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SOLUTION-PHASE-PEPTIDE SYNTHESIS WITHOUT PURIFICATION OF COLUMN CHROMATOGRAPHY AND RECRYSTALLIZATION BY PROTECTING AMINO ACID ESTERS WITH PHOSPHINYL CHLORIDE

Guanghai An,^a Wei Zhou,^b Xiaokang Xu,^a Yi Pan,^b and Guigen Li^{a,b*}

^a Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, Texas 79409-106, USA; ^b Institute of Chemistry & BioMedical Sciences, Nanjing University, Nanjing 210093, P. R. China; E-mail: guigen.li@ttu.edu

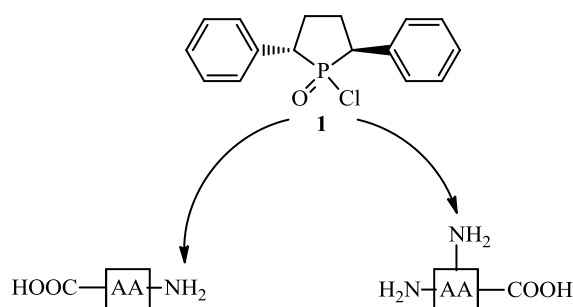
Abstract – Biphenyl phosphinyl chloride (Bpp-Cl) has been successfully applied for the amino acid GAP (Group-Assisted Purification) protection. The resulting *N*-protected amino acid esters have been readily converted into the corresponding amino acids and peptides through GAP operation. Biphalin, enkephalin derivatives and the fragments of surfaxin have also been synthesized *via* GAP work-up by avoiding column chromatography. The GAP protecting group (Bpp) can be recovered and can implement the former phosphonyl groups in peptide synthesis.

The market of pharmaceuticals of peptides and peptidomimetics has been growing rapidly in the past several decades.^{1,2} So far, there have been approximately 60 peptide-based pharmaceutical products and over 600 more in different phases of clinical and preclinical trials.³ To accelerate the peptide drug discovery process, Merrifield established the pioneering work on solid-phase-peptide synthesis (SPPS),⁴ which has solved the tedious purification encountered at each step in solution phase peptide synthesis, although it still faces challenges such as scale-up, steric hindrance for longer peptides and resin expenses.⁵⁻⁷ For many cases, solution-phase-peptide synthesis cannot be completely replaced due to its faster rates, the use of less coupling reagents, easier production of longer peptides, less racemization and side-chain reactions, etc.⁶⁻⁹ To benefit from both SPPS and solution phase peptide synthesis, an alternative method was developed by using soluble polymers as the templates for peptide synthesis.^{10,11} This method enables the peptide synthesis to be performed in the solution phase but the work-up in a solid-phase manner, or, through convenient extractions. However, the latter method needs soluble polymers of high molecular weights, which makes it inconvenient to produce large amounts of peptides

of much lower molecular weights than polymeric templates. In addition, it often needs long periods to generate solid or crystalline products by carefully controlling solidification/crystallization conditions. Obviously, the integration of these known methods would be anticipated to facilitate highly practical peptide synthesis.

Recently, we have developed a new concept called Group-Assisted Purification (GAP) chemistry¹²⁻¹⁵ which is to introduce well engineered functional groups onto reaction substrates for synthetic reactions; these functional group can result in solid products that can be purified simply by washing with common solvents or co-solvents without using column chromatography and recrystallization. The GAP chemistry has also been employed as a new strategy to for biphalin peptide synthesis successfully.^{15,16} This synthesis was started from *O*-phosphonyl protected tyrosine and followed a stepwise protocol in the *N* → *C* direction. To extend the GAP synthesis of a larger scope of peptides, further study of converting *N*-phosphonyl amino acid esters to acids were thus carried out. Herein, we would like to report an *N*-phosphinyl protection group for peptide synthesis via stepwise protocol in the *N* → *C* direction in GAP chemistry operation.

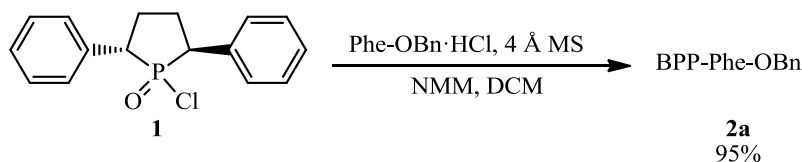
During the *N* → *C* peptide elongation, the α -amino moiety of the initial amino acid was protected by *N*-phosphinyl group. More functionalized amino acids such as lysine and ornithine usually require additional protection. We envisioned that phosphinyl protecting group, as a GAP protection group,¹⁴ would show greater stability under cleavage conditions of methyl esters and benzyl esters as compared to its phosphonyl counterpart. Biphenyl phosphinyl group **1** (Bpp-Cl)¹⁴ was chosen to replace traditional *N*-protecting groups such as Fmoc, Boc and Cbz for the general use (Scheme 1).



Scheme 1. Protection for GAP Peptide Synthesis

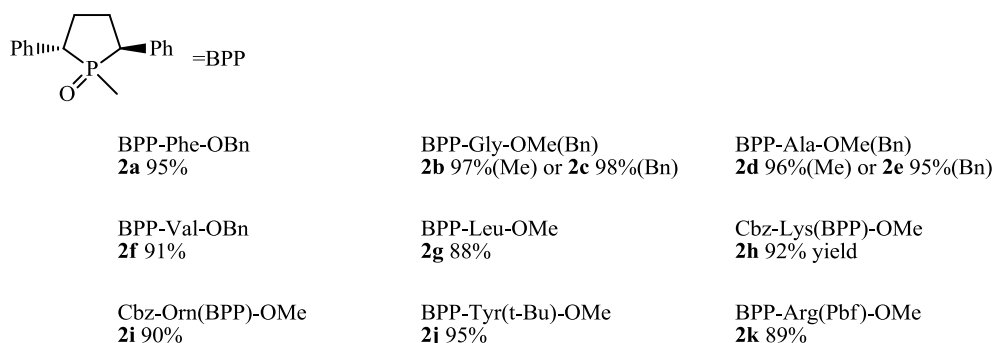
The protection of phenylalanine benzyl ester was chosen for the initial experiment. The slurry of phenylalanine benzyl ester hydrochloride in dichloromethane (DCM) was added by *N*-methylmorpholine (NMM, 2.5 equiv.) and 4 Å MS. By cooling the mixture to 0 °C, the Bpp-Cl (**1**) was added dropwise under inert gas to the resulting solution. The resulting mixture was warmed to room temperature and

stirred overnight for complete consumption of the starting materials. The pure protected amino acid esters **2a** was obtained in 95% yields by extraction and trituration with ether or dichloromethane/hexane (Scheme 2). It was found that either decreasing or increasing the temperature of Bpp-Cl addition resulted in a lower yield of **2a**. The 4 Å molecular sieves were also found to be important for the reaction.



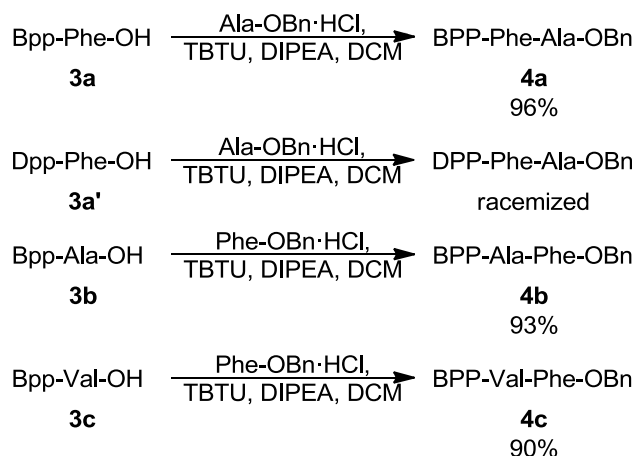
Scheme 2. Protection of Phenylalanine Benzyl Ester with Phosphinyl Chloride **1**

With the optimized condition in hand, different amino acid esters were protected with phosphinyl chloride **1** (Scheme 3). Employing the hindrance amino acid esters slightly decreased the yields (**2d**, **2e**, **2f**, **2g** and **2k** in Scheme 3). Lysine and ornithine also afforded branched-protected products in good yields (**2h** and **2i** in Scheme 3). All the *N*-phosphinyl amino acid esters were purified with standard manner as above.



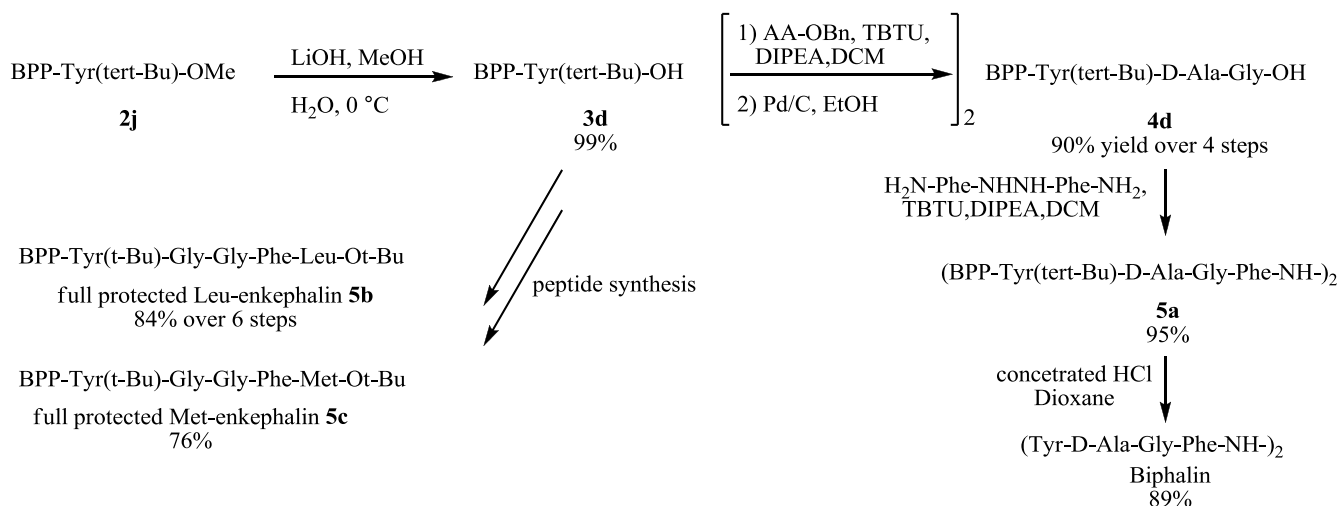
Scheme 3. Protection of Amino Acid Esters with Phosphinyl Chloride **1**

These *N*-biphenylphosphinyl amino acid esters were readily converted into corresponding acid by either hydrolysis (methyl ester) or hydrogenation (benzyl ester), which overcome the sluggish cleavage reaction of *N*-phosphonyl amino acid esters.¹³ To study the peptide coupling efficiency, Bpp-Phe-OH (**3a**), Bpp-Ala-OH (**3b**) and more steric hindrance amino acid, Bpp-Val-OH (**3c**), were subjected to the standard coupling conditions for dipeptide synthesis. All the dipeptide was obtained *via* the GAP work-up in good yields (Scheme 4). Meanwhile, the known diphenylphosphinyl group **1'**(Ph₂PO-)¹⁷ was also tested in this synthesis; the coupling of Dpp-Phe-OH **3a'** with alanine benzyl ester hydrochloride resulted in racemization (Scheme 4). The bulkiness of the Bpp group may prevent the deprotonation of amino acid's α-H, which provided the configuration maintenance.



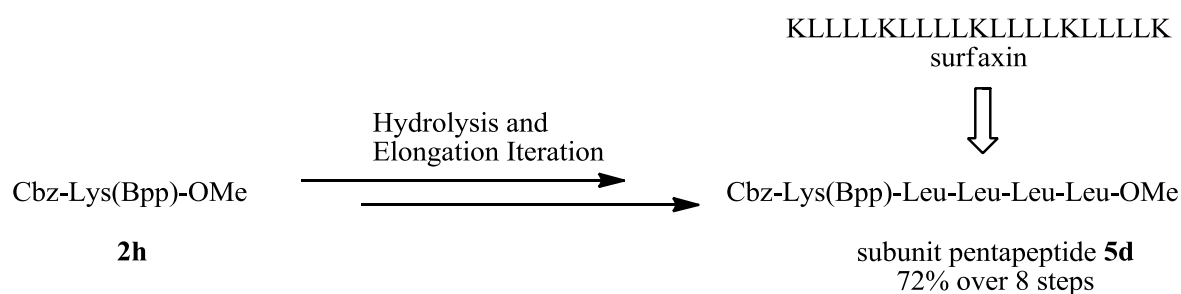
Scheme 4. Dipeptide Coupling Results

Encouraged by the above results, we started to investigate the peptide chain extension. Symmetric linear biphalin¹⁸ was synthesized *via* the GAP protocol (Scheme 5).

Scheme 5. Biphalin Synthesis *via* GAP Operation

Bpp-Tyr (*tert*-Bu)-OMe (**2j**) was hydrolyzed to Bpp-Tyr (*tert*-Bu)-OH (**3d**) in almost quantitative yield. Temperature was found to be important to maintain the L configuration in tyrosine during the hydrolysis. The following peptide elongation and benzyl ester deprotection proceeded efficiently to afford the Bpp-Tyr (*tert*-Bu)-D-Ala-Gly-OH **4d** in 90% yield. The coupling of tripeptide with H₂N-Phe-NHNH-Phe-NH₂ afforded the full protected biphalin **5a** in 95% yields. Final deprotection with concentrated hydrochloric acid gave the biphalin in 89% yields. The Bpp-OH precipitated out during the reaction process, and can be recovered simply by filtration. All the reaction products were purified by simple extraction or filtration after washing. Similarly, two neuro linear peptides, full protected Leu-enkephalin and Met-enkephalin¹⁹ were synthesized *via* the above GAP protocol (Scheme 5). The iterative ester cleavage and peptide elongation gave Leu-enkephalin and Met-enkephalin in 84% and 76% yields, respectively.

We then study the GAP synthesis of subunit peptide KLLLL (5d) of surfaxin, which is a FDA approved drug in prevention of RDS in premature infants and meconium aspiration syndrome.² The peptide contains four leucines and one lysine; and it is a hydrophobic peptide with the tendency of gelation during the synthesis. The branch-protected Lysine 2h was utilized as the first amino acid in GAP synthesis routine (Scheme 6). Through our GAP synthesis process, the iterative ester cleavage and peptide elongation afforded the final pentapeptide in 72% yield. The present GAP protocol using the phosphinyl group in the synthesis afforded high solubility of protecting peptide in the dichloromethane. The better solubility gave no racemization or difficulty occurring for purification. Because of the homogeneous property, the reaction was rapid and occurred to completion well as monitored by ³¹P NMR. These results demonstrated that both phosphinyl protection on α -amino and branched-amino groups can result in a GAP peptide synthesis.



Scheme 6. GAP Synthesis of Pentapeptide 5d

In summary, we have demonstrated an efficient linear peptide synthesis *via* Group-Assisted Purification protocol by using biphenyl phosphinyl group 1(Bpp). Both α - and β - branch protected peptides have been synthesized in good chemical yields. The recyclization of diphenyl phosphinyl group during the final cleavage and high purity of resulting unprotected peptides have been achieved. Elongation difficulties, such as hindrance amino acid, gelation, hydrophobic peptide and sulfur containing peptide, can be overcome by this GAP protocol. Further applications to synthesize other bioactive peptides and peptide drugs will be under investigation in our laboratories.

EXPERIMENTAL

The ¹H NMR, ¹³C NMR and ³¹P NMR spectra were recorded on a Jeol eclipse 400 SS (operating at 400 MHz for proton) at ambient temperature. The ESI-MS and ESI-HRMS were recorded with an Exactive (Thermo Scientific, San Jose, CA) mass spectrometer.

Starting Materials and Reagents:

Dry dichloromethane (DCM) was bought from Acros Organics and used directly. *N,N*-Dimethylformamide (DMF) was dried over 4 Å molecule sieves powder. 4 Å Molecular sieves

powder was activated by heating in oven at 120 °C for 24 h. Triethylamine was stand over KOH solid overnight and distilled before use. Tetrahydrofuran (THF) was distilled from Na metal before use. All the other chemicals were bought from commercial source and used directly after arrival.

General Procedure for Protection of amino acid ester with Bpp-Cl:

Bpp-OH (1 g) was suspended in DCM (10 mL) and was cooled to 0 °C. Oxalyl chloride was added to the solution via slow addition. After stirring at 0 °C for 1 h, the reaction solution was allowed to warm to room temperature and stirred for overnight. After the reaction completed, the reaction solution was evaporated and the residue was washed with hexane (3 times) and dried under vacuum to afford the Bpp-Cl as a white solid.

To a mixture of NMM (3 mmol), amino acid ester (2 mmol) and DCM (10 mL) was added Bpp-Cl (1 mmol) in DCM (5 mL) solution at 0 °C. The resulting mixture was stirred at room temperature for 24 h. The mixture was then filtered through celite, washed with by 5% citric acid aqueous solution, sat. aqueous NaHCO₃ solution, H₂O and then brine, dried over Na₂SO₄, filtered, and evaporated to give a white solid.

Compound **2a**: a white solid, 484.1 mg, 95% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.42 – 7.30 (m, 4H), 7.29 – 7.15 (m, 9H), 7.13 – 7.06 (m, 3H), 6.96 – 6.86 (m, 2H), 6.67 – 6.58 (m, 2H), 5.19 (dd, *J* = 15.1, 10.5 Hz, 1H), 4.72 (q, *J* = 12.6 Hz, 2H), 3.40 – 3.30 (m, 2H), 3.29 – 3.21 (m, 1H), 2.39 – 1.89 (m, 6H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 173.27, 138.22 (d, *J* = 5.1 Hz), 138.03 (d, *J* = 5.9 Hz), 136.84, 135.98, 129.38, 129.20, 129.15, 129.05, 128.70, 128.59 (d, *J* = 1.8 Hz), 128.36, 128.32, 128.28, 126.99, 126.61, 99.99, 65.97, 54.84, 48.88, 45.53, 29.85, 26.86, 26.75. ³¹P NMR (162 MHz, DMSO-*d*₆) δ 54.98. ESI-HRMS calcd. for C₃₂H₃₂NO₃PNa ([M+Na]⁺): 532.2018; found 532.2020.

Compound **2b**: a white solid, 333 mg, 97% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.33 – 7.13 (m, 10H), 5.12 – 4.89 (m, 1H), 3.43 (d, *J* = 2.4 Hz, 3H), 3.30 – 3.16 (m, 1H), 3.10 – 2.90 (m, 1H), 2.62 – 2.49 (m, 1H), 2.34 – 1.92 (m, 1H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 171.79 (d, *J* = 6.0 Hz), 138.19 (d, *J* = 5.1 Hz), 137.65 (d, *J* = 5.8 Hz), 129.33 (d, *J* = 5.3 Hz), 128.82 (d, *J* = 2.2 Hz), 128.58 (d, *J* = 1.6 Hz), 128.28 (d, *J* = 4.7 Hz), 126.77 (d, *J* = 2.7 Hz), 126.66 (d, *J* = 2.2 Hz), 52.01, 48.44 (d, *J* = 77.2 Hz), 44.77 (d, *J* = 77.2 Hz), 40.87, 30.16 (d, *J* = 10.3 Hz), 27.21 (d, *J* = 11.2 Hz). ³¹P NMR (162 MHz, DMSO-*d*₆) δ 55.65. ESI-HRMS calcd. for C₁₉H₂₂NO₃PNa ([M+Na]⁺): 366.1235; found 366.1232.

Compound **2c**: a white solid, 411 mg, 98% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.37 – 7.13 (m, 15H), 5.10 – 4.98 (m, 1H), 4.94 (s, 2H), 3.30 – 3.19 (m, 2H), 3.12 – 2.97 (m, 1H), 2.60 (ddd, *J* = 17.9, 9.0, 5.1 Hz, 1H), 2.36 – 1.91 (m, 4H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 171.22 (d, *J* = 6.0 Hz), 138.15 (d, *J* = 5.1 Hz), 137.64 (d, *J* = 5.8 Hz), 136.31, 129.30 (d, *J* = 5.3 Hz), 128.92, 128.83 (d, *J* = 2.3 Hz), 128.59, 128.57, 128.50, 128.28 (d, *J* = 4.7 Hz), 126.79 (d, *J* = 2.7 Hz), 126.65 (d, *J* = 2.3 Hz), 66.26, 48.43 (d, *J* = 77.2 Hz), 44.79 (d, *J* = 77.3 Hz), 41.09, 30.10 (d, *J* = 9.5 Hz), 27.24 (d, *J* = 10.9 Hz). ³¹P NMR (162 MHz,

DMSO- d_6) δ 55.66. ESI-HRMS calcd. for $C_{25}H_{26}NO_3PNa$ ($[M+Na]^+$): 442.1548; found 442.1545.

Compound **2d**: a white solid, 341.2mg, 96% yield. 1H NMR (400 MHz, DMSO- d_6) δ 7.57 – 6.95 (m, 10H), 5.04 (dd, $J = 14.5, 10.3$ Hz, 1H), 3.33 – 2.97 (m, 6H), 2.35 – 1.79 (m, 4H), 0.91 (d, $J = 6.9$ Hz, 3H). ^{13}C NMR (100 MHz, DMSO- d_6): δ 174.01 (d, $J = 6.7$ Hz), 138.31 (d, $J = 5.2$ Hz), 137.54 (d, $J = 5.8$ Hz), 129.26 (d, $J = 5.2$ Hz), 128.67 (d, $J = 2.1$ Hz), 128.57 (d, $J = 1.8$ Hz), 128.16 (d, $J = 4.6$ Hz), 126.60 (d, $J = 2.0$ Hz), 126.53 (d, $J = 2.4$ Hz), 51.92, 48.31 (d, $J = 76.5$ Hz), 48.22, 44.86 (d, $J = 76.6$ Hz), 29.81 (d, $J = 10.1$ Hz), 27.45 (d, $J = 10.9$ Hz), 22.89. ^{31}P NMR (162 MHz, DMSO- d_6) δ 54.62. ESI-HRMS calcd. for $C_{20}H_{24}NO_3PNa$ ($[M+Na]^+$): 380.1391; found 380.1388.

Compound **2e**: a white solid, 411.8 mg, 95% yield. 1H NMR (400 MHz, DMSO- d_6) δ 7.41 – 7.06 (m, 15H), 5.06 (dd, $J = 15.9, 10.3$ Hz, 1H), 4.94 (q, $J = 12.6$ Hz, 2H), 3.27 – 3.03 (m, 3H), 2.37 – 1.82 (m, 4H), 0.49 (d, $J = 7.2$ Hz, 3H). ^{13}C NMR (100 MHz, DMSO- d_6): δ 174.53, 138.13, 138.07 (d, $J = 2.6$ Hz), 136.50, 129.11 (d, $J = 5.2$ Hz), 128.89, 128.77 (d, $J = 2.0$ Hz), 128.54 (d, $J = 1.7$ Hz), 128.45, 128.34 (d, $J = 4.6$ Hz), 128.22, 126.79, 126.56, 66.16, 48.56 (d, $J = 76.0$ Hz), 48.48, 45.39 (d, $J = 76.1$ Hz), 29.48 (d, $J = 9.4$ Hz), 27.07 (d, $J = 12.3$ Hz), 19.91 (d, $J = 5.9$ Hz). ^{31}P NMR (162 MHz, DMSO- d_6) δ 55.29. ESI-HRMS calcd. for $C_{26}H_{28}NO_3PNa$ ($[M+Na]^+$): 456.1704; found 456.1708.

Compound **2f**: a white solid, 420 mg, 91% yield. 1H NMR (400 MHz, DMSO- d_6) δ 7.36 – 7.08 (m, 15H), 4.95 (s, 2H), 4.60 (dd, $J = 14.3, 10.8$ Hz, 1H), 3.47 – 3.33 (m, 1H), 3.24 (dd, $J = 13.5, 7.3$ Hz, 1H), 3.11 (ddd, $J = 10.8, 8.3, 5.0$ Hz, 1H), 2.33 – 1.93 (m, 4H), 1.26 – 1.16 (m, 1H), 0.37 (d, $J = 6.9$ Hz, 3H), 0.29 (d, $J = 6.8$ Hz, 3H). ^{13}C NMR (100 MHz, DMSO- d_6): δ 173.56 (d, $J = 1.6$ Hz), 138.48 (d, $J = 5.1$ Hz), 137.94 (d, $J = 5.9$ Hz), 136.46, 129.18 (d, $J = 5.2$ Hz), 128.88, 128.70 (d, $J = 2.3$ Hz), 128.63, 128.54, 128.48 (d, $J = 1.8$ Hz), 126.64 (d, $J = 2.7$ Hz), 126.44 (d, $J = 2.3$ Hz), 100.00, 66.14, 58.18, 48.63 (d, $J = 79.9$ Hz), 45.87 (d, $J = 73.5$ Hz), 32.03 (d, $J = 5.8$ Hz), 29.72 (d, $J = 9.7$ Hz), 27.09 (d, $J = 11.1$ Hz), 18.63, 18.30. ^{31}P NMR (162 MHz, DMSO- d_6) δ 55.22. ESI-HRMS calcd. for $C_{28}H_{32}NO_3PNa$ ($[M+Na]^+$): 484.2018; found 484.2020.

Compound **2g**: a white solid, 351.5 mg, 88% yield. 1H NMR (400 MHz, DMSO- d_6) δ 7.43 – 7.01 (m, 10H), 4.90 (dd, $J = 16.2, 10.7$ Hz, 1H), 3.33 – 3.13 (m, 6H), 2.35 – 1.81 (m, 4H), 1.40 (dt, $J = 13.1, 6.5$ Hz, 1H), 1.13 (t, $J = 7.0$ Hz, 2H), 0.64 (dd, $J = 6.6, 1.8$ Hz, 3H), 0.57 (dd, $J = 6.5, 1.8$ Hz, 3H). ^{13}C NMR (100 MHz, DMSO- d_6): δ 173.92 (d, $J = 4.7$ Hz), 138.34 (d, $J = 5.4$ Hz), 137.62 (d, $J = 5.4$ Hz), 129.25 (d, $J = 5.1$ Hz), 128.66 (d, $J = 2.1$ Hz), 128.48 (d, $J = 1.8$ Hz), 128.09 (d, $J = 4.7$ Hz), 126.56, 126.39, 51.70, 50.67, 48.22 (d, $J = 78.2$ Hz), 45.55 (d, $J = 62.6$ Hz), 45.16 (d, $J = 12.1$ Hz), 29.66 (d, $J = 10.5$ Hz), 27.43 (d, $J = 10.8$ Hz), 24.00, 23.08, 22.37. ^{31}P NMR (162 MHz, DMSO- d_6) δ 54.27. ESI-HRMS calcd. for $C_{23}H_{30}NO_3PNa$ ($[M+Na]^+$): 422.1861; found 422.1859.

Compound **2h**: a white solid, 504.7 mg, 92% yield. 1H NMR (400 MHz, DMSO- d_6) δ 7.62 (d, $J = 7.7$ Hz, 1H), 7.44 – 6.98 (m, 15H), 4.99 (s, 2H), 4.39 (dd, $J = 14.2, 6.7$ Hz, 1H), 3.84 (td, $J = 8.4, 5.4$ Hz, 1H),

3.62 – 3.43 (m, 3H), 3.26 – 3.00 (m, 2H), 2.35 – 1.71 (m, 6H), 1.47 – 1.16 (m, 2H), 1.07 – 0.75 (m, 4H). ^{13}C NMR (100 MHz, DMSO- d_6): δ 173.45, 156.64, 138.43 (d, $J = 3.3$ Hz), 138.37 (d, $J = 3.6$ Hz), 137.46, 129.25 (d, $J = 5.0$ Hz), 128.89, 128.69 (d, $J = 2.2$ Hz), 128.55 (d, $J = 1.7$ Hz), 128.38, 128.33, 128.26, 126.56, 66.00, 54.31, 52.31, 48.67 (d, $J = 76.6$ Hz), 45.36 (d, $J = 77.1$ Hz), 39.19, 31.52 (d, $J = 5.5$ Hz), 30.81, 29.99 (d, $J = 10.2$ Hz), 27.87 (d, $J = 10.4$ Hz), 23.08. ^{31}P NMR (162 MHz, DMSO- d_6) δ 54.48. ESI-HRMS calcd. for $\text{C}_{31}\text{H}_{37}\text{N}_2\text{O}_5\text{PNa}$ ($[\text{M}+\text{Na}]^+$): 571.2338; found 571.2336.

Compound **2i**: a white solid, 481.1 mg, 90% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 7.60 (d, $J = 7.7$ Hz, 1H), 7.35 – 7.15 (m, 15H), 4.98 (s, 2H), 4.53 – 4.38 (m, 1H), 3.84 – 3.66 (m, 1H), 3.64 – 3.46 (m, 3H), 3.25 – 3.05 (m, 2H), 2.28 – 1.84 (m, 6H), 1.48 – 1.14 (m, 2H), 1.06 – 0.78 (m, 2H). ^{13}C NMR (100 MHz, DMSO- d_6): δ 173.36, 156.58, 138.34 (t, $J = 5.2$ Hz), 137.45, 129.22 (d, $J = 5.0$ Hz), 129.12 (d, $J = 5.1$ Hz), 128.89, 128.72 (d, $J = 2.2$ Hz), 128.60, 128.56 (d, $J = 1.7$ Hz), 128.55, 128.36 (d, $J = 2.1$ Hz), 128.29 (d, $J = 2.9$ Hz), 126.57 (d, $J = 2.3$ Hz), 66.00, 54.34, 52.31, 48.64 (d, $J = 76.5$ Hz), 45.33 (d, $J = 77.2$ Hz), 38.94, 29.90 (d, $J = 9.9$ Hz), 28.68 (d, $J = 5.0$ Hz), 28.38, 27.81 (d, $J = 10.6$ Hz). ^{31}P NMR (162 MHz, DMSO- d_6) δ 54.72. ESI-HRMS calcd. for $\text{C}_{30}\text{H}_{35}\text{N}_2\text{O}_5\text{PNa}$ ($[\text{M}+\text{Na}]^+$): 557.2181; found 557.2180.

Compound **2j**: a white solid, 480.2 mg, 95% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 7.41 – 7.07 (m, 10H), 6.89 – 6.56 (m, 4H), 4.79 (dd, $J = 15.3, 9.9$ Hz, 1H), 3.41 – 3.31 (m, 2H), 3.27 – 3.23 (m, 1H), 3.20 (s, 3H), 2.61 – 2.49 (m, 1H), 2.31 – 1.86 (m, 1H), 1.32 – 1.04 (m, 9H). ^{13}C NMR (100 MHz, DMSO- d_6): δ 174.10 (d, $J = 1.9$ Hz), 154.05, 138.28 (d, $J = 4.9$ Hz), 137.99 (d, $J = 6.0$ Hz), 131.57, 129.84, 129.18 (d, $J = 5.2$ Hz), 129.01 (d, $J = 2.3$ Hz), 128.58 (d, $J = 1.8$ Hz), 128.34 (d, $J = 4.7$ Hz), 126.89, 126.62, 123.94, 78.19, 54.85, 51.45, 48.46 (d, $J = 79.4$ Hz), 45.04 (d, $J = 75.3$ Hz), 40.71, 29.81 (d, $J = 9.5$ Hz), 29.04, 26.83 (d, $J = 10.3$ Hz). ^{31}P NMR (162 MHz, DMSO- d_6) δ 55.35. ESI-HRMS calcd. for $\text{C}_{30}\text{H}_{36}\text{NO}_4\text{PNa}$ ($[\text{M}+\text{Na}]^+$): 528.2280; found 528.2283.

Compound **2k**: a white solid, 618.3 mg, 89% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 7.32 – 7.13 (m, 10H), 7.11 (br, 1H), 6.35 (br, 2H), 4.88 (dd, $J = 15.2, 10.7$ Hz, 1H), 3.42 (s, 3H), 3.39 – 3.30 (m, 1H), 3.27 – 3.18 (m, 1H), 3.08 (s, 1H), 2.93 (s, 2H), 2.69 – 2.57 (m, 2H), 2.45 (s, 3H), 2.40 (s, 3H), 2.33 – 1.96 (m, 7H), 1.36 (s, 6H), 0.83 – 0.73 (m, 4H). ^{13}C NMR (100 MHz, DMSO- d_6): δ 174.63 (d, $J = 2.0$ Hz), 157.99, 138.30, 131.94, 129.18, 129.12, 128.74, 128.54, 128.28, 126.73, 126.57, 124.86, 123.14, 116.82, 100.00, 99.54, 86.84, 52.50, 51.97, 45.19, 43.03, 39.04, 34.72, 34.46, 31.49, 28.82, 25.31, 22.59, 19.50, 18.13, 14.50, 12.84. ^{31}P NMR (162 MHz, DMSO- d_6) δ 55.36. ESI-HRMS calcd. for $\text{C}_{36}\text{H}_{47}\text{N}_4\text{O}_6\text{PSNa}$ ($[\text{M}+\text{Na}]^+$): 717.2852; found 717.2849.

General procedure for deprotection of benzyl esters:

To a flask, **Bpp-attached Amino Acid benzyl ester** or **Bpp-attached peptide benzyl ester** (1.0 mmol) was charged with Pd/C (10%, 0.2 g) in absolute EtOH solution. The reaction underwent under hydrogen

for 6 h and the reaction mixture was filter through the Celite and the filtrate cake was washed by EtOH for 3 times. Collect all filtrates. Evaporate to get the corresponding acid.

General procedure for deprotection of methyl esters:

To a flask, **Bpp-attached Amino Acid methyl ester** or **Bpp-attached peptide methyl ester** (1.0 mmol) was suspended in a 10 mL MeOH/H₂O (*v:v*, 9:1) solution. The mixture was cooled to 0 °C followed by addition of LiOH·H₂O (5 mmol). The reaction was allowed to warm to room temperature and stirred overnight. HCl (1.0 M aqueous solution) was added to neutralize the reaction. The water phase was extracted by DCM for 3 times. Combine all organic phase. Dry over Na₂SO₄ and evaporate to get the corresponding acid.

Compound **3a**: a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.87 (br, 1H), 7.51 – 6.97 (m, 15H), 6.72 – 6.66 (m, 2H), 4.74 – 4.49 (m, 1H), 2.35 – 1.84 (m, 7H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 174.53 (d, *J* = 2.7 Hz), 138.72 (d, *J* = 5.2 Hz), 138.05 (d, *J* = 5.7 Hz), 137.94, 129.76, 129.25 (d, *J* = 5.1 Hz), 128.96 (d, *J* = 2.1 Hz), 128.96 (d, *J* = 2.1 Hz), 128.50 (d, *J* = 1.8 Hz), 128.33 (d, *J* = 4.5 Hz), 128.23, 126.79 (d, *J* = 2.7 Hz), 126.45 (d, *J* = 2.2 Hz), 126.41, 55.46, 54.91, 48.34 (d, *J* = 79.3 Hz), 45.41 (d, *J* = 74.9 Hz), 29.99 (d, *J* = 9.9 Hz), 26.93 (d, *J* = 10.6 Hz). ³¹P NMR (162 MHz, DMSO-*d*₆) δ 54.29. ESI-HRMS calcd. for C₂₅H₂₆NO₃PNa ([M+Na]⁺): 442.1548; found 442.1551.

Compound **3b**: a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.37 (s, 1H), 7.43 – 7.08 (m, 10H), 4.86 (s, 1H), 3.23 (dd, *J* = 12.8, 7.1 Hz, 1H), 3.12 (d, *J* = 3.3 Hz, 1H), 3.02 (d, *J* = 6.4 Hz, 1H), 2.35 – 1.87 (m, 4H), 0.46 (d, *J* = 7.1 Hz, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 175.94, 138.24, 138.09, 129.16 (d, *J* = 5.1 Hz), 128.74, 128.53, 128.45, 126.77, 126.55, 55.46, 48.69, 48.61 (d, *J* = 76.0 Hz), 45.30 (d, *J* = 76.0 Hz), 29.55 (d, *J* = 10.2 Hz), 27.42 (d, *J* = 10.6 Hz), 20.17. ³¹P NMR (162 MHz, DMSO-*d*₆) δ 55.47. ESI-HRMS calcd. for C₁₉H₂₂NO₃PNa ([M+Na]⁺): 366.1235; found 366.1232.

Compound **3c**: a white solid. ¹H NMR (400 MHz, CDCl₃) δ 7.35 – 7.17 (m, 10H), 3.58 (ddd, *J* = 22.8, 12.2, 7.4 Hz, 1H), 3.41 (td, *J* = 10.5, 4.3 Hz, 1H), 3.12 (td, *J* = 12.9, 7.5 Hz, 1H), 2.68 (t, *J* = 11.6 Hz, 1H), 2.57 – 2.02 (m, 4H), 1.59 (td, *J* = 13.0, 6.4 Hz, 1H), 0.51 (d, *J* = 6.8 Hz, 3H), 0.44 (d, *J* = 6.8 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃): δ 176.77, 136.64, 136.38, 128.96, 128.87, 128.59, 127.95, 127.09, 126.74, 58.50, 47.51 (d, *J* = 63.5 Hz), 46.73 (d, *J* = 60.1 Hz), 31.76, 30.45 (d, *J* = 10.4 Hz), 27.25 (d, *J* = 10.4 Hz), 18.51, 17.26. ³¹P NMR (162 MHz, CDCl₃) δ 55.87. ESI-HRMS calcd. for C₂₁H₂₆NO₃PNa ([M+Na]⁺): 394.1548; found 394.1552.

Compound **3a'**: a white solid. ¹H NMR (400 MHz, CDCl₃) δ 13.20 (br, 1H), 8.00 – 7.64 (m, 2H), 7.52 – 7.09 (m, 13H), 3.99 – 3.56 (m, 2H), 3.18 (d, *J* = 13.5 Hz, 1H), 2.99 – 2.66 (m, 1H). ¹³C NMR (100 MHz, CDCl₃): δ 174.33, 137.24, 132.78 (d, *J* = 10.0 Hz), 132.47, 132.14, 131.88 (d, *J* = 9.8 Hz), 130.83, 130.11, 129.77, 128.76, 128.57 (d, *J* = 8.6 Hz), 128.41, 126.97, 55.83, 40.79 (d, *J* = 9.9 Hz). ³¹P NMR (162 MHz,

CDCl₃) δ 26.77.

General Procedure for Peptide Coupling Reactions:

Diisopropylethylamine (DIPEA) (2.4 mmol), **Bpp-attached Amino Acid I** (1 mmol) and **Amino Acid II** (1.2 mmol) were dissolved in 5 mL of dry DCM. The mixture was cooled to 0 °C. TBTU (1.2 mmol) was added in portion at 0 °C. The resulting mixture was stirred at 0 °C for 2 h and room temperature for 10 h. The mixture was then washed by 5% citric acid aqueous solution, sat. aqueous NaHCO₃ solution, H₂O and then brine. The organic phase was dried over Na₂SO₄, filtered and evaporated to give corresponding protected peptide as a white solid.

Compound **4a**: a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.05 (d, *J* = 7.1 Hz, 1H), 7.39 – 7.10 (m, 17H), 7.02 – 6.98 (m, 3H), 6.63 (dd, *J* = 6.6, 2.9 Hz, 2H), 4.98 (s, 2H), 4.64 (dd, *J* = 15.4, 10.7 Hz, 1H), 4.11 (t, *J* = 7.2 Hz, 1H), 3.48 – 3.41 (m, 1H), 2.31 – 1.89 (m, 6H), 0.92 (d, *J* = 7.2 Hz, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 172.57, 172.16 (d, *J* = 2.0 Hz), 138.06 (d, *J* = 5.4 Hz), 137.88, 137.83, 136.46, 129.77, 129.21 (d, *J* = 5.0 Hz), 129.02 (d, *J* = 2.1 Hz), 128.90, 128.48, 128.46, 128.41, 128.18, 128.16, 126.88, 126.55, 126.34, 66.30, 53.85, 48.22 (d, *J* = 78.7 Hz), 47.99, 45.68 (d, *J* = 74.8 Hz), 29.58 (d, *J* = 10.4 Hz), 27.15 (d, *J* = 10.4 Hz), 17.33. ³¹P NMR (162 MHz, DMSO-*d*₆) δ 56.44. ESI-HRMS calcd. for C₃₅H₃₇N₂O₄PNa ([M+Na]⁺): 603.2389; found 603.2392.

Compound **4b**: a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.16 (d, *J* = 7.1 Hz, 1H), 7.37 – 7.06 (m, 20H), 6.97 (dd, *J* = 6.2, 3.2 Hz, 2H), 4.98 – 4.76 (m, 3H), 4.29 (q, *J* = 7.1 Hz, 1H), 3.11 (dd, *J* = 16.4, 9.0 Hz, 1H), 2.66 (d, *J* = 7.1 Hz, 2H), 2.31 – 1.93 (m, 4H), 0.33 (d, *J* = 6.9 Hz, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 174.02 (d, *J* = 3.1 Hz), 171.51, 138.00 (d, *J* = 5.8 Hz), 137.90 (d, *J* = 5.3 Hz), 137.00, 136.12, 129.94, 129.56, 129.21 (d, *J* = 5.2 Hz), 129.15, 128.96, 128.86, 128.84 (d, *J* = 0.8 Hz), 128.82, 128.77, 128.56 (d, *J* = 1.8 Hz), 128.50, 128.41 (d, *J* = 4.7 Hz), 128.33, 127.07, 126.84 (d, *J* = 2.9 Hz), 126.64, 66.34, 54.25, 48.75, 48.45 (d, *J* = 77.6 Hz), 45.41 (d, *J* = 76.1 Hz), 37.23, 29.49 (d, *J* = 10.3 Hz), 27.36 (d, *J* = 10.3 Hz), 19.52 (d, *J* = 4.9 Hz). ³¹P NMR (162 MHz, DMSO-*d*₆) δ 56.59. ESI-HRMS calcd. for C₃₅H₃₇N₂O₄PNa ([M+Na]⁺): 603.2389; found 603.2391.

Compound **4c**: a white solid. ¹H NMR (400 MHz, MeOH-*d*₄) δ 8.05 (d, *J* = 7.4 Hz, 1H), 7.40 – 7.10 (m, 20H), 7.10 – 7.01 (m, 2H), 5.04 – 4.93 (m, 2H), 4.63 – 4.55 (m, 1H), 3.62 (dd, *J* = 15.1, 10.5 Hz, 1H), 3.49 – 3.34 (m, 1H), 3.24 – 3.14 (m, 2H), 2.95 – 2.82 (m, 2H), 2.47 – 2.08 (m, 4H), 1.38 (tt, *J* = 9.0, 4.6 Hz, 1H), 0.44 (d, *J* = 6.8 Hz, 3H), 0.29 (d, *J* = 6.8 Hz, 3H). ¹³C NMR (100 MHz, MeOH-*d*₄): δ 173.73 (d, *J* = 2.3 Hz), 171.32, 136.77 (d, *J* = 6.0 Hz), 136.45 (d, *J* = 5.9 Hz), 136.34, 135.54, 134.84, 133.88, 129.14, 128.93, 128.82, 128.71, 128.64 (d, *J* = 4.0 Hz), 128.59 (d, *J* = 2.5 Hz), 128.50, 128.37, 128.28, 128.20 (t, *J* = 3.0 Hz), 128.14 (d, *J* = 2.0 Hz), 128.02, 127.91 (d, *J* = 4.8 Hz), 127.58, 126.71 (d, *J* = 2.8

Hz), 126.62, 126.33 (d, $J = 2.5$ Hz), 67.83, 66.62, 58.22 (d, $J = 2.1$ Hz), 53.97 (d, $J = 18.2$ Hz), 46.26, 36.68 (d, $J = 90.4$ Hz), 31.98 (d, $J = 4.5$ Hz), 29.82 (d, $J = 10.8$ Hz), 26.97 (d, $J = 11.4$ Hz), 17.81, 16.40. ^{31}P NMR (162 MHz, MeOH-*d*₄) δ 57.34. ESI-HRMS calcd. for C₃₇H₄₁N₂O₄P Na ([M+Na]⁺): 631.2702; found 631.2799.

Compound **5a**: a white solid. ^1H NMR (400 MHz, DMSO-*d*₆) δ 10.14 (s, 2H), 8.14 (s, 2H), 7.72 (t, $J = 8.7$ Hz, 4H), 7.37 – 7.14 (m, 30H), 6.67 (d, $J = 8.3$ Hz, 4H), 6.58 (d, $J = 8.2$ Hz, 4H), 5.32 – 5.13 (m, 2H), 4.63 – 4.44 (m, 2H), 3.95 – 3.84 (m, 2H), 3.23 (dd, $J = 16.9, 6.4$ Hz, 4H), 3.05 – 2.96 (m, 4H), 2.86 – 2.73 (m, 4H), 2.65 (s, 2H), 2.35 – 1.90 (m, 12H), 1.16 (s, 18H), 0.82 – 0.77 (m, 6H). ^{13}C NMR (100 MHz, DMSO-*d*₆): δ 172.77, 172.70, 170.63, 169.00, 153.80, 138.11, 137.94 (d, $J = 5.4$ Hz), 137.75 (d, $J = 5.6$ Hz), 132.15, 130.11, 129.79, 129.14, 129.09, 128.89, 128.78, 128.62, 128.54, 126.85, 126.61, 123.79, 78.07, 56.03, 52.78, 48.27, 48.24 (d, $J = 82.0$ Hz), 44.44 (d, $J = 75.5$ Hz), 42.37, 38.77, 38.47, 29.70 (d, $J = 7.7$ Hz), 29.01, 27.84, 17.67. ^{31}P NMR (162 MHz, DMSO-*d*₆) δ 56.36. ESI-HRMS calcd. for C₈₆H₁₀₃N₁₀O₁₂P₂ ([M+H]⁺): 1529.7232; found 1529.7226.

Compound **5b**: a white solid. ^1H NMR (400 MHz, DMSO-*d*₆) δ 8.25 – 8.16 (br, 2H), 7.82 (t, $J = 5.7$ Hz, 1H), 7.58 (d, $J = 8.5$ Hz, 1H), 7.34 – 7.13 (m, 15H), 6.68 (d, $J = 8.4$ Hz, 2H), 6.58 (d, $J = 8.4$ Hz, 2H), 5.22 (dd, $J = 12.9, 10.0$ Hz, 1H), 4.49 (td, $J = 9.1, 4.0$ Hz, 1H), 4.09 (dd, $J = 13.9, 8.2$ Hz, 1H), 3.48 (dd, $J = 16.9, 6.3$ Hz, 1H), 3.42 – 3.30 (m, 2H), 3.24 – 3.11 (m, 2H), 3.01 – 2.89 (m, 2H), 2.83 – 2.72 (m, 2H), 2.44 – 1.86 (m, 6H), 1.62 – 1.41 (m, 3H), 1.35 (d, $J = 5.5$ Hz, 9H), 1.18 (s, 9H), 0.84 (d, $J = 6.5$ Hz, 3H), 0.78 (d, $J = 6.4$ Hz, 3H). ^{13}C NMR (101 MHz, DMSO-*d*₆) δ 173.45, 171.97, 171.67, 169.69, 168.87, 153.84, 138.29, 138.01 (d, $J = 4.9$ Hz), 137.45 (d, $J = 5.8$ Hz), 132.03, 130.10, 129.76, 129.17, 129.12, 128.99, 128.97, 128.53, 126.95, 126.74, 126.65, 123.70, 80.98, 78.09, 56.25, 53.80, 51.72, 47.98 (d, $J = 80.9$ Hz), 44.39 (d, $J = 75.4$ Hz), 43.92, 43.52, 42.68, 42.31, 38.17, 29.86, 29.05, 28.13, 27.36, 24.74, 23.24 (d, $J = 4.7$ Hz), 21.97. ^{31}P NMR (162 MHz, DMSO-*d*₆) δ 56.48. ESI-HRMS calcd. for C₅₂H₆₉N₅O₈P ([M+H]⁺): 922.4884; found 922.4878.

Compound **5c**: a white solid. ^1H NMR (400 MHz, DMSO-*d*₆) δ 8.24 – 8.17 (br, 2H), 7.84 (t, $J = 5.7$ Hz, 1H), 7.61 (d, $J = 8.5$ Hz, 1H), 7.35 – 7.16 (m, 15H), 6.71 – 6.66 (m, 2H), 6.59 (dd, $J = 8.9, 2.2$ Hz, 2H), 5.24 (dd, $J = 13.1, 9.8$ Hz, 1H), 4.46 (td, $J = 9.1, 4.1$ Hz, 1H), 4.28 – 4.14 (m, 1H), 3.48 (dd, $J = 16.8, 6.4$ Hz, 1H), 3.32 – 3.26 (m, 2H), 3.25 – 3.13 (m, 2H), 3.00 – 2.91 (m, 2H), 2.80 – 2.72 (m, 2H), 2.44 – 2.07 (m, 6H), 1.98 (s, 3H), 1.89 – 1.76 (m, 2H), 1.38 – 1.33 (m, 11H), 1.18 (d, $J = 4.1$ Hz, 9H). ^{13}C NMR (100 MHz, DMSO-*d*₆): δ 173.51, 171.75, 171.21, 169.76, 168.94, 153.85, 138.32, 137.99 (d, $J = 5.0$ Hz), 137.43 (d, $J = 6.2$ Hz), 132.03, 130.11, 129.73, 129.18, 129.13, 128.98, 128.57, 126.93, 126.77, 126.67, 123.70, 81.25, 78.09, 56.34, 53.94, 52.25, 48.02 (d, $J = 80.1$ Hz), 44.40 (d, $J = 74.7$ Hz), 42.67, 42.36, 38.06, 31.00, 30.04, 29.77 (d, $J = 8.3$ Hz), 29.05, 28.13, 28.00, 27.46 (d, $J = 10.7$ Hz), 15.03. ^{31}P NMR (162 MHz, DMSO-*d*₆) δ 56.59. ESI-HRMS calcd. for C₅₁H₆₇N₅O₈PS ([M+H]⁺): 940.4448; found

940.4442.

Compound **5d**: a white solid. ^1H NMR (400 MHz, DMSO- d_6) δ 8.12 (d, $J = 6.2$ Hz, 1H), 7.93 – 7.68 (m, 3H), 7.32 – 7.13 (m, 16H), 4.97 (s, 2H), 4.52 – 4.04 (m, 6H), 3.80 (s, 1H), 3.55 (d, $J = 2.3$ Hz, 3H), 3.09 (d, $J = 7.7$ Hz, 1H), 2.32 – 1.82 (m, 7H), 1.40 (d, $J = 25.1$ Hz, 13H), 0.78 (dd, $J = 11.9, 9.0$ Hz, 28H). ^{13}C NMR (100 MHz, DMSO- d_6): δ 173.26, 172.43, 172.32, 172.21, 171.87, 138.42, 138.37, 137.56, 129.27, 129.22, 128.86, 128.69, 128.55, 128.36, 128.31, 128.14, 126.61, 65.84, 55.44, 55.19, 52.31, 51.35, 51.04, 50.59, 49.27 – 47.93 (m), 44.96, 41.38, 41.24, 41.09, 39.27, 39.22, 31.95, 31.89, 31.80, 30.04, 29.96, 28.00, 27.90, 24.62, 24.56, 24.48, 23.53, 23.31, 23.11, 22.23, 21.70. ^{31}P NMR (162 MHz, DMSO- d_6) δ 54.51. ESI-HRMS calcd. for $\text{C}_{55}\text{H}_{82}\text{N}_6\text{O}_9\text{P}$ ($[\text{M}+\text{H}]^+$): 1001.5881; found 1001.5875.

General Procedure for Final Cleavage of Bpp-Group and Recovery:

Bpp-protected peptide (0.130 mmol) was dissolved in dioxane (2 mL) and concentrated HCl (4 mL). The reaction mixture was stirred at room temperature until the reaction completed. After the completion, the Bpp-OH was filtered and the aqueous solution was evaporated below 30 °C to afford peptide as a solid with the recycled Bpp-OH.

Biphalin: a white solid. ^1H NMR (400 MHz, DMSO- d_6) δ 8.64 (dd, $J = 19.5, 7.6$ Hz, 2H), 8.38 – 8.22 (m, 8H), 7.26 – 7.16 (m, 10H), 6.96 (d, $J = 8.4$ Hz, 4H), 6.66 (d, $J = 8.4$ Hz, 4H), 4.56 – 4.46 (m, 1H), 4.40 – 4.30 (m, 1H), 4.19 (dt, $J = 17.9, 7.2$ Hz, 2H), 3.93 (d, $J = 4.7$ Hz, 2H), 2.90 (ddd, $J = 20.5, 17.4, 4.5$ Hz, 6H), 2.70 – 2.54 (m, 2H), 0.99 (t, $J = 6.6$ Hz, 6H). ESI-HRMS calcd. for $\text{C}_{46}\text{H}_{57}\text{N}_{10}\text{O}_{10}$ ($[\text{M}+\text{H}]^+$): 909.4259; found 909.4253.

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