

Supporting information for:

Human Carbonic Anhydrase II as Scaffold for Artificial Transfer Hydrogenase

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Solvents and reagents

Materials and reagents were purchased at the highest commercially available grade and used without further purification.

Solvents used for reactions correspond to the quality “puriss”. For analytical and preparative high performance liquid chromatography (HPLC), HPLC-grade solvents were used. The water used for reactions was filtered using a Barnstead ultrapure water system.

Separation and purification methods

Reactions were monitored by thin layer chromatography (TLC) using Merck silica gel 60 F₂₅₄ plates. Flash chromatography was performed using Merck silica gel 60, particle size 40-63 μm . Compounds were visualized using UV (254 and/or 366 nm) with a UV-lamp from Camag.

High performance liquid chromatography was performed on Agilent 1100 Series with UV-Vis detection.

Spectroscopic methods

^1H , ^{13}C and ^{19}F -NMR spectra were recorded (295 K) on Bruker Avance DRX-500 or DPX-400 MHz spectrometers. Solvents for NMR were obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Chemical shifts (δ) are reported in ppm using trimethylsilylne or the residual solvent peaks as a reference and coupling constants (J) are reported in Hertz (Hz). The multiplicity's are abbreviated as: s = singlet, d = doublet, t = triplet, m = multiplet and br = broad.

The esterase activity screening and competitive displacement assays were performed using a Tecan Safire spectrophotometer using NUNC 96-well plates. The data were analyzed with gnuplot^{S1} (Version 4.2) software using a least-square fitting.

Spectrometric methods

The mass spectra (MS) were recorded on an Esquire 3000 plus (Bruker) for Electron Spray Ionisation (ESI) and/or a Finnigan MAT 8400 for Fast Atom Bombardment (FAB).

High resolution mass spectrometry (HRMS) was recorded on a Bruker FTMS 4.7T BioAPEX II.

Other methods

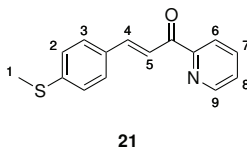
The elemental analysis (EA) was measured on a Analysator 240 from Perkin-Elmer or a vario MICRO cube from Elementar.

Ligand-inhibitors synthesis

Compounds bispy **1** was synthesized according to literature protocols.^{S2}

bipy **2**

(*E*)-3-(4-(Methylthio)phenyl)-1-(pyridin-2-yl)prop-2-en-1-one (**21**)



To 4-(methylthio)benzaldehyde (5.61 mL, 42.0 mmol, 1.1 eq) in a 1.0 M aqueous solution of NaOH (30 mL) and MeOH (90 mL), 2-acetylpyridine (4.49 mL, 40.0 mmol, 1.0 eq) was added. After 3 h of stirring at room temperature, the solution was stored at 4 °C overnight. The yellow precipitate was filtered and washed with H₂O and cold MeOH. The solvent was removed under reduced pressure. The isolated product was recrystallized from MeOH to obtain **21** (5.30 g, 20.8 mmol, 52%) as a yellow-green solid.

TLC : *n*-hexane/EtOAc (1/1); *R_f* = 0.51.

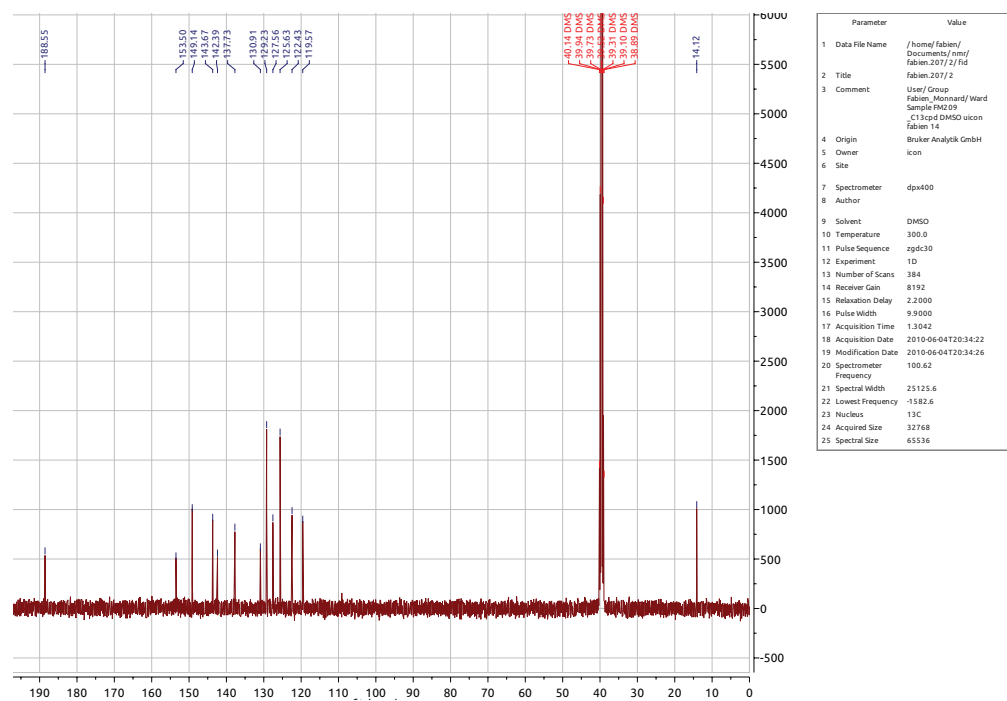
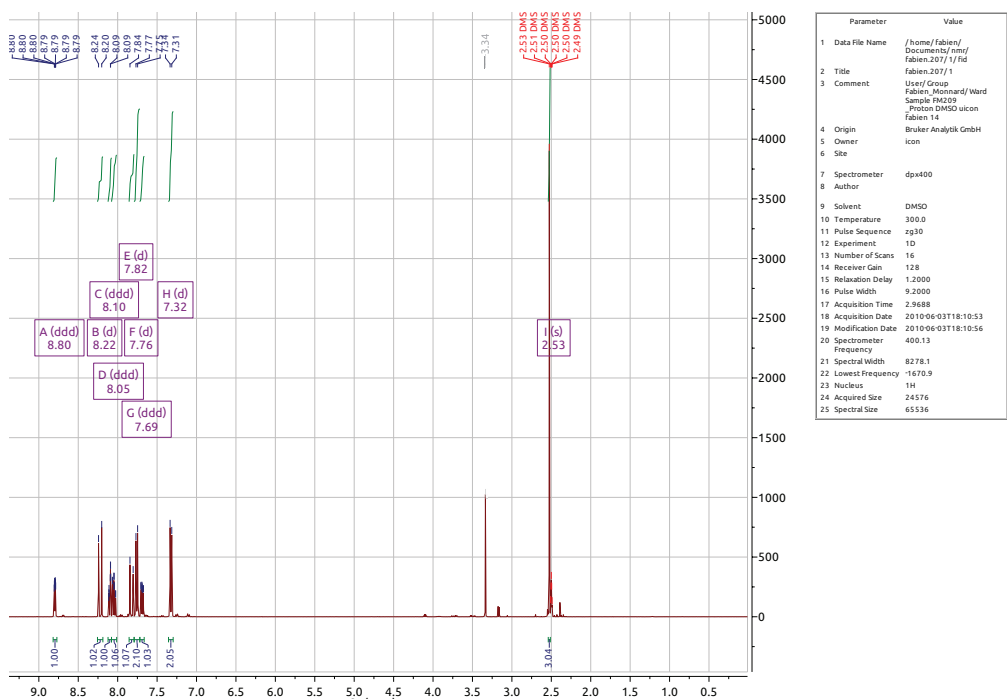
mp 86.1 °C

¹H NMR (400 MHz, DMSO-*d*₆, 20 °C, δ): 8.80 (ddd, *J* = 4.7, 1.7, 0.9 Hz, 1H, **H**⁹), 8.22 (d, *J* = 16.1 Hz, 1H, **H**⁴), 8.10 (ddd, *J* = 7.9, 1.4, 1.0 Hz, 1H, **H**⁶), 8.05 (ddd, *J* = 7.9, 7.4, 1.7 Hz, 1H, **H**⁷), 7.82 (d, *J* = 16.1 Hz, 1H, **H**⁵), 7.76 (d, *J* = 8.3 Hz, 2H, **H**²), 7.69 (ddd, *J* = 7.4, 4.7, 1.4 Hz, 1H, **H**⁸), 7.32 (d, *J* = 8.4 Hz, 2H, **H**³), 2.53 (s, 3H, **H**¹).

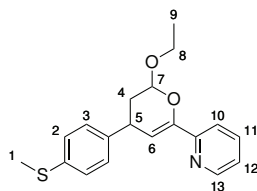
¹³C NMR (101 MHz, DMSO-*d*₆, 20 °C, δ): 188.6, 153.5, 149.1, 143.7, 142.4, 137.7, 130.9, 129.2, 127.6, 125.6, 122.4, 119.6, 14.1.

HRMS (ESI, pos.) *m/z*: [M+H]⁺ calcd for C₁₅H₁₄NOS, 256.0796; found, 256.0793.

EA Anal. calcd for C₁₅H₁₃NOS: C, 70.56%; H, 5.13%; N, 5.49%. Found: C, 70.30%; H, 5.17%; N, 5.59%.



2-(6-Ethoxy-4-(4-(methylthio)phenyl)-5,6-dihydro-2H-pyran-2-yl)pyridine (22)



22

Compound **21** (2.0 g, 7.8 mmol, 1.0 eq) and yttrium (III) hexafluoroacetylacetonate (0.10 g, 0.1 mmol, 0.02 eq) were dissolved in THF (40 mL) and 4 Å molecular sieves (2 g) were added. Ethyl vinyl ether (7.5 mL, 78.3 mmol, 10.0 eq) was added, and the reaction was stirred under nitrogen at room temperature for 3 days. The sieves were removed by filtration over celite. The solvent was removed under reduced pressure, and the brown oil was purified by flash gel chromatography (*n*-hexane/EtOAc, 3/1) to obtain **22** (1.87 g, 5.7 mmol, 73%) as a colorless oil.

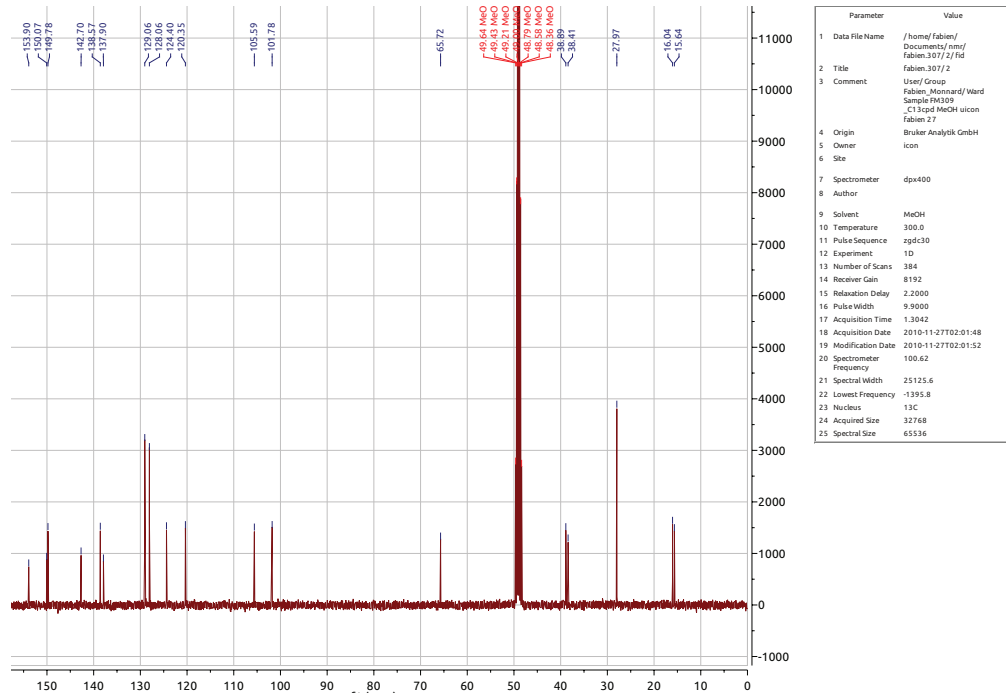
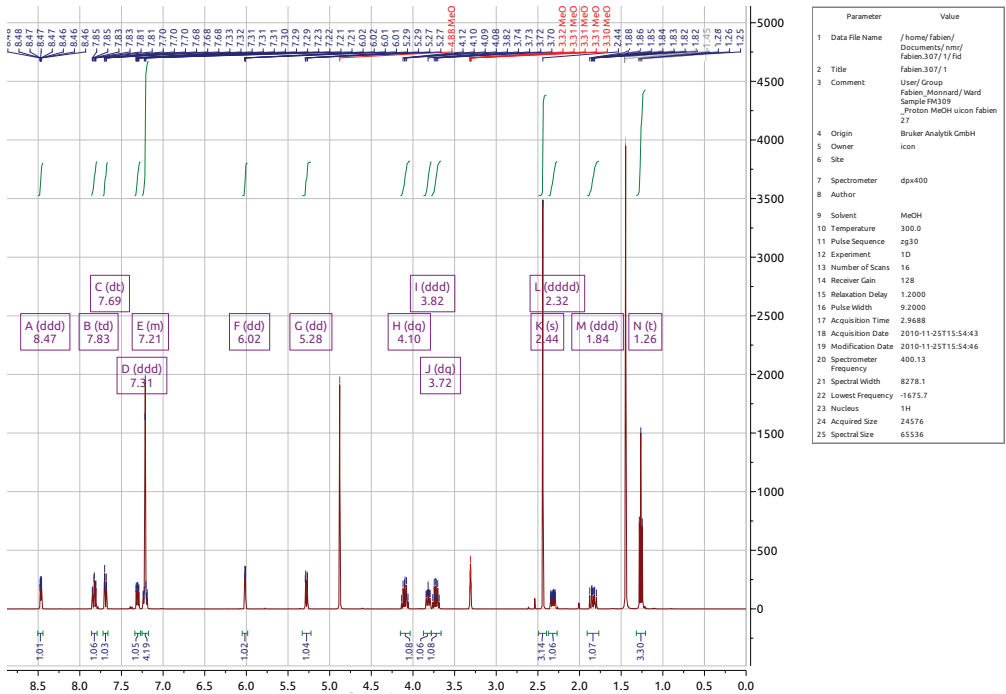
TLC : *n*-hexane/EtOAc (1/1); R_f = 0.66.

¹H NMR (400 MHz, CD₃OD, 20 °C, δ): 8.46 (ddd, J = 4.8, 1.8, 0.9 Hz, 1H, **H**¹³), 7.83 (td, J = 7.8, 1.8 Hz, 1H, **H**¹¹), 7.69 (dt, J = 8.0, 1.1 Hz, 1H, **H**¹⁰), 7.31 (ddd, J = 7.6, 4.9, 1.2 Hz, 1H, **H**¹²), 7.21 (m, 4H, **H**^{2,3}), 6.02 (dd, J = 2.9, 1.2 Hz, 1H, **H**⁶), 5.28 (dd, J = 8.6, 2.0 Hz, 1H, **H**⁷), 4.10 (dq, J = 9.6, 7.1 Hz, 1H, **H**⁸), 3.82 (ddd, J = 9.9, 6.7, 2.8 Hz, 1H, **H**⁵), 3.72 (dq, J = 9.6, 7.1 Hz, 1H, **H**^{8'}), 2.44 (s, 3H, **H**¹), 2.32 (dddd, J = 13.2, 6.8, 2.1, 1.3 Hz, 1H, **H**⁴), 1.84 (ddd, J = 13.2, 10.3, 8.6 Hz, 1H, **H**^{4'}), 1.25 (t, J = 7.1 Hz, 3H, **H**⁹).

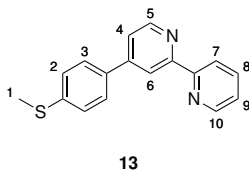
¹³C NMR (101 MHz, CD₃OD, 20 °C, δ): 153.9, 150.0, 149.8, 142.7, 138.6, 137.9, 129.1, 128.0, 124.4, 120.3, 105.6, 101.8, 65.7, 38.9, 38.4, 16.0, 15.7.

MS (ESI, pos.) m/z (relative intensity): [M+H]⁺ calcd for C₁₉H₂₂NO₂S, 328.1; found, 328.2 (100) [M+H]⁺, 350.0 (6) [M+Na]⁺.

EA Anal. calcd for C₁₉H₂₁NO₂S: C, 69.69%; H, 6.46%; N, 4.28%. Found: C, 69.79%; H, 6.51%; N, 4.29%.



(4-(4-(Methylthio)phenyl)-2,2'-bipyridine (13)



Compound **12** (1.50 g, 4.5 mmol, 1.0 eq) was dissolved in ACN (10 mL) and $\text{H}_2\text{NOH} \cdot \text{HCl}$ (3.18 g, 45.7 mmol, 10.4 eq) was added. The mixture was refluxed for 6 h, during which time a yellow precipitate formed. The solution was allowed to cool to room temperature, and the ACN was removed under reduced pressure to give an orange solid. A saturated aqueous solution of NaOH/NaCl (30 mL) and CH_2Cl_2 (30 mL) were added to the solid, and the mixture was stirred vigorously until all the solid had dissolved. The organic layer was extracted with CH_2Cl_2 (3 x 20 mL), dried over Na_2SO_4 , and the solvent was removed under reduced pressure. The resulting solid was washed with MeOH to yield **13** (583 mg, 2.1 mmol, 46%) as a white powder.

TLC : $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (9/1); $R_f = 0.77$.

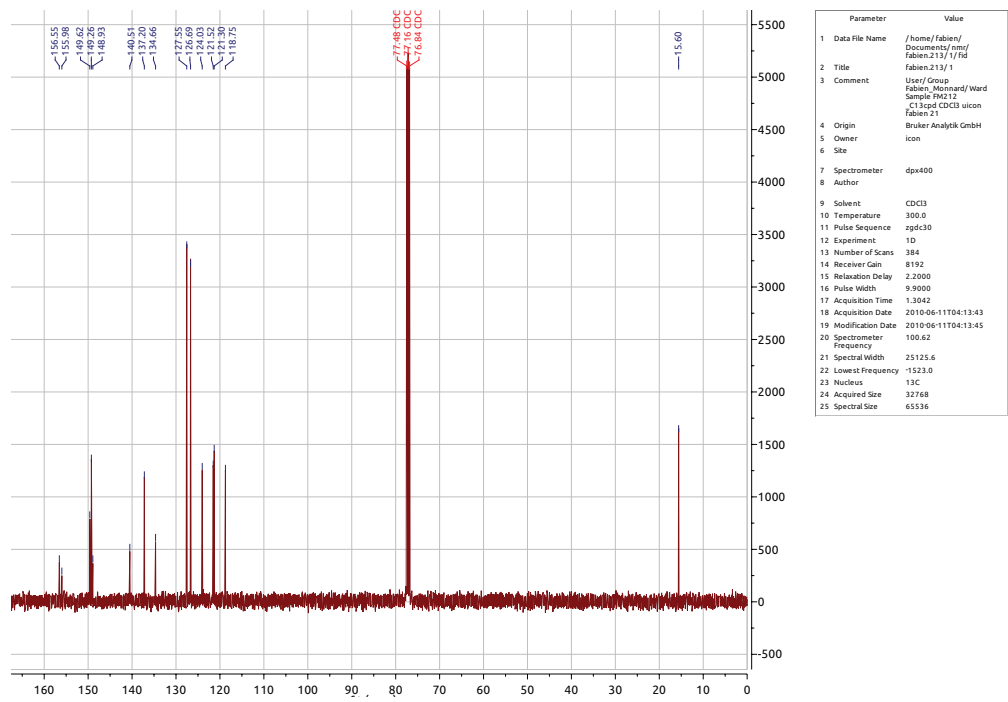
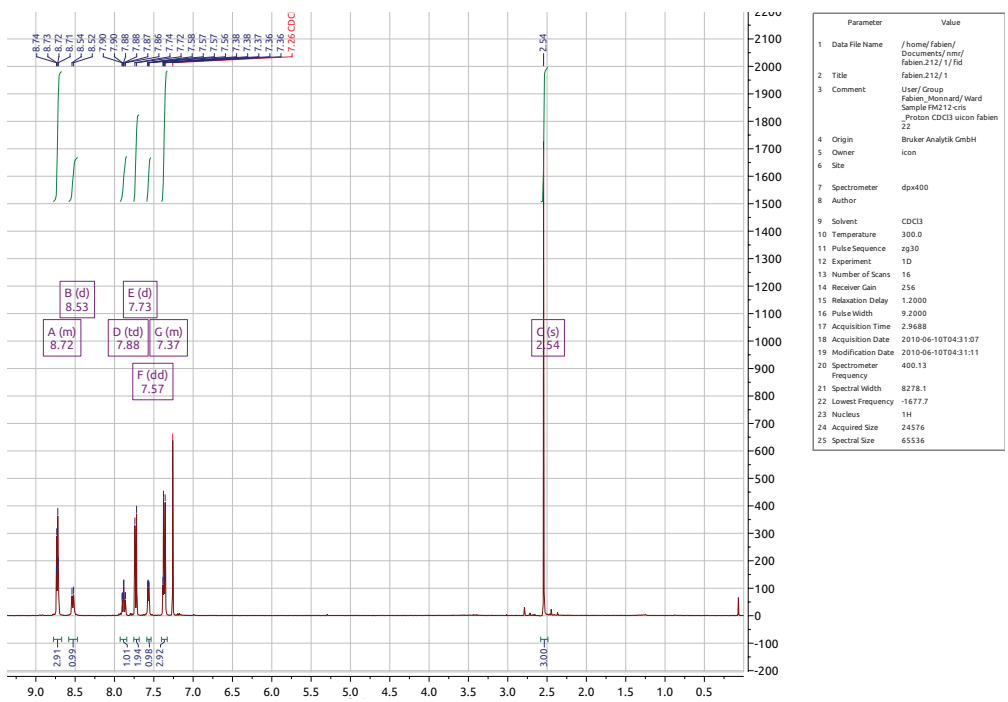
mp 139.1 °C

^1H NMR (400 MHz, CDCl_3 , 20 °C, δ): 8.72 (m, 3H, $\text{H}^{5,6,10}$), 8.53 (d, $J = 8.0$ Hz, 1H, H^7), 7.88 (td, $J = 7.8, 1.8$ Hz, 1H, H^8), 7.73 (d, $J = 8.5$ Hz, 2H, H^3), 7.57 (dd, $J = 5.2, 1.9$ Hz, 1H, H^4), 7.37 (m, 3H, $\text{H}^{2,9}$), 2.54 (s, 3H, H^1).

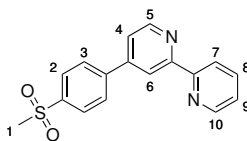
^{13}C NMR (101 MHz, CDCl_3 , 20 °C, δ): 156.5, 156.0, 149.6, 149.3, 149.0, 140.5, 137.2, 134.6, 127.5, 126.7, 124.0, 121.5, 121.3, 118.7, 15.9.

HRMS (ESI, pos.) m/z : $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{17}\text{H}_{15}\text{N}_2\text{S}$, 279.0955; found, 279.0951.

EA Anal. calcd for $\text{C}_{17}\text{H}_{14}\text{N}_2\text{S} \cdot \frac{1}{6}\text{H}_2\text{O}$: C, 72.57%; H, 5.13%; N, 9.96%. Found: C, 72.50%; H, 5.23%; N, 10.19%.



(4-(4-(Methylsulfonyl)phenyl)-2,2'-bipyridine (23)



13

Compound **13** (249 mg, 0.9 mmol, 1.0 eq) was dissolved in CH₂Cl₂ (20 mL). Finely ground KMnO₄/MnO₂ (4.0 g, 1/1) was added to the solution over a period of 0.5 h. The mixture was stirred at room temperature for 3 days. After completion of the reaction, the product was filtered through celite and CH₂Cl₂ was removed under reduced pressure to obtain **23** (220 mg, 0.7 mmol, 79%) as a white powder which was used with no further purification.

TLC : CH₂Cl₂/MeOH (9/1); R_f = 0.83.

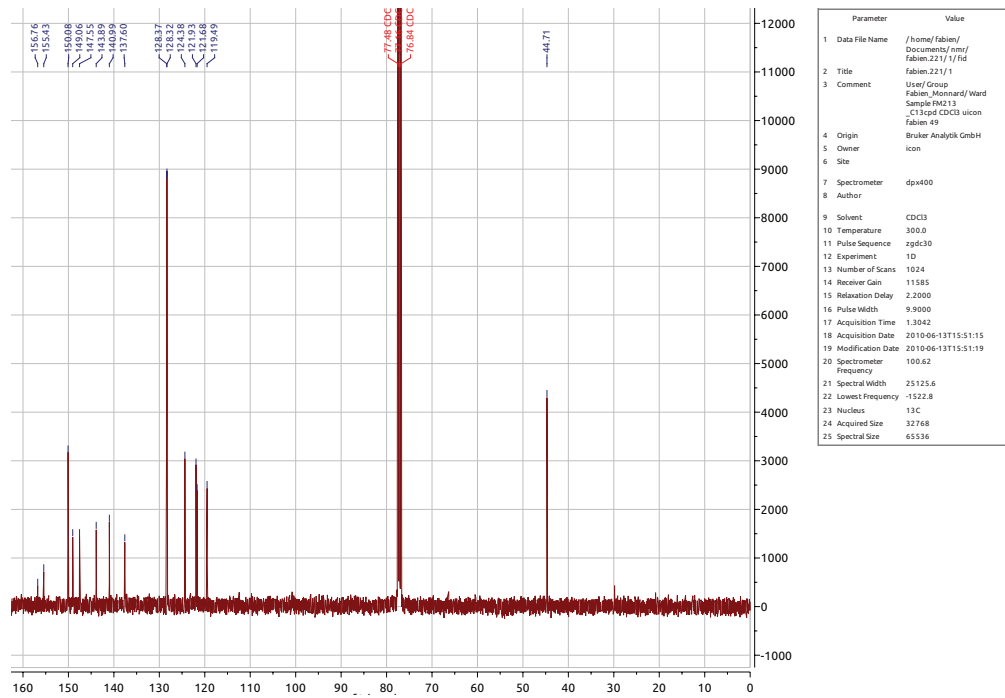
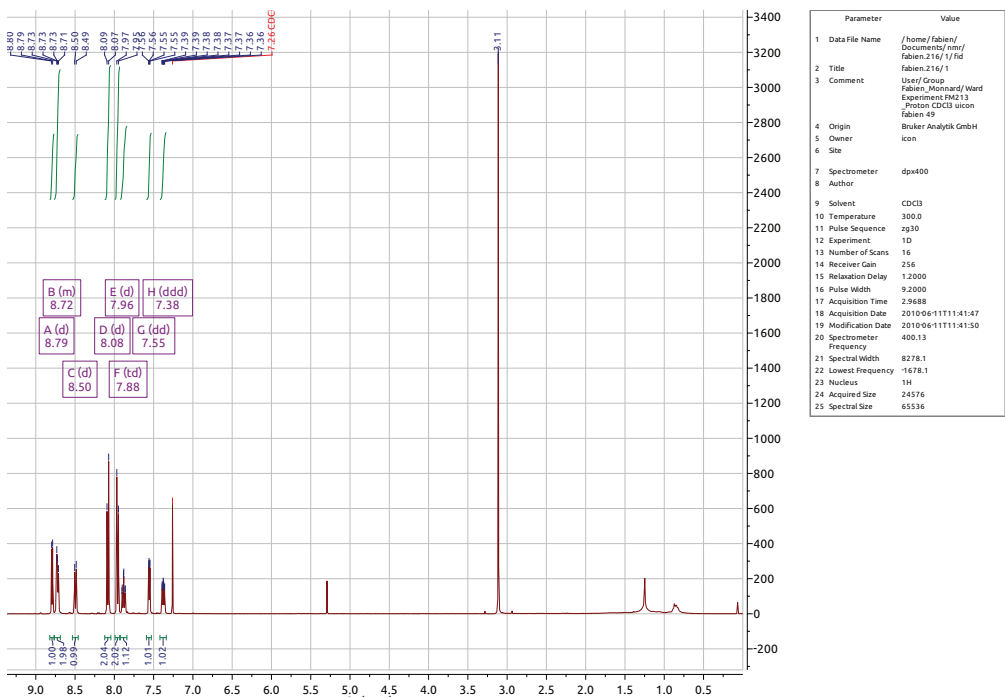
mp 170.8 °C

¹H NMR (400 MHz, CDCl₃, 20 °C, δ): 8.79 (d, J = 5.1 Hz, 1H, **H**⁵), 8.72 (m, 2H, **H**^{6,10}), 8.50 (d, J = 8.0 Hz, 1H, **H**⁷), 8.08 (d, J = 8.4 Hz, 2H, **H**²), 7.96 (d, J = 8.4 Hz, 2H, **H**³), 7.88 (td, J = 7.8, 1.8 Hz, 1H, **H**⁸), 7.55 (dd, J = 5.1, 1.8 Hz, 1H, **H**⁴), 7.38 (ddd, J = 7.6, 4.8, 1.2 Hz, 1H, **H**⁹), 3.11 (s, 3H, **H**¹).

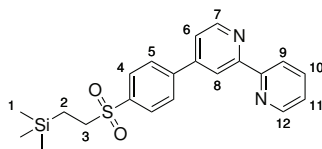
¹³C NMR (101 MHz, CDCl₃, 20 °C, δ): 156.8, 155.4, 150.1, 149.1, 147.6, 143.9, 141.0, 137.6, 128.4, 128.3, 124.4, 121.9, 121.7, 119.5, 44.7.

HRMS (ESI, pos.) m/z : [M+H]⁺ calcd for C₁₇H₁₅N₂O₂S, 311.0854; found, 311.0850.

EA Anal. calcd for C₁₇H₁₄N₂O₂S · $\frac{1}{3}$ H₂O: C, 64.54%; H, 4.67%; N, 8.85%. Found: C, 64.40%; H, 4.42%; N, 8.81%.



(4-(4-((2-(Trimethylsilyl)ethyl)sulfonyl)phenyl)-2,2'-bipyridine (24)



24

Diisopropylamine (0.89 mL, 6.3 mmol, 1.3 eq) in THF (5 mL) was cooled to 0 °C and *n*-butyllithium (2.2 M solution in cyclohexane) (2.6 mL, 5.8 mmol, 1.2 eq) was added. The solution was stirred at 0 °C for 5 min, and cooled to -70 °C with a dry ice/acetone bath. A solution of **23** (1.5 g, 4.8 mmol, 1.0 eq) in dry THF (5 mL) was added dropwise over a period of 10 min. The reaction mixture was stirred for 1 h. Chloromethyltrimethylsilane (0.94 mL, 6.8 mmol, 1.4 eq) was added dropwise and the reaction was stirred at room temperature for 2 d. The reaction was quenched with a 1 M aqueous solution of HCl (2 mL), and the aqueous phase extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine and dried over MgSO₄, and the solvent was removed under reduced pressure to obtain **24** (1.61 g, 4.0 mmol, 84%) as a red-brown solid.

TLC : CH₂Cl₂/MeOH (9/1); R_f = 0.74.

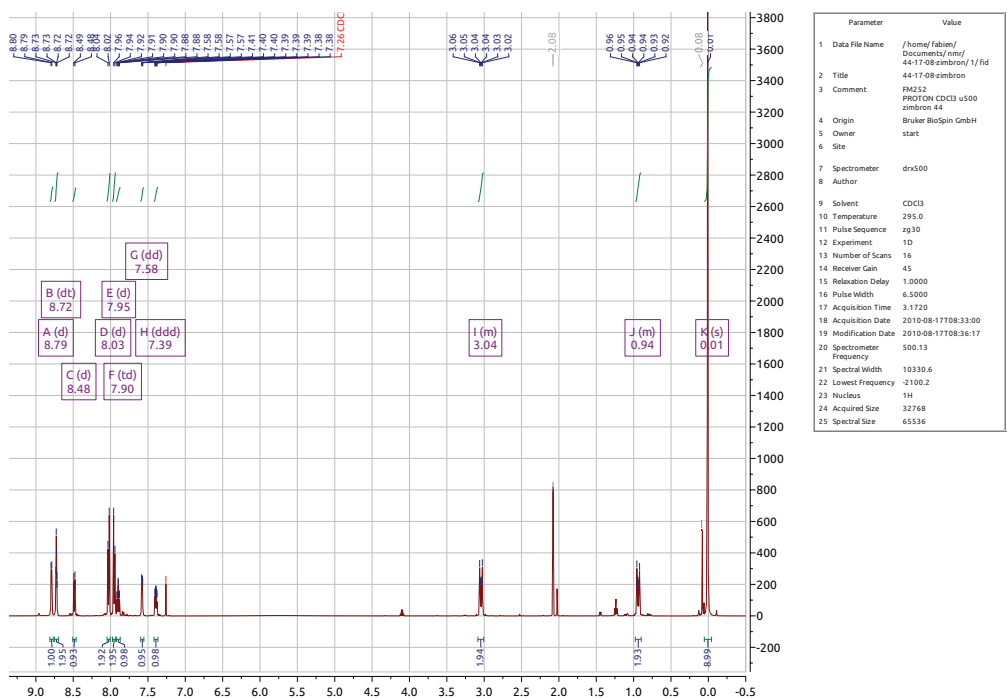
mp 93.1 °C

¹H NMR (500 MHz, CDCl₃, 20 °C, δ): 8.79 (d, *J* = 5.1 Hz, 1H, **H**⁷), 8.73 (b, 2H, **H**^{8,12}), 8.48 (d, *J* = 7.9 Hz, 1H, **H**⁹), 8.03 (d, *J* = 8.4 Hz, 2H, **H**⁴), 7.95 (d, *J* = 8.4 Hz, 2H, **H**⁵), 7.90 (td, *J* = 7.7, 1.8 Hz, 1H, **H**¹⁰), 7.58 (dd, *J* = 5.1, 1.8 Hz, 1H, **H**⁶), 7.39 (ddd, *J* = 7.4, 4.8, 1.2 Hz, 1H, **H**¹¹), 3.04 (m, 2H, **H**³), 0.94 (m, 2H, **H**²), 0.01 (s, 9H, **H**¹).

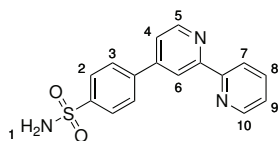
¹³C NMR (101 MHz, CDCl₃, 20 °C, δ): 156.3, 155.1, 149.9, 148.9, 147.8, 143.5, 139.2, 137.9, 129.2, 128.2, 124.5, 122.1, 121.9, 119.7, 53.0, 9.3, -1.9.

HRMS (ESI-MS, pos.) *m/z*: [M+H]⁺ calcd for C₂₁H₂₅N₂O₂SSi, 397.1406; found, 397.1398.

EA Anal. calcd for C₂₁H₂₄N₂O₂SSi · $\frac{2}{3}$ H₂O: C, 61.73%; H, 6.25%; N, 6.86%. Found: C, 61.36%; H, 6.14%; N, 6.56%.



(4-((2,2'-Bipyridine)4-yl)benzenesulfonamide (bipy 2)



bipy 2

To a solution of **24** (262 mg, 0.7 mmol, 1.0 eq) in dry THF (5 mL) a 1 M solution of TBAF in THF (3.87 mL, 3.9 mmol, 4.3 eq) was added. The mixture was refluxed for 1 h, and cooled to room temperature. A solution of sodium acetate (523 mg, 6.4 mmol, 9.7 eq) in 2 mL of water and hydroxyl-amine-*O*-sulfonic acid (729 mg, 6.5 mmol, 9.8 eq) were added sequentially, and the mixture was stirred overnight at room temperature. The reaction mixture was quenched by adding H₂O (10 mL), and extracted with EtOAc (3 x 30 mL). The organic fractions were washed sequentially with saturated NaHCO₃ solution, water and brine. The organic layer was dried over Na₂SO₄, and the solvent was removed under reduced pressure. The product was purified by flash chromatography (CH₂Cl₂/MeOH, gradient 0 to 5 %) to obtain bipy **2** (149 mg, 0.5 mmol, 72%) as a white solid.

TLC : CH₂Cl₂/MeOH (9/1); R_f = 0.38.

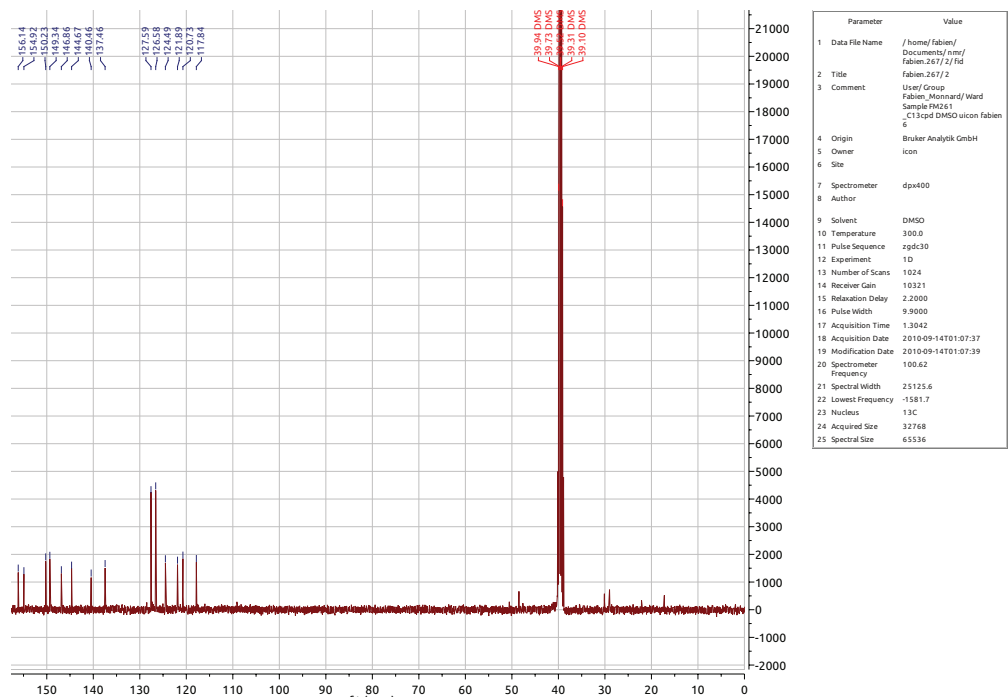
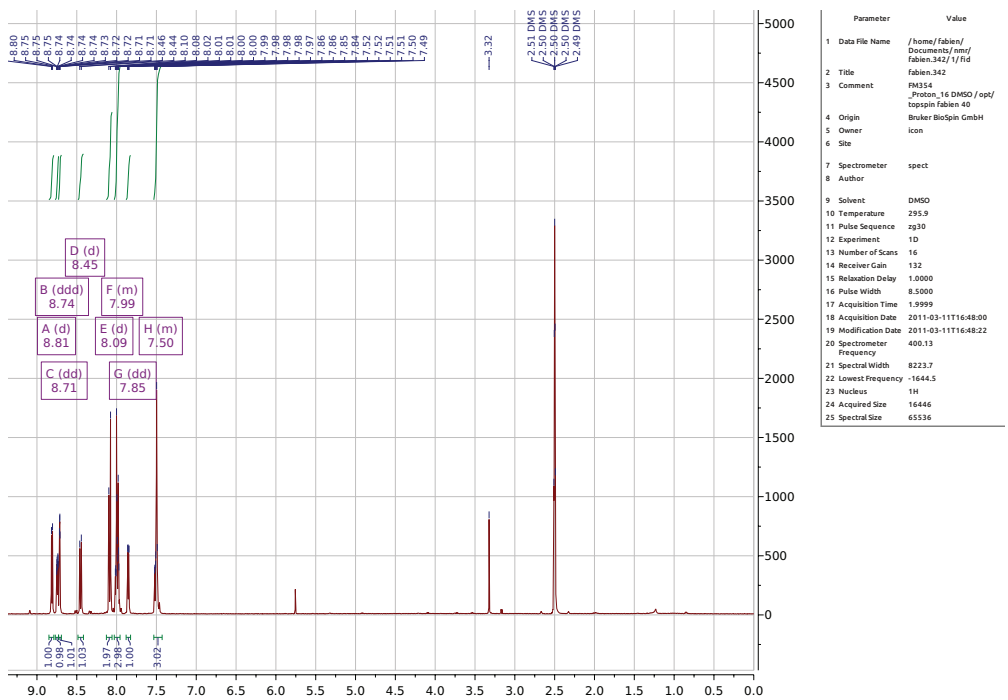
mp 232.2 °C

¹H NMR (400 MHz, DMSO-*d*₆, 20 °C, δ): 8.81 (d, *J* = 5.1 Hz, 1H, **H**⁵), 8.74 (ddd, *J* = 4.6, 1.7, 0.9 Hz, 1H, **H**¹⁰), 8.71 (d, *J* = 1.5 Hz, 1H, **H**⁶), 8.45 (d, *J* = 8.0 Hz, 1H, **H**⁷), 8.09 (d, *J* = 8.5 Hz, 2H, **H**²), 7.99 (m, 3H, **H**^{3,8}), 7.85 (dd, *J* = 5.1, 1.9 Hz, 1H, **H**⁴), 7.50 (m, 3H, **H**^{1,9}).

¹³C NMR (101 MHz, DMSO-*d*₆, 20 °C, δ): 156.2, 154.9, 150.2, 149.4, 146.9, 144.7, 140.5, 137.5, 127.6, 126.6, 124.5, 121.9, 120.7, 117.9.

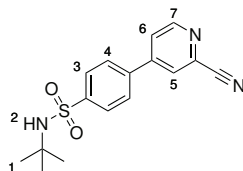
HRMS (ESI, pos.) *m/z*: [M+H]⁺ calcd for C₁₆H₁₄N₃O₂S, 312.0806; found, 312.0803.

EA Anal. calcd for C₁₆H₁₃N₃O₂S: C, 61.72%; H, 4.21%; N, 13.50%. Found: C, 61.41%; H, 4.20%; N, 13.32%.



pico 3

N-(*tert*-Butyl)-4-(2-cyanopyridin-4-yl)benzenesulfonamide (**25**)



25

4-Chloro-2-pyridinecarbonitrile (182 mg, 1.3 mmol, 0.7 eq) and 4-(*tert*-butylaminosulphonyl) benzene-boronic acid (496 mg, 1.9 mmol, 1.0 eq) were dissolved in THF (8 mL). Tetrakis(triphenylphosphine)-palladium (136 mg, 0.1 mmol, 0.06 eq) and an aqueous solution (2 mL) of Na₂CO₃ (381 mg, 3.6 mmol, 1.9 eq) were added. The reaction mixture was heated at 80 °C for 6 h. The cooled crude mixture was poured onto water (50 mL), and extracted with CH₂Cl₂ (3 x 50 mL). The combined organic layers were dried over Na₂SO₄. The product was purified by flash chromatography (*n*-hexane/EtOAc 1/1). The solvent was removed under reduced pressure to obtain **25** (325 mg, 1.0 mmol, 80%) as a pale brown powder.

TLC : *n*-hexane/EtOAc (1/1); R_f = 0.54.

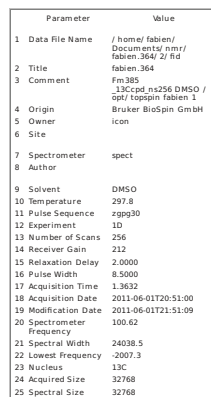
mp 156.2 °C

¹H NMR (400 MHz, DMSO-*d*₆, 20 °C, δ): 8.85 (dd, *J* = 5.2, 0.8 Hz, 1H, **H**⁷), 8.51 (dd, *J* = 2.0, 0.8 Hz, 1H, **H**⁵), 8.15 (dd, *J* = 5.2, 1.9 Hz, 1H, **H**⁶), 8.10 (d, *J* = 8.5 Hz, 2H, **H**³), 7.97 (d, *J* = 8.6 Hz, 2H, **H**⁴), 7.70 (s, 1H, **H**²), 1.12 (s, 9H, **H**¹).

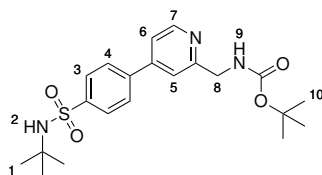
¹³C NMR (101 MHz, DMSO-*d*₆, 20 °C, δ): 151.9, 147.1, 145.6, 138.3, 133.5, 127.9, 127.0, 126.8, 125.2, 117.5, 53.5, 29.8.

HRMS (ESI-MS, pos.) *m/z*: [M+Na]⁺ calcd for C₁₆H₁₇N₃O₂SNa, 338.0939; found, 338.0941.

EA Anal. calcd for C₁₆H₁₇N₃O₂S · $\frac{1}{3}$ EtOAc: C, 60.39%; H, 5.75%; N, 12.19%. Found: C, 60.31%; H, 5.58%; N, 12.30%.



***N*-(*tert*-Butyl)-4-(2-(aminomethyl)pyridin-4-yl)benzenesulfonamide (**26**)**



26

LiAlH₄ (104 mg, 2.7 mmol, 2.2 eq) was added to dry THF (10 mL). The reaction mixture was allowed to stir until a homogeneous slurry had formed. AlCl₃ (267 mg, 1.6 mmol, 1.6 eq) was then added, the reaction mixture was cooled to 0 °C, and a solution of **25** (382 mg, 1.2 mmol, 1.0 eq) in THF (10 mL) was added dropwise. The reaction was allowed to warm to room temperature, and was stirred for 2 h. The reaction was quenched with a 2 M aqueous solution of NaOH (10 mL). The product was extracted with EtOAc (3 x 50 mL), and the combined organic layers were washed with brine and dried over Na₂SO₄. The solvent was removed under reduced pressure, and the obtained orange solid was dissolved in CH₂Cl₂ (30 mL) and di-*tert*-butyl dicarbonate (528 mg, 2.4 mmol, 2.0 eq) was added. The solution was stirred overnight at room temperature. The solvent was removed, and the product was purified by flash gel chromatography (*n*-hexane/EtOAc, 1/1) to obtain **26** (328 mg, 0.8 mmol, 65%) as a colorless oil.

TLC : *n*-hexane/EtOAc (1/1); R_f = 0.17.

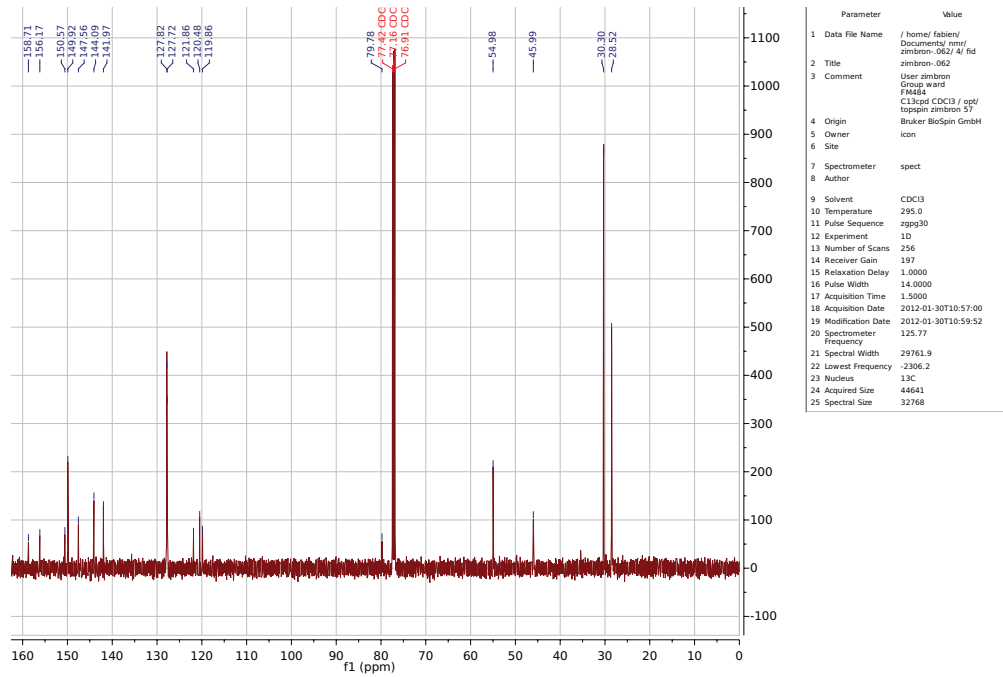
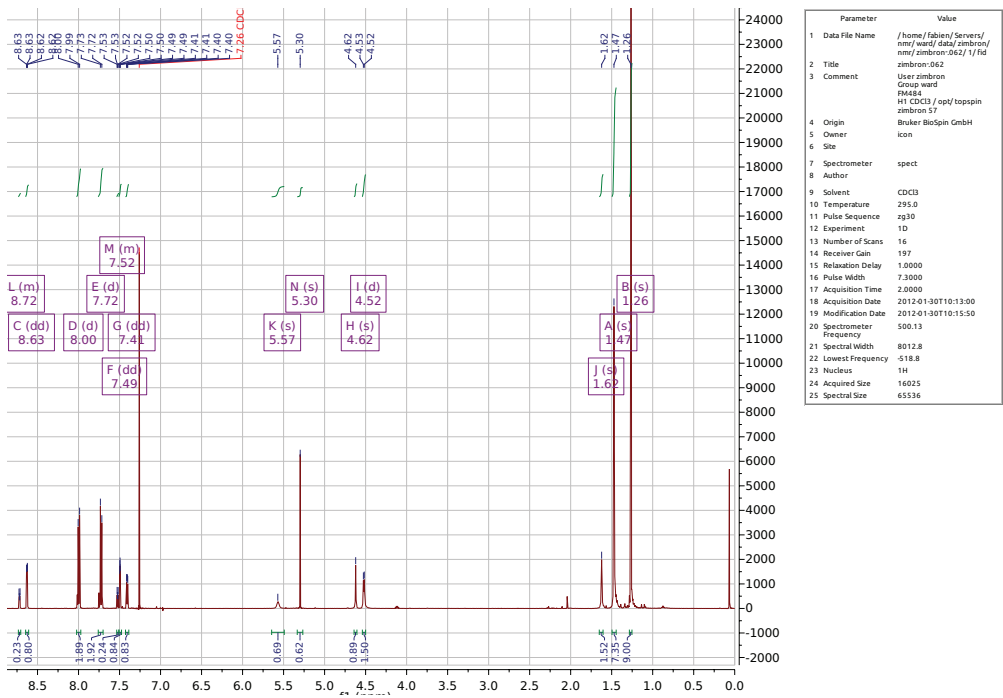
mp 81.5 °C

¹H NMR (500 MHz, DMSO-*d*₆, 20 °C, δ): 8.72m, 8.63M (dd, *J* = 5.2, 0.8 Hz, 1H, **H**⁷), 8.00 (d, *J* = 8.7 Hz, 2H, **H**³), 7.72 (d, *J* = 8.7 Hz, 2H, **H**⁴), 7.52m, 7.49M (dd, *J* = 1.8, 0.8 Hz, 1H, **H**⁵), 7.41 (dd, *J* = 5.2, 1.8 Hz, 1H, **H**⁶), 5.57 (s, 1H, **H**⁹), 5.30 (s, 1H, **H**²), 4.62m, 4.52M (d, *J* = 5.5 Hz, 2H, **H**⁸), 1.62m, 1.47M (s, 9H, **H**¹⁰), 1.26 (s, 9H, **H**¹).

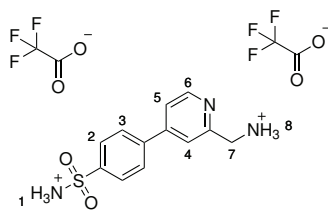
¹³C NMR (126 MHz, DMSO-*d*₆, 20 °C, δ): 158.7, 156.2, 150.6, 149.9, 147.6, 144.1, 141.9, 127.8, 127.7, 121.9, 120.5, 119.8, 79.8, 54.9, 45.9, 30.3, 28.5.

MS (ESI-MS, pos.) *m/z*: [M+H]⁺ calcd for C₂₁H₃₀N₃O₄S, 420.2; found, 420.2.

EA Anal. calcd for $C_{21}H_{29}N_3O_4S \cdot \frac{1}{4}CH_2Cl_2$: C, 57.81%; H, 6.82%; N, 9.26%. Found:
C, 57.87%; H, 6.72%; N, 9.22%.



4-(2-(Aminomethyl)pyridin-4-yl)benzenesulfonamide (pico 3)



pico 3

A few drops of anisole were added to a solution of compound **26** (410 mg, 1.3 mmol, 1.0 eq) in TFA (5 mL). The solution was stirred overnight at room temperature. After removal of TFA under a gentle stream of N₂, the product was dissolved in a mixture of CH₂Cl₂/MeOH, and precipitated with diethyl ether. The solvent was removed by filtration and **pico 3** (545 mg, 1.0 mmol, 76%) was obtained as a pale brown solid.

TLC : *n*-hexane/EtOAc (1/1); R_f = 0.17.

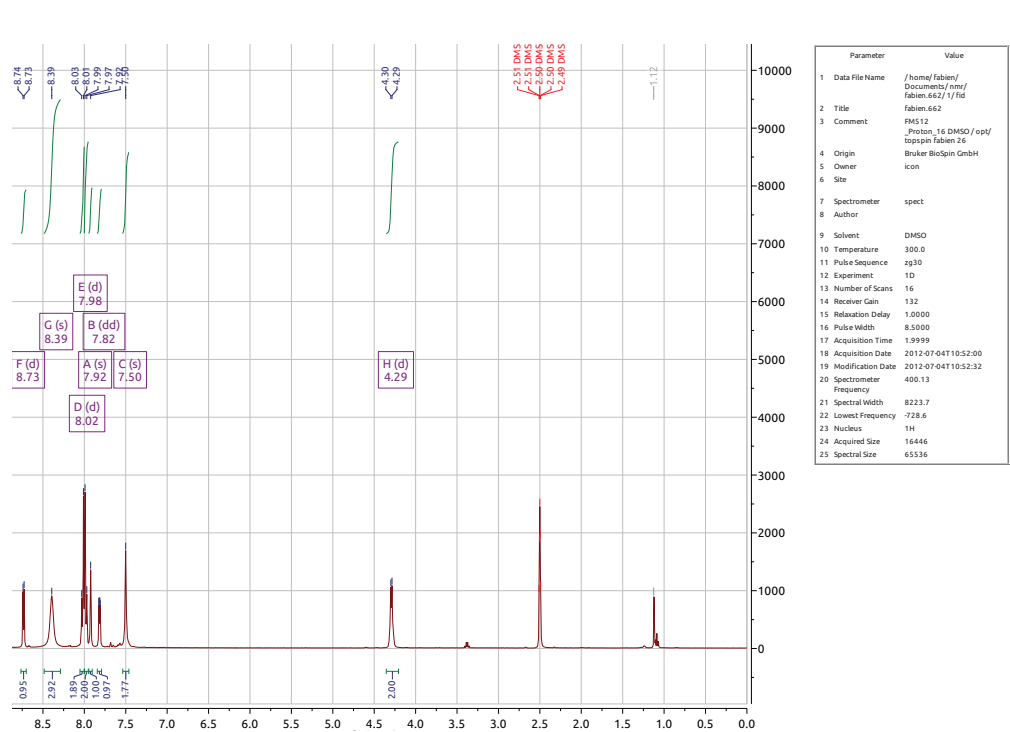
mp 165.2 °C

¹H NMR (400 MHz, DMSO-*d*₆, 20 °C, δ): 8.73 (d, *J* = 5.2 Hz, 1H, **H**⁶), 8.39 (b, 3H, **H**⁸), 8.02 (d, *J* = 8.6 Hz, 2H, **H**²), 7.98 (d, *J* = 8.6 Hz, 2H, **H**³), 7.92 (s, 2H, **H**⁴), 7.82 (dd, *J* = 5.3, 1.8 Hz, 1H, **H**⁵), 7.50 (s, 2H, **H**¹), 4.29 (d, *J* = 5.8 Hz, 2H, **H**⁷).

¹³C NMR (101 MHz, DMSO-*d*₆, 20 °C, δ): 154.2, 149.7, 146.5, 144.8, 139.8, 127.5, 126.5, 120.9, 120.2, 42.8.

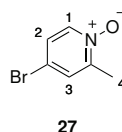
HRMS (ESI-MS, pos.) *m/z*: [M+H]⁺ calcd for C₁₂H₁₄N₃O₂S, 264.0806; found, 264.0802.

EA Anal. calcd for C₁₂H₁₃N₃O₂S · 2 TFA: C, 39.11%; H, 3.08%; N, 8.55%. Found: C, 39.30%; H, 3.24%; N, 8.62%.



pico 4-H

4-Bromo-2-methylpyridine-*N*-oxide (**27**)



4-Bromo-2-methylpyridine (3.00 mg, 17.4 mmol, 1.0 eq) and 3-chloroperoxybenzoic acid (4.80 mg, 27.8 mmol, 1.6 eq) in CH₂Cl₂ (20 mL) were stirred overnight at room temperature. A 2 M aqueous solution of Na₂CO₃ (20 mL) was added, and the reaction mixture was stirred for 1 h. The aqueous layer was extracted with CH₂Cl₂ (3 x 30 mL), and the combined organic layers were washed with brine and dried over Na₂SO₄. The solvent was removed under reduced pressure to obtain **27** (2.40 g, 12.8 mmol, 73%) as a yellow oil.

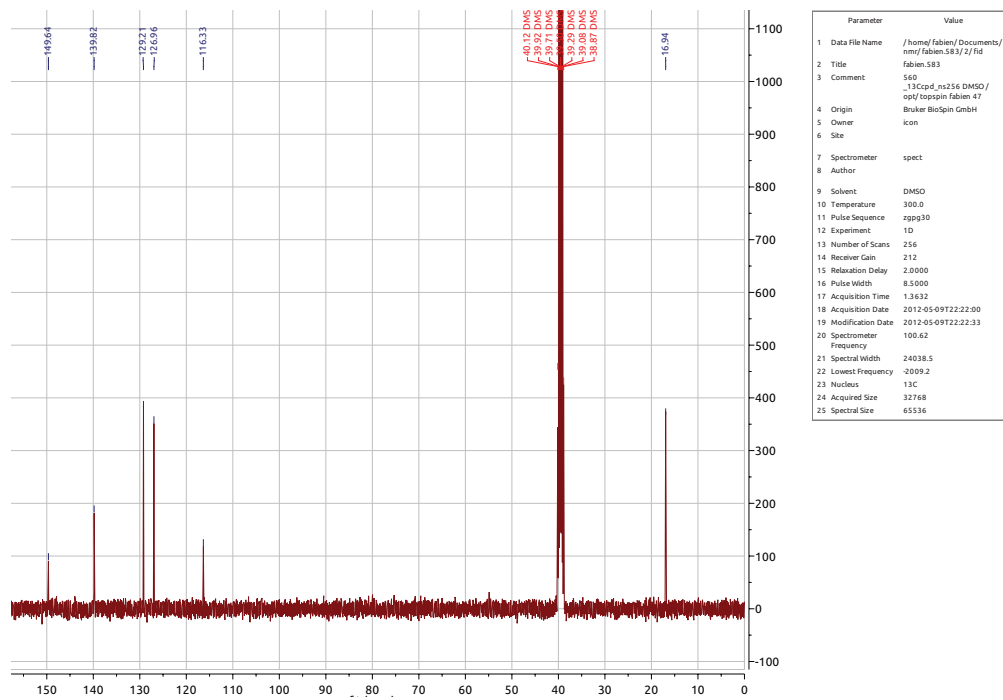
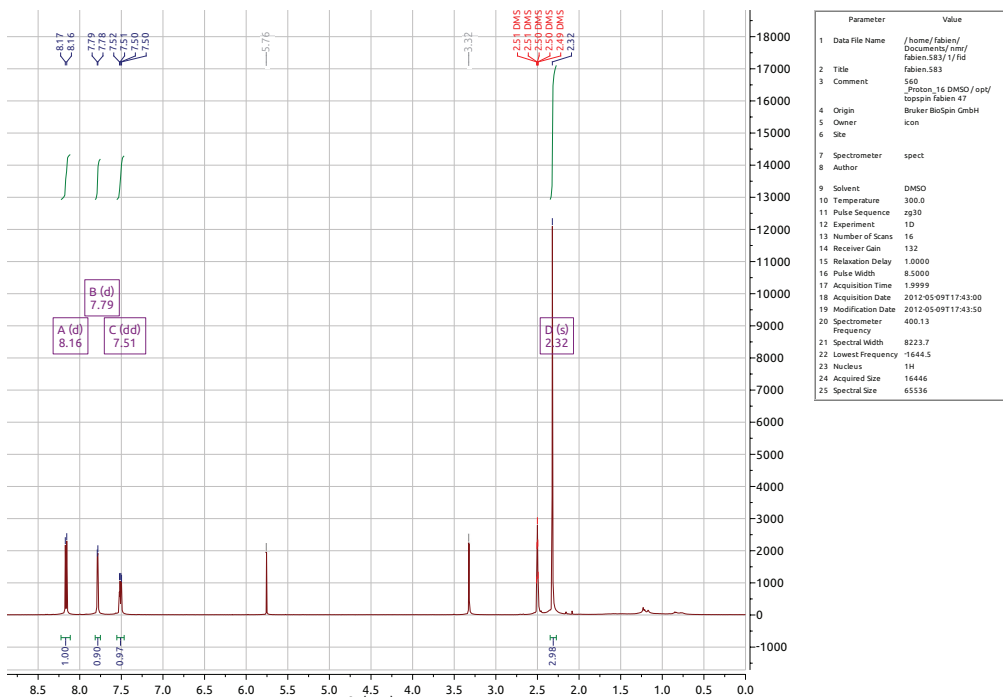
TLC : CH₂Cl₂/MeOH (9/1); R_f = 0.72.

¹H NMR (400 MHz, DMSO-*d*₆, 20 °C, δ): 8.16 (d, *J* = 6.9 Hz, 1H, **H**¹), 7.79 (d, *J* = 2.9 Hz, 1H, **H**³), 7.51 (dd, *J* = 6.9, 3.0 Hz, 1H, **H**²), 2.32 (s, 3H, **H**⁴).

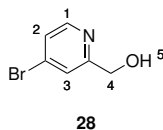
¹³C NMR (101 MHz, DMSO-*d*₆, 20 °C, δ): 149.6, 139.8, 129.2, 126.9, 116.3, 16.9.

HRMS (ESI-MS, pos.) *m/z*: [M+H]⁺ calcd for C₆H₇BrNO, 187.9711; found, 187.9704.

EA Anal. calcd for C₆H₆BrNO · $\frac{1}{4}$ H₂O: C, 37.43%; H, 3.40%; N, 7.28%. Found: C, 37.51%; H, 3.57%; N, 6.96%.



4-Bromo-2-hydroxymethylpyridine (**28**)



Compound **27** (2.40 g, 12.8 mmol, 1.0 eq) was dissolved in dry CH₂Cl₂ (10 mL) and the solution was cooled to 0 °C. Trifluoroacetic acid anhydride (15 mL) was added. When the vigorous thermal reaction had ceased, the orange mixture was stirred at room temperature for 30 min and then refluxed for 3 h. An aqueous saturated solution of NaHCO₃ was carefully added. The aqueous layer was extracted with CH₂Cl₂ (3 x 30 mL), and the combined organic layers were washed with brine, and dried over Na₂SO₄. The solvent was removed under reduced pressure to obtain **28** (1.90 g, 10.1 mmol, 79%) as a brown oil.

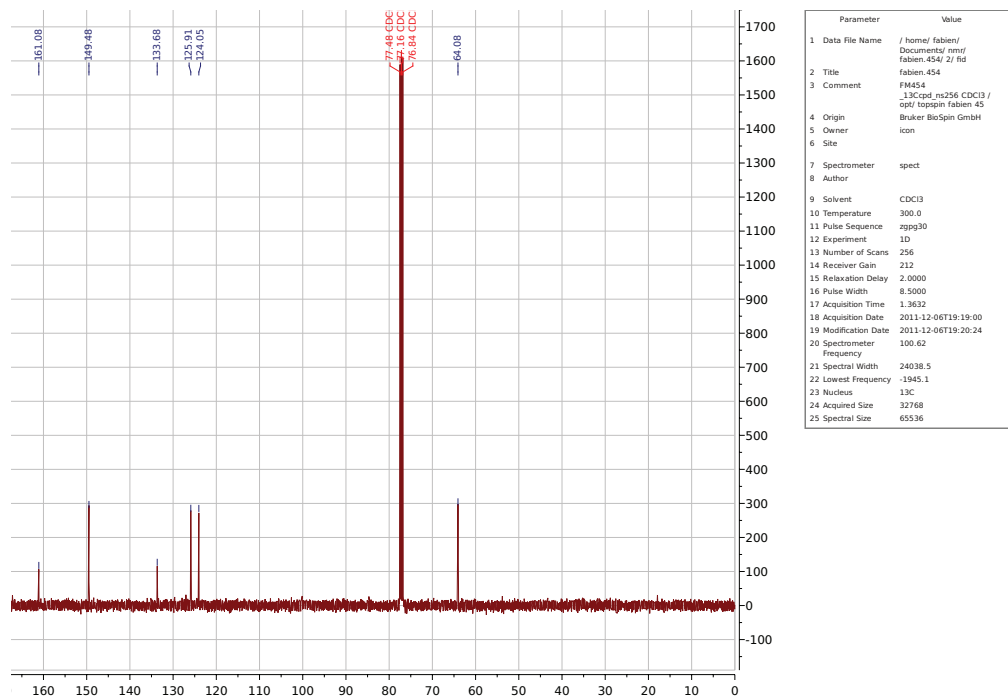
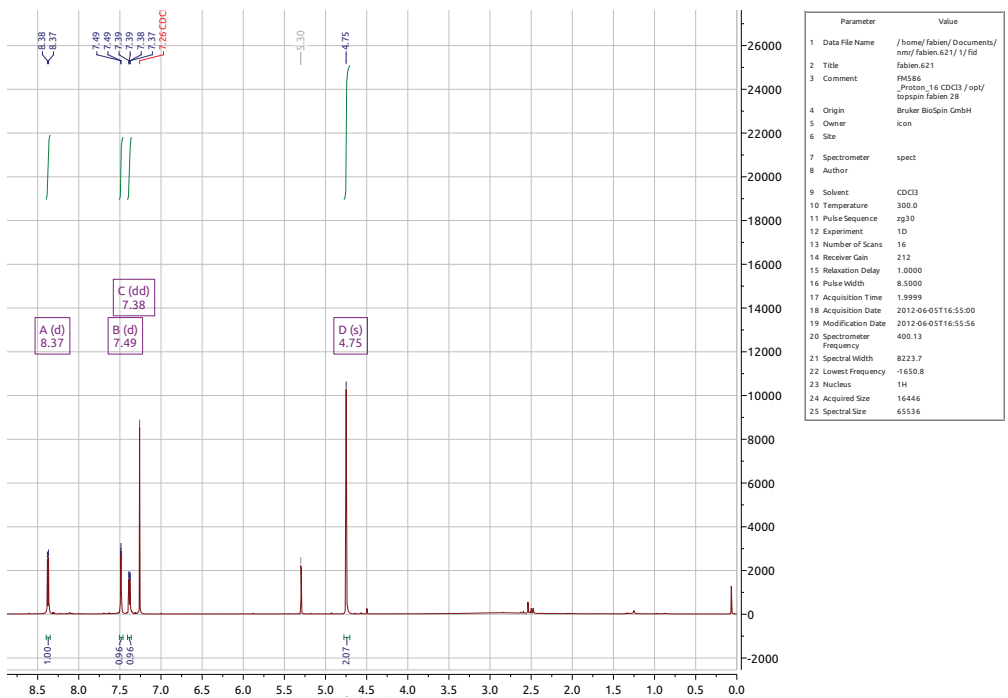
TLC : *n*-hexane/EtOAc (1/1); *R_f* = 0.25.

¹H NMR (400 MHz, CDCl₃, 20 °C, δ): 8.37 (d, *J* = 5.3 Hz, 1H, **H**¹), 7.49 (d, *J* = 1.1 Hz, 1H, **H**³), 7.38 (dd, *J* = 5.4, 1.7 Hz, 1H, **H**²), 4.75 (s, 2H, **H**⁴).

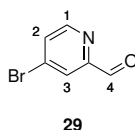
¹³C NMR (101 MHz, CDCl₃, 20 °C, δ): 161.1, 149.5, 133.7, 125.9, 124.1, 64.1.

HRMS (ESI-MS, pos.) *m/z*: [M+H]⁺ calcd for C₆H₇BrNO, 187.9711; found, 187.9704.

EA Anal. calcd for C₆H₆BrNO · $\frac{1}{4}$ H₂O: C, 37.43%; H, 3.40%; N, 7.28%. Found: C, 37.28%; H, 3.18%; N, 7.13%.



4-Bromo-2-pyridine aldehyde (**29**)



Compound **28** (1.90 g, 10.1 mmol, 1.0 eq) was dissolved in chloroform (15 mL) and MnO_2 (8.78 g, 101.0 mmol, 10.0 eq) was added. The reaction mixture was refluxed for 2 h. Then the solid material was removed by filtration over celite, and the solvent was removed under reduced pressure to obtain **29** (1.07 g, 5.75 mmol, 57%) as a brown oil.

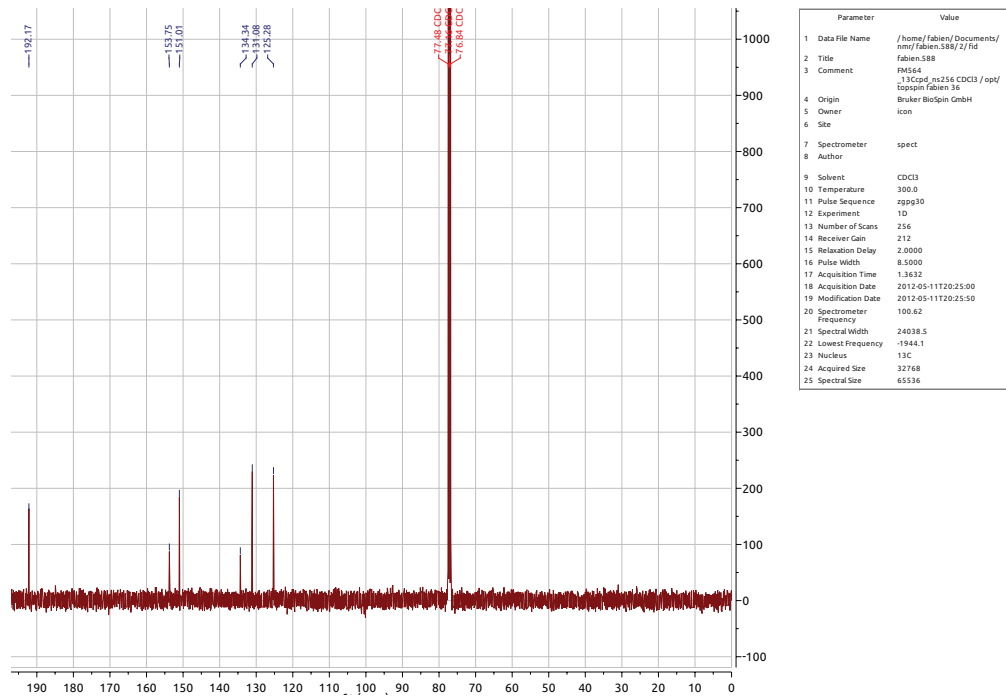
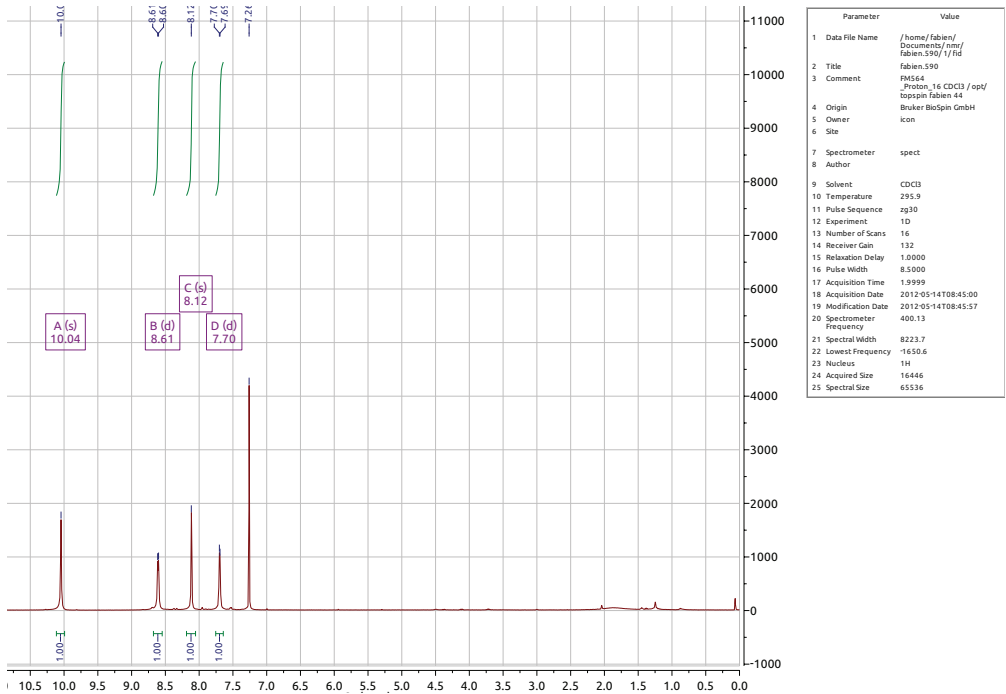
TLC : *n*-hexane/EtOAc (1/1); R_f = 0.58.

^1H NMR (400 MHz, CDCl_3 , 20 °C, δ): 10.04 (s, 1H, H^4), 8.61 (d, J = 5.3 Hz, 1H, H^1), 8.12 (s, 1H, H^3), 7.70 (d, J = 3.7 Hz, 1H, H^2).

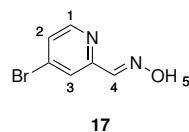
^{13}C NMR (101 MHz, CDCl_3 , 20 °C, δ): 192.1, 153.7, 151.0, 134.3, 131.8, 125.3.

MS (FAB-MS, pos.) m/z (relative intensity): $[\text{M}]^+$ calcd for $\text{C}_6\text{H}_4\text{BrNO}$, 184.9; found, 157.0 (100) $[\text{M}-\text{COH}]^+$, 185.0 (12) $[\text{M}]^+$.

EA Anal. calcd for $\text{C}_6\text{H}_4\text{BrNO} \cdot \frac{1}{2}\text{CHCl}_3$: C, 36.96%; H, 2.62%; N, 6.63%. Found: C, 37.27%; H, 2.39%; N, 6.63%.



4-Bromo-2-pyridine ketoxime (17)



To compound **29** (1.07 g, 5.8 mmol, 1.0 eq) in MeOH (15 mL), NaHCO₃ (0.77 g, 9.2 mmol, 1.6 eq) and NH₂OH · HCl (1.40 g, 20.1 mmol, 3.5 eq) were added. The reaction mixture was stirred overnight at room temperature before diluting with EtOAc (30 mL), and washing with NaHCO₃ solution and brine. The organic layer was dried over Na₂SO₄, and the solvent was removed under reduced pressure to obtain **17** (0.98 g, 4.9 mmol, 85%) as a yellow solid.

TLC : *n*-hexane/EtOAc (1/1); R_f = 0.50.

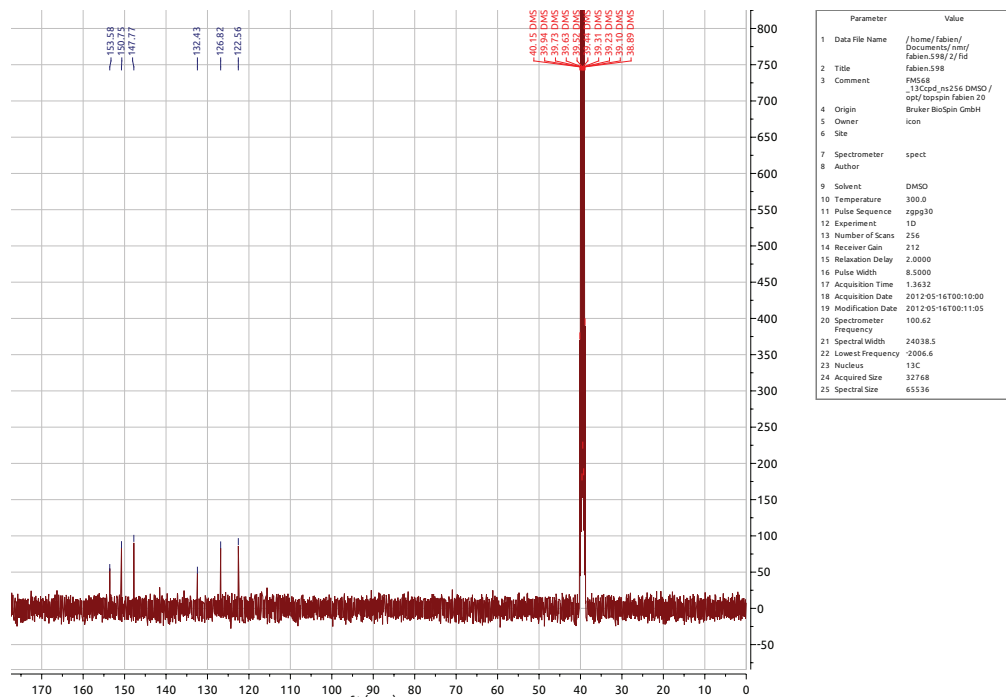
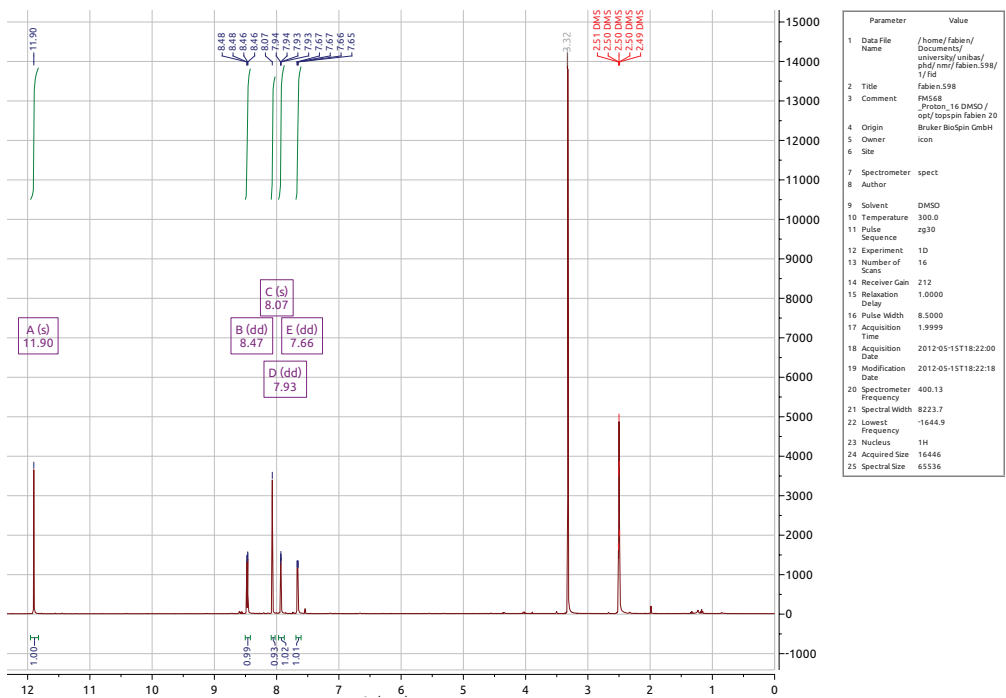
mp 151.7 °C

¹H NMR (400 MHz, DMSO-*d*₆, 20 °C, δ): 11.90 (s, 1H, **H**⁴), 8.47 (dd, *J* = 5.4, 0.6 Hz, 1H, **H**¹), 8.07 (s, 1H, **H**⁵), 7.93 (dd, *J* = 2.0, 0.6 Hz, 1H, **H**³), 7.66 (dd, *J* = 5.3, 1.9 Hz, 1H, **H**²).

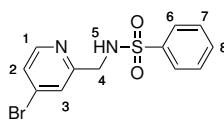
¹³C NMR (101 MHz, DMSO-*d*₆, 20 °C, δ): 153.6, 150.8, 147.7, 132.4, 126.8, 122.5.

MS (FAB-MS, pos.) *m/z*: [M]⁺ calcd for C₆H₅BrN₂O, 199.9; found, 200.0.

EA Anal. calcd for C₆H₅BrN₂O · $\frac{1}{10}$ EtOAc: C, 36.63%; H, 2.79%; N, 13.35%. Found: C, 36.50%; H, 2.77%; N, 13.06%.



***N*-[**(4-Bromopyridin-2-yl)methyl**]benzenesulfonamide (**18**)**



18

Zinc dust (1.37 g, 21.0 mmol, 6.0 eq) was added in several portions to a solution of compound **17** (700 mg, 3.5 mmol, 1.0 eq) in TFA (15 mL) at 0 °C. The reaction mixture was stirred for 15 min, and added to a mixture of a 2 M aqueous solution of NaOH (20 mL) and CH₂Cl₂ (20 mL). The insoluble material was removed by filtration, and the organic layer was separated. The organic layer was washed with water, brine and dried over Na₂SO₄. The solvent was removed under reduced pressure. The reduced product was dissolved in CH₂Cl₂ (15 mL) and DIPEA (0.70 mL, 4.2 mmol, 1.2 eq) was added. The reaction mixture was stirred at 25 °C for 10 min. It was then cooled to 0 °C, and benzenesulfonyl chloride (0.29 mL, 2.2 mmol, 0.7 eq) was added dropwise. The resulting solution was allowed to warm up to 25 °C, and was stirred overnight. The reaction mixture was diluted with CH₂Cl₂ (20 mL), washed with water and brine before drying over Na₂SO₄. The product was purified by flash chromatography (*n*-hexane/EtOAc, 1/1) to afford **18** (280 mg, 0.9 mmol, 25%) as a pale yellow solid.

TLC : *n*-hexane/EtOAc (1/1); *R_f* = 0.32.

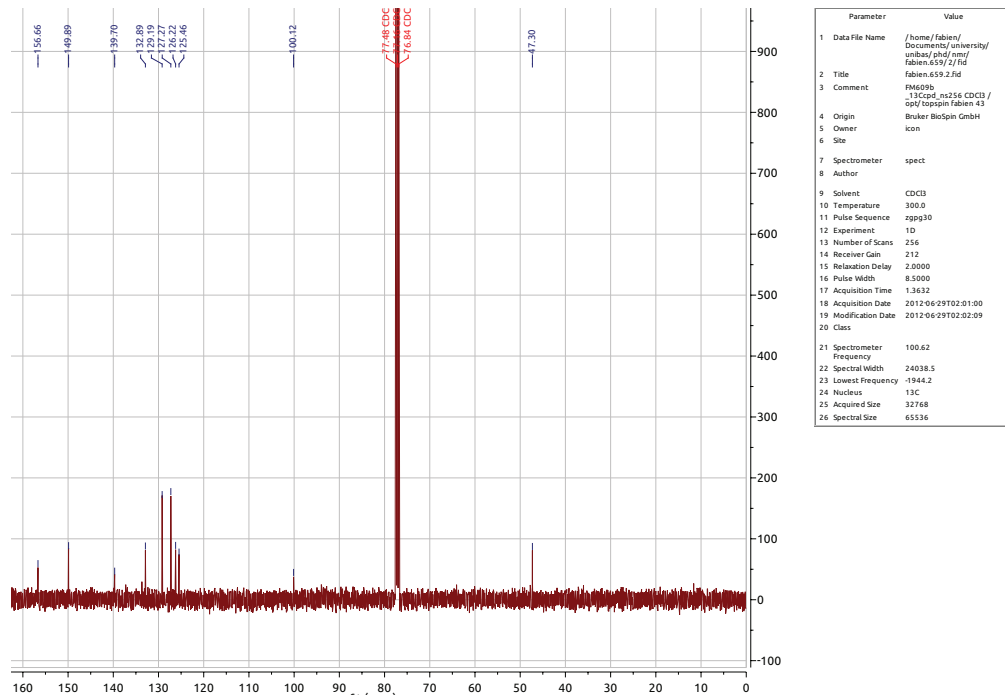
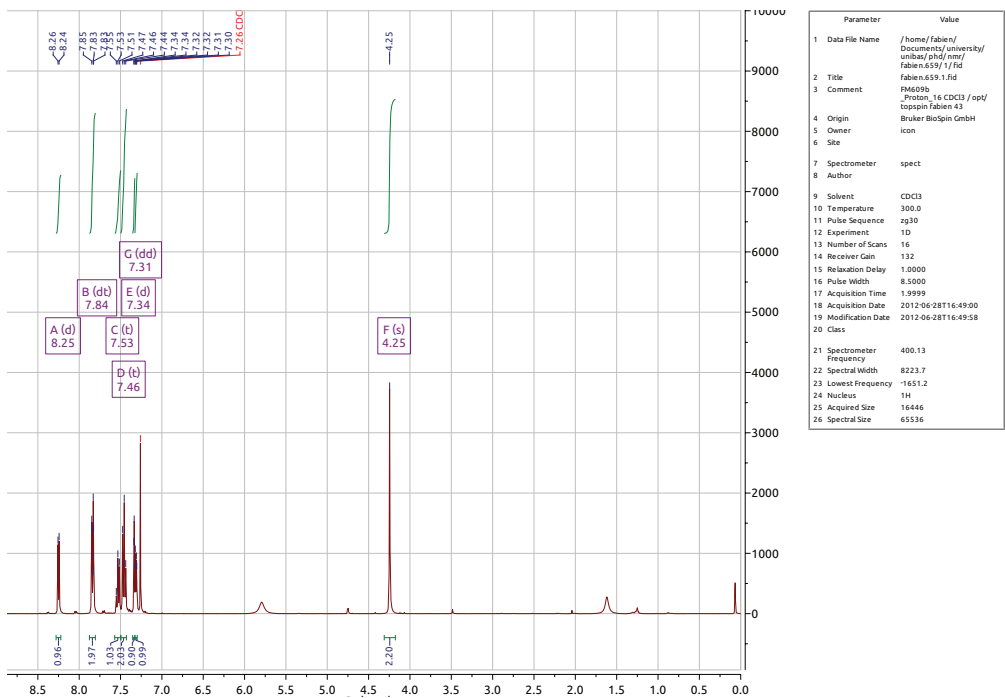
mp 105.2 °C

¹H NMR (400 MHz, CDCl₃, 20 °C, δ): 8.25 (d, *J* = 5.2 Hz, 1H, **H**¹), 7.84 (dt, *J* = 7.2, 1.4 Hz, 2H, **H**⁶), 7.53 (t, *J* = 7.4 Hz, 1H, **H**⁸), 7.46 (t, *J* = 7.5 Hz, 2H, **H**⁷), 7.34 (d, *J* = 1.4 Hz, 1H, **H**³), 7.31 (dd, *J* = 5.3, 1.7 Hz, 2H, **H**²), 4.25 (s, 2H, **H**⁴).

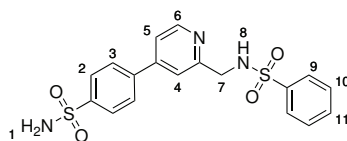
¹³C NMR (101 MHz, CDCl₃, 20 °C, δ): 156.7, 149.9, 139.7, 132.9, 129.2, 127.3, 126.2, 125.4, 100.1, 47.3.

MS (ESI-MS, pos.) *m/z* (relative intensity): [M+H]⁺ calcd for C₁₂H₁₂BrN₂O₂S, 326.9; found, 327.0 (100) [M+H]⁺, 349.0 (37) [M+Na]⁺.

EA Anal. calcd for C₁₂H₁₁BrN₂O₂S: C, 44.05%; H, 3.39%; N, 8.56%. Found: C, 44.15%; H, 3.47%; N, 8.61%.



***N*-((4-(4-Sulfamoylphenyl)pyridin-2-yl)methyl)benzenesulfonamide (pico 4-H)**



pico 4-H

A mixture of 4-sulfamoylphenylboronic acid pinacol ester (207 mg, 0.7 mmol, 1.2 eq), **18** (200 mg, 0.6 mmol, 1.0 eq), tetrakis(triphenylphosphine)palladium (40 mg, 0.03 mmol, 0.05 eq), Na₂CO₃ (191 mg, 1.8 mmol, 2.9 eq), water (1.5 mL) and 1,4-dioxane (1.5 mL) were heated in a sealed vial in a microwave reactor at 150 °C for 15 min. The reaction mixture was diluted with CH₂Cl₂ (30 mL), and washed with water and brine. The organic layer was dried over Na₂SO₄ and the solvent was removed under reduced pressure. The product was purified by flash chromatography (CH₂Cl₂/MeOH, 3%) to obtain pico **4-H** (160 mg, 0.4 mmol, 64%) as a colorless solid.

TLC : CH₂Cl₂/MeOH (9/1); R_f = 0.51.

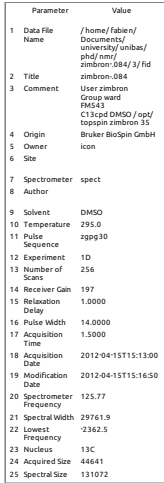
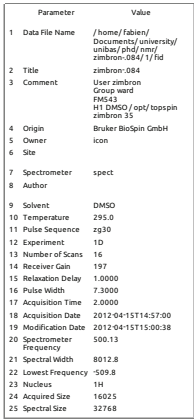
mp 157.8 °C

¹H NMR (500 MHz, DMSO-*d*₆, 20 °C, δ): 8.52 (d, *J* = 6.1 Hz, 1H, **H**⁶), 8.35 (t, *J* = 6.3 Hz, 1H, **H**⁸), 7.96 (d, *J* = 8.5 Hz, 2H, **H**²), 7.86 (d, *J* = 8.4 Hz, 2H, **H**³), 7.79 (d, *J* = 6.9 Hz, 2H, **H**⁹), 7.55 (m, 1H, **H**^{1,4,5,10,11}), 4.22 (d, *J* = 6.1 Hz, 2H, **H**⁷).

¹³C NMR (126 MHz, DMSO-*d*₆, 20 °C, δ): 157.8, 149.6, 146.1, 144.6, 140.7, 140.3, 132.3, 129.1, 127.4, 126.5, 126.4, 120.1, 119.3, 48.1.

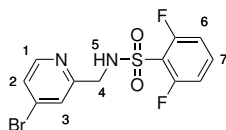
MS (ESI-MS, pos.) *m/z*: [M]⁺ calcd for C₁₈H₁₈N₃O₄S₂, 404.1; found, 404.1.

EA Anal. calcd for C₁₈H₁₇N₃O₄S₂ · $\frac{1}{3}$ H₂O: C, 52.80%; H, 4.35%; N, 10.26%. Found: C, 52.61%; H, 4.23%; N, 10.42%.



pico 5-H

N-[(4-Bromopyridin-2-yl)methyl]-2,6-difluorobenzene-1-sulfonamide (**19**)



19

Zinc dust (1.93 g, 29.5 mmol, 6.0 eq) was added in several portions to a solution of compound **17** (987 mg, 4.9 mmol, 1.0 eq) in TFA (15 mL) at 0 °C. The reaction mixture was stirred for 15 min, and added to a mixture of a 2 M aqueous solution of NaOH (20 mL) and CH₂Cl₂ (20 mL). The insoluble material was removed by filtration, and the organic layer was separated. The organic layer was washed with water, brine and dried over Na₂SO₄. The solvent was removed under reduced pressure. The reduced product was dissolved in CH₂Cl₂ (15 mL) and DIPEA (0.59 mL, 4.3 mmol, 0.9 eq) was added. The reaction mixture was stirred at 25 °C for 10 min. It was then cooled to 0 °C, and 2,6-difluorobenzenesulfonyl chloride (917 mg, 7.3 mmol, 1.1 eq) was added dropwise. The resulting solution was allowed to reach 25 °C, and was stirred overnight. The reaction mixture was diluted with CH₂Cl₂ (20 mL), washed with water and brine before drying over Na₂SO₄. The product was purified by flash chromatography (*n*-hexane/EtOAc, 1/1) to afford **19** (698 mg, 1.9 mmol, 39%) as a pale yellow solid.

TLC : *n*-hexane/EtOAc (1/1); *R_f* = 0.27.

mp 129.5 °C

¹H NMR (400 MHz, CDCl₃, 20 °C, δ): 8.32 (d, *J* = 5.5 Hz, 1H, **H¹**), 7.61 (d, *J* = 1.9 Hz, 1H, **H³**), 7.47 (m, 2H, **H^{2,7}**), 6.98 (t, *J* = 8.5 Hz, 1H, **H⁶**), 6.49 (t, *J* = 5.9 Hz, 1H, **H⁵**), 4.52 (d, *J* = 5.8 Hz, 2H, **H⁴**).

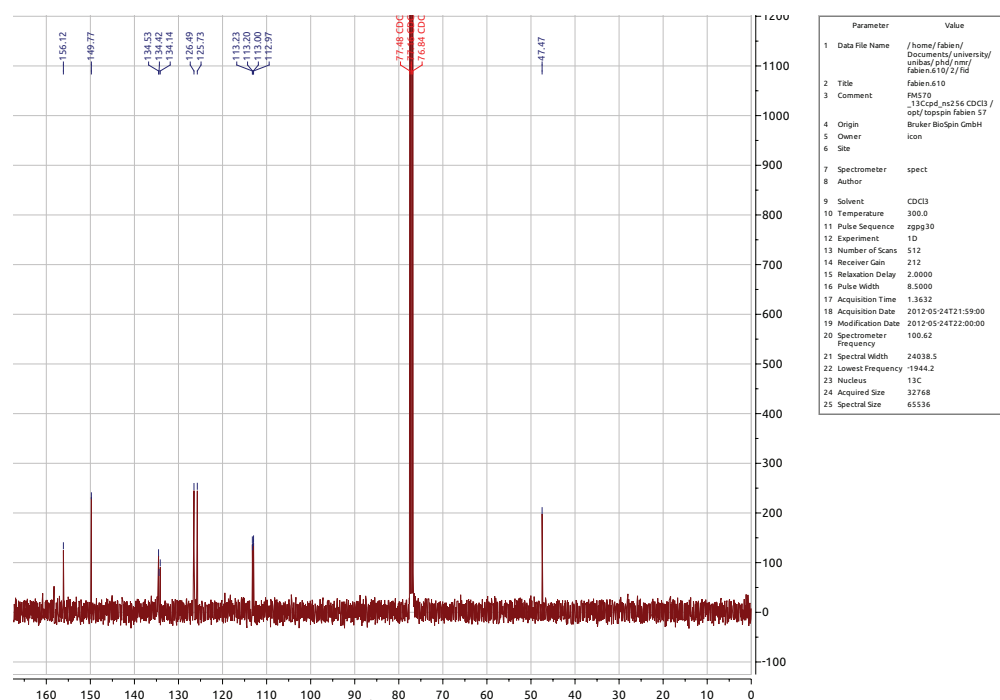
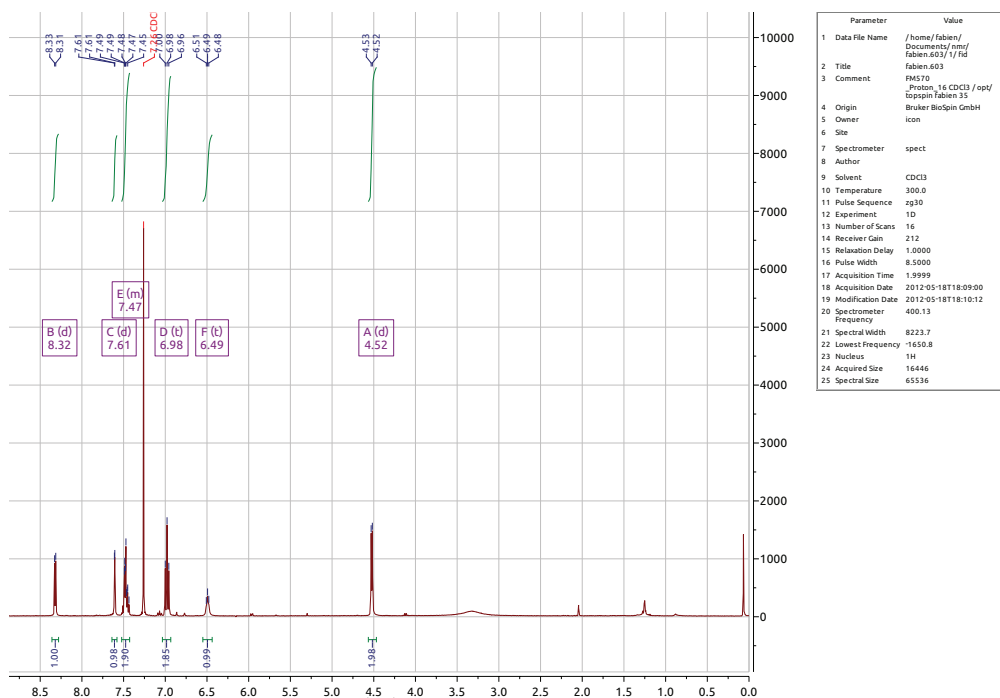
¹³C NMR (101 MHz, CDCl₃, 20 °C, δ): 156.1, 149.7, 134.5, 134.4, 134.1, 126.5, 125.7, 113.2, 113.0, 47.5.

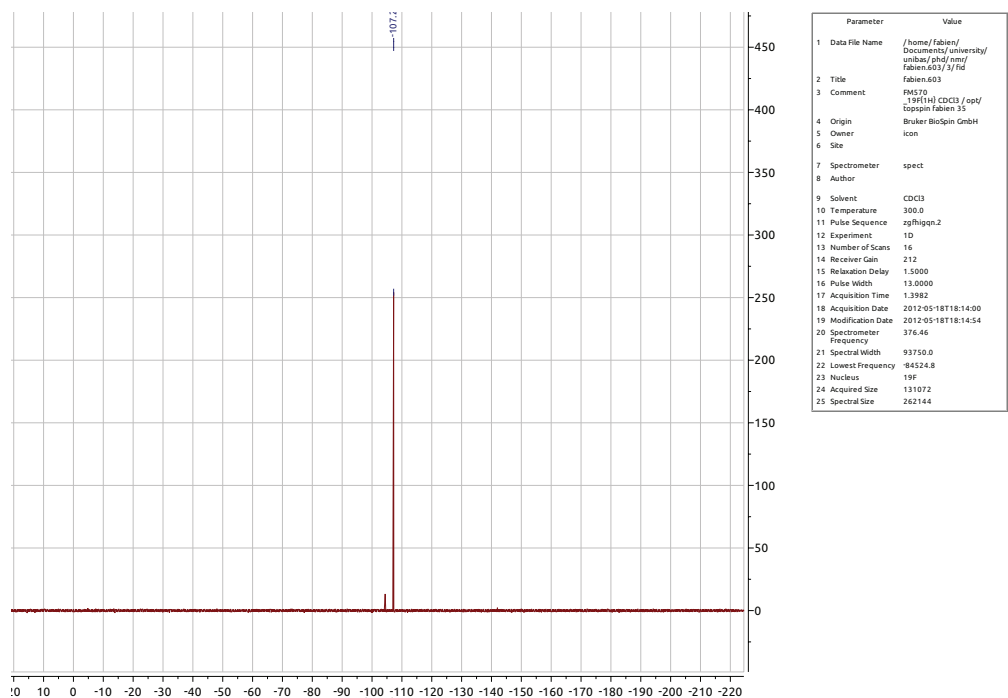
¹⁹F NMR (376 MHz, CDCl₃, 20 °C, δ): -107.2.

MS (ESI-MS, pos.) *m/z* (relative intensity): [M+H]⁺ calcd for C₁₂H₁₀BrF₂N₂O₂S, 363.0;

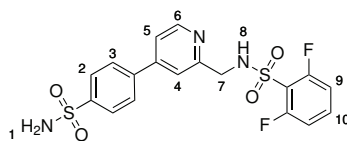
found, 363.0 (100) $[M+H]^+$, 385.0 (100) $[M+Na]^+$.

EA Anal. calcd for $\text{C}_{12}\text{H}_9\text{BrF}_2\text{N}_2\text{O}_2\text{S}$: C, 39.69%; H, 2.50%; N, 7.71%. Found: C, 39.44%; H, 2.65%; N, 7.63%.





2,6-Difluoro-N-((4-(4-sulfamoylphenyl)pyridin-2-yl)methyl)benzenesulfonamide (pico 5-H)



pico 5-H

A mixture of 4-sulfamoylphenylboronic acid pinacol ester (147 mg, 0.5 mmol, 1.2 eq), **19** (160 mg, 0.4 mmol, 1.0 eq), tetrakis(triphenylphosphine)palladium (20 mg, 0.02 mmol, 0.05 eq), Na₂CO₃ (136 mg, 1.3 mmol, 2.9 eq), water (1.2 mL) and 1,4-dioxane (1.2 mL) were heated in a sealed vial in a microwave reactor at 150 °C for 15 min. The reaction mixture was diluted with CH₂Cl₂ (30 mL), and washed with water and brine. The organic layer was dried over Na₂SO₄, and the solvent was removed under reduced pressure. The product was purified by flash chromatography (CH₂Cl₂/MeOH, 3%) to obtain pico **5-H** (82 mg, 0.2 mmol, 42%) as a colorless solid.

TLC : CH₂Cl₂/MeOH (9/1); R_f = 0.45.

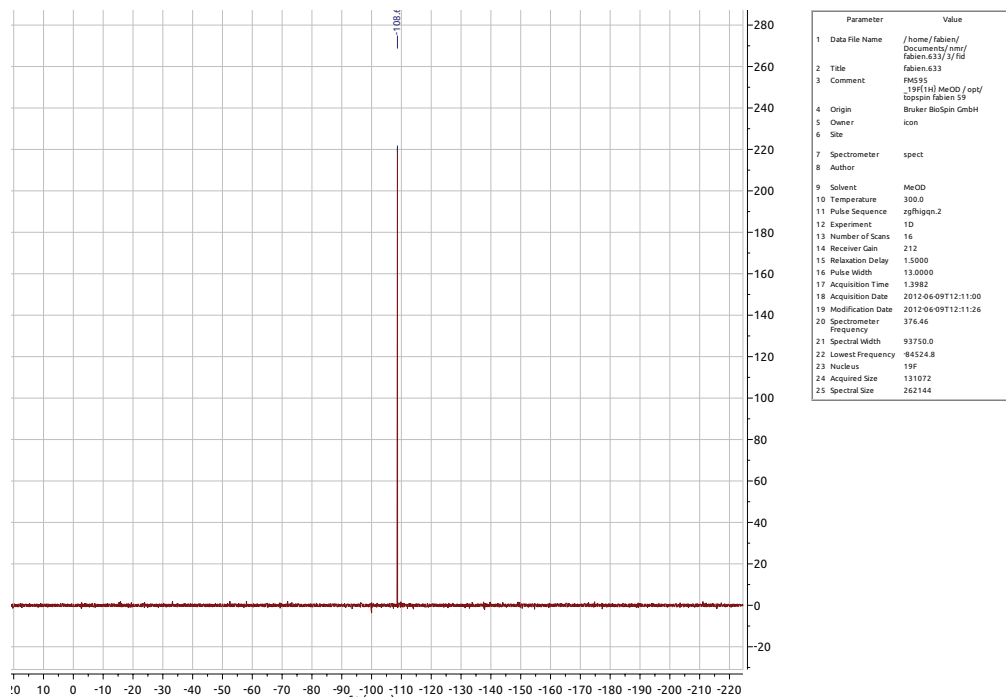
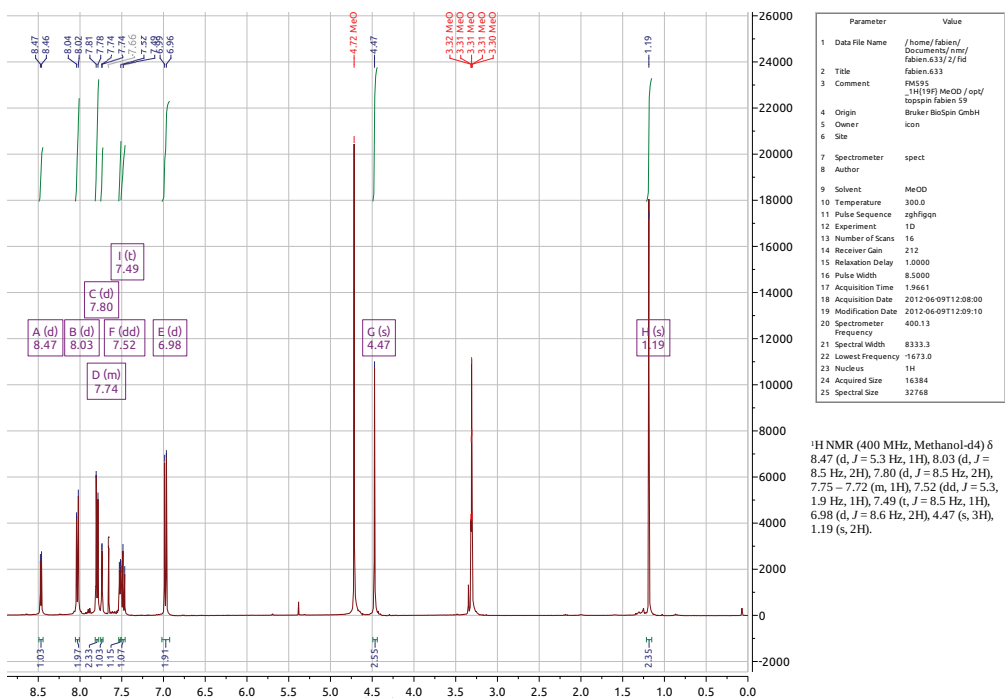
mp 183.9 °C

¹H NMR (400 MHz, CD₃OD, 20 °C, δ): 8.47 (d, *J* = 5.3 Hz, 1H, **H**⁶), 8.03 (d, *J* = 8.5 Hz, 2H, **H**²), 7.80 (d, *J* = 8.5 Hz, 2H, **H**³), 7.74 (d, *J* = 1.3 Hz, 1H, **H**⁴), 7.52 (dd, *J* = 5.3, 1.9 Hz, 1H, **H**⁵), 7.47 (t, *J* = 8.5 Hz, 1H, **H**¹⁰), 6.98 (d, *J* = 8.6 Hz, 2H, **H**⁹), 4.47 (s, 2H, **H**⁷), 1.19 (s, 2H, **H**¹),

¹⁹F NMR (376 MHz, CD₃OD, 20 °C, δ): -108.9.

MS (ESI-MS, pos.) *m/z*: [M+H]⁺ calcd for C₁₈H₁₆F₂N₃O₄S₂, 440.0; found, 440.1.

EA Anal. calcd for C₁₈H₁₅F₂N₃O₄S₂ · 2 H₂O: C, 45.47%; H, 4.03%; N, 8.84%. Found: C, 45.67%; H, 3.86%; N, 8.45%.



Complex synthesis

General procedures

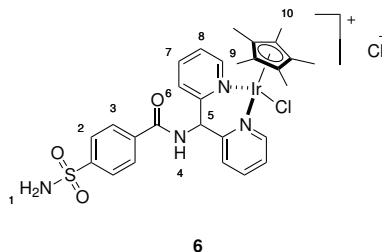
Procedure 1

Bipyridine derivative complexes were synthesized according a modification of the procedure published by Mann.^{S3} The ligand (0.28 mmol, 2.0 eq) and the metal dimer (0.14 mmol, 1.0 eq) were dissolved in ACN (5 mL). The resulting solution was purged with nitrogen for 20 min, and then refluxed for 4 h. The volume of the reaction was reduced to 2 mL, and the resulting solid was filtered and washed with a small amount of cold ACN. The solid was dried under vacuum to afford the desired complex.

Procedure 2

The picolylamine-derivative complexes were synthesized according a modification of the procedure published by Çetinkaya.^{S4} The ligand (0.28 mmol, 2.0 eq) and the metal dimer (0.14 mmol, 1.0 eq) were dissolved in EtOH (5 mL), and the resulting solution was purged with nitrogen for 20 min. The reaction mixture was refluxed for 2 h and the solvent was removed under reduced pressure. The solid was washed with a small amount of CH₂Cl₂, and dried to afford the desired complex.

[(cp*)Ir(6)Cl]Cl (6)



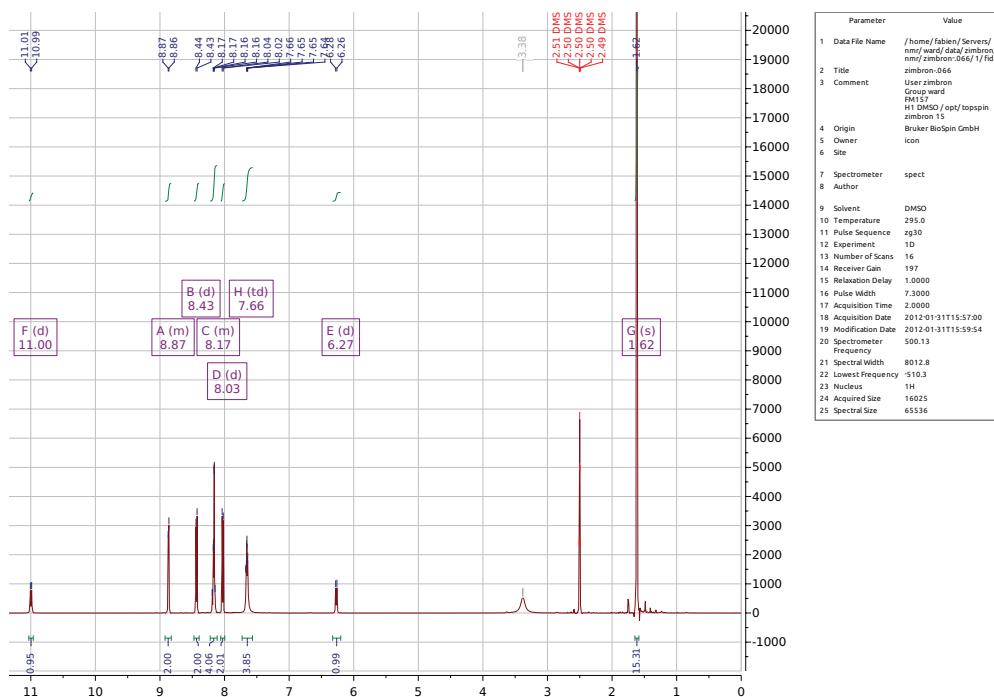
Following procedure 1, compound **6** was obtained as a yellow solid (45 mg, 0.06 mmol, 49%).

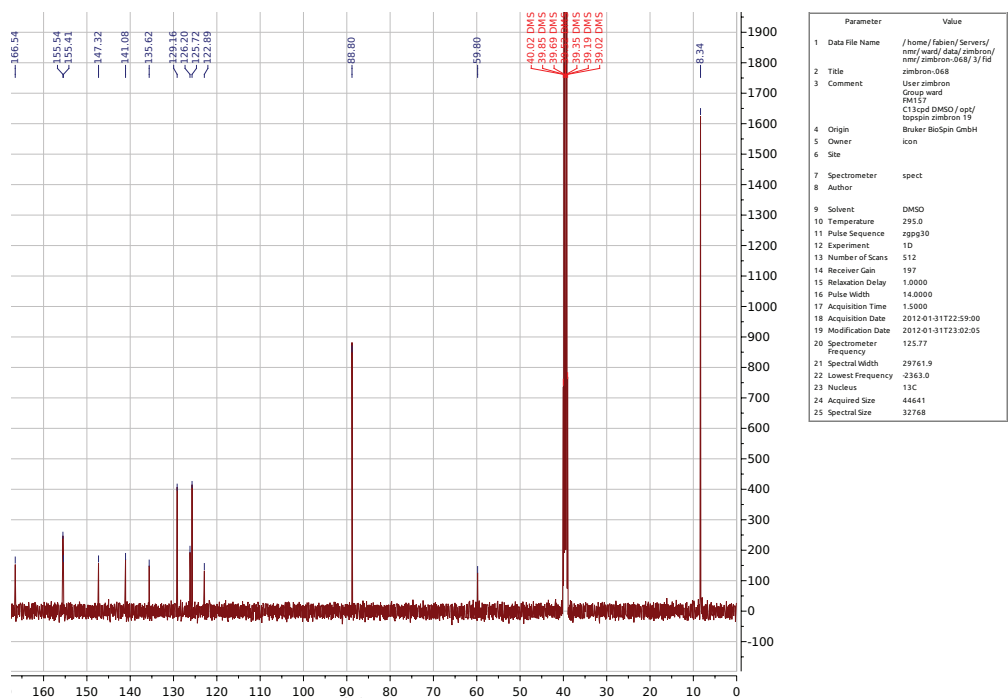
¹H NMR (400 MHz, DMSO-*d*₆, 20 °C, δ): 11.00 (d, J = 9.9 Hz, 1H, **H**⁴), 8.87 (m, 2H, **H**⁹), 8.43 (d, J = 8.5 Hz, 2H, **H**²), 8.17 (m, 4H, **H**^{6,7}), 8.03 (d, J = 8.5 Hz, 2H, **H**³), 7.66 (m, 4H, **H**^{1,8}), 6.27 (d, J = 9.7 Hz, 1H, **H**⁵), 1.62 (s, 15H, **H**¹⁰).

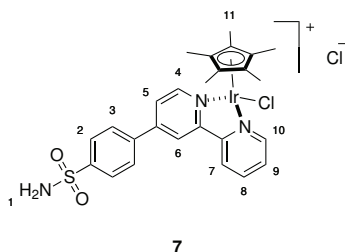
¹³C NMR (100 MHz, DMSO-*d*₆, 20 °C, δ): 166.5, 155.5, 155.4, 147.3, 141.1, 135.6, 129.1, 126.2, 125.7, 122.9, 88.8, 59.8, 8.34.

HRMS (ESI-MS, pos.) m/z : [M-Cl]⁺ calcd for C₂₈H₃₁ClIrN₄O₃S, 731.1; found, 731.2.

EA Anal. calcd for C₂₈H₃₁Cl₂IrN₄O₃S · 2.1H₂O: C, 41.80%; H, 4.41%; N, 6.96%. Found: C, 41.43%; H, 3.97%; N, 7.06%.





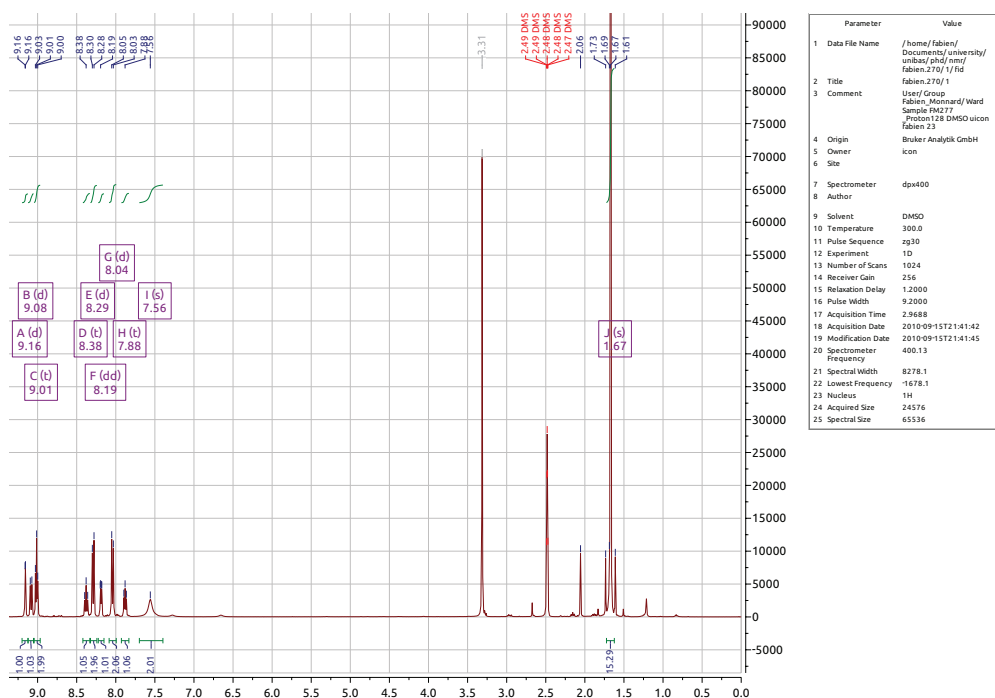


Following procedure 1, compound **7** was obtained as a yellow solid (37 mg, 0.05 mmol, 54%).

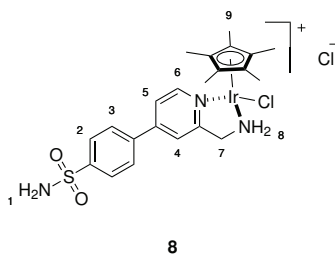
¹H NMR (400 MHz, DMSO-*d*₆, 20 °C, δ): 9.18 (d, *J* = 1.3 Hz, 1H, **H**⁶), 9.10 (d, *J* = 8.1 Hz, 1H, **H**⁷), 9.03 (t, *J* = 5.9 Hz, 2H, **H**^{4,10}), 8.40 (t, *J* = 7.8 Hz, 1H, **H**⁸), 8.31 (d, *J* = 8.4 Hz, 1H, **H**²), 8.21 (dd, *J* = 6.0, 1.7 Hz, 1H, **H**⁵), 8.06 (d, *J* = 8.4 Hz, 2H, **H**³), 7.90 (t, *J* = 6.5 Hz, 1H, **H**⁹), 7.58 (s, 2H, **H**¹), 1.69 (s, 15H, **H**¹¹).

HRMS (ESI-MS, pos.) *m/z*: [M-Cl]⁺ calcd for C₂₆H₂₈ClIrN₃O₇S, 674.1220; found, 674.1198.

EA Anal. calcd for $\text{C}_{26}\text{H}_{28}\text{Cl}_2\text{IrN}_3\text{O}_2\text{S} \cdot 2\text{H}_2\text{O}$: C, 41.88%; H, 4.33%; N, 5.63%. Found: C, 41.54%; H, 4.33%; N, 5.69%.



[(cp^{*})Ir(pico 3)Cl]Cl (8**)**



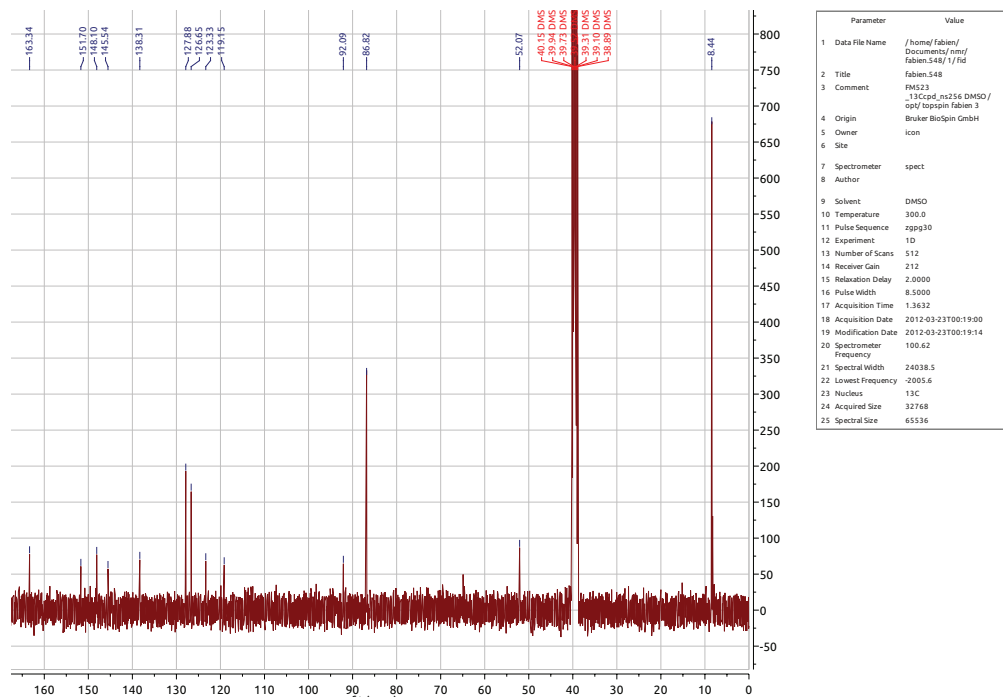
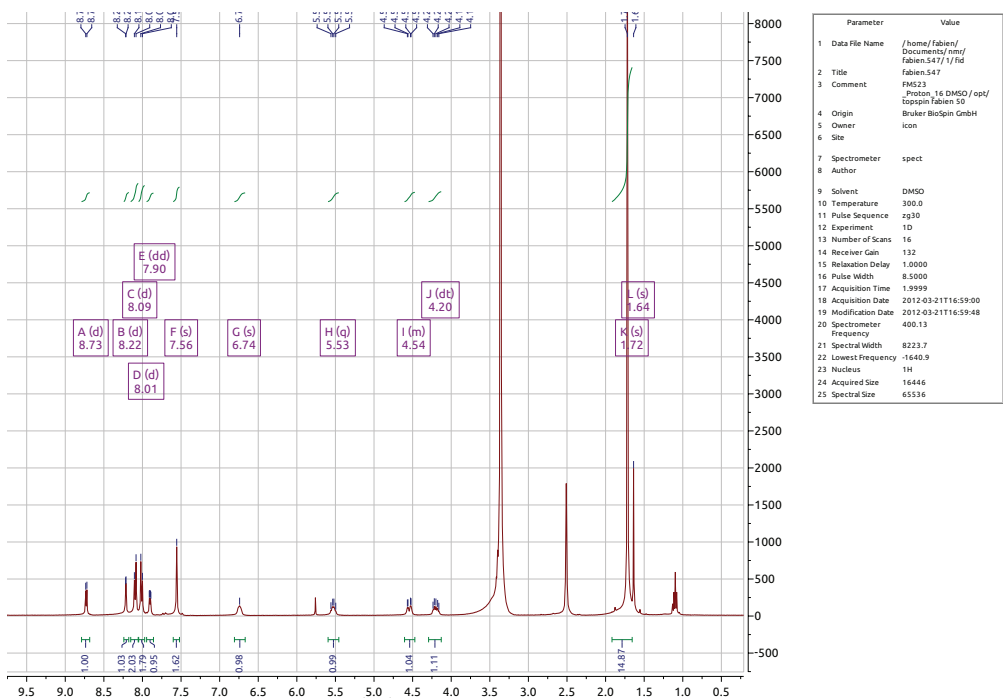
Following procedure 2, complex **8** was obtained as a pale orange-yellow solid (24 mg, 0.05 mmol, 42%).

¹H NMR (400 MHz, DMSO-*d*₆, 20 °C, δ): 8.73 (d, J = 6.1 Hz, 1H, **H**⁶), 8.22 (d, J = 2.1 Hz, 1H, **H**⁴), 8.09 (d, J = 8.6 Hz, 2H, **H**²), 8.01 (d, J = 8.5 Hz, 2H, **H**³), 7.90 (dd, J = 6.2, 2.1 Hz, 1H, **H**⁵), 7.56 (b, 1H, **H**⁸), 5.53 (q, J = 9.3 Hz, 1H, **H**^{8'}), 4.54 (dd, J = 16.2, 2.9 Hz, 1H, **H**⁷), 4.20 (dt, J = 15.2, 6.5 Hz, 1H, **H**^{7'}), 1.72 (s, 15H, **H**⁹).

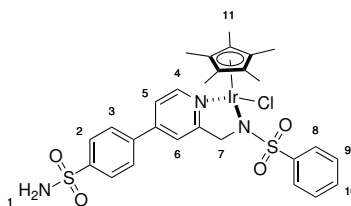
¹³C NMR (101 MHz, DMSO-*d*₆, 20 °C, δ): 163.3, 151.7, 148.1, 145.5, 138.3, 127.8, 126.6, 123.3, 119.2, 90.1, 86.8, 52.1, 8.4.

HRMS (ESI-MS, pos.) m/z (relative intensity): [M-Cl]⁺ calcd for C₂₂H₂₇ClIrN₃O₂S, 625.1; found, 590.2 (100) [M-2Cl-H]⁺, 626.2 (81) [M-Cl]⁺.

EA Anal. calcd for C₂₂H₂₇Cl₂IrN₃O₂S · 3.3H₂O: C, 36.69%; H, 4.70%; N, 5.84%. Found: C, 36.57%; H, 4.32%; N, 5.45%.



[(cp*)Ir(pico 4)Cl]Cl (9**)**



9

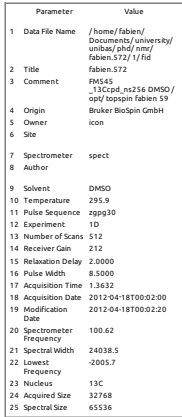
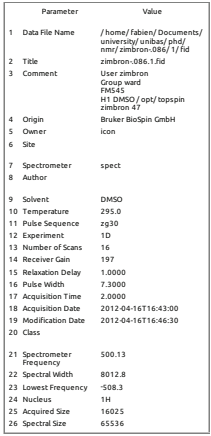
Following procedure 2, K₂CO₃ (7 mg, 0.05 mmol, 0.6 eq) was added in the reaction mixture, complex **9** was obtained as a pale orange-yellow solid (49 mg, 0.1 mmol, 62%).

¹H NMR (500 MHz, DMSO-*d*₆, 20 °C, δ): 8.53 (m, 1H, **H**⁴), 8.36 (t, *J* = 6.3 Hz, 1H, **H**¹⁰), 7.96 (d, *J* = 8.4 Hz, 2H, **H**²), 7.86 (d, *J* = 8.4 Hz, 2H, **H**³), 7.79 (d, *J* = 6.9 Hz, 2H, **H**⁸), 7.54 (m, 8H, **H**^{1,5,6,9}), 4.22 (d, *J* = 6.2 Hz, 2H, **H**⁷), 1.63 (s, 15H, **H**¹¹).

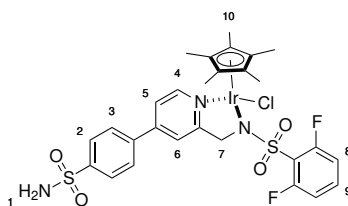
¹³C NMR (101 MHz, DMSO-*d*₆, 20 °C, δ): 157.8, 149.6, 146.1, 144.6, 140.6, 140.3, 132.3, 129.1, 127.4, 126.5, 126.4, 120.1, 119.2, 92.1, 48.0, 8.3.

HRMS (ESI-MS, pos.) *m/z*: [M-Cl]⁺ calcd for C₂₈H₃₁IrN₃O₄S₂, 730.1; found, 730.2.

EA Anal. calcd for C₂₈H₃₁Cl₂IrN₃O₄S₂ · 0.25K₂CO₃: C, 40.62%; H, 3.74%; N, 5.03%. Found: C, 40.95%; H, 3.69%; N, 5.43%.



[(cp*)Ir(pico 5)Cl]Cl (10**)**



10

Following procedure 2, K_2CO_3 (13 mg, 0.09 mmol, 1.0 eq) was added in the reaction mixture, complex **10** was obtained as a pale orange-yellow solid (24 mg, 0.05 mmol, 42%).

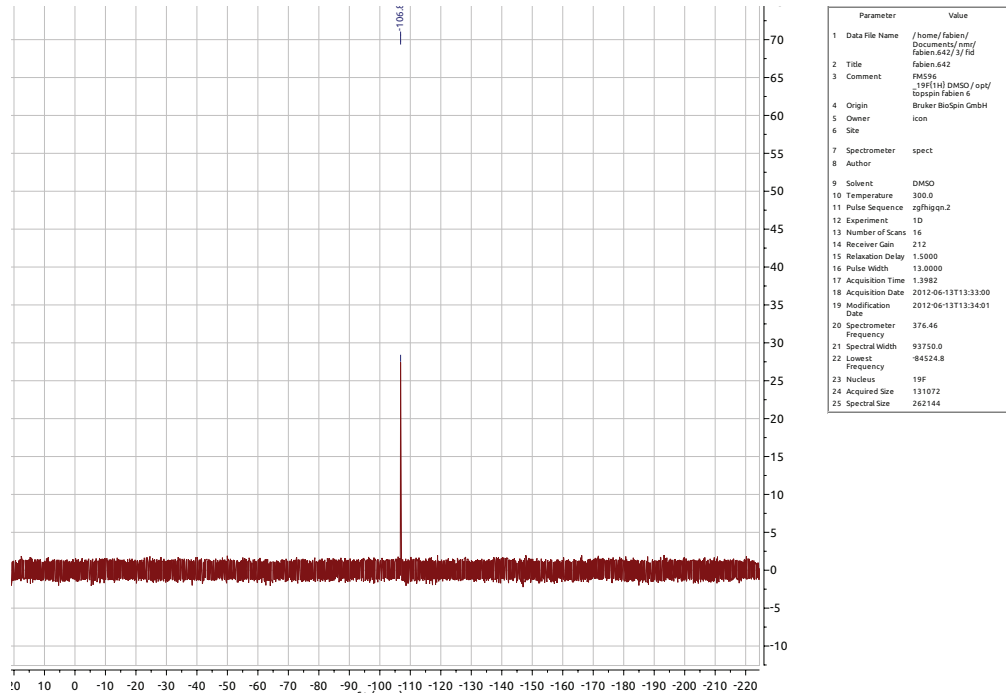
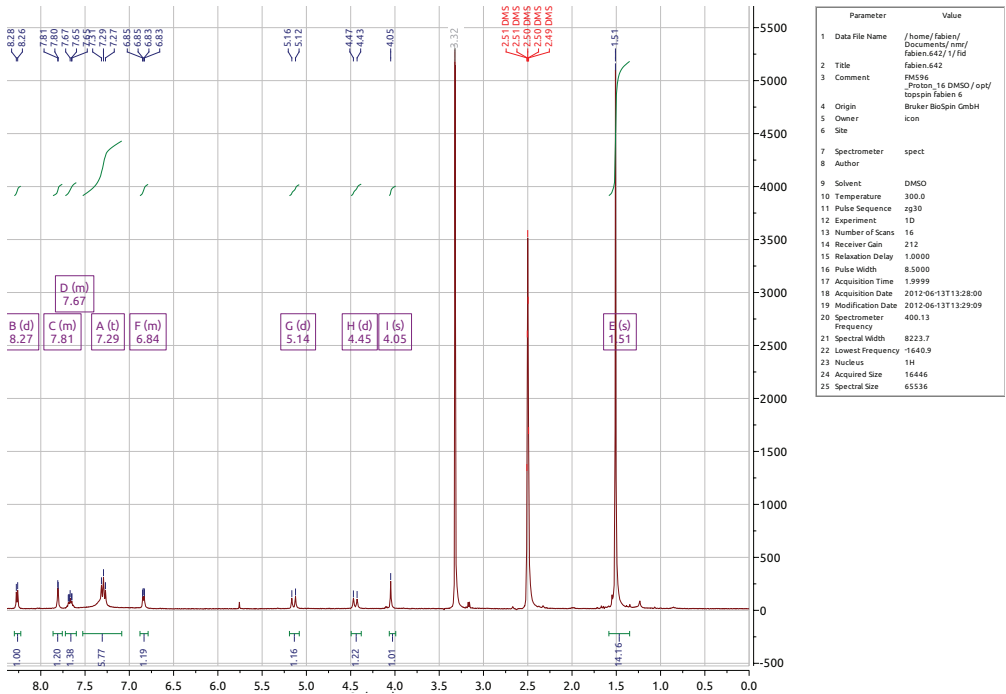
^1H NMR (400 MHz, $\text{DMSO}-d_6$, 20 °C, δ): 8.27 (d, $J = 6.1$ Hz, 1H, H^4), 7.81 (m, 1H, H^9), 7.67 (m, 1H, H^6), 7.29 (b, 6H, $\text{H}^{2,3,8}$), 6.84 (d, $J = 6.8$ Hz, 1H, H^5), 5.14 (d, $J = 16.7$ Hz, 1H, H^7), 4.45 (d, $J = 16.6$ Hz, 1H, $\text{H}^{7'}$), 1.51 (s, 15H, H^{10}).

^{19}F NMR (376 MHz, $\text{DMSO}-d_6$, 20 °C, δ): 106.8.

MS (ESI-MS, pos.) m/z : $[\text{M}-\text{Cl}]^+$ calcd for $\text{C}_{28}\text{H}_{29}\text{ClF}_2\text{IrN}_3\text{O}_4\text{S}_2$, 766.1; found, 766.1.

EA Anal. calcd for $\text{C}_{28}\text{H}_{29}\text{ClF}_2\text{IrN}_3\text{O}_4\text{S}_2 \cdot 5\text{H}_2\text{O} \cdot 0.9\text{K}_2\text{CO}_3$: C, 34.17%; H, 3.87%; N, 4.14%.

Found: C, 33.82%; H, 3.52%; N, 4.08%.



Biology

Expression of human carbonic anhydrase II in *Escherichia coli*

Plasmid encoding human carbonic anhydrase isozyme II (hCAII) and containing a T7 RNA polymerase promoter and an ampicillin resistance gene (pACA) was a generous gift from Carol Fierke, Michigan University.^{S5} The construct of this plasmid has a serine residue at position 2 instead of an alanine, with no effect on protein expression or catalytic properties. A 50 mL test tube containing 15 mL of Luria-Bertani broth (LB: 10 g/L bactotryptone, 5 g/L bacto yeast extract, and 10 g/L NaCl, containing 100 µg/mL ampicillin and 34 µg/mL chloramphenicol) was inoculated with one medium-sized colony (*E. coli* BL21(DE3)pLysS transformant). After 6 to 7 h of incubation at 37 °C at 250 rpm, it was spun down (3,000 rpm, 5 min) and the cells resuspended in 60 mL of fresh LB medium in a 125 mL Erlenmeyer baffled-flask. After overnight growth at 37 °C and 250 rpm, this pre-culture was spun down (3,000 rpm, 5 min) and the cells resuspended in 20% v/v glucose. This two steps inoculum and pre-culture method allowed to reach a high cell density to inoculate the main culture. One liter minimal medium cultures (1X M9 salts, 0.4% w/v glucose, 0.2 mL of 0.3 M ZnSO₄, and 100 µg/mL ampicillin and 34 µg/mL chloramphenicol) were carried out in 3 liters Erlenmeyer baffled-flasks, and were inoculated with the pre-culture, which was prepared as described above. The temperature was kept constant at 37 °C, until induction time. Agitation was set at 250 rpm to keep the culture broth homogeneous. Cultures were incubated for 3 hours (or until OD₆₀₀ = 0.8 – 1.0), and were induced by addition of 250 µM IPTG and 450 µM ZnSO₄. The temperature of the shaker was set to 25 °C. Three hours later, 8 µg/mL phenylmethylsulfonyl fluoride (PMSF, prepared in isopropanol) was added to the culture broth, to inhibit serine proteases growth. After additional growth for 3 h at 25 °C, cells were harvested (4,400 rpm, 4 °C for 15 min). Cells were lysed by activating the gene encoding T7 lysozyme using three cycles of “freeze/thaw”. Lysed cells were then resuspended in 25 mL lysis buffer (50 mM Tris-SO₄, pH 8.6, 50 mM NaCl, 0.5 mM ZnSO₄, and 10 µg/mL PMSF). The resuspended cells were incubated under vigorous shaking (approx. 300 rpm) at room temperature for 1 h; DNase I (1 µg/L final concentration) was added, and cells were left for another hour under the same conditions, as previously described,

until complete digestion of nucleic acids. The resuspension was centrifuged (10,000 rpm, 45 min at 4 °C). The process (resuspension in 25 mL lysis buffer and 30 min incubation) was repeated another two times to maximise the recovery of hCA II variant. Extractions were pooled and finally, the remaining pellet was deactivated and discarded. The protein was purified to homogeneity in one-step purification by sulfonamide affinity chromatography.^{S6} The column was equilibrated with activity buffer (50 mM Tris-SO₄, pH 8.6 and 0.5 mM ZnSO₄), and the enzyme was loaded onto the sulfonamide column producing agarose-bound enzyme. The column was first washed with two column volumes (CV) of activity buffer, and then with wash buffer (50 mM Na₂SO₄, 50 mM NaClO₄, and 25 mM Tris at pH 8.8) to remove any unbound contaminants. The bound enzyme was eluted by addition of 200 mM NaClO₄ and 100 mM NaCH₃COO, at pH 5.6, and 5 mL eluted fractions were collected. The purified variants were examined by SDS-PAGE, and protein concentrations were determined from the absorbance at 280 nm (extinction coefficient, $\epsilon = 50,483 \text{ cm}^{-1}\text{M}^{-1}$). This expression protocol yielded ~400 mg/L of pure protein (after lyophilisation) in 1 L working volume of culture in shake-flasks.

ESI mass spectrometry of protein

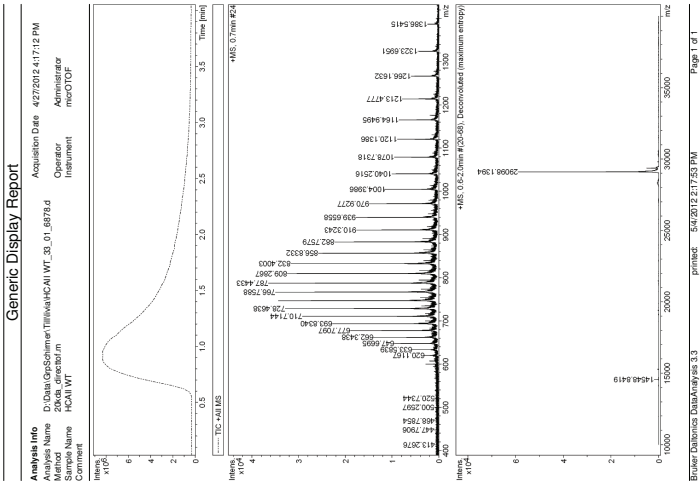


Figure S1: ESI mass spectrometry of wild type human Carbonic Anhydrase II

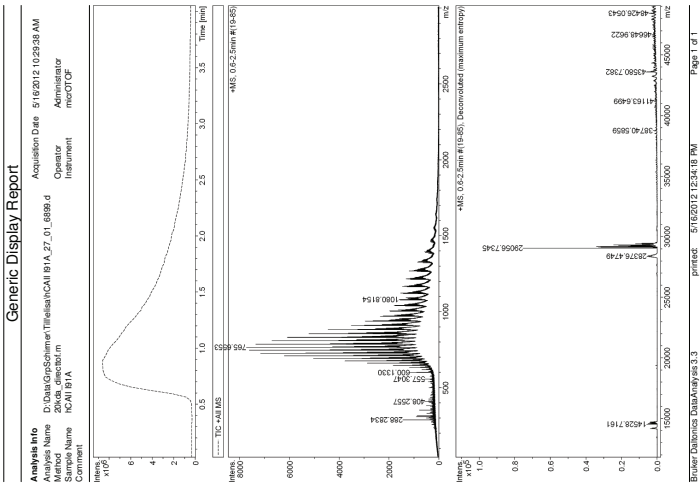


Figure S2: ESI mass spectrometry of I91A human Carbonic Anhydrase II

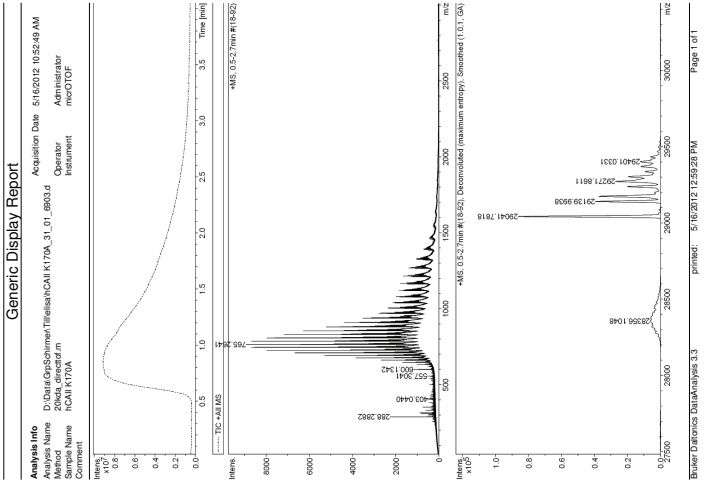


Figure S3: ESI mass spectrometry of K170A human Carbonic Anhydrase II

General procedure for hCA II inhibition profiling

Esterase activity screenig assay

All steady-state measurements were performed according the procedure previously described.^{S2,S5,S7,S8}

$$v = \frac{v_o \cdot K_i}{K_i + ([I]_t - 0.5\{([I]_t + [E]_t + K_i) - \sqrt{([I]_t + [E]_t + K_i)^2 - 4 \cdot [I]_t \cdot [E]_t}\})} \quad (1)$$

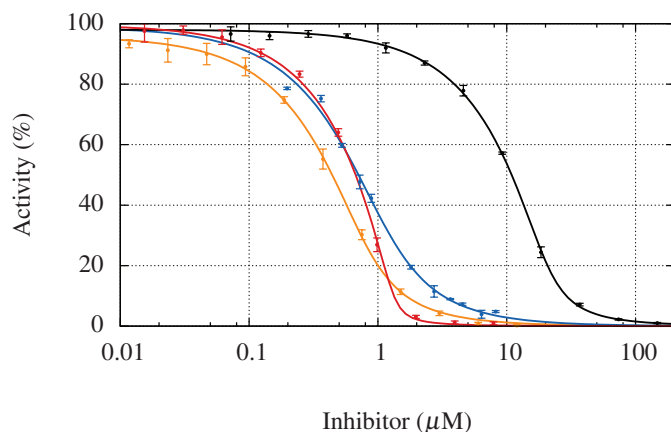


Figure S4: Steady-state kinetic data for the inhibition of hCA II iridium metal complexes. The initial rates of the enzyme-catalyzed hydrolysis of *p*-nitrophenyl acetate substrate were measured as a function of inhibitor concentration. [Enzyme] = 1 μM, [*p*-nitrophenyl acetate] = 0.5 mM. The solid, smooth lines are the best fits of the data according to equation 1 for the K_i of **6** (●), **7** (●), **8** (●), **10** (●).

Competitive displacement assay

All steady-state measurements were performed according the procedure previously described.^{S9}

$$F_{tot} = \frac{F_{obs} - F_{ini}}{F_{end} - F_{ini}} = \frac{1}{1 + (K_{DNSA}/[DNSA])(1 + [I]/K_d)} \quad (2)$$

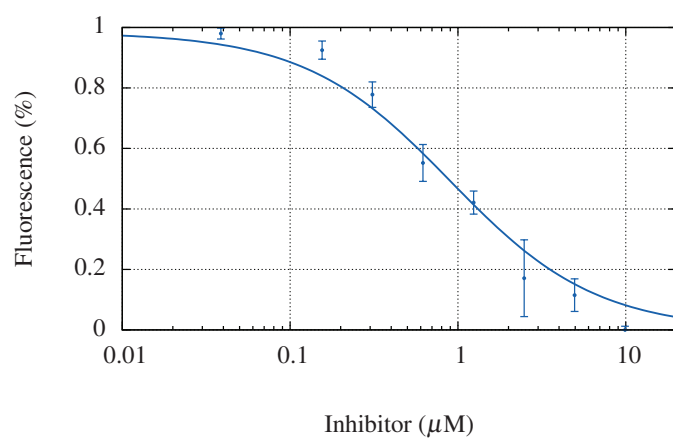


Figure S5: Competitive displacement assay data for the inhibition of WT hCA II. The initial rates of the enzyme-catalyzed hydrolysis of *p*-nitrophenyl acetate substrate were measured as a function of inhibitor concentration. [Enzyme] = 1 μ M, [*p*-nitrophenyl acetate] = 0.5 mM. The solid, smooth lines are the best fits of the data according to equation 2 for the K_d of **9** (●).

Catalysis

Lyophilized hCA II corresponding to 0.4 mM final concentration of free binding sites (~ 2.5 mg) was weighed into vials. The reaction buffer (MOPS 1.2 mM buffer, 3 M sodium formate, pH 7.5, 200 μ L) was added, and the mixture was stirred until all the protein was dissolved. The metal complex stock solution was added (final concentration 0.3 mM; 0.8 equivalents [Ru] or [Ir] vs. hCA II free binding sites), and the mixture was stirred for 30 minutes. Finally, the substrate stock solution was added (4 μ L, final concentration 20 mM). The tubes were placed in a magnetically stirred multireactor, and were heated up to 40 °C if required.^{S10}

Michaelis-Menten kinetics

Reaction setup^{S11}

Human Carbonic Anhydrase II (~ 2.5 mg, final concentration: 0.4 mM hCA II) was dissolved in a MOPS/formate solution (100 μ L, pH 7.5, 0.4 mM and 3 M respectively) and [Ir] catalyst (8.75 μ L of DMSO stock solution, final concentration: 0.35 mM [Ir]) was added. The resulting ATHase solution was mixed for 15 min at 25 °C (100 rpm).

Reactions were started by adding an appropriate volume of the substrate stock solution (100 μ L, final concentration between 150 and 1 mM) to the tubes. After 20, 40 and 60 minutes, respectively, reaction aliquots (50 μ L) were removed and added to a glutathione solution (40 μ L, 0.25 M) to stop the catalytic reaction.

Work up and analysis

Water (300 μ L) was added to aliquots, and HPLC sample were prepared by adding 200 μ L of the previous solution in water (500 μ L). Conversions were determined using an Eclipse XDB-C18 column (5 μ m, 4.6 x 150 mm) and water/MeOH/TFA 87:13:0.1 as an eluent at a flow of 1 ml/min and 25 °C (t_R 6,7-dimethoxy-1-methyl-1,2,3,4-tetrahydroisoquinoline = 11.9 min, t_R 1-methyl-6,7-dimethoxy-3,4-dihydroisoquinoline = 16.3 min,). The response factor used for the conversion determination is 1.26 at 280 nm.

X-ray structure

Crystallization

Crystals of ligand-free carbonic anhydrase II were obtained by hanging drop vapor diffusion technique. A volume of 3.2 μL of crystallization buffer (2.6 M $(\text{NH}_4)_2\text{SO}_4$, 50 mM Tris- SO_4 , pH 7.9) was mixed with 0.8 μL of protein solution (20 mg/mL human Carbonic Anhydrase II, 50 mM Tris- SO_4 , 1 mM 4-chloromercuribenzoic acid, pH 7.9). The droplet was equilibrated against a reservoir of 500 μL crystallization buffer at 293 K. Protein crystals grew within 24 hours. For the subsequent ligand soaking, ligand-free Carbonic Anhydrase II crystals were cross-linked in an atmosphere of 25% aqueous glutaraldehyde for 15 minutes using hanging-drop vapor diffusion technique as described by Lusty *et al.*^{S12} Cross-linked crystals were transferred into a ligand solution (0.1 mM **9**, 0.5% DMSO, 2.6 M $(\text{NH}_4)_2\text{SO}_4$, 50 mM Tris- SO_4 , pH 7.9) and soaked for 1 hour at 293 K. Soaked crystals were shock-frozen in liquid nitrogen.

Data Collection and Structure Solution

Diffraction data of soaked hCA II crystals were collected at the Swiss Light Source (SLS) beam line PXIII at a wavelength of 1.0000 Å and a temperature of 100 K to a resolution of 1.31 Å. The reflections were indexed, processed and scaled using software XDS and XSCALA.^{S13} (Table S1) The structure was solved by molecular replacement (software PHASER)^{S14} using as a model the structure with PDB code 3PKY devoid of all ligand and water molecules. Software phenix.refine (PHENIX package)^{S15} was used for the structure refinement. Geometry restraints for complex **9** were obtained from a related structure in the Cambridge Structural Database (CSD code SIFLOJ). Coordinates and restraints were adapted using program REEL (PHENIX package).^{S15} Electron densities and structures were visualized using software COOT.^{S16} Figures were drawn with PYMOL.^{S17}

Table S1: Data Processing and refinement of crystal $[(\eta^5\text{-Cp}^*)\text{Ir}(\text{pico } \mathbf{4})\text{Cl}] \mathbf{9} \subset \text{hCA II}$.

Data Processing	
Resolution ($\text{\AA}$)	40.0 - 1.3
Space Group	$P2_1$
Cell dimensions	$a=42.5 \text{ \AA}$, $b=41.6 \text{ \AA}$, $c=72.5 \text{ \AA}$, $\beta=103.8^\circ$
R_{merge} (%)	7.8 (126.0)*
No. unique reflections	49267
Multiplicity	3.2 (2.9)
Completeness	82.9 (43.2)
$I/\sigma(I)$	9.3 (1.3)
CC (1/2)	99.8 (51.4)
Structure Refinement	
$R_{\text{work}}/R_{\text{free}}$	0.168/0.193
B-factors ($\text{\AA}^2$)	
Protein	18.6
Ligand (9TH)	27.1
Water	32.2
R.m.s. deviations	
Bond lengths ($\text{\AA}$)	0.018
Bond angles ($^\circ$)	1.790

* values in parenthesis are for the highest resolution bin (1.38-1.31 $\text{\AA}$)

Overall structure, 4-chloromercuribenzoic acid and water binding sites

The molecular replacement procedure yielded one molecule of hCA II in the asymmetric unit of the monoclinic crystal. As observed previously,^{S2} the first two C-terminal residues could not be refined, probably due to the high flexibility of the C-terminus. In close proximity to Cys206 strong signals of difference density were detected in the F_o-F_c omit map (30 σ) and in the anomalous difference density map (20 σ). In analogy to a structure solved earlier,^{S10} the 4-chloromercuribenzoic acid of the buffer solution was modeled in this position. Finally, a total of 167 water and two glycerol molecules were modeled in the structure.

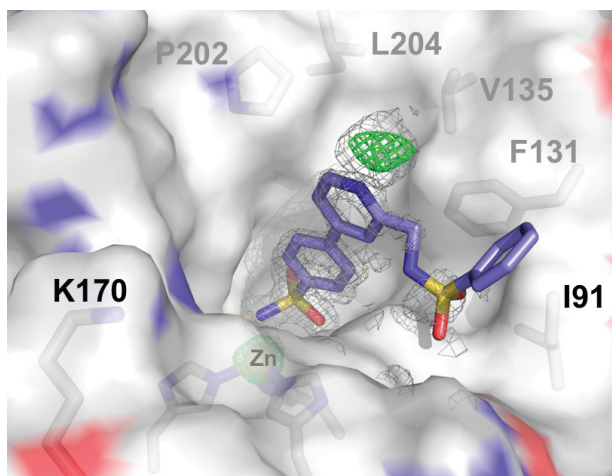


Figure S6: Close up view of the proposed *anticlinal* ligand conformation in the iridium-free ATHase **9** $\subset$ hCA II. The electron density of the F_o-F_c omit map (gray color) and the anomalous difference density (green color) are contoured at 3 σ and 4 σ , respectively (as also shown in Figure 3).

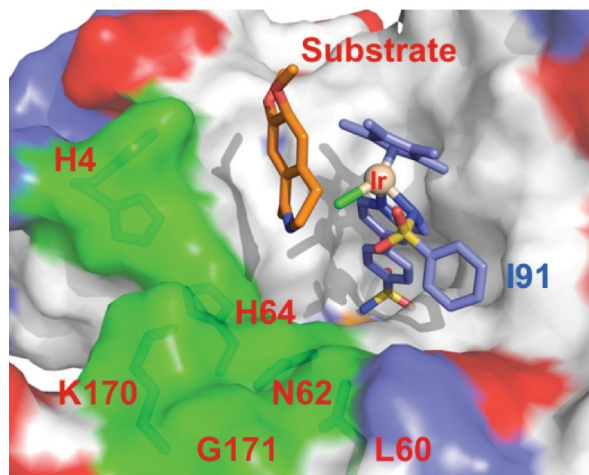


Figure S7: Qualitative model of substrate (orange) binding to ATHase **9** $\subset$ hCA II based on the X-ray structure. Residues of the presumed substrate-binding site that are potential targets for genetic optimization are colored in green.

References

- (S1) Williams, T.; Kelley, C.; many others, Gnuplot 4.4: an interactive plotting program. <http://gnuplot.sourceforge.net/>, 2010.
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