Supplementary Information

Hybrid Ligand/Alkylating Agents Targeting Telomeric G-Quadruplex Structures

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Inventory

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Supplementary Figures

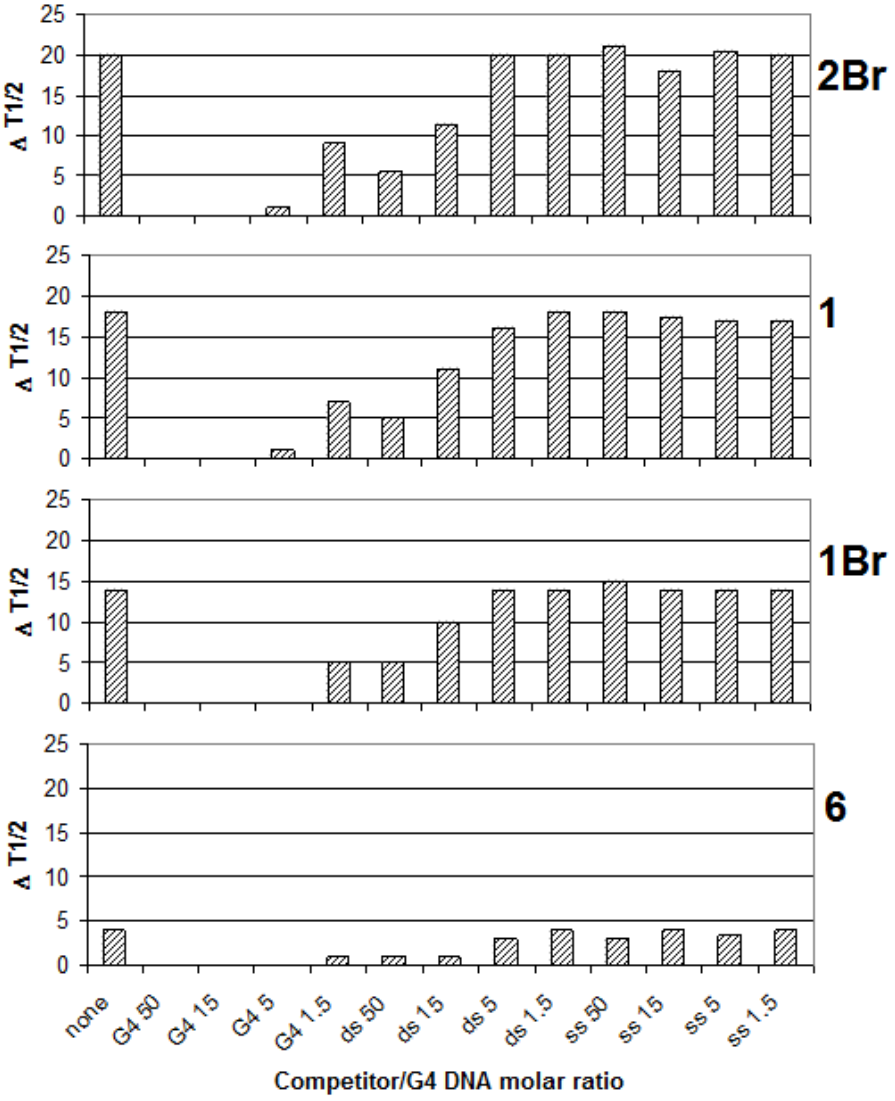
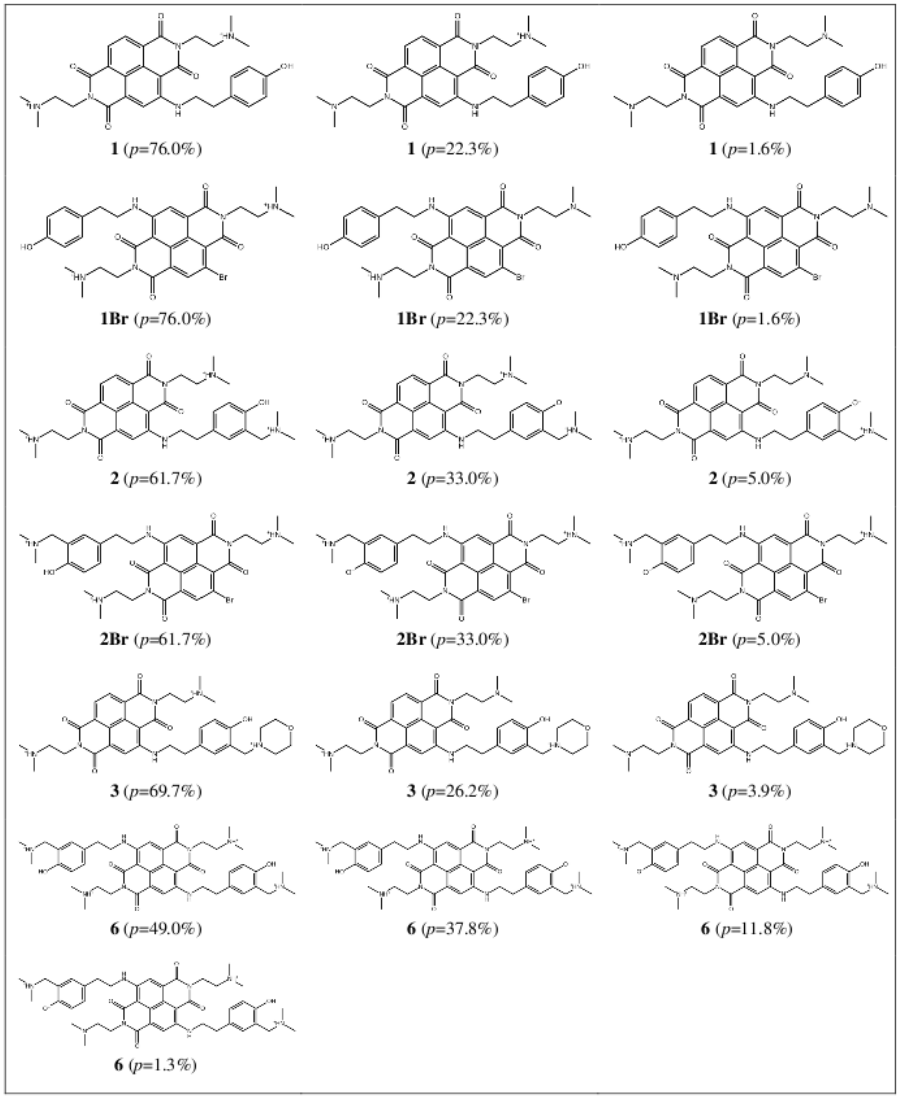


Figure S1. FRET-based competition assay. Binding of NDIs to the fluorescence-labelled F21T oligo was challenged by non-fluorescent duplex (ds), single-stranded scrambled G-4 DNA (ss), or a telomere non-related G-4 forming DNA (G4). Data for the NDIs **2Br**, **1**, **1Br** and **6** are shown.

A



B

Compound	BASASA	$SASA_{Min}$	$SASA_{Max}$
1	127.87	122.43	141.09
1Br	114.46	108.85	127.24
2	134.36	126.69	147.52
2Br	122.02	113.22	136.74
3	115.41	105.54	127.28
6	110.44	96.90	133.32

Figure S2. (A) 2D structure and existence probability (*p*) of each protomer/tautomer related to **1**, **1Br**, **2**, **2Br**, **3** and **6** NDIs. (B) Average (BASASA), minimum ($SASA_{Min}$) and maximum ($SASA_{Max}$) values related to the solvent accessible surface area (expressed in $Å^2$) calculated on **1**, **1Br**, **2**, **2Br**, **3** and **6** NDIs.

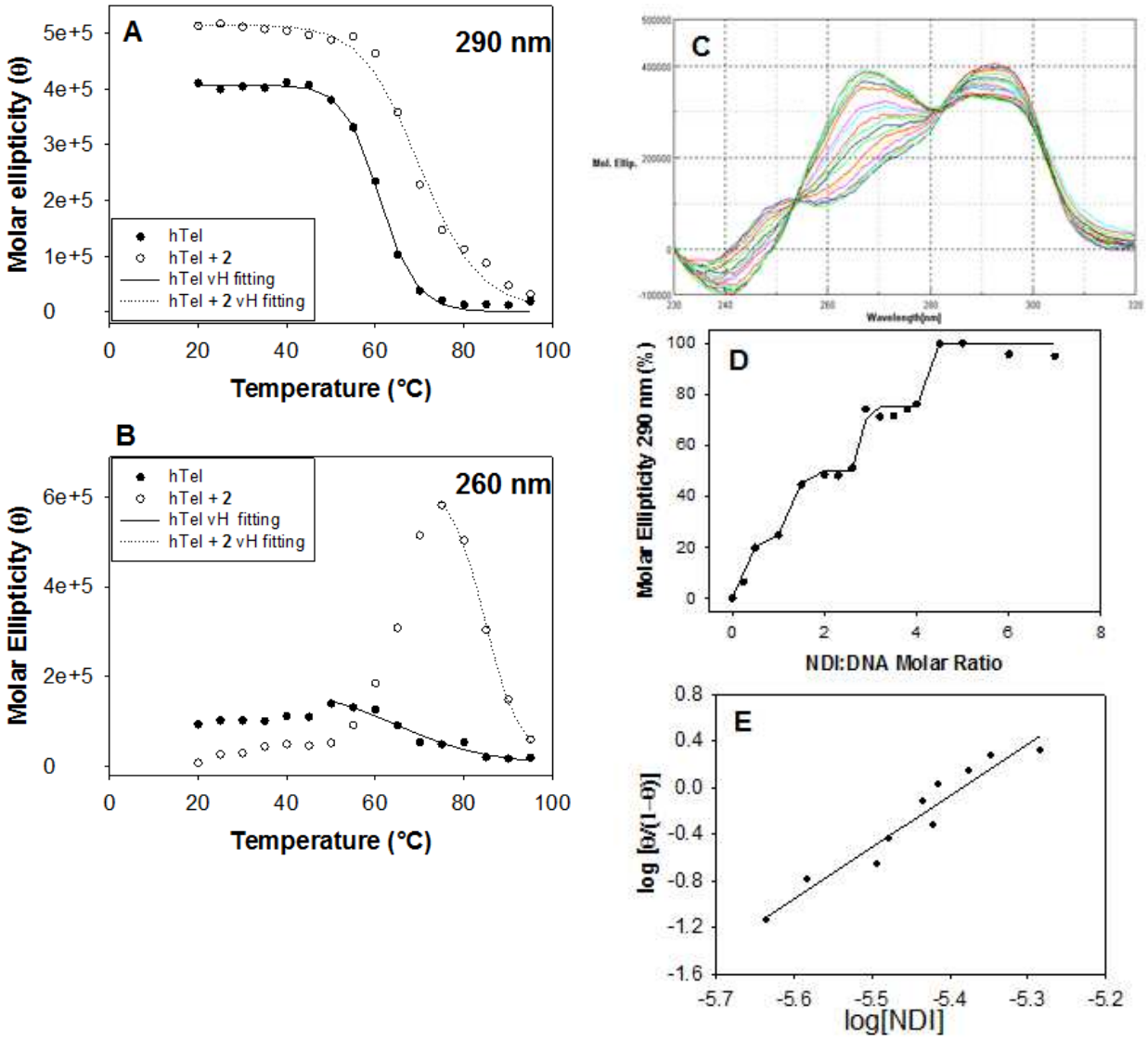


Figure S3. CD analysis of the thermal unfolding of hTel DNA in the absence (•) or presence (○) of compound **2** in the temperature range of 20-95°C. **(A)** Molar ellipticity at 290 nm, **(B)** molar ellipticity at 260 nm. Experimental data were fitted with the van't Hoff (vH) equation to obtain T_m values. (C-E) Stoichiometry of NDI binding to hTel DNA analyzed by CD. (C) Increasing molar ratio of NDI **2Br** were incubated with hTel DNA after oligonucleotide annealing. Relative molar ellipticity values at 290 nm were plotted against NDI molar ratio. (D) Increasing molar ratio of 2 were incubated with hTel DNA before oligonucleotide annealing. Shown are overlaid CD spectra. (E) Hill plot for the binding of NDI 2 to G-4 DNA; θ represents the fraction of NDI-bound G-4 and [NDI] the molar concentration of free NDI.

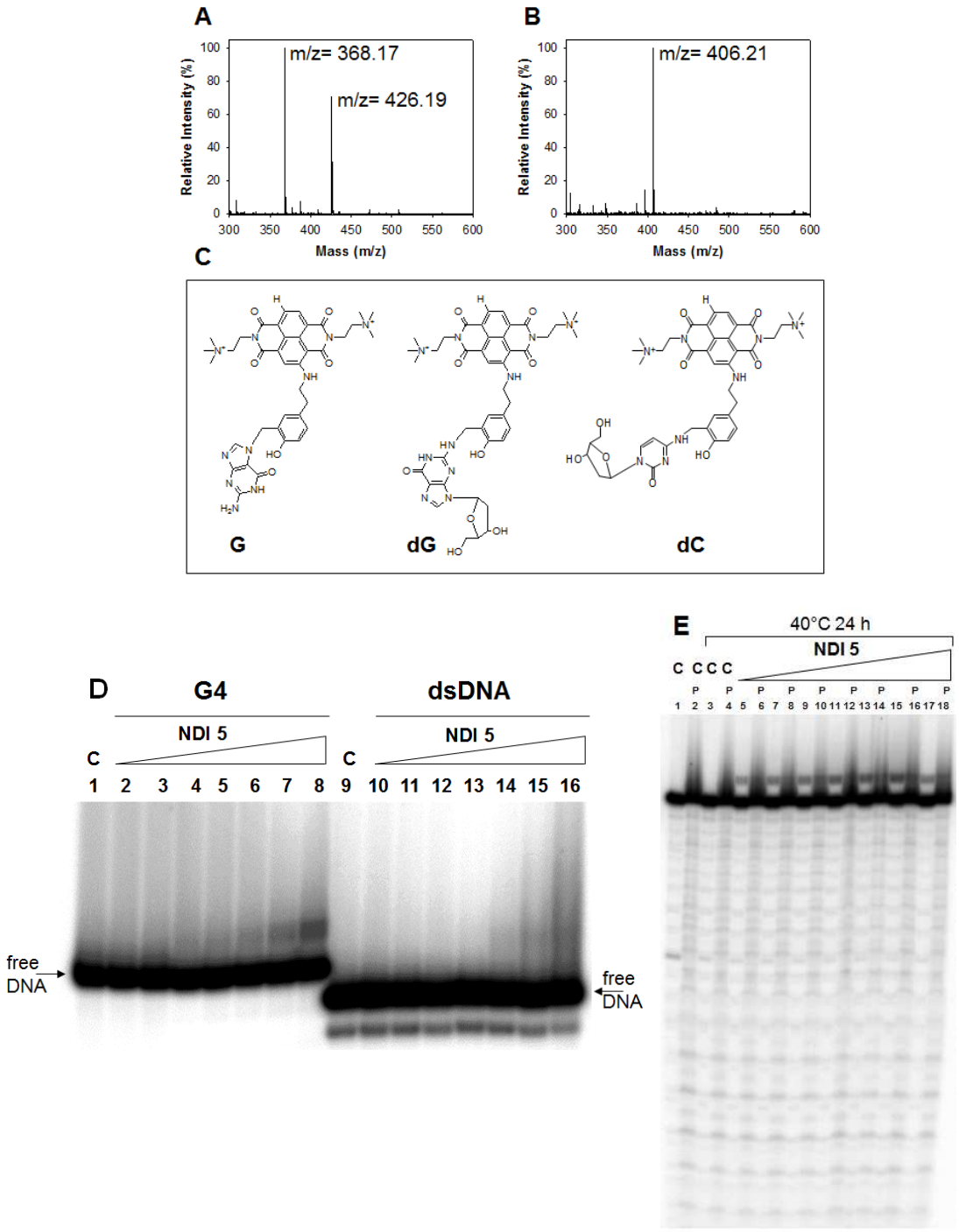


Figure S4. Alkylation effects of NDIs **5.** (**A**) and (**B**) Mass spectra of the HPLC-separated adducts of compound **5** with dG and dC, respectively. (**C**) Rational adduct structures suggested by MS data and literature data. G is the adduct that alkylates N7 of dG and induce glycoside bond cleavage. dG and dC are adducts of the NDI with dG and

dC at purine/pyrimidine nucleophilic sites that do not result in depurination (**D**) Alkylation of NDI **5** on G-4 and dsDNA. The P^{32} 5'-end-labelled hTel oligo was incubated with increasing amounts of NDIs (0.003- 50 mM) at 40°C to activate alkylation. The alkylated DNA (alk) was separated from the unreacted DNA (free) by 20% polyacrylamide 7 M urea denaturing gel. (**E**) The **5**-alkylated hTel P^{32}-5'-end labelled DNA and its non-alkylated control were treated with increasing amounts of piperidine at 90°C for 30 min. After reaction samples were ethanol precipitated and loaded onto 20% polyacrylamide denaturing gel.

Supplementary Table

Table S1. TaqMan® assays used throughout the study.

Gene	GeneBank, accession number	TaqMan® assays	Assay location
c-myc	NM_002467.4	Hs00153408_m1	1325
hTERT	NM_198253.2	Hs00972656_m1	2638

Supplemental Experimental Procedures

General Procedures.

^{1}H, ^{13}C-NMR spectra were recorded on a 300 MHz spectrometer and the chemical shifts are reported relative to TMS. The structures of new compounds were deduced from the results of ^{1}H, and $^{13}CNMR$. Elemental analyses were made on a Carlo Erba CNH analyzer, model 19106. The NDIs **8** and **9** have been synthesized according to published procedure.[1] **11** has been purchased from Sigma-Aldrich.

Synthesis of intermediates and final ligands:

N-(3-((dimethylamino)methyl)-4-hydroxyphenethyl)amine (12).[3] 3.95 g (28.8 mmol) of tyramine (**11**) were dissolved in 25 ml of THF. 9.42 g (43.2 mmol) of di-*tert*-butyl dicarbonate in THF solution (5ml) were added slowly dropwise at the stirring solution. The resulting suspension was allowed to stand at room temperature for 2 hours, then the solvent was removed under vacuum. The resulting crude product was solved in $CHCl_3$ and washed with a water solution of $NaHCO_3$. The organic phases were collected, dried with sodium sulphate and the solvent was removed under vacuum to give 4.9 g of N-(4-hydroxyphenethyl)*tert*-butylcarbamate (brown/yellow oil, 97%, yield). The compound did not require any further purification. Its spectroscopic properties were in agreement with those reported in the literature.[2] ^{1}H NMR(200 MHz, $CDCl_3$) δ(ppm): 7.02 (d, 2H, J=8.0 Hz); 6.80 (d, 2H, J=8.0 Hz); 3.35 (m, 2H); 2.71 (t, 2H, J=6.6 Hz); 1.46 (s, 9H).

N-(4-hydroxyphenethyl)*tert*-butylcarbamate (2.46 g, 10.4 mmol) was dissolved in 15 ml of anhydrous EtOH. Then 0.33g (10.9 mmol) of paraformaldehyde and 3.0 g (11.8 mmol) of a solution of dimethylamine in EtOH (20%) were added. This mixture was heated under reflux and nitrogen atmosphere for 2 h. The mixture was concentrated under vacuum and a yellow oil was obtained. The oil was purified by column chromatography (eluent: EtOAc : MeOH = 8:2 v/v) to give the pure product, N-(3-((dimethylamino)methyl)-4-hydroxyphenethyl)*tert*-butylcarbamate as a yellow oil (1.4 g, yield 47%). R_f 0.25 (EtOAc-MeOH 8:2 v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ(ppm): 9.28 (bs, 1H); 6.95 (d, 1H, J=6.3 Hz); 6.77 (d; 1H, J=6.3 Hz); 6.74 (s, 1H); 4.63 (bs, 1H); 3.30 (m, 2H); 2.67 (t, 2H, J=7.6 Hz); 2.32 (s, 6H); 1.43 (s, 12H). ^{13}C NMR ($CDCl_3$) δ(ppm): 156.3; 155.8; 129.1; 128.8; 128.6; 121.7; 115.9; 79.0; 62.6; 44.3; 41.9; 35.2; 28.3.

300 mg of N-(3-((dimethylamino)methyl)-4-hydroxyphenethyl)*tert*-butylcarbamate was deprotected dissolving it in a trifluoroacetic acid (1.94 ml, 13 mmol) and dichloromethane (10 ml) solution and stirring in the presence of triethylsilane (0.8 ml, 2.5 mmol), at r.t. After stirring 2h, the solvent was removed under vacuum, the oily residue was dissolved in CH_2Cl_2 and washed twice with a 10% solution of $NaHCO_3$. The organic layer was dried over MgSO4 and the solvent removed under vacuum to give product **12** as oil.[3] The yield was almost quantitative (93 %) using triethylsilane as carbocation scavenger.

^{1}H NMR (200 MHz, $CDCl_3$) δ(ppm): 7.28 (s, 1H); 6.94 (d, 1H, J=8.6 Hz); 6.74 (d, 1H, J= 8.5 Hz); 6.73 (s, 1H); 6.05 (bs, 1H); 3.57 (s, 2H); 2.86 (t, 2H, J=6.24 Hz); 2.62 (m, 2H); 2.28 (s, 6H).

N-(3-((morpholino)methyl)-4-hydroxyphenethyl)amine (13): N-(4-hydroxyphenethyl)*tert*-butylcarbamate (2.46 g, 10.4 mmol) was dissolved in 15 ml of anhydrous EtOH. Then 0.33 g (10.9 mmol) of paraformaldehyde and 1.02 g (11.8 mmol) of morpholine were added. This mixture was heated under reflux and nitrogen atmosphere for 2 h. After cooling, the mixture was concentrated under vacuum and a yellow oil was obtained. The oil was purified by column chromatography (eluent: EtOAc:MeOH=8:2 v/v) to give pure N-(3-((morpholino)methyl)-4-hydroxyphenethyl)*tert*-butylcarbamate (yellow oil, 1.74g, yield 50%). R_f 0.15 (EtOAc-MeOH 8:2 v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ(ppm): 6.97 (d, 1H, J=6.6 Hz); 6.79 (d, 1H, J=8.8 Hz); 6.73 (s, 1H); 4.67 (bs, 1H); 3.74 (bs, 4H); 3.66 (s, 2H); 3.29 (bs, 2H); 2.69 (bs, 2H); 2.56 (bs, 4H); 1.43 (s, 9H).

300 mg of N-(3-((morpholino)methyl)-4-hydroxyphenethyl)*tert*-butylcarbamate was deprotected by stirring it in a solution of trifluoroacetic acid (1.94 ml, 13 mmol) and dichloromethane (10 ml), in the presence of triethylsilane (0.8 ml, 2.5 mmol), at r.t. The work up was identical to that reported above for the synthesis of **12**, giving **13** as oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ(ppm): 7.01 (d, 1H, J=6.6 Hz); 6.80 (.d, 1H, J=5.8 Hz); 6.76 (s, 1H); 3.79 (m, 4H); 3.69 (s, 2H); 2.90 (m, 2H); 2.66 (m, 2H); 2.58 (m, 4H). Elemental analysis (%) calcd. for $C_{13}H_{20}N_2O_2$: C, 66.07; H, 8.53; N, 11.85; O, 13.54. Found C, 65.98; H, 8.42; N, 11.87.

N-(3-((trimethylamino)methyl)-4-hydroxyphenethyl)amine (14): N-(3-((dimethylamino)methyl)-4-hydroxyphenethyl)*tert*-butylcarbamate (1.47 g, 5.0 mmol) was suspended in 50 ml of CH_3CN and CH_3I (0.33g, 10.0 mmol) was added. After heating 3 h at 80°C, under nitrogen, the reaction was cooled at r.t. and the solvent was

removed under vacuum to give N-(3-((trimethylamino)methyl)-4-hydroxyphenethyl)*tert*-butylcarbamate as a yellow solid (2.07g, yield 95%). M.p. dec. > 90°C. ^{1}H NMR (200 MHz, $CDCl_3$) δ(ppm): 7.28 (m, 2H); 7.11 (m, 1H); 4.66 (bs, 2H); 3.30 (m, 2H); 3.23 (s, 9H); 2.73 (m, 2H); 1.44 (s, 9H). ^{13}C NMR (CD_3OD) δ(ppm): 159.3; 157.5; 136.1; 134.5; 132.4; 117.5; 115.8; 65.86; 53.8; 42.3; 35.7; 28.3.

300 mg of N-(3-((trimethylamino)methyl)-4-hydroxyphenethyl)*tert*-butylcarbamate was deprotected in a solution of trifluoroacetic acid (1.94 ml, 13 mmol) and dichloromethane (10 ml) in the presence of triethylsilane (0.8 ml, 2.5 mmol), at r.t. After stirring 2h, the solvent was removed under vacuum. HCl 1M (2 ml) was added to the residue. Solvent evaporation under vacuum afforded the adduct **14** as hydrochloride (91%, yield). M.p. dec. > 90°C. ^{1}H NMR (200 MHz, $CDCl_3$) δ(ppm): 7.39 (bs, 1H); 7.31 (d, 1H, J=7.3 Hz); 6.97 (d, 1H, J= 8.04 Hz); 4.62 (bs, 2H); 4.52 (bs, 1H); 3,70 (m, 2H); 3.15 (s, 9H); 2.95 (m, 2H). Elemental analysis (%) calcd. for $C_{12}H_{22}Cl_2N_2O$: C, 51.25; H, 7.89; Cl, 25.21; N, 9.96; O, 5.69. Found C, 51.20; H, 7.95; Cl, 25.15; N, 10.03.

***N*,*N*'-Bis-((dimethylamino)ethylamino)-2-((4-hydroxyphenyl)ethylamino)-1,4-5,8-naphthalenetetracarboxylic bisimide dihydrochloride** (**1**·2HCl): Red solid. M.p. dec.>350°C. ^{1}H NMR(CD_3OD, 300 MHz): δ=8.40 (d, 1H, J=7.8 Hz); 8.13 (d. 1H, J=7.8 Hz); 7.97 (s, 1H); 7.23 (d, 1H, J=8.5 Hz); 6.77 (d, 1H, J=8.5 Hz); 4.48 (m, 4H); 3.78 (t, 2H; J=6.8 Hz); 3.54 (m, 4H); 3.03 (s, 12 H); 3.01 (bs, 2H). ^{13}C NMR (CD_3OD): δ=167.5; 165.2; 165.0; 164.7; 157.7; 153.8; 132.4; 131.5; 131.0; 130.6; 129.0; 127.5; 125.6; 124.5; 121.5; 120.8; 117.0; 100.4; 57.8; 57.4; 46.1; 44.5; 44.4; 37.1; 36.8; 36.0. Elemental analysis (%) calcd. for $C_{30}H_{35}Cl_2N_5O_5$: C, 58.44; H, 5.72; Cl, 11.50; N, 11.36; O, 12.98. Found C, 58.36; H, 5.70; Cl, 11.42; N, 11.21.

***N,N'*-Bis-((dimethylamino)ethylamino)-2-bromo-6-((4-hydroxyphenyl)ethylamino) -1,4-5,8-naphthalenetetracarboxylic bisimide dihydrochloride** (**1Br**·2HCl): Red solid. M.p. dec.>350°C. ^{1}H NMR (CD_3OD, 300 MHz): δ=8.54 (s, 1H); 8.06 (s. 1H); 7.25 (d, 1H, J=8.5 Hz); 6.80 (d, 1H, J=8.5 Hz); 4.49 (m, 4H); 3.80 (t, 2H, J=6.9 Hz); 3.55 (m, 4H); 3.05 (s, 12H); 2.86 (bs, 2H). ^{13}C NMR (CD_3OD): δ=167.2; 163.8; 163.1; 157.7; 153.2; 138.8; 134.4; 131.6; 131.1; 130.6; 130.0; 128.5; 124.4, 122.2; 121.2; 117.0; 100.5; 79.2; 57.6; 57.2; 46.2; 44.5; 37.6; 37.0; 35.8. Elemental analysis (%) calcd. for $C_{30}H_{34}BrCl_2N_5O_5$: C, 51.81; H, 4.93; Br, 11.49; Cl, 10.20; N, 10.07; O, 11.50. Found C, 51.84; H, 5.00; Br, 11.46; Cl, 10.28; N, 10.12.

Exhaustive methylation of the amines 2, 2Br: The amine hydrochlorides (**2**·3HCl and **2Br**·3HCl) 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 (2.9 mmol) was suspended in 50 ml of CH_3CN and 1.2 g (8.5 mmol) of CH_3I were added. This suspension was heated under reflux and nitrogen atmosphere for 3 h. The reaction mixture turned dark red, within few minutes. The reaction was cooled down at r.t. and a red solid formation was observed by addition of Et_2O (50 ml) to the reaction mixture. The suspension was filtered and washed with CH_3CN to give the quaternary ammonium salts **5** and **5Br**.

***N,N'*-Bis-((trimethylamino)ethylamino)-2-(2-(3-(trimethylamino)methyl-4-hydroxy phenyl)ethylamino)-1,4-5,8-naphthalenetetracarboxylic bisimide triiodide (5):** Red solid. Yield 97% M.p. dec.>350°C. ^{1}H NMR(D_2O, 300 MHz): δ=8.24 (d, 1H, J=7.8 Hz); 7.98 (d, 1H, J=7.9 Hz); 7.54 (s, 1H); 7.33 (d, 1H, J=1.8 Hz); 7.28 (d, 1H, J=6 Hz); 6.83 (d, 1H, J=8.8 Hz); 4.46 (m, 4H); 4.24 (s, 2H); 3.77 (t, 2H, J=5.77 Hz); 3.57 (m, 4H), 3.24

(s, 18H); 2.94 (s, 9H), 2.63 (bs, 2H). ^{13}C NMR (D_2O): δ=164.6; 163.4; 163.2; 162.7; 155.1; 152.1; 135.0; 133.4; 131.0; 130.3; 128.7; 126.0; 125.0, 124.2; 122.0; 120.5; 118.4; 116.7; 114.6; 98.2; 63.6; 62.2; 61.8; 53.4; 52.4; 44.3; 38.6; 34.2; 33.8. Elemental analysis (%) calcd. for $C_{36}H_{49}I_3N_6O_5$: C, 42.12; H, 4.81; I, 37.09; N, 8.19; O, 7.79. Found C, 42.14; H, 4.78; I, 37.12; N, 8.22.

***N*,*N*'-Bis-((trimethylamino)ethylamino)-2-bromo-6-(2-(3-(trimethylamino)methyl-4-hydroxyphenyl)ethylamino)-1,4-5,8-naphthalenetetracarboxylic bisimide triiodide (5Br):** Blue solid. Yield 97% M.p. dec.>350°C. ^{1}H NMR (D_2O, 300 MHz): δ=8.54 (s, 1H), 7.78 (s, 1H); 7.28 (m, 2H); 6.82 (d, 1H, J=8.5 Hz); 4.53 (m, 4H); 4.27 (s, 2H); 3.85 (m, 2H); 3.63 (m, 4H); 3.27 (s, 18H); 2.95 (s, 9H); 2.65 (bs, 2H). ^{13}C NMR (D_2O): δ=165.0; 163.8; 161.0; 162.5; 155.0; 151.8; 135.0; 133.5; 131.1; 130.6; 126.0, 124.0; 123.2; 122.2; 120.3; 116.7; 114.6; 99.0; 63.7; 62.3; 62.0; 53.4; 52.4; 45.7; 38.6; 34.5; 34.0. Elemental analysis (%) calcd. for $C_{36}H_{48}BrI_3N_6O_5$: C, 39.11; H, 4.38; Br, 7.23; I, 34.44; N, 7.60; O, 7.24 Found C, 39.18; H, 4.41; Br, 7.22; I, 34.39; N, 7.64.

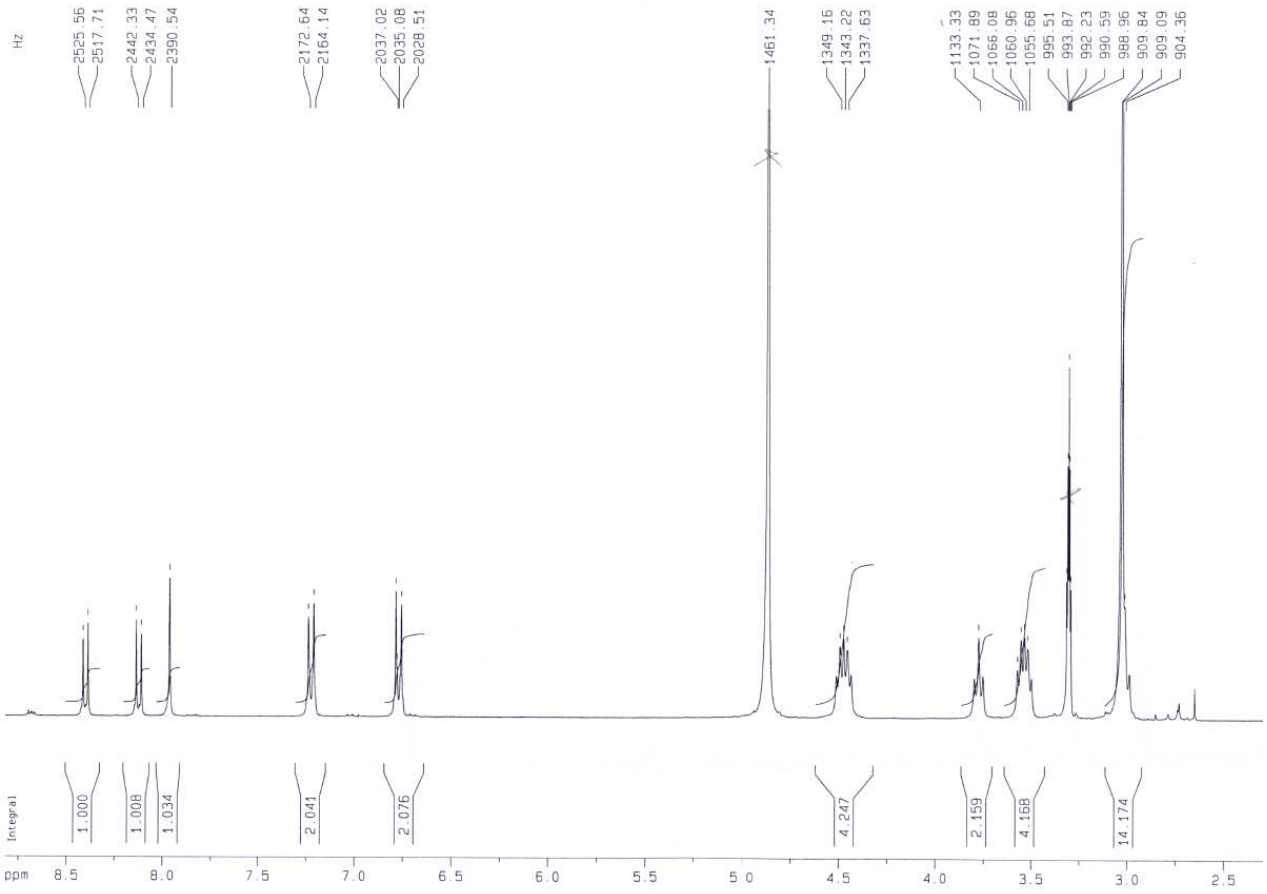
***N*,*N*'-Bis-((dimethylamino)ethylamino)-2-(2-(3-(N-morpholino)methyl-4-hydroxy phenyl)ethylamino)-1,4-5,8-naphthalenetetracarboxylic bisimide trihydrochloride (3·3HCl):** Red solid. Yield 12%. M.p. dec. >350°C. ^{1}H NMR (CD_3OD, 300 MHz) δ=8.63 (d, 1H, J=7.7 Hz); 8.37 (d, 1H, J=7.7 Hz); 8.23 (s, 1H); 7.39 (s, 1H); 7.32 (dd, 1H, J=8.3, 2.0 Hz); 6.88 (d, 1H, J=8.3 Hz); 4.59-4.53 (m, 4H); 4.36 (s, 2H); 4.03-3.98 (m, 2H); 3.94-3.90 (m, 2H); 3.80-3.72 (m, 2H); 3.57 (bs, 4H); 3.42-3.39 (m, 2H); 3.32-3.21 (m, 4H); 3.04 (s, 12H). Elemental analysis (%) calcd. for $C_{35}H_{45}Cl_3N_6O_6$: C, 55.89; H, 6.03; Cl, 14.14; N, 11.17; O, 12.76. Found C, 55.92; H, 6.08; Cl, 14.11; N, 11.15.

***N,N'*-Bis-((dimethylamino)ethylamino)-2-bromo-6-(2-(3-(N-morpholino)methyl-4-hydroxyphenyl)ethylamino)-1,4-5,8-naphthalenetetracarboxylic bisimide trihydrochloride** (**3Br**·3HCl): Violet solid. Yield 45%. M.p. dec. >350°C. ^{1}H NMR(CD_3OD, 300 MHz) δ=8.58 (s, 1H); 8.14 (s, 1H); 7.44 (s, 1H); 7.37 (d, 1H, J=8.2, 2.0 Hz); 6.93 (d, 1H, J=8.2 Hz); 4.55 (m, 4H); 4.4 (s, 2H); 4.06-4.03 (m, 2H); 3.90-3.87 (m, 2H); 3.85-3.80 (m, 2H); 3.61-3.57 (m, 4H); 3.47-3.43 (m, 2H); 3.34-3.31 (m, 2H); 3.26-3.22 (m, 2H); 3.05 (s, 12H). ^{13}C NMR (CD_3OD): δ=167.3; 163.9; 163.2; 157.4; 153.3; 139.0; 135.0; 140.0; 131.4; 130.2; 128.7; 124.8; 124.7; 123.1; 122.1; 121.2; 117.3; 117.0; 101.0; 65.1; 57.5; 57.3; 57.2; 53.1; 46.0; 45.0; 44.4; 37.6; 36.9; 35.8. Elemental analysis (%) calcd. for $C_{35}H_{44}BrCl_3N_6O_6$: C, 50.59; H, 5.34; Br, 9.62; Cl, 12.80; N, 10.11; O, 11.55. Found C, 51.03; H, 5.30; Br, 9.67; Cl, 12.86; N, 10.09.

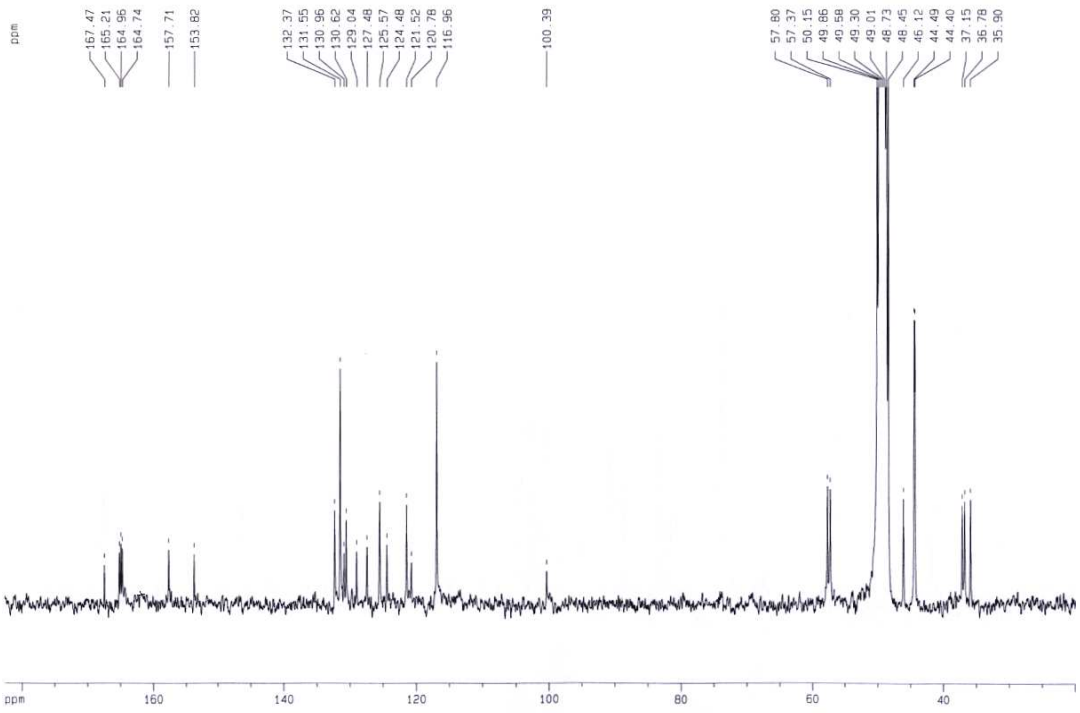
***N,N'*-Bis-((dimethylamino)ethylamino)-2-(2-(3-(trimethylamino)methyl-4-hydroxy phenyl)ethylamino)-1,4-5,8-naphthalenetetracarboxylic bisimide trihydrochloride** (**4**·2HCl): Red solid. Yield 6%. M.p. dec. >350°C. ^{1}H NMR (CD_3OD, 300 MHz): δ=8.67 (d, 1H, J=7.7 Hz); 8.38 (d, 1H, J=7.7 Hz); 8.27 (s, 1H); 7.36 (bs, 1H); 7.33 (d, 1H, J=8.6 Hz); 6.91 (d, 1H, J=8.6 Hz); 4.59 (bs, 4H); 4.31 (s, 2H); 3.93 (t, 2H, J=6.8 Hz); 3.60 (bs, 4H); 3.07 (bs, 2H); 3.06 (s, 15H); 2.87 (s, 6H). ^{13}C NMR (CD_3OD): δ=168.0; 165.6; 165.4; 165.1; 162.0; 161.6; 157.0; 154.1; 134.1; 133.8; 132.61; 131.6; 131.4; 129.7; 128.0; 125.8; 125.0; 121.6; 118.2; 117.0; 101.0; 58.7; 57.9; 57.5; 46.0; 44.5; 44.4; 43.6; 37.2; 36.8; 36.0. Elemental analysis (%) calcd. for $C_{34}H_{45}Cl_3N_6O_5$: C, 56.39; H, 6.26; Cl, 14.69; N, 11.61; O, 11.05. Found C, 56.43; H, 6.23; Cl, 14.62; N, 11.66.

3. NMR spectra

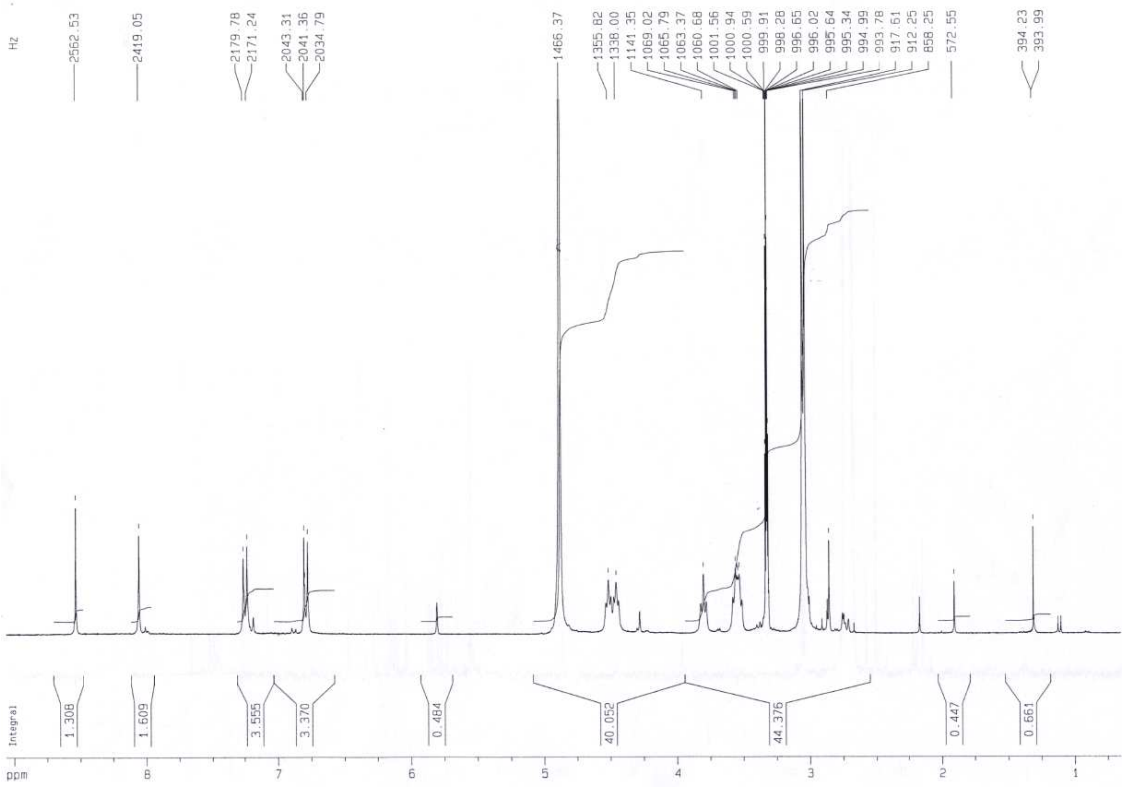
300 MHz ^{1}H NMR spectra of 1 CD_3OD



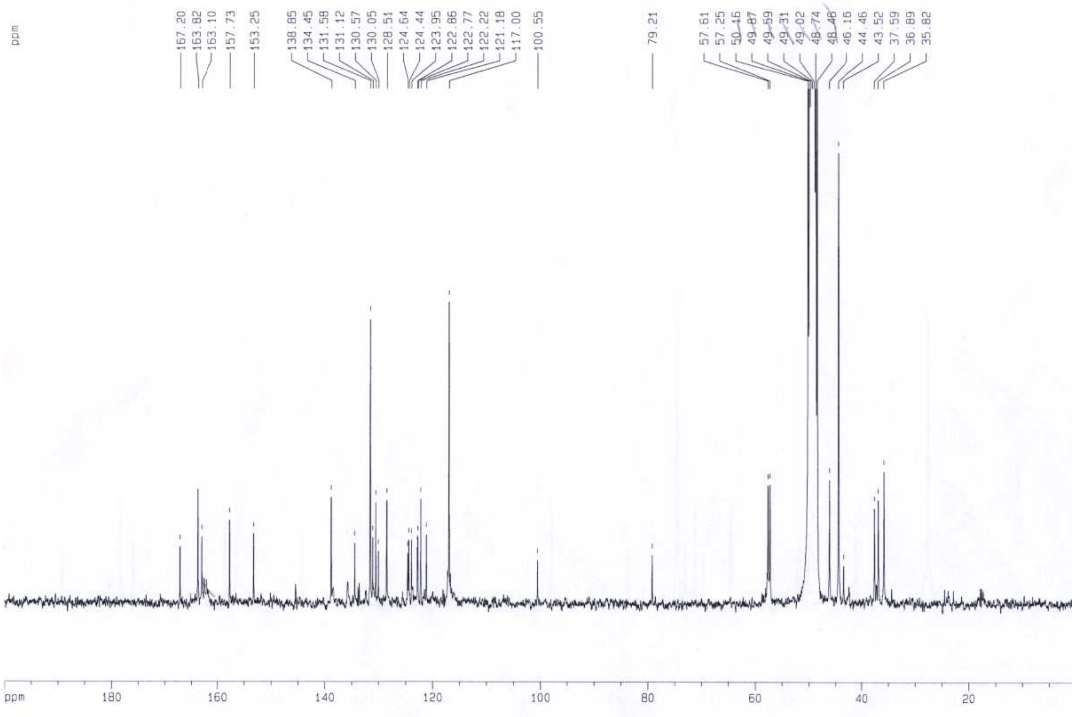
75 MHz ^{13}C NMR spectra of 1 CD_3OD



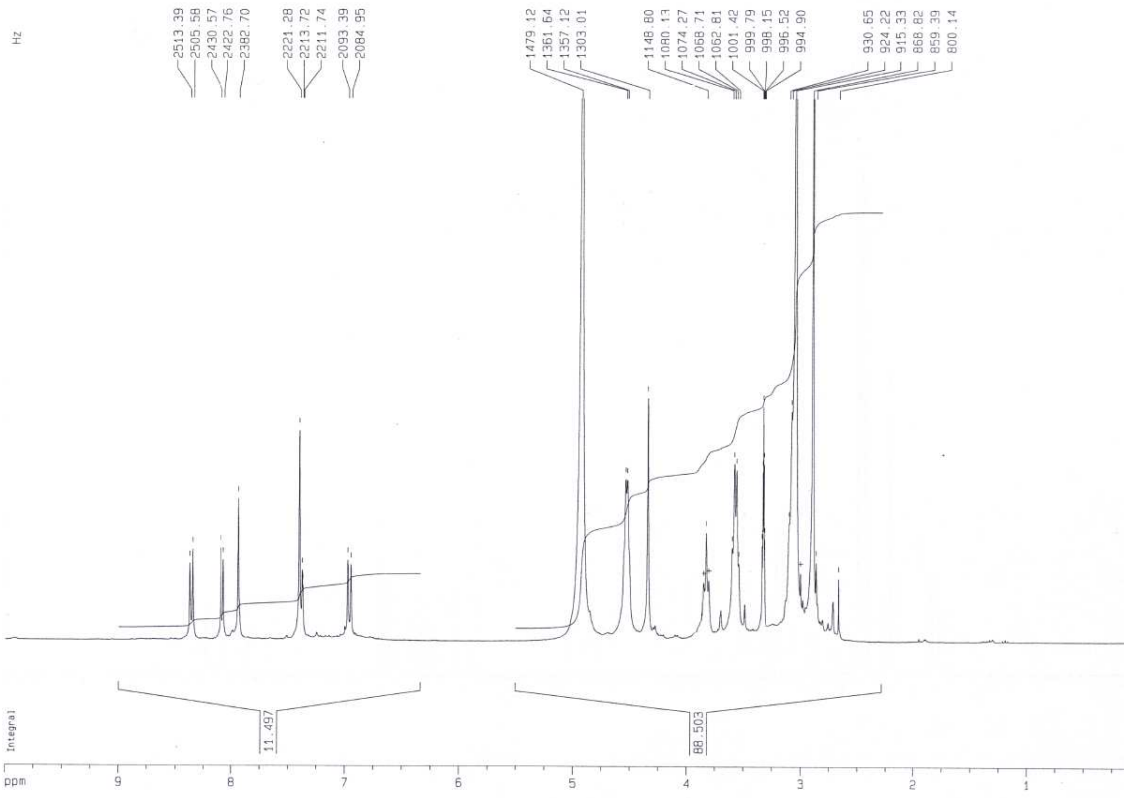
300 MHz ^{1}H NMR spectra of 1Br CD_3OD



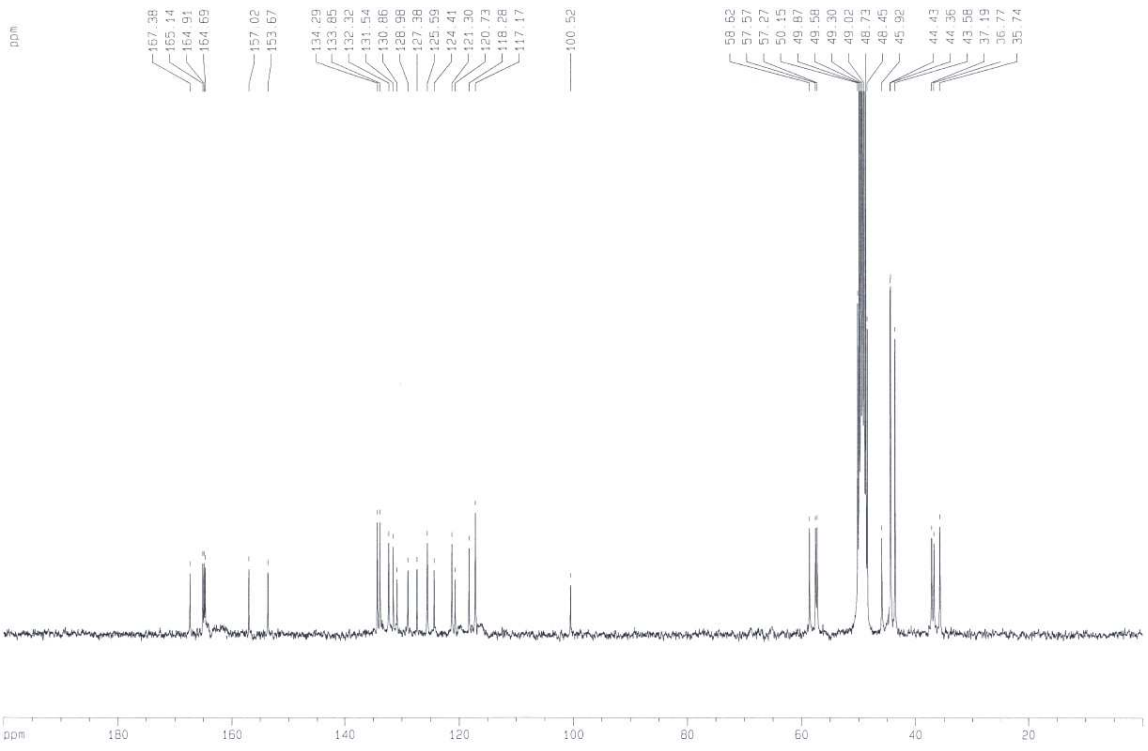
75 MHz ^{13}C NMR spectra of 1Br CD_3OD



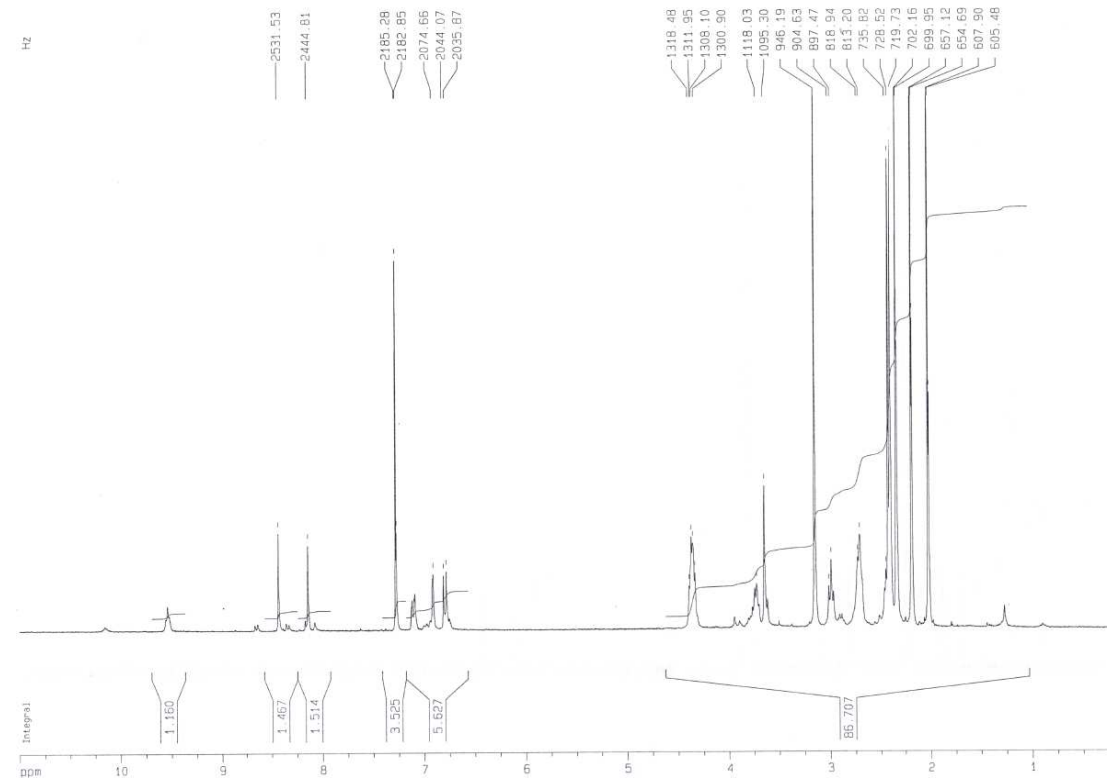
300 MHz ^{1}H NMR spectra of 2 CD_3OD



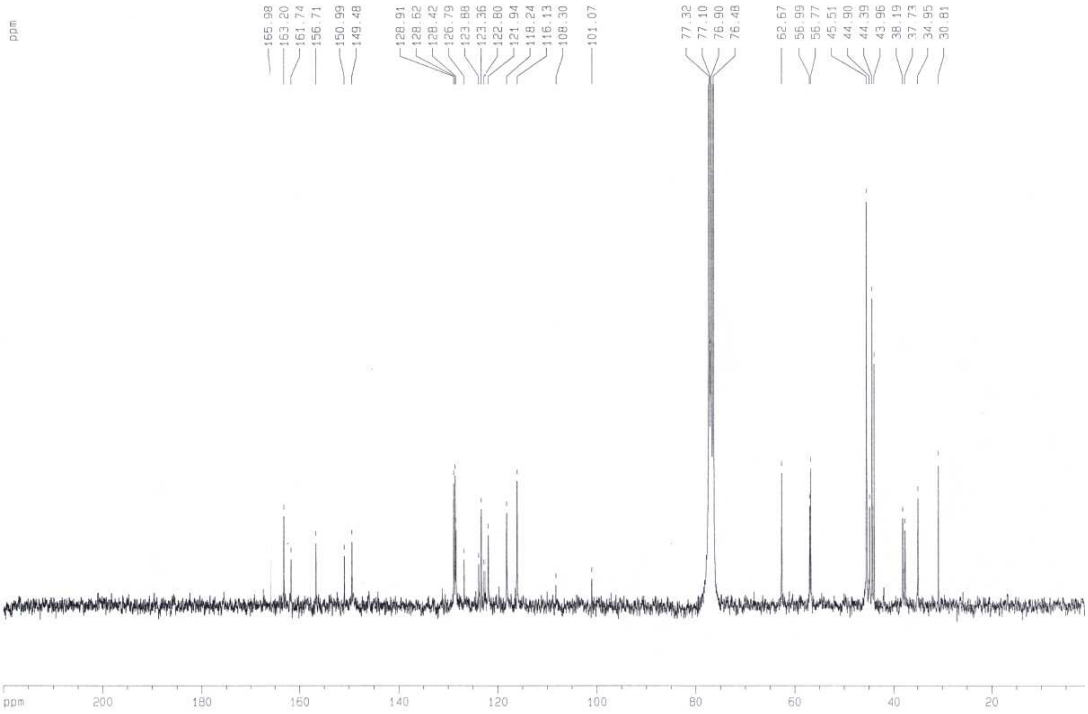
75 MHz ^{13}C NMR spectra of 2 CD_3OD



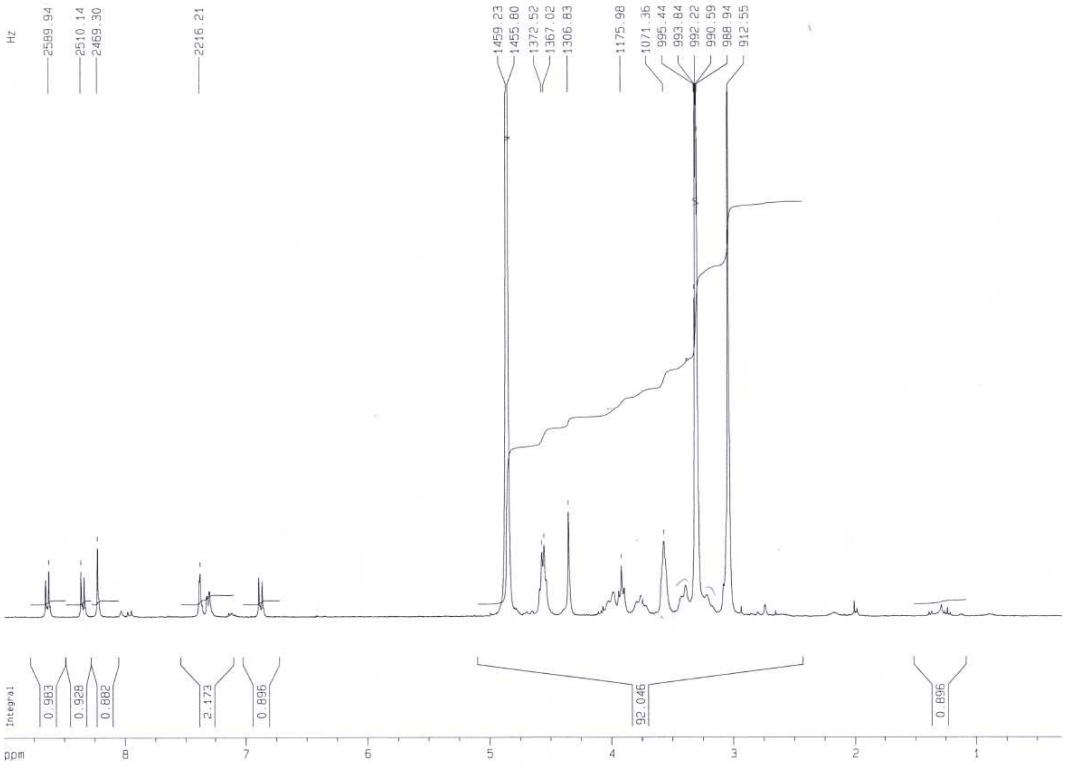
300 MHz ^{1}H NMR spectra of 2Br $CDCl_3$



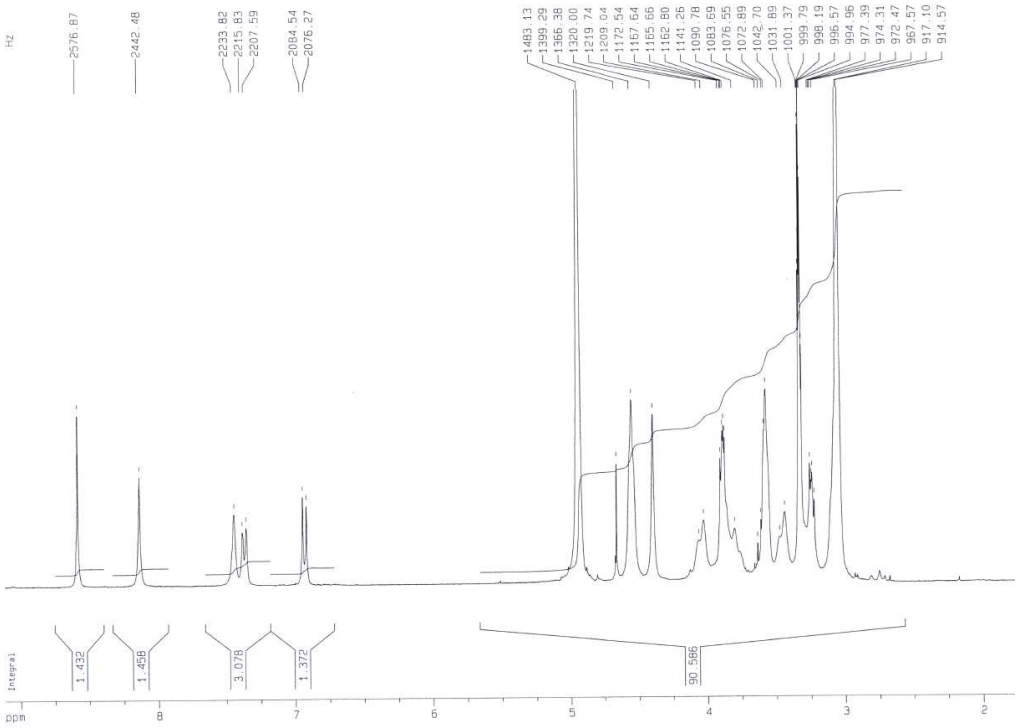
75 MHz ^{13}C NMR spectra of 2Br $CDCl_3$



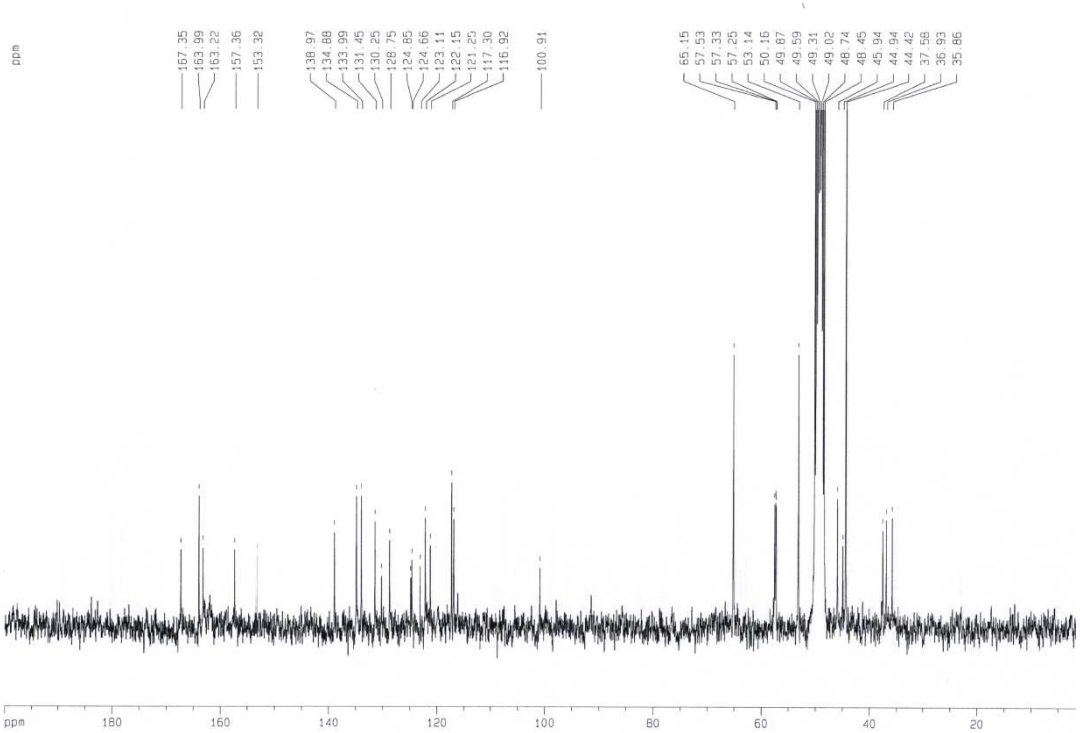
300 MHz ^{1}H NMR spectra of 3 CD_3OD



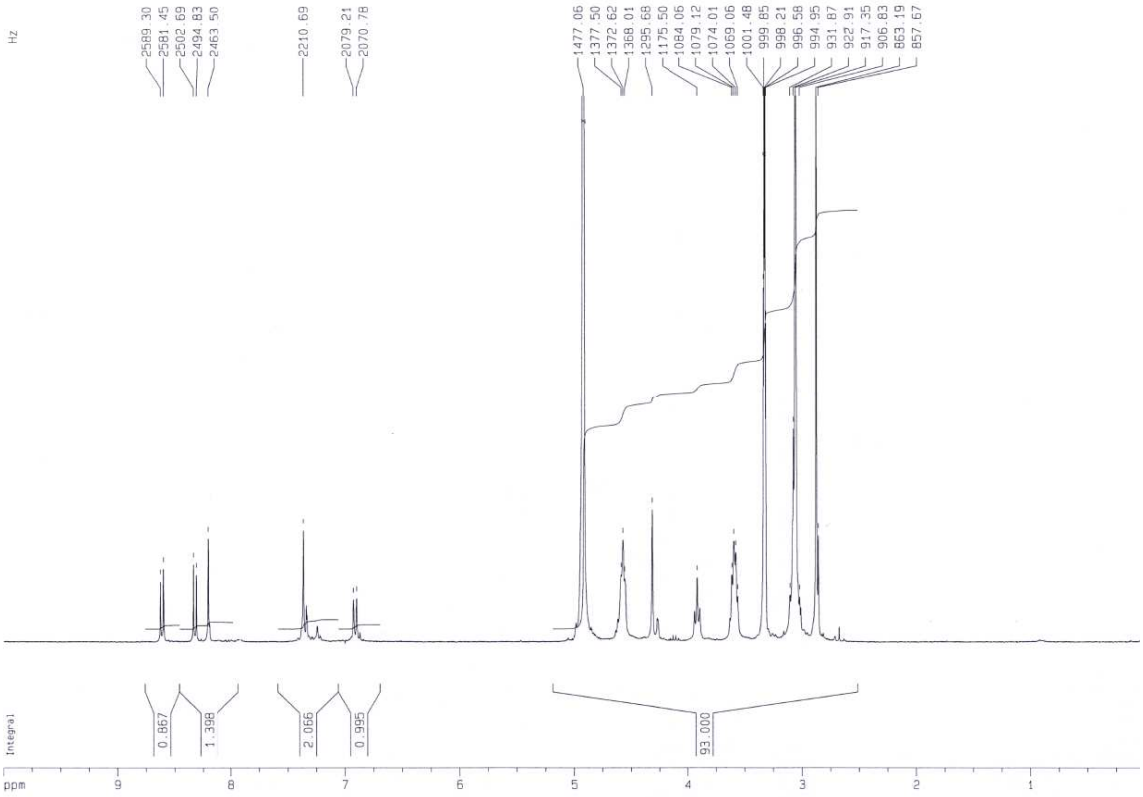
300 MHz ^{1}H NMR spectra of 3Br CD_3OD



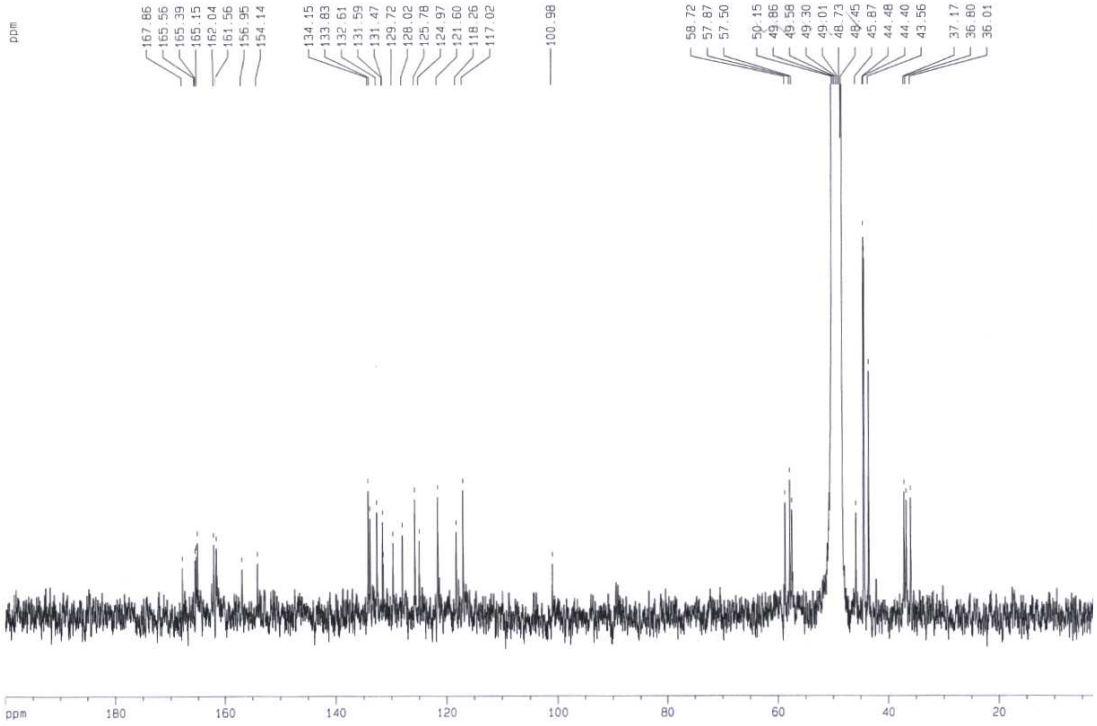
75 MHz ^{13}C NMR spectra of 3Br CD_3OD



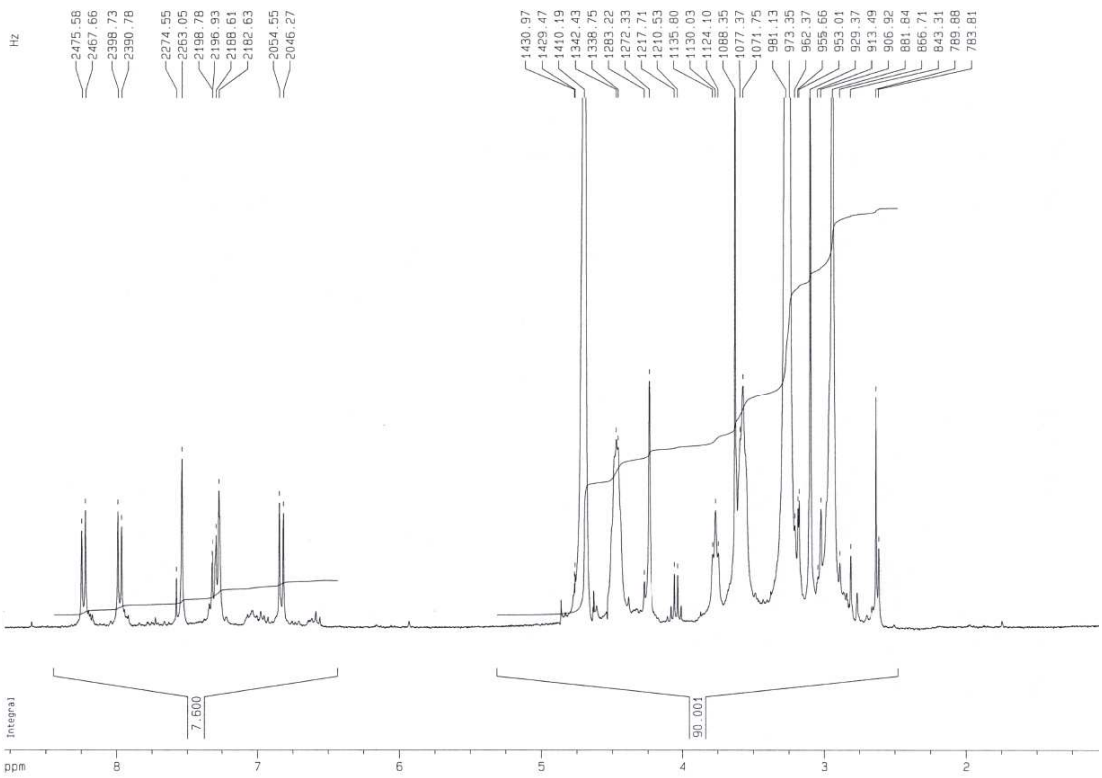
300 MHz ^{1}H NMR spectra of 4 CD_3OD



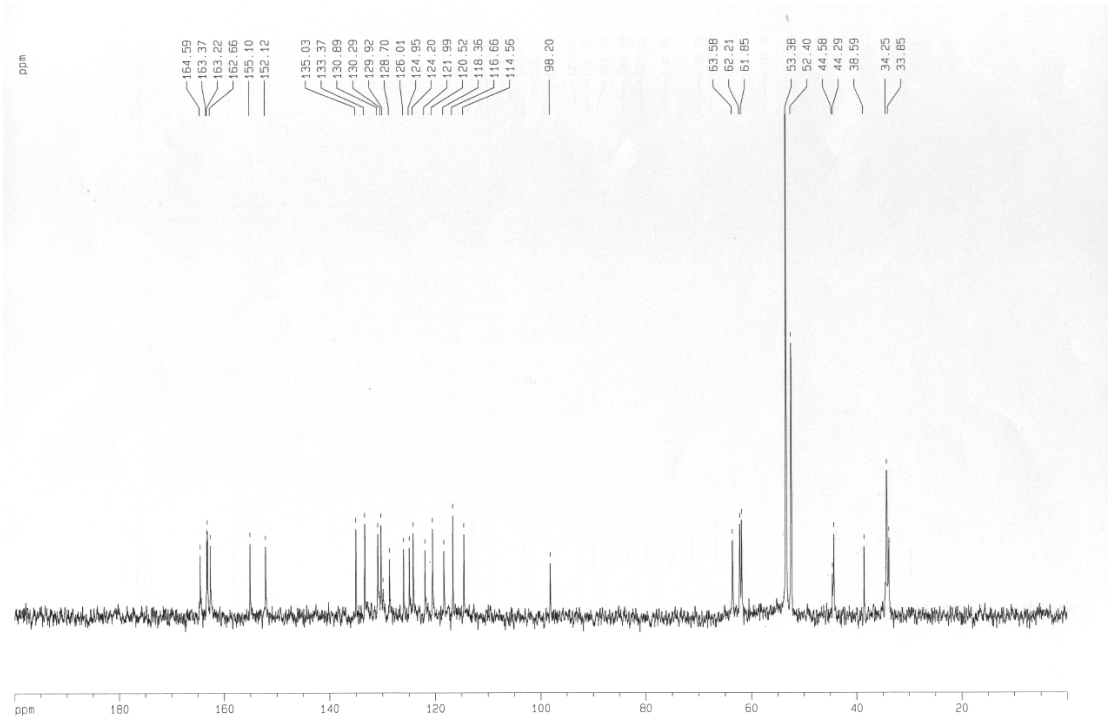
75 MHz ^{13}C NMR spectra of 4 CD_3OD



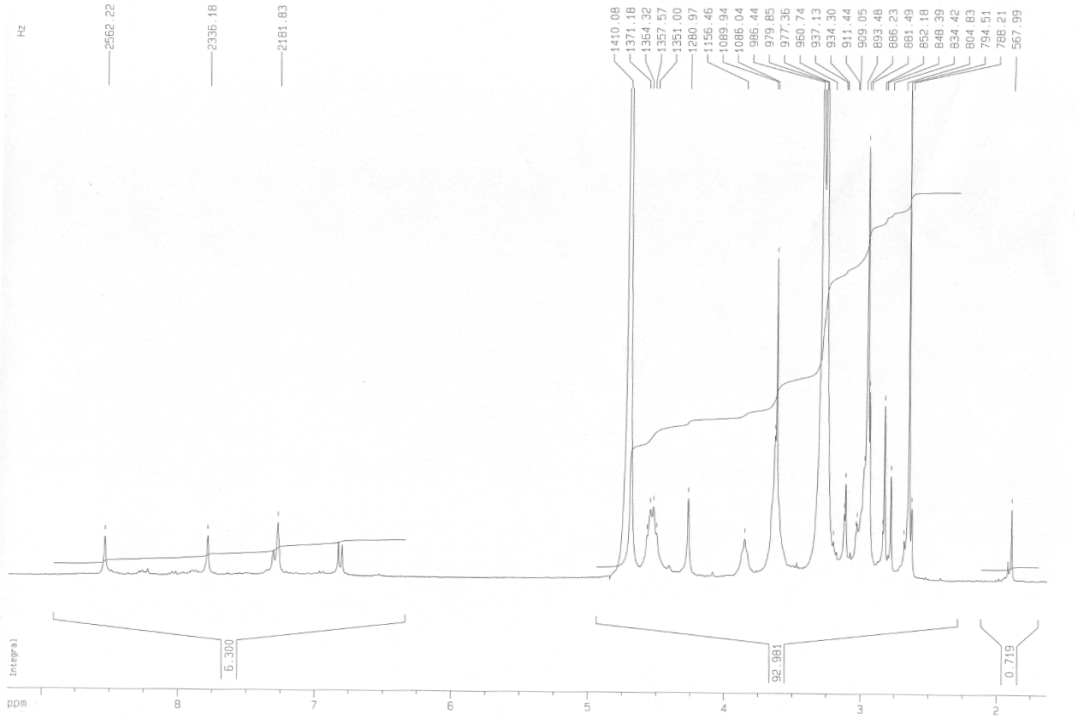
300 MHz ^{1}H NMR spectra of 5 D_2O



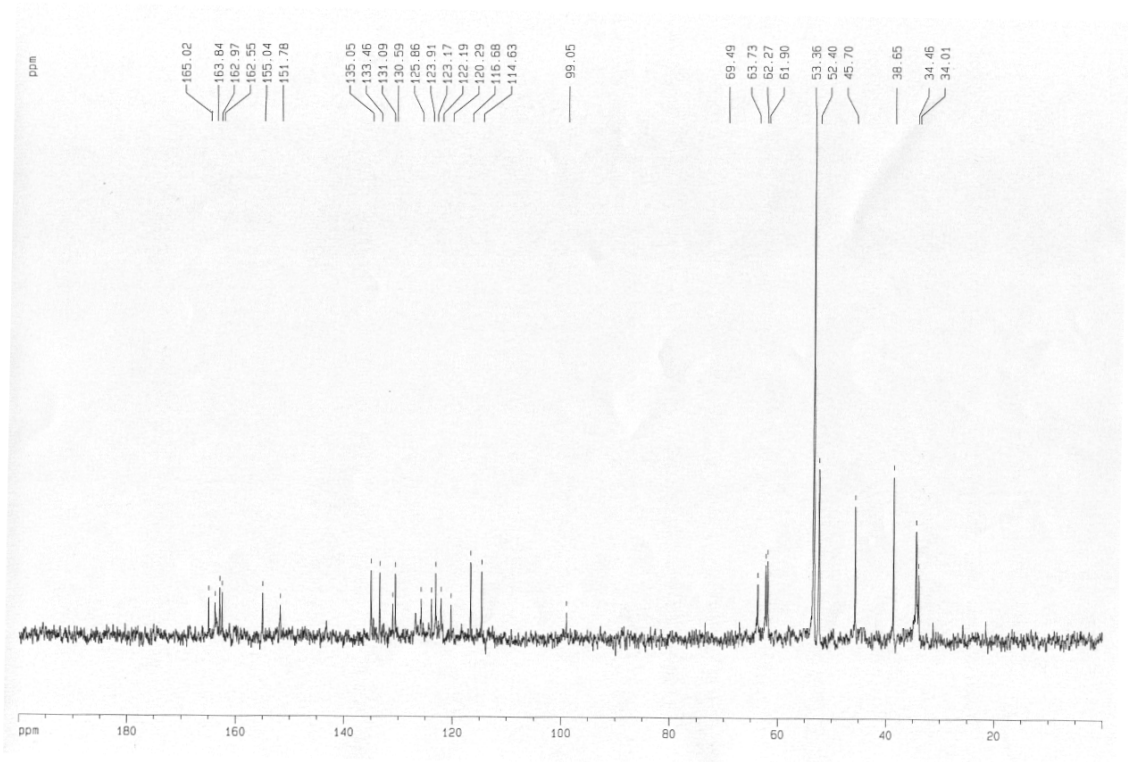
75 MHz ^{13}C NMR spectra of 5 D_2O



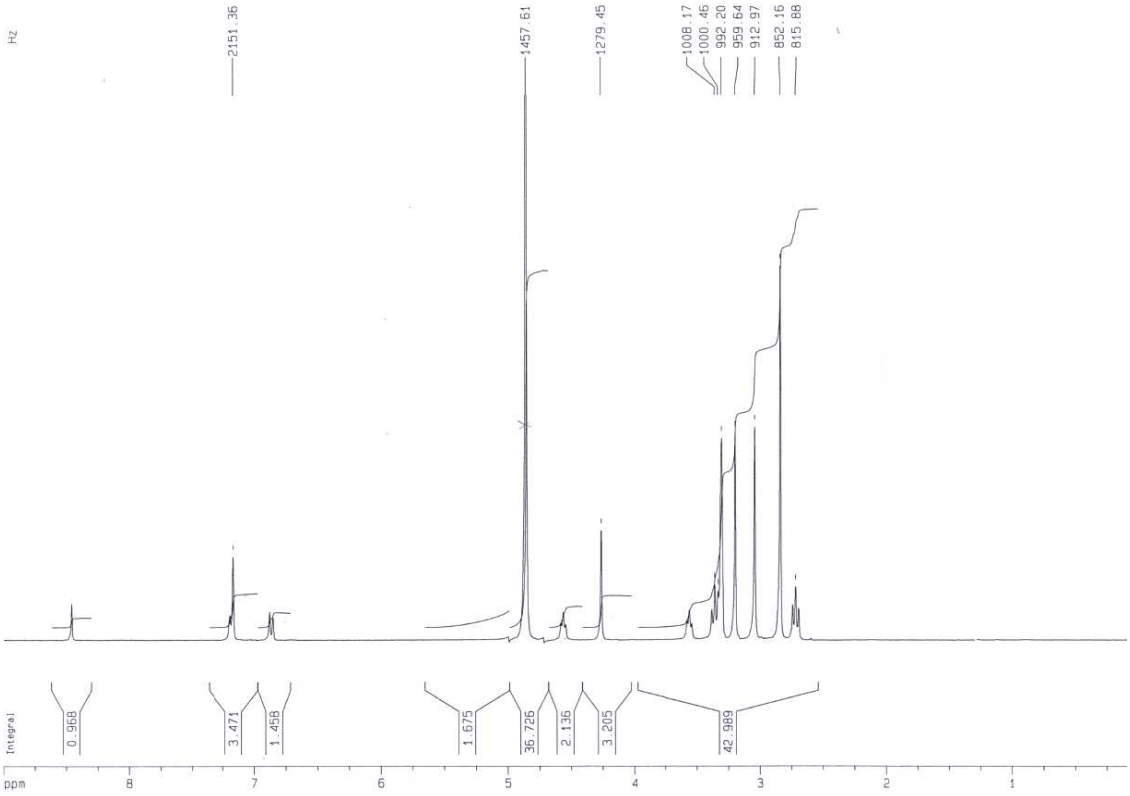
300 MHz ^{1}H NMR spectra of 5Br D_2O



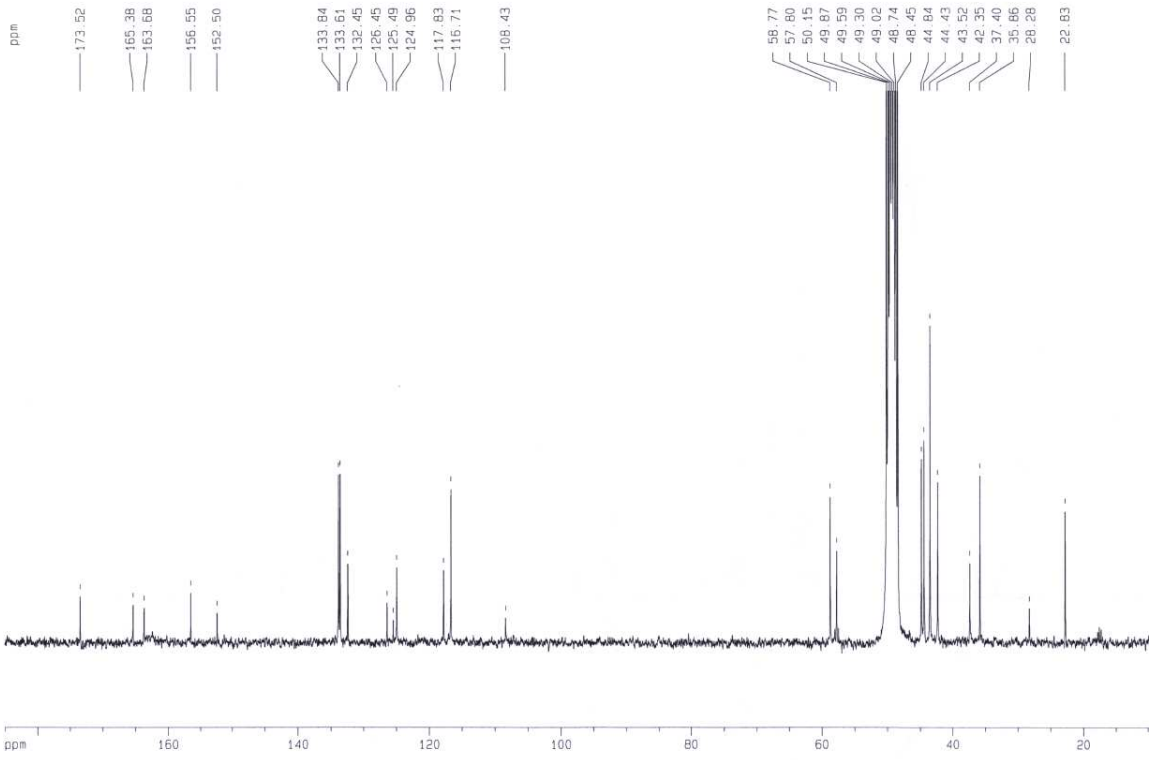
75 MHz ^{13}C NMR spectra of 5Br D_2O



300 MHz ^{1}H NMR spectra of 6 CD_3OD



75 MHz ^{13}C NMR spectra of 6 CD_3OD



Nucleotide and oligonucleotide alkylation.

To measure the alkylation properties of **5**, **5Br** and **4** towards 2'-deoxynucleotides, 200 nmol of drug and 1800 nmol of the nucleoside dG, dA, dC or dT were incubated for 24 h at 60 °C in 50 mM potassium phosphate buffer, pH 7.4. The mixtures were loaded on a HPLC C18 reverse phase column (Eclipse XDB C18 column, Agilent Technologies), using solvent A (CH_3CN/TFA 0.01%) and solvent B (H_2O/TFA 0.01%) as elution buffers, with a gradient 0-100% solvent A in 20 min and 100 µl injection. Peaks were detected at 260 nm, and the adduct peaks were collected in Eppendorf tubes and dried at room temperature in Speed Vac UniVapo 100 H (UniEquip, Martinsried, Germany). Liquid chromatography mass spectrometry was performed on a Time-of-Flight mass analyser (Mariner ESI-TOF, Applied Biosystems, CA). Positive ion mass spectra were acquired on the ESI-TOF instrument by directly injecting 7 μl of analyte solutions in CH_3CN:H_2O:HCOOH (50:49.5:0.5) at room temperature and with a 0.16 ml/min flow rate. The nozzle temperature was 140 °C, while a constant flow of N_2 gas was kept at 0.35 l min ± 1 to facilitate the spray. A three-point external calibration provided typical 100 ppm accuracy. The oligonucleotide alkylation and exonuclease I digestion were performed in conditions previously reported (Nadai et al. 2011).

Evaluation of telomerase activity. Telomerase activity was measured on 1 µg of protein by the telomeric-repeat amplification protocol (TRAP) using the TRAPeze kit (Chemicon International, Canada), according to manufacturer's protocol. Each reaction product was amplified in the presence of a 36-bp internal TRAP assay standard (ITAS). A TSR8 quantitation standard (which serves as a standard to estimate the amount of product extended by telomerase in a given protein extract) was included for each set of TRAP

assays. PCR amplification products were then resolved by polyacrylamide gel electrophoresis and visualized by autoradiography. Quantification of data was performed according to manufacturer's instructions.

Immunoblotting analyses. Fourty μg of protein extracts were fractioned by SDS-PAGE and transferred onto Hybond nitrocellulose membranes (GE Healthcare). Filters were blocked in PBS-Tween-20 in 5% skim milk and probed with antibodies raised against $p21^{waf1}$and γ-H2AX (Abcam, Cambridge, UK). Bound antibodies were detected by SuperSignal® West PICO chemiluminescent detections system (Thermo Scientific, Rockford, IL) after being probed with secondary horseradish peroxidase-linked antibodies (GE Healthcare). β-actin was used as equal protein loading control.

Statistical analysis. Data quantification is reported as mean values ± s.d. from at least three independent experiments. Two-sided Student's *t* test was used to analyze the differences in total 3'-overhang amount and TRF2 and hPOT1 binding to telomeres. *P* values <0.05 were considered statistically significant.

References

1) M. Nadai, F. Doria, M. Di Antonio, G. Sattin, L. Germani, C. Percivalle, M. Palumbo, R.S. Richter and M. Freccero, *Biochimie*, 2011, **93**, 1328

2) P. V. Czarnecki, A. Kampert, S. Barbe and J. C. Tiller, *Tetrahedron Letters*, **2011**, 52, 3551–3554.

3) M. Di Antonio, F. Doria, S.-N. Richter, C. Bertipaglia, M. Mella, C. Sissi, M. Palumbo and M. Freccero, *J. Am. Chem.Soc.*, **2009**, 131, 13132.