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## An efficient methodology to introduce *o*-(Aminomethyl) phenyl-boronic acids into peptides: alkylation of secondary amines

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General Methods. For automated, Fmoc amino solid-phase peptide synthesis, Ala, Pbf (Arg), Trt (Cys), Gly, Leu, Boc (Lys), Phe, and Boc (Thr) were purchased for P3 biosystems. Fmoc-Lys( $N_{\varepsilon}$ , Me)-OH was purchased from Chem. Pep. Inc. Fmoc-Lys( $N_3$ )-OH was purchased from Chem-Impex, Inc. Cysteamine 2-ClTrt (0.95 mmol/g) resin and Fmoc-Tyr(tBu)-Wang resin (0.46 mmol/g) were purchased from AnaSpec, Inc. Fmoc-Ala-Wang (100-200 mesh, 0.72 mmol/g) was purchased NovaBiochem. DMF, DCM, piperidine used for automated, solid-phase peptide synthesis were purchased from Fisher Scientific and Sigma-Aldrich. *N*, *N'*-dimethylethylenediamine, chloroacetyl chloride, and sodium iodide, N-methyl propargyl amine, copper iodide, and sodium ascorbate were purchased from Sigma-Aldrich. *o*-(bromomethyl) phenylboronic acid was purchased from Combi-Blocks. EZ-Link<sup>TM</sup> NHS-PEG<sub>4</sub>-Biotin was purchased from Thermo Scientific.

A Prelude peptide synthesizer (Protein Technologies, Inc.) was used for automated-solid phase synthesis of the peptides. For the longer peptides, a Liberty Blue microwave peptide synthesizer was used. Preparative HPLC purification of peptides was performed using an Agilent Zorbax SB-C<sub>18</sub> Prep HT column 21.2 x 250 mm. Analytical HPLC characterization of peptides was performed using an Agilent Zorbax column 4.6 x 250 mm; 1 mL/min, 5-95% MeCN (0.1 % TFA) in 35 min (RT). A Gemini C<sub>18</sub> 3.5 micron 2.1 x 50 mm was used for online separation; 0.7 mL/min, 5-95% MeCN (0.1 % formic acid) in 12 min (RT). An Agilent Technologies 6530 Accurate Mass QTofLC/MS was used for high-resolution mass spectra of purified peptides. Solvents used were HPLC grade. For small molecule organic compounds, a reverse-phase CombiFlash was used for purification if necessary using a RediSepRf High Performance 30 g reverse-phase column, 25 mL/min, 5-95% MeOH in 40 mins (RT).

General Procedure (A): synthesis of dimeric peptides and  $N_e$ -methyl lysine peptides—Fmoc-CysAla-Wang resin, Fmoc-Lys(N<sub>3</sub>)Ala-S-Resin, Fmoc- Lys(N<sub>e</sub>, Me)Ala-Wang, and biotinylated N<sub>e</sub>-methyl lysine peptides resin (100 µmol) were synthesized by automated sequential coupling of N<sub>a</sub>-Fmoc-amino acid (0.1 M) in DMF in the presence of *N*,*N*,*N*,*N*-Tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU, 0.15 M) and Hünig's base (0.2 M) with gentle nitrogen bubbling for 30 minutes at room temperature. A total of three repetitions were performed for each amino acid building block incorporated, followed by DMF (3 mL, 3 min, 3x) and DCM (3 mL, 3 min, 3x) washes. For peptides longer than three amino acid residues, a 0.8 M LiCl wash (3 mL, 3 min, 3x) was included after swelling with DCM. After the synthesis, resin was washed with glacial AcOH (5 mL, 3x), DCM (5 mL, 3x), and MeOH (5 mL, 3x). Resins were placed under vacuum overnight. Fmoc-Cys Ala was cleaved from the resin using trifluoroacetic acid (TFA), triisopropylsilane, 1,2ethanedithiol (EDT), and nanopure water (94: 1.0: 2.5: 2.5) (4 hrs) for Fmoc-CysAla-OH. TFA was evaporated and the remaining oil was precipitated with diethyl ether at 0° C. No further purification of the crude peptide was performed. General Procedure (B): solution-phase alkylation procedure with o-(bromomethyl)phenylboronic acid— Peptides were dissolved in a mixture of H<sub>2</sub>O and MeCN 0.6 mL (1:1 v/v). To this solution, 0.1 mL of Hünig's base was added. If the peptide precipitated, 0.15 mL of MeOH was added. Solution was further diluted with 0.2 mL of MeCN, followed by addition of the appropriate 3.5 equivalents of *o*-(bromomethyl)phenylboronic acid. The reaction was allowed to stir overnight at RT. The following day, additional boronic acid was added, followed by addition of 0.05 mL of Hünig's base. The reaction incubated at RT for an additional 2 hrs. Preparative HPLC was used to purify peptides. Purified samples were placed on the rotary evaporator to remove MeOH. The aqueous remnants were frozen and lyophilized overnight.

*General Procedure (C):* solid-phase modification of the N-terminus with EZ-Link<sup>™</sup> NHS-PEG<sub>4</sub>-Biotin NHS-PEG<sub>4</sub>-Biotin: Post automated solid phase synthesis, Fmoc group was removed with 3 mL of piperidine (20% v. in DMF, 3 mins, 3x) followed by washes with DMF (3 mL, 3 min, 3x), DCM (3 mL, 3 min, 3x), and 0.8 M LiCl (3 mL, 3 min, 3x). A 1.5 mL solution of EZ-Link<sup>™</sup> NHS-PEG<sub>4</sub>-Biotin was introduced with 0.5 mL solution of 1.2 M Hünig's base. Gentle stirring under nitrogen was performed for 1 at RT, followed by washes with DMF (3 mL, 3 min, 3x), DCM (3 mL, 3 min, 3x), and 0.8 M LiCl (3 mL, 3 min, 3x). Coupling of biotin to peptide was repeated a total of three times. Resins were washed with glacial AcOH (5 mL, 3x), DCM (5 mL, 3x), and MeOH (5 mL, 3x), and resins were placed under vacuum overnight. Peptides were cleaved with trifluoroacetic acid (TFA), triisopropylsilane, and nanopure water (95: 2.5: 2.5) (4 hrs), followed by removal of TFA, and precipitated with diethyl ether at 0° C. No further purification of the crude peptide was performed.

For preperative HPLC, the following conditions were used: Agilent Zorbax SB-C18 Prep HT column 21.2 x 250 mm; 10 mL/min, 5-95% MeOH in 90 mins.



*Synthesis of tert-butyl (2-(2-iodo-N-methylacetamido)ethyl)(methyl)carbamate (2)*—Under nitrogen, Boc-anhydride (0.011 mole) was dissolved in 20 mL of DCM and added dropwise to a solution of *N*, *N'* dimethylethylenediamine (0.034 mole) dissolved in 30 mL of DCM at RT, overnight. The DCM was removed via rotary evaporation. Residual oil was washed with EtOAc, followed by washes with water and brine. Ethyl acetate was evaporated and a colorless oil remained. No further purification was performed. The crude product (1g) was dissolved in 20 mL of dry DCM and placed in an ice bath, and under nitrogen chloroacetyl chloride (0.0058 mmol) was introduced slowly. The solution turned a dark brown. The reaction was allowed to come to RT and stirred overnight. DCM was removed via rotary evaporation. The remaining oil was dissolved in EtOAc, and washed once with distilled water, followed by three washes with brine. The organic layer was dried with Na<sub>2</sub>SO<sub>4</sub>. After filtration, the ethyl acetate was removed by rotary evaporation. The compound was purified by normal-phase combi-flash (SI-1). Isolated product (0.11 mole) was dissolved in 1 mL of acetone followed by the introduction of NaI (0.22 mole). Precipitate formed after ten minutes. The reaction was stirred overnight at RT. The acetone was removed via rotary evaporation, followed by three washes with brine. No further purification was required. Yield 73%. LRMS-ESI/APC<sup>+/-</sup> (MeOH/H<sub>2</sub>O): calcd.: m/z = 379.2; found m/z = 379.05 [M+Na]<sup>+</sup>.





*N*, *N*'-dimethylethylenediamine (2.0 mmol) was dissolved in a solution of MeCN, MeOH, DMF 1.4 mL (71/14/14) (v/v/v), followed by addition of 2-bromomethyl)phenylboronic (4.0 mmol). The reaction was stirred overnight at RT. The compound was purified via reverse-phase combi-flash. Purified yield 16%. Chemical formula:  $C_{18}H_{26}B_2N_2O_{4.}$ , HRMS-ESI<sup>+</sup> (MeOH/H<sub>2</sub>O): calcd.: *m/z* = 319.20310; found: *m/z* = 319.20060 [M-2H<sub>2</sub>O+H]<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, Acetonitrile-*d*<sub>3</sub>)  $\delta$  7.72 (dt, *J* = 7.4, 1.0 Hz, 1H), 7.66 – 7.63 (m, 1H), 7.61 – 7.58 (m, 1H), 7.38 – 7.23 (m, 6H), 5.00 (d, *J* = 0.8 Hz, 1H), 4.86 (s, 2H), 4.52 (s, 1H), 3.89 (s, 7H), 3.30 (d, *J* = 0.8 Hz, 2H), 2.78 – 2.70 (m, 1H).





*Synthesis of Fmoc-C(B)A* (**3**)—Fmoc-CA-OH (0.07 mmol) was dissolved in 0.3 mL of H<sub>2</sub>O, followed by addition of 0.1 mL of MeOH:TEA:Pyr:H<sub>2</sub>O (7:1 :1:1) (v/v/v). A solution of compound **3** (0.07 mmol) in 0.5 mL MeCN:H<sub>2</sub>O (69:31) (v/v) was introduced. The boc protecting group was removed using trifluoroacetic acid (TFA), triisopropylsilane, and nanopure water (95: 2.5: 2.5) (4 hrs), followed by removal of TFA, and precipitated with diethyl ether at 0° C. No further purification of the peptide was performed. The peptide was alkylated with *o*-(bromomethyl)phenylboronic acid. The peptide was purified via preparative HPLC. MeOH was removed by rotary evaporation, followed by freezing and lyophilization of aqueous remnants. Purified yield 6%. Chemical formula:  $C_{34}H_{41}BN_4O_8S$ ; HRMS-ESI\* (MeOH/H<sub>2</sub>O): calcd:: m/z = 659.2711; found: m/z = 659.2712 [M-2H<sub>2</sub>O+H]<sup>+</sup>. <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.30 (s, 1H), 7.82 (d, *J* = 7.6 Hz, 2H), 7.65 (t, *J* = 6.8 Hz, 2H), 7.38 (t, *J* = 7.4 Hz, 2H), 7.33 – 7.25 (m, 3H), 7.16 (d, *J* = 7.3 Hz, 1H), 4.25 (t, *J* = 6.6 Hz, 2H), 4.20 – 4.11 (m, 1H), 3.94 (d, *J* = 7.0 Hz, 1H), 3.88 (s, 4H), 3.72 (d, *J* = 10.9 Hz, 1H), 3.57 – 3.39 (m, 2H), 3.40 – 3.30 (m, 1H), 2.91 (dd, *J* = 13.8, 5.2 Hz, 1H), 2.71 (d, *J* = 14.0 Hz, 3H), 2.68 – 2.58 (m, 4H), 2.24 (s, 2H), 2.16 (s, 1H), 1.18 (dd, *J* = 7.0, 1.7 Hz, 3H). <sup>13</sup>C NMR (126 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  170.31, 156.90, 144.37, 144.36, 144.25, 141.36, 135.41, 135.40, 130.34, 130.14, 128.60, 128.02, 126.00, 120.83, 66.76, 63.25, 63.23, 54.97, 54.97, 52.75, 50.19, 47.26, 47.26, 44.12, 41.44, 40.45, 40.45, 40.06, 40.02, 39.90, 39.87, 39.84, 39.73, 39.70, 39.67, 39.56, 39.53, 39.51, 39.40, 39.37, 39.34, 39.23, 39.20, 39.17, 39.04, 39.01, 35.87, 34.48, 34.12, 18.92, 18.91.







L	682.25560	682.25580	1	40203.08	C34H39BN407S	(M+Na)+	
I	683.25740	683.25530	1	13898.59	C34H39BN4O7S	(M+Na)+	
I	684.26040	684.25610	1	3726.04	C34H39BN4O7S	(M+Na)+	
Γ	685.27310	685.25750	1	1567.39	C34H39BN4O7S	(M+Na)+	
I	686.29420	686.25920	1	578.84	C34H39BN407S	(M+Na)+	

SI-Figure 5. HRMS data for compound 3.

-3.1 -6.25 -22.79 -51.11



SI-Figure 6. Purity check for compound 3. Retention time: 21.952 min.



Synthesis of Fmoc-Lys( $N_3$ , B)Ala-SH (4)—Fmoc-Lys( $N_3$ )Ala-S-Resin-Mixing of solvents, preparation of click solutions, and click reaction was done under nitrogen. Resin (100 µmol) and alkyne (1 eq) were combined with 1 mL of DMF followed by the addition of 0.2 mL solution of Cul (0.5 eq), tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA) (1 eq), and sodium ascorbate (1 eq) in DMF and stirred for 1 hr at RT. The reaction solution mixture was drained from the resin and fresh reaction solution was introduced two more times. The resin was washed with DMF (3 mL, 3 min, 3x), DCM (3 mL, 3 min, 3x), glacial AcOH (5 mL, 3x), DCM (5 mL, 3x), and MeOH (5 mL, 3x). The resin was then placed under vacuum overnight, and cleaved using trifluoroacetic acid (TFA), triisopropylsilane, 1,2-ethanedithiol (EDT), and nanopure water (94: 1.0: 2.5: 2.5) (4 hrs). TFA was evaporated and remaining oil was precipitated with diethyl ether at 0° C. Peptide was purified via preparative HPLC. MeOH was removed by rotary evaporation, followed by freezing and lyophilization of aqueous remnants. Purified yield 14%. Chemical formula: C<sub>37</sub>H<sub>46</sub>BN<sub>7</sub>O<sub>6</sub>S; HRMS-ESI<sup>+</sup> (MeOH/H<sub>2</sub>O): calcd.: *m/z* = 750.3222; found: m/z = 750.3228 [M+Na]<sup>+</sup>. <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 8.30 (s, 1H), 7.97 (d, J = 6.7 Hz, 1H), 7.84 (t, J = 6.8 Hz, 2H), 7.65 (q, J = 6.2 Hz, 2H), 7.58 (d, J = 7.1 Hz, 1H), 7.39 (q, J = 7.0 Hz, 2H), 7.29 (q, J = 7.5, 7.0 Hz, 2H), 7.22 (dd, J = 8.7, 6.6 Hz, 1H), 7.15 (q, J = 6.8 Hz, 1H), 4.30 (t, J = 6.8 Hz, 2H), 4.22 (dd, J = 7.4, 4.0 Hz, 2H), 4.16 (td, J = 6.9, 3.3 Hz, 2H), 3.93 (dd, J = 9.4, 4.9 Hz, 1H), 3.65 (d, J = 4.4 Hz, 2H), 3.33 (dt, J = 13.5, 6.8 Hz, 1H), 3.25 (dt, J = 13.4, 6.6 Hz, 1H), 2.78 - 2.63 (m, 2H), 2.03 (d, J = 4.5 Hz, 3H), 1.77 (d, J = 9.3 Hz, 3H), 1.64 (s, 1H), 1.52 (d, J = 11.8 Hz, 1H), 1.35 - 1.19 (m, 4H), 1.16 (d, J = 7.1 Hz, 3H). <sup>13</sup>C NMR (126 MHz, DMSO-*d*<sub>6</sub>) δ 172.78, 172.08, 156.53, 144.13, 144.03, 143.25, 141.86, 141.07, 134.48, 129.44, 129.21, 129.09, 128.16, 127.57, 127.19, 125.65, 124.80, 121.78, 120.51, 72.44, 66.14, 61.71, 60.55, 54.72, 49.83, 49.70, 48.63, 47.02, 42.26, 40.28, 40.11, 40.02, 39.94, 39.86, 39.77, 39.69, 39.60, 39.52, 39.43, 39.35, 39.26, 39.18, 39.07, 39.02, 38.16, 37.41, 35.91, 31.38, 29.67, 22.76, 18.51.







SI-Figure 9. HRMS for compound 4.



SI-Figure 10. Purity check for compound 4. Retention time: 34.443 min.



*Synthesis of Fmoc-Lys(N<sub>ε</sub>, Me, B)A-OH* (**5**)—General procedure B was used for 0.054 mmol of starting material. Purified yield 60 %. Chemical formula: C<sub>32</sub>H<sub>38</sub>BN<sub>3</sub>O<sub>7</sub>; HRMS-ESI<sup>+</sup> (MeOH/H<sub>2</sub>O): calcd.: *m/z* = 610.27010; found: *m/z* = 610.2685 [M+Na]<sup>+</sup>. Negative HRMS-ESI<sup>-</sup> (MeOH/H<sub>2</sub>O): calcd.: *m/z* = calcd. 586.2736; found: *m/z* = 586.2727 [M-H]<sup>-</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.23 (s, 1H), 7.84 (dd, *J* = 7.6, 3.1 Hz, 2H), 7.68 – 7.61 (m, 2H), 7.50 (dt, *J* = 7.1, 1.1 Hz, 1H), 7.39 (tt, *J* = 7.5, 1.6 Hz, 2H), 7.35 – 7.30 (m, 2H), 7.30 – 7.26 (m, 1H), 7.23 (ddd, *J* = 7.2, 5.3, 3.4 Hz, 1H), 7.18 (d, *J* = 7.2 Hz, 1H), 4.63 (s, 2H), 4.28 – 4.15 (m, 3H), 4.04 (q, *J* = 7.2 Hz, 1H), 3.93 (dd, *J* = 9.0, 5.1 Hz, 1H), 3.66 (q, *J* = 7.3 Hz, 2H), 3.50 – 3.45 (m, 1H), 3.43 – 3.37 (m, 1H), 2.19 – 2.12 (m, 3H), 1.61 (dt, *J* = 13.8, 7.0 Hz, 1H), 1.49 (h, *J* = 7.6 Hz, 2H), 1.20 (dd, *J* = 9.4, 7.1 Hz, 4H).





MS Spectrum	Peak List					
Obs. m/z	Calc. m/z	Charge	Abund	Formula	Ion/Isotope	Tgt Mass Error (ppm)
585.27530	585.27660	1	4800.18	C32H38BN307	(M-H)-	2.35
586.27270	586.27360	1	24297.9	C32H38BN3O7	(M-H)-	1.48
587.27560	587.27650	1	8683.08	C32H38BN3O7	(M-H)-	1.67
588.27470	588.27930	1	2756.03	C32H38BN3O7	(M-H)-	7.8
589.27210	589.28190	1	588.39	C32H38BN307	(M-H)-	16.68
590.27610	590.28450	1	182.32	C32H38BN3O7	(M-H)-	14.21

SI-Figure 12. HRMS data for compound 5.



SI-Figure 13. Purity for compound 6. Retention time: 22.944 min.

![](_page_19_Figure_0.jpeg)

*Synthesis of Biotin-ArgThrArgLys*( $N_{\varepsilon}$ , *Me*, *B*)*LeuLys*( $N_{\varepsilon}$ , *Me*, *B*)*PheLys*( $N_{\varepsilon}$ , *Me*, *B*)*Tyr-OH* (**6**)— General procedure B was used for 0.022 mmol of starting material. Purified yield 7.5 %. Chemical formula:  $C_{91}H_{142}B_2N_{20}O_{23}S$ ; HRMS-ESI<sup>+</sup> (MeOH/H<sub>2</sub>O): m/z = 701.0625; found: m/z =found: m/z = 586.2727 [M-3H<sub>2</sub>O+3H]<sup>3</sup>. <sup>1</sup>H NMR (500 MHz, DMSO- $d_6$ )  $\delta$  8.29 (s, 8H), 7.65 – 7.50 (m, 3H), 7.33 – 7.03 (m, 18H), 6.93 (d, J = 7.8 Hz, 3H), 6.58 (d, J = 7.9 Hz, 3H), 4.51 – 4.30 (m, 2H), 3.57 (q, J = 8.5, 7.5 Hz, 5H), 3.46 (d, J = 8.9 Hz, 9H), 3.37 (t, J = 5.7 Hz, 2H), 3.24 (s, 1H), 3.20 – 3.11 (m, 2H), 3.04 (dt, J = 26.2, 7.3 Hz, 6H), 2.91 (s, 5H), 2.83 – 2.63 (m, 7H), 2.42 – 2.24 (m, 10H), 2.23 – 2.00 (m, 12H), 1.70 – 1.32 (m, 29H), 1.29 – 1.05 (m, 14H), 1.04 – 0.90 (m, 3H), 0.78 (dtd, J = 33.8, 18.2, 17.3, 5.5 Hz, 9H).

![](_page_20_Figure_0.jpeg)

![](_page_21_Figure_0.jpeg)

![](_page_21_Figure_1.jpeg)

Obs. m/z	Calc. m/z	Charge	Abund	Formula	Ion/Isotope	Tgt Mass Error (ppm)
189.06950			123801.57			
700.72720	700.73010	3	1229.77	C103H154B3N21O22S	(M+3H)+3	4.25
701.06380	701.06250	3	4590.75	C103H154B3N21O22S	(M+3H)+3	-1.95
701.39700	701.39500	3	20871.28	C103H154B3N21O22S	(M+3H)+3	-2.9
701.73030	701.72780	3	41272.51	C103H154B3N21O22S	(M+3H)+3	-3.59
702.06390	702.06160	3	40435.42	C103H154B3N21O22S	(M+3H)+3	-3.21
702.39720	702.39550	3	29841.61	C103H154B3N21O22S	(M+3H)+3	-2.4
702.73060	702.72940	3	16789.51	C103H154B3N21O22S	(M+3H)+3	-1.58
703.06180	703.06330	3	10529.62	C103H154B3N21O22S	(M+3H)+3	2.2
703.39300	703.39730	3	6336.79	C103H154B3N21O22S	(M+3H)+3	6.00
703.72440	703.73120	3	3439.32	C103H154B3N21O22S	(M+3H)+3	9.68
704.05510	704.06520	3	2118.36	C103H154B3N21022S	(M+3H)+3	14.3

SI-Figure 15. HRMS data for compound 6.

![](_page_21_Figure_4.jpeg)

SI-Figure 16. Purity check for compound 6. Retention time: 19.648 min

![](_page_22_Figure_0.jpeg)

Synthesis of Biotin-ArgThrArgLys( $N_{e}$ , Me, B)LeuLys( $N_{e}$ , Me, B)PheGlyTyr-OH (**7**)— General procedure B was used for 0.021 mmol of starting material. Purified yield 11 %. Chemical formula:  $C_{91}H_{142}B_2N_{20}O_{23}S$ ; HRMS-ESI<sup>+</sup> (MeOH/H<sub>2</sub>O): calcd.: m/z = 951.0221; found: m/z = 951.0258 [M-3H<sub>2</sub>O+2H]<sup>2+</sup>. HRMS-ESI<sup>-</sup> (MeOH/H<sub>2</sub>O): calcd.: m/z = calcd. 949.0075; found: m/z = 949.0051 [M-2H<sub>2</sub>O-2H]<sup>2-</sup>. <sup>1</sup>H NMR (499 MHz, DMSO- $d_6$ )  $\delta$  8.35 (s, 8H), 8.16 (s, 4H), 7.59 (s, 3H), 7.20 – 7.05 (m, 4H), 6.94 (q, J = 8.8 Hz, 2H), 6.54 (d, J = 8.3 Hz, 1H), 4.34 – 4.27 (m, 1H), 4.13 (dd, J = 7.9, 4.3 Hz, 1H), 3.42 – 3.35 (m, 3H), 2.91 (s, 1H), 2.81 – 2.65 (m, 1H), 2.57 (d, J = 12.6 Hz, 2H), 2.45 – 2.17 (m, 1H), 2.12 – 1.99 (m, 13H), 1.69 (d, J = 17.0 Hz, 1H), 1.63 – 1.55 (m, 1H), 1.54 – 1.34 (m, 18H), 1.33 – 1.22 (m, 1H), 1.18 (d, J = 7.1 Hz, 5H), 1.00 (td, J = 14.0, 12.8, 6.7 Hz, 4H), 0.86 (d, J = 6.1 Hz, 1H), 0.80 (t, J = 5.6 Hz, 4H), 0.74 (dd, J = 10.5, 4.6 Hz, 1H). <sup>13</sup>C NMR (126 MHz, DMSO- $d_6$ )  $\delta$  142.07, 133.86, 129.20, 129.17, 127.81, 127.04, 74.53, 57.89.

![](_page_23_Figure_0.jpeg)

![](_page_24_Figure_0.jpeg)

SI-Figure 18. Carbon NMR spectra spectra for compound 7.

![](_page_25_Figure_0.jpeg)

SI-Figure 19. C/H correlation NMR for compound 7.

![](_page_26_Figure_0.jpeg)

SI-Figure 20. HRMS data for compound 7.

![](_page_27_Figure_0.jpeg)

SI-Figure 21. Purity check for compound 7. Retention time: 17.995.

![](_page_28_Figure_0.jpeg)

Synthesis of Biotin-ArgThrArgLys( $N_{e}$ , Me, B)LeuGlyPheLys( $N_{e}$ , Me, B)Tyr-OH (**8**)— General procedure B was used for 0.015 mmol of starting material. Purified yield 19 %. Chemcial formula:  $C_{91}H_{142}B_2N_{20}O_{23}S$ ; HRMS-ESI<sup>+</sup> (MeOH/H<sub>2</sub>O): calcd.: m/z = calcd. 634.6855; found: m/z = 634.6832 [M-2H<sub>2</sub>O+3H]<sup>3+</sup>.<sup>1</sup>H NMR (500 MHz, DMSO- $d_6$ )  $\delta$  8.34 (s, 9H), 7.84 (t, J = 5.5 Hz, 1H), 7.66 (s, 1H), 7.37 – 6.87 (m, 3H), 6.58 (d, J = 8.4 Hz, 3H), 6.41 (s, 1H), 6.36 (s, 1H), 4.32 – 4.28 (m, 1H), 4.12 (d, J = 7.2 Hz, 1H), 3.99 (d, J = 24.1 Hz, 1H), 3.21 – 3.14 (m, 2H), 3.10 – 2.89 (m, 1H), 2.85 – 2.78 (m, 2H), 2.39 – 2.29 (m, 1H), 2.21 (q, J = 0.5 Hz, 2H), 1.63 – 1.35 (m, 6H), 1.33 – 1.09 (m, 3H), 1.01 (dd, J = 15.0, 6.0 Hz, 4H), 0.84 (ddd, J = 23.6, 11.2, 5.7 Hz, 8H). <sup>13</sup>C NMR (126 MHz, DMSO- $d_6$ )  $\delta$  179.31, 172.13, 165.36, 162.71, 157.24, 129.20, 127.96, 69.75, 63.36, 61.04, 59.20, 55.40, 39.12, 35.09, 30.67, 30.65, 28.17, 28.02, 25.24.

![](_page_29_Figure_0.jpeg)

![](_page_30_Figure_0.jpeg)

SI-Figure 23. Carbon NMR spectra for compound 8.

![](_page_31_Figure_0.jpeg)

SI-Figure 24. Purity check for compound 8. Retention time: 18.240 min.

![](_page_32_Figure_0.jpeg)

Synthesis of Biotin-ArgThrArgLys(Ne, Me, B)LeuGlyPheGlyTyr-OH (9)— General procedure B was used for 0.015 mmol of starting material. Purified yield 45 %. Chemical formula:  $C_{79}H_{124}BN_{19}O_{21}S$ ; HRMS-ESI<sup>+</sup> (MeOH/H<sub>2</sub>O): calcd.: m/z = 567.6386; found: m/z = found 567.6403 [M-H<sub>2</sub>O+3H]<sup>3+</sup>. HRMS-ESI<sup>-</sup> (MeOH/H<sub>2</sub>O): calcd.: m/z = 848.9370; found: m/z = 848.9370 [M-H<sub>2</sub>O-2H]<sup>2-</sup>. <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 8.35 (s, 8H), 8.13 (s, 1H), 7.92 – 7.57 (m, 2H), 7.55 (d, J = 7.2 Hz, 1H), 7.38 (d, J = 7.6 Hz, 1H), 7.32 - 7.26 (m, 3H), 7.26 - 7.18 (m, 7H), 7.18 - 7.02 (m, 2H), 6.94 (d, J = 8.3 Hz, 3H), 6.59 (d, J = 7.9 Hz, 3H), 6.39 (d, J = 28.5 Hz, 2H), 4.88 (s, 1H), 4.76 (s, 1H), 4.64 (d, J = 4.2 Hz, 1H), 4.58 (s, 2H), 4.36 (s, 1H), 4.32 - 4.27 (m, 1H), 4.20 (s, 3H), 4.13 (s, 2H), 4.02 (s, 2H), 3.88 - 3.70 (m, 3H), 3.59 (d, J = 6.4 Hz, 2H), 3.49 (d, J = 2.6 Hz, 10H), 3.39 (t, J = 6.0 Hz, 2H), 3.31 (s, 1H), 3.28 (d, J = 5.1 Hz, 5H), 3.22 - 3.15 (m, 3H), 3.04 (s, 6H), 2.94 (d, J = 12.8 Hz, 1H), 2.82 (dd, J = 12.5, 5.1 Hz, 2H), 2.74 (s, 2H), 2.58 (d, J = 12.4 Hz, 1H), 2.46 – 2.28 (m, 2H), 2.22 – 2.20 (m, 2H), 1.47 (d, J = 25.5 Hz, 20H), 1.35 -1.16 (m, 2H), 1.02 (d, J = 8.1 Hz, 4H), 0.83 (d, J = 22.7 Hz, 8H). <sup>13</sup>C NMR (126 MHz, DMSO- $d_6$ )  $\delta$  172.45, 172.13, 171.90, 171.66, 171.53, 171.15, 170.33, 170.15, 168.71, 168.71, 167.56, 166.37, 162.73, 157.29, 157.29, 155.45, 143.52, 142.89, 142.27, 138.01, 137.98, 134.00, 133.99, 133.73, 133.25, 130.13, 129.09, 128.93, 128.56, 128.33, 128.05, 127.37, 126.95, 126.81, 126.19, 126.04, 125.72, 125.63, 125.35, 114.66, 73.89, 73.37, 73.33, 73.24, 73.20, 69.76, 69.70, 69.67, 69.55, 69.45, 69.15, 66.76, 66.34, 61.05, 59.22, 58.44, 57.64, 57.59, 57.52, 57.49, 57.47, 55.88, 55.40, 51.87, 40.78, 40.34, 39.45, 39.13, 38.45, 37.19, 35.85, 35.09, 30.97, 30.79, 30.65, 30.47, 28.66, 28.18, 28.03, 25.25, 24.73, 24.12, 22.97, 21.60, 21.47, 20.08, 19.76, 18.69.

![](_page_33_Figure_0.jpeg)

![](_page_34_Figure_0.jpeg)

![](_page_35_Figure_0.jpeg)

SI-Figure 27. HRMS data for compound 9.

![](_page_36_Figure_0.jpeg)

SI-Figure 28. Purity check for compound 9.Retention time: 17.995 min.

![](_page_37_Figure_0.jpeg)

*Synthesis of Biotin-GlyThrGlyLys*(*N<sub>ν</sub>*, *Me*, *B*)*LeuLys*(*N<sub>ν</sub>*, *Me*, *B*)*PheLys*(*N<sub>ν</sub>*, *Me*, *B*)*Tyr-OH* (**10**)— General procedure B was used for 0.015 mmol of starting material. Purified yield 25 %. HRMS-ESI<sup>+</sup> (MeOH/H<sub>2</sub>O): calcd.: *m/z* = 635.3418; found: *m/z* = 635.3437 [M-H<sub>2</sub>O+3H]<sup>3+</sup>. Chemical formula: C<sub>95</sub>H<sub>142</sub>B<sub>3</sub>N<sub>15</sub>O<sub>25</sub>S; HRMS-ESI<sup>+</sup> (MeOH/H<sub>2</sub>O): calcd.: *m/z* = 950.9938; found: *m/z* = 949.9907 [M-3H<sub>2</sub>O-2H]<sup>2-</sup>. <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 8.30 (s, 6H), 7.64 (d, *J* = 7.2 Hz, 3H), 7.34 – 7.19 (m, 4H), 7.15 (d, *J* = 8.8 Hz, 12H), 6.94 (d, *J* = 7.5 Hz, 3H), 6.59 (d, *J* = 7.8 Hz, 3H), 4.52 (d, *J* = 1.8 Hz, 1H), 4.49 – 4.39 (m, 1H), 4.31 (t, *J* = 6.4 Hz, 1H), 4.13 (dd, *J* = 7.8, 4.3 Hz, 2H), 4.00 (t, *J* = 5.3 Hz, 1H), 3.38 (t, *J* = 5.9 Hz, 2H), 3.25 (d, *J* = 1.7 Hz, 1H), 3.20 – 3.13 (m, 2H), 3.11 – 2.98 (m, 1H), 2.93 (d, *J* = 9.7 Hz, 1H), 2.83 – 2.67 (m, 2H), 2.57 (d, *J* = 12.5 Hz, 1H), 2.50 (p, *J* = 1.8 Hz, 11H), 2.45 – 2.31 (m, 6H), 2.21 – 2.17 (m, 1H), 1.94 (s, 1H), 1.66 – 1.56 (m, 1H), 1.55 – 1.33 (m, 13H), 1.27 (q, *J* = 7.8 Hz, 1H), 1.22 (dd, *J* = 6.6, 2.2 Hz, 9H),, 1.07 – 0.94 (m, 4H), 0.93 – 0.68 (m, 6H). <sup>13</sup>C NMR (126 MHz, DMSO-*d*<sub>6</sub>) δ 234.84, 234.82, 180.21, 172.94, 171.36, 170.97, 169.88, 169.35, 165.77, 163.23, 155.70, 142.16, 141.70, 135.07, 133.88, 130.93, 130.55, 129.64, 129.64, 129.41, 129.07, 129.07, 128.30, 127.61, 127.16, 126.84, 115.00, 109.87, 74.38, 72.40, 70.05, 69.99, 69.93, 69.84, 69.81, 69.32, 69.32, 66.89, 66.72, 63.79, 62.93, 61.35, 60.46, 59.50, 57.78, 55.79, 55.72, 53.45, 42.42, 41.85, 40.32, 40.14, 38.71, 36.16, 35.40, 31.77, 31.29, 31.11, 30.97, 30.95, 30.91, 30.87, 30.84, 30.80, 30.77, 30.71, 30.61, 30.46, 28.51, 28.31, 25.55, 25.15, 24.43, 23.26, 21.79, 19.82, 17.80, 12.84, 4.89, -15.08, -15.08.

![](_page_38_Figure_0.jpeg)

![](_page_39_Figure_0.jpeg)

![](_page_40_Figure_0.jpeg)

SI-Figure 31. HRMS data for compound 10.

![](_page_41_Figure_0.jpeg)

SI-Figure 32. Purity check for compound 10. Retention time: 19.861 min.

![](_page_42_Figure_0.jpeg)

11

*Synthesis of Biotin-ArgThrLeuLys(N<sub>e</sub>, Me, B)ArgLys(N<sub>e</sub>, Me, B)PheLys(N<sub>e</sub>, Me, B)Tyr-OH* (**11**)— General procedure B was used for 0.022 mmol of starting material. Purified yield 17 %. HRMS-ESI<sup>+</sup> (MeOH/H<sub>2</sub>O): calcd.: *m/z* = 701.3950; found: *m/z* = 701.3926 [M-H<sub>2</sub>O+3H]<sup>3+</sup>. Chemical formula: C<sub>103</sub>H<sub>160</sub>B<sub>3</sub>N<sub>21</sub>O<sub>25</sub>S. HRMS-ESI<sup>+</sup> (MeOH/H<sub>2</sub>O): calcd.: *m/z* = 1048.57700; found: *m/z* = 1048.56380 [M-3H<sub>2</sub>O-2H]<sup>2-</sup>. <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 8.28 (s, 5H), 7.64 (dt, *J* = 19.1, 8.7 Hz, 3H), 7.41 – 6.99 (m, 18H), 6.94 (d, *J* = 8.0 Hz, 2H), 6.60 (d, *J* = 8.0 Hz, 2H), 4.59 – 4.41 (m, 2H), 4.39 – 4.28 (m, 1H), 4.11 (d, *J* = 14.8 Hz, 97H), 3.56 (q, *J* = 14.4, 10.9 Hz, 2H), 3.45 (d, *J* = 7.9 Hz, 12H), 3.38 (d, *J* = 6.0 Hz, 2H), 3.16 (t, *J* = 6.3 Hz, 2H), 3.10 – 2.96 (m, 6H), 2.84 – 2.61 (m, 7H), 2.57 (d, *J* = 12.7 Hz, 1H), 2.45 – 2.10 (m, 10H), 2.05 (t, *J* = 7.4 Hz, 2H), 1.79 – 1.30 (m, 28H), 1.27 – 1.21 (m, 1H), 1.06 – 0.88 (m, 3H), 0.77 (dt, *J* = 27.5, 7.3 Hz, 8H). <sup>13</sup>C NMR (126 MHz, DMSO-*d*<sub>6</sub>) δ 235.31, 175.67, 174.39, 173.56, 173.06, 172.72, 172.43, 171.79, 171.67, 171.22, 167.42, 164.16, 160.27, 160.01, 157.35, 157.30, 155.95, 142.27, 138.02, 135.25, 134.31, 131.05, 130.22, 129.87, 129.81, 129.19, 128.94, 128.54, 127.66, 127.24, 127.23, 118.79, 116.42, 115.59, 110.38, 74.96, 70.39, 70.37, 70.31, 70.18, 69.57, 67.28, 67.03, 62.00, 60.24, 60.13, 58.19, 56.29, 56.15, 54.43, 53.62, 52.39, 49.34, 42.70, 41.00, 40.48, 40.28, 40.17, 39.25, 37.84, 37.10, 36.54, 35.91, 31.41, 29.10, 29.08, 28.92, 28.67, 25.97, 25.43, 24.87, 24.21, 23.63, 23.47, 23.47, 23.47, 23.34, 23.33, 22.07, 20.17.

S-43

![](_page_43_Figure_0.jpeg)

![](_page_44_Figure_0.jpeg)

![](_page_45_Figure_0.jpeg)

![](_page_46_Figure_0.jpeg)

![](_page_46_Figure_1.jpeg)

MS Spectrum Peak List								
Obs. m/z	Calc. m/z	Charge	Abund	Formula	Ion/Isotope	Tgt Mass Error (ppm)		
130.15910			34928.27					
700.73060	700.73010	3	175.78	C103H154B3N21O22S	(M+3H)+3	-0.61		
701.06380	701.06250	3	969.65	C103H154B3N21O22S	(M+3H)+3	-1.85		
701.39260	701.39500	3	3391.49	C103H154B3N21O22S	(M+3H)+3	3.39		
701.73030	701.72780	3	8844.23	C103H154B3N21O22S	(M+3H)+3	-3.49		
702.06400	702.06160	3	8637.74	C103H154B3N21O22S	(M+3H)+3	-3.4		
702.39610	702.39550	3	5805.99	C103H154B3N21O22S	(M+3H)+3	-0.75		
702.73060	702.72940	3	3515.3	C103H154B3N21O22S	(M+3H)+3	-1.62		
703.06250	703.06330	3	1875.76	C103H154B3N21O22S	(M+3H)+3	1.16		
700 00700	700 00700	-	000 65	C1001115 100101 0000	(11.011).0	14.50		

![](_page_46_Figure_3.jpeg)

Obs. m/z	Calc. m/z	Charge	Abund	Formula	Ion/Isotope	Tgt Mass Error (ppm)
1048.56380	1048.57700	2	34	C103H154B3N21O22S	(M-2H)-2	12.66
1049.06980	1049.07550	2	149.97	C103H154B3N21O22S	(M-2H)-2	5.43
1049.56920	1049.57420	2	648.83	C103H154B3N21O22S	(M-2H)-2	4.77
1050.06840	1050.07350	2	1438.84	C103H154B3N21O22S	(M-2H)-2	4.91
1050.56820	1050.57430	2	1428.76	C103H154B3N21O22S	(M-2H)-2	5.82
1051.06990	1051.07510	2	936.71	C103H154B3N21O22S	(M-2H)-2	5
1051.56820	1051.57600	2	542.3	C103H154B3N21O22S	(M-2H)-2	7.4
1052.06280	1052.07680	2	352.52	C103H154B3N21O22S	(M-2H)-2	13.34
1052.55960	1052.57770	2	209.53	C103H154B3N21O22S	(M-2H)-2	17.16

SI-Figure 36. HRMS data for compound 11.

![](_page_47_Figure_0.jpeg)

SI-Figure 37. Purity check for compound 11. Retention time: 21.621 min.

![](_page_48_Figure_0.jpeg)

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*Synthesis of Biotin-ArgThrPheLys*( $N_{e}$ , *Me*, *B*)*LeuLys*( $N_{e}$ , *Me*, *B*)*ArgLys*( $N_{e}$ , *Me*, *B*)*Tyr-OH* (**12**)—General procedure B was used for 0.022 mmol of starting material. Purified yield 22 %. Chemical formula:  $C_{103}H_{160}B_3N_{21}O_{25}S$ ; HRMS-ESI<sup>+</sup> (MeOH/H<sub>2</sub>O): calcd.: *m/z* = 701.3950; found: *m/z* = found 701.3975 [M-H<sub>2</sub>O+3H]<sup>3+</sup> HRMS-ESI<sup>-</sup> (MeOH/H<sub>2</sub>O): calcd.: *m/z* = calcd. 1050.0735; found: *m/z* = 1050.0684 [M-3H<sub>2</sub>O-2H]<sup>2-</sup>. <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.28 (s, 8H), 7.65 – 7.57 (m, 3H), 7.31 – 7.20 (m, 11H), 7.15 (t, *J* = 11.0 Hz, 16H), 6.85 (d, *J* = 8.1 Hz, 2H), 6.62 – 6.50 (m, 3H), 4.51 (s, 1H), 4.49 (s, 1H), 4.31 (dd, *J* = 7.8, 5.0 Hz, 2H), 4.22 – 4.09 (m, 2H), 3.98 (s, 2H), 3.65 – 3.51 (m, 4H), 3.50 – 3.46 (m, 12H), 3.44 (s, 4H), 3.37 (t, *J* = 5.8 Hz, 3H), 3.24 (d, *J* = 1.3 Hz, 1H), 3.16 (t, *J* = 5.9 Hz, 3H), 3.11 – 2.87 (m, 4H), 2.79 (dd, *J* = 12.6, 5.2 Hz, 3H), 2.20 – 2.17 (m, 1H), 1.95 – 1.91 (m, 1H), 1.59 (s, 7H), 1.54 – 1.32 (m, 26H), 1.28 (p, *J* = 7.1 Hz, 1H), 1.18 (d, *J* = 18.2 Hz, 8H), 0.95 (d, *J* = 6.3 Hz, 4H), 0.81 (dd, *J* = 22.7, 6.6 Hz, 9H).

![](_page_49_Figure_0.jpeg)

![](_page_50_Figure_0.jpeg)

SI-Figure 39. HRMS for compound 12.

![](_page_51_Figure_0.jpeg)

SI-Figure 40. Purity check for compound 12. Retention time: 18.251 min.