Supporting Information

Site-selective Intramolecular Hydrogen-bonding Interactions in Phosphorylated Serine and Threonine Dipeptides

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Synthesis of Compounds

General. ¹H and ¹³C NMR spectra were recorded on a Bruker Avance 500 NMR spectrometer. Chemical shifts (δ) and coupling constants (J) are reported in parts per million (ppm) and hertz (Hz), respectively. ¹H NMR spectra are referenced to TMS (tetramethylsilane in DMSO-*d*₆) as an internal standard. ¹³C NMR spectra are referenced to solvent (¹³C: DMSO-*d*₆, δ 39.50 ppm) as an internal standard. ³¹P NMR spectra are referenced to phosphoric acid as an external standard (100% phosphoric acid in the inner tube and CDCl₃ in the outer tube; ³¹P: H₃PO₄, δ 0.0018 ppm). High-resolution mass spectra (HRMS) were recorded on a JEOL JMS-AX505WA or JMS-600W mass spectrometer using fast atom bombardment (FAB) or chemical ionization (CI) techniques, respectively. Thin-layer chromatography (TLC) was performed on silica gel 60 F₂₅₄ precoated plates (0.25 mm thickness, Merck, Darmstadt). Flash chromatography was carried out on silica gel 60 (230–400 mesh, Merck). Reagent-grade chemicals were purchased from Aldrich, Fluka, and TCI and used as received unless otherwise specified.

Ac-L-Ser(Ac)-OMe (6a). To a suspension of **5a**-HCl (H-Ser-OMe-HCl, 5 g, 32.1 mmol, Fluka) in CH₂Cl₂ (60 mL) were added triethylamine (TEA, 6.7 mL, 48.1 mmol), acetic anhydride (15.2 mL, 161 mmol), and 4-(dimethylamino)pyridine (DMAP, 195 mg, 1.60 mmol) at 0 °C. After stirring at room temperature for 4 h, the reaction mixture was quenched with H₂O and extracted with CH₂Cl₂. The combined organic layers were washed with H₂O, dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by flash chromatography (MeOH/CH₂Cl₂ = 1:9) to give **6a** (3.2 g, 49%) as a white solid. TLC (MeOH/CH₂Cl₂ = 1:15) *R*_f = 0.58; ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.43 (d, *J* = 7.5 Hz, 1H), 4.59 (ddd, *J* = 7.9, 5.6, 4.9 Hz, 1H), 4.26 (dd, *J* = 11.5, 4.5 Hz, 1H), 4.19 (dd, *J* = 11.5, 6.0 Hz, 1H), 3.65 (s, 3H), 2.01 (s, 3H), 1.87 (s, 3H); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 170.04, 169.97, 169.49, 62.83, 52.14, 51.00, 22.18, 20.53; HRMS (FAB+) for C₈H₁₃NO₅Na (*M*Na⁺), calcd 226.0691, found 226.0685.

Ac-L-Ser-NHMe (1a).¹ To **6a** (3.02 g, 14.9 mmol) was added methylamine (40% in H₂O, 10.2 mL, 131 mmol) at 0 °C. After stirring at room temperature for 6 h, the reaction mixture was concentrated in vacuo. The residue was purified by flash chromatography (MeOH/CH₂Cl₂ = 1:5) to give **1a** (2.0 g, 84%) as a white solid. TLC (MeOH/CH₂Cl₂ = 1:7) *R*_f = 0.38; ¹H NMR (500 MHz, DMSO-*d*₆) δ 7.84 (d, *J* = 8.0 Hz, 1H), 7.75 (q, *J* = 4.5 Hz, 1H), 4.82 (t, *J* = 5.5 Hz, 1H), 4.19 (dt, *J* = 7.8, 5.6 Hz, 1H), 3.52 (t, *J* = 5.8 Hz, 2H), 2.57 (d, *J* = 4.5 Hz, 3H), 1.86 (s, 3H); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 170.49, 169.27, 61.62, 55.12, 25.55, 22.62; HRMS (CI+) for C₆H₁₃N₂O₃ (*M*H⁺), calcd 161.0926, found 161.0925.

Ac-L-Ser(PO(OBzl)₂)-NHMe (7a).²⁻⁴ To a stirred solution of **1a** (1.07 g, 6.68 mmol) and dibenzyl *N,N*-diisopropylphosphoramidite (2.70 mL, 8.04 mmol) in DMF (6 mL) was added 1*H*-tetrazole (~0.45 M in CH₃CN, 45.7 mL, 20.6 mmol). After stirring at room temperature for 30 min, the reaction mixture was cooled to -40 °C and a solution of 3-chloroperoxybenzoic acid (*m*-CPBA, ≤77%, 1.84 g, 8.21 mmol) in CH₂Cl₂ (20 mL) was added. After stirring below -20 °C for 10 min, the reaction mixture was quenched with 10% aqueous Na₂S₂O₅ and extracted with CH₂Cl₂. The combined organic layers were washed with 10% aqueous Na₂S₂O₅, 5% NaHCO₃ (× 2), and then 1 N HCl. The organic layer was dried over Na₂SO₄ and concentrated in vacuo. The residue was purified by flash chromatography (MeOH/CH₂Cl₂ = 1:20) to give **7a** (973 mg, 35%) as a colorless oil. TLC (MeOH/CH₂Cl₂ = 1:15) *R*_f = 0.30; ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.23 (d, *J* = 8.5 Hz, 1H), 8.04 (q, *J* = 4.2

Hz, 1H), 7.40–7.34 (m, 10H), 5.01 (d, $J = 7.5$ Hz, 4H), 4.55 (dt, $J = 8.3, 5.6$ Hz, 1H), 4.13 (dt, $J = 10.3, 6.1$ Hz, 1H), 4.10 (dt, $J = 10.5, 5.5$ Hz, 1H), 2.59 (d, $J = 5.0$ Hz, 3H), 1.87 (s, 3H); ^{13}C NMR (125 MHz, DMSO- d_6) δ 169.52, 168.66, 135.95 (d, $J = 7.4$ Hz), 128.45, 128.34, 127.81, 127.79, 68.53 (d, $J = 5.5$ Hz), 68.50 (d, $J = 5.7$ Hz), 66.69 (d, $J = 5.7$ Hz), 52.47 (d, $J = 8.4$ Hz), 25.65, 22.53; ^{31}P NMR (200 MHz, DMSO- d_6) δ -0.96; HRMS (FAB+) for $\text{C}_{20}\text{H}_{25}\text{N}_2\text{O}_6\text{PNa}$ (MNa^+), calcd 443.1348, found 443.1359.

Ac-L-Ser(PO_3H_2)-NHMe (2a).^{2–4} To a solution of **7a** (521 mg, 1.24 mmol) in AcOH/MeOH (1:9, 4 mL) was added 10% Pd/C (136 mg). After stirring at room temperature under 1 atm H_2 for complete reaction, the reaction mixture was filtered through Celite 545 and concentrated in vacuo. The residue was dissolved in H_2O and washed successively with diethyl ether and CH_2Cl_2 ($\times 3$). The aqueous layer was concentrated in vacuo to give **2a** (254 mg, 85%) as a colorless oil. ^1H NMR (500 MHz, DMSO- d_6) δ 8.16 (d, $J = 8.0$ Hz, 1H), 7.93 (q, $J = 4.5$ Hz, 1H), 4.38 (dt, $J = 7.5, 5.5$ Hz, 1H), 3.94 (dt, $J = 10.5, 6.5$ Hz, 1H), 3.91 (dt, $J = 10.0, 6.5$ Hz, 1H), 2.58 (d, $J = 5.0$ Hz, 3H), 1.87 (s, 3H); ^{13}C NMR (125 MHz, DMSO- d_6) δ 169.44, 169.34, 64.78 (d, $J = 4.7$ Hz), 53.27 (d, $J = 7.4$ Hz), 25.67, 22.62; ^{31}P NMR (200 MHz, DMSO- d_6) δ -0.49; HRMS (FAB+) for $\text{C}_6\text{H}_{14}\text{N}_2\text{O}_6\text{P}$ (MH^+), calcd 241.0589, found 241.0591.

Ac-L-Thr(Ac)-OMe (6b). To a suspension of **5b**-HCl (H-Thr-OMe·HCl, 25 g, 147 mmol, Novabiochem, La Jolla, CA) in CH_2Cl_2 (300 mL) were added TEA (30.7 mL, 220 mmol), acetic anhydride (69 mL, 730 mmol), and DMAP (897 mg, 7.34 mmol) at 0 °C. After stirring at room temperature for 4 h, the reaction mixture was quenched with H_2O and extracted with CH_2Cl_2 . The combined organic layers were washed with H_2O , dried over Na_2SO_4 , and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc/*n*-hexane = 1:2) to give **6b** (20 g, 62%) as a white solid. TLC (EtOAc/*n*-hexane = 2:1) $R_f = 0.33$; ^1H NMR (500 MHz, DMSO- d_6) δ 8.34 (d, $J = 8.5$ Hz, 1H), 5.20 (qd, $J = 6.3, 3.8$ Hz, 1H), 4.59 (dd, $J = 8.5, 3.5$ Hz, 1H), 3.63 (s, 3H), 1.98 (s, 3H), 1.93 (s, 3H), 1.15 (d, $J = 6.0$ Hz, 3H); ^{13}C NMR (125 MHz, DMSO- d_6) δ 170.15, 169.95, 169.44, 69.28, 55.02, 52.06, 22.11, 20.70, 16.53; HRMS (CI+) for $\text{C}_9\text{H}_{16}\text{NO}_5$ (MH^+), calcd 218.1028, found 218.1028.

Ac-L-Thr-NHMe (1b).¹ To **6b** (9.3 g, 42.8 mmol) was added methylamine (40% in H_2O , 37.0 mL, 476 mmol) at 0 °C. After stirring at room temperature for 6 h, the reaction mixture

was concentrated in vacuo. The residue was purified by flash chromatography (MeOH/CH₂Cl₂ = 1:12) to give **1b** (6.4 g, 86%) as a white solid. TLC (MeOH/CH₂Cl₂ = 1:12) *R*_f = 0.21; ¹H NMR (500 MHz, DMSO-*d*₆) δ 7.67 (q, *J* = 4.5 Hz, 1H), 7.64 (d, *J* = 9.0 Hz, 1H), 4.77 (d, *J* = 5.5 Hz, 1H), 4.08 (dd, *J* = 8.5, 4.0 Hz, 1H), 3.97 (qdd, *J* = 6.5, 5.5, 4.0 Hz, 1H), 2.58 (d, *J* = 4.5 Hz, 3H), 1.90 (s, 3H), 1.00 (d, *J* = 6.5 Hz, 3H); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 170.69, 169.53, 66.27, 58.37, 25.55, 22.65, 20.09; HRMS (CI⁺) for C₇H₁₅N₂O₃ (MH⁺), calcd 175.1082, found 175.1081.

Ac-L-Thr(PO(OBzl)₂)-NHMe (7b).²⁻⁴ To a stirred solution of **1b** (2.92 g, 16.8 mmol) and dibenzyl *N,N*-diisopropylphosphoramidite (6.61 mL, 19.7 mmol) in DMF (18 mL) was added 1*H*-tetrazole (~0.45 M in CH₃CN, 112 mL, 50.4 mmol). After stirring at room temperature for 30 min, the reaction mixture was cooled to -40 °C and a solution of *m*-CPBA ($\leq$ 77%, 4.51 g, 20.1 mmol) in CH₂Cl₂ (50 mL) was added. After stirring below -20 °C for 10 min, the reaction mixture was quenched with 10% aqueous Na₂S₂O₅ and extracted with CH₂Cl₂. The combined organic layers were washed with 10% aqueous Na₂S₂O₅, 5% aqueous NaHCO₃ ($\times$ 2), and then 1 N HCl. The organic layer was dried over Na₂SO₄ and concentrated in vacuo. The residue was purified by flash chromatography (MeOH/CH₂Cl₂ = 1:20) to give **7b** (5.12 g, 70%) as a white solid. TLC (MeOH/CH₂Cl₂ = 1:15) *R*_f = 0.26; ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.09 (d, *J* = 9.0 Hz, 1H), 7.97 (q, *J* = 4.3 Hz, 1H), 7.40–7.33 (m, 10H), 5.02 (dd, *J* = 11.8, 7.8, 1H), 4.99 (dd, *J* = 11.5, 6.5 Hz, 1H), 4.98 (dd, *J* = 11.8, 7.8 Hz, 1H), 4.96 (dd, *J* = 11.8, 7.3 Hz, 1H), 4.87 (qdd, *J* = 6.5, 6.5, 4.0 Hz, 1H), 4.47 (ddd, *J* = 9.1, 3.6, 2.4 Hz, 1H), 2.56 (d, *J* = 4.5 Hz, 3H), 1.93 (s, 3H), 1.22 (d, *J* = 6.5 Hz, 3H); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 169.90, 169.01, 136.10 (d, *J* = 7.3 Hz), 136.08 (d, *J* = 7.3 Hz), 128.40, 128.25, 128.22, 127.72, 127.64, 75.31 (d, *J* = 5.5 Hz), 68.36 (d, *J* = 5.7 Hz), 68.23 (d, *J* = 5.7 Hz), 56.43 (d, *J* = 8.3 Hz), 25.59, 22.49, 18.14; ³¹P NMR (200 MHz, DMSO-*d*₆) δ -1.69; HRMS (FAB⁺) for C₂₁H₂₇N₂O₆PNa (MNa⁺), calcd 457.1504, found 457.1517; HRMS (FAB⁺) for C₂₁H₂₈N₂O₆P (MH⁺), calcd 435.1685, found 435.1684.

Ac-L-Thr(PO₃H₂)-NHMe (2b).²⁻⁴ To a solution of **7b** (401 mg, 0.923 mmol) in AcOH/MeOH (1:9, 5 mL) was added 10% Pd/C (95 mg). After stirring at room temperature under 1 atm H₂ for complete reaction, the reaction mixture was filtered through Celite 545 and concentrated in vacuo. The residue was dissolved in H₂O and washed successively with diethyl ether and CH₂Cl₂ ($\times$ 3). The aqueous layer was concentrated in vacuo to give **2b** (198

mg, 84%) as a white solid. ^1H NMR (500 MHz, $\text{DMSO-}d_6$) δ 7.86 (q, J = 3.5 Hz, 1H), 7.85 (d, J = 8.0 Hz, 1H), 4.50 (dq, J = 7.8, 6.1, 6.1 Hz, 1H), 4.28 (dd, J = 8.3, 5.3 Hz, 1H), 2.58 (d, J = 4.5 Hz, 3H), 1.89 (s, 3H), 1.18 (d, J = 6.0 Hz, 3H); ^{13}C NMR (125 MHz, $\text{DMSO-}d_6$) δ 169.46, 169.35, 72.14 (d, J = 5.5 Hz), 57.32 (d, J = 6.5 Hz), 25.61, 22.54, 18.25; ^{31}P NMR (200 MHz, $\text{DMSO-}d_6$) δ -0.86; HRMS (FAB+) for $\text{C}_7\text{H}_{16}\text{N}_2\text{O}_6\text{P}$ (MH^+), calcd 255.0746, found 255.0749.

Ac-D-Ser(Ac)-OMe (9a). To a suspension of **8a**-HCl (H-D-Ser-OMe-HCl, 5 g, 32.1 mmol, Aldrich) in CH_2Cl_2 (60 mL) were added TEA (6.7 mL, 48.1 mmol), acetic anhydride (15.2 mL, 161 mmol), and DMAP (195 mg, 1.60 mmol) at 0 °C. After stirring at room temperature for 4 h, the reaction mixture was quenched with H_2O and extracted with CH_2Cl_2 . The combined organic layers were washed with H_2O , dried over Na_2SO_4 , and concentrated in vacuo. The residue was purified by flash chromatography ($\text{MeOH}/\text{CH}_2\text{Cl}_2$ = 1:9) to give **9a** (3.0 g, 46%) as a white solid. TLC ($\text{MeOH}/\text{CH}_2\text{Cl}_2$ = 1:15) R_f = 0.52; ^1H NMR (500 MHz, $\text{DMSO-}d_6$) δ 8.43 (d, J = 8.0 Hz, 1H), 4.59 (ddd, J = 7.5, 5.5, 5.0 Hz, 1H), 4.26 (dd, J = 11.5, 4.5 Hz, 1H), 4.19 (dd, J = 11.5, 6.0 Hz, 1H), 3.65 (s, 3H), 2.01 (s, 3H), 1.87 (s, 3H); ^{13}C NMR (125 MHz, $\text{DMSO-}d_6$) δ 170.05, 169.97, 169.49, 62.83, 52.15, 51.00, 22.18, 20.53; HRMS (FAB+) for $\text{C}_8\text{H}_{13}\text{NO}_5\text{Na}$ (MNa^+), calcd 226.0691, found 226.0698.

Ac-D-Ser-NHMe (3a).¹ To **9a** (3.0 g, 14.8 mmol) was added methylamine (40% in H_2O , 10.2 mL, 131 mmol) at 0 °C. After stirring at room temperature for 6 h, the reaction mixture was concentrated in vacuo. The residue was purified by flash chromatography ($\text{MeOH}/\text{CH}_2\text{Cl}_2$ = 1:5) to give **3a** (1.4 g, 59%) as a white solid. TLC ($\text{MeOH}/\text{CH}_2\text{Cl}_2$ = 1:7) R_f = 0.32; ^1H NMR (500 MHz, $\text{DMSO-}d_6$) δ 7.86 (d, J = 8.0 Hz, 1H), 7.76 (q, J = 4.5 Hz, 1H), 4.83 (t, J = 5.0 Hz, 1H), 4.19 (dt, J = 7.7, 5.8 Hz, 1H), 3.52 (t, J = 5.3 Hz, 2H), 2.57 (d, J = 5.0 Hz, 3H), 1.86 (s, 3H); ^{13}C NMR (125 MHz, $\text{DMSO-}d_6$) δ 170.50, 169.28, 61.63, 55.13, 25.56, 22.64; HRMS (CI+) for $\text{C}_6\text{H}_{13}\text{N}_2\text{O}_3$ (MH^+), calcd 161.0926, found 161.0927.

Ac-D-Ser(PO(OBzl)₂)-NHMe (10a).²⁻⁴ To a stirred solution of **3a** (3.06 g, 19.1 mmol) and dibenzyl *N,N*-diisopropylphosphoramidite (7.73 mL, 23.0 mmol) in DMF (15 mL) was added 1*H*-tetrazole (~0.45 M in CH_3CN , 112 mL, 50.4 mmol). After stirring at room temperature for 30 min, the reaction mixture was cooled to -40 °C and a solution of *m*-CPBA ($\leq 77\%$, 5.27 g, 23.5 mmol) in CH_2Cl_2 (20 mL) was added. After stirring below -20 °C for 10

min, the reaction mixture was quenched with 10% aqueous $\text{Na}_2\text{S}_2\text{O}_5$ and extracted with CH_2Cl_2 . The combined organic layers were washed with 10% aqueous $\text{Na}_2\text{S}_2\text{O}_5$, 5% aqueous NaHCO_3 ($\times 2$), and then 1 N HCl. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography ($\text{MeOH}/\text{CH}_2\text{Cl}_2 = 1:20$) to give **10a** (4.42 g, 55%) as a colorless oil. TLC ($\text{MeOH}/\text{CH}_2\text{Cl}_2 = 1:15$) $R_f = 0.30$; ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 8.23 (d, $J = 8.0$ Hz, 1H), 8.04 (q, $J = 4.5$ Hz, 1H), 7.40–7.34 (m, 10H), 5.01 (d, $J = 7.5$ Hz, 4H), 4.55 (dt, $J = 8.0, 5.5$ Hz, 1H), 4.13 (dt, $J = 10.2, 5.9$ Hz, 1H), 4.10 (dt, $J = 10.7, 5.6$ Hz, 1H), 2.59 (d, $J = 4.5$ Hz, 3H), 1.87 (s, 3H); ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$) δ 169.52, 168.66, 135.95 (d, $J = 7.4$ Hz), 128.45, 128.35, 127.81, 127.80, 68.53 (d, $J = 5.5$ Hz), 68.50 (d, $J = 5.7$ Hz), 66.69 (d, $J = 5.7$ Hz), 52.46 (d, $J = 8.3$ Hz), 25.66, 22.53; ^{31}P NMR (200 MHz, $\text{DMSO}-d_6$) δ -0.96 ; HRMS (FAB+) for $\text{C}_{20}\text{H}_{26}\text{N}_2\text{O}_6\text{P}$ (MH^+), calcd 421.1529, found 421.1527.

Ac-D-Ser(PO_3H_2)-NHMe (4a).^{2–4} To a solution of **10a** (4.42 g, 10.5 mmol) in AcOH/MeOH (1:9, 15 mL) was added 10% Pd/C (1.15 g). After stirring at room temperature under 1 atm H_2 for complete reaction, the reaction mixture was filtered through Celite 545 and concentrated in vacuo. The residue was dissolved in H_2O and washed successively with diethyl ether and CH_2Cl_2 ($\times 3$). The aqueous layer was concentrated in vacuo to give **4a** (2.50 g, 99%) as a colorless oil. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 8.19 (d, $J = 8.0$ Hz, 1H), 7.93 (q, $J = 4.5$ Hz, 1H), 4.36 (dt, $J = 7.5, 5.5$ Hz, 1H), 3.93 (dt, $J = 10.0, 6.0$ Hz, 1H), 3.90 (dt, $J = 10.5, 6.0$ Hz, 1H), 2.58 (d, $J = 4.5$ Hz, 3H), 1.87 (s, 3H); ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$) δ 169.44, 169.40, 64.74 (d, $J = 4.5$ Hz), 53.35 (d, $J = 7.4$ Hz), 25.68, 22.62; ^{31}P NMR (200 MHz, $\text{DMSO}-d_6$) δ -0.23 ; HRMS (FAB+) for $\text{C}_6\text{H}_{14}\text{N}_2\text{O}_6\text{P}$ (MH^+), calcd 241.0589, found 241.0598.

Ac-D-Thr(Ac)-OMe (9b). To a suspension of **8b**-HCl ($\text{H-D-Thr-OMe}\cdot\text{HCl}$, 10.0 g, 59.0 mmol, Bachem, Bubendorf) in CH_2Cl_2 (150 mL) were added TEA (12.3 mL, 88.2 mmol), acetic anhydride (27.8 mL, 294 mmol), and DMAP (360 mg, 2.95 mmol) at 0 °C. After stirring at room temperature for 4 h, the reaction mixture was quenched with H_2O and extracted with CH_2Cl_2 . The combined organic layers were washed with H_2O , dried over Na_2SO_4 , and concentrated in vacuo. The residue was purified by flash chromatography ($\text{EtOAc}/n\text{-hexane} = 1:2$) to give **9b** (10.0 g, 78%) as a white solid. TLC ($\text{EtOAc}/n\text{-hexane} = 2:1$) $R_f = 0.27$; ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 8.34 (d, $J = 8.5$ Hz, 1H), 5.20 (qd, $J = 6.4$,

3.6 Hz, 1H), 4.59 (dd, $J = 8.5, 3.5$ Hz, 1H), 3.63 (s, 3H), 1.98 (s, 3H), 1.93 (s, 3H), 1.15 (d, $J = 6.0$ Hz, 3H); ^{13}C NMR (125 MHz, DMSO- d_6) δ 170.16, 169.97, 169.45, 69.29, 55.02, 52.08, 22.12, 20.72, 16.55; HRMS (CI+) for $\text{C}_9\text{H}_{16}\text{NO}_5$ (MH^+), calcd 218.1028, found 218.1030.

Ac-D-Thr-NHMe (3b).¹ To **9b** (9.6 g, 44.2 mmol) was added methylamine (40% in H_2O , 38.0 mL, 489 mmol) at 0 °C. After stirring at room temperature for 6 h, the reaction mixture was concentrated in vacuo. The residue was purified by flash chromatography (MeOH/ CH_2Cl_2 = 1:9) to give **3b** (6.9 g, 90%) as a white solid. TLC (MeOH/ CH_2Cl_2 = 1:12) R_f = 0.21; ^1H NMR (500 MHz, DMSO- d_6) δ 7.66 (q, $J = 4.5$ Hz, 1H), 7.64 (d, $J = 8.5$ Hz, 1H), 4.76 (d, $J = 5.0$ Hz, 1H), 4.08 (dd, $J = 8.8, 3.8$ Hz, 1H), 3.97 (qdd, $J = 6.5, 5.5, 4.0$ Hz, 1H), 2.58 (d, $J = 4.5$ Hz, 3H), 1.90 (s, 3H), 1.00 (d, $J = 6.5$ Hz, 3H); ^{13}C NMR (125 MHz, DMSO- d_6) δ 170.68, 169.51, 66.26, 58.36, 25.54, 22.63, 20.08; HRMS (CI+) for $\text{C}_7\text{H}_{15}\text{N}_2\text{O}_3$ (MH^+), calcd 175.1082, found 218.1081.

Ac-D-Thr(PO(OBzl) $_2$)-NHMe (10b).²⁻⁴ To a stirred solution of **3b** (577 mg, 3.31 mmol) and dibenzyl N,N -diisopropylphosphoramidite (1.42 mL, 4.23 mmol) in DMF (8 mL) was added 1H-tetrazole (~0.45 M in CH_3CN , 29.4 mL, 13.2 mmol). After stirring at room temperature for 30 min, the reaction mixture was cooled to -40 °C and a solution of m -CPBA ($\leq 77\%$, 965 mg, 4.31 mmol) in CH_2Cl_2 (20 mL) was added. After stirring below -20 °C for 10 min, the reaction mixture was quenched with 10% aqueous $\text{Na}_2\text{S}_2\text{O}_5$ and extracted with CH_2Cl_2 . The combined organic layers were washed with 10% aqueous $\text{Na}_2\text{S}_2\text{O}_5$, 5% aqueous NaHCO_3 ($\times 2$), and then 1 N HCl. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography (MeOH/ CH_2Cl_2 = 1:20) to give **10b** (967 mg, 67%) as a white solid. TLC (MeOH/ CH_2Cl_2 = 1:15) R_f = 0.26; ^1H NMR (500 MHz, DMSO- d_6) δ 8.09 (d, $J = 9.0$ Hz, 1H), 7.98 (q, $J = 4.3$ Hz, 1H), 7.40–7.33 (m, 10H), 5.02 (dd, $J = 11.8, 7.8$ Hz, 1H), 4.99 (dd, $J = 11.5, 7.0$ Hz, 1H), 4.98 (dd, $J = 11.8, 7.8$ Hz, 1H), 4.96 (dd, $J = 11.8, 7.3$ Hz, 1H), 4.87 (qdd, $J = 6.5, 6.5, 4.0$ Hz, 1H), 4.47 (ddd, $J = 9.3, 3.3, 2.5$ Hz, 1H), 2.56 (d, $J = 4.5$ Hz, 3H), 1.93 (s, 3H), 1.22 (d, $J = 6.5$ Hz, 3H); ^{13}C NMR (125 MHz, DMSO- d_6) δ 169.90, 169.02, 136.11 (d, $J = 7.4$ Hz), 136.09 (d, $J = 7.4$ Hz), 128.41, 128.26, 128.23, 127.72, 127.64, 75.32 (d, $J = 5.5$ Hz), 68.36 (d, $J = 5.7$ Hz), 68.24 (d, $J = 5.5$ Hz), 56.43 (d, $J = 8.3$ Hz), 25.59, 22.50, 18.14; ^{31}P NMR (200 MHz, DMSO- d_6) δ -1.69; HRMS (FAB+) for $\text{C}_{21}\text{H}_{27}\text{N}_2\text{O}_6\text{PNa}$ (MNa^+), calcd 457.1504, found 457.1517.

Ac-D-Thr(PO₃H₂)-NHMe (4b).²⁻⁴ To a solution of **10b** (410 mg, 0.944 mmol) in AcOH/MeOH (1:9, 5 mL) was added 10% Pd/C (100 mg). After stirring at room temperature under 1 atm H₂ for complete reaction, the reaction mixture was filtered through Celite 545 and concentrated in vacuo. The residue was dissolved in H₂O and washed successively with diethyl ether and CH₂Cl₂ (× 3). The aqueous layer was concentrated in vacuo to give **4b** (237 mg, 99%) as a white solid. ¹H NMR (500 MHz, DMSO-*d*₆) δ 7.94 (d, *J* = 6.5 Hz, 1H), 7.90 (q, *J* = 2.5 Hz, 1H), 4.45 (m, 1H), 4.23 (t, *J* = 5.5 Hz, 1H), 2.58 (d, *J* = 4.0 Hz, 3H), 1.89 (s, 3H), 1.16 (d, *J* = 6.0 Hz, 3H); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 169.51, 169.41, 71.65, 57.69 (d, *J* = 4.7 Hz), 25.61, 22.54, 18.33; ³¹P NMR (200 MHz, DMSO-*d*₆) δ -0.45; HRMS (FAB+) for C₇H₁₆N₂O₆P (MH⁺), calcd 255.0746, found 255.0749.

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