A Proline-Based Phosphine Template for Staudinger Ligation

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Instrumentation: ^1H NMR spectra, ^{13}C NMR, and ^{31}P NMR were recorded at 300 MHz (Varian, VX 300) and are reported in parts per million (ppm) on the δ scale relative to residual CHCl_3 (δ 7.25 for ^1H and δ 77.0 for ^{13}C). NMR experiments were performed at room temperature. All reported melting points for solid materials were measured by Fisher-Johns melting point apparatus and not corrected. Mass spectra were recorded using an electrospray ionization mass spectrometry (ESI, Thermo Finnigan LCQ Advantage). Mass data were reported in units of m/z for $[\text{M}+\text{H}]^+$ or $[\text{M}+\text{Na}]^+$. Infrared spectra were recorded on a Thermo Scientific Nicolet iS10 (Thin film) and reported in units of cm^{-1} .

Solvents and Reagents: Reagents and solvents employed were of the highest grade available. Reagent grade solvents were used for either chromatography or extraction without further purification before use. Tetrahydrofuran (THF) and Dichloromethane (DCM) were directly used from a solvent purifier (Pure Solv, Innovative Technology, Inc.). *N*-hydroxybenzotriazole (HOBt), *N,N'*-diisopropylcarbodiimide (DIC), *N,N'*-dicyclohexylcarbodiimide (DCC) and all protected and unprotected natural amino acids were purchased from Advanced ChemTech and used as received. Benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphates (BOP) was purchased from BACHEM. Chlorodiphenylphosphine was purchased from TIC. Triethylamine (TEA) was purchased from Aldrich and freshly distilled before use. Redistilled diisopropyl ethylamine (DIEA) was purchased from Aldrich and used without further purification.

Chromatography: The progress of the reactions was monitored by analytical thin layer chromatography (VWR, TLC 60 F₂₅₄ plates). Plates were visualized first with UV (254 nm) and then illuminated by CAM stain (2.5 g of ammonium molybdate tetrahydrate and 1 g of cerium ammonium sulfate in a solution of 10% sulfuric acid in water) or ninhydrin solution (0.3 % ninhydrin in a solution of 3 % acetic acid in ethanol). Flash column chromatography was performed using silica gel (230-400 mesh). The solvent compositions for all separations are on a volume/volume (v/v) basis.

Experimental Procedures and Characterization of Compounds

Preparation of proline-based phosphine substrates 3a-3d

The general synthetic scheme is illustrated below:

Scheme S1. Synthesis of proline-based diphenylphosphine esters **3b-3d**.

To a solution of commercially available (S)-proline methyl ester hydrogen chloride **S3a** (1.20 g, 7.25 mmol) in DCM (45 mL, $c = 0.16$ M) was added TEA (2.02 mL, 14.5 mmol) and PPh_2Cl (2.60 mL, 14.5 mmol) at 0 °C. The resulting mixture was stirred and warmed to room temperature for 12 h. After completion of the reaction (monitored by TLC), the heterogeneous mixture was filtered with DCM to remove the triethylammonium salt. The filtrate was evaporated by rotary evaporation, and extracted with EtOAc (20 mL). The organic layer was washed with water and brine, dried over MgSO_4 . Flash column chromatography on silica gel (pre-neutralized with 1% TEA in hexane) with 10:1 hexanes-EtOAc gave the desired product **3a** as sticky oil (1.64 g, 72 %). ^1H NMR (300 MHz, CDCl_3) δ 7.59 – 7.27 (m, 10H), 4.22 (ddd, $J = 8.8, 6.0, 3.5$ Hz, 1H), 3.63 (s, 3H), 3.17 – 3.06 (m, 1H), 2.88 (m, 1H), 2.22 – 1.70 (m, 4H); ^{31}P NMR (122 MHz, CDCl_3) δ 49.31; ^{13}C NMR (75 MHz, CDCl_3) δ 176.00, 138.93, 132.88, 132.61, 132.23, 131.98, 128.90, 128.40, 128.33, 128.27, 128.19, 65.28, 64.87, 51.96, 47.64, 47.57, 31.80, 31.71, 25.90. IR (thin film) 3056.1, 2953.9, 2868.1, 1744.4, 1425.7, 1205.0, 1164.1, 1115.1, 743.2, 698.3 cm^{-1} ; MS (ESI) m/z calcd for $\text{C}_{18}\text{H}_{21}\text{NO}_2\text{P}$ $[\text{M}+\text{H}]^+$ 314.1, found 314.1.

Boc-proline (3.77 g, 17.5 mmol), DCC (3.62 g, 17.5 mmol) and DMAP (89.2 mg, 0.730 mmol) were combined and dissolved in DCM (100 mL, $c = 0.15$ M). The reaction mixture was stirred for 10 min at room temperature followed by the addition of phenol (1.37 g, 14.6 mmol) in DCM (10 mL). The reaction mixture was stirred at room temperature for 1 h and no remaining SM was monitored by TLC. The resulting mixture was filtered with DCM. The filtrate was concentrated to about 30 mL and cooled down. The white precipitate was filtered again. The organic layer was extracted with EtOAc (2×20 mL) and washed with sat. NaHCO_3 (3×20 mL), water (2×30 mL) and brine. The solvent was dried with MgSO_4 and concentrated. A flash column chromatography with 6:1 Hex/EtOAc on a silica gel gave a white solid **S2b** (92%). A part of the white solid was directly dissolved in EtOAc (25 mL) and then HCl (g) prepared by mixing of $\text{NH}_4\text{Cl}/\text{H}_2\text{SO}_4$ was bubbled for 30 min at room temperature. After removing excess HCl (g) by bubbling with Ar, the reaction mixture was concentrated by rotary evaporation then dried *in vacuo* to give a sticky solid **S3b** (100%). To a solution of L-proline phenyl ester HCl salt (1.80 g, 7.92 mmol) in DCM (25 mL) was added TEA (5.52 mL, 39.6 mmol). The solution became a heterogeneous mixture. To the resulting mixture was added PPh_2Cl (1.42 mL, 7.92 mmol) dropwise at 0°C . After stirring 3 h at 0°C , no remaining SM was monitored by TLC. The reaction mixture was filtered, concentrated and extracted with EtOAc. The organic layer was washed with sat. NaHCO_3 , water and brine. The organic layers were dried with MgSO_4 and filtered, evaporated by rotary evaporation. The concentrated crude product was purified by flash column chromatography on silica gel (pre-neutralized by 1 % TEA in Hexane) with 10% EtOAc in hexanes to obtain sticky colorless oil (2.51 g, 81 %).

^1H NMR (300 MHz, CDCl_3) δ 7.59 – 7.15 (m, 13H), 7.07 – 6.95 (m, 2H), 4.47 (ddd, $J = 8.3, 6.3, 3.8$ Hz, 1H), 3.21 (dddd, $J = 8.7, 7.5, 5.1, 1.0$ Hz, 1H), 2.95 (dtd, $J = 9.4, 7.0, 2.4$ Hz, 1H), 2.36 – 2.14 (m, 2H), 2.09 – 1.93 (m, 1H), 1.83 (m, 1H); ^{31}P NMR (122 MHz, CDCl_3) δ 49.99; ^{13}C NMR (75 MHz, CDCl_3) δ 174.0, 150.9, 138.8, 138.7, 138.6, 132.9, 132.7, 132.2, 131.9, 129.5, 129.0, 128.5, 128.3, 128.2, 125.9,

121.6, 65.6, 65.1, 47.8, 47.7, 32.0, 31.9, 25.9; IR (thin film) 3068.3, 3052.0, 2962.1, 2941.7, 2868.1, 1764.8, 1598.2, 1486.9, 1437.9, 1192.7, 1160.0, 1119.2, 743.2, 698.3 cm^{-1} ; MS (ESI) m/z calcd for $\text{C}_{23}\text{H}_{23}\text{NO}_2\text{P}$ $[\text{M}+\text{H}]^+$ 376.1, found 376.2.

Boc-Pro-OH (2.35 g, 10.9 mmol), DCC (2.25 g, 10.9 mmol), and DMAP (111 mg, 0.908 mmol) were combined and dissolved in 50 mL of DMF. The resulting mixture was stirred for 10 min at room temperature and cooled to 0 °C. To the solution was added freshly distilled thiophenol (1.00 g, 9.08 mmol). The reaction mixture was stirred overnight and then 20 mL of water was added to quench the reaction. The heterogeneous mixture was filtered (rinsed with EtOAc) to remove DCU. The combined organic filtrate was washed with sat. NaHCO_3 (3×20 mL), water, and brine. It was then dried over with MgSO_4 , filtered, and concentrated. Purification by flash column chromatography gave a colorless oil **S2c**. The resulting oil was dissolved in EtOAc (25 mL) and then HCl (g) prepared by mixing of $\text{NH}_4\text{Cl}/\text{H}_2\text{SO}_4$ was bubbled for 30 min at room temperature. After removing excess HCl (g) by bubbling with Ar, the mixture was concentrated by rotary evaporation to give sticky solid then dried *in vacuo* to give a white solid **S3c** (1.55 g, 70%). To a solution of **S3c** (1.48 g, 6.07 mmol) in DCM (25 mL, $c = 0.30$ M) was added TEA (2.11 mL, 15.2 mmol) and PPh_2Cl (1.23 g, 6.07 mmol) at 0 °C. The resulting mixture was stirred for 4 h at room temperature. The reaction mixture was filtered to remove the triethylammonium salt. The concentrated crude product was extracted with EtOAc and washed with sat. NaHCO_3 , water and brine. The organic layers were dried with MgSO_4 and filtered. Purification by flash column chromatography on silica gel (pre-neutralized by 1% TEA in hexanes) with 5% EtOAc in hexanes gave colorless sticky oil **3c** (1.52 g, 64%). ^1H NMR (300 MHz, CDCl_3) δ 7.71 – 7.57 (m, 2H), 7.50 – 7.30 (m, 13H), 4.40 (ddd, $J = 11.6, 7.5, 4.5$ Hz, 1H), 3.44 – 3.17 (m, 1H), 2.95 (m, 1H), 2.14 (ddd, $J = 9.3, 8.2, 6.2$ Hz, 2H), 2.02 – 1.79 (m, 1H), 1.79 – 1.50 (m, 1H); ^{31}P NMR (122 MHz, CDCl_3) δ 56.30; ^{13}C NMR (75 MHz, CDCl_3) δ 204.3, 138.4, 138.1, 137.8, 134.8, 133.2, 132.9, 132.5, 132.2, 129.3, 129.3, 129.2, 129.2,

128.6, 128.6, 128.5, 128.3, 128.3, 73.2, 72.8, 48.4, 48.3, 32.73, 32.6, 25.8; IR (thin film) 3071.7, 3059.0, 2973.3, 2941.5, 2865.3, 1694.2, 1583.1, 1478.3, 1437.1, 1091.1, 1068.9, 1024.4, 976.8, 745.1, 691.2 cm^{-1} ; MS (ESI) m/z calcd for $\text{C}_{23}\text{H}_{23}\text{NOPS}$ $[\text{M}+\text{H}]^+$ 392.1, found 392.2.

Compound **3d** was synthesized following the same protocol as for compound **3c**. The product was obtained after flash column chromatography on silica gel (pre-neutralized by 1% hexanes) as colorless oil (1.4 g, 69%). ^1H NMR (300 MHz, CDCl_3) δ 7.67 – 7.49 (m, 2H), 7.47 – 7.29 (m, 8H), 4.27 (m, 1H), 3.20 (m, 2H), 2.89 – 2.74 (m, 2H), 2.09 – 1.97 (m, 1H), 1.93 (m, 1H), 1.81 – 1.67 (m, 1H), 1.66 – 1.49 (m, 1H), 1.21 (t, $J = 7.4$ Hz, 3H). ^{31}P NMR (122 MHz, CDCl_3) δ 55.15; ^{13}C NMR (75 MHz, CDCl_3) δ 206.47, 138.54, 133.19, 132.91, 132.44, 132.19, 129.28, 128.52, 128.49, 128.44, 128.27, 128.19, 73.17, 72.75, 55.98, 48.11, 48.00, 32.60, 32.53, 25.80, 14.84; IR (thin film) 3047.9, 2966.2, 2925.3, 2855.8, 1679.0, 1429.7, 1090.6, 1045.6, 980.2, 743.2, 698.3 cm^{-1} ; MS (ESI) m/z calcd for $\text{C}_{19}\text{H}_{23}\text{NOPS}$ $[\text{M}+\text{H}]^+$ 344.1, found 344.3.

The reaction between **3a** and azide **4** (Scheme 2)

To a solution of diphenylphosphine proline methyl ester **3a** (150 mg, 0.479 mmol) in 3:1 mixture of THF/phosphate buffer (2 mL) was added a solution of benzyl azide **4** (70.1 mg, 0.5 mmol) in the same solvent (2.8 mL) at room temperature. The reaction was monitored by TLC and completed within 30 min at room temperature. The reaction mixture was diluted with ether (20 mL) and washed with water, brine. The organic layer was then dried with MgSO_4 and concentrated. Purification by flash column chromatography with 5% MeOH in DCM gave a major product as a sticky solid. Based on NMR data (^1H , ^{13}C , ^{31}P , COSY) and mass, we believe the product was the protonated azaylide **6** (below).

Product **6**: (107 mg, 55 %). mp 37-40 °C, ^1H NMR (300 MHz, CDCl_3) δ 9.31 (br-s, 1H), 8.00 – 7.78 (m, 4H), 7.74 – 7.41 (m, 6H), 7.38 – 7.06 (m, 5H), 4.32 (ddd, $J = 9.2, 6.7, 2.6$ Hz, 1H), 4.28 – 4.14 (m, 2H), 3.75 (s, 1H), 3.43 (s, 3H), 3.44 (m, 1H), 3.32 (m, 1H), 2.32 – 2.12 (m, 1H), 2.03 – 1.82 (m, 3H); ^{31}P NMR (122 MHz, CDCl_3) δ 43.11; ^{13}C NMR (75 MHz, CDCl_3) δ 173.8, 139.1, 139.0, 134.7, 134.6, 134.6, 134.5, 133.7, 133.6, 133.5, 133.5, 129.9, 129.7, 129.6, 129.4, 128.7, 127.9, 127.5, 123.4, 123.3, 121.7, 121.6, 61.6, 61.5, 52.4, 49.6, 49.5, 45.9, 31.6, 31.6, 25.7, 25.6; MS (ESI) m/z calcd for the cation $\text{C}_{25}\text{H}_{28}\text{N}_2\text{O}_2\text{P}$ $[\text{M}]^+$ 419.2, found 419.1.

The desired ligation product **7** was obtained using a similar protocol as described above. The reaction temperature was maintained at 50 °C for 1h. Purification by flash column chromatography gave **7** as a white solid. When THF was the solvent, the yield was 32%. When DMF was the solvent, the yield was 40%. When THF/ CH_3CN (1:1) was the solvent, the yield was 38%. Compound **7**: mp 43-46 °C; ^1H NMR (300 MHz, CDCl_3) δ 8.18 (t, $J = 5.9$ Hz, 1H), 7.75 (m, 4H), 7.62 – 7.18 (m, 11H), 4.51 – 4.30 (m, 2H), 4.07 (ddd, $J = 8.1, 5.8, 2.2$ Hz, 1H), 3.29 – 3.05 (m, 2H), 2.52 – 2.32 (m, 1H), 2.17 – 1.97 (m, 1H), 1.95 – 1.84 (m, 2H); ^{31}P NMR (122 MHz, CDCl_3) δ 28.44; ^{13}C NMR (75 MHz, CDCl_3) δ 172.94, 138.67, 132.53, 132.48, 132.44, 132.39, 132.36, 132.26, 132.23, 129.13, 129.07, 128.97, 128.90, 128.82, 128.04, 127.49, 62.60, 62.57, 47.95, 47.90, 43.84, 31.29, 31.21, 25.36, 25.27; IR (thin film) 3260.4, 3060.2, 2941.7, 2872.2, 1653.5, 1511.5, 1437.9, 1167.1, 1102.8, 1062.0, 726.9, 694.2 cm^{-1} ; MS (ESI) m/z calcd for $\text{C}_{24}\text{H}_{26}\text{N}_2\text{O}_2\text{P}$ $[\text{M}+\text{H}]^+$ 405.2, found 405.2.

The reactions between 3b-3d and benzyl azide 4 (Table 1)

General procedure: to a solution of diphenylphosphine proline phenyl ester **3b** (103 mg, 0.274 mmol) in 3:1 mixture of DMF/pH 7.4 phosphate buffer (2 mL) was added a solution of benzyl azide (38 mg, 0.28 mmol) in the same solvent (1 mL) at room temperature. The reaction was monitored by TLC and it was completed within 30 min at room temperature. The reaction mixture was extracted with ethyl acetate (2 × 10 mL). The combined organic layers were washed with water and brine then dried over MgSO₄ and concentrated. Purification by flash column chromatography with 3% MeOH in DCM gave the desired product **7** (106 mg, 96 %).

The reactions using **3c** and **3d** followed the same procedure. Corresponding yields were shown in Table 1.

³¹P NMR signals of compound 3a, 6, and 7:

Figure S1. ³¹P NMR signals of compounds **3a** (blue, $\delta = 49.4$), **6** (green, $\delta = 43.1$), and **7** (red, $\delta = 28.4$).

Procedure for pH dependence analysis (Figure S1)

The reactions were carried out using the same procedure described above for Table 1. Phosphate buffers with varying pH values (5.5, 6.5, 7.5, 8.5) were used as indicated. The yields were reported in Figure S1.

Figure S1 pH dependence of the Staudinger ligation using substrates 3b-3d. Reactions were performed with 1:1 ratio of phosphine and azide (0.10 M) in a mixture of DMF/phosphate buffer (20mM, varying pH).

General procedure of kinetics analysis by ^{31}P NMR spectrometry (Figure 1)

Stock solutions of phosphine, azide, and the internal standard (triphenylphosphine oxide) were prepared by following a literature procedure.¹ In a flame-dried 1.00 mL volumetric tube was placed phosphine with anhydrous CD_3CN followed by vortexing until the phosphine was completely dissolved. The final phosphine concentration was 0.031 M. The flask containing phosphine stock solution was capped and immediately used with stock solutions of the azide (3.12 M) and triphenylphosphine oxide (0.31 M).

To a 5 mm NMR tube 100 μL each of the phosphine and phosphine oxide stock solutions were combined using a micropipette followed by double-distilled water (30 μL , 1.7 mmol) and 270 μL of CD_3CN (final volume = 500 μL). The azide stock solution (100 μL) was added immediately to the NMR tube before the start of kinetic runs. Volumes of azide and CD_3CN were adjusted accordingly in

experiments. ^{31}P NMR experiments were performed with a Varian VX-300 spectrometer at 20 °C. Each scan was acquired about 3 min intervals for 30 min. ^{31}P NMR spectra were processed using the MestReNova data processing software. Peak areas were integrated automatically and normalized to the internal standard, triphenylphosphine oxide. Data were plotted and analyzed using Origin 8.1.5 (Figure 2-B).

Preparation of azido amino acids and peptides (8a-8h and S4)

Azido amino acids were prepared following the literature procedures.^{2,3} To prepare azido peptides, 1.1 equiv. of the corresponding azido amino acid and 1.1 equiv. of HOBt (124 mg, 0.914 mmol) were combined and dissolved in DMF (total $c = 0.1$ M) at room temperature. To the reaction flask was added 1.1 equiv. of DIC and the reaction was stirred for 5 min at room temperature. To the resulting mixture was added the corresponding protected amino acid at room temperature. The reaction was stirred for overnight at room temperature. The reaction mixture was then transferred to a separation funnel and rinsed with EtOAc (15 mL). The organic layer was washed with water (2 $\times$), NaHCO_3 (2 $\times$), and brine. The solution was dried with MgSO_4 , filtered, and concentrated. Purification by flash column chromatography gave corresponding product.

Compound **8a** was synthesized as white solid (72% yield). mp 64-67 °C; ^1H NMR (300 MHz, CDCl_3) δ 7.28 (td, $J = 8.0, 7.3, 4.6$ Hz, 8H), 7.13 (dd, $J = 7.4, 2.1$ Hz, 2H), 6.55 (t, $J = 5.8$ Hz, 1H), 4.53 – 4.31 (m, 2H), 4.25 (dd, $J = 7.7, 4.4$ Hz, 1H), 3.36 (dd, $J = 14.0, 4.4$ Hz, 1H), 3.09 (dd, $J = 14.0, 7.7$ Hz, 1H); ^{13}C NMR (75 MHz, CDCl_3) δ 168.63, 137.58, 136.22, 129.81, 128.97, 128.91, 127.98, 127.89, 127.47, 65.72, 43.73, 38.73; IR (thin film) 3325.8, 3064.2, 3031.6, 2099.9, 2067.2, 1654.1, 1536.0, 1491.0, 1254.0, 1209.1, 747.3, 698.3 cm^{-1} ; MS (ESI) m/z calcd for $\text{C}_{16}\text{H}_{16}\text{N}_4\text{NaO}$ $[\text{M}+\text{Na}]^+$ 303.1, found 303.1.

Compound **8b** was obtained as colorless oil (77% yield). ^1H NMR (300 MHz, CDCl_3) δ 7.45 – 7.16 (m, 5H), 6.67 (br-s, 1H), 4.44 (d, J = 5.7 Hz, 2H), 3.99 (dd, J = 9.3, 4.0 Hz, 1H), 2.00 – 1.57 (m, 3H), 0.99 (d, J = 2.3 Hz, 3H), 0.97 (d, J = 2.4 Hz, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ 170.05, 137.88, 129.02, 127.99, 127.92, 63.27, 43.76, 41.61, 25.25, 23.32, 21.74; IR (thin film) 3289.0, 3084.7, 3064.2, 3031.6, 2953.9, 2929.4, 2864.0, 2113.5, 1650.4, 1536.0, 1258.1, 1225.4, 698.3 cm^{-1} ; MS (ESI) m/z calcd for $\text{C}_{13}\text{H}_{18}\text{N}_4\text{NaO}$ $[\text{M}+\text{Na}]^+$ 269.1, found 269.0.

Compound **8c** was obtained as white solid (69% yield). mp 72-74 $^{\circ}\text{C}$; ^1H NMR (300 MHz, CDCl_3) δ 7.45 – 7.18 (m, 10H), 6.69 (t, J = 5.8 Hz, 1H), 5.08 (s, 2H), 4.83 (t, J = 6.1 Hz, 1H), 4.44 (d, J = 5.9 Hz, 2H), 4.01 (dd, J = 6.9, 4.9 Hz, 1H), 3.19 (q, J = 6.6 Hz, 2H), 1.92 (m, 2H), 1.65 – 1.34 (m, 4H); ^{13}C NMR (75 MHz, CDCl_3) δ 169.26, 156.63, 137.83, 136.79, 129.04, 128.75, 128.34, 128.01, 127.96, 66.87, 64.41, 43.75, 40.88, 31.98, 29.74, 22.64; IR (thin film) 3305.3, 3060.1, 3027.4, 2921.2, 2864.0, 2091.7, 1698.2, 1652.1, 1535.9, 1454.2, 1266.2, 747.4 cm^{-1} ; MS (ESI) m/z calcd for $\text{C}_{21}\text{H}_{25}\text{N}_5\text{NaO}_3$ $[\text{M}+\text{Na}]^+$ 418.2, found 418.2.

Compound **8d** was obtained as white solid (84% yield). mp 80-83 $^{\circ}\text{C}$; ^1H NMR (300 MHz, Chloroform- d) δ 7.44 – 7.15 (m, 5H), 7.02 (t, J = 5.5 Hz, 1H), 5.74 (d, J = 80.4 Hz, 2H), 4.24 (dd, J = 8.1, 4.4 Hz, 1H), 4.05 – 3.75 (m, 2H), 3.33 (dd, J = 14.0, 4.4 Hz, 1H), 3.05 (dd, J = 14.0, 8.1 Hz, 1H); ^{13}C NMR (75 MHz,

CDCl₃) δ 171.20, 169.81, 136.20, 129.64, 128.99, 127.58, 65.16, 42.83, 38.62; IR (thin film) 3399.3, 3305.3, 3235.9, 3186.8, 3064.2, 3027.5, 2095.8, 1679.0, 1650.4, 1609.5, 1556.4, 1413.4, 1229.5, 747.3, 698.3 cm⁻¹; MS (ESI) m/z calcd for C₁₁H₁₃N₅NaO₂ [M+Na]⁺ 270.1, found 270.1.

Compound **8e** was obtained as white solid (65% yield). mp 51-53 °C; ¹H NMR (300 MHz, CDCl₃) δ 7.45 – 7.23 (m, 5H), 6.96 (t, J = 5.6 Hz, 1H), 5.07 (s, 2H), 4.02 (dd, J = 5.5, 2.8 Hz, 2H), 4.01 (m, 1H), 3.71 (s, 3H), 3.19 (q, J = 6.4 Hz, 2H), 1.88 (h, J = 7.3 Hz, 2H), 1.50 (m, 4H); ¹³C NMR (75 MHz, CDCl₃) δ 170.10, 169.94, 156.70, 136.81, 128.72, 128.31, 66.83, 64.02, 52.67, 42.21, 41.25, 31.80, 22.47; IR (thin film) 3305.3, 2966.2, 2941.7, 2868.1, 2104.0, 1752.6, 1683.1, 1646.3, 1548.2, 1274.5, 1225.4 cm⁻¹; MS (ESI) m/z calcd for C₁₇H₂₃N₅NaO₅ [M+Na]⁺ 400.2, found 400.1.

Compound **8f** was obtained in 66% yield. ¹H NMR (300 MHz, CDCl₃) δ 7.35 – 7.18 (m, 3H), 7.13 – 7.04 (m, 2H), 4.96 – 4.76 (m, 1H), 3.89 (dd, J = 9.2, 4.6 Hz, 1H), 3.74 (s, 2H), 3.18 (dd, J = 13.9, 5.6 Hz, 1H), 3.06 (dd, J = 13.9, 6.6 Hz, 1H), 1.89 – 1.38 (m, 3H), 0.93 (d, J = 6.4 Hz, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 171.83, 169.88, 135.72, 129.44, 128.86, 127.48, 62.90, 53.17, 52.73, 41.34, 38.09, 25.13, 23.23, 21.70; IR (thin film) 3297.2, 3064.2, 3031.6, 2958.0, 2868.1, 2112.1, 1744.4, 1662.7, 1519.6, 1433.8, 1213.2, 739.2, 698.3 cm⁻¹; MS (ESI) m/z calcd for C₁₆H₂₂N₄NaO₃ [M+Na]⁺ 341.2, found 341.1.

N₃-Leu-Lys(Boc)-Phe-OMe (S4): To a solution of Fmoc-Lys(Boc)-OH (1.26 g, 2.68 mmol) in DCM (20 mL) was added DIC (0.72 mL, 2.68 mmol) followed by DIEA (0.47 mL, 0.682 mmol) at room temperature. The mixture was stirred for 10 min and then HCl H-Phe-OMe (0.482 mg, 2.24 mmol) was added. The resulting mixture was stirred overnight and filtered to remove DIU with DCM (20 mL). The filtrate was washed with sat. NaHCO₃ (2 × 10 mL). The organic layers were concentrated to about 10 mL and then DBU (0.63 mL, 4.47 mmol) was directly added to remove Fmoc group. The reaction mixture was stirred for 30 min at room temperature. The reaction mixture was transferred into a column packed with a normal silica gel with DCM. The elution with 1:1 hexanes –EtOAc removed DBF and then 10% MeOH in DCM gave the crude Fmoc-off compound. After evaporation by rotary evaporation, the crude product was used without further purification. To a solution of N₃-Leu-OH (447 mg, 3.13 mmol) in DCM (15 mL) was added DIC (0.84 mL, 3.13 mmol) at room temperature. The mixture was stirred for 10 min followed by the crude product, H-Lys(Boc)-Phe-OMe, in DCM (10 mL) at room temperature. The reaction was complete within 1 h (monitored by TLC). The reaction mixture was extracted with DCM and the organic layers were washed with sat. NaHCO₃ and brine. After rotary evaporation, flash column chromatography with 1:1 hexanes-EtOAc gave a product as a white solid (1.14 g, 93% over 3 steps). mp 63-65 °C; ¹H NMR (300 MHz, CDCl₃) δ 7.34 – 7.15 (m, 3H), 7.15 – 7.04 (m, 2H), 6.94 (d, *J* = 8.1 Hz, 1H), 6.79 (d, *J* = 7.8 Hz, 1H), 4.86 – 4.78 (m, 1H), 4.74 (t, *J* = 6.0 Hz, 1H), 4.43 (td, *J* = 7.7, 5.8 Hz, 1H), 3.80 (dd, *J* = 8.7, 4.9 Hz, 1H), 3.70 (s, 3H), 3.19 – 2.91 (m, 4H), 1.79 – 1.62 (m, 5H), 1.50 (m, 1H), 1.40 (s, 9H), 1.35 – 1.15 (m, 4H), 0.94 (d, *J* = 6.4 Hz, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 171.92, 171.14, 170.17, 156.30, 135.90, 129.38, 128.82, 127.38, 79.27, 62.64, 60.63, 53.49, 52.86, 52.64, 41.33, 40.19, 37.87, 32.23, 29.69, 28.64, 25.16, 23.32, 22.55, 21.69, 14.41; IR (thin film) 3343.8, 2958.4, 2929.9,

2869.2, 2095.0, 1738.2, 1681.1, 1645.4, 1524.1, 1442.0, 1363.5, 1274.3, 1249.4, 1203.0, 1163.7, 703.4 cm^{-1} ; MS (ESI) m/z calcd for $\text{C}_{27}\text{H}_{43}\text{N}_6\text{O}_6$ $[\text{M}+\text{H}]^+$ 547.3, found 547.4.

N_3 -Leu-Lys(Boc)-Phe-OMe **S4** (400 mg, 0.732 mmol) was directly dissolved in EtOAc (20 mL) and then HCl (g) prepared by mixing of $\text{NH}_4\text{Cl}/\text{H}_2\text{SO}_4$ was bubbled for 30 min at room temperature. After removing excess HCl (g) by bubbling with Ar. During the rotary evaporation of the organic layers, white solid was formed. The solid was filtered with EtOAc to obtain a white solid (348 mg, 98%). mp 162-166 $^\circ\text{C}$; ^1H NMR (300 MHz, D_2O) δ 7.25 – 6.87 (m, 5H), 4.50 (dd, $J = 9.1, 5.3$ Hz, 1H), 4.21 (dd, $J = 8.1, 6.4$ Hz, 1H), 3.74 (dd, $J = 8.3, 5.8$ Hz, 1H), 3.52 (s, 3H), 2.99 (dd, $J = 13.9, 5.3$ Hz, 1H), 2.91 – 2.70 (m, 3H), 1.73 – 1.02 (m, 9H), 0.68 (dd, $J = 6.1, 2.4$ Hz, 6H); ^{13}C NMR (75 MHz, D_2O) δ 173.10, 172.76, 171.94, 136.51, 129.23, 128.61, 126.98, 60.94, 54.16, 52.97, 39.93, 39.30, 36.80, 31.11, 26.51, 24.74, 22.34, 22.18, 21.49; IR (thin film) 3315.8, 3032.9, 2947.7, 2862.5, 2088.8, 1734.3, 1638.9, 1523.0, 1444.6, 1209.4, 1172.0 cm^{-1} ; MS (ESI) m/z calcd for $\text{C}_{22}\text{H}_{35}\text{N}_6\text{O}_4$ $[\text{M}+\text{H}]^+$ 447.3, found 447.3.

N_3 -Leu-Lys(Boc)-Phe-OMe **S4** (299 mg, 0.561 mmol) was dissolved in a THF/MeOH/ H_2O (3:1:1) solution (10 mL) and LiOH was added (118 mg, 2.81 mmol). The resulting solution was stirred overnight and then diluted with THF. The solution was acidified to pH 2 by addition of 1N HCl. The resulting solution was extracted with EtOAc, the combined organic layers were dried with MgSO_4 and

concentrated *in vacuo* to give as a white foam (288 mg, 99%). mp 49-52 °C; ^1H NMR (300 MHz, CDCl_3) δ 11.29 (br-s, 1H), 7.67 (d, J = 8.6 Hz, 1H), 7.37 – 6.96 (m, 6H), 5.08 – 4.66 (m, 2H), 4.42 (m, 1H), 3.19 (m, 1H), 3.01 (m, 3H), 2.73 (m, 1H), 2.12 (m, 1H), 1.77 (m, 1H), 1.60 (m, 1H), 1.41 (m, 11H), 1.31 – 1.05 (m, 4H), 0.93 (m, 6H); ^{13}C NMR (75 MHz, CDCl_3) δ 197.83, 176.11, 171.13, 160.40, 136.13, 129.59, 128.73, 128.67, 127.21, 53.52, 53.13, 45.58, 37.52, 31.93, 29.63, 28.60, 24.38, 23.39, 22.74, 21.02; IR (thin film) 3322.6, 3067.0, 3032.9, 2957.9, 2934.0, 2872.7, 2112.6, 1717.3, 1669.6, 1519.6, 1545.8, 1362.8, 1250.3, 1168.6 cm^{-1} ; MS (ESI) m/z calcd for $\text{C}_{26}\text{H}_{41}\text{N}_6\text{O}_6$ $[\text{M}+\text{H}]^+$ 533.3, found 533.4.

General Procedure for Staudinger Ligation of substrates **9a-9i**

To a solution of diphenylphosphine proline phenyl ester **3b** in 3:1 DMF-phosphate buffer (pH 7.4) was added 1.1 equiv. of the corresponding azido amino acid or peptide in the same solvent system at room temperature. The final concentration of **3b** was 0.1~0.08 M. The reaction was completed usually within 30 min at room temperature. Work-up procedure was the same as mentioned before. Purification by flash column chromatography with 3~10 % MeOH in DCM gave the desired products.

Compound **9a** was obtained as white solid (91% yield). mp 53-57 °C; ^1H NMR (300 MHz, CDCl_3) δ 8.38 (t, J = 5.9 Hz, 1H), 7.99 (s, 1H), 7.64 (m, 2H), 7.52 (m, 2H), 7.47 – 7.32 (m, 5H), 7.32 – 7.07 (m, 12H), 6.93 – 6.76 (m, 2H), 4.89 (dt, J = 9.7, 6.2 Hz, 1H), 4.58 – 4.32 (m, 2H), 3.97 (td, J = 6.7, 4.3 Hz, 1H), 3.44 – 3.23 (m, 1H), 3.17 – 3.02 (m, 2H), 2.94 (m, 1H), 2.16 – 2.00 (m, 2H), 1.83 – 1.64 (m, 1H), 1.52 (m, 1H); ^{31}P NMR (122 MHz, CDCl_3) δ 30.15; ^{13}C NMR (75 MHz, CDCl_3) δ 172.68, 171.24, 138.77, 136.81, 132.87, 132.83, 132.71, 132.68, 132.35, 132.23, 131.91, 131.77, 129.70, 129.67, 129.14, 128.83, 128.53, 128.34, 127.17, 127.14, 119.88, 115.74, 62.00, 61.96, 53.57, 49.93, 43.74, 43.71, 38.20, 32.14, 32.06,

25.25, 25.16; IR (thin film) 3391.1, 3264.5, 3060.2, 3027.5, 2917.1, 2868.1, 1651.5, 1491.0, 1433.8, 1176.4, 1119.2, 726.9, 698.3 cm^{-1} ; MS (ESI) m/z calcd for $\text{C}_{33}\text{H}_{35}\text{N}_3\text{O}_3\text{P}$ $[\text{M}+\text{H}]^+$ 552.2, found 552.2.

Compound **9b** was obtained as white solid (92% yield). mp 58-61 °C; ^1H NMR (300 MHz, CDCl_3) δ 8.06 (t, $J = 6.0$ Hz, 1H), 7.84 – 7.58 (m, 5H), 7.58 – 7.17 (m, 11H), 4.66 – 4.26 (m, 3H), 4.06 (ddd, $J = 8.4, 5.7, 2.8$ Hz, 1H), 3.19 (ddd, $J = 8.0, 5.6, 4.0$ Hz, 2H), 2.35 – 2.05 (m, 2H), 1.96 – 1.80 (m, 3H), 1.71 – 1.50 (m, 2H), 1.09 – 0.76 (m, 6H); ^{31}P NMR (122 MHz, CDCl_3) δ 29.17; ^{13}C NMR (75 MHz, CDCl_3) δ 172.79, 172.36, 138.93, 132.72, 132.68, 132.34, 132.21, 132.07, 131.94, 129.66, 129.33, 129.17, 129.00, 128.66, 128.03, 127.22, 62.41, 62.37, 49.42, 49.38, 43.56, 41.73, 31.99, 31.91, 25.53, 25.47, 25.38, 23.47, 21.89; IR (thin film) 3264.5, 3060.2, 2953.9, 2929.4, 2872.2, 1654.5, 1540.1, 1433.8, 1176.4, 1119.2, 718.7, 690.1 cm^{-1} ; MS (ESI) m/z calcd for $\text{C}_{30}\text{H}_{37}\text{N}_3\text{O}_3\text{P}$ $[\text{M}+\text{H}]^+$ 518.2, found 518.2.

Compound **9c** was obtained as colorless oil (91% yield). ^1H NMR (300 MHz, CDCl_3) δ 8.00 (t, $J = 6.0$ Hz, 1H), 7.89 (d, $J = 8.8$ Hz, 1H), 7.70 (m, 4H), 7.59 – 7.12 (m, 16H), 5.23 (t, $J = 5.8$ Hz, 1H), 5.03 (s, 2H), 4.47 (m, 3H, CH_2Ph overlapped with Lys(H_α)), 4.04 (ddd, $J = 8.3, 5.6, 2.6$ Hz, 1H), 3.19 – 3.10 (m, 4H, Pro(H_δ) overlapped with Lys(H_ϵ)), 2.27 (m, 1H), 2.16 – 1.69 (m, 5H), 1.52 (m, 2H), 1.38 (m, 2H); ^{31}P NMR (122 MHz, CDCl_3) δ 29.34; ^{13}C NMR (75 MHz, CDCl_3) δ 173.04, 171.71, 156.74, 138.85, 136.91,

132.75, 132.71, 132.34, 132.21, 132.13, 131.99, 129.32, 129.16, 129.03, 128.99, 128.70, 128.67, 128.26, 128.20, 128.06, 127.33, 66.66, 62.40, 62.36, 53.67, 49.17, 43.59, 40.71, 32.19, 31.70, 29.34, 25.49, 25.40, 22.83; IR (thin film) 3293.1, 3060.2, 2941.7, 2864.0, 1699.4, 1651.4, 1523.7, 1437.9, 1249.3, 735.1, 694.2 cm^{-1} ; MS (ESI) m/z calcd for $\text{C}_{38}\text{H}_{44}\text{N}_4\text{O}_5\text{P}$ $[\text{M}+\text{H}]^+$ 667.3, found 667.2.

Compound **9d** was obtained colorless oil (94% yield). ^1H NMR (300 MHz, CDCl_3) δ 8.47 (t, $J = 6.0$ Hz, 1H), 7.69 – 7.46 (m, 3H), 7.39 (m, 6H), 7.32 – 7.09 (m, 10H), 6.96 – 6.80 (m, 1H), 4.90 (dt, $J = 9.8, 6.0$ Hz, 1H), 4.59 – 4.32 (m, 2H), 3.95 (q, $J = 5.9$ Hz, 1H), 3.37 (dd, $J = 13.8, 6.3$ Hz, 1H), 3.19 – 2.97 (m, 2H), 2.91 (m, 1H), 2.14 – 2.01 (m, 2H), 1.75 (m, 1H), 1.62 – 1.39 (m, 1H); ^{13}C NMR (75 MHz, CDCl_3) δ 172.50, 171.16, 157.02, 138.94, 136.92, 132.81, 132.78, 132.65, 132.62, 132.35, 132.22, 131.88, 131.75, 131.39, 130.27, 129.72, 129.67, 129.28, 129.11, 128.94, 128.89, 128.79, 128.63, 128.49, 128.36, 127.09, 119.92, 115.73, 62.02, 61.98, 53.48, 50.01, 49.97, 43.66, 38.19, 32.12, 32.04, 25.21, 25.12; IR (thin film) 3395.2, 3268.6, 3060.2, 3027.5, 2937.6, 1652.4, 1503.3, 1433.8, 1176.4, 1119.2, 910.8, 726.9, 698.3 cm^{-1} ; MS (ESI) m/z calcd for $\text{C}_{28}\text{H}_{32}\text{N}_4\text{O}_4\text{P}$ $[\text{M}+\text{H}]^+$ 519.2, found 519.1.

Compound **9e** was obtained as white solid (90% yield). mp 61-64 $^{\circ}\text{C}$; ^1H NMR (300 MHz, CDCl_3) δ 8.20 (t, $J = 5.7$ Hz, 1H), 7.89 – 7.69 (m, 5H), 7.65 – 7.39 (m, 6H), 7.27 (m, 5H), 5.40 (t, $J = 5.9$ Hz, 1H), 5.03 (s, 2H), 4.50 (td, $J = 8.1, 4.7$ Hz, 1H), 4.18 – 4.02 (m, 2H), 3.92 (dd, $J = 17.7, 5.5$ Hz, 1H), 3.65 (s, 3H), 3.28 – 2.99 (m, 4H), 2.35 – 2.06 (m, 3H), 2.01 – 1.68 (m, 5H), 1.49 (ddt, $J = 21.2, 14.3, 6.3$ Hz, 3H); ^{31}P

NMR (122 MHz, CDCl₃) δ 30.15; ¹³C NMR (75 MHz, CDCl₃) δ 173.09, 172.45, 170.53, 156.80, 136.94, 132.84, 132.37, 132.24, 132.13, 132.00, 129.36, 129.19, 129.01, 128.66, 128.28, 128.19, 66.64, 62.22, 62.18, 53.31, 52.37, 41.41, 40.61, 31.80, 29.32, 25.60, 25.51, 22.45; IR (thin film) 3290.8, 3052.0, 2941.7, 2876.3, 1748.5, 1711.7, 1661.3, 1519.6, 1433.8, 1205.0, 1176.4, 1119.2, 731.0, 698.3 cm⁻¹; MS (ESI) *m/z* calcd for C₃₄H₄₂N₄O₇P [M+H]⁺ 649.3, found 649.2.

Compound **9f** was obtained as colorless oil (89% yield). ¹H NMR (300 MHz, CDCl₃) δ 7.83 (m, 5H), 7.66 (d, *J* = 8.0 Hz, 1H), 7.59 – 7.38 (m, 5H), 7.29 – 7.11 (m, 5H), 4.83 (td, *J* = 7.9, 5.8 Hz, 1H), 4.40 (td, *J* = 9.0, 4.8 Hz, 1H), 4.04 (ddd, *J* = 8.6, 6.6, 2.4 Hz, 1H), 3.63 (s, 2H), 3.24 – 3.08 (m, 4H, Pro(H_δ) overlapped with Phe(H_β)), 2.31 (m, 1H), 2.13 – 2.01 (m, 1H), 1.82 (m, 2H), 1.68 – 1.36 (m, 3H), 0.88 (d, *J* = 5.5 Hz, 3H), 0.85 (d, *J* = 5.5 Hz, 3H); ³¹P NMR (122 MHz, CDCl₃) δ 29.21; ¹³C NMR (75 MHz, CDCl₃) δ 172.70, 172.16, 172.14, 136.92, 132.70, 132.66, 132.60, 132.58, 132.45, 132.19, 132.06, 129.57, 129.53, 129.30, 129.13, 129.11, 128.94, 128.58, 126.97, 62.06, 62.02, 53.70, 52.44, 52.37, 49.17, 49.13, 41.48, 37.83, 31.51, 31.43, 25.43, 25.34, 25.22, 23.29, 22.07; IR (thin film) 3268.6, 3056.1, 2953.9, 2864.0, 1740.3, 1658.6, 1519.6, 1442.0, 1205.0, 1172.3, 1119.2, 722.8, 694.2 cm⁻¹; MS (ESI) *m/z* calcd for C₃₃H₄₁N₃O₅P [M+H]⁺ 590.3, found 590.2.

Compound **9g** was obtained as a pale yellow solid (78% yield). mp 62-64 °C; ^1H NMR (300 MHz, DMSO_3) δ 8.62 – 8.30 (m, 2H), 8.09 (d, J = 8.2 Hz, 1H), 8.04 – 7.67 (m, 6H), 7.67 – 7.33 (m, 5H), 7.20 (m, 6H), 4.44 (m, 1H), 4.38 – 4.11 (m, 1H), 3.99 – 3.69 (m, 1H), 3.55 (m, 3H), 3.21 – 3.05 (m, 1H), 3.05 – 2.83 (m, 3H), 2.67 (m, 2H), 2.03 (m, 1H), 1.93 – 1.03 (m, 13H), 0.95 – 0.74 (m, 6H); ^{31}P NMR (122 MHz, CDCl_3) δ 30.67; IR (thin film) 3238.0, 3030.8, 2949.3, 2923.4, 2871.6, 1742.8, 1650.2, 1528.1, 1439.3, 1206.1, 1172.8, 1124.7, 736.0, 699.0 cm^{-1} ; MS (ESI) m/z calcd for $\text{C}_{39}\text{H}_{53}\text{N}_5\text{O}_6\text{P}$ $[\text{M}+\text{H}]^+$ 718.4, found 718.4.

Compound **9h** was obtained as a white solid (90% yield). mp 110-112 °C; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 10.12 (br-s, 1H), 8.17 – 7.65 (m, 5H), 7.50 (m, 5H), 7.32 – 6.93 (m, 7H), 6.73 (m, 1H), 6.09 (br-s, 1H), 4.34 (m, 1H), 4.14 (m, 1H), 3.54 – 3.28 (m, 2H), 3.09 (m, 1H), 2.94 – 2.59 (m, 4H), 2.08 – 1.82 (m, 1H), 1.60 (m, 3H), 1.34 (s, 9H), 1.32 – 0.57 (m, 16H); ^{31}P NMR (122 MHz, CDCl_3) δ 30.53; IR (thin film) 3291.8, 3064.6, 2958.6, 2935.8, 2867.7 1659.8, 1606.8 1512.1, 1497.0, 1436.4, 1356.9, 1247.1, 1163.8, 1110.8, 732.2, 694.0 cm^{-1} ; MS (ESI) m/z calcd for $\text{C}_{43}\text{H}_{58}\text{N}_5\text{NaO}_8\text{P}$ $[\text{M}+\text{Na}]^+$ 826.4, found 826.4.

Compound **9i** was obtained as a white solid (100% yield). mp 212-216 °C; ^1H NMR (300 MHz, CDCl_3) δ 10.24 (s, 1H), 7.84 (m, 4H), 7.73 – 7.65 (m, 2H), 7.62 – 7.43 (m, 6H), 7.33 (t, J = 7.8 Hz, 2H), 7.16 – 7.05 (m, 1H), 4.19 (ddd, J = 7.9, 5.5, 2.1 Hz, 1H), 3.31 – 3.10 (m, 2H), 2.66 – 2.51 (m, 1H), 2.16 – 1.81 (m, 4H); ^{31}P NMR (122 MHz, CDCl_3) δ 29.93; ^{13}C NMR (75 MHz, CDCl_3) δ 171.12, 138.68, 132.76,

132.72, 132.68, 132.34, 132.21, 129.31, 129.18, 129.14, 129.09, 129.01, 124.22, 119.85, 63.02, 62.98, 48.45, 48.41, 30.68, 30.59, 25.34, 25.24; IR (thin film) 3248.4, 3189.8, 3125.1, 3050.7, 3031.3, 3015.1, 2963.3, 2869.5, 1682.1, 1594.7, 1552.6, 1467.6, 1439.4, 1258.2, 1212.9, 1180.6, 1112.6, 727.6, 692.0 cm^{-1} ; MS (ESI) m/z calcd for $\text{C}_{23}\text{H}_{24}\text{N}_2\text{O}_2\text{P}$ $[\text{M}+\text{H}]^+$ 391.2, found 391.3.

Synthesis of PPh_2 -Hyp[Gly(Me)]-OPh, compound **10**

Scheme S2. Synthesis of a *trans*-hydroxyproline diphosphine derivative **10**

(a) DCC, Fmoc-Sar-OH, DMAP, DCM, rt, overnight (b) HCl(g) , EtOAc; and then PPh_2Cl , TEA, DCM, 0 $^\circ\text{C}$ (c) DBU, DCM, rt

Hyp[Fmoc-Gly(Me)]-OPh (S6**):** Fmoc-(Me)Gly-OH (**S5**, 3.12 g, 10.0 mmol), DMAP (55.7 mg, 0.456 mmol), and DCC (2.07 g, 10.0 mmol) were combined and dissolved in DCM (70 mL) at room temperature. The mixture was stirred for 5-10 min and then N-Boc-*trans*-4-hydroxyproline phenyl ester (2.80 g, 9.11 mmol) in DCM (20 mL) was added into the mixture. The reaction was stirred for 12 h. The mixture was then filtered and rinsed with DCM. The filtrate was evaporated by rotary evaporation and

diluted with EtOAc (30 mL). The resulting white precipitate was filtered. The filtrate was washed with NaHCO_3 (2×20 mL), water, and brine. The crude product was concentrated and purified by flash column chromatography with 2:1 hexanes-EtOAc to give the product as white form (3.06 g, 56 %). mp 54-55 °C; $R_f = 0.72$, 2:1 Hexanes-EtOAc; *The NMR spectra are reported for a dynamic equilibrium between two rotamers*: ^1H NMR (300 MHz, CDCl_3) δ 7.76 (dd, $J = 7.5, 2.1$ Hz, 2H), 7.58 (m, 2H), 7.46 – 7.18 (m, 7H), 7.11 (m, 2H), 5.50 – 5.27 (m, 1H), 4.70 – 4.34 (m, 3H), 4.34 – 3.70 (m, 5H), 3.02 (m, 3H), 2.70 – 2.27 (m, 2H), 1.48 (d, $J = 5.6$ Hz, 9H).; ^{13}C NMR (75 MHz, CDCl_3) δ 171.05, 169.25, 156.93, 156.08, 153.73, 150.60, 144.11, 141.54, 129.81, 129.69, 127.98, 127.34, 127.31, 126.36, 126.24, 125.30, 125.01, 121.62, 121.24, 120.25, 81.22, 81.03, 73.81, 73.00, 68.23, 67.73, 60.62, 58.18, 58.11, 52.28, 51.03, 50.58, 47.47, 47.37, 36.95, 36.25, 35.75, 28.59, 14.46; IR (thin film) 3068.3, 2978.4, 2929.4, 1748.5, 1703.5, 1478.8, 1446.2, 1397.0, 1360.3, 1184.6, 1143.7, 1066.1, 743.2 cm^{-1} ; MS (ESI) m/z calcd for $\text{C}_{34}\text{H}_{36}\text{N}_2\text{NaO}_8$ $[\text{M}+\text{Na}]^+$ 623.2, found 623.2.

PPh₂-Hyp[Fmoc-Gly(Me)]-OPh (S7): A solution of Boc-Hyp[Fm-Gly(Me)]-OPh **S6** (2.58 g, mmol) in EtOAc (25 mL) was bubbled with HCl (g) generated by mixing of ammonium chloride and sulfuric acid for 1 h at room temperature. Then the reaction mixture was bubbled with Ar (g) to remove excess HCl (g) and the organic solvent was removed under vacuum to afford the desired product as white solid. Part of the salt (1.88 g, 3.14 mmol) was dissolved in 20 mL of dry DCM and to it triethylamine (1.31 mL, 3.0 mmol) was added. The mixture was cooled in an ice-water bath, and then chlorodiphenylphosphine (0.62 mL, 3.45 mmol) was added dropwise. The heterogeneous reaction mixture was stirred at 0 °C for 1.5 h. After the precipitate was removed by filtration the filtrate was washed with water, NaHCO_3 (2×20 mL), and brine. The organic layer was dried with MgSO_4 and concentrated. Purification by flash column chromatography with 4:1 hexanes-EtOAc gave the product as white form (1.73 g, 81 %). mp 46-48 °C; $R_f = 0.49$, 2:1 hexanes-EtOAc; *The NMR spectra are reported for a dynamic equilibrium between two*

rotamers: ^1H NMR (300 MHz, CDCl_3) δ 7.76 (t, $J = 8.7$ Hz, 2H), 7.60 (d, $J = 7.4$ Hz, 1H), 7.55 – 7.17 (m, 17H), 7.07 – 6.94 (m, 2H), 5.32 (m, 1H), 4.67 – 4.49 (m, 1H), 4.42 (m, 2H), 4.33 – 3.99 (m, 2H), 3.92 – 3.55 (m, 2H), 3.47 (m, 1H), 3.09 (m, 1H), 2.96 (m, 3H), 2.46 (m, 2H).; ^{31}P NMR (122 MHz, CDCl_3) δ 50.36; ^{13}C NMR (75 MHz, CDCl_3) δ 169.20, 168.94, 167.11, 167.05, 156.99, 156.12, 149.98, 144.16, 144.09, 144.06, 141.51, 129.84, 128.03, 127.96, 127.93, 127.88, 127.33, 127.04, 125.29, 125.04, 124.98, 121.43, 120.29, 120.23, 120.19, 73.12, 68.22, 67.67, 60.64, 58.45, 51.30, 51.01, 50.52, 47.41, 47.31, 36.33, 35.84, 34.81, 21.31, 14.44; IR (thin film) 3060.2, 2929.4, 2851.8, 1751.5, 1721.4, 1589.1, 1478.8, 1446.1, 1237.7, 1143.7, 747.3, 694.2 cm^{-1} ; MS (ESI) m/z calcd for $\text{C}_{41}\text{H}_{38}\text{N}_2\text{O}_6$ $[\text{M}+\text{H}]^+$ 685.2, found 685.2.

PPh₂-Hyp[Gly(Me)]-OPh (10): To a solution of PPh₂-Hyp[Fm-Gly(Me)]-OPh **S7** (1.42 g, 2.07 mmol) in DCM (20 mL) was added DBU (0.37 mL, 2.48 mmol) at room temperature under Ar. The reaction mixture was stirred for 1.5 h at room temperature. Without concentration, the mixture was transferred to a column packed with silica gel with DCM. Dibenzofulvene (DBF) was removed with 100% DCM and the product was purified with 5% MeOH in DCM. The product was obtained as sticky colorless oil (809 mg, 85 %). $R_f = 0.32$, 5% MeOH in DCM; ^1H NMR (300 MHz, CDCl_3) δ 7.55 – 7.41 (m, 4H), 7.41 – 7.30 (m, 8H), 7.22 (m, 2H), 7.05 – 6.93 (m, 2H), 5.34 (q, $J = 3.6$ Hz, 1H), 4.61 (td, $J = 7.4, 5.3$ Hz, 1H), 3.48 (ddd, $J = 11.8, 4.3, 1.4$ Hz, 1H), 3.36 – 3.08 (m, 3H), 2.56 – 2.44 (m, 2H), 2.41 (s, 3H); ^{31}P NMR (122 MHz, CDCl_3) δ 50.77; ^{13}C NMR (75 MHz, CDCl_3) δ 173.14, 172.09, 150.73, 138.58, 138.07, 132.78, 132.54, 132.51, 132.28, 131.03, 130.88, 129.63, 129.23, 129.16, 129.06, 128.91, 128.57, 128.49, 128.41, 126.17, 121.58, 75.07, 64.37, 63.94, 53.58, 53.50, 52.72, 37.53, 37.44, 36.38; IR (thin film) 3052.6, 2935.2, 2862.2, 2792.3, 1741.8, 1592.6, 1481.5, 1433.9, 1192.7, 1160.9, 1129.2, 1024.4, 748.3, 694.4 cm^{-1} ; MS (ESI) m/z calcd for $\text{C}_{26}\text{H}_{28}\text{N}_2\text{O}_4\text{P}$ $[\text{M}+\text{H}]^+$ 463.2, found 463.2.

Compound **11a** was synthesized following the same protocol described for Table 2. The product was obtained as colorless oil (91% yield). $R_f = 0.15$, 5% MeOH in DCM; ^1H NMR (300 MHz, CDCl_3) δ 8.28 (br-s, 1H), 7.86 – 7.65 (m, 4H), 7.60 – 7.13 (m, 11H), 5.30 (br-s, 1H), 4.50 – 4.19 (m, 3H), 3.51 – 3.13 (m, 4H), 2.75 (m, 2H), 2.44 (s, 3H), 2.39 – 2.22 (m, 1H); ^{31}P NMR (122 MHz, CDCl_3) δ 28.94; ^{13}C NMR (75 MHz, CDCl_3) δ 174.06, 150.97, 138.87, 132.98, 132.71, 132.24, 131.99, 129.57, 129.05, 128.53, 128.45, 128.35, 128.27, 125.95, 121.69, 65.61, 65.19, 47.80, 47.73, 32.01, 31.93, 25.96. IR (thin film) 3260.4, 3052.0, 2933.5, 2864.0, 1736.2, 1666.7, 1552.3, 1433.8, 1176.3, 1119.2, 726.9, 698.3 cm^{-1} ; MS (ESI) m/z calcd for $\text{C}_{27}\text{H}_{31}\text{N}_3\text{O}_4\text{P}$ $[\text{M}+\text{H}]^+$ 492.2, found 492.2.

Compound **11b** was obtained as white solid (86% yield). mp 40-43 $^{\circ}\text{C}$; $R_f = 0.32$, 10% MeOH in DCM; ^1H NMR (300 MHz, CDCl_3) δ 7.73 (m, 6H), 7.61 – 7.31 (m, 7H, NH), 7.24 (m, 5H), 5.26 (br-s, 1H, Pro(H_γ)), 4.61 – 4.30 (m, 3H, CH_2Ph , Leu(H_α)), 4.24 (m, 1H, Pro(H_α)), 3.59 – 3.04 (m, 4H, Gly(H_α), Pro(H_δ)), 2.45 (m, 5H, Gly(Me), Pro(H_β)), 1.93 – 1.71 (m, 1H, Leu(H_β)), 1.56 (m, 2H, Leu(H_β), Leu(H_γ)), 0.90 (d, $J = 4.2$ Hz, 3H, Leu(CH_3)), 0.88 (d, $J = 4.2$ Hz, 3H, Leu(CH_3)); ^{31}P NMR (122 MHz, CDCl_3) δ 30.35; ^{13}C NMR (75 MHz, CDCl_3) δ 172.14, 172.08, 171.38, 138.81, 133.04, 132.95, 132.57, 132.45, 132.18, 132.05, 129.29, 129.12, 128.69, 127.97, 127.29, 77.50, 74.24, 60.62, 54.35, 52.68, 43.54, 41.36, 37.31, 25.40, 23.35, 21.98; IR (thin film) 3272.6, 3056.1, 3023.4, 2953.9, 2929.4, 2864.0, 1740.3, 1654.5, 1540.1, 1433.8, 1176.4, 1119.2, 722.8, 698.3 cm^{-1} ; MS (ESI) m/z calcd for $\text{C}_{33}\text{H}_{42}\text{N}_4\text{O}_5\text{P}$ $[\text{M}+\text{H}]^+$ 605.3, found 605.2.

Compound **11c** was obtained as white solid (83% yield). mp 60-62 °C; R_f = 0.27, 10% MeOH in DCM; ^1H NMR (300 MHz, CDCl_3) δ 7.83 (m, 6H), 7.66 – 7.39 (m, 6H), 7.33 (d, J = 7.9 Hz, 1H), 7.28 – 7.09 (m, 5H), 5.28 (br-s, 1H, Pro(H_γ)), 4.81 (td, J = 7.7, 5.6 Hz, 1H, Phe(H_α)), 4.28 (m, 2H, Pro(H_α)+Leu(H_α)), 3.64 (s, 2H), 3.39 (m, 1H, Pro(H_δ)), 3.30 (d, J = 3.6 Hz, 2H), 3.26 (m, 1H, Pro(H_δ)), 3.16 (dd, J = 13.9, 5.7 Hz, 2H, Phe(H_β)), 3.07 (dd, J = 13.9, 7.7 Hz, 2H, Phe(H_β)), 2.66 – 2.50 (m, 1H, Pro(H_β)), 2.46 (s, 3H), 2.35 (m, 1H, Pro(H_β)), 1.70 – 1.28 (m, 3H, Leu(H_β + H_γ)), 0.82 (t, J = 6.4 Hz, 6H, Leu(2Me)); ^{31}P NMR (122 MHz, CDCl_3) δ 30.47; ^{13}C NMR (75 MHz, CDCl_3) δ 172.13, 172.00, 171.82, 170.99, 136.66, 132.96, 132.92, 132.89, 132.75, 132.62, 132.33, 132.19, 131.14, 130.79, 129.54, 129.43, 129.28, 129.20, 129.11, 129.03, 128.60, 127.03, 77.48, 74.67, 74.59, 60.08, 54.10, 54.05, 53.51, 52.63, 52.47, 52.43, 40.94, 37.90, 36.60, 36.10, 25.05, 23.15, 22.10. IR (Thin film) 3272.6, 3060.2, 3027.5, 2953.9, 2925.3, 2868.1, 1736.2, 1662.7, 1540.1, 1433.8, 1176.4, 1115.1, 739.2, 698.3 cm^{-1} ; MS (ESI) m/z calcd for $\text{C}_{36}\text{H}_{46}\text{N}_4\text{O}_7\text{P}$ $[\text{M}+\text{H}]^+$ 677.3, found 677.2.

Control experiments to examine the reactivity of **3b** and **10** towards –SH and –NH₂

To a 3:1 DMF/phosphate-buffer solution of phosphine **3b** or **10** was added 10 equiv. of lysine and cysteine separately. The reaction was stirred at room temperature and monitored by TLC for 6 h. Both phosphines did not react with lysine or cysteine under this reaction condition. Starting materials were fully recovered after 6 h.

Procedure of one-pot reaction to give **12** and **13** (Scheme 3)

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To a solution of **3b** (16.6 mg, 0.0440 mmol) in 0.5 mL 3:1 DMF-phosphate buffer was added a solution of **4** (6.50 mg, 0.049 mmol) in the same solvent 0.4 mL at room temperature. After 30 min, conc. HCl was added to the reaction flask (total $c = 6$ M). The deprotection was monitored completed within 20 min. The reaction mixture was transferred into a sep-funnel and diluted with water. The mixture was extracted with EtOAc one time. The aqueous layer was adjusted to pH 8-9 with 10% NaOH, and then extracted with ethyl acetate twice (2×10 mL). The combined organic layers were dried over Na_2SO_4 , filtered and concentrated *in vacuo* to afford a desired amine as pale yellow oil (15 mg, 84 %). ^1H NMR (300 MHz, CDCl_3) δ 7.97 (br-s, 1H), 7.45 – 7.15 (m, 5H), 4.43 (d, $J = 6.0$ Hz, 2H), 3.79 (dd, $J = 9.1, 5.3$ Hz, 1H), 2.99 (dt, $J = 10.2, 6.8$ Hz, 1H), 2.86 (dt, $J = 10.2, 6.3$ Hz, 1H), 2.31 (br-s, 1H), 2.26 – 2.07 (m, 1H), 2.03 – 1.88 (m, 1H), 1.79 – 1.64 (m, 2H); ^{13}C NMR (75 MHz, CDCl_3) δ 175.32, 138.90, 128.84, 127.80, 127.51, 60.81, 47.49, 43.13, 31.06, 26.46; IR (thin film) 3313.5, 3064.2, 3027.5, 2958.0, 2945.7, 2876.3, 1654.5, 1511.5, 14461.1, 731.0, 698.3 cm^{-1} ; MS (ESI) m/z calcd for $\text{C}_{12}\text{H}_{17}\text{N}_2\text{O}$ $[\text{M}+\text{H}]^+$ 205.1, found 205.1.

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Compound **13** was synthesized following the same protocol for compound **12**. Without extraction, the crude product was subjected to flash column chromatography on silica gel with 20 to 30% MeOH in DCM to obtain a white solid (72 % yield). mp 149-153 $^{\circ}\text{C}$; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 8.71 (d, $J = 8.1$ Hz, 1H), 8.28 (m, 1H), 7.72 (m, 1H), 7.48 (m, 1H), 7.26 (m, 5H), 5.26 (s, 1H), 4.34 (m, 1H), 4.25 (s, 2H), 3.90 (m, 1H), 3.90 – 3.71 (m, 2H), 3.09 (m, 2H), 2.53 (s, 3H), 2.24 (m, 1H), 1.98 (m, 1H), 1.65 – 1.38 (m, 3H), 0.97 – 0.75 (m, 6H); MS (ESI) m/z calcd for $\text{C}_{21}\text{H}_{33}\text{N}_4\text{O}_4$ $[\text{M}+\text{H}]^+$ 405.3, found 405.2.

Protein synthesis

To synthesize azide-containing Z-domain protein, *E. coli* genehog strain was cotransformed with plasmids pET-Z-TAG7 (Amp^R) and pEvol-AzF (Cm^R).⁴ Cells were first grown in LB media (5 mL) supplemented with ampicillin (100 mg/mL) and chloramphenicol (34 mg/mL). The 5-mL culture was then transferred to 100 mL LB with appropriate antibiotics and grown at 37 °C to give an OD₆₀₀ value of 0.6. Gene expression was then induced by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG, final concentration 0.4 mM), L-arabinose (final concentration 0.2%), and 4-azido-L-phenylalanine (final concentration 1 mM). After overnight incubation at 30 °C, the cells were harvested by centrifugation and lysed by sonication. The Z-domain protein was purified by Ni-NTA affinity chromatography under native conditions. The purified protein was characterized by mass spectrometry, SDS-PAGE, and used directly in the subsequent reactions. ESI-MS analyses of the mutant Z-domain protein gave an observed average mass of 7822 Da, which is in agreement with the calculated mass of 7822 Da (without N-terminal methionine) for the azido-containing Z-domain mutant.

Coomassie-stained SDS-PAGE of Z-domain mutant expressed in *E. coli* BL21(DE3).

Lane M, molecular weight marker; lane 1, crude lysate; lane 2, flow through; lane 3, wash fraction 1 (with binding buffer); lane 4-6, wash fraction 2 (with wash buffer); lane 7-9, elution.

Ligation reaction of azido-containing Z-domain protein

The phosphine reagent **3b** in 0.5 mL of DMF (500 μM) was added to 0.5 mL of the protein solution in 20 mM phosphate buffer, pH 7.4. The reaction mixture was incubated at room temperature with gentle shaking for 4 hours, and was subsequently subjected to LC-ESI analysis. ESI-MS analysis showed one

major fragment that has a molecular mass of 8093 Da, which corresponds to the expected mass of the conjugated product (calculated mass, 8093 Da, without N-terminal methionine).

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300MHz ¹H NMR for PPh₂-Pro-OMe **3a** in CDCl₃ (ppm)

300MHz ^1H NMR for $\text{PPh}_2\text{-Pro-OPh}$ **3b** in CDCl_3 (ppm)

300MHz ^1H NMR for PPh_2 -Pro-SPh **3c** in CDCl_3 (ppm)

300MHz ^1H NMR for PPh_2 -Pro-SEt **3d** in CDCl_3 (ppm)

75MHz ^{13}C NMR for $\text{PPh}_2\text{-Pro-SEt } \mathbf{3d}$ in CDCl_3 (ppm)

300MHz ¹H NMR for compound **6** in CDCl₃ (ppm)

75MHz ^{13}C NMR for compound **6** in CDCl_3 (ppm)

300MHz 2D NMR (COSY) for compound **6** in CDCl₃ (ppm)

300MHz ^1H NMR for compound **7** in CDCl_3 (ppm)

300MHz ^1H NMR for compound **8a** in CDCl_3 (ppm)

75MHz ¹³C NMR for compound **8a** in CDCl₃ (ppm)

300MHz ^1H NMR for compound **8b** in CDCl_3 (ppm)

75MHz ^{13}C NMR for compound **8b** in CDCl_3 (ppm)

300MHz ¹H NMR for compound **8c** in CDCl₃ (ppm)

75MHz ¹³C NMR for compound **8c** in CDCl₃ (ppm)

300MHz ¹H NMR for compound **8d** in CDCl₃ (ppm)

75MHz ^{13}C NMR for compound **8d** in CDCl_3 (ppm)

300MHz ¹H NMR for compound **8e** in CDCl₃ (ppm)

75MHz ^{13}C NMR for compound **8f** in CDCl_3 (ppm)

75MHz ^{13}C NMR for compound **S4** in CDCl_3 (ppm)

300MHz ^1H NMR for compound **8g** in D_3O (ppm)

75MHz ^{13}C NMR for compound **S4** in D_3O (ppm)

300MHz ^1H NMR for compound **8h** in CDCl_3 (ppm)

75MHz ^{13}C NMR for compound **8h** in CDCl_3 (ppm)

300MHz ^1H NMR for compound **9a** in CDCl_3 (ppm)

75MHz ¹³C NMR for compound **9a** in CDCl₃ (ppm)

300MHz ^1H NMR for compound **9b** in CDCl_3 (ppm)

9b

75MHz ^{13}C NMR for compound **9b** in CDCl_3 (ppm)

300MHz ^1H NMR for compound **9c** in CDCl_3 (ppm)

300MHz ^1H NMR for compound **9d** in CDCl_3 (ppm)

300MHz ^1H NMR for compound **9e** in CDCl_3 (ppm)

300MHz ^1H NMR for compound **9f** in CDCl_3 (ppm)

9g

300MHz ^1H NMR for compound **9g** in $\text{DMSO}-d_6$ (ppm)

300MHz ^1H NMR for compound **9h** in $\text{DMSO}-d_6$ (ppm)

300MHz ^1H NMR for compound **9i** in CDCl_3 (ppm)

75MHz ^{13}C NMR for compound **9i** in CDCl_3 (ppm)

300MHz ^1H NMR for compound **S5** in CDCl_3 (ppm)

300MHz ^1H NMR for compound **S6** in CDCl_3 (ppm)

75MHz ^{13}C NMR for compound **S6** in CDCl_3 (ppm)

300MHz ^1H NMR for compound **10** in CDCl_3 (ppm)

75MHz ^{13}C NMR for compound **10** in CDCl_3 (ppm)

300MHz ¹H NMR for compound **11a** in CDCl₃ (ppm)

75MHz ^{13}C NMR for compound **11a** in CDCl_3 (ppm)

300MHz ^1H NMR for compound **11b** in CDCl_3 (ppm)

75MHz ¹³C NMR for compound **11b** in CDCl₃ (ppm)

300MHz ^1H NMR for compound **11c** in CDCl_3 (ppm)

75MHz ¹³C NMR for compound **11c** in CDCl₃ (ppm)

300MHz ¹H NMR for compound **12** in CDCl₃ (ppm)

75MHz ¹³C NMR for compound **12** in CDCl₃ (ppm)

300MHz ¹H NMR for compound **13** in DMSO-*d*₆ (ppm)