Base-Modified PNA-Graphene Oxide Platform as a Turn-On Fluorescence Sensor for the Detection of Human Telomeric Repeats

Pramod M. Sabale, Jerrin Thomas George, Seergazhi G. Srivatsan*

Department of Chemistry, Indian Institute of Science Education and Research, Dr. Homi Bhabha Road, Pashan, Pune 411008, India

Electronic Supplementary Information

1. Materials

Di-*tert*-butyl dicarbonate, ethylenediamine, ethyl bromoacetate, TFA, chloroacetyl chloride and sodium nitrate were purchased from Spectrochem. Sulphuric acid, hydrochloric acid, hydrogen peroxide and $KMnO_4$ were purchased from Rankem India. Barium chloride was purchased from Merck, India. 5-Iodouracil, benzo[*b*]furan and *bis*(triphenylphosphine)-palladium (II) chloride, HOBt, HBTU, *N*,*N*-diisopropylethylamine (DIPEA), 1,2-ethanedithiol, thioanisole, trifluoromethanesulfonic acid (TFMSA), and graphite flakes were obtained from Sigma-Aldrich. Boc-protected *aeg*-PNA monomers were purchased from ASM Research Chemicals. 4-Methylbenzhydrylamine•HCl (MBHA) resin LL (100-200 mesh) was obtained from Novabiochem. DNA oligonucleotides were purchased from Integrated DNA Technologies, Inc. Oligonucleotides were purified by polyacrylamide gel electrophoresis (PAGE) under denaturing conditions and desalted on Sep-Pak Classic C18 cartridges (Waters Corporation). Autoclaved water was used in all biochemical reactions and fluorescence measurements.

2. Instrumentation

NMR spectra were recorded on a 400 MHz Jeol ECS-400. All mass measurements were recorded on an Applied Biosystems 4800 Plus MALDI-TOF/TOF analyzer and Water Synapt G2 high definition mass spectrometers. PNA oligomers were purified using Dionex ICS 3000 HPLC. Absorption spectra were recorded on a Shimadzu UV-2600 spectrophotometer. UV-thermal melting analysis of duplexes was performed on a Cary 300Bio UV-Vis spectrophotometer. Steady-state fluorescence experiments were carried out in a micro fluorescence cuvette (Hellma, path length 1.0 cm) on a Fluoromax-4 spectrofluorometer (Horibha Scientific). Time-resolved fluorescence experiments were carried out on a TCSPC instrument (Horiba JobinYvon, Fluorolog 3). All CD spectra were recorded on JASCO J-815 CD spectrometer. Powder X-ray diffraction spectrum of GO was recorded using Bruker D8 Advance X-ray powder diffractometer (Cu Kα radiation, λ = 1.541Å). Raman spectra (532 nm excitation) were recorded using LabRAM HR 800 Raman spectrometer (Horiba Scientific).

3. MALDI-TOF mass measurements

Molecular weight of PNA oligomers were determined using Applied Biosystems 4800 Plus MALDI-TOF/TOF analyser. 1 μL of a ~150 μM stock solution of PNA oligomer was combined with 2,5-dihydroxybenzoic acid (DHB) or α-cyano-4-hydroxycinnamic acid (CHCA) matrix. The samples were spotted on a plate and air dried before mass analysis. For MALDI-TOF mass analysis of PNA oligomers see Table S1.

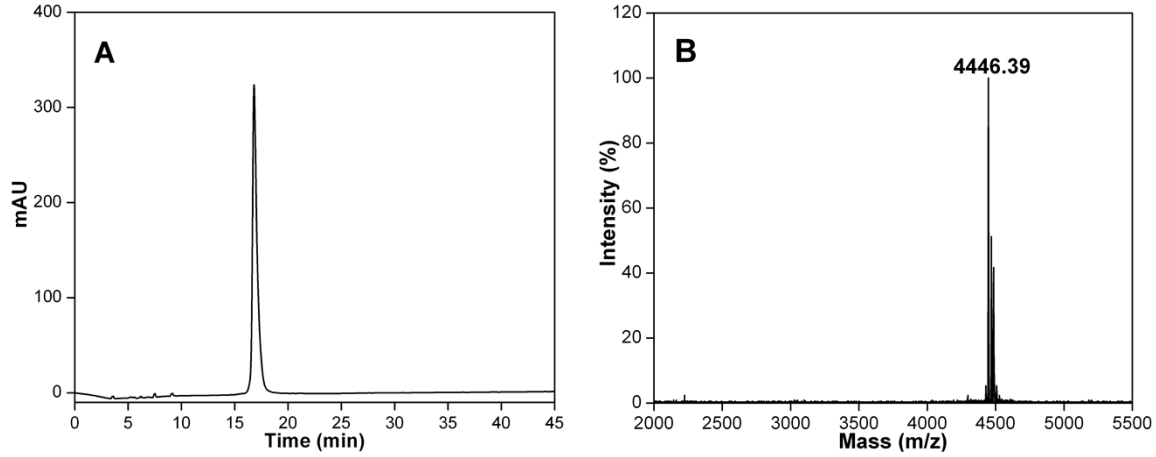


Fig. S1 Representative HPLC and MALDI-TOF mass spectrum of a modified PNA oligomer. (**A**) RP-HPLC chromatogram of PNA oligomer **7**. Mobile phase A: 5% acetonitrile in H_2O containing 0.1% TFA. Mobile phase B: 50% acetonitrile in H_2O containing 0.1% TFA. Flow rate: 3 mL/min. Gradient: 0–50% B in 25 min and 50–100% B in 20 min. (**B**) MALDI-TOF mass spectrum of PNA oligomer **7.** Calculated for $[M+H]^+$ = 4446.33 and observed = 4446.39. See Table S1 for mass data.

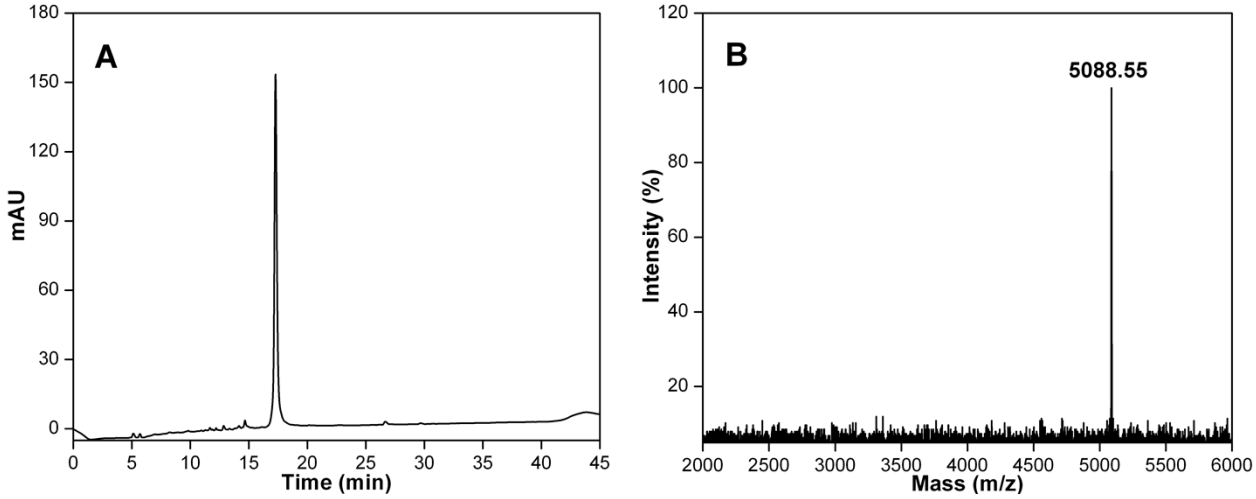


Fig. S2 (**A**) RP-HPLC chromatogram of PNA telomere probe **14.** Mobile phase A: 5% acetonitrile in H_2O containing 0.1% TFA, mobile phase B: 50% acetonitrile in H_2O containing 0.1% TFA. Flow rate: 3 mL/min. Gradient: 0–50% B in 25 min and 50–100% B in 20 min. (**B**) MALDI-TOF mass spectrum of PNA telomere probe **14**. Calculated for $[M+H]^+$ = 5088.01 and observed = 5088.55.

Table S1 ε_{260} and MALDI-TOF mass analysis of PNA oligomers

PNA	Sequence[a]	ε_{260} ($M^{-1}cm^{-1}$)	Calculated mass	Observed mass
6	CGATCAGTGACTAGCKK	15.4 x 10^4	4344.24 $[M+H]^+$	4344.67
7	CGATCAG**5**GACTAGCKK	15.7 x 10^4	4446.33 $[M+H]^+$	4446.39
13	CCCTAACCCTAACCCTAAKK	16.7 x 10^4	5007.90 $[M+Na]^+$	5007.15
14	CCC**5**AACCCTAACCCTAAKK	17.1 x 10^4	5088.01 $[M+H]^+$	5088.55

[a]PNA sequences are written from N to C terminus.

4. Quantum yield determination of PNA monomer 5 in various solvents

Quantum yield of PNA monomer **5** in different solvents relative to 2-aminopurine standard was determined using the following equation.

$$\Phi_{F(x)} = (A_s/A_x)\,(F_x/F_s)\,(n_x/n_s)^2\,\Phi_{F(s)}$$

Where s is the standard, x is the PNA monomer, A is the absorbance at excitation wavelength, F is the area under the emission curve, n is the refractive index of the solvent, and Φ_F is the quantum yield.

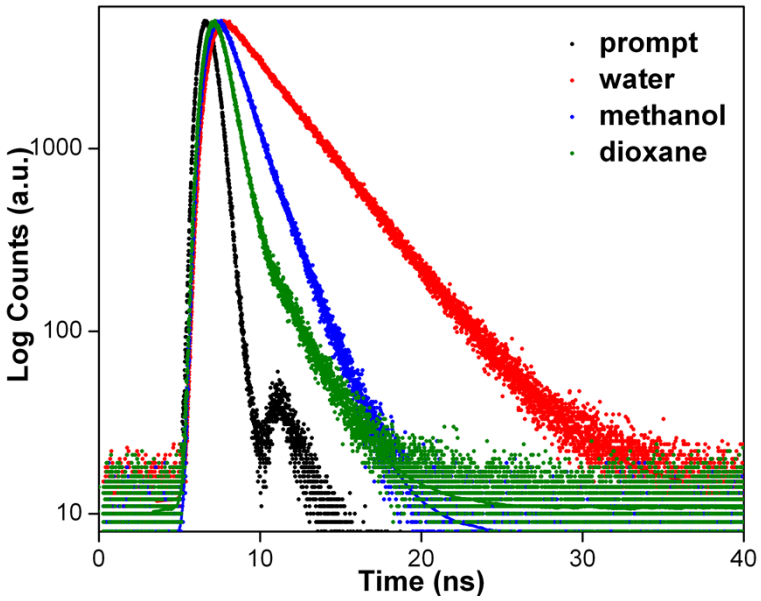


Fig. S3 Excited state decay profile of PNA monomer **5** (5.0 μM) in solvents of different polarity. Samples were excited using 320 nm LED source. Laser profile is shown in black (prompt). Curve fits are shown in solid lines.

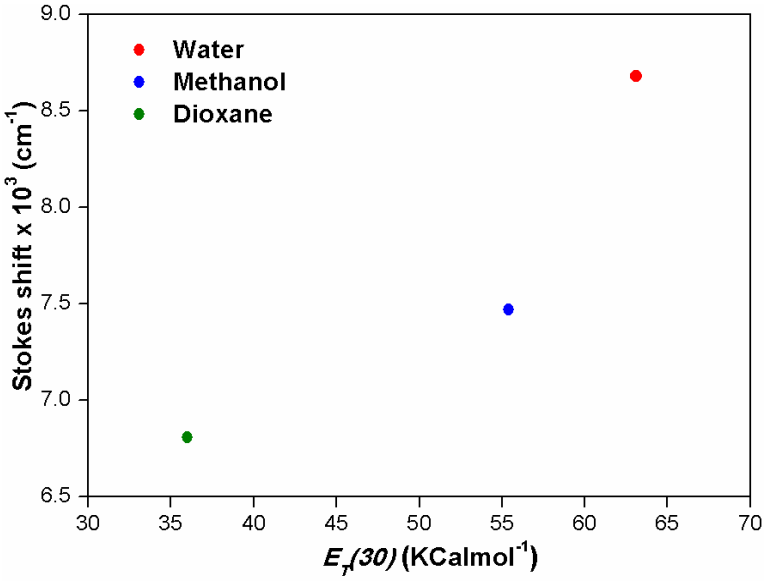


Fig. S4 Plot of Stokes shift versus $E_T(30)$ (microscopic solvent polarity parameter) for PNA monomer **5**.

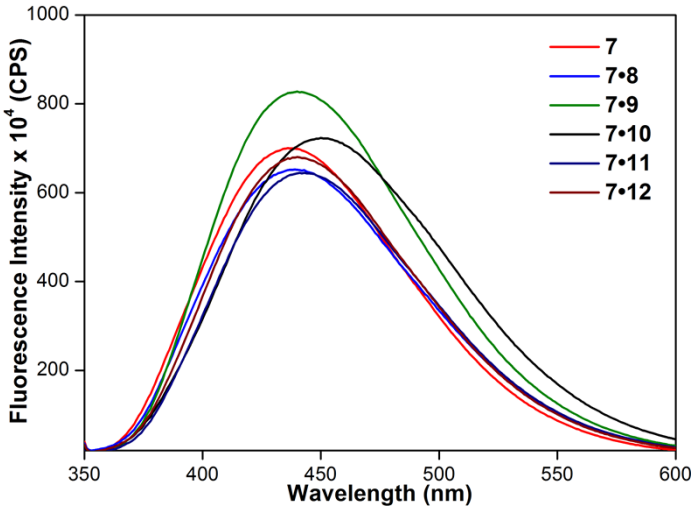


Fig. S5 Fluorescence spectra of modified PNA oligomer **7** (2.0 µM) and corresponding duplexes (2.0 µM) in 10 mM phosphate buffer (pH 7.1, 100 mM NaCl, 0.1 mM EDTA). Samples were excited at 330 nm, and excitation and emission slit widths were maintained at 10 nm and 12 nm respectively. For emission maximum of PNA **7** and duplexes made of **7** see Table S2.

Table S2 Emission maximum of PNA **7** and duplexes made of **7**

Sample	λ_{em}	$I_{rel}{}^a$
7	436	1.00
7•8	439	0.93
7•9	440	1.18
7•10	450	1.03
7•11	443	0.92
7•12	440	0.97

[a]Relative emission intensity with respect to ssPNA **7**

Table S3 T_m values of control and modified PNA-DNA and DNA-DNA duplexes

Control unmodified duplex	T_m (°C)	Fluorescently modified duplex	T_m (°C)
6•8	77.0 ± 0.1	**7•8**	73.3 ± 0.5
13•15	71.2 ± 0.8	**14•15**	71.0 ± 1.0
15•18	59.3 ± 0.9		

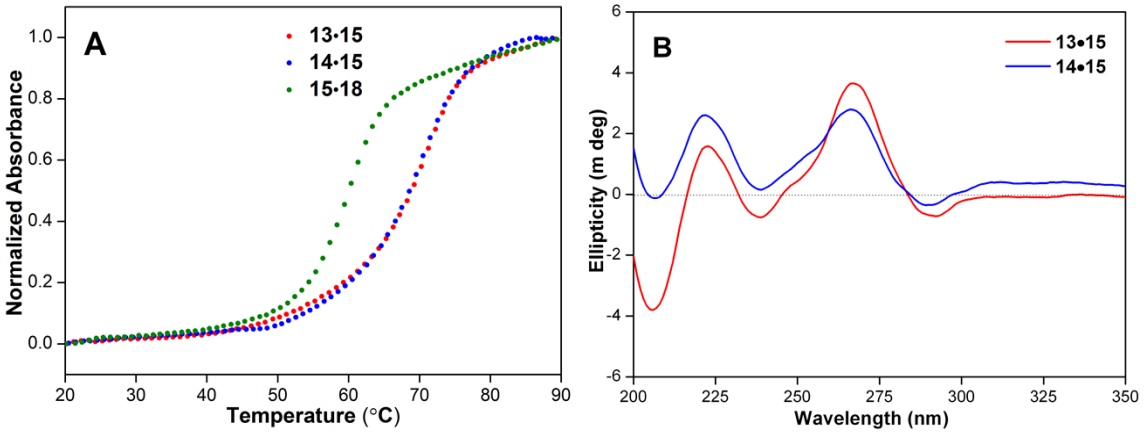


Fig. S6 (**A**) UV-thermal melting analysis (2.0 µM) of control unmodified (**13•15**) and fluorescently modified (**14•15**) PNA-DNA duplexes and corresponding DNA-DNA duplex (**15•18**) in 10 mM phosphate buffer (pH 7.1, 100 mM NaCl, 0.1 mM EDTA). For T_m values see Table S3. (**B**) CD spectra (5.0 µM) of control unmodified **13•15** and fluorescently modified **14•15** duplexes in 10 mM phosphate buffer (pH 7.1, 100 mM NaCl, 0.1 mM EDTA). A 1:1 mixture of PNA and DNA in the above mentioned buffer was heated at 90 ºC for 3 min and cooled slowly to room temperature. The samples were placed on crused ice bath for at least 1 hr before thermal melting and CD analyses.

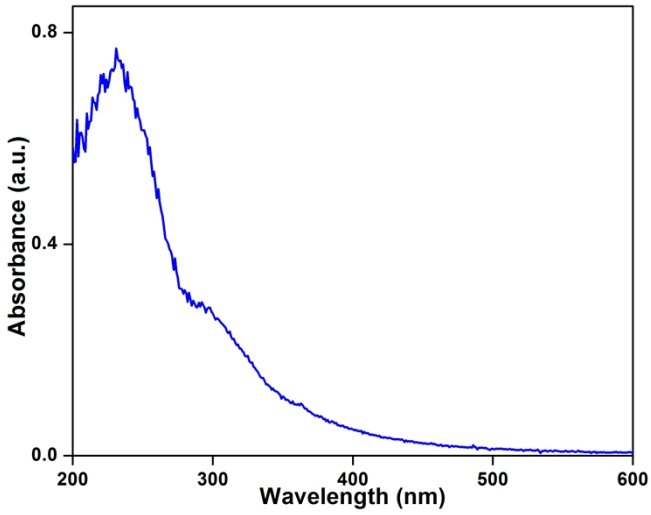


Fig. S7 UV-Vis absorption spectrum of GO dispersion in water. A peak at ~235 nm and a shoulder at ~292 nm match with the literature report for GO dispersion in water.[S1]

Table S4 Emission maximum of PNA probe **14**, PNA-GO complex (**14**-GO) and PNA-GO complex in the presence of telomere target **15** and control DNA **8**.

Sample[a]	λ_{em} (nm)	I_{rel}[b]
14	409	2.8
14-GO	409	1.0
14-GO + **15**	422	3.7
14-GO + **8**	406	1.2
GO + **14•15**	422	4.8
GO + **14•8**	406	1.1
14•15	427	4.9

[a]Duplexes **14•15** and **14•8** were prepared by heating a mixture of PNA **14** (100 nM) and DNA **15** or **8** (1.0 μM) in 10 mM phosphate buffer (pH 7.1, 100 mM NaCl, 0.1 mM EDTA) at 90 °C for 3 min. Samples were cooled to RT slowly and kept on an ice bath for ~60 min. [b]Relative emission intensity with respect to PNA-GO complex.

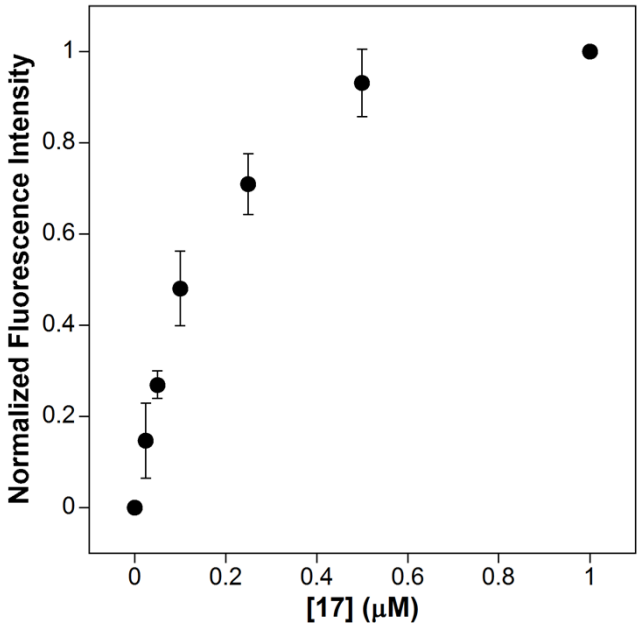


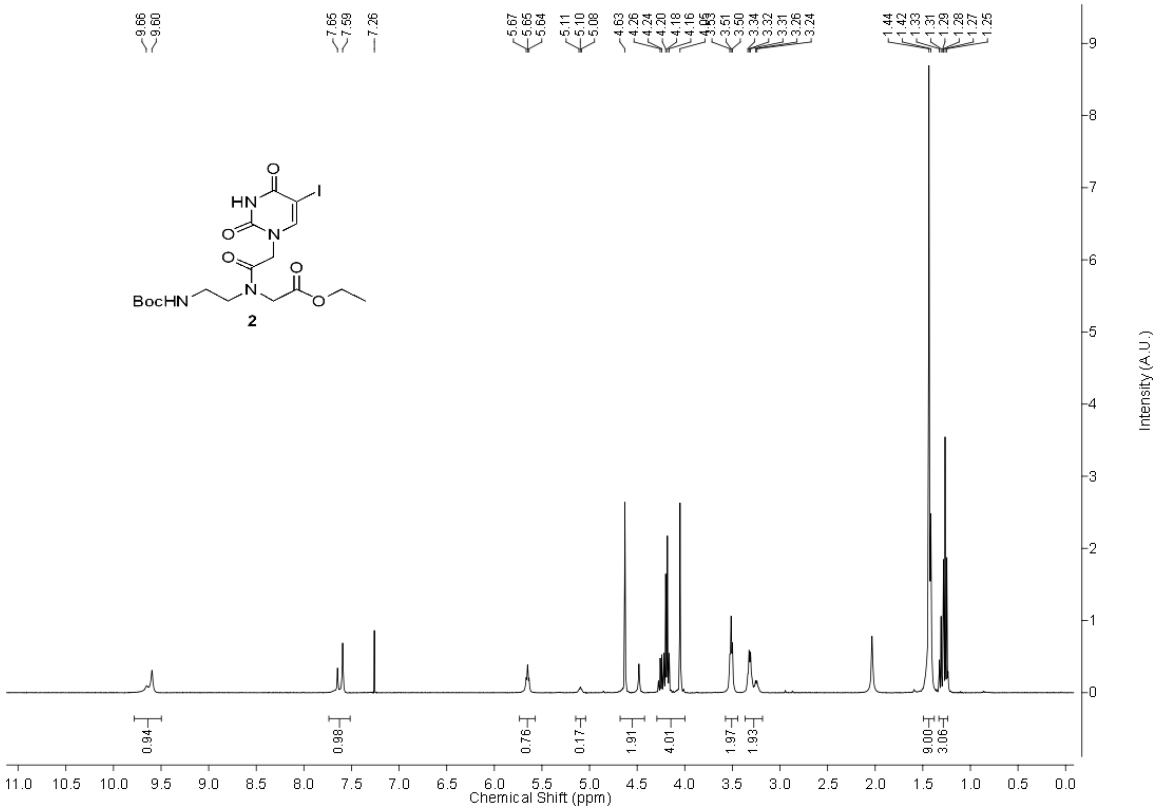
Fig. S8 A plot of normalized fluorescence intensity at λ_{em} = 422 nm versus increasing concentration of telomere DNA **17.** Conc. of PNA **14** (50 nM), GO (0.5 μg/mL) and DNA **17** (25−1000 nM). Samples were excited at 330 nm and excitation and emission slit widths were kept at 10 nm and 12 nm, respectively. A linear correaltion was obatined until 100 nM of DNA **17** ($y = 4.74x + 0.02$, $R^2 = 0.99$). The detection limit was calculated based on three times the signal-to-noise level using the following equation.[S2]

$$\text{Detection limit} = 3\sigma/S$$

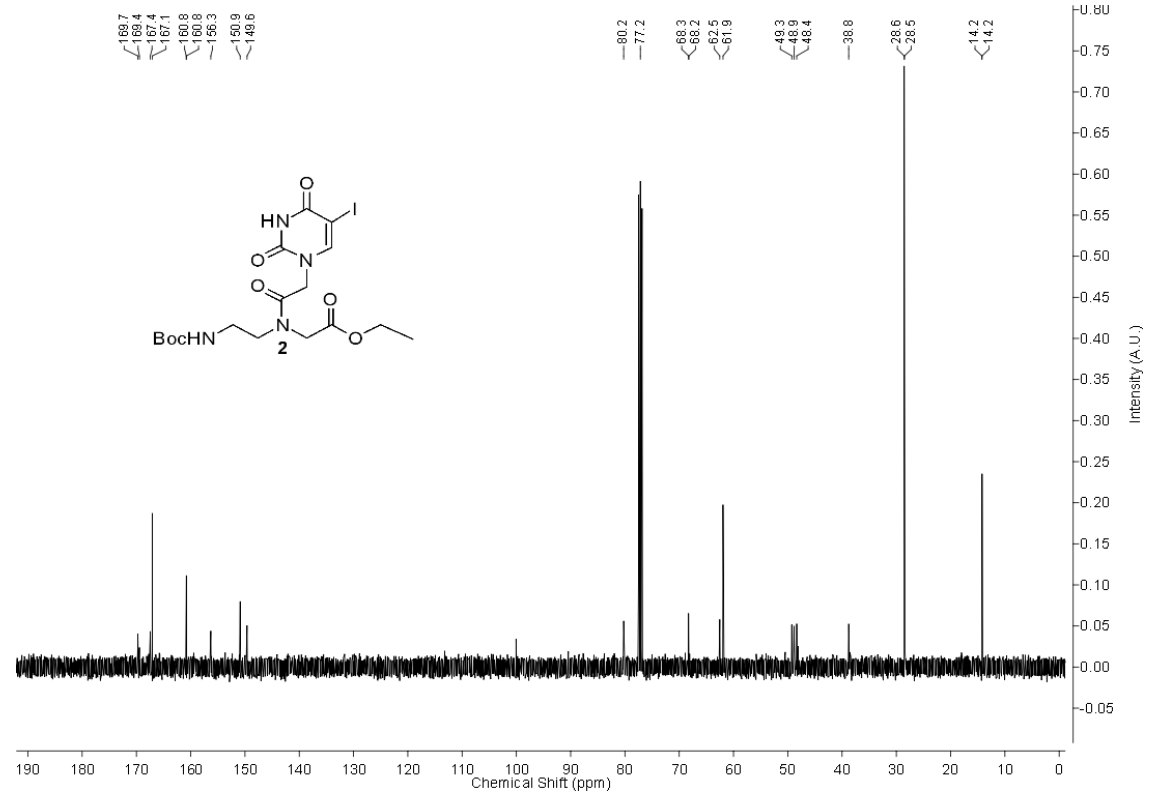
Where, σ is the variation in fluoresence intensity of PNA-GO complex in the absence of telomere DNA target and S is the slope of the linear fit.

5. NMR Spectra

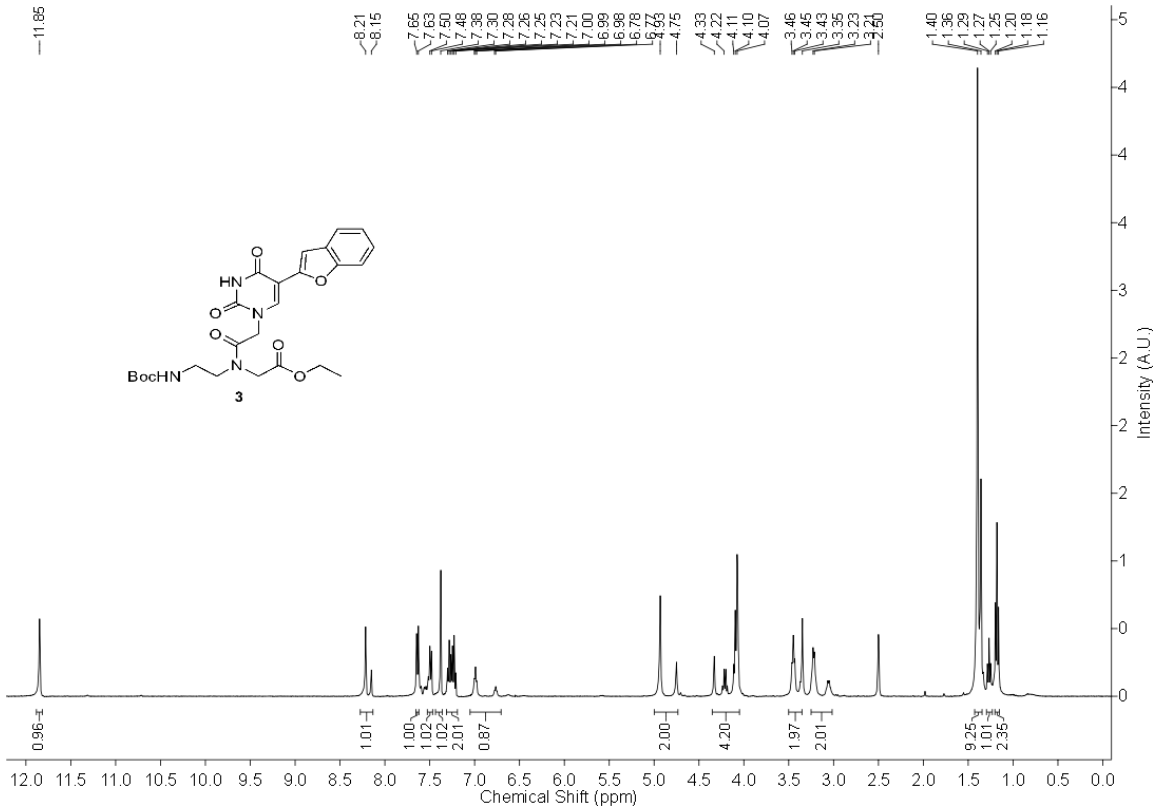
^{1}H-NMR of compound **2** in $CDCl_3$



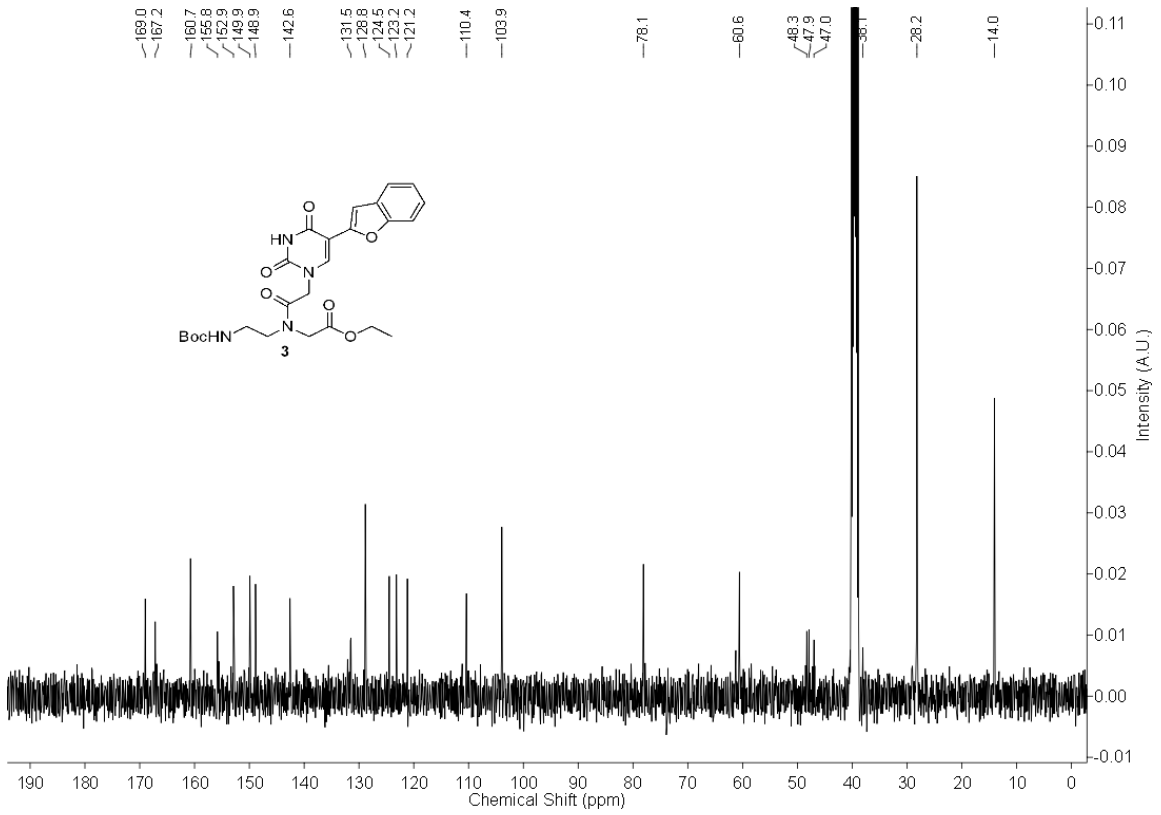
^{13}C-NMR of compound **2** in $CDCl_3$



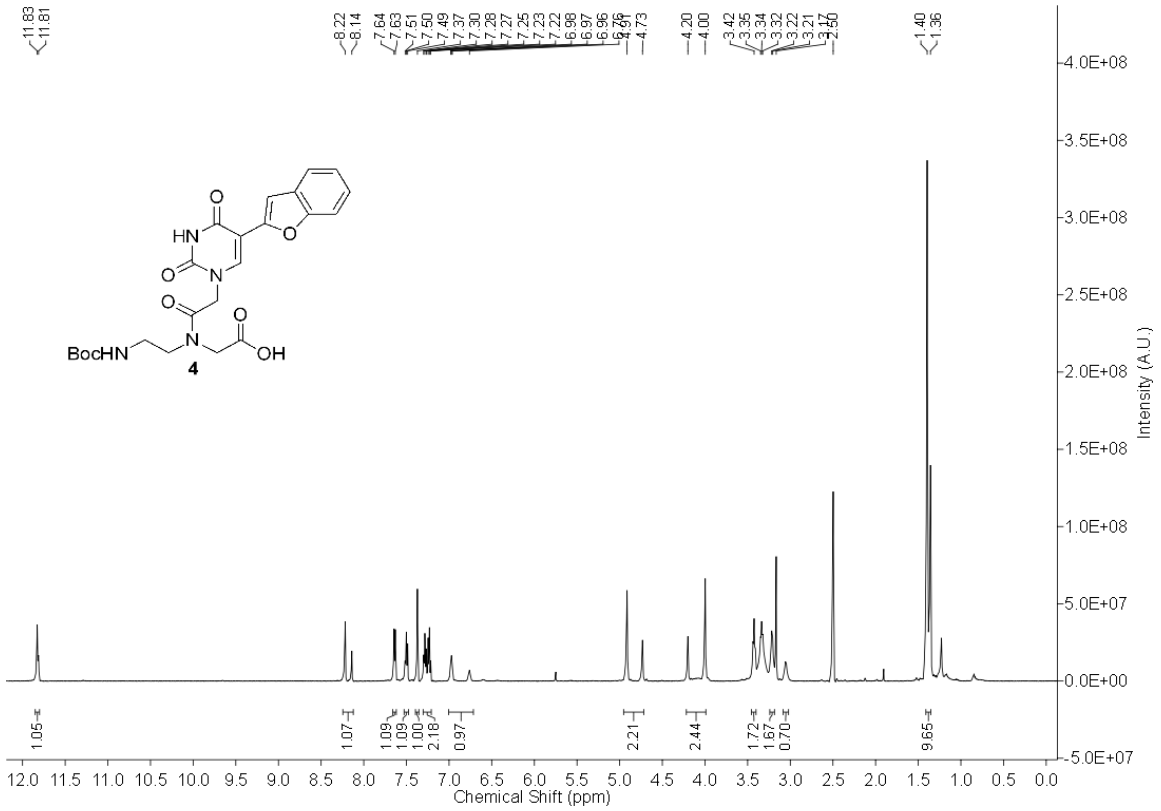
[1]H-NMR of compound **3** in d_6-DMSO



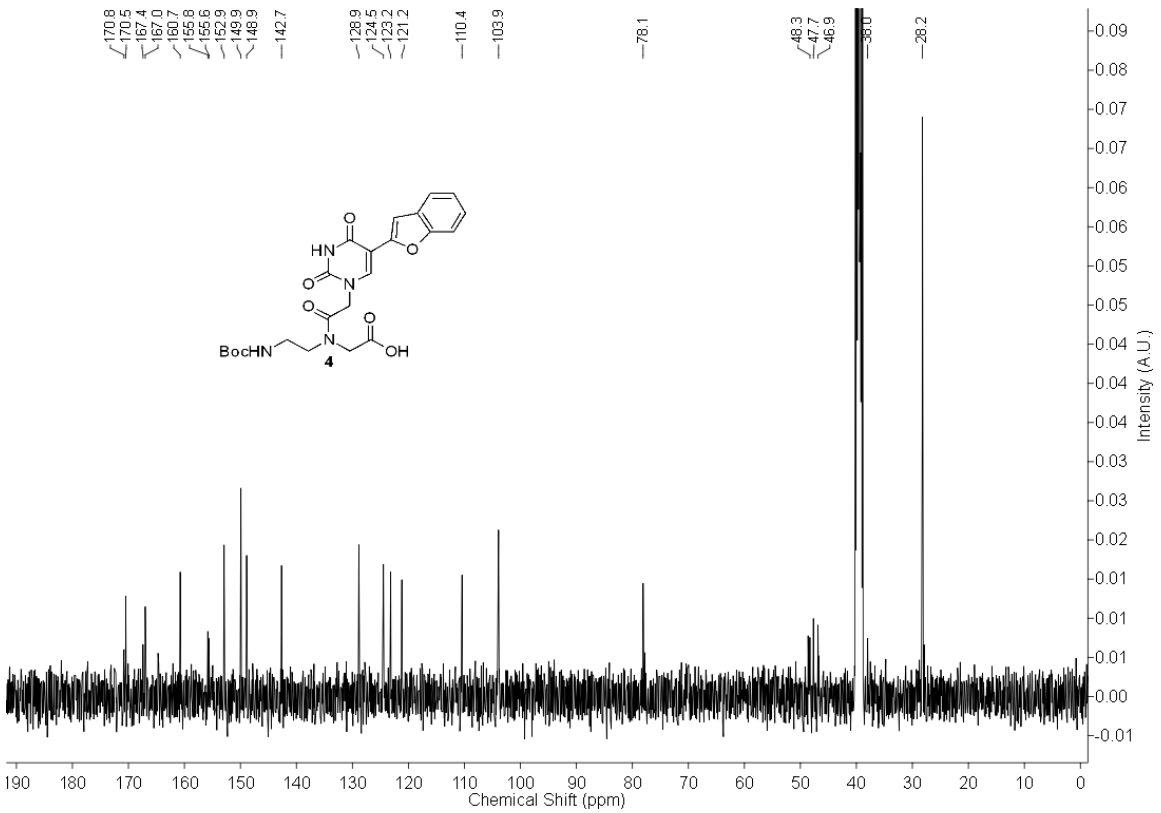
[13]C-NMR of compound **3** in d_6-DMSO



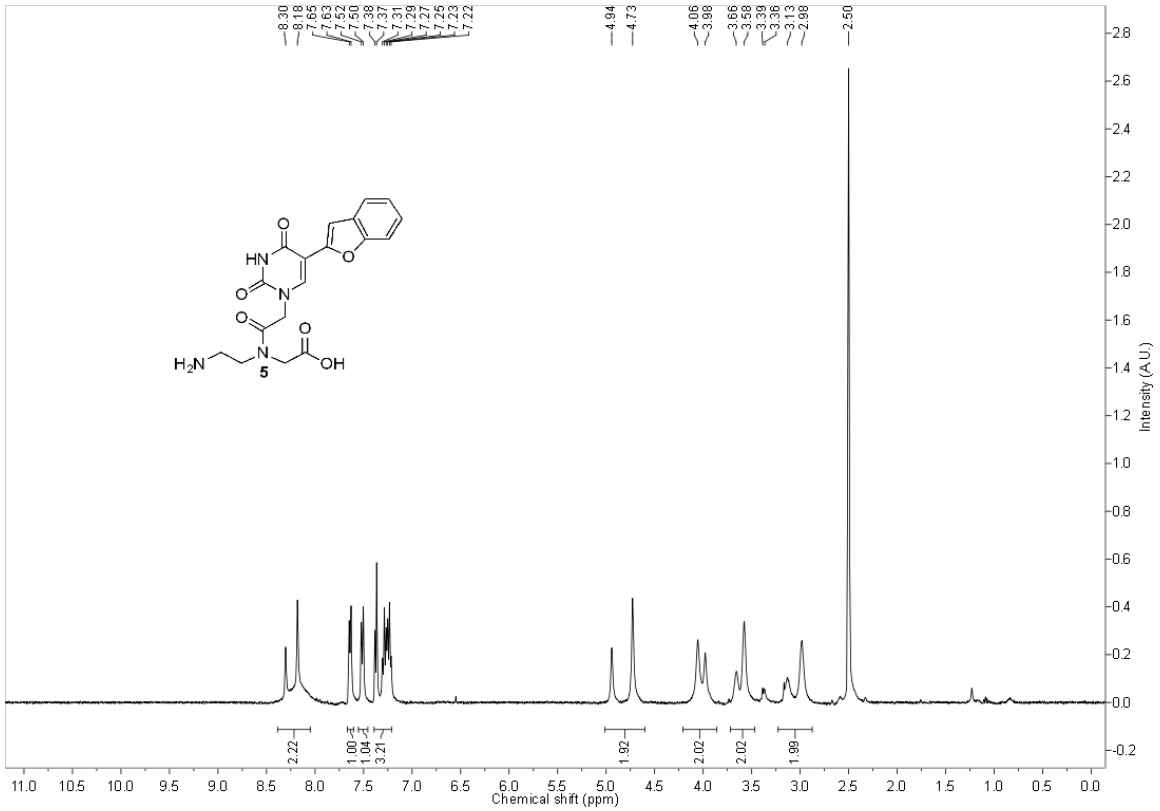
^{1}H-NMR of compound **4** in d_6-DMSO



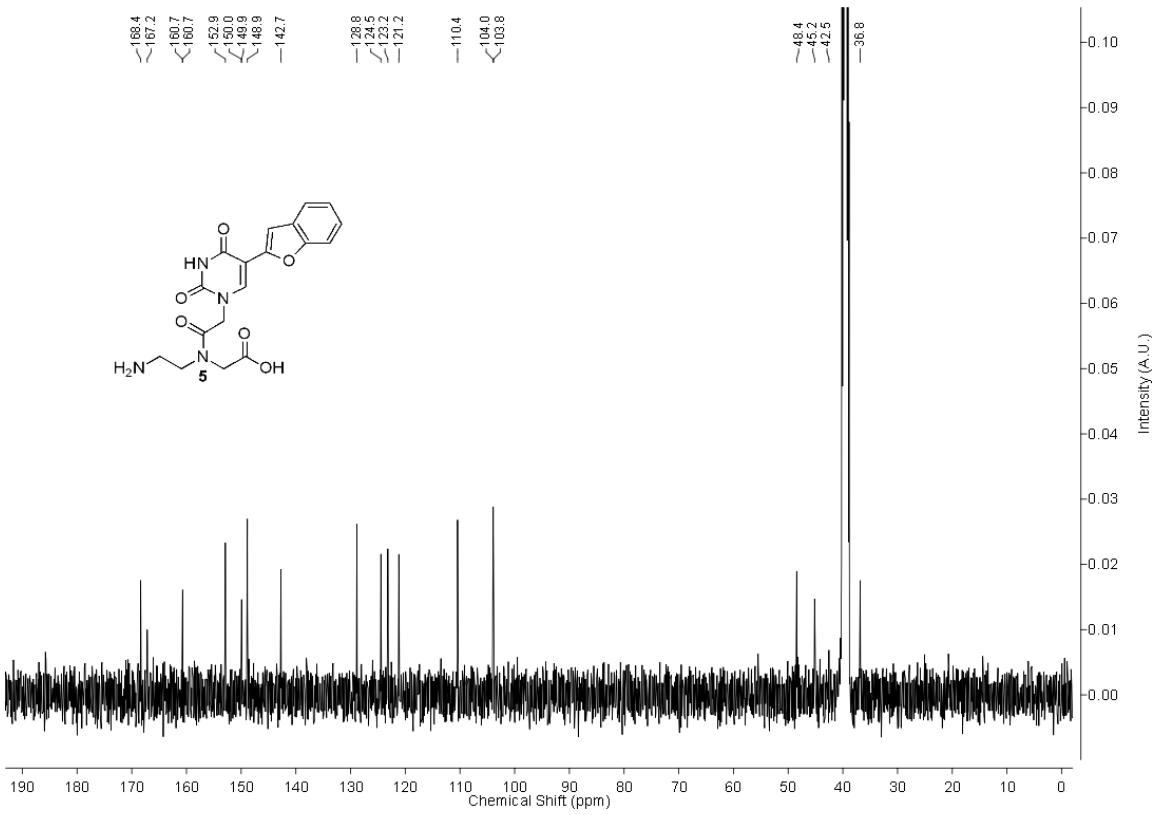
^{13}C-NMR of compound **4** in d_6-DMSO



^{1}H-NMR of compound **5** in d_6-DMSO



^{13}C-NMR of compound **5** in d_6-DMSO



6. References

S1. Z. Luo, Y. Lu, L. A. Somers and A. T. C. Johnson, *J. Am. Chem. Soc*., 2009, **131**, 898–899.
S2. (*a*) C.-H. Lu, H.-H. Yang, C.-L. Zhu, X. Chen and G.-N. Chen, *Angew. Chem. Int. Ed.*, 2009, **48**, 4785–4787; (*b*) P.-J. J. Huang and J. Liu, *Anal. Chem.*, 2012, **84**, 4192−4198; (*c*) S. Guo, D. Du, L. Tang, Y. Ning, Q. Yao and G.-J. Zhang, *Analyst*, 2013, **138**, 3216–3220.