Supplementary information

Development of Sulfonamide Photoaffinity Inhibitors for Probing Cellular γ-Secretase

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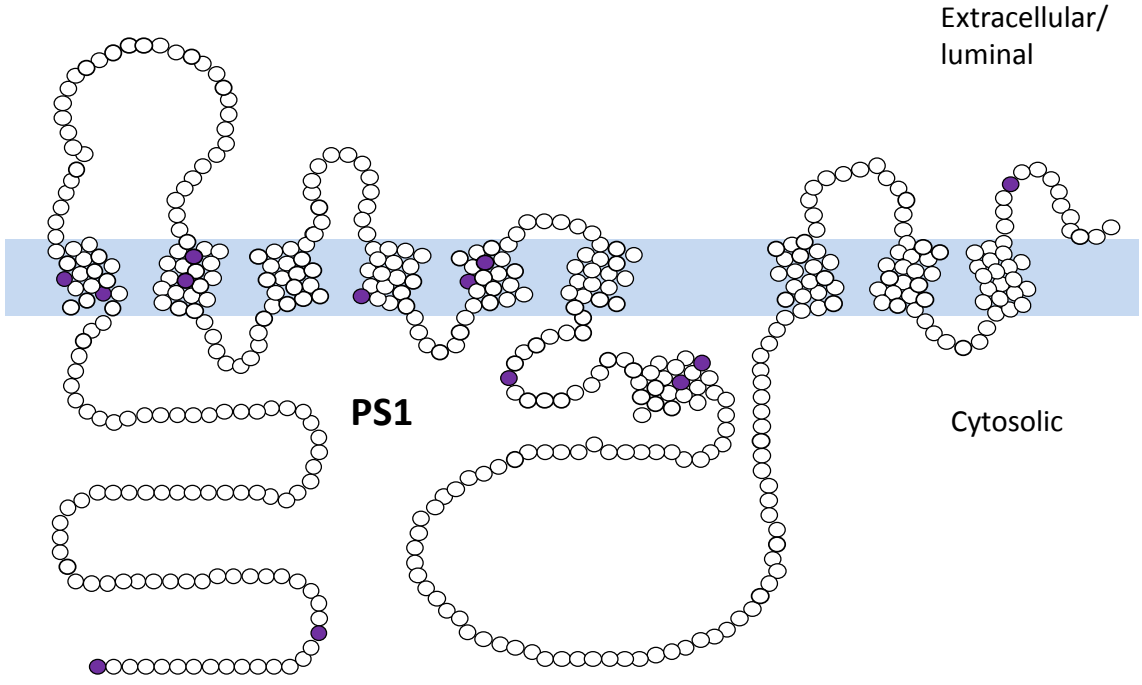
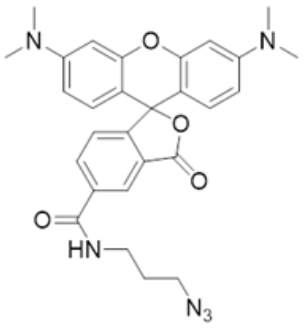
Supplemental Figures

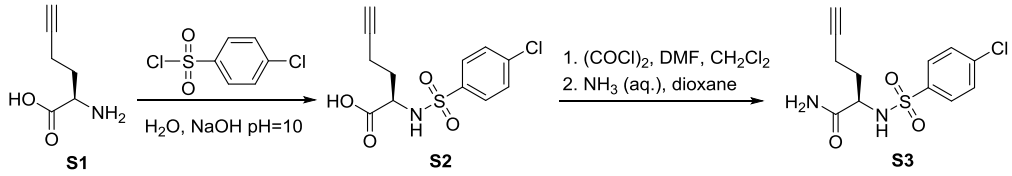


Supplemental Figure 1. Schematic representation of PS1 with 13 residues marked by purple solid circles at positions of 1, 16, 84, 93, 139, 146, 210, 228, 233, 270, 292, 298 and 457.



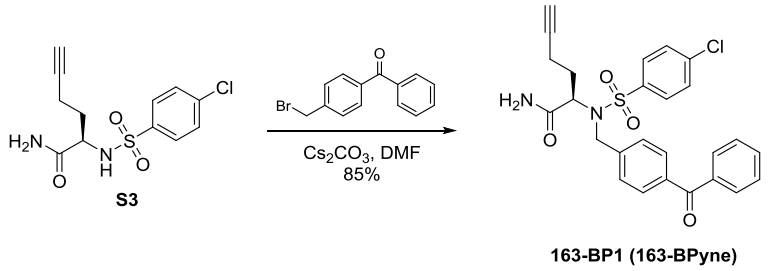
Supplemental Figure 2. Structure of TAMRA-azide

General Information: All commercially available reagents and solvents were used as received. Reactions were monitored by thin layer chromatography (TLC) performed on Analtech, Inc. silica gel GF 250 µm plates and were visualized with ultraviolet (UV) light (254 nm), $KMnO_4$ and/or ninhydrin staining or by UPLC-MS (Waters Acquity, ESCI (ESI +/-, APCI +/-). Silica gel flash chromatography was performed with RediSep Rf or Biotage disposable normal phase silica gel flash columns on a CombiFlash Rf system from Teledyne Isco, Inc. or a Biotage Horizon automatic purification system. Proton, Carbon and Fluorine nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR and ^{19}F NMR) spectra were recorded on a Varian-Inova 400 (400 MHz, 101 MHz and 377 MHz, respectively), Varian-Inova 600 (600 MHz and 151 MHz, respectively), a Bruker 400 (400 MHz, 101 MHz and 377 MHz, respectively), or a Bruker 500 (500 MHz and 126 MHz, respectively) spectrometer. Chemical shifts are reported in ppm relative to $CHCl_3$ or MeOH (i.e. ^{1}H NMR δ = 7.26 and ^{13}C NMR δ = 77.0 or ^{1}H NMR δ = 3.31 and ^{13}C NMR δ = 49.1, respectively). The peak shapes are denoted as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br., broad. High-resolution mass spectra (HRMS) were acquired on an Agilent model 6220 time-of-flight (TOF) mass spectrometer in positive or negative electrospray ionization (ESI) mode.



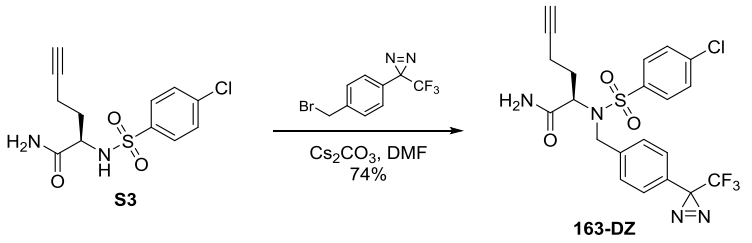
(*R*)-2-(4-chlorophenylsulfonamido)hex-5-ynoic acid (S2): D-Homopropargylglycine **S1**[1] (1500 mg, 11.8 mmol) and 4-chlorobenzenesulphonyl chloride (3480 mg, 16.5 mmol) were dissolved in deionized water (175 mL) under a nitrogen atmosphere. 1 M NaOH was added until pH 10 was reached. The mixture was allowed to react for 16 hours at room temperature kept at pH 10 with 1 M NaOH. The clear mixture was washed with *tert*-butylmethylether (2 x 10 mL). The aqueous layer was then acidified to pH 2-3 using 1 N HCl, extracted with EtOAc (2 x 45 mL). The combined organics were washed with water (20 mL) and brine (20 mL) before being dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to afford the title compound **S2** (2800 mg, 79%) as an off-white solid. This material was used in the next step without further purification.

(***R*)-2-(4-chlorophenylsulfonamido)hex-5-ynamide (S3)**: To a solution of **S2** (2800 mg, 9.3 mmol) in CH_2Cl_2 (50 mL) was added oxalyl chloride (2M solution in CH_2Cl_2, 2.5 mL, 18.6 mmol) and DMF (3 drops) and the solution was heated to reflux for 30 minutes. The solution was evaporated to dryness and the crude acid chloride was solvated into dioxanes (10 mL) then NH_4OH (aq) (1.24 mL, 46.5 mmol) was added and the mixture was allowed to stir for 48 hours. Upon completion, the solution was evaporated to dryness and solvated with EtOAc/THF (2:3, 60 mL) and was washed with water (10 mL) and brine (10 mL) before being dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to afford the title compound **S3** (2000 mg, 71%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.06 (s, 1H), 7.77 (d, *J* = 8.6, 2H), 7.62 (d, *J* = 8.6, 2H), 7.29 (s, 1H), 7.03 (s, 1H), 3.71, (m, 1H), 2.73 (m, 1H), 2.05 (m, 2H), 1.71 (m, 1H), 1.57 (m, 1H); HPLC Purity: 96%.

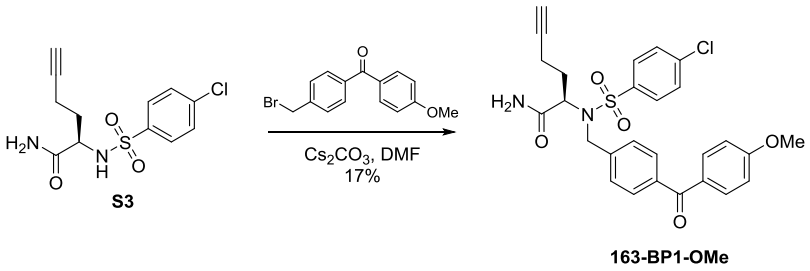


(*R*)-2-(*N*-(4-benzoylbenzyl)-4-chlorophenylsulfonamido)hex-5-ynamide (163-BP1/163-BPyne)[1]: Alkynyl sulfonamide **S3** (26 mg, 0.086 mmol) was dissolved in anhydrous DMF (0.5 mL) and cooled to 0 °C. To this solution was added 4-(bromomethyl)-benzophenone (26.1 mg, 0.095 mmol) and Cs_2CO_3 (36.9 mg, 0.112 mmol) and the solution was then warmed to room temperature. The reaction was judged complete by TLC analysis (~16 hours) and the reaction mixture was diluted with EtOAc (10 mL) and washed with water (10 mL). The aqueous layer was back-extracted with EtOAc (3 x 5 mL) and the combined organics were washed with water (10 mL), 1 M LiCl (10 mL) and brine (10 mL) before being dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The resulting oil was purified by silica gel flash column chromatography eluting with EtOAc/Heptane (10 – 50%) to afford the title compound **163-BP1** (36 mg, 85%) as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 – 7.70 (m, 6H), 7.59 (tt, *J* = 6.7, 1.4 Hz, 1H), 7.50 – 7.41 (m, 6H), 6.37 (bs, 1H), 5.59 (bs, 1H), 4.60 (dd, *J* = 8.6, 5.5 Hz, 1H), 4.62 (d, *J* = 15.7 Hz, 1H), 4.51 (d, *J* = 16.0 Hz, 1H), 2.21 – 2.07 (m, 3H), 2.01 – 1.97 (m, 1H), 1.52 – 1.38 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.1, 170.8, 140.8, 139.8, 138.1, 137.4, 137.1, 132.5, 130.3,

130.0, 129.6, 128.7, 128.5, 128.3, 82.4, 70.3, 58.0, 48.5, 27.8, 15.5; HRMS calcd for $C_{26}H_{24}ClN_2O_4S$ (M+H) 495.1140, found 495.1126; HPLC Purity: 99%.

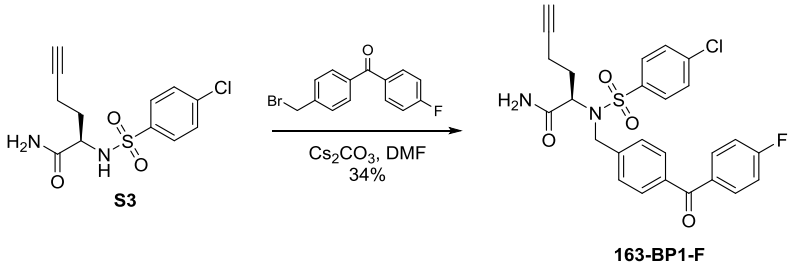


(*R*)-2-((4-chloro-*N*-(4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)benzyl)phenyl)sulfonamido)hex-5-ynamide (163-DZ): To a solution of alkynyl sulfonamide **S3** (100 mg, 0.33 mmol) and 3-(4-(bromomethyl)phenyl)-3-(trifluoromethyl)-3H-diazirine[2] (111 mg, 0.398 mmol) in anhydrous DMF (3.3 mL) was added Cs_2CO_3 (131 mg, 0.398 mmol). The reaction mixture was stirred at room temperature for 19 hours. EtOAc (10 mL) was added and the mixture was washed with water (3 x 10 mL). The organic fraction was dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by silica gel flash chromatography eluting with EtOAc/heptane (0 – 80%) to afford the title compound **163-DZ** (122 mg, 74% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 – 7.65 (m, 2H), 7.48 – 7.42 (m, 2H), 7.35 (d, *J* = 8.4 Hz, 2H), 7.11 (d, *J* = 8.2 Hz, 2H), 6.30 (br. s., 1H), 5.22 (br. s., 1H), 4.59 (dd, *J* = 5.4, 8.6 Hz, 1H), 4.57 – 4.37 (m, 2H), 2.21 – 2.13 (m, 1H), 2.13 – 2.06 (m, 2H), 1.99 (t, *J* = 2.5 Hz, 1H), 1.45 – 1.35 (m, 1H); LRMS (M+H) 499.1; HPLC Purity: > 99%.

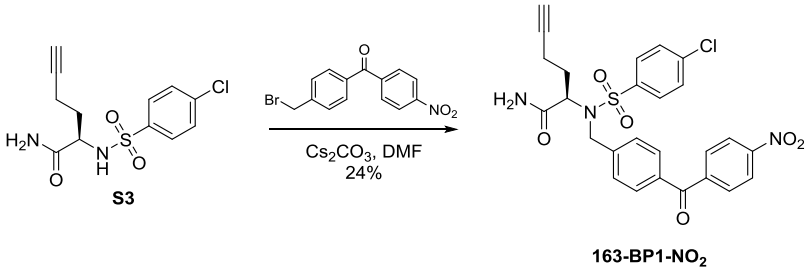


(*R*)-2-(4-chloro-*N*-(4-(4-methoxybenzoyl)benzyl)phenylsulfonamido)hex-5-ynamide (163-BP1-OMe): Alkynyl sulfonamide **S3** (163 mg, 0.54 mmol) was dissolved in anhydrous DMF (3 mL) and cooled to 0 °C. To this solution was added 4-(bromomethyl)-4'-methoxybenzophenone[3] (150 mg, 0.49 mmol) and Cs_2CO_3 (322 mg, 0.99 mmol) and the solution was then warmed to room temperature. The reaction was judged complete by TLC analysis (~16 hours) and the reaction mixture was diluted with EtOAc (10 mL) and washed with water (10 mL). The aqueous layer was back-extracted with EtOAc (3 x 5 mL) and the combined organics were washed with water (10 mL), 1 M LiCl (10 mL) and brine (10 mL) before being dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The resulting oil was purified by silica gel flash column chromatography eluting with EtOAc/Heptane (10 – 50%) to afford the title compound **163-BP1-OMe** (45 mg, 17%) as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, *J* = 8.6 Hz, 2H), 7.74 (d, *J* = 8.6 Hz, 2H), 7.67 (d, *J* = 8.0 Hz, 2H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.42 (d, *J* = 8.0 Hz, 2H), 6.96 (d, *J* = 8.6 Hz, 2H), 6.37 (bs, 1H), 5.52 (bs, 1H), 4.69 – 4.45 (m, 3H), 3.88 (s, 3H), 2.25 – 2.06 (m, 3H), 1.99 (t, *J* = 2.2 Hz, 1H), 1.54 – 1.37 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.9, 170.8, 163.3, 140.1,

139.8, 138.1, 137.8, 132.5, 129.9, 129.6, 128.7, 128.5, 113.6, 82.4, 70.3, 58.0, 55.5, 48.5, 27.8, 15.5; HRMS calcd for $C_{27}H_{26}ClN_2O_5S$ (M+H) 525.1245, found 525.1238; HPLC Purity: 98%.

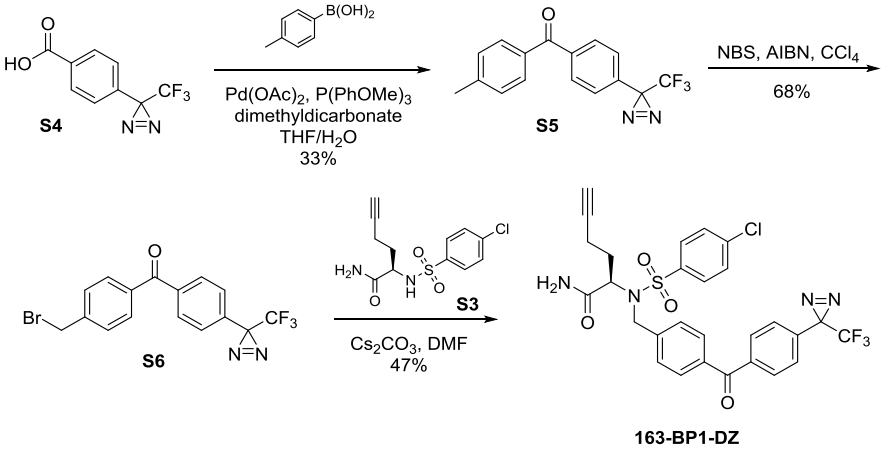


(*R*)-2-(4-chloro-*N*-(4-(4-fluorobenzoyl)benzyl)phenylsulfonamido)hex-5-ynamide (163-BP1-F): Alkynyl sulfonamide **S3** (56.3 mg, 0.188 mmol) was dissolved in anhydrous DMF (1 mL) and cooled to 0 °C. To this solution was added 4-(bromomethyl)-4'-fluorobenzophenone[4] (50 mg, 0.175 mmol) and Cs_2CO_3 (110 mg, 0.34 mmol) and the solution was then warmed to room temperature. The reaction was judged complete by TLC analysis (~16 hours) and the reaction mixture was diluted with EtOAc (10 mL) and washed with water (10 mL). The aqueous layer was back-extracted with EtOAc (3 x 5 mL) and the combined organics were washed with water (10 mL), 1 M LiCl (10 mL) and brine (10 mL) before being dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The resulting oil was purified by silica gel flash column chromatography eluting with EtOAc/Heptane (10 – 50%) to afford the title compound **163-BP1-F** (30 mg, 34%) as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.78 (m, 2H), 7.78 – 7.72 (m, 2H), 7.72 – 7.66 (m, 2H), 7.51 – 7.42 (m, 4H), 7.20 – 7.13 (m, 2H), 6.36 (bs, 1H), 5.43 (bs, 1H), 4.70 – 4.55 (m, 2H), 4.55 – 4.46 (m, 1H), 2.23 – 2.07 (m, 3H), 2.02 – 1.98 (m, 1H), 1.50 – 1.38 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.6, 170.6, 165.4 (d, J_{CF} = 253 Hz), 140.8, 139.9, 138.1, 137.0, 133.6 (d, J_{CF} = 2.8 Hz), 132.6 (d, J_{CF} = 9.5 Hz), 130.1, 129.6, 128.7, 128.6, 115.5 (d, J_{CF} = 22 Hz), 82.4, 70.3, 57.9, 48.5, 27.7, 15.5; ^{19}F NMR (377 MHz, $CDCl_3$) δ -105.6 (s, 1F); HRMS calcd for $C_{26}H_{23}ClFN_2O_4S$ (M+H) 513.1046, found 513.1031; HPLC Purity: 87%.



(*R*)-2-(4-chloro-*N*-(4-(4-nitrobenzoyl)benzyl)phenylsulfonamido)hex-5-ynamide (163-BP1-NO_2): Alkynyl sulfonamide **S3** (86.7 mg, 0.29 mmol) was dissolved in anhydrous DMF (2 mL) and cooled to 0 °C. To this solution was added 4-(bromomethyl)-4'-nitrobenzophenone[5] (85 mg, 0.27 mmol) and Cs_2CO_3 (173 mg, 0.53 mmol) and the solution was then warmed to room temperature. The reaction was judged complete by TLC analysis (~16 hours) and the reaction mixture was diluted with EtOAc (10 mL) and washed with water (10 mL). The aqueous layer was back-extracted with EtOAc (3 x 5 mL) and the combined organics were washed with water (10 mL), 1 M LiCl (10 mL) and brine (10 mL) before being dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The resulting oil was purified by silica gel

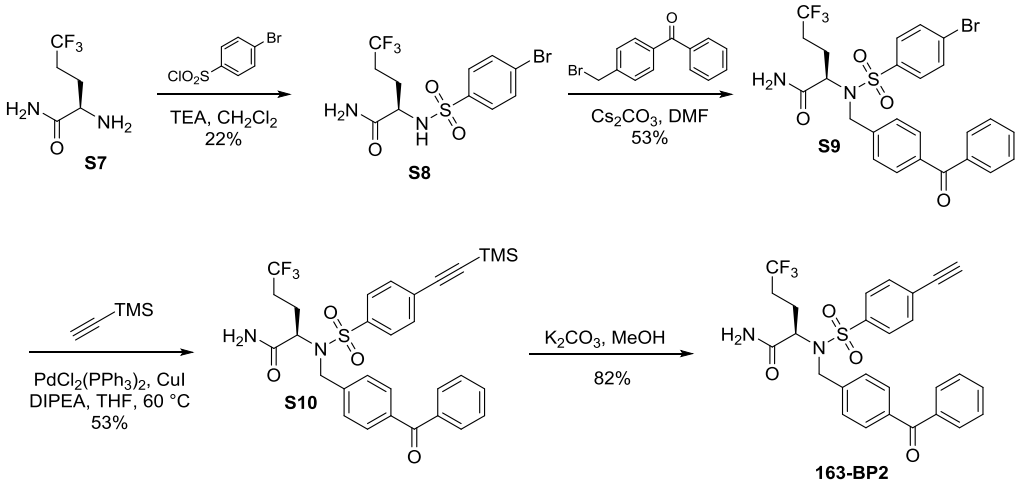
flash column chromatography eluting with EtOAc/Heptane (10 – 50%) to afford the title compound **163-BP1-NO$_2$** (35 mg, 24%) as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (d, *J* = 8.6 Hz, 2H), 7.84 (d, *J* = 8.6 Hz, 2H), 7.71 (d, *J* = 8.6 Hz, 2H), 7.67 (d, *J* = 8.2 Hz, 2H), 7.43 (d, *J* = 8.2 Hz, 4H), 6.28 (bs, 1H), 5.30 (bs, 1H), 4.61 (d, *J* = 16.0 Hz, 1H), 4.57 – 4.52 (m, 1H), 4.42 (d, *J* = 16.0 Hz, 1H), 2.16 – 2.00 (m, 3H), 1.97 – 1.92 (m, 1H), 1.42 – 1.30 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.2, 170.5, 149.9, 142.7, 142.1, 140.0, 138.0, 135.8, 130.7, 130.3, 129.7, 128.8, 128.7, 123.6, 82.3, 70.4, 57.8, 48.4, 27.7, 15.5; HRMS calcd for $C_{26}H_{23}ClN_3O_6S$ (M+H) 540.0991, found 540.0972; HPLC Purity: 97%.



***p*-tolyl(4-(3-(trifluoromethyl)-3H-diazirin-3-yl)phenyl)methanone (S5)**: 4-(3-(Trifluoromethyl)-3H-diazirin-3-yl)benzoic acid **S4** (536 mg, 2.33 mmol) was dissolved in THF (11.6 mL) and brought to 0 °C. To the solution was added *p*-tolylboronic acid (380 mg, 2.80 mmol), followed by $P(PhOMe)_3$ (60.5 mg, 0.163 mmol), dimethyldicarbonate (625 mg, 4.66 mmol), and then $Pd(OAc)_2$ (16.2 mg, 0.07 mmol). Water (0.11 mL) was added and the solution was degassed with nitrogen for 5 minutes at this temperature. The solution was brought to room temperature and stirred overnight. The solution became dark overnight and was then diluted with EtOAc (50 mL) and water (50 mL). The organic fraction was washed with water (50 mL), and then brine (50 mL). The organic fraction was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by silica gel flash chromatography eluting with CH_2Cl_2/heptane (0 – 50%) to afford the title compound **S5** (232 mg, 33% yield) as a beige solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 – 7.77 (m, 2H), 7.73 – 7.65 (m, 2H), 7.29 (d, *J* = 8.3 Hz, 4H), 2.45 (s, 3H).

(4-(bromomethyl)phenyl)(4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)phenyl)methanone (S6): A solution of **S5** (0.232 g, 0.762 mmol) in CCl_4 (3.05 mL) was heated to 70 °C and freshly recrystalized *N*-bromosuccinimide (0.164 g, 0.914 mmol) was added and stirred for 10 min, then AIBN (3.0 mg, 0.0108 mmol) was added and the reaction was refluxed for 2 hours. A precipitate formed and was filtered, and washed with CH_2Cl_2. The filtrate was concentrated under reduced pressure to afford a white residue. The residue was purified by silica gel flash chromatography eluting with 5% CH_2Cl_2 in heptane to afford **S6** (199 mg, 68%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 – 7.79 (m, 2H), 7.78 – 7.72 (m, 2H), 7.55 – 7.47 (m, 2H), 7.34 – 7.27 (m, 2H), 4.53 (s, 2H); LRMS (M+H) 383.1.

(*R*)-2-((4-chloro-*N*-(4-(4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)benzoyl)benzyl)phenyl)sulfonamido)hex-5-ynamide (163-BP1-DZ): To a solution of alkynyl sulfonamide **S3** (71 mg, 0.24 mmol) and **S6** (90 mg, 0.24 mmol) in anhydrous DMF (2.4 mL) was added Cs_2CO_3 (93 mg, 0.28 mmol). The reaction mixture was stirred at room temperature for 17 hours. EtOAc (10 mL) was added and the mixture was washed with water (3 x 10 mL). The organic fraction was dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by silica gel flash chromatography eluting with EtOAc/heptane (0 – 80%) to afford the title compound **163-BP1-DZ** (67 mg, 47% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 – 7.78 (m, 2H), 7.78 – 7.74 (m, 2H), 7.73 – 7.69 (m, 2H), 7.50 – 7.47 (m, 2H), 7.47 – 7.43 (m, 2H), 7.30 (d, *J* = 8.1 Hz, 2H), 6.34 (br. s., 1H), 5.24 (br. s., 1H), 4.68 – 4.42 (m, 3H), 2.23 – 2.08 (m, 3H), 2.00 (t, *J* = 2.5 Hz, 1H), 1.48 – 1.37 (m, 1H); LRMS (M+H) 603.2; HPLC Purity: > 99%.



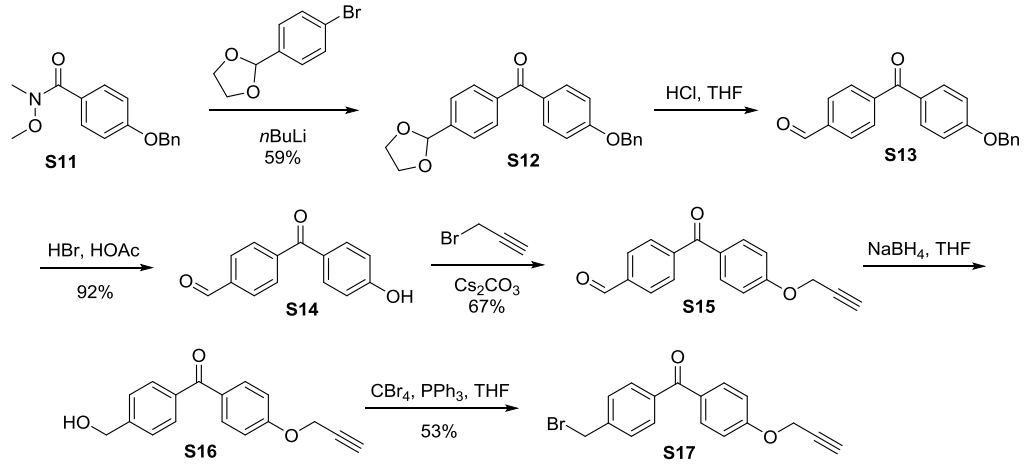
(*R*)-2-((4-bromophenyl)sulfonamido)-5,5,5-trifluoropentanamide (S8): To a solution of **S7**[6] (235 mg, 1.14 mmol) in CH_2Cl_2 (5.4 mL) was added triethylamine (356 mg, 0.49 mL, 3.41 mmol) followed by 4-bromobenzenesulfonyl chloride (320 mg, 1.25 mmol). The reaction mixture was stirred at room temperature for 15 minutes upon which the reaction was judged complete by LCMS. The reaction mixture was quenched with saturated $NaHCO_3$ and then extracted with CH_2Cl_2. The organic fraction was dried with anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by silica gel flash chromatography eluting with EtOAc/Heptane (20 – 100%) to afford the title compound **S8** (98 mg, 22% yield) as a solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 – 7.65 (m, 4H), 5.61 (br. s., 1H), 5.43 (d, *J* = 6.9 Hz, 1H), 5.35 (br. s., 1H), 3.84 – 3.77 (m, 1H), 2.26 – 2.14 (m, 2H), 2.10 – 1.99 (m, 1H), 1.86 – 1.75 (m, 1H); LRMS (M+H) 389.1.

(*R*)-2-((*N*-(4-benzoylbenzyl)-4-bromophenyl)sulfonamido)-5,5,5-trifluoropentanamide (S9): To a solution of **S8** (98 mg, 0.25 mmol) and 4-(bromomethyl)benzophenone (121 mg, 0.44 mmol) in DMF (0.88 mL) was added Cs_2CO_3 (290 mg, 0.88 mmol) and the reaction mixture was stirred at room temperature for 1 hour. The mixture was diluted with EtOAc and washed with water (4 x 10 mL). The combined organic fraction was dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by silica gel flash chromatography eluting with EtOAc/Heptane (10 – 100%) to afford the title compound **S9** (110 mg, 43% yield) as a white solid. ^{1}H NMR (400 MHz,

$CDCl_3$) δ 7.80 – 7.71 (m, 6H), 7.70 – 7.61 (m, 3H), 7.52 – 7.42 (m, 4H), 6.22 (br. s., 1H), 5.27 (br. s., 1H), 4.63 – 4.48 (m, 2H), 4.41 – 4.35 (m, 1H), 2.24 – 2.09 (m, 1H), 2.04 – 1.94 (m, 1H), 1.90 – 1.76 (m, 1H), 1.51 – 1.45 (m, 1H); LRMS (M+H) 583.2.

(*R*)-2-((*N*-(4-benzoylbenzyl)-4-((trimethylsilyl)ethynyl)phenyl)sulfonamido)-5,5,5-trifluoropentanamide (S10): To a solution of **S9** (171 mg, 0.29 mmol) in THF (3 mL) was added trimethylsilylacetylene (33 mg, 0.34 mmol), $PdCl_2(PPh_3)_2$ (10.7 mg, 0.0150 mmol), CuI (2.9 mg, 0.0150 mmol) and DIPEA (114 mg, 0.879 mmol). The reaction was heated to 60 °C for 20 hours and then the mixture was concentrated under reduced pressure. The crude residue was purified by silica gel flash chromatography eluting with EtOAc/Heptane (10 – 100%) to afford the title compound **S10** (94 mg, 53% yield) as a solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 – 7.68 (m, 6H), 7.62 – 7.56 (m, 3H), 7.52 – 7.42 (m, 4H), 6.19 (br. s., 1H), 5.23 (br. s., 1H), 4.60 – 4.49 (m, 2H), 4.37 (t, *J* = 7.1 Hz, 1H), 2.20 – 2.08 (m, 1H), 2.03 – 1.92 (m, 1H), 1.89 – 1.72 (m, 1H), 1.50 – 1.45 (m, 1H), 0.27 (s, 9H); LRMS (M+H) 601.4.

(*R*)-2-((*N*-(4-benzoylbenzyl)-4-ethynylphenyl)sulfonamido)-5,5,5-trifluoropentanamide (163-BP2): To a solution of **S10** (94 mg, 0.16 mmol) in MeOH (3.2 mL) was added K_2CO_3 (50 mg, 0.36 mmol) in one portion and the reaction mixture was stirred at room temperature for 1 hour upon which the reaction was judged complete by LCMS. The reaction mixture was concentrated under reduced pressure and EtOAc was added and washed with water. The organic fraction was dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by silica gel flash chromatography eluting with EtOAc/Heptane (0 – 100%) to afford the title compound **163-BP2** (23 mg, 82% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 – 7.68 (m, 6H), 7.63 (d, *J* = 8.5 Hz, 2H), 7.59 (d, *J* = 7.5 Hz, 1H), 7.53 – 7.42 (m, 4H), 6.22 (br. s., 1H), 5.34 (br. s., 1H), 4.65 – 4.47 (m, 2H), 4.38 (dd, *J* = 7.0, 8.2 Hz, 1H), 3.31 (s, 1H), 2.15 (dtd, *J* = 5.6, 8.8, 14.0 Hz, 1H), 2.05 – 1.90 (m, 1H), 1.88 – 1.73 (m, 1H), 1.56 – 1.43 (m, 1H); LRMS (M+H) 529.4.



(4-(1,3-dioxolan-2-yl)phenyl)(4-(benzyloxy)phenyl)methanone (S12): To a solution of 2-(4-bromophenyl)-1,3-dioxolane (20000 mg, 87 mmol) in THF (300 mL) was added dropwise 2.5 M *n*BuLi (36 mL, 87 mmol) at -70 °C. Then the mixture was stirred at -70 °C for 40 minutes. A solution of **S11**[7] (11800 mg, 43.5 mmol) in THF (100 mL) was added dropwise, after addition, the mixture was stirred at -70 °C

for 1 hour. TLC (Petroleum ether/EtOAc=3/1) showed the reaction was completed. The mixture was quenched with saturated NH_4Cl (50 mL) and extracted with EtOAc (2 x 100 mL), the organic layer was concentrated under reduced pressure and the residue was purified by silica gel flash chromatography to afford the title compound **S12** (10000 mg, 59%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.8 Hz, 2H), 7.75 (d, *J* = 8.1 Hz, 2H), 7.58 (d, *J* = 8.1 Hz, 2H), 7.44 – 7.32 (m, 5H), 7.02 (d, *J* = 8.8 Hz, 2H), 5.88 (s, 1H), 5.14 (s, 2H), 4.13 – 4.07 (m, 4H); LRMS (M+H) 361.0.

4-(4-(benzyloxy)benzoyl)benzaldehyde (S13): To a solution of compound **S12** (10000 mg, 27.7 mmol) in THF (200 mL) was added concentrated HCl (30 mL), then the mixture was refluxed for 4 hours. TLC (Petroleum ether/EtOAc=3/1) indicated the reaction was completed. The mixture was concentrated in vacuo and the residue was dissolved in EtOAc (150 mL), washed with saturated $NaHCO_3$ and brine, the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to afford the title compound **S13** (9000 mg) as a yellow oil which was used with no further purification.

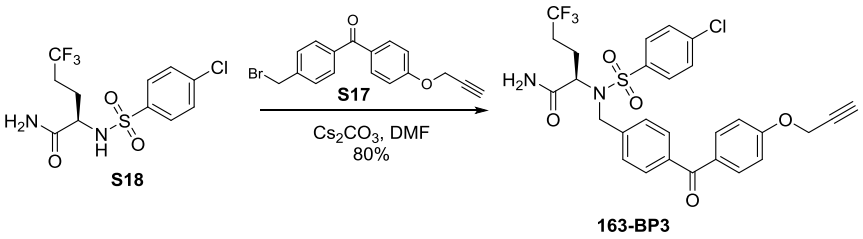
4-(4-hydroxybenzoyl)benzaldehyde (S14): A solution of compound **S13** (9000 mg, 28 mmol) in HBr/HOAc (100 mL) was stirred at room temperature for 3 hours. TLC (Petroleum ether/EtOAc=3/1) indicated the reaction was completed. The mixture was concentrated in vacuum, H_2O (200 mL) was added and the mixture was extracted with EtOAc (2 x 100 mL), the organic layer was concentrated under reduced pressure and the residue was purified by silica gel flash chromatography to afford the title compound **S14** (5800 mg, 92%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.54 (s, 1H), 10.13 (s, 1H), 8.05 (d, *J* = 8.1 Hz, 2H), 7.83 (d, *J* = 8.1 Hz, 2H), 7.68 (d, *J* = 8.7 Hz, 2H), 6.91 (d, *J* = 8.7 Hz, 2H); LRMS (M-H) 225.2.

4-(4-(prop-2-yn-1-yloxy)benzoyl)benzaldehyde (S15): To a solution of compound **S14** (5200 mg, 23 mmol) in DMF (200 mL) was added K_2CO_3 (3200 mg, 23 mmol) in one portion. Then the mixture was stirred at room temperature for 20 minutes. Propargyl bromide (2700 mg, 23 mmol) was added and the mixture was stirred at room temperature for 2 hours. TLC (Petroleum ether/EtOAc=3/1) indicated the reaction was completed. H_2O (200 mL) was added and the mixture was extracted with EtOAc (2 x 100 mL), the organic layer was concentrated under reduced pressure and the residue was purified by silica gel flash chromatography to afford the title compound **S15** (4000 mg, 67%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.12 (s, 1H), 7.98 (d, *J* = 8.1 Hz, 2H), 7.87 (d, *J* = 8.1 Hz, 2H), 7.82 (d, *J* = 8.8 Hz, 2H), 7.06 (d, *J* = 8.8 Hz, 2H), 4.78 (s, 2H), 2.56 (s, 1H); LRMS (M+H) 265.2; HPLC Purity: 98.6%.

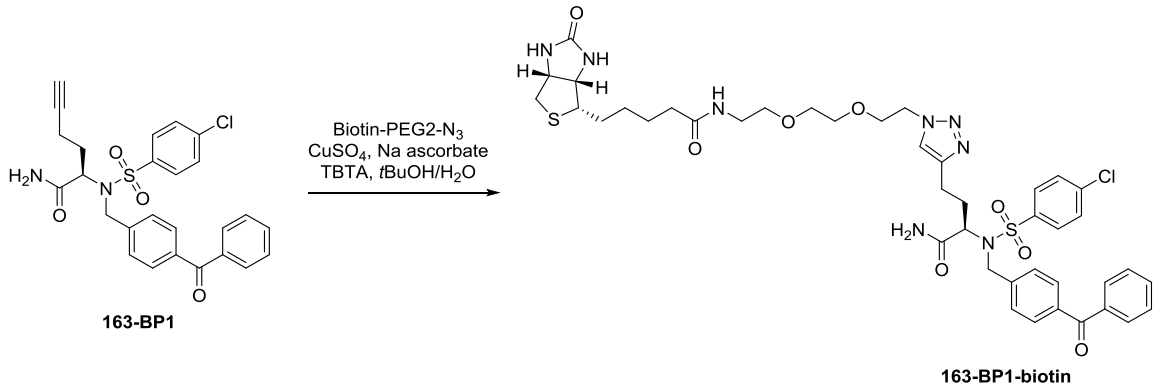
(4-(hydroxymethyl)phenyl)(4-(prop-2-yn-1-yloxy)phenyl)methanone (S16): To a solution of **S15** (5000 mg, 18.9 mmol) in THF (250 mL) was added sodium borohydride (720 mg, 18.4 mmol) portion-wise at 0 °C and stirred for 30 minutes at room temperature. TLC indicated **S15** was consumed. The mixture was quenched with saturated NH_4Cl solution (100 mL) and extracted with EtOAc (2 x 150 mL). The combined organic solution was dried over Na_2SO_4 and concentrated under reduced pressure to afford the title compound **S16** (5000 mg) which was used in the next step without purification.

(4-(bromomethyl)phenyl)(4-(prop-2-yn-1-yloxy)phenyl)methanone (**S17**): To the solution of **S16** (5000 mg, 18.8 mmol) in CH_2Cl_2 (150 mL) was added PPh_3 (5900 mg, 22.5 mmol), followed by the addition of CBr_4 (7460 mg, 22.5 mmol) and the reaction was stirred at the room temperature for 3 hours. TLC

showed complete consumption of **S16**, the reaction was evaporated and the crude mixture was dissolved in EtOAc and washed with $NaHCO_3$ (aq) and H_2O and then brine solution, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure and the crude compound was purified by silica gel flash chromatography to afford the title compound **S17** (3300 mg, 53%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (d, *J* = 8.7 Hz, 2H), 7.73 (d, *J* = 8.8 Hz, 2H), 7.49 (d, *J* = 8.8 Hz, 2H), 7.04 (d, *J* = 8.7 Hz, 2H), 4.77 (s, 2H), 4.53 (s, 2H), 2.56 (s, 1H); LRMS (M+H) 328.0; HPLC Purity: 95.1%

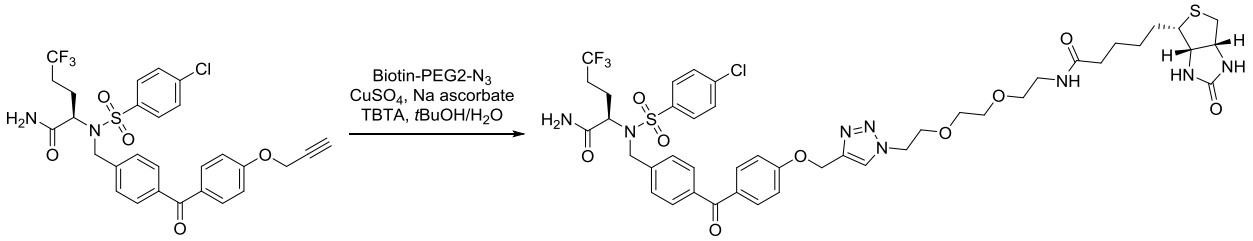


(*R*)-2-((4-chloro-*N*-(4-(4-(prop-2-yn-1-yloxy)benzoyl)benzyl)phenyl)sulfonamido)-5,5,5-trifluoropentanamide (163-BP3): A solution of **S18**[6] (57 mg, 0.16 mmol) in anhydrous DMF (0.83 mL) was cooled to 0 °C. To this solution was added **S17** (60 mg, 0.18 mmol) and Cs_2CO_3 (65 mg, 0.20 mmol) and the mixture was then warmed to room temperature. The reaction was judged complete by TLC analysis (~4 hours) and the reaction mixture was diluted with EtOAc (10 mL) and washed with water (15 mL). The aqueous layer was back-extracted with EtOAc (3 x 10 mL) and the combined organics were washed with water (10 mL), 1 M LiCl (10 mL) and brine (10 mL) before being dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The resulting crude residue was purified by silica gel flash chromatography eluting with EtOAc/heptane (10 – 40%) to afford the title compound **163-BP3** (78 mg, 80% yield) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.86 – 7.79 (m, 2H), 7.80 – 7.72 (m, 2H), 7.67 – 7.62 (m, 2H), 7.61 – 7.56 (m, 2H), 7.56 – 7.51 (m, 2H), 7.13 – 7.05 (m, 2H), 4.84 (s, 4H), 4.56 (dd, *J* = 5.9, 8.6 Hz, 1H), 3.01 (t, *J* = 2.3 Hz, 1H), 2.16 – 1.90 (m, 3H), 1.75 – 1.63 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 197.2, 173.5, 163.1, 143.8, 140.6, 140.0, 138.7, 133.6, 131.9, 131.0, 130.6, 130.4, 129.7, 127.2 (q, $^1J_{CF}$ = 275.1 Hz), 115.8, 79.3, 77.6, 60.2, 57.0, 49.7, 31.8 (q, $^2J_{CF}$ = 29.3 Hz), 24.5; ^{19}F NMR (377 MHz, CD_3OD) δ -68.00 (t, *J* = 10.2 Hz, 3F); HRMS calcd for $C_{28}H_{25}ClF_3N_2O_5S$ (M+H) 593.1119, found 593.1123; HPLC Purity: 95%.



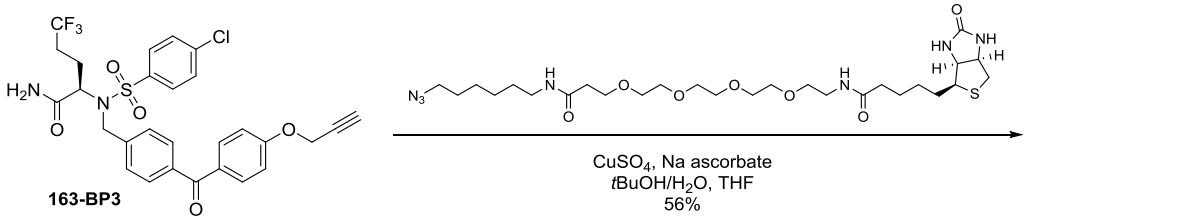
***N*-(2-(2-(2-(4-((R)-4-amino-3-((*N*-(4-benzoylbenzyl)-4-chlorophenyl)sulfonamido)-4-oxobutyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethyl)-5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-**

yl)pentanamide (163-BP1-biotin): A solution of **163-BP1** (4.2 mg, 8.5 µmol), biotin-PEG2-N_3[8] (3.4 mg, 8.5 µmol), copper sulfate pentahydrate (0.1 mg, 0.42 µmol), sodium ascorbate (0.33mg, 1.68 µmol) and Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine ligand (0.22mg, 0.42 µmol) in *t*BuOH/H_2O (50:50) was stirred at room temperature overnight. The mixture was filtered and purified by HPLC to afford the title compound **163-BP1-biotin** as a white solid. (m/z = 929.16 for Cl^- salt, m/z = 917.19 for Na^+ salt).



163-BP3 163-BP3-biotin

(*R*)-2-((4-chloro-*N*-(4-(4-((1-(2-(2-(2-(5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)ethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzoyl)benzyl)phenyl)sulfonamido)-5,5,5-trifluoropentanamide (163-BP3-biotin): A solution of **163-BP3** (5.0 mg, 8.5 µmol), biotin-PEG2-N_3 (3.4 mg, 8.5 µmol), copper sulfate pentahydrate (0.1 mg, 0.42 µmol), sodium ascorbate (0.33mg, 1.68 µmol) and Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine ligand (0.22mg, 0.42 µmol) in *t*BuOH/H_2O (50:50) was stirred at room temperature overnight. The mixture was filtered and purified by HPLC to afford the title compound **163-BP3-biotin** as a white solid. (m/z = 1027.4 for Cl^- salt, m/z = 1015.49 for Na^+ salt).



163-BP3

163-BP3-L-biotin

***N*-(6-(4-((4-(4-(((*N*-((*R*)-1-amino-5,5,5-trifluoro-1-oxopentan-2-yl)-4-chlorophenyl)sulfonamido)methyl)benzoyl)phenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)hexyl)-1-(5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)-3,6,9,12-tetraoxapentadecan-15-amide (163-BP3-L-biotin)**: To the mixture of **163-BP3** (35 mg, 0.059 mmol) and biotin-PEG4-N_3[9] (36 mg, 0.059 mmol) in *t*BuOH-H_2O (1:1, 0.75 mL) and THF (0.75 mL) were added copper sulfate pentahydrate (1.5 mg, 0.0059 mmol) and sodium ascorbate (1 M in H_2O, 5 drops) at room temperature and stirred for 18 hours. TLC indicated complete consumption of starting material and the

reaction mixture was concentrated under reduced pressure. The mixture was diluted with water and extracted with EtOAc (2x). The combined organic fraction was washed with water and brine, dried with anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude residue was purified by preparative TLC to afford the title compound **163-BP3-L-biotin** (40mg, 56% yield) as an off-white solid. ^{1}H NMR (400 MHz, CD_3OD) δ = 8.12 (s, 1H), 7.85 (d, *J* = 7.4 Hz, 2H), 7.79 (d, *J* = 7.6 Hz, 2H), 7.68 – 7.64 (m, 2H), 7.61 – 7.47 (m, 4H), 7.16 (d, *J* = 7.6 Hz, 2H), 5.30 (s, 2H), 4.82 (s, 2H), 4.60 – 4.53 (m, 1H), 4.51 – 4.40 (m, 3H), 4.29 (dd, *J* = 4.4, 7.5 Hz, 1H), 3.71 (t, *J* = 5.8 Hz, 2H), 3.61 (d, *J* = 6.7 Hz, 12H), 3.53 (t, *J* = 5.2 Hz, 2H), 3.35 (t, *J* = 5.5 Hz, 2H), 3.22 – 3.12 (m, 3H), 2.91 (dd, *J* = 4.2, 12.8 Hz, 1H), 2.69 (d, *J* = 12.7 Hz, 1H), 2.42 (t, *J* = 5.9 Hz, 2H), 2.21 (t, *J* = 7.2 Hz, 2H), 2.16 – 1.85 (m, 6H), 1.81 – 1.53 (m, 5H), 1.53 – 1.21 (m, 7H); LRMS (M+H) 1208.6; HPLC Purity: 96%.

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