SUPPORTING INFORMATION

Mapping the Protein Interaction Landscape for Fully Functionalized Small-Molecule Probes in Human Cells

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Table S1. Full proteomic data sets for quantitative MS (SILAC) probe versus probe studies. Data sets are provided as tabs within the accompanying Excel file. Each detected tryptic peptide is listed with its corresponding charge state in parentheses and the salt bump LC segment in which it was detected in brackets.

Tab legend.
Soluble Targets: *list of probe targets from soluble proteome of PC3 cells.*
8 versus **3** soluble: *proteomic data from soluble proteome for probe **8** versus probe **3** comparison.*
22 versus **3** soluble: *proteomic from soluble proteome data for probe **22** versus probe **3** comparison.*
24 versus **3** soluble: *proteomic data from soluble proteome for probe **24** versus probe **3** comparison.*
26 versus **3** soluble: *proteomic data from soluble proteome for probe **26** versus probe **3** comparison.*
31 versus **3** soluble: *proteomic data from soluble proteome for probe **31** versus probe **3** comparison.*
55 versus **3** soluble: *proteomic data from soluble proteome for probe **55** versus probe **3** comparison.*
Membrane targets of **3** (**26** versus **3**): *list of probe targets from membrane proteome of PC3 cells which preferentially engage probe **3** in comparison to probe **26**.*
26 versus **3** membrane: *proteomic data from membrane proteome for probe **26** versus probe **3** comparison.*
3 versus **3** no UV soluble: *proteomic data from soluble proteome for probe **3** versus probe **3** (no UV) comparison.*
24 versus **24** no UV soluble: *proteomic data from soluble proteome for probe **24** versus probe **24** (no UV) comparison.*
31 versus **31** no UV soluble: *proteomic data from soluble proteome for probe **31** versus probe **31** (no UV) comparison.*

Table S2. Full proteomic data sets for quantitative MS (SILAC) competitor studies. Data sets from soluble PC3 proteomes for competition experiments with non-clickable competitor analogues are provided as tabs within the accompanying Excel file. Each detected tryptic peptide is listed with its corresponding charge state in parentheses and the salt bump LC segment in which it was detected in brackets.

Tab legend

3 versus **66**: *proteomic data from soluble proteome for probe **3** versus probe **66** comparison.*
8 versus **62**: *proteomic data from soluble proteome for probe **8** versus probe **62** comparison.*
22 versus **71**: *proteomic data from soluble proteome for probe **22** versus probe **71** comparison.*
24 versus **60**: *proteomic data from soluble proteome for probe **24** versus probe **60** comparison.*
26 versus **70**: *proteomic data from soluble proteome for probe **26** versus probe **70** comparison.*
31 versus **64**: *proteomic data from soluble proteome for probe **31** versus probe **64** comparison.*

Table S3. Candidate promiscuous ligand-binding proteins, defined as proteins that show strong competition with non-clickable analogues of most test probes, but did not show substantial enrichment with any of the test probes in comparisons to probe **3**. This set of probe targets also showed ratios of ~1 and 20 in **3** versus **3** and **3** versus no-UV control experiments, respectively.

Protein Name	**31**:(**31** + **64**)*	**26**:(**26** + **70**)*	**8**:(**8** + **62**)*	**24**:(**24** + **60**)*	**3**:(**3** + **66**)*	**3**:**3**	**3**:(**3** + No-UV)
PTGR1	3.2	1.8	3.7	2.9	2.7	1.0	20
PTGR2	3.0	4.9	3.8	2.7	1.7	1.1	20
SPR	2.3	1.3	2.9	12	3.1	0.9	20
NAMPT	2.2	1.9	2.2	3.0	2.2	1.1	20
VDAC1	6.7	1.9	4.4	0.8	2.7	0.8	20
VDAC2	3.5	2.1	3.5	0.8	2.5	0.9	20

*Competition experiments performed as described in the main text with 10 μM probe and 20 μM non-clickable analogue.

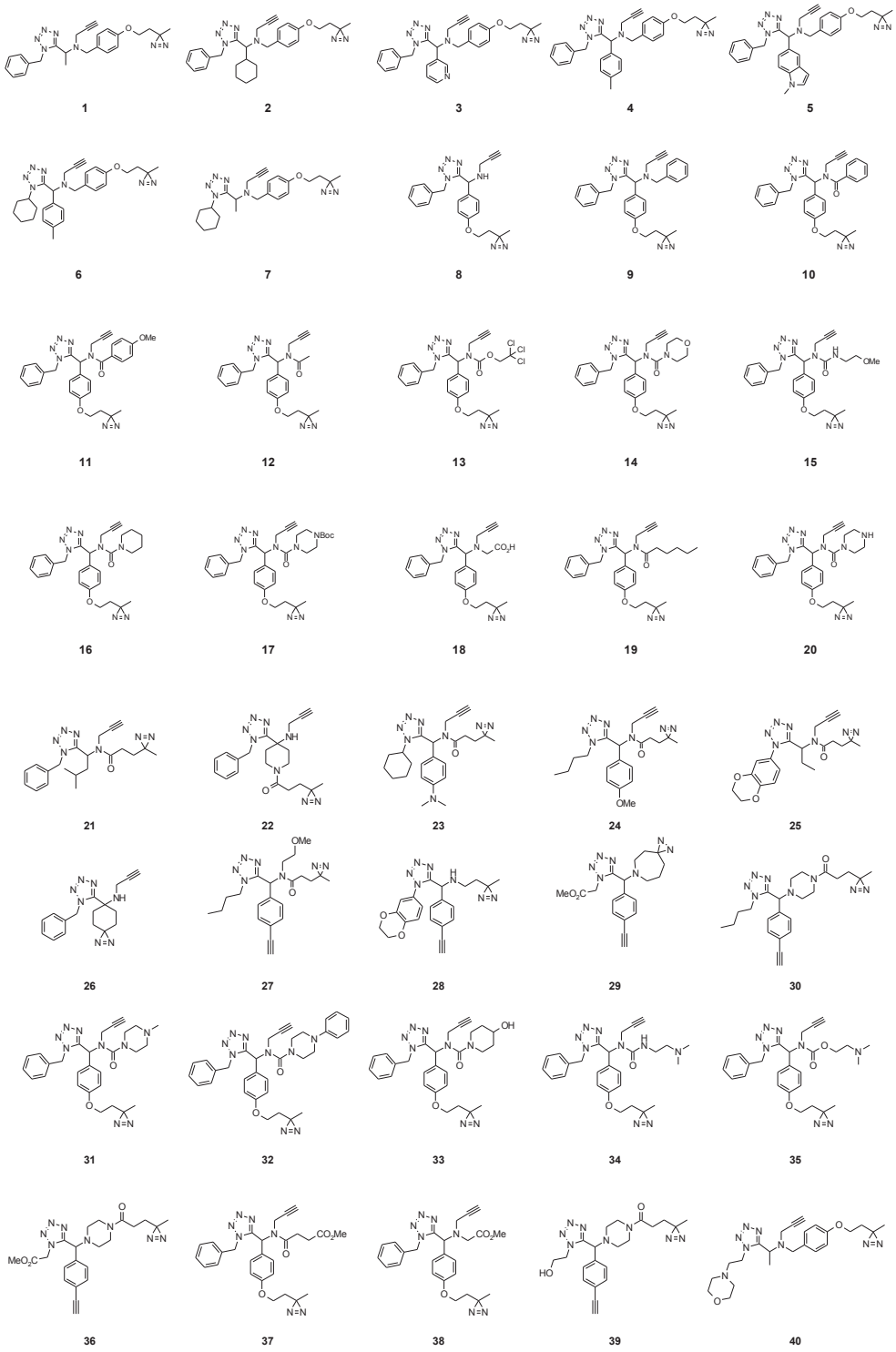


Figure S1. Structures of fully-functionalized probes (continued on next page).

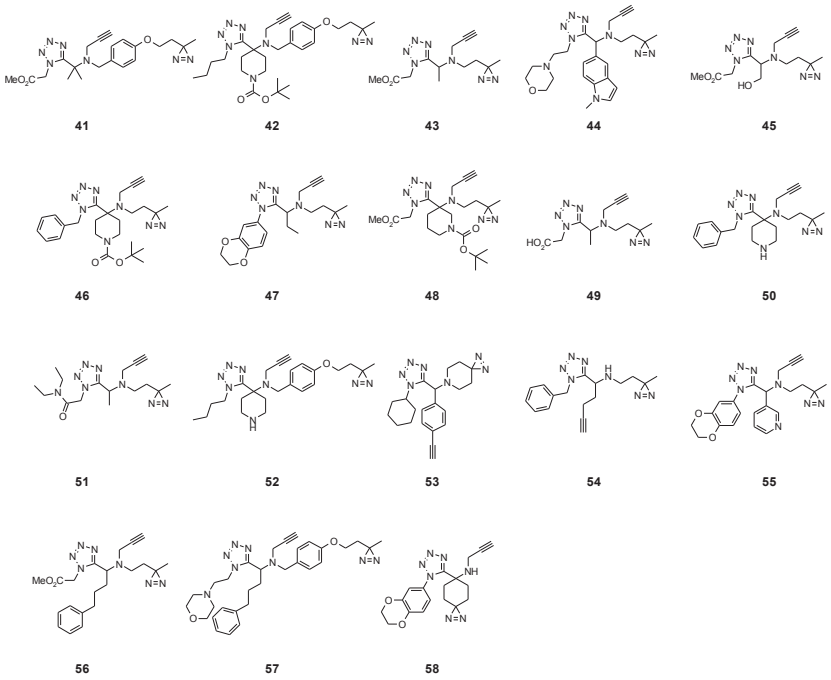


Figure S1 (cont.). Structures of fully-functionalized probes.

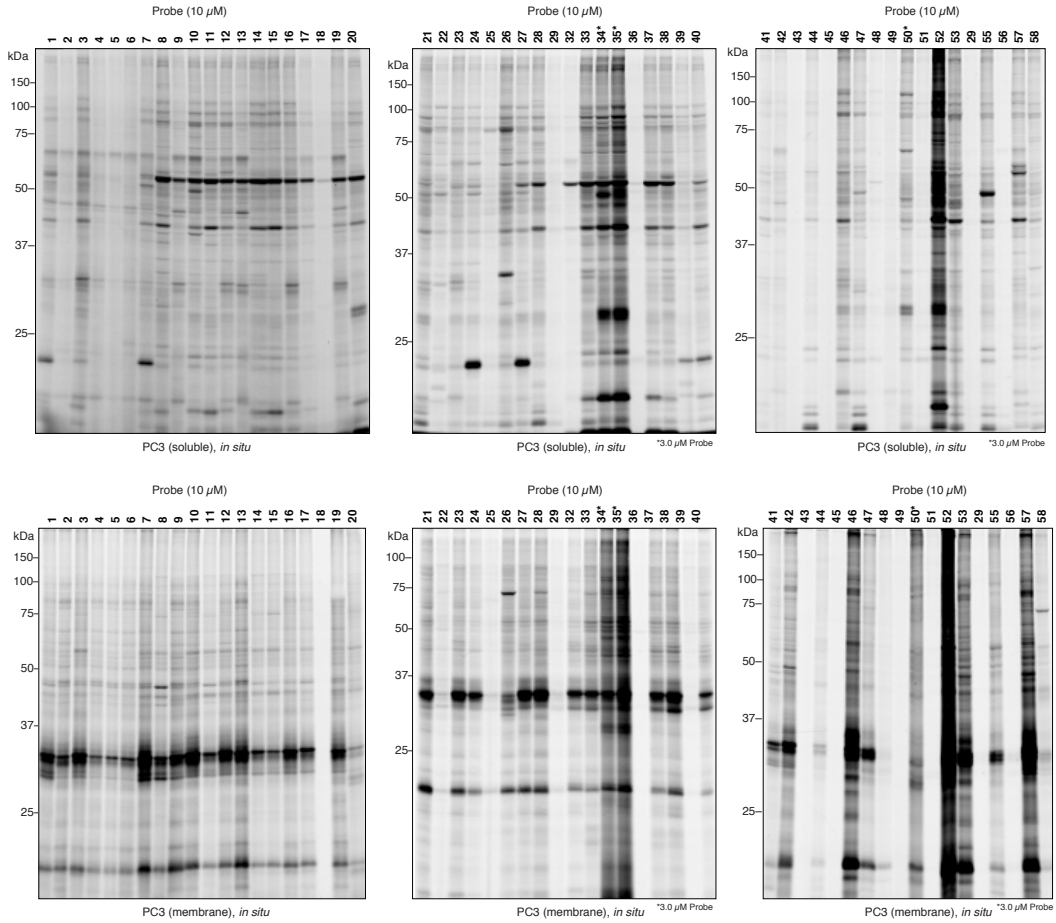


Figure S2. Gel-based profiles of *in situ* protein labeling events for membrane and soluble fractions of PC3 cells treated with probes. Unless otherwise specified, PC3 cells were treated for 30 min with each probe (10 μM) for 30 min at 37 ^{o}C and subsequently irradiated with UV light (365 nm, 10 min, 4 ^{o}C). Membrane and soluble fractions were prepared from cell lysates following centrifugation at 100,000 g and the probe-labeled proteins conjugated to a Rh-N_3 tag using CuAAC and analyzed by SDS-PAGE and fluorescence scanning.

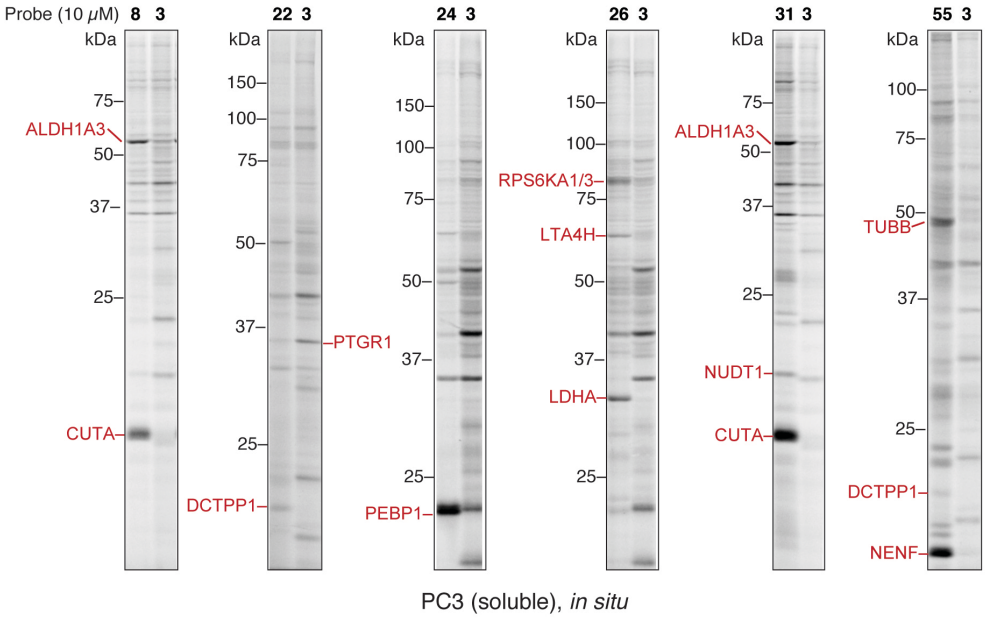


Figure S3. Comparative gel profiles for *in situ* protein labeling events of test probes (**8**, **22**, **24**, **26**, **31** and **55)** versus **3** in the soluble fraction of PC3 cells. Gel bands are labeled with putative protein identifications based on a correlation of MS-based probe selectivity data and the predicted molecular weights of each protein target.

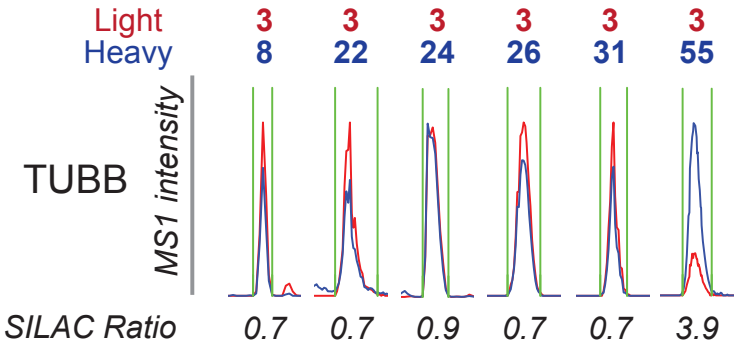


Figure S4. Representative MS1 peptide traces for tubulin (TUBB) showing preferential labeling by **55** versus **3**, but not by other test probes. Peptide chromatograms correspond to the $[K.LAVNMVPFPR.L]^{2+}$ peptide [m/z = 572.3208 (light) and 577.3249 (heavy)].

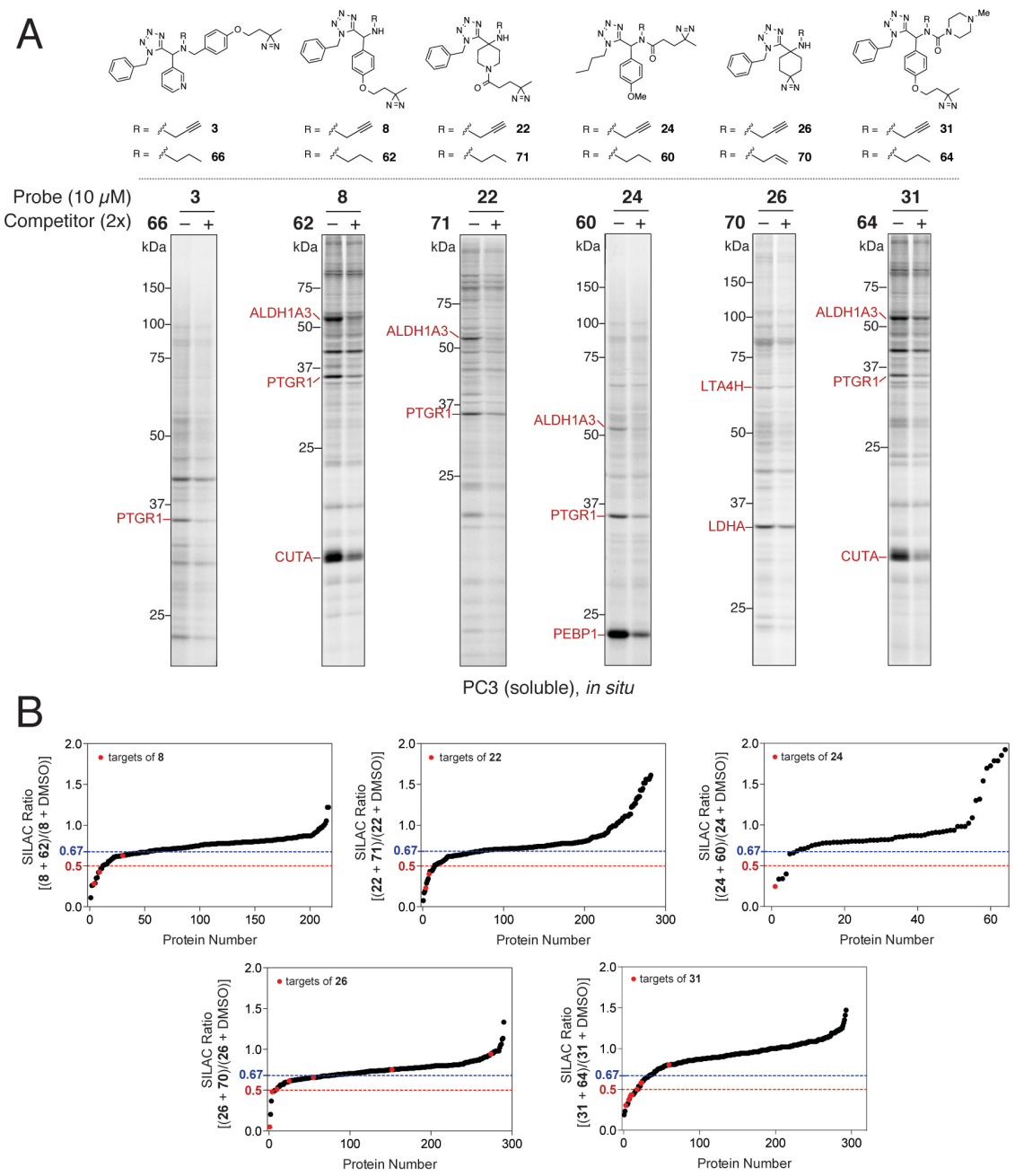
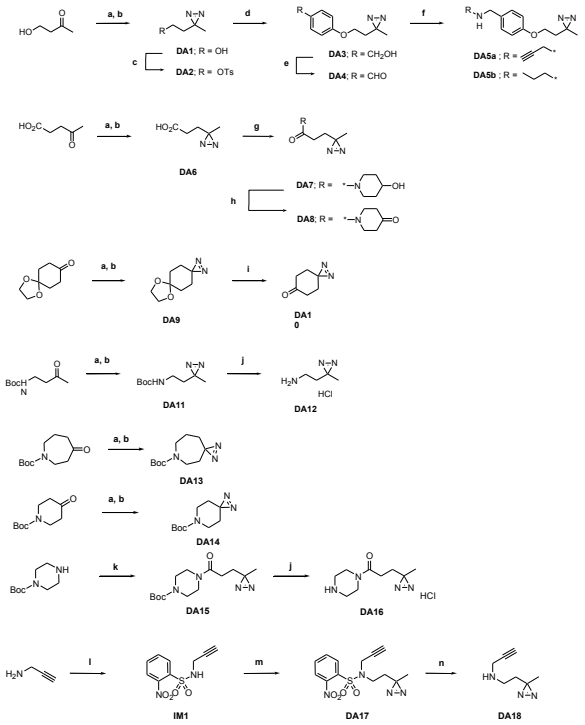


Figure S5. Gel- and MS-based profiles for *in situ* competition of test probes with their non-clickable analogs. **A**, Structures of test probes and their non-clickable competitors; and gel-based profiles for *in situ* competition experiments involving treatment of PC3 cells with the indicated probe (10 μM) and DMSO or non-clickable competitors (20 μM) for 30 min prior to UV irradiation, lysis, conjugation with Rh-N_3 by CuAAC and analysis by SDS-PAGE and fluorescence gel imaging. **B**, SILAC ratio plots for total proteins identified in competition experiments with non-clickable competitors. Data points in red indicate preferred targets of each probe, identified from test probe-versus-**3** comparative experiments (see **Figures 3A** and **S3**). The red and blue dashed lines mark boundaries for substantial (two-fold or greater) and partial (between 1.5-2.0 fold) competition by the non-clickable analogues, respectively.

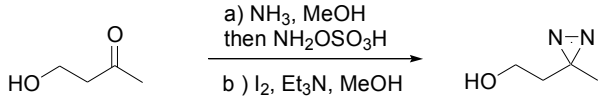
II. EXPERIMENTAL SECTION

General Methods: All chemicals, including anhydrous solvents, were obtained from commercial suppliers and were used without further purification. Merck silica gel TLC plates (0.25 mm, 60 F_{254}) were used to monitor reactions and visualized under UV light (254 nm) or by staining with potassium permanganate ($KMnO_4$), cerium ammonium molybdenate, phosphomolybdic acid or anisaldehyde. Flash chromatography was performed using SiliaFlash F60 silica gel (40–63 μm, 60 Å). NMR spectra were recorded at room temperature on Bruker DRX-500, Varian Inova-400 or Bruker DRX-600 (5 mm DCH Cryoprobe) instruments. Chemical shifts are recorded in ppm relative to tetramethylsilane (TMS) or solvent signals [$CDCl_3$ (^{13}C, 77.16 ppm), MeOD-d_4 (^{13}C, 49.0)] with peaks being reported as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant (Hz). High-resolution mass spectra (HRMS) were obtained on an Agilent LC/MSD TOF mass spectrometer by electrospray ionization–time-of-flight (ESI-TOF). IR experiments were recorded on Thermo Nicolet 380 FT-IR spectrometer. Cell culture media and supplements were obtained through CellGro or Omega Scientific. Photocrosslinking was performed with a Spectrolinker XL-1000 (Spectroline).

Synthesis of diazirine building blocks DA1-DA18

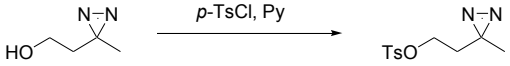


Scheme 1. **a)** 7N NH_3 in MeOH; *then* NH_2OSO_3H, MeOH; **b)** I_2, Et_3N, MeOH; **c)** *p*-TsCl, Py; **d)** 4-hydroxy benzylalcohol, Cs_2CO_3, DMF; **e)** SO_3•Py, Et_3N, DMSO, EtOAc; **f)** propargylamine, $NaBH(OAc)_3$, CH_3CN, CH_2Cl_2, **g)** 4-hydroxypiperidine, EDC•HCl, HOAt, DMF; **h)** SO_3•Py, *i*Pr_2NEt, DMSO, EtOAc; **i)** 5% HCl,THF; **j)** HCl in 1,4-dioxane, MeOH; **k**) **DA6**, EDC•HCl, HOAt, DMF **l)** 2-nitrobenzenesulfonyl chloride, Et_3N, CH_2Cl_2; **m) DA2**, K_2CO_3, DMF; **n)** LiOH•H_2O, $HS(CH_2)_2CO_2H$, DMF.



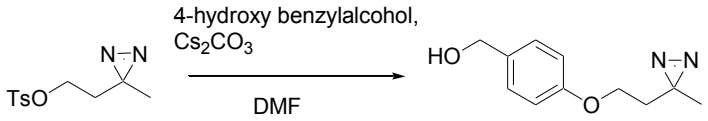
DA1

2-(3-Methyl-3H-diaziren-3-yl)ethanol (DA1). A round-bottom flask containing 4-hydroxy-2-butanone (6.2 g, 69 mmol) was cooled to 0 °C under N_2 and 7N NH_3 in MeOH (70 ml) was added slowly. After 3 h, an anhydrous methanolic solution of NH_2OSO_3H (8.5 g, 75 mmol) was added dropwise at 0 °C. The resulting solution was allowed to warm to room temperature overnight. The mixture was evaporated to dryness in the reaction vessel under a stream of dry N_2, and the remaining residue was then resuspended in anhydrous MeOH and the insoluble material was filtered away. The filter paper was washed with additional MeOH and the total filtrate was then concentrated under reduced pressure and re-dissolved in anhydrous MeOH (50 ml) in an amber flask. The solution was cooled to 0 °C, and Et_3N (15 mL) was added. I_2 (7.2 g, 28 mmol) was then added in small portions until a dark brown color persisted in the solution for more than 10 minutes indicating complete oxidation of the diaziridine intermediate. The solution was then diluted with EtOAc and the organic phase was washed successively with 1N HCl and then sat. aq. $Na_2S_2O_3$. The organic phase was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* in an amber flask to yield the title compound as a pale yellow oil (1.4 g, 20%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.54 (t, *J* = 6.0 Hz, 2H), 1.65 (t, *J* = 6.0 Hz, 2H), 1.09 (s, 3H); HRMS (ESI-TOF) calc'd for $C_4H_9N_2O$ [M + H]$^+$, 101.0709; found, 101.0706.



DA1 **DA2**

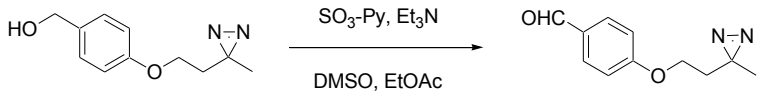
2-(3-Methyl-3*H*-diaziren-3-yl)ethyl 4-methylbenzenesulfonate (DA2). To a stirred solution of **DA1** (300 mg, 3.0 mmol) in pyridine (2.5 mL) was added *p*-toluenesulfonyl chloride (800 mg, 4.2 mmol) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was diluted with EtOAc, washed with 1N HCl, sat. aq. $NaHCO_3$, brine, and then dried over anhydrous Na_2SO_4. Evaporation of the solvent gave the title compound as a pale yellow oil (760 mg, 100%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, *J* = 8.6 Hz, 2H), 7.37 (d, *J* = 8.0 Hz, 2H), 3.96 (t, *J* = 8.0 Hz, 2H), 2.46 (s, 3H), 1.68 (t, *J* = 6.4 Hz, 2H), 1.01 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{11}H_{15}N_2O_3S$ [M + H]$^+$, 255.0798; found, 255.0797.



DA2 **DA3**

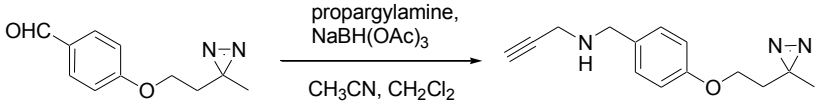
{4-[2-(3-Methyl-3*H*-diaziren-3-yl)ethoxy]phenyl}methanol (DA3). To a solution of **DA2** (76 mg, 3.00 mmol) in DMF (3 mL) was added Cs_2CO_3 (2.9 g, 9.0 mmol) and 4-hydroxy benzylalcohol (560 mg, 4.5 mmol) at room temperature. After being stirred at 80 °C for 1 h, the reaction mixture was cooled and diluted with EtOAc. The organic layer was then washed with H_2O, brine, dried over anhydrous Na_2SO_4

and concentrated under reduced pressure. Purification of the crude reaction mixture by chromatography on silica gel (30–50% EtOAc/hexanes) yielded the title compound as a yellow oil (290 mg, 46% from **DA1**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (d, J = 8.4 Hz, 2H), 6.89 (d, J = 8.4 Hz, 2H), 4.63 (s, 2H), 3.88 (t, J = 6.4 Hz, 2H), 1.82 (t, J = 6.4 Hz, 2H), 1.12 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{11}H_{15}N_2O_2$ [M + H]$^+$, 229.0947; found, 229.0947.



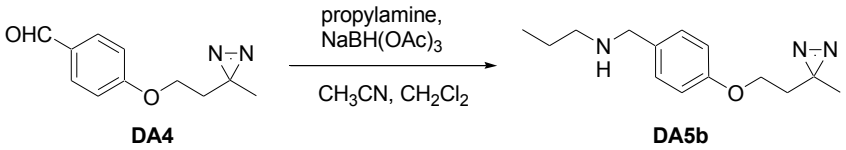
DA3 **DA4**

4-[2-(3-Methyl-3*H*-diaziren-3-yl)ethoxy]benzaldehyde (DA4). To a stirred solution of the alcohol **DA3** (290 mg, 1.4 mmol) in EtOAc (5 mL) and Et_3N (1.2 mL, 8.3 mmol) was added SO_3•Py (660 mg, 4.1 mmol) in DMSO (2.5 mL) at 0 °C under an argon atmosphere. After being stirred at room temperature for 20 min, the reaction was quenched with 1N HCl. The reaction mixture was extracted with EtOAc, washed with sat. aq. $NaHCO_3$ and brine and then dried over anhydrous Na_2SO_4. The solvent was removed by evaporation and the resulting residue was purified by column chromatography on silica gel (10–50% EtOAc in hexanes) to give the title compound as a pale yellow oil (250 mg, 87%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.89 (s, 1H), 7.84 (d, J = 8.4 Hz, 2H), 6.98 (d, J = 8.4 Hz, 2H), 3.96 (t, J = 6.4 Hz, 2H), 1.87 (t, J = 6.4 Hz, 2H), 1.14 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{11}H_{13}N_2O_2$ [M + H]$^+$, 205.0971; found, 205.0977.



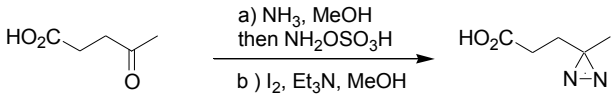
DA4 **DA5a**

***N*-{4-[2-(3-Methyl-3*H*-diaziren-3-yl)ethoxy]benzyl}-2-propyn-1-amine (DA5a).** To the stirred solution of **DA4** (200 mg, 0.98 mmol) and propargylamine (160 mg, 2.9 mmol) in CH_3CN: CH_2Cl_2 (2.0 mL, 1:1) was added $NaBH(OAc)_3$ (620 mg, 2.9 mmol) at 0 °C under a N_2 atmosphere. After being stirred at room temperature for additional 15 h, the reaction mixture was diluted with EtOAc, washed with H_2O, sat. aq. $NaHCO_3$, brine, and dried over anhydrous Na_2SO_4. The organic layer was evaporated and the resulting residue was purified by column chromatography on silica gel (25–50% EtOAc/hexanes) to give the title compound as a colorless oil (180 mg, 75%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 (d, J = 8.5 Hz, 2H), 6.85 (d, J = 8.5 Hz, 2H), 3.87 (t, J = 6.5 Hz, 2H), 3.82 (s, 2H), 3.41 (t, J = 2.5 Hz, 2H), 2.25 (t, J = 2.5 Hz, 1H), 1.80 (t, J = 6.5 Hz, 2H), 1.11 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{14}H_{19}N_3O$ [M + H]$^+$, 244.1450; found, 244.1459.



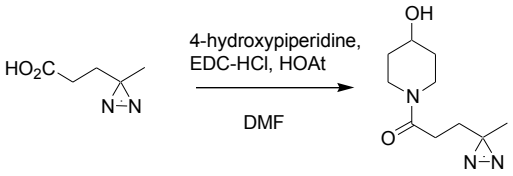
DA4 **DA5b**

***N*-{4-[2-(3-Methyl-3*H*-diaziren-3-yl)ethoxy]benzyl}-1-propanamine (DA5b).** The title compound was prepared from **DA4** (100 mg, 0.49 mmol), propylamine (0.12 mL, 1.5 mmol), $NaBH(OAc)_3$ (310 mg, 1.5 mmol) in 1:1 in CH_3CN:CH_2Cl_2 (0.4 mL, 1:1) according to the same procedure as described for the preparation of **DA5a** from **DA4** as a colorless oil (110 mg, 91%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.23 (d, *J* = 8.4 Hz, 2H), 6.83 (d, *J* = 8.4 Hz, 2H), 3.85 (t, *J* = 6.6 Hz, 2H), 3.71 (s, 2H), 2.57 (t, *J* = 7.2 Hz, 2H), 1.79 (t, *J* = 6.6 Hz, 2H), 1.56-1.48 (m, 2H), 1.11 (s, 3H), 0.91 (t, *J* = 7.8 Hz, 3H); HRMS (ESI-TOF) calc'd for $C_{14}H_{22}N_3O$ $[M + H]^+$, 248.1757; found, 248.1754.



DA6

3-(3-Methyl-3H-diaziren-3-yl)propanoic acid (DA6). The title compound was prepared from levulinic acid (14 g, 0.12 mol), NH_2OSO_3H (22 g, 200 mmol) and 7N NH_3 methanolic solution (140 mL) in MeOH (120 mL) and I_2 (14 g, 56 mmol), Et_3N (30 mL) in MeOH (100 mL) according to the same procedure used in the preparation of **DA1** and isolated as a yellow oil (3.8 g, 20%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.24 (t, *J* = 8.0 Hz, 2H), 1.73 (t, *J* = 8.0 Hz, 2H), 1.06 (s, 3H); HRMS (ESI-TOF) calc'd for $C_5H_8N_2O_2Na$ $[M + Na]^+$, 151.0478 ; found, 151.0471.

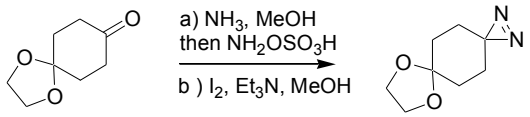


DA6 **DA7**

1-(4-Hydroxy-1-piperidinyl)-3-(3-methyl-3*H*-diaziren-3-yl)-1-propanone (DA7). To a stirred solution of 4-hydroxypiperidine (200 mg, 2.0 mmol), **DA6** (300 mg, 2.3 mmol) and HOAt (130 mg, 0.98 mmol) in DMF (3.0 mL) was added EDC•HCl (750 mg, 3.9 mmol) at room temperature under N_2. After being stirred at room temperature for 3 h, the reaction mixture was cooled then diluted with EtOAc, washed with H_2O, sat. aq. $NaHCO_3$, brine, and dried over anhydrous Na_2SO_4. The organic layer was evaporated and the resulting residue was purified by column chromatography on silica gel (EtOAc) to give the title compound as a colorless oil (180 mg, 43%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.02 (m, 1H), 3.94 (m, 1H), 3.71 (m, 1H), 3.20-3.15 (m, 2H), 2.16-2.09 (m, 2H), 1.94-1.83 (m, 3H), 1.80-1.75 (m, 2H), 1.55-1.49 (m, 2H), 1.05 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{10}H_{18}N_3O_2$ $[M + H]^+$, 212.1393; found, 212.1393.

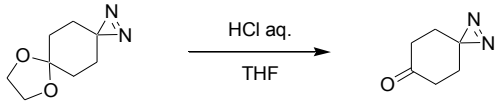
DA7 **DA8**

1-[3-(3-Methyl-3*H*-diaziren-3-yl)propanoyl]-4-piperidinone (DA8). To a stirred solution of the alcohol **DA7** (180 mg, 0.83 mmol) in EtOAc (2.5 mL) and *i*Pr_2NEt (0.85 mL, 5.0 mmol) was added a solution of SO_3•Py (400 mg, 2.5 mmol) in DMSO (1.3 mL) at 0 °C under an argon atmosphere. After being stirred at room temperature for 20 min, the reaction was quenched with 1N HCl. The reaction mixture was extracted with EtOAc, washed with sat. aq. $NaHCO_3$, brine, and dried over anhydrous Na_2SO_4. The solvent was removed by evaporation and the resulting residue was purified by column chromatography on silica gel (33–100% EtOAc/hexanes) to give the title compound as a yellow oil (139 mg, 80%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.90 (t, *J* = 6.4 Hz, 2H), 3.72 (t, *J* = 6.2 Hz, 2H), 2.51-2.48 (m, 4H), 2.18 (t, *J* = 7.2 Hz, 2H), 1.86 (t, *J* = 7.2 Hz, 2H), 1.08 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{10}H_{16}N_3O_2$ $[M + H]^+$, 210.1237; found, 210.1241.



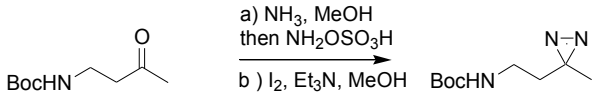
DA9

7,10-Dioxa-1,2-diazadispiro[2.2.4.2]dodec-1-ene (DA9). The title compound was prepared from 1,4-cyclohexanedione monoethylene acetal (600 mg, 3.9 mmol), 7N NH_3 in MeOH (3 mL), NH_2OSO_3H (520 mg, 4.6 mmol), MeOH (2 mL), I_2 (810 mg, 3.2 mmol) and Et_3N (0.8 mL) in MeOH (5 mL) according to the same procedure used in the preparation of **DA1** and isolated as a colorless oil (350 mg, 53%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.99 (s, 4H), 1.85 (t, *J* = 6.0 Hz, 4H), 1.33 (t, *J* = 6.0 Hz, 4H); HRMS (ESI-TOF) calc'd for $C_8H_{13}N_2O_2$ $[M + H]^+$, 169.0971; found, 169.0972.



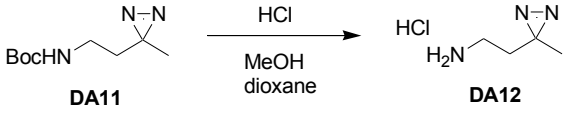
DA9 **DA10**

1,2-Diazaspiro[2.5]oct-1-en-6-one (DA10) A solution of **DA9** (33 mg, 0.19 mmol) in THF (0.3 mL) was treated with 5% HCl (aq.) (0.3 mL). After being stirred at 50 °C for 1.5 h, the reaction mixture was cooled, then diluted with Et_2O, washed with H_2O, sat. aq. $NaHCO_3$, brine, and dried over anhydrous Na_2SO_4. Removal of the organic solvent to dryness to give the title compound as a colorless oil (12 mg, 50%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.60 (t, *J* = 6.0 Hz, 4H), 1.59 (t, *J* = 6.0 Hz, 4H); HRMS (ESI-TOF) calc'd for $C_6H_9N_2O$ $[M + H]^+$, 125.0709; found, 125.0729.



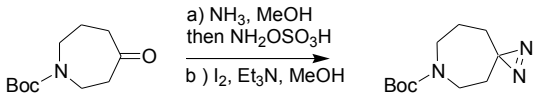
DA11

***tert*-Butyl [2-(3-methyl-3*H*-diaziren-3-yl)ethyl]carbamate (DA11).** The title compound was prepared from *N*-Boc-4-amino-2-butanone (1.1 g, 6.0 mmol), 7N NH_3 in MeOH (12 mL), NH_2OSO_3H (810 mg, 7.2 mmol), MeOH (6 mL), I_2 (680 mg, 2.7 mmol) and Et_3N (2.5 mL) in MeOH (5 mL) according to the same procedure used in the preparation of **DA1** and isolated as a colorless oil (360 mg, 30%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.57 (m, 1H), 3.10-3.02 (m, 2H), 1.55 (t, *J* = 6.0 Hz, 2H), 1.45 (s, 9H), 1.05 (s, 3H); HRMS (ESI-TOF) calc'd for $C_9H_{18}N_3O_2$ $[M + H]^+$, 200.1393; found, 200.1393.



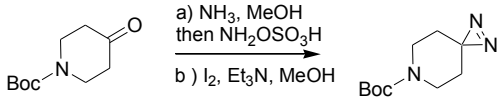
DA11 **DA12**

2-(3-Methyl-3*H*-diaziren-3-yl)ethanamine hydrochloride (DA12) A solution of **DA11** (35 mg, 0.18 mmol) in MeOH (0.15 mL) was treated with 4N HCl in 1,4-dioxane (0.15 mL) at 0 °C. After being stirred for 30 min, the reaction mixture was evaporated to dryness to give the title compound as a white powder (24 mg, 100%): ^{1}H NMR (400 MHz, CD_3OD) δ 2.95 (t, *J* = 8.0 Hz, 2H), 1.73 (t, *J* = 8.0 Hz, 2H), 1.11 (s, 3H); HRMS (ESI-TOF) calc'd for $C_4H_{10}N_3$ $[M + H]^+$, 100.0869; found, 100.0870.



DA13

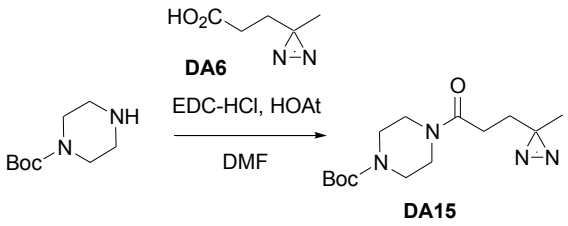
***tert*-Butyl 1,2,6-triazaspiro[2.6]non-1-ene-6-carboxylate (DA13).** The title compound was prepared from *N*-Boc-hexahydro-1*H*-azepin-4-one (850 mg, 4.0 mmol), 7N NH_3 in MeOH (9 mL), NH_2OSO_3H (540 mg, 4.8 mmol), MeOH (4 mL), I_2 (710 mg, 2.8 mmol) and Et_3N (1.5 mL) in MeOH (3 mL) according to the same procedure used in the preparation of **DA1** and isolated as a colorless oil (470 mg, 52% in 2 steps): ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.55-3.41 (m, 4H), 1.71-1.52 (m, 2H), 1.51 and 1.47 (s, 9H), 1.41 (m, 1H), 1.35-1.32 (m, 3H); HRMS (ESI-TOF) calc'd for $C_{11}H_{20}N_3O_2$ $[M + H]^+$, 226.1550; found, 226.1550.



DA14

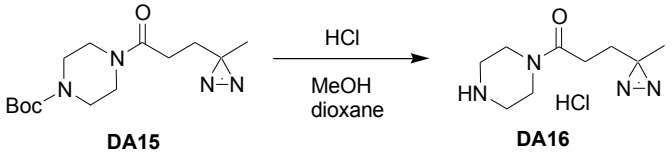
***tert*-Butyl 1,2,6-triazaspiro[2.5]oct-1-ene-6-carboxylate (DA14).** The title compound was prepared from 1-Boc-4-piperidone (200 mg, 1.0 mmol), 7N NH_3 in MeOH (1 mL), NH_2OSO_3H (140 mg, 1.2 mmol), MeOH (1 mL), I_2 (210 mg, 0.83 mmol) and Et_3N (0.2 mL) in MeOH (1 mL) according to the same procedure used in the preparation of **DA1** and isolated as a colorless oil (69 mg, 33% in 2 steps): ^{1}H NMR

(500 MHz, $CDCl_3$) δ 3.62-3.58 (m, 4H), 1.50 (s, 9H), 1.27-1.24 (m, 4H); HRMS (ESI-TOF) calc'd for $C_{10}H_{18}N_3O_2$ $[M + H]^+$, 212.1393; found, 212.1389.



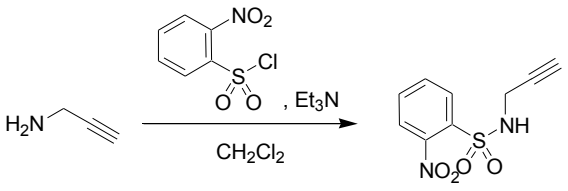
DA15

***tert*-Butyl 4-[3-(3-methyl-3*H*-diaziren-3-yl)propanoyl]-1-piperazinecarboxylate (DA15).** To a stirred solution of 1-Boc-piperazine (300 mg, 1.6 mmol), **DA6** (230 mg, 1.8 mmol) and HOAt (110 mg, 0.81 mmol) in DMF (3.2 mL) was added EDC•HCl (620 mg, 3.2 mmol) at room temperature under N_2. After being stirred at room temperature for 3 h, the reaction mixture was cooled then diluted with EtOAc, washed with H_2O, sat. aq. $NaHCO_3$, brine, and dried over anhydrous Na_2SO_4. The organic layer was evaporated and the resulting residue was purified by column chromatography on silica gel (33–50% EtOAc/hexanes) to give the title compound as a white powder (180 mg, 38%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.59-3.55 (m, 2H), 3.45-3.38 (m, 6H), 2.11 (t, *J* = 7.8 Hz, 2H), 1.82-1.78 (m, 2H), 1.47 (s, 9H), 1.07 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{14}H_{25}N_3O_3$ $[M + H]^+$, 297.1921; found, 297.1922.



DA15 DA16

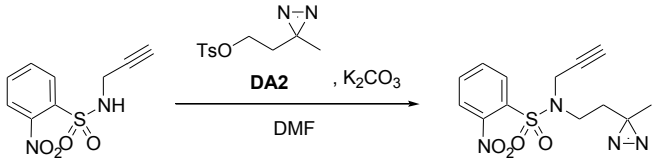
3-(3-Methyl-3*H*-diaziren-3-yl)-1-(1-piperazinyl)-1-propanone hydrochloride (DA16). A solution of compound **DA15** (70 mg, 0.24 mmol) in MeOH (0.4 mL) was treated with 4N HCl in 1,4-dioxane (0.4 mL) at 0 °C. After being stirred at room temperature for 30 min, the reaction mixture was evaporated to dryness to give the title compound as white powder (55 mg, 100%): ^{1}H NMR (400 MHz, CD_3OD) δ 3.90-3.81 (m, 4H), 3.29-3.22 (m, 4H), 2.36 (t, *J* = 7.6 Hz, 2H), 1.72 (t, *J* = 7.6 Hz, 2H), 1.06 (s, 3H); HRMS (ESI-TOF) calc'd for $C_9H_{17}N_4O$ $[M + H]^+$, 197.1397; found, 197.1396.



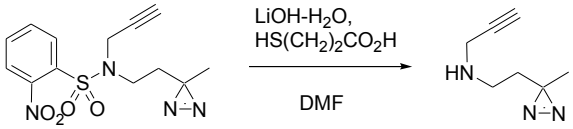
IM1

2-Nitro-*N*-(2-propyn-1-yl)benzenesulfonamide (IM1). To a stirred solution of propargylamine (1.0 g, 18 mmol) and Et_3N (2.5 mL, 18 mmol) in CH_2Cl_2 (15 mL) was added 2-nitrobenzenesulfonyl chloride (3.80 g, 17 mmol) at 0 °C under N_2. After being stirred at room temperature 3 h, the reaction was diluted with CH_2Cl_2, washed with 2N HCl, H_2O, brine, and dried over anhydrous Na_2SO_4. The solvent was removed by

evaporation and washed with EtOAc/hexanes to give the title compound as an ivory powder (3.9 g, 95%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (m, 1H), 7.91 (m, 1H), 7.78-7.74 (m, 2H), 5.71 (bs, 1H), 4.05-4.00 (m, 2H), 1.98 (t, *J* = 2.4 Hz, 1H); HRMS (ESI-TOF) calc'd for $C_9H_9N_2O_4S$ $[M + H]^+$, 241.0278; found, 241.0279.



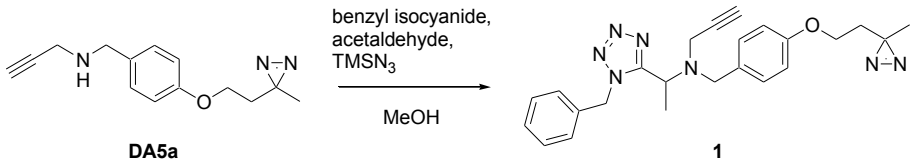
***N*-[2-(3-Methyl-3*H*-diaziren-3-yl)ethyl]-2-nitro-*N*-(2-propyn-1-yl)benzenesulfonamide (DA17).** To a solution of **DA2** (760 mg, 3.0 mmol) in DMF (6 mL) were added K_2CO_3 (1.2 g, 9.0 mmol) and **IM1** (720 mg, 3.0 mmol) at room temperature. After being stirred at 80 ^{o}C for 3 h, the reaction mixture was cooled, and diluted with EtOAc, washed with H_2O, brine, dried over anhydrous Na_2SO_4, and concentrated by evaporation. Purification by column chromatography on silica gel (33% EtOAc/hexanes) yielded the title compound as a colorless oil (420 mg, 43%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (m, 1H), 7.80-7.65 (m, 3H), 4.19 (d, *J* = 3.0 Hz, 2H), 3.45-341 (m, 2H), 2.17 (t, *J* = 2.4 Hz, 1H), 1.65-1.61 (m, 2H), 1.07 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{13}H_{15}N_4O_4S$ $[M + H]^+$, 323.0808; found, 323.0803.



***N*-[2-(3-Methyl-3*H*-diaziren-3-yl)ethyl]-2-propyn-1-amine (DA18).** To a stirred solution of **DA17** (1.4 g, 4.3 mmol) in DMF (9 mL) was added LiOH•H_2O (538 mg, 13 mmol) and 3-mercaptopropionic acid (0.55 ml, 6.3 mmol) at room temperature. After being stirred at room temperature for 2 h, the reaction mixture was diluted with EtOAc, washed with H_2O, sat. aq. $NaHCO_3$, brine, and dried over anhydrous Na_2SO_4 and concentrated by evaporation. Purification by column chromatography on silica gel (10–20% EtOAc/hexanes) yielded the title compound as a colorless oil (250 mg, 42%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.40 (d, *J* = 2.4 Hz, 1H), 2.56 (t, *J* = 7.2 Hz, 2H), 2.21 (t, *J* = 2.4 Hz, 1H), 1.57 (t, *J* = 7.2 Hz, 2H), 1.38 (bs, 1H), 1.05 (s, 3H); HRMS (ESI-TOF) calc'd for $C_7H_{12}N_3$ $[M + H]^+$, 138.1026; found, 138.1029.

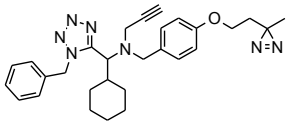
Synthesis of fully-functionalized probe library

Representative Ugi-azide condensation reaction: **Method A**



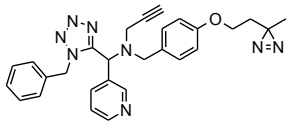
DA5a **1**

***N*-[1-(1-Benzyl-1*H*-tetrazol-5-yl)ethyl]-*N*-{4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]benzyl}-2-propyn-1-amine (1).** To a stirred solution of **DA5a** (30 mg, 0.12 mmol), acetaldehyde (6.9 µL, 0.12 mmol) and benzylisocyanide (14 mg, 0.12 mmol) in MeOH (0.3 mL) was added $TMSN_3$ (10 µL, 0.12 mmol) at 0 °C under N_2. After being stirred at room temperature for 3 h, the reaction mixture was cooled then diluted with EtOAc, washed with H_2O, sat. aq. $NaHCO_3$, brine, and dried over anhydrous Na_2SO_4. The organic layer was evaporated and the resulting residue was purified by column chromatography on silica gel (25–50% EtOAc/hexanes) to give the title compound as a colorless oil (21 mg, 40%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38-7.29 (m, 3H), 7.17-7.10 (m, 2H), 7.06 (d, *J* = 6.8 Hz, 2H), 6.82 (d, *J* = 6.8 Hz, 2H), 5.68 (d, *J* = 12.0 Hz, 1H), 5.67 (d, *J* = 12.0 Hz, 1H), 4.43 (m, 1H), 3.84 (t, *J* = 6.4 Hz, 2H), 3.67 (d, *J* = 10.8 Hz, 1H), 3.48 (d, *J* = 10.8 Hz, 1H), 3.28 (s, 2H), 2.26 (t, *J* = 2.4 Hz, 1H), 1.80 (t, *J* = 6.4 Hz, 2H), 1.57-1.53 (m, 3H), 1.12 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{24}H_{28}N_7O$ $[M + H]^+$, 430.2350; found 430.2366.



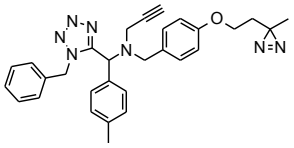
2

***N*-[(1-benzyl-1*H*-tetrazol-5-yl)(cyclohexyl)methyl]-*N*-{4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]benzyl}-2-propyn-1-amine (2).** The title compound was prepared from **DA5a** (30 mg, 0.12 mmol), cyclohexanecarboxaldehyde (15 µL, 0.12 mmol), benzylisocyanide (14 mg, 0.12 mmol), MeOH (0.3 mL), and $TMSN_3$ (10 µL, 0.12 mmol) according to **Method A** and isolated as a colorless oil (41 mg, 67%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35-7.32 (m, 3H), 7.22 (d, *J* = 8.5 Hz, 2H), 7.19-7.11 (m, 2H), 6.86 (d, *J* = 8.5 Hz, 2H), 5.75 (d, *J* = 15.5 Hz, 1H), 5.37 (d, *J* = 15.5 Hz, 1H), 4.12 (m, 1H), 3.91 (m, 1H), 3.87 (t, *J* = 6.5 Hz, 2H), 3.42 (m, 1H), 3.29 (m, 1H), 3.05 (m, 1H), 2.33 (m, 1H), 2.22 (m, 1H), 2.18 (t, *J* = 2.4 Hz, 1H), 1.82 (t, *J* = 6.5 Hz, 2H), 1.73 (m, 1H), 1.60-1.55 (m, 2H), 1.35 (m, 1H), 1.25 (m, 1H), 1.22 (s, 3H), 1.08-0.85 (m, 3H), 0.72 (m, 1H); HRMS (ESI-TOF) calc'd for $C_{29}H_{36}N_7O$ $[M + H]^+$, 498.2976; found, 498.2988.



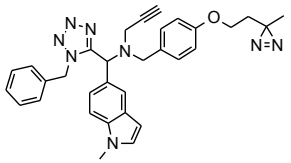
3

***N*-[(1-Benzyl-1*H*-tetrazol-5-yl)(3-pyridinyl)methyl]-*N*-{4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]benzyl}-2-propyn-1-amine (3).** The title compound was prepared from **DA5a** (30 mg, 0.12 mmol), 3-pyridinecarboxaldehyde (12 μL, 0.12 mmol), benzylisocyanide (14 mg, 0.12 mmol), MeOH (0.3 mL), and $TMSN_3$ (10 μL, 0.12 mmol) according to **Method A** and isolated as a colorless oil (41 mg, 68%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.52 (m, 1H), 8.34 (m, 1H), 7.86 (d, *J* = 7.8 Hz, 1H), 7.35-7.22 (m, 4H), 7.15 (d, *J* = 8.4 Hz, 2H), 7.07 (d, *J* = 6.6 Hz, 2H), 6.81 (d, *J* = 8.4 Hz, 2H), 5.71 (d, *J* = 15.6 Hz, 1H), 5.59 (d , *J* = 15.6 Hz, 1H), 5.28 (s, 1H), 3.84 (t, *J* = 6.6 Hz, 2H), 3.54 (d, *J* = 12.6 Hz, 1H), 3.41 (d, *J* = 12.6 Hz, 1H), 3.27 (dd, *J* = 18.0, 2.4 Hz, 1H), 3.16 (dd, *J* = 18.0 Hz, 2.4 Hz, 1H), 2.28 (t, *J* = 2.4 Hz, 1H), 1.80 (t, *J* = 6.6 Hz, 2H), 1.11 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 157.64, 153.41, 149.56, 136.45, 132.64, 130.53, 129.59, 128.98, 128.81, 128.64, 127.07, 123.27, 114.11, 77.96, 73.90, 62.48, 56.55, 52.91, 50.95, 38.64, 33.82, 29.24, 23.82, 19.78; IR (cm^{-1}) 3286, 3032, 2924, 2850, 1585, 1610, 1509, 1473, 1453, 1425, 1385, 1300, 1241, 1102, 1025, 829, 753, 716, 667; HRMS (ESI-TOF) calc'd for $C_{28}H_{29}N_8O$ $[M + H]^+$, 493.2459; found, 493.2477.



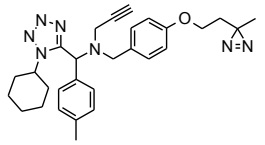
4

***N*-[(1-Benzyl-1*H*-tetrazol-5-yl)(4-methylphenyl)methyl]-*N*-{4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]benzyl}-2-propyn-1-amine (4).** The title compound was prepared from **DA5a** (30 mg, 0.12 mmol), 4-methyl benzaldehyde (15 μL, 0.12 mmol), benzylisocyanide (14 mg, 0.12 mmol), MeOH (0.3 mL), and $TMSN_3$ (10 μL, 0.12 mmol) according to **Method A** and isolated as colorless oil (33 mg, 53%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33-7.18 (m, 7H), 7.12-7.06 (m, 4H), 6.78 (d, *J* = 8.5 Hz, 2H), 5.58 (d, *J* = 15.5 Hz, 1H), 5.44 (d, *J* = 15.5 Hz, 1H), 5.22 (s, 1H), 3.84 (t, *J* = 6.0 Hz, 2H), 3.57 (d, *J* = 13.0 Hz, 1H), 3.42 (d, *J* = 13.0 Hz, 1H), 3.29 (m, 1H), 3.17 (m, 1H), 2.30 (s, 3H), 2.16 (t, *J* = 2.4 Hz, 1H), 1.80 (t, *J* = 6.0 Hz, 2H), 1.11 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{30}H_{32}N_7O$ $[M + H]^+$, 506.2663; found, 506.2676.



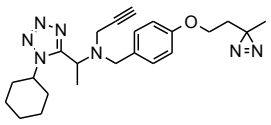
5

***N*-[(1-Benzyl-1*H*-tetrazol-5-yl)(1-methyl-1*H*-indol-5-yl)methyl]-*N*-{4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]benzyl}-2-propyn-1-amine (5).** The title compound was prepared from **DA5a** (20 mg, 0.082 mmol), 1-Methyl-1*H*-indole-5-carboxaldehyde (13.0 mg, 0.082 mmol), benzyl isocyanide (10 µL, 0.082 mmol), MeOH (0.2 mL), and $TMSN_3$ (6.7 µL, 0.082 mmol) according to **Method A** and isolated as colorless oil (20 mg, 45%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 (m, 1H), 7.37 (m, 1H), 7.34-7.25 (m, 4H), 7.24-7.20 (m, 2H), 7.16-7.10 (m, 2H), 7.06 (m, 1H), 6.80 (d, *J* = 8.8 Hz, 2H), 6.41 (m, 1H), 5.62 (d, *J* = 15.6 Hz, 1H), 5.37 (d, *J* = 15.6 Hz, 1H), 5.32 (s, 1H), 3.82 (t, *J* = 6.0 Hz, 2H), 3.78 (s, 3H), 3.60 (d, *J* = 12.8 Hz, 1H), 3.43 (d, *J* = 12.8 Hz, 1H), 3.34 (m, 1H), 3.17 (m, 1H), 2.14 (t, *J* = 2.4 Hz, 1H), 1.78 (t, *J* = 6.0 Hz, 2H), 1.11 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{32}H_{33}N_8O$ $[M + H]^+$, 545.2772; found, 545.2791.



6

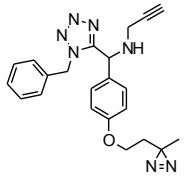
***N*-[(1-cyclohexyl-1*H*-tetrazol-5-yl)(4-methylphenyl)methyl]-*N*-{4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]benzyl}-2-propyn-1-amine (6)**. The title compound was prepared from **DA5a** (20 mg, 0.082 mmol), 4-methyl benzaldehyde (9.7 µL, 0.082 mmol), cyclohexyl isocyanide (10 µL, 0.082 mmol), MeOH (0.2 mL), and $TMSN_3$ (6.7 µL, 0.082 mmol) according to **Method A** and isolated as colorless oil (20 mg, 45%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42 (d, *J* = 8.0 Hz, 2H), 7.23 (d, *J* = 8.0 Hz, 2H), 7.18 (d, *J* = 7.5 Hz, 2H), 6.84 (d, *J* = 7.5 Hz, 2H), 5.37 (s, 1H), 4.41 (m, 1H), 3.84 (t, *J* = 6.0 Hz, 2H), 3.71 (d, *J* = 13.5 Hz, 1H), 3.52 (d, *J* = 13.5 Hz, 1H), 3.31-3.29 (m, 2H), 2.33 (s, 3H), 2.28 (t, *J* = 2.5 Hz, 1H), 2.06-1.91 (m, 3H), 1.90-1.85 (m, 2H), 1.80 (t, *J* = 6.5 Hz, 2H), 1.75 (m, 1H), 1.65 (m, 1H), 1.45-1.28 (m, 3H), 1.11 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{29}H_{36}N_7O$ $[M + H]^+$, 498.2976; found, 498.2996.



7

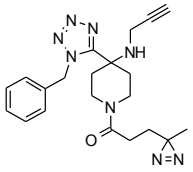
***N*-[1-(1-Cyclohexyl-1*H*-tetrazol-5-yl)ethyl]-*N*-{4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]benzyl}-2-propyn-1-amine (7)**. The title compound was prepared from **DA5a** (20 mg, 0.082 mmol), acetaldehyde (4.6 µL, 0.082 mmol), cyclohexyl isocyanide (10 µL, 0.082 mmol), MeOH (0.2 mL), and $TMSN_3$ (6.7 µL, 0.082 mmol) according to **Method A** and isolated as colorless oil (10 mg, 29%): ^{1}H NMR (500 MHz,

$CDCl_3$) δ 7.14 (d, *J* = 8. 5 Hz, 2H), 6.84 (d, *J* = 8.5 Hz, 2H), 4.53 (m, 1H), 4.46 (m, 1H), 3.84 (t, *J* = 6.5 Hz, 2H), 3.76 (d, *J* = 13.5 Hz, 1H), 3.47 (d, *J* = 13.5 Hz, 1H), 3.28-3.24 (m, 2H), 2.26 (t, *J* = 2.5 Hz, 1H), 2.15-2.00 (m, 2H), 1.95 (m, 1H), 1.87-1.84 (m, 2H), 1.82 (t, *J* = 6.5 Hz, 2H), 1.79 (m, 1H), 1.65 (m, 1H), 1.62 (d, *J* = 7.0 Hz, 3H), 1.49-1.22 (m, 3H), 1.12 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{23}H_{32}N_7O$ [M + H]$^+$, 422.2663; found, 422.2666.



8

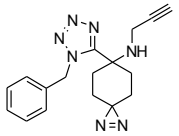
***N*-[(1-Benzyl-1*H*-tetrazol-5-yl){4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]phenyl}methyl]-2-propyn-1-amine (8)**. The title compound was prepared from propargylamine (230 mg, 4.2 mmol), **DA4** (680 mg, 3.3 mmol), benzyl isocyanide (0.36 mL, 2.7 mmol), MeOH (6 mL), and $TMSN_3$ (0.29 mL, 3.6 mmol) **Method A** and isolated as a colorless oil (830 mg, 70%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32-7.27 (m, 3H), 7.13 (d, *J* = 8.4 Hz, 2H), 7.07-7.03 (m, 2H), 6.81 (d, *J* = 8.4 Hz, 2H), 5.55 (d, *J* = 15.2 Hz, 1H), 5.26 (s, 1H), 5.25 (d, *J* = 15.2 Hz, 1H), 3.82 (t, *J* = 6.4 Hz, 2H), 3.37 (dd, *J* = 17.2, 2.8 Hz, 1H), 3.29 (dd, *J* = 17.2, 2.8 Hz, 1H), 2.23 (bs, 1H), 2.16 (t, *J* = 2.4 Hz, 1H), 1.81 (t, *J* = 6.4 Hz, 2H), 1.11 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 159.01, 155.50, 133.32, 129.36, 129.18, 128.91, 128.85, 127.67, 115.17, 80.65, 72.78, 63.12, 55.02, 51.17, 35.87, 34.34, 24.38, 20.41; IR (cm^{-1}) 3289, 3033, 2924, 2878, 1609, 1585, 1510, 1452, 1387, 1304, 1244, 1176, 1107, 1066, 1018, 897, 825, 722, 696, 667; HRMS (ESI-TOF) calc'd for $C_{22}H_{24}N_7O$ [M + H]$^+$, 402.2037; found, 402.2039.



22

1-[4-(1-Benzyl-1*H*-tetrazol-5-yl)-4-(2-propyn-1-ylamino)-1-piperidinyl]-3-(3-methyl-3*H*-diaziren-3-yl)-1-propanone (22). The title compound was prepared from propargylamine (9.6 µL, 0.15 mmol), **DA8** (31 mg, 0.15 mmol), benzylisocyanide (18 µL, 0.15 mmol), MeOH (0.2 mL), and $TMSN_3$ (12 µL, 0.15 mmol) according to **Method A** and isolated as a colorless oil (25 mg, 45%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37-7.32 (m, 3H), 7.20-7.17 (m, 2H), 5.96-5.89 (m, 2H), 3.81 (m, 1H), 3.57-3.46 (m, 2H), 3.19-3.06 (m, 3H), 2.44 (m, 1H), 2.14 (t, *J* = 2.4 Hz, 1H), 2.12-2.02 (m, 3H), 1.78-1.73 (m, 3H), 1.66-1.62 (m, 2H), 1.04 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.66, 155.99, 134.51, 129.29, 128.83, 127.29, 80.91, 72.13, 55.07, 52.23, 42.02, 37.95, 35.49, 35.37, 32.16, 29.62, 27.14, 25.65, 20.23; IR (cm^{-1}) 3293, 3003, 2959,

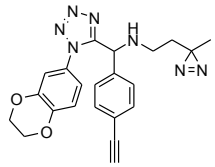
2923, 2862, 1629, 1496, 1440, 1357, 1277, 1217, 1144, 1096, 1003, 969, 751, 725, 700, 663; HRMS (ESI-TOF) calc'd for $C_{21}H_{27}N_8O$ $[M + H]^+$, 407.2302; found, 407.2301.



26

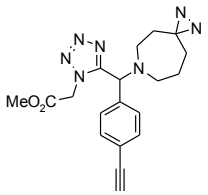
6-(1-Benzyl-1*H*-tetrazol-5-yl)-*N*-(2-propyn-1-yl)-1,2-diazaspiro[2.5]oct-1-en-6-amine (26). The title compound was prepared from propargylamine (13 μL, 0.20 mmol), **DA10** (24 mg, 0.20 mmol), benzylisocyanide (24 μL, 0.20 mmol), MeOH (0.5 mL), and $TMSN_3$ (16 μL, 0.20 mmol) according to **Method A** and isolated as a colorless oil (32 mg, 51%) ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.36-7.30 (m, 3H), 7.25-7.19 (m, 2H), 5.95 (s, 2H), 3.16-3.14 (m, 2H), 2.40-2.36 (m, 2H), 2.16 (t, *J* = 2.0 Hz, 1H), 1.98-1.92 (m, 2H), 1.70 (bs, 1H), 1.35-1.30 (m, 2H), 1.21-1.13 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 156.74, 134.86, 129.38, 128.88, 127.56, 81.16, 72.25, 55.75, 52.45, 33.71, 32.59, 27.23, 27.12; IR (cm^{-1}) 3290, 3033, 2947, 2852, 1574, 1497, 1440, 1411, 1258, 1157, 1111, 727, 701, 658; HRMS (ESI-TOF) calc'd for $C_{17}H_{20}N_7$ $[M + H]^+$, 322.1775; found, 322.1776.

Representative Ugi-azide condensation reaction: **Method B**



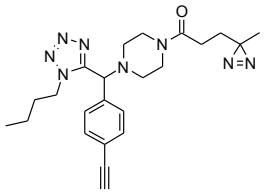
28

***N*-{[1-(2,3-dihydro-1,4-benzodioxin-6-yl)-1*H*-tetrazol-5-yl](4-ethynylphenyl)methyl}-2-(3-methyl-3*H*-diaziren-3-yl)ethanamine (28).** A solution of **DA12** (20 mg, 0.15 mmol), 4-ethynylbenzaldehyde (20 mg, 0.15 mmol), Et_3N (21 μL, 0.15 mmol) and 2,3-dihydro-6-isocyano-1,4-benzodioxine (24 mg, 0.15 mmol) in MeOH (0.3 mL) was treated with $TMSN_3$ (12 μL, 0.15 mmol) at room temperature. After being stirred for 3 h, the reaction mixture was diluted with EtOAc, washed with 1N HCl, sat. aq. $NaHCO_3$, brine, and dried over anhydrous Na_2SO_4. The solvent was removed by evaporation and the resulting residue was purified by column chromatography on silica gel (33–50% EtOAc/hexanes) to give the title compound as a colorless oil (22 mg, 36%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.44 (d, *J* = 8.4 Hz, 2H), 7.20 (d, *J* = 8.4 Hz, 2H), 6.93 (d, *J* = 8.4 Hz, 1H), 6.75 (d, *J* = 2.4 Hz, 1H), 6.55 (dd, *J* = 8.4, 2.4 Hz, 1H), 4.93 (s, 1H), 4.35-4.30 (m, 4H), 3.10 (s, 1H), 2.34 (m, 1H), 2.28 (m, 1H), 2.25 (bs, 1H), 1.55-1.51 (m, 2H), 0.99 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{22}H_{22}N_7O_2$ $[M + H]^+$, 416.1829; found, 416.1828.



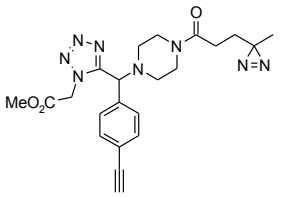
29

Methyl {5-[(4-ethynylphenyl)(1,2,6-triazaspiro[2.6]non-1-en-6-yl)methyl]-1*H*-tetrazol-1-yl}acetate (29). **DA13** (68 mg, 0.30 mmol) in MeOH (0.3 mL) was treated with 4N HCl in 1,4-dioxane (0.3 mL) at 0 °C under N_2. After being stirred for 30 min, the reaction mixture was evaporated to dryness. To a stirred solution of de-protected **DA13** (49 mg, 0.30 mmol) was added 4-ethynylbenzaldehyde (39 mg, 0.30 mmol), Et_3N (42 µL, 0.30 mmol) and methyl isocyanoacetate (27 µL, 0.30 mmo) in MeOH (0.6 mL) followed by $TMSN_3$ (24.5 µL, 0.30 mmol) at 0 °C under N_2. After being stirred at room temperature for 15 h, the reaction mixture was cooled and subsequently diluted with EtOAc, washed with H_2O, sat. aq. $NaHCO_3$ and brine, and dried over anhydrous Na_2SO_4. The organic layer was evaporated and the remaining residue was purified by column chromatography on silica gel (33–50% EtOAc/hexanes) to give the title compound as a colorless oil (10 mg, 8.8%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.52 (d, *J* = 7.8 Hz, 2H), 7.38 (d, *J* = 7.8 Hz, 2H), 5.45 (d, *J* = 17.4 Hz, 1H), 5.32 (s, 1H), 5.25 (d, *J* = 17.4 Hz, 1H), 3.77 (s, 3H), 3.13 (t, 1H), 2.81-2.68 (m, 4H), 1.75-1.65 (m, 2H), 1.31-1.20 (m, 4H); HRMS (ESI-TOF) calc'd for $C_{19}H_{22}N_7O_2$ $[M + H]^+$, 380.1829; found, 380.1838.



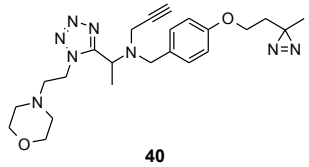
30

1-{4-[(1-Butyl-1*H*-tetrazol-5-yl)(4-ethynylphenyl)methyl]-1-piperazinyl}-3-(3-methyl-3*H*-diaziren-3-yl)-1-propanone (30). The title compound was prepared from **DA16** (58 mg, 0.25 mmol), 4-ethynylbenzaldehyde (33 mg, 0.25 mmol), *n*-butyl isocyanide (26 µL, 0.25 mmol), Et_3N (24 µL, 0.25 mmol), MeOH (0.6 mL) and $TMSN_3$ (20 µL, 0.25 mmol) according to **Method B** and isolated as a colorless oil (17 mg, 16%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.50 (d, *J* = 8.4 Hz, 2H), 7.37 (d, *J* = 8.4 Hz, 2H), 4.94 (s, 1H), 4.25 (t, *J* = 7.2 Hz, 2H), 3.72-3.59 (m, 2H), 3.47-3.39 (m, 2H), 3.13 (s, 1H), 2.67-2.55 (m, 2H), 2.42-2.34 (m, 2H), 2.06 (t, *J* = 7.2 Hz, 2H), 1.80-1.68 (m, 4H), 1.35-1.25 (m, 2H), 1.03 (s, 3H), 0.90 (t, *J* = 7.8 Hz, 3H); HRMS (ESI-TOF) calc'd for $C_{23}H_{31}N_8O$ $[M + H]^+$, 435.2615; found, 435.2626.



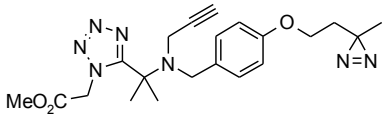
36

Methyl {5-[(4-ethynylphenyl){4-[3-(3-methyl-3*H*-diaziren-3-yl)propanoyl]-1-piperazinyl}methyl]-1*H*-tetrazol-1-yl}acetate (36). The title compound was prepared from **DA16** (300 mg, 1.3 mmol), 4-ethynylbenzaldehyde (170 mg, 1.3 mmol), methyl isocyanoacetate (0.12 mL, 1.3 mmol), Et_3N (0.18 mL, 1.3 mmol) and $TMSN_3$ (0.10 mL, 1.3 mmol) in MeOH (5 mL) according to **Method B** and isolated as a colorless oil (57 mg, 10%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (d, *J* = 8.4 Hz, 2H), 7.34 (d, *J* = 8.4 Hz, 2H), 5.28 (d, *J* = 17.6 Hz, 1H), 5.13 (d, *J* = 17.6 Hz, 1H), 5.01 (s, 1H), 3.74 (s, 3H), 3.65-3.55 (m, 2H), 3.45-3.38 (m, 2H), 3.13 (s, 1H), 2.55-2.49 (m, 2H), 2.45-2.36 (m, 2H), 2.04 (t, *J* = 8.0 Hz, 2H), 1.74 (t, *J* = 8.0 Hz, 2H), 1.05 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{22}H_{27}N_8O_3$ $[M + H]^+$, 451.2201; found, 451.2195.



40

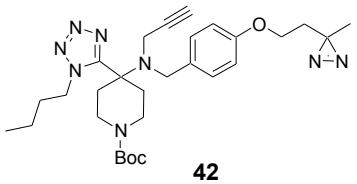
***N*-{4-[2-(3-Methyl-3*H*-diaziren-3-yl)ethoxy]benzyl}-*N*-(1-{1-[2-(4-morpholinyl)ethyl]-1*H*-tetrazol-5-yl}ethyl)-2-propyn-1-amine (40).** The title compound was prepared from **DA5a** (20 mg, 0.082 mmol), acetaldehyde (4.6 μL, 0.082 mmol), 2-morpholinoethyl isocyanide (11 μL, 0.082 mmol) and $TMSN_3$ (6.7 μL, 0.082 mmol) in MeOH (0.25 mL) according to **Method A** and isolated as a yellow oil (13 mg, 33%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.14 (d, *J* = 8.5 Hz, 2H), 6.83 (d, *J* = 8.5 Hz, 2H), 4.51-4.43 (m, 3H), 3.83 (t, *J* = 6.5 Hz, 2H), 3.72 (d, *J* = 13.5 Hz, 1H), 3.62-3.56 (m, 4H), 3.51 (d, *J* = 13.5 Hz, 1H), 3.29-3.26 (m, 2H), 2.86-2.72 (m, 2H), 2.43-2.30 (m, 4H), 2.41 (t, *J* = 2.5 Hz, 1H), 1.82 (t, *J* = 6.0 Hz, 2H), 1.66 (d, *J* = 7.0 Hz, 3H), 1.12 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{23}H_{33}N_8O_2$ $[M + H]^+$, 453.2721; found, 453.2719.



41

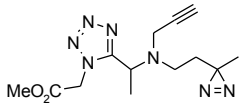
Methyl (5-{2-[{4-[2-(3-methyl-3H-diaziren-3-yl)ethoxy]benzyl}(2-propyn-1-yl)amino]-2-propanyl}-1H-tetrazol-1-yl)acetate(41). The title compound was prepared from **DA5a** (20 mg, 0.082 mmol), acetone (6.0 μL, 0.082 mmol), methyl isocyanoacetate (7.5 μL, 0.082 mmol), and $TMSN_3$ (6.7 μL, 0.082 mmol) in MeOH (0.25 mL) according to **Method A** and isolated as a colorless oil (5.2 mg, 15%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.07 (d, *J* = 8.5 Hz, 2H), 6.83 (d, *J* = 8.5 Hz, 2H), 5.56 (s, 2H), 3.85 (t, *J* = 6.0 Hz, 2H),

3.76 (s, 3H), 3.65 (s, 2H), 3.25-3.23 (m, 2H), 2.29 (t, J = 2.5 Hz, 1H), 1.82 (t, J = 6.0 Hz, 2H), 1.73 (s, 6H), 1.12 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{21}H_{28}N_7O_3$ $[M + H]^+$, 426.2248; found, 426.2250.



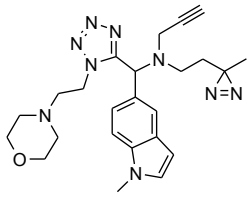
42

***tert*-Butyl 4-(1-butyl-1H-tetrazol-5-yl)-4-[{4-[2-(3-methyl-3H-diaziren-3-yl)ethoxy]benzyl}(2-propyn-1-yl)amino]-1-piperidinecarboxylate (42).** The title compound was prepared from amine **DA5a** (20 mg, 0.082 mmol), 1-Boc-4-piperidone (16 mg, 0.082 mmol), *n*-butyl isocyanide (8.6 µL, 0.082 mmol), and $TMSN_3$ (6.7 µL, 0.082 mmol) in MeOH (0.25 mL) according to **Method A** and isolated as a colorless oil (16 mg, 36%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.02 (d, J = 6.6 Hz, 2H), 6.81 (d, J = 6.6 Hz, 2H), 4.52-4.48 (m, 2H), 4.18-4.00 (m, 2H), 3.84 (t, J = 6.3 Hz, 2H), 3.72-3.67 (m, 2H), 3.28-2.24 (m, 2H), 3.02 (m, 1H), 2.78 (m, 1H), 2.47-2.30 (m, 4H), 2.26 (t, J = 2.4 Hz, 1H), 1.97-1.90 (m, 2H), 1.81 (t, J = 6.3 Hz, 2H), 1.48 (s, 9H), 1.45-1.42 (m, 2H), 1.11 (s, 3H), 0.99 (t, J = 7.4 Hz, 3H); HRMS (ESI-TOF) calc'd for $C_{29}H_{43}N_8O_3$ $[M + H]^+$, 551.3452; found, 551.3455.



43

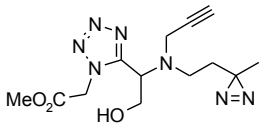
Methyl [5-(1-{[2-(3-methyl-3H-diaziren-3-yl)ethyl](2-propyn-1-yl)amino}ethyl)-1H-tetrazol-1-yl]acetate (43). The title compound was prepared from **DA18** (30 mg, 0.22 mmol), acetaldehyde (0.22 mL, 1M in MeOH, 0.22 mmol), methyl isocyanoacetate (20 µL, 0.22 mmol), and $TMSN_3$ (18 µL, 0.22 mmol) in MeOH (0.4 mL) according to **Method A** and isolated as colorless oil (29 mg, 43%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.64 (d, J = 17.6 Hz, 1H), 5.43 (d, J = 17.6 Hz, 1H), 4.48 (q, J = 6.9 Hz, 1H), 3.81 (s, 3H), 3.24-3.22 (m, 2H), 2.50 (m, 1H), 2.33 (m, 1H), 2.25 (t, J = 2.4 Hz, 1H), 1.58 (d, J = 6.9 Hz, 3H), 1.49 (m, 1H), 1.34 (m, 1H), 0.85 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{13}H_{20}N_7O_2$ $[M + H]^+$, 306.1673; found, 306.1668.



44

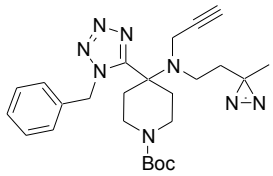
***N*-[2-(3-methyl-3*H*-diaziren-3-yl)ethyl]-*N*-[(1-methyl-1*H*-indol-5-yl){1-[2-(4-morpholinyl)ethyl]-1*H*-tetrazol-5-yl}methyl]-2-propyn-1-amine (44).** The title compound was prepared from **DA18** (15 mg, 0.11 mmol), 1-methyl-1*H*-indole-5-carboxaldehyde (17 mg, 0.11 mmol), 2-morpholinoethyl isocyanide (15

μL, 0.11 mmol), and $TMSN_3$ (8.9 μL, 0.11 mmol) in MeOH (0.2 mL) according to **Method A** and isolated as a colorless oil (1.9 mg, 3.7%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.63 (d, *J* = 1.6 Hz, 1H), 7.42-7.25 (m, 2H), 7.08 (d, *J* = 0.8 Hz, 1H), 6.44 (d, *J* = 0.8 Hz, 1H), 5.40 (s, 1H), 4.38 (t, *J* = 7.1 Hz, 2H), 3.79 (s, 3H), 3.58-3.55 (m, 4H), 3.49-3.35 (m, 2H), 2.75-2.55 (m, 4H), 2.48-2.33 (m, 4H), 2.27 (t, *J* = 2.3 Hz, 1H), 1.49 (t, *J* = 7.5 Hz, 2H), 0.92 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{24}H_{32}N_9O$ $[M + H]^+$, 462.2724; found, 462.2720.



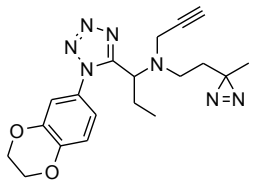
45

Methyl [5-(2-hydroxy-1-{[2-(3-methyl-3H-diaziren-3-yl)ethyl](2-propyn-1-yl)amino}ethyl)-1H-tetrazol-1-yl]acetate (45). The title compound was prepared from **DA18** (30 mg, 0.22 mmol), glycolaldehyde dimer (26 mg, 0.22 mmol), methyl isocyanoacetate (20 μL, 0.22 mmol), and $TMSN_3$ (18 μL, 0.22 mmol) in MeOH (0.4 mL) according to **Method A** and isolated as a colorless oil (31 mg, 45%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.56 (d, *J* = 17.7 Hz, 1H), 5.46 (d, *J* =17.7 Hz, 1H), 4.34 (m, 1H), 4.26 (m, 1H), 4.19 (m, 1H), 3.82 (s, 3H), 3.48-3.22 (m, 2H), 2.82 (m, 1H), 2.70-2.52 (m, 2H), 2.25 (t, *J* = 2.5 Hz, 1H), 1.57-1.46 (m, 2H), 0.94 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{13}H_{20}N_7O_3$ $[M + H]^+$, 322.1622; found, 322.1622.



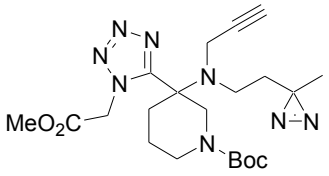
46

***tert*-Butyl 4-(1-benzyl-1*H*-tetrazol-5-yl)-4-{[2-(3-methyl-3*H*-diaziren-3-yl)ethyl](2-propyn-1-yl)amino}-1-piperidinecarboxylate (46)**. The title compound was prepared from **DA18** (30 mg, 0.22 mmol), 1-Boc-4-piperidone (44 mg, 0.22 mmol), benzyl isocyanide (27 μL, 0.22 mmol), and $TMSN_3$ (18 μL, 0.22 mmol) in MeOH (0.4 mL) according to **Method A** and isolated as a white powder (52 mg, 50%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36-7.32 (m, 3H), 7.15-7.11 (m, 2H), 5.95 (s, 2H), 3.88-3.80 (m, 2H), 3.30-3.24 (m, 2H), 2.98 (m, 1H), 2.70-2.35 (m, 3H), 2.25 (t, *J* = 2.4 Hz, 1H), 2.24-1.95 (m, 4H), 1.53-1.48 (m, 2H), 1.41 (s, 9H), 0.90 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{25}H_{35}N_8O_2$ $[M + H]^+$, 479.2877; found, 479.2883.



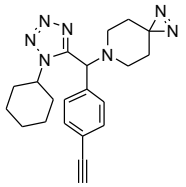
47

***tert*-Butyl- *N*-{1-[1-(2,3-dihydro-1,4-benzodioxin-6-yl)-1*H*-tetrazol-5-yl]propyl}-*N*-[2-(3-methyl-3*H*-diaziren-3-yl)ethyl]-2-propyn-1-amine (47).** The title compound was prepared from **DA18** (15 mg, 0.11 mmol), propionaldehyde (7.9 μL, 0.11 mmol), 2,3-dihydro-6-isocyano-1,4-benzodioxine (18 mg, 0.11 mmol), and $TMSN_3$ (8.9 μL, 0.11 mmol) in MeOH (0.2 mL) according to **Method A** and isolated as colorless oil (22 mg, 54%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.10 (m, 1H), 7.03-7.01 (m, 2H), 4.37-4.33 (m, 4H), 3.95 (m, 1H), 3.35 (d, *J* = 2.4 Hz, 2H), 2.70-2.66 (m, 2H), 2.24-2.10 (m, 2H), 2.07 (t, *J* = 2.4 Hz, 1H), 1.35 (t, *J* = 7.3 Hz, 2H), 0.91 (s, 3H), 0.83 (t, *J* = 7.4 Hz, 3H); HRMS (ESI-TOF) calc'd for $C_{19}H_{24}N_7O_2$ $[M + H]^+$, 382.1986; found, 382.1989.



48

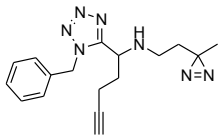
***tert*-Butyl-3-[1-(2-methoxy-2-oxoethyl)-1*H*-tetrazol-5-yl]-3-{[2-(3-methyl-3*H*-diaziren-3-yl)ethyl](2-propyn-1-yl)amino}-1-piperidinecarboxylate (48)**. The title compound was prepared from amine **DA18** (15 mg, 0.11 mmol), 1-Boc-3-piperidone (65 mg, 0.33 mmol), methyl isocyanoacetate (30 μL, 0.33 mmol), and $TMSN_3$ (27 μL, 0.33 mmol) in MeOH (0.6 mL) according to **Method A** and isolated as a colorless oil (22 mg, 15%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.68 (d, *J* = 17.2 Hz, 1H), 5.36 (d, *J* = 17.2 Hz, 1H), 4.37 (d, *J* = 13.6 Hz, 1H), 3.82 (m, 1H), 3.80 (s, 3H), 3.40-3.20 (m, 3H), 2.87 (m, 1H), 2.30 (m, 1H), 2.25 (t, *J* = 2.4 Hz, 1H), 2.10 (m, 1H), 1.70-1.30 (m, 6H), 1.71 (s, 9H), 0.96 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{21}H_{33}N_8O_4$ $[M + H]^+$, 461.2619; found, 461.2620.



53

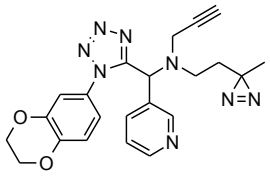
6-[(1-Cyclohexyl-1*H*-tetrazol-5-yl)(4-ethynylphenyl)methyl]-1,2,6-triazaspiro[2.5]oct-1-ene (53) **DA14** (34 mg, 0.16 mmol) was treated with 4N HCl in 1,4-dioxane (0.4 mL) at 0 °C under N_2. After being stirred for 30 min, the reaction mixture was evaporated to dryness. The title compound was prepared from

above described de-protected **DA14** (0.16 mmol), MeOH (0.4 mL), 4-ethynylbenzaldehyde (21 mg, 0.16 mmol), cyclohexyl isocyanide (20 μL, 0.16 mmol), Et_3N (23 μL, 0.16 mmol), MeOH (0.4 mL), and $TMSN_3$ (13 μL, 0.16 mmol) according to the same procedure as described for the preparation of **29** from **DA13**. Using this procedure, the title compounds was isolated as a white powder (6.7 mg, 11%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52-7.48 (d, *J* = 8.5 Hz, 2H), 7.41 (d, *J* = 8.5 Hz, 2H), 5.12 (s, 1H), 4.46 (m, 1H), 3.12 (s, 1H), 2.82-2.75 (m, 2H), 2.55-2.53 (m, 2H), 2.05-1.82 (m, 5H), 1.80-1.65 (m, 3H), 1.45-1.29 (m, 6H); HRMS (ESI-TOF) calc'd for $C_{21}H_{26}N_7$ $[M + H]^+$, 376.2244; found, 376.2248.



54

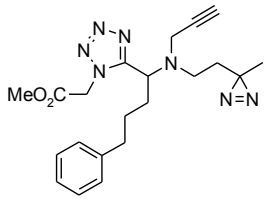
1-(1-Benzyl-1*H*-tetrazol-5-yl)-*N*-[2-(3-methyl-3*H*-diaziren-3-yl)ethyl]-4-pentyn-1-amine (54) To a stirred solution of 4-pentyn-1-ol (300 mg, 3.6 mmol) in CH_2Cl_2 (3.6 mL) and Et_3N (3.0 mL, 21 mmol) was added a solution of SO_3•Py (1.7 g, 11 mmol) in DMSO (1.9 mL) at 0 °C under argon. After being stirred at room temperature for 20 min, the reaction was quenched with 1N HCl. The reaction mixture was extracted with CH_2Cl_2, washed with sat. aq. $NaHCO_3$ and brine, and then dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated *in vacuo* to give 4-pentynal as colorless oil (200 mg, 70%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.81 (t, *J* = 2.4 Hz, 1H), 2.69 (t, *J* = 7.2 Hz, 2H), 2.52 (dt, *J* = 7.2, 2.4 Hz, 2H), 2.00 (t, *J* = 2.4 Hz, 1H). To a stirred solution of 4-pentynal (16 mg, 0.20 mmol), **DA12** (24 mg, 0.18 mmol), Et_3N (25 μL, 0.18 mmol) and benzyl isocyanide (21 μL, 0.18 mmol) in MeOH (0.3 mL) was added $TMSN_3$ (14 μL, 0.18 mmol) at 0 °C under N_2. After being stirred at room temperature for 15 h, the reaction mixture was cooled then diluted with EtOAc, washed with H_2O, sat. aq. $NaHCO_3$ and brine, and then dried over anhydrous Na_2SO_4. The organic layer was evaporated and the resulting residue was purified by column chromatography on silica gel (33–50% EtOAc/hexanes) to give the title compound as a colorless oil (8.5 mg, 15%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41-7.35 (m, 3H), 7.28-7.19 (m, 2H), 5.74 (d, *J* = 15.6 Hz, 1H), 5.62 (d, *J* = 15.6 Hz, 1H), 4.09 (m, 1H), 2.43 (m, 1H), 2.22-2.10 (m, 2H), 1.92 (t, *J* = 2.4 Hz, 1H), 1.98-1.90 (m, 2H), 1.88-1.78 (m, 1H), 1.44-1.24 (m, 3H), 0.92 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{17}H_{22}N_7$ $[M + H]^+$, 324.1931; found, 324.1920.



55

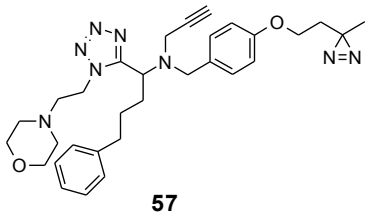
***N*-{[1-(2,3-dihydro-1,4-benzodioxin-6-yl)-1*H*-tetrazol-5-yl](3-pyridinyl)methyl}-*N*-[2-(3-methyl-3H-diaziren-3-yl)ethyl]-2-propyn-1-amine (55).** The title compound was prepared from **DA5a** (28 mg, 0.20

mmol), 3-pyridinecarboxaldehyde (22 mg, 0.20 mmol), 2,3-dihydro-6-isocyano-1,4-benzodioxine (33 mg, 0.20 mmol), and $TMSN_3$ (17 μL, 0.20 mmol) and MeOH (0.4 mL) according to **Method A** and isolated as a colorless oil (18 mg, 20%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.60 (d, *J* = 1.8 Hz, 1H), 8.55 (m, 1H), 7.93 (m, 1H), 7.34 (m, 1H), 7.01 (d, *J* = 8.4 Hz, 1H), 6.91 (m, 1H), 6.79 (dd, *J* = 8.4, 2.4 Hz, 1H), 5.26 (s, 1H), 4.39-4.32 (m, 4H), 3.50 (dd, *J* = 18.0, 2.4 Hz, 1H), 3.35 (dd, *J* = 18.0, 2.4 Hz, 1H), 2.63 (m, 1H), 2.53 (m, 1H), 2.12 (t, *J* = 2.4 Hz, 1H), 1.44 (m, 1H), 1.31 (m, 1H), 0.88 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.74, 150.53, 150.49, 146.05, 144.44, 137.23, 131.91, 126.24, 124.13, 119.16, 118.54, 115.50, 77.80, 74.81, 64.68, 64.56, 58.17, 44.61, 40.02, 32.88, 24.79, 19.83; IR (cm^{-1}) 3287, 3059, 2926, 2877, 1593, 1510, 1459, 1427, 1313, 1282, 1254, 1219, 1189, 1121, 1063, 957, 931, 897, 878, 814, 753, 713, 668; HRMS (ESI-TOF) calc'd for $C_{22}H_{23}N_8O_2$ $[M + H]^+$, 431.1938; found, 431.1933.



56

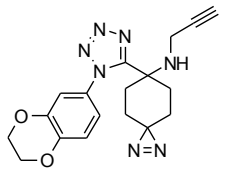
Methyl [5-(1-{[2-(3-methyl-3H-diaziren-3-yl)ethyl](2-propyn-1-yl)amino}-4-phenylbutyl)-1H-tetrazol-1-yl]acetate (56). The title compound was prepared from **DA18** (28 mg, 0.20 mmol), 4-phenylbutyraldehyde (30 mg, 0.20 mmol), methyl isocyanoacetate (19 μL, 0.20 mmol), and $TMSN_3$ (17 μL, 0.20 mmol) in MeOH (0.4 mL) according to **Method A** and isolated as a colorless oil (30 mg, 35%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30-7.25 (m, 2H), 7.20-7.12 (m, 3H), 5.52 (d, *J* = 17.5 Hz, 1H), 5.40 (d, *J* = 17.5 Hz, 1H), 4.27 (dd, *J* = 11.0, 4.0 Hz, 1H), 3.78 (s, 3H), 3.23 (d, *J* = 2.5 Hz, 2H), 2.71-2.64 (m, 2H), 2.52 (m, 1H), 2.31 (m, 1H), 2.26 (m, 1H), 2.22 (t, *J* = 2.5 Hz, 1H), 1.94 (m, 1H), 1.80 (m, 1H), 1.56 (m, 1H), 1.40 (m, 1H), 1.26 (m, 1H), 0.79 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{21}H_{28}N_7O_2$ $[M + H]^+$, 410.2299; found, 410.2294.



57

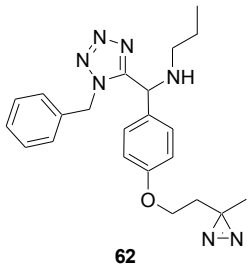
***N*-{4-[2-(3-Methyl-3*H*-diaziren-3-yl)ethoxy]benzyl}-1-{1-[2-(4-morpholinyl)ethyl]-1*H*-tetrazol-5-yl}-4-phenyl-*N*-(2-propyn-1-yl)-1-butanamine (57).** The title compound was prepared from **DA5a** (20 mg, 0.082 mmol), 4-phenylbutyraldehyde (12 mg, 0.082 mmol), 2-morpholinoethyl isocyanide (11 μL, 0.082 mmol) and $TMSN_3$ (6.7 μL, 0.082 mmol) in MeOH (0.3 mL) according to **Method A** and isolated as a colorless oil (26 mg, 56%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30-7.26 (m, 2H), 7.22-7.15 (m, 3H), 7.09 (d, *J* = 8.4 Hz, 2H), 6.81 (d, *J* = 8.4 Hz, 2H), 4.43-4.30 (m, 2H), 4.23 (m, 1H), 3.82 (t, *J* = 6.0 Hz, 2H), 3.67-

3.63 (m, 2H), 3.57-3.49 (m, 4H), 3.29 (dd, *J* = 17.4, 2.4 Hz, 1H), 3.19 (dd, *J* = 17.4, 2.4 Hz, 1H), 2.79 (m, 1H), 2.76-2.67 (m, 3H), 2.39-2.26 (m, 4H), 2.21 (t, *J* = 2.4 Hz, 1H), 2.19-2.14 (m, 2H), 1.83 (t, *J* = 6.0 Hz, 2H), 1.69-1.58 (m, 2H), 1.12 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{31}H_{41}N_8O_2$ [M + H]$^+$, 557.3598; found, 557.3611.



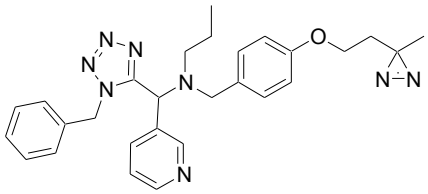
58

6-[1-(2,3-Dihydro-1,4-benzodioxin-6-yl)-1*H*-tetrazol-5-yl]-*N*-(2-propyn-1-yl)-1,2-diazaspiro[2.5]oct-1-en-6-amine (58). The title compound was prepared from proparglylamine (27 µL, 0.42 mmol), **DA10** (37 mg, 0.30 mmol), 2,3-dihydro-6-isocyano-1,4-benzodioxine (48 mg, 0.30 mmol), and $TMSN_3$ (24 µL, 0.30 mmol) in MeOH (0.5 mL) according to **Method A** and isolated as a colorless oil (4.0 mg, 4.0%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.06 (d, *J* = 2.5Hz, 1H), 7.02-6.95 (m, 2H), 4.37-4.32 (m, 4H), 3.30 (d, *J* = 2.5 Hz, 2H), 2.31-2.28 (m, 2H), 2.10 (t, *J* = 2.5 Hz, 1H), 2.05-2.00 (m, 2H), 1.68-1.64 (m, 3H), 1.01-0.91 (m, 2H); HRMS (ESI-TOF) calc'd for $C_{18}H_{20}N_7O_2$ [M + H]$^+$, 366.1673; found, 366.1672.



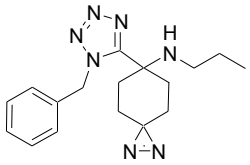
62

***N*-[(1-Benzyl-1*H*-tetrazol-5-yl){4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]phenyl}methyl]-1-propanamine (62).** The title compound was prepared from propylamine (89 µL, 1.1 mmol), **DA4** (200 mg, 0.98 mmol), benzyl isocyanide (0.12 mL, 0.98 mmol), and $TMSN_3$ (88 µL, 1.1 mmol) in MeOH (2 mL) according to **Method A** and isolated as a yellow oil (250 mg, 62%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.33-7.28 (m, 3H), 7.12 (d, *J* = 8.4 Hz, 2H), 7.04-7.01 (m, 2H), 6.80 (d, *J* = 8.4 Hz, 2H), 5.52 (d, *J* = 15.0 Hz, 1H), 5.31 (d, *J* = 15.0 Hz, 1H), 4.99 (s, 1H), 3.82 (t, *J* = 6.4 Hz, 2H), 2.41-2.33 (m, 2H), 1.81 (t, *J* = 6.4 Hz, 2H), 1.77 (bs, 1H), 1.45-1.28 (m, 2H), 1.12 (s, 3H), 0.83 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 158.68, 156.15, 133.56, 130.12, 129.14, 128.81, 128.74, 127.53, 115.07, 63.09, 57.13, 51.08, 49.71, 34.35, 24.38, 23.05, 20.40, 11.78; IR (cm^{-1}) 3321, 3065, 2957, 2929, 2873, 1609, 1585, 1509, 1453, 1358, 1244, 1176, 1110, 1068, 1018, 897, 823, 722, 698; HRMS (ESI-TOF) calc'd for $C_{22}H_{28}N_7O$ [M + H]$^+$, 406.2350; found, 406.2346.



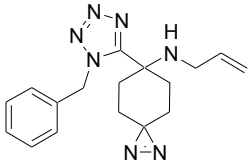
66

***N*-[(1-Benzyl-1*H*-tetrazol-5-yl)(3-pyridinyl)methyl]-*N*-{4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]benzyl}-1-propanamine (66).** The title compound was prepared from **DA5b** (30 mg, 0.12 mmol), 3-pyridinecarboxaldehyde (11 µL, 0.12 mmol), benzyl isocyanide (15 µL, 0.12 mmol), and $TMSN_3$ (9.9 µL, 0.12 mmol) in MeOH (0.3 mL) according to **Method A** and isolated as a yellow oil (31 mg, 52%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.51 (m, 1H), 8.31 (m, 1H), 7.57 (d, *J* = 8.4 Hz, 1H), 7.24-7.12 (m, 6H), 6.90 (d, *J* = 8.4 Hz, 2H), 6.72 (d, *J* = 7.2 Hz, 2H), 5.45 (d, *J* = 15.6 Hz, 1H), 5.13 (d, *J* = 15.6 Hz, 1H), 5.10 (s, 1H), 3.88 (t, *J* = 6.0 Hz, 2H), 3.57 (s, 2H), 2.78 (m, 1H), 2.49 (m, 1H), 1.86 (t, *J* = 6.0 Hz, 2H), 1.41-1.34 (m, 2H), 1.14 (s, 3H), 0.71 (t, *J* = 7.8 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 158.22, 153.39, 150.65, 149.51, 137.02, 132.97, 131.18, 130.17, 129.14, 128.98, 127.46, 123.42, 114.91, 63.13, 55.31, 54.22, 51.64, 51.19, 34.40, 29.84, 24.43, 21.38, 20.45, 11.63; IR (cm^{-1}) 3031, 2958, 2928, 2871, 1609, 1584, 1509, 1471, 1453, 1423, 1385, 1299, 1239, 1169, 1063, 1024, 828, 751, 714, 667; HRMS (ESI-TOF) calc'd for $C_{28}H_{33}N_8O$ $[M + H]^+$, 497.2772; found, 497.2779.



67

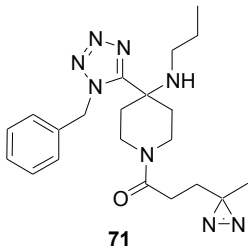
6-(1-Benzyl-1*H*-tetrazol-5-yl)-*N*-propyl-1,2-diazaspiro[2.5]oct-1-en-6-amine (67). The title compound was prepared from propylamine (20 µL, 0.24 mmol), **DA10** (50 mg, 0.30 mmol), benzyl isocyanide (29 µL, 0.24 mmol), and $TMSN_3$ (19 µL, 0.24 mmol) in MeOH (0.3 mL) according to **Method A** and isolated as a colorless oil (41 mg, 53%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34-7.30 (m, 3H), 7.16-7.10 (m, 2H), 5.98 (s, 2H), 2.42-2.30 (m, 2H), 2.19 (t, *J* = 7.2 Hz, 2H), 2.00-1.92 (m, 2H), 1.45-1.20 (m, 7H), 0.89 (t, *J* = 7.6 Hz, 3H); HRMS (ESI-TOF) calc'd for $C_{17}H_{24}N_7$ $[M + H]^+$, 326.2088; found, 326.2087.



70

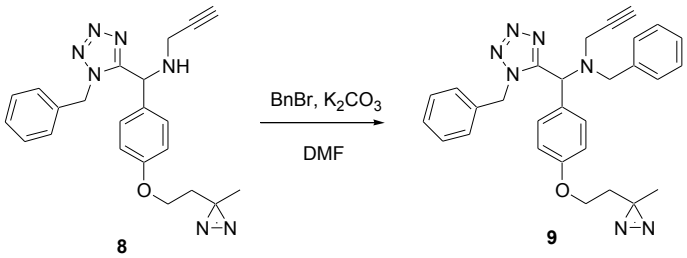
***N*-Allyl-6-(1-benzyl-1*H*-tetrazol-5-yl)-1,2-diazaspiro[2.5]oct-1-en-6-amine (70).** The title compound was prepared from allylamine (18 µL, 0.24 mmol), **DA10** (50 mg, 0.30 mmol), benzyl isocyanide (29 µL, 0.24 mmol), and $TMSN_3$ (19 µL, 0.24 mmol) in MeOH (0.3 mL) according to **Method A** and isolated as a

colorless oil (46 mg, 60%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36-7.27 (m, 3H), 7.19-7.14 (m, 2H), 5.97 (s, 2H), 5.72 (m, 1H), 5.16 (dd, *J* = 17.2, 1.6 Hz, 1H), 5.08 (dd, *J* = 10.4 Hz, 1.6 Hz, 1H), 2.94-2.90 (m, 2H), 2.41-2.32 (m, 2H), 1.98-1.90 (m, 2H), 1.42 (bs, 1H), 1.35-1.26 (m, 2H), 1.22-1.15 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 157.01, 135.63, 134.80, 129.15, 128.58, 127.13, 116.52, 55.36, 51.94, 45.48, 33.67, 27.06, 26.99; IR (cm^{-1}) 3314, 3070, 2947, 2850, 1574, 1497, 1440, 1412, 1252, 1158, 1109, 996, 959, 922, 726, 700; HRMS (ESI-TOF) calc'd for $C_{17}H_{22}N_7$ $[M + H]^+$, 324.1931; found, 324.1934.



71

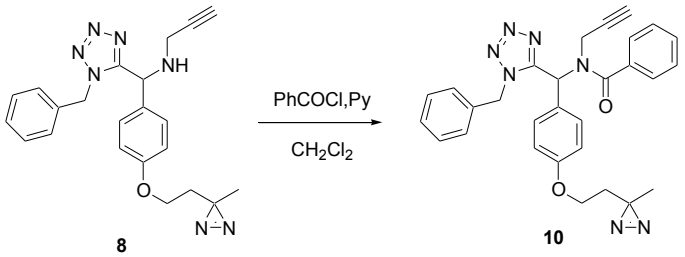
(1-[4-(1-Benzyl-1*H*-tetrazol-5-yl)-4-(propylamino)-1-piperidinyl]-3-(3-methyl-3*H*-diaziren-3-yl)-1-propanone) (71). The title compound was prepared from propylamine (12 µL, 0.15 mmol), **DA8** (31 mg, 0.15 mmol), benzylisocyanide (18 µL, 0.15 mmol), $TMSN_3$ (12 µL, 0.15 mmol) and MeOH (0.2 mL) according to **Method A** and isolated as a white powder (30 mg, 49%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.36-7.31 (m, 3H), 7.15-7.10 (m, 2H), 6.00 (d, *J* = 15.2 Hz, 1H), 5.91 (d, *J* = 15.2 Hz, 1H), 3.80 (m, 1H), 3.53-3.47 (m, 2H), 3.14 (m, 1H), 2.42 (m, 1H), 2.30 (m, 1H), 2.17-2.00 (m, 4H), 1.75 (t, *J* = 8.4 Hz, 2H), 1.74-1.61 (m, 2H), 1.37-1.25 (m, 2H), 1.08 (bs, 1H), 1.03 (s, 3H), 0.84 (t, *J* = 7.8 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.66, 156.69, 134.70, 129.24, 128.70, 126.94, 54.74, 51.82, 44.31, 42.12, 38.08, 35.74, 35.58, 29.67, 27.17, 25.66, 23.68, 20.22, 11.91; IR (cm^{-1}) 3325, 3032, 2959, 2927, 2872, 1633, 1496, 1440, 1382, 1276, 1215, 1136, 1001, 751, 725, 696, 664; HRMS (ESI-TOF) calc'd for $C_{21}H_{31}N_8O$ $[M + H]^+$, 411.2615; found, 411.2617.



8 **9**

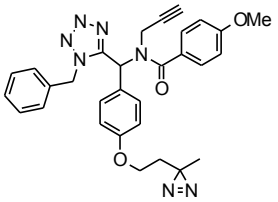
***N*-Benzyl-*N*-[(1-benzyl-1*H*-tetrazol-5-yl){4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]phenyl}methyl]-2-propyn-1-amine (9)** To a stirred solution of amine **8** (30 mg, 0.075 mmol) and K_2CO_3 (31 mg, 0.22 mmol) in DMF (0.3 mL) was added benzyl bromide (11 µL, 0.090 mmol) at room temperature under N_2. After being stirred at 80 °C for an additional 15 h, the reaction mixture was cooled and then diluted with EtOAc, washed with H_2O, sat. aq. $NaHCO_3$ and brine, and dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated *in vacuo* and the remaining residue was purified by preparative TLC (33%

EtOAc/hexanes) to give the title compound as a white powder (19 mg, 52%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35-7.18 (m, 10H), 7.10-7.08 (m, 2H), 6.78 (d, *J* = 8.5 Hz, 2H), 5.57 (d, *J* = 15.5 Hz, 1H), 5.49 (d, *J* = 15.5 Hz, 1H), 5.23 (s, 1H), 3.82 (t, *J* = 6.5 Hz, 2H), 3.63 (d, *J* = 13.5 Hz, 1H), 3.49 (d, *J* = 13.5 Hz, 1H), 3.30 (dd, *J* = 17.5, 2.0 Hz, 1H), 3.19 (dd, *J* = 17.5, 2.0 Hz, 1H), 2.18 (t, *J* = 2.5 Hz, 1H), 1.80 (t, *J* = 6.5 Hz, 2H), 1.11 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{29}H_{30}N_7O$ $[M + H]^+$, 492.2506; found, 492.2515.



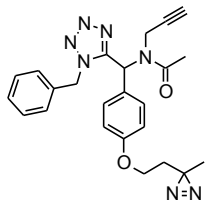
8 **10**

***N*-[(1-benzyl-1*H*-tetrazol-5-yl){4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]phenyl}methyl]-*N*-(2-propyn-1-yl)benzamide (10)** To a stirred solution of **8** (30 mg, 0.075 mmol) and pyridine (18 µL, 0.22 mmol) in CH_2Cl_2 (0.3 mL) was added benzoyl chloride (10 µL, 0.090 mmol) at room temperature under N_2. After being stirred for 1 h, the reaction mixture was diluted with EtOAc, washed with 1N HCl (aq.), H_2O, sat. aq. $NaHCO_3$ and brine, and dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated *in vacuo* and the remaining residue was purified by preparative TLC (33% EtOAc/hexanes) to give the title compound as a pale yellow oil (29 mg, 77%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55-7.35 (m, 5H), 7.28-6.97 (m, 8H), 6.80 (d, *J* = 8.5 Hz, 2H), 5.54 (m, 2H), 4.38 (dd, *J* = 18.5, 2.5 Hz, 1H), 4.18 (m, 1H), 3.90-3.80 (m, 2H), 1.83 (t, *J* = 6.5 Hz, 2H), 1.82 (t, *J* = 2.4 Hz, 1H), 1.12 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{29}H_{28}N_7O_2$ $[M + H]^+$, 506.2299; found, 506.2309.



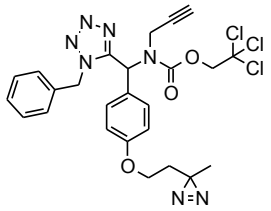
11

***N*-[(1-Benzyl-1*H*-tetrazol-5-yl){4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]phenyl}methyl]-4-methoxy-*N*-(2-propyn-1-yl)benzamide (11).** The title compound was prepared from amine **8** (30 mg, 0.075 mmol), 4-methoxylbenzoyl chloride (12 µL, 0.090 mmol), pyridine (18 µL, 0.22 mmol) and CH_2Cl_2 (0.3 mL) according to the same procedure as described for the preparation of **10** from **8**. Using this procedure, the title compound was isolated as a colorless oil (32 mg, 80%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55 (d, *J* = 8.0 Hz, 2H), 7.30-7.05 (m, 8H), 6.89 (d, *J* = 8.5 Hz, 2H), 6.78 (d, *J* = 8.5 Hz, 2H), 5.55-5.52 (m, 2H), 4.41 (dd, *J* = 18.5, 2.5 Hz, 1H), 4.20 (m, 1H), 3.88-3.80 (m, 2H), 3.84 (s, 3H), 1.84 (t, *J* = 2.5 Hz, 1H), 1.82 (t, *J* = 6.0 Hz, 2H), 1.12 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{30}H_{30}N_7O_3$ $[M + H]^+$, 536.2405; found, 536.2404.



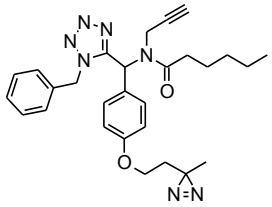
12

***N*-[(1-Benzyl-1*H*-tetrazol-5-yl){4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]phenyl}methyl]-*N*-(2-propyn-1-yl)acetamide (12).** The title compound was prepared from amine **8** (30 mg, 0.075 mmol), acetyl chloride (6.4 μL, 0.090 mmol), pyridine (18 μL, 0.22 mmol), and CH_2Cl_2 (0.3 mL) according to the same procedure as described for the preparation of **10** from **8**. Using this procedure, the title compound was isolated as a colorless oil (31 mg, 94%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26-7.23 (m, 3H), 7.20 (s, 1H), 7.15-7.08 (m, 2H), 7.07 (d, *J* = 8.5 Hz, 2H), 6.77 (d, *J* = 8.5 Hz, 2H), 5.52 (m, 2H), 4.25 (dd, *J* = 19.5, 2.5 Hz, 1H), 4.16 (d, *J* = 19.5, 2.5 Hz, 1H), 3.85-3.78 (m, 2H), 2.26 (s, 3H), 1.94 (t, *J* = 2.5 Hz, 1H), 1.81 (t, *J* = 6.5 Hz, 2H), 1.12 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{24}H_{26}N_7O_2$ $[M + H]^+$, 444.2142; found, 444.2141.



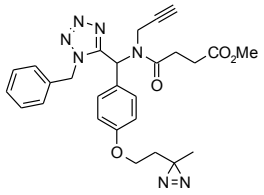
13

2,2,2-trichloroethyl [(1-Benzyl-1*H*-tetrazol-5-yl){4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]phenyl}methyl]2-propyn-1-ylcarbamate (13). To a stirred solution of amine **8** (250 mg, 0.62 mmol) in EtOAc (1 mL) and sat. aq. $NaHCO_3$ (3.0 ml) was added 2,2,2-trichloroethyl chloroformate (0.12 mL, 0.87 mmol) at 0 °C under N_2. After being stirred for 1 h at room temperature, the reaction mixture was diluted with EtOAc, washed with H_2O and brine, and dried over anhydrous Na_2SO_4. The organic layer was evaporated and the resulting residue was purified by column chromatography on silica gel (10–25% EtOAc/hexanes) to give the title compound as a pale yellow oil (210 mg, 59%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30-7.20 (m, 3H), 7.15-7.00 (m, 4H), 6.85-6.65 (m, 3H), 5.50-5.49 (s, 2H), 4.89 (s, 2H), 4.82 (m, 1H), 4.20 (m, 1H), 3.89-3.79 (m, 2H), 1.90-1.82 (m, 2H), 1.83 (t, *J* = 2.5 Hz, 1H), 1.13 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{25}H_{25}Cl_3N_7O_3$ $[M + H]^+$, 576.1079; found, 576.1106.



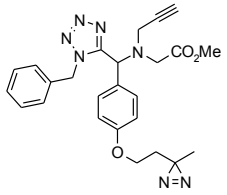
19

***N*-[(1-benzyl-1*H*-tetrazol-5-yl){4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]phenyl}methyl]-*N*-(2-propyn-1-yl)hexanamide (19).** The title compound was prepared from amine **8** (40 mg, 0.10 mmol), hexanoyl chloride (17 μL, 0.12 mmol), pyridine (18 μL, 0.22 mmol) and CH_2Cl_2 (0.3 mL) according to the same procedure as described for the preparation of **10** from **8**. Using this procedure, the title compound was isolated as a colorless oil (35 mg, 71%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29-7.21 (m, 4H), 7.15-7.11 (m, 2H), 7.07 (d, *J* = 8.5 Hz, 2H), 6.75 (d, *J* = 8.5 Hz, 2H), 5.57 (d, *J* = 15.5 Hz, 1H), 5.52 (d, *J* = 15.5 Hz, 1H), 4.28 (dd, *J* = 19.0, 2.5 Hz, 1H), 4.15 (dd, *J* = 19.0, 2.5 Hz, 1H), 3.86-3.78 (m, 2H), 2.54 (m, 1H), 2.43 (m, 1H), 1.93 (t, *J* = 2.5 Hz, 1H), 1.81 (t, *J* = 6.0 Hz, 2H), 1.72-1.66 (m, 2H), 1.40-1.31 (m, 4H), 1.11 (s, 3H), 0.91 (t, *J* = 7.5 Hz, 3H); HRMS (ESI-TOF) calc'd for $C_{28}H_{34}N_7O_2$ $[M + H]^+$, 500.2768; found, 500.2773.



37

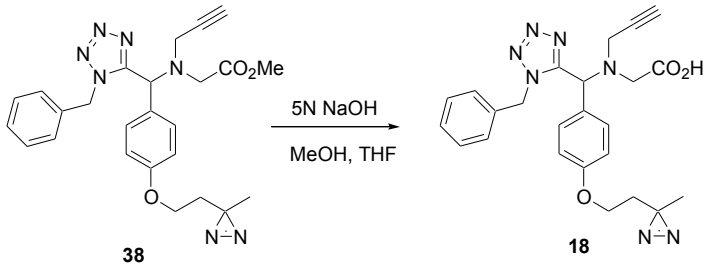
Methyl 4-{[(1-benzyl-1*H*-tetrazol-5-yl){4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]phenyl}methyl](2-propyn-1-yl)amino}-4-oxobutanoate (37). The title compound was prepared from amine **8** (30 mg, 0.075 mmol), methyl succinyl chloride (14 μL, 0.11 mmol), pyridine (18 μL, 0.22 mmol) and CH_2Cl_2 (0.3 mL) according to the same procedure as described for the preparation of **10** from **8**. Using this procedure, the title compound was isolated as a colorless oil (15 mg, 39%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25-7.20 (m, 4H), 7.13-7.09 (m, 2H), 7.05 (d, *J* = 8.4 Hz, 2H), 6.76 (d, *J* = 8.4 Hz, 2H), 5.58 (d, *J* = 15.6 Hz, 1H), 5.57 (d, *J* = 15.6 Hz, 1H), 4.29 (dd, *J* = 19.2, 2.4 Hz, 1H), 4.18 (dd, *J* = 19.2, 2.4 Hz, 1H), 3.85-3.78 (m, 2H), 3.71 (s, 3H), 2.91 (m, 1H), 2.79-2.67 (m, 3H), 1.98 (t, *J* = 2.4 Hz, 1H), 1.81 (t, *J* = 6.0 Hz, 2H), 1.12 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{27}H_{30}N_7O_4$ $[M + H]^+$, 516.2354; found, 516.2345.



38

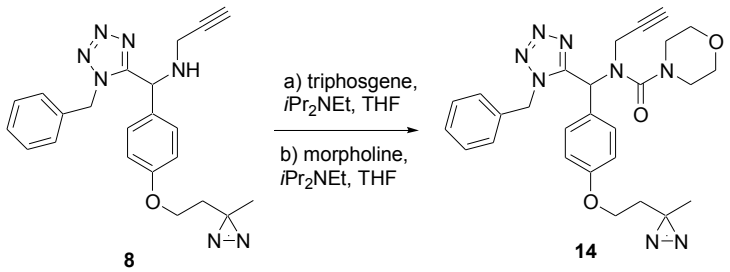
Methyl {[(1-benzyl-1*H*-tetrazol-5-yl){4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]phenyl}methyl](2-propyn-1-yl)amino}acetate (38). To a stirred solution of amine **8** (30 mg, 0.075 mmol) and K_2CO_3 (31 mg, 0.22 mmol) in DMF (0.3 mL) was added methyl bromoacetate (8.3 μL, 0.090 mmol) at room temperature under N_2. After being stirred for 16 h at 80 °C, the reaction mixture was cooled to room temperature and was diluted with EtOAc, washed with H_2O, brine, and dried over anhydrous Na_2SO_4. The organic layer was evaporated and the resulting residue was purified on preparative TLC (33%

EtOAc/hexanes) to give the title compound as a colorless oil (15 mg, 42%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32-7.26 (m, 3H), 7.24 (d, *J* = 9.0 Hz, 2H), 7.18-7.10 (m, 2H), 6.75 (d, *J* = 9.0 Hz, 2H), 5.72 (d, *J* = 15.5 Hz, 1H), 5.53 (d, *J* = 15.5 Hz, 1H), 5.47 (s, 1H), 3.84 (t, *J* = 6.0 Hz, 2H), 3.63 (s, 3H), 3.52 (d, *J* = 17.0 Hz, 1H), 3.51 (dd, *J* = 17.0, 2.0 Hz, 1H), 3.41 (dd, *J* = 17.0, 2.0 Hz, 1H), 3.35 (d, *J* = 17.0 Hz, 1H), 2.17 (t, *J* = 2.5 Hz, 1H), 1.80 (t, *J* = 6.0 Hz, 2H), 1.11 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{25}H_{28}N_7O_3$ $[M + H]^+$, 474.2248; found, 474.2243.



38 18

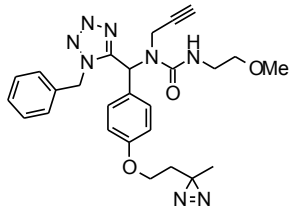
{[(1-Benzyl-1*H*-tetrazol-5-yl){4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]phenyl}methyl](2-propyn-1-yl)amino}acetic acid (18). A solution of **38** (15 mg, 0.032 mmol) in MeOH (0.15 mL) and THF (0.03 mL) was treated with 5N NaOH (0.030 mL) at room temperature for 10 h. The reaction mixture was acidified with 2N HCl and then extracted with EtOAc. The organic layer was washed with brine, and dried over anhydrous Na_2SO_4. The organic layer was evaporated and the resulting residue was purified by preparative TLC ($CHCl_3$/MeOH/AcOH, 20/1/0.3) to give the title compound as a pale yellow oil (10 mg, 69%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35-7.25 (m, 3H), 7.20-6.95 (m, 4H), 6.80-6,74 (m, 2H), 5.58 (d, *J* = 15.0 Hz, 1H), 5.24 (m, 1H), 5.12 (m, 1H), 3.82 (t, *J* = 6.0 Hz, 2H), 3.55-3.07 (m, 4H), 2.17 (t, *J* = 2.5 Hz, 1H), 1.83 (t, *J* = 6.0 Hz, 2H), 1.12 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{24}H_{26}N_7O_3$ $[M + H]^+$, 460.2092; found, 460.2098.



8 14

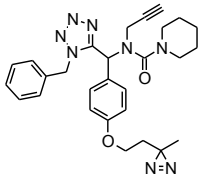
***N*-[(1-Benzyl-1*H*-tetrazol-5-yl){4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]phenyl}methyl]-*N*-(2-propyn-1-yl)-4-morpholinecarboxamide (14).** A solution of amine **8** (250 mg, 0.62 mmol) in THF (2 mL) was treated with *i*Pr_2NEt (0.32 mL, 1.9 mmol) and triphosgene (93 mg, 0.31 mmol), and the reaction mixture was stirred for 30 min at 0 °C. The mixture was poured into H_2O and extracted with EtOAc. The organic layer was washed with H_2O and brine, dried over anhydrous Na_2SO_4 and concentrated under reduced

pressure. A portion of this intermediate (0.13 mmol) was dissolved in THF (0.6 mL), and *i*Pr_2NEt (0.064 mL, 0.38 mmol), DMAP (15 mg, 0.13 mmol) and morpholine (13 μL, 0.15 mmol) were added. The mixture was stirred for 30 min at 60 °C and poured into a mixture of EtOAc and 1N HCl, washed with H_2O and brine and dried over anhydrous Na_2SO_4. After filtration, the eluent was evaporated and the resulting residue was purified on preparative TLC (50% EtOAc/hexanes) to give the title compound as pale yellow oil (55 mg, 86%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28-7.19 (m, 3H), 7.08-7.03 (m, 4H), 6.69 (d, *J* = 8.5 Hz, 2H), 5.95 (s, 1H), 5.63 (d, *J* = 15.0 Hz, 1H), 5.57 (d, *J* = 15.0 Hz, 1H), 4.01 (dd, *J* = 18.5, 2.5 Hz, 1H), 3.91 (dd, *J* = 18.5, 2.5 Hz, 1H), 3.80 (t, *J* = 6.5 Hz, 2H), 3.72-2.64 (m, 5H), 3.44-3.37 (m, 3H), 2.05 (t, *J* = 2.5 Hz, 1H), 1.80 (t, *J* = 6.5 Hz, 2H), 1.11 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{27}H_{31}N_8O_3$ $[M + H]^+$, 515.2514; found, 515.2527.



15

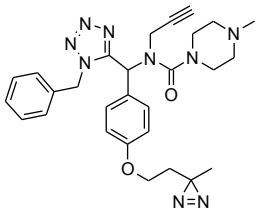
1-[(1-Benzyl-1*H*-tetrazol-5-yl){4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]phenyl}methyl]-3-(2-methoxyethyl)-1-(2-propyn-1-yl)urea (15). The title compound was prepared from **8** (50 mg, 0.13 mmol), triphosgene (1.9 mg, 0.063 mmol), *i*Pr_2NEt (0.064 mL, 0.38 mmol), 2-methoxyethylamine (13 μL, 0.15 mmol), DMAP (15 mg, 0.13 mmol) and THF (0.6 mL) according to the same procedure as described for the preparation of **14** from **8**. Using this procedure, the title compound was isolated as a colorless oil (47 mg, 75%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23-7.20 (m, 3H), 7.18-7.14 (m, 2H), 7.04 (s, 1H), 7.01 (d, *J* = 9.0 Hz, 2H), 6.74 (d, *J* = 9.0 Hz, 2H), 5.63 (d, *J* = 15.5 Hz, 1H), 5.59 (d, *J* = 15.5 Hz, 1H), 5.54 (bs, 1H), 4.13 (dd, *J* = 19.0, 2.5 Hz, 1H), 4.01 (dd, *J* = 19.0, 2.5 Hz, 1H), 3.85-3.80 (m, 2H), 3.55-3.43 (m, 4H), 3.36 (s, 3H), 2.16 (t, *J* = 2.4 Hz, 1H), 1.80 (t, *J* = 6.5 Hz, 2H), 1.11 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{26}H_{31}N_8O_3$ $[M + H]^+$, 503.2514; found, 503.2516.



16

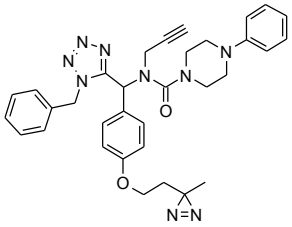
***N*-[(1-Benzyl-1*H*-tetrazol-5-yl){4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]phenyl}methyl]-*N*-(2-propyn-1-yl)-1-piperidinecarboxamide (16).** The title compound was prepared from **8** (50 mg, 0.13 mmol), triphosgene (1.9 mg, 0.063 mmol), *i*Pr_2NEt (0.064 mL, 0.38 mmol), piperidine (15 μL, 0.15 mmol), DMAP (15 mg, 0.13 mmol) and THF (0.6 mL) according to the same procedure as described for the preparation of **14** from **8**. Using this procedure, the title compound was isolated as a colorless oil (48 mg, 75%): ^{1}H NMR

(500 MHz, $CDCl_3$) δ 7.28-7.18 (m, 3H), 7.09-7.05 (m, 2H), 7.05 (d, *J* = 8.5 Hz, 2H), 6.68 (d, *J* = 8.5 Hz, 2H), 5.90 (s, 1H), 5.71 (d, *J* = 15.5 Hz, 1H), 5.62 (d, *J* = 15.5 Hz, 1H), 3.93 (dd, *J* = 18.5, 2.5 Hz, 1H), 3.84 (dd, *J* = 18.5, 2.5 Hz, 1H), 3.79 (t, *J* = 6.0 Hz, 2H), 3.41-3.32 (m, 4H), 2.05 (t, *J* = 2.5 Hz, 1H), 1.79 (t, *J* = 6.5 Hz, 2H), 1.62-1.51 (m, 6H), 1.10 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{28}H_{33}N_8O_2$ [M + H]$^+$, 513.2721; found, 513.2728.



31

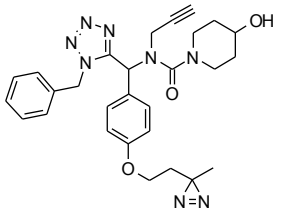
***N*-[(1-Benzyl-1*H*-tetrazol-5-yl){4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]phenyl}methyl]-4-methyl-*N*-(2-propyn-1-yl)-1-piperazinecarboxamide (31).** The title compound was prepared from **8** (30 mg, 0.075 mmol), triphosgene (11 mg, 0.037 mmol), *i*Pr_2NEt (38 μL, 0.22 mmol), 1-methylpiperazine (10 μL, 0.090 mmol), DMAP (9.1 mg, 0.075 mmol), and THF (0.3 mL) according to the same procedure as described for the preparation of **14** from **8**. Using this procedure, the title compound was isolated as a colorless oil (15 mg, 38%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.27-7.21 (m, 3H), 7.07-7.02 (m, 4H), 6.68 (d, *J* = 8.4 Hz, 2H), 5.93 (s, 1H), 5.67 (d, *J* = 15.6 Hz, 1H), 5.59 (d, *J* = 15.6 Hz, 1H), 4.01 (dd, *J* = 18.6, 2.4 Hz, 1H), 3.91 (dd, *J* = 18.6, 2.4 Hz, 1H), 3.79 (t, *J* = 6.4 Hz, 2H), 3.51-3.40 (m, 4H), 2.47-2.34 (m, 4H), 2.30 (s, 3H), 2.05 (t, *J* = 2.4 Hz, 1H), 1.80 (t, *J* = 6.4 Hz, 2H), 1.11 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.27, 159.00, 155.45, 133.64, 131.00, 129.10, 128.72, 127.80, 125.97, 114.70, 78.39, 73.07, 63.02, 54.84, 54.33, 51.32, 46.24, 46.17, 38.67, 34.30, 24.35, 20.40; IR (cm^{-1}) 3293, 3039, 2933, 2853, 2795, 1649, 1610, 1510, 1455, 1416, 1247, 1223, 1175, 1152, 1002, 946, 828, 722, 663; HRMS (ESI-TOF) calc'd for $C_{28}H_{34}N_9O_2$ [M + H]$^+$, 528.2830; found, 528.2826.



32

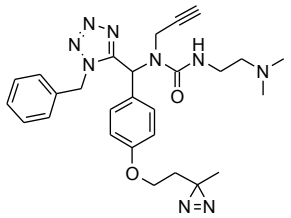
***N*-[(1-benzyl-1*H*-tetrazol-5-yl){4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]phenyl}methyl]-4-phenyl-*N*-(2-propyn-1-yl)-1-piperazinecarboxamide (32).** The title compound was prepared from **8** (30 mg, 0.075 mmol), triphosgene (11 mg, 0.037 mmol), *i*Pr_2NEt (38 μL, 0.22 mmol), 1-phenylpiperazine (14 μL, 0.090 mmol), DMAP (9.1 mg, 0.075 mmol) and THF (0.3 mL) according to the same procedure as described for the preparation of **14** from **8**. Using this procedure, the title compound was isolated as a yellow oil (17 mg,

38%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32-7.20 (m, 5H), 7.09-7.04 (m, 4H), 6.93-6.86 (m, 3H), 6.70 (d, *J* = 8.8 Hz, 2H), 5.99 (s, 1H), 5.62 (d, *J* = 15.6 Hz, 1H), 5.58 (d, *J* = 15.6 Hz, 1H), 4.10 (dd, *J* = 18.4, 2.4 Hz, 1H), 3.98 (dd, *J* = 18.4, 2.4 Hz, 1H), 3.80 (t, *J* = 6.0 Hz, 2H), 3.65-3.51 (m, 4H), 3.24-3.10 (m, 4H), 2.05 (t, *J* = 2.4 Hz, 1H), 1.80 (t, *J* = 6.0 Hz, 2H), 1.11 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{33}H_{36}N_9O_2$ [M + H]$^+$, 590.2986; found, 590.2985.



33

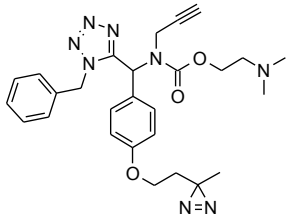
***N*-[(1-benzyl-1*H*-tetrazol-5-yl){4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]phenyl}methyl]-4-hydroxy-*N*-(2-propyn-1-yl)-1-piperidinecarboxamide (33).** The title compound was prepared from **8** (30 mg, 0.075 mmol), triphosgene (11 mg, 0.037 mmol), *i*Pr_2NEt (38 μL, 0.22 mmol), 4-hydroxypiperidine (9.1 mg, 0.090 mmol), DMAP (9.1 mg, 0.075 mmol) and THF (0.3 mL) according to the same procedure as described for the preparation of **14** from **8**. Using this procedure, the title compound was isolated as a colorless oil (22 mg, 55%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23-7.20 (m, 3H), 7.08-7.00 (m, 4H), 6.67 (d, *J* = 8.8 Hz, 2H), 5.89 (s, 1H), 5.69 (d, *J* = 15.6 Hz, 1H), 5.59 (d, *J* = 15.6 Hz, 1H), 3.94-3.85 (m, 3H), 3.81-3.71 (m, 4H), 3.19-3.08 (m, 2H), 2.07 (t, *J* = 2.4 Hz, 1H), 1.95-1.83 (m, 2H), 1.80 (t, *J* = 6.0 Hz, 2H), 1.53-1.44 (m, 2H), 1.11 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{28}H_{33}N_8O_3$ [M + H]$^+$, 529.2670; found, 529.2670.



34

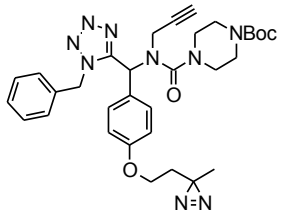
1-[(1-Benzyl-1*H*-tetrazol-5-yl){4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]phenyl}methyl]-3-[2-(dimethylamino)ethyl]-1-(2-propyn-1-yl)urea (34). The title compound was prepared from **8** (30 mg, 0.075 mmol), triphosgene (11 mg, 0.037 mmol), *i*Pr_2NEt (38 μL, 0.22 mmol), 2-dimethylaminoethylamine (9.8 μL, 0.090 mmol), DMAP (9.1 mg, 0.075 mmol) and THF (0.3 mL) according to the same procedure as described for the preparation of **14** from **8**. Using this procedure, the title compound was isolated as a yellow oil (12 mg, 32%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24-7.15 (m, 5H), 7.06 (s, 1H), 7.02 (d, *J* = 8.8 Hz, 2H), 6.75 (d, *J* = 8.8 Hz, 2H), 5.87 (m, 1H), 5.66-5.57 (m, 2H), 4.12 (dd, *J* = 18.8, 2.4 Hz, 1H), 4.01 (dd, *J* = 18.8, 2.4 Hz, 1H), 3.84-3.80 (m, 2H), 3.43-3.25 (m, 2H), 2.50-2.43 (m, 2H), 2.25 (s, 6H), 2.10 (t, *J*

= 2.4 Hz, 1H), 1.83 (t, *J* = 6.4 Hz, 2H), 1.12 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{27}H_{34}N_9O_2$ [M + H]$^+$, 516.2830; found, 516.2826.



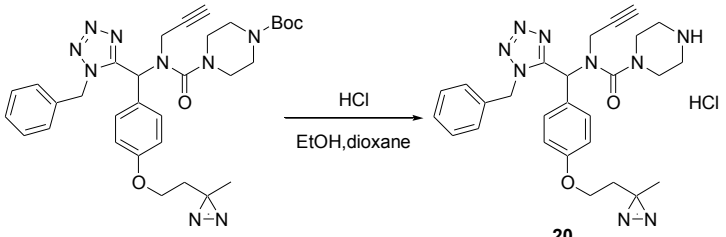
35

2-(Dimethylamino)ethyl [(1-benzyl-1*H*-tetrazol-5-yl){4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]phenyl}methyl]2-propyn-1-ylcarbamate (35). The title compound was prepared from **8** (30 mg, 0.075 mmol), triphosgene (11 mg, 0.037 mmol), *i*Pr_2NEt (38 µL, 0.22 mmol), 2-dimethylaminoethanol (9.0 µL, 0.090 mmol), DMAP (9.1 mg, 0.075 mmol) and THF (0.3 mL) according to the same procedure as described for the preparation of **14** from **8**. Using this procedure, the title compound was isolated as a white solid (15 mg, 38%): ^{1}H NMR (400 MHz, CD_3OD) δ 7.32-7.27 (m, 3H), 7.19-7.09 (m, 4H), 6.89 (d, *J* = 8.8 Hz, 2H), 6.82 (s, 1H), 5.66-5.59 (m, 2H), 4.33-4.07 (m, 4H), 3.94-3.90 (m, 2H), 2.85-2.68 (m, 2H), 2.40-2.22 (m, 6H), 2.20 (t, *J* = 2.4 Hz, 1H), 1.82 (t, *J* = 6.0 Hz, 2H), 1.12 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{27}H_{33}N_8O_3$ [M + H]$^+$, 517.2670; found, 517.2666.



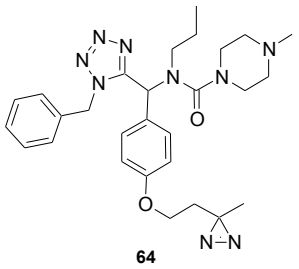
17

***tert*-Butyl 4-{[(1-benzyl-1*H*-tetrazol-5-yl){4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]phenyl}methyl](2-propyn-1-yl)carbamoyl}-1-piperazinecarboxylate (17).** The title compound was prepared from **8** (100 mg, 0.25 mmol), triphosgene (37 mg, 0.13 mmol), *i*Pr_2NEt (0.19 mL, 0.75 mmol), 1-Boc-piperazine (56 mg, 0.30 mmol), DMAP (31 mg, 0.25 mmol), and THF (1.2 mL) according to the same procedure as described for the preparation of probe **14** from probe **8**. Using this procedure, the title compound was isolated as a colorless oil (73 mg, 95%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25-7.22 (m, 3H), 7.08-7.03 (m, 4H), 6.70 (d, *J* = 9.0 Hz, 2H), 5.94 (s, 1H), 5.61 (d, *J* = 15.5 Hz, 1H), 5.56 (d, *J* = 15.5 Hz, 1H), 4.01 (dd, *J* = 18.0, 2.0 Hz, 1H), 3.91 (dd, *J* = 18.0, 2.0 Hz, 1H), 3.80 (t, *J* = 6.5 Hz, 2H), 3.51-3.34 (m, 8H), 2.05 (t, *J* = 2.5 Hz, 1H), 1.80 (t, *J* = 6.5 Hz, 2H), 1.46 (s, 9H), 1.11 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{32}H_{40}N_9O_4$ [M + H]$^+$, 614.3198; found, 614.3212.



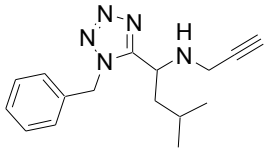
17 20

***N*-[(1-Benzyl-1*H*-tetrazol-5-yl){4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]phenyl}methyl]-*N*-(2-propyn-1-yl)-1-piperazinecarboxamide hydrochloride (20).** To a stirred solution of **17** (31 mg, 0.050 mmol) in EtOH (0.3 mL) was added 4N HCl in 1,4-dioxane (0.10 mL, 0.4 mmol) at 0 °C under N_2. After being stirred for 3 h at room temperature, the reaction mixture was diluted with EtOAc. The precipitate was collected by filtration to give the title compound as a white powder (8.0 mg, 31%): ^{1}H NMR (500 MHz, CD_3OD) δ 7.33-7.26 (m, 3H), 7.16 (d, *J* = 8.5 Hz, 2H), 7.10-7.06 (m, 2H), 6.82 (d, *J* = 8.5 Hz, 2H), 6.04 (s, 1H), 5.69-5.60 (m, 2H), 4.18 (dd, *J* = 18.5, 2.5, 1H), 3.89 (t, *J* = 6.5 Hz, 2H), 3.83 (dd, *J* = 18.5, 2.5 Hz, 1H), 3.45-3.38 (m, 4H), 3.30-3.27 (m, 4H), 2.69 (t, *J* = 2.4 Hz, 1H), 1.81 (t, *J* = 6.5 Hz, 2H), 1.11(s, 3H); HRMS (ESI-TOF) calc'd for $C_{27}H_{32}N_9O_2$ $[M + H]^+$, 514.2673; found, 514.2692.



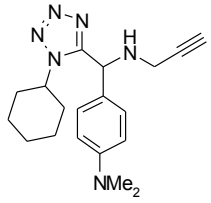
64

***N*-[(1-Benzyl-1*H*-tetrazol-5-yl){4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]phenyl}methyl]-1-propanamine (64)** The title compound was prepared from **62** (50 mg, 0.12 mmol) first by formation of the corresponding carbamyl chloride using triphosgene (18 mg, 0.062 mmol) and *i*Pr_2NEt (63 µL, 0.37 mmol) in THF (0.5 mL). The carbamyl chloride was then treated with 1-methylpiperazine (16 µL, 0.15 mmol), DMAP (15 mg, 0.12 mmol) and *i*Pr_2NEt (63 µL, 0.37 mmol) in THF (0.5 mL) according to the same procedure as described for the preparation of **14** from **8**. Using this procedure, the title compound was isolated as a yellow oil (47 mg, 52%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.30-7.22 (m, 3H), 7.08-7.06 (m, 2H), 6.99 (d, *J* = 8.4 Hz, 2H), 6.70 (d, *J* = 8.4 Hz, 2H), 6.03 (s, 1H), 5.63 (d, *J* = 15.6 Hz, 1H), 5.58 (d, *J* = 15.6 Hz, 1H), 3.80 (t, *J* = 6.6 Hz, 2H), 3.43-3.29 (m, 4H), 3.10 (m, 1H), 3.02 (m, 1H), 2.40-2.33 (m, 4H), 2.28 (s, 3H), 1.80 (t, *J* = 6.0 Hz, 2H), 1.23 (m, 1H), 1.13 (m, 1H), 1.12 (s, 3H), 0.65 (t, *J* = 7.8 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 164.14, 158.72, 155.74, 133.74, 130.28, 129.14, 128.77, 127.79, 127.30, 114.72, 63.02, 54.94, 54.47, 51.25, 49.69, 46.38, 46.24, 34.34, 24.36, 20.96, 20.40, 11.46; IR (cm^{-1}) 2934, 2873, 2793, 1641, 1612, 1510, 1457, 1415, 1365, 1292, 1248, 1175, 1140, 1069, 1003, 722; HRMS (ESI-TOF) calc'd for $C_{28}H_{38}N_9O_2$ $[M + H]^+$, 532.3143; found, 532.3138.



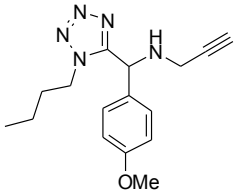
IM2

1-(1-Benzyl-1*H*-tetrazol-5-yl)-3-methyl-*N*-(2-propyn-1-yl)-1-butanamine (IM2) To a stirred solution of propargylamine (23 μL, 0.36 mmol), isobutylaldehyde (39 μL, 0.36 mmol) and benzyl isocyanide (44 μL, 0.36 mmol) in MeOH (0.5 mL) was added $TMSN_3$ (29 μL, 0.36 mmol) at 0 °C under N_2. After being stirred at room temperature for 3 h, the reaction mixture was cooled and diluted with EtOAc, washed with H_2O, sat. aq. $NaHCO_3$, brine, and dried over anhydrous Na_2SO_4. The organic layer was evaporated and the resulting residue was purified by column chromatography on silica gel (33–50% EtOAc/hexanes) to give **IM2** as a colorless oil (93 mg, 91%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28-7.26 (m, 3H), 7.25-7.19 (m, 2H), 5.76 (d, *J* = 15.5 Hz, 1H), 5.66 (d, *J* = 15.5 Hz, 1H), 4.37 (m, 1H), 3.33 (dd, *J* = 17.5, 2.5 Hz, 1H), 3.04 (dd, *J* = 17.5, 2.5 Hz, 1H), 2.19 (t, *J* = 2.5 Hz, 1H), 1.58 (bs, 1H), 1.56 (m, 1H), 1.44 (m, 1H), 1.36 (m, 1H), 0.80 (d, *J* = 7.0 Hz, 3H), 0.70 (d, *J* = 7.0 Hz, 3H); HRMS (ESI-TOF) calc'd for $C_{16}H_{22}N_5$ $[M + H]^+$, 284.1870; found, 284.1865.



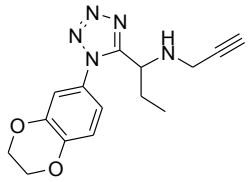
IM3

4-[(1-Cyclohexyl-1*H*-tetrazol-5-yl)(2-propyn-1-ylamino)methyl]-*N,N*-dimethylaniline (IM3). The title compound was prepared from propargylamine (18 μL, 0.28 mmol), cyclohexyl isocyanide (35 μL, 0.28 mmol), 4-dimethylaminobenzaldehyde (42 mg, 0.28 mmol) and $TMSN_3$ (23 μL, 0.28 mmol) according to the same procedure as described for the preparation of **IM2**. Using this procedure, the title compound was isolated as a yellow oil (79 mg, 83%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.18 (d, *J* = 9.0 Hz, 2H), 6.66 (d, *J* = 9.0 Hz, 2H), 5.35 (s, 1H), 4.21 (m, 1H), 3.46 (dd, *J* = 17.0, 2.5 Hz, 1H), 3.39 (dd, *J* = 17.0, 2.5 Hz, 1H), 2.94 (s, 6H), 2.27 (t, *J* = 2.5 Hz, 1H), 1.98-1.72 (m, 4H), 1.70-1.42 (m, 3H), 1.50-1.18 (m, 4H); HRMS (ESI-TOF) calc'd for $C_{19}H_{27}N_6$ $[M + H]^+$, 339.2292; found, 339.2295.



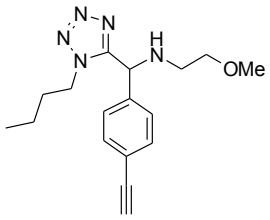
IM4

***N*-[(1-Butyl-1*H*-tetrazol-5-yl)(4-methoxyphenyl)methyl]-2-propyn-1-amine (IM4).** The title compound was prepared from propargylamine (18 μL, 0.28 mmol), *n*-butyl isocyanide (26 μL, 0.25 mmol), 4-methoxybenzaldehyde (38 mg, 0.28 mmol) and $TMSN_3$ (23 μL, 0.28 mmol) according to the same procedure as described for the preparation of **IM2**. Using this procedure, the title compound was isolated as a colorless oil (75 mg, 98%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (d, *J* = 8.5 Hz, 2H), 6.89 (d, *J* = 8.5 Hz, 2H), 5.40 (s, 1H), 4.20-4.10 (m, 2H), 3.80 (s, 3H), 3.47 (dd, *J* = 17.0, 2.5 Hz, 1H), 3.39 (dd, *J* = 17.0, 2.5 Hz, 1H), 2.29 (t, *J* = 2.5 Hz, 1H), 1.73-1.55 (m, 3H), 1.30-1.20 (m, 2H), 0.86 (t, *J* = 7.5 Hz, 3H); HRMS (ESI-TOF) calc'd for $C_{16}H_{22}N_5O$ $[M + H]^+$, 300.1819; found, 300.1814.



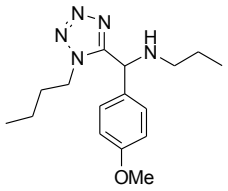
IM5

***N*-{1-[1-(2,3-Dihydro-1,4-benzodioxin-6-yl)-1*H*-tetrazol-5-yl]propyl}-2-propyn-1-amine (IM5).** The title compound was prepared from propargylamine (18 μL, 0.28 mmol), 2,3-dihydro-6-isocyano-1,4-benzodioxine (45 mg, 0.28 mmol), propionaldehyde (20 μL, 0.28 mmol) and $TMSN_3$ (23 μL, 0.28 mmol) according to the same procedure as described for the preparation of **IM2**. Using this procedure, the title compound was isolated as a colorless oil (51 mg, 61%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.05 (d, *J* = 2.5 Hz, 1H), 7.02 (d, *J* = 8.5 Hz, 1H), 6.96 (dd, *J* = 8.5, 2.5 Hz, 1H), 4.35-4.31 (m, 4H), 4.10 (m, 1H), 3.40 (dd, *J* = 17.0, 2.5 Hz, 1H), 3.33 (dd, *J* = 17.0, 2.5 Hz, 1H), 2.12 (t, *J* = 2.5 Hz, 1H), 1.88-1.75 (m, 3H), 0.85 (t, *J* = 7.0 Hz, 3H); HRMS (ESI-TOF) calc'd for $C_{15}H_{18}N_5O_2$ $[M + H]^+$, 300.1455; found, 300.1459.



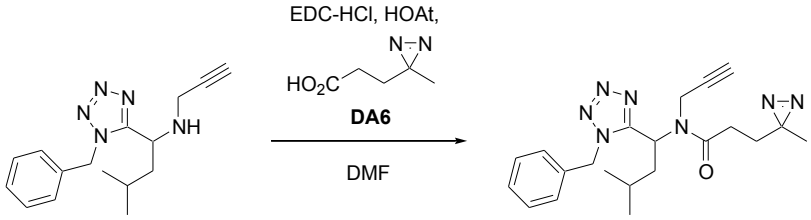
IM6

***N*-[(1-Butyl-1*H*-tetrazol-5-yl)(4-ethynylphenyl)methyl]-2-methoxyethanamine (IM6).** The title compound was prepared from 2-methoxyethylamine (24 μL, 0.28 mmol), *n*-butyl isocyanide (29 μL, 0.28 mmol), 4-ethynyl benzaldehyde (36 mg, 0.28 mmol) and $TMSN_3$ (23 μL, 0.28 mmol) according to the same procedure as described for the preparation of **IM2**. Using this procedure, the title compound was isolated as a colorless oil (55 mg, 63%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.49 (d, *J* = 7.8 Hz, 2H), 7.34 (d, *J* = 7.8 Hz, 2H), 5.31 (s, 1H), 4.20 (t, *J* = 7.8 Hz, 2H), 3.53-3.49 (m, 2H), 3.33 (s, 3H), 3.10 (s, 1H), 2.78 (m, 1H), 2.72 (m, 1H), 2.43 (bs, 1H), 1.68-1.55 (m, 2H), 1.30-1.20 (m, 2H), 0.86 (t, *J* = 7.2 Hz, 3H); HRMS (ESI-TOF) calc'd for $C_{17}H_{24}N_5O$ $[M + H]^+$, 314.1975; found, 314.1975.



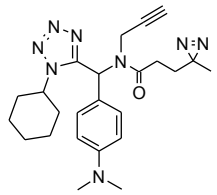
IM7

***N*-[(1-Butyl-1*H*-tetrazol-5-yl)(4-methoxyphenyl)methyl]-1-propanamine (IM7).** The title compound was prepared from propylamine (25 μL, 0.30 mmol), 4-methoxybenzaldehyde (36 μL, 0.30 mmol), *n*-butyl isocyanide (31 μL, 0.30 mmol) and $TMSN_3$ (25 μL, 0.30 mmol) in MeOH (0.6 mL) according to the same procedure as described for the preparation of **IM2**. Using this procedure, the title compound was isolated as a colorless oil (47 mg, 52%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 (d, *J* = 8.5 Hz, 2H), 6.87 (d, *J* = 8.5 Hz, 2H), 5.14 (s, 1H), 4.19 (t, *J* = 7.5 Hz, 2H), 3.79 (s, 3H), 2.53 (m, 1H), 2.48 (m, 1H), 1.97 (bs, 1H), 1.67-1.60 (m, 4H), 1.27-1.22 (m, 2H), 0.91 (t, *J* = 7.0 Hz, 3H), 0.86 (t, *J* = 7.0 Hz, 3H); HRMS (ESI-TOF) calc'd for $C_{16}H_{26}N_5O$ $[M + H]^+$, 304.2132; found, 304.2136.



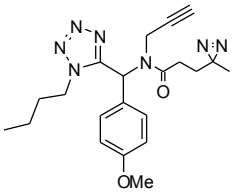
IM2 **21**

***N*-[1-(1-Benzyl-1*H*-tetrazol-5-yl)-3-methylbutyl]-3-(3-methyl-3*H*-diaziren-3-yl)-*N*-(2-propyn-1-yl)propanamide (21)** To a stirred solution of amine **IM2** (40 mg, 0.14 mmol), **DA6** (18 mg, 0.14 mmol) and HOAt (9.6 mg, 0.071 mmol) in DMF (0.5 mL) was added EDC•HCl (54 mg, 0.28 mmol) at room temperature under N_2. After being stirred at 60 °C for an additional 10 h, the reaction mixture was cooled then diluted with EtOAc, washed with H_2O, sat. aq. $NaHCO_3$, brine, and dried over anhydrous Na_2SO_4. The organic layer was evaporated and the resulting residue was purified by column chromatography on silica gel (33–50% EtOAc/hexanes) to give the title compound as a colorless oil (25 mg, 45%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37-7.32 (m, 3H), 7.27-7.20 (m, 2H), 6.20 (m, 1H), 5.61 (d, *J* = 15.5 Hz, 1H), 5.56 (d, *J* = 15.5 Hz, 1H), 3.97 (dd, *J* = 19.0, 2.5 Hz, 1H), 3.66 (dd, *J* = 19.0, 2.5 Hz, 1H), 2.28 (m, 1H), 2.19 (t, *J* = 2.5 Hz, 1H), 2.08 (m, 1H), 1.90-1.72 (m, 4H), 1.46 (m, 1H), 1.05 (s, 3H), 0.80 (d, *J* = 6.5 Hz, 3H), 0.75 (d, *J* = 6.5 Hz, 3H); HRMS (ESI-TOF) calc'd for $C_{21}H_{28}N_7O$ $[M + H]^+$, 394.2350; found, 394.2350.



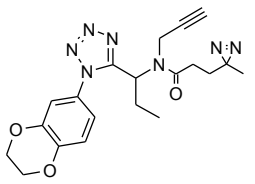
23

(*N*-{(1-Cyclohexyl-1*H*-tetrazol-5-yl)[4-(dimethylamino)phenyl]methyl}-3-(3-methyl-3*H*-diaziren-3-yl)-*N*-(2-propyn-1-yl)propanamide) (23). The title compound was prepared from **IM3** (75 mg, 0.22 mmol), **DA6** (31 mg, 0.24 mmol), HOAt (15 mg, 0.11 mmol) and EDC•HCl (85 mg, 0.44 mmol) according to the same procedure as described for the preparation of **21** from **IM2**. Using this procedure, the title compound was isolated as a yellow oil (30 mg, 30%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.17 (s, 1H), 7.05 (d, *J* = 9.0 Hz, 2H), 6.65 (d, *J* = 9.0 Hz, 2H), 4.30 (dd, *J* = 19.0, 2.5 Hz, 1H), 4.21 (dd, *J* = 19.0, 2.5 Hz, 1H), 4.18 (m, 1H), 2.96 (s, 6H), 2.48 (m, 1H), 2.34 (m, 1H), 2.25 (m, 1H), 2.01 (t, *J* = 2.5 Hz, 1H), 2.00-1.80 (m, 3H), 1.79-1.75 (m, 3H), 1.70-1.65 (m, 2H), 1.41 (m, 1H), 1.30-1.21 (m, 2H), 1.05(s, 3H); HRMS (ESI-TOF) calc'd for $C_{24}H_{33}N_8O$ $[M + H]^+$, 449.2772; found, 449.2775.



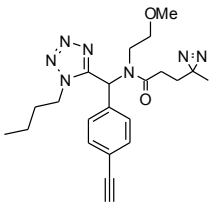
24

***N*-[(1-Butyl-1*H*-tetrazol-5-yl)(4-methoxyphenyl)methyl]-3-(3-methyl-3*H*-diaziren-3-yl)-*N*-(2-propyn-1-yl)propanamide (24).** The title compound was prepared from **IM4** (71 mg, 0.24 mmol), **DA6** (33 mg, 0.26 mmol), HOAt (16 mg, 0.12 mmol) and EDC•HCl (91 mg, 0.48 mmol) according to the same procedure as described for the preparation of **21** from **IM2**. Using this procedure, the title compound was isolated as a colorless oil (30 mg, 31%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.26 (s, 1H), 7.20 (d, *J* = 7.8 Hz, 2H), 6.89 (d, *J* = 7.8 Hz, 2H), 4.36-4.16 (m, 4H), 3.82 (s, 3H), 2.45 (m, 1H), 2.32 (m, 1H), 2.00 (t, *J* = 2.4 Hz, 1H), 1.84-1.72 (m, 4H), 1.34-1.23 (m, 2H), 1.05 (s, 3H), 0.86 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.76, 160.54, 153.62, 130.73, 125.37, 114.78, 78.84, 72.38, 55.70, 49.98, 47.69, 34.91, 31.69, 29.72, 28.21, 25.67, 20.29, 19.87, 13.66; IR (cm^{-1}) 3291, 3010, 2960, 2934, 2873, 1651, 1611, 1585, 1512, 1445, 1413, 1306, 1251, 1204, 1177, 1116, 1030, 931, 871, 844, 751, 663; HRMS (ESI-TOF) calc'd for $C_{21}H_{28}N_7O_2$ $[M + H]^+$, 410.2299; found, 410.2298.



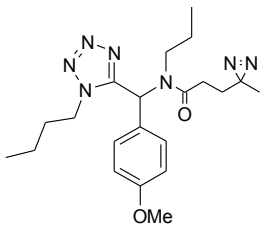
25

***N*-{1-[1-(2,3-dihydro-1,4-benzodioxin-6-yl)-1*H*-tetrazol-5-yl]propyl}-3-(3-methyl-3*H*-diaziren-3-yl)-N-(2-propyn-1-yl)propanamide (25).** The title compound was prepared from **IM5** (45 mg, 0.15 mmol), **DA6** (21 mg, 0.17 mmol), HOAt (10 mg, 0.075 mmol), and EDC•HCl (58 mg, 0.30 mmol) according to the same procedure as described for the preparation of **21** from **IM2**. Using this procedure, the title compound was isolated as a colorless oil as a colorless oil (32 mg, 54%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.00 (d, *J* = 8.5 Hz, 1H), 6.94 (d, *J* = 2.5 Hz, 1H), 6.89 (dd, *J* = 8.5, 2.5 Hz, 1H), 5.94 (t, *J* = 8.0 Hz, 1H), 4.35-4.28 (m, 4H), 4.24 (dd, *J* = 19.0, 2.5 Hz, 1H), 4.03 (dd, *J* = 19.0, 2.5 Hz, 1H), 2.32 (m, 1H), 2.21 (t, *J* = 2.5 Hz, 1H), 2.13-2.05 (m, 3H), 1.78-1.65 (m, 2H), 1.04 (s, 3H), 0.88 (t, *J* = 7.0 Hz, 3H); HRMS (ESI-TOF) calc'd for $C_{20}H_{24}N_7O_3$ $[M + H]^+$, 410.1935; found, 410.1933.



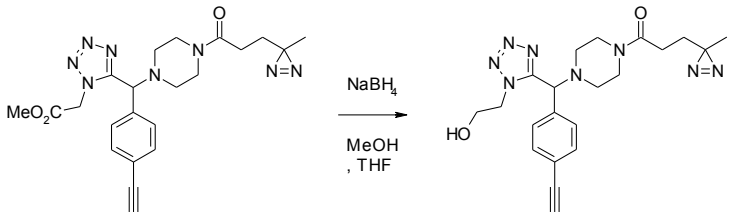
27

***N*-[(1-Butyl-1*H*-tetrazol-5-yl)(4-ethynylphenyl)methyl]-N-(2-methoxyethyl)-3-(3-methyl-3H-diaziren-3-yl)propanamide (27).** The title compound was prepared from **IM6** (53 mg, 0.17 mmol), **DA6** (24 mg, 0.19 mmol), HOAt (12 mg, 0.085 mmol), and EDC•HCl (65 mg, 0.34 mmol) according to the same procedure as described for the preparation of **21** from **IM2**. Using this procedure, the title compound was isolated as a colorless oil (32 mg, 54%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.51 (d, *J* = 7.8 Hz, 2H), 7.35 (s, 1H), 7.34 (d, *J* = 7.8 Hz, 2H), 4.40 (m, 1H), 4.31 (m, 1H), 3.79 (m, 1H), 3.57 (m, 1H), 3.15 (s, 1H), 3.11 (m, 3H), 2.89 (m, 1H), 2.82 (m, 1H), 2.44 (m, 1H), 2.32 (m, 1H), 1.79-1.72 (m, 4H), 1.30-1.23 (m, 2H), 1.03 (s, 3H), 0.86 (t, *J* = 7.2 Hz, 3H); HRMS (ESI-TOF) calc'd for $C_{22}H_{30}N_7O_2$ $[M + H]^+$, 424.2455; found, 424.2459.



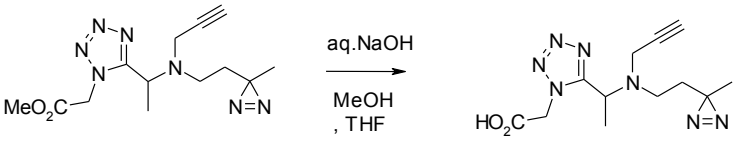
60

***N*-[(1-butyl-1*H*-tetrazol-5-yl)(4-methoxyphenyl)methyl]-3-(3-methyl-3*H*-diaziren-3-yl)-*N*-propylpropanamide (60).** The title compound was prepared from **IM7** (30 mg, 0.099 mmol), **DA6** (15 mg, 0.12 mmol), HOAt (6.7 mg, 0.049 mmol) and EDC•HCl (38 mg, 0.20 mmol) in DMF (0.3 mL) according to the same procedure as described for the preparation of **21** from **IM2**. Using this procedure, the title compound was isolated as a colorless oil (18 mg, 44%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.25 (d, *J* = 8.4 Hz, 2H), 7.17 (s, 1H), 6.90 (d, *J* = 8.4 Hz, 2H), 4.31 (m, 1H), 4.24 (m, 1H), 3.83 (s, 3H), 3.48 (m, 1H), 3.28 (m, 1H), 2.22 (m, 1H), 2.15 (m, 1H), 1.86-1.66 (m, 4H), 1.30-1.17 (m, 3H), 1.04 (s, 3H), 0.84 (t, *J* = 7.8 Hz, 3H), 0.77 (m, 1H), 0.65 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.58, 160.35, 154.06, 130.91, 126.02, 114.70, 55.67, 50.31, 47.57, 47.30, 31.61, 29.80, 27.77, 25.83, 23.67, 20.36, 19.81, 13.65, 11.63; IR (cm^{-1}) 3057, 2961, 2933, 2873, 1642, 1611, 1539, 1512, 1460, 1442, 1419, 1289, 1252, 1179, 1122, 1032, 872, 842, 740; HRMS (ESI-TOF) calc'd for $C_{21}H_{32}N_7O_2$ [M + H]$^+$, 414.2612; found, 414.2616.



36 **39**

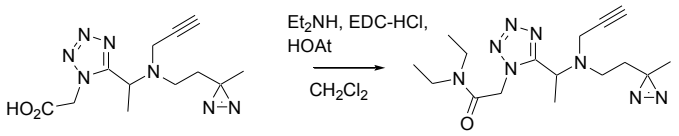
1-(4-{(4-Ethynylphenyl)[1-(2-hydroxyethyl)-1*H*-tetrazol-5-yl]methyl}-1-piperazinyl)-3-(3-methyl-3*H*-diaziren-3-yl)-1-propanone (39). To a stirred solution of **36** (40 mg, 0.089 mmol) in MeOH (0.25 mL) was added $NaBH_4$ (10 mg, 0.27 mmol) at 0 °C under N_2. After being stirred at 50 °C for 3 h, the reaction was quenched with H_2O. The reaction mixture was extracted with EtOAc three times. The combined organic layers were washed with brine, and dried over $MgSO_4$. The organic layer was evaporated and the resulting residue was purified by column chromatography on silica gel (100% EtOAc) to give the title compound as a colorless oil (19 mg, 51%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.50 (d, *J* = 7.8 Hz, 2H), 7.43 (d, *J* = 7.8 Hz, 2H), 5.16 (s, 1H), 4.56 (m, 1H), 4.39 (m, 1H), 4.10-4.04 (m, 2H), 3.67-3.57 (m, 2H), 3.43-3.39 (m, 2H), 3.12 (s, 1H), 2.76 (bs, 1H), 2.64 (m 1H), 2.56 (m, 1H), 2.45-2.40 (m, 2H), 2.07-2.02 (m, 2H), 1.74 (t, *J* = 7.8 Hz, 2H), 1.02 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{21}H_{27}N_8O_2$ [M + H]$^+$, 423.2251; found, 423.2247.



43 **49**

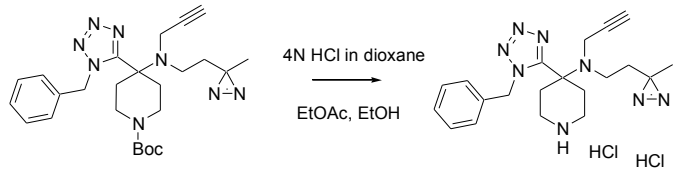
[5-(1-{[2-(3-Methyl-3*H*-diaziren-3-yl)ethyl](2-propyn-1-yl)amino}ethyl)-1*H*-tetrazol-1-yl]acetic acid (49). A solution of **43** (24 mg, 0.078 mmol) in THF (0.1 mL) and MeOH (0.4 mL) was treated with 2N NaOH (0.1 mL), and the reaction mixture was stirred for 3 h at room temperature. The mixture was poured

into ice cold 2N HCl and extracted with EtOAc twice. The organic layer was washed with H_2O and brine, dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure. The residue was resdissolved in EtOAc and passed through a pad of silica gel to give the title compound as a colorless oil (19 mg, 84%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (bs, 1H), 5.64 (d, *J* = 17.8 Hz, 1H), 5.43 (d, *J* =17.8 Hz, 1H), 4.63 (q, *J* = 6.7 Hz, 1H), 3.40 (d, *J* = 2.4 Hz, 2H), 2.63 (m, 1H), 2.49 (m, 1H), 2.42 (t, *J* = 2.4 Hz, 1H), 1.62 (d, *J* = 6.8 Hz, 3H), 1.56 (m, 1H), 1.52 (m, 1H), 0.88 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{12}H_{18}N_7O_2$ $[M + H]^+$, 292.1516; found, 292.1520.



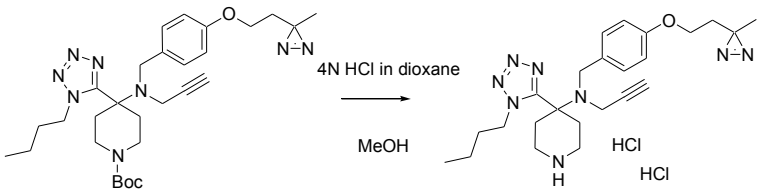
49 **51**

***N,N*-diethyl-2-[5-(1-{[2-(3-methyl-3*H*-diaziren-3-yl)ethyl](2-propyn-1-yl)amino}ethyl)-1*H*-tetrazol-1-yl]acetamide (51).** To a stirred solution of **49** (15 mg, 0.052 mmol), Et_2NH (11 µL, 0.10 mmol) and HOAt (3.5 mg, 0.026 mmol) in CH_2Cl_2 (0.5 mL) was added EDC•HCl (25 mg, 0.13 mmol) at room temperature under N_2. After being stirred at room temperature for 10 h, the reaction mixture was cooled and then diluted with EtOAc, washed with H_2O, sat. aq. $NaHCO_3$, brine, and dried over anhydrous Na_2SO_4. The organic layer was evaporated and the resulting residue was purified by column chromatography on silica gel (25–50% EtOAc/hexanes) to give the title compound as a colorless oil (11 mg, 61%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.70 (d, *J* = 16.4 Hz, 1H), 5.55 (d, *J* = 16.4 Hz, 1H), 4.59 (m, 1H), 3.50-3.43 (m, 4H), 3.23 (d, *J* = 2.4 Hz, 2H), 2.55 (m, 1H), 2.38 (m, 1H), 2.20 (t, *J* = 2.4 Hz, 1H), 1.59 (d, *J* = 6.8 Hz, 3H), 1.55 (m, 1H), 1.34 (t, *J* = 7.2 Hz, 3H), 1.51 (m, 1H), 1.16 (t, *J* = 7.2 Hz, 3H), 0.79 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{16}H_{27}N_8O$ $[M + H]^+$, 347.2302; found, 347.2310.



46 **50**

4-(1-Benzyl-1*H*-tetrazol-5-yl)-*N*-[2-(3-methyl-3*H*-diaziren-3-yl)ethyl]-*N*-(2-propyn-1-yl)-4-piperidinamine dihydrochloride (50). To a stirred solution of **46** (40 mg, 0.084 mmol) in EtOH (0.4 mL) and EtOAc (0.2 mL) was added 4N HCl in 1,4-dioxane (0.20 mL, 0.80 mmol) at 0 °C under N_2. After being stirred for 1 h at room temperature, the reaction mixture was concentrated and triturated with hexanes and EtOAc. The precipitate was collected by filtration to give the title compound as a white powder (31 mg, dihydrochloric acid salt, 81%): ^{1}H NMR (400 MHz, CD_3OD) δ 7.48-7.38 (m, 3H), 7.27-7.24 (m, 2H), 6.02 (s, 2H), 3.46 (d, *J* = 2.4 Hz, 2H), 3.35-3.28 (m, 2H), 3.05-2.96 (m, 2H), 2.81 (t, *J* = 2.4 Hz, 1H), 2.57 (t, *J* = 7.6 Hz, 2H), 2.51-2.48 (m, 4H), 1.48 (t, *J* = 7.2 Hz, 2H), 0.95 (s, 3H); HRMS (ESI-TOF) calc'd for $C_{20}H_{27}N_8$ $[M + H]^+$, 379.2353; found, 379.2365.



42 **52**

4-(1-Butyl-1*H*-tetrazol-5-yl)-*N*-{4-[2-(3-methyl-3*H*-diaziren-3-yl)ethoxy]benzyl}-*N*-(2-propyn-1-yl)-4-piperidinamine dihydrochloride (52). To a stirred solution of **42** (10 mg, 0.018 mmol) in MeOH (0.3 mL) was added 4N HCl in 1,4-dioxane (0.10 mL, 0.40 mmol) at 0 °C under N_2. After being stirred for 1 h at room temperature, the reaction mixture was concentrated and triturated with hexanes and EtOAc. The precipitate was collected by filtration to give the title compound as white powder (5.5 mg, dihydrochloric acid salt, 58%): ^{1}H NMR (600 MHz, CD_3OD) δ 7.18 (d, *J* = 8.5 Hz, 2H), 6.91 (d, *J* = 8.5 Hz, 2H), 4.63 (t, *J* = 7.8 Hz, 2H), 3.91 (t, *J* = 6.0 Hz, 2H), 3.82 (s, 2H), 3.55-3.51 (m, 2H), 3.34 (s, 2H), 3.18-3.14 (m, 2H), 2.82 (t, *J* = 2.3 Hz, 1H), 2.81-2.76 (m, 2H), 2.69-2.65 (m, 2H), 2.02-1.98 (m, 2H), 1.81 (t, *J* = 6.0 Hz, 2H), 1.58-1.55 (m, 2H), 1.11 (s, 3H), 1.03 (t, *J* = 7.4 Hz, 3H); HRMS (ESI-TOF) calc'd for $C_{24}H_{35}N_8O$ $[M + H]^+$, 451.2928; found, 451.2927.

Cell culture and *in situ* labeling

PC3 cells were grown at 37 °C under a humidified 5% CO_2 atmostphere, in a culture medium consisting of RPMI (Cellgro) supplemented with 10% FBS (Gemini), penicillin, streptomycin and glutamine (PSQ; Cellgro). For SILAC experiments, the culture medium was replaced with SILAC RPMI (Thermo) supplemented instead with 10% dialyzed FBS, PSQ and 100 μg/ml [$^{13}C_6$,$^{15}N_4$]-L-arginine•HCl and [$^{13}C_6$,$^{15}N_2$]-L-lysine•HCl (Sigma-Aldrich). Cells were passaged at least six times in isotope-containing medium before being used for analysis by liquid chromatography–tandem mass spectrometry (LC-MS/MS). All probe stock solutions were made at 10 mM in DMSO .

Photocrosslinking and gel-based analysis

For *in situ* labeling, PC3 cells were plated in 6 cm dishes and grown to ~100% confluency, washed with PBS, and treated with 3-10 μM compound (in 2 mL serum free media). After 30 min at 37 ºC, cells were placed at 4 ºC and subsequently irradiated with 365 nm light for 10 min at 4 ºC. Cells were then harvested by washing 2x with cold PBS (pH 7.5) and then scraped into cold PBS. Cell pellets were isolated by centrifugation (1400 x g, 3 min), resuspended in PBS, lysed by probe sonication and centrifuged (100,000 x g, 45 min) to provide the soluble (supernatant) and membrane (pellet) fractions. Protein concentrations were determined using the BCA protein assay (Bio-Rad) using a microplate reader. For conjugation to rhodamine azide, 50 μg of lysate was treated 6 μL of a pre-mixed solution: 3μL of 1.7 mM Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine in 4:1 DMSO:*t*-BuOH, 1.0 μL of 50 mM $CuSO_4$ in H_2O, 1.0 μL of 50 mM tris(2-carboxyethyl)phosphine hydrochloride (TCEP) in PBS, 1.0 μL of 1.25 mM rhodamine-azide in DMSO. After 1 h at room temperature, samples were mixed with 4X SDS loading buffer and 25 μL of the click reaction mixture was loaded into each gel lane and resolved using SDS-PAGE. Imaging was achieved using in-gel fluorescent scanning on a Hitachi FMBIO-II flatbed fluorescence scanner.

Photocrosslinking and protein profiling using mass spectrometry

Using a modified protocol from previous work,[1] we performed quantitative proteomics experiments using SILAC (stable isotope labeling by amino acids in cell culture) methods as follows. PC3 cells were passaged six times in RPMI minus L-Lysine and L-Arginine (Thermo) supplemented with 10% dialyzed FBS (Gemini) and 100 mg/L [$^{13}C_6$,$^{15}N_4$] L-Arginine•HCl and 100 mg/L [$^{13}C_6$,^{15}N] L-Lysine•HCl (Aldrich) or L-Arginine•HCl and L-Lysine•HCl (Sigma) and cell aliquots were frozen and replaced periodically. Heavy and light PC3 cells were plated in 10 cm dishes and grown to 100% confluency, washed with PBS, and treated with 10 μM compound (in 4 mL serum free media). For probe-probe comparison experiments, heavy cells were treated with test probe (10 μM) and light cells were treated with probe **3** (10 μM). For competition experiments, heavy cells were treated with test probe (10 μM) and alkane/alkene competitor (20 μM), and light cells with test probe (10 μM) and DMSO. For UV light control experiments, heavy and light cells were treated with 10 μM of test probe and only heavy cells were irradiated with UV light. For control experiments, heavy and light cells were both treated with test probe (10 μM). Cells were incubated

with compound(s) at 37 ºC for 30 min, irradiated with 365 nm light for 10 min at 4 ºC, harvested, lysed, and centrifuged (100,000 x g, 45 min) as described above to provide the soluble and membrane fractions. Heavy and light proteomes were combined in equal amounts (1.0 mg of proteome each) and diluted with DPBS to a final volume of 1.0 mL. Click chemistry reagents were added to achieve the following final concentrations: 1.0 mM $CuSO_4$, 100 μM TBTA, 1.0 mM TCEP, and 100 μM biotin-azide. After mixing for 1 h at room temperature, MeOH (2.0 mL), $CHCl_3$ (0.5 mL) and DPBS (1.0 mL) were added to the reaction mixture and the cloudy mixture was centrifuged at 5000x rpm for 15 min yielding a precipitated protein disc between the aqueous and organic layers. The top and bottom liquid phases were aspirated, leaving the protein precipitate. The protein disc was then washed with MeOH:$CHCl_3$ (3 x 2mL, 1:1). MeOH (2.0 mL) was then added and the solution was sonicated to yield a cloudy mixture. $CHCl_3$ (0.5 mL) was added and the solution was centrifuged (5000 rpm, 10 min), aspirated, and the pellet was solubilized in urea (450 μL, 6 M in DPBS). Membrane fractions were supplemented with 1% SDS. The solution was treated with TCEP (10 mM final concentration, neutralized to pH 7) for 30 minutes at 37°C, followed by iodoacetamide (20 mM final concentration) for 30 minutes at room temperature. The sample was diluted 10-fold in PBS and SDS (10%) was added to achieve a final concentration of 0.2%. Streptavidin beads (Thermo) (100 μL slurry) were added and the mixture was rotated for 2 hours at room temperature. Beads were washed with 0.5% SDS in DPBS (3 x 10 mL) and then DPBS (3 x 10 mL) before transferring to screw-top eppendorf tubes and centrifuged (1000 rpm, 2 min) into a pellet. Following aspiration of the supernatant, the beads were resuspended in urea (2.0 M in DPBS) supplemented with $CaCl_2$ (1.0 mM final concentration) and sequence grade porcine trypsin (Promega). Following overnight digestion at 37°C, the tryptic digest solution (supernatant) was removed from the beads following centrifugation (1000 rpm, 2 min) and acidified with 5% formic acid. The tryptic digests were stored at –20 ºC until analyzed by LC/LC-MS/MS.

Mass spectrometry and data analysis

Mass spectrometry was performed using a Thermo Orbitrap Velos mass spectrometer, using a previously described protocol[2,3]. Peptides were eluted using a five-step multidimensional LC-MS (MudPIT[4]) protocol (using 0%, 25%, 50%, 80% and 100% salt bumps of 500 mM aqueous ammonium acetate, followed by an increasing gradient of aqueous acetonitrile and 0.1% formic acid in each step), and data were collected in data-dependent acquisition mode (two MS1 microscans (400–1800 mass to charge ratio (m/z)) and 30 data-dependent fragmentation (MS2) scans) with dynamic exclusion enabled (repeat count of 1, exclusion duration of 20 s) with monoisotopic precursor selection enabled. All other parameters were left at default values. Prolucid searches allowed for variable oxidation of methionine (+15.9949 m/z), static modification of cysteine residues (+57.0215 m/z; iodoacetamide alkylation) and accepted only half or fully tryptic peptides. Each data set was independently searched with light and heavy parameter files; for the light search, all other amino acids were left at default masses; for the heavy search, static modifications on lysine (+8.0142 m/z) and arginine (+10.0082 m/z) were specified. The precursor-ion mass tolerance was set to 50 ppm and the fragment-ion mass tolerance was the default assignment of 0. The data were searched using a

human reverse-concatenated nonredundant (gene-centric) FASTA database that assembled from the Uniprot database (www.uniprot.org). The resulting matched MS2 spectra were assembled into protein identifications, then filtered using DTASelect (version 2.0.47), and only half-tryptic or fully tryptic peptides were accepted for identification, and only fully-tryptic peptides were considered for quantification. Peptides were restricted to a specified false positive rate of 1%. Redundant peptide identifications common between multiple proteins were allowed, but the database was restricted to a single consensus splice variant. SILAC ratios were quantified using in-house software as described (CIMAGE[2]). Briefly, extracted MS1 ion chromatograms (± 10 ppm) from both 'light' and 'heavy' target peptide masses (m/z) were generated using a retention time window (± 10 min) centered on the time when the peptide ion was selected for MS/MS fragmentation, and subsequently identified. Next, the ratio of the peak areas under the light and heavy signals (signal-to-noise ratio > 2.5) are calculated. Computational filters used to ensure that the correct peak-pair is used for quantification include a co-elution correlation score filter ($R^2 \geq 0.8$), removing target peptides with bad co-elution profile, and an 'envelope correlation score' filter ($R^2 > 0.8$) that eliminates target peptides whose predicted pattern of the isotopic envelope distribution does not match the experimentally observed high-resolution MS1 spectrum. Also, peptides detected as singletons, where only the heavy or light isotopically labeled peptide was detected and sequenced, but which passed all other filtering parameters, were given a standard ratio of 20, which is the maximum SILAC ratio reported here.

All reported SILAC results from test probe (**8**, **22**, **24**, **26**, **31** and **55**) versus probe **3** experiments represent data combined from two separate biological replicates. The soluble and membrane fractions from each biological replicate were run separately to improve protein coverage. Identified proteins were included in the final quantitative datasets if they exhibited three or more unique and quantified peptides and if they were detected in both biological replicate experiments. Probe targets were defined as proteins that complied with at least one of the two following criteria: 1) the protein exhibited a median test probe:probe **3** (heavy:light) SILAC ratio of > 3 in both replicate experiments; or II) the protein exhibited a mean test probe:probe **3** SILAC ratio of > 3 across both replicates and a standard deviation of the mean of < 67% of the mean ratio value. In addition to the described filters, in the membrane proteome study, proteins also found in the soluble proteome were removed to limit the analysis to *bona fide* membrane-associated proteins. Filtered data sets and probe targets are shown in Table S1. Single quantitative proteomic studies were performed for the competition experiments comparing SILAC signals in test probe (**8**, **22**, **24**, **26** and **31**) versus test probe + 2X non-clickable analogues (**62**, **71**, **60**, **70**, and **64**). Identified proteins were included in the final quantitative datasets if they exhibited three or more unique and quantified peptides. Filtered data sets are shown in Table S2. Representative probe targets were confirmed to show: 1) strong enrichment (heavy:light ratios > 10) in control experiments comparing probe-treated PC3 cells with UV-light exposure (heavy) to probe-treated PC3 cells without UV-light exposure (light); and 2) approximately 1:1 ratios in control experiments where heavy and light-labeled PC3 cells were treated with equal concentrations of the same test probe (evaluated for probe **24** and **31**; **Table S1**).

Recombinant expression and labeling

HEK293T cells were grown to 60–70% confluency in 6-well dishes in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS. cDNAs encoding human PEBP1 (OpenBiosystems Cat. #MHS6278-202826063) and CUTA (OpenBiosystems Cat. #MHS6278-202828255) were purchased and subcloned into pcDNA3.1 (C-terminal Myc-his epitope tag). The vectors containing these genes or empty vector control ("mock") were transfected into the cells using X-tremeGENE HP and the manufactures protocols. After 48 h, cells were labeled *in situ* with 1.0 μM probe according to already described methods (*see above*). SDS-PAGE (4–20% gradient) was also performed on cell lysates which had not been subjected to click chemistry conditions to determine the expression levels for each protein of interest. These gels were analyzed by Western blot using commercial mouse anti-Myc (CALIBIOCHEM, cat#OP10) and mouse anti-GAPDH antibodies (6C5, Santa Cruz Biotechnology, sc-32233).

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NMR spectra

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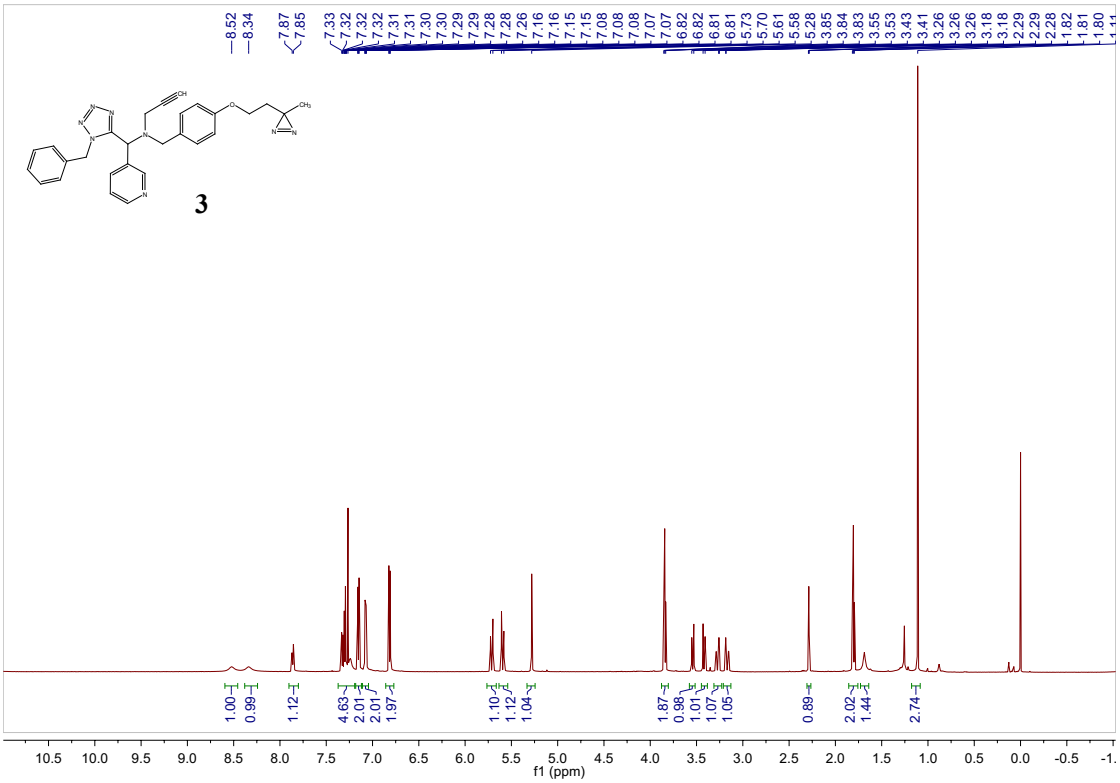

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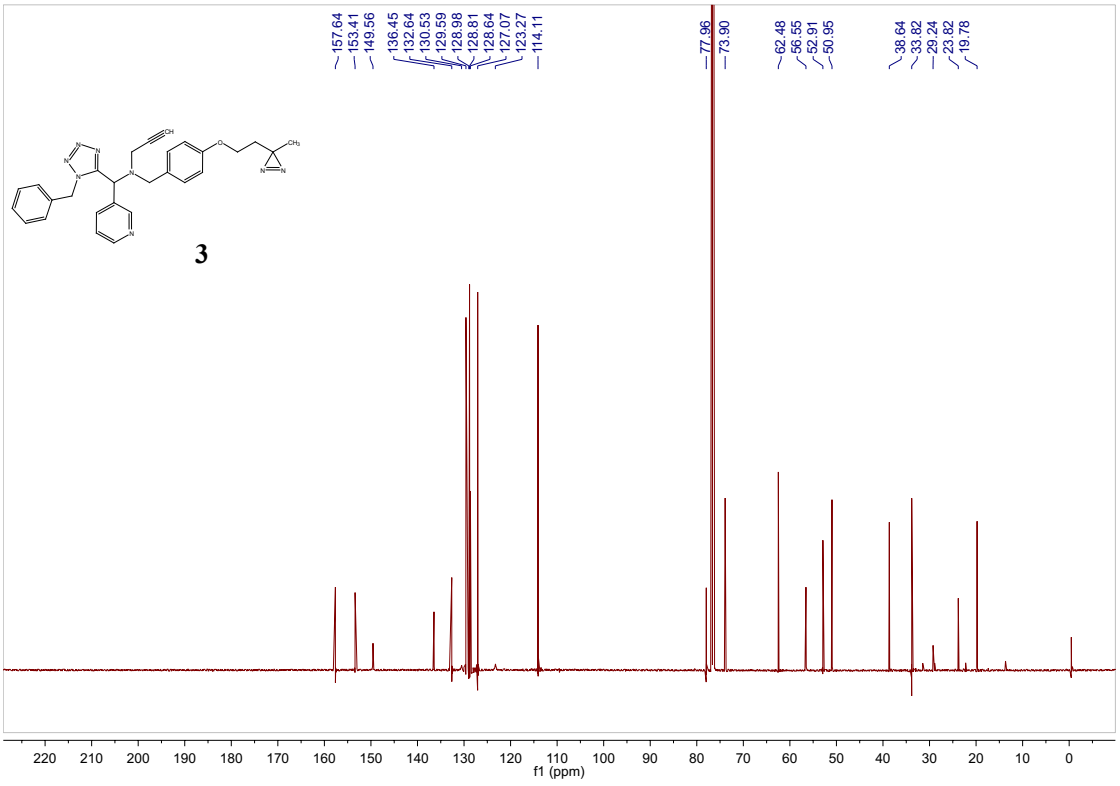

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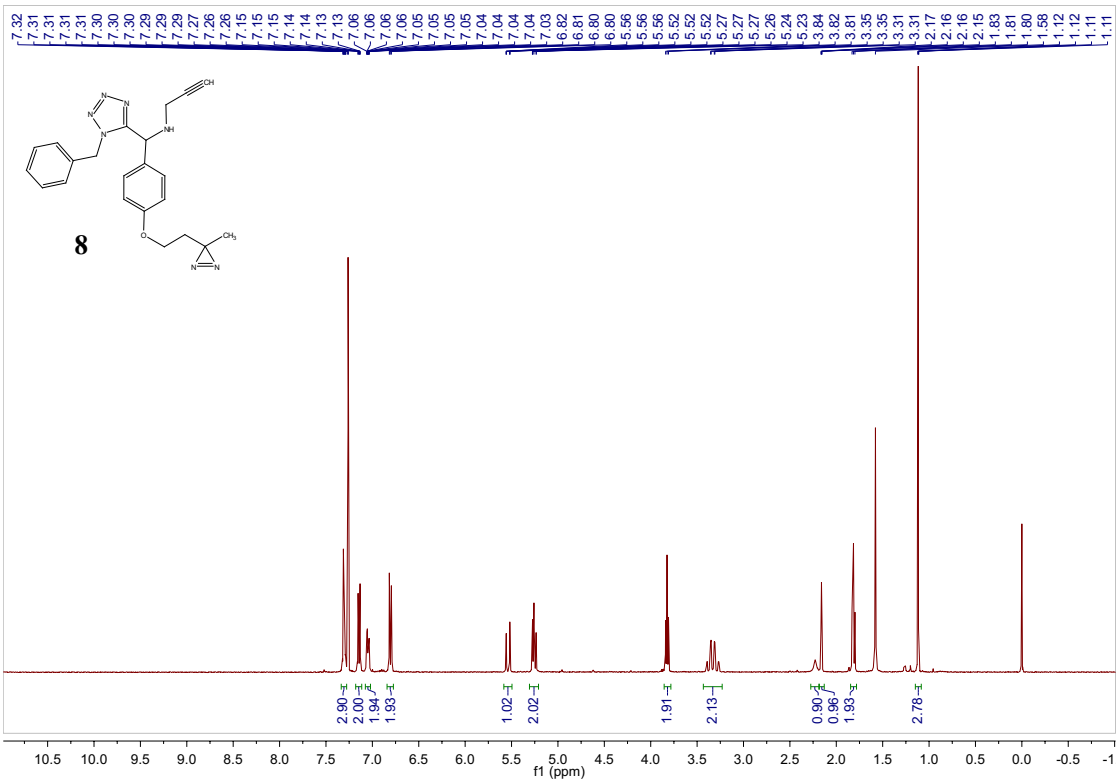

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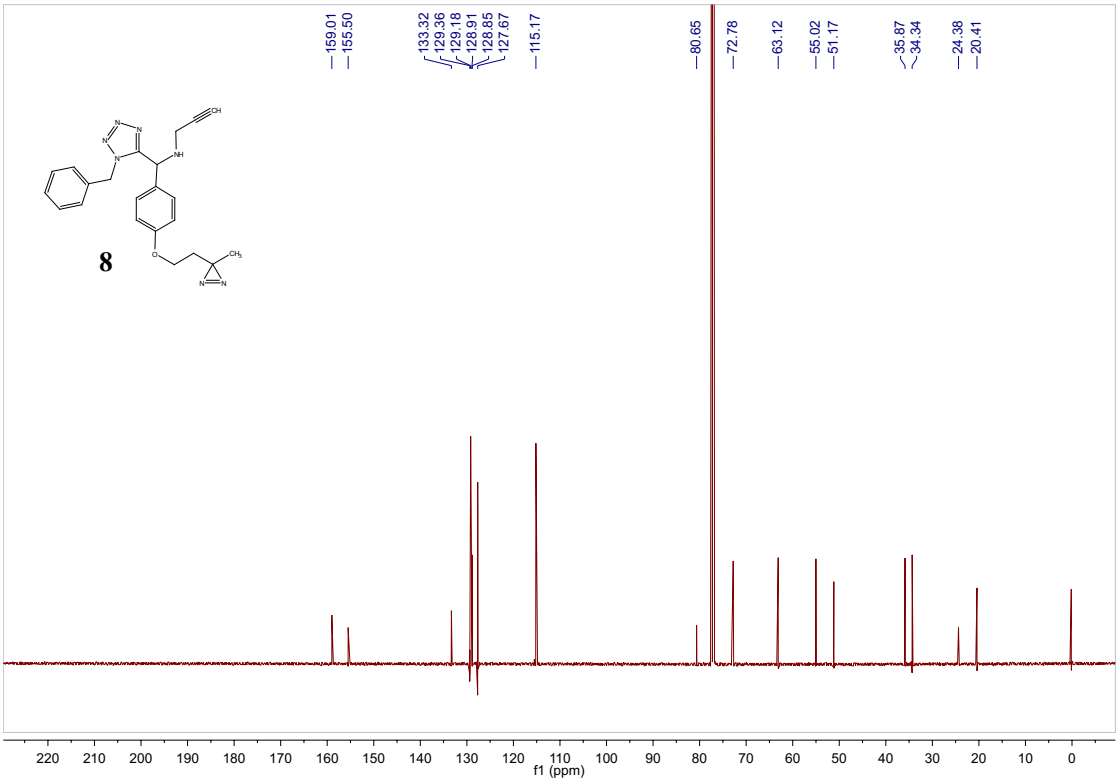

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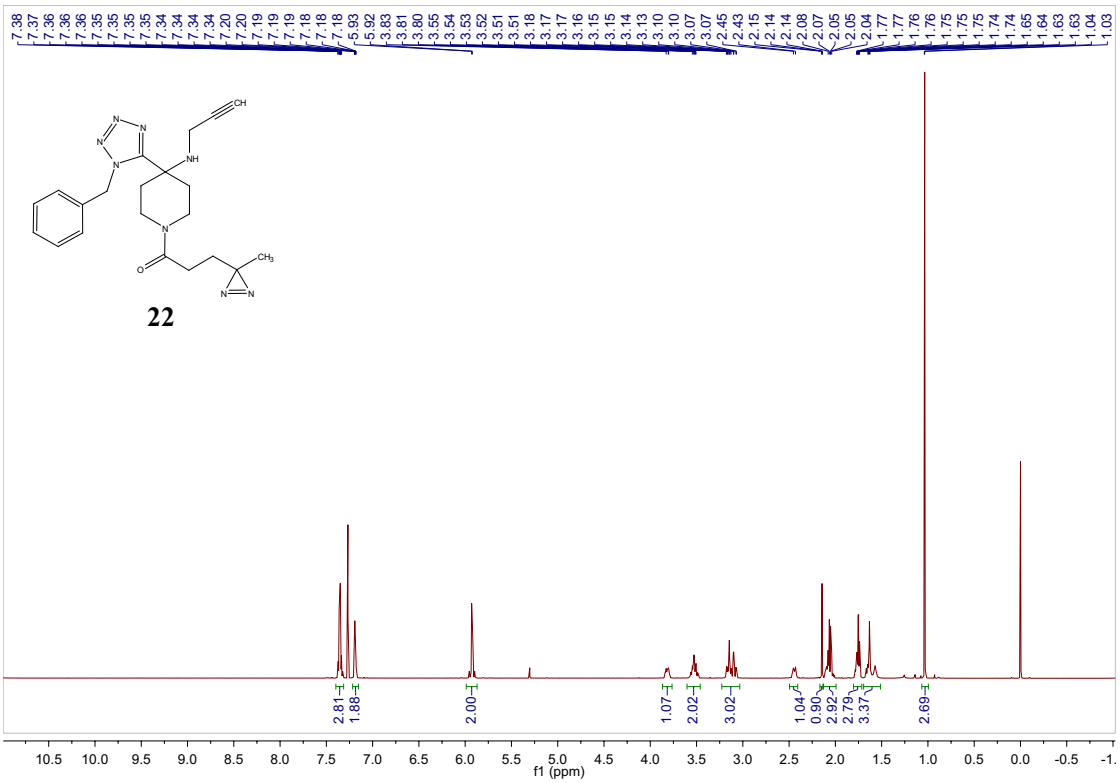

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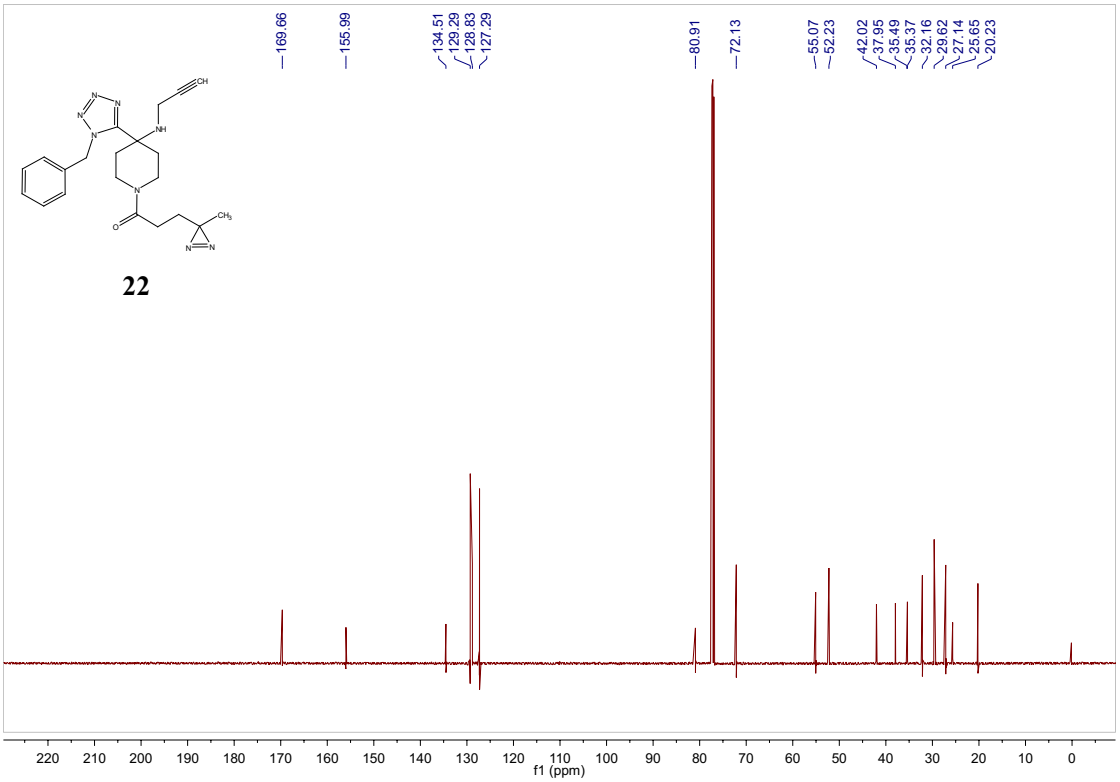

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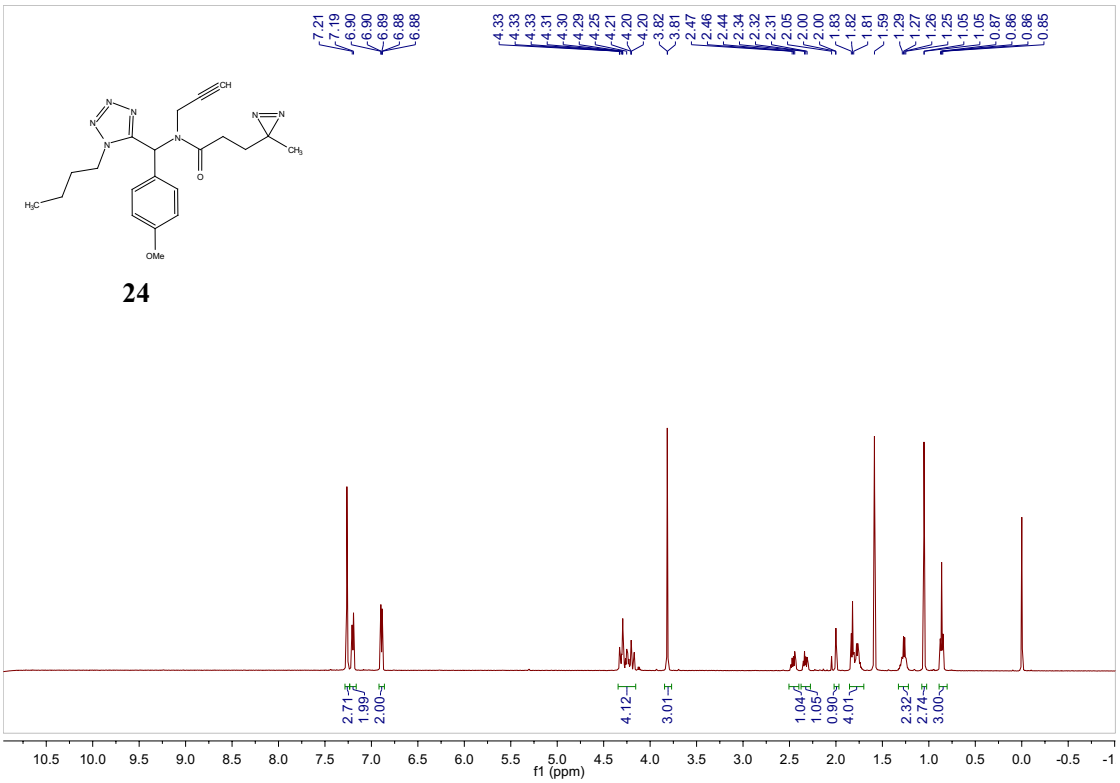

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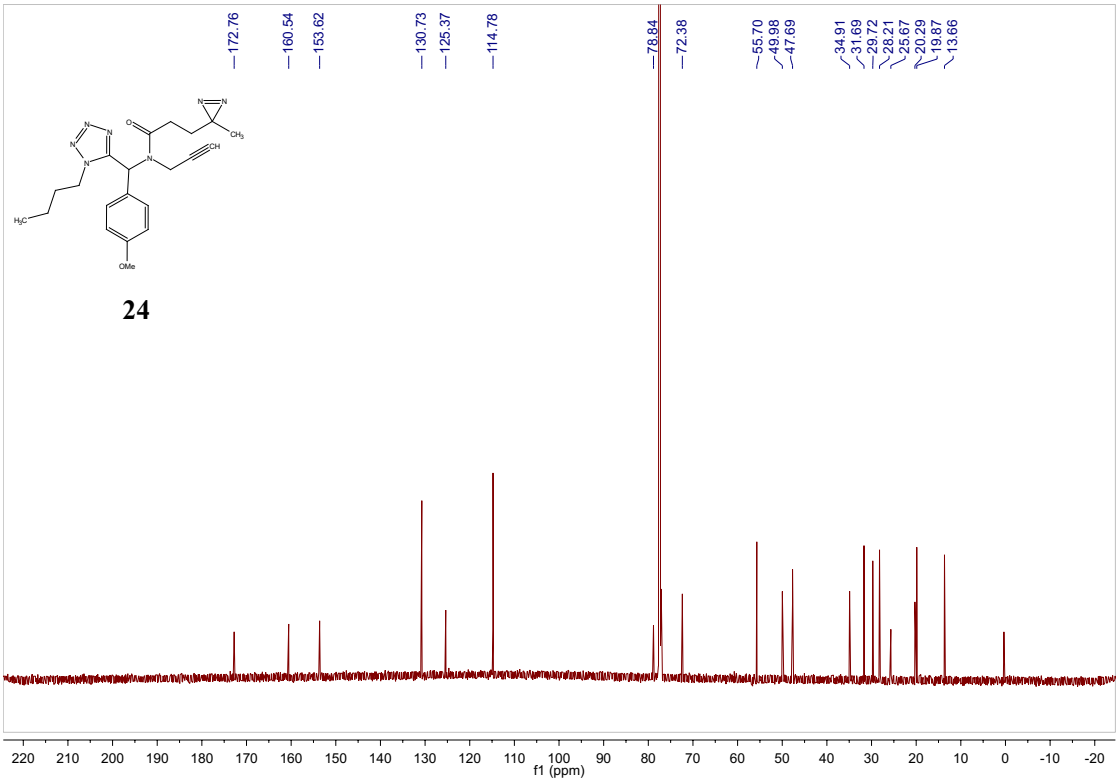

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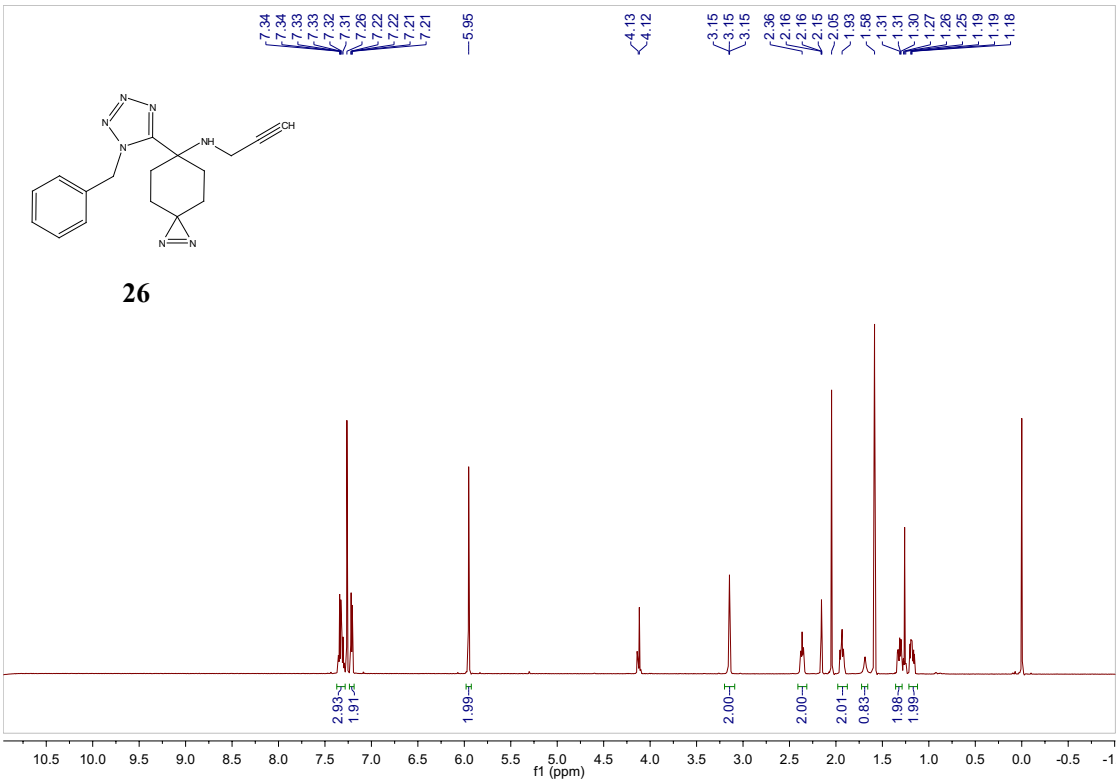

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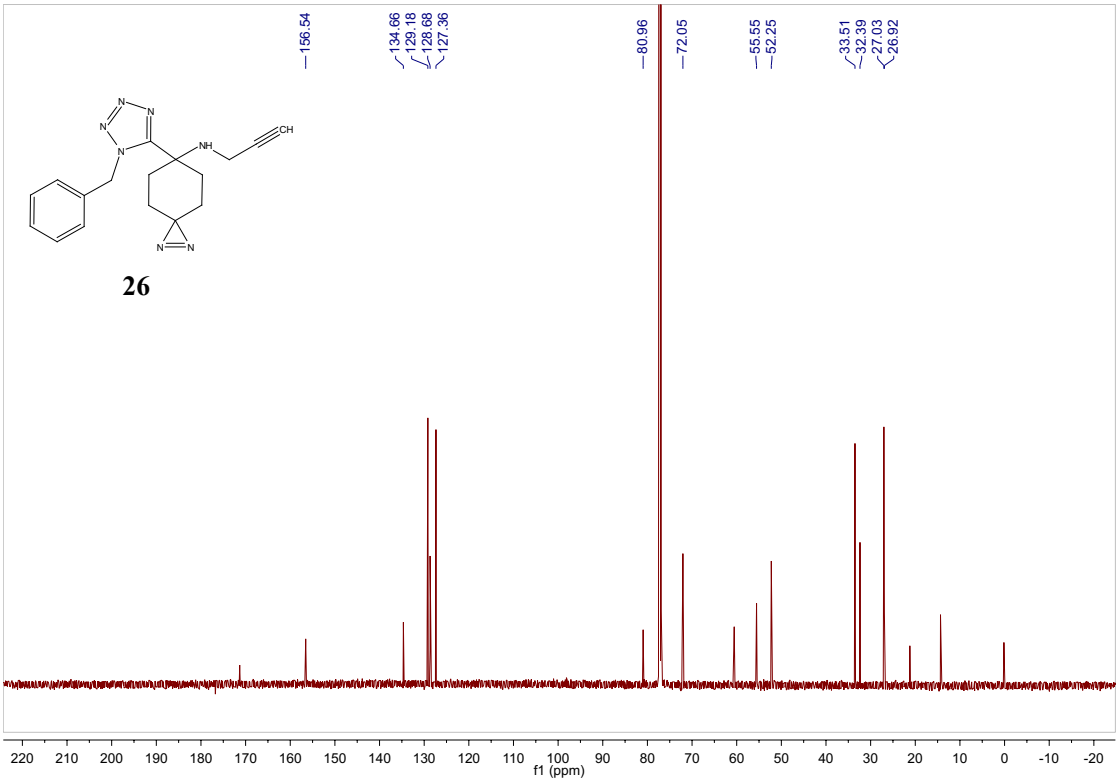

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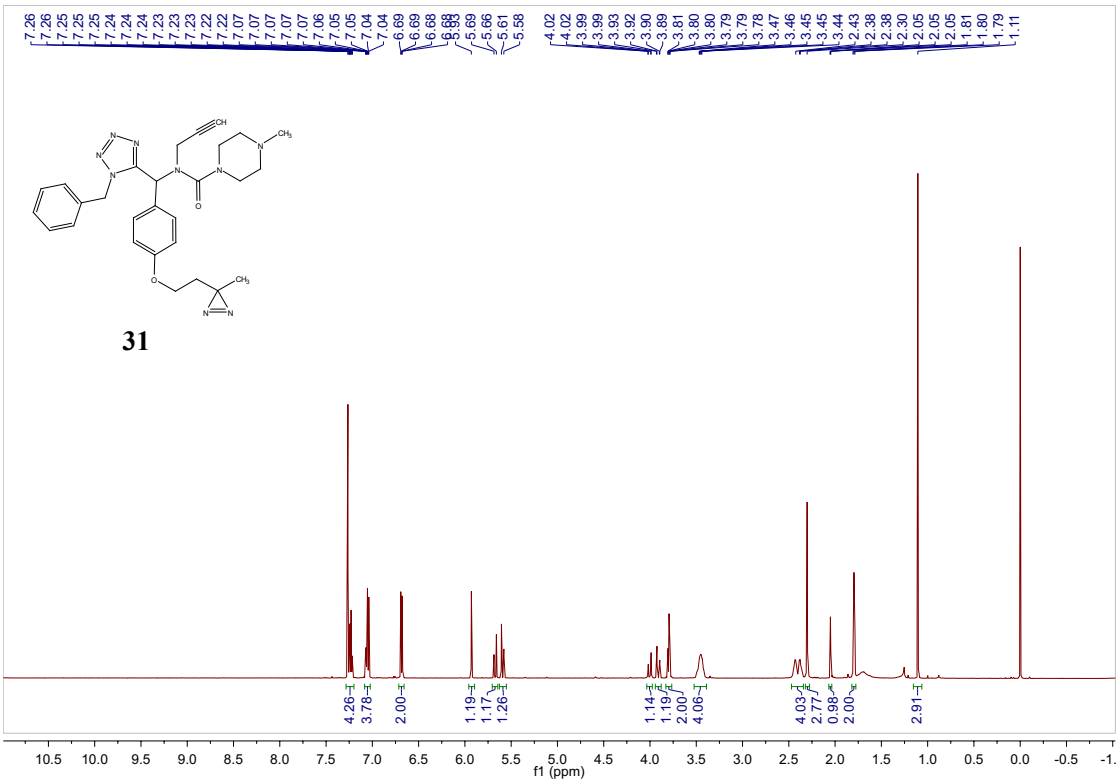

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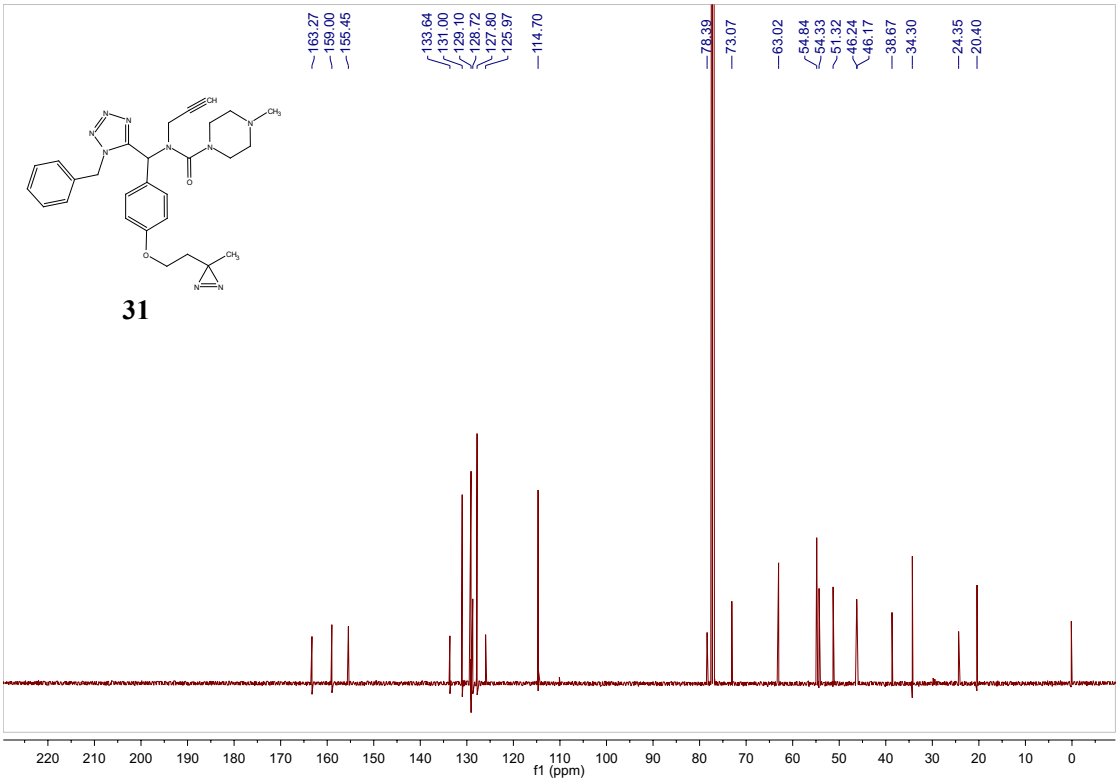

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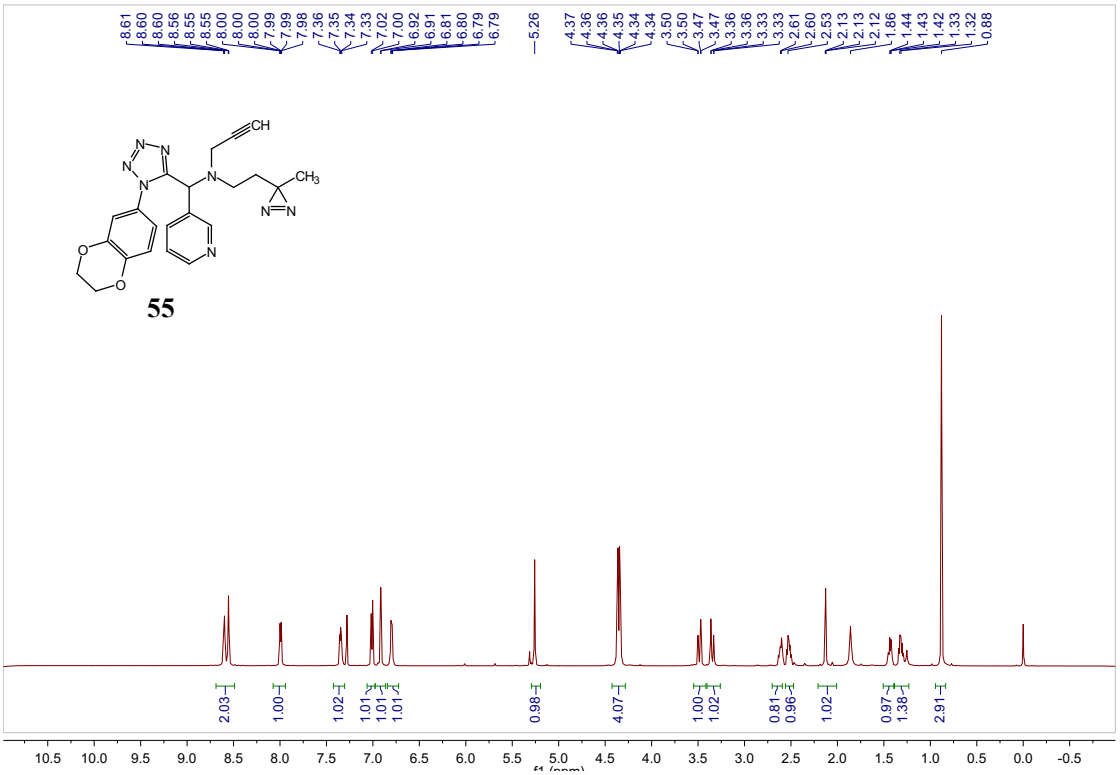

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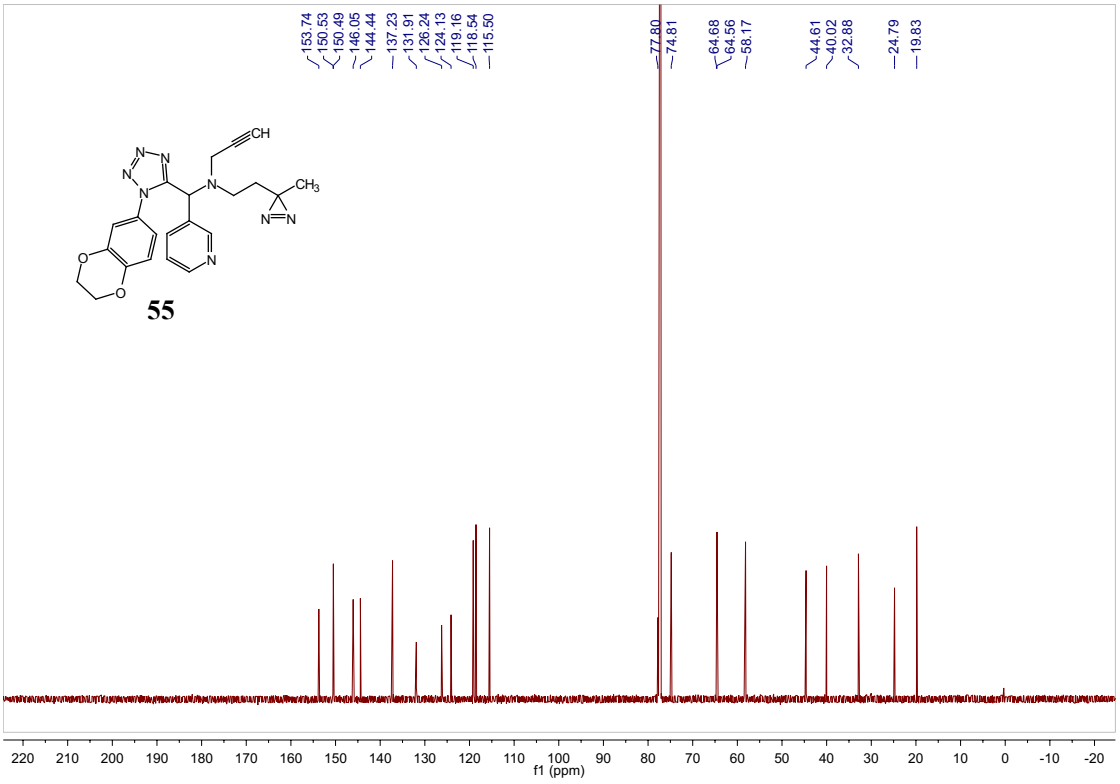

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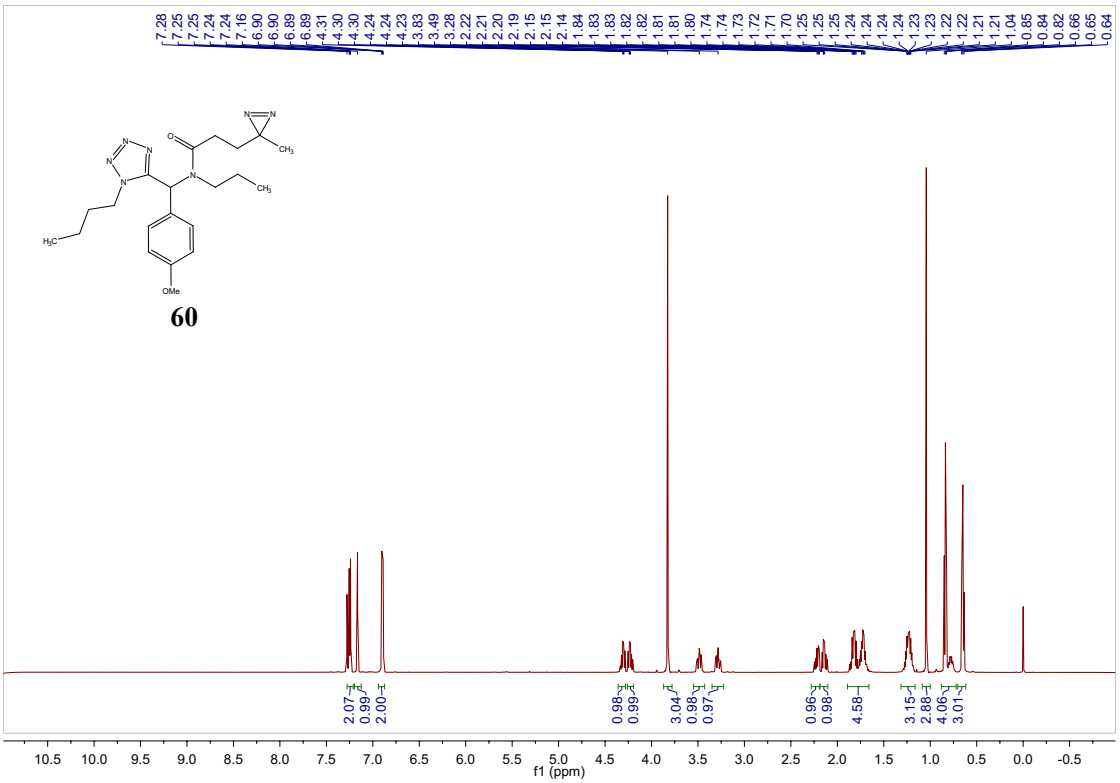

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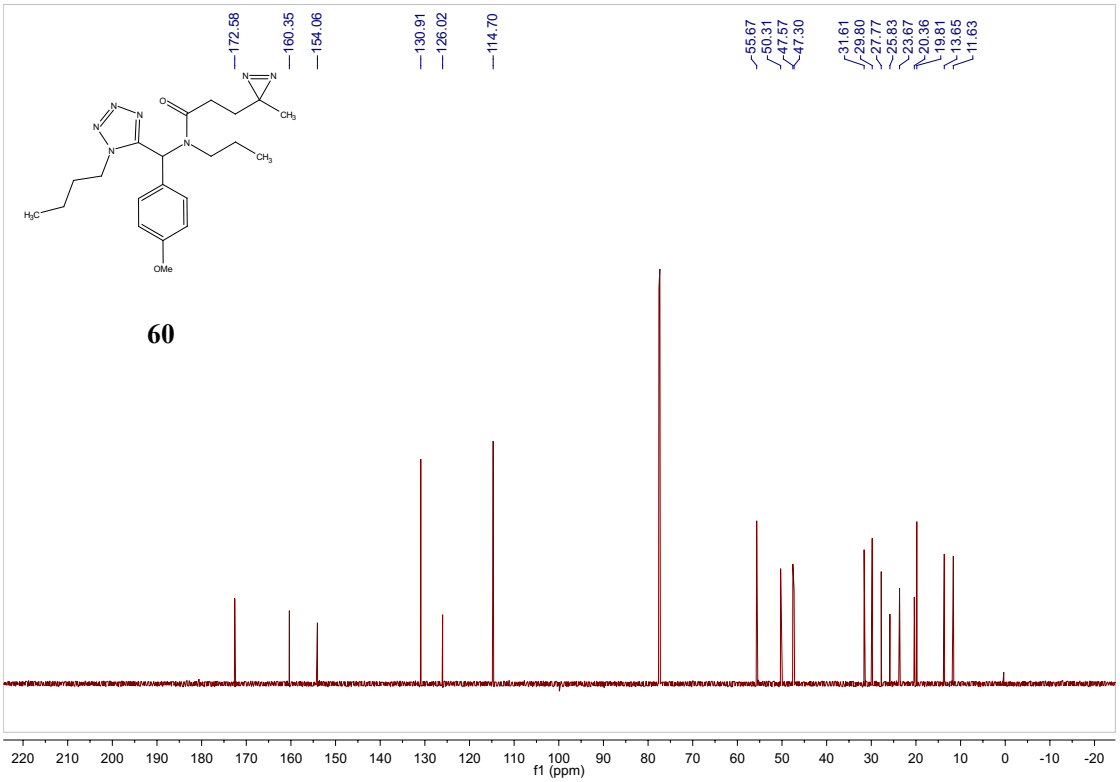

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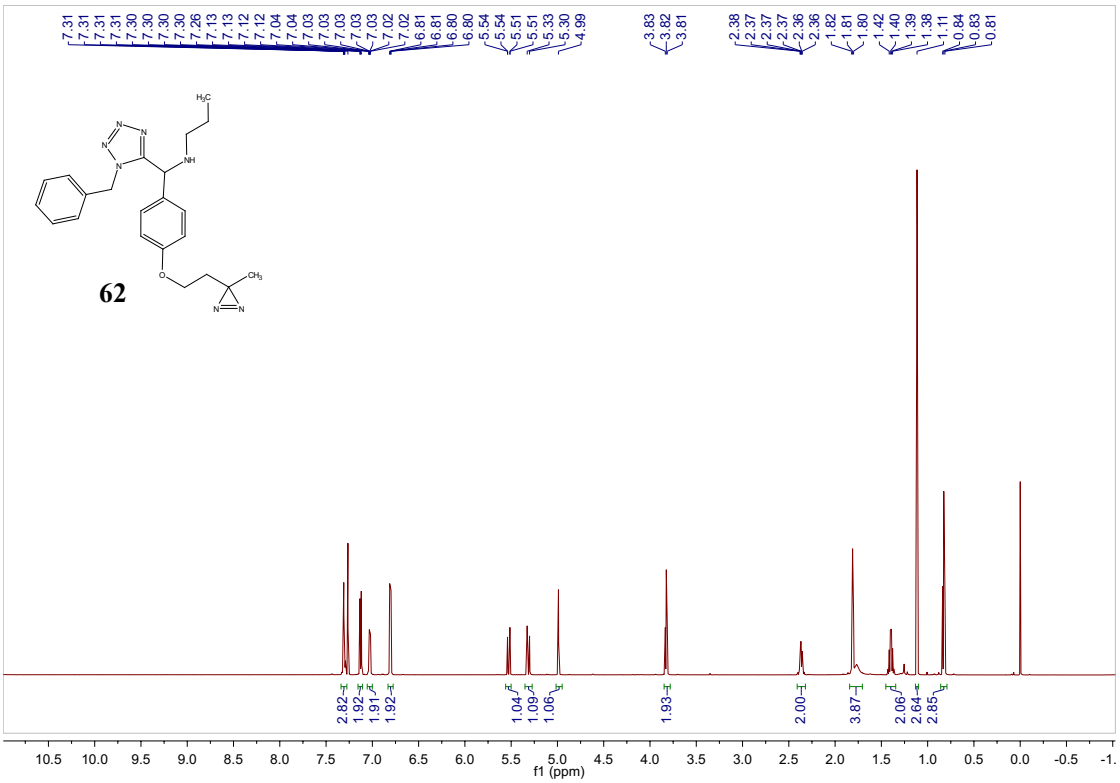

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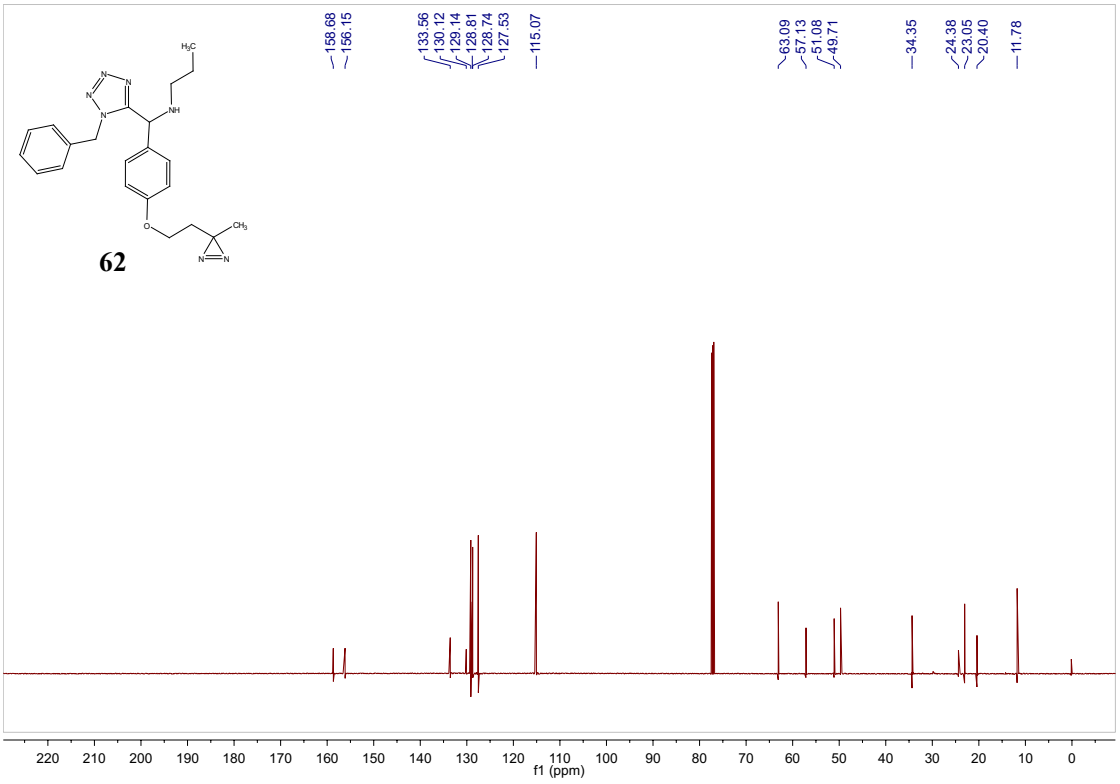

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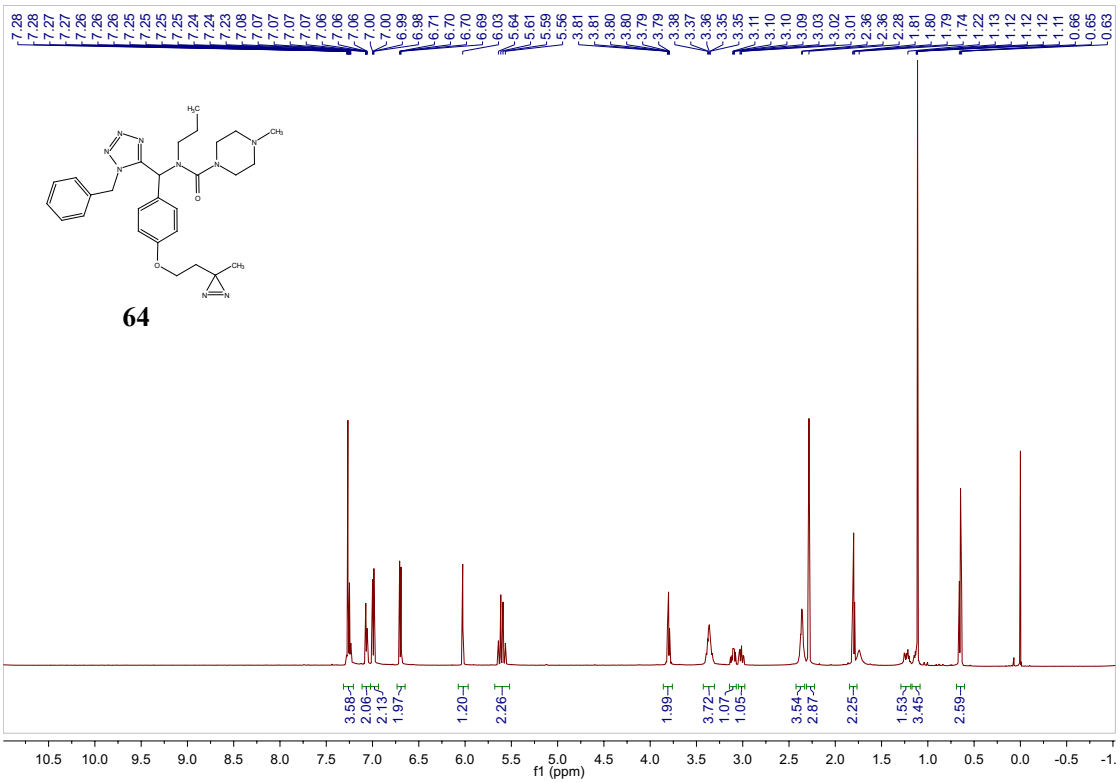

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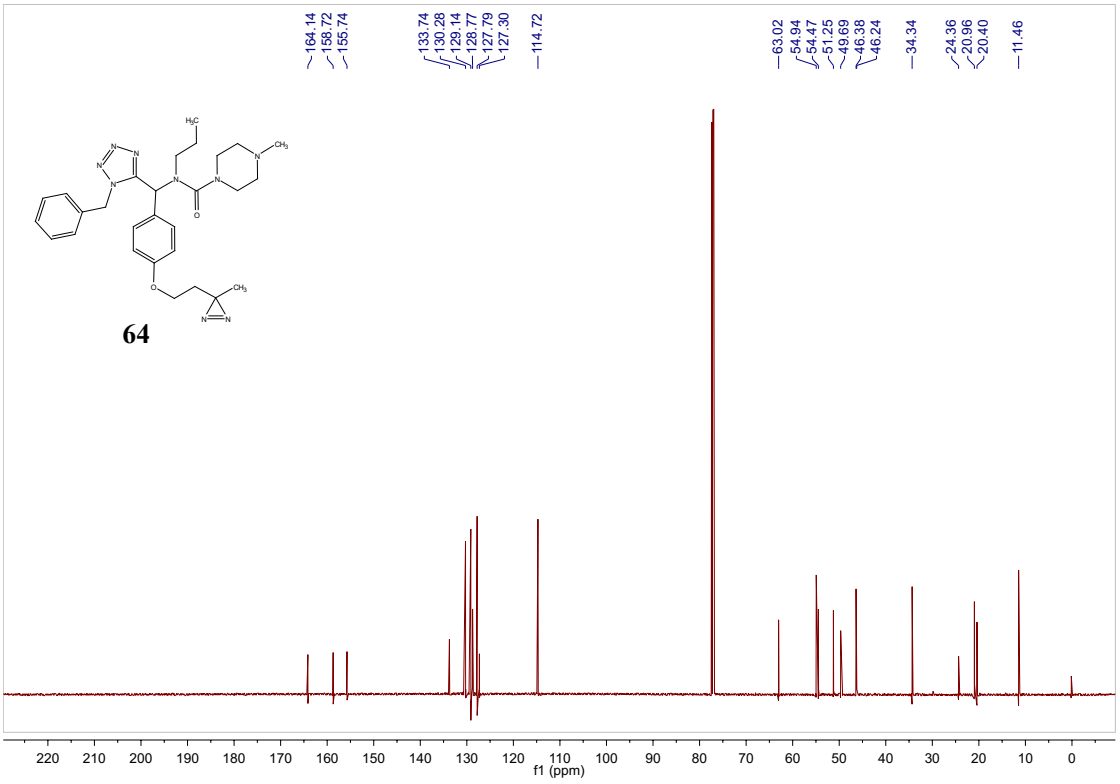

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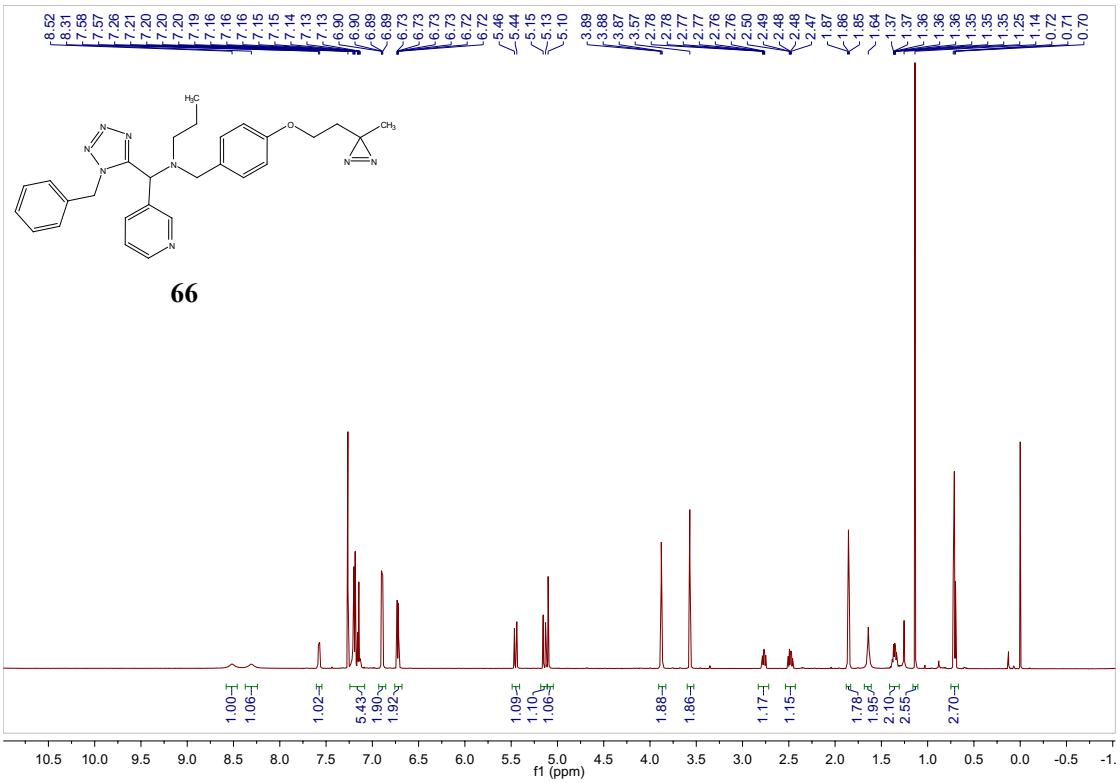

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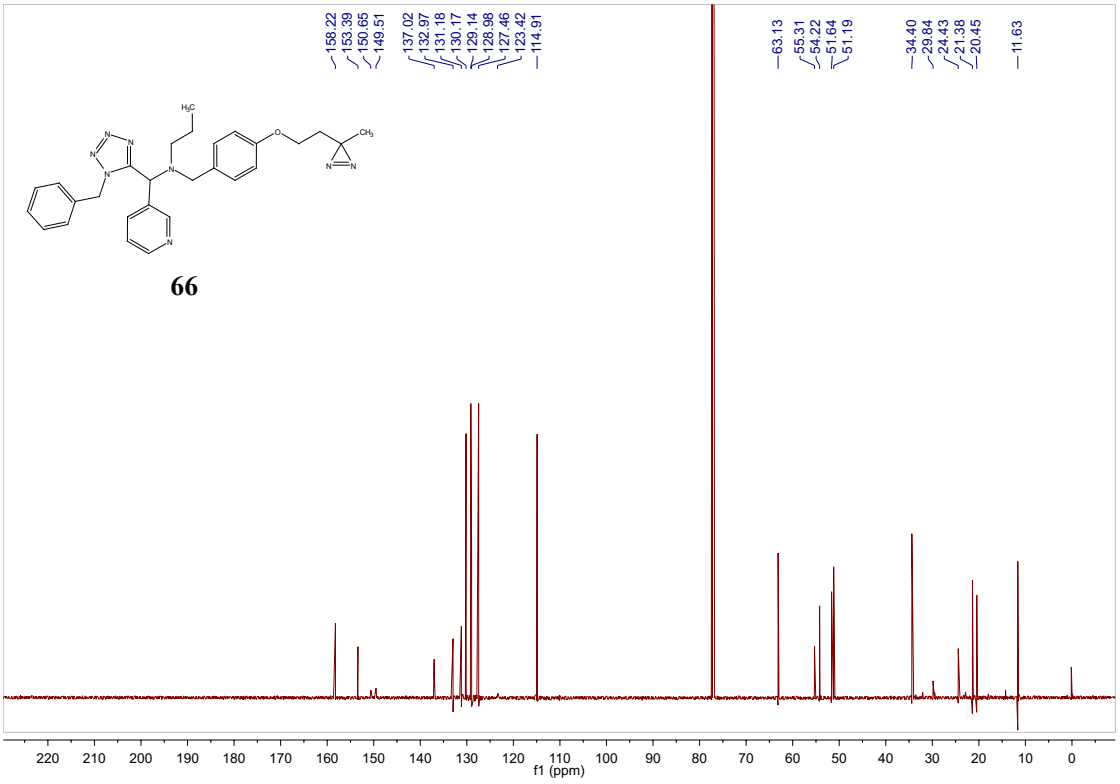

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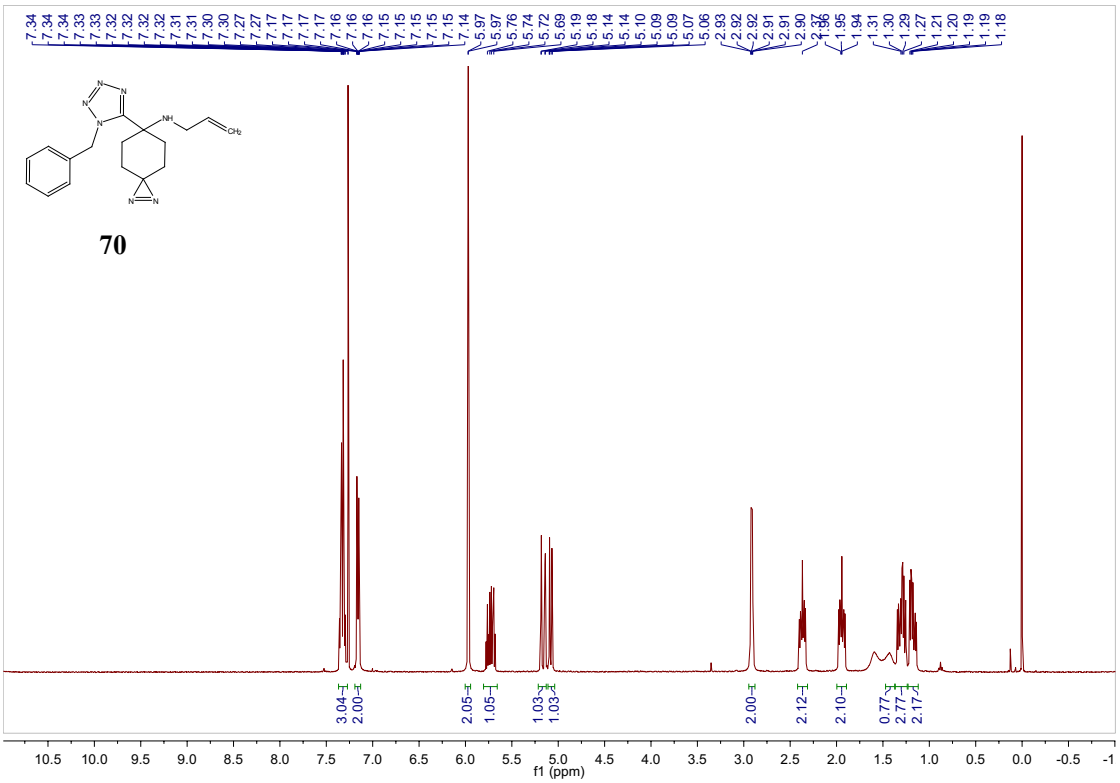

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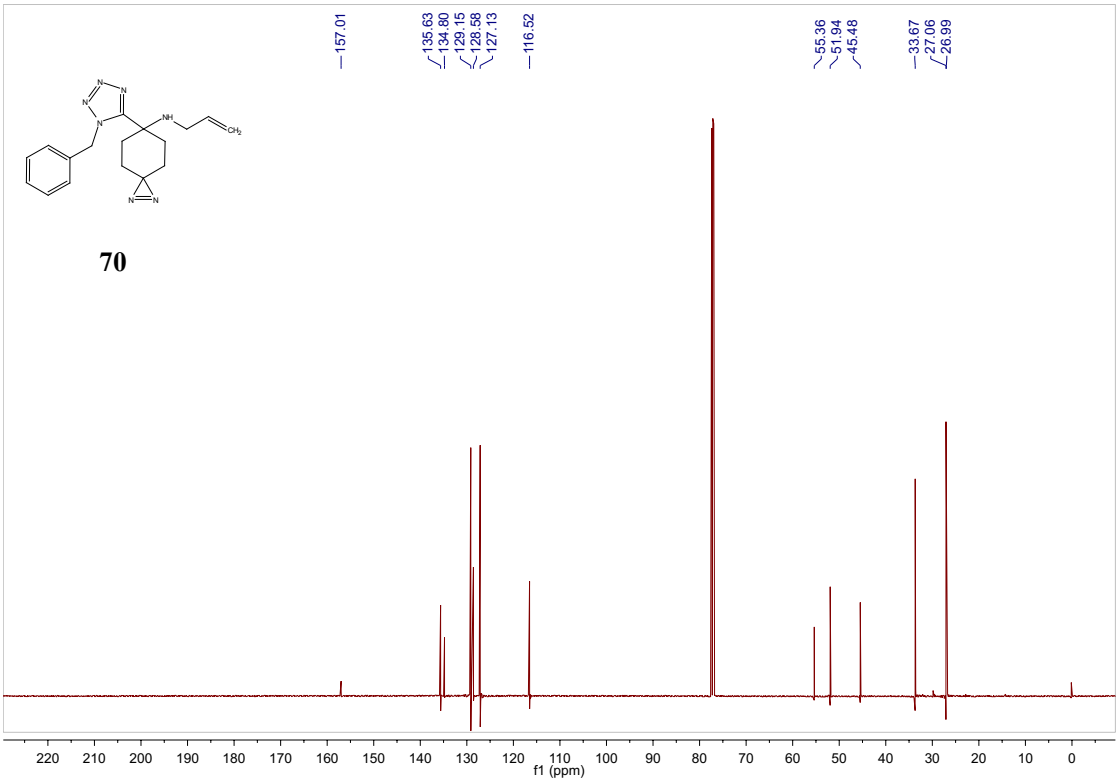

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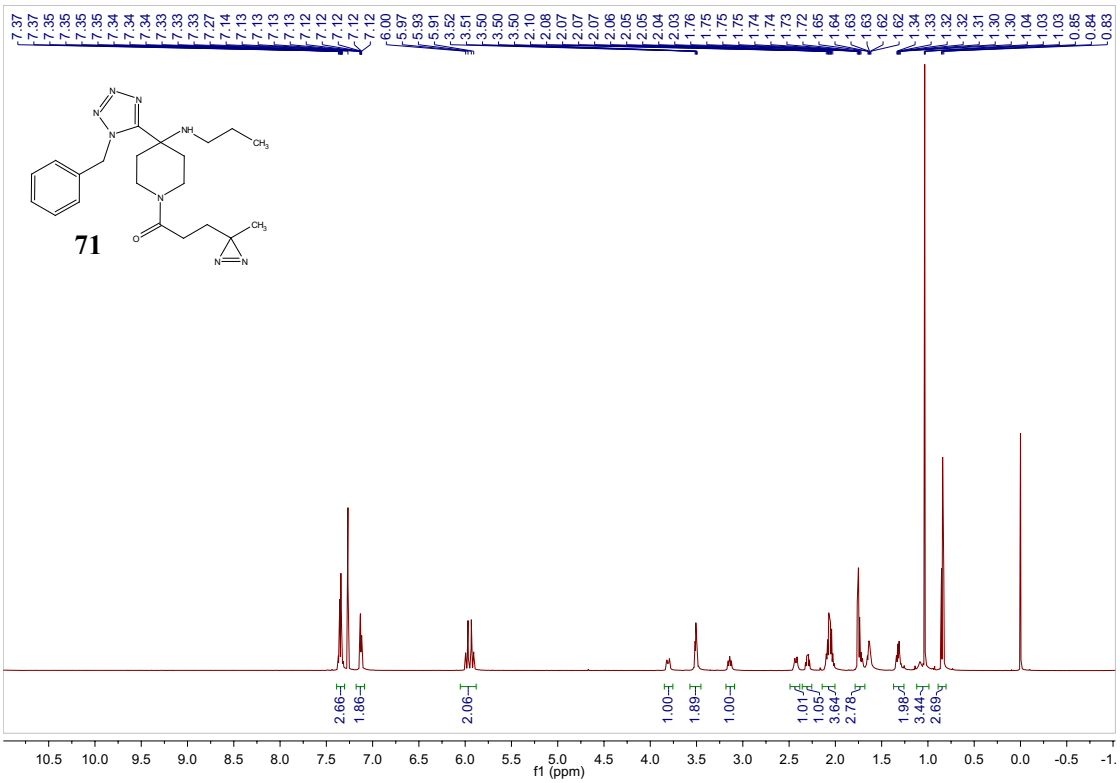

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