Supporting Information I

"Click" synthesis of vinyl sulfone-containing small molecule inhibitors targeting caspases

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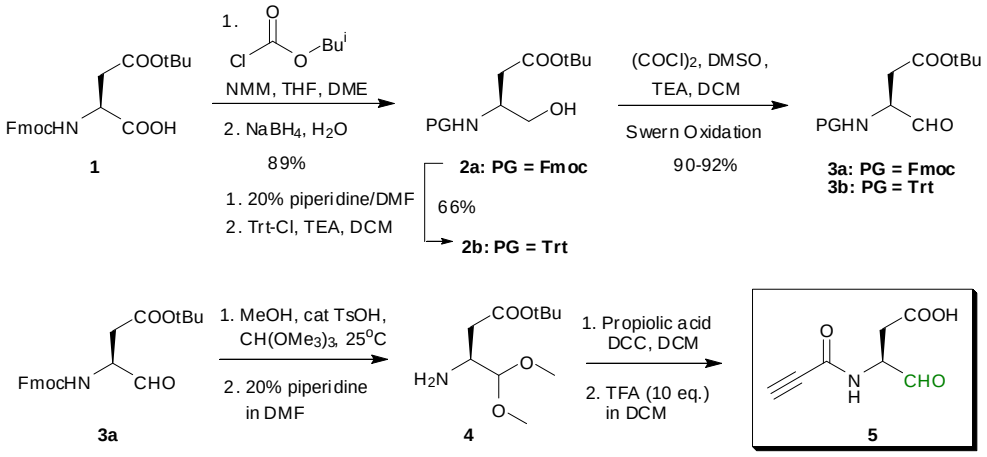
1. General Information

All chemicals were purchased from commercial vendors and used without further purification, unless otherwise noted. All solvents used in the synthesis are reagent grade and distilled. All reactions were carried out under an inert atmosphere using flame-dried apparatus, unless otherwise stated. ^{1}H & ^{13}C NMR spectra were taken on a Bruker ACF-300/500 MHz NMR spectrometer, using $CDCl_3$, CD_3OD or $(CD_3)_2SO$ as the solvent. Chemical shifts are reported in parts per million referenced with respect to residual solvent ($CDCl_3$ = 7.26 ppm, CD_3OD = 3.31 and $(CD_3)_2SO$ = 2.50). ESI mass spectra were obtained using a Finnigan/Mat TSQ7000 spectrometer. LC-MS spectra were recorded using Shimadzu LCMS IT-TOF. Extent of reaction was monitored by Thin Layer Chromatography (TLC) using Merck 60 F_{254} pre-coated silica gel plates with fluorescent indicator UV254. After the plates were subjected to elution in the TLC chamber, the spots were visualized under UV light or using the appropriate stain (I_2, $KMNO_4$, ninhydrin, p-anisaldehyde). Flash column chromatography was carried out using Merck silica gel (0.040-0.063).

2. Chemical synthesis

2.1 Synthesis of Asp-containing aldehyde warhead



Scheme S1. Synthesis of Asp-containing aldehyde warhead

The synthesis of Asp-containing aldehyde warhead involves 4 steps. The Fmoc-protected amino acid **1** was initially activated using isobutylchloroformate, followed by reduction of the mixed anhydride to the amino alcohol **2a**.[1] Swern oxidation gave the aldehyde intermediate **3a**, which was then treated with trimethyl orthoformate and Fmoc-deprotection condition (20% piperidine/DMF) to generate dimethyl acetal **4**. Finally, the alkyne handle was installed through DCC coupling with propiolic acid, affording the final aldehyde warhead **5**.[2]

*Procedure for the conversion of **1** to **5**:*

(*S*)-*tert*-butyl 3-(((9H-fluoren-9-yl)methoxy)carbonylamino)-4-hydroxybutanoate **(2a)**:

Synthesized based on previously published procedures.[1] Yield = 91%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.76 (d, ^3J = 7.4 Hz, 2H), 7.59 (d, ^3J = 7.4 Hz, 2H), 7.40 (t, ^3J = 7.2 Hz, 2H), 7.31 (t, ^3J = 7.4 Hz, 2H), 5.51(d, ^3J = 7.4 Hz, 1H), 4.40 (d, ^3J = 6.8 Hz, 2H), 4.21 (t, ^3J = 6.8 Hz, 1H), 4.02 (s, 1H), 3.71, (s, 1H), 2.55 (s, 1H), 1.45 (s, 9H).

(*S*)-*tert*-butyl 3-(((9H-fluoren-9-yl)methoxy)carbonylamino)-4-oxobutanoate **(3a)**:

Dry DMSO (3.95 g, 50.5 mmol) was dissolved under argon at -45 ºC in anhydrous CH_2Cl_2 (60 mL), and oxalyl chloride (3.5 g, 27.5 mmol) was added dropwise (immediate gas production). After 5 min, a solution of **2a** (7.1 g, 22.9 mmol) in anhydrous CH_2Cl_2 (35 mL) was added dropwise at -45 ºC and the white suspension was stirred for 30 min. Following the addition of *i*Pr_2EtN (9.2 g, 71 mmol), the mixture was allowed to warm up to -20 ºC, where stirring was continued for another 30 min. The solution was diluted with CH_2Cl_2 (200 mL) and extracted with H_2O (50 ml), 1N $NaHSO_4$ (50 ml), and again with H_2O (3 x 50 mL). The organic phase was dried (Na_2SO_4) and evaporated to give a yellow oil (7.0 g, 95%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.45 (s, 9H), 2.79 (dd, J_1= 4.7 Hz, J_2= 17.0 Hz, 1H), 2.96 (dd, J_1= 4.7 Hz, J_2= 17.6 Hz, 1H), 4.24 (t, J = 6.9 Hz, 1H), 4.42 (m, 2H), 5.89 (d, J = 8.2 Hz, 1H), 7.32 (t, J = 7.6 Hz, 2H), 7.40 (t, J =

7.6 Hz, 2H), 7.60 (d, *J* = 7.6 Hz, 2H), 7.78 (d, *J* = 7.6 Hz, 2H), 9.65 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.0, 35.7, 47.1, 56.5, 67.3, 82.3, 120.0, 123.8, 125.0, 127.1, 127.7 (t), 141.3, 143.6 (d), 149.7, 156.2, 170.2, 198.7.

(*S*)-*tert*-butyl 3-amino-4,4-dimethoxybutanoate **(4)**: [1]

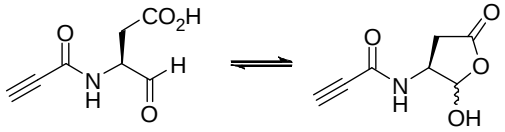
At r.t., trimethyl orthoformate (11.2 g, 105 mmol) and TsOH (100 mg) were added to a solution of Fmoc-Asp-(OtBu)-H (6.50 g, 21.1 mmol) in MeOH (30 mL). After 4 hrs, the mixture was evaporated, the residue dissolved in CH_2Cl_2 (200 ml), and the solution extracted with 5% $NaHCO_3$ (50 mL) and H_2O (50 mL). The extraction of the aqueous layer with CH_2Cl_2 (200 mL) was repeated. The combined organic phase was dried (Na_2SO_4) and evaporated and the residue purified by flash column chromatography (AcOEt:hexane (1:4) with 0.5% Et_3N) (6.5 g, 83%). Yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (s, 9H), 2.52 (m, 2H), 3.43 (d, *J* = 3.8 Hz, 6H), 4.23 (t, *J* = 6.9 Hz, 2H), 4.38 (t, *J* = 6.9 Hz, 2H), 5.38 (d, *J* = 8.8 Hz, 1H), 7.31 (t, *J* = 6.9 Hz, 2H), 7.40 (t, *J* = 6.9 Hz, 2H), 7.60 (d, *J* = 7.6 Hz, 2H), 7.76 (d, *J* = 7.6 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.0, 35.6, 47.2, 49.7, 55.4, 56.0, 66.8, 81.0, 105.0, 119.9, 125.1 (d), 127.0, 127.6, 141.3, 143.9, 155.9, 170.8.

The Fmoc-Asp(OtBu)-H dimethyl acetal (4.22 g, 10 mmol) was dissolved in 20 mL of piperidine/DMF (1:4) and stirred at room temperature for 2 h, and was concentrated *in vacuo*. After column chromatography, NH_2-Asp(OtBu)-H dimethyl acetal was obtained as a clear yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.44 (s, 9H), 2.22 (dd, J_1= 8.8 Hz, J_2= 15.8 Hz, 1H), 2.51 (dd, J_1= 3.8 Hz, J_2= 16.4 Hz, 1H), 3.26 (m, 1H), 3.40 (d, *J* = 8.2 Hz, 6H), 4.12 (d, *J* = 5.7 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.1, 38.5, 49.9, 54.8, 55.2, 80.5, 107.4, 171.8.

(*S*)-4-oxo-3-propiolamidobutanoic acid **(5)**:[2]

Propynoic acid (310 μL, 5.0 mmol) was added to a stirred solution of DCC (1.23 g, 5.0 mmol) in 15 mL DCM at 0°C and stirred for 10 mins. NH_2-Asp(OtBu)-H dimethyl acetal **4** (1.10 g, 0.5 mmol) was pre-dissolved in 5 mL of DCM and added dropwise to the reaction mixture, followed by stirring for 1 h at rt. The white solid was removed by filtration and the filtrate was concentrated and purified by flash column (50% EA/Hex) to afford the pure product as a yellow solid (0.67 g, 49.6%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.50 (d, *J* = 7.3 Hz, 1H), 4.46-4.40 (m, 1H), 4.35 (d, *J* = 4.2 Hz, 1H), 3.39 (d, *J* = 2.9 Hz, 6H), 2.79 (s, 1H), 2.49-2.47 (m, 2H), 1.43 (s, 9H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 170.5, 151.6, 104.3 81.2, 73.4, 55.9, 55.3, 52.8, 48.1, 36.0, 34.9, 27.9.

The yellow oil was re-dissolved in 10 mL of TFA/DCM/H_2O (4/4/2) and the mixture was stirred overnight at rt. The solvent was removed under reduced pressure and the product was dried in vacuo to afford the final warhead as a yellow oil.



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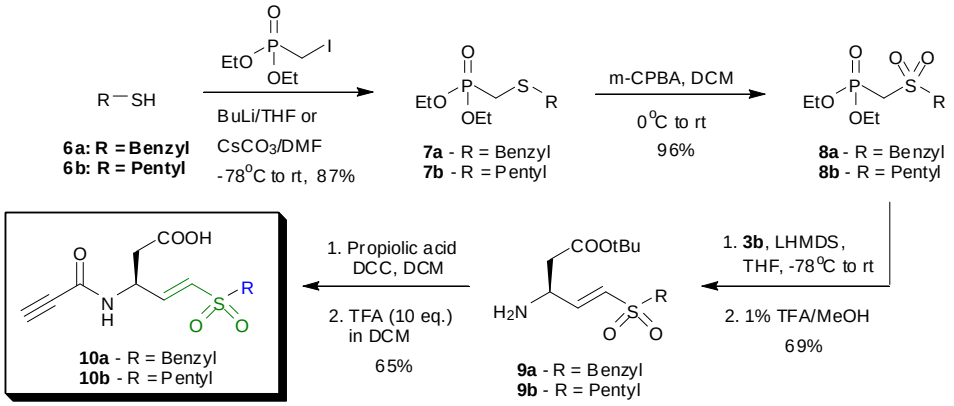
Exist as an isomeric mixture[3]. ^{1}H NMR cannot be properly interpreted. LCMS shows a broad peak corresponding to the correct molecular mass, TOF-MS: *m/z* 170.039 $[M + H]^+$. (See Supporting Information II).

2.2 Synthesis of Asp-containing vinyl sulfone warheads



Scheme S2. Synthesis of Asp-containing vinyl sulfone warheads

The syntheses of Asp-containg vinyl sulfone warheads involve 6 steps. The key step being the Horner-Wadsworth-Emmons condensation between the trityl-protected aspartic aldehyde **3b** and the P_1'-containing sulfones **8**. Aldehyde **3b** was synthesized similar to intermediate **3a** (Scheme S1), except that the Fmoc protecting group was replaced with trityl to give a Trityl-protected aldehyde **3b**. To synthesize the P_1'-containing sulfones **8**, a S_N2 substitution reaction was carried out on the respective thiols **6** to give sulphides **7**. Following which, the benzyl and pentyl sulphides **7** were oxidized to the corresponding sulfones using *m*-CPBA.

*Procedure for the conversion of **2a** to **3b**:*

(*S*)-*tert*-butyl 4-hydroxy-3-(tritylamino)butanoate **(2b)**:

Fmoc-Asp-alcohol **1** (27 g, 68 mmol) was dissolved in 200 mL of 20% piperidine in dimethylformamide (DMF). After stirring the reaction mixture for 1 h, the solvent was evaporated under reduced pressure to dryness and used directly for next step without purification. Protection with trityl was carried out as previously reported.[4] Overall yield = 66%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ7.55 (d, ^3J = 7.2 Hz, 6H), 7.27-7.16 (m, 9H), 3.40 (dd, ^3J = 10Hz, 4Hz, 1H), 3.13 (m, ^3J = 11 Hz, 5.9 Hz, 1H), 2.97 (m, 1H), 2.55 (s, 1H), 1.83 (m, 2H), 1.38 (s, 9H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ72.2, 146.6, 128.5, 127.9, 126.4, 80.6, 70.9, 65.1, 50.9, 38.1, 27.9. ESI-MS: *m/z* 440.2 $[M + Na]^+$, 857.0 $[2M + Na]^+$.

(*S*)-*tert*-butyl 4-oxo-3-(tritylamino)butanoate **(3b)** [5]:

Oxalyl chloride (5 mL, 56.4 mmol) was dissolved in 150 mL of DCM and cooled to -50°C. DMSO (6.8 mL, 95.5 mmol) was pre-dissolved in 5 mL of DCM and added dropwise. After stirring for 15 mins, Trt-Asp-OH **2b** (18 g, 43.4 mmol) was pre-dissolved in 20 mL of DCM and added dropwise over 10 mins. Upon stirring for another 15 mins, TEA was added and the reaction mixture was allowed to warm naturally to room temperature over 1 hour. The reaction was quenched with 100 mL water, and re-extracted

with 4 × 30 mL DCM. The organic layer was combined, washed with brine, dried over anhydrous Na_2SO_4 and concentrate under reduced pressure to afford the product as a yellow oil (17.4 g, 90%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.17 (s, 1H), 7.56 (d, 6H), 7.53-7.21 (m, 9H), 3.37 (m, 1H), 2.60 (m, 2H), 2.13 (dd, ^3J = 6 Hz, 1H), 1.43 (s, 9H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 204.2, 170.7, 145.9, 120.5, 128.2, 126.7, 81.3, 71.1, 58.4, 38.7, 28.0. ESI-MS: *m/z* 438.1 $[M + Na]^+$, 852.7 $[2M + Na]^+$.

*Procedure for the conversion of **6** to **8**:*

Diethyl benzylthiomethylphosphonate **(7a)** [6]:

Benzyl mercaptan **6a** (5.67 mL, 44 mmol) was dissolved in 100 mL of tetrahydrofuran (THF) and BuLi (32 mL, 52 mmol) was added dropwise at -78°C. The reaction was allowed to stir for 1 h. Following that, diethyl iodomethylphosphonate (7.41 mL, 40 mmol) was added dropwise and stirred for 4 h. The reaction was quenched with 30 mL of 2M NH_4Cl and the aqueous layer was extracted with 4 × ether, 1 × brine, dried with anhydrous Na_2SO_4, concentrated under reduced pressure and purified by flash column chromatography (30% EA/Hex) to afford the product as a colourless oil (95%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.34-7.25 (m, 5H), 4.16 (m, ^3J = 7 Hz, 4H), 3.90 (s, 2H), 2.55 (d, ^3J = 13 Hz, 2H), 1.34 (t, 6H).

Diethyl pentylthiomethylphosphonate **(7b)** [5]:

1-pentanethiol **6b** (6.8 mL, 55 mmol) was dissolved in 150 mL of DMF and $CsCO_3$ (9.27 g, 50 mmol) was added carefully at rt. The reaction was allowed to stir for 1 h. Subsequently, diethyl iodomethylphosphonate (32.6 mL, 100 mmol) was added dropwise and stirred overnight. The reaction was quenched with 50 mL of 1M HCl and DMF was removed. The crude product was re-dissolved in EA and washed with 2 × 1M HCl, 2 × H_2O, 1 × brine, dried with anhydrous Na_2SO_4, concentrated under reduced pressure and purified by flash column chromatography (30% EA/Hex) to give the product as a colourless oil (90%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 4.21-4.11 (m, 4H), 2.71 (d, ^3J = 11.8 Hz, 2H), 2.69 (d, ^3J = 15.4 Hz, 2H), 1.57 (m, 2H), 1.33 (m, 9H), 0.89 (t, ^3J = 7 Hz, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ = 62.5, 33.5, 30.8, 28.6, 25.2, 22.2, 16.3, 13.8.

Diethyl benzylsulfonylmethylphosphonate **(8a)**/Diethyl pentylsulfonylmethyphosphonate **(8b)** [5]:

Compound **7a** (10.9 g, 40 mmol) was dissolved in 200 mL DCM at 0 °C. Meta-chloroperoxybenzoic acid (m-CPBA) (20.7 g, 120 mmol) was added carefully and the reaction mixture was allowed to stir overnight at rt. The white solid was filtered and the filtrate was washed with 1M NaOH, H_2O and brine, dried with anhydrous Na_2SO_4 and concentrated under reduced pressure to afford the product as a colourless oil.

(8a): Yield = 94%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.52 (m, ^3J = 3.6 Hz, 2.3 Hz, 2H), 7.39 (m, ^3J = 3.9 Hz, 2.5Hz, 3H), 4.60 (s, 2H), 4.24 (m, ^3J = 7 Hz, 4H), 3.39 (d, ^3J = 16 Hz, 2H), 1.37 (t, 6H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 130.9, 129.0, 128.9, 128.0, 63.6, 60.2, 48.7, 46.9, 16.2. ESI-MS: *m/z* 329.1 $[M + Na]^+$, 634.8 $[2M + Na]^+$.

(8b): Yield = 98%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 4.24 (m, ^3J = 7.2 Hz, 4H), 3.57 (d, ^3J = 16.6 Hz, 2H), 3.33 (t, ^3J = 8Hz, 2H), 1.86 (m, 2H), 1.45-1.35 (m, 10H), 0.92 (t, ^3J = 7 Hz, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 63.6, 54.5, 50.2, 30.3, 22.0, 21.5, 16.2, 13.6. ESI-MS: *m/z* 287.1 $[M + H]^+$, 309.2 $[M + Na]^+$, 594.9 $[M + Na]^+$.

*General procedure for the conversion of **8** to **10**:*

(*S*, *E*)-tert-butyl 3-amino-5-(benzylsulfonyl)pent-4-enoate **(9a)**/(*S*, *E*)-tert-butyl 3-amino-5-(pentylsulfonyl)pent-4-enoate **(9b)**:[5]

Benzyl sulphone **8a** (8.58 g, 28 mmol) was dissolved in 100 mL THF at -78°C. LHMDS (36 ml, 36 mmol) was added dropwise and the mixture was allowed to stir for 1 h. Trt-Asp-H **3** (11.6 g, 28 mmol) was pre-dissolved in 10 mL of THF and added dropwise to the reaction mixture. After stirring for 1.5 h, the reaction was quenched by 100 mL of saturated NH_4Cl, extracted with ethyl acetate, then washed with H_2O and brine, dried with anhydrous Na_2SO_4 and concentrated under reduced pressure to give the crude product as a yellow oil. The crude product was then dissolved in 100 mL of 1% trifluoroacetic acid (TFA) in methanol (MeOH) and stirred at rt for 1 h. The TFA/MeOH solvent was removed under reduced pressure and the crude product was purified by flash column chromatography (80% EA/Hex) to afford the pure product as a yellow oil.

(9a): Yield = 66%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.37 (m, 5H), 6.69 (dd, ^3J = 15 Hz, 5Hz, 1H), 6.42 (dd, ^3J = 15 Hz, 1.62 Hz, 1H), 4.23 (s, 2H), 3.88 (m, 1H), 2.31 (qd, ^2J = 27 Hz, ^3J = 12 Hz, 8.4 Hz, 5 Hz, 2H), 1.45 (s, 9H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 169.9, 151.8, 130.8, 128.9, 128.8, 128.0, 126.7, 81.5, 61.3, 49.0, 42.0, 28.0. ESI-MS: *m/z* 325.8 [M + H]+, 348.0 $[M + Na]^+$, 650.9 $[2M + 1]^+$, 672.8 $[2M + Na]^+$.

(9b): Yield = 74%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.72, (qd, ^3J = 15 Hz, 5 Hz, 1.5 Hz, 2H), 3.99 (m, 1H), 2.95 (t, ^3J = 7.7 Hz), 2.46 (qd, ^3J = 16Hz, 8.2 Hz, 4.8 Hz, 2H), 1.76 (m, 2H), 1.46 (s, 9H), 1.39 (m, 4H), 0.91 (t, ^3J = 7Hz). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 170.0, 150.6, 127.8, 81.6, 54.6, 49.1, 42.3, 30.4, 28.0, 22.1, 13.7. ESI-MS: *m/z* = 305.7 $[M + H]^+$, 328.0 $[M + Na]^+$, 611.0 $[2M + H]^+$, 632.8 $[2M + Na]^+$.

(*S*, *E*)-5-(benzylsulfonyl)-3-propiolamidopent-4-enoic acid **(10a)**/(*S*, *E*)-5-(pentylsulfonyl)
-3-propiolamidopent-4-enoic acid **(10b)**:[2]

Propynoic acid (0.4 mL, 6.45 mmol) was added to a stirred solution of DCC (1.523 g, 7.38 mmol) in 15 mL DCM at 0°C and stirred for 10 mins. NH_2-Asp(tBu)-VS **9** (2 g, 6.15 mmol) was pre-dissolved in 5 mL of DCM and added dropwise to the reaction mixture, followed by stirring for 1 h at rt. The white solid was removed by filtration and the filtrate was concentrated and purified by flash column (40% EA/Hex) to afford the pure product as a yellow oil. The yellow oil was re-dissolved in 10 mL of TFA (3 mL, 40 mmol) in DCM and the mixture was stirred overnight at rt. The TFA/DCM solvent was removed under reduced pressure and the product was dried *in vacuo* to afford the final warhead as a yellow solid.

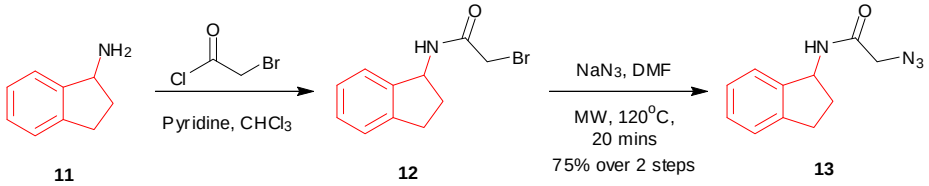
(10a): Yield = 65%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.38 (s, 5H), 6.53 (qd, ^3J = 13 Hz, 10 Hz, 5 Hz, 2H), 4.38 (s, 2H), 3.68 (s, 1H), 2.60 (m, 2H). ESI-MS: *m/z* 319.9 $[M - H]^+$, 640.9 $[2M - 1]^+$.

(10b): Yield = 67%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.72 (qd, ^3J = 15 Hz, 5.2 Hz, 1.5 Hz, 2H), 5.02 (m, 1H), 3.67 (s, 1H), 3.08 (t, ^3J = 5.8 Hz, 2H), 2.73 (m, ^3J = 7 Hz, 2H), 1.71 (m, 4 Hz, 2H), 1.45-1.37 (m, 4H), 0.94 (t, ^3J = 7 Hz, 3H). ESI-MS: *m/z* 300.0 $[M - H]^+$, 600.9 $[2M - H]^+$.

2.2 Synthesis of azide building blocks

The synthesis of azide building blocks was carried out either through a two-step solution-phase route[6] or a solid-phase method based on similar chemistry (full details of the solid phase strategy will be published in due course).

Procedure for solution-phase synthesis of azides from amines:



Scheme S3 Solution-phase synthesis of azide building block **13**.

2-azido-N-(2,3-dihydro-1H-inden-1-yl)acetamide **(13)**:[7]

1-aminoindane (0.642 mL, 5 mmol) was dissolved in distilled chloroform (15 mL). 2-bromoacetyl chloride (0.839 mL, 5.5 mmol) was then slowly added at 0 ^{o}C, followed by addition of pyridine (0.445 mL, 5.5 mmol). The reaction mixture was allowed to stir for 1 h at rt. The organic layer was extracted with $NaHCO_3$ (2 × 5 mL), water (2 × 5 mL), brine (1 × 5 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. Without purification, the product **12** obtained was dissolved in DMF (20 mL), followed by the addition of sodium azide (0.6501 g, 10 mmol). The reaction mixture was heated at 100^{o}C with microwave for 20 min. At the end of the reaction, 50 mL of EA was added and the organic layer was washed with $NaHCO_3$ (2 × 20 mL), water (2 × 20 mL), brine (1 × 20 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to yield the azide **13** in 75% yield. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.29 (s, 4H), 5.50 (q, ^{3}J = 7.7 Hz, 1H), 4.05 (s, 2H), 2.97 (m, 2H), 2.60 (m, 1H), 1.83 (m, 1H). ESI-MS: *m/z* 217.0 $[M + H]^+$, 239.1 $[M + Na]^+$, 413.2 $[2M + H]^+$, 454.8 $[2M + Na]^+$, 215.3 $[M – H]^+$.

*Azides(*characterization will be reported in another publication*) used in this study (Synthesized by solid-phase chemistry)* ***14*** *and* ***15****:*

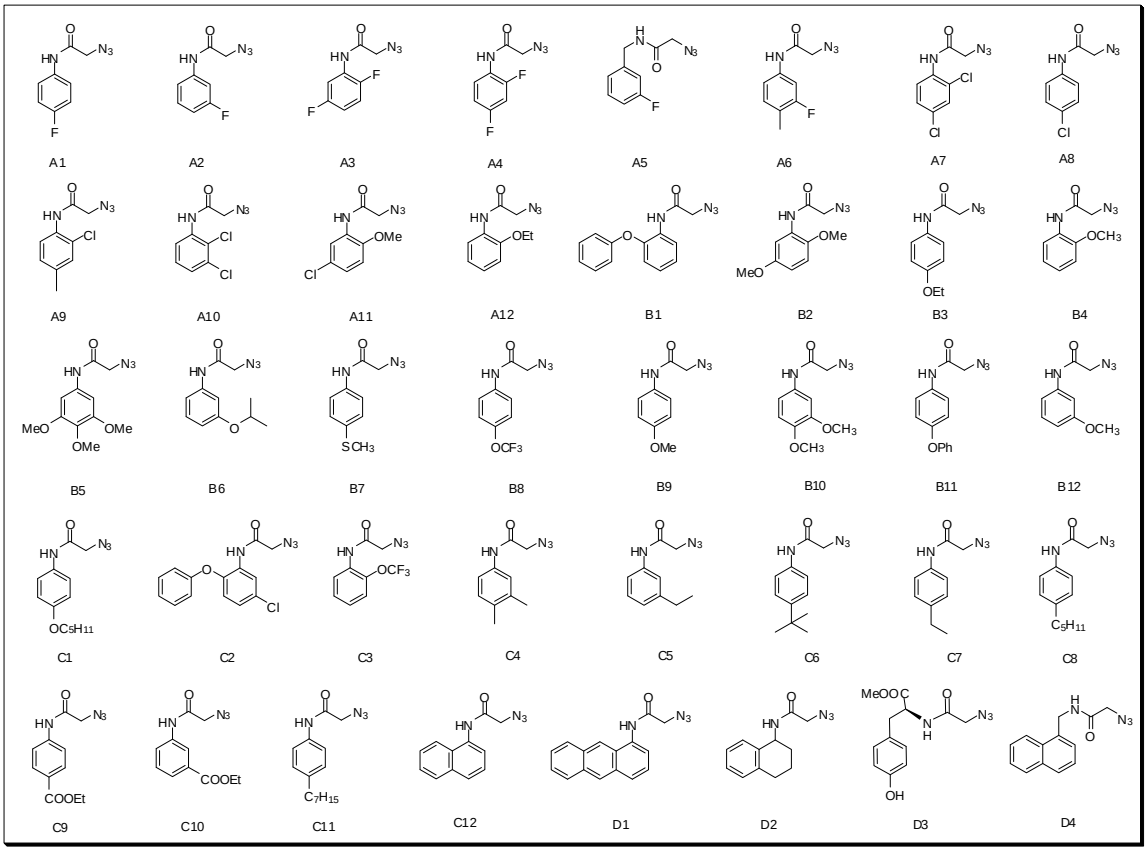


Figure S1. Structures of the 40 azide building blocks **14** used in this study.

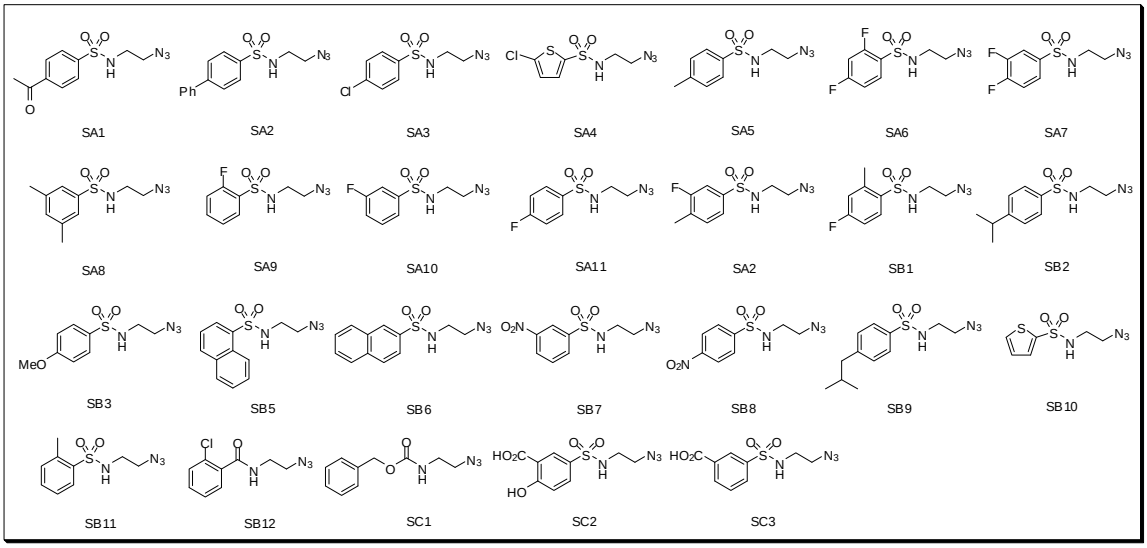
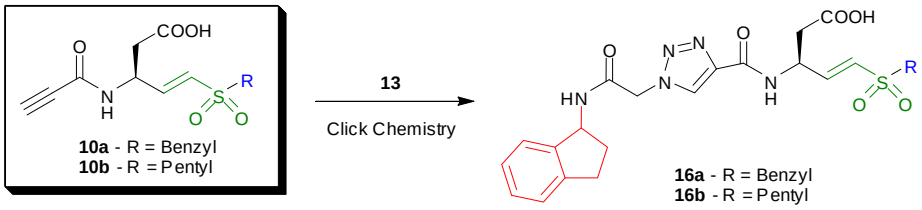


Figure S2. Structures of the 26 sulfonamide azide building blocks **15** used in this study.

3. Click Chemistry

3.1 Optimization of click chemistry conditions



Scheme S5. Click assembly of model pair, alkyne warheads **10** and azide **13.**

Table S1. Optimization conditions for click chemistry.

Entry	Azide	Alkyne	Catalyst	Base / Additive	Solvent	Duration	% Yield*	% purity*
1	**13** 1 eq	**10a** 1 eq	$CuSO_4.5H_2O$ 0.05 eq	Na Asc. 0.2 eq	tBuOH:H_2O 1:1	> 4 days	50	50
2	**13** 1 eq	**10a** 1 eq	$CuSO_4.5H_2O$ 0.05 eq	Na Asc. 0.2 eq	DCM:H_2O 1:1	1 day	>95	>95
3	**13** 1 eq	**10a** 1 eq	$CuSO_4.5H_2O$ 0.05 eq	Na Asc. 0.2 eq	DMSO:H_2O 1:1	> 2 days	60	60
4	**13** 1 eq	**10a** 1 eq	CuI 1 eq	DIEA 2 eq	tBuOH:H_2O 1:1	2 days	>95	90
5	**13** 1 eq	**10a** 1 eq	CuI 1 eq	DIEPA 2 eq	DMSO:H_2O 1:1	2 days	>95	60
6	**13** 1 eq	**10a** 1 eq	CuI 1 eq	DIEPA 2 eq	Toluene	2 days	>95	40
7	**13** 1 eq	**10a** 1 eq	CuI 1 eq	DIEPA 2 eq	MeOH	2 days	>95	50
8	**13** 1 eq	**10a** 1 eq	CuBr 0.2 eq	DBU 3 eq	Toluene	> 4 days	-	-
9	**13** 1 eq	**10a** 1 eq	Cu/C 0.05 eq	TEA 1.5 eq	Dioxane	> 4 days	-	-
10	**13** 1 eq	**10b** 1 eq	$CuSO_4.5H_2O$ 0.05 eq	Na Asc. 0.2 eq	DCM:H_2O 1:1	1 day	>95	>95

*Based on LCMS results. % yield is estimated base on consumption/conversion of the limiting starting material.

*General procedure for click chemistry optimization **(16)***

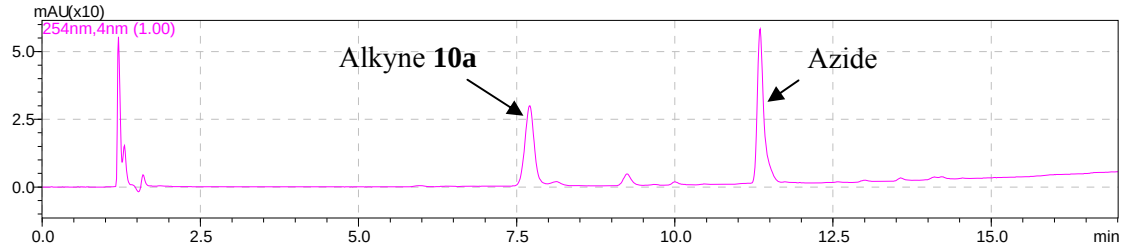
To a 500 µL solution of solvent in a 2 mL eppendof tube, were added 50 µL of alkyne warhead **10a** (100 mM), 50 µL of azides **9** (100 mM), 2.5 µL of $CuSO_4.5H_2O$ (100 mM) with 10 µL of sodium ascorbate (100 mM) or CuI/CuBr (50 mM) with DIEA/DBU (2 µL). The tubes were sealed with parafilm tape, shaken at rt and monitored for 4 days. A sample of each of the reaction mixtures was taken out everyday and concentrated, re-dissolved in 200 µL of DMSO, and followed by LC-MS analysis.
(16a): TOF-MS: *m/z* 538.147 $[M + H]^+$.

(16b): TOF-MS: *m/z* 518.184 $[M + H]^+$.

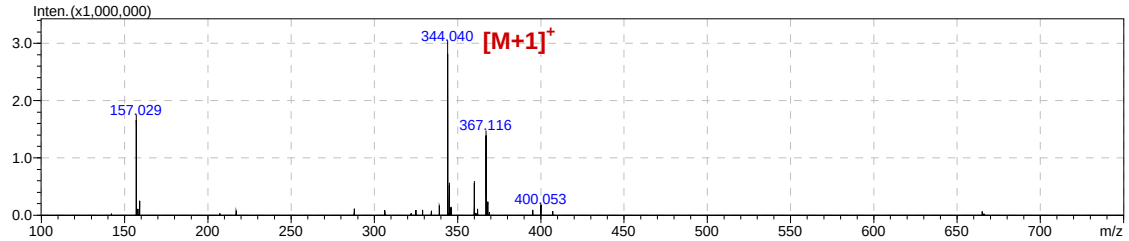
3.1 LC-MS profiles of click chemistry optimization

All conditions are based on:
0 – 10 min: 20% B (1% TFA in ACN) → 80% B
10 – 15 min: 80% B (1% TFA in ACN) → 100% B
15 – 17 min: 100% B

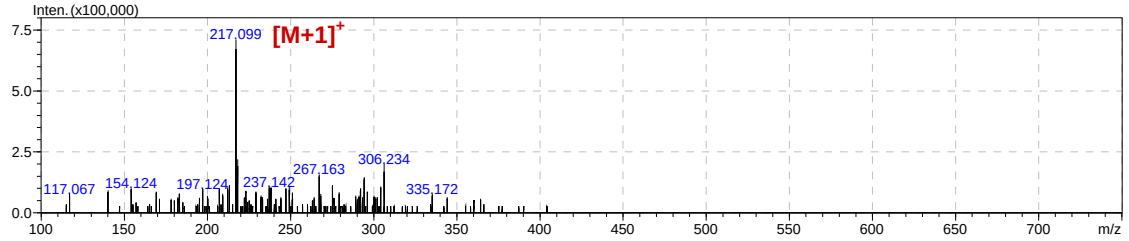
Time = 0 h



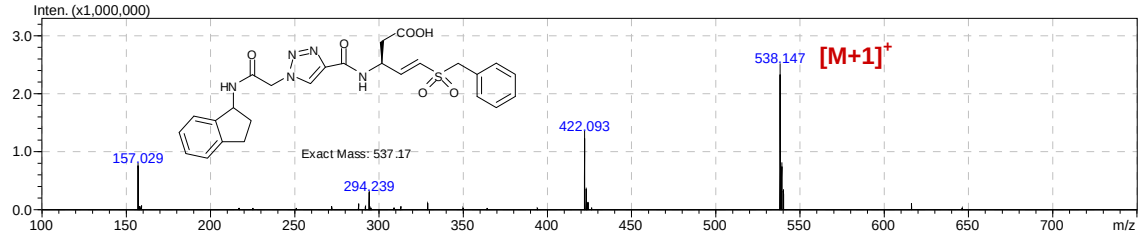
Alkyne:

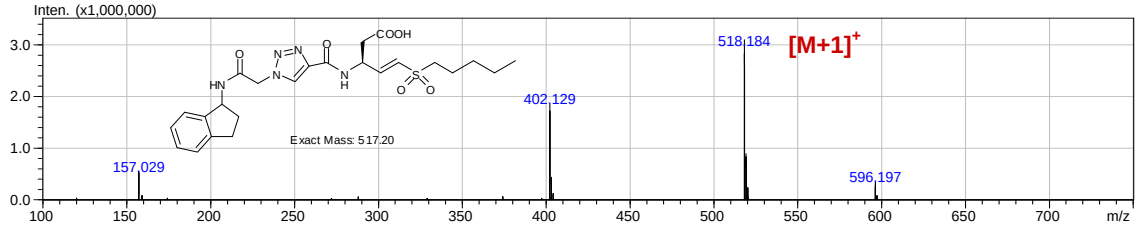


Azide:



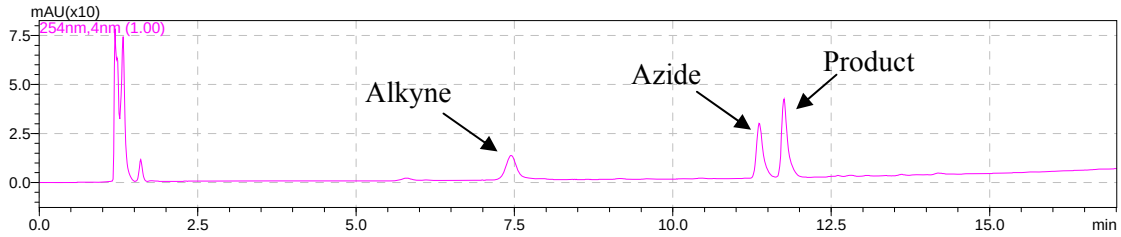
Product:





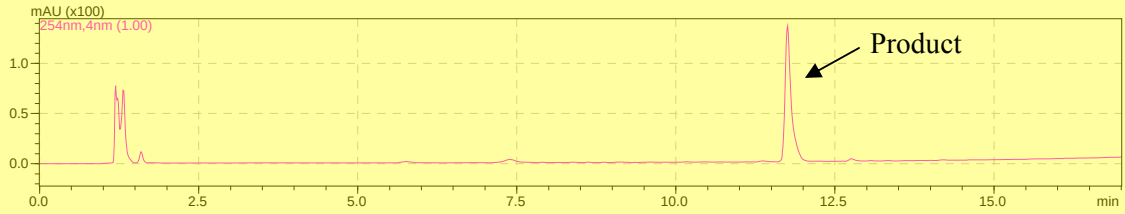
Entry 1: $CuSO_4.5H_2O$ (0.05 eq) + Na ascorbate (0.2 eq) + $tBuOH/H_2O$

After 4 days:



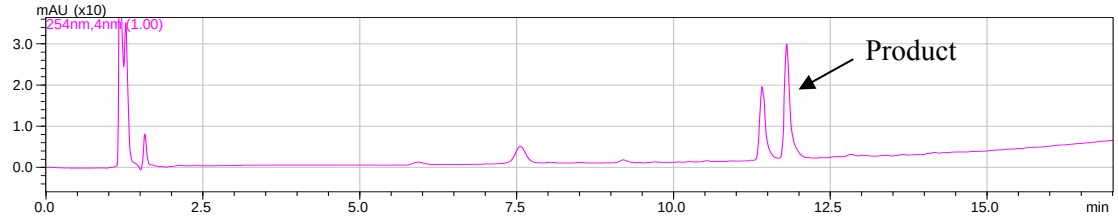
Entry 2: $CuSO_4.5H2O$ (0.05 eq) + Na ascorbate (0.2 eq) + DCM/H_2O

After 1 day:



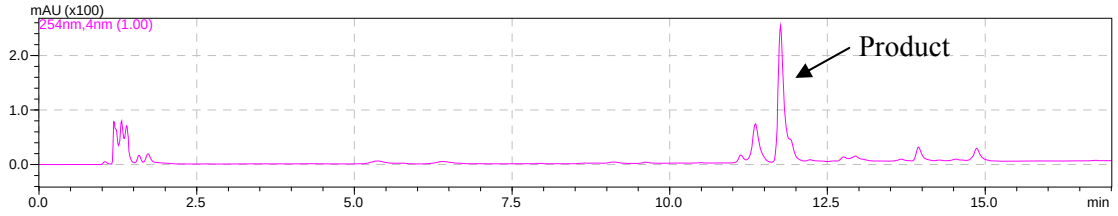
Entry 3: $CuSO_4.5H_2O$ (0.05 eq) + Na ascorbate (0.2 eq) + $DMSO/H_2O$

After 2 days:



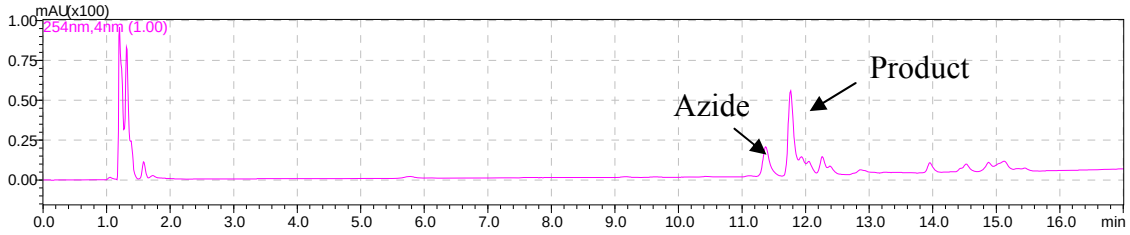
Entry 4: CuI (1 eq) + DIEA (2 eq) + $tBuOH/H_2O$

After 4 days:



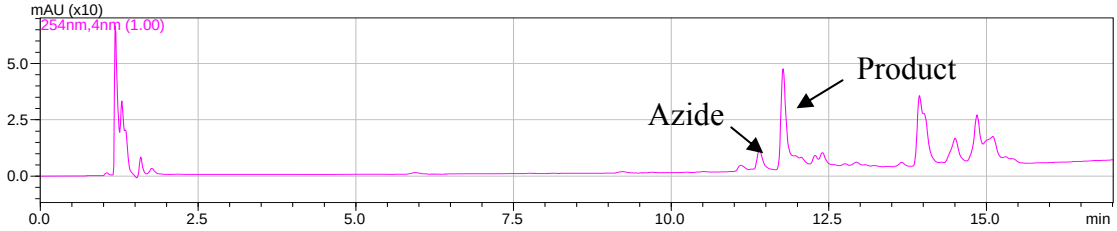
Entry 5: CuI (1 eq) + DIEA (2 eq) + $DMSO/H_2O$

After 2 days:



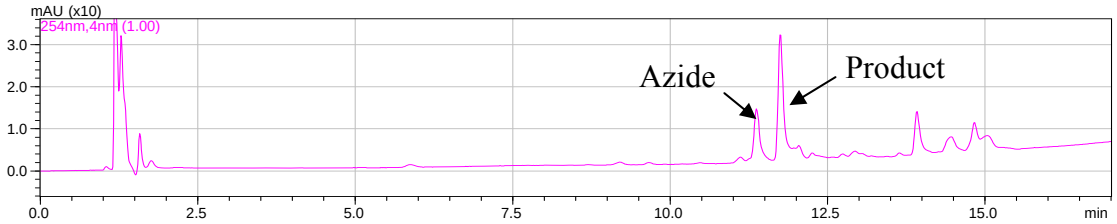
Entry 6: CuI (1 eq) + DIEA (2 eq) + Toluene

After 2 days:



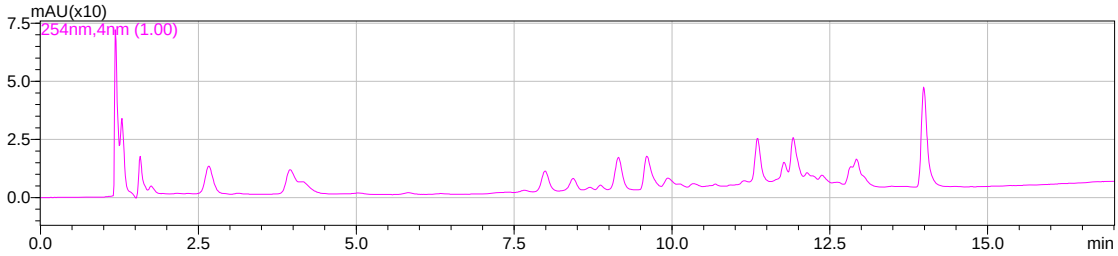
Entry 7: CuI (1 eq) + DIEA (2 eq) + MeOH

After 2 days:



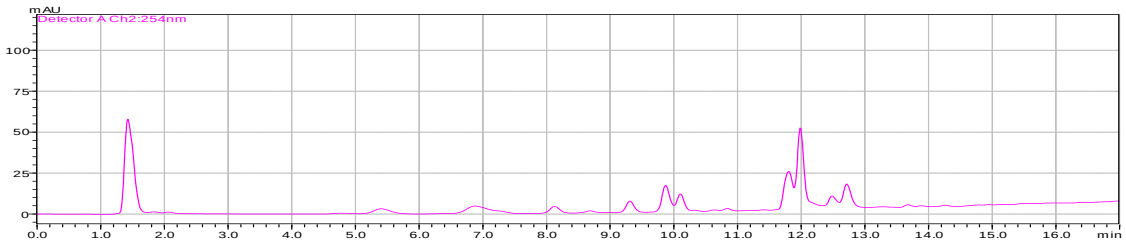
Entry 8: CuBr (1 eq) + DBU (3 eq) + Toluene

After 2 days: **No product, no MS**



Entry 9: Cu/C (0.05 eq) + TEA (1.5 eq) + Dioxane

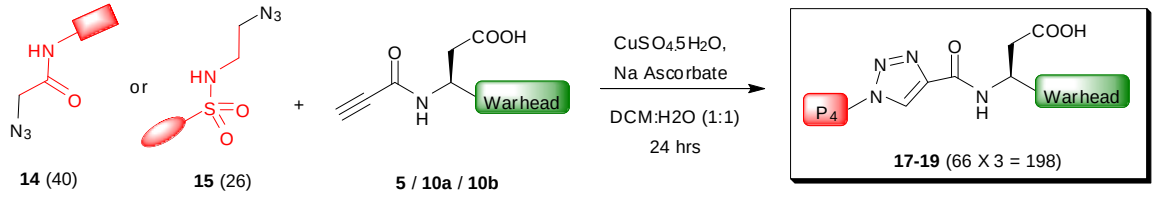
After 2 days: **No product, no MS**



Entry 10: $CuSO_4$.5H2O (0.05 eq) + Na ascorbate (0.2 eq) + DCM/H_2O

After 1 day:

3.2 Click chemistry assembly of inhibitor library



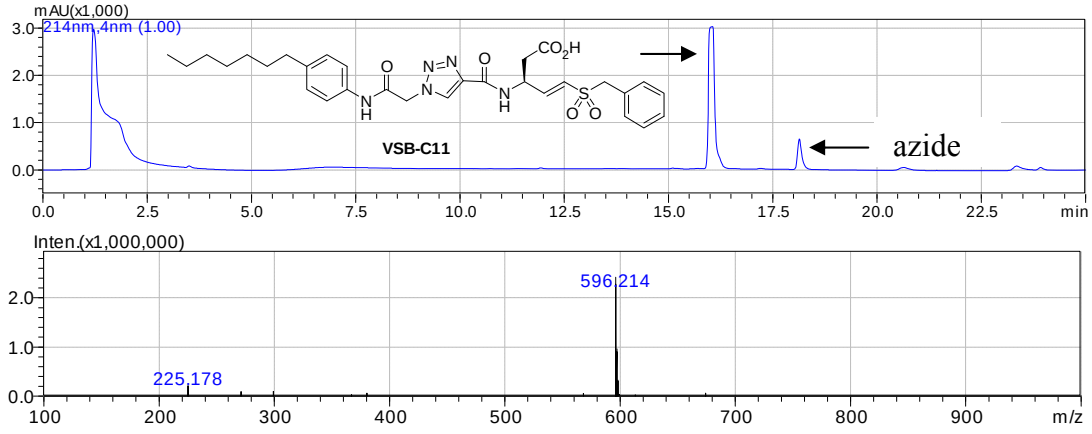
Scheme S6. Click assembly of azide building blocks **14-15** with alkyne warheads **5/10**.

*General procedure for click chemistry assembly of caspase inhibitors **(17-18):***

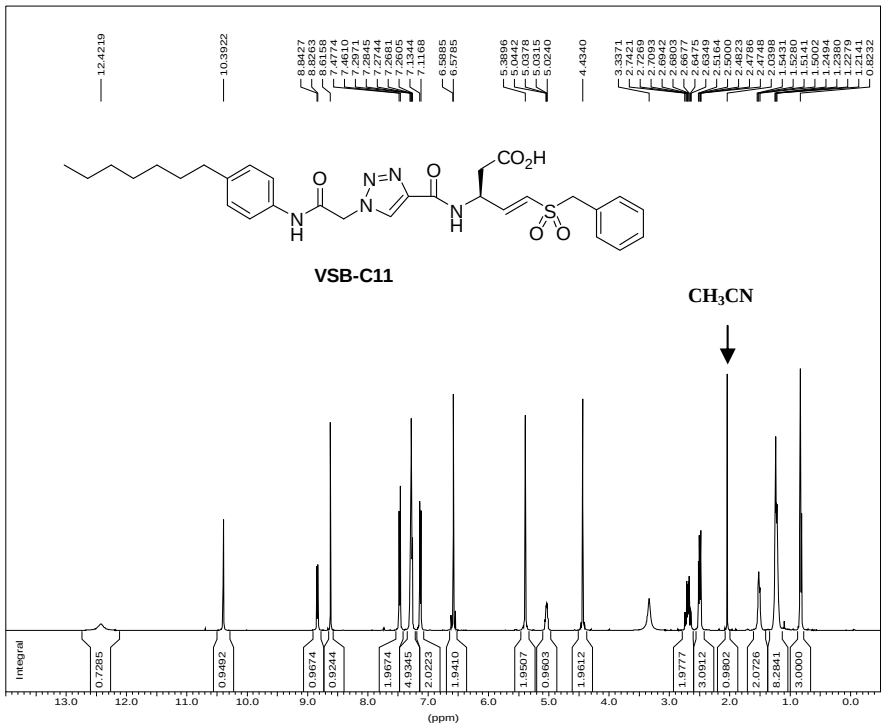
To a 100 µL solution of DCM/H_2O (1:1) in a 384-well microplate, were added 10 µL of alkyne warheads **5/10a/10b** (25 mM), 5 µL of 66 azides **14-15** (assumed to be 50 mM, 40% yield from solid phase synthesis), 2 µL of $CuSO_4.5H_2O$ (12.5 mM) and 2 µL of sodium ascorbate (50 mM). The plate was then capped tightly with silicon placemat and shaken at rt for at least 24 h. The solvent was removed by *HT-4X Vacuum Evaporator Genevac* and the "clicked" products **17-19** were re-dissolved in 125 µL of DMSO (calculated final concentration of inhibitor = 2 mM) and analyzed by LC-MS. (see **Supporting Information II**).

Characterizations of selected click products

(17): VSB-C11
^{1}H NMR (500 MHz, DMSO-d_6) δ 0.82 (m, 3H), 1.21-1.25 (m, 8H), 1.53 (m, 2H), 2.48 (m, 2H), 2.68 (m, 2H), 4.43 (s, 2H), 5.03 (m, 1H), 5.39 (s, 2H), 6.58 (d, J = 5.0 Hz, 2H), 7.12 (d, J = 8.8 Hz, 2H), 7.27 (m, 5H), 7.47 (d, J = 8.2 Hz, 2H), 8.62 (s, 1H), 8.83 (d, J = 8.2 Hz, 2H), 10.39 (s, 1H), 12.4 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 13.9, 22.0, 28.5, 30.9, 31.2, 34.5, 37.2, 46.0, 52.4, 59.5, 128.1, 128.2, 128.3, 128.5, 128.7, 131.2, 136.0, 137.8, 142.1, 147.0, 159.1, 163.6, 171.5. LCMS found for $C_{30}H_{37}N_5O_6S$ $(M + H)^+$: m/z = 596.214.



VSB-C11 in DMSO-d61H AMX500



*** Current Data Parameters ***

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EXPNO	:	1
PROCNO	:	1
*** Acquisition Parameters ***		
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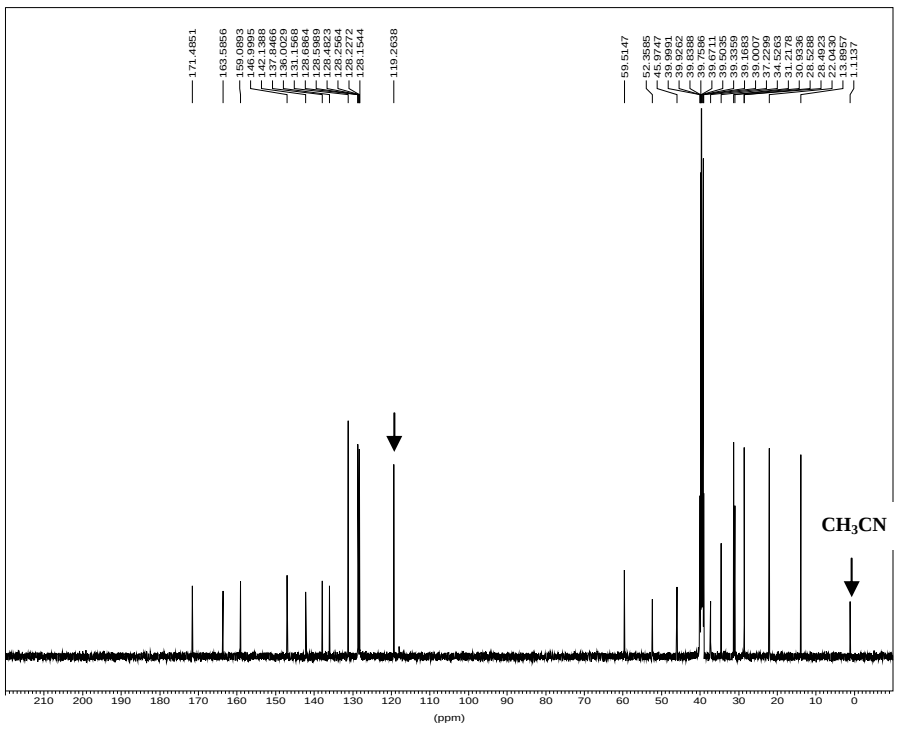
DATE_d	:	Oct 03 2007
DS	:	0
NS	:	8
O1	:	3088.51 Hz
O2	:	3088.51 Hz
O3	:	3088.51 Hz
SFO1	:	500.1330885 MHz
SFO2	:	500.1330885 MHz
SFO3	:	500.1330885 MHz
SOLVENT	:	DMSO
*** 1D NMR Plot Parameters ***		
SR	:	15.33 Hz

VSB-C11 in DMSO-d6 13C AMX500



*** Current Data Parameters ***

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PROCNO	:	1
*** Acquisition Parameters ***		
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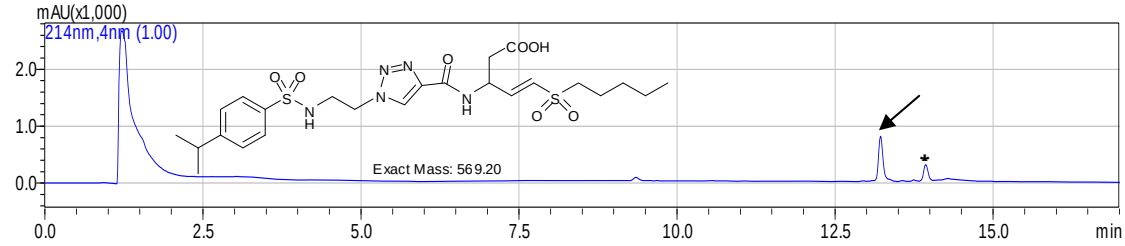
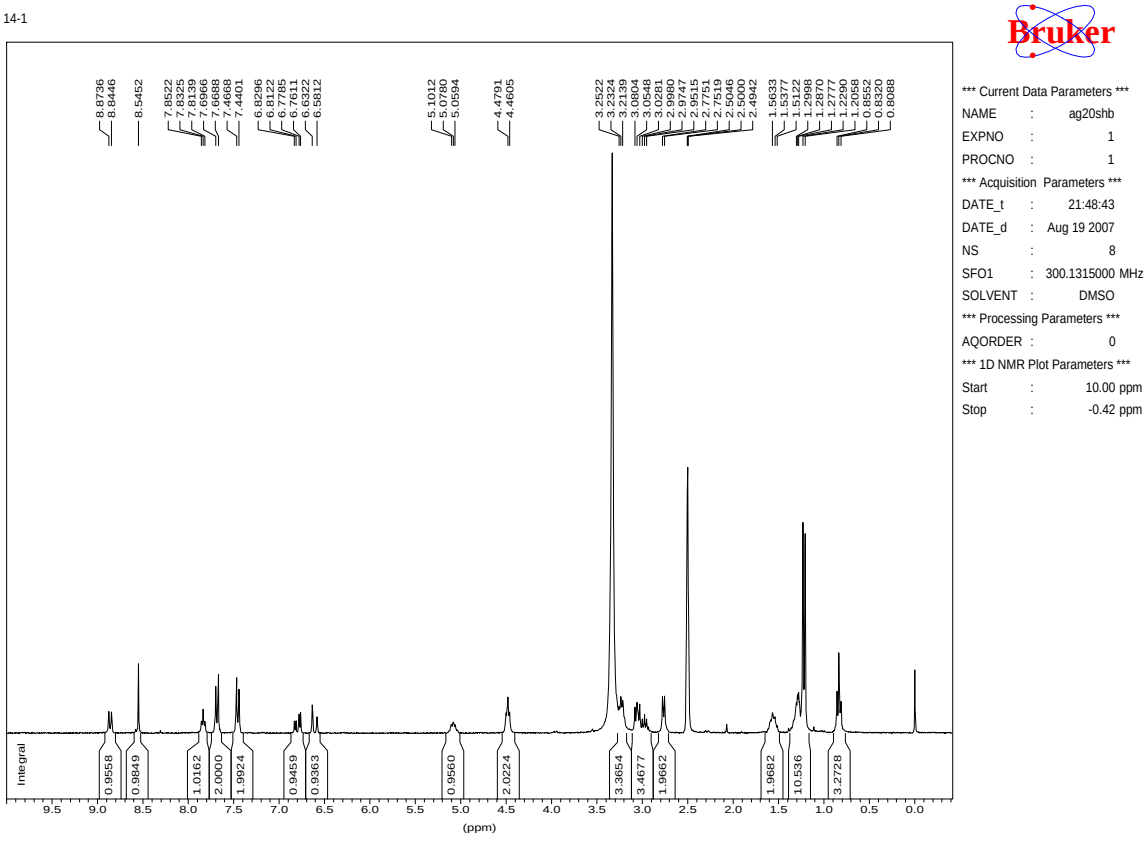
DATE_d	:	Oct 03 2007
DS	:	0
NS	:	300
O1	:	13204.57 Hz
O2	:	2000.52 Hz
O3	:	12575.30 Hz
SFO1	:	125.7709936 MHz
SFO2	:	500.1320005 MHz
SFO3	:	125.7703643 MHz
SOLVENT	:	DMSO
*** 1D NMR Plot Parameters ***		
SR	:	62.01 Hz

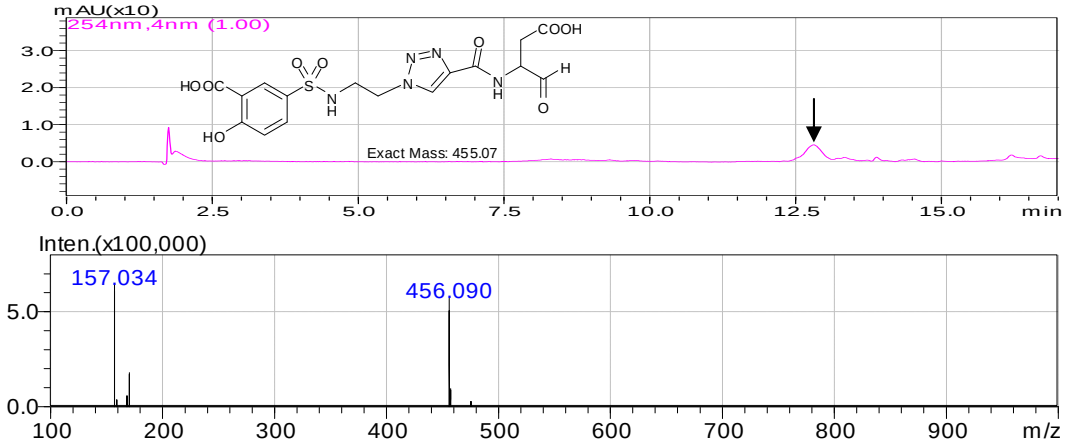
(18): VSP-SB2

^{1}H NMR: δ 8.86 (d, ^{3}J = 8.7 Hz, 1H), 8.55 (s, 1H), 7.83 (t, ^{3}J = 5.91 Hz, 3H), 7.83 (d, ^{3}J = 5.9 Hz, 2H), 7.67 (d, ^{3}J = 8.34,2H), 7.45 (d, ^{3}J = 8.1Hz, 2H), 6.80 (m, 1H), 6.60 (m, 1H), 5.08 (m, 1H), 4.47 (m, 2H), 3.22 (m, 2H), 3.05 (m, 3H), 2.76 (m, 2H), 2.50 (m, 2H), 1.54 (m, 2H), 1.29-1.21 (m, 10H), 0.83 (t, ^{3}J = 6.9 Hz, 3H).





(19): Ald-SC2
TOF-MS: *m/z* 456.090 $[M + H]^+$.



4. General procedure for caspase screening

4.1 Expression and Purification of His-Tagged Caspases

Recombinant caspase-3 and caspase-7, tagged with penta-His at their C-terminal, were expressed using the corresponding pET expression system (obtained from www.addgene.com) in the *E. coli* strain, BL21(DE3). Further repression of the expression system was used using pLysS. The transformed E.*coli* host was grown in Luria Bertani (LB) medium supplemented with 100 µg/mL of ampicillin and 34 µg/mL chloramphenicol at 37 ^{o}C in a 150 rpm shaker and their expression induced with isopropyl thiogalactosidase (IPTG). Both caspases were induced and expressed with 0.2 mM of IPTG, an OD_{600} of 0.6-0.8 for induction, and an expression temperature of 30 ^{o}C. The expression time was 7 hours for caspase-3 and 18 hrs for caspase-7. Cells were harvested by centrifugation (3220 *g*, 40 min, 4 ^{o}C) and resuspended in chilled resuspension buffer (50 mM Hepes, 10% glycerol, 300 mM NaCl, 0.05% Chaps, 25 mM imidazole, 0.1 mM PMSF, 5 mM β-ME, *p*H 8.0). 10-20 mL of resuspension buffer was used per liter of culture volume. The resuspended pellet was either purified immediately or stored at -20 ^{o}C or -80 ^{o}C.

Resuspended pellets were lysed by sonication on ice. Cell debris was pelleted down by centrifugation (29817 *g*, 20 min, 4 ^{o}C) in 1.5 mL eppendorf tubes. Clear supernatant, containing the soluble caspases, was incubated with 0.5-1 mL of nickel-nitrilotriacetic acid (Ni-NTA) agarose beads (Qiagen, Germany) per 1 liter of culture volume. Incubation time of approximately 30 min was sufficient for both caspases. All purification procedures were carried out on ice or at 4 ^{o}C. The purification column was pre-equilibrated with one-column volume of wash buffer (50 mM Tris, 500 mM NaCl, *p*H 8.0), prior to loading with the supernatant. Flow-through was collected. The column was washed with wash buffer with at least five column volumes. Proteins were eluted with a buffer containing 50 mM Hepes, 10% glycerol, 100 mM NaCl, 300 mM imidazole, 5 mM β-ME, *p*H 7.0 in 1-2 mL fractions. Protein concentration of each fraction was determined by Bio-Rad protein assay (Bio-Rad USA) and the purity of the eluants was analyzed using 15% SDS-PAGE Gel. A Coomassie Blue stain was used to visualize the separation bands of the caspases. An SDS-PAGE gel was also electroblotted onto a polyvinylidene difluoride (PVDF) membrane and western blotting with penta-His HRP conjugate and QIAexpress Anti-His HRP conjugate blocking buffer kit (Qiagen, Germany) was used. The membrane was visualized with an Enhanced ChemiLuminescent (ECL) Plus Western Blotting Detection kit (Amersham Pharmacia Biotech, USA).

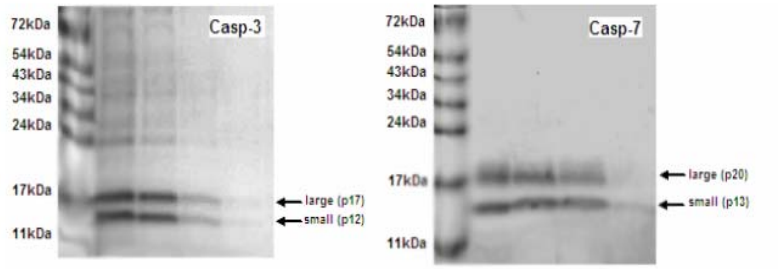


Figure S3. Western blot gel for expression of caspase-3 and -7, showing the successful expression and purification of both subunits of each casapse.

4.3 General Microplate Screening of Pentyl, Benzyl, and Aldehyde Warhead Inhibitors (17-19).

From the "clicked" inhibitor masterplate (2 mM) in a 384-well microplate, two 10-fold serial dilutions with water were carried out using *Sciclone ALH 3000* Liquid Handler (Caliper Life Sciences, USA) to give working plates of 200 uM and 20 uM. 5 µL of the individual inhibitor was transferred into each well of a black 384-well assay plate using *Sciclone ALH 3000* Liquid Handler. An enzyme mastermix containing 1980 µL of 2.5X reaction buffer (25 mM Pipes, 250 mM NaCl, 2.5 mM EDTA, 25% EDTA, 0.25% Chaps, 10 mM DTT, *p*H 7.2), 1980 µL of caspase 3/7 protein solution diluted in reaction buffer (4 nM in a 50 µL total volume), and 2970 µL of distilled water was first prepared. 35 uL of the mixture was added to each of the 384-wells using a MultiDrop Combi bulk dispenser (Thermo Scientific, USA). The enzyme-inhibitor mix was then incubated at 4^{o}C for 1 hr. A background fluorescence scan of the enzyme-inhibitor mix was taken using a *Tecan Infinite F200* microplate reader at excitation of 400 nm and emission of 505 nm prior to the addition of substrate. 10 µL of Ac-DEVD-AFC (AnaSpec, USA) caspase substrate (5 µM in a 50 µL total volume) diluted with water was then added to all the wells using the bulk dispenser to make up a final reaction volume of 50 µL. The fluorescence signal of each well was recorded over a one hour time-course and analyzed by *Treeview*. Commercially available caspase 3/7 inhibitor, Ac-DEVD-CHO, was used as a positive control (denote "pos" in **Figure S4**). Other controls ("neg": no inhibitor; "WH": warhead only) and duplicates of the preliminary microplate screening was performed. IC_{50} values of several selected inhibitors found in the preliminary microplate screenings were calculated by fitting the resulting dose-dependent data with the PRISM software (GraphPad, USA).

4.4 Fingerprints of caspase-3 and -7 against 198 inhibitors

The inhibitory potency of each inhibitor was calculated using normalization against reactions performed without inhibitor:

$$\left(1 - \frac{\text{Average Intensity with Inhibitor}}{\text{Average Intensity without Inhibitor}}\right) \times 100\%$$

The results were visualized using TreeView, producing heat maps or 'barcodes' that displayed the most potent inhibitors in bright red. The fingerprints of caspase-3 and -7 are provided in **Figure S5**.

Figure S4. Fingerprint scale of relative inhibition.

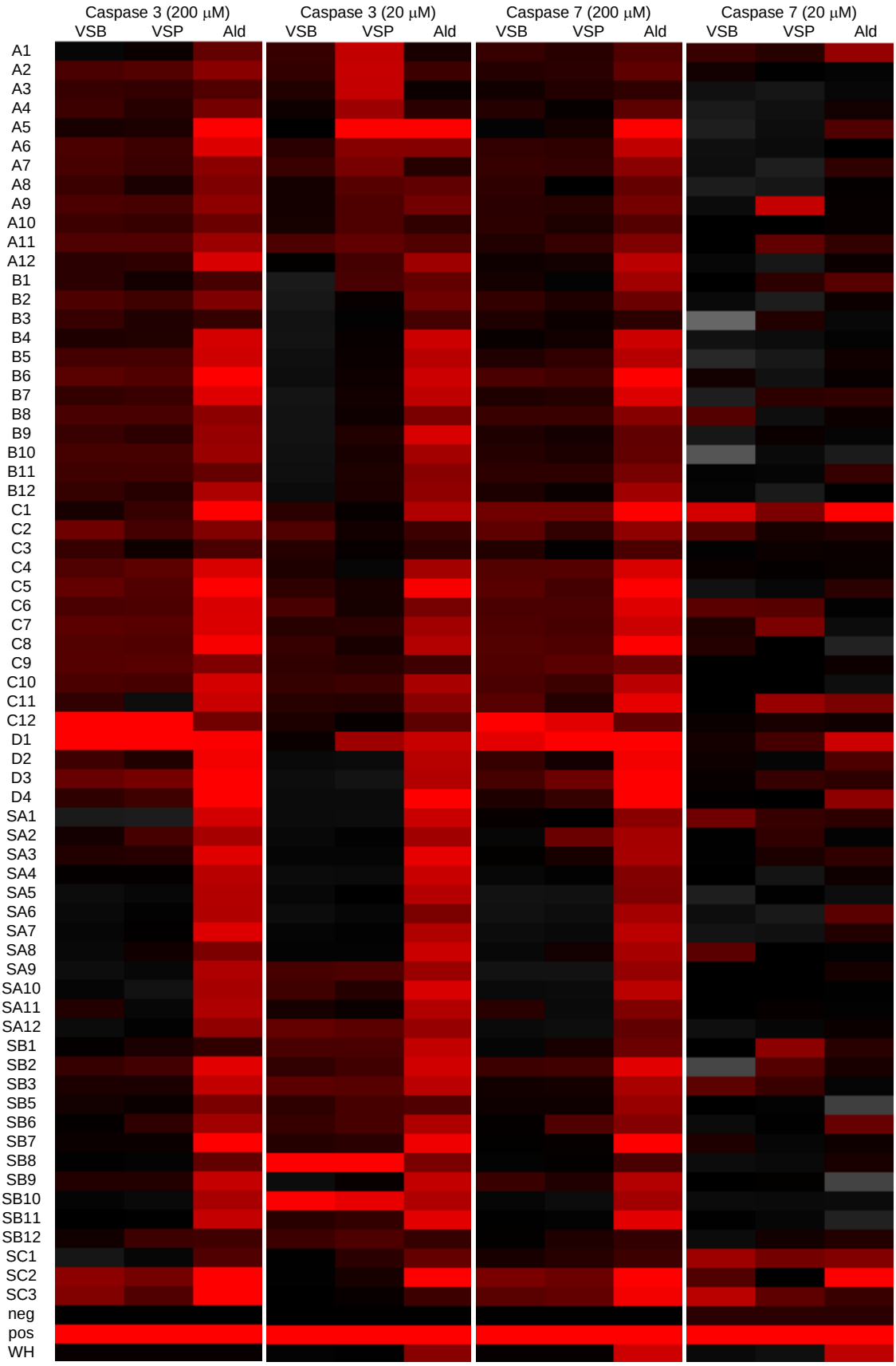


Figure S5. Inhibitor fingerprints of caspase-3 and -7 against the 198-member "click" inhibitors **17-19**.

4.5 IC_{50} Measurement

More comprehensive measurements were performed to evaluate the potency of selected inhibitors within the library set. The IC_{50} measurements were performed using dose-dependent reactions by varying the concentrations of the inhibitor, under the same enzyme-concentration. Briefly, a two-fold dilution series from approximately 200 μM to 1.5 μM (final concentration) was prepared for each inhibitor to be tested in black flat-bottom polypropylene 384-well plates (Nunc, USA). Screening was carried out using as described in section **4.3**. The IC_{50} was calculated by fitting the fluorescent outputs obtained using the Graphpad Prism software v.4.03 (GraphPad, San Diego). Results are shown in **Figure S6**.

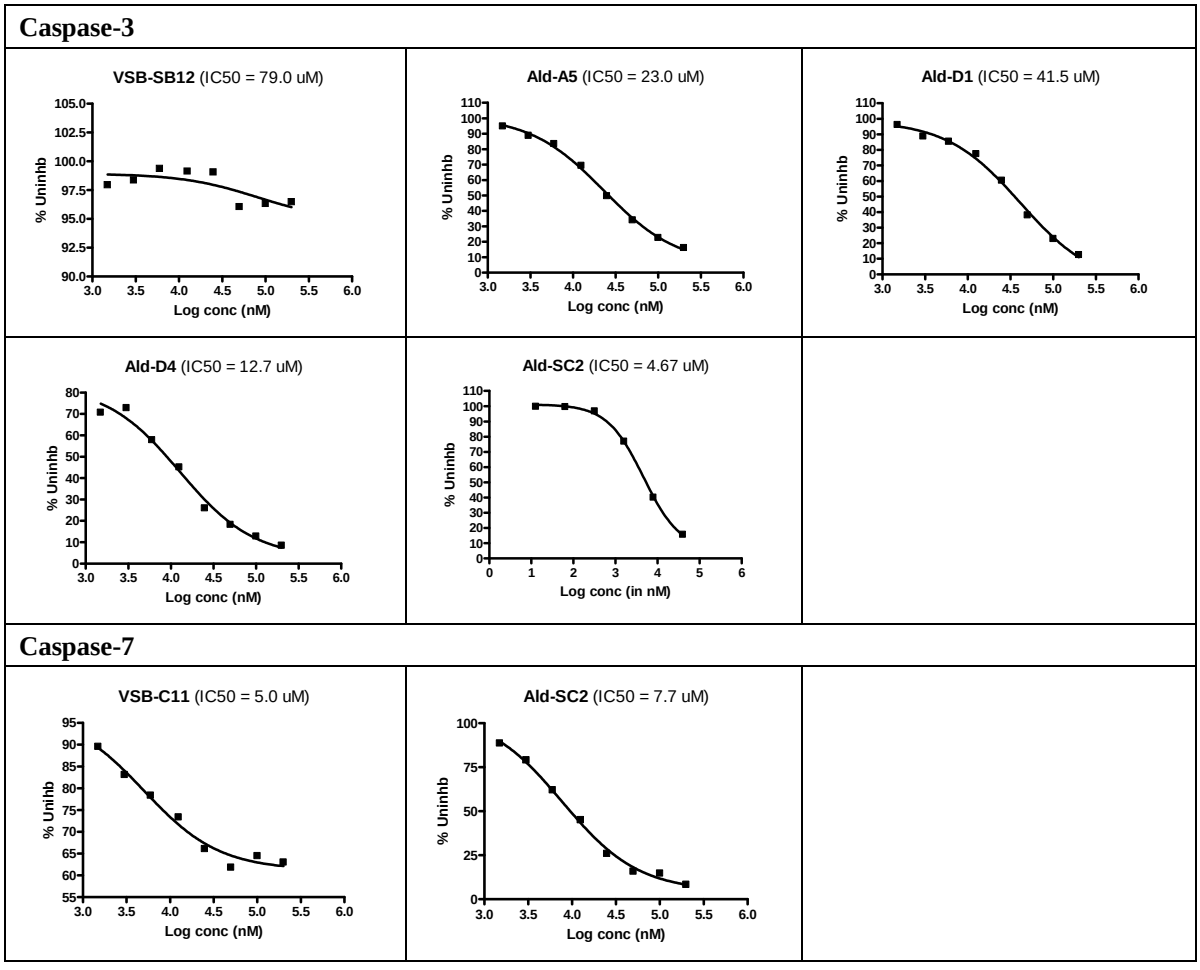


Figure S6. IC50 values of selected hits against caspase-3 and -7.

5. Docking simulations

In order to visualize the binding configurations, we docked Ald-SC2 against caspase-3 and VSB-C11 against caspase-7 several using the Sybyl™ 7.3 software, on the FlexX suite. Inhibitor structures were drawn and using the "Sketch Molecule" option and hydrogens were added. The structures were minimized using 1000 iterations at 0.05 kcal/mol Å to relieve any torsional strain and formal charges were assigned. Water molecules were removed from the protein structure, and the docking sphere was set at 15 Å in the enzyme active site. Applying these criteria, the docking was performed for 30 iterations, with the most optimized configuration for selected probes displayed in **Figure S7**. Protein surface representation was rendered by PyMol. As would be expected, the optimized docking configuration of the inhibitor/enzyme complex shows the P_1 aspartic acid residue in both inhibitors formed extensive interactions with the active-site resudles of the enzyme. In the case of Ald-SC2/Caspase 3 complex, two of the nitrogens in the triazole ring (formed by click chemistry) form H-bonds with Ser205 located in the enzyme active site.

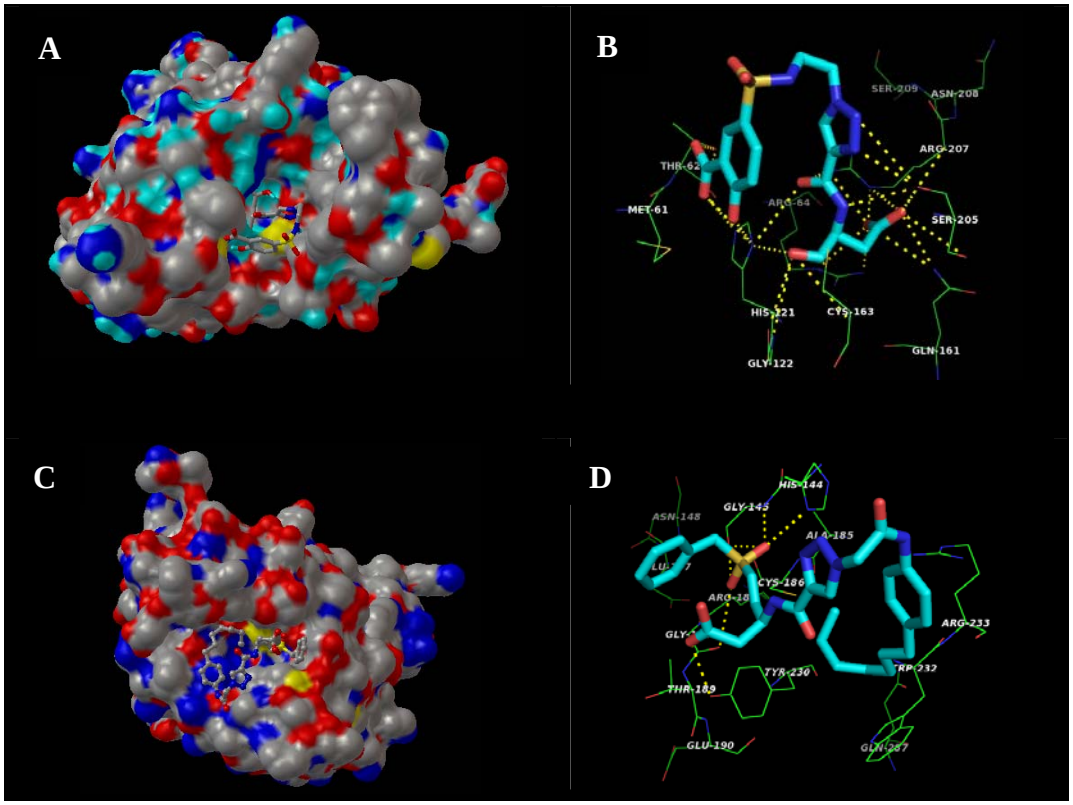


Figure S7. *In silico* docking displays the possible binding mode of the inhibitors Ald-SC2 against caspase-3 (**A** and **B**) and VSB-C11 against caspase-7 (**C** and **D**). Docking was performed using Sybyl v7.3 (Tripos, Missouri, USA) with molecular surface images generated using PyMol (DeLano Scientific LLC). Hydrogen bonds are shown as yellow lines.

6. References

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