Supporting Information

Diversity-Oriented Submonomer Synthesis of Azapeptide Mediated by Mitsunobu Reaction

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1. Experimental Section

1.1 General experimental procedures.

**Chemicals** were used as received from commercial sources without further purification unless stated otherwise. Wang resin (0.96 mmol/g) and Rink amide resin (0.67 mmol/g) was purchased from GL Biochem (Shanghai) Ltd, and the manufacturer’s reported loading of the resin was used in the calculation of the yields of the final products. Reagents including 2-nitrobenzaldehyde, hydrazine hydrate, N,N'-Disuccinimidyl carbonate (DSC), N,N-diisopropylethyamine (DIEA), triphenylphosphine, diisopropyl azodicarboxylate (DIAD), sodium dithionite, tetrabutylammonium hydrogen sulfate, hydroxylamine hydrochloride, m-phenylenediamine, bistrichloromethyl carbonate (BTC), 2,4,6-collidine, p-hydroxybenzaldehyde, tert-butyl(dimethyl)silyl chloride, imidazole, lithium aluminium hydride, 3-aminopropan-1-ol, di-tert-butyl dicarbonate piperidine, pyridine, 4-dimethylaminopyridine (DMAP), acetic anhydride, formic acid (FA), isopropanol, benzyl alcohol, cyclopropyl carbinol and anhydrous solvents tetrahydrofuran (THF) were purchased from Adamas-beta®. Fmoc amino acids and coupling reagents including HOBT and diisopropylcarbodiimide (DIC) were purchased from GL Biochem (Shanghai) Ltd. All solvents were obtained from Chronchem™. Analyses by LC-MS were performed on a Waters™ Acquity SQD Series instrument with ESI ion-source, single quadrupole mass detection and positive and negative mode ionization. Analyses of crude peptide samples and purified peptide were determined with a Phenomenex Aeris™ C_{18} column (pore size: 100 Å, particle size: 2.6 μm; 75×4.6 mm) with a flow rate of 0.5 mL/min using a proper linear gradient MeOH in water containing 0.1% FA or Phenomenex Aeris™ C_{18} column (pore size: 100 Å, particle size: 5 μm; 150×4.6 mm) with a flow rate of 0.8 mL/min using a proper linear gradient of CH₃CN or MeOH in water containing 0.1% FA. Preparative RP-HPLC was conducted on a Shimadzu™ LC-20AP instrument with a reverse-phase Phenomenex Aeris™ C_{18} column (pore size: 100 Å, particle size: 5μm; 150×21.2. mm) at a flow rate of 10 mL/min and monitored with a UV detector at 220 nm and 254 nm. Linear gradient of 10-60% of MeOH in water containing 0.1% FA was used for purification of the peptides. Chiral HPLC analyses of samples were determined with a Chiralcel IA-H with a flow rate of 1 mL/min or 0.5 mL/min using a proper isocratic elution i-PrOH in Hexane. HRMS of the purified peptides was performed on a Bruker™ solariX FTMS mass spectrometer with ESI ion-source.

**Fmoc-based peptide syntheses** were performed under standard conditions on an automated shaker using Wang resin (0.96 mmol/g) or Rink amide resin. Couplings of amino acids (3 equiv) were performed in DMF using HOBT (3 equiv) and DIC (3 equiv) as coupling reagent. Fmoc deprotections were performed by treating the resin with 20% piperidine in DMF for 30 min. Resin was washed after each coupling and deprotection step sequentially with DMF (3×10 mL ), MeOH (3×10 mL), and DCM (3×10 mL).
Cleavage test of resin-bound peptide After Fmoc group removal, a sample of peptide bound resin (3-5 mg) was treated with a freshly made solution of TFA/H$_2$O/TES (95:2.5:2.5, v/v/v, 0.5 mL) for 1 h at room temperature. The cleavage mixture was filtered and then concentrated and crude peptide was precipitated with cold ether (1.5 mL). Crude peptide samples were agitated on a vortex shaker, and spun in a centrifuge. Decantation of the supernatant left a pellet, which was dissolved in 20% MeCN/H$_2$O (1 mg/mL) and subjected to LC-MS analysis.

Deprotection and cleavage of aza-peptide from the resin After Fmoc group removal, Wang resin-bound peptide was deprotected and cleaved from the support using a freshly made solution of TFA/H$_2$O/TES (95:2.5:2.5, v/v/v, 20 mL/g of peptide resin) at rt for 2 h. The resin was filtered and rinsed with 2 mL of TFA. The filtrate and rinses were concentrated until a crude oil persisted, from which a precipitate was obtained by addition of cold ether (10-15 mL). After centrifugation (1200 rpm for 10 min), the supernatant was removed and the crude peptide was taken up in aqueous acetonitrile (10% v/v) and freeze-dried to a solid prior to analysis and purification.

1.2 Synthesis of aza-dipeptide 1b-d.

Tert-butyl (2-(2-nitrobenzylidene)hydrazine-1-carbonyl)-L-phenylalaninate 1b: A solution of N,N'-disuccinimidyl carbonate (2.57 g, 10 mmol) in 40 mL of DMF/DCM (1:1, v/v) was treated dropwise with a solution of 2-nitrobenzylidene hydrazine (1.65 g, 10 mmol) in 10 mL of DCM over 15 min, stirred for 2 h at rt. Then L-Phe-O'Bu·HCl (2.58 g, 10 mmol) was added. The mixture was treated with DIEA (4.96 mL, 30 mmol), stirred for 12 h at rt. After reaction completed, the mixture was washed sequentially with 1M KHSO$_4$, saturated NaHCO$_3$ and brine. The volatiles were evaporated under reduced pressure and the residue was purified by Al$_2$O$_3$ column chromatography (EtOAc/hexanes, 1:3 to 3:1, v/v). 1b was obtained as yellow solid (2.00 g, 48%): mp 117.4-117.8 °C; $R_f=0.26$ (EtOAc/hexanes, 1:3, v/v); $[\alpha]_D^{20}+9.53$ (c 1.0, CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$) δ 9.21 (s, 1H), 8.28 (s, 1H), 8.02 (dd, $J=8.2, 1.2$ Hz, 1H), 7.94 (dd, $J=7.9, 1.4$ Hz, 1H), 7.62 (t, $J=7.7$ Hz, 1H), 7.50 (ddd, $J=8.4, 7.3, 1.4$ Hz, 1H), 7.34 – 7.21 (m, 5H), 6.59 (d, $J=8.3$ Hz, 1H), 4.79 (dt, $J=8.1, 5.9$ Hz, 1H), 3.25 – 3.12 (m, 2H), 1.42 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 170.83, 154.84, 147.90, 136.27, 133.25, 129.75, 129.64, 128.86, 128.35, 128.23, 126.87, 124.78, 82.27, 53.95, 38.72, 27.97; HRMS m/z calcd for C$_{21}$H$_{24}$N$_4$NaO$_5$ [M+Na]$^+$ 435.16389, found 435.16538.
Tert-butyl (2-(3-nitrobenzylidene)hydrazine-1-carbonyl)-L-phenylalaninate 1c: was synthesized using DSC (2.57 g, 10 mmol), 3-nitrobenzylidene hydrazine (1.65 g, 10 mmol), L-Phe-OtBu·HCl (2.58 g, 10 mmol) and DIEA (4.96 mL, 30 mmol) according to the procedure described above for 1b. The crude product was purified by Al₂O₃ column chromatography (EtOAc/hexanes, 3:1 to 1:3, v/v). 1c was obtained as white solid (2.63 g, 64%): mp 159.8-160.3 °C; R_f = 0.46 (EtOAc/hexanes, 1:1, v/v); [α]D^20 = -15.3 (c 0.1, CHCl₃); 1H NMR (400 MHz, CDCl₃) δ 9.75 (d, J = 4.5 Hz, 1H), 8.37 (t, J = 2.0 Hz, 1H), 8.20 (ddd, J = 8.2, 2.4, 1.1 Hz, 1H), 7.89 (dt, J = 7.7, 1.3 Hz, 1H), 7.78 (s, 1H), 7.56 (t, J = 8.0 Hz, 1H), 7.39 – 7.25 (m, 5H), 6.58 (d, J = 8.3 Hz, 1H), 4.77 (dt, J = 8.3, 6.0 Hz, 1H), 3.27 – 3.14 (m, 2H), 1.45 (s, 9H); 13C NMR (100 MHz, CDCl₃) δ 170.65, 155.48, 148.63, 138.54, 136.14, 135.85, 132.09, 129.65, 129.52, 128.51, 127.11, 123.96, 121.45, 82.28, 54.05, 38.61, 27.99; HRMS m/z calcd for C₂₁H₂₄N₄NaO₅ [M+Na]⁺ 435.16389, found 435.16517.

Tert-butyl (2-(4-nitrobenzylidene)hydrazine-1-carbonyl)-L-phenylalaninate 1d: was synthesized using DSC (2.57 g, 10 mmol), 4-nitrobenzylidene hydrazine (1.65 g, 10 mmol), L-Phe-OtBu·HCl (2.58 g, 10 mmol) and DIEA (4.96 mL, 30 mmol) according to the procedure described above for 1b. The crude product was purified by Al₂O₃ column chromatography (EtOAc/hexanes, 3:1 to 1:3, v/v). 1c was obtained as yellow solid (1.98 g, 48%): mp 164.8-165.3 °C, R_f = 0.46 (EtOAc/hexanes, 1:1, v/v); [α]D^20 = +8.7 (c 1, CHCl₃); 1H NMR (400 MHz, CDCl₃) δ 10.90 (s, 1H), 8.19 (d, J = 8.3 Hz, 2H), 7.81 (s, 1H), 7.67 (d, J = 8.4 Hz, 2H), 7.25 (t, J = 10.1 Hz, 4H), 6.68 (d, J = 8.2 Hz, 1H), 4.76 (q, J = 6.5 Hz, 1H), 3.19 (tt, J = 13.9, 6.7 Hz, 2H), 1.43 (s, 9H); 13C NMR (150 MHz, CDCl₃) δ 170.65, 155.67, 148.63, 138.54, 136.14, 135.85, 132.09, 129.65, 129.52, 128.51, 127.11, 123.96, 121.45, 82.28, 54.05, 38.61, 27.99; HRMS m/z calcd for C₂₁H₂₄N₄NaO₅ [M+Na]⁺ 435.16389, found 435.16550.

1.3 Synthesis of azadipeptide 2b-2d using Mitsunobu Reaction

Representative synthesis of azadipeptide using Mitsunobu reaction in solution:

Tert-butyl (1-methyl-2-(2-nitrobenzylidene)hydrazine-1-carbonyl)-L-phenylalaninate 2b: To a round-bottomed flask was added triphenylphosphine (525 mg, 2 mmol) in 3 mL of THF under nitrogen. The solution is cooled to 0 °C and sequentially added a solution of 1b (413 mg, 1mmol) in THF (1mL), DIAD (394 μL, 2 mmol) in THF (500 μL), and Methanol (122 μL, 3mmol) in THF (500 μL). The mixture was stirred at rt for 5 h. The volatiles were evaporated under reduced pressure and the residue was purified by Al₂O₃ column chromatography (EtOAc/hexanes, 1:3 to 3:1, v/v). 2b was obtained as yellow oil (305 mg, 71%): R_f = 0.45.
(EtOAc/hexanes, 1:3, v/v); \([\alpha]_D^{20} +2.30\) (c 0.1, CHCl₃); \(^1\)H NMR (400 MHz, CDCl₃) \(\delta\) 8.05 (s, 1H), 8.01 (dd, \(J = 8.5, 7.4, 1.4\) Hz, 1H), 7.48 (dd, \(J = 8.5, 8.3, 1.4\) Hz, 1H), 7.32 – 7.26 (m, 2H), 7.25 – 7.20 (m, 3H), 6.99 (d, \(J = 8.3\) Hz, 1H), 4.76 (dt, \(J = 8.3, 5.9\) Hz, 1H), 3.38 (s, 3H), 3.21 (dd, \(J = 13.8, 5.6\) Hz, 1H), 3.12 (dd, \(J = 13.8, 6.3\) Hz, 1H), 1.42 (s, 9H);

\(^{13}\)C NMR (100 MHz, CDCl₃) \(\delta\) 170.94, 154.71, 148.03, 136.39, 133.26, 131.69, 129.60, 129.27, 128.39, 127.95, 126.85, 124.82, 82.05, 54.52, 38.59, 28.70, 27.99; HRMS m/z calcd for C\(_{22}\)H\(_{26}\)N\(_4\)NaO\(_5\) + \([M+Na]^+\) 449.17954, found 449.18130;

Chiral HPLC analysis: Chiralcel IA-H (Hexane/i-PrOH) = 70:30, flow rate = 1.0 mL/min, wave length = 254 nm, \(t_R = 4.894\) min (2b), \(t_R = 5.551\) min (enantiomer of 2b).

**Tert-butyl (1-methyl-2-(3-nitrobenzylidene)hydrazine-1-carbonyl)-L-phenylalaninate 2c** was synthesized using azadipeptide 1c (413 mg, 1mmol), triphenylphosphine (525 mg, 2 mmol), DIAD (394 μL, 2 mmol) and Methanol (122 μL, 3mmol) according to the procedure described above for 2b. The crude product was purified by Al\(_2\)O₃ column chromatography (EtOAc/hexane, 3:1 to 1:3, v/v). 2c was obtained as yellow oil (158 mg, 37%): \(R_f = 0.26\) (EtOAc/hexanes, 1:3, v/v); \([\alpha]_D^{20} – 11.0\) (c 0.1, CHCl₃); \(^1\)H NMR (400 MHz, CDCl₃) \(\delta\) 8.34 (t, \(J = 1.9 \)Hz, 1H), 8.21 – 8.14 (m, 1H), 7.85 (d, \(J = 7.7\) Hz, 1H), 7.58 – 7.50 (m, 2H), 7.33 – 7.23 (m, 5H), 7.00 (d, \(J = 8.1\) Hz, 1H), 4.73 (dt, \(J = 8.1, 6.0\) Hz, 1H), 3.36 (s, 3H), 3.17 (qd, \(J = 13.8, 6.0\) Hz, 2H), 1.43 (s, 9H); \(^{13}\)C NMR (100 MHz, CDCl₃) \(\delta\) 170.91, 154.70, 148.67, 136.59, 136.34, 133.58, 131.71, 129.66, 129.49, 128.48, 126.97, 123.55, 121.58, 82.07, 54.67, 38.52, 28.54, 27.98; HRMS m/z calcd for C\(_{22}\)H\(_{26}\)N\(_4\)NaO\(_5\) + \([M+Na]^+\) 449.17954, found 449.18130.

**Tert-butyl (1-methyl-2-(4-nitrobenzylidene)hydrazine-1-carbonyl)-L-phenylalaninate 2d** was synthesized using aza-dipeptide 1d (413 mg, 1mmol), triphenylphosphine (525 mg, 2 mmol), DIAD (394 μL, 2 mmol) and Methanol (122 μL, 3mmol) according to the procedure described above for 2b. The crude product was purified by Al\(_2\)O₃ column chromatography (EtOAc/hexane, 3:1 to 1:3, v/v). 2d was obtained as yellow oil (241 mg, 57%): \(R_f = 0.24\) (EtOAc/hexanes, 1:3, v/v); \([\alpha]_D^{20} +6.00\) (c 0.1, CHCl₃); \(^1\)H NMR (400 MHz, CDCl₃) \(\delta\) 8.20 (d, \(J = 8.4\) Hz, 2H), 7.62 (d, \(J = 8.4\) Hz, 2H), 7.49 (s, 1H), 7.36 – 7.26 (m, 3H), 7.24 – 7.19 (m, 2H), 7.00 (d, \(J = 8.4\) Hz, 1H), 4.74 (dt, \(J = 8.2, 5.7\) Hz, 1H), 3.36 (s, 3H), 3.26 – 3.05 (m, 2H), 1.43 (s, 9H); \(^{13}\)C NMR (100 MHz, CDCl₃) \(\delta\) 170.86, 154.59, 147.72, 140.77, 136.22, 133.44, 129.59, 128.48, 127.06, 127.02, 124.05, 82.18, 54.39, 38.44, 28.58, 28.00; HRMS m/z calcd for C\(_{22}\)H\(_{26}\)N\(_4\)NaO\(_5\) + \([M+Na]^+\) 449.17954, found 449.18072.
1.4 Synthesis of dipeptide 2e-n using Mitsunobu Reaction

**Tert-butyl (1-isopropyl-2-(2-nitrobenzylidene)hydrazine-1-carbonyl)-L-phenylalaninate 2e** was synthesized using aza-dipeptide 1b (100 mg, 0.24 mmol), triphenylphosphine (126 mg, 0.48 mmol), DIAD (85 μL, 0.48 mmol) and isopropanol (55 μL, 0.72 mmol) according to the procedure described above for 2b. The crude product was purified by Al₂O₃ column chromatography (EtOAc/hexanes, 10:1 to 1:1, v/v). 2e was obtained as yellow oil (78 mg, 71%): R₇ = 0.55 (EtOAc/hexanes, 1:3, v/v); [α]D²⁰ –5.3 (c 0.1, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.36 (s, 1H), 8.02 (dd, J = 8.2, 1.2 Hz, 1H), 7.73 (dd, J = 7.9, 1.4 Hz, 1H), 7.58 (td, J = 7.7, 1.3 Hz, 1H), 7.46 (ddd, J = 8.5, 7.4, 1.5 Hz, 1H), 7.28 – 7.19 (m, 5H), 6.95 (d, J = 8.3 Hz, 1H), 5.06 (hept, J = 7.2 Hz, 1H), 4.71 (dt, J = 8.3, 5.9 Hz, 1H), 3.22 – 3.08 (m, 2H), 1.48 (d, J = 2.3 Hz, 3H), 1.46 (d, J = 2.3 Hz, 3H), 1.41 (s, 9H); ¹³C NMR (100 MHz, CDCl₃) δ 170.99, 154.91, 147.90, 136.51, 133.38, 131.76, 130.22, 129.62, 129.07, 128.34, 127.58, 126.77, 124.84, 81.90, 54.56, 45.83, 38.58, 27.98, 18.86; HRMS calcd for C₂₄H₃₀N₄NaO₅ [M+Na]⁺ 477.21084, found 477.21208.

**Tert-butyl (1-benzyl-2-(2-nitrobenzylidene)hydrazine-1-carbonyl)-L-phenylalaninate 2f** was synthesized using aza-dipeptide 1b (100 mg, 0.24 mmol), triphenylphosphine (126 mg, 0.48 mmol), DIAD (85 μL 0.48 mmol) and benzyl alcohol (75 μL, 0.72 mmol) according to the procedure described above for 2b. The crude product was purified by Al₂O₃ column chromatography (EtOAc/hexanes, 10:1 to 1:1, v/v). Propargylamine 2f was obtained as yellow oil (74 mg, 61%): R₇ = 0.55 (EtOAc/hexanes, 1:3, v/v); [α]D²⁰ +9.2 (c 1, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.09 (s, 1H), 7.93 (dd, J = 8.3, 1.3 Hz, 1H), 7.76 (dd, J = 8.0, 1.5 Hz, 1H), 7.62 – 7.51 (m, 1H), 7.42 (td, J = 7.9, 7.4, 1.5 Hz, 1H), 7.37 – 7.21 (m, 10H), 7.10 (d, J = 8.4 Hz, 1H), 5.29 – 5.14 (m, 2H), 4.84 (dt, J = 8.5, 6.0 Hz, 1H), 3.27 – 3.19 (m, 2H), 1.46 (s, 9H); ¹³C NMR (100 MHz, CDCl₃) δ 170.94, 155.12, 147.86, 136.43, 135.00, 133.18, 132.85, 129.66, 129.44, 129.30, 128.87, 128.43, 127.77, 127.48, 126.95, 126.87, 124.76, 82.11, 54.72, 45.70, 38.64, 28.03; HRMS m/z calcd for C₂₈H₃₀N₄NaO₅ [M+Na]⁺ 525.21084, found 525.21245.

**Tert-butyl (1-(4-(benzyloxy)benzyl)-2-(2-nitrobenzylidene)hydrazine-1-carbonyl)-L-phenylalaninate 2g** was synthesized using aza-dipeptide 1b (100 mg, 0.24 mmol), triphenylphosphine (126 mg, 0.48 mmol), DIAD (85 μL 0.48 mmol) and 4-(benzyloxy)phenyl)methanol (154 mg, 0.72 mmol) according to the procedure described above for 2b. The crude product was purified by Al₂O₃ column chromatography (EtOAc/hexanes,
10:1 to 1:1, v/v). 2g was obtained as yellow oil (93 mg, 64%): \( R_f = 0.29 \) (EtOAc/hexanes, 1:3, v/v); [\( \alpha \)]$_{D}^{20}$ +4.3 (c 0.1, CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.15 (s, 1H), 7.95 (dd, $J = 8.2, 1.3$ Hz, 1H), 7.77 (dd, $J = 7.9, 1.4$ Hz, 1H), 7.56 (td, $J = 7.6, 1.3$ Hz, 1H), 7.47 – 7.22 (m, 12H), 7.22 – 7.14 (m, 2H), 7.08 (d, $J = 8.4$ Hz, 1H), 6.98 – 6.89 (m, 2H), 5.20 (d, $J = 16.2$ Hz, 1H), 5.10 (d, $J = 16.2$ Hz, 1H), 5.03 (s, 2H), 4.84 (dt, $J = 8.4, 6.0$ Hz, 1H), 3.30 – 3.15 (m, 2H), 1.46 (s, 9H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 170.95, 158.19, 155.12, 147.87, 136.94, 134.45, 133.17, 132.64, 129.66, 129.50, 129.25, 128.53, 128.42, 127.90, 127.71, 127.47, 127.29, 126.86, 124.78, 115.24, 82.09, 70.04, 54.72, 45.11, 38.66, 28.03; HRMS m/z calcd for C$_{35}$H$_{36}$N$_4$NaO$_6$ [M+Na]$^+$ 631.25271, found 631.25319.

**Tert-butyl (1-((tert-butoxycarbonyl)amino)propyl)-2-(2-nitrobenzyldiene)hydrazine-1-carbonyl)-L-phenylalaninate** 2h was synthesized using aza-dipeptide 1b (100 mg, 0.24 mmol), triphenylphosphine (126 mg, 0.48 mmol), DIAD (85 µL, 0.48 mmol) and tert-butyl Boc-3-aminopropanol (126 mg, 0.72 mmol) according to the procedure described above for 2b. The crude product was purified by Al$_2$O$_3$ column chromatography (EtOAc/hexanes, 10:1 to 1:1, v/v). 2h was obtained as yellow oil (102 mg, 75%): $R_f = 0.62$ (EtOAc/hexanes, 1:3, v/v); [\( \alpha \)]$_{D}^{20}$ +5.3 (c 1, CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.08 (s, 1H), 8.02 (d, $J = 8.2$ Hz, 1H), 7.75 (d, $J = 7.9$ Hz, 1H), 7.61 (t, $J = 7.6$ Hz, 1H), 7.49 (t, $J = 7.9$ Hz, 1H), 7.23 (dd, $J = 19.6, 6.2$ Hz, 5H), 6.95 (d, $J = 8.3$ Hz, 1H), 5.45 (t, $J = 6.3$ Hz, 1H), 4.71 (dt, $J = 8.3, 6.0$ Hz, 1H), 4.03 (q, $J = 6.4$ Hz, 2H), 3.13 (q, $J = 7.5, 6.4$ Hz, 2H), 3.06 (q, $J = 6.3$ Hz, 2H), 1.74 (q, $J = 6.0$ Hz, 2H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 170.89, 156.07, 155.46, 147.92, 136.27, 133.39, 132.10, 129.57, 129.54, 129.49, 128.42, 128.05, 126.91, 124.87, 82.13, 78.91, 54.60, 38.44, 38.15, 36.76, 28.42, 27.96, 25.51; HRMS m/z calcd for C$_{29}$H$_{40}$N$_5$O$_7$ [M+H]$^+$ 570.29223, found 570.29386.

**Tert-butyl (1-(cyclopropylmethyl)-2-(2-nitrobenzyldiene)hydrazine-1-carbonyl)-L-phenylalaninate** 2i was synthesized using aza-dipeptide 1b (100 mg, 0.24 mmol), triphenylphosphine (126 mg, 0.48 mmol), DIAD (85 µL, 0.48 mmol) and cyclopropylmethanol (58 µL, 0.72 mmol) according to the procedure described above for 2b. The crude product was purified by Al$_2$O$_3$ column chromatography (EtOAc/hexanes, 10:1 to 1:1, v/v). 2i was obtained as yellow oil (61 mg, 54%): $R_f = 0.69$ (EtOAc/hexanes, 1:3, v/v); [\( \alpha \)]$_{D}^{20}$ +3.0 (c 0.1, CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.25 (s, 1H), 8.02 (dd, $J = 8.2, 1.3$ Hz, 1H), 7.81 (dd, $J = 7.9, 1.4$ Hz, 1H), 7.61 (td, $J = 7.7, 1.3$ Hz, 1H), 7.48 (ddd, $J = 8.6, 7.3, 1.5$ Hz, 1H), 7.32 – 7.20 (m, 5H), 6.98 (d, $J = 8.3$ Hz, 1H), 4.78 – 4.68 (m, 1H), 3.89 (d, $J = 6.6$ Hz, 2H), 3.16 (qd, $J = 13.8, 5.9$ Hz, 2H), 1.42 (s, 9H), 1.17 – 1.05 (m, 1H), 0.58 – 0.49 (m, 2H), 0.45 – 0.33 (m, 2H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 170.96, 154.96, 147.98, 136.46, 133.31, 131.51, 129.91, 129.62, 129.19, 128.36, 127.92, 126.81, 124.81, 81.96, 54.63, 45.21, 38.57, 27.98, 8.18, 4.20, 4.16; HRMS m/z calcd for C$_{25}$H$_{30}$N$_4$NaO$_5$ [M+Na]$^+$ 489.21084, found 489.21218.

**Tert-butyl (1-allyl-2-(2-nitrobenzyldiene)hydrazine-1-carbonyl)-L-phenylalaninate** 2j was synthesized using aza-dipeptide 1b (100 mg, 0.24 mmol), triphenylphosphine (126 mg, 0.48 mmol), DIAD (85 µL, 0.48...
mmol) and allyl alcohol (49 μL, 0.72 mmol) according to the procedure described above for 2b. The crude product was purified by Al₂O₃ column chromatography (EtOAc/hexanes, 10:1 to 1:1, v/v). 2j was obtained as yellow oil (88 mg, 81%): R₇ = 0.54 (EtOAc/hexanes, 1:3, v/v); [α]D° +1.5 (c 1, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.07 (s, 1H), 7.98 (dd, J = 8.2, 1.2 Hz, 1H), 7.78 (dd, J = 7.9, 1.4 Hz, 1H), 7.58 (t, J = 7.6 Hz, 1H), 7.50 – 7.41 (m, 1H), 7.27 (d, J = 6.9 Hz, 3H), 7.24 – 7.16 (m, 2H), 7.00 (d, J = 8.4 Hz, 1H), 5.74 (ddt, J = 17.3, 10.0, 4.8 Hz, 1H), 5.19 (dd, J = 34.4, 13.9 Hz, 2H), 4.75 (dt, J = 8.3, 5.9 Hz, 1H), 4.69 – 4.53 (m, 2H), 3.16 (qd, J = 13.8, 5.9 Hz, 2H), 1.42 (s, 9H).

Tert-butyl (2-(2-nitrobenzylidene)-1-(prop-2-yn-1-yl)hydrazine-1-carbonyl)-L-phenylalaninate 2k was synthesized using aza-dipeptide 1b (100 mg, 0.24 mmol), triphenylphosphine (126 mg, 0.48 mmol), DIAD (85 μL, 0.48 mmol) and homopropargyl alcohol (55 μL, 0.72 mmol) according to the procedure described above for 2b. The crude product was purified by Al₂O₃ column chromatography (EtOAc/hexanes, 10:1 to 1:1, v/v). 2k was obtained as yellow oil (44 mg, 39%): R₇ = 0.60 (EtOAc/hexanes, 1:3, v/v); [α]D° –1.5 (c 1, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.18 (s, 1H), 8.02 (d, J = 8.2 Hz, 1H), 7.76 (d, J = 7.9 Hz, 1H), 7.61 (t, J = 7.6 Hz, 1H), 7.49 (t, J = 7.7 Hz, 1H), 7.26 – 7.18 (m, 5H), 6.93 (d, J = 8.3 Hz, 1H), 4.73 (dt, J = 8.4, 5.9 Hz, 1H), 4.17 (hept, J = 7.5 Hz, 2H), 3.15 (tt, J = 13.7, 6.5 Hz, 2H), 2.50 (td, J = 7.4, 2.6 Hz, 2H), 1.99 (d, J = 2.7 Hz, 1H), 1.41 (s, 9H).

13C NMR (100 MHz, CDCl₃) δ 170.85, 154.38, 147.96, 136.34, 133.40, 132.11, 129.61, 129.52, 128.72, 128.38, 128.14, 126.85, 124.85, 117.62, 82.05, 54.53, 44.07, 38.55, 27.98; HRMS m/z calcd for C₂₅H₂₉N₄O₅ [M+Na]⁺ 487.19519, found 487.19659.

Tert-butyl (1-cyclohexyl-2-(2-nitrobenzylidene)hydrazine-1-carbonyl)-L-phenylalaninate 2l was synthesized using aza-dipeptide 1b (100 mg, 0.24 mmol), triphenylphosphine (126 mg, 0.48 mmol), DIAD (85 μL, 0.48 mmol) and 4-pentenol (74 μL, 0.72 mmol) according to the procedure described above for 2b. The crude product was purified by Al₂O₃ column chromatography (EtOAc/hexanes, 10:1 to 1:1, v/v). 2l was obtained as yellow oil (50 mg, 43%): R₇ = 0.55 (EtOAc/hexanes, 1:3, v/v); [α]D° –7.0 (c 1, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.06 (s, 1H), 8.00 (dd, J = 8.2, 1.4 Hz, 1H), 7.77 (dd, J = 8.0, 1.4 Hz, 1H), 7.58 (td, J = 7.6, 1.3 Hz, 1H), 7.46 (ddd, J = 8.6, 7.3, 1.5 Hz, 1H), 7.27 (d, J = 6.1 Hz, 1H), 7.23 – 7.16 (m, 3H), 6.94 (d, J = 8.3 Hz, 1H), 5.83 (ddt, J = 16.8, 9.9, 6.6 Hz, 1H), 5.11 – 5.00 (m, 2H), 5.00 – 4.95 (m, 1H), 4.72 (dt, J = 8.3, 6.0 Hz, 1H), 3.96 (dd, J = 8.7, 6.5 Hz, 2H), 3.20 – 3.08 (m, 2H), 2.17 – 2.05 (m, 2H), 1.73 – 1.64 (m, 2H), 1.41 (s, 9H). ¹³C NMR (100 MHz, CDCl₃) δ 170.96, 154.70, 147.89, 136.34, 133.40, 132.11, 129.61, 129.52, 128.38, 128.14, 126.85, 124.85, 117.62, 80.37, 70.47, 54.49, 39.69, 38.55, 27.98, 16.11; HRMS m/z calcd for C₂₆H₃₃N₄O₅ [M+Na]⁺ 481.24566, found 481.24566.
**Tert-butyl (2-(2-nitrobenzylidene)-1-(pyridin-3-ylmethyl)hydrazine-1-carbonyl)-L-phenylalaninate 2m**

was synthesized using aza-dipeptide 1b (100 mg, 0.24 mmol), triphenylphosphine (126 mg, 0.48 mmol), DIAD (85 μL, 0.48 mmol) and pyridin-3-ylmethanol (70 μL, 0.72 mmol) according to the procedure described above for 2b. The crude product was purified by Al₂O₃ column chromatography (EtOAc/hexanes, 10:1 to 1:1, v/v).

2m was obtained as yellow oil (65 mg, 54%): R_f = 0.42 (EtOAc/hexanes, 1:1, v/v); [α]_D²⁰ = +3.2 (c 1, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.50 (d, J = 2.3 Hz, 1H), 8.48 – 8.44 (m, 1H), 8.03 (d, J = 2.5 Hz, 1H), 7.29 – 7.49 (m, 2H), 7.90 (dt, J = 7.8, 2.5 Hz, 1H), 7.92 (t, J = 7.4, 5.5 Hz, 1H), 7.43 – 7.37 (m, 5H), 7.72 – 7.67 (m, 1H), 7.66 – 7.58 (m, 1H), 7.49 (tdd, J = 7.4, 5.5, 3.3 Hz, 4H), 7.43 – 7.37 (m, 5H), 7.27 (dd, J = 6.9, 2.1 Hz, 1H), 7.21 (d, J = 7.2 Hz, 3H), 7.05 (d, J = 8.4 Hz, 1H), 5.26 – 5.12 (m, 2H), 4.78 (dt, J = 8.1, 6.1 Hz, 1H), 3.18 (dd, J = 6.2, 3.7 Hz, 2H), 1.42 (d, J = 2.3 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 170.81, 154.91, 148.98, 148.59, 136.27, 134.85, 133.85, 133.03, 132.08, 130.75, 129.09, 128.54, 127.89, 126.93, 124.86, 123.69, 82.22, 54.68, 43.05, 38.53, 28.00; HRMS m/z calcd for C₂₇H₂₉N₅NaO₅ [M+Na]⁺ 526.20609, found 526.20757.

**Tert-butyl (1-((S)-sec-butyl)-2-((E)-2-nitrobenzylidene)hydrazine-1-carbonyl)-L-phenylalaninate 2n**

was synthesized using aza-dipeptide 1b (100 mg, 0.24 mmol), triphenylphosphine (125.9 mg, 0.48 mmol), DIAD (84.7 μL, 0.48 mmol) and R-(-)-2-Butanol (66.1 μL, 0.72 mmol) according to the procedure described above for 2b. The crude product was purified by Al₂O₃ column chromatography (EtOAc/hexanes, 10:1 to 1:1, v/v).

2n was obtained as yellow oil (71 mg, 63%): R_f = 0.45 (EtOAc/hexanes, 1:5, v/v); [α]_D²⁰ = -4.1 (c 1, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.65 (s, 1H), 8.01 (dd, J = 7.9, 1.5 Hz, 1H), 7.88 (dd, J = 8.1, 1.3 Hz, 1H), 7.54 (t, J = 7.6 Hz, 1H), 7.42 (td, J = 7.8, 7.4, 1.5 Hz, 1H), 7.29 (dd, J = 7.8, 5.9 Hz, 2H), 7.24 – 7.18 (m, 3H), 6.39 (d, J = 8.5 Hz, 1H), 5.05 (q, J = 6.2 Hz, 1H), 4.45 (dt, J = 8.6, 6.2 Hz, 1H), 3.09 (dd, J = 6.2, 3.8 Hz, 2H), 1.72 – 1.59 (m, 2H), 1.22 (d, J = 6.2 Hz, 3H), 0.92 (t, J = 7.5 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 170.81, 154.91, 148.98, 148.59, 136.27, 134.85, 133.85, 133.03, 132.08, 130.75, 129.09, 128.54, 127.89, 126.93, 124.86, 123.69, 82.22, 54.68, 43.05, 38.53, 28.00; HRMS m/z calcd for C₂₅H₃₃N₄O₅ [M+H]⁺ 469.24455, found 469.24584; Chiral HPLC analysis: Chiralcel IA-H (Hexane/i-PrOH) = 98:2, flow rate = 0.5 mL/min, wave length = 254 nm, t_R = 21.322 min.

**Tert-butyl (1-((R)-sec-butyl)-2-((E)-2-nitrobenzylidene)hydrazine-1-carbonyl)-L-phenylalaninate 2n**

was synthesized using the same procedure as described for 2n, but (S)-(+)/2-Butanol was used. The crude product was purified by Al₂O₃ column chromatography (EtOAc/hexanes, 10:1 to 1:1, v/v). The product was obtained as yellow oil (68 mg, 60%): R_f = 0.46 (EtOAc/hexanes, 1:5, v/v); [α]_D²⁰ = -9.5 (c 1, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.65 (s, 1H), 8.01 (dd, J = 7.9, 1.5 Hz, 1H), 7.88 (dd, J = 8.1, 1.3 Hz, 1H), 7.54 (t, J = 7.6 Hz, 1H), 7.42 (td, J = 7.8, 7.4, 1.5 Hz, 1H), 7.29 (dd, J = 7.8, 5.9 Hz, 2H), 7.24 – 7.18 (m, 3H), 6.39 (d, J = 8.5 Hz, 1H), 5.05 (q, J = 6.2 Hz, 1H), 4.45 (dt, J = 8.6, 6.2 Hz, 1H), 3.09 (dd, J = 6.2, 3.8 Hz, 2H), 1.72 – 1.59 (m, 2H), 1.22 (d, J = 6.2 Hz, 3H), 0.92 (t, J = 7.5 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 170.79, 161.39, 148.36, 145.59, 145.49, 136.27, 134.85, 133.85, 133.03, 132.08, 130.75, 129.09, 128.54, 128.40, 126.88, 124.24, 82.01, 74.74, 55.11, 39.34, 28.88, 27.93, 19.32, 9.51; HRMS m/z calcd for C₂₅H₃₃N₄O₅ [M+H]⁺ 469.24455, found 469.24584; Chiral HPLC analysis: Chiralcel IA-H (Hexane/i-PrOH) = 98:2, flow rate = 0.5 mL/min, wave length = 254 nm, t_R = 21.322 min.
136.19, 132.44, 130.38, 129.57, 128.88, 128.42, 128.46, 126.92, 124.24, 81.97, 74.83, 55.17, 39.19, 28.90, 27.93, 19.41, 9.56. HRMS m/z calcld for C_{25}H_{32}N_{4}NaO_{5} [M+Na]^+ 491.2264, found 491.2276; Chiral HPLC analysis: Chiralcel IA-H (Hexane/i-PrOH) = 98:2, flow rate = 0.5 mL/min, wave length = 254 nm, $t_R = 23.808$ min.

Chiral HPLC analysis of the Mitsunobu reaction product of azadipeptide 1b with (a) (R)-sec-butanol, (b) (S)-sec-butanol, and (c) racemic sec-butanol.

### 1.5 Synthesis of dipeptide 3a-f and 4a-f using Mitsunobu Reaction

**Representative synthesis of azadipeptide using Mitsunobu reaction on solid phase:**

**2-Nitrobenzylidene-azaAla-Phe-NH$_3$ 3a:** Vacuum dried semicarbazone resin 1e (~208 mg, 0.2 mmol) was suspended in anhydrous THF (3 mL) in a sealed flask under nitrogen. Solutions of methanol (122 μL, 3 mmol) in anhydrous THF (1 mL), PPh$_3$ (525 mg, 2 mmol) in anhydrous THF (1 mL), and DIAD (394 μL, 2 mmol) in anhydrous THF (1 mL) were sequentially added to the resin mixture, which was shaken on an automated shaker for 5 h, and filtered. After filtration, the resin was washed sequentially with DMF (3 × 10 mL), MeOH (3 × 10 mL), THF (3 × 10 mL), and DCM (3 × 10 mL). Examination by LC-MS of a cleaved resin sample showed excellent conversion (96%);
2-Nitrobenzylidene azadipeptide 3b-f and 4a-f were synthesized using the same protocol as described for 3a.

2-Nitrobenzylidene-azaAla-Phe-NH₂ 3a: LC-MS (60-95% MeOH, 14 min) R.T. = 7.55 min; Conversion 96%; HRMS m/z calcd for C₁₅H₂₀N₅O₄ [M+H] + 370.15098, found 370.15206; LC-MS (60-95% MeOH, 14 min) R.T. = 7.55 min;

2-Nitrobenzylidene-azaPhe-Phe-NH₂ 3b: LC-MS (60-95% MeOH, 14 min) R.T. = 10.73 min; Conversion 96%; HRMS m/z calcd for C₂₄H₂₄N₅O₄ [M+H] + 446.18228, found 446.18370.
2-Nitrobenzylidene-azaOrn-Phe-NH$_2$ 3c: LC-MS (40-95% MeOH, 14 min) R.T. = 7.27 min; Conversion 95%; HRMS m/z calcd for C$_{20}$H$_{25}$N$_6$O$_4$ [M+H]$^+$ 413.19318, found 413.19426.

2-Nitrobenzylidene-cyclopropylmethyl-azaGly-Phe-NH$_2$ 3d: LC-MS (60-95% MeOH, 14 min) R.T. = 10.22 min; Conversion 96%; HRMS m/z calcd for C$_{21}$H$_{24}$N$_5$O$_4$ [M+H]$^+$ 410.18228, found 410.18297.

2-Nitrobenzylidene-azaVal-Phe-NH$_2$ 3e: LC-MS (60-95% MeOH, 14 min) R.T. = 9.75 min; Conversion 87% (reaction was repeated for once); HRMS m/z calcd for C$_{20}$H$_{24}$N$_5$O$_4$ [M+H]$^+$ 398.18336, found 398.18228.
2-Nitrobenzylidene-azaLle-Phe-NH₂ 3f: LC-MS (60-95% MeOH, 14 min) R.T. = 10.70 min; Conversion 92%; HRMS m/z calcd for C₂₁H₂₈N₅O₄ [M+H]⁺ 412.19793, found 412.19877.

2-Nitrobenzylidene-azaAla-Phe-OH 4a: LC-MS (60-95% MeOH, 14 min) R.T. = 9.23 min; Conversion 98%; HRMS m/z calcd for C₁₈H₁₉N₄O₅ [M+H]⁺ 371.13500, found 371.13568.

2-Nitrobenzylidene-azaPhe-Phe-OH 4b: LC-MS (60-95% MeOH, 14 min) R.T. = 11.83 min; Conversion 97%; HRMS m/z calcd for C₂₄H₂₃N₄O₅ [M+H]⁺ 447.16630, found 447.16768.
2-Nitrobenzylidene-azaOrn-Phe-OH 4c: LC-MS (40-95% MeOH, 14 min) R.T. = 8.60 min; Conversion 92%; HRMS m/z calcd for C_{20}H_{24}N_{5}O_{5} [M+H]^+ 414.17720, found 414.17767.

2-Nitrobenzylidene-cyclopropylmethyl-azaGly-Phe-OH 4d: LC-MS (40-95% MeOH, 14 min) R.T. = 11.53 min; Conversion 82%; HRMS m/z calcd for C_{21}H_{25}N_{4}O_{5} [M+H]^+ 425.18195, found 425.18275.

2-Nitrobenzylidene-azaVal-Phe-OH 4e: LC-MS (60-95% MeOH, 14 min) R.T. = 11.03 min; Conversion 85% (reaction was repeated for once); HRMS m/z calcd for C_{20}H_{21}N_{4}O_{5} [M+H]^+ 399.16630, found 399.16764.
2-Nitrobenzylidene-azaIle-Phe-OH 4f: LC-MS (60-95% MeOH, 15 min) R.T. = 11.88 min; Conversion 100%; HRMS m/z calcd for C₂₁H₂₅N₄O₅ [M+H]^+ 413.18195, found 413.18342.

1.6 Synthesis of [aza-Xaa³]-Ang-(1-7) analogs 10a-d

Wang resin supported NH₂-Tyr(O'Bu)-Ile-His(Trt)-Pro was prepared by standard SPPS on Polystyrene Wang resin (0.96 mmol/g, typically ~208 mg per peptide).

Synthesis of [aza-Val³]-Ang-(1-7) 10a as a representative for the synthesis of [aza-Xaa³]-Ang-(1-7) analogs using Mitsunobu reaction

Representative synthesis of nitrobenzylidene semicarbazone-protected aza-Gly on solid phase:

2-Nitrobenzylidene semicarbazone resin 5: A solution of N,N'-disuccinimidy carbonate (205 mg, 0.8 mmol) in 3 mL of DMF/DCM (1:1, v/v) was treated dropwise with a solution of 2-nitrobenzylidene hydrazine (132 mg, 0.8 mmol) in 1 mL of DCM over 15 min, stirred for 2 h at rt, and then transferred to a syringe tube equipped with teflon™ filter, stopper and stopcock containing swollen NH₂-Tyr(O'Bu)-Ile-His(Trt)-Pro resin (~208 mg, 0.2 mmol). The resin mixture was treated with DIEA (207 μL, 1.2 mmol), shaken on an automated shaker for 12 h. After filtration, the resin was washed sequentially with DMF (3 × 10 mL), MeOH (3 × 10 mL), THF (3 ×
10 mL), and DCM (3 × 10 mL). Examination by LC-MS of a cleaved resin sample showed complete coupling and gave semicarbazone 5 in good purity.

Representative alkylation of nitrobenzylidene semicarbazone-protected aza-Gly using Mitsunobu reaction on solid phase:

2-Nitrobenzylidene semicarbazone resin 6a: Vacuum dried semicarbazone resin 5 (~208 mg, 0.2 mmol) was suspended in anhydrous THF (3 mL) in a sealed flask under nitrogen. Solutions of isopropanol (229 μL, 3 mmol) in anhydrous THF (1 mL), PPh₃ (525 mg, 2 mmol) in anhydrous THF (1 mL), and DIAD (394 μL, 2 mmol) in anhydrous THF (1 mL) were sequentially added to the resin mixture, which was shaken on an automated shaker for 5 h, and filtered. After filtration, the resin was washed sequentially with DMF (3 × 10 mL), MeOH (3 × 10 mL), THF (3 × 10 mL), and DCM (3 × 10 mL). The above procedure was repeated for once. Examination by LC-MS of a cleaved resin sample showed complete conversion and gave semicarbazone 6a in good purity. 2-Nitrobenzylidene semicarbazone 6b-d were synthesized using the same procedure as described for 6a using corresponding alcohols and the alkylation reaction does not need to be repeated.

Representative reduction of nitrobenzylidene semicarbazone to aminobenzylidene on solid phase:

2-Aminobenzylidene semicarbazone resin 7a: A solution of sodium dithionite (696 mg, 4 mmol), potassium carbonate (774 mg, 5.6 mmol), and TBAHS (136 mg, 0.4 mmol) in water (4 mL) and DCM (4 mL) was added to a syringe tube equipped with teflon™ filter, stopper and stopcock containing swollen semicarbazone resin 6a (~208 mg, 0.2 mmol) and the reaction slurry was shaken at room temperature for 2 h. After filtration, the resin was washed sequentially with DCM/water (1:1, v/v), DMF (3 × 10 mL), THF (3 × 10 mL), MeOH (3 × 10 mL), and DCM (3 × 10 mL). Examination by LC-MS of a cleaved resin sample showed complete conversion and gave semicarbazone 7a in good purity. 2-Aminobenzylidene semicarbazone 7b-d were synthesized using the same procedure as described for 7a.

Representative removal of aminobenzylidene semicarbazone on solid phase:

Semicarbazide 8a: Semicarbazone 7a (~208 mg, 0.2 mmol) was treated with a solution of NH₂OH.HCl (278 mg, 4 mmol) and m-phenylenediamine (433 mg, 4 mmol) in EtOH (5.3 mL), and heated with sonication at 60 °C for 12 h in a sealed syringe tube equipped with teflon™ filter, stopper and stopcock. The resin was filtered and washed with 10% DIEA:DMF (3 × 10 mL), DCM/water (1:1, v/v), DMF (3 × 10 mL), THF (3 × 10 mL), MeOH (3 × 10 mL), and DCM (3 × 10 mL). Examination by LC-MS of a cleaved resin sample showed complete conversion and gave semicarbazide 8a in good purity. Semicarbazide 8b-d were synthesized using the same procedure as described for 8a.

Representative amino acylation of semicarbazide on solid phase:
Azahexapeptide resin 9a: Fmoc-Arg(Pbf)-OH (649 mg, 1 mmol) in 6 mL of DCM was treated with BTC (99 mg, 0.33 mmol, CAUTION: BTC may liberate small quantities of highly toxic phosgene, and must be handled with extreme care in a fumehood) and 2,4,6-Collidine (684 μL, 4.6 mmol). After stirring for 5 min the mixture was transferred to a syringe tube equipped with teflon™ filter, stopper and stopcock containing swollen resin 8a (~208 mg, 0.2 mmol). The resin was shaken on an automated shaker for 12 h. After filtration, the resin was washed sequentially with DMF (3 × 10 mL), MeOH (3 × 10 mL), THF (3 × 10 mL), and DCM (3 × 10 mL). Examination by LC-MS of a cleaved resin sample showed the coupling was incomplete. The coupling procedure was repeated for once and still yield incomplete coupling with only a conversion of 8%. No further optimization was executed and the resin was used for next peptide coupling. Azahexapeptide resin 9b-d were synthesized using the same procedure as described for 9a.

Asp-Arg-azaVal-Tyr-Ile-His-Pro-OH (10a): After Fmoc-removal of azahexapeptide 9a and standard peptide coupling with Fmoc-Asp(OtBu)-OH, [aza-Val³]-Ang-(1-7) 10a was obtained. After final cleavage from resin, the crude product was analyzed by LC-MS with a crude purity of 2%. The crude product was purified by preparative RP-HPLC and the purity was ascertained by LC-MS. [Aza-Xaa³]-Ang-(1-7) analogs 10b-d were synthesized using the same procedure as described for 10a.

Asp-Arg-azaVal-Tyr-Ile-His-Pro-OH (10a): LC-MS (10-60% MeOH, 15 min) R.T. = 7.00 min; (5-50% MeCN, 15 min) R.T. = 5.07 min; HRMS m/z calcd for C₄₀H₆₂N₁₃O₁₁ [M+H]+ 900.46863, found 900.47159

Asp-Arg-azaAla-Tyr-Ile-His-Pro-OH (10b): LC-MS (10-60% MeOH, 15 min) R.T. = 5.78 min; (5-50% MeCN, 15 min) R.T. = 4.50 min; HRMS m/z calcd for C₃₈H₅₈N₁₃O₁₁ [M+H]+ 872.43733, found 872.44010

Asp-Arg-azaPhe-Tyr-Ile-His-Pro-OH (10c): LC-MS (10-60% MeOH, 15 min) R.T. = 9.77 min; (5-50% MeCN, 15 min) R.T. = 6.60 min; HRMS m/z calcd for C₄₄H₆₂N₁₃O₁₁ [M+H]+ 948.46863, found 948.46939

Asp-Arg-cyclopropylmethyl-azaGly-Tyr-Ile-His-Pro-OH (10d): LC-MS (10-60% MeOH, 15 min) R.T. = 7.60 min; (5-50% MeCN, 15 min) R.T. = 5.37 min; HRMS m/z calcd for C₄₁H₆₂N₁₃O₁₁ [M+H]+ 912.46863, found 912.46706

1.7 Synthesis of [aza-Xaa⁴]-Ang-(1-7) analogs 11a-d and [Aza-Xaa⁵]-Ang-(1-7) analogs 12a-d
[Aza-Xaa]^4^-Ang-(1-7) peptide analogs 11a-d and [Aza-Xaa]^5^-Ang-(1-7) peptide analogs 12a-d were synthesized following the same procedures above as described for the synthesis of azapeptide 12a by varying the position of the aza-residue. The peptides were purified by preparative RP-HPLC and product purity was ascertained by LC-MS.

Asp-Arg-Val-azaTyr-Ile-His-Pro-OH (11a): LC-MS (10-60% MeOH, 15 min) R.T. = 6.60 min; (5-50% MeCN, 15 min) R.T. = 4.90 min; HRMS m/z calcd for C_{40}H_{62}N_{13}O_{11} [M+H]^+ 900.46863, found 900.46869

Asp-Arg-Val-azaAla-Ile-His-Pro-OH (11b): LC-MS (10-60% MeOH, 15 min) R.T. = 5.25 min; (5-50% MeCN, 15 min) R.T. = 4.18 min; HRMS m/z calcd for C_{34}H_{58}N_{13}O_{10} [M+H]^+ 808.44241, found 808.44373

Asp-Arg-Val-azaPhe-Ile-His-Pro-OH (11c): LC-MS (10-60% MeOH, 15 min) R.T. = 8.97 min; (5-50% MeCN, 15 min) R.T. = 6.07 min; HRMS m/z calcd for C_{40}H_{62}N_{13}O_{10} [M+H]^+ 884.47371, found 884.47509

Asp-Arg-Val-cyclopropylmethyl-azaGly-Ile-His-Pro-OH (11d): LC-MS (10-60% MeOH, 15 min) R.T. = 6.87 min; (5-50% MeCN, 15 min) R.T. = 4.95 min; HRMS m/z calcd for C_{37}H_{62}N_{13}O_{10} [M+H]^+ 848.47371, found 848.47498

Asp-Arg-Val-Tyr-azaIle-His-Pro-OH (12a): LC-MS (10-60% MeOH, 15 min) R.T. = 6.12 min; (5-50% MeCN, 15 min) R.T. = 4.75 min; HRMS m/z calcd for C_{40}H_{62}N_{13}O_{11} [M+H]^+ 900.46863, found 900.46706

Asp-Arg-Val-Tyr-azaAla-His-Pro-OH (12b): LC-MS (10-60% MeOH, 15 min) R.T. = 3.92 min; (5-50% MeCN, 15 min) R.T. = 3.62 min; HRMS m/z calcd for C_{37}H_{56}N_{13}O_{11} [M+H]^+ 858.42168, found 858.42278

Asp-Arg-Val-Tyr-azaPhe-His-Pro-OH (12c): LC-MS (10-60% MeOH, 15 min) R.T. = 7.87 min; (5-50% MeCN, 15 min) R.T. = 5.75 min; HRMS m/z calcd for C_{43}H_{60}N_{13}O_{11} [M+H]^+ 934.45298, found 934.45619

Asp-Arg-Val-Tyr-cyclopropylmethyl-azaGly-His-Pro-OH (12d): LC-MS (10-60% MeOH, 15 min) R.T. = 5.62 min; (5-50% MeCN, 15 min) R.T. = 4.52 min; HRMS m/z calcd for C_{40}H_{60}N_{13}O_{11} [M+H]^+ 898.45298, found 898.45388
2. Crystal structure of azadipeptide 2b
3. $^1$H-NMR and $^{13}$C-NMR spectra of compounds 1b-d, 2b-n
2m

\begin{align*}
N & N \\
O & O
\end{align*}
4. Analytical LC-MS chromatograms of purified azapeptides 10a-d, 11a-d and 12a-d

LC-MS chromatogram of [azaVal]$^3$-Ang-(1-7) 10a in a linear gradient of 10-60% of MeOH in H$_2$O containing 0.1% FA over 15 min, R.T. = 7.00 min

LC-MS chromatogram of [azaVal]$^3$-Ang-(1-7) 10a in a linear gradient of 5-50% of MeCN in H$_2$O containing 0.1% FA over 15 min, R.T. = 5.07 min
LC-MS chromatogram of [azaAla\textsuperscript{3}]-Ang-(1-7) 10b in a linear gradient of 10-60% of MeOH in H\textsubscript{2}O containing 0.1% FA over 15 min, R.T. = 5.78 min

LC-MS chromatogram of [azaAla\textsuperscript{3}]-Ang-(1-7) 10b in a linear gradient of 5-50% of MeCN in H\textsubscript{2}O containing 0.1% FA over 15 min. LC-MS (5-50% MeCN, 15 min) R.T. = 4.50 min
LC-MS chromatogram of [azaPhe]-Ang-(1-7) 10c in a linear gradient of 10-60% of MeOH in H₂O containing 0.1% FA over 15 min, R.T. = 9.77 min

LC-MS chromatogram of [azaPhe]-Ang-(1-7) 10c in a linear gradient of 10-60% of MeCN in H₂O containing 0.1% FA over 15 min, R.T. = 6.60 min
LC-MS chromatogram of [cyclopropymethyl-azaGly]-Ang-(1-7) 10d in a linear gradient of 10-60% of MeOH in H2O containing 0.1% FA over 15 min, R.T. = 7.60 min

LC-MS chromatogram of [cyclopropymethyl-azaGly]-Ang-(1-7) 10d in a linear gradient of 5-50% of MeCN in H2O containing 0.1% FA over 15 min, R.T. = 5.37 min
LC-MS chromatogram of [azaTyr]-Ang-(1-7) 11a in a linear gradient of 10-60% of MeOH in H₂O containing 0.1% FA over 15 min, R.T. = 6.60 min

LC-MS chromatogram of [azaTyr]-Ang-(1-7) 11a in a linear gradient of 5-50% of MeCN in H₂O containing 0.1% FA over 15 min, R.T. = 4.90 min
LC-MS chromatogram of [azaAla⁸]-Ang-(1-7) 11b in a linear gradient of 10-60% of MeOH in H₂O containing 0.1% FA over 15 min, R.T. = 5.25 min

LC-MS chromatogram of [azaAla⁸]-Ang-(1-7) 11b in a linear gradient of 5-50% of MeCN in H₂O containing 0.1% FA over 15 min, R.T. = 4.18 min
LC-MS chromatogram of [azaPhe]-Ang-(1-7) 11c in a linear gradient of 10-60% of MeOH in H₂O containing 0.1% FA over 15 min, R.T. = 8.97 min

LC-MS chromatogram of [azaPhe]-Ang-(1-7) 11c in a linear gradient of 5-50% of MeCN in H₂O containing 0.1% FA over 15 min, R.T. = 6.07 min
LC-MS chromatogram of [cyclopropylmethyl-azaGly]-Ang-(1-7) 11d in a linear gradient of 10-60% of MeOH in H₂O containing 0.1% FA over 15 min, R.T. = 6.87 min

LC-MS chromatogram of [cyclopropylmethyl-azaGly]-Ang-(1-7) 11d in a linear gradient of 10-60% of MeCN in H₂O containing 0.1% FA over 15 min, R.T. = 4.95 min
LC-MS chromatogram of [azalle⁵]-Ang-(1-7) **12a** in a linear gradient of 10-60% of MeOH in H₂O containing 0.1% FA over 15 min, R.T. = 6.12 min

LC-MS chromatogram of [azalle⁵]-Ang-(1-7) **12a** in a linear gradient of 5-50% of MeCN in H₂O containing 0.1% FA over 15 min, R.T. = 4.75 min
LC-MS chromatogram of [azaAla₅]-Ang-(1-7) **12b** in a linear gradient of 10-60% of MeOH in H₂O containing 0.1% FA over 15 min, R.T. = 3.92 min

LC-MS chromatogram of [azaAla₅]-Ang-(1-7) **12b** in a linear gradient of 5-50% of MeCN in H₂O containing 0.1% FA over 15 min, R.T. = 3.62 min
LC-MS chromatogram of [azaPhe\(^5\)]-Ang-(1-7) \textbf{12c} in a linear gradient of 10-60\% of MeOH in H\(_2\)O containing 0.1\% FA over 15 min, R.T. = 7.87 min

LC-MS chromatogram of [azaPhe\(^5\)]-Ang-(1-7) \textbf{12c} in a linear gradient of 5-50\% of MeCN in H\(_2\)O containing 0.1\% FA over 15 min, R.T. = 5.75 min
LC-MS chromatogram of [cyclopropylmethyl-azaGly]-Ang-(1-7) \textbf{12d} in a linear gradient of 10-60\% of MeOH in H\textsubscript{2}O containing 0.1\% FA over 15 min, R.T. = 5.62 min

LC-MS chromatogram of [cyclopropylmethyl-azaGly]-Ang-(1-7) \textbf{12d} in a linear gradient of 5-50\% of MeCN in H\textsubscript{2}O containing 0.1\% FA over 15 min, R.T. = 4.52 min