Electronic Supplementary Material (ESI) for Chemical Science.


# 3-Bromotetrazine: Labelling of Macromolecules via Monosubstituted Bifunctional *s*-Tetrazines

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## Experimental Procedures

### General Information, Materials and Equipment

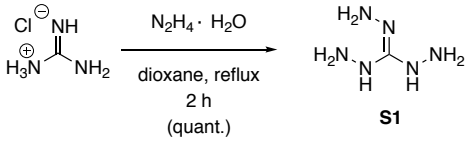
All chemicals were purchased from Sigma-Aldrich, Acros, Alfa Aesar or Fluka and were used without further purification. All reactions were carried out in flame dried glassware under a nitrogen atmosphere. Solvents applied for chemical transformations were either puriss. quality or HPLC grade solvents. For work-up and purification, solvents were distilled from technical grade. All synthetic transformations have been monitored by either thin layer chromatography (TLC), $^{1}$H-NMR spectroscopy or UHPLC/ESI-MS. TLC was performed on Merck silica gel 60 $F_{254}$ plates (0.25 mm thickness) precoated with a fluorescent indicator. The developed plates were examined under UV light and stained with ceric ammonium molybdate or potassium permanganate followed by heating. GC/EI-MS measurements were performed on a Finnigan Trace GC ultra from Thermo Electron Corporation with EI (electron ionization), Zebron ZB-5MS (30 m) column and Finnigan Trace DSQ. Concentration under reduced pressure was performed by rotary evaporation at 40 °C. Flash chromatography was performed using silica gel 60 (230-400 mesh) from Sigma-Aldrich with a forced flow eluent at 0.3-0.5 bar pressure. All $^{1}$H, $^{13}$C-NMR and $^{19}$F spectra were recorded using Bruker 300 MHz ($^{1}$H) or Bruker 400 MHz ($^{1}$H) & 101 MHz ($^{13}$C) or Bruker 500 MHz ($^{1}$H) & 126 MHz ($^{13}$C) spectrometers at 25 °C. Chemical shifts (δ-values) are reported in ppm, spectra were calibrated related to solvents residual proton chemical shifts ($CHCl_3$, δ = 7.26; methanol-*d3*, δ = 3.31; DMSO-$d_5$, δ = 2.50) and solvents residual carbon chemical shifts ($CDCl_3$, δ = 77.16; methanol-*d4*, δ = 49.00; DMSO-$d_6$, δ = 39.52), multiplicity is reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet or unresolved and coupling constant *J* in Hz. $^{19}$F spectra were referenced to the internal standard $CFCl_3$. IR spectra were recorded on a Varian 800 FT-IR ATR spectrophotometer. Intensities are reported as follows: very strong (vs), strong (s), medium (m), weak (w) and very weak (vw). The absorptions are reported in $cm^{-1}$. RP-HPLC was conducted with a *Prominence* modular HPLC instrument (*Shimadzu*) coupled to a *SPD-20A* UV/Vis detector (*Shimadzu*) using a reversed-phase column (*Gemini-NX* C18, 5 µm, 10 Å, 150 mm x 4.6 mm; *Phenomenex*) for analytical HPLC. The Prep-HPLC was equipped with a *CBM-20A* system controller, a *LC-20AP* solvent delivery unit, a *DGU-20A* degassing unit and a *FRC-10A* fraction collector (all *Shimadzu*). The following solvents were used: $H_2O$ + 0.1 % HCOOH (A), MeCN + 0.1 % HCOOH (B). All high-resolution mass spectra (HRMS-ESI) were recorded by the mass spectrometry service at the University of Zürich on a Finnigan MAT95 mass spectrometer or (for EI) on a *DFS* double-focusing (BE geometry) magnetic sector mass spectrometer (*ThermoFisher Scientific*, Bremen, Germany). Mass spectra were measured with electron ionization (EI) at 70 eV, solid probe inlet, a source temperature of 200°C, an acceleration voltage of 5 kV, and a resolution of 10'000. The instrument was scanned between e.g. *m/z* 300 and 350 at scan rate of 100-200 s / decade in the electric scan mode. Perfluorokerosene (*PFK, Fluorochem*, Derbyshire, UK) served for calibration or were analyzed with an Acquity UPLC (*Waters,* Milford, USA) connected to an Acquity eλ detector and a maXis QToF high-resolution mass- spectrometer (*Bruker Daltonics*, Bremen, Germany). Separation was performed with an Acquity BEH C18 HPLC column (1.7 µm particle size, 2 x 100 mm, Waters) kept at 30 °C. The mobile phase was consisting of A: $H_2O$ 0.1% HCOOH and B: $CH_3CN$ + 0.1% HCOOH. A linear gradient was run from 5 to 98% B within 5 min followed by flushing with 98% B for 1 min at 400 µl $min^{-1}$ flow rate. UV spectra were recorded between 200 and 600 nm at 1.2 nm resolution and 20 points $s^{-1}$. The mass spectrometer was operated in the positive (negative) electrospray ionization mode at 4'000 V (–4'000 V) capillary voltage, –500 V (500 V) endplate offset, with a $N_2$ nebulizer pressure of 1.6 bar and dry gas flow of 8l $min^{-1}$ at 200°C. Spectra were

acquired in the mass range from m/z 50 to 2'000 at 20'000 resolution (full width at half maximum) and 1.5 Hz rate. The mass analyzer was calibrated prior to analysis between m/z 158 and 1450 using a 2 mM solution of sodium formate at a resolution of 20'000 and a mass accuracy below 2 ppm. UV-Vis spectra were recorded on a *Shimadzu UV-1800* spectrometer. Melting points (M.p.) were determined using a *Büchi B-545* apparatus in open capillaries and are uncorrected. Specific optical rotation was measured on *a JASCO P-2000 Polarimeter*, measured at the indicated temperature. UV-Vis spectra were recorded on a Shimadzu UV-1800 spectrophotometer with a spectral width of 200 to 800 nm. Fluorescence measurements were carried out on a *Perkin Elmer* Luminescence Spectrometer LS 50 B with an excitation wavelength corresponding to the absorption maximum in the UV-Vis spectrum and a spectral width from 300 to 800 nm. X-ray diffraction data were recorded using a *Rigaku Oxford Diffraction SuperNova* area-detector diffractometer. The impact sensitivity tests were carried out according to STANAG 4489[1]modified instruction[2] using a BAM (Bundesanstalt für Materialforschung) drophammer.[3] The friction sensitivity tests were carried out according to STANAG 4487[4] modified instruction[5] using the BAM friction tester. The classification of the tested compounds results from the "UN Recommendations on the Transport of Dangerous Goods".[6] Sensitivity towards electrical discharge was tested using the Electric Spark Tester ESD2010 EN.[7] Differential thermal analysis (DTA) measurements to determine the decomposition temperatures of compound **S1** – **S5**, **2** and **3** were performed at a heating rate of 5 °C $min^{-1}$ with an OZM Research DTA 552-Ex instrument. Gel electrophoresis was conducted using Mini-PROTEAN TGX Stain-Free™ precast gels purchased from Bio-Rad at 100 V.

## Synthetic Procedures

*Caution! Several compounds presented herein have a very high nitrogen content and thus might potentially have energetic properties. Thus, caution is of need when handling the compounds, however, in our hands, none of the presented compounds proved to have any dangerous properties and all compounds were safe to handle in large scale. Additionally, the safety evaluation of compounds **S1** – **S5**, **2** and **3** towards impact, friction and electrostatics showed no energetic properties.*

**Hydrazinecarbohydrazonhydrazide (S1)**[8]




Guanidine hydrochloride (25.0 g, 262 mmol, 1.00 equiv) was suspended in 1,4-dioxane (130 mL). Hydrazine monohydrate (43.3 mL, 891 mmol, 3.40 equiv) was added and a clear solution formed. The mixture was heated to reflux for 2 h and during this time the product precipitated as a white solid. Then, the suspension was filtered and the filter cake was rinsed with 1,4-dioxane (100 mL). The solid was dried under high vacuum affording compound **S1** (27.3 g, 262 mmol, quant.) as a colorless solid.

**$^{13}$C-NMR** ($D_2O$, 101 MHz): $\delta$ = 159.5 ppm.

The obtained analytical data are consistent with the values reported in the literature.[8]

**Sensitivity Tests:** BAM impact: > 40 J; BAM friction: >360 N; ESD: > 1.5 J (at grain size <100 μm).

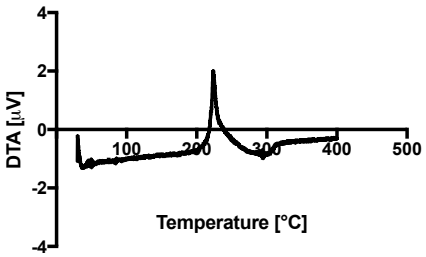
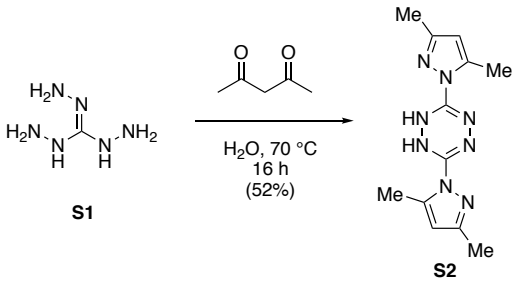



**Figure 1.** DTA Anaylsis of hydrazinecarbohydrazonhydrazide (**S1**).

**Dihydrotetrazine S2**[8]



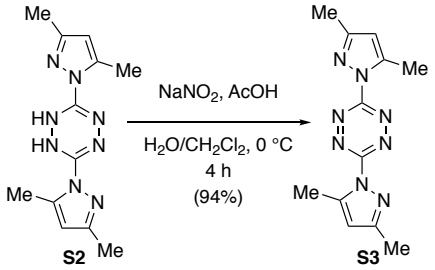

Compound **S1** (16.3 g, 157 mmol, 1.00 equiv) was suspended in water (130 mL) and acetylacetone (32.2 mL, 314 mmol, 2.00 equiv) was added dropwise over 15 min. The mixture was stirred for 18 h at 70 °C. The yellow suspension was then filtered and the filter cake was rinsed with cold water (100 mL). The residue was dried under high vacuum to afford dihydrotetrazine **S2** (22.0 g, 81.0 mmol, 52%) as a yellow solid.

**$^{1}$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 8.05 (br s, 2H), 5.97 (s, 2H), 2.49 (s, 6H), 2.22 (s, 6H) ppm.

The obtained analytical data are consistent with the values reported in the literature.[8]

**Sensitivity Tests:** BAM impact: > 40 J; BAM friction: >360 N; ESD: > 1.5 J (at grain size <100 μm).

**Tetrazine S3**[8]



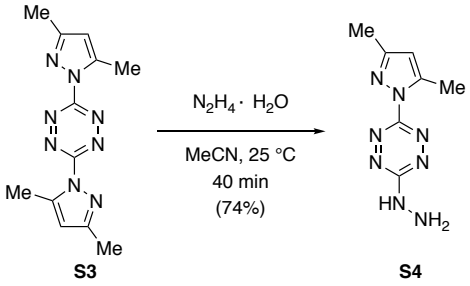

Dihydrotetrazine **S2** (29.5 g, 108 mmol, 1.00 equiv) was suspended in $CH_2Cl_2$ (125 mL) and cooled to 0 °C. A solution of $NaNO_2$ (22.6 g, 324 mmol, 3.00 equiv) in water (250 mL) was added dropwise over 5 min. Then acetic acid (15.4 mL, 270 mmol, 2.50 equiv) was added dropwise. The mixture was stirred for 3.5 h, turning bright red over time (*caution: evolution of nitrous gases!*). After completion of the reaction, the layers were separated and the aqueous layer was extracted with $CH_2Cl_2$ (5 x 200 mL). The combined organic layers were washed with an aqueous potassium carbonate solution (5% wt/wt; 200 mL), dried over sodium sulfate, filtered and concentrated. The obtained bright red solid was dried under high vacuum to afford tetrazine **S3** (27.5 g, 102 mmol, 94%).

**$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 6.19 (s, 2H), 2.71 (s, 6H), 2.39 (s, 6H) ppm.

The obtained analytical data are consistent with the values reported in the literature.[8]

**Sensitivity Tests:** BAM impact: > 40 J; BAM friction: >360 N; ESD: > 1.5 J (at grain size <100 μm).

**Tetrazine S4**[9]



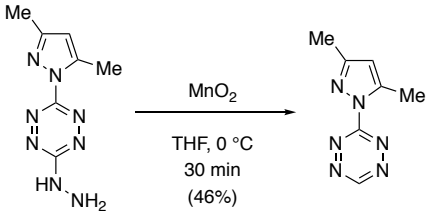

Tetrazine **S3** (24.7 g, 91.4 mmol, 1.00 equiv) was dissolved in MeCN (230 mL) and hydrazine monohydrate (4.44 mL, 91.4 mmol, 1.00 equiv) was added dropwise. Immediate formation of a red solid could be observed and after 40 min, the suspension was filtered. The filter cake was washed with toluene (100 mL) and the residue was dried under high vacuum to afford tetrazine **S4** (13.9 g, 67.4 mmol, 74%) as a bright red solid.

**$^1$H-NMR** (DMSO-*d6*, 400 MHz): $\delta$ = 9.78 (s, 1H), 6.19 (s, 1H), 4.68 (s, 2H), 2.38 (s, 3H), 2.22 (s, 3H) ppm.

The obtained analytical data are consistent with the values reported in the literature.[9]

**Sensitivity Tests:** BAM impact: > 40 J; BAM friction: >360 N; ESD: > 1.5 J (at grain size <100 μm).

**Tetrazine S5**[9]




**S4** **S5**

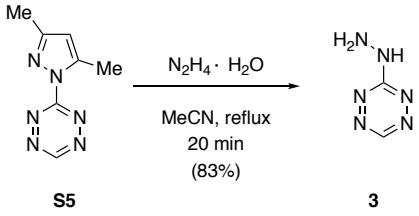
Activated manganese dioxide (35.2 g, 404 mmol, 6.00 equiv) was suspended in THF (270 mL) and the mixture was cooled to 0 °C. Compound **S4** (13.9 g, 67.4 mmol, 1.00 equiv) was added in portions over 15 min. The mixture was stirred for 30 min at 0 °C and was then filtered through a pad of celite. The filter cake was rinsed with $CH_2Cl_2$ (100 mL) and the solvent was removed under reduced pressure. The obtained solid was filtered through a short silica column eluting with $CH_2Cl_2$ and the solvent was removed under reduced pressure to afford tetrazine **S5** (5.40 g, 30.7 mmol, 47%) as a red solid.

**$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.19 (s, 1H), 6.21 (s, 1H), 2.74 (s, 3H), 2.39 (s, 3H) ppm.

The obtained analytical data are consistent with the values reported in the literature.[9]

**Sensitivity Tests:** BAM impact: > 40 J; BAM friction: >360 N; ESD: > 1.5 J (at grain size <100 μm).

**Tetrazine 3**[9]




**S5** **3**

Tetrazine **S5** (5.41 g, 30.7 mmol, 1.00 equiv) was added to MeCN (150 mL) and hydrazine monohydrate (1.64 mL, 33.8 mmol, 1.10 equiv) was added dropwise. The mixture was heated to reflux for 20 min, before the solvent was removed under reduced pressure. The residue was washed with diethyl ether (200 mL) and dried under high vacuum to afford tetrazine **3** (2.85 g, 25.4 mmol, 83%) as a red solid.

**$^1$H-NMR** (DMSO-*d6*, 400 MHz): $\delta$ = 9.75 (s, 1H), 9.55 (br s, 1H), 4.59 (br s, 2H) ppm.

The obtained analytical data are consistent with the values reported in the literature.[9]

**Sensitivity Tests:** BAM impact: > 40 J; BAM friction: >360 N; ESD: > 1.5 J (at grain size <100 μm).

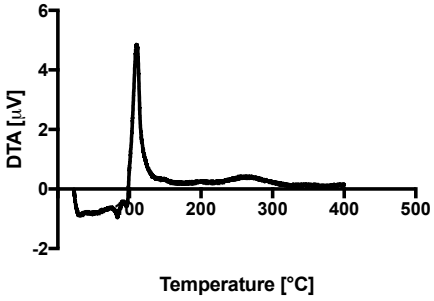
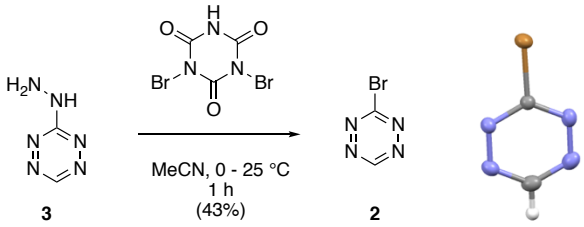



**Figure 2.** DTA Anaylsis of 3-hydrazinotetrazine (**3**).

**3-Bromotetrazine (2)**




Tetrazine **3** (1.38 g, 12.3 mmol, 1.00 equiv) was added to MeCN (23 mL) and cooled to 0 °C. Dibromoisocyanuric acid (5.51 g, 18.5 mmol, 1.50 equiv) was added in portions over 10 min. Then, the bright orange suspension was allowed to warm to 25 °C and stirred for 1 h. The suspension was filtered through a pad of celite covered with silica gel and the filter cake was rinsed with $CH_2Cl_2$ until the filtrate became colorless. The solvent was removed under a stream of nitrogen (*caution: product is volatile!*). The crude product was then purified via flash column chromatography on silica gel ($CH_2Cl_2$) to afford 3-bromotetrazine (**2**) (854 mg, 5.30 mmol, 43%) as a bright orange, crystalline solid.

**$R_f$** ($CH_2Cl_2$) = 0.7 (orange spot); **melting point** = 70 – 72 °C; **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.34 (s, 1H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 101 MHz): $\delta$ = 164.2, 158.0 ppm; **IR** (neat): $\tilde{\nu}$ = 3081 (w), 1228 (s), 1208 (vs), 882 (vs) $cm^{-1}$; **elemental analysis**: calculated for $C_2HN_4Br$: C = 14.92, H = 0.63, N = 34.81; found: C = 14.72, H = 0.64, N = 34.32.

**Sensitivity Tests:** BAM impact: > 40 J; BAM friction: >360 N; ESD: > 1.08 J (at grain size <100 μm).

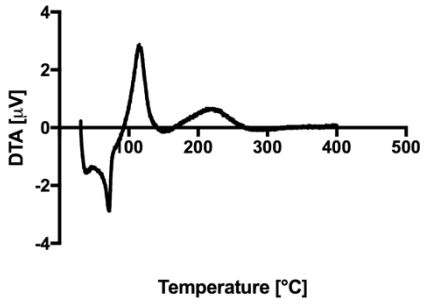
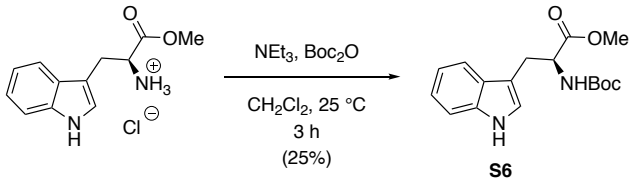



**Figure 1.** DTA Anaylsis of 3-bromotetrazine (**2**).

**Synthesis of Amino Acid Precursors**

**Boc-Trp-OMe (S6)**[10]

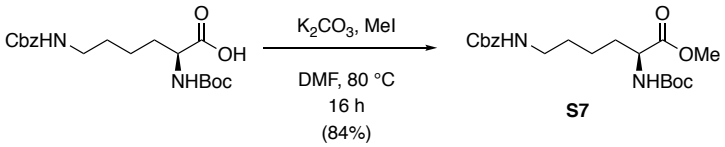



Tryptophan methyl ester hydrochloride (1.00 g, 3.93 mmol, 1.00 equiv) was dissolved in $CH_2Cl_2$ (15 mL) and $NEt_3$ (2.79 mL, 19.7 mmol, 5.00 equiv) was added, followed by $Boc_2O$ (1.26 mL, 5.90 mmol, 1.50 equiv). The mixture was stirred for 3 h and then the reaction mixture was concentrated. The crude material was purified via flash column chromatography on silica gel (30% EtOAc in pentane) to afford Boc-Trp-OMe (**S6**) (309 mg, 0.971 mmol, 25%) as a white solid.

**$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 8.10 (br s, 1H), 7.57 (d, *J* = 7.9 Hz, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.19 (t, *J* = 8.1 Hz, 1H), 7.12 (t, *J* = 7.8 Hz, 1H), 7.00 (s, 1H), 5.08 (d, *J* = 8.3 Hz, 1H), 4.66 (d, *J* = 8.5 Hz, 1H), 3.68 (s, 3H), 3.29 (dd, *J* = 5.8, 2.8 Hz, 1H), 1.43 (s, 9H) ppm.

The obtained analytical data are consistent with the values reported in the literature.[10]

**Boc-Lys(Cbz)-OMe (S7)**[11]

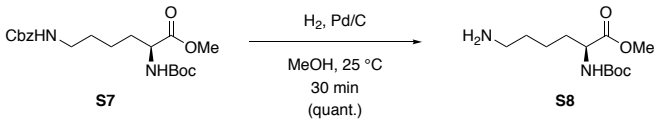



Boc-Lys(Cbz)-OH (768 mg, 2.02 mmol, 1.00 equiv) was dissolved in DMF (10.1 mL). $K_2CO_3$ (568 mg, 4.24 mmol, 2.10 equiv) was added followed by MeI (0.28 mL, 4.44 mmol, 2.20 equiv). The mixture was heated to 80 °C and was stirred for 16 h. Then, water (100 mL) and EtOAc (100 mL) were added and the mixture was stirred until two clear layers were formed. The layers were separated and the aqueous layer was extracted with EtOAc (2 x 50 mL). The combined organic layers were washed with brine (2 x 50 mL), dried over sodium sulfate, filtered and concentrated. The crude product was purified via flash column chromatography on silica gel (40% EtOAc in pentane) to afford Boc-Lys(Cbz)-OMe (**S7**) (666 mg, 1.69 mmol, 84%) as a colorless oil.

**$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 7.38–7.29 (m, 5H), 5.09–5.05 (m, 3H), 4.78 (br s, 1H), 4.32–4.26 (m, 1H), 3.73 (s, 3H), 3.19 (q, *J* = 6.6 Hz, 2H), 1.86–1.75 (m, 1H), 1.69–1.59 (m, 1H), 1.54–1.49 (m, 2H), 1.43 (s, 9H), 1.40–1.34 (m, 2H) ppm.

The obtained analytical data are consistent with the values reported in the literature.[11]

**Boc-Lys-OMe (S8)**[12]

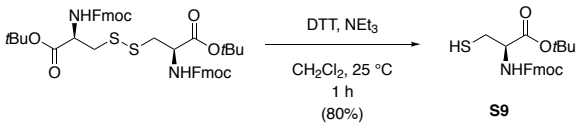



Boc-Lys(Cbz)-OMe (**S7**) (667 mg, 1.69 mmol, 1.00 equiv) was dissolved in MeOH (16.9 mL) and Pd/C (10% Pd; 66.7 mg, 10 wt%) was added. The system was purged with hydrogen gas and the suspension was then stirred for 30 min. The reaction mixture was filtered through a pad of celite and the filter cake was rinsed with EtOAc (20 mL). The solvent was removed to afford Boc-Lys-OMe (**S8**) (439 mg, 1.69 mmol, quant.) as a clear, colorless oil. The material was used in the next step without purification.

**$[\alpha]_D^{20}$** = –20.43 (c = 1.11, MeOH); **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 5.02 (d, *J* = 8.4 Hz, 1H), 4.33–4.26 (m, 1H), 3.74 (s, 3H), 2.69 (t, *J* = 6.7 Hz, 2H), 1.84–1.77 (m, 1H); 1.68–1.59 (m, 1H), 1.48–1.35 (m, 15H) ppm.

The obtained analytical data are consistent with the values reported in the literature.[12]

**Fmoc-Cys-O*t*Bu (S9)**[13]

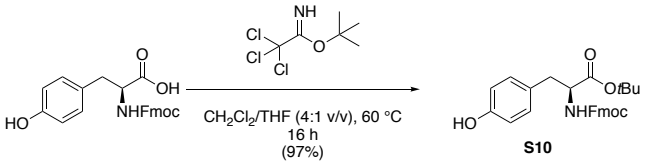



(Fmoc-Cys-O*t*Bu)$_2$ (100 mg, 0.125 mmol, 1.00 equiv) was dissolved in $CH_2Cl_2$ (2 mL) and dithiothreitol (28.9 mg, 0.188 mmol, 1.50 equiv) was added followed by freshly distilled triethylamine (over $CaH_2$) (26.4 µL, 0.188 mmol, 1.50 equiv). The mixture was stirred for 1 h, and was then diluted with $CH_2Cl_2$ (50 mL). The layers were separated and the aqueous layer was washed with saturated aqueous sodium bicarbonate solution (2 x 100 mL) and water (2 x 100 mL). The organic layer was dried over sodium sulfate, filtered and concentrated to afford Fmoc-Cys-O*t*Bu (**S9**) (80.0 mg, 0.20 mmol, 80%) as a colorless oil. The crude product was used in the next step without further purification.

**$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 7.77 (d, *J* = 7.5 Hz, 2H), 7.61 (d, *J* = 7.5 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.32 (t, *J* = 7.5 Hz, 2H), 5.68 (d, *J* = 7.3 Hz, 1H), 4.55–4.54 (m, 1H), 4.42 (t, *J* = 7.0 Hz, 2H), 4.24 (t, *J* = 7.0 Hz, 1H), 2.99 (dt, *J* = 9.3, 3.5 Hz, 2H), 1.50 (s, 9H) ppm.

The obtained analytical data are consistent with the values reported in the literature.[13]

**Fmoc-Tyr-O*t*Bu (S10)**[14]



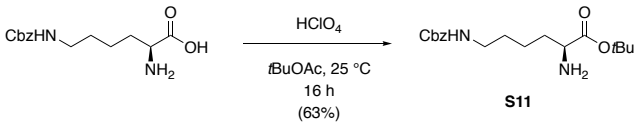
**S10**

Fmoc-Tyr-OH (1.00 g, 2.48 mmol, 1.00 equiv) was dissolved in a mixture of $CH_2Cl_2$/THF (4:1 v/v; 10 mL) and cooled to 0 °C. *Tert*-butyl trichloroacetimidate (1.33 mL, 7.44 mmol, 3.00 equiv) was added dropwise. The mixture was allowed to warm to 25 °C and was then heated to 60 °C. After 16 h, $CH_2Cl_2$ (100 mL) was added and the organic layer was washed with aqueous sodium bicarbonate solution (2.5 wt% in water; 2 x 100 mL). The organic layer was dried over sodium sulfate, filtered and concentrated. The crude product was purified via flash column chromatography (20 – 30% EtOAc in pentane) to afford Fmoc-Tyr-O*t*Bu (**S10**) (1.11 g, 2.42 mmol, 97%) as a sticky, colorless solid.

$[\alpha]_D^{20}$ = +12.66 (c = 0.885, $CHCl_3$); **$^1$H-NMR** (MeOH-$d_4$, 400 MHz): $\delta$ = 7.79 (d, *J* = 7.5 Hz, 2H), 7.61 (d, *J* = 7.5 Hz, 2H), 7.39 (t, *J* = 7.5 Hz, 2H), 7.32–7.28 (m, 2H), 7.04 (d, *J* = 8.4 Hz, 2H), 6.70 (d, *J* = 8.4 Hz, 2H), 4.35–4.23 (m, 3H), 4.18 (t, *J* = 7.1 Hz, 1H), 2.98 (dd, *J* = 13.9, 6.1 Hz, 1H), 2.83 (dd, *J* = 13.9, 8.8 Hz, 1H), 1.41 (s, 9H) ppm.

The obtained analytical data are consistent with the values reported in the literature.[14]

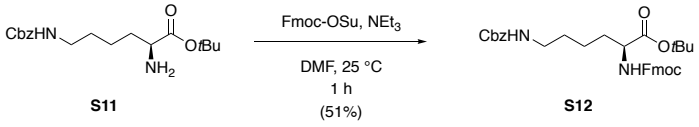
**$H_2$N-Lys(Cbz)-O*t*Bu (S11)**[15]



**S11**

$H_2$N-Lys(Cbz)-OH (4.29 g, 15.3 mmol, 1.00 equiv) was added to *tert*-butyl acetate (53 mL). Perchloric acid (70% in water; 2 mL) was added and the mixture was stirred for 16 h. Then, the mixture was extracted with water (100 mL) and aqueous HCl (0.5 M; 150 mL). The combined aqueous layers were basified with aqueous $K_2CO_3$ solution (10 wt%) to a pH of 9. The aqueous layer was extracted with $CH_2Cl_2$ (4 x 100 mL) and the combined organic layers were dried over sodium sulfate, filtered and concentrated to afford $H_2$N-Lys(Cbz)-O*t*Bu (**S11**) (3.26 g, 9.69 mmol, 63%) as a colorless oil. The crude product was used in the next step without purification.

**Fmoc-Lys(Cbz)-O*t*Bu (S12)**

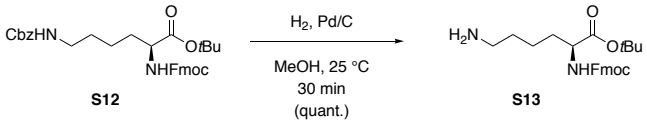


**S11** **S12**

$H_2$N-Lys(Cbz)-O*t*Bu (**S11**) (4.17 g, 12.4 mmol, 1.00 equiv) was dissolved in DMF (41 mL) and Fmoc-OSu (4.60 g, 13.6 mmol, 1.10 equiv) was added followed by freshly distilled triethylamine (over $CaH_2$) (1.74 mL, 1.25 mmol, 1.00 equiv). The mixture was stirred for 1 h, before the mixture was diluted with $CH_2Cl_2$ (100 mL) and water (100 mL). The layers were separated and the organic layer was washed with water (6 x 100 mL). The organic layer was dried over sodium sulfate, filtered and concentrated. The crude product was purified via flash column chromatography (30% EtOAc in pentane) to afford Fmoc-Lys(Cbz)-O*t*Bu (**S12**) (3.87 g, 6.93 mmol, 56%) as a colorless oil.

$R_f$ (40% EtOAc in pentane) = 0.37 ($KMnO_4$, UV); $[\alpha]_D^{20}$= –10.68 (c = 2.43, MeOH); **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 7.77 (d, *J* = 7.5 Hz, 2H), 7.61 (d, *J* = 7.5 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.33–7.29 (m, 7H), 5.37 (d, *J* = 8.3 Hz, 1H), 5.15–5.04 (m, 2H), 4.81 (br s, 1H), 4.40 (d, *J* = 7.4 Hz, 2H), 4.28–4.20 (m, 2H), 3.23–3.16 (m, 2H), 1.88–1.79 (m, 1H), 1.72–1.62 (m, 1H), 1.59–1.52 (m, 2H), 1.47–1.30 (m, 11H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 101 MHz): $\delta$ = 171.7, 156.6, 156.1, 144.1, 143.9, 141.4, 136.7, 128.6, 128.2, 127.8, 127.2, 125.2, 120.1, 82.3, 67.1, 66.7, 54.2, 47.3, 40.8, 32.6, 29.6, 28.2, 22.3 ppm; **IR** (neat): $\tilde{\nu}$ = 3336 (w), 2929 (w), 1699 (vs), 1520 (s), 1451 (m), 1245 (vs), 1153 (vs), 738 (vs) $cm^{-1}$; **HRMS** (ESI) for $C_{33}H_{39}N_2O_6^+$ $[M+H]^+$: calculated: 559.2803; found: 559.2805.

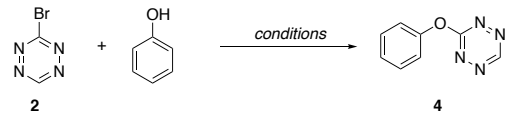
**Fmoc-Lys-O*t*Bu (S13)**




Fmoc-Lys(Cbz)-O*t*Bu (**S12**) (693 mg, 1.24 mmol, 1.00 equiv) was dissolved in MeOH (7 mL) and Pd/C (10% Pd; 69 mg, 10 wt%) was added. The system was purged with hydrogen for 5 min and was then stirred for further 30 min. After 30 min, the mixture was filtered through a pad of celite and the filter cake was rinsed with $CH_2Cl_2$ (50 mL) and the solvent was removed via a stream of nitrogen to afford Fmoc-Lys-O*t*Bu (**S13**) (526 mg, 1.24 mmol, quant.) as a colorless oil. The product proved to be very unstable and was used directly after synthesis to avoid degradation.

## Substrate Scope

**Table S1.** Screening the nucleophilic aromatic substitution using phenol.

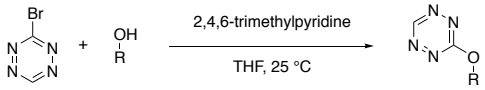



**2** **4**

| Entry | Base | Solvent | Equiv phenol | t [min] | Yield [%] |
|---|---|---|---|---|---|
| 1 | DBU | $CH_2Cl_2$ | 4 | 10 | 22 |
| 2 | DBU | MeCN | 4 | 10 | 17 |
| 3 | DBU | THF | 1.2 | 10 | 37 |
| 4 | DBU | THF | 2 | 10 | 32 |
| 5 | DBU | THF | 4 | 10 | 56 |
| 6 | collidine[a] | THF | 4 | 10 | 67 |
| 7 | collidine[a,b] | THF | 1.1 | 10 | 56 |

a) collidine = 2,4,6-trimethylpyridine; b) reverse addition of 3-bromotetrazine (**2**).

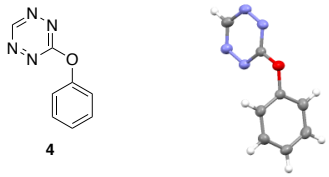
### General Procedure for the Functionalization of Hydroxy Groups (GP1)




**2**

The respective alcohol (0.171 mmol 1.10 equiv) was dissolved in THF (0.75 mL) and 2,4,6-trimethylpyridine (20.6 µL, 0.155 mmol, 1.00 equiv) was added. 3-Bromotetrazine (**2**) (25.0 mg, 0.155 mmol, 1.00 equiv) was dissolved in THF (0.75 mL) and the resulting solution was added dropwise to the alcohol. After completion of the reaction, the solvent was removed via a stream of nitrogen and the crude product was purified via flash column chromatography on silica gel.

### 3-Phenoxy-*s*-tetrazine (4)

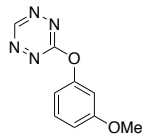


**4**

Compound **4** was synthesized according to **GP1**. After 20 min, the solvent was removed under a stream of nitrogen and the crude material was purified via flash column chromatography on silica gel (50% $CH_2Cl_2$ in pentane) to afford tetrazine **4** (18.0 mg, 103 µmol, 66%) as a red solid.

**$R_f$** (50% $CH_2Cl_2$ in pentane) = 0.26 (pink spot, UV); **melting point** = 111 – 113 °C; **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.16 (s, 1H), 7.53–7.48 (m, 2H), 7.38–7.34 (m, 1H), 7.31–7.28 (m, 2H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 101 MHz): $\delta$ = 169.1, 156.8, 151.8, 130.4, 127.0, 121.1 ppm; **IR** (neat): $\tilde{\nu}$ = 1589 (w), 1493 (m), 1443 (vs), 1364 (vs), 1202 (m), 1117 (m), 767 (s) $cm^{-1}$; **HRMS** (EI) for $C_8H_6N_4O^+$: calculated: 174.0542; found: 174.0533.

**3-(3-Methoxyphenoxy)-*s*-tetrazine (5)**

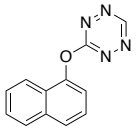



**5**

Compound **5** was synthesized according to **GP1**. After 20 min, the solvent was removed under a stream of nitrogen and the crude material was purified via flash column chromatography on silica gel (50% $CH_2Cl_2$ in pentane) to afford tetrazine **5** (23.4 mg, 115 µmol, 74%) as a red solid.

**$R_f$** (40% $Et_2O$ in pentane) = 0.50 (pink spot, UV); **melting point** = 73 – 75°C; **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.16 (s, 1H), 7.39 (t, *J* = 8.2 Hz, 1H), 6.89 (m, 2H), 6.83 (t, *J* = 2.3 Hz, 1H), 3.83 (s, 3H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 101 MHz): $\delta$ = 169.1, 161.3, 156.8, 152.7, 130.8, 113.1, 112.7, 107.2, 55.7 ppm; **IR** (neat): $\tilde{\nu}$ = 1590 (w), 1620 (w), 1432 (vs), 1357 (vs), 1147 (s), 1109 (s), 1039 (m), 931 (m) $cm^{-1}$; **HRMS** (ESI) for $C_9H_8N_4O_2Na^+$ $[M+Na]^+$: calculated: 227.0539; found: 227.0539.

**3-(naphthalen-1-yloxy)-*s*-tetrazine (S14)**

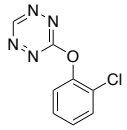



**S14**

Compound **S14** was synthesized according to **GP1**. After 20 min, the solvent was removed under a stream of nitrogen and the crude material was purified via flash column chromatography on silica gel (20% $Et_2O$ in pentane) to afford tetrazine **S14** (25.0 mg, 111 µmol, 72%) as a pink solid.

**$R_f$** (40% $Et_2O$ in pentane) = 0.48 (pink spot, UV); **melting point** = 128 – 129 °C; **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.17 (s, 1H); 7.96 – 7.87 (m, 3H), 7.59 – 7.49 (m, 3H), 7.43 (d, *J* = 7.6 Hz, 1H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 101 MHz): $\delta$ = 169.5, 156.8, 147.7, 135.1, 128.4, 127.2, 127.1, 127.0, 126.3, 125.6, 120.9, 117.4 ppm; **IR** (neat): $\tilde{\nu}$ = 1600 (w), 1430 (s), 1354 (vs), 1227 (w), 1070 (m), 771 (s) $cm^{-1}$; **HRMS** (EI) for $C_{12}H_8N_4O^+$: calculated: 224.0698; found: 224.0693.

**3-(2-Chlorophenoxy)-*s*-tetrazine (6)**

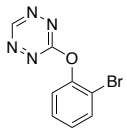



**6**

Compound **6** was synthesized according to **GP1**. After 20 min, the solvent was removed under a stream of nitrogen and the crude material was purified via flash column chromatography on silica gel (10% $Et_2O$ in pentane) to afford tetrazine **6** (27.5 mg, 132 µmol, 85%) as a red solid.

**$R_f$** (10% $Et_2O$ in pentane) = 0.19 (pink spot, UV); **melting point** = 57 – 58 °C; **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.18 (s, 1H), 7.54 (dd, J = 7.9, 1.6 Hz, 1H), 7.44–7.31 (m, 3H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 101 MHz): $\delta$ = 168.4, 157.1, 147.8, 131.2, 128.7, 128.2, 126.8, 123.2 ppm; **IR** (neat): $\tilde{\nu}$ = 1475 (m), 1431 (vs), 1352 (vs), 1218 (m), 1120 (m), 1060 (s), 931 (m), 762 $cm^{-1}$ (s); **HRMS** (EI) for $C_8H_5N_4OCl^+$: calculated: 208.0152; found: 208.0142.

**3-(2-Bromophenoxy)-*s*-tetrazine (S15)**

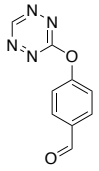


**S15**

Compound **S15** was synthesized according to **GP1**. After 20 min, the solvent was removed under a stream of nitrogen and the crude material was purified via flash column chromatography on silica gel (20% $Et_2O$ in pentane) to afford tetrazine **S15** (28.0 mg, 111 µmol, 72%) as a red solid.

**$R_f$** (20% $Et_2O$ in pentane) = 0.25 (pink spot, UV); **melting point** = 73 – 74 °C; **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ =10.19 (s, 1H), 7.72 (dd, J = 8.0, 1.5 Hz, 1H), 7.46 (ddd, J = 8.1, 7.4, 1.6 Hz, 1H), 7.35 (dd, J = 8.1, 1.6 Hz, 1H), 7.29–7.25 (m, 1H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 101 MHz): $\delta$ = 168.4, 157.1, 149.1, 134.2, 129.4, 128.5, 123.3, 115.9 ppm; **IR** (neat): $\tilde{\nu}$ = 1470 (w), 1431 (vs), 1353 (vs), 1215 (m), 1046 (w), 932 (m) $cm^{-1}$; **HRMS** (EI) for $C_8H_5N_4OBr^+$: calculated: 251.9647; found: 251.9641.

**4-((*s*-Tetrazin-3-yl)oxy)benzaldehyde (S16)**

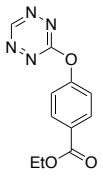


**S16**

Compound **S16** was synthesized according to **GP1**. After 20 min, the solvent was removed under a stream of nitrogen and the crude material was purified via flash column chromatography on silica gel (70% $CH_2Cl_2$ in pentane) to afford tetrazine **S16** (9.4 mg, 46.0 µmol, 30%) as a pink solid.

**$R_f$** (70% $CH_2Cl_2$ in pentane) = 0.29 (pink spot, UV); **melting point** = 99 – 100 °C; **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.23 (s, 1H), 10.06 (s, 1H), 8.07–8.03 (m, 2H), 7.50–7.47 (m, 2H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 101 MHz): $\delta$ = 190.7, 168.7, 157.2, 156.1, 134.9, 132.0, 121.9 ppm; **IR** (neat): $\tilde{\nu}$ = 1696 (vs), 1600 (w), 1455 (m), 1438 (s), 1355 (vs), 1217 (m), 907 (s), 731 (s) $cm^{-1}$; **HRMS** (EI) for $C_9H_6N_4O_2^+$: calculated: 202.0491; found: 202.0484.

**Ethyl 4-((*s*-tetrazin-3-yl)oxy)benzoate (S17)**

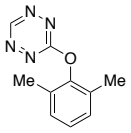


**S17**

Compound **S17** was synthesized according to **GP1**. After 20 min, the solvent was removed under a stream of nitrogen and the crude material was purified via flash column chromatography on silica gel (70% $CH_2Cl_2$ in pentane) to afford tetrazine **S17** (20.0 mg, 81.0 µmol, 52%) as a pink solid.

**$R_f$** (100% $CH_2Cl_2$) = 0.27 (pink spot, UV); **melting point** = 89 – 91 °C; **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.20 (s, 1H), 8.21 (d, *J* = 8.9 Hz, 2H), 7.38 (d, *J* = 8.9 Hz, 2H), 4.40 (q, *J* = 7.1 Hz, 2H), 1.41 (t, *J* = 7.1 Hz, 3H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 126 MHz): $\delta$ = 168.8, 165.6, 157.1, 155.1, 132.0, 129.3, 121.1, 61.5, 14.5 ppm; **IR** (neat): $\tilde{\nu}$ = 1714 (s), 1604 (m), 1437 (s), 1356 (vs), 1275 (vs), 1208 (m), 1165 (w), 1115 (s), 932 (m) $cm^{-1}$; **HRMS** (ESI) for $C_{11}H_{10}N_4O_3Na^+$ $[M+Na]^+$: calculated: 269.0645; found: 269.0642.

**3-(2,6-Dimethylphenoxy)-*s*-tetrazine (S18)**

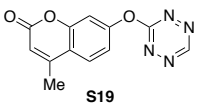


**S18**

Compound **S18** was synthesized according to **GP1**. After 20 min, the solvent was removed under a stream of nitrogen and the crude material was purified via flash column chromatography on silica gel (40 – 50% $CH_2Cl_2$ in pentane) to afford tetrazine **S18** (12.4 mg, 61.0 µmol, 39%) as a red solid.

**$R_f$** (50% $CH_2Cl_2$ in pentane) = 0.33 (pink spot, UV); **melting point** = 62 – 64 °C; **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.14 (s, 1H), 7.16 (m, 3H), 2.16 (s, 6H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 101 MHz): $\delta$ = 168.2, 156.9, 149.1, 130.0, 129.4, 127.0, 16.5 ppm; **IR** (neat): $\tilde{\nu}$ = 1434 (vs), 1356 (vs), 1170 (m), 1088 (m), 928 (m) $cm^{-1}$; **HRMS** (EI) for $C_{10}H_{10}N_4O^+$: calculated: 202.0855; found: 202.0849.

**7-((*S*-tetrazin-3-yl)oxy)-4-methyl-2*H*-chromen-2-one (S19)**

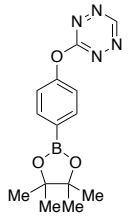


**S19**

Compound **S19** was synthesized according to **GP1**. After 20 min, the solvent was removed under a stream of nitrogen and the crude material was purified via flash column chromatography on silica gel (100% $CH_2Cl_2$) to afford tetrazine **S19** (9.5 mg, 37.0 µmol, 24%) as a pink solid.

**$R_f$** (100% $CH_2Cl_2$) = 0.1 (pink spot, UV); **melting point** = 188 – 189 °C; **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.22 (s, 1H), 7.75 (d, *J* = 8.7 Hz, 1H), 7.32 (d, *J* = 2.4 Hz, 1H) 7.26 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.33 (m, 1H), 2.48 ppm (d, *J* = 1.2 Hz, 3H); **$^{13}$C-NMR** ($CDCl_3$, 126 MHz): $\delta$ = 168.8, 160.2, 157.3, 154.7, 153.8, 151.8, 126.4, 118.9, 117.4, 115.3, 110.2, 18.9 ppm; **IR** (neat): $\tilde{\nu}$ = 3087 (m), 1737 (s), 1618 (s), 1435 (vs), 1357 (vs), 1260 (m), 1112 (m), 845 (m) $cm^{-1}$; **HRMS** (ESI) for $C_{12}H_8N_4O_3Na^+$ $[M+Na]^+$: calculated: 279.0489; found: 279.0485.

**3-(4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy)-*s*-tetrazine (7)**

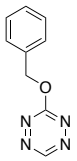


**7**

Compound **7** was synthesized according to **GP1**. After 48 h, the solvent was removed under a stream of nitrogen and the crude material was purified via flash column chromatography on silica gel (30% $Et_2O$ in pentane) to afford tetrazine **7** (24.5 mg, 82.0 µmol, 53%) as a pink solid.

**$R_f$** (30% $Et_2O$ in pentane) = 0.39 (pink spot, UV); **melting point** = 131 – 133 °C; **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.16 (s, 1H), 7.96 (d, *J* = 8.6 Hz, 2H), 7.29 (d, *J* = 8.6 Hz, 2H), 1.36 (s, 12H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 126 MHz): $\delta$ = 169.0, 156.9, 154.2, 137.1, 120.3, 84.2, 25.0 ppm (carbon attached to the boron could not be observed); **IR** (neat): $\tilde{\nu}$ = 1603 (m), 1437 (s), 1354 (vs), 1202 (w), 1088 (m), 1019 (w), 857 (w) $cm^{-1}$; **HRMS** (EI) for $C_{14}H_{17}N_4O_3B^+$: calculated: 300.1394; found: 300.1382.

**3-(Benzyloxy)-*s*-tetrazine (S20)**

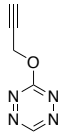


**S20**

Compound **S20** was synthesized according to **GP1**. After 3 h, the solvent was removed under a stream of nitrogen and the crude material was purified via flash column chromatography on silica gel (30% $Et_2O$ in pentane) to afford tetrazine **S20** (9.0 mg, 48.0 µmol, 31%) as a red solid.

**$R_f$** (30% $Et_2O$ in pentane) = 0.55 (pink spot); **melting point** = 84 – 85 °C **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.06 (s, 1H), 7.56–7.55 (m, 2H), 7.44–7.36 (m, 3H), 5.72 (s, 2H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 101 MHz): $\delta$ = 167.9, 156.2, 134.3, 129.2, 128.9, 128.9, 71.3 ppm; **IR** (neat): $\tilde{\nu}$ = 1500 (w), 1470 (vs), 1452 (vs), 1353 (vs), 1346 (vs), 1193 (m), 970 (vs), 936 (s), 764 (vs) $cm^{-1}$; **HRMS** (ESI) for $C_9H_8N_4ONa^+$ $[M+Na]^+$: calculated: 211.0590; found: 211.0590.

**3-(Prop-2-yn-1-yloxy)-*s*-tetrazine (8)**

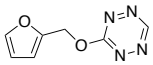


**8**

Compound **8** was synthesized according to **GP1**. After 4 h, the solvent was removed under a stream of nitrogen and the crude material was purified via flash column chromatography on silica gel (30% $Et_2O$ in pentane) to afford tetrazine **8** (8.0 mg, 59.0 µmol, 38%) as a red oil.

**$R_f$** (30% $Et_2O$ in pentane) = 0.35 (pink spot); **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.13 (s, 1H), 5.31 (s, 2H), 2.61 (s, 1H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 101 MHz): $\delta$ = 167.3, 156.6, 77.1, 76.2, 57.1 ppm; **IR** (neat): $\tilde{\nu}$ = 3284 (m), 1469 (vs), 1335 (vs), 996 (s), 939 (s) $cm^{-1}$; **HRMS** (EI) for $C_5H_4N_4O^+$: calculated: 136.0385; found: 136.0377.

**3-(Furan-2-ylmethoxy)-*s*-tetrazine (S21)**

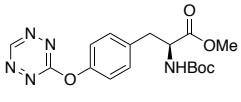


**S21**

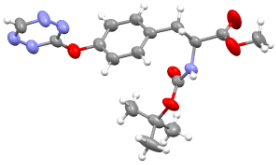
Compound **S21** was synthesized according to **GP1**. After 24 h, the solvent was removed under a stream of nitrogen and the crude material was purified via flash column chromatography on silica gel (20% $Et_2O$ in pentane) to afford tetrazine **S21** (7.0 mg, 39.0 µmol, 25%) as a red oil.

**$R_f$** (20% $Et_2O$ in pentane) = 0.39 (pink spot); **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.08 (s, 1H), 7.48 (dd, *J* = 1.9, 0.8 Hz, 1H), 6.63 (d, *J* = 0.7 Hz, 1H), 6.41 (dd, *J* = 3.3, 1.9 Hz, 1H), 5.69 (s, 2H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 126 MHz): $\delta$ = 167.7, 156.3, 147.7, 144.2, 112.7, 110.9, 62.9 ppm; **IR** (neat): $\tilde{\nu}$ = 1468 (vs), 1337 (vs), 1192 (m), 1919 (m), 752 (m) $cm^{-1}$; **HRMS** (EI) for $C_7H_6N_4O_2^+$: calculated: 178.0491; found: 178.0481.

**Boc-Tyr(Tet)-OMe (23)**



**23**

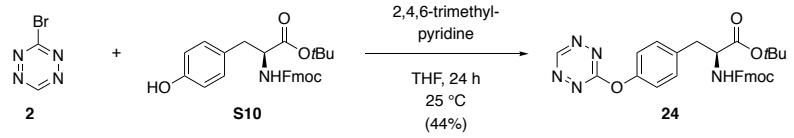


Compound **23** was synthesized according to **GP1**. After 4 h, the solvent was removed under a stream of nitrogen and the crude material was purified via flash column chromatography on silica gel (10% $Et_2O$ in pentane) to afford tetrazine **23** (42.0 mg, 112 µmol, 72%) as a pink solid.

**$R_f$** (10% $Et_2O$ in pentane) = 0.47 (pink spot, UV); **melting point** = 120 °C; **$[\alpha]_D^{20}$** = +46.18 (c = 0.775, $CHCl_3$); **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.18 (s, 1H), 7.31–7.24 (m, 4H), 5.06 (d, *J* = 8.3 Hz, 1H), 4.65 (q, *J* = 6.8 Hz, 1H), 3.77 (s, 3H), 3.21 (dd, *J* = 13.9, 5.8 Hz, 1H), 3.13 (dd, *J* = 13.9, 6.4 Hz, 1H), 1.46 (s, 9H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 101 MHz): $\delta$ = 172.2,

169.0, 156.9, 155.2, 150.8, 135.1, 131.2, 121.1, 80.3, 54.5, 52.5, 38.0, 28.4 ppm; **IR** (neat): $\tilde{\nu}$ = 3358 (br w), 2979 (br w), 1742 (m), 1710 (vs), 1507 (m), 1437 (vs), 1359 (vs), 1202 (m), 1165 (s), 732 (m) $cm^{-1}$; **HRMS** (ESI) for $C_{17}H_{21}N_5O_5Na^+$ $[M+Na]^+$: calculated: 398.1435; found: 398.1436.

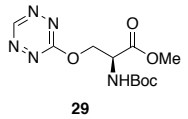
**Fmoc-Tyr(Tet)-O*t*Bu (24)**




Fmoc-Tyr-O*t*Bu (**S10**) (78.4 mg, 0.171 mmol, 1.10 equiv) was dissolved in THF (0.75 mL) and 2,4,6-trimethylpyridine (20.6 µL, 0.155 mmol, 1.00 equiv) was added. To this solution, a solution of 3-bromotetrazine (**2**) in THF (0.75 mL) was added dropwise. The mixture was stirred for 24 h. Then, the mixture was diluted with diethyl ether (50 mL) and washed with water (50 mL). The layers were separated and the organic layer was washed with aqueous sodium hydroxide solution (1 M; 50 mL). The organic layer was dried over sodium sulfate, filtered and concentrated. The crude product was purified via flash column chromatography (2% $Et_2O$ in $CH_2Cl_2$) to afford tetrazine **24** (37 mg, 69.0 µmol, 45%) as a pink oil.

$\mathbf{R_f}$ (2% $Et_2O$ in $CH_2Cl_2$) = 0.27 (pink spot, UV); $\mathbf{[\alpha]_D^{20}}$ = +22.35 (c = 0.73, $CHCl_3$); **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.14 (s, 1H), 7.75 (d, *J* = 7.5 Hz, 2H), 7.58 (dd, *J* = 7.5, 1.5 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.33–7.26 (m, 4H), 7.21 (d, *J* = 8.3 Hz, 2H), 5.36 (d, *J* = 8.0 Hz, 1H), 4.58 (q, *J* = 6.6 Hz, 1H), 4.47 (dd, *J* = 10.6, 7.1 Hz, 1H), 4.37 (dd, *J* = 10.6, 6.9 Hz, 1H), 4.22 (t, *J* = 6.9 Hz, 1H), 3.15 (d, *J* = 6.1 Hz, 2H), 1.43 (s, 9H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 101 MHz): $\delta$ = 170.4, 168.9, 156.7, 155.5, 150.6, 143.9, 143.8, 141.3, 141.3, 135.0, 131.2, 127.7, 127.1, 125.1, 125.0, 120.9, 120.0, 120.0, 82.8, 66.9, 55.1, 47.2, 38.0, 28.0 ppm; **IR** (neat): $\tilde{\nu}$ = 3343 (br w), 1712 (vs), 1506 (m), 1436 (vs), 1358 (vs), 1202 (m), 1152 (s), 737 (vs) $cm^{-1}$; **HRMS** (ESI) for $C_{30}H_{29}N_5O_5Na^+$ $[M+Na]^+$: calculated: 562.2061; found: 562.2066.

**Boc-Ser(Tet)-OMe (29)**

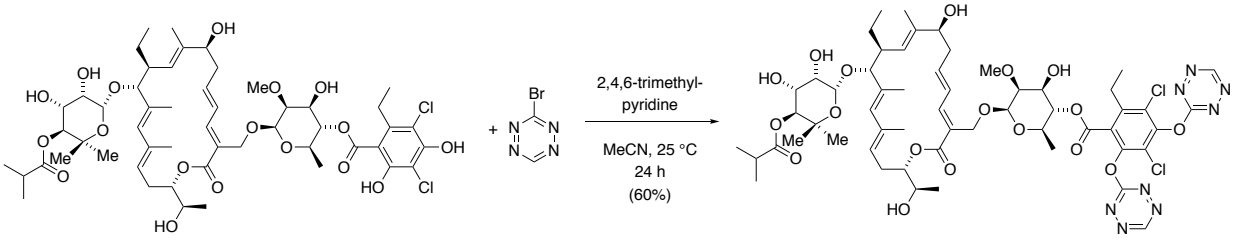



**29**

Boc-Ser(Tet)-OMe **29** was synthesized according to **GP1**. After 24 h, the solvent was removed via a stream of nitrogen and the crude product was purified via flash column chromatography on silica gel (30% EtOAc in pentane) to afford tetrazine **29** (22.0 mg, 73.0 µmol, 47%) as a red oil.

$\mathbf{R_f}$ (30% EtOAc in pentane) = 0.27 (pink spot, UV); $\mathbf{[\alpha]_D^{20}}$ = +6.73 (c = 0.275, MeOH); **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.09 (s, 1H), 5.53 (d, *J* = 8.3 Hz, 1H), 5.05–4.95 (m, 2H), 4.86, 4.84 (m, 1H), 3.81 (s, 3H), 1.44 (s, 9H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 126 MHz): $\delta$ = 169.7, 167.8, 156.5, 155.3, 80.8, 69.5, 53.2, 52.9, 28.4 ppm; **IR** (neat): $\tilde{\nu}$ = 3358 (br w), 2978 (w), 1749 (m), 1712 (vs), 1479 (vs), 1447 (s), 1349 (vs), 1164 (vs), 1056 (m), 1018 (w), 940 (w) $cm^{-1}$; **HRMS** (ESI) for $C_{11}H_{17}N_5O_5Na^+$ $[M+Na]^+$: calculated: 322.1122; found: 322.1119.

**Di-Tet-Fidaxomicin (33)**

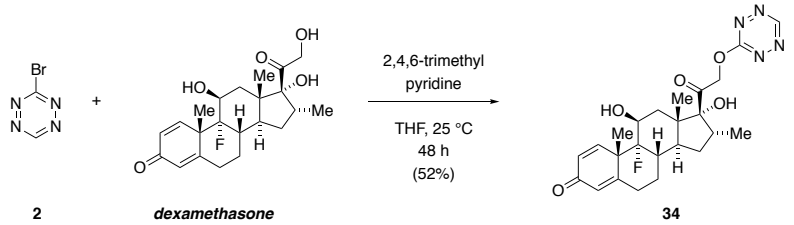



***fidaxomicin*** **2** **33**

Fidaxomicin (16.4 mg, 15.5 µmol, 1.00 equiv) was dissolved in MeCN (0.5 mL) and 2,4,6-trimethylpyridine (4.13 µL, 31 µmol, 2.00 equiv) was added. Then, 3-bromotetrazine (**2**) (12.5 mg, 77.5 µmol, 5.00 equiv), dissolved in MeCN (0.5 mL), was added dropwise. After 16 h, additional 3-bromotetrazine (**2**) (12.5 mg, 77.5 µmol, 5.00 equiv) and 2,4,6-trimethylpyridine (4.13 µL, 31 µmol, 2.00 equiv) were added. After a total of 24 h, the reaction was complete and the solvent was removed via a stream of nitrogen. The crude product was purified via flash column chromatography (5% MeOH in $CH_2Cl_2$) to afford di-tet-fidaxomicin (**33**) (11.3 mg, 9.00 µmol, 58%) as a pink oil.

**$R_f$** (5% MeOH in $CH_2Cl_2$) = 0.28 (pink spot, UV); **$[\alpha]_D^{20}$** = –20.32 (c = 0.505, MeOH); **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.44 (s, 1H), 10.40 (s, 1H), 7.22 (d, *J* = 11.4 Hz, 1H), 6.60–6.53 (m, 1H), 5.94 (ddd, *J* = 14.6, 9.4, 4.8 Hz, 1H), 5.83 (s, 1H), 5.56 (t, *J* = 8.3 Hz, 1H), 5.15 (d, *J* = 10.5 Hz, 1H), 5.01 (d, *J* = 10.2 Hz, 1H), 4.93 (t, *J* = 9.6 Hz, 1H), 4.72–4.68 (m, 2H), 4.59 (d, *J* = 11.5 Hz, 1H), 4.54 (s, 1H), 4.40 (d, *J* = 11.5 Hz, 1H),4.24–4.23 (m, 1H), 4.02 (t, *J* = 6.4 Hz, 1H), 3.93 (d, *J* = 3.3 Hz, 1H), 3.75–3.70 (m, 2H), 3.50 (s, 3H), 3.44–3.41 (m, 2H), 3.06–2.97 (m, 2H), 2.76–2.67 (m, 3H), 2.59 (p, *J* = 7.0 Hz, 1H), 2.52–2.38 (m, 3H), 2.04–1.96 (m, 1H), 1,81 (s, 3H), 1.76 (s, 3H), 1.66 (s, 3H), 1.32–1.28 (m, 5H), 1.19–1.12 (m, 18H), 0.88 (t, *J* = 7.4 Hz, 1H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 126 MHz): $\delta$ = 178.4, 169.5, 169.1, 168.9, 165.1, 159.5, 159.1, 147.8, 146.2, 145.6, 143.7, 142.0, 137.1, 137.0, 136.4, 134.6, 129.5, 128.8, 128.5, 126.9, 125.6, 124.6, 122.0, 102.1, 97.2, 94.3, 82.4, 78.6, 78.2, 75.9, 74.5, 73.5, 73.2, 72.5, 71.1, 70.5, 68.3, 63.9, 62.2, 42.5, 37.3, 35.4, 28.7, 28.3, 26.9, 26.4, 20.2, 19.5, 19.1, 18.7, 18.2, 17.5, 15.4, 14.3, 13.9, 11.3 ppm; **IR** (neat): $\tilde{\nu}$ = 3474 (br m), 2976 (m), 2934 (m), 1737 (s), 1339 (vs), 1072 (s), 1032 (m), 928 (w), 795 (w) $cm^{-1}$; **HRMS** (ESI) for $C_{56}H_{74}N_8O_{18}Cl_2Na^+$ $[M+Na]^+$: calculated: 1239.4390; found: 1239.4389.

**Tet-Dexamethasone (34)**




**2** ***dexamethasone*** **34**

Dexamethasone (73.0 mg, 0.186 mmol, 1.20 equiv) was dissolved in THF (0.75 mL) and collidine was added (41.3 µL, 0.310 mmol, 2.00 equiv). Then, 3-bromotetrazine (**2**) (25.0 mg, 0.155 mmol, 1.00 equiv), in THF (0.75 mL) was added dropwise. After 48 h, the solvent was removed via a stream of nitrogen and the crude product was purified via flash column chromatography on silica gel (5% MeOH in $CH_2Cl_2$) to afford tetrazine **34** (38.0 mg, 80.0 µmol, 52%) as a pink oil.

**$R_f$** (5% MeOH in $CH_2Cl_2$) = 0.42 (pink spot, UV); **$[\alpha]_D^{20}$** = +154 (c = 1.245, MeOH); **$^{19}$F-NMR** ($CDCl_3$, 376 Hz): $\delta$ = –165.93 ppm; **$^1$H-NMR** ($CDCl_3$, 500 MHz): $\delta$ = 10.04 (s, 1H), 7.25 (d, *J* = 10.1 Hz, 1H), 6.36 (dd, *J* = 10.1, 1.9 Hz, 1H), 6.11 (d, *J* = 1.7 Hz, 1H), 5.67 (d, *J* = 17.6 Hz, 1H), 5.54 (d, *J* = 17.6 Hz, 1H), 4.42 (d, *J* = 9.7 Hz, 1H), 3.12–3.04 (m, 1H), 2.65–2.59 (m, 1H), 2.49–2.35 (m, 3H), 2.23 (br s, 1H), 2.20–2.14 (m, 1H), 1.93 (br s, 1H), 1.86–1.73 (m, 3H), 1.59–1.51 (m, 4H), 1.27–1.22 (m, 2H), 1.06 (s, 3H), 0.98 (d, *J* = 7.3 Hz, 3H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 126 MHz): $\delta$ = 203.9, 186.9, 167.6, 166.5, 156.2, 152.5, 129.9, 125.2, 101.1 (d, *J* = 176 Hz), 91.5, 72.7, 72.3 (d, *J* = 38.8 Hz), 49.0, 48.5 (d, *J* = 22.7 Hz), 44.3, 36.9, 36.7, 34.3 (d, *J* = 19.5 Hz), 32.3, 31.2, 27.5, 23.0, 16.7, 14.8 ppm; **IR** (neat): $\tilde{\nu}$ = 3440 (br m), 1944 (m), 1728 (m), 1662 (vs), 1616 (m), 1470 (s), 1377 (m), 1035 (m), 890 (m), 732 (s) $cm^{-1}$; **HRMS** (ESI) for $C_{24}H_{30}N_4O_5F^+$ $[M+H]^+$: calculated: 473.2195; found: 473.2200.

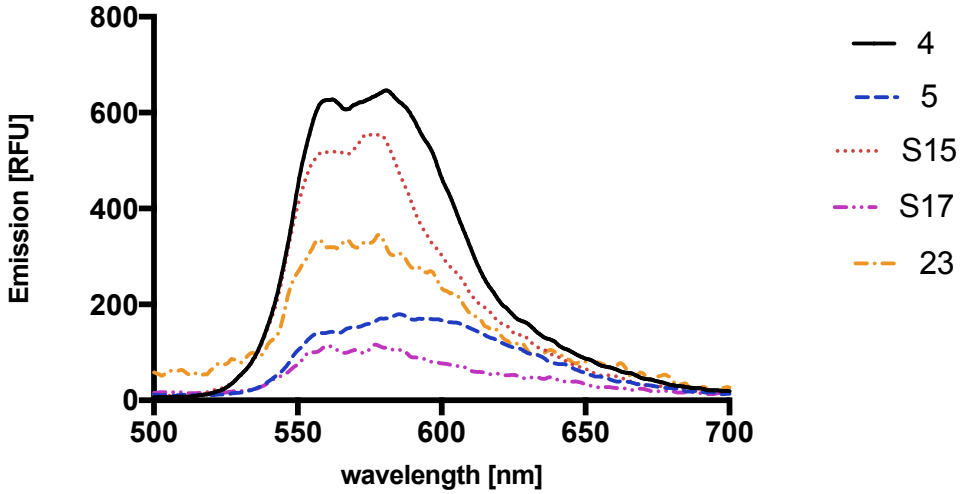



**Figure 4.** Fluorescence emission spectra of phenoxytetrazines **4**, **5**, **S15**, **S17**, **23** (15 μM in MeOH).

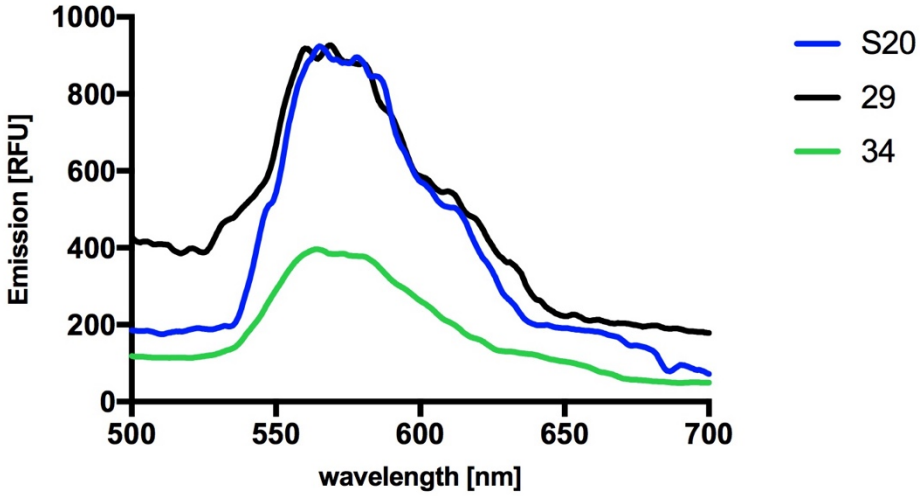
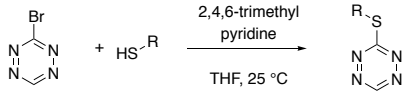


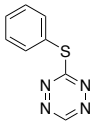

**Figure 5.** Fluorescence emission spectra of ether functionalized tetrazines **S20**, **29**, **34** (15 μM in MeOH).

**General Procedure for the Functionalization of Sulfides (GP2)**




The respective thiol (0.171 mmol, 1.10 equiv) was dissolved in THF (0.75 mL) and 2,4,6-trimethylpyridine (20.6 µL, 0.155 mmol, 1.00 equiv) was added. Then, 3-bromotetrazine (**2**) (25.0 mg, 0.155 mmol, 1.00 equiv), dissolved in THF (0.75 mL), was added to the previously obtained solution over 10 min. After the reaction was complete, the solvent was removed via a stream of nitrogen and the crude product was purified via flash column chromatography on silica gel.

**3-(Phenylthio)-*s*-tetrazine (S22)**

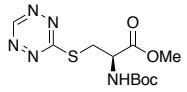



**S22**

Thioether **S22** was synthesized according to **GP2**. After 20 min, the solvent was removed via a stream of nitrogen and the crude product was purified via flash column chromatography (15% $Et_2O$ in pentane) to afford tetrazine **S22** (10.0 mg, 53.0 µmol, 34%) as a red oil.

**$R_f$** (20% $Et_2O$ in pentane) = 0.43 (red spot, UV); **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 9.97 (s, 1H), 7.70–7.67 (m, 2H), 7.55–7.47 (m, 3H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 126 MHz): $\delta$ = 179.3, 156.2, 135.7, 130.8, 130.1, 125.3 ppm; **IR** (neat): $\tilde{\nu}$ = 1476 (s), 1442 (s), 1393 (s), 1215 (vs), 1023 (m), 884 (s), 746 (s) $cm^{-1}$; **HRMS** (EI) for $C_8H_6N_4S^+$: calculated: 190.0313; found: 190.0305.

**Boc-Cys(Tet)-OMe (25)**

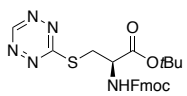



**25**

Thioether **25** was synthesized according to **GP2**. After 1 h 20 min, the solvent was removed via a stream of nitrogen and the crude product was purified via flash column chromatography (40% $Et_2O$ in pentane) to afford tetrazine **25** (45.0 mg, 143 µmol, 92%) as a red oil.

**$R_f$** (40% $Et_2O$ in pentane) = 0.24 (pink spot, UV); **$[\alpha]_D^{20}$** = +48.56 (c = 0.815, $CHCl_3$); **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.00 (s, 1H), 5.37 (d, *J* = 8.1 Hz, 1H), 4.79 (q, *J* = 5.6 Hz, 1H), 3.97 (dd, *J* = 14.0, 5.0 Hz, 1H), 3.78 (s, 3H), 3.70 (dd, *J* = 14.2 Hz, 6.5 Hz, 1H), 1.42 (s, 9H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 101 MHz): $\delta$ = 177.8, 170.8, 156.2, 155.1, 80.7, 53.1, 52.6, 32.9, 28.4 ppm; **IR** (neat): $\tilde{\nu}$ = 3364 (br, m), 2978 (w), 1744 (m), 1710 (vs), 1510 (m), 1367 (m), 1217 (vs), 1161 (vs), 1054 (w), 888 (w) $cm^{-1}$; **HRMS** (ESI) for $C_{11}H_{17}N_5O_4SNa^+$ $[M+Na]^+$: calculated: 338.0893; found: 338.0889.

**Fmoc-Cys(Tet)-O*t*Bu (26)**



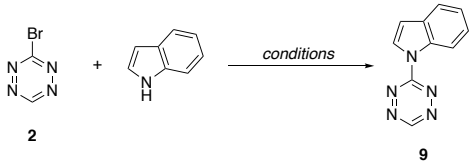

**26**

Thioether **26** was synthesized according to **GP2**. After 3 h, the solvent was removed via a stream of nitrogen and the crude product was purified via flash column chromatography (40% $Et_2O$ in pentane) to afford tetrazine **26** (61.0 mg, 127 µmol, 82%) as a red oil.

**$R_f$** (40% $Et_2O$ in pentane) = 0.23 (pink spot, UV); **$[\alpha]_D^{20}$** = +20.24 (c = 1.885, $CHCl_3$); **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 9.90 (s, 1H), 7.77 (d, *J* = 7.7 Hz, 2H), 7.59–7.51 (m, 2H), 7.41–7.38 (m, 2H), 7.31–7.27 (m, 2H), 5.68 (d, *J* = 7.5 Hz, 1H), 4.76 (q, *J* = 6.1 Hz, 1H), 4.43–4.33 (m, 2H), 4.20 (t, *J* = 7.2 Hz, 1H), 4.09 (dd, *J* = 14.1, 4.6 Hz, 1H), 3.71 (dd, *J* = 14.1, 6.2 Hz, 1H), 1.50 (s, 9H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 101 MHz): $\delta$ = 177.7, 168.7, 156.0, 155.6, 143.7, 141.3, 127.7, 127.1, 125.0, 120.0, 83.8, 67.3, 53.7, 47.1, 32.4, 28.0 ppm; **IR** (neat): $\tilde{\nu}$ = 1707 (s), 1512 (m), 1450 (w), 1342 (m), 1218 (vs), 1152 (s),

1051 (m), 889 (w), 759 (m), 738 (vs) $cm^{-1}$; **HRMS** (ESI) for $C_{24}H_{25}N_5O_4SNa^+$ $[M+Na]^+$: calculated: 502.1519; found: 338.0889.

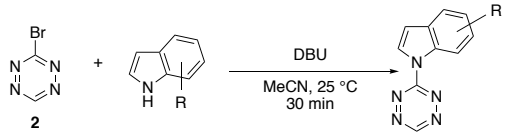
**Table S2.** Screening the nucleophilic aromatic substitution using indole.




| Entry | Base | Equiv base | Solvent | t [h] | Yield [%] |
|---|---|---|---|---|---|
| 1 | $Et_3N$ | 1.5 | MeCN | 1 | traces |
| 2 | $Et_3N$ | 1.5 | $CH_2Cl_2$ | 2 | traces |
| 3 | $Et_3N$ | 1.5 | THF | 2 | decomp. |
| 4 | $Et_3N$ | 1.5 | MeCN | 3 | traces |
| 5 | DBU | 1.2 | MeCN | 1 | 38 |
| 6 | DBU | 1.2 | MeCN | 0.5 | 41 |
| 7[a] | DBU | 1.0 | MeCN | 0.5 | 51 |
| 8 | 2,6-lutidine | 1.2 | MeCN | 4 | decomp. |
| 9[a,b] | DBU | 1.0 | MeCN | 0.5 | 55 |

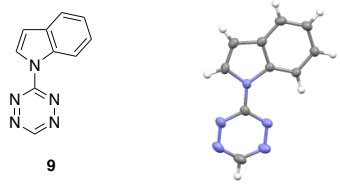
a) reverse addition of 3-bromotetrazine; b) 25 mg scale.

**General Procedure for the Functionalization of Nitrogen-Heterocycles (GP3)**




The respective *N*-heterocycle (0.186 mmol, 1.20 equiv) was dissolved in MeCN (0.75 mL) and DBU (23.1 µL, 0.155 mmol, 1.00 equiv) was added. Then, 3-bromotetrazine (**2**) (25.0 mg, 0.155 mmol, 1.00 equiv) was dissolved in MeCN (0.75 mL) and the resulting solution was added dropwise over 10 min to the previously obtained solution. After completion of the reaction, the mixture was filtered through a pad of celite and the filter cake was rinsed with $CH_2Cl_2$ (50 mL). The solution was concentrated and the resulting crude product was purified via flash column chromatography.

**1-(*S*-tetrazin-3-yl)-1*H*-indole (9)**

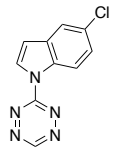



Indole **9** was synthesized according to **GP3**. After 30 min, the reaction mixture was filtered through a pad of celite covered with silica gel and the filter pad was rinsed with $CH_2Cl_2$ (50 mL). The solvent was removed under reduced pressure and the crude product was purified via flash column chromatography on silica gel (4% EtOAc in pentane) to afford tetrazine **9** (16.7 mg, 85.0 µmol, 55%) as a red solid.

$\mathbf{R_f}$ (4% EtOAc in pentane) = 0.32 (red spot, UV, CAM); **melting point** = 103 °C; **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.08 (s, 1H), 8.70 (dd, *J* = 8.3, 0.9 Hz, 1H), 8.33 (dd, *J* = 3.8, 0.4 Hz, 1H), 7.67 (ddd, *J* = 7.7, 1.4, 0.8 Hz, 1H), 7.44–7.40 (m, 1H), 7.36–7.32 (m, 1H), 6.88 (dd, *J* = 3.8, 0.8 Hz, 1H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 126 MHz): $\delta$ = 161.1, 156.2, 135.1, 131.7, 125.2,

124.3, 124.1, 121.6, 116.8, 111.0 ppm; **IR** (neat): $\tilde{\nu}$ = 1500 (vs), 1484 (vs), 1355 (w), 1213 (m), 1092 (m), 749 (m) $cm^{-1}$; **HRMS** (EI) for $C_{10}H_7N_5^+$: calculated: 197.0701; found: 197.0696.

**5-Chloro-1-(*s*-tetrazin-3-yl)-1*H*-indole (10)**

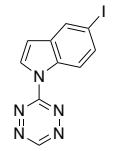


**10**

Indole **10** was synthesized according to **GP3**. After 30 min, the reaction mixture was filtered through a pad of celite covered with silica gel and the filter pad was rinsed with $CH_2Cl_2$ (50 mL). The solvent was removed under reduced pressure and the crude product was purified via flash column chromatography (4% EtOAc in pentane) to afford tetrazine **10** (22.8 mg, 98.0 µmol, 63%) as a red solid.

$R_f$ (4% EtOAc in pentane) = 0.20 (red spot, UV, CAM); **melting point** = 157 – 158 °C; **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.12 (s, 1H), 8.63 (dt, *J* = 8.9, 0.7 Hz, 1H), 8.38 (dd, *J* = 3.8, 0.5 Hz, 1H), 7.64 (dd, *J* = 2.1, 0.6 Hz, 1H), 7.39 (ddd, *J* = 8.9, 2.1, 0.5 Hz, 1H), 6.83 (dd, *J* = 3.8, 0.8 Hz, 1H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 126 MHz): $\delta$ = 160.9, 156.4, 133.3, 132.9, 129.8, 125.6, 125.3, 121.2, 117.7, 110.2 ppm; **IR** (neat): $\tilde{\nu}$ = 1495 (vs), 1478 (vs), 1335 (w), 1205 (m), 963 (w), 931 (w) $cm^{-1}$; **HRMS** (EI) for $C_{10}H_6N_5Cl^+$: calculated: 231.0312; found: 231.0311.

**5-Iodo-1-(*s*-tetrazin-3-yl)-1*H*-indole (S23)**

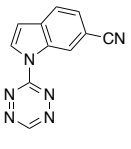


**S23**

Indole **S23** was synthesized according to **GP3**. After 30 min, the reaction mixture was filtered through a pad of celite covered with silica gel and the filter pad was rinsed with $CH_2Cl_2$ (50 mL). The solvent was removed under reduced pressure and the crude product was purified via flash column chromatography (20% $Et_2O$ in pentane) to afford tetrazine **S23** (13.6 mg, 42.0 µmol, 27%) as an orange solid.

$R_f$ (20% $Et_2O$ in pentane) = 0.43 (red spot, UV, CAM); **melting point** = 178 °C; **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.12 (s, 1H), 8.48 (d, *J* = 8.8 Hz, 1H), 8.32 (d, *J* = 3.8 Hz, 1H), 8.00 (d, *J* = 1.8 Hz, 1H), 7.69 (dd, *J* = 8.8, 1.8 Hz, 1H), 6.81 (d, *J* = 3.7 Hz, 1H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 101 MHz): $\delta$ = 160.8, 156.2, 134.1, 133.8, 133.6, 130.3, 125.0, 118.4, 109.7, 88.2 ppm; **IR** (neat): $\tilde{\nu}$ = 1494 (vs), 1477 (vs), 1329 (m), 1202 (m), 1091 (m), 963 (w), 930 (w), 808 (m) $cm^{-1}$; **HRMS** (EI) for $C_{10}H_6N_5I^+$: calculated: 322.9668; found: 322.9663.

**1-(*S*-tetrazin-3-yl)-1*H*-indole-6-carbonitrile (11)**

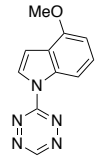


**11**

Indole **11** was synthesized according to **GP3**. After 30 min, the reaction mixture was filtered through a pad of celite covered with silica gel and the filter pad was rinsed with $CH_2Cl_2$ (50 mL). The solvent was removed under reduced pressure and the crude product was purified via flash column chromatography (20% EtOAc in pentane) to afford tetrazine **11** (20.0 mg, 90.0 µmol, 58%) as a red solid.

$R_f$ (20% EtOAc in pentane) = 0.30 (red spot, UV, CAM); **melting point** = 202 – 203 °C; **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.21 (s, 1H), 9.11 (s, 1H), 8.56 (d, *J* = 3.8 Hz, 1H), 7.77 (d, *J* = 8.1 Hz, 1H), 7.60 (dd, *J* = 8.1, 1.4 Hz, 1H), 6.98 (d, *J* = 3.7 Hz, 1H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 126 MHz): $\delta$ = 160.8, 156.7, 134.8, 133.9, 127.5, 127.0, 122.3, 121.1, 119.6, 110.5, 108.1 ppm; **IR** (neat): $\tilde{\nu}$ = 2224 (m), 1524 (w), 1475 (vs), 1466 (vs), 1360 (s), 1261 (m), 1213 (m), 1080 (m), 930 (m) $cm^{-1}$; **HRMS** (EI) for $C_{11}H_6N_6^+$: calculated: 222.0654; found: 222.0653.

**5-Methoxy-1-(*s*-tetrazin-3-yl)-1*H*-indole (S24)**

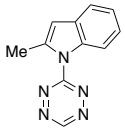



**S24**

Indole **S24** was synthesized according to **GP3**. After 30 min, the reaction mixture was filtered through a pad of celite covered with silica gel and the filter pad was rinsed with $CH_2Cl_2$ (50 mL). The solvent was removed under reduced pressure and the crude product was purified via flash column chromatography (40% $CH_2Cl_2$ in pentane) to afford tetrazine **S24** (17.0 mg, 75.0 µmol, 48%) as red solid.

**$R_f$** (40% $CH_2Cl_2$ in pentane) = 0.50 (red spot, UV, CAM); **melting point** = 143 °C; **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.07 (s, 1H), 8.30 (d, *J* = 8.5 Hz, 1H), 8.23 (d, *J* = 3.8 Hz, 1H), 7.34 (t, *J* = 8.2 Hz, 1H), 7.00 (d, *J* = 3.8 Hz, 1H), 6.78 (d, *J* = 8.1 Hz, 1H), 3.98 (s, 3H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 101 MHz): $\delta$ = 161.1, 156.1, 153.3, 136.2, 126.1, 122.7, 121.9, 109.7, 108.0, 104.5, 55.6 ppm; **IR** (neat): $\tilde{\nu}$ = 1589 (m), 1468 (vs), 1358 (s), 1287 (s), 1265 (s), 1060 (s), 980 (m), 746 (vs) $cm^{-1}$; **HRMS** (EI) for $C_{11}H_9N_5O^+$: calculated: 227.0807; found: 227.0800.

**2-Methyl-1-(*s*-tetrazin-3-yl)-1*H*-indole (S25)**

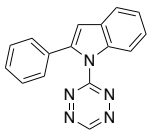



**S25**

Indole **S25** was synthesized according to **GP3**. After 2 h, the reaction mixture was filtered through a pad of celite covered with silica gel and the filter pad was rinsed with $CH_2Cl_2$ (50 mL). The solvent was removed under reduced pressure and the crude product was purified via flash column chromatography (30 – 50% $CH_2Cl_2$ in pentane) to afford tetrazine **S25** (8.4 mg, 40.0 µmol, 26%) as a red oil.

**$R_f$** (40% $CH_2Cl_2$ in pentane) = 0.20 (red spot, UV, CAM); **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.13 (s, 1H), 8.40–8.38 (m, 1H), 7.56–7.53 (m, 1H), 7.33–7.28 (m, 2H), 6.59 (m, 1H), 2.79 (s, 3H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 126 MHz): $\delta$ = 162.5, 155.7, 137.3, 136.4, 130.3, 123.8, 123.7, 120.1, 114.7, 110.4, 17.1 ppm; **IR** (neat): $\tilde{\nu}$ = 1601 (w), 1572 (w), 1457 (vs), 1403 (w), 1214 (m), 1129 (m), 932 (w) $cm^{-1}$; **HRMS** (EI) for $C_{11}H_9N_5^+$: calculated: 211.0850; found: 211.0858.

**2-Phenyl-1-(*s*-tetrazin-3-yl)-1*H*-indole (S26)**

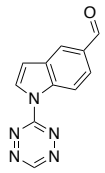



**S26**

Indole **S26** was synthesized according to **GP3**. After 2 h, the reaction mixture was filtered through a pad of celite covered with silica gel and the filter pad was rinsed with $CH_2Cl_2$ (50 mL). The solvent was removed under reduced pressure and the crude product was purified via flash column chromatography (40% $CH_2Cl_2$ in pentane) to afford tetrazine **S26** (8.4 mg, 31.0 µmol, 20%) as a red oil.

**$R_f$** (30% $CH_2Cl_2$ in pentane) = 0.09 (red spot, UV, CAM); **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.07 (s, 1H), 8.31 (ddd, *J* = 7.4, 1.9, 0.7 Hz, 1H), 7.72–7.69 (m, 1H), 7.41–7.34 (m, 5H), 7.28–7.26 (m, 2H), 6.95 (d, *J* = 0.8 Hz, 1H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 126 MHz): $\delta$ = 162.7, 155.9, 140.3, 137.6, 132.8, 130.3, 128.7, 128.4, 128.2, 125.0, 124.1, 121.3, 113.5, 111.6 ppm; **IR** (neat): $\tilde{\nu}$ = 1454 (vs), 1446 (vs), 1394 (m), 1345 (s), 1220 (m), 1173 (m), 913 (m), 745 (s) $cm^{-1}$; **HRMS** (EI) for $C_{16}H_{11}N_5^+$: calculated: 273.1014; found: 273.1014.

**1-(*S*-tetrazin-3-yl)-1*H*-indole-5-carbaldehyde (S27)**

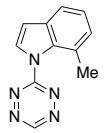


**S27**

Indole **S27** was synthesized according to **GP3**. After 30 min, the reaction mixture was filtered through a pad of celite covered with silica gel and the filter pad was rinsed with $CH_2Cl_2$ (50 mL). The solvent was removed under reduced pressure and the crude product was purified via flash column chromatography (30% EtOAc in pentane) to afford tetrazine **S27** (5.0 mg, 22.0 µmol, 14%) as a red solid.

**$R_f$** (30% EtOAc in pentane) = 0.44 (red spot, UV, CAM); **melting point** = 202 – 203 °C; **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.20 (s, 1H), 10.13 (s, 1H), 8.90 (d, *J* = 8.7 Hz, 1H), 8.49 (d, *J* = 3.8 Hz, 1H), 8.22 (s, 1H), 7.99 (dd, *J* = 8.7, 1.6 Hz, 1H), 7.04 (d, *J* = 3.9 Hz, 1H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 126 MHz): $\delta$ = 192.0, 161.2, 156.7, 138.4, 132.8, 132.0, 126.2, 126.2, 124.5, 117.2, 111.3 ppm; **IR** (neat): $\tilde{\nu}$ = 1691 (vs), 1609 (w), 1478 (vs), 1335 (m), 1218 (w), 1077 (w), 918 (w) $cm^{-1}$; **HRMS** (EI) for $C_{11}H_7N_5O^+$: calculated: 225.0651; found: 225.0644.

**7-Methyl-1-(*s*-tetrazin-3-yl)-1*H*-indole (S28)**

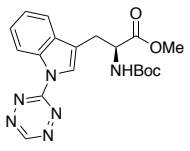


**S28**

Indole **S28** was synthesized according to **GP3**. After 30 min, the reaction mixture was filtered through a pad of celite covered with silica gel and the filter pad was rinsed with $CH_2Cl_2$ (50 mL). The solvent was removed under reduced pressure and the crude product was purified via flash column chromatography (40% $CH_2Cl_2$ in pentane) to afford tetrazine **S28** (9.0 mg, 43.0 µmol, 28%) as a red oil.

**$R_f$** (40% $CH_2Cl_2$ in pentane) = 0.26 (red spot, UV, CAM); **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.12 (s, 1H), 7.99 (d, *J* = 3.7 Hz, 1H), 7.54 (d, *J* = 7.6 Hz, 1H), 7.29–7.19 (m, 2H), 6.91 (d, *J* = 3.70 (s, 1H), 2.41 (s, 3H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 126 MHz): $\delta$ = 161.5, 156.2, 134.5, 132.3, 128.7, 128.0, 124.8, 124.0, 119.3, 110.9, 22.6 ppm; **IR** (neat): $\tilde{\nu}$ = 1457 (vs), 1352 (m), 1232 (w), 1071 (w), 786 (m) $cm^{-1}$; **HRMS** (EI) for $C_{11}H_9N_5^+$: calculated: 211.0858; found: 211.0850.

**Boc-Trp(Tet)-OMe (21)**

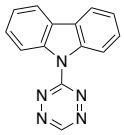


**21**

Indole **21** was synthesized according to **GP3**. After 30 min, the reaction mixture was filtered through a pad of celite covered with silica gel and the filter pad was rinsed with $CH_2Cl_2$ (50 mL). The solvent was removed under reduced pressure and the crude product was purified via flash column chromatography (20% EtOAc in pentane) to afford tetrazine **21** (26.5 mg, 66.0 µmol, 43%) as a red solid.

**$R_f$** (20% EtOAc in pentane) = 0.18 (red spot, UV, CAM); **melting point** = 123 – 124 °C; **$[\alpha]_D^{20}$** = +72.30 (c = 0.47, $CHCl_3$); **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.06 (s, 1H), 8.70 (d, *J* = 8.3 Hz, 1H), 8.15 (s, 1H), 7.62 (d, *J* = 7.6 Hz, 1H), 7.43 (t, *J* = 7.4 Hz, 1H), 7.35 (t, *J* = 7.6 Hz, 1H), 5.21 (d, *J* = 8.0 Hz, 1H), 4.73 (q, *J* = 6.3 Hz, 1H), 3.73 (s, 3H), 3.42–3.27 (m, 2H), 1.44 (s, 9H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 126 MHz): $\delta$ = 172.3, 160.8, 156.0, 155.2, 135.2, 131.9, 125.6, 124.0, 122.3, 119.6, 119.2, 116.9, 80.3, 53.8, 52.7, 28.5, 28.2 ppm; **IR** (neat): $\tilde{\nu}$ = 3355 (br m), 2879 (w), 1735 (m), 1685 (s), 1492 (s), 1470 (vs), 1366 (m), 1250 (m), 1163 (s), 1100 (s), 746 (s) $cm^{-1}$; **HRMS** (ESI) for $C_{19}H_{22}N_6O_4Na^+$ [M+Na]$^+$: calculated: 421.1595; found: 421.1591.

**9-(*S*-tetrazin-3-yl)-9*H*-carbazole (12)**

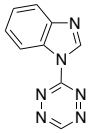


**12**

Carbazole **12** was synthesized according to **GP3**. After 30 min, the reaction mixture was filtered through a pad of celite covered with silica gel and the filter pad was rinsed with $CH_2Cl_2$ (50 mL). The solvent was removed under reduced pressure and the crude product was purified via flash column chromatography (30% $CH_2Cl_2$ in pentane) to afford tetrazine **12** (32.0 mg, 129 µmol, 83%) as a red solid.

**$R_f$** (30% $Et_2O$ in pentane) = 0.56 (red spot, UV, CAM); **melting point** = 179 °C; **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.12 (s, 1H), 8.85 (d, *J* = 8.5 Hz, 2H), 8.08 (dd, *J* = 7.6, 1.4 Hz, 2H), 7.54 (dd, *J* = 8.5, 7.3 Hz, 2H), 7.45 (td, *J* = 7.5, 1.0 Hz, 2H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 126 MHz): $\delta$ = 163.0, 155.3, 138.1, 127.6, 127.1, 124.4, 120.0, 117.3 ppm; **IR** (neat): $\tilde{\nu}$ = 1495 (s), 1460 (vs), 1337 (m), 1212 (m), 750 (s) $cm^{-1}$; **HRMS** (EI) for $C_{14}H_9N_5^+$: calculated: 247.0858; found: 247.0850.

**1-(*S*-tetrazin-3-yl)-1*H*-benzo[*d*]imidazole (S29)**

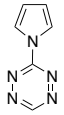


**S29**

Imidazole **S29** was synthesized according to **GP3**. After 30 min, the reaction mixture was filtered through a pad of celite covered with silica gel and the filter pad was rinsed with $CH_2Cl_2$ (50 mL). The solvent was removed under reduced pressure and the crude product was purified via flash column chromatography (40% EtOAc in pentane) to afford tetrazine **S29** (23.0 mg, 116 µmol, 75%) as a red solid.

**$R_f$** (40% EtOAc in pentane) = 0.40 (red spot, UV, CAM); **melting point** = 166 – 167 °C; **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.28 (s, 1H), 9.19 (s, 1H), 8.55–8.53 (m, 1H), 7.91–7.89 (m, 1H), 7.52–7.44 (m, 2H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 101 MHz): $\delta$ = 160.4, 157.7, 145.1, 140.2, 130.9, 126.1, 125.5, 121.3, 115.8 ppm; **IR** (neat): $\tilde{\nu}$ = 1608 (w), 1470 (vs), 1296 (s), 1246 (m), 1207 (s), 1097 (m), 737 (m) $cm^{-1}$; **HRMS** (EI) for $C_9H_6N_6^+$: calculated: 198.0654; found: 198.0645.

**3-(1*H*-pyrrol-1-yl)-*s*-tetrazine (S30)**

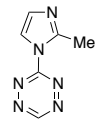


**S30**

Pyrrole **S30** was synthesized according to **GP3**. After 30 min, the reaction mixture was filtered through a pad of celite covered with silica gel and the filter pad was rinsed with $CH_2Cl_2$ (50 mL). The solvent was removed under reduced pressure and the crude product was purified via flash column chromatography (5% $Et_2O$ in pentane) to afford tetrazine **S30** (2.0 mg, 14.0 µmol, 9%) as an orange solid.

**$R_f$** (5% $Et_2O$ in pentane) = 0.31 (orange spot); **melting point** = 113 °C; **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.10 (s, 1H), 7.92–7.90 (m, 2H), 6.52–6.51 (m, 2H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 126 MHz): $\delta$ = 159.6, 157.3, 119.1, 115.0 ppm; **IR** (neat): $\tilde{\nu}$ = 3147 (vs), 1519 (vs), 1378 (m), 1257 (w), 1070 (m), 932 (s), 744 (vs) $cm^{-1}$; **HRMS** (EI) for $C_6H_5N_5^+$: calculated: 147.0545; found: 147.0537.

**3-(2-methyl-1*H*-imidazol-1-yl)-*s*-tetrazine (S31)**

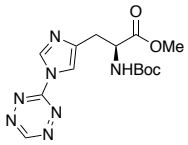


**S31**

Imidazole **S31** was synthesized according to **GP3**. After 30 min, the reaction mixture was filtered through a pad of celite covered with silica gel and the filter pad was rinsed with $CH_2Cl_2$ (50 mL). The solvent was removed under reduced pressure and the crude product was purified via flash column chromatography (50% EtOAc in pentane) to afford tetrazine **S31** (4.0 mg, 25.0 µmol, 16%) as a red oil.

**$R_f$** (50% EtOAc in pentane) = 0.20 (red spot, UV, CAM); **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.26 (s, 1H), 8.05 (d, *J* = 1.8 Hz, 1H), 7.14 (d, *J* = 1.8 Hz, 1H), 2.92 (s, 3H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 101 MHz): $\delta$ = 160.5, 157.5, 147.4, 130.2, 117.8, 18.4 ppm; **IR** (neat): $\tilde{\nu}$ = 1553 (w), 1509 (w), 1458 (vs), 1285 (s), 1137 (m), 981 (w), 926 (w) cm$^{-1}$; **HRMS** (EI) for $C_6H_6N_6^+$: calculated: 162.0654; found: 162.0651.

**Boc-His(Tet)-OMe (22)**



**22**

Histidine **22** was synthesized according to **GP3**. After 30 min, the reaction mixture was filtered through a pad of celite covered with silica gel and the filter pad was rinsed with $CH_2Cl_2$ (50 mL). The solvent was removed under reduced pressure and the crude product was purified via flash column chromatography (50% EtOAc in pentane) to afford tetrazine **22** (15.0 mg, 43.0 µmol, 28%) as a red oil.

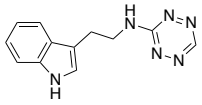
**$R_f$** (50% EtOAc in pentane) = 0.26 (red spot, UV, CAM); **$[\alpha]_D^{20}$** = +35.94 (c = 1.16, $CHCl_3$); **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.10 (s, 1H), 8.65 (s, 1H), 7.76 (s, 1H), 5.64 (d, *J* = 8.3 Hz, 1H), 4.63–4.58 (m, 1H), 3.69 (s, 3H), 3.13–3.08 (m, 2H), 1.38 (s, 9H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 101 MHz): $\delta$ = 172.2, 158.5, 158.3, 155.4, 141.4, 135.6, 113.3, 79.9, 53.0, 52.4, 30.5, 28.3 ppm; **IR** (neat): $\tilde{\nu}$ = 2977 (w), 1743 (m), 1708 (vs), 1479 (vs), 1366 (m), 1308 (m), 1164 (s), 1061 (m), 919 (w) cm$^{-1}$; **HRMS** (ESI) for $C_{14}H_{20}N_7O_4^+$ $[M+H]^+$: calculated: 350.1571; found: 350.1576.

**General Procedure for the Functionalization of Amines (GP4)**


**2**

The amine (0.310 mmol, 2.00 equiv) was dissolved in THF (0.75 mL) and 3-bromotetrazine (**2**) (25.0 mg, 0.155 mmol, 1.00 equiv), dissolved in THF (0.75 mL), was added dropwise over 10 min to the previously obtained solution. The solvent was removed via a stream of nitrogen and the crude material was purified via flash column chromatography.

***N*-(2-(1*H*-indol-3-yl)ethyl)-*s*-tetrazin-3-amine (13)**



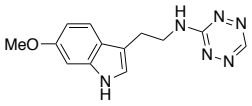
**13**

Tetrazine **13** was synthesized according to **GP4**. After 30 min, the reaction mixture was filtered through a pad of celite and the filter cake was rinsed with EtOAc (50 mL). The solvent was removed and the crude product was purified via flash column chromatography (50% EtOAc in pentane) to afford tetrazine **13** (32 mg, 134 µmol, 86%) as an orange solid.

**$R_f$** (50% EtOAc in pentane) = 0.48 (orange spot, UV); **melting point** = 183 °C; **$^1$H-NMR** (DMSO-*d6*, 400 MHz): $\delta$ = 10.83 (br s, 1H), 9.72 (s, 1H), 8.70 (t, *J* = 5.9 Hz, 1H), 7.57 (d, *J* = 7.9 Hz, 1H), 7.35 (d, *J* = 8.1 Hz, 1H), 7.21 (d, *J* = 2.3 Hz, 1H),

7.06 (dd, *J* = 8.1, 6.9 Hz, 1H), 6.98 (dd, *J* = 7.9, 7.0 Hz, 1H), 3.70 (q, *J* = 6.5 Hz, 2H), 3.03 (t, *J* = 7.5 Hz, 2H) ppm; **$^{13}$C-NMR** (DMSO-*d6*, 101 MHz): $\delta$ = 162.6, 152.6, 136.2, 127.2, 122.9, 120.9, 118.3, 118.2, 111.4, 111.3, 41.1, 24.3 ppm; **IR** (neat): $\tilde{\nu}$ = 3251 (br m), 1591 (s), 1505 (m), 1338 (m), 1100 (m), 1075 (m), 960 (s), 743 (vs) cm$^{-1}$; **HRMS** (EI) for $C_{12}H_{12}N_6^+$: calculated: 240.1123; found: 240.1113.

***N*-(2-(6-methoxy-1*H*-indol-3-yl)ethyl)-*s*-tetrazin-3-amine (14)**

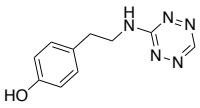


**14**

Tetrazine **14** was synthesized according to **GP4**. After 30 min, the reaction mixture was filtered through a pad of celite and the filter cake was rinsed with EtOAc (50 mL). The solvent was removed and the crude product was purified via flash column chromatography (50% EtOAc in pentane) to afford tetrazine **14** (32.0 mg, 118 µmol, 76%) as a brown solid.

**R$_f$** (50% EtOAc in pentane) = 0.52 (orange spot, UV); **melting point** = 167 °C; **$^1$H-NMR** (DMSO-*d6*, 400 MHz): $\delta$ = 10.62 (br s, 1H), 9.72 (s, 1H), 8.68 (t, *J* = 5.9 Hz, 1H), 7.43 (d, *J* = 8.6 Hz, 1H), 7.05 (d, *J* = 2.2 Hz, 1H), 6.83 (d, *J* = 2.2 Hz, 1H), 6.66 (dd, *J* = 8.6, 2.3 Hz, 1H), 3.75 (s, 3H), 3.69 (q, *J* = 6.6 Hz, 2H), 2.98 (t, *J* = 7.5 Hz, 2H) ppm; **$^{13}$C-NMR** (DMSO-*d6*, 101 MHz): $\delta$ = 162.6, 155.5, 153.6, 136.9, 121.6, 121.4, 118.8, 111.3, 108.5, 94.5, 55.1, 41.1, 24.4 ppm; **IR** (neat): $\tilde{\nu}$ = 3230 (br m), 1632 (m), 1589 (vs), 1501 (m), 1456 (m), 1257 (m), 1160 (vs), 967 (vs) cm$^{-1}$; **HRMS** (EI) for $C_{13}H_{14}N_6O^+$: calculated: 270.1229; found: 270.1220.

**4-(2-((*S*-tetrazin-3-yl)amino)ethyl)phenol (15)**

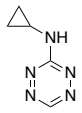


**15**

Tetrazine **15** was synthesized according to **GP4**. After 30 min, the solvent was removed via a stream of nitrogen and the crude product was purified via flash column chromatography (5% MeOH in $CH_2Cl_2$) to afford tetrazine **15** (23.2 mg, 107 µmol, 69%) as an orange solid.

**R$_f$** (5% MeOH in $CH_2Cl_2$) = 0.65 (orange spot, UV); **melting point** = 209 – 210 °C; **$^1$H-NMR** (DMSO-*d6*, 400 MHz): $\delta$ = 9.71 (s, 1H), 9.17 (s, 1H), 8.63 (t, *J* = 5.8 Hz, 1H), 7.05 (d, *J* = 8.4 Hz, 2H), 6.68 (d, *J* = 8.4 Hz, 2H), 3.59 (q, *J* = 6.4 Hz, 2H), 2.79 (t, *J* = 7.7 Hz, 2H) ppm; **$^{13}$C-NMR** (DMSO-*d6*, 101 MHz): $\delta$ = 200.3, 193.4, 190.3, 167.2, 166.6, 152.8, 79.7, 71.1 ppm; **IR** (neat): $\tilde{\nu}$ = 3097 (br s), 1614 (w), 1593 (vs), 1514 (vs), 1455 (s), 1345 (m9; 1230 (m), 1211 (m), 1071 (w), 833 (s) cm$^{-1}$; **HRMS** (EI) for $C_{10}H_{11}N_5O^+$: calculated: 217.0964; found: 217.0960.

***N*-cyclopropyl-*s*-tetrazin-3-amine (S32)**

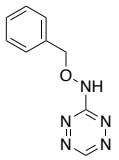


**S32**

Tetrazine **S32** was synthesized according to **GP4**. After 30 min, the solvent was removed via a stream of nitrogen and the crude product was purified via flash column chromatography (50% – 60% $Et_2O$ in pentane) to afford tetrazine **S32** (19.0 mg, 139 µmol, 90%) as a red solid.

**R$_f$** (60% $Et_2O$ in pentane) = 0.43 (orange spot, UV); **melting point** = 98 – 99 °C; **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 9.70 (s, 1H), 6.27 (br s, 1H), 2.97–2.91 (m, 1H), 1.00–0.95 (m, 2H), 0.71–0.67 (m, 2H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 126 MHz): $\delta$ = 164.2, 153.9, 23.8, 7.7 ppm; **IR** (neat): $\tilde{\nu}$ = 3242 (s), 3099 (m), 1574 (vs), 1494 (vs), 1381 (m), 1121 (s), 1029 (w), 957 (s), 944 (s) cm$^{-1}$; **HRMS** (EI) for $C_5H_7N_5^+$: calculated: 137.0701; found: 137.0694.

***O*-benzyl-*N*-(*s*-tetrazin-3-yl)hydroxylamine (S33)**

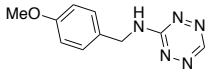


**S33**

Tetrazine **S33** was synthesized according to **GP4**. After 30 min, the solvent was removed via a stream of nitrogen and the crude product was purified via flash column chromatography (30% – 40% $Et_2O$ in pentane) to afford tetrazine **S33** (18.2 mg, 90.0 µmol, 58%) as a red solid.

**$R_f$** (30% $Et_2O$ in pentane) = 0.27 (red spot, UV); **melting point** = 87 °C; **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 9.88 (s, 1H), 8.35 (br s, 1H), 7.50–7.48 (m, 2H), 7.43–7.37 (m, 3H), 5.13 (s, 2H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 126 MHz): $\delta$ = 166.1, 155.9, 135.1, 129.5, 129.1, 128.8, 78.9 ppm; **IR** (neat): $\tilde{\nu}$ = 3153 (br m), 2945 (m), 2875 (m), 1550 (s), 1498 (vs), 1465 (s), 1383 (m), 1133 (s), 955 (vs), 868 (s) $cm^{-1}$; **HRMS** (EI) for $C_9H_9N_5O^+$: calculated: 203.0807; found: 203.0798.

***N*-(4-methoxybenzyl)-*s*-tetrazin-3-amine (S34)**

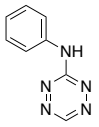


**S34**

Tetrazine **S34** was synthesized according to **GP4**. After 2 h, the solvent was removed via a stream of nitrogen and the crude product was purified via flash column chromatography ($CH_2Cl_2$) to afford tetrazine **S34** (32.0 mg, 147 µmol, 95%) as an orange solid.

**$R_f$** (20% EtOAc in pentane) = 0.28 (orange spot, UV); **melting point** = 132 – 134 °C; **$^1$H-NMR** (DMSO-*d6*, 400 MHz): $\delta$ = 9.75 (s, 1H), 9.08 (t, *J* = 6.2 Hz, 1H), 7.31 (d, *J* = 8.7 Hz, 2H), 6.90 (d, *J* = 8.7 Hz, 2H), 4.57 (d, *J* = 6.3 Hz, 2H), 3.72 (s, 3H) ppm; **$^{13}$C-NMR** (DMSO-*d6*, 101 MHz): $\delta$ = 200.2, 196.0, 190.5, 167.8, 166.4, 151.4, 92.7, 80.6 ppm; **IR** (neat): $\tilde{\nu}$ = 3079 (w), 1580 (vs), 1512 (vs), 1442 (m), 1248 (vs), 1227 (s), 957 (vs), 817 (s) $cm^{-1}$; **HRMS** (EI) for $C_{10}H_{11}N_5O^+$: calculated: 217.0964; found: 217.0953.

***N*-phenyl-*s*-tetrazin-3-amine (16)**



**16**

Tetrazine **16** was prepared according to **GP4**. After 30 min, the solvent was removed via a stream of nitrogen and the crude product was purified via flash column chromatography on silica gel (80% $CH_2Cl_2$ in pentane to 100% $CH_2Cl_2$) to afford tetrazine **16** (23.3 mg, 135 µmol, 87%) as an orange, crystalline solid.

**$R_f$** ($CH_2Cl_2$) = 0.29 (red spot, UV); **melting point** = 199 – 202°C; **$^1$H-NMR** (DMSO-$d_6$, 400 MHz): $\delta$ = 10.84 (br s, 1H), 9.96 (s, 1H), 7.74 (d, *J* = 7.8 Hz, 2H), 7.40 (t, *J* = 7.6 Hz, 2H), 7.12 (t, *J* = 7.4 Hz, 1H) ppm; **$^{13}$C-NMR** (DMSO-$d_6$, 101 MHz): $\delta$ = 162.1, 153.7, 138.0, 128.9, 123.5, 120.1 ppm; **IR** (neat): $\tilde{\nu}$ = 3274 (m), 3113 (w), 1612 (s), 1575 (vs), 1508 (vs), 1449 (w), 1121 (w), 946 (m) $cm^{-1}$; **HRMS** (ESI) for $C_8H_7N_5Na^+$ $[M+Na]^+$: calculated: 196.0594; found: 196.0594.

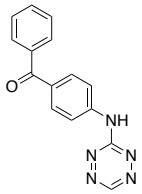
***N*-(4-fluorophenyl)-*s*-tetrazin-3-amine (17)**

**17**

Tetrazine **17** was synthesized according to **GP4**. After 30 min, the solvent was removed via a stream of nitrogen and the crude product was purified via flash column chromatography on silica gel (25% EtOAc in pentane) to afford tetrazine **17** (17.0 mg, 89.0 µmol, 57%) as a red, crystalline solid.

**$R_f$** (20% EtOAc in pentane) = 0.37 (red spot, UV); **melting point** = 271 – 273 °C; **$^{19}$F-NMR** (DMSO-$d_6$, 376 MHz): $\delta$ = –119.07 ppm; **$^{1}$H-NMR** (DMSO-$d_6$, 400 MHz): $\delta$ = 10.85 (s, 1H), 9.96 (s, 1H), 7.75–7.72 (m, 2H), 7.24 (t, $J$ = 8.9 Hz, 2H) ppm; **$^{13}$C-NMR** (DMSO-$d_6$, 101 MHz): $\delta$ = 162.0, 159.5 (d, $J$ = 240 Hz), 153.7, 134.4 (d, $J$ = 2.6 Hz), 122.4 (d, $J$ = 7.8 Hz), 115.6 ppm (d, $J$ = 22.4 Hz); **IR** (neat): $\tilde{\nu}$ = 3244 (w), 3082 (m), 2922 (m), 1621 (m), 1571 (s), 1510 (vs), 1418 (m), 1251 (s), 1121 (m), 957 (m), 830 (s) cm$^{-1}$; **HRMS** (EI) for $C_8H_6N_5F^+$: calculated: 191.0607; found: 191.0602.

**(4-((*S*-tetrazin-3-yl)amino)phenyl)(phenyl)methanone (S35)**

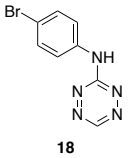


**S35**

Tetrazine **S35** was synthesized according to **GP4**. After 24 h, the solvent was removed via a stream of nitrogen and the crude product was purified via flash column chromatography on silica gel (25% EtOAc in pentane) to afford tetrazine **S35** (10.0 mg, 36.0 µmol, 23%) as an orange solid.

**$R_f$** (20% EtOAc in pentane) = 0.22 (red spot, UV); **melting point** = 196 – 198 °C; **$^{1}$H-NMR** (DMSO-$d_6$, 400 MHz): $\delta$ = 11.30 (s, 1H), 10.08 (s, 1H), 7.96 (d, $J$ = 8.9 Hz, 2H), 7.84 (d, $J$ = 8.9 Hz, 2H), 7.75 (d, $J$ = 7.2 Hz, 2H), 7.67 (t, $J$ = 7.4 Hz, 1H), 7.57 (t, $J$ = 7.5 Hz, 2H) ppm; **$^{13}$C-NMR** (DMSO-$d_6$, 101 MHz): $\delta$ = 194.5, 162.2, 154.1, 142.5, 137.5, 132.3, 131.2, 129.4, 128.5, 118.9 ppm; **IR** (neat): $\tilde{\nu}$ = 3267 (w), 1652 (vs), 1603 (s), 1558 (m), 1501 (s), 1319 (s), 1278 (vs) 1116 (vs), 1055 (vs), 938 (vs), 797 (s) cm$^{-1}$; **HRMS** (EI) for $C_{15}H_{11}N_5ONa^+$: calculated: 300.0856; found: 300.0853.

***N*-(4-bromophenyl)-*s*-tetrazin-3-amine (18)**

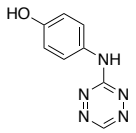


**18**

Tetrazine **18** was synthesized according to **GP4**. After 30 min, the solvent was removed via a stream of nitrogen and the crude product was purified via flash column chromatography on silica gel (25% EtOAc in pentane) to afford tetrazine **18** (29.0 mg, 115 µmol, 74%) as a red solid.

**$R_f$** (20% EtOAc in pentane) = 0.48 (red spot, UV); **melting point** = 214 – 216°C; **$^{1}$H-NMR** (DMSO-$d_6$, 400 MHz): $\delta$ = 10.98 (br s, 1H), 10.00 (s, 1H), 7.73 (d, $J$ = 8.9 Hz, 2H), 7.59 (d, $J$ = 8.9 Hz, 2H) ppm; **$^{13}$C-NMR** (DMSO-$d_6$, 101 MHz): $\delta$ = 162.0, 153.8, 137.5, 131.7, 121.8, 115.1 ppm; **IR** (neat): $\tilde{\nu}$ = 3256 (w), 3106 (w), 1620 (m), 1586 (vs), 1523 (vs), 1447 (s), 1358 (m), 1242 (vs), 958 (vs), 835 (vs) cm$^{-1}$; **HRMS** (EI) for $C_8H_6N_5Br^+$: calculated: 250.9807; found: 250.9801.

**4-((*S*-tetrazin-3-yl)amino)phenol (19)**

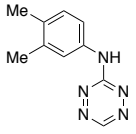


**19**

Tetrazine **19** was synthesized according to **GP4**. After 1 h, the solvent was removed via a stream of nitrogen and the crude product was purified via flash column chromatography on silica gel (25% EtOAc in pentane) to afford tetrazine **19** (11.0 mg, 58.0 µmol, 37%) as a red-brown solid.

**R$_f$** (20% EtOAc in pentane) = 0.11 (red spot, UV); **melting point** = 271 – 273 °C; **$^1$H-NMR** (DMSO-$d_6$, 400 MHz): $\delta$ = 10.51 (br s), 9.85 (s, 1H), 9.34 (s, 1H), 7.47 (d, $J$ = 8.8 Hz, 2H), 6.79 (d, $J$ = 8.9 Hz, 2H) ppm; **$^{13}$C-NMR** (DMSO-$d_6$, 101 MHz): $\delta$ = 161.9, 154.0, 153.3, 129.1, 122.5, 115.3 ppm; **IR** (neat): $\tilde{\nu}$ = 3256 (w), 3106 (w), 1620 (m), 1586 (vs), 1523 (vs), 1447 (s), 1358 (w), 1242 (vs), 1179 (m), 958 (vs), 835 (vs) cm$^{-1}$; **HRMS** (EI) for $C_8H_7N_5O^+$: calculated: 189.0651; found: 189.0642.

***N*-(3,4-dimethylphenyl)-*s*-tetrazin-3-amine (S36)**

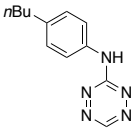


**S36**

Tetrazine **S36** was synthesized according to **GP4**. After 2 h, the solvent was removed via a stream of nitrogen and the crude product was purified via flash column chromatography on silica gel (10% EtOAc in pentane) to afford tetrazine **S36** (21.0 mg, 104 µmol, 67%) as a red solid.

**R$_f$** (20% EtOAc in pentane) = 0.64 (red spot, UV); **melting point** = 157 – 158 °C; **$^1$H-NMR** (DMSO-$d_6$, 400 MHz): $\delta$ = 10.67 (br s, 1H), 9.91 (s, 1H), 7.48 (d, $J$ = 2.3 Hz, 1H), 7.46 (dd, $J$ = 8.1, 2.4 Hz, 1H), 7.15 (d, $J$ = 8.1 Hz, 1H), 2.23 (s, 3H), 2.20 (s, 3H) ppm; **$^{13}$C-NMR** (DMSO-$d_6$, 101 MHz): $\delta$ = 162.0, 153.4, 136.5, 135.6, 131.5, 129.7, 121.4, 117.8, 19.7, 18.8 ppm; **IR** (neat): $\tilde{\nu}$ = 3272 (w), 3109 (w), 2921 (w), 1618 (m), 1606 (s), 1563 (s), 1494 (vs), 1457 (s), 1112 (m), 1051 (m), 943 (s), 885 (w) cm$^{-1}$; **HRMS** (EI) for $C_{10}H_{11}N_5^+$: calculated: 201.1014; found: 201.1005.

***N*-(4-butylphenyl)-*s*-tetrazin-3-amine (20)**

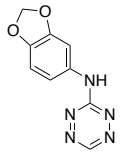


**20**

Tetrazine **20** was synthesized according to **GP4**. After 2 h, the solvent was removed via a stream of nitrogen and the crude product was purified via flash column chromatography on silica gel (40% EtOAc in pentane) to afford tetrazine **20** (22.0 mg, 96.0 µmol, 62%) as a red solid.

**R$_f$** (20% EtOAc in pentane) = 0.76 (red spot, UV); **melting point** = 174 – 177 °C; **$^1$H-NMR** (DMSO-$d_6$, 400 MHz): $\delta$ = 10.74 (br s, 1H), 9.92 (s, 1H), 7.63 (d, $J$ = 8.5 Hz, 2H), 7.21 (d, $J$ = 8.5 Hz, 2H), 2.56 (t, $J$ = 7.6 Hz, 2H), 1.58–1.51 (m, 2H), 1.35–1.26 (m, 2H), 0.90 (t, $J$ = 7.3 Hz, 3H) ppm; **$^{13}$C-NMR** (DMSO-$d_6$, 101 MHz): $\delta$ = 162.0, 153.5, 137.7, 135.6, 128.6, 120.2, 34.2, 33.2, 21.7, 13.8 ppm; **IR** (neat): $\tilde{\nu}$ = 3245 (w), 1077 (m), 1923 (m), 1612 (s), 1557 (s), 1502 (vs), 1463 (vs), 1393 (m), 1237 (w), 1116 (s) 961 (s), 842 (vs) cm$^{-1}$; **HRMS** (EI) for $C_{12}H_{15}N_5^+$: calculated: 229.1327; found: 229.1322.

***N*-(benzo[*d*][1,3]dioxol-5-yl)-*s*-tetrazin-3-amine (S37)**

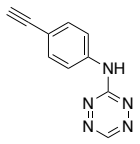


**S37**

Tetrazine **S37** was synthesized according to **GP4**. After 20 h, the solvent was removed via a stream of nitrogen and the crude product was purified via flash column chromatography on silica gel (40% EtOAc in pentane) to afford tetrazine **S37** (17.0 mg, 78.0 µmol, 50%) as a red solid.

**R$_f$** (20% EtOAc in pentane) = 0.37 (red spot, UV); **melting point** = 231 – 234 °C; **$^1$H-NMR** (DMSO-$d_6$, 400 MHz): $\delta$ = 10.69 (br s, 1H), 9.91 (s, 1H), 7.32 (d, $J$ = 2.2 Hz, 1H), 7.15 (dd, $J$ = 8.4, 2.2 Hz, 1H), 6.95 (d, $J$ = 8.4 Hz, 1H), 6.03 (s, 2H) ppm; **$^{13}$C-NMR** (DMSO-$d_6$, 101 MHz): $\delta$ = 162.0, 153.5, 147.3, 143.5, 132.0, 113.6, 108.2, 102.6, 101.2 ppm; **IR** (neat): $\tilde{\nu}$ = 1638 (w), 1580 (vs), 1502 (vs), 1455 (vs), 1261 (m), 1194 (m), 1034 (s), 924 (vs), 807 (s), 787 (s) cm$^{-1}$; **HRMS** (EI) for $C_9H_7N_5O_2^+$: calculated: 217.0600; found: 217.0591.

***N*-(4-ethynylphenyl)-*s*-tetrazin-3-amine (S38)**

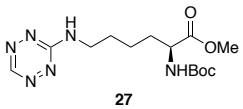


**S38**

Tetrazine **S38** was synthesized according to **GP4**. After 2 h, the solvent was removed via a stream of nitrogen and the crude product was purified via flash column chromatography on silica gel (10% EtOAc in pentane) to afford tetrazine **S38** (5.0 mg, 25.0 µmol, 16%) as a red solid.

**R$_f$** (20% EtOAc in pentane) = 0.44 (red spot, UV); **melting point** = 179 – 183 °C; **$^1$H-NMR** (DMSO-$d_6$, 400 MHz): $\delta$ = 11.06 (br s, 1H), 10.02 (s, 1H), 7.79 (d, $J$ = 8.7 Hz, 2H), 7.52 (d, $J$ = 8.7 Hz, 2H), 4.12 (s, 1H) ppm; **$^{13}$C-NMR** (DMSO-$d_6$, 101 MHz): $\delta$ = 162.0, 153.9, 138.8, 132.5, 119.6, 116.1, 83.5, 80.1 ppm; **IR** (neat): $\tilde{\nu}$ = 3271 (m), 2922 (m), 1604 (vs), 1552 (m), 1496 (vs), 1464 (w), 838 (vs) cm$^{-1}$; **HRMS** (EI) for $C_{10}H_7N_5^+$: calculated: 197.0701; found: 197.0692.

**Boc-Lys(Tet)-OMe (27)**

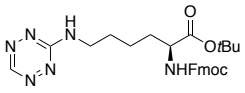


**27**

Tetrazine **27** was synthesized according to **GP4**. After 2 h, the solvent was removed via a stream of nitrogen and the crude product was purified via flash column chromatography (40% EtOAc in pentane) to afford tetrazine **27** (45.0 mg, 132 µmol, 85%) as a red oil.

**R$_f$** (40% EtOAc in pentane) = 0.29 (orange spot, UV); **$[\alpha]_D^{20}$** = –12.54 (c = 0.865, MeOH); **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 9.63 (s, 1H), 5.99 (br s, 1H), 5.07 (br s, 1H), 4.32 (br s, 1H), 3.74 (s, 3H), 3.64–3.58 (m, 2H), 1.85–1.62 (m, 6H), 1.44 (s, 9H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 101 MHz): $\delta$ = 173.3, 163.1, 155.6, 153.4, 80.2, 53.2, 52.5, 41.1, 32.8, 28.5, 28.5, 22.7 ppm; **IR** (neat): $\tilde{\nu}$ = 3319 (br m), 2934 (m), 1741 (m), 1697 (vs), 1568 (vs), 1505 (s), 1455 (w), 1366 (s), 1163 (vs), 955 (m) cm$^{-1}$; **HRMS** (ESI) for $C_{14}H_{24}N_6O_4Na^+$ [M+Na]$^+$: calculated: 363.1751; found: 363.1744.

**Fmoc-Lys(Tet)-O*t*Bu (28)**



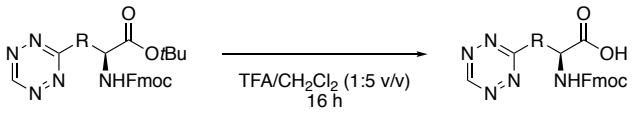

**28**

Tetrazine **28** was synthesized according to **GP4**. After 2 h, the solvent was removed via a stream of nitrogen and the crude product was purified via flash column chromatography (40% EtOAc in pentane) to afford tetrazine **28** (43.0 mg, 85.0 µmol, 55%) as a red oil.

**$R_f$** (40% EtOAc in pentane) = 0.62 (orange spot, UV); **$[\alpha]_D^{20}$** = –11.65 (c = 0.52, MeOH); **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 9.62 (s, 1H), 7.77 (d, *J* = 7.5 Hz, 2H), 7.62–7.60 (m, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.33–7.29 (m, 2H), 6.00 (br s, 1H), 5.39 (d, *J* = 8.3 Hz, 1H), 4.47–4.37 (m, 2H), 4.32–4.27 (m, 1H), 4.23 (t, *J* = 7.0 Hz, 1H), 3.64–3.54 (m, 2H), 1.89–1.52 (m, 6H), 1.47 (s, 9H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 126 MHz): $\delta$ = 171.6, 163.2, 156.2, 153.5, 144.0, 144.0, 141.5, 127.8, 127.2, 125.3, 125.2, 120.1, 82.5, 67.2, 54.0, 47.3, 41.1, 32.9, 28.5, 28.2, 22.5 ppm; **IR** (neat): $\tilde{\nu}$ = 3308 (br m), 2935 (m), 1714 (vs), 1568 (vs), 1450 (m), 1368 (w), 1078 (m); 1154 (vs), 956 (m), 739 (vs) $cm^{-1}$; **HRMS** (ESI) for $C_{27}H_{33}N_6O_4Na^+$ $[M+H]^+$: calculated: 505.2558; found: 505.2564.

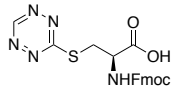
## Deprotection of Fmoc protected amino acid *tert*-butyl esters

**General Procedure for the Deprotection *tert*-Butyl Protected Amino Acids**




The respective amino acid *tert*-butyl ester was dissolved in $CH_2Cl_2$ and TFA (5:1 v/v) was added dropwise. The mixture was stirred for 16 h, then water (50 mL) and $CH_2Cl_2$ (50 mL) were added and the layers were separated. The aqueous layer was extracted with $CH_2Cl_2$ (50 mL) and the combined organic layers were dried over sodium sulfate, filtered and concentrated. The crude product was purified via flash column chromatography on silica gel to afford the free acid.

**Fmoc-Cys(Tet)-OH (30)**

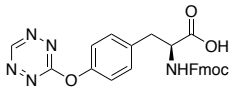



**30**

Fmoc-Cys(Tet)-O*t*Bu (**26**) (41.9 mg, 87.4 µmol, 1.00 equiv) was dissolved in $CH_2Cl_2$ (5 mL) and TFA (1 mL) was added dropwise. The mixture was stirred for 16 h, then water (50 mL) and $CH_2Cl_2$ (50 mL) were added and the layers were separated. The aqueous layer was extracted with $CH_2Cl_2$ (50 mL) and the combined organic layers were dried over sodium sulfate, filtered and concentrated. The crude product was purified via flash column chromatography on silica gel (5 – 10% MeOH in $CH_2Cl_2$) to afford thiol **30** (26.6 mg, 64.0 µmol, 73%) as a slightly pink solid.

**$R_f$** (10% MeOH in $CH_2Cl_2$) = 0.13 (pink spot, UV); **melting point** = 209 °C (decomp.); **$[\alpha]_D^{20}$** = –8.63 (c = 1.215, MeOH); **$^1$H-NMR** (MeOH-$d_4$, 400 MHz): $\delta$ = 10.02 (s, 1H), 7.78 (d, *J* = 7.6 Hz, 2H), 7.59 (t, *J* = 7.0 Hz, 2H), 7.37 (t, *J* = 7.4 Hz, 2H), 7.27 (t, *J* = 7.4 Hz, 2H), 4.48 (dd, *J* = 8.3, 4.3 Hz, 1H), 4.39 (dd, *J* = 10.5, 6.9 Hz, 1H), 4.28 (dd, *J* = 10.5, 6.9 Hz, 1H), 4.20–4.13 (m, 2H), 3.55 (dd, *J* = 13.9, 8.2 Hz, 1H) ppm; **$^{13}$C-NMR** (MeOH-$d_4$, 101 MHz): $\delta$ = 179.4, 158.5, 157.3, 145.2, 142.5, 128.7, 128.1, 128.1, 126.1, 126.1, 120.9, 68.0, 55.9, 33.6 ppm; **IR** (neat): $\tilde{\nu}$ = 3374 (br vs), 2475 (w), 1686 (s), 1590 (vs), 1450 (s), 1405 (vs), 1220 (vs), 1035 (w), 890 (w) cm$^{-1}$; **HRMS** (ESI) for $C_{20}H_{17}N_5O_4S^-$ [M-H]$^-$: calculated: 422.0928; found: 422.0928.

**Fmoc-Tyr-OH (31)**

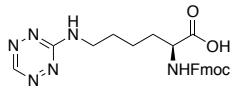



**31**

Fmoc-Tyr(Tet)-O*t*Bu (**24**) (23.0 mg, 42.6 µmol, 1.00 equiv) was dissolved in $CH_2Cl_2$ (2.5 mL) and TFA (0.5 mL) was added dropwise. The mixture was stirred for 16 h, then water (50 mL) and $CH_2Cl_2$ (50 mL) were added and the layers were separated. The aqueous layer was extracted with $CH_2Cl_2$ (50 mL) and the combined organic layers were dried over sodium sulfate, filtered and concentrated. The crude product was purified via flash column chromatography on silica gel (10% MeOH in $CH_2Cl_2$) to afford ether **31** (10.2 mg, 21.0 µmol, 49%) as a slightly pink solid.

**$R_f$** (10% MeOH in $CH_2Cl_2$) = 0.4 (pink spot, UV); **melting point** = 223 – 225 °C; **$[\alpha]_D^{20}$**= +9.79 (c = 0.39, MeOH); **$^1$H-NMR** (MeOH-$d_4$, 400 MHz): $\delta$ = 10.23 (s, 1H), 7.80 (d, *J* = 7.5 Hz, 2H), 7.64 (d, *J* = 7.5 Hz, 2H), 7.41–7.22 (m, 8H), 4.42 (dd, *J* = 10.5, 6.8 Hz, 1H), 4.36 (dd, *J* = 8.5, 4.7 Hz, 1H), 4.27 (dd, *J* = 10.4, 6.8 Hz, 1H), 4.19 (t, *J* = 6.6 Hz, 1H), 3.30 (dd, *J* = 13.8, 8.6 Hz, 1H), 3.04 (dd, *J* = 13.8, 8.6 Hz, 1H) ppm; **$^{13}$C-NMR** (MeOH-$d_4$, 126 MHz): $\delta$ = 170.4, 158.3, 158.0, 152.1, 145.3, 145.2, 142.6, 137.8, 132.1, 128.8, 128.1, 126.2, 126.1, 121.9, 120.9, 67.8, 57.9, 38.2 ppm; **IR** (neat): $\tilde{\nu}$ = 3387 (br s), 2479 (w), 1692 (m), 1580 (m), 1507 (w), 1437 (vs), 1360 (vs), 1200 (w), 1053 (w), 933 (w), 741 (m) cm$^{-1}$; **HRMS** (ESI) for $C_{26}H_{21}N_5O_5Na^+$ [M+Na]$^+$: calculated: 506.1435; found: 506.1434.

**Fmoc-Lys(Tet)-OH (32)**



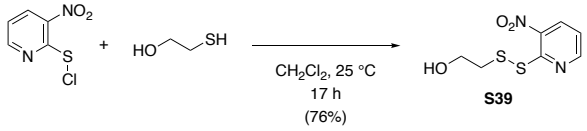
**32**

Fmoc-Lys(Tet)-O*t*Bu (**28**) (40.0 mg, 79.3 µmol, 1.00 equiv) was dissolved in $CH_2Cl_2$ (4 mL) and TFA (0.8 mL) was added dropwise. The mixture was stirred for 16 h, then water (50 mL) and $CH_2Cl_2$ (50 mL) were added and the layers were separated. The aqueous layer was extracted with $CH_2Cl_2$ (50 mL) and the combined organic layers were dried over sodium sulfate, filtered and concentrated. The crude product was purified via flash column chromatography on silica gel (10% MeOH in $CH_2Cl_2$) to afford ether **32** (31.0 mg, 69.0 µmol, 87%) as an orange oil.

**$R_f$** (10% MeOH in $CH_2Cl_2$) = 0.2 (orange spot, UV); **$[\alpha]_D^{20}$** = +0.43 (c = 1.265, MeOH); **$^1$H-NMR** (MeOH-$d_4$, 400 MHz): $\delta$ = 9.53 (s, 1H), 7.79 (d, *J* = 7.5 Hz, 2H), 7.65 (dd, *J* = 7.4, 5.4 Hz, 2H), 7.37 (t, *J* = 7.4 Hz, 2H), 7.29 (t, *J* = 7.5 Hz, 2H), 4.41–4.30 (m, 2H), 4.20 (t, *J* = 6.7 Hz, 1H), 4.08 (dd, *J* = 9.0, 4.7 Hz, 1H), 3.52–3.45 (m, 2H), 1.94–1.28 (m, 6H) ppm; **$^{13}$C-NMR** (MeOH-$d_4$, 101 MHz): $\delta$ = 164.5, 158.7, 153.6, 145.3, 145.2, 142.6, 128.8, 128.1, 126.2, 126.2, 120.9, 67.8, 56.1, 41.6, 32.7, 29.4, 24.3 ppm; **IR** (neat): $\tilde{\nu}$ = 3362 (br s), 2481 (br s), 2076 (m), 1686 (w), 1554 (m), 1358 (w), 1115 (s), 970 (vs) $cm^{-1}$; **HRMS** (ESI) for $C_{23}H_{24}N_6O_4Na^+$ $[M+Na]^+$: calculated: 471.1751; found: 471.1753.

## Synthesis of Chemical Probes

**Disulfide S39**[16]

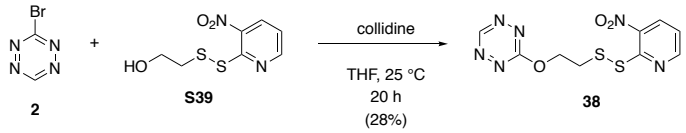



3-Nitro-2-pyridinesulfenyl chloride (379 mg, 1.99 mmol, 1.40 equiv) was dissolved in $CH_2Cl_2$ (10 mL) and cooled to 0 °C. Then, 2-mercaptoethanol (0.10 mL, 1.42 mmol, 1.00 equiv) in $CH_2Cl_2$ (2 mL) was added dropwise over 15 min. The reaction was allowed to warm to 25 °C and was stirred for 17 h. Then, the solvent was removed under reduced pressure and the crude product was purified via flash column chromatography (5% MeOH in $CH_2Cl_2$) to afford the disulfide **S39** (251 mg, 1.08 mmol, 76%) as a yellow oil.

$^1$H-NMR ($CDCl_3$, 400 MHz): $\delta$ = 8.79 (d, *J* = 4.5 Hz, 1H), 8.49 (dd, *J* = 8.2, 1.6 Hz, 1H), 7.39 (dd, *J* = 8.2, 4.6 Hz, 1H), 3.66 (t, *J* = 5.2 Hz, 2H), 2.97 (*J* = 5.2 Hz, 2H) ppm.

The obtained analytical data are consistent with the values reported in the literature.[16]

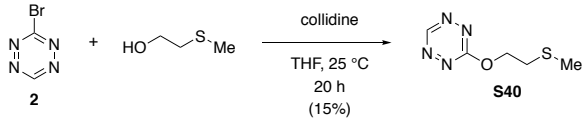
**Disulfide 38**




Disulfide **S39** (146 mg, 0.628 mmol, 1.10 equiv) was dissolved in THF (1.5 mL) and collidine (76.0 μL, 0.571 mmol, 1.00 equiv) was added. Then, 3-bromotetrazine (**2**) (91.9 mg, 0.571 mmol, 1.00 equiv) in THF (1.5 mL) was added dropwise. The mixture was stirred for 20 h, then the solvent was removed via a stream of nitrogen and the crude product was purified via flash column chromatography (2% diethyl ether in $CH_2Cl_2$) to afford disulfide **38** (49.0 mg, 0.157 mmol, 28%) as a red oil.

**$R_f$** (1% diethyl ether in $CH_2Cl_2$) = 0.58 (pink spot, UV); **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.07 (s, 1H), 8.83 (dd, *J* = 4.7, 1.5 Hz, 1H), 8.54 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.39 (dd, *J* = 8.1, 4.5 Hz, 1H), 4.98 (t, *J* = 6.6 Hz, 2H), 3.41 (t, *J* = 6.6 Hz, 2H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 101 MHz): $\delta$ = 167.8, 156.9, 156.3, 153.8, 143.0, 134.0, 121.2, 67.8, 36.3 ppm; **IR** (neat): $\tilde{\nu}$ = 1559 (m), 1581 (s), 1514 (s), 1478 (vs), 1342 (vs)1259 (w), 1055 (w), 856 (w), 745 (w) $cm^{-1}$; **HRMS** (ESI) for $C_9H_9N_6O_3S_2^+$ $[M+H]^+$: calculated: 313.0172; found: 313.0171.

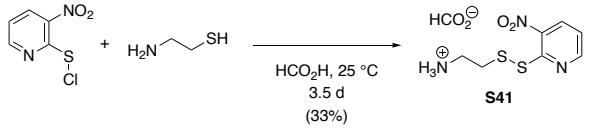
**Thioether S40**




2-(Methylthio)ethanol (15.0 μL, 0.171 mmol, 1.10 equiv) was dissolved in THF (0.75 mL) and collidine was added. Then, 3-bromotetrazine (**2**) (25 mg, 0.155 mmol, 1.00 equiv) in THF (0.75 mL) was added dropwise. The mixture was stirred for 20 h and then the solvent was removed via a stream of nitrogen. The crude product was purified via flash column chromatography (40% diethyl ether in pentane) to afford thioether **S40** (4.00 mg, 23.0 μmol, 15%) as a pink oil.

**$R_f$** (40% diethyl ether in pentane) = 0.4 (pink spot); **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 10.07 (s, 1H), 4.86 (t, *J* = 6.9 Hz, 2H), 3.03 (t, *J* = 6.9 Hz, 2H), 2.25 (s, 3H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 101 MHz): $\delta$ = 167.9, 156.3, 68.6, 32.4, 16.2 ppm; **IR** (neat): $\tilde{\nu}$ = 2920 (w), 1479 (vs), 1449 (s), 1345 (vs), 1054 (w), 941 (m) $cm^{-1}$; **HRMS** (EI) for $C_5H_8N_4OS^+$: calculated: 172.0419; found: 172.0410.

**Disulfide S41**[17]

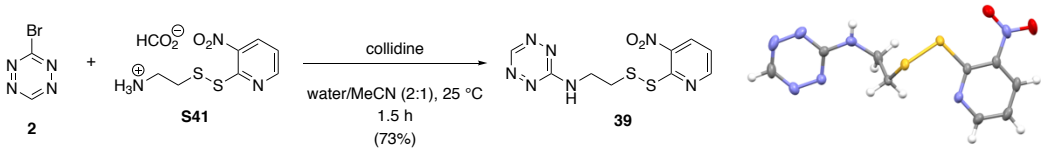


2-Aminoethanethiol hydrochloride (100 mg, 0.88 mmol, 1.00 equiv) was dissolved in formic acid (9 mL) and 3-nitro-2-pyridinesulfenyl chloride (168 mg, 0.88 mmol, 1.00 equiv) was added in one portion and the mixture was stirred for 3.5 d. Then, diethyl ether (50 mL) was added and the precipitate was filtered off and dried under vacuum to afford thioether **S41** (80.0 mg, 0.288 mmol, 33%) as a yellow solid.

**$^1$H-NMR** ($D_2O$, 400 MHz): $\delta$ = 8.86 (dd, *J* = 4.7, 1.6 Hz, 1H), 8.71 (dd, *J* = 8.3, 1.5 Hz, 1H), 7.60 (dd, *J* = 8.3, 4.7 Hz, 1H), 3.30 (t, *J* = 6.1 Hz, 2H), 3.17 (t, *J* = 6.1 Hz, 2H) ppm.

The obtained analytical data are consistent with the values reported in the literature.[17]

**Disulfide 39**




Disulfide **S41** (47.9 mg, 0.179 mmol, 1.00 equiv) was dissolved in water (1.5 mL) and collidine was added followed by 3-bromotetrazine (**2**) (28.8 mg, 0.179 mmol, 1.00 equiv) in MeCN (0.75 mL). The mixture was stirred for 1.5 h and then water (20 mL) and $CH_2Cl_2$ (20 mL) were added. The layers were separated and the aqueous layer was extracted with $CH_2Cl_2$ (2 x 50 mL). The combined organic layers were dried over sodium sulfate, filtered and concentrated. The crude product was purified via flash column chromatography ($CH_2Cl_2$ – 2% MeOH in $CH_2Cl_2$) to afford disulfide **39** (40.4 mg, 0.13 mmol, 73%) as a red solid.

**$R_f$** (2% MeOH in $CH_2Cl_2$) = 0.43 (pink spot); **melting point** = 145 – 148 °C; **$^1$H-NMR** ($CDCl_3$, 400 MHz): $\delta$ = 9.65 (s, 1H), 9.12 (dd, *J* = 4.6, 1.6 Hz, 1H), 8.57 (dd, *J* = 8.2, 1.6 Hz, 1H), 8.01 (br s, 1H), 7.47 (dd, *J* = 8.2, 4.6 Hz, 1H), 3.88 (m, 2H), 3.26–3.23 (m, 2H) ppm; **$^{13}$C-NMR** ($CDCl_3$, 101 MHz): $\delta$ = 163.0, 157.6, 154.0, 153.6, 143.2, 134.3, 121.6, 39.2, 39.1 ppm; **IR** (neat): $\tilde{\nu}$ = 3261 (w), 1578 (vs), 1557 (vs), 1514 (s), 1396 (m), 1341 (s), 1120 (w), 1066 (w), 954 (w), 744 (m) cm$^{-1}$; **HRMS** (ESI) for $C_9H_{10}N_7O_2S_2^+$ $[M+H]^+$: calculated: 312.0332; found: 312.0329.

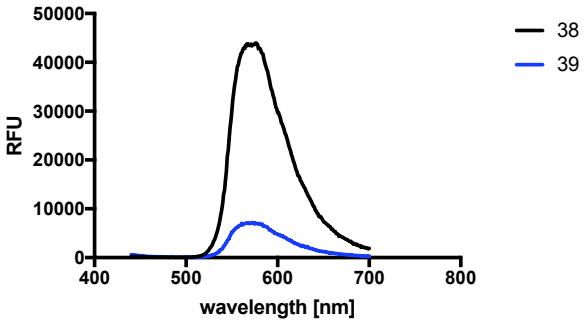
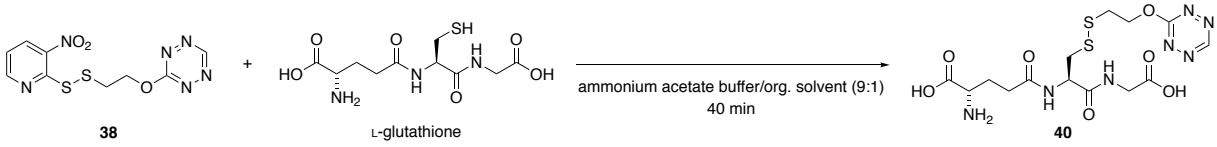
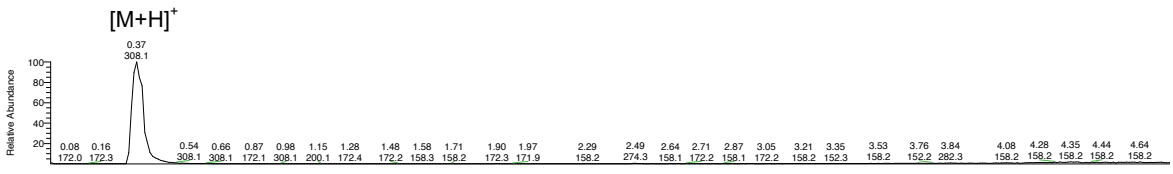



**Figure 6.** Spectroscopic Data for compounds **38** and **39**.

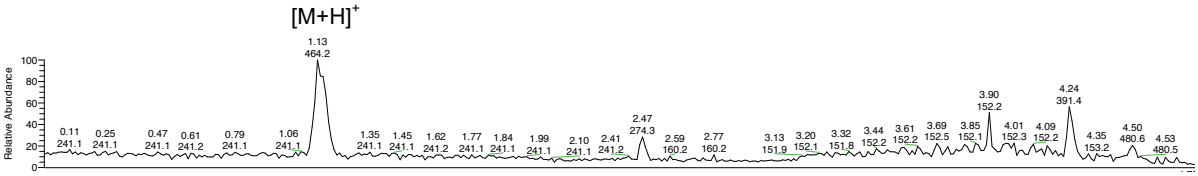
**Control Experiments using L-Glutathione**



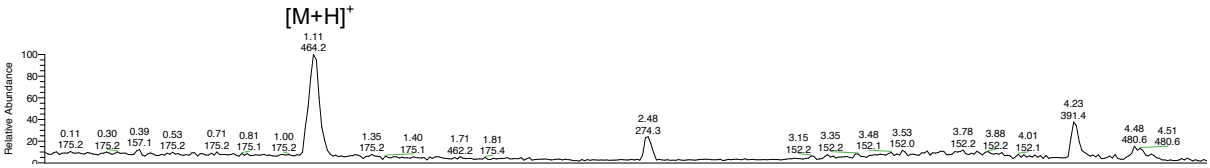

UHPLC trace of L-glutathione



HPLC trace of reaction mixture at pH = 5.15 with 10% acetonitrile




HPLC trace of reaction mixture at pH = 4.15 with 10% DMSO




**HRMS** (ESI) for $C_{14}H_{20}N_7O_7S_2^-$ [M-H]$^-$: calculated: 462.0871; found: 462.0871.

**HRMS/HRMS of labelled L-glutathione**

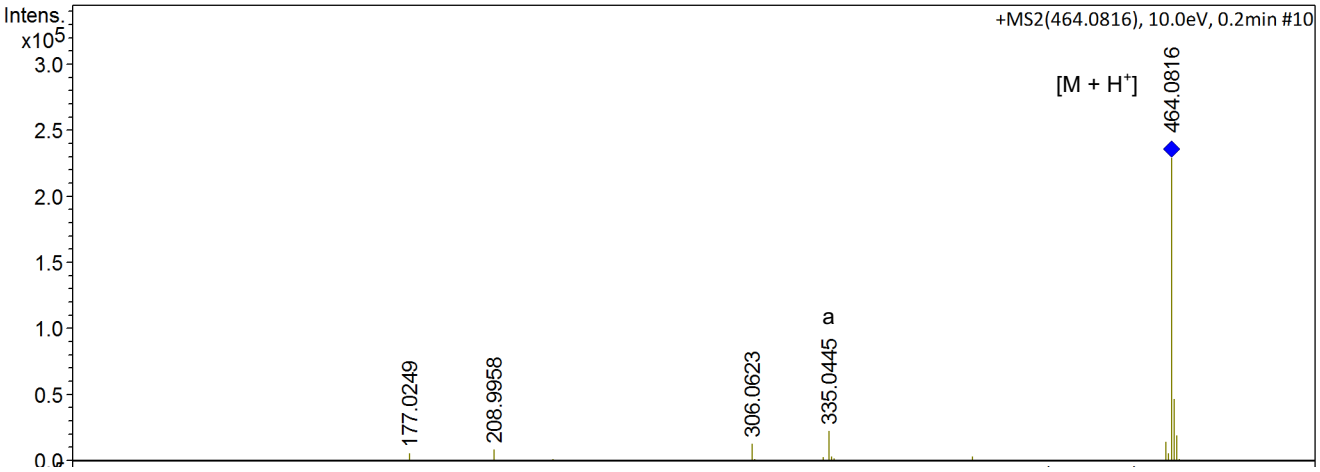
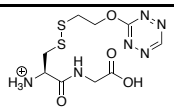
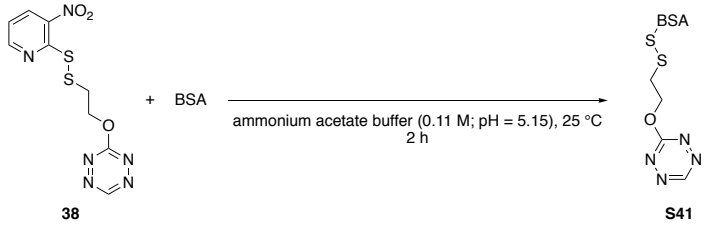



**Table S3.** MS/MS fragments.

| fragment | pseudomolecular ion | calculated mass | found mass | proposed structure |
|---|---|---|---|---|
| a | $[C_9H_{15}N_6O_4S_2]^+$ | 335.0591 | 335.0445 |  |

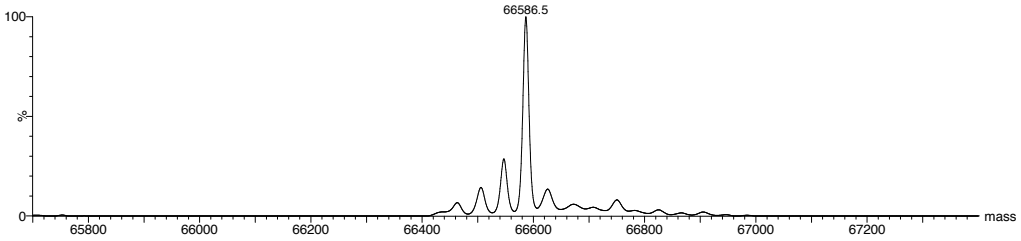
**Functionalization of BSA with disulfide 38**

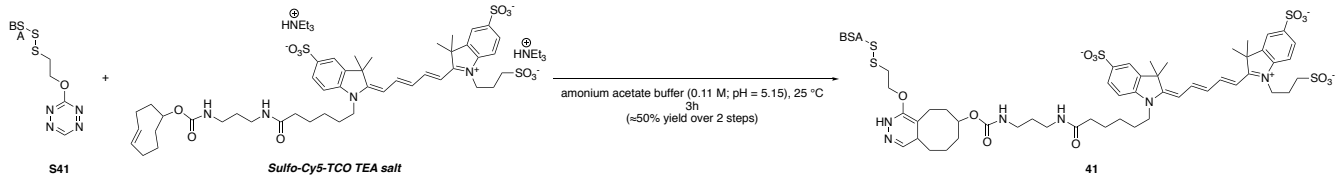



**38** **S41**

BSA (50 mg, 0.75 µmol, 1.00 equiv) was dissolved in ammonium acetate buffer (0.11 M, pH = 5.15) (49.5 mL). Then, disulfide **38** (1.18 mg, 3.77 µmol, 5.00 equiv), dissolved in MeCN (0.5 mL) was added dropwise and the mixture was stirred for 2 h. After 2 h, full conversion to the monofunctionalized BSA derivative **S41** was observed by ESI-MS of a sample taken from the reaction mixture.

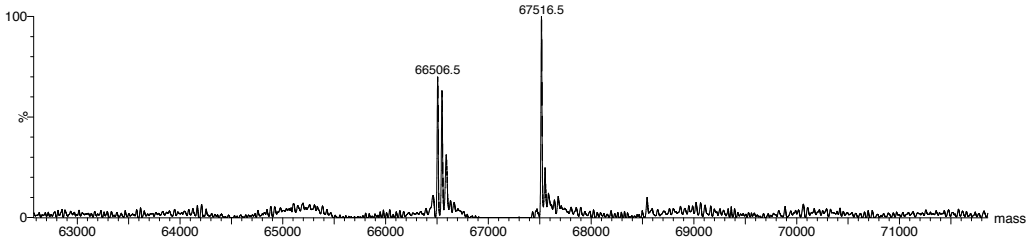
Deconvoluted ESI-MS Spectra of **S41**.




An aliquot (5 mL) of the above solution was removed and treated with Sulfo-Cy5-TCO triethylamine salt (0.44 mg, 0.375 μmol, 5.00 equiv). After 3 h, the reaction was complete and analysis with ESI-MS showed full conversion to the desired product **41**. To determine the yield, a gel electrophoresis was run on an aliquot (7.5 μL) of the reaction mixture.

Deconvoluted ESI-MS Spectra of **41**.



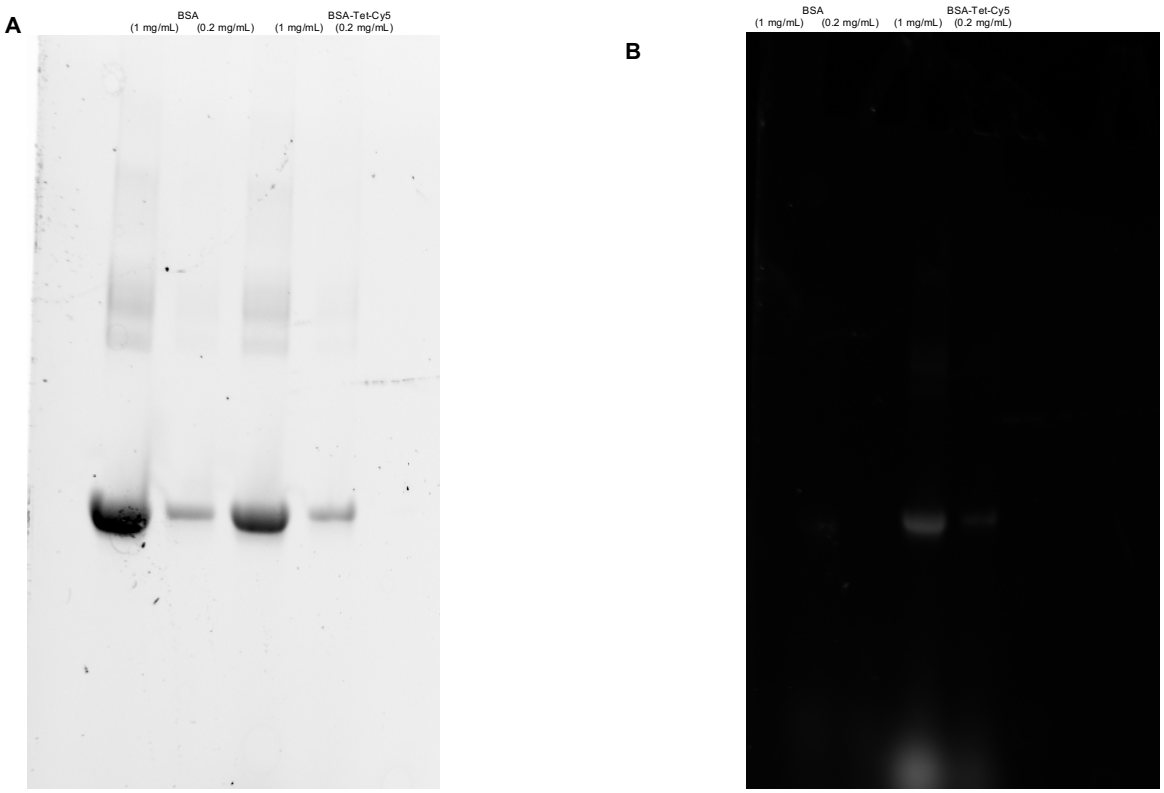




**Figure 7.** A) Stain free gel analysis of BSA and the BSA-Tet-Cy5 conjugate; B) In-gel fluorescence measurement of respective gel.

## Whole Cell Labelling

### Whole cell labeling of MCF7 cells without TCEP activation

Untreated microscope glass coverslips were placed in the wells of a six-well plate and cells were seeded at a density of 200000 cells/well in suitable medium (2 mL, Minimum essential medium eagle + 10% FBS + 1% pen/strep + 0.01 mg/mL human recombinant insulin). The six-well plate was placed in the incubator (37 °C, 90% RH, 5% $CO_2$) and cells were allowed to attach for 21 h. After incubation, the old medium was removed and MEM (1 mL) spiked with 20% of a 4% solution of paraformaldehyde was added to the cells for 2 min before aspirating. Subsequently, samples were fixed by treatment with a 4% solution of paraformaldehyde (1 mL) for 15 min, followed by rinsing with PBS (1 x 1 mL). Staining of the cell nuclei was performed by incubation of the fixed cells with DAPI (c = 2.0 µg/mL in $H_2O$) at 25 °C for 15 min in the dark followed by washing with $H_2O$ (2 x 1 mL). Further the cells were stained either with **38** (c = 10 µg/mL in $H_2O$) or **S40** (c = 10 µg/mL) at 25 °C for 30 min in the dark followed by rinsing with $H_2O$ (2 x 1 mL). The cells were then incubated with a solution of Sulfo-Cy5-TCO (7.1 µg/mL in $H_2O$) at 25 °C for 30 min in the dark followed by washing with $H_2O$ (2 x 1 mL). In order to probe dye accumulation by unspecific non-covalent binding to the probes, cells were incubated with 6-aminofluorescein (20 µg/mL in $H_2O$) at 25 °C for 30 min. After washing twice with $H_2O$ (2 x 1 mL), the coverslips were removed from the six-well plates, mounted with *Prolong Diamond Antifade Mountant* and air dried in the dark before imaging.

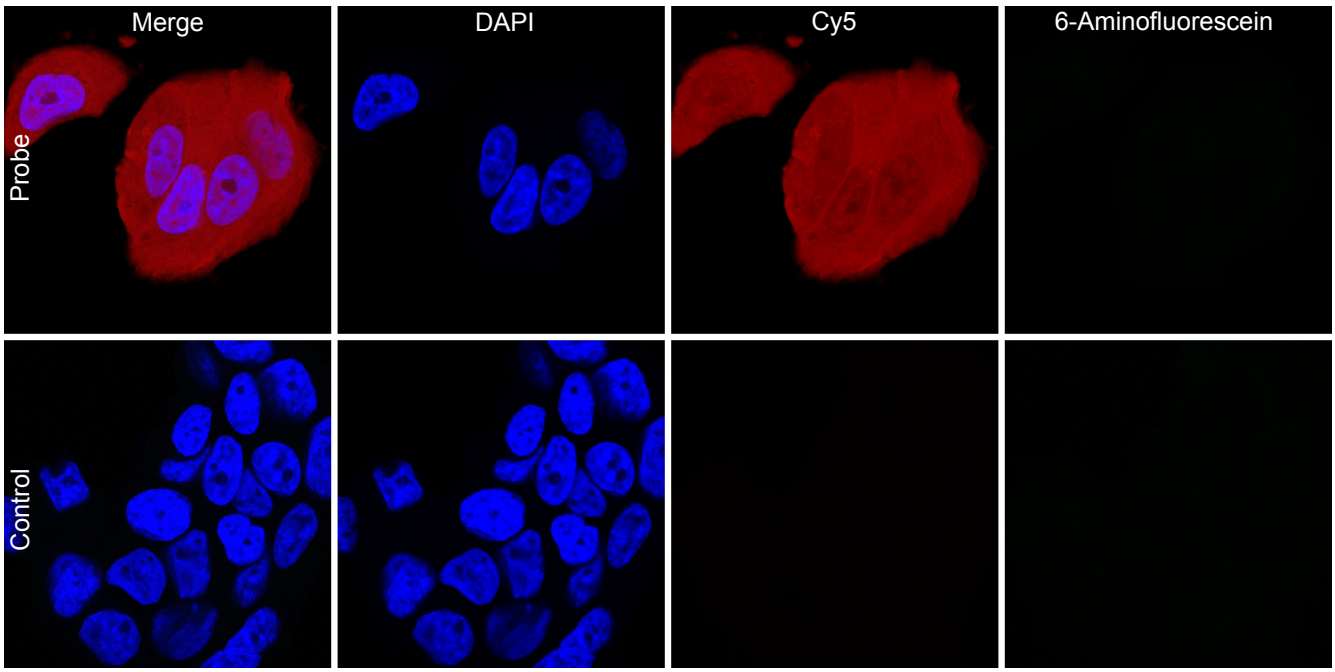



**Figure 8.** Confocal microscopy images (Leica SP8 inverse FALCON) of MCF7 cells stained with DAPI and Cy5 and incubated with 6-aminofluorescein. DAPI channel: Excitation: 405 nm, laser power: 2%, gain: 22% / 22% (probe/control); Cy5 channel: Excitation: 640 nm, laser power: 1%, gain: 21% / 21% (probe/control); 6-aminofluorescein: Excitation: 490 nm, laser power: 2%, gain: 100% / 100% (probe/control).

### Whole cell labeling of MCF7 cells with TCEP activation

Untreated microscope glass coverslips were placed in the wells of a six-well plate and cells were seeded at a density of 200000 cells/well in suitable medium (2 mL, Minimum essential medium eagle + 10% FBS + 1% pen/strep + 0.01 mg/mL human recombinant insulin). The six-well plate was then placed in the incubator (37 °C, 90% RH, 5% $CO_2$) and cells were allowed to attach for 21 h. After incubation, the old medium was removed and MEM (1 mL) spiked with 20% of a 4% solution of paraformaldehyde was added to the cells for 2 min before aspirating. Subsequently, samples were fixed by treatment with a 4% solution of paraformaldehyde (1 mL) for 15 min, followed by rinsing with PBS (1 x 1 mL). Staining of the cell nuclei was performed by incubation of the fixed cells with DAPI (c = 2.0 µg/mL in $H_2O$) at 25 °C for 15 min in the dark followed by washing with $H_2O$ (2 x 1 mL). To activate the accessible disulfide bonds of the cells, they were incubated in a solution of TCEP (1 mL, 0.1 mM in $H_2O$) at 25 °C for 30 min followed by rinsing with $H_2O$ (2 x 1 mL). Further the cells were stained either with **38** (c = 10 µg/mL in $H_2O$) or **S40** (c = 10 µg/mL in $H_2O$) at 25 °C for 30 min in the dark followed by rinsing with $H_2O$ (2 x 1 mL). The cells were then incubated with a solution of Sulfo-Cy5-TCO (7.1 µg/mL in $H_2O$) at 25 °C for 30 min in the dark followed by washing with $H_2O$ (2 x 1 mL). In order to probe dye accumulation by unspecific non-covalent binding to the probes, cells were incubated with 6-aminofluorescein (20 µg/mL in $H_2O$) at 25 °C for 30 min.

After washing twice with $H_2O$ (2 x 1 mL), the coverslips were removed from the six-well plates, mounted with *Prolong Diamond Antifade Mountant* and air dried in the dark before imaging.

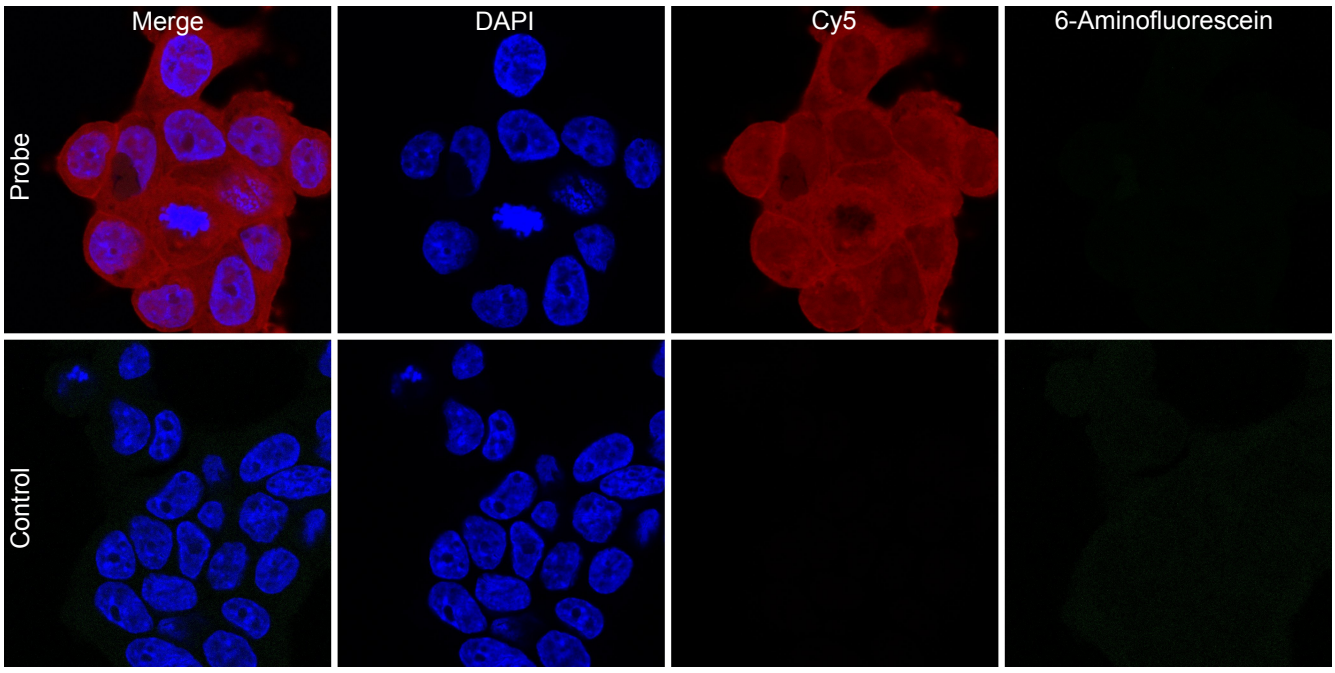



**Figure 9.** Confocal microscopy images (Leica SP8 inverse FALCON) of MCF7 cells stained with DAPI and Cy5 and incubated with 6-aminofluorescein. DAPI channel: Excitation: 405 nm, laser power: 2%, gain: 40% / 40% (probe/control); Cy5 channel: Excitation: 640 nm, laser power: 0.5%, gain: 20% / 20% (probe/control); 6-aminofluorescein: Excitation: 490 nm, laser power: 2%, gain: 100% / 500% (probe/control).

## Targeting of L-glutathione in Cyanobacteria extracts

### Sources, Cultivation and Isolation of Cyanobacteria.

The strain *Microcystis aeruginosa* (*M. aeruginosa*) EAWAG 127a is part of the cyanobacteria collection of the University of Zürich, previously located at EAWAG (Swiss Federal Institute of Aquatic Science and Technology). The strain was grown in a 60 L batch reactor (Z medium) with a light/dark cycle of 12:12 h and continuous airflow. Part of the culture (0.5 L) was collected, centrifuged (4500 × g, 30 min) and the supernatant was discarded. To the cyanobacteria pellet, an aq. MeOH solution. (50%, 200 mL) was added, the mixture was sonicated (3 x 3 min), centrifuged (4500 × g, 30 min) and filtered, and the procedure was repeated twice with an aq. MeOH solution. (80%, 200 mL). The filtrates were combined, evaporated under reduced pressure at 40°C, and freeze dried to obtain 29.5 mg of the crude extract.

### Functionalization in Cyanobacteria Extract.

The functionalization of L-glutathione with the tetrazine probe **38** was performed in the presence of the *M. aeruginosa* EAWAG 127a crude extract. Stock solutions of L-glutathione (200 µg/mL) in $H_2O$ and tetrazine probe **38** (2 mg/mL) in MeCN were prepared, and the crude extract was dissolved in MeCN:$H_2O$ (1:1; v/v) at a concentration of 3 mg/mL. The experiment was performed by mixing the crude extract solution (100 µL), the tetrazine probe (8.1 µL) and the L-glutathione (40 µL) stock solution for 5 min at RT. Control experiments were achieved using only the crude extract solution with MeCN (8.1 µL) and $H_2O$ (40 µL), the crude extract solution with tetrazine probe **38** (8.1 µL) solution and $H_2O$ (40 µL), and the crude extract solution with L-glutathione (40 µL) solution and MeCN (8.1 µL). The results were analyzed by UHPLC MS using a solvent system composed of $H_2O$ + 0.1% HCOOH (A) and MeCN + 0.1% HCOOH (B). The gradient varied from 5 to 95 % of B in 3.5 min, from 95 to 100 % in 0.05 min, and the column was washed with 100 % B for 1.24 min. The functionalized L-glutathione with the tetrazine probe was detected 1.00 min.

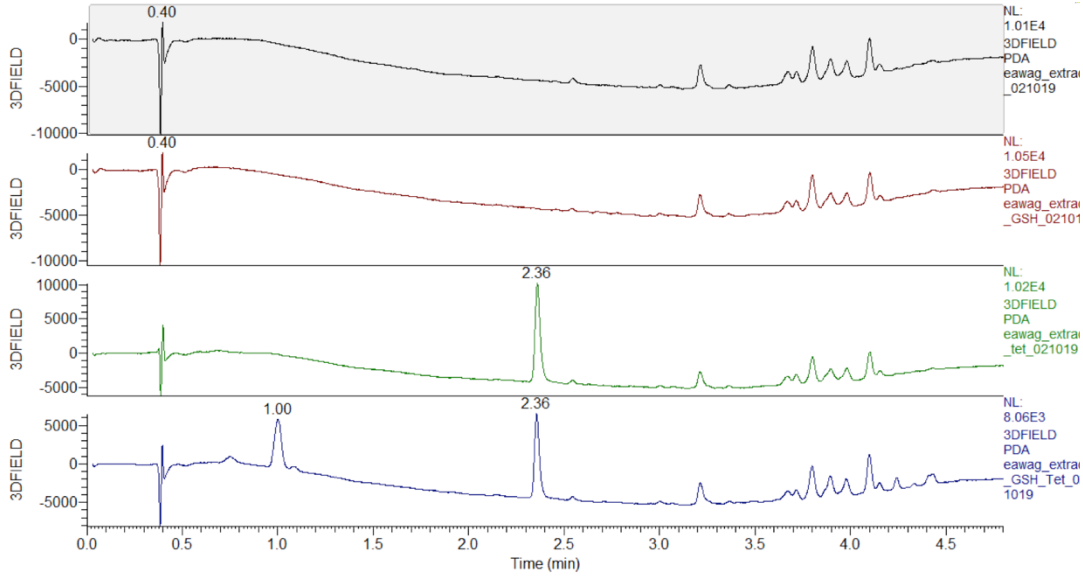



**Figure 10**. HPLC UV chromatograms monitored over the wavelength range of 500 to 520 nm of the L-glutathione functionalization with tetrazine in the presence of a complex matrix as cyanobacteria extract (*M. aeruginosa* EAWAG 127a). From top to bottom experiments 1) with only the crude extract, 2) with the crude extract and L-glutathione, 3) with the crude extract and tetrazine probe **38**, and 4) with the crude extract, tetrazine probe **38** and L-glutathione. The peak at 1.0 min is the functionalized L-glutathione.

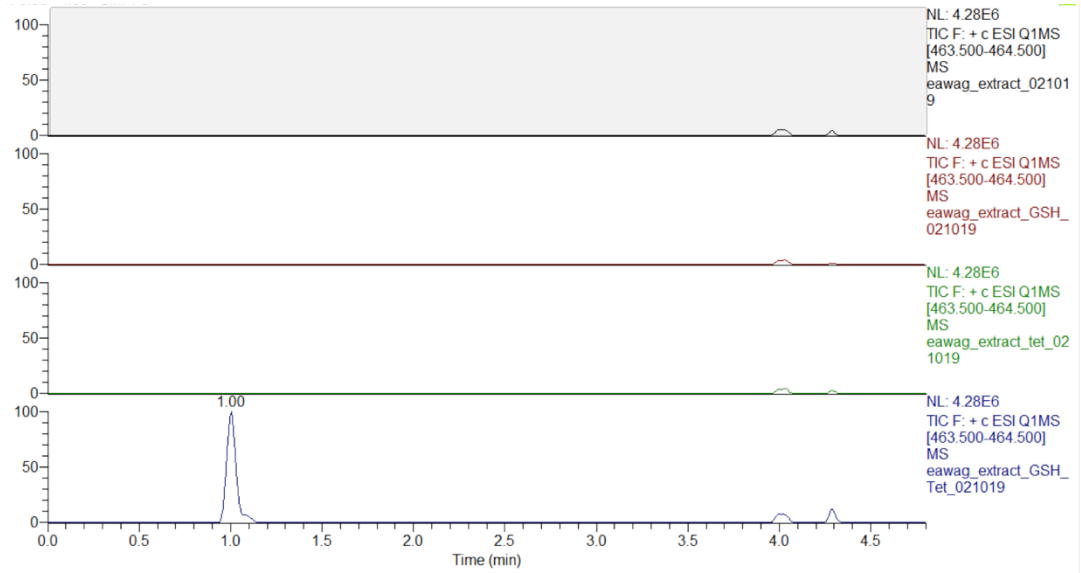
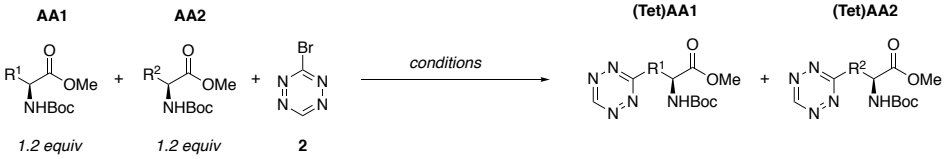



**Figure 11.** HPLC MS chromatograms in SIM mode (464 Da) of the L-glutathione functionalization with tetrazine in the presence of a complex matrix as cyanobacteria extract (*M. aeruginosa* EAWAG 127a). From top to bottom experiments 1) with only the crude extract, 2) with the crude extract and L-glutathione, 3) with the crude extract and tetrazine probe **38**, and 4) with the crude extract, tetrazine probe **38** and L-glutathione. The peak at 1.00 min is the functionalized L-glutathione.

**Competition Experiments Between Amino Acids**

To establish a reactivity profile of 3-bromotetrazine (**2**) towards amino acids, competition experiments between amino acids were conducted




The two different amino acids (0.186 mmol, 1.20 equiv) were dissolved in the specified solvent and base (0.155 mmol, 1.00 equiv) was added. Then, 3-bromotetrazine (**2**) (25.0 mg, 0.155 mmol, 1.00 equiv) in specified organic solvent, was added. After completion of the reaction, the solvent was removed and the crude material was purified via flash column chromatography on silica gel.

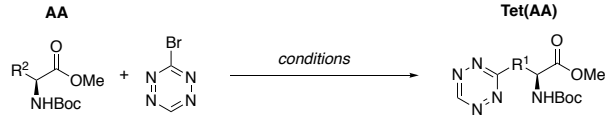
**Table S4.** Competition experiments between amino acids.

| entry | AA1 | AA2 | base | solvent | T [°C] | yield (Tet)AA1 [%][a] | yield (Tet)AA2 [%][b] |
|---|---|---|---|---|---|---|---|
| **1** | Boc-Ser-OMe | Boc-Trp-OMe | DBU | MeCN | 25 | 0 | 15 |
| **2** | Boc-Ser-OMe | Boc-Cys-OMe | collidine | THF | 25 | 0 | 92 |
| **3** | Boc-Trp-OMe | Boc-Cys-OMe | collidine | THF | 25 | 0 | 82 |
| **4** | Boc-Lys-OMe | Boc-Cys-OMe | collidine | THF | 25 | 28 | 42 |
| **5** | Boc-Lys-OMe | Boc-Cys-OMe | collidine | THF | 0 | 23 | 62 |
| **6** | Boc-Lys-OMe | Boc-Cys-OMe | collidine | THF | –20 | 21 | 63 |
| **7** | Boc-Lys-OMe | Boc-Cys-OMe | -[c] | THF | 25 | 17 | 45 |
| **8** | Boc-Lys-OMe | Boc-Cys-OMe | - | $NH_4OAc$[d]/ 10% MeCN | 25 | 0 | 57 |
| **9** | Boc-Lys-OMe | Boc-Cys-OMe | - | $NH_4OAc$[e]/ 10% MeCN | 25 | 0 | 76 |
| **10** | Boc-Tyr-OMe | Boc-Cys-OMe | collidine | THF | 25 | 0 | 98 |
| **11** | Boc-His-OMe | Boc-Cys-OMe | collidine | THF | 25 | 0 | 82 |
| **12** | Boc-Lys-OMe | Boc-Tyr-OMe | collidine | THF | 25 | 72 | 0 |
| **13** | Boc-His-OMe | Boc-Tyr-OMe | collidine | THF | 25 | 70 | 0 |
| **14** | Boc-His-OMe | Boc-Tyr-OMe | collidine | MeCN/$H_2O$ (1:1) | 25 | 70 | 10 |

[a] isolated yield; [b] isolated yield; [c] 2 equiv of Boc-Lys-OMe were used; [d] ammonium acetate buffer (0.11 M, pH = 5.15); [e] ammonium acetate buffer (0.11 M, pH = 4.15).

## Functionalization of Amino Acids in Aqueous/Organic Solvent Systems and Aqueous Buffers

**Table S5.** Nucleophilic Aromatic Substitution in Aqueous/Organic Solvent Systems and Aqueous Buffers.



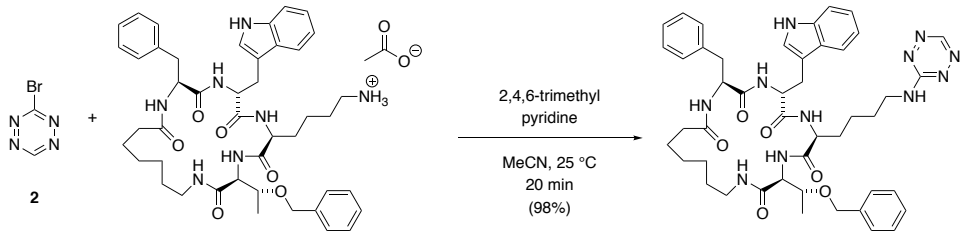

**2**

| entry | AA | solvent | Base | T [°C] | yield (Tet)AA [%][a] |
|---|---|---|---|---|---|
| **1** | Boc-Cys-OMe | MeCN | collidine | 25 | 85 |
| **2** | Boc-Cys-OMe | MeCN (80)/water (20) | collidine | 25 | 72 |
| **3** | Boc-Cys-OMe | MeCN (40)/water (40) | collidine | 25 | 68 |
| **4** | Boc-Cys-OMe | MeCN (50)/water (50) | collidine | 25 | 87 |
| **5** | Boc-Cys-OMe | $NH_4OAc$[b] | - | 25 | 83 |
| **6** | Boc-Cys-OMe | $NH_4OAc$[c] | - | 25 | 81 |
| **7** | Boc-Lys-OMe | MeCN | -[d] | 25 | 74 |
| **8** | Boc-Lys-OMe | MeCN (50)/water (50) | -[d] | 25 | 43 |
| **9** | Boc-Lys-OMe | MeCN (50)/water (50) | collidine | 25 | 54 |

[a] isolated yield; [b] ammonium acetate buffer (0.11 M, pH = 5.15); [c] ammonium acetate buffer (0.11 M, pH = 4.15) [d] 2 equiv of Boc-Lys-OMe were used.

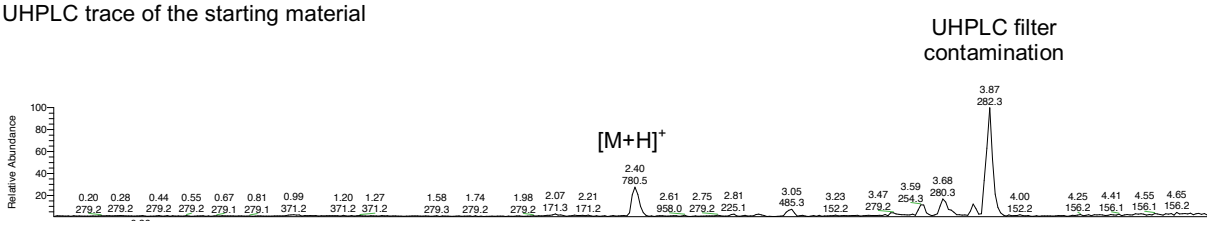
## Functionalization of Peptides and Proteins

### Tet-Cyclosomatostatin (35)

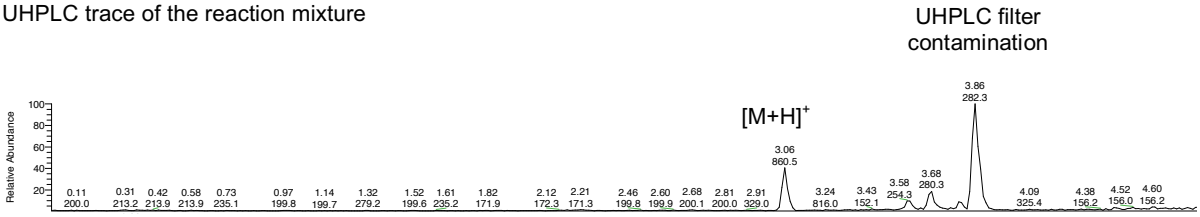



Cyclo-(7-aminoheptanoyl-Phe-D-Trp-Lys-Thr(Bzl)) acetate salt (5.0 mg, 5.95 µmol, 1.00 equiv) (cyclosomatostatin) was dissolved in MeCN (5 mL) and collidine (1.58 µL, 11.9 µmol, 2.00 equiv) was added. Then 3-bromotetrazine (**2**) (4.79 mg, 29.8 µmol, 5.00 equiv) in MeCN (0.5 mL) was added dropwise. After 30 min, the solvent was removed and the crude product was purified via flash column chromatography (5% MeOH in $CH_2Cl_2$) to afford tetrazine **35** (5.00 mg, 5.83 µmol, 98%) as a red solid.

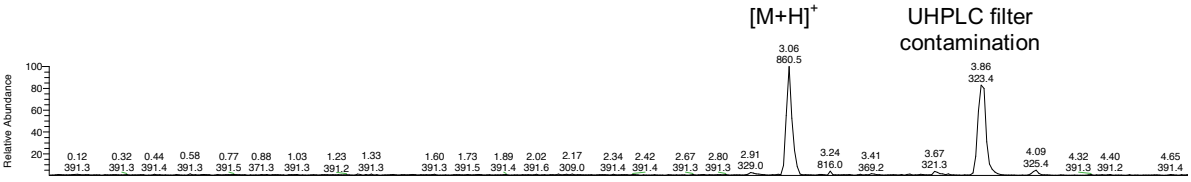
UHPLC trace of the starting material




UHPLC trace of the reaction mixture




UHPLC trace of the purified material




**HRMS** (ESI) for $C_{46}H_{57}N_{11}O_6Na^+$ $[M+Na]^+$: calculated: 882.4385; found: 882.4373.

**HRMS/HRMS analysis of starting material product:**

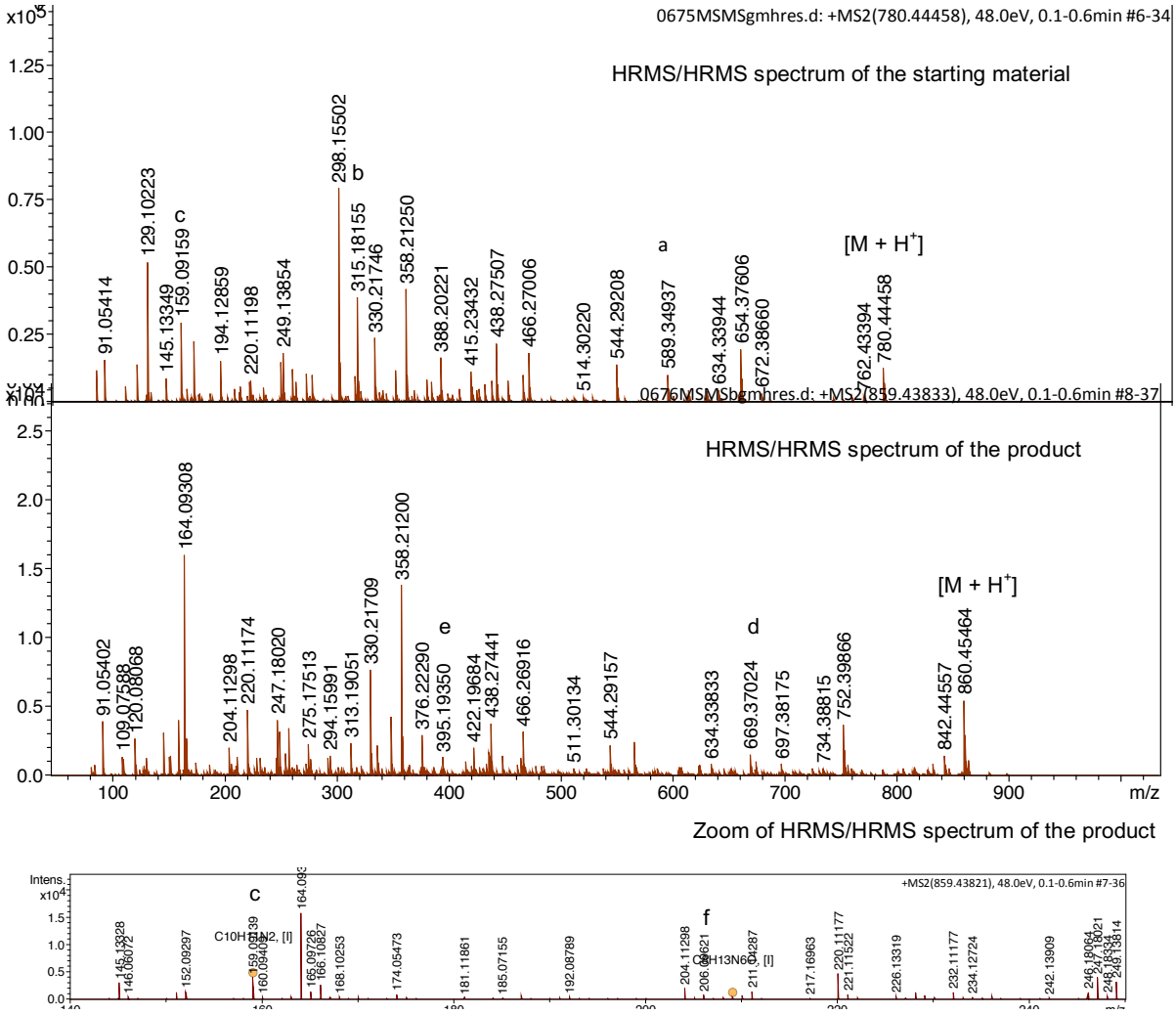
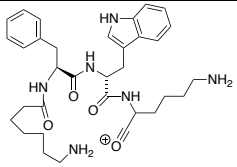
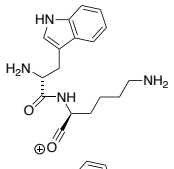
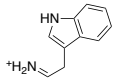
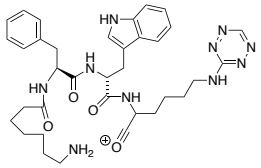
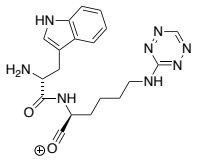
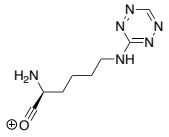
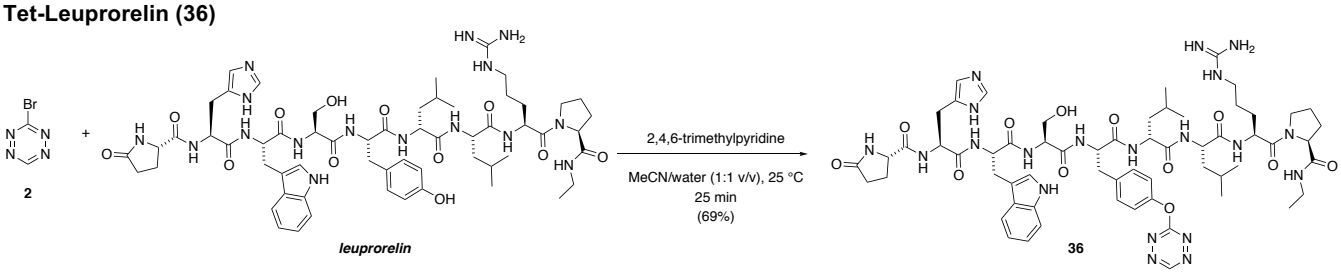



**Table S6.** MS/MS fragments.

| fragment | pseudomolecular ion | calculated mass | found mass | proposed structure |
|---|---|---|---|---|
| a | $[C_{33}H_{45}N_6O_4]^+$ | 589.3497 | 589.3494 |  |
| b | $[C_{17}H_{23}N_4O_2]^+$ | 315.1816 | 315.1816 |  |
| c | $[C_{10}H_{11}N_2]^+$ | 159.0917 | 159.0916 |  |
| d | $[C_{35}H_{45}N_{10}O_4]^+$ | 669.3620 | 669.3702 |  |

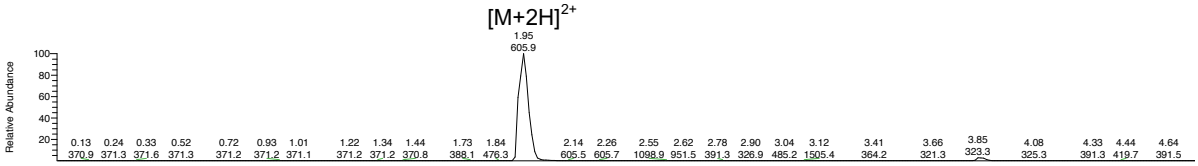
| | | | | |
|---|---|---|---|---|
| e | $C_{19}H_{23}N_8O_2^+$ | 395.1938 | 395.1935 |  |
| f | $C_8H_{13}N_6O^+$ | 209.1145 | 209.1145 |  |

**Tet-Leuprorelin (36)**

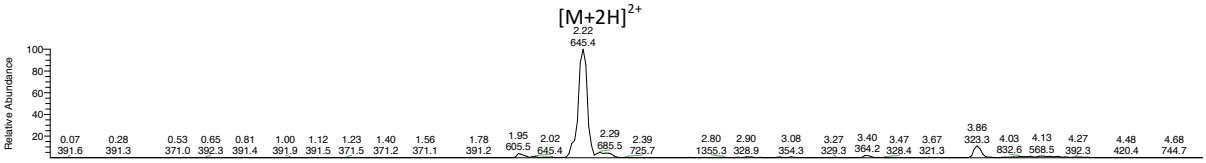



Leuprorelin (4.1 mg, 3.39 µmol, 1.00 equiv) was dissolved in water (2 mL) and collidine (0.1 M in MeCN; 33.9 µL, 3.39 µmol, 1.00 equiv) was added. Then, 3-bromotetrazine (**2**) (2.73 mg, 17.0 µmol, 5.00 equiv) in MeCN (2 mL) was added dropwise. After 25 min, the reaction was complete and the solution was lyophilized. The obtained slightly pink powder was purified via preparative HPLC to afford tetrazine **36** (3.0 mg, 2.33 µmol, 69%) as a pink powder.

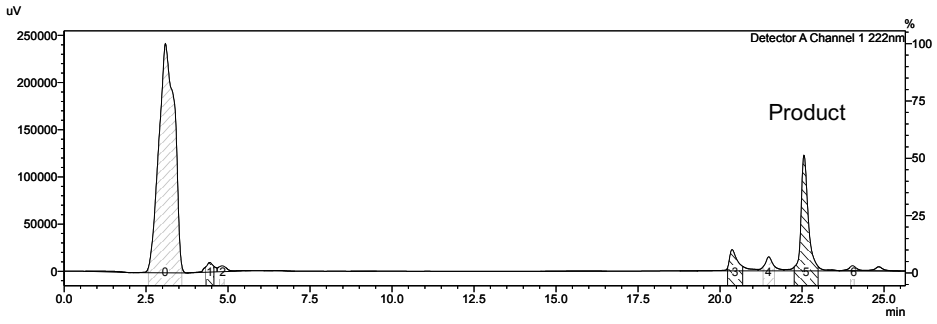
UHPLC-MS chromatogram of the starting material




UHPLC-MS chromatogtam of the reaction mixture




HPLC-UV chromatogram from the preperative HPLC-purification




**HRMS** (ESI) for $C_{61}H_{85}N_{20}O_{12}^+$ [M+H]$^+$: calculated: 1289.6650; found: 1289.6654.

**HRMS/HRMS analysis of starting material and product:**

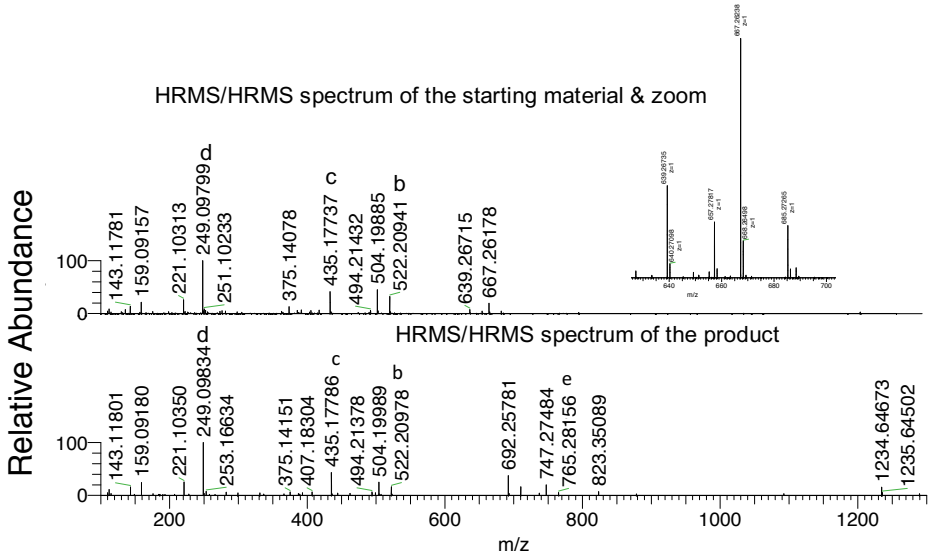
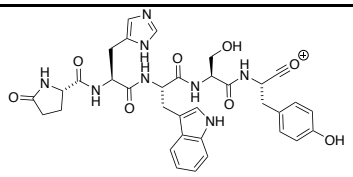
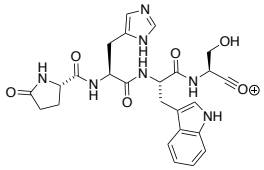
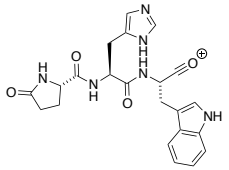
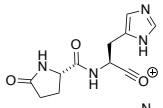
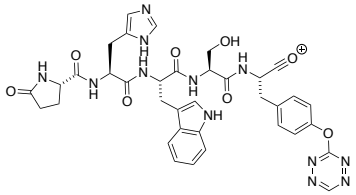
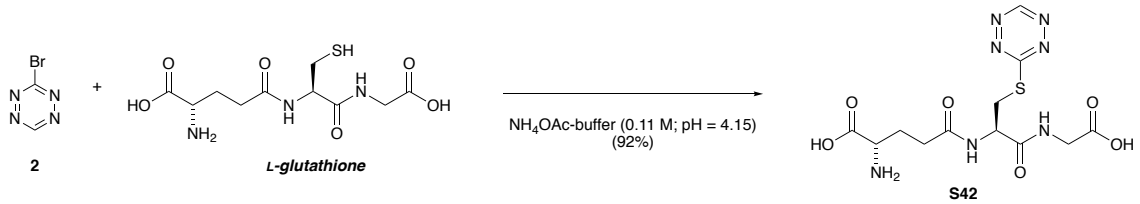



**Table S7.** MS/MS fragments.

| fragment | pseudomolecular ion | calculated mass | found mass | proposed structure |
|---|---|---|---|---|
| a | $[C_{34}H_{37}N_8O_8]^+$ | 685.2729 | 685.2727 |  |
| b | $[C_{25}H_{28}N_7O_6]^+$ | 522.2096 | 522.2094 |  |
| c | $[C_{22}H_{23}N_6O_4]^+$ | 435.1775 | 435.1774 |  |
| d | $[C_{11}H_{13}N_4O_3]^+$ | 249.0982 | 249.0980 |  |
| e | $[C_{36}H_{37}N_{12}O_8]^+$ | 765.2852 | 765.2816 |  |

**Tet-L-Glutathione (S42)**

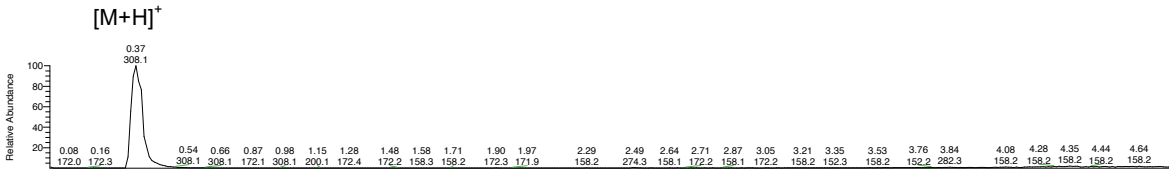



**2** ***L-glutathione*** **S42**

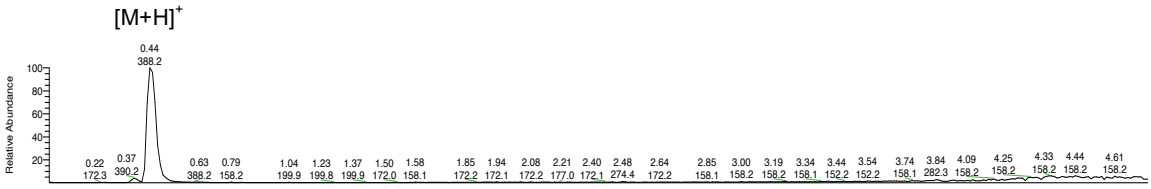
L-Glutathione (47.6 mg, 0.155 mmol, 1.00 equiv) was dissolved in ammonium acetate buffer (0.11 M; pH = 4.15; 3 mL) and 3-bromotetrazine (**2**) (25.0 mg, 0.115 mmol, 1.00 equiv) in MeCN (0.3 mL) was added dropwise. The mixture was stirred for 1 h at 25 °C and then the solution was purified directly via reverse-phase flash column chromatography (water) to obtain pure tet-L-glutathione (**S42**) (55.0 mg, 0.142 mmol, 92%) as a red fluffy solid after lyophilizing of the aqueous solution.

**$^1$H-NMR** ($D_2O$, 400 MHz): $\delta$ = 10.18 (s, 1H), 4.94 (dd, *J* = 8.3, 4.9 Hz, 1H), 4.04 (dd, *J* = 14.6, 5.0 Hz, 1H), 3.92 (s, 2H), 3.78 (t, *J* = 6.3 Hz, 1H), 3.62 (dd, *J* =14.6, 84 Hz, 1H), 2.50 (td, *J* = 7.1, 6.5, 2H), 2.12 (td, *J* = 7.3, 5.4 Hz, 2H) ppm; **$^{13}$C-NMR** ($D_2O$, 126 MHz): $\delta$ = 178.1, 175.8, 175.1, 174.6, 172.5, 156.7, 54.9, 53.0, 43.0, 32.2 (2 x C), 27.0 ppm; **HRMS** (ESI) for $C_{12}H_{18}N_7O_6S^+$ $[M+H]^+$: calculated: 388.1034; found: 388.1034.

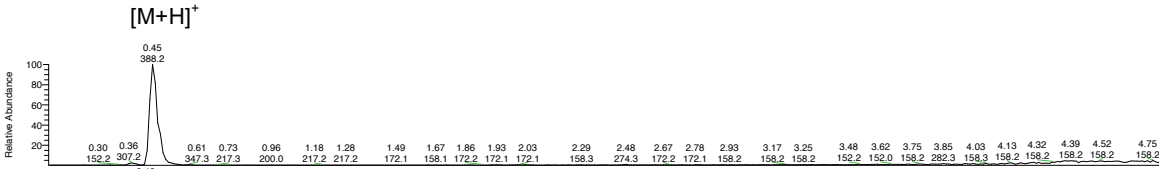
UHPLC-MS chromatogram of L-glutathione




UHPLC-MS chromatogram of the reaction mixture

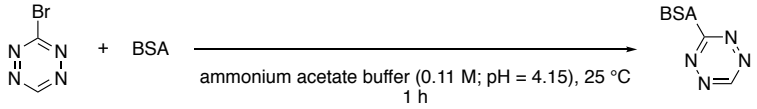



UHPLC-MS chromatogram of the purified product



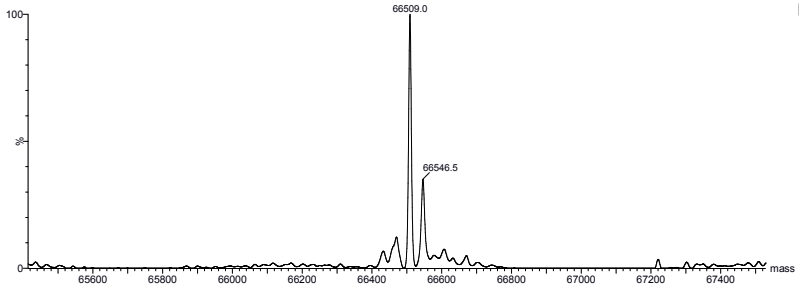
**Functionalization of Bovine Serum Albumin (BSA)**

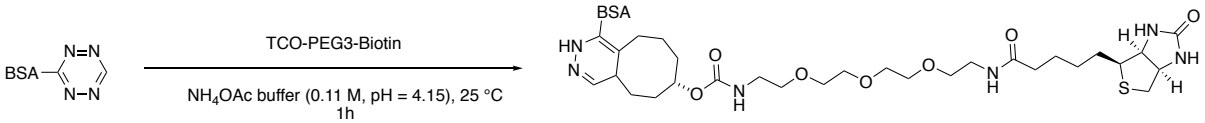
*Ellman`s Assay*:[18] To assess the amount of free sulfhydryl groups present in BSA, an Ellman`s Assay was carried out. 250 µL of a 300 µM BSA solution in borate buffer was added to 2.5 mL of borate buffer (pH = 8.6). 50 µL of a 10 mM 5,5`-dithio-bis-(2-nitrobenzoic acid) (DTNB) solution in borate buffer was added delivering a BSA concentration of 26.8 µM and a DTNB concentration of 178 µM. The mixture was stirred for 15 min, turning yellow over time. Then, a UV-Vis spectrum was recorded and the absorption at 412 nm was measured to be 0.1351. With a coefficient of extinction of 5-mercapto-2-nitrobenzoic acid of $\varepsilon$ = 14150 L · mol$^{-1}$ · cm$^{-1}$ the concentration of free thiol groups in the BSA studied was calculated to be 0.36 free sulfhydryl groups per BSA molecule.
This result was verified using cysteine as a standard. 250 µL of a 300 µM cysteine solution in borate buffer was added to 2.5 mL of borate buffer (pH = 8.6). 50 µL of a 10 mM 5,5`-dithio-bis-(2-nitrobenzoic acid) (DTNB) solution in borate buffer was added delivering a BSA concentration of 26.8 µM and a DTNB concentration of 178 µM. The mixture was stirred for 15 min, turning yellow over time. Then, a UV-Vis spectrum was recorded and the absorption at 412 nm was measured to be 0.4125. With a coefficient of extinction of 5-mercapto-2-nitrobenzoic acid of $\varepsilon$ = 14150 L · mol$^{-1}$ · cm$^{-1}$ the concentration of free thiol groups in cysteine was calculated to be 1.08 free sulfhydryl groups per cysteine molecule, thus verifying the results obtained previously.




One-Pot Functionalization of BSA: BSA (50.0 mg, 0.75 µmol, 1.00 equiv) was dissolved in ammonium acetate buffer (0.11 M, pH = 4.15) (49.5 mL). Then, 3-bromotetrazine (**2**) (0.12 mg, 0.75 µmol, 1.00 equiv), dissolved in MeCN (0.5 mL) was added dropwise and the mixture was stirred for 1 h. After 1 h, full conversion to the monofunctionalized BSA was observed by ESI-MS of a sample from the reaction mixture.

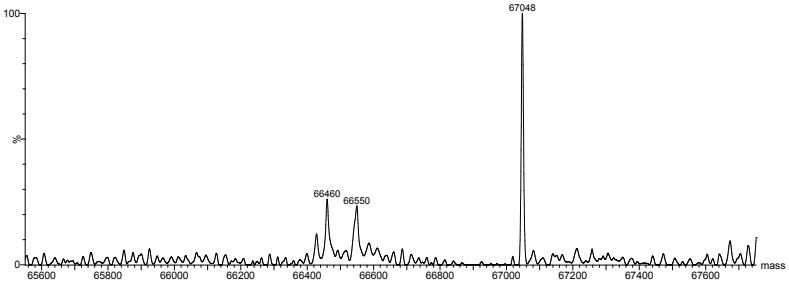
Deconvoluted ESI-MS Spectra of functionalized BSA.




Then, TCO-PEG3-Biotin (0.43 mg, 0.75 µmol, 1.00 equiv) was added. After 1 h, the reaction was complete and the sample was analyzed with ESI-MS. Full conversion to the desired mass was observed.

Deconvoluted ESI-MS Spectra of biotinolyted BSA.



**Determination of the rate constant under pseudo-first order conditions**

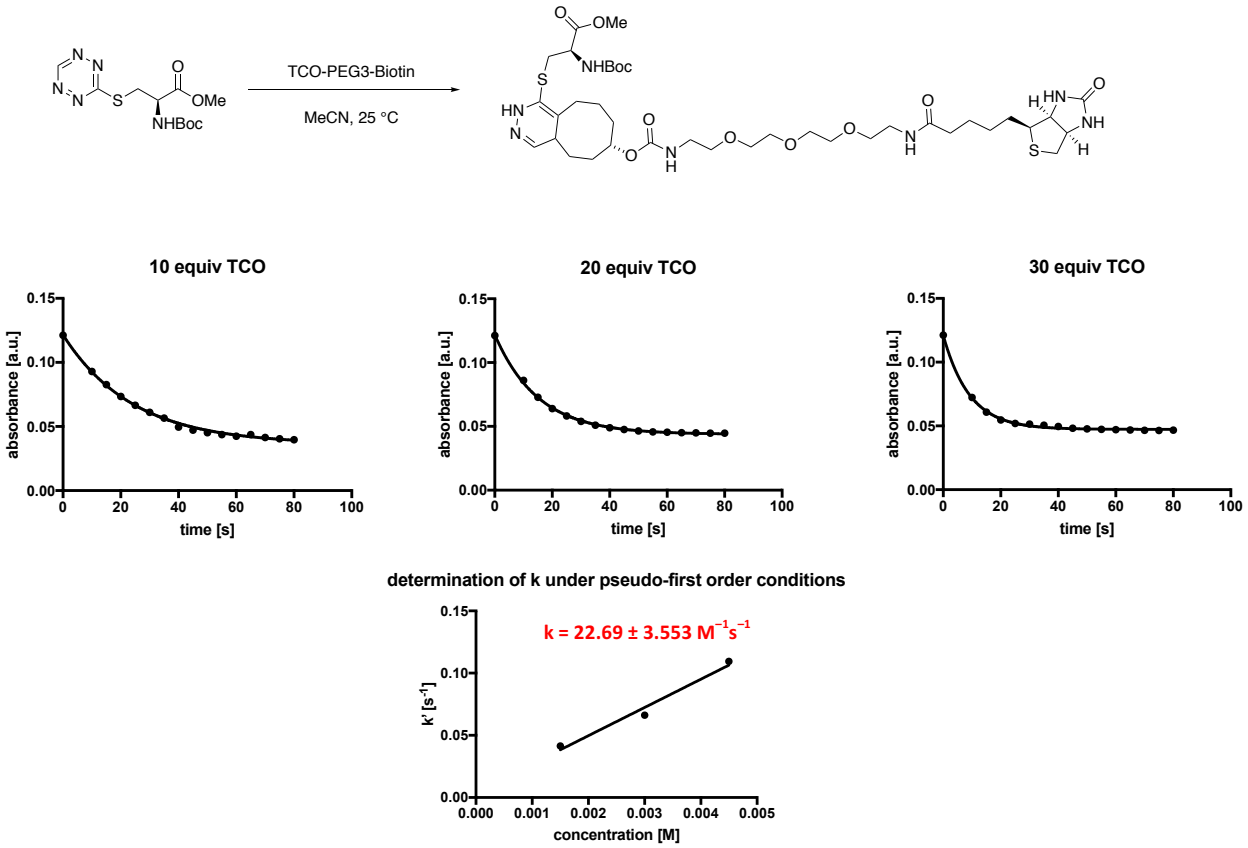
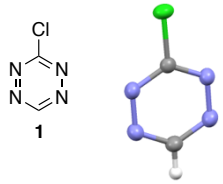



**Figure 12.** Determination of the second-order rate constant k between Boc-Cys(Tet)-OMe (**25**) and TCO-PEG3-Biotin under pseudo-first order conditions. Boc-Cys(Tet)-OMe (300 μM in MeCN) was mixed with different concentrations of TCO-PEG3-Biotin (1.5 mM to 4.5 mM in MeCN) in a UV-quartz cuvette. After 10 sec reaction time, the UV/Vis measurement was started, measuring the absorbance between 570 and 470 nm. The reaction course was monitored by following the decreasing absorbance at 524 nm. The obtained values were fitted to a single exponential equation using Prism 7, thus determining k`. These obtained values were plotted against the concentrations of TCO-PEG3-Biotin and subjected to a linear fit. The rate constant k = 22.69 ± 3.553 $M^{-1}s^{-1}$ was obtained as the slope of the resulting linear fit.

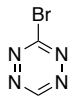
## Crystallographic Data




**1**

**Table S8.** Crystallographic Data for **1**.

| | |
|---|---|
| Crystallized from | $CH_2Cl_2$ |
| Empirical formula | $C_2HClN_4$ |
| Formula weight [g mol$^{-1}$] | 116.52 |
| Crystal colour, habit | orange, plate |
| Crystal dimensions [mm] | 0.02 x 0.13 x 0.26 |
| Temperature [K] | 160(1) |
| Crystal system | orthorhombic |
| Space group | *Pbca* (#61) |
| *Z* | 8 |
| Reflections for cell determination | 3146 |
| $2\theta$ range for cell determination [°] | 14–149 |
| *a* [Å] | 11.2724(5) |
| *b* [Å] | 6.2790(4) |
| *c* [Å] | 12.5272(7) |
| *V* [Å$^3$] | 886.67(9) |
| *F*(000) | 464 |
| $D_X$ [g cm$^{-3}$] | 1.746 |
| $\mu$(Cu $K\alpha$) [mm$^{-1}$] | 6.417 |
| Scan type | $\omega$ |
| $2\theta_{(max)}$ [°] | 149.0 |
| Transmission factors (min; max) | 0.382; 0.858 |
| Total reflections measured | 4312 |
| Symmetry independent reflections | 904 |
| $R_{int}$ | 0.019 |
| Reflections with $I > 2\sigma(I)$ | 883 |
| Reflections used in refinement | 903 |
| Parameters refined | 68 |
| Final $R(F)$ [$I > 2\sigma(I)$ reflections] | 0.0281 |
| $wR(F^2)$ (all data) | 0.0772 |
| Weights: | $w = [\sigma^2 (F_O^2) + (0.0494P)^2 + 0.2063P]^{-1}$ where $P = (F_O^2 + 2F_C^2)/3$ |
| Goodness of fit | 1.096 |
| Final $\Delta_{max}/\sigma$ | 0.001 |
| $\Delta\rho$ (max; min) [e Å$^{-3}$] | 0.16; -0.34 |




**2**

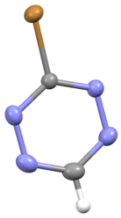
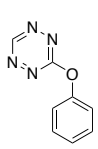


**Table S9.** Crytallographic Data for **2**.

| | |
|---|---|
| Crystallized from | $CH_2Cl_2$ |
| Empirical formula | $C_2HBrN_4$ |
| Formula weight [g mol$^{-1}$] | 160.98 |
| Crystal colour, habit | orange, plate |
| Crystal dimensions [mm] | 0.05 x 0.08 x 0.26 |
| Temperature [K] | 160(1) |
| Crystal system | orthorhombic |
| Space group | *Pbca* (#61) |
| *Z* | 8 |
| Reflections for cell determination | 2980 |
| 2$\theta$ range for cell determination [°] | 6–147 |
| *a* [Å] | 12.7468(5) |
| *b* [Å] | 5.60166(19) |
| *c* [Å] | 12.9507(4) |
| *V* [Å$^3$] | 924.72(6) |
| *F*(000) | 608 |
| $D_X$ [g cm$^{-3}$] | 2.313 |
| $\mu$(Cu $K\alpha$) [mm$^{-1}$] | 10.963 |
| Scan type | $\omega$ |
| $2\theta_{(max)}$ [°] | 147.9 |
| Transmission factors (min; max) | 0.481; 1.000 |
| Total reflections measured | 4719 |
| Symmetry independent reflections | 926 |
| $R_{int}$ | 0.024 |
| Reflections with $I > 2\sigma(I)$ | 904 |
| Reflections used in refinement | 926 |
| Parameters refined | 65 |
| Final $R(F)$ [$I > 2\sigma(I)$ reflections] | 0.0185 |
| $wR(F^2)$ (all data) | 0.0516 |
| Weights: | $w = [\sigma^2(F_O^2) + (0.0285P)^2 + 0.4717P]^{-1}$ where $P = (F_O^2 + 2F_c^2)/3$ |
| Goodness of fit | 1.101 |
| Secondary extinction coefficient | 0.00063(9) |
| Final $\Delta_{max}/\sigma$ | 0.001 |
| $\Delta\rho$ (max; min) [e Å$^{-3}$] | 0.32; -0.43 |



**4**

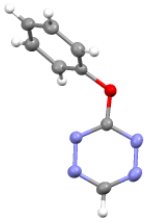
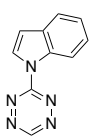


**Table S10.** Crystallographic Date for **4**.

| | |
|---|---|
| Crystallized from | MeCN |
| Empirical formula | $C_8H_6N_4O$ |
| Formula weight [g mol$^{-1}$] | 174.17 |
| Crystal colour, habit | red, tablet |
| Crystal dimensions [mm] | 0.06 x 0.12 x 0.25 |
| Temperature [K] | 160(1) |
| Crystal system | monoclinic |
| Space group | $P2_1/c$ (#14) |
| $Z$ | 4 |
| Reflections for cell determination | 5042 |
| $2\theta$ range for cell determination [°] | 7–57 |
| $a$ [Å] | 10.7358(3) |
| $b$ [Å] | 5.14454(12) |
| $c$ [Å] | 14.9437(4) |
| $\beta$ [°] | 105.056(3) |
| $V$ [Å$^3$] | 797.02(4) |
| $F$(000) | 360 |
| $D_X$ [g cm$^{-3}$] | 1.451 |
| $\mu$(Mo $K\alpha$) [mm$^{-1}$] | 0.104 |
| Scan type | $\omega$ |
| $2\theta_{(max)}$ [°] | 58.5 |
| Transmission factors (min; max) | 0.866; 1.000 |
| Total reflections measured | 9349 |
| Symmetry independent reflections | 1936 |
| $R_{int}$ | 0.017 |
| Reflections with $I > 2\sigma(I)$ | 1614 |
| Reflections used in refinement | 1936 |
| Parameters refined | 119 |
| Final $R(F)$ [$I > 2\sigma(I)$ reflections] | 0.0345 |
| $wR(F^2)$ (all data) | 0.0868 |
| Weights: | w = $[\sigma^2 (F_O^2) + (0.0344P)^2 + 0.2119P]^{-1}$ where $P = (F_O^2 + 2F_C^2)/3$ |
| Goodness of fit | 1.048 |
| Secondary extinction coefficient | 0.012(2) |
| Final $\Delta_{max}/\sigma$ | 0.001 |
| $\Delta\rho$ (max; min) [e Å$^{-3}$] | 0.20; -0.19 |
| $\sigma$ ($d_{(C–C)}$) [Å] | 0.0016 – 0.0018 |



**9**

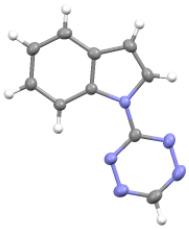
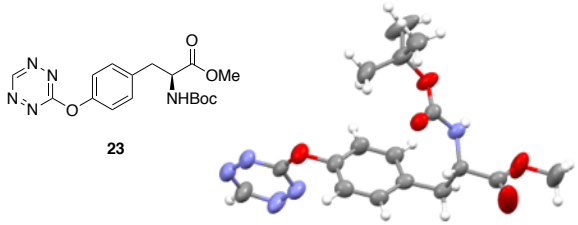


**Table S11.** Crystallographic Data for **9**.

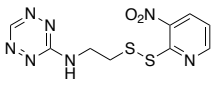
| | |
|---|---|
| Crystallized from | $Et_2O$ |
| Empirical formula | $C_{10}H_7N_5$ |
| Formula weight [g mol$^{-1}$] | 197.21 |
| Crystal colour, habit | red, tablet |
| Crystal dimensions [mm] | 0.06 x 0.14 x 0.16 |
| Temperature [K] | 160(1) |
| Crystal system | orthorhombic |
| Space group | *Fdd*2 (#43) |
| *Z* | 16 |
| Reflections for cell determination | 6471 |
| 2$\theta$ range for cell determination [°] | 9–148 |
| *a* [Å] | 38.7645(3) |
| *b* [Å] | 11.93147(14) |
| *c* [Å] | 7.70847(8) |
| *V* [Å$^3$] | 3565.30(6) |
| *F*(000) | 1632 |
| $D_X$ [g cm$^{-3}$] | 1.470 |
| $\mu$(Cu *K*$\alpha$) [mm$^{-1}$] | 0.794 |
| Scan type | $\omega$ |
| $2\theta_{(max)}$ [°] | 148.1 |
| Transmission factors (min; max) | 0.907; 1.000 |
| Total reflections measured | 8381 |
| Symmetry independent reflections | 1702 |
| $R_{int}$ | 0.013 |
| Reflections with $I > 2\sigma(I)$ | 1689 |
| Reflections used in refinement | 1702 |
| Parameters refined; restraints | 137; 1 |
| Final $R(F)$ [$I > 2\sigma(I)$ reflections] | 0.0228 |
| $wR(F^2)$ (all data) | 0.0608 |
| Weights: | $w = [\sigma^2 (F_O^2) + (0.0379P)^2 + 1.6158P]^{-1}$ where $P = (F_O^2 + 2F_C^2)/3$ |
| Goodness of fit | 1.072 |
| Secondary extinction coefficient | 0.00026(5) |
| Final $\Delta_{max} / \sigma$ | 0.001 |
| $\Delta\rho$ (max; min) [e Å$^{-3}$] | 0.15; -0.11 |
| $\sigma(d_{(C-C)})$ [Å] | 0.002 |




**23**

**Table S12.** Crystallographic Data for **23**.

| | |
|---|---|
| Crystallized from | $Et_2O$ |
| Empirical formula | $C_{17}H_{21}N_5O_5$ |
| Formula weight [g mol$^{-1}$] | 375.39 |
| Crystal colour, habit | pink, needle |
| Crystal dimensions [mm] | 0.03 x 0.04 x 0.28 |
| Temperature [K] | 160(1) |
| Crystal system | monoclinic |
| Space group | *C*2 (#5) |
| *Z* | 4 |
| Reflections for cell determination | 3168 |
| 2$\theta$ range for cell determination [°] | 8–142 |
| *a* [Å] | 32.663(2) |
| *b* [Å] | 5.1610(2) |
| *c* [Å] | 11.0162(6) |
| $\beta$ [°] | 94.704(5) |
| *V* [Å$^3$] | 1850.79(17) |
| *F*(000) | 792 |
| $D_X$ [g cm$^{-3}$] | 1.347 |
| $\mu$(Cu *K*$\alpha$) [mm$^{-1}$] | 0.849 |
| Scan type | $\omega$ |
| $2\theta_{(max)}$ [°] | 146.3 |
| Transmission factors (min; max) | 0.565; 1.000 |
| Total reflections measured | 13382 |
| Symmetry independent reflections | 3606 |
| $R_{int}$ | 0.064 |
| Reflections with $I > 2\sigma(I)$ | 2965 |
| Reflections used in refinement | 3606 |
| Parameters refined; restraints | 252; 2 |
| Final $R(F)$ [$I > 2\sigma(I)$ reflections] | 0.0541 |
| $wR(F^2)$ (all data) | 0.1556 |
| Weights: | $w = [\sigma^2 (F_O^2) + (0.0850P)^2 + 0.5336P]^{-1}$ where $P = (F_O^2 + 2F_C^2)/3$ |
| Goodness of fit | 1.039 |
| Final $\Delta_{max} / \sigma$ | 0.000 |
| $\Delta\rho$ (max; min) [e Å$^{-3}$] | 0.29; -0.17 |
| $\sigma$ ($d_{(C–C)}$) [Å] | 0.005 – 0.008 |



**39**

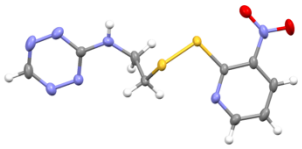
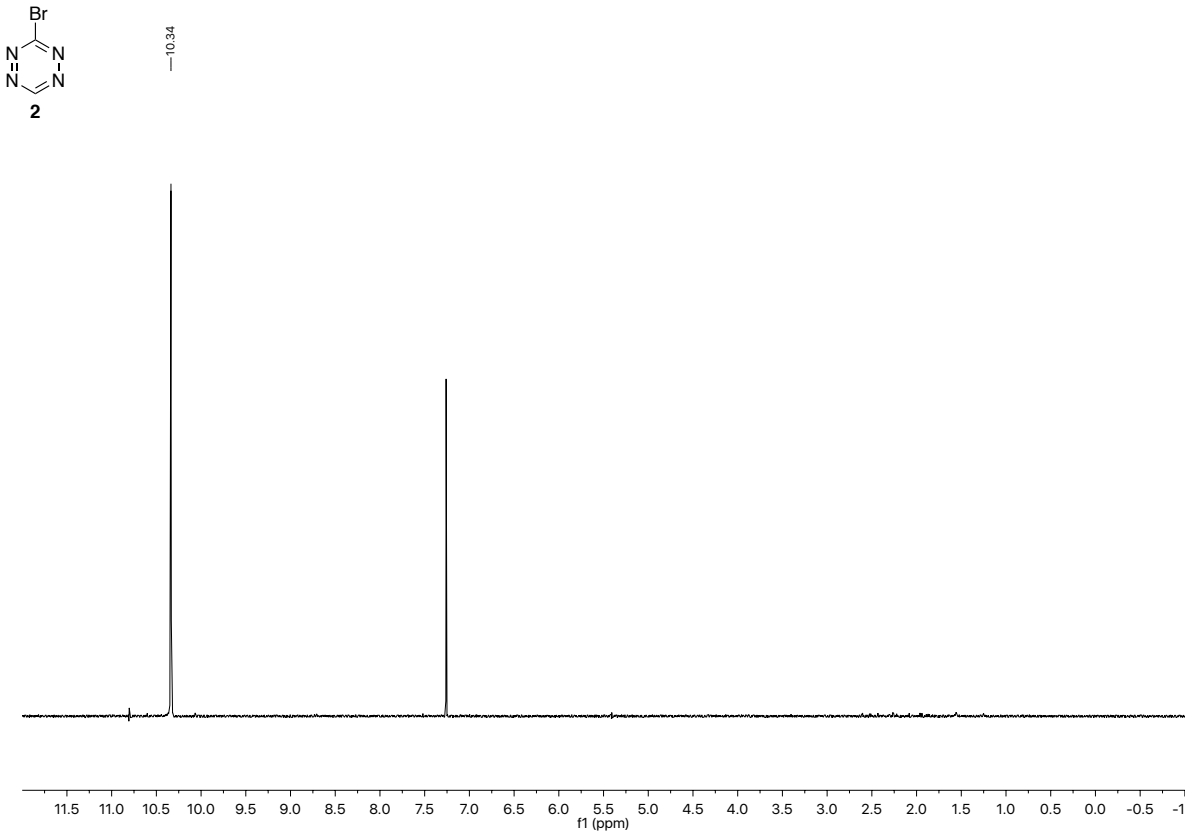


**Table S13.** Crystallographic Data for **39**.

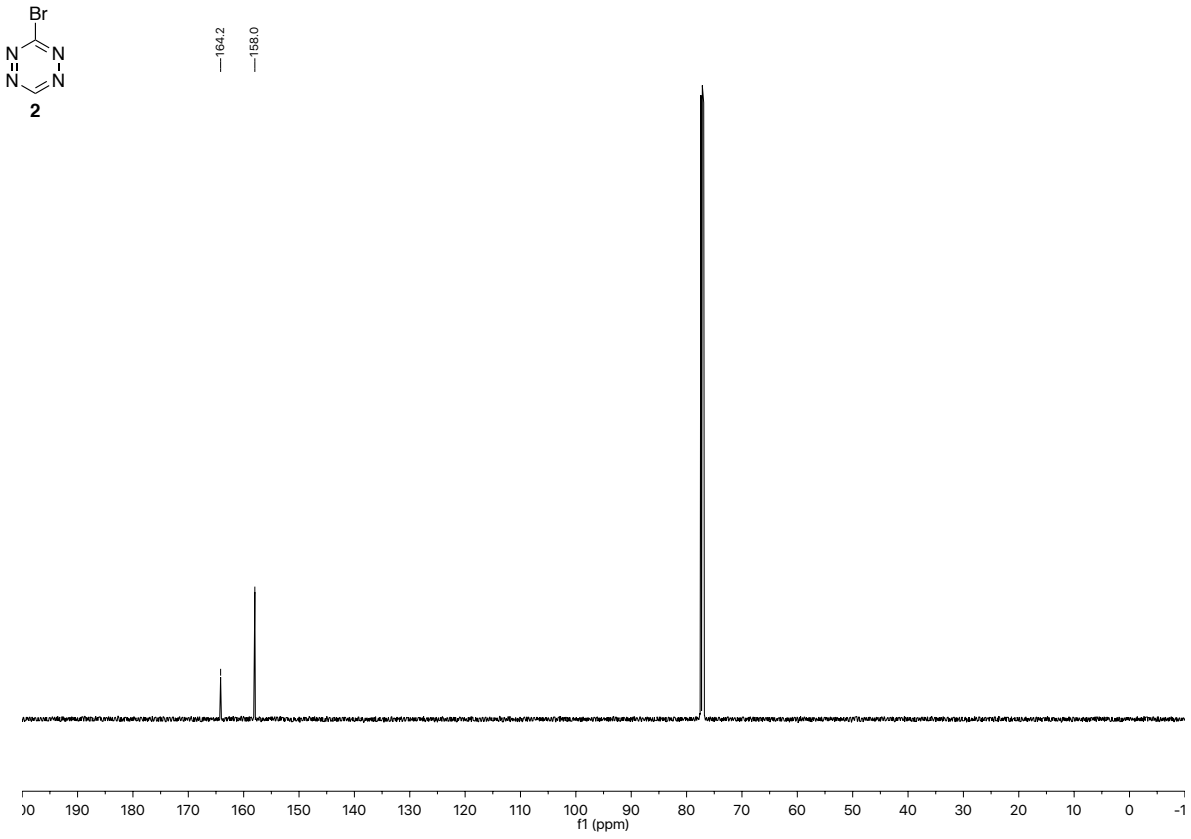
| | |
|---|---|
| Crystallized from | $CH_2Cl_2$ / MeCN |
| Empirical formula | $C_9H_9N_7O_2S_2$ |
| Formula weight [g mol$^{-1}$] | 311.35 |
| Crystal colour, habit | red, prism |
| Crystal dimensions [mm] | 0.12 x 0.20 x 0.23 |
| Temperature [K] | 160(1) |
| Crystal system | monoclinic |
| Space group | *C*2/*c* (#15) |
| *Z* | 8 |
| Reflections for cell determination | 10728 |
| 2$\theta$ range for cell determination [°] | 6–61 |
| *a* [Å] | 17.7417(3) |
| *b* [Å] | 6.22991(9) |
| *c* [Å] | 23.2377(3) |
| $\beta$ [°] | 93.9389(13) |
| *V* [Å$^3$] | 2562.38(7) |
| *F*(000) | 1280 |
| $D_X$ [g cm$^{-3}$] | 1.614 |
| $\mu$(Cu *K*$\alpha$) [mm$^{-1}$] | 0.429 |
| Scan type | $\omega$ |
| $2\theta_{(max)}$ [°] | 60.5 |
| Transmission factors (min; max) | 0.927; 1.000 |
| Total reflections measured | 16497 |
| Symmetry independent reflections | 3564 |
| $R_{int}$ | 0.019 |
| Reflections with $I > 2\sigma(I)$ | 3204 |
| Reflections used in refinement | 3564 |
| Parameters refined | 186 |
| Final $R(F)$ [$I > 2\sigma(I)$ reflections] | 0.0266 |
| $wR(F^2)$ (all data) | 0.0704 |
| Weights: | $w = [\sigma^2 (F_O^2) + (0.0311P)^2 + 1.7110P]^{-1}$ where $P = (F_O^2 + 2F_C^2)/3$ |
| Goodness of fit | 1.060 |
| Secondary extinction coefficient | 0.0007(2) |
| Final $\Delta_{max} / \sigma$ | 0.001 |
| $\Delta\rho$ (max; min) [e Å$^{-3}$] | 0.32; -0.22 |
| $\sigma$ ($d_{(C–C)}$) [Å] | 0.0015 – 0.0019 |

**NMR Spectra**

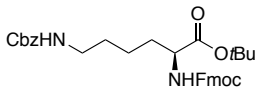
$^{1}$H-NMR, $CDCl_3$, 400 MHz




$^{13}$C-NMR, $CDCl_3$, 101 MHz



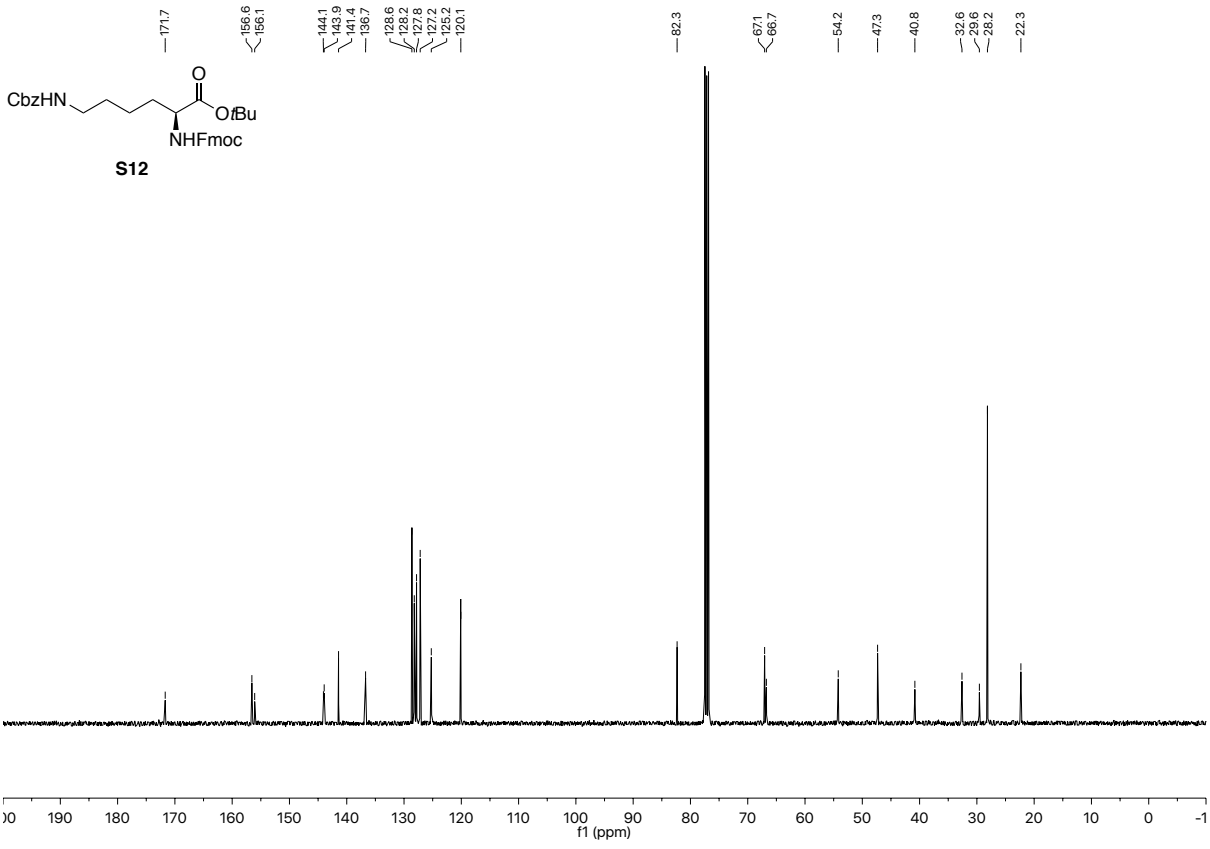
$^{1}$H-NMR, $CDCl_3$, 400 MHz




**S12**

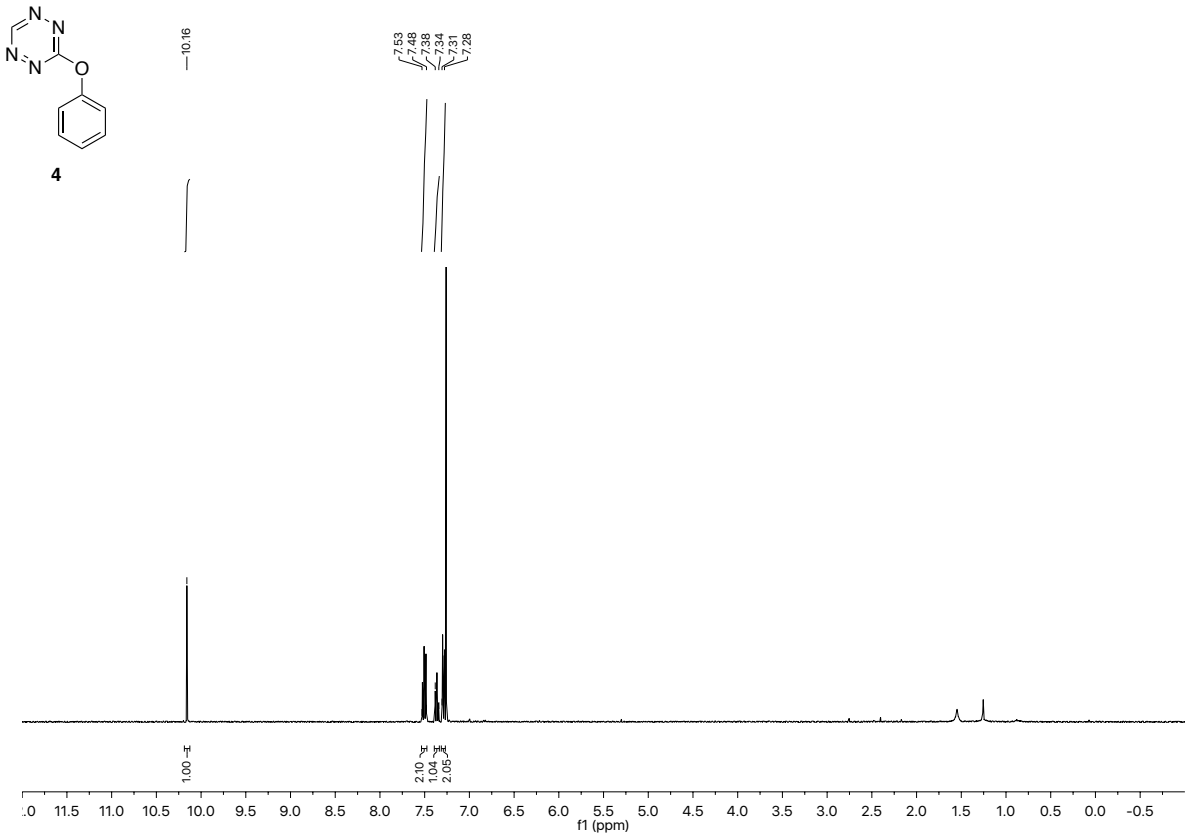



$^{13}$C-NMR, $CDCl_3$, 101 MHz



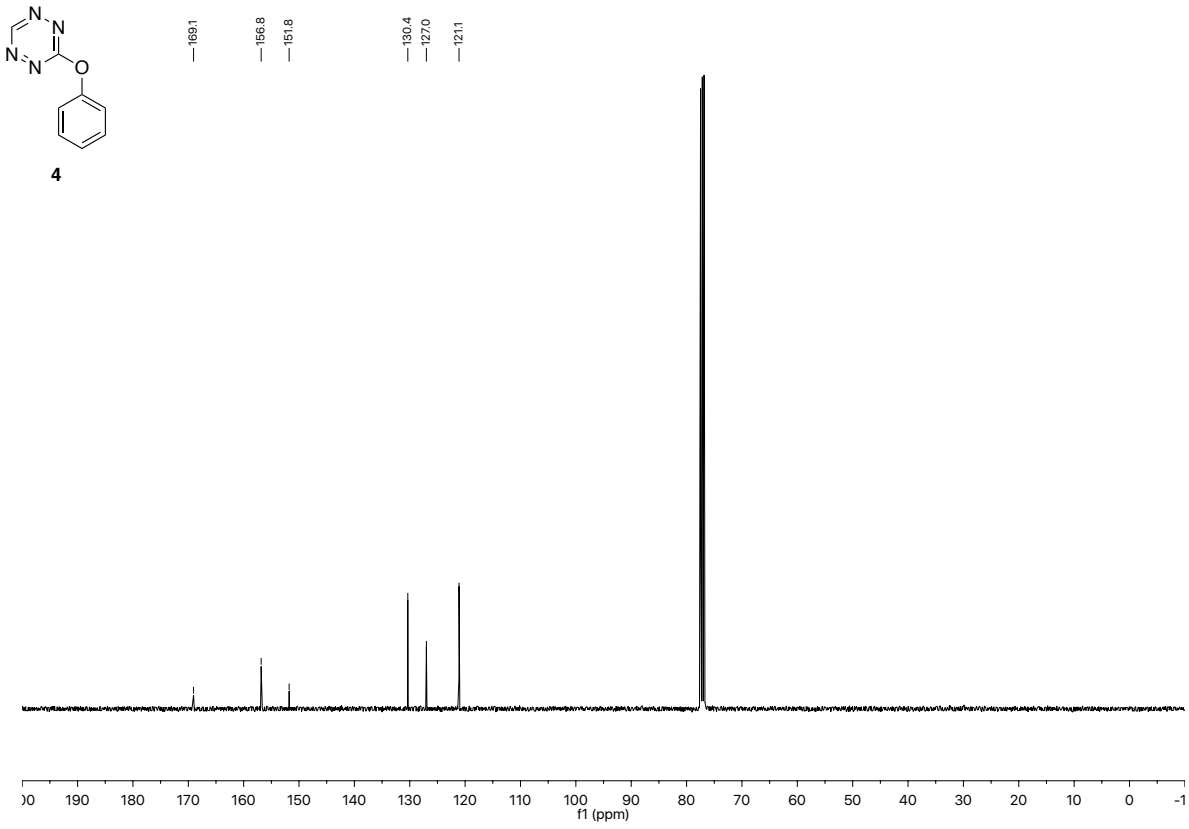

**S12**

$^{1}$H-NMR, $CDCl_3$, 400 MHz



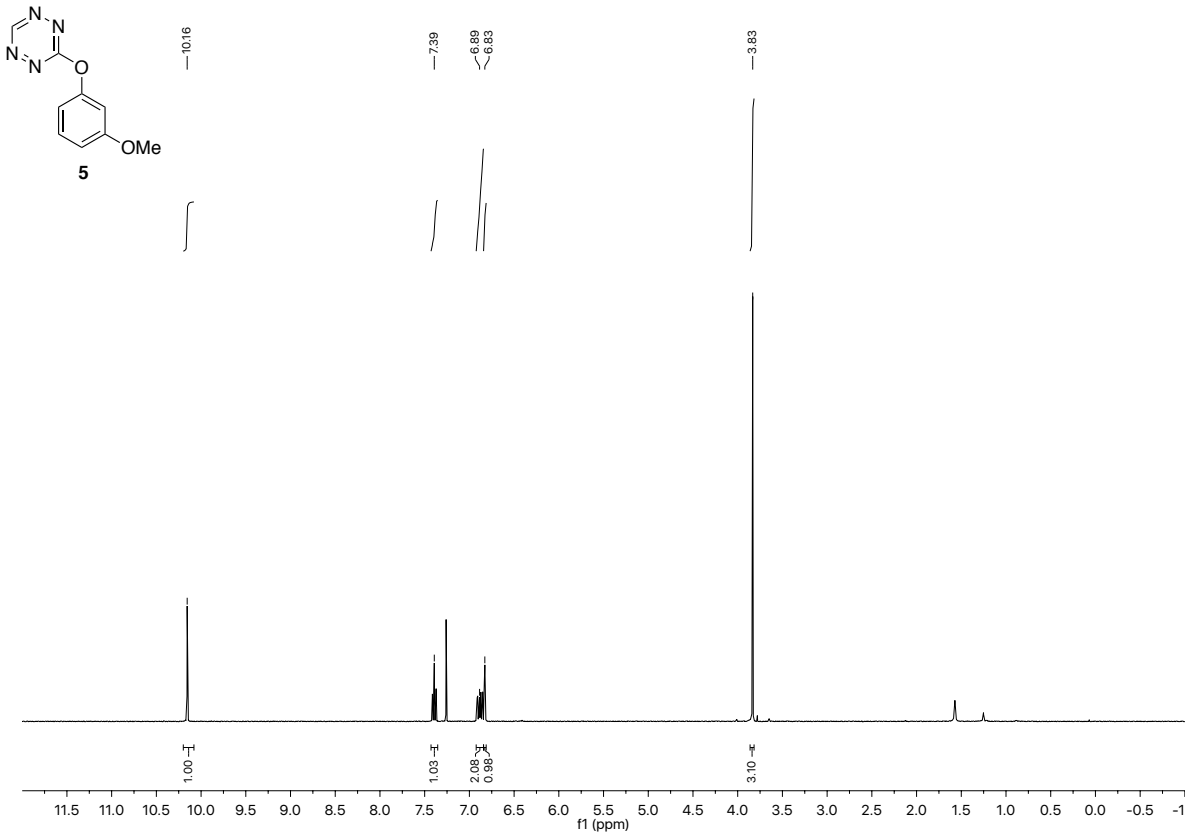

**4**

$^{13}$C-NMR, $CDCl_3$, 101 MHz

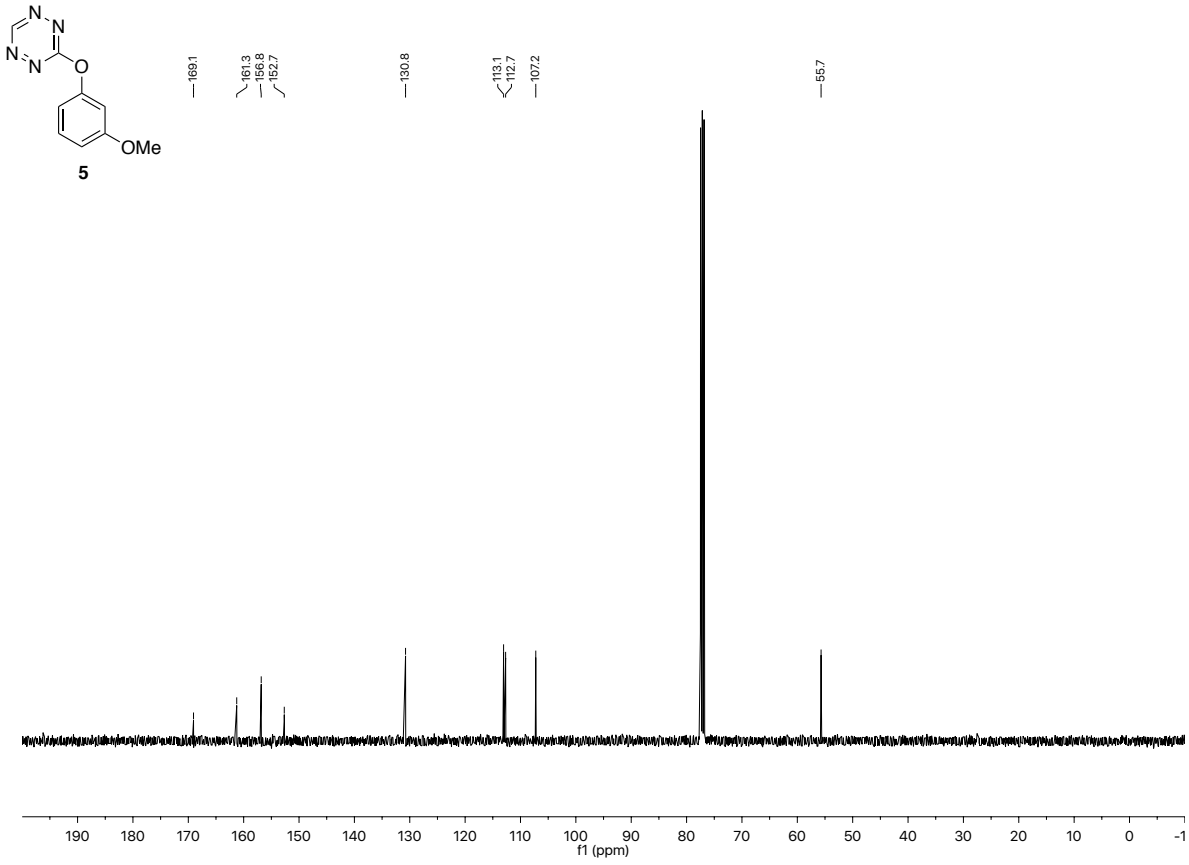



**4**

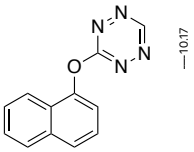
$^{1}$H-NMR, $CDCl_3$, 400 MHz




$^{13}$C-NMR, $CDCl_3$, 101 MHz



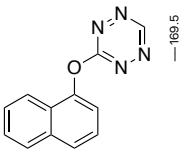
$^{1}$H-NMR, $CDCl_3$, 400 MHz



**S14**

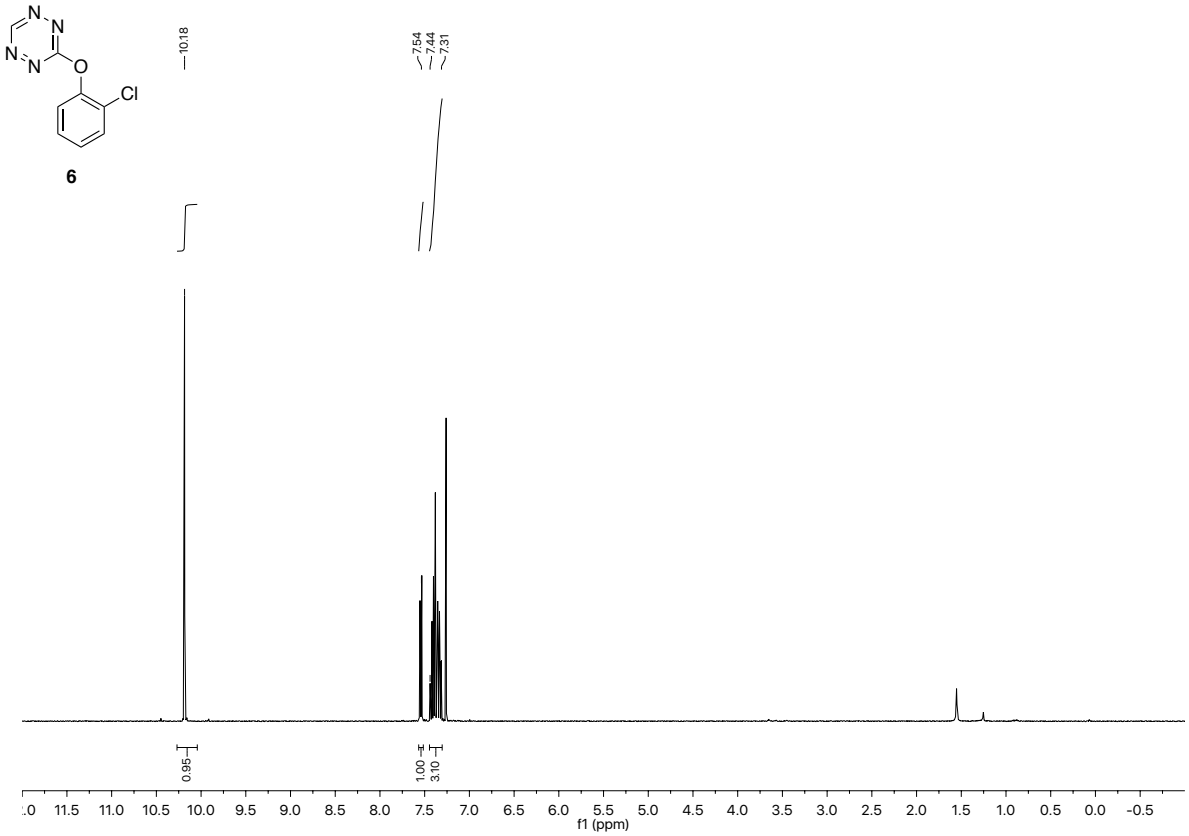



$^{13}$C-NMR, $CDCl_3$, 101 MHz



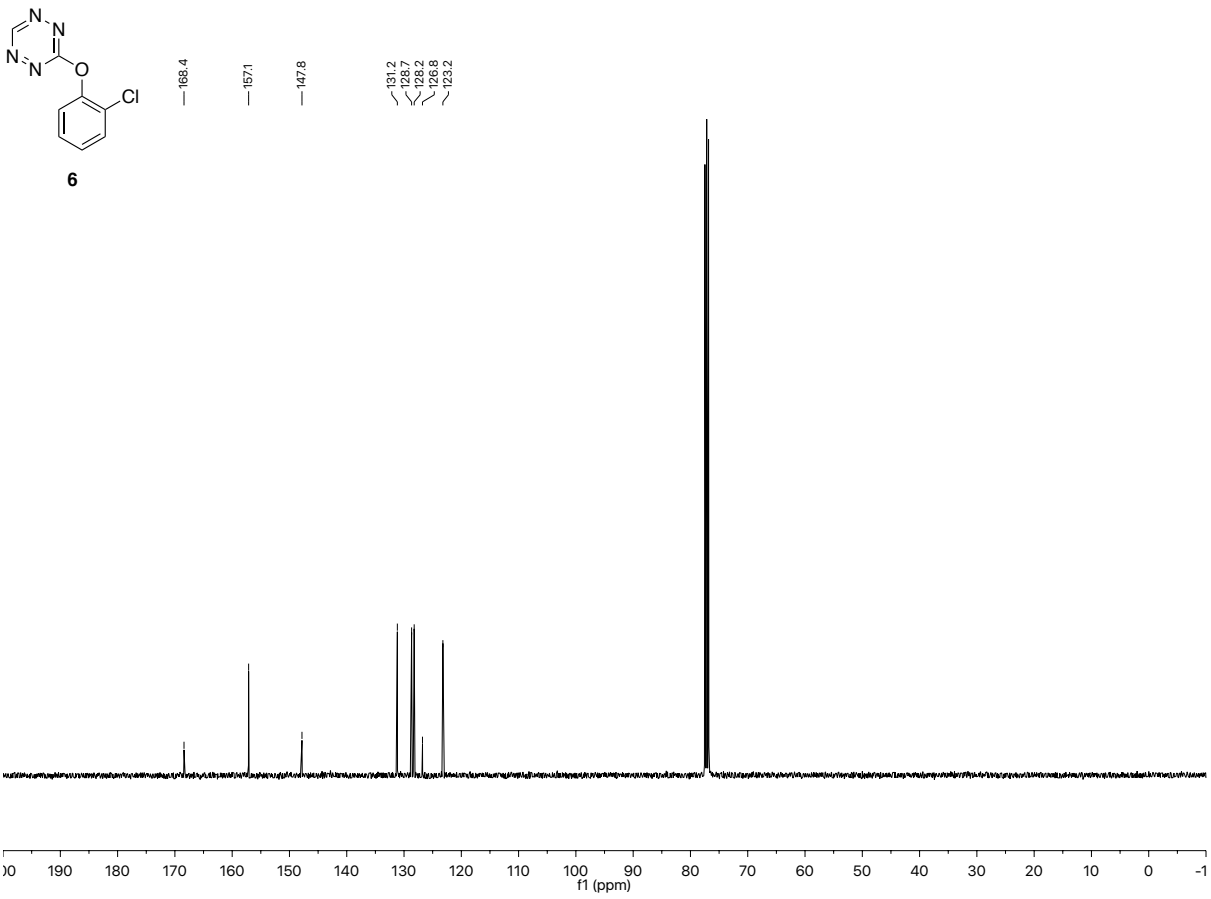
**S14**

$^{1}$H-NMR, $CDCl_3$, 400 MHz



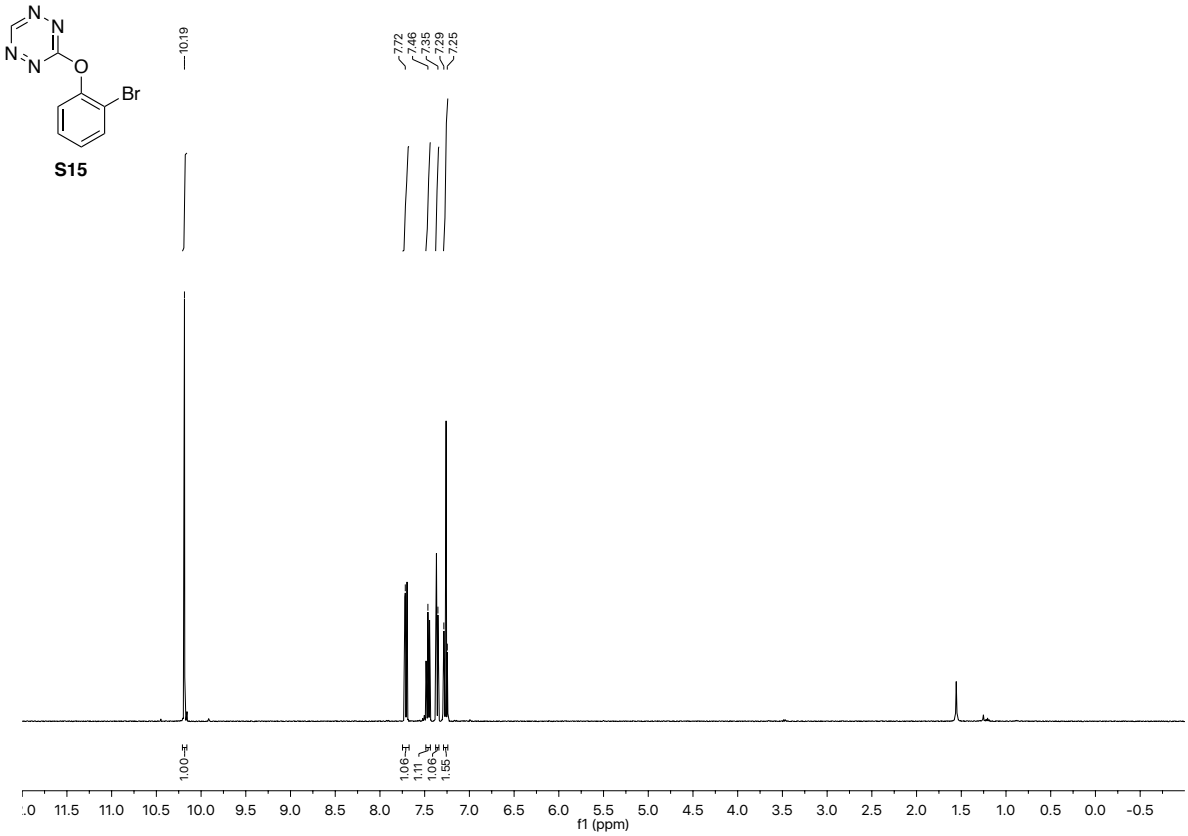

6

$^{13}$C-NMR, $CDCl_3$, 101 MHz

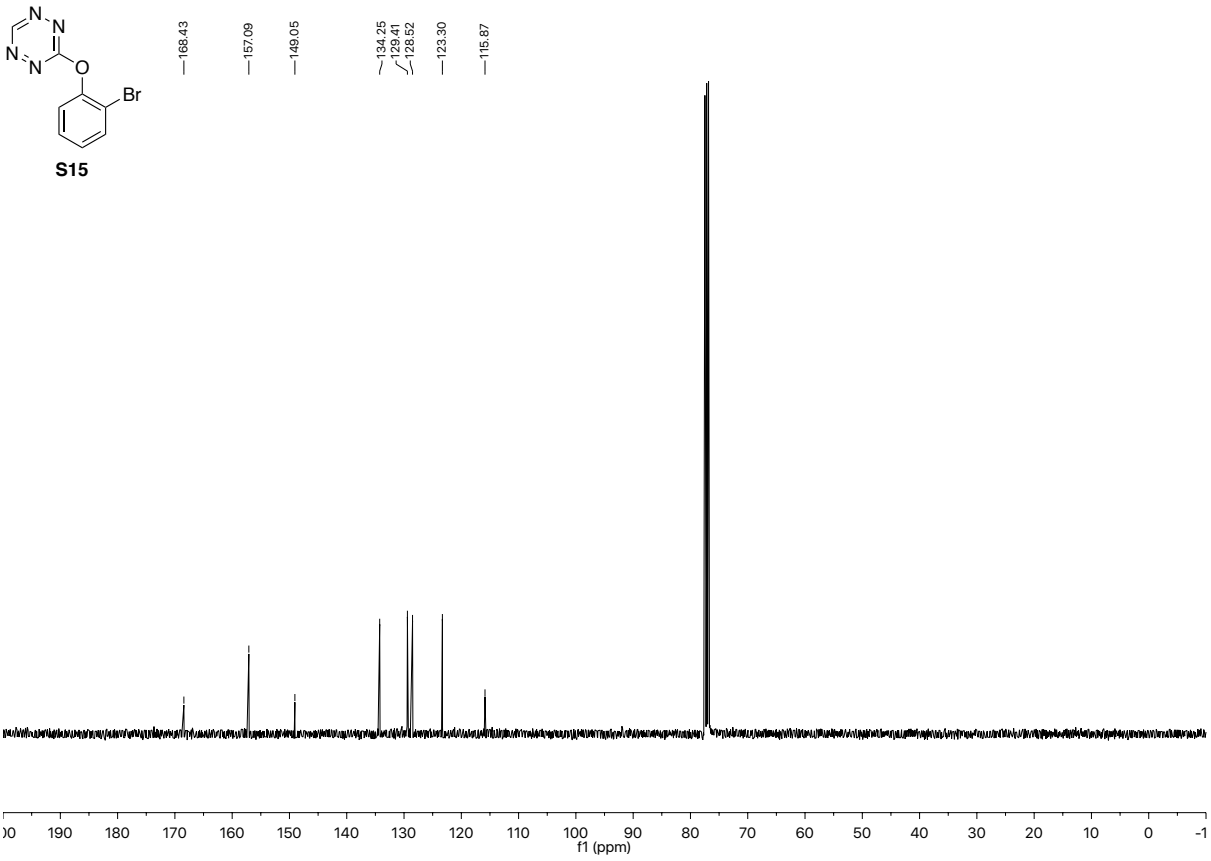



6

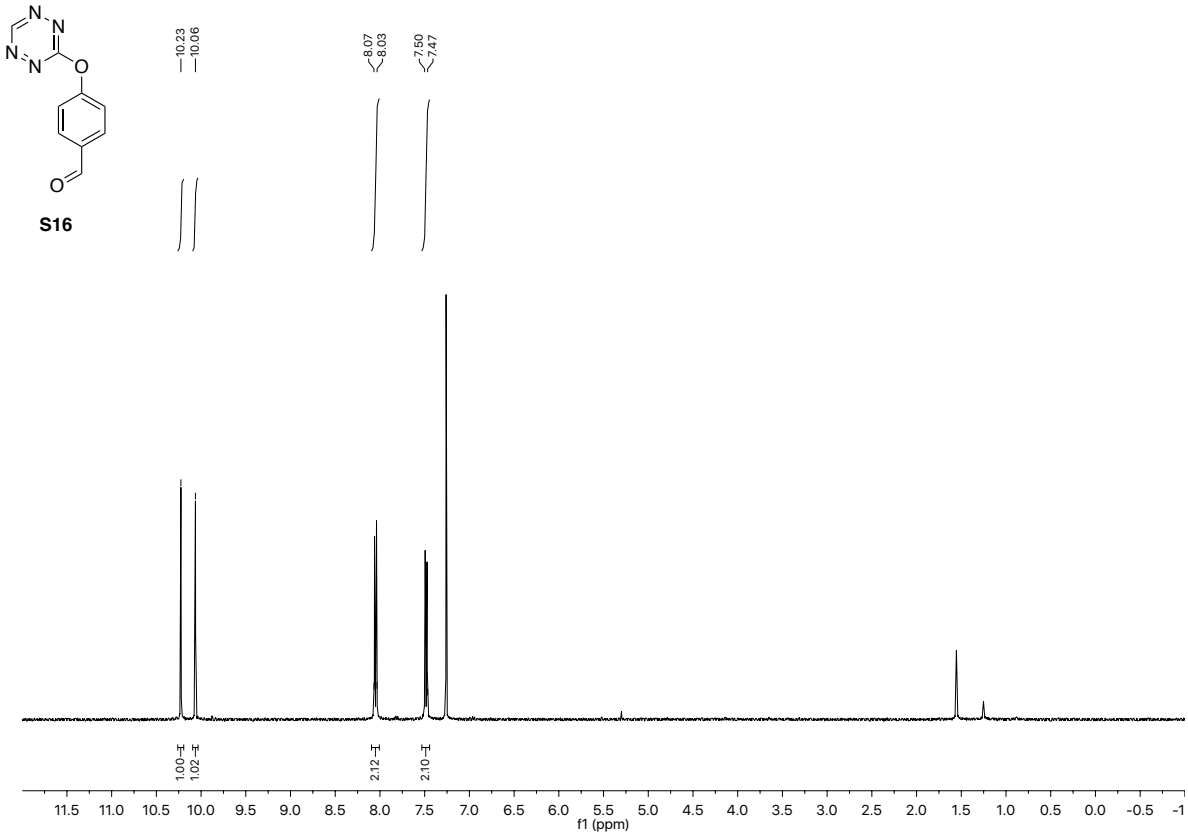
$^{1}$H-NMR, $CDCl_3$, 400 MHz




$^{13}$C-NMR, $CDCl_3$, 101 MHz

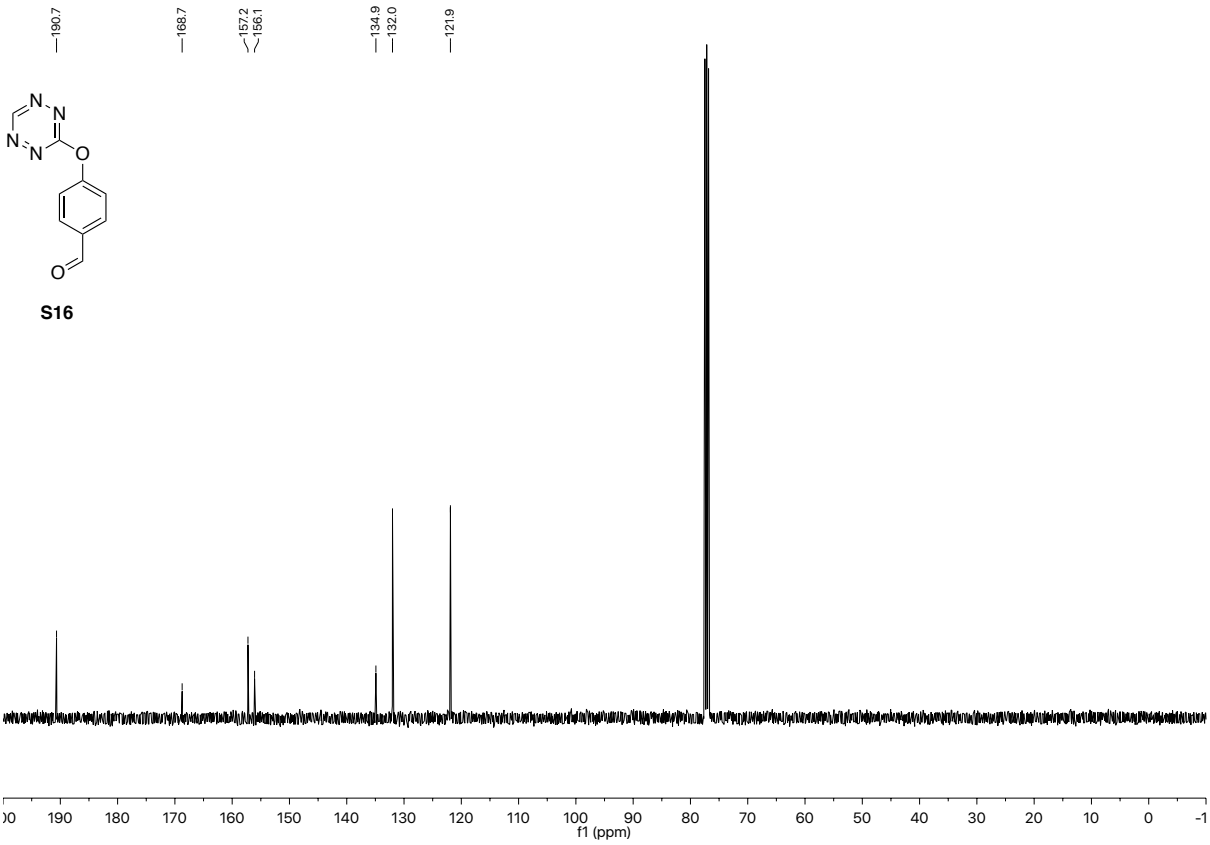


$^{1}$H-NMR, $CDCl_3$, 400 MHz



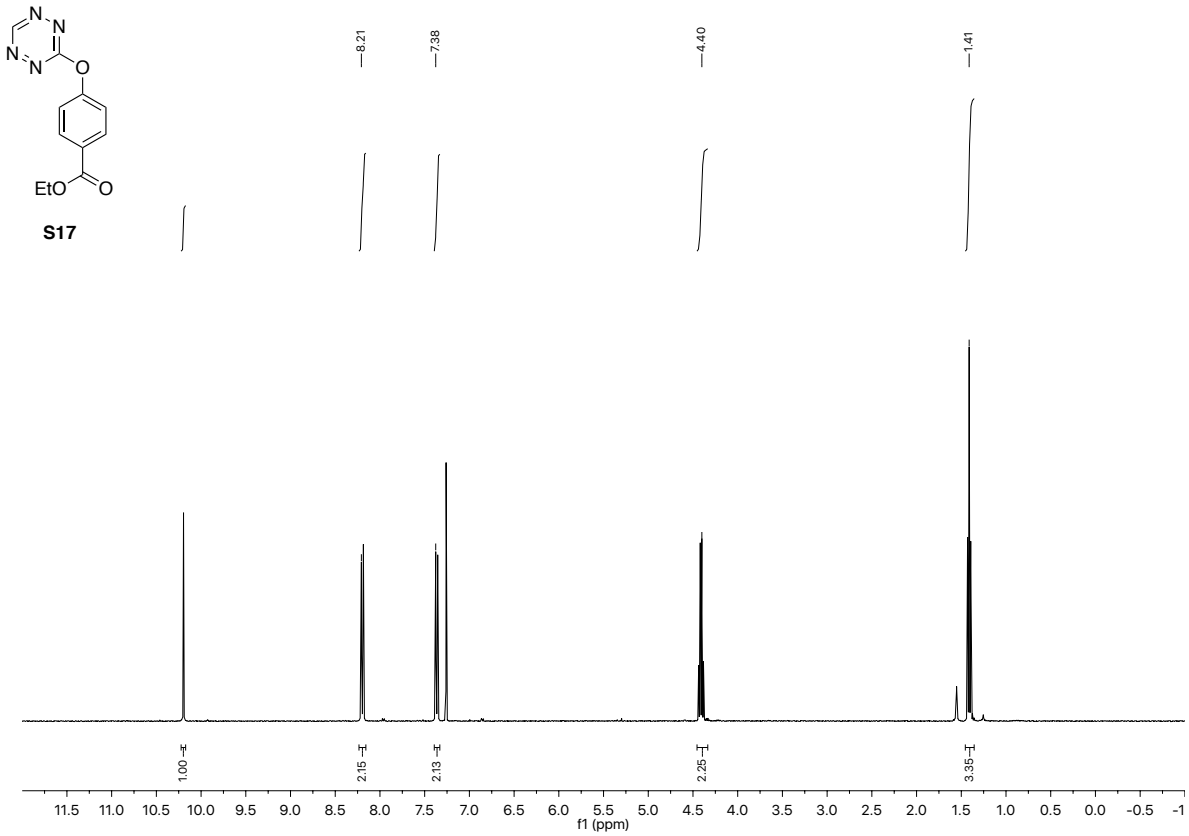

**S16**

$^{13}$C-NMR, $CDCl_3$, 101 MHz



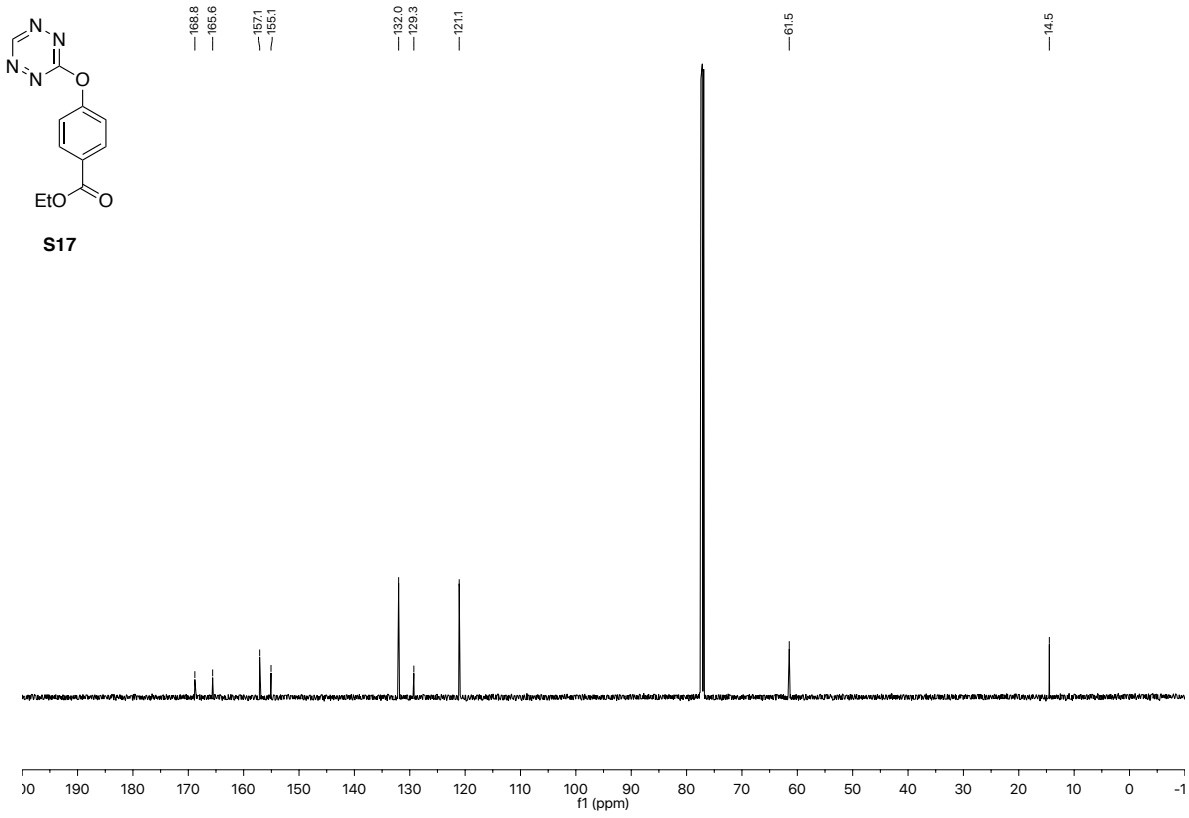

**S16**

$^{1}$H-NMR, $CDCl_3$, 400 MHz



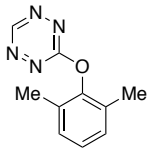

**S17**

$^{13}$C-NMR, $CDCl_3$, 126 MHz




**S17**

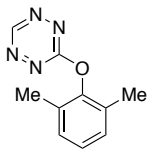
$^{1}$H-NMR, $CDCl_3$, 400 MHz



**S18**

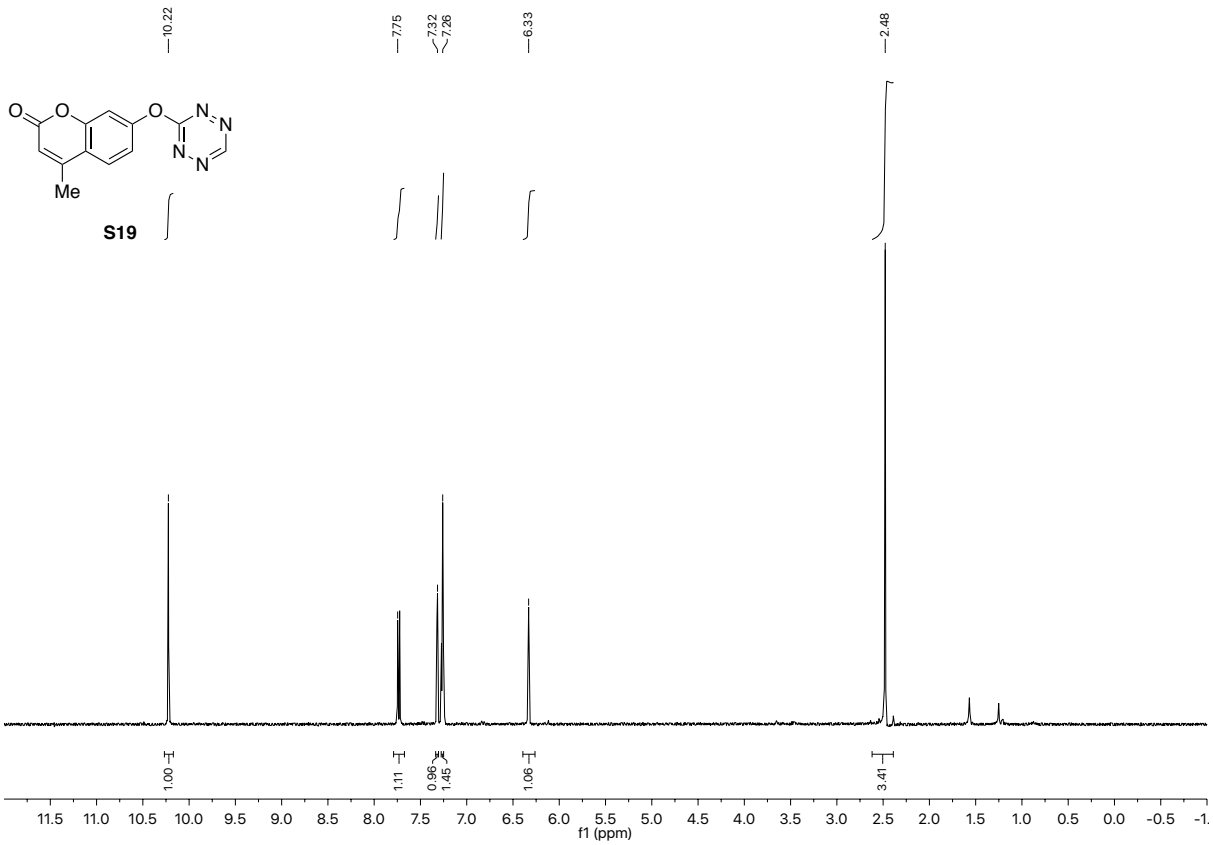



$^{13}$C-NMR, $CDCl_3$, 101 MHz

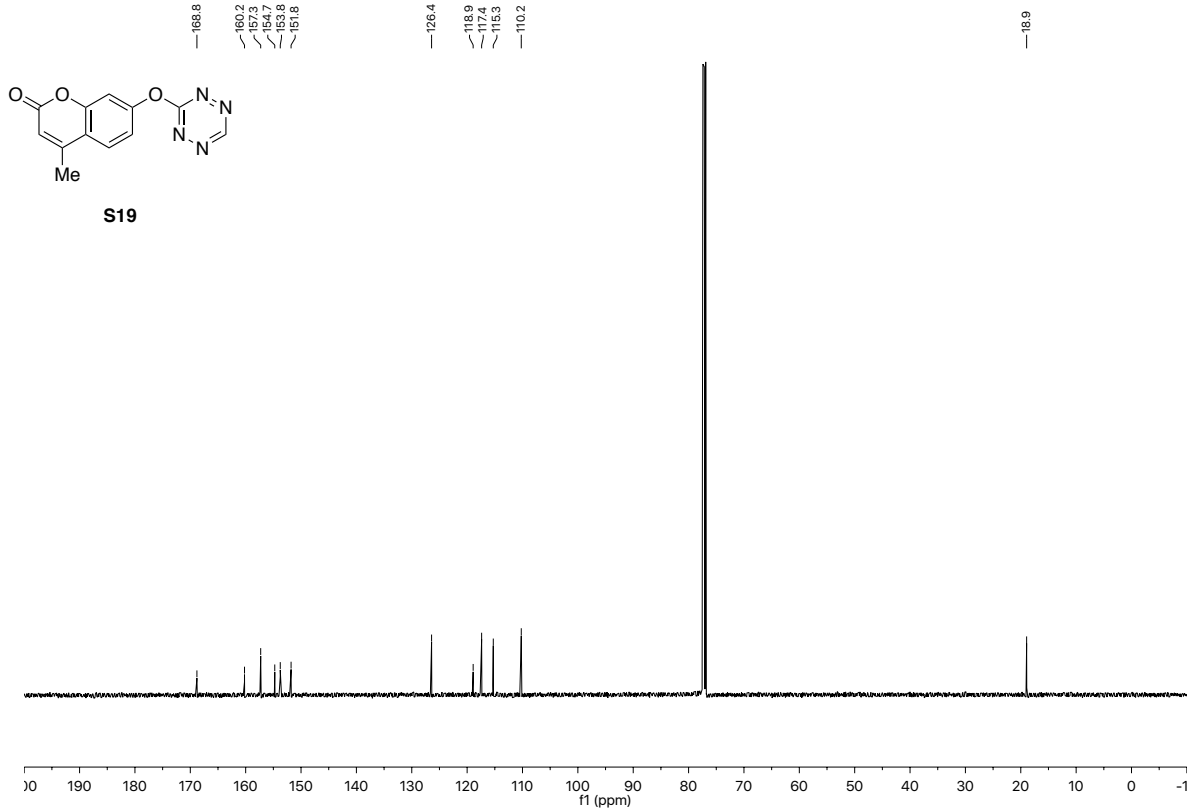


**S18**

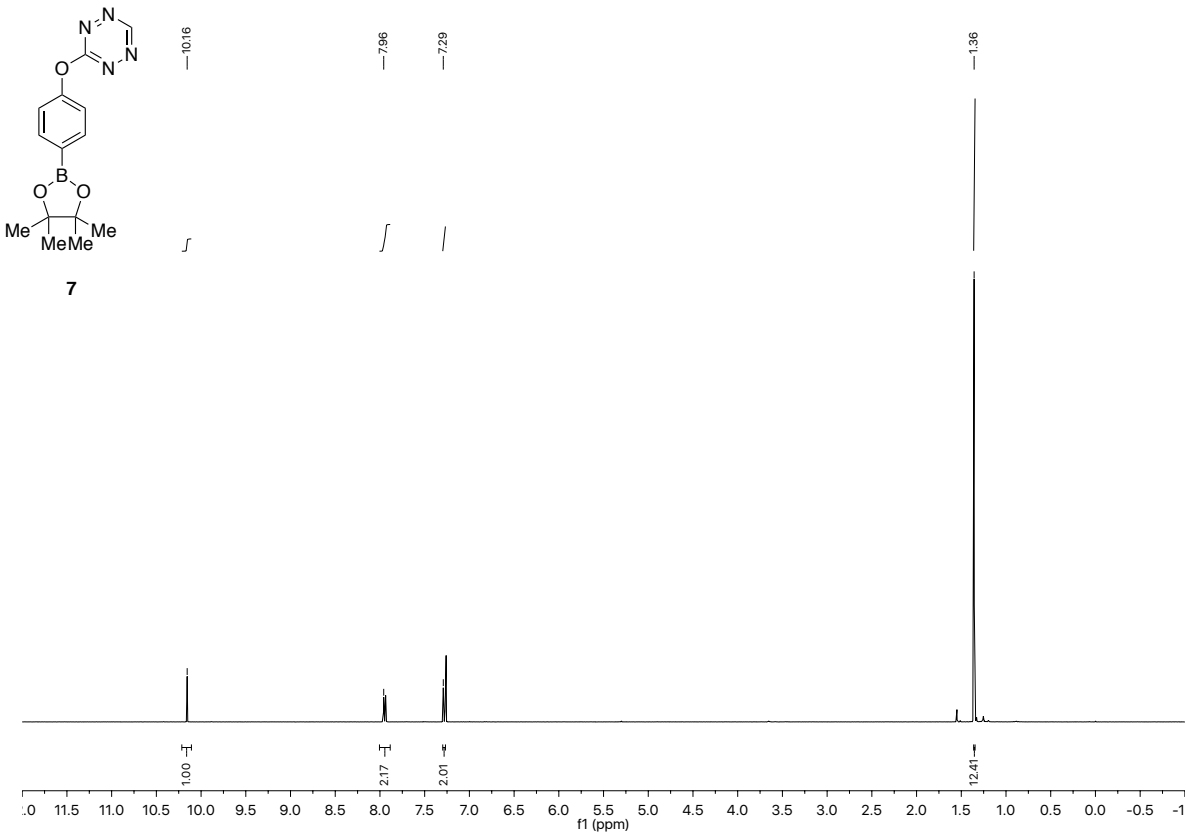
$^{1}$H-NMR, $CDCl_3$, 400 MHz




$^{13}$C-NMR, $CDCl_3$, 126 MHz

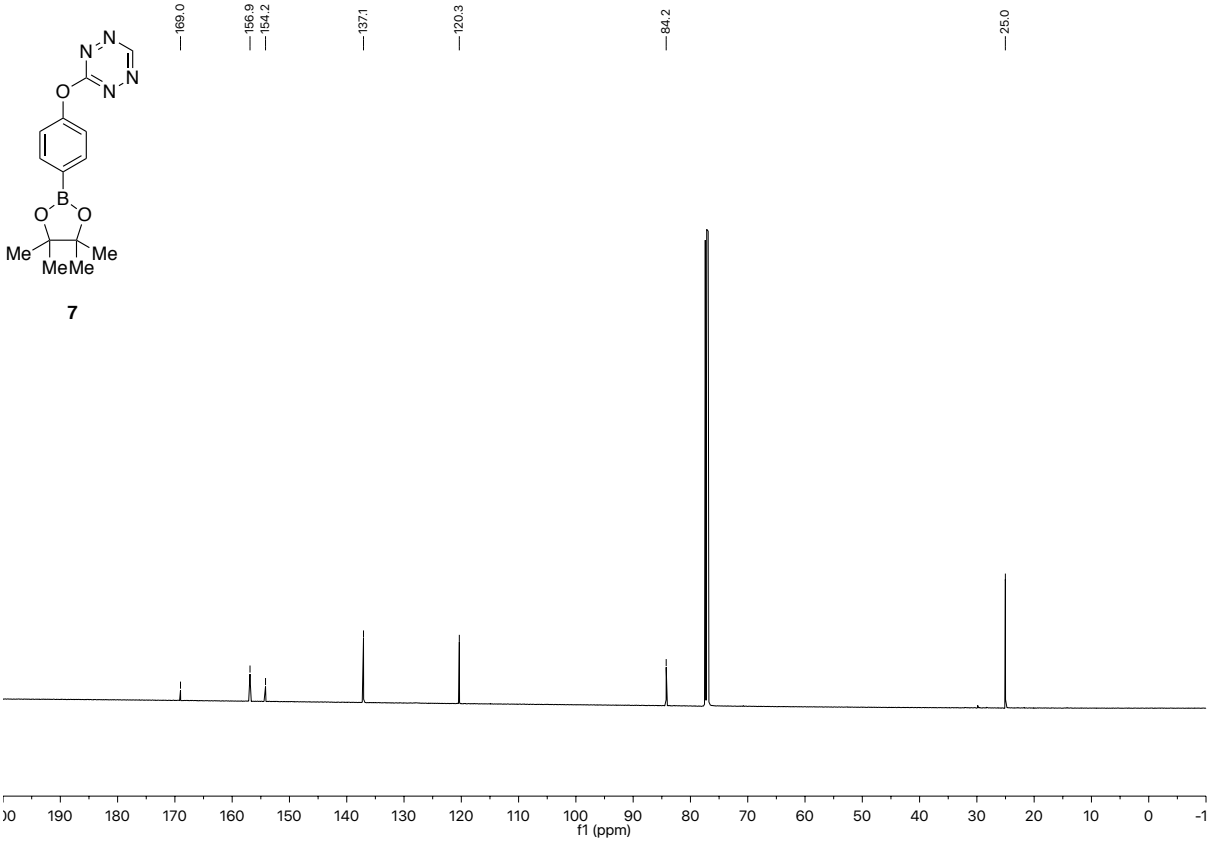


$^{1}$H-NMR, $CDCl_3$, 400 MHz



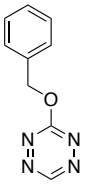

**7**

$^{13}$C-NMR, $CDCl_3$, 126 MHz




**7**

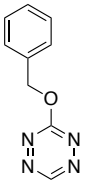
$^{1}$H-NMR, $CDCl_3$, 400 MHz



**S20**

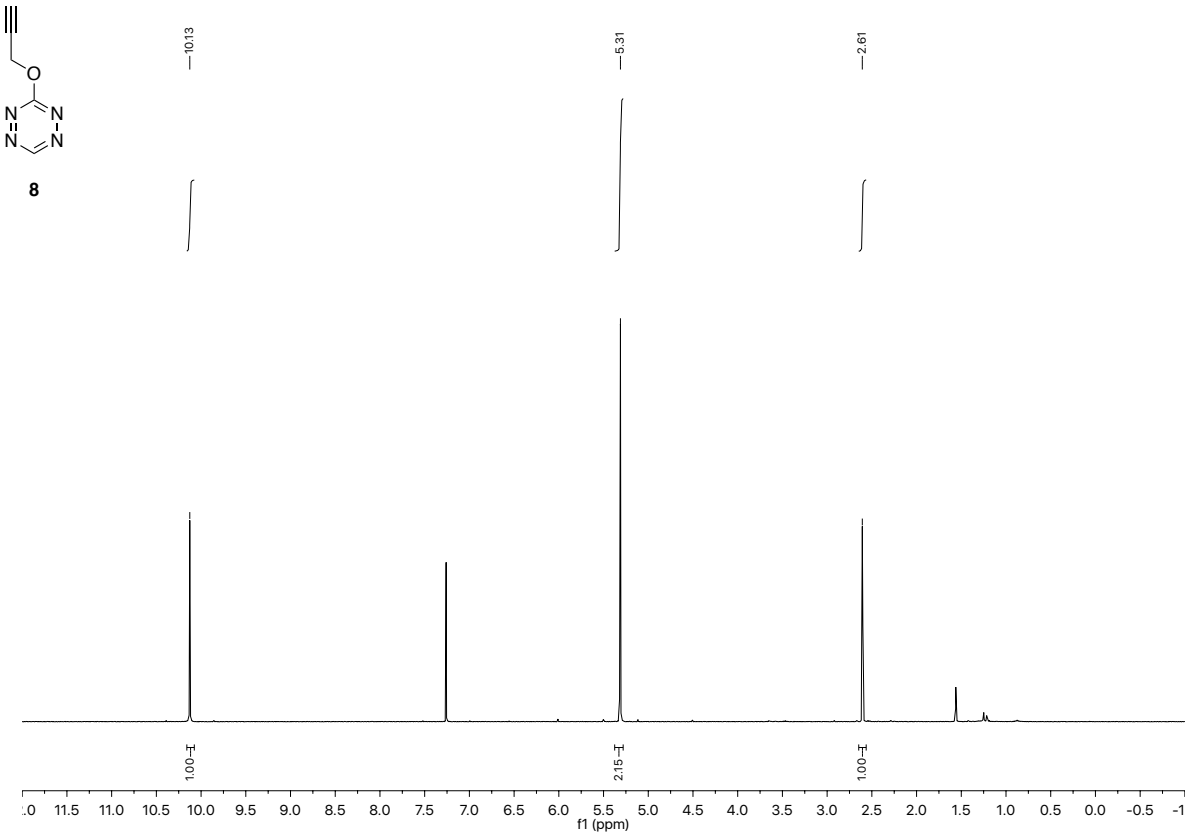



$^{13}$C-NMR, $CDCl_3$, 101 MHz



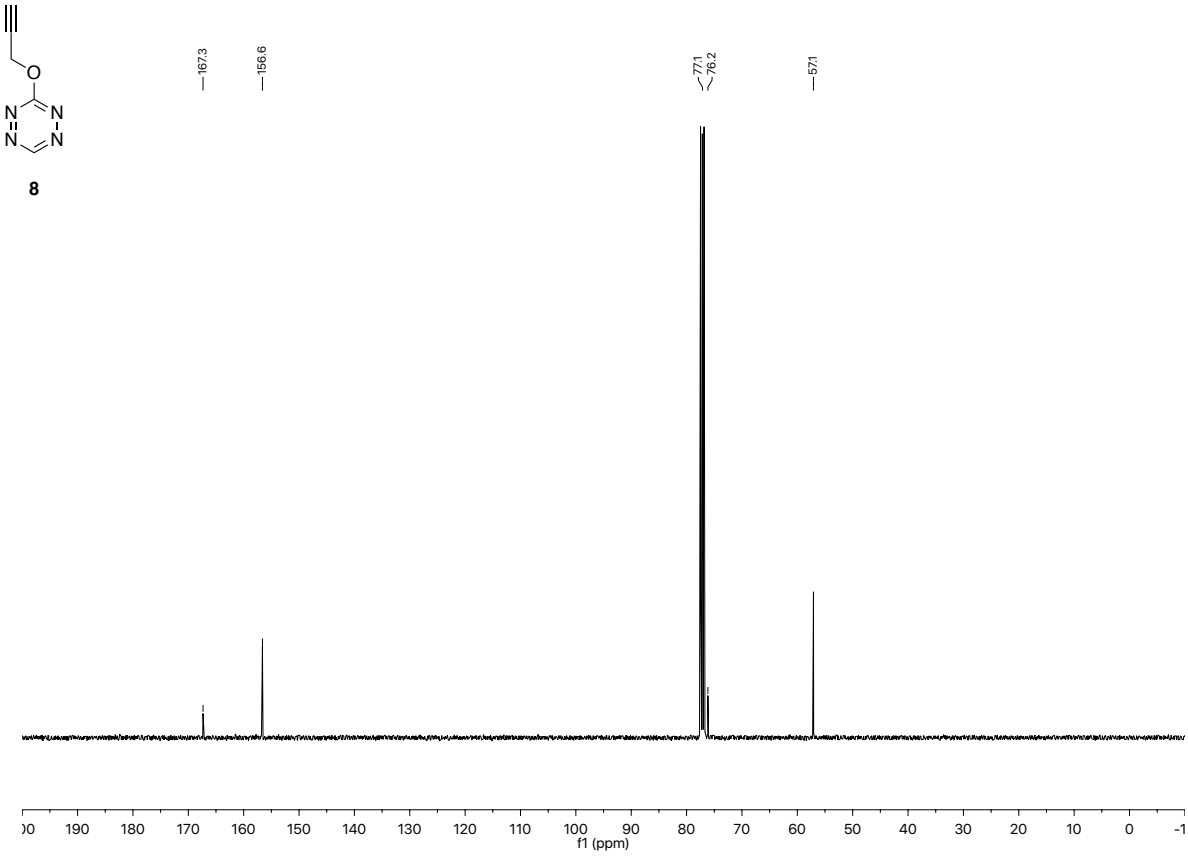
**S20**

$^{1}$H-NMR, $CDCl_3$, 400 MHz



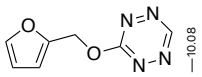

**8**

$^{13}$C-NMR, $CDCl_3$, 101 MHz




**8**

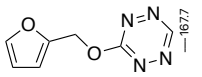
$^{1}$H-NMR, $CDCl_3$, 400 MHz



**S21**

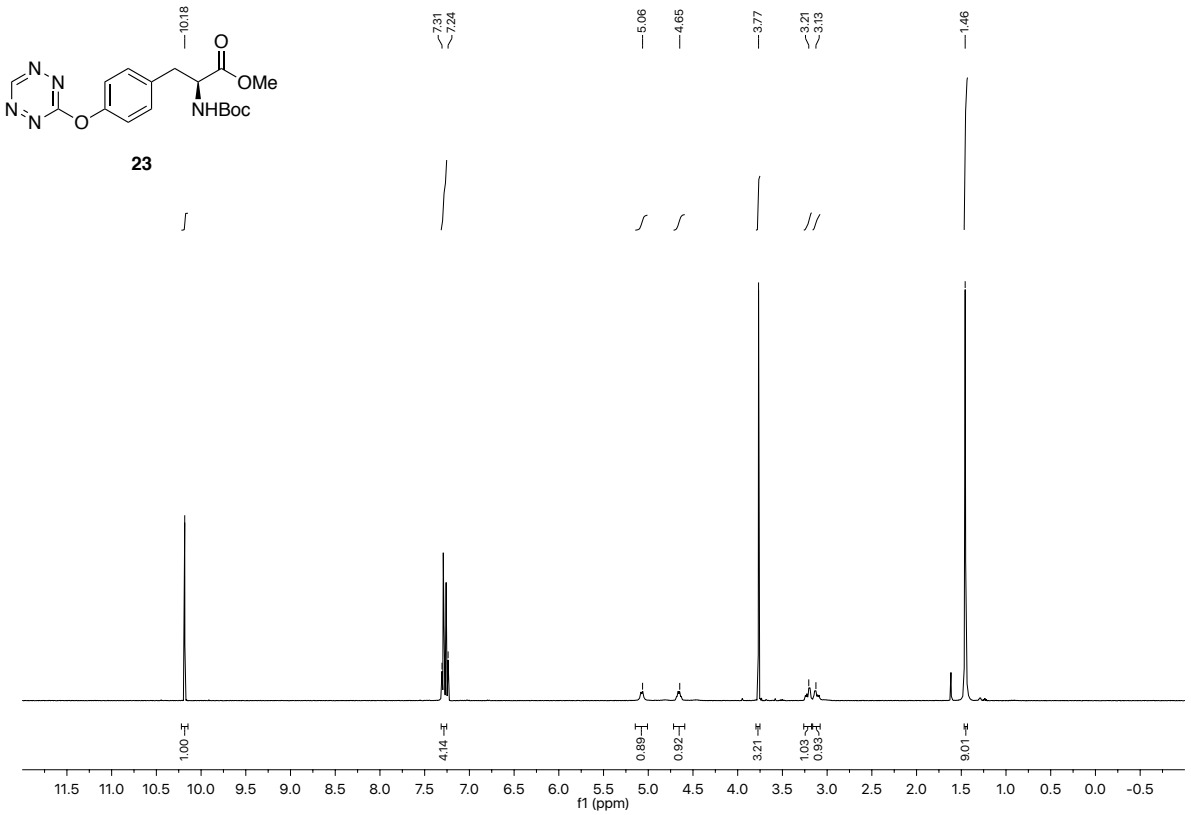



$^{13}$C-NMR, $CDCl_3$, 126 MHz



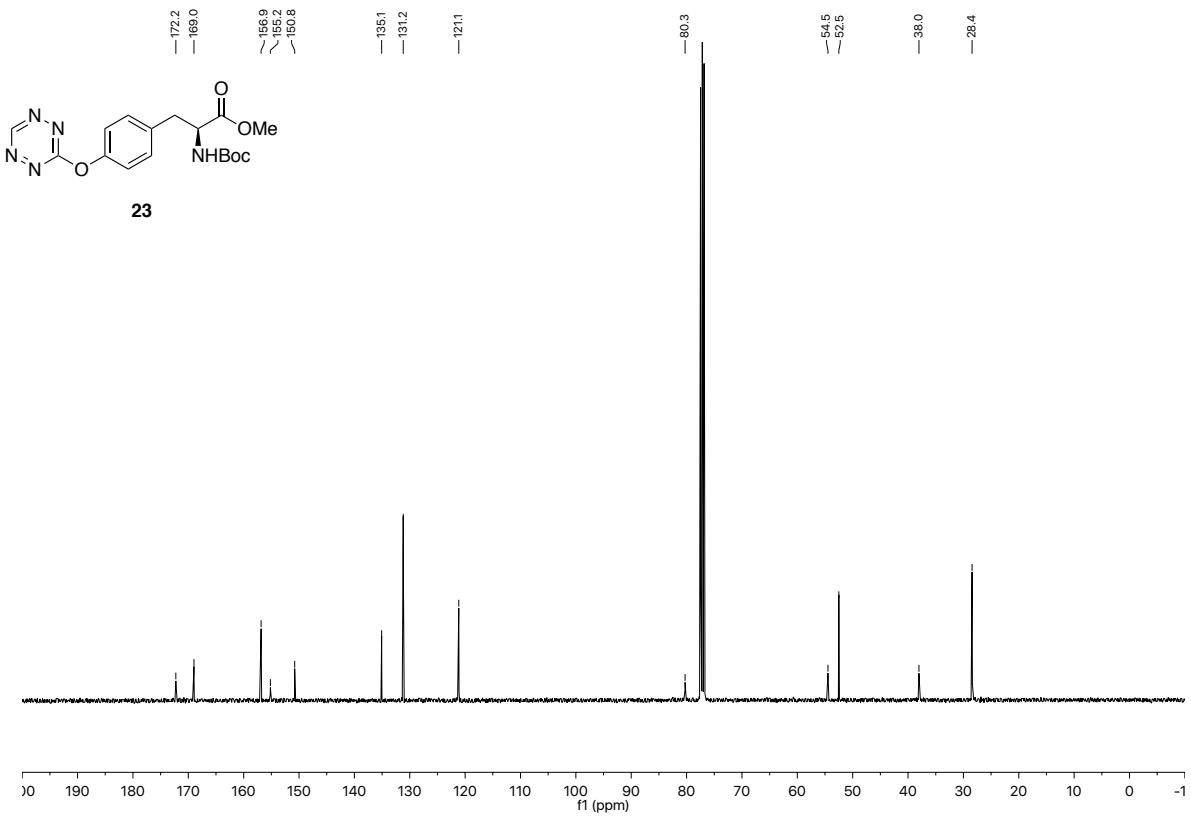
**S21**

$^{1}$H-NMR, $CDCl_3$, 400 MHz



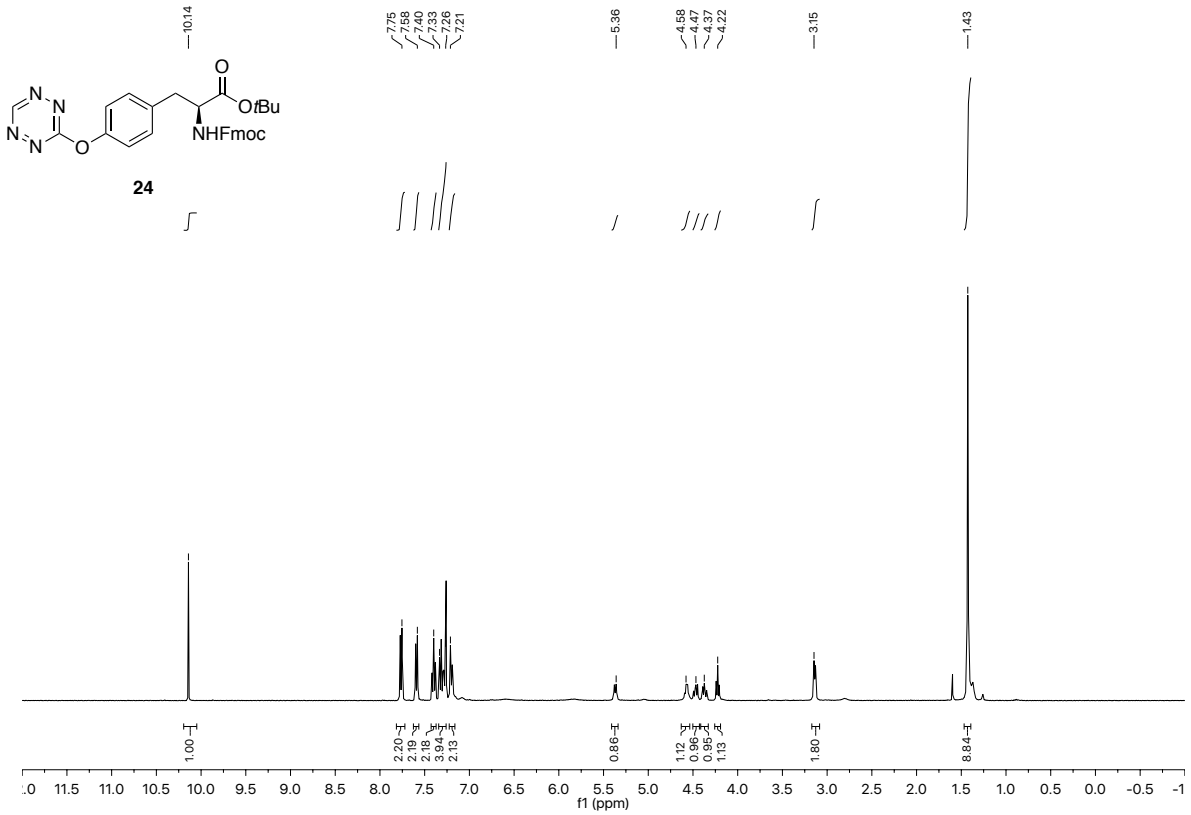

**23**

$^{13}$C-NMR, $CDCl_3$, 101 MHz



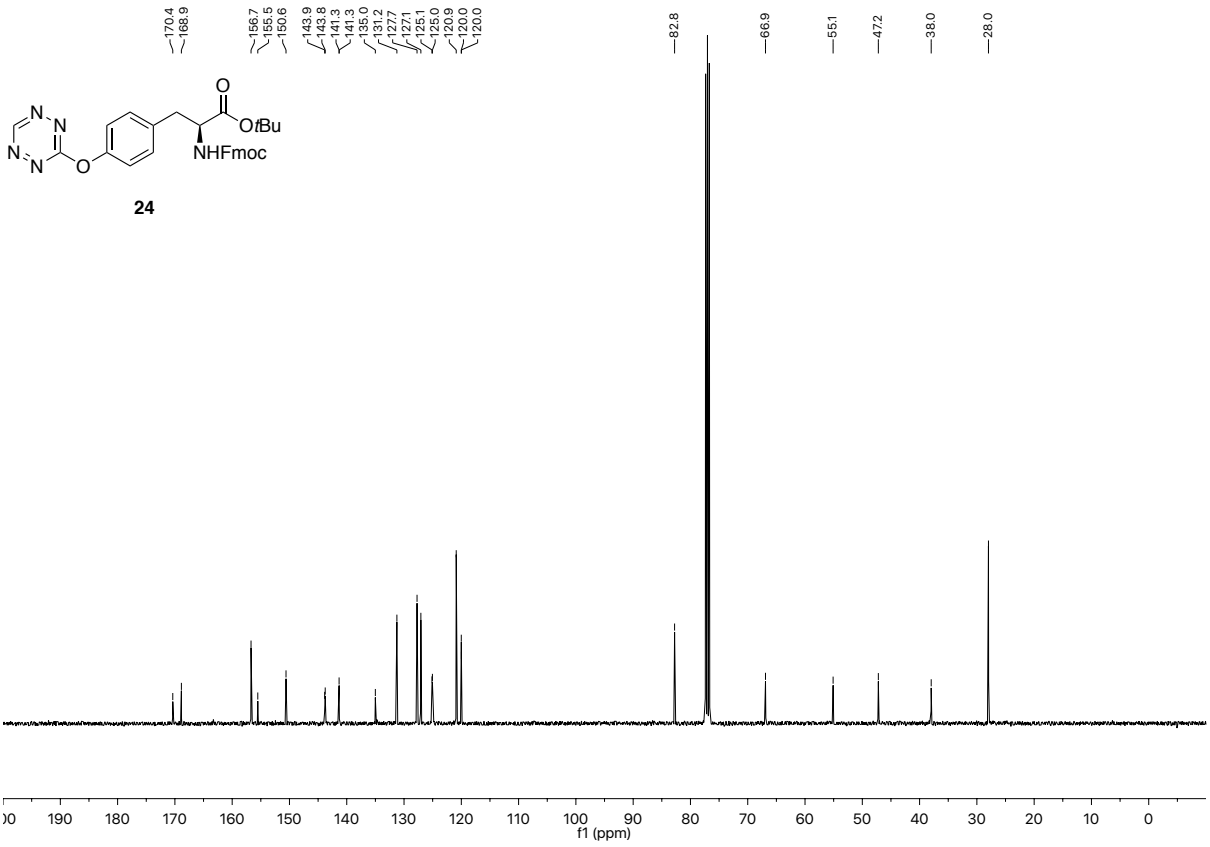

**23**

$^{1}$H-NMR, $CDCl_3$, 400 MHz



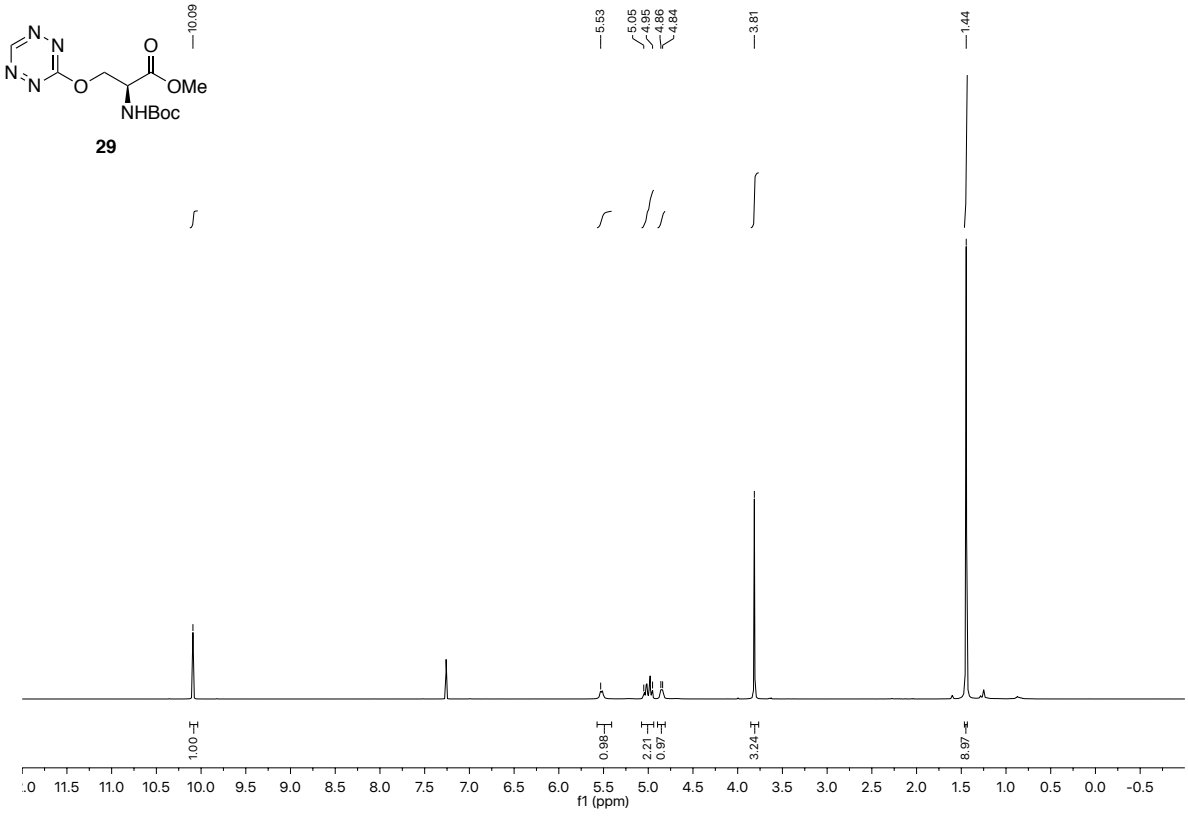

**24**

$^{13}$C-NMR, $CDCl_3$, 101 MHz

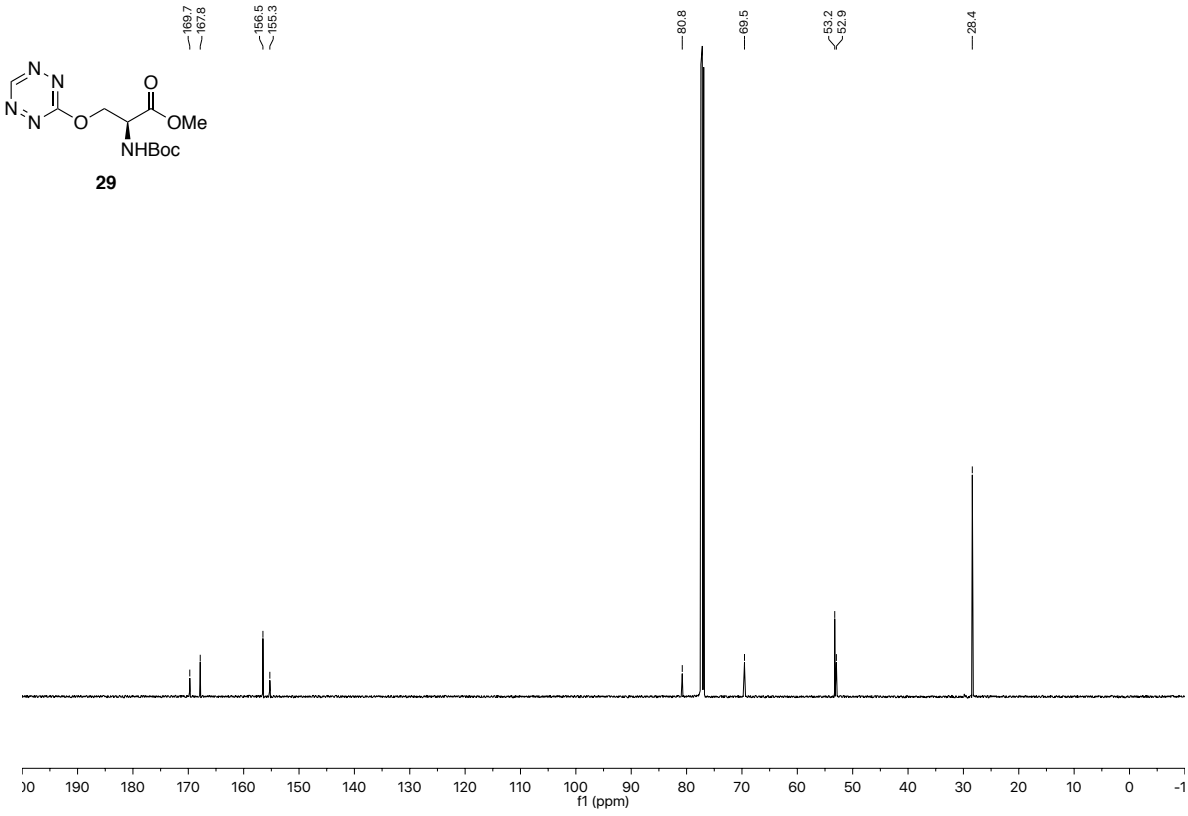



**24**

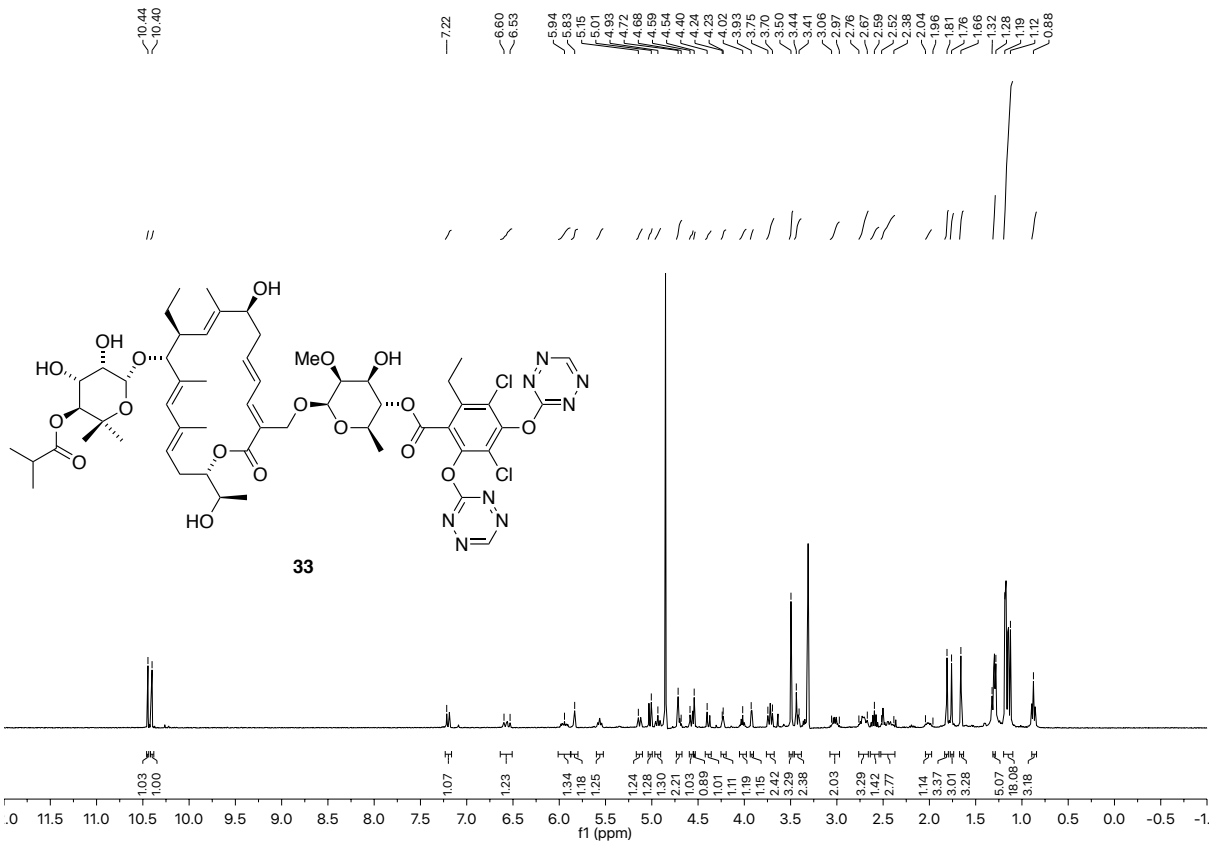
$^{1}$H-NMR, $CDCl_3$, 400 MHz




$^{13}$C-NMR, $CDCl_3$, 126 MHz

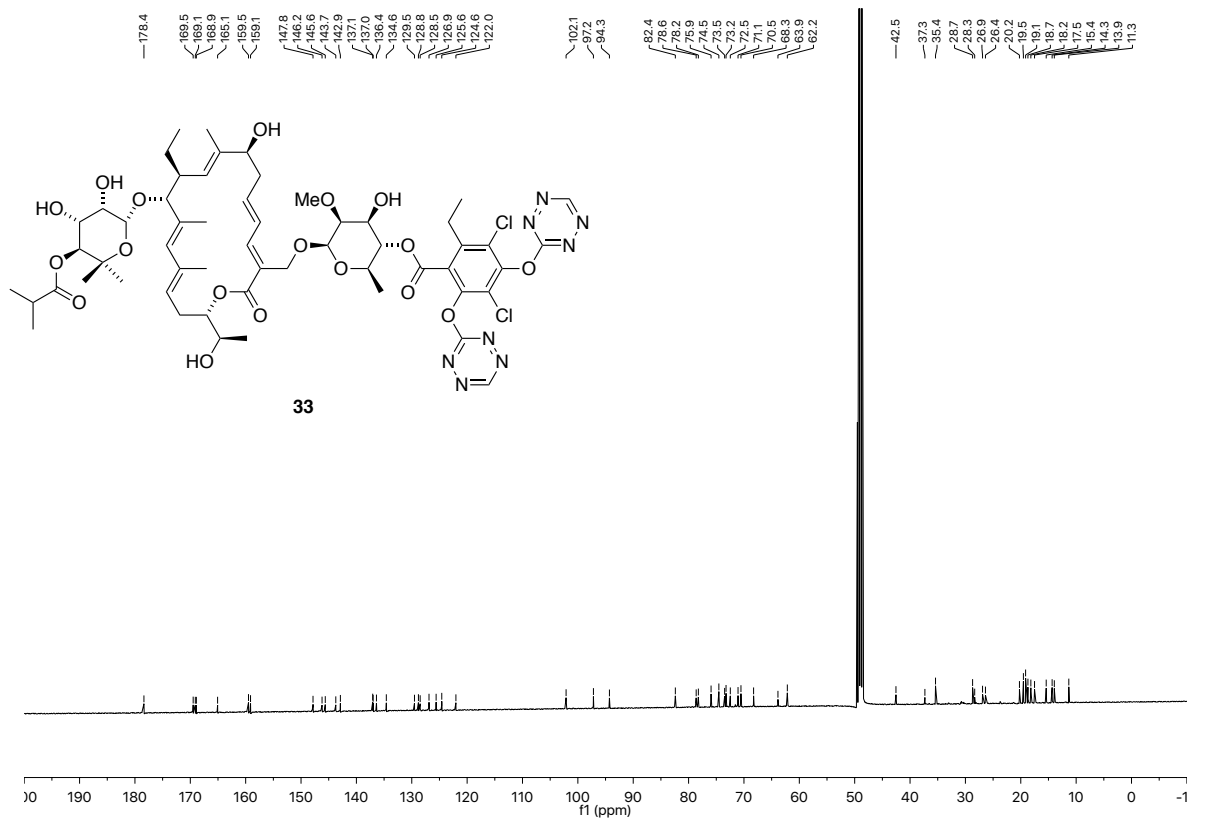


$^{1}$H-NMR, $CDCl_3$, 400 MHz



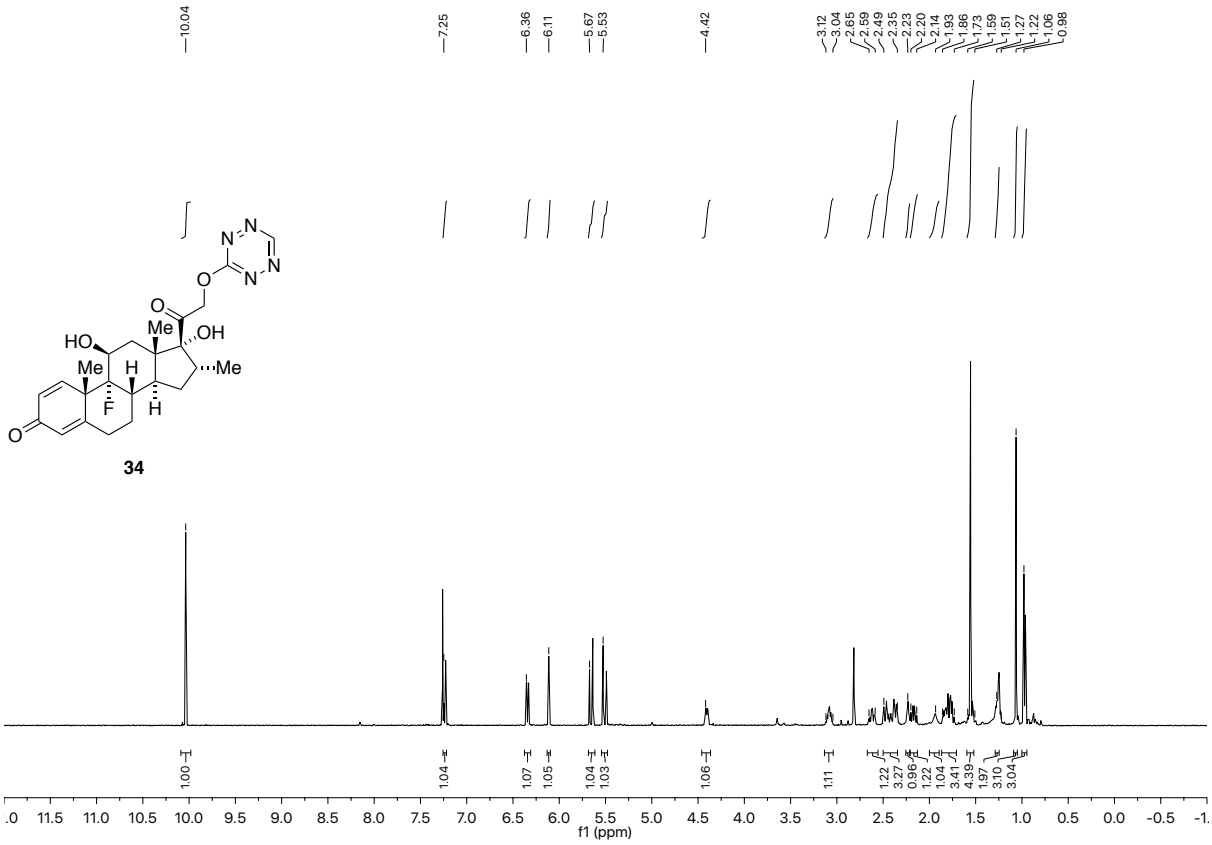

**33**

$^{13}$C-NMR, $CDCl_3$, 126 MHz



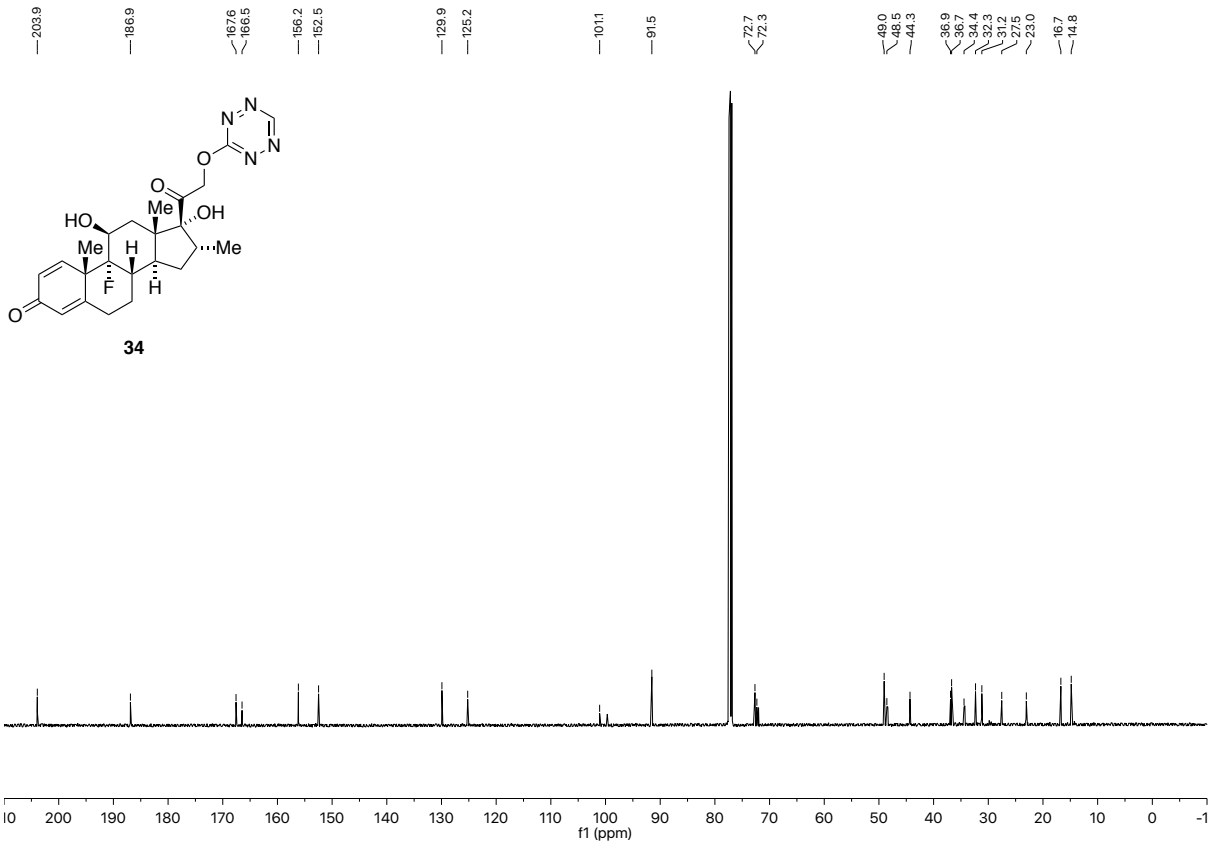

**33**

$^{1}$H-NMR, $CDCl_3$, 500 MHz



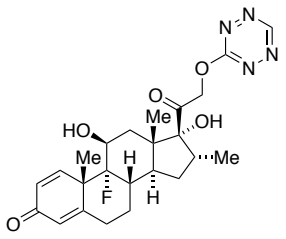

**34**

$^{13}$C-NMR, $CDCl_3$, 126 MHz



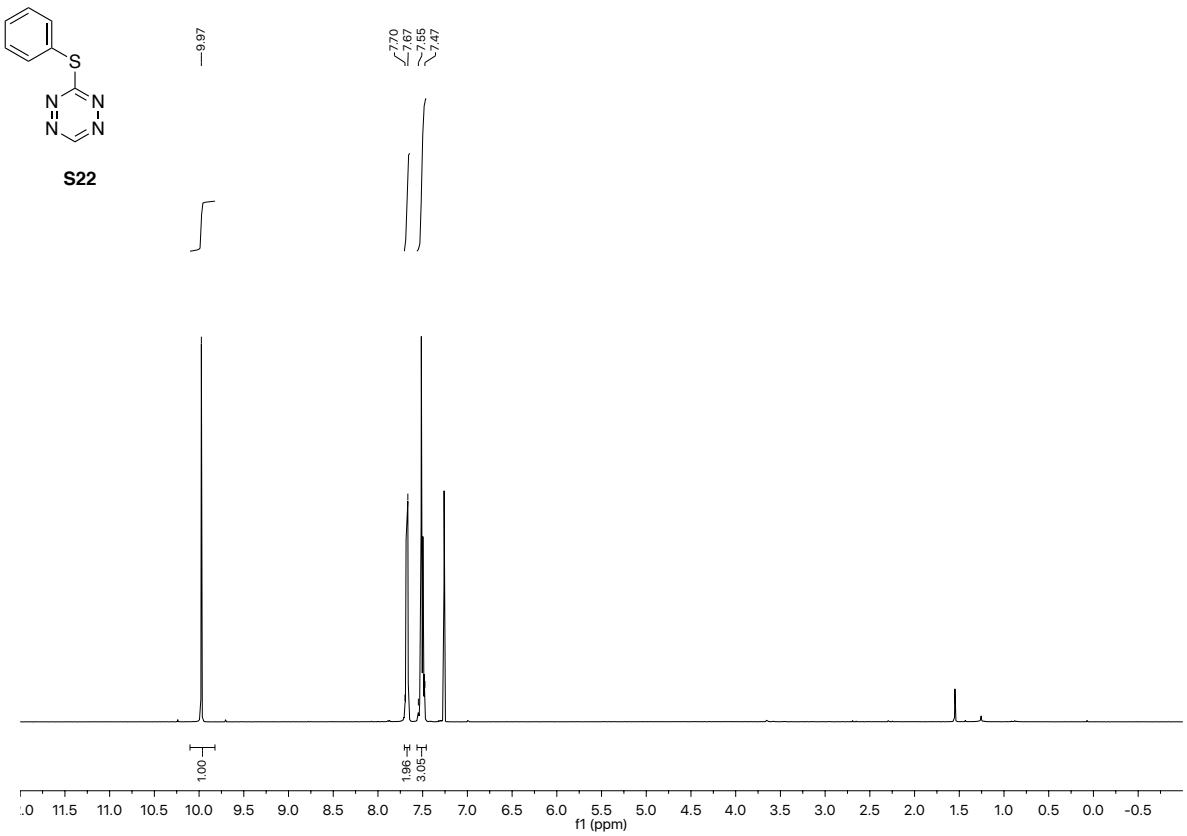

**34**

$^{13}$C-NMR, $CDCl_3$, 376 MHz

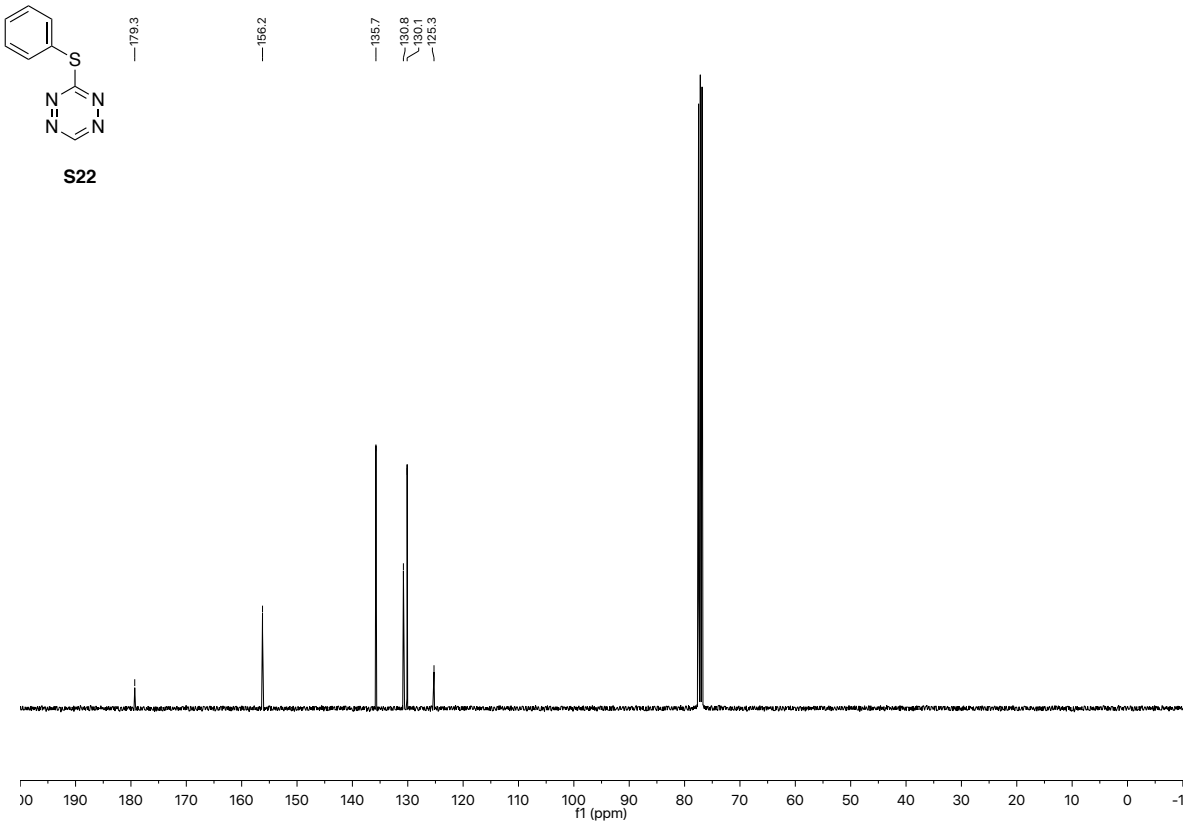



**34**

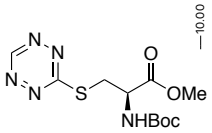
$^{1}$H-NMR, $CDCl_3$, 500 MHz




$^{13}$C-NMR, $CDCl_3$, 126 MHz



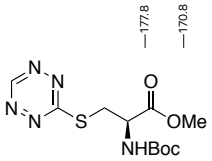
$^{1}$H-NMR, $CDCl_3$, 400 MHz



**25**

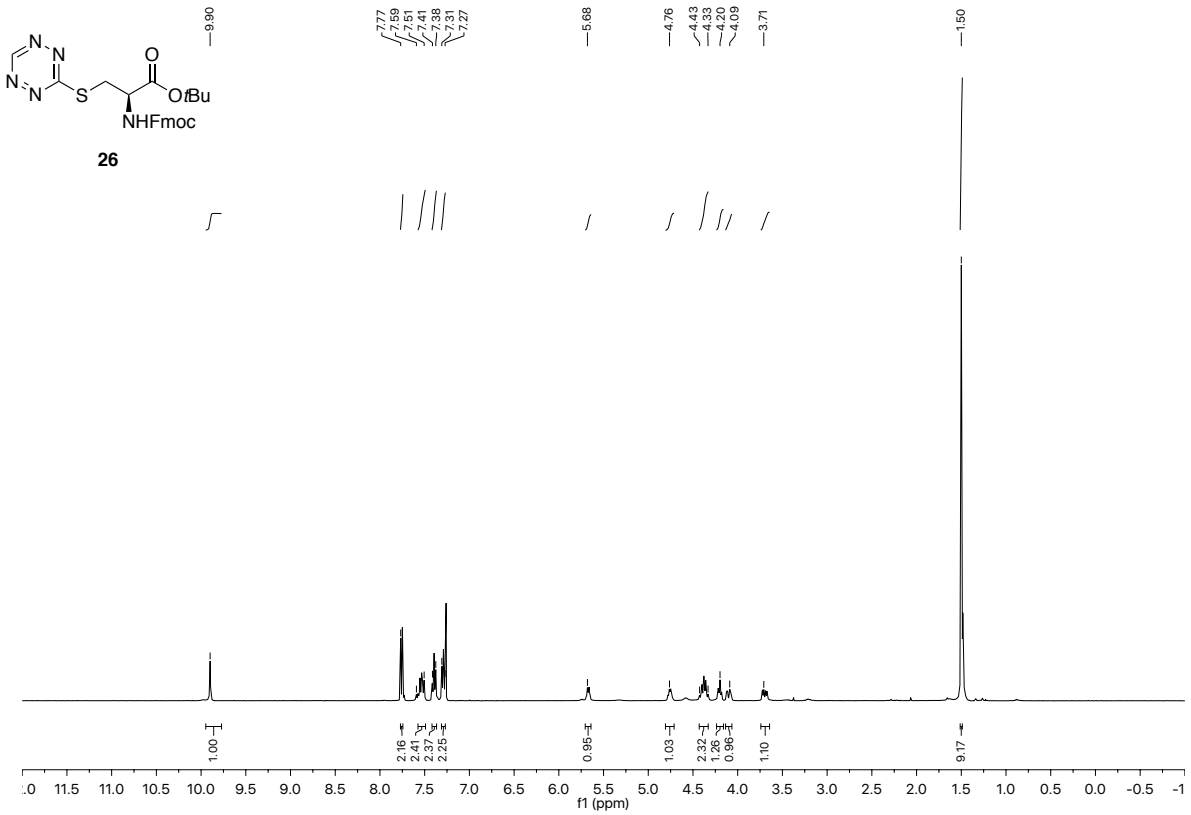



$^{13}$C-NMR, $CDCl_3$, 101 MHz



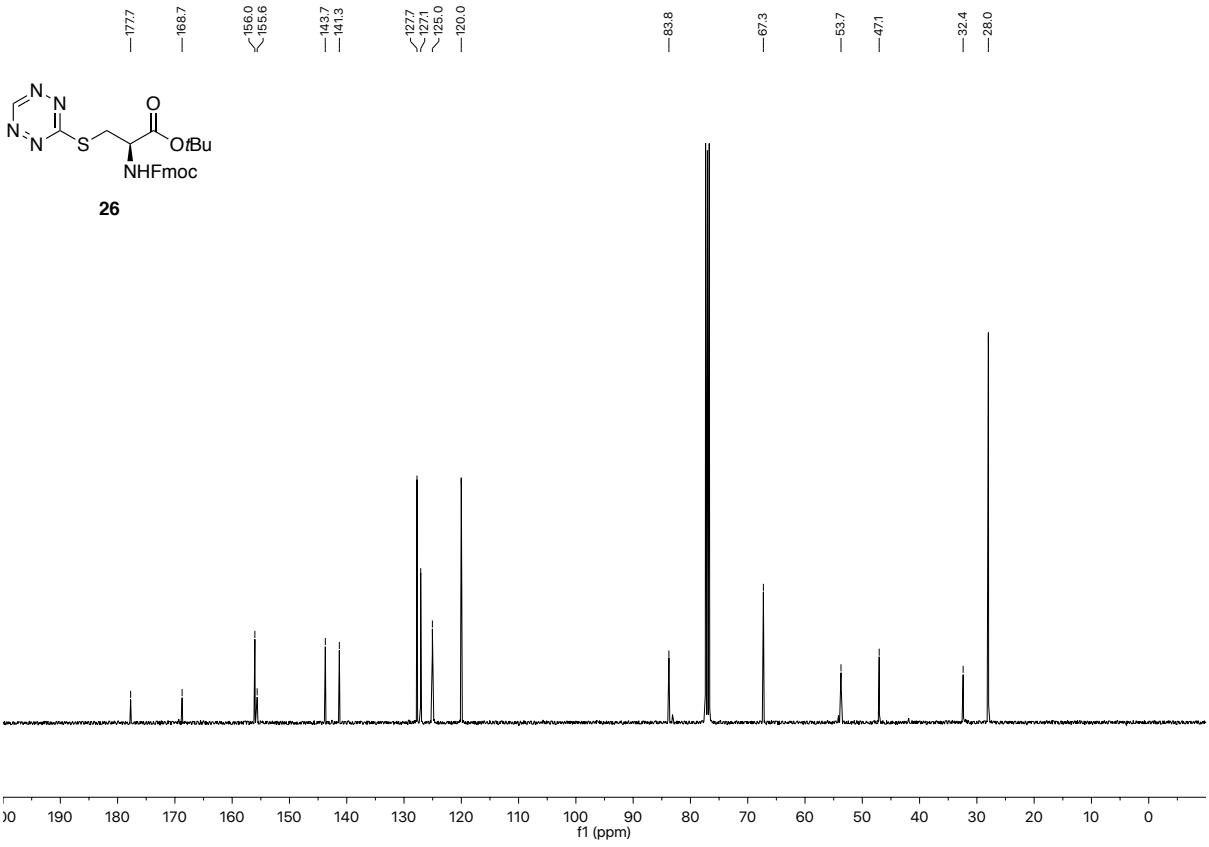
**25**

$^{1}$H-NMR, $CDCl_3$, 400 MHz



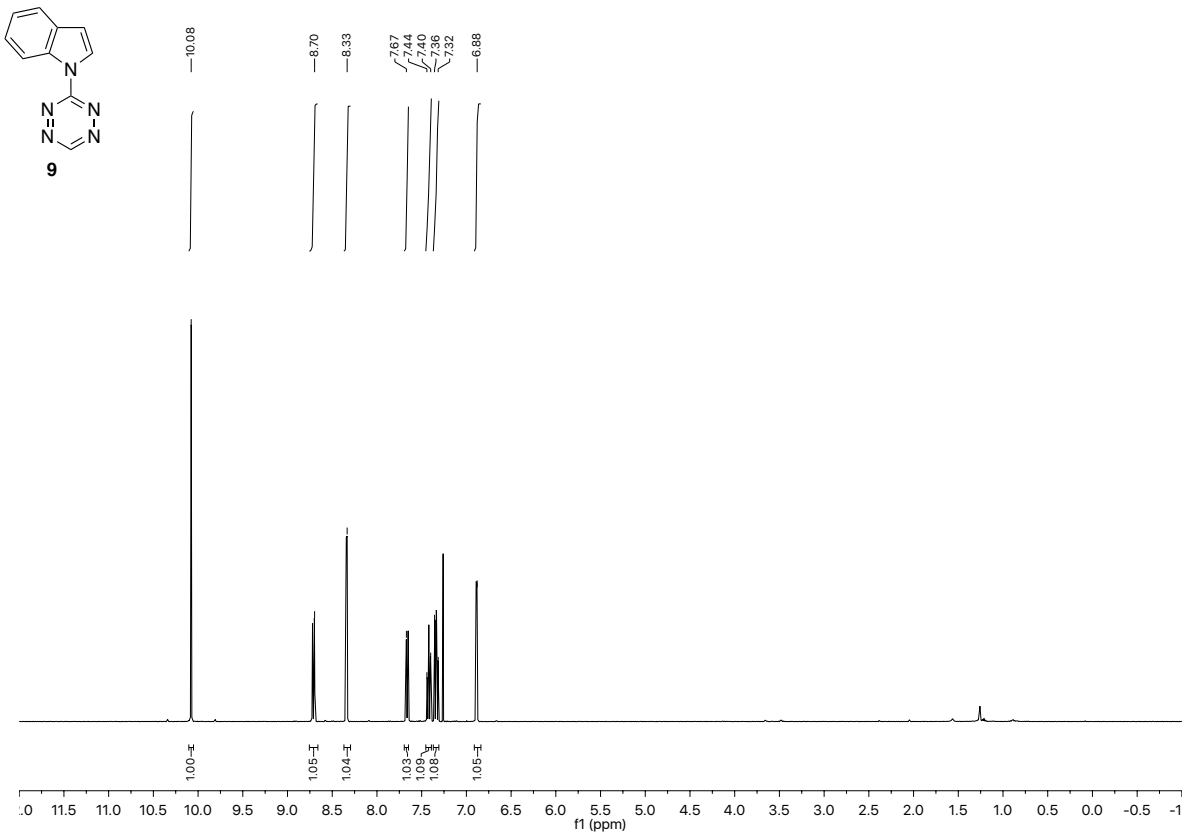

**26**

$^{13}$C-NMR, $CDCl_3$, 101 MHz



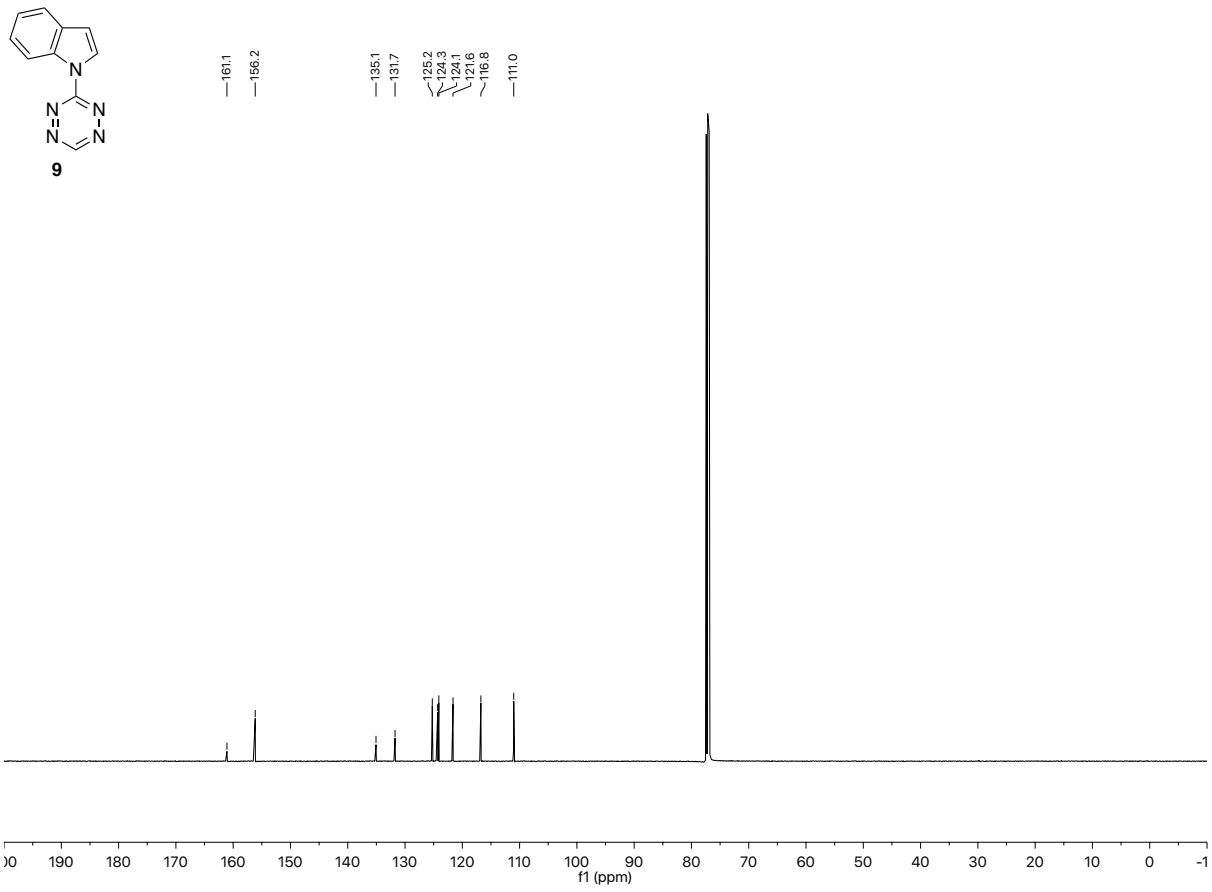

**26**

$^{1}$H-NMR, $CDCl_3$, 400 MHz



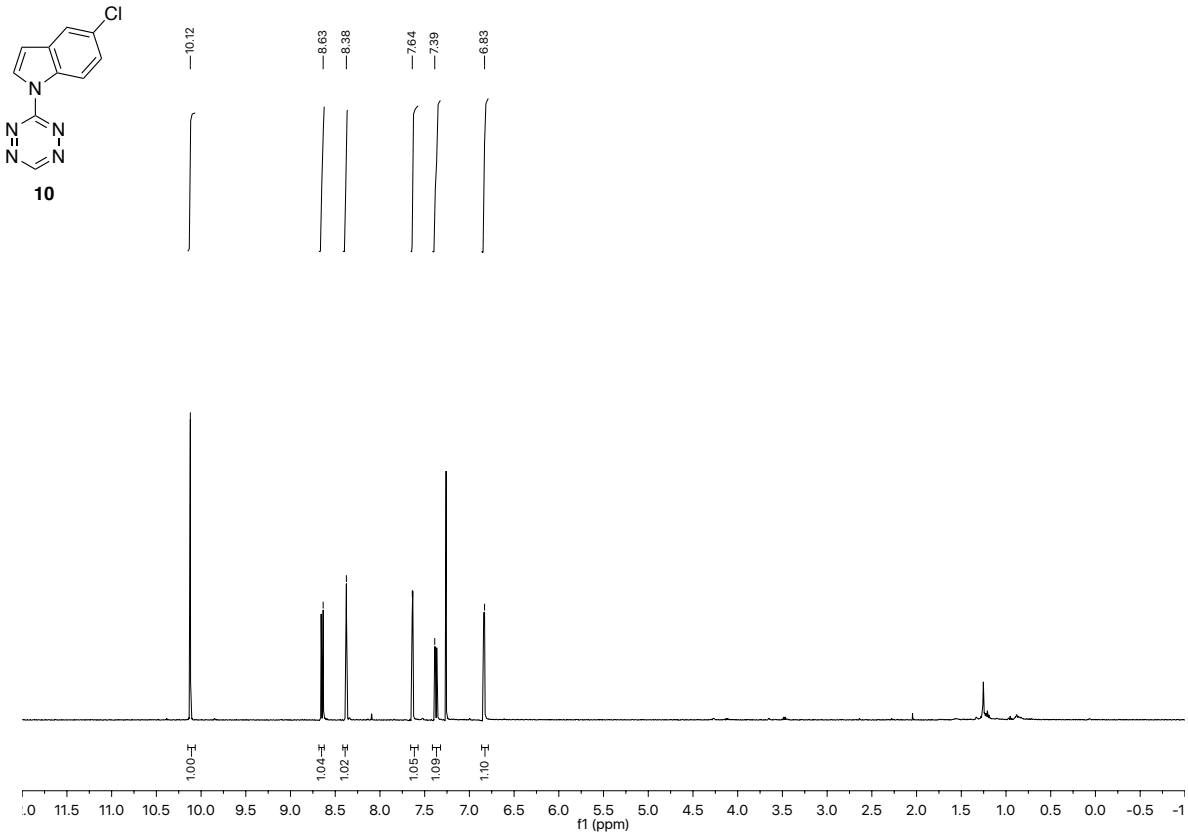

**9**

$^{13}$C-NMR, $CDCl_3$, 126 MHz

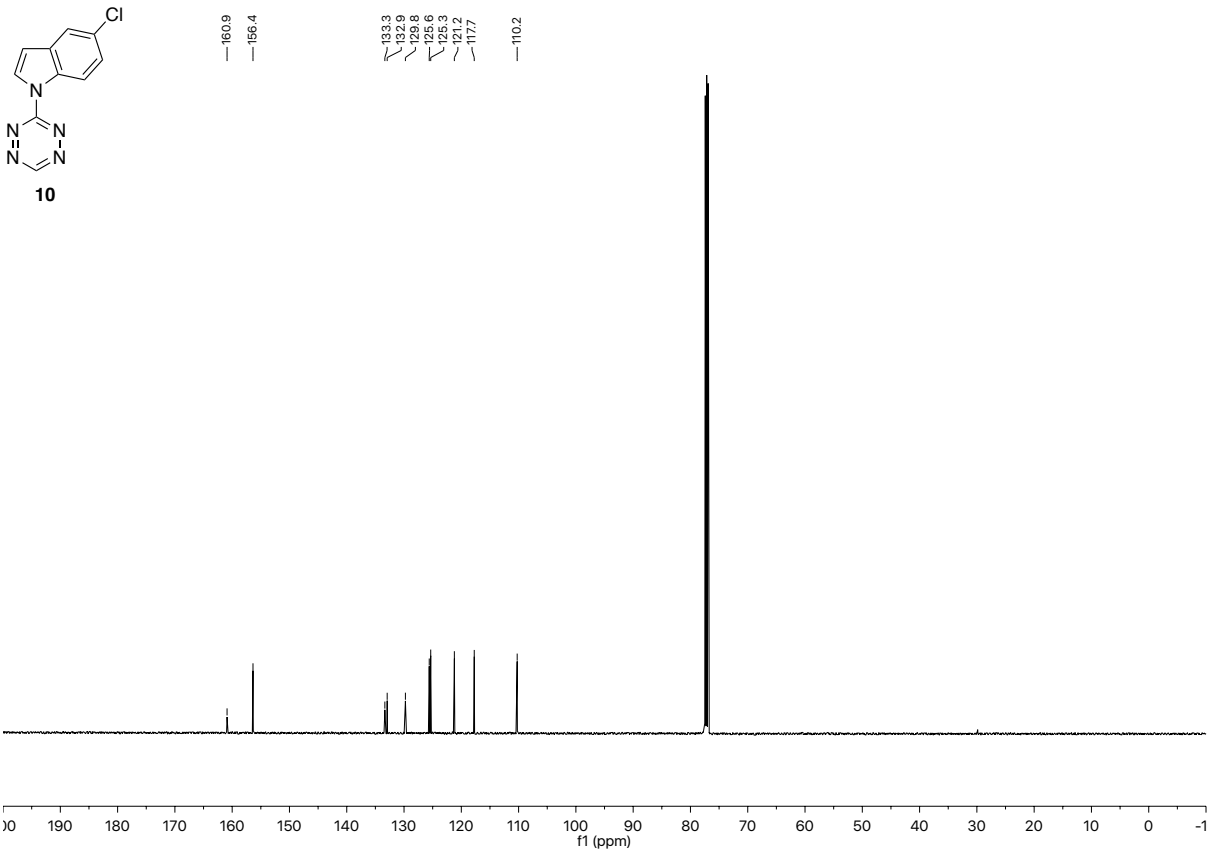



**9**

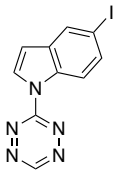
$^{1}$H-NMR, $CDCl_3$, 400 MHz




$^{13}$C-NMR, $CDCl_3$, 126 MHz



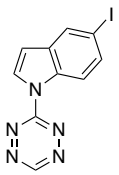
$^{1}$H-NMR, $CDCl_3$, 400 MHz



**S23**

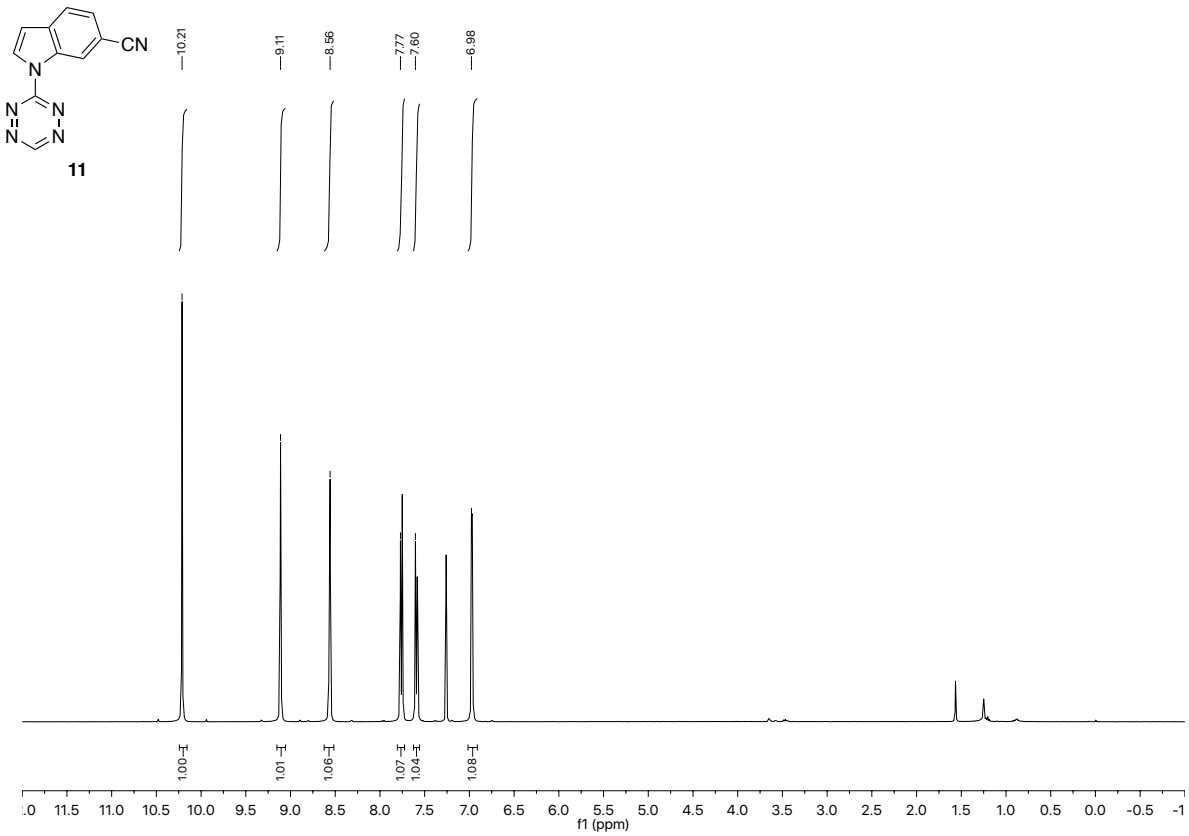



$^{13}$C-NMR, $CDCl_3$, 101 MHz

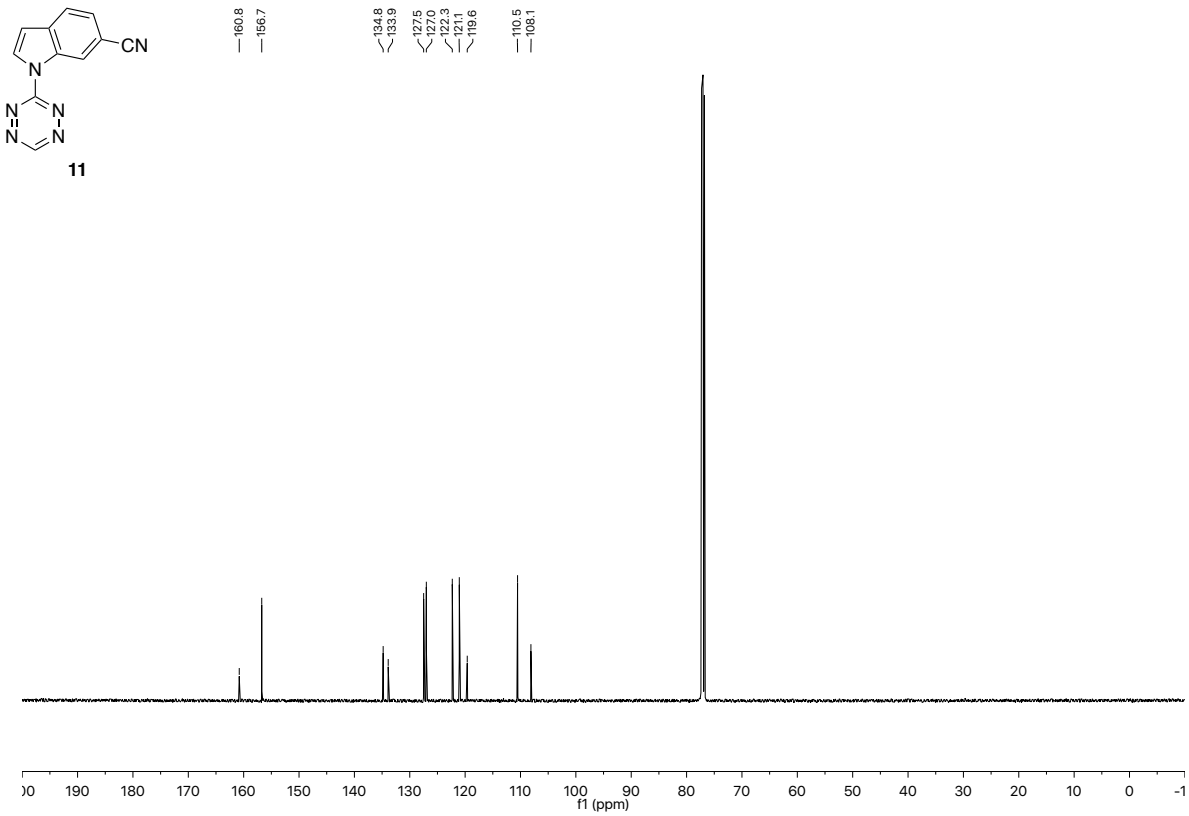


**S23**

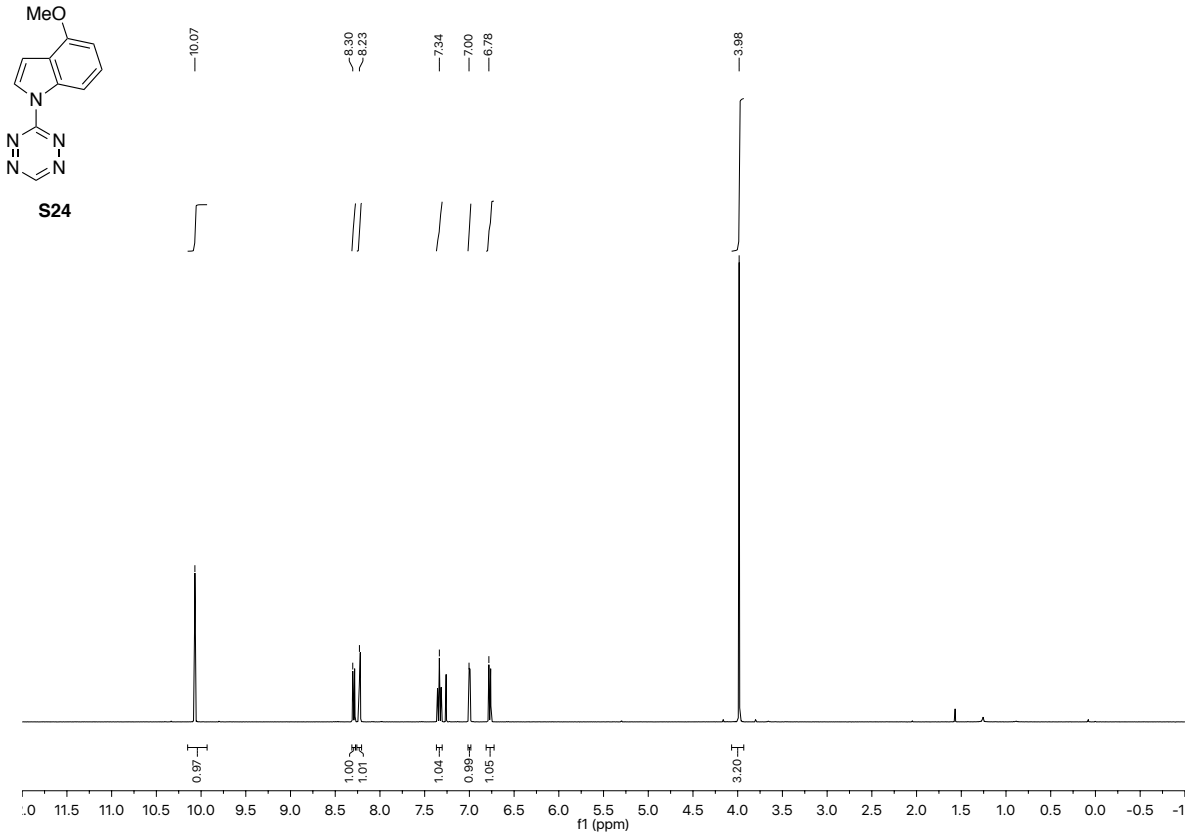
$^{1}$H-NMR, $CDCl_3$, 400 MHz



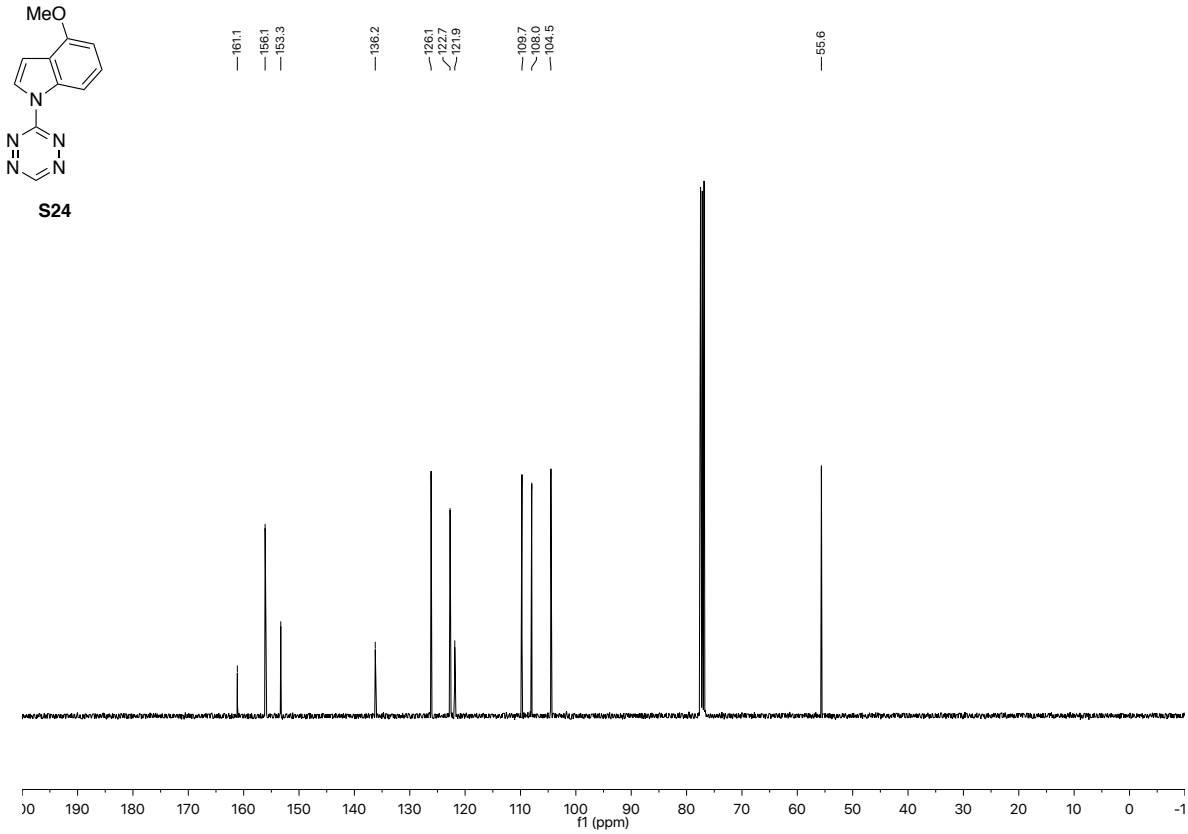

$^{13}$C-NMR, $CDCl_3$, 126 MHz



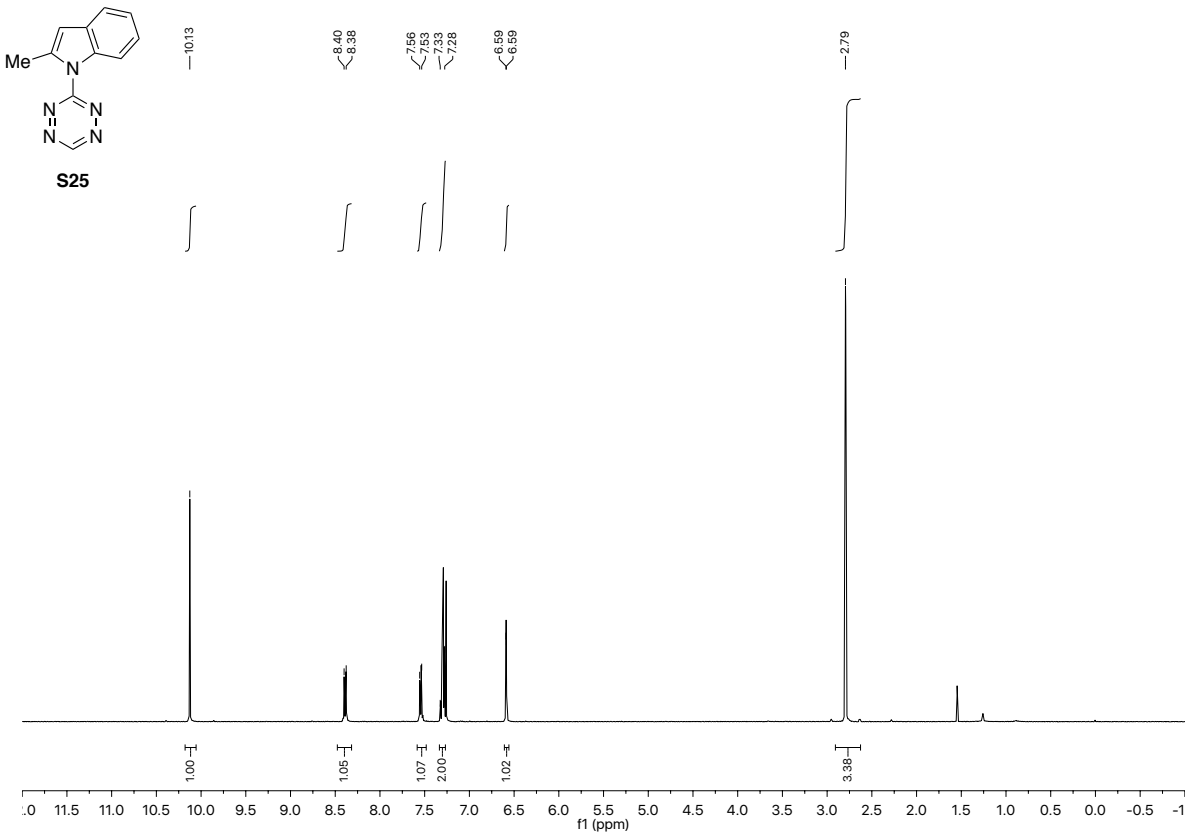
$^{1}$H-NMR, $CDCl_3$, 400 MHz



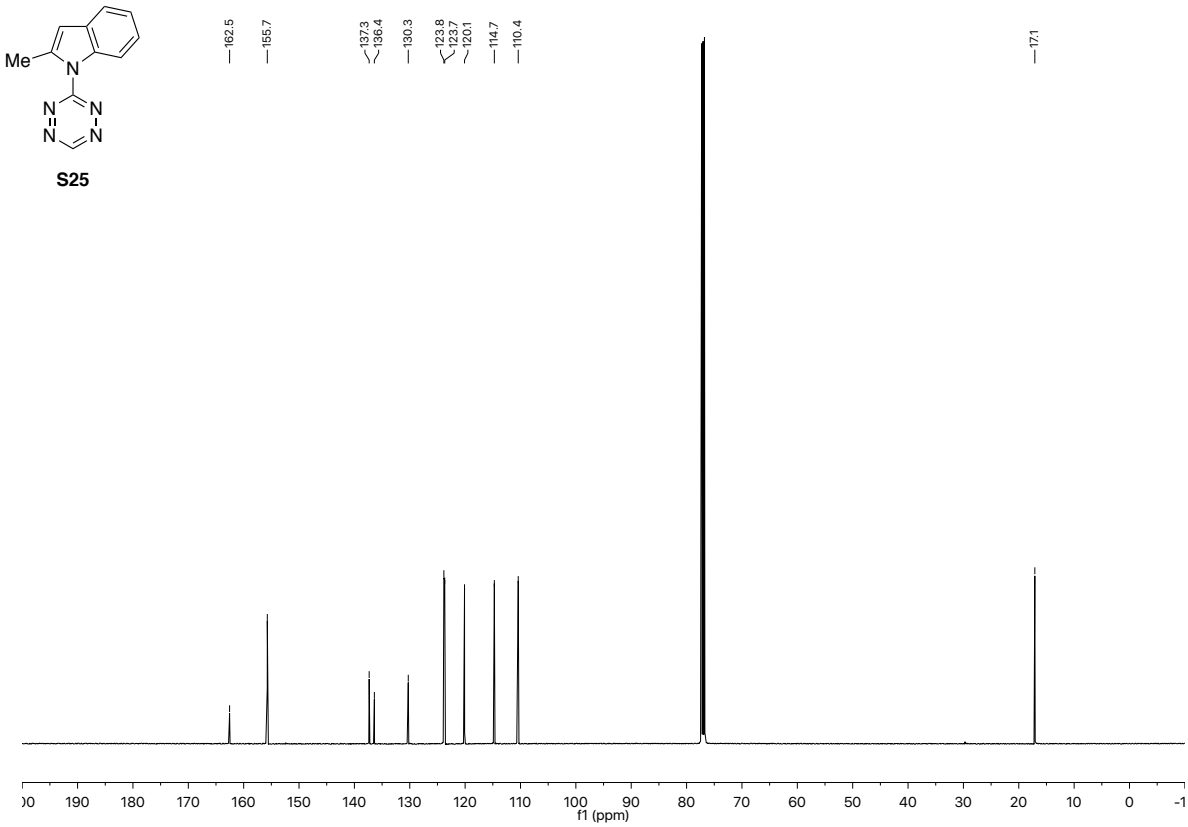

$^{13}$C-NMR, $CDCl_3$, 101 MHz



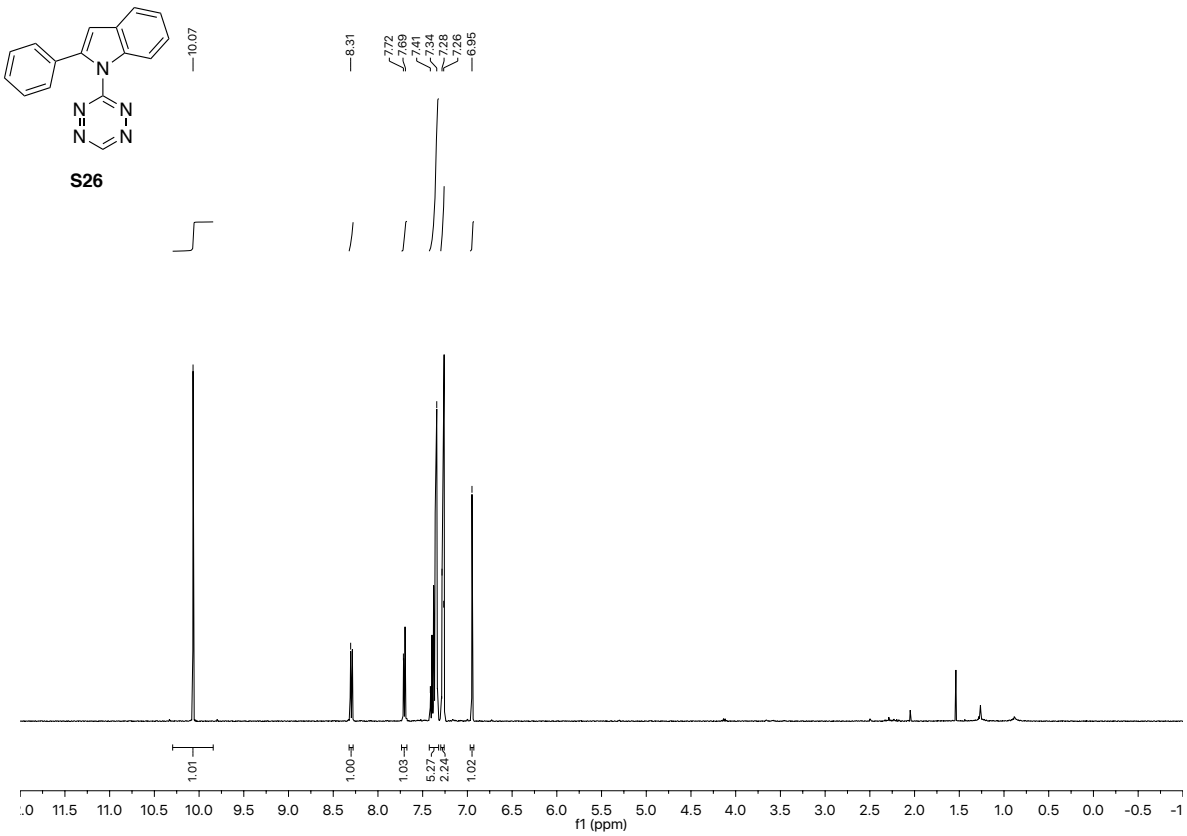
$^{1}$H-NMR, $CDCl_3$, 400 MHz




$^{13}$C-NMR, $CDCl_3$, 126 MHz

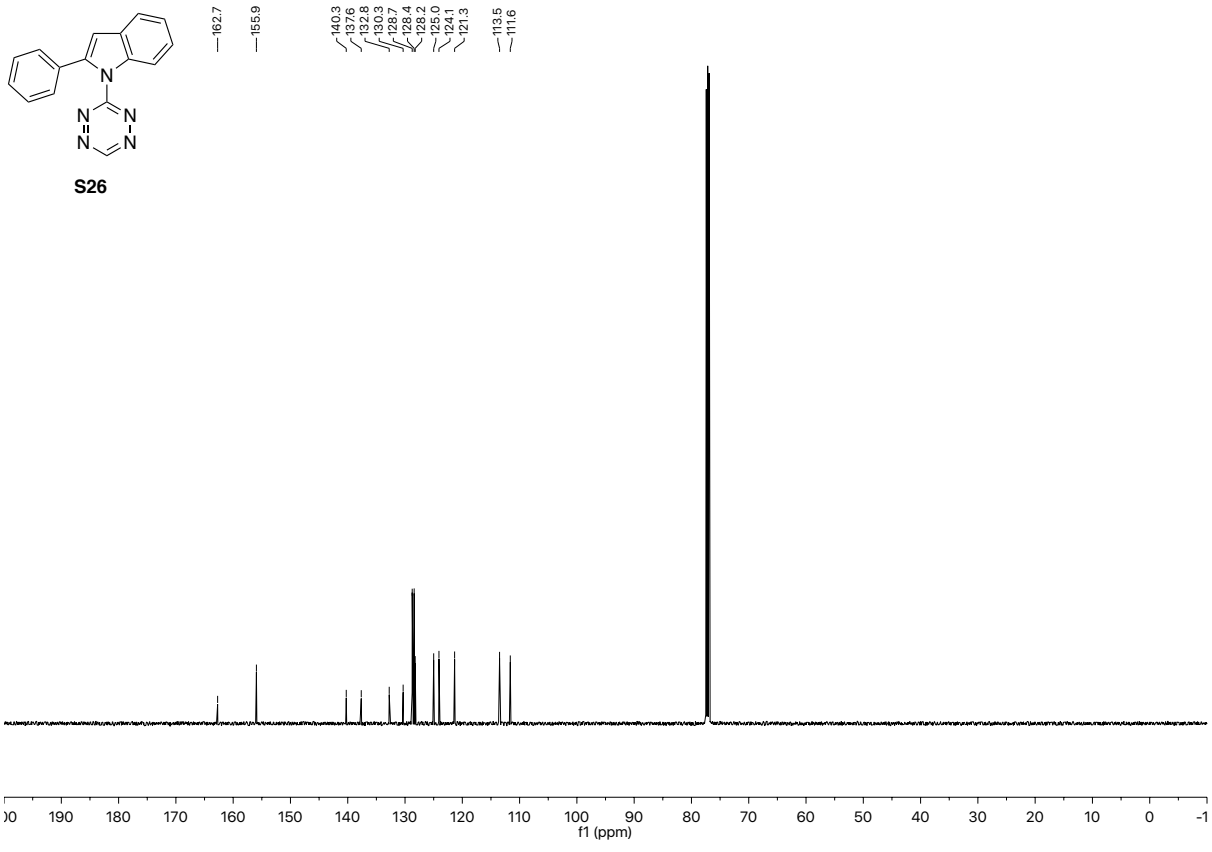


$^{1}$H-NMR, $CDCl_3$, 400 MHz



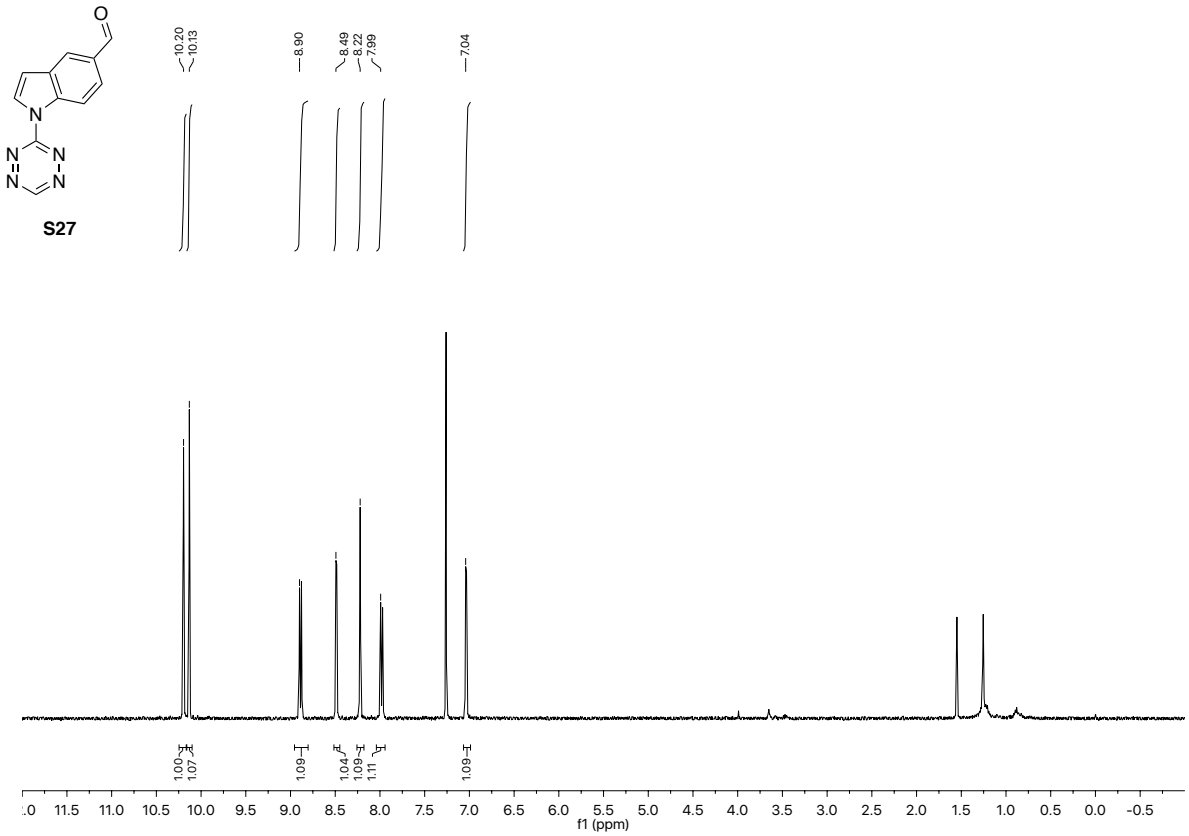

**S26**

$^{13}$C-NMR, $CDCl_3$, 126 MHz



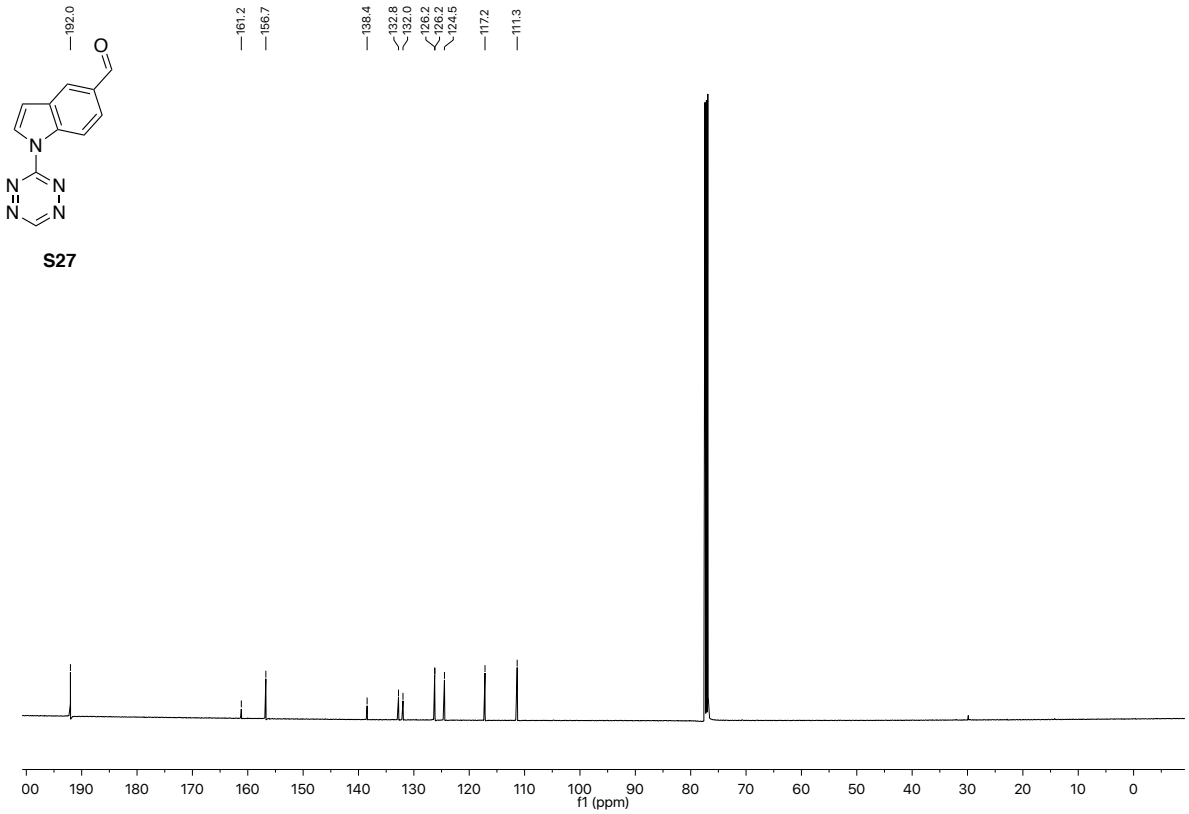

**S26**

$^{1}$H-NMR, $CDCl_3$, 400 MHz



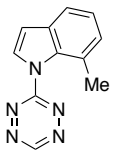

**S27**

$^{13}$C-NMR, $CDCl_3$, 126 MHz




**S27**

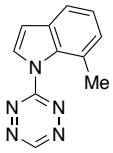
$^{1}$H-NMR, $CDCl_3$, 400 MHz



**S28**

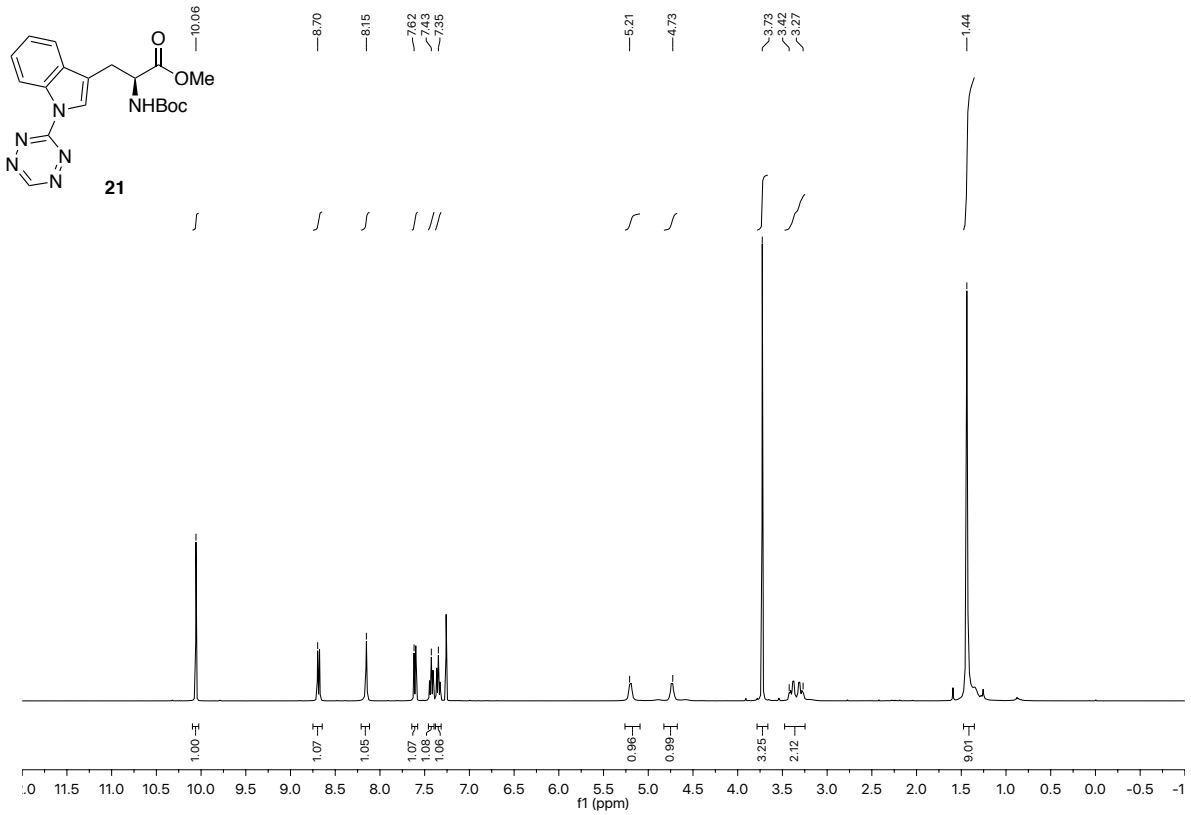



$^{13}$C-NMR, $CDCl_3$, 126 MHz



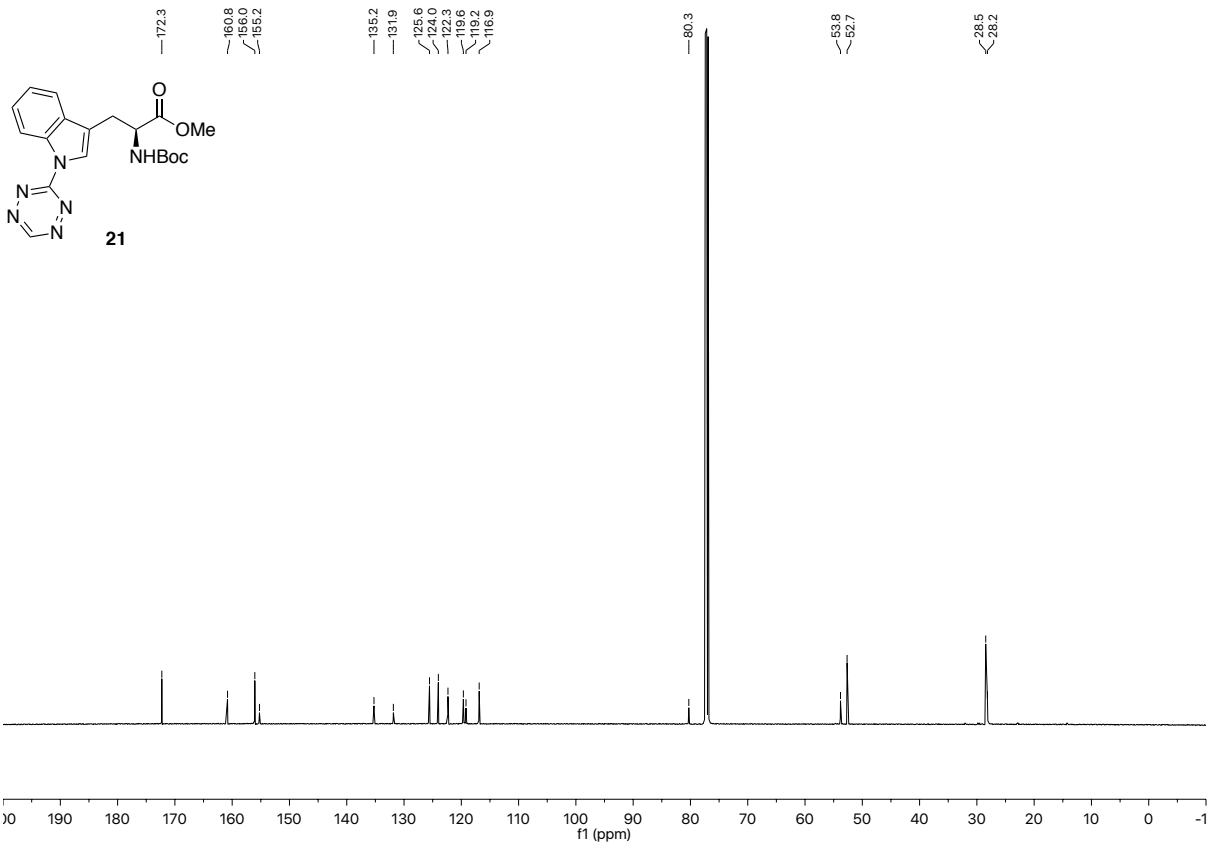
**S28**

$^{1}$H-NMR, $CDCl_3$, 400 MHz



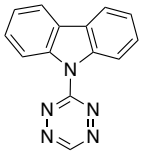

**21**

$^{13}$C-NMR, $CDCl_3$, 126 MHz




**21**

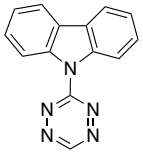
$^{1}$H-NMR, $CDCl_3$, 400 MHz



**12**

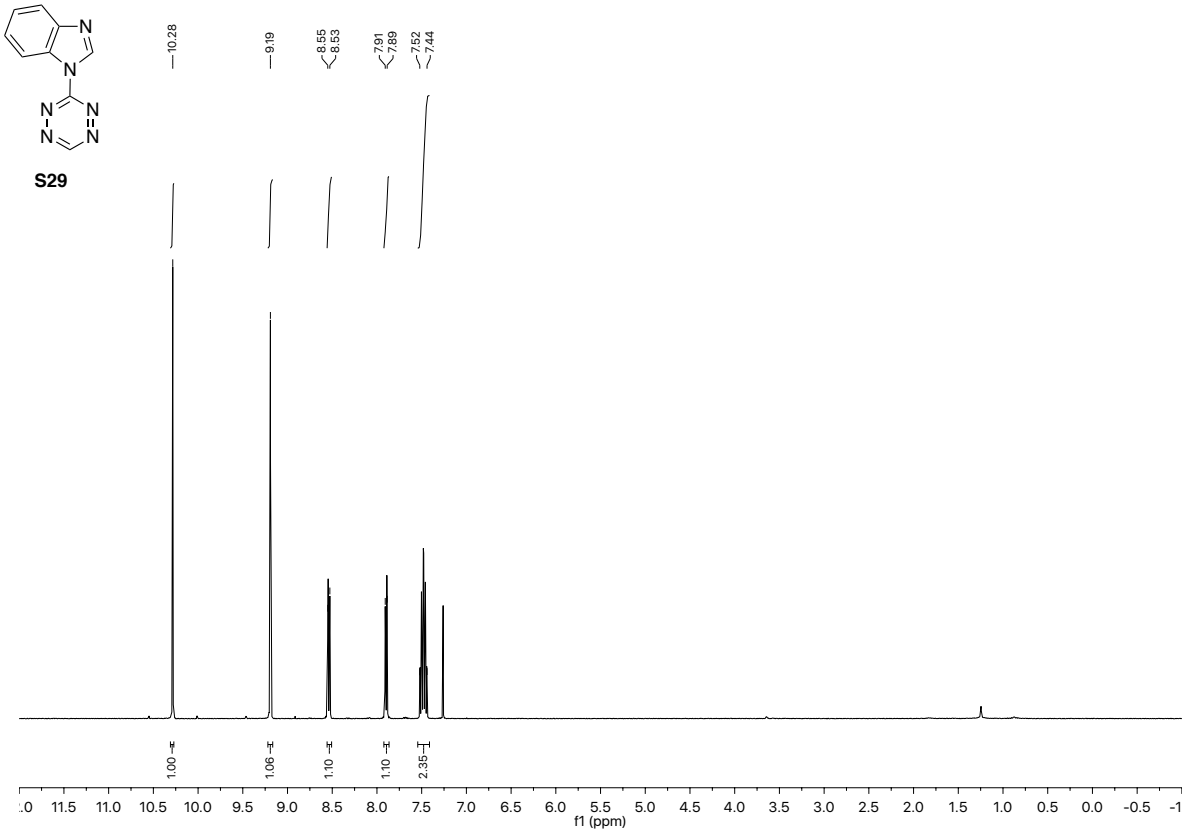



$^{13}$C-NMR, $CDCl_3$, 126 MHz



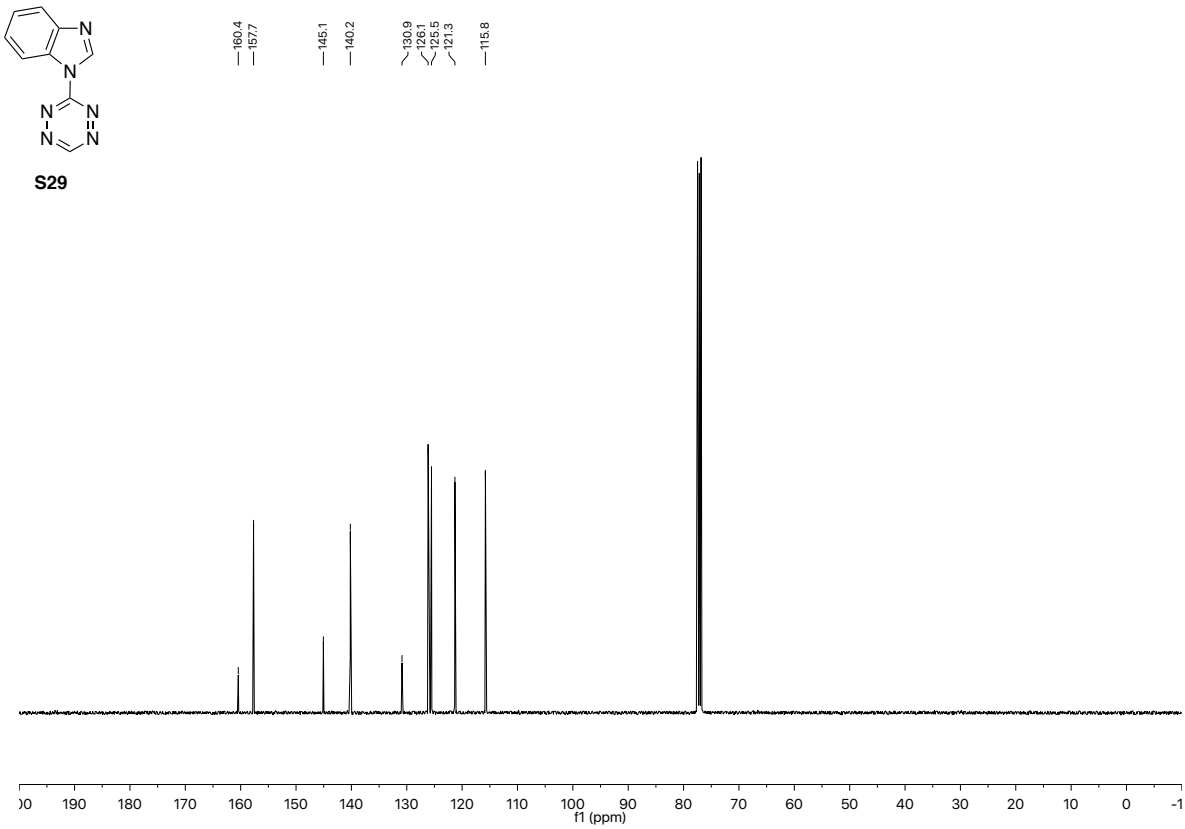
**12**

$^{1}$H-NMR, $CDCl_3$, 400 MHz



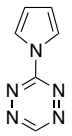

**S29**

$^{13}$C-NMR, $CDCl_3$, 101 MHz




**S29**

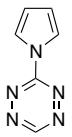
$^{1}$H-NMR, $CDCl_3$, 400 MHz



**S30**

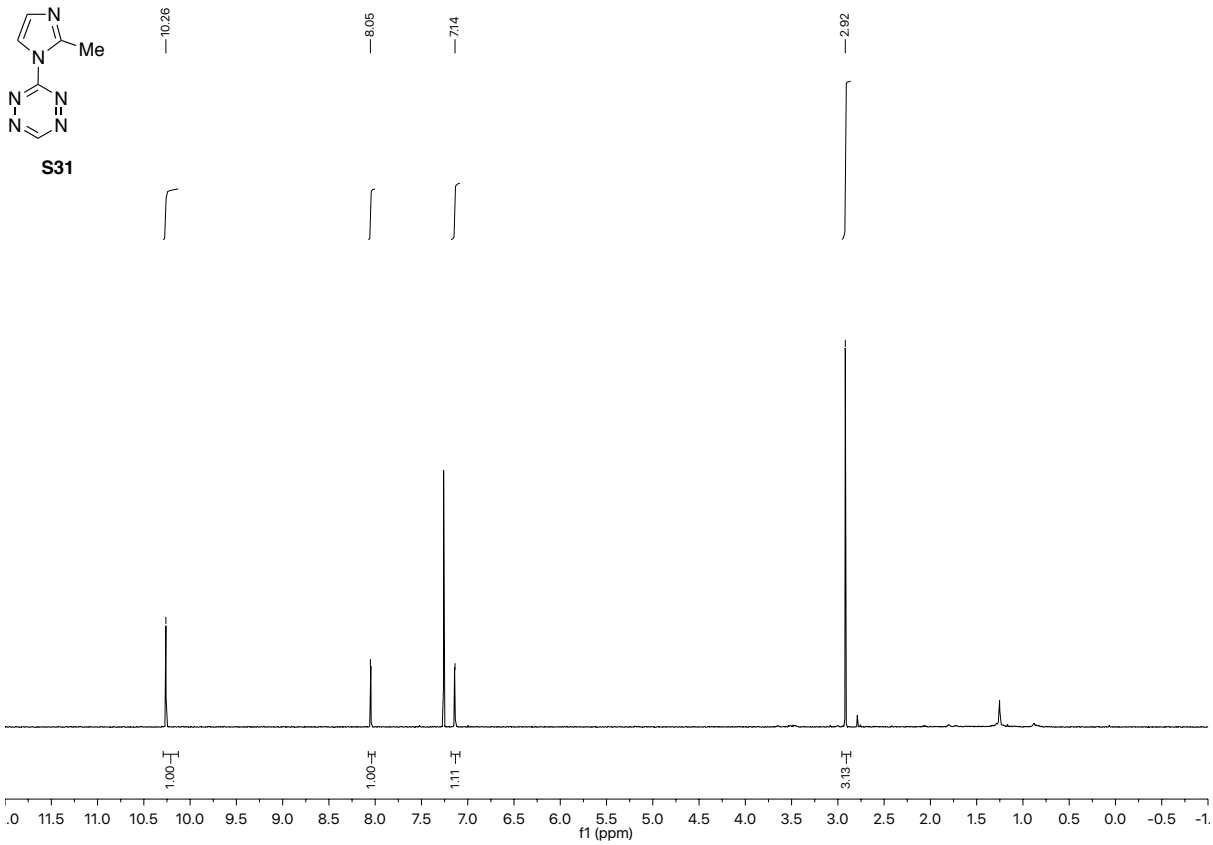



$^{13}$C-NMR, $CDCl_3$, 126 MHz

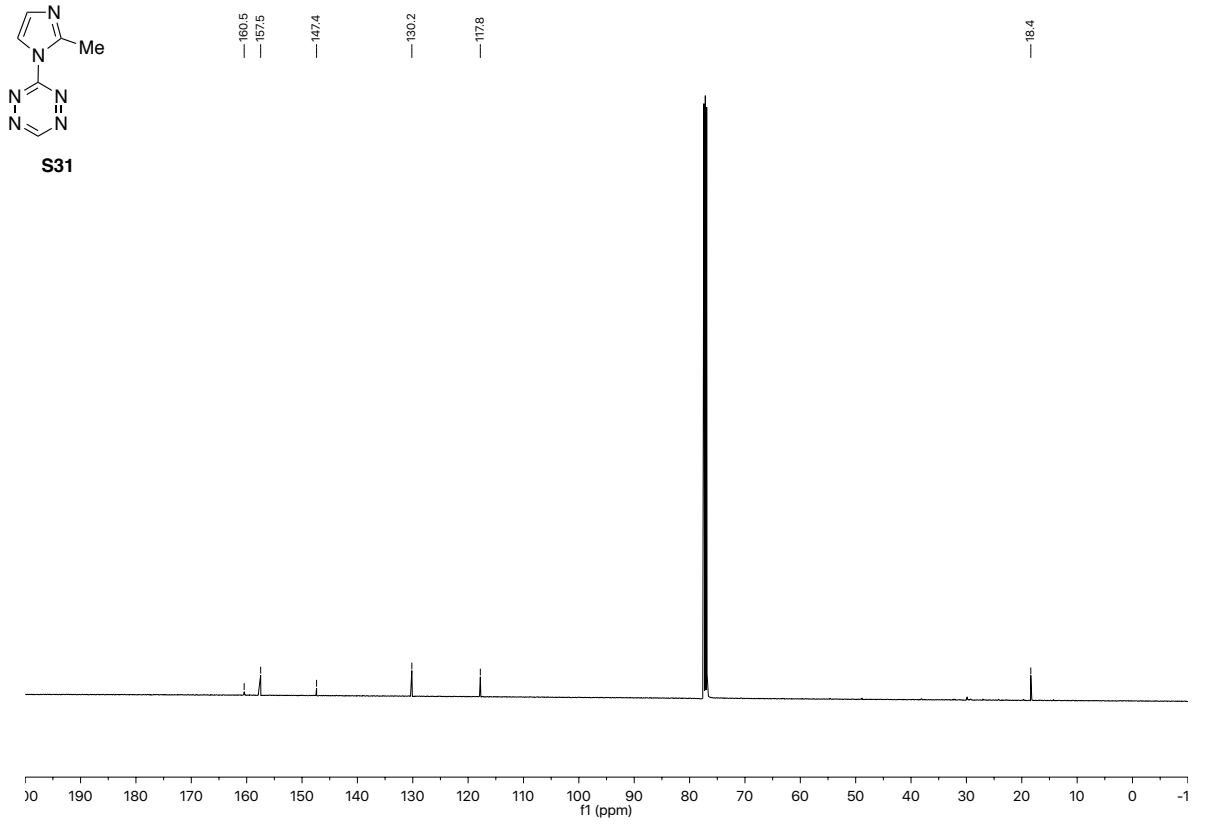


**S30**

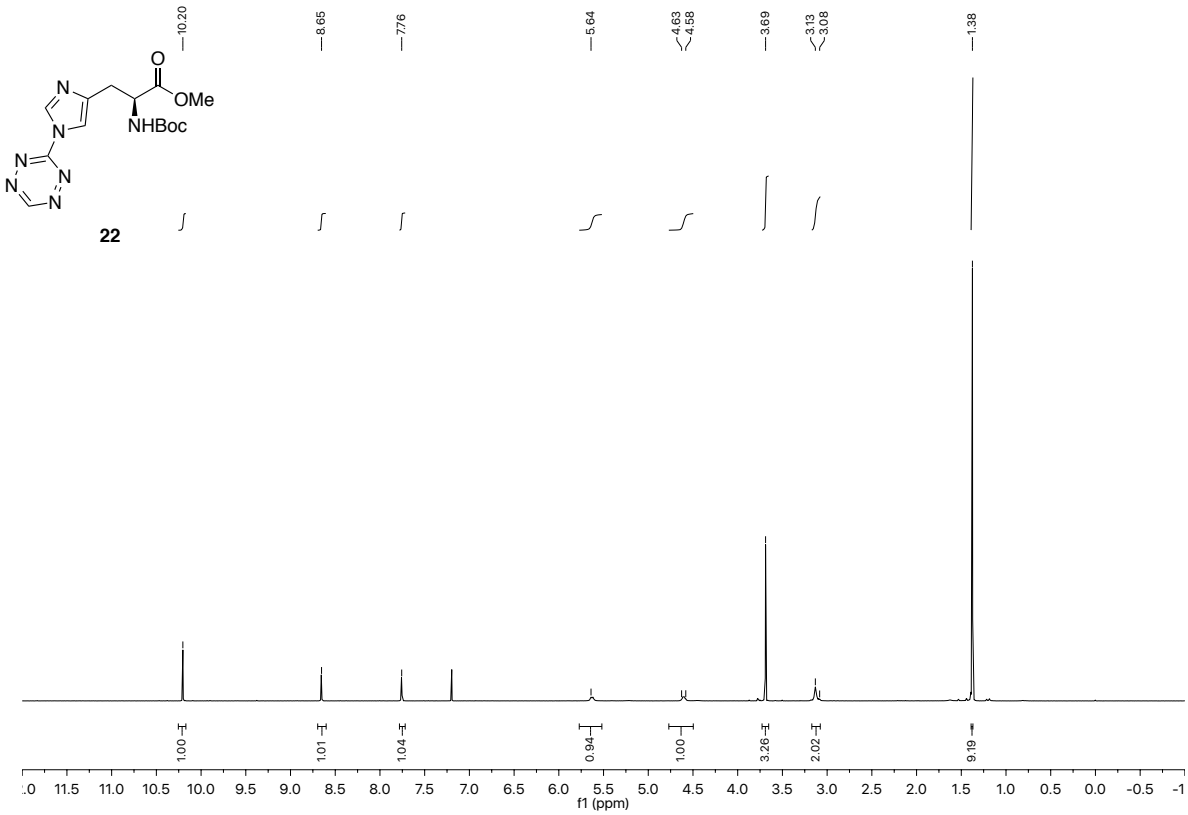
$^{1}$H-NMR, $CDCl_3$, 400 MHz




$^{13}$C-NMR, $CDCl_3$, 101 MHz

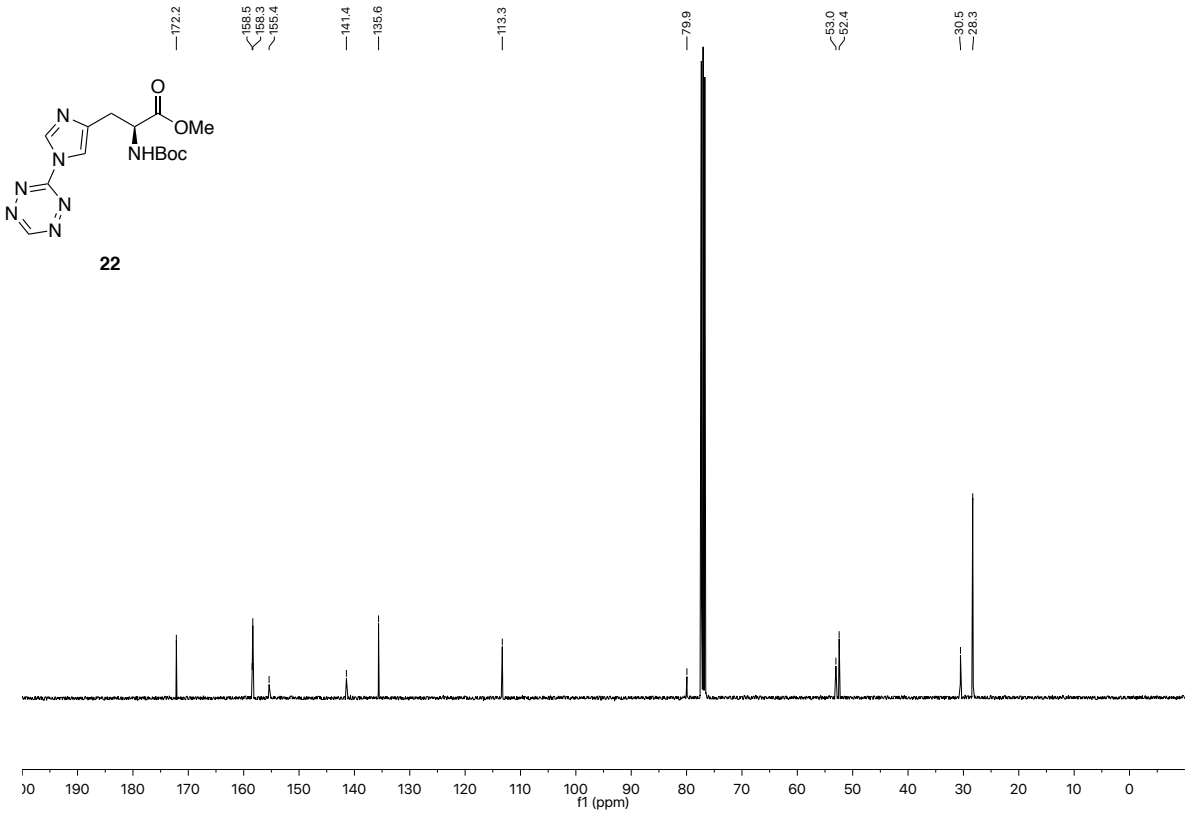


$^{1}$H-NMR, $CDCl_3$, 400 MHz



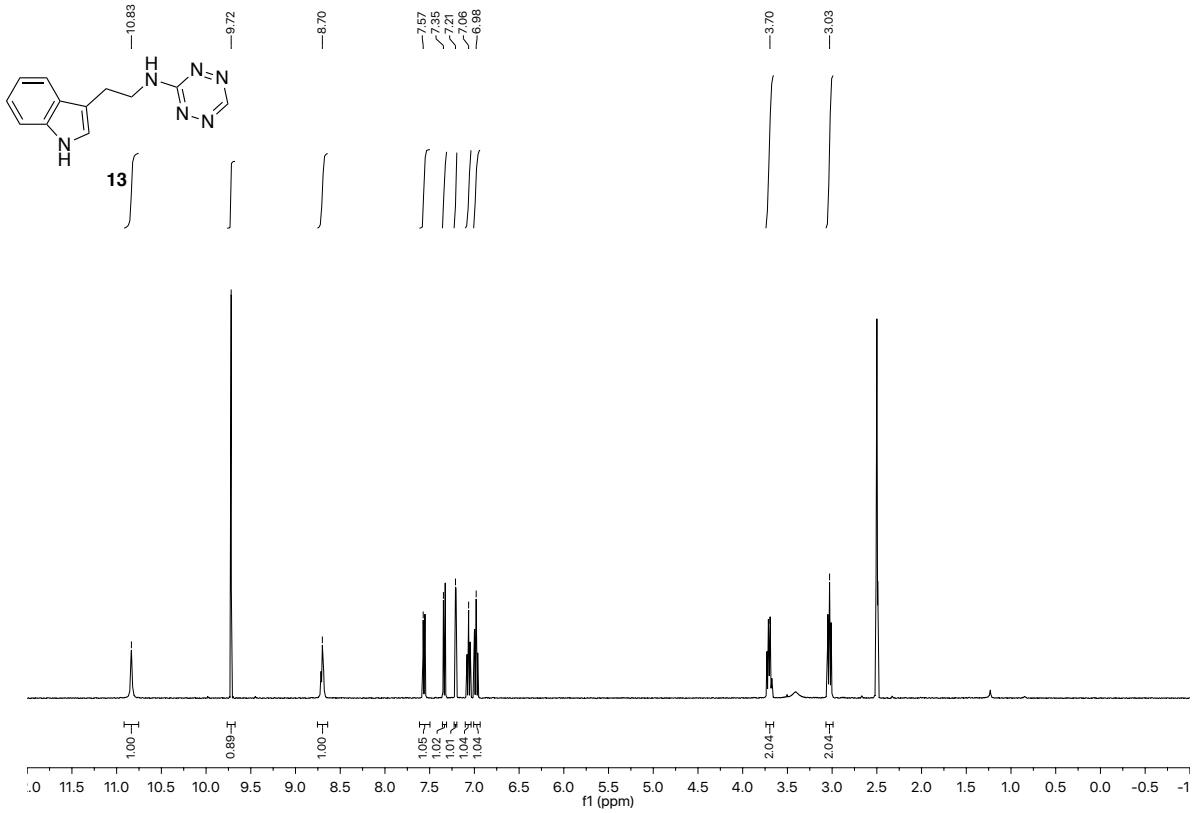

**22**

$^{13}$C-NMR, $CDCl_3$, 101 MHz

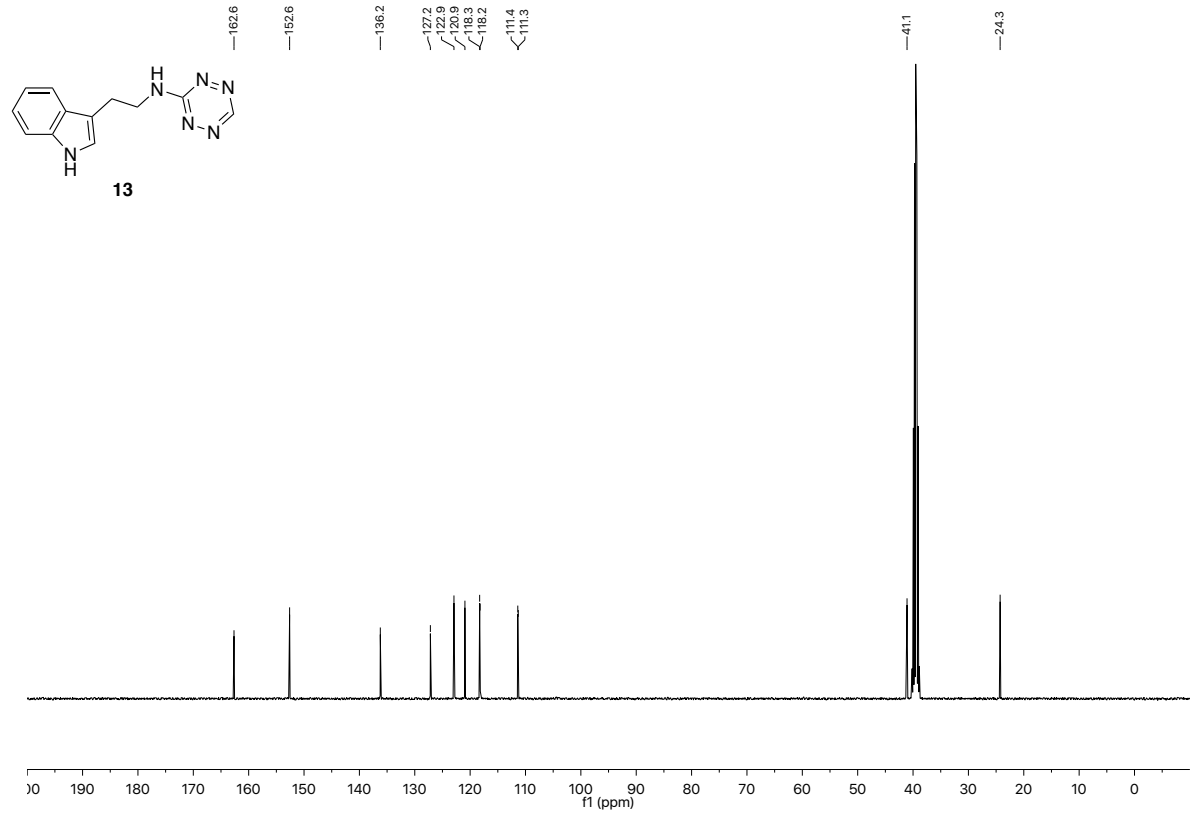



**22**

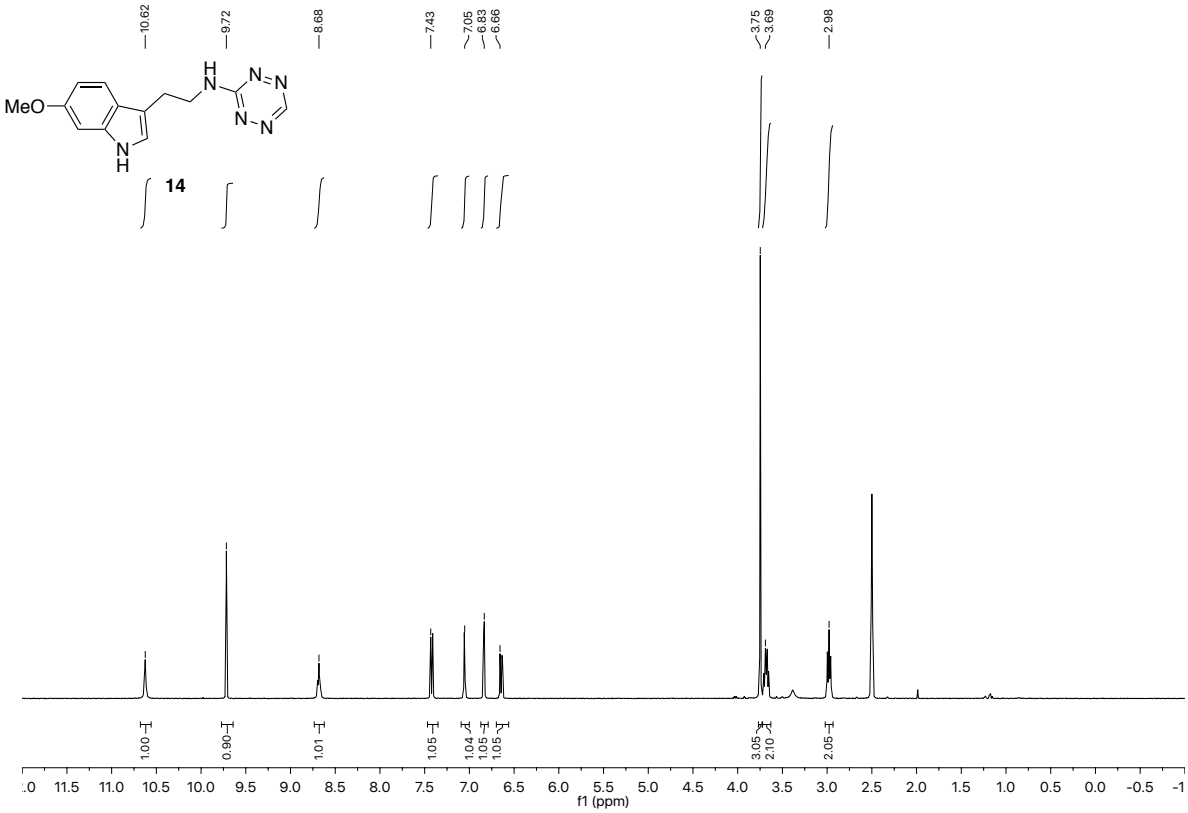
$^{1}$H-NMR, DMSO-*d6*, 400 MHz



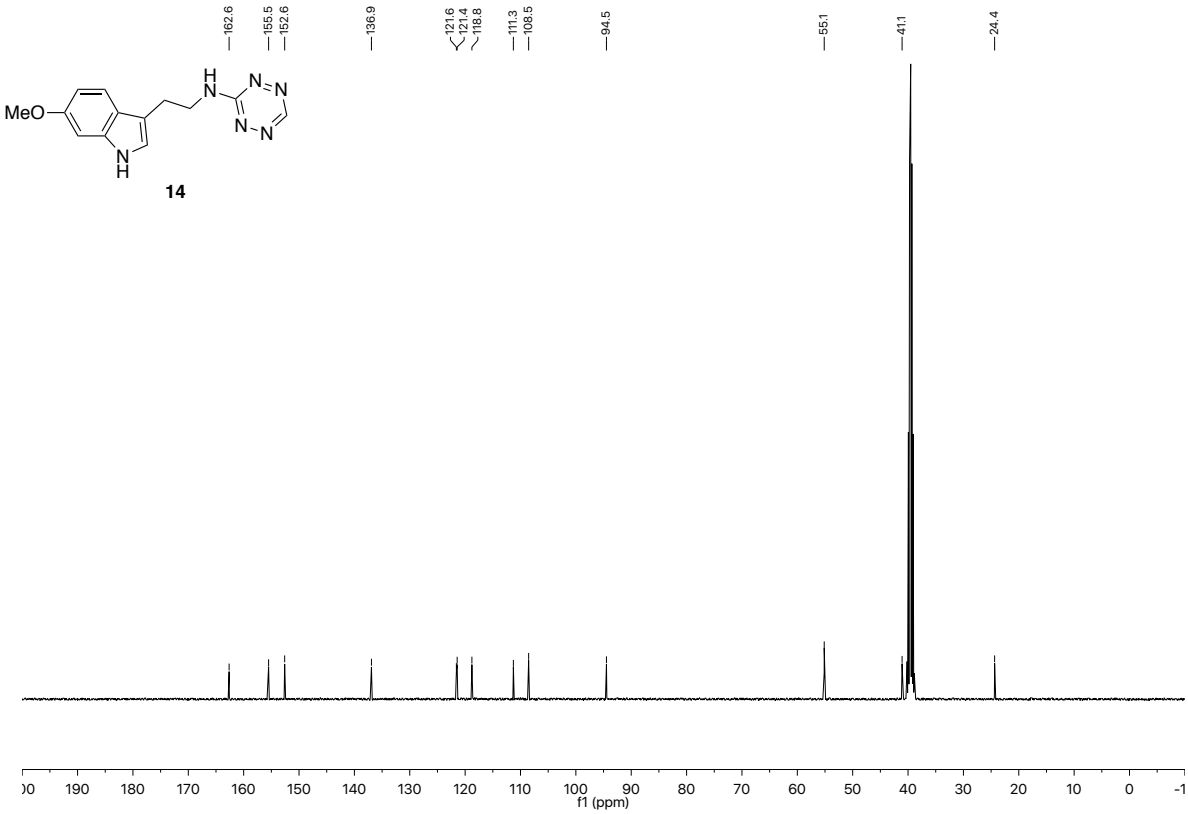

$^{13}$C-NMR, DMSO-*d6*, 101 MHz



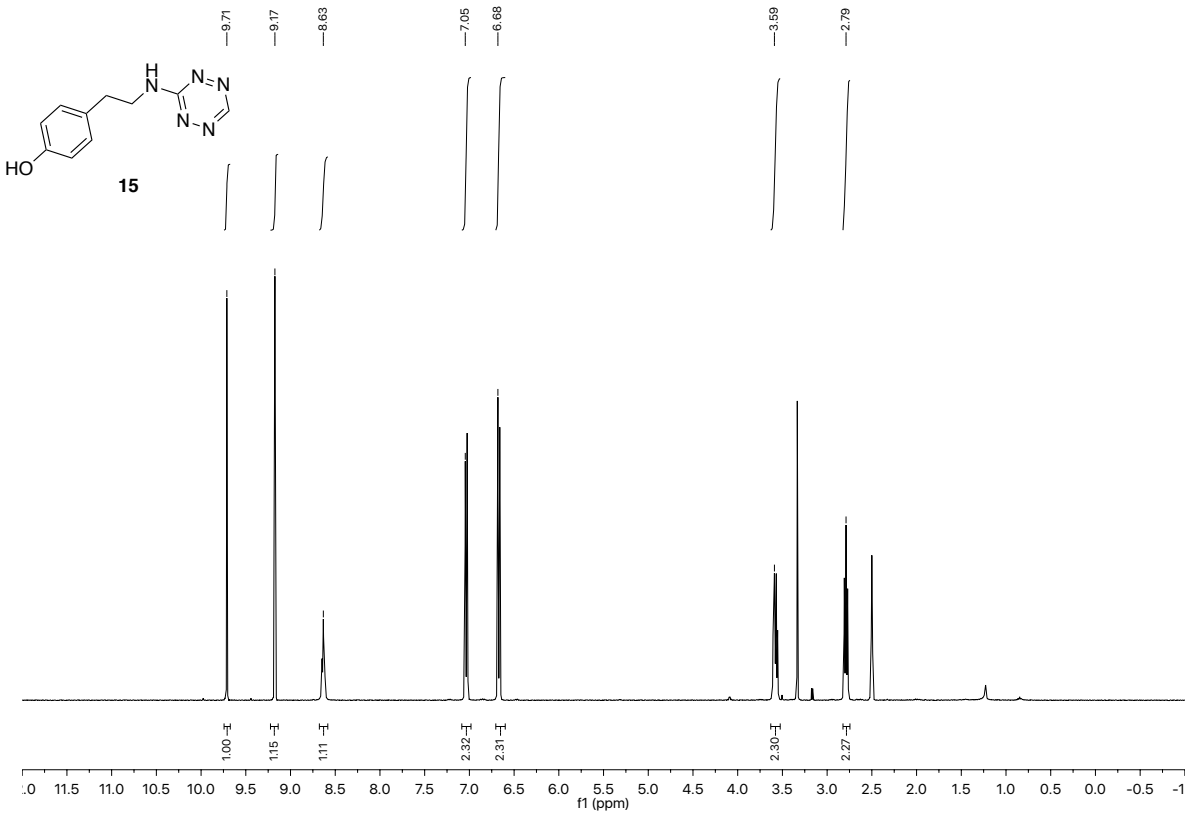
$^{1}$H-NMR, DMSO-*d6*, 400 MHz



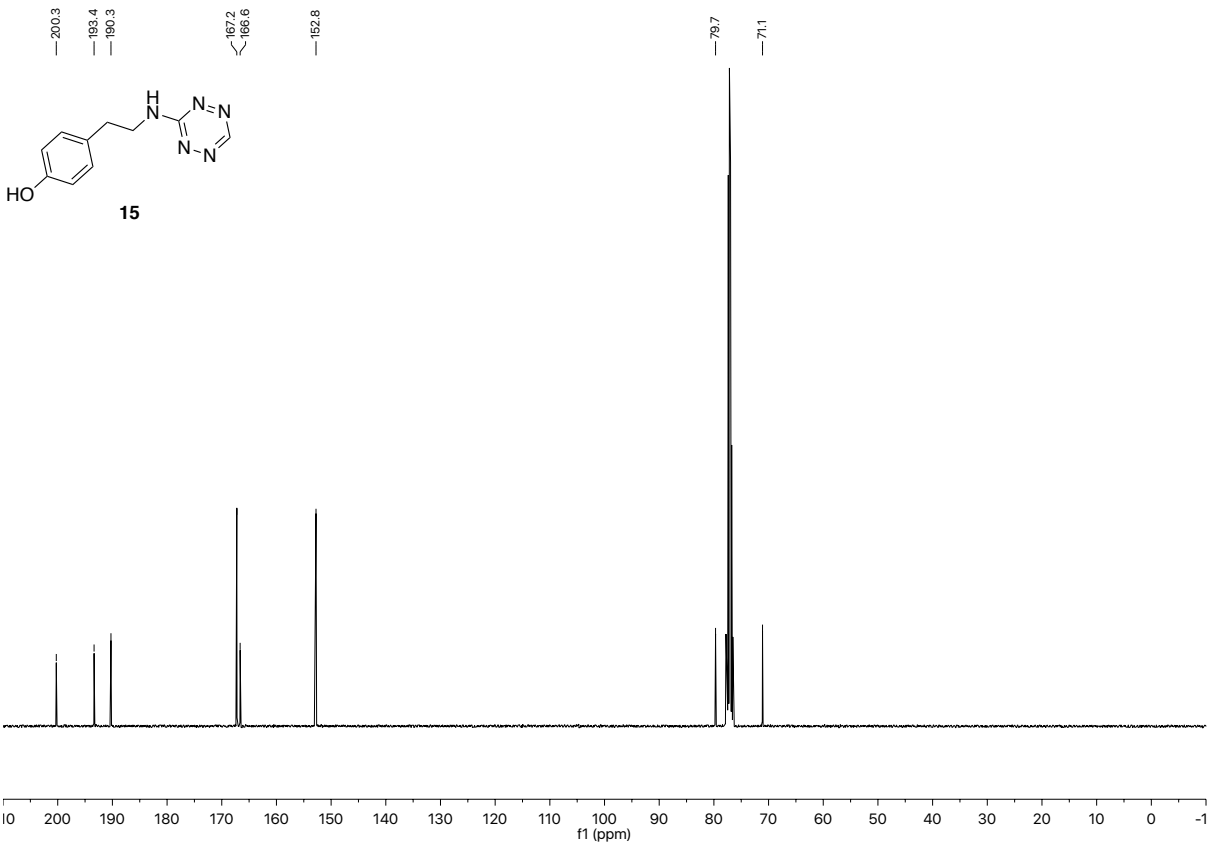

$^{13}$C-NMR, DMSO-*d6*, 101 MHz



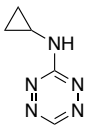
$^{1}$H-NMR, DMSO-*d6*, 400 MHz




$^{13}$C-NMR, DMSO-*d6*, 101 MHz



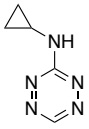
$^{1}$H-NMR, $CDCl_3$, 400 MHz



**S32**

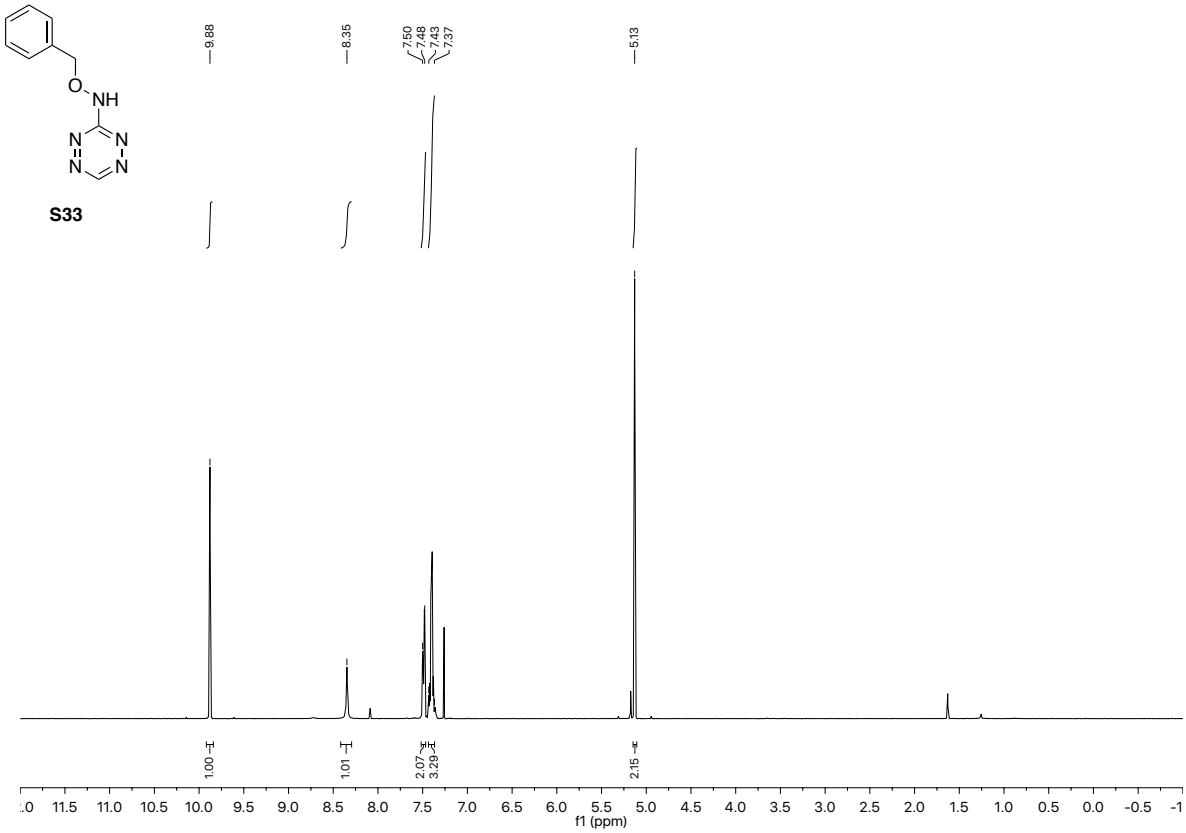



$^{13}$C-NMR, $CDCl_3$, 126 MHz



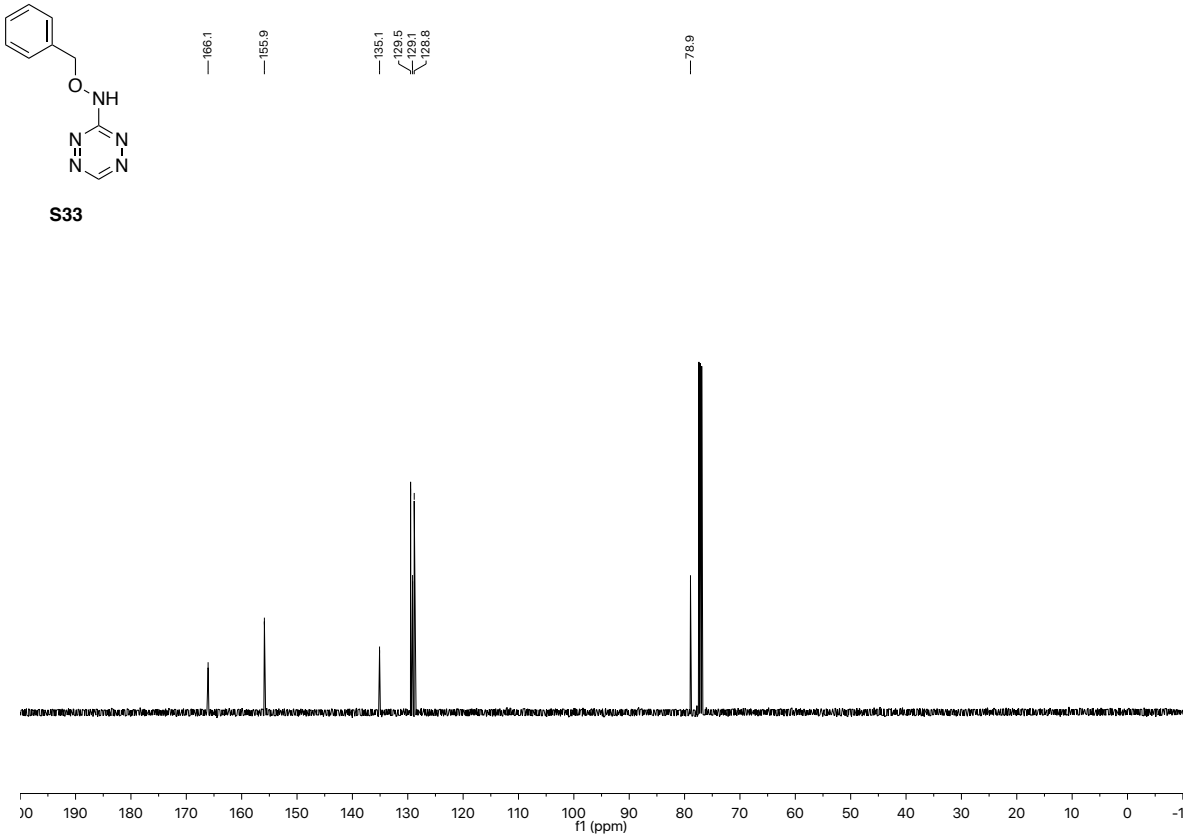
**S32**

$^{1}$H-NMR, $CDCl_3$, 400 MHz



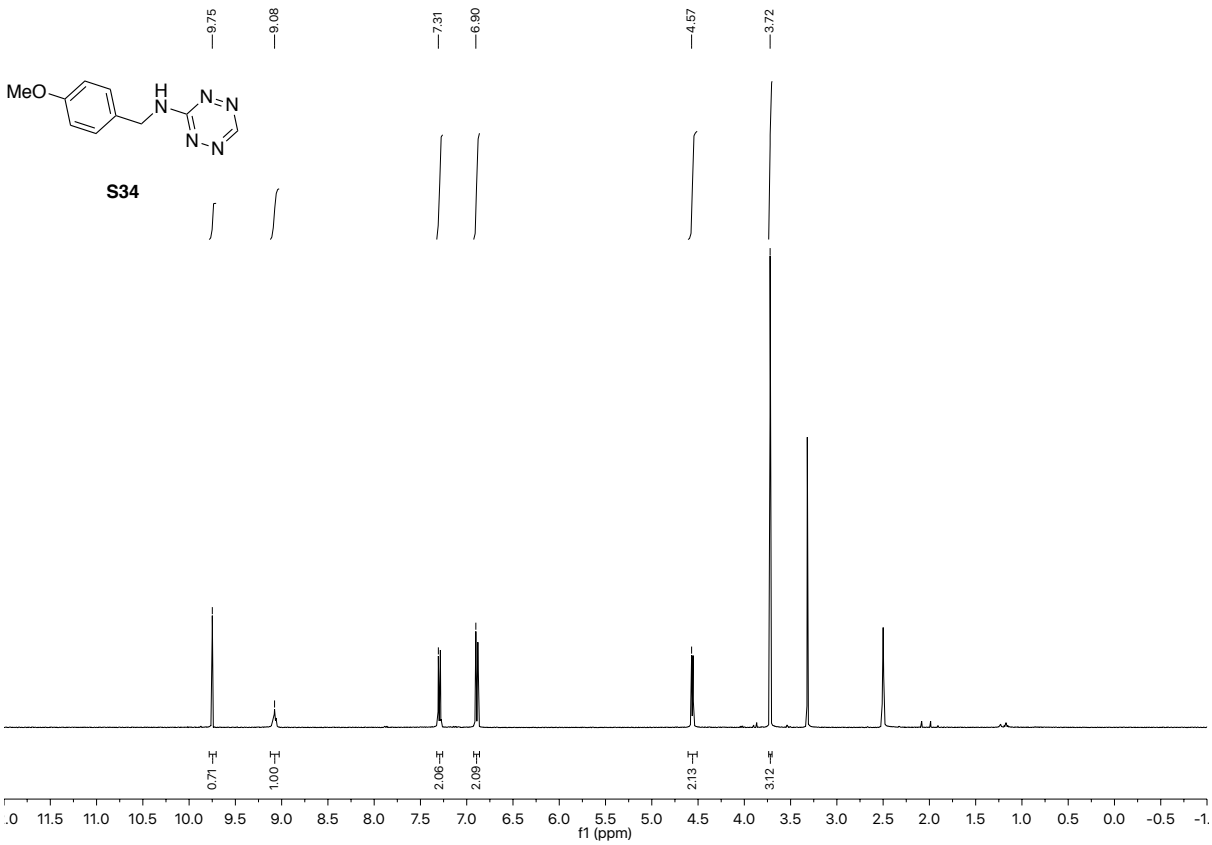

**S33**

$^{13}$C-NMR, $CDCl_3$, 126 MHz

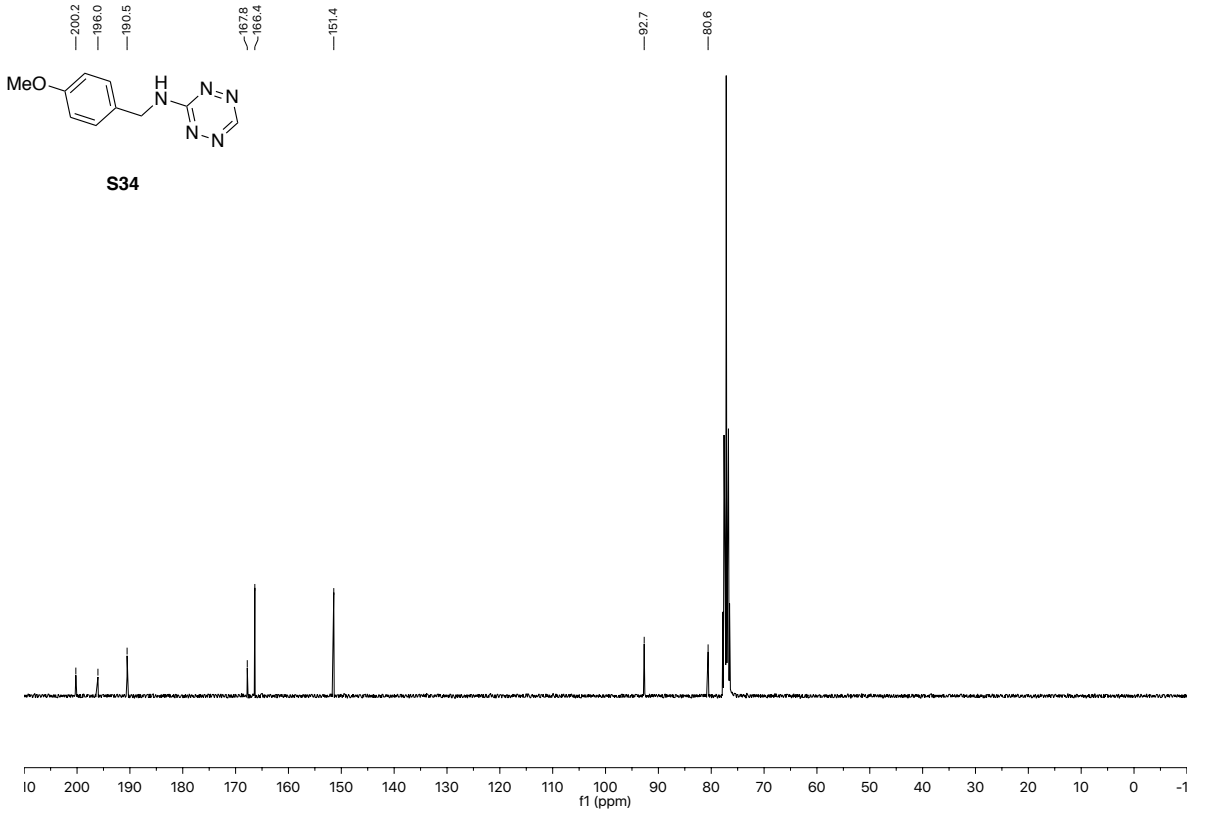



**S33**

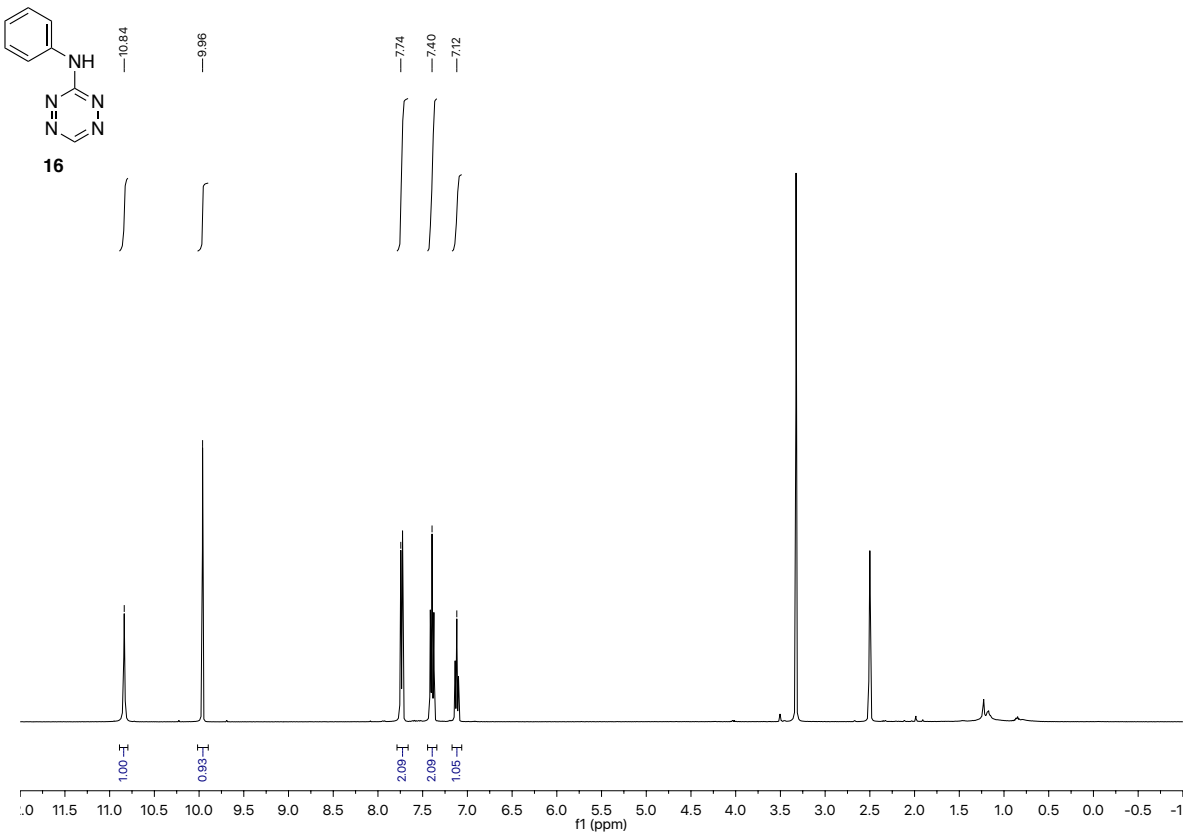
$^{1}$H-NMR, DMSO-*d6*, 400 MHz



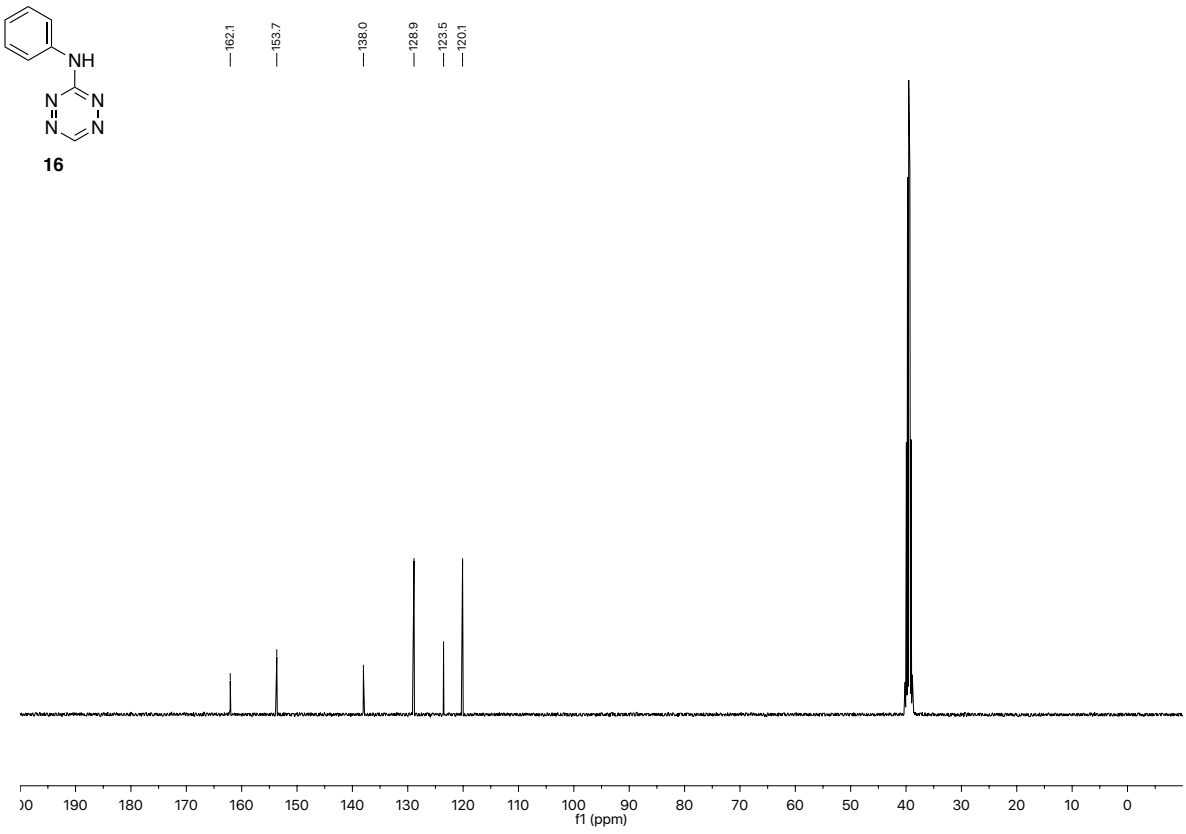

$^{13}$C-NMR, DMSO-*d6*, 101 MHz



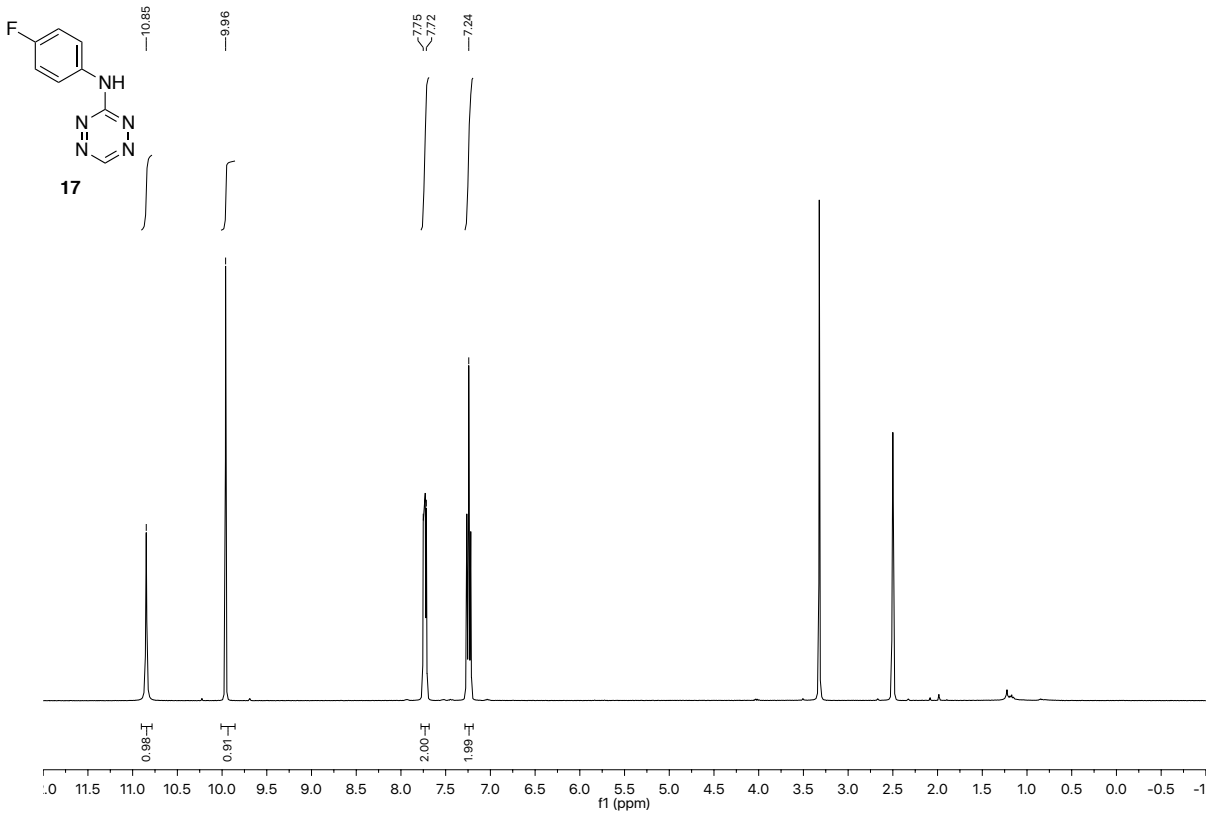
$^{1}$H-NMR, DMSO-*d6*, 400 MHz



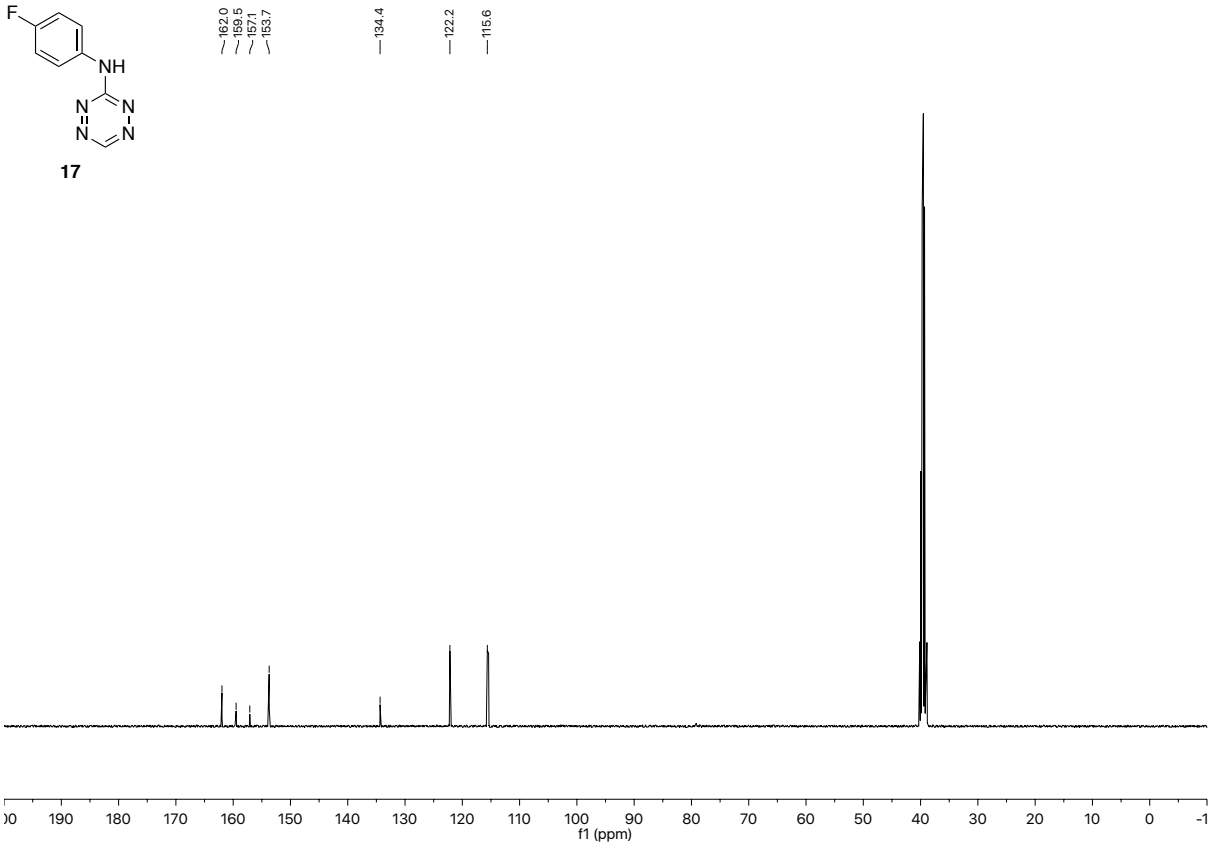

$^{13}$C-NMR, DMSO-*d6*, 101 MHz



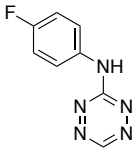
$^{1}$H-NMR, DMSO-*d6*, 400 MHz




$^{13}$C-NMR, DMSO-*d6*, 101 MHz

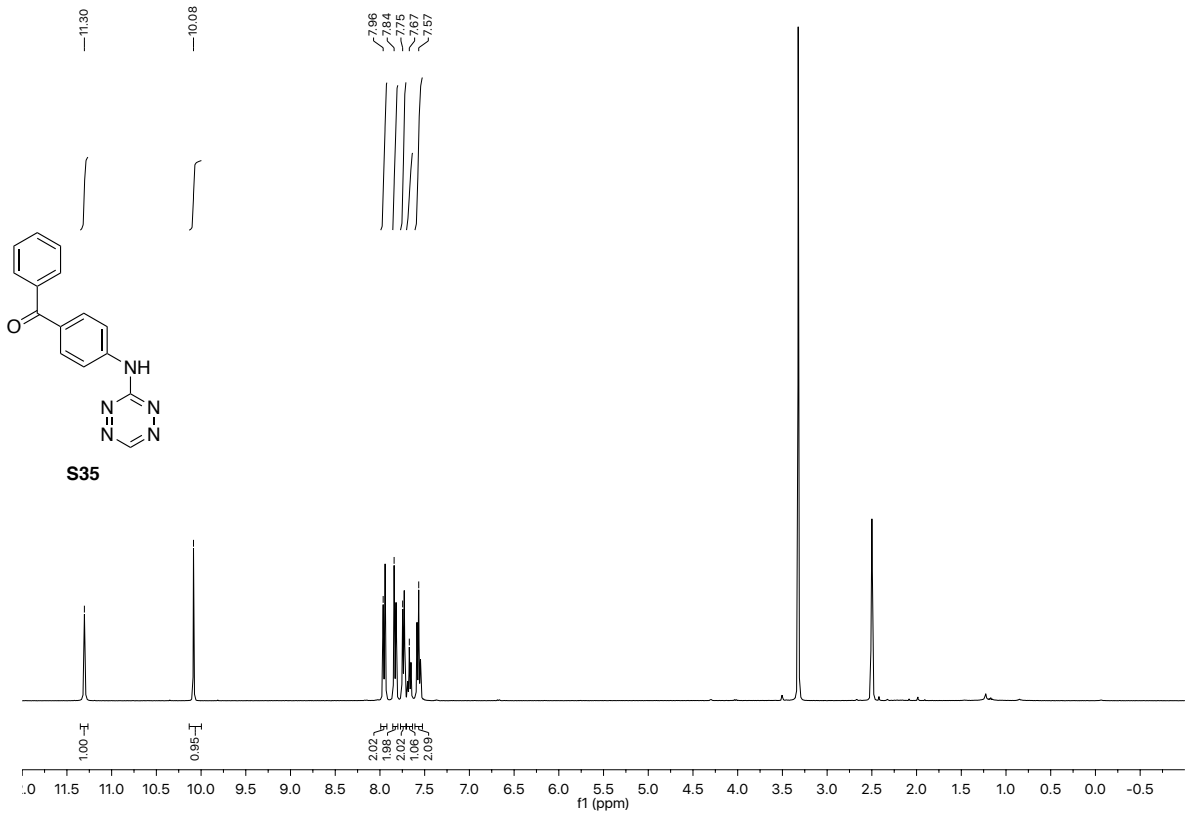


$^{19}$F-NMR, DMSO-*d6*, 376 MHz



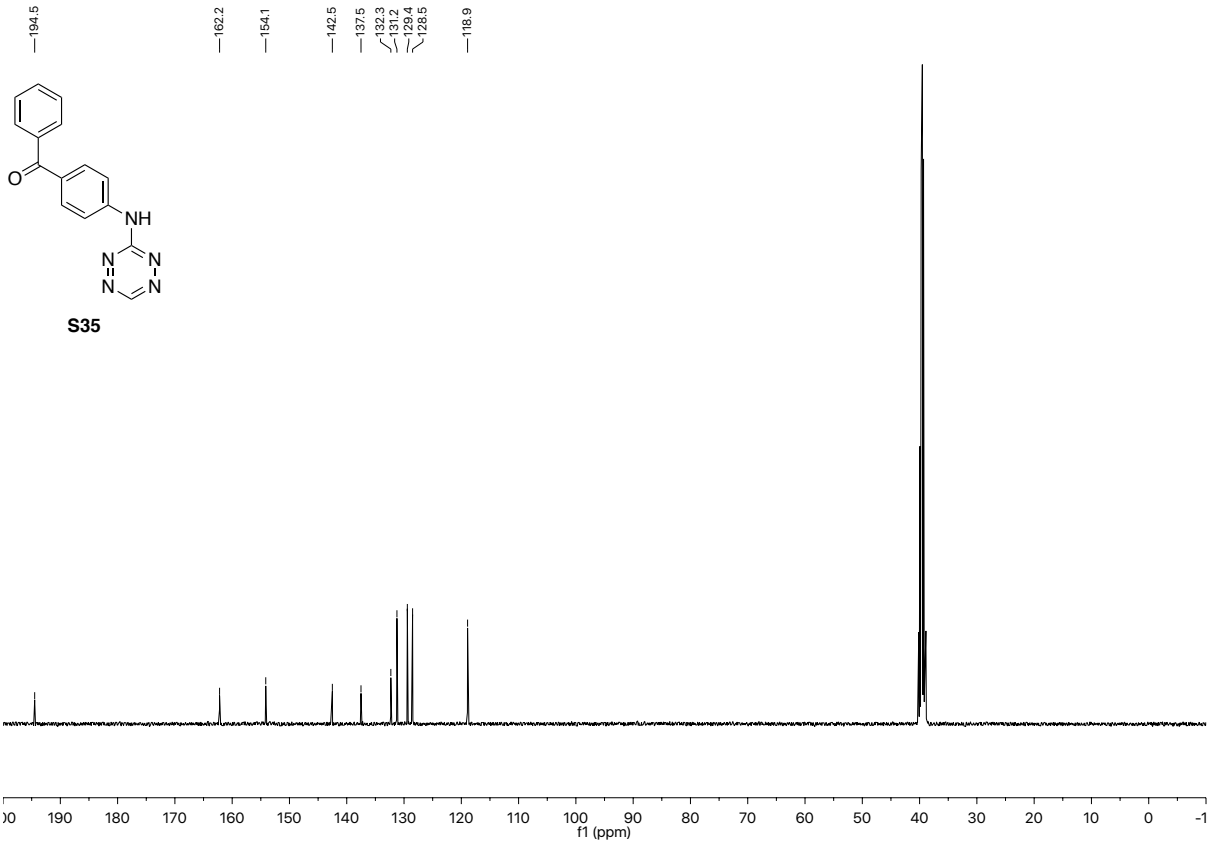

**17**

$^{1}$H-NMR, DMSO-*d6*, 400 MHz



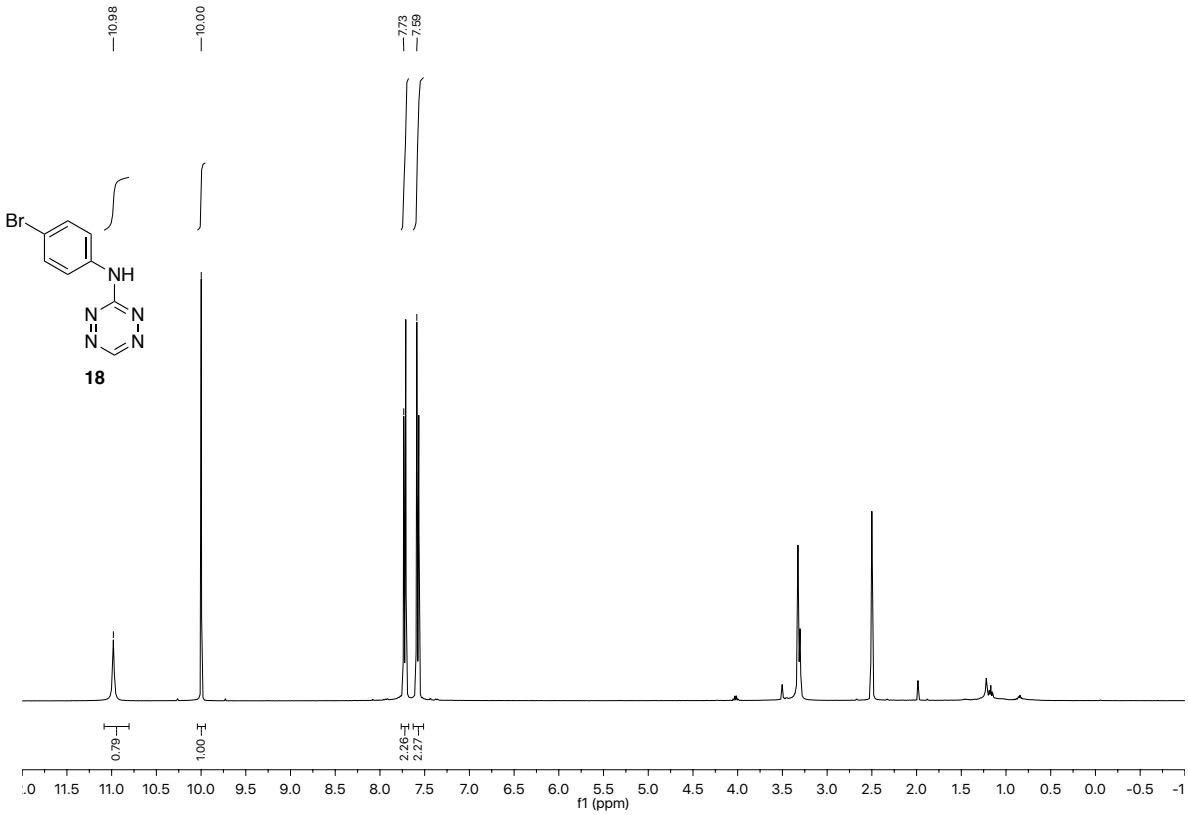

**S35**

$^{13}$C-NMR, DMSO-*d6*, 101 MHz

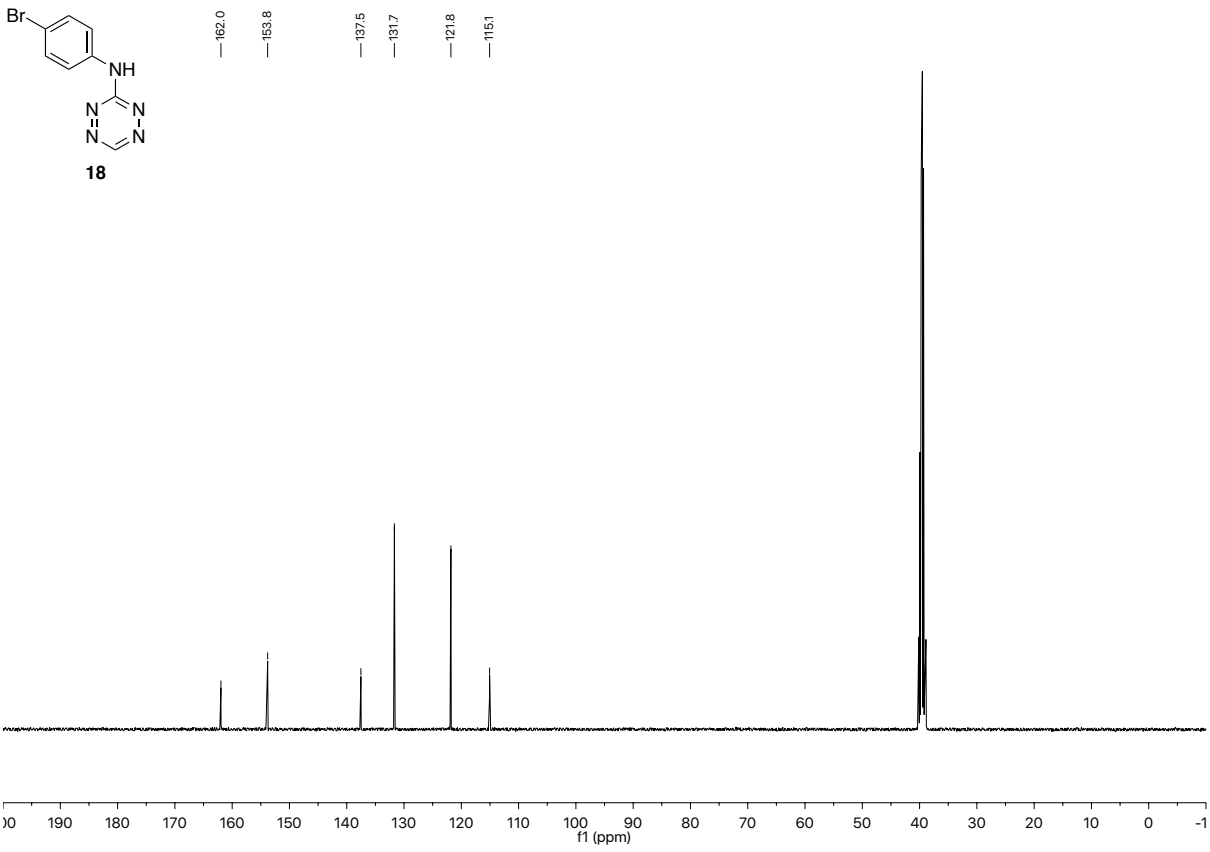



**S35**

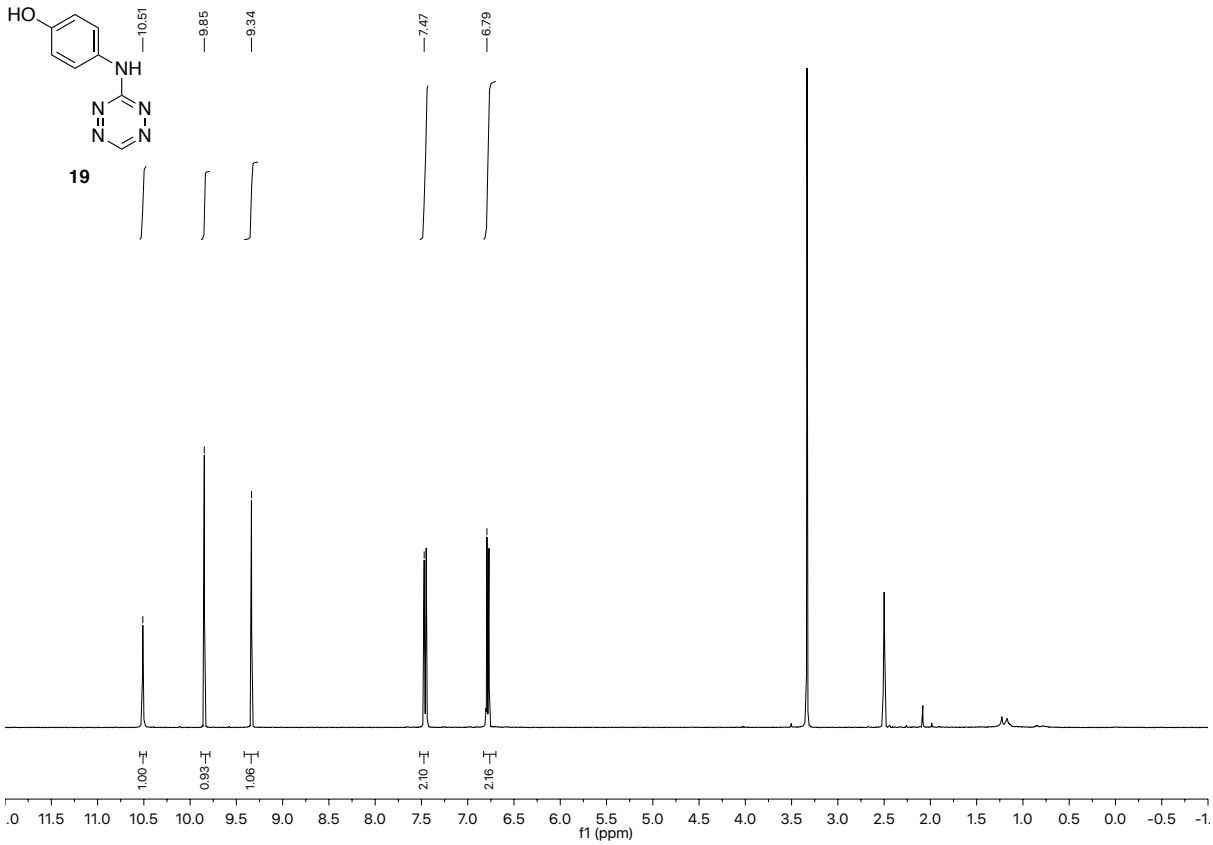
$^{1}$H-NMR, DMSO-*d6*, 400 MHz



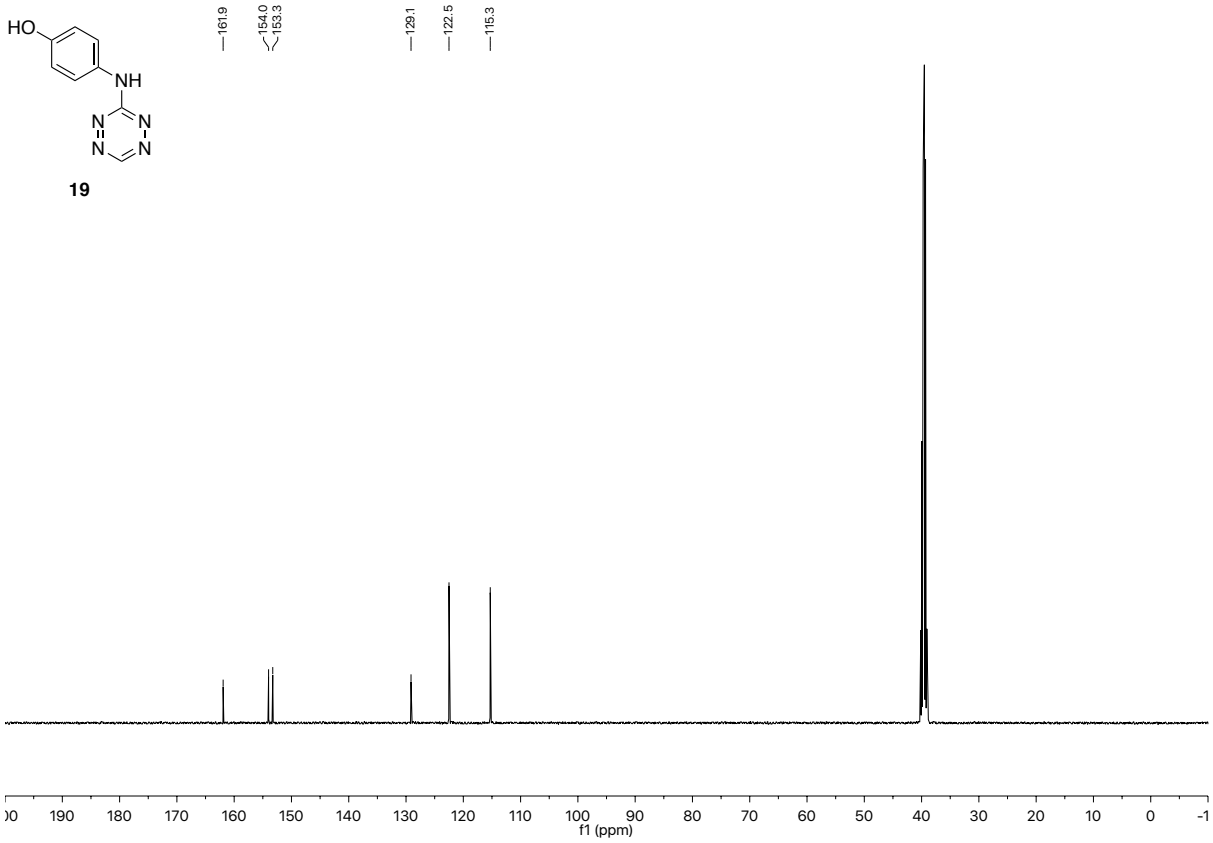

$^{13}$C-NMR, DMSO-*d6*, 101 MHz



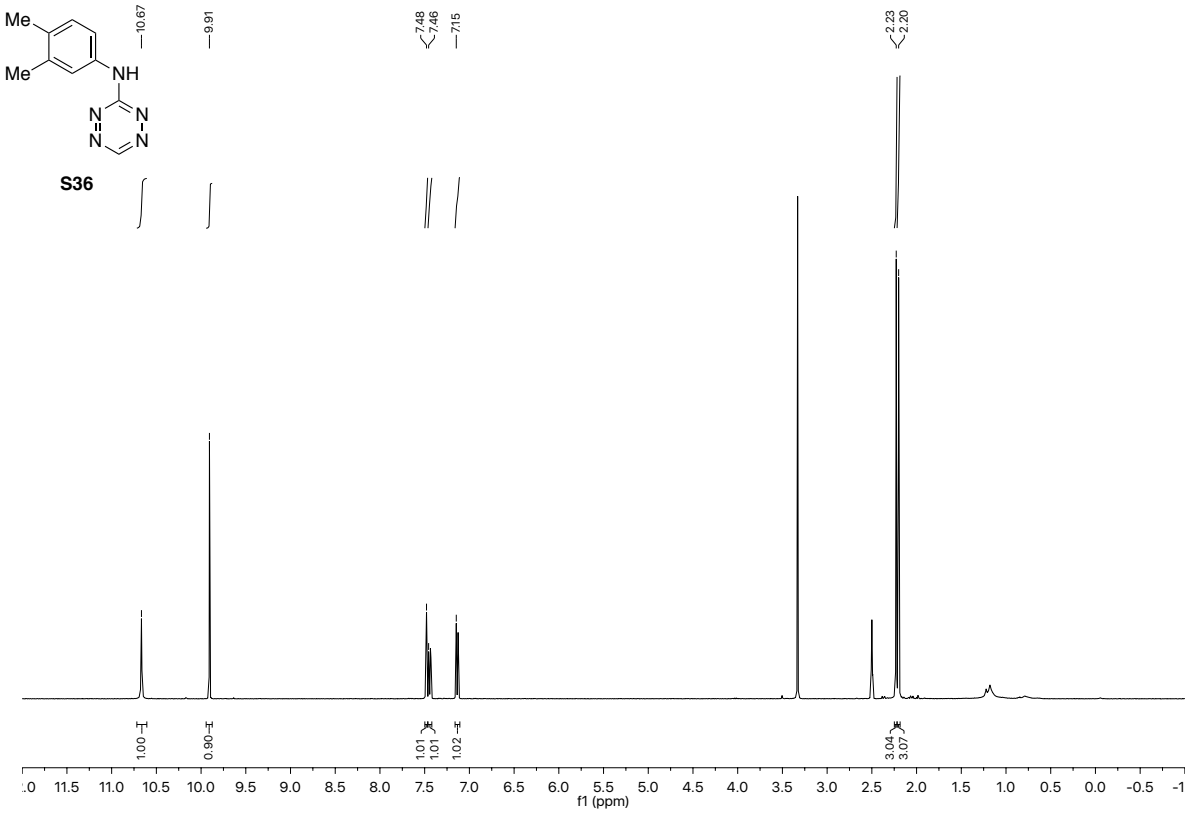
$^{1}$H-NMR, DMSO-*d6*, 400 MHz



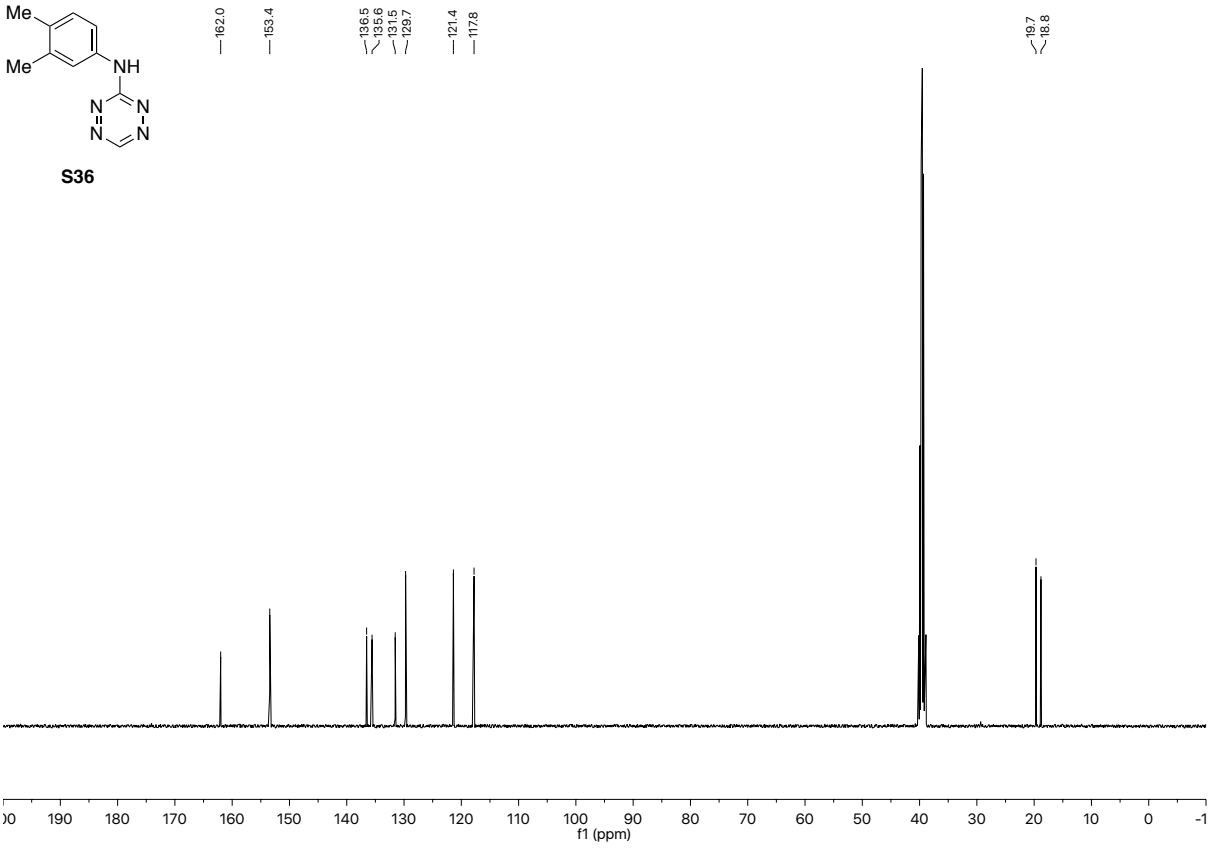

$^{13}$C-NMR, DMSO-*d6*, 101 MHz



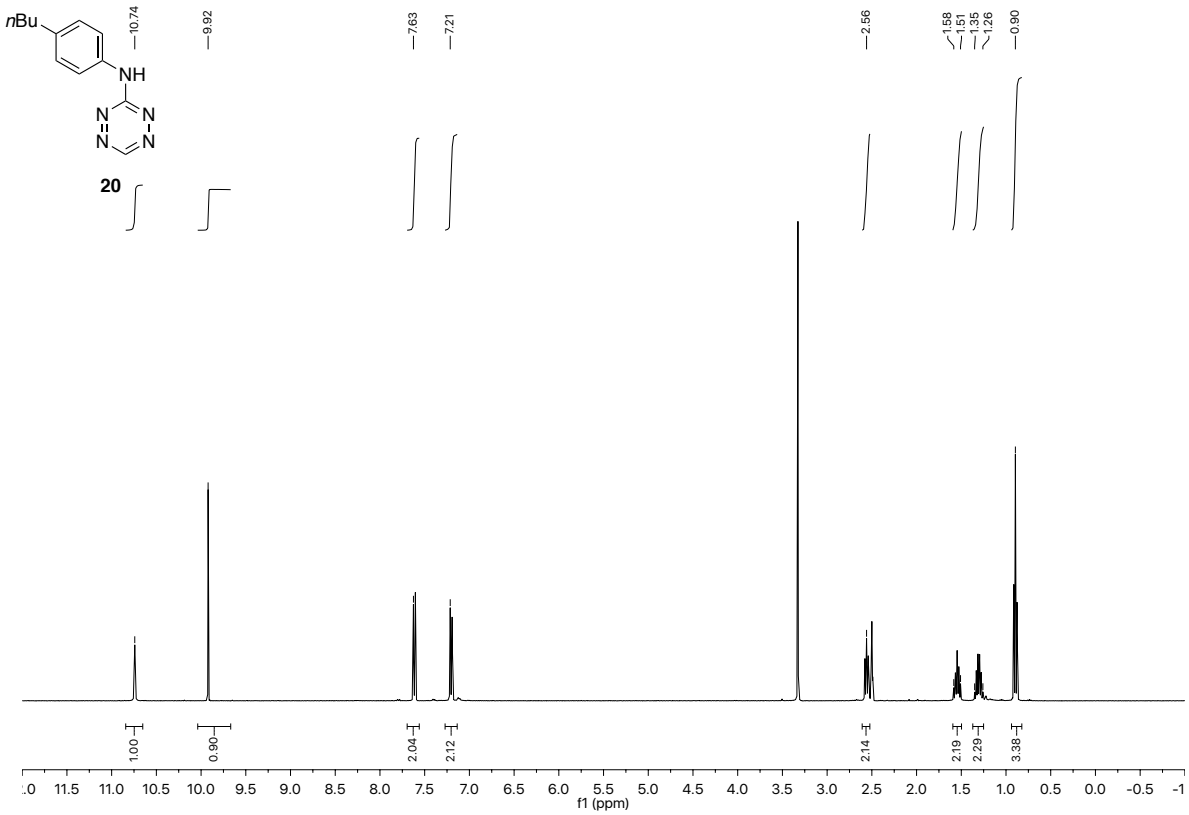
$^{1}$H-NMR, DMSO-*d6*, 400 MHz



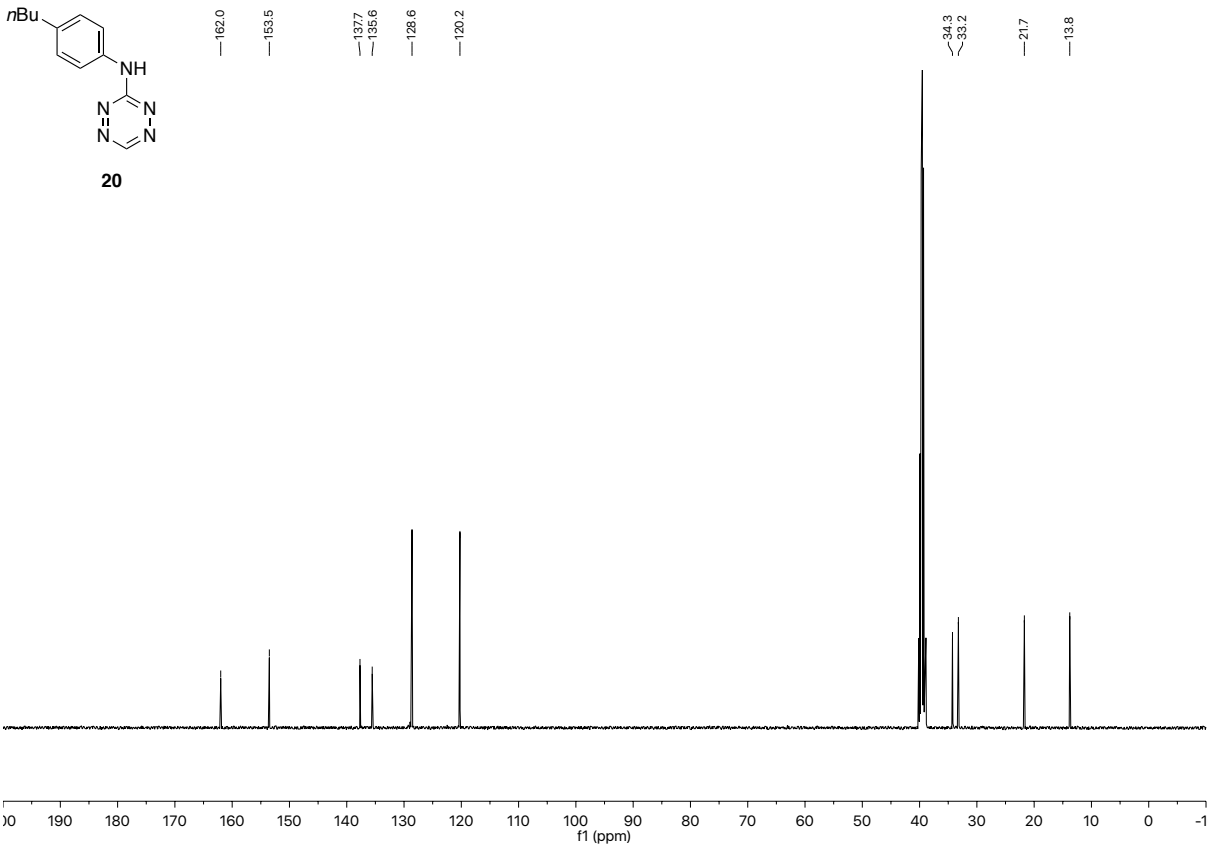

$^{13}$C-NMR, DMSO-*d6*, 101 MHz



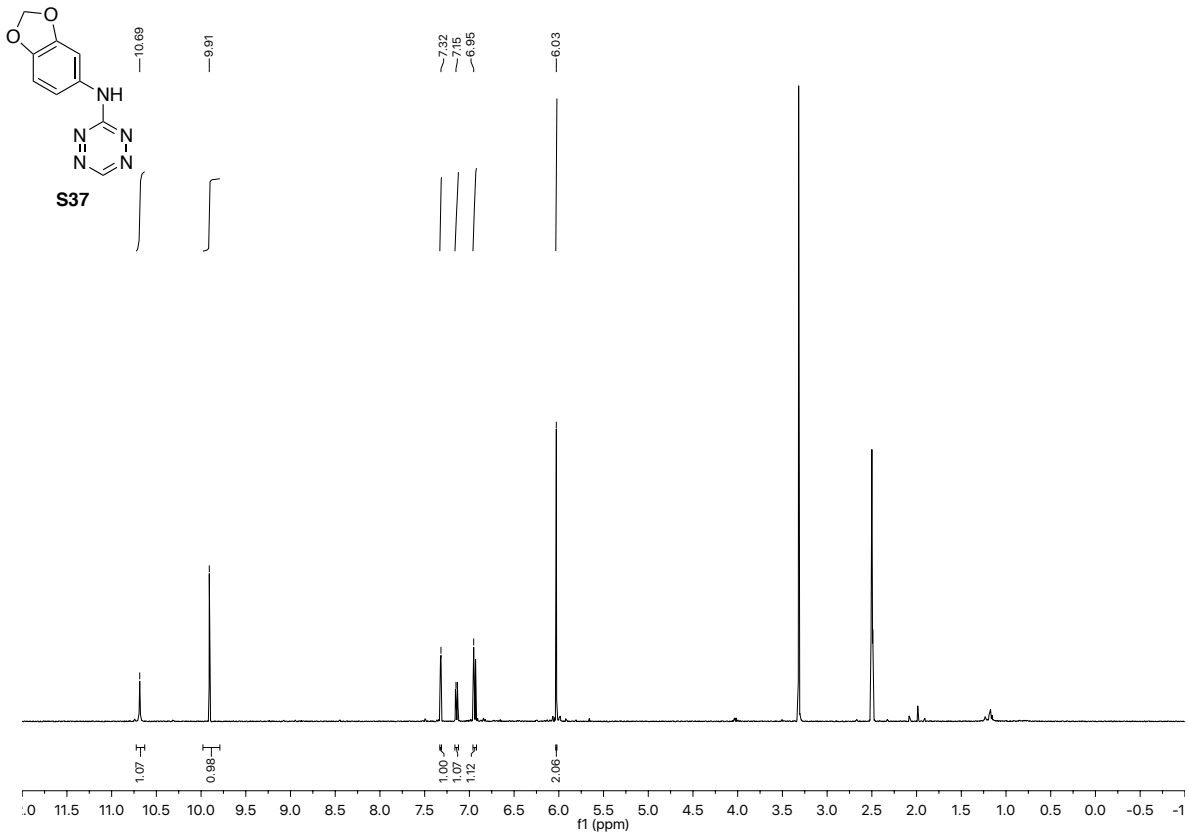
$^{1}$H-NMR, DMSO-*d6*, 400 MHz




$^{13}$C-NMR, DMSO-*d6*, 101 MHz

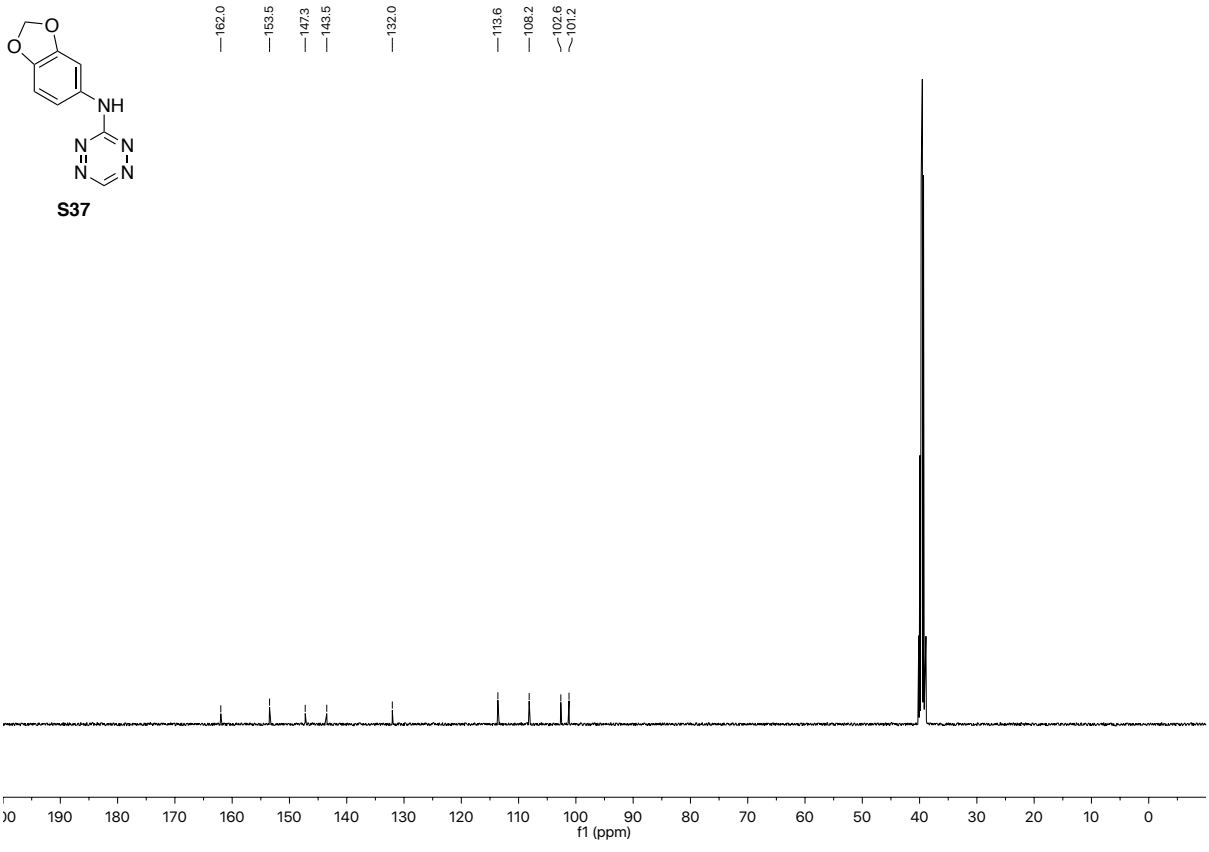


$^{1}$H-NMR, DMSO-*d6*, 400 MHz



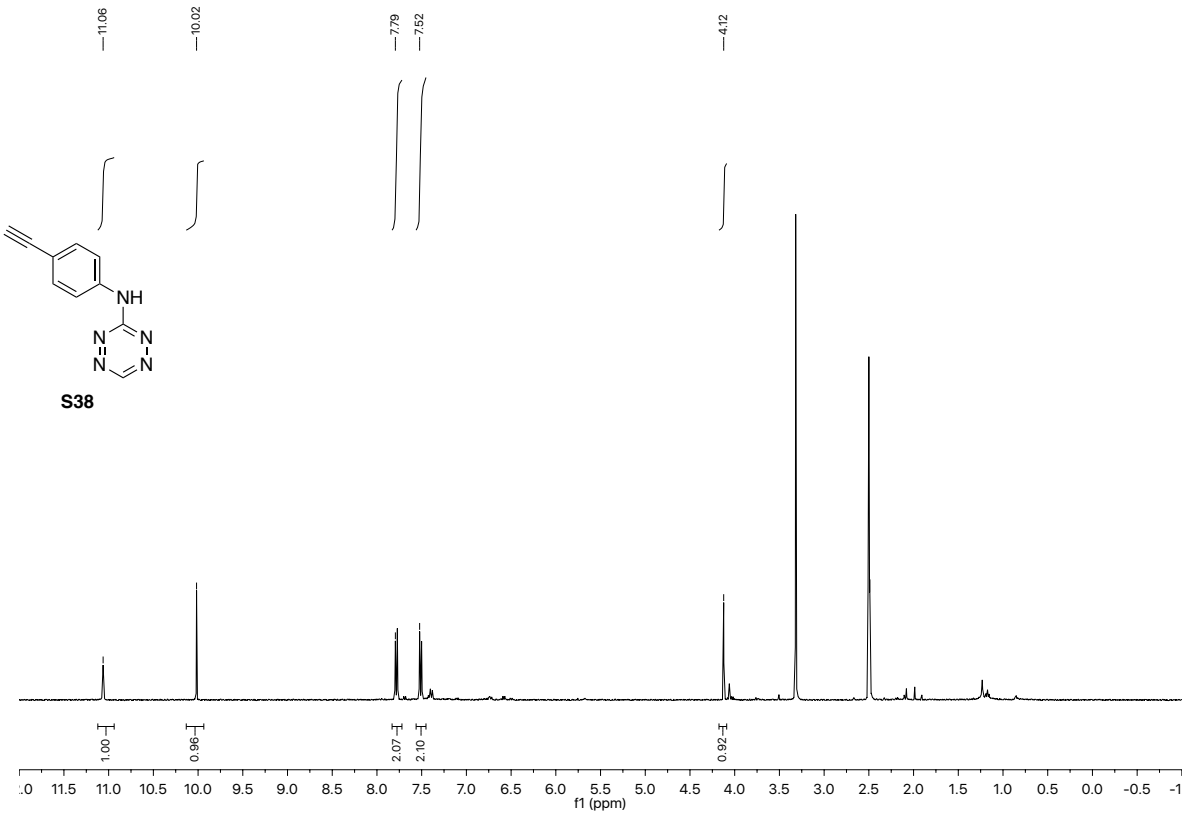

**S37**

$^{13}$C-NMR, DMSO-*d6*, 101 MHz



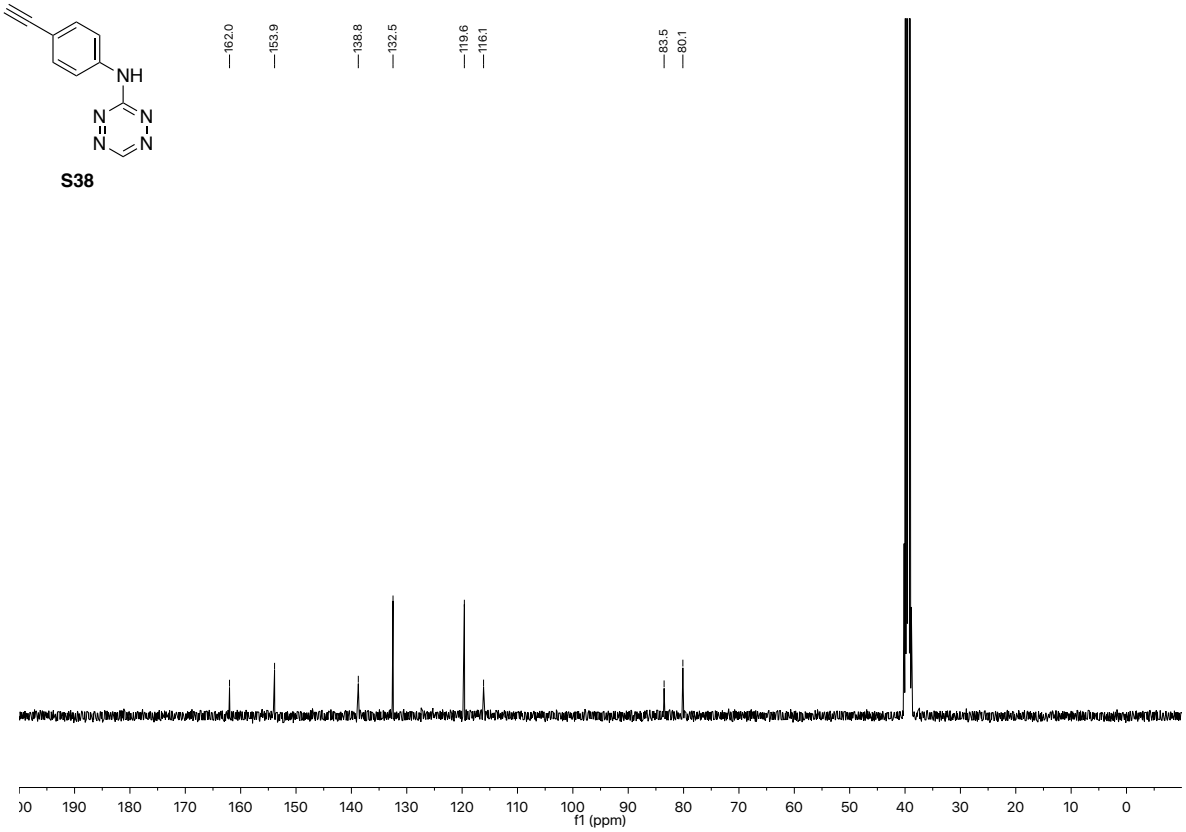

**S37**

$^{1}$H-NMR, DMSO-*d6*, 400 MHz



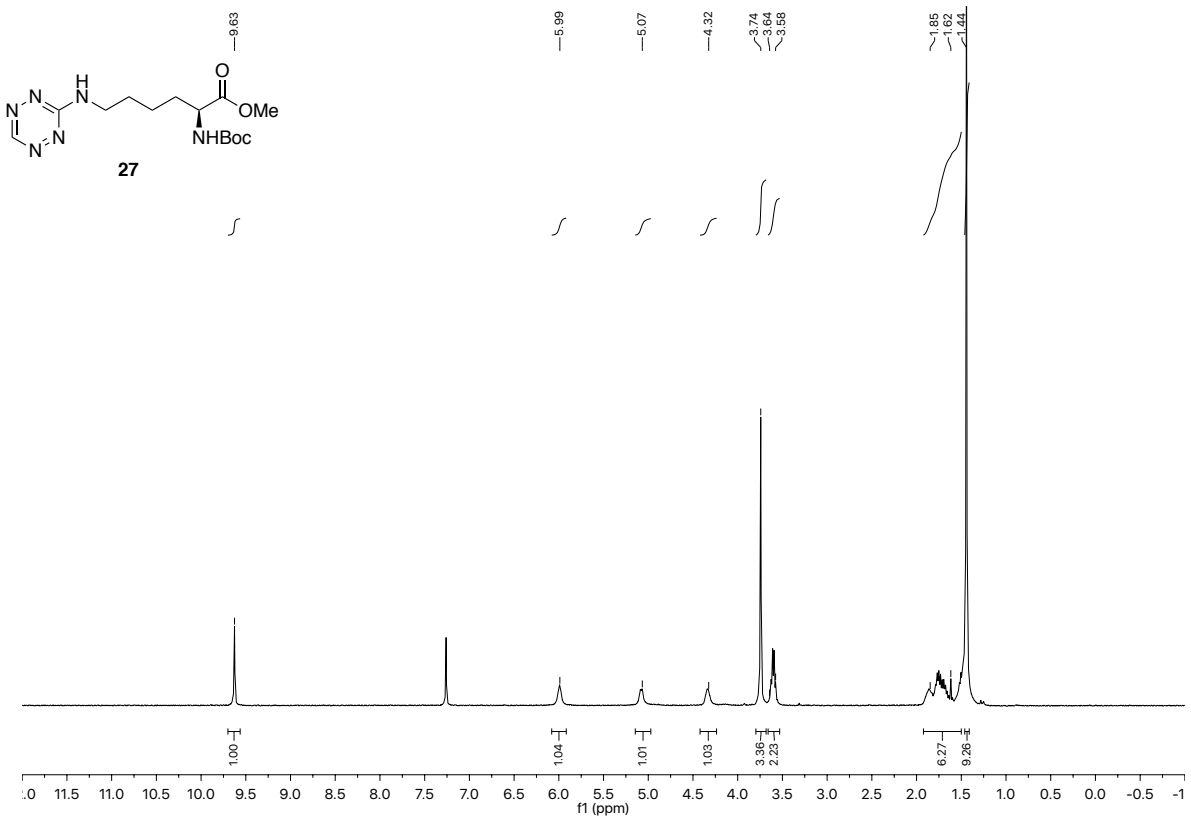

**S38**

$^{13}$C-NMR, DMSO-*d6*, 101 MHz



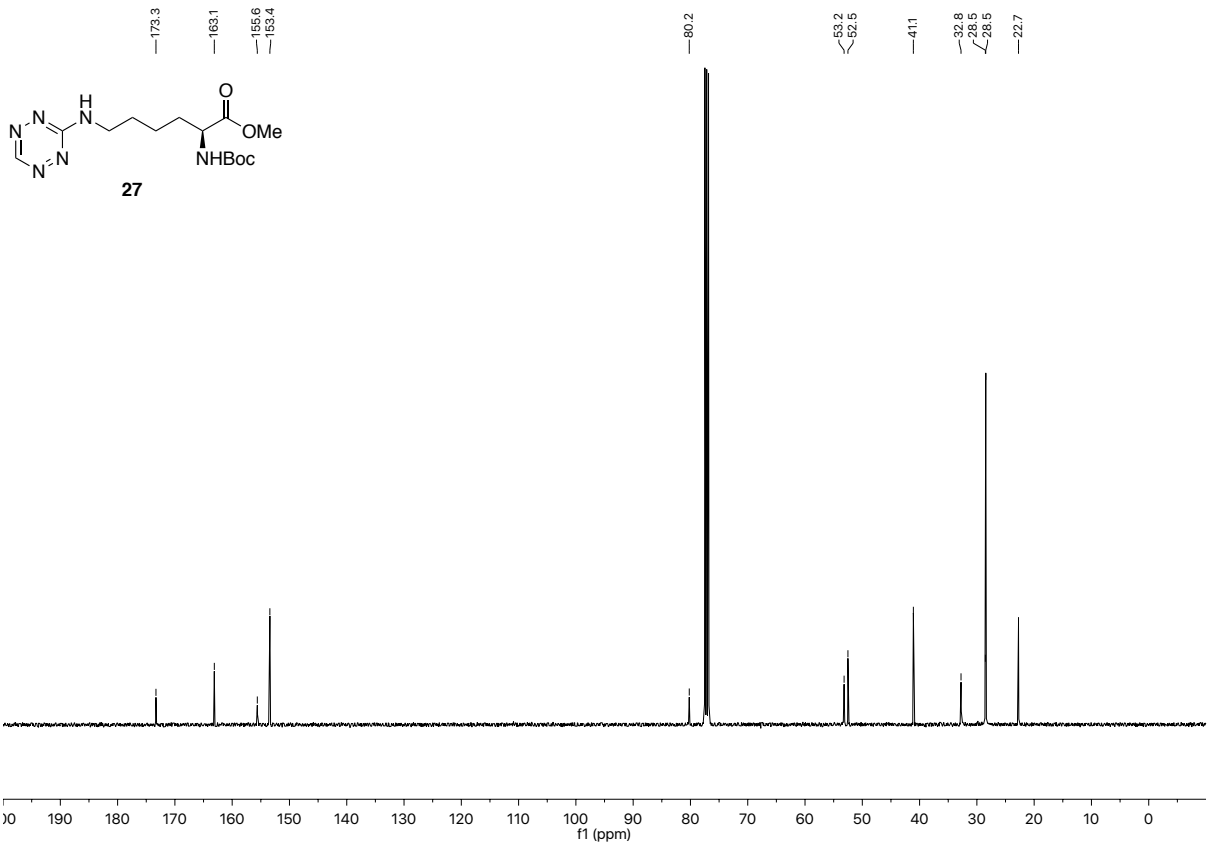

**S38**

$^{1}$H-NMR, $CDCl_3$, 400 MHz



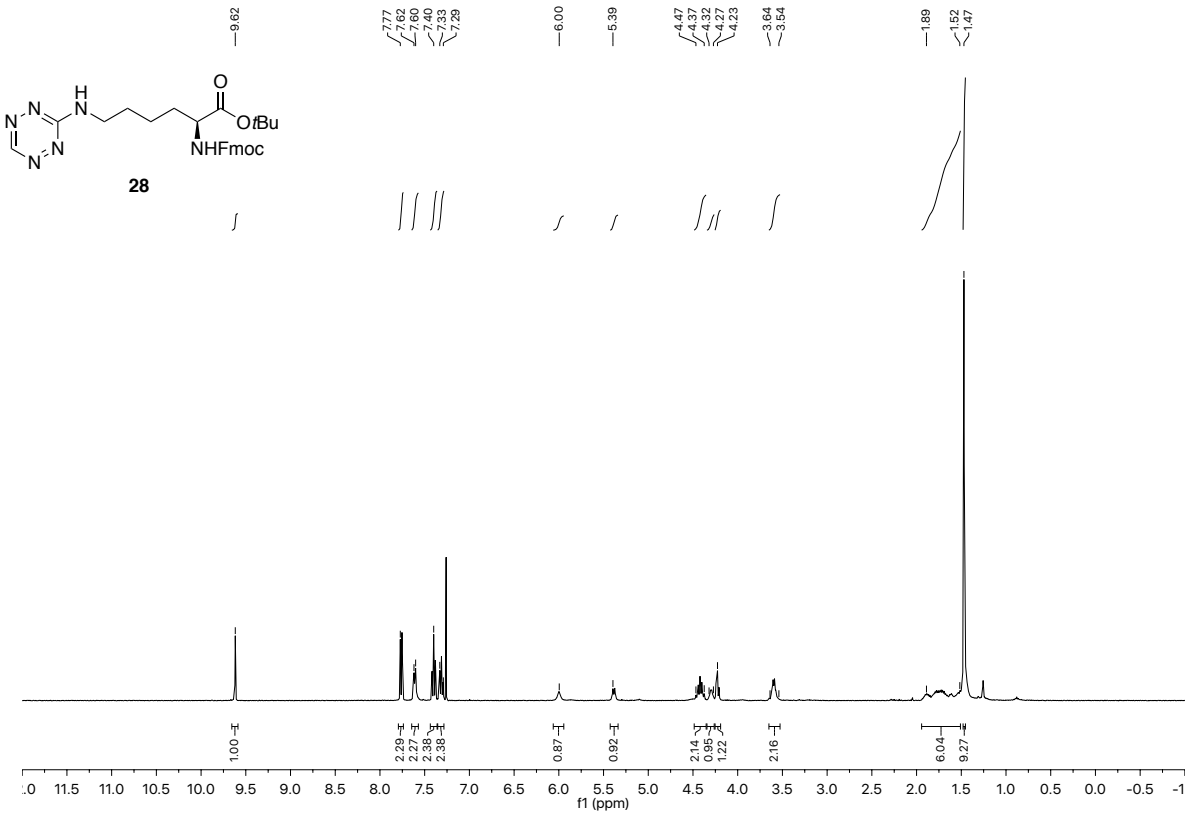

**27**

$^{13}$C-NMR, $CDCl_3$, 101 MHz



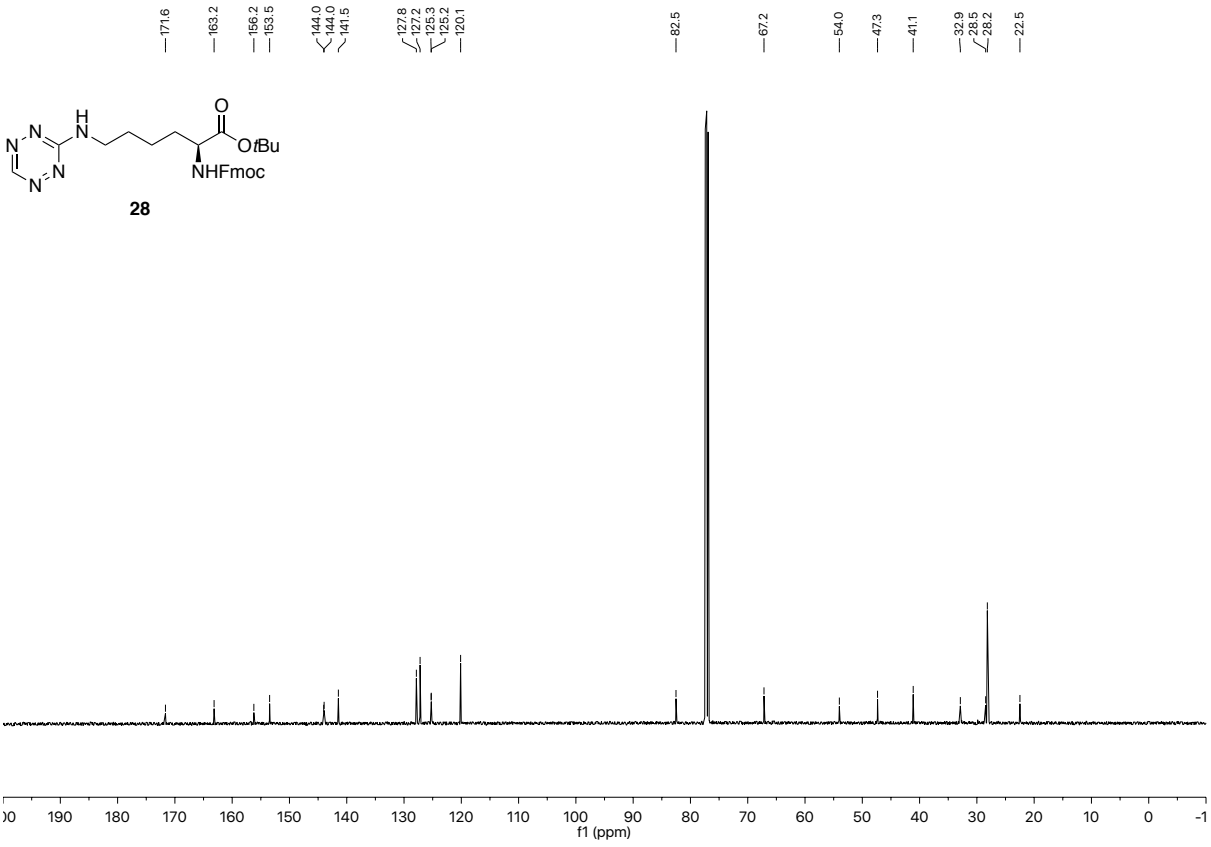

**27**

$^{1}$H-NMR, $CDCl_3$, 400 MHz



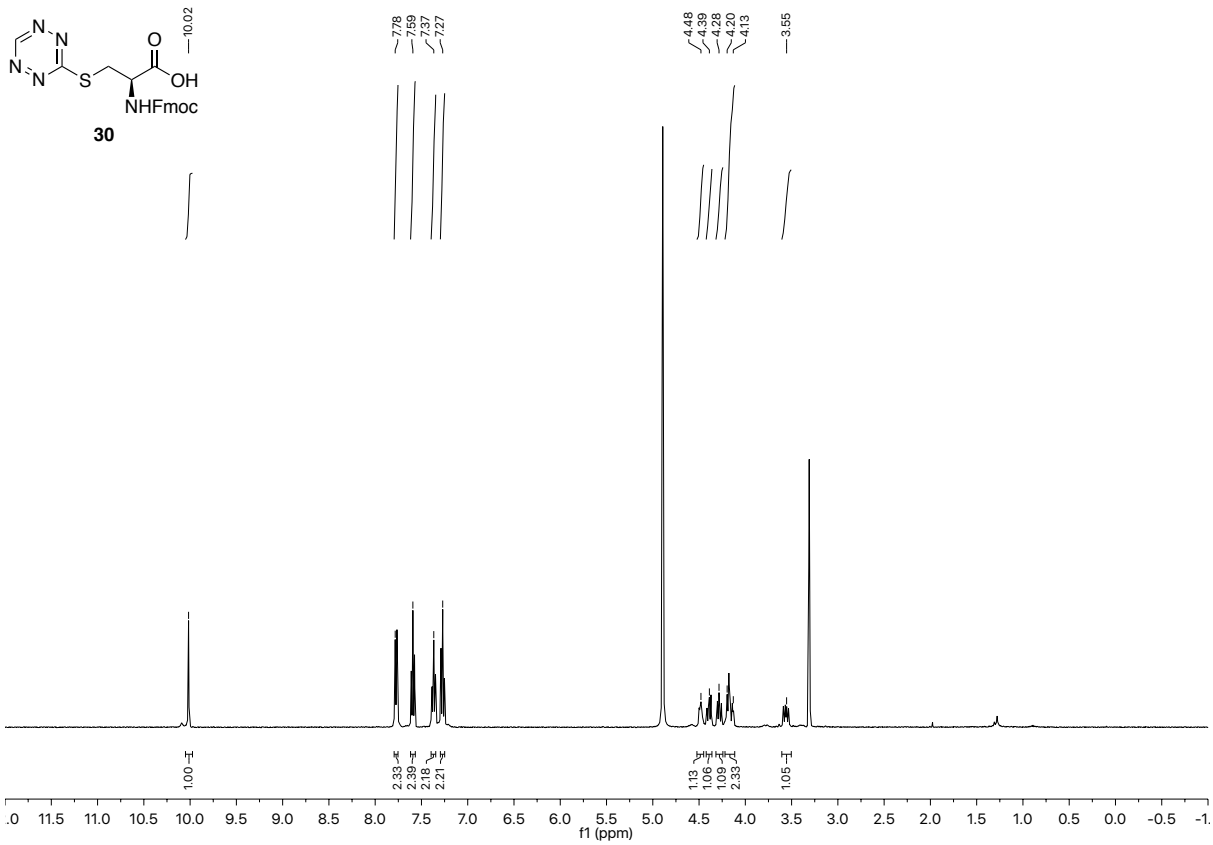

**28**

$^{13}$C-NMR, $CDCl_3$, 126 MHz



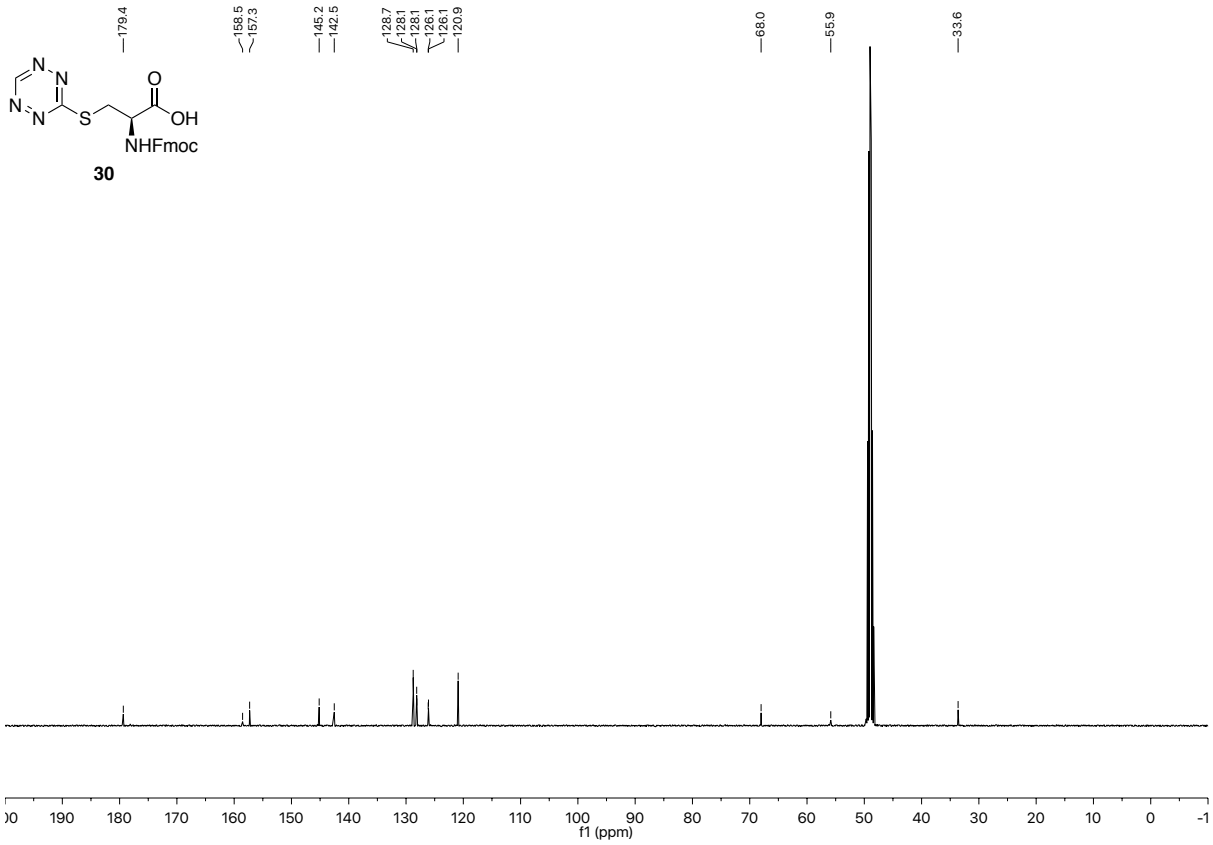

**28**

$^{1}$H-NMR, MeOH-*d4*, 400 MHz



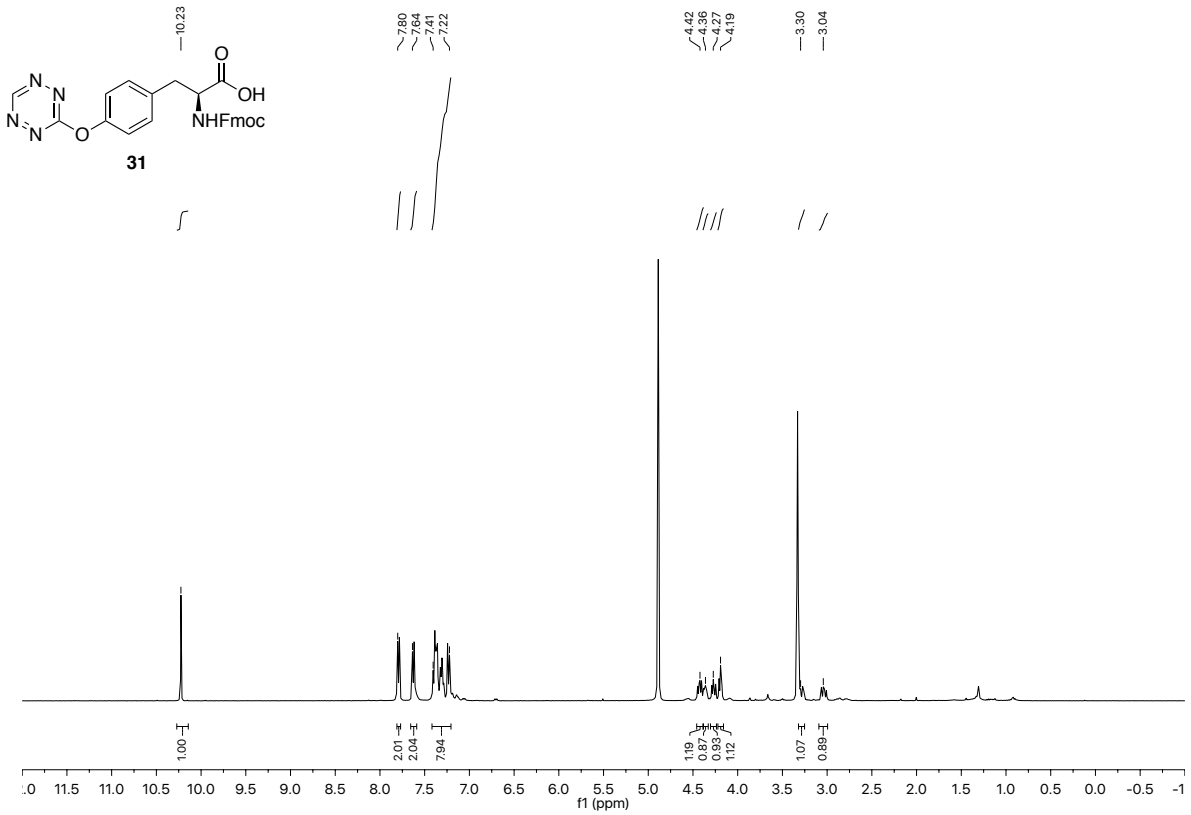

**30**

$^{13}$C-NMR, MeOH-*d4*, 101 MHz



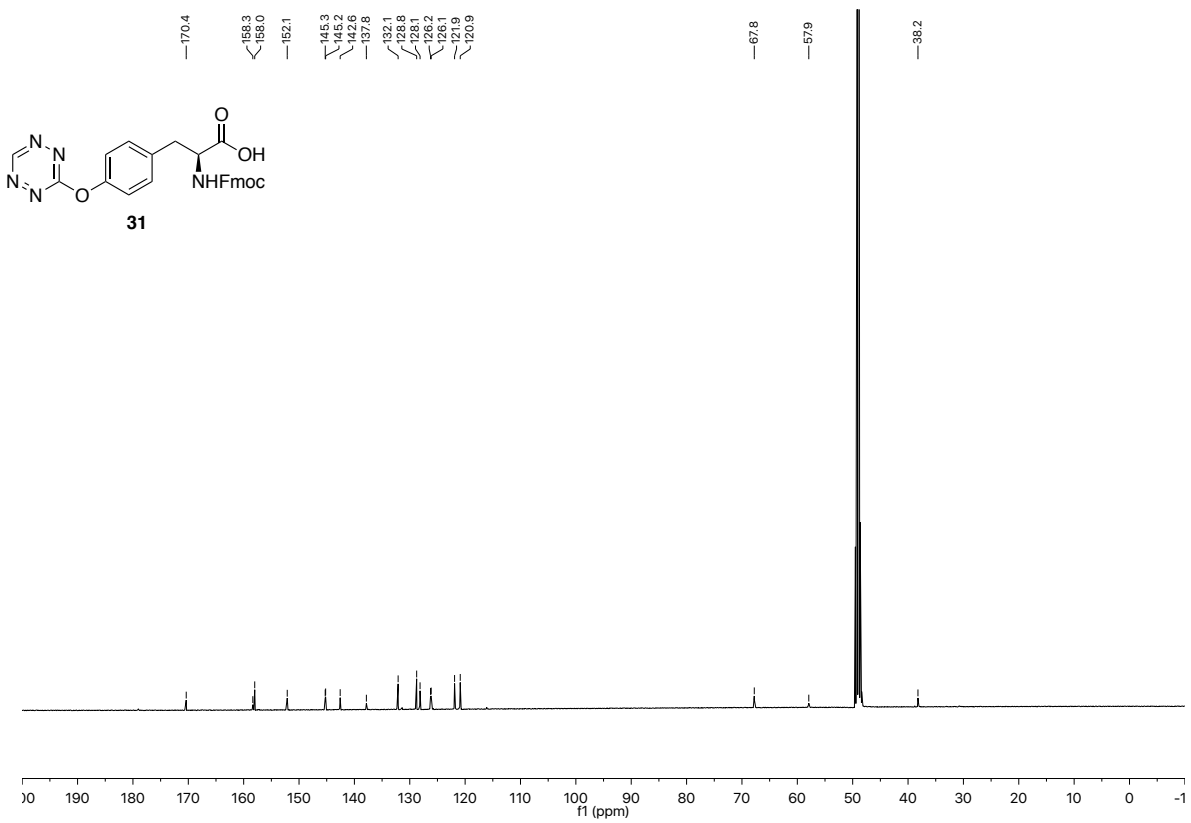

**30**

$^{1}$H-NMR, MeOH-*d4*, 400 MHz



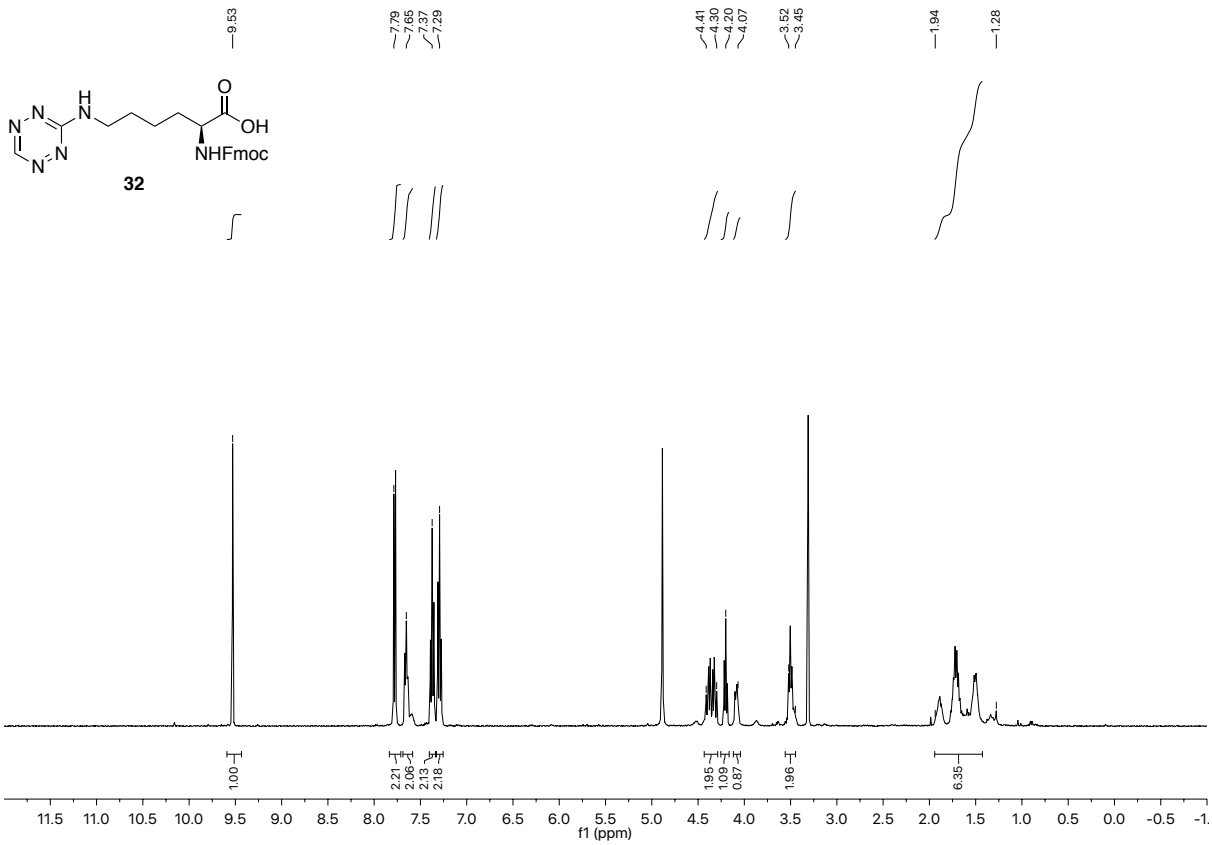

**31**

$^{13}$C-NMR, MeOH-*d4*, 126 MHz

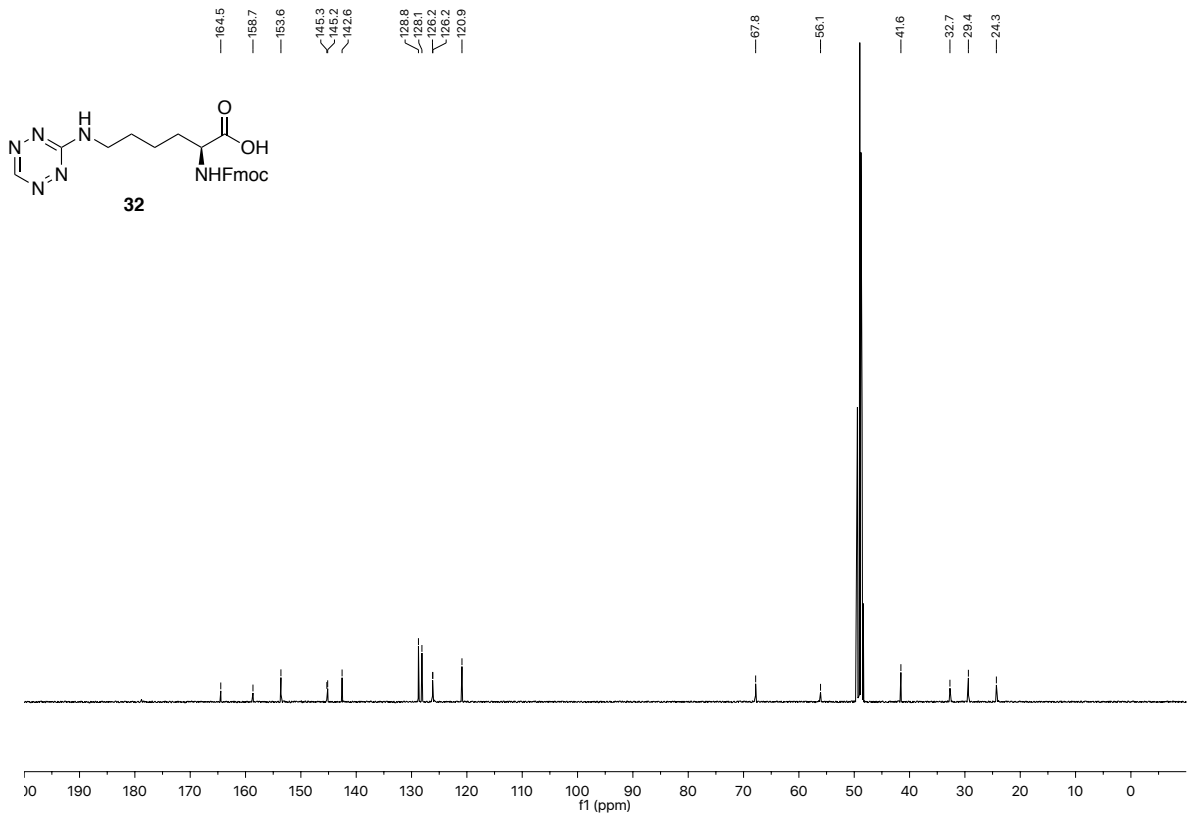



**31**

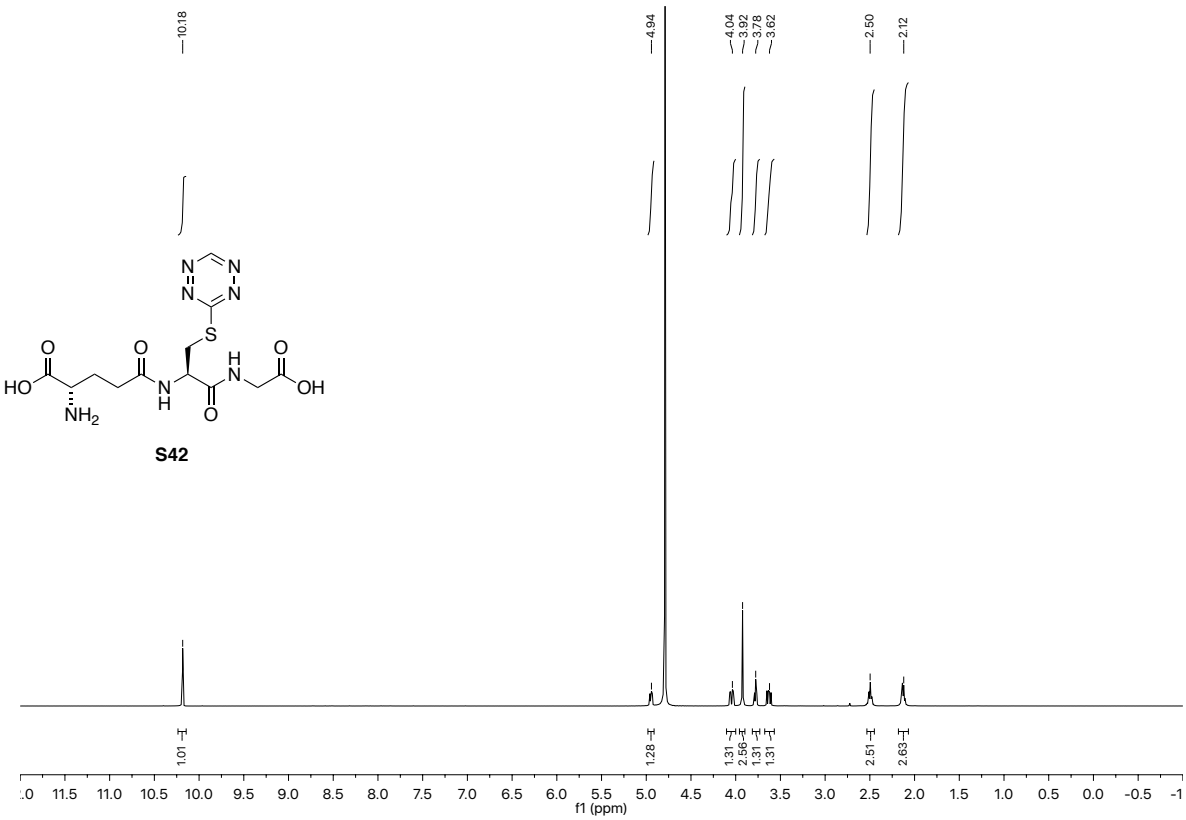
$^{1}$H-NMR, MeOH-*d4*, 400 MHz




$^{13}$C-NMR, MeOH-*d4*, 101 MHz

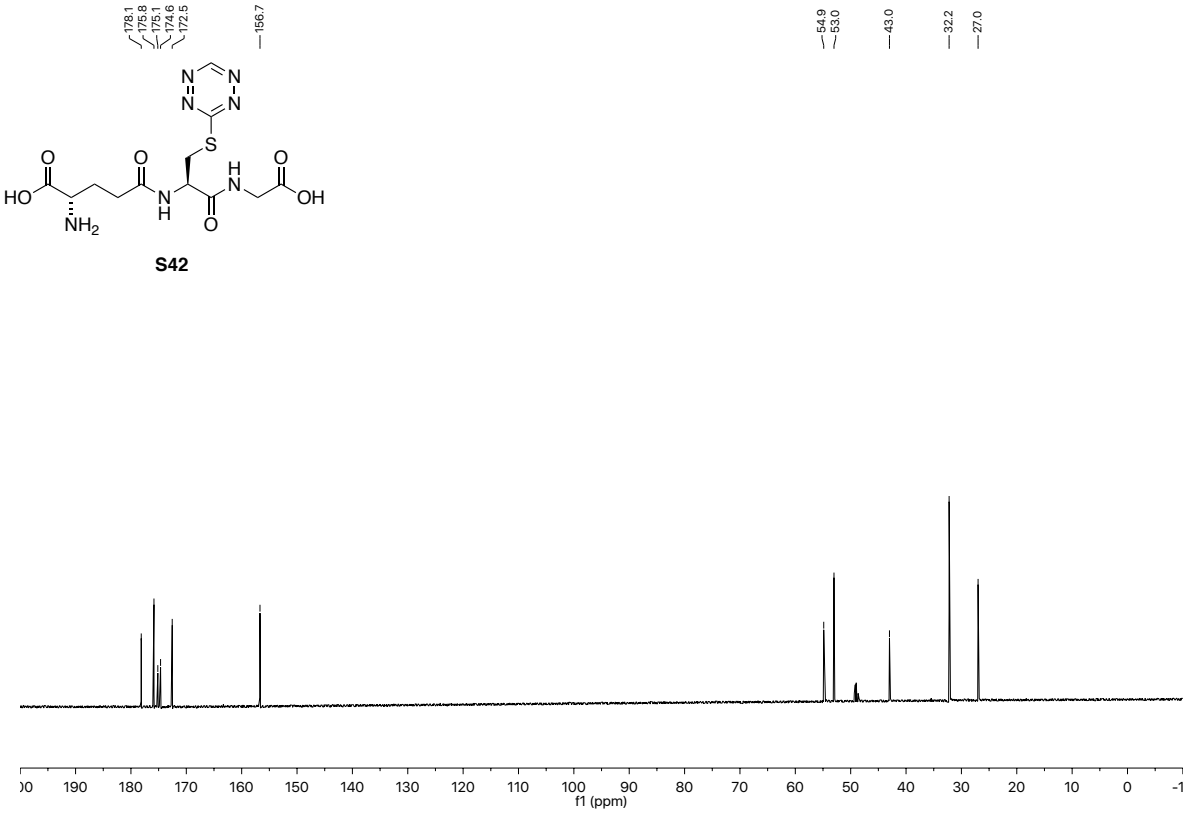


$^{1}$H-NMR, $D_2O$, 400 MHz



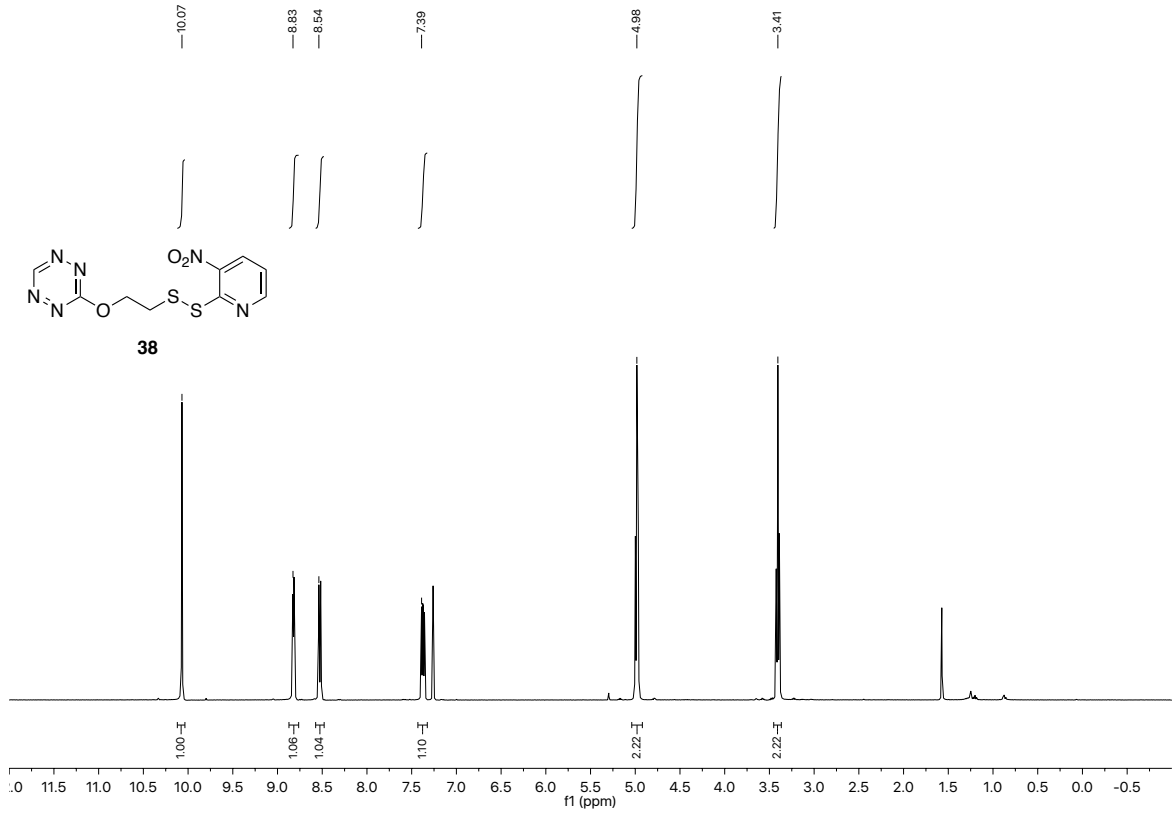

**S42**

$^{13}$C-NMR, $D_2O$, 126 MHz

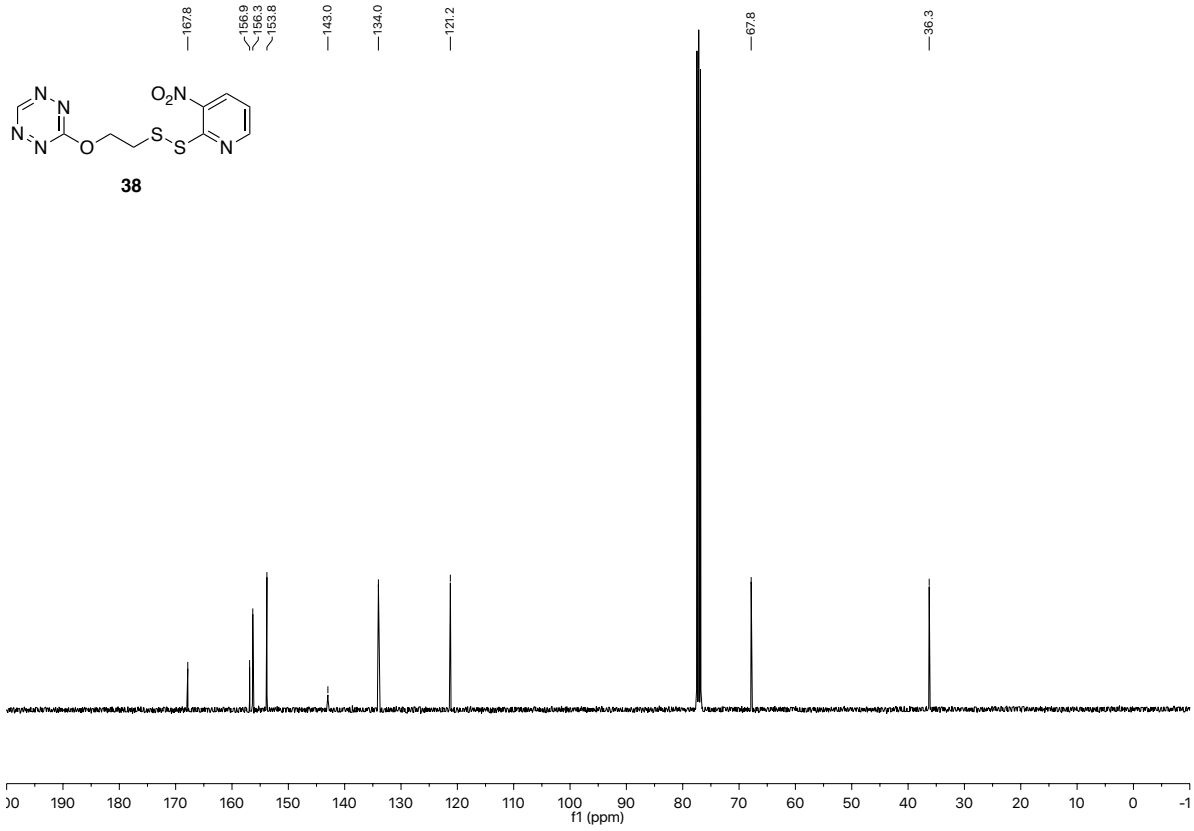



**S42**

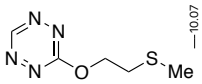
$^{1}$H-NMR, $CDCl_3$, 400 MHz




$^{13}$C-NMR, $CDCl_3$, 101 MHz



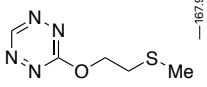
$^{1}$H-NMR, $CDCl_3$, 400 MHz



**S41**

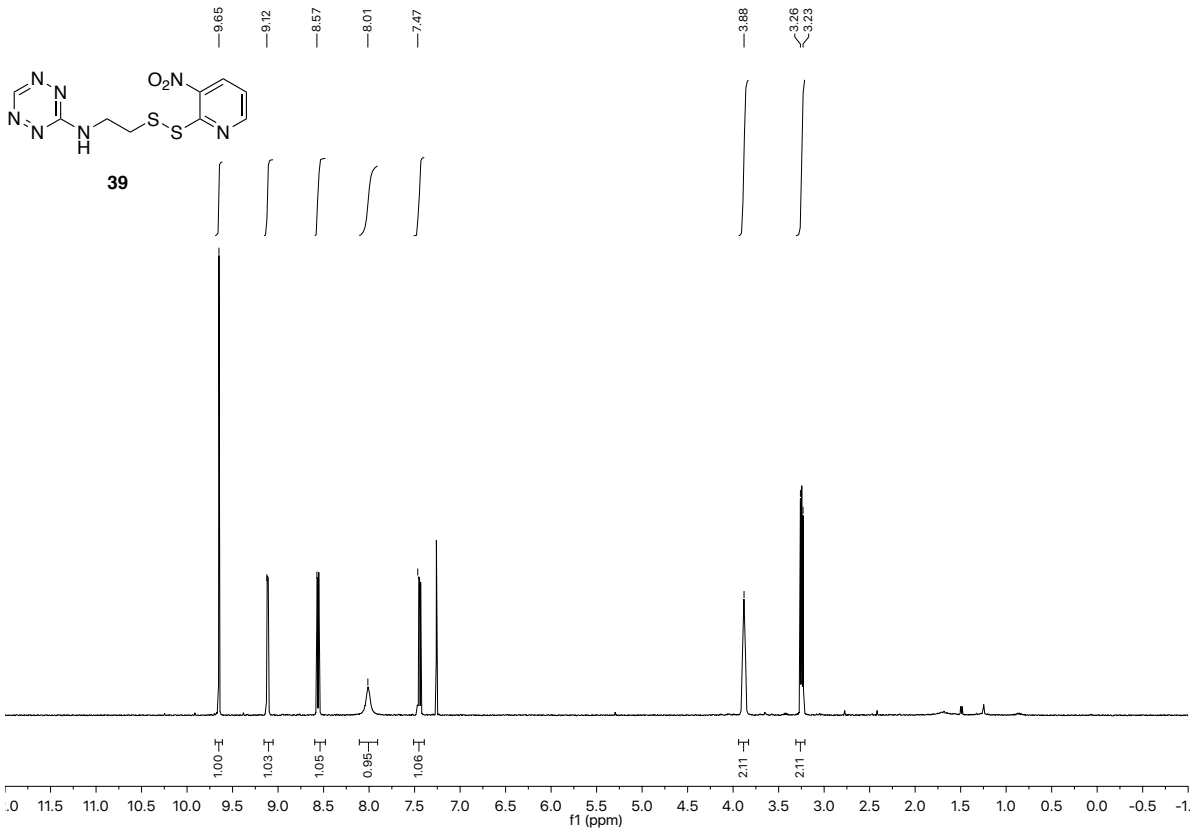



$^{13}$C-NMR, $CDCl_3$, 101 MHz



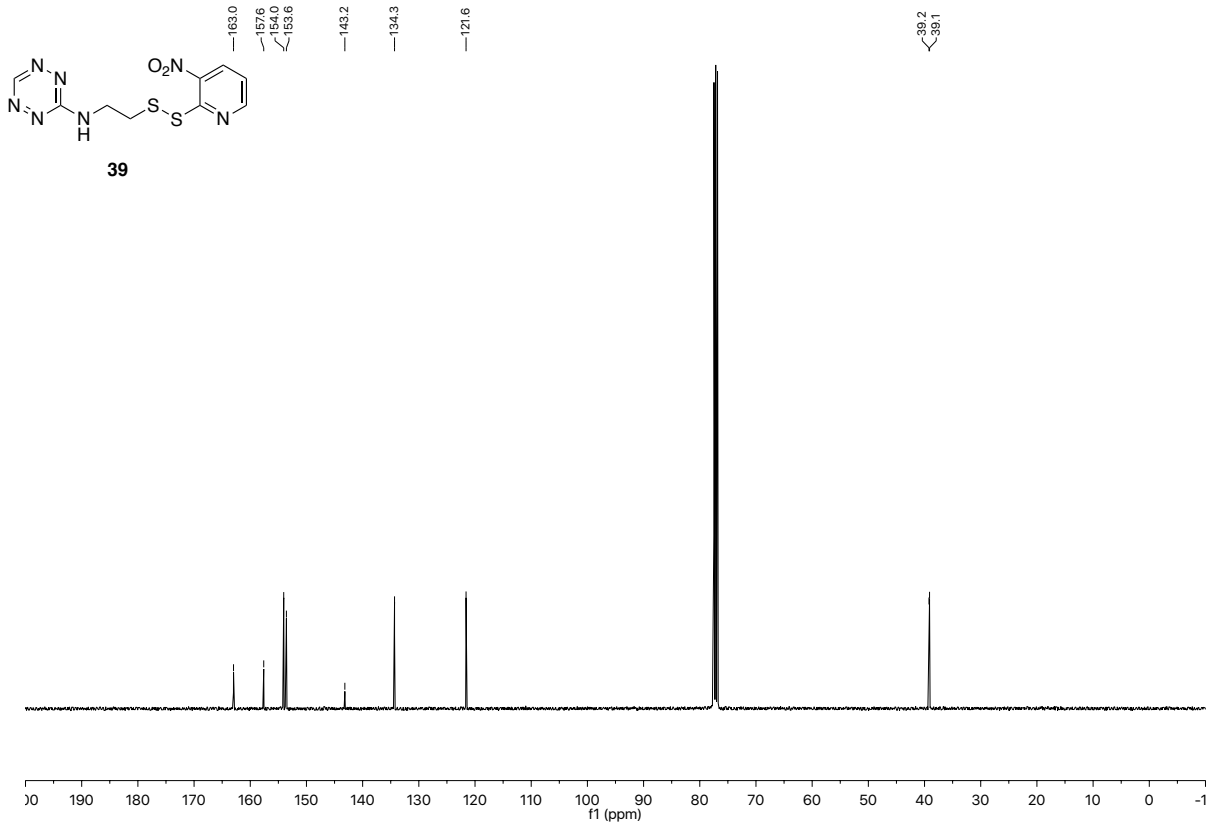
**S41**

$^{1}$H-NMR, $CDCl_3$, 400 MHz




**39**

$^{13}$C-NMR, $CDCl_3$, 101 MHz




**39**

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6 Impact: Insensitive > 40 J, less sensitive ≥ 35 J, sensitive ≥ 4 J, very sensitive ≥ 3 J; friction: Insensitive > 360 N, less sensitive = 360 N, sensitive < 360 N a. > 80 N, very sensitive ≤ 80 N, extreme sensitive ≤ 10 N; according to the UN recommendations on the transport of dangerous goods. (+) Indicates: not safe for transport.
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