otherwise noted, at 100 MHz. Silica gel column chromatography was performed with silica gel 60 N (spherical, neutral, 63-210 μm, or Merck). MS data were obtained with MDSSCIEX QSTAR systems.

(1S, 2S, 3R, 5S)-Pinanediol proline boronate (2)

A flame dried round bottom flask equipped with a magnetic stir bar was charged with N-Boc-pyrrolidine (10 g, 58 mmol, 1 eq) and dry THF (40 mL) under a nitrogen atmosphere. The clear colorless solution was cooled to -78 °C and a solution of s-BuLi (64 mL of a 1.0 M solution in cyclohexane, 64 mmol) was added slowly over a 30 minute period. The light orange colored solution was stirred at -78 °C for 3 hours followed by treatment with $B(OMe)_3$ (15 mL, 175 mmol) after which the cooling bath was removed and the clear colorless solution slowly warmed to 0 °C. Upon reaching 0 °C, the reaction was quenched with a small amount of water (~2 mL), allowed to warm to room temp then extracted into 2 N NaOH (100 mL) and backwashed with additional EtOAc (60 mL). The aqueous phase was acidified to pH 3 by the addition of 2 N HCl and then extracted with EtOAc (3 x 60 mL). The organic extracts were combined and dried over Na_2SO_4 and concentrated to produce the free boronic acid 9 g as a sticky white solid. Without further purification the boronic acid was dissolved in EtOAc (60 mL) and with constant stirring (+)-pinanediol (7.0 g, 41 mmol) was added at room temperature. After 18 h the ether was removed and the (+)-pinanediol boronic ester was purified by column chromatography (silica gel, 6:1 hexanes/EtOAc) to give a clear thick oil (12.1 g, 34.8 mmol) 60% yield in two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.50 – 4.15 (m, 1H), 3.38 (dt, *J* = 13.8, 6.1 Hz, 2H), 3.12 (ddd, *J* = 25.1, 15.8, 8.4 Hz, 1H), 2.33 (dd, *J* = 12.3, 10.3 Hz, 1H), 2.20 (s, 1H), 2.10 – 1.69 (m, 7H), 1.45 (d, *J* = 7.3 Hz, 9H), 1.41 (s, 3H), 1.28 (s, 3H), 0.84 (s, 3H).

(1S, 2S, 3R, 5S)-Pinanediol L-proline boronate hydrochloride (3)

Compound **2** (12.1 g, 34.8 mmol) was dissolve in a small amount of EtOAc and HCl/EtOAc (2.5N, 25 mL) was added. After 12 h, racemic hydrochloride was precipitated as white solid 7.9 g. Crystallization and isolation of the desired isomer was performed by dissolving the HCl salt in a minimal amount of dichloromethane (60 mL) with gentle heating to facilitate a homogenous solution followed by continuous stirring for 8 hours to yield a fluffy white precipitate that was collected by vacuum filtration, dried and then dissolved in minimal 2-propanol (50 mL) with gentle heating until homogenous. The alcoholic solution was stirred overnight and the resulting white precipitate was collected by vacuum filtration affording isomeric pure **3** as a white solid 3.2g. mp: 143-147℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (s, 1H), 4.43 (d, *J* = 8.3 Hz, 1H), 3.43 (s, 2H), 3.22 (s, 1H), 2.37 – 2.18 (m, 3H), 2.09 – 1.90 (m, 6H), 1.45 (s, 3H), 1.29 (s, 3H), 1.13 (d, *J* = 11.0 Hz, 1H), 0.83 (s, 3H).

N-(2-Pyrazinylcarbonyl)-L-phenylalanine methyl ester (6a)

To pyrazine-2-carboxylic acid (1 mmol) in absolute THF (30 mL) at 0 □ was added DCC (1 mmol) and HOBt (1.2 mmol), and the mixture was reacted for 40 mins. Then methyl L-phenylalaninate hydrochloride (1 mmol) and 4-Methylmorpholine (NMM) were added and the react was performed until TLC showed the starting materials disappeared (12h). After the reaction finished, the resulting solid was filtered and the solvent was removed under vacuum. The white solid was dissolved in 50 mL ethyl acetate and washed with 5% $NaHCO_3$, 10% citric acid, 5% $NaHCO_3$, and brine, dried over anhydrous Na_2SO_4, filtered, and evaporated to provide methyl ester **6a** as colorless oil 0.28 g (98% yield); mp: 152 - 155℃. ^{1}H-NMR ($CDCl_3$, 400MHz) 3.26 (m, 2H), 3.75 (s, 3H), 5.09 (m, 1H), 7.15~7.32 (m, 5H), 8.23 (d, 1H), 8.52 (d, 1H), 8.74 (d, 1H), 9.37 (s, 1H).

(1, 2, 3, 4-tetrahydroisoquinoline-2-carbonyl)-L-phenylalanine methyl ester (6b)

To a solution of L-phenylalanine methyl ester hydrochloride (10.1 mmol) in THF (8 mL) and di-chloromethane (40 mL) was added triethylamine (9.3 mmol) and N,N'-carbonyldiimidazole (CDI, 15.2 mmol). The solution was stirred at room temperature for 2 h. Then, the reaction mixture was poured into water, and the mixture was washed with brine twice, and the organic phase was evaporated to provide crude intermediate. The intermediate was dissolved in dichloromethane (40 mL), and 1, 2, 3, 4-tetrahydroisoquinoline was then added. The reaction mixture was stirred at room temperature for 24 h until completion as monitored by TLC, and then concentrated under reduced pressure. The residue was dissolved in ethyl acetate (50 mL) and washed with 10% citric acid, 5% $NaHCO_3$, and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuum to provide methyl ester **6b** as colorless oil, 90% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 – 7.01 (m, 4H), 5.23 (d, J = 8.2 Hz, 1H), 4.58 (dt, J = 8.6, 4.4 Hz, 1H), 4.54 (d, J = 1.7 Hz, 2H), 3.73 (s, 3H), 3.66 (ddd, J = 11.9, 6.7, 5.0 Hz, 1H), 3.53 (ddd, J = 12.2, 7.0, 5.0 Hz, 1H), 2.93 – 2.73 (m, 2H), 1.75 (dq, J = 8.0, 6.3 Hz, 1H), 1.68 – 1.50 (m, 2H), 0.95 (d, J = 6.5 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.3, 157.1, 135.0, 133.3, 128.3, 126.6, 126.3, 52.2, 45.4, 41.8, 41.2, 29.0, 24.9, 22.9, 21.9.

Methyl (morpholine-4-carbonyl)-L-phenylalaninate (6c)

Using the same procedure as **6b**, colorless oil, 75% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (dq, *J* = 14.0, 6.9 Hz, 3H), 7.10 (d, *J* = 7.3 Hz, 2H), 4.90 (d, *J* = 7.6 Hz, 1H), 4.79 (q, *J* = 6.3 Hz, 1H), 3.72 (s, 3H), 3.64 (t, *J* = 5.0 Hz, 4H), 3.38 – 3.22 (m, 4H), 3.17 – 3.04 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.0, 156.7, 136.2, 129.2, 128.5, 127.0, 66.4, 54.3, 52.2, 44.0, 38.3.

Methyl (S)-3-(naphthalen-2-yl)-2-(1, 2, 3, 4-tetrahydroisoquinoline-2-carboxamido) propanoate (6d)

Using the same procedure as **6b**, colorless oil, 77% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 – 7.65 (m, 3H), 7.57 (d, *J* = 1.6 Hz, 1H), 7.44 (dt, *J* = 6.3, 3.5 Hz, 2H), 7.27 – 7.22 (m, 1H), 7.16 (dt, *J* = 7.4, 3.8 Hz, 2H), 7.13 – 7.09 (m, 1H), 7.07 – 7.02 (m, 1H), 5.02 (d, *J* = 7.5 Hz, 1H), 4.94 (dt, *J* = 7.5, 5.8 Hz, 1H), 4.58 – 4.39 (m, 2H), 3.72 (s, 3H), 3.59 (ddd, *J* = 12.0, 6.6, 5.1 Hz, 1H), 3.49 (ddd, *J* = 12.2, 6.7, 5.2 Hz, 1H), 3.37 – 3.23 (m, 2H), 2.80 (q, *J* = 5.3 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.3, 156.6, 135.0, 134.0, 133.5, 133.3, 132.5, 128.4, 128.2, 128.11, 127.7, 127.6, 127.5, 126.8, 126.5, 126.4, 126.2, 125.8, 54.6, 52.4, 45.5, 41.3, 38.7, 29.0.

Methyl (S)-2-(3-benzyl-3-methylureido)-3-(naphthalen-2-yl) propanoate (6e)

Using the same procedure as **6b**, colorless oil, 82% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 – 7.65 (m, 3H), 7.56 – 7.40 (m, 3H), 7.28 – 7.07 (m, 6H), 4.90 (dq, *J* = 12.8, 7.5, 7.0 Hz, 2H), 4.51 – 4.36 (m, 2H), 3.72 (s, 3H), 3.32 (dd, *J* = 13.7, 5.2 Hz, 1H), 3.24 (dd, *J* = 13.7, 5.6 Hz, 1H), 2.80 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.2, 157.4, 137.5, 133.8, 133.4, 132.4, 128.6, 128.2, 128.0, 127.7, 127.6, 127.3, 127.3, 127.2, 126.1, 125.7, 54.5, 52.3, 52.2, 38.5, 34.2.

N-Cbz-L-naphthylalanine methyl ester (6f)

L-naphthylalanine methyl ester hydrochloride (0.53 g, 2 mmol) was added to an ice bath of 8 mL of toluene followed by addition of benzyl chloroformate (0.35 mL, 2.4 mmol) and sodium carbonate solution (1N, 2.5 mL) was added dropwise with vigorous stirring. Then the reaction was carried out overnight. The layers were separated and the aqueous layer was extracted three times with DCM. The organic phase was successively washed with 10% citric acid, 5% $NaHCO_3$ and brine, and was dried over anhydrous Na_2SO_4, evaporated to give **6f** (0.65 g) in 90% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.68 (m, 3H), 7.55 (s, 1H), 7.49 – 7.42 (m, 2H), 7.30 – 7.18 (m, 6H), 5.28 (d, *J* = 7.8 Hz, 1H), 5.14 – 5.02 (m, 2H), 4.75 (q, *J* = 6.3 Hz, 1H), 3.71 (s, 3H), 3.26 (qd, *J* = 13.9, 5.9 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.0, 155.7, 136.2, 133.4, 133.3, 132.5, 128.5, 128.3, 128.2, 128.1, 128.1, 127.7, 127.6, 127.4, 127.2, 126.2, 125.8, 67.0, 54.9, 52.4, 38.4.

N-(2-Pyrazinylcarbonyl)-L-phenylalanine (7a)

The product **6a** (1 mmol) was dissolved in 10 mL THF and was added 3N LiOH dropwise at 0 ℃ until TLC showed methyl ester **6a** was disappeared completely (1 h). The reaction solution was acidified to pH 2. The precipitate was filtered and washed with water until pH value reached 6 - 7. After dryness, the acid **7a** was obtained with high yield and used exactly without further purification, 93% yield; mp: 166 - 169℃. ^{1}H NMR (400 MHz, $CDCl_3$) **δ**9.37 (s, 1H), 8.76 (s, 1H), 8.53 (s, 1H), 8.22 (d, J = 8.1 Hz, 1H), 7.25 (dt, J = 16.6, 7.2 Hz, 5H), 5.12 (dd, J = 13.8, 6.2 Hz, 1H), 3.31 (dd, J = 20.6, 14.1, 6.0 Hz, 2H).

N-((S)-1-oxo-3-phenyl-1-((R)-2-((3aS,4S,6S,7aR)-3a,5,5,7a-tetramethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)pyrrolidin-1-yl)propan-2-yl)pyrazine-2-carboxamide (8a)

To a stirred suspension of **7a** (1.0 mmol) in CH_2Cl_2 (15 mL) were added 1-(3 (dimethyl amino) propyl)-3-ethylcarbodiimide hydrochloride (EDCI, 1.2 mmol) and 1-hydroxybenzotriazole monohydrate (HOBt, 1.1 mmol). Pinanediol proline boronate hydrochloride **3** (1.0 mmol, prepared according to the literature[25]) and DIPEA (0.36 mL, 2.4 mmol) were then added. The mixture was stirred at room temperature for 24 h and solvent was removed under vacuum. The residue was dissolved in 20 mL of ethyl acetate and was successively washed with 10% citric acid, 5% $NaHCO_3$ and brine. The organic phase was dried over anhydrous Na_2SO_4 and then was evaporated to give crude product. The crude product was purified by silica gel chromatography (ethyl acetate: petroleum ether = 1:3) to give product **8a** as colorless oil, 71% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.37 (d, J = 1.3 Hz, 1H), 8.75 (d, J = 2.3 Hz, 1H), 8.57 (s, 2H), 5.05 (td, J = 9.3, 5.5 Hz, 1H), 4.37 – 4.14 (m, 1H), 3.30 (dd, J = 16.2, 9.2 Hz, 1H), 3.18 (dd, J = 12.8, 5.5 Hz, 1H), 3.13 – 3.06 (m, 1H), 3.05 – 2.97 (m, 1H), 2.60 – 2.47 (m, 1H), 2.36 – 2.24 (m, 1H), 2.06 (ddd, J = 18.3, 9.2, 4.0 Hz, 1H), 1.88 (ddd, J = 20.1, 14.2, 8.0 Hz, 7H), 1.72 – 1.55 (m, 1H), 1.39 (s, 4H), 1.24 (s, 3H), 0.82 (d, J = 4.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.6, 161.4, 146.3, 143.7, 143.5, 141.8, 135.5, 128.7, 127.6, 126.2, 85.0, 77.0, 51.4, 50.3, 45.4, 39.3, 38.7, 37.4, 34.6, 27.7, 26.3, 26.0, 25.2, 23.2, 0.1.

N-((S)-1-oxo-3-phenyl-1-((R)-2-((3aS,4S,6S,7aR)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)pyrrolidin-1-yl)propan-2-yl)-3,4-dihydroisoquinoline-2(1H)-carboxamide (8b)

Using the same procedure as **8a**, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (d, *J* = 7.2 Hz, 2H), 7.28 – 7.20 (m, 3H), 7.20 – 7.05 (m, 4H), 5.70 (d, *J* = 7.9 Hz, 1H), 4.82 (q, *J* = 8.1 Hz, 1H), 4.51 (s, 2H), 4.38 (d, *J* = 8.5 Hz, 1H), 3.65 (dt, *J* = 11.9, 5.8 Hz, 1H), 3.54 (p, *J* = 6.7, 6.1 Hz, 2H), 3.08 (qd, *J* = 13.4, 6.3 Hz, 3H), 2.82 (q, *J* = 5.2 Hz, 2H), 2.43 (dq, *J* = 20.5, 11.5, 10.0 Hz, 2H), 2.23 (dt, *J* = 11.5, 6.3 Hz, 1H), 2.09 (t, *J* = 5.4 Hz, 1H), 1.95 (d, *J* = 11.2 Hz, 3H), 1.77 (q, *J* = 6.7, 6.1 Hz, 2H), 1.68 – 1.57 (m, 1H), 1.48 (s, 3H), 1.40 (d, *J* = 10.6 Hz, 1H), 1.31 (s, 3H), 0.88 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.4, 156.7, 136.9, 135.0, 133.3, 130.1, 128.4, 128.2, 126.6, 126.5, 126.4, 126.3, 85.8, 77.7, 53.7, 51.3, 46.4, 45.5, 41.1, 39.9, 39.6, 38.2, 35.5, 29.0, 28.7, 27.4, 27.1, 27.1, 26.3, 24.1.

N-((S)-1-oxo-3-phenyl-1-((R)-2-((3aS,4S,6S,7aR)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)pyrrolidin-1-yl)propan-2-yl)morpholine-4-carboxamide (8c)

Using the same procedure as **8a**, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 – 7.30 (m, 2H), 7.28 – 7.20 (m, 3H), 5.48 (d, *J* = 7.9 Hz, 1H), 4.76 (td, *J* = 8.2, 5.4 Hz, 1H), 4.36 (dd, *J* = 8.7, 1.9 Hz, 1H), 3.64 (t, *J* = 4.9 Hz, 4H), 3.48 (dt, *J* = 10.7, 5.7 Hz, 1H), 3.40 – 3.25 (m, 4H), 3.12 – 2.94 (m, 3H), 2.48 (q, *J* = 8.2 Hz, 1H), 2.44 – 2.33 (m, 1H), 2.24 – 2.18 (m, 1H), 2.08 (t, *J* = 5.4 Hz, 1H), 1.99 – 1.87 (m, 3H), 1.81 – 1.73 (m, 2H), 1.69 – 1.54 (m, 1H), 1.46 (s, 3H), 1.38 (d, *J* = 10.8 Hz, 1H), 1.30 (s, 3H), 0.87 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.2, 156.9, 136.8, 130.2, 128.3, 126.7, 86.0, 77.9, 66.6, 53.6, 51.5, 46.5, 44.1, 40.0, 39.7, 38.4, 35.6, 28.80 27.4, 27.3, 27.2, 26.4, 24.2.

N-((S)-3-(naphthalen-2-yl)-1-oxo-1-((R)-2-((3aS,4S,6S,7aR)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)pyrrolidin-1-yl)propan-2-yl)-3,4-dihydroisoquinoline-2(1H)-carboxamide (8d)

Using the same procedure as **8a**, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, *J* = 9.6 Hz, 3H), 7.73 (d, *J* = 8.4 Hz, 1H), 7.52 (d, *J* = 8.3 Hz, 1H), 7.46 – 7.38 (m, 2H), 7.19 – 7.14 (m, 2H), 7.13 – 7.05 (m, 2H), 5.71 (d, *J* = 8.0 Hz, 1H), 4.90 (td, *J* = 8.6, 4.7 Hz, 1H), 4.52 (s, 2H), 4.39 (d, *J* = 8.3 Hz, 1H), 3.65 (dt, *J* = 12.0, 5.8 Hz, 1H), 3.54 (dt, *J* = 12.3, 5.9 Hz, 1H), 3.52 – 3.42 (m, 1H), 3.24 (qd, *J* = 13.1, 6.9 Hz, 2H), 3.06 (dd, *J* = 10.2, 7.1 Hz, 1H), 2.89 – 2.69 (m, 2H), 2.39 (dq, *J* = 17.8, 9.4 Hz, 2H), 2.22 (dt, *J* = 11.7, 6.0 Hz, 1H), 2.11 (t, *J* = 5.4 Hz, 1H), 1.93 – 1.82 (m, 1H), 1.74 – 1.54 (m, 2H), 1.50 (s, 3H), 1.41 (d, *J* = 10.7 Hz, 1H), 1.30 (s, 3H), 0.88 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.5, 156.7, 135.0, 134.5, 133.5, 133.3, 132.3, 128.4, 128.4, 127.8, 127.8, 127.6, 126.5, 126.4, 126.3, 125.7, 125.4, 85.9, 77.8, 77.3, 53.6, 51.4, 46.4, 45.5, 41.1, 40.2, 39.7, 38.3, 35.6, 29.0, 28.8, 27.3, 27.2, 27.0, 26.3, 24.1.

1-benzyl-1-methyl-3-((S)-3-(naphthalen-2-yl)-1-oxo-1-((R)-2-((3aS,4S,6S,7aR)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl) pyrrolidin-1-yl) propan-2-yl) urea (8e)

Using the same procedure as **8a**, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 – 7.75 (m, 3H), 7.74 (d, *J* = 8.4 Hz, 1H), 7.49 (d, *J* = 8.4 Hz, 1H), 7.47 – 7.40 (m, 2H), 7.32 – 7.18 (m, 4H), 7.15 (d, *J* = 6.9 Hz, 2H), 5.44 (d, *J* = 7.9 Hz, 1H), 4.93 (q, *J* = 7.6 Hz, 1H), 4.57 – 4.38 (m, 2H), 4.38 (d, *J* = 8.7 Hz, 1H), 3.52 (t, *J* = 8.7 Hz, 1H), 3.27 – 3.10 (m, 2H), 3.13 – 3.04 (m, 1H), 2.80 (s, 3H), 2.54 (q, *J* = 8.7 Hz, 1H), 2.41 (t, *J* = 11.5 Hz, 1H), 2.20 (d, *J* = 7.8 Hz, 1H), 2.10 (t, *J* = 5.0 Hz, 1H), 1.96 (d, *J* = 12.4 Hz, 2H), 1.78 – 1.60 (m, 3H), 1.62 – 1.50 (m, 1H), 1.48 (s, 3H), 1.39 (d, *J* = 10.4 Hz, 1H), 1.31 (s, 3H), 0.89 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.3, 157.5, 137.8, 134.4, 133.4, 132.3, 128.6, 128.5, 128.4, 127.8, 127.7, 127.6, 127.4, 127.2, 125.7, 125.4, 53.4, 52.0, 51.4, 46.4, 40.0, 39.6, 38.3, 35.6, 34.0, 28.7, 27.2, 27.2, 27.1, 26.3, 24.1.

Pyrazine-2-carbonyl-L-phenylalanine-L-proline boronic acid (I-1)

To a solution of **7** (0.45 mmol) in methanol (10 mL) and water (5 mL) was added phenylboronic acid (0.68 mmol). Then hexane (15 mL) was added and the bi-phasic mixture was stirred vigorously. HCl (4 N, 2 mmol) was dropped into react mixture and the hexane layer was periodically removed and replaced with fresh hexane 6 times over a 24-hour period. The methanol/aqueous layer was separated and was extracted with dichloromethane three times, and dried over anhydrous Na_2SO_4. Then the solvent was evaporated to give crude product and the white foam **I-1** was isolated by crystallization in hexane and ethyl acetate, (120 mg, 32% yield of two steps); mp: 103 - 107℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.27 (s, 1H), 8.64 (s, 2H), 8.48 (s, 1H), 7.32-7.13 (m, 5H), 5.03 (m, 1H), 3.32–2.90 (m, 3H), 2.72 (dt, J = 16.1, 13.0 Hz, 1H), 2.56 (s, 1H), 1.91 –1.59 (m, 2H), 1.53 (s, 1H), 1.41 (d, J = 14.3 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.2, 162.6, 147.4, 147.2, 144.6, 142.8, 135.8, 129.4, 128.7, 127.3, 51.1, 45.8, 39.5, 39.3, 27.3, 25.9. HRMS (ESI) calcd for $C_{19}H_{22}BN_4O_3$: 365.1782 [(M-H_2O+CH_2+H)$^+$], found: 365.1783.

(1, 2, 3, 4-tetrahydroisoquinoline-2-carbonyl)-L-phenylalanine-L-proline boronic acid (I-2)

Using the same procedure as **I-1**, white solid, 41% yield; mp: 123 - 126 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.32 (d, *J* = 7.4 Hz, 2H), 7.24 (t, *J* = 7.3 Hz, 2H), 7.14 (m, 5H), 4.51 – 4.45 (m, 1H), 4.43 (s, 2H), 3.73 (t, *J* = 7.6 Hz, 1H), 3.58 – 3.43 (m, 2H), 3.38 (q, *J* = 8.1 Hz, 1H), 3.01 (d, *J* = 12.0 Hz, 1H), 2.91 – 2.81 (m, 2H), 2.76 – 2.62 (m, 2H), 2.00 – 1.84 (m, 2H), 1.82 – 1.73 (m, 1H), 1.68 – 1.55 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.7, 157.4, 139.2, 135.2, 134.4, 129.8, 129.0, 128.5, 126.7, 126.6, 126.4, 54.4, 48.2, 46.6, 45.6, 41.5, 36.8, 28.4, 27.4, 27.3. HRMS (ESI) calcd for $C_{24}H_{29}BN_3O_3$: 418.2297 [(M-H_2O+CH_2+H)$^+$], found 418.2305.

Morpholine-4-carbonyl-L-phenylalanine-L-proline boronic acid (I-3)

Using the same procedure as **I-1**, white solid, 37% yield; mp: 143 - 146 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.32 – 7.22 (m, 4H), 7.22 – 7.15 (m, 1H), 4.43 (dd, *J* = 10.2, 4.1 Hz, 1H), 3.71 (td, *J* = 9.6, 8.8, 3.1 Hz, 1H), 3.52 – 3.41 (m, 4H), 3.37 (q, *J* = 9.1 Hz, 1H), 3.30 – 3.15 (m, 5H), 2.99 (dd, *J* = 13.9, 4.0 Hz, 1H), 2.89 (dd, *J* = 10.0, 7.0 Hz, 1H), 2.80 (dd, *J* = 13.8, 10.2 Hz, 1H), 2.02 – 1.70 (m, 3H), 1.68 – 1.54 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.6, 157.7, 139.2, 129.8, 128.5, 126.6, 66.3, 54.4, 46.6, 44.3, 36.9, 27.4, 27.3. HRMS (ESI) calcd for $C_{19}H_{27}BN_3O_4$: 372.2089 [(M-H_2O+CH_2+H)$^+$], found 372.2080.

(1, 2, 3, 4-tetrahydroisoquinoline-2-carbonyl)-L-(2-naphthyl)-alanine-L-proline boronic acid (I-4)

Using the same procedure as **I-1**, white solid, 52% yield; mp: 127 - 130 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.88 – 7.74 (m, 4H), 7.56 – 7.39 (m, 3H), 7.20 – 7.03 (m, 4H), 4.59 (dd, *J* = 10.4, 3.8 Hz, 1H), 4.42 (s, 2H), 3.76 (t, *J* = 8.3 Hz, 1H), 3.59 – 3.37 (m, 3H), 3.25 – 3.15 (m, 1H), 3.06 – 2.96 (m, 1H), 2.91 (t, *J* = 8.6 Hz, 1H), 2.77 – 2.60 (m, 2H), 2.00 – 1.72 (m, 3H), 1.67 – 1.58 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.6, 157.4, 136.9, 135.1, 134.4, 133.4, 132.1, 129.0, 128.6, 128.0, 127.8, 126.7, 126.5, 126.4, 126.3, 125.8, 54.3, 46.6, 45.7, 41.5, 37.1, 28.4, 27.4, 27.3. HRMS (ESI) calcd for $C_{28}H_{31}BN_3O_3$: 468.2453 [(M-H_2O+CH_2+H)$^+$], found 468.2443.

(N-methylbenzylamine-N-carbonyl)-L-(2-naphthyl)-alanine-L-proline boronic acid (I-5)

Using the same procedure as **I-1,** white solid, 33% yield; mp: 121 - 124 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.91 – 7.85 (m, 1H), 7.85 – 7.77 (m, 3H), 7.57 – 7.42 (m, 3H), 7.18 – 7.06 (m, 3H), 6.94 (d, *J* = 7.3 Hz, 2H), 4.69 (dd, *J* = 10.1, 4.1 Hz, 1H), 4.45 – 4.17 (m, 2H), 3.75 (t, *J* = 7.4 Hz, 1H), 3.46 (q, *J* = 8.1 Hz, 1H), 3.21 (dd, *J* = 12.4, 2.9 Hz, 1H), 3.06 – 2.88 (m, 2H), 2.67 (s, 3H), 1.99 – 1.74 (m, 3H), 1.71 – 1.56 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.5, 157.9, 138.8, 136.8, 133.4, 132.2, 128.7, 128.6, 128.0, 127.9, 127.8, 127.5, 127.2, 126.3, 125.8, 54.2, 51.4, 46.7, 37.2, 34.1, 27.4, 27.3. HRMS (ESI) calcd for $C_{27}H_{31}BN_3O_3$: 456.2452 [(M-H_2O+CH_2+H)$^+$], found 456.2457.

N-Cbz-L-(2-naphthyl)-alanine-L-proline boronic acid (I-6)

Using the same procedure as **I-1,** white solid, 41% yield; mp: 105 - 108 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.87 (d, *J* = 7.4 Hz, 1H), 7.85 – 7.78 (m, 3H), 7.55 – 7.42 (m, 3H), 7.28 – 7.12 (m, 5H), 4.95 – 4.80 (m, 2H), 4.51 (dd, *J* = 10.5, 3.5 Hz, 1H), 3.67 (t, *J* = 8.7 Hz, 1H), 3.47 (q, *J* = 8.5 Hz, 1H), 3.20 (dd, *J* = 13.8, 3.4 Hz, 1H), 2.97 – 2.83 (m, 2H), 1.99 – 1.73 (m, 3H), 1.69 – 1.52 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.4, 156.3, 137.4, 136.4, 133.4, 132.2, 128.7, 128.4, 128.1, 128.0, 127.9, 127.8, 127.8, 126.4, 125.9, 65.6, 54.5, 46.8, 37.1, 27.4, 27.3. HRMS (ESI) calcd for $C_{26}H_{28}BN_2O_4$: 443.2137 [(M-H_2O+CH_2+H)$^+$], found 443.2141.

(S)-4-Methyl-2-(pyrazine-2-carboxamido) pentanoic acid (7g)

Using the same procedure as **7a,** white solid, mp: 136-138℃. ^{1}H-NMR (DMSO-d_6, 400MHz) δ 0.88~0.91 (t，6H)，1.57~1.64(m，2H),1.81~1.91(m, 1H)，4.48~4.55(m，1H)，8.77(q，1H)，8.90~8.95(dd，2H)，9.19(d，1H)，12.81(s，1H).

(S)-Methyl 2-((S)-4-methyl-2-(pyrazine-2-carboxamido) pentanamido)-3- (naphthalen-2-yl) propanoate (9g)

Using the same procedure as **6a**, colorless oil, 73% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.21 (d, *J* = 1.3 Hz, 1H), 8.66 (d, *J* = 2.4 Hz, 1H), 8.36 (dd, *J* = 2.3, 1.5 Hz, 1H), 8.04 (d, *J* = 8.7 Hz, 1H), 7.72 – 7.56 (m, 3H), 7.51 (s, 1H), 7.32 (dd, *J* = 6.2, 3.3 Hz, 2H), 7.24 – 7.05 (m, 2H), 5.02 (dd, *J* = 13.6, 6.8 Hz, 1H), 4.76 (td, *J* = 8.6, 5.9 Hz, 1H), 3.74 (s, 3H), 3.25 (ddd, *J* = 20.8, 14.0, 6.2 Hz, 2H), 1.76 (dt, *J* = 15.6, 4.9 Hz, 1H), 1.66 (dt, *J* = 12.0, 5.5 Hz, 2H), 0.90 (t, *J* = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.9, 171.3, 162.9, 147.3, 144.2, 143.7, 142.6, 133.2, 132.3, 128.1, 128.1, 127.5, 127.2, 126.0, 125.6, 53.1, 52.4, 51.5, 40.9, 38.0, 24.8, 22.9, 22.1.

(S)-Methyl 2-((S)-4-methyl-2-(pyrazine-2-carboxamido) pentanamido)-3- phenylpropanoate (9h)

Using the same procedure as **6a**, colorless oil, 79% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.33 (d, *J* = 1.4 Hz, 1H), 8.75 (d, *J* = 2.4 Hz, 1H), 8.52 (dd, *J* = 2.4, 1.5 Hz, 1H), 8.14 (d, *J* = 8.8 Hz, 1H), 7.16 – 6.88 (m, 6H), 4.83 (ddt, *J* = 17.4, 8.7, 6.0 Hz, 2H), 3.07 (ddd, *J*

= 32.4, 13.9, 6.1 Hz, 2H), 1.86 – 1.58 (m, 3H), 0.91 (t, *J* = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.8, 171.3, 162.9, 147.5, 144.4, 143.9, 142.7, 135.6, 129.2, 128.4, 126.8, 53.2, 52.4, 51.5, 41.0, 37.8, 24.8, 22.9, 22.1.

(S)-Methyl 3-phenyl-2-((S)-3-phenyl-2-(pyrazine-2-carboxamido) propanamido)-propanoate (9i)

Using the same procedure as **6a**, colorless oil, 81% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.29 (d, *J* = 1.1 Hz, 1H), 8.75 (d, *J* = 2.4 Hz, 1H), 8.63 – 8.46 (m, 1H), 8.34 (d, *J* = 8.4 Hz, 1H), 7.27 – 7.15 (m, 5H), 7.11 – 6.91 (m, 5H), 6.77 (d, *J* = 7.8 Hz, 1H), 4.92 (ddd, *J* = 19.9, 14.5, 6.6 Hz, 2H), 3.70 (s, 3H), 3.27 – 3.13 (m, 2H), 3.05 (ddd, *J* = 30.3, 13.8, 6.1 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.4, 170.1, 162.9, 147.5, 144.4, 143.8, 142.7, 136.3, 135.6, 129.4, 129.1, 128.6, 128.4, 127.0, 126.9, 54.2, 53.4, 52.4, 38.2, 37.9.

(S)-Methyl 3-(naphthalen-2-yl)-2-((S)-3-phenyl-2-(pyrazine-2-carboxamido)-propanamido) propanoate (9j)

Using the same procedure as **6a**, colorless oil, 83% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.17 (d, *J* = 1.3 Hz, 1H), 8.66 (d, *J* = 2.4 Hz, 1H), 8.36 (dd, *J* = 2.2, 1.6 Hz, 1H), 8.27 (d, *J* = 8.5 Hz, 1H), 7.76 – 7.53 (m, 3H), 7.47 – 7.33 (m, 3H), 7.21 – 7.09 (m, 3H), 6.88 (d, *J* = 7.7 Hz, 1H), 5.01 (dq, *J* = 14.1, 6.9 Hz, 2H), 3.73 (s, 3H), 3.36 – 3.11 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.4, 170.2, 162.8, 147.4, 144.2, 143.6, 142.6, 136.3, 133.2, 133.1, 132.3, 129.4, 128.6, 128.2, 128.0, 127.5, 127.1, 127.0, 126.0, 125.6, 54.2, 53.2, 52.4, 38.2, 38.1.

(S)-Methyl 4-methyl-2-((S)-4-methyl-2-(pyrazine-2-carboxamido) pentanamido) pentanate (9k)

Using the same procedure as **6a**, colorless oil, 68% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.37 (d, *J* = 1.2 Hz, 1H), 8.74 (d, *J* = 2.4 Hz, 1H), 8.51 (dd, *J* = 2.3, 1.6 Hz, 1H), 8.24 (d, *J* = 8.8 Hz, 1H), 6.97 (d, *J* = 8.1 Hz, 1H), 4.68 (dtd, *J* = 91.7, 8.4, 5.5 Hz, 2H), 3.71 (s, 3H), 1.97 – 1.26 (m, 6H), 0.92 (t, *J* = 5.9 Hz, 6H), 0.82 (d, *J* = 6.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.2, 171.4, 163.0, 147.5, 144.4, 144.0, 142.8, 52.2, 51.6, 50.8, 41.4, 41.2, 24.8, 24.7, 22.9, 22.6, 22.1, 21.9.

(S)-Methyl-2-((S)-2-(((benzyloxy)carbonyl)amino)-4-methylpentanamido)-3- (naphthalen-2-yl)propanoate (9m)

Using the same procedure as **6a**, colorless oil, 93% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.73 (m, 3H), 7.60 (s, 1H), 7.54 – 7.44 (m, 2H), 7.41 – 7.18 (m, 6H), 6.67 (d, *J* = 7.1 Hz, 1H), 5.24 (d, *J* = 8.2 Hz, 1H), 5.17 – 4.88 (m, 3H), 4.22 (d, *J* = 4.0 Hz, 1H), 3.72 (s, 3H), 3.29 (ddd, *J* = 33.7, 13.8, 5.9 Hz, 2H), 1.74 – 1.56 (m, 2H), 1.53 – 1.41 (m, 1H), 0.90 (d, *J* = 5.5 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.9, 171.9, 156.1, 136.2, 133.4, 133.3, 132.5, 128.5, 128.2, 128.2, 128.0, 127.7, 127.3, 126.2, 125.8, 67.0, 53.5, 53.2, 52.4, 41.2, 38.0, 24.6, 22.8, 21.9.

(S)-Methyl 2-((S)-2-(((benzyloxy)carbonyl)amino)-4-methylpentanamido)-4-methyl pentanoate (9n)

Using the same procedure as **6a**, colorless oil, 88% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 – 7.29 (m, 5H), 6.47 (s, 1H), 5.30 (s, 1H), 5.12 (s, 2H), 4.61 (td, *J* = 8.6, 5.0 Hz, 1H), 4.25 (d, *J* = 4.8 Hz, 1H), 3.74 (s, 3H), 1.77 – 1.60 (m, 4H), 1.60 – 1.45 (m, 2H), 0.94 (dd, *J* = 9.5, 5.9 Hz, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.2, 172.0, 156.2, 136.2, 128.5, 128.2, 128.0, 67.0, 53.4, 52.3, 50.7, 41.4, 24.8, 24.6, 22.9, 22.8, 22.1, 21.9.

Methyl (1, 2, 3, 4-tetrahydroisoquinoline-2-carbonyl)-L-phenylalanyl-L-phenylalaninate (9o)

Using the same procedure as **6a**, colorless oil, 65% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 – 7.06 (m, 12H), 6.98 (d, *J* = 6.6 Hz, 2H), 6.82 (d, *J* = 7.5 Hz, 1H), 5.15 (d, *J* = 7.4 Hz, 1H), 4.81 – 4.71 (m, 1H), 4.65 (q, *J* = 6.9 Hz, 1H), 4.43 (s, 2H), 3.66 (s, 3H), 3.54 (dt, *J* = 11.8, 5.8 Hz, 1H), 3.45 (dt, *J* = 12.1, 5.8 Hz, 1H), 3.15 – 2.92 (m, 4H), 2.79 (d, *J* = 4.2 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.8, 171.5, 156.8, 136.9, 135.8, 134.9, 133.1, 129.5, 129.2, 128.6, 128.4, 128.4, 127.0, 126.9, 126.8, 126.5, 126.4, 55.4, 53.4, 52.3, 45.4, 41.3, 38.3, 37.9, 28.9.

Methyl (S)-3-(naphthalen-2-yl)-2-((S)-3-phenyl-2-(1, 2, 3, 4-tetrahydroisoquinoline-2-carboxamido) propanamido) propanoate (9p)

Using the same procedure as **6a**, colorless oil, 77% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 – 7.66 (m, 1H), 7.63 (t, *J* = 7.8 Hz, 2H), 7.42 (s, 1H), 7.36 (dd, *J* = 6.2, 3.2 Hz, 2H), 7.25 – 7.06 (m, 9H), 7.03 – 6.93 (m, 2H), 5.05 (d, *J* = 7.4 Hz, 1H), 4.88 (q, *J* = 6.5 Hz, 1H), 4.69 (q, *J* = 6.8 Hz, 1H), 4.32 (d, *J* = 3.6 Hz, 2H), 3.66 (s, 3H), 3.34 (ddq, *J* = 24.0, 12.1, 5.8 Hz, 2H), 3.22 (dd, *J* = 13.8, 5.8 Hz, 1H), 3.13 (dt, *J* = 13.4, 6.5 Hz, 2H), 3.02 (dd, *J* = 13.7, 6.9 Hz, 1H), 2.67 (q, *J* = 5.7 Hz, 2H).

Methyl (1, 2, 3, 4-tetrahydroisoquinoline-2-carbonyl)-L-phenylalanyl-L-tyrosinate (9q)

Using the same procedure as **6a**, colorless oil, 77% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 – 7.13 (m, 7H), 7.11 – 7.08 (m, 1H), 7.06 – 7.01 (m, 1H), 6.87 (d, *J* = 7.9 Hz, 1H), 6.82 (d, *J* = 8.3 Hz, 2H), 6.63 (d, *J* = 8.3 Hz, 2H), 5.25 (d, *J* = 7.8 Hz, 1H), 4.74 (q, *J* = 6.3 Hz, 1H), 4.67 (q, *J* = 7.1 Hz, 1H), 4.42 (q, *J* = 15.6 Hz, 2H), 3.66 (s, 3H), 3.54 (dt, *J* = 11.9, 5.7 Hz, 1H), 3.42 (dt, *J* = 12.2, 5.9 Hz, 1H), 3.03 (d, *J* = 6.9 Hz, 2H), 2.98 (dd, *J* = 14.0, 5.4 Hz, 1H), 2.88 (dd, *J* = 14.0, 6.5 Hz, 1H), 2.76 (h, *J* = 10.3 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.0, 171.6, 156.9, 155.7, 136.7, 134.9, 133.1, 130.3, 129.4, 128.5, 128.3, 126.9, 126.8, 126.4, 126.4, 115.6, 55.4, 53.7, 52.3, 45.4, 41.3, 38.6, 37.1, 28.8.

Methyl (1, 2, 3, 4-tetrahydroisoquinoline-2-carbonyl)-L-phenylalanyl-L-leucinate (9r)

Using the same procedure as **6a**, colorless oil, 64% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 – 7.08 (m, 9H), 6.85 (d, *J* = 7.9 Hz, 1H), 4.85 (dt, *J* = 20.4, 7.3 Hz, 2H), 4.52 (s, 2H), 4.41 (td, *J* = 8.5, 5.6 Hz, 1H), 3.70 (s, 3H), 3.63 (ddd, *J* = 11.8, 6.5, 5.1 Hz, 1H), 3.53

(ddd, *J* = 12.2, 6.7, 5.3 Hz, 1H), 3.14 (dd, *J* = 13.9, 5.8 Hz, 1H), 3.04 (dd, *J* = 13.9, 6.8 Hz, 1H), 2.86 (td, *J* = 6.1, 3.1 Hz, 2H), 1.75 – 1.58 (m, 2H), 1.58 – 1.44 (m, 1H), 0.91 (dd, *J* = 6.3, 4.0 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.0, 171.8, 157.0, 135.9, 135.0, 133.2, 129.2, 128.4, 128.4, 127.0, 126.8, 126.5, 126.4, 53.2, 52.6, 52.3, 45.4, 41.3, 41.3, 38.0, 29.0, 24.8, 22.9, 22.3.

Methyl (1,2,3,4-tetrahydroisoquinoline-2-carbonyl)-L-leucinate (6s)

Using the same procedure as **6b**, colorless oil, 88% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 – 7.07 (m, 4H), 5.14 (d, *J* = 8.0 Hz, 1H), 4.58 (t, *J* = 7.8 Hz, 1H), 4.55 (s, 2H), 3.73 (s, 3H), 3.67 (dt, *J* = 11.5, 5.6 Hz, 1H), 3.55 (dt, *J* = 12.0, 5.8 Hz, 1H), 2.84 (q, *J* = 6.2 Hz, 2H), 1.74 (dp, *J* = 13.3, 6.6 Hz, 1H), 1.67 – 1.50 (m, 2H), 0.96 (s, 3H), 0.94 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.3, 157.1, 135.0, 133.3, 128.3, 126.6, 126.4, 52.2, 52.2, 45.4, 41.8, 41.2, 29.0, 24.9, 22.9, 22.0.

Methyl (S)-2-((S)-4-methyl-2-(1,2,3,4-tetrahydroisoquinoline-2-carboxamido) pentanamido)-3-(naphthalen-2-yl) propanoate (9s)

Using the same procedure as **6a**, colorless oil, 72% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 – 7.63 (m, 3H), 7.59 (s, 1H), 7.41 – 7.33 (m, 2H), 7.26 – 7.22 (m, 1H), 7.19 (dt, *J* = 7.3, 3.7 Hz, 2H), 7.16 – 7.11 (m, 1H), 7.08 (dd, *J* = 5.3, 3.6 Hz, 1H), 6.99 (d, *J* = 7.8 Hz, 1H), 4.92 (q, *J* = 6.9 Hz, 1H), 4.82 (d, *J* = 8.0 Hz, 1H), 4.46 – 4.36 (m, 3H), 3.50 (dt, *J* = 11.8, 5.8 Hz, 1H), 3.41 (dt, *J* = 12.1, 5.9 Hz, 1H), 3.32 (dd, *J* = 13.9, 5.7 Hz, 1H), 3.19 (dd, *J* = 13.9, 6.9 Hz, 1H), 2.77 (t, *J* = 5.4 Hz, 2H), 1.72 – 1.60 (m, 2H), 1.50 (q, *J* = 8.7, 8.2 Hz, 1H), 0.89 (t, *J* = 5.8 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.1, 171.9, 157.0, 135.0, 133.5, 133.4, 133.2, 132.4, 128.4, 128.1, 128.0, 127.6, 127.6, 127.3, 126.7, 126.4, 126.4, 126.0, 125.6, 53.2, 52.7, 52.4, 45.3, 41.2, 41.2, 38.1, 29.0, 24.9, 22.9, 22.2.

Methyl (benzyl (methyl) carbamoyl)-L-leucinate (6t)

Using the same procedure as **6b**, colorless oil, 70% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (t, *J* = 7.5 Hz, 2H), 7.23 (d, *J* = 7.8 Hz, 3H), 5.03 (d, *J* = 8.0 Hz, 1H), 4.54 (q, *J* = 7.8 Hz, 1H), 4.48 (s, 2H), 3.70 (s, 3H), 2.88 (s, 3H), 1.71 – 1.44 (m, 3H), 0.92 (t, *J* = 5.7 Hz, 6H).^{13}C NMR (101 MHz, $CDCl_3$) δ 175.0, 157.9, 137.7, 128.6, 127.3, 127.2, 52.3, 52.1, 52.1, 41.6, 34.2, 24.8, 22.9, 21.9.

Methyl (S)-2-((S)-2-(3-benzyl-3-methylureido)-4-methylpentanamido)-3-(naphthalen-2-yl) propanoate (9t)

Using the same procedure as **6a**, colorless oil, 77% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 – 7.72 (m, 3H), 7.62 (s, 1H), 7.49 – 7.41 (m, 2H), 7.35 – 7.21 (m, 4H), 7.16 (d, *J* = 7.2 Hz, 2H), 4.94 (q, *J* = 6.6 Hz, 1H), 4.75 (d, *J* = 7.6 Hz, 1H), 4.46 – 4.36 (m, 2H), 4.26 (d, *J* = 16.0 Hz, 1H), 3.71 (s, 3H), 3.34 (dd, *J* = 13.9, 5.7 Hz, 1H), 3.23 (dd, *J* = 13.8, 6.7 Hz, 1H), 2.77 (s, 3H), 1.62 (dt, *J* = 12.9, 6.6 Hz, 1H), 1.53 (dt, *J* = 12.8, 6.5 Hz, 1H), 1.49 – 1.37 (m, 1H), 1.33 (dd, *J* = 19.5, 11.1 Hz, 1H), 0.87 (d, *J* = 6.2 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.1, 171.9, 157.9, 137.6, 133.6, 133.4, 132.4, 128.7, 128.1, 127.7, 127.6, 127.4, 127.3, 127.1, 126.0, 125.8, 53.2, 52.9, 52.3, 52.1, 50.0, 38.0, 34.4, 24.7, 23.0, 22.0.

(+)-Pinanediol-N-(2-pyrazinecarbonyl)-L-leucine-L-(2-naphthyl)-alanine-L-proline boronate (8g)

Using the same procedure as **8a**, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.33 (s, 1H), 8.71 (d, *J* = 2.4 Hz, 1H), 8.44 (d, *J* = 1.4 Hz, 1H), 8.05 (d, *J* = 8.8 Hz, 1H), 7.75 – 7.59 (m, 4H), 7.43 (d, *J* = 8.3 Hz, 1H), 7.34 (dd, *J* = 9.1, 5.2 Hz, 2H), 7.02 (d, *J* = 8.0 Hz, 1H), 4.94 (dt, *J* = 13.7, 7.0 Hz, 1H), 4.66 (dd, *J* = 15.3, 7.7 Hz, 1H), 4.36 (d, *J* = 7.4 Hz, 1H), 3.52 – 3.39 (m, 1H), 3.29 – 3.00 (m, 3H), 2.54 (dd, *J* = 17.1, 8.8 Hz, 1H), 2.44 – 2.31 (m, 1H), 2.19 (dd, *J* = 10.9, 5.5 Hz, 1H), 2.08 (t, *J* = 5.3 Hz, 1H), 1.97 – 1.86 (m, 3H), 1.68 (dd, *J* = 16.0, 10.9 Hz, 4H), 1.57 (ddd, *J* = 21.5, 10.6, 4.4 Hz, 1H), 1.47 (s, 3H), 1.42 – 1.35 (m, 1H), 1.29 (s, 3H), 1.25 (dd, *J* = 8.5, 4.7 Hz, 1H), 0.91 (t, *J* = 6.1 Hz, 6H), 0.87 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.9, 168.8, 162.8, 147.3, 144.4, 144.0, 142.6, 133.7, 133.3, 132.2, 128.5, 128.1, 127.8, 127.6, 127.4, 125.7, 125.4, 85.9, 77.8, 52.1, 51.7, 51.3, 46.4, 41.5, 39.6, 39.4, 38.2, 35.6, 28.7, 27.1, 27.0, 26.3, 24.8, 24.1, 23.1, 21.8.

(+)-Pinanediol-N-(2-pyrazinecarbonyl)-L-leucine-L-phenylalanine-L-proline boronate (8h)

Using the same procedure as **8a**, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.35 (d, *J* = 1.4 Hz, 1H), 8.70 (d, *J* = 2.4 Hz, 1H), 8.46 (dd, *J* = 2.4, 1.5 Hz, 1H), 8.11 (d, *J* = 8.7 Hz, 1H), 7.28 – 7.09 (m, 7H), 4.98 – 4.59 (m, 2H), 4.36 – 4.26 (m, 1H), 3.58 – 3.44 (m, 1H), 3.19 – 2.91 (m, 3H), 2.00 – 1.92 (m, 1H), 1.90 – 1.77 (m, 4H), 1.74 – 1.62 (m, 4H), 1.40 (s, 3H), 1.31 (d, *J* = 1.9 Hz, 1H), 1.26 (s, 3H), 1.02 – 0.92 (m, 2H), 0.95 – 0.87 (m, 6H), 0.84 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.2, 169.2, 162.9, 147.2, 144.4, 142.6, 136.0, 129.9, 128.2, 126.8, 126.7, 85.9, 77.8, 52.4, 51.8, 51.3, 46.6, 41.4, 39.5, 38.8, 38.2, 35.4, 28.6, 27.2, 27.1, 26.2, 24.8, 24.0, 23.1, 21.7.

(+)-Pinanediol-N-(2-pyrazinecarbonyl)-L-phenylalanine-L-phenylalanine-L-proline boronate (8i)

Using the same procedure as **8a**, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.34 (d, *J* = 1.3 Hz, 1H), 8.74 (d, *J* = 2.4 Hz, 1H), 8.51 (dd, *J* = 2.3, 1.5 Hz, 1H), 8.24 (d, *J* = 8.4 Hz, 1H), 7.26 – 7.08 (m, 10H), 6.76 (t, *J* = 14.5 Hz, 1H), 4.90 – 4.74 (m, 2H), 4.40 – 4.29 (m, 1H), 3.22 – 3.08 (m, 0H), 3.03 – 2.85 (m, 0H), 2.53 (dd, *J* = 17.6, 8.1 Hz, 1H), 2.43 – 2.30 (m, 1H), 2.20 (dd, *J* = 10.7, 6.1 Hz, 1H), 2.06 (dd, *J* = 10.2, 4.8 Hz, 1H), 1.98 – 1.91 (m, 2H), 1.79 (dt, *J* = 16.2, 8.1 Hz, 2H), 1.62 (ddd, *J* = 12.1, 11.1, 5.6 Hz, 1H), 1.44 (s, 3H), 1.30 (s, 3H), 0.87 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.5, 168.4, 162.7, 147.4, 144.4, 142.7, 136.2, 130.0, 129.5, 129.3, 128.5, 128.2, 127.0, 126.6, 85.9, 77.8, 54.3, 52.4, 51.3, 46.4, 39.2, 38.4, 38.2, 35.5, 28.6, 27.2, 27.1, 26.2, 24.1.

(+)-Pinanediol-N-(2-pyrazinecarbonyl)-L-phenylalanine-L-(2-naphthyl)-alanine-L-proline boronate (8j)

Using the same procedure as **8a**, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.31 (d, *J* = 1.3 Hz, 1H), 8.71 (d, *J* = 2.2 Hz, 1H), 8.44 (dd, *J* = 2.2, 1.5 Hz, 1H), 8.22 (d, *J* = 8.4 Hz, 1H), 7.74 (dd, *J* = 7.0, 4.9 Hz, 3H), 7.54 – 7.34 (m, 4H), 7.25 – 7.15 (m, 5H), 7.03 (d, *J* = 7.9 Hz, 1H), 4.99 – 4.80 (m, 2H), 3.41 (t, *J* = 6.9 Hz, 1H), 3.15 (ddt, *J* = 18.7, 15.6, 4.7 Hz, 5H), 2.55 (dd, *J* = 17.3, 8.6 Hz, 1H), 2.46 –

2.35 (m, 1H), 2.19 (dd, *J* = 10.4, 5.9 Hz, 1H), 2.11 (s, 1H), 1.94 (dd, *J* = 10.0, 2.0 Hz, 3H), 1.72 (dd, *J* = 14.4, 7.6 Hz, 2H), 1.63 – 1.53 (m, 1H), 1.48 (s, 3H), 1.31 (s, 3H), 0.89 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.7, 168.7, 162.7, 147.3, 144.3, 143.9, 142.7, 136.2, 133.7, 133.4, 132.3, 129.3, 128.6, 128.4, 128.0, 127.7, 127.5, 127.0, 125.8, 125.4, 86.0, 77.8, 54.3, 52.2, 51.4, 46.5, 39.6, 39.3, 38.3, 38.3, 35.6, 28.6, 27.1, 27.1, 26.3, 24.1.

(+)-Pinanediol-N-(2-pyrazinecarbonyl)-L-leucine-L-leucine-L-proline boronate (8k)

Using the same procedure as **8a**, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.37 (d, *J* = 1.3 Hz, 1H), 8.74 (d, *J* = 2.4 Hz, 1H), 8.56 – 8.51 (m, 1H), 8.16 (d, *J* = 8.8 Hz, 1H), 6.72 (d, *J* = 8.5 Hz, 1H), 4.89 – 4.50 (m, 2H), 4.32 – 4.22 (m, 1H), 3.85 – 3.73 (m, 1H), 3.42 (dd, *J* = 16.1, 9.2 Hz, 1H), 3.18 (dd, *J* = 9.9, 7.0 Hz, 1H), 2.30 (dd, *J* = 8.2, 5.7 Hz, 1H), 2.15 – 2.00 (m, 3H), 2.00 – 1.93 (m, 2H), 1.91 – 1.82 (m, 2H), 1.73 – 1.61 (m, 4H), 1.56 – 1.48 (m, 2H), 1.38 (s, 3H), 1.26 (s, 3H), 0.91 (ddd, *J* = 21.7, 10.1, 6.6 Hz, 12H), 0.82 (s, 3H).

(+)-Pinanediol-N-Cbz-L-phenylalanine-L-(2-naphthyl)-alanine-L-proline boronate (8l)

Using the same procedure as **8a**, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 – 7.73 (m, 4H), 7.53 – 7.42 (m, 3H), 7.38 – 7.20 (m, 9H), 7.15 (d, *J* = 6.6 Hz, 2H), 6.93 (d, *J* = 7.7 Hz, 1H), 5.35 (d, *J* = 8.3 Hz, 1H), 5.07 (q, *J* = 12.3 Hz, 2H), 4.89 (td, *J* = 8.6, 4.8 Hz, 1H), 4.50 (d, *J* = 7.0 Hz, 1H), 4.38 (d, *J* = 7.3 Hz, 1H), 3.39 – 3.28 (m, 1H), 3.21 (dd, *J* = 12.9, 7.9 Hz, 1H), 3.16 – 3.00 (m, 4H), 2.49 – 2.36 (m, 2H), 2.23 (dt, *J* = 8.8, 5.9 Hz, 1H), 2.12 (d, *J* = 5.2 Hz, 1H), 2.00 – 1.87 (m, 3H), 1.72 – 1.60 (m, 2H), 1.51 (s, 3H), 1.33 (s, 3H), 0.91 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.2, 168.6, 155.8, 136.3, 133.9, 133.5, 132.4, 129.3, 128.6, 128.6, 128.5, 127.6, 126.9, 125.8, 125.5, 85.9, 77.8, 67.0, 56.0, 52.3, 51.4, 46.4, 39.6, 38.3, 35.6, 28.7, 27.2, 27.0, 26.3, 24.1.

(+)-Pinanediol-N-Cbz-L-leucine-L-(2-naphthyl)-alanine-L-proline boronate (8m)

Using the same procedure as **8a**, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.74 (m, 4H), 7.74 (d, *J* = 8.4 Hz, 1H), 7.51 – 7.42 (m, 3H), 7.36 (t, *J* = 7.9 Hz, 5H), 7.02 (d, *J* = 7.8 Hz, 1H), 5.36 (d, *J* = 8.5 Hz, 1H), 5.18 – 5.08 (m, 2H), 4.93 (dd, *J* = 12.8, 8.3 Hz, 1H), 4.39 (d, *J* = 7.6 Hz, 1H), 4.34 – 4.20 (m, 1H), 3.41 – 3.32 (m, 1H), 3.30 – 3.21 (m, 1H), 3.18 – 3.01 (m, 2H), 2.50 – 2.37 (m, 2H), 2.29 – 2.19 (m, 1H), 2.13 (t, *J* = 5.4 Hz, 1H), 2.03 – 1.95 (m, 2H), 1.93 – 1.86 (m, 1H), 1.64 (ddd, *J* = 18.4, 13.6, 8.3 Hz, 4H), 1.51 (s, 3H), 1.33 (s, 3H), 0.93 (d, *J* = 7.7 Hz, 6H), 0.90 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.7, 168.8, 156.1, 136.4, 133.9, 133.4, 132.4, 128.6, 128.5, 128.3, 128.1, 128.1, 127.8, 127.6, 125.8, 125.5, 86.0, 77.8, 67.0, 53.7, 52.3, 51.4, 46.5, 41.9, 39.6, 38.3, 35.6, 28.7, 27.2, 27.0, 26.3, 24.6, 24.1, 23.2, 21.7.

(+)-Pinanediol-N-Cbz-L-leucine-L-leucine-L-proline boronate (8n)

Using the same procedure as **8a**, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 – 7.29 (m, 5H), 6.83 (d, *J* = 8.4 Hz, 1H), 5.46 (d, *J* = 8.8 Hz, 1H), 5.09 (dd, *J* = 31.5, 12.3 Hz, 2H), 4.78 (dd, *J* = 13.9, 8.4 Hz, 1H), 4.36 – 4.19 (m, 2H), 3.78 (t, *J* = 7.6 Hz, 1H), 3.42 (dd, *J* = 16.4, 9.0 Hz, 1H), 3.18 (dd, *J* = 16.0, 8.1 Hz, 1H), 2.32 (dd, *J* = 12.5, 10.1 Hz, 1H), 2.15 (dd, *J* = 11.2, 5.5 Hz, 1H), 2.09 – 1.96 (m, 3H), 1.91 – 1.82 (m, 3H), 1.69 – 1.48 (m, 6H), 1.39 (s, 3H), 1.27 (s, 3H), 0.92 (dd, *J* = 9.2, 5.7 Hz, 12H), 0.83 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.8, 170.0, 156.0, 136.4, 128.5, 128.0, 85.8, 77.9, 66.8, 53.6, 51.2, 48.8, 46.7, 41.9, 39.5, 38.2, 35.5, 28.6, 27.2, 27.1, 26.2, 24.6, 24.3, 24.1, 23.2, 23.2, 22.3, 21.7.

N-((S)-1-oxo-1-(((S)-1-oxo-3-phenyl-1-((R)-2-((3aS,4S,6S,7aR)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)pyrrolidin-1-yl) propan-2-yl) amino)-3-phenylpropan-2-yl)-3,4-dihydroisoquinoline-2(1H)-carboxamide (8o)

Using the same procedure as **8a**, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 – 7.12 (m, 14H), 7.10 (q, *J* = 4.9 Hz, 1H), 6.80 (d, *J* = 7.9 Hz, 1H), 4.92 (d, *J* = 7.5 Hz, 1H), 4.76 (q, *J* = 8.1 Hz, 1H), 4.63 (q, *J* = 6.5 Hz, 1H), 4.53 – 4.37 (m, 2H), 4.34 (d, *J* = 8.9 Hz, 1H), 3.60 – 3.53 (m, 1H), 3.46 (dt, *J* = 12.2, 6.5 Hz, 1H), 3.37 (dt, *J* = 10.9, 6.1 Hz, 1H), 3.14 – 3.04 (m, 3H), 2.97 (dd, *J* = 10.0, 7.0 Hz, 2H), 2.83 (d, *J* = 5.3 Hz, 1H), 2.56 (q, *J* = 8.2 Hz, 1H), 2.36 (dt, *J* = 8.5, 5.1 Hz, 1H), 2.20 (dt, *J* = 10.4, 6.1 Hz, 1H), 2.06 (t, *J* = 5.4 Hz, 1H), 1.96 – 1.91 (m, 2H), 1.83 – 1.74 (m, 2H), 1.68 – 1.60 (m, 1H), 1.44 (s, 3H), 1.37 (d, *J* = 10.8 Hz, 1H), 1.30 (s, 3H), 0.86 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.0, 168.6, 156.6, 136.9, 136.4, 135.0, 133.2, 130.0, 129.5, 128.6, 128.5, 128.3, 128.1, 126.8, 126.6, 126.4, 126.4, 85.9, 77.8, 55.3, 52.3, 51.4, 46.4, 45.4, 41.2, 39.6, 39.1, 38.5, 38.2, 35.5, 29.0, 28.6, 27.2, 27.1, 26.3, 24.1.

N-((S)-1-(((S)-3-(naphthalen-2-yl)-1-oxo-1-((R)-2-((3aS,4S,6S,7aR)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)pyrrolidin-1-yl)propan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)-3,4-dihydroisoquinoline-2(1H)-carboxamide (8p)

Using the same procedure as **8a**, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 – 7.72 (m, 2H), 7.70 (s, 1H), 7.68 (d, *J* = 8.4 Hz, 1H), 7.45 – 7.36 (m, 3H), 7.28 – 7.06 (m, 9H), 7.08 – 7.01 (m, 1H), 6.91 (d, *J* = 8.0 Hz, 1H), 4.92 (d, *J* = 7.6 Hz, 1H), 4.86 (dt, *J* = 8.3, 4.2 Hz, 1H), 4.66 (q, *J* = 6.6 Hz, 1H), 4.49 – 4.31 (m, 3H), 3.55 – 3.45 (m, 1H), 3.46 – 3.30 (m, 2H), 3.21 – 3.01 (m, 5H), 2.76 (q, *J* = 5.2 Hz, 2H), 2.53 (q, *J* = 8.7 Hz, 1H), 2.44 – 2.34 (m, 1H), 2.19 (dt, *J* = 10.9, 6.4 Hz, 1H), 1.96 – 1.90 (m, 2H), 1.75 – 1.52 (m, 2H), 1.61 – 1.49 (m, 1H), 1.46 (s, 3H), 1.38 (d, *J* = 10.7 Hz, 1H), 1.30 (s, 3H), 0.87 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.2, 168.6, 156.6, 136.8, 135.0, 134.0, 133.4, 133.2, 132.3, 129.5, 128.6, 128.5, 128.4, 128.3, 128.2, 127.7, 127.5, 126.8, 126.6, 126.4, 125.7, 125.4, 85.9, 77.8, 55.3, 52.2, 51.4, 46.4, 45.4, 41.2, 39.7, 39.3, 38.5, 38.3, 35.6, 28.9, 28.7, 27.2, 27.1, 26.3, 24.1.

N-((S)-1-(((S)-3-(4-hydroxyphenyl)-1-oxo-1-((R)-2-((3aS,4S,6S,7aR)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl) pyrrolidin-1-yl) propan-2-yl) amino)-1-oxo-3-phenylpropan-2-yl)-3,4-dihydroisoquinoline-2(1H)-carboxamide (8q)

Using the same procedure as **8a**, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (s, 1H), 7.25 – 7.02 (m, 12H), 6.94 (d, *J* = 8.1 Hz, 1H), 6.68 (d, *J* = 8.3 Hz, 2H), 5.05 (d, *J* = 7.5 Hz, 1H), 4.73 (q, *J* = 7.6 Hz, 1H), 4.62 (q, *J* = 6.8 Hz, 1H), 4.52 – 4.32 (m, 2H), 4.32 (d, *J* = 7.8 Hz, 1H), 3.54 (dd, *J* = 12.3, 5.9 Hz, 1H), 3.48 – 3.36 (m, 2H), 3.16 – 3.07 (m, 1H), 3.04 (d, *J* = 6.8 Hz, 2H), 2.95 – 2.70 (m, 5H), 2.40 – 2.29 (m, 1H), 2.04 (t, *J* = 5.3 Hz, 1H), 1.96 (dd, *J* = 10.8, 4.9 Hz, 1H), 1.94 – 1.74 (m, 4H), 1.71 – 1.62 (m, 1H), 1.42 (s, 3H), 1.33 (d, *J* = 10.8 Hz, 1H), 1.28 (s, 3H), 0.84 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.4, 168.9, 156.7, 155.5, 136.7, 135.0, 133.2, 131.0, 129.4, 128.6, 128.5, 128.3, 127.5, 126.8, 126.7, 126.4, 115.3, 85.9, 77.8, 55.4, 52.5, 51.4, 46.5, 45.4, 41.2, 39.6, 38.5, 38.2, 38.1, 35.5, 28.9, 28.6, 27.1, 26.3, 24.0.

N-((S)-1-(((S)-4-methyl-1-oxo-1-((R)-2-((3aS,4S,6S,7aR)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl) pyrrolidin-1-yl) pentan-2-yl) amino)-1-oxo-3-phenylpropan-2-yl)-3,4-dihydroisoquinoline-2(1H)-carboxamide (8r)

Using the same procedure as **8a**, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.05 (m, 9H), 6.85 (d, *J* = 8.2 Hz, 1H), 5.20 (d, *J* = 7.2 Hz, 1H), 4.71 (dq, *J* = 13.8, 7.4, 6.9 Hz, 2H), 4.48 (q, *J* = 15.8 Hz, 2H), 4.27 (d, *J* = 8.5 Hz, 1H), 3.74 (t, *J* = 8.7 Hz, 1H), 3.60 (dt, *J* = 12.6, 5.8 Hz, 1H), 3.51 (p, *J* = 6.2 Hz, 1H), 3.42 (q, *J* = 9.2, 8.6 Hz, 1H), 3.22 – 3.13 (m, 1H), 3.15 – 2.95 (m, 2H), 2.81 (p, *J* = 7.8, 6.3 Hz, 2H), 2.38 – 2.25 (m, 1H), 2.17 – 2.05(m, 3H), 1.99 – 1.84 (m, 4H), 1.82 – 1.72 (m, 1H), 1.68 – 1.42 (m, 3H), 1.39 (s, 3H), 1.33 (d, *J* = 10.7 Hz, 1H), 1.27 (s, 3H), 0.89 (t, *J* = 6.7 Hz, 6H), 0.83 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.4, 169.7, 156.6, 136.8, 135.0, 133.3, 129.5, 128.4, 128.3, 126.7, 126.6, 126.4, 85.8, 77.8, 55.2, 51.2, 48.8, 46.6, 45.4, 41.6, 41.2, 39.6, 38.6, 38.2, 35.5, 29.0, 28.6, 27.4, 27.2, 27.1, 26.2, 24.3, 24.1, 23.1, 22.3.

N-((S)-4-methyl-1-(((S)-3-(naphthalen-2-yl)-1-oxo-1-((R)-2-((3aS,4S,6S,7aR)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl) pyrrolidin-1-yl) propan-2-yl) amino)-1-oxopentan-2-yl)-3,4-dihydroisoquinoline-2(1H)-carboxamide (8s)

Using the same procedure as **8a**, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 – 7.70 (m, 3H), 7.69 (d, *J* = 8.4 Hz, 1H), 7.47 (d, *J* = 8.4 Hz, 1H), 7.40 – 7.36 (m, 2H), 7.27 (s, 1H), 7.23 – 7.13 (m, 3H), 7.15 – 7.08 (m, 1H), 6.91 (d, *J* = 8.0 Hz, 1H), 4.91 (dd, *J* = 15.7, 7.7 Hz, 1H), 4.85 (d, *J* = 8.2 Hz, 1H), 4.50 (s, 2H), 4.48 – 4.29 (m, 2H), 3.74 (t, *J* = 5.0 Hz, 1H), 3.61 (dt, *J* = 11.4, 5.6 Hz, 1H), 3.50 (dt, *J* = 12.1, 5.9 Hz, 1H), 3.38 (t, *J* = 8.4 Hz, 1H), 3.21 (d, *J* = 8.8 Hz, 1H), 3.13 (dd, *J* = 12.9, 4.5 Hz, 1H), 3.11 – 3.02 (m, 1H), 2.85 (q, *J* = 5.9 Hz, 2H), 2.50 – 2.38 (m, 2H), 2.27 – 2.16 (m, 1H), 2.10 (t, *J* = 4.9 Hz, 1H), 2.00 – 1.92 (m, 2H), 1.89 – 1.83 (m, 2H), 1.73 – 1.59 (m, 3H), 1.49 (s, 3H), 1.43 (d, *J* = 7.6 Hz, 1H), 1.31 (s, 3H), 0.95 – 0.86 (m, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.7, 168.8, 157.0, 135.1, 134.0, 133.4, 133.4, 132.3, 128.5, 128.3, 127.8, 127.5, 126.6, 126.4, 125.7, 125.4, 85.9, 77.8, 77.2, 68.0, 52.9, 52.2, 51.4, 46.4, 45.4, 42.2, 41.2, 39.6, 39.6, 38.3, 35.6, 29.0, 28.7, 27.2, 26.9, 26.3, 25.6, 24.8, 24.1, 23.2, 22.0.

(S)-2-(3-benzyl-3-methylureido)-4-methyl-N-((S)-3-(naphthalen-2-yl)-1-oxo-1-((R)-2-((3aS,4S,6S,7aR)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl) pyrrolidin-1-yl) propan-2-yl) pentanamide (8t)

Using the same procedure as **8a**, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, *J* = 8.0 Hz, 3H), 7.73 (d, *J* = 8.3 Hz, 1H), 7.49 (d, *J* = 8.4 Hz, 1H), 7.46 – 7.39 (m, 2H), 7.33 (t, *J* = 7.0 Hz, 2H), 7.29 – 7.16 (m, 4H), 6.95 (d, *J* = 7.7 Hz, 1H), 4.89 (q, *J* = 7.7 Hz, 1H), 4.69 (d, *J* = 7.7 Hz, 1H), 4.44 (s, 2H), 4.36 (dd, *J* = 16.2, 8.5 Hz, 2H), 3.43 – 3.33 (m, 1H), 3.23 (dd, *J* = 13.5, 8.6 Hz, 1H), 3.13 (dd, *J* = 13.5, 4.8 Hz, 1H), 3.07 (dd, *J* = 9.9, 7.5 Hz, 1H), 2.84 (s, 3H), 2.44 (p, *J* = 10.8, 9.8 Hz, 2H), 7.24 – 2.16 (m, 1H), 2.11 (d, *J* = 5.4 Hz, 1H), 2.05 (s, 1H), 1.97 (d, *J* = 11.6 Hz, 2H), 1.92 – 1.83 (m, 1H), 1.74 – 1.60 (m, 3H), 1.50 (s, 3H), 1.44 (d, *J* = 12.8 Hz, 1H), 1.31 (s, 3H), 0.88 (d, *J* = 5.6 Hz, 6H), 0.85 (d, *J* = 6.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.6, 168.8, 157.8, 137.7, 134.1, 133.4, 132.3, 128.7, 128.6, 128.33, 127.80, 127.73, 127.53, 127.33, 127.20, 125.71, 125.43, 85.9, 77.8, 77.2, 53.2, 52.2, 52.1, 51.4, 46.4, 41.8, 39.6, 39.5, 38.3, 35.6, 34.4, 34.0, 28.7, 27.2, 27.1, 26.3, 25.6, 25.0, 24.7, 24.1, 23.2, 21.8.

N-(2-Pyrazinecarbonyl)-L-leucine-L-(2-naphthyl)-alanine-L-proline boronic acid (II-1)

Using the same procedure as **I-1**, white solid, 36% yield; mp: 123 - 126 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.25 – 9.14 (m, 1H), 8.89 (d, *J* = 2.4 Hz, 1H), 8.70 (d, *J* = 1.3 Hz, 1H), 8.65 – 8.52 (m, 1H), 7.90 – 7.66 (m, 4H), 7.40 (ddd, *J* = 9.3, 7.4, 5.3 Hz, 3H), 5.00 – 4.75 (m, 1H), 4.55 (td, *J* = 9.3, 4.8 Hz, 1H), 3.50 – 3.32 (m, 1H), 3.31 – 2.92 (m, 3H), 1.92 – 1.21 (m, 7H), 0.89 – 0.63 (m, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.3, 168.7, 162.6, 144.0, 143.8, 128.5, 128.1, 127.8, 126.3, 126.1, 125.7, 51.9, 46.8, 41.8, 27.4, 24.7, 23.3, 22.1, 21.8. HRMS (ESI) calcd for $C_{29}H_{35}BN_5O_4$: 528.2782 [(M-H_2O+CH_2+H) $^+$], found 528.2780.

N-(2-Pyrazinecarbonyl)-L-leucine-L-phenylalanine-L-proline boronic acid (II-2)

Using the same procedure as **I-1**, white solid, 34% yield; mp: 134 - 136 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.19 (d, *J* = 1.2 Hz, 1H), 8.89 (dd, *J* = 5.5, 2.4 Hz, 1H), 8.74 (dd, *J* = 5.0, 1.5 Hz, 1H), 8.67 – 8.55 (m, 1H), 7.37 – 7.03 (m, 5H), 4.81 – 4.48 (m, 2H), 3.03 – 2.72 (m, 3H), 1.90 – 1.41 (m, 6H), 0.94 – 0.74 (m, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.3, 168.9, 162.6, 148.2, 144.6, 144.0, 143.8, 138.4, 129.8, 128.4, 126.6, 51.8, 46.8, 41.8, 37.3, 27.4, 27.2, 24.8, 23.4, 22.2, 22.1. HRMS (ESI) calcd for $C_{25}H_{33}BN_5O_4$: 478.2625 [(M-H_2O+CH_2+H) $^+$], found 478.2623.

N-(2-Pyrazinecarbonyl)-L-phenylalanine-L-phenylalanine-L-proline boronic acid (II-3)

Using the same procedure as **I-1**, white solid, 39% yield; mp: 174 - 176 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.12 (dd, J = 9.7, 2.5 Hz, 1H), 8.90 – 8.84 (m, 1H), 8.71 (d, J = 2.0 Hz, 1H), 8.57 (dd, J = 12.9, 8.5 Hz, 1H), 7.31 (dd, J = 11.9, 5.9 Hz, 2H), 7.26 – 7.09 (m, 8H), 4.97 – 4.61 (m, 2H), 3.03 (tdd, J = 34.2, 16.6, 9.4 Hz, 4H), 2.89 – 2.64 (m, 2H), 1.95 – 1.45 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.3, 170.1, 168.9, 162.4, 148.3, 144.4, 143.8, 138.4, 137.3, 129.8, 128.5, 128.4, 126.8, 60.2, 53.8, 52.4, 37.4, 27.2, 21.2. HRMS (ESI) calcd for $C_{28}H_{31}BN_5O_4$: 512.2468 [(M-H_2O+CH_2+H)$^+$], found 512.2466.

N-(2-Pyrazinecarbonyl)-L-phenylalanine-L-(2-naphthyl)-alanine-L-proline boronic acid (II-4)

Using the same procedure as **I-1**, white solid, 42% yield; mp: 197 - 199 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.12 (s, 1H), 8.86 (s, 1H), 8.67 (d, J = 5.3 Hz, 1H), 8.58 (d, J = 8.4 Hz, 1H), 7.78 (dd, J = 14.8, 7.1 Hz, 4H), 7.51 – 7.31 (m, 3H), 7.23 – 6.97 (m, 5H), 4.82 (dd, J = 23.9, 16.6 Hz, 2H), 3.25 (m, 2H), 3.16 – 2.85 (m, 4H), 1.75 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.1, 168.7, 162.4, 148.2, 144.4, 143.8, 137.6, 133.4, 129.7, 128.4, 128.1, 127.9, 127.9, 126.8, 126.2, 125.7, 54.1, 46.9, 38.0, 37.6, 27.5, 27.2. HRMS (ESI) calcd for $C_{32}H_{33}BN_5O_4$: 562.2626 [(M-H_2O+CH_2+H)$^+$], found 562.2618.

N-(2-Pyrazinecarbonyl)-L-leucine-L-leucine-L-proline boronic acid (II-5)

Using the same procedure as **I-1**, white solid, 27% yield; mp: 102 - 104 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.19 (d, J = 1.2 Hz, 1H), 8.90 (d, J = 2.3 Hz, 1H), 8.76 (dd, J = 2.3, 1.5 Hz, 1H), 8.70 (d, J = 9.0 Hz, 1H), 8.23 (d, J = 8.2 Hz, 1H), 4.59 (dtd, J = 13.9, 8.9, 4.9 Hz, 2H), 3.63 (m, 1H), 2.83 (m, 1H), 2.07 – 1.29 (m, 10H), 1.00 – 0.77 (m, 12H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.5, 169.5, 162.7, 148.3, 144.7, 144.0, 143.9, 51.7, 48.9, 46.6, 42.0, 27.4, 27.3, 24.8, 24.4, 23.8, 23.5, 22.1, 22.0. HRMS (ESI) calcd for $C_{22}H_{35}BN_5O_4$: 444.2780 [(M-H_2O+CH_2+H)$^+$], found 444.2774.

N-Cbz-L-phenylalanine-L-(2-naphthyl)-alanine-L-proline boronic acid (II-6)

Using the same procedure as **I-1**, white solid, 47% yield; mp: 155 - 157 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.93 – 7.70 (m, 4H), 7.46 (ddd, J = 19.4, 18.7, 7.0 Hz, 4H), 7.31 (dt, J = 12.9, 7.2 Hz, 3H), 7.26 – 7.11 (m, 7H), 5.11 – 4.86 (m, 2H), 4.79 (dt, J = 24.5, 11.4 Hz, 1H), 4.40 – 4.17 (m, 1H), 3.39 – 2.57 (m, 6H), 2.01 – 1.37 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.7, 171.4, 168.8, 156.1, 137.4, 133.5, 132.3, 129.6, 128.8, 128.5, 128.1, 127.9, 127.9, 126.7, 65.7, 56.5, 38.0, 37.6, 27.5, 27.2. HRMS (ESI) calcd for $C_{35}H_{37}BN_3O_5$: 590.2827 [(M-H_2O+CH_2+H)$^+$], found 590.2815.

N-Cbz-L-leucine-L-(2-naphthyl)-alanine-L-proline boronic acid (II-7)

Using the same procedure as **I-1**, white solid, 43% yield; mp: 161 - 163 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.92 – 7.65 (m, 4H), 7.52 – 7.16 (m, 9H), 5.11 – 4.92 (m, 2H), 4.91 – 4.72 (m, 1H), 4.00 (dd, J = 13.3, 9.7 Hz, 1H), 3.44 – 2.78 (m, 4H), 1.99 – 1.02 (m, 7H), 0.89 – 0.56 (m, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.5, 172.2, 170.7, 168.8, 156.2, 137.4, 133.4, 132.4, 128.8, 128.2, 128.1, 128.1, 126.4, 126.2, 65.8, 53.6, 46.8, 41.2, 27.5, 27.2, 24.5, 23.3, 21.6. HRMS (ESI) calcd for $C_{32}H_{39}BN_3O_5$: 556.2983 [(M-H_2O+CH_2+H)$^+$], found 556.2973.

N-Cbz-L-leucine-L-leucine-L-proline boronic acid (II-8)

Using the same procedure as **I-1**, white solid, 37% yield; mp: 138 - 141 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.44 – 7.20 (m, 6H), 5.10 – 4.93 (m, 2H), 4.74 – 4.42 (m, 1H), 4.07 (d, J = 4.5 Hz, 1H), 3.59 (s, 1H), 3.30 (d, J = 7.4 Hz, 1H), 2.07 – 1.72 (m, 3H), 1.62 (d, J = 5.4 Hz, 3H), 1.54 – 1.26 (m, 4H), 0.93 – 0.79 (m, 12H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.6, 169.6, 156.3, 137.5, 128.8, 128.0, 65.8, 53.6, 48.8, 46.6, 41.2, 27.3, 24.7, 24.2, 23.8, 23.5, 22.1, 21.8. HRMS (ESI) calcd for $C_{25}H_{39}BN_3O_5$: 472.2982 [(M-H_2O+CH_2+H)$^+$], found 472.2981.

(1, 2, 3, 4-tetrahydroisoquinoline-2-carbonyl)-L-phenylalanine-L-phenylalanine-L-proline boronic acid (II-9)

Using the same procedure as **I-1**, white solid, 38% yield; mp: 135 - 138 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ7.27 (d, J = 7.3 Hz, 2H), 7.23 – 7.07 (m, 12H), 4.75 – 4.62 (m, 1H), 4.49 – 4.39 (m, 2H), 4.38 – 4.32 (m, 1H), 3.52 – 3.42 (m, 2H), 3.29 (q, J = 9.2, 8.6 Hz, 1H), 3.07 (dd, J = 13.9, 4.5 Hz, 1H), 2.96 – 2.87 (m, 2H), 2.82 – 2.61 (m, 4H), 1.95 – 1.68 (m, 3H), 1.66 – 1.49 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.3, 169.0, 157.2, 138.9, 138.3, 135.2, 135.2, 134.4, 129.9, 129.8, 129.7, 129.7, 128.9, 128.5, 128.5, 128.4, 126.7, 126.7, 126.6, 126.5, 56.0, 52.3, 46.8, 45.6, 41.5, 37.6, 37.5, 28.5, 27.4, 27.1. HRMS (ESI) calcd for $C_{33}H_{38}BN_4O_4$: 565.2981 [(M-H_2O+CH_2+H)$^+$], found 565.2976.

(1, 2, 3, 4-tetrahydroisoquinoline-2-carbonyl)-L-phenylalanine-L-(2-naphthyl)-alanine-L-proline boronic acid (II-10)

Using the same procedure as **I-1**, white solid, 52% yield; mp: 144 - 147 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.84 – 7.76 (m, 3H), 7.72 (d, J = 8.4 Hz, 1H), 7.46 – 7.40 (m, 3H), 7.22 – 7.07 (m, 9H), 4.82 – 4.75 (m, 1H), 4.42 – 4.30 (m, 3H), 3.63 – 3.55 (m, 1H), 3.43 (t, J = 6.0 Hz, 2H), 3.33 (q, J = 8.4 Hz, 1H), 3.26 (dd, J = 14.1, 4.6 Hz, 1H), 2.98 – 2.84 (m, 3H), 2.81 – 2.75 (m, 1H), 2.72 – 2.59 (m, 2H), 1.91 – 1.70 (m, 3H), 1.61 (ddd, J = 9.8, 6.8, 3.1 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.3, 172.2, 168.8, 157.2, 138.9, 136.0, 135.2, 134.4, 133.5, 132.2, 129.7, 129.6, 128.9, 128.6, 128.4, 128.0, 128.0, 127.9, 127.8, 126.7, 126.6, 126.5, 126.5, 126.2, 125.8, 56.1, 52.2, 46.8, 45.6, 41.5, 37.6, 28.5, 27.4, 27.2. HRMS (ESI) calcd for $C_{37}H_{40}BN_4O_4$: 615.3137 [(M-H_2O+CH_2+H)$^+$], found 615.3122.

(1, 2, 3, 4-tetrahydroisoquinoline-2-carbonyl)-L-phenylalanine-L-tyrosine-L-proline boronic acid (II-11)

Using the same procedure as **I-1**, white solid, 54% yield; mp: 235 - 238 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ7.22 – 7.18 (m, 3H), 7.17 – 7.09 (m, 6H), 7.07 (d, *J* = 8.3 Hz, 2H), 6.63 (d, *J* = 8.3 Hz, 2H), 4.57 (dd, *J* = 8.6, 5.1 Hz, 1H), 4.51 – 4.28 (m, 3H), 3.59 (s, 1H), 3.47 (q, *J* = 5.7 Hz, 2H), 3.24 (q, *J* = 9.2, 8.8 Hz, 1H), 3.00 – 2.86 (m, 3H), 2.85 – 2.73 (m, 2H), 3.72 – 3.61 (m, 3H), 1.93 – 1.67 (m, 3H), 1.59 (ddd, *J* = 19.7, 9.5, 4.0 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.2, 169.2, 157.2, 156.0, 138.9, 135.2, 134.4, 134.3, 130.7, 129.7, 129.7, 128.9, 128.4, 128.4, 126.7, 126.6, 126.5, 115.3, 56.0, 52.6, 48.2, 46.8, 45.6, 41.6, 37.6, 36.7, 28.5, 27.4, 27.1. HRMS (ESI) calcd for $C_{33}H_{38}BN_4O_5$: 581.2930 [(M-H_2O+CH_2+H)$^+$], found 581.2928.

(1, 2, 3, 4-tetrahydroisoquinoline-2-carbonyl)-L-phenylalanine-L-leucine-L-proline boronic acid (II-12)

Using the same procedure as **I-1**, white solid, 38% yield; mp: 141 - 144 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.00 (d, *J* = 8.2 Hz, 1H), 7.33 – 7.06 (m, 9H), 6.57 (d, *J* = 8.4 Hz, 1H), 4.58 – 4.51 (m, 1H), 4.50 – 4.35 (m, 3H), 3.67 – 3.59 (m, 1H), 3.49 (dt, *J* = 10.2, 6.6 Hz, 2H), 3.36 (q, *J* = 8.9 Hz, 1H), 3.01 (dd, *J* = 13.6, 3.9 Hz, 1H), 2.91 – 2.78 (m, 2H), 2.75 – 2.61 (m, 2H), 1.97 – 1.68 (m, 3H), 1.65 – 1.38 (m, 4H), 0.86 (d, *J* = 5.2 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.5, 169.6, 157.3, 139.0, 135.2, 134.4, 129.7, 129.0, 128.4, 126.7, 126.6, 126.4, 56.0, 48.9, 48.1, 46.6, 45.7, 41.6, 40.8, 37.7, 28.5, 27.3, 27.2, 24.4, 23.7, 22.2. HRMS (ESI) calcd for $C_{30}H_{40}BN_4O_4$: 531.3137 [(M-H_2O+CH_2+H)$^+$], found 531.3123.

(1, 2, 3, 4-tetrahydroisoquinoline-2-carbonyl)-L-leucine-L-(2-naphthyl)-alanine-L-proline boronic acid (II-13)

Using the same procedure as **I-1**, white solid, 42% yield; mp: 136 - 139 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ7.86 – 7.77 (m, 2H), 7.79 – 7.67 (m, 2H), 7.49 – 7.40 (m, 3H), 7.21 – 7.11 (m, 4H), 4.83 – 4.72 (m, 1H), 4.47 (s, 2H), 4.19 – 4.10 (m, 1H), 3.53 (t, *J* = 5.3 Hz, 2H), 3.36 (q, *J* = 8.9, 8.4 Hz, 1H), 3.28 – 3.19 (m, 1H), 2.98 – 2.85 (m, 2H), 2.79 – 2.69 (m, 2H), 1.95 – 1.69 (m, 3H), 1.68 – 1.35 (m, 4H), 0.79 (d, *J* = 6.4 Hz, 3H), 0.72 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.3, 168.8, 157.4, 135.9, 135.2, 134.4, 133.4, 132.2, 129.0, 128.6, 128.1, 128.0, 127.8, 126.7, 126.6, 126.5, 126.2, 125.7, 53.3, 51.9, 46.8, 45.7, 41.5, 41.0, 37.5, 28.7, 27.4, 27.2, 24.6, 23.4, 21.8. HRMS (ESI) calcd for $C_{34}H_{42}BN_4O_4$: 581.3294 [(M-H_2O+CH_2+H)$^+$], found 581.3307.

(N-methylbenzylamine-N-carbonyl)-L-leucine-L-(2-naphthyl)-alanine-L-proline boronic acid (II-14)

Using the same procedure as **I-1**, white solid, 39% yield; mp: 132 - 134 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.90 – 7.73 (m, 5H), 7.51 – 7.42 (m, 3H), 7.33 (t, *J* = 7.4 Hz, 2H), 7.27 – 7.21 (m, 1H), 7.17 (d, *J* = 7.2 Hz, 2H), 4.81 (q, *J* = 6.3, 4.8 Hz, 1H), 4.41 (q, *J* = 16.0 Hz, 2H), 4.11 (dd, *J* = 10.6, 4.6 Hz, 1H), 3.64 (t, *J* = 7.9 Hz, 1H), 3.37 (q, *J* = 8.1 Hz, 1H), 3.27 (dd, *J* = 14.0, 4.6 Hz, 1H), 2.98 – 2.87 (m, 2H), 2.74 (s, 3H), 1.97 – 1.68 (m, 3H), 1.67 – 1.57 (m, 1H), 1.49 – 1.34 (m, 2H), 1.20 – 1.13 (m, 1H), 0.74 (dd, *J* = 22.0, 6.4 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.2, 168.8, 157.9, 138.9, 135.8, 133.4, 132.2, 128.9, 128.8, 128.6, 128.2, 128.0, 127.8, 127.6, 127.3, 126.2, 125.8, 53.6, 51.7, 51.5, 48.3, 46.8, 41.0, 37.7, 34.3, 27.4, 27.2, 24.6, 23.4, 21.7. HRMS (ESI) calcd for $C_{33}H_{42}BN_4O_4$: 569.3294 [(M-H_2O+CH_2+H)$^+$], found 569.3310.

4.2 Biological assay

The enzymatic activities of the proteasome were assayed using The Proteasome-Glo™ Cell-Based Assays kit (Promega, USA). The Proteasome-Glo™ Cell-Based Reagents each contain a specific luminogenic proteasome substrate in a buffer optimized for cell permeabilization, proteasome activity and luciferase activity. These peptide substrates are Suc-LLVYaminoluciferin (Succinyl-leucine-leucine-valine-tyrosine-aminoluciferin), Z-LRRaminoluciferin (Z-leucine-arginine-arginine-aminoluciferin) and Z-nLPnLDaminoluciferin (Z-norleucine-proline-norleucine-aspartate-aminoluciferin) for the chymotrypsin-like, trypsin-like and caspase-like activities, respectively. The trypsinlike assay also contains two inhibitors to reduce nonspecific protease activities. Briefly, acute human myeloid leukemic cell line (HL60, 5,000 cells/well) were plated in 20μl/well in a 384-well plate. Cells were then equilibrated at 37°C, 5% CO_2 for 2 hours. Serial dilutions of compounds were prepared in culture medium, and 5μl of each dilution was added to wells. The cells were incubated with the drug for 2 hours at 37°C, 5% CO_2 before 25μl/well of each Proteasome-Glo™ Cell-Based Reagent was added. The relative luminescence units (RLU) were measured using the Multimode Microplate Reader Varioskan Flash (Thermo Scientific, USA) after 15 minutes and compared with the RLU of solvent control, and IC_{50} was calculated from the curves generated by plotting the percentage of the solvent control versus the test concentration on a logarithmic scale using SigmaPlot 10.0 software.

Cell growth inhibitory tests. MGC-803 or other cell lines, and a standard MTT assay was used to measure cell growth. In brief, a suspension of 3000 cells/150 μL of medium was added to each well of 96-well plates and allowed to grow. Twenty-four hours later, drugs prepared in medium at 10 different concentrations were added to the corresponding plates at a volume of 50 μL per well, and the plates were incubated for 72 h with drugs. Then 20 μL of a solution of 5 mg/ml MTT were added to each well and incubated for another 4 h at 37 °C. Plates were then centrifuged at 1000 rpm at 4 °C for 5 min, and the medium was carefully discarded. The formazan crystals were dissolved in 100 μL of DMSO and absorbance was read on an Infinite M200 (Tecan, Austria) microplate reader at 540 nm.

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Supplementary Material

The original data of ^{1}H NMR and ^{13}C NMR of all products are supplied. The supplementary data files are to be used as an aid for the refereeing of the paper only.

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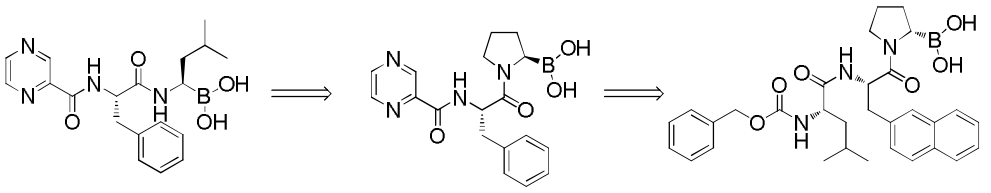
Highlights

- Two series of peptide-boronic acids as proteasome were designed and synthesized.
- Most compounds showed significant antiproliferative activity against MGC803.
- Compound **II-7** exhibited potent activity against 5 cancer cell lines.
- Compound **II-7** showed excellent subunit selectivity over proteasome comparing with Bortezomib.

Graphical abstract:



Bortezomib

II-7

3 Cell lines inhibition: IC_{50} < 10 nM

HL-60 inhibition: IC_{50} < 1 nM

Improved subunit selectivty over proteasome