

Supporting Information

α,β -Unsaturated N-acylpyrrole peptidyl derivatives: new proteasome inhibitors

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Chemistry

General procedures

Synthesis of 1,1'-carbonylpyrrole

75 mmol of pyrrole was added to a suspension of N,N'-carbonyldiimidazole (25 mmol) in toluene and the mixture was stirred for 2 hours at 110 °C. The solvent was then evaporated using reduced pressure and the residue was washed with Et₂O. The 1,1'-carbonylpyrrole obtained was conserved under Argon (Bergman, J.; Carlsson, R.; Sjöberg B. The reaction of Indole Grignard reagent with Phosgene. A Facile synthesis of Indole-3-carboxylic Acid Derivatives. *J. Heterocyclic Chem.* **1977**, *14*, 1123-1134).

Synthesis of pyrrolylmethylenetriphenylphosphorane (ylide)

PhLi (d-butyl ether solution, 36 mmol) was added to a suspension of methyltriphenylphosphonium bromide (12.9 g, 36 mmol) in THF (14 mL) at 0 °C. The mixture was stirred at 25 °C for 60 min to obtain methylenetriphenylphosphorane. Subsequently, the mixture was cooled to -78 °C, at which the solution of 1,1'-carbonyldipyrrole (1.92 g, 12 mmol) in THF (18 mL) was added. The cooling bath was removed and the reaction was stirred overnight at room temperature. H₂O was then added and the mixture was extracted with ethyl acetate/CH₂Cl₂=5/1 (x3). The organic layers were washed with brine and dried over MgSO₄. After evaporation, the residue was purified by silica gel flash column chromatography (AcOEt/ petroleum ether = 2/1) to yield the ylide.²⁷

Synthesis of Boc-Leu-al

N_α-Boc-protected leucine aldehyde was obtained as describe in the literature.²⁶

Synthesis of Boc-Leu-VAP

Boc-Leu-al (3 mmol) was added to a suspension of the ylide (4 mmol) in toluene (10 mL). The suspension was stirred for 48 h at 110 °C, then cooled to room temperature. The reaction mixture was evaporated under reduced pressure, and the residue was purified by silica gel flash chromatography (AcOEt/petroleum ether 1/5) to generate Boc-Leu-VAP in a 76% yield.²⁷ ¹H NMR (CDCl₃) δ 0.95 (d, 6H); 1.39-1.47 (m, 19H); 1.63-1.72 (m, 3H); 4.43 (m, 1H); 6.32 (dd, 2H); 6.65 (d, J = 16.1, 1H); 7.09 (dd, J = 16.1, 1H); 7.37 (dd, 2H). M+H⁺ = 307.1.

Solide-phase synthesis

The synthesis of N-terminal peptide intermediates was performed using a Wang resin (0.7 mmol/g derivatization grade) with the condensed N-terminal Fmoc-protected residue. Fmoc de-protection was obtained suspending the resin for 60 min in a 20% piperidine in DMF mixture. After washing the resin with DMF and Et₂O for three times, 4 equivalents of the carboxylic component, WSC and HOBt were poured onto the resin in DMF (5 mL/g) and the suspension was shaken for 8 h at room temperature while acylation occurred.

Reagent residues were removed by washing the resin with DMF for at least three times. When the sequence was complete, treatment with TFA and scavengers (H₂O, triethylsilane) at room temperature for 1 h was used to remove the pseudopeptides from the solid support. The residue obtained after evaporation under pressure and centrifugation with Et₂O was coupled with the pharmacophore unit H-Leu-VAP (obtained after TFA de-protection) to generate the desired structures.

Coupling with WSC/HOBt

The amino component (1 mmol), NMM (1 mmol), HOBt (1.1 mmol) and WSC (1.1 mmol) were added in this order to a solution of the carboxyl component (1 mmol) in DMF (10 mL) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and then for 18 h at room temperature and the solution was subsequently diluted with EtOAc (100 mL) and washed consecutively with HCl 0.1 N, brine, NaHCO₃ and brine. The organic phase was dried (MgSO₄), filtered and evaporated to dryness, and the residue was treated with Et₂O and the resulting solid separated by centrifugation.

TFA de-protection

t-Butyloxycarbonyl protective group was removed by treating intermediates with aqueous 90% TFA (1:10, w/v) for 30-40 min. Following evaporation, the residue was treated with Et₂O and the resulting solid was separated by centrifugation.

HMB-Leu-Leu-Leu-VAP (2). ¹H NMR (CDCl₃): δ 0.97-1.05 (m, 18H); 1.77-1.85 (m, 9H); 2.41(s, 3H); 4.29 (m, 1H); 4.51-4.57 (m, 2H); 5.02 (bs, 1H); 5.99 (d, 2H); 6.18 (d, J = 16.5, 1H); 6.48 (d, 2H); 6.72 (dd, J = 16.7, 1H); 6.92-7.18 (m, 3H); 7.98 (bs, 3H).

Z-NH-(CH₂)₇-CO-Val-Ser-Leu-VAP (8). ¹H NMR (CDCl₃): δ 0.98-1.03 (m, 12H); 1.27-1.33 (m, 6H); 1.53-1.59 (m, 6H); 1.84 (m, 1H); 2.02 (s, 1H); 2.24 (t, 2H); 2.71 (m, 1H); 2.99 (t, 2H); 4.08 (m, 2H); 4.28 (m, 1H); 4.50-4.71(m, 2H); 5.28 (s, 2H); 6.02(d, 2H); 6.17 (d, J = 16.2, 1H); 6.52(d, 2H); 6.76 (dd, J = 16.4, 1H); 7.08(m, 5H); 7.92 (bs, 4H).

Table 1S. Analytical data and physicochemical properties of the novel pseudotripeptides **1-8**.

N°	Compound	HPLC		^a [α] _D ²⁰	m.p. (°C)	M+H ⁺
		K ¹ (a)	K ¹ (b)			
1	Z -Leu-Leu-Leu-VAP	6.52	7.47	- 10.9	130-133	567.35
2	HMB-Leu-Leu-Leu-VAP	5.94	6.72	- 14.9	138-140	567.35
3	Z-NH-(CH ₂) ₅ -CO-Leu-Leu-Leu-VAP	7.35	8.04	- 9.2	128-130	680.36
4	Z-NH-(CH ₂) ₇ -CO-Leu-Leu-Leu-VAP	7.91	8.63	- 8.5	118-121	708.39
5	Z-Val-Ser-Leu-VAP	5.08	6.95	- 18.7	140-142	527.28
6	HMB-Val-Ser-Leu-VAP	4.80	6.21	- 22.3	145-148	527.28
7	Z-NH-(CH ₂) ₅ -CO-Val-Ser-Leu-VAP	5.92	7.43	- 11.4	141-143	640.36
8	Z-NH-(CH ₂) ₇ -CO-Val-Ser-Leu-VAP	6.33	7.97	- 12.9	135-137	668.39

^a c = 1, MeOH.

Molecular modeling

The docking simulation was performed by utilizing the crystal structure of the complex proteasome-epoxomicin and proteasome-spirolactacystin available in the Protein Data Bank.³² Hydrogen atoms were added to the inner part of the proteasome and the energy of the structure was minimized keeping fixed the atoms of the mainframe. Molecule **8** was placed in the β 1-binding site delimited by Thr1, Asp17, Lys33, Ser129, Asp166 and Ser169 residues.³¹ The program used was MOE-Dock integrated in the MOE system of programs [MOE, Chemical Computing Group, release 2009.10]. The final poses were given a score on the base of the value assumed by the enthalpic contribution to the free energy of binding (called Affinity dG scoring).

DFT quantum-mechanical calculations have been performed to optimize the geometry of molecule **6** by using the Dmol(3) code of the MaterialStudio system of programs [Materials Studio 4.4 version, Accelrys Software Inc., 2008]

Figure 1S. Superposition of the eight molecules of Table 1. The dotted van der Waals surfaces of **6** and **8** are drawn in pink and green, respectively.

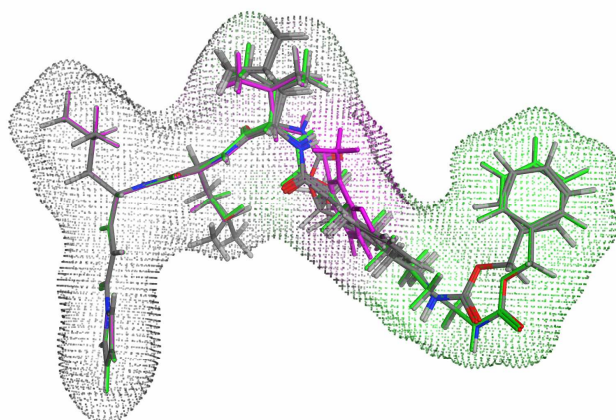


Figure 2S. Equilibrium geometry of Molecule 6

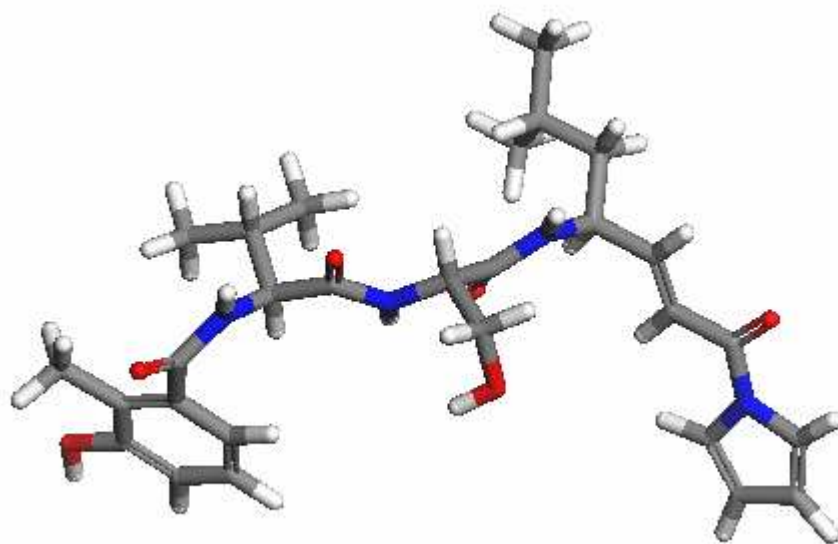


Figure 3S. Molecule **8** (green) into the $\beta 5$ catalytic site. The spiro-lactacystin ligand is shown in yellow.

