Electronic Supplementary Information

The *N*-Biphenyldihydroisoquinolinium Scaffold as a Novel Motif for Selective Fluorimetric Detection of Quadruplex DNA

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1. Equipment

The NMR spectra were recorded with a JEOL ECZ 500 (^{1}H: 500 MHz, ^{13}C: 125 MHz) at room temperature (T = 25 °C). The ^{1}H and ^{13}C NMR spectra were referenced to $CDCl_3$ [δ(^{1}H) = 7.26 ppm, δ(^{13}C) = 77.0 ppm] or CD_3OD [δ(^{1}H) = 3.31 ppm, δ(^{13}C) = 49.0 ppm] and processed with the software MestReNova.

Preparative HPLC was carried out on a Jacso HPLC unit (PU-1580 plus) in combination with UV/Vis detection at 200–680 nm (Jacso MD-2010 plus diode array detector) at room temperature. Experiments for purification of the compounds were carried out on a SymmetryPrep™ C18 column (19 x 300 mm, 7 µm, Waters) at a flow rate of 10 mL min^{-1}; mobile phases: (A) 90% H_2O with 10% MeCN (0.05% TFA) and (B) 90% MeCN with 10% H_2O, 0.05% TFA.

Elemental analysis data were determined by Rochus Breuer (Organic Chemistry I, University of Siegen) on a HEKAtech EUROEA combustion analyzer. The ESI mass spectra were measured on a Bruker Daltonics micOTOF Focus (driving current: 4.5 kV, capillary temperature: 200 °C) and an orbitrap mass spectrometer Thermo Fisher Exactive (driving current: 4 kV, capillary temperature: 300 °C, injection rate: 10 µL min^{-1}) or with a Finnigan LCQ Deca (voltage 6 kV, surge gas argon, capillary temperature 200 °C, auxiliary gas nitrogen).

The absorption spectra were measured in Hellma quartz glass cuvettes 110-QS (d = 10 mm) with a Varian Cary 100 Bio absorption spectrometer. The emission spectra were recorded with a Varian Cary Eclipse fluorescence spectrometer in Hellma quartz glass cuvettes 114F-QS and 115 F-QS (d = 10 mm). For melting temperature measurements the implemented Thermal temperature program was used. The LD spectra were recorded with a Chirascan spectrometer (Applied Photophysics). The samples were oriented in a rotating Couette with a shear gradient of 1200 s^{-1}. All measurements were recorded at a temperature of T = 20 °C as adjusted with a thermostat. The sample solutions for the DNA experiments were mixed with a reaction vessel shaker Top-Mix 11118 (Fisher Bioblock Scientific). E-Pure® water was obtained with an ultrapure water system D4632-33 (Wilhelm Werner GmbH, Leverkusen, D); filters: D 0835, D0803 and D 5027 (2 x). The pH values were measured with a QpH 70 (Merck).

2. Materials

Commercially available reagents were obtained from Acros Organics [$LiAlH_4$, $POCl_3$, 4-(Dimethylamino)pydridine], Carbolution Chemicals GmbH (*L*-Alanin, Di-*tert*-butyldicarbonate), Thermo Fisher Scientific Inc. (TsCl, Cs_2CO_3, I_2), Alfa Aesar GmbH & Co KG [3,5-diemthoxybenzyl bromide, $CuBrSMe_2$, $Pd(PPh_3)_4$, 1,4-dibromobenzene], Biomers [**22AG** d(5´-AGGGTTAGGGTTAGGGTTAGGG-3´), **c-kras** (5´-AGGGCGGTGTGGGAAGAGGGA-3´), **c-kit** (5´-AGGGAGGGCGCTGGGAGGAGGAGGG-3´), **c-myc** (5´-TGAGGGTGGGTAGGGZG-

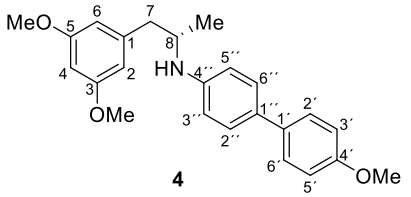
GGTAA-3´), **h-ras1** (5'-TCGGGTTGCGGGCGCAGGGCACGGGCG-3'), **F21T** [Fluo-d(GGGTTAGGGGTAGGGTTAGGG)-Tamra; Fluo = fluoresceine; Tamra = tetramethyl-rhodamine], **FmycT** [Fluo-d(TGAGGGTGGGTAGGGTGGGTAA)-Tamra], **FkitT** [Fluo-d(AGGGAGGGCGGGAGGAGGG)-Tamra], **FkrasT** [Fluo-d(AGGGCGGTGGAAGAGGGA)-Tamra]; HPLC purified, quality control by MS: MALDI-TOF; were used without further purification], Carl Roth GmbH + Co. KG (trifluoroacetic acid, KOH), Chempur GmbH [(4-methoxyphenyl)boronic acid], Merck KGaA [Magnesium, KOtBu, acetyl chloride, Calf thymus DNA (ct DNA, type I; highly polymerized sodium salt; were used without further purification; ε = 12824 $cm^{-1} M^{-1}$)], BLD Pharmatech Ltd. [$Pd_2(dba)_3$], VWR Chemicals International GmbH (Na_2SO_4), Chemsolute Laboratory Chemicals ($NaHCO_3$). (*S*)-2-aminopropan-1-ol, tert-Butyl (*S*)-(1-hydroxypropan-2-yl)carbamate, tert-Butyl (*S*)-2-methylaziridine-1-carboxylate, tert-Butyl (S)-(1-(3,5-dimethoxyphenyl)propan-2-yl)carbamate and 4-bromo-4'-methoxy-1,1'-biphenyl were obtained synthetically and used for the further synthesis steps.[1,2] Technical-grade solvents were distilled prior use. THF and Et_2O were stirred under reflux with a Na wire and distilled prior to use. Acetonitrile was stirred with powdered CaH_2 (2 g/ L) for 10 min under reflux and then distilled off quickly. The distillate was mixed with P_2O_5 (2 g/ L), stirred again for 10 min under reflux and distilled and stored over a molecular sieve (3 Å) and under argon. Toluene was dried overnight with $CaCl_2$ before use, decanted from the desiccant and distilled. The distillate was stored over molecular sieve (3 Å) and under argon. Column chromatography was carried out with silica gel 60 M (0.0063–0.25 mm) from Macherey Nagel GmbH & Co. KG. or Sephadex™ (LH-20) from GE Healthcare.

3. Methods

Reaction solutions were stirred with a magnetic stirring bar. Reaction temperatures refer to the medium that surrounded the reaction vessel. Solvents were usually removed under reduced pressure at 40–50 °C with a rotatory evaporator. Room temperature (rt) was approximately 22 °C. Air- and/or water-sensitive reactions were carried out under inert atmosphere with Schlenk equipment.

4. Synthesis

(*S*)-*N*-(1-(3,5-Dimethoxyphenyl)propan-2-yl)-4'-methoxy-[1,1'-biphenyl]-4-amine (**4**)



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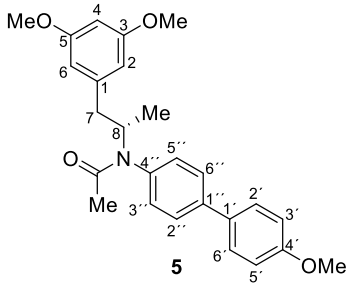
Argon was bubbled softly through a solution of (*S*)-1-(3,5-dimethoxyphenyl)propan-2-amine (51.9 mg, 266 µmol), 4-bromo-4'-methoxy-1,1'-biphenyl (70.0 mg, 266 µmol) and KOtBu (59.7 mg, 532 µmol) in toluene (20 mL) for 15 min. *rac*-BINAP (3.31 mg, 5.32 µmol) and $Pd_2(dba)_3$ (2.43 mg, 2.66 µmol) were added to the solution. The reaction mixture was stirred under reflux for 2 d, diluted with ethyl acetate (10 mL) and filtered, and the solvent was removed under reduced pressure. After purification by column chromatography (SiO_2, *n*-hexane/ethyl acetate 5:1, Rf = 0.36), product **4** was obtained as a white solid [75.5 mg, 253 µmol, 96%, mp >230 °C (dec.)]. – ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 1.19 (d, ^3J = 6 Hz, 3 H, 8-C**H**$_3$), 2.68 (dd, ^3J = 13 Hz, ^3J = 7 Hz, 1 H, 7-C**H**$_2$), 2.89 (dd, ^3J = 13 Hz, ^3J = 5 Hz, 1 H, 7-C**H**$_2$), 3.78 (s, 6 H, 2 x OC**H**$_3$), 3.79–3.82 (m, 1 H, 8-H), 3.83 (s, 3 H, OC**H**$_3$), 6.34–6.36 (m, 1 H, 4-H), 6.37–6.38 (m, 2H, 2-H, 6-H), 6.69–6.72 (m, 2 H, 3´´-H, 5´´-H), 6.94–6.97 (m, 2H, 3´-H, 5´-H), 7.39–7.42 (m, 2 H, 2´´-H, 6´´-H), 7.46–7.49 (m, 2H, 2´-H, 6´-H). – ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) = 20.5 (8-**C**H$_3$), 42.7 (C7), 49.5 (C8), 55.4 (2 x O**C**H$_3$), 55.8 (O**C**H$_3$), 98.7 (C4), 107.8 (C2, C6), 113.8 (C3´´, C5´´), 114.3 (C3´, C5´), 127.4 (C2´, C6´), 127.8 (C2´´, C6´´), 130.1 (C1´´), 134.1 (C1´), 140.9 (C1), 146.2 (C4´´), 158.2 (C4´), 160.8 (C3, C5). – El. Anal. for $C_{24}H_{27}NO_3$, calc. (%): C 76.36, H 7.21, N 3.71; found (%): C 76.25, H 7.26, N 3.45.

(*S*)-*N*-(1-(3,5-Dimethoxyphenyl)propan-2-yl)
-*N*-(4'-methoxy-[1,1'-biphenyl]-4-yl)acetamide (**5**)

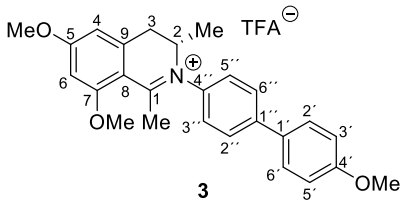


5

To a solution of arylamine **4** (65.2 mg, 173 µmol) and DMAP (63.3 mg, 518 µmol) in toluene (15 mL) was added acetyl chloride (40.7 mg, 518 mmol, 37.0 µL) and the reaction mixture was stirred for 2 h under reflux. After cooling to room temperature, the reaction mixture was mixed with a saturated K_2CO_3 solution (5 mL), diluted with water (20 mL) and extracted with ethyl

acetate (3 x 30 mL). The combined organic layers were dried with Na_2SO_4 and filtered, and the solvent was removed under reduced pressure. After column-chromatographic purification (SiO_2, *n*-hexane/ethyl acetate 1:3, Rf = 0.40), product **5** was obtained as a colorless oil (68.7 mg, 163 µmol, 95%). – ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 1.10 (d, ^3J = 7 Hz, 3H, 8-C**H**$_3$), 1.79 (s, 3H, NCO-C**H**$_3$), 2.48 (dd, ^3J = 14 Hz, ^3J = 9 Hz, 1H, 7-C**H**$_2$), 2.97 (dd, ^3J = 14 Hz, ^3J = 6 Hz, 1H, 7-C**H**$_2$), 3.78 (s, 6H, 2 x OC**H**$_3$), 3.85 (s, 3H, OC**H**$_3$), 5.07–5.12 (m, 1H, 8-H), 6.33 (t, ^3J = 3 Hz, 1H, 4-H), 6.40 (d, ^3J = 2 Hz, 2H, 2-H, 6-H), 6.98–7.00 (m, 2 H, 3´-H, 5´-H), 7.03–7.05 (m, 2H, 3´´-H, 5´´-H), 7.53–7.57 (m, 4 H, 2´-H, 6´-H, 2´´-H, 6´´-H). – ^{13}C-NMR (150 MHz, $CDCl_3$): δ (ppm) = 18.5 (8-**C**H$_3$), 23.8 (NCO-**C**H$_3$), 41.6 (C7), 51.9 (C8), 55.4 (2 x O**C**H$_3$), 55.5 (O**C**H$_3$), 98.8 (C4), 107.1 (C2, C6), 114.5 (C3´, C5´), 127.5 (C2´´, C6´´), 128.2 (C2´, C6´), 130.3 (C3´´, C5´´), 132.4 (C1´), 138.5 (C4´´), 140.9 (C1´´), 141.4 (C1), 159.7 (C4´), 160.9 (2 x **C**-OCH$_3$), 170.6 (**C**=O). – MS (ESI$^+$): *m/z* (%) = 442 (100) [M+Na]$^+$.

(*S*)-6,8-Dimethoxy-2-(4'-methoxy-[1,1'-biphenyl]-4-yl)-1,3-dimethyl-3,4-dihydroisoquinolin-2-ium (**3**)



3

To a solution of acetamide **5** (68.7 mg, 165 µmol) in acetonitrile (10 mL), $POCl_3$ (127 mg, 828 µmol, 77.4 µL) was added under argon atmosphere and the reaction mixture was stirred for 2 h under reflux. The solution was diluted with water (30 mL) and acetonitilr was removed under reduced pressure. The remaining residue was extracted with chloroform (3 x 50 mL) and the combined organic layers were dried with Na_2SO_4 and filtered. The solvent was removed under reduced pressure. After purification by column chromatography (Sephadex-LH50, MeOH + 0.05% TFA), product **3** was obtained as a yellow viscous oil (62.3 mg, 125 µmol, 76%). – ^{1}H-NMR (500 MHz, CD_3OD): δ (ppm) = 1.31 (d, ^3J = 7 Hz, 3H, 2-C**H**$_3$), 2.63 (s, 3H, 1-C**H**$_3$), 3.08 (dd, ^3J = 17 Hz, ^3J = 3 Hz, 1H, 3-C**H**$_2$), 3.70 (dd, ^3J = 17 Hz, ^3J = 7 Hz, 1H, 3-C**H**$_2$), 3.80 (s, 3H, 4´COC**H**$_3$), 4.03 (s, 3H, 7CO-C**H**$_3$), 4.06 (s, 3H, 5COC**H**$_3$), 4.45–4.50 (m, 1H, 2 H), 6.74–6.76 (m, 2H, 4-H, 6-H), 7.06–7.10 (m, 2H, 3´-H, 5´-H), 7.50 (br. s, 2H, 2´´-H, 6´´-H), 7.66–7.69 (m, 2H, 2´-H, 6´-H), 7.89 (br. s, 2H, 3´´-H, 5´´-H). – ^{13}C-NMR (150 MHz, $CDCl_3$): δ (ppm) = 14.8 (2-**C**H$_3$), 25.0 (1-**C**H$_3$), 34.8 (C3), 55.8 (4´-O**C**H$_3$), 56.9 (7-O**C**H$_3$), 57.0 (5-O**C**H$_3$), 61.0 (C2), 98.9 (C6), 109.1 (C4), 110.9 (C8), 115.6 (C3´, C5´), 127.5 (C2´´, C6´´), 128.9 (3´´C, 5´´C), 129.3 (C2´, C6´), 132.6 (C1´), 140.8 (C4´´), 141.9 (C9), 144.6 (C1´´), 161.7 (C4´), 166.1 (5C), 170.5 (7C), 176.1 (C1). – HRMS (ESI$^+$): *m/z* (%) = 470 (100) [M+H]$^+$. – El. Anal. for $C_{28}H_{28}F_3NO_5$ x H_2O, calc. (%): C 63.03, H 5.67, N 2.63; found (%): C 63.41, H 6.37, N 2.71.

5. Photometric and fluorimetric DNA titrations

Preparation of buffer solutions

For the preparation of buffer solutions E-Pure® water (18 MΩ cm) and biochemistry-grade chemicals were used. The buffer solution was filtered through a membrane filter (pore size 0.45 µM; Carl Roth GmbH, Karlsruhe) before use and kept under exclusion of light at 4 °C for a maximum of four weeks. The following concentrations were used:

BPE (biphosphate EDTA) buffer: 6.0 mM Na_2HPO_4, 2.0 mM NaH_2PO_4, 1.0 mM Na_2EDTA; pH 7.0.

Potassium phosphate buffer: 25 mM K_2HPO_4, 25 mM KH_2PO_4, 70 mM KCl; pH = 7.0.

Buffer for thermal DNA denaturation analysis: $c(Li(CH_3)_2AsO_2 \times 3H_2O)$ = 10 mM, c(KCl) = 10.0 mM, c(LiCl) = 90 mM, pH = 7.2.

Preparation of DNA solutions

The DNA (approximately 1–2 mg/mL) was dissolved in BPE buffer and kept at 4 °C for 24 h under exclusion of light. In the case of ct DNA, the solution was filtered through a membrane filter before use (pore size 0.45 µM; Carl Roth GmbH, Karlsruhe). After dilution with BPE buffer (1:20), the concentration was determined photometrically [ct DNA: ε_{264} = 12824 cm^{-1} M^{-1}, λ = 260 nm; c in base pairs].[3]

Preparation of the oligonucleotide solutions

The lyophilized oligonucleotide strands **F21T**, **FkitT**, **FmycT** and **FkrasT** were treated with Li-cacodylate or ODN buffer, heated for 5 min at 90 °C in a water bath, then cooled to room temperature overnight and stored at –25 °C; c = 50 µM.

22AG, **c-myc**, **c-kit** and **c-kras** were dissolved in potassium phosphate buffer, heated for 5 min at 90 °C in a water bath, then cooled to room temperature overnight and stored at –25 °C. The concentration of **22AG**, **c-myc**, **c-kit**, **c-kras** in the solution were c = 300 μM.

Preparation of ligand solution

For spectroscopic measurements a stock solution of the ligand **3** (c = 1.00 mM) was prepared in MeOH and kept under exclusion of light at 4 °C. It was shown by photometric analysis of a sample of **3** in neutral buffer solution, stored at room temperature and in the dark, that the compound remains stable without detectable decomposition after 7d.

Sample preparation, measurement, and measurement parameters

Starting from the stock solution of compound 3 (in MeOH, c = 1.00 mM), a solution (1 mL) with a concentration of c = 20 µM was prepared by removing MeOH with an N_2 gas stream by

evaporation and dissolving and homogenizing the residue in the corresponding phosphate buffer solution (1 mL). The experiments were performed with a sample volume of V = 700 µL. Absorbance and fluorescence measurements were performed at the same concentration and initial volume, in order to correct the fluorescence spectra for the change in absorption at the corresponding excitation wavelength. First, an absorption or emission spectrum of the ligand solution was recorded. After addition of the titrant solution and an equilibration time of 1.5 min the solution was analyzed with photometric or fluorimetric measurements. After each titration step, an absorption or fluorescence spectrum was recorded, and the concentration of DNA was increased until no more changes in the spectra were observed. Photometric titrations were measured from 240 nm to 500 nm at a rate of 120 nm min^{-1} at 20 °C. The wavelength range of the fluorimetric titration was selected according to the emission bands of the ligands. Excitation wavelengths were selected based on the photometric titration. Analogous to photometric titration, the emission spectra were recorded at 20 °C with 120 nm min^{-1}. The slit widths for emission and excitation wavelength were 5 nm in all measurements. The detector voltage was between 600–700 V depending on the intensity of the fluorescence. The spectra were modified with the smoothing function "moving average" with a factor of 5 with the Origin software.[4]

Determination of the binding constant K_b

The binding constants were calculated according to the method of Stootman and Aldrich (eq. S1).[5] They were determined by fitting of the respective saturation factors SF to the DNA concentration c_{DNA} obtained from the photometric titration spectra (eq. S1).

$$\mathrm{SF} = \frac{1}{2}\, R \left\{ A + B + c\mathrm{DNA} - \sqrt{(A + B + c\mathrm{DNA})^2 - 4\, B\, c\mathrm{DNA}} \right\} \qquad \text{eq. S1}$$

The variable R refers to an instrumental response sensitivity dependent variable, A is the reciprocal binding constant $1/K_b$, B is equal to the concentration of the ligand multiplied with the number of independent binding sites per DNA.

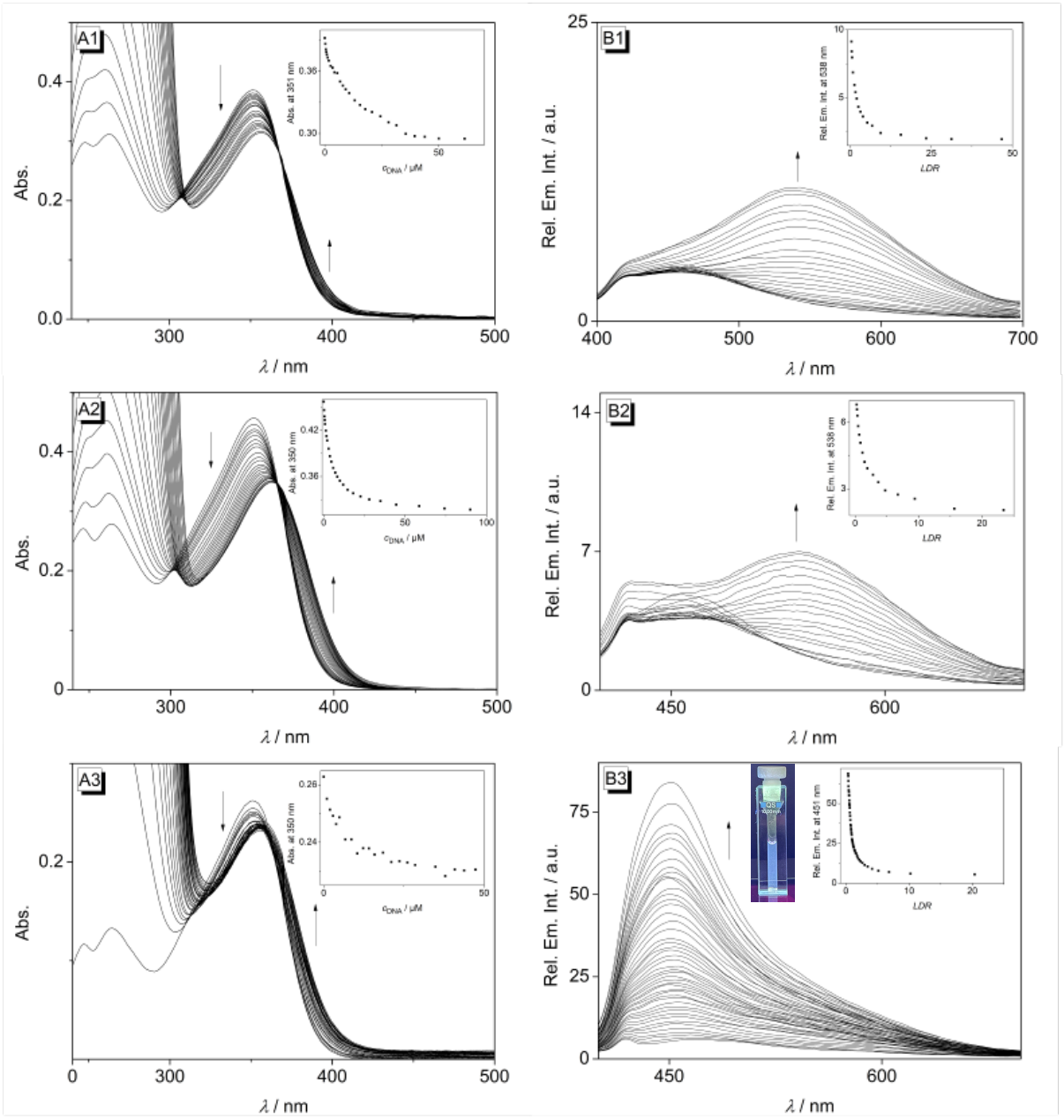


Fig. S1. Photometric (A) and fluorimetric (B) titration of **3** (c = 20 µM) with **c-kras** (1), **c-myc** (2)**, and h-ras1** (3) in K-phosphate buffer (c_{K+} = 95 mM, pH = 7.0). The arrows indicate the development of the absorption and emission bands during the titration. Inset (A): plot of the absorption at λ = 351 nm or λ = 350 nm versus c_{DNA}. Inset (B): plot of the emission at λ = 538 nm versus ligand-DNA ratio *LDR*.

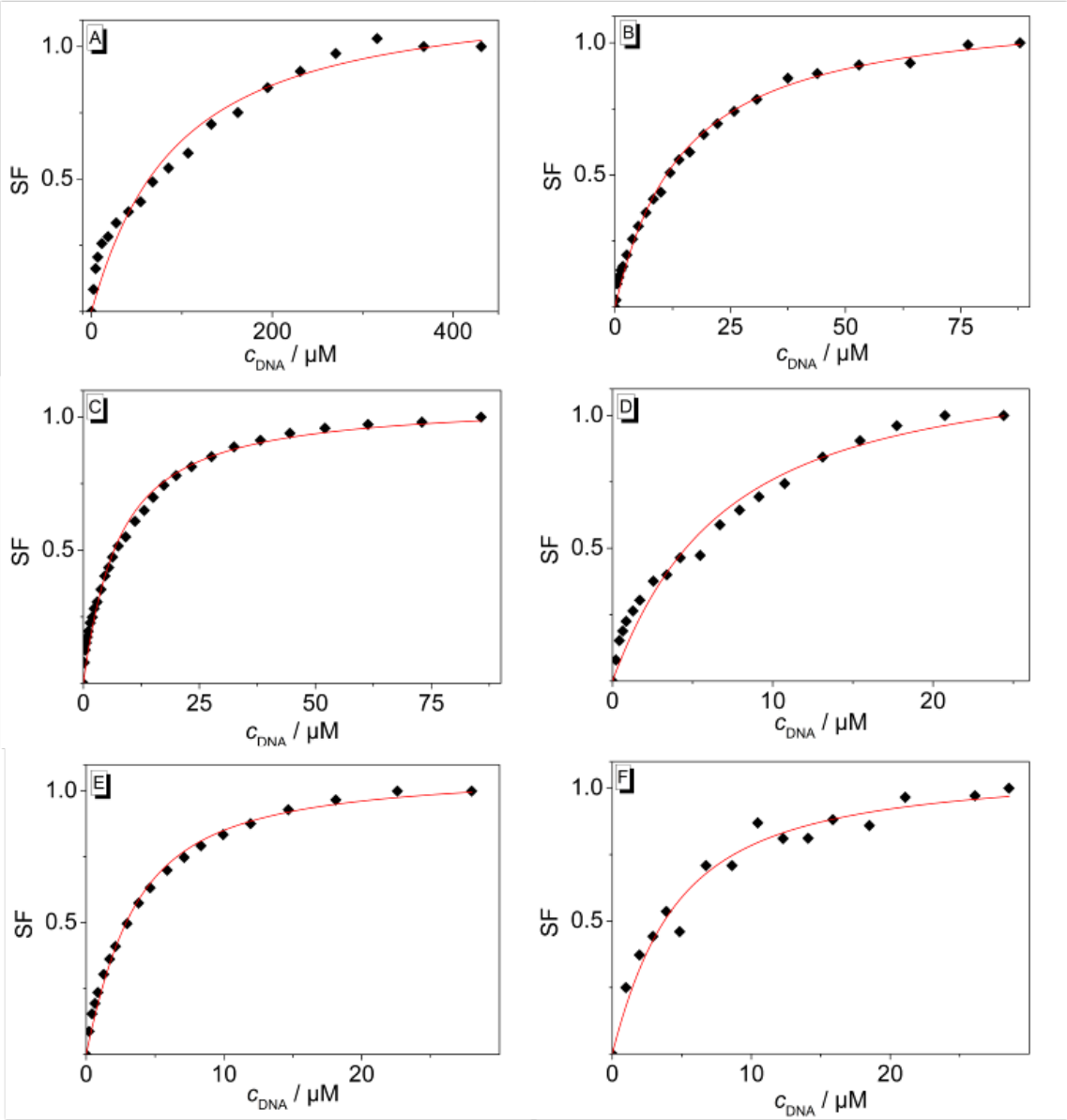


Fig. S2. Fitting curves of binding isotherms obtained from photometric titrations of **3** with ct DNA (A), **22AG** (B), **c-kit** (C), **c-kras** (D), **c-myc** (E), and **h-ras1** (F). Red lines represent the best fits of the experimental data to the theoretical model with a 1:1 stoichiometry (eq. S1); SF = saturation factor.

6. DNA melting-temperature analysis

Sample preparation and measurement

For the denaturation experiments, stock solutions of the ligand **3** (20 µM) were prepared in Na-cacodylate buffer and the DNA-forms **F21T**, **FmycT**, **FkrasT**, **FkitT** (50 µM) was added. Four samples with increasing ligand concentration (0.00, 1.00, 5.00, 10.0) according to Table S1 were prepared.

Table S1. Composition of the analyte solution for the DNA denaturation experiments.

Sample[a]	c_L / µM	V_L / µL[b]	*LDR*	V_{Puffer} / µL
1	0.00	0	0.00	960
2	2.00	20	1.00	940
3	10.0	100	5.00	860
4	20.0	200	10.0	760

[a] c_{DNA} = 2 µM; V_{DNA} = 40.0 µL; [b] c_L = 100 µM.

Parameters of the DNA melting temperature measurements

For the fluorimetric detection excitation was performed at λ_{ex} = 470 nm and the change of the emission was recorded at λ_{em} = 515 nm as a function of temperature. The slit width was 5 nm for emission and excitation, the detector voltage was 600 V. The implemented temperature program included the following steps:

1: Heating from 20 °C auf 90 °C with 2.5 °C min^{-1}

2: Maintaining the temperature at 90 °C for 5 min

3: Cooling to 10 °C with 1.0 °C min^{-1}

4: Maintaining the temperature at 10 °C for 5 min

5: Heating from 10 °C to 90 °C with 0.2 °C min^{-1} and measuring of the emission

During the complete measurement, the cuvette chamber was flushed with a N_2 gas stream to avoid condensation of water on the cuvette at temperatures below 20 °C.

Determination of the melting temperatures

To determine the melting temperature, the normalised fluorescence intensity I_{norm} was plotted versus temperature. According to equation S2, I_{norm} was calculated from the measured fluorescence intensity at the respective temperature T and the fluorescence intensities I_{min} and I_{max}. A temperature range of 20 °C to 90 °C was used, with I_{min} representing the fluorescence intensity at 20 °C and I_{max} the fluorescence intensity at 90 °C.

$$I_{norm} = \frac{I_T - I_{min}}{I_{max}}$$ eq. S2

The numerical 1st derivatives of the melting curves were calculated, and the maxima for determining T_m were obtained with the Gauss function as implemented in the Origin software. The change of the melting temperature ΔT_m was calculated according to equation S3 and plotted as a function of the ligand-DNA ratio (LDR = c_{Ligand} = c_{DNA}).

$$\Delta T_m = T_m(DNA - Ligand) - T_m(DNA)$$ eq. S3

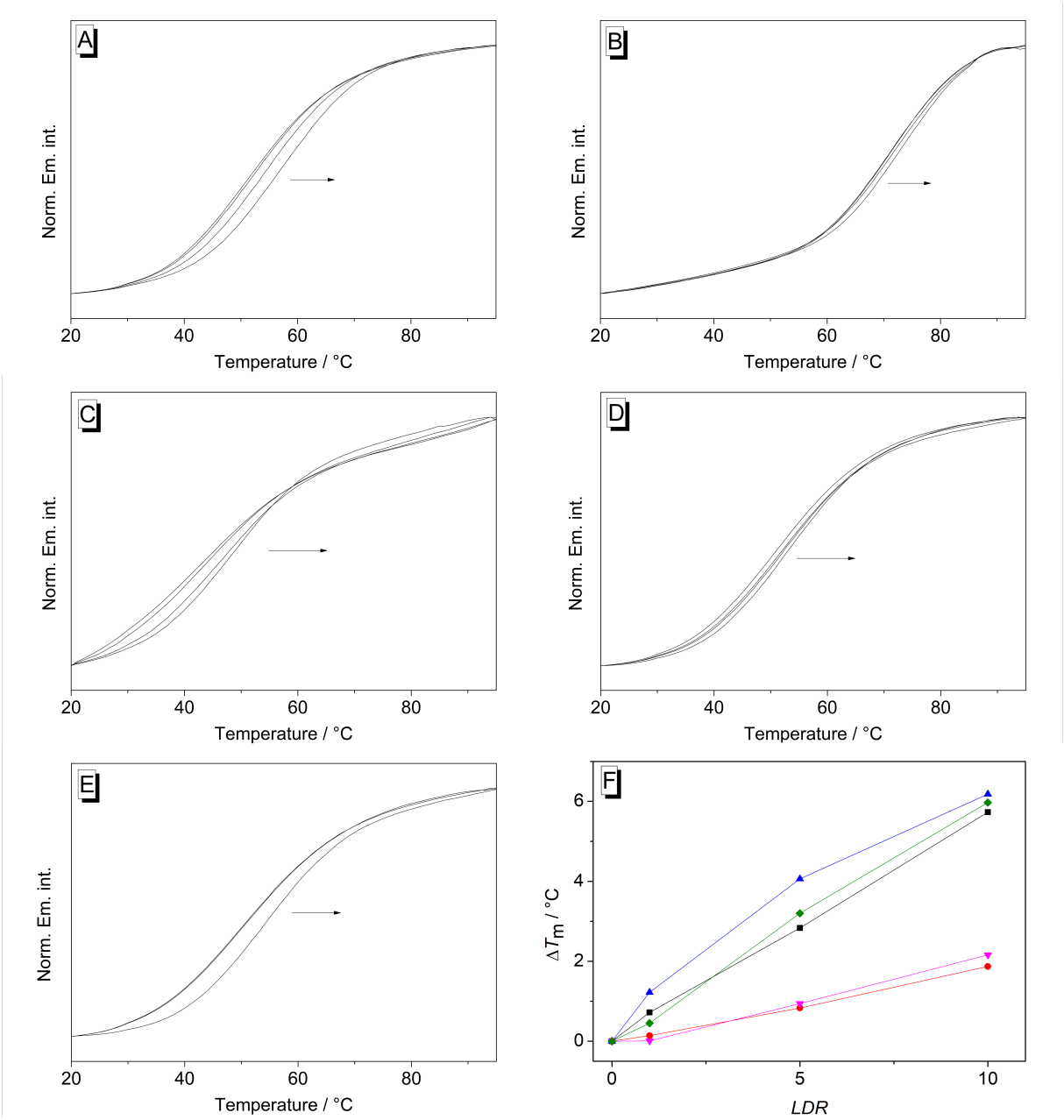


Fig. S3. Normalised DNA melting curves of **F21T** (A), **FmycT** (B), **FkrasT** (C), **FkitT** (D) **FkitT** + **ds 26** (E) (c = 2 μM) with **3** at *LDR* 0.00, 1.00, 5.00 and 10.0; λ_{ex} 515 nm; and plot of ΔT_m of G4-DNA **F21T** (black), **FmycT** (magenta), **FkrasT** (blue), **FkitT** (red), and **FkitT** + **ds26** (green) in the presence of **3** versus different *LDR* (F) in Na-cacodylate buffer (c_{Na+} = 10 mM, pH 7.2). The arrows indicate the shift of the melting curves with increasing *LDR*.

7. LD-spectroscopic analysis

For LD-spectroscopic analysis, a stock solution of DNA in K-phosphate buffer with a concentration of 200 µM was prepared. The ligand solution was taken directly from the stock solution (c = 1.00 mM) in MeOH and the solvent was removed with a vigorous N_2 gas stream. For the LD measurement five samples with constant DNA concentration (20 µM) and increasing ligand concentrations (*LDV*: 0.20, 0.40, 0.60, 0.80, 1.00, 1.60, 2.00) were prepared in K-phosphate buffer (Table S2). The LD spectra were recorded from 200 to 500 nm with a measuring speed of 1.0 nm/s at 20 °C in a cuvette with a rotating cuvette (LD) with 1 mm path length. The shear gradient was 1200 s^{-1}. The spectra were processed with the software Origin with the implemented smoothing function "moving average" with a factor of 5.[2]

Table S2. Composition of the solutions for LD-spectroscopic investigations of ligand-DNA interactions with ligand **3**.

Sample[a]	c_{Ligand} /µM	V_{Ligand} / µL[b]	*LDR*[c]
1	0	0	0
2	4	8	0.20
3	10	20	0.50
4	20	40	1.00
5	30	60	1.50

[a]c_{DNA} = 20 µM, V_{DNA} = 200 µL, V_{buffer} = 1.800 mL; [b]c_0 (ligand) = 1.00 mM in K-phosphate buffer; [c]*LDR* = ligand DNA ratio.

8. Molecular docking

The structure parameters of the G4-DNA **c-kit** were taken from the Protein Data Bank (PDB, https://www.rcsb.org/; PDB ID: 2O3M, sequence: 5′-AGGGAGGGCGCTGGGAGGAGGG-3′). The geometry optimizations of the ligand was made with Avogadro.[6] The binding interactions between the ligand and **c-kit** were analyzed with Autodock Vina.[7] Polar hydrogen were added and nonpolar hydrogens were merged. The biaryl axes and aliphatic bonds were defined as rotatable. The active site box was 30 Å × 30 Å × 30 Å.

9. Investigation of the absorption and emission properties of 3

For the investigation of the absorption properties solutions of the compound **3** were prepared from a stock solution (*c* = 1 mM) in different solvents. To prepare the samples *c* = 10 µL of the solvent of the stock solution was removed by evaporation with a stream of N_2 gas. The residue was dissolved in the respective solvent (1 ml) and homogenized with a reaction vessel shaker Top-Mix 11118 (Fisher Bioblock Scientific). The absorption spectra were measured in a range of 200–500 nm at a rate of 120 nm / min at 20 °C. To determine the fluorescence quantum yields, solutions of **3** were prepared from the stock solution by dilution. Their concentration was

adjusted, such that an absorption of 0.1 at the excitation wavelength (λ_{ex} = 286 nm) was obtained. Naphthalene was used as standard (Φ_{fl} = 0.23 in cyclohexane).[120] Aerobic oxygen was removed from the samples by carefully bubbling N_2 gas through the solution for 3 min before each measurement. The fluorescence spectra were measured at a detector voltage of 700 V at a speed of 120 nm min^{-1} at 20 °C. The slit widths for emission and excitation wavelength were 5 nm in all cases. After integration of the fluorescence bands, the relative quantum yield Φ_{fl} was calculated with equation 1.

$$\Phi_{fl} = \frac{J_X A_X}{J_S A_S} \frac{n_X^2}{n_S^2} \Phi_{fl,\,S} \qquad \text{eq. S4}$$

Here, the indices x and S refer to the sample under investigation and the standard, respectively. Φ_{fl} denotes the quantum yield, *J* the integrals of the fluorescence bands, *A* the absorption at the excitation wavelength and the parameter *n* the refractive index of the corresponding solution. The spectra were processed with the software Origin.[4]

10. Viscosity dependence of fluorescence

The viscosity of the medium was systematically varied by investigating the solvent with different viscosity (glycerol/water mixtures: 0%, 40%, 70%, 80%, 90% and 100%) at constant temperature (20 °C) with glycerol solution [$\eta_{20°C}$(glycerol) = 1499 cP, $\eta_{20°C}$(water) = 1.005 cP] as solvent (Figure S4A). Additionally, the sample with a glycerol content of 100% was heated from 20–90 °C [$\eta_{90°C}$(glycerol) = 21.3 cP] (Figure S4B). For the fluorimetric detection excitation was performed at λ_{ex} = 350 nm and the change of the emission was recorded at λ_{em} = 524 nm as a function of temperature. The slit width was 5 nm for emission and excitation, the detector voltage was 600 V. The implemented temperature program included the following steps: Heating from 20 °C to 90 °C with 0.3 °C min^{-1} and measuring of the emission. The spectra were processed with the software Origin.[4]

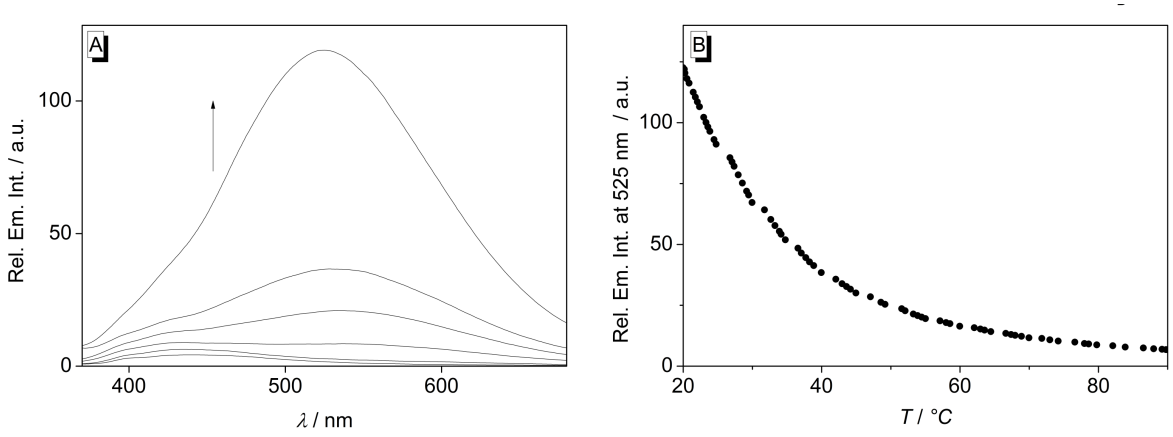


Fig. S4. Fluorescence spectra of derivative **3** (*c* = 10 µM, λ_{ex} = 350 nm) in glycerol/water mixtures with increasing glycerol content from 0% to 100% (A) . Arrows indicate the change in emission intensity with increasing glycerol content. Temperature-dependent fluorescence intensity of **3** (*c* = 10 µM, λ_{ex}= 350 nm) in glycerol (B).

11. NMR spectra

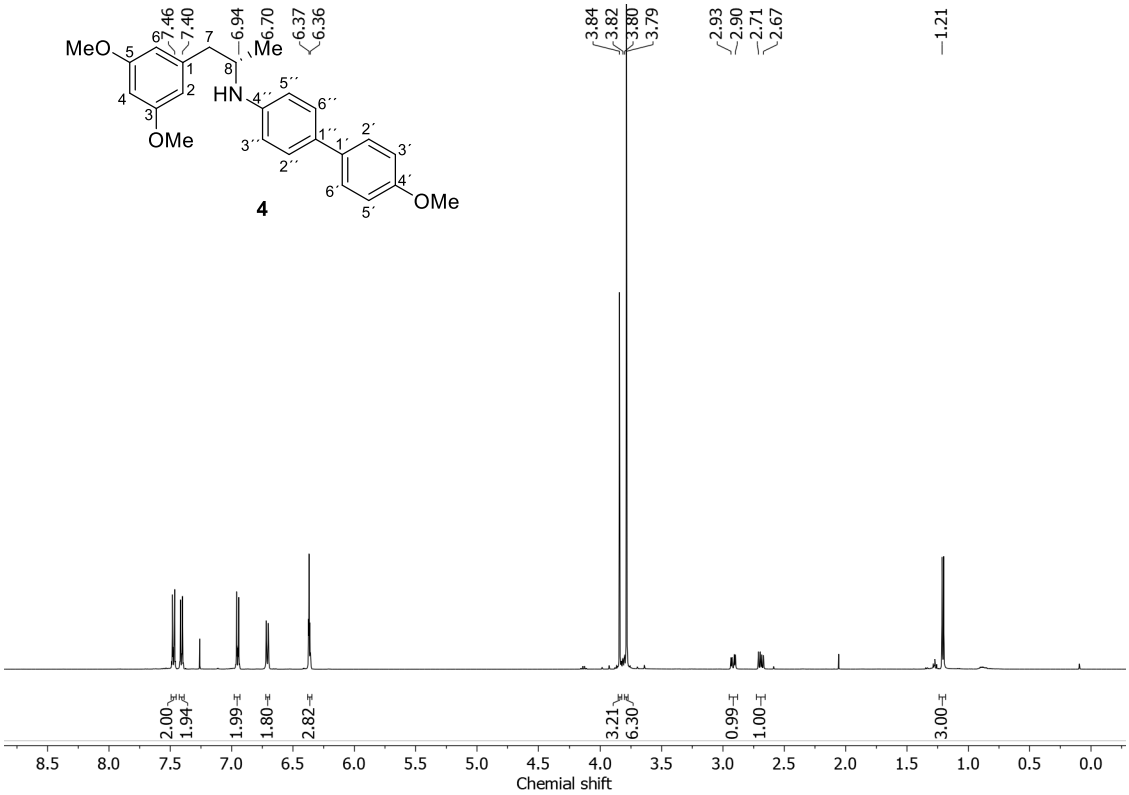


Fig. S5. ^{1}H NMR spectrum (500 MHz) of **4** in $CDCl_3$.

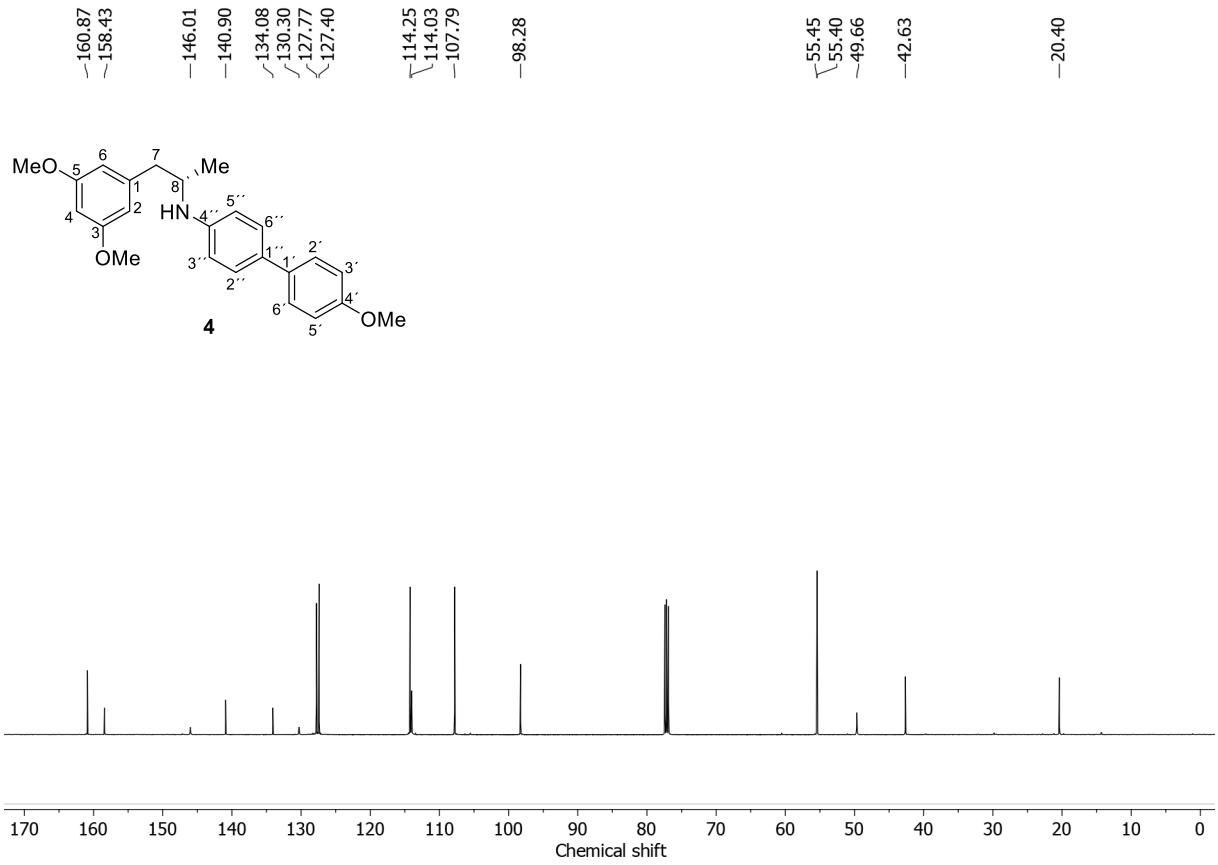


Fig. S6. ^{13}C NMR spectrum (500 MHz) of **4** in $CDCl_3$.

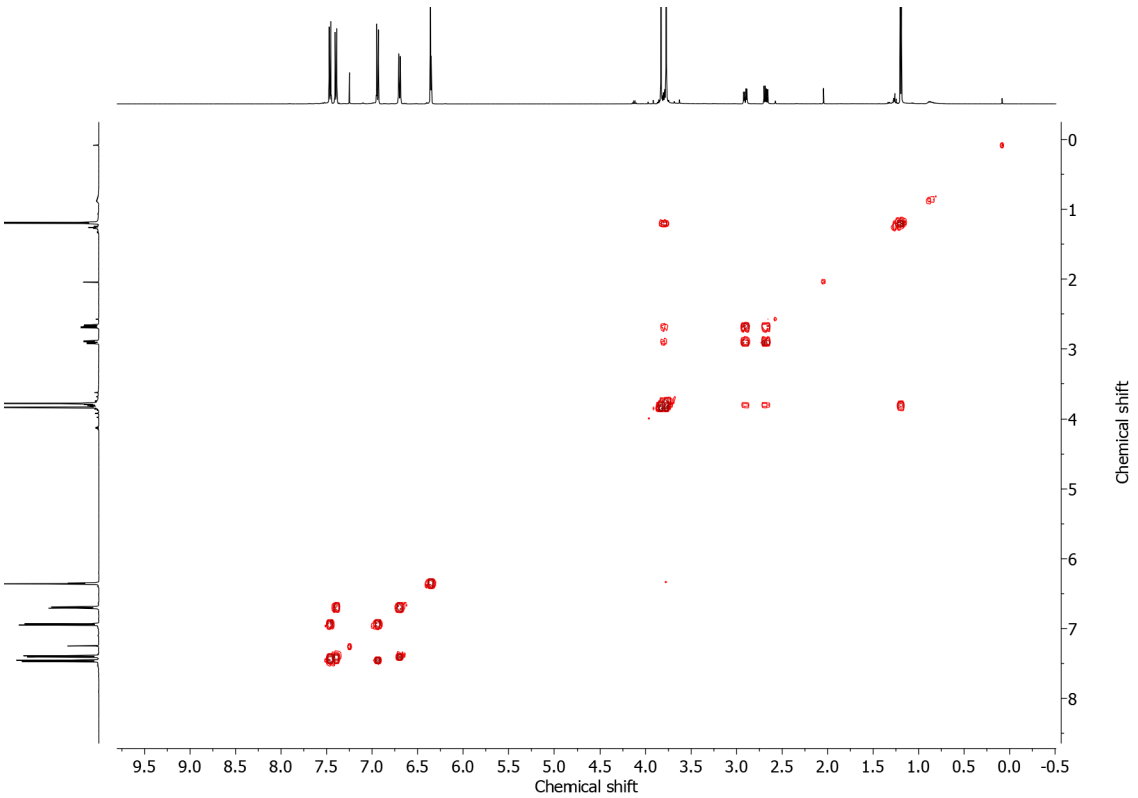


Figure S7. COSY NMR spectrum (500 MHz) of **4** in $CDCl_3$.

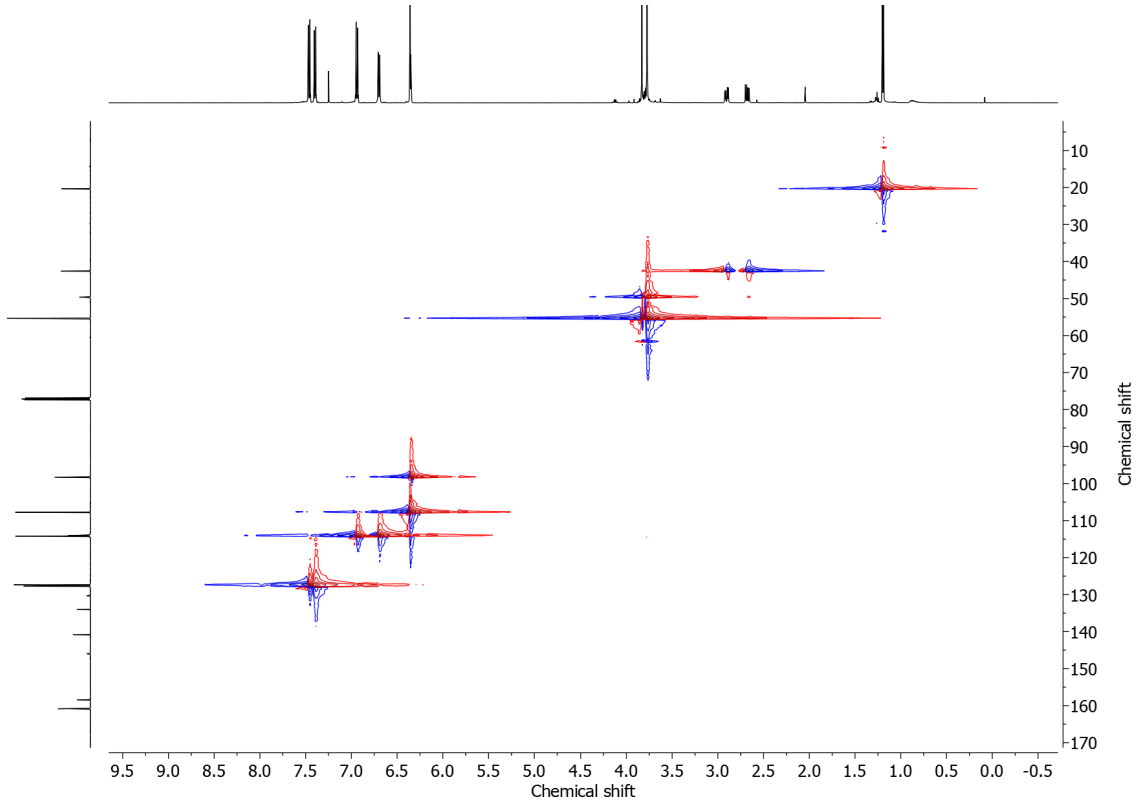


Fig. S8. HSQC NMR spectrum (500 MHz) of **4** in $CDCl_3$.

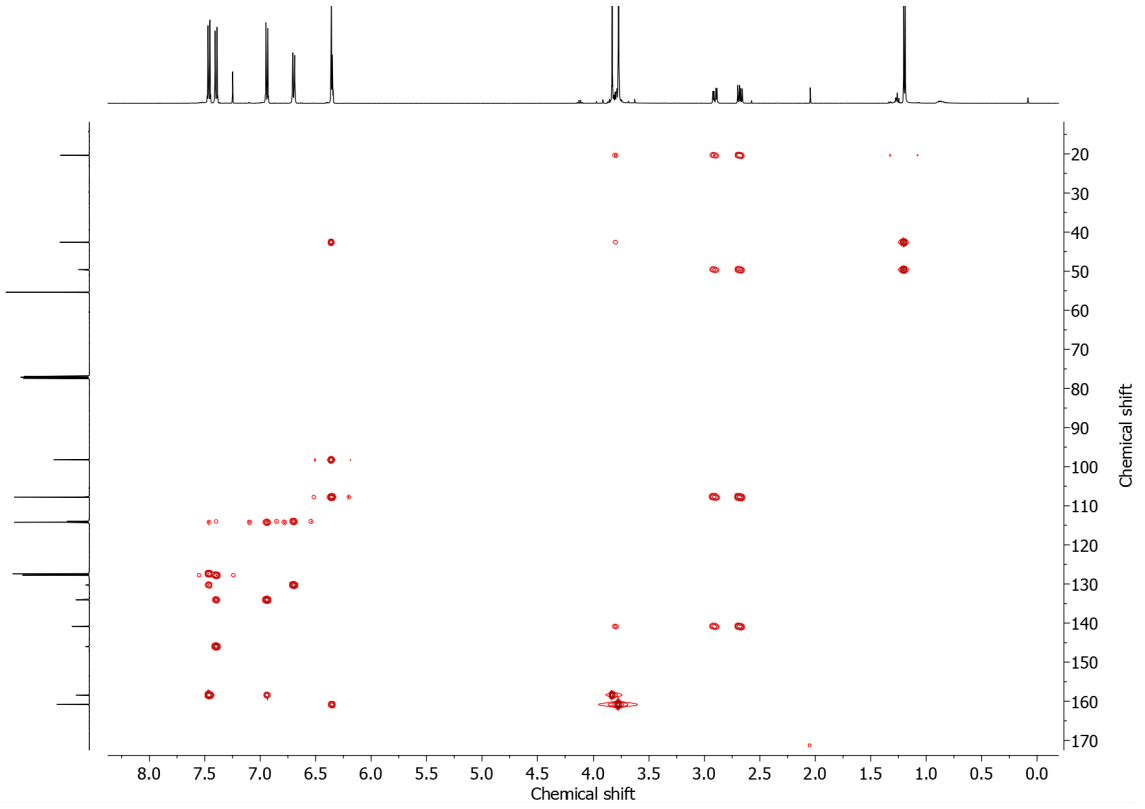


Fig. S9. HMBC NMR spectrum (500 MHz) of **4** in $CDCl_3$.

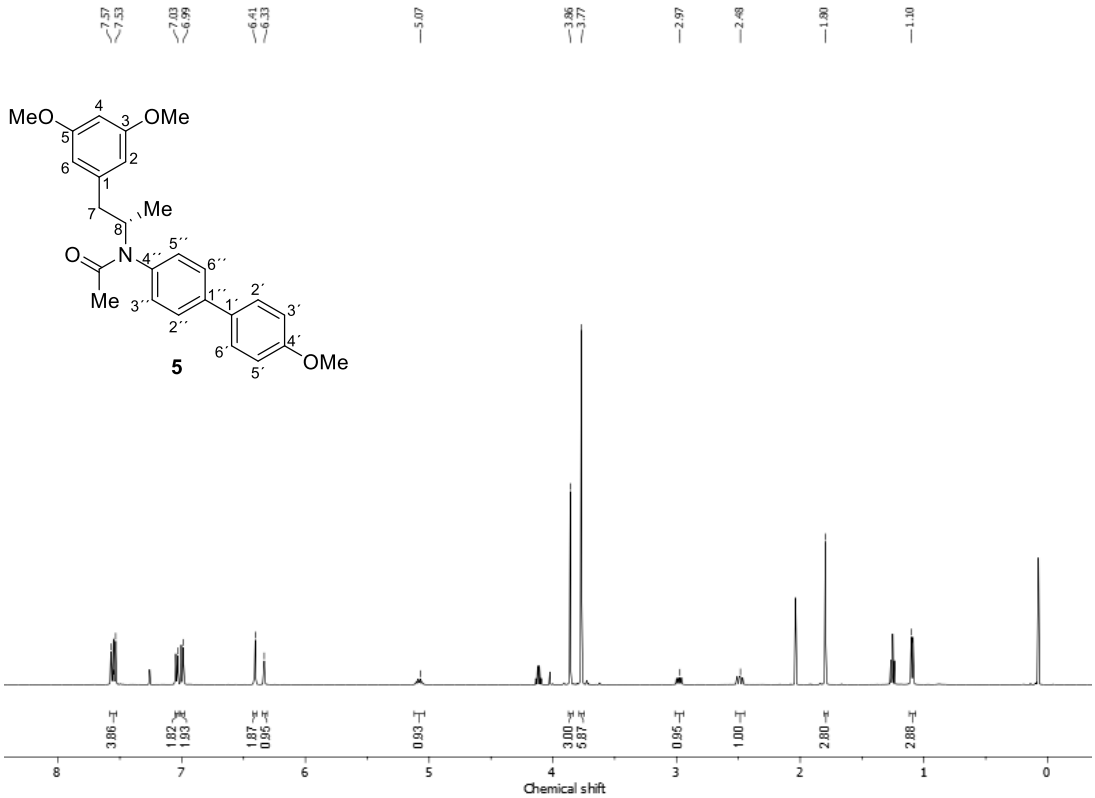


Fig. S10. ^{1}H NMR spectrum (500 MHz) of **5** in $CDCl_3$.

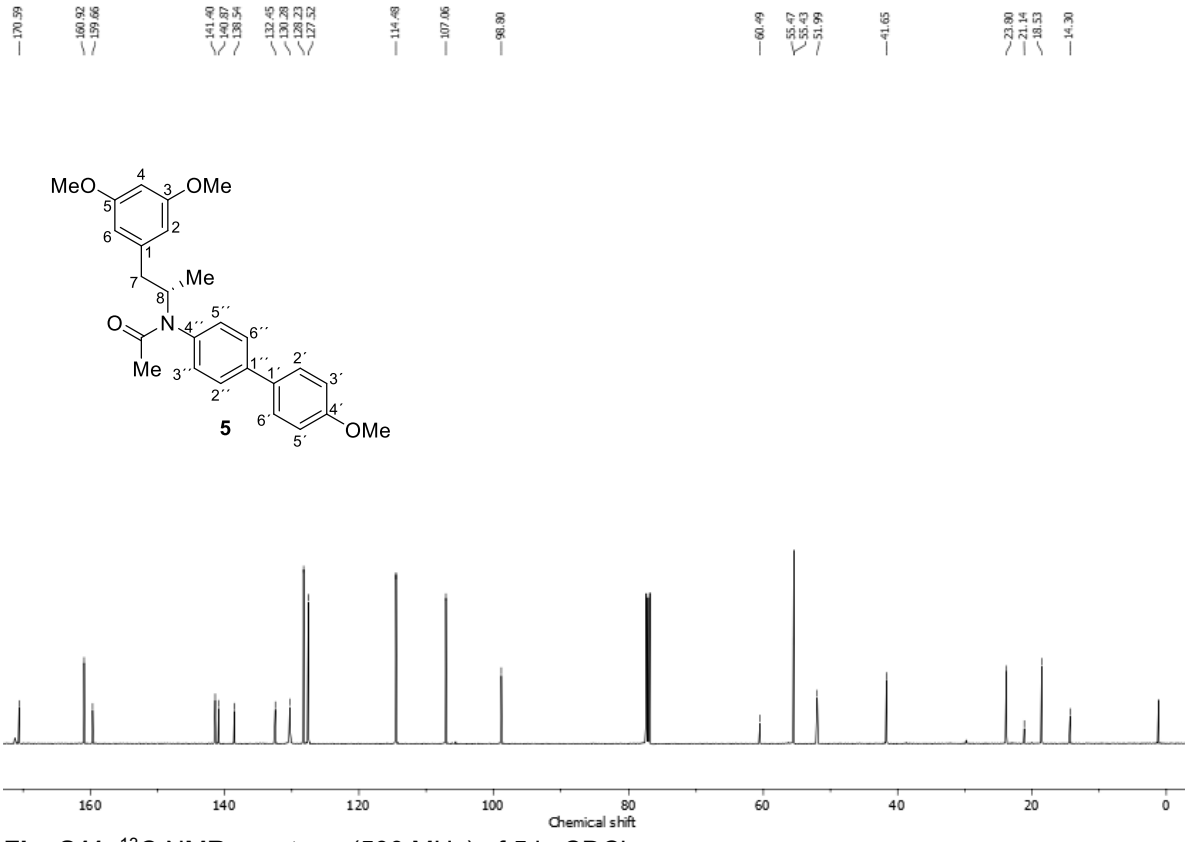


Fig. S11. ^{13}C NMR spectrum (500 MHz) of **5** in $CDCl_3$.

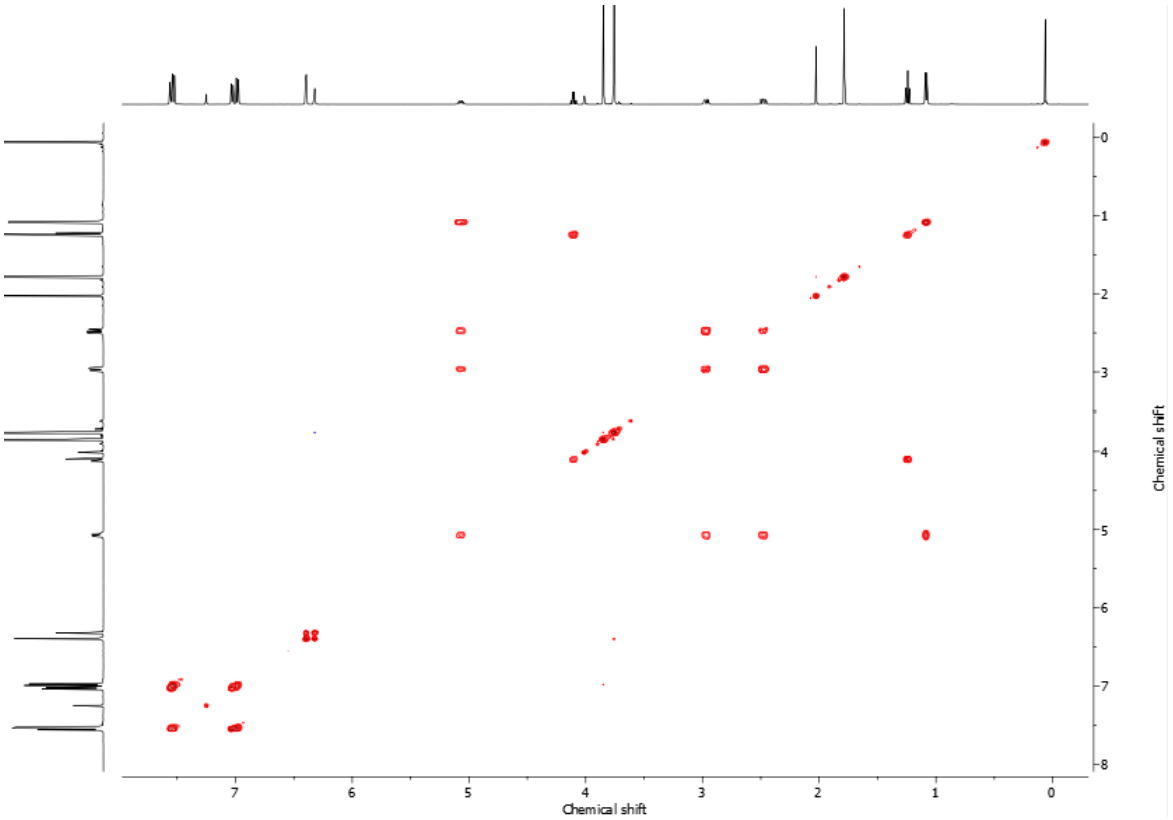


Fig. S12. COSY NMR spectrum (500 MHz) of **5** in $CDCl_3$.

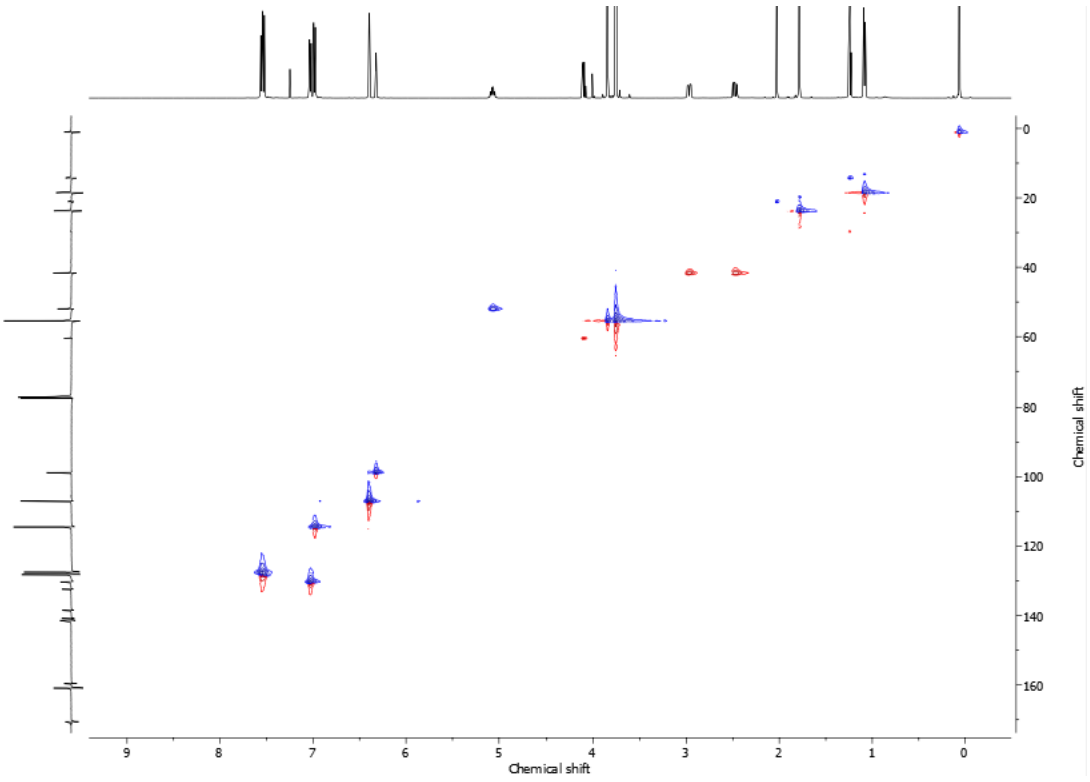


Fig. S13. HSQC NMR spectrum (500 MHz) of **5** in $CDCl_3$.

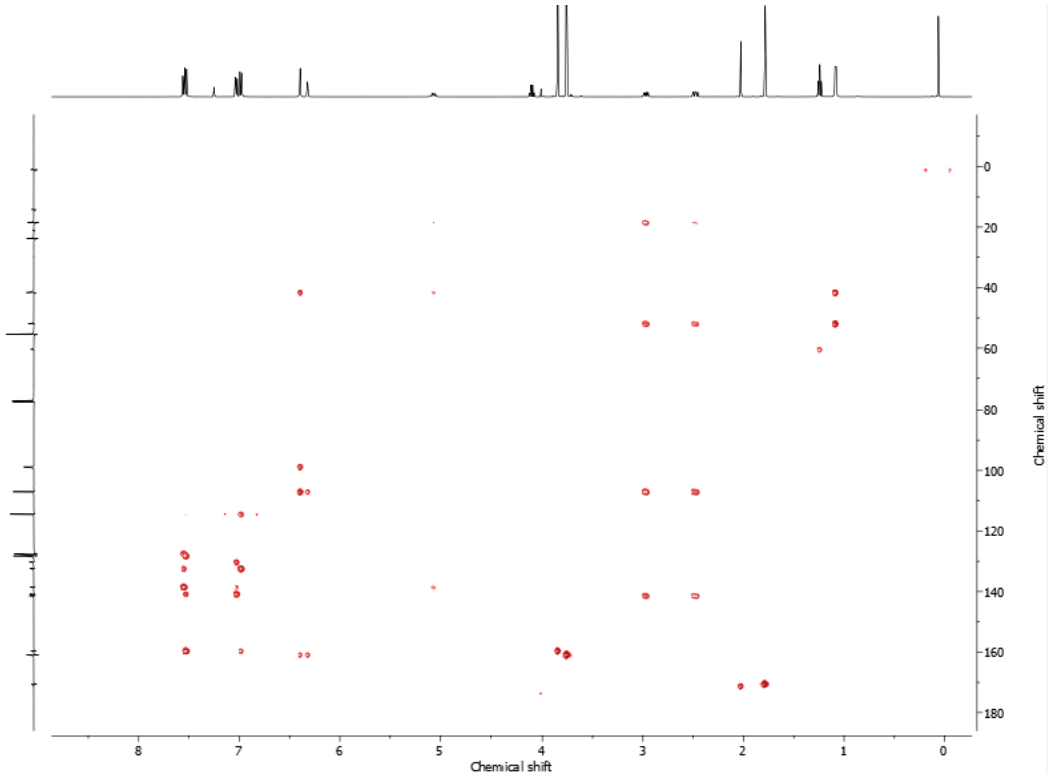


Fig. S14. HMBC NMR spectrum (500 MHz) of **5** in $CDCl_3$.

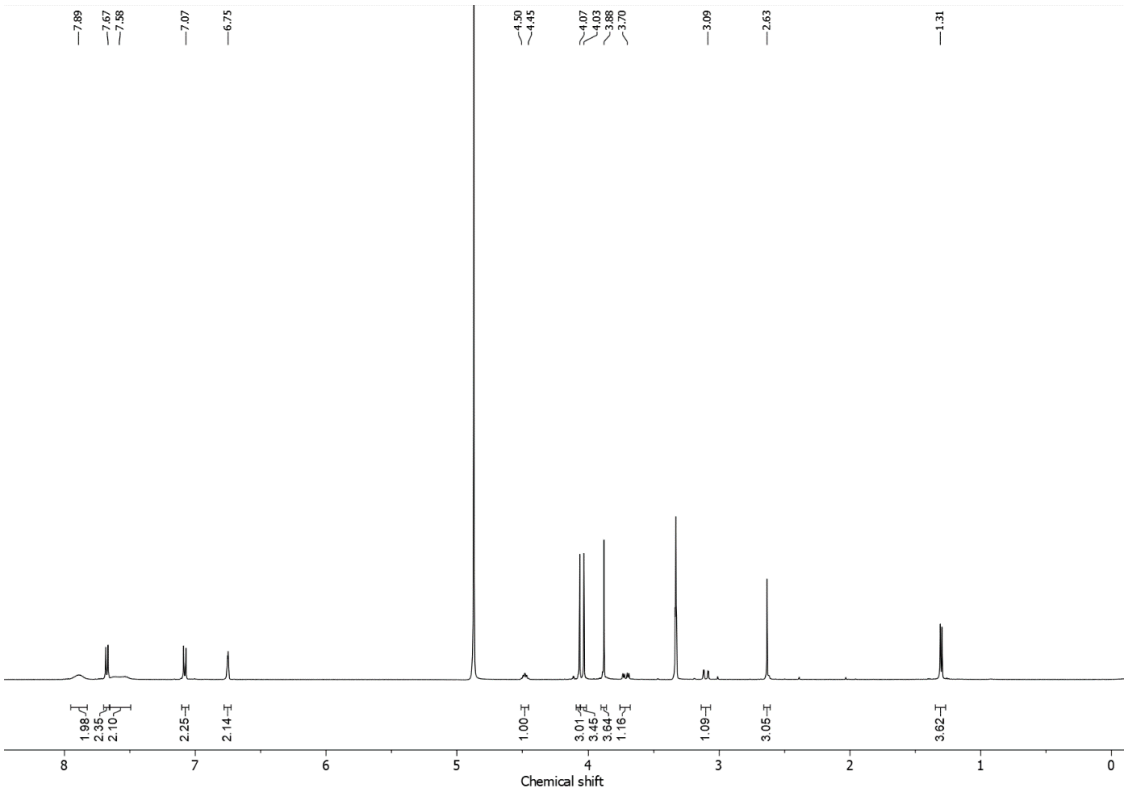


Fig. S15. ^1H NMR spectrum (500 MHz) of **3** in CD_3OD.

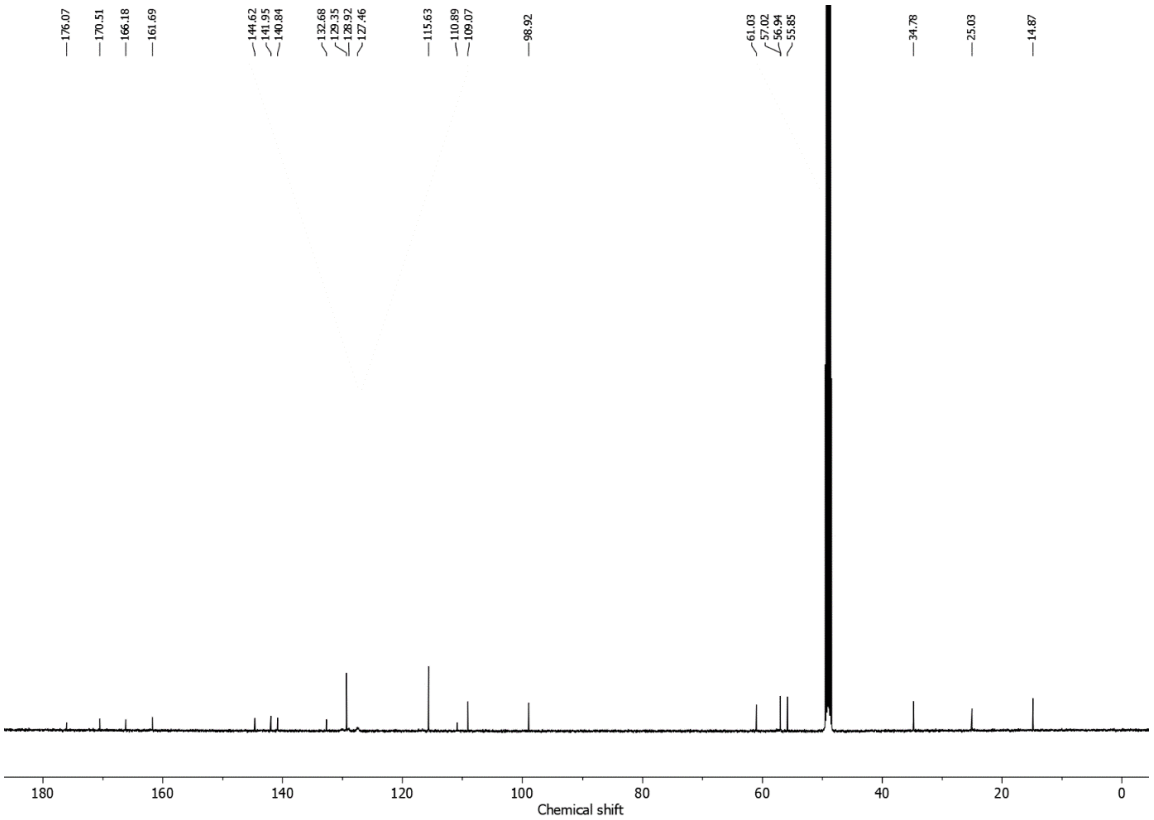


Fig. S16. ^{13}C NMR spectrum (500 MHz) of **3** in CD_3OD.

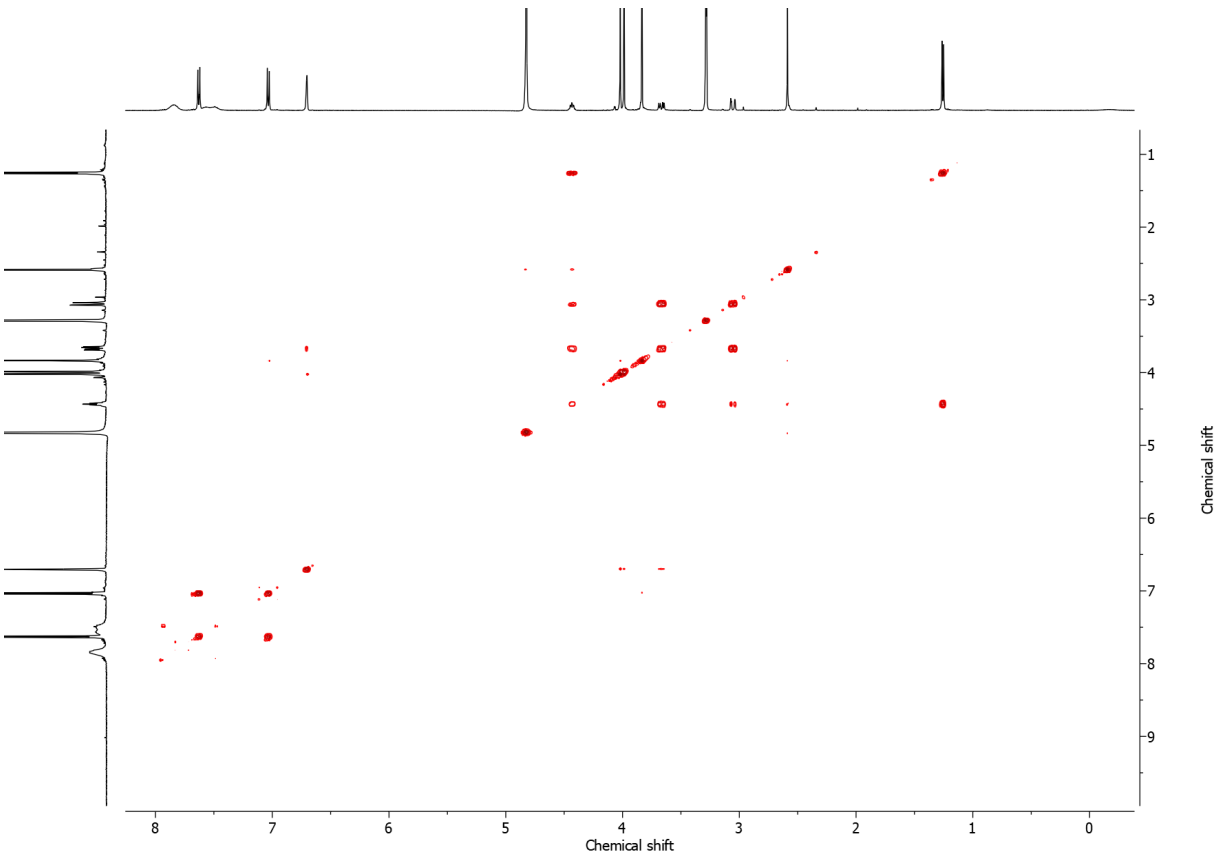


Fig. S17. COSY spectrum (500 MHz) of **3** in CD_3OD.

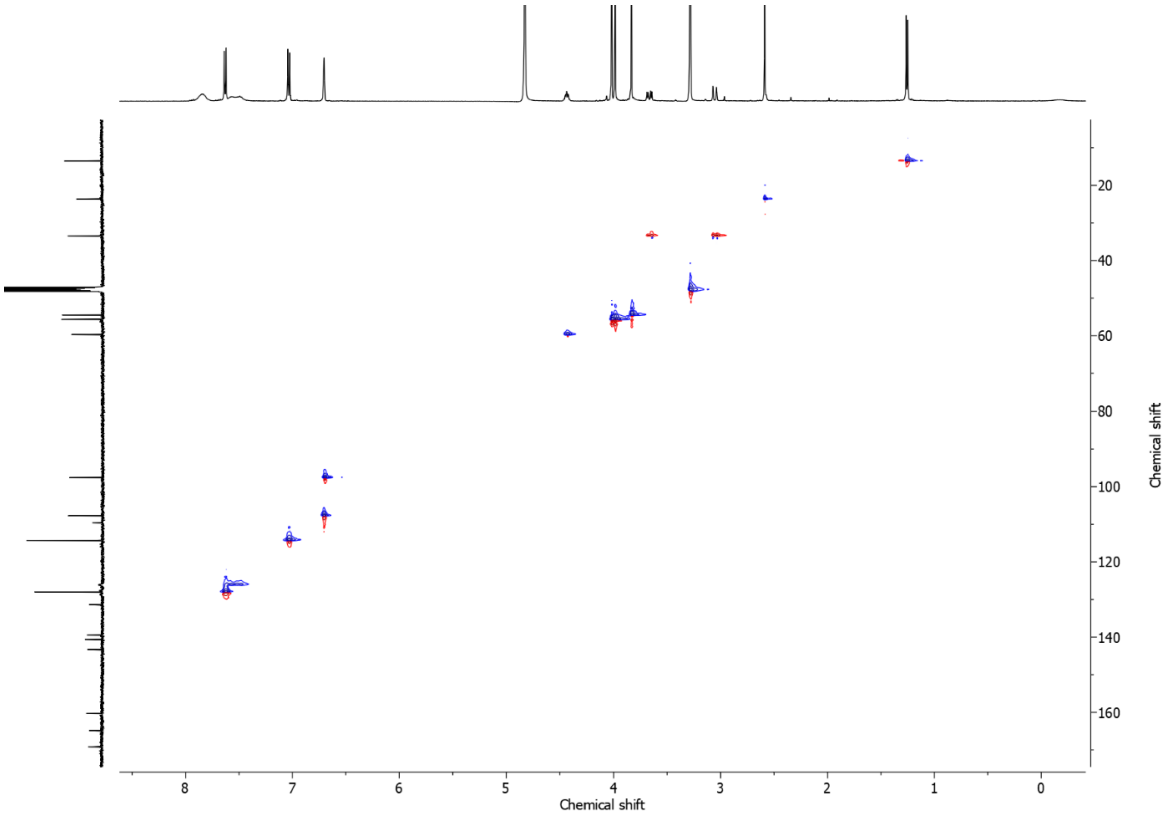


Fig. S18. HSQC spectrum (500 MHz) of **3** in CD_3OD.

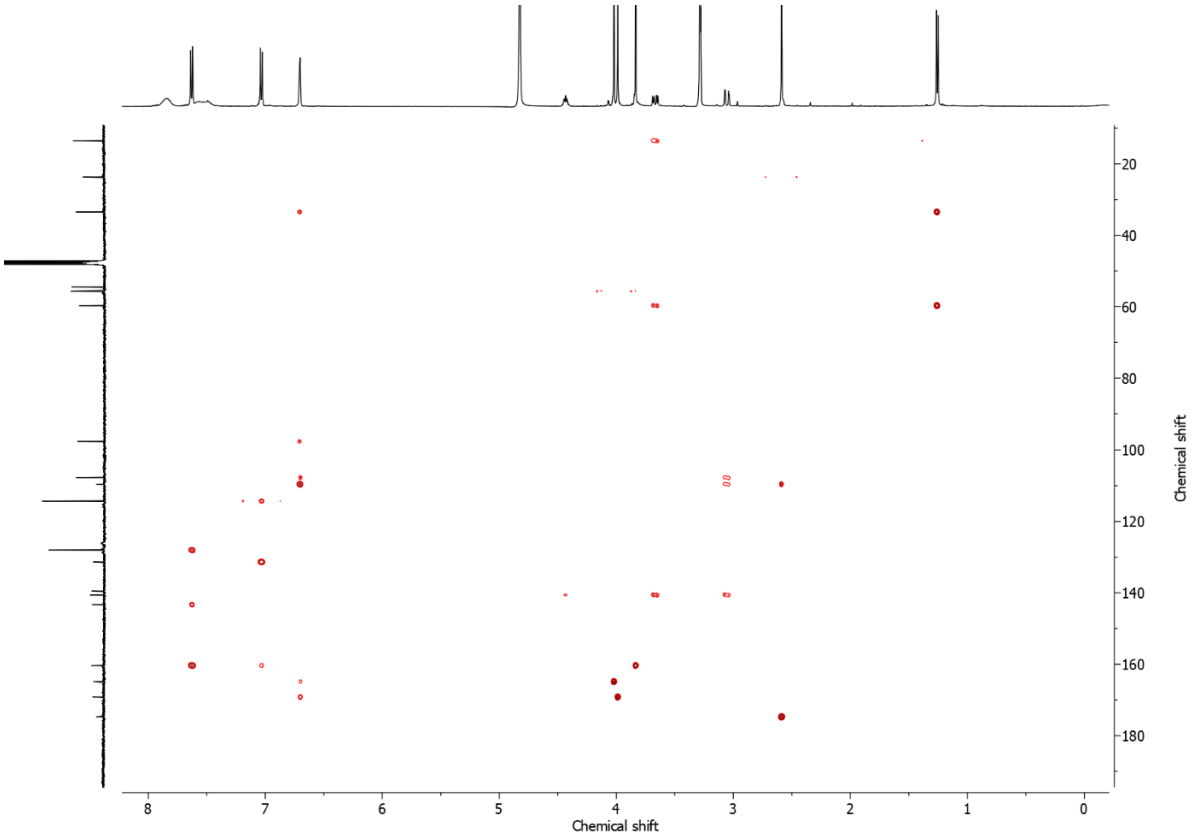


Fig. S19. HMBC spectrum (500 MHz) of **3** in CD_3OD.

12. References

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