Reversible Synthesis and Characterisation of Dynamic Imino Analogues of Trimethine and Pentamethine Cyanine Dyes.

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SUPPORTING INFORMATION

1 SYNTHESIS

1.1 General experimental

Solvents were of HPLC or reagent quality and purchased commercially. Starting material were purchased commercially and used without further purification. Compounds were characterized using ^{1}H and ^{13}C NMR that were recorded on a Bruker Avance DRX 400 spectrometer at 400 and 100.6 MHz, respectively. Chemical shifts are reported as δ values (ppm) with reference to the residual solvent peaks. Products were purified by flash column chromatography on silica gel. Melting points were measured on a Büchi B-540 apparatus. IR spectra were recorded on a Nicolet 6700 FT-IR spectrometer from Thermo scientific.

1.2 Synthesis of Building blocks

2-Amino-1-methylbenzothiazolium iodide (1)
2-Aminobenzothiazole (3.5 g, 23 mmol) was dissolved in acetonitrile (50 mL) then iodomethane (2 mL) was added. The solution was stirred at 45 °C for 12 hours. After cooling to room temperature, Et_2O (50 mL) was added and the white suspension was filtered. The precipitate was washed with diethylether and dried under vacuum. Compound **1** was obtained pure as a white solid (6.75 g, 100%, mp 227-228 °C).
^{1}H NMR (400 MHz, DMSO) δ 10.0 (s, 2 H), 8.0 (d, *J* = 7.9 Hz, 1 H), 7.69 (d, *J* = 7.9 Hz, 1 H), 7.6 (t, *J* = 7.5 Hz, 1H), 7.44 (t, *J* =7.5 Hz, 1 H), 3.75 (s, 3 H).
^{13}C NMR (100 MHz, DMSO) δ 167.9, 138.9, 127.7, 125.1, 123.4, 122.2, 122.1, 113.3, 32.2
HRMS: *m/z*: calcd for $C_8H_9N_2S^+$: 165.0481; found 165.0475.

(*Z*)-2-(1,3,3-Trimethylindolin-2-ylidene)acetaldehyde (2)
(Chloromethylene)dimethylammonium chloride (1.66 g, 13 mmol) was dissolved in CH_2Cl_2 (20 mL) and was stirred at room temperature for 10 minutes. Then a solution of 1,2,3,3-tetramethyl-3*H*-indole (2.3 g, 13 mmol) in CH_2Cl_2 (20 mL) was added dropwise. After 15 minutes under stirring at room temperature, an aqueous solution of 10 % NaOH (40 mL) was added carefully at 0 °C. After 1h, the organic solution was extracted and washed 3 times with water and then dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography on silica gel (Et_2O, Rf = 0.3 in Et_2O). Compound **2** was obtained pure as a white powder (1 g, 40 %, mp 116-117 °C).
^{1}H NMR (400 MHz, DMSO) δ 9.9 (d, *J* = 8.8 Hz, 1 H), 7.41 (d, *J* = 7.5 Hz, 1 H), 7.28 (dt, *J* = 7.5, 1 Hz, 1 H), 7.1 (d, *J* = 7.5 Hz, 1 H), 7.02 (dt, *J* = 7.5, 0.6 Hz, 1 H), 5.3 (d, *J* = 8.8 Hz, 1H), 3.24 (s, 3H), 1.6 (s, 6 H)
^{13}C NMR (100 MHz, DMSO) δ 185.3, 172.8, 143.3, 139.1, 127.8, 121.9, 121.8, 108.4, 98.3, 46.8, 29.3, 28.8
IR (cm^{-1}) 1629, 1573, 1490, 1467, 1396, 1184.
HRMS: *m/z*: calcd for $C_{13}H_{16}NO^+$: 202.1226; found 202.1221

(2*E*,4*Z*)-4-(1,3,3-Trimethylindolin-2-ylidene)but-2-enal (3)
1,2,3,3-Tetramethyl-3*H*-indolium iodide (1 g, 3.3 mmol), malonaldehydebis(phenylimine) monohydrochloride (859 mg, 3.3 mmol) and sodium acetate (600 mg) were suspended in acetic anhydride (40 mL). After 30 minutes under stirring at room temperature, Et_2O (200 mL) was added then the green dark precipitate was filtered off solution and washed with Et_2O. The solid was subsequently washed with CH_2Cl_2 and the filtrate was collected and concentrated off under vacuum. The crude residue was purified by flash chromatography on

silica gel (CH_2Cl_2/MeOH 98:2, Rf = 0.1) affording the desired activated hemicyanine pure as a brown foam (400 mg, 32 %).
^{1}H NMR (400 MHz, DMSO) δ 8.88 (d, *J* = 13.1 Hz, 1 H), 8.5 (dd, *J* = 15, 11.3 Hz, 1 H), 7.79 (d, *J* = 7.5 Hz), 7.72 (d, 7.5 Hz, 1 H), 7.44-7.69 (m, 7 H), 6.85 (d, *J* = 15 Hz, 1 H), 5.51 (dd, *J* = 13.1, 11.3 Hz, 1 H), 3.81 (s, 3 H), 2 (s, 3H), 1.7 (s , 6 H).
^{13}C NMR (100 MHz, DMSO) δ 181, 170.1, 156.8, 147.7, 143.3, 142.3, 138.3, 130.9, 130, 129.2, 129.1, 128.9, 128.6, 123.2, 119.4, 114.6, 113.3, 112.3, 51.5, 33.6, 23.7.
This freshly prepared activated hemicyanine (400 mg, 1.05 mmol) was dissolved in THF (20 mL) then a solution of 10 % NaOH (60 mL) was added. The brown mixture was stirred at room temperature for 10 h then CH_2Cl_2 was added. The organic solution was extracted and washed with water, dried over Na_2SO_4 and concentrated under vacuum. The crude was purified by flash chromatography on silica gel (CH_2Cl_2/ Et_2O 1:1, Rf= 0.3 in Et_2O). Compound **3** was obtained pure as brown/orange oil (238 mg, 100 %).
^{1}H NMR (400 MHz, DMSO) δ 9.45 (d, *J* = 8.3 Hz, 1 H), 7.86 (dd, *J* =13.9, 13 Hz, 1 H), 7.37 (d, *J* = 7.2 Hz, 1 H), 7.24 (t, *J* = 7.2 Hz, 1 H), 7 (d, *J* = 7.2 Hz, 1H), 6.96 (t, *J* = 7.2 Hz, 1H), 5.85 (dd, *J* = 13.9, 8.3 Hz, 1 H), 5.68 (d, *J* = 13 Hz, 1 H), 3.26 (s, 3 H), 1.59 (s, 3 H)
^{13}C NMR (100 MHz, DMSO) δ 191.6, 164.9, 150, 143.4, 138.4, 127.4, 122.1, 121.2, 120.7, 107.3, 94.2, 45.8, 28.7, 27.4
IR (cm^{-1}) 1656, 1595, 1537, 1492, 1466, 1392, 1184, 1120.
HRMS: *m/z*: calcd for $C_{15}H_{18}NO^+$: 228.1383; found 228.1391

1.3 Synthesis and isolation of cyanine dye analogues

3-Methyl-2-((*E*)-(*Z*)-2-(1,3,3-trimethylindolin-2-ylidene)ethylidene)amino)benzo[d] thiazol- 3-ium iodide (4)
(*Z*)-2-(1,3,3-Trimethylindolin-2-ylidene)acetaldehyde **2** (845 mg, 4.2 mmol) and 2-amino-1-methylbenzothiazolium iodide **1** (1.23 g, 4.2 mmol) were added to a mixture of toluene (30 mL) and DMF (8 mL). The orange suspension was stirred at 95 °C for 16 h in a flask equipped with a Dean Starck apparatus. After cooling down to room temperature, Et_2O (200 mL) was added and the precipitate was isolated by filtration. The solid was washed with cold $CHCl_3$ and the filtrate was concentrated under vacuum. The crude residue was finally purified by a short column chromatography on silica gel (AcOEt then AcOEt/MeOH 200:5, AcOEt/MeOH 200:10, Rf= 0.1 in AcOEt/MeOH 8:2) affording the desired compound **4** pure as a bright orange/red solid (101 mg, 5 %, mp 223-224 °C).
^{1}H NMR (400 MHz, DMSO) δ 8.56 (d, *J* = 12.1 Hz, 1 H), 8.1 (d, *J* = 7.8 Hz, 1 H), 7.83 (d, *J* = 7.4 Hz, 1 H), 7.75 (d, *J* = 7.4 Hz, 1H), 7.65 (m, 2 H), 7.53 (t, *J* = 7.4 Hz, 1 H), 7.51 (t, *J* = 7..8 Hz, 1 H), 7.44 (t, *J* = 7.4 Hz, 1 H), 6.55 (d, *J* = 12.1 Hz), 3.92 (s, 3 H), 3.81 (s, 3 H), 1.73 (s, 6H)
^{13}C NMR (100 MHz, DMSO) δ 179.4, 170.8, 161.6, 142.1, 141.4, 139.3, 128.5, 128.1, 126.5, 125.4, 124.3, 123.4, 123.1, 122.4, 113.9, 112.6, 102.6, 102, 50.1, 32.2, 27.6
IR (cm^{-1}) 1619, 1591, 1544, 1490, 1479, 1452, 1392, 1359, 1336, 1265, 1116.
HRMS: *m/z*: calcd for $C_{21}H_{22}N_3S^+$: 348.1529; found 348.1512.

3-Methyl-2-((*E*)-((2*E*-4*Z*)-4-(1,3,3-trimethylindolin-2-ylidene)but-2-enylidene)amino) benzo[*d*]thiazol-3-ium iodide (5)
1,3,3-Trimethyl-2-((1*E*,3*E*)-4-(*N*-phenylacetamido)buta-1,3-dienyl)-3*H*-indolium chloride (280 mg, 0.73 mmol) and 2-amino-1-methylbenzothiazolium iodide (213 mg, 0.73 mmol) were dissolved in DMF (30 mL). The solution was stirred at 100 °C for 16 h. The green solution became red after few minutes. After cooling down to room temperature, Et_2O (200

mL) was added and the precipitate formed was washed with Et_2O. $CHCl_3$ (100 mL) was then added, the filtrate was collected and the solvents were evaporated off. The purple crude residue was purified by column chromatography on silica gel (CH_2Cl_2 then CH_2Cl_2/MeOH 200:5, 200:10, Rf= 0.2 in AcOEt/MeOH 8:2) affording the desired compound **5** pure as a purple solid (12.4 mg, 4 %, mp 225-226 °C)

^{1}H NMR (400 MHz, DMSO) δ 8.5 (dd, *J* = 14.2, 11.8 Hz, 1 H), 8.33 (d, *J* = 11.8 Hz, 1 H), 7.98 (d, *J* = 7.5 Hz, 1 H), 7.74 (d, *J* = 7.5 Hz, 1 H), 7.68 (d, 8 Hz, 1 H), 7.63 (d, *J* = 8 Hz, 1 H), 7.58 (t, *J* = 8 Hz, 1H), 7.52 (t, *J* = 8 Hz, 1H), 7.43 (t, *J* = 7.5 Hz, 1 H) 7.41 (t, *J* = 7.5 Hz, 1 H), 6.75 (d, *J* = 14.2 Hz, 1 H), 6.6 (t, *J* = 11.8 Hz, 1 H), 3.81 (s, 3H), 3.79 (s, 3H), 1.72 (s, 6 H)

^{13}C NMR (100 MHz, DMSO) δ 177.8, 161.8, 142.1, 142, 139.4, 128.5, 128.4, 127.8, 126.7, 124.3, 123, 122.9, 122.5, 121.7, 121.4, 112.5, 107.7, 50.1, 32.2, 27.8

IR (cm^{-1}) 1617, 1595, 1542, 1494, 1478, 1452, 1366, 1358, 1332, 1310, 1264, 1117.

HRMS: *m/z*: calcd for $C_{23}H_{24}N_3S^+$: 374.1685; found 374.1681.

^{1}H NMR experiments were carried out from amine **1** and aldehyde **3** (10mM each in d_6-DMSO). As for the formation of compound **4**, the reaction of **1** with **3** led to the slow formation of the imine analogue **5** of a Cy5 that can also be visualised by a colour change of the NMR solution from bright yellow to dark purple. After 72h, a plateau was reached that corresponded to the formation of c.a. 15% of compound **5**. To unambiguously characterise the generated imine, a sample was isolated by flash-chromatography and analysed by NMR (Figure S1). The signals corresponding to the four protons from the polymethine/imine chain were assigned using a COSY ^{1}H NMR. As for the Cy3 analogue **4**, the Cy5 analogue **5** is present in solution as a unique isomer. Coupling constants *J* of 11.8 Hz between H_a and H_b and H_b and H_c and 14.2 Hz between H_c and H_d also suggested an all-*trans* conformation for the imine/polymethine chain which was subsequently confirmed by a NOESY experiment.

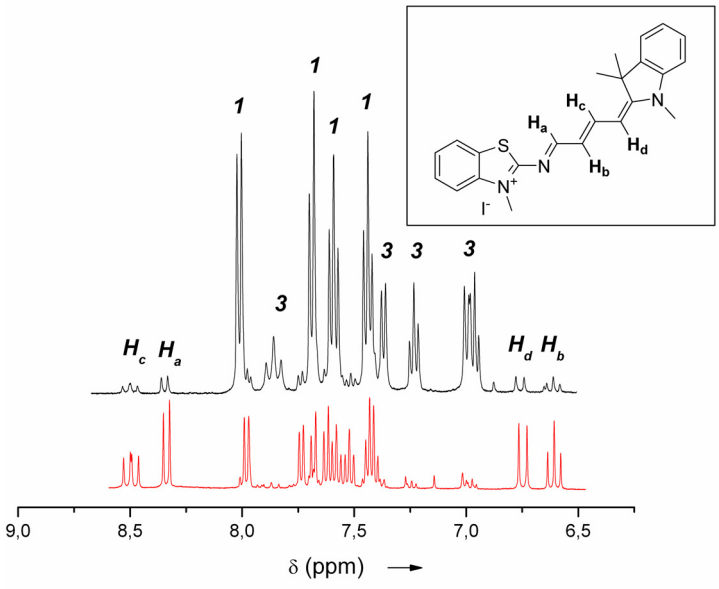


Figure S1. ^{1}H NMR spectrum of a mixture of amine **1** and aldehyde **3** after reacting for 72h at 50°C in d_6 DMSO (top) and ^{1}H NMR spectrum of isolated Cy5 imine analogue **5** (bottom). Structure of compound **5** is represented in the inset.

1.4 Synthesis of C3 and C5 cyanine dyes.

For comparison, unsymmetrical trimethine (C3) and pentamethine (C5) cyanine dyes, direct analogues of imines **4** and **5**, were prepared according to literature procedures (Hamer, F.M. *The Cyanine dyes and related compounds* (1964) in Interscience: New York, London). Absorption and emission spectra of analytically pure samples are reported in Figure S2.

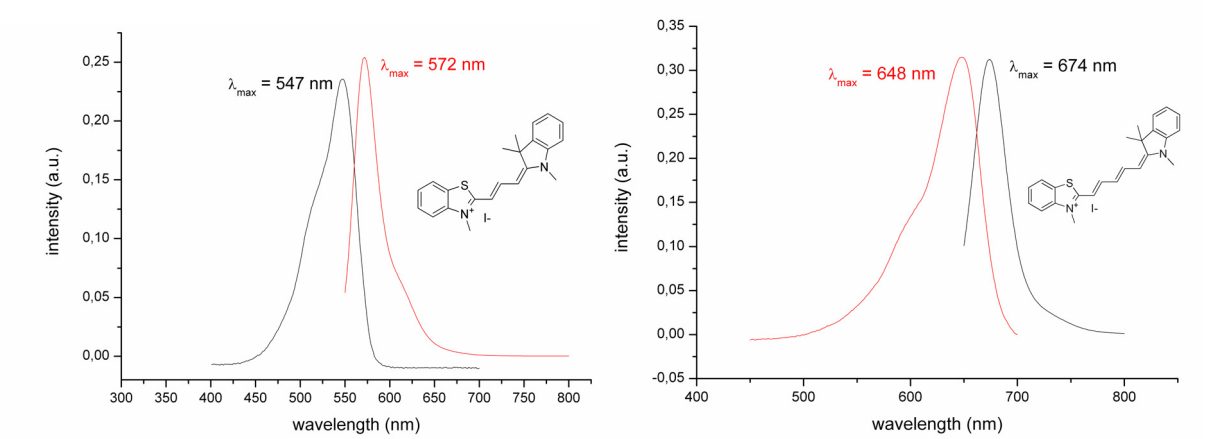


Figure S2. Absorption and fluorescence emission spectra of unsymmetrical trimethine (C3) and pentamethine (C5) cyanine dyes in DMSO solutions.

1.5 Kinetic studies.

In order to gain further insight into the kinetics of imine formation, we used fluorescence spectroscopy to monitor the formation of imine **4** from a mixture of amine **1** and Fisher's base aldehyde **2** either at room temperature or at 60 °C. Briefly, a mixture of compounds **1** and **2** (25 mM each) in DMSO was stirred at room temperature or at 60°C and the formation of imine **4** was quantitatively monitored by fluorescence spectroscopy (λexc = 480 nm) until equilibrium was reached (see spectrum below). At room temperature, thermodynamic equilibrium was reached after 48h while increasing temperature to 60°C resulted not only in a different distribution at equilibrium (5-fold increase of the amount of imine **4** formed), equilibrium which was also reached much faster (less than 24h).

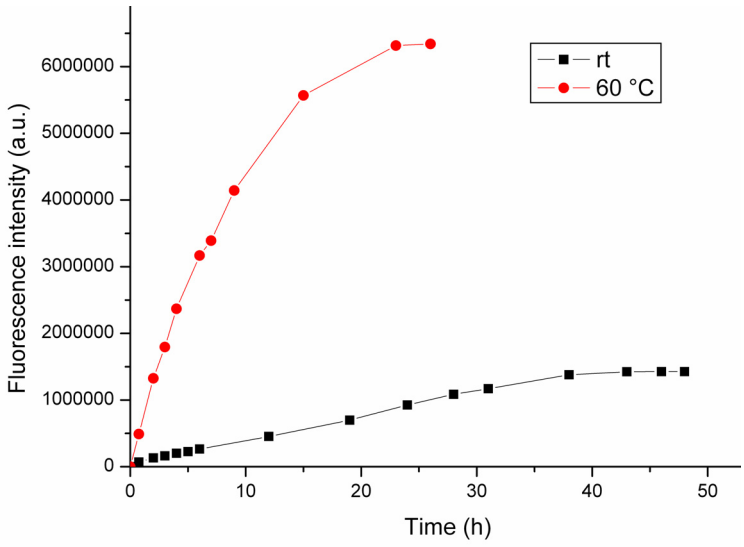


Figure S3. Kinetics of formation of imine **4** from a stoichiometric mixture of amine **1** and aldehyde **2** (25mM each) at room temperature (black squares) or 60°C (red circles)

2 SPECTROSCOPIC STUDIES

2.1 General Experimental

UV spectra were recorded on a UV-Visible Varian Cary 3 spectrophotometer in a 1 mm pathlength cuvette. Fluorescence emission spectra were recorded in quartz cells (with a 5 mm pathlength) at 20 °C on a Jobin Yvon Fluorolog 3.22 instrument. The excitation and emission bandwidths were fixed to 3 nm and 3 nm respectively.

2.2 Fluorescence Quantum Yields (QY)

Quantum yields of aldehydes and imines **2-5** in DMSO were determined relative to either quinine sulfate in 0.5M H_2SO_4, Fluorescein in 0.1M NaOH or Sulforhodamine 101 in ethanol assuming quantum yields of 0.55, 0.92 and 1.0, respectively (Standards purchased from *Invitrogen*). For fluorescence measurements, the total absorption of the samples never exceeded 0.06, making the inner filter effect negligible.
Fluorescence quantum yields (QY) were calculated according to the following equation :

$$\phi_X = \phi_{ST} (Grad_X / Grad_{ST})(\eta_X / \eta_{ST})^2$$

Where the subscripts ST and X denote standard and sample respectively, ϕ is the fluorescence quantum yield, *Grad* the gradient from the plot of integrated fluorescence intensity *vs* absorbance, and η the refractive index of the solvent.

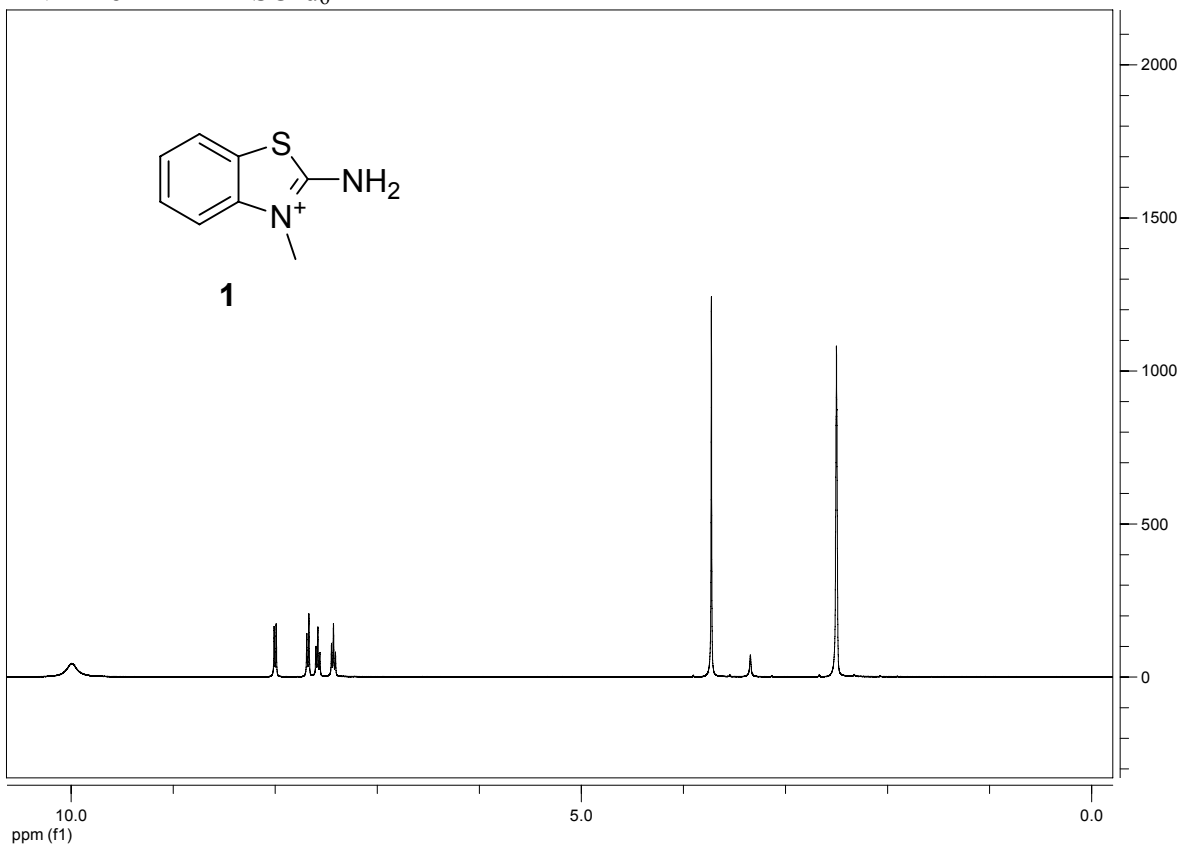
3 DENSITY FUNCTIONAL THEORY CALCULATIONS

All DFT calculations were carried out with the program GAUSSIAN 03. The charge and multiplicity of the system are both 1. The initial geometry of the dyes corresponds to that shown in Scheme 1 (main text), i.e. the configuration with all double bonds in a *trans* conformation with the sulphur atom being *cis* to the methine chain. We used Becke's three-parameter hybrid exchange functional (B3)[i] with the correlation functional of Lee, Yang, and Parr (LYP)[ii]. The geometry of the ground states was optimised with the basis set 6-31G(d); it is planar for all dyes (with the exception of the methyl groups, of course). The vertical excitation energies were calculated by single-point TD-DFT calculations using the basis set 6-311G(d) and the polarisable continuum model (PCM) of Tomasi and coworkers[iii] with a dielectric constant $\varepsilon = 46.7$ (DMSO). The reported values for vertical excitation in the text are scaled values, ΔE_{scaled}, that have been obtained by applying the correction scheme of Champagne *et al.*: $\Delta E_{scaled} = -0.6017$ eV + 1.0941 ΔE_{calc} (with ΔE_{calc} being the excitation energy extracted from the TD-DFT calculations).

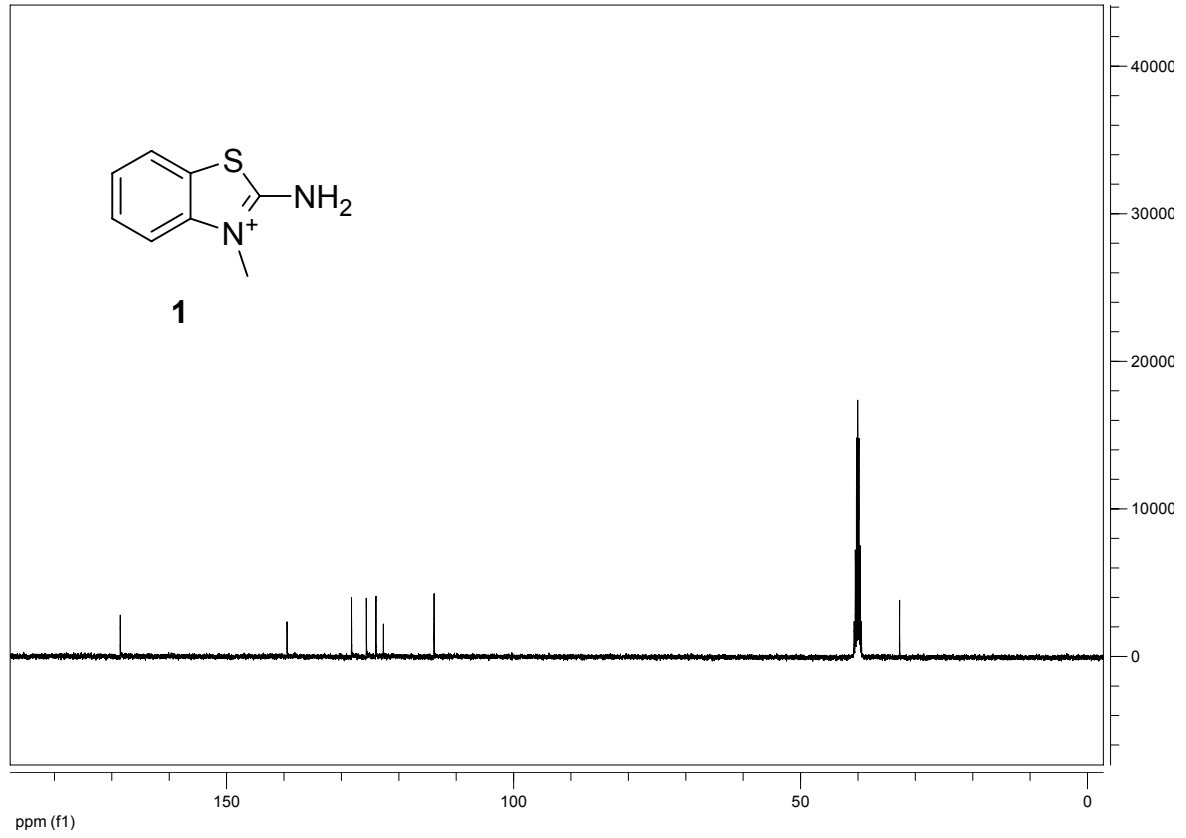
i) Becke, A. D..; *J. Chem. Phys.* **1993**, *98*, 5648.
ii) Lee, C.; Yang, W.; and Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.
iii) Miertus, S.; Scrocco, E.; and J. Tomasi, J. *Chem. Phys.* **1981**, *55*, 117.

4 NMR SPECTRA

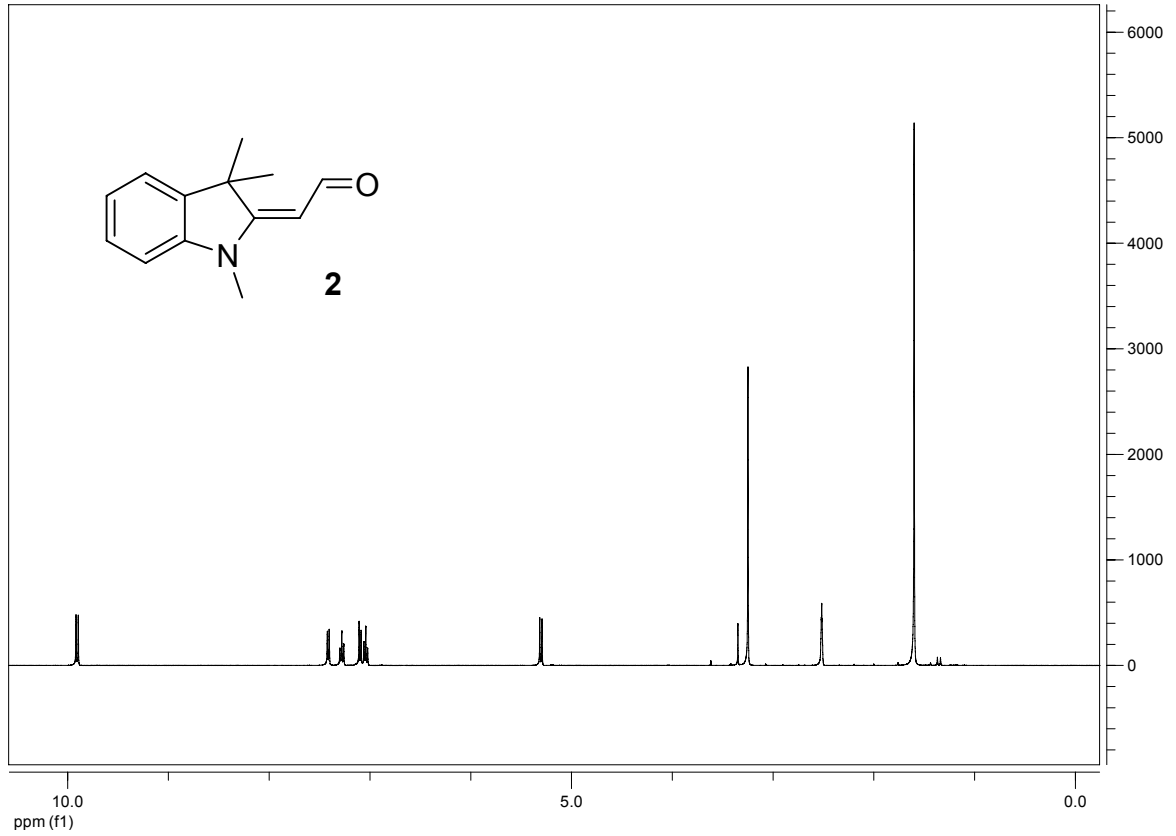
^{1}H NMR of **1** in DMSO-d_6



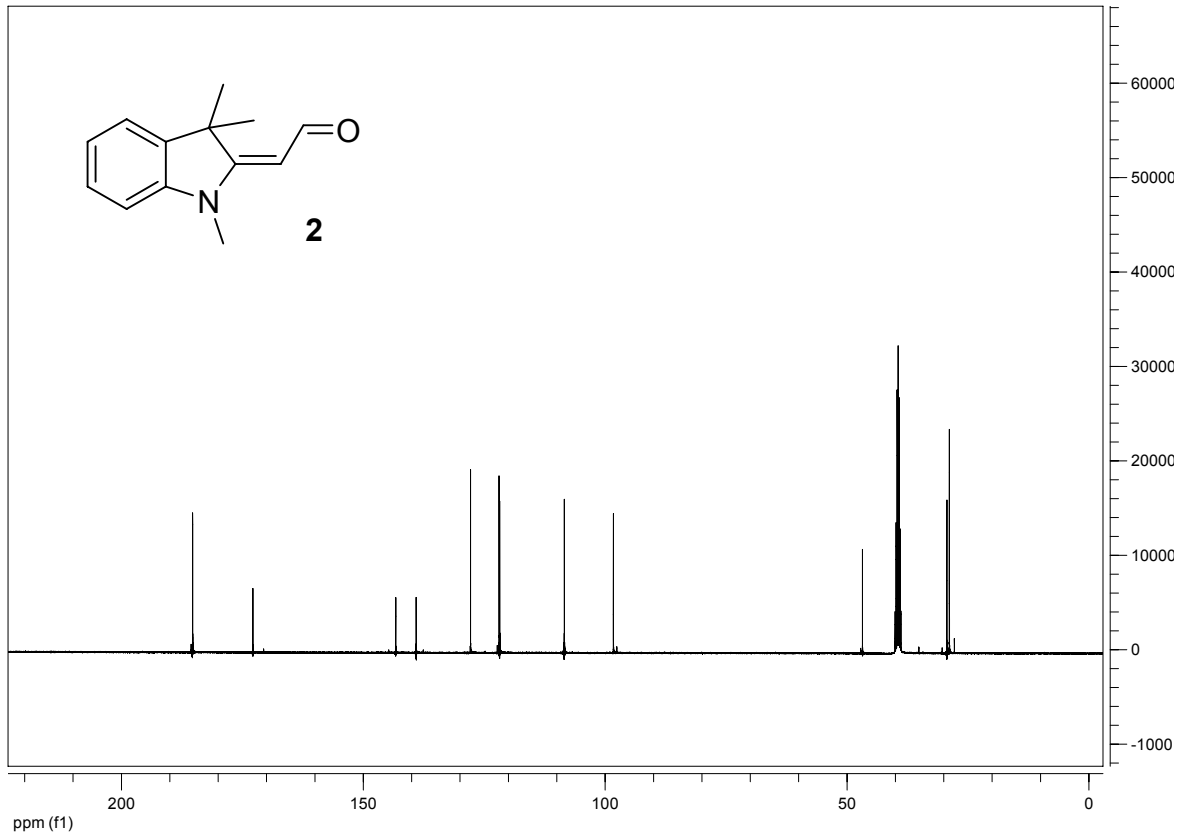
^{13}C NMR of **1** in DMSO-d_6



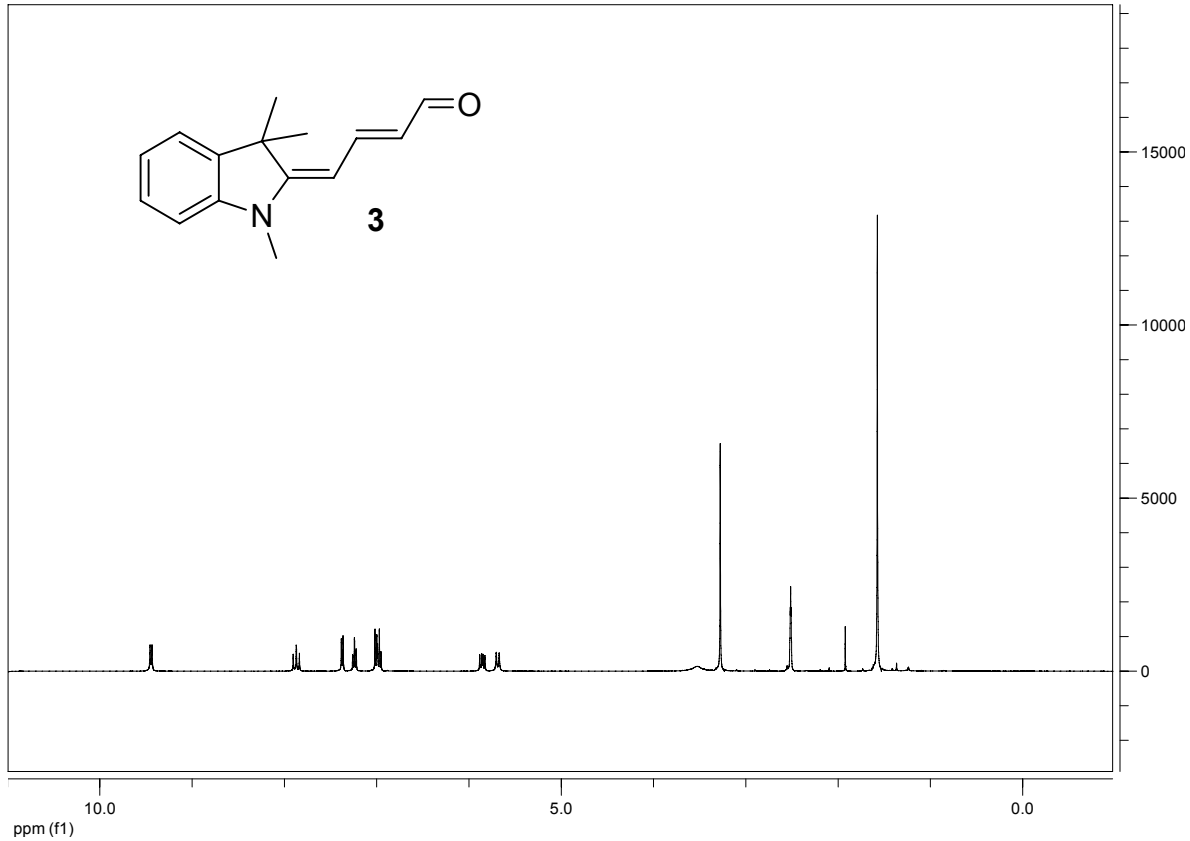
^{1}H NMR of aldehyde **2** in DMSO-d_6



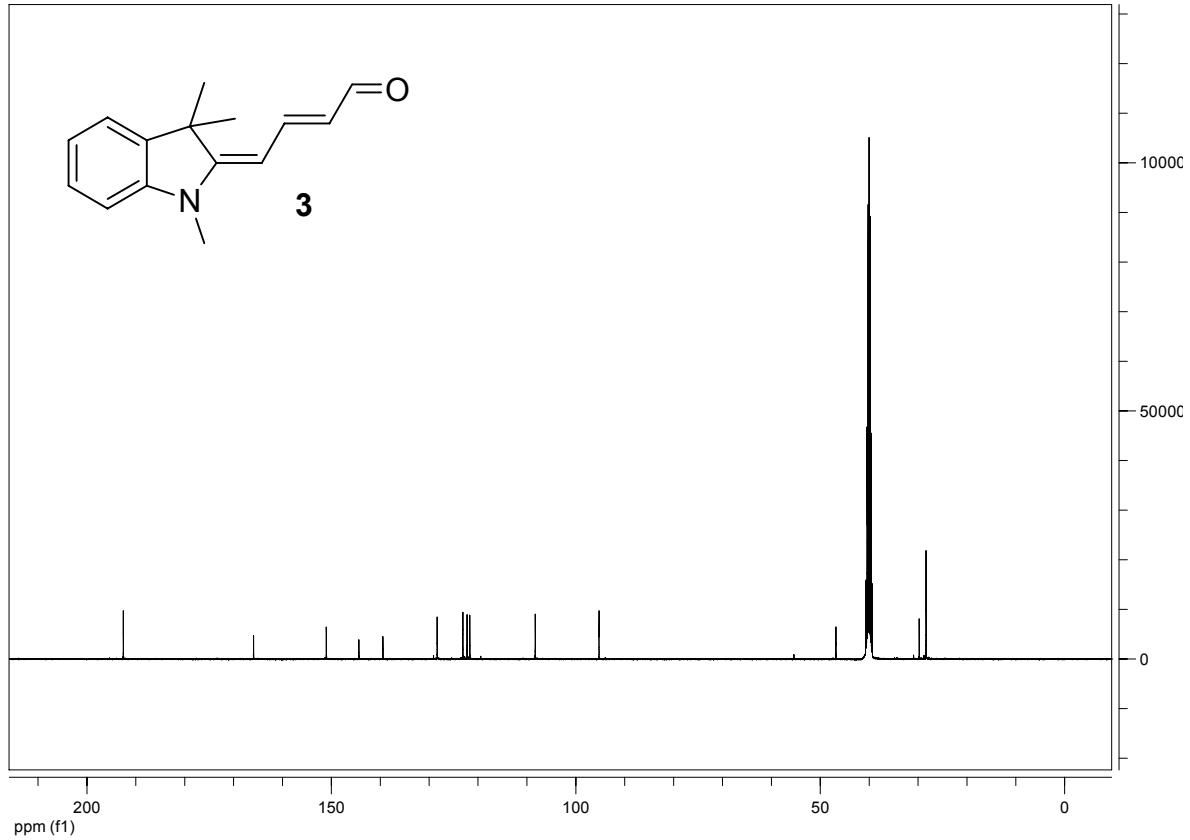
^{13}C NMR of aldehyde **2** in DMSO-d_6



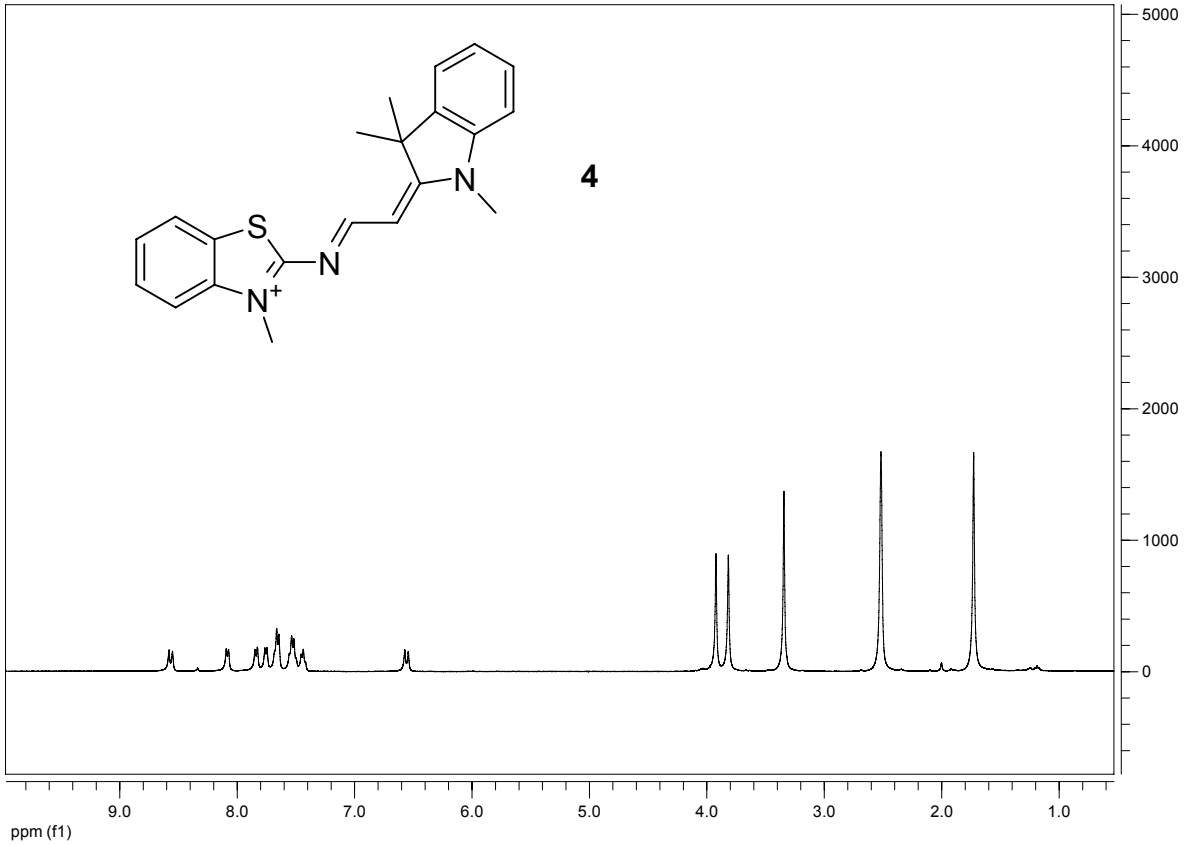
^{1}H NMR of aldehyde **3** in DMSO-d_6



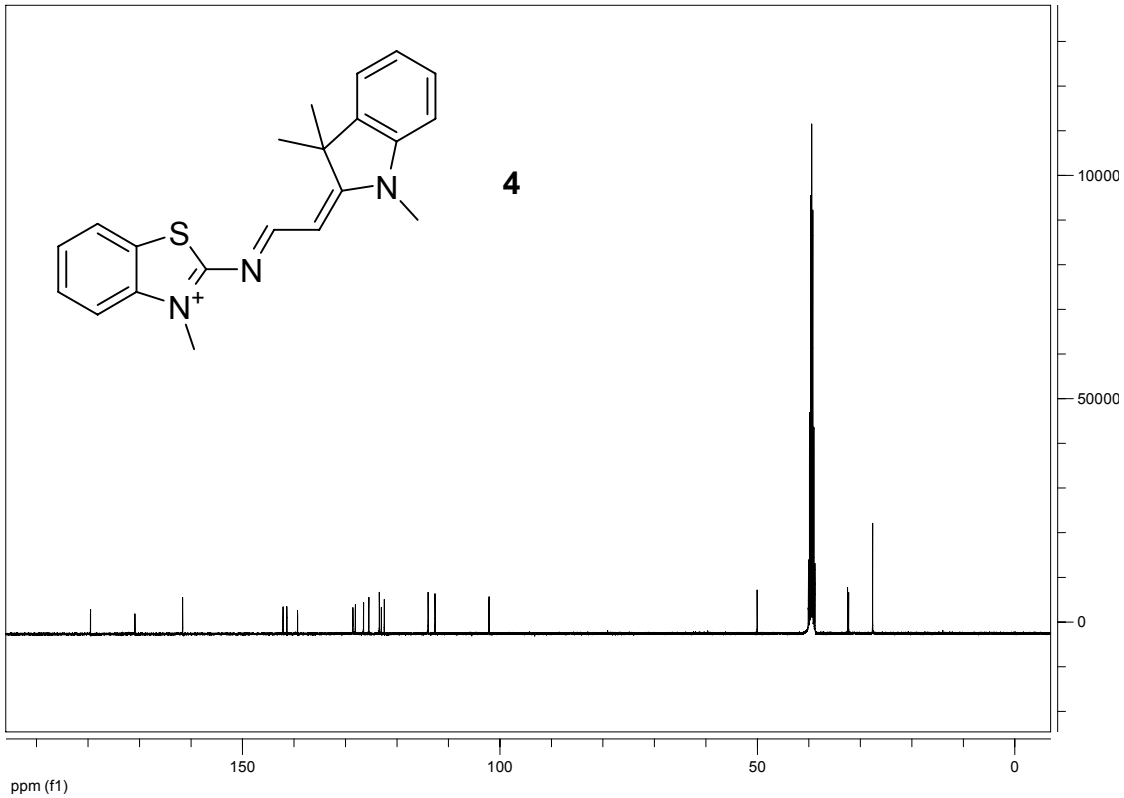
^{13}C NMR of aldehyde **3** in DMSO-d_6



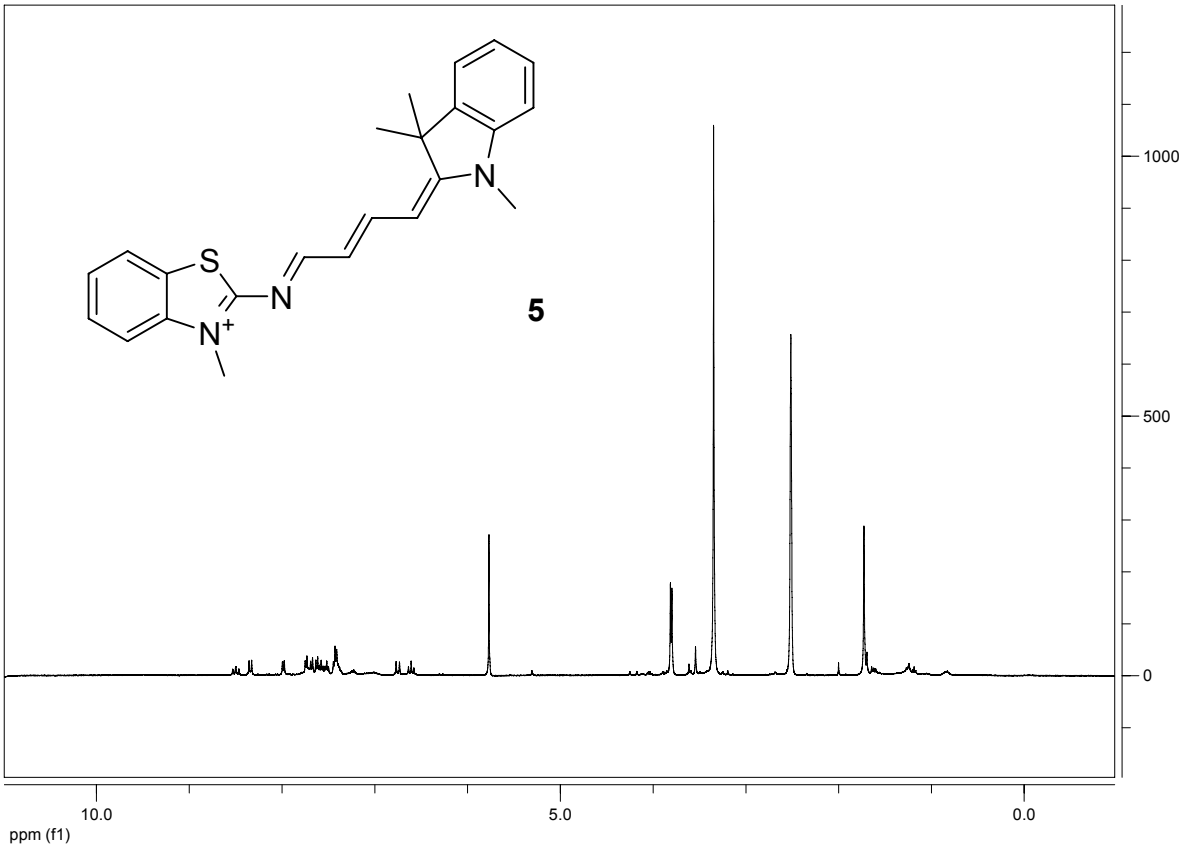
^{1}H NMR of cyanine **4** in DMSO-d_6



^{13}C NMR of cyanine **4** in DMSO-d_6



^{1}H NMR of cyanine **5** in DMSO-d_6 *(additional signals in the ^{1}H and ^{13}C NMRs correspond to the amine **1** and aldehyde **3** and result from a partial hydrolysis of the imine bond during the NMR acquisitions)*



^{13}C NMR of cyanine **5** in DMSO-d_6