# Supporting Information

# 2-Azo-, 2-diazocine-thiazols and 2-azo-imidazoles as photoswitchable kinase inhibitors: limitations and pitfalls of the photoswitchable inhibitor approach

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## I. Molecular Modelling

Molecular modeling was performed on a DELL Precision T5500 eight core workstation. For visualization Maestro, version 10.6, 2016 (Schrödinger LLC, New York, NY, USA) was used. Protein crystal structures were prepared prior to docking by the Protein Preparation Wizard[1] synchronizing the following modules: Epik, version 3.6, 2016[2]; Impact, version 7.1, 2016; Prime, version 4.4, 2016.[3] In order to achieve high Enrichment-factors, the common refinement protocol by Sastry *et al.* [1] has been adjusted: the process involved assignment of bond orders, addition of hydrogen atoms, identification of disulfide bonds, and the conversion of artifical selenomethionines to methionines (default settings). Missing side chains were filled in using Prime. Missing loops have not been detected. Water molecules beyond 5 Å from hetero atoms have been deleted automatically. H-bond optimization was performed in a standard sampling, the Root-mean-square deviation for atomic positions cutoff for heavy atoms in subsequent protein minimization was set to 0.3 Å Ligands were prepared to generate energetically minimized three-dimensional coordinates with an extended cutoff by MacroModel, version 11.2, 2016 (Schrödinger LLC). Ionization and tautomeric states were estimated at pH 7 ± 2 by LigPrep, version 3.8, 2016 (Schrödinger LLC) ,[1] utilizing Hammet and Taft methodology-based Epik[2]. Additionally, Epik state penalties ($kcal \cdot mol^{-1}$) were calculated for each ligand to quantify the energetic cost for state transition in solution.[1] In order to indicate ligand flexibility, up to 50 bioactive conformers per ligand were identified and prioritized utilizing the conformational search module in the fast mode (ConfGen, version 3.6, 2016, Schrödinger LLC).[4] Receptor grid generation was generated with Glide, version 7.1, 2016 (Schrödinger LLC).[5] For ligand docking and screening the Glide XP workflow was used.[5] Energetically minimized ligand conformations were docked into the active site of the protein; possible binding poses were determined and subsequently ranked based on their calculated binding affinities.

## II. Analytical equipment

### NMR spectroscopy

NMR spectra were measured in deuterated solvents (Deutero). To reference the NMR spectra the following solvent signals were used:[6]

| solvent | degree of deuteration | $^{1}$H signal | $^{13}$C signal |
|---|---|---|---|
| acetone-$d_6$ | 99.8 % | 2.05 (quintet) | 29.84 (septet) |
| chloroform-$d_1$ | 99.8 % | 7.26 (s) | 77.16 (triplet) |
| DMSO-$d_6$ | 99.8 % | 2.50 (s) | 39.52 (septet) |

NMR measurements were performed with a Bruker DRX 500 ($^{1}$H NMR: 500 MHz, $^{13}$C NMR: 125 MHz, $^{19}$F NMR: 470 MHz) and a Bruker AV 600 ($^{1}$H NMR: 600 MHz, $^{13}$C NMR: 150 MHz).

### Melting point

Melting points were measured with a Melting Point B-560 (Büchi) in melting point tubes.

### Mass spectrometry

The high resolution (HR-EI) mass spectra were measured with an AccuTOF GCv 4G (Joel) with ionization energy of 70 eV and the high resolution (HR-ESI) mass spectra were measured with a Thermo Fischer Q Exactive Plus MS, Hybrid Quadrupol-Orbitrap. MALDI-TOF mass spectra were measured with a Bifex III, Fa. Bruker-Daltonics, accelerating voltage: 19 kV, wavelength of ionization laser: 337 nm, applied matrix: 4-chlor-α-cyanocinnamic acid (Cl-CCA).

### IR spectroscopy

Infrared spectra were measured on a Perkin-Elmer 1600 Series FT-IR spectrometer with an A531-G Golden-Gate-Diamond-ATR-unit. Signals were abbreviated with w, m, s for weak, medium and strong signal intensity. Broad signals are additionally abbreviated with br.

### UV-Vis spectroscopy

UV-Vis spectra were measured with a Lambda 14 spectrometer (Perkin-Elmer) with a (Büchi) thermostat. Quartz cuvettes of 1 cm optical path length were used.

### Chromatography stationary phases

For column chromatography purifications silica gel (Merck, particle size 0.040-0.063 mm) was used. Flash column chromatography purfications were performed on a Biotage® type isolera one with Biotage® Ultra cartidges (Biotage®, HP-Sphere™, particle diameter: 25 µm, cartridges sizes: 10 g, 25 g, 50 g and 100 g). $R_f$ values were determined by thin layer chromatography on Polygeram® SilG/UV254 (Macherey Nagel, 0.2 mm particle size).

**Light sources**

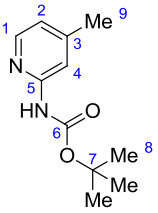
UV/vis and $^{1}$H NMR:

The irradiation of the samples was performed with LEDs with a wavelength of 420 nm, 435 nm and 525 nm from SAHLMANN PHOTOCHEMICAL SOLUTIONS with followed specifications:

- 420 nm: 3 x Roithner SMB1N-420H, FWHM = 15 nm, *P* (opt) = 3 x 420 mW
- 435 nm: 3 x Roithner APG2C1-435, FWHM = 16 nm, *P* (opt) = 3 x 380 mW
- 525 nm: 3 x NCSG219-V1, FWHM = 36 nm, *P* (opt) = 3 x 450 mW

## III. Syntheses

### III. 1 Synthesis of *tert*-butyl-(4-methylpyridin-2-yl) carbamate (6)




2-Amino-4-methylpyridin (**5**, 2.00 g, 18.5 mmol) and di-*tert*-butyl dicarbonate (4.40 g, 20.3 mmol) were dissolved in 200 mL *tert*-butanol and stirred for 18 h at 30 °C. Afterwards, 250 mL water was added to the solution and after 15 min the formed precipitate was filtered off. The precipitate was washed with water, dried in vacuo and purified by flash column chromatography (cyclohexane/ethyl acetate, ethyl acetate: 12 % → 100 %) on silica. A colourless, crystalline solid was obtained (2.63 g, 12.6 mmol, 68 %[*]) (Lit.:[7] 84 %).

**melting point:** 120 °C

**$R_f$ :** 0.6 (cyclohexane/ethyl acetate, 1:3).

**$^1$H NMR** (600 MHz, acetone-$d_6$): □ = 9.55 (bs, 1 H, N*H*), 8.18 (d, $^3J$ = 5.3 Hz, 1 H, *H*-1), 7.85 (s, 1 H, *H*-4), 6.87 (d, $^3J$ = 5.3 Hz, *H*-2), 2.35 (s, 3 H, *H*-9), 1.53 (s, 9 H, *H*-8) ppm.

**$^{13}$C NMR** (150 MHz, acetone-$d_6$): □ = 153.0 (*C*-6), 152.6 (*C*-5), 149.3 (*C*-3), 147.4 (*C*-1), 119.1 (*C*-2), 112.4 (*C*-4), 79.7 (*C*-7), 27.5 (*C*-8), 20.4 (*C*-9) ppm.

**IR** (ATR): ῡ = 2975 (m), 1720 (vs), 1612 (m), 1575 (s), 1531 (m), 1423 (m), 1365 (m), 1276 (s), 1231 (s), 1154 (vs), 1119 (s), 1058 (m), 996 (m), 887 (w), 867 (m), 816 (vs), 766 (vs), 745 (s), 626 (m), 524 (m) $cm^{-1}$.

**MS** (EI, 70 eV): m/z (%) = 208 (100) $[M]^+$, 209 (14) $[M]^+$.

**MS** (EI, HR, 70 eV): $C_{11}H_{16}N_2O_2$, m/z = calc.: 208.1212, found: 208.1211.

[*]After purification, traces of di-*tert*-butyl dicarbonate could not be separated from the product **6** as indicated by the slightly higher integral in the $^1$H NMR for the *tert*-butyl group.

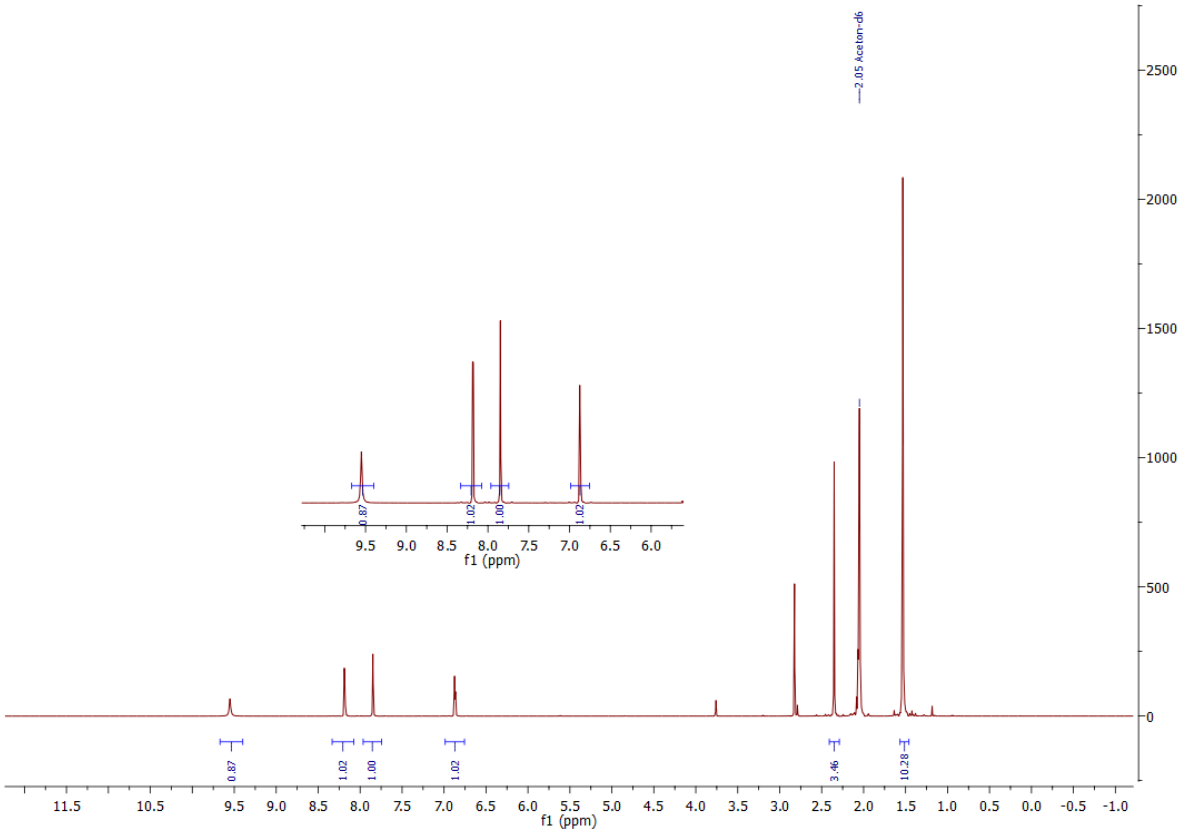



**Figure S1**: $^{1}$H NMR spectrum of compound **6** measured in deuterated acetone.

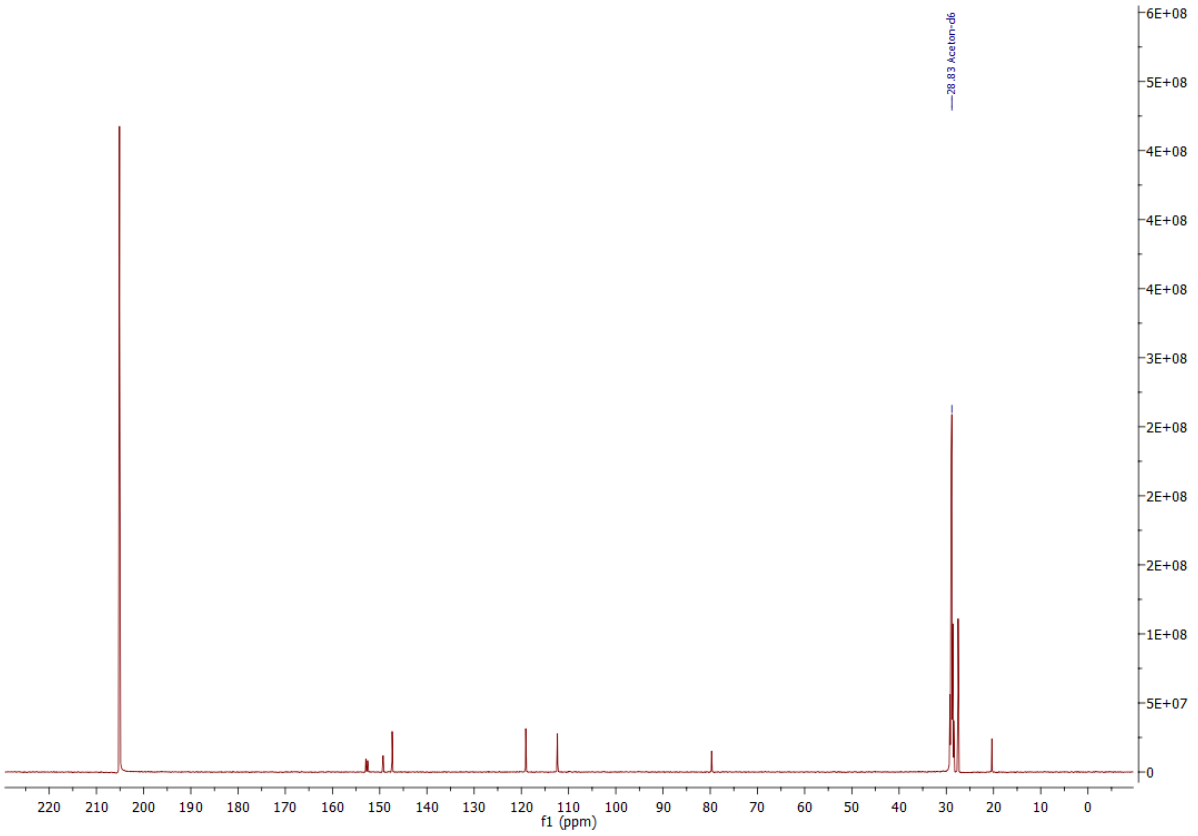
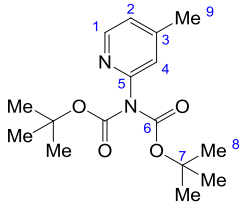



**Figure S2**: $^{13}$C NMR spectrum of compound **6** measured in deuterated acetone.

### III.2. Synthesis of bis-*tert*-butyl-(4-methylpyridin-2-yl) dicarbamate (7)




*tert*-Butyl-(4-methylpyridin-2-yl) carbamate (**6**, 5.50 g, 26.4 mmol) was dissolved in 50 mL THF, DMAP (1.61 g, 13.2 mmol) and di-*tert*-butyl dicarbonate ( 6.34 g, 29.0 mmol) were added and the solution was stirred for 48 h at room temperature. Additional 0.5 eq. di-*tert* butyl dicarbonate ( 3.17 g, 14.5 mmol) was added and the solution was stirred for 24 h at room temperature. The solvent was evaporated, the crude product was filtered over silica (cyclohexane/ethyl acetate, 1:1) and purified by flash column chromatography (cyclohexane/ethyl acetate, ethyl acetate: 12 % → 100 % ) on silica. A colorless solid was obtained (8.05 g, 26.1 mmol, 99 %[*]).

**melting point:** 66 °C.

**$R_f$:** 0.6 (cyclohexane/ethyl acetate, 1:3).

**$^1$H NMR** (500 MHz, $CDCl_3$): □ = 8.36 (d, $^3J$ = 5.1 Hz, 1 H, *H*-1), 7.10 (bs, 2 H, *H*-2, *H*-4), 2.40 (s, 3 H, *H*-9), 1.545 (s, 18 H, *H*-8) ppm.

**$^{13}$C NMR** (125 MHz, $CDCl_3$): □ = 151.6 (*C*-6), 151.5 (*C*-3), 151.2 (*C*-5), 147.3 (*C*-1), 123.8 (*C*-2 oder *C*-4), 123.2 (*C*-4 oder *C*-2), 83.4 (*C*-7), 27.9 (*C*-8), 21.1 (*C*-9) ppm.

**IR** (ATR): ϑ = 2991 (w), 2165 (w), 1745 (s), 1723 (s), 1608 (m), 1558 (w), 1482 (w), 1409 (w), 1368 (m), 1309 (s), 1272 (m), 1250 (s), 1155 (s), 1119 (vs), 1061 (m), 1038 (m), 996 (w), 895 (w), 855 (m), 838 (m), 781 (m), 768 (m), 740 (m), 720 (w), 671 (w), 644 (w), 586 (w), 421 (m), 501 (m), 475 (m), 455 (m ) $cm^{-1}$.

**MS** (EI, 70 eV): m/z (%) = 308 (2) $[M]^+$.

**MS** (HR-ESI): m/z (%) = $[C_{16}H_{25}N_2O_4]^+$, m/z = calc.: 309.1809, found.: 309.1804.

[*]After purification, traces of di-*tert*-butyl dicarbonate could not be separated from the product **7** as indicated by the slightly higher integral in the $^1$H NMR for the *tert*-butyl group.

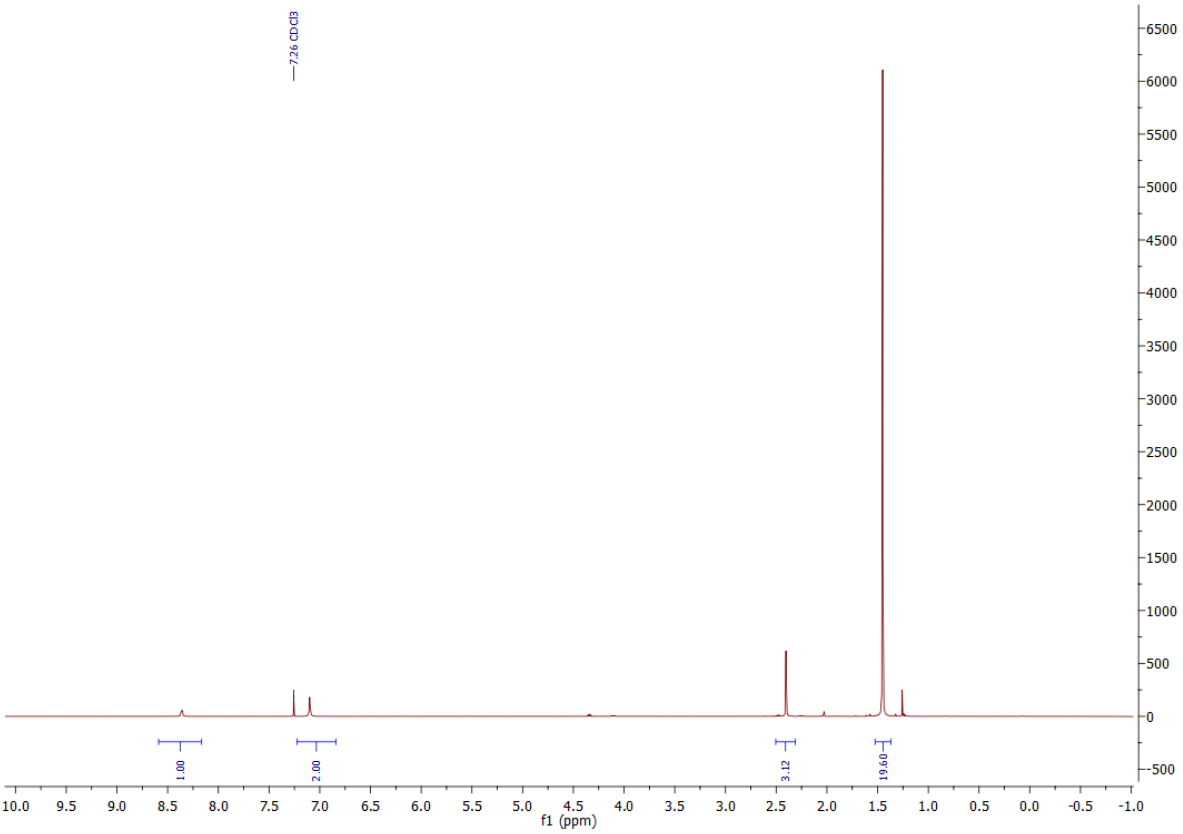



**Figure S3**: $^{1}$H NMR spectrum of compound **7** measured in deuterated chloroform.

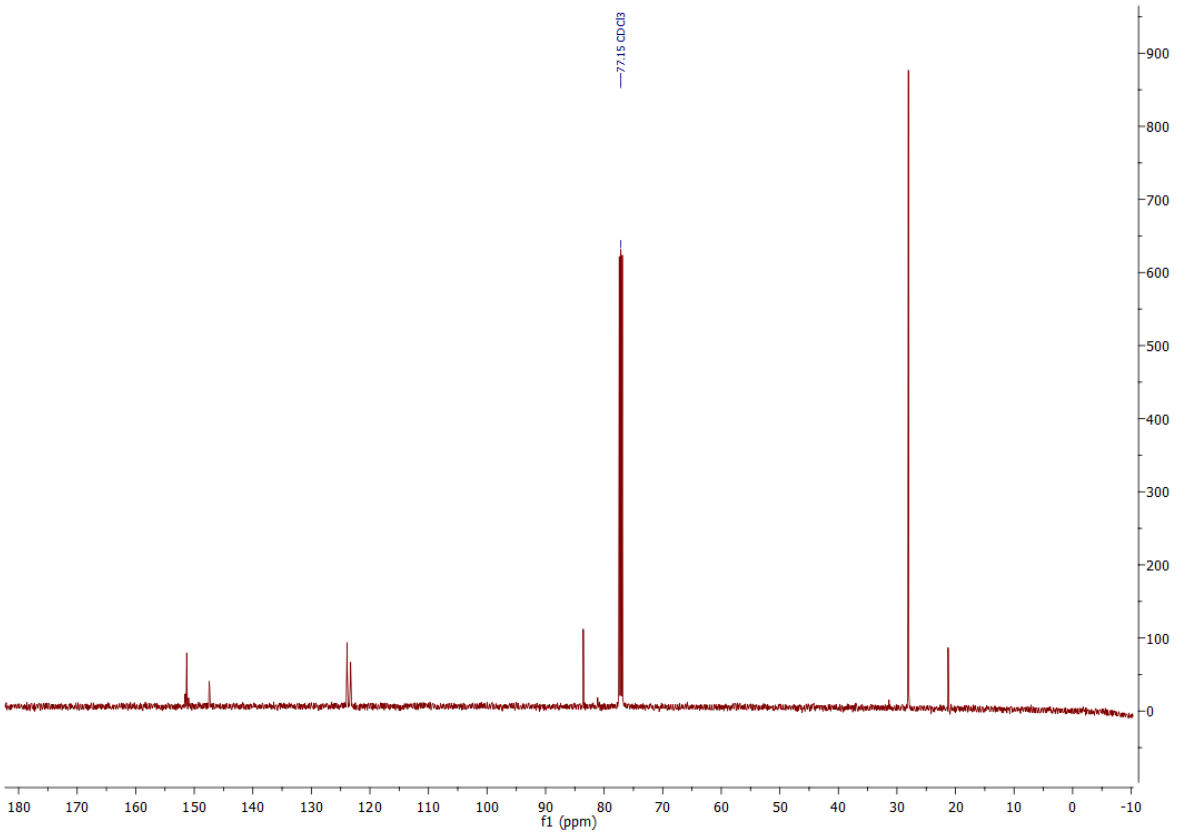
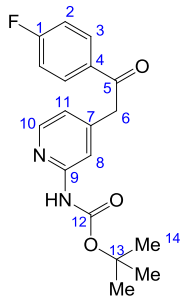



**Figure S4**: $^{13}$C NMR spectrum of compound **7** measured in deuterated chloroform.

**III.3 Synthesis of *tert*-butyl-(4-2-[4-fluorophenyl]-2-oxoethylpyridin-2-yl) carbamate (9)**




Bis-*tert*-butyl-(4-methylpyridin-2-yl) dicarbamate (**7**, 8.28 g, 26.9 mmol) and ethyl-4-fluorobenzoate (**7**, 3.94 mL, 26.9 mmol) were dissolved in 12 mL dry THF under nitrogen atmosphere and cooled to 0 °C. NaHMDS (26.9 mL, 2 M in THF) was added dropwise, the solution was then stirred for 2 h at 0 °C and 1 h at room temperature. 100 mL water was added and the mixture was extracted with ethyl acetate (3 x 50 mL). The organic layers were dried over magnesium sulfate, filtered and the solvent was evaporated. The crude product was recrystallized from methanol to obtain colourless crystals (5.33 g, 16.1 mmol, 60 %).

**melting point:** 146°C

**$^{1}$H NMR** (500 MHz, $CDCl_3$): □ = 8.63 (bs, 1 H, N*H*), 8.23 (d, $^{3}J$ = 5.3 Hz, 1 H, *H*-10), 8.02 ($m_c$, 2 H, *H*-3), 7.93 (bs, 1 H, *H*-8), 7.14 ($m_c$, 2 H, *H*-2), 6.85 (dd, $^{3}J$ = 5.3 Hz, $^{4}J$ = 1.6 Hz, 1 H, *H*-11), 4.25 (s, 2 H, *H*-6), 1.53 (s, 9 H, *H*-14) ppm.

**$^{13}$C NMR** (125 MHz, $CDCl_3$): □ = 194.4 (*C*-5), 166.1 (d, $^{1}J_{CF}$ = 255.3 Hz, *C*-1), 152.9 (*C*-12), 152.8 (*C*-9), 148.0 (*C*-10), 145.8 (*C*-7), 132.9 (d, $^{4}J_{CF}$ = 3.1 Hz, *C*-4), 131.3 (d, $^{3}J_{CF}$ = 9.4 Hz, *C*-3), 119.6 (*C*-11), 81.2 (*C*-13), 45.2 (*C*-6), 28.5 (*C*-14) ppm.

**$^{19}$F NMR** (470 MHz, $CDCl_3$): □ = -104.4 ppm.

**IR** (ATR): ϑ = 2974 (m), 1725 (m), 1689 (m), 1579 (m), 1528 (w), 1505 (w), 1437 (m), 1409 (w), 1366 (w), 1324 (w), 1292 (w), 1274 (w), 1225 (s), 1210 (m), 1155 (vs), 1118 (m), 1059 (w), 997 (s), 834 (s), 816 (w), 768 (s), 740 (w), 623 (w), 591 (w), 560 (s), 489 (w) $cm^{-1}$.

**MS** (EI, 70 eV): m/z (%) = 330 (1) $[M]^+$, 123 (100) $[M\text{-}C_{11}H_{15}N_2O_2]^+$.

**MS** (EI, HR, 70 eV): $C_{18}H_{19}FN_2O_3$, m/z = calc.: 330.1380, found: 330.1371.

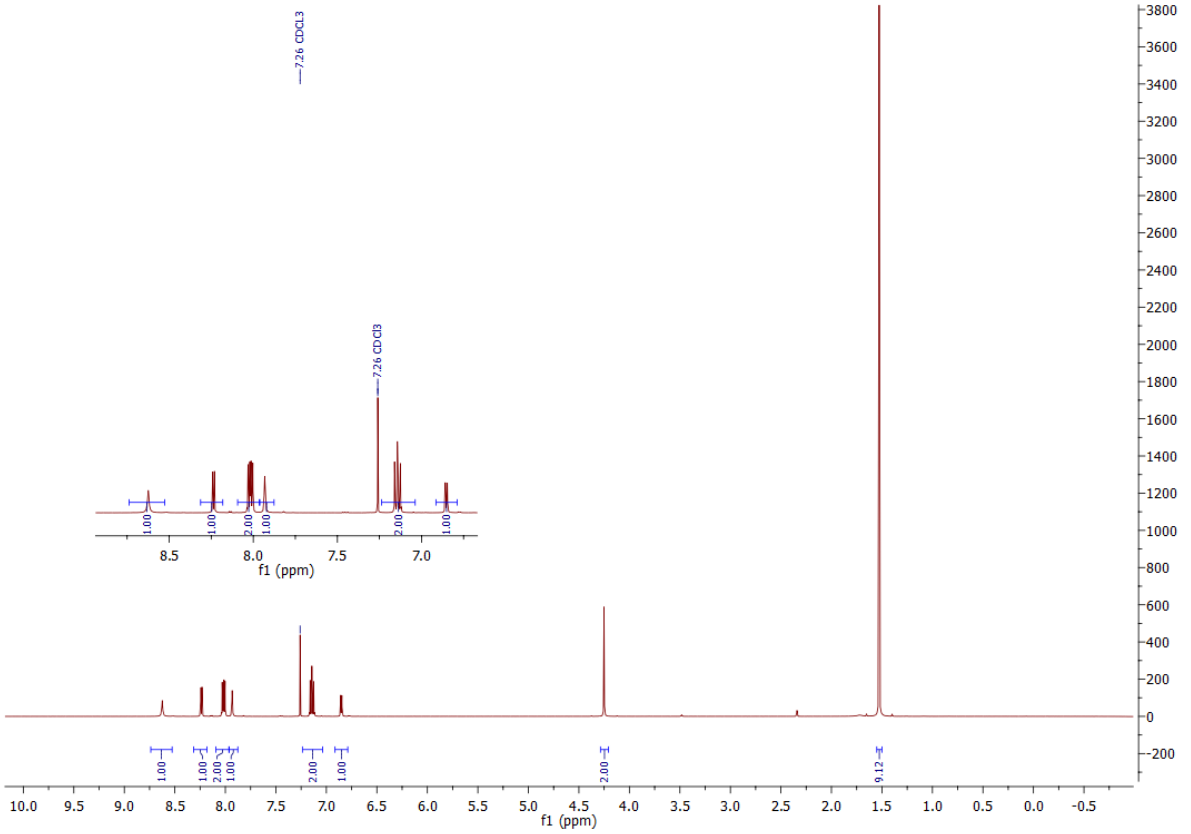



**Figure S5**: $^{1}$H NMR spectrum of compound **9** measured in deuterated chloroform.

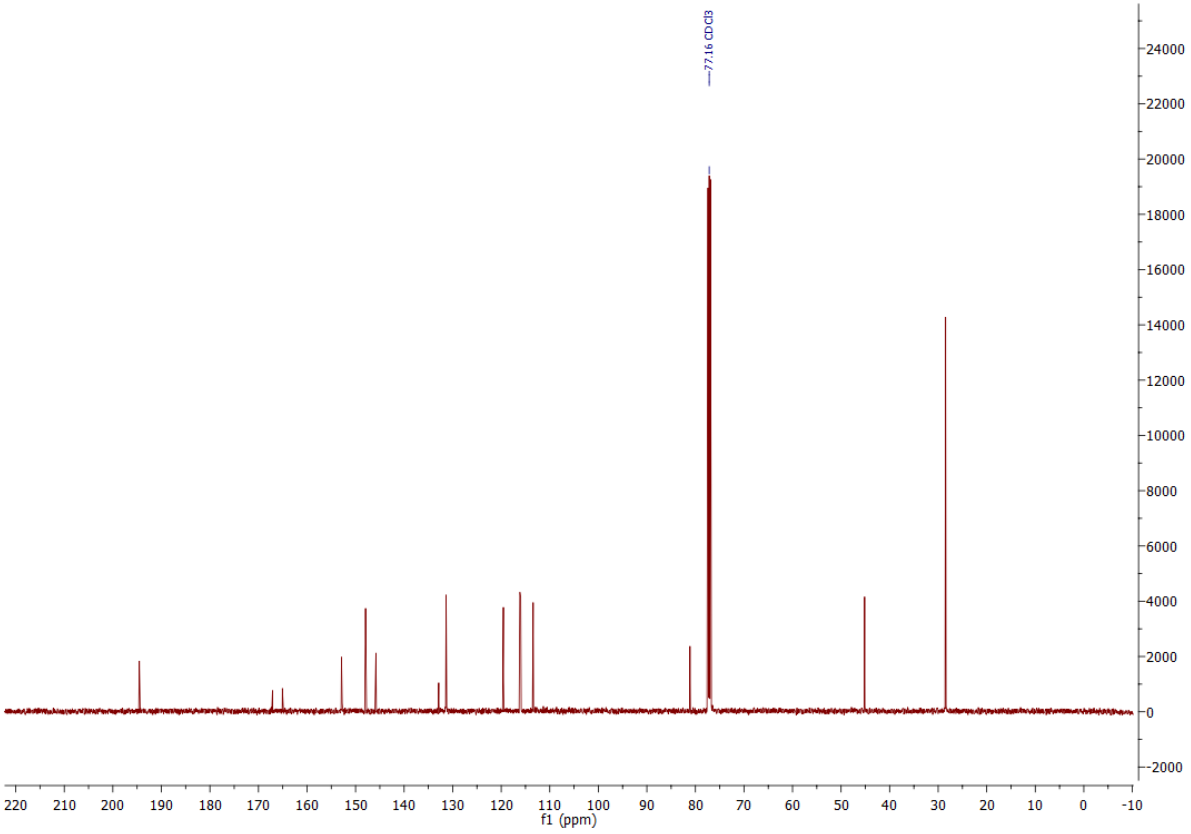



**Figure S6**: $^{13}$C NMR spectrum of compound **9** measured in deuterated chloroform.

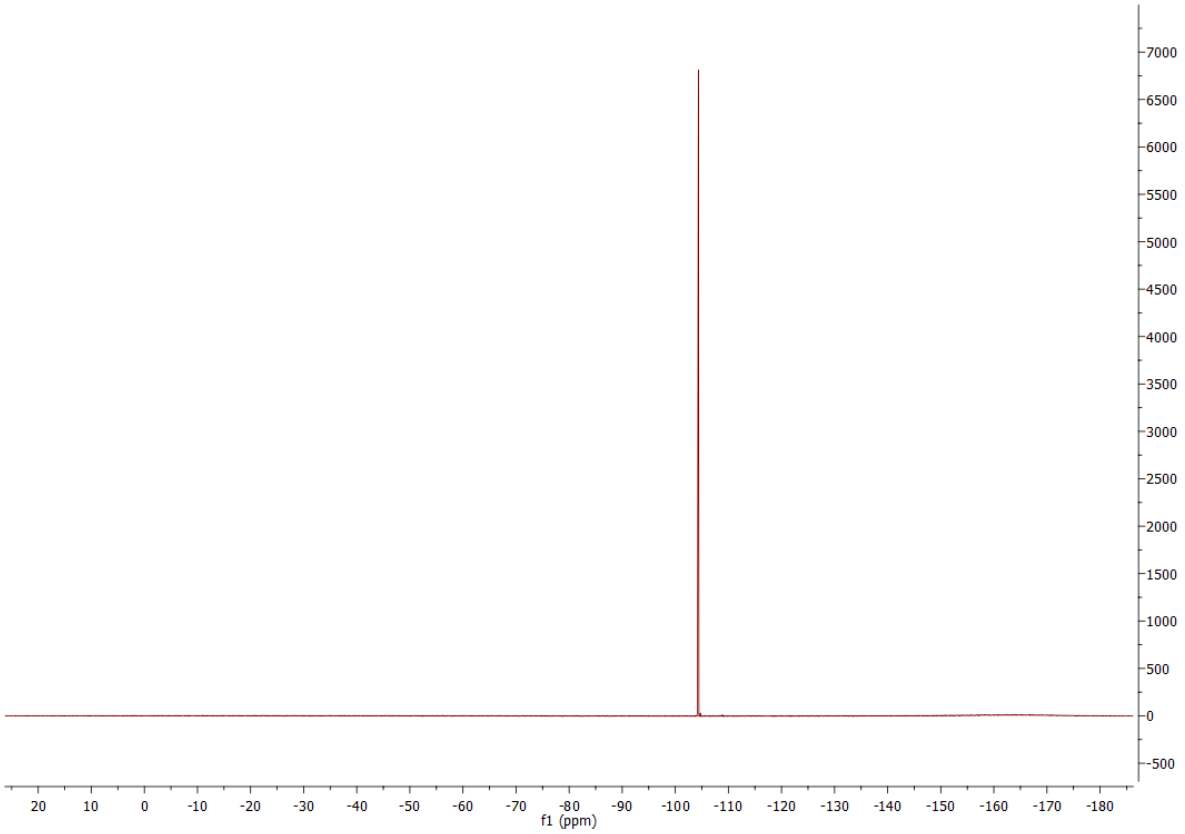
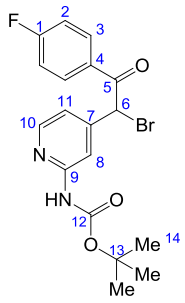



**Figure S7**: $^{19}$F NMR spectrum of compound **9** measured in deuterated chloroform.

**III.4 Synthesis of *tert*-butyl (4-(1-bromo-2-(4-fluorophenyl)-2-oxoethyl)pyridin-2-yl) carbamate (10)**




*tert*-Butyl-(4-2-[4-fluorophenyl]-2-oxoethylpyridin-2-yl) carbamate (**9**, 2.00 g, 6.06 mmol) was dissolved in a mixture of 40 mL DCM and 25 mL acetic acid. NBS (1.08 g, 6.06 mmol) was added and the solution was stirred for 26 h at room temperature. The reaction mixture was diluted with water and washed with 100 mL 1 M sodium hydroxide solution. The organic layer was dried over magnesium sulfate, filtered and the solvent was evaporated to obtain a colourless solid (2.41 g, 5.89 mmol, 97 %).

**melting point:** 187 °C.

**$^{1}$H NMR** (500 MHz, $CDCl_3$): □ = 8.37 (bs, 1 H, N*H*), 8.29 (d, $^{3}J$ = 5.3 Hz, 1 H, *H*-10),8.11 (s, 1 H, *H*-8), 8.04 ($m_c$, 2 H, *H*-3), 7.19-7.13 (m, 3 H, *H*-2, *H*-11), 6.16 (s, 1 H, *H*-6), 1.53 (s, 9 H, *H*-14) ppm.

13**C NMR** (125 MHz, $CDCl_3$): □ = 188.9 (*C*-5), 166.4 (d, $^{1}J_{CF}$ = 258.3 Hz, *C*-1), 152.8 (*C*-9), 152.62 (*C*-12), 148.4 (*C*-10), 146.6 (*C*-7), 132.1 (d, $^{3}J_{CF}$ = 9.6 Hz, *C*-3), 130.3 (d, $^{4}J_{CF}$ = 3.0 Hz, *C*-4), 119.2 (*C*-11), 116.3 (d, $^{2}J_{CF}$ = 22.0 Hz, *C*-2), 112.2 (*C*-8), 81.5 (*C*-13), 47.54 (*C*-6), 28.4 (*C*-14) ppm.

**$^{19}$F NMR** (470 MHz, $CDCl_3$): □ = -102.8 ppm.

**IR** (ATR): ϑ = 2980 (w), 1726 (m), 1680 (m), 95 (m), 1574 (s), 1530 (m), 1507 (w), 14136 (m), 1391 (w), 1365 (m), 1293 (m), 998 (m), 852 (m), 832 (m), 780 (s), 749 (m), 655 (w), 565 (vs), 498 (m), 928 (w), 461 (w) $cm^{-1}$.

**MS** (EI, 70 eV): m/z (%) = 123 (100) $[M\text{-}C_{11}H_{14}BrN_2O_2]^+$, 212 (19) $[M\text{-}C_{11}H_{13}O_2]^+$, 229 (80) $[M\text{-}C_5H_9O_2Br]^+$, 255 (6) $[M\text{-}C_4H_9OBr]^+$, 273 (12) $[M\text{-}C_4H_9Br]^+$, 329 (4) $[M\text{-}Br]^+$, 335 (3) $[M\text{-}C_4H_9O]^+$, 351 (2) $[M\text{-}C_4H_9]^+$, 408 (1) $[M]^+$.

**MS** (ESI, HR,): $[C_{18}H_{19}BrFN2O_3S]^+$, m/z = calc.: 409.0558, found: 409.0560.

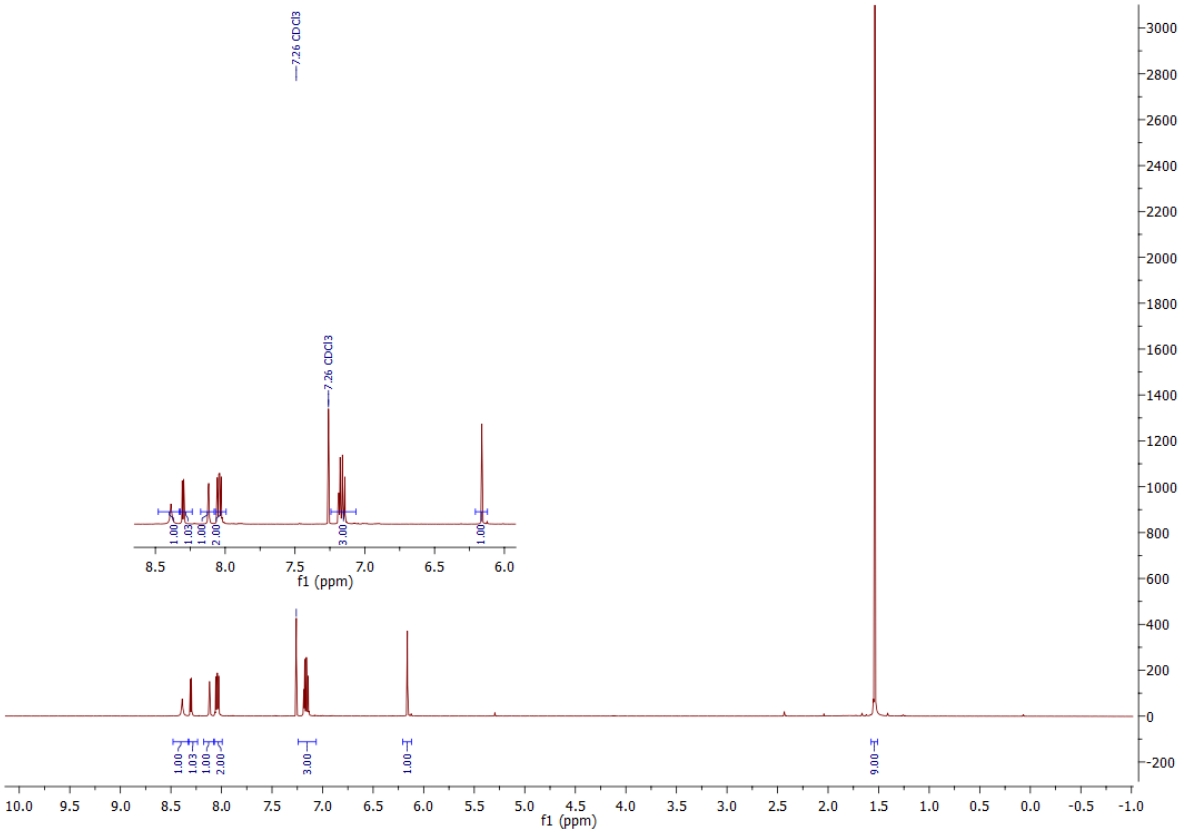



**Figure S8**: $^{1}$H NMR spectrum of compound **10** measured in deuterated chloroform.

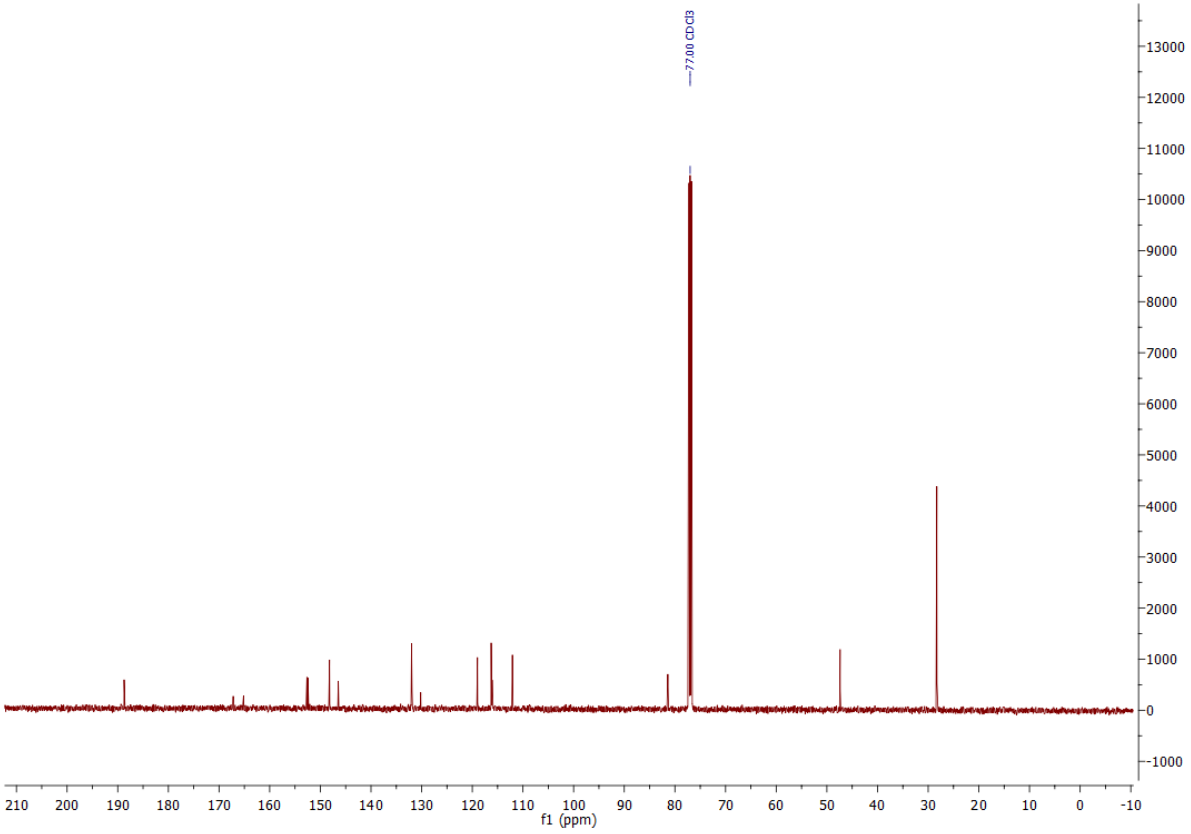



**Figure S9**: $^{13}$C NMR spectrum of compound **10** measured in deuterated chloroform.

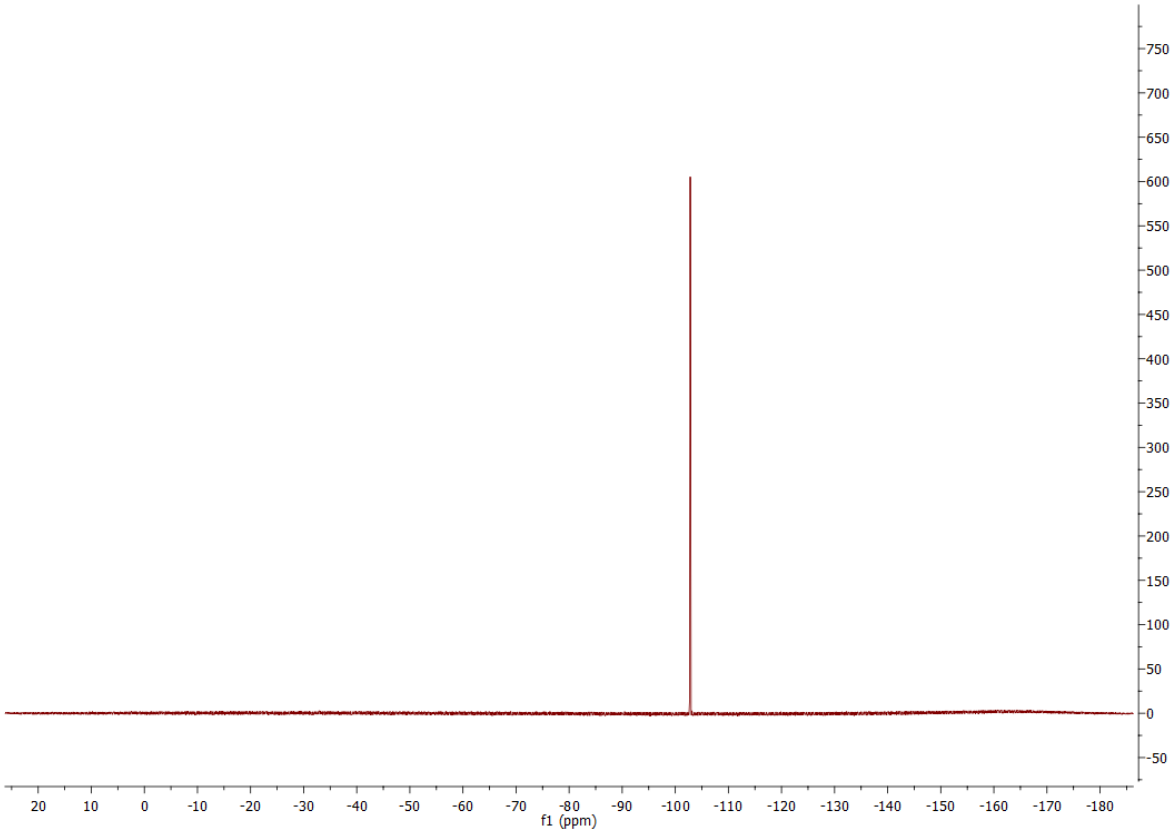
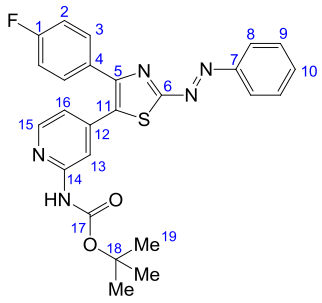



**Figure S10**: $^{19}$F NMR spectrum of compound **10** measured in deuterated chloroform.

**III.5 Synthesis of (*E*)-*tert*-butyl (4-(4-(4-fluorophenyl)-2-(phenyldiazenyl)thiazol-5-yl)pyridin-2-yl)carbamate (12)**



*tert*-Butyl (4-(1-bromo-2-(4-fluorophenyl)-2-oxoethyl)pyridin-2-yl) carbamate (**10**, 500 mg, 1.22 mmol) and 1-phenyl-thiocarbazide (**11**, 224 mg, 1.34 mmol) were dissolved in 10 mL dry THF under nitrogen atmosphere. After addition of 0.35 mL acetic acid the reaction mixture was heated for 30 min at 80 °C. Then another 0.35 mL acetic acid was added and the mixture was heated 1 h at 80 °C. After cooling to room temperature the reaction mixture was neutralized with saturated sodium hydrogen carbonate solution, diluted with 70 mL THF and stirred at room temperature for 2 h while air was bubbled through the solution. The reaction mixture was poured onto water, extracted with dichloromethane (3 x 50 mL), dried over magnesium sulfate, filtered and the solvent was evaporated in vacuo. The crude product was purified by column chromatography (dichloromethane) using basic aluminium oxide as stationary phase to yield an orange solid (241 mg, 0.51 mmol, 42 %).

**melting point:** 224 °C.

**$^{1}$H NMR** (500 MHz, $CDCl_3$): δ = 8.18, (dd, $^{3}J$ = 5.3 Hz, $^{5}J$ = 0.6 Hz, 1 H, *H*-15), 8.15 (s, 1 H, *H*-13), 8.07-8.02 (m, 2 H, *H*-8), 7.97 (s, 1 H, N*H*), 7.61 ($m_c$, 2 H, *H*-3), 7.58-7.53 (m, 3 H, *H*-9, *H*-10), 7.06 ($m_c$, 2 H, *H*-2), 6.86 (dd, $^{3}J$ = 5.3 Hz, $^{4}J$ = 1.6 Hz, 1 H, *H*-16) ppm.

**$^{13}$C NMR** (125 MHz, $CDCl_3$): δ = 175.4 (*C*-17), 163.1 (d, $^{1}J_{CF}$ = 248.3 Hz, *C*-1), 153.1 (*C*-12), 152.3 (*C*-6), 151.8 (*C*-7), 151.7 (*C*-5), 148.4 (*C*-15), 142.0 (*C*-14), 133.5 (*C*-10), 133.0 (*C*-11), 131.3 (d, $^{3}J_{CF}$ = 8.49 Hz, *C*-3), 130.1 (d, $^{4}J_{CF}$ = 3.6 Hz, *C*-4), 129.6 (*C*-9), 124.3 (*C*-8), 118.4 (*C*-16), 115.7 (d, $^{2}J_{CF}$ = 21.8 Hz, *C*-2), 112.4 (*C*-13), 81.6 (*C*-18), 28.4 (*C*-19) ppm.

**$^{19}$F NMR** (470 MHz, $CDCl_3$): δ = -112.4 ppm.

**IR** (ATR): ύ = 2969 (w), 1720 (s), 1604 (s), 1573 (s), 1525 (s), 1497 (m), 1452 (w), 1419 (s), 1404 (s), 1366 (m), 1296 (m), 1270 (s), 1222 (vs), 1151 (vs), 1122 (s), 1094 (m), 1057 (s), 1015 (m), 996 (m), 928 (w), 883 (m), 856 (s), 842 (vs) $cm^{-1}$.

**MS** (EI, 70 eV): m/z (%) = 123 (100) $[M\text{-}C_{11}H_{14}BrN_2O_2]^+$, 212 (19) $[M\text{-}C_{11}H_{13}O_2]^+$, 229 (80) $[M\text{-}C_5H_9O_2Br]^+$, 255 (6) $[M\text{-}C_4H_9OBr]^+$, 273 (12) $[M\text{-}C_4H_9Br]^+$, 329 (4) $[M\text{-}Br]^+$, 335 (3) $[M\text{-}C_4H_9O]^+$, 351 (2) $[M\text{-}C_4H_9]^+$, 408 (1) $[M]^+$.

**MS** (EI, HR, 70 eV): $C_{25}H_{22}FN_5O_2S$, m/z = calc.: 475.1478, found: 475.1470.

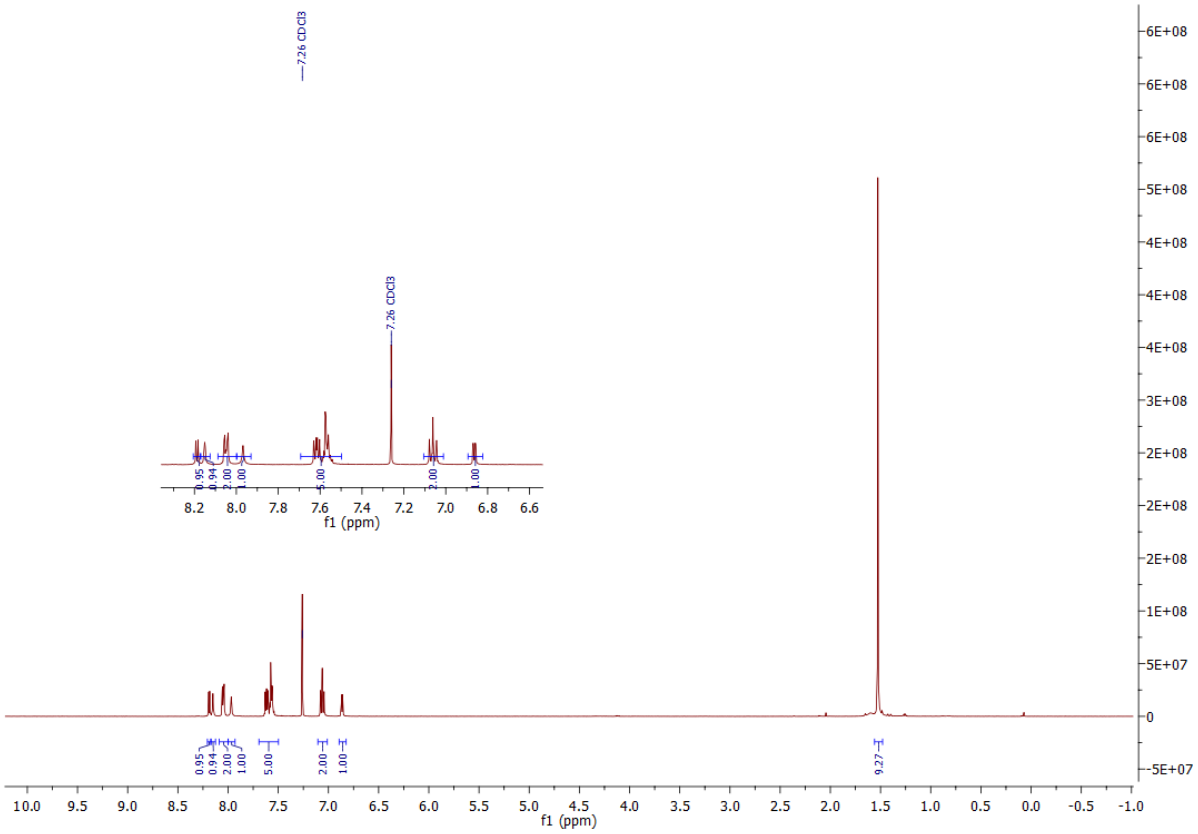



**Figure S11**: $^{1}$H NMR spectrum of compound **12** measured in deuterated chloroform.

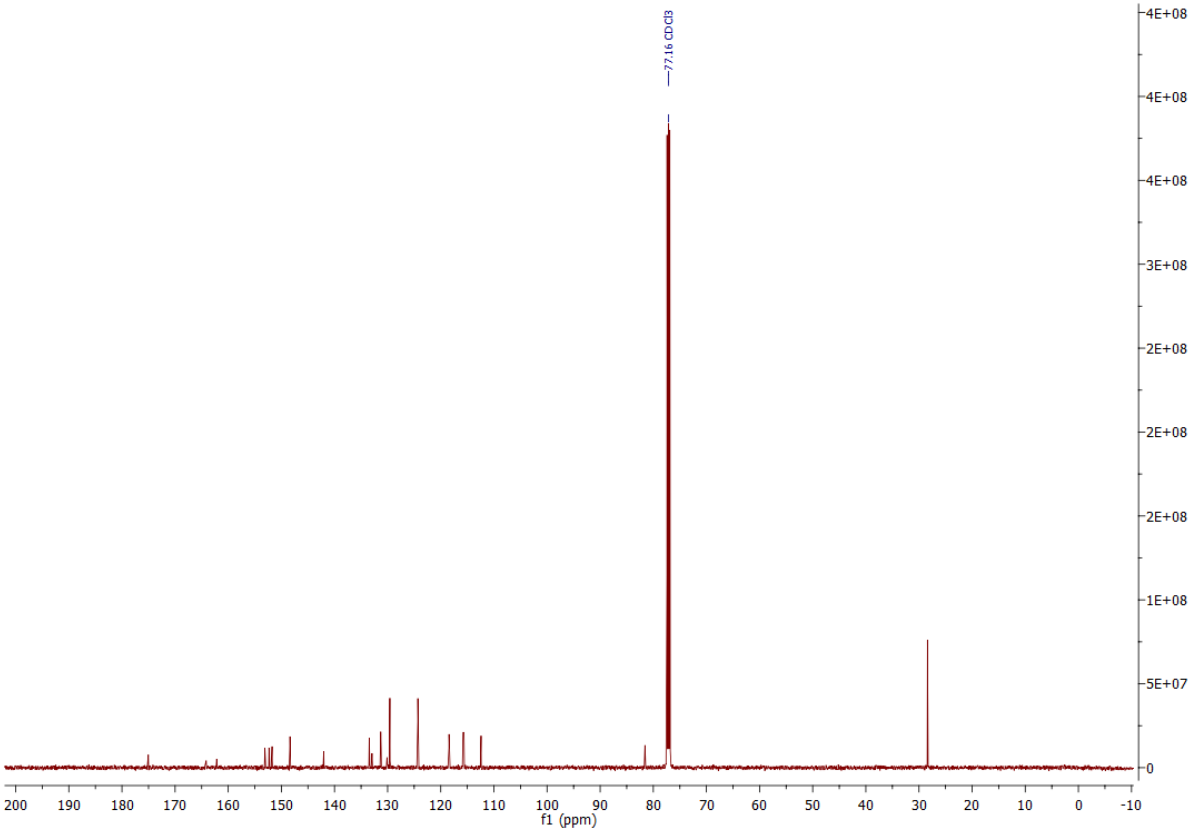



**Figure S12**: $^{13}$C NMR spectrum of compound **12** measured in deuterated chloroform.

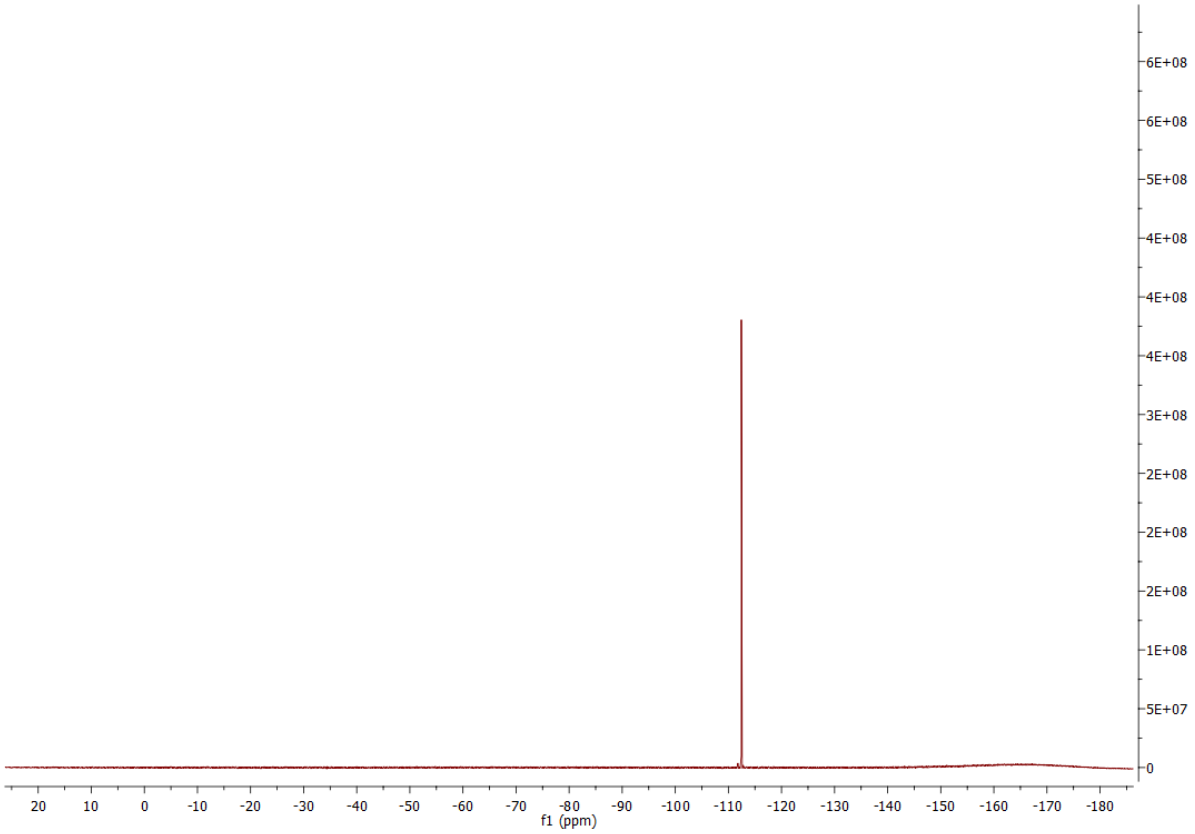
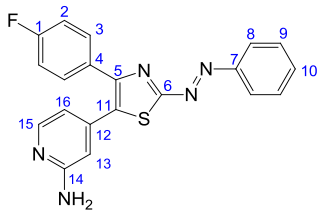



**Figure S13**: $^{19}$F NMR spectrum of compound **12** measured in deuterated chloroform.

### III.6 Synthesis of (*E*)-4-(4-(4-fluorophenyl)-2-(phenyldiazenyl)thiazol-5-yl)pyridin-2-amine (13)



(*E*)-*tert*-Butyl (4-(4-(4-fluorophenyl)-2-(phenyldiazenyl)thiazol-5-yl)pyridin-2-yl)carbamate (**12**, 201 mg, 0.42 mmol) was dissolved in 15 mL ethyl acetate, 10 mL of a 6 M hydrogen chloride solution was added and the reaction mixture was heated for 6 h at 55 °C. After cooling to room temperature the reaction mixture was neutralized with a 6 M sodium hydroxide solution and extracted with ethyl acetate (2 x 60 mL). The combined organic layers were dried over magnesium sulfate, filtered and the solvent was evaporated to yield a red, crystalline solid (153 mg, 0.41 mmol, 97 %).

**melting point:** 241 °C.

**$^{1}$H NMR** (600 MHz, $CDCl_3$): δ = 8.06 (d, $^{3}J$ = 5.1 Hz, 1 H, *H*-15), 8.05-8.02 (m, 2 H, *H*-8), 7.64 ($m_c$, 2 H, *H*-3), 7.16-7.11 (m, 3 H, *H*-9, *H*-10), 7.06 ($m_c$, 2 H, *H*-2), 6.64 (dd, $^{3}J$ = 5.1 Hz, $^{4}J$ = 1.5 Hz 1 H, *H*-16), 6.52 (s, 1 H, *H*-13), 4.54 (s, 2 H, $NH_2$) ppm.

**$^{13}$C NMR** (125 MHz, $CDCl_3$): δ = 174.7 (*C*-6), 163.1 (d, $^{1}J_{CF}$ = 249.9 Hz, *C*-1), 159.0 (*C*-12), 151.7 (*C*-7) 151.4 (*C*-5), 149.2 (*C*-15), 141.4 (*C*-14), 133.4 (*C*-11), 133.0 (*C*-10), 131.2 ( $^{3}J_{CF}$ = 8.0 Hz, *C*-3), 130.1 (*C*-4), 129.6 (*C*-9), 124.3 (*C*-8), 115.7 (d, $^{2}J_{CF}$ = 22.2 Hz, *C*-2), 114.4 (*C*-16), 108.3 (*C*-13) ppm.

**$^{19}$F NMR** (470 MHz, $CDCl_3$): δ = -112.3 ppm.

**IR** (ATR): ύ = 3408 (w), 3319 (m), 3214 (m), 1636 (s), 1597 (s), 1544 (s), 1527 (m), 1498 (m), 1453 (m), 1422 (s), 1302 (w), 1235 (s), 1154 (m), 1093 (w), 1073 (w), 1014 (m), 981 (m), 931 (w), 888 (w), 838 (vs), 810 (s), 775 (s), 755 (s), 687 (vs), 650 (w), 574 (s), 530 (s), 491 (vs), 468 (s), 444 (m), 419 (m) $cm^{-1}$.

**MS** (EI, HR, 70 eV): $C_{20}H_{14}FN_5S$, m/z = calc.: 375.0954, found: 375.0957.

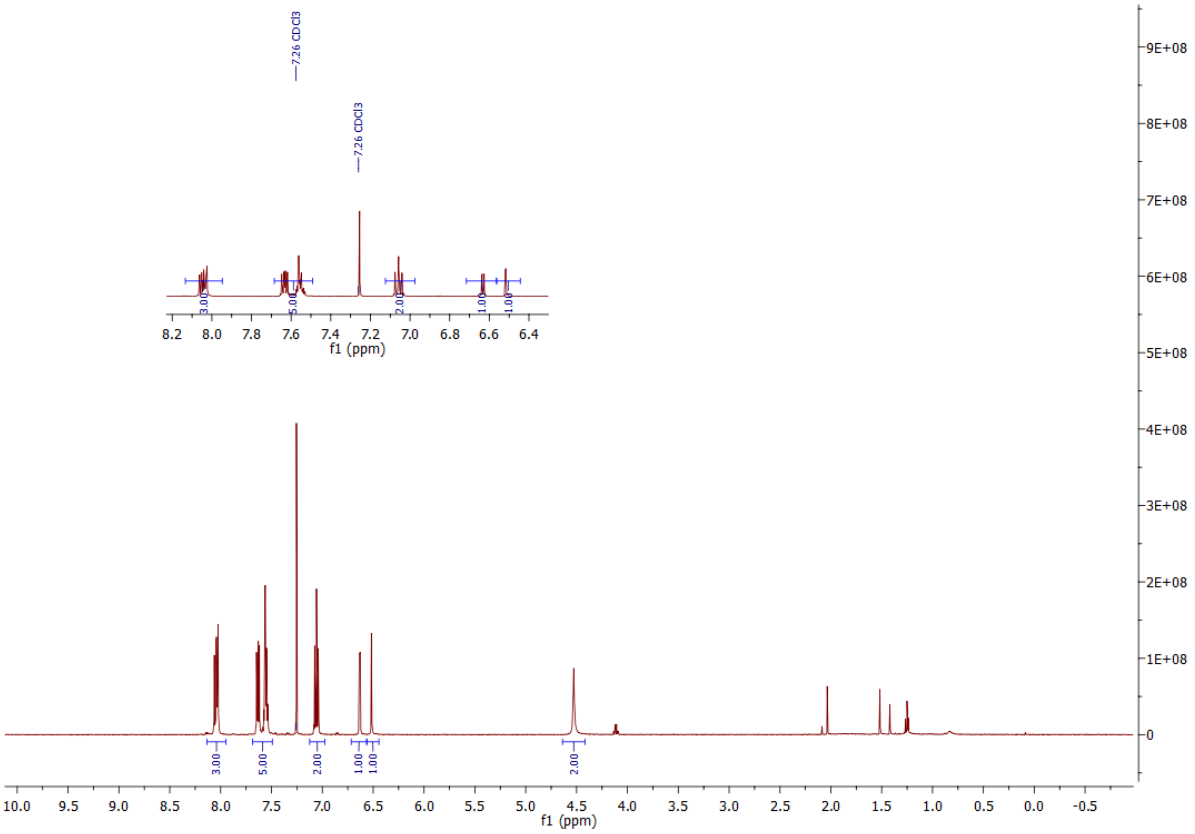



**Figure S14**: $^{1}$H NMR spectrum of compound **13** measured in deuterated chloroform.

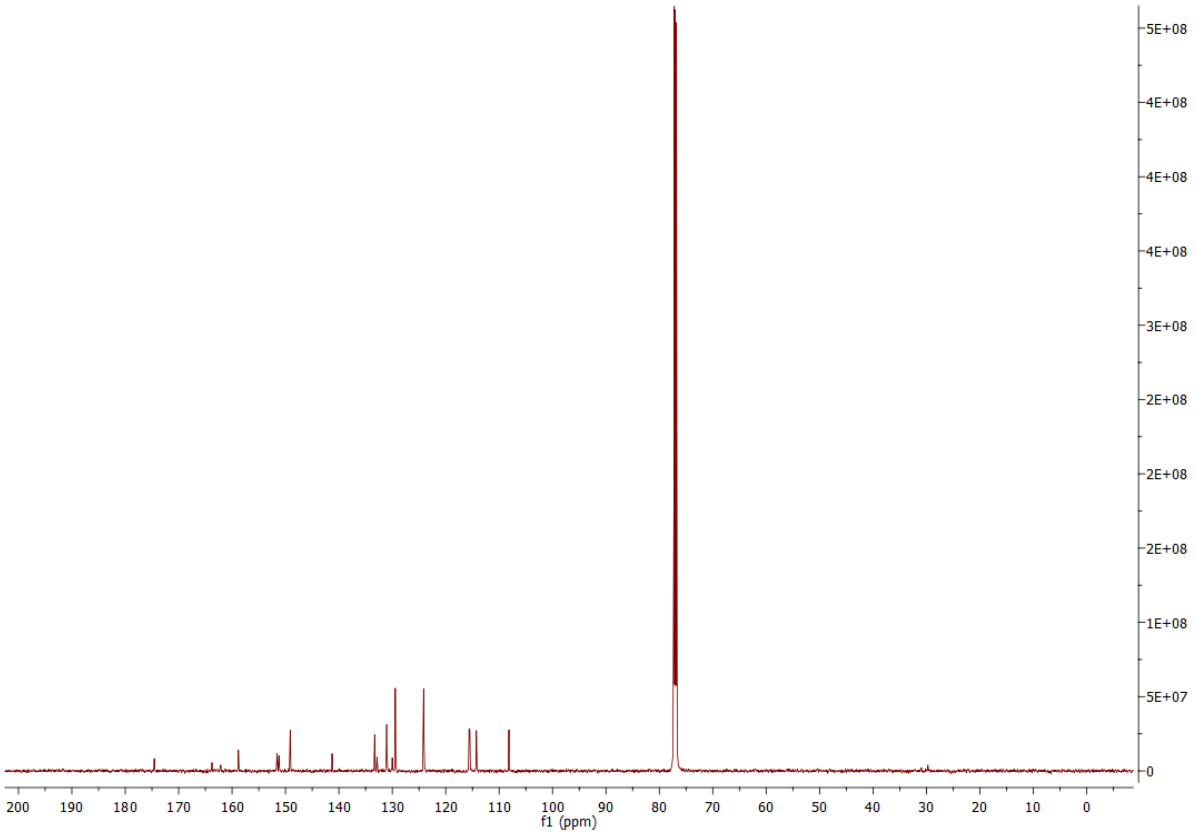



**Figure S15**: $^{13}$C NMR spectrum of compound **13** measured in deuterated chloroform.

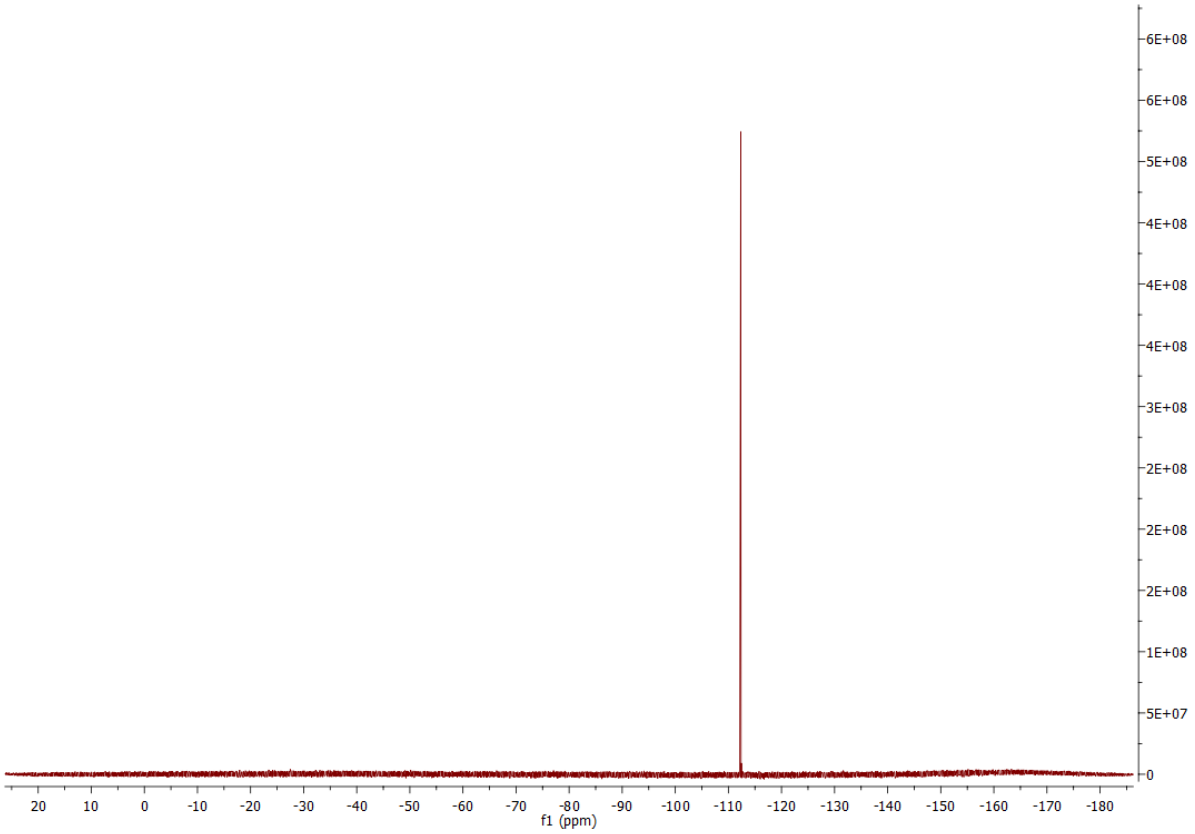
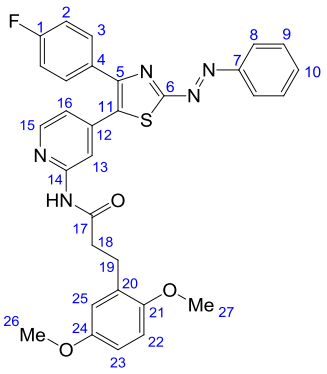



**Figure S16**: $^{19}$F NMR spectrum of compound **13** measured in deuterated chloroform.

**III.7 Synthesis of (*E*)-3-(2,5-dimethoxyphenyl)-N-(4-(4-(4-fluorophenyl)-2-(phenyldiazenyl)-thiazol-5-yl)pyridin-2-yl)propanamide (2)**




(*E*)-4-(4-(4-Fluorophenyl)-2-(phenyldiazenyl)thiazol-5-yl)pyridin-2-amine (**13**, 144 mg, 0.38 mmol) and 3-(2,5-dimethoxyphenyl)propionic acid (**14**, 122 mg, 0.58 mmol) were dissolved in 15 mL dry ethyl acetate under nitrogen atmosphere. T3P® (0.87 mL, 1.46 mmol, 50 % in ethyl acetate) and DIPEA (0.27 mL, 1.59 mmol) were added and the reaction mixture was refluxed for 24 h. After cooling to room temperature the reaction mixture was diluted with water and extracted with ethyl acetate (3 x 50 mL). The combined organic layers were washed with 70 mL saturated sodium chloride solution, dried over magnesium sulfate, filtered and the solvent was evaporated. The crude product was filtered over silica gel (cyclohexane/ethyl acetate, 1:1) and then purified by flash column chromatography (cyclohexane/ethyl acetate, ethyl acetate: 12 % → 100 %) on silica. The product fraction was recrystallized from acetonitrile to yield an orange solid (104 mg, 0.18 mmol, 48 %).

**melting point:** 177 °C.

**$R_f$:** 0.42 (cyclohexane/ethyl acetate, 1:1).

**$^1$H NMR** (500 MHz, $CDCl_3$): δ = 8.44 (s, 1 H, *H*-13), 8.24 (s, 1 H, N*H*), 8.16 (dd, $^3J$ = 5.1 Hz, $^5J$ = 0.6 Hz 1 H, *H*-15), 8.07-8.03 (m, 2 H, H-8), 7.61 ($m_c$, 2 H, H-3), 7.59-7.53 (m, 3 H, *H*-9, *H*-10), 7.06 ($m_c$, 2 H, *H*-2), 6.92 (dd, $^3J$ = 5.1 Hz, $^4J$ = 1.6 Hz 1 H, *H*-16), 6.80-7.75 (m, 2 H, *H*-22, *H*-25), 6.75-6.70 (m, 1 H, *H*-23), 3.80 (s, 3 H, *H*-27), 3.74 (s, 3 H, *H*-26), 3.01 ($m_c$, 2 H, *H*-19), 2.69 (m*c*, 2 H, *H*-18) ppm.

**$^{13}$C NMR** (125 MHz, $CDCl_3$): δ = 175.2 (*C*-6), 171.5 (*C*-17), 163.2 (d, $^1J_{CF}$ = 248.5 Hz, *C*-1), 153.7 (*C*-24), 152.4 (*C*-14), 151.8 (*C*-7), 151.7 (*C*-21, *C*-5), 148.3 (*C*-15), 142.2 (*C*-12), 133.5 (*C*-10), 132.7 (*C*-11), 131.3 ( $^3J_{CF}$ = 8.3 Hz, *C*-3), 130.1 ( $^4J_{CF}$ = 3.3 Hz, *C*-4), 129.8 (*C*-20), 129.58 (*C*-9), 124.3 (*C*-8), 119.6 (*C*-16), 116.6 (*C*-25), 115.8 (d, $^2J_{CF}$ = 22.0 Hz, *C*-2), 114.0 (*C*-13), 112.0 (*C*-23), 111.3 (*C*-22), 55.9 (*C*-27), 55.8 (*C*-26), 33.0 (*C*-18), 26.7 (*C*-19) ppm.

**$^{19}$F NMR** (470 MHz, $CDCl_3$): δ = -112.2 ppm.

**IR** (ATR): ύ = 3264 (w), 2943 (w), 2829 (w), 2167 (w), 1695 (m), 1602 (m), 1558 (m), 1530 (m), 1502 (s), 1418 (s), 1313 (w), 1289 (s), 1219 (vs), 1173 (m), 1148 (m), 1047 (s), 966 (w), 903 (w), 840 (s), 802 (s), 810 (s), 773 (s), 685 (s), 649 (w), 624 (w), 574 (m), 535 (m), 515 (w), 500 (w), 460 (w), 420 (w), 404 (w) $cm^{-1}$.

**MS** (MALDI-MS-TOF): m/z = 568 $[M+1]^+$.

**MS** (EI, HR, 70 eV): $C_{31}H_{26}FN_5O_3S$, m/z =calc.: 567.1740, found: 567.1738.

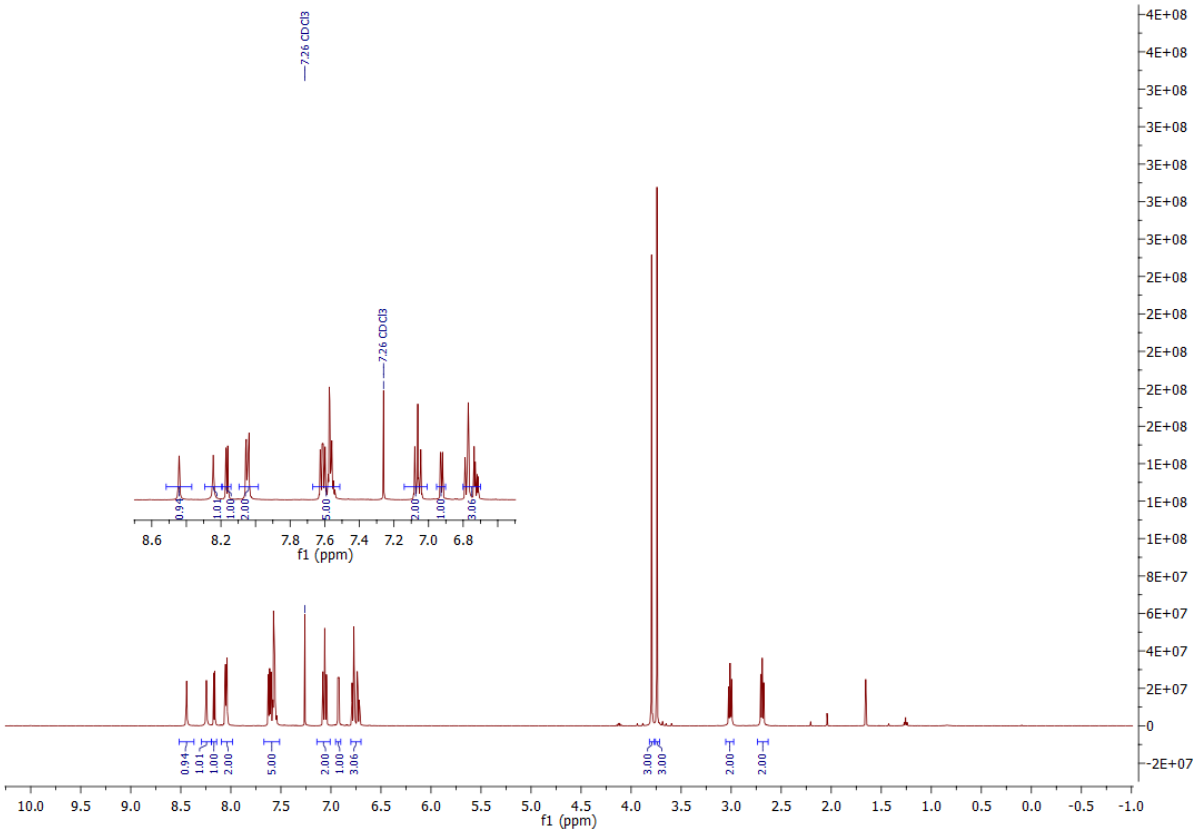



**Figure S17**: $^{1}$H NMR spectrum of compound **2** measured in deuterated chloroform.

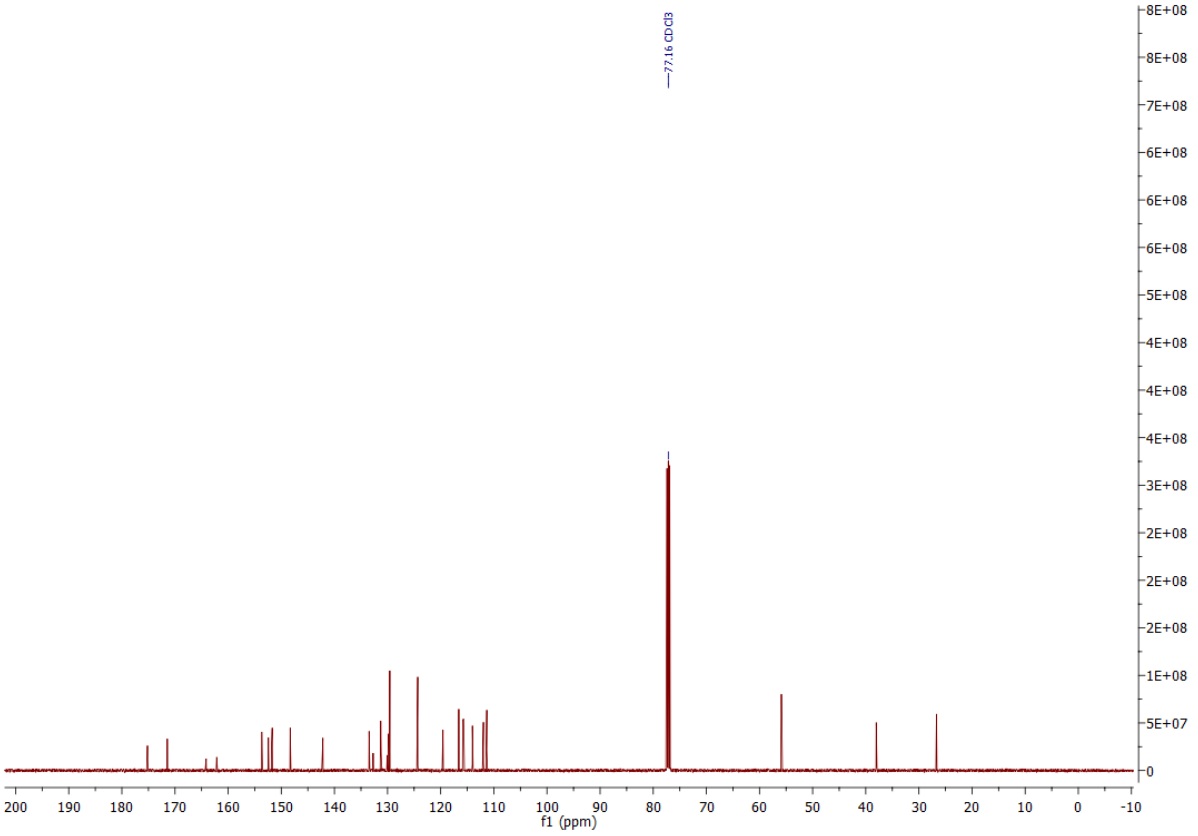



**Figure S18**: $^{13}$C NMR spectrum of compound **2** measured in deuterated chloroform.

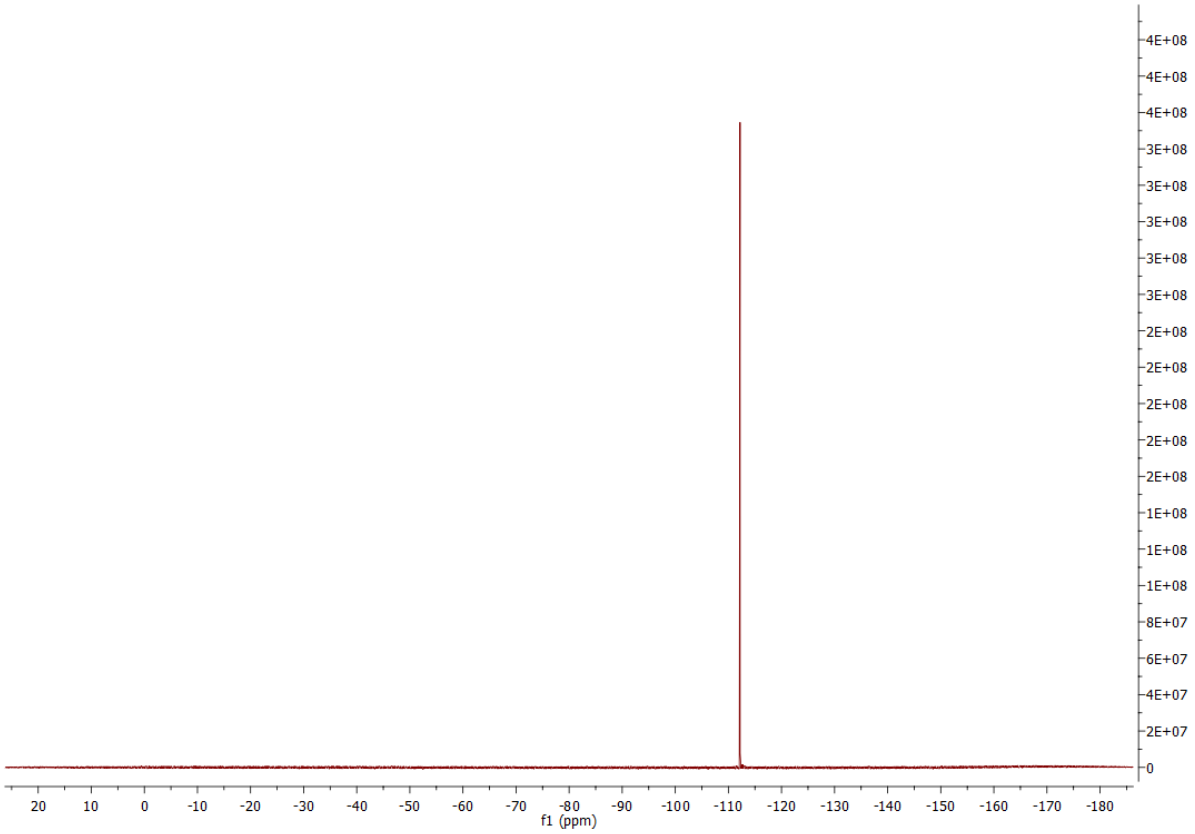
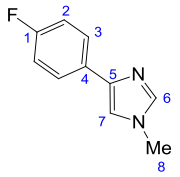



**Figure S19**: $^{19}$F NMR spectrum of compound **2** measured in deuterated chloroform.

### III.8 Synthesis of 4-(4-fluorophenyl)-1-methyl-1*H*-imidazole (17)




(4-Fluorophenyl)boronic acid (**16**, 347 mg, 2.48 mmol), Pd[dppf]$Cl_2$ (36 mg, 0.05 mmol, 4 mol %), caesium fluoride (283 mg, 1.86 mmol) and benzyltriethylammonium chloride (23 mg, 0.10 mmol, 4 mol %) were placed in a flask under nitrogen evacuated and refilled with nitrogen gas three times. Bromo-1-methyl-1*H*-imidazole (**15**, 200 mg, 1.24 mmol) and 10 mL of a 1:1 mixture of toluene and water was added and the mixture was stirred at 90 °C for 24 h. After cooling to room temperature the reaction mixture was diluted with 20 mL water and extracted with ethyl acetate (2 x 50 mL). The combined organic layers were dried over magenisum sulfate, filtered and the solvent was evaporated. The crude product was filtered over silica gel (ethyl acetate) and then purified by flash column chromatography (cyclohexane/ethyl acetate, ethyl acetate: 18 % → 100 %) on silica to obtain a pale powder (183 mg, 1.04 mmol, 84 %).

**melting point:** 121 °C.

**$R_f$:** 0.25 (cyclohexane/ethyl acetate, 1:3).

**$^1$H NMR** (500 MHz, $CDCl_3$): δ = 7.70 ($m_c$, 2 H, *H*-3), 7.43 (s, 1 H, *H*-6), 7.08 (d, $^4J$ = 1.3 Hz, 1 H, *H*-7), 7.04 ($m_c$, 2 H, H-2), 3.68 (s, 3 H, H-8) ppm.

**$^{13}$C NMR** (125 MHz, $CDCl_3$): δ = 162.0 (d, $^1J_{CF}$ = 245 Hz, *C*-1), 141.7 (*C*-5), 138.1 (*C*-6), 130.6 ($^4J_{CF}$ = 3.1 Hz, *C*-4), 126.4 (d, $^3J_{CF}$ = 7.9 Hz, *C*-3), 115.7 (*C*-7), 115.5 (d, $^2J_{CF}$ = 21.6 Hz, *C*-2), 33.6 (*C*-8) ppm.

**$^{19}$F NMR** (470 MHz, $CDCl_3$): δ = -116.1 ppm.

**IR** (ATR): ύ = 2949 (w), 2364 (w), 1597 (w), 1558 (m), 1508 (m), 1489 (s), 1423 (w), 1356 (w), 1307 (w), 1291 (w), 1206 (s), 1154 (s), 1091 (m), 1062 (m), 1043 (w), 1013 (w), 942 (s), 837 (s), 825 (vs) 812 (s), 786 (m), 767 (s), 764 (s), 745 (s), 722 (w), 696 (s), 631 (m), 629 (w), 616 (s), 609 (s), 589 (s), 567 (m), 523 (m), 516 (m) $cm^{-1}$.

**MS** (EI, 70 eV): m/z (%) = 176 (100) $[M]^+$, 161 (90) $[M\text{-}CH_3]^+$.

**MS** (EI, HR, 70 eV): $C_{10}H_9FN_5$, m/z =calc.: 176.0750, found: 176.0748.

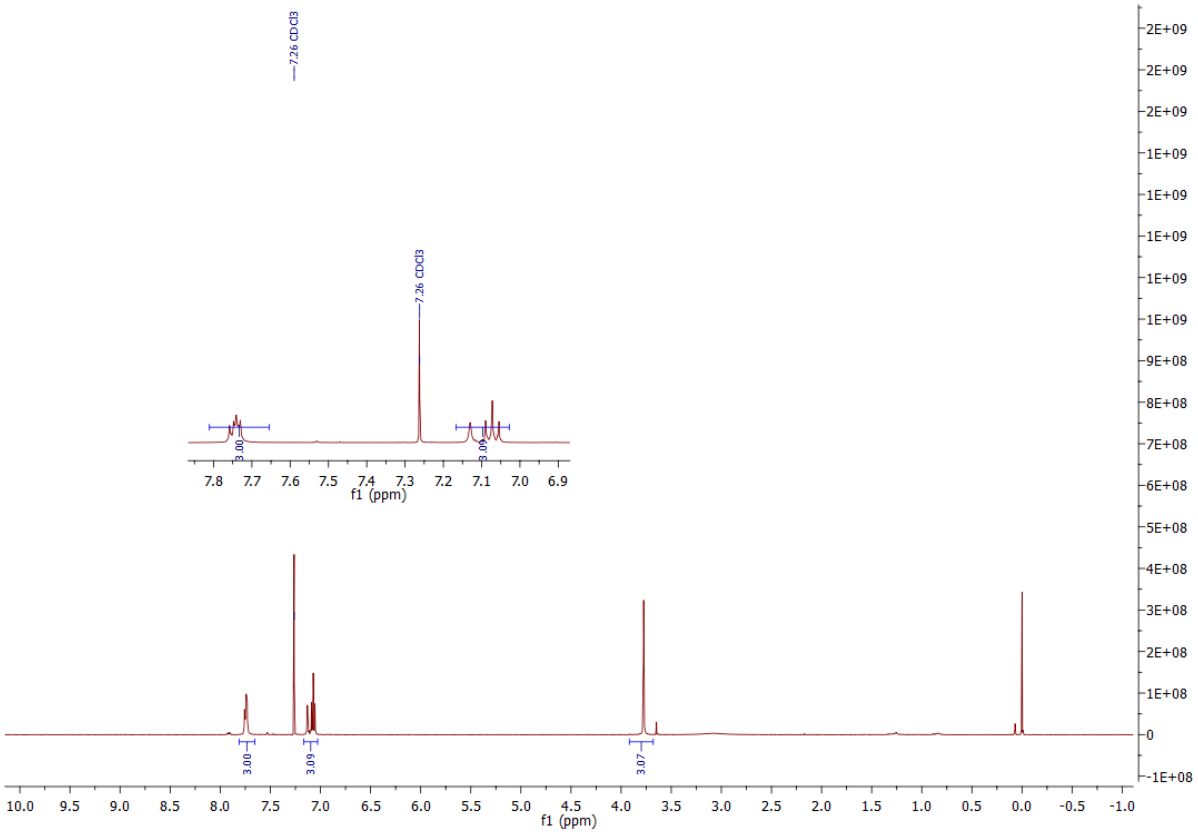



**Figure S20**: $^{1}$H NMR spectrum of compound **17** measured in deuterated chloroform.

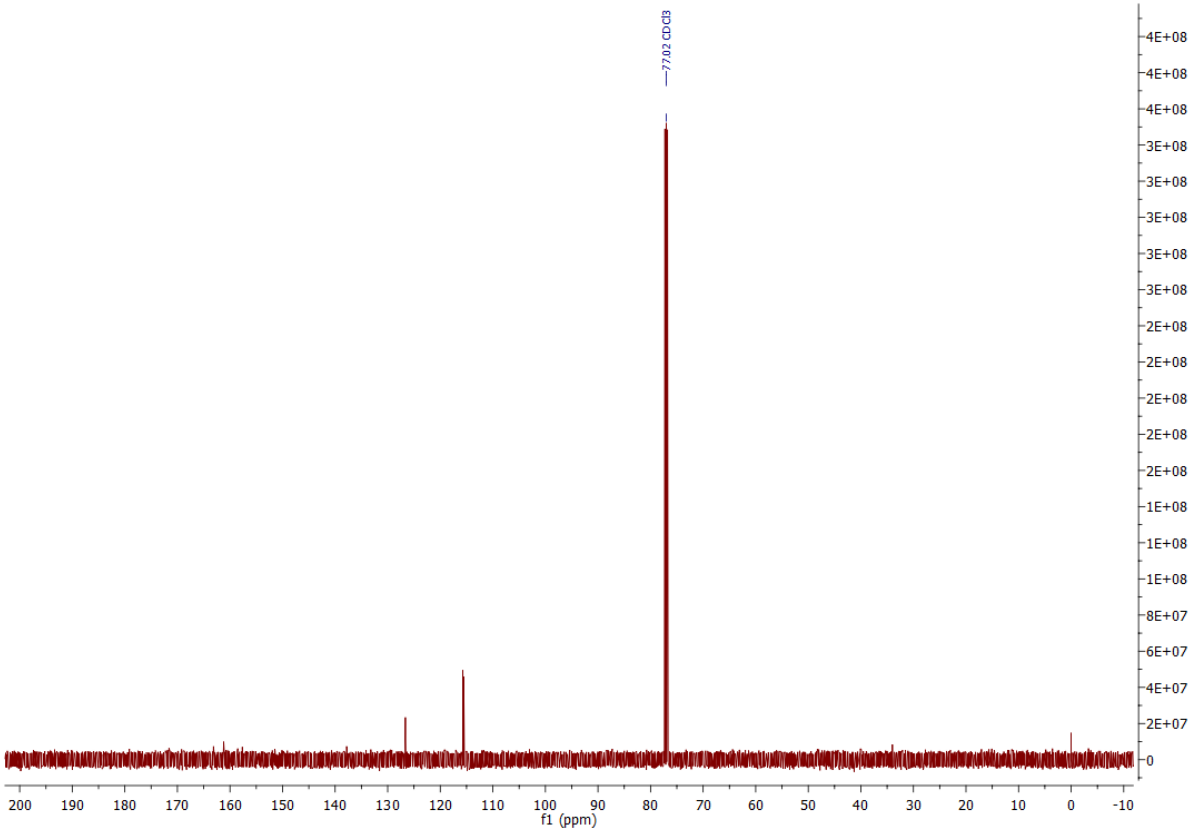



**Figure S21**: $^{13}$C NMR spectrum of compound **17** measured in deuterated chloroform.

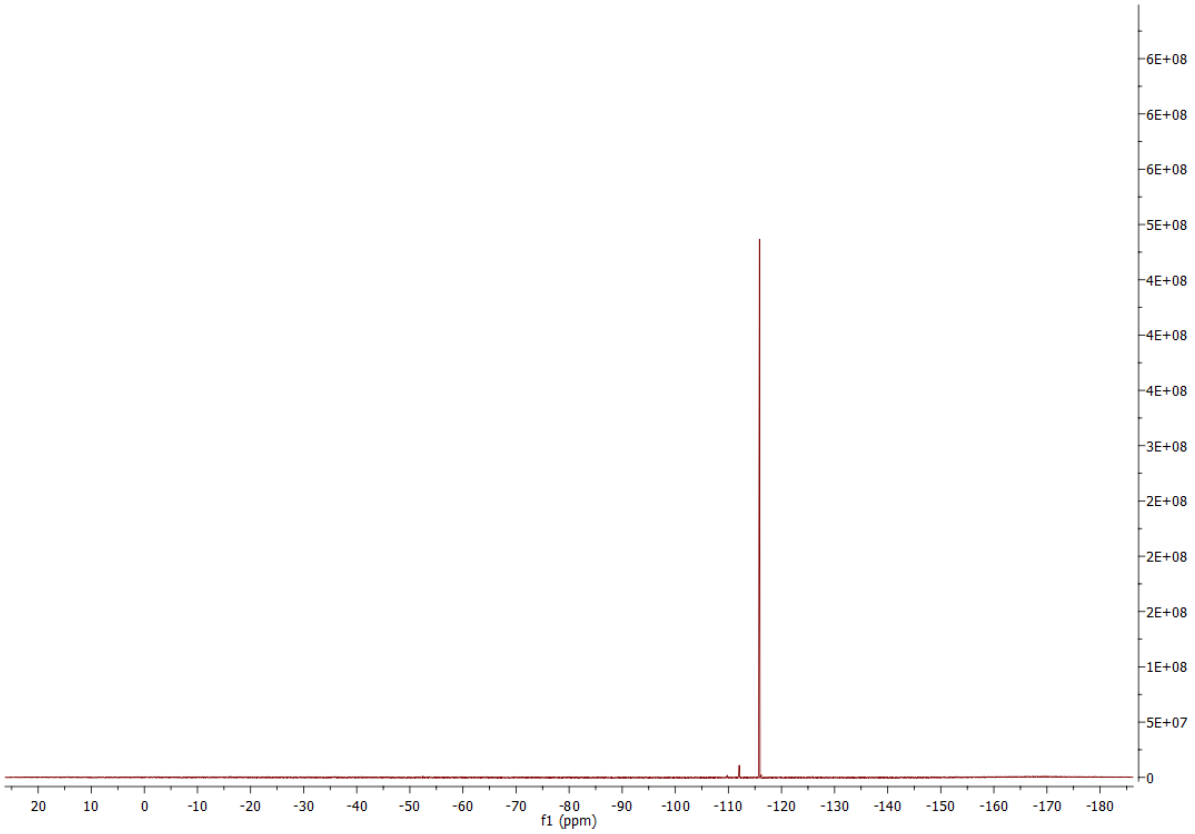
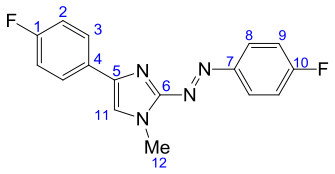



**Figure S22**: $^{19}$F NMR spectrum of compound **17** measured in deuterated chloroform.

### III.9 Synthesis of (*E*)-4-(4-fluorophenyl)-2-((4-fluorophenyl)diazenyl)-1-methyl-1*H*-imidazole (19)




4-(4-Fluorophenyl)-1-methyl-1*H*-imidazole (**17**, 322 mg, 1.83 mmol) was dissolved in 15 mL dry THF under nitrogen atmosphere and cooled to -78 °C. *n*-BuLi (0.73 mL, 1.83 mmol, 2.5 M in *n*-hexane) was added dropwise and the mixture was stirred for 30 min at -78 °C. 4-Fluoro-benzenediazonium tetrafluoroborat (**18**, 225 mg, 1.17 mmol) was added and the reaction mixture was stirred for 18 h, while slowly allowed to warm to room temperature. The reaction mixture was diluted with 15 mL saturated sodium bicarbonate solution and the organic layer was washed with saturated brine solution (3 x 20 mL). The mixture was then extracted with DCM (3 x 30 mL), dried over magnesium sulfate, filtered and the solvent was evaporated. Purified twice by flash column chromatography (cyclohexane/ethyl acetate, ethyl acetate: 6 % → 50 %, DCM/*n*-pentane, DCM: 12 % → 100 % ) to yield an orange, crystalline solid (100 mg, 0.34 mmol, 19 %).

**melting point:** 208 °C.

**$R_f$:** 0.16 (cyclohexane/ethyl acetate, 3:1).

**$^1$H NMR** (500 MHz, acetone-$d_6$): δ = 8.05 ($m_c$, 2 H, *H*-8), 7.96 ($m_c$, 2 H, *H*-3), 7.93 (s, 1 H, *H*-11), 7.36 (m*c*, 2 H, *H*-9), 7.18 (m*c*, 2 H, *H*-2), 4.14 (s, 3 H, ($m_c$, 2 H, *H*-12) ppm.

**$^{13}$C NMR** (125 MHz, acetone-$d_6$): δ = 165.3 (d, $^1J_{CF}$ = 251 Hz, *C*-10), 163.2 (d, $^1J_{CF}$ = 244 Hz, *C*-1), 153.1 (*C*-6), 151.2 (d, $^4J_{CF}$ = 3.1 Hz, *C*-7), 142.3 (*C*- 4), 131.5 (d, $^5J_{CF}$ = 3.1 Hz, *C*-5), 127.7 (d, $^3J_{CF}$ = 8.0 Hz, *C*-3), 125.8 (d, $^3J_{CF}$ = 9.0 Hz, *C*-8), 122.0 (*C*-11), 117.1 (d, $^2J_{CF}$ = 22.9 Hz, *C*-9), 116.2 ($^2J_{CF}$ = 21.9 Hz, *C*-2), 33.6 (*C*-12) ppm.

**$^{19}$F NMR** (470 MHz, acetone-$d_6$): δ = -110.8, -116.6 ppm.

**IR** (ATR): ύ = 2251 (w), 2007 (w), 1900 (w), 1589 (m), 1551 (m), 1500 (m),1425 (m), 1404 (m), 1307 (m), 1289 (m), 1274 (m), 1214 (s), 1155 (m), 1138 (m),1091 (m), 1053 (m), 1006 (m), 958 (m), 929 (m), 840 (vs), 812 (m), 773 (s), 727 (m), 718 (m), 687 (m)$cm^{-1}$.

**MS** (EI, 70 eV): m/z (%) = 298 (19) $[M]^+$, 175 (3) $[M\text{-}CH_6FN_2]^+$, 95 (100) $[M\text{-}C_{10}H_8FN_4]^+$.

**MS** (EI, HR, 70 eV): $C_{16}H_{12}F_2N_4$, m/z =calc.: 298.1030, found: 298.1024.

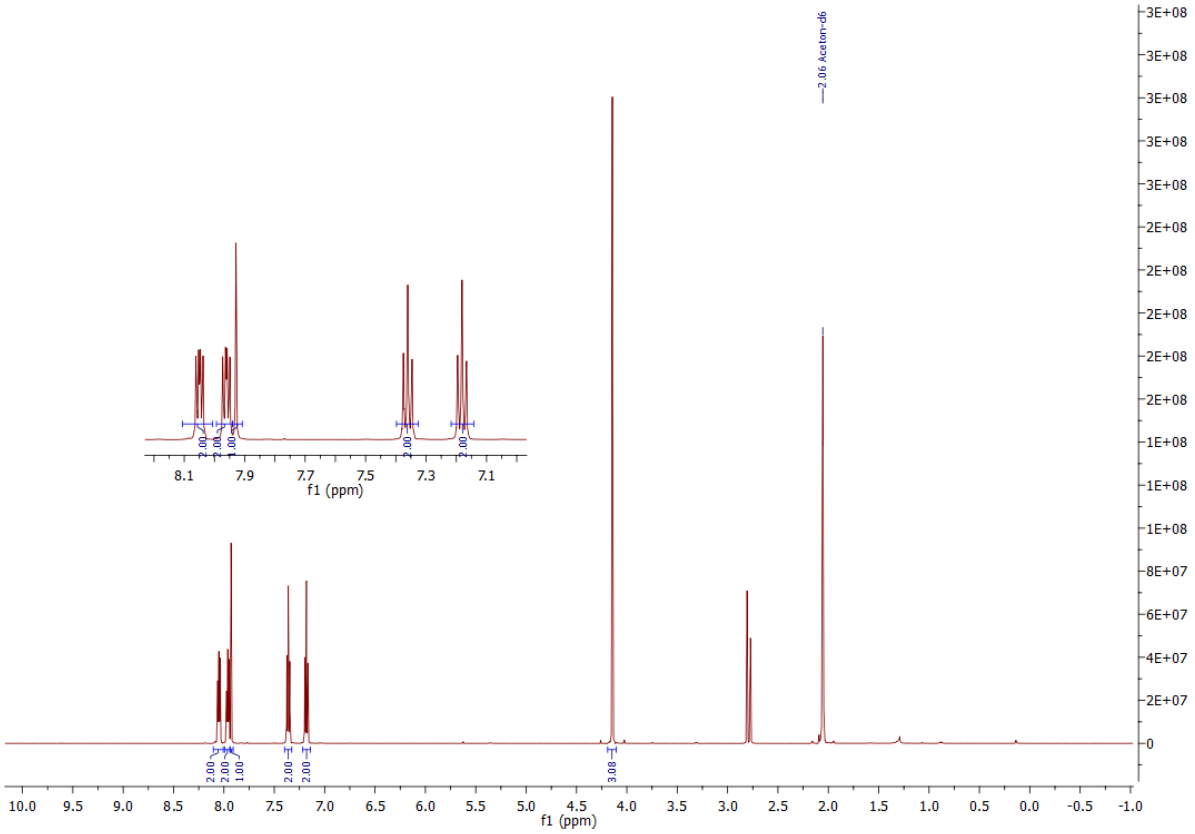



**Figure S23**: $^{1}$H NMR spectrum of compound **19** measured in deuterated acetone

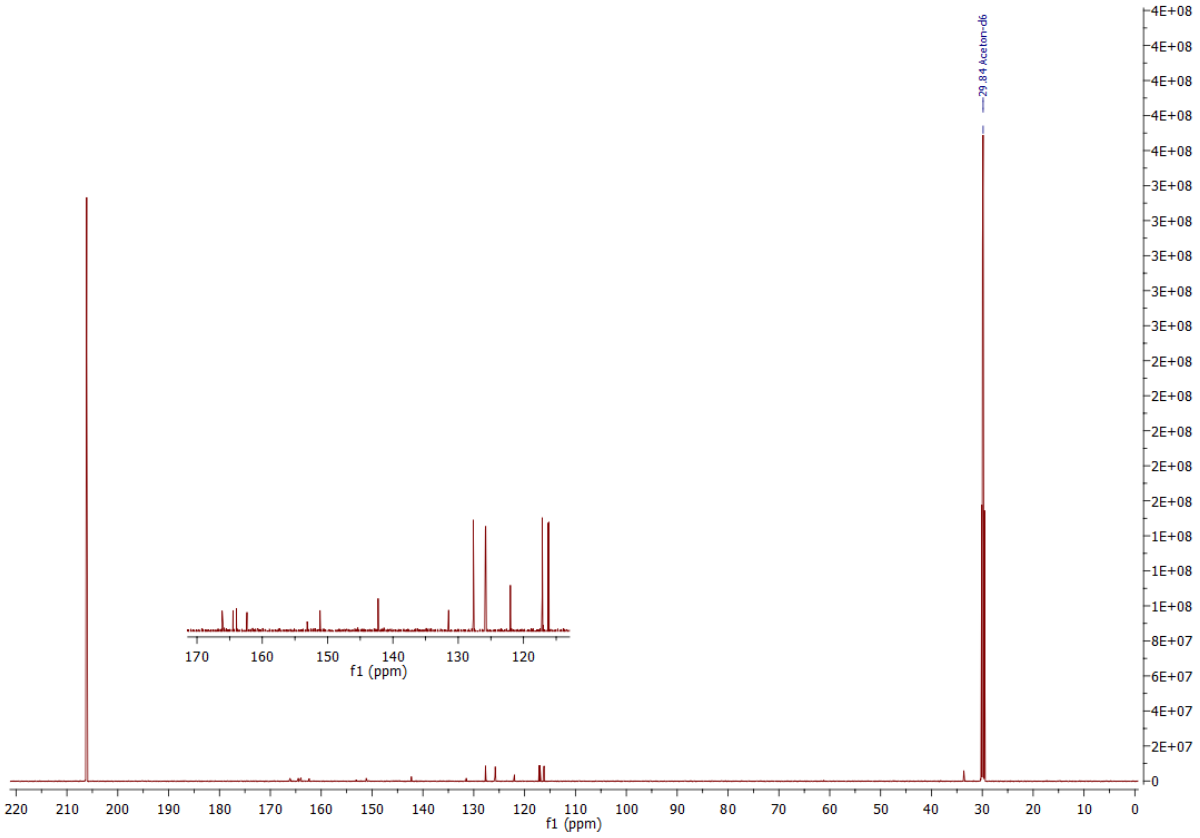



**Figure S24**: $^{13}$C NMR spectrum of compound **19** measured in deuterated acetone.

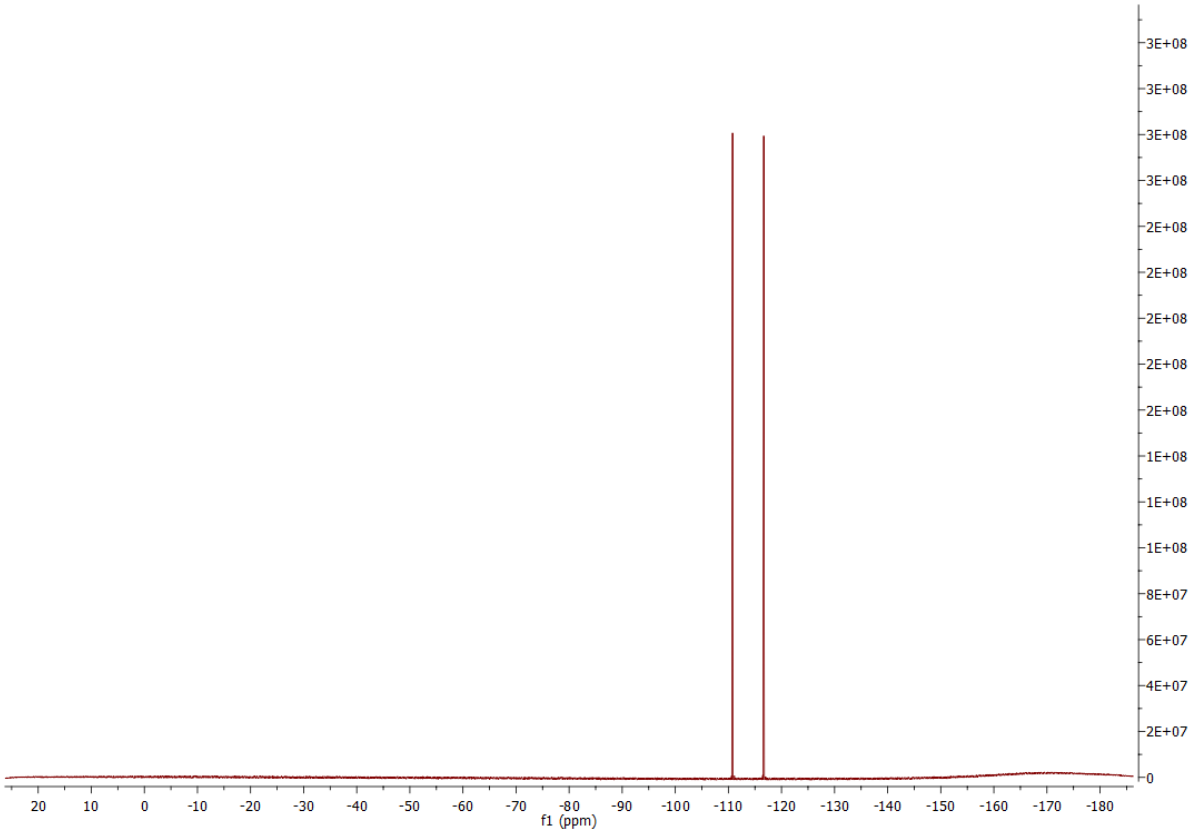
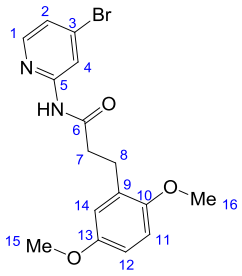



**Figure S25**: $^{19}F$ NMR spectrum of compound **19**.

### III.10 Synthesis of *N*-(4-bromopyridin-2-yl)-3-(2,5-dimethoxyphenyl)propanamide (21)




2-Amino-4-bromopyridine (**20**, 412 mg, 2.38 mmol) and 3-(2,5-Ddimethoxyphenyl)propioic acid (**14**, 500 mg, 2.38 mmol) were dissolved in 60 mL dry ethyl acetate. T3P® (5.68 mL, 9.5 mmol, 50% solution in ethyl acetate) and DIPEA (1.75 mL, 10.3 mmol) were added and the solution was stirred for 23 h at 85 °C. After cooling to room temperature the mixture was diluted with 50 mL water, the organic layer separated and the water layer was extracted with 50 mL ethyl acetate. The combined organic layers were washed with 100 mL saturated brine solution, dried over magnesium sulfate, filtered and the solvent was evaporated. The crude product was filtered over silica (cyclohexane/ethyl acetate, 1:1) and recrystallized from cyclohexane/methanol (1:2) to yield colourless crystals (498 mg, 1.36 mmol, 57 %).

**melting point:** 133 °C.

**$R_f$:** 0.49 (cyclohexane/ethyl acetate, 1:1).

**$^1$H NMR** (500 MHz, $CDCl_3$): δ = 8.50 (bs, 1 H, *H*-4), 8.11 (bs, 1 H, N*H*), 8.05 (d, $^3J$ = 5.3 Hz, 1 H, *H*-1), 7.18 (dd, $^3J$ = 5.3 Hz, $^4J$ = 1.5 Hz, 1 H, *H*-2), 6.79-6.75 (m, 2 H, *H*-11, *H*-14), 6.72 (dd, $^3J$ = 8.7 Hz, $^4J$ = 3.1 Hz, 1 H, *H*-12), 3.80 (s, 3 H, *H*-16), 3.74 (s, 3 H, *H*-15), 3.00 ($m_c$, 2 H, *H*-8), 2.68 ($m_c$, 2 H, *H*-7) ppm.

**$^{13}$C NMR** (125 MHz, $CDCl_3$): δ = 171.3 (*C*-6), 153.6 (*C*-13), 152.0 (*C*-5), 151.6 (*C*-10), 148.1 (*C*-1), 134.7 (*C*-3), 129.6 (*C*-9), 123.0 (*C*-2), 117.1 (*C*-4), 116.4 (*C*-14), 111.9 *C*-12), 111.2 (*C*-11), 55.8 (*C*-15), 55.7 (*C*-16), 37.9 (*C*-7), 26.6 (*C*-8) ppm.

**IR** (ATR): ύ = 3237 (w), 3199 (w), 3067 (w), 2996 (w), 2961 (w), 2837 (w), 1692 (s), 1575 (vs), 1520 (s), 1473 (w), 1463 (m), 1445 (m), 1427 (m), 1405 (s), 1366 (m), 1294 (m), 1278 (m), 1252 (m), 1221 (s), 1168 (s), 1125 (m), 1108 (w), 1094 (m), 1054 (m), 1030 (s), 1006 (m), 962 (w), 931 (m), 894 (w), 867 (m), 797 (s), 761 (m), 738 (m), 708 (m), 690 (vs) $cm^{-1}$.

**MS** (EI, 70 eV): m/z (%) = 364 (37) $[M]^+$, 334 (88) $[M\text{-}CH_3O]^+$, 172 (74) $[M\text{-}C_{11}H_{14}O_3]^+$.

_.
**MS** (EI, HR, 70 eV): $C_{16}H_{12}F_2N_4$, m/z = calc.: 364.0423, found: 364.0434.

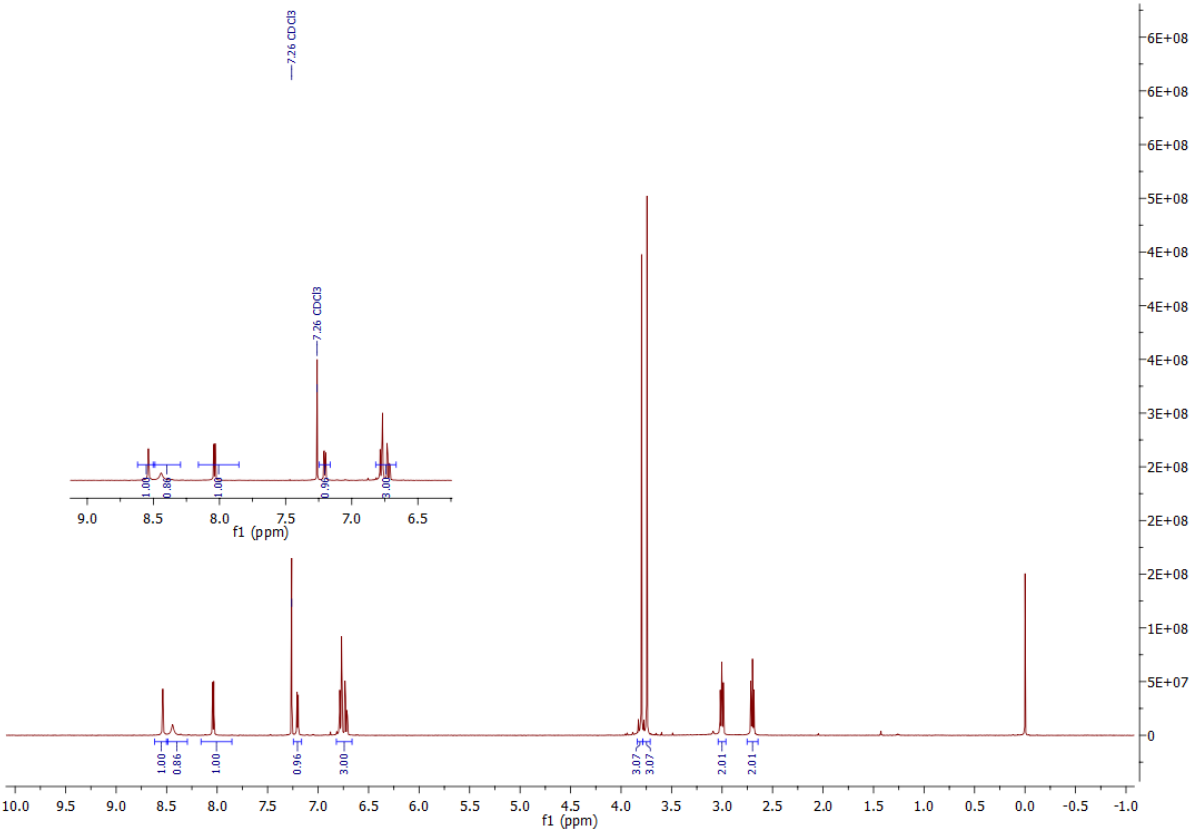



**Figure S26**: $^{1}$H NMR spectrum of compound **21** measured in deuterated chloroform.

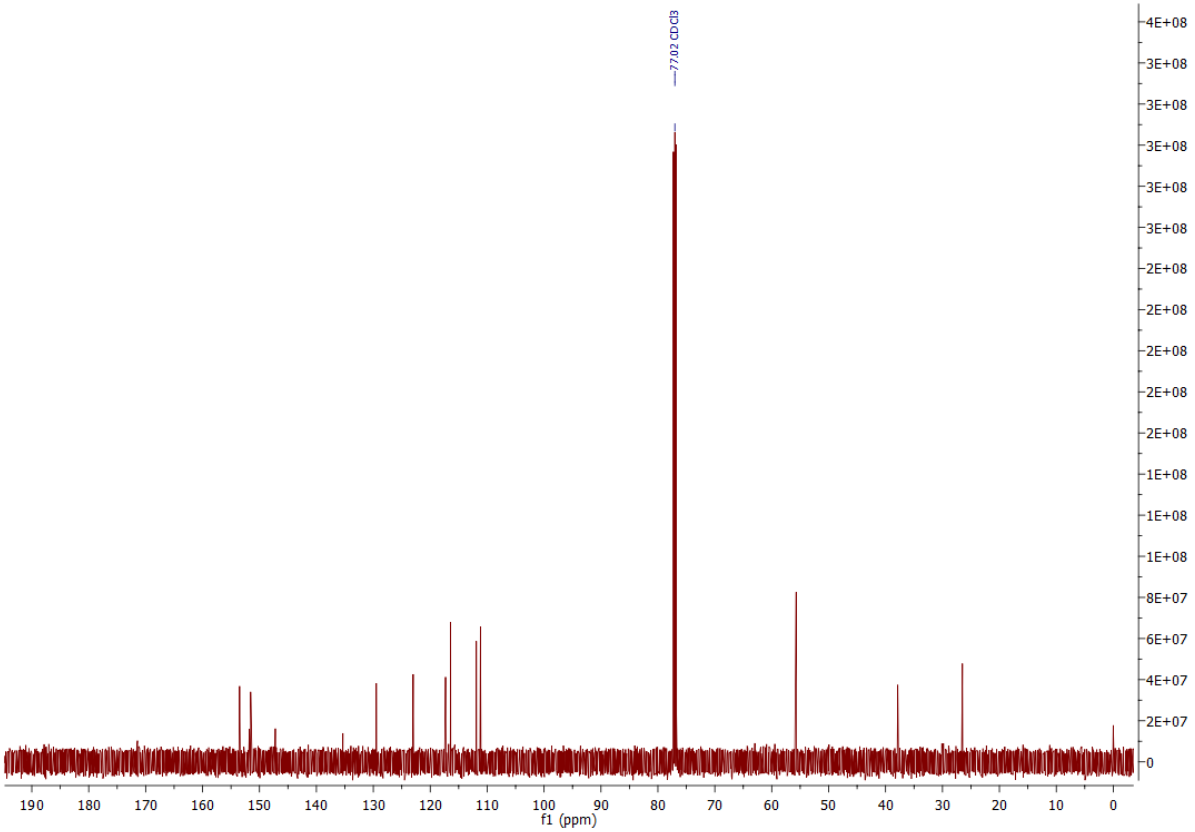
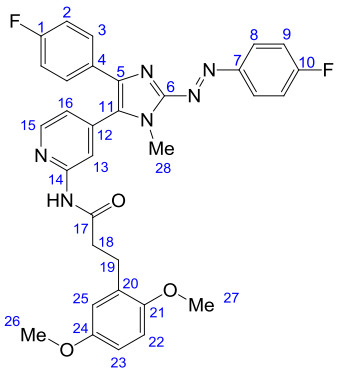



**Figure S27**: $^{13}$C NMR spectrum of compound **21** measured in deuterated chloroform.

**III.11 Synthesis of *(E)*-3-(2,5-dimethoxyphenyl)-*N*-(4-(4-(4-fluorophenyl)-2-((4-fluorophenyl)-diazenyl)-1-methyl-1*H*-imidazol-5-yl)pyridin-2-yl)propanamide (3)**



(*E*)-4-(4-Fluorophenyl)-2-((4-fluorophenyl)diazenyl)-1-methyl-1*H*-imidazol (**19**, 98 mg, 0.33 mmol), *N*-(4-bromopyridin-2-yl)-3-(2,5-dimethoxyphenyl)propanamide (**21**, 242 mg, 0.66 mmol), palladium acetate (2.3 mg, 0.01 mmol, 3 mol%) and potassium carbonate (134 mg, 0.99 mmol) were dissolved in 5 mL DMAc and heated for 18 h at 150 °C under nitrogen atmosphere. After cooling to room temperature the mixture was diluted with ethyl acetate and the solvent was evaporated. The crude product was filtered over silica (cyclohexane/ethyl acetate, 1:1) and then purified by flash column chromatography (cyclohexane/ethyl acetate, ethyl acetate: 12 % → 100 %) on silica. The product fraction was recrystallized from acetonitrile to yield red crystals (35 mg, 589 $\mu$mol, 18 %).

**melting point:** 177 °C.

**$R_f$:** 0.18 (cyclohexane/ethyl acetate, 3:1).

$^1$**H NMR** (500 MHz, acetone-$d_6$): δ = 9.48 (bs, 1 H, N*H*), 8.30 (dd, $^3J$ = 5.0 Hz, $^5J$ = 0.8 Hz, 1 H, *H*-15), 8.28 (bs, 1 H, *H*-13), 7.96 ($m_c$, 2 H, *H*-8), 7.48 ($m_c$, 2 H, *H*-3), 7.25 ($m_c$, 1 H, *H*-9), 7.06 (dd, $^3J$ = 5.0 Hz, $^4J$ = 1.6 Hz, 1 H, *H*-16), 6.94 ($m_c$, 2 H, *H*-2), 6.73 (d, $^3J$ = 8.8 Hz, *H*-22), 6.70 (d, $^4J$ = 3.1 Hz, 1 H, *H*-25), 6.59 (dd, $^3J$ = 8.8 Hz, $^4J$ = 3.1 Hz, 1 H, *H*-23), 3.87 (s, 3 H, *H*-28), 3.65 (s, 3 H, *H*-27), 3.57 (s, 3 H, *H*-26), 2.83 ($m_c$, 2 H, *H*-19), 2.68 ($m_c$, 2 H, *H*-18) ppm.

$^{13}$**C NMR** (125 MHz, acetone-$d_6$): δ = 172.3 (*C*-17), 164.5 (d, $^1J_{CF}$ = 251 Hz, *C*-10), 162.1 (d, $^1J_{CF}$ = 240 Hz, *C*-1), 153.4 (*C*-24), 153.3 (*C*-12), 152.3 (*C*-6), 151.7 (*C*-21), 150.2 (d, $^4J_{CF}$ = 2.9 Hz, *C*-7), 149.5 (*C*-15), 139.5 (*C*-14), 138.9 (*C*-5), 130.8 (*C*-11), 130.4 (*C*-20), 130.2 (d, $^4J_{CF}$ = 3.1 Hz, *C*-4), 129.5 (d, $^3J_{CF}$ = 8.2 Hz, *C*-3), 125.7 (d, $^3J_{CF}$ = 9.0 Hz, *C*-8), 120.9 (*C*-16), 117.1 (d, $^2J_{CF}$ = 23.2 Hz, *C*-9), 116.4 (*C*-25), 115.9 (d, $^2J_{CF}$ = 22.2 Hz, *C*-2), 114.8 (*C*-13), 112.0 (*C*-22), 111.7 (*C*-23), 55.2 (*C*-26), 55.7 (*C*-27), 36.5 (*C*-18), 31.9 (*C*-28), 25.7 (*C*-19) ppm.

$^{19}$**F NMR** (470 MHz, $CDCl_3$): δ = -110.3, -116.2 ppm.

**IR** (ATR): ύ = 3261 (w), 2942 (w), 2168 (w), 1974 (w), 1662 (m), 1604 (m), 1592 (m), 1544 (m), 1498 (s), 1451 (m), 1421 (m), 1399 (m), 1316 (m), 1284 (w), 1221 (vs), 1178 (m), 1155 (m), 1135 (m), 1092 (m), 1047 (m), 932 (w), 840 (s), 814 (m), 798 (m), 776 (m), 743 (m), 710 (m), 696 (m), 665 (m) $cm^{-1}$.

**MS** (EI, 70 eV): m/z (%) = 582 (22) $[M]^+$, 553 (41) $[M\text{-}CH_3HO]^+$, 521 (10) $[M\text{-}C_2H_6O_2]^+$, 444 (3) $[M\text{-}C_8H_{10}O_2]^+$, 389 (21) $[M\text{-}C_{11}H_{14}O_3]^+$, 95 (100) $[C_{26}H_{24}FN_6O_3]^+$.

**MS** (EI, HR, 70 eV): $C_{32}H_{28}F_2N_6O_3$, m/z = calc.: 582.2191, found.: 582.2162.

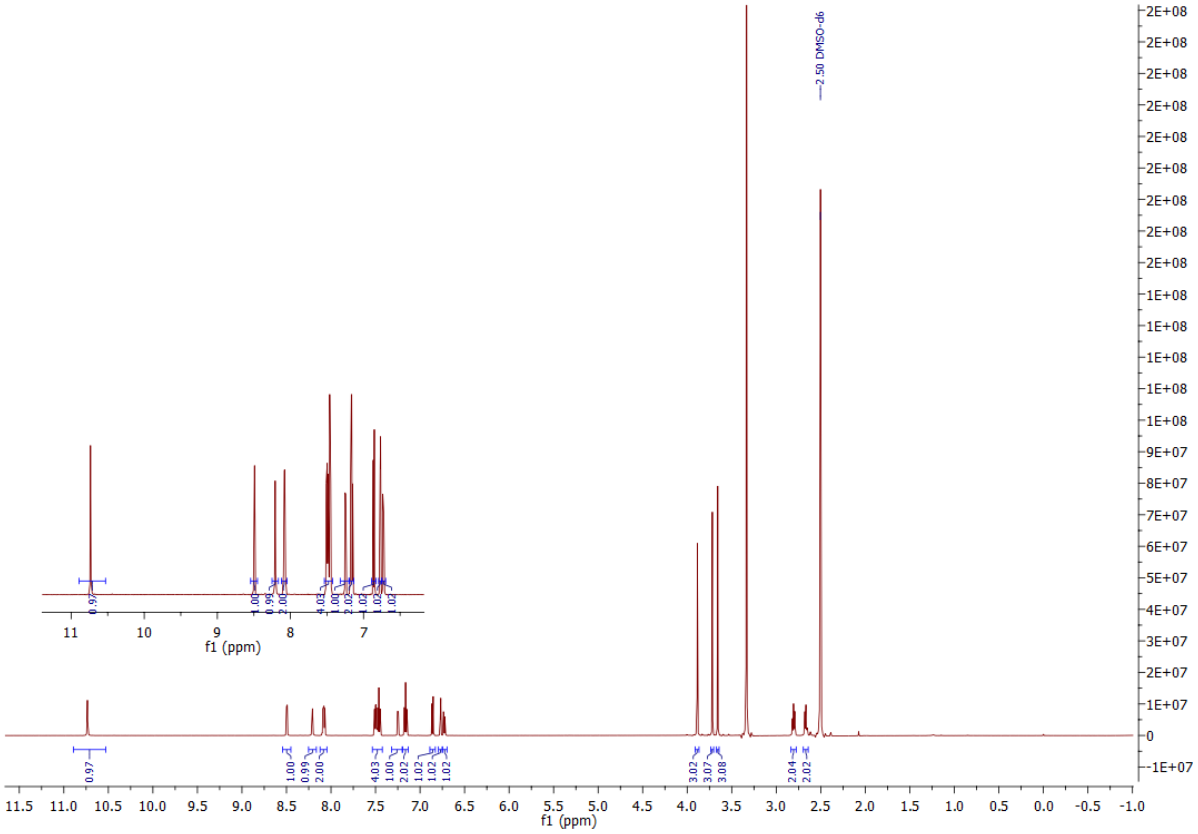



**Figure S28**: $^{1}$H NMR spectrum of compound **3** measured in deuterated DMSO.

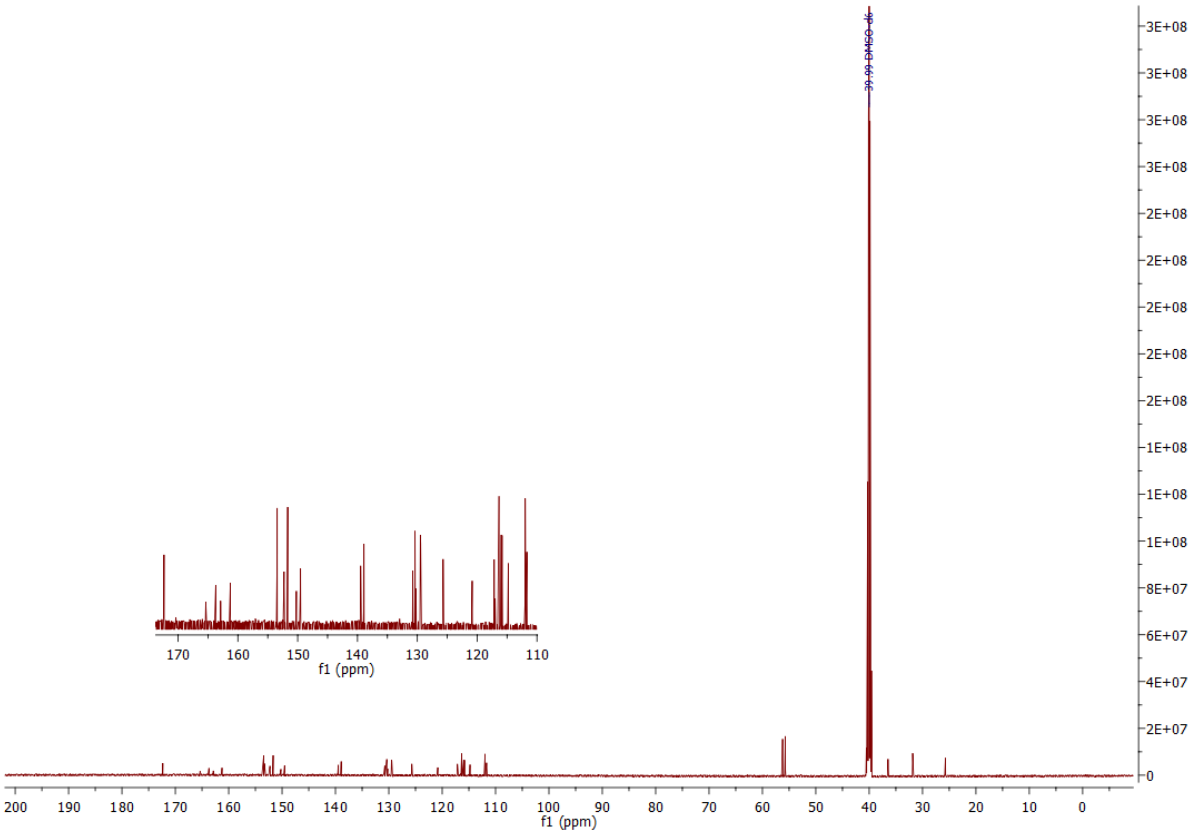



**Figure S29**: $^{13}$C NMR spectrum of compound **3** measured in deuterated DMSO.

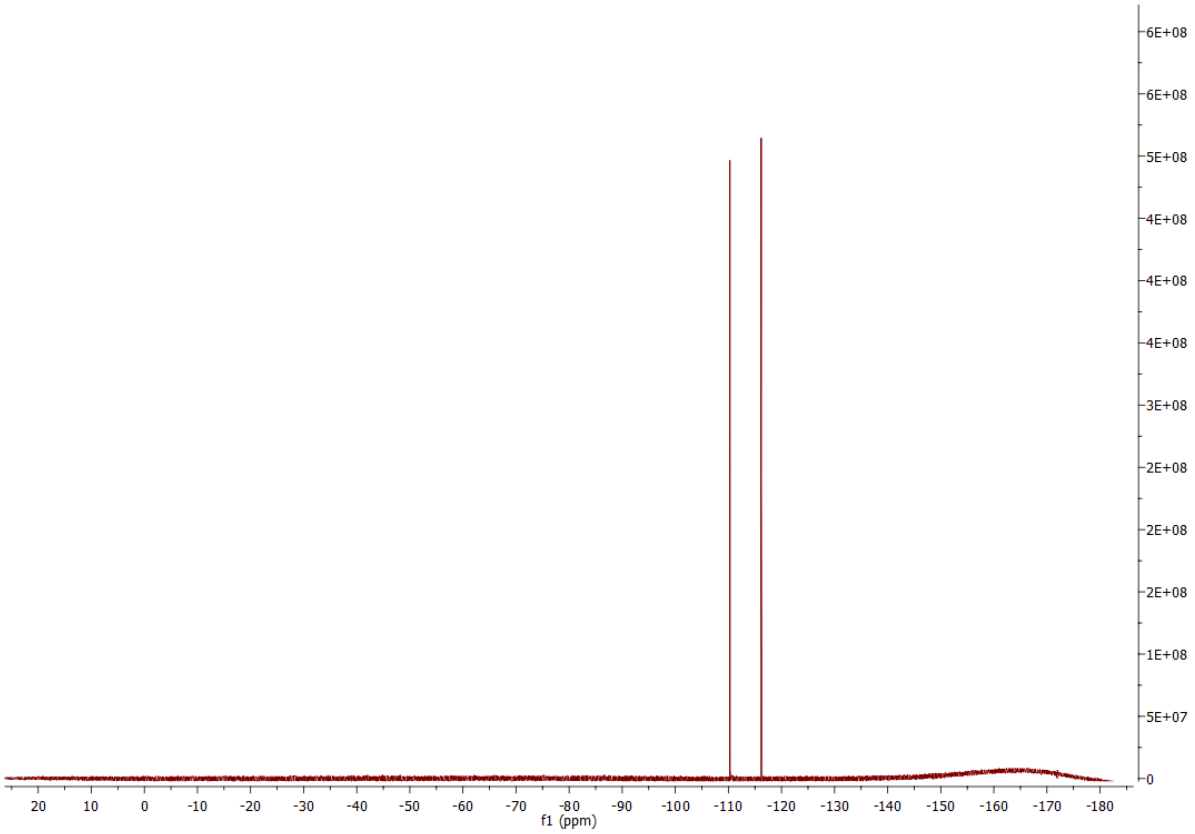
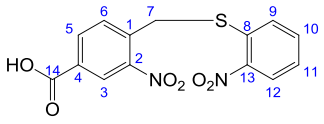



**Figure S30**: $^{19}$F NMR spectrum of compound **3** measured in deuterated chloroform.

### III.12 Synthesis of 3-nitro-4-(((2-nitrophenyl)thio)methyl)benzoic acid (24)




1,2-Bis(2-nitrophenyl)disulfane (**23**, 2.37 g, 7.68 mmol) was dissolved in 60 mL dry THF under nitrogen atmosphere. Sodium borohydride (581 mg, 15.4 mmol) was added (solution turned immediately red to dark red) and the mixture was heated for 1 h at 60 °C. The solution was then cooled to 0 °C and 4-(bromomethyl)-3-nitrobenzoic acid (**22**, 4.00 g, 15.4 mmol) was added (solution decolorized immediately to a bright yellow). The solution was stirred for 30 min at 0 °C and was poured carefully onto ice. The solvent was evaporated and the crude product was purified by flash column chromatography (ethyl acetate/ethanol, ethanol: 0 % → 100 %) on silica. The product fraction was dissolved in acetone and precipitated with cyclohexane. A yellow solid could be obtained (1.78 g, 5.32 mmol, 35 %).

**melting point:** 189 °C.

**$R_f$ :** 0.04 (DCM).

**$^1$H-NMR** (500 MHz, DMSO-$d_6$)): □ = 8.41 (d, $^4J$ = 1.51 Hz, 1 H, *H*-3), 8.17 (dd, $^3J$ = 7.15 Hz, $^4J$ = 1.35 Hz, 1 H, *H*-9), 8.09 (dd, $^3J$ = 7.83 Hz, $^4J$ = 1.51 Hz, 1 H, *H*-5), 7.76-7.78 (m, 2 H, *H*-10, *H*-12), 7.64 (d, $^3J$ = 7.83 Hz, 1 H, *H*-6), 7.43 ($m_c$, 1 H, *H*-11), 4.67 (s, 2 H, *H*-7) ppm.

**$^{13}$C-NMR** (125 MHz, DMSO-$d_6$): □ = 167.9 (*C*-4), 148.5 (*C*-2), 146.5 (*C*-8), 135.4 (*C*-13), 134.8 (*C*-10), 134.1 (*C*-5), 132.3 (*C*-6), 131.2 (*C*-1), 128.8 (*C*-12), 126.5 (*C*-11), 126.2 (*C*-9), 125.8 (*C*-3), 34.2 (*C*-7) ppm.

**IR** (ATR): ῡ = 2552 (w), 1683 (vs), 1620 (m), 1591 (m), 1568 (m), 1539 (m), 1504 (s), 1423 (m), 1337 (s), 1319 (s), 1299 (vs), 1272 (s), 1260 (s), 1175 (m), 1106 (m), 1059 (m), 1047 (m), 916 (m), 883 (m), 858 (m), 819 (m), 783 (m), 725 (vs), 702 (s), 681 (m), 631 (m), 533 (m) $cm^{-1}$.

**MS** (HR-ESI): m/z (%) = $[C_{14}H_9N_2O_6S]^+$, m/z = calc.: 333.0187, found: 333.0192.

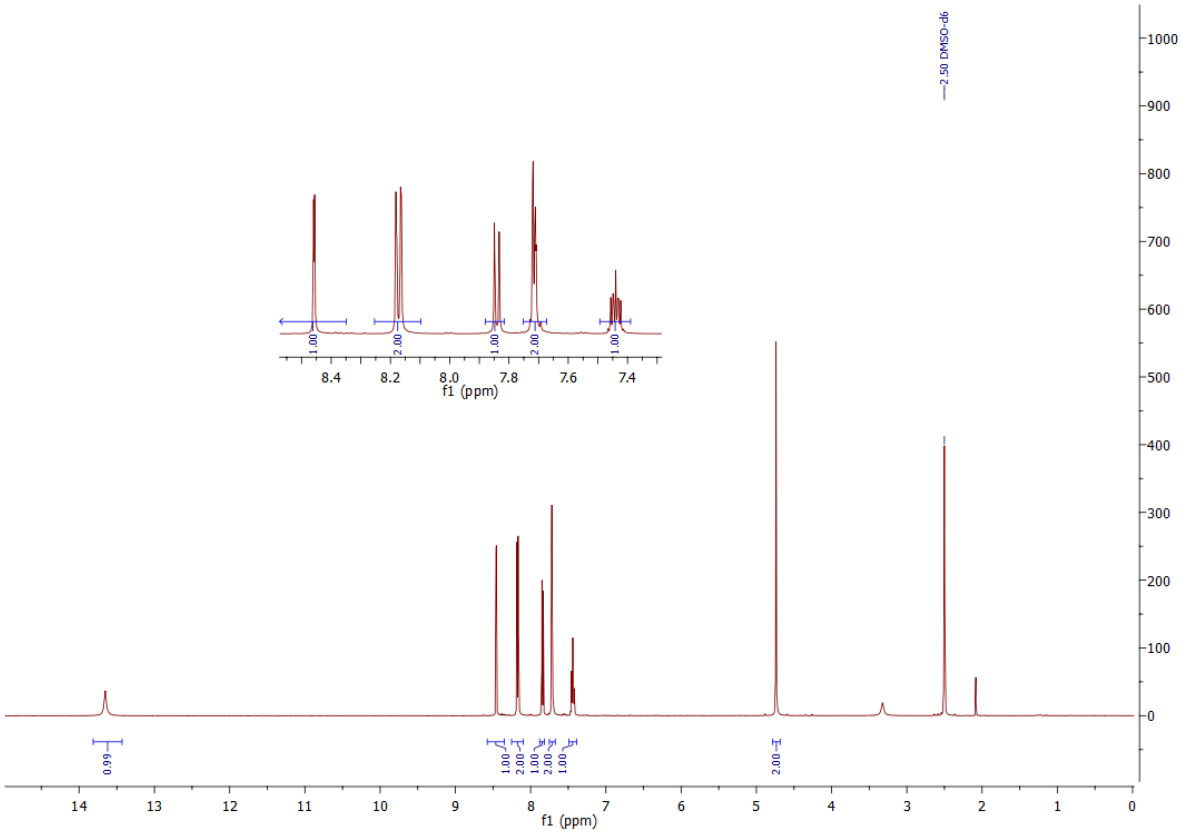



**Figure S31**: $^{1}$H NMR spectrum of compound **24** measured in deuterated DMSO.

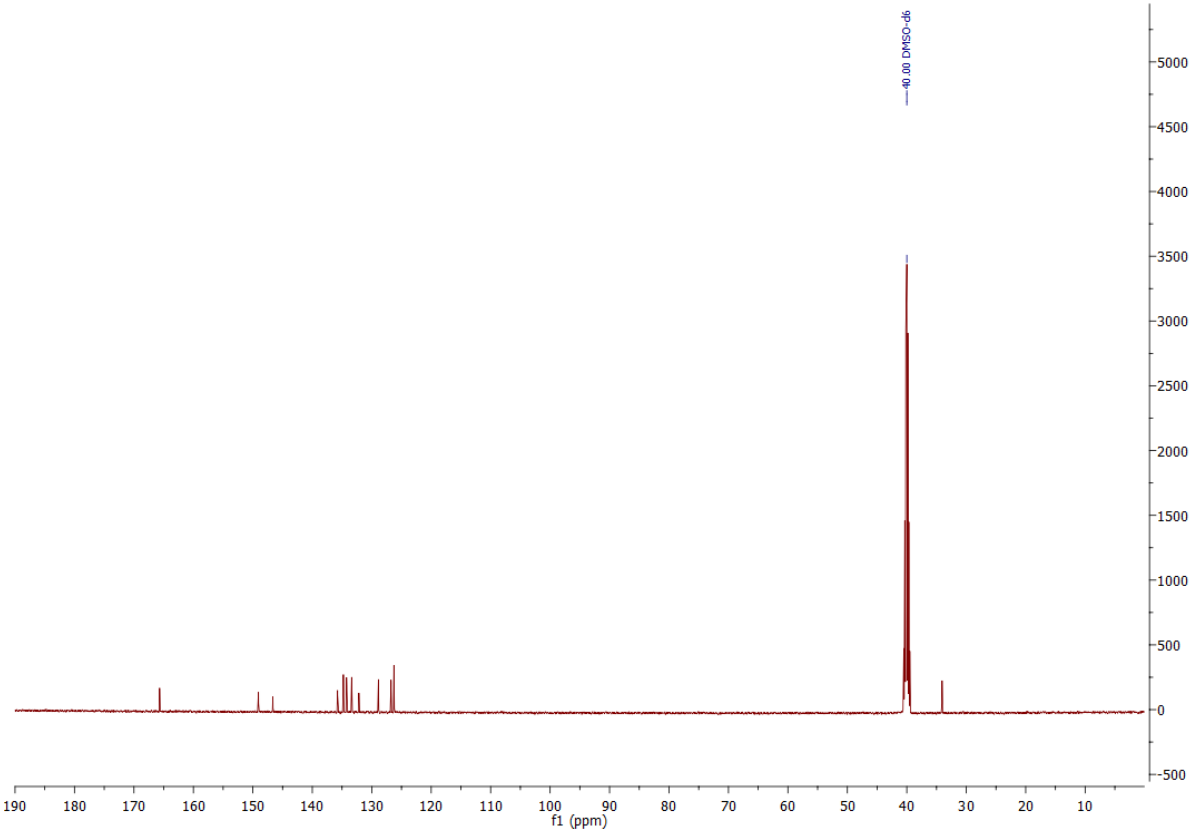
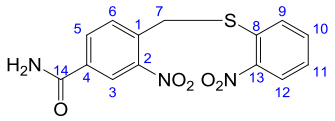



**Figure S32**: $^{13}$C NMR spectrum of compound **24** measured in deuterated DMSO.

### III.13 Synthesis of 3-nitro-4-(((2-nitrophenyl)thio)methyl)benzamide (25)



3-Nitro-4-(((2-nitrophenyl)thio)methyl)benzoic acid (**24**, 1.05 g, 3.14 mmol) was suspended in 15 mL dry ethyl acetate. Oxalyl chloride (0.30 mL, 3.45 mmol) and a drop of DMF were added and the mixture was stirred for 16 h at room temperature under nitrogen atmosphere. Afterwards the mixture was dropped carefully to 0 °C cooled ammonia solution (25 % in water). The mixture was stirred for 15 min, the formed precipitate was filtered off, washed with water and dried in vacuo to obtain a yellow solid (998 mg, 3.00 mmol, 95 %).

**melting point:** 186 °C.

**$^{1}$H NMR** (600 MHz, DMSO-$d_6$): □ = 8.49 (d, $^{4}J$ = 1.7 Hz, 1 H, *H*-3), 8.26 (bs,1 H, N$H_2$), 8.18 (d, $^{3}J$ = 8.1 Hz, 1 H, *H*-9), 8.13 (dd, $^{3}J$ = 8.1 Hz, $^{4}J$ = 1.7 Hz, 1 H, *H*-5), 7.81 (d, $^{3}J$ = 8.1 Hz, 1 H, *H*-6), 7.75-7.70 (m, 2 H, *H*-10, *H*-12), 7.69 (bs, 1 H, N$H_2$), 7.44 ($m_c$, 1 H, *H*-11), 4.72 (s, 2 H, *H*-7) ppm.

**$^{13}$C NMR** (150 MHz, DMSO-$d_6$): □ = 165.4 (*C*-14), 148.5 (*C*-2), 146.2 (*C*-13), 135.1 (*C*-4), 134.5 (*C*-8), 134.3 (*C*-12), 133.6 (*C*-1), 132.6 (*C*-6), 132.2 (*C*-5), 128.4 (*C*-10), 126.3 (*C*-11), 125.9 (*C*-9), 124.2 (*C*-3), 33.5 (*C*-7) ppm.

**IR** (ATR): ῡ =3444 (w), 3106 (w), 1673 (m), 1623 (w), 1592 (w), 1564 (w), 1529 (s), 1504 (s), 1439 (w), 1393 (m), 1334 (vs), 1307 (vs), 1252 (m), 1159 (w), 1125 (m), 1062 (w), 1046 (w), 908 (w), 866 (w), 817 (m), 779 (m), 731 (vs), 721 (vs), 690 (m), 679 (w), 655 (w), 625 (w), 542 (s) $cm^{-1}$.

**MS** (EI, 70 eV): m/z (%) = 333 (1) $[M]^+$, 179 (56) $[M-C_6H_4NO_2S]^+$.

**MS** (EI, HR, 70 eV): $C_{14}H_{11}N_3O_5S$, m/z = calc.: 333.0419, found: 333.0413.

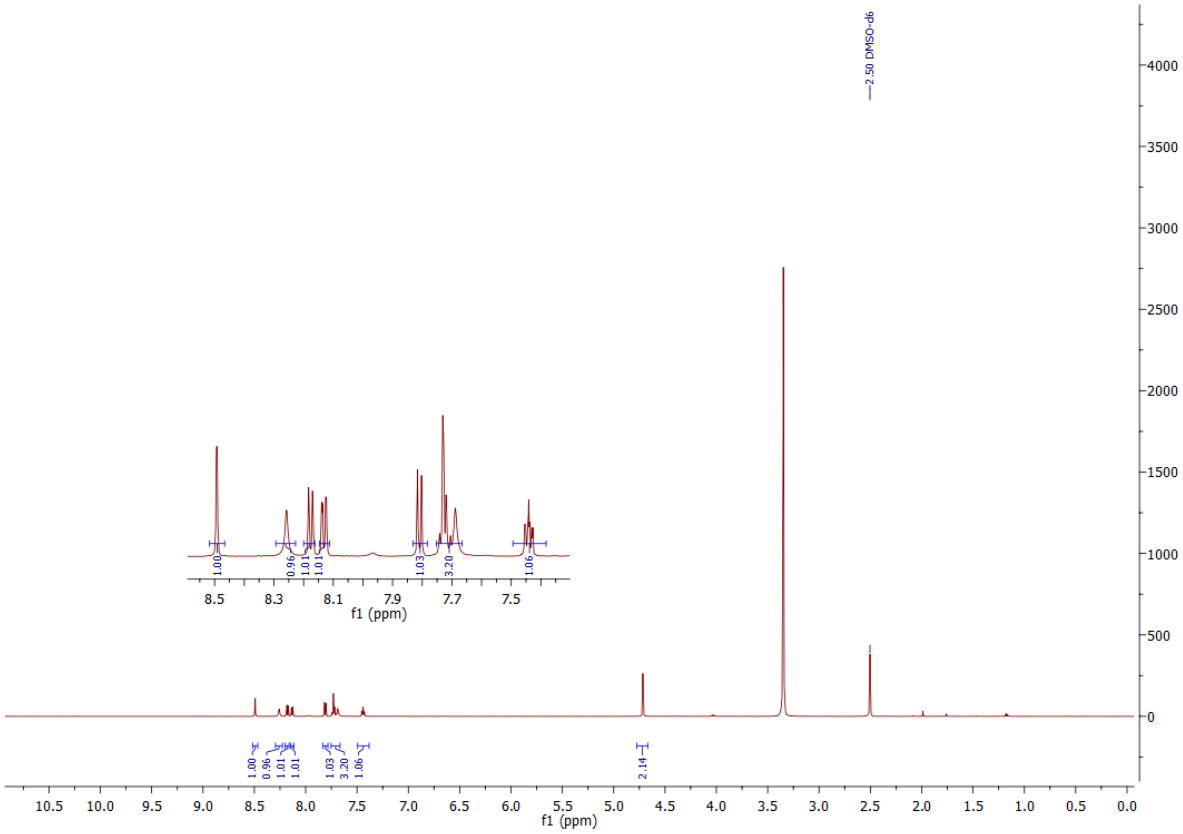



**Figure S33**: $^{1}$H NMR spectrum of compound **25** measured in deuterated DMSO.

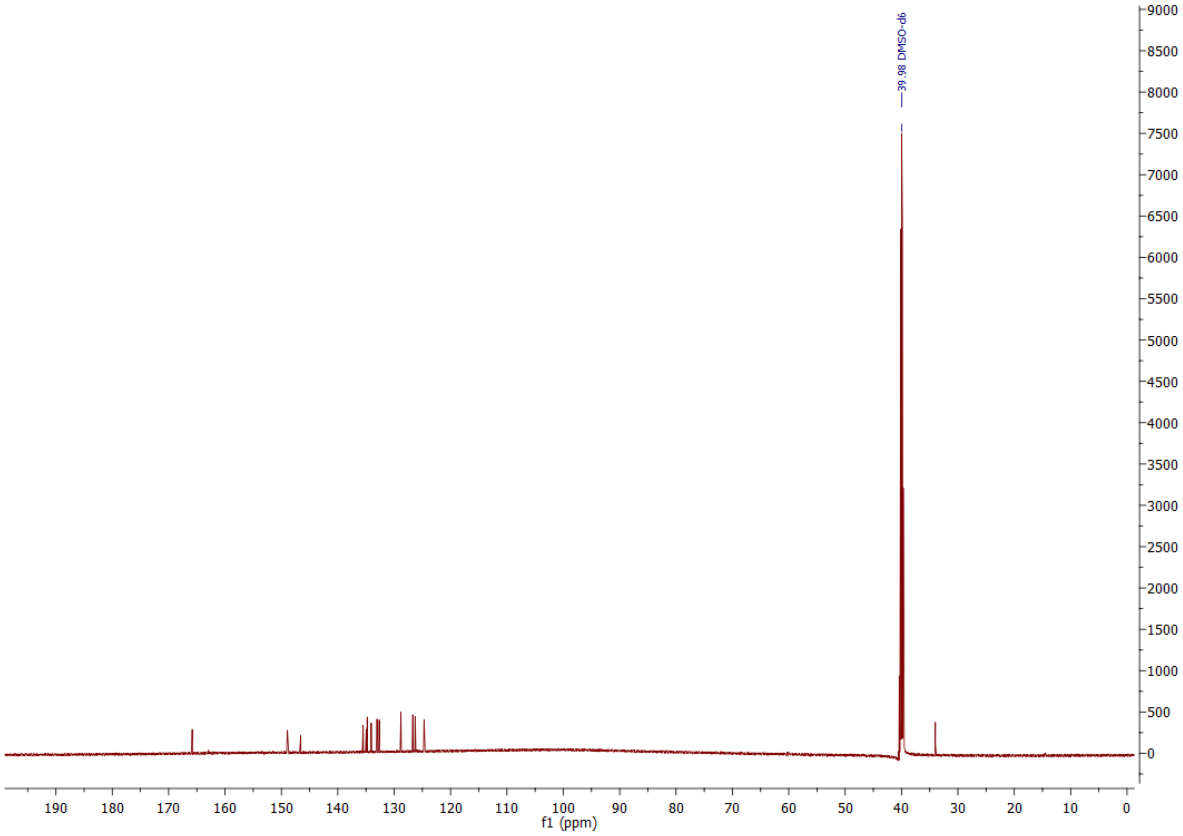
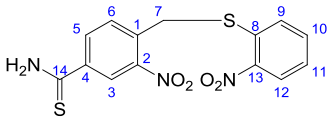



**Figure S34**: $^{13}$C NMR spectrum of compound **25** measured in deuterated DMSO.

### III.14 Synthesis of 3-nitro-4-(((2-nitrophenyl)thio)methyl)benzothioamide (26)




3-Nitro-4-(((2-nitrophenyl)thio)methyl)benzamide (**25**, 629 mg, 1.89 mmol) was dissolved in 27 mL dry THF under nitrogen atmosphere. Lawesson´s reagent (386 mg, 0.95 mmol) was added and the mixture was refluxed for 3 h. The solvent was evaporated and the crude product was purified by flash column chromatography (cyclohexane/ethyl acetate, ethyl acetate: 12 % → 100 %) on silica. A yellow solid was obtained (517 mg, 1.48 mmol, 78 %).

**melting point:** 173 °C.

**$R_f$:** 0.33 (cyclohexane/ethyl acetate, 1:1).

**$^1$H NMR** (500 MHz, DMSO-$d_6$): □ = 10.2 (bs, 1 H, N*H*$_2$), 9.78 (bs, 1 H, N*H*$_2$), 8.51 (dd, $^4J$ = 2.0 Hz, 1 H, *H*-3), 8.18 (dd, $^3J$ = 8.0 Hz, $^4J$ = 1.6 Hz, 1 H, *H*-9), 8.11 (dd, $^3J$ = 8.1 Hz, $^4J$ = 2.0 Hz, 1 H, *H*-5), 7.77 (d, $^3J$ = 8.1 Hz, 1 H, *H*-6), 7.75-7.70 (m, 2 H, *H*-10, *H*-12), 7.44 ($m_c$, 1 H, *H*-11), 4.71 (s, 2 H, *H*-7) ppm.

**$^{13}$C-NMR** (125 MHz,DMSO-$d_6$): □ = 197.2 (*C*-14), 148.4 (*C*-13), 146.4 (*C*-2), 140.3 (*C*-4), 135.1 (*C*-8), 134.8 (*C*-10), 133.7 (*C*-1), 132.7 (*C*-6), 132.0 (*C*-5), 128.6 (*C*-12), 126.6 (*C*-9), 126.3 (*C*-11), 124.6 (*C*-3), 25.6 (*C*-7) ppm.

**IR** (ATR): ϑ = 3388 (w), 3123 (w), 1594 (w), 15664 (w), 1528 (s), 1505 (vs), 1443 (m), 1418 (m), 1334 (vs), 1307 (vs), 1252 (s), 1106 (m), 1062 (m), 942 (s), 898 (m), 814 (m), 731 (vs), 691 (s), 654 (m), 641 (m), 528 (m), 491 (m), 463 (m), 448 (m), 420 (m) $cm^{-1}$.

**MS** (HR-ESI): m/z (%) = $[C_{14}H_{10}N_3O_4S_2]^-$, m/z = calc.: 348.0118, found: 348.0116.

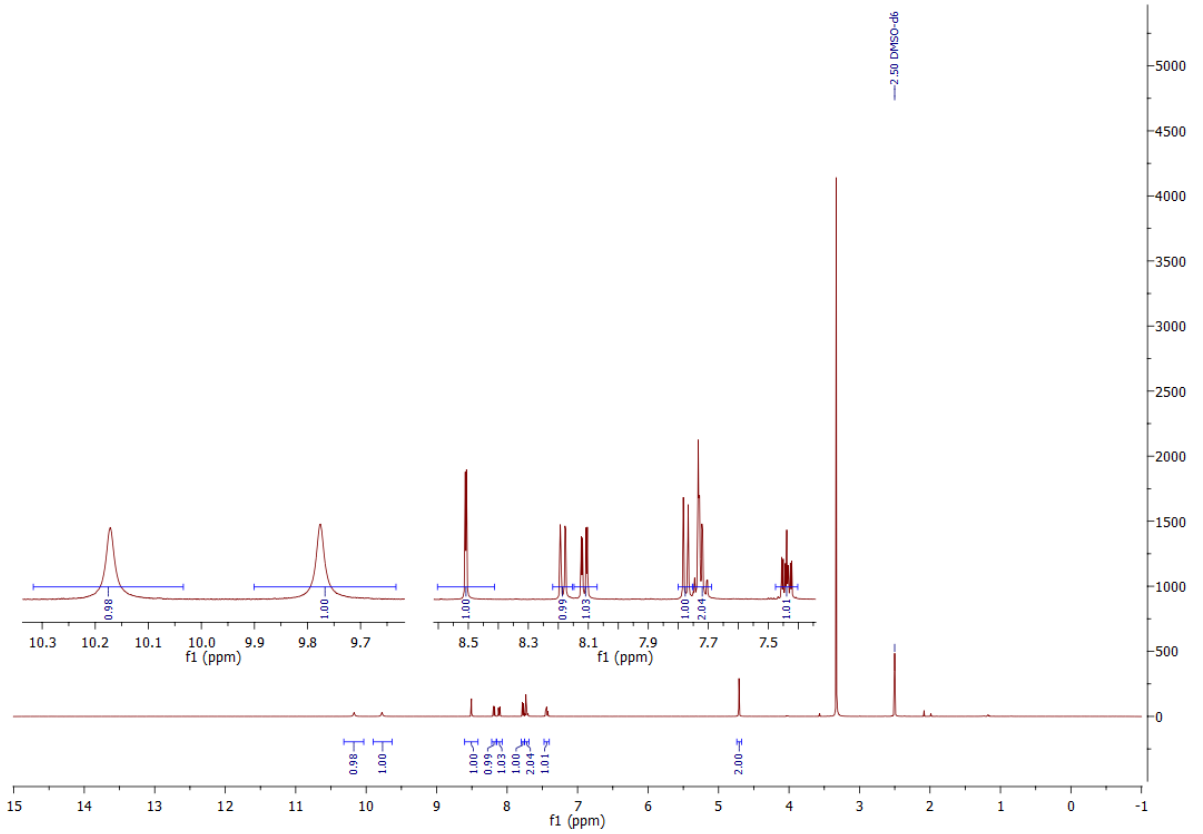



**Figure S35**: $^{1}$H NMR spectrum of compound **26** measured in deuterated DMSO.

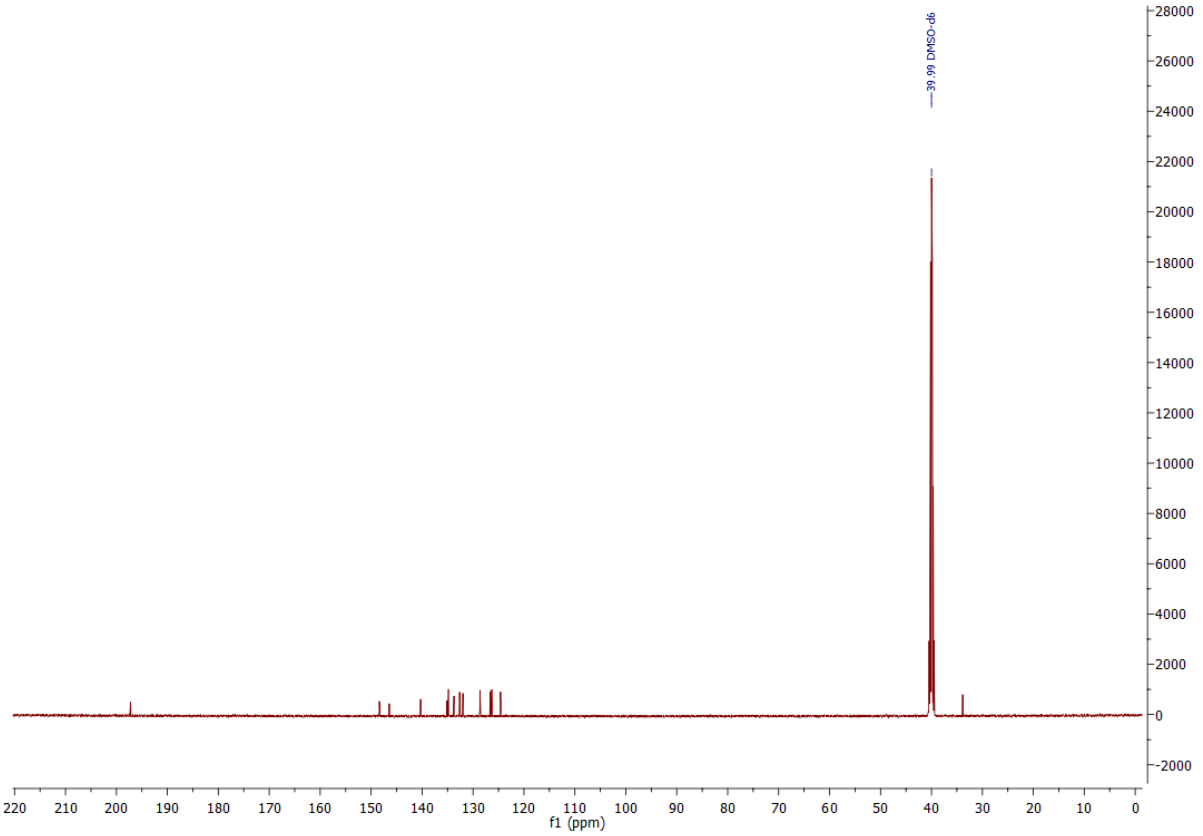
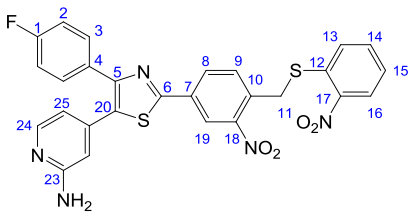



**Figure S36**: $^{13}$C NMR spectrum of compound **26** measured in deuterated DMSO.

### III.15 Sythesis of 4-(4-(4-fluorophenyl)-2-(3-nitro-4-(((2-nitrophenyl)thio)methyl)phenyl)-thiazol-5-yl)pyridin-2-amine (27)



*tert*-butyl(4-(1-bromo-2-(4-fluorophenyl)-2-oxoethyl)pyridin-2-yl) carbamate (**10**, 827 mg, 2.02 mmol) and 3-nitro-4-(((2-nitrophenyl)thio)methyl)benzothioamide (**26**, 705 mg, 2.02 mmol) were suspended in 20 mL acetonitrile and heated at 80 °C for 14 h. After cooling to room temperature the formed precipitate was filtered off, washed with cold acetonitrile and dried in vacuo. The crude product was suspended in 60 mL acetic acid, 6 mL of a 6 M hydrogen chloride solution was added and the mixture was heated for 7 h at 80 °C. After cooling to room temperature the mixture was poured onto water and extracted with ethyl acetate (3 x 100 mL). The organic layers were dried over magnesium sulfate, filtered and reduced in vacuo to about 50 mL. 200 mL of a mixture of water/*n*-pentane (1:1) was added to the residue and left for crystallization at room temperature for 18 h. The formed precipitate was filtered off, washed with water and dried in vacuo to yield a yellow solid (403 mg, 0.72 mmol, 36 %).

**melting point:** 186 °C.

**$^{1}$H NMR** (500 MHz, DMSO-$d_6$): δ = 8.59 (d, $^{4}J$ = 1.9 Hz, 1 H, *H*-19), 8.27 (dd, $^{3}J$ = 8.1 Hz, $^{4}J$ = 1.9 Hz, 1 H, *H*-8), 8.19 (dd, $^{3}J$ = 8.3 Hz, $^{4}J$ = 1.3 Hz, 1 H, *H*-16), 7.92 (d, $^{3}J$ = 5.3 Hz, 1 H, *H*-24), 7.87 (d, $^{3}J$ = 8.1 Hz, 1 H, *H*-9), 7.78-7.70 (m, 2 H, *H*-13 , *H*-14), 7.63 ($m_c$, 2 H, *H*-3), 7.45 ($m_c$, 1 H, *H*-15), 7.26 ($m_c$, 2 H, *H*-2), 6.46 (bs, 1 H, *H*-22), 6.41 (dd, $^{3}J$ = 5.3 Hz, $^{4}J$ = 1.6 Hz, 1 H, *H*-25), 6.15 (bs, 1 H, N$H_2$), 4.74 (bs, 2 H, *H*-11) ppm.

**$^{13}$C NMR** (125 MHz, DMSO-$d_6$): δ = 162.6 (d, $^{1}J_{CF}$ = 247 Hz, *C*-1), 162.7 (*C*-6), 160.8 (*C*-21), 150.8 (*C*-5), 149.7 (*C*-18), 149.3 (*C*-24), 146.7 (*C*-17), 139.7 (*C*-23), 134.9 (*C*-12), 134.8 (*C*-14), 134.0 (*C*-9), 133.6 (*C*-7), 133.0 (*C*-10), 132.9 (*C*-20), 131.4 (d, $^{3}J_{CF}$ = 8.3 Hz, *C*-3), 131.3 (*C*-8), 130.7 (d, $^{4}J_{CF}$ = 3.4 Hz, *C*-4), 128.9 (*C*-13), 126.8 (*C*-15), 126.3 (*C*-16), 122.7 (*C*-19), 116.0 (d, $^{2}J_{CF}$ = 22.3 Hz, *C*-2), 112.1 (*C*-25), 107.8 (*C*-22), 34.0 (*C*-11) ppm.

**$^{19}$F NMR** (470 MHz, DMSO-$d_6$): δ = -112.8 ppm.

**IR** (ATR): ϑ = 3322 (w), 3181 (w), 1593 (m), 1567 (w), 1542 (m), 1512 (vs), 1422 (m), 1407 (m), 1336 (vs), 1305 (m), 1226 (m), 1156 (m), 1128 (w), 1045 (w), 1014 (w), 886 (m), 853 (m), 837 (m), 816 (m), 784 (m), 734 (s), 650 (m), 578 (m), 540 (m), 506 (m), 459 (m), 431 (m), 408 (m) $cm^{-1}$.

**MS** (MALDI-MS-TOF): m/z = 560 $[M+H]^+$.

**MS** (HR-ESI): m/z (%) = $[C_{27}H_{19}FN_5O_4S_2]^+$, m/z = calc.: 560.0857, found.: 560.0852.

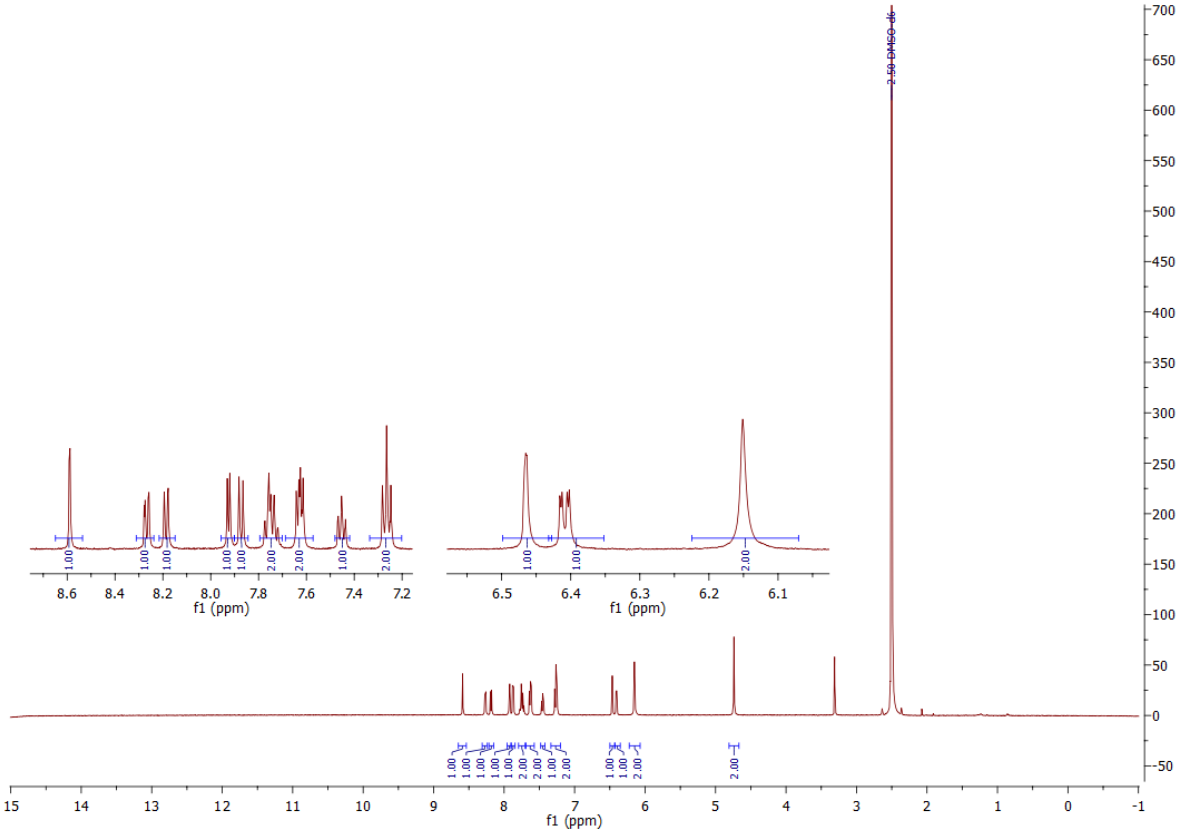



**Figure S37**: $^{1}$H NMR spectrum of compound **27** measured in deuterated DMSO.

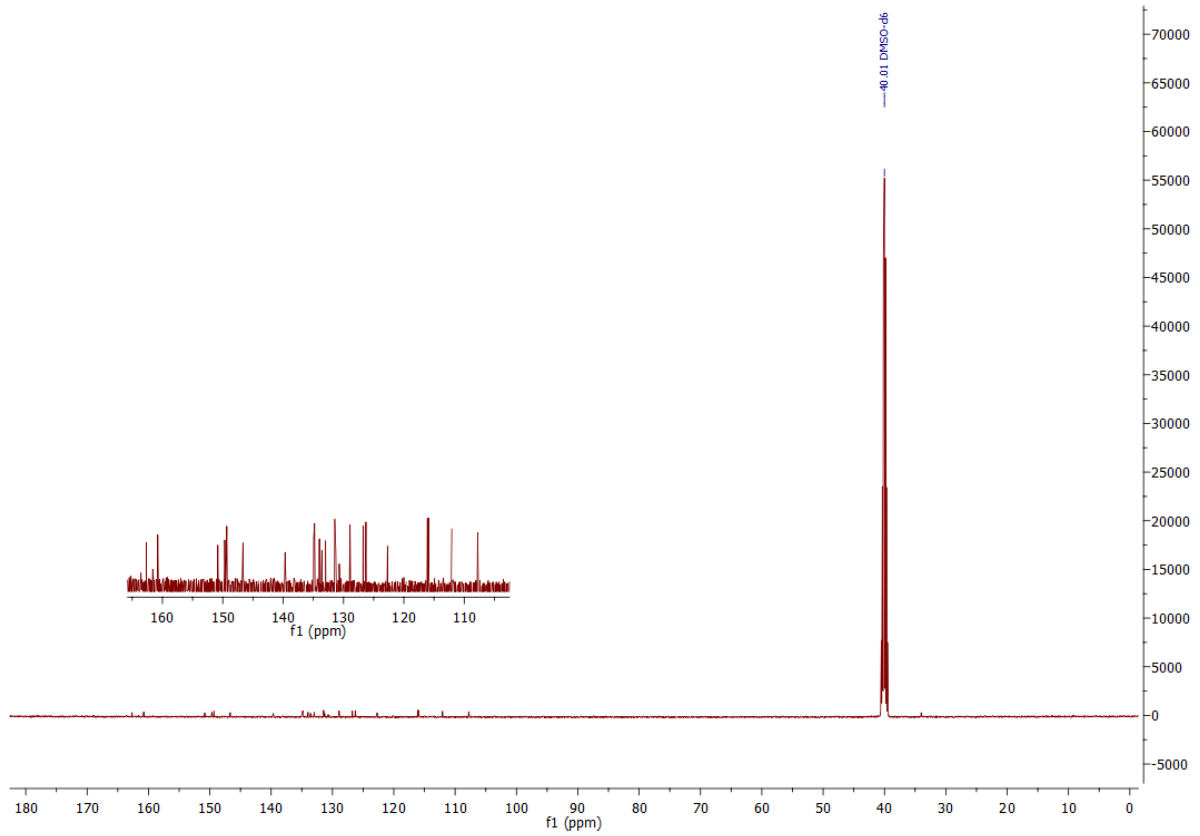



**Figure S38**: $^{13}$C NMR spectrum of compound **27** measured in deuterated DMSO.

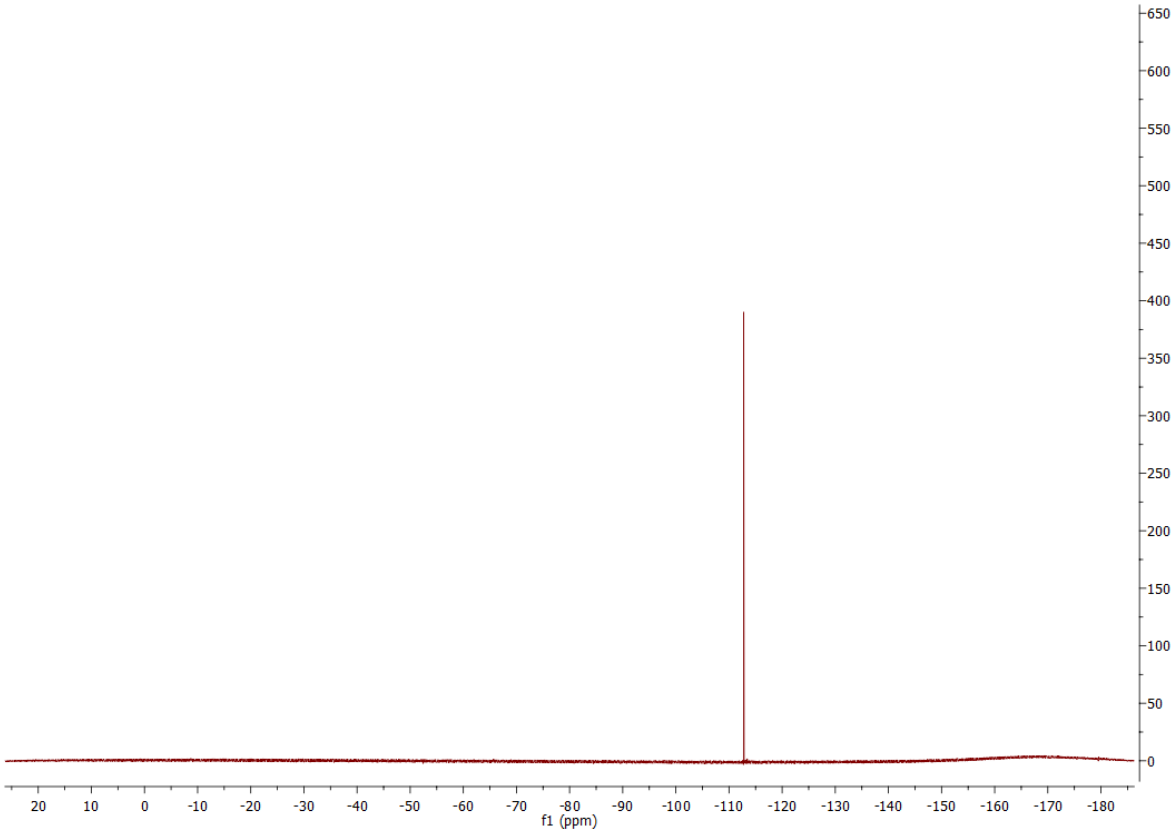
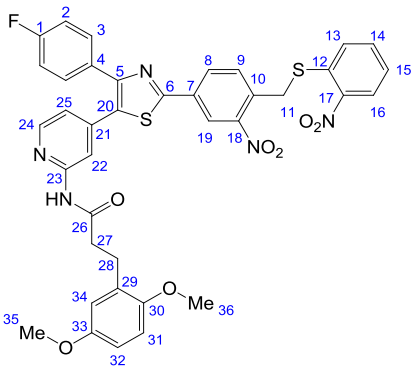



**Figure S39**: $^{19}F$ NMR spectrum of compound **27** measured in deuterated DMSO.

**III.16 Synthesis of 3-(2,5-dimethoxyphenyl)-*N*-(4-(4-(4-fluorophenyl)-2-(3-nitro-4-(((2-nitrophenyl)thio)methyl)phenyl)thiazol-5-yl)pyridin-2-yl)propanamide (28)**



4-(4-(4-Fluorophenyl)-2-(3-nitro-4-(((2-nitrophenyl)thio)methyl)phenyl)-thiazol-5-yl)pyridin-2-amine (**27**, 67 mg, 0.12 mmol) and 3-(2,5-dimethoxyphenyl)propionic acid (**14**, 29 mg, 0.14 mmol) were suspended in 5 mL ethyl acetate under nitrogen atmosphere. DIPEA (0.09 mL, 5.42 mmol) and T3P® (0.27 mL, 10.3 mmol, 50 % in ethyl acetate) were added and the mixture was refluxed for 24 h. The solution was washed with water and extracted with ethyl acetate. The combined organic layers were washed with saturated sodium chloride solution, dried over magnesium sulfate, filtered and the solvent was evaporated. After cooling to room temperature the mixture was washed with 20 mL water and extracted with ethyl acetate (2 x 20 mL). The combined organic layers were washed with sodium chloride solution (2 x 20 mL), dried over magnesium sulfate, filtered and the solvent was evaporated. The crude product was first filtered over silica (cyclohexane/ethyl acetate, 1:1) and then purified by flash chromatography (dichloromethane/ethyl acetate, ethyl acetate: 0 % → 10 %) on silica to obtain a yellow solid (33 mg, 0.04 mmol, 37 %).

**melting point:** decomposition at 86 °C.

**$R_f$:** 0.34 (dichloromethane /ethyl acetate, ethyl acetate: 3 %).

**$^1$H NMR** (600 MHz, $CDCl_3$): δ = 8.68 (d, $^4J$ = 1.8 Hz, 1 H, *H*-19), 8.40 (s, 1 H, *H*-22), 8.20 (dd, $^3J$ = 8.2 Hz, $^4J$ = 1.4 Hz, 1 H, *H*-16), 8.16 (d, $^3J$ = 5.2 Hz, 1 H, *H*-24), 8.14 (dd, $^3J$ = 8.1 Hz, $^4J$ = 1.9 Hz, 1 H, *H*-8), 8.07 (bs, 1 H, N*H*), 7.73 (d, $^3J$ = 8.4 Hz, 1 H, *H*-9), 7.60-7.52 (m, 3 H, *H*-3, *H*-14), 7.38 (dd, $^3J$ = 8.2 Hz, $^4J$ = 0.6 Hz, 1 H, *H*-13), 7.33 (t, $^3J$ = 7.8 Hz, 1 H, *H*-15), 7.06 ($m_c$, 2 H, *H*-2), 6.89 (dd, $^3J$ = 5.2 Hz, $^4J$ = 1.6 Hz, 1 H, *H*-25), 6.81-6.77 (m, 2 H, *H*-34, *H*-31), 6.73 (dd, $^3J$ = 8.8 Hz, $^4J$ = 3.1 Hz, 1 H, *H*-32), 4.62 (s, 2 H, *H*-11), 3.81 (s, 3 H, *H*-36), 3.75 (s, 3 H, *H*-35), 3.01 ($m_c$, 2 H, *H*-28), 2.69 ($m_c$, 2 H, *H*-27) ppm.

**$^{13}$C NMR** (150 MHz, $CDCl_3$): δ = 171.3 (*C*-26), 163.0 (d, $^1J_{CF}$ = 249 Hz, *C*-1), 163.2 (*C*-6), 153.3 (*C*-33), 152.3 (d, $^5J_{CF}$ = 26.5 Hz, *C*-5), 151.8 (*C*-23), 151.6 (*C*-30), 149.5 (*C*-18), 148.3 (*C*-24), 146.9 (*C*-17), 141.6 (*C*-21), 135.4 (*C*-12), 134.2 (*C*-7), 133.7 (*C*-14), 132.8 (*C*-9), 132.4 (*C*-10), 131.6 (*C*-20), 131.1 (d, $^3J_{CF}$ 8.4 Hz, *C*-3), 130.7 (*C*-8), 129.9 (*C*-4), 129.6 (*C*-29), 127.9 (*C*-13), 126.1 (*C*-16), 125.9 (*C*-15), 123.1 (*C*-19), 119.4 (*C*-25), 116.5 (*C*-34), 115.7 (d, $^2J_{CF}$ = 22.3 Hz, *C*-2), 113.8 (*C*-22), 111.8 (*C*-32), 111.2 (*C*-31), 55.8 (*C*-36), 55.7 (*C*-35), 37.9 (*C*-28), 34.7 (*C*-11), 26.6 (*C*-27) ppm.

**$^{19}$F NMR** (470 MHz, $CDCl_3$): δ = -112.3 ppm.

**IR** (ATR): ῡ = 2935 (w), 2834 (w), 2251 (w), 2037 (w), 1696 (m), 1597 (m), 1554 (m), 1500 (s), 1457 (m), 1401 (s), 1338 (s), 1305 (m), 1221 (vs), 1157 (m), 1107 (m), 1045 (m), 840 (s), 806 (m), 780 (m), 732 (s), 710 (m) $cm^{-1}$.

**MS** (MALDI-MS-TOF): m/z = 752 $[M+H]^+$.

**MS** (HR-ESI): m/z (%) = $[C_{39}H_{31}FN_5O_7S_2]^+$, m/z = calc.: 752.1643, found.: 752.1637.

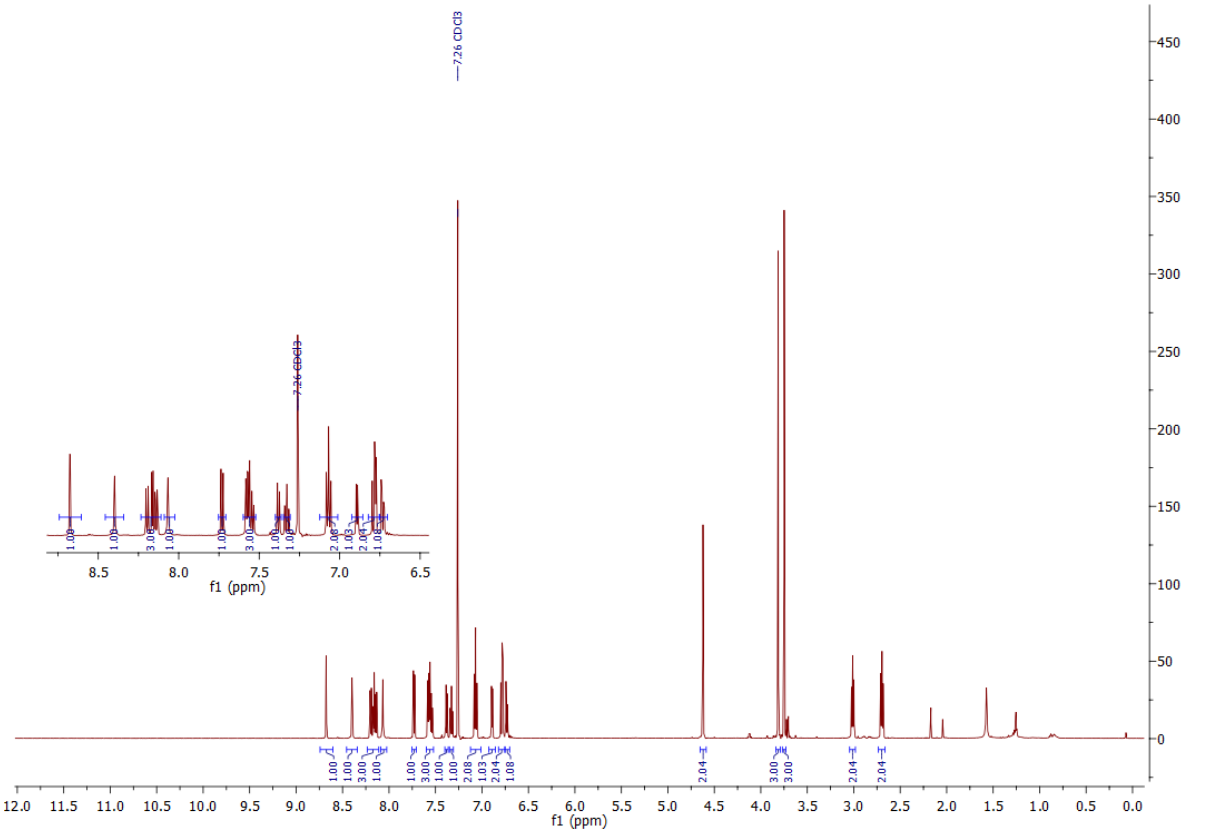



**Figure S40**: $^{1}$H NMR spectrum of compound **28** measured in deuterated chloroform.

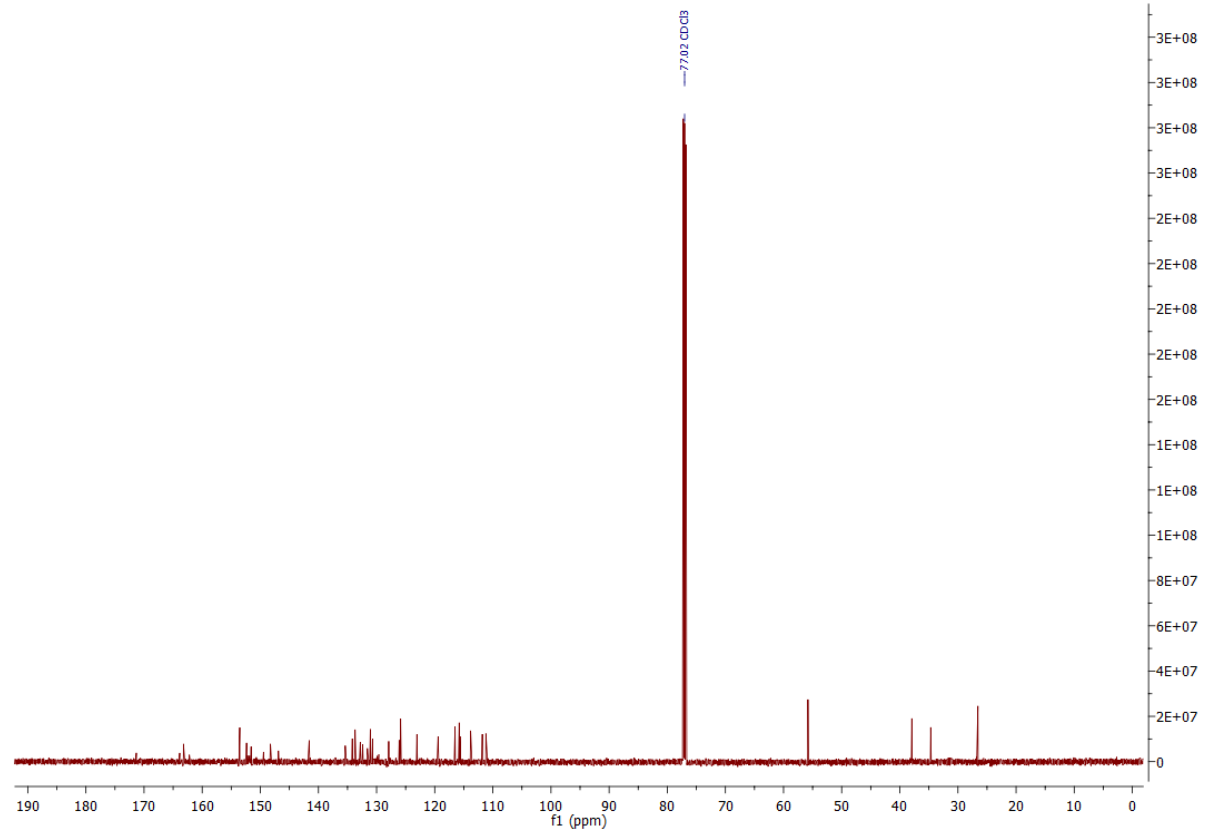



**Figure S41**: $^{13}$C NMR spectrum of compound **28** measured in deuterated chloroform.

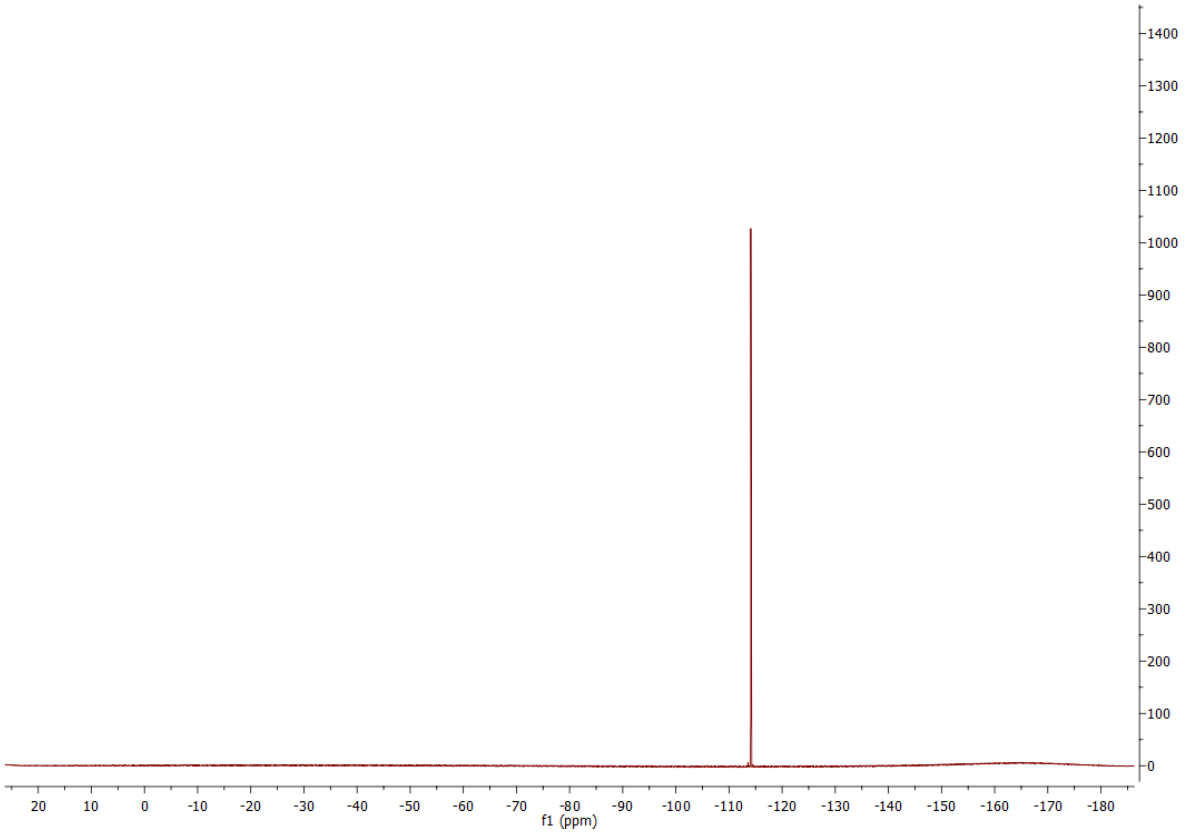
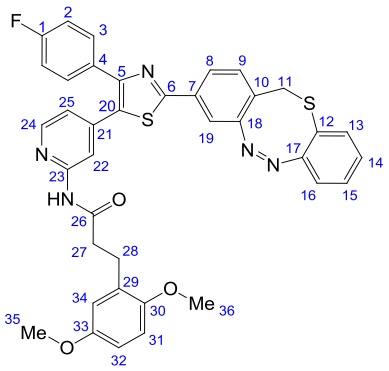



**Figure S42**: $^{19}$F NMR spectrum of compound **28** measured in deuterated chloroform.

**III.17 Synthesis of (*Z*)-*N*-(4-(2-(12*H*-dibenzo[*b*,*f*][1,4,5]thiadiazocin-3-yl)-4-(4-fluorophenyl)-thiazol-5-yl)pyridin-2-yl)-3-(2,5-dimethoxyphenyl)propanamide (29)**



3-(2,5-Dimethoxyphenyl)-*N*-(4-(4-(4-fluorophenyl)-2-(3-nitro-4-(((2-nitro-phenyl)thio)methyl)-phenyl)thiazol-5-yl)pyridin-2-yl)propanamide (**28**, 707 mg, 0.94 mmol) was dissolved in 34 mL DCM and 91 mL of a mixture of methanol (58 %), triethylamine (25 %), water (16 %) and formic acid (1 %) was added. After addition of lead powder (2.92 g, 14.1 mmol) the reaction mixture was stirred vigorous for 24 h at room temperature. Another portion lead powder (2.92 g, 14.1 mmol) was added and the mixture was stirred vigorous for additional 24 h at room temperature. The reaction mixture was reduced in vacuo, the residue was poured onto water and extracted with DCM (3 x 100 mL). The combined organic layers were dried over magnesium sulfate, filtered and the solvent was evaporated. The crude product was filtered over silica (ethyl acetate) and purified by flash chromatography (cyclohexane/ethyl acetate, ethyl acetate: 12 % → 100 %) on silica. The product fraction was then purified again by flash chromatography (cyclohexane/ethyl acetate, ethyl acetate: 6 % → 66 %) on silica (column: MACHERY-NAGEL, 15 $\mu$m particle diameter) to yield 17 mg of pure product. The main fraction obtained after the first flash chromatography could be identified as impure azoxy compound (*E*)-3-(5-(2-(3-(2,5-dimethoxyphenyl)propanamido)pyridin-4-yl)-4-(4-fluorophenyl)thiazol-2-yl)-12*H*-dibenzo[*b*,*f*][1,4,5]thiadiazocine 6-oxide using mass spectrometry and was reduced in the next step to the diazocine compound **28** without further purification:
The crude azoxy fraction (486 mg) was suspended in 87 mL of a mixture of methanol (58 %), triethylamine (25 %), water (16 %) and formic acid (1 %). Lead powder (2.10 g,10.1 mmol) was added and the suspension was treated with ultrasound for 1 h under cooling with ice. Additional lead powder was added (2.10 g, 10.1 mmol) and the suspension was treated with ultrasound for 1 h under cooling with ice. The reaction mixture was filtered, reduced in vacuo, poured onto water and extracted with DCM (3 x 100 mL). The combined organic layers were dried over magnesium sulfate, filtered and the solvent reduced in vacuo to estimated 30 mL. 40 mL of a 0.1 M methanolic sodium hydroxide solution and copper(I) chloride (55.0 mg, 0.56 mmol) were added to the residue and stirred at room temperature for 18 h while air was bubbled through the solution. The residue was suspended in DCM and washed with water. The organic layer was dried over magnesium sulfate, filtered and the solvent was evaporated. The crude product was purified by flash column chromatography (cyclohexane/ethyl acetate, ethyl acetate: 6 % → 66 %) on silica to yield a yellow solid (overall yield: 79 mg, 0.11 mmol, 12 %).

**melting point:** 219 °C.

**$R_f$:** 0.49 (cyclohexane/ethyl acetate, 1:1).

**$^1$H NMR** (600 MHz, DMSO-$d_6$): δ = 10.6 (s, 1 H, N*H*), 8.27 (dd, $^3J$ = 5.2 Hz, $^5J$ = 0.6 Hz, 1 H, *H*-24), 8.22 (bs, 1 H, *H*-22), 7.79 (dd, $^3J$ = 8.0 Hz, $^4J$ = 1.9 Hz, 1 H, *H*-8), 7.55 ($m_c$, 2 H, *H*-3), 7.52 (d, $^4J$ = 1.9 Hz, 1 H, *H*-19), 7.45 (d, $^3J$ = 8.0 Hz, 1 H, *H*-9), 7.26 (dt, 3*J* = 7.7 Hz, $^4J$ = 1.3 Hz, 1 H, *H*-15), 7.23 ($m_c$, 2 H, *H*-2), 7.16 (dd, $^3J$ = 7.9 Hz, $^4J$ = 1.1 Hz, 1 H, *H*-13), 6.94-6.90 (m, 2 H, *H*-25, *H*-16), 6.86 (d, $^3J$ = 8.8 Hz, 1 H, *H*-31), 6.77 (d, $^4J$ = 3.0 Hz, 1 H, *H*-34), 6.73 (dd, $^3J$ = 8.8 Hz,$^4J$ = 3.0 Hz, 1 H, *H*-32), 4.05 (d, $^2J$ = 11.7 Hz, 1 H, *H*-11), 3.97 (d, $^2J$ = 11.7 Hz, 1 H, *H*-11), 3.72 (s, 3 H, *H*-36), 3.66 (s, 3 H, *H*-35), 2.80 ($m_c$, 2 H, *H*-28), 2.64 ($m_c$, 2 H, *H*-27) ppm.

**$^{13}$C NMR** (150 MHz, DMSO-$d_6$): δ = 171.7 (*C*-26), 164.3 (*C*-6), 162.1 (d, $^1J_{CF}$= 246.2 Hz, *C*-1), 157.4 (*C*-18), 157.0 (*C*-17), 152.8 (*C*-23), 152.7 (*C*-33), 151.1 (*C*-30), 150.9 (*C*-5), 148.6 (*C*-24), 140.4 (*C*-21), 133.2 (*C*-13), 132.2 (*C*-7), 131.0 (d, $^3J_{CF}$ = 8.3 Hz, *C*-3), 130.7 (*C*-20), 130.7 (*C*-9), 130.0 (d, $^4J_{CF}$ = 3.2 Hz, *C*-4),129.8 (*C*-29), 128.3 (*C*-15), 127.4 (*C*-14), 126.6 (*C*-10), 125.4 (*C*-8), 121.0 (*C*-12), 119.3 (*C*-16), 118.6 (*C*-25), 115.7 (*C*-34), 115.5 (d, $^2J_{CF}$ = 21.5 Hz, *C*-2), 114.2 (*C*-19), 112.8 (*C*-22), 111.4 (*C*-31), 111.1 (*C*-32), 55.6 (*C*-36), 55.1 (*C*-35), 35.8 (*C*-28), 33.6 (*C*-11), 25.1 (*C*-27) ppm.

**$^{19}$F NMR** (470 MHz, acetone-$d_6$): δ = -112.2 ppm.

**IR** (ATR): ϑ = 3291 (w), 2252 (w), 1737 (w), 1675 (m), 1603 (m), 1556 (m), 1502 (m), 1457 (m), 1418 (m), 1225 (vs), 1176 (m), 1113 (m), 1046 (m), 867 (w), 846 (m), 825 (m), 805 (m), 761 (m), 737 (m), 697 (m), 637 (w), 573 (w), 525 (w), 435 (w) $cm^{-1}$.

**MS** (MALDI-MS-TOF): m/z = 688 $[M-H]^+$.

**MS** (EI, 70 eV): m/z (%) = 688 (7) $[M]^+$, 655 (36) $[M-CH_3O]^+$, 495 (39) $[M-C_{11}H_{14}O_3]^+$, 463 (100) $[M-C_{13}H_{16}N_2S]^+$.

**MS** (EI, HR, 70 eV): $C_{38}H_{30}FN_5O_3S_2$, m/z = calc.: 687.1774, found: 687.1752.

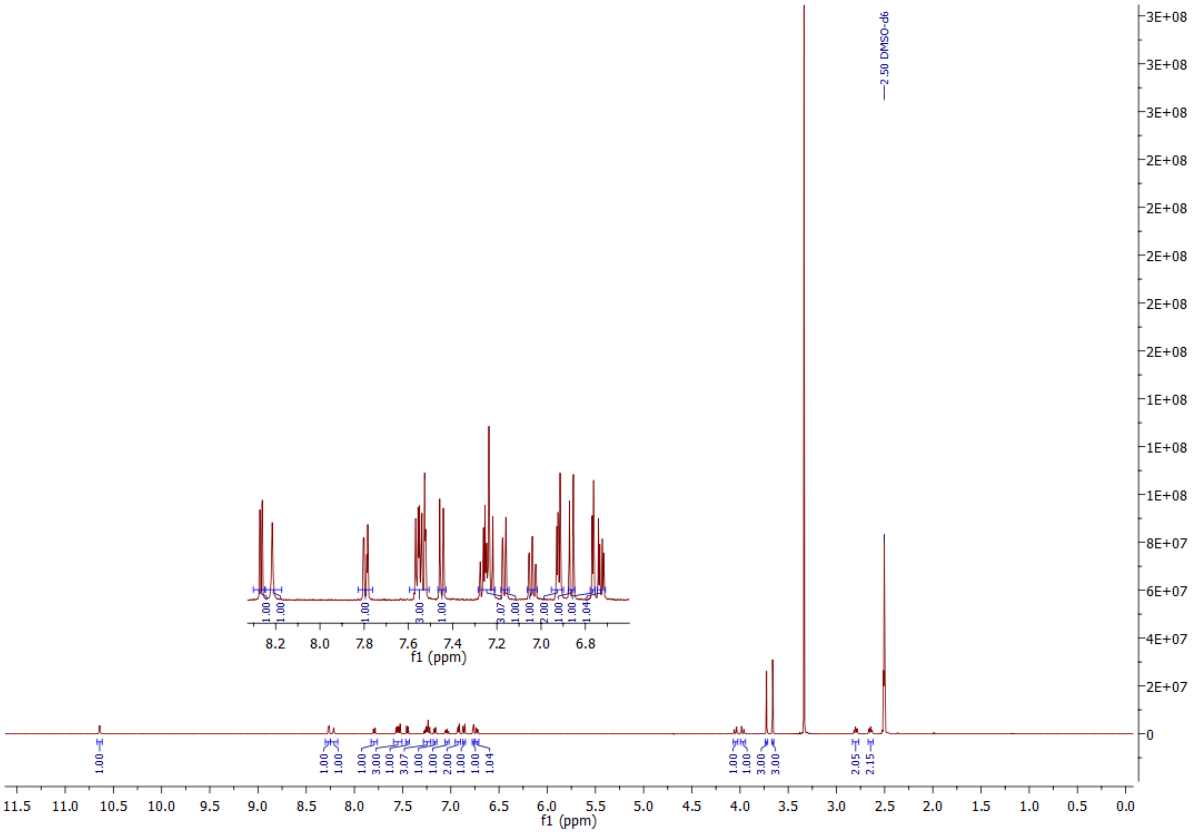



**Figure S43**: $^{1}$H NMR spectrum of compound **29** measured in deuterated DMSO.

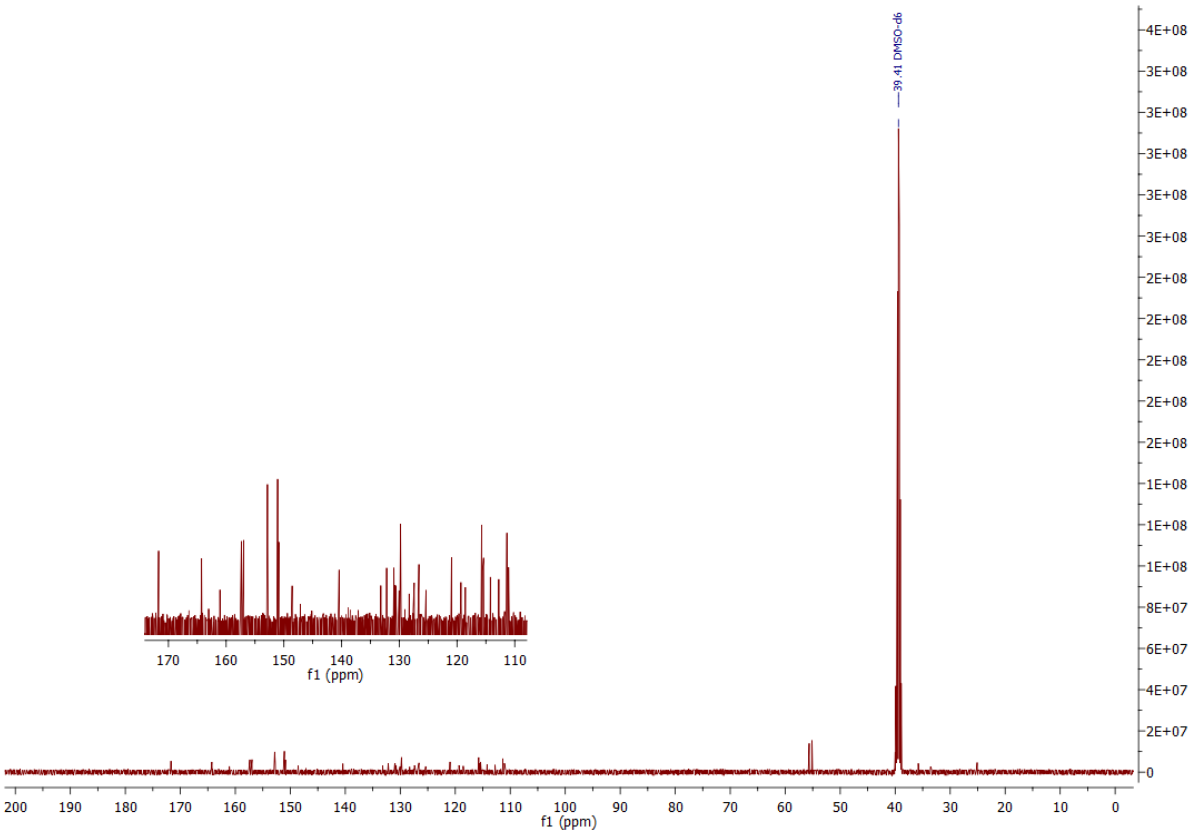



**Figure S44**: $^{13}$C NMR spectrum of compound **29** measured in deuterated DMSO.

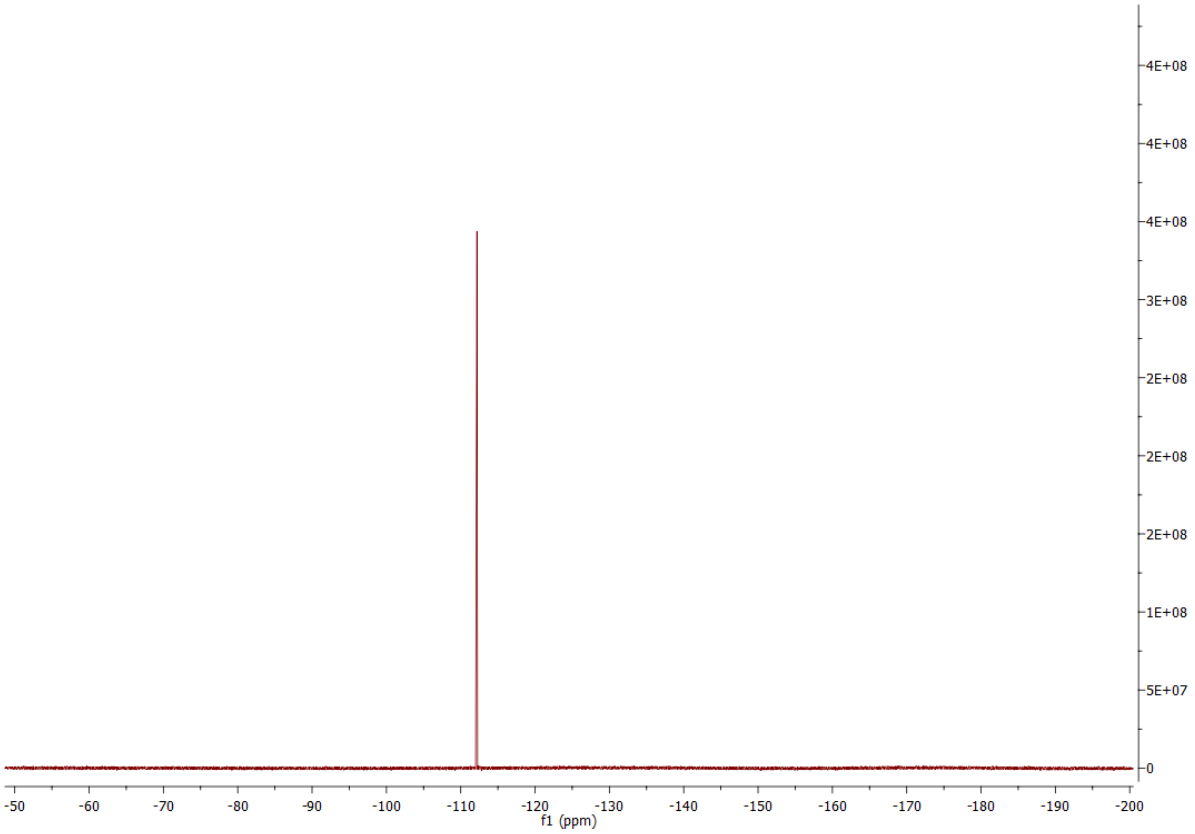



**Figure S45**: $^{19}$C NMR spectrum of compound **29** measured in deuterated DMSO.

## IV. Photochemical characterization of compounds 2-4

IV.1 Photostationary state and thermal half-life and of 2-azo-thiazole **2**

To determine the photostationary state of compound **2**, $^{1}$H NMR spectra were measured in deuterated DMSO at 300 K before and after irradiation with 435 nm for 15 min. To ensure a homogenous mixture during irradiation, the NMR tube was manually shaken every 2 min. It could be shown, that the 2-azo-thiazole **2** is converted to the *Z*-isomer with a yield of 29 %. Back isomerization with 525 nm showed a residual 19 % of the *Z*-isomer.

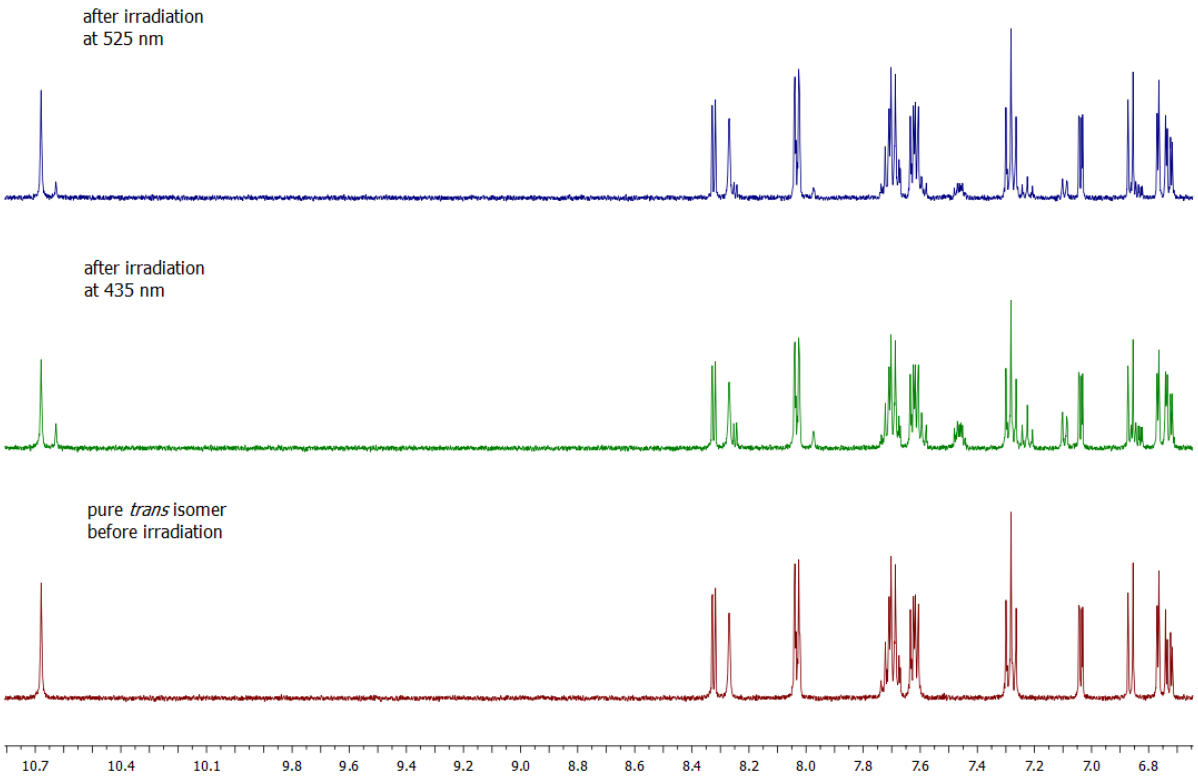



**Figure S46**: Aromatic region of the $^{1}$H NMR spectra of 2-azo-thiazole **2** before (red) irradiation, after irradiation with 435 nm (green) and after back isomerization with 525 nm (blue).

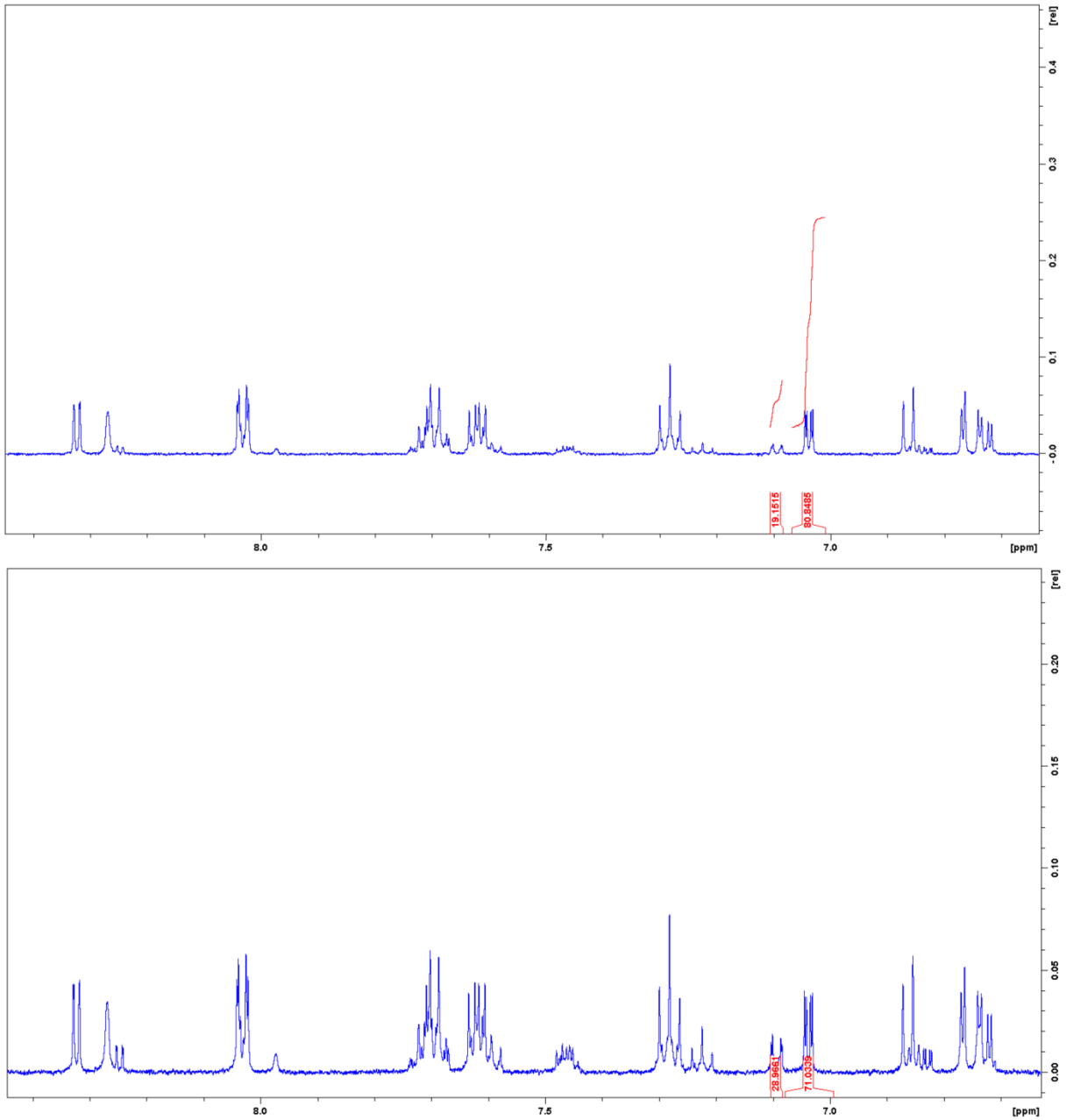



**Figure S47**: *Below*: Photostationary state of 2-azo-thiazole **2** after irradiation with 435 nm (*Z*: 29 %, *E:* 71 %); *Above*: Photostationary state of 2-azo-thiazole **2** after irradiation with 525 nm (*Z*: 19 %, *E*: 81 %).

To determine the thermal half-life, a solution of compound **2** in DMSO was irradiated with 435 nm for 1 min and UV/vis spectra were recorded every 2 min at 27 °C. The absorption maxima were plotted as a function of time to identify the half-live of 13.1min (figure S48).

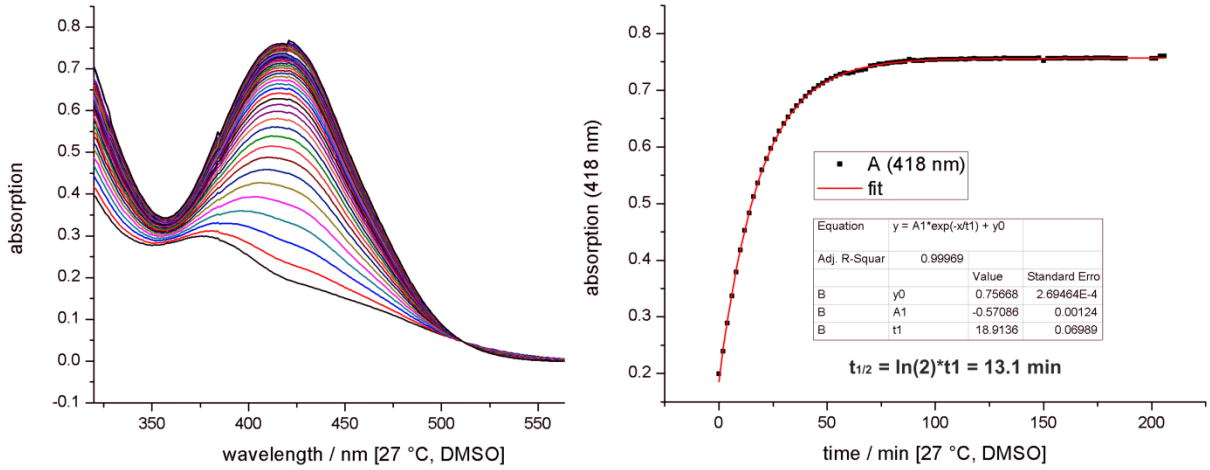



**Figure S48**: *Left*: UV/vis spectra of 2-azo-thiazol 2 recorded every 2 min after irradiation with 435 nm at 27 °C. *Right*: Absorption at 418 nm plotted as a function of time (black dots) with exponential fit (red line).

IV.2 Photostationary state and thermal half-life and of 2-azo-imidazole **3**

To determine the photostationary state of compound **3**, $^{1}$H NMR spectra were measured in deuterated DMSO at 300 K before and after irradiation with 420 nm for 15 min. To ensure a homogenous mixture during irradiation, the NMR tube was manually shaken every 2 min. It could be shown, that the 2-azo-imidazole **3** is converted to the *Z*-isomer with a yield of 85 %. Back isomerization with 525 nm showed a residual 10 % of the *Z*-isomer.

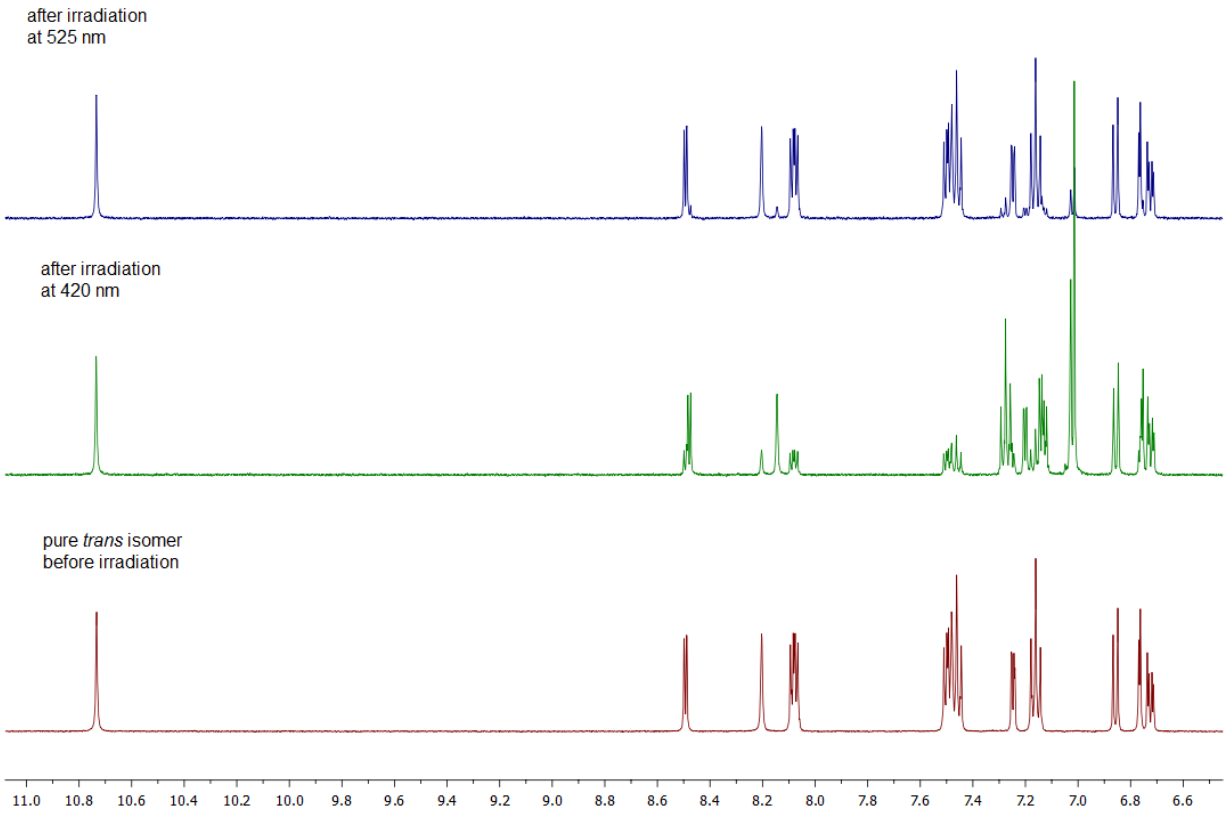



**Figure S49**: Aromatic region of the $^{1}$H NMR spectra of 2-azo-imidazole **3** before (red) irradiation, after irradiation with 420 nm (green) and after back isomerization with 525 nm (blue).

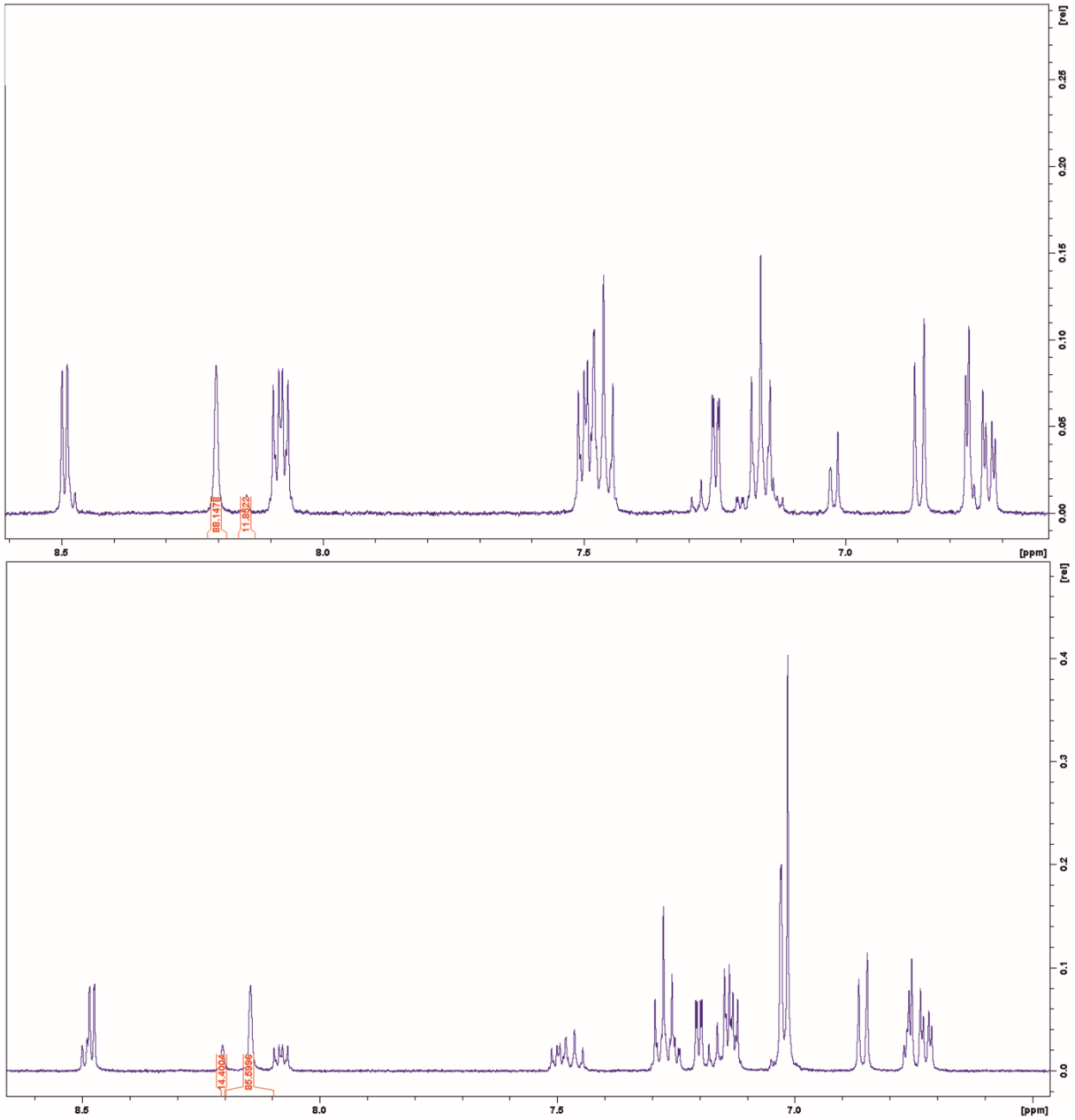



**Figure S50**: *Below*: Photostationary state of 2-azo-imidazole **3** after irradiation with 420 nm (*Z*: 85 %, *E*: 15 %;) *Above*: Photostationary state of 2-azo-imidazole **3** after irradiation with 525 nm (*Z*: 12 %, *E*: 88 %).

To determine the thermal half-life, a solution of compound **3** in deuterated DMSO was irradiated with 420 nm for 10 min and $^{1}$H NMR spectra at 300 K were recorded every 6.5 min. The integral percentage of the *Z*-isomer was plotted as a function of time to identify the half-live of 2.4 h (figure S51).

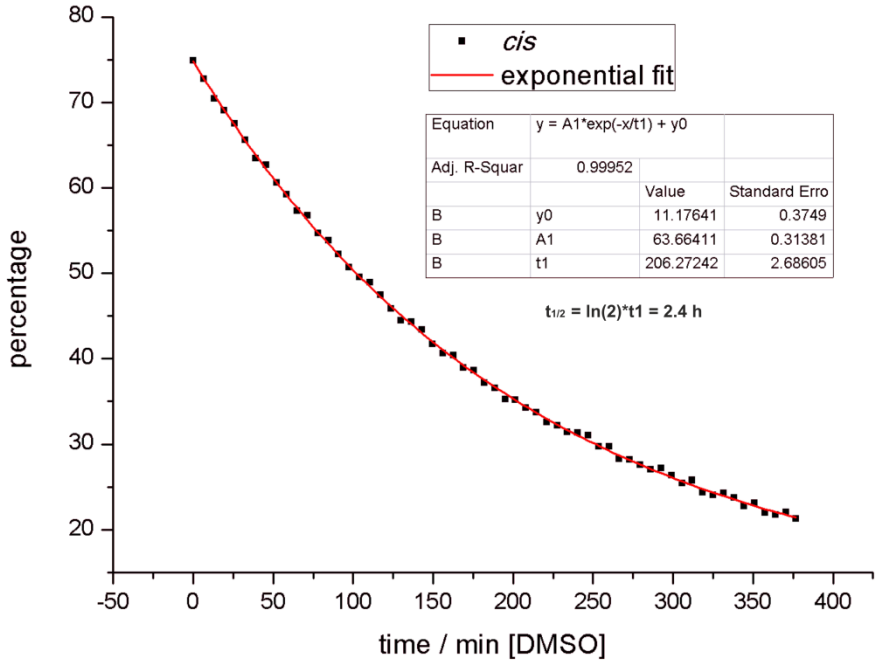



**Figure S51**: Integral percentage of the *Z*-isomer of compound **3** plotted as a function of time (black dots) with exponential fit (red line).

IV.3 Photostationary state and thermal half-life and of 2-diazocine-thiazole **4**

To determine the photostationary state of compound **4** $^{1}$H NMR spectra were measured in deuterated DMSO at 300 K before and after irradiation with 405 nm for 15 min. To ensure a homogenous mixture during irradiation, the NMR tube was manually shaken every 2 min. It could be shown, that the 2-diazocine-thiazole **4** is converted to the *E*-isomer with a yield of 47 %. Back isomerization with 525 nm is achieved quantitatively.

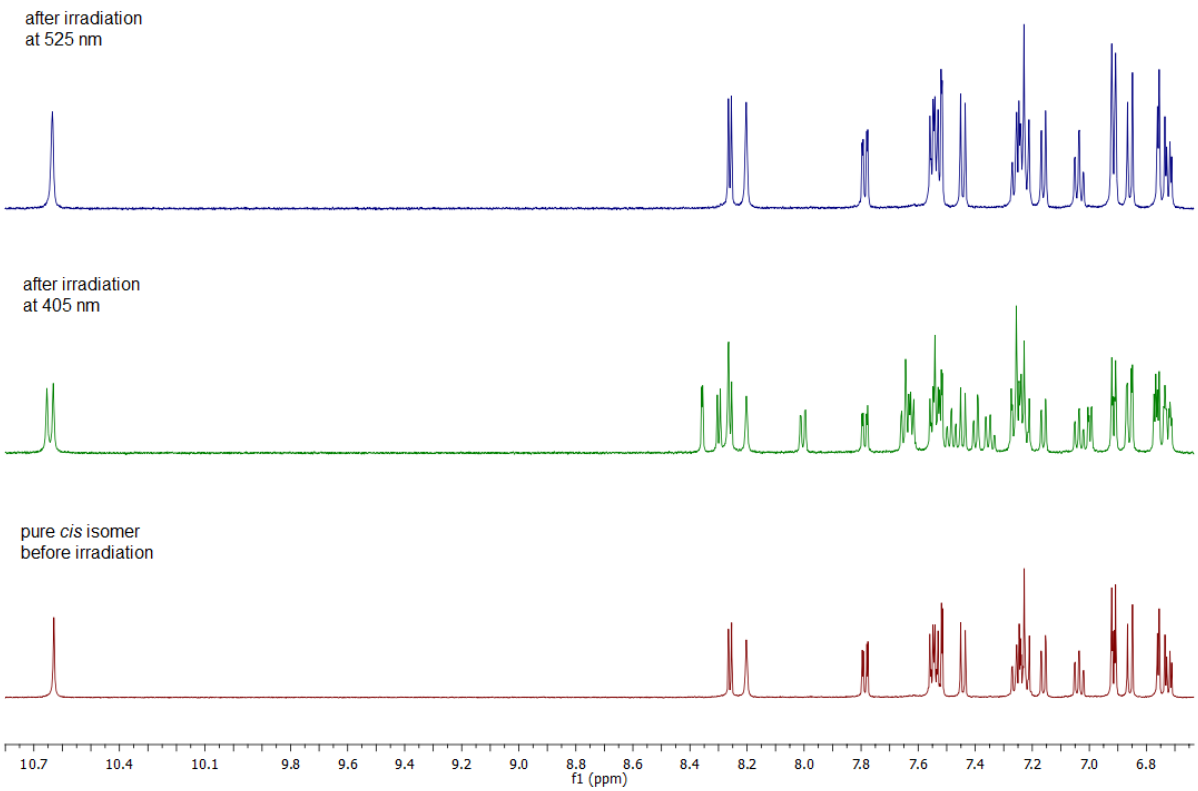



**Figure S52**: Aromatic region of the $^{1}$H NMR spectra of 2-diazocine-thiazol **4** before (red) irradiation, after irradiation with 405 nm (green) and after back isomerization with 525 nm (blue).

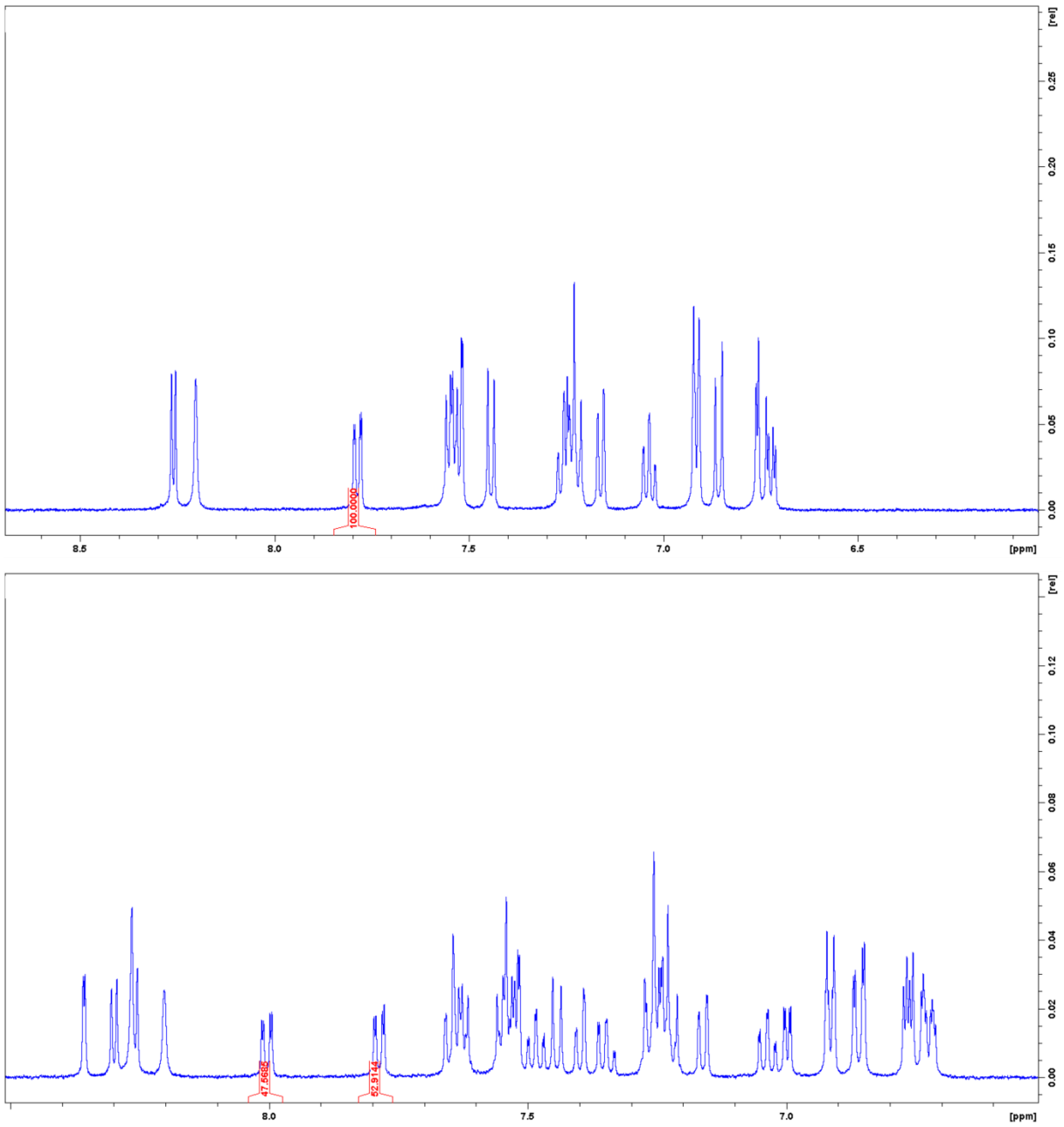



**Figure S53**: *Below*: Photostationary state of 2-diazocine-thiazol **4** after irradiation with 405 nm (*E*: 47 %, *Z*: 53 %); *Above*: Photostationary state of 2-diazocine-thiazol **4** after irradiation with 525 nm (*E:* 0 %, *Z*: 100 %).

To determine the thermal half-life, a solution of compound **4** in deuterated DMSO was irradiated with 405 nm for 10 min and $^{1}$H NMR spectra at 300 K were recorded every 30 min. The integral percentage of the *E*-isomer was plotted as a function of time to identify the half-live of 3.2 d (figure S54).

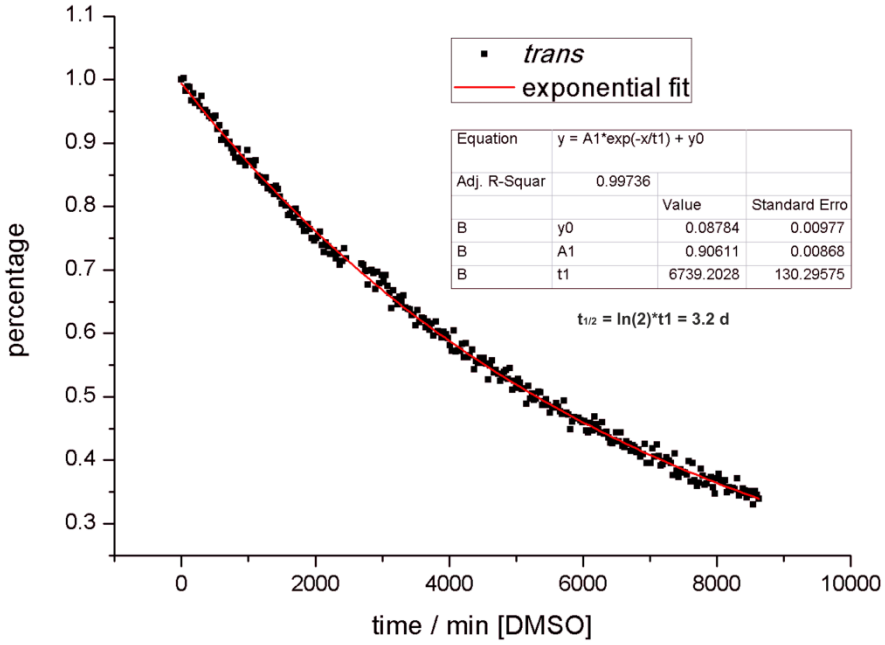



**Figure S54**: Integral percentage of the *E*-isomer of compound **4** plotted as a function of time (black dots) with exponential fit (red line).

<u>UV/vis spectra of compound **4**:</u>

The UV absorption of the diazocine moiety of 2-diazocine-thiazole **4** is relatively weak compared to the UV absorption of the residual molecule. In the UV/vis spectra below, displayed from 250-600 nm, the diazocine absorption using a concentration of 50 µM is bearly visible. Therefore, for the enlarged area displayed from 400-700 nm a higher concentration of 290 µM was used to ensure satisfying resultion of the diazocine absorption.

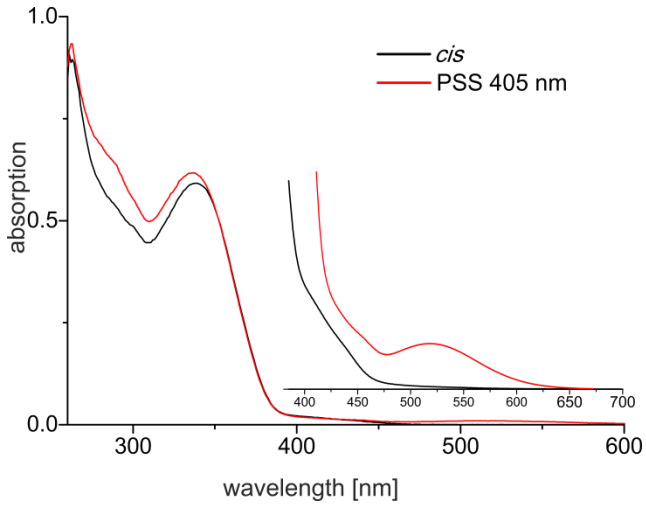



**Figure S54**: UV/vis spectra of compound **4** displayed from 250-600 nm (50 µM, DMSO).The enlarged area was measured at a higher concentration to ensure satisfying resolution of the diazocine absorption (290 µM, DMSO).

**V. Activity based kinase assays**

p38α MAPK assays:

N-terminally His6-tagged wild type p38α MAPK was expressed and purified as described previously.[8] Subsequently, purified wild type p38α MAPK was activated with constitutively active $MKK6^{S207E/T211E}$ (Thermo Scientific, Lot# 877061F) in activation buffer (50 mM Tris, 10 mM $MgCl_2$, 1 mM ATP, 1 mM DTT, 0.001 % Tween 20, pH 7.4) at 37 °C for 90 min with shaking at 400 rpm. Afterwards, the samples were dialyzed overnight at 4 °C against storage buffer (20 mM HEPES, 50 mM NaCl, 5 % glycerol, pH 7.1), concentrated to ~0.2 mg/mL and stored at -80 °C for further use.

Activity-based $IC_{50}$ measurements were carried out using the HTRF® KinEASE™ assay from Cisbio according to the manufacturer's instructions. Therefore, 0.04 ng activated p38α MAPK per well were incubated in the presence of analyte (6-fold dilution series, 20 µM – 0.071 nM, final DMSO concentration 2 %) in reaction buffer (50 mM HEPES, 0.1 mM $Na_3VO_4$, 0.02 % $NaN_3$, 0.01 % (w/v) BSA, 10 mM $MgCl_2$, 1 mM $MnCl_2$, 1 mM DTT, 0.01 % Triton X-100, pH 7.0) in microtiter plates (Greiner Bio-One, 384 well, black, flat bottom) for 60 min at ambient temperature. Plates were illuminated separately with the specific wavelengths (420 nm, 435 nm) intermittently every 5 min. The kinase reaction was started by addition of ATP/substrate solution (100 µM ATP, 1 µM GST-ATF2) and allowed to react for 10 min at room temperature with intermittent illumination. Afterwards, the reaction was stopped by addition of detection solution (50 mM HEPES, 0.1 % (w/v) BSA, 800 mM KF, 20 mM EDTA, 0.666 nM anti-phospho-ATF2-Eu(K) antibody, 100 nM anti-GST-d2 antibody, pH 7.0) and incubation for 60 min at room temperature. The entire procedure was carried out in the dark to enable isomer enrichment induced by specific illumination. Finally, fluorescence was detected at emission wavelengths of 620 nm and 665 nm 60 µs after excitation at 317 nm using an EnVision 2104 plate reader (Perkin Elmer). The acceptor/donor ratios ($fl_{665\ nm}/fl_{620\ nm}$) were calculated and background fluorescence was subtracted. Kinase activity was calculated in percent of signals for lowest inhibitor concentration and plotted against logarithm of analyst concentration. Sigmoidal fitting (*log(inhibitor) vs. response – variable slope*) and calculation of relative $IC_{50}$ values and maximal inhibitory effect (bottom plateau) were performed using GraphPad Prism®(7.03). Each assay was carried out three times in double measurements.

Found residual kinase activity at the plateau for some compounds is contributed to the poor solubility of the tested compounds and a difference of water solubility for the *Z*- and *E*-isomer. At higher concentrations precipitation can take place causing a residual kinase activity due to lower compound concentration.

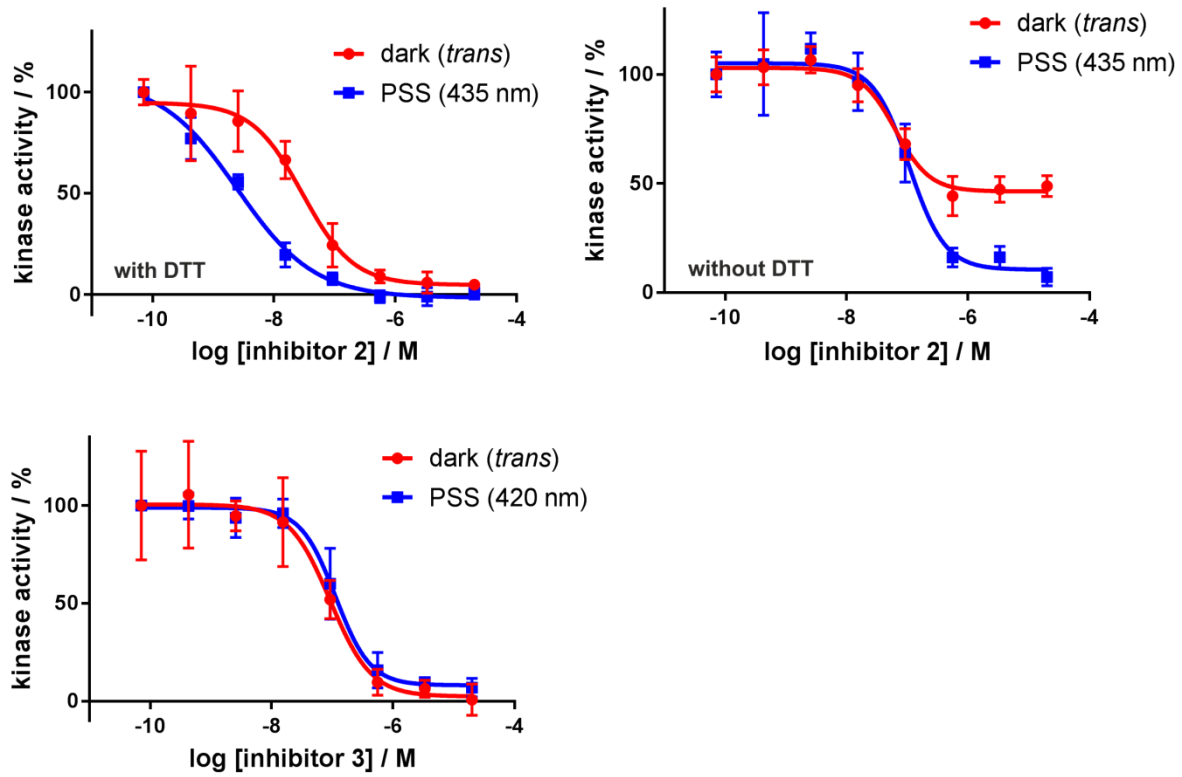



**Figure S55**: *In vitro* dose-response data for p38α for compound **2** (above) and compound **3** (bottom). For compound **2** the dose-response data with DTT (above left) and without DTT (above right) are compared. The red curves show the data for the *E*-isomers (without irradiation) and the blue curves the data for the PSS (after irradiation). n = 3, ± SD.

CK1δ assays:

*Generation of Expression Vectors*

Human CK1δ transcription variant 1 (CK1δ TV1_NM_001893.4) has been amplified from the cDNA obtained from HT1080 cell line, using CK1δ_forward-primer: 5'-GGA TCC ATG GAG CTG AGA GTC GGG AAC AG-3' and CK1δ_reverse primer: 5'-GGA TCC TCA TCG GTG CAC GAC AGA CTG A-3'. The CK1δ DNA construct was cloned into pSC-A cloning vector (Agilent Technologies, Munich, Germany) before being subcloned into pGEX6-P3 expression vector (GE Healthcare, Munich, Germany) with BamHI enzyme, to generate plasmid pGEX6-P3-GST-CK1δ TV1 (FP1417).

*Overexpression and Purification of Glutathion S-Transferase Fusion CK1δ TV1 Protein*

Overexpression and purification of GST-CK1δ TV1 protein has been performed as described before.[9] Briefly, XL1-Blue supercompetent bacteria, previously transformed with pGEX6-P3-GST-CK1δ TV1 (FP1417), have been inoculated in 50 ml LB-medium (1 % (w/v) NaCl, 1 % (w/v) trypton/pepton, 0.5 % (w/v) yeast extract in $dH_2O$, sterilized by autoclaving, supplemented with 100 µg/ml ampicillin) and incubated ON at 37°C (in shaking conditions, ~ 140 rpm). Bacterial suspension has been diluted with 50 ml of LB-medium and incubated for ~1 h at 37°C, until an OD600 of 0.7-0.9 was reached. The expression of the GST-fusion protein (GST-CK1δ TV1) has been induced by adding IPTG (0.5 mM) to the culture and incubating ON at 15°C (in shaking conditions, ~ 140 rpm). Bacteria have been collected by centrifugation and pellet has been frozen at -80°C for about 20 min. Pellet has been resuspended with 10 ml of NP-40 lysis buffer (20 mM Tris-HCl [pH 7.6], 150 mM NaCl, 10 % (v/v) glycerol, 0.5 % (v/v) NP-40, 1 mM DTT, 1 mM EDTA, 1 mM EGTA, 50 µM benzamidine, 25 µg/ml aprotinin) and transferred in a fresh Sorvall-tube where 2.5 mg of lysozyme has been added. After 30 min on ice, lysates have been diluted with additional 10 ml of NP-40 lysis buffer. Sonication (2 sec, level 3; Ultrasonic Sonifier 250; Branson, Danbury, USA), has been performed to fragment the DNA and samples have been centrifuged (10000 rpm, 4°C, 30 min). Supernatant has been transferred in a fresh tube and 600 µl (1:1 in PBS) of Glutathione Sepharose® 4 Fast Flow beads (GE Healthcare, Munich, Germany) have been added and incubated for 2 h-ON at 4°C under rotating conditions. GST-fusion proteins can bind to the glutathione sepharose beads which have been washed three times with washing buffer 1 (lysis buffer with 0.2 mM NaCl) and subsequently twice with of washing buffer 2 (20 mM Tris-HCl [pH 7.6], 50 mM NaCl, 10 % (v/v) glycerol, 1 mM EDTA, 25 µg/ml aprotinin), each time it was centrifuged at maximal speed at 4°C for 1 min. GST-fusion proteins have been eluted with 600 µl of elution buffer (50 mM Tris-HCl [pH 7.0], 0.1% (w/v) reduced glutathione, 1 mM EDTA, 25 µg/ml aprotinin) in rotating conditions (4°C, 30 min), and collected after centrifugation (maximal speed, 4°C, 1 min). The elution step has been repeated two further times with 300 µl and then 150 µl of elution buffer and centrifuged after respectively 20 min and 10 min. Dialysis was not

performed in order to preserve kinase activity. Purified GST-CK1δ TV1 fusion protein has been then stored at -80°C after shock freezing in liquid nitrogen.

*Kinase Assays*

*In vitro* kinase assays have been performed to calculate $IC_{50}$ values of the compounds for CK1δ. Each reaction was carried out using 2 µCi $^{32}$P-γ-ATP in kinase buffer containing 25 mM Tris-HCl (pH = 7.5), 10 mM $MgCl_2$, 100 µM EDTA, and 10 µM ATP. Potential inhibitor compounds were used in a dilution series ranging from 10 µM to 5 nM final reaction concentration, which was prepared by serial dilution in DMSO, in order to calculate the $IC_{50}$ values. Recombinant human GST-CK1δ TV1 (expressed and purified as GST fusion protein as described earlier) was used as sources of enzyme, while α-casein (C6780; Sigma-Aldrich) was used as substrate. Kinase reactions were incubated for 30 min at 30 °C and then stopped with loading dye (50 mM Tris-HCl (pH = 6.8), 5 % (v/v) β-mercaptoethanol (MSH), 10 % (v/v) glycerol, 2 % (w/v) SDS, 0.1 % (w/v) bromphenol blue). Subsequently, reactions were separated by SDS-PAGE and phosphorylated protein bands were visualized on dried gels by autoradiography. The phosphorylated substrate protein bands were excised and phosphorylation was quantified by Cherenkov counting. Kinase activity was calculated in percent of control without inhibitor (DMSO only) and plotted against logarithm of analyst concentration. Sigmoidal fitting (*log(inhibitor) vs. response – variable slope*) and calculation of relative $IC_{50}$ values and maximal inhibitory effect (bottom plateau) were performed using GraphPad Prism®(7.03). Each assay was carried out three times in double measurements.

Found residual kinase activity at the plateau for some compounds is contributed to the poor solubility of the tested compounds and a difference of water solubility for the *Z*- and *E*-isomer. At higher concentrations precipitation can take place causing a residual kinase activity due to lower compound concentration.

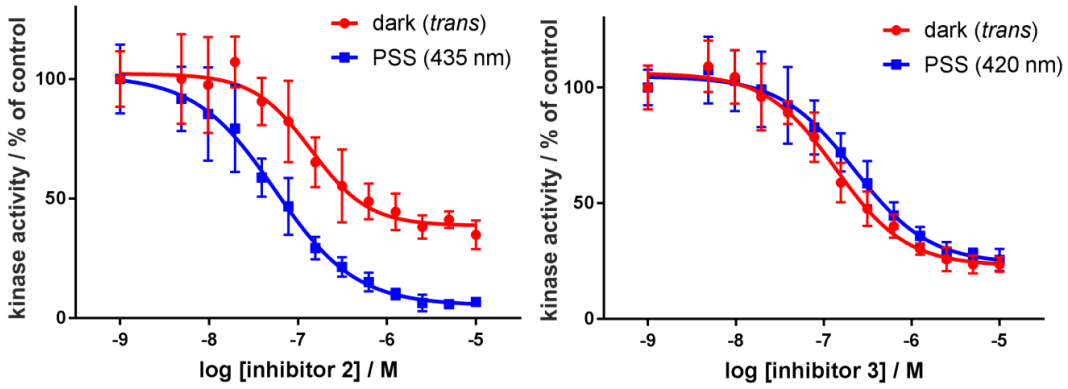



**Figure S56**: *In vitro* dose-response data for CK1δ for compound **2** (left) and compound **3** (right). The red curves show the data for the *E*-isomers (without irradiation) and the blue curves the data for the PSS (after irradiation). n = 3, ± SD.

## VI. Reduction of 2-azo-thiazole 2 with DTT

The reduction of compound **2** with DTT was investigated using $^{1}$H NMR spectroscopy. The 2-azo-thiazole **2** was dissolved in deuterated DMSO and a $^{1}$H NMR spectrum was recorded (blue spectrum). Then an approximately 3 mM DTT solution in deuterated DMSO was added and a second $^{1}$H NMR spectrum was recorded (red spectrum). In this spectrum a second signal set emerges, which could be assigned to the reduced azobenzene **31** with high resolution mass spectrometry (MS (HR-ESI): m/z (%) = $[C_{31}H_{29}FN_5O_3S]^+$, m/z = calc.: 570.1970, found: 570.1966). After addition of a great excess of DTT the 2-azo-thiazole **2** is reduced quantitatively to the corresponding hydrazine **31**.

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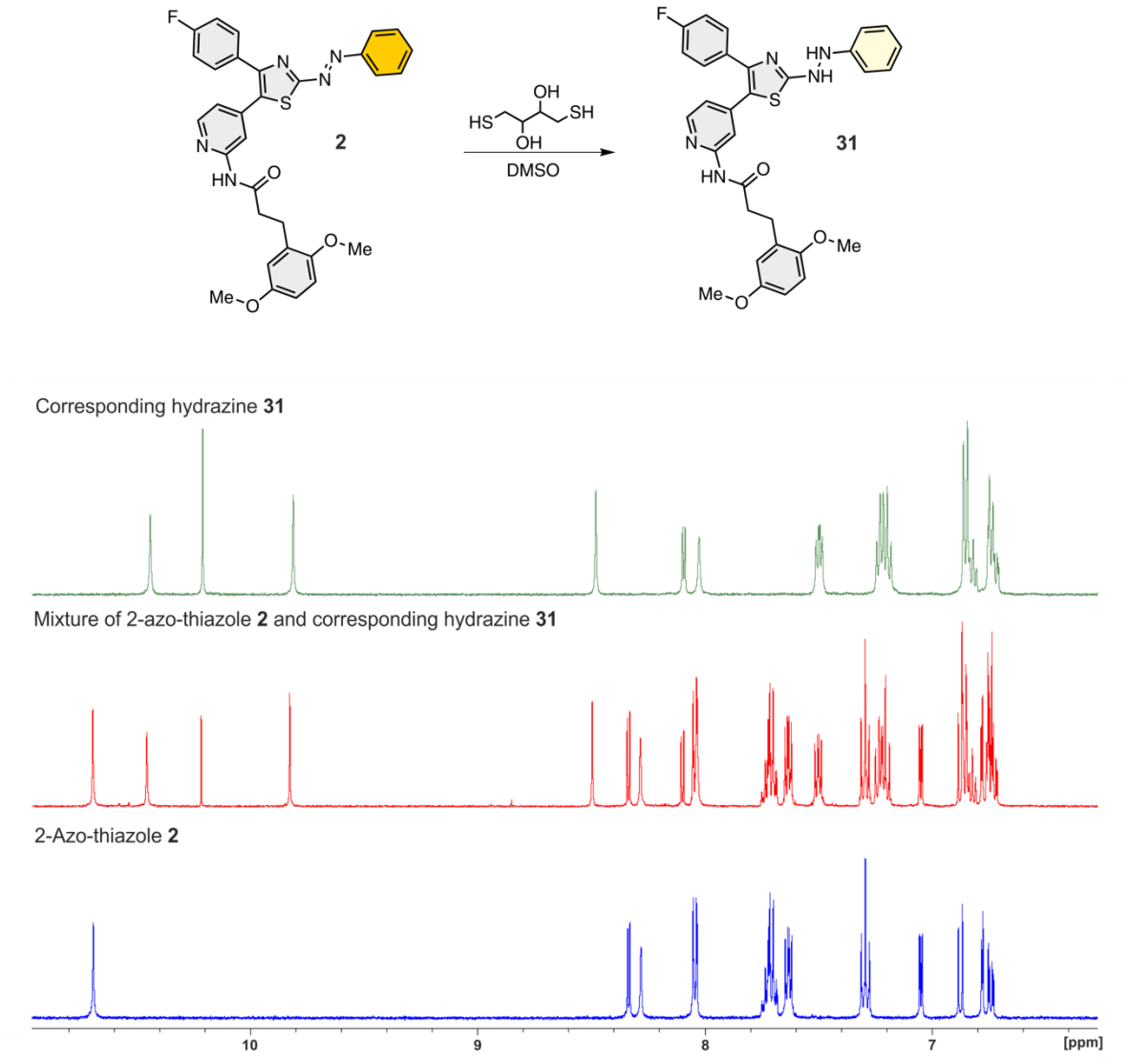



**Figure S57**: Aromatic region in the $^{1}$H NMR of compound **2** in deuterated DMSO without DTT (blue), after addition of approximately 3 mM DTT (red) and after addition of a great excess DTT.

The reduction process was also investigated using UV/vis spectroscopy. Therefore a 50 µM solution of compound **2** in DMSO was prepared and a UV/vis spectrum was recorded (figure S58, black line). Afterwards a 3 mM DTT solution in DMSO was added and in an interval of 3 min new spectra were recorded. From these spectra the decrease of the maximum at 424 nm was plotted as a function of time to identify the half-live of 3.3 min. Due to the very fast reduction the value is an approximation.

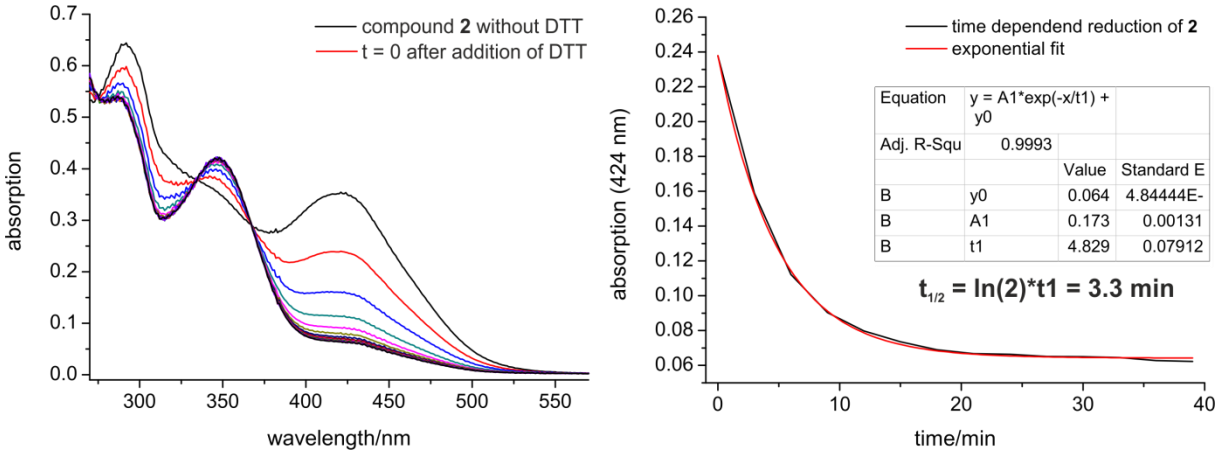



**Figure S58:** *Left*: UV/vis spectra of a 50 µM solution of compound **2** in DMSO before addition of DTT (black line) and after addition of 3 mM DTT solution in DMSO (red line shows t = 0). After addition of DTT every 3 min a spectrum was recorded. *Right*: Absorption at 424 nm plotted as a function of time (black line) with exponential fit (red line).

## VII. Crystallographic data of compound 13

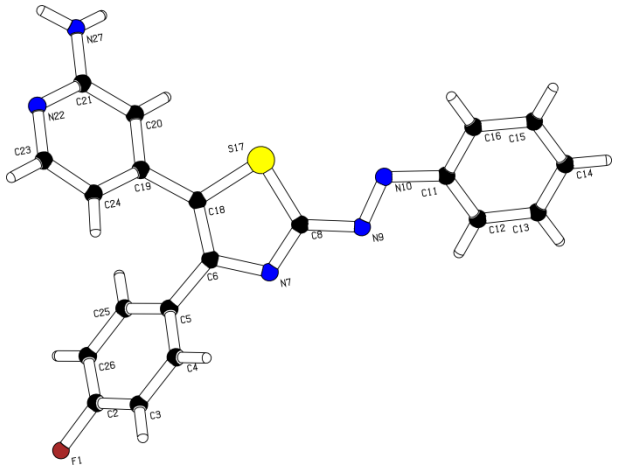



**Table S1. Crystal data and structure refinement for compound 3**

| | |
|---|---|
| Empirical formula | $C_{20}H_{14}FN_5S$ |
| Formula weight | 375.42 |
| Temperature | - 80 °C |
| Wavelength | 1.54178 (Cu-Kalfa) |
| Crystal system | Monoclinic |
| Space group | $P2_1/c$ |
| Unit cell dimensions | a = 10.0319(6) Å |
| | b = 8.6937(6) Å β= 95.512(4)°. |
| | c = 20.5271(10) Å |
| Volume | 1781.98(18) $Å^3$ |
| Z | 4 |
| Density (calculated) | 1.399 $g/cm^3$ |
| Absorption coefficient | $\mu$ = 1.827 $mm^{-1}$ |
| F(000) | 776.0 |
| Crystal size | 0.020 x 0.030 x 0.160 $mm^3$ |
| Theta range for data collection | 4 to 67.6 °. |
| Index ranges | -11<=h<=11, -10<=k<=9, -24<=l<=21 |
| Reflections collected | 13377 |
| Independent reflections | 3179 [R(int) = 0.035] |
| Completeness to theta = 67.6° | 98.5 % |
| Refinement method | Full-matrix least-squares on $F^2$ |
| Data / restraints / parameters | 3179 / 0 / 251 |
| Goodness-of-fit on $F^2$ | 0.963 |
| Final R indices [I>2sigma(I)] | R1 = 0.0361, wR2 = 0.0901 |
| R indices (all data) | R1 = 0.0615 |
| Largest diff. peak and hole | 0.20 and -0.30 e.$Å^-$ |

Comments

A numerical absorption correction was performed (Tmin(max: 0.832/0.963). All non-hydrogen atoms were refined anisotropic The C-H H atoms were positioned with idealized geometry and refined isotropic using a riding model. The N-H H atoms were localized and refined.

**Table S2. Atomic coordinates and equivalent displacement parameters ($Å^2$)**
**$U(eq) = (1/3)*\sum\sum_{ij} a_i*a_j*a_ia_j$**

| atom | X | Y | Z | $U_{eq}$ |
|---|---|---|---|---|
| F1 | 1.0908(1) | 0.9228(2) | 0.41646(8) | 0.0686(7) |
| C2 | 0.9637(2) | 0.8815(3) | 0.4274(1) | 0.0430(8) |
| C3 | 0.8591(3) | 0.9332(3) | 0.3850(1) | 0.0491(9) |
| C4 | 0.7306(2) | 0.8874(3) | 0.3948(1) | 0.0384(8) |
| C5 | 0.7082(2) | 0.7927(2) | 0.44748(9) | 0.0249(6) |
| C6 | 0.5706(2) | 0.7395(2) | 0.45601(9) | 0.0222(6) |
| N7 | 0.4961(2) | 0.6784(2) | 0.40198(7) | 0.0245(5) |
| C8 | 0.3788(2) | 0.6340(2) | 0.41709(9) | 0.0242(6) |
| N9 | 0.2874(2) | 0.5636(2) | 0.37010(8) | 0.0281(6) |
| N10 | 0.1772(2) | 0.5340(2) | 0.39263(8) | 0.0287(5) |
| C11 | 0.0844(2) | 0.4498(2) | 0.34929(9) | 0.0277(6) |
| C12 | 0.1214(2) | 0.3630(3) | 0.2972(1) | 0.0404(8) |
| C13 | 0.0253(3) | 0.2784(3) | 0.2601(1) | 0.0489(9) |
| C14 | -0.1066(3) | 0.2831(3) | 0.2734(1) | 0.0454(8) |
| C15 | -0.1429(3) | 0.3698(3) | 0.3250(1) | 0.0522(9) |
| C16 | -0.0466(2) | 0.4517(3) | 0.3638(1) | 0.0441(8) |
| S17 | 0.34866(5) | 0.66117(6) | 0.49802(2) | 0.0258(1) |
| C18 | 0.5075(2) | 0.7388(2) | 0.51291(9) | 0.0214(6) |
| C19 | 0.5500(2) | 0.8007(2) | 0.57893(8) | 0.0214(6) |
| C20 | 0.5051(2) | 0.7320(2) | 0.63386(9) | 0.0235(6) |
| C21 | 0.5362(2) | 0.8000(2) | 0.69583(9) | 0.0254(6) |
| N22 | 0.6089(2) | 0.9294(2) | 0.70392(8) | 0.0276(5) |
| C23 | 0.6546(2) | 0.9901(2) | 0.65014(9) | 0.0270(6) |
| C24 | 0.6284(2) | 0.9328(2) | 0.58770(9) | 0.0256(6) |
| C25 | 0.8169(2) | 0.7460(3) | 0.4901(1) | 0.0319(7) |
| C26 | 0.9462(2) | 0.7899(3) | 0.4801(1) | 0.0410(8) |
| N27 | 0.4900(2) | 0.7380(3) | 0.75005(9) | 0.0417(7) |

**Table S3. Anisotropic displacement parameter**

| atom | $U_{11}$ | $U_{22}$ | $U_{33}$ | $U_{12}$ | $U_{13}$ | $U_{23}$ |
|---|---|---|---|---|---|---|
| F1 | 0.0342(9) | 0.089(1) | 0.088(1) | -0.0254(8) | 0.0301(8) | 0.0152(10) |
| C2 | 0.030(1) | 0.052(2) | 0.051(1) | -0.016(1) | 0.020(1) | -0.014(1) |
| C3 | 0.053(2) | 0.057(2) | 0.041(1) | -0.018(1) | 0.021(1) | 0.006(1) |
| C4 | 0.037(1) | 0.049(1) | 0.030(1) | -0.007(1) | 0.0074(10) | 0.007(1) |
| C5 | 0.027(1) | 0.029(1) | 0.0197(9) | -0.0038(9) | 0.0071(8) | -0.0040(8) |
| C6 | 0.024(1) | 0.024(1) | 0.0183(9) | -0.0001(8) | 0.0020(8) | 0.0019(8) |
| N7 | 0.0274(9) | 0.0270(9) | 0.0190(7) | -0.0035(7) | 0.0025(7) | 0.0013(7) |
| C8 | 0.027(1) | 0.026(1) | 0.0187(9) | -0.0013(9) | 0.0012(8) | 0.0006(8) |
| N9 | 0.029(1) | 0.0311(10) | 0.0231(8) | -0.0023(8) | -0.0013(7) | 0.0006(7) |
| N10 | 0.0279(10) | 0.0308(9) | 0.0269(9) | -0.0024(8) | -0.0008(7) | 0.0007(7) |
| C11 | 0.029(1) | 0.028(1) | 0.0251(10) | -0.0032(9) | -0.0037(8) | 0.0009(8) |
| C12 | 0.034(1) | 0.045(1) | 0.042(1) | -0.002(1) | 0.000(1) | -0.012(1) |
| C13 | 0.050(2) | 0.051(2) | 0.045(1) | -0.007(1) | 0.000(1) | -0.017(1) |
| C14 | 0.046(2) | 0.048(1) | 0.040(1) | -0.015(1) | -0.012(1) | 0.002(1) |
| C15 | 0.034(1) | 0.068(2) | 0.054(1) | -0.016(1) | 0.002(1) | -0.006(1) |
| C16 | 0.038(1) | 0.055(2) | 0.040(1) | -0.009(1) | 0.006(1) | -0.009(1) |
| S17 | 0.0246(3) | 0.0328(3) | 0.0205(2) | -0.0053(2) | 0.0042(2) | -0.0011(2) |
| C18 | 0.022(1) | 0.0218(10) | 0.0207(9) | -0.0024(8) | 0.0033(8) | 0.0022(8) |
| C19 | 0.021(1) | 0.025(1) | 0.0191(9) | 0.0021(8) | 0.0033(8) | 0.0002(7) |
| C20 | 0.025(1) | 0.025(1) | 0.0210(9) | -0.0029(8) | 0.0053(8) | -0.0016(8) |
| C21 | 0.029(1) | 0.028(1) | 0.0200(9) | 0.0009(9) | 0.0056(8) | 0.0008(8) |
| N22 | 0.033(1) | 0.0279(10) | 0.0228(8) | -0.0022(8) | 0.0054(7) | -0.0037(7) |
| C23 | 0.028(1) | 0.026(1) | 0.0276(10) | -0.0055(9) | 0.0073(8) | -0.0035(8) |
| C24 | 0.028(1) | 0.026(1) | 0.0234(10) | -0.0026(9) | 0.0076(8) | 0.0004(8) |
| C25 | 0.029(1) | 0.038(1) | 0.028(1) | -0.0008(10) | 0.0052(9) | 0.0001(9) |
| C26 | 0.027(1) | 0.050(2) | 0.046(1) | -0.002(1) | 0.005(1) | -0.008(1) |
| N27 | 0.067(1) | 0.042(1) | 0.0185(9) | -0.019(1) | 0.0120(9) | -0.0032(8) |

**Table S4. Hydrogen coordinates and isotropic displacement parameters ($Å^2$).**

| atom | X | Y | Z | $U_{iso}$ |
|---|---|---|---|---|
| H3 | 0.87471 | 0.99916 | 0.34964 | 0.0589 |
| H4 | 0.65713 | 0.92053 | 0.3655 | 0.0461 |
| H12 | 0.21188 | 0.36185 | 0.28713 | 0.0485 |
| H13 | 0.05046 | 0.21656 | 0.22515 | 0.0587 |
| H14 | -0.17244 | 0.2266 | 0.24697 | 0.0544 |
| H15 | -0.23398 | 0.37351 | 0.3341 | 0.0627 |
| H16 | -0.07109 | 0.50895 | 0.40026 | 0.0529 |
| H20 | 0.45385 | 0.63995 | 0.62973 | 0.0282 |
| H23 | 0.7092 | 1.07935 | 0.65561 | 0.0324 |
| H24 | 0.66284 | 0.98193 | 0.55154 | 0.0307 |
| H25 | 0.80232 | 0.68301 | 0.52653 | 0.0383 |
| H26 | 1.02042 | 0.75734 | 0.509 | 0.0492 |
| H27A | 0.459(3) | 0.639(3) | 0.748(1) | 0.049(5) |
| H27B | 0.513(2) | 0.783(3) | 0.788(1) | 0.049(5) |

## VIII. Expression, purification and crystal structure determination of p38α and CK1δ complexes

p38α:

The protein was expressed and purified as described previously.[10] Briefly, N-terminally His6-tagged p38α was expressed in E. coli BL21(DE3) overnight at 18°C and purified by $Ni^{2+}$-NTA-affinity, anion exchange and size exclusion chromatography (SEC) after proteolytic removal of the affinity tag. For crystallization in the absence of DTT, the final purification step (SEC) was performed in the absence of DTT.

The protein was crystallized after incubation for 2 h at 4 °C with the various compounds (40 µl protein at 10 mg/ml and 1µl 50mM compound in DMSO). Crystals grew after mixing 1.5 µl protein solution and 0.5 µl reservoir solution (100 mM MES pH 5.6-6.2, 20-30% PEG4000 and 40 mM BOG, vapor diffusion, hanging drop) and were harvested and flash-cooled in liquid $N_2$ after cryo-protection with 25% PEG400. All data was collected at beamline PXII X10SA (Paul Scherrer Institute, Villigen, Switzerland) from a single crystal each. The datasets were integrated with XDS and scaled with XSCALE.[11] Molecular replacement was performed with Phaser[12] using pdb 5n63 as starting model. The model was rebuild using Wincoot[13] and refined with Phenix.[14] The Dundee PRODRG server was used to generate ligand topology files.[15] All figures were prepared using PyMol.[16] Data collection and refinement statistics are shown in table S5.

CK1δ:

CK1δ structures in complex with the inhibitors **2** and **3** were obtained as described previously.[17,18] Briefly, N-terminally His6-tagged CK1δ was expressed in *E.coli* BL21 TaKaRa 2 cells (Clontech) and subsequently purified by immobilized metal ion affinity and size exclusion chromatography. DTT was removed before CK1δ was co-crystallized with compound **2** using a PD 10 desalting column (GE Healthcare). Crystals grew in conditions with precipitant solutions containing 0.2 M malonate, pH 6.0, 20 % PEG3350 in case of compound **2** or 0.2 M sodium formate, pH 7.0 and 20 % PEG3350 in case of compound **3**. Prior to freezing, crystals were cryo-protected using the respective precipitant solution supplemented with 15 % PEG400. Diffraction date was collected at beamline PXIII X06DA (Paul Scherrer Institute, Villigen, Switzerland) and processed using XDS (2). For structure determination, model building and refinement, the phenix software package was used. The model 5MQV was used for molecular replacement. All figures werte prepared using PyMol (7). Data collection and refinement statistics are shown in table S6.

## IX. Data collection and structure refinement of protein crystallization

### IX.1 Protein crystallization with p38α

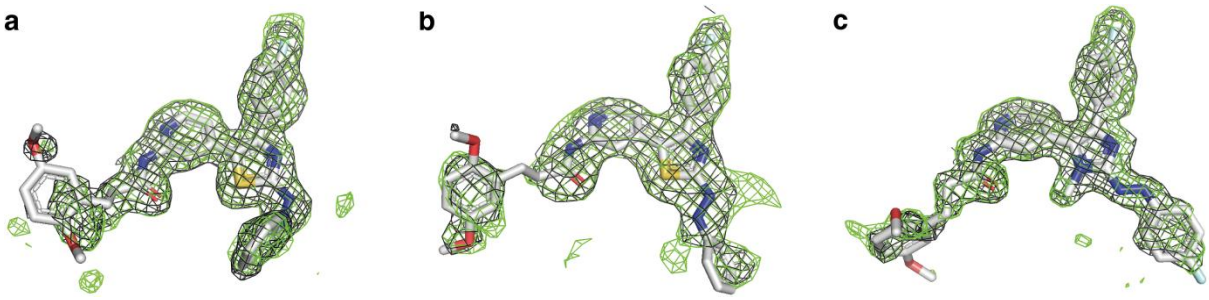



**Figure S59:** Electron density maps surrounding the co-crystallized compounds **31** (a), **2** (b) and **3** (c). The $2F_O$-$F_C$ maps are contoured at an rmsd of 1.0 (black), the $F_O$-$F_C$ simulated annealing omit maps at an rmsd of 2.5 (green). Note the minor additional different configurations of the diarylazo moiety observed in compound **2**.

**Table S5**: Data collection and structure refinement results of protein crystallization with p38α.

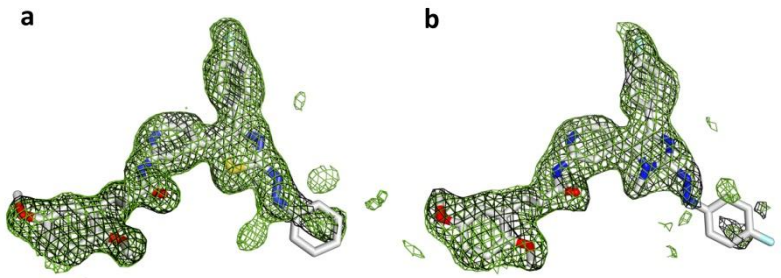
| | p38α: compound **31**, with DTT | p38α: compound **2**, without DTT | p38α: compound **3** |
|---|---|---|---|
| PDB ID | 6HWT | 6HWU | 6HWV |
| **Data collection**[a,b] | | | |
| Space group | $P2_12_12_1$ | $P2_12_12_1$ | $P2_12_12_1$ |
| Wavelength (Å) | 0.977928 | 0.99967 | 1.0 |
| Cell dimensions | | | |
| a, b, c (Å) | 69.51, 69.81, 74.57 | 66.67, 74.69, 77.24 | 69.0, 70.0, 74.7 |
| α, β, γ (°) | 90, 90, 90 | 90, 90, 90 | 90, 90, 90 |
| Resolution (Å) | 49.3 - 1.7 (1.8 - 1.7) | 49.7 - 2.3 (2.4 - 2.3) | 49.1 - 1.7 (1.8 - 1.7) |
| No. of unique reflections | 39982 (6261) | 17697 (2052) | 40470 (6261) |
| Redundancy | 8.4 (8.6) | 13.1 (13.4) | 13.1 (13.3) |
| I / σI | 17.0 (2.7) | 15.1 (2.3) | 20.6 (3.0) |
| Completeness (%) | 98.5 (100) | 100 (100) | 100 (100) |
| $R_{meas}$ (%) | 6.9 (85.5) | 12.5 (109.2) | 8.1 (84.0) |
| **Refinement** | | | |
| Resolution (Å) | 49.3 - 1.7 (1.74-1.7) | 49.7 - 2.3 (2.4 - 2.3) | 37.4 - 1.7 (1.74 - 1.7) |
| No. reflections | 39952 | 17691 | 40463 |
| $R_{work}$ / $R_{free}$ (%) | 20.2 / 23.5 (31.3 / 32.5) | 19.1 / 24.7 (25.4 / 32.1) | 18.0 / 19.7 (28.1 / 31.0) |

| | | | |
|---|---|---|---|
| No. atoms | | | |
| Protein | 2745 | 2693 | 2754 |
| Ligand/ion | 101 | 101 | 103 |
| Water | 205 | 65 | 270 |
| B-factors | | | |
| Protein | 38.0 | 54.7 | 26.7 |
| Ligand/ion | 61.9 | 94.6 | 60.4 |
| Water | 45.9 | 49.2 | 37.3 |
| R.m.s. deviations | | | |
| Bond lengths (Å) | 0.005 | 0.005 | 0.006 |
| Bond angles (°) | 0.965 | 0.879 | 0.956 |
| Ramachandran Plot | | | |
| Outliers (%) | 0 | 0 | 0 |
| Allowed (%) | 1.78 | 2.13 | 1.77 |
| Favored (%) | 98.22 | 97.87 | 98.23 |

[a] Data collection statistics refer to merged Friedel pairs. [b] Diffraction data from a single crystal was used to determine the complex structure.

Crystallographic statistics of p38α MAPK in complex compound **31** (pdb 6HWT), compound **2** (pdb 6HWU) or compound **3** (pdb 6HWV) are given. Values in parenthesis are for the highest resolution shell.

**IX.2 Protein crystallization with CK1δ**




**S60:** Electron density maps surrounding the compounds **2** (a) and **3** (b) bound to CK1δ. The $2F_O$-$F_C$ maps are contoured at an rmsd of 1.0 (black), the $F_O$-$F_C$ simulated annealing omit maps at an rmsd of 2.5 (green).

**Table S6**: Data collection and structure refinement results of protein crystallization with CK1δ.

| | CK1δ: compound **2** | CK1δ: compound **3** |
|---|---|---|
| PDB ID | 6HMR | 6HMP |
| **Data collection**[a,b] | | |
| Space group | $P2_1$ | $P2_12_12_1$ |
| wavelength (Å) | 1.000 | 1.0000 |
| Cell dimensions | | |
| a, b, c (Å) | 48.8, 74, 89.5 | 48.8, 72.2, 89.1 |
| α, β, γ (°) | 90, 102.8, 90 | 90, 103.2, 90 |
| Resolution (Å) | 46.38 -1.78 (1.85-1.79) | 46.4 - 2.04 (2.11 – 2.04) |
| No. of unique reflections | 57955 (5655) | 37921 (3190) |
| Redundancy | 7 (7) | 8.2 (4.3) |
| I / σI | 11.2 (.2) | 12.8 (1.09) |

| | | |
|---|---|---|
| Completeness (%) | 97.4 (95.23) | 98.3 (84.7) |
| $R_{meas}$ (%) | 14(187.5) | 13.2 (125.7) |
| **Refinement** | | |
| Resolution (Å) | 46.38 – 1.78 (1.85 – 1.78) | 46.4 – 2.04 (2.1 – 2.04) |
| No. reflections | 57929 | 37896 |
| $R_{work}$ / $R_{free}$ (%) | 17.6 / 20.1 (33.0 / 33.8) | 19.0 / 22.2 (32.3 / 37.0) |
| No. atoms | | |
| Protein | 4634 | 4597 |
| Ligand/ion | 96 | 96 |
| Water | 462 | 229 |
| B-factors | | |
| Protein | 36.2 | 43.3 |
| Ligand/ion | 35.6 | 42.9 |
| Water | 39.54 | 71.9 |
| R.m.s. deviations | | |
| Bond lengths (Å) | 0.007 | 0.005 |
| Bond angles (°) | 0.82 | 0.67 |
| Ramachandran Plot | | |
| Outliers (%) | 0.18 | 0.18 |
| Allowed (%) | 2.32 | 2.16 |
| Favored (%) | 97.5 | 97.66 |

[a] Data collection statistics refer to merged Friedel pairs. [b] Diffraction data from single crystal was used to determine the complex structure.

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