# Supporting Information I

## Small Molecule Microarray-facilitated Screening of Affinity-based Probes (A*f*BPs) for γ-secretase

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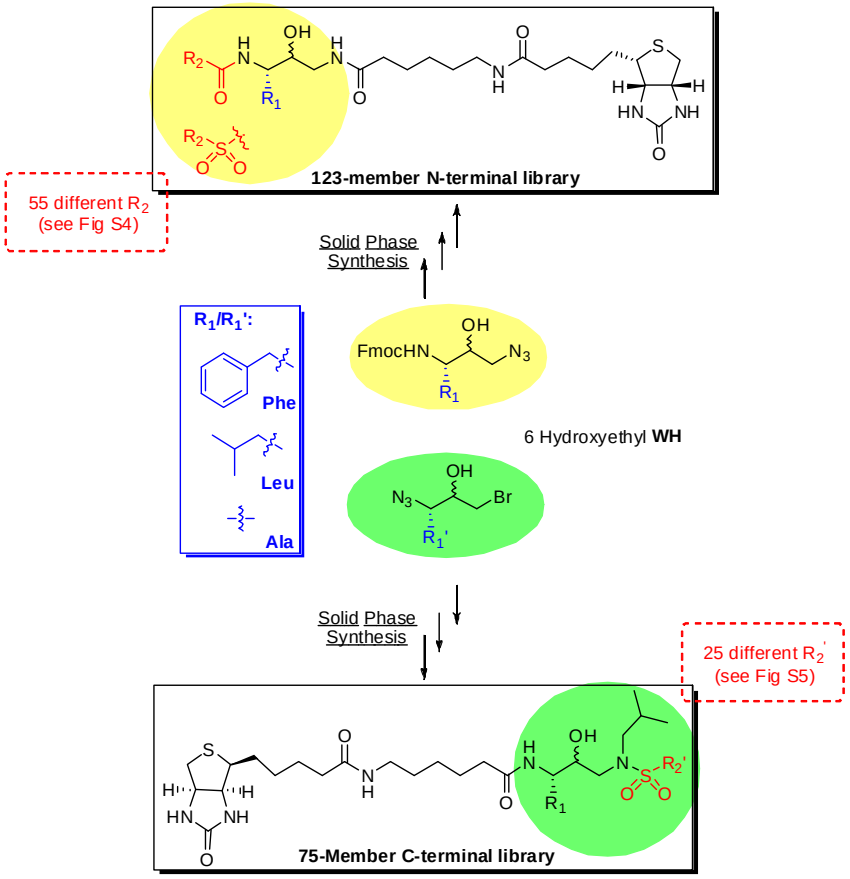
## 1. General

All chemicals were purchased from commercial vendors and used without further purification, unless otherwise noted. $^1$H NMR spectra was recorded on a Bruker model *Avance* 300 MHz, 500 MHz or DPX-300 NMR spectrameter. Chemical shifts are reported in parts per million referenced with relative to residual solvent ($CDCl_3$=7.26 ppm, DMSO-$d_6$ = 2.5 ppm and $CD_3OD$ = 3.31 ppm) or from internal standard tetramethylsilane (TMS = 0.00 ppm). The following abbreviations were used in reporting spectra: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets. All reactions were carried out under $N_2$ atmosphere, unless otherwise stated. Concentration in vacuo was performed on a Büchi rotary evaporator. HPLC grade solvents were used for all the reactions. HPLC profiles and ESI mass spectra were acquired in the positive or negative mode by using a Shimadzu IT-TOF. HAP and mutants H34A, S37A, E278K were obtained from the Yada Lab (University of Guelph, Canada).[1] Fluorescence scanning of SDS-PAGE gels was carried out with Typhoon 9200 fluorescence gel scanner (GE), and where applicable, the bands were quantified with the software installed on the instrument. Plain glass slides were purchased from Sigma Aldrich (USA), and modified to generate the corresponding avidin-coated surface as previously described.[2] The numbering of the residures in the inhibititors synthesized for this study ($P_2$, $P_1$, $P_1'$ and $P_2'$) is based on the standard protease/substrate nomemclature, where residues in the substrate/inhibitor is numbered 1, 2, 3…, according to their relative positions from the scissile bond (non-prime and prime residues are given to those located either on the left or right side of the scissile bond, respectively).

## 2. Chemical Synthesis




**Scheme S1.** Overall design and synthesis of the 198-member, biotin-containing small molecules targeting aspartic proteases. Each member contains a hydroxylethyl "core" group which is a well-known transitionstate (TS) analog of aspartic proteases. The biotin tag in each molecule was used for microarray immobilization.

### 2.1 General procedure for the synthesis of N-terminal azide scaffolds **4(a-c).**

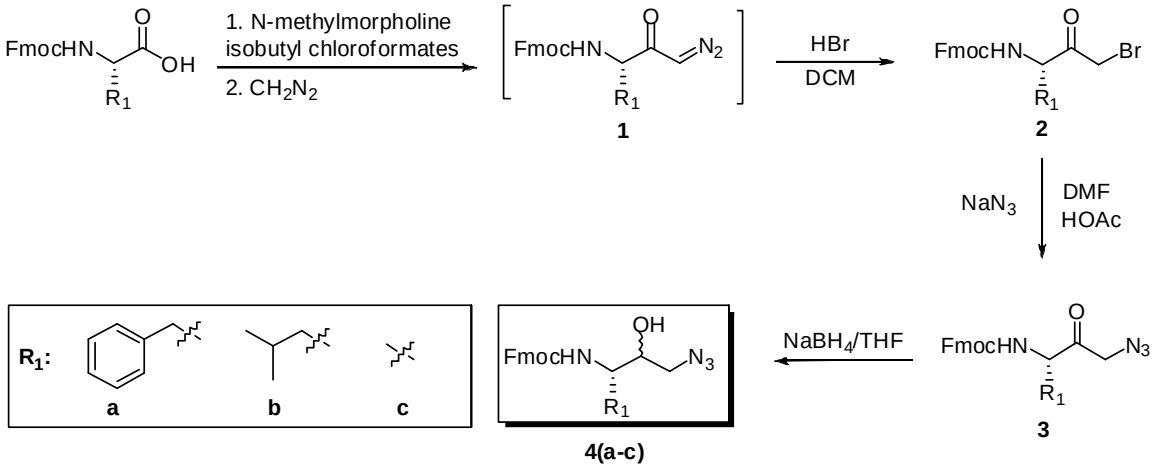



**Figure S1.** Synthesis of the N-terminal hydroxylethyl TS warheads **4(a-c)**.

*2.1.1 General procedure for the synthesis of bromomethyl ketones **2(a-c):***

Compound **2** was synthesized according to published procedures.[3] To a stirring solution of the Fmoc-protection amino acid and N-methylmorpholine (6 mmol each) in THF (40 mL) was added isobutyl chloroformate (6 mmol) at -40 $^{o}$C. After 15 min, the reaction mixture was filtered. Diazomethane in ether (freshly prepared from 1.33 g, 6.2 mmol of 1-methyl-3-nitrol-nitrosoguanidine and 1.8 mL of 40 wt% aqueous KOH in 10 mL of EtOH) was added to the filtrate slowly and the mixture was warmed up to room temperature slowly. After completion of the reaction, the solution was concentrated *in vacuo* to obtain diazo ketone **1** which was dissolved in 30 mL of $CH_2Cl_2$. Subsequently 48% HBr (6 mmol) was then added slowly at 0 $^{o}$C and stir for further 15 min. The reaction mixture was finally diluted with EtOAc (40 mL) and washed with saturated sodium bicarbonate, then with brine. The organic layer was dried over anhydrous $Na_2SO_4$, filtered, and concentrated under reduced pressure to afford bromomethyl ketone **2(a-f)** (80-88% yield).

N-α-Fmoc-phenylalanyldiazomethane (**1a**):
Yield = 85%. $^{1}$H-NMR (500 MHz, $CDCl_3$) δ 3.05 (d, *J* = 6.95 Hz, 2H), 4.21 (t, *J* = 6.00 Hz, 1H), 4.41-4.43 (m, 3H), 5.13 (s, 1H), 5.29 (s, 1H), 7.17 (d, *J* = 4.17 Hz, 1H), 7.23-7.32 (m, 6H), 7.40 (t, *J* = 4.53 Hz, 2H), 7.54 (t, *J* = 6.42 Hz, 2H), 7.78 (d, *J* = 7.49 Hz, 2H); ESI-MS: m/z $[M+Na]^+$ = 434.134.

N-α-Fmoc-phenylalanylbromomethylketone (**2a**):
Yield = 86%. $^{1}$H-NMR (500 MHz, $CDCl_3$) δ 3.09-3.14 (m, 2H), 3.77-3.93 (m, 2 H), 4.17 (d, *J* = 7.32 Hz, 1H), 4.42 (d, *J* = 6.30 Hz, 2H), 4.78 (d, *J* = 6.95 Hz, 1H), 5.31 (d, *J* = 6.95 Hz, 1H), 7.13 (d, *J* = 6.95 Hz , 4H), 7.25-7.32 (m, 5H), 7.40 (t, *J* = 7.55 Hz, 2H), 7.53 (t, *J* = 7.88, Hz, 2H), 7.76 (d, *J* = 7.55 Hz, 2H); $^{13}$C-NMR (125 MHz, $CDCl_3$) δ 200.3, 158.7, 143.5, 141.3, 135.5, 129.1, 127.7-124.7(m), 119.9, 74.1, 58.8, 54.0, 18.8; ESI-MS: m/z $[M+Na]^+$ =486.06.

N-α-Fmoc-isobutylalanylbromomethylketone (**2b**):
Yield = 89%. $^{1}$H-NMR (300 MHz, $CDCl_3$) δ 0.93 (d, *J* = 10.02 Hz, 6H), 1.43 (t, *J* = 6.16 Hz, 2 H), 1.61 (t, *J* = 6.16 Hz, 2 H), 3.98 (d, *J* = 4.44 Hz, 2H), 4.18 (t, *J* = 6.33 Hz, 1H), 4.43 (t, *J* = 9.05 Hz 2H), 4.52 (t, *J* = 12.66 Hz, 1H), 7.30-7.55 (m, 4H), 7. 65 (t, *J* = 3.36 Hz, 2H), 7.75 (d, *J* = 7.56 Hz, 2H).

N-α-Fmoc-methylalanylbromomethylketone (**2c**):
Yield = 83%. $^{1}$H-NMR (300 MHz, $CDCl_3$) δ 1.40 (d, *J* = 7.42 Hz, 3H), 3.93-4.00(m, 1H), 4.18-4.25 (m, 2H), 4.38-4.66 (m, 3H), 5.39 (s, 2H), 7.29-7.43 (m, 4H), 7.59 (d, *J* = 5.07 Hz, 2H), 7.77 (d, *J* = 7.41 Hz, 2H); $^{13}$C-NMR (75 MHz, $CDCl_3$) δ 201.5, 143.6, 141.3, 127.7, 127.6, 127.0, 124.9, 119.9, 66.8, 53.5, 47.1, 45.9, 31.3, 18.7, 17.8, 17.5.

2.1.2 General procedure for the synthesis of α-Azido Ketones **3(a-c)**:

α-Azido Ketones were synthesized according to published procedure with some modifications.[6] To a solution of the α-bromo ketone **2** (5 mmol) and acetic acid (10 mmol) in DMF was added $NaN_3$ (7.5 mmol) in water at 0 °C. After stirring for overnight at room

temperature, the reaction was quenched with lots of water and the aqueous layer was extracted with EtOAc. The combined organic extracts were washed with brine, dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure. Purification of the crude product by silica-gel chromatography with 80:20 hexane/ethyl acetate afforded α-Azido Ketones **3** (85-90% yield).

N-α-Fmoc-phenylalanylazidoketone (**3a**):
Yield = 79%. $^1$H-NMR (300 MHz, $CDCl_3$) δ 3.03 (d, *J* = 3.39 Hz, 2H), 3.64 (d, *J* = 11.34 Hz, 1H), 3.90 (d, *J* = 11.34 Hz, 1H), 4.18 (t, *J* = 6.32 Hz, 1H), 4.44 (d, *J* = 6.42 Hz, 2H), 4.53-4.56 (m, 1H), 5.23 (d, *J* = 7.08 Hz, 1H), 7.12 (d, *J* = 6.90 Hz, 2H), 7.28-7.33 (m, 5H), 7.40 (t, *J* = 4.17 Hz, 3H), 7.54 (t, *J* = 5.10 Hz, 2H), 7.77 (d, *J* = 7.56 Hz, 2H); $^{13}$C-NMR (75 MHz, $CDCl_3$) δ 203.4, 155.8, 143.6, 141.1, 135.2, 129.0, 127.8-127.0(m), 124.9, 120.0, 66.9, 58.7, 56.6, 47.2, 37.6; ESI-MS: m/z $[M+Na]^+$ = 449.166.

N-α-Fmoc-isobutylalanylalanylazidoketone (**3b**):
Yield = 87%. $^1$H-NMR (500 MHz, $CDCl_3$) δ 0.94 (d, *J* = 1.90 Hz, 6H), 1.38-1.43 (m, 1H), 1.52-1.56 (m, 2H), 3.96 (d, *J* = 8.30 Hz, 2H), 4.37 (t, *J* = 8.20 Hz, 1H), 4.38-4.51 (m, 3H), 5.11 (d, *J* = 7.55 Hz, 1H), 7.31-7.34 (m, 2H), 7.41 (t, *J* = 7.57 Hz, 2H), 7.58 (t, *J* = 6.95 Hz, 2H), 7.77 (d, *J* = 7.55 Hz, 2H).

N-α-Fmoc-methylalanylazidoketone (**3c**):
Yield = 75%. $^1$H-NMR (500 MHz, $CDCl_3$) δ 1.35 (d, *J* = 6.90 Hz, 3H), 4.03 (d, *J* = 2.50 Hz, 2H), 4.20 (t, *J* = 6.30 Hz, 1H), 4.39-4.44 (m, 2H), 4.48-4.52 (m, 1H), 5.34 (d, *J* = 6.95 Hz, 1H), 7.32 (t, *J* = 6.95 Hz, 2H), 7.41 (t, *J* = 7.55 Hz, 2H), 7.59 (t, *J* = 6.30 Hz, 2H), 7.77 (d, *J* = 7.55 Hz, 2H); $^{13}$C-NMR (125 MHz, $CDCl_3$) δ 201.6, 144.5, 141.3, 126.9, 126.6, 126.0, 124.9, 119.6, 66.7, 53.1, 47.0, 45.9, 31.2, 18.7, 17.9, 17.7.

2.1.3 General procedure for the synthesis of α-Azido alcohols **4(a-c)**:

α-Azido Ketones **3** (5 mmol) was dissolved in 20 ml of THF followed by addition of sodium borohydride (6 mmol) in 2 ml $H_2O$ at 0 $^oC$. The reaction mixture was stirred for 1 h and neutralized with aqueous 1 N HCl. After extraction, the organic layer was washed with brine and dried over anhydrous $Na_2SO_4$, and concentrated under reduced pressure. Purification by silica-gel chromatography with 85:15 hexane/EtOAc afforded bromomethyl alcohol **4** (50-70%).

N-α-Fmoc-phenylalanylazidoalcohol (**4a**):
Yield = 55%. $^1$H-NMR (500 MHz, $CDCl_3$) δ 2.84-2.93 (m, 2H), 3.26 (t, *J* = 6.30 Hz, 1H), 3.35 (s, 1H), 3.69-3.89 (m, 2H), 4.12-4.17 (m, 1H), 4.40-4.43 (m, 2H), 4.76 (d, *J* = 6.95 Hz, 1H), 5.07 (d, *J* = 8.80 Hz, 1H), 7.14-7.31 (m, 7H), 7.40 (t, *J* = 7.25 Hz, 2H), 7.46-7.55 (m, 2H), 7.75 (d, *J* = 6.95 Hz, 2H); $^{13}$C-NMR (125 MHz, $CDCl_3$) δ 156.5, 143.7, 141.4, 137.0, 129.3-126.7(m), 124.9, 119.9, 72.2, 66.5, 54.9, 54.1, 47.3, 38.3; LC-MS: m/z $[M+Na]^+$ = 451.175.

N-α-Fmoc-isobutylalanylalanylazidoalcohol (**4b**):
Yield =96%. $^1$H-NMR (500 MHz, $CDCl_3$) δ 0.91 (d, *J* = 3.80 Hz, 6H), 1.24-1.36 (m, 2H), 1.48-1.57 (m, 1H), 3.19-3.31 (m, 2H), 3.71-3.76 (m, 2H), 4.19 (t, *J* = 5.97 Hz, 1H), 4.45-4.50 (m, 1H), 4.52-4.56 (m, 1H), 4.74 (t, *J* = 8.15 Hz, 1H), 4.86 (t, *J* = 9.50 Hz, 1H), 7.31 (t, *J* = 7.25 Hz,

2H), 7.39 (t, *J* = 6.95 Hz, 2H), 7.57 (d, *J* = 6.30 Hz, 2H), 7.75 (d, *J* = 7.55 Hz, 2H), $^{13}$C-NMR (125 MHz, $CDCl_3$) δ 156.7, 143.7, 141.4, 127.7, 127.0, 124.8, 119.9, 73.5, 66.3, 53.7, 52.4, 47.4, 24.6, 23.2, 21.5; LC-MS: m/z $[M+Na]^+$ = 417.189.

N-α-Fmoc-methylalanylazidoalcohol (**4c**):
Yield = 75%. $^1$H-NMR (300 MHz, $CDCl_3$) δ 1.18 (d, *J* = 5.91 Hz, 3H), 3.25-3.33(m, 2H), 3.65-3.79 (m, 2H), 4.24 (t, *J* = 6.24 Hz, 1H), 4.55 (d, *J* = 6.72 Hz, 1H), 5.00 (s, 1H), 7.33-7.47 (m, 4H), 7.62 (d, *J* = 7.41 Hz, 2H), 7.81 (d, *J* = 7.56 Hz, 2H); $^{13}$C-NMR (75 MHz, $CDCl_3$) δ 157.5, 142.6, 139.4, 137.4, 128.9, 127.3, 121.4, 120.0, 109.6, 72.0, 53.3, 48.9, 16.7**.**

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## 2.2 General procedure for the synthesis of C-terminal azide scaffolds **8 (a-c)**.

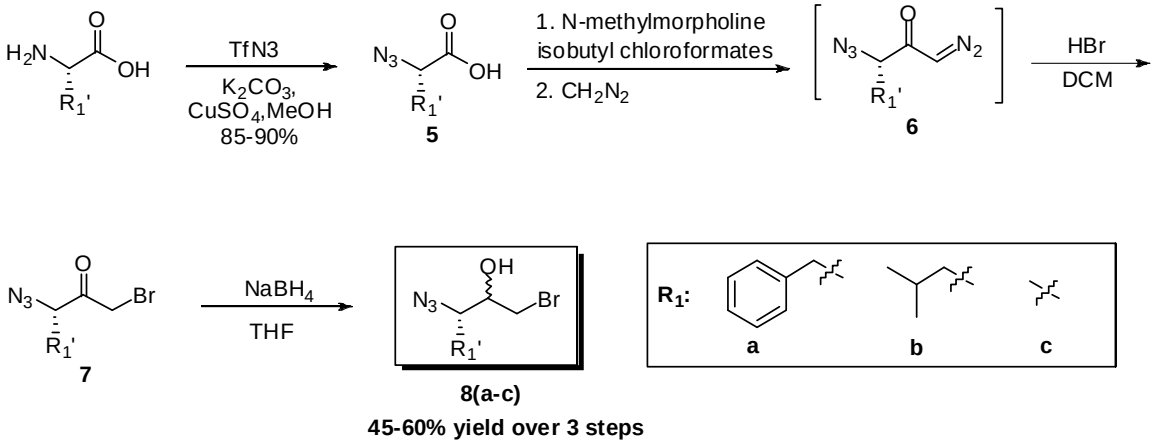



**Figure S2.** Synthesis of the C-terminal warhead scaffolds building block **8(a-c).**

2.2.1 General procedure for the synthesis of α-azido acid **5(a-c):**

The diazo transfer reaction was performed according to the reported procedure.[4] L-Amino acids (10 mmol, 1.0 eqv) was combined with $K_2CO_3$ (15 mmol, 1.5 eqv) and $CuSO_4$ pentahydrate (0.1 mmol, 0.01 eqv), distilled $H_2O$ (20 mL), and $CH_3OH$ (40 mL). Triflyl azide in $CH_2Cl_2$ (20 mmol, 2 equiv.) freshly prepared according to the reported procedure was added, and the resulting mixture was stirred at ambient temperature overnight. Subsequently, the organic solvents were removed under reduced pressure and the aqueous slurry was diluted with $H_2O$ (50 mL). This slurry was then acidified to pH 6 with 2 N HCl aqueous solution and diluted with 0.25 M, pH 6.2 phosphate buffer (50 mL) and extracted with EtOAc to remove the sulfonamide byproduct. The aqueous phase was then acidified to pH 2 with 2 N HCl aqueous solution. The product was obtained from another round of EtOAc extractions. The EtOAc extracts were combined, dried with anhydrous $Na_2SO_4$ and evaporated to dryness giving the α-azido acid 5**(a-c)** in 70-85% yield.

2-Azido-3-phenylpropanoic acid (**5a**)
Yield = 84%. $^1$H-NMR (300 MHz, $CDCl_3$) δ 3.04 (dd, *J* = 13.98, 8.88 Hz, 1H), 3.24 (dd, *J* = 13.98, 4.92 Hz, 1H), 4.13-4.18 (m, 1H), 7.25-7.33 (m, 5H); $^{13}$C-NMR (125 MHz, $CDCl_3$) δ 175.6, 135.6, 129.2, 128.7, 127.4, 63.1, 37.5; LC-MS: m/z $[M-H]^-$ =190.1.

2-azido-4-methylpentanoic acid (**5b**)
Yield = 73%. $^{1}$H-NMR (300 MHz, $CDCl_3$) δ 0.99 (d, *J* = 6.95 Hz, 6H), 1.68-1.78 (m, 2H), 1.80-1.85 (m, 1H), 3.86-3.89 (m, 1H), 10.77 (br s, 1H); LC-MS: m/z 2$[M]^+$ =313.0.

2-Azidopropanoic acid (**5c**)
Yield = 75%. $^{1}$H-NMR (500 MHz, $CDCl_3$) δ 1.54 (d, *J* = 6.95 Hz, 3H), 4.02-4.06 (m, 1H), 10.6 (br s, 1H); $^{13}$C-NMR (125 MHz, $CDCl_3$) δ 176.9, 57.0, 16.5.

2.2.2 General procedure for the synthesis of α-azido bromomethyl ketones **7(a-c)**:

Isobutyl chloroformate (9.6 mmol, 1.2 eqv) was added to a solution of α-azido acid **5(a-c)**(8.0 mmol, 1.0 eqv) and N-methylmorpholine (9.6 mmol, 1.2 eqv) in THF (20 mL) at -40 $^{o}$C, and the reaction mixture was stirred for 15 min. The reaction mixture was then filtered and diazomethane (4.0 eqv, freshly prepared from 1-methyl-3-nitro1-nitrosoguanidine and of 40 wt% aqueous KOH in EtOH) was added slowly into the filtrate. The reaction mixture was stirred for 2-3h at 0 $^{o}$C. After completion of the reaction, the solvent was evaporated *in vacuo* and the residue was dissolved in DCM followed by the treatment with 48% HBr aq (1.2 eqv) at 0 $^{o}$C. The reaction mixture was then stirred for 3 h. After completion of the reaction, it was diluted with 15 wt% aqueous citric acid and extracted with DCM. The organic layer was washed with saturated sodium bicarbonate and brine. Finally, it was dried over anhydrous sodium sulfate, filtered, concentrated and purified by silica-gel chromatography with 80:20 hexane/EtOAc afforded α-azido bromomethyl ketones **7(a-c)** in 60-93%.

3-azido-1-bromo-4-phenylbutan-2-one（**7a**）
Yield = 74%. $^{1}$H-NMR (300 MHz, $CDCl_3$) δ 2.99 (d, *J* = 8.55 Hz, 1H), 3.25 (d, *J* = 5.58 Hz, 1H), 3.86 (d, *J* = 4.59 Hz, 1H), 3.95 (d, *J* = 6.06 Hz, 1H), 4.35-4.40 (m, 1H), 7.25-7.36 (m, 5H); $^{13}$C-NMR (75 MHz, $CDCl_3$) δ 198.3, 135.3, 129.2-128.6(m), 127.5, 66.8, 37.5, 32.3.

3-azido-1-bromo-5-methylhexan-2-one (**7b**)
Yield = 65%. $^{1}$H-NMR (300 MHz, $CDCl_3$) δ 0.73-1.17 (m, 6H), 1.62 (t, *J* = 7.48 Hz, 2H), 1.80-1.88 (m, 1H), 3.38 (t, *J* = 5.91 Hz, 1H), 4.05 (d, *J* = 6.27 Hz, 1H), 4.19 (d, *J* = 7.68 Hz, 1H); $^{13}$C-NMR (75 MHz, $CDCl_3$) δ 198.9, 64.7, 40.6, 32.7, 25.4, 23.5, 22.7.

3-azido-1-bromo-5-methy-2-one (**7c**)
Yield = 60%. $^{1}$H-NMR (500 MHz, $CDCl_3$) δ 1.52 (d, *J* = 6.30 Hz, 3H), 4.08-4.11(m, 1H), 4.29-4.31(m, 1H); $^{13}$C-NMR (125 MHz, $CDCl_3$) δ 198.8, 61.0, 31.2, 15.8.

2.2.3 General procedure for the synthesis of α-azido bromomethyl alcohol **8(a-c)**:

To the solution of α-azido bromomethyl ketones **7(a-c)** (8 mmol, 1 eqv) in 20 mL of 95:5 THF/$H_2O$, sodium borohydride (12 mmol. 1.5 eqv) was added gradually at 0 ℃. The reaction mixture was stirred for 1 h and then was neutralized with aqueous 1N HCl. After extraction with EtOAc, the organic extracts were washed with brine, dried with $Na_2SO_4$, and concentrated under reduced pressure. Purification by silica-gel chromatography with 80:20 hexanes/EtOAc afforded compound **8(a-d)** as white solid.

α-Azido bromomethyl-Phe-alcohol (**8a**)

Yield = 75%. $^{1}$H-NMR (300 MHz, $CDCl_3$) δ 2.40 (d, *J* = 4.44 Hz, 1H), 2.80-3.18 (m, 2H), 3.48-3.52 (m, 1H), 3.65 (d, *J* = 6.09 Hz, 1H), 7.29-7.35 (m, 5H); $^{13}$C-NMR (75 MHz, $CDCl_3$) δ 136.9, 136.6-128.6 (m), 127.0, 126.9, 72.1, 65.9, 64.8, 37.0, 35.2. LC-MS: m/z $[M+Na]^+$=293.990.

α-Azido bromomethyl-Leu-alcohol (**8b**)

Yield = 90%. $^{1}$H-NMR (500 MHz, $CDCl_3$) δ 0.97 (d, *J* = 2.50 Hz, 6H), 1.38-1.49 (m, 1H), 1.60-1.69 (m, 1H), 1.73-1.84 (m, 1H), 2.53 (d, *J* = 5.65 Hz, 1H), 3.31-3.54 (m, 2H), 3.56-3.72 (m, 1H); $^{13}$C-NMR (125 MHz, $CDCl_3$) δ 73.4, 62.9, 39.8, 36.6, 25.3, 23.3, 21.8. LC-MS: m/z 2$[M]^+$=388.089.

α-Azido bromomethyl-Ala-alcohol (**8c**)

Yield = 80%. $^{1}$H-NMR (500 MHz, $CDCl_3$) δ 1.36 (d, *J* = 6.57 Hz, 3H), 2.37 (t, *J* = 5.43 Hz, 1H), 3.41-3.78 (m, 4H); $^{13}$C-NMR (125 MHz, $CDCl_3$) δ 73.7, 59.3, 36.5, 34.9, 15.6, 14.9. LC-MS: m/z 2[M]=388.089.

## 2.3 Synthesis of the biotin linkers (**9** & **14**):

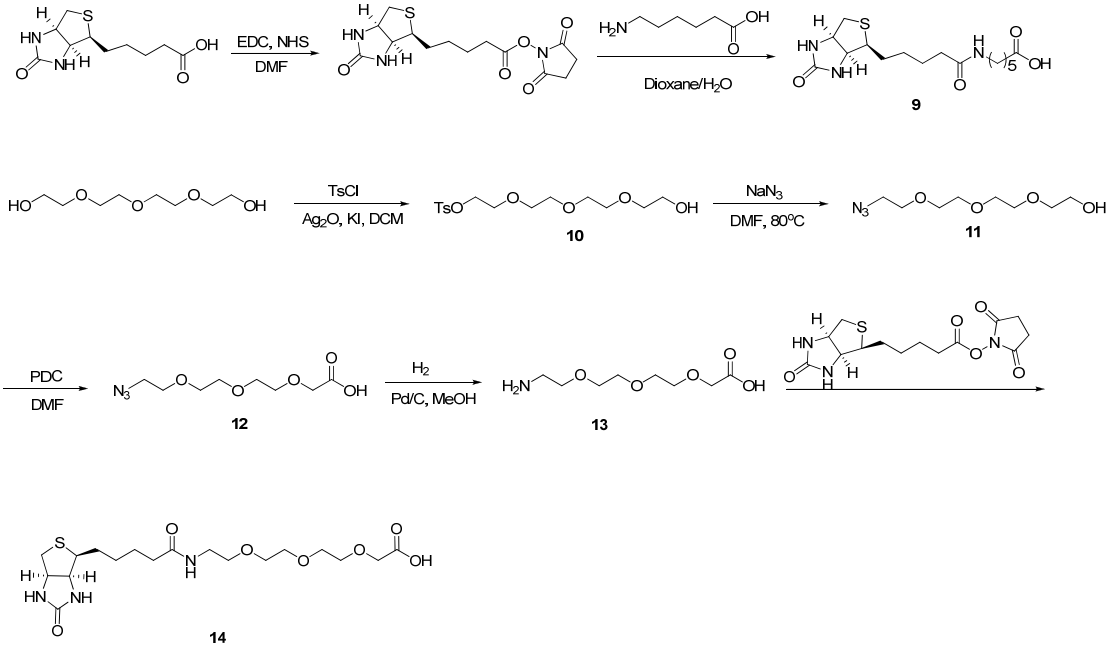



**Figure S3.** Synthesis of two biotin liners**.**

2.3.1. Biotin linker (**9**).

Compound **9** was synthesized according to previous report with some modification.[5] To a solution of D-biotin (12 g, 50 mmol) in DMF was added N-hydroxysuccinimide (7 g, 60 mmol) and EDC (11.5 g, 60 mmol). The reaction was allowed to proceed overnight. The resulting mixture was dried in vacuo to remove DMF. The gel-like residue was recrystallized from

EtOH/Acetic acid/$H_2O$ (95:1:4) to afford biotin-NHS as a white solid (14 g; 80% yield). To a solution of 6-aminohexanoic acid (1.3 g, 10 mmol) in 30 ml of 1:1 Dioxane/water co-solvent, the pH of the solution was adjusted to 8~8.5 by using a 4 M NaOH solution at 0℃. Biotin-NHS (3.4 g, 10 mmol) was subsequently added, and the reaction mixture was stirred at room temperature overnight. The resulting gelatinous solid formed was added ether, stirred for 5 minutes. The supernatant ether was decanted followed by addition of acetone and adjusting the pH of the resulting solution to 3 using 2 M HCl at 0 oC. Finally, the solid was filtered, washed several times with MeOH to afford the pure compound **9** as a white solid (2.85 g, 80%). $^1$H-NMR (300 MHz, $CDCl_3$) δ 1.23-1.62 (m, 12H), 2.04(t, *J* = 7.41 Hz, 2H), 2.19 (t, *J* = 7.23 Hz, 2H), 2.57 (d, *J* = 12.15 Hz, 1H), 2.82 (dd, *J* = 12.48, 4.92 Hz, 1H), 2.97-3.16 (m, 3H), 4.13 (t, *J* = 4.29 Hz, 1H), 4.30 (t, *J* = 7.38 Hz, 1H), 6.43 (s, 2H), 7.73 (t, *J* = 5.28 Hz, 1H); $^{13}$C-NMR (75 MHz, $CDCl_3$) δ 174.8, 172.2, 163.1, 61.4, 59.6, 55.8, 38.6, 35.6, 33.9, 29.2, 28.5, 26.3, 25.7, 24.6; LC-MS: m/z $[M+H]^+$=357.282.

2.3.2. Biotin linker (**14**).

Compound **14** was synthesized according to published procedures with some modifications,[5] as follows.

(**10**). To a stirred solution of diol (1 mmol) in $CH_2Cl_2$ (10mL) was added fresh $Ag_2O$ (0.35, 1.5mmol), TsCl (0.21g, 1.1mmol), and KI (O.033g, 0.2 mmol). The reaction mixture was stirred at room temperature for 8h, then filtered through a small pad of silica gel, and washed with EtOAc. Evaporation of the solvent, followed by column chromatography, gave the desired monotosylate product **10** (Yield = 70%). $^1$H-NMR (300 MHz, $CDCl_3$) δ 2.43 (s, 3H), 3.27(s, 1H), 3.57-3.68 (m, 14H), 4.15 (t, *J* = 4.44 Hz, 2H), 7.34 (d, *J* = 8.22 Hz, 2H), 7.78 (d, *J* = 8.04 Hz, 2H); $^{13}$C-NMR (75 MHz, $CDCl_3$) δ 144.4, 132.4, 129.4, 127.4, 72.0, 70.0-68.9(m), 68.1, 60.9, 21.1.

(**11**). To a solution of **10** (10 mmol) in DMF, $NaN_3$ (30 mmol) was added. The reaction was then stirred for 8h at 80 ℃. After completation of reaction, DMF was firstly removed under high vacuum, and the residue was purified by column chromatography to afford the desired product in 80% yield. $^1$H-NMR (300 MHz, $CDCl_3$) δ 3.39 (t, *J* = 5.10 Hz, 2H), 3.59 (t, *J* = 4.92 Hz, 2H), 3.67-3.71(m, 10H); $^{13}$C-NMR (75 MHz, $CDCl_3$) δ 72.4, 69.8-70.4(m), 61.3, 50.4.

(**12**). To a solution of **11** (10 mmol) in DMF, Pyridinium dichromate (PDC**)** (50 mmol) was added at 0 ℃. Then the reaction was stirred overnight at room temperature. After completation of reaction, a lot of brine was added to quench the reaction followed by EA extraction. The extrate was dried by $Na_2SO_4$, filtrated and evaporated under high vacuum. Finally the residue was purified by column chromatography to afford the desired product 12 in 55% yield. $^1$H-NMR (300 MHz, $CDCl_3$) δ 3.40 (t, *J* =4.65 Hz, 2H), 3.68-3.72 (m, 10H), 4.14 (s, 2H), 8.02 (s, 1H); $^{13}$C-NMR (75 MHz, $CDCl_3$) δ 172.1, 69.5-72.1(m), 68.0, 63.5, 50.2.

(**13** & **14**). To a solution of **12** (10 mmol) in MeOH, Pd/C (20%w) was added at room temperature. The reaction was then stirred overnight. After completation of reaction, Pd/C was filtrated by celite. The filtrate was evaporated and the residue was purified by column chromatography to afford compound **13** in 70% yield. To a solution of above PEG amino acid in

30 ml of 1:1 Dioxane/water co-solvent, the pH of the solution was adjusted to 8~8.5 by using a 4 M NaOH solution at 0 $^{o}$C. Biotin-NHS (3.4 g, 10 mmol) was subsequently added, and the reaction mixture was stirred at room temperature overnight. The resulting gelatinous solid formed was added ether, stirred for 5 minutes. The supernatant ether was decanted followed by addition of acetone and adjusting the pH of the resulting solution to 3 using 2 M HCl at 0 ℃. Finally, the solid was filtered, washed several times with MeOH to afford the pure compound **14** as a white solid in 85% yield. $^{1}$H-NMR (300 MHz, $CDCl_3$) δ 1.34-1.69 (m, 6H), 2.15 (t, *J* = 6.99 Hz, 2H), 2.56-2.61(m, 1H), 2.77-2.92 (m, 2H), 3.11-3.15 (m, 4H), 3.22-3.66 (m, 8H), 4.05 (s, 2H), 4.21-4.25 (m, 2H), 4.40-4.79 (m, 2H); $^{13}$C-NMR (75 MHz, $CDCl_3$) δ 176.2, 174.23, 167.7, 70.2-71.7(m), 69.2, 63.3, 61.6, 56.9, 41.0, 40.4, 36.7, 36.2, 33.8, 31.4, 29.5, 26.7, 25.6; LC-MS: m/z $[M+H]^{+}$=434.134.

## 2.4. Solid-phase synthesis of the 198-member small molecule library

The 198-member small molecule library was synthesized using DHP resin (GL Biochem, Shanghai, China). The synthesis of the N-terminal library (123 compounds in total) was carried out as shown in Figure S4. The synthesis of the C-terminal library (75 compounds in total) was carried out as shown in Figure S5.

### 2.4.1. General procedures for solid-phase synthesis of 123-member, N-terminal small molecule library.

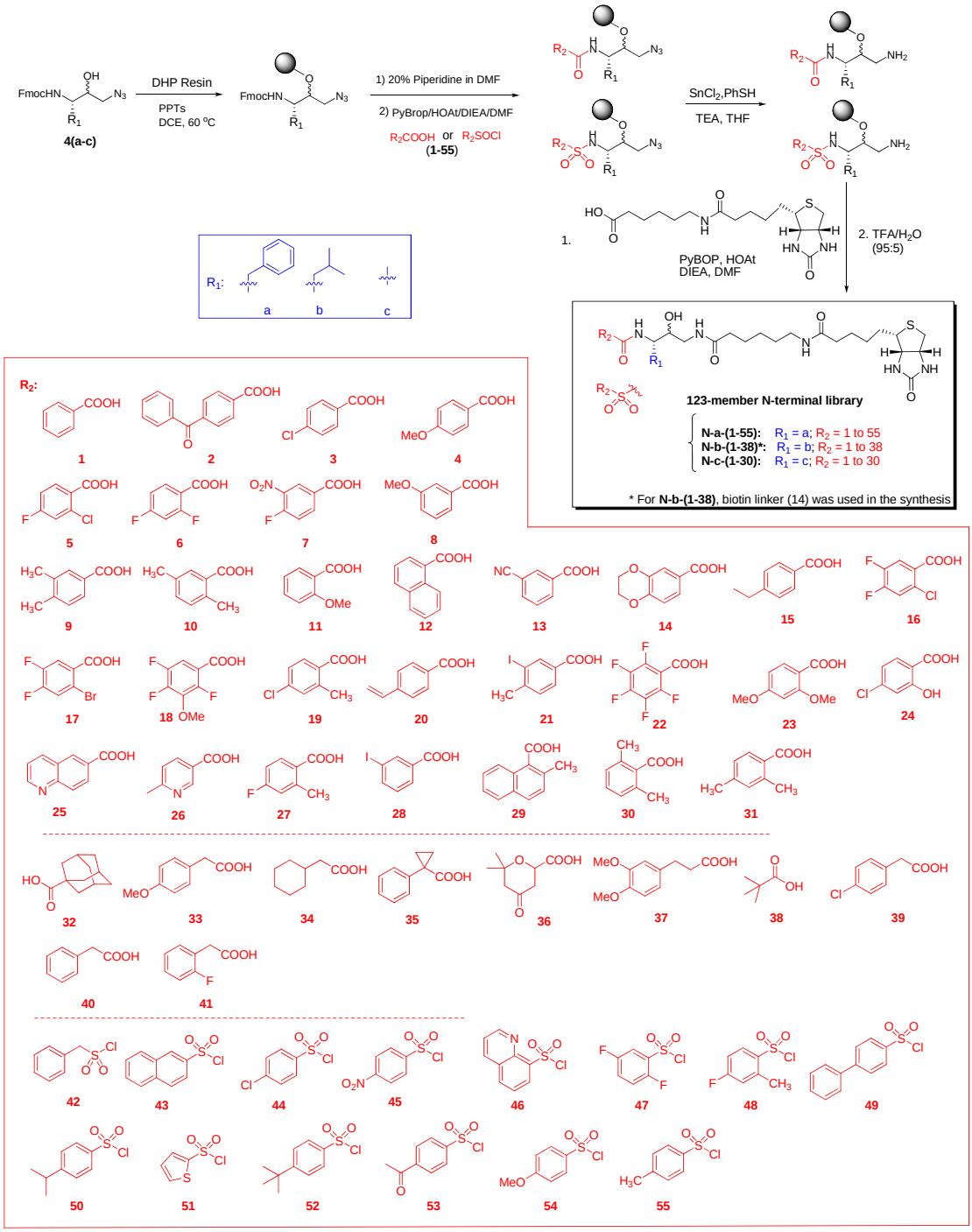



**Figure S4.** Solid-phase synthesis of N-terminal library in solid phase**.**

**Support-bound α-Azido alcohols (a-c)**. According to published procedures with some modifications,[4,6,7] HM DHP resin (40 mg) was swelled in DCE (5 mL) for 2 h. The solvent was then removed, followed by the addition of α-Azido alcohols **4(a-c)** (4.0 eq) in DCE (10 mL) and PPTs (Pyridinium p-toluenesulfonate) (1.5 eq) at room temperature. Then the reaction mixture was strirred for 12 h at 60 $^{o}$C. The resulting resin was washed with NMP (3×), THF (3×), $CH_2Cl_2$ (3×) and $Et_2O$ (3×) and dried *in vacuo*, then stored at – 20 $^{o}$C.

**Deprotection of Fmoc group.** To a 50 ml plastic tube was added a solution of 20% piperidine in NMP and the resulting resin. The mixture was shaken for 2 h. The solvent was then removed and washed with NMP (3×), THF (3×), $CH_2Cl_2$ (3×) and $Et_2O$ (3×) and dried *in vacuo*. The completeness of the reaction was monitored by ninhydrin test. Blue beads indicate the presence of primary amine and the completeness of the reaction.

**Coupling of different acids or sulfonyl chlorides.** The resulting resin firstly was swelled in THF for 2 h. The solvent was then removed. To the resin was added a preactived solution containing the corresponding acid (4.0 eq), PyBOP (4.0 eq), HOAt (4.0 eq) and DIPEA (8.0 eq) in THF or a solution of the corresponding sulfonyl chloride (4.0 eq) and DIEA (8.0 eq) in dry THF. The reaction mixture was shaken overnight at room temperature and the resin was washed with NMP (3×), THF (3×), $CH_2Cl_2$ (3×) and $Et_2O$ (3×) and dried under vacuum. The completeness of the reaction was also monitored by ninhydrin test. Negative test indicate the absence of primary amine and the completeness of the reaction.

**Reduction of azido group to primary amine.** Reduction of the azide was accomplished using 0.2 M $SnCl_2$, 0.8 M PhSH, and 1.0 M $Et_3N$ in THF (1 mL) for 4 h. The resin was then washed with 50 vol% aqueous THF solution (3×), THF (3×), $CH_2Cl_2$ (3×) and ether (3×), and then dried *in vacuo*.

**Acylation of the primary amine with the biotin linker (9 or 14).** The resulting support-bound amine was then acylated in a preactived solution of biotin acid **9** (4.0 eq), PyBOP (4.0 eq), HOAt (4.0 eq) and DIPEA (8.0 eq) in THF. The reaction mixture was shaken overnight at room temperature and the resin was washed with NMP (3×), THF (3×), $CH_2Cl_2$ (3×) and $Et_2O$ (3×) and dried under vacuum. The completeness of the reaction was monitored by ninhydrin test. ***For the 33 compounds **N-b-(1-33)**, biotin linker 14 was used instead in the synthesis.****

**Cleavage of small molecules from the solid support.** Dried resin from above reaction was treated with a 3 ml solution containing TFA (95%) and $H_2O$ (5%) and the mixture was shaken for 20 min at r.t followed by filtration. The resin was rinsed with DCM (2×). The combined filtrates were concentrated to dryness to afford the 123-member N-terminal library as shown in Figure S4. The resulting compounds were then directly checked by LC-MS

2.4.2. General procedure for solid-phase synthesis of the 75-member, C-terminal library.

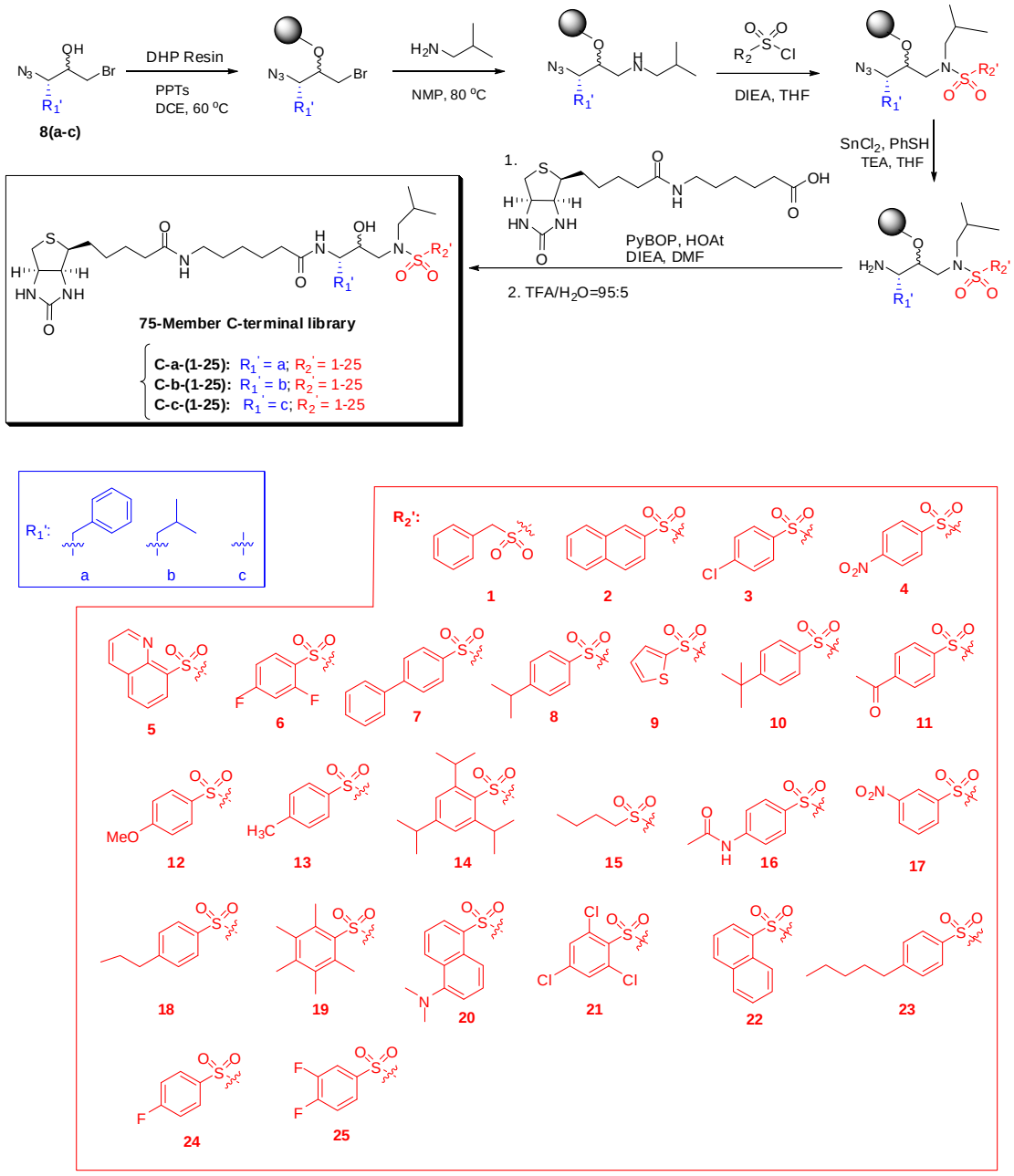



**Figure S5.** Solid-phase synthesis of the 75-member, C-terminal library**.**

**Support-bound bromomethyl alcohol (a-c).** HM DHP resin (40 mg) was swelled in DCE (5 mL) for 2 h. The solvent was then removed followed by the addition of bromomethyl alcohols **8(a-c)** (4.0 eq) in DCE (10 mL) and PPTs (Pyridinium p-toluenesulfonate) (1.5 eq) at room temperature. The reaction mixture was strirred for 12 h at 60 °C. The resulting resin was washed with NMP (3×), THF (3×), $CH_2Cl_2$ (3×) and $Et_2O$ (3×) and dried *in vacuo*, then stored at -20 °C.

**Synthesis of C-terminal library.** Support-bound bromomethyl alcohol was added to a vial followed by addition of a solution of *i*-butylamine (10 eqv) in NMP (10 mL). The vial was then

sealed and the reaction mixture was heated at 80 °C for 36 h. The resin was then washed with NMP (3×), THF (3×), $CH_2Cl_2$ (3×), and ether (3×), and then dried *in vacuo*. Acylation with the sulfonyl chloride (4.0 eqv) and DIEA (8.0 eqv) in dry THF was carried out overnight. The resin was then washed with NMP (3×), THF (3×), $CH_2Cl_2$ (3×), and ether (3×), and then dried *under vacuum*. The resulting resin was treated with 95:5 TFA/$H_2O$ for 20 min followed by filtration. The resin was then rinsed with 95:5 TFA/$H_2O$ and $CH_2Cl_2$. The combined filtrates were concentrated to dryness to afford the 75-member C-terminal library as shown in Figure S5. The resulting compounds were then directly checked by LC-MS.

## 2.5. "Click" assembly of A*f*BPs targeting γ-secretase.

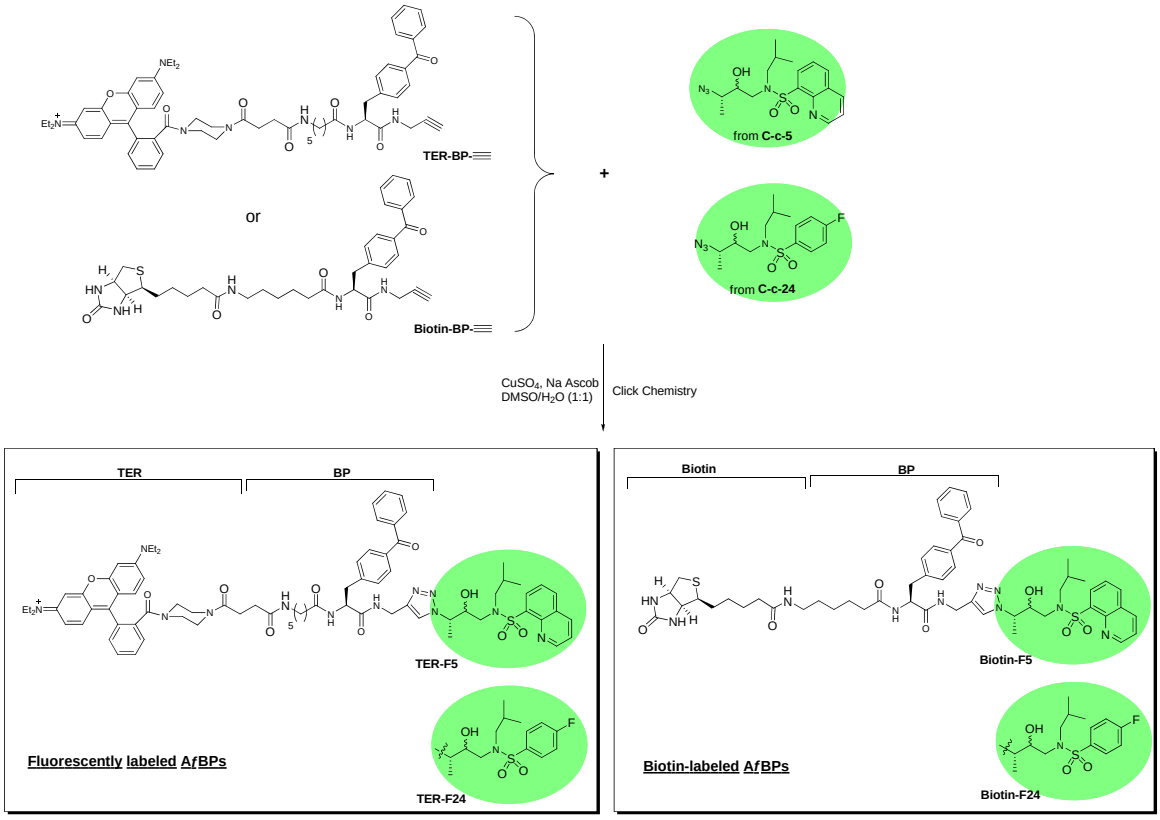



**Figure S6.** "Click" assembly of the fluorescently labeled and biotin-containing affinity-based probes (A*f*BPs).

Synthesis of the above 4 probes by "Click chemistry" followed previously published procedures with minor modifications as indicated below.[8] The TER-BP-alkyne and Biotin-BP-alkyne linkers were obtained as previously described.[8] The alkyne (1.2 eq) and the azide (1.0 eq; final concentration: 10.0 mM) were dissolved in a minimal amount of DMSO. A mixture of DMSO/$H_2O$ solution (1:1; 2 mL) was subsequently added and the reaction was shaken for a few minutes to obtain a clear solution. The "click chemistry" was initiated by sequential addition of catalytic amounts of sodium ascorbate (0.4 eq) and $CuSO_4$ (0.1 eq). The reaction was continued with shaking at room temperature for another 12 h. The reaction product was then directly

analyzed by LC-MS; results indicated the complete consumption of the azide and quantitative formation of the triazole final product in all cases. The final probes were subsequently purified by prep-HPLC and characterized/confirmed by NMR and LC-MS (the amount of the TER probes obtained was too small to be characterized by NMR). A negative control probe, **TER-A-31**, was also synthesized and characterized similarly.

**Biotin-F5** $^{1}$H-NMR (300 MHz, DMSO-d6) δ 0.70 (d, *J* = 5.85 Hz, 6H), 0.78 (d, *J* = 5.55 Hz, 2H), 0.90-1.04 (m, 3H), 1.23-1.55 (m, 10H), 1.97-2.03 (m, 6H), 2.27 (s, 2H), 2.66-3.00 (m, 10H), 3. 95-4.10 (m, 1H), 4.25-4.27 (m, 2H), 4.55-4.71 (m, 3H), 5.55(s, 1H), 6.50 (s, 1H), 6.79 (s, 1H), 7.37 (d, *J* = 8.19 Hz, 3H), 7.52 (d, *J* = 6.99 Hz, 2H), 7.50-7.66 (m, 6H), 7.75-7.85 (m, 4H), 8.06 (s, 2H), 8.54 (s, 2H); LC-MS: m/z $[M+H]^{+}$=1023.150.

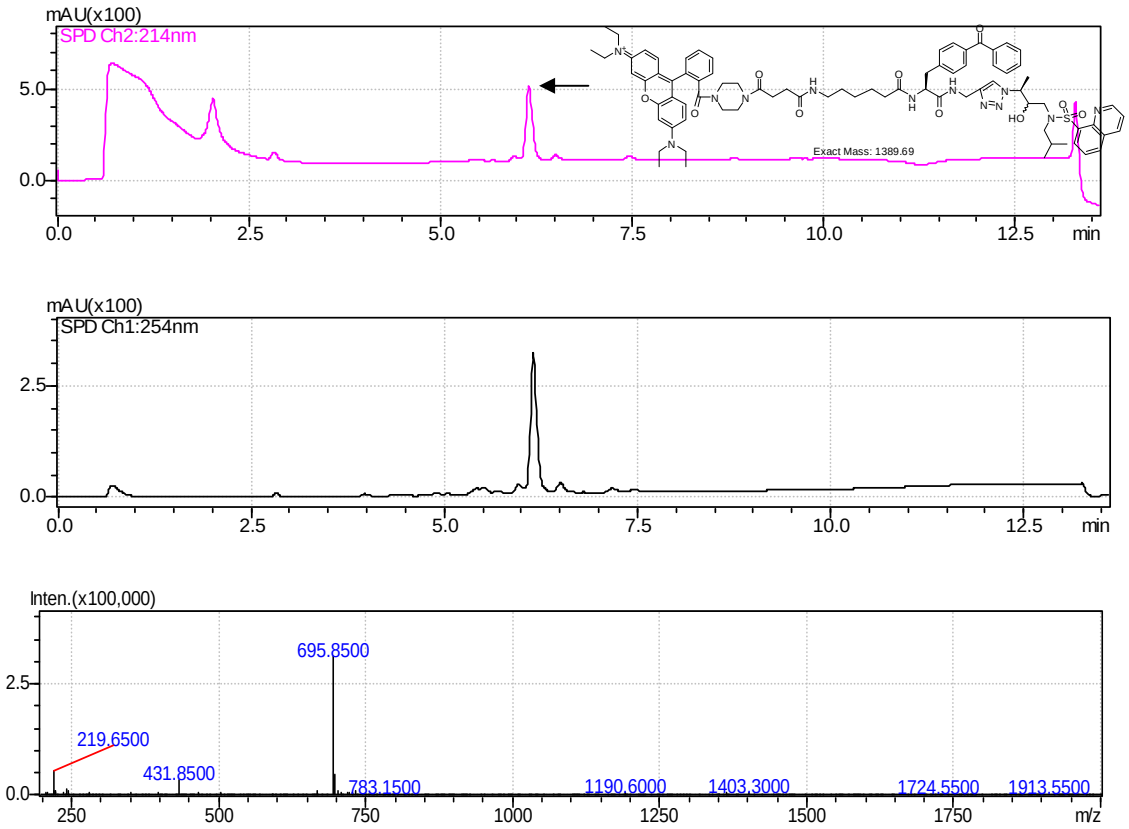
**Biotin-F24** $^{1}$H-NMR (300 MHz, DMSO-d6) δ 0.72 (d, *J* = 5.85 Hz, 6H), 0.78 (d, *J* = 6.15 Hz, 2H), 1.13-1.41(m, 16H), 2.00 (t, *J* = 6.99 Hz, 4H), 2.66-2.78 (m, 6H), 2.85-3.15 (m, 24H), 2.29 (s, 2H), 5. 71 (s, 2H), 6.91 (s, 2H), 7.08 (s, 2H), 7.37 (s, 2H), 7.50-7.65 (m, 3H), 7.66-7.75 (m, 4H), 7.81 (s, 1H); LC-MS: m/z $[M+H]^{+}$= 990.150.

## LC-MS characterizations of the probes:

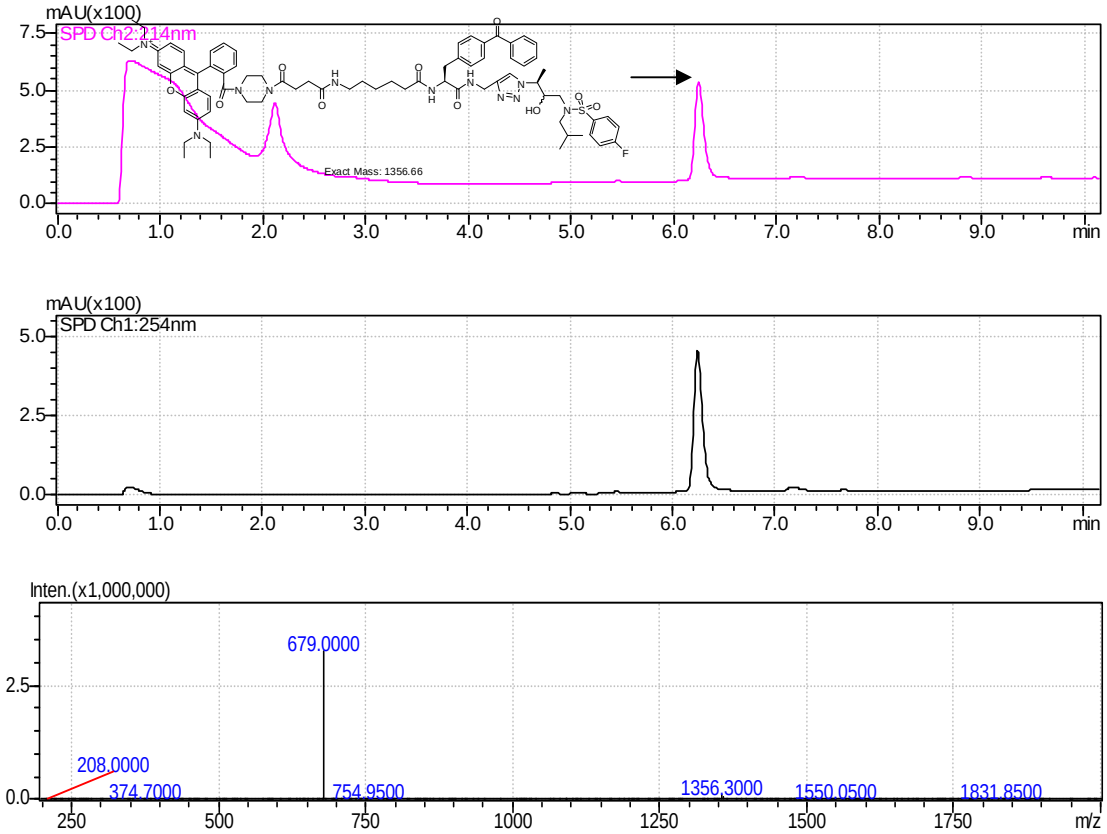
HPLC conditions: 0-100% B for 10 mins, then 100% B for 2 mins.

(Solvent A: 100% $H_2O$ with 0.1 % TFA; Solvent B: 100% $CH_3CN$ with 0.1 % TFA)

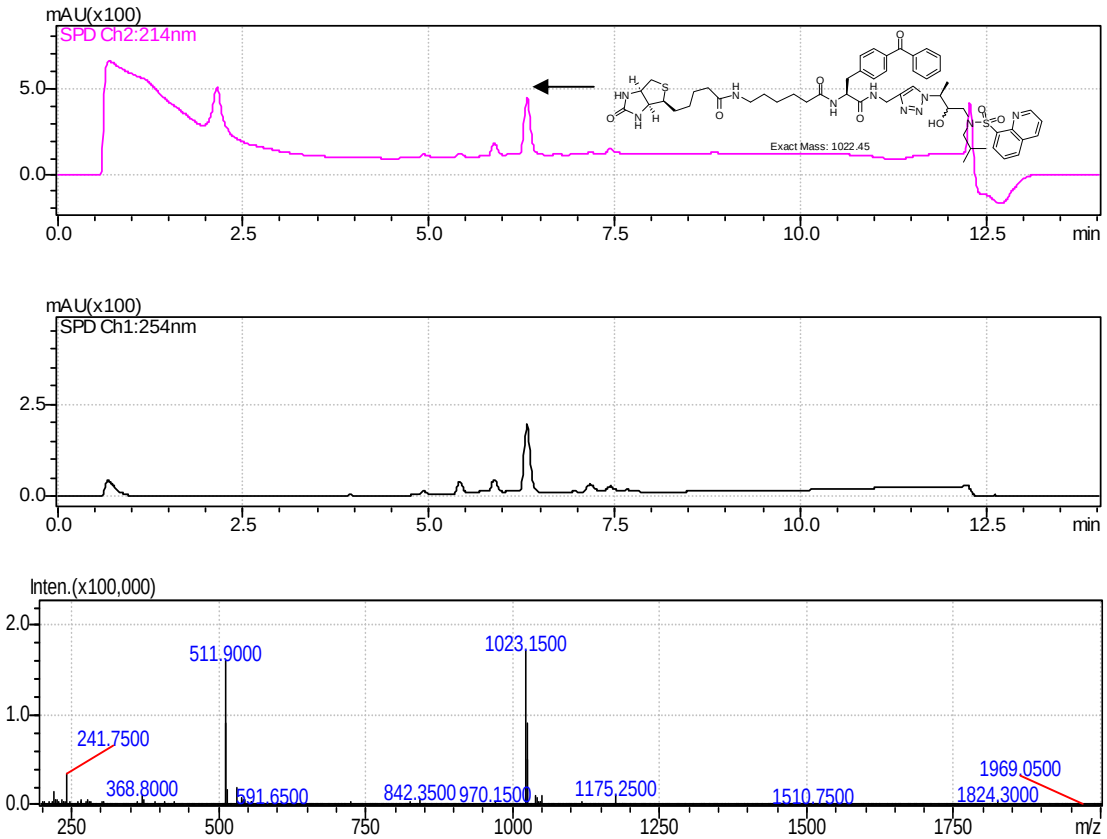
**TER-F5**



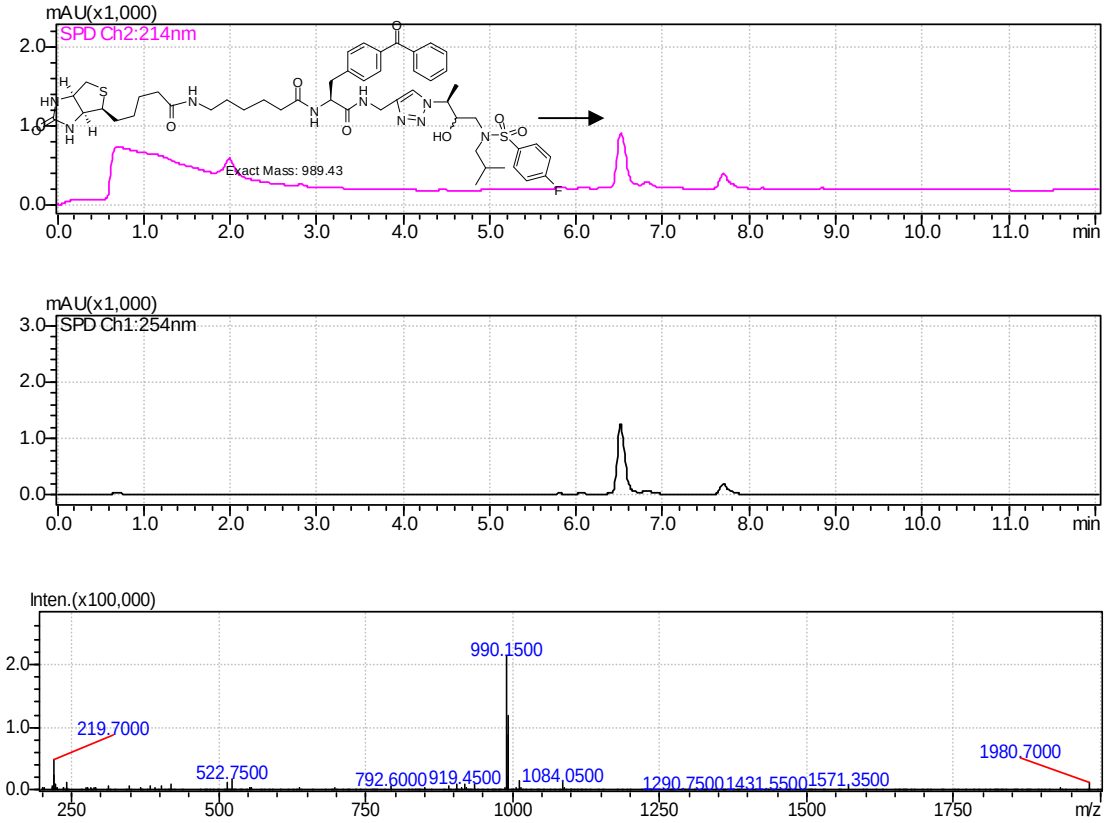
**TER-F24**




**Biotin-F5**



**Biotin-F24**


mAU(x1,000)
SPD Ch2:214nm
Exact Mass: 989.43
mAU(x1,000)
SPD Ch1:254nm
Inten.(x100,000)
990.1500
219.7000
522.7500
792.6000
919.4500
1084.0500
1290.7500
1431.5500
1571.3500
1980.7000
m/z

## 3. Screening and Biology Experiments

### 3.1. Preparation of avidin slides

25 mm × 75 mm glass slides (Sigma-Aldrich) were cleaned in piranha solution (sulfuric acid/hydrogen peroxide, 7:3). An amine functionality was incorporated onto the slides by silanization using a solution of 3% (aminopropyl)triethoxysilane in 2% water and 95% ethanol. After 1-2 h incubation, the slides were washed with ethanol and cured at 150 ºC for at least 2 h. The resulting amine slides were incubated in a solution of 180 mM succinic anhydride in DMF for 30 min before being transferred to a boiling water bath for 2 min. The slides were rinsed with ethanol and dried under a stream of nitrogen. The carboxylic acid derivatized slide surface was activated with a solution of 100 mM of HBTU (O-Benzotriazole-N, N, N', N'-tetramethyl-uronium-hexafluoro-phosphate), 200 mM DIEA, and 100 mM N-hydroxysuccinimide in DMF, thus generating the NHS-derivatized slides. These surfaces were reacted with a solution of 1 mg/mL avidin in 10 mM $NaHCO_3$ (pH 9) for 40 min, washed with water, air dried. The unreacted NHS groups were quenched with a solution of 2 mM aspartic acid in a 0.5 M $NaHCO_3$ buffer, pH 9. These slides were washed with water, dried and stored at 4 ºC, ready for printing.

### 3.2 Microarray preparation

All compounds stocks were prepared in a 1:1 DMSO/PBS spotting solution (to 5 mM final concentration), and were distributed in 384-well plates. All compounds were shown to be completely soluble in this spotting solution. One slide was spotted on an ESI SMA arrayer (Ontario, Canada) with the print head installed with 4 Stealth SMP8B Microspotting pins (Telechem, U.S.A.). Spots generated were of approximately 350 μm diameter and were printed with a spot-spot spacing of 450 μm. The pins were rinsed in between samples using two cycles of wash (for 10 s) and sonication (for 10 s) in reservoirs containing 70 % ethanol followed by drying under reduced pressure (for 10 s). The slides were allowed to stand for 1 h on the printer platform and stored at 4 ºC until use. Before incubation with the labeled proteins or lysates, the slides were rinsed with PBS (pH 7.4) for 10 min and blocked with PBS-containing 1 % BSA for 1 h. For studies with the 198-member library, all compounds were spotted on the same slide in duplicate.

### 3.3 Cell culture

γ-30 cell line was cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and 1% penicillin/streptomycin/puromycin/zeocine/geneticin/hygromycin). Cells were incubated at 37 °C in a humidified atmosphere containing 5% $CO_2$. Subsequently, the cells were harvested with mammalian cell lysis buffer (50 mM HEPES, pH7.4, 1 mM EDTA, 100 mM NaCl, 10% Glycerol). Protein concentration was determined using the Bradford protein assay (Bio-Rad). Equal proteins were loaded and separated on 12% SDS-PAGE gels and transferred to PVDF membrane. The Western blots were done using anti-PS antibodies.

### 3.4 Preparation of Membrane Fractions from γ-30 cell lysates

The cellular lysates were centrifuged at 800×*g* for 10 min to remove nuclei and cell debris. The postnuclear supernatant was further fractionated by sequential centrifugation at 100000×g for

1 h .The collected pellet were resuspended in HEPES buffer with 1% CHAPSO followed by centrifugation at 100000×g for further 1 h. The supernant was collected with addition of 20% glycerol and stored at -80 ℃ until use. All centrifugation steps were carried out at 4 ℃.

### 3.5 Protein labeling and screening on the small molecule microarray

Protein or lysate samples were minimally labeled with either Cy3 or Cy5 N-hydroxysuccinimide ester (Amersham, GE Healthcare, USA) for 1 h on ice, following the manufactor's protocols and our previously published procedures.[2] The unreacted dye was quenched with a 10-fold molar excess of hydroxylamine for a further 1 h. The excess dye was further removed by extensive dialysis at 4 °C overnight (Amersham, GE Healthcare, USA), or Microcon spin-column. After analysis by SDS-PAGE gel to ensure successful labeling and purity, the labeled proteins or lysates were reconstituted in a final corresponding buffer. In a standard microarray experiment, the labeled protein (1 μM; 30 μL) or lysates (2 μg, 30 μL) was applied to the array. For denaturing experiments, the labeled protein was firsted boiled for 10 min, cooled before being applied to the small molecule microarray. For dual-color screening experiments, an equal amount of a Cy3-labeled protein and a Cy5-labeled protein were mixed and applied together to the slide. The samples were incubated with the array in a humidified chamber for 1 h at room temperature, before repeated rinses with PBS + 0.05% Tween 20, typically 3 x 10 min washes with gentle shaking. Slides were scanned using an ArrayWoRx microarray scanner installed with the relevant filters (Cy3: λex/em = 548/595 nm; Cy5: λex/em = 633/685 nm).

## 4. Results and Discussion

(a)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Cc-14 | Cc-16 | CC-18 | Cc-23 | Cc-25 | Cc-15 | Cc-17 | Cc-19 | Cc-24 | | |
| Cc-4 | Cc-6 | Cc-8 | Cc-10 | Cc-12 | Cc-5 | Cc-7 | Cc-9 | Cc-11 | Cc-13 | |
| Ca-20 | Cb-21 | Cb-23 | Cb-25 | Cc-2 | Ca-21 | Cb-22 | Cb-24 | Cc-1 | Cc-3 | |
| Ca-10 | Ca-12 | Ca-14 | Ca-16 | Ca-18 | Ca-11 | Ca-13 | Ca-15 | Ca-17 | Ca-19 | |
| Nc-44 | Ca-2 | Ca-4 | Ca-6 | Ca-8 | Ca-1 | Ca-3 | Ca-5 | Ca-7 | Ca-9 | |
| Nb-50 | Nb-52 | Nb-31 | Nc-2 | Nc-42 | Nb-51 | Nb-53 | Nc-1 | Nc-3 | Nc-43 | |
| Nb-37 | Nb-40 | Nb-42 | Nb-44 | Nb-47 | Nb-38 | Nb-55 | Nb-43 | Nb-46 | Nb-49 | |
| Na-45 | Na-47 | Nb-20 | Nb-22 | Nb-33 | Na-46 | Na-48 | Nb-21 | Nb-32 | Nb-34 | |
| Na-35 | Na-37 | Na-39 | Na-41 | Na-43 | Na-36 | Na-38 | Na-40 | Na-42 | Na-44 | |
| Na-25 | Na-27 | Na-29 | Na-31 | Na-33 | Na-26 | Na-28 | Na-30 | Na-32 | Na-34 | |
| Cb-15 | Cb-17 | Cb-19 | Cc-20 | Cc-22 | Cb-16 | Cb-18 | Cb-20 | Cc-21 | | |
| Cb-5 | Cb-7 | Cb-9 | Cb-11 | Cb-13 | Cb-6 | Cb-8 | Cb-10 | Cb-12 | Cb-14 | |
| Nc-40 | Ca-22 | Ca-24 | Cb-1 | Cb-3 | Nc-55 | Ca-23 | Ca-25 | Cb-2 | Cb-4 | |
| Nc-18 | Nc-18 | Nc-22 | Nc-33 | Nc-37 | Nc-19 | Nc-21 | Nc-32 | Nc-34 | Nc-38 | |
| Nc-6 | Nc-10 | Nc-12 | Nc-14 | Nc-16 | Nc-8 | Nc-11 | Nc-13 | Nc-15 | Nc-17 | |
| Nb-12 | Nb-14 | Nb-16 | Nb-16 | Nc-4 | Nb-13 | Nb-15 | Nb17 | Nb-19 | Nc-5 | |
| Na-55 | Nb-2 | Nb-4 | Nb-6 | Nb-10 | Nb-1 | Nb-3 | Nb-5 | Nb-8 | Nb-11 | |
| Na-21 | Na-23 | Na-49 | Na-51 | Na-53 | Na-22 | Na-24 | Na-50 | Na-52 | Na-54 | |
| Na-11 | Na-13 | Na-15 | Na-17 | Na-19 | Na-12 | Na-14 | Na-16 | Na-18 | Na-20 | |
| Na-1 | Na-3 | Na-5 | Na-7 | Na-9 | Na-2 | Na-4 | Na-6 | Na-8 | Na-10 | |

(b)

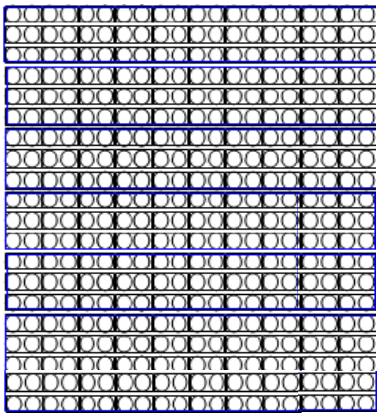


**Figure S6.** (a) The arrangement of 198-member library on microarrary; (b) The spotting format (duplicate) of all the microarrays used in this study, unless otherwise specified.

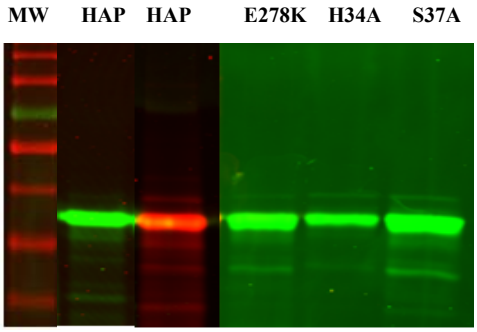



**Figure S7**. Fluorescence gel images of the dye-labeled HAP and its mutants used in this study. Cy3-and Cy-5 labeled proteins were false-colored in Green and Red, respectively.

## 4.1. Fingerprint of HAP and its mutants

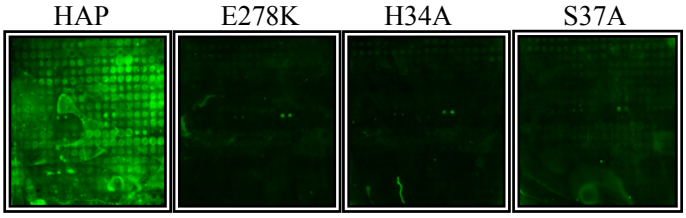



**Figure S8.** Small molecules microarray immobilized with the 198-member library was screened against fluorescently labeled HAP and its active-site mutants. Results indicated the ability of HAP binding to SMM was lost after mutation.

## 4.2. Characterization of A*f*BPs with γ-30 cell lysates

Based on the microarray screening results, two stronger binders, **C-c-5** and **C-c-24**, were identified and their corresponding A*f*BPs (both TER and Biotin versions) were synthesized using "Click Chemistry" as shown in Figure S6. A negative control probe **TER-A31** (from the spot **N-a-31** from the SMM screening which showed no binding and was deemed negative) was also synthesized. Subsequently, in-gel fluorescence labeling and pull-down experiments were carried out with these probes against mammalian cell lysates prepared from the γ-30 cell line. Firstly, the three TER-containing probes, **TER-F5**, **TER-F24** and **TER-A-31** (negative control), were used to label γ-30 cell lysates, Details of the cell lysate labeling experiment are described below: a desired lysate amount (15 μg of total proteins as determined by Bradford assay) was incubated with the probes (5 μM final concentration; 5 % DMSO) in HEPES buffer for 30 min at RT. After 30 min incubation, samples were irradiated on ice for 25 min using a B100A lamp (UVP) at a distance of 5 cm. After irradiation, samples were boiled for 10 min with 4 μL of 6 x SDS loading buffer, resolved on a 12% SDS-PAGE followed by in-gel fluorescence scanning with a Typhoon 9200 gel scanner (Fig S10(a)). Western blotting was carried out with anti-PS1 (Santa Cruz Biotechnology).

To further validate the labeling of PS-NTF by the two probes, pull-down from labeled lysates experiments were carried out as described below using the biotin version of the probe: 2 mg of lysate were labeled by 5 μM probe **F24** in a 2-ml reaction using above described conditions. After labeling, the lysates in the reaction were acetone precipitated and resolubilized in 0.1% SDS in PBS with brief sonication. This resuspended sample was then incubated with avidin-agarose beads

(100 μL/mg protein; Pierce) at RT for 30 min. After centrifugation, supernatant were removed and the beads were washed with 1% SDS in PBS for 4 times. After washing, the beads were boiled in elution buffer (200 mM Tris pH 6.8, 400 mM DTT, 8% SDS). This pull-down sample from labeled lysates was then separated on 12% SDS PAGE gel together with pull down sample from un-labeled lysates. After SDS-PAGE gels separation, proteins were then transferred to a PVDF membrane and subsequently blocked with 2.5% (w/v) BSA/PBST. Membranes were incubated for 1 h at RT with neutravidin conjugated HRP (1:10000) or anti-PS-NTF (1/5000; Cat# C1245). After wash with PBST for three times, blots were further incubated with appropriate secondary antibody for 1 hr at RT. After incubation, blot was washed again with PBST for 3 times and the SuperSignal West Pico kit (Pierce) was used to develop the blot. The results are shown in the maintext (Figure 2).

## 5. Summary of Characterizations of the 198-member Library

Table S1. The 123-member, N-terminal Library

| # | ID | Structure (Figure S4) | | LCMS | | |
|---|---|---|---|---|---|---|
| | | R1 (AA) | R2 (acid) | % purity | Cal MW | Obs MW |
| 1 | **N-a-1** | Phe | 1 | >90 | 623.31 | 624.238 |
| 2 | **N-a-2** | Phe | 2 | >90 | 727.34 | 728.246 |
| 3 | **N-a-3** | Phe | 3 | ~85 | 657.28 | 658.191 |
| 4 | **N-a-4** | Phe | 4 | >90 | 653.32 | 654.238 |
| 5 | **N-a-5** | Phe | 5 | ~80 | 675.27 | 676.179 |
| 6 | **N-a-6** | Phe | 6 | ~85 | 659.30 | 660.209 |
| 7 | **N-a-7** | Phe | 7 | ~50 | 686.29 | 688.193 |
| 8 | **N-a-8** | Phe | 8 | >90 | 653.32 | 654.238 |
| 9 | **N-a-9** | Phe | 9 | ~70 | 651.36 | 652.258 |
| 10 | **N-a-10** | Phe | 10 | >90 | 651.35 | 652.261 |
| 11 | **N-a-11** | Phe | 11 | ~85 | 653.32 | 654.247 |
| 12 | **N-a-12** | Phe | 12 | >90 | 673.33 | 674.241 |
| 13 | **N-a-13** | Phe | 13 | >90 | 648.31 | 649.226 |
| 14 | **N-a-14** | Phe | 14 | >90 | 681.32 | 682.225 |
| 15 | **N-a-15** | Phe | 15 | >90 | 651.35 | 652.261 |
| 16 | **N-a-16** | Phe | 16 | ~80 | 693.26 | 694.167 |
| 17 | **N-a-17** | Phe | 17 | >90 | 737.21 | 740.110 |
| 18 | **N-a-18** | Phe | 18 | ~80 | 707.30 | 708.203 |
| 19 | **N-a-19** | Phe | 19 | >90 | 671.29 | 672.200 |
| 20 | **N-a-20** | Phe | 20 | ~85 | 649.33 | 650.243 |
| 21 | **N-a-21** | Phe | 21 | >90 | 763.23 | 764.119 |
| 22 | **N-a-22** | Phe | 22 | - | 713.27 | - |
| 23 | **N-a-23** | Phe | 23 | ~80 | 683.34 | 684.249 |
| 24 | **N-a-24** | Phe | 24 | >90 | 673.27 | 674.244 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 25 | **N-a-25** | Phe | 25 | ~90 | 674.33 | 674.243 |
| 26 | **N-a-26** | Phe | 26 | ~50 | 638.33 | 639.244 |
| 27 | **N-a-27** | Phe | 27 | ~80 | 655.32 | 656.238 |
| 28 | **N-a-28** | Phe | 28 | ~80 | 749.21 | 750.108 |
| 29 | **N-a-29** | Phe | 29 | ~85 | 687.35 | 688.259 |
| 30 | **N-a-30** | Phe | 30 | ~70 | 651.35 | 652.261 |
| 31 | **N-a-31** | Phe | 31 | ~80 | 651.35 | 652.257 |
| 32 | **N-a-32** | Phe | 32 | ~50 | 681.39 | 682.299 |
| 33 | **N-a-33** | Phe | 33 | >90 | 667.34 | 668.255 |
| 34 | **N-a-34** | Phe | 34 | ~60 | 643.38 | 644.291 |
| 35 | **N-a-35** | Phe | 35 | ~50 | 663.35 | 664.260 |
| 36 | **N-a-36** | Phe | 36 | ~50 | 673.35 | 672.244 |
| 37 | **N-a-37** | Phe | 37 | >90 | 711.37 | 712.272 |
| 38 | **N-a-38** | Phe | 38 | ~70 | 603.35 | 604.270 |
| 39 | **N-a-39** | Phe | 39 | ~70 | 671.29 | 673.223 |
| 40 | **N-a-40** | Phe | 40 | - | 637.33 | - |
| 41 | **N-a-41** | Phe | 41 | ~85 | 655.32 | 656.238 |
| 42 | **N-a-42** | Phe | 42 | ~85 | 673.30 | 675.204 |
| 43 | **N-a-43** | Phe | 43 | ~80 | 709.30 | 710.203 |
| 44 | **N-a-44** | Phe | 44 | ~80 | 693.24 | 694.15 |
| 45 | **N-a-45** | Phe | 45 | - | 704.27 | - |
| 46 | **N-a-46** | Phe | 46 | ~85 | 710.29 | 711.198 |
| 47 | **N-a-47** | Phe | 47 | ~80 | 695.26 | 696.165 |
| 48 | **N-a-48** | Phe | 48 | ~60 | 691.29 | 692.196 |
| 49 | **N-a-49** | Phe | 49 | >90 | 735.31 | 736.216 |
| 50 | **N-a-50** | Phe | 50 | >90 | 701.33 | 702.242 |
| 51 | **N-a-51** | Phe | 51 | >90 | 665.24 | 666.150 |
| 52 | **N-a-52** | Phe | 52 | ~70 | 715.34 | 716.245 |
| 53 | **N-a-53** | Phe | 53 | >90 | 701.29 | 702.199 |
| 54 | **N-a-54** | Phe | 54 | >90 | 689.29 | 690.204 |
| 55 | **N-a-55** | Phe | 55 | >90 | 673.30 | 674.211 |
| 56 | **N-b-1** | Leu | 1 | ~80 | 665.35 | 666.258 |
| 57 | **N-b-2** | Leu | 2 | ~70 | 769.37 | 770.262 |
| 58 | **N-b-3** | Leu | 3 | ~70 | 699.31 | 700.208 |
| 59 | **N-b-4** | Leu | 4 | ~80 | 695.36 | 696.259 |
| 60 | **N-b-5** | Leu | 5 | ~70 | 717.30 | 718.206 |
| 61 | **N-b-6** | Leu | 6 | ~80 | 701.33 | 702.231 |
| 62 | **N-b-8** | Leu | 8 | ~70 | 695.36 | 696.256 |
| 63 | **N-b-10** | Leu | 10 | ~80 | 693.38 | 694.283 |
| 64 | **N-b-11** | Leu | 11 | ~80 | 695.36 | 696.259 |
| 65 | **N-b-12** | Leu | 12 | - | 751.33 | - |
| 66 | **N-b-13** | Leu | 13 | ~80 | 690.34 | 691.246 |
| 67 | **N-b-14** | Leu | 14 | ~70 | 723.35 | 724.247 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 68 | **N-b-15** | Leu | 15 | ~70 | 693.38 | 694.282 |
| 69 | **N-b-16** | Leu | 16 | ~70 | 569.16 | 570.153 |
| 70 | **N-b-17** | Leu | 17 | - | 815.20 | - |
| 71 | **N-b-18** | Leu | 18 | ~70 | 749.33 | 750.222 |
| 72 | **N-b-19** | Leu | 19 | ~80 | 713.32 | 714.227 |
| 73 | **N-b-20** | Leu | 20 | ~80 | 691.36 | 692.264 |
| 74 | **N-b-21** | Leu | 21 | ~60 | 805.26 | 806.143 |
| 75 | **N-b-22** | Leu | 22 | - | 791.27 | - |
| 76 | **N-b-32** | Leu | 32 | ~70 | 723.42 | 746.299 |
| 77 | **N-b-33** | Leu | 33 | ~80 | 709.37 | 710.278 |
| 78 | **N-b-34** | Leu | 34 | ~70 | 685.41 | 686.312 |
| 79 | **N-b-37** | Leu | 37 | ~85 | 753.40 | 754.294 |
| 80 | **N-b-38** | Leu | 38 | ~60 | 645.38 | 646.292- |
| 81 | **N-b-40** | Leu | 40 | ~70 | 679.36 | 680．265 |
| 82 | **N-b-55** | Leu | 55 | ~70 | 715.33 | 716.229 |
| 83 | **N-b-42** | Leu | 42 | - | 715.33 | - |
| 84 | **N-b-43** | Leu | 43 | ~70 | 751.33 | 752.231 |
| 85 | **N-b-44** | Leu | 44 | ~60 | 735.27 | 736.172 |
| 86 | **N-b-46** | Leu | 46 | ~60 | 752.32 | 753.215 |
| 87 | **N-b-47** | Leu | 47 | - | 737.29 | - |
| 88 | **N-b-49** | Leu | 49 | ~60 | 777.34 | 778.241 |
| 89 | **N-b-50** | Leu | 50 | ~60 | 743.36 | 744.255 |
| 90 | **N-b-51** | Leu | 51 | ~80 | 707.27 | 708.174 |
| 91 | **N-b-52** | Leu | 52 | ~70 | 757.38 | 758.269 |
| 92 | **N-b-53** | Leu | 53 | ~70 | 743.32 | 744.217 |
| 93 | **N-b-31** | Leu | 31 | ~80 | 693.38 | 694.332 |
| 94 | **N-c-1** | Ala | 1 | ~70 | 547.28 | 548.299 |
| 95 | **N-c-2** | Ala | 2 | ~80 | 651.31 | 652.310 |
| 96 | **N-c-3** | Ala | 3 | ~60 | 581.24 | 582.284 |
| 97 | **N-c-4** | Ala | 4 | ~80 | 577.29 | 578.301 |
| 98 | **N-c-5** | Ala | 5 | ~70 | 599.23 | 600.245 |
| 99 | **N-c-6** | Ala | 6 | ~70 | 583.26 | 584.279 |
| 100 | **N-c-8** | Ala | 8 | ~80 | 577.29 | 578.340 |
| 101 | **N-c-10** | Ala | 10 | ~70 | 575.31 | 576.330 |
| 102 | **N-c-11** | Ala | 11 | ~80 | 577.29 | 578.301 |
| 103 | **N-c-12** | Ala | 12 | - | 597.30 | - |
| 104 | **N-c-13** | Ala | 13 | ~80 | 572.28 | 573.288 |
| 105 | **N-c-14** | Ala | 14 | ~70 | 605.29 | 606.305 |
| 106 | **N-c-15** | Ala | 15 | ~70 | 575.31 | 576.327 |
| 107 | **N-c-16** | Ala | 16 | ~50 | 617.23 | 618.247 |
| 108 | **N-c-17** | Ala | 17 | - | 661.17 | - |
| 109 | **N-c-18** | Ala | 18 | ~40 | 631.27 | 632.251 |
| 110 | **N-c-19** | Ala | 19 | ~50 | 595.26 | 596.270 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 111 | **N-c-20** | Ala | 20 | ~40 | 573.30 | 574.333 |
| 112 | **N-c-21** | Ala | 21 | ~90 | 687.20 | 688.212 |
| 113 | **N-c-22** | Ala | 22 | - | 637.24 | - |
| 114 | **N-c-32** | Ala | 32 | - | 603.35 | - |
| 115 | **N-c-33** | Ala | 33 | ~80 | 591.31 | 592.331 |
| 116 | **N-c-34** | Ala | 34 | ~40 | 567.35 | 568.328 |
| 117 | **N-c-37** | Ala | 37 | ~60 | 635.34 | 636.382 |
| 118 | **N-c-38** | Ala | 38 | ~60 | 527.31 | 528.328 |
| 119 | **N-c-40** | Ala | 40 | - | 597.27 | - |
| 120 | **N-c-55** | Ala | 55 | - | 597.27 | - |
| 121 | **N-c-42** | Ala | 42 | - | 633.27 | - |
| 122 | **N-c-43** | Ala | 43 | ~50 | 617.21 | 618.272 |
| 123 | **N-c-44** | Ala | 44 | - | 634.26 | - |

"-" = Not characterized.

Table S2. The 75-member, C-terminal Library

| # | ID | Structure (Figure S5) | | LCMS | | |
|---|---|---|---|---|---|---|
| | | R1' (AA) | R2' (Sulfonyl chloride) | % purity | Cal MW | Obs MW |
| 1 | **C-a-1** | Phe | 1 | - | 674.27 | - |
| 2 | **C-a-2** | Phe | 2 | ~70 | 765.36 | 766.370 |
| 3 | **C-a-3** | Phe | 3 | >90 | 749.30 | 750.318 |
| 4 | **C-a-4** | Phe | 4 | - | 760.33 | - |
| 5 | **C-a-5** | Phe | 5 | ~80 | 766.35 | 767.375 |
| 6 | **C-a-6** | Phe | 6 | ~60 | 751.32 | 752.341 |
| 7 | **C-a-7** | Phe | 7 | ~70 | 791.38 | 792.392 |
| 8 | **C-a-8** | Phe | 8 | ~70 | 757.39 | 758.410 |
| 9 | **C-a-9** | Phe | 9 | ~50 | 721.30 | 722.318 |
| 10 | **C-a-10** | Phe | 10 | ~70 | 771.41 | 772.422 |
| 11 | **C-a-11** | Phe | 11 | ~60 | 757.35 | 758.362 |
| 12 | **C-a-12** | Phe | 12 | - | 745.35 | - |
| 13 | **C-a-13** | Phe | 13 | ~80 | 729.36 | 730.369 |
| 14 | **C-a-14** | Phe | 14 | - | 841.48 | - |
| 15 | **C-a-15** | Phe | 15 | - | 695.38 | - |
| 16 | **C-a-16** | Phe | 16 | >90 | 772.37 | 773.381 |
| 17 | **C-a-17** | Phe | 17 | - | 760.33 | - |
| 18 | **C-a-18** | Phe | 18 | ~50 | 757.39 | 758.409 |
| 19 | **C-a-19** | Phe | 19 | - | 785.42 | - |
| 20 | **C-a-20** | Phe | 20 | ~50 | 808.40 | 809.415 |
| 21 | **C-a-21** | Phe | 21 | - | 817.23 | - |
| 22 | **C-a-22** | Phe | 22 | ~60 | 765.36 | 766.372 |
| 23 | **C-a-23** | Phe | 23 | ~80 | 785.42 | 786.430 |
| 24 | **C-a-24** | Phe | 24 | ~50 | 733.33 | 734.345 |
| 25 | **C-a-25** | Phe | 25 | ~50 | 751.32 | 752.333 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 26 | **C-b-1** | Leu | 1 | ~70 | 695.38 | 696.352- |
| 27 | **C-b-2** | Leu | 2 | ~80 | 731.38 | 732.391 |
| 28 | **C-b-3** | Leu | 3 | ~85 | 715.32 | 716.335 |
| 29 | **C-b-4** | Leu | 4 | - | 726.34 | - |
| 30 | **C-b-5** | Leu | 5 | ~80 | 732.37 | 733.380 |
| 31 | **C-b-6** | Leu | 6 | ~30 | 717.34 | 718.354 |
| 32 | **C-b-7** | Leu | 7 | ~80 | 757.39 | 758.406 |
| 33 | **C-b-8** | Leu | 8 | - | 723.41 | - |
| 34 | **C-b-9** | Leu | 9 | - | 687.32 | - |
| 35 | **C-b-10** | Leu | 10 | ~60 | 737.42 | 738.438 |
| 36 | **C-b-11** | Leu | 11 | ~70 | 723.37 | 724.390 |
| 37 | **C-b-12** | Leu | 12 | ~70 | 711.37 | 712.408 |
| 38 | **C-b-13** | Leu | 13 | ~80 | 695.38 | 696.383 |
| 39 | **C-b-14** | Leu | 14 | - | 807.50 | - |
| 40 | **C-b-15** | Leu | 15 | - | 661.39 | - |
| 41 | **C-b-16** | Leu | 16 | ~80 | 738.38 | 739.405 |
| 42 | **C-b-17** | Leu | 17 | - | 726.34 | - |
| 43 | **C-b-18** | Leu | 18 | ~80 | 723.41 | 724.417 |
| 44 | **C-b-19** | Leu | 19 | - | 751.44 | - |
| 45 | **C-b-20** | Leu | 20 | - | 774.42 | - |
| 46 | **C-b-21** | Leu | 21 | - | 783.24 | - |
| 47 | **C-b-22** | Leu | 22 | ~60 | 731.38 | 732.389 |
| 48 | **C-b-23** | Leu | 23 | ~80 | 751.44 | 752.441 |
| 49 | **C-b-24** | Leu | 24 | ~70 | 699.35 | 700.364 |
| 50 | **C-b-25** | Leu | 25 | ~85 | 717.34 | 718.357 |
| 51 | **C-c-1** | Ala | 1 | - | 653.33 | - |
| 52 | **C-c-2** | Ala | 2 | >90 | 689.33 | 690.338 |
| 53 | **C-c-3** | Ala | 3 | >90 | 673.27 | 674.281 |
| 54 | **C-c-4** | Ala | 4 | - | 684.30 | - |
| 55 | **C-c-5** | Ala | 5 | ~70 | 690.32 | 691.332 |
| 56 | **C-c-6** | Ala | 6 | ~85 | 675.29 | 676.307 |
| 57 | **C-c-7** | Ala | 7 | >90 | 715.34 | 716.352 |
| 58 | **C-c-8** | Ala | 8 | - | 681.36 | - |
| 59 | **C-c-9** | Ala | 9 | ~90 | 645.27 | 646.280 |
| 60 | **C-c-10** | Ala | 10 | >95 | 695.38 | 696.386 |
| 61 | **C-c-11** | Ala | 11 | >90 | 681.32 | 682.339 |
| 62 | **C-c-12** | Ala | 12 | >90 | 669.32 | 670.341 |
| 63 | **C-c-13** | Ala | 13 | >95 | 653.33 | 654.33 |
| 64 | **C-c-14** | Ala | 14 | - | 765.45 | - |
| 65 | **C-c-15** | Ala | 15 | - | 619.34 | - |
| 66 | **C-c-16** | Ala | 16 | ~80 | 696.33 | 697.343 |
| 67 | **C-c-17** | Ala | 17 | - | 684.30 | - |
| 68 | **C-c-18** | Ala | 18 | >90 | 681.38 | 682.369 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 69 | **C-c-19** | Ala | 19 | ~90 | 709.39 | 710.409 |
| 70 | **C-c-20** | Ala | 20 | ~90 | 732.37 | 733.389 |
| 71 | **C-c-21** | Ala | 21 | - | 741.20 | - |
| 72 | **C-c-22** | Ala | 22 | ~90 | 689.33 | 690.335 |
| 73 | **C-c-23** | Ala | 23 | ~90 | 709.39 | 710.397 |
| 74 | **C-c-24** | Ala | 24 | >90 | 657.30 | 658.312 |
| 75 | **C-c-25** | Ala | 25 | ~90 | 675.29 | 676.309 |

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