Electronic Supplementary Information

# Optical control of GIRK channels using visible light

Julie B. Trads,[a,b] Jessica Burgstaller,[c] Laura Laprell,[a] David B. Konrad,[a]
Luis de la Osa de la Rosa,[a] C. David Weaver,[d] Herwig Baier,[c]
Dirk Trauner*[a] and David M. Barber*[a]

[a] *Department of Chemistry and Center for Integrated Protein Science, Ludwig Maximilians University Munich, Butenandtstraße 5-13, 81377 Munich, Germany*

[b] *Center for DNA Nanotechnology, Department of Chemistry and iNANO, Aarhus University, Gustav Wieds Vej 14, 8000 Aarhus C, Denmark*

[c] *Max Planck Institute of Neurobiology, Am Klopferspitz 18, 82152 Martinsried, Germany*

[d] *Department of Pharmacology and Institute of Chemical Biology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232, USA*

*dirk.trauner@lmu.de*

*david.barber@cup.uni-muenchen.de*

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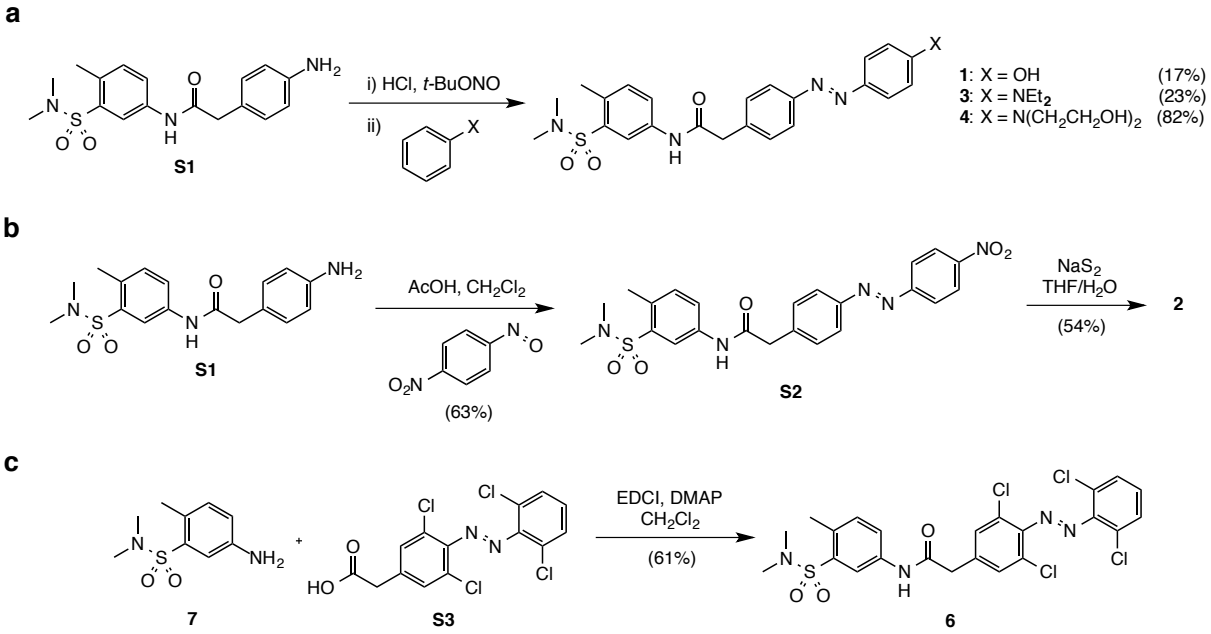



**Fig. S1 Synthesis of compounds 1-4 and 6.** a) Compounds **1**, **3** and **4** were prepared by azo coupling reactions of aniline **S1** with the appropriate aromatic coupling partner. b) Compound **2** was prepared starting from aniline **S1** via a Mills reaction with 4-nitronitrosobenzene followed by reduction of the nitro group using sodium sulfide. c) Compound **6** was prepared from aniline **7** and carboxylic acid **S3** using EDCI and DMAP. Compounds **7**, **S1** and **S3** were prepared according to the literature procedures.[1, 2] All of the analytical data was in accordance with the published values.

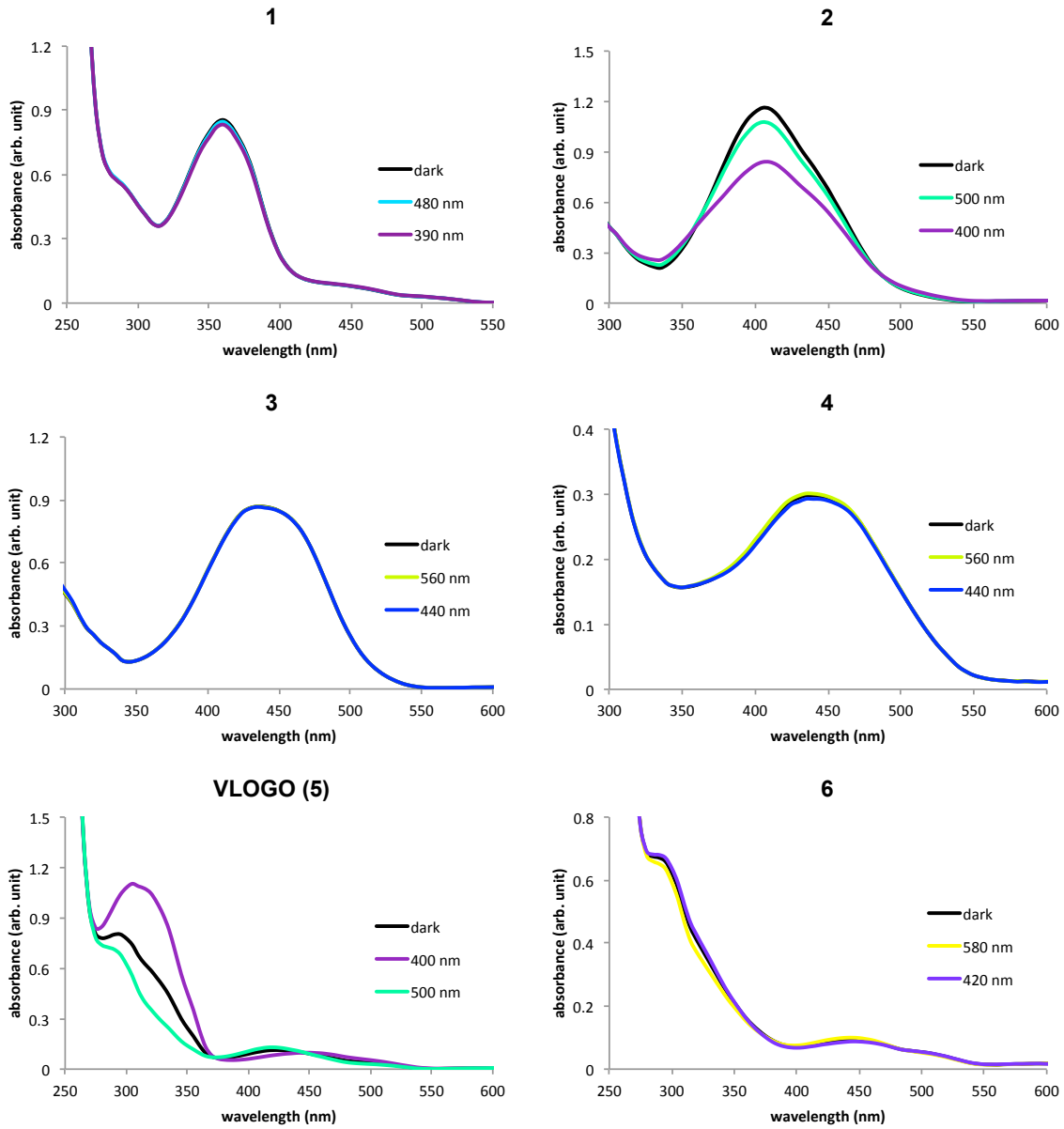



**Fig. S2 UV-Vis absorption spectra of compounds 1-6**. Solutions of **1-6** in DMSO (50 μM) were placed in a 1 mL quartz cuvette (10 mm diameter). A light-fibre cable connected to a Till Photonics Polychrome 5000 monochromator was placed in the cuvette until it penetrated the surface of the solution. Illumination was screened from wavelengths 340–500 nm in 10 nm steps (**1**) and 340-600 nm in 20 nm steps (**2-6**) going from higher to lower wavelengths. Every wavelength was applied for 5 min before a UV-Vis spectrum was recorded. Illumination conditions that afforded the highest *trans*-isomer and *cis*-isomer enrichment are shown in Fig. S2.

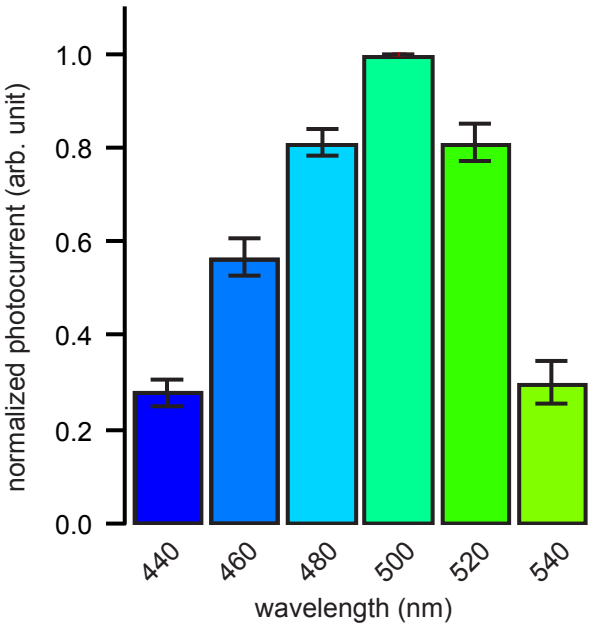



**Fig. S3 Action spectrum of VLOGO.** When using **VLOGO** (10 μM) it was consistently shown that illuminating at 500 nm provided the largest change in current when using 400 nm as the ON wavelength (n=6 cells). Values represent mean ± SEM.

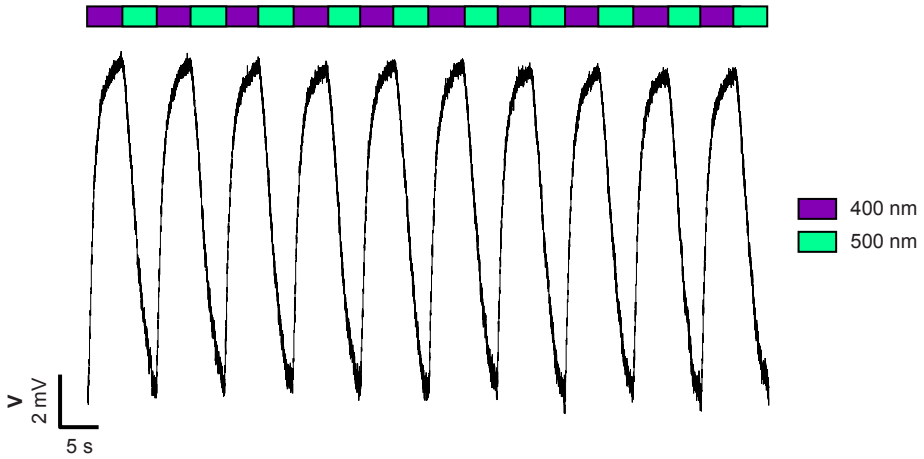



**Fig. S4 Current-clamp photoswitching of VLOGO.** The photoswitching of **VLOGO** (10 μM) is highly reproducible whilst in current clamp mode.

| | GIRK1/2 | | | | GIRK1/4 | | | | GIRK2 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $EC_{50}$ | SEM | $\%E_{max}$ | SEM | $EC_{50}$ | SEM | $\%E_{max}$ | SEM | $EC_{50}$ | SEM | $\%E_{max}$ | SEM |
| **1** | – | – | – | – | – | – | – | – | – | – | – | – |
| **2*** | >2.5 | – | >12 | – | – | – | – | – | – | – | – | – |
| **3** | 3.6 | 0.06 | 78.9 | 4.6 | 6.7 | 0.10 | 118.4 | 7.9 | – | – | – | – |
| **4** | – | – | – | – | – | – | – | – | – | – | – | – |
| **VLOGO** | 2.0 | 0.11 | 49.4 | 4.7 | 1.9 | 0.06 | 30.9 | 5.2 | – | – | – | – |
| **6*** | – | – | – | – | – | – | – | – | – | – | – | – |

**Table S1 Potency, efficacy and selectivity of compounds 1-6**. Shown are the potency and efficacy values obtained from testing **compounds 1-6** using the thallium flux assay on cell lines stably expressing GIRK1/2, GIRK1/4 and GIRK2. Efficacy values were normalised to the maximum activity observed using **ML297** (10 μM) on GIRK1/2 expressing cells. Error values shown are the SEM obtained from three independent experiments. In instances where values are described as greater than a certain value (e.g. **2** $EC_{50}$ >2.5 μM), the compound displayed clear concentration-dependent efficacy but either the potency of the compound or the solubility of the compound was too low to obtain an accurate estimate of the potency and efficacy. * These compounds exhibited a small amount of activity that could not be easily quantified.

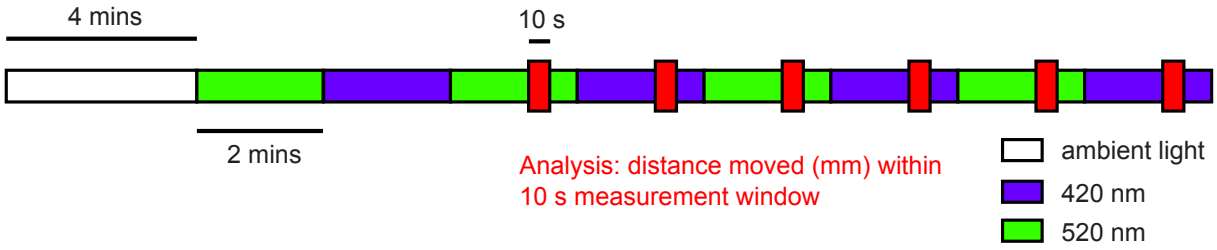



**Fig. S5 Protocol used for the evaluation of VLOGO in zebrafish larvae.** The zebrafish larvae were exposed to ambient light for an initial 4 minutes. Then four cycles of green light (520 nm) and violet light (420 nm) were applied at 2 minute intervals. The red bars indicate the 10 seconds within each interval of the last 3 cycles where the distance moved by the zebrafish larvae was analysed to calculate the average value for each zebrafish.

# Methods

**HEK293T Cell Electrophysiology.** HEK293T cells were incubated in Dulbecco´s MEM supplemented with 10% FBS and split at 80-90% confluency. For detachment, the growth medium was removed, the cells were washed with calcium and magnesium free PBS buffer and then treated with trypsin solution 2 min at 37 °C. The detached cells were diluted with growth medium and singularised by pipetting. For transfection, acid-etched coverslips were coated with poly-L-lysin and placed in a 24-well plate. 40,000 cells were added to each well in 500 µL standard growth medium. DNA (per coverslip: 350 ng GIRK1/2 and 50 ng YFP) was mixed with 1 µL polyplus jetprime in 50 µL jetprime buffer. After standing at room temperature for 10-15 min, the DNA-mix was added to the cells shortly after seeding them into the abovementioned 24-well-plate. After 3-5 hours, the medium was exchanged for standard growth medium. Cells were used for electrophysiological recordings 24-48 hours post transfection.

Whole-cell patch clamp experiments were performed using a standard electrophysiology setup equipped with a HEKA Patch Clamp EPC10 USB amplifier and PatchMaster software (HEKA Electronik). Micropipettes were generated from a Science Products GB200-F-8P with filament pipettes using a vertical puller. Resistance varied between 3-7 MΩ. The extracellular solution contained in mM: 115 NaCl, 50 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 11 glucose and 5 HEPES (KOH to pH 7.4). The intracellular solution contained in mM: 4 NaCl, 107 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 EGTA, 2 MgATP, 0.3 $Na_2GTP$ and 5 HEPES (KOH to pH 7.2). The holding potential for voltage-clamp experiments was −60 mV. All compounds were diluted into the extracellular solution from 100 mM DMSO stock solutions. Illumination during electrophysiology experiments was provided by a TILL Photonics Polychrome 5000 monochromator.

**Hippocampal Neuron Electrophysiology.** All animal procedures were performed in accordance with the guidelines of the Regierung Oberbayern. Horizontal slices were prepared from C57Bl6JRj mice (postnatal day 10-13). Following decapitation, the brain was rapidly removed and transferred to an ice-cold saline solution composed of (in mM) 87 NaCl, 75 sucrose, 25 $NaHCO_3$, 2.5 KCl, 1.25 $NaH_2PO_4$, 0.5 $CaCl_2$, 7 $MgCl_2$, 25 glucose saturated with carbogen (95% $O_2$/5% $CO_2$). Slices (300 µM thick) were cut with a vibratome (NPI Electronic), incubated at 34 °C for 1 hour in saline solution and then kept at room temperature for up to 6 hours before being used in patch-clamp recordings. Experiments were carried out

in ACSF composed of (in mM) 125 NaCl, 26 $NaHCO_3$, 2.5 KCl, 1.25 $NaH_2PO_4$, 2 $CaCl_2$, 1 $MgCl_2$ and 20 glucose saturated with carbogen at room temperature.

Hippocampal neurons were patched using glass electrodes (Science Products) with a resistance of 6–9 MΩ. Current-clamp recordings were carried out using the following intracellular solution (in mM): 140 K-gluconate, 10 HEPES, 12 KCl, 4 NaCl, 4 MgATP, 0.4 $Na_2GTP$. Recordings were made with an EPC 10 USB amplifier, controlled by the Patchmaster software (HEKA). Data was filtered at 2.9 and 10 kHz. Data was analyzed using the Patcher's Power Tools (MPI Göttingen) and IgorPro (Wavemetrics). **VLOGO** was diluted into the extracellular solution from a 100 mM DMSO stock solution. Photoswitching was achieved through a microscope coupled monochromator (Poly V, FEI).

**Thallium Flux Assays.** HEK293 cells co-expressing human GIRK1/2 and GIRK1/4 were constructed as previously described.[3] For the GIRK2 expressing cell line, HEK293 cells (ATCC) were transfected with GIRK2 in pCMV6-A-puro (Origene) using FuGene 6 (Promega) transfection reagent. All cells were maintained in Minimal Essential Medium, Alpha Medium (Mediatech) supplemented with 10% FBS (Sigma-Aldrich) and GlutaGro (Mediatech), referred to henceforth as cell culture medium. Cell culture medium was further supplemented with selection antibiotics, as appropriate.

Thallium flux assays, test compound solubilisation, serial dilutions and data analysis were performed as previously described.[3] with the following exceptions: the plate reader used was a Panoptic, WaveFront Biosciences, data were acquired at 1 Hz (excitation 494 ± 10 nm, emission 538 ± 20 nm), the thallium stimulus buffer contained in mM: 125 $NaHCO_3$, 1.8 $CaSO_4$, 1 $MgSO_4$, 5 glucose, 2 $Tl_2SO_4$, 10 HEPES (pH 7.4) and the slopes of vehicle control-subtracted data were calculated from five data points beginning with the point 2 seconds after thallium stimulus buffer addition. The potency and efficacy values depicted in the Figure 3 and Table S1 were obtained from three independent experiments.

**Zebrafish Maintenance and Care.** Adult zebrafish (*Danio rerio*) were maintained and bred at 28 °C on a 14/10 h light/dark cycle. All animal procedures were performed in accordance with approved protocols. All experiments were performed using larvae 5-7 days post fertilisation.

**Behavioral Tests in *Danio rerio* Zebrafish Larva.** For experiments, *Danio rerio* zebrafish larva at 5-7 days after fertilisation were placed in a 96-well plate at a density of one animal/well in fish water for video recording. To correct for possible sources of variability, all behavioral

assays were performed under carefully controlled experimental conditions including timings, location and setup for stimulation and video recording. 4 minutes of an initial adaptation period were followed by a 16 minute behavioral trial. Light-dependent behavior was induced by changes in illumination between intervals of 2 minutes violet light (420 nm) and 2 minutes green light (520 nm). Basal motility was measured prior to the addition of **VLOGO**, **ML297** and DMSO as control. 100 μM of compounds or vehicle (1% DMSO) were added and motility was measured again after 60 min incubation. Light cycles were repeated consecutively four times. Zebrafish motility was measured as distance moved during a representative 10 second period within each interval of violet and green light in the last three cycles of the protocol. Animal tracking was obtained using the Noldus DanioVision system. The action of **VLOGO** (n=18 zebrafish), **ML297** (n=15 zebrafish) and DMSO (n=15 zebrafish) was averaged over the declared number of individual fish and quantified as an increase or decrease in distance moved (in mm) compared to pre-drug baseline. Illumination was provided by a M420L3 (violet 420 nm, 1000 mA, 750 mW) mounted LED driven by a LEDD1B T-Cube LED Driver (1200 mA) using ACL2520U-A lenses (Thorlabs) and a UHP-Mic-LED-520 (green 520 nm, 900 mW) ultra high power LED (Prizmatix).

## References


1. D. M. Barber, M. Schönberger, J. Burgstaller, J. Levitz, C. D. Weaver, E. Y. Isacoff, H. Baier and Dirk Trauner, *Chem. Sci.*, 2016, **7**, 2347–2352.
2. D. B. Konrad, J. A. Frank and D. Trauner, *Chem. Eur. J.*, 2016, **22**, 4364–4368.
3. K. Kaufmann, I. Romaine, E. Days, C. Pascual, A. Malik, L. Yang, B. Zou, Y. Du, G. Sliwoski, R. D. Morrison, J. Denton, C. M. Niswender, J. S. Daniels, G. A. Sulikowski, X. Xie, C. W. Lindsley and C. D. Weaver, *ACS Chem. Neurosci.*, 2013, **4**, 1278−1286.

## Methods for Chemical Synthesis

**General Experimental Techniques.** All reactions were conducted using oven-dried glassware (120 °C) under a positive pressure of nitrogen with magnetic stirring unless otherwise stated. Liquid reagents and solvents were added via syringe or oven-dried stainless steel cannulas through rubber septa. Solids were added under inert gas counter flow or were dissolved in specified solvents prior to addition. Low temperature reactions were carried out in a Dewar vessel filled with the appropriate cooling agent e.g. $H_2O$/ice (0 °C). Reactions using temperatures above room temperature were conducted using a heated oil bath. Yields refer to spectroscopically pure compounds unless otherwise stated.

**Solvents and Reagents.** Tetrahydrofuran (THF) was distilled under a nitrogen atmosphere from Na/benzophenone prior to use. Dichloromethane ($CH_2Cl_2$) and Hünig's base (DIPEA) were distilled under a nitrogen atmosphere from $CaH_2$ prior to use. Dry *N*,*N*-dimethylformamide (DMF), methanol (MeOH) and toluene (PhMe) were purchased from commercial suppliers and used as received. Solvents for extraction and flash column chromatography were purchased in technical grade purity and distilled under reduced pressure prior to use. All other reagents and solvents were purchased from commercial suppliers and used as received.

**Chromatography.** Reactions and chromatography fractions were monitored by qualitative thin-layer chromatography (TLC) on silica gel $F_{254}$ TLC plates from Merck KGaA. Analytes on the glass plates were visualized by irradiation with UV light and by immersion of the TLC plate in an appropriate staining solution followed by heating with a hot-air gun. The following staining solutions were applied: Hanessian's (CAM) staining solution [$Ce(SO_4)_2$ (5.0 g), $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (25 g), concentrated aqueous $H_2SO_4$ (50 mL) and $H_2O$ (450 mL)]; potassium permanganate staining solution [$KMnO_4$ (3.0 g), $K_2CO_3$ (20 g), 5% aqueous NaOH (5.0 mL) and $H_2O$ (300 mL)]; ninhydrin staining solution [ninhydrin (20.0 g) and ethanol (600 mL)]. Flash column chromatography was performed using silica gel, particle size 40–63 μm (eluents are given in parenthesis).

**Compound Naming.** Compound names were generated using ACD/I-Lab software according to standard IUPAC nomenclature.

**Melting Points.** Melting points were measured on an EZ-Melt apparatus form Stanford Research Systems and are uncorrected.

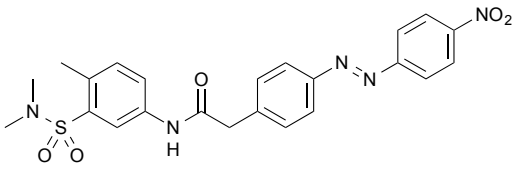
**NMR Spectroscopy.** NMR spectra were measured on Bruker Avance III HD 800 MHz and 400 MHz spectrometers operating at 800 MHz and 400 MHz for proton nuclei, 200 MHz and 100 MHz for carbon nuclei and 376 MHz for fluorine nuclei. The $^{1}H$ and $^{13}C$ NMR shifts are reported in ppm related to the chemical shift of tetramethylsilane. $^{1}H$ NMR shifts were calibrated to residual solvent resonances: $CDCl_3$ (7.26 ppm), $CD_3OD$ (3.31 ppm), $(CD_3)_2SO$ (2.50 ppm), $C_6D_6$ (7.16 ppm) and THF-$d_8$ (1.73 ppm). $^{13}C$ NMR shifts were calibrated to the centre of the multiplet signal of the residual solvent resonance: $CDCl_3$ (77.16 ppm), $CD_3OD$ (49.00 ppm), $(CD_3)_2SO$ (39.52 ppm), $C_6D_6$ (128.06 ppm) and THF-$d_8$ (25.37). The $^{19}F$ NMR shifts are reported in ppm related to the chemical shift of trichlorofluoromethane. NMR spectroscopic data are reported as follows: Chemical shift in ppm (multiplicity, coupling constants, integration). The multiplicities are abbreviated with s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Except for multiplets, the chemical shift of all signals is reported as the centre of the resonance range. Additionally to $^{1}H$ and $^{13}C$ NMR measurements, 2D NMR techniques such as homonuclear correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond coherence (HMBC) were used to assist the compound identification process. Coupling constants *J* are reported in Hz. All raw fid files were processed and the spectra analysed using the program MestReNova 9.0 from Mestrelab Research S. L.

**Infrared Spectroscopy.** IR spectra were recorded on a PerkinElmer Spectrum BX II FT-IR instrument equipped with an ATR unit. The measured wave numbers are reported in $cm^{-1}$.

**Mass Spectrometry.** All high-resolution mass spectra (HRMS) were recorded by the LMU Mass Spectrometry Service. HRMS were recorded on MAT 95 (EI) and MAT 90 (ESI) spectrometers from Thermo Finnigan GmbH. The method used is reported in the experimental section.

**UV-Vis Spectroscopy.** UV-Vis spectra were recorded on a Varian Cary 50 Scan UV-Vis spectrometer using Helma SUPRASIL precision cuvettes (10 mm light path). Illumination was provided by a TILL Photonics Polychrome 5000 monochromator.

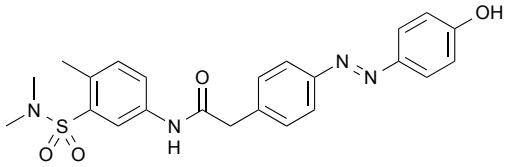
**Synthesis and characterisation of *N*-[3-(dimethylsulfamoyl)-4-methylphenyl]-2-{4-[(*E*)-(4-nitrophenyl)diazenyl]phenyl}acetamide (S2)**



To a solution of compound **S1** (94 mg, 0.250 mmol) in a mixture of $CH_2Cl_2$/AcOH (10 mL, 1:1) at r.t. was added 1-nitro-4-nitrosobenzene (190 mg, 1.25 mmol). The reaction mixture was stirred at r.t. for 1 h and then concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel eluting with *i*-Hex/EtOAc (2:1 → 6:4) to afford compound **S2** (76 mg, 63%) as an orange solid.

**mp**: 198 – 200 °C; **TLC** (*i*-Hex/EtOAc, 6:4): $R_f$ = 0.21 (UV/CAM); **$^1$H NMR** (400 MHz, $(CD_3)_2SO$): δ 10.52 (s, 1H), 8.47 – 8.40 (m, 2H), 8.13 – 8.03 (m, 3H), 7.99 – 7.92 (m, 2H), 7.77 (dd, *J* = 8.0, 2.5 Hz, 1H), 7.64 – 7.56 (m, 2H), 7.40 – 7.35 (m, 1H), 3.82 (s, 2H), 2.72 (s, 6H), 2.48 (s, 3H); **$^{13}$C NMR** (100 MHz, $(CD_3)_2SO$): δ 168.8, 155.1, 150.7, 148.4, 140.9, 137.3, 135.6, 133.3, 131.5, 130.6, 125.1, 123.4, 123.2, 122.9, 119.5, 43.0, 36.9, 19.6; **IR** (thin film): 3275, 1660, 1607, 1589, 1523, 1491, 1455, 1388, 1342, 1252, 1189, 1153, 1142, 1107, 1059, 956, 912, 863, 829, 729 $cm^{-1}$; **HRMS** (ESI, *m/z*): [$(M+H)^+$] calcd. for $C_{23}H_{24}N_5O_5S^+$, 482.1493; found 482.1498; **UV-Vis**: $\lambda_{max}$ = 350 nm (50 μM in DMSO).

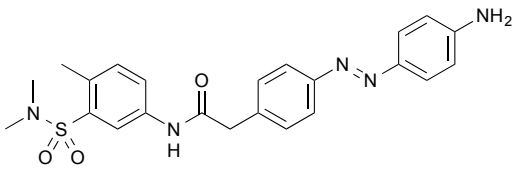
**Synthesis and characterisation of *N*-[3-(dimethylsulfamoyl)-4-methylphenyl]-2-{4-[(*E*)-(4-hydroxyphenyl)diazenyl]phenyl}acetamide (1)**



To a solution of compound **S1** (50 mg, 0.140 mmol) in MeOH (5.0 mL) at 0 °C was added conc. HCl (0.10 mL) dropwise. *t*-Butyl nitrite (17 mg, 0.150 mmol, 20 μL, 90% purity) was added dropwise and the resulting solution was stirred at 0 °C for 1 h. The diazonium salt was then added dropwise to a flask containing a solution of phenol (13 mg, 0.140 mmol) in aq. NaOH (2.0 mL, 6 M) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h. The reaction mixture was adjusted to pH = 7 using aq. HCl (5 M) and extracted with EtOAc (2 × 10 mL). The combined organic extracts were dried over $Na_2SO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography on silica gel eluting with *i*-Hex/EtOAc (1:1) afforded compound **1** (11 mg, 17%) as a pale yellow oil.

**TLC** (*i*-Hex/EtOAc, 1:1): $R_f$ = 0.34 (UV/CAM); **$^1$H NMR** (400 MHz, THF-$d_8$): δ 9.53 (br s, 1H), 9.09 (br s, 1H), 7.98 – 7.89 (m, 2H), 7.84 – 7.77 (m, 4H), 7.52 – 7.45 (m, 2H), 7.25 (d, *J* = 8.0 Hz, 1H), 6.91 – 6.83 (m, 2H), 3.71 (s, 2H), 2.75 (s, 6H), 2.53 (s, 3H); **$^{13}$C NMR** (100 MHz, THF-$d_8$): δ 169.3, 161.9, 152.7, 147.2, 139.0, 138.8, 137.6, 133.8, 132.8, 130.7, 125.7, 123.6, 123.3, 121.0, 116.5, 44.5, 37.4, 20.2; **IR** (neat): 3331, 2959, 2361, 1666, 1598, 1528, 1504, 1465, 1438, 1388, 1309, 1273, 1139, 1100, 1059, 1014, 957, 844, 796, 730, 704 $cm^{-1}$; **HRMS** (ESI, *m/z*): [$(M+H)^+$] calcd. for $C_{23}H_{25}N_4O_4S^+$, 453.1591; found 453.1595; **UV-Vis**: $\lambda_{max}$ = 360 nm (50 μM in DMSO).

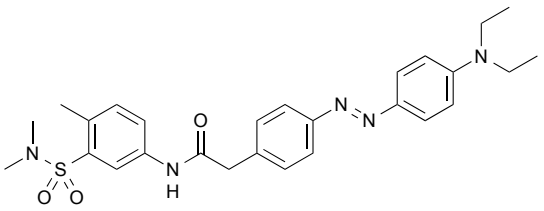
**Synthesis and characterisation of 2-{4-[(*E*)-(4-aminophenyl)diazenyl]phenyl}-*N*-[3-(dimethylsulfamoyl)-4-methylphenyl]acetamide (2)**



To a solution of compound **S1** (24 mg, 0.050 mmol) in THF/$H_2O$ (4.0 mL, 3:1) at r.t. was added sodium sulfide (44 mg, 0.600 mmol). The reaction mixture was sealed in a pressure tube and was stirred at 90 °C for 4 h. The resulting mixture was cooled to r.t., quenched with aq. NaOH (10 mL, 2.0 M) and extracted with EtOAc (2 × 20 mL). The combined organic extracts were washed with sat. aq. $NaHCO_3$ (20 mL), dried over $Na_2SO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography on silica gel eluting with *i*-Hex/EtOAc (2:1 → 1:1) afforded compound **2** (12.3 mg, 54%) as an orange oil.

**TLC** (*i*-Hex/EtOAc, 1:1): $R_f$ = 0.19 (UV/CAM); **$^1$H NMR** (400 MHz, $CD_3OD$): δ 8.16 (d, *J* = 2.5 Hz, 1H), 7.82 – 7.66 (m, 5H), 7.51 – 7.43 (m, 2H), 7.33 (d, *J* = 8.5 Hz, 1H), 6.83 – 6.75 (m, 2H), 3.76 (s, 2H), 2.80 (s, 6H), 2.55 (s, 3H); **$^{13}$C NMR** (100 MHz, $CD_3OD$): δ 172.1, 153.1, 152.5, 146.0, 138.3, 137.4, 134.4, 134.3, 131.0, 126.3, 124.9, 123.3, 121.8, 115.9, 44.4, 37.6, 20.3; **IR** (thin film): 3372, 1652, 1626, 1600, 1492, 1429, 1383, 1308, 1139, 1059, 955, 837, 729 $cm^{-1}$; **HRMS** (ESI, *m/z*): [$(M+H)^+$] calcd. for $C_{23}H_{26}N_5O_3S^+$, 452.1751; found 452.1756; **UV-Vis**: $\lambda_{max}$ = 405 nm (50 μM in DMSO).

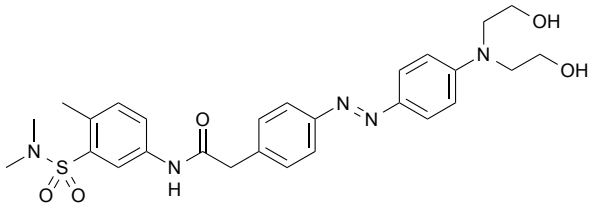
**Synthesis and characterisation of 2-(4-{(*E*)-[4-(diethylamino)phenyl]diazenyl}phenyl)-*N*-[3-(dimethylsulfamoyl)-4-methylphenyl]acetamide (3)**



To a solution of compound **S1** (50 mg, 0.140 mmol) in MeOH (2.0 mL) at 0 °C was added conc. HCl (0.10 mL) dropwise. *t*-Butyl nitrite (17 mg, 0.150 mmol, 20 μL, 90% purity) was added dropwise and the resulting solution was stirred at 0 °C for 30 min. The diazonium salt was then added dropwise to a flask containing a solution of *N*,*N*-diethylaniline (22 mg, 0.150 mmol, 24 μL) in MeOH (2.0 mL) and conc. HCl (0.10 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 2 h and then at r.t. for 14 h. The reaction was quenched with sat. aq. $NaHCO_3$ (10 mL), diluted with $H_2O$ (10 mL) and extracted with EtOAc (2 × 20 mL). The combined organic extracts were dried over $Na_2SO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography on silica gel eluting with *i*-Hex/EtOAc (2:1) afforded compound **3** (16 mg, 23%) as a red solid.

**mp**: 54 – 55 °C; **TLC** (*i*-Hex/EtOAc, 2:1): $R_f$ = 0.22 (UV/CAM); **$^1$H NMR** (400 MHz, $CDCl_3$): δ 8.04 – 7.69 (m, 6H), 7.44 (d, *J* = 8.0 Hz, 2H), 7.23 (t, *J* = 8.0 Hz, 2H), 6.96 – 6.87 (m, 1H), 6.73 (br s, 1H), 3.82 (s, 2H), 3.46 (q, *J* = 7.0 Hz, 4H), 2.82 – 2.77 (m, 6H), 2.56 – 2.52 (m, 3H), 1.23 (t, *J* = 7.0 Hz, 6H); **$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 170.1, 169.4, 159.2, 136.4 – 135.9 (m), 133.5, 130.8, 130.3, 126.1 – 126.0 (m), 124.3 – 124.0 (m), 123.0 – 122.6 (m), 121.0 – 120.8 (m), 114.8, 111.6 – 110.9 (m), 55.4, 45.2 – 44.7 (m), 37.6 – 37.2 (m), 20.5 – 20.1 (m), 13.1 – 12.5 (m); **IR** (neat): 3320, 2970, 1666, 1596, 1512, 1447, 1389, 1311, 1271, 1247, 1194, 1155, 1135, 1076, 1059, 1033, 1011, 953, 822, 793, 730 $cm^{-1}$; **HRMS** (ESI, *m/z*): [$(M+H)^+$] calcd. for $C_{27}H_{34}N_5O_3S^+$, 508.2377; found 508.2381; **UV-Vis**: $\lambda_{max}$ = 435 nm (50 μM in DMSO).

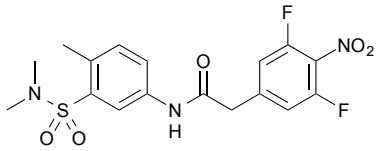
**Synthesis and characterisation of 2-{4-[(*E*)-{4-[bis(2-hydroxyethyl)amino]phenyl}diazenyl] phenyl}-*N*-[3-(dimethylsulfamoyl)-4-methylphenyl]acetamide (4)**



To a solution of compound **S1** (50 mg, 0.140 mmol) in MeOH (2.0 mL) at 0 °C was added conc. HCl (0.10 mL) dropwise. *t*-Butyl nitrite (17 mg, 0.150 mmol, 20 μL, 90% purity) was added dropwise and the resulting solution was stirred at 0 °C for 30 min. The diazonium salt was then added dropwise to a flask containing a solution of *N*-phenyldiethanolamine (30 mg, 0.150 mmol) in MeOH (2.0 mL) and conc. HCl (0.10 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 2 h and then at r.t. for 14 h. The reaction was quenched with sat. aq. $NaHCO_3$ (10 mL), diluted with $H_2O$ (10 mL) and extracted with EtOAc (2 × 20 mL). The combined organic extracts were dried over $Na_2SO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography on silica gel eluting with $CH_2Cl_2$/MeOH (99:1 → 95:5) afforded compound **4** (62 mg, 82%) as a red oil.

**TLC** (EtOAc): $R_f$ = 0.35 (UV/CAM); **$^1$H NMR** (400 MHz, $CD_3OD$): δ 8.16 (d, *J* = 2.5 Hz, 1H), 7.84 – 7.75 (m, 4H), 7.71 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.51 – 7.44 (m, 2H), 7.35 (d, *J* = 8.5 Hz, 1H), 6.91 – 6.84 (m, 2H), 3.83 – 3.73 (m, 6H), 3.71 – 3.63 (m, 4H), 2.81 (s, 6H), 2.56 (s, 3H); **$^{13}$C NMR** (100 MHz, $CD_3OD$): δ 172.1, 153.5, 152.2, 144.7, 138.3, 138.1, 137.4, 134.4, 134.3, 130.9, 126.1, 124.9, 123.3, 121.8, 112.7, 60.3, 55.0, 44.4, 37.6, 20.3; **IR** (thin film): 3328, 2926, 1670, 1598, 1513, 1391, 1315, 1141, 1059, 956, 825, 733 $cm^{-1}$; **HRMS** (ESI, *m/z*): [$(M+H)^+$] calcd. for $C_{27}H_{34}N_5O_5S^+$, 540.2275; found 540.2280; **UV-Vis**: $\lambda_{max}$ = 440 nm (50 μM in DMSO).

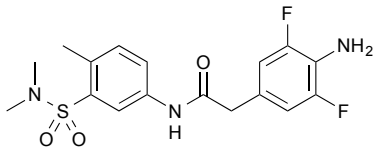
**Synthesis and characterisation of 2-(3,5-difluoro-4-nitrophenyl)-*N*-[3-(dimethylsulfamoyl)-4-methylphenyl]acetamide (9)**




To a suspension of compound **8** (450 mg, 2.07 mmol) in $CH_2Cl_2$ (10 mL) at 0 °C was added a solution of oxalyl chloride (1.08 mL, 2.17 mmol, 2.0 M in $CH_2Cl_2$) and DMF (4 drops). The resulting mixture was warmed to r.t., stirred for 30 min and then concentrated under a stream of nitrogen. The residue was dissolved in DMF (10 mL) and then added dropwise to a solution of compound **7** (386 mg, 1.80 mmol) in DMF (10 mL) and DIPEA (465 mg, 3.60 mmol, 627 μL) at 0 °C. The resulting mixture was warmed to r.t. and stirred for 16 h. The reaction mixture was diluted with $H_2O$ (100 mL), brine (20 mL) and extracted with $Et_2O$ (2 × 100 mL). The combined organic extracts were washed with a mixture of $H_2O$/brine (5:1, 2 × 120 mL), dried over $Na_2SO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography on silica gel eluting with *i*-Hex/EtOAc (2:1 → 6:4) afforded compound **9** (380 mg, 56%) as a pale yellow oil.

**TLC** (*i*-Hex/EtOAc, 6:4): $R_f$ = 0.27 (UV/ninhydrin); **$^1$H NMR** (400 MHz, THF-$d_8$): δ 9.67 (br s, 1H), 7.95 (d, *J* = 2.5 Hz, 1H), 7.87 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.37 – 7.22 (m, 3H), 3.79 (s, 2H), 2.75 (s, 6H), 2.53 (s, 3H); **$^{13}$C NMR** (100 MHz, THF-$d_8$): δ 167.7, 155.1 (dd, *J* = 258.5, 2.5 Hz), 144.2 (t, *J* = 10.0 Hz), 138.3, 137.7, 133.9, 133.3, 123.7, 121.1, 115.3 – 114.8 (m), 43.5, 37.6, 20.7; **$^{19}$F NMR** (376 MHz, THF-$d_8$): δ −112.0 (d, *J* = 9.0 Hz); **IR** (thin film): 3329, 2934, 1672, 1625, 1596, 1534, 1491, 1446, 1389, 1364, 1328, 1159, 1142, 1057, 957, 842, 732 $cm^{-1}$; **HRMS** (ESI, *m/z*): [$(M-H)^-$] calcd. for $C_{17}H_{16}F_2N_3O_5S^-$, 412.0784; found 412.0789.

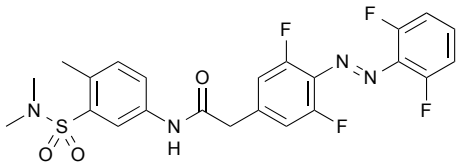
**Synthesis and characterisation of 2-(4-amino-3,5-difluorophenyl)-*N*-[3-(dimethylsulfamoyl) -4-methylphenyl]acetamide (10)**




To a solution of compound **9** (50 mg, 0.120 mmol) in MeOH/EtOAc (1:1, 10 mL) at r.t. was added Pd/C (4.9 mg, 10 wt. % Pd labelling). The reaction vessel was flushed with hydrogen (×3) and the resulting mixture was stirred at r.t. for 16 h. The reaction mixture was filtered through a pad of Celite® washing with MeOH (20 mL). The filtrate was concentrated under reduced pressure and purified by flash column chromatography on silica gel eluting with *i*-Hex/EtOAc (6:4) to afford compound **10** (41 mg, 89%) as a colourless oil.

**TLC** (*i*-Hex/EtOAc, 6:4): $R_f$ = 0.32 (UV/ninhydrin); **$^1$H NMR** (400 MHz, $CD_3OD$): δ 8.13 (d, *J* = 2.5 Hz, 1H), 7.68 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.32 (d, *J* = 8.5 Hz, 1H), 6.87 – 6.82 (m, 2H), 3.55 (s, 2H), 2.80 (s, 6H), 2.54 (s, 3H); **$^{13}$C NMR** (100 MHz, $CD_3OD$): δ 172.1, 153.3 (dd, *J* = 239.5, 9.0 Hz), 138.2, 137.4, 134.4, 134.3, 124.9, 124.7 (t, *J* = 9.0 Hz), 121.8, 113.0 – 112.4 (m), 43.4, 37.6, 20.3; **$^{19}$F NMR** (376 MHz, $CD_3OD$) δ −134.19 – −134.23 (m); **IR** (thin film): 3454, 3364, 2931, 1651, 1606, 1591, 1524, 1491, 1451, 1432, 1386, 1325, 1141, 1059, 955, 830, 731 $cm^{-1}$; **HRMS** (ESI, *m/z*): [$(M+H)^+$] calcd. for $C_{17}H_{20}F_2N_3O_3S^+$, 384.1188; found 384.1193.

**Synthesis and characterisation of 2-{4-[(2,6-difluorophenyl)diazenyl]-3,5-difluoro phenyl}-*N*-[3-(dimethylsulfamoyl)-4-methylphenyl]acetamide (VLOGO)**

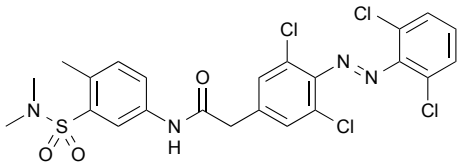


To a solution of compound **10** (38 mg, 0.100 mmol) in a mixture of PhMe/AcOH (2.4 mL, 1:1 mL) at r.t. was added TFA (0.2 mL) and 1,3-difluoro-2-nitrosobenzene (**11**) (72 mg, 0.500 mmol). The reaction mixture was stirred at r.t. for 24 h and then concentrated under a stream of nitrogen. The resulting residue was diluted with EtOAc (10 mL), $H_2O$ (10 mL) and sat. aq. $NaHCO_3$ (5 mL). The organic layer was decanted and the aqueous layer was extracted with EtOAc (10 mL). The combined organic extracts were dried over $Na_2SO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography on silica gel eluting with *i*-Hex/EtOAc (2:1 → 1:1) afforded **VLOGO** (8.3 mg, 16%, 51:49 mixture of geometric isomers) as a yellow oil. Further elution of the silica gel column with *i*-Hex/EtOAc (1:1) afforded an additional fraction of **VLOGO** (7.2 mg, 14%, 68:32 mixture of geometric isomers) as a yellow oil.

**VLOGO Fraction 1**: All characterisation data reported corresponds to the mixture of geometric isomers (51:49). **TLC** (*i*-Hex/EtOAc, 1:1): $R_f$ = 0.46 (UV/$KMnO_4$); **$^1$H NMR** (800 MHz, $CDCl_3$): δ 8.18 – 8.13 (m, 1H), 8.12 – 8.06 (m, 1H), 8.06 – 8.01 (m, 2H), 7.83 (d, *J* = 2.5 Hz, 1H), 7.77 (d, *J* = 2.5 Hz, 1H), 7.37 (tt, *J* = 8.5, 5.5 Hz, 1H), 7.29 (t, *J* = 8.5 Hz, 2H), 7.21 (tt, *J* = 8.5, 6.0 Hz, 1H), 7.17 – 7.13 (m, 2H), 7.09 – 7.03 (m, 2H), 6.96 – 6.92 (m, 2H), 6.87 (t, *J* = 8.0 Hz, 2H), 3.77 (s, 2H), 3.65 (s, 2H), 2.82 (s, 6H), 2.80 (s, 6H), 2.58 (s, 3H), 2.57 (s, 3H); **$^{13}$C NMR** (200 MHz, $CDCl_3$): δ 167.7, 167.7, 155.7 (dd, *J* = 261.0, 3.5 Hz), 151.8 (ddd, *J* = 254.5, 17.0, 5.5 Hz), 139.4 (t, *J* = 10.0 Hz), 137.5 (t, *J* = 9.0 Hz), 136.3, 136.2, 135.5, 133.9, 133.7, 133.7, 132.3 – 131.8 (m), 131.7 (t, *J* = 10.5 Hz), 131.4 – 130.7 (m), 130.2 (t, *J* = 9.5 Hz), 124.5, 124.4, 121.1, 113.8 (dd, *J* = 21.0, 2.5 Hz), 113.3 (dd, *J* = 21.0, 2.5 Hz), 112.8 (dd, *J* = 20.0, 3.5 Hz), 112.3 (dd, *J* = 20.0, 3.5 Hz), 44.1, 43.6, 37.4, 37.3, 20.2, 20.2; **$^{19}$F NMR** (376 MHz, $CDCl_3$): δ −118.7 (dt, *J* = 9.5, 5.0 Hz), −119.33 – −119.52 (m), −120.2 (d, *J* = 9.5 Hz), −121.19 (dd, *J* = 9.5, 6.0 Hz); **IR** (thin film): 3326, 3093, 2936, 1692, 1668, 1613, 1589, 1527, 1490, 1471, 1441, 1389, 1325, 1281, 1242, 1158, 1141, 1044, 1016, 957, 784, 732 $cm^{-1}$; **HRMS** (ESI, *m/z*): [$(M+H)^+$] calcd. for $C_{23}H_{21}F_4N_4O_3S^+$, 509.1265; found 509.1268; **UV-Vis**: $\lambda_{max}$ = 305 nm (50 μM in DMSO).

**VLOGO Fraction 2**: All characterisation data reported corresponds to the mixture of geometric isomers (68:32). **TLC** (*i*-Hex/EtOAc, 1:1): $R_f$ = 0.39 (UV/$KMnO_4$); **$^1$H NMR** (800 MHz, $CDCl_3$): δ 8.06 – 7.93 (m, 2H *major* and 2H *minor*), 7.84 (d, *J* = 2.5 Hz, 1H, *minor*), 7.77 (d, *J* = 2.5 Hz, 1H, *major*), 7.37 (tt, *J* = 8.5, 6.0 Hz, 1H, *minor*), 7.32 – 7.26 (m, 1H *major* and 1H *minor*), 7.21 (tt, *J* = 8.5, 6.0 Hz, 1H, *major*), 7.16 – 7.12 (m, 2H, *minor*), 7.09 – 7.04 (m, 2H, *minor*), 6.93 (d, *J* = 8.5 Hz, 2H, *major*), 6.87 (t, *J* = 8.0 Hz, 2H, *major*), 3.77 (s, 2H, *minor*), 3.65 (s, 2H, *major*), 2.82 (s, 6H, *minor*), 2.80 (s, 6H, *major*), 2.58 (s, 3H, *minor*), 2.57 (s, 3H, *major*); **$^{13}$C NMR** (200 MHz, $CDCl_3$): δ 167.6 (*minor*), 167.6 (*major*), 155.7 (dd, *J* = 261.5, 4.0 Hz, *minor*), 151.8 (ddd, *J* = 254.5, 12.0, 5.5 Hz, *major*), 139.2 (t, *J* = 10.0 Hz, *minor*), 137.41 (t, *J* = 9.0 Hz, *major*), 136.2 (*minor*), 136.1 (*major*), 135.7, 133.9, 133.8 (*major*), 133.8 (*minor*), 132.2 – 131.8 (m), 131.7 (t, *J* = 10.0 Hz, *minor*), 131.2 – 130.7 (m), 130.2 (t, *J* = 9.5 Hz, *major*), 124.4 (*minor*), 124.4 (*major*), 121.1, 113.9 (dd, *J* = 21.5, 2.5 Hz, *minor*), 113.3 (dd, *J* = 20.5, 3.0 Hz, *major*), 112.8 (dd, *J* = 20.0, 4.0 Hz, *minor*), 112.3 (dd, *J* = 19.5, 3.5 Hz, *major*), 44.1 (*minor*), 43.7 (*major*), 37.4 (*minor*), 37.3 (*major*), 20.3 (*minor*), 20.2 (*major*); **$^{19}$F NMR** (376 MHz, $CDCl_3$): δ −118.6 (dt, *J* = 9.5, 5.0 Hz, *major*), −119.3 – −119.5 (m, *major*), −120.1 (d, *J* = 9.5 Hz, *minor*), −121.2 (dd, *J* = 9.5, 5.5 Hz, *minor*); **IR** (thin film): 3328, 3097, 2930, 1670, 1625, 1613, 1589, 1527, 1490, 1471, 1442, 1389, 1325, 1281, 1242, 1159, 1142, 1045, 1016, 957, 911, 830, 785, 733 $cm^{-1}$; **HRMS** (ESI, *m/z*): [$(M+H)^+$] calcd. for $C_{23}H_{21}F_4N_4O_3S^+$, 509.1265; found 509.1268; **UV-Vis**: $\lambda_{max}$ = 305 nm (50 μM in DMSO).

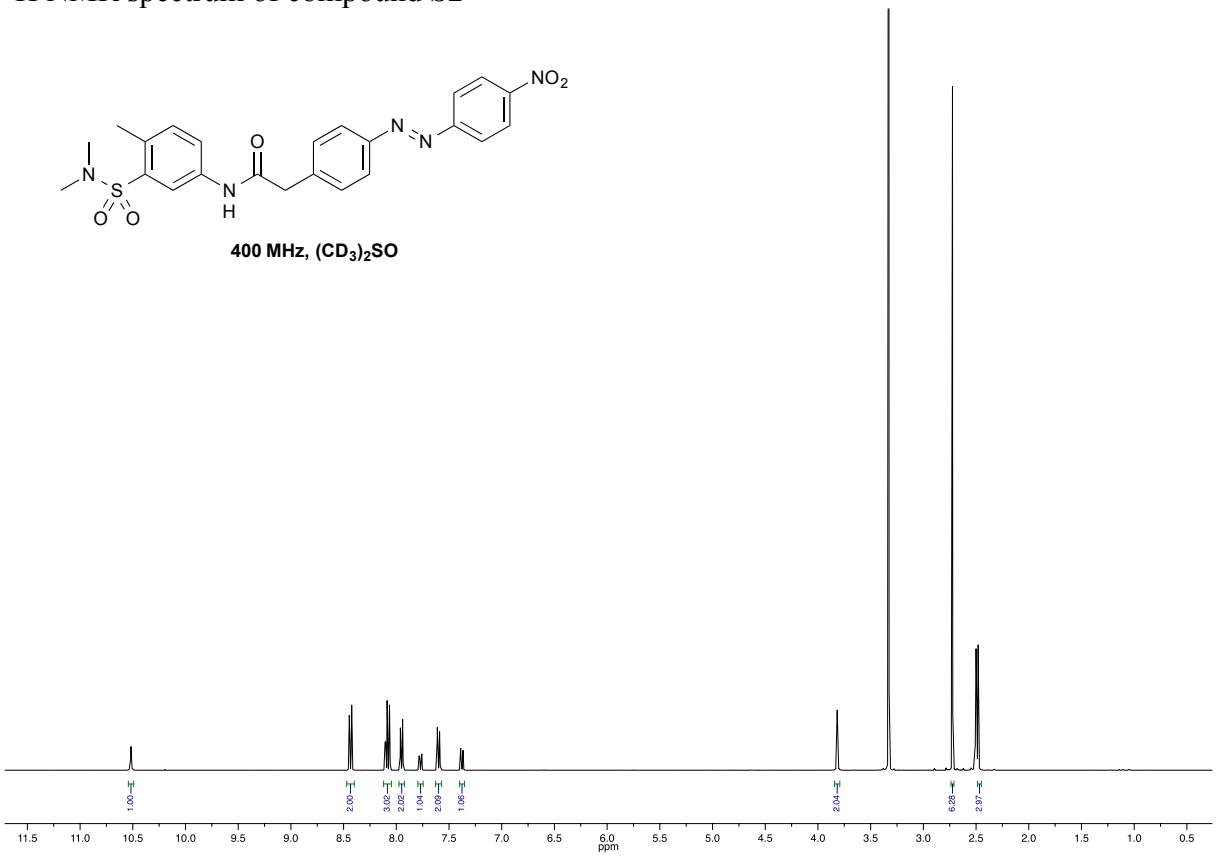
**Synthesis and characterisation of 2-{3,5-dichloro-4-[(2,6-dichlorophenyl)diazenyl] phenyl}-*N*-[3-(dimethylsulfamoyl)-4-methylphenyl]acetamide (6)**



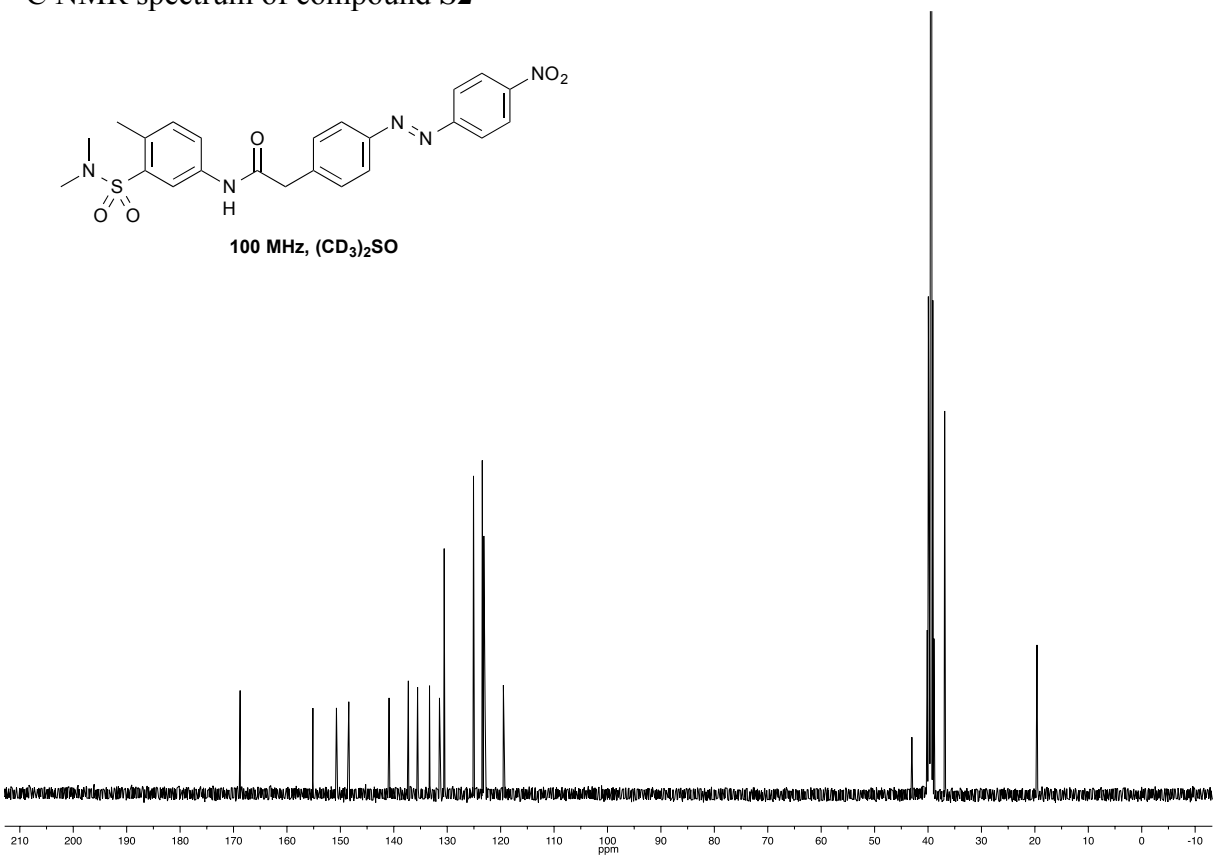
To a solution of compound **S3** (61 mg, 0.162 mmol) in $CH_2Cl_2$ (9.60 mL) at r.t. was added compound **7** (29 mg, 0.135 mmol), EDCI (78 mg, 0.405 mmol) and DMAP (66 mg, 0.540 mmol). The reaction mixture was stirred at r.t. for 3 h. The resulting mixture was diluted with $CH_2Cl_2$ (60 mL) and washed with aq. $KHSO_4$ (20 mL, 3% solution). The organic layer was separated and washed with sat. aq. $NaHCO_3$ (20 mL) and brine (20 mL). The organic layer was dried over $Na_2SO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography on silica gel eluting with pentane/EtOAc (3:1) afforded compound **6** (47 mg, 61%, 69:31 mixture of geometric isomers) as a red oil.

All characterisation data reported corresponds to the mixture of geometric isomers (69:31). **TLC** (pentane/EtOAc, 2:1): $R_f$ = 0.29 (UV); **$^1$H NMR** (400 MHz, ($C_6D_6$): δ 8.55 (s, 1H, *major*), 8.36 (s, 1H, *minor*), 8.32 – 8.19 (m, 1H *major* and 1H *minor*), 8.15 – 8.09 (m, 1H, *major*), 8.05 – 7.98 (m, 1H, *minor*), 7.41 (s, 2H, *major*), 7.21 (s, 2H, *minor*), 6.94 – 6.84 (m, 3H *major* and 1H *minor*), 6.69 (d, *J* = 8.0 Hz, 2H, *minor*), 6.36 (t, *J* = 8.0 Hz, 1H, *major*), 6.29 (t, *J* = 8.0 Hz, 1H, *minor*), 3.37 (s, 2H, *major*), 3.24 (s, 2H, *minor*), 2.44 (s, 3H, *major*), 2.41 (s, 3H, *minor*), 2.35 (s, 6H, *major*), 2.33 (s, 6H, *minor*); **$^{13}$C NMR** (100 MHz, $C_6D_6$): δ 168.0 (*major*), 167.7 (*minor*), 148.8 (*minor*), 148.2 (*major*), 147.6 (*minor*), 146.7 (*major*), 138.1 (*major*), 137.6 (*minor*), 137.5 (*major*), 137.3 (*minor*), 135.8 (*major* and *minor*), 134.0 (*major*), 134.0 (*minor*), 133.2 (*major* and *minor*), 130.7 (*major*), 130.5 (*minor*), 129.6 (*major*), 129.4 (*major* and *minor*), 129.3 (*minor*), 127.9 (*major*), 127.5 (*major*), 126.2 (*minor*), 126.1 (*minor*), 124.4 (*major*), 124.3 (*minor*), 121.2 (*major*), 121.2 (*minor*), 43.0 (*major*), 42.7 (*minor*), 36.9 (*major*), 36.8 (*minor*), 20.3 (*major*), 20.2 (*minor*); **IR** (neat): 3324, 3070, 2924, 1667, 1590, 1563, 1525, 1490, 1456, 1434, 1388, 1319, 1254, 1201, 1138, 1057, 954, 907, 872, 828, 778, 728 $cm^{-1}$; **HRMS** (EI, *m/z*): [$M^+$] calcd. for $C_{23}H_{20}Cl_4N_4O_3S^+$, 572.0005; found 572.0009; **UV-Vis**: $\lambda_{max}$ = 295 nm (50 μM in DMSO).

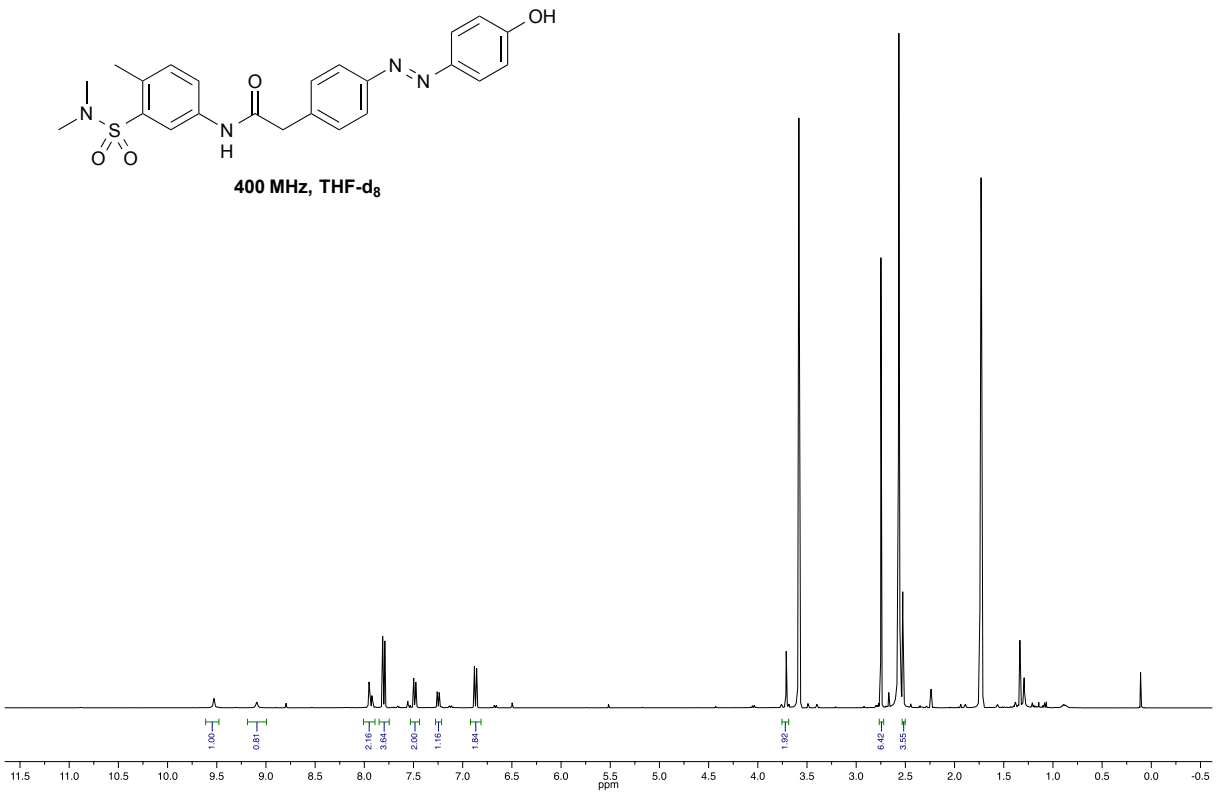
$^{1}$H NMR spectrum of compound **S2**



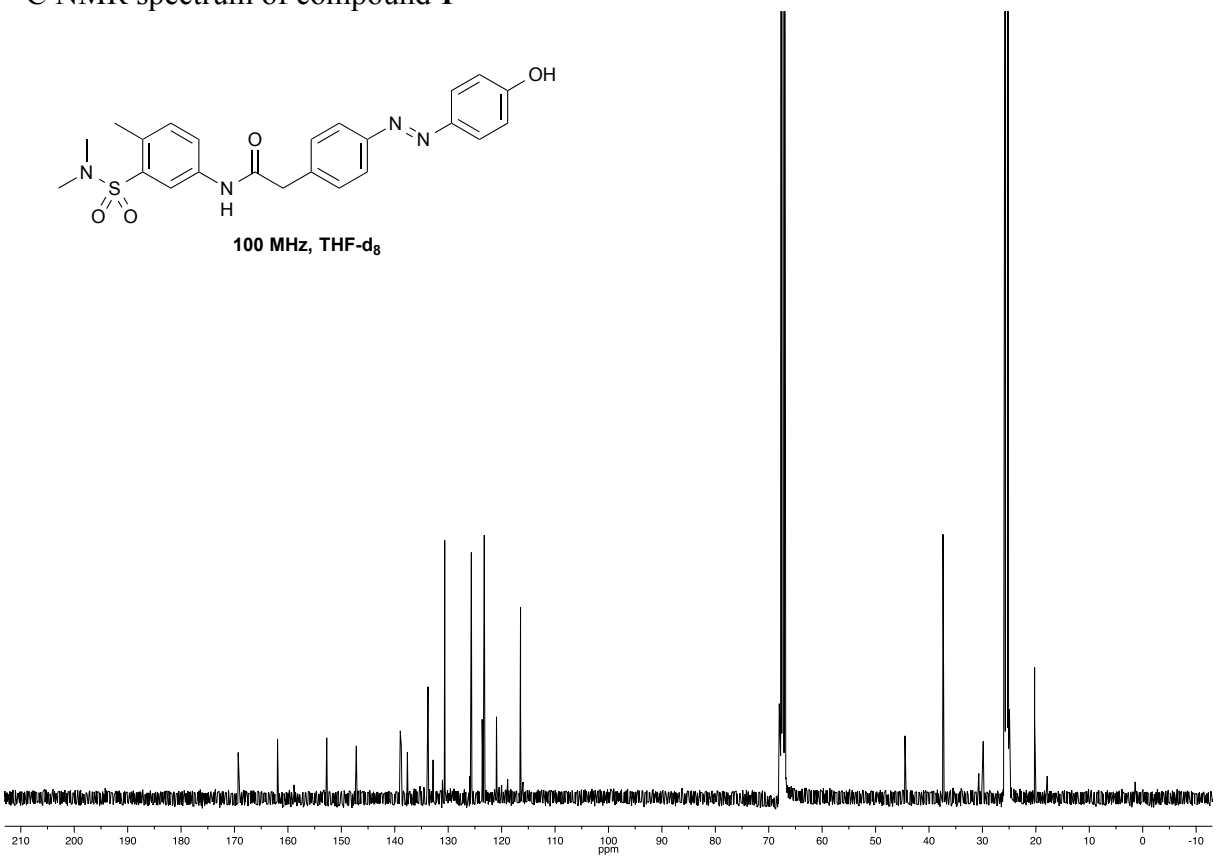

$^{13}$C NMR spectrum of compound **S2**



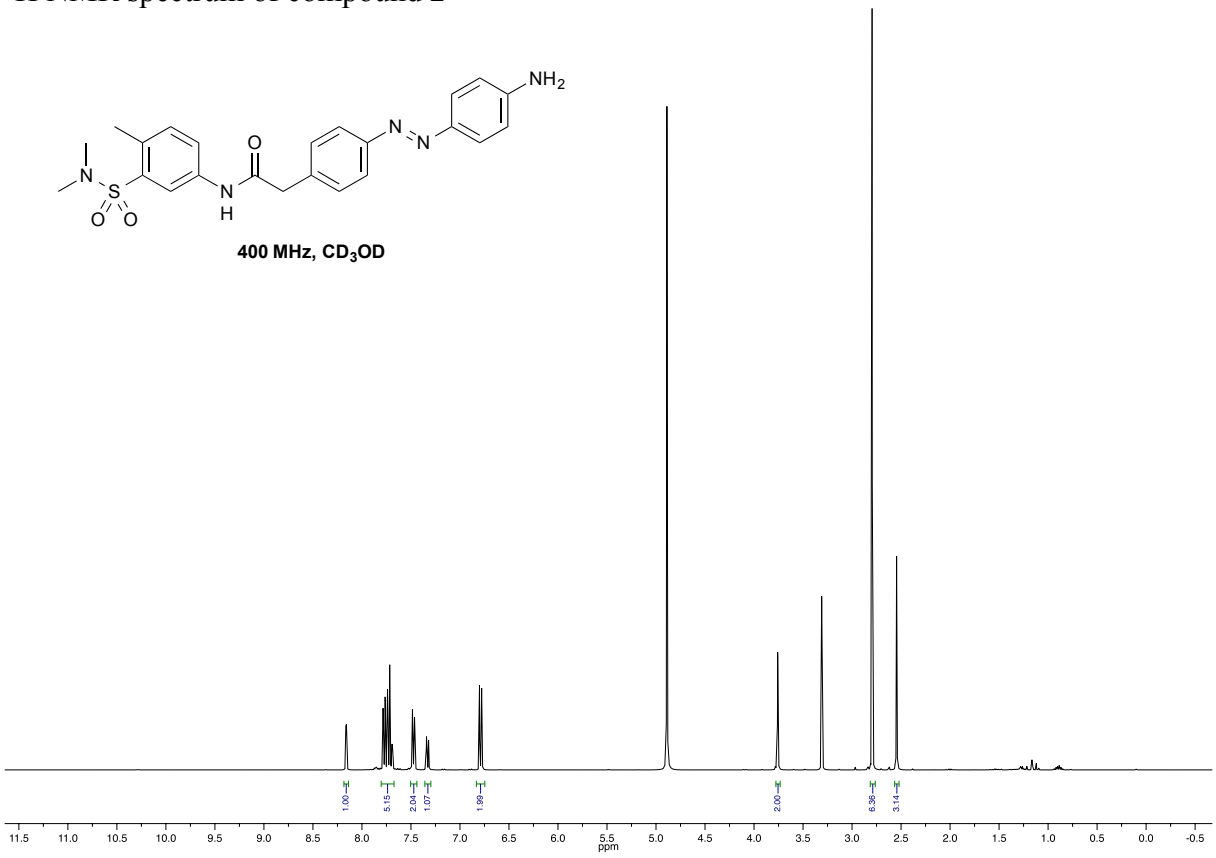
$^{1}$H NMR spectrum of compound **1**



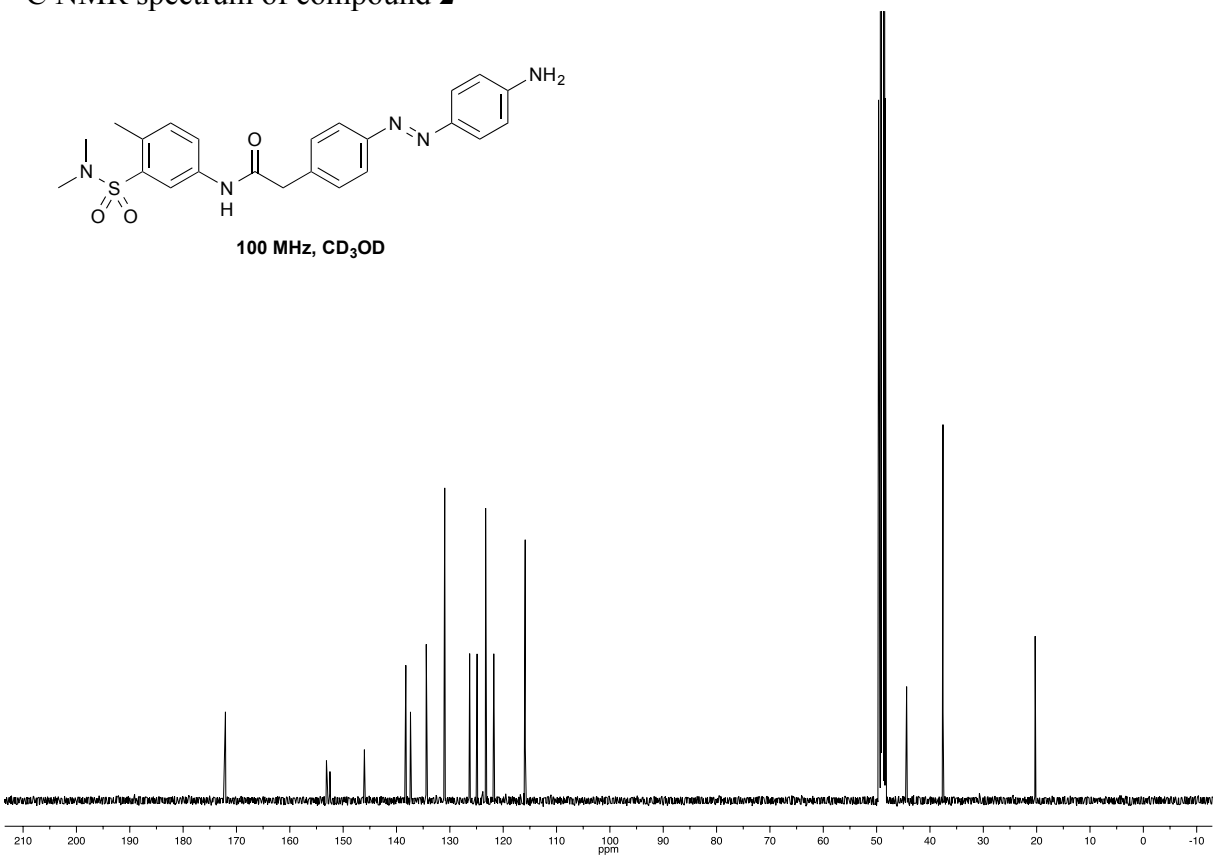

$^{13}$C NMR spectrum of compound **1**



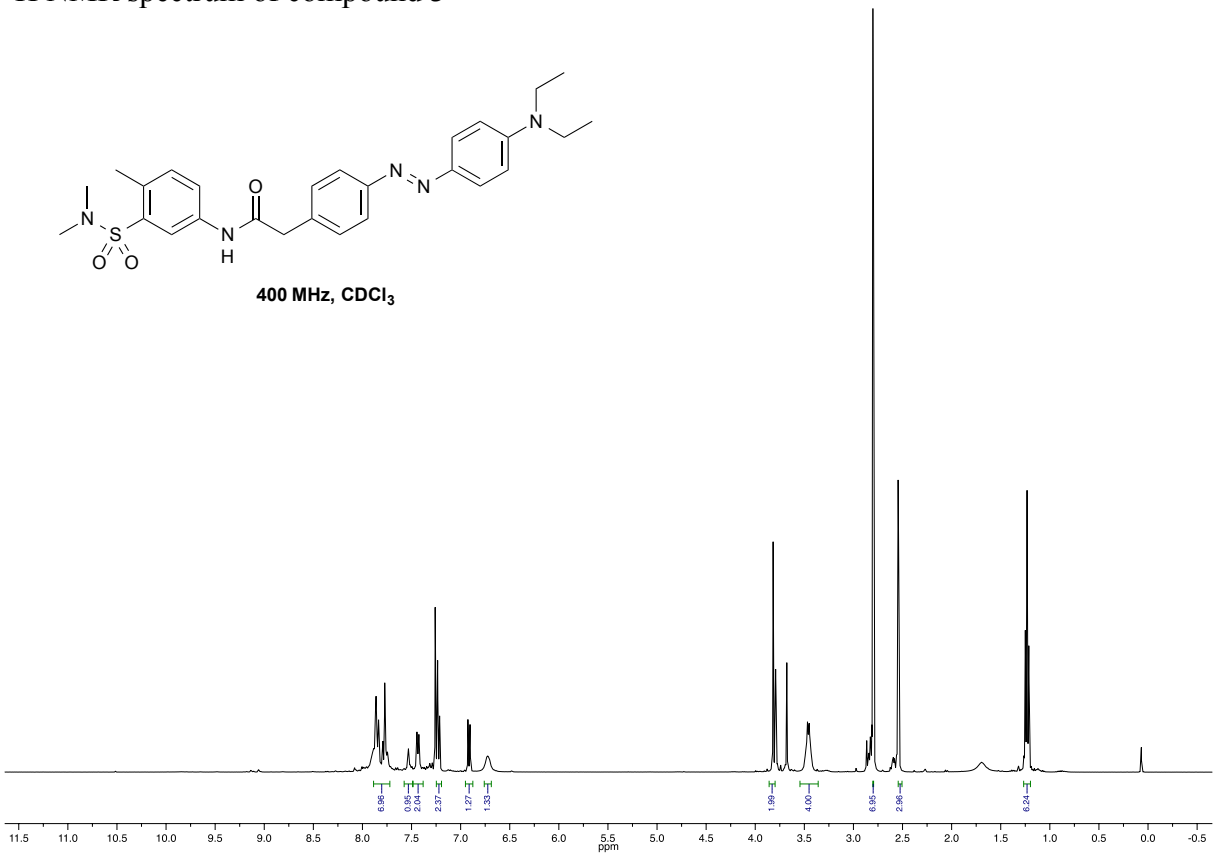
$^{1}$H NMR spectrum of compound **2**



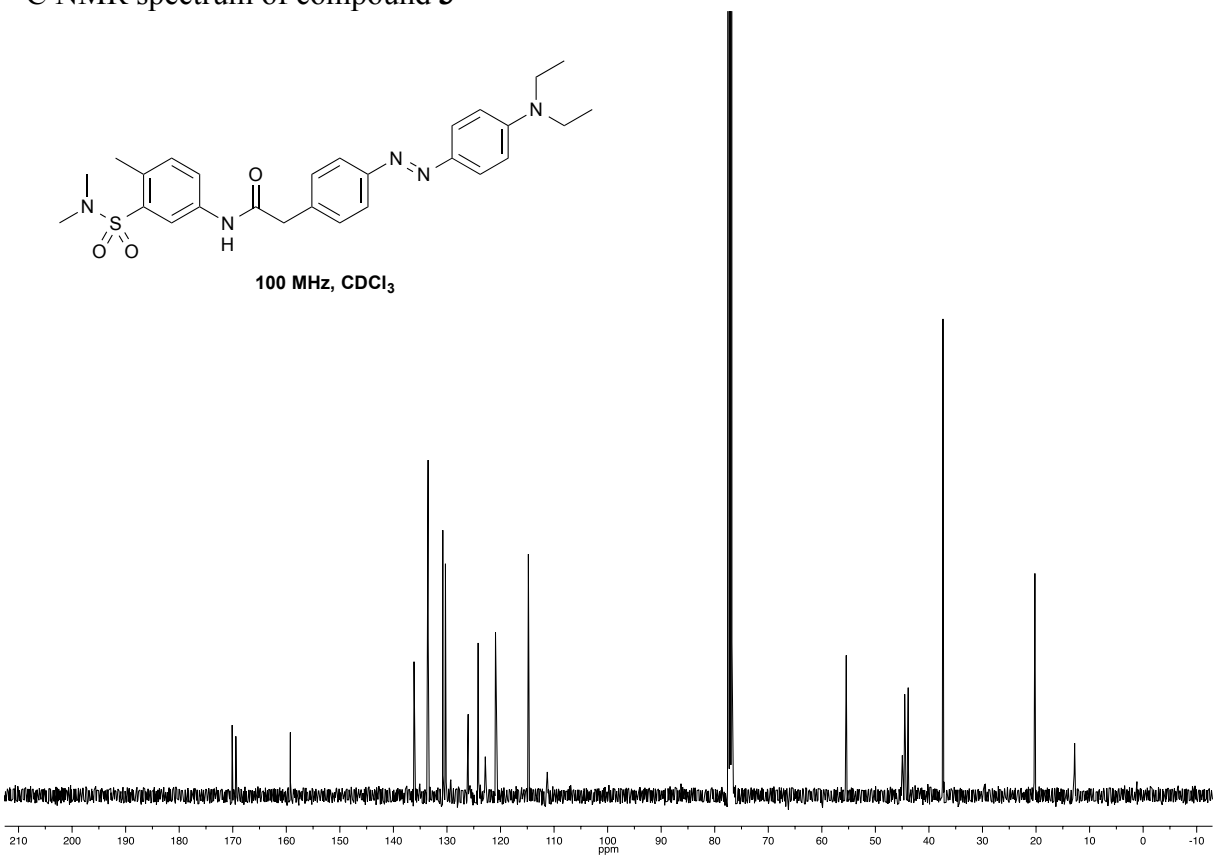

$^{13}$C NMR spectrum of compound **2**



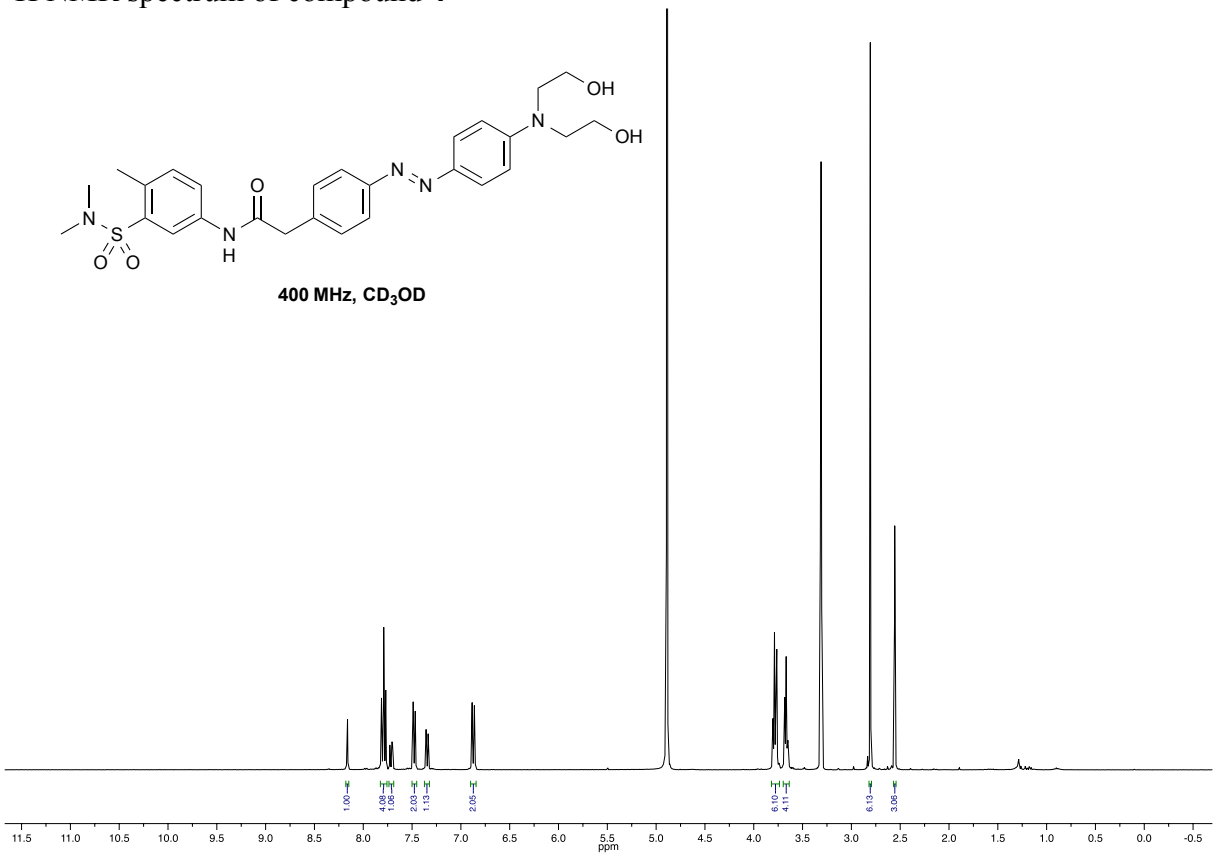
$^{1}$H NMR spectrum of compound **3**



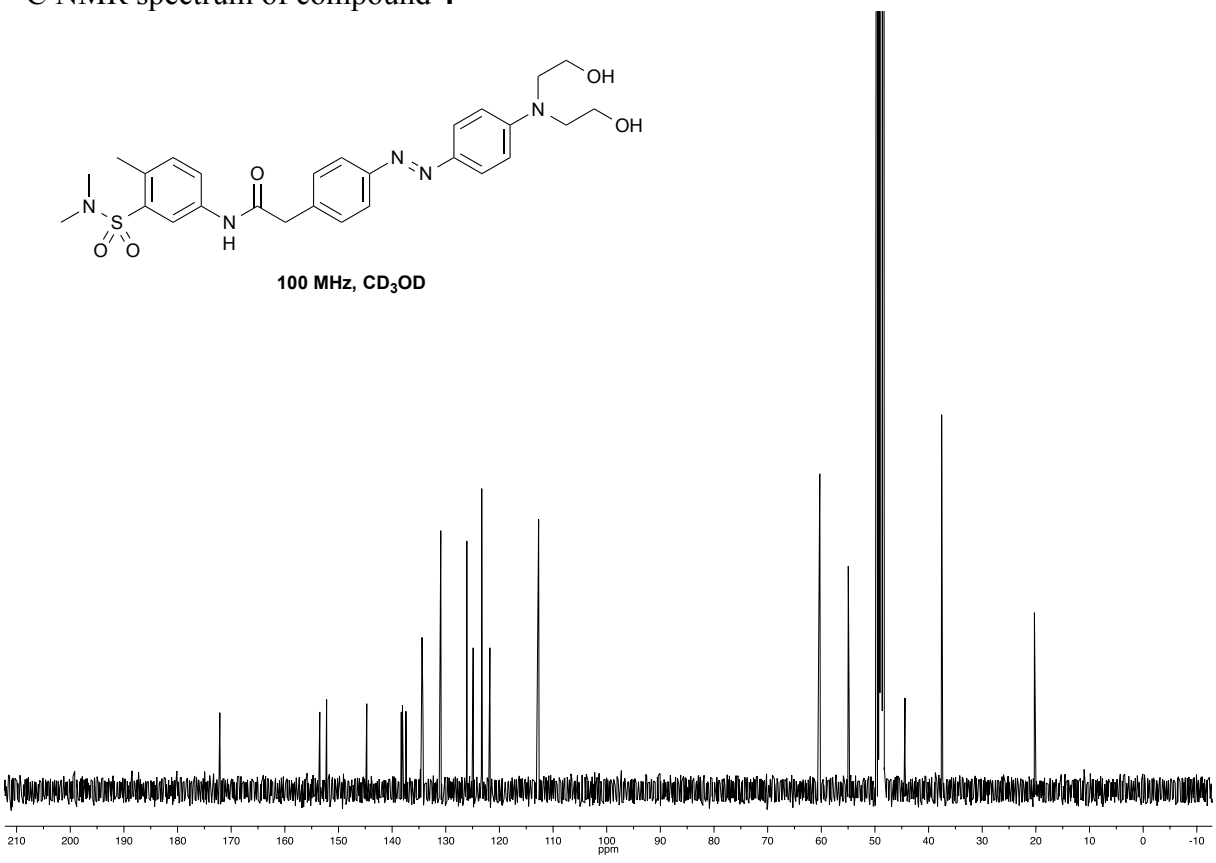

$^{13}$C NMR spectrum of compound **3**



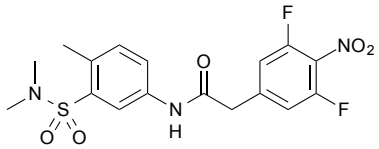
$^{1}$H NMR spectrum of compound **4**




$^{13}$C NMR spectrum of compound **4**



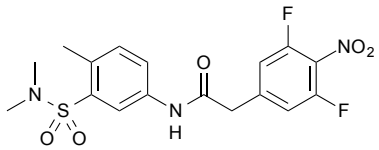
$^{1}$H NMR spectrum of compound **9**




**400 MHz, THF-$d_8$**

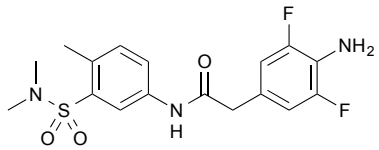



$^{13}$C NMR spectrum of compound **9**




**100 MHz, THF-$d_8$**

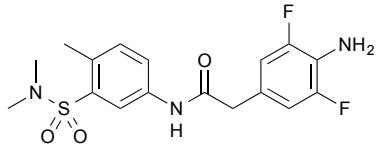
$^{1}$H NMR spectrum of compound **10**




**400 MHz, $CD_3OD$**

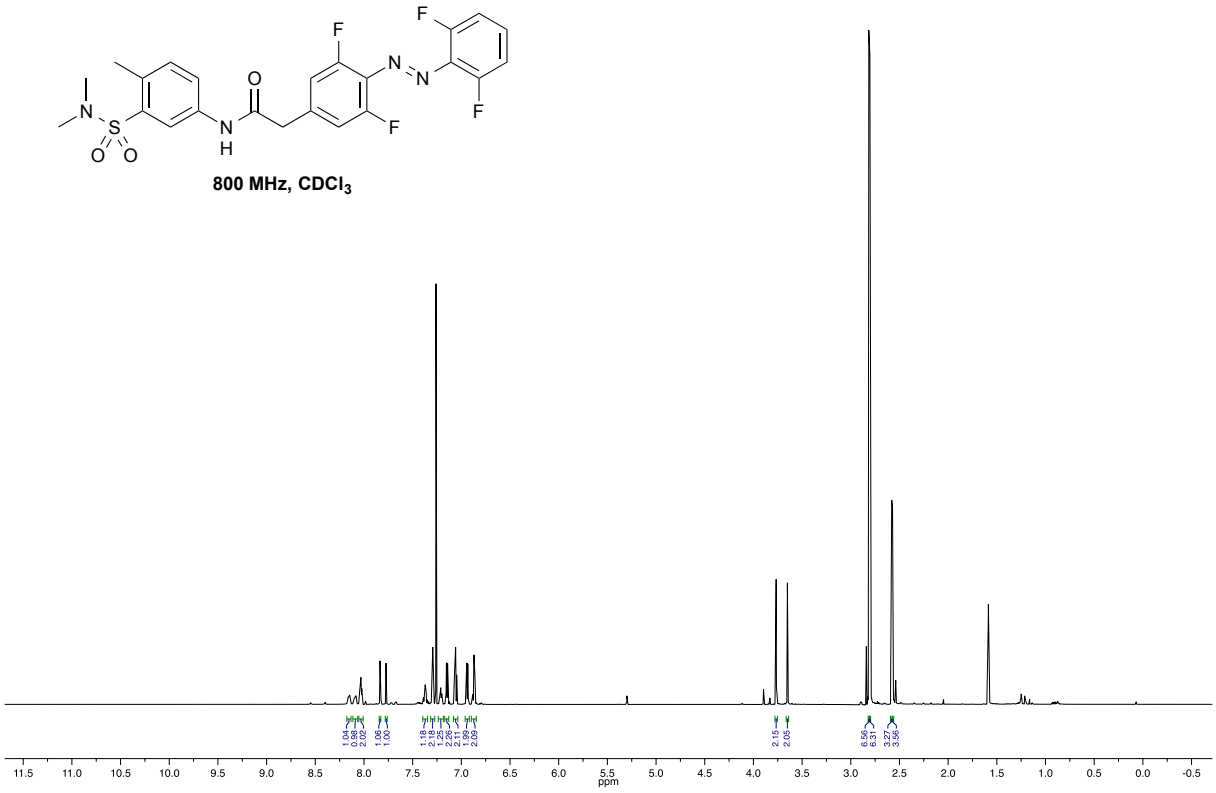



$^{13}$C NMR spectrum of compound **10**

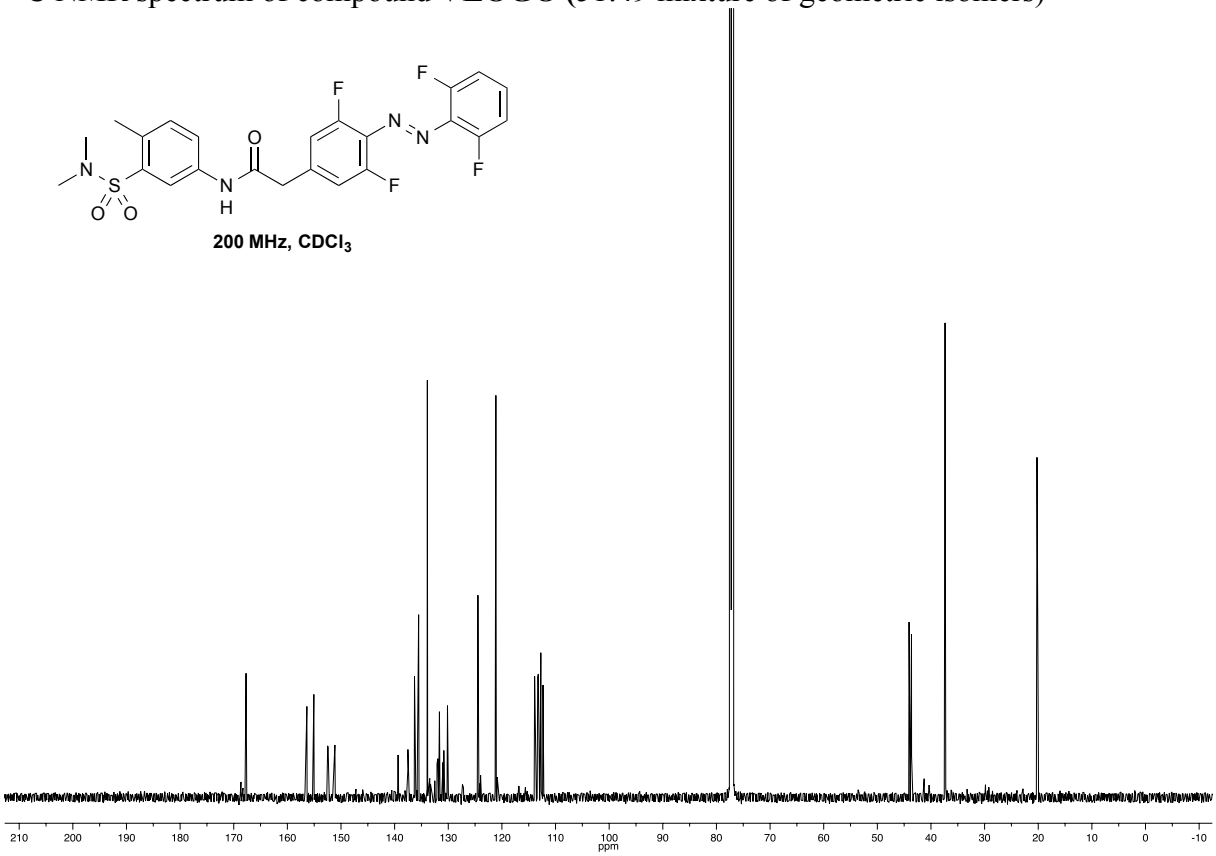



**100 MHz, $CD_3OD$**

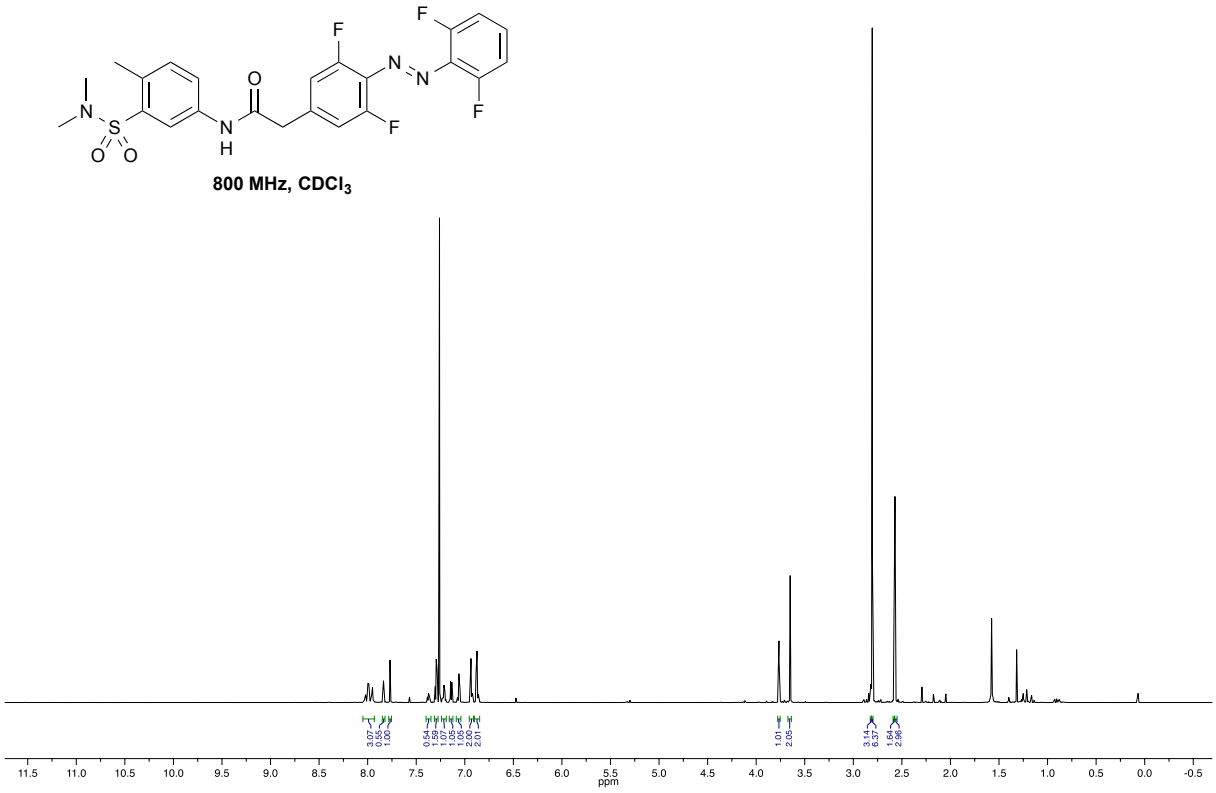
[1]H NMR spectrum of compound **VLOGO** (51:49 mixture of geometric isomers)



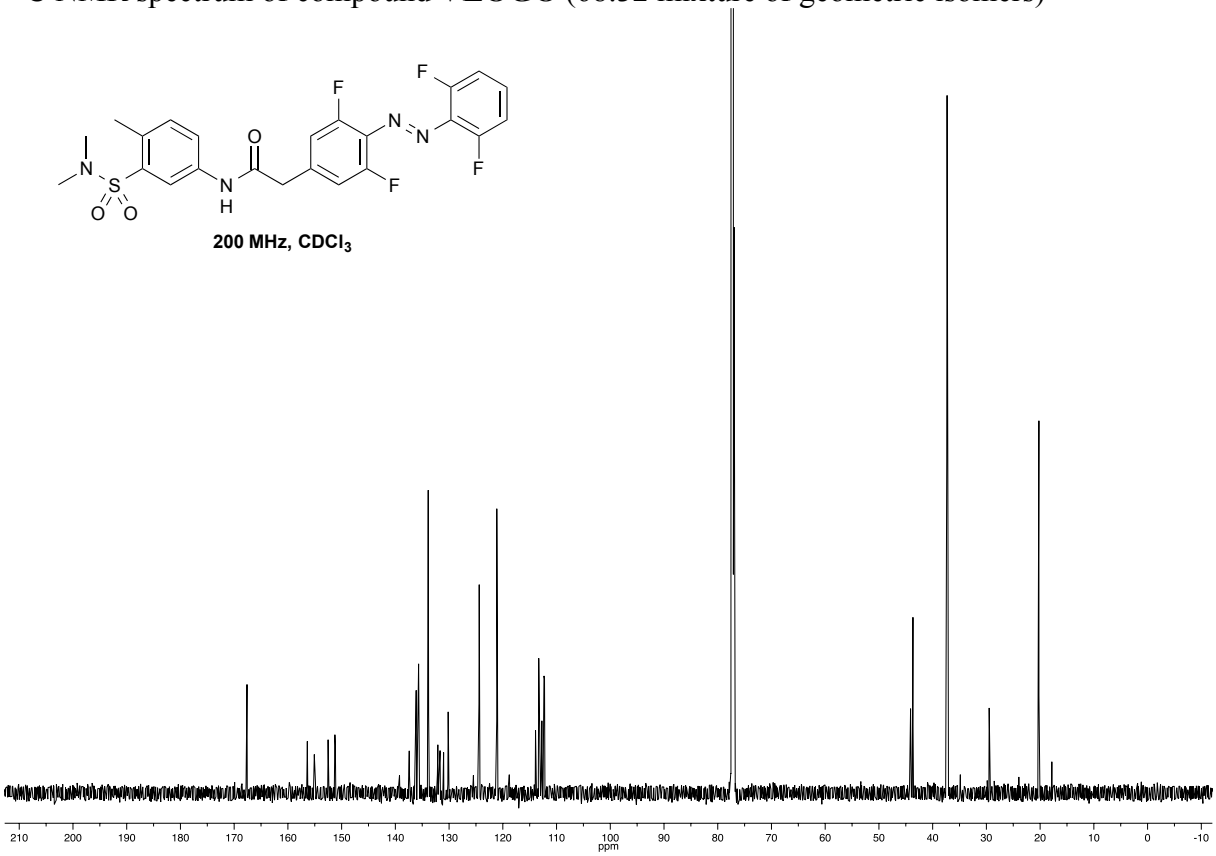

[13]C NMR spectrum of compound **VLOGO (**51:49 mixture of geometric isomers)



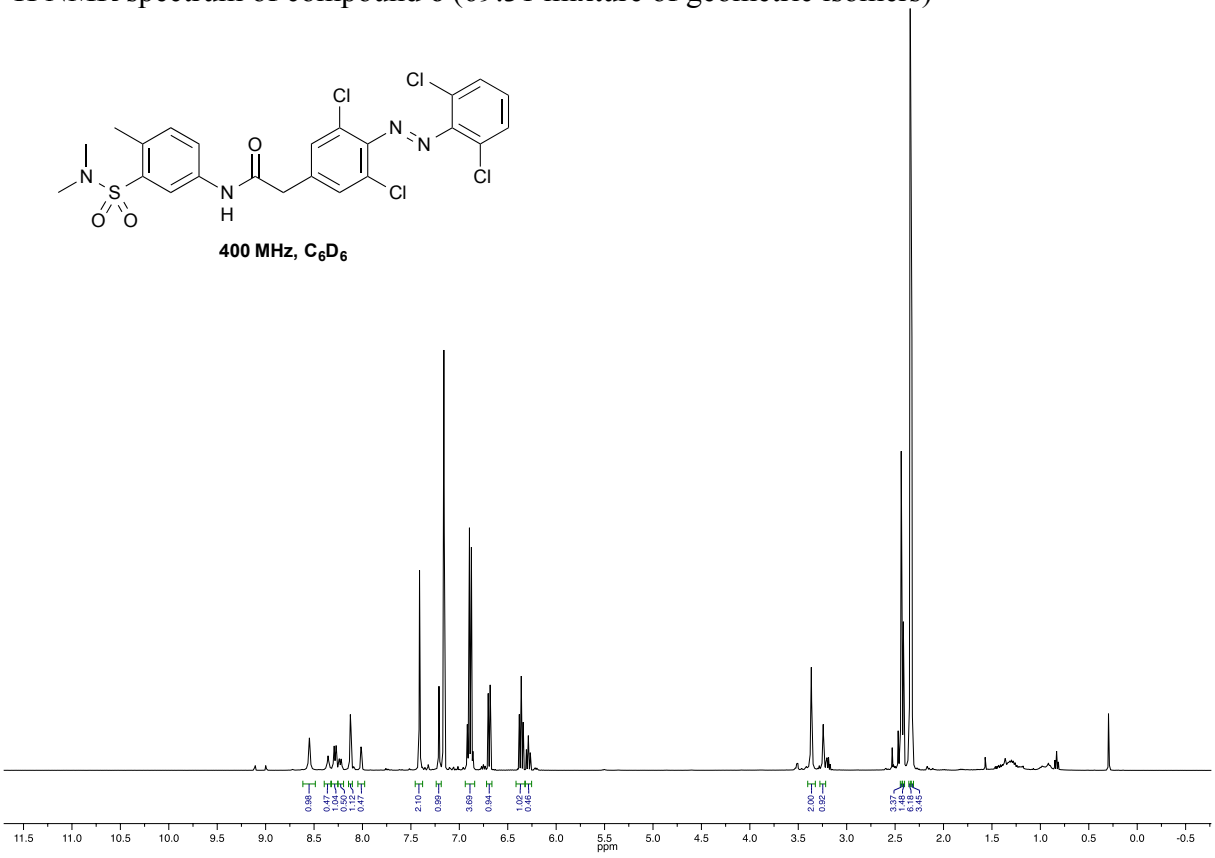
$^{1}$H NMR spectrum of compound **VLOGO** (68:32 mixture of geometric isomers)




$^{13}$C NMR spectrum of compound **VLOGO** (68:32 mixture of geometric isomers)



$^{1}$H NMR spectrum of compound **6** (69:31 mixture of geometric isomers)




$^{13}$C NMR spectrum of compound **6** (69:31 mixture of geometric isomers)