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

Simon D. Schnell, Lukas V. Hoff, Advaita Panchagnula, Maximilian H. H. Wurzenberger, Thomas M. Klapötke, Simon Sieber, Anthony Linden, Karl Gademann

a Department of Chemistry, University of Zurich, Winterthurer Strasse 190, 8057 Zurich, Switzerland
b Department of Chemistry, Ludwig-Maximilians-University, Butenandtstrasse 5-13, 81377 Munich, Germany
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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 (CDCl$_3$, δ = 7.26; methanol-d$_3$, δ = 3.31; DMSO-d$_6$, δ = 2.50) and solvents residual carbon chemical shifts (CDCl$_3$, δ = 77.16; methanol-d$_4$, 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$_2$O + 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 e.i. 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$_2$O 0.1% HCOOH and B: CH$_3$CN + 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 44891 modified instruction2 using a BAM (Bundesanstalt für Materialforschung) drophammer.3 The friction sensitivity tests were carried out according to STANAG 44874 modified instruction5 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]

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

\[^{13}\text{C-NMR (D}_2\text{O, 101 MHz): } \delta = 159.5 \text{ ppm.}\]

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 Analysis of hydrazinecarbohydrazonhydrazide (S1).

Dihydrotetrazine S2[8]

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

\[^{1}\text{H-NMR (CDCl}_3\text{, 400 MHz): } \delta = 8.05 \text{ (br s, 2H), } 5.97 \text{ (s, 2H), } 2.49 \text{ (s, 6H), } 2.22 \text{ (s, 6H) ppm.}\]

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 CH₂Cl₂ (125 mL) and cooled to 0 °C. A solution of NaNO₂ (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 CH₂Cl₂ (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 (CDCl₃, 400 MHz): δ = 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): δ = 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).
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 CH₂Cl₂ (100 mL) and the solvent was removed under reduced pressure. The obtained solid was filtered through a short silica column eluting with CH₂Cl₂ and the solvent was removed under reduced pressure to afford tetrazine S5 (5.40 g, 30.7 mmol, 47%) as a red solid.

\[ ^1H-NMR \text{ (CDCl}_3\text{, 400 MHz): } \delta = 10.19 \text{ (s, 1H), 6.21 (s, 1H), 2.74 (s, 3H), 2.39 (s, 3H) ppm.} \]

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\[9\]

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.

\[ ^1H-NMR \text{ (DMSO-}d_6\text{, 400 MHz): } \delta = 9.75 \text{ (s, 1H), 9.55 (br s, 1H), 4.59 (br s, 2H) ppm.} \]

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).

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**Figure 2.** DTA Analysis of 3-hyrazinotetrazine (3).
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 CH₂Cl₂ 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 (CH₂Cl₂) to afford 3-bromotetrazine (2) (854 mg, 5.30 mmol, 43%) as a bright orange, crystalline solid.

Rₓ (CH₂Cl₂) = 0.7 (orange spot); melting point = 70 – 72 °C; ¹H-NMR (CDCl₃, 400 MHz): δ = 10.34 (s, 1H) ppm; ¹³C-NMR (CDCl₃, 101 MHz): δ = 164.2, 158.0 ppm; IR (neat): ν = 3081 (w), 1228 (s), 1208 (vs), 882 (vs) cm⁻¹; elemental analysis: calculated for C₂H₄N₄Br: 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 Analysis of 3-bromotetrazine (2).](image)

Synthesis of Amino Acid Precursors

Boc-Trp-OMe (S6)

Tryptophan methyl ester hydrochloride (1.00 g, 3.93 mmol, 1.00 equiv) was dissolved in CH₂Cl₂ (15 mL) and NEt₃ (2.79 mL, 19.7 mmol, 5.00 equiv) was added, followed by Boc₂O (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.

¹H-NMR (CDCl₃, 400 MHz): δ = 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.
The obtained analytical data are consistent with the values reported in the literature.

Boc-Lys(Cbz)-OMe (S7)$^{[1]}$

\[
\text{CbzHN} \xrightarrow{\text{K}_2\text{CO}_3, \text{Mel}} \text{O} \xrightarrow{\text{DMF, 80 °C}} \text{OMe}
\]

Boc-Lys(Cbz)-OH (768 mg, 2.02 mmol, 1.00 equiv) was dissolved in DMF (10.1 mL). K$_2$CO$_3$ (568 mg, 4.24 mmol, 2.10 equiv) was added followed by Mel (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 × 50 mL). The combined organic layers were washed with brine (2 × 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.$^{[1]}$

Boc-Lys-OMe (S8)$^{[2]}$

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.$^{[2]}$

Fmoc-Cys-OfBu (S9)$^{[3]}$

(Fmoc-Cys-OfBu)$_2$ (100 mg, 0.125 mmol, 1.00 equiv) was dissolved in CH$_2$Cl$_2$ (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 then was diluted with CH$_2$Cl$_2$ (50 mL). The layers were separated and the aqueous layer was washed with saturated aqueous sodium bicarbonate solution (2 × 100 mL) and water (2 × 100 mL). The organic layer was dried over sodium sulfate, filtered and concentrated to afford Fmoc-Cys-OfBu (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.$^{[3]}$
Fmoc-Tyr-OtBu (S10)\(^{[14]}\)

\[
\begin{align*}
\text{Fmoc-Tyr-OH (1.00 g, 2.48 mmol, 1.00 equiv) was dissolved in a mixture of CH}_2\text{Cl}_2/\text{THF (4:1 v/v; 10 mL) and cooled to 0 °C. tert-Butyl trichloroacetimidate (1.33 mL, 7.44 mmol, 3.00 equiv) was added dropwise. The mixture was allowed to warm to 25 °C and was then heated to 60 °C. After 16 h, CH}_2\text{Cl}_2 (100 mL) was added and the organic layer was washed with aqueous sodium bicarbonate solution (2.5 wt% in water; 2 x 100 mL). The organic layer was dried over sodium sulfate, filtered and concentrated to afford Fmoc-Tyr-OtBu (S10) (1.11 g, 2.42 mmol, 97%) as a sticky, colorless solid.}
\end{align*}
\]

\[\text{[a]}\text{P}^{[2]} = +12.66 (c = 0.885, \text{CHCl}_3); \text{^1H-NMR (MeOH-d}_4, 400 MHz); \delta = 7.79 (d, J = 7.5 Hz, 2H), 7.61 (d, J = 7.5 Hz, 2H), 7.39 (t, J = 7.5 Hz, 2H), 7.32–7.28 (m, 2H), 7.04 (d, J = 8.4 Hz, 2H), 6.70 (d, J = 8.4 Hz, 2H), 4.35–4.23 (m, 3H), 4.18 (t, J = 7.1 Hz, 1H), 2.98 (dd, J = 13.9, 6.1 Hz, 1H), 2.83 (dd, J = 13.9, 8.8 Hz, 1H), 1.41 (s, 9H) ppm.}
\]

The obtained analytical data are consistent with the values reported in the literature.\(^{[14]}\)

\[
\begin{align*}
\text{H}_2\text{N-Lys(Cbz)-OtBu (S11)}^{[15]} & \quad \text{H}_2\text{N-Lys(Cbz)-OH (4.29 g, 15.3 mmol, 1.00 equiv) was added to tert-butyl acetate (53 mL). Perchloric acid (70% in water; 2 mL) was added and the mixture was stirred for 16 h. Then, the mixture was extracted with water (100 mL) and aqueous HCl (0.5 M; 150 mL). The combined aqueous layers were basified with aqueous K}_2\text{CO}_3 solution (10 wt%) to a pH of 9. The aqueous layer was extracted with CH}_2\text{Cl}_2 (4 x 100 mL) and the combined organic layers were dried over sodium sulfate, filtered and concentrated to afford H}_2\text{N-Lys(Cbz)-OtBu (S11) (3.26 g, 9.69 mmol, 63%) as a colorless oil. The crude product was used in the next step without purification.}
\end{align*}
\]

Fmoc-Lys(Cbz)-OtBu (S12)

\[
\begin{align*}
\text{H}_2\text{N-Lys(Cbz)-OtBu (S11) (4.17 g, 12.4 mmol, 1.00 equiv) was dissolved in DMF (41 mL) and Fmoc-OSu (4.60 g, 13.6 mmol, 1.10 equiv) was added followed by freshly distilled triethylamine (over CaH}_2 (1.74 mL, 1.25 mmol, 1.00 equiv). The mixture was stirred for 1 h, before the mixture was diluted with CH}_2\text{Cl}_2 (100 mL) and water (100 mL). The layers were separated and the organic layer was washed with water (6 x 100 mL). The organic layer was dried over sodium sulfate, filtered and concentrated. The crude product was purified via flash column chromatography (30% EtOAc in pentane) to afford Fmoc-Lys(Cbz)-OtBu (S12) (3.87 g, 6.93 mmol, 56%) as a colorless oil.}
\end{align*}
\]

\[
\begin{align*}
\text{R}_f (40% \text{EtOAc in pentane}) = 0.37 (\text{KMN}_{\text{O}_3, \text{UV); [a]}\text{P}^{[2]} = -10.68 (c = 2.43, \text{MeOH); ^1H-NMR (CDCl}_3, 400 MHz); \delta = 7.77 (d, J = 7.5 Hz, 2H), 7.61 (d, J = 7.5 Hz, 2H), 7.40 (t, J = 7.4 Hz, 2H), 7.33–7.29 (m, 2H), 7.37–7.39 (m, 7H), 5.37 (d, J = 8.3 Hz, 1H), 5.15–5.04 (m, 2H), 4.81 (br s, 1H), 4.40 (d, J = 7.4 Hz, 2H), 4.28–4.20 (m, 2H), 3.23–3.16 (m, 2H), 1.88–1.79 (m, 1H), 1.72–1.62 (m, 1H), 1.59–1.52 (m, 2H), 1.47–1.30 (m, 11H) ppm; ^13C-NMR (CDCl}_3, 101 MHz); \delta = 171.7, 156.6, 156.1, 144.1, 143.9, 141.4, 136.7, 128.6, 128.2, 127.8, 127.2, 125.2, 120.1, 82.3, 67.1, 66.7, 54.2, 47.3, 40.8, 32.6, 29.6, 28.2, 22.3 ppm; IR (neat); \bar{\nu} = 3336 (w), 2929 (w), 1699 (vs), 1520 (s), 1451 (m), 1245 (vs), 1153 (vs), 738 (vs) cm}^{-1}; \text{HRMS (ESI) for C}_{32}H_{39}N_{10}O_{6} [M+H]^+: \text{calculated: 559.2803; found: 559.2805.}
\end{align*}
\]
Fmoc-Lys-OrBu (S13)

Fmoc-Lys(Cbz)-OrBu (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$_2$Cl$_2$ (50 mL) and the solvent was removed via a stream of nitrogen to afford Fmoc-Lys-OrBu (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.

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</tbody>
</table>

<sup>a</sup> collidine = 2,4,6-trimethylpyridine; <sup>b</sup> reverse addition of 3-bromotetrazine (2).

General Procedure for the Functionalization of Hydroxy Groups (GP1)

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)

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% CH₂Cl₂ in pentane) to afford tetrazine 4 (18.0 mg, 103 µmol, 66%) as a red solid.

R<sub>f</sub> (50% CH₂Cl₂ in pentane) = 0.26 (pink spot, UV); melting point = 111 – 113 °C; <sup>1</sup>H-NMR (CDCl₃, 400 MHz): δ = 10.16 (s, 1H), 7.53–7.48 (m, 2H), 7.38–7.34 (m, 1H), 7.31–7.28 (m, 2H) ppm; <sup>13</sup>C-NMR (CDCl₃, 101 MHz): δ = 169.1, 156.8, 151.8, 130.4, 127.0, 121.1 ppm; IR (neat): ν = 1589 (w), 1493 (m), 1443 (vs), 1364 (vs), 1202 (m), 1117 (m), 767 (s) cm⁻¹; HRMS (EI) for C₈H₆N₄O⁺: calculated: 174.0542; found: 174.0533.
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$_2$Cl$_2$ in pentane) to afford tetrazine 5 (23.4 mg, 115 µmol, 74%) as a red solid. 

R$_f$ (40% Et$_2$O 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): $\nu$ = 1590 (w), 1620 (w), 1432 (vs), 1357 (vs), 1147 (s), 1109 (s), 1039 (s) cm$^{-1}$; HRMS (ESI) for C$_9$H$_8$N$_4$O$_2$Na$^+$ [M+Na]$^+$: calculated: 227.0539; found: 227.0539.

3-(naphthalen-1-yloxy)-s-tetrazine (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$_2$O in pentane) to afford tetrazine S14 (25.0 mg, 111 µmol, 72%) as a pink solid. 

R$_f$ (40% Et$_2$O 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): $\nu$ = 1600 (w), 1430 (s), 1354 (vs), 1227 (w), 1070 (m), 771 (s) cm$^{-1}$; HRMS (EI) for C$_{12}$H$_8$N$_4$O$^+$: calculated: 224.0698; found: 224.0693.

3-(2-Chlorophenoxy)-s-tetrazine (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$_2$O in pentane) to afford tetrazine 6 (27.5 mg, 132 µmol, 85%) as a red solid.

R$_f$ (10% Et$_2$O 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): $\nu$ = 1475 (m), 1431 (vs), 1352 (vs), 1218 (m), 1120 (m), 1060 (s), 931 (m), 762 cm$^{-1}$ (s); HRMS (EI) for C$_8$H$_5$N$_4$OCl$^+$: calculated: 208.0152; found: 208.0142.
3-(2-Bromophenoxy)-s-tetrazine (S15)

![Structure of 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<sub>2</sub>O in pentane) to afford tetrazine S15 (28.0 mg, 111 µmol, 72%) as a red solid.

R<sub>f</sub> (20% Et<sub>2</sub>O in pentane) = 0.25 (pink spot, UV); melting point = 73 – 74 °C; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz): δ = 10.19 (s, 1H), 7.72 (dd, J = 8.0, 1.5 Hz, 1H), 7.46 (dd, J = 8.1, 1.6 Hz, 1H), 7.35 (dd, J = 8.1, 1.6 Hz, 1H), 7.29 – 7.25 (m, 1H) ppm; <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 101 MHz): δ = 168.4, 157.1, 149.1, 134.2, 129.4, 128.5, 123.3, 115.9 ppm; IR (neat): ν = 1470 (w), 1431 (vs), 1353 (vs), 1215 (m), 1046 (w), 932 (m) cm<sup>-1</sup>; HRMS (EI) for C<sub>8</sub>H<sub>5</sub>N<sub>4</sub>OBr+: calculated: 251.9647; found: 251.9641.

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

![Structure of 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<sub>2</sub>Cl<sub>2</sub> in pentane) to afford tetrazine S16 (9.4 mg, 46.0 µmol, 30%) as a pink solid.

R<sub>f</sub> (70% CH<sub>2</sub>Cl<sub>2</sub> in pentane) = 0.29 (pink spot, UV); melting point = 99 – 100 °C; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz): δ = 10.23 (s, 1H), 10.06 (s, 1H), 8.07 – 8.03 (m, 2H), 7.50 – 7.47 (m, 2H) ppm; <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 101 MHz): δ = 190.7, 168.7, 157.2, 156.1, 134.9, 132.0, 121.9 ppm; IR (neat): ν = 1696 (vs), 1600 (w), 1455 (m), 1438 (s), 1355 (vs), 1217 (m), 907 (s), 731 (s) cm<sup>-1</sup>; HRMS (EI) for C<sub>9</sub>H<sub>6</sub>N<sub>4</sub>O<sub>2</sub>: calculated: 202.0491; found: 202.0484.

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

![Structure of 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<sub>2</sub>Cl<sub>2</sub> in pentane) to afford tetrazine S17 (20.0 mg, 81.0 µmol, 52%) as a pink solid.

R<sub>f</sub> (100% CH<sub>2</sub>Cl<sub>2</sub>) = 0.27 (pink spot, UV); melting point = 89 – 91 °C; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz): δ = 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; <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 126 MHz): δ = 168.8, 165.6, 157.1, 155.1, 132.0, 129.3, 121.1, 61.5, 14.5 ppm; IR (neat): ν = 1714 (s), 1604 (m), 1437 (s), 1356 (vs), 1275 (vs), 1208 (m), 1165 (w), 1115 (s), 932 (m) cm<sup>-1</sup>; HRMS (ESI) for C<sub>11</sub>H<sub>10</sub>N<sub>4</sub>O<sub>3</sub>Na<sup>+</sup>[M+Na<sup>+</sup>]: calculated: 269.0645; found: 269.0642.
3-(2,6-Dimethylphenoxy)-s-tetrazine (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% CH₂Cl₂ in pentane) to afford tetrazine S18 (12.4 mg, 61.0 µmol, 39%) as a red solid.

Rf (50% CH₂Cl₂ in pentane) = 0.33 (pink spot, UV); melting point = 62 – 64 °C; ¹H-NMR (CDCl₃, 400 MHz): δ = 10.14 (s, 1H), 7.16 (m, 3H), 2.16 (s, 6H) ppm; ¹³C-NMR (CDCl₃, 101 MHz): δ = 168.2, 156.9, 149.1, 130.0, 129.4, 127.0, 16.5 ppm; IR (neat): v = 1434 (vs), 1356 (vs), 1170 (m), 1088 (m), 928 (m) cm⁻¹; HRMS (EI) for C₁₀H₁₀N₄O⁺: calculated: 202.0855; found: 202.0849.

7-((S-tetrazin-3-yl)oxy)-4-methyl-2H-chromen-2-one (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% CH₂Cl₂) to afford tetrazine S19 (9.5 mg, 37.0 µmol, 24%) as a pink solid.

Rf (100% CH₂Cl₂) = 0.1 (pink spot, UV); melting point = 188 – 189 °C; ¹H-NMR (CDCl₃, 400 MHz): δ = 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); ¹³C-NMR (CDCl₃, 126 MHz): δ = 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): v = 3087 (m), 1737 (s), 1618 (s), 1435 (vs), 1357 (vs), 1260 (m), 1112 (m), 845 (m) cm⁻¹; HRMS (ESI) for C₁₂H₈N₄O₃Na⁺ [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)

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% Et₂O in pentane) to afford tetrazine 7 (24.5 mg, 82.0 µmol, 53%) as a pink solid.

Rf (30% Et₂O in pentane) = 0.39 (pink spot, UV); melting point = 131 – 133 °C; ¹H-NMR (CDCl₃, 400 MHz): δ = 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; ¹³C-NMR (CDCl₃, 126 MHz): δ = 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): v = 1603 (m), 1437 (s), 1354 (vs), 1202 (w), 1088 (m), 1019 (w), 857 (w) cm⁻¹; HRMS (EI) for C₁₄H₁₇N₄O₃B⁺: calculated: 300.1394; found: 300.1382.
3-(Benzyloxy)-s-tetrazine (S20)

\[
\begin{array}{c}
\text{N} \quad \text{N} \\
\text{O} \quad \text{N} \\
\text{S20} \\
\end{array}
\]

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% Et₂O in pentane) to afford tetrazine S20 (9.0 mg, 48.0 µmol, 31%) as a solid.

\( R_f (30\%\text{ Et}_2\text{O in pentane}) = 0.55 \text{ (pink spot); melting point } = 84 – 85 ^\circ\text{C} \) ¹H-NMR (CDCl₃, 400 MHz): \( \delta = 10.06 \text{ (s, 1H), 7.56–7.55 \text{ (m, 2H), 7.44–7.36 \text{ (m, 3H), 5.72 \text{ (s, 2H) ppm;}} ¹³\text{C-NMR (CDCl₃, 101 MHz): } \delta = 167.9, 156.2, 134.3, 129.2, 128.9, 128.9, 71.3 \text{ ppm; IR (neat): } \nu = 1500 \text{ (w), 1470 \text{ (vs), 1452 \text{ (vs), 1353 \text{ (vs), 1346 \text{ (vs), 1193 \text{ (m), 970 \text{ (vs), 936 \text{ (s), 764 \text{ (vs) cm}^{-1}; HRMS (EI) for } C_{10}H_{15}N_{2}O_{2}^{+}[M+Na]^+: calculated: 211.0590; found: 211.0590.} } \}

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

\[
\begin{array}{c}
\text{N} \quad \text{N} \\
\text{O} \\
\text{S20} \\
\end{array}
\]

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% Et₂O in pentane) to afford tetrazine 8 (8.0 mg, 59.0 µmol, 38%) as a solid.

\( R_f (30\%\text{ Et}_2\text{O in pentane}) = 0.35 \text{ (pink spot); } ¹H\text{-NMR (CDCl}_3, 400 \text{ MHz): } \delta = 10.13 \text{ (s, 1H), 5.31 \text{ (s, 2H), 2.61 \text{ (s, 1H) ppm; } ¹³\text{C-NMR (CDCl}_3, 101 \text{ MHz): } \delta = 167.3, 156.7, 77.1, 76.2, 75.7 \text{ ppm; IR (neat): } \nu = 3284 \text{ (m), 1469 \text{ (vs), 1335 \text{ (vs), 996 \text{ (s), 939 \text{ (s) cm}^{-1; HRMS (EI) for } C_{10}H_{15}N_{2}O_{2}^{+}: calculated: 136.0385; found: 136.0377.} } \}

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

\[
\begin{array}{c}
\text{O} \quad \text{N} \\
\text{O} \quad \text{N} \\
\text{S20} \\
\end{array}
\]

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% Et₂O in pentane) to afford tetrazine S21 (7.0 mg, 39.0 µmol, 25%) as a solid.

\( R_f (20\%\text{ Et}_2\text{O in pentane}) = 0.39 \text{ (pink spot); } ¹H\text{-NMR (CDCl}_3, 400 \text{ MHz): } \delta = 10.08 \text{ (s, 1H), 7.48 \text{ (dd, } J = 1.9, 0.8 \text{ Hz, 1H), 6.63 \text{ (d, } J = 0.7 \text{ Hz, 1H), 6.41 \text{ (dd, } J = 3.3, 1.9 \text{ Hz, 1H), 5.69 \text{ (s, 2H) ppm; } ¹³\text{C-NMR (CDCl}_3, 126 \text{ MHz): } \delta = 167.7, 156.9, 147.7, 144.9, 112.7, 110.9, 62.9 \text{ ppm; IR (neat): } \nu = 1468 \text{ (vs), 1337 \text{ (vs), 1192 \text{ (m), 919 \text{ (m), 752 \text{ (m) cm}^{-1; HRMS (EI) for } C_{10}H_{15}N_{2}O_{2}^{+}: calculated: 172.0491; found: 172.0481.} } \}

Boc-Tyr(Tet)-OMe (23)

\[
\begin{array}{c}
\text{O} \quad \text{N} \\
\text{O} \\
\text{S20} \\
\end{array}
\]

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% Et₂O in pentane) to afford tetrazine 23 (42.0 mg, 112 µmol, 72%) as a pink solid.

\( R_f (10\%\text{ Et}_2\text{O in pentane}) = 0.47 \text{ (pink spot, UV); melting point } = 120 ^\circ\text{C; } [\alpha]_D^{20} = +46.18 \text{ (c = 0.775, CHCl}_3; ¹H\text{-NMR (CDCl}_3, 400 \text{ MHz): } \delta = 10.18 \text{ (s, 1H), 7.31–7.24 \text{ (m, 4H), 5.06 \text{ (d, } J = 8.3 \text{ Hz, 1H), 4.65 \text{ (q, } J = 6.8 \text{ Hz, 1H), 3.77 \text{ (s, 3H), 3.21 \text{ (dd, } J = 13.9, 5.8 \text{ Hz, 1H), 3.13 \text{ (dd, } J = 13.9, 6.4 \text{ Hz, 1H), 1.46 \text{ (s, 9H) ppm; } ¹³\text{C-NMR (CDCl}_3, 101 \text{ MHz): } \delta = 172.2, } \}

S16
The solvent was removed via a stream of nitrogen. The crude product was purified via flash column chromatography (5% trimethylpyridine (4.13 mL, 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% EtOAc in CH2Cl2) to afford tetrazine 24 (37 mg, 77.5 µmol, 45%) as a red oil.

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

**Di-Tet-Fidaxomicin (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) was added. 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 CH2Cl2) to afford di-tet-fidaxomicin (33) (11.3 mg, 9.00 µmol, 58%) as a pink oil.
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 CH₂Cl₂) to afford tetrazine 34 (38.0 mg, 80.0 µmol, 52%) as a pink oil.

R₂ (5% MeOH in CH₂Cl₂) = 0.42 (pink spot, UV); [α]₂⁰D = +154 (c = 1.245, MeOH); ¹H-NMR (CDCl₃, 400 MHz): δ = 10.44 (s, 1H), 10.40 (s, 1H), 7.22 (d, J = 11.4 Hz, 1H), 6.80–6.53 (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; ¹³C-NMR (CDCl₃, 126 MHz): δ = 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, 129.5, 128.9, 128.5, 128.3, 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): ν = 3474 (br m), 2976 (m), 2934 (m), 1737 (vs), 1761 (s), 1737 (w), 1724 (s), 1669 (vs), 1665 (s), 1645 (s), 1575 (s), 1534 (s), 1473 (s), 1451 (s), 1339 (vs), 1072 (s), 1032 (m), 928 (w), 795 (w) cm⁻¹; HRMS (ESI) for C₃₀H₂₆O₃N₆F₂⁺ [M+Na⁺]: 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)

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% EtO in pentane) to afford tetrazine S22 (10.0 mg, 53.0 µmol, 34%) as a red oil.

Boc-Cys(Tet)-OMe (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% EtO in pentane) to afford tetrazine 25 (45.0 mg, 143 µmol, 92%) as a red oil.

Fmoc-Cys(Tet)-OBu (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% EtO in pentane) to afford tetrazine 26 (61.0 mg, 127 µmol, 82%) as a red oil.
1051 (m), 889 (w), 759 (m), 738 (vs) cm⁻¹; HRMS (ESI) for C₂₄H₂₅N₅O₄SNa⁺ [M+Na⁺]: calculated: 502.1519; found: 538.0889.

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

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<th>Base</th>
<th>Equiv base</th>
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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 CH₂Cl₂ (50 mL). The solution was concentrated and the resulting crude product was purified via flash column chromatography.

1-(S-tetrazin-3-yl)-1H-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 CH₂Cl₂ (50 mL). The solvent was removed under reduced pressure and the crude product was purified via flash column chromatography on silica gel (4% EIOAc in pentane) to afford tetrazine 9 (16.7 mg, 85.0 µmol, 55%) as a red solid.

Rf (4% EIOAc in pentane) = 0.32 (red spot, UV, CAM); melting point = 103 °C; ¹H-NMR (CDCl₃, 400 MHz): δ = 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; ¹³C-NMR (CDCl₃, 126 MHz): δ = 161.1, 156.2, 135.1, 131.7, 125.2,
124.3, 124.1, 121.6, 116.8, 111.0 ppm; IR (neat): ν = 1500 (vs), 1484 (vs), 1355 (w), 1213 (m), 1092 (m), 749 (m) cm⁻¹; HRMS (EI) for C₁₀H₈N₃Cl⁺: calculated: 197.0701; found: 197.0696.

5-Chloro-1-(s-tetrazin-3-yl)-1H-indole (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 CH₂Cl₂ (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.

Rf (4% ETOAc in pentane) = 0.20 (red spot, UV, CAM); melting point = 157 – 158 °C; ¹H-NMR (CDCl₃, 400 MHz): δ = 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; ¹³C-NMR (CDCl₃, 126 MHz): δ = 160.9, 156.4, 133.3, 132.9, 129.8, 125.6, 125.3, 121.2, 117.7, 110.2 ppm; IR (neat): ν = 1495 (vs), 1478 (vs), 1335 (w), 1235 (w), 1205 (m), 963 (w), 931 (w) cm⁻¹; HRMS (EI) for C₁₀H₈N₃Cl⁺: calculated: 231.0312; found: 231.0311.

5-Iodo-1-(s-tetrazin-3-yl)-1H-indole (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 CH₂Cl₂ (50 mL). The solvent was removed under reduced pressure and the crude product was purified via flash column chromatography (20% Et₂O in pentane) to afford tetrazine S23 (13.6 mg, 42.0 µmol, 27%) as an orange solid.

Rf (20% Et₂O in pentane) = 0.43 (red spot, UV, CAM); melting point = 178 °C; ¹H-NMR (CDCl₃, 400 MHz): δ = 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; ¹³C-NMR (CDCl₃, 101 MHz): δ = 160.8, 156.2, 134.1, 134.8, 133.6, 130.3, 125.0, 118.4, 109.7, 88.2 ppm; IR (neat): ν = 1494 (vs), 1477 (vs), 1329 (m), 1235 (w), 1205 (m), 963 (w), 930 (w), 808 (m) cm⁻¹; HRMS (EI) for C₁₀H₈N₃I⁺: calculated: 322.9668; found: 322.9663.

1-(s-Tetrazin-3-yl)-1H-indole-6-carbonitrile (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 CH₂Cl₂ (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.

Rf (20% ETOAc in pentane) = 0.30 (red spot, UV, CAM); melting point = 202 – 203 °C; ¹H-NMR (CDCl₃, 400 MHz): δ = 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; ¹³C-NMR (CDCl₃, 126 MHz): δ = 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): ν = 2224 (m), 1524 (w), 1475 (vs), 1466 (vs), 1360 (s), 1261 (m), 1213 (m), 1080 (m), 930 (m) cm⁻¹; HRMS (EI) for C₁₁H₈N₅Cl⁺: calculated: 222.0654; found: 222.0653.
5-Methoxy-1-(s-tetrazin-3-yl)-1H-indole (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 CH2Cl2 (50 mL). The solvent was removed under reduced pressure and the crude product was purified via flash column chromatography (40% CH2Cl2 in pentane) to afford tetrazine S24 (17.0 mg, 75.0 µmol, 48%) as a red solid.

Rf (40% CH2Cl2 in pentane) = 0.50 (red spot, UV, CAM); melting point = 143 °C; 1H-NMR (CDCl3, 400 MHz): δ = 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; 13C-NMR (CDCl3, 101 MHz): δ = 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): v = 1589 (m), 1468 (vs), 1358 (s), 1287 (s), 1265 (s), 1206 (s), 1100 (s), 980 (m), 746 (vs) cm⁻¹; HRMS (EI) for C11H10N2O: calculated: 227.0807; found: 227.0800.

2-Methyl-1-(s-tetrazin-3-yl)-1H-indole (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 CH2Cl2 (50 mL). The solvent was removed under reduced pressure and the crude product was purified via flash column chromatography (30 – 50% CH2Cl2 in pentane) to afford tetrazine S25 (8.4 mg, 40.0 µmol, 26%) as a red oil.

Rf (40% CH2Cl2 in pentane) = 0.20 (red spot, UV, CAM); 1H-NMR (CDCl3, 400 MHz): δ = 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; 13C-NMR (CDCl3, 126 MHz): δ = 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): v = 1601 (w), 1572 (w), 1457 (m), 1403 (w), 1214 (m), 1129 (m), 932 (w) cm⁻¹; HRMS (EI) for C13H11N3: calculated: 211.0850; found: 211.0856.

2-Phenyl-1-(s-tetrazin-3-yl)-1H-indole (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 CH2Cl2 (50 mL). The solvent was removed under reduced pressure and the crude product was purified via flash column chromatography (40% CH2Cl2 in pentane) to afford tetrazine S26 (8.4 mg, 31.0 µmol, 20%) as a red oil.

Rf (30% CH2Cl2 in pentane) = 0.09 (red spot, UV, CAM); 1H-NMR (CDCl3, 400 MHz): δ = 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; 13C-NMR (CDCl3, 126 MHz): δ = 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): v = 1454 (vs), 1446 (vs), 1394 (m), 1345 (s), 1220 (m), 1173 (m), 913 (m), 745 (s) cm⁻¹; HRMS (EI) for C18H17N5: calculated: 273.1014; found: 273.1014.
1-(S-tetrazin-3-yl)-1H-indole-5-carbaldehyde (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 CH₂Cl₂ (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.

Rf (30% EtOAc in pentane) = 0.44 (red spot, UV, CAM); melting point = 202 – 203 °C; ¹³C-NMR (CDCl₃, 400 MHz): δ = 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; ¹³C-NMR (CDCl₃, 126 MHz): δ = 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): ν = 1691 (vs), 1609 (w), 1478 (vs), 1335 (m), 1218 (w), 1077 (w), 918 (w) cm⁻¹; HRMS (EI) for C₁₇H₁₆N₃O: calculated: 225.0651; found: 225.0644.

7-Methyl-1-(S-tetrazin-3-yl)-1H-indole (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 CH₂Cl₂ (50 mL). The solvent was removed under reduced pressure and the crude product was purified via flash column chromatography (40% CH₂Cl₂ in pentane) to afford tetrazine S28 (9.0 mg, 43.0 µmol, 28%) as a red oil.

Rf (40% CH₂Cl₂ in pentane) = 0.26 (red spot, UV, CAM); ¹³C-NMR (CDCl₃, 400 MHz): δ = 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; ¹³C-NMR (CDCl₃, 126 MHz): δ = 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): ν = 1457 (vs), 1352 (m), 1324 (w), 1071 (w), 786 (m) cm⁻¹; HRMS (EI) for C₁₉H₁₄N₃O⁺: calculated: 211.0858; found: 211.0850.

Boc-Trp(Tet)-OMe (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 CH₂Cl₂ (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.

Rf (20% EtOAc in pentane) = 0.18 (red spot, UV, CAM); melting point = 123 – 124 °C; [α]D²⁰ = +72.30 (c = 0.47, CHCl₃); ¹³C-NMR (CDCl₃, 400 MHz): δ = 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; ¹³C-NMR (CDCl₃, 126 MHz): δ = 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): ν = 3355 (br m), 2879 (w), 1734 (m), 1685 (s), 1492 (s), 1470 (vs), 1366 (m), 1250 (m), 1163 (s), 1100 (s), 746 (s) cm⁻¹; HRMS (ESI) for C₁₉H₂₀N₄O⁺[M+Na]+: calculated: 421.1595; found: 421.1591.
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 CH₂Cl₂ (50 mL). The solvent was removed under reduced pressure and the crude product was purified via flash column chromatography (30% CH₂Cl₂ in pentane) to afford tetrazine 12 (32.0 mg, 129 µmol, 83%) as a red solid.

\[ R_f (30\% \text{Et}_2\text{O in pentane}) = 0.56 \ (\text{red spot, UV, CAM}); \text{melting point} = 179 \degree \text{C}; \] ¹H-NMR (CDCl₃, 400 MHz): \[ \delta = 10.12 \ (s, 1H), 8.85 \ (d, J = 8.5 \text{ Hz}, 2H), 8.08 \ (dd, J = 7.6, 1.4 \text{ Hz}, 2H), 7.54 \ (dd, J = 8.5, 7.3 \text{ Hz}, 2H), 7.45 \ (td, J = 7.5, 1.0 \text{ Hz}, 2H) \text{ ppm}; \] ¹³C-NMR (CDCl₃, 126 MHz): \[ \delta = 163.0, 155.3, 138.1, 127.6, 127.1, 124.4, 120.0, 117.3 \text{ ppm}; \] IR (neat): \[ \nu = 1495 \ (s), 1460 \ (vs), 1337 \ (m), 1212 \ (m), 750 \ (s) \text{ cm}^{-1}; \] HRMS (EI) for C₁₄H₉N₅⁺: calculated: 247.0858; found: 247.0850.

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 CH₂Cl₂ (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.

\[ R_f (40\% \text{EtOAc in pentane}) = 0.40 \ (\text{red spot, UV, CAM}); \text{melting point} = 166 - 167 \degree \text{C}; \] ¹H-NMR (CDCl₃, 400 MHz): \[ \delta = 10.28 \ (s, 1H), 9.19 \ (s, 1H), 8.55-8.53 \ (m, 1H), 7.91-7.89 \ (m, 1H), 7.52-7.44 \ (m, 2H) \text{ ppm}; \] ¹³C-NMR (CDCl₃, 101 MHz): \[ \delta = 160.4, 157.7, 145.1, 140.2, 130.9, 126.1, 125.5, 121.3, 115.8 \text{ ppm}; \] IR (neat): \[ \nu = 1608 \ (w), 1470 \ (vs), 1296 \ (s), 1246 \ (m), 1207 \ (s), 1097 \ (m), 737 \ (m) \text{ cm}^{-1}; \] HRMS (EI) for C₉H₆N₆⁺: calculated: 198.0654; found: 198.0645.

Pyrrrole 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 CH₂Cl₂ (50 mL). The solvent was removed under reduced pressure and the crude product was purified via flash column chromatography (5% EtOAc in pentane) to afford tetrazine S30 (2.0 mg, 14.0 µmol, 9%) as an orange solid.

\[ R_f (5\% \text{EtOAc in pentane}) = 0.31 \ (\text{orange spot}); \text{melting point} = 113 \degree \text{C}; \] ¹H-NMR (CDCl₃, 400 MHz): \[ \delta = 10.10 \ (s, 1H), 7.92-7.90 \ (m, 2H), 6.52-6.51 \ (m, 2H) \text{ ppm}; \] ¹³C-NMR (CDCl₃, 126 MHz): \[ \delta = 159.6, 157.3, 119.1, 115.0 \text{ ppm}; \] IR (neat): \[ \nu = 3147 \ (vs), 1519 \ (vs), 1378 \ (m), 1257 \ (w), 1070 \ (m), 932 \ (s), 744 \ (vs) \text{ cm}^{-1}; \] HRMS (EI) for C₆H₅N₅⁺: calculated: 147.0545; found: 147.0537.
3-{2-methyl-1H-imidazol-1-yl}-s-tetrazine (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 CH₂Cl₂ (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.

IR (neat): \( \nu = 1553 \text{ (w)}, 1509 \text{ (w)}, 1458 \text{ (vs)}, 1285 \text{ (s)}, 1137 \text{ (m)}, 981 \text{ (w)}, 926 \text{ (w)} \) cm⁻¹; HRMS (EI) for C₆H₆N₆+: calculated: 162.0654; found: 162.0651.

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 CH₂Cl₂ (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.

\[ \text{IR (neat): } \sigma = 2977 \text{ (w)}, 1743 \text{ (m)}, 1708 \text{ (vs)}, 1479 \text{ (vs)}, 1366 \text{ (m)}, 1308 \text{ (m)}, 1164 \text{ (s)}, 1061 \text{ (m)}, 919 \text{ (w)} \text{ cm}^{-1}; \] HRMS (ESI) for C₁₄H₂₀N₇O₄+: calculated: 350.1571; found: 350.1576.

General Procedure for the Functionalization of Amines (GP4)

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.

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 EIOAc (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.

\[ \text{IR (neat): } \sigma = 10.83 \text{ (br s, 1H)}, 9.72 \text{ (s, 1H)}, 8.70 \text{ (t, } J = 5.9 \text{ Hz, 1H)}, 7.57 \text{ (d, } J = 7.9 \text{ Hz, 1H)}, 7.35 \text{ (d, } J = 8.1 \text{ Hz, 1H)}, 7.21 \text{ (d, } J = 2.3 \text{ Hz, 1H}), \]
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 EIOAc (50 mL). The solvent was removed and the crude product was purified via flash column chromatography (50% EIOAc in pentane) to afford tetrazine 14 (32.0 mg, 118 µmol, 76%) as a brown solid.

\[ \text{Tetrazine 14} \]

\[ \text{\textcolor{red}{MeO-O-\text{C6H4}-O-\text{N32}}} \]

\[ \text{\textcolor{red}{\text{N}}}_{\text{833}}(s) \text{ cm}^{-1}; \text{IR (neat): } \nu = 3097 \text{ (br s), } 1614 \text{ (w), } 159 \text{ (s), } 1514 \text{ (vs), } 1501 \text{ (s), } 1456 \text{ (m), } 1257 \text{ (m), } 1160 \text{ (vs), } 967 \text{ (vs) cm}^{-1}; \text{HRMS (EI) for } \text{C}_{13} \text{H}_{13} \text{N}_{6} \text{O}^+: \text{calculated: } 240.1123; \text{found: } 240.1113. \]

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

4-[2-((S-tetrazin-3-yl)amino)ethyl]phenol (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 CHCl\(_3\)) to afford tetrazine 15 (23.2 mg, 107 µmol, 69%) as an orange solid.

\[ \text{Tetrazine 15} \]

\[ \text{\textcolor{red}{\text{N}}}_{\text{644}}(s); \text{IR (neat): } \nu = 3242 \text{ (m), } 1574 \text{ (vs), } 1455 \text{ (s), } 1345 \text{ (m), } 1230 \text{ (m), } 1211 \text{ (m), } 1071 \text{ (w), } 833 \text{ (s) cm}^{-1}; \text{HRMS (EI) for } \text{C}_{10} \text{H}_{14} \text{N}_{6} \text{O}^+: \text{calculated: } 217.0964; \text{found: } 217.0960. \]

**N-cyclopropyl-s-tetrazin-3-amine (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\(_2\)O in pentane) to afford tetrazine S32 (19.0 mg, 139 µmol, 90%) as a red solid.

\[ \text{Tetrazine S32} \]

\[ \text{\textcolor{red}{\text{N}}}_{\text{197}}(s); \text{IR (neat): } \nu = 3242 \text{ (s), } 1574 \text{ (vs), } 1494 \text{ (vs), } 1381 \text{ (m), } 1121 \text{ (s), } 1029 \text{ (w), } 957 \text{ (s), } 944 \text{ (s) cm}^{-1}; \text{HRMS (EI) for } \text{C}_{8} \text{H}_{16} \text{N}_{6}^+: \text{calculated: } 137.0701; \text{found: } 137.0694. \]
O-benzyl-N-(s-tetrazin-3-yl)hydroxylamine (S33)

![Diagram](attachment://image.png)

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% EtO in pentane) to afford tetrazine **S33** (18.2 mg, 90.9 µmol, 58%) as a red solid.

\[ R_f (30\% \text{ Et}_2\text{O in pentane}) = 0.27 \text{ (red spot, UV); melting point} = 87^\circ\text{C}; ^1\text{H-NMR} (\text{CDCl}_3, 400 \text{ 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) \text{ ppm}; ^13\text{C-NMR} (\text{CDCl}_3, 126 \text{ MHz}): \delta = 166.1, 155.9, 135.1, 129.5, 129.1, 128.8, 78.9 \text{ ppm}; IR (neat): \nu = 3153 \text{ (br m), 2945 (m), 2875 (m), 1550 (s), 1498 (vs), 1465 (s), 1383 (m), 1133 (s), 955 (vs), 868 (s) cm}^{-1}; \text{HRMS (EI) for C}_{9}H_{9}N_{5}O^+: calculated: 203.0807; found: 203.0798.}

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

![Diagram](attachment://image.png)

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\textsubscript{2}Cl\textsubscript{2}) to afford tetrazine **S34** (32.0 mg, 147 µmol, 95%) as an orange solid.

\[ R_f (20\% \text{ EtOAc in pentane) = 0.28 \text{ (orange spot, UV); melting point} = 132 – 134^\circ\text{C}; ^1\text{H-NMR} (\text{DMSO-d}_6, 400 \text{ 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) \text{ ppm}; ^13\text{C-NMR} (\text{DMSO-d}_6, 101 \text{ MHz}): \delta = 200.2, 196.0, 190.5, 167.8, 166.4, 151.4, 92.7, 80.6 \text{ ppm}; IR (neat): \nu = 3079 \text{ (w), 1580 (vs), 1512 (vs), 1449 (w), 1248 (m), 1227 (s), 957 (vs), 817 (s) cm}^{-1}; \text{HRMS (EI) for C}_{10}H_{11}N_{5}O: calculated: 217.0964; found: 217.0953.}

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

![Diagram](attachment://image.png)

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\textsubscript{2}Cl\textsubscript{2} in pentane to 100% CH\textsubscript{2}Cl\textsubscript{2}) to afford tetrazine **16** (23.3 mg, 135 µmol, 87%) as an orange, crystalline solid.

\[ R_f (\text{CH}_2\text{Cl}_2) = 0.29 \text{ (red spot, UV); melting point} = 199 – 202^\circ\text{C}; ^1\text{H-NMR} (\text{DMSO-d}_6, 400 \text{ 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) \text{ ppm}; ^13\text{C-NMR} (\text{DMSO-d}_6, 101 \text{ MHz}): \delta = 162.1, 153.7, 138.0, 128.9, 123.5, 120.1 \text{ ppm}; IR (neat): \nu = 3274 (m), 3113 (w), 1612 (s), 1575 (vs), 1508 (w), 1449 (w), 1121 (w), 946 (m) cm}^{-1}; \text{HRMS (ESI) for C}_{8}H_{7}N_{5}\text{Na}^+[M+Na]^+: calculated: 196.0594; found: 196.0594.}
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.

Rf (20% EtOAc in pentane) = 0.37 (red spot, UV); melting point = 271 – 273 °C; \(^{19}\text{F-NMR}\) (DMSO-\(d_6\), 376 MHz): \(\delta = -119.07\) ppm; \(^{1}\text{H-NMR}\) (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; \(^{13}\text{C-NMR}\) (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): \(\nu = 3244\) (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\(_8\)H\(_6\)N\(_5\)F\(^+\): calculated: 191.0607; found: 191.0602.

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.

Rf (20% EtOAc in pentane) = 0.48 (red spot, UV); melting point = 214 – 216°C; \(^{1}\text{H-NMR}\) (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; \(^{13}\text{C-NMR}\) (DMSO-\(d_6\), 101 MHz): \(\delta = 162.0, 153.8, 137.5, 131.7, 121.8, 115.1\) ppm; IR (neat): \(\nu = 3256\) (w), 3106 (m), 1620 (m), 1586 (vs), 1523 (vs), 1447 (s), 1358 (m), 1242 (vs), 958 (vs), 835 (vs) cm\(^{-1}\); HRMS (EI) for C\(_9\)H\(_9\)N\(_5\)Br\(^+\): calculated: 250.9807; found: 250.9801.
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.

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

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

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

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

\[ R_f \ (20\% \text{ EtOAc in pentane}) = 0.76 \ (\text{red spot, UV}) ; \; \text{melting point} = 174 - 177 ^\circ \text{C} ; \; ^1\text{H-NMR (DMSO-}d_6, 400 \text{ MHz): } \delta = 10.74 \ (\text{br s}), \ 9.92 \ (\text{s, 1H}), \ 7.63 \ (\text{d}, \ J = 8.5 \text{ Hz, 2H}), \ 7.21 \ (\text{d}, \ J = 8.5 \text{ Hz, 2H}), 2.56 \ (\text{t}, \ J = 7.6 \text{ Hz, 2H}), 1.58-1.51 \ (\text{m, 2H}), \ 1.35-1.26 \ (\text{m, 2H}), \ 0.90 \ (\text{t}, \ J = 7.3 \text{ Hz, 3H}) \text{ ppm} ; \; ^{13}\text{C-NMR (DMSO-}d_6, 101 \text{ MHz): } \delta = 162.0, \ 153.5, \ 137.7, \ 135.6, \ 128.6, \ 120.2, \ 34.2, \ 33.2, \ 21.7, \ 13.8 \text{ ppm} ; \; \text{IR (neat): } \nu = 3245 \ (\text{w}), 1077 \ (\text{m}), 1923 \ (\text{m}), 1612 \ (\text{s}), 1557 \ (\text{s}), 1502 \ (\text{vs}), 1463 \ (\text{vs}), 1393 \ (\text{m}), 1237 \ (\text{w}), 1116 \ (\text{s}), 961 \ (\text{s}), 842 \ (\text{vs}) \; ; \; \text{HRMS (EI) for } C_{12}H_{15}N_5^+: \text{calculated: } 229.1327 ; \text{found: } 229.1322. \]
**N-(benzo[d][1,3]dioxol-5-yl)-s-tetrazin-3-amine (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.

Rf (20% EtOAc in pentane) = 0.37 (red spot, UV); **melting point** = 231 – 234 °C; **1H-NMR** (DMSO-d6, 400 MHz): δ = 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; **13C-NMR** (DMSO-d6, 101 MHz): δ = 162.0, 153.5, 147.3, 143.5, 132.0, 113.6, 108.2, 102.6, 101.2 ppm; **IR** (neat): ν = 1638 (w), 1580 (vs), 1502 (vs), 1455 (vs), 1261 (m), 1194 (m), 1034 (s), 924 (vs), 807 (s) cm⁻¹; **HRMS** (EI) for C9H7N5O2+: calculated: 217.0600; found: 217.0591.

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

Rf (20% EtOAc in pentane) = 0.44 (red spot, UV); **melting point** = 179 – 183 °C; **1H-NMR** (DMSO-d6, 400 MHz): δ = 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; **13C-NMR** (DMSO-d6, 101 MHz): δ = 162.0, 153.9, 138.8, 132.5, 119.6, 116.1, 83.5, 80.1 ppm; **IR** (neat): ν = 3271 (m), 2922 (m), 1604 (vs), 1552 (m), 1496 (vs), 1464 (w), 838 (vs) cm⁻¹; **HRMS** (EI) for C10H7N5: calculated: 197.0701; found: 197.0692.

**Boc-Lys(Tet)-OMe (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 on silica gel (40% EtOAc in pentane) to afford tetrazine 27 (45.0 mg, 132 µmol, 85%) as a red oil.

Rf (40% EtOAc in pentane) = 0.29 (orange spot, UV); [α]D²⁰ = -12.54 (c = 0.865, MeOH); **1H-NMR** (CDCl₃, 400 MHz): δ = 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; **13C-NMR** (CDCl₃, 101 MHz): δ = 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): ν = 3319 (br m), 2934 (m), 1741 (m), 1697 (vs), 1505 (s), 1455 (w), 1366 (s), 1163 (vs), 955 (m) cm⁻¹; **HRMS** (ESI) for C₁₄H₂₄N₆O₄Na⁺ [M+Na]⁺: calculated: 363.1751; found: 363.1744.
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.

Rf (40% EtOAc in pentane) = 0.62 (orange spot, UV); [α]D20 = −11.65 (c = 0.52, MeOH); 1H-NMR (CDCl3, 400 MHz): δ = 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; 13C-NMR (CDCl3, 126 MHz): δ = 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): v = 3308 (br m), 2935 (m), 1714 (vs), 1568 (vs), 1450 (m), 1368 (w), 1078 (m); 1154 (vs), 958 (m), 739 (vs) cm−1; HRMS (ESI) for C27H33N6O4Na+ [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$_2$Cl$_2$ and TFA (5:1 v/v) was added dropwise. The mixture was stirred for 16 h, then water (50 mL) and CH$_2$Cl$_2$ (50 mL) were added and the layers were separated. The aqueous layer was extracted with CH$_2$Cl$_2$ (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)**

Fmoc-Cys(Tet)-OBut (26) (41.9 mg, 87.4 µmol, 1.00 equiv) was dissolved in CH$_2$Cl$_2$ (5 mL) and TFA (1 mL) was added dropwise. The mixture was stirred for 16 h, then water (50 mL) and CH$_2$Cl$_2$ (50 mL) were added and the layers were separated. The aqueous layer was extracted with CH$_2$Cl$_2$ (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% MeOH in CH$_2$Cl$_2$) to afford thiol 30 (26.6 mg, 64.0 µmol, 73%) as a slightly pink solid.

**R$_f$** (10% MeOH in CH$_2$Cl$_2$) = 0.13 (pink spot, UV); **melting point** = 209 °C (decomp.); [a]$^\text{D}$ = −8.63 (c = 1.215, MeOH): $^1$H-NMR (MeOH-$d_4$, 400 MHz): δ = 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): δ = 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$_2$O$_7$S$^-$ [M-H]$^-$: calculated: 422.0928; found: 422.0928.

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

Fmoc-Tyr(Tet)-OBut (24) (23.0 mg, 42.6 µmol, 1.00 equiv) was dissolved in CH$_2$Cl$_2$ (2.5 mL) and TFA (0.5 mL) was added dropwise. The mixture was stirred for 16 h, then water (50 mL) and CH$_2$Cl$_2$ (50 mL) were added and the layers were separated. The aqueous layer was extracted with CH$_2$Cl$_2$ (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$_2$Cl$_2$) to afford ether 31 (10.2 mg, 21.0 µmol, 49%) as a slightly pink solid.

**R$_f$** (10% MeOH in CH$_2$Cl$_2$) = 0.4 (pink spot, UV); **melting point** = 223 – 225 °C; [α]$^\text{D}$ = +9.79 (c = 0.39, MeOH); $^1$H-NMR (MeOH-$d_4$, 400 MHz): δ = 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): δ = 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$_{20}$H$_{17}$N$_2$O$_7$Na$^+$ [M+Na]$^+$: calculated: 506.1435; found: 506.1434.
Fmoc-Lys(Tet)-OH (32)

Fmoc-Lys(Tet)-OtBu (28) (40.0 mg, 79.3 µmol, 1.00 equiv) was dissolved in CH₂Cl₂ (4 mL) and TFA (0.8 mL) was added dropwise. The mixture was stirred for 16 h, then water (50 mL) and CH₂Cl₂ (50 mL) were added and the layers were separated. The aqueous layer was extracted with CH₂Cl₂ (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 CH₂Cl₂) to afford ether 32 (31.0 mg, 69.0 µmol, 87%) as an orange oil.

Rf (10% MeOH in CH₂Cl₂) = 0.2 (orange spot, UV); [α]D = +0.43 (c = 1.265, MeOH). ¹H-NMR (MeOH-d₄, 400 MHz): δ = 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; ¹³C-NMR (MeOH-d₄, 101 MHz): δ = 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 (heat): ν = 3362 (br s), 2981 (br s), 1686 (w), 1554 (m), 1358 (w), 1115 (s), 970 (vs) cm⁻¹; HRMS (ESI) for C₂₃H₂₄N₆O₄Na⁺ [M+Na]⁺: calculated: 471.1751; found: 471.1753.
Synthesis of Chemical Probes

Disulfide S39[16]

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 CH₂Cl₂) to afford disulfide 38 (49.0 mg, 0.157 mmol, 28%) as a red oil.

Rf (1% diethyl ether in CH₂Cl₂) = 0.58 (pink spot, UV); 1H-NMR (CDCl₃, 400 MHz): δ = 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; 13C-NMR (CDCl₃, 101 MHz): δ = 167.8, 156.9, 156.3, 153.8, 143.0, 134.0, 121.2, 67.8, 36.3 ppm; IR (neat): ν = 1559 (m), 1581 (s), 1514 (s), 1478 (vs), 1342 (vs)1259 (w), 1055 (w), 856 (w), 745 (w) cm⁻¹; HRMS (ESI) for C₂₀H₂₂N₄O₅S₂⁺ [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.

Rf (40% diethyl ether in pentane) = 0.4 (pink spot); 1H-NMR (CDCl₃, 400 MHz): δ = 10.07 (s, 1H), 8.86 (t, J = 6.9 Hz, 2H), 3.03 (t, J = 6.9 Hz, 2H), 2.25 (s, 3H) ppm; 13C-NMR (CDCl₃, 101 MHz): δ = 167.9, 156.3, 68.6, 32.4, 16.2 ppm; IR (neat): ν = 2920 (w), 1479 (vs), 1449 (s), 1345 (vs), 1054 (w), 941 (m) cm⁻¹; HRMS (EI) for C₁₀H₁₄N₂O₂S⁺: 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-pyridinesulfonyl 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$_2$O, 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$_2$Cl$_2$ (20 mL) were added. The layers were separated and the aqueous layer was extracted with CH$_2$Cl$_2$ (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$_2$Cl$_2$ – 2% MeOH in CH$_2$Cl$_2$) to afford disulfide 39 (40.4 mg, 0.13 mmol, 73%) as a red solid.

R$_f$ (2% MeOH in CH$_2$Cl$_2$) = 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): $\nu^\prime$ = 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$_6$H$_{12}$N$_2$O$_5$S$_2$$^+[M+H]^+$: calculated: 312.0332; found: 312.0329.

![Figure 6. Spectroscopic Data for compounds 38 and 39.](image)

**Control Experiments using l-Glutathione**

![UHPLC trace of l-glutathione](image)

S36
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/HRMS of labelled L-glutathione

Table S3. MS/MS fragments.

<table>
<thead>
<tr>
<th>fragment</th>
<th>pseudomolecular ion</th>
<th>calculated mass</th>
<th>found mass</th>
<th>proposed structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>[C₇H₃₂N₄O₄S₂]^−</td>
<td>335.0591</td>
<td>335.0445</td>
<td></td>
</tr>
</tbody>
</table>

**Functionalization of BSA with disulfide 38**

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 (1.16 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% CO₂) 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 H₂O) at 25 °C for 15 min in the dark followed by washing with H₂O (2 x 1 mL). Further the cells were stained either with 38 (c = 10 μg/mL in H₂O) or S40 (c = 10 μg/mL) at 25 °C for 30 min in the dark followed by rinsing with H₂O (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 H₂O) at 25 °C for 30 min. After washing twice with H₂O (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.

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% CO₂) 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 H₂O) at 25 °C for 15 min in the dark followed by washing with H₂O (2 x 1 mL). Further the cells were stained either with 38 (c = 10 μg/mL in H₂O) or S40 (c = 10 μg/mL in H₂O) at 25 °C for 30 min followed by rinsing with H₂O (2 x 1 mL). Further the cells were stained either with 38 (c = 10 μg/mL in H₂O) or S40 (c = 10 μg/mL in H₂O) at 25 °C for 30 min in the dark followed by rinsing with H₂O (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 H₂O) at 25 °C for 30 min.
After washing twice with H$_2$O (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 H₂O and tetrazine probe 38 (2 mg/mL) in MeCN were prepared, and the crude extract was dissolved in MeCN:H₂O (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 H₂O (40 µL), the crude extract solution with tetrazine probe 38 (8.1 µL) solution and H₂O (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 H₂O + 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.](image-url)
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.

<table>
<thead>
<tr>
<th>entry</th>
<th>AA1</th>
<th>AA2</th>
<th>base</th>
<th>solvent</th>
<th>T [°C]</th>
<th>yield (Tet)AA1 [%]</th>
<th>yield (Tet)AA2 [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Boc-Ser-OMe</td>
<td>Boc-Trp-OMe</td>
<td>DBU</td>
<td>MeCN</td>
<td>25</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>Boc-Ser-OMe</td>
<td>Boc-Cys-OMe</td>
<td>collidine</td>
<td>THF</td>
<td>25</td>
<td>0</td>
<td>92</td>
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<tr>
<td>3</td>
<td>Boc-Trp-OMe</td>
<td>Boc-Cys-OMe</td>
<td>collidine</td>
<td>THF</td>
<td>25</td>
<td>0</td>
<td>82</td>
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<tr>
<td>4</td>
<td>Boc-Lys-OMe</td>
<td>Boc-Cys-OMe</td>
<td>collidine</td>
<td>THF</td>
<td>25</td>
<td>28</td>
<td>42</td>
</tr>
<tr>
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<td>Boc-Cys-OMe</td>
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<tr>
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<td>Boc-Cys-OMe</td>
<td>collidine</td>
<td>THF</td>
<td>-20</td>
<td>21</td>
<td>63</td>
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<td>collidine</td>
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<td>45</td>
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<td>Boc-Cys-OMe</td>
<td>-</td>
<td>NH\textsubscript{4}OAc\textsuperscript{c}/10% MeCN</td>
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<td>57</td>
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<tr>
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<td>Boc-Lys-OMe</td>
<td>Boc-Cys-OMe</td>
<td>-</td>
<td>NH\textsubscript{4}OAc\textsuperscript{c}/10% MeCN</td>
<td>25</td>
<td>0</td>
<td>76</td>
</tr>
<tr>
<td>10</td>
<td>Boc-Tyr-OMe</td>
<td>Boc-Cys-OMe</td>
<td>collidine</td>
<td>THF</td>
<td>25</td>
<td>0</td>
<td>98</td>
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<tr>
<td>11</td>
<td>Boc-His-OMe</td>
<td>Boc-Cys-OMe</td>
<td>collidine</td>
<td>THF</td>
<td>25</td>
<td>0</td>
<td>82</td>
</tr>
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<td>12</td>
<td>Boc-Lys-OMe</td>
<td>Boc-Tyr-OMe</td>
<td>collidine</td>
<td>THF</td>
<td>25</td>
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<td>72</td>
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<td>13</td>
<td>Boc-His-OMe</td>
<td>Boc-Tyr-OMe</td>
<td>collidine</td>
<td>THF</td>
<td>25</td>
<td>0</td>
<td>0</td>
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<tr>
<td>14</td>
<td>Boc-His-OMe</td>
<td>Boc-Tyr-OMe</td>
<td>collidine</td>
<td>MeCN/H\textsubscript{2}O (1:1)</td>
<td>25</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>entry</th>
<th>AA</th>
<th>solvent</th>
<th>Base</th>
<th>T [°C]</th>
<th>yield (TetAA [%])</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Boc-Cys-OMe</td>
<td>MeCN</td>
<td>collidine</td>
<td>25</td>
<td>85</td>
</tr>
<tr>
<td>2</td>
<td>Boc-Cys-OMe</td>
<td>MeCN (80)/water (20)</td>
<td>collidine</td>
<td>25</td>
<td>72</td>
</tr>
<tr>
<td>3</td>
<td>Boc-Cys-OMe</td>
<td>MeCN (40)/water (40)</td>
<td>collidine</td>
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<td>68</td>
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<tr>
<td>4</td>
<td>Boc-Cys-OMe</td>
<td>MeCN (50)/water (50)</td>
<td>collidine</td>
<td>25</td>
<td>87</td>
</tr>
<tr>
<td>5</td>
<td>Boc-Cys-OMe</td>
<td>NH₄OAc[^b]</td>
<td>-</td>
<td>25</td>
<td>83</td>
</tr>
<tr>
<td>6</td>
<td>Boc-Cys-OMe</td>
<td>NH₄OAc[^c]</td>
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<td>7</td>
<td>Boc-Lys-OMe</td>
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<td>-[^d]</td>
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<td>74</td>
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<tr>
<td>8</td>
<td>Boc-Lys-OMe</td>
<td>MeCN (50)/water (50)</td>
<td>-[^d]</td>
<td>25</td>
<td>43</td>
</tr>
<tr>
<td>9</td>
<td>Boc-Lys-OMe</td>
<td>MeCN (50)/water (50)</td>
<td>collidine</td>
<td>25</td>
<td>54</td>
</tr>
</tbody>
</table>

[^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.
Cyclo-(7-aminooctanoyl-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 CH₂Cl₂) 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/HRMS analysis of starting material product:

Table S6. MS/MS fragments.

<table>
<thead>
<tr>
<th>fragment</th>
<th>pseudomolecular ion</th>
<th>calculated mass</th>
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<th>proposed structure</th>
</tr>
</thead>
<tbody>
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<tr>
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<tr>
<td>c</td>
<td>[C_{10}H_{11}N_{2}]^+</td>
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<tr>
<td>d</td>
<td>[C_{20}H_{14}N_{6}O_{4}]^+</td>
<td>669.3620</td>
<td>669.3702</td>
<td></td>
</tr>
</tbody>
</table>
e  C\textsubscript{11}H\textsubscript{15}N\textsubscript{4}O\textsubscript{2}\textsuperscript{−}  395.1938  395.1935

f  C\textsubscript{6}H\textsubscript{9}N\textsubscript{5}O\textsuperscript{−}  209.1145  209.1145
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 chromatogram of the reaction mixture**

**HPLC-UV chromatogram from the preparative HPLC-purification**

**HRMS (ESI)** for C_{36}H_{52}N_{25}O_{12}^{+}[M+H]^{+}: calculated: 1289.6650; found: 1289.6654.
HRMS/HRMS analysis of starting material and product:

<table>
<thead>
<tr>
<th>Fragment</th>
<th>Pseudomolecular Ion</th>
<th>Calculated Mass</th>
<th>Found Mass</th>
<th>Proposed Structure</th>
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<td>765.2852</td>
<td>765.2816</td>
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</tbody>
</table>

Table S7. MS/MS fragments.
**Tet-\(\text{L-}\)Glutathione (S42)**

\(\text{L-Glutathione}(47.6\text{ mg},\ 0.155\text{ mmol},\ 1.00\text{ equiv})\) was dissolved in ammonium acetate buffer (0.11 M; \(\text{pH} = 4.15;\ 3\text{ mL}\)) 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.

**\(^1\text{H-NMR} (\text{D}_2\text{O},\ 400\text{ MHz})\)**: \(\delta = 10.18\text{ (s, 1H)}\), 4.94 (dd, \(J = 8.3,\ 4.9\text{ Hz},\ 1H\)), 4.04 (dd, \(J = 14.6,\ 5.0\text{ Hz},\ 1H\)), 3.92 (s, 2H), 3.78 (t, \(J = 6.3\text{ Hz},\ 1H\)), 3.62 (dd, \(J =14.6,\ 84\text{ Hz},\ 1H\)), 2.50 (td, \(J = 7.1,\ 6.5\text{ Hz},\ 2H\)), 2.12 (td, \(J = 7.3,\ 5.4\text{ Hz},\ 2H\)) ppm; \(^{13}\text{C-NMR} (\text{D}_2\text{O},\ 126\text{ MHz})\): \(\delta = 178.1,\ 175.8,\ 175.1,\ 174.6,\ 172.5,\ 156.7,\ 54.9,\ 53.0,\ 43.0,\ 32.2\) (2 x C), 27.0 ppm; HRMS (ESI) for \(\text{C}_2\text{H}_9\text{N}_2\text{O}_5\text{S}^+\ [\text{M+H}]^+\); 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**\(^{(1)}\) 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 \(\varepsilon = 14150 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}\) 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 \(\varepsilon = 14150 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}\) 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 biotinyloleyed 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 $k = 22.69 \pm 3.553 \text{ M}^{-1}\text{s}^{-1}$ was obtained as the slope of the resulting linear fit.
### Crystallographic Data

#### Table S8. Crystallographic Data for 1.

<table>
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<tr>
<th>Property</th>
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</thead>
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<tr>
<td>Crystallized from</td>
<td>CH₂Cl₂</td>
</tr>
<tr>
<td>Empirical formula</td>
<td>C₂HClN₄</td>
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<tr>
<td>Formula weight [g mol⁻¹]</td>
<td>116.52</td>
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<td>Crystal colour, habit</td>
<td>orange, plate</td>
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<tr>
<td>Crystal dimensions [mm]</td>
<td>0.02 x 0.13 x 0.26</td>
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<tr>
<td>Temperature [K]</td>
<td>160(1)</td>
</tr>
<tr>
<td>Crystal system</td>
<td>orthorhomic</td>
</tr>
<tr>
<td>Space group</td>
<td>Pbcc (No.61)</td>
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<tr>
<td>Z</td>
<td>8</td>
</tr>
<tr>
<td>Reflections for cell determination</td>
<td>3146</td>
</tr>
<tr>
<td>2θ range for cell determination [*]</td>
<td>14–149</td>
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<tr>
<td>a [Å]</td>
<td>11.2724(5)</td>
</tr>
<tr>
<td>b [Å]</td>
<td>6.2790(4)</td>
</tr>
<tr>
<td>c [Å]</td>
<td>12.5272(7)</td>
</tr>
<tr>
<td>V [Å³]</td>
<td>886.67(9)</td>
</tr>
<tr>
<td>F(000)</td>
<td>464</td>
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<td>D₂ [g cm⁻³]</td>
<td>1.746</td>
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<tr>
<td>ρ(Cu Kα) [mm⁻¹]</td>
<td>6.417</td>
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<td>Scan type</td>
<td>ω</td>
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<td>2θ forω [*]</td>
<td>149.0</td>
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<td>Transmission factors (min; max)</td>
<td>0.382; 0.858</td>
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<td>Total reflections measured</td>
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<td>Symmetry independent reflections</td>
<td>904</td>
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<tr>
<td>Rstd</td>
<td>0.019</td>
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<tr>
<td>Reflections with I &gt; 2σ(I)</td>
<td>883</td>
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<tr>
<td>Reflections used in refinement</td>
<td>903</td>
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<tr>
<td>Parameters refined</td>
<td>68</td>
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<td>Final R(F) [I &gt; 2σ(I) reflections]</td>
<td>0.0281</td>
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<tr>
<td>wR(F²) (all data)</td>
<td>0.0772</td>
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<tr>
<td>Weights:</td>
<td>w = [σ²(F₀²) + (0.0494P)² + 0.2063P]⁻¹ where P = (F₀² + 2Fᵡ²)²/3</td>
</tr>
<tr>
<td>Goodness of fit</td>
<td>1.096</td>
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<tr>
<td>Final Δr/σ</td>
<td>0.001</td>
</tr>
<tr>
<td>Δρ (max; min) [e Å⁻³]</td>
<td>0.16; -0.34</td>
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Table S9. Crystallographic Data for 2.

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<th>Property</th>
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<td>Crystallized from</td>
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<td>Empirical formula</td>
<td>C₂HBrN₄</td>
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<td>Formula weight [g mol⁻¹]</td>
<td>160.98</td>
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<tr>
<td>Crystal colour, habit</td>
<td>orange, plate</td>
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<tr>
<td>Crystal dimensions [mm]</td>
<td>0.05 x 0.08 x 0.26</td>
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<tr>
<td>Temperature [K]</td>
<td>160(1)</td>
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<tr>
<td>Crystal system</td>
<td>orthorhombic</td>
</tr>
<tr>
<td>Space group</td>
<td>Pbca (#61)</td>
</tr>
<tr>
<td>Z</td>
<td>8</td>
</tr>
<tr>
<td>Reflections for cell determination</td>
<td>2980</td>
</tr>
<tr>
<td>2θ range for cell determination [°]</td>
<td>6–147</td>
</tr>
<tr>
<td>a [Å]</td>
<td>12.7468(5)</td>
</tr>
<tr>
<td>b [Å]</td>
<td>5.60166(19)</td>
</tr>
<tr>
<td>c [Å]</td>
<td>12.9507(4)</td>
</tr>
<tr>
<td>V [Å³]</td>
<td>924.72(6)</td>
</tr>
<tr>
<td>F(000)</td>
<td>608</td>
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<tr>
<td>Dₓ [g cm⁻³]</td>
<td>2.313</td>
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<td>μ(Cu Kα) [mm⁻¹]</td>
<td>10.963</td>
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<td>Scan type</td>
<td>ω</td>
</tr>
<tr>
<td>2θ(ω max) [°]</td>
<td>147.9</td>
</tr>
<tr>
<td>Transmission factors (min; max)</td>
<td>0.481; 1.000</td>
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<tr>
<td>Total reflections measured</td>
<td>4719</td>
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<td>Symmetry independent reflections</td>
<td>926</td>
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<tr>
<td>( R_{int} )</td>
<td>0.024</td>
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<tr>
<td>Reflections with ( I &gt; 2σ(I) )</td>
<td>904</td>
</tr>
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<td>Reflections used in refinement</td>
<td>926</td>
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<tr>
<td>Parameters refined</td>
<td>65</td>
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<tr>
<td>Final ( R(F) ) ( I &gt; 2σ(I) ) reflections]</td>
<td>0.0185</td>
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<tr>
<td>( wR(F^2) ) (all data)</td>
<td>0.0516</td>
</tr>
<tr>
<td>Weights:</td>
<td>( w = \frac{1}{σ^2(F_o^2) + (0.0285P)^2 + 0.4717P^{-1}} ) ( \text{where:} \ P = (F_o^2 + 2F_c^2)/3 )</td>
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<tr>
<td>Goodness of fit</td>
<td>1.101</td>
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<tr>
<td>Secondary extinction coefficient</td>
<td>0.00063(9)</td>
</tr>
<tr>
<td>Final ( Δσ/σ )</td>
<td>0.001</td>
</tr>
<tr>
<td>( Δρ ) (max; min) [e Å⁻³]</td>
<td>0.32; -0.43</td>
</tr>
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</table>
Table S10. Crystallographic Date for 4.

- Crystallized from: MeCN
- Empirical formula: C₈H₆N₄O
- 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: P₂₁/c (№14)
- Z: 4
- Reflections for cell determination: 5042
- 2θ range for cell determination [°]: 7–57
- a [Å]: 10.7358(3)
- b [Å]: 5.14454(12)
- c [Å]: 14.9437(4)
- β [°]: 105.056(3)
- V [Å³]: 797.02(4)
- F(000): 360
- Dₓ [g cm⁻³]: 1.451
- μ(Mo Kα) [mm⁻¹]: 0.104
- Scan type: w
- 2θ(max) [°]: 58.5
- Transmission factors (min; max): 0.866; 1.000
- Total reflections measured: 9349
- Symmetry independent reflections: 1936
- Rint: 0.017
- Reflections with I > 2σ(I): 1614
- Reflections used in refinement: 1936
- Parameters refined: 119
- Final R(P) [I > 2σ(I) reflections]: 0.0345
- wR²(F²): (all data): 0.0868
- Goodness of fit: 1.048
- Secondary extinction coefficient: 0.012(2)
- Final Δρ(max; min) [e Å⁻³]: 0.20; -0.19
- σ(d(230)) [Å]: 0.0016 – 0.0018

\[ w = \frac{\sum (F_o^2)^2}{\sum (F_o^2)} + (0.0344P)^2 + 0.2119P]^{-1} \text{ where } P = (F_o^2 + 2F_c^2)/3 \]
Table S1. Crystallographic Data for 9.

<table>
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<th>Property</th>
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<tr>
<td>Crystallized from</td>
<td>Et$_2$O</td>
</tr>
<tr>
<td>Empirical formula</td>
<td>C$_{10}$H$_7$N$_5$</td>
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<tr>
<td>Formula weight [g mol$^{-1}$]</td>
<td>197.21</td>
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<td>Crystal colour, habit</td>
<td>red, tablet</td>
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<tr>
<td>Crystal dimensions [mm]</td>
<td>0.06 x 0.14 x 0.16</td>
</tr>
<tr>
<td>Temperature [K]</td>
<td>160(1)</td>
</tr>
<tr>
<td>Crystal system</td>
<td>orthorhombic</td>
</tr>
<tr>
<td>Space group</td>
<td>Fdd2 (443)</td>
</tr>
<tr>
<td>Z</td>
<td>16</td>
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<tr>
<td>Reflections for cell determination</td>
<td>6471</td>
</tr>
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<td>2Θ range for cell determination [*]</td>
<td>9–148</td>
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<td>a [Å]</td>
<td>38.7645(3)</td>
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<td>b [Å]</td>
<td>11.93147(14)</td>
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<tr>
<td>c [Å]</td>
<td>7.70847(8)</td>
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<tr>
<td>V [Å$^3$]</td>
<td>3565.30(6)</td>
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<td>F(000)</td>
<td>1632</td>
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<tr>
<td>D$_x$ [g cm$^{-3}$]</td>
<td>1.470</td>
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<td>μ(Cu Kα) [mm$^{-1}$]</td>
<td>0.794</td>
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<td>Scan type</td>
<td>w</td>
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<td>2θ(max) [*]</td>
<td>148.1</td>
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<td>Transmission factors (min; max)</td>
<td>0.907; 1.000</td>
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<td>Total reflections measured</td>
<td>8381</td>
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<td>Symmetry independent reflections</td>
<td>1702</td>
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<td>$R_{int}$</td>
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<td>Reflections with I &gt; 2σ(I)</td>
<td>1689</td>
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<td>Reflections used in refinement</td>
<td>1702</td>
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<td>Parameters refined; restraints</td>
<td>137; 1</td>
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<td>Final $R(P)$</td>
<td>$l &gt; 2σ(l)$ reflections</td>
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<td>$wR(P^2)$ (all data)</td>
<td>0.0608</td>
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<td>Weights:</td>
<td>$w = [σ^2(Fo) + (0.0379P)^2 + 1.6158P]^{-1}$ where P = $(Fo)^2 + 2Fc)^2)/3$</td>
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<tr>
<td>Goodness of fit</td>
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<tr>
<td>Secondary extinction coefficient</td>
<td>0.00026(5)</td>
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<td>Final $\Delta_{max} / \sigma$</td>
<td>0.001</td>
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<tr>
<td>$\Delta$σ (max; min) [e Å$^{-3}$]</td>
<td>0.15; -0.11</td>
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<tr>
<td>$\sigma$ (d$_{C-C}$) [Å]</td>
<td>0.002</td>
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**Table S12. Crystallographic Data for 23.**

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<td>Crystallized from</td>
<td>EtO</td>
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<tr>
<td>Empirical formula</td>
<td>C\textsubscript{17}H\textsubscript{21}N\textsubscript{5}O\textsubscript{5}</td>
</tr>
<tr>
<td>Formula weight [g mol\textsuperscript{−1}]</td>
<td>375.39</td>
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<td>Crystal colour, habit</td>
<td>pink, needle</td>
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<td>Crystal dimensions [mm]</td>
<td>0.03 x 0.04 x 0.28</td>
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<td>Temperature [K]</td>
<td>160(1)</td>
</tr>
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<td>Crystal system</td>
<td>monoclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>C2 (#5)</td>
</tr>
<tr>
<td>Z</td>
<td>4</td>
</tr>
<tr>
<td>Reflections for cell determination</td>
<td>3168</td>
</tr>
<tr>
<td>2θ range for cell determination [°]</td>
<td>8–142</td>
</tr>
<tr>
<td>a [Å]</td>
<td>32.663(2)</td>
</tr>
<tr>
<td>b [Å]</td>
<td>5.1610(2)</td>
</tr>
<tr>
<td>c [Å]</td>
<td>11.0162(6)</td>
</tr>
<tr>
<td>β [°]</td>
<td>94.704(5)</td>
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<tr>
<td>V [Å\textsuperscript{3}]</td>
<td>1850.79(17)</td>
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<tr>
<td>F(000)</td>
<td>792</td>
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<td>D\textsubscript{x} [g cm\textsuperscript{−3}]</td>
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<td>μ(Cu Kα) [mm\textsuperscript{−1}]</td>
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<td>ω</td>
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<td>2θ(max) [°]</td>
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<tr>
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<td>3606</td>
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<td>R\textsubscript{int}</td>
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<td>Parameters refined; restraints</td>
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<td>Final R(F) [I &gt; 2σ(I) reflections]</td>
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<tr>
<td>wR(F\textsuperscript{2}) (all data)</td>
<td>0.1556</td>
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</tbody>
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Weights:  
\( w = \frac{1}{\sigma^2(F_o^2) + (0.0850P)^2 + 0.5336P} \) where  
\( P = (F_o^2 + 2F_c^2)/3 \)

Goodness of fit  
1.039

Final \( \lambda_{max} / \sigma \)  
0.000

\( δ P \) (max; min) [e Å\textsuperscript{−3}]  
0.29; -0.17

\( σ(d_{C-Cl}) \) [Å]  
0.005 – 0.008
Table S13. Crystallographic Data for 39.

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<td>Crystallized from</td>
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<td>Formula weight [g mol⁻¹]</td>
<td>311.35</td>
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<td>Crystal colour, habit</td>
<td>red, prism</td>
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<td>Crystal dimensions [mm]</td>
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</tr>
<tr>
<td>Temperature [K]</td>
<td>160(1)</td>
</tr>
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<td>Crystal system</td>
<td>monoclinic</td>
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<td>Space group</td>
<td>C2/c (#15)</td>
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<td>Z</td>
<td>8</td>
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<td>Reflections for cell determination</td>
<td>10728</td>
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<td>2θ range for cell determination [*]</td>
<td>6–61</td>
</tr>
<tr>
<td>a [Å]</td>
<td>17.7417(3)</td>
</tr>
<tr>
<td>b [Å]</td>
<td>6.22991(9)</td>
</tr>
<tr>
<td>c [Å]</td>
<td>23.2377(3)</td>
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<td>β [*]</td>
<td>93.9389(13)</td>
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<td>V [Å³]</td>
<td>2562.38(7)</td>
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<tr>
<td>F(000)</td>
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<td>Dₓ [g cm⁻³]</td>
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</tr>
<tr>
<td>μ(Cu Kα) [mm⁻¹]</td>
<td>0.429</td>
</tr>
<tr>
<td>Scan type</td>
<td>ω</td>
</tr>
<tr>
<td>2θ(ω) range [*]</td>
<td>60.5</td>
</tr>
<tr>
<td>Transmission factors (min; max)</td>
<td>0.927; 1.000</td>
</tr>
<tr>
<td>Total reflections measured</td>
<td>16497</td>
</tr>
<tr>
<td>Symmetry independent reflections</td>
<td>3564</td>
</tr>
<tr>
<td>Rint</td>
<td>0.019</td>
</tr>
<tr>
<td>Reflections with I &gt; 2σ(I)</td>
<td>3204</td>
</tr>
<tr>
<td>Reflections used in refinement</td>
<td>3564</td>
</tr>
<tr>
<td>Parameters refined</td>
<td>186</td>
</tr>
<tr>
<td>Final R(P) [I &gt; 2σ(I) reflections]</td>
<td>0.0266</td>
</tr>
<tr>
<td>wR(F²) (all data)</td>
<td>0.0704</td>
</tr>
<tr>
<td>Weights:</td>
<td></td>
</tr>
<tr>
<td>Goodness of fit</td>
<td>1.060</td>
</tr>
<tr>
<td>Secondary extinction coefficient</td>
<td>0.0007(2)</td>
</tr>
<tr>
<td>Final Δρ / σ</td>
<td>0.001</td>
</tr>
<tr>
<td>Δρ (max; min) [e Å⁻³]</td>
<td>0.32; -0.22</td>
</tr>
<tr>
<td>σ (d(C–C)) [Å]</td>
<td>0.0015 – 0.0019</td>
</tr>
</tbody>
</table>
NMR Spectra

$^1$H-NMR, CDCl$_3$, 400 MHz

$^{13}$C-NMR, CDCl$_3$, 101 MHz
$\text{H-NMR, CDCl}_3, 400 \text{ MHz}$

$\text{C-NMR, CDCl}_3, 101 \text{ MHz}$
**$^1$H-NMR, CDCl$_3$, 400 MHz**

![1H-NMR spectrum](image)

**$^{13}$C-NMR, CDCl$_3$, 101 MHz**

![13C-NMR spectrum](image)
$^1$H-NMR, CDCl$_3$, 400 MHz

$^{13}$C-NMR, CDCl$_3$, 101 MHz
$^1$H-NMR, CDCl$_3$, 400 MHz

$^{13}$C-NMR, CDCl$_3$, 101 MHz

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$^1$H-NMR, CDCl$_3$, 400 MHz

$^{13}$C-NMR, CDCl$_3$, 101 MHz
$^1\text{H-NMR, CDCl}_3$, 400 MHz

$^{13}\text{C-NMR, CDCl}_3$, 101 MHz
$^1$H-NMR, CDCl$_3$, 400 MHz

$^{13}$C-NMR, CDCl$_3$, 126 MHz
$^1$H-NMR, CDCl$_3$, 400 MHz

$^{13}$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

$^{13}$C-NMR, CDCl$_3$, 126 MHz
$^1$H-NMR, CDCl$_3$, 400 MHz

![NMR Spectrum](image)

$^{13}$C-NMR, CDCl$_3$, 101 MHz

![NMR Spectrum](image)
$^1$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

$^{13}$C-NMR, CDCl$_3$, 101 MHz
$^1$H-NMR, CDCl$_3$, 400 MHz

$^{13}$C-NMR, CDCl$_3$, 101 MHz
\[ \text{H-NMR, CDCl}_3, \ 400 \text{ MHz} \]

\[ \text{C-NMR, CDCl}_3, \ 126 \text{ MHz} \]
\[^1\text{H-NMR, CDCl}_3, 400 \text{ MHz}\]

\[^{13}\text{C-NMR, CDCl}_3, 126 \text{ MHz}\]

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\(^1\text{H-NMR, CDCl}_3, 500 \text{ MHz}\)

\(^{13}\text{C-NMR, CDCl}_3, 126 \text{ MHz}\)
$^{13}$C-NMR, CDCl$_3$, 376 MHz

[Chemical structure image]
$^1$H-NMR, CDCl$_3$, 500 MHz

$^13$C-NMR, CDCl$_3$, 126 MHz
$^1$H-NMR, CDCl$_3$, 400 MHz

$^{13}$C-NMR, CDCl$_3$, 101 MHz
$\mathrm{^1H-NMR}$, $\mathrm{CDCl}_3$, 400 MHz

$\mathrm{^{13}C-NMR}$, $\mathrm{CDCl}_3$, 101 MHz
$^1$H-NMR, CDCl$_3$, 400 MHz

$^{13}$C-NMR, CDCl$_3$, 126 MHz
$^1$H-NMR, CDCl$_3$, 400 MHz

$^{13}$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

\(^{1}\)C-NMR, CDCl\(_3\), 101 MHz
$\text{H-NMR, CDCl}_3$, 400 MHz

$\text{C-NMR, CDCl}_3$, 126 MHz
$^1$H-NMR, CDCl$_3$, 400 MHz

$^{13}$C-NMR, CDCl$_3$, 126 MHz
$^1$H-NMR, CDCl$_3$, 400 MHz

$^{13}$C-NMR, CDCl$_3$, 126 MHz
$^1$H-NMR, CDCl$_3$, 400 MHz

$^{13}$C-NMR, CDCl$_3$, 126 MHz
$^1$H-NMR, CDCl$_3$, 400 MHz

$^{13}$C-NMR, CDCl$_3$, 126 MHz
$^1$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
\[ ^1\text{H-NMR, CDCl}_3, \, 400 \, \text{MHz} \]

\[ ^{13}\text{C-NMR, CDCl}_3, \, 126 \, \text{MHz} \]
\(^1\text{H-NMR, CDCl}_3, 400 \text{ MHz}\)

\(^{13}\text{C-NMR, CDCl}_3, 101 \text{ MHz}\)
$^1$H-NMR, CDCl$_3$, 400 MHz

$^1$H-NMR, CDCl$_3$, 400 MHz

$^{13}$C-NMR, CDCl$_3$, 101 MHz

$^{13}$C-NMR, CDCl$_3$, 101 MHz
$^1$H-NMR, DMSO-d$_6$, 400 MHz

$^{13}$C-NMR, DMSO-d$_6$, 101 MHz
\[^1\text{H-NMR, DMSO-}d_6, 400 \text{ MHz}\]

\[^{13}\text{C-NMR, DMSO-}d_6, 101 \text{ MHz}\]
$^1$H-NMR, CDCl$_3$, 400 MHz

$^{13}$C-NMR, CDCl$_3$, 126 MHz
$^1$H-NMR, CDCl$_3$, 400 MHz

$^{13}$C-NMR, CDCl$_3$, 126 MHz
$^1$H-NMR, DMSO-$d_6$, 400 MHz

$^{13}$C-NMR, DMSO-$d_6$, 101 MHz
$^1$H-NMR, DMSO-$d_6$, 400 MHz

$^{13}$C-NMR, DMSO-$d_6$, 101 MHz
$^{19}$F-NMR, DMSO-$d_6$, 376 MHz
\textsuperscript{1}H-NMR, DMSO-\textit{d}6, 400 MHz

\begin{figure}
\centering
\includegraphics[width=\textwidth]{1H_NMR.png}
\end{figure}

\textsuperscript{13}C-NMR, DMSO-\textit{d}6, 101 MHz

\begin{figure}
\centering
\includegraphics[width=\textwidth]{13C_NMR.png}
\end{figure}
$^1$H-NMR, DMSO-$d_6$, 400 MHz

$^{13}$C-NMR, DMSO-$d_6$, 101 MHz
$^1$H-NMR, DMSO-$d_6$, 400 MHz

$^{13}$C-NMR, DMSO-$d_6$, 101 MHz
$^1$H-NMR, DMSO-$d_6$, 400 MHz

$^{13}$C-NMR, DMSO-$d_6$, 101 MHz
$^1$H-NMR, DMSO-d$_6$, 400 MHz

$^{13}$C-NMR, DMSO-d$_6$, 101 MHz
$^1$H-NMR, DMSO-$d_6$, 400 MHz

$^{13}$C-NMR, DMSO-$d_6$, 101 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, MeOH-$d_4$, 400 MHz

$^{13}$C-NMR, MeOH-$d_4$, 101 MHz
\( ^1H\text{-NMR, MeOH-d}_4, 400 \text{ MHz} \)

\[
\text{HN\text{-moc} OH}
\]

\( ^{13}C\text{-NMR, MeOH-d}_4, 126 \text{ MHz} \)

\[
\text{HN\text{-moc} OH}
\]
$^1$H-NMR, MeOH-$d_4$, 400 MHz

$^{13}$C-NMR, MeOH-$d_4$, 101 MHz
\(^1\)H-NMR, CDCl\(_3\), 400 MHz

\[^{13}\text{C}\text{-NMR, CDCl}_3, 101 MHz\]

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\(^1\)H-NMR, CDCl\(_3\), 400 MHz

\(^1^3\)C-NMR, CDCl\(_3\), 101 MHz
$^1$H-NMR, CDCl$_3$, 400 MHz

$^{13}$C-NMR, CDCl$_3$, 101 MHz
References

2 WIWEB-Standardarbeitsanweisung 4-5.1.02, Ermittlung der Explosionsgefährlichkeit, hier der Schlagempfindlichkeit mit dem Fallhammer, Nov. 8, 2002.
3 http://www.bam.de
5 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.
6 http://www.ozm.cz