Selective fluorimetric detection of pyrimidine nucleotides in neutral aqueous solution with a styrylpyridine-based cyclophane

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Supporting Information

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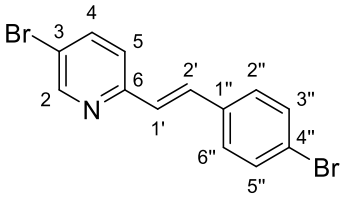
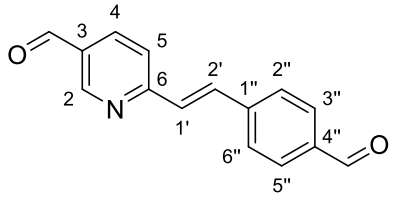
1. Synthesis

(*E*)-5-Bromo-2-[2-(4-bromophenyl)ethenyl]pyridine (1)[1]



A mixture of 4-bromobenzaldehyde (2.07 g, 11.2 mmol), 5-bromo-2-methylpyridine (1.92 g, 11.2 mmol), $Ca(OTf_2)$ (189 mg, 560 µmol), and Bu_4NPF_6 (87.0 mg, 220 µmol) was stirred under argon gas atmosphere at 130 °C for 5 d in a closed vessel. The reaction mixture was treated with acetone (50 mL). The remaining solid was filtered off and washed with cold acetone (3 x 1 mL) to give the product **1** as colorless crystalline plates (1.13 g, 3.34 mmol, 30%); mp 189–190 °C. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.08 (d, ^3J = 16 Hz, 1H, 1'-H), 7.26 (d, ^3J = 8 Hz, 1H, 5-H), 7.43 (d, ^3J = 8 Hz, 2H, 2''-H, 6''-H), 7.50 (d, ^3J = 8 Hz, 2H, 3''-H, 5''-H), 7.56 (d, ^3J = 16 Hz, 1H, 2'-H), 7.78 (dd, ^3J = 8, ^4J = 2 Hz, 1H, 4-H), 8.64 (d, ^4J = 2 Hz, 1H, 2-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 119.1 (C3), 122.7 (C4''), 123.4 (C5), 127.5 (C1'), 128.8 (C2'', C6''), 132.1 (C3'', C5''), 132.4 (C2'), 135.5 (C1''), 139.3 (C4), 151.0 (C2), 153.9 (C6).

(*E*)-6-[2-(4-Formylphenyl)ethenyl]-3-pyridine carbaldehyde (2)



To a solution of **1** (250 mg, 740 µmol) in anhydrous THF (6 mL) was added *n*-BuLi (2.5 M in hexane, 710 µL, 1.78 mmol) at –78 °C under N_2 atmosphere, and the reaction mixture was stirred at –78 °C for 5 min. DMF (580 µL, 7.38 mmol) was added dropwise, and the mixture was stirred until room temperature was reached. A saturated aq. NH_4Cl solution (20 mL) was added to the mixture, and the product was extracted with Et_2O (3 x 20 mL) and $CHCl_3$ (3 x 20 mL). The combined organic layers were dried with Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The product was crystallized from $CHCl_3$/hexane to give **2** as fine yellow needles (111 mg, 468 µmol, 63%); mp 195–196 °C. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.35 (d, ^3J = 16 Hz, 1H, 1'-H), 7.55 (d, ^3J = 8 Hz, 1H, 5-H), 7.77 (d, ^3J = 8 Hz, 2H, 3''-H), 7.90 (d, ^3J = 16 Hz, 1H, 2'-H), 7.92 (d, ^3J = 8 Hz, 2H, 2''-H), 8.18 (dd, ^3J = 8, ^4J = 2 Hz, 1H, 4-H), 9.07 (d, ^4J = 2 Hz, 1H, 2-H), 10.03 (s, 1H, C4'-C**H**O), 10.11 (s, 1H, C3-C**H**O). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 123.0 (C5), 128.0 (C3'', C5''), 129.9 (C1'), 130.2 (C3), 130.3 (C2'', C6''), 135.1 (C2'), 136.4 (C4''), 136.7 (C4), 141.8 (C1''), 152.4 (C2), 159.7 (C6), 190.1 (C3-**C**HO), 191.5 (C5'-**C**HO). – MS (ESI$^+$): *m*/*z* (%) = 238 (100) [M + H]$^+$. – El. Anal. for $C_{15}H_{11}NO_2$, calc. (%): C 75.94, H 4.67, N 5.90, found C 75.86, H 4.69, N 5.87.

2. Determination of fluorescence quantum yields

Solutions were prepared for each measurement from stock solutions of the cyclophane **4** in MeOH (*c* = 1.0 mM). Aliquots of the stock solution were thoroughly evaporated under a stream of nitrogen, and the residue was redissolved in the respective solvent. For the detection of fluorescence spectra, the excitation slit was adjusted to 10 nm and the emission slit to 5 nm, and the excitation wavelengths were fixed at 280 nm. The relative fluorescence quantum yields, Φ_{fl}, of **4** were determined under identical conditions (detection wavelength, excitation wavelength, detector voltage, slit bandwidths, collection rate). The quantum yield, Φ_{fl}, was determined according to eq. 1.

$$\Phi_{fl,\,X} = \frac{F_X A_S}{F_S A_X} \frac{n_X^2}{n_S^2} \Phi_{fl,\,S} \qquad \text{(eq. 1)}$$

The indices X and S indicate the analyte (X) and standard (S) solution.
Φ_{fl} = Fluorescence quantum yield.
F = Integral of the emission band.
A = Absorbance at the excitation wavelength.
n = Refraction index of the solution.
Measurements were performed with naphthalene in cyclohexane as standard [Φ = 0.23][2]
The estimated error is ca. 10% of the given values.

3. Determination of binding constants

From the binding isotherms derived from the fluorimetric titrations, the binding constants were determined with the software SpecFit/32TM (Spectrum Software Associates, West Marlborough, MA, U.S.A.). The binding interactions were treated as host–guest interactions between the nucleotides (guest) and the cyclophane **4** (host), with the host providing a distinct number of binding sites. The experimental values were fitted to the theoretical model by the least square method with a Levenberg–

Marquardt algorithm as implemented in the software program. In all cases, satisfactory fits were obtained for 1:1 complexes (Figure S1).

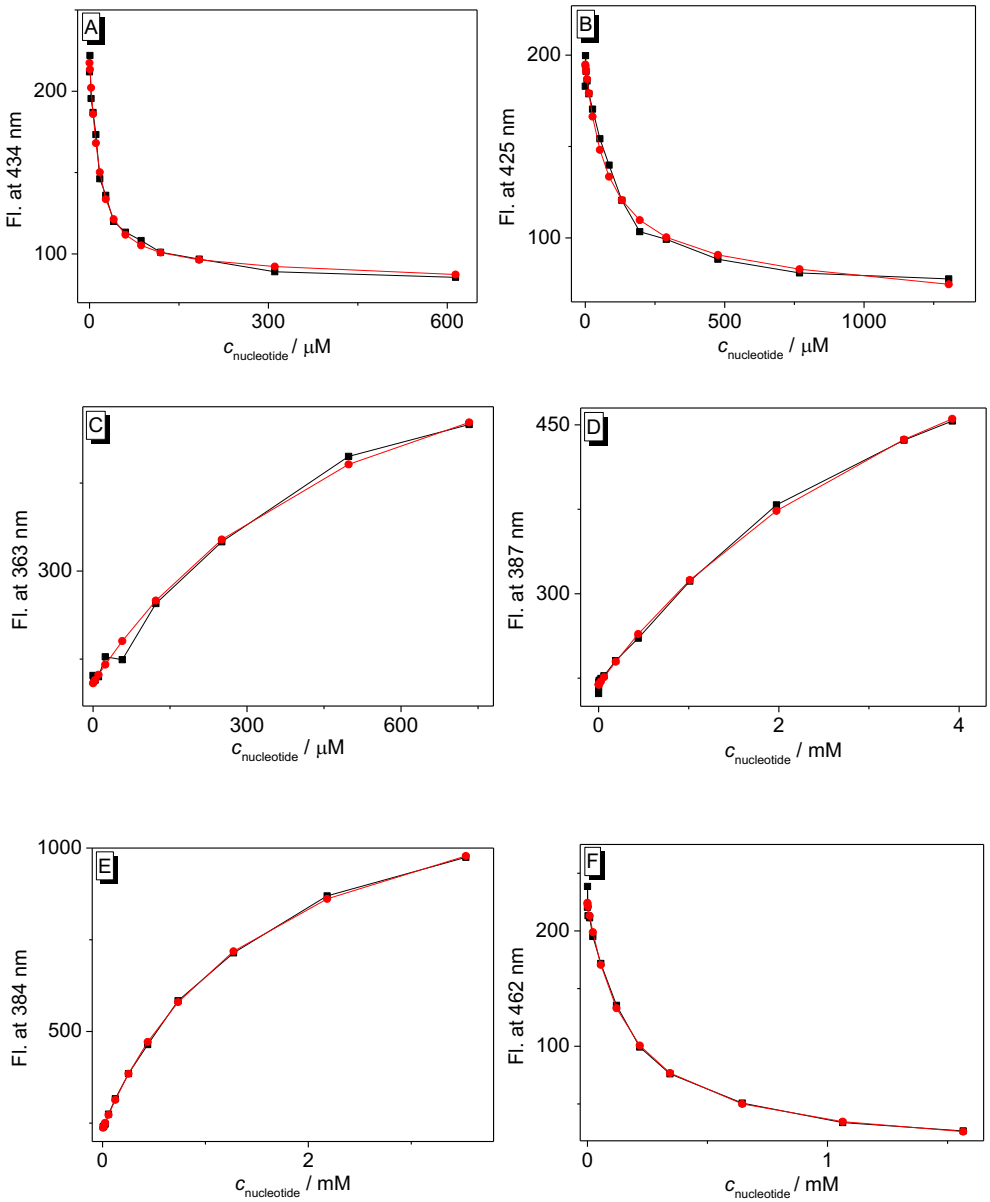


Figure S1. Plot of fluorescence intensity of cyclophane **4** at selected wavelength versus concentration of nucleotide as obtained from fluorimetric titrations of nucleotides ATP (A), AMP (B), TTP (C), CMP (D), TMP (E) and dGMP (F) to **4** in cacodylate buffer (pH 7.2). Red lines: fitting of the experimental data to the theoretical model. Black squares: experimental data.

4. Determination of the limit of detection

The limit of detection (LOD) of **4** for TMP, TTP and CMP were determined from equation 2.[3,4]

$$LOD = 3\,\sigma / S \qquad \text{(eq. 2)}$$

σ is the standard deviation for the measurement of 20 reference samples; S is the slope derived from the linear range of the fluorimetric titrations of **4** with TMP, TTP and CMP.

The corresponding values of standard deviation, linear range and detection limits are shown in Table S1 and calculated from the data shown in Fig. 3.

Table S1. Calculation of the Limit of Detection (LOD) from Fluorimetric Titrations.

Nucleotide	σ[a]	linear range / µM	LOD[b] / µM
TMP	0.06	0–26.9	0.09
TTP	0.01	0–122	0.02
CMP	0.004	0–197	0.04

[a]σ = standard deviation, [b]LOD = limit of detection

5. NMR spectra

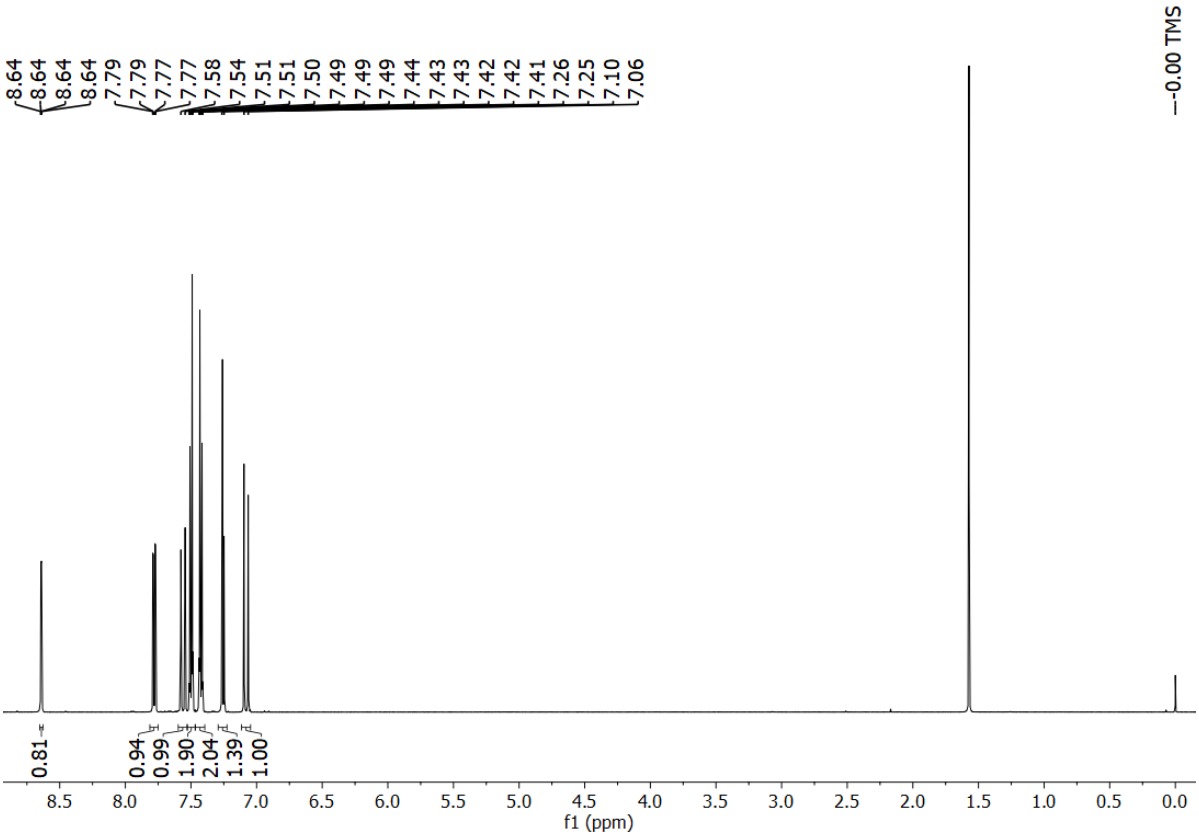


Figure S2. ^{1}H NMR spectrum (500 MHz) of **1** in $CDCl_3$.

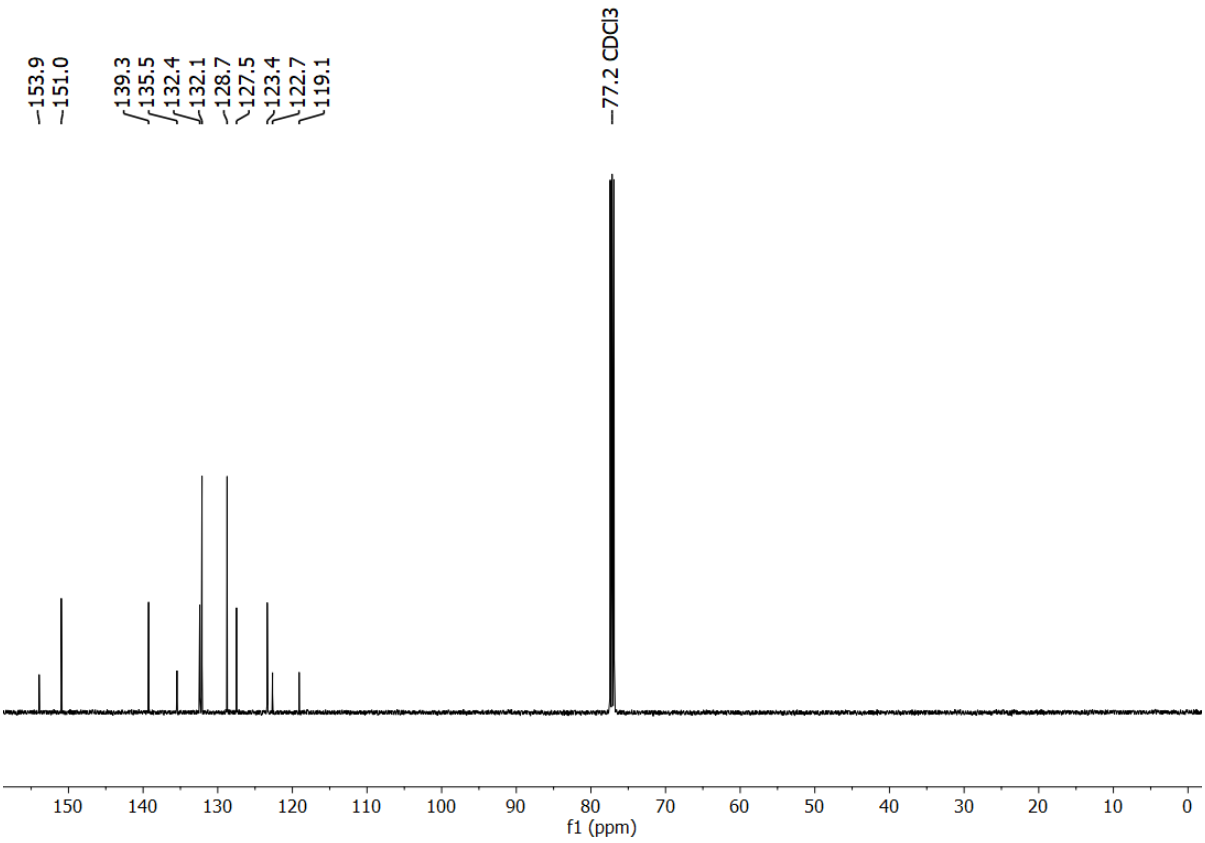


Figure S3. $^{13}C\{^{1}H\}$-NMR spectrum of **1** (125 MHz) in $CDCl_3$.

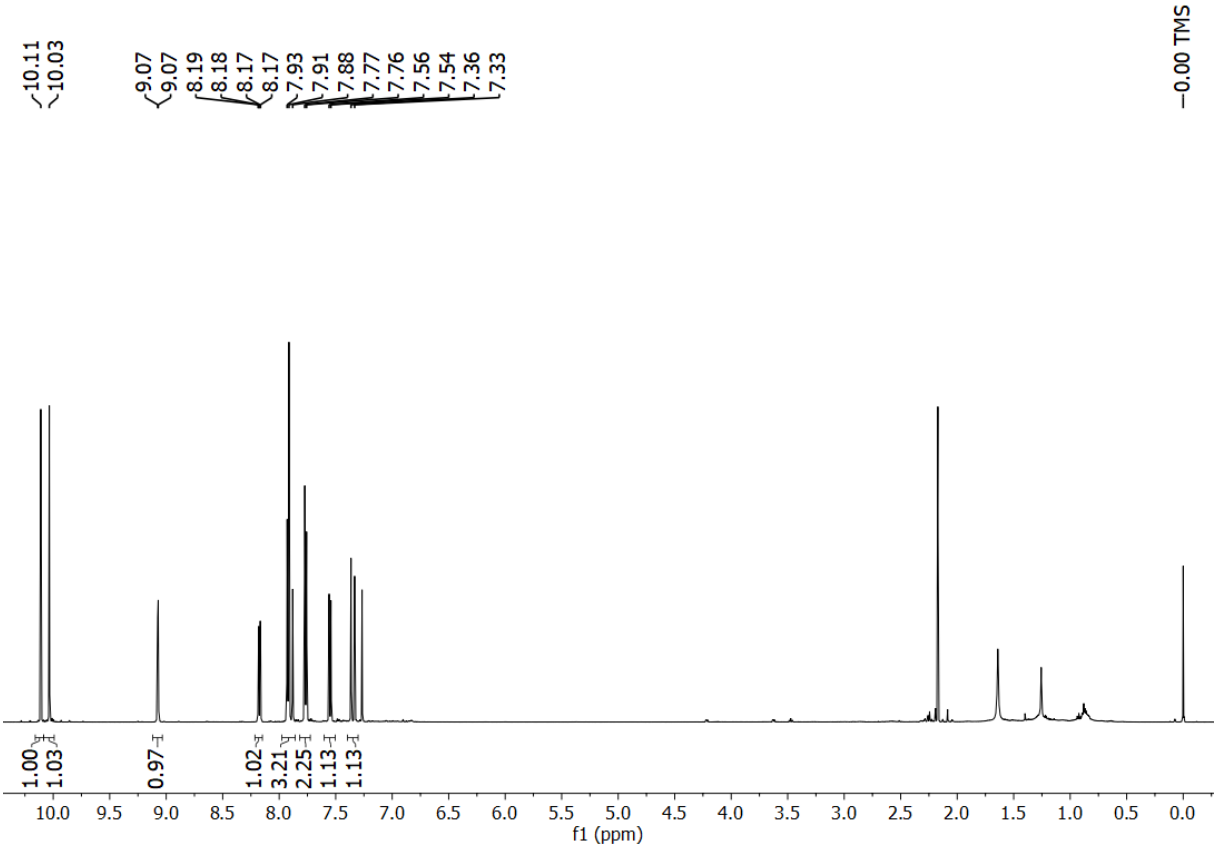


Figure S4. ^{1}H NMR spectrum of **2** (500 MHz) in $CDCl_3$.

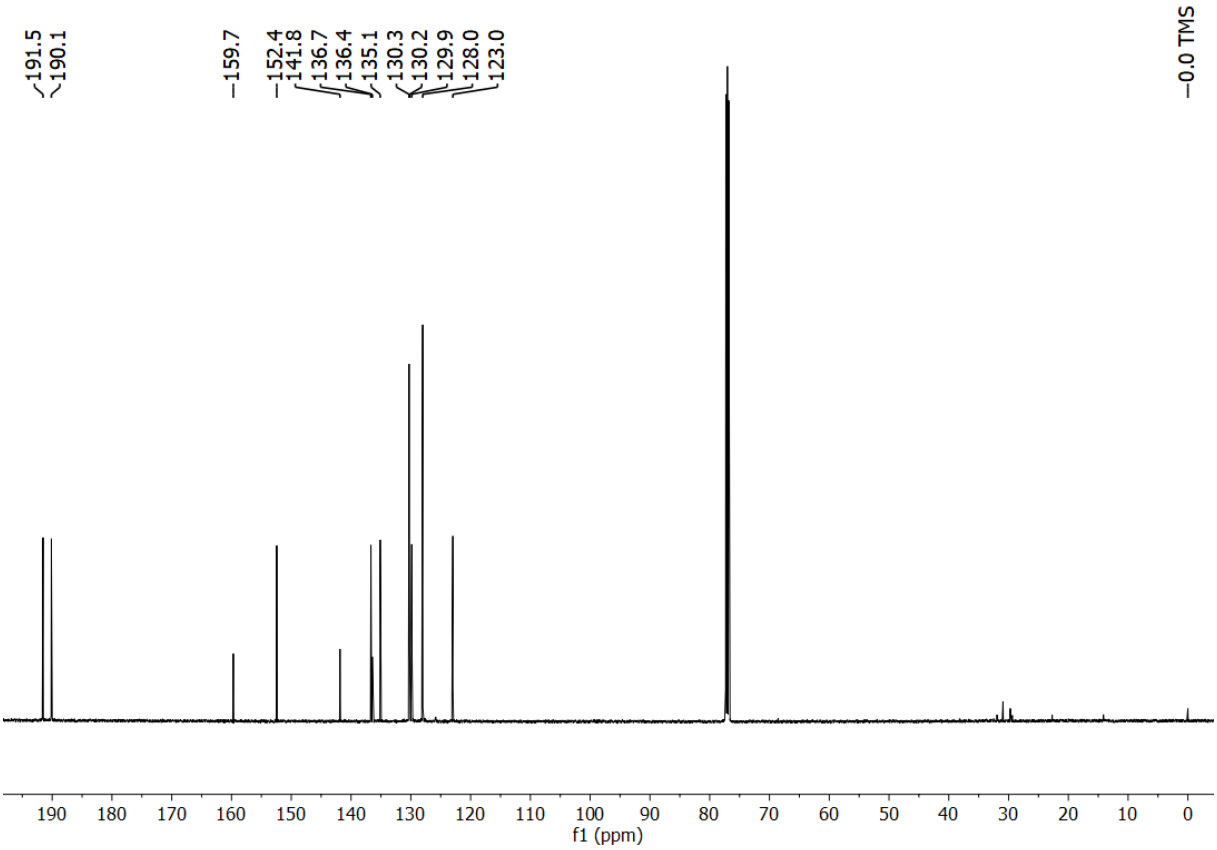


Figure S5. $^{13}C\{^{1}H\}$-NMR spectrum of **2** (125 MHz) in $CDCl_3$.

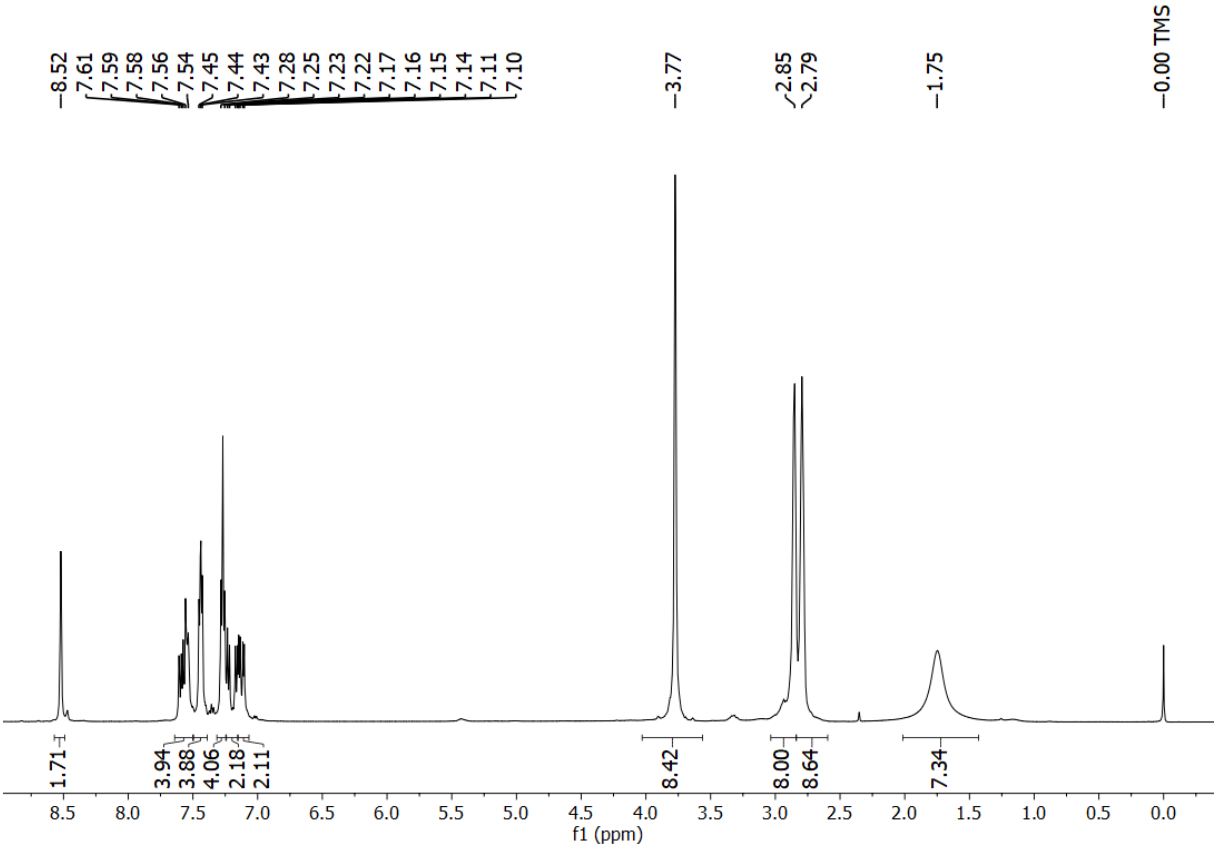


Figure S6. ^{1}H NMR spectrum of **4** (500 MHz) in $CDCl_3$.

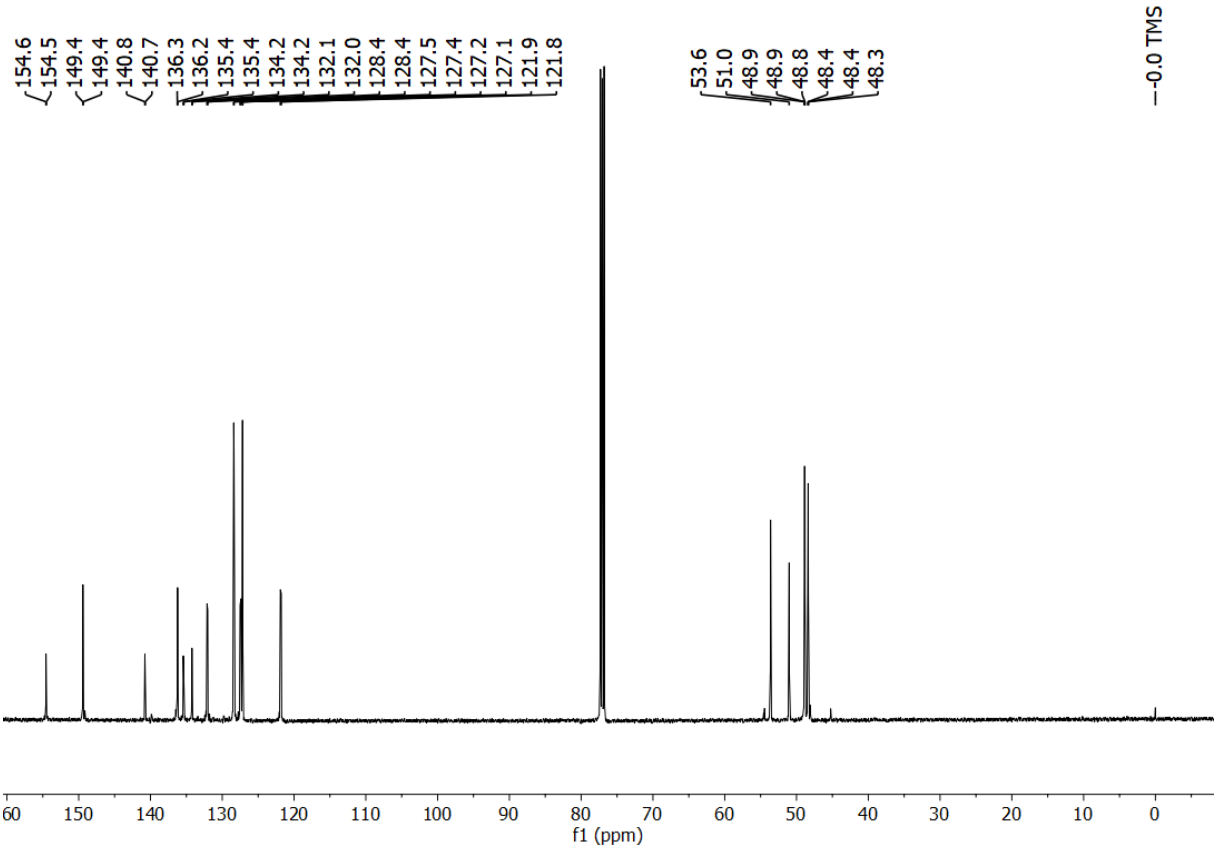


Figure S7. $^{13}C\{^{1}H\}$-NMR spectrum of **4** (125 MHz) in $CDCl_3$.

6. References

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