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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of macrocyclic α-ketoamide as a selective and reversible immunoproteasome inhibitor

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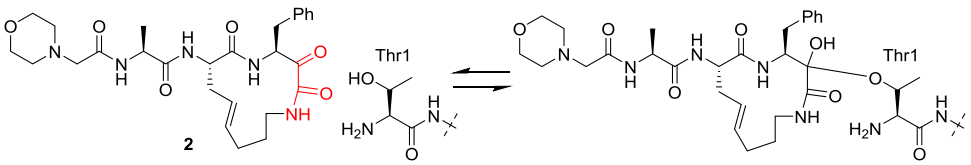
Abstract

In recent years, the human immunoproteasome has emerged as an attractive therapeutic target for various diseases, leading to a growing interest in the discovery of immunoproteasome inhibitors that selectively target specific subunits. Herein we report the design, synthesis, and evaluation of a new immunoproteasome inhibitor that feature a macrocyclic ring containing an internal α-ketoamide warhead. This compound is a selective and reversible inhibitor of immunoproteasome subunits β1i and β5i and shows essentially no inhibition of constitutive proteasome subunits.

Graphical Abstract



Keywords Immunoproteasome · Immunoproteasome inhibitors · Ketoamide · Macrocyclic ketoamide · Reversible covalent inhibitors

Abbreviations

UPS	ubiquitin-proteasome system;
CP	core particle;
RP	regulatory particle;
LMP2	low-molecular mass protein-2;
MECL1	multicatalytic endopeptidase complex-like 1;
LMP7	low-molecular mass protein-7;
DMP	Dess-Martin periodinane;
HBTU	N,N,N′,N′-tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate;
IIDQ	2-isobutoxy-1-isobutoxycarbonyl-1,2-dihydroquin-oline;
NOE	nuclear overhauser effect;
EDC	N-ethyl-N′-(3-dimethylaminopropyl) carbodiimide.

Introduction

The 26S proteasome is a key player in the ubiquitin-proteasome system (UPS) that maintains protein homeostasis [1, 2]. It consists of the proteolytic 20S core particle (CP) and two 19S regulatory particles (RP). In the eukaryotic CP, only three of the seven β subunits, namely β1, β2, and β5, are catalytically active because of the presence of an N-terminal threonine. While the constitutive proteasomes are present in all eukaryotic cells, the immunoproteasomes, a special type of proteasomes, are normally expressed in monocytes and lymphocytes [3]. Moreover, immunoproteasomes are highly expressed in immune cells and non-immune cells under conditions of stress and inflammation [3]. The immunoproteasomes and constitutive proteasomes

This paper is dedicated to Professor Robert Vince on the occasion of his 80th birthday.

Supplementary information The online version of this article (https://doi.org/10.1007/s00044-020-02678-2) contains supplementary material, which is available to authorized users.

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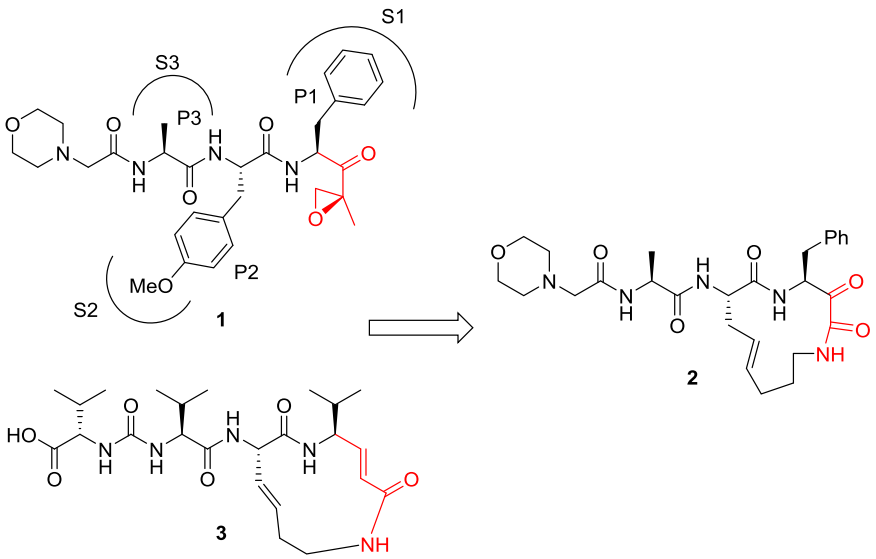


Fig. 1 Design of reversible immunoproteasome inhibitors based on a macrocyclic α-ketoamide warhead

share the same structural architecture; but in the immunoproteasomes, the three catalytically active subunits of constitutive proteasomes, β1c, β2c and β5c, are replaced with β1i (low-molecular mass protein-2, LMP2), β2i (multicatalytic endopeptidase complex-like 1, MECL1), and β5i (low-molecular mass protein-7, LMP7), respectively [4].

The proteasome is a well-established anticancer target as evidenced by the FDA approval of three proteasome inhibitors: bortezomib, ixazomib, and carfilzomib for multiple myeloma [5]. In recent years, the immunoproteasome has emerged as a potential therapeutic target for various diseases including cancer, autoimmune disorders, and obesity [6, 7]. Therefore, there is a growing interest in the discovery of immunoproteasome inhibitors that selectively target specific subunits [8, 9]. Majority of the immunoproteasome inhibitors reported are those that selectively target β5i, including α′,β′-epoxyketones [10, 11], thiasyrbactins [12], peptides with a reactive sidechain [13], N,C-capped dipeptides [14], and nonpeptidic inhibitors [15, 16]. For β1i inhibitors, UK-101 [17] with an expoxyketone warhead, aldehyde-based IPSI-001 [18], and boronic acid-based ML604440 [19] are among the first reported. Recent studies have revealed that structural modifications in the peptide backbone of α′,β′-epoxyketones can be used to elicit β1i selectivity [20, 21].

ONX-0914/PR-957 (**1**, Fig. 1) is the prototype of selective β5i inhibitors that feature an α′,β′-epoxyketone warhead. Compound **1** is moderately selective against β5i over β5c [10]. Its selectivity has been mainly attributed to a bulky phenyl group at the P1 position that is accommodated by the larger S1 pocket of β5i but not that of β5c [10]. Based on structural studies, it has been postulated that the α′,β′-epoxyketone warhead adopts a bent conformation before it reacts irreversibly with Thr1 to form a six- or seven-membered morpholine ring [10, 22, 23]. Herein we report the design, synthesis, and evaluation of a new immunoproteasome inhibitor that feature a macrocyclic ring containing an internal α-ketoamide warhead.

Materials and methods

Chemistry

General procedures

All commercial reagents were used as provided unless otherwise indicated. An anhydrous solvent dispensing system (J. C. Meyer) using two packed columns of neutral alumina was used for drying THF, Et_2O, and CH_2Cl_2, whereas two packed columns of molecular sieves were used to dry DMF. Solvents were dispensed under argon. Flash chromatography was performed with RediSep R_f silica gel columns on a Teledyne ISCO CombiFlash® R_f system using the solvents as indicated. Nuclear magnetic resonance spectra were recorded on a Varian 600 MHz with Me_4Si or signals from residual solvent as the internal standard for ^1H. Chemical shifts are reported in ppm, and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br s (broad singlet), and dd (double doublet). Values given for coupling constants are first order. High resolution mass spectra were recorded on an Agilent TOF II TOF/MS instrument equipped with either an ESI or APCI interface.

tert-Butyl (S)-(4-Cyano-3-oxo-1-phenyl-4-(triphenyl-λ^5-phosphanylidene)butan-2-yl)carbamate (5)

[24] To a solution of Boc-Phe-OH (**4**, 3.61 g, 13.6 mmol) in CH_2Cl_2 (100 mL) was added EDC·HCl (2.61 g, 13.6 mmol)

and DMAP (41.6 mg, 0.340 mmol). After the resulting mixture was stirred at rt for 1 h, (triphenylphosphoranylidene)acetonitrile (4.10 g, 13.6 mmol) was added and the solution was stirred overnight. The reaction was quenched with sat. $NaHCO_3$ and extracted with CH_2Cl_2 (×3). After the combined organic layer was dried over Na_2SO_4 and filtered, the filtrate was concentrated in vacuo and the residue was purified by flash column chromatography using EtOAc/hexanes (50%) to afford product **5** as a white solid (5.50 g, 74%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.68–7.60 (m, 3H), 7.60–7.46 (m, 12H), 7.30–7.16 (m, 5H), 5.19 (br s, 1H), 5.11 (br s, 1H), 3.38–3.27 (m, 1H), 3.06–3.97 (m, 1H), 1.38 (s, 9H). HRMS (ESI^+) calcd for $C_{34}H_{34}N_2O_3P$ $(M + H)^+$ 549.2302, found 549.2309. $[\alpha]_D^{25}$ − 35.6 ($c = 1.0$, $CHCl_3$).

Methyl (2R,3S)-3-((tert-Butoxycarbonyl)amino)-2-hydroxy-4-phenylbutanoate (6a) and Methyl (2S,3S)-3-((tert-Butoxycarbonyl)amino)-2-hydroxy-4-phenylbutanoate (6b)

A solution of **5** (5.79 g, 10.5 mmol) in CH_2Cl_2 (100 mL) was treated with O_3 at –78 °C until the color of solution remained yellow-blue. The excess O_3 was removed by purging with N_2 at –78 °C until the blue color faded. To this yellow solution was added slowly $NaBH_4$ (600 mg, 15.9 mmol) and MeOH (20 mL). After the reaction mixture being warmed up to rt, the solvents were removed in vacuo and the residue was purified by flash chromatography over silica gel using EtOAc/hexanes (10%) to afford products **6a** (816 mg, 25%) and **6b** (1.40 g, 43%) as white solids. Compound **6a**, ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.32–7.26 (m, 4H), 7.24–7.20 (m, 1H), 4.85 (d, $J =$ 9.3 Hz, 1H), 4.30–4.23 (m, 1H), 4.06 (d, $J = 3.6$ Hz, 1H), 3.74 (s, 3H), 3.24 (d, $J = 4.7$ Hz, 1H), 2.97–2.87 (m, 2H), 1.38 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.4, 155.2, 137.5, 129.4, 128.5, 126.6, 79.6, 70.2, 54.2, 52.8, 38.2, 28.2. HRMS (ESI^+) calcd for $C_{16}H_{24}NO_5$ $(M + H)^+$ 332.1468, found 332.1461. $[\alpha]_D^{23}$ − 61.2 ($c = 1.0$, $CHCl_3$); Compound **6b**, ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.29–7.26 (m, 2H), 7.22–7.18 (m, 3H), 4.89 (d, $J = 7.1$ Hz, 1H), 4.35–4.29 (m, 2H), 3.59 (s, 3H), 3.38 (d, $J = 4.1$ Hz, 1H), 2.85–2.75 (m, 2H), 1.38 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.1, 155.5, 137.0, 129.5, 128.3, 126.6, 79.8, 72.5, 54.3, 52.5, 35.7, 28.2. HRMS (ESI^+) calcd for $C_{16}H_{24}NO_5$ $(M + H)^+$ 332.1468, found 332.1468. $[\alpha]_D^{23}$ − 18.0 ($c = 1.0$, $CHCl_3$).

tert-Butyl ((2S,3R)-3-Hydroxy-4-oxo-4-(pent-4-en-1-ylamino)-1-phenylbutan-2-yl)carbamate (7a)

To a solution of **6a** (170 mg, 0.550 mmol) in MeOH (4 mL) and H_2O (2 mL) was added LiOH (66.0 mg, 2.75 mmol), and the solution was stirred at rt for 1 h. The reaction was quenched with sat. NH_4Cl and extracted with CH_2Cl_2 (×3). After the combined organic layer was dried over Na_2SO_4 and filtered, the filtrate was concentrated in vacuo. To the solution of the residue in THF (8 mL) were added triphosgene (64.1 mg, 0.216 mmol) and triethylamine (131 mg, 1.29 mmol). After 5 min, 4-penten-1-amine (110 mg, 1.29 mmol) was added and the solution was stirred for 10 min. The reaction was then quenched with sat. $NaHCO_3$ and extracted with CH_2Cl_2 (×3). After the combined organic layer was dried over Na_2SO_4 and filtered, the filtrate was concentrated in vacuo and the residue was purified by flash column chromatography using EtOAc/hexanes (40%) to afford product **7a** as a white solid (178 mg, 76%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.31–7.27 (m, 2H), 7.25–7.20 (m, 3H), 6.84 (br s, 1H), 5.82–5.74 (m, 1H), 5.55 (br s, 1H), 5.11 (br s, 1H), 5.02 (d, $J = 17.2$ Hz, 1H), 4.97 (d, $J = 10.2$ Hz, 1H), 4.08 (dd, $J_1 = 6.4$ Hz, $J_2 = 2.3$ Hz, 1H), 3.97 (br s, 1H), 3.35–3.27 (m, 1H), 3.26–3.19 (m, 1H), 3.14–3.07 (m, 1H), 3.06–3.00 (m, 1H), 2.10–2.04 (m, 2H), 1.63–1.56 (m, 2H), 1.37 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.5, 157.5, 138.1, 137.6, 129.3, 128.5, 126.6, 115.3, 80.4, 73.9, 55.6, 38.6, 36.4, 30.9, 28.7, 28.2. HRMS (ESI^+) calcd for $C_{20}H_{31}N_2O_4$ $(M + H)^+$ 363.2278, found 363.2273. $[\alpha]_D^{25}$ − 25.2 ($c = 1.0$, $CHCl_3$).

tert-Butyl ((2S,3S)-3-Hydroxy-4-oxo-4-(pent-4-en-1-ylamino)-1-phenylbutan-2-yl)carbamate (7b)

Compound **7b** was prepared in a manner similar to **7a** (white solid, 82%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.30–7.24 (m, 2H), 7.22–7.18 (m, 3H), 7.06 (br s, 1H), 5.84–5.75 (m, 1H), 5.57 (br s, 1H), 5.06 (br s, 1H), 5.04 (d, $J = 17.2$ Hz, 1H), 4.99 (d, $J = 10.2$ Hz, 1H), 4.26 (br s, 1H), 4.05 (br s, 1H), 3.34–3.22 (m, 2H), 3.06–2.99 (m, 1H), 2.95–2.88 (m, 1H), 2.13–2.06 (m, 2H), 1.66–1.58 (m, 2H), 1.38 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.7, 157.9, 138.0, 137.5, 129.3, 128.5, 126.5, 115.3, 80.5, 75.1, 57.1, 38.5, 35.4, 31.0, 28.7, 28.2. HRMS (ESI^+) calcd for $C_{20}H_{31}N_2O_4$ $(M + H)^+$ 363.2278, found 363.2271. $[\alpha]_D^{25}$ − 58.8 ($c = 1.0$, $CHCl_3$).

(2R,3S)-3-Amino-2-hydroxy-N-(pent-4-en-1-yl)-4-phenylbutanamide (8a)

A solution of **7a** (192 mg, 0.530 mmol) in CH_2Cl_2 (5 mL) was treated with TFA (2 mL), and the solution was stirred at rt for 2 h. The reaction was quenched with sat. $NaHCO_3$ and extracted with CH_2Cl_2 (×3). After the combined organic layer was dried over Na_2SO_4 and filtered, the filtrate was concentrated in vacuo and the residue was purified by flash

column chromatography using MeOH/DCM (5%) to afford product **8a** as a white solid (116 mg, 83%). ^{1}H NMR (600 MHz, CD_3OD) δ 7.31–7.27 (m, 2H), 7.26–7.23 (m, 2H), 7.22–7.18 (m, 1H), 5.86–5.78 (m, 1H), 5.03 (d, J = 17.1 Hz, 1H), 4.95 (d, J = 10.1 Hz, 1H), 3.88 (d, J = 2.4 Hz, 1H), 3.35–3.25 (m, 2H), 3.23–3.16 (m, 1H), 2.87 (dd, J_1 = 13.4 Hz, J_2 = 7.1 Hz, 1H), 2.68 (dd, J_1 = 13.4 Hz, J_2 = 7.8 Hz, 1H), 2.12–2.05 (m, 2H), 1.65–1.58 (m, 2H). ^{13}C NMR (150 MHz, CD_3OD) δ 175.8, 140.2, 139.1, 130.4, 129.6, 127.4, 115.5, 73.7, 56.4, 41.2, 39.6, 32.2, 29.7. HRMS (ESI$^+$) calcd for $C_{15}H_{23}N_2O_2$ $(M + H)^+$ 263.1754, found 263.1752. $[\alpha]_D^{25}$ 20.0 (c = 1.0, MeOH).

(2S,3S)-3-Amino-2-hydroxy-N-(pent-4-en-1-yl)-4-phenylbutanamide (8b)

Compound **8b** was prepared in a manner similar to **8a** (white solid, 81%). ^{1}H NMR (600 MHz, CD_3OD) δ 7.38–7.32 (m, 2H), 7.31–7.24 (m, 3H), 5.93–5.85 (m, 1H), 5.09 (d, J = 17.1 Hz, 1H), 5.02 (d, J = 10.1 Hz, 1H), 4.14 (d, J = 3.6 Hz, 1H), 3.40–3.35 (m, 1H), 3.34–3.24 (m, 2H), 2.87 (dd, J_1 = 13.6 Hz, J_2 = 3.8 Hz, 1H), 2.62 (dd, J_1 = 13.6 Hz, J_2 = 9.9 Hz, 1H), 2.19–2.13 (m, 2H), 1.72–1.65 (m, 2H). ^{13}C NMR (150 MHz, CD_3OD) δ 175.0, 140.2, 139.0, 130.4, 129.5, 127.4, 115.5, 75.6, 56.6, 39.5, 38.8, 32.2, 29.7. HRMS (ESI$^+$) calcd for $C_{15}H_{23}N_2O_2$ $(M + H)^+$ 263.1754, found 263.1755. $[\alpha]_D^{25}$ − 24.6 (c = 1.0, MeOH).

tert-Butyl ((S)-1-(((2S,3R)-3-Hydroxy-4-oxo-4-(pent-4-en-1-ylamino)-1-phenylbutan-2-yl)amino)-1-oxopent-4-en-2-yl) carbamate (9a)

A solution of (*S*)-*N*-Boc-allylglycine (159 mg, 0.740 mmol), **8a** (97 mg, 0.37 mmol) and IIDQ (224 mg, 0.740 mmol) in THF (30 mL) was stirred at rt for 24 h. The reaction was quenched with sat. $NaHCO_3$ and extracted with CH_2Cl_2 (×3). After the combined organic layer was dried over Na_2SO_4 and filtered, the filtrate was concentrated in vacuo and the residue was purified by flash column chromatography using EtOAc/hexanes (40%) to afford product **9a** as a white solid (150 mg, 88%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.29–7.25 (m, 2H), 7.24–7.17 (m, 4H), 6.97 (t, J = 5.6 Hz, 1H), 5.89 (br s, 1H), 5.82–5.74 (m, 1H), 5.54–5.45 (m, 1H), 5.09–5.00 (m, 3H), 4.99–4.93 (m, 2H), 4.29–4.23 (m, 1H), 4.11–4.04 (m, 2H), 3.32–3.25 (m, 1H), 3.25–3.18 (m, 1H), 3.17–3.09 (m, 1H), 2.97–2.90 (m, 1H), 2.37–2.27 (m, 2H), 2.11–2.04 (m, 2H), 1.64–1.57 (m, 2H), 1.42 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.6, 172.6, 155.3, 137.9, 137.7, 132.8, 129.4, 128.7, 126.8, 119.3, 115.5, 80.4, 73.3, 55.5, 54.0, 38.8, 36.9, 35.9, 31.2, 28.7, 28.4. HRMS (ESI$^+$) calcd for $C_{25}H_{38}N_3O_5$ $(M + H)^+$ 460.2806, found 460.2806. $[\alpha]_D^{25}$ − 30.6 (c = 1.6, $CHCl_3$).

tert-Butyl ((S)-1-(((2S,3S)-3-Hydroxy-4-oxo-4-(pent-4-en-1-ylamino)-1-phenylbutan-2-yl)amino)-1-oxopent-4-en-2-yl) carbamate (9b)

Compound **9b** was prepared in a manner similar to **9a** (white solid, 89%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.30–7.25 (m, 2H), 7.23–7.18 (m, 3H), 6.95 (t, J = 5.5 Hz, 1H), 6.64 (d, J = 6.8 Hz, 1H), 5.84–5.75 (m, 1H), 5.54–5.43 (m, 1H), 5.38 (br s, 1H), 5.10–5.01 (m, 3H), 5.00–4.96 (m, 1H), 4.79 (d, J = 6.4 Hz, 1H), 4.33–4.28 (m, 1H), 4.23 (br s, 1H), 4.06 (br s, 1H), 3.33–3.20 (m, 2H), 3.07–2.94 (m, 2H), 2.41–2.31 (m, 2H), 2.13–2.06 (m, 2H), 1.66–1.58 (m, 2H), 1.41 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.6, 171.2, 155.5, 137.6 (2C), 132.5, 129.2, 128.6, 126.7, 119.4, 115.3, 80.7, 74.3, 56.7, 53.9, 38.7, 36.3, 35.1, 31.1, 28.6, 28.3. HRMS (ESI$^+$) calcd for $C_{25}H_{38}N_3O_5$ $(M + H)^+$ 460.2806, found 460.2807. $[\alpha]_D^{25}$ − 33.4 (c = 1.0, MeOH).

tert-Butyl ((3R,4S,7S,E)-4-Benzyl-3-hydroxy-2,6-dioxo-1,5-diazacyclotridec-9-en-7-yl)carbamate (10a)

A solution of **9a** (125 mg, 0.272 mmol) and Nitro-Grela catalyst (36.5 mg, 0.0545 mmol) in toluene (150 mL) was stirred at rt for 24 h. After the solvent was removed in vacuo, the residue was purified by flash chromatography using EtOAc/hexanes (80%) to afford product **10a** as a white solid (62 mg, 53%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.30–7.25 (m, 2H), 7.23–7.16 (m, 3H), 5.66–5.60 (m, 1H), 5.36–5.27 (m, 1H), 4.37 (br s, 1H), 4.12–4.04 (m, 2H), 3.62–3.55 (m, 1H), 3.25–3.17 (m, 1H), 3.16–3.05 (m, 2H), 2.81–2.73 (m, 1H), 2.32–2.24 (m, 2H), 2.14–2.06 (m, 1H), 1.83–1.73 (m, 1H), 1.68–1.59 (m, 1H), 1.37 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.7, 172.7, 154.7, 138.0, 136.2, 129.3, 128.8, 126.8, 122.2, 80.7, 74.6, 57.5, 54.5, 40.8, 40.6, 35.6, 34.3, 33.2, 28.4. HRMS (ESI$^+$) calcd for $C_{23}H_{34}N_3O_5$ $(M + H)^+$ 432.2493, found 432.2486. $[\alpha]_D^{25}$ − 17.2 (c = 1.0, $CHCl_3$).

tert-Butyl ((3S,4S,7S,E)-4-Benzyl-3-hydroxy-2,6-dioxo-1,5-diazacyclotridec-9-en-7-yl)carbamate (10b)

Compound **10b** was prepared in a manner similar to **10a** (white solid, 51%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.32–7.27 (m, 4H), 7.24–7.18 (m, 1H), 6.82 (br s, 1H), 6.18 (br s, 1H), 5.81 (br s, 1H), 5.56–5.48 (m, 1H), 5.37–5.30 (m, 1H), 5.05 (d, J = 7.4 Hz, 1H), 4.43–4.34 (m, 1H), 4.10–3.99 (m, 2H), 3.71–3.62 (m, 1H), 3.44–3.36 (m, 1H), 3.17–3.09 (m, 1H), 3.08–3.00 (m, 1H), 2.49–2.41 (m, 1H), 2.34–2.23 (m, 2H), 2.18–2.08 (m, 1H), 1.89–1.80 (m, 1H), 1.63–1.55 (m, 1H), 1.39 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.7, 171.6, 154.8, 137.0, 134.9, 129.3, 128.8, 126.9, 123.9, 80.2, 74.5, 58.4, 54.1, 40.1, 38.2, 36.2, 32.8,

28.3, 27.6. HRMS (ESI^+) calcd for $C_{23}H_{34}N_3O_5$ $(M+H)^+$ 432.2493, found 432.2493. $[\alpha]_D^{24}$ − 138.1 ($c = 0.32$, $CHCl_3$).

(3R,4S,7S,E)-7-Amino-4-benzyl-3-hydroxy-1,5-diazacyclotridec-9-ene-2,6-dione (11a)

A solution of **10a** (62 mg, 0.144 mmol) in CH_2Cl_2 (5 mL) was treated with TFA (2 mL), and the solution was stirred at rt for 2 h. The reaction was quenched with sat. $NaHCO_3$ and extracted with CH_2Cl_2 (×3). After the combined organic layer was dried over Na_2SO_4 and filtered, the filtrate was concentrated in vacuo and the residue was purified by flash column chromatography using MeOH/DCM (5%) to afford product **11a** as a white solid (31 mg, 65%). ^1H NMR (600 MHz, $CDCl_3$) δ 7.87 (d, $J = 5.7$ Hz, 1H), 7.46 (d, $J = 8.2$ Hz, 1H), 7.30–7.25 (m, 2H), 7.23–7.18 (m, 3H), 6.91 (d, $J = 6.9$ Hz, 1H), 5.59–5.53 (m, 1H), 5.38–5.32 (m, 1H), 4.15–4.07 (m, 2H), 3.73–3.65 (m, 1H), 3.52–3.47 (m, 1H), 3.38–3.32 (m, 1H), 3.15–3.10 (m, 1H), 3.07–3.01 (m, 1H), 2.77–2.70 (m, 1H), 2.33–2.26 (m, 1H), 2.21–2.15 (m, 1H), 2.12–2.03 (m, 1H), 1.86–1.79 (m, 1H), 1.65–1.56 (m, 1H), 1.22 (br s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.8, 172.8, 138.4, 135.6, 129.1, 128.5, 126.7, 121.9, 75.5, 58.0, 54.3, 40.6, 36.6, 35.0, 33.1, 28.1. HRMS (ESI^+) calcd for $C_{18}H_{26}N_3O_3$ $(M+H)^+$ 332.1969, found 332.1970. $[\alpha]_D^{24}$ − 5.8 ($c = 1.0$, $CHCl_3$).

(3S,4S,7S,E)-7-Amino-4-benzyl-3-hydroxy-1,5-diazacyclotridec-9-ene-2,6-dione (11b)

Compound **11b** was prepared in a manner similar to **11a** (white solid, 65%). ^1H NMR (600 MHz, CD_3OD) δ 7.36–7.28 (m, 4H), 7.21 (t, $J = 7.1$ Hz, 1H), 5.62–5.55 (m, 1H), 5.48–5.39 (m, 1H), 4.38–4.33 (m, 1H), 3.98–3.94 (m, 1H), 3.55–3.48 (m, 1H), 3.37–3.27 (m, 2H), 3.11–3.05 (m, 1H), 2.99–2.93 (m, 1H), 2.30–2.21 (m, 3H), 2.19–2.11 (m, 1H), 1.83–1.76 (m, 1H), 1.73–1.65 (m, 1H). ^{13}C NMR (150 MHz, CD_3OD) δ 178.7, 173.9, 139.6, 134.8, 130.5, 129.5, 127.6, 126.6, 74.9, 58.6, 55.6, 41.5, 40.0, 38.4, 33.7, 28.4. HRMS (ESI^+) calcd for $C_{18}H_{26}N_3O_3$ $(M+H)^+$ 332.1969, found 332.1975. $[\alpha]_D^{24}$ − 21.1 ($c = 1.0$, MeOH).

tert-Butyl ((S)-1-(((3R,4S,7S,E)-4-Benzyl-3-hydroxy-2,6-dioxo-1,5-diazacyclotridec-9-en-7-yl)amino)-1-oxopropan-2-yl)carbamate (12a)

To a solution of Boc-Ala-OH (19.9 mg, 0.105 mmol) in CH_2Cl_2 (3 mL) were added HBTU (39.8 mg, 0.105 mmol) and TEA (17.7 mg, 0.175 mmol). After the resulting mixture was stirred at rt for 1 h, **11a** (29.0 mg, 0.0876 mmol) was added, and the solution was stirred overnight. The reaction was quenched with sat. $NaHCO_3$ and extracted with CH_2Cl_2 (×3). After the combined organic layer was dried over Na_2SO_4 and filtered, the filtrate was concentrated in vacuo and the residue was purified by flash column chromatography using EtOAc/hexanes (80%) to afford product **12a** as a white solid (42 mg, 95%). ^1H NMR (600 MHz, CD_3OD) δ 7.28–7.22 (m, 4H), 7.16 (t, $J = 6.5$ Hz, 1H), 5.64–5.57 (m, 1H), 5.39–5.32 (m, 1H), 4.60–4.55 (m, 1H), 4.42–4.36 (m, 1H), 4.07–3.99 (m, 1H), 3.92–3.89 (m, 1H), 3.49–3.42 (m, 1H), 3.02–2.96 (m, 1H), 2.94–2.83 (m, 2H), 2.47–2.34 (m, 2H), 2.21–2.11 (m, 2H), 1.79–1.72 (m, 1H), 1.71–1.64 (m, 1H), 1.43 (s, 9H), 1.27 (d, $J = 7.1$ Hz, 3H). ^{13}C NMR (150 MHz, CD_3OD) δ 175.1, 174.9, 171.5, 157.6, 139.4, 135.1, 130.4, 129.4, 127.5, 124.7, 80.7, 72.3, 54.3, 54.2, 51.5, 41.5, 39.7, 36.0, 32.5, 28.7, 27.9, 18.1. HRMS (ESI^+) calcd for $C_{26}H_{39}N_4O_6$ $(M+H)^+$ 503.2864, found 503.2857. $[\alpha]_D^{22}$ − 67.8 ($c = 1.0$, MeOH).

tert-Butyl ((S)-1-(((3S,4S,7S,E)-4-Benzyl-3-hydroxy-2,6-dioxo-1,5-diazacyclotridec-9-en-7-yl)amino)-1-oxopropan-2-yl)carbamate (12b)

Compound **12b** was prepared in a manner similar to **12a** (white solid, 54%). ^1H NMR (600 MHz, DMSO-d_6) δ 7.80 (d, $J = 7.1$ Hz, 1H), 7.65 (d, $J = 7.1$ Hz, 1H), 7.46–7.41 (m, 1H), 7.26–7.19 (m, 4H), 7.19–7.14 (m, 1H), 7.01 (d, $J = 7.4$ Hz, 1H), 5.60–5.53 (m, 2H), 5.35–5.28 (m, 1H), 4.33–4.24 (m, 1H), 4.15–4.09 (m, 1H), 3.99–3.89 (m, 1H), 3.68–3.62 (m, 1H), 3.22–3.16 (m, 1H), 3.12–3.03 (m, 2H), 2.83–2.77 (m, 1H), 2.27–2.19 (m, 1H), 2.19–2.13 (m, 1H), 2.12–2.02 (m, 2H), 1.66–1.56 (m, 2H), 1.37 (s, 9H), 1.13 (d, $J = 6.6$ Hz, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.8, 171.2, 171.0, 154.9, 138.2, 133.0, 129.5, 128.0, 126.0, 124.3, 78.1, 72.1, 54.4, 51.8, 49.7, 40.1, 36.3, 35.4, 32.1, 28.2, 26.9, 18.0. HRMS (ESI^+) calcd for $C_{26}H_{39}N_4O_6$ $(M+H)^+$ 503.2864, found 503.2856. $[\alpha]_D^{22}$ − 106.8 ($c = 0.1$, MeOH).

(S)-N-((3R,4S,7S,E)-4-Benzyl-3-hydroxy-2,6-dioxo-1,5-diazacyclotridec-9-en-7-yl)-2-(2-morpholinoacetamido) propanamide (13a)

A solution of **12a** (42 mg, 0.0837 mmol) in CH_2Cl_2 (4 mL) was treated with TFA (2 mL), and the solution was stirred at rt for 2 h. The reaction was quenched with sat. $NaHCO_3$ and extracted with CH_2Cl_2 (×3). The combined organic layer was dried over Na_2SO_4 and filtered, and the filtrate was concentrated in vacuo. After the residue was dissolved in CH_2Cl_2 (5 mL), 4-morpholineacetic acid·TFA (23.8 mg, 0.0985 mmol), HBTU (37.3 mg, 0.0985 mmol) and TEA (33.2 mg, 0.328 mmol) were added. After being stirred at rt for 1 h, the reaction was quenched with sat. $NaHCO_3$ and extracted with CH_2Cl_2 (×3). After the combined organic layer was dried over Na_2SO_4 and filtered, the filtrate was concentrated in vacuo and the residue was purified by flash column chromatography using MeOH/DCM (5%) to afford

product **13a** as a white solid (25 mg, 56%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.67 (d, $J = 7.7$ Hz, 1H), 7.60 (d, $J = 6.5$ Hz, 1H), 7.28–7.16 (m, 5H), 7.13 (d, $J = 7.1$ Hz, 1H), 7.07 (br s, 1H), 6.13 (br s, 1H), 5.60–5.53 (m, 1H), 5.36–5.29 (m, 1H), 4.68–4.63 (m, 1H), 4.58–4.51 (m, 1H), 4.29 (s, 1H), 4.05 (d, $J = 4.8$ Hz, 1H), 3.74 (s, 4H), 3.43–3.34 (m, 1H), 3.32–3.24 (m, 1H), 3.17–3.11 (m, 1H), 3.10–3.03 (m, 1H), 3.01 (s, 2H), 2.60–2.47 (m, 5H), 2.36–2.29 (m, 1H), 2.25–2.17 (m, 1H), 2.13–2.05 (m, 1H), 1.71–1.60 (m, 2H), 1.29 (d, $J = 6.4$ Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.4 (2C), 171.7, 170.1, 137.7, 135.7, 129.2, 128.5, 126.6, 122.4, 72.9, 66.8, 61.7, 55.6, 53.7, 53.3, 48.4, 40.5, 36.6, 35.5, 32.3, 28.1, 18.4. HRMS (ESI$^+$) calcd for $C_{27}H_{40}N_5O_6$ $(M + H)^+$ 530.2973, found 530.2975. $[\alpha]_D^{23} - 76.6$ ($c = 1.0$, MeOH).

(S)-N-((3S,4S,7S,E)-4-Benzyl-3-hydroxy-2,6-dioxo-1,5-diazacyclotridec-9-en-7-yl)-2-(2-morpholinoacetamido) propanamide (13b)

Compound **13b** was prepared in a manner similar to **13a** (white solid, 71%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.00 (d, $J = 7.4$ Hz, 1H), 7.86 (br s, 1H), 7.80 (d, $J = 8.0$ Hz, 1H), 7.44 (t, $J = 5.3$ Hz, 1H), 7.25–7.21 (m, 4H), 7.20–7.15 (m, 1H), 5.60–5.53 (m, 2H), 5.36–5.29 (m, 1H), 4.37–4.28 (m, 2H), 4.14–4.08 (m, 1H), 3.68–3.64 (m, 1H), 3.60 (br s, 4H), 3.22–3.15 (m, 1H), 3.13–3.07 (m, 1H), 3.04 (dd, $J_1 = 13.5$ Hz, $J_2 = 5.4$ Hz, 1H), 2.96 (d, $J = 14.8$ Hz, 1H), 2.89 (d, $J = 14.8$ Hz, 1H), 2.83 (dd, $J_1 = 13.5$ Hz, $J_2 = 7.1$ Hz, 1H), 2.43 (br s, 4H), 2.28–2.20 (m, 1H), 2.19–2.12 (m, 1H), 2.12–2.01 (m, 2H), 1.61 (br s, 2H), 1.17 (d, $J = 6.8$ Hz, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.3 (2C), 171.2, 171.0, 138.3, 133.0, 129.6, 128.0, 126.0, 124.4, 72.2, 66.2, 61.3, 54.5, 53.2, 52.0, 47.4, 40.1, 36.4, 35.2, 32.1, 26.8, 18.7. HRMS (ESI$^+$) calcd for $C_{27}H_{40}N_5O_6$ $(M + H)^+$ 530.2973, found 530.2965. The optical rotation of **13b** was not determined due to its low solubility in common organic solvents.

(S)-N-((4S,7S,E)-4-Benzyl-2,3,6-trioxo-1,5-diazacyclotridec-9-en-7-yl)-2-(2-morpholinoacetamido)propanamide (2)

To a solution of **13a** (13 mg, 0.0245 mmol) in CH_2Cl_2 (3 mL) was added DMP (31.2 mg, 0.0736 mmol). After 10 min, the reaction was quenched with sat. $NaHCO_3$ and extracted with CH_2Cl_2 (×3). After the combined organic layer was dried over Na_2SO_4 and filtered, the filtrate was concentrated in vacuo and the residue was purified by flash column chromatography using acetone/CH_2Cl_2 (50%) to afford product **2** as a white solid (11.5 mg, 89%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.85 (br s, 1H), 7.73 (br s, 1H), 7.72 (br s, 1H), 7.31–7.24 (m, 2H), 7.24–7.16 (m, 3H), 6.96 (br s, 1H), 5.43–5.31 (m, 2H), 4.57–4.49 (m, 2H), 4.41–4.35 (m, 1H), 3.75 (s, 4H), 3.68–3.59 (m, 1H), 3.44–3.37 (m, 1H), 3.36–3.27 (m, 1H), 3.20–3.10 (m, 1H), 3.02 (s, 2H), 2.53 (s, 4H), 2.44–2.36 (m, 1H), 2.36–2.29 (m, 1H), 2.28–2.20 (m, 1H), 2.19–2.09 (m, 1H), 1.77–1.68 (m, 1H), 1.67–1.58 (m, 1H), 1.28 (d, $J = 6.6$ Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 195.4, 172.0 (2 C), 169.5, 162.5, 136.2, 135.6, 129.2, 128.8, 127.2, 122.7, 67.1, 62.0, 60.0, 53.9, 52.6, 48.3, 40.3, 37.6, 37.2, 32.7, 27.7, 19.4. HRMS (ESI$^+$) calcd for $C_{27}H_{38}N_5O_6$ $(M + H)^+$ 528.2817, found 528.2814. $[\alpha]_D^{25}$ 171 ($c = 0.58$, $CHCl_3$).

Biological activity

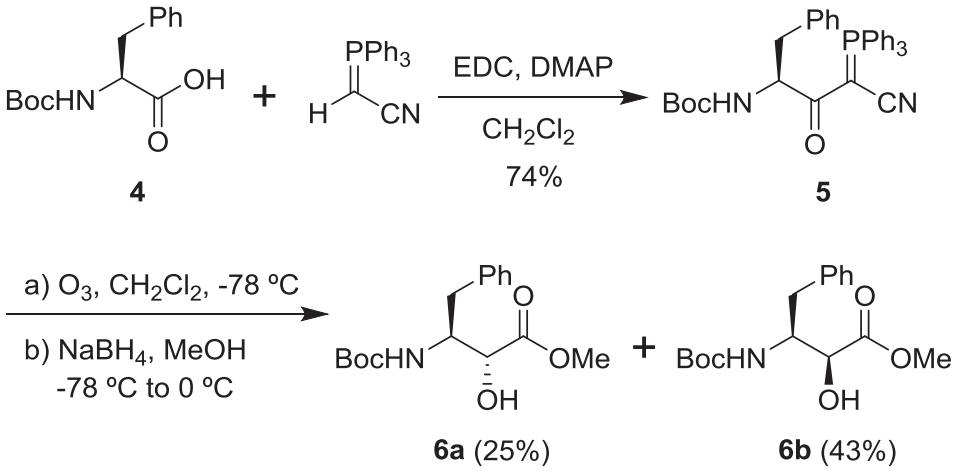
Biochemical assay

Human S20 constitutive proteasome and immunoproteasome were purchased from BostonBiochem. Substrates Suc-LLVY-AMC, Ac-RLR-AMC, and Z-LLE-AMC (BostonBiochem) were used for constitutive proteasome subunits β5c, β2c, and β1c, respectively. Substrates Ac-ANW-AMC, Ac-RLR-AMC, and Ac-PAL-AMC (BostonBiochem) were used for immunoproteasome subunits β5i, β2i, and β1i, respectively. Reactions were carried out in a black 384 well non-binding surface microplate at the final volume of 50 µL. Inhibitors (0.046–100 µM final concentrations) were mixed with either 4 nM constitutive proteasome or 1 nM immunoproteasome in reaction buffer (50 mM HEPES pH 7.5, 0.5 mM EDTA, and 0.01% SDS, 45 µL) in a 384-well plate and incubated for 40 min at 37 °C. A second set of reaction without enzyme was also prepared as a control. After the preincubation, 100 µM of the appropriate substrate was added to each well for a total of 50 µL and 1.5% DMSO (1% from the inhibitor and 0.5% from the substrate that was diluted from 20 mM in DMSO to 1 mM in water prior to addition). The plate was mixed for 30 s at 1500 RPM and the reactions were monitored on an i3 multimode plate reader (Molecular Devices) using an excitation of 380 nm and an emission of 460 nm to detect the release of byproduct 7-amino-4-methylcoumarin (AMC). The reactions were monitored for 1 h at 25 °C and the linear portion of the reaction was used to determine the IC_{50} value. The initial velocities were fit using Prism (GraphPad) to the log(inhibitor) vs. response–variable slope (four parameter model) with the top and bottom fixed at 1 and 0, respectively.

Determination of reversibility

Compound **2** was tested against the human S20 immunoproteasome using substrates Ac-ANW-AMC and Ac-PAL-AMC for immunoproteasome subunits β5i and β1i, respectively. Compound **2** was incubated with immunoproteasome (100 nM) in reaction buffer (50 mM HEPES pH 7.5, 0.5 mM EDTA, 0.01% SDS) at a final volume of 10 µL.

Scheme 1 Synthesis of intermediates **6a** and **6b**



The concentrations of compound **2** were 10 µM and 3 µM using the substrates Ac-ANW-AMC and Ac-PAL-AMC, respectively. In addition, a positive control using 1% DMSO instead of an inhibitor was included. The inhibitor-immunoproteasome mixtures were incubated for 40 min at 37 °C. After preincubation, 0.5 µL of the reactions containing DMSO, compound or water (negative control) were added to a 384 well black non-binding surface plate containing master mix (49 µL) consisting of 100 µM substrate in reaction buffer and an additional 0.5 µL DMSO. Reactions containing compound **2** were also added to wells containing preincubation concentrations of inhibitors instead of the additional DMSO (undiluted inhibitor control). The plate was read for 3.5 h at rt monitoring ex. 380 em. 460.

All graphs were generated using Prism 5 (GraphPad) and data fit lines were added for visualization purposes only.

Results and discussion

Design and chemical synthesis

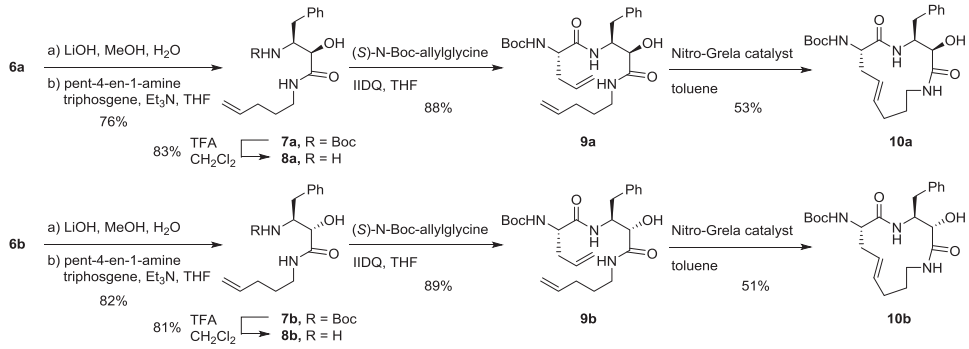
As our initial effort to discover selective and reversible immunoproteasome inhibitors, we designed compound **2**, in which a phenyl group was placed at the P1 position to elicit immunoproteasome selectivity like compound **1** [10]. We also adopted the tail of compound **1**, in which the small *L*-alanine was optimal for β5i selectivity while the morpholine was originally introduced to improve aqueous solubility [10]. To enforce a bent conformation, we introduced a macrocyclic ring similar to the one in natural product syringolin A (**3**, Fig. 1) [25]. Syringolin A belongs to syrbactins, a group of proteasome inhibitors that contain a macrocyclic, internal Michael acceptor warhead [26]. Syringolin A and its analogues have been actively sought for their anticancer applications through total syntheses [27–30] and further structural manipulation [12, 31–34]. To avoid potential side effects associated with irreversible inhibition [35], we incorporated reversible α-ketoamide as a warhead within the macrocyclic ring. Acyclic ketoamides have been investigated as reversible proteasome inhibitors [36–38]. Furthermore, ketoamide-based HCV protease inhibitors have been approved by the FDA, attesting ketoamide's clinical safety [39].

While acyclic α-ketoamides have been incorporated in inhibitors of a wide range of targets including HDAC, HCV NS3/4A protease and proteasome [40], to our best knowledge there is no report on the synthesis and drug design application of macrocyclic rings containing an internal α-ketoamide even though they are present in several natural products [40]. To prepare compound **2**, we have developed a synthetic strategy (Schemes 1–3) in which a ring-closing metathesis was used to construct the requisite macrocyclic ring. Furthermore, to avoid α-ketoamides being exposed to various synthetic conditions, we used α-hydroxylamide as an α-ketoamide precursor which was oxidized by mild Dess-Martin periodinane (DMP) at the late stage of the synthetic sequence.

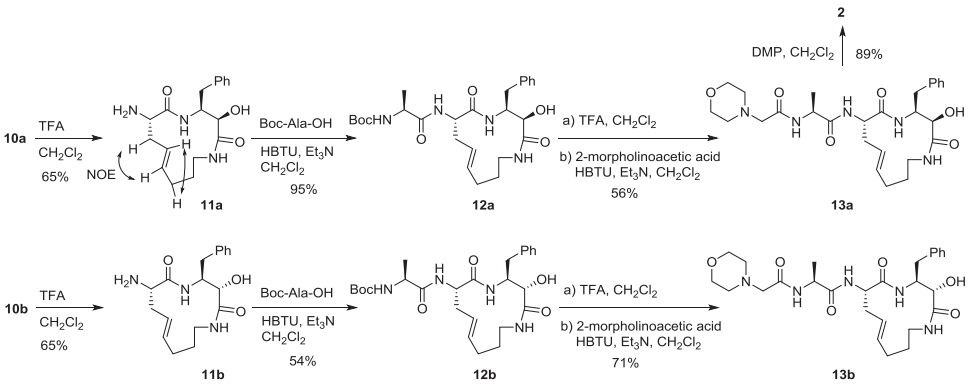
To obtain the requisite α-hydroxylamides, Boc-Phe-OH was first coupled with 2-(triphenyl-λ^5-phosphanylidene) acetonitrile to give ketone **5** (Scheme 1) [24]. Under ozonolysis conditions at low temperature, compound **5** was converted into a diketo nitrile intermediate, which could be trapped as an α-ketomethyl ester and α-ketoamide in the presence of methanol and an amine, respectively. Unfortunately, we observed severe racemization under these conditions. Nevertheless, ozonolysis at low temperature followed by in situ reduction with $NaBH_4$ in the presence of methanol gave a mixture of α-hydroxylmethyl esters **6a** and **6b**, which were readily separated by flash column chromatography. The absolute stereochemistry of **6a** and **6b** was established by comparison of their measured optical rotation values and NMR spectra with those reported for **6a** [41].

With these two isomers available, they were individually subjected to the subsequent chemical transformations as

Scheme 2 Synthesis of cyclic intermediates **10a** and **10b**



Scheme 3 Synthesis of compound **2**



shown in Scheme 2. After methyl ester **6a** underwent hydrolysis, the resulting acid was coupled with pent-4-en-1-amine in an amide formation reaction mediated by triphosgene, a reagent that proved to be advantageous over conventional coupling reagents such as *N,N,N′,N′*-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU). The Boc protective group in β-amino acid **7a** was then removed to give free amine **8a**, which underwent an 2-isobutoxy-1-isobutoxycarbonyl-1,2-dihydroquinoline (IIDQ)-mediated amide formation with commercially available (*S*)-*N*-Boc-allylglycine to afford di-olefin **9a** in high yields. IIDQ was adopted because it has been successfully used to effect a coupling reaction between a hydroxylamine and a protected vinyl glycine during a total synthesis of syringolin A and B [28]. With di-olefin **9a** available, macrocyclic **10a** was obtained in good yields after a key ring-closing metathesis catalyzed by Nitro-Grela catalyst, which has been used in an efficient synthesis of HCV protease inhibitor BILN 2061 via ring-closing metathesis macrocyclization [42]. As depicted in Scheme 2, methyl ester **6b** was also converted into the corresponding isomeric macrocyclic α-hydroxylamide **10b** in an identical synthetic sequence.

Deprotection of **10a** led to free amine **11a**, whose *trans*-double bond was determined by nuclear overhauser effect (NOE) (Scheme 3). Free amine **11b** was also obtained after deprotection. With a macrocyclic ring in place, **11a** and **11b** were ready for introduction of a tail. Amine **11a** was coupled with protected alanine Boc-Ala-OH to give **12a**, which subsequently underwent deprotection and amide formation with 2-morpholinoacetic acid to give macrocyclic α-hydroxylamide **13a**. Isomeric **13b** was obtained in an identical sequence. Preparation of compound **2** was finally accomplished by brief oxidation of alcohol **13a** with DMP while alcohol **13b** was not used for such purpose due to its surprisingly low solubility in common organic solvents.

Table 1 Inhibition of constitutive proteasome and immunoproteasome subunits

Compound	IC_{50} (μM)					
	β_{1i}	β_{2i}	β_{5i}	β_{1c}	β_{2c}	β_{5c}
2	0.29 ± 0.02	33.5 ± 3.3	2.05 ± 0.22	>100	>100	>100
13a	>100	>100	>100	>100	>100	>100
13b	>100	>100	>100	>100	>100	>100
1	76%[a]	9%[a]	91%[a]	5%[a]	28%[a]	52%[a]

[a]Percentage of inhibition at 1 μM.

Biological activity

Compounds **2** as well as intermediates **13a** and **13b** were tested against human constitutive proteasome and immunoproteasome subunits and their IC_{50} values were listed in Table 1. Compound **2** possessed IC_{50} values of ~300 nM and 2 μM against immunoproteasome subunits β1i and β5i,

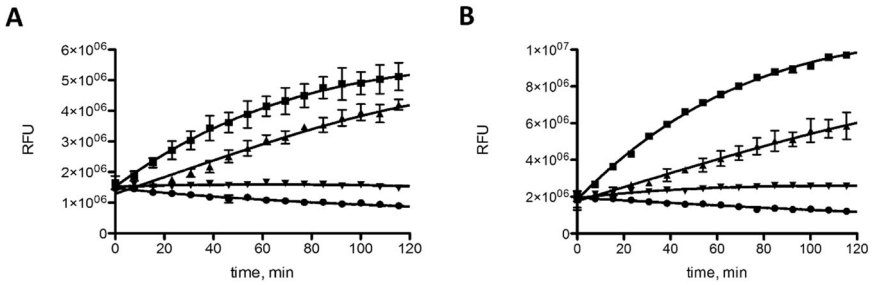


Fig. 2 Determination of the reversibility of immunoproteasome inhibitors **2**. **A** Reversibility of inhibition of β5i by **2**. Ac-ANW-AMC was used at 100 μM and compound **2** was used at 0 (■), 10 μM diluted to 0.1 μM (▲), 10 μM (▼) and 0 without enzyme (●). **B** Reversibility of inhibition of β1i by **2**. Ac-PAL-AMC was used at 100 μM and compound **2** was used at 0 (■), 3 μM diluted to 0.03 μM (▲), 3 μM (▼) and 0 without enzyme (●). Unless noted, all reactions contained 50 mM HEPES (pH 7.5), 0.5 mM EDTA, 0.01% SDS, and a final concentration of 1 nM of human S20 immunoproteasome (0 nM for negative control)

respectively, indicative of a slight preference for β1i over β5i. Furthermore, it showed high selectivity (>100-fold) against β1i over β2i. More remarkably, compound **2** exhibited essentially no inhibition of constitutive proteasome subunits β5c, β2c or β1c at 100 μM. This profile compared favorably with compound **1** in terms of immunoselectivity (β1i vs. β1c and β5i vs. β5c) even though a precise comparison was difficult due to different modes of inhibition (reversible vs. irreversible). α-Hydroxylamides **13a** and **13b** displayed no activity against either constitutive proteasome or immunoproteasome subunits, indicating that the α-ketoamide warhead was responsible for inhibitory activities.

To determine the reversibility of compound **2**, it was tested under "jump dilution" conditions [43]. In this technique, an enzyme incubated with an inhibitor at a high concentration (generally 10× IC_{50}, expected to inhibit 91% of enzyme activity) is promptly diluted 100-fold and the reaction is monitored under diluted conditions that are expected to inhibit 9% of activity. Slow recovery of the β1i and β5i activity (Fig. 2) indicated that compound **2** was indeed a reversible inhibitor.

Conclusions

In summary, we have reported a concise synthesis of compound **2** that features a macrocyclic ring containing an internal α-ketoamide warhead. Compound **2** is a selective and reversible inhibitor of immunoproteasome subunits β1i and β5i and shows essentially no inhibition of constitutive proteasome subunits. To our best knowledge, our current work is the first synthesis and use of macrocyclic, internal α-ketoamide in drug design. Also importantly, compound **2** represents a new chemotype of selective and reversible immunoproteasome inhibitors. Our synthetic strategy will allow us to explore structural features based on the template of compound **2** and identify those that elicit activity and selectivity in our future effort to develop new immunoproteasome inhibitors.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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