Supporting Information

A naphthalene diimide dyad for fluorescence switch-on detection of G-quadruplexes

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Supplemental Experimental Procedures

Materials and General Procedures.

Reagents, solvents and chemicals were purchased from Alfa Aesar, or Sigma-Aldrich and were used as supplied without further purification. TLC analysis was carried out on silica gel (Merck 60F-254) with visualization at 254 and 366 nm. HPLC analysis and purifications were performed using two different HPLC: Waters system combining a Delta 600 PUMP, a 2489 UV/VIS detector and Fraction Collector III (for preparative and analytical) and an Agilent system SERIES 1260 (for analytical). The analytical column was XSelect CSH Phenyl-Hexyl (150 x 4.6 mm) (Waters). The preparative column was XSelect CSH Prep Phenyl-Hexyl 5μm (150 x 30 mm) (Waters). Flows were 1 ml/min or 1.4 ml/min for analytical and 27 ml/min for preparative. For the analytical analysis were used the two following method A, B: (Aqueous solvent: 0.1% trifluoroacetic acid in water; Organic solvent: Acetonitrile; Method A= 1 ml/min, Gradient: 95% aqueous, gradually to 55% aqueous over 12 minutes and at the end an isocratic flow over 4 minutes; Method B= 1.4 ml/min, Gradient: 90% aqueous, gradually to 75% aqueous over 20 minutes and at the end an isocratic flow over 4 minutes).Preparative HPLC were performed using un upgrade of the analytical method. ^{1}H-, ^{13}C-NMR spectra were recorded on a Bruker ADVANCE 300 MHz spectrometer. The potentiometric titrations were made with a Radiometer TitraLab 90 titration system. UV/Vis spectra were run on a Varian Cary 100 SCAN spectrophotometer with quartz cuvettes of the appropriate path length (0.1-1 cm) at 25.0 ± 0.1 °C. Emission spectra were recorded on a Perkin Elmer LS 50B instrument.

Synthesis of intermediates and final ligands:

The NDIs**2**, **3** and**4**have been synthesized according to the publishedprocedure.[S1] For the following nucleophilic aromatic substitution (S_NAr) was performed an efficient protocol, in order to synthesized the ligand **6** as major product with good yield.

Nucleophilic Aromatic Substitution: Synthesis of 5 and 6. The NDI **4** (0.5 mmol) was dissolved into 40 ml of acetonitrile in a round bottom flask together with 1.5 mmol of 1,7-diaminoheptane, the mixture was stirred at 85°C for 5 h under argon. The resulting red solution was concentrated under vacuum and a red solid was obtained. The crude product was analysed by HPLC chromatography, (CH_3CN:H_2O 0.1%TFA) according to analytical method A, afforded the

NDIs**5**(20%) and**6**(80%).The reaction mixture was used without further purification for the next reductive debromination step.

***N,N*'-Bis-((dimethylamino)propylamino)-2-bromo-6-((amino)heptylamino)-1,4-5,8-naphthalenetetracarboxylic bisimide trihydrochloride**(**5**·3HCl)**.**The collected crude from the previous step was purified by preparative HPLC chromatography (CH_3CN:H_2O 0.1%TFA), HCl 1M solution was added to each chromatographic portion. Solvent evaporation under vacuum afforded the product. Red solid; m.p. dec.>200°C. ^{1}H-NMR (300 MHz, CD_3OD):8.05 (s, 1H), 7.79 (s, 1H), 4.13 (bs, 2H); 4.06 (bs, 2H), 3.58 (s, 2H), 3.36 (bs, 4H); 3.00 (bs, 14H); 2.21 (bs, 4H); 1.91 (bs, 2H); 1.81 (bs, 2H); 1.60 (bs, 6H).^{13}C-NMR (75 MHz, CD_3OD):166.6; 162.9; 162.8; 162.2; 152.6; 138.2; 129.1; 127.8; 123.7; 123.6; 121.8; 121.6; 121.0; 100.0;57.0; 56.8; 44.6; 43.9; 41.1; 39.7; 39.0; 30.5; 30.3; 28.9; 28.3; 27.8; 24.9; 24.8.

Mild reductive dehalogenation for the synthesis of 6. The crude(400 mg) was suspended in a solution of 15 ml of acetonitrile and 15 ml of water, out-gassed by argon and stirred at r.t.. Sodium dithionite (500 mg) was added and the new mixture was stirred. After 2 hours the reaction was worked up bubbling molecular oxygen, then the solution was basified with $NaHCO_3$ and extracted with three portions of $CHCl_3$. The organic layer was dried under vacuum to give the pure red product**6**.

***N,N*'-Bis-((dimethylamino)propylamino)-6-((amino)heptylamino)-1,4-5,8-naphthalenetetracarboxylic bisimide trihydrochloride**(**6**·3HCl)**.** The collected solid was purified by preparative HPLC chromatography (CH_3CN:H_2O 0.1%TFA), HCl 1M solution was added to each chromatographic portion. Solvent evaporation under vacuum afforded the product. Red solid; m.p. dec.>200°C. ^{1}H-NMR (300 MHz, CD_3OD):7.87 (d, *J*=7.7 Hz, 1H), 7.62 (d, *J*=7.7 Hz, 1H), 7.48 (s, 1H), 4.04-3.98 (m, 4H); 3.98-3.34 (m, 6H),3.01 (bs, 14H); 2.18 (bs, 4H); 1.84 (bs, 4H); 1.60 (bs, 6H).^{13}C-NMR (75 MHz, CD_3OD): 166.5; 164.1; 163.7; 163.6; 152.9; 131.9; 129.6; 128.1; 126.4; 125.1; 123.3; 120.6; 119.4; 99.5; 56.9; 56.8; 44.6; 43.9; 41.3; 41.2; 39.3; 38.7; 30.5; 30.3; 28.8; 28.3; 27.8; 24.9.

Nucleophilic Aromatic Substitution: Synthesis of 7. The NDI **6** (0.25 mmol) was dissolved into 50 ml of acetonitrile in a round bottom flask together with 0.35 mmol of **4**, the mixture was stirred at 85°C for 24 h under argon. The reaction mixture was cooled to room temperature to induce the precipitation of the product. The crude solid was collected and purifiedby preparative HPLC chromatography, (CH_3CN/H_2O 0.1%TFA, preparative method), HCl 1M solution was added to each chromatographic portion. Solvent evaporation under vacuum afforded the

adduct**7**(23%yield).**NDI**: **7**·4HCl:Red solid; m.p. dec.>200°C. ^{1}H-NMR (300 MHz, D_2O): 7.92 (m, 2H), 7.68 (m, 2H), 7.42(bs, 1H);3.95 (m, 8H), 3.50 (m, 2H), 3.38 (m, 2H); 3.21 (bs, 8H); 2.88 (s, 24H); 2.06 (m, 8H); 1.90 (m, 4H); 1.72 (bs, 6H). ^{13}C-NMR (75 MHz, D_2O): 164.8; 164.6; 163.1; 162.8; 162.4; 162.3; 161.5; 161.4; 151.4; 151.0; 130.6; 127.9; 127.0; 126.2; 125.5; 124.5; 124.0; 121.3; 119.5; 118.2; 117.6; 114.3; 110.4; 97.8; 97.7;55.1; 55.0; 46.5; 42.9; 42.6; 38.1; 37.7; 37.3; 37.1; 28.5; 26.5; 22.7; 22.6.

Microwave assisted (MW) nucleophilic aromatic substitution: Synthesis of 1. The NDI **7** (0.5 mmol) was dissolved into 4 ml of N,N-dimethyl-propan-1,3-diamine. The mixture was stirred and heated in a microwave reactor, according to a closed vessel protocol, at 150 °C, 250 psi, 200 W, for 3 min. The resulting dark-violet solution was cooled at r.t. to induce crystallization of the product. The resulting blue powder was filtered and washed by water afforded **1**(Yield 64%). Further HPLC preparative purification (CH_3CN:H_2O and 0.1% CF_3COOH as eluent), and final trifluoroacetate-chloride anion exchange, by addition of 1 ml HCl 1M and final lyophilisation,yielded **1** as penta-hydrochloride (**1**x5HCl). Dark violet crystals. M.P. dec. >200°C. ^{1}H-NMR (300 MHz, D_2O): δ=8.67 (m, 2H); 8.46 (s, 1H); 8.36 (m, 2H); 4.31 (m, 8H); 3.82 (m, 2H); 3.66 (m, 4H); 3.40 (m, 2H); 3.28 (m, 8H); 2.98 (s, 30H); 2.30 (m, 10H); 1.97 (m, 2H); 1.87 (m, 2H); 1.54 (bs, 6H). ^{13}C-NMR (75 MHz, D_2O): δ=165.6; 165.0; 164.5; 162.8; 161.2; 160.7; 160.5; 152.6; 151.0; 129.3; 127.0; 125.2; 124.2; 122.1; 120.4; 100.2; 98.3;55.2; 42.2; 39.2; 36.4; 28.0; 27.6; 26.1; 25.5; 25.1; 23.6; 22.3; 22.2.Anal. Calcd for $C_{60}H_{87}Cl_5N_{12}O_8$: C, 56.23; H, 6.84; Cl, 13.83; N, 13.11; O, 9.99. Found: C, 55.87; H, 6.91; N, 13.02.

Exhaustive methylation: Synthesis of 1a. The NDI **1** purified as hydrochlorides were dissolved in a $NaHCO_3$ solution and extracted 3 times with CH_2Cl_2. The recovered organic layers have been dried on Na_2SO_4 and the solvent evaporated under reduced pressure. The collected amine (2.5 mmol) was suspended in 50 ml of CH_3CN and 1.2 g (8.5 mmol) of CH_3I were added. This suspension was stirred under nitrogen atmosphere for 12 h. After this period the solvent was removed under vacuum, yielded **1a** as penta-iodide salt (Yield 99%). Dark violet crystals. M.P. dec. >200°C. ^{1}H-NMR (300 MHz, D_2O recorded at 65°C): δ=8.63 (d, *J*=7.8 Hz, 1H);8.38(d, *J*=7.8 Hz, 1H); 8.28 (s, 1H); 8.23 (s, 1H); 8.02 (s, 1H); 4.58 (m, 8H); 3.99 (m, 6H); 3.89 (m, 10H); 3.63 (s, 9H); 3.55 (s, 36H); 2.74 (m, 2H); 2.62 (bs, 8H); 2.33 (bs, 2H); 2.13 (bs, 6H).^{13}C-NMR (75 MHz, D_2O): δ=165.0; 163.4; 162.7; 162.3; 161.8; 147.8; 130.2; 128.0; 126.3; 124.5; 123.8; 121.5; 119.6; 118.2; 118.0;116.8; 114.3; 110.4; 97.9; 63.8; 52.9; 52.8;46.5; 42.9; 39.4; 37.6; 28.8; 27.0; 22.6; 21.3.Anal. Calcd for $C_{65}H_{97}I_5N_{12}O_8$: C, 43.16; H, 5.40; I, 35.07; N, 9.29; O, 7.07. Found: C, 43.03; H, 5.58; N, 9.39.

Absorption and fluorescence spectra: UV-visible absorption spectra were recorded on a standard Perkin Elmer λ650 spectrophotometer. Fluorescence spectra were measured using 1 nm steps and 0.5-1 s dwell time. Slits were kept as narrow as possible to 4-8 nm in excitation and 4-8 nm in emission. Where necessary a cut-off filter was used. Right angle detection was used. All the measurements were carried out at 295 K in quartz cuvettes with path length of 1 cm. All fluorescence spectra have been obtained for air-equilibrated solutions absorbing less than 0.1 at all wavelengths to avoid inner filter effects and re-absorption of emission. Furthermore, they have been corrected for wavelength dependent response of the monochromator/PMT couple. The compound $Ru(bpy)_3Cl_3.xH_2O$ dissolved in air-equilibrated water with known fluorescence quantum yield (Φ_F) of 0.028 was used as standard for the determination of the fluorescence quantum yield of the NDI samples. The Φ_F value obtained for the compound **3** in buffer of pH 2 was used as reference to determine the fluorescence quantum yield of dyad excited at 600 nm. Using the same solvents for all compounds and iso-absorbing solutions at the excitation wavelength no corrections had to be made for absorbance neither solvent refraction index and we calculated the fluorescence quantum yields, Φ_F, using the formula below, with A being the integrated area of the corrected fluorescence spectra:

$$\Phi_F = \Phi_F^{ref} \times A/A^{ref} \qquad (1)$$

Fluorescence lifetimes: Fluorescence decays in solution were measured in air-equilibrated solutions for excitation at 637 nm (Hamamatsu pulsed laser with 1 MHz repetition rate) using a time-correlated single photon counting system (TCSPC) (IBH Consultants Ltd., Glasgow, UK) with a resolution of 55 ps per channel. Photons were detected in right angle configuration at 690 nm with a cut-off filter. Fluorescence decay profiles were analyzed with a least-squares method, using multiexponential decay functions (eq. 2) and deconvolution of the instrumental response function. The software package was provided by IBH Consultants Ltd.

The fitting function used is:

$$I(t) = b + \Sigma_j a_j e(-t/\tau_j) \qquad (2)$$

The fractional intensity and the average fluorescence lifetime are calculated according to the following equations:

$$f_i = a_i\tau_i / \Sigma_j a_j\tau_j \qquad \tau_{av} = \Sigma_j f_j\tau_j$$

Sample Preparation for Titration Studies. For the spectroscopic measurements a 10 mM K^+ phosphate buffer of pH 7.0 was used, with 100 mM KCl/NaCl. Excess of K^+ mimics physiological

conditions of cellular compartments where K^+ is abundant. The DNA stock solution was heated at 90 °C for 15 min and then cooled down to room temperature before use. Concentration of the DNA stock solution was determined spectrophotometrically with 0.1 cm cuvets. Compound **1** was dissolved up to a concentration of 7×10^{-5} M in buffer. Aliquots of the dyad and DNA solutions dissolved in the same buffer were mixed together to prepare samples of varying molar ratio. Solutions were kept stirring in the dark for ca. 1 hour before starting the measurements. Water was purified by passage through a Millipore MilliQ system (Millipore SpA, Milan, Italy).

Multiwavelength Global analysis: The best complexation model and the association constants were determined by means of a multivariate global analysis of multiwavelength fluorescence data, analyzing a set of spectra corresponding to different **1**-DNA mixtures. We used the program ReactLab™ Equilibria (Jplus Consulting Pty Ltd). The procedure is based on singular value decomposition (SVD) and non linear regression modelling by the Levenberg-Marquardt method. The analysis also afforded the individual fluorescence spectra of the complexes. The software allows comparison of experimental data and calculated values to evaluate goodness of the fit.

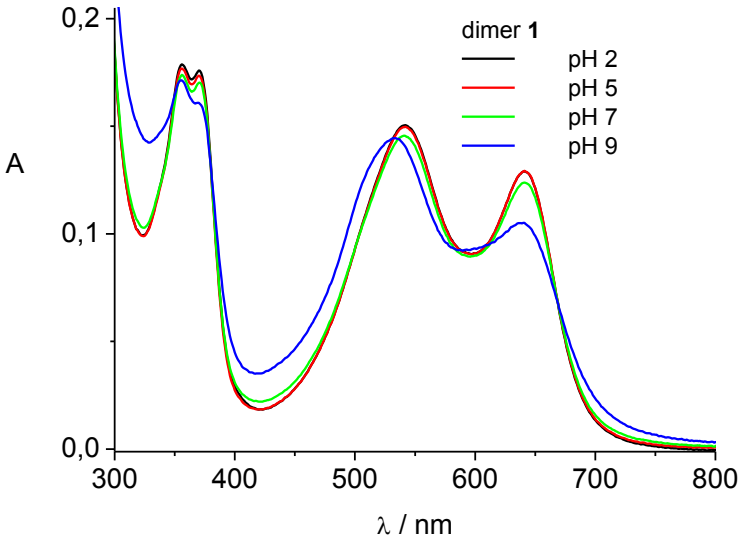


Fig. S1 Absorption spectra of the dimer **1**at different pH

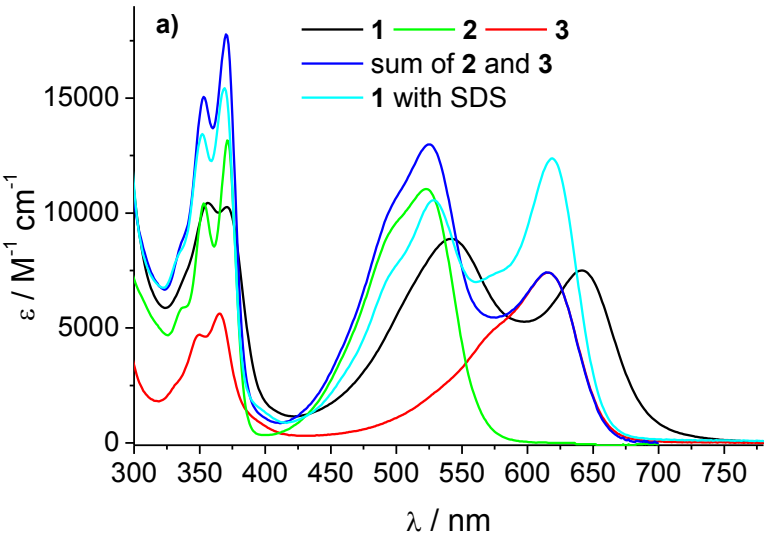


Fig. S2 Absorption spectra of the monomers **2** and **3**, the dimer **1** with and without SDS.

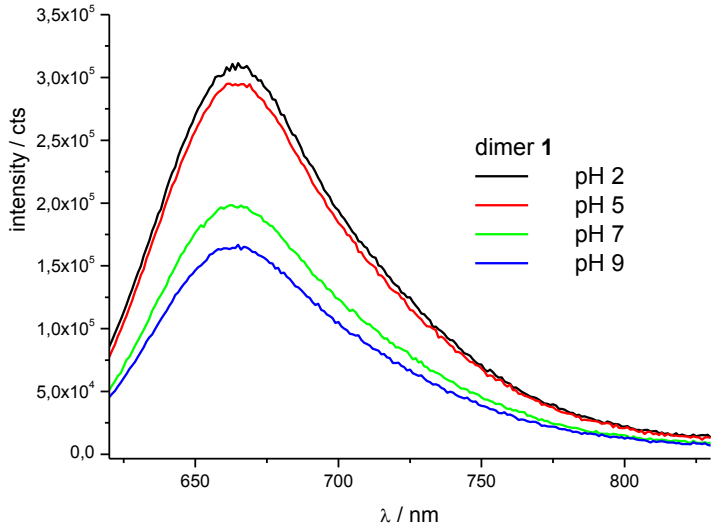


Fig. S3 Fluorescence spectra of **1** at different pH values normalized for absorbance at 610 nm, the excitation wavelength.

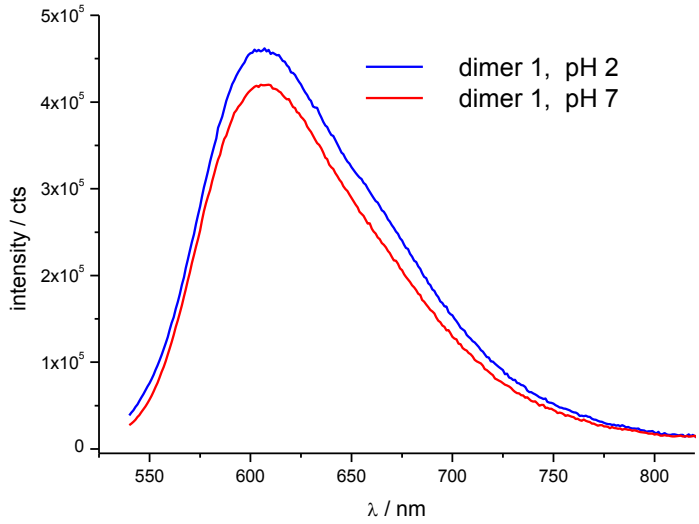


Fig. S4 Fluorescence spectra of **1** at different pH values normalized for absorbance at 525 nm, the excitation wavelength.

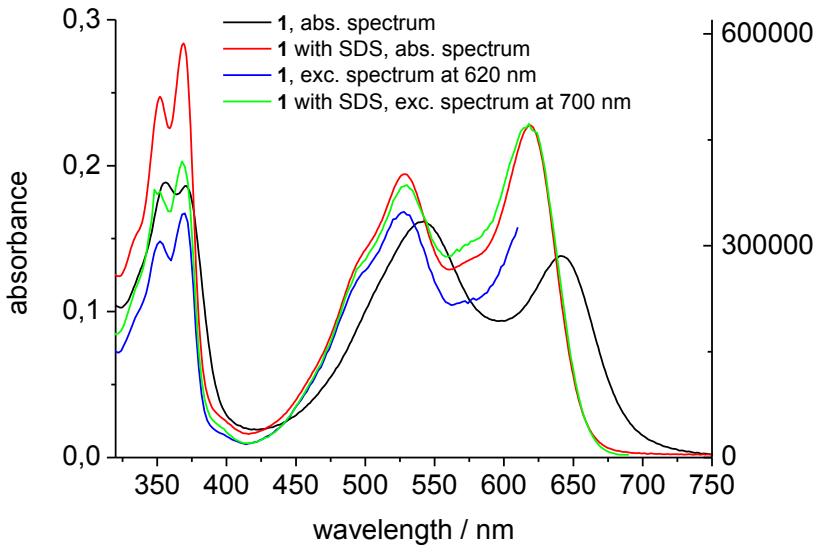


Fig. S5 Comparison of absorption spectra and fluorescence excitation spectra of **1**with and without SDS.

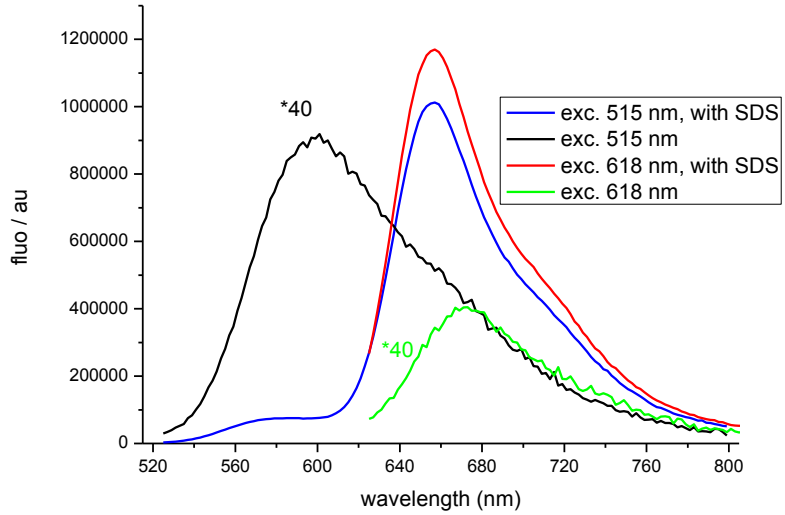


Fig. S6 Fluorescence spectra of the dimer with and without SDS.

Table S1. Fluorescence lifetimes and preexponential factors of **1** in the presence of SDS for excitation at 373 nm

Entry	**Samples**	**τ_1**	**a_1**	**τ_2**	**a_2**
		λexc= 373 nm, λem= 690 nm., Cut off filter at 645 nm			
1	**1** +SDS	6.47 ns	-	-	-
		λexc= 373 nm, λem= 570 nm., Cut off filter at 550 nm			
2	**1**+SDS	0.97 nsec	0.02	7.58 ns	0.03

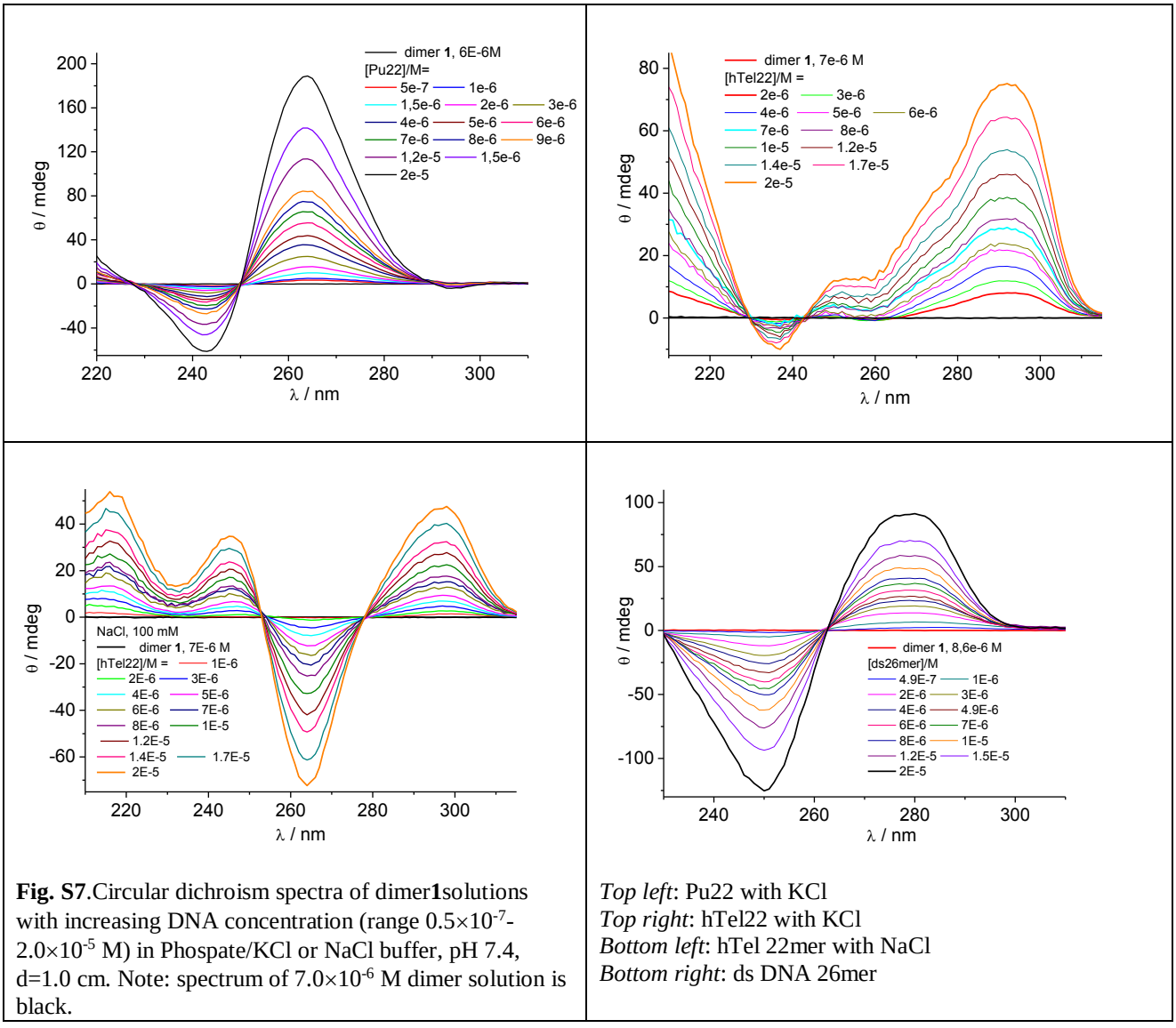


Fig. S7.Circular dichroism spectra of dimer**1**solutions with increasing DNA concentration (range 0.5×10^{-7}-2.0×10^{-5} M) in Phospate/KCl or NaCl buffer, pH 7.4, d=1.0 cm. Note: spectrum of 7.0×10^{-6} M dimer solution is black.

Top left: Pu22 with KCl
Top right: hTel22 with KCl
Bottom left: hTel 22mer with NaCl
Bottom right: ds DNA 26mer

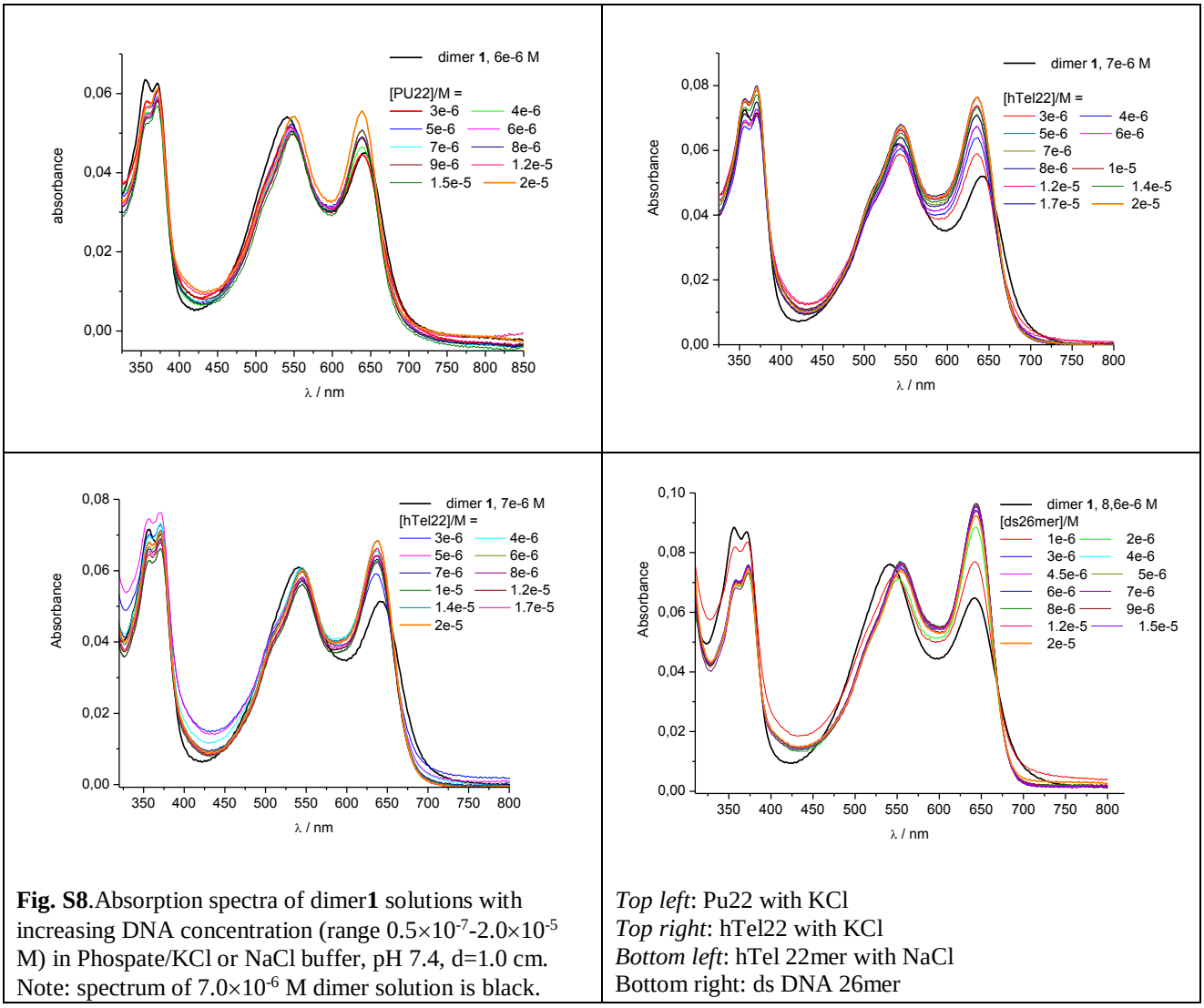


Fig. S8.Absorption spectra of dimer**1** solutions with increasing DNA concentration (range 0.5×10^{-7}-2.0×10^{-5} M) in Phospate/KCl or NaCl buffer, pH 7.4, d=1.0 cm. Note: spectrum of 7.0×10^{-6} M dimer solution is black.

Top left: Pu22 with KCl
Top right: hTel22 with KCl
Bottom left: hTel 22mer with NaCl
Bottom right: ds DNA 26mer

Figure S9 below shows the calculated fluorescence spectra of the free dimer as well as the complexes. M indicates the DNA while L indicates the ligand, dimer **1**. The area corresponding to the product ε × Φ is also reported.

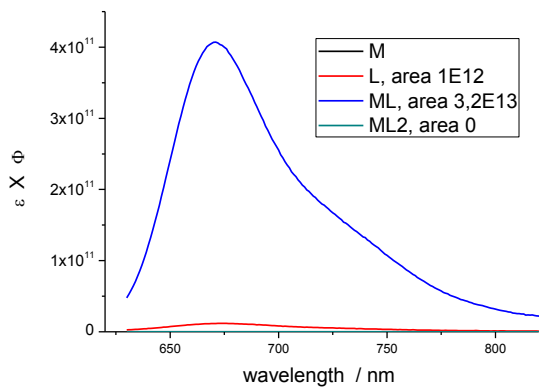


Fig. S9a Calculated fluorescence spectra of dimer **1** and Pu22 complexes for the binding constants with pK values 5.89 ± 0.01 (ML complex) and 12.66 ± 0.01 (ML2 complex) (K^+ solution).

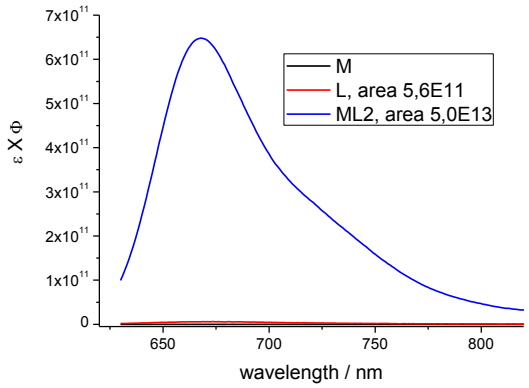


Fig. S9b Calculated fluorescence spectra of dimer **1**and hTel22 complex for the binding constant with pK value 11.65 ± 0.02 (K^+ rich solution)

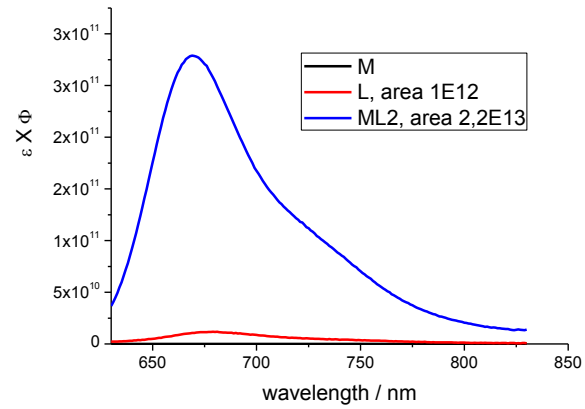


Fig. S9c Calculated fluorescence spectra of dimer **1**and hTel22 complex for the binding constant with pK value 11.32 ± 0.02 (Na^+ rich solution)

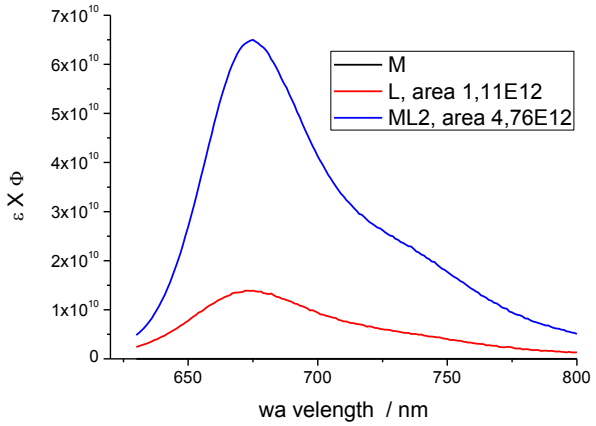
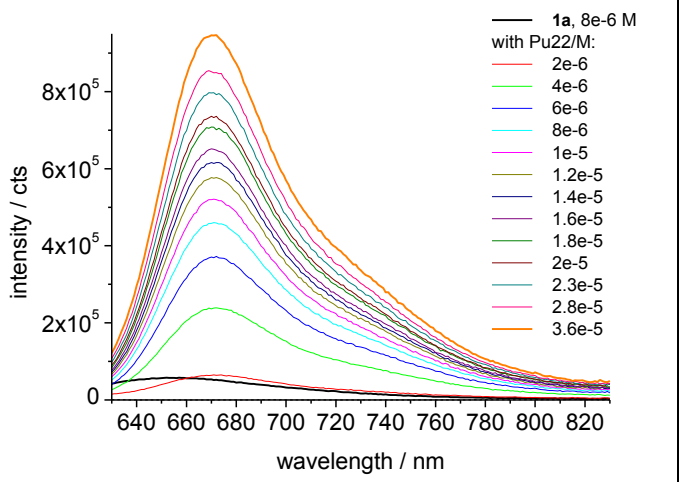


Fig. S9d Calculated fluorescence spectra of dimer **1**and ds 26mer complex for the binding constant with pK value 12.75 ± 0.02

Table S2. Fluorescence lifetimes of the dimer **1** in the presence of excess DNA, for excitation at 373 nm

DNA	τ_{av} / ns	τ_{long} / ns
Pu22	3.92	4.97
hTel22/KCl	4.40	5.24
hTel22/NaCl	3.18	4.84
ds 26mer	1.76	3.74



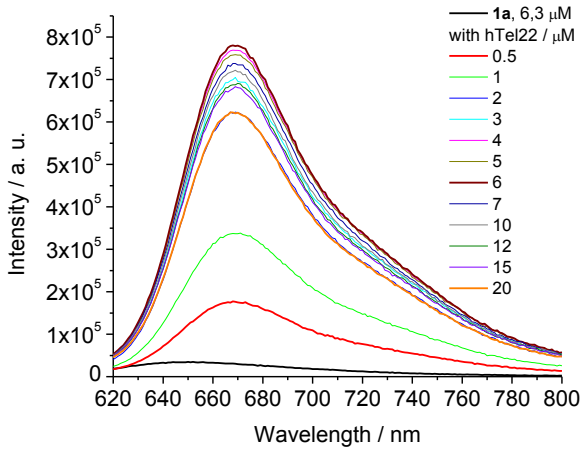


Fig. S10 Fluorescence spectra of **1a** solutions with increasing DNA concentrations in phospate buffer/KCl, pH 7.0. Spectrum of dimer **1a** solution is black. Right: Pu22 with 100 mM KCl; left: hTel22 with 100 mM KCl.

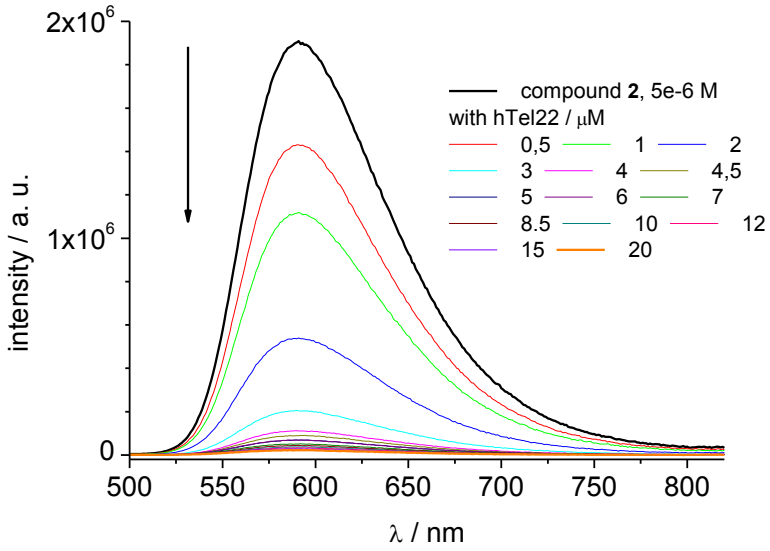
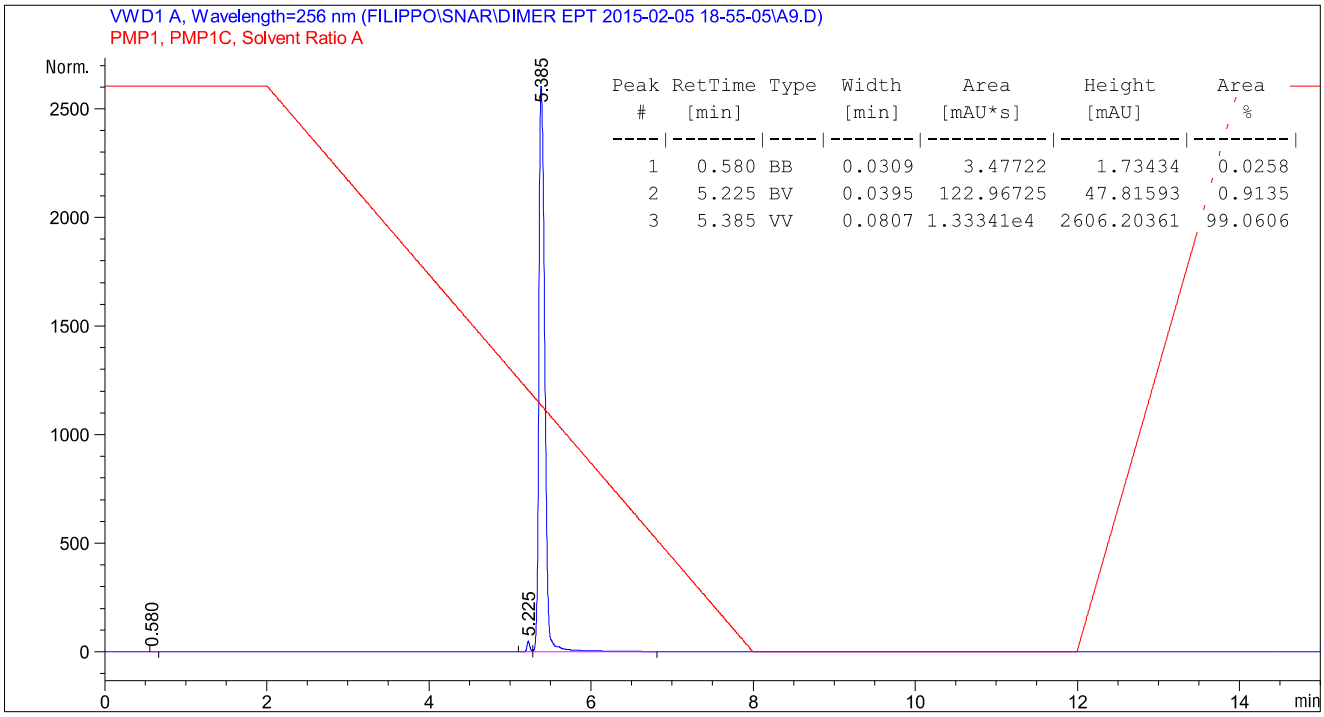


Fig. S11 Fluorescence spectra of solutions of **2** with increasing hTel22 concentrations in phospate buffer/KCl, pH 7.0. Spectrum of **2** solution is black; hTel22 with 100 mM KCl

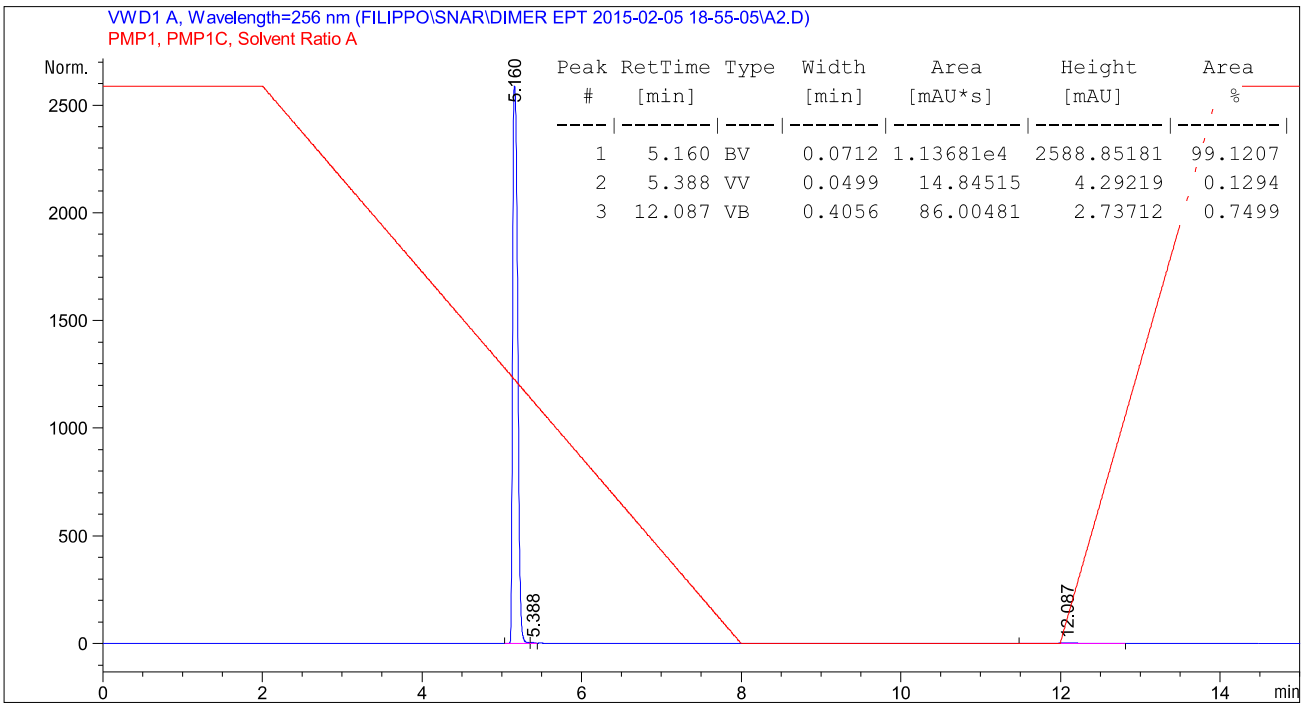
HPLC PURITY DATA:

***N,N'*-Bis-((dimethylamino)propylamino)-2-bromo-6-((amino)heptylamino)-1,4-5,8-naphthalenetetracarboxylic bisimide trihydrochloride(5·3HCl).**
Analytical Method-B



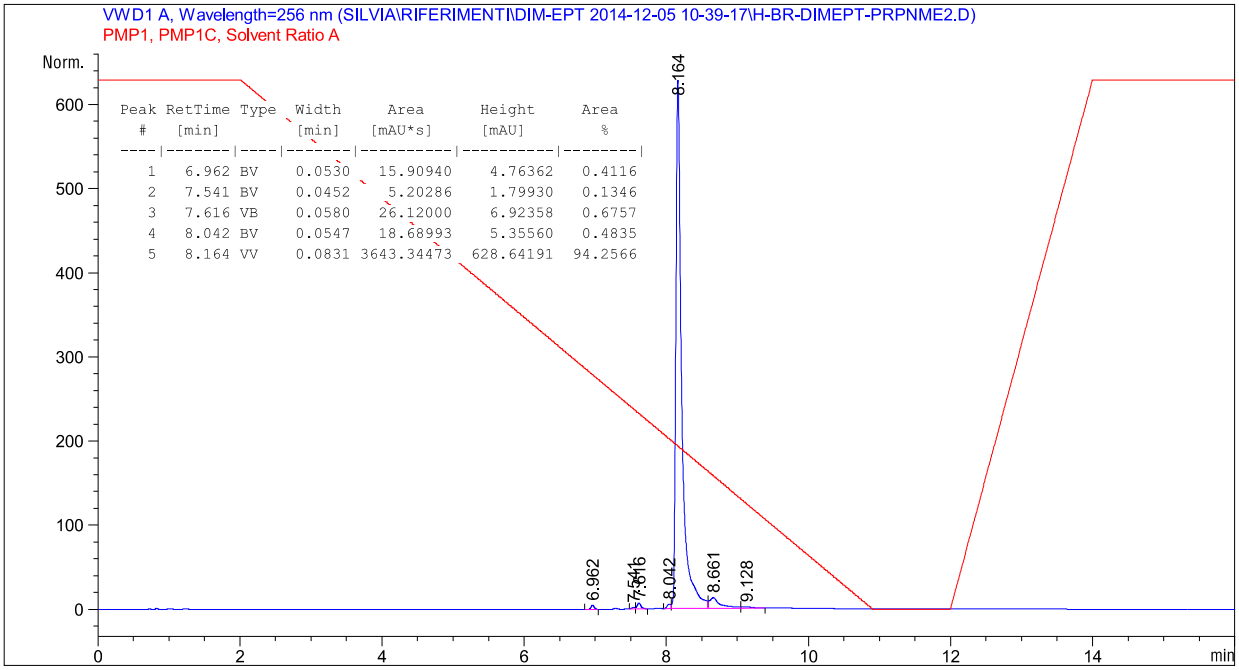
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.580	BB	0.0309	3.47722	1.73434	0.0258
2	5.225	BV	0.0395	122.96725	47.81593	0.9135
3	5.385	VV	0.0807	1.33341e4	2606.20361	99.0606

***N,N'*-Bis-((dimethylamino)propylamino)-6-((amino)heptylamino)-1,4-5,8-naphthalenetetracarboxylic bisimide trihydrochloride(6·3HCl).**
Analytical Method-B

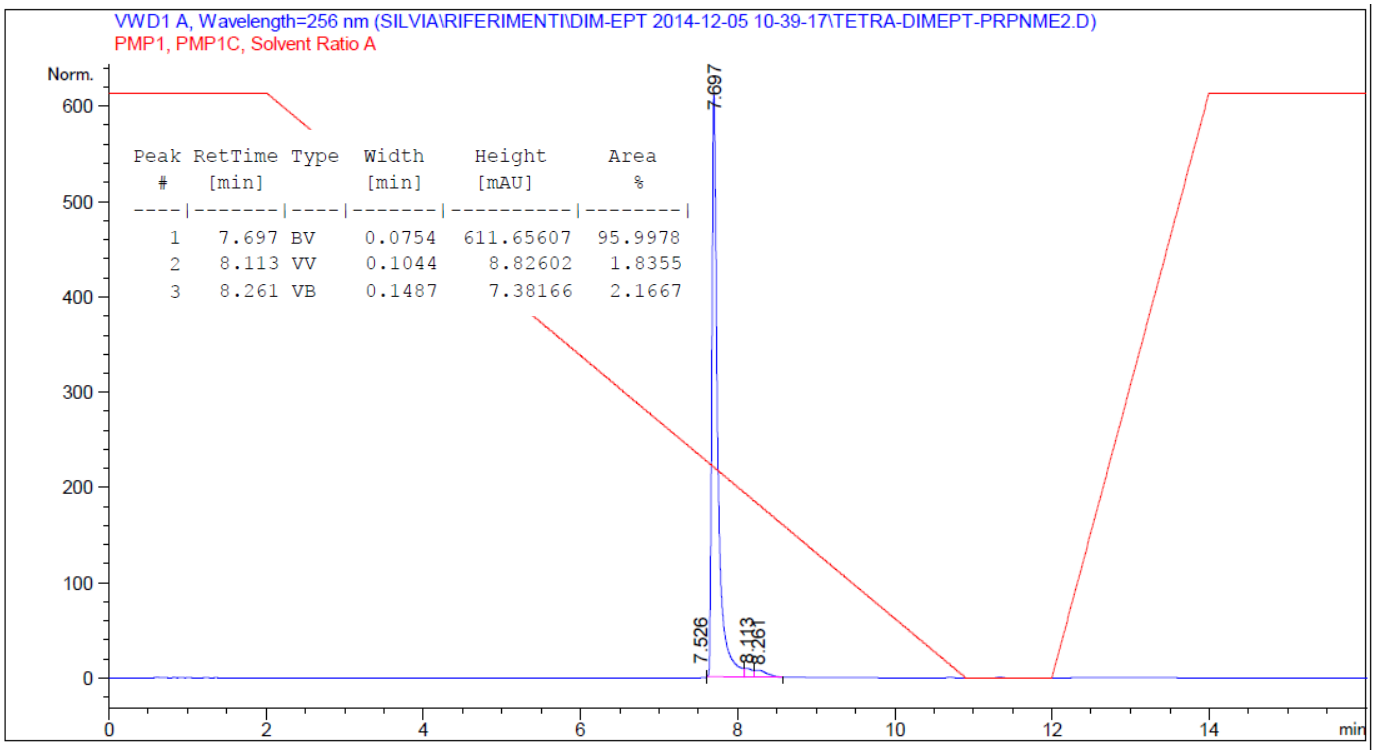


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.160	BV	0.0712	1.13681e4	2588.85181	99.1207
2	5.388	VV	0.0499	14.84515	4.29219	0.1294
3	12.087	VB	0.4056	86.00481	2.73712	0.7499

NDI7
Analytical Method-A

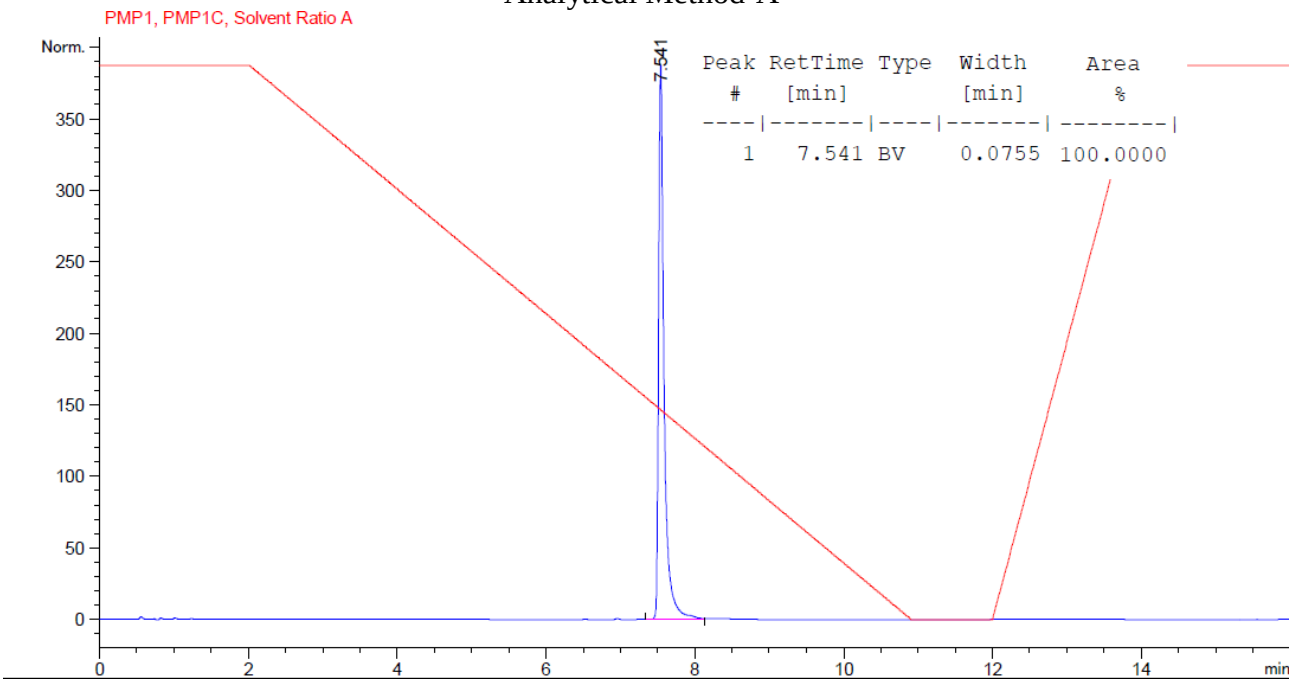


NDI1
Analytical Method-A



NDI1a

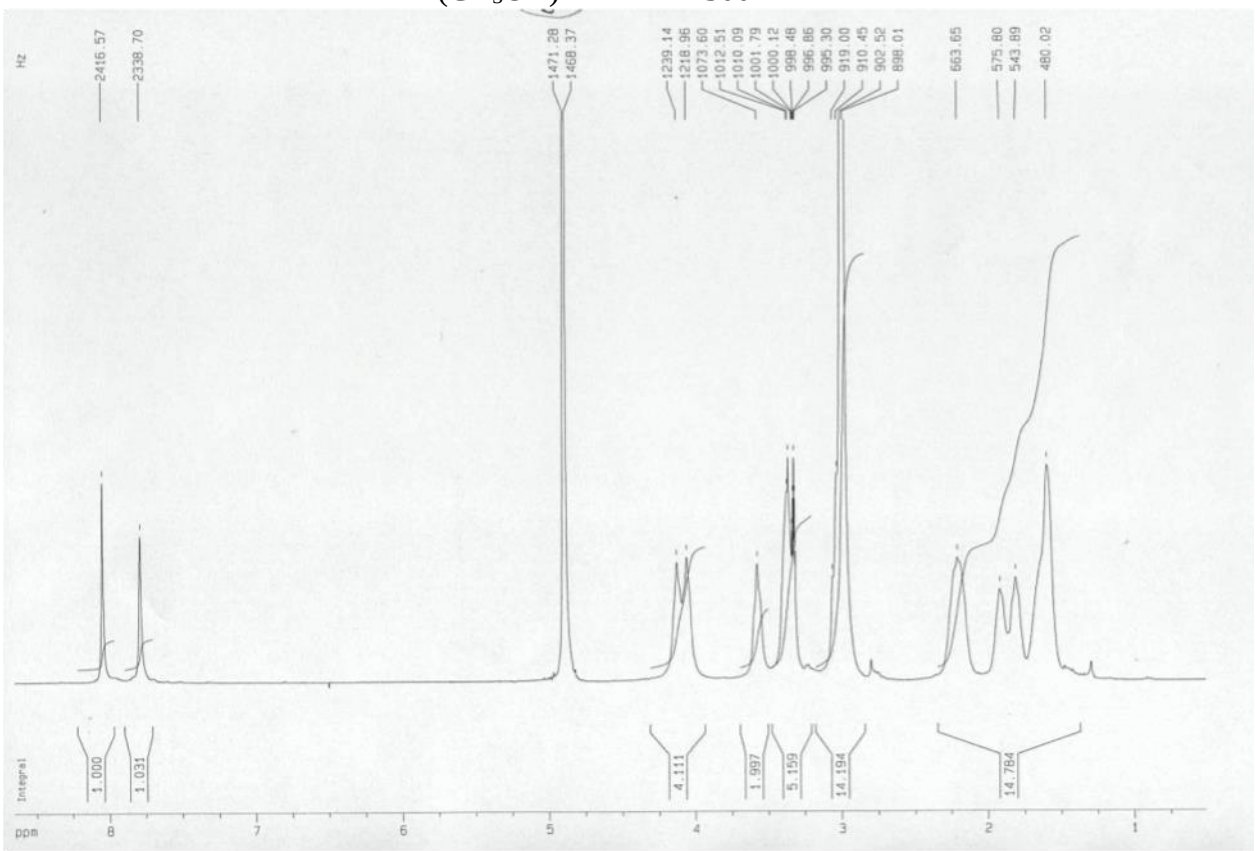
Analytical Method-A



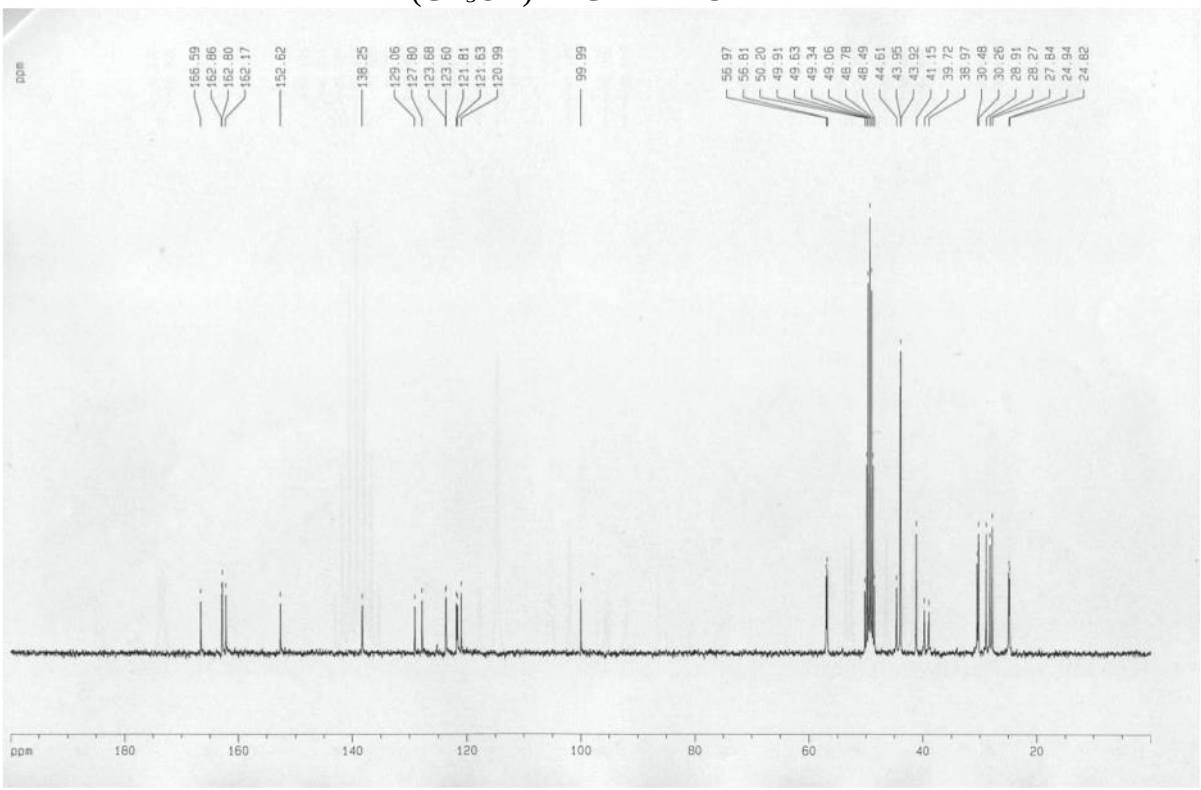
NMR Spectra:

***N,N'*-Bis-((dimethylamino)propylamino)-2-bromo-6-((amino)heptylamino)-1,4-5,8-naphthalenetetracarboxylic bisimide trihydrochloride(5·3HCl).**

(CD_3OD) ^1H NMR 300MHz

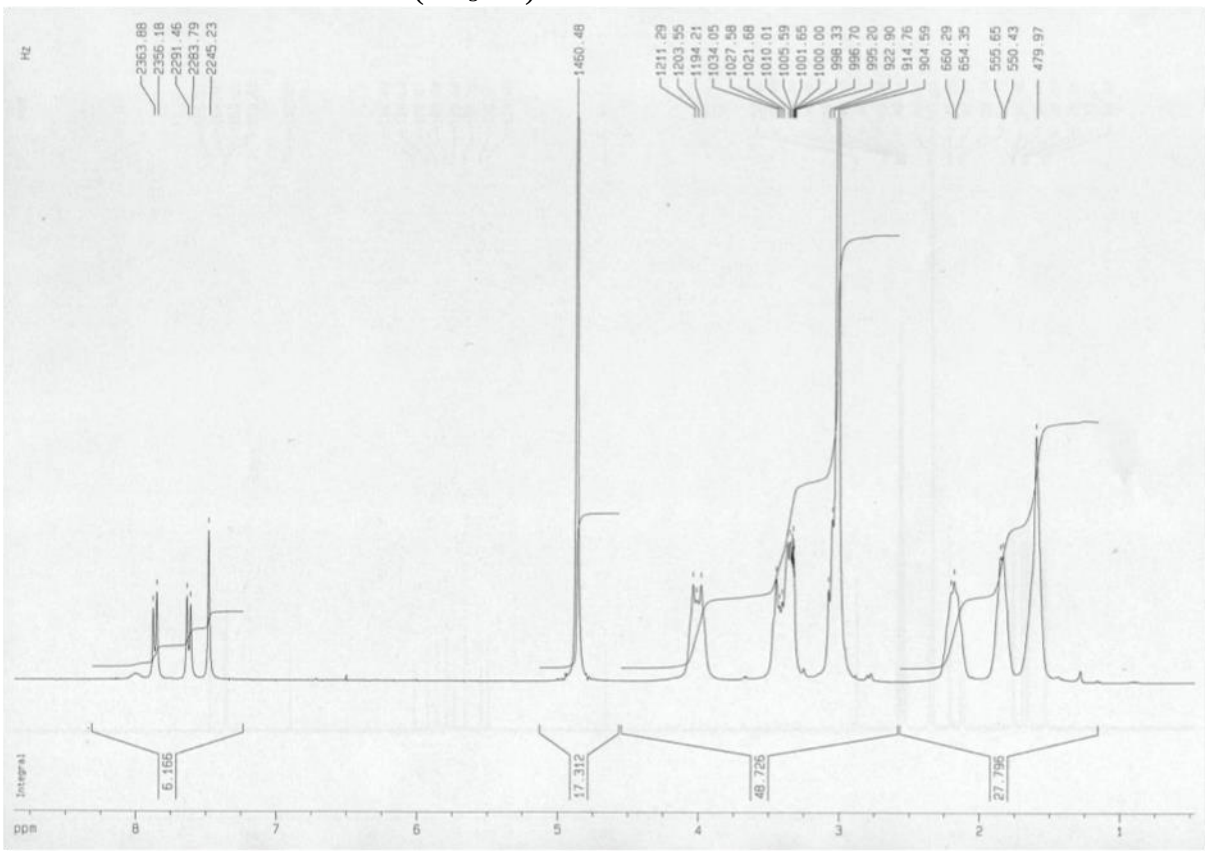


(CD_3OD) ^{13}C NMR 75MHz

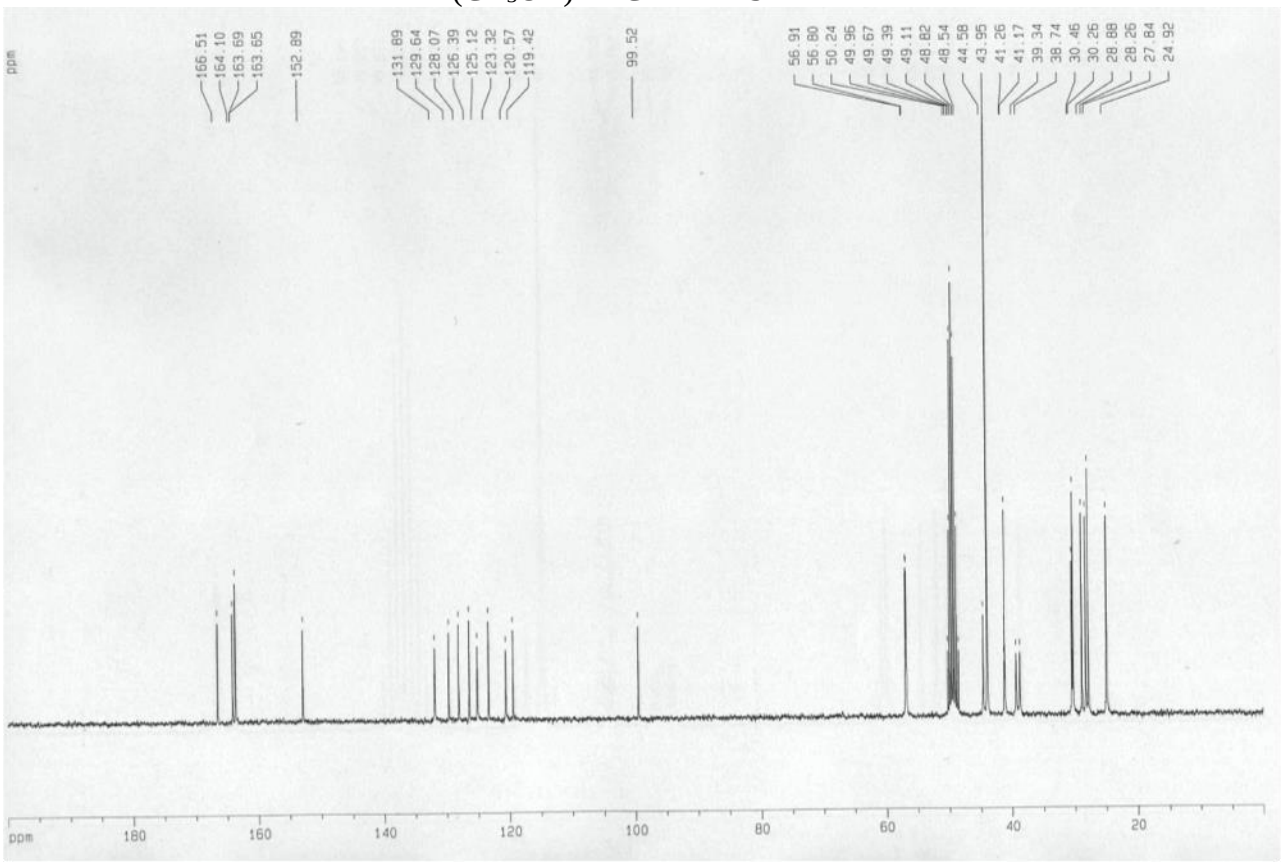


***N*,*N*'-Bis-((dimethylamino)propylamino)- 6-((amino)heptylamino)-1,4-5,8-naphthalenetetracarboxylic bisimide trihydrochloride**(**6**·3HCl)**.**

(CD_3OD) ^{1}H NMR 300MHz

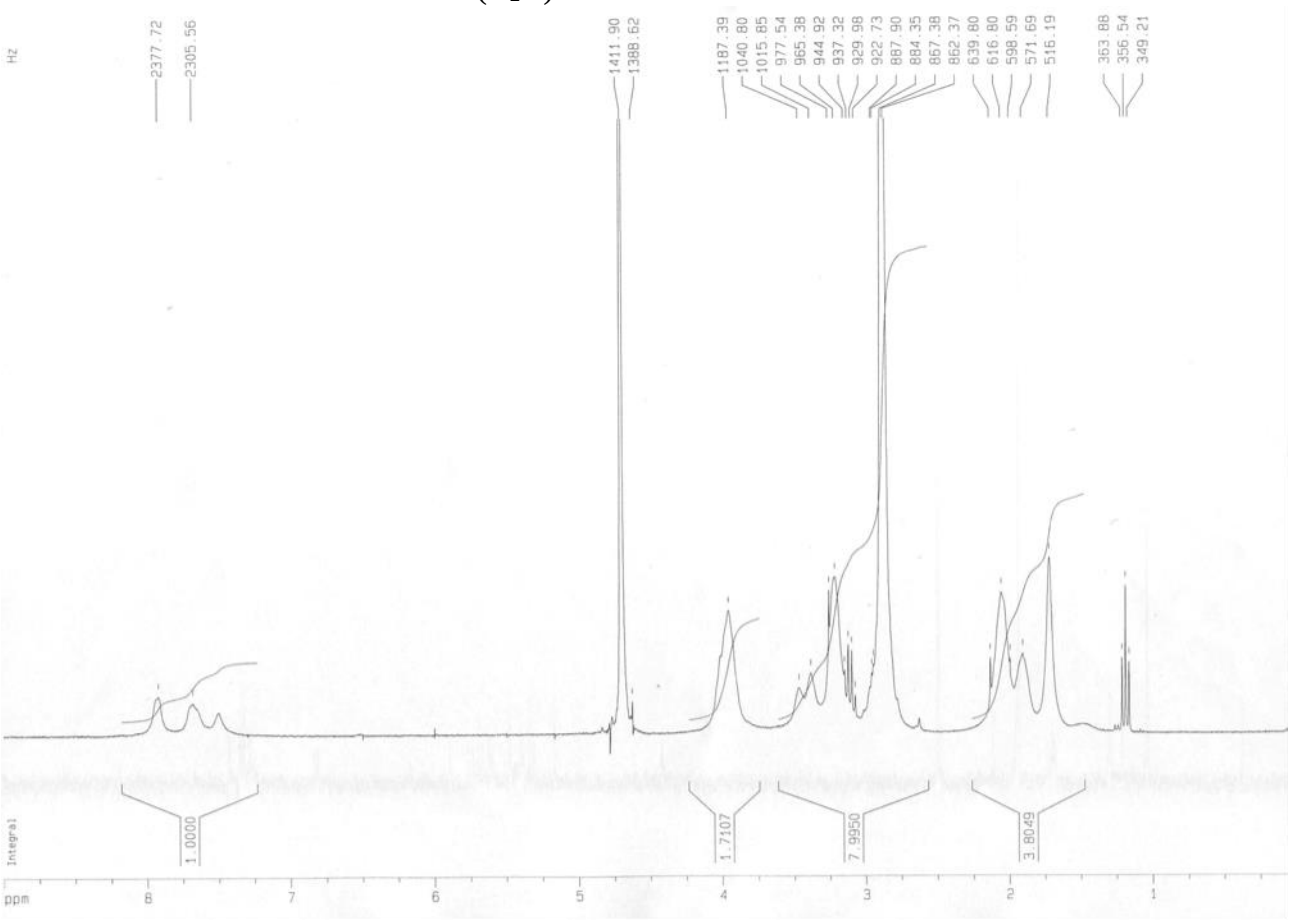


(CD_3OD) ^{13}C NMR 75MHz

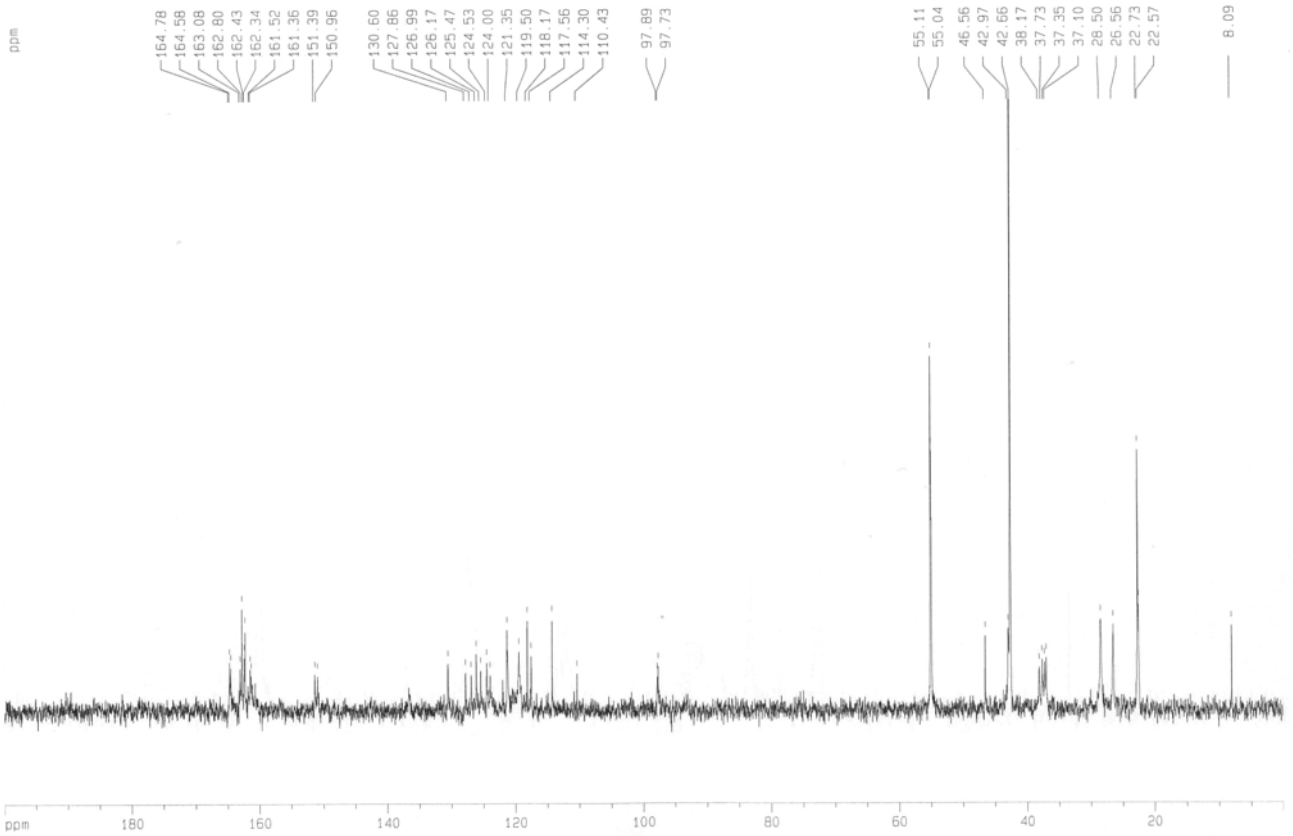


(**7**·3HCl)

(D_2O) ^{1}H NMR 300MHz

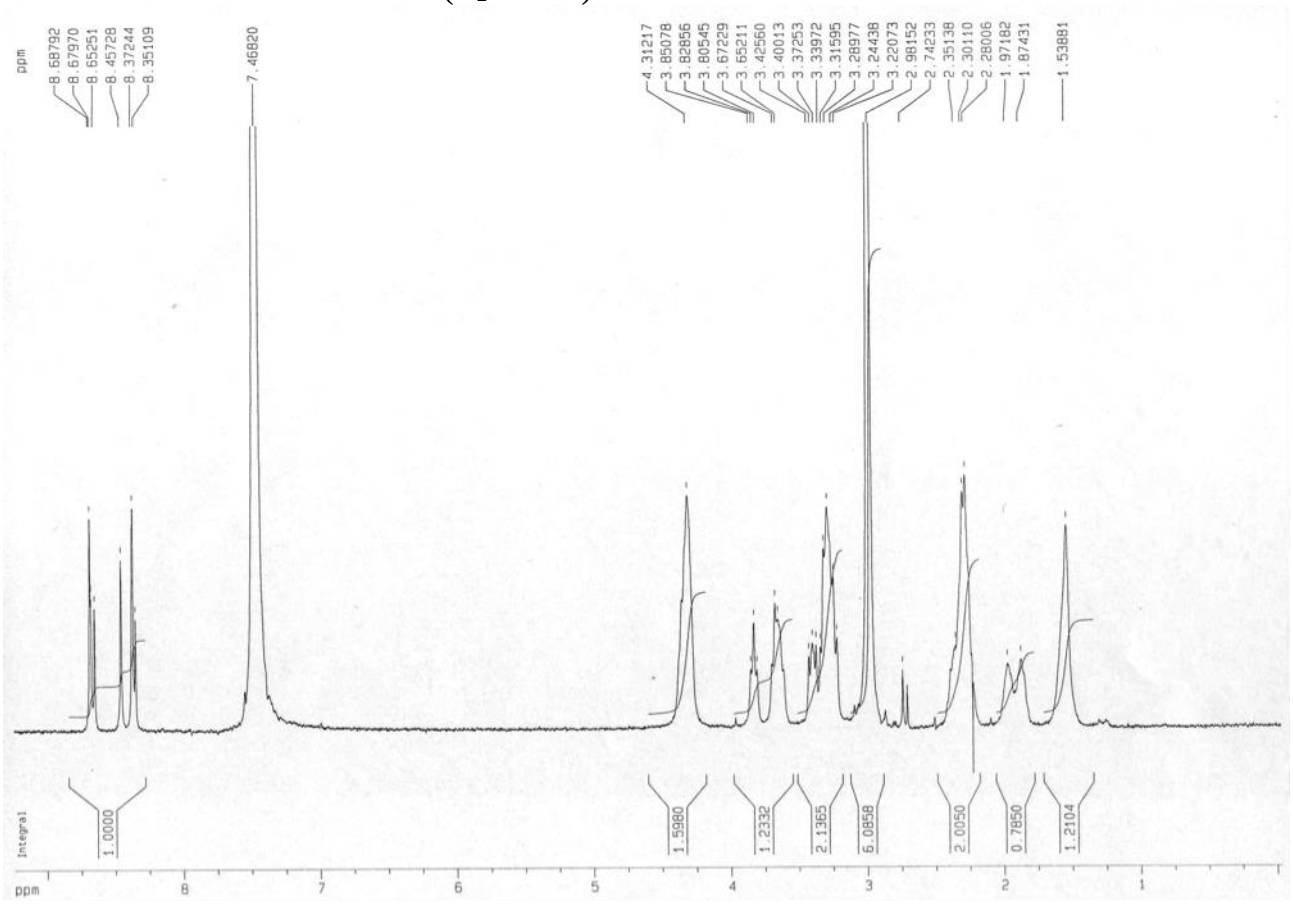


(D_2O) ^{13}C NMR 75MHz

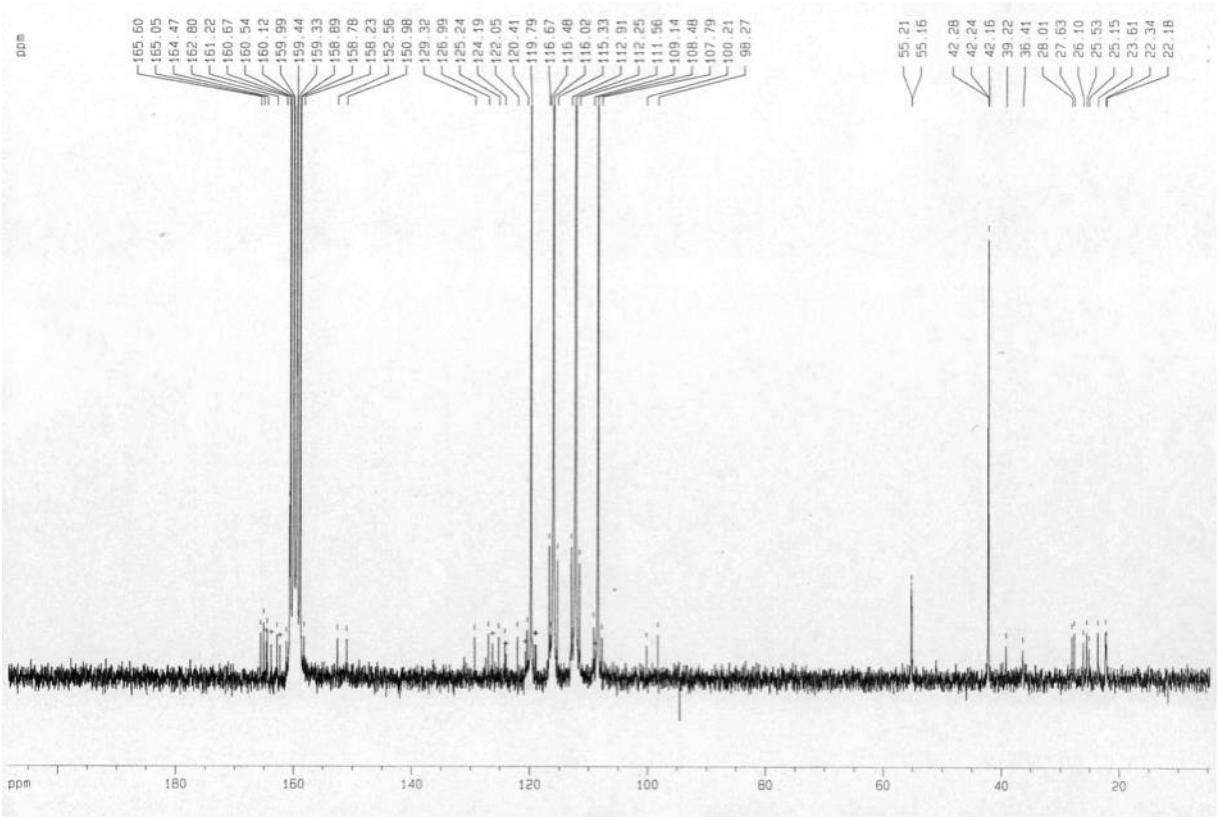


(**1**·3HCl)

(D_2O-TFA) ^{1}H NMR 300MHz

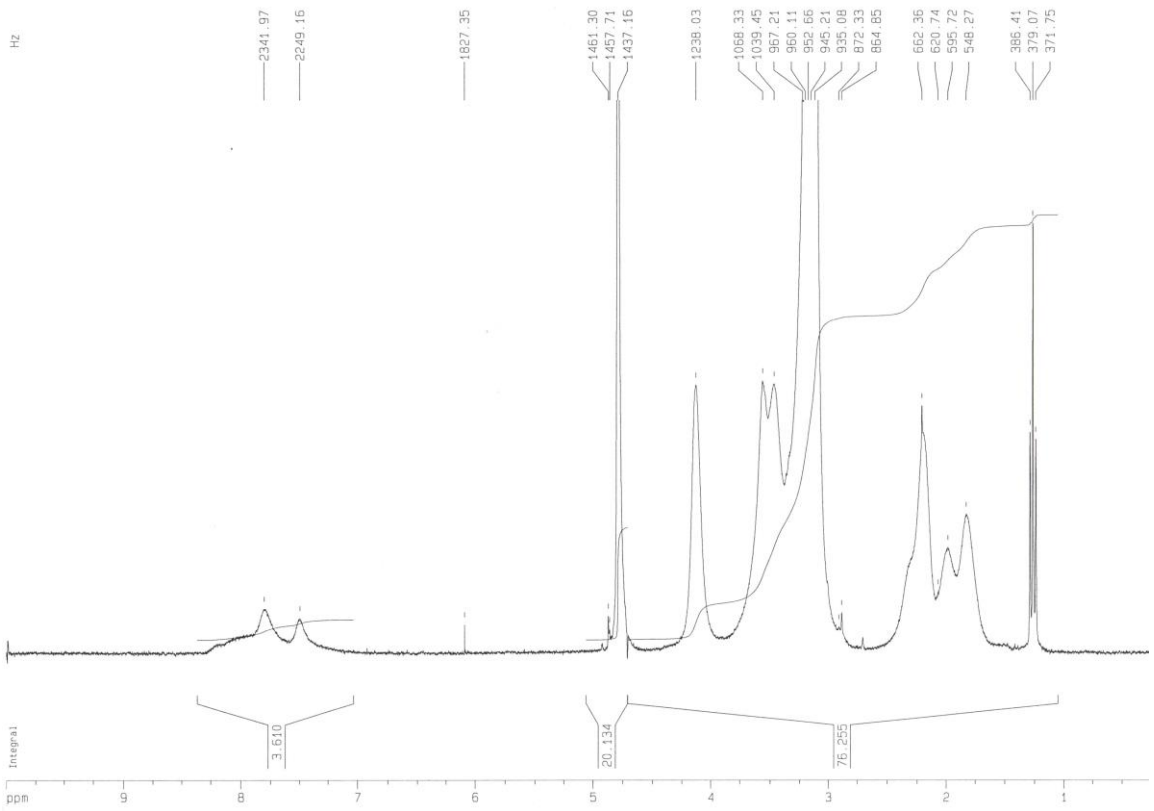


(D_2O-TFA) ^{13}C NMR 75MHz

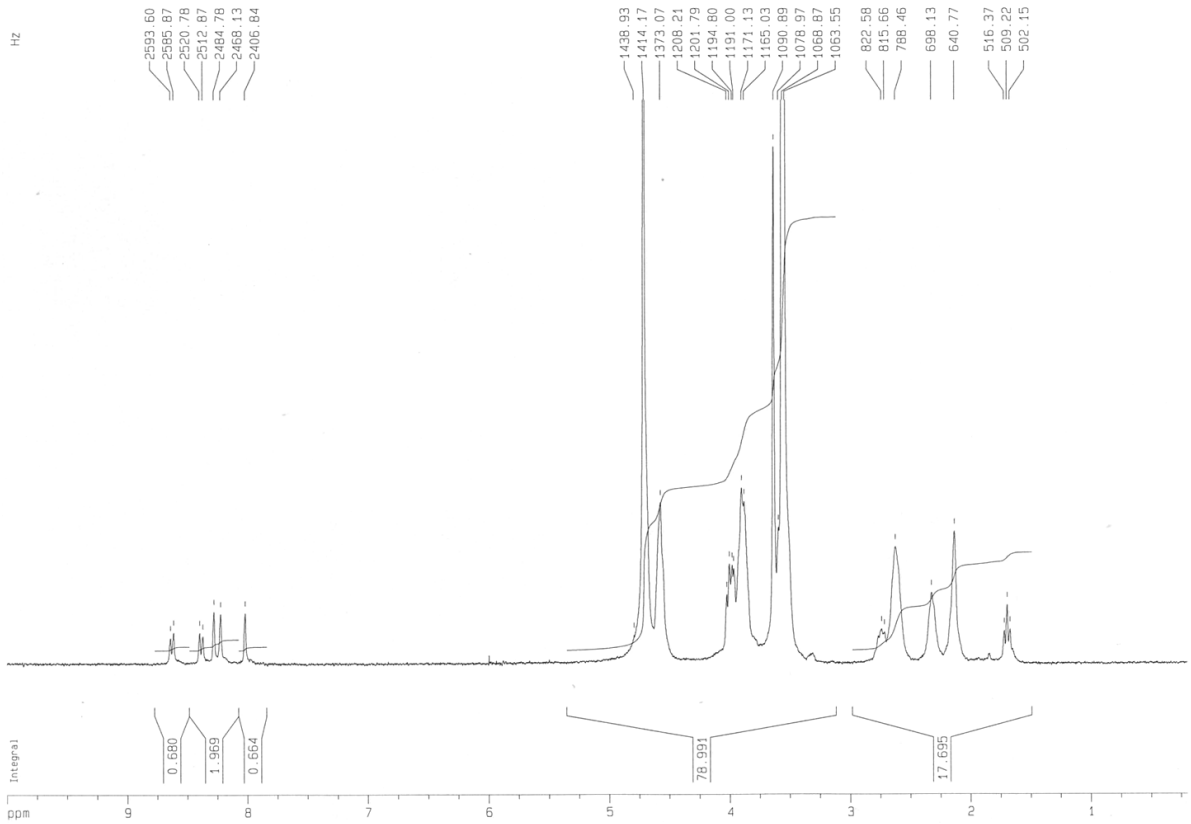


(**1a**)

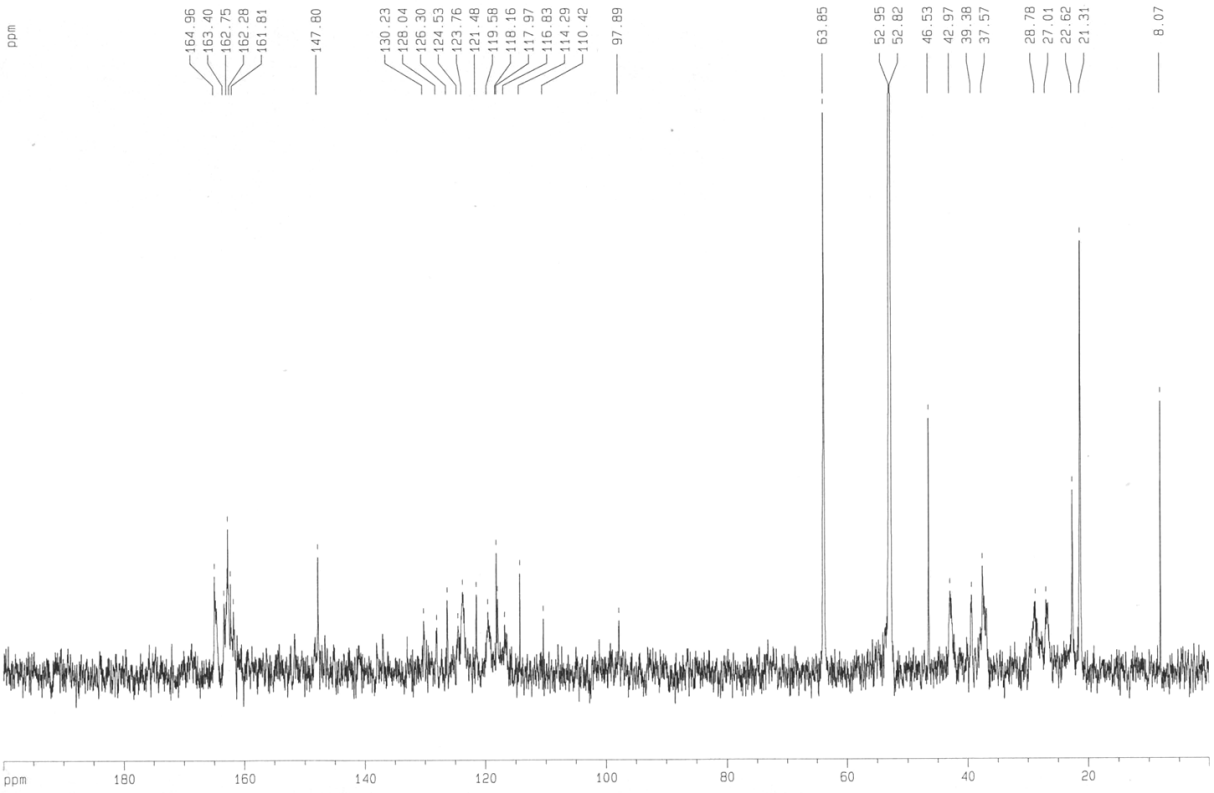
(D_2O-recorded at 25°C) ^{1}H NMR 300MHz



(D_2O-recorded at 65°C) ^1H NMR 300MHz



(D_2O-recorded at 25°C) ^{13}C NMR 75MHz



References

S1. F. Doria, I. Manet, V. Grande, S. Monti and M. Freccero, *J. Org. Chem.* 2013, **78**, 8065.