Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information for:

Dual fluorescent deoxyguanosine mimics for FRET detection of G-quadruplex folding

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Table of Contents

Experimental Section

Materials

$Pd(OAc)_2$, 3,3′,3″-phosphanetriyltris(benzenesulfonic acid) trisodium salt (TPPTS), *N*,*N*-dimethylformamide diethyl acetal, 4,4′-dimethoxytrityl chloride, 2-cyanoethyl N,N-diisopropylchlorophosphoramidite, 2-bromobenzo[*b*]thiophene, (trimethylsilyl)acetylene (TMSA) and other commercial products used for ^{vBth}dG and phosphoramidite synthesis were used as received. Unmodified TBA oligonucleotides and complementary strands were purchased from Sigma Genosys and were purified by Sigma using polyacrylamide gel electrophoresis. All unmodified phosphoramidites (bz-dA-CE, ac-dC-CE, dmf-dG-CE and dT-CE), activator (0.25 M 5-(ethylthio)-1*H*-tetrazole in CH_3CN), oxidizing agent (0.02 M I_2 in THF/pyridine/H_2O, 70/20/10, v/v/v), deblock (3% dichloroacetic acid in dichloromethane), cap A (THF/2,6-lutidine/acetic anhydride), cap B (methylimidazole in THF), and 1000 Å controlled pore glass (CPG) solid supports were purchased from BioAutomation or Glen Research.

Methods

NMR spectra were recorded at room temperature on a 400 MHz Bruker spectrometer in either $CDCl_3$ or DMSO-d_6 referenced to TMS (0 ppm) or the respective solvent. The quantum yield of ^{vBth}dG was determined in 10mM MOPS buffer (pH 7, 100mM NaCl) using the comparative method. Quinine bisulfate (Φ_{fl} = 0.546 in 0.5 M H_2SO_4) served as the fluorescence quantum yield standard. High-resolution mass spectra were obtained with an Agilent LC-UHD quadrupole time-of-flight (Q-Tof) instrument using electrospray ionization. The 8-furyl-dG (^{Fur}dG) phosphoramidite was available in our laboratory and was synthesized from ^{Fur}dG as previously outlined.[1] All mTBA oligonucleotides were prepared on a 1 μmol scale using a BioAutomation MerMade 12 automatic DNA synthesizer. Standard phosphoramidites

were prepared in dry acetonitrile (1 mM) and coupling reactions consisted of a 70 μL injection volume with a 60 s coupling time. For modified phosphoramidites (^{Fur}dG and ^{vBth}dG) the injection volume from the 1mM acetonitrile solutions was the same (70 μL), but the coupling time was increased to 120 s. Upon completion of DNA synthesis, the crude mTBA oligonucleotide solutions were deprotected and cleaved from their solid support in aqueous ammonium hydroxide at 55 °C for 15 h, filtered using syringe filters (PVDF 0.22 μm) and concentrated under diminished pressure. Samples were then resuspended in Milli-Q water (18.2 MΩ) and purified using an Agilent HPLC instrument equipped with an autosampler, a diode array detector, fluorescence detector and autocollector. Separation was carried out at 50 °C using a 5 μm reversed-phase (RP) semi-preparative C18 column (100 × 10 mm) with a flow rate of 3.5 mL/min, and various gradients of buffer B in buffer A (buffer A = 95:5 aqueous 50 mM TEAA, pH 7.2/acetonitrile; buffer B = 30:70 aqueous 50 mM TEAA, pH 7.2/acetonitrile). Yields of the mTBA samples were estimated from integration of the semi-preparative HPLC trace. Following lyophilisation of the collected fractions, the mTBA samples were analyzed using analytical HPLC to check for purity. Analytical HPLC separation was performed using a Clarity Oligo-RP C18 column (4.6 mm x 50 mm, 3um, Phenomenex, Torrance, CA). Buffer A contained 100 mM TEAA (pH 7.2) and 5% CH_3CN (v/v). Buffer B contained 100 mM TEAA (pH 7.2) and 20% CH_3CN (v/v). The flow rate for this method was maintained at 0.5 mL/min using a multistep gradient protocol. From 0-3 min, hold A and B at 73 and 27% respectively. From 3-23 min, a linear program to 100% B; 23-25 min, hold at 100% B; 25-27 min linear program to 73 and 27% A and B respectively; 27-35 min hold at 73% A and 27% B

for column equilibration. Integration of the analytical HPLC chromatograms indicated purity levels >85% for the mTBA samples.

MS experiments for identification of the mTBA oligonucleotides were conducted on a Bruker amaZon quadrupole ion trap SL spectrometer. Oligonucleotide samples were prepared in 90% Milli-Q filtered water/10% methanol containing 0.1 mM ammonium acetate. Masses were acquired in the negative ionization mode with an electrospray ionization source. mTBA quantification was performed with UV−vis. The extinction coefficient at 260 nm of unmodified TBA was obtained from the following website: http://www.idtdna.com/analyzer/applications/ oligoanalyzer. The mTBA samples were assumed to have the same extinction coefficient as unmodified TBA (143,300 M^{-1} cm^{-1}).

All melting temperatures (T_m) of TBA oligonucleotides were measured using a Cary 300-Bio UV-Vis spectrophotometer equipped with a 6 x 6 multicell Peltier block-heating unit using Hellma 114-QS 10 mm light path cells. Oligonucleotide samples were prepared in 50 mM phosphate buffer, pH 7, with 100 mM MCl (M = Na^+ or K^+), using equivalent amounts (3.0 μM) of the unmodified or mTBA oligonucleotide and its complementary strand. The UV absorption at 260 nm (for duplex formation) and 295 nm (for GQ formation) was monitored as a function of temperature and consisted of forward-reverse scans from 10 to 90 ºC at a heating rate of 0.5 ^oC/min, which was repeated five times. The T_m values were determined using hyperchromicity calculations provided in the Thermal software.

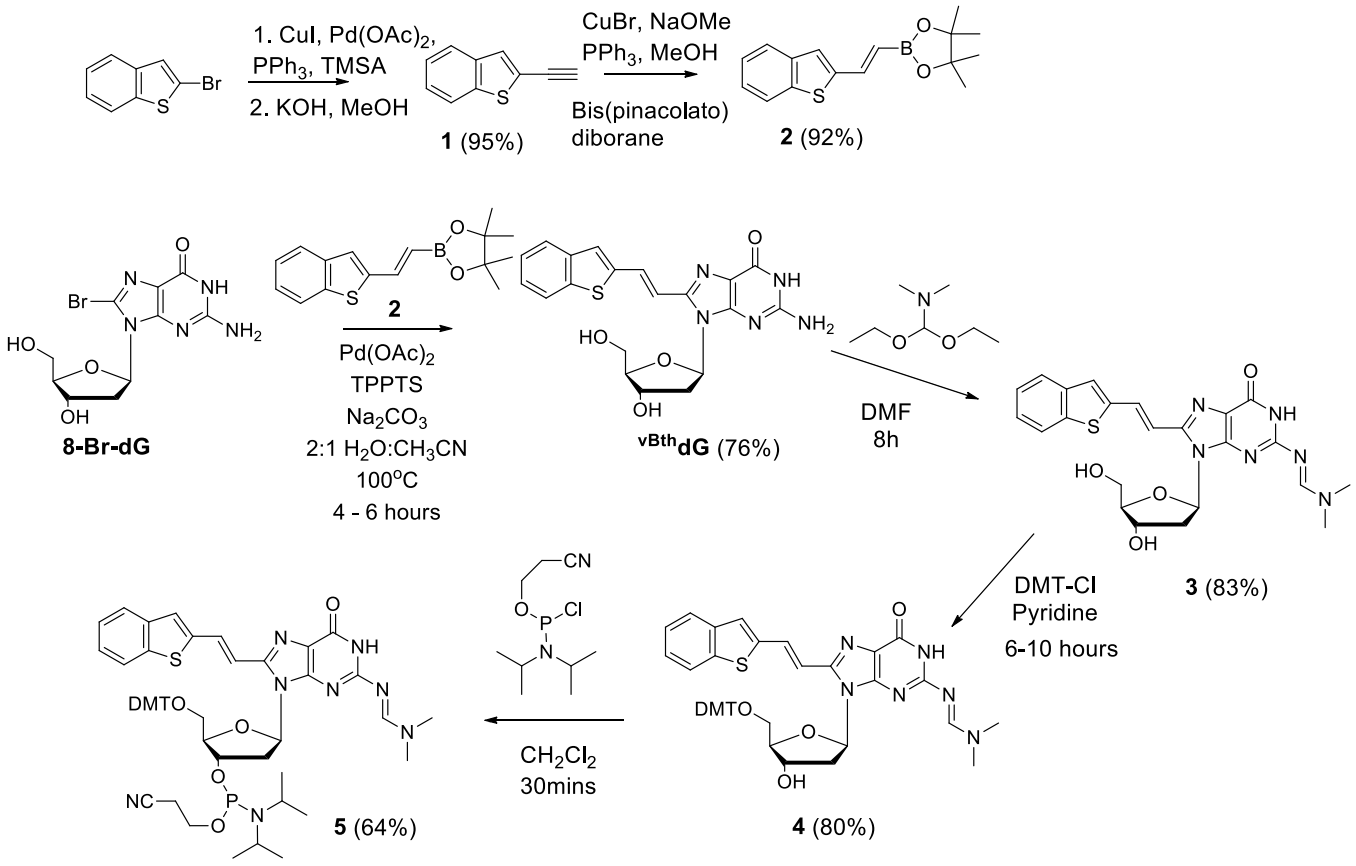
Circular dichroism (CD) spectra were recorded on a Jasco J-815 CD spectropolarimeter equipped with a 1 x 6 Multicell block thermal controller and a water circulator unit. Spectra were collected at 10 ^oC between 200 and 400 nm, with a

bandwidth of 1 nm and scanning speed at 100 nm/min. All fluorescence spectra were recorded on a Cary Eclipse Fluorescence spectrophotometer equipped with a 1 x 4 Multicell block Peltier stirrer and temperature controller. Excitation and emission spectra (5 nm slit-width) were recorded at 10 ^{o}C.

mTBA samples were annealed with equivalent amounts of complementary strand (5′-CCAACCACACCAACC-3′) to produce duplex samples with a final concentration of 3 μM in 50 mM Na^{+}-phosphate, pH 7.0 with 100 mM NaCl buffer. Excitation and emission spectra were recorded for both 8aryldG modified bases corresponding to ^{Fur}dG and ^{vBth}dG. Bovine thrombin was titrated in 0.25 M aliquots until no further fluorescent response was detected. Fluorescence titration data was transformed into binding isotherms by calculating fraction bound using: Fraction Bound= $(F_{obs}\text{-}Fi)/(F_{max}\text{-}F_i)$, where F_{obs} = observed fluorescence intensity, F_i is the initial fluorescence intensity and F_{max} is the fluorescence intensity of the mTBA when fully bound by thrombin. Fraction bound vs. [titrant] were plotted in order to generate a binding isotherm. K_d values were obtained using SigmaPlot Version 11.0 by subjecting the binding isotherm to a one site saturation ligand binding analysis. K_a values were calculated as: $K_a = 1/K_d$.

^{vBth}dG and phosphoramidite synthesis.

The 8-vinyl-benzo[b]thieny-dG (^{vBth}dG) phosphoramidite was synthesized as outlined in Scheme 1. The requisite boronic ester **2** required for Suzuki coupling with 8-Br-dG to afford ^{vBTh}dG was prepared starting from 2-bromo-benzo[*b*]thiophene. Sonogashira coupling of the bromoarene with TMSA[2] afforded the ethynylarene derivative **1** in 95%. A Cu-catalyzed hydroboration[3] of **1** produced the desired boronic ester **2** for subsequent Pd-catalyzed coupling to 8-Br-dG to afford ^{vBth}dG. The ^{vBth}dG nucleoside was then converted into the phosphoramidite **5** using previously published strategies.[1]



Scheme 1 Synthesis of ^{vBth}dG and its phosphoramidite.

2-ethynylbenzo[*b*]thiophene (1)

To a mixture of 2-bromo-benzo[b[thiophene (6 mmol), triphenylphosphine (0.6 mmol), palladium acetate (0.02 mmol), and copper iodide (0.36 mmol) combined under argon, 30 ml of 2:1 triethylamine:THF was added with stirring. This mixture was allowed to stir for 15 minutes at room temperature before the addition of TMSA (9 mmol). This reaction mixture was stirred at room temperature until completion as monitored by TLC. Solvents were removed under reduced pressure, hexanes was added and then filtered. The resulting filtrate was concentrated under reduced pressure producing a brown oil. The product was dissolved in 5 ml of methylene chloride, 15 ml of 1M KOH in methanol was then added and the reaction was stirred overnight at

room temperature. The solvent was removed under reduced pressure, water was added and the product was extracted with diethyl ether. The ether was removed and column was performed, yielding the desired product (Yield 95 %). ^{1}H (NMR) (300 MHz, $CDCl_3$), δ: 7.78 (m, 2H), 7.55 (s, 1H), 7.40 (m, J=9.5 Hz, 2H), 3.49 (s, 1H); ^{13}C NMR ($CDCl_3$) (100.6 MHz), δ: 140.2, 138.7, 130.0, 125.7, 124.8, 124.0, 122.0, 91.3, 90.3, 83.0. Spectra obtained matched the published ^{1}H NMR data.[2]

(*E*)-2-(2-(benzo[*b*]thiophen-2-yl)vinyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (2)

A mixture of bis(pinacolato)diboron (6.6 mmol), sodium methoxide (1.2 mmol), triphenylphosphine (0.36 mmol) and copper bromide (0.3 mmol) were stirred under argon for 30 minutes at room temperature in dry THF. To this mixture **1**(6 mmol) and methanol (12 mmol) were added. The reaction mixture was stirred at room temperature until completion. The solvents were then removed under reduced pressure, hexanes was added and then filtered. The resulting filtrate was concentrated under reduced pressure. The resulting oil was purified via column producing the desired product (Yield 92 %). ^{1}H (NMR) (300 MHz, $CDCl_3$), δ: 7.75 (m, 1H), 7.70 (m, 1H), 7.57 (d, *J*=18 Hz, 1H), 7.29 (m, 2H), 7.25 (s, 1H), 6.02 (d, *J*= 9.0 Hz, 1H), 1.32 (s, 12H); ^{13}C NMR ($CDCl_3$) (100.6 MHz), δ: 143.9, 142.3, 140.0, 139.6, 125.3, 125.1, 124.5, 124.1, 122.4, 83.6, 24.8. HRMS calcd for $C_{16}H_{19}BO_2S^+$ [M+H$^+$] 287.1269; found 287.1275. Spectra obtained matched the published ^{1}H NMR and ^{13}C NMR data.[2]

8-(2″-vinylbenzo[b]thiophene)-2'-dG (^{vBth}dG)

$Pd(OAc)_2$ (0.15 mmol), TPPTS (0.3 mmol), Na_2CO_3 (15 mmol), and 8-vinyl-benzo[b]thienyl boronic ester **2** (3.6 mmol) were placed in a round bottomed flask fitted with a condenser and reverse filled with argon. Degassed 2:1 H_2O:CH_3CN (35 mL) solution was added and the solution was heated to reflux for 4-5 hours. Following completion the mixture was

diluted with 200 mL of H_2O and pH was adjusted to 7.5 with 1.0 M aqueous HCl. The mixture was then cooled to 0°C, filtered, washed with DCM and dried to yield product. (Yield 76%). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 10.77 (bs, 1H), 7.90 (m, 1H), 7.81 (m, 1H), 7.81 (d, J = 15.2 Hz, 1H), 7.66 (s, 1H), 7.37 (m, 2H), 7.18 (d, J = 15.6 Hz, 1H), 6.58 (s, 2H), 6.35 (m, 1H), 5.38 (d, J= 4.1, 1H), 5.17 (t, J=10.4, 1H), 4.36 (m, 1H), 3.83 (m, J= 7.5, 1H), 3.70 (m, 2H), 2.60 (m, 1H), 2.13 (m, 1H); ^{13}C NMR (600 MHz, DMSO-d_6) δ = 156.88, 153.93, 152.29, 143.84, 141.89, 140.27, 139.17, 126.77, 126.22, 125.92, 125.35, 124.36, 122.86, 117.74, 117.19, 87.69, 82.83, 70.96, 62.07, C2' obscured by DMSO solvent. HRMS calcd for $C_{23}H_{24}N_6O_4S^+$ [M+H$^+$] 426.1237; found 426.1223.

***N*^{2}-(dimethylformamidyl)-8-(2″-vinylbenzo[b]thiophene)-2′-dG (3)**

The nucleoside ^{vBth}dG (3.3 mmol) was placed in a round bottomed flask (RBF) and reverse filled with argon. 15 mL of dry DMF was added followed by dimethylformamide-diethyl-acetal (2.7 mL, 13.5 mmol) and the mixture was allowed to stir until completion overnight at room temperature. The reaction mixture was then evaporated to dryness and the solid washed with MeOH and dried to yield the final product. (83% yield). ^{1}H NMR (400MHz, DMSO-d_6) δ = 11.48 (bs, 1H), 8.59 (s, 1H), 7.93 (m, 1H), 7.87 (d, J=15.5 Hz, 1H), 7.83 (m, 1H), 7.70 (s, 1H), 7.38 (m, 2H), 7.20 (d, J=15.6 Hz, 1H), 6.48 (t, J=14.77 Hz, 1H), 5.44 (d, J=4.5 Hz, 1H), 5.11 (t, J=10.9 Hz, 1H), 4.48 (m, 1H), 3.84 (m, 1H), 3.76 (m, 1H), 3.67 (m, 1H), 3.18 (s, 3H), 3.05 (s, 3H), 2.71 (m, 1H), 2.21, (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): 158.6, 157.7, 157.5, 150.9, 144.9, 141.8, 140.3, 139.2, 127.4, 126.5, 126.0, 125.4, 124.4, 122.9, 120.3, 117.42, 87.6, 82.7, 70.8, 61.9, 41.2, 35.2. HRMS Calcd for $C_{23}H_{24}N_6O_4S^+$ [M+H$^+$]: 481.1659; found 481.1651.

5′-*O*-(4,4′-Dimethoxytrityl)-N^2-(dimethylformamidyl)-8-(2′′-vinylbenzo[b]thiophene) -2′-dG (4)

Compound **3** (2.7 mmol) was co-evaporated from dry pyridine (3 x 5 mL) in a RBF. The RBF was then fitted with a constant pressure dropping funnel, reverse filled with argon, and 7 mL of dry pyridine (or DMF) was added to the RBF and cooled to 0^{o}C. A DMT-Cl (1.28 g, 3.78 mmol) pyridine (5 mL) solution was added to the dropping funnel under argon and allowed to add dropwise over 30 min. The reaction was allowed to stir at room temperature under argon and was monitored by TLC. Upon completion, the mixture was diluted with methylene chloride (10 mL) and washed with water (2 x 10 mL). TEA (1 mL) was added and the mixture was evaporated to yield an oil. The oil was then loaded onto a silica column and run with 95:5 CH_2Cl_2:TEA to elute unreacted DMT material; product was then eluted with MeOH:CH_2Cl_2:TEA (5:90:5).(80% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 11.53 (bs, 1H), 8.56 (s, 1H), 7.95 (d, J=15.3, 1H), 7.95 (m, 1H), 7.88 (m, 1H), 7.70 (s, 1H), 7.45 (m, 2H), 7.32 (m, 2H), 7.17 (m, 7H), 7.10 (d, J = 15.5 Hz, 1H), 6.80 (m, 4H), 6.62 (dd, J = 7.7 Hz and 4.8 Hz, 1H), 5.53 (bs, 1H), 4.63 (m, 1H), 3.99 (m, 1H), 3.67 (s, 3H), 3.66 (s, 3H), [3.76 (m, 1H), 3.67 (m, 1H), 3.28 (m, 1H)] predicted based on starting material, 3.17 (s, 3H), 3.11 (s, 3H), 2.37 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ= 158.5, 157.9, 156.0, 150.7, 145.9, 144.7, 141.6, 140.0, 139.2, 135.7, 130.03, 129.96, 128.8, 128.1, 127.9, 126.9, 126.0, 125.3, 124.7, 123.9, 122.2, 120.4, 116.0, 113.2, 86.6, 84.8, 82.6, 73.0, 64.6, 55.2, 41.4, 39.4, 35.2. HRMS calcd for [M + H^+] 783.2966, found 783.2961.

3′-O-[(2-Cyanoethoxy)(diisopropylamino)phosphino]-5′-*O*-(4,4′-dimethoxytrityl)-N^2-(dimethylformamidyl)-8-(2′′-vinylbenzo[b]thiophene) -2′-dG (5)

Compound **4** (0.706 mmol) was co-evaporated from dry THF (3 x 5 mL), reverse filled with argon and dissolved in 10 mL dry, degassed CH_2Cl_2. To this was added dry, degassed TEA

(0.4 mL, 2.83 mmol) and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.24 mL, 1.06 mmol). The reaction was monitored via TLC and, upon completion (20-40 min) the solvent was removed under reduced pressure, redissolved in minimal DCM and re-precipitation by dripping into hexanes at -78°C (64% yield). The amidite **5** was ~ 71% pure based on ^{31}P NMR analysis with a P(V) derivative (δ 14.3 ppm) as the major impurity. ^{31}P NMR (121 MHz, $CDCl_3$) δ = 149.0, 148.9. HRMS calcd for $C_{53}H_{59}N_8O_7PS^+$ [M+H$^+$]: 983.4044; found 983.4029.

Table S1 Yields and ESI-MS analysis for mTBA.

Site (D;A)	yield (%)[a]	M.W. calcd.[b]	M.W. found[c]
D, 10	69	4789.8	4789.6
D, 14	76	4789.8	4790.0
A, 6	52	4881.8	4882.5
A, 5	49	4881.8	4883.0
10;2	24	4947.8	4949.0
14;2	30	4947.8	4948.8
10;6	40	4947.8	4947.8
14;6	61	4947.8	4948.5
10;5	32	4947.8	4948.0

[a] Yield derived from integration of the semi-preparative HPLC trace following deprotection of the mTBA with concentrated ammonium hydroxide at 55 ° C for 15 h. [b] M.W. of neutral species. [c] ESI-MS analysis and mass deconvoluted for neutral species.

References

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2. B. Blanco, A. Sedes, A. Peón, H. Lamb and A. R. Hawkins, *Org. Biomol. Chem*. 2012, **10**, 3662-3676.

3. R. Alfaro, A. Parra, J. Alemán, J. L. G. Ruano and M. Tortosa, *J. Am. Chem. Soc*. 2012, **134**, 15165-15168.

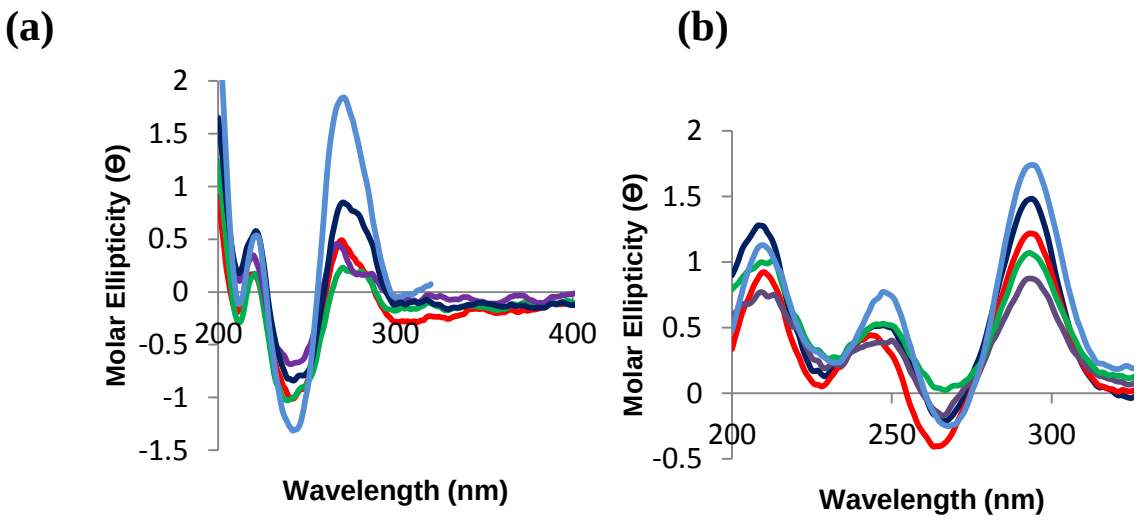

Fig S1 CD spectra of ^{vBth}dG (A) and ^{Fur}dG (D) mTBA (a) Duplex with complementary strand = (5'-CCAACCACACCAACC). (b) GQ. D;A light blue = 10;5, dark blue = 14;6, red = 10;6, green 14;2, purple 10;2.

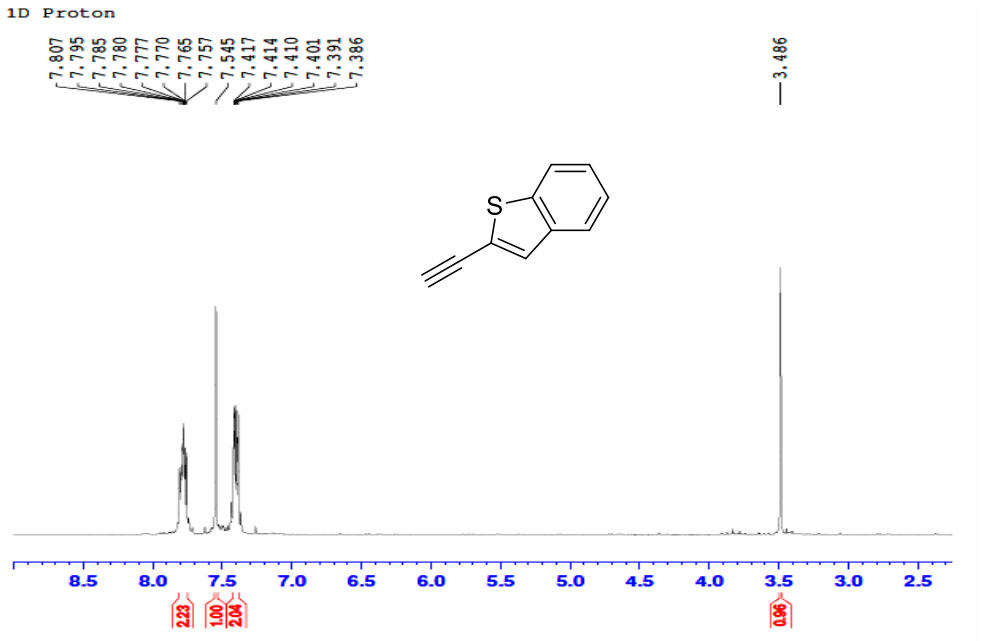

Fig S2 ^{1}H NMR of 2-ethynylbenzo[b]thiophene (**1**) in $CDCl_3$

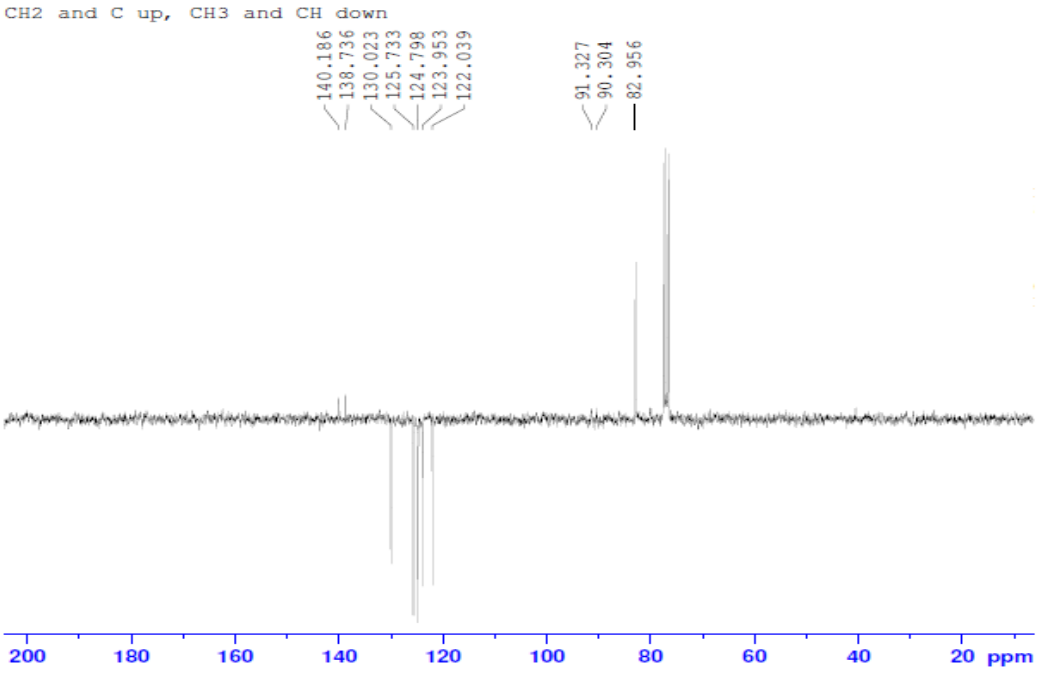

Fig S3 ^{13}C NMR of 2-ethynylbenzo[b]thiophene (**1**) in $CDCl_3$

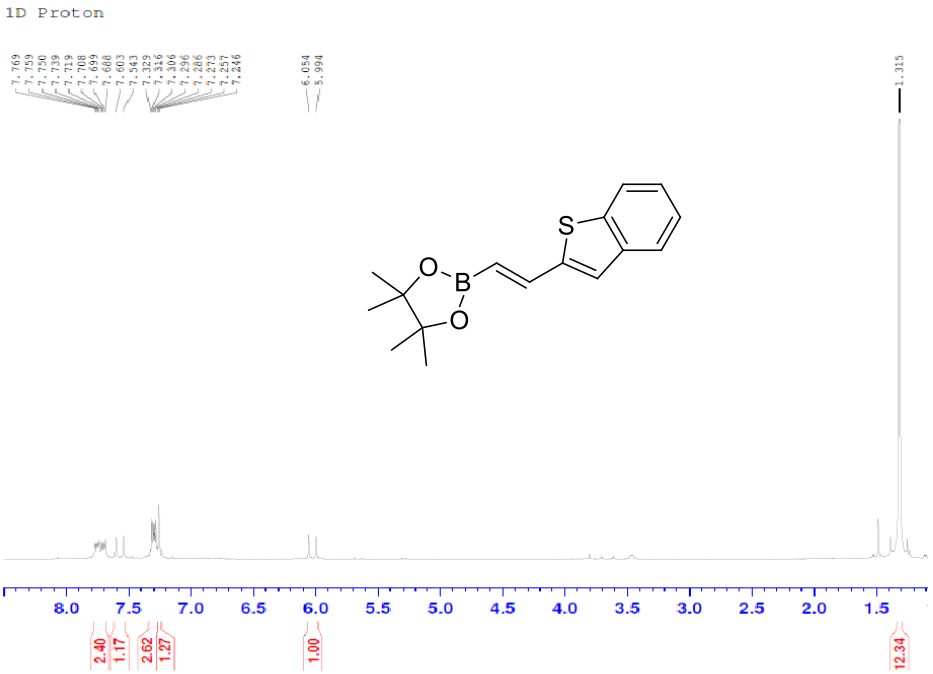

Fig S4 ^{1}H NMR of 9-(2-ethynylbenzo[*b*]thiophene) boronic acid pinacol ester (**2**) in $CDCl_3$

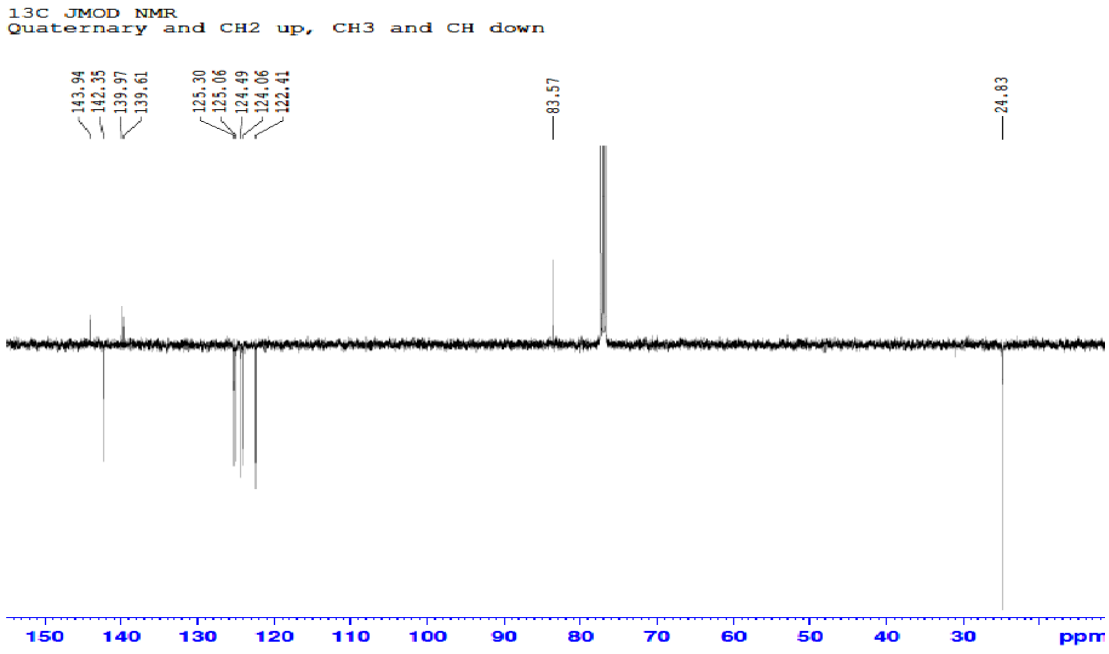

Fig S5 ^{13}C NMR of 9-(2-ethynylbenzo[*b*]thiophene) boronic acid pinacol ester (**2**) in $CDCl_3$

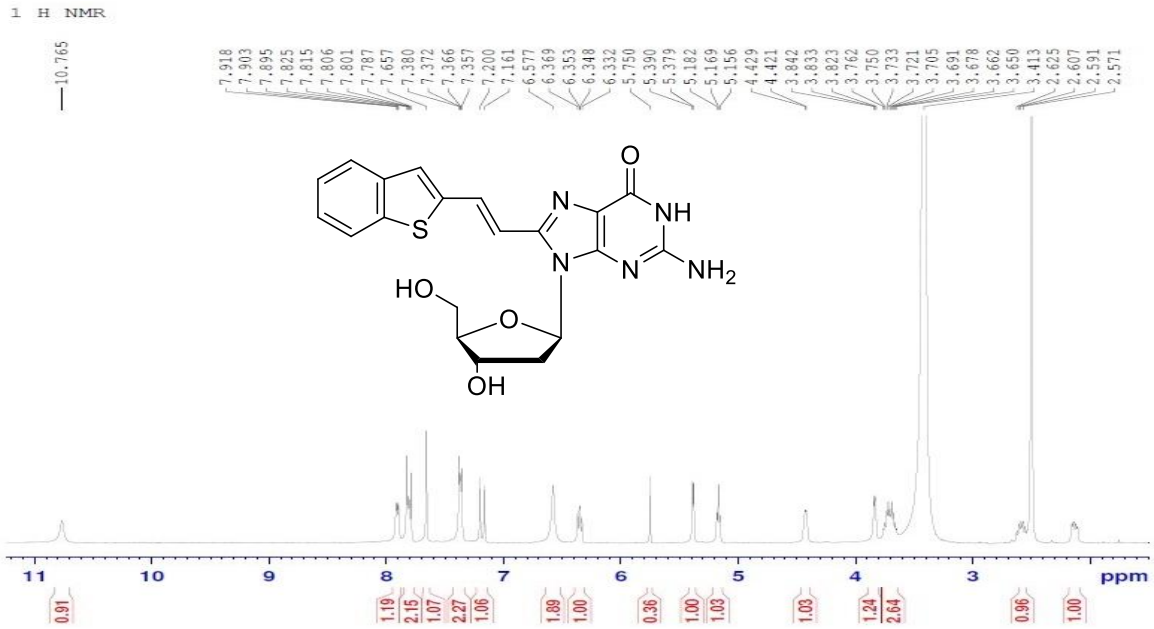

Fig S6 ^{1}H NMR spectrum ^{vBth}dG in DMSO-d_6

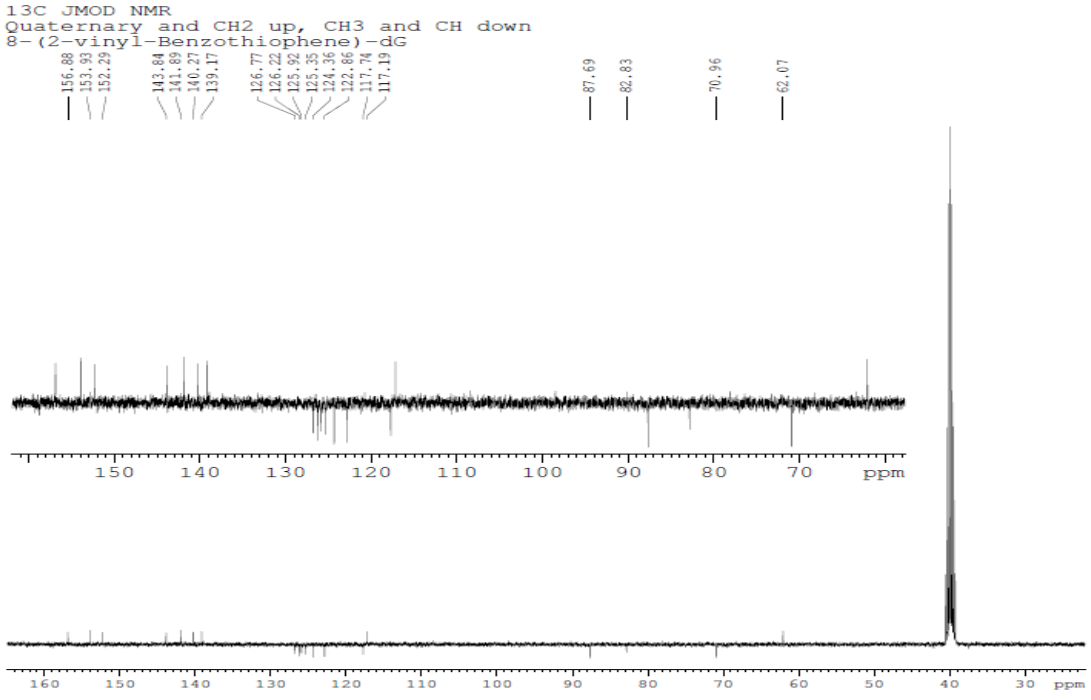

Fig S7 ^{13}C NMR spectrum of ^{vBth}dG in DMSO-d_6

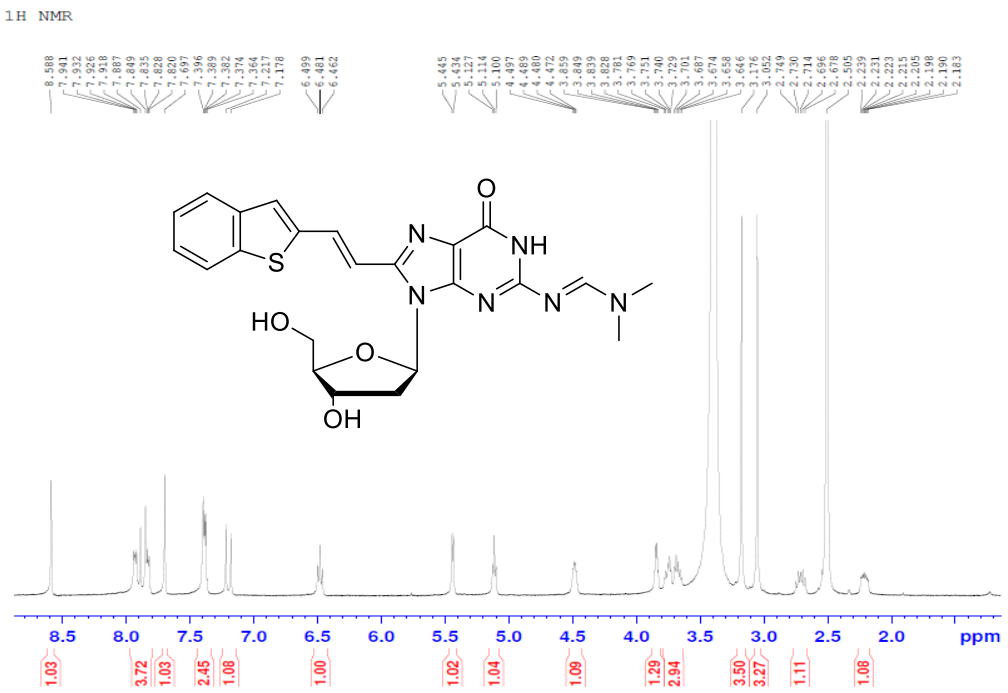

Fig S8 ^{1}H NMR spectrum of **3** in DMSO-d_6

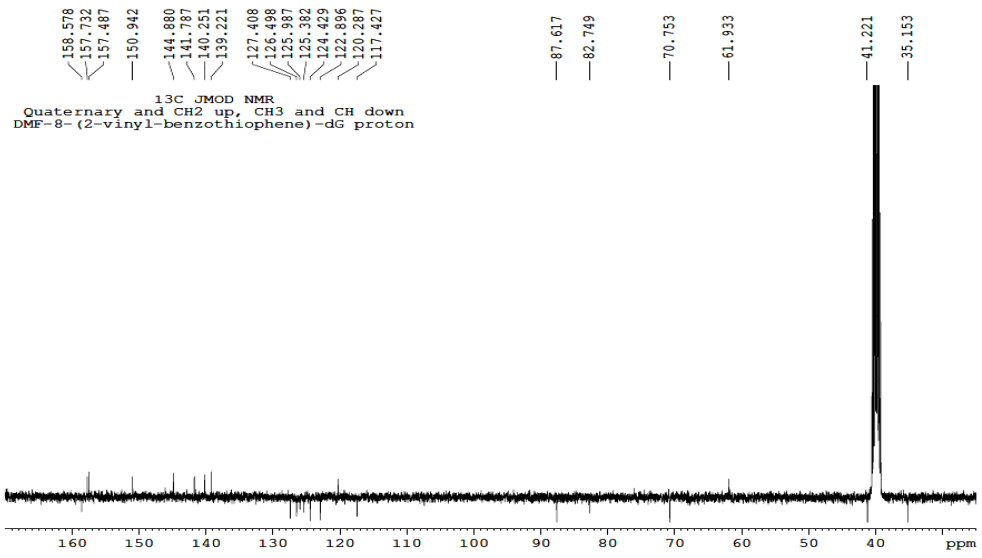

Fig S9 ^{13}C NMR spectrum of **3** in DMSO-d_6

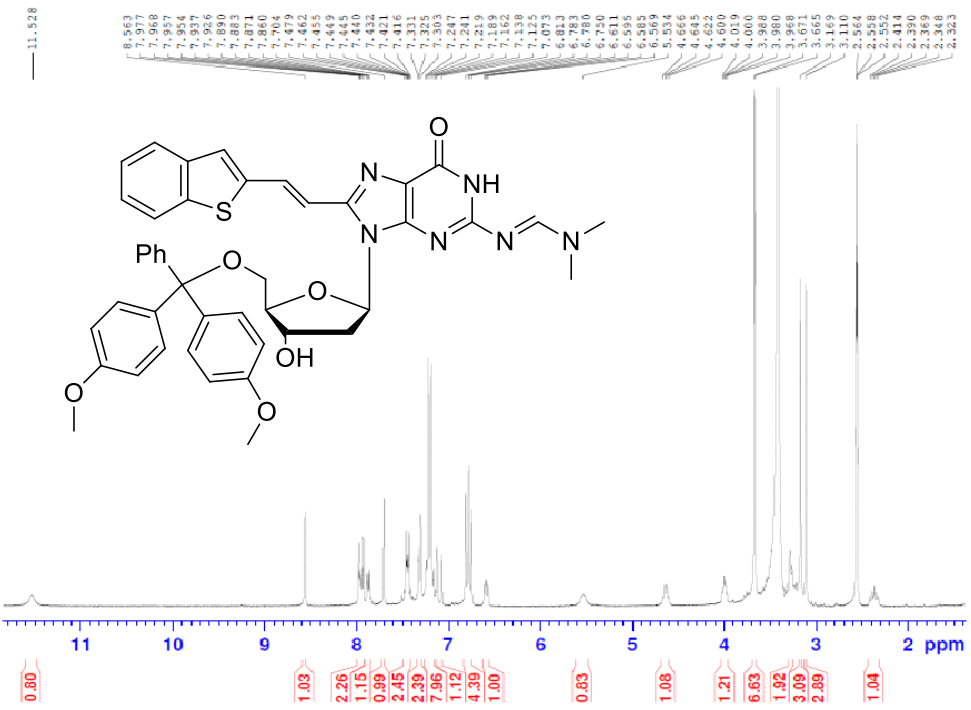

Fig S10 ^{1}H NMR spectrum of **4** in $CDCl_3$

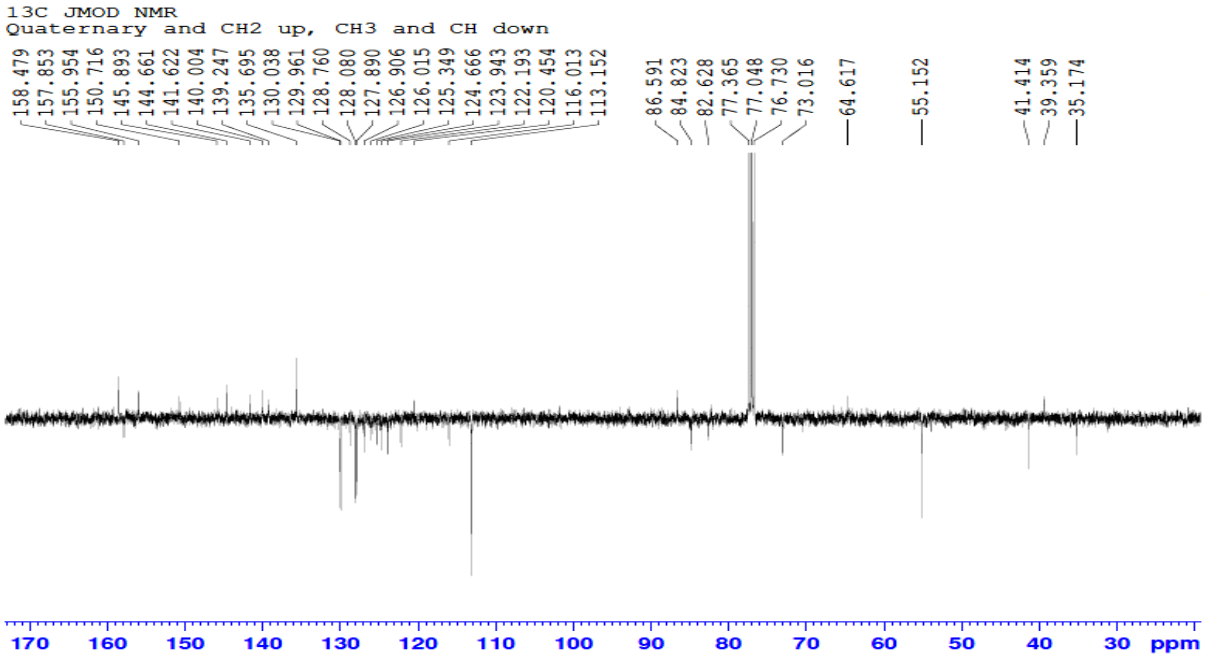

Fig S11 ^{13}C NMR spectrum of **4** in $CDCl_3$

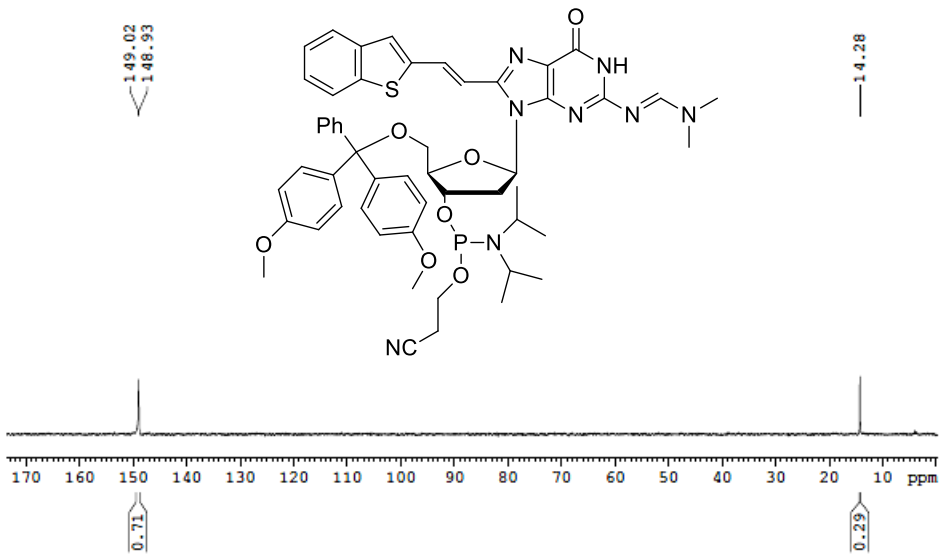

Fig S12 ^{31}P NMR spectrum of **5** in $CDCl_3$

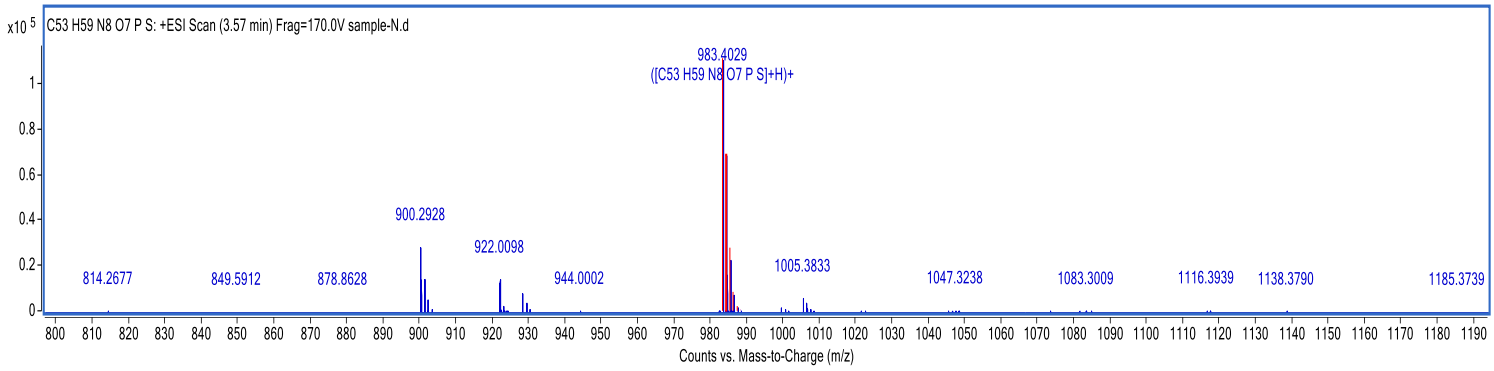

Fig S13 ESI$^+$-HRMS spectrum of **5**

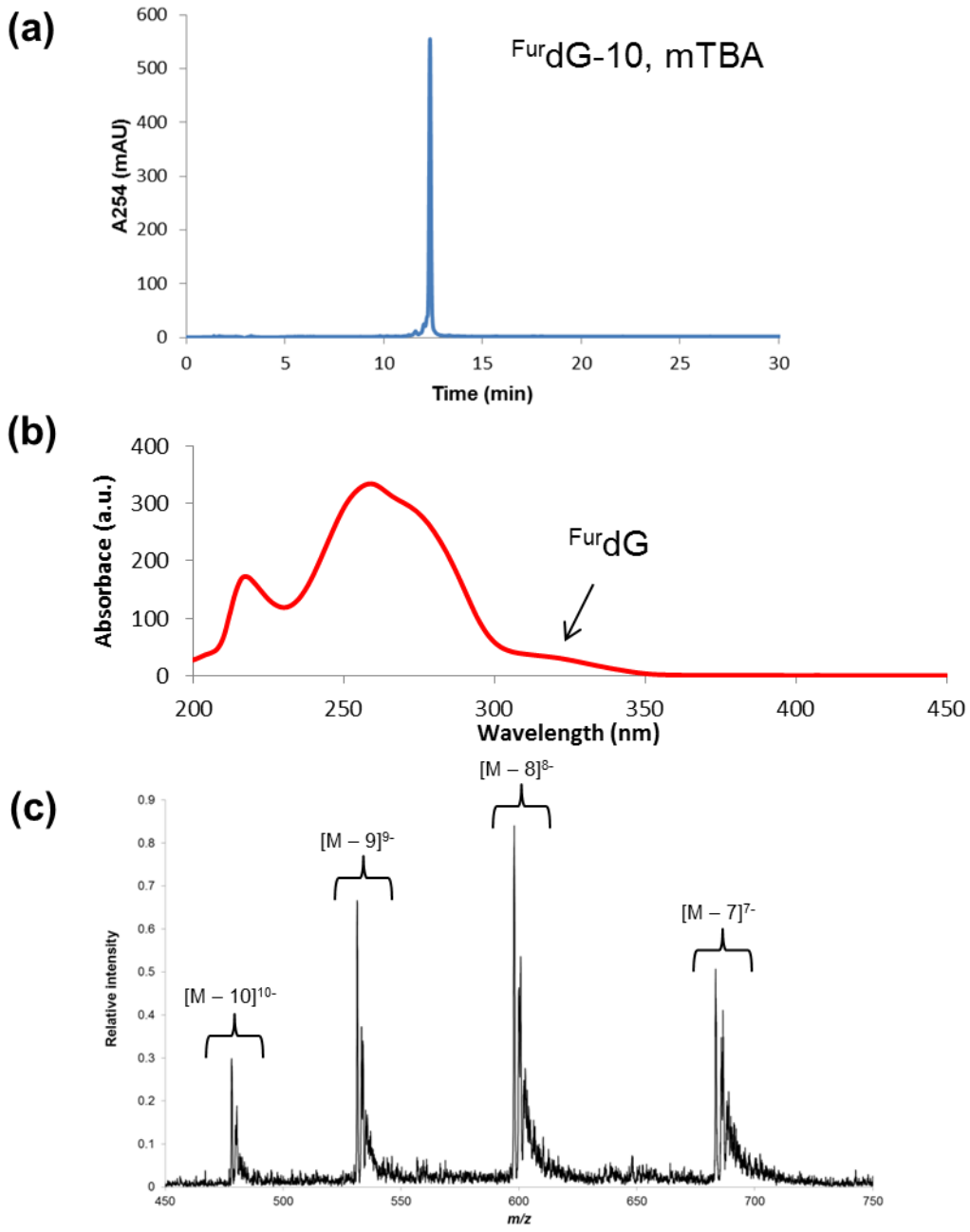

Fig S14 HPLC, UV-vis and ESI-MS analysis of ^{Fur}dG-10, mTBA, (a) analytical RP-HPLC chromatogram, (b) UV-vis spectrum, (c) ESI-MS spectrum

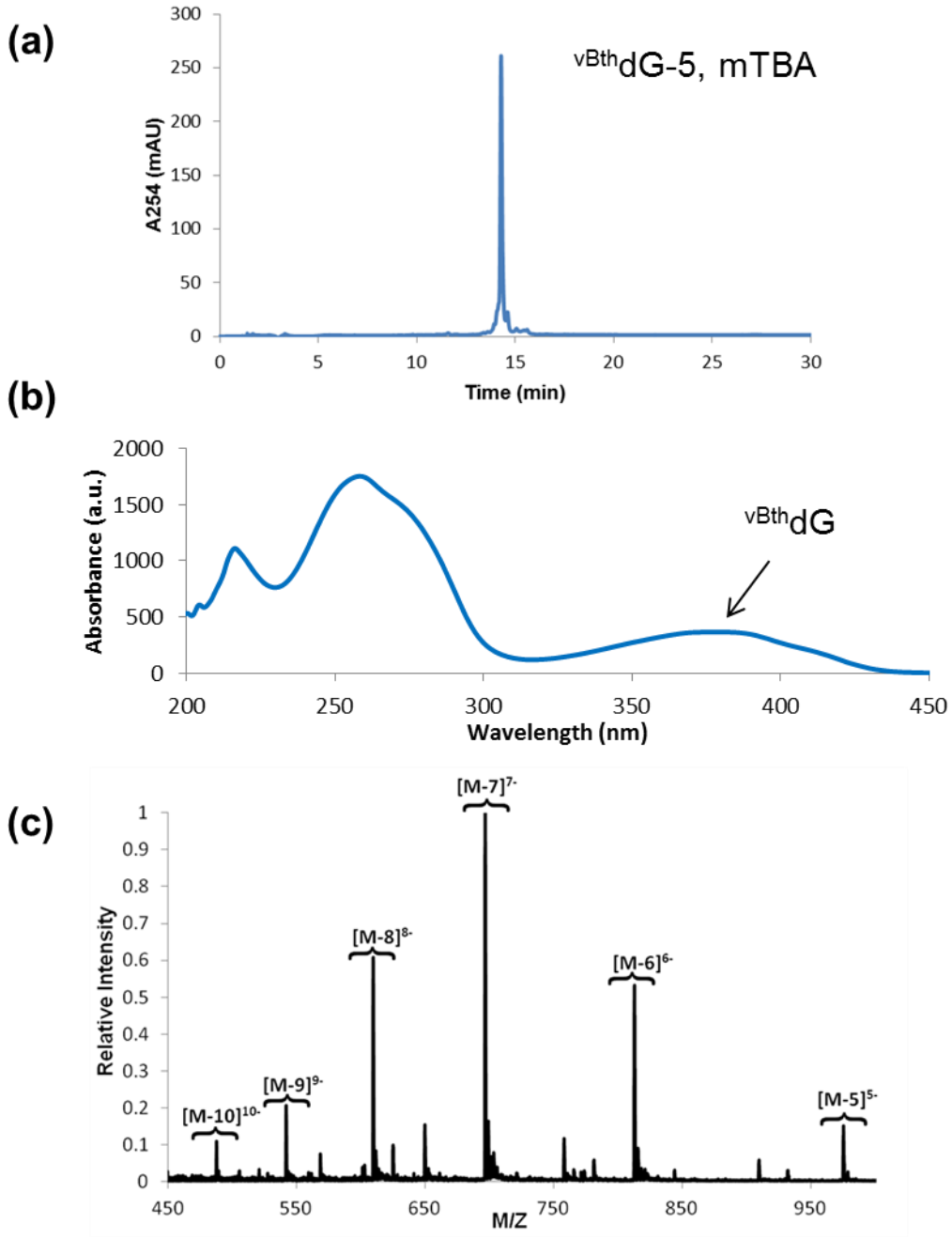

Fig S15 HPLC, UV-vis and ESI-MS analysis of ^{vBth}dG-5, mTBA, (a) analytical RP-HPLC chromatogram, (b) UV-vis spectrum, (c) ESI-MS spectrum

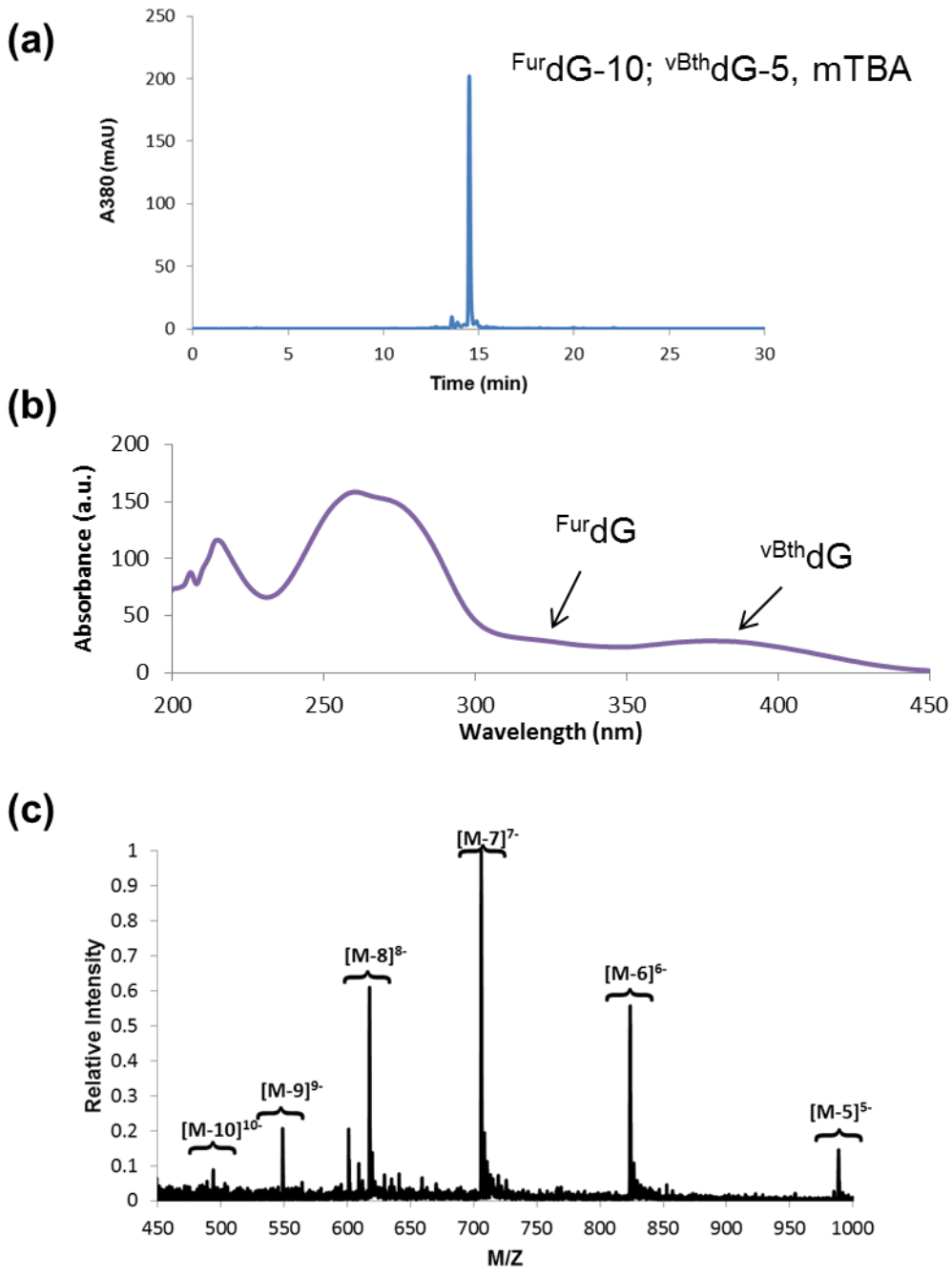

Fig S16 HPLC, UV-vis and ESI-MS analysis of ^{Fur}dG-10; ^{vBth}dG-5, mTBA, (a) analytical RP-HPLC chromatogram, (b) UV-vis spectrum, (c) ESI-MS spectrum