Supplemental Information for:

Deuterated oxazines are bright near-infrared fluorophores for mitochondrial imaging and single molecule spectroscopy

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1. General

All chemical reagents and anhydrous solvents for synthesis were purchased from commercial suppliers (Sigma-Aldrich, Fluka, Acros, Fluorochem, TCI) and were used without further purification or distillation. If necessary, solvents were degassed either by freeze-pump-thaw or by bubbling N_2 through the vigorously stirred solution for several minutes.

NMR spectra were recorded at 300 K in deuterated solvents on a Bruker AVANCE III HD 400 equipped with a CryoProbe or on Bruker AV-III spectrometers using either a cryogenically cooled 5 mm TCI-triple resonance probe equipped with one-axis self-shielded gradients or room-temperature 5 mm broadband probe and calibrated to residual solvent peaks ($^{1}H/^{13}C$ in ppm): DMSO-d_6 (2.50/39.52), MeOD-d_4 (3.31/49.00), $CDCl_3$ (7.26/77.16). Multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, br = broad, m = multiplet. Coupling constants *J* are reported in Hz. NOTE: Spectra are reported based on appearance, not on theoretical multiplicities derived from structural information.

LC-MS were performed on an Agilent 1260 Infinity II LC System equipped with Agilent SB-C18 column (1.8 µm, 2.1 × 50 mm). Buffer A: 0.1% FA in H_2O Buffer B: 0.1% FA acetonitrile. The typical gradient was *Method A*: from 10% B for 0.5 min → gradient to 95% B over 5 min → 95% B for 0.5 min → gradient to 99% B over 1 min with 0.6 mL/min flow. *Method B*: from 30% B for 0.5 min → gradient to 95% B over 5 min → 95% B for 0.5 min → gradient to 99% B over 1 min with 0.6 mL/min flow. *Method C*: from 50% B for 0.5 min → gradient to 95% B over 5 min → 95% B for 0.5 min → gradient to 99% B over 1 min with 0.6 mL/min flow. Retention times (t_R) are given in minutes (min). Chromatograms were imported into Graphpad Prism8 and purity was determined by calculating AUC ratios.

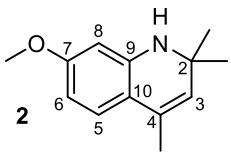
High resolution ESI-MS spectra were recorded on a *Waters H-class* instrument equipped with a quaternary solvent manager, a *Waters* column manager with an *Acquity* UPLC BEH C18 (1.7 µm, 2.1 mm x 50 mm) RP column. Samples were eluted with a flow rate of 0.3 mL/min using the following gradient: Buffer A: 0.01% FA in H_2O and Buffer B: 0.01% FA in MeCN. 5% B 0-1 min, 5 to 95% B 1-7 min. 95% B 7-8.5 min. Mass analysis was conducted with a *Waters XEVO G2-XS* QTof analyzer.

Preparative or semi-preparative HPLC were performed an Agilent 1260 Infinity II LC System equipped with columns as followed: preparative column –Reprospher 100 C18 columns (10 µm: 50 x 30 mm at 20 mL/min flow rate; semi-preparative column – 5 µm: 250 x 10 mm at 4 mL/min flow rate. Eluents A (0.1% TFA in H_2O) and B (0.1% TFA in MeCN) were applied as a linear gradient. Peak detection was performed at maximal absorbance wavelength.

Flash column chromatography (FCC) was performed on a *CombiFlash® nextgen 300+* with pre-packed silica columns (0.040–0.063 mm, *Macherey-Nagel*). Reactions and chromatography fractions were monitored by thin layer chromatography (TLC) on *Macherey-Nagel* on silica 60 with UV_{254} fluorescent indicator. The spots were visualized either under UV light at 254 nm and/or 366 nm or with appropriate staining method (iodine, *para*-anisaldehyde, $KMnO_4$) followed by heating.

2. Synthesis

2.1 1,2-Dihydro-7-methoxy-2,2,4-trimethyl-quinoline (2)



2

$C_{13}H_{17}NO$
$M = 203.3\ g.mol^{-1}$

Under N_2 atmosphere, in a flame dried round bottom flask ytterbium (III)-trifluoromethansulfonate (1.30 g, 2.10 mmol, 20 mol%) was dried *in vacuo*. The dried solid was added to a solution of *m*-anisidine (**1**) (2.60 g, 21.0 mmol, 1.0 equiv.) in acetone (80 mL). The yellow mixture was stirred at r.t. during 24h. The resulting reaction mixture was concentrated under vacuum. The dark yellow, oily residue obtained was taken up in EtOAc (100 mL), washed with water (70 mL), dried with brine (70 mL) and over $MgSO_4$. Then, finally purified by flash column chromatography (40 g, gradient from Cy to Cy/EtOAc 50:50). The desired product (**2**) was obtained (2.78 g, 13.7 mmol, $\eta = 65\%$) as a white solid.

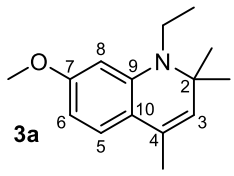
R_f(Cy/EtOAc, 9:1): 0.56

^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm] = 6.99 (d, *J* = 8.4 Hz, 1H, H-**5**), 6.24 (d, *J* = 6.8 Hz, 1H, H-**6**), 6.14 (s, 1H, H-**8**), 5.21 (s, 1H, H-**3**), 3.75 (s, 3H, OCH_3), 1.97 (d, *J* = 1.2 Hz, 3H, CH_3), 1.30 (s, 6H, C(CH_3)$_2$).

HRMS (ESI): *m/z* calculated for $C_{13}H_{18}NO^+$ $[M+H]^+$: 204.1383, found: 204.1376.

^{1}H NMR was in accordance with literature.[1]

2.2 1-Ethyl-1,2-dihydro-7-methoxy-2,2,4-trimethyl-quinoline (3a)



$C_{15}H_{21}NO$
M = 231.3 g.mol^{-1}

Under N_2 atmosphere, in a flame dried tube, ethyl iodide (100 µL, 1.18 mmol, 1.2 equiv.) was added dropwise at r.t to a suspension of compound (**2**) (200 mg, 980 µmol, 1.0 equiv.) and K_2CO_3 (136 mg, 980 µmol, 1.0 equiv.) in anhydrous DMF (800 µL). The yellow reaction mixture was sealed and stirred at 100 °C during 14h. The green solution obtained was cooled down to r.t. and concentrated under high vacuum. The crude residue was then diluted with a mixture of EtOAc (10 mL) and water (5 mL), and the aqueous phase was extracted with EtOAc (5×5 mL). The combined organic layers were washed with brine and dried over $MgSO_4$. All volatiles were removed using a rotary evaporator and the resulting dark yellow oil was purified by flash column chromatography with silica gel (15 g, gradient from Cy to Cy/EtOAc 80:20). The alkylated compound (**3a**) was obtained as a colorless oil (140 mg, 605 µmol, η = 62%).

R_f(Cy/EtOAc, 95:5): 0.67

^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm] = 6.96 (d, *J* = 8.2 Hz, 1H, H-**5**), 6.13 (dd, *J* = 8.1 Hz, *J* = 1.9 Hz, 1H, H-**6**), 6.07 (d, *J* = 1.9 Hz, 1H, H-**8**), 5.09 (s, 1H, H-**3**), 3.79 (s, 3H, OC*H*$_3$), 3.30 (q, *J* = 7.0 Hz, 2H, C*H*$_2$CH$_3$), 1.94 (s, 3H, CC*H*$_3$), 1.30 (s, 6H, C(C*H*$_3$)$_2$), 1.20 (t, *J* = 7.0 Hz, CH$_2$C*H*$_3$).

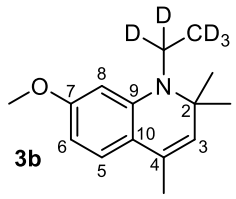
^{13}C NMR (150 MHz, $CDCl_3$): δ [ppm] = 160.7 (Cq, C-**7**), 145.1 (Cq, C-**9**), 127.5 (Cq, C-**4**), 127.15 (CH, C-**3**), 124.6 (CH, C-**5**), 116.7 (Cq, C-**10**), 98.9 (CH, C-**6**), 97.65 (CH, C-**8**), 57.0 (Cq, C-**2**), 55.25 (CH$_3$, O*C*H$_3$), 38.3 (CH$_2$, N*C*H$_2$CH$_3$), 28.8 (CH$_3$, C(*C*H$_3$)$_2$), 18.9 (CH$_3$, C*C*H$_3$), 14.4 (CH$_3$, NCH$_2$*C*H$_3$).

HRMS (ESI): *m/z* calculated for $C_{15}H_{22}NO^+$ $[M+H]^+$: 232.1696, found: 232.1691.

LC-MS (*Method C*) calculated for $C_{15}H_{22}NO^+$ $[M+H]^+$: 232.2, found 232.1; $\mathbf{t_R}$ = 4.00 min.

Analyses were in accordance with the literature.[2]

2.3 d_5-1-Ethyl-1,2-dihydro-7-methoxy-2,2,4-trimethyl-quinoline (3b)



$C_{15}H_{16}D_5NO$
M = 236.2 g.mol^{-1}

Under N_2 atmosphere, in a flame dried tube, bromethan-d_5 (235 µL, 3.15 mmol, 1.6 equiv.) was added dropwise to a suspension of 1,2-dihydro-7-methoxy-2,2,4-trimethyl-quinoline (**2**) (401 mg, 1.97 mmol, 1.0 equiv.) and K_2CO_3 (273 mg, 1.97 mmol, 1.0 equiv.) in anhydrous DMF (1.60 mL). The yellow reaction mixture was sealed then stirred at 100 °C during 24 h. The dark green solution obtained was cooled down to r.t. and concentrated under high vacuum. The crude residue was then diluted with a mixture of EtOAc (15 mL) and water (10 mL). The aqueous phase was extracted with EtOAc (5×5 mL). The combined organic layers were dried over $MgSO_4$. The solvent was removed using a rotary evaporator and the resulting dark brown oil was purified by flash column chromatography (15 g SiO_2, gradient from PE to PE/Et_2O 95:5) and the alkylated product (**3b**) (212 mg, 878 µmol, η = 46%) was obtained as a pale-yellow oil.

R_f(PE/Et_2O, 95:5): 0.59; (Cy/EtOAc, 99:1): 0.46

^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm] = 6.96 (d, *J* = 8.3 Hz, 1H, H-**5**), 6.135 (dd, *J* = 8.5 Hz, *J* = 2.5 Hz, 1H, H-**6**), 6.06 (d, *J* = 2.3 Hz, 1H, H-**8**), 5.09 (s, 1H, H-**3**), 3.79 (s, 3H, OC*H*$_3$), 1.94 (s, 3H, CC*H*$_3$), 1.30 (s, 6H, C(C*H*$_3$)$_2$).

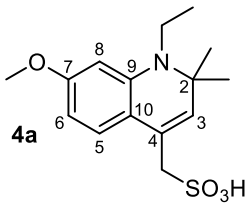
^{13}C NMR (150 MHz, $CDCl_3$): δ [ppm] = 160.7 (Cq, C-**7**), 145.2 (Cq, C-**9**), 127.5 (Cq, C-**4**), 127.1 (CH, C-**3**), 124.6 (CH, C-**5**), 116.7 (Cq, C-**10**), 98.9 (CH, C-**6**), 97.6 (CH, C-**8**), 57.0 (Cq, C-**2**), 55.2 (CH_3, O*C*H$_3$), 28.8 (CH_3, C(*C*H$_3$)$_2$), 18.9 (CH_3, C*C*H$_3$).

HRMS (ESI): *m/z* calculated for $C_{15}H_{17}D_5NO^+$ $[M+H]^+$: 237.2010, found: 237.2031.

LC-MS (*Method C*) calculated for $C_{15}H_{17}D_5NO^+$ $[M+H]^+$: 237.2, found 237.2; $\mathbf{t_R}$ = 4.20 min.

NOTE: A second fraction was collected after FCC, and starting material was recovered (174 mg, ~40 % conversion).

2.4 (1-Ethyl-7-methoxy-2,2-dimethyl-1,2-dihydroquinolin-4-yl)methanesulfonic acid (4a)



$C_{15}H_{21}NO_4S$
M = 311.4 g.mol^{-1}

In a round bottom flask, at 0 ºC, 1-ethyl-1,2-dihydro-7-methoxy-2,2,4-trimethyl-quinoline (**3a**) (50 mg, 216 µmol, 1 equiv.) was dissolved in a mixture of sulfuric acid and fuming sulfuric acid (400 µL, 4:1 v/v), and stirred at r.t. during 20h. The reaction was carefully poured onto ice in Erlenmeyer cooled in an ice bath then quenched with an aqueous solution of NaOH (1M) until pH 12 was reached. Unreacted starting material was removed by extraction with chloroform (3 × 10 mL). The aqueous phase was lyophilized the residue obtained was suspended in ethanol filtered to separate the product from the sodium sulfate salt. The filtrate was concentrated and purified by flash column chromatography (15 g SiO_2, gradient from DCM to DCM/MeOH 8:2) and the expected sulfonated compound (**4a**) was (46 mg, 148 µmol, η = 67%) as a colorless oil.

R$_f$(DCM/MeOH, 8:2): 0.20

^{1}H NMR (600 MHz, DMSO-d6): δ [ppm] = 7.18 (d, *J* = 8.4 Hz, 1H, H-**5**), 6.05 (dd, *J* = 8.5 Hz, *J* = 2.4 Hz, 1H, H-**6**), 5.95 (d, *J* = 2.1 Hz, 1H, H-**8**), 5.29 (s, 1H, H-**3**), 3.69 (s, 3H, OC*H*$_3$), 3.41 (s, 2H, C*H*$_2$SO$_3$H), 3.27 (q, *J* = 7.0 Hz, 2H, C*H*$_2$CH$_3$), 1.25 (s, 6H, C(C*H*$_3$)$_2$), 1.11 (t, *J* = 6.9 Hz, 1H, CH$_2$C*H*$_3$).

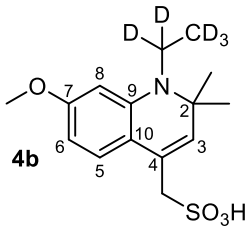
HRMS (ESI): *m/z* calculated for $C_{15}H_{22}NO_4S^+$ $[M+H]^+$: 312.1264, found: 312.1252.

LC-MS (*Method C*) calculated for $[M+H]^+$: 312.1, found 312.2; $\mathbf{t_R}$ = 4.40 min.

Analyses were in accordance with the literature.[3]

NOTE: The organic layer was concentrated then purified by HPLC (from 10 to 95% MeCN/H_2O, linear gradient, with constant 0.1% v/v TFA additive). Starting material (conversion >80%) was recovered and another fraction of compound (**4a**) was isolated (1 mg, 3.21 µmol, η = 1 %).

2.5 (d_5-1-Ethyl-7-methoxy-2,2-dimethyl-1,2-dihydroquinolin-4-yl)methanesulfonic acid (4b)



$C_{15}H_{16}D_5NO_4S$
M = 316.4 g.mol^{-1}

In a round bottom flask, at 0 ºC, 1-ethyl-1,2-dihydro-7-methoxy-2,2,4-trimethyl-quinoline (**3a**) (37 mg, 157 µmol, 1 equiv.) was dissolved in a mixture of sulfuric acid and fuming sulfuric acid (600 µL, 4:1 v/v), and stirred at r.t. during 20h. The reaction was carefully poured onto ice cooled in an ice bath and quenched with an aqueous solution of NaOH (1M) until pH 12 was reached. The aqueous phase was lyophilized the residue obtained was suspended in ethanol filtered to separate the product from the sodium sulfate salt. The filtrate was concentrated and purified by flash column chromatography (25 g SiO_2, gradient from DCM to DCM/MeOH 8:2) and the expected sulfonated compound (**4a**) was (20 mg, 63.2 µmol, η = 66%) as a colorless oil.

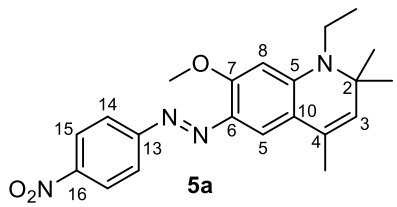
R$_f$ (DCM/MeOH, 7:3): 0.40

^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm] = 7.16 (d, *J* = 8.4 Hz, 1H, H-**5**), 6.04 (dd, *J* = 8.4, *J* = 2.4 Hz, 1H, H-**6**), 5.93 (d, *J* = 2.1 Hz, 1H, H-**8**), 5.29 (s, 1H, H-**3**), 3.68 (s, 3H, OC*H*$_3$), 3.43 (s, 2H, CH_2, C*H*$_2$SO$_3$H), 1.24 (s, 6H, C(C*H*$_3$)$_2$).

^{13}C NMR (150 MHz, $CDCl_3$): δ [ppm] = 159.8 (Cq, C-**7**), 144.6 (Cq, C-**9**), 130.0 (CH, C-**3**), 125.9 (CH, C-**5**), 125.3 (Cq, C-**4**), 115.3 (Cq, C-**10**), 99.0 (CH, C-**6**), 96.6 (CH, C-**8**), 56.4 (Cq, C-**2**), 54.7 (CH_3, O*C*H$_3$), 54.0 (CH_2, *C*H$_2$SO$_3$H), 28.2 (CH_3, C(*C*H$_3$)$_2$).

LC-MS (*Method A*) calculated for $C_{15}H_{17}D_5NO_4S^+$ $[M+H]^+$: 317.1, found 317.2; **t$_R$** = 1.90 min.

2.6 (*E*)-Ethyl-7-methoxy-2,2,4-trimethyl-6-((4-nitrophenyl)diazinyl)-1,2-dihydroquinoline (5a)



5a

$C_{21}H_{24}N_4O_3$
M = 380.4 g.mol^{-1}

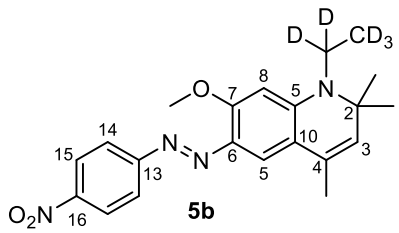
A solution of 1-ethyl-1,2-dihydro-7-methoxy-2,2,4-trimethyl-quinoline (**3a**) (61 mg, 260 µmol, 1.0 equiv.) in MeOH (5 mL) was added on a suspension of *p*-nitrobenzenediazonium tetrafluoroborate (63 mg, 270 µmol, 1.0 equiv.) in an aqueous of H_2SO_4 (10 % in 5.0 mL) under vigorous stirring. The formation of a dark red precipitate was immediately observed. The reaction mixture was stirred for 1 h at r.t. The solution was carefully basified with solid K_2CO_3. The dark red precipitate was collected via vacuum filtration and washed with small portions of water. The precipitate was dried, yielding the diazene (**5a**) (82 mg, 216 µmol, η = 82%) as a purple solid. The obtained compound was used for the next step without further purification.

^{1}H NMR (600 MHz, DMSO-d_6): δ [ppm] = 8.31 (d, *J* = 9.0 Hz, 2H, H-**15**), 7.83 (d, *J* = 9.0 Hz, 2H, H-**14**), 7.52 (s, 1H, H-**5**), 6.17 (s, 1H, H-**8**), 5.47 (s, 1H, H-**3**), 4.00 (s, 3H, OC*H*$_3$), 3.61 (q, *J* = 6.8 Hz, 2H, C*H*$_2$CH$_3$), 1.94 (s, 3H, CC*H*$_3$), 1.40 (s, 6H, C(C*H*$_3$)$_2$), 1.25 (t, *J* = 7.0 Hz, 3H, CH$_2$C*H*$_3$).

^{13}C NMR (150 MHz, DMSO-d_6): δ [ppm] = 161.45 (Cq, C-**7**), 156.9 (Cq, C-**16**), 150.6 (Cq, C-**6**), 145.8 (Cq, C-**13**), 132.4 (Cq, C-**9**), 129.6 (CH, C-**3**), 125.35 (Cq, C-**4**), 125.0 (CH, C-**15**), 121.9 (CH, C-**14**), 115.9 (Cq, C-**10**), 111.2 (CH, C-**5**), 93.3 (CH, C-**8**), 58.9 (Cq, C-**2**), 56.1 (CH$_3$, O*C*H$_3$), 38.8 (CH$_2$, N*C*H$_2$CH$_3$), 29.0 (CH$_3$, C(*C*H$_3$)$_2$), 18.2 (CH$_3$, C*C*H$_3$), 13.65 (CH$_3$, NCH$_2$*C*H$_3$).

LC-MS (*Method C*) calculated for $C_{21}H_{25}N_4O_3^+$ $[M+H]^+$: 381.2, found 381.2; $\mathbf{t_R}$ = 3.78 min.

2.7 d_5-(*E*)-Ethyl-7-methoxy-2,2,4-trimethyl-6-((4-nitrophenyl)diazinyl)-1,2-dihydroquinoline (5b)



5b

$C_{21}H_{19}D_5N_4O_3$
M = 385.2 g.mol^{-1}

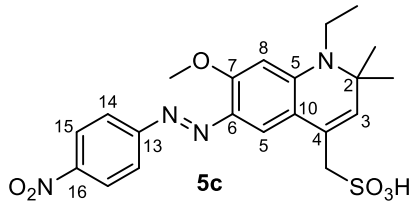
A solution of d_5-1-ethyl-1,2-dihydro-7-methoxy-2,2,4-trimethyl-quinoline (**3b**) (63 mg, 270 µmol, 1.0 equiv.) in MeOH (6.0 mL) was added on a suspension of *p*-nitrobenzenediazonium tetrafluoroborate (65 mg, 280 µmol, 1.0 equiv.) in an aqueous solution of H_2SO_4 (10 % in 5.0 mL) under vigorous stirring. The formation of a dark red precipitate was immediately observed. The reaction mixture was stirred for 1 h at r.t. Then the solution was carefully basified with solid K_2CO_3. The dark red precipitate was collected via vacuum filtration and washed with small portions of water. The precipate was dried, yielding the deuterated diazene (**5b**) (82 mg, 213 µmol, η = 78%) as a purple solid. The obtained compound was used for the next step without further purification.

^{1}H NMR (600 MHz, DMSO-d_6): δ [ppm] = 8.31 (d, *J* = 8.95 Hz, 2H, H-**15**), 7.83 (d, *J* = 8.9 Hz 2H, H-**14**), 7.52 (s, 1H, H-**5**), 6.16 (s, 1H, H-**8**), 5.43 (br s, 1H, H-**3**), 3.99 (s, 3H, OC*H*$_3$), 1.92 (s, 3H, CC*H*$_3$), 1.38 (s, 6H, C(C*H*$_3$)$_2$).

^{13}C NMR (150 MHz, DMSO-d_6): δ [ppm] = 161.25 (Cq, C-**7**), 157.2 (Cq, C-**16**), 150.1 (Cq, C-**6**), 145.8 (Cq, C-**13**), 132.4 (Cq, C-**9**), 129.3 (CH, C-**3**), 125.3 (Cq, C-**4**), 124.9 (CH, C-**15**), 122.1 (CH, C-**14**), 115.45 (Cq, C-**10**), 111.0 (CH, C-**5**), 93.2 (CH, C-**8**), 58.6 (Cq, C-**2**), 56.0 (CH_3, O*C*H$_3$), 29.0 (CH_3, C(*C*H$_3$)$_2$), 18.2 (CH_3, C*C*H$_3$).

LC-MS (*Method C*) calculated for $C_{21}H_{20}D_5N_4O_3^+$ $[M+H]^+$: 386.2, found 386.2; $\mathbf{t_R}$ = 3.73 min.

2.8 (*E*)-(1-Ethyl-7-methoxy-2,2-dimethyl-6-((4-nitrophenyl)diazenyl)-1,2-dihydroquinolin-4-yl)methanesulfonic acid (5c)



$C_{21}H_{24}N_4O_6S$
M = 460.1 g.mol^{-1}

A solution of (1-ethyl-7-methoxy-2,2-dimethyl-1,2-dihydroquinolin-4-yl)methanesulfonic acid (**4a**) (20 mg, 87.0 µmol, 1.0 equiv.) in MeOH (2.0 mL) was added on a suspension of *p*-nitrobenzenediazonium tetrafluoroborate (20.8 mg, 87.0 mmol, 1.0 equiv.) in an aqueous solution of H_2SO_4 (10 % in 5.0 mL) under vigorous stirring. The formation of a dark red precipitate was immediately observed. The reaction mixture was stirred for 1 h at r.t. Then the solution was carefully basified with solid K_2CO_3. The dark red precipitate was collected via vacuum filtration and washed with small portions of water. The precipate was dried, yielding the deuterated diazene (**5b**) (37 mg, 80.4 mmol, η = 93%) as a purple solid. The obtained compound was used for the next step without further purification.

R*f*(DCM/MeOH, 8:2): 0.65

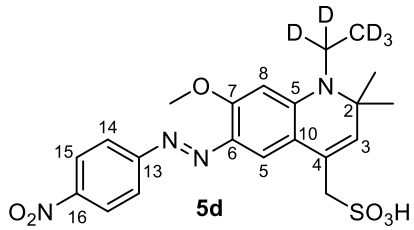
^{1}H NMR (600 MHz, DMSO-d_6): δ [ppm] = 8.30 (d, *J* = 9.0 Hz, 1H, H-**15**), 7.84 (d, *J* = 9.0 Hz, 1H, H-**14**), 7.77 (s, 1H, H-**5**), 6.16 (s, 1H, H-**8**), 5.60 (s, 1H, H-**3**), 3.98 (s, 3H, OC*H*$_3$), 3.59 (q, *J* = 7.0 Hz, 2H, NC*H*$_2$CH$_3$), 3.47 (s, 2H, C*H*$_2$SO$_3$H), 1.39 (s, 6H, (CH$_3$)$_2$), 1.25 (q, *J* = 6.8 Hz, 3H, NCH$_2$C*H*$_3$).

^{13}C NMR (150 MHz, DMSO-d_6): δ [ppm] = 160.9 (Cq, C-**7**), 157.3 (Cq, C-**16**), 150.5 (Cq, C-**6**), 145.7 (Cq, C-**13**), 132.5 (Cq, C-**9**), 132.0 (CH, C-**3**), 124.8 (CH, C-**15**), 124.0 (Cq), 122.1 (CH, C-**14**), 114.9 (Cq), 112.6 (CH, C-**5**), 93.2 (CH, C-**8**), 58.5 (Cq, C-**2**), 55.9 (CH$_3$, O*C*H$_3$), 52.9 (CH$_2$, *C*H$_2$SO$_3$H), 38.6 (CH$_2$, N*C*H$_2$CH$_3$), 28.9 (CH$_3$, C(*C*H$_3$)$_2$), 13.6 (CH$_3$, NCH$_2$*C*H$_3$).

HRMS (ESI): *m/z* calculated for $C_{21}H_{25}N_4O_6S^+$ $[M+H]^+$: 461.1489, found: 461.1485.

LC-MS (*Method A*) calculated for $C_{21}H_{25}N_4O_6S^+$ $[M+H]^+$: 461.3, found 461.3; $\mathbf{t_R}$ = 4.36 min.

2.9 (*E*)-(1-(Ethyl-d5)-7-methoxy-2,2-dimethyl-6-((4-nitrophenyl)diazenyl)-1,2-dihydroquinolin-4-yl)methanesulfonic acid (5d)



$C_{21}H_{19}D_5N_4O_6S$
M = 465.2 g.mol^{-1}

A solution of (d_5-1-ethyl-7-methoxy-2,2-dimethyl-1,2-dihydroquinolin-4-yl)methanesulfonic acid (**4b**) (12.5 mg, 52.9 µmol, 1.0 equiv.) in MeOH (6.0 mL) was added on a suspension of *p*-nitrobenzenediazonium tetrafluoroborate (12.5 mg, 52.9 mmol, 1.0 equiv.) in an aqueous solution of H_2SO_4 (10 % in 5.0 mL) under vigorous stirring. The formation of a dark red precipitate was immediately observed. The reaction mixture was stirred for 1 h at r.t. Then the solution was carefully basified with solid K_2CO_3. The dark red precipitate was collected via vacuum filtration and washed with small portions of water. The precipitate was dried, yielding the deuterated diazene (**5b**) (16 mg, 34.4 µmol, η = 65%) as a purple solid. The obtained compound was used for the next step without further purification.

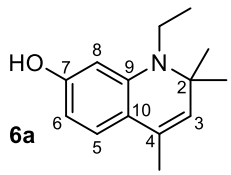
R_f(DCM/MeOH, 8:2): 0.62

^{1}H NMR (600 MHz, DMSO-d_6): δ [ppm] = 8.30 (d, *J* = 8.8 Hz, 2H, H-**15**), 7.84 (d, *J* = 8.9 Hz, 2H, H-**14**), 7.76 (s, 1H, H-**5**), 6.15 (s, 1H, H-**8**), 5.59 (s, 1H, H-**3**), 3.98 (s, 3H, OC*H*$_3$), 3.46 (s, 2H, C*H*$_2$SO$_3$H), 1.38 (s, 6H, (CH$_3$)$_2$).

HRMS (ESI): *m/z* calculated for $C_{21}H_{20}D_5N_4O_6S^+$ $[M+H]^+$: 466.1803, found: 466.1797.

LC-MS (*Method A*) calculated for $[M+H]^+$: 466.3, found 466.3; $\mathbf{t_R}$ = 4.97 min.

2.10 1-Ethyl-2,2,4-trimethyl-1,2-dihydroquinoline-7-ol (6a)



6a

$C_{14}H_{19}NO$
M = 217.3 g.mol^{-1}

Under N_2 atmosphere, in a flame dried round bottom flask, a solution of boron tribromide (1M) (900 µL, 900 µmol, 3.0 equiv.) was added dropwise on a solution of 1-ethyl-1,2-dihydro-7-methoxy-2,2,4-trimethyl-quinoline (**3a**) (70 mg, 300 µmol, 1.0 equiv.) was dissolved in dry DCM (2.0 mL) and under inert conditions. The brown reaction mixture was stirred at r.t. for 2.5 h. Afterwards the mixture was diluted with DCM (8.0 mL) and cooled down with an ice bath. The cooled solution was quenched with a sat. $NaHCO_3$ solution (8.0 mL). The organic layer was washed with H_2O (2×10 mL), brine solution (2×10 mL) and finally dried over $MgSO_4$. The combined organic layers were concentrated *in vacuo* yielding a dark brown solid. The obtained crude product was purified by flash column chromatography (15 g SiO_2, gradient from Cy to Cy/EtOAc 80:20). The demethylated product (**6a**) (48 mg, 225 µmol, η = 73%) was obtained as a brown oil.

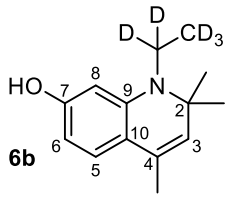
R_f(Cy/EtOAc, 80:20): 0.47

^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm] = 6.89 (d, *J* = 8.1 Hz, 1H, H-**5**), 6.02-6.04 (m, 2H, H-**6** and H-**8**), 5.08 (s, 1H, H-**3**), 4.61 (br s, 1H, OH), 3.25-3.33 (m, 2H, C*H*$_2$CH$_3$), 1.93 (s, 3H, CC*H*$_3$), 1.30 (s, 6H, C(C*H*$_3$)$_2$), 1.20 (t, *J* = 7.1 Hz, -CH$_2$C*H*$_3$).

LC-MS (*Method A*) calculated for $C_{14}H_{20}NO^+$ $[M+H]^+$: 218.2, found 218.2; $\mathbf{t_R}$ = 4.80 min.

^{1}H NMR in accordance with literature.[4]

2.11 d_5-1-Ethyl-2,2,4-trimethyl-1,2-dihydroquinoline-7-ol (6b)



$C_{14}H_{14}D_5NO$
M = 222.3 g.mol^{-1}

Under N_2 atmosphere, in a flame dried round bottom flask, a solution of boron tribromide (1M) (950 µL, 950 µmol, 3.0 equiv.) was added dropwise on a solution of d_5-1-ethyl-1,2-dihydro-7-methoxy-2,2,4-trimethyl-quinoline (**3b**) (74 mg, 310 µmol, 1.0 equiv.) was dissolved in dry DCM (2.0 mL). The brown reaction mixture was stirred at r.t. for 2.5 h. Afterwards, the reaction mixture was diluted with DCM (8.0 mL) and cooled down with an ice bath. The cooled solution was quenched with a saturated $NaHCO_3$ solution (8.0 mL). The organic layer was washed with H_2O (2×10 mL), brine (2×10 mL) and finally dried over $MgSO_4$. The obtained organic solution was concentrated *in vacuo* yielding a dark brown solid. The crude product was purified by flash column chromatography (15 g SiO_2, gradient from Cy to Cy/EtOAc 80:20) yielding to the desired product (**5b**) (55 mg, 247 µmol, η = 80%) as a brown oil.

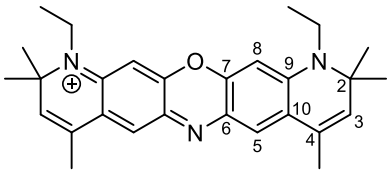
R_f(Cy/EtOAc, 80:20): 0.51

^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm] = 6.895 (d, *J* = 6.7 Hz, 1H, H-**5**), 6.04 (d, *J* = 7.0 Hz, 1H, H-**6**), 6.01 (s, 1H, H-**8**), 5.08 (s, 1H, H-**3**), 4.63 (br s, 1H, O*H*), 1.93 (s, 3H, CCH_3), 1.30 (s, 6H, C(CH_3)$_2$).

LC-MS (*Method A*) calculated for $C_{14}H_{15}D_5NO^+$ $[M+H]^+$: 223.2, found 223.2; $\mathbf{t_R}$ = 4.79 min.

2.12 1,11-Diethyl-2,2,4,8,10,10-hexamethyl-10,11-dihydro-2*H*-dipyrido-[3,2*b*:2',3'-*i*]phenoxazin-1-ium (Oxazine700-d0)

Oxazine700-d0



$C_{28}H_{34}N_3O^+$
M = 428.6 g.mol^{-1}

Under N_2 atmosphere, in a flame dried round bottom flask 1-ethyl-2,2,4-trimethyl-1,2-dihydroquinoline-7-ol (**6a**) (17.0 mg, 80.0 µmol, 1.0 equiv.) and (*E*)-ethyl-7-methoxy-2,2,4-trimethyl-6-((4-nitrophenyl) diazinyl)-1,2-dihydroquinoline (**5a**) (29.9 mg, 80.0 µmol, 1.0 equiv.) were dissolved in acetic acid (1.0 mL). The dark red reaction mixture was stirred for 2 h at 80 °C. Toluene (4×3 mL) was added, the reaction mixture was concentrated to dryness and then azeotroped. The dark blue residue was purified by flash column chromatography (15 g SiO_2, gradient from DCM to DCM/MeOH 80:20). Fractions, which contained the main product were further purified by HPLC (from 30 to 95% MeCN/H_2O, linear gradient, with constant 0.1% v/v TFA additive). The desired product (**Oxazine700-d0**) was obtained as a dark blue solid (11.0 mg, 25.7 µmol, η = 32%).

R_f(DCM/MeOH, 95:5): 0.67

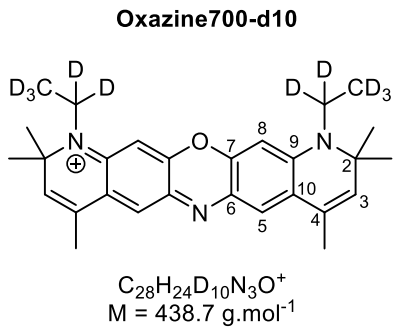
^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm] = 7.35 (s, 2H, H-**5**), 6.91 (s, 2H, H-**8**), 5.63 (s, 2H, H-**3**), 3.81 (q, *J* = 7.1 Hz, 4H, NC*H*$_2$CH$_3$), 2.08 (d, *J* = 1.1 Hz, 6H, CC*H*$_3$), 1.55 (s, 12H, C(C*H*$_3$)$_2$), 1.43 (t, *J* = 7.0 Hz, 6H, NCH$_2$C*H*$_3$).

^{13}C NMR (150 MHz, $CDCl_3$): δ [ppm] = 153.1 (Cq, C-**9**), 149.9 (Cq, C-**6**), 134.8 (Cq, C-**7**), 133.8 (CH, C-**3**), 126.5 (Cq, C-**10**), 125.9 (Cq, C-**4**), 124.8 (CH, H-**5**), 96.6 (CH, C-**8**), 61.4 (Cq, C-**2**), 41.5 (CH$_2$, N*C*H$_2$CH$_3$), 29.5 (CH$_3$, C(*C*H$_3$)$_2$), 18.7 (CH$_3$, C*C*H$_3$), 13.7 (CH$_3$, NCH$_2$*C*H$_3$).

HRMS (ESI): *m/z* calculated for $C_{28}H_{34}N_3O^+$ $[M]^+$: 428.2697, found: 428.2702.

LC-MS (*Method C*) calculated for $[M]^+$: 248.3, found 248.3; $\mathbf{t_R}$ = 4.51 min.

2.13 d_{10}-1,11-Diethyl-2,2,4,8,10,10-hexamethyl-10,11-dihydro-2*H*-dipyrido-[3,2*b*-:2', 3'-*i*]phenoxazin-1-ium (Oxazine700-d10)



$C_{28}H_{24}D_{10}N_3O^+$
M = 438.7 g.mol^{-1}

In a flame dried round bottom flask d_5-1-ethyl-2,2,4-trimethyl-1,2-dihydroquinoline-7-ol (**6b**) (19.0 mg, 85.0 µmol, 1.0 equiv.) and d_5-(*E*)-ethyl-7-methoxy-2,2,4-trimethyl-6-((4-nitrophenyl)diazinyl)-1,2-dihydroquinoline (**4b**) (33.0 mg, 85.0 µmol, 1.0 equiv.) were dissolved in acetic acid (1.0 mL) under nitrogen atmosphere. The dark red reaction mixture was stirred for 2 h at 80 °C. Acetic acid was removed by azeotroping with toluene (4×3 mL) under rotary evaporation to give a dark blue residue. The crude product was directly purified HPLC (from 30 to 95% MeCN/H_2O, linear gradient, with constant 0.1% v/v TFA additive). The desired product (**Oxazine700-d10**) was obtained as a dark blue solid (9.0 mg, 20.5 µmol, η = 24%).

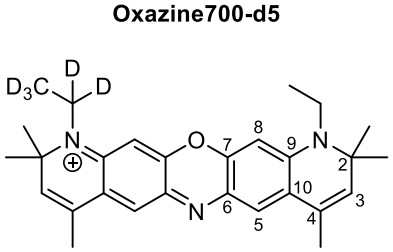
^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm] = 7.36 (s, 2H, H-**5**), 6.84 (s, 2H, H-**8**), 5.63 (s, 2H, H-**3**), 2.08 (s, 6H, CCH_3), 1.55 (s, 12H, C(CH_3)$_2$).

^{13}C NMR (150 MHz, $CDCl_3$): δ [ppm] = 152.9 (Cq, C-**9**), 149.75 (Cq, C-**6**), 134.7 (Cq, C-**7**), 133.7 (CH, C-**3**), 126.3 (Cq, C-**10**), 125.8 (Cq, C-**4**), 124.7 (CH, H-**5**), 96.4 (CH, C-**8**), 61.2 (Cq, C-**2**), 29.4 (CH_3, C(CH_3)$_2$), 18.6 (CH_3, CCH_3).

HRMS (ESI): *m/z* calculated for $C_{28}H_{24}D_{10}N_3O^+$ $[M]^+$: 438.3325, found: 438.3325.

LC-MS (*Method A*) calculated for $[M]^+$: 438.3, found 438.5; $\mathbf{t_R}$ = 3.90 min.

2.14 d_5-1,11-Diethyl-2,2,4,8,10,10-hexamethyl-10,11-dihydro-2*H*-dipyrido- [3,2-*b*:2', 3'-*i*]phenoxazin-1-ium (Oxazine700-d5)



$C_{28}H_{29}D_5N_3O^+$
M = 433.6 g.mol^{-1}

In a flame dried round bottom flask 1-ethyl-2,2,4-trimethyl-1,2-dihydroquinoline-7-ol (**6a**) (18.8 mg, 86.0 µmol, 1.0 equiv.) and d_5-(*E*)-ethyl-7-methoxy-2,2,4-trimethyl-6-((4-nitrophenyl) diazinyl)-1,2-dihydroquinoline (**4b**) (33.0 mg, 85.0 µmol, 1.0 equiv.) were dissolved in acetic acid (1.0 mL) under nitrogen atmosphere. The dark red reaction mixture was stirred for 2 h at 80 °C. Acetic acid was removed by azeotroping with toluene (4×3 mL) under rotary evaporation to give a dark blue residue. The crude product was purified by flash column chromatography (15 g SiO_2, gradient from DCM to DCM/MeOH 80:20). Fractions, which contained the main product were further purified by HPLC (from 30 to 95% MeCN/H_2O, linear gradient, with constant 0.1% v/v TFA additive). The desired product (**Oxazine700-d5**) was obtained as a dark blue solid (2.3 mg, 5.3 µmol, η = 6%).

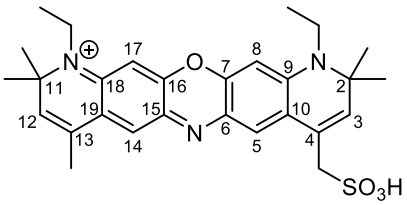
R_f(DCM/MeOH, 95:5): 0.64

^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm] = 7.35 (s, 2H, H-**5**), 6.895 (s, 2H, H-**8**), 5.63 (s, 2H, H-**3**), 3.765 (q, *J* = 7.1 Hz, 2H, NCH_2CH_3), 2.08 (s, 6H, CCH_3), 1.55 (s, 12H, $C(CH_3)_2$), 1.42 (t, *J* = 7.0 Hz, NCH_2CH_3).

LC-MS (*Method B*) calculated for $C_{28}H_{30}D_5N_3O^+$ $[M]^+$: 433.3, found 433.3; $\mathbf{t_R}$ = 5.24 min.

2.15 1,11-Diethyl-2,2,4,10,10-pentamethyl-8-(sulfomethyl)-10,11-dihydro-2*H*-dipyrido[3,2-*b*:2',3'-*i*]phenoxazin-1-ium (SulfoOxazine700-d0)

SulfoOxazine700-d0



$C_{28}H_{34}N_3O_4S^+$
M = 508.7 g.mol^{-1}

In a flame dried round bottom flask 1-ethyl-2,2,4-trimethyl-1,2-dihydroquinoline-7-ol (**6a**) (4.5 mg, 21.0 µmol, 1.0 equiv.) and d_5-(*E*)-ethyl-7-methoxy-2,2,4-trimethyl-6-(4-nitrophenyl) diazinyl)-1,2-dihydroquinoline (**4c**) (10 mg, 21.0 µmol, 1.0 equiv.) were dissolved in acetic acid (1.0 mL) under nitrogen atmosphere. The dark red reaction mixture was stirred for 3 h at 80 °C. Acetic acid was removed by azeotroping with toluene (4×3 mL) under rotary evaporation to give a dark blue residue. The crude product was directly purified HPLC (from 30 to 95% MeCN/H_2O, linear gradient, with constant 0.1% v/v TFA additive). The desired product (**SulfoOxazine700-d0**) was obtained as a dark blue solid (4.5 mg, 8.85 µmol, η = 45%).

R_f(DCM/MeOH, 9:1): 0.75

^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm] = 7.92 (s, 1H, H-**5**), 7.34 (s, 1H, H-**14**), 6.58 (s, 1H, H-**8**), 6,53 (s, 1H, H-**17**) 6.10 (s, 1H, H-**3**), 5.60 (s, 1H, H-**12**), 4.06 (s, 2H, CH_2, C$H_2$$SO_3H$), 3.72 – 3.65 (m, 4H, NC$H_2$$CH_3$), 2.07 (s, 3H, CH_3, CCH_3), 1.59 (s, 6H, C(CH_3)$_2$), 1.56 (s, 6H, C(CH_3)$_2$), 1.36-1.43 (m, 6H, NCH_2CH_3).

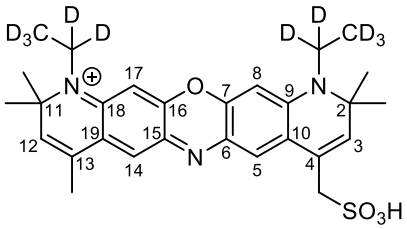
^{13}C NMR (150 MHz, $CDCl_3$): δ [ppm] = 154.0 (Cq), 152.5 (Cq), 149.4 (Cq), 149.2 (Cq), 137.8 (CH, C-**3**), 135.8 (Cq), 134.3 (Cq), 133.2 (CH, C-**12**), 127.2 (CH, H-**5**), 126.2 (Cq), 126.0 (Cq), 125.8 (Cq), 125.5 (CH, H-**14**), 124.0 (Cq), 95.6 (CH, C-**8** or C-**17**), 95.5 (CH, C-**8** or C-**17**), 62.0 (Cq, C-**2** or C-**11**), 61.2 (Cq, C-**2** or C-**11**), 53.1 (CH_2, *C*$H_2$$SO_3H$), 41.5 ($CH_2$, N*C*$H_2$$CH_3$), 41.2 ($CH_2$, N*C*$H_2$$CH_3$), 29.7 ($CH_3$, C(*C*$H_3$)$_2$), 29.3($CH_3$, C(*C*$H_3$)$_2$), 18.8 ($CH_3$, C*C*$H_3$), 13.8 ($CH_3$, NCH_2*C*H_3), 13.7 (CH_3, NCH_2*C*H_3).

HRMS (ESI): *m/z* calculated for $C_{28}H_{34}N_3O_4S^+$ $[M]^+$: 508.2265, found: 508.2262.

LC-MS (*Method A*) calculated for $[M]^+$: 508.4, found 508.4; $\mathbf{t_R}$ = 5.14 min.

2.16 1,11-Bis(ethyl-d5)-2,2,4,10,10-pentamethyl-8-(sulfomethyl)-10,11-dihydro-2*H*-dipyrido[3,2-*b*:2',3'-*i*]phenoxazin-1-ium (SulfoOxazine700-d10)

SulfoOxazine700-d10



$C_{28}H_{24}D_{10}N_3O_4S^+$
M = 518.7 g.mol^{-1}

In a flame dried round bottom flask 1 d_5-1-ethyl-2,2,4-trimethyl-1,2-dihydroquinoline-7-ol (**6b**) (4.5 mg, 20.2 µmol, 1.0 equiv.) and (*E*)-(1-(ethyl-d5)-7-methoxy-2,2-dimethyl-6 (4-nitrophenyl)diazenyl)-1,2-dihydroquinolin-4-yl) methanesulfonic acid (**4d**) (10 mg, 21.5 µmol, 1.0 equiv.) were dissolved in acetic acid (1.0 mL) under nitrogen atmosphere. The dark red reaction mixture was stirred for 3 h at 80 °C. Acetic acid was removed by azeotroping with toluene (4×3 mL) under rotary evaporation to give a dark blue residue. The crude product was directly purified HPLC (from 30 to 95% MeCN/H_2O, linear gradient, with constant 0.1% v/v TFA additive). The desired product (**SulfoOxazine700-d10**) was obtained as a dark blue solid (2.3 mg, 7.71 µmol, η = 22%).

R_f(DCM/MeOH, 9:1): 0.70

^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm] = 7.92 (s, 1H, H-**5**), 7.33 (s, 1H, H-**14**), 6.57 (s, 1H, H-**8**), 6.51 (s, 1H, H-**17**) 6.11 (s, 1H, H-**3**), 5.61 (d, *J* = 1.5 Hz, 1H, H-**12**), 4.08 (s, 2H, CH_2, C*H*$_2$SO$_3$H), 2.07 (s, 3H, CH_3, CC*H*$_3$), 1.57 (s, 6H, C(C*H*$_3$)$_2$), 1.56 (s, 6H, C(C*H*$_3$)$_2$).

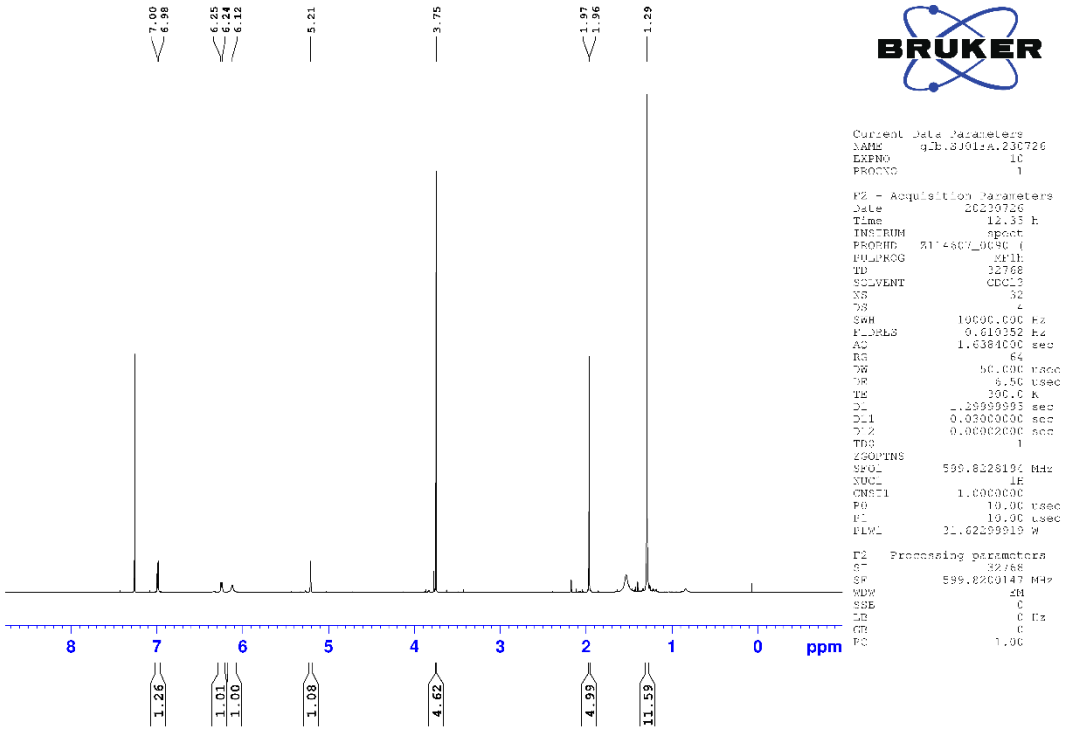
^{13}C NMR (150 MHz, $CDCl_3$): δ [ppm] = 154.0 (Cq), 152.5 (Cq), 149.4 (Cq), 149.2 (Cq), 137.8 (CH, C-**3**), 135.7 (Cq), 134.3 (Cq), 133.2 (CH, C-**12**), 127.3 (CH, H-**5**), 126.1 (Cq), 126.0 (Cq), 125.7 (Cq), 125.5(CH, H-**14**), 123.9 (Cq), 95.5 (CH, C-**8** or C-**17**), 95.3 (CH, C-**8** or C-**17**), 61.9 (Cq, C-**2** or C-**11**), 61.2 (Cq, C-**2** or C-**11**), 53.1 (CH_2, *C*H$_2$SO$_3$H), 29.7 (CH_3, C(*C*H$_3$)$_2$), 29.2 (CH_3, C(*C*H$_3$)$_2$), 18.8 (CH_3, C*C*H$_3$).

HRMS (ESI): *m/z* calculated for $C_{28}H_{34}N_3O_4S^+$ $[M]^+$: 518.2892, found: 518.2925.

LC-MS (*Method A*) calculated for $[M]^+$: 518.3, found 518.3; $\mathbf{t_R}$ = 4.83 min.

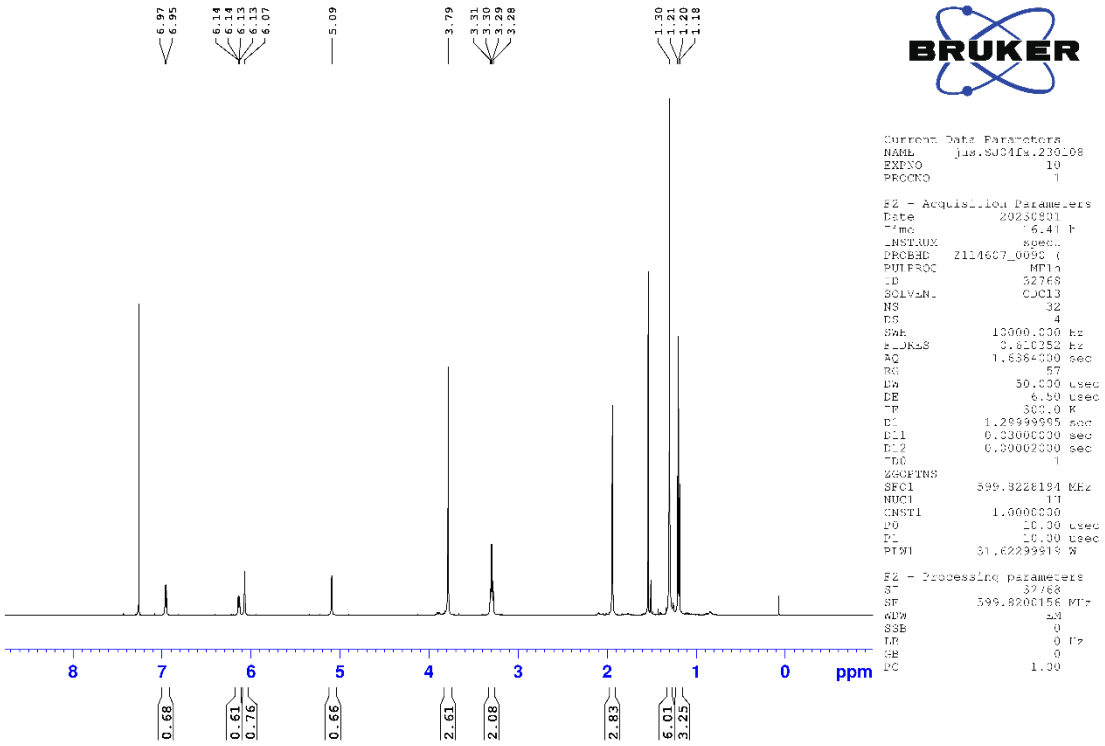
3. NMR spectra

3.1. 1,2-Dihydro-7-methoxy-2,2,4-trimethyl-quinoline (2)

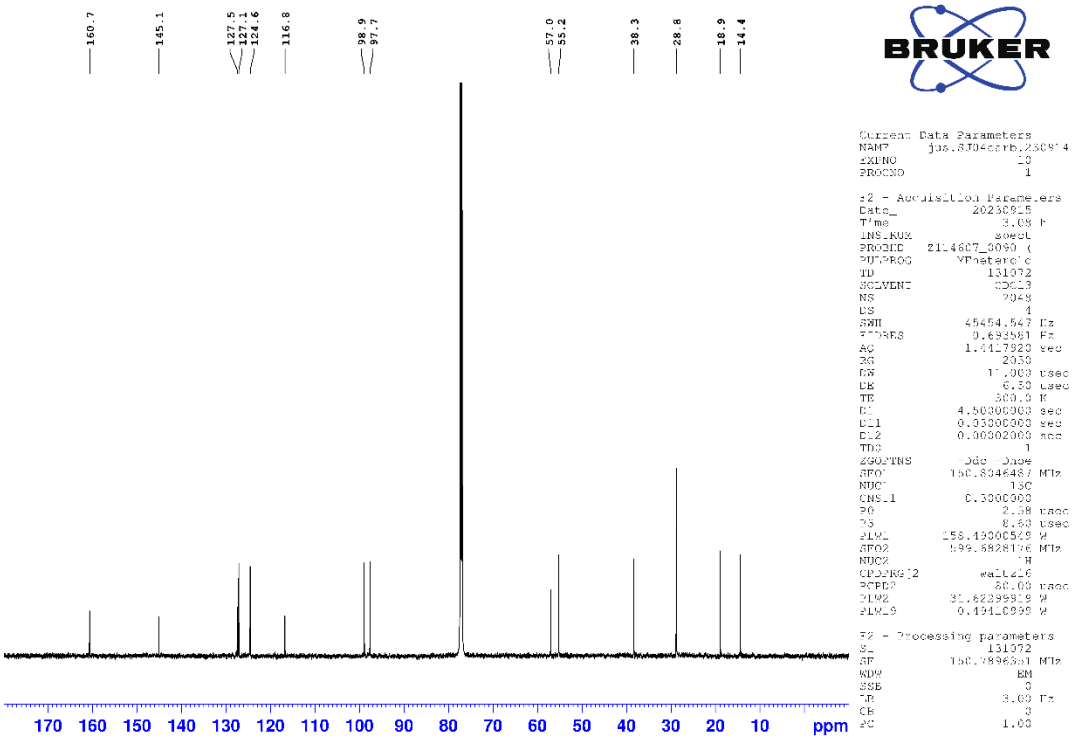


^{1}H NMR (600 MHz) of (**2**) in $CDCl_3$.

3.2. 1-Ethyl-1,2-dihydro-7-methoxy-2,2,4-trimethyl-quinoline (3a)

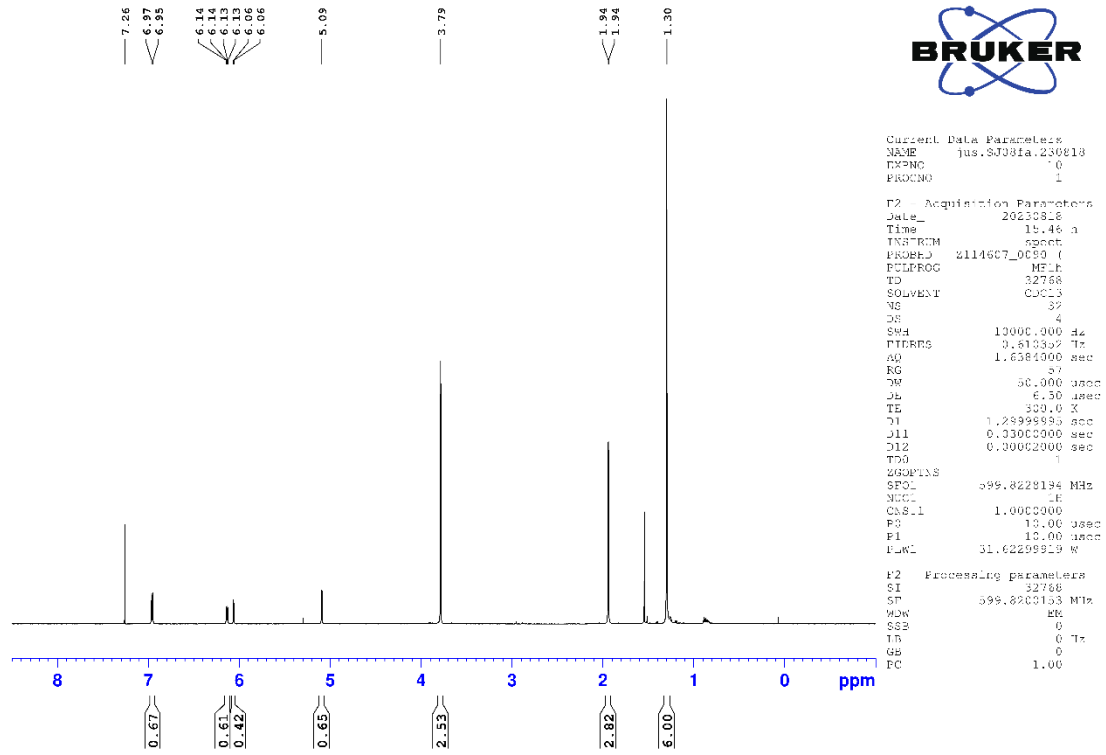


^{1}H NMR (600 MHz) of (**3a**) in $CDCl_3$.

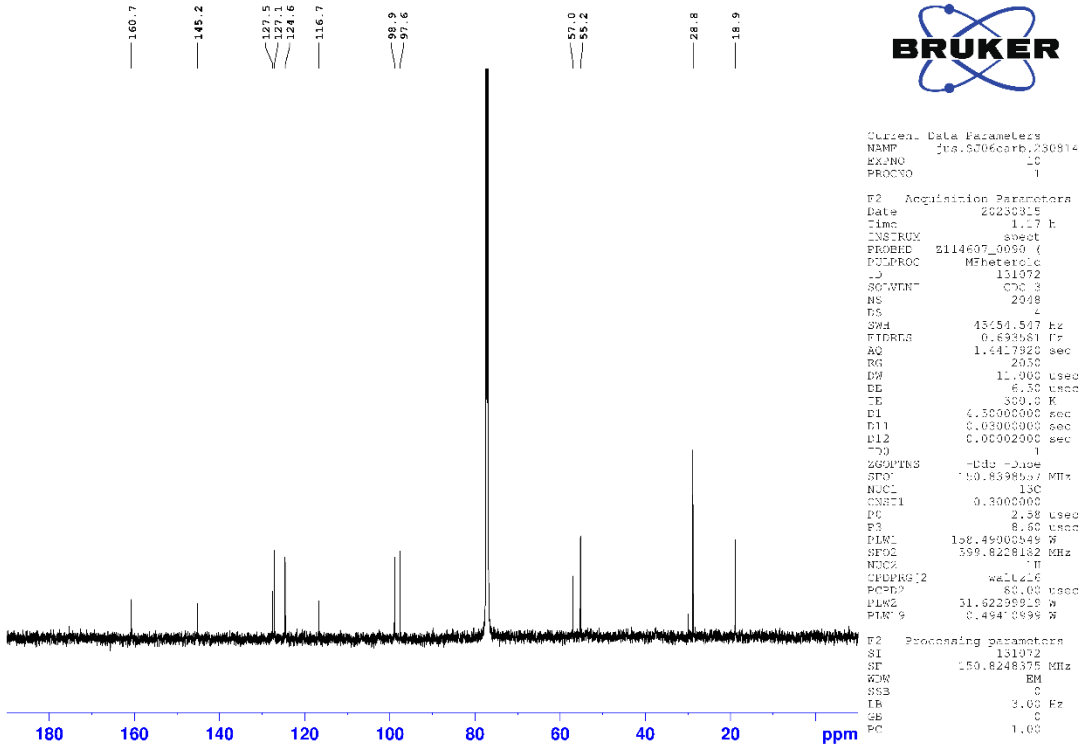


^{13}C NMR (150MHz) of (**3a**) in $CDCl_3$.

3.3. d5-1-Ethyl-1,2-dihydro-7-methoxy-2,2,4-trimethyl-quinoline (3b)

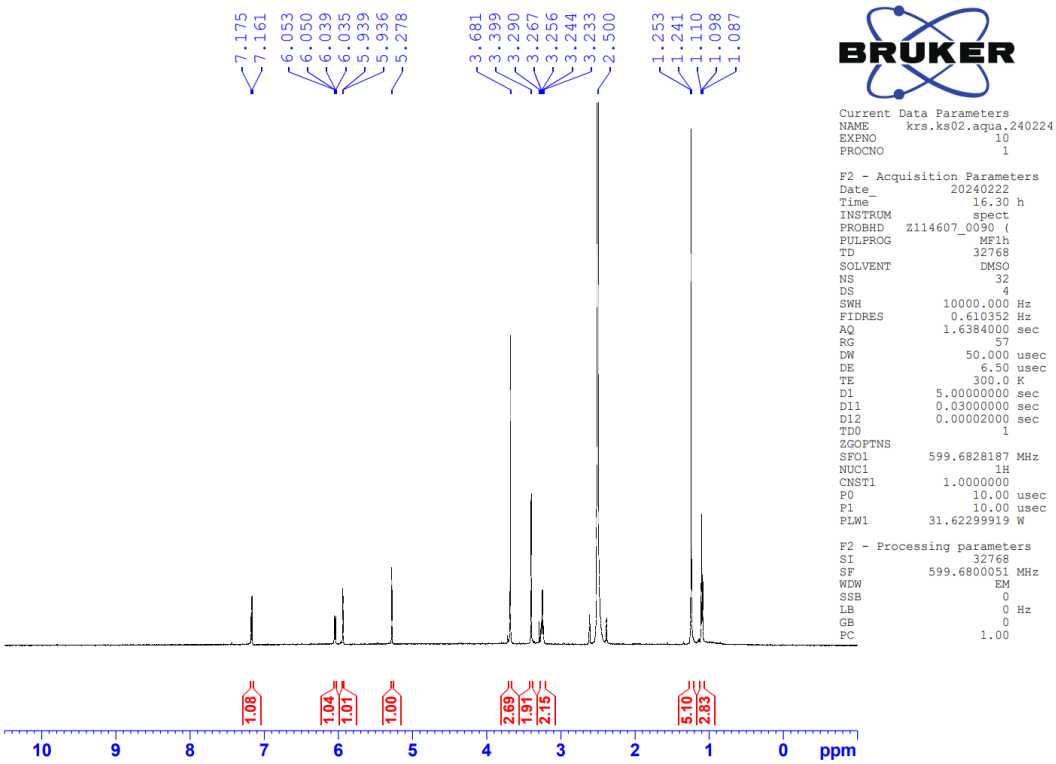


^{1}H NMR (600 MHz) of (**3b**) in $CDCl_3$.



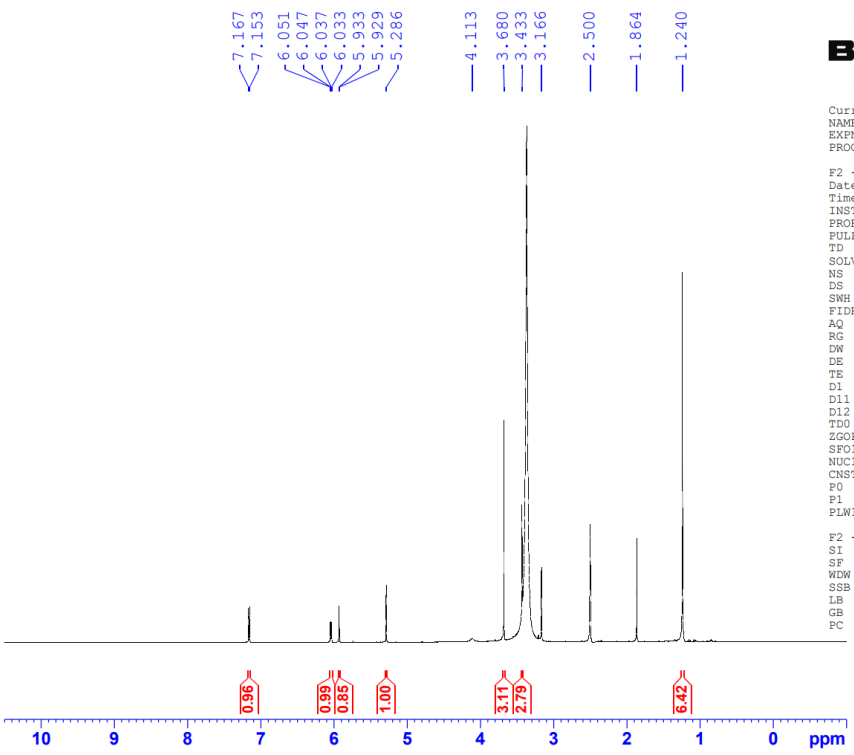
^{13}C NMR (150MHz) of (**3b**) in $CDCl_3$.

3.4. (1-Ethyl-7-methoxy-2,2-dimethyl-1,2-dihydroquinolin-4-yl)methanesulfonic acid (4a)



^{1}H NMR (600 MHz) of (**4a**) in DMSO-d_6.

3.5. (d5-1-Ethyl-7-methoxy-2,2-dimethyl-1,2-dihydroquinolin-4-yl)methanesulfonic acid (4b)

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F2 - Processing parameters
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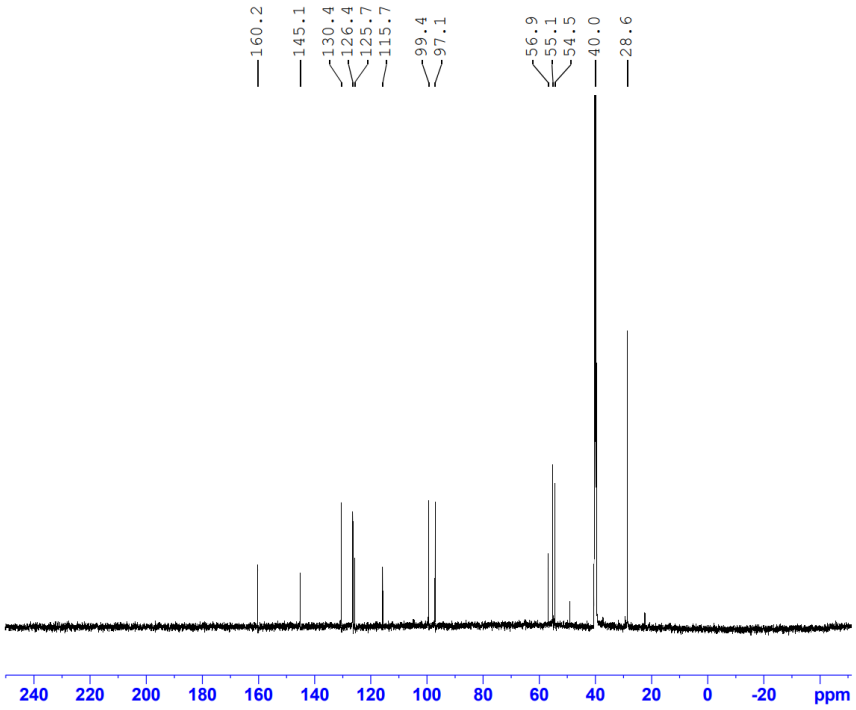
^{1}H NMR (600 MHz) of (**4b**) in DMSO-d_6.

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DS                     4
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RG                  2050
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TE                 300.0 K
D1              2.50000000 sec
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D12             0.00002000 sec
TD0                    1
ZGOPTNS         -Ddc -Dnoe
SFO1           150.8046487 MHz
NUC1                   13C
CNST1           1.0000000
P0                   8.60 usec
P3                   8.60 usec
PLW1          158.49000549 W
SFO2           599.6828176 MHz
NUC2                   1H
CPDPRG[2          waltz16
PCPD2               80.00 usec
PLW2           31.62299919 W
PLW19           0.49410939 W

F2 - Processing parameters
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SF            150.7896564 MHz
WDW                   EM
SSB                    0
LB                  3.00 Hz
GB                     0
PC                  1.00
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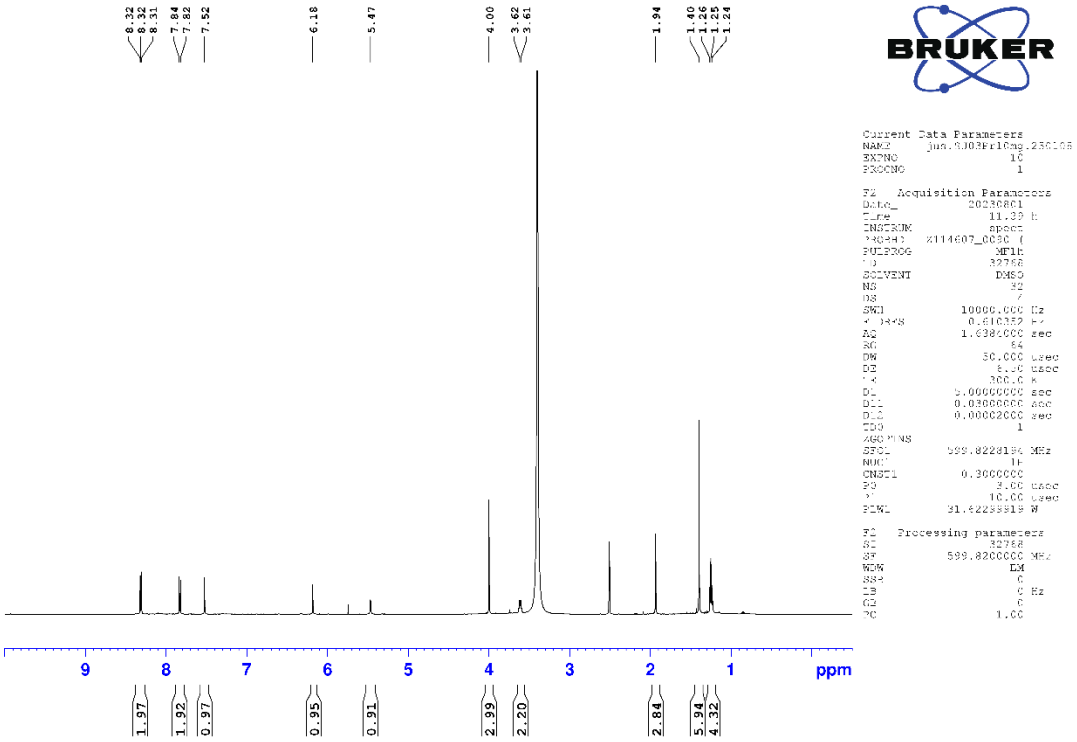
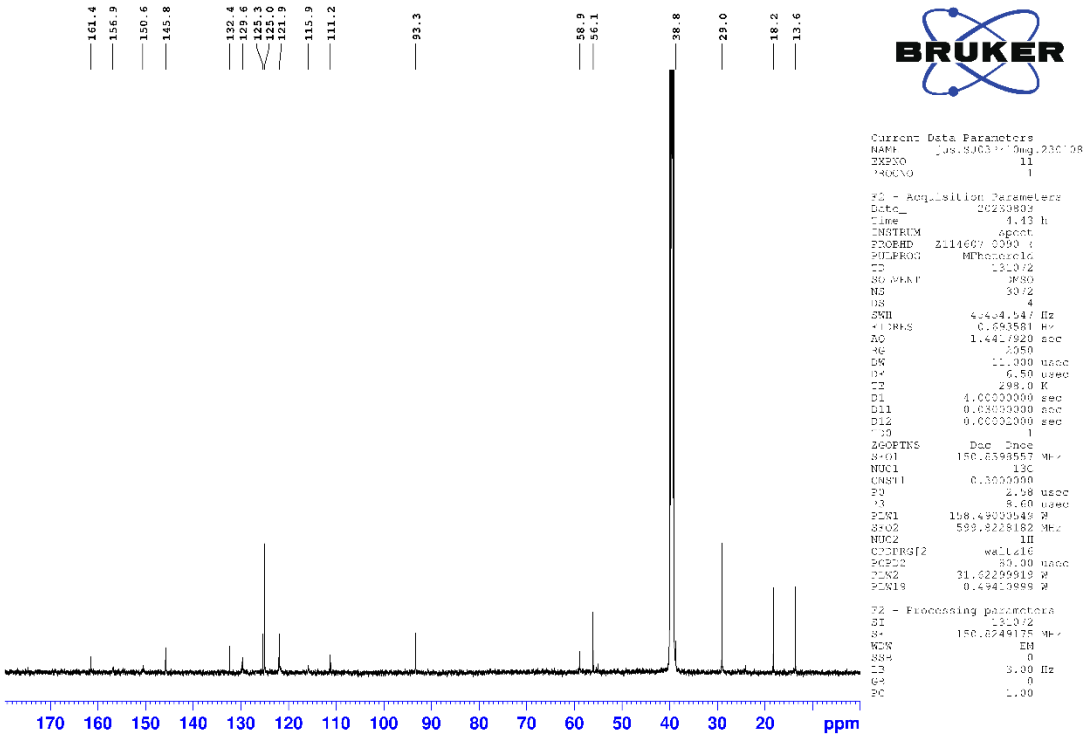
^{13}C NMR (150MHz) of (**4b**) in DMSO-d_6.

3.6. (*E*)-Ethyl-7-methoxy-2,2,4-trimethyl-6-((4-nitrophenyl)diazinyl)-1,2-dihydroquinoline (5a)

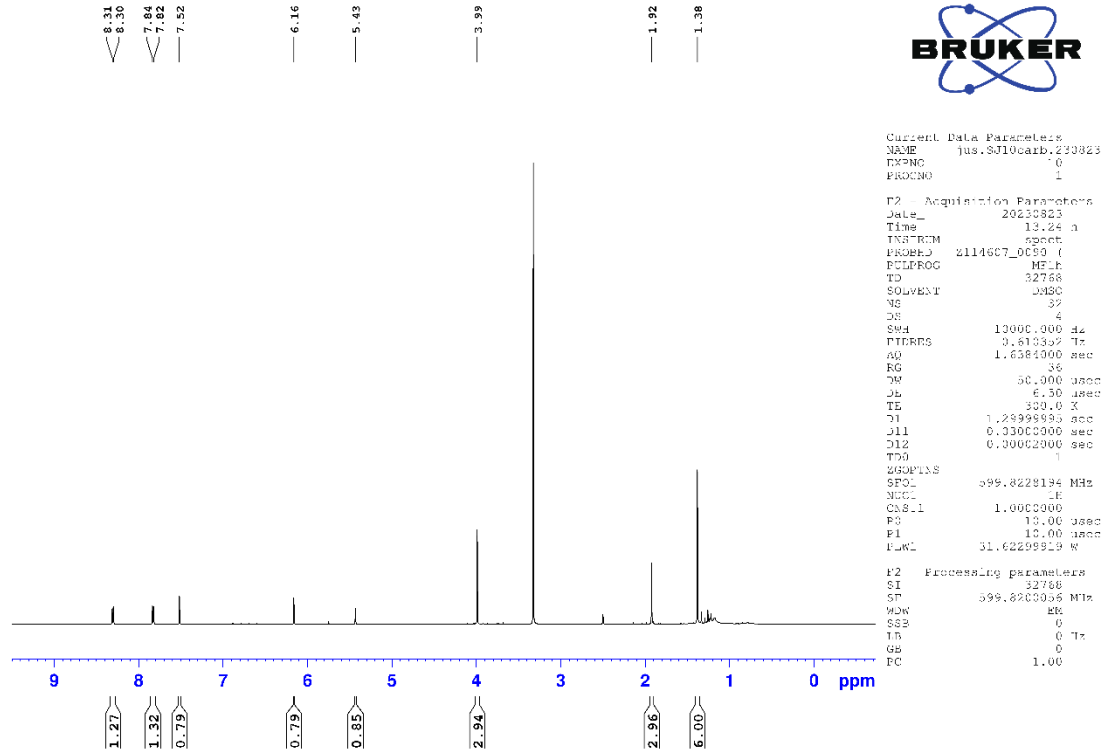


^{1}H NMR (600 MHz) of (**5b**) in DMSO-d_6.

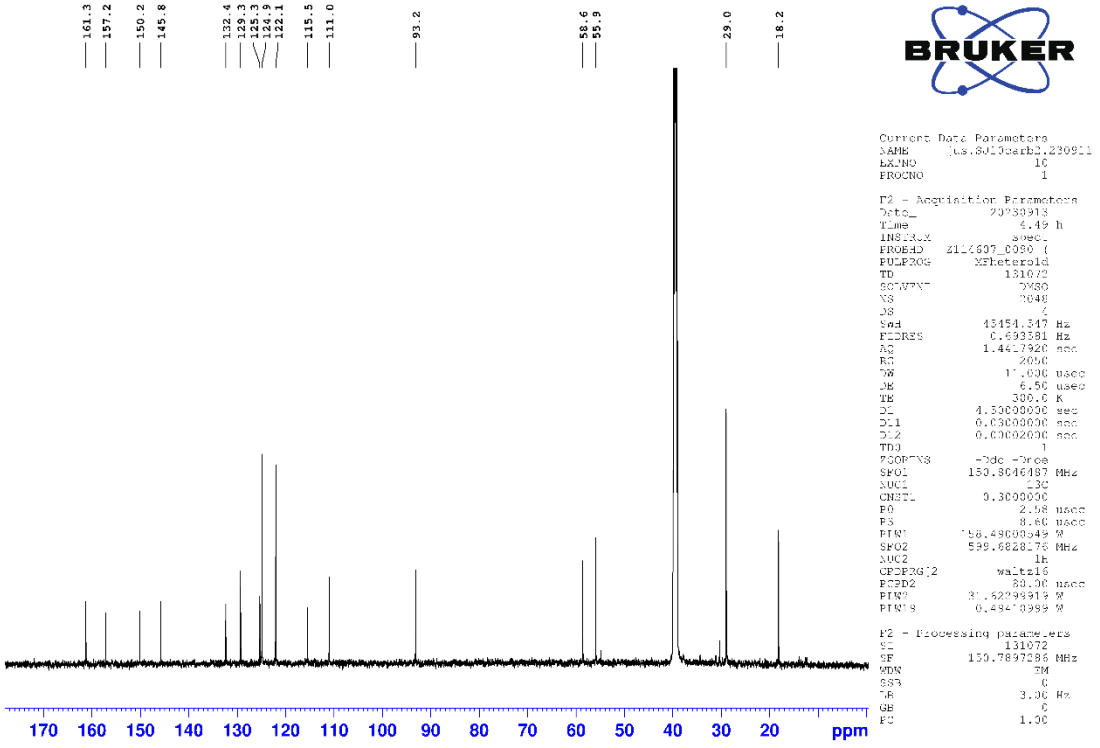


^{13}C NMR (150MHz) of (**5a**) in DMSO-d_6.

3.7. d5-(*E*)-Ethyl-7-methoxy-2,2,4-trimethyl-6-((4-nitrophenyl)diazinyl)-1,2-dihydroquinoline (5b)

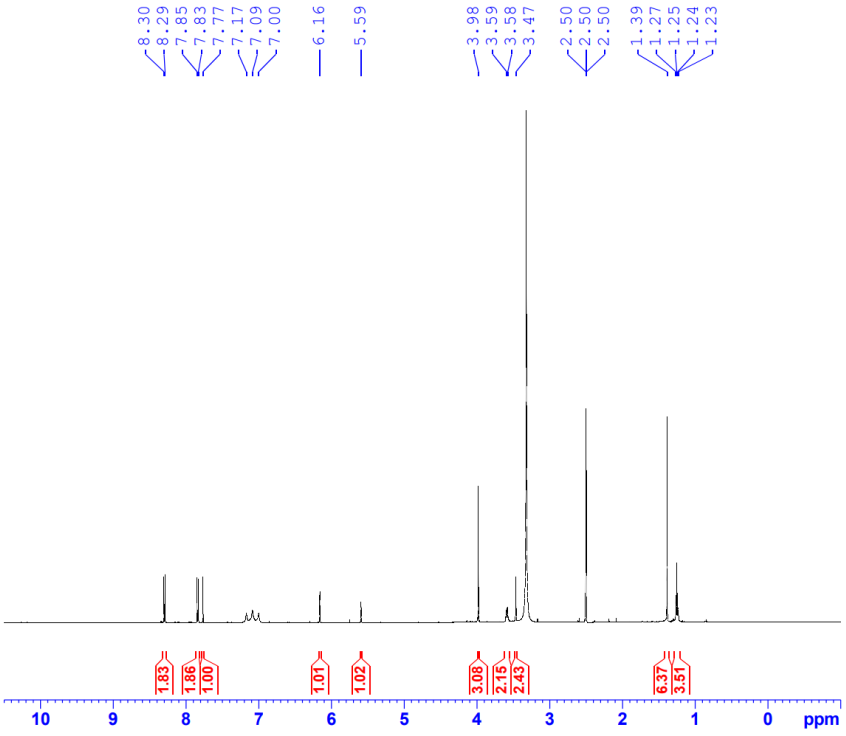


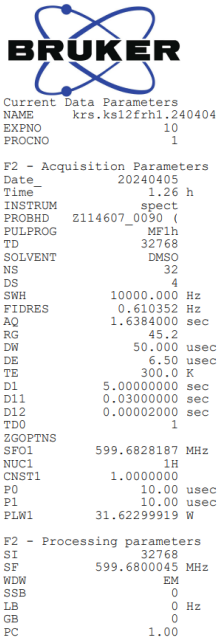
H NMR (600 MHz) of (**5b**) in DMSO-d_6.



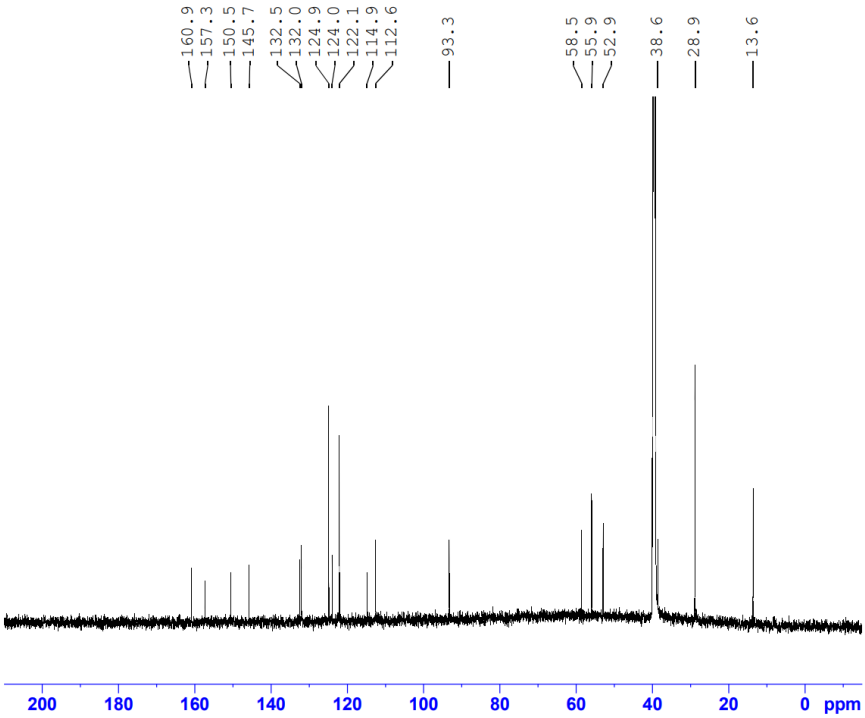
^{13}C NMR (150MHz) of (**5b**) in DMSO-d_6.

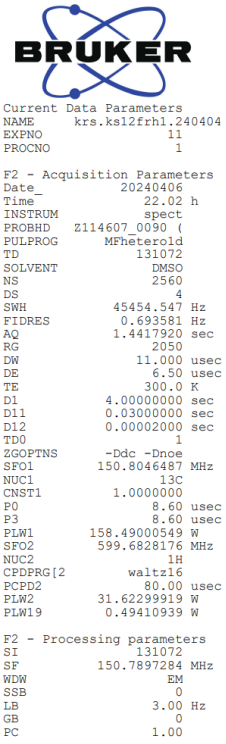
3.8. (*E*)-(1-Ethyl-7-methoxy-2,2-dimethyl-6-((4-nitrophenyl)diazenyl)-1,2-dihydroquinolin-4-yl)methanesulfonic acid (5c)





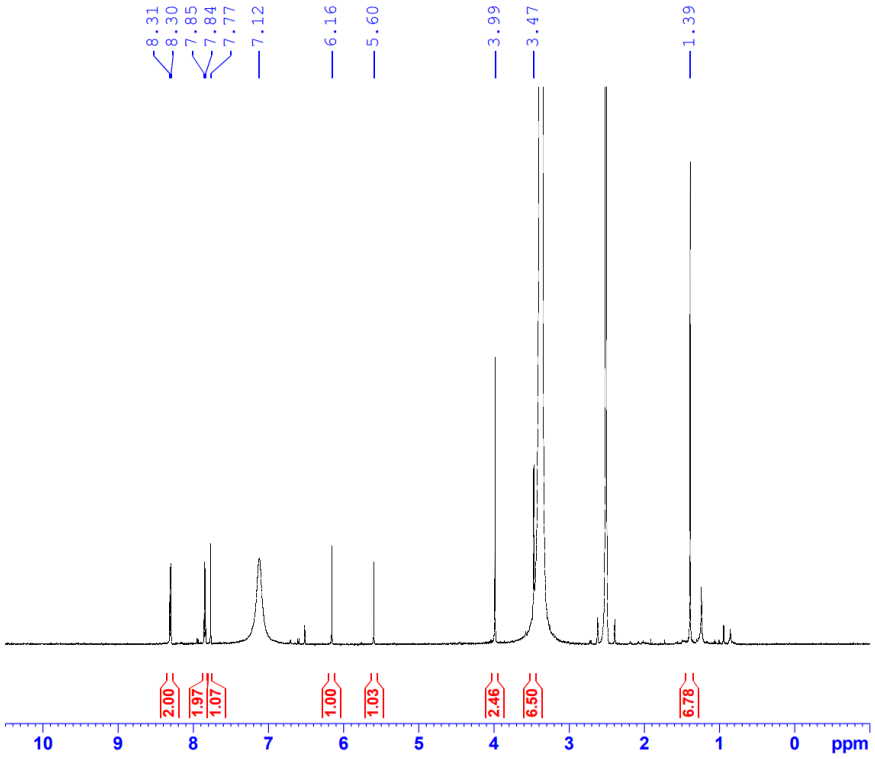
^{1}H NMR (600 MHz) of (**5c**) in DMSO-d$_6$.

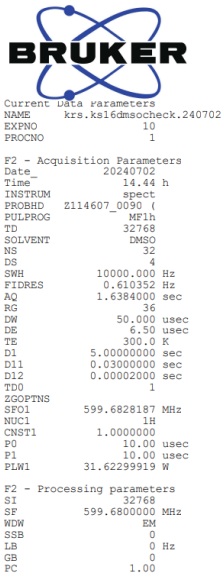




^{13}C NMR (150MHz) of (**5c**) in DMSO-d$_6$.

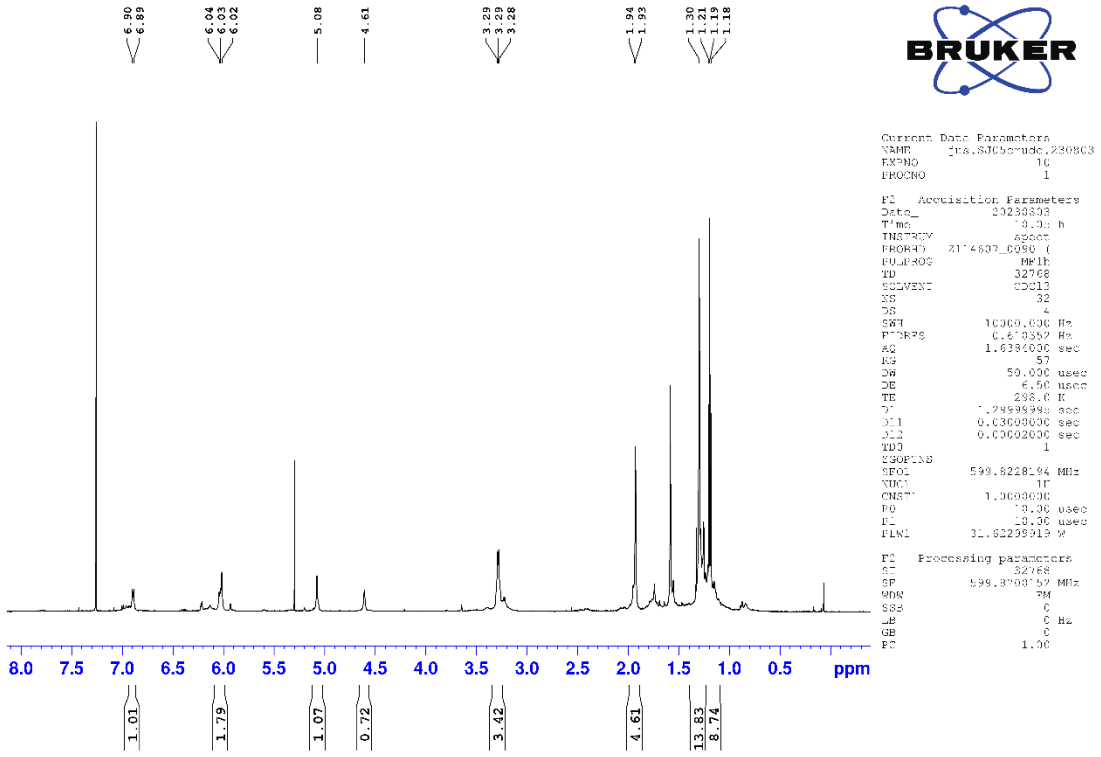
3.9. (*E*)-(1-(Ethyl-d5)-7-methoxy-2,2-dimethyl-6-((4-nitrophenyl)diazenyl)-1,2-dihydroquinolin-4-yl)methanesulfonic acid (5d)





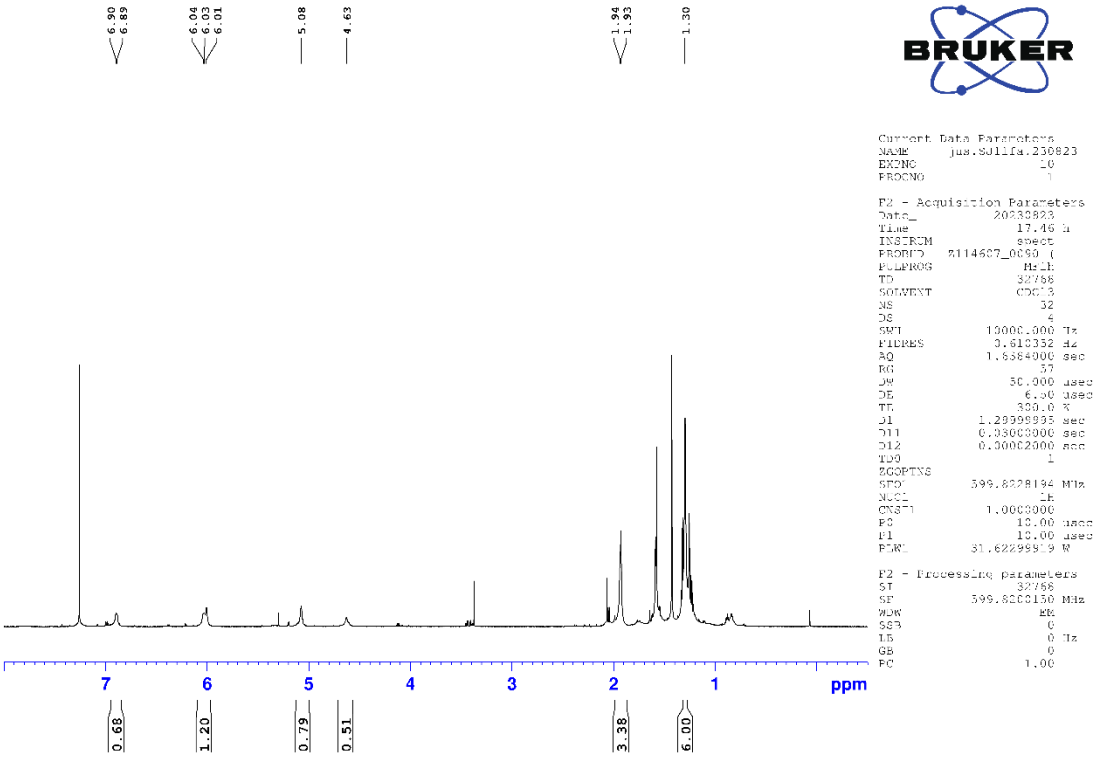
^{1}H NMR (600 MHz) of (**5d**) in DMSO-d_6.

3.10. 1-Ethyl-2,2,4-trimethyl-1,2-dihydroquinoline-7-ol (6a)



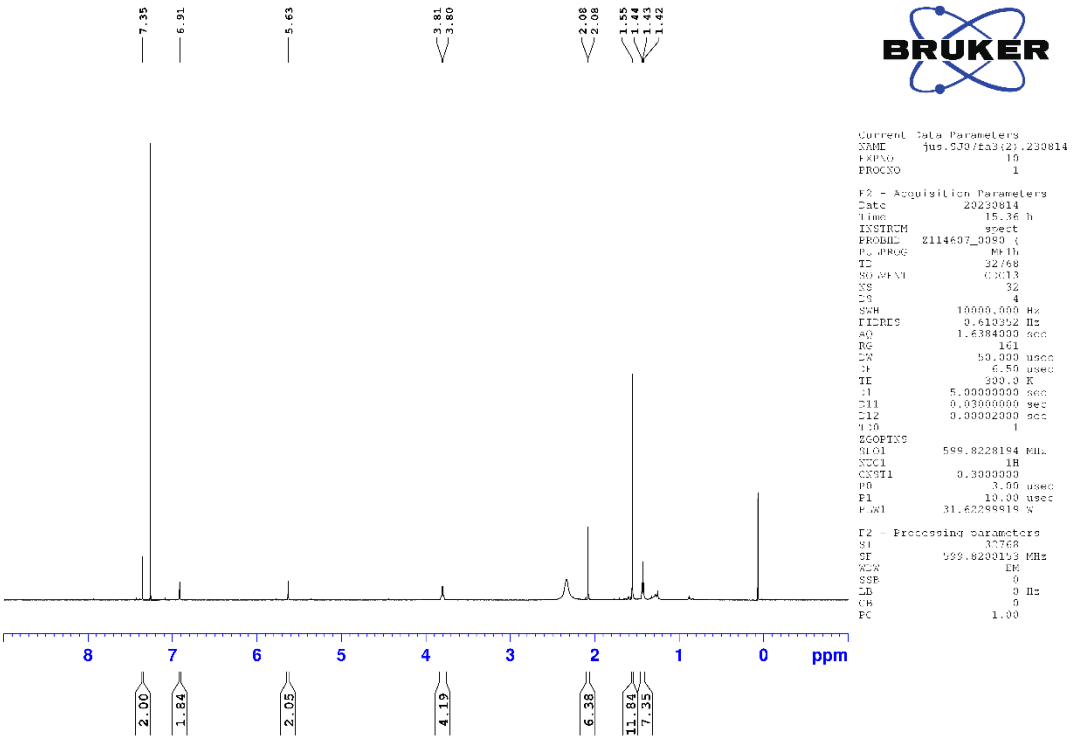
^{1}H NMR (600 MHz) of (**6a**) in $CDCl_3$.

3.11. d5-1-Ethyl-2,2,4-trimethyl-1,2-dihydroquinoline-7-ol (6b)

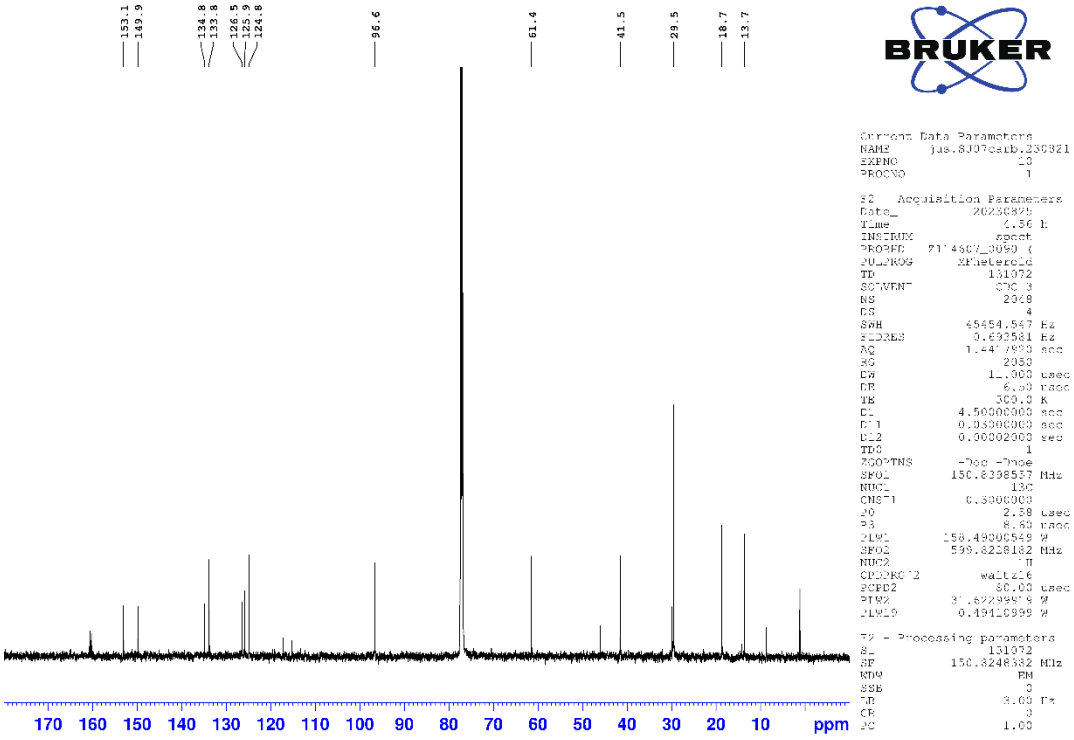


^{1}H NMR (600 MHz) of (**6b**) in $CDCl_3$.

3.12. 1,11-Diethyl-2,2,4,8,10,10-hexamethyl-10,11-dihydro-2*H*-dipyrido-[3,2*b*:2',3'-*i*]phenoxazin-1-ium (Oxazine700-d0)

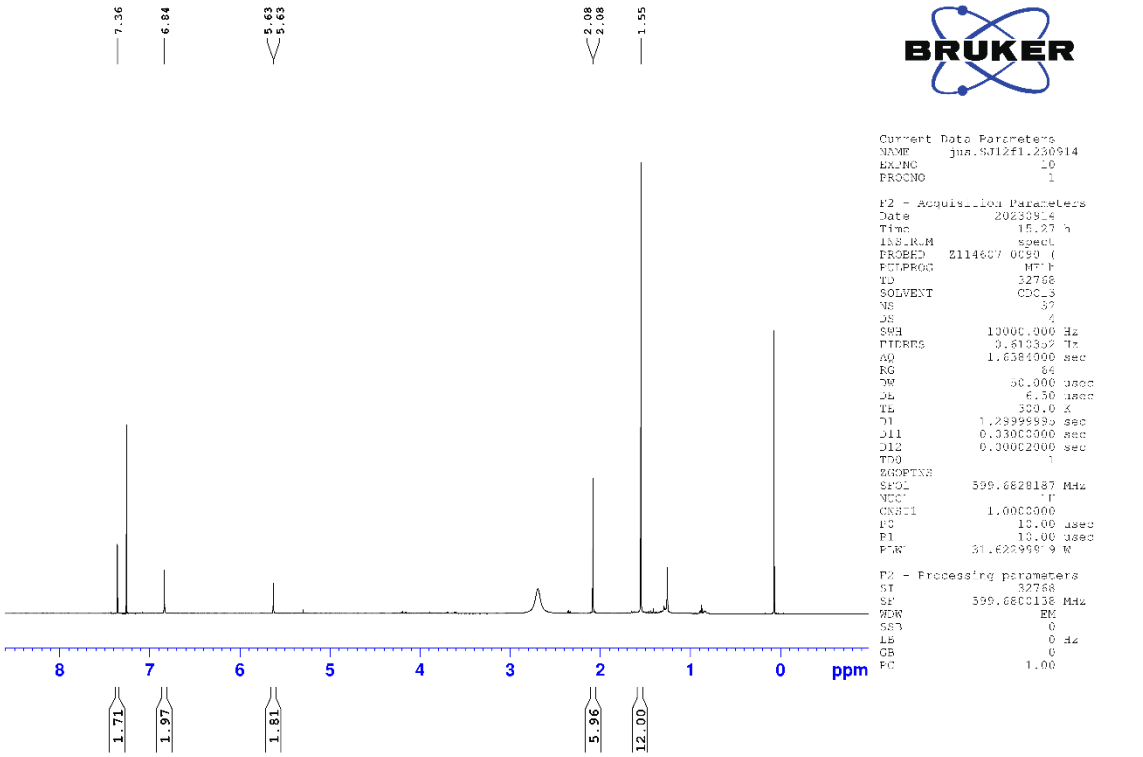


^{1}H NMR (600 MHz) of (**Oxazine700-d0**) in $CDCl_3$.

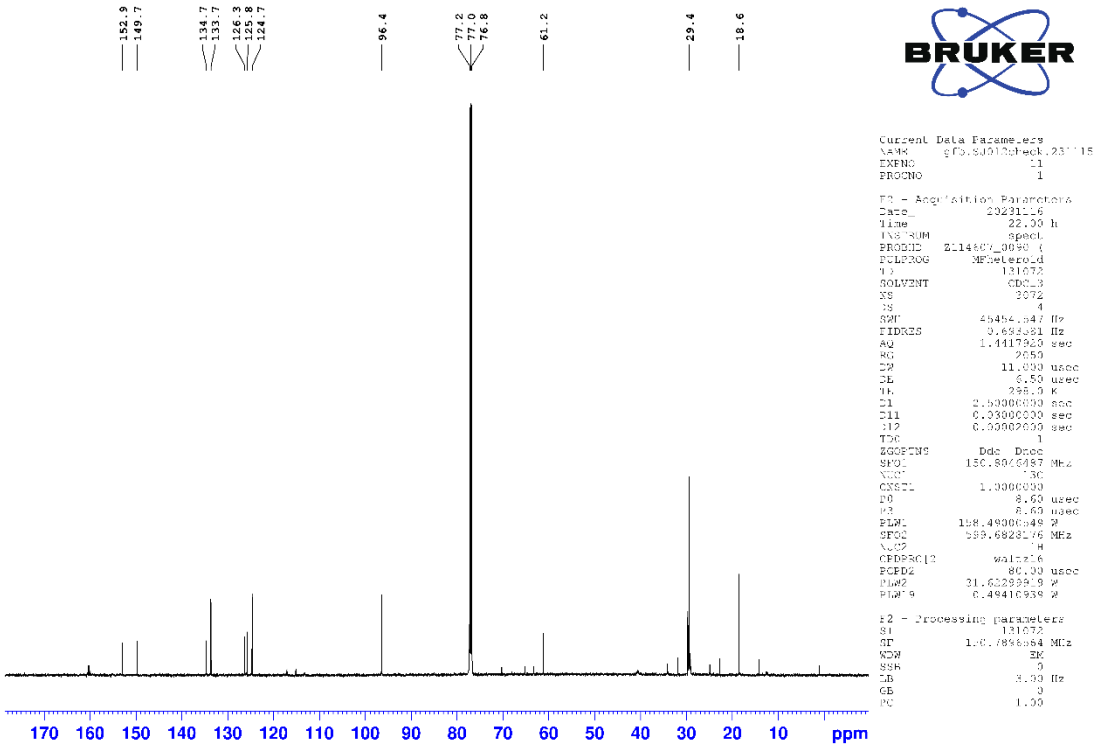


^{13}C NMR (150MHz) of (**Oxazine700-d0**) in $CDCl_3$.

3.13. d10-1,11-Diethyl-2,2,4,8,10,10-hexamethyl-10,11-dihydro-2*H*-dipyrido-[3,2-*b*:2', 3'-*i*]phenoxazin-1-ium (Oxazine700-d10)

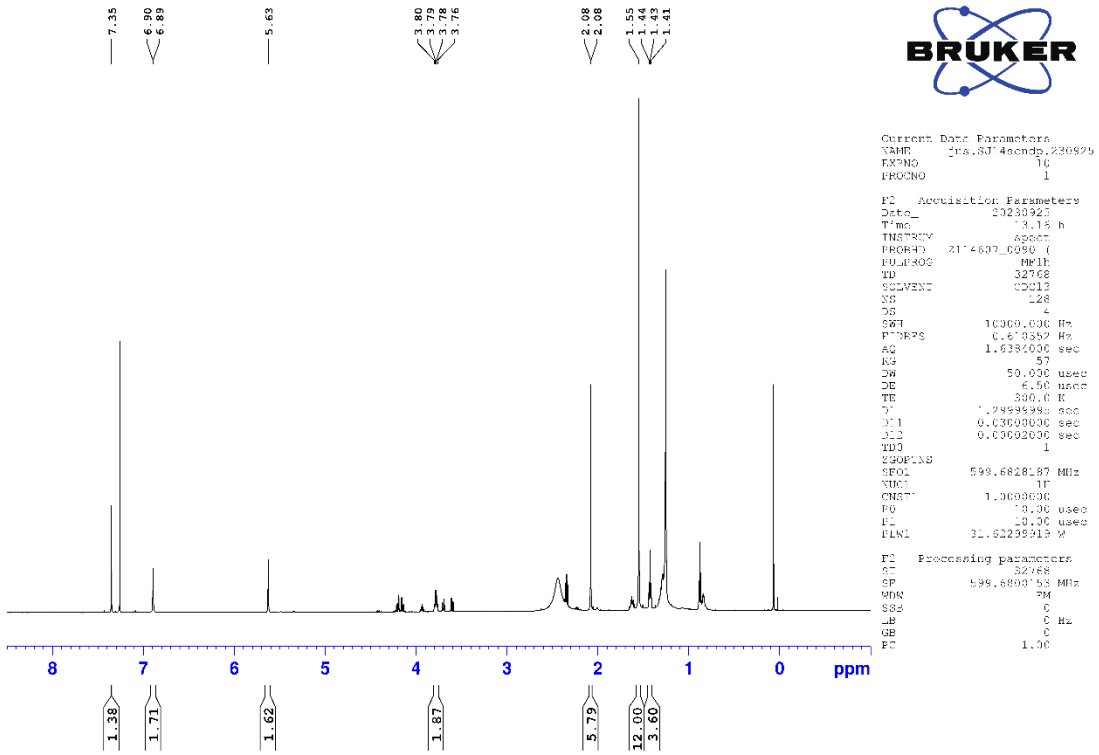


^{1}H NMR (600 MHz) of (**Oxazine700-d10**) in $CDCl_3$.



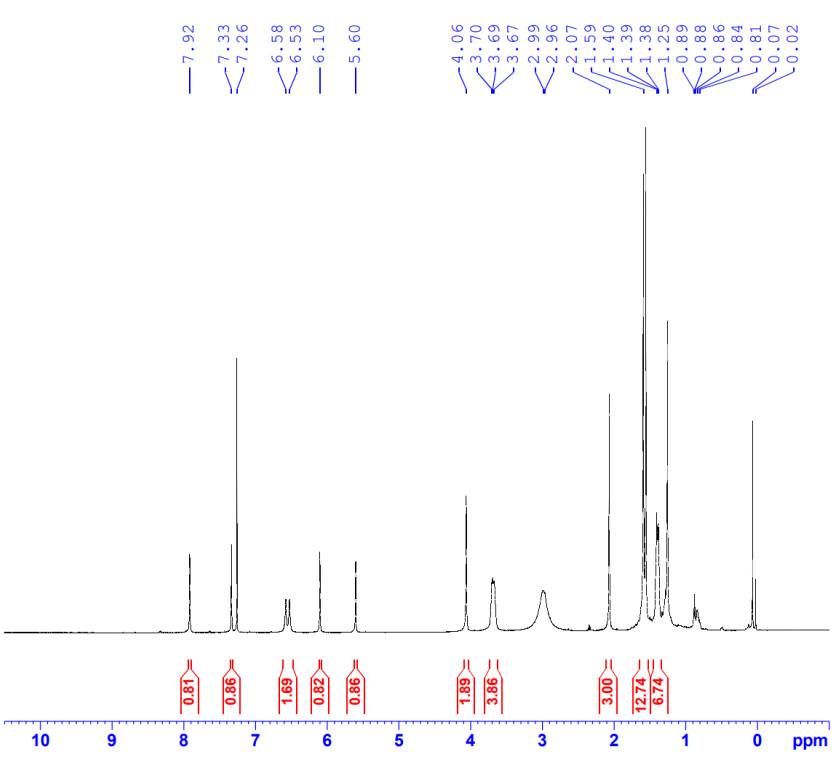
^{13}C NMR (150MHz) of (**Oxazine700-d10**) in $CDCl_3$.

3.14. d5-1,11-Diethyl-2,2,4,8,10,10-hexamethyl-10,11-dihydro-2*H*-dipyrido- [3,2-*b*:2', 3'-*i*]phenoxazin-1-ium (Oxazine700-d5)



^{1}H NMR (600 MHz) of (**Oxazine700-d10**) in $CDCl_3$.

3.15. 1,11-Diethyl-2,2,4,10,10-pentamethyl-8-(sulfomethyl)-10,11-dihydro-2*H*-dipyrido[3,2-*b*:2',3'-*i*]phenoxazin-1-ium (SulfoOxazine700-d0)

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F2 - Processing parameters
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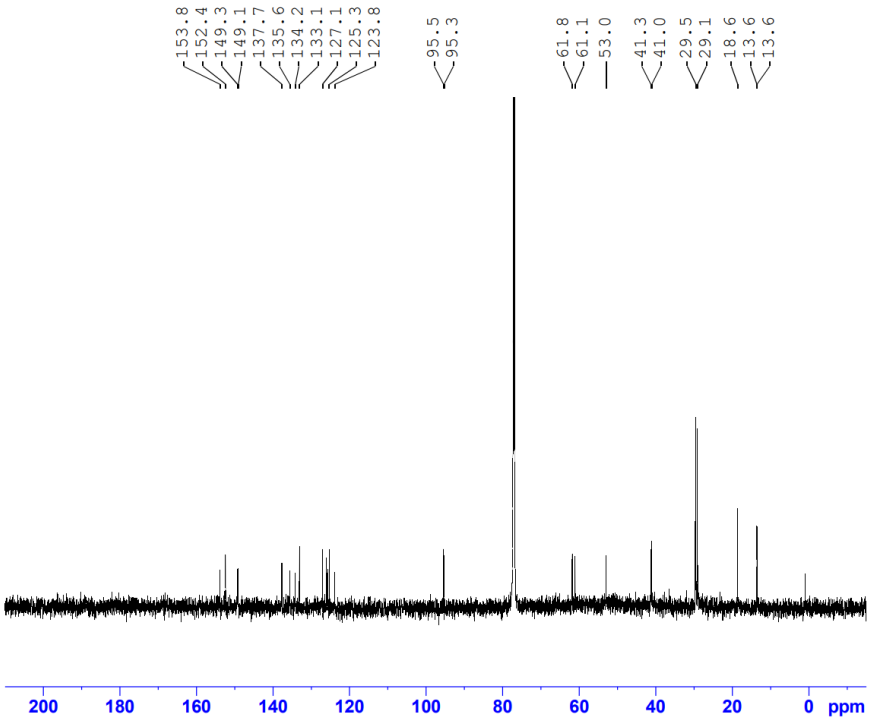
^{1}H NMR (600 MHz) of (**SulfoOxazine700-d0**) in $CDCl_3$.

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F2 - Processing parameters
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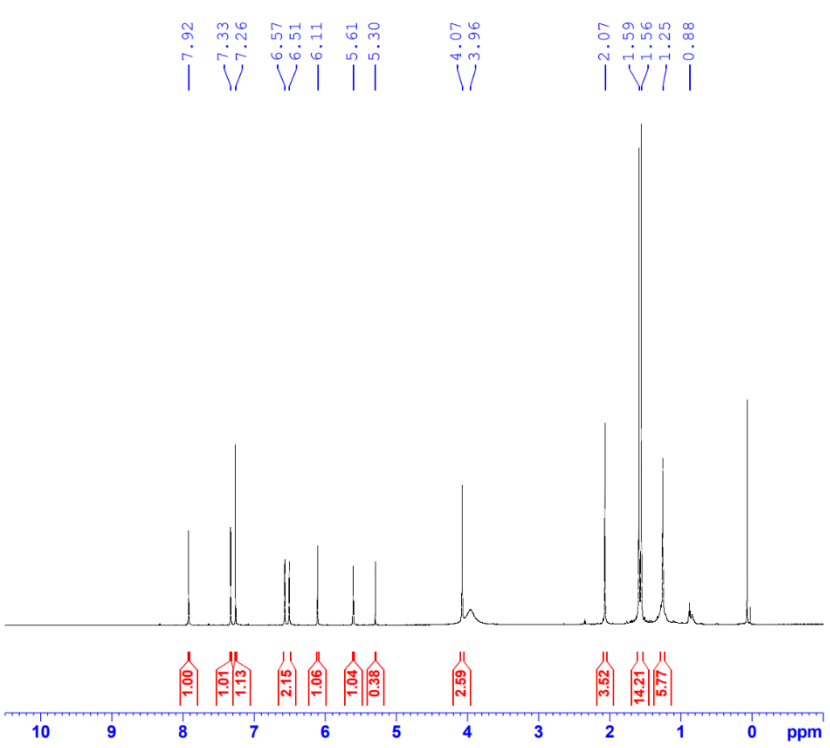
^{13}C NMR (150 MHz) of (**SulfoOxazine700-d0**) in $CDCl_3$.

3.16. 1,11-Bis(ethyl-d5)-2,2,4,10,10-pentamethyl-8-(sulfomethyl)-10,11-dihydro-2*H*-dipyrido[3,2-*b*:2',3'-*i*]phenoxazin-1-ium (SulfoOxazine700-d10)

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DS                      4
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RG                     57
DW                  50.000 usec
DE                   6.50 usec
TE                  300.0 K
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TD0                    1
ZGOPTNS
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NUC1                   1H
CNST1            1.0000000
P0                  10.00 usec
P1                  10.00 usec
PLW1            31.62299919 W

F2 - Processing parameters
SI                  32768
SF              599.6800160 MHz
WDW                    EM
SSB                     0
LB                      0 Hz
GB                      0
PC                   1.00
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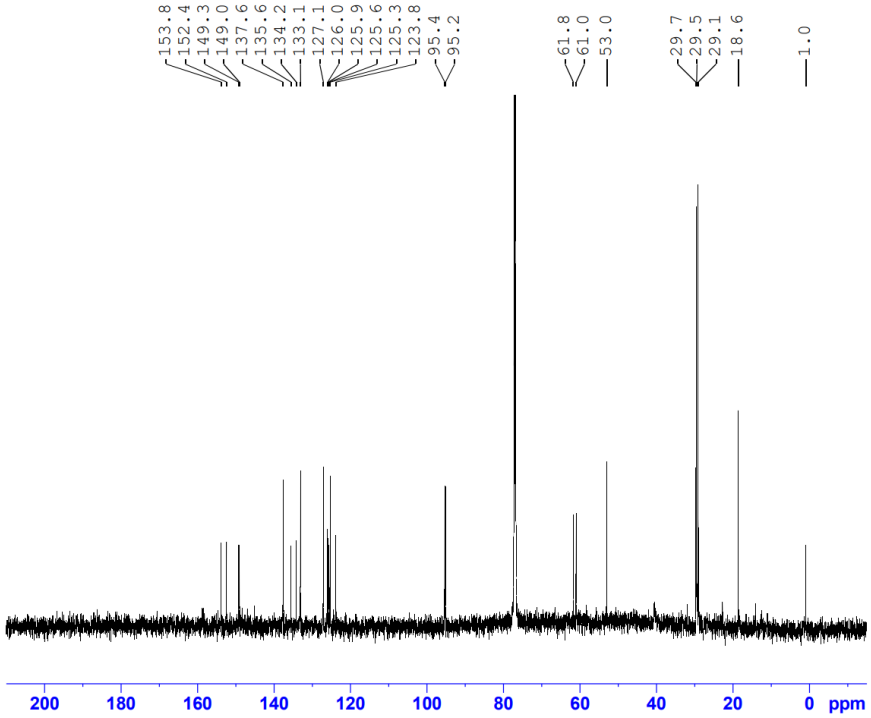
^{1}H NMR (600 MHz) of (**SulfoOxazine700-d10**) in $CDCl_3$.

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PROCNO                 1

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CNST1            1.0000000
P0                   8.60 usec
P3                   8.60 usec
PLW1           158.49000549 W
SFO2            599.6828176 MHz
NUC2                   1H
CPDPRG[2           waltz16
PCPD2                80.00 usec
PLW2            31.62299919 W
PLW19            0.49410939 W

F2 - Processing parameters
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WDW                    EM
SSB                     0
LB                   3.00 Hz
GB                      0
PC                   1.00
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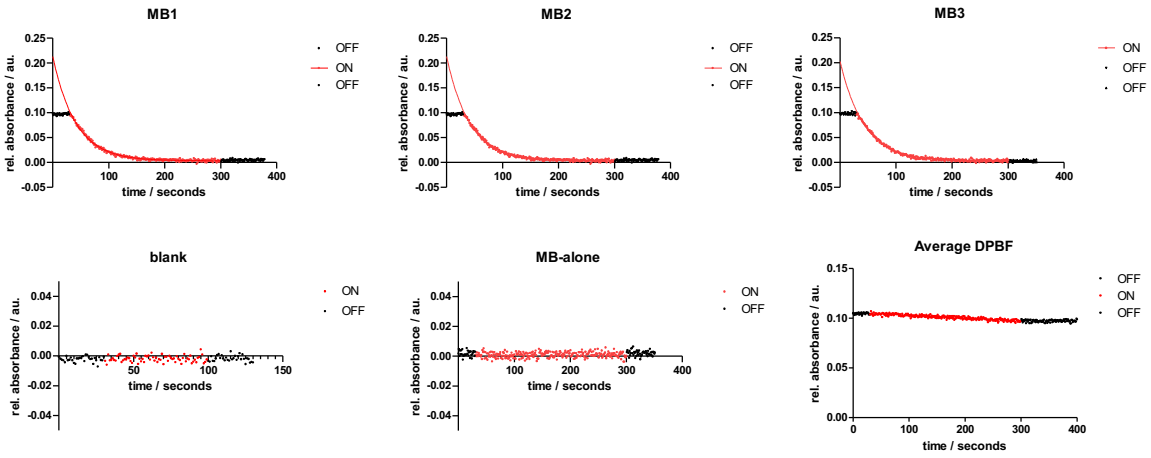
^{13}C NMR (150 MHz) of (**SulfoOxazine700-d10**) in $CDCl_3$.

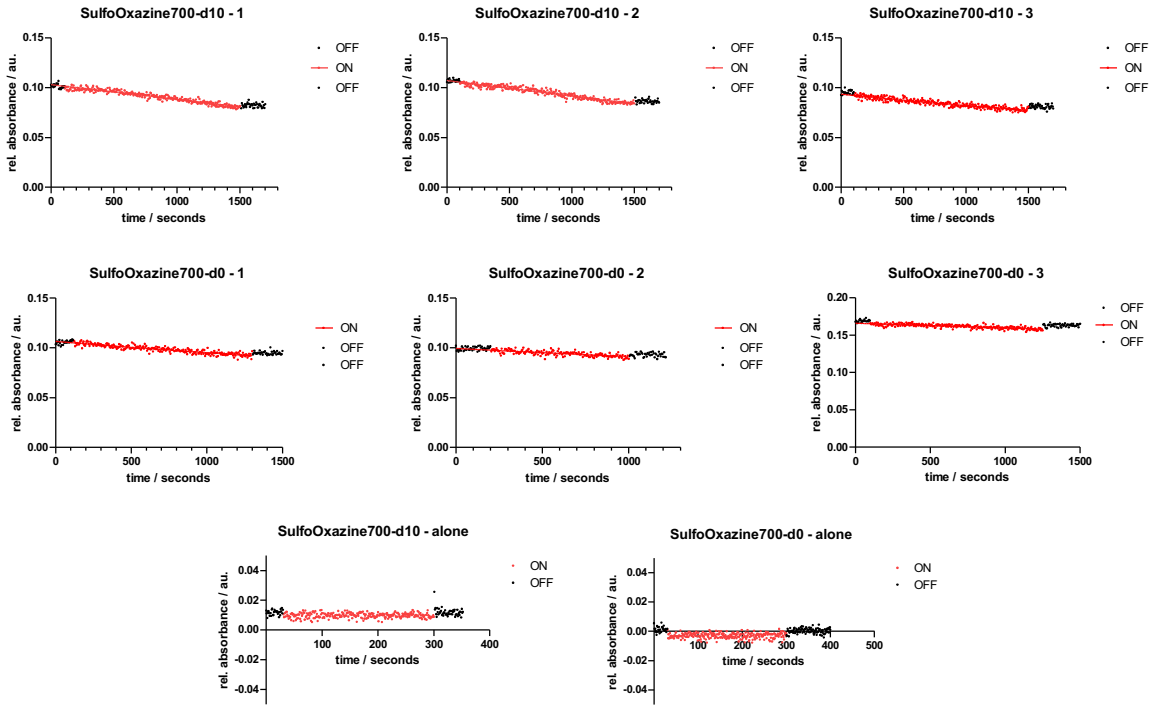
4. Quantum Yield Determination

Absolute fluorescence quantum yields (Φ) were measured using Quantaurus-QY Absolute PL (model C11347-11) with a 150 W xenon light source using a clear window quartz cuvette with long quartz tube mouth (10 mm light path) from Hamamatsu. This instrument uses an integrating sphere to determine photons absorbed and emitted by a sample. Measurements were carried out using dilute samples (A < 0.1) and self-absorption corrections were performed using the instrument software. We validated the quantum yield values using fluorophores with established quantum yield values (i.e. fluorescein in 0.1 M NaOH; measured: 0.88; literature: 0.86– 0.92).

Singlet oxygen quantum yield $\Phi_{\text{singlet oxygen}}$ measurements were carried out on a JASCO V-550 UV/Vis/NIR spectrophotometer with a Hellma quartz glass cuvette (10 mm pathlength). 1,3-diphenylisobenzofuran (DPBF; 97% purity, Vendor: ChemPur) was used as a detector molecule for trapping singlet oxygen. The measurements were carried out with end-concentrations: DPBF = 18.5 µM, ATTO700= 10 µM, **SulfoOxazine700-d10** = 10 µM in ethanol. Light for singlet oxygen generation was delivered by a CoolLED pE-4000 with intensity $I_{635\,nm}$ = 60.0 µW/mm^2. Singlet oxygen kinetics were recorded at 410 nm (for DBPF) under annotated illumination in 1 sec steps. Results were exported and plotted in GraphPad Prism 10 and kinetic traces were fitted monoexponentially in GraphPad Prism 10 and plotted in GraphPad Prism 10.



Supplementary Figure S1: top row: methylene blue and DBPF in ethanol. Control experiments: blank (only ethanol), methylene-blue (without DBPF) in ethanol, DPBF in ethanol (vehicle). Abs at 410 nm, no irradiation during 30s then irritation at 635 nm during 270 s, then no irradiation. Exponential fitting was performed from 30–300 seconds.



Supplementary Figure S2: Top row: SulfoOxazine700-d10 and DPBF in ethanol. Middle row: SulfoOxazine700-d0 and DPBF in ethanol. Control experiments: SulfoOxazine700-d10 in ethanol, SulfoOxazine700-d0 in ethanol, each without DBPF. Abs at 410 nm, no irradiation during 100 s then irritation at 635 nm during at least 1200 s, then no irradiation.

The $\Phi_{\text{singlet oxygen}}$ was calculated by multiplying the ROS QY of methylene blue (MB) ($\Phi_{\text{singlet oxygen}}$ = 0.52 in ethanol)[5] with the slope m of the different samples over the slope of methylene blue by plotting the natural logarithm of absorbance before exposure time (0) against the absorbance after exposure time (t) using equation (1), with m representing the slope of a linear fit:

$$\mathrm{m} = \ln\frac{A(0)}{A(t)} = \ln(A0) - (\ln(A0) + \ln(e^{-kt})) = kt$$

	n = 1	n = 2	n = 3	average	$\Phi_{\text{singlet oxygen}}$
k (Methylene blue)	0.0219	0.02445	0.0231	0.0231	0.52
k (SulfoOxazine700-d10)	1.16×10^{-6}	1.49×10^{-6}	0.000279	9.39×10^{-5}	0.002
k (SulfoOxazine700-d0)	0.000175	1.04×10^{-5}	2.54×10^{-6}	5.93×10^{-5}	0.001
k (vehicle)	0.000293	0.000707	1.73e-007	3.33×10^{-4}	/

Finally, the average was used to calculated $\Phi_{\text{singlet oxygen}}$ of different samples.

5. pH sensitivity

All measurements were taken at ambient temperature (23 ± 2 °C). Fluorescent molecules were prepared as stock solutions in DMSO and diluted to 200 nM in potassium phosphate buffer (120 mM) supplemented with NaCl (50 mM). Fluorescence spectra were acquired in quadruplicates on a black flat bottom 96-well plate on a TECAN infinite 2000Pro plate reader. Experiments were run in quadruplicate and plotted in GraphPad Prism 10.

6. Cell culture and confocal microscopy

CHO-K1 cells were cultured in growth medium (DMEM, Glutamax, 4.5 g *D*-glucose, 10% FCS, 1% PS; Invitrogen) at 37 °C and 5% CO_2. 50 000 cells per well were seeded on 8-well µL slides (Ibidi) previously coated with poly-*L*-lysine (Aldrich, mol wt 70 000–150 000). The next day, cells were incubated with dyes at a concentration of 1 µM in growth medium at 37 °C and 5% CO_2 and imaged directly.

Imaging was performed on a NIKON-CSU-X1 (Nikon Ti Eclipse with automatic stage and Perfect Focus System, equipped with Yokogawa spinning disk (CSU-X1, 1000 scan/s) and EMCCD Camera (AU-888, 13 um pixel) and laser: 405 (120 mW), 488 (200 mW), 638 (200 mW) for fast confocal imaging, objective: 40x air NA 0.95. Emission signals were captured at λ = 700 nm with a 75 nm bandpass.

7. WST-1 Assay

CHO-K1 cells were seeded (12000 cells/well) in two clear 96 well plates and incubated overnight in 100 µL DMEM supplemented with 10% FBS at 37 °C, 5% CO_2. The following day, 1 µM or 10 µM of dye, 10 µM of staurosporin or vehicle (volume corresponding to dye volume) were prepared in full media and then added in six replicates to the cells along with six wells containing no cells. One plate was immediately placed in the dark in the incubator, while another plate was illuminated for 10 min with a LED torchlight (Alonefire X004, 500 luminous flux, 650 nm of light) placed 7 cm above the plate. After illumination, the plate was placed back in the incubator. After 24-hour incubation 10 µL WST-1 (#MK400, Takara Bio) was added to the cells according to the manufacturer's instructions and left to incubate at 37 °C, 5% CO_2. Absorbance was read after 2.5 hours on a TECAN INFINITE M PLEX plate reader (λ_{Abs} = 440 nm) and corrected by subtraction ($\lambda_{Abs\ correct}$ = 660 nm). The data was normalized to "no treatment" (=1.0) plotted using GraphPad Prism 10.

8. Bleaching and fluorescence life-time

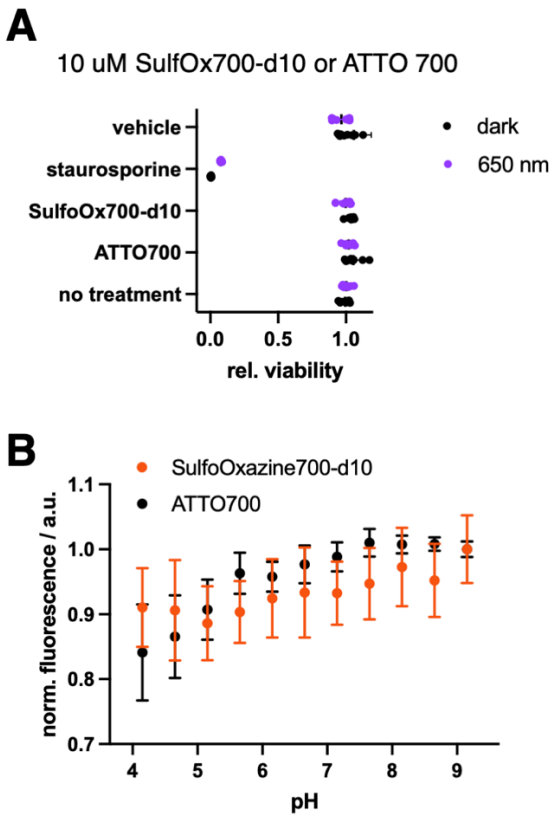
Drops of 10-20 µl of 1µM fluorophore were spotted in 8 Well IBIDI dish. Fluorescence lifetime microscopy was performed using a Leica SP8 with FALCON (Leica Microsystems) equipped with a pulsed white-light excitation laser (80 MHz repetition rate (NKT Photonics)), a 100× objective (HC PL APO CS2 100×/1.40 NA oil), a temperature controlled chamber at room temperature and operated by LAS X. ATTO700 and oxazines were excited using λ = 640 nm, emission signals were captured at λ = 680–784 nm using a Hybrid detector producing FLIM images of 512 × 512 pxl with

303 nm per pxl and 10 frame repetitions. Fluorescence lifetime decay curves from selected regions were fitted with one exponential function and the lifetime is reported for each region. Multiple images were acquired with 100% laser power at $\lambda = 640$ nm, and images integrated to obtain bleaching curves.

9. Single molecule measurements

The PicoQuant MicroTime 200 time-resolved fluorescence system based on an inverted Olympus IX73 microscope (Olympus, Tokyo, Japan) with a 60× Plan-Apo/1.4-NA water-immersion objective using a 690 nm excitation laser was used. Excitation light was split with a dichroic mirror at 488/561/685 nm and detected using a 740/60 band pass. The pinhole was set to 100 µm. For each sample 10 30-second autocorrelation curves were collected before fitting and analyzing with a single component 3D diffusion and triplet state equation integrated in the SynPhoTime 64 software.

10. Supplemental Figures



Supplementary Figure S3. A) WST-1 assay to assess cell viability of CHO-K1 cells after 1 µM exposure to ATTO700 or 10 µM SulfoOxazine700-d10 (± light) over night, normalized to non-treated cells in the dark. Scatter dot plot, 2 biological replicates, n = 5-6. B) pH sensitivity of ATTO700 and SulfoOxazine700-d10 in potassium phosphate buffer (120 mM) supplemented with NaCl (50 mM).

11. References

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