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Supporting Information

Development of Fluorescent Probes Specific for Parallel-Stranded G-Quadruplexesby Library Approach

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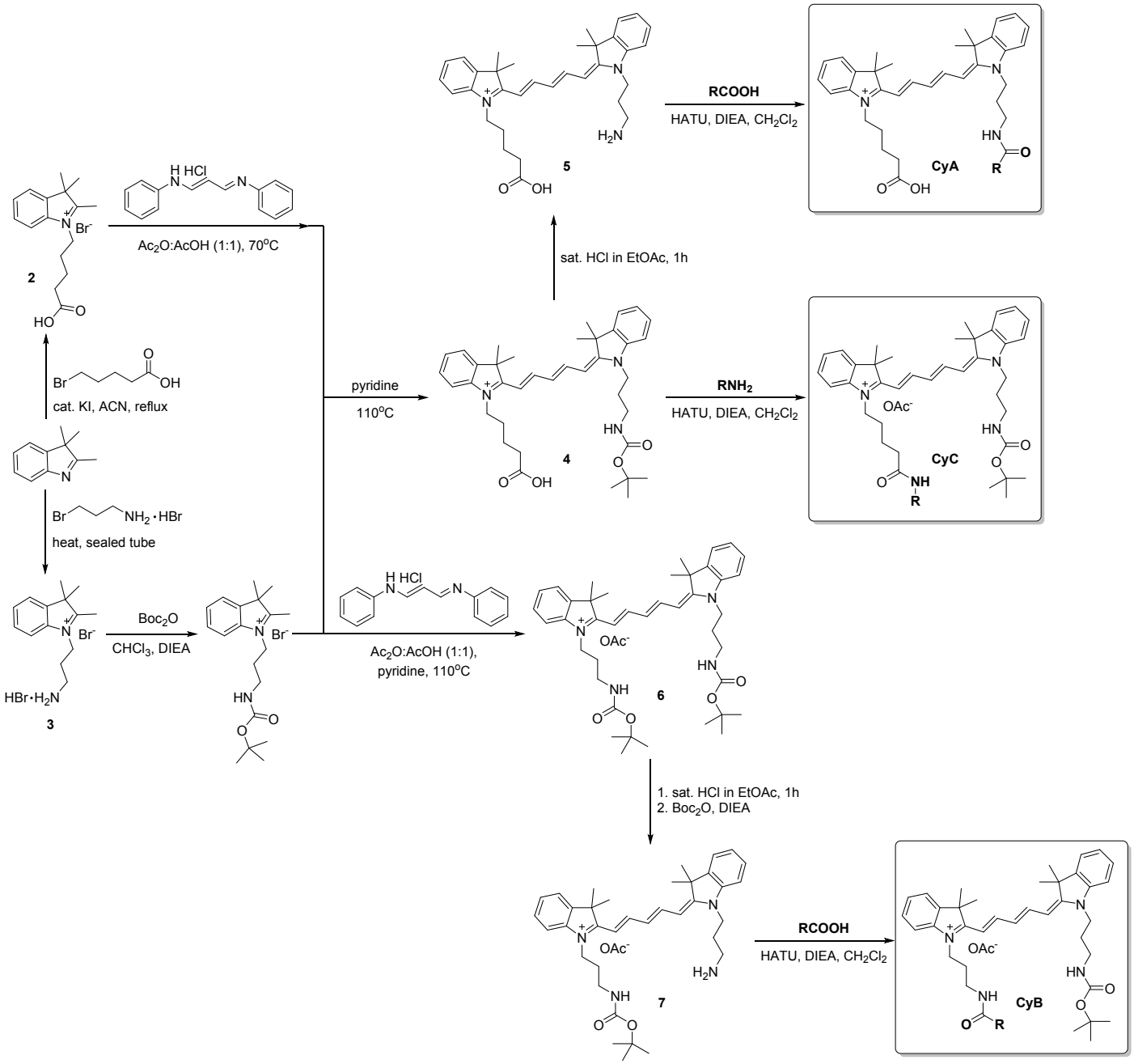
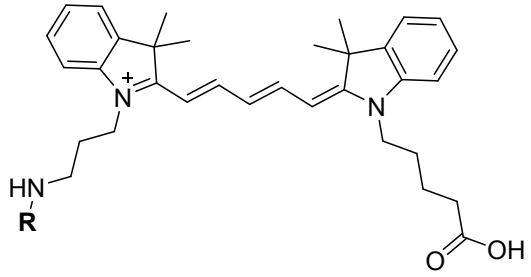
EXPERIMENTAL PROCEDURES

Materials

All commercially available reagents and oligonucleotides (dsDNA, ssDNA and RNA) were purchased from Sigma-Aldrich, Alfa Aesar or TCI, and used as received unless otherwise stated. Anhydrous solvents were purchased from Alfa Aesar and used without further purification.

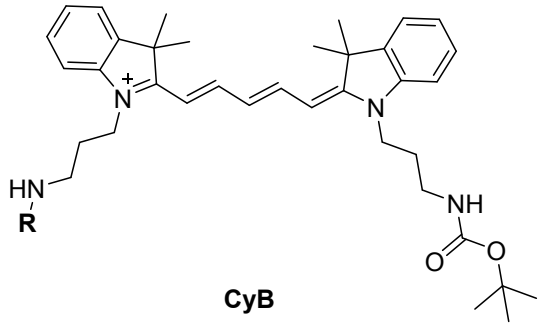
Synthesis


Ac2O:AcOH (1:1), 70°C
cat. KI, ACN, reflux
heat, sealed tube
Boc2O
CHCl3, DIEA
pyridine
110°C
sat. HCl in EtOAc, 1h
RCOOH
HATU, DIEA, CH2Cl2
RNH2
HATU, DIEA, CH2Cl2
Ac2O:AcOH (1:1),
pyridine, 110°C
1. sat. HCl in EtOAc, 1h
2. Boc2O, DIEA
RCOOH
HATU, DIEA, CH2Cl2
CyA
CyB
CyC
2
3
4
5
6
7



CyA

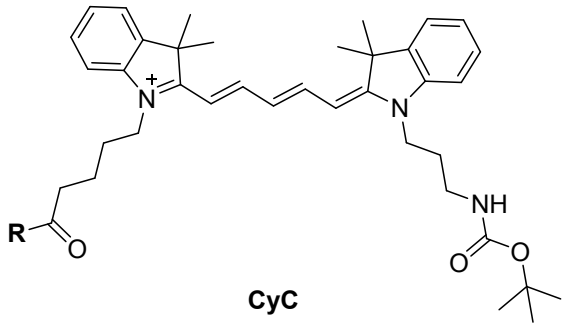
General procedure for the preparation of CyA library: A solution of the corresponding carboxylic acid building block (37 μmol, 2 eq.) in CH_2Cl_2(1 mL) was treated with DIEA (6.4 μL, 37 μmol, 2 eq.) and HATU (14 mg, 37 μmol, 2 eq.) and stirred for 5 min at rt. **5** (5.1 mg, 18 μmol, 1 eq.) was subsequently added and the reaction stirred at rt to completion. The resulting mixture were purified by reverse-phase liquid chromatography on a C-18 column (H_2O:ACN (30% to 99% ACN gradient)) or flash column chromatography on silica gel (CH_2Cl_2:MeOH (94:6)) to afford the respective **CyA** compounds as blue solids. The characterization of **CyA** compounds were performed by LC-MS (**Table S1**).



CyB

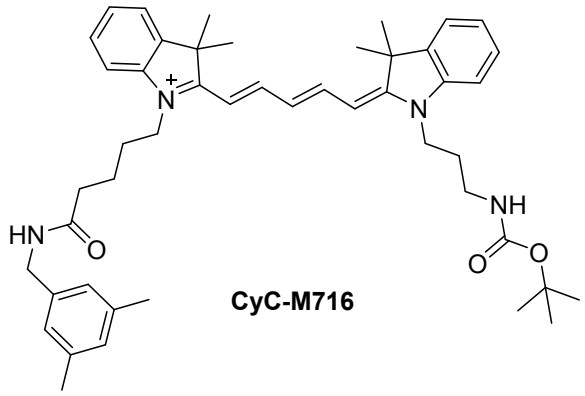
General procedure for the preparation of CyB library: A solution of the corresponding carboxylic acid or sulfonyl chloride building block (37 μmol, 2 eq.) in CH_2Cl_2(1 mL) was treated with DIEA (6.4 μL, 37 μmol, 2 eq.) and HATU (14 mg, 37 μmol, 2 eq.) and stirred for 5 min at rt. **7** (11 mg, 18 μmol, 1 eq.) was subsequently added and the reaction stirred at rt to completion. The resulting mixture were purified by reverse-phase liquid chromatography on a C-18 column (H_2O:ACN (30% to 99% ACN gradient)) or flash column chromatography on silica gel

(CH_2Cl_2:MeOH (94:6)) to afford the respective **CyB** compounds as blue solids. The characterization of **CyB** compounds were performed by LC-MS (**Table S2**).



CyC

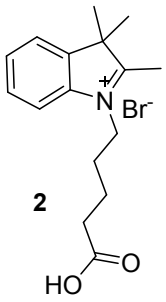
General procedure for the preparation of CyC library:A solution of **4** (11 mg, 18μmol, 1 eq.) in CH_2Cl_2(1 mL) wastreated DIEA (6.4 μL, 37 μmol, 2 eq.) and HATU (14 mg, 37 μmol, 2 eq.) and stirred at rt for 5 min. The corresponding amine building block (37 μmol, 2 eq.) was subsequently added and the reaction mixture was stirred at rtto completion.The resulting mixture were purified by reverse-phase liquid chromatography on a C-18 column (H_2O:ACN (30% to 99% ACN gradient)) or flash column chromatography on silica gel (CH_2Cl_2:MeOH (94:6)) to afford the respective **CyC** compounds as blue solids. The characterization of **CyC** compounds were performed by LC-MS (**Table S3**).



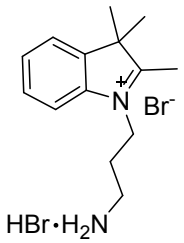
CyC-M716

2-((1*E*,3*E*)-5-((*E*)-1-(3-((*tert*-butoxycarbonyl)amino)propyl)-3,3-dimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-1-(5-((3,5-dimethylbenzyl)amino)-5-oxopentyl)-3,3-dimethyl-3*H*-indol-1-ium acetate (CyC-M716):^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (t, *J* = 13.0 Hz, 1H),

7.74 (t, *J* = 12.8 Hz, 1H), 7.39 – 7.30 (m, 5H), 7.21 (td, *J* = 7.4, 2.3 Hz, 2H), 7.09 (d, *J* = 7.8 Hz, 3H), 7.00 (d, *J* = 12.6 Hz, 1H), 6.96 (s, 2H), 6.80 (s, 1H), 6.41 (d, *J* = 13.4 Hz, 1H), 6.28 (d, *J* = 13.8 Hz, 1H), 4.38 (d, *J* = 5.6 Hz, 2H), 4.04 (t, *J* = 7.9 Hz, 2H), 3.97 (t, *J* = 7.5 Hz, 2H), 3.33 (s, 2H), 2.50 (t, *J* = 6.9 Hz, 2H), 2.22 (s, 6H), 2.07 – 1.99 (m, 2H), 1.91 – 1.85 (m, 2H), 1.85 – 1.79 (m, 2H), 1.67 (s, 6H), 1.67 (s, 6H), 1.43 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.0, 172.3, 156.6, 152.7, 142.2, 142.1, 141.0, 138.9, 138.0, 129.0, 128.9, 128.8, 128.0, 127.8, 126.0, 125.3, 125.2, 123.1, 122.7, 122.3, 122.2, 110.8, 110.7, 108.6, 104.5, 79.3, 49.3, 49.2, 49.1, 44.1, 43.6, 42.4, 35.4, 28.6, 28.2, 28.2, 27.6, 26.9, 22.7, 21.4; HRMS ($C_{47}H_{61}N_4O_3$): Calc. $[M]^+$: 729.4738, Found $[M]^+$: 729.4768.

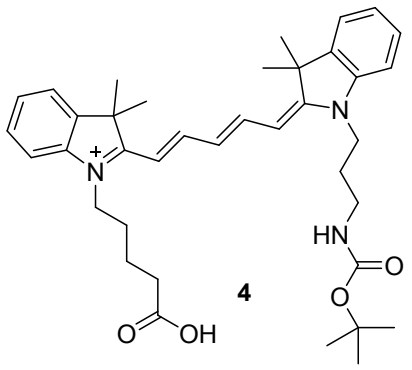


1-(4-carboxybutyl)-2,3,3-trimethyl-3*H*-indol-1-ium bromide (2):To a solution of 2,3,3-trimethyl-3*H*-indole (2.0 mL, 12.5 mmol, 1 eq.) in ACN(120 mL),5-bromovaleric acid (2.5 g, 13.8 mmol, 1.1 eq.) was added and refluxed with continuous stirring for 20 h in the presence of KI (200mg, 0.1 eq.). The resulting mixture was dried *in vacuo*and washed sequentially washed with Et_2O, acetone and chloroform. The crude solid was recrystallized in acetone and MeOH to obtain **2**as a white solid (3.8 g, 11.1mmol, 90% yield).^{1}H NMR (500 MHz, MeOD) δ7.94 – 7.89 (m, 1H), 7.81 – 7.76 (m, 1H), 7.68 – 7.63 (m, 2H), 4.57 (t, *J* = 7.7 Hz, 2H), 2.43 (t, *J* = 7.1 Hz, 2H), 2.11 – 1.95 (m, 2H), 1.78 (dt, *J* = 7.1, 4.9 Hz, 2H), 1.63 (s, 6H); ^{13}C NMR (126 MHz, MeOD) δ 198.0, 176.6, 143.4, 142.6, 131.2, 130.5, 124.7, 116.6, 56.0, 49.1, 33.9, 28.2, 22.9, 22.8; HRMS ($C_{16}H_{22}NO_2$): Calc. $[M]^+$: 260.1645, Found $[M]^+$: 260.1651.



3

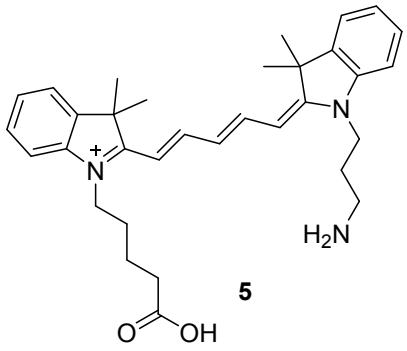
1-(3-aminopropyl)-2,3,3-trimethyl-3*H*-indol-1-ium bromide hydrobromide (3):3-bromopropylamine hydrobromide (2.7 g, 12.5 mmol, 1 eq.) was added in a sealed tube containing 2,3,3-trimethyl-3*H*-indole (2.0 mL, 12.5 mmol, 1 eq.) under N_2 atmosphere and gently heated to 120°C in an oil bath. The mixture was stirred at 120°C for 10 h. Upon completion, the mixture was cooled rt. to form a solid cake that was sequentially washed with Et_2O, Et_2O:$CHCl_3$ (1:1) andacetoneto obtain **3** as a pale pink solid. (4.0 g, 10.5 mmol, yield 85%).^{1}H NMR (500 MHz, DMSO-d_6) δ 8.06 (d, J = 6.5 Hz, 1H), 7.98 (s, 3H), 7.86 (d, J = 7.1 Hz, 1H), 7.68 – 7.64 (m, 1H), 7.64 – 7.60 (m, 1H), 4.59 (t, J = 7.4 Hz, 2H), 3.06 (tt, J = 7.4, 7.2, Hz, 2H), 2.87 (s, 3H), 2.18 (t, J = 7.2 Hz, 2H), 1.56 (s, 6H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 197.4, 141.9, 141.1, 129.4, 128.9, 123.6, 115.4, 54.3, 45.2, 39.5, 36.1, 25.2, 22.1, 14.4; HRMS ($C_{14}H_{21}N_2$): Calc. $[M]^+$: 217.1699, Found $[M]^+$: 217.1706.



4

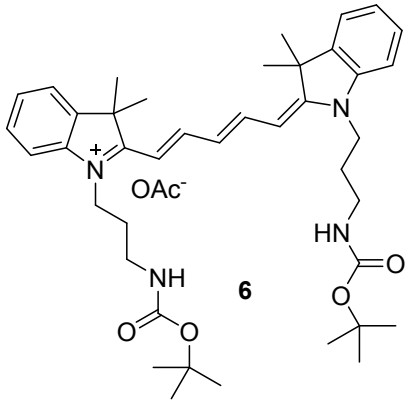
5-(2-((1*E*,3*E*)-5-((*E*)-1-(3-((*tert*-butoxycarbonyl)amino)propyl)-3,3-dimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-3,3-dimethyl-3*H*-indol-1-ium-1-yl)pentanoate(4):To a solution of **3** (2.3 g, 6mmol, 1 eq.) in dry $CHCl_3$(180 mL) was added DIEA (5.3 mL, 30mmol, 5 eq.) and

Boc anhydride (3.3 g, 15 mmol, 2.5 eq.) and stirred at rt for 2 h. The reaction mixture was diluted with CH_2Cl_2, washed with water, and concentrated *in vacuo* to afford crude 1-(3-((*tert*-butoxycarbonyl)amino)propyl)-2,3,3-trimethyl-3*H*-indol-1-ium bromide as a brownish liquidthat was used immediately without furtherpurification.A separate solution of *N*-((1*E*,3*E*)-3-(phenylimino)prop-1-en-1-yl)aniline hydrochloride (1.1 g, 5 mmol, 1 eq.) in AcOH:Ac_2O (1:1, 4 mL) was added 2 (1.7 g, 5 mmol, 1 eq.) and heated at70^oC for 70 min and then cooled to rt. The crude 1-(3-((*tert*-butoxycarbonyl)amino)propyl)-2,3,3-trimethyl-3*H*-indol-1-ium bromide (≈2.3 g) and pyridine (2 mL) were added to the reaction mixtureand further stirred at 110^oC for 1 h. Upon completion, the reaction was diluted with water, neutralized with $NaHCO_3$and extracted with CH_2Cl_2 (3 x 200 mL). The crude mixture was purified by flash column chromatography on silica gel (CH_2Cl_2:MeOH (50:2) to afford **4**as a blue solid (2.0 g, 2.9mmol, and 60% yield).^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (t, *J* = 12.9 Hz, 1H), 7.71 (t, *J* = 12.9 Hz, 1H), 7.43 – 7.30 (m, 6H), 7.23 (d, *J* = 7.4 Hz, 1H), 7.19 (t, *J* = 7.7 Hz, 1H), 7.11 (d, *J* = 8.0 Hz, 1H), 7.04 (d, *J* = 7.9 Hz, 1H), 6.99 (d, *J* = 12.7 Hz, 1H), 6.44 (d, *J* = 13.2 Hz, 1H), 4.20 (t, *J* = 7.9 Hz, 2H), 4.10 – 3.99 (m, 2H), 3.40 (t, *J* = 6.5 Hz, 2H), 2.70 – 2.66 (m, 2H), 2.14 – 2.05 (m, 2H), 1.87 (m, 4H), 1.68 (s, 6H), 1.66 (s, 6H), 1.43 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.6, 172.9, 171.5, 156.8, 153.5, 152.4, 142.3, 142.1, 141.24, 140.9, 130.0, 128.9, 128.8, 128.0, 125.3, 124.8, 122.3, 111.0, 110.3, 105.7, 104.2, 79.1, 77.2, 53.9, 49.3, 49.0, 43.9, 38.2, 34.0, 28.6, 28.3, 28.2, 27.6, 25.9, 22.8; HRMS ($C_{38}H_{49}N_3O_4$): Calc. $[M+H]^+$: 612.3796, Found $[M]^+$: 612.3809.



5

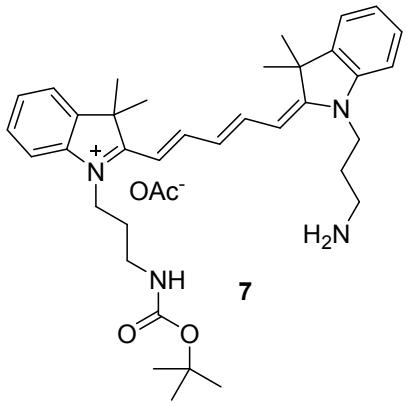
5-(2-((1*E*,3*E*)-5-((*E*)-1-(3-aminopropyl)-3,3-dimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-3,3-dimethyl-3*H*-indol-1-ium-1-yl)pentanoate (5):Compound **4** (1 g, 1.6 mmol) was dissolved in CH_2Cl_2(*ca.*5 mL) andtreated with saturated HCl in EtOAc (50 mL) for 1h at r.t to obtain **5**asa blue solid 5 (580 mg, 1.1 mmol, 68% yield). ^{1}H NMR (500 MHz, xx) δ ; ^{13}C NMR (126 MHz, $CDCl_3$) δ ; HRMS ($C_{33}H_{42}N_3O_2^+$): Calc. $[M]^+$: 512.3272, Found $[M]^+$:512.3331.



6

1-(3-((tert-butoxycarbonyl)amino)propyl)-2-((1*E*,3*E*)-5-((*E*)-1-(3-((*tert*-butoxycarbonyl)amino)propyl)-3,3-dimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-3,3-dimethyl-3*H*-indol-1-ium acetate (6):Crude 1-(3-((*tert*-butoxycarbonyl)amino)propyl)-2,3,3-trimethyl-3*H*-indol-1-ium bromide (≈3.5 g) was synthesized as a brownish liquidfollowing the procedure described for the synthesis of **4**.*N*-((1*E*,3*E*)-3-(phenylimino)prop-1-en-1-yl)aniline hydrochloride (1.1 g, 5 mmol, 1 eq.) and the crude 1-(3-((*tert*-butoxycarbonyl)amino)propyl)-2,3,3-trimethyl-3*H*-indol-1-ium bromide (3.4 g, 10 mmol, 2 eq.) were mixed in AcOH:Ac_2O

(1:1, 4 mL) in presence of pyridine (2 mL)and heated at 110^{o}C for 70 min and then cooled to rt. Upon completion, the reaction was diluted with water, neutralized with $NaHCO_3$ and extracted with CH_2Cl_2 (3 x 200 mL). The crude mixture was purified by flash column chromatography on silica gel (CH_2Cl_2:MeOH (50:2) to afford **6** as a blue solid 2.1 g, 2.8 mmol, and 70% yield). ^{1}H NMR (500 MHz, xx) δ ; ^{13}C NMR (126 MHz, $CDCl_3$) δ ; HRMS ($C_{41}H_{57}N_4O_4^+$): Calc. $[M]^+$: 669.4374, Found $[M]^+$: 669.4501.



7

2-((1*E*,3*E*)-5-((E)-1-(3-aminopropyl)-3,3-dimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-1-(3-((*tert*-butoxycarbonyl)amino)propyl)-3,3-dimethyl-3*H*-indol-1-ium acetate (7):Compound **6** (1 g, 1.3 mmol) was treated with saturated HCl in EtOAc for 2h at r.t to obtain a blue solid which was subsequently redissolved in dry $CHCl_3$ (100 mL) and added DIEA (270 µL, 2.1 mmol, 1 eq.) and Boc anhydride (0.46 g, 2.1 mmol, 1 eq.) for 1h at rt. The reaction mixture was diluted with CH_2Cl_2, washed with water, and concentrated *in vacuo*.Flash column chromatography on silica gel (CH_2Cl_2:MeOH (50:4) returned**7**as a blue solid(400 mg, 0.63 mmol and 48 % yield). ^{1}H NMR (500 MHz, xx) δ ; ^{13}C NMR (126 MHz, $CDCl_3$) δ ; HRMS ($C_{36}H_{49}N_4O_2$): Calc. $[M]^+$: 569.3850, Found $[M]^+$:569.3659.

Nucleic Acids

DNA oligonucleotides were synthesized and structurally verified by NMR spectroscopy as previously reported[1-7]. dsDNA, ssDNA and RNA were purchased from Sigma Aldrich (product number D8515, D8899 and R7250 respectively) and used as such. The nucleic acids were dissolved in buffer (20mM K_2HPO_4/KH_2PO_4, 100 mM KCl, pH 7.0). DNA concentration was expressed in strand molarity using a nearest-neighbour approximation for the absorption coefficients of the unfolded species.

Methods

Spectroscopic and quantum yield data were measured on a Spectra Max M2 spectrophotometer (Molecular Devices). Data analysis was performed using Origin 8.0. Analytical characterization was performed on a HPLC-MS (Agilent-1200 series) with a DAD detector and a single quadrupole mass spectrometer (6130 series) with an ESI probe. Analytical HPLC method: eluents, A: H_2O (0.1% HCOOH), B: CH_3CN (0.1% HCOOH), gradient 5% B to 95% B (10 min). Reverse-phase Phenomenex C_{18} Luna column (4.6 x 50 mm^2, 3.5 μm particle size), flow rate: 1 mL/min. ^1H and ^{13}C NMR spectra were recorded on Bruker ACF300 (300 MHz) and AMX500 (500 MHz) spectrometers and reported in δ (ppm). ^1H NMR spectra were referenced to solvent residual signals as the ^1H internal reference ($CDCl_3$ (δ = 7.26 ppm), DMSO-d_6 (δ = 2.50 ppm), CD_3OD (δ = 3.31)); ^{13}C NMR spectra were referenced to solvent resonances as the ^{13}C internal reference ($CDCl_3$ (δ = 77.0 ppm), DMSO-d_6 (δ = 39.5ppm), CD_3OD (δ = 49.0 ppm)); High resolution mass spectra (ESI) were obtained on a Finnigan/MAT 95XL-T spectrometer.

Quantum yield calculation

Quantum yields were calculated by measuring the integrated emission area of the fluorescent spectra in its respective solvents and comparing to the area measured for Cy5 (Φ_F =0.28) in EtOH (η = 1.361) when excited at 600 nm. Quantum yields were calculated using the equation:

$$\Phi_F^{sample} = \Phi_F^{reference}\left(\frac{F^{sample}}{F^{reference}}\right)\left(\frac{n^{sample}}{n^{reference}}\right)^2\left(\frac{f^{reference}}{f^{sample}}\right)$$

where F represents the integrated intensities of the emission spectra, n is the refractive index of the solvent, and f is absorption factor($f = 1 - 10^{-A}$, where A = absorbance) at the excitation wavelength selected for reference and samples. Emission was integrated between 630 and 750 nm.

Fluorescent life time measurement

Fluorescence lifetime measurements were carried out using a time correlated single photon counting (TCSPC) spectrometer (IBH, UK). In the present work, 595 nm diode lasers (~100 ps, 1MHz repetition rate) were used for excitation. From the measured decay traces, the lifetime parameters were calculated using 1 exponential and time calibration is 5.486969E-02 ns/ch.

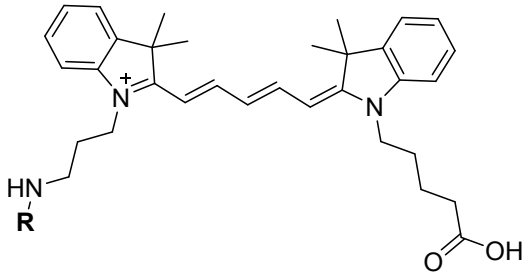
Molecular Modeling

The coordinates of *J19* structures were retrieved from the Protein Data Bank (ID code 2LE6). The **CyC-M716** structure was optimized using the Gaussian03 program (B3LYP/6-31G* level). By using Auto-dock 4.2, docking studies were carried out with the Lamarckian genetic algorithm following the procedure developed for G-quadruplex DNA and ligand docking[8, 9]. Two rounds of simulation were performed. In the first round, simulated annealing was used to find a rough binding mode of **CyC-M716** with 50 runs while keeping all other parameters default. The search

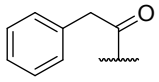
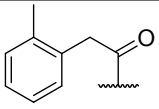
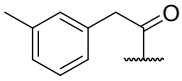
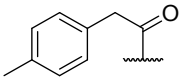
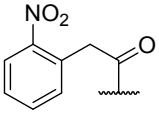
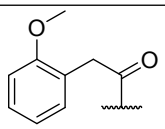
space was subsequently reduced and another 200 runs were conducted to get a more precise result. Following the docking studies of **CyC-M716** with *J19*quadruplex DNA, all the figures were rendered using PyMOL v0.99 (http://www.pymol.org).

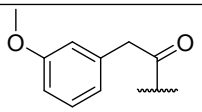
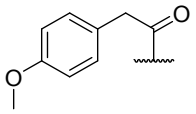
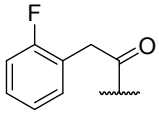
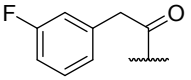
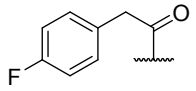
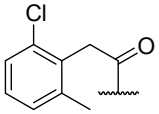
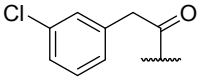
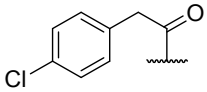
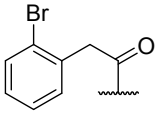
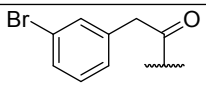
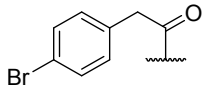
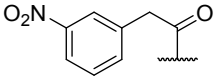
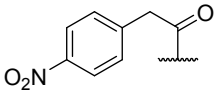
SUPPORTING FIGURES AND TABLES

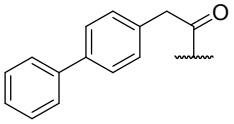
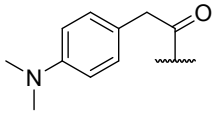
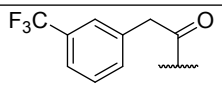
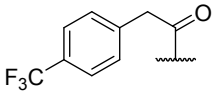
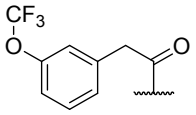
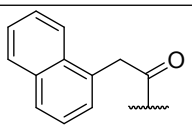
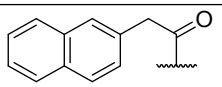
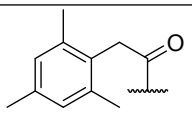
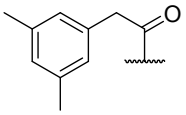
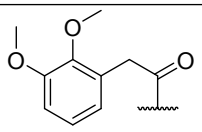
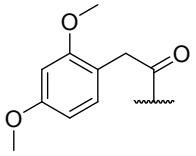
Table S1. Chemical structures and characterization data for the **CyA**library. Concentration = 100μM in DMSO.

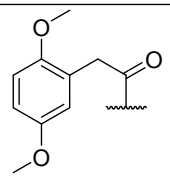
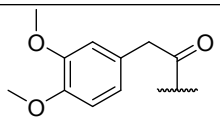
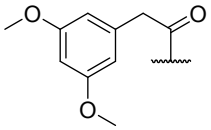
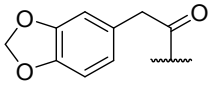
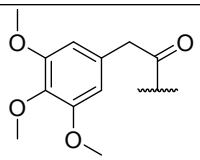
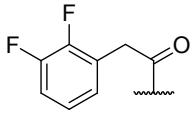
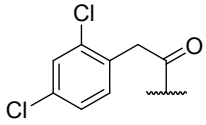
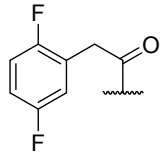
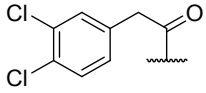
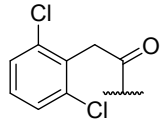


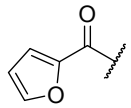
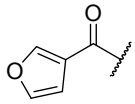
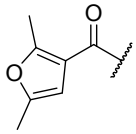
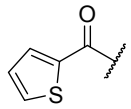
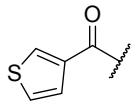
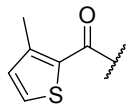
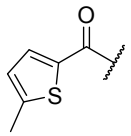
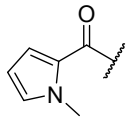
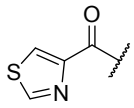
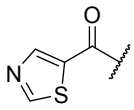
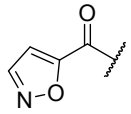
CyA

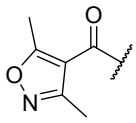
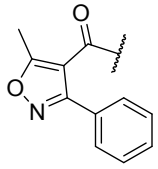
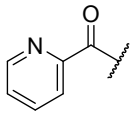
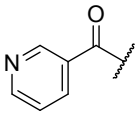
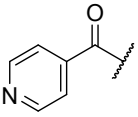
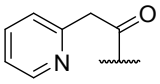
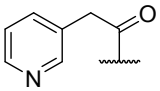
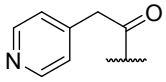
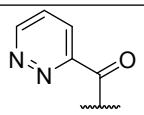
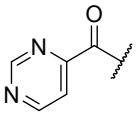
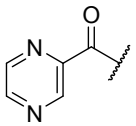
Compound	R	M^+ (Calc.)	M^+ (Meas.)	λ_{max} Abs (nm)	λ_{max}, Em (nm)	Φ	Purity at 254 nm
CyA-C330		630.84	630.60	648	675	0.28	83.7
CyA-C446		644.86	644.60	654	676	0.25	87.4
CyA-C341		644.86	644.55	649	672	0.25	88.4
CyA-C331		644.86	644.60	649	673	0.28	90.9
CyA-C322		675.84	675.60	649	675	0.19	100
CyA-C447		660.86	660.60	649	673	0.27	78.1

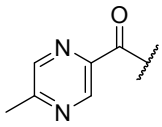
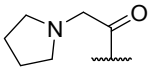
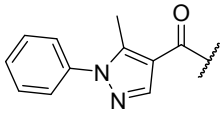
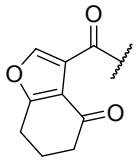
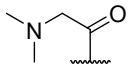
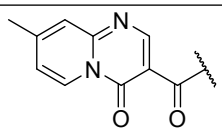
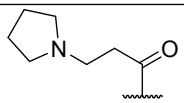
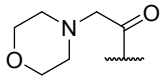
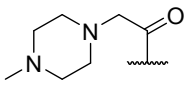
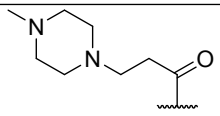
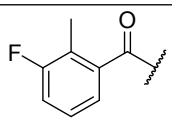
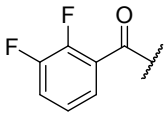
Compound	R	M^+ (Calc.)	M^+ (Meas.)	λ_{max} Abs (nm)	λ_{max}, Em (nm)	Φ	Purity at 254 nm
CyA-C448		660.86	660.60	648	673	0.23	72.9
CyA-C449		660.86	660.60	649	674	0.24	81.9
CyA-C450		648.83	648.55	643	675	0.25	100
CyA-C451		648.83	648.55	650	673	0.23	99.6
CyA-C452		648.83	648.60	649	675	0.26	100
CyA-C350		665.28	664.55	649	674	0.29	95.8
CyA-C453		665.28	664.55	648	675	0.27	80.8
CyA-C454		665.28	664.55	649	673	0.29	85.0
CyA-C455		709.73	709.50	649	676	0.26	100
CyA-C456		709.73	709.50	649	675	0.23	58.1
CyA-C345		709.53	709.50	649	671	0.23	95.3
CyA-C340		675.84	675.60	649	673	0.19	100
CyA-C457		675.84	675.60	649	674	0.18	84.6

Compound	R	M^+ (Calc.)	M^+ (Meas.)	λ_{max} Abs (nm)	λ_{max}, Em (nm)	Φ	Purity at 254 nm
CyA-C458		706.93	706.60	649	675	0.25	95.8
CyA-C459		673.91	673.65	649	676	0.21	91.1
CyA-C460		698.84	698.60	649	673	0.26	90.4
CyA-C461		698.84	698.60	649	671	0.26	97.7
CyA-C462		714.83	714.60	648	671	0.27	95.7
CyA-C463		680.90	680.60	649	674	0.25	91.2
CyA-C464		680.90	680.60	649	675	0.23	94.4
CyA-C465		672.92	672.65	649	674	0.27	100
CyA-C466		658.89	658.60	649	672	0.23	88.6
CyA-C467		690.89	690.55	649	675	0.22	91.7
CyA-C468		690.89	690.55	649	673	0.21	75.2

Compound	R	M^+ (Calc.)	M^+ (Meas.)	λ_{max} Abs (nm)	λ_{max}, Em (nm)	Φ	Purity at 254 nm
CyA-C469		690.89	690.60	649	675	0.26	96.4
CyA-C470		690.89	690.60	649	673	0.22	89.0
CyA-C471		690.89	690.60	649	672	0.22	94.9
CyA-C342		674.85	674.60	649	673	0.26	94.1
CyA-C472		720.92	720.60	649	673	0.23	90.4
CyA-C473		666.82	666.55	648	673	0.26	90.0
CyA-C474		699.73	698.50	650	672	0.26	92.2
CyA-C475		666.82	666.55	649	673	0.29	99.3
CyA-C476		699.73	698.50	649	672	0.28	90.0
CyA-C477		699.73	698.50	648	674	0.23	94.8

Compound	R	M^+ (Calc.)	M^+ (Meas.)	λ_{max} Abs (nm)	λ_{max}, Em (nm)	Φ	Purity at 254 nm
CyA-C195		606.77	605.55	650	675	0.33	91.3
CyA-C478		606.77	605.55	650	674	0.29	97.7
CyA-C479		634.83	633.60	649	672	0.26	86.1
CyA-C161		622.84	621.55	649	670	0.27	76.1
CyA-C480		622.84	621.55	650	671	0.26	100
CyA-C481		636.87	635.55	648	671	0.27	90.0
CyA-C482		636.87	635.55	649	672	0.28	97.1
CyA-C483		619.81	619.60	649	674	0.25	95.2
CyA-C484		623.83	623.55	649	675	0.22	72.8
CyA-C485		623.83	623.55	649	673	0.27	88.3
CyA-C486		607.76	607.55	650	672	0.27	87.7

Compound	R	M^+ (Calc.)	M^+ (Meas.)	λ_{max} Abs (nm)	λ_{max}, Em (nm)	Φ	Purity at 254 nm
CyA-C487		635.81	634.60	648	672	0.25	86.5
CyA-C488		697.88	696.65	649	673	0.26	90.4
CyA-C375		617.80	617.55	649	675	0.28	92.5
CyA-C197		617.80	616.60	649	674	0.24	94.5
CyA-C435		617.80	617.55	649	674	0.24	85.2
CyA-C489		631.83	631.60	649	673	0.23	90.5
CyA-C490		631.83	631.60	319	674	0.23	94.5
CyA-C491		631.83	631.60	649	674	0.28	95.8
CyA-C492		618.79	618.55	649	674	0.26	94.5
CyA-C493		618.79	618.55	649	675	0.23	92.6
CyA-C398		618.79	618.55	650	674	0.24	91.5

Compound	R	M^+ (Calc.)	M^+ (Meas.)	λ_{max} Abs (nm)	λ_{max}, Em (nm)	Φ	Purity at 254 nm
CyA-C494		632.81	632.60	650	674	0.25	94.9
CyA-C495		625.87	625.65	649	673	0.25	91.8
CyA-C496		696.90	695.60	650	674	0.28	92.0
CyA-C497		674.85	674.60	650	673	0.22	86.6
CyA-C498		597.81	597.60	649	673	0.23	89.6
CyA-C499		699.85	699.60	650	673	0.27	90.8
CyA-C500		637.87	636.60	649	673	0.21	87.9
CyA-C501		639.85	639.65	649	673	0.22	95.2
CyA-C502		652.89	652.65	648	674	0.21	85.3
CyA-C503		666.91	666.65	649	672	0.23	90.8
CyA-C504		648.83	648.60	648	672	0.23	88.3
CyA-C505		652.79	652.55	649	675	0.28	88.6

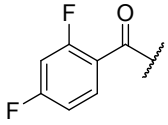
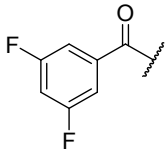
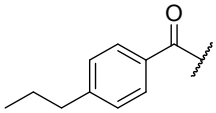
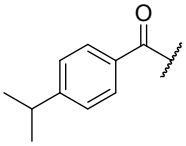
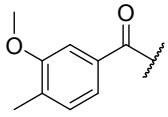
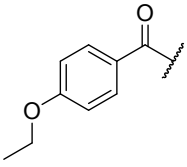
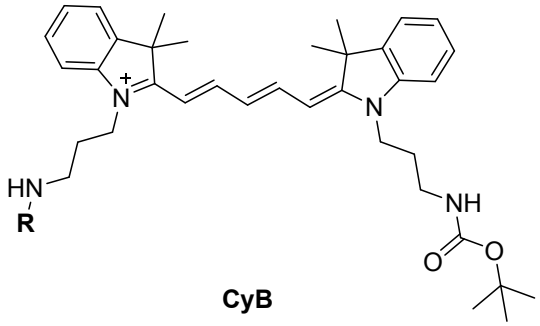
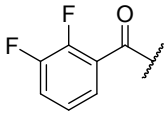
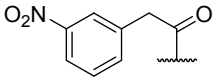
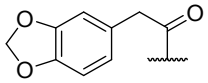
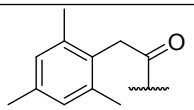
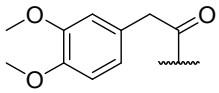
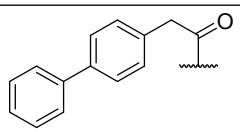
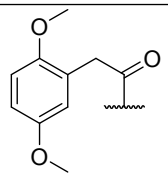
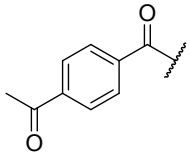
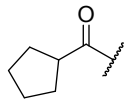
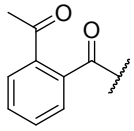
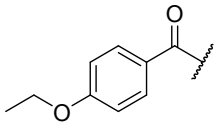
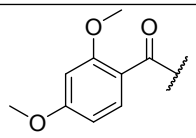
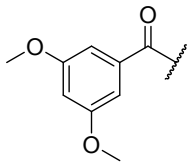
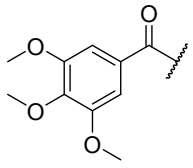
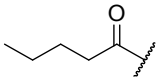
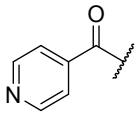
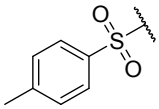
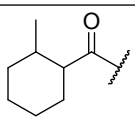
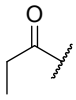
Compound	R	M^+ (Calc.)	M^+ (Meas.)	λ_{max} Abs (nm)	λ_{max}, Em (nm)	Φ	Purity at 254 nm
CyA-C506		652.79	652.55	649	676	0.26	83.5
CyA-C507		652.79	652.55	649	673	0.28	89.2
CyA-C508		658.89	658.65	649	673	0.26	87.0
CyA-C433		658.89	657.70	648	672	0.25	86.7
CyA-C509		660.86	659.70	649	673	0.27	84.5
CyA-C510		660.86	659.65	648	672	0.28	86.7

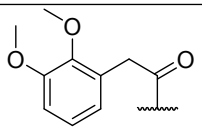
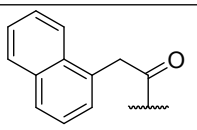
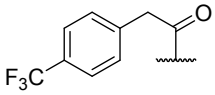
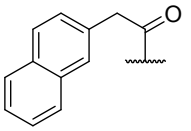
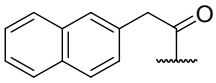
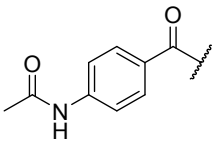
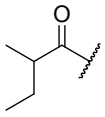
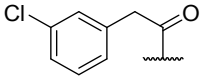
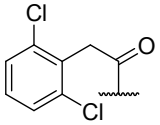
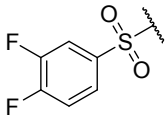
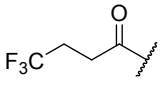
Table S2. Chemical structures and characterization data for the **CyB**library. Concentration = 100μM in DMSO.

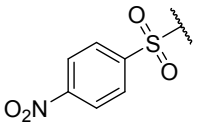
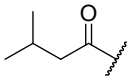
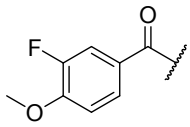
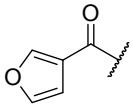
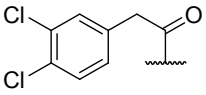
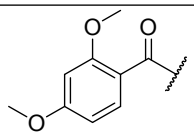
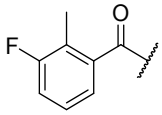
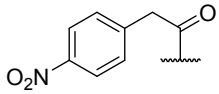
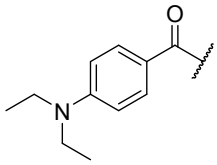
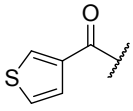
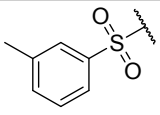


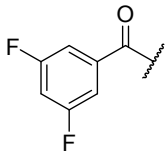
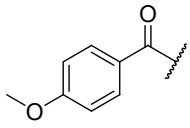
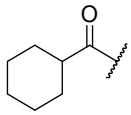
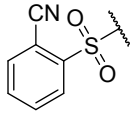
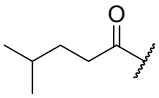
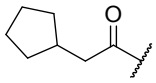
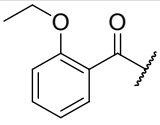
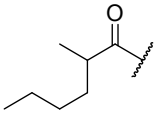
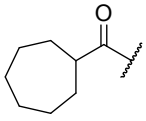
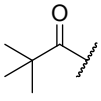
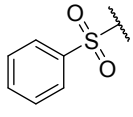
CyB

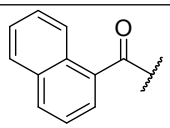
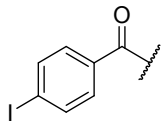
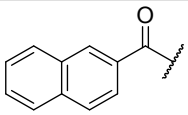
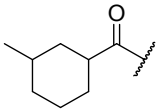
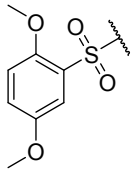
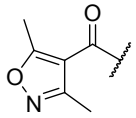
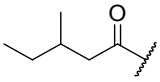
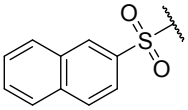
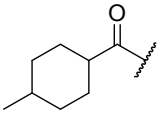
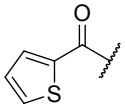
Compound	R	M^+ (Calc.)	M^+ (Meas.)	λ_{max} Abs (nm)	λ_{max}, Em (nm)	Φ	Purity at 254 nm
CyB-C505		709.89	709.60	648	670	0.28	98.7
CyB-C340		732.93	732.65	648	670	0.29	77.9
CyB-C342		731.94	731.65	648	670	0.28	83.9
CyB-C465		730.02	729.70	648	670	0.29	86.0
CyB-C470		747.99	747.65	648	670	0.22	77.3
CyB-C458		764.03	763.70	648	670	0.26	86.4
CyB-C469		747.99	747.85	648	670	0.28	86.2
CyB-C358		715.95	715.65	648	670	0.25	95.3

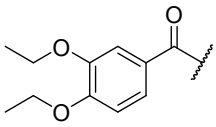
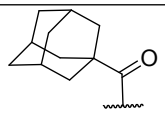
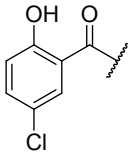
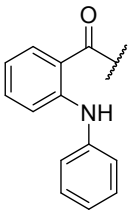
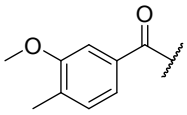
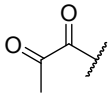
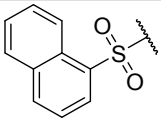
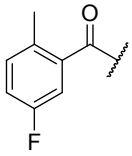
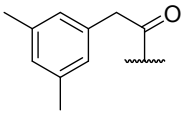
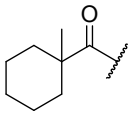
Compound	R	M^+ (Calc.)	M^+ (Meas.)	λ_{max} Abs (nm)	λ_{max}, Em (nm)	Φ	Purity at 254 nm
CyB-C511		665.93	665.65	648	670	0.28	94.0
CyB-C512		715.95	715.65	648	670	0.24	100
CyB-C510		717.96	717.65	648	670	0.28	76.5
CyB-C468		747.99	747.65	648	670	0.23	85.3
CyB-C471		747.99	747.65	648	670	0.28	85.8
CyB-C472		778.01	777.65	648	670	0.28	83.9
CyB-C513		653.92	653.70	648	670	0.29	77.6
CyB-C435		674.90	674.65	648	670	0.22	100
CyB-C317		778.06	777.55	648	670	0.22	100
CyB-C814		693.98	693.70	648	670	0.26	84.8
CyB-C010		625.87	625.65	648	670	0.22	87.7

Compound	R	M^+ (Calc.)	M^+ (Meas.)	λ_{max} Abs (nm)	λ_{max}, Em (nm)	Φ	Purity at 254 nm
CyB-C467		747.99	747.65	648	670	0.24	86.0
CyB-C463		737.99	737.65	648	670	0.29	70.0
CyB-C461		755.93	755.65	648	670	0.20	88.4
CyB-C863		737.99	737.60	648	670	0.28	85.4
CyB-C464		737.99	737.65	648	670	0.28	93.3
CyB-C518		730.96	730.65	648	670	0.27	97.9
CyB-C515		653.92	653.65	648	670	0.29	68.9
CyB-C453		722.38	721.60	648	670	0.28	80.0
CyB-C477		756.83	755.55	648	670	0.23	86.4
CyB-C905		745.94	745.60	648	670	0.25	100
CyB-C516		679.89	679.60	648	670	0.29	92.2

Compound	R	M^+ (Calc.)	M^+ (Meas.)	λ_{max} Abs (nm)	λ_{max}, Em (nm)	Φ	Purity at 254 nm
CyB-C636		840.00	839.50	648	670	0.27	64.5
CyB-C517		653.92	653.65	648	670	0.28	74.3
CyB-C518		721.93	721.60	648	670	0.28	70.9
CyB-C478		663.87	663.60	648	670	0.23	84.2
CyB-C476		756.83	755.55	648	670	0.23	83.5
CyB-C519		750.03	749.60	648	670	0.24	93.7
CyB-C504		705.93	705.60	648	670	0.21	77.7
CyB-C457		732.93	732.60	648	670	0.29	85.6
CyB-C520		745.03	744.70	648	670	0.20	88.9
CyB-C480		679.94	679.60	648	670	0.29	80.2
CyB-C817		623.87	623.55	648	670	0.26	100

Compound	R	M^+ (Calc.)	M^+ (Meas.)	λ_{max} Abs (nm)	λ_{max}, Em (nm)	Φ	Purity at 254 nm
CyB-C507		709.89	709.60	648	670	0.28	94.4
CyB-C306		719.93	719.60	648	670	0.24	90.5
CyB-C521		679.96	679.70	648	670	0.21	95.2
CyB-C1445		734.97	734.60	648	670	0.26	86.2
CyB-C522		667.95	667.65	648	670	0.27	92.7
CyB-C523		679.96	679.70	648	670	0.25	91.4
CyB-C524		717.96	717.65	648	670	0.24	60.3
CyB-C525		667.95	667.70	648	670	0.24	95.4
CyB-C526		693.98	693.70	648	670	0.23	61.0
CyB-C527		653.92	653.65	648	670	0.22	92.1
CyB-C916		709.96	709.60	648	670	0.23	93.7

Compound	R	M^+ (Calc.)	M^+ (Meas.)	λ_{max} Abs (nm)	λ_{max}, Em (nm)	Φ	Purity at 254 nm
CyB-C463		647.89	647.55	648	670	0.26	100
CyB-C528		723.97	723.65	648	670	0.26	87.2
CyB-C529		701.96	701.65	648	670	0.25	78.1
CyB-C530		723.97	723.60	648	670	0.23	89.6
CyB-C514		693.98	693.70	648	670	0.22	81.2
CyB-C1446		770.01	769.60	648	670	0.23	100
CyB-C487		692.91	692.65	648	670	0.26	92.2
CyB-C392		667.95	667.65	648	670	0.26	82.7
CyB-C445		760.01	759.60	648	670	0.28	90.2
CyB-C531		693.98	693.85	648	670	0.23	84.1
CyB-C161		679.94	679.60	648	670	0.25	84.2

Compound	R	M^+ (Calc.)	M^+ (Meas.)	λ_{max} Abs (nm)	λ_{max}, Em (nm)	Φ	Purity at 254 nm
CyB-C532		762.01	761.65	648	670	0.23	67.5
CyB-C133		732.03	731.70	648	670	0.21	75.3
CyB-C533		724.35	724.55	648	670	0.20	79.2
CyB-C534		765.02	764.65	648	670	0.27	81.7
CyB-C509		717.96	717.60	648	670	0.22	94.5
CyB-C535		637.85	637.65	648	670	0.26	91.4
CyB-C767		760.01	759.55	648	670	0.25	100
CyB-C536		705.93	705.60	648	670	0.23	92.3
CyB-C466		715.99	715.65	648	670	0.22	100
CyB-C537		693.98	693.65	648	670	0.24	95.6

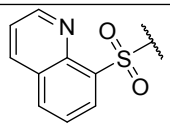
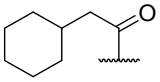
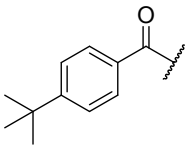
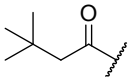
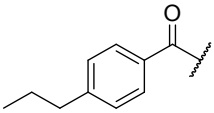
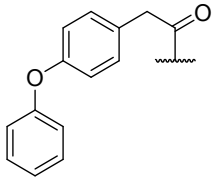
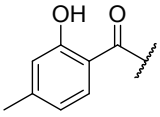
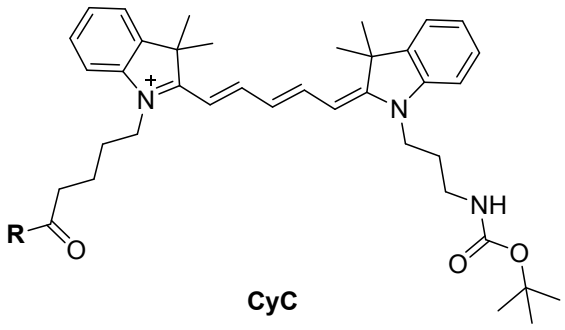
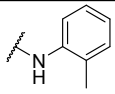
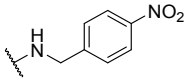
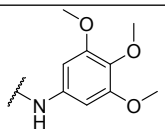
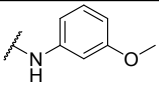
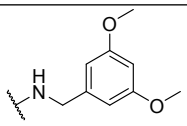
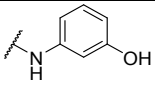
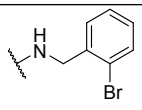
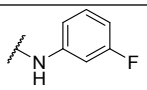
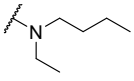
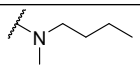
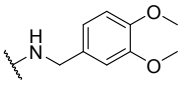
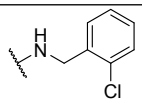
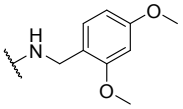
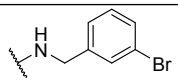
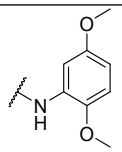
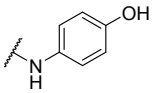
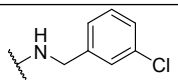
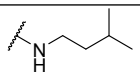
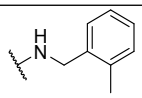
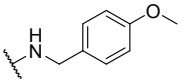
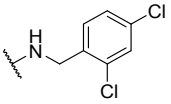
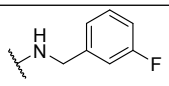
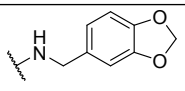
Compound	R	M^+ (Calc.)	M^+ (Meas.)	λ_{max} Abs (nm)	λ_{max}, Em (nm)	Φ	Purity at 254 nm
CyB-C907		761.01	760.55	648	670	0.21	97.8
CyB-C434		693.98	693.65	648	670	0.27	97.5
CyB-C545		730.02	729.65	648	670	0.28	96.6
CyB-C538		667.95	667.65	648	670	0.27	87.3
CyB-C508		715.99	715.65	648	670	0.25	94.7
CyB-C539		780.03	779.60	648	670	0.23	90.6
CyB-C540		703.93	703.60	648	670	0.21	82.2

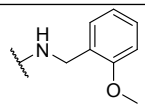
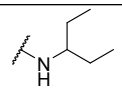
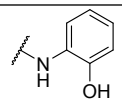
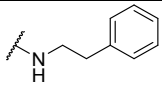
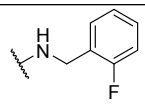
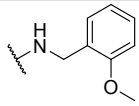
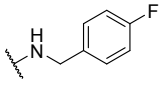
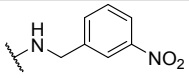
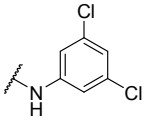
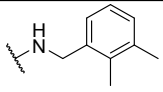
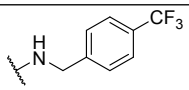
Table S3. Chemical structures and characterization data for the **CyC** library. Concentration = 100μM in DMSO.

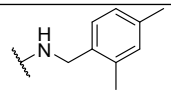
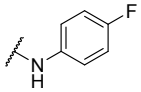
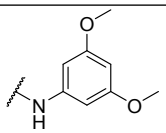
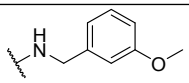
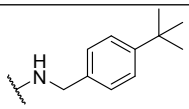
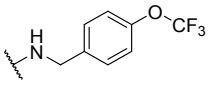
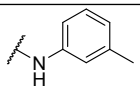
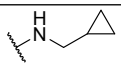
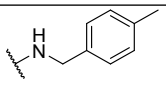
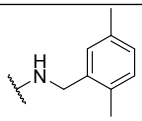
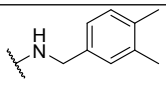
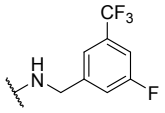
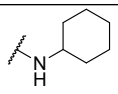


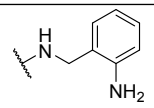
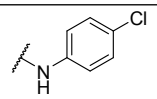
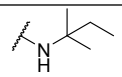
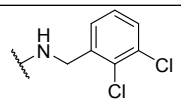
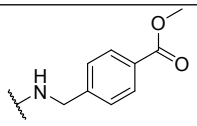
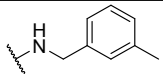
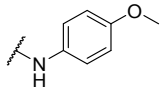
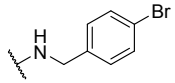
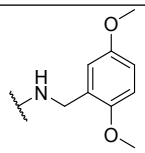
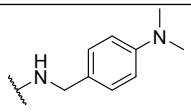
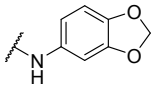
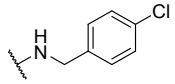
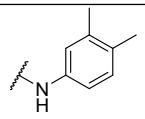
CyC

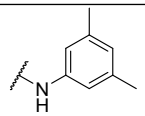
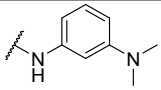
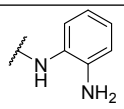
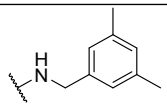
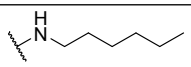
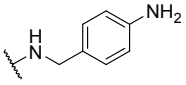
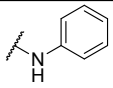
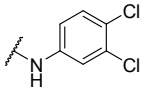
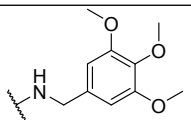
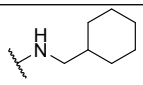
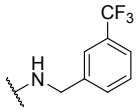
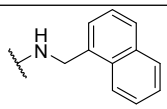
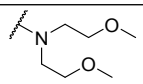
Compound	R	M^+ (Calc.)	M^+ (Meas.)	λ_{max} Abs (nm)	λ_{max}, Em (nm)	Φ	Purity at 254 nm
CyC-M129		701.96	701.60	648	670	0.25	83.7
CyC-M361		746.96	746.60	648	670	0.25	87.4
CyC-M505		778.01	777.60	648	670	0.19	88.4
CyC-M152		717.96	717.60	648	670	0.18	90.9
CyC-M706		761.46	761.65	648	670	0.29	100
CyC-M127		703.42	703.65	648	670	0.29	78.1
CyC-M329		779.35	779.50	648	670	0.18	72.9
CyC-M143		705.42	705.60	648	670	0.20	81.9
CyC-M188		695.49	695.65	648	670	0.28	100
CyC-M206		681.47	681.65	648	670	0.28	99.6

Compound	R	M^+ (Calc.)	M^+ (Meas.)	λ_{max} Abs (nm)	λ_{max}, Em (nm)	Φ	Purity at 254 nm
CyC-M319		761.46	761.65	648	670	0.28	100
CyC-M364		735.40	735.60	648	670	0.24	95.8
CyC-M358		761.46	761.65	648	670	0.20	80.8
CyC-M341		779.35	779.55	648	670	0.27	85.0
CyC-M202		747.45	747.65	648	670	0.25	100
CyC-M123		703.42	703.60	648	670	0.27	58.1
CyC-M403		735.40	735.60	648	670	0.23	95.3
CyC-M442		681.47	681.65	648	670	0.26	100
CyC-M101		715.46	715.65	648	670	0.29	84.6
CyC-M381		731.45	731.65	648	670	0.23	95.8
CyC-M531		769.36	769.55	648	670	0.23	91.1
CyC-M220		719.43	719.60	648	670	0.23	90.4
CyC-M49		745.43	745.60	648	670	0.25	97.7

Compound	R	M^+ (Calc.)	M^+ (Meas.)	λ_{max} Abs (nm)	λ_{max}, Em (nm)	Φ	Purity at 254 nm
CyC-M388		731.45	731.60	648	670	0.28	95.7
CyC-M425		681.47	681.65	648	670	0.25	91.2
CyC-M118		703.42	703.55	648	670	0.30	94.4
CyC-M211		715.46	715.65	648	670	0.24	100
CyC-M375		719.43	719.60	648	670	0.21	88.6
CyC-M182		681.47	681.65	648	670	0.26	91.7
CyC-M71		717.44	717.60	648	670	0.18	75.2
CyC-M50		667.46	667.65	648	670	0.29	96.4
CyC-M218		719.43	719.60	648	670	0.22	89.0
CyC-M148		746.43	746.60	648	670	0.24	94.9
CyC-M215		665.44	665.70	648	670	0.22	94.1
CyC-M172		755.35	755.55	648	670	0.24	90.4
CyC-M707		729.47	729.70	648	670	0.27	90.0
CyC-M330		769.43	769.65	648	670	0.22	92.2

Compound	R	M^+ (Calc.)	M^+ (Meas.)	λ_{max} Abs (nm)	λ_{max}, Em (nm)	Φ	Purity at 254 nm
CyC-M708		729.47	729.70	648	670	0.27	99.3
CyC-M138		705.42	705.65	648	670	0.20	90.0
CyC-M709		747.45	747.55	648	670	0.21	94.8
CyC-M387		731.45	731.65	648	670	0.24	91.3
CyC-M522		757.51	757.70	648	670	0.26	97.7
CyC-M115		785.43	785.65	648	670	0.21	86.1
CyC-M133		701.44	701.65	648	670	0.21	76.1
CyC-M575		665.44	665.65	648	670	0.20	100
CyC-M396		715.46	715.70	648	670	0.24	90.0
CyC-M710		730.47	729.70	648	670	0.25	97.1
CyC-M711		729.47	729.70	648	670	0.23	95.2
CyC-M718		787.42	787.85	648	670	0.31	72.8
CyC-M712		681.47	681.70	648	670	0.27	88.3
CyC-M164		693.47	693.65	648	670	0.26	87.7

Compound	R	M^+ (Calc.)	M^+ (Meas.)	λ_{max} Abs (nm)	λ_{max}, Em (nm)	Φ	Purity at 254 nm
CyC-M713		716.45	716.70	648	670	0.27	86.5
CyC-M126		721.39	721.60	648	670	0.21	90.4
CyC-M422		681.47	681.70	648	670	0.28	92.5
CyC-M714		769.37	769.60	648	670	0.23	94.5
CyC-M715		759.45	759.65	648	670	0.24	85.2
CyC-M398		715.46	715.70	648	670	0.26	90.5
CyC-M151		717.44	717.65	648	670	0.17	94.5
CyC-M452		779.35	779.55	648	670	0.24	95.8
CyC-M181		761.46	761.65	648	670	0.24	94.5
CyC-M318		744.48	744.65	648	670	0.24	92.6
CyC-M379		731.42	731.65	648	670	0.28	91.5
CyC-M221		735.40	735.65	648	670	0.20	94.9
CyC-M122		715.46	715.70	648	670	0.28	91.8

Compound	R	M^+ (Calc.)	M^+ (Meas.)	λ_{max} Abs (nm)	λ_{max}, Em (nm)	Φ	Purity at 254 nm
CyC-M290		715.46	715.70	648	670	0.22	92.0
CyC-M636		730.47	730.70	648	670	0.26	86.6
CyC-M231		702.44	702.65	648	670	0.28	89.6
CyC-M716		729.47	729.70	648	670	0.25	90.8
CyC-M439		695.49	695.70	648	670	0.28	87.9
CyC-M411		716.45	716.70	648	670	0.24	95.2
CyC-M156		687.43	687.65	648	670	0.21	85.3
CyC-M170		755.35	755.55	648	670	0.31	90.8
CyC-M356		791.47	791.70	648	670	0.24	88.3
CyC-M382		707.49	707.65	648	670	0.26	88.6
CyC-M447		769.43	769.60	648	670	0.29	83.5
CyC-M542		751.48	751.65	648	670	0.28	89.2
CyC-M426		727.48	727.65	648	670	0.25	87.0

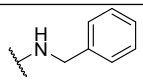
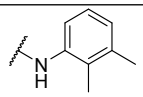
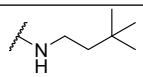
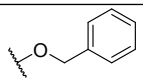
Compound	R	M^+ (Calc.)	M^+ (Meas.)	λ_{max} Abs (nm)	λ_{max}, Em (nm)	Φ	Purity at 254 nm
CyC-M374		701.44	701.65	648	670	0.27	86.7
CyC-M117		715.46	715.65	648	670	0.22	84.5
CyC-M92		695.49	695.70	648	670	0.25	86.7
CyC-O374		702.43	702.	648	670	0.23	96.5

Table S4. Photophysical properties of **CyC-M716** in various solvents.

Solvent [1]	Dielectric constant, ε	Viscosity (η/mPa s)	$\lambda_{abs,max}$ (nm)	$\lambda_{fluo,max}$ (nm)	Φ_F
buffer	80.10	0.89	640	660	0. 10
J19	80.10	0.89	640	670	0.69
DMSO	46.70	2.00	650	675	0.25
ACN	38.00	0.92	640	665	0.23
PEG 400	12.40	90.00	645	675	0.82
Toluene	2.38	0.55	655	690	0.16

[1]Buffer: 20 mM KH_2PO_4/K_2HPO_4, 100 mMKCl, pH 7.0; *J19*: 100μM in buffer; DMSO: dimethyl sulfoxide; ACN: acetonitrile; PEG 400: polyethylene glycol 400.

Table S5. DNA sequences used in this study.

Name	Sequence (5' → 3')	Structural Features
J19	GIGT GGGT GGGTGGGT	propeller loops,parallel, dimer
T95-2T	TT GGGT GGGTGGGTGGGT	propeller loops, parallel, monomer
A95	GGGA GGGAGGGAGGGA	propeller loops, parallel, dimer
C95	GGGC GGGCGGGCGGGC	propeller loops, parallel, dimer
93del	GGGG TGGG AGGA GGGT	propeller loops, parallel, interlocked dimer
c-kit1	AGGG AGGG CGCT GGGA GGAG GG	Mixture of loops, parallel,snap-back
Pu24T	TGAG GGTG GTGA GGGT GGGG AAGG	Mixture of loops, parallel,snap-back
Oxy	GGGG TTTT GGGG	antiparallel
HT	TT GGGTTA GGGTTAGGGTTA GGGA	hybrid

Table S6. Binding parameters of *G4s* with **CyC-M716** derived from fluorescent titration and auto-docking.

Sample	K_d (μM)
CyC-M716-*J19*	20.63±0.02
CyC-M716-*C95*	22.33±0.04
CyC-M716-*A95*	21.33±0.04
CyC-M716-*T95-2T*	18.59±0.02
CyC-M716-*93del*	51.84±0.03
CyC-M716-Kit	/
CyC-M716-Pu24t	/
CyC-M716-Oxy	/
CyC-M716-*HT*	*-24.23±0.01
CyC-M716-ctDNA	-27.92±0.03
CyC-M716-RNA	-26.38±0.05
#**Groove-binding A**	15.55
#**Groove-binding B**	24.08

/: the data can't be given from fluorescent titration; #: results from anto-docking. -: data were fitting by fluorescent decreasing

Table S7. Fitting parameters for the **ICSPC** decays of DNA sequence (λ_{ex}= 595nm,λ_{em} = 665 nm) in 20 mM $KH_2PO_4/K_2HPO_4$and 100 mMKCl (pH=7.0).Calculated using 1 exponential model.

Sample	Lifetime (ns)
CyC-M716	0.81±0.02
CyC-M716-ctDNA	1.57±0.03
CyC-M716-*HT*	1.58±0.02
CyC-M716-*93del*	1.84±0.02
CyC-M716-*T95-2T*	2.22±0.01
CyC-M716-*J19*	2.26±0.03

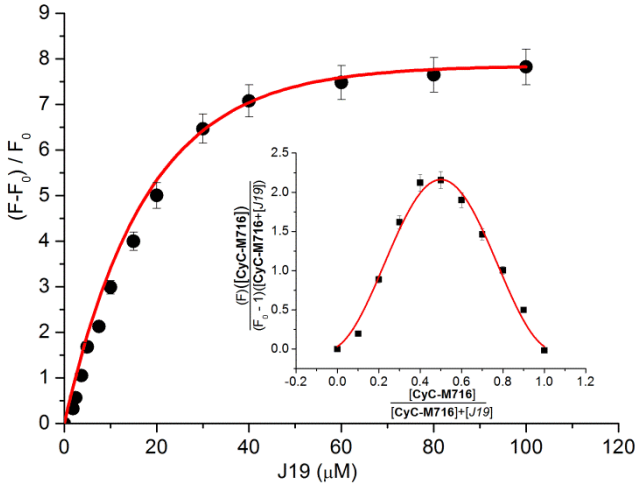


Fig.S1 Fluorescence fold change upon addition of J19 (0 – 100 μM). F0 and Fmax are the fluorescent maximum intensities of the CyC-M716 in the absence and presence of J19 respectively. Inset: Job plot analysis. CyC-M716 was mixed with J19 at different ratios while maintaining total concentration at 20 μM in buffer. λ_{ex}: 600 nm, λ_{em}: 665 nm. Values are represented as means (n = 3). Measurements were taken at room temperature (RT).

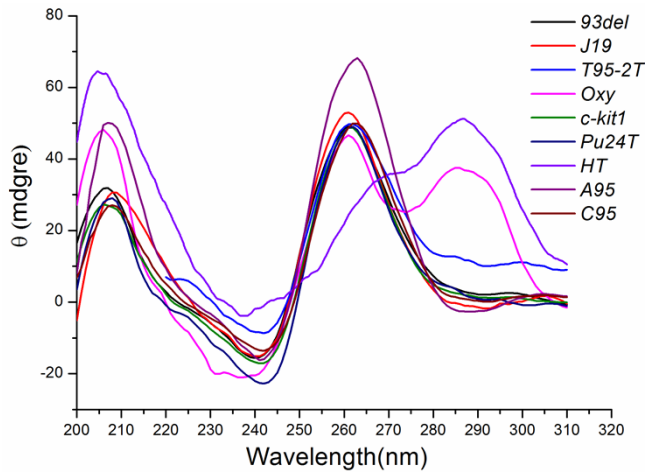


Fig. S2. CD signatures of G4-forming oligonucleotides. Oligonucleotides dissolved in buffer (20 mM K_2HPO_4/KH_2PO_4, 100 mM KCl, pH 7.0).

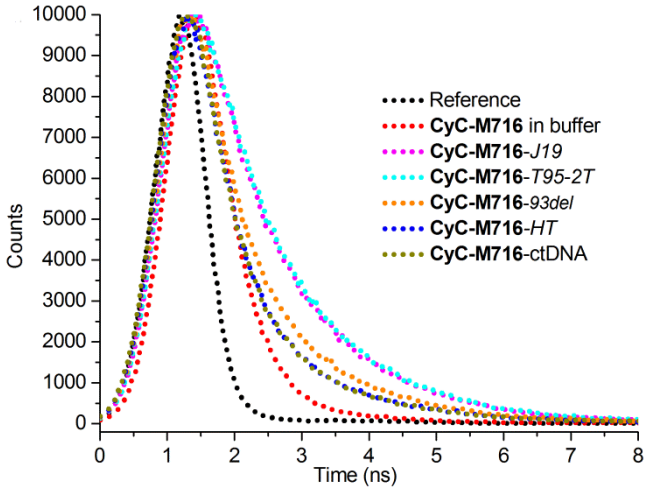


Fig. S3 Fluorescence decay traces of **CyC-M716** (10 μM) monitored at 665 nm in the absence and presence of different oligonucleotides (40 μM). λ_{ex}: 595 nm.

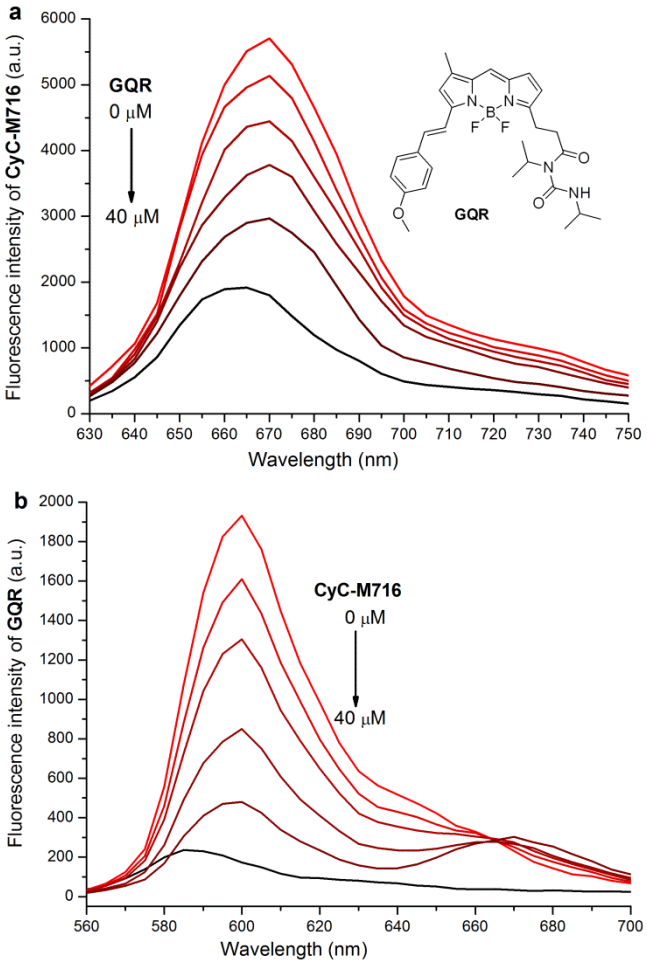


Fig. S4: (a) Systematic addition of GQR (0, 5, 10, 20, 40 μM) to solution of *J19*-**CyC-M716** (10 μM). λ_{ex}: 600 nm (b) The reverse titration of **CyC-M716** (0, 5, 10, 20, 40 μM) to solution of *J19*-GQR (10 μM)**.** λ_{ex}: 360 nm.

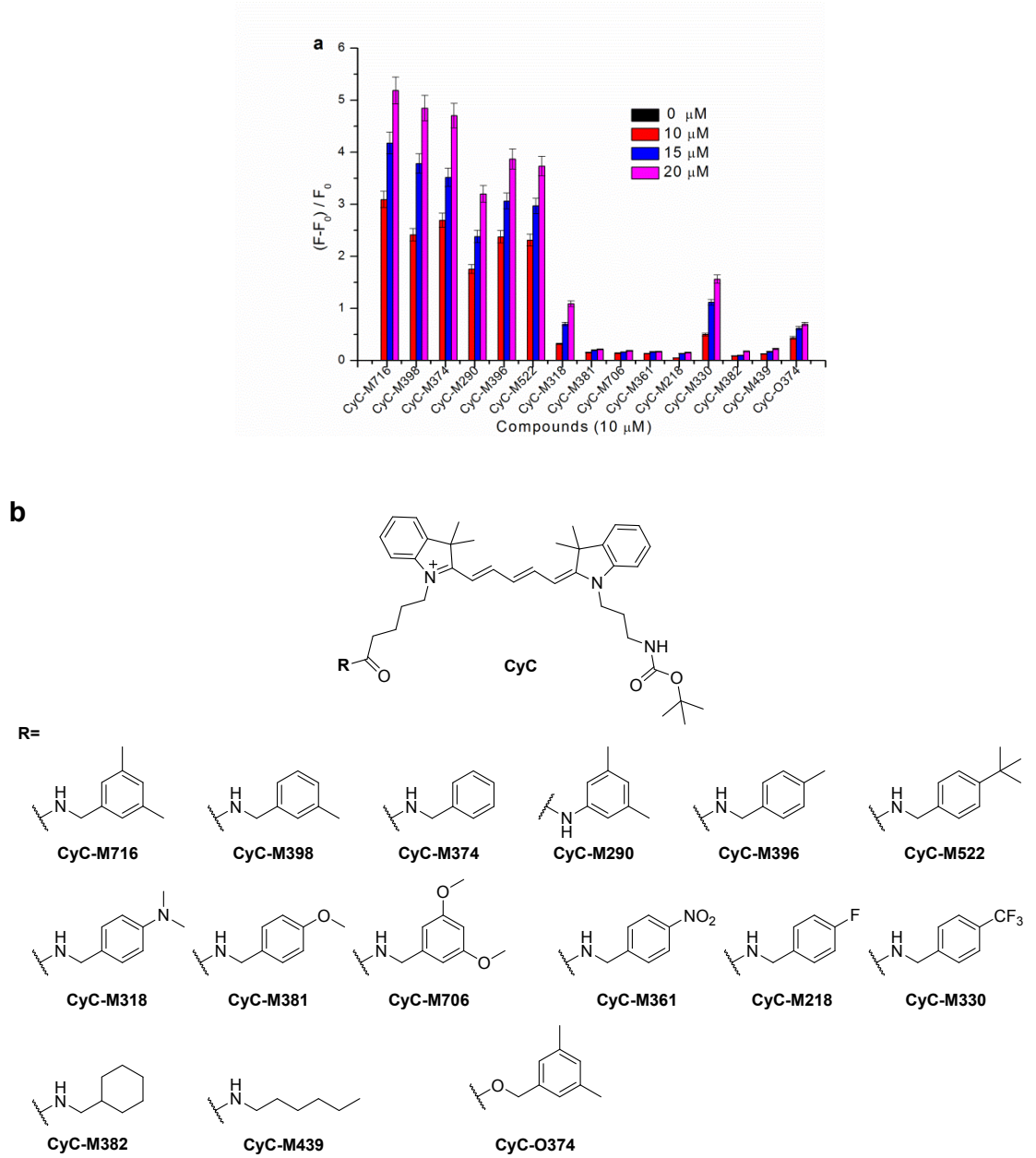
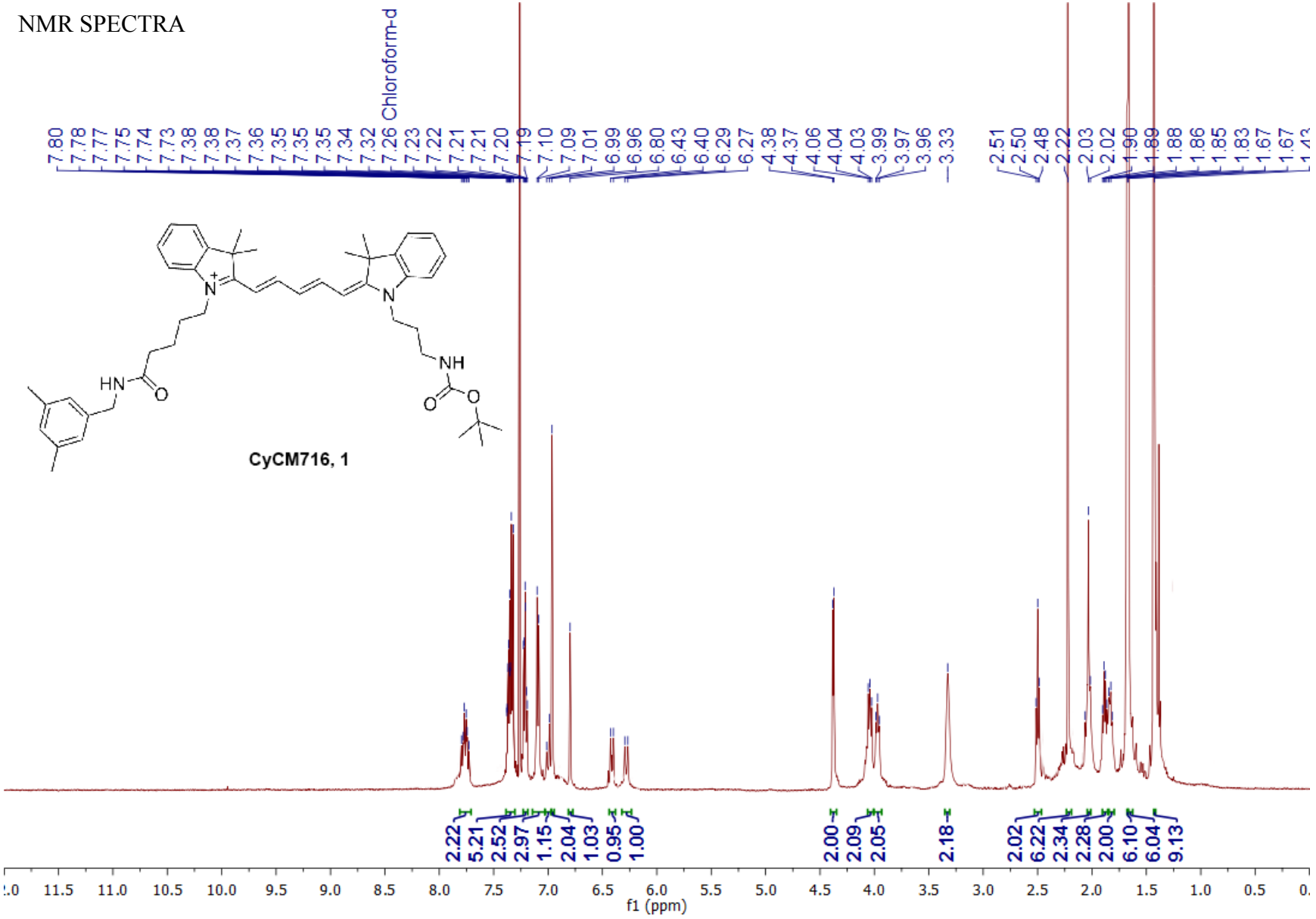
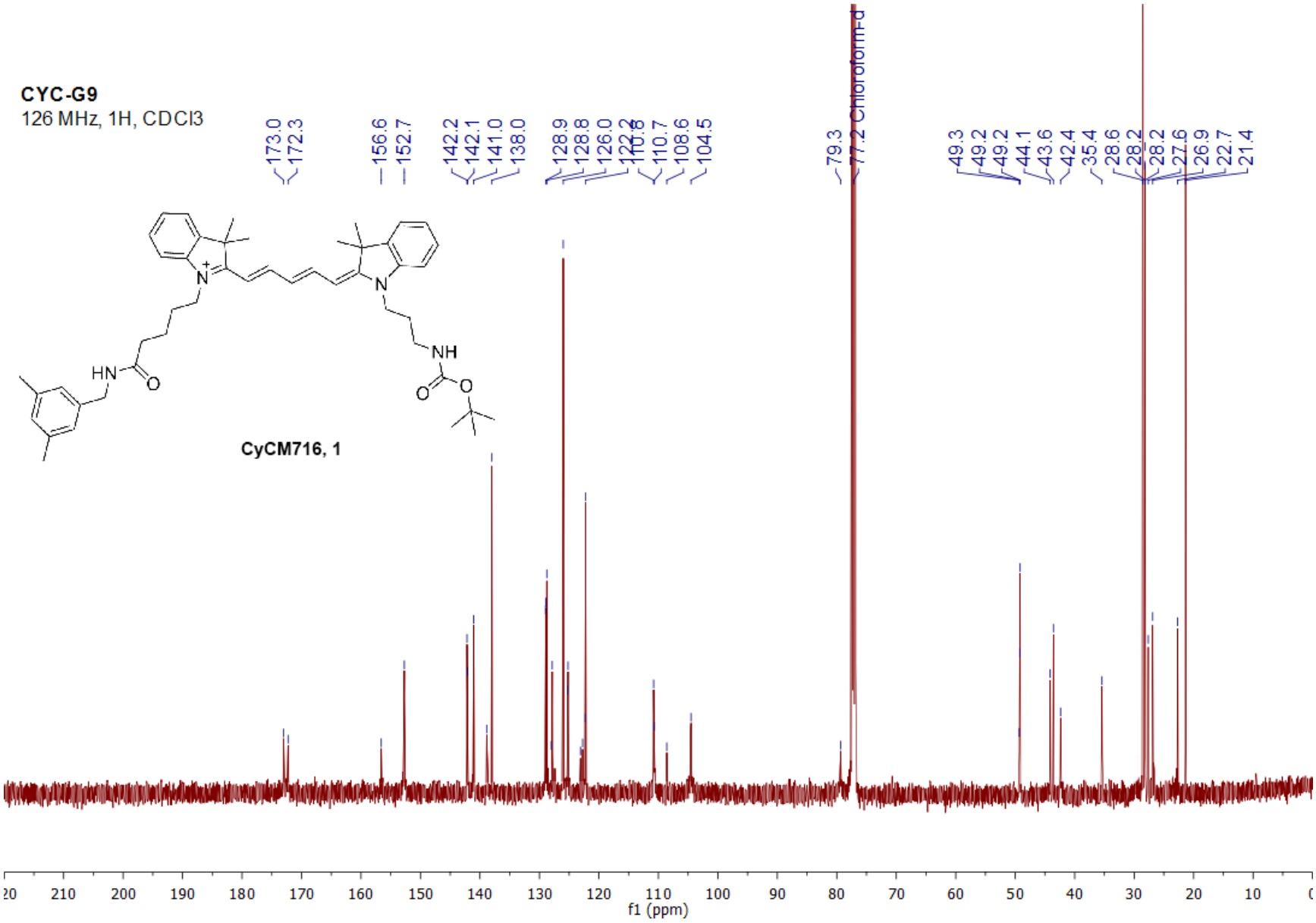
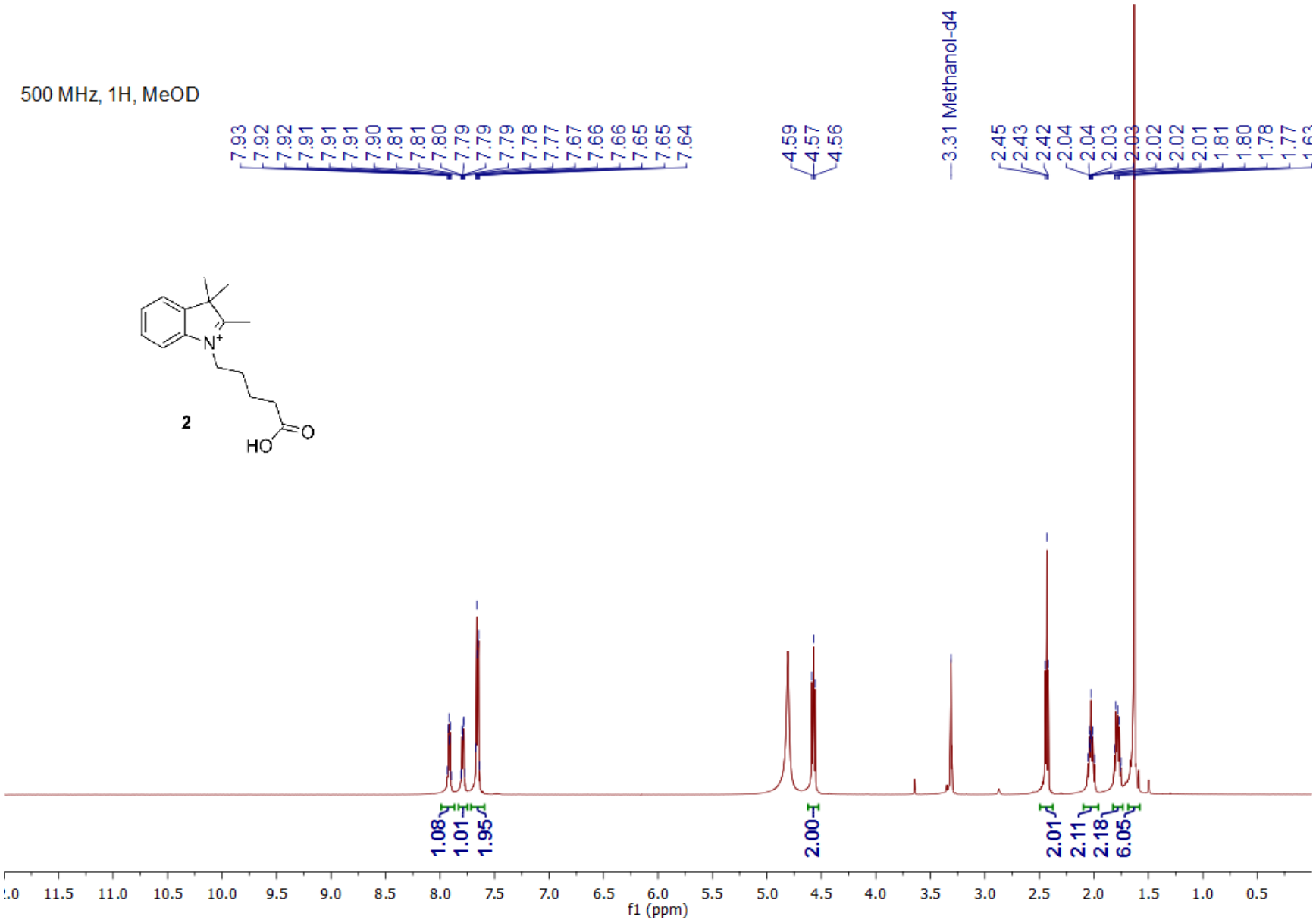


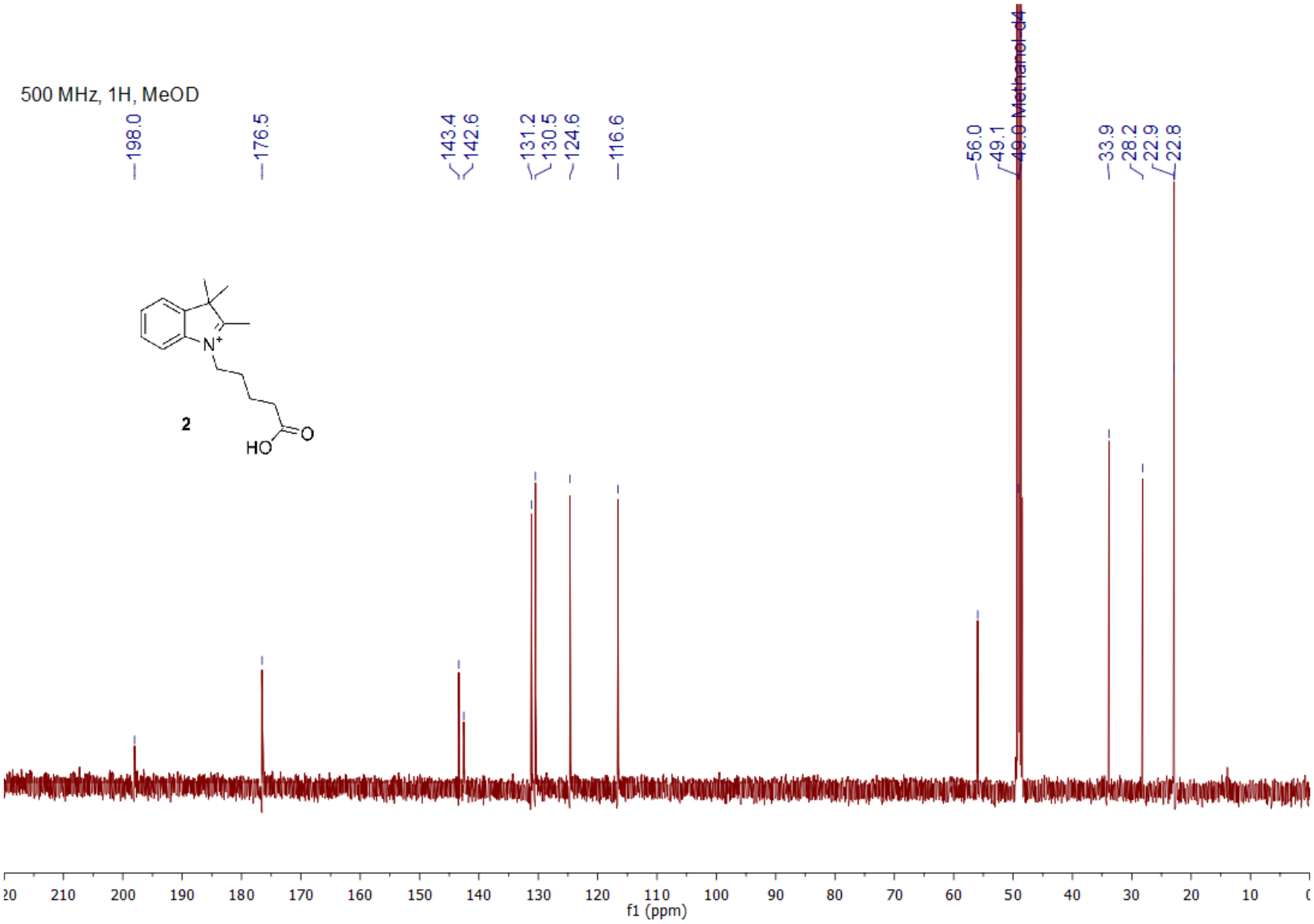
Fig. S5: (a) Fluorescence spectra of **CyC** compounds (10 μM) upon incubation with serial dilutions of *J19* (0, 10, 15 and 20 μM). λ_{ex}: 600 nm, λ_{em}: 665 nm. F_0 is the fluorescence intensity of dyes in buffer. (b)The structure of compounds tested.

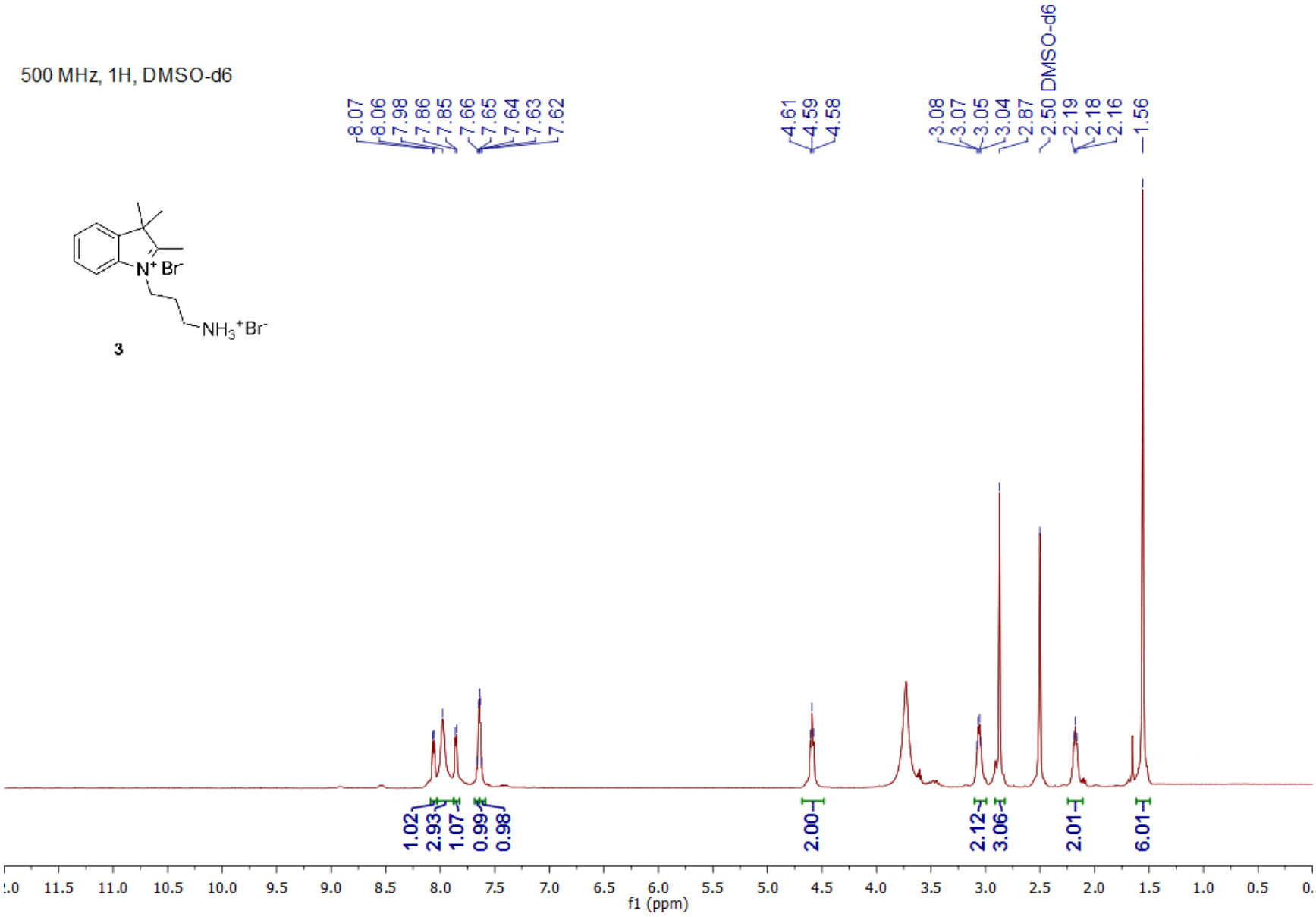
NMR SPECTRA

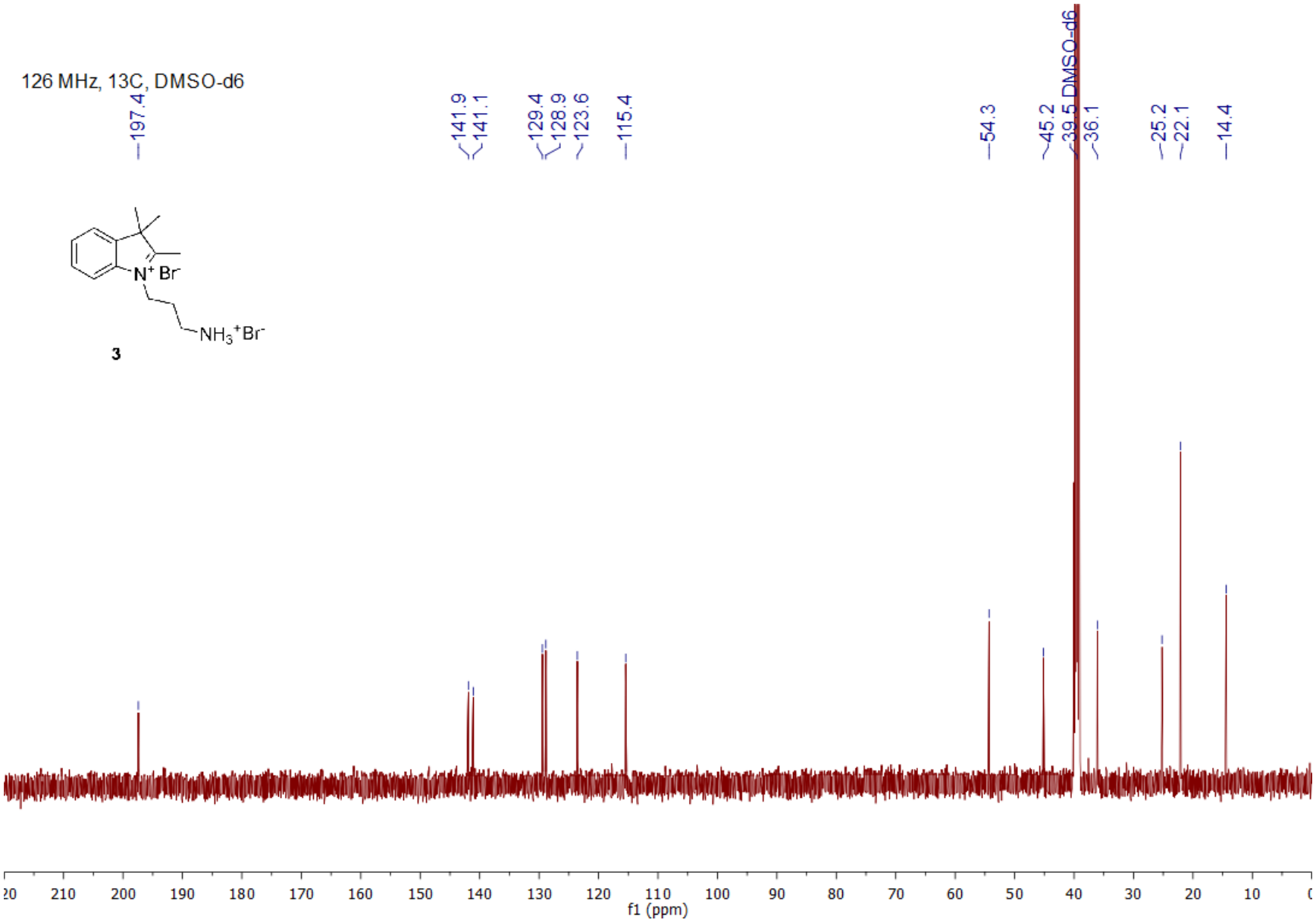

Chloroform-d
7.80 7.78 7.77 7.75 7.74 7.73 7.38 7.38 7.37 7.36 7.35 7.35 7.35 7.34 7.32 7.26 7.23 7.22 7.21 7.21 7.20 7.19 7.10 7.09 7.01 6.99 6.96 6.80 6.43 6.40 6.29 6.27 4.38 4.37 4.06 4.04 4.03 3.99 3.97 3.96 3.33 2.51 2.50 2.48 2.22 2.03 2.02 1.90 1.89 1.88 1.86 1.85 1.83 1.67 1.67
HN
O
N
NH
O
O
CyCM716, 1
2.22 5.21 2.52 2.97 1.15 2.04 1.03 0.95 1.00 2.00 2.09 2.05 2.18 2.02 6.22 2.34 2.28 2.00 6.10 6.04 9.13
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)

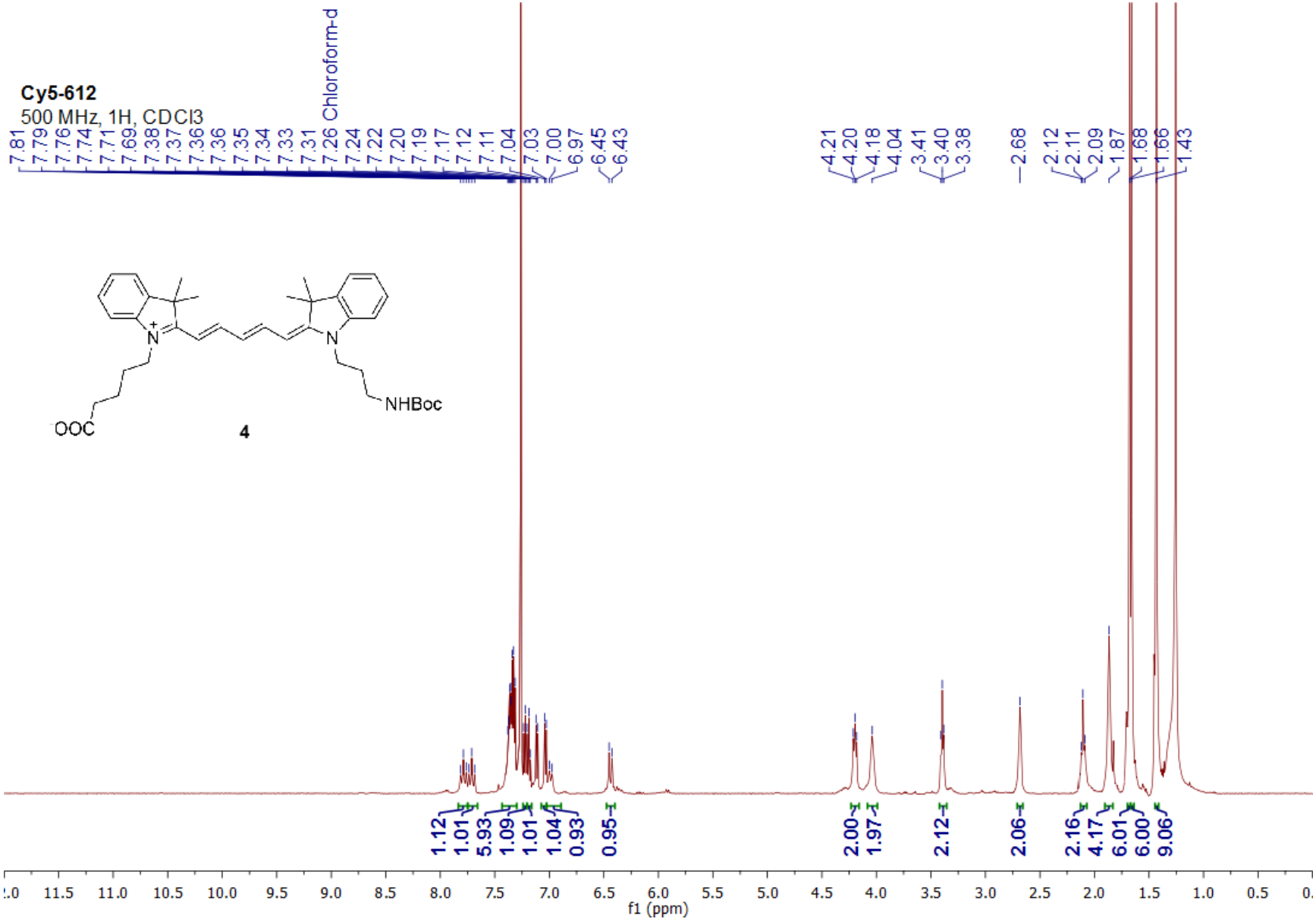

CYC-G9
126 MHz, 1H, CDCl3
173.0
172.3
156.6
152.7
142.2
142.1
141.0
138.0
128.9
128.8
126.0
122.2
110.8
110.7
108.6
104.5
79.3
77.2 Chloroform-d
49.3
49.2
49.2
44.1
43.6
42.4
35.4
28.6
28.2
28.2
27.6
26.9
22.7
21.4
HN
O
NH
O
O
CyCM716, 1
f1 (ppm)

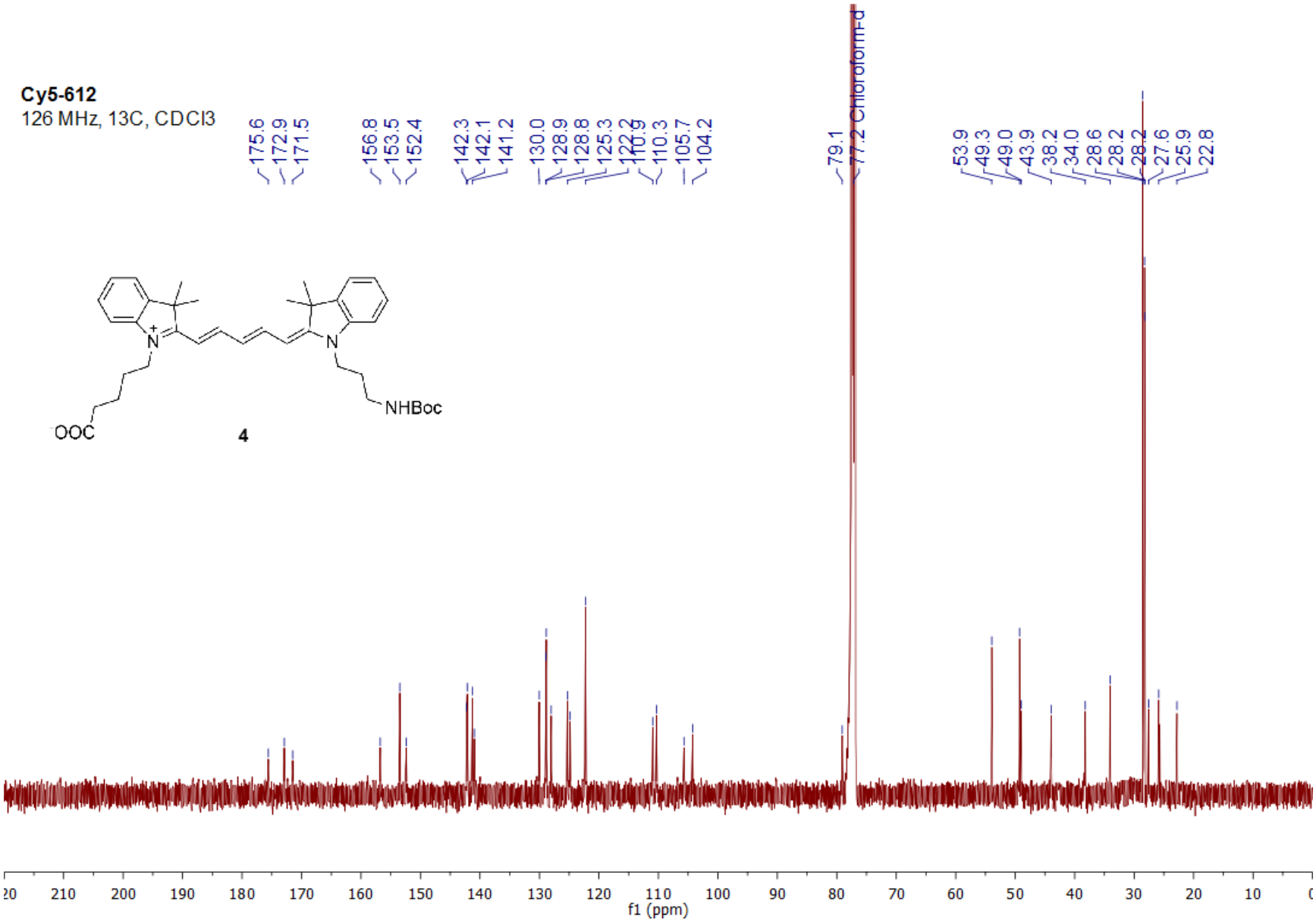

500 MHz, 1H, MeOD
7.93
7.92
7.92
7.91
7.91
7.91
7.90
7.81
7.81
7.80
7.79
7.79
7.79
7.78
7.77
7.67
7.66
7.66
7.65
7.65
7.64
4.59
4.57
4.56
3.31 Methanol-d4
2.45
2.43
2.42
2.04
2.04
2.03
2.03
2.02
2.02
2.01
1.81
1.80
1.78
1.77
2
HO
O
N+
1.08
1.01
1.95
2.00
2.01
2.11
2.18
6.05
f1 (ppm)


500 MHz, 1H, MeOD
198.0
176.5
143.4
142.6
131.2
130.5
124.6
116.6
56.0
49.1
49.0 Methanol-d4
33.9
28.2
22.9
22.8
2
HO
O
N+
f1 (ppm)


500 MHz, 1H, DMSO-d6
8.07
8.06
7.98
7.86
7.85
7.66
7.65
7.64
7.63
7.62
4.61
4.59
4.58
3.08
3.07
3.05
3.04
2.87
2.50 DMSO-d6
2.19
2.18
2.16
1.56
N+ Br-
NH3+Br-
3
1.02
2.93
1.07
0.99
0.98
2.00
2.12
3.06
2.01
6.01
f1 (ppm)


126 MHz, 13C, DMSO-d6
197.4
141.9
141.1
129.4
128.9
123.6
115.4
54.3
45.2
39.5 DMSO-d6
36.1
25.2
22.1
14.4
N+ Br-
NH3+Br-
3
f1 (ppm)


Cy5-612
500 MHz, 1H, CDCl3
Chloroform-d
7.81 7.79 7.76 7.74 7.71 7.69 7.38 7.37 7.36 7.36 7.35 7.34 7.33 7.31 7.26 7.24 7.22 7.20 7.19 7.17 7.12 7.11 7.04 7.03 7.00 6.97 6.45 6.43
4.21 4.20 4.18 4.04 3.41 3.40 3.38 2.68 2.12 2.11 2.09 1.87 1.68 1.66 1.43
OOC
NHBoc
4
1.12 1.01 5.93 1.09 1.01 1.04 0.93 0.95 2.00 1.97 2.12 2.06 2.16 4.17 6.01 6.00 9.06
f1 (ppm)


Cy5-612
126 MHz, 13C, CDCl3
175.6
172.9
171.5
156.8
153.5
152.4
142.3
142.1
141.2
130.0
128.9
128.8
125.3
122.2
110.9
110.3
105.7
104.2
79.1
77.2 Chloroform-d
53.9
49.3
49.0
43.9
38.2
34.0
28.6
28.2
28.2
27.6
25.9
22.8
N
N
NHBoc
OOC
4
f1 (ppm)

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