Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Investigating γ-secretase protein interactions in live cells using active site-directed clickable dual-photoaffinity probes

T. Eric Ballard,[*,[a],[b],†] Heather E. Murrey,[[a]] Kieran F. Geoghegan,[[c]] Christopher W. am Ende,[[b]] Douglas S. Johnson[*,[a]]

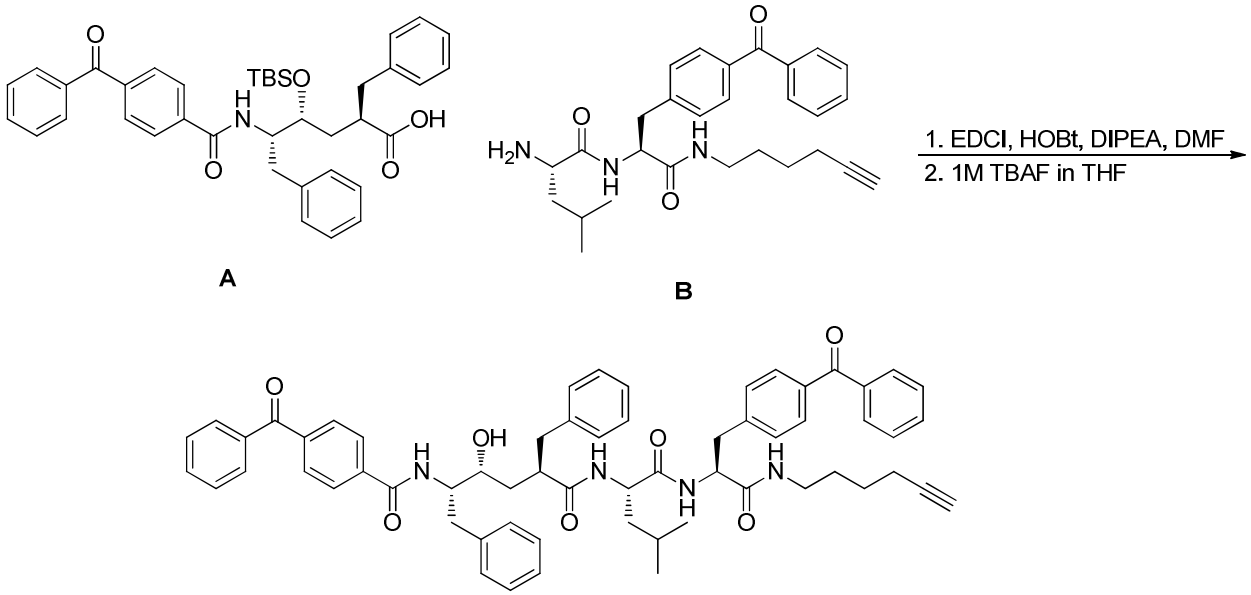
[a] Neuroscience Medicinal Chemistry and Chemical Biology, Pfizer Worldwide Research and Development, Cambridge, MA 02139 (USA)
[b] Neuroscience Medicinal Chemistry, Pfizer Worldwide Research and Development, Groton, CT 06340 (USA)
[c] Structural Biology and Biophysics, Pfizer Worldwide Research and Development, Groton, CT 06340 (USA)
[†] Current Address: Pharmacokinetics, Dynamics and Metabolism
Pfizer Worldwide Research and Development, Groton, CT 06340 (USA)

Supporting Information

General methods for chemical synthesis

Starting materials and reagents were purchased from Aldrich or VWR and used as received. Analytical thin-layer chromatography (TLC) was performed on glass plates, precoated with silica gel. Flash chromatography was carried out using silica gel 60 (Redisep) as the stationary phase. Both analytical and preparative high performance liquid chromatography (HPLC) was performed on reversed phase-HPLC (RPHPLC) instruments, using C_{18} columns and a binary solvent system (MeCN and H_2O with 0.1% CF_3CO_2H). High resolution mass spectrometry was carried out using Agilent (6220) LC-MS TOF using (pH 3.5) 10 mM ammonium formate in H_2O as mobile phase A1 and 50:50 methanol:acetonitrile as mobile phase B1. ^{1}H NMR spectra were recorded on a Varian 400 MHz spectrometer. Chemical shifts are reported in ppm relative to the signals corresponding to the residual non-deuterated solvents ($CDCl_3$: δ 7.26 ppm, CD_3OD: δ 3.31 ppm and DMSO-d_6: δ 2.50 ppm / 298 K).

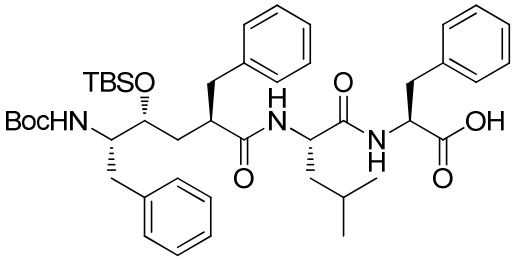
1. Chemical Synthesis of Probes



A B

L646-BPyne (5)

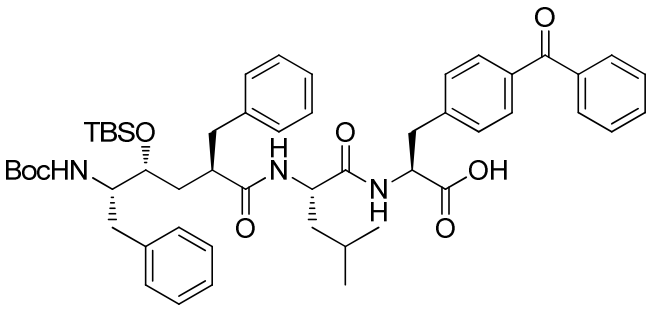
L646-BPyne (5). To a vial containing **A** (70 mg, 0.11 mmol), **B** (56 mg, 0.12 mmol), HOBt (22 mg, 0.17 mmol) and EDCI (32 mg, 0.17 mmol) was added DMF (3.0 ml) and DIPEA (0.06 ml, 0.33 mmol). The solution was stirred at room temperature for 17 h then diluted with EtOAc (50 ml), washed with sat. $NaHCO_3$ (2 x 20 ml), water (2 x 20 ml), brine (20 ml), dried ($MgSO_4$), filtered, and then evaporated to dryness under reduced pressure to afford the crude amide product as an off-white solid (110 mg, 93%) which was used in the next step without further purification. To a round bottom flask containing the crude amide (110 mg, 0.10 mmol) was added 1 M TBAF in THF (2.7 ml, 2.7 mmol) and the solution was allowed to stir for 2 h. Water (10 ml) was added and the solution was extracted with CH_2Cl_2 (3 x 15 ml). The organic layers were combined and then washed with water (3 x 10 ml), dried ($MgSO_4$), filtered and evaporated to dryness under reduced pressure. The resulting crude solid was purified by flash column chromatography (Phenomenex HILIC (Diol): 5-95% EtOH/Heptane) to afford the title compound L646-DiBPyne (**5**) as a white solid (80 mg, 75%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 (d, J = 9.0 Hz, 1H), 7.91 - 7.83 (m, 2H), 7.82 - 7.75 (m, 2H), 7.71 - 7.66 (m, 3H), 7.65 - 7.55 (m, 5H), 7.54 - 7.44 (m, 3H), 7.34 - 7.29 (m, 3H), 7.20 - 6.99 (m, 9H), 4.88 (d, J = 5.9 Hz, 1H), 4.46 (td, J = 8.3, 6.1 Hz, 1H), 4.20 - 4.10 (m, 1H), 4.10 - 3.97 (m, 1H), 3.54 (bs, 1H), 3.05 -2.97 (m, 2H), 2.96 - 2.73 (m, 3H), 2.72 - 2.60 (m, 3H), 2.54 (dd, J = 13.3, 6.2 Hz, 1H), 2.04 (td, J = 6.8, 2.7 Hz, 2H), 1.65 (d, J = 7.4 Hz, 1H), 1.62 - 1.52 (m, 1H), 1.51 - 1.39 (m, 1H), 1.39 - 1.16 (m, 6H), 0.73 (d, J = 6.2 Hz, 3H), 0.68 (d, J = 6.2 Hz, 3H); HRMS calcd for $C_{61}H_{64}N_4O_7$ (M+H) 965.4848, found 965.4874; HPLC Purity: >99%.



16

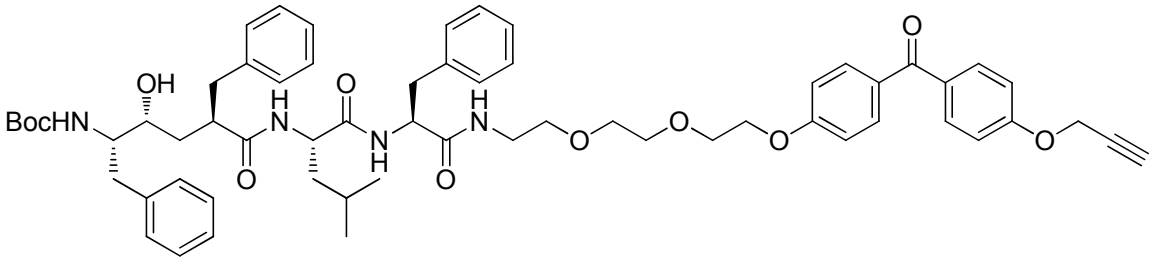
L458-carboxylic acid (16). To a stirred solution of the methyl ester of Leu-Phe **14** (167 mg, 0.57 mmol) and DMF (7.5 ml) at 0 °C was added HATU (223 mg, 0.57 mmol), OTBS-carboxylic acid **13** (200 mg, 0.38 mmol) and DIPEA (0.26 ml, 1.5 mmol). The solution was allowed to warm to RT slowly upon which TLC indicated consumption of starting material (2 h). The solution was diluted with EtOAc (30 ml) and washed with 0.5 N HCl (10 ml), after which the aqueous layer was back extracted with EtOAc (2 x 30 ml). The organics were combined and washed with water (10 ml), sat. $NaHCO_3$ (10 ml), 1 N LiCl (10 ml), brine (10 ml), dried (Na_2SO_4), filtered, and then evaporated to dryness under reduced pressure. The resulting crude solid was purified by flash column chromatography (ISCO: 0-40% EtOAc/Heptane) to obtain the intermediate L458-methyl ester (280 mg, 92%) as a white solid. To a 0 °C stirred solution of L458-methyl ester (150 mg, 0.187 mmol) and THF/MeOH (2 ml:2 ml) was added 1 N LiOH (0.37 ml, 0.37 mmol). The reaction was judged complete by TLC analysis (~1.5 h) and the solution was slowly acidified to pH 4 using 6 M HCl. The resulting suspension was extracted with EtOAc (3 x 20 ml) and the combined organics were washed with brine (10 ml), dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure to afford the title compound **16** as a white solid (146 mg, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 - 6.98 (m, 12H), 6.88 (d, J = 7.2 Hz, 2H), 4.94 - 4.90 (m, 1H), 4.68 - 4.71 (m, 1H), 4.60 (d, J = 8.4 Hz, 1H), 3.79 - 3.73 (m, 2H), 3.17 - 2.99 (m, 3H), 2.86 - 2.83 (m, 1H), 2.62 - 2.48 (m, 2H), 2.46 - 2.27 (m, 2H), 1.91 - 1.84 (m, 2H), 1.68 - 1.56 (m, 2H), 1.53 - 1.44 (m, 2H), 1.30 (s, 9H), 0.91 (s, 9H), 0.80 - 0.73 (m,

6H), 0.08 (s, 3H), 0.03 (s, 3H); LRMS calcd for $C_{61}H_{64}N_4O_7$ (M+Na) 810.5, found 810.5; HPLC Purity: 95.6%.



17

L505-carboxylic acid (17). Prepared using the same procedure as described for compound **16**. In brief, the methyl ester of Leu-Bpa **15** (167 mg, 0.57 mmol) afforded 188 mg (95%) of the title compound **17** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 - 7.38 (m, 7H), 7.34 - 7.08 (m, 10H), 6.90 (d, J = 6.8 Hz, 2H), 4.89 - 4.71 (m, 1H), 4.66 - 4.59 (m, 2H), 3.85 - 3.63 (m, 3H), 3.36 - 3.01 (m, 3H), 2.98 - 2.74 (m, 1H), 2.66 - 2.29 (m, 2H), 1.90 - 1.77 (m, 2H), 1.79 - 1.40 (m, 7H), 1.29 (s, 9H), 0.90 (s, 9H), 0.78 (bs, 6H), 0.06 (s, 3H), 0.02 (s, 3H); LRMS calcd for $C_{61}H_{64}N_4O_7$ (M+Na) 810.5, found 810.5; HPLC Purity: 97.6%.



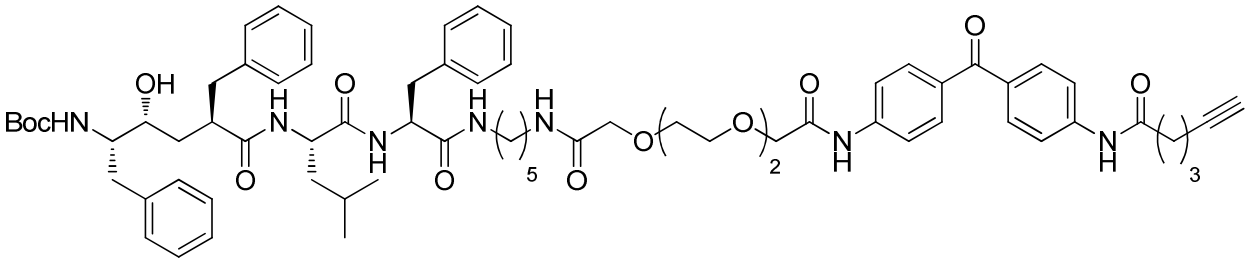
L458-BPyne-Short (7)

L458-BPyne-Short (7). To a 0 °C stirred solution of L458-carboxylic acid **16** (37 mg, 0.05 mmol) and DMF (0.5 ml) was added propargylbenzophenone-PEG_2 amine **18** (30 mg, 0.07

mmol), HATU (28 mg, 0.07 mmol) and DIPEA (0.025 ml, 0.14 mmol). The solution was slowly allowed to warm to RT and the reaction was judged complete by TLC analysis (22 h). The solution was diluted with CH_2Cl_2 (20 ml) and washed with water, after which the aqueous layer was back extracted with CH_2Cl_2 (2 x 10 ml). The organics were combined and washed with water (10 ml), 1 N LiCl (10 ml), brine (10 ml), dried (Na_2SO_4), filtered, and then evaporated to dryness under reduced pressure. The resulting crude oil was purified by flash column chromatography (ISCO: 0-10% MeOH/ CH_2Cl_2) to obtain the intermediate L458-BPyne-Short-OTBS (45 mg, 83%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (t, *J* = 6.3 Hz, 2H), 7.36 - 7.12 (m, 17H), 7.05 (d, *J* = 7.0 Hz, 2H), 6.97 (d, *J* = 6.6 Hz, 2H), 6.45 (bs, 1H), 6.34 (bs, 1H), 6.21 (d, *J* = 7.2 Hz, 1H), 4.78 (s, 2H), 4.63 - 4.47 (m, 1H), 4.25 - 4.20 (m, 3H), 4.00 - 3.75 (m, 3H), 3.73 - 3.26 (m, 8H), 3.11 - 3.05 (m, 1H), 2.90 - 2.75 (m, 3H), 2.68 - 2.64 (m, 1H), 2.58 (bs, 1H), 2.59 - 2.42 (m, 2H), 1.85 - 1.60 (m, 4H), 1.55 - 1.40 (m, 2H), 1.34 (s, 9H), 1.33 - 1.28 (m, 1H), 0.91 (s, 9H), 0.85 (m, 3H), 0.81 (m, 3H), 0.05 (s, 3H), 0.03 (s, 3H); LRMS calcd for $C_{67}H_{88}N_4O_{11}Si$ (M+Na) 1175.6, found 1175.8.

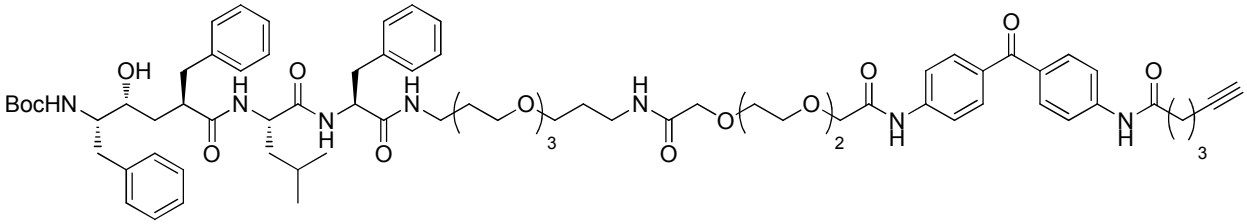
To a stirred solution of L458-BPyne-Short-OTBS (22 mg, 0.02 mmol) and THF (0.1 ml) at 0 °C was added 1 M TBAF in THF (0.38 ml, 0.38 mmol) dropwise and the solution was allowed to slowly warm to room temperature. The reaction was judged complete by TLC analysis (8 h) and the solution was diluted with EtOAc (15 ml) and then washed with sat. NH_4Cl (10 ml), brine (10 ml), dried (Na_2SO_4), filtered, and then evaporated to dryness under reduced pressure. The resulting crude oil was purified by flash column chromatography (ISCO: 0-100% EtOAc/Heptane or 0-10% MeOH/ CH_2Cl_2) to afford the title compound L458-BPyne-Short (**7**) as a white solid (14.3 mg, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (t, *J* = 8.7 Hz, 4H), 7.36 - 7.07 (m, 15H), 7.28 (d, *J* = 8.6 Hz, 2H), 6.93 (d, J = 8.6 Hz, 2H), 6.57 (bs, 1H), 5.93 (bs, 1H),

4.90 (d, *J* = 8.4 Hz, 1H), 4.76 (s, 2H), 4.65 (q, *J* = 7.8 Hz, 1H), 4.19 (t, *J* = 4.2 Hz, 2 H), 4.06 (bs, 1H), 3.87 (d, *J* = 3.9 Hz, 2H), 3.79 (bs, 1H), 3.72 - 3.59 (m, 4H), 3.58 - 3.44 (m, 4H), 3.38 - 3.15 (m, 2H), 2.94 - 2.77 (m, 3H), 2.76 - 2.59 (m, 3H), 2.58 - 2.55 (m, 1H), 1.72 (bs, 2H), 1.51 - 1.39 (m, 2H), 1.32 (bs, 9H), 1.25 (bs, 2H), 0.82 (d, *J* = 5.9 Hz, 3H), 0.76 (d, *J* = 5.7 Hz, 3H); HRMS calcd for $C_{61}H_{74}N_4O_{11}$ (M+H) 1039.5427, found 1039.5455; HPLC Purity: 96.8%.



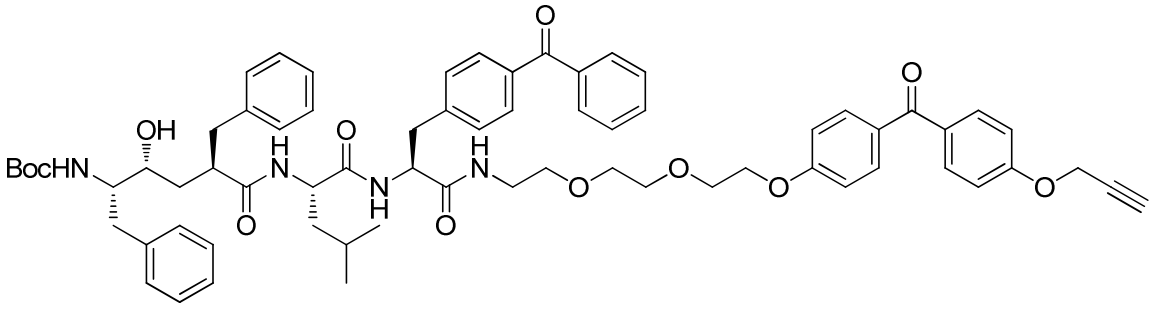
L458-BPyne-Medium (9)

L458-BPyne-Medium (9). Prepared using the same procedure as described for compound **7**. In brief, diaminopentane-PEG_2benzophenone alkyne **21** (29 mg, 0.05 mmol) afforded 40 mg (71%) of the intermediate OTBS compound as a white solid. The intermediate silyl-protected alcohol (13.5 mg, 0.01 mmol) was deprotected with 1 M TBAF in THF to afford the title compound L458-BPyne-Medium (**9**) as a white solid (10.3 mg, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.00 (bs, 1H), 8.22 (bs, 1H), 7.80 - 7.65 (m, 8H), 7.31 - 7.15 (m, 10H), 7.15 - 7.04 (m, 6H), 6.83 (bs, 1H), 6.78 (bs, 1H), 6.59 (bs, 1H), 6.22 (bs, 1H), 4.89 (d, *J* = 8.0 Hz, 1H), 4.64 - 4.54 (m, 1H), 4.15 (s, 2H), 3.97 (s, 2H), 3.81 - 3.78 (m, 2H), 3.77 - 3.71 (m, 4H), 3.67 - 3.61 (m, 2H), 3.30 - 3.04 (m, 6H), 3.02 - 2.92 (m, 2H), 2.89 - 2.59 (m, 6H), 2.55 (t, *J* = 7.2 Hz, 2H), 2.32 (td, *J* = 6.7, 2.5 Hz, 2H), 2.00 (t, *J* = 2.4 Hz, 1H), 1.99 - 1.93 (m, 2H), 1.83 - 1.64 (m, 4H), 1.45 - 1.35 (m, 4H), 1.33 (s, 9H), 1.32 - 1.19 (m, 2H), 0.80 (d, *J* = 5.9 Hz, 3H), 0.74 (d, *J* = 6.1 Hz, 3H); HRMS calcd for $C_{71}H_{91}N_7O_{13}$ (M+H) 1250.6748, found 1250.6750; HPLC Purity: >99%.



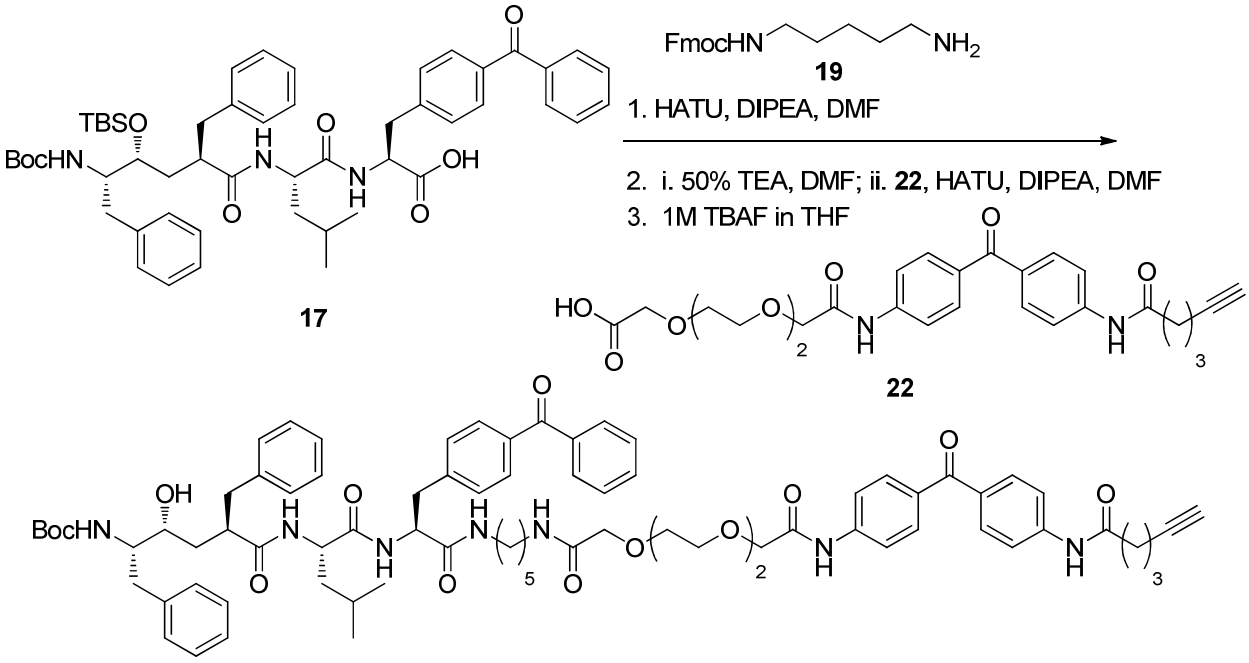
L458-BPyne-Long (11)

L458-BPyne-Long (11). Prepared using the same procedure as described for compound **7**. In brief, diaminoPEG$_3$-PEG$_2$benzophenone alkyne **20** (38 mg, 0.05 mmol) afforded 13.5 mg (71%) of the intermediate OTBS compound as a colorless oil. The intermediate silyl-protected alcohol (13.5 mg, 0.01 mmol) was deprotected with 1 M TBAF in THF to afford the title compound L458-BPyne-Long (**11**) as a white solid (11.6 mg, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.96 (s, 1H), 8.15 (s, 1H), 7.82 - 7.65 (m, 8H), 7.29 - 7.15 (m, 10H), 7.15 - 7.06 (m, 6H), 7.00 (bs, 2H), 6.72 (t, *J* = 5.0 Hz, 1H), 6.22 (d, *J* = 6.3 Hz, 1H), 4.82 (d, *J* = 8.2 Hz, 1H), 4.61 - 4.49 (m, 1H), 4.15 (s, 2H), 4.13 - 4.04 (m, 1H), 3.97 (s, 2H), 3.82 - 3.76 (m, 2H), 3.76 - 3.69 (m, 6H), 3.67 - 3.63 (m, 1H), 3.62 - 3.51 (m, 6H), 3.51 - 3.43 (m, 4H), 3.41 - 3.36 (m, 2H), 3.36 - 3.27 (m, 3H), 3.25 - 3.06 (m, 2H), 2.96 (dt, *J* = 12.6, 4.5 Hz, 2H), 2.88 (dd, *J* = 13.6, 7.5 Hz, 2H), 2.83 - 2.75 (m, 1H), 2.75 - 2.59 (m, 2H), 2.55 (t, *J* = 7.2 Hz, 2H), 2.37 - 2.28 (m, 2H), 2.01 (t, *J* = 2.6 Hz, 1H), 1.96 (quin, *J* = 7.0 Hz, 2H), 1.82 - 1.60 (m, 6H), 1.42 - 1.36 (m, 1H), 1.33 (s, 9H), 1.29 - 1.23 (m, 2H), 0.82 (d, *J* = 6.3 Hz, 3H), 0.76 (d, *J* = 6.3 Hz, 3H); HRMS calcd for $C_{76}H_{101}N_7O_{16}$ (M+NH_4) 1385.7643, found 1385.7658; HPLC Purity: 95.8%.



L505-BisBPyne-Short (8)

L505-BisBPyne-Short (8). Prepared using the same procedure as described for compound **7**. In brief, L505-carboxylic acid **17** (27 mg, 0.03 mmol), propargylbenzophenone-PEG_2 amine **18** (19 mg, 0.05 mmol) afforded 33 mg (88%) of the intermediate OTBS compound as a white solid. The intermediate silyl-protected alcohol (33 mg, 0.025 mmol) was deprotected with 1 M TBAF in THF to afford the title compound L505-BisBPyne-Short (**8**) as a white solid (28 mg, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 - 7.69 (m, 6H), 7.57 (d, J = 7.0 Hz, 1H), 7.46 (t, J = 6.8 Hz, 2H), 7.33 - 7.16 (m, 12H), 7.12 (bs, 2H), 7.04 (d, J = 8.8 Hz, 2H), 6.93 (d, J = 8.8 Hz, 2H), 6.67 (bs, 1H), 5.84 (bs, 1H), 4.87 (bs, 1H), 4.74 (s, 2H), 4.74 - 4.70 (m, 1H), 4.16 (bs, 2H), 4.07 - 4.01 (m, 1H), 3.88 (bs, 2H), 3.82 - 3.76 (m, 1H), 3.73 - 3.65 (m, 4H), 3.63 - 6.54 (m, 4H), 3.44 - 3.36 (m, 1H), 3.34 - 3.27 (m, 1H), 2.99 - 2.79 (m, 3H), 2.79 - 2.61 (m, 3H), 2.57 (d, J = 2.3 Hz, 1H), 1.77 - 1.71 (m, 2H), 1.46 - 1.48 (m, 2H), 1.33 (s, 9H), 1.33 - 1.20 (m, 2H), 0.83 (d, J = 4.7 Hz, 3H), 0.77 (d, J = 4.9 Hz, 3H); HRMS calcd for $C_{68}H_{78}N_4O_{12}$ (M+H) 1143.5689, found 1143.5713; HPLC Purity: 93.8%.



L505-BisBPyne-Medium (10)

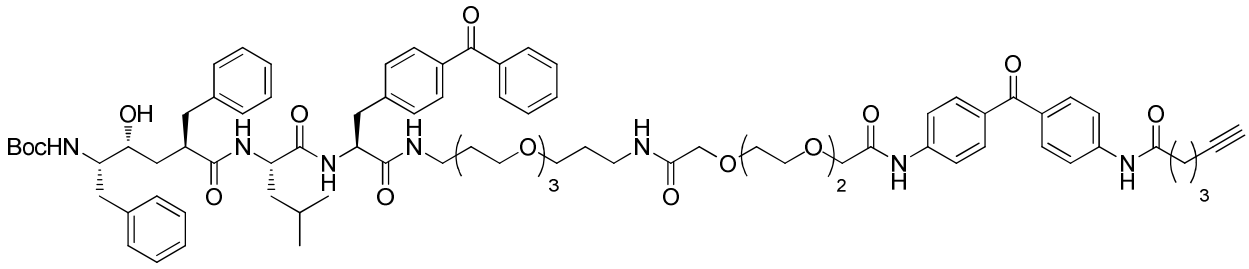
L505-BisBPyne-Medium (10). To a stirred solution of L505-carboxylic acid **17** (73 mg, 0.08 mmol) and DMF (0.8 ml) at 0 °C was added mono-Fmoc 1,5-diaminopentane **19** (44 mg, 0.12 mmol), HATU (48 mg, 0.12 mmol) and DIPEA (0.06 ml, 0.33 mmol). The solution was slowly warmed to RT and the reaction was judged complete by TLC analysis (24 h). The solution was diluted with EtOAc (30 ml), washed with 0.5 N HCl (10 ml) after which the aqueous layer was back extracted with EtOAc (20 ml). The organics were combined and washed with water (10 ml), sat. $NaHCO_3$ (10 ml), 1 N LiCl (10 ml), brine (10 ml), dried (Na_2SO_4), filtered, and then evaporated to dryness under reduced pressure. The resulting crude solid was purified by flash column chromatography (ISCO: 0-75% EtOAc/Heptane) to obtain the intermediate L505-Fmoc-pentylamine (70 mg, 71%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 7.2 Hz, 2H), 7.72 (d, J = 7.6 Hz, 2H), 7.62 (d, J = 7.4 Hz, 2H), 7.57 (t, J = 7.2 Hz, 1H), 7.45 (t, J = 7.4 Hz, 2H), 7.40 (t, J = 7.1 Hz, 2H), 7.34 - 7.19 (m, 12H), 7.15 (d, J = 7.4 Hz, 2H), 7.02 (d, J = 6.6 Hz, 2H), 6.63 (bs, 1H), 6.46 (bs, 1H), 5.97 (d, J = 8.0 Hz, 1H), 5.13 (bs, 1H), 4.60 (d, J = 7.8 Hz,

1H), 4.41 - 4.37 (m, 2H), 4.23 (t, *J* = 6.4 Hz, 1H), 4.16 - 4.12 (m, 1H), 3.83 (d, *J* = 12.1 Hz, 1H), 3.76 - 3.68 (m, 1H), 3.38 - 3.28 (m, 2H), 3.23 - 3.13 (m, 2H), 2.86 - 2.68 (m, 3H), 2.61 - 2.42 (m, 4H), 1.84 - 1.80 (m, 1H), 1.69 - 1.58 (m, 2H), 1.56 - 1.45 (m, 3H), 1.45 - 1.37 (m, 3H), 1.35 - 1.26 (m, 2H), 1.33 (s, 9H), 0.91 (s, 9H), 0.82 (d, *J* = 5.5 Hz, 3H), 0.75 (bs, 3H), 0.05 (s, 3H), 0.03 (s, 3H); LRMS calcd for $C_{72}H_{91}N_5O_9Si$ (M+Na) 1220.65, found 1220.7.

L505-Fmoc-pentylamine was solvated in DMF (1.2 ml) and TEA (1.2 ml) was added in one portion with stirring. The solution was judged complete by TLC analysis (18 h) and concentrated to dryness under reduced pressure with the aid of toluene. The resulting crude film of L505-pentylamine was used in the next step without further purification. In a similar manner as above using L505-pentylamine (47 mg, 0.05 mmol), PEG_2benzophenone alkyne carboxylic acid **22** (37 mg, 0.07 mmol) afforded 32 mg (43%) of the intermediate OTBS compound as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.98 (bs, 1H), 7.87 (bs, 1H), 7.81 - 7.63 (m, 9H), 7.61 - 7.54 (m, 1H), 7.48 - 7.42 (m, 2H), 7.31 - 7.20 (m, 7H), 7.16 - 7.11 (m, 2H), 7.01 - 6.96 (m, 2H), 6.75 (bs, 1H), 6.62 (bs, 1H), 6.56 (bs, 1H), 6.17 (bs, 1H), 4.63 - 4.56 (m, 1H), 4.16 (s, 2H), 3.99 (s, 2H), 3.84 - 3.78 (m, 2H), 3.78 - 3.71 (m, 4H), 3.35 - 3.05 (m, 6H), 2.91 - 2.73 (m, 4H), 2.71 - 2.66 (m, 2H), 2.61 - 2.53 (m, 2H), 2.52 - 2.41 (m, 1H), 2.38 - 2.31 (m, 2H), 2.07 - 1.93 (m, 2H), 1.86 - 1.74 (m, 2H), 1.70 - 1.62 (m, 2H), 1.52 - 1.37 (m, 6H), 1.33 (s, 9H), 1.29 - 1.21 (m, 6H), 0.91 (s, 9H), 0.82 (m, 3H), 0.76 (m, 3H), 0.05 - 0.00 (m, 6H); LRMS calcd for $C_{84}H_{109}N_7O_{14}Si$ (M+Na) 1490.77, found 1491.0.

The intermediate silyl-protected alcohol (23 mg, 0.016 mmol) was deprotected with 1 M TBAF in THF to afford the title compound L505-BisBPyne-Medium (**10**) as a white solid (18 mg, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.00 (s, 1H), 7.99 (s, 1H), 7.80 - 7.62 (m, 10H), 7.61 - 7.56 (m, 1H), 7.48 - 7.43 (m, 2H), 7.31 - 7.21 (m, 12H), 7.15 - 7.09 (m, 2H), 7.01 - 6.96 (m, 2H),

6.80 (bs, 1H), 6.70 (bs, 1H), 6.09 (bs, 1H), 4.94 - 4.88 (m, 1H), 4.73 - 4.64 (m, 1H), 4.16 (s, 2H), 4.04 - 3.99 (m, 1H), 3.98 (s, 2H), 3.84 - 3.79 (m, 2H), 3.79 - 3.72 (m, 4H), 3.72 - 3.66 (m, 2H), 3.41 - 3.31 (m, 1H), 3.29 - 3.09 (m, 5H), 3.02 - 2.63 (m, 7H), 2.57 (t, J = 7.2 Hz, 2H), 2.34 (td, J = 6.6, 2.3 Hz, 2H), 2.01 (t, J = 2.4 Hz, 1H), 1.99 - 1.96 (m, 2H), 1.83 - 1.72 (m, 4H), 1.45 - 1.36 (m, 4H), 1.34 (s, 9H), 1.32 - 1.19 (m, 2H), 0.81 (d, J = 6.4 Hz, 3H), 0.75 (d, J = 6.4 Hz, 3H); HRMS calcd for $C_{78}H_{95}N_7O_{14}$ (M+H) 1354.7010, found 1354.7016; HPLC Purity: 93.4%.



L505-BisBPyne-Long (12)

L505-BisBPyne-Long (12). Prepared using the same procedure as described for compound **7**. In brief, L505-carboxylic acid **17** (39 mg, 0.045 mmol), DiaminoPEG$_3$-PEG$_2$benzophenone alkyne **20** (38 mg, 0.05 mmol) afforded 11 mg (16%) of the intermediate OTBS compound as a light yellow oil. The intermediate silyl-protected alcohol (11 mg, 0.007 mmol) was deprotected with 1 M TBAF in THF to afford the title compound L505-BisBPyne-Long (**12**) as a white solid (8.2 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.96 (s, 1H), 8.03 (s, 1H), 7.80 - 7.64 (m, 10H), 7.57 (t, J = 7.5 Hz, 1H), 7.45 (t, J = 7.4 Hz, 2H), 7.31 - 7.14 (m, 12H), 7.14 - 7.08 (m, 3H), 7.01 (bs, 1H), 6.90 (bs, 1H), 6.22 (bs, 1H), 4.82 (d, J = 8.1 Hz, 1H), 4.67 - 4.61 (m, 1H), 4.15 (s, 2H), 4.13 - 4.06 (m, 1H), 3.97 (s, 2H), 3.82 - 3.77 (m, 2H), 3.76 - 3.69 (m, 6H), 3.67 - 3.63 (m, 1H), 3.62 - 3.39 (m, 12H), 3.36 - 3.28 (m, 3H), 3.28 - 3.16 (m, 2H), 3.00 - 2.87 (m, 4H), 2.86 - 2.77 (m, 1H), 2.76 - 2.63 (m, 2H), 2.55 (t, J = 7.2 Hz, 2H), 2.36 - 2.29 (m, 2H), 2.01 (t, J = 2.5 Hz, 1H), 1.96 (quin, J = 7.0 Hz, 2H), 1.82 - 1.62 (m, 6H), 1.42 - 1.36 (m, 1H), 1.33 (s, 9H), 1.32 - 1.23 (m,

2H), 0.82 (d, J = 6.5 Hz, 3H), 0.77 (d, J = 6.3 Hz, 3H); HRMS calcd for $C_{83}H_{105}N_7O_{17}$ (M+NH_4) 1489.7905, found 1489.7906; HPLC Purity: >99%.

2. Biological Methods

2.1 Labeling of HeLa membranes with Clickable Photoprobes – Western Blot Analysis

HeLa cell membranes (800 µg in 1.2 ml PBS containing 1X Halt protease inhibitor (Thermo Scientific)) were treated with L458 (**1**) or DMSO control for 30 min followed by the photoprobe at the designated concentrations for 1 h at 37 °C and then UV-irradiated (365 nm) for 30 min at 4 °C. Following photocrosslinking, the membranes were precipitated by ultracentrifugation at 125,000 x *g* for 30 min and re-suspended in PBS (900 µL) with the aid of a Qiagen TissueLyser II (3 min, 25 shakes/sec). CHAPSO (0.25%) was added, followed by the click chemistry reagents (1 mM TCEP, 1 mM $CuSO_4$, 0.1 mM TBTA (DMSO:*t*-Butanol 1:4), and 50 – 100 µM biotin azide) and the mixture was shaken for 1.5 h at RT. The reactions were transferred to centrifuge tubes, diluted with PBS (3 ml) and ultracentrifuged at 100,000 x *g* for 30 min at 4 °C. The resulting pellets were resuspended in PBS (2 x 200 µL) with the aid of a Qiagen TissueLyser II, diluted to 1X RIPA buffer (1 ml) and solubilized overnight with rotation agitation followed by centrifugation at 20,000 x *g* for 10 min at 4 °C. The supernatant was then added to 100 µL of pre-washed streptavidin magnetic beads (Pierce) and incubated with rotation for 2.5 h at room temperature. The beads were washed with 1X RIPA (3 x 500 µL) and then transferred to new eppendorf tubes with PBS (100 µL). Biotinylated proteins were eluted with ~30 µL elution buffer (2X LDS, 2 mM Biotin) for 10 min at 70 °C and then loaded onto an SDS-PAGE gel for protein separation (4-12% Bis-Tris NuPAGE SDS with MES running buffer (Life

Technologies)). After electrophoresis, proteins were transferred to a nitrocellulose membrane and blotted for PS1-NTF, PS1-CTF or nicastrin.

2.2 Labeling of HeLa membranes with Clickable Photoprobes – In-gel Fluorescence

The procedure in 2.1 was followed using biotin-TAMRA-azide (50-100 μM) during the click reaction. After gel electrophoresis, gels were visualized on a Typhoon 9210 flat-bed scanner using a TAMRA filter set.

2.3 Labeling of live cells with Clickable Photoprobes – Western Blot and MS Analysis

HeLa and HEK293 ANPP.8 cells[1] were maintained in Dulbecco's Modified Eagle Medium (DMEM, Life Technologies) supplemented with 10% fetal bovine serum (Life Technologies), 20 mM L-glutamine (Life Technologies), 20 Units/ml penicillin, and 20 μg/ml streptomycin at 37 ˚C / 5% CO2. Cells were plated at ~4 x 10^5 cells/well in 6-well plates (or ~4 x 10^6 for MS experiments) and allowed to reach 90-100% confluence. The cells were treated with L458 (**1**) or DMSO control for 30 min followed by the photoprobe at the designated concentrations for 1-2.5 h at 37 °C / 5% CO_2 and then UV-irradiated (365 nm) for 15 min at 4 °C. Following photocrosslinking, cells were lysed by sonication (6 x 6W pulses) with Halt protease inhibitor cocktail (Thermo Scientific) added to 1X. Membranes were precipitated by centrifugation at 110,000 x *g* for 30 min, and re-suspended in PBS with the aid of a Qiagen TissueLyser II (3 min, 25 shakes/sec). CHAPSO (0.25%) was added, followed by the click chemistry reagents (1 mM TCEP, 1 mM $CuSO_4$, 0.1 mM TBTA, and 0.05 mM biotin-TAMRA-azide) and the mixture was shaken for 1.5 h at RT. The membranes were pelleted by centrifugation at 100,000 x *g* for 30 min, washed once with PBS and solubilized overnight in RIPA buffer (1 ml) followed by affinity

enrichment with Streptavidin magnetic beads (Pierce). Biotinylated proteins were eluted with 2 mM biotin in 2X LDS sample buffer (Life Technologies) and separated on a 4-12% NuPAGE Bis-Tris gel in MES running buffer (Life Technologies). After electrophoresis, proteins were transferred to a nitrocellulose membrane and blotted for PS1-NTF, PS1-CTF or nicastrin, or protein bands were visualized on a Typhoon 9210 flat-bed scanner using a TAMRA filter set. The gels were then silver stained (Silver Quest – Invitrogen) to determine protein concentration and alignment for MS studies. A repeat, unstained gel was sectioned into 14 equal parts per lane for both the competition and non-competition lanes and each gel piece was dehydrated and dried, then rehydrated in 75 µL of 25 mM NH_4HCO_3, 4.5 mM DTT, and incubated at 50 °C for 20 min. The samples were then cooled and treated with 100 mM iodoacetamide (8.3 µL) for 20 min followed by 25 mM NH_4HCO_3 (25 µL) containing 1 µg trypsin and incubated overnight at 37 °C. The digest was removed, and gel pieces were further extracted with 100 µL and 50 µL of 50% CH_3CN, 5% TFA, sequentially. Each combined digest was dried and then re-dissolved in 0.1% formic acid (15 µL). LC-MS was performed on a LTQ Orbitrap XL with 8 µL injections with the order of analysis of C14-C1 followed by one wash cycle and N14-N1 to minimize risks from carryover. Mascot searches were run vs. UniProtHuman and an additional database of common contaminants.[2]

3. Supplemental Figures

Figure S1. L505-BisBPyne and L458-BPyne analogue photolabelling of γ-secretase components in HeLa cell membrane preparations. Click chemistry performed using biotin-TAMRA-azide.

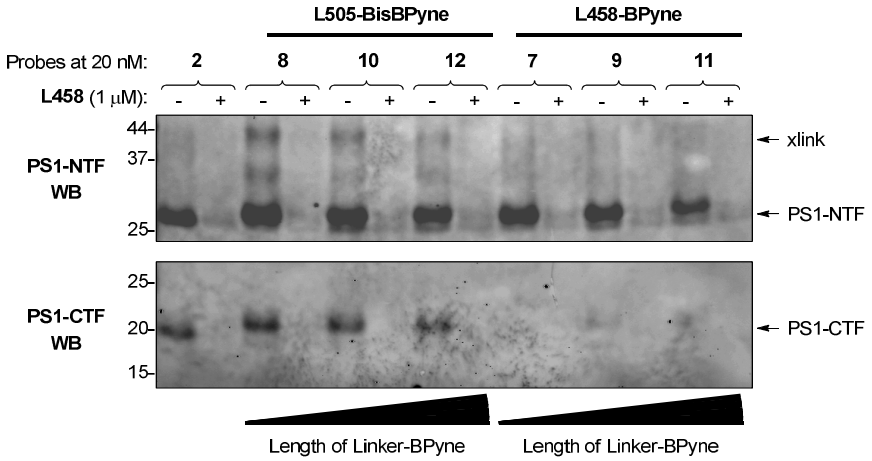


Figure S2. L505-BisBPyne-Medium (**10**) photolabelling in live cells.

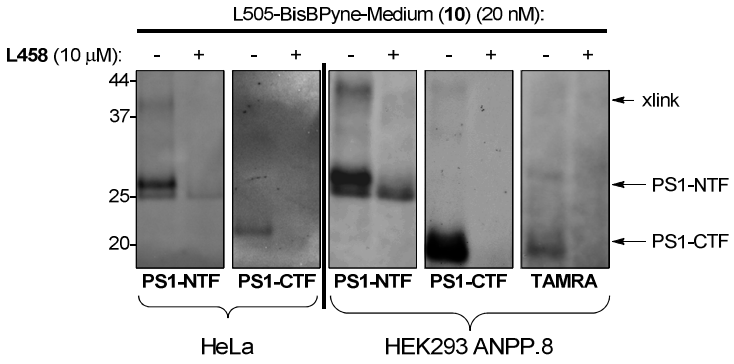


Figure S3. L505-BisBPyne-Long (**12**) photolabelling in live cells.

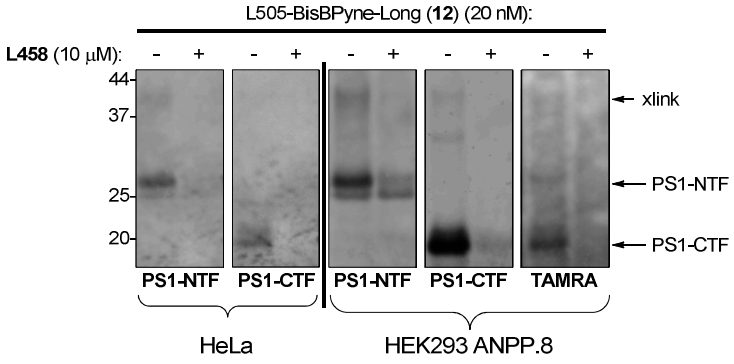


Figure S4. L505-BisBPyne-Short (**8**) photolabelling in living primary cortical neurons.

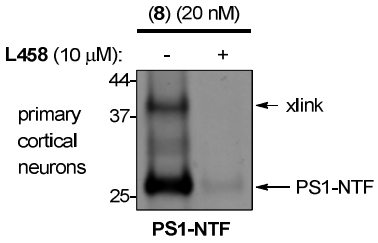
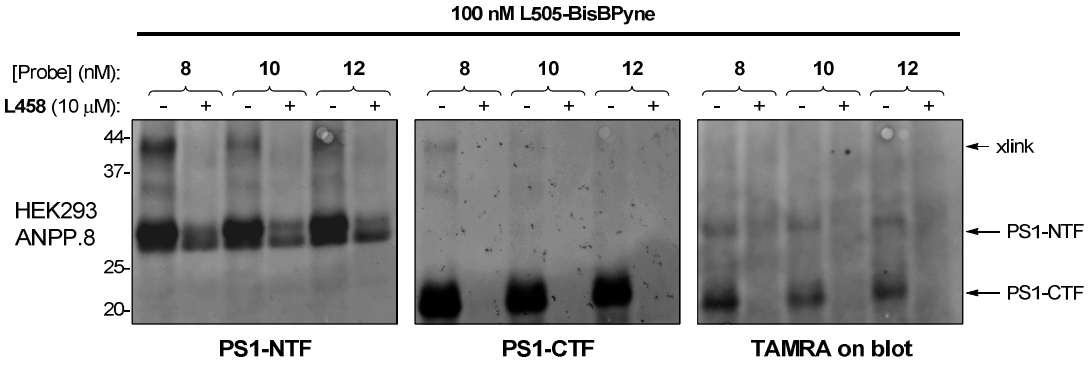


Figure S5. Photolabelling of γ-secretase components with L505-BisBPyne analogues in live HEK293 ANPP.8 cells at 37 °C. After a 2.5 hour probe incubation period, HEK293 ANPP.8 cells were exposed to UV irradiation without cooling for 15 minutes.



[1] S. H. Kim, T. Ikeuchi, C. Yu, S. S. Sisodia. *J Biol Chem*, 2003, **278**, 33992–34002.
[2] D. N. Perkins, D. J. C. Pappin, D. M. Creasy, J. S. Cottrell. *Electrophoresis*, 1999, **20**, 3551-3567.