Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Multicarbazole scaffolds for selective G-quadruplex binding

Arnold Ou,[1] Aurore Guédin,[2] Brian W. Skelton,[1,3] Samir Amrane,[2] Cameron W. Evans,[1] Marck Norret,[1] Iyer K. Swaminathan,[1] Jean-Louis Mergny[2] and Nicole Smith*[1]

1. School of Molecular Sciences, The University of Western Australia, 35 Stirling HW, Crawley-6009
2. *ARNA Laboratory, IECB, Institution Université Bordeaux, INSERM U1212, CNRS UMR 5320, Pessac, France*
3. The Centre for Microscopy, Characterisation and Analysis, The University of Western Australia, 35 Stirling HW, Crawley-6009

All reactions were carried out under argon or nitrogen with Schlenk techniques unless otherwise stated. Glassware was dried in the oven prior to reactions. Chemicals were purchased from Sigma-Aldrich, Merck, Acros, Alfa Aesar or Chem Supply and were used as is, unless otherwise stated. Dimethylformamide was stored over 3 Å molecular sieves and under argon. Nuclear Magnetic Resonance (NMR) were conducted on the Bruker AV400, AV500 or AV600. Multiplicities are abbreviated as the following; singlet (s), doublet (d), triplet (t), multiplet (m), broad (br), doublet of doublets (dd). NMR spectra were calibrated to residue solvent peak.[S1] Deuterated solvents was purchased from Sigma-Aldrich and was stored over 3 Å molecular sieves. 3-(chloropropyl)amines,[S2] 1,3,5-Tris(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzene[S3] and 3-bromocarbazole[S4] were prepared according to literature procedures. High resolution mass spectrometry was conducted on a Waters LCT Premier XE mass spectrometer. Compounds **2a-h** were weighed out into vials and dissolved in dioxane (1-2 mL). Multicarbazole salts were prepared by the dropwise addition of 4M HCl in dioxane (Alfra Aesar) in dioxane solution, until no more precipitate formed. The solvent was then removed in vacuo to afford a powder (off white to brown). **2a-h**•3HCl salts were dissolved in molecular biology grade dimethylsulfoxide (Sigma-Aldrich) to make 10 mM and 100 mM stock solutions and stored in the freezer until required. Compounds were dissolved in water (milliQ) to the appropriate concentration for each reaction.

For Forster resonance energy transfer (FRET), Oligonucleotides were purchased from Eurogentec or Integrated DNA Technologies and resuspended in nuclease free water to make 100 µM stock solutions. Compounds (1, 2a-h) were dissolved in molecular biology grade dimethylsulfoxide (Sigma-Aldrich) to make 10 mM and 100 mM stock solutions. All stock solutions were stored at -20 °C until required. All oligonucleotides were then further diluted in the relevant buffer and pre-annealed prior to conducting FRET assays. Preannealing of the oligonucleotides consisted of heating at 95 °C for 2 min and then cooling on ice .

The FRET assays were performed as a high throughput screen in a 96-well format on a Agilent Stratagene Mx3005P real-time PCR machine. After a first equilibration step at 25 °C for 5 min, a stepwise increase of 1 °C every minute for 71 cycles to reach 96 °C was performed and measurements were made after each “cycle” with excitation at 492 nm and detection at 516 nm. Final analysis of the data was carried out using Excel and KaleidagGraph software. Emission of FAM was normalized between 0 and 1, and apparent Tm was defined as the temperature for which the normalized emission is 0.5. Each well was duplicated and contained a total reaction

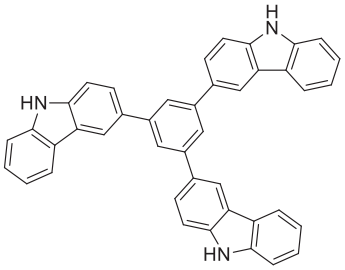
volume of 25 µL, with the labelled oligonucleotide (0.2 mM) in K^+ or Na^+ buffer in the absence or presence of compound (1, 5 and 10 mM). K^+ buffer contained lithium cacodylate (10 mM) at pH 7.2, KCl (10 mM) and LiCl (90 mM) and Na+ buffer contained lithium cacodylate (10 mM) at pH 7.2 and NaCl (100 mM).

FRET competition experiments were conducted in K^+ buffer with 0.2 mM of *F21T* in the presence of compound (5 µM) and ds26 competitor (15 µM or 50 µM).

Circular dichroism (CD) titration was performed on the Jasco J-1500 CD Spectrometer using quartz cells (Hellma Analytics) with a path length of 10 mm. DNA solution (10 mM potassium chloride, 90 mM lithium chloride, 10 mM lithium cacodylate at pH 7.2, and 4 µM 22Ag or 22Kras) was annealed prior to CD titration, then 500 µL was transferred into each cell. The CD spectra was an average of 3 runs, with background (buffer) subtracted. Scan rate was 100 nm/min and temperature set at 20 °C

NMR titrations were performed in a 3 mm NMR tube, at 25 °C, with 250 µL of sample (100 µM 22Kras, 20 mM potassium phosphate pH 6.5 and 70 mM potassium chloride,) in 10% D_2O in H_2O, on the Bruker 600 MHz. The DNA was annealed prior to NMR (95 °C for 2 minutes and then cool on ice). The water signal was suppressed with excitation sculpting. Compound was added in the appropriate amount after each experiment.

Preparation of multicarbazoles



1,3,5-Tri(carbazol-3-yl)benzene (1)

In a Schlenk tube, 1,3,5-tris(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzene (500 mg, 1.1 mmol), 3-bromocarbazole (821 mg, 3.4 mmol), cesium carbonate (3.81 g, 11.7 mmol), degassed dioxane/water (5:1, 25 mL), (dppf)$PdCl_2$ (124 mg, 0.17 mmol) and stir bar was added. The flask was sealed under argon and the suspension was then stirred at 90 °C overnight. The reaction was quenched the next day with water (20 mL), extracted with dichloromethane (3 x 25 mL), dried over anhydrous sodium sulfate and then concentrated. The residue was purified by silica gel column chromatography (hexane:ethyl acetate, 6:4) to afford the desired product as an off-white solid (221 mg, 35%). Crystals suitable for X-ray diffraction were prepared by vapor diffusion (room temperature) of methanol into a concentrated solution of **1** in dimethyl sulfoxide.

^{1}H NMR (500 MHz, dmso-d_6): δ 11.35 (s, 3H), 8.73 (s, 3H), 8.33 (d, *J* = 7.6 Hz, 3H), 8.08 (s, 3H), 7.99 (d, *J* = 8.4 Hz, 3H), 7.64 (d, *J* = 8.3 Hz, 3H), 7.53 (d, *J* = 7.9 Hz, 3H), 7.42 (t, *J* = 7.4 Hz, 3H), 7.20 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (125 MHz, dmso-d_6): δ 142.6, 140.3, 139.4, 131.4, 125.7, 125.1, 123.4, 123.2, 122.7, 120.1, 118.9, 118.6, 111.3, 111.1.

HRMS (ESI+): m/z calculated for $C_{42}H_{28}N_3$ $[M+H]^+$ 574.2283 and found 574.2283.

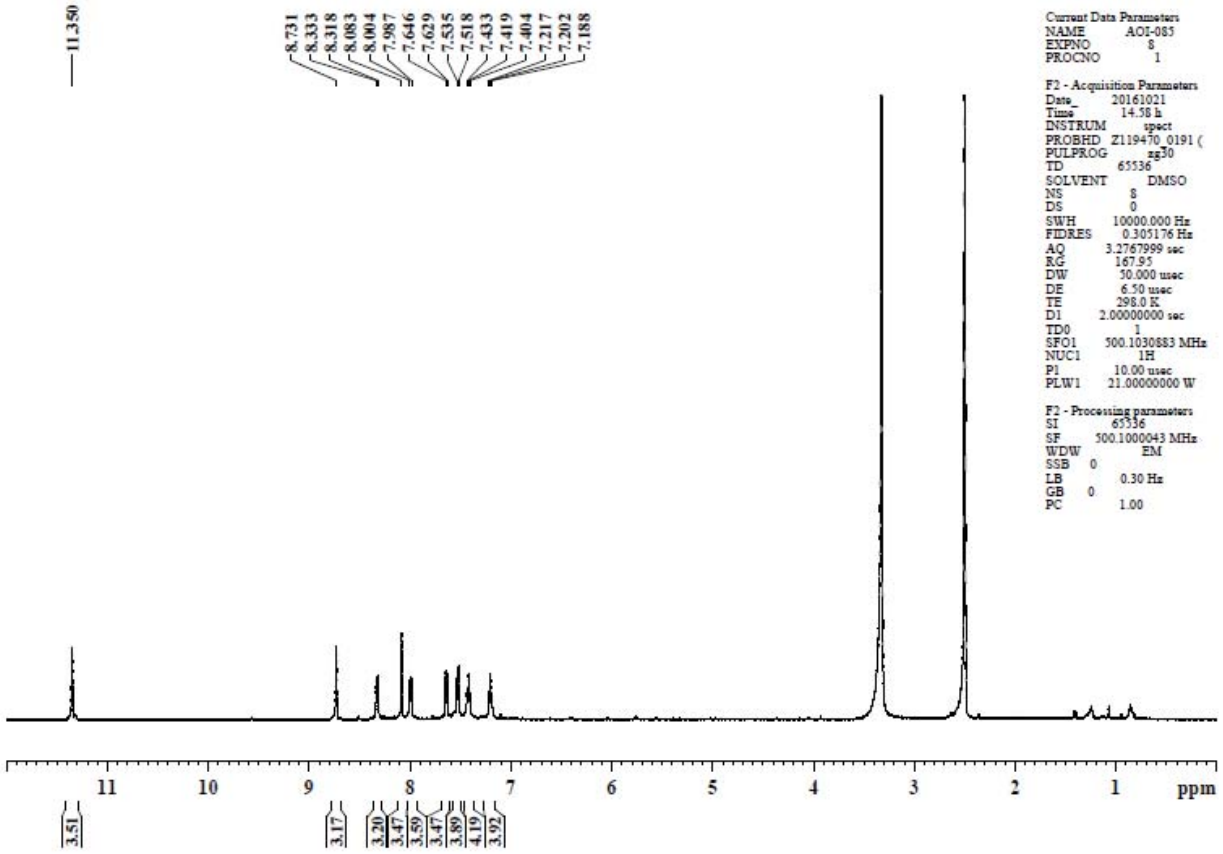


Figure S1: ^{1}HNMR of compound **1**

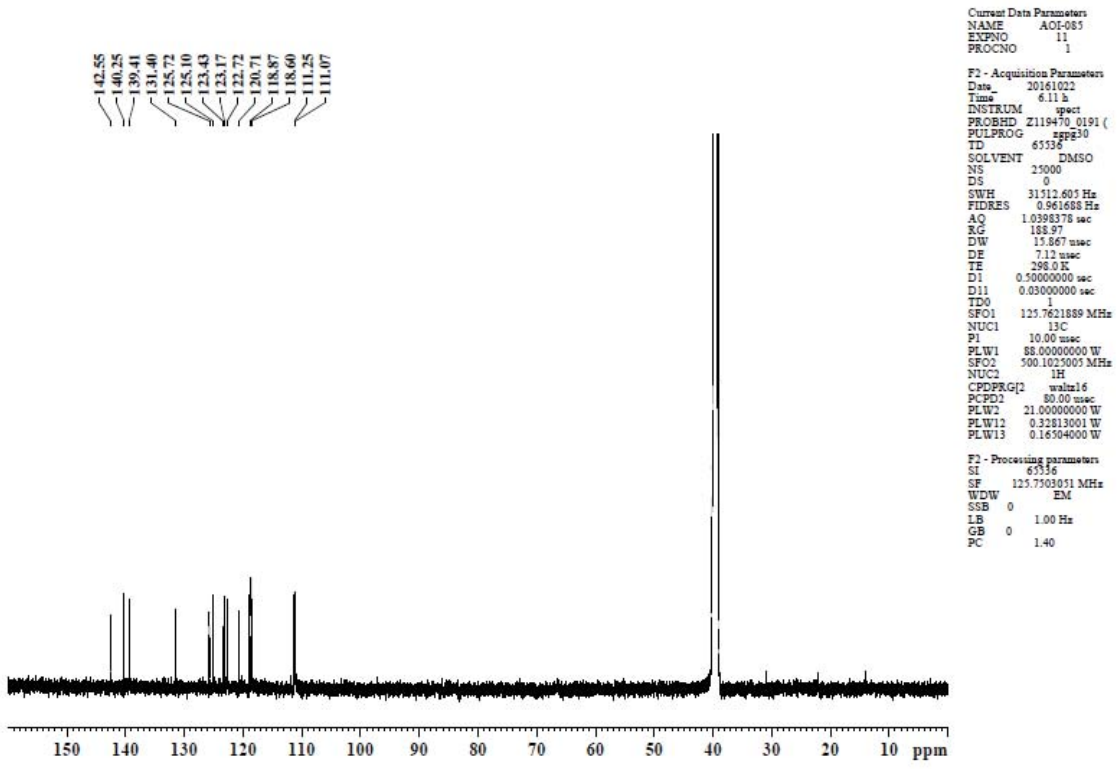


Figure S2: ^{13}CNMR of compound **1**

Crystallography

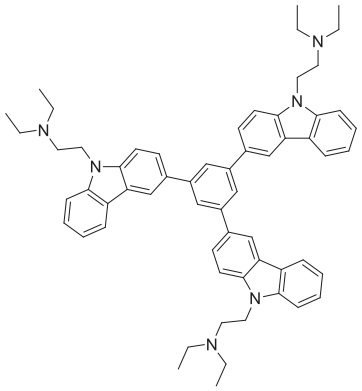
The crystal data for **1** are summarized in Table S1 with the structure depicted in Scheme 1, where ellipsoids have been drawn at the 50% probability level. Crystallographic data for the structure were collected at 100(2) K on an Oxford Diffraction Gemini diffractometer using Cu Kα radiation. Following multi-scan absorption corrections and solution by direct methods, the structure was refined against F^2 with full-matrix least-squares using the program SHELXL-2014[S5.] The solvent was modelled as a methanol molecule disordered about a $\bar{3}$ centre. Methanol hydrogen atoms were located from residual electron density. All remaining hydrogen atoms were added at calculated positions and refined by use of riding models with isotropic displacement parameters based on those of the parent atoms. Anisotropic displacement parameters were employed throughout for the non-hydrogen atoms. The CCDC deposit (CCDC No: 1838265) contains supplementary crystallographic data, and can be obtained free of charge *via* http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge

Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K.; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk.

Table S1. Crystal data and structure refinement for **1**.

Empirical formula	$C_{42.50}H_{29}N_3O_{0.50}$
Formula weight	589.69
Temperature	100(2) K
Wavelength	1.54184 Å
Crystal system	Trigonal
Space group	$R\bar{3}$
Unit cell dimensions	a = 21.9820(5) Å
	b = 21.9820(5) Å
	c = 10.3781(3) Å
Volume	4342.9(2) $Å^3$
Z	6
Density (calculated)	1.353 Mg/m^3
μ	0.623 mm^{-1}
Crystal size	0.146 x 0.094 x 0.042 mm^3
θ range for data collection	4.022 to 67.341°.
Index ranges	-25<=h<=16, -18<=k<=26, -12<=l<=5
Reflections collected	3874
Independent reflections	1719 [R(int) = 0.0285]
Completeness to θ = 67.341°	99.0 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	1.000/ 0.9355
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	1719 / 13 / 156
Goodness-of-fit on F^2	1.074
Final R indices [I>2σ(I)]	R1 = 0.0402, wR2 = 0.1053
R indices (all data)	R1 = 0.0506, wR2 = 0.1116
Largest diff. peak and hole	0.186 and -0.168 $e.Å^{-3}$

Procedure for N-alkylation of multicarbazoles.



2,2',2''-(3,3',3''-(benzene-1,3,5-triyl)tris(9H-carbazole-9,3-diyl))tris(N,N-diethylethanamine) (2a)

In a 5 mL round bottom flask containing **1** (20 mg, 0.035 mmol) and dmf (1 mL), sodium hydride (15 mg, 0.35 mmol) was added at 0 °C. The yellow suspension was stirred for 10 minutes under argon, prior to the addition of 2-(chloroethyl)diethylamine (20 mg, 0.12 mmol). The flask was then heated at 95 °C under an atmosphere of argon, overnight. The next day, the reaction was checked with thin layer chromatography and if the reaction has not completed, sodium hydride was added and stirred until completion. Once completed, the reaction is filtered through Celite/cotton and the solvent was removed. The residue was imbedded on a small silica plug with dichloromethane (2 mL), and washed with mL hexanes/ethyl acetate (3 x 25 mL, 6:4). The product was then eluted with chloroform:methanol:$NH_4OH_{(aq)}$ (65:30:5) and the solvent was removed to give the desired product as an off-white waxy solid (18 mg, 59%).

^{1}H NMR (500 MHz, dcm-d_2): 8.56 (d, *J* = 1.5 Hz, 3H), 8.22 (d, *J* = 7.8, 3H), 8.08 (s, 3H), 7.99-7.97 (m, 3H), 7.63 (d, J = 8.5 Hz, 3H), 7.52-7.51 (m, 6H), 7.29-7.26 (m, 3H), 4.50 (t, *J* = 7.1 Hz, 6H), 2.94-2.91 (m, 6H), 2.70-2.66 (m, 12H), 1.05 (t, *J* = 7.1 Hz, 18H).

^{13}C NMR (125 MHz, dcm-d_2): 143.5, 141.4, 140.5, 132.9, 126.3, 125.8, 124.8, 123.8, 123.4, 120.8, 119.44, 119.36, 109.5, 109.4, 51.5, 48.0, 42.7, 12.1.

HRMS (ESI+): m/z calculated for $C_{60}H_{67}N_7$ $[M+H]^+$ 871.5427 and found 871.5396.

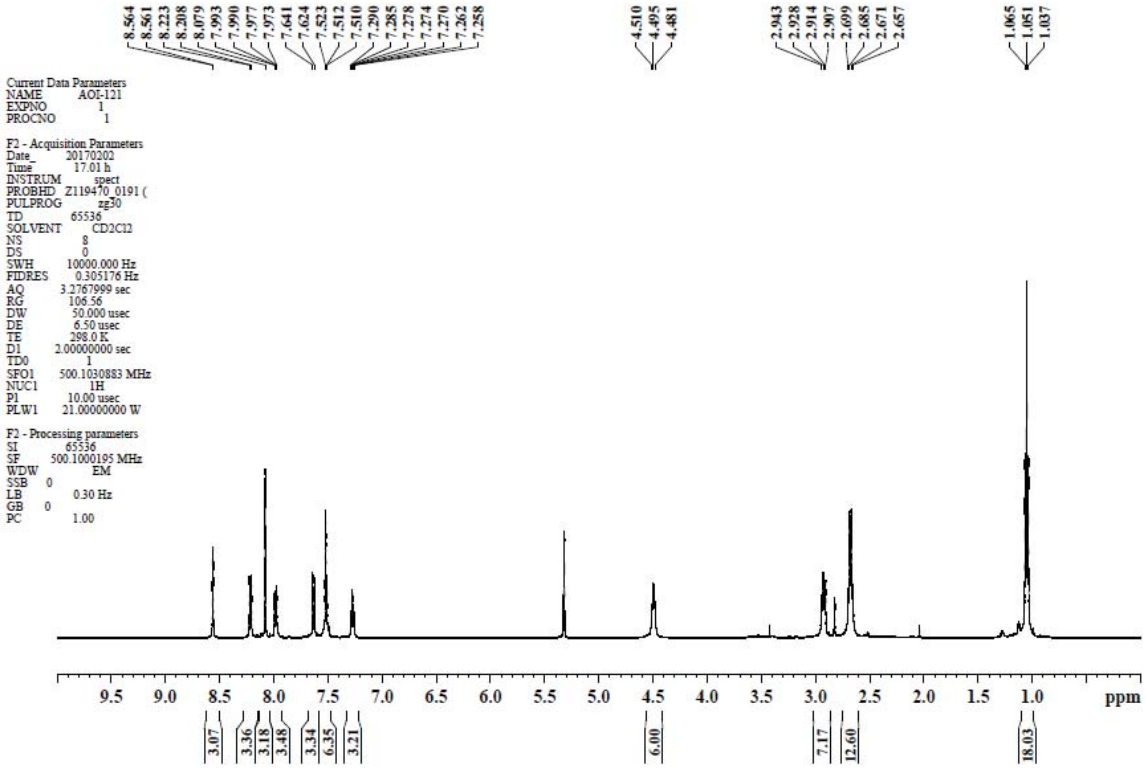


Figure S3: ^{1}HNMR of compound **2a**

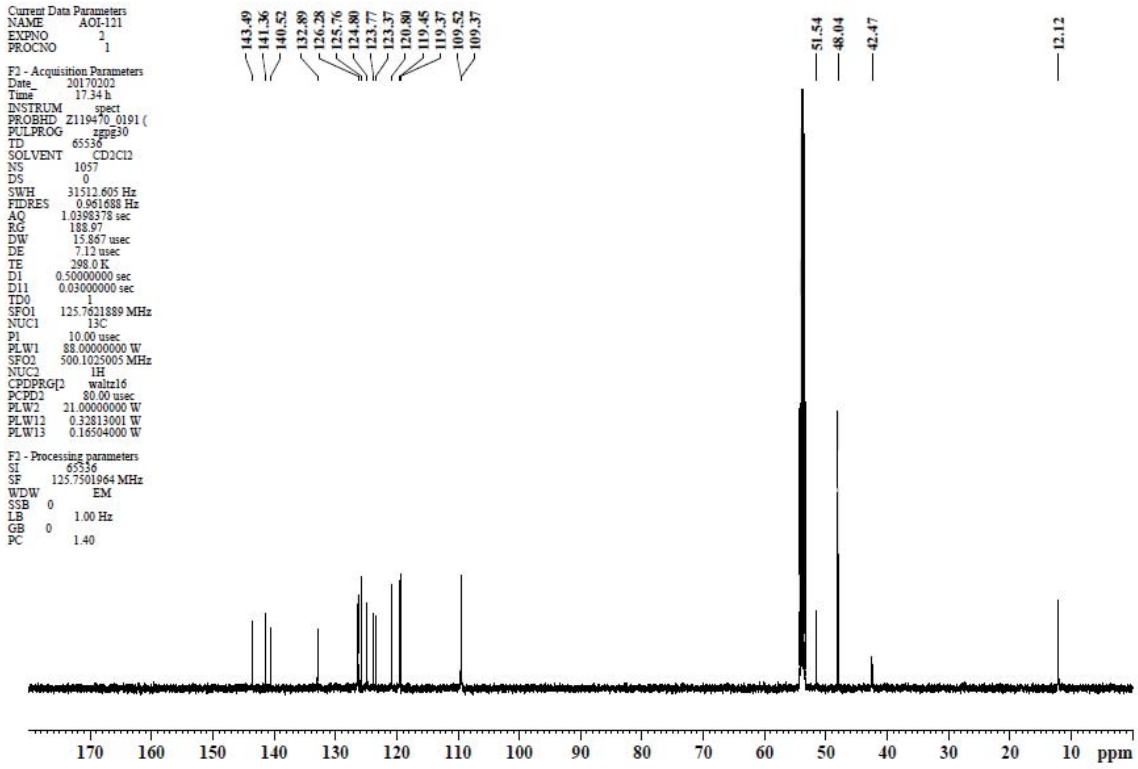
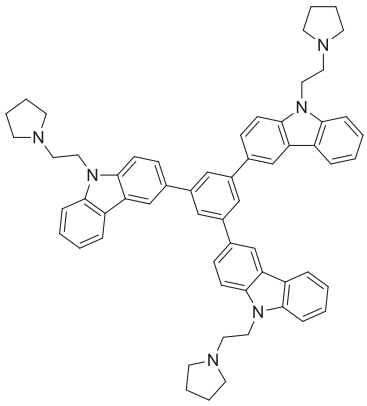


Figure S4: ^{13}CNMR of compound **2a**



1,3,5-tris(9-(2-(pyrrolidin-1-yl)ethyl)-9H-carbazol-3-yl)benzene (2b)

2b was prepared following the procedure of **2a**. Off-white solid (23 mg, 76%).

^{1}H NMR (500 MHz, dcm-d_2): 8.56 (d, *J* = 1.2 Hz, 3H), 8.21 (d, *J* = 7.7 Hz, 3H), 8.07 (s, 3H), 7.99-9.77 (m, 3H), 7.64 (d, *J* = 8.5 Hz, 3H), 7.54-7.51 (m, 6H), 7.29-7.26 (m, 3H), 4.56 (t, *J* = 7.4 Hz, 6H), 3.00 (t, *J* = 7.4 Hz, 6H), 2.69 (br, 12H), 1.83-1.80 (m, 12H).

^{13}C NMR (125 MHz, dcm-d_2): 143.5, 141.3, 140.5, 133.0, 126.3, 125.8, 124.8, 123.8, 123.4, 120.8, 119.5, 119.4, 109.5, 109.3, 54.8, 54.5, 42.8, 24.0.

HRMS (ESI+): m/z calculated for $C_{60}H_{61}N_6$ $[M+H]^+$ 865.4956 and found 865.4919.

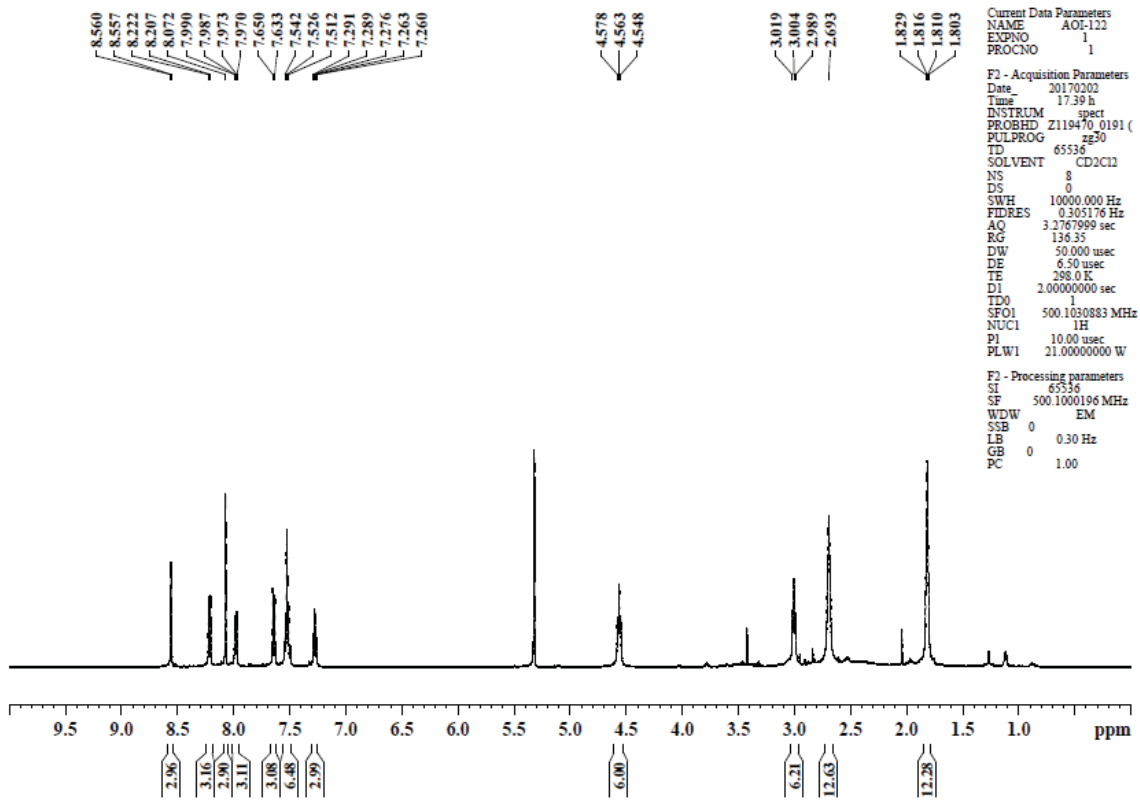


Figure S5: ^{1}HNMR of compound **2b**

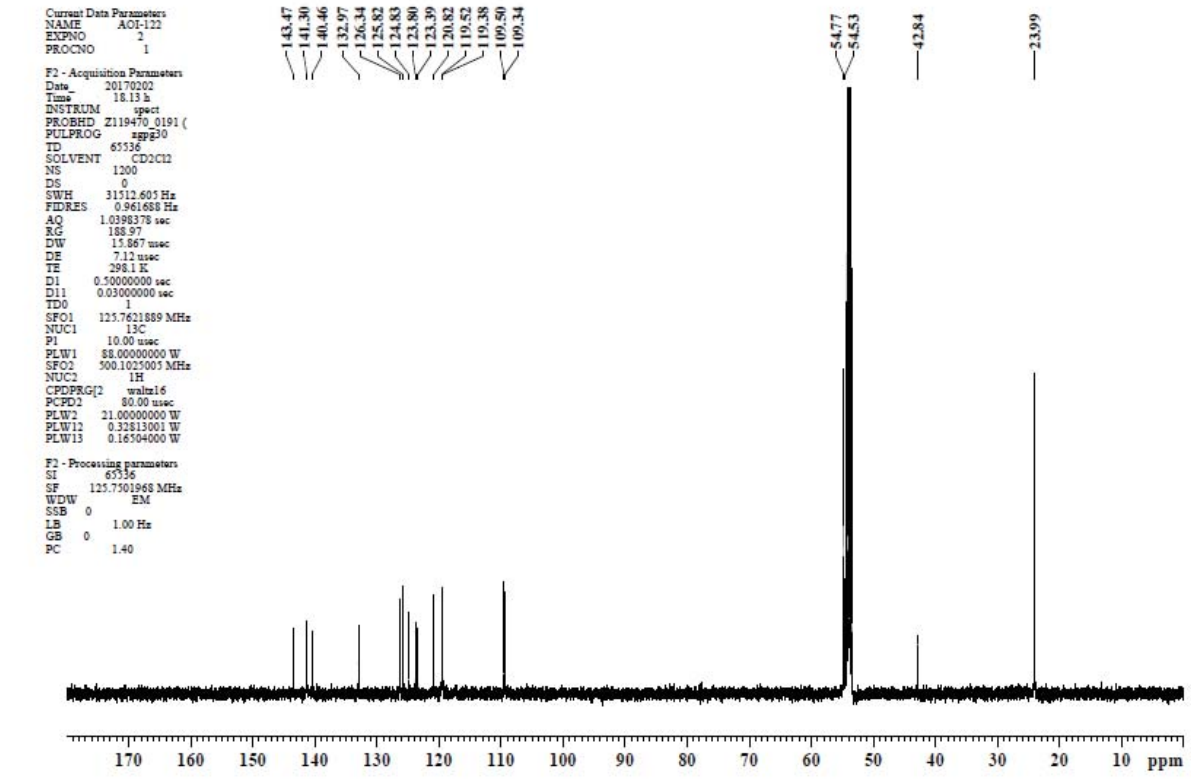
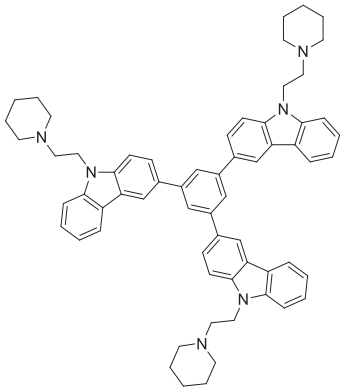


Figure S6: ^{13}CNMR of compound **2b**



1,3,5-tris(9-(2-(piperidin-1-yl)ethyl)-9H-carbazol-3-yl)benzene (2c)

2c was prepared following the procedure of **2a**. Off-white solid (26 mg, 50%).

^{1}H NMR (500 MHz, dcm-d_2): 8.55 (d, *J* = 1.5 Hz, 3H), 8.21 (d, *J* = 7.8 Hz, 3H), 8.07 (s, 3H), 7.98-7.96 (m, 3H), 7.62 (d, *J* = 8.5, 3H), 7.53-7.4 (m, 6H), 7.28-7.25 (m, 3H), 4.52 (t, *J* = 7.1 Hz, 6H), 2.79 (t, *J* = 7.2 Hz, 6H), 2.56 (br, 12H), 1.63-1.59 (br, 12H), 1.47-1.46 (m, 6H).

^{13}C NMR (125 MHz, dcm-d_2): 143.5, 141.4, 140.5, 132.9, 126.3, 125.8, 124.8, 123.8, 123.4, 120.8, 119.4, 119.3, 109.5, 109.4, 57.4, 55.4, 41.6, 26.4, 24.6.

HRMS (ESI+): m/z calculated for $C_{63}H_{67}N_6$ $[M+H]^+$ 907.5427 and found 907.5447.

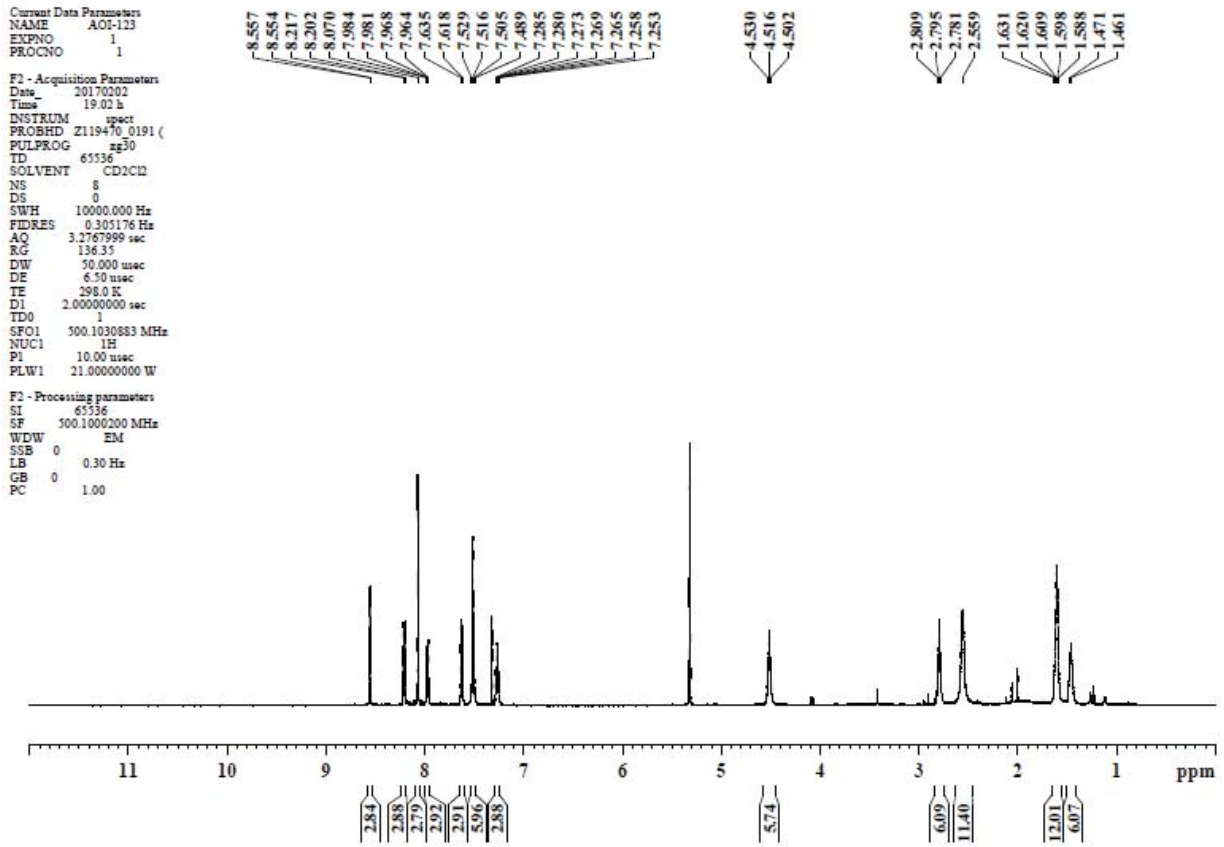


Figure S7: ^{1}HNMR of compound **2c**

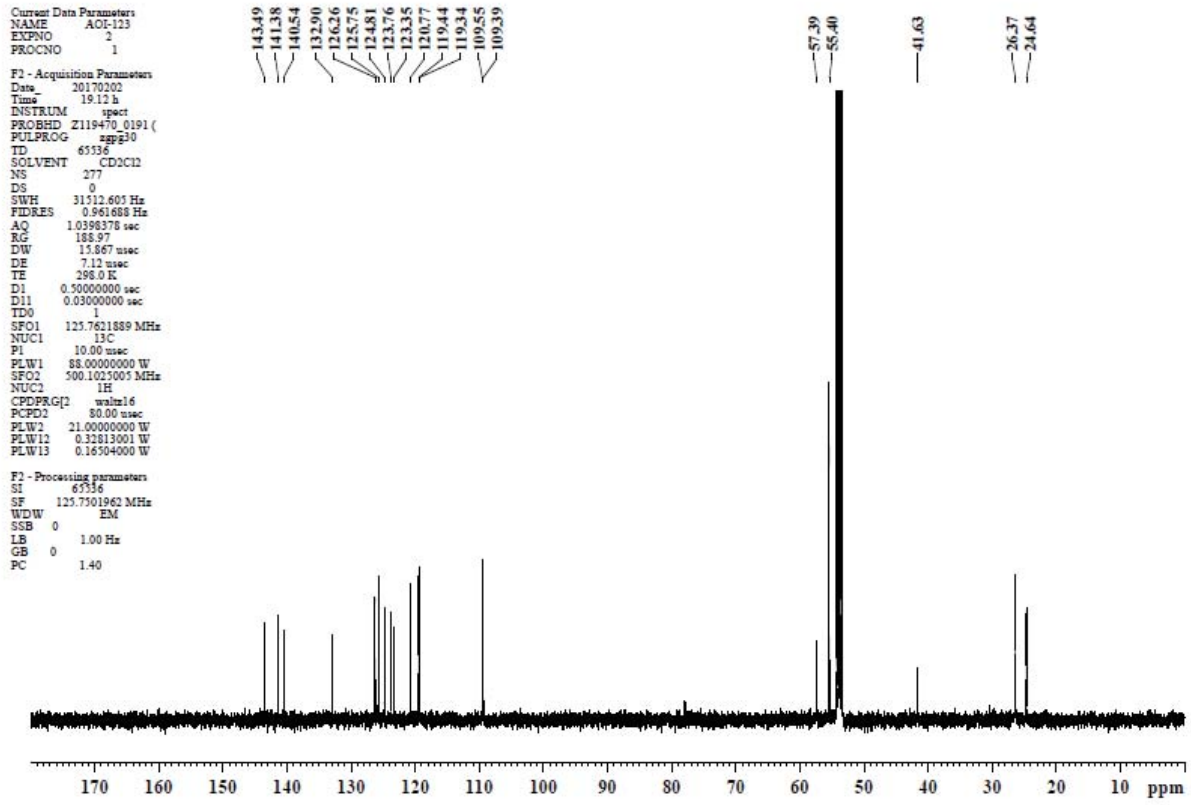
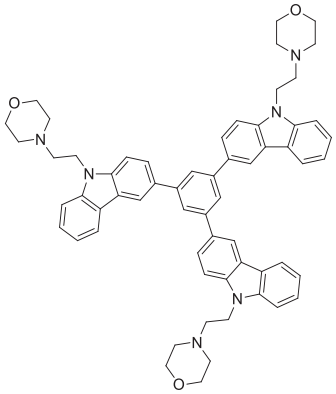


Figure S8: ^{13}CNMR of compound **2c**



1,3,5-tris(9-(2-morpholinoethyl)-9H-carbazol-3-yl)benzene (2d)

2d was prepared following the procedure of **2a**. Off-white solid (22 mg, 69%).

^{1}H NMR (500 MHz, dcm-d_2): 8.56 (s, 3H), 8.21 (d, *J* = 7.7 Hz, 3H), 8.08 (s, 3H), 7.97 (d, *J* = 7.2 Hz, 3H), 7.60 (d, *J* = 8.4 Hz, 3H), 7.51-7.49 (m, 6H), 7.29-7.27 (m, 3H), 4.50 (t, *J* = 7.1 Hz, 6H), 3.67 (t, *J* = 4.4 Hz, 18H), 2.83 (t, *J* = 7.1 Hz, 6H), 2.56 (br, 12H).

^{13}C NMR (125 MHz, dcm-d_2): 143.5, 141.3, 140.5, 132.9, 126.3, 127.8, 124.8, 123.8, 123.4, 120.8, 119.5, 119.4, 109.5, 109.3, 67.3, 57.2, 54.5, 41.5.

HRMS (ESI+): m/z calculated for $C_{60}H_{61}N_6O_3$ $[M+H]^+$ 913.4805 and found 913.4863

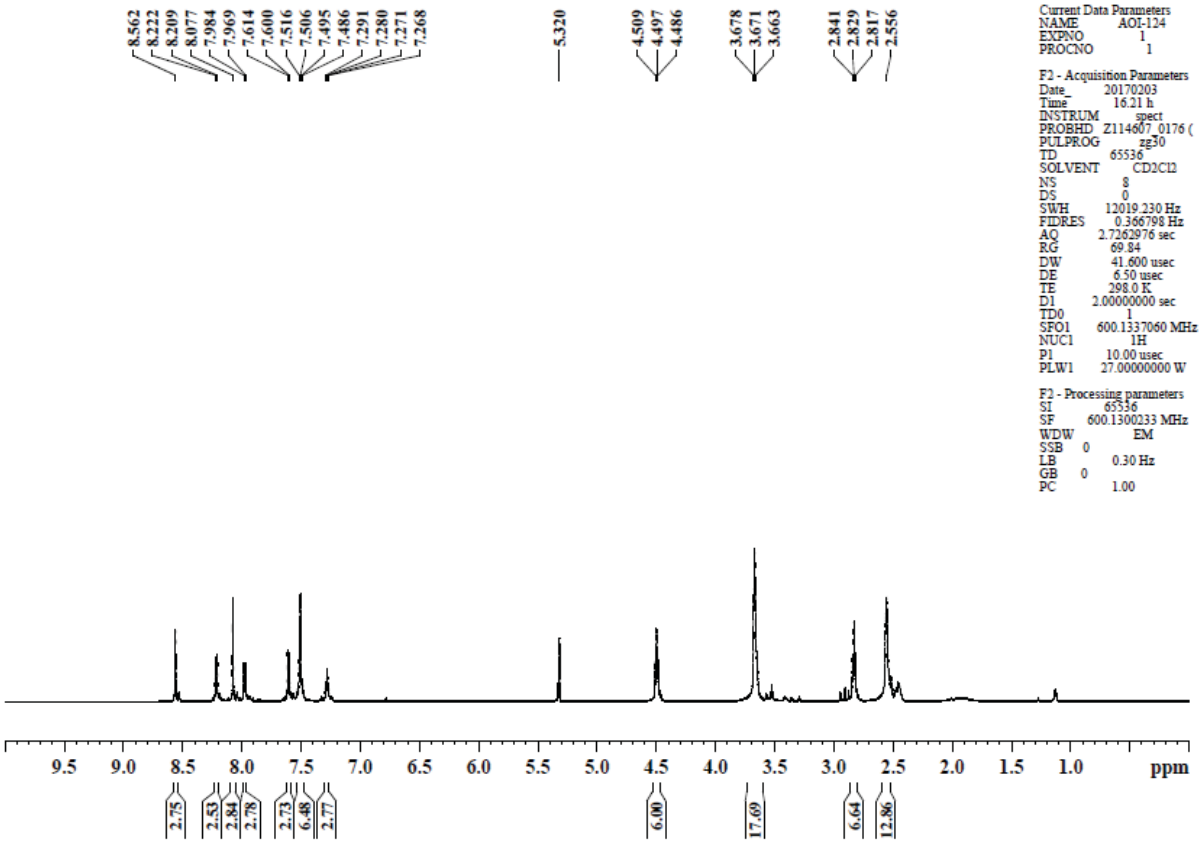


Figure S9: ^{1}HNMR of compound **2d**

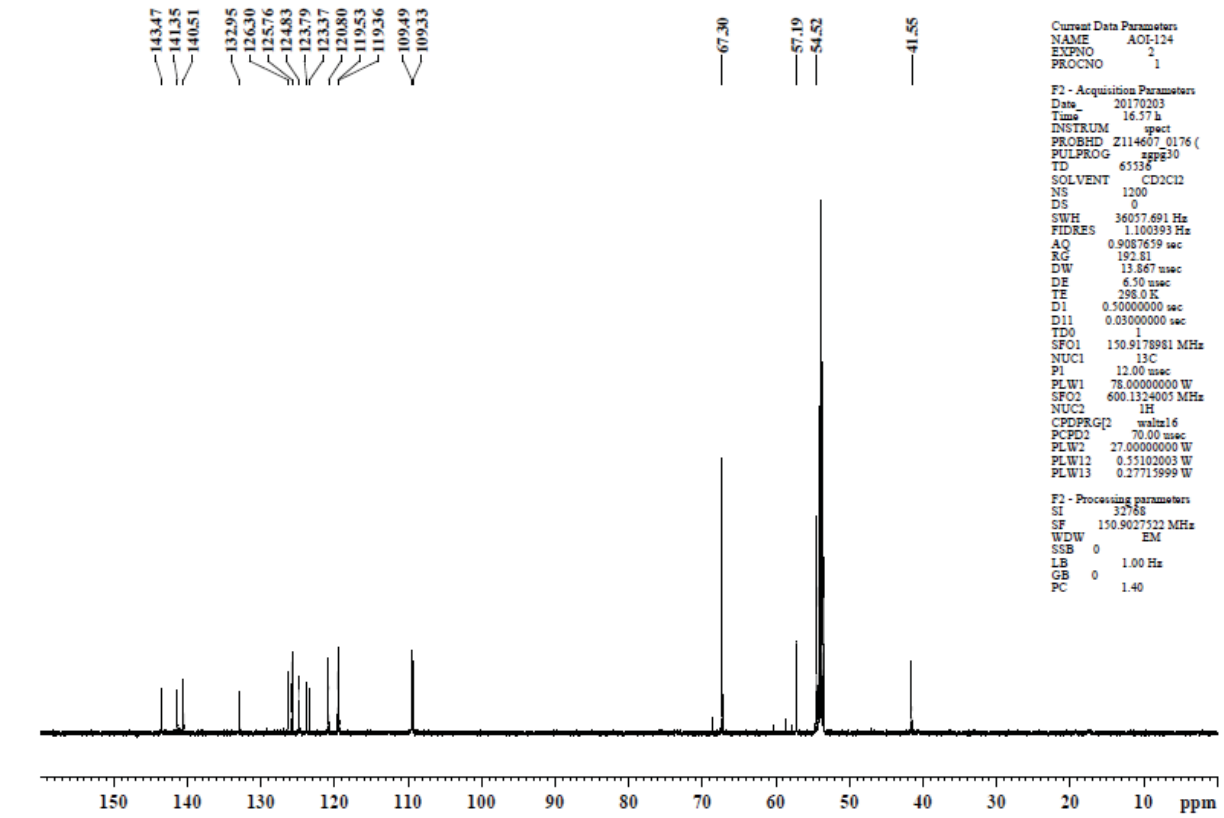
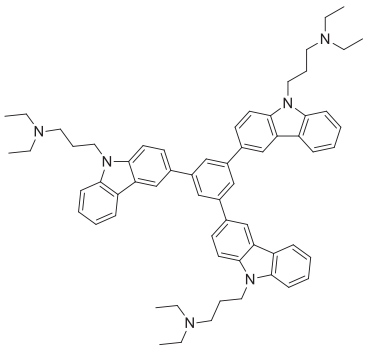


Figure S10: ^{13}CNMR of compound **2d**



3,3',3''-(3,3',3''-(benzene-1,3,5-triyl)tris(9H-carbazole-9,3-diyl))tris(N,N-diethylpropan-1-amine) (2e)

2e was prepared following the procedure of **2a**. Obtained as an off-white solid after purification (21 mg, 66%)

^{1}H NMR (500 MHz, dcm-d_2): 8.56 (d, *J* = 1.4 Hz, 3H), 8.22 (d, *J* = 7.7 Hz, 3H), 8.08 (s, 3H), 7.99-7.97 (m, 3H), 7.65 (d, *J* = 8.5 Hz, 3H), 7.55-7.49 (m, 6H), 7.29-7.26 (m, 3H), 4.74 (t, *J* = 7.1, 6H), 2.64-2.58 (m, 18H), 2.14 (m, 6H), 1.06 (t, *J* = 7.2 Hz, 18H).

^{13}C NMR (125 MHz, dcm-d_2): 143.5, 141.4, 140.5, 132.8, 126.3, 125.7, 124.8, 123.8, 123.4, 120.8, 119.4, 119.3, 109.6, 109.5, 50.5, 46.9, 41.5, 26.5, 11.3.

HRMS (ESI+): m/z calculated for $C_{63}H_{73}N_6$ $[M+H]^+$ 913.5897 and 913.5887

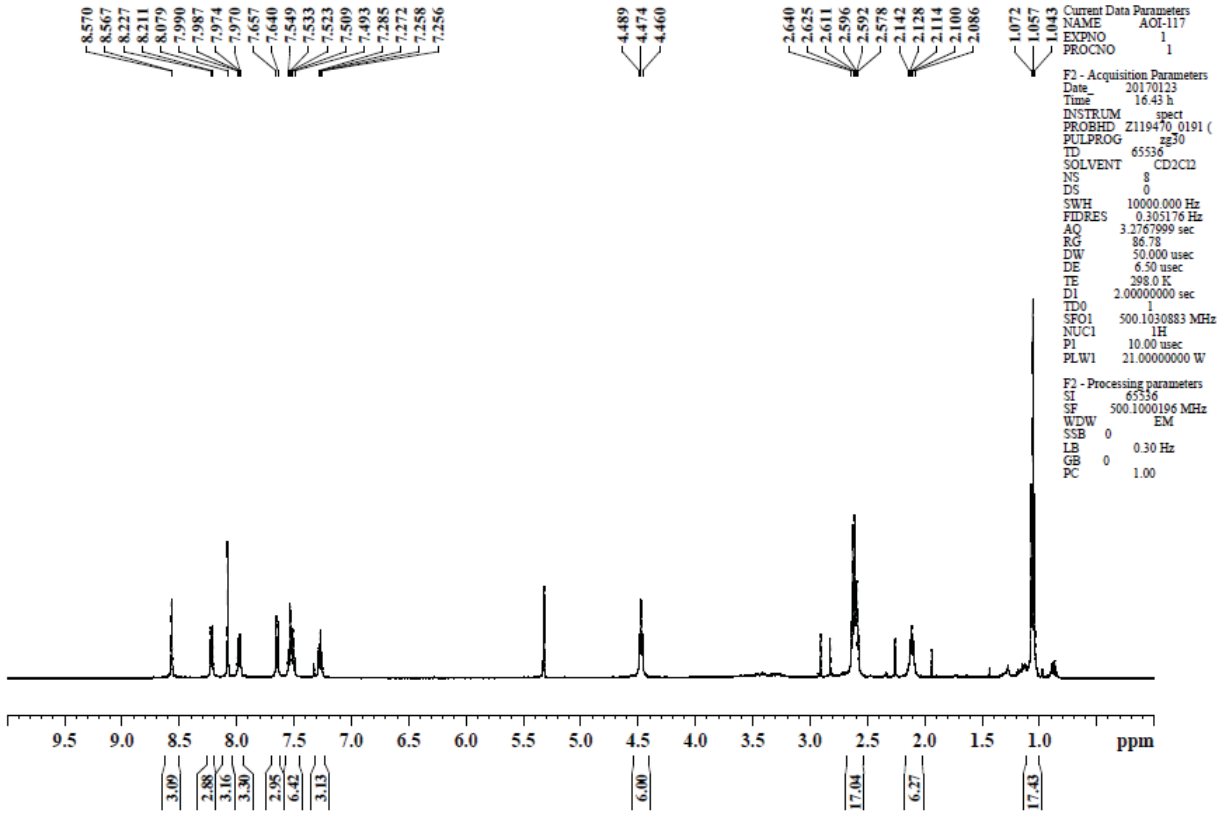


Figure S11: ^{1}HNMR of compound **2e**

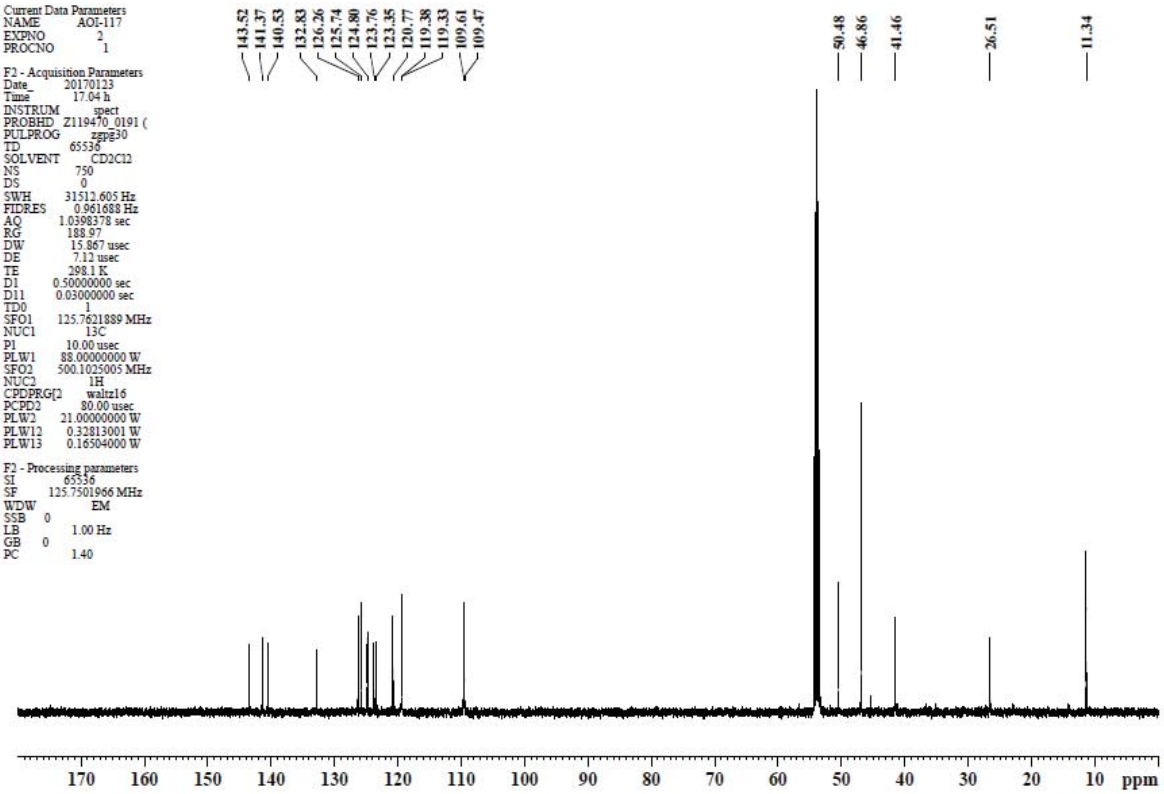
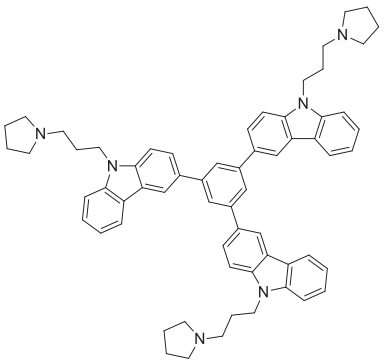


Figure S12: ^{13}CNMR of compound **2e**



1,3,5-tris(9-(3-(pyrrolidin-1-yl)propyl)-9H-carbazol-3-yl)benzene (2f)

2f was prepared following the procedure of **2a.** Off white solid (15 mg, 47%)

^{1}**H NMR** (500 MHz, dcm-d2): 8.56 (d, J = 1.4 Hz, 3H), 8.21 (d, J = 7.7 Hz, 3H), 8.07 (s, 3H), 7.97 (dd, J = 8.4Hz, 1.6 Hz, 3H), 7.67 (d, J = 8.5 Hz, 3H), 7.55 (d, J = 8.2 Hz, 3H), 7.51-7.49 (m, 3H), 7.29-7.25 (m, 3H), 4.51 (t, J = 6.5 Hz, 6H), 2.60-2.55 (m, 18H), 2.18-2.16 (m, 6H), 1.86-1.81 (br, 12H).

^{13}**C NMR** (125 MHz, dcm-d2): 143.5, 141.5, 140.7, 132.8, 126.3, 125.8, 124.8, 123.7, 123.3, 120.7, 119.4, 119.3, 109.7, 109.5, 67.4, 41.2, 27.4, 23.9.

HRMS (ESI+): m/z calculated for $C_{66}H_{67}N_6$ $[M+H]^+$ 907.5427 and found 907.5408.

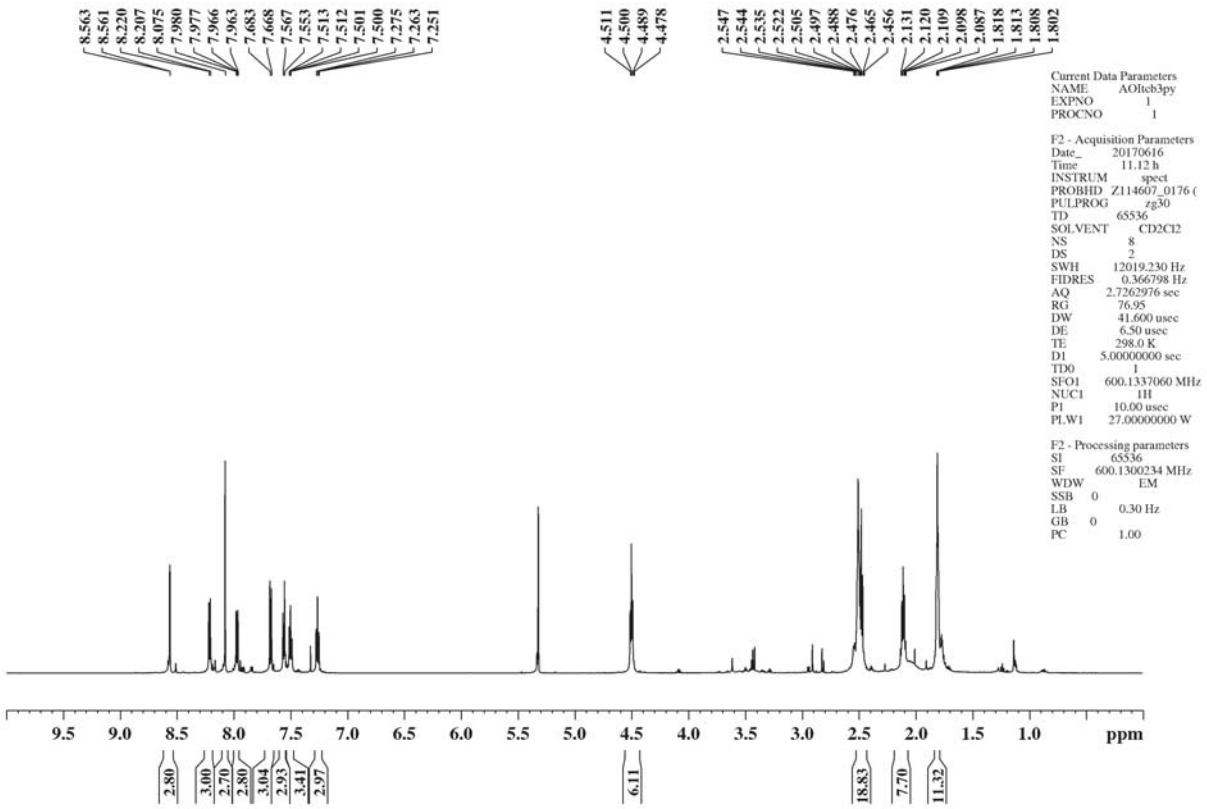


Figure S13: ^{1}HNMR of compound **2f**

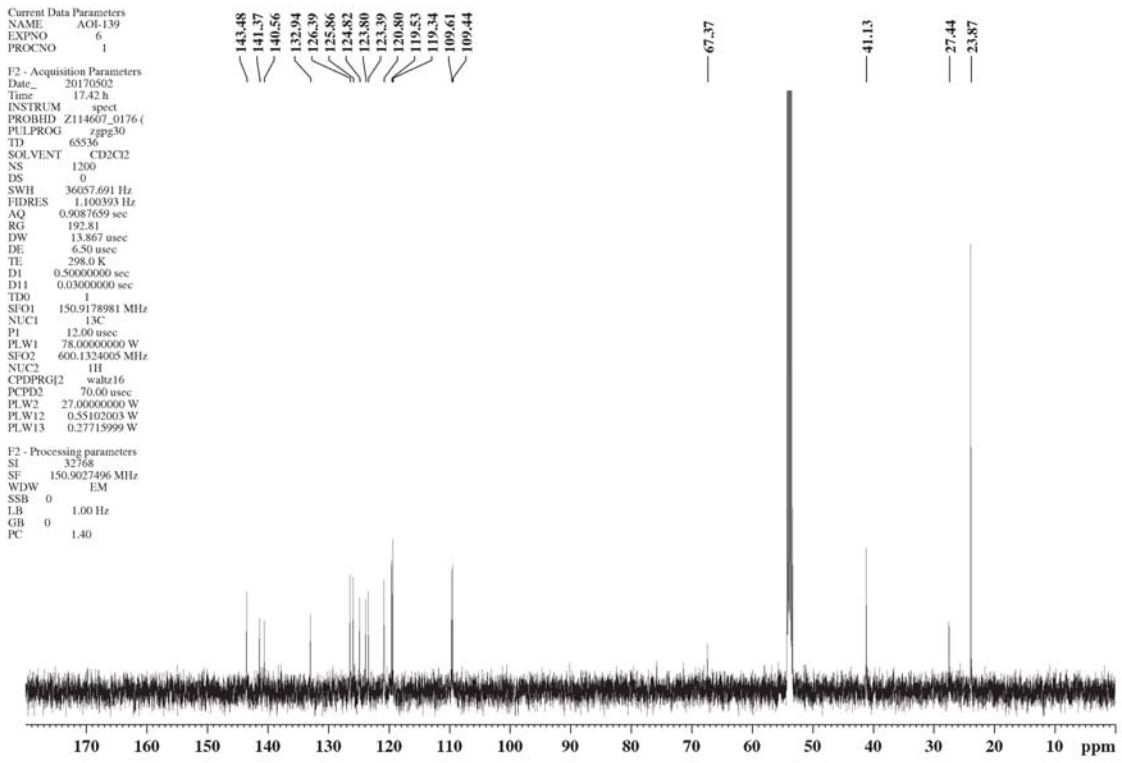
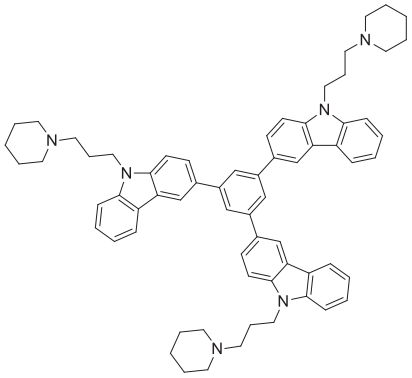


Figure S14: ^{13}CNMR of compound **2f**



1,3,5-tris(9-(3-(piperidin-1-yl)propyl)-9H-carbazol-3-yl)benzene (2g)

2g was prepared following the procedure of **2a**. Isolated as off-white solid (10 mg, 30%).

^{1}H NMR (500 MHz, dcm-d2): 8.56-8.55 (m, 3H), 8.21 (d, *J* = 7.7 Hz, 3H), 8.07 (s, 3H), 7.98-7.96 (m, 3H), 7.68 (d, *J* = 8.5 Hz, 3H), 7.57 (d, *J* = 8.3 Hz, 3H), 7.52-7.48 (m, 3H), 7.28-7.25 (m, 3H), 4.49 (t, *J* = 6.6 Hz, 6H), 5.53-2.31 (m, 14H), 1.67 (t, *J* = 5.4, 12H), 1,48 (br, 6H).

^{13}C NMR (125 MHz, dcm-d2): 143.6, 141.5, 140.7, 132.8, 126.2, 125.7, 124.8, 123.7, 123.4, 120.7, 119.4, 119.3, 109.8, 109.6, 55.9, 54.7, 41.1, 26.2, 24.7.

HRMS (ESI+): m/z calculated for $C_{66}H_{73}N_6$ $[M+H]^+$ 949.5897 and found 949.5883.

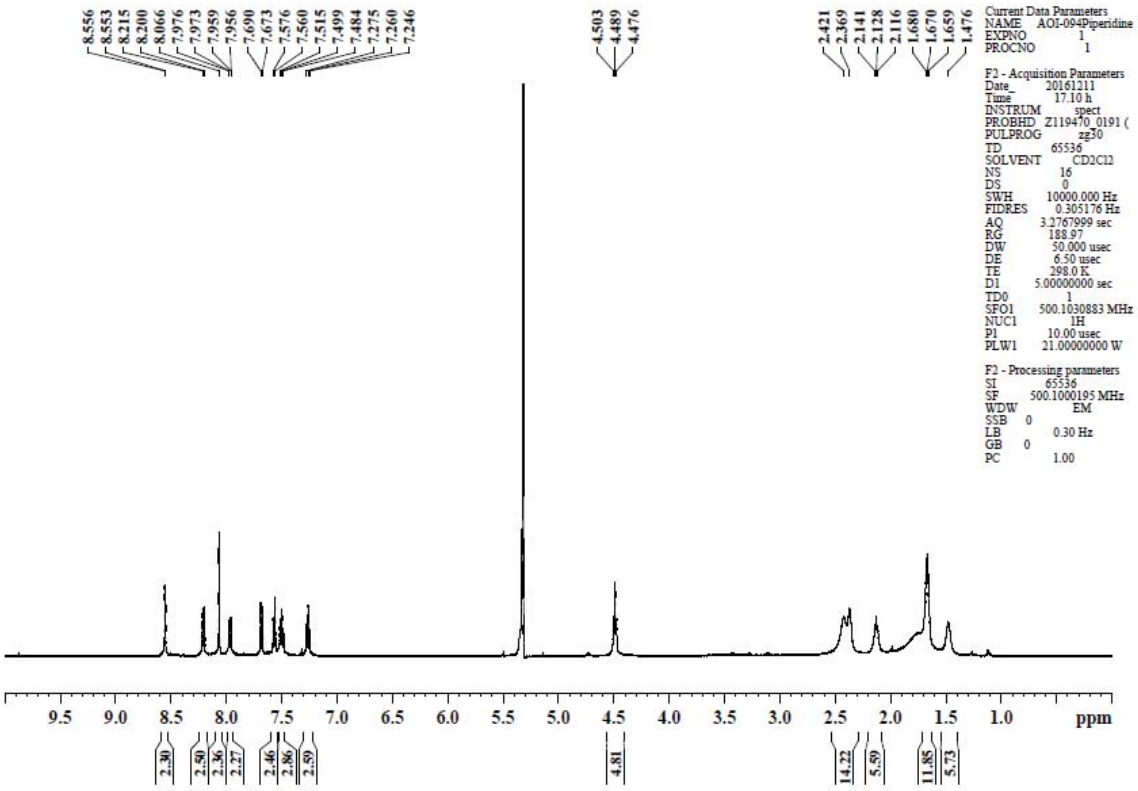


Figure S15: ^{1}HNMR of compound **2g**

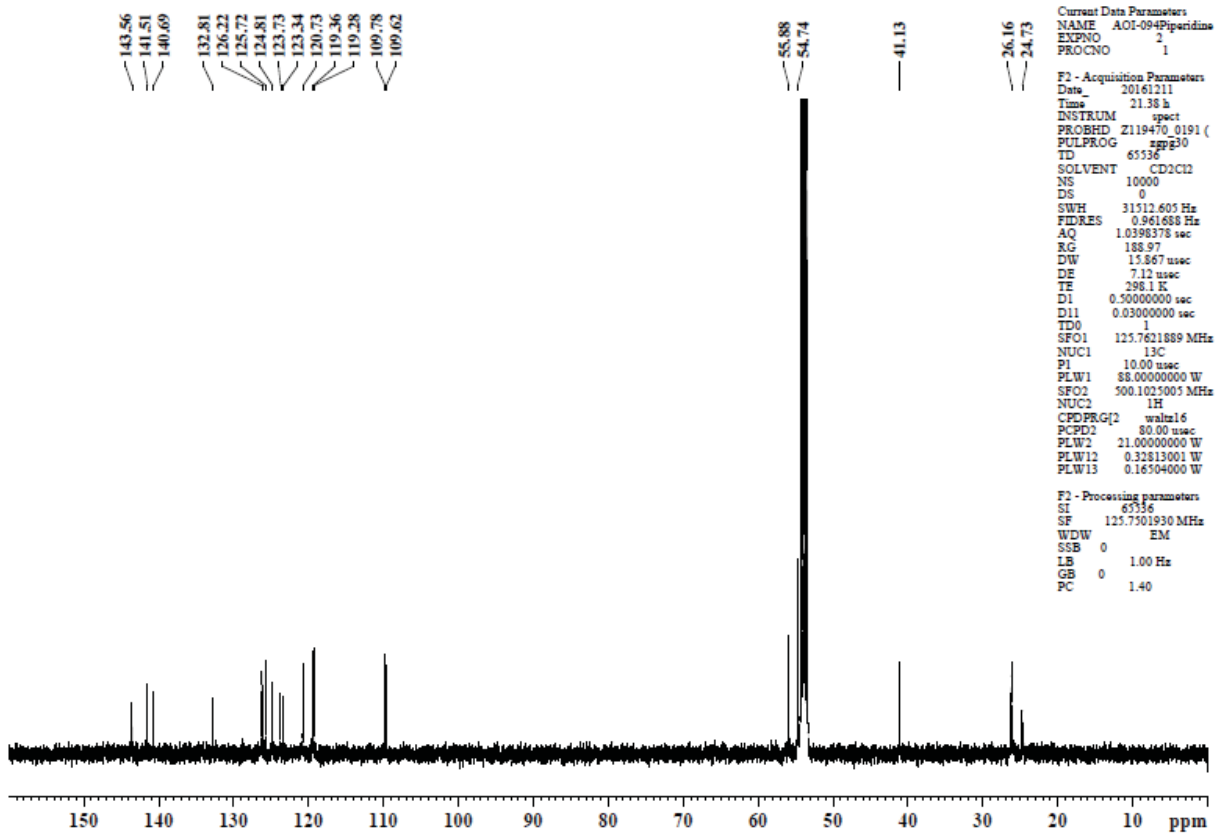
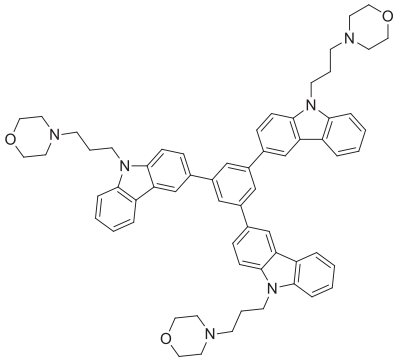


Figure S16: ^{13}CNMR of compound **2g**



1,3,5-tris(9-(3-morpholinopropyl)-9H-carbazol-3-yl)benzene (2h)

2h was prepared following the procedure of **2a.** Off white solid (23 mg, 80%)

^{1}**H NMR** (500 MHz, dcm-d2): 8.57 (d, *J* = 1.5 Hz, 3H), 8.22 (d, *J* = 7.6 Hz, 3H), 8.08 (s, 3H), 7.97 (dd, *J* = 8.5 Hz, 1.8 Hz, 3H), 7.68 (d, *J* = 8.5 Hz, 3H), 7.57 (d, *J* = 8.2 Hz, 3H), 7.52-7.48 (m, 3H), 7.29-7.25 (m, 3H), 4.50 (t, *J* = 6.6 Hz, 6H), 3.74 (t, *J* = 9.2 Hz, 12H), 2.41 (br, 12H), 2.34 (t, *J* = 6.5 Hz, 6H), 2.13-2.08 (m, 6H).

^{13}C NMR (125 MHz, dcm-d2): 143.5, 131.5, 140.7, 132.8, 126.2, 125.7, 124.8, 123.7, 123.3, 120.7, 119.4, 119.3, 109.7, 109.6, 67.3, 55.6, 54.0, 40.9, 25.8.

HRMS (ESI+): m/z calculated for C63H67N6O3 $[M+H]^+$ 955.5275 and found 955.5289.

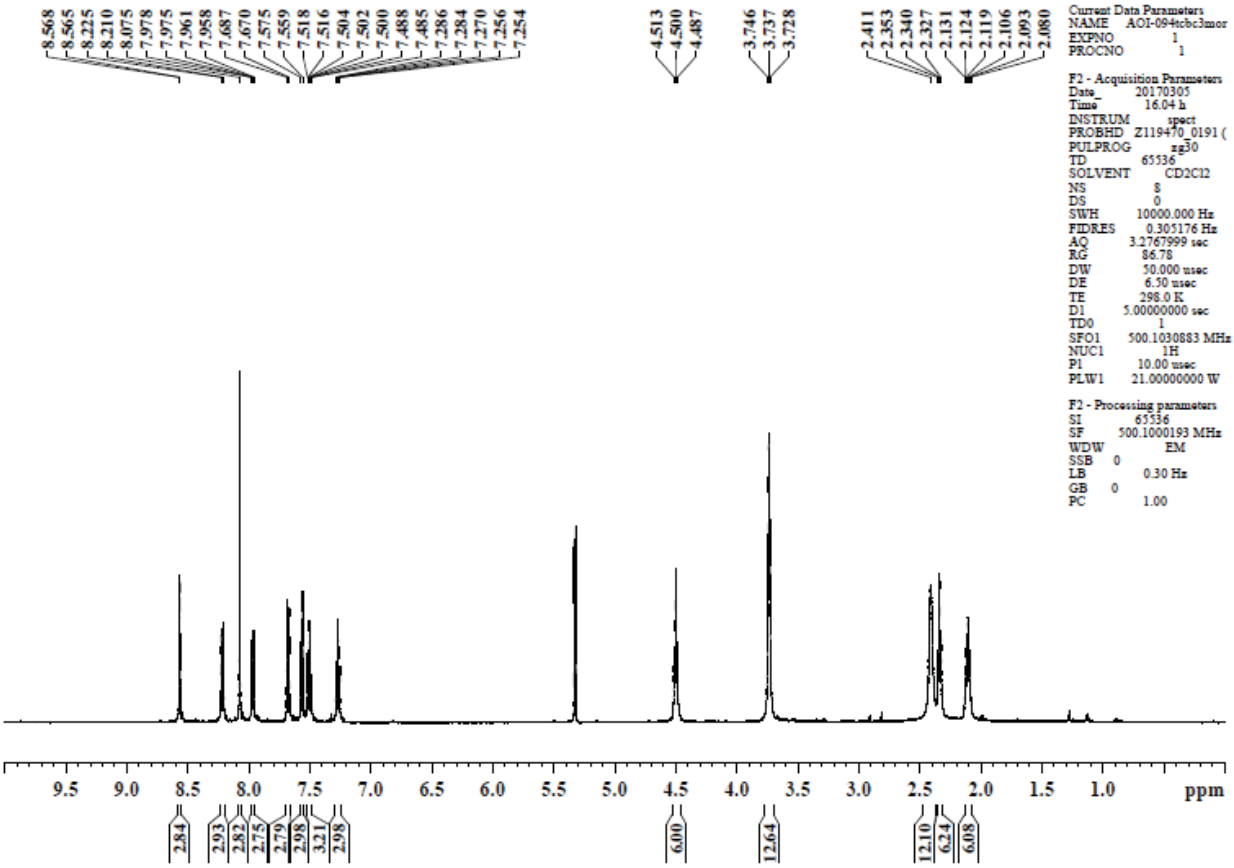


Figure S17: ^{1}HNMR of compound **2h**

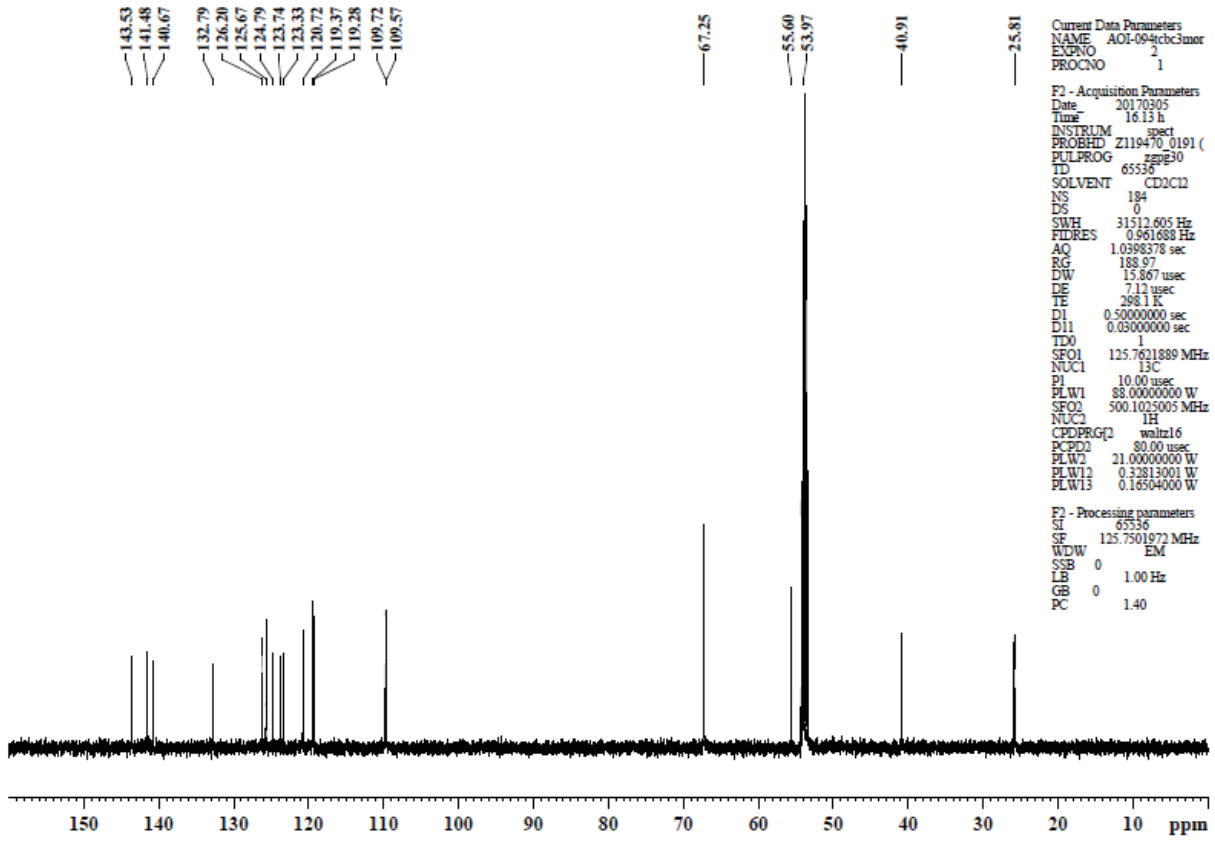


Figure S18: ^{13}CNMR of compound **2h**

Biophysical Assays

Table S2 – Table of oligonucleotides for FRET and CD

Name	Sequence	Structure	Species	Region
F21T	5'-FAM-GGGTTAGGGTTAGGGTTAGGG-TAMRA-3'	K^+ Hybrid Na^+: Antiparallel basket	Human	Human Telomeric repeat
FmycT	5'-FAM-TTGAGGGTGGGTAGGGTGGGTAA-TAMRA-3'	Parallel	Human	*c-Myc* Promotor
FHIV32T	5'-FAM-CAGGGAGGCGTGGC2TGGGCGGGA-TAMRA-3'	Polymorphic	Virus	HIV *PRO2* gene
F21RT	5'-FAM-rGrGrGrUrUrArGrGrGrUrUrArGrGrGrUrUrArGrGrG-TAMRA-3'	Parallel	Human	Human telomeric repeat containing RNA
F21CTAT	5'-FAM-GGGCTAGGGCTAGGGCTAGGG-TAMRA-3'	Anti-parallel	Human	Human telomeric repeat variant
FKrasT	5'-FAM-AGGGCGGTGTGGGAAGAGGGA-TAMRA-3'	Parallel	Human	*KRAS* promotor
FHIV321T	5'-FAM-TTGGCCTGGGCGGGACTGGGA-TAMRA-3'	Anti-Parallel	Virus	HIV *PRO1* gene
FdxT	5'-FAM-TATAGCTATA-hexaethyleneglycol-TATAGCTATA-TAMRA-3'	Intramolecular duplex (hairpin)		
ds26	5'-AGAGAGTTAGTTAGTTAGTTAGTTAGTTAGTTAGTTAG-3'	Duplex DNA		
22Ag	5'-AGGGTTAGGGTTAGGGTTAGGG-3'	K^+ Hybrid Na^+: antiparallel basket	Human	Human telomeric repeat
22Kras	5'-AGGGCGGTGTGGGAATAGGGAA-3'	Parallel	Human	*KRAS* promotor

Table S3: Ligand induced ΔTm (˚C) of *F21T* in 100 mM Na^+ buffer

	ΔTm (°C) *F21T* (0.2 μM)		
Ligand	1 μM	5 μM	10 μM
1	NS	5.9 ± 1.4	3.0 ± 0.04
2a	2.2 ± 0.3	5.0 ± 0.7	6.8 ± 0.1
2b	NS	1.3 ± 0.06	14.6 ± 0.5
2c	NS	2.0 ± 1.1	8.2 ± 1.0
2d	NS	NS	NS
2e	2.8 ± 0.1	9.0 ± 0.04	10.3 ± 0.1
2f	2.0 ± 0.9	14.1 ± 0.3	18.8 ± 1.5
2g	4.0 ± 0.04	13.5 ± 0.6	15.2 ± 0.2
2h	NS	2.1 ± 0.5	3.7 ± 0.1

NS = No stabilization (ΔTm <1 °C)

Circular Dichroism Titration

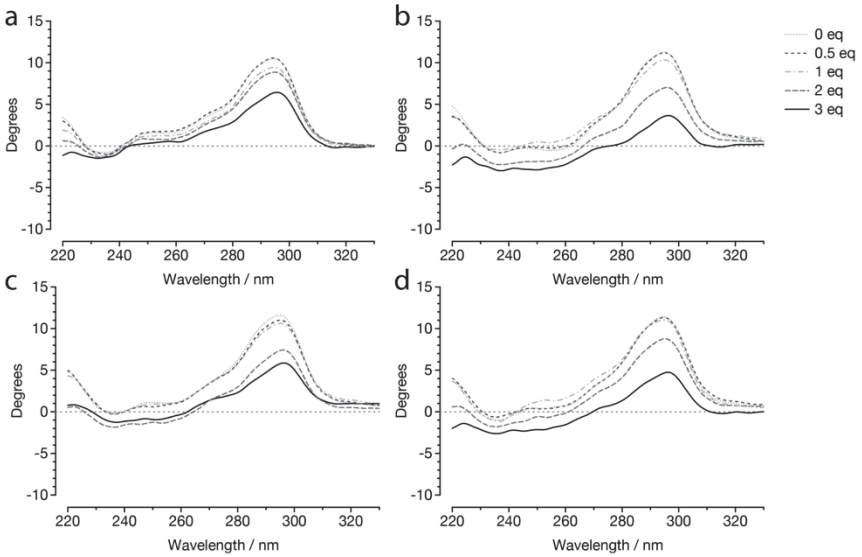


Figure S19: Circular dichroism titration with 22Ag. a) **2b**; b) **2e**; c) **2f**; d) **2g**

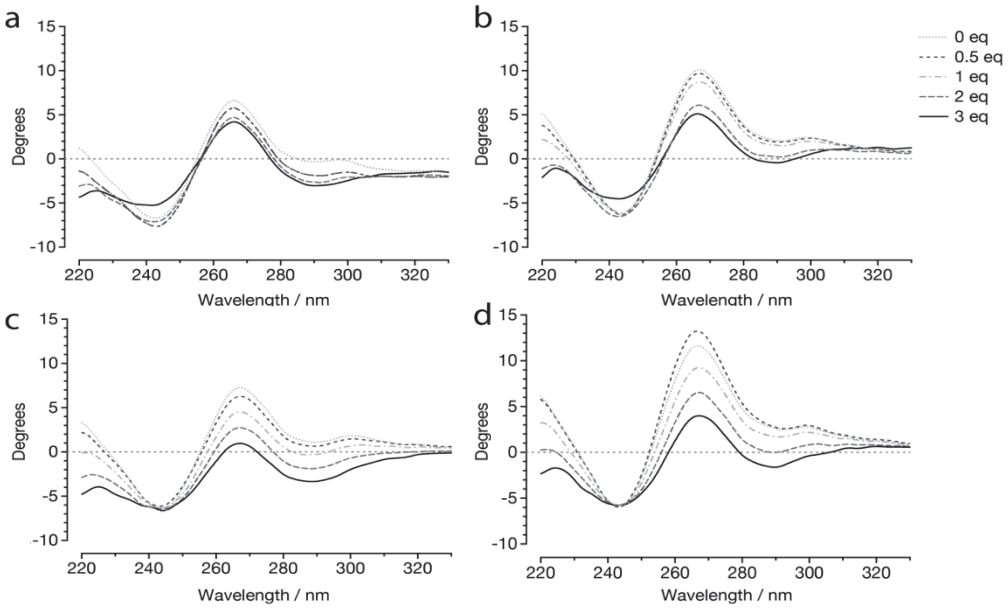


Figure S20: Circular dichroism titration with 22KRAS. a) **2b**; b) **2e**; c) **2f**; d) **2g**

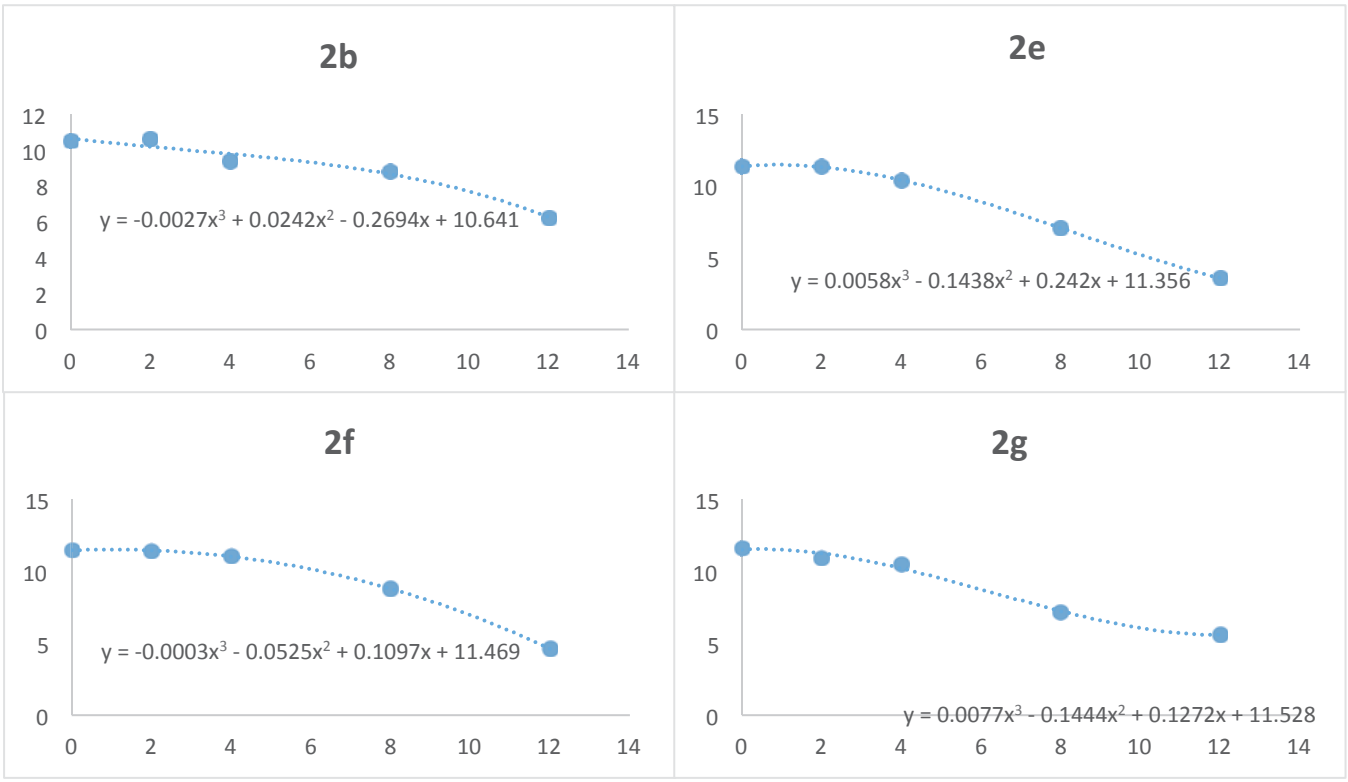


Figure S21: Plot of molar ellipticity versus concentration of *22Ag*, fitted to a polynomial

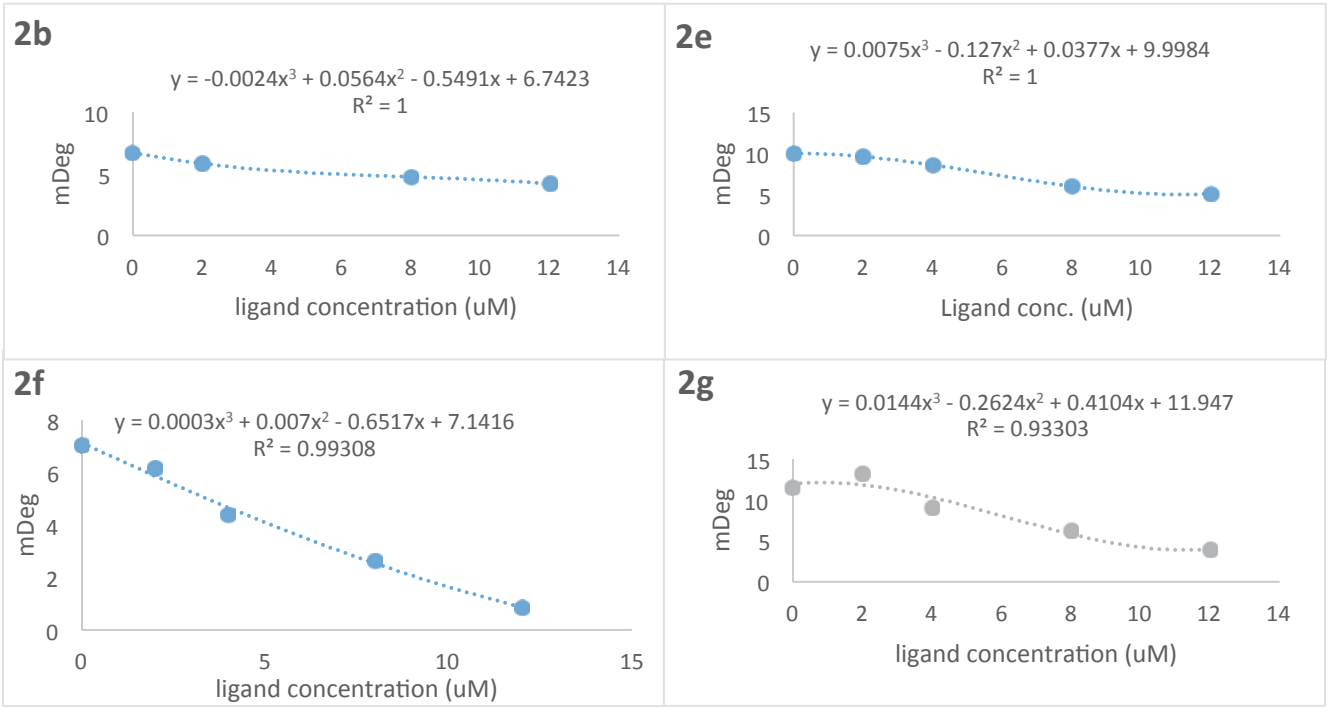


Figure S22: Plot of molar ellipticity versus concentration of *22KRAS*, fitted to a polynomial

Table S4: Plots of [D]50% of **2b**, **2e**, **2f** and **2g**

Ligand	22Ag [D]50% (uM)	22KRAS [D]50% (uM)
2b	**16.9**	**14.4**
2e	**9.5**	**11.1**
2f	**11.2**	**6.0**
2g	**10.6**	**8.05**

NMR titration

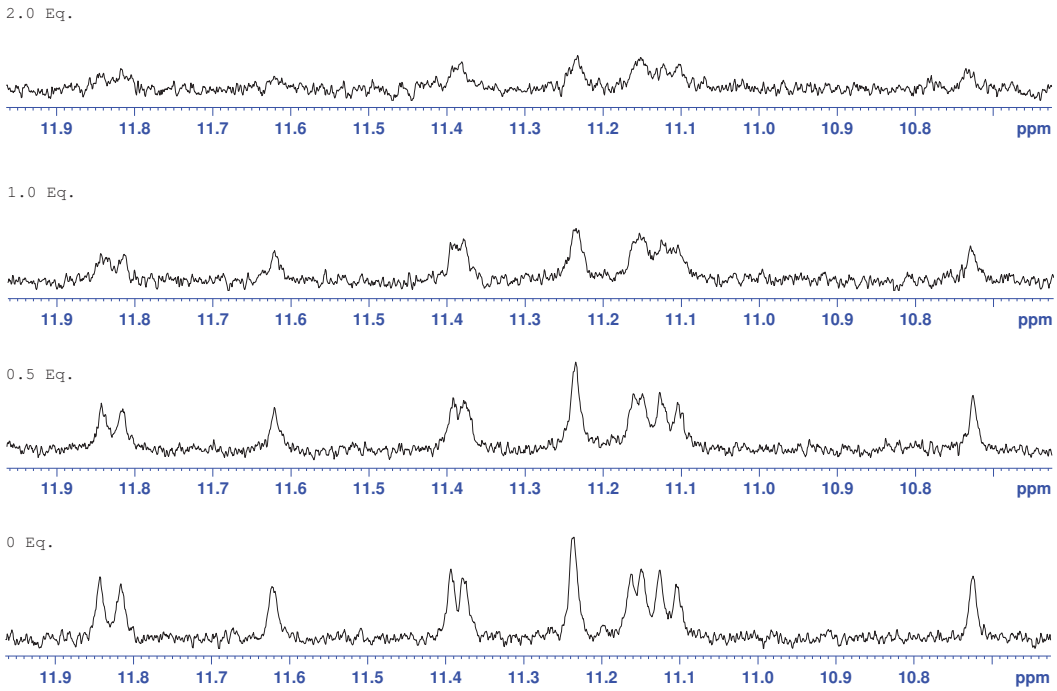


Figure S23: NMR titration of 22Kras with **2f.**3HCl (0.5-2.0 equivalents).

FRET Competition

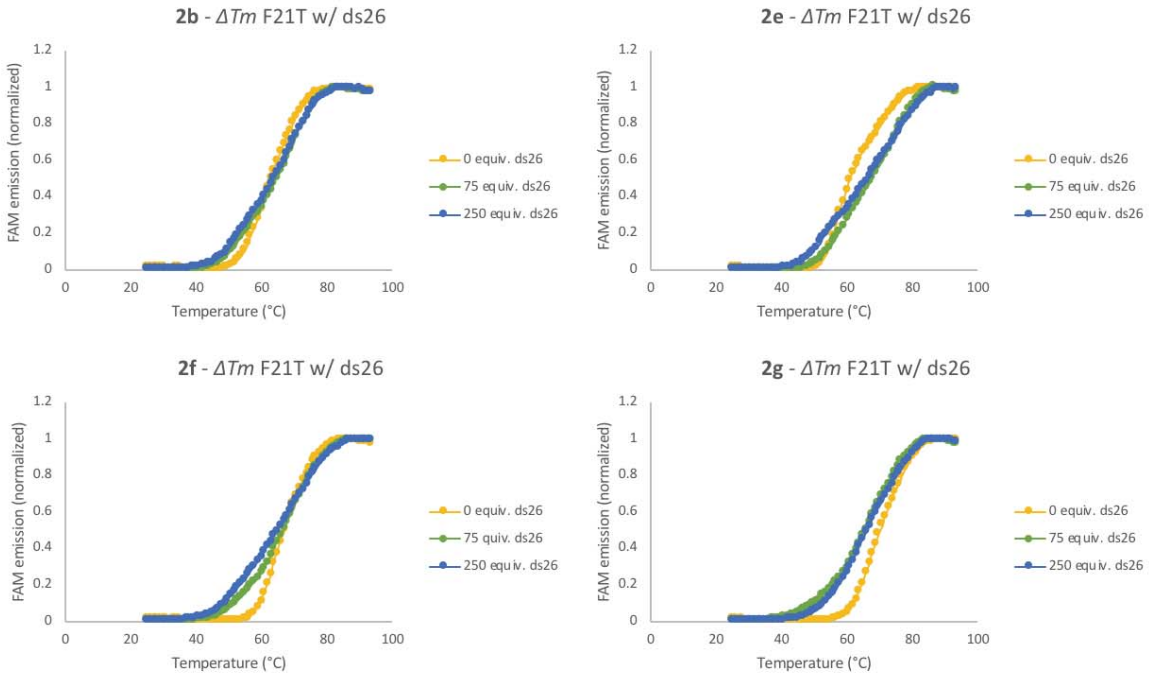


Figure S24: Plots of normalized fluorescence vs temperature for F21T in the absence and presence of ds26 competitor

References

S1. G.R. Fulmer, A.J.M. Miller, N.H. Sherden, H.E. Gottlieb, A. Nudelman, B.M. Stoltz, J.E. Bercaw and K.I. Goldberg, *Oranometallics*, **2010**, *29,* 2176.

S2. S.-J. Zhu, H.-Z. Ying, Y. Wu, N. Qiu, T. Liu, B. Yang, X.-W. Dong and Y.-Z. Hu, *RSC Adv.*, **2015**, *5*, 103172.

S3. Y.-B. Zhang, H. Furukawa, N. Ko, W. Nie, H. J. Park, S. Okajima, K. E. Cordova, H. Deng, J. Kim and O.M. Yaghi, *J. Am. Chem. Soc.,* **2015**, *137*, 2641.

S4. K. Sun, X. Xie, Y. Liu, W. Jiang, X. Ban, B. Huang and Y. Sun, *J. Mater. Chem. C*, **2016**, 4, 8973.

S5. G.M. Sheldrick, *Acta Cryst. C*, **2015**, *71*, 3.