DOTASQ as a prototype of nature-inspired G-quadruplex ligand

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- Supporting Information -

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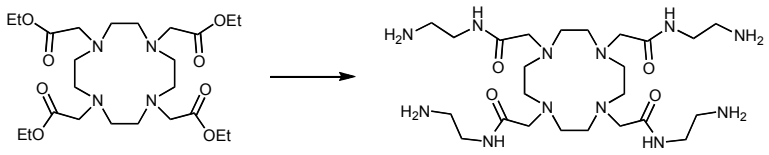
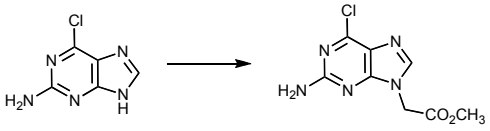
Synthesis Part – Generality:

^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 using TMS as internal standard. Deuterated solvents ($CDCl_3$, [D_6]DMSO) were purchased from SDS. The following abbreviations are used: singlet (s), doublet (d), doubled doublet (dd), triplet (t), doubled triplet (td), quintuplet (quint), sextuplet (sext) and multiplet (m). Mass spectrometry services were provided by PACSMUB (Plateforme d'Analyse Chimique et de Synthèse Moléculaire), at the *Université de Bourgogne*, Dijon; the following abbreviations are used: ESI for Electrospray ionization, MALDI for matrix assisted desorption ionization and TOF for time of flight. TLC analysis was carried out on silica gel (Merck 60F-254) with visualization at 254 and 366 nm. Preparative flash chromatography was carried out with Merck silica gel (Si 60, 40-63 μm). Reagents and chemicals were purchased from Sigma-Aldrich unless otherwise stated. Solvents were purchased from SDS. Dichloromethane (CH_2Cl_2 or DCM), chloroform ($CHCl_3$), methanol (MeOH) were distilled from calcium hydride, toluene was distilled from sodium.

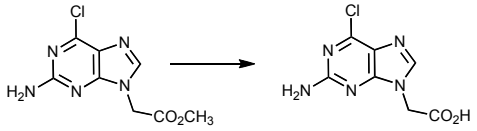
Part 1. Synthesis of DOTASQ-C_1



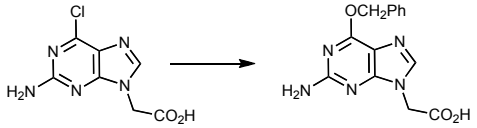
A solution of DOTAEt (506.5 mg, 0.98 mmol, 1.0 eq) in ethylene diamine (3.2 mL) is stirred at 20°C for 4 days under inert atmosphere. The mixture is then concentrated under reduced pressure and the resulting sluggish residue is taken up in CH_3CN (10 mL); the mixture is concentrated under reduced pressure and this protocol is repeated 3 times. The final compound, obtained as a white powder, is washed several times with CH_3CN (2 x 20 mL) and finally dried under reduced pressure (508.0 mg, 0.89 mmol, chemical yield = 91%). **MS** (ESI): m/z = 573.433 $[M+H]^+$; 595.422 $[M+Na]^+$ (100%).



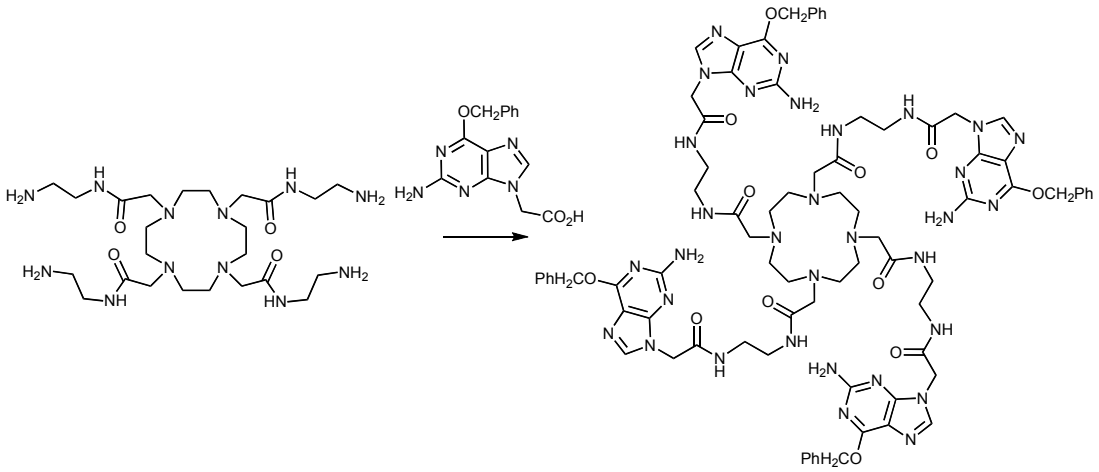
To a cold (4°C) solution of 2-amino-6-chloropurine (1.19 g, 7.02 mmol, 1.0 eq) in DMF (15 mL) is added NaH (60% dispersed, 280.6 mg, 7.02 mmol, 1.0 eq). The mixture is stirred till complete homogenization, and methyl bromoacetate (1.13 g, 7.37 mmol, 1.1 eq) is added. The yellow mixture is stirred at 20°C for 16 hours under inert atmosphere. After concentration under reduced pressure, the final compound is precipitated upon addition of water (50 mL). The solid is collected by filtration, washed successively with water (40 mL) and ethanol (10 mL) and dried under reduced pressure. The final compound is obtained as a white solid. Retreatment of mother liquors (2 days at 20°C) leads to the crystallization of the final product as colorless needles; 1.05 g of the final product are thus obtained (4.36 mmol, chemical yield = 62 %). **^{1}H NMR** (300 MHz, DMSO, 298K) δ (ppm): 8.10 (s, 1H); 7.00 (s, 2H); 5.01 (s, 2H); 3.71 (s, 3H); **^{13}C{^{1}H} NMR** (75 MHz, DMSO, 298 K) δ (ppm): 168.1 (C=O); 159.9 (C-NH_2); 154.2; 149.4 (C-Cl); 143.4 (C-H); 122.9; 52.5 (CH_3); 44.9 (CH_2); **MS** (MALDI-TOF): m/z = 241.583 $[M]^{+\cdot}$



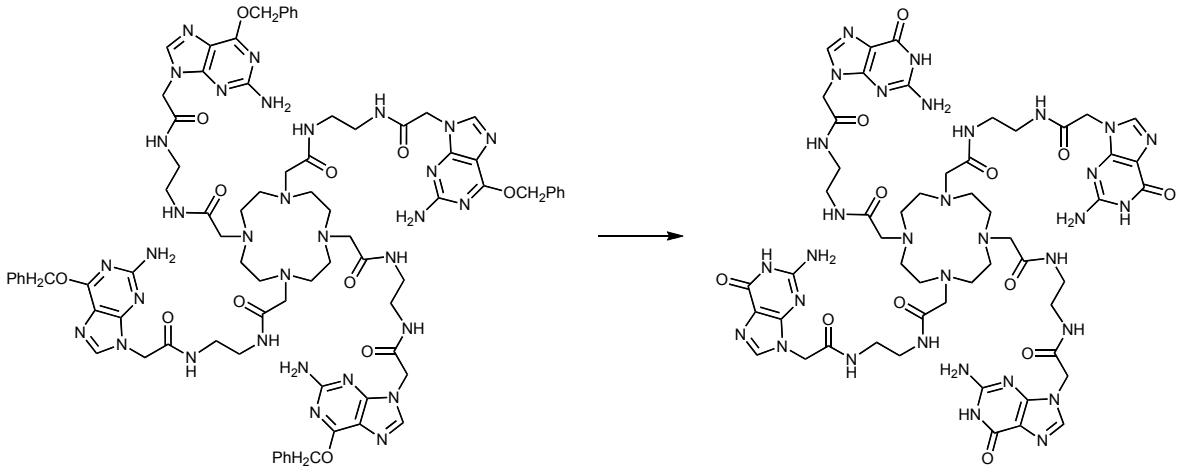
To a solution of the starting material (769.3 mg, 3.18 mmol) in dioxane (14 mL) is added a solution of LiOH (1M, 14 mL). The mixture is stirred at 20°C for 3 hours and then it is acidified (at pH = 5) by adding a solution of HCl (1M). The mixture is then concentrated under reduced pressure; the resulting residue is solved in a saturated solution of $NaHCO_3$ (10 mL), and the aqueous phase is extracted with ethyl acetate (40 mL). The resulting aqueous phase is acidified at pH = 1 by adding a solution of HCl (1M). The white precipitate formed is collected by filtration and dried under reduced pressure; the final compound is obtained as a white solid (481.4 mg, 2.12 mmol, chemical yield = 67 %). **^{1}H NMR** (300 MHz, DMSO, 298K) δ (ppm): 13.39 (s, 1H); 8.09 (s, 1H); 6.97 (s, 2H); 4.85 (s, 2H); **^{13}C{^{1}H} NMR** (75 MHz, DMSO, 298 K) δ (ppm): 169.0 (C=O); 159.9 (C-NH_2); 154.2; 143.6 (C-H); 44.1 (CH_2); **MS** (MALDI-TOF): m/z = 226.596 $[M]^{+\cdot}$ (100%).



To a solution of the starting material (514.7 mg, 2.26 mmol, 1.0 eq) in benzyl alcohol (25 mL) are added K_2CO_3 (343.8 mg, 2.49 mmol, 1.1 eq) and DABCO (50.7 mg, 0.45 mmol, 0.2 eq). The mixture is stirred overnight at 85°C. The sluggish orange residue is solved in water (30 mL) and the resulting aqueous phase is extracted with ethyl acetate (30 mL); the aqueous phase is acidified at pH = 2 by a solution of HCl (1M). The white precipitate formed is extracted by ethyl acetate (2 x 40 mL) and the resulting solution is concentrated under reduced pressure. The final product is obtained as a white solid (563.5 mg, 1.88 mmol, chemical yield = 83 %). **^{1}H NMR** (300 MHz, DMSO, 298K) δ (ppm): 7.82 (s, 1H); 7.53 - 7.35 (m, 5H); 6.49 (s, 2H); 5.50 (s, 2H); 4.79 (s, 2H); **^{13}C{^{1}H} NMR** (75 MHz, DMSO, 298 K) δ (ppm): 187.2 (C=O); 169.3 (C-OBn); 159.8 (C-NH_2); 140.2 (C-H); 142.5; 136.7; 128.4; 128.0; 126.6; 126.4; 113.2; 66.8 (O-CH_2); 62.8 (-CH_2-); **MALDI-TOF**: m/z = 299.639 $[M]^{+\cdot}$ (100%)

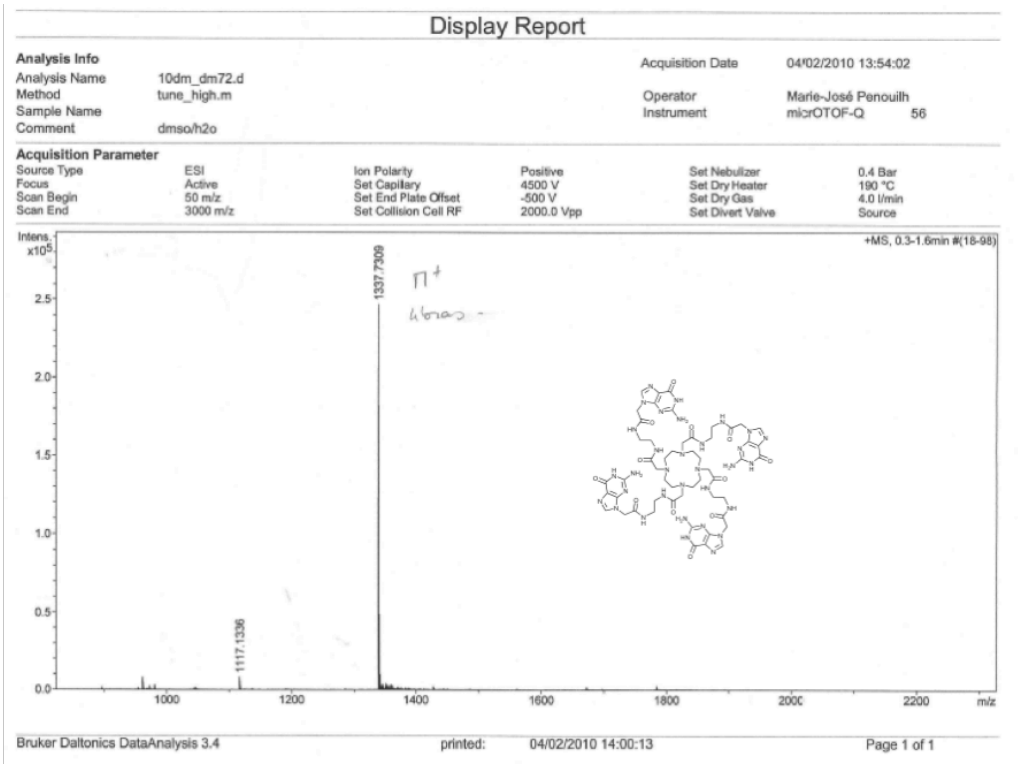


To a solution of the starting material (80.1 mg, 0.14 mmol, 1.0 eq) in DMF (11 mL) is added the protected purine (209.3 mg, 0.70 mmol, 5.0 eq). HOBt (94.6 mg, 0.70 mmol, 5.0 eq) and EDCI (134.2 mg, 0.70 mmol, 5.0 eq) are thus added and the resulting mixture is stirred at 20°C for 4 days. After concentration under reduced pressure the yellow oily residue is taken 'up in water/ethyl acetate (1/1, 20 mL). The formed white precipitate is collected by filtration, and the resulting solid is solved in methanol (10 mL). The resulting mixture is concentrated under reduced pressure and the final compound is obtained as a white solid (149.5 mg, 0.088 mmol, chemical yield = 63%). **MS** (ESI): m/z = 1719.8 $[M+Na]^+$; **MS** (MALDI-TOF): m/z = 1697.734 $[M]^{+\cdot}$ (100%)

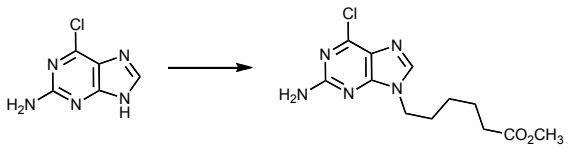


Protected **DOTASQ-*C*$_1$** (140.0 mg, 0.08 mmol) is taken up in a solution of MeOH saturated by HCl (4 mL). The mixture is stirred at 20°C for 1 hour. The final product is precipitated by addition of diethyl ether (80 mL). **DOTASQ-*C*$_1$** is obtained as a white solid (108.5 mg, 0.08

mmol, chemical yield = 99%). **MS** (ESI): m/z = 1337.6 $[M]^+$; 1359.6 $[M+Na]^+$; 1375.5 $[M+K]^+$; **MS** (MALDI-TOF): m/z = 1337.592 $[M]^{+\cdot}$ (100%).

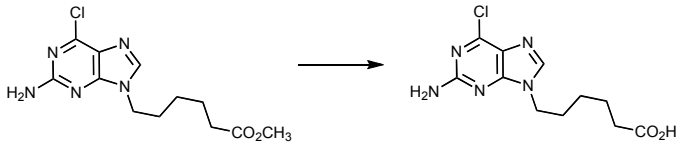


Part 2. Synthesis of DOTASQ-C_5

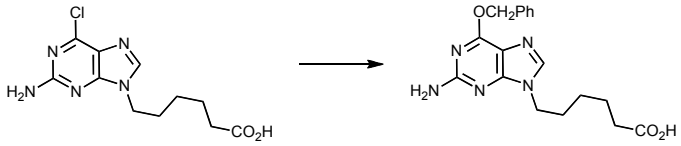


To a cold (4°C) solution of 2-amino-6-chloropurine (1.01 g, 5.95 mmol, 1.0 eq) in DMF (20 mL) is added NaH (60% dispersed, 237.9 mg, 5.95 mmol, 1.0 eq). The mixture is stirred till complete homogenization, and methyl 6-bromohexanoate (1.31 g, 6.24 mmol, 1.1 eq) is added. The yellow mixture is stirred at 20°C for 16 hours under inert atmosphere. After concentration under reduced pressure, the final compound is precipitated upon addition of water (70 mL). The solid is collected by filtration, washed successively with water (60 mL) and ethanol (10 mL) and dried under reduced pressure. The final compound is obtained as a white solid (1.42 g, 4.76 mmol, chemical yield = 80%). **^{1}H NMR** (300 MHz, DMSO, 298K) δ (ppm): 8.20 (s, 1H); 6.96 (s, 2H); 4.09 (t, 2H, ^{3}J = 7.20 Hz); 2.35 (t, 2H, ^{3}J = 7.35 Hz); 1.83

(m, 2H, ^3J = 7.40 Hz); 1.60 (m, 2H, ^3J = 7.50 Hz); 1.31 (m, 2H); **$^{13}C\{^1H\}$ NMR** (75 MHz, DMSO, 298 K) δ (ppm): 173.2 (C=O); 159.7 (C-NH_2); 154.0; 149.2 (C-Cl); 143.2 (C-H); 123.3; 51.1 (-OCH_3); 42.8 (-CH_2-); 33.0 (-CH_2-); 28.6 (-CH_2-); 25.4 (-CH_2-); 23.8 (-CH_2-); **MS** (MALDI): m/z = 297.709 $[M^{35}Cl]^{+\cdot}$ (100%); 299.710 $[M^{37}Cl]^{+\cdot}$ (38%); 319.703 $[M+Na]^+$ (30%).

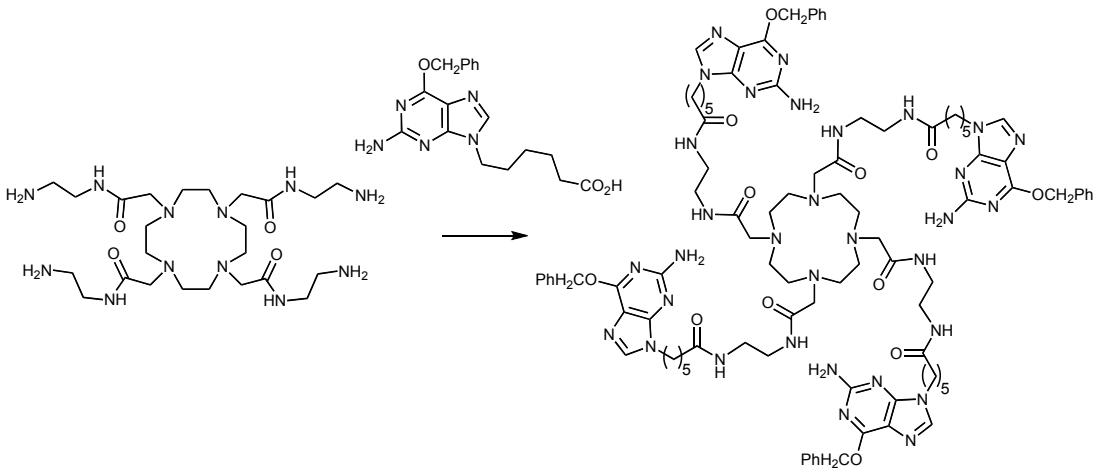


To a solution of the starting material (1.38 mg, 4.63 mmol) in dioxane (28 mL) is added a solution of LiOH (1M, 18 mL). The mixture is stirred at 20°C for 3 hours and then it is acidified (at pH = 5) by adding a solution of HCl (1M). The mixture is then concentrated under reduced pressure; the resulting residue is solved in a saturated solution of $NaHCO_3$ (20 mL), and the aqueous phase is extracted with ethyl acetate (20 mL). The resulting aqueous phase is acidified at pH = 1 by adding a solution of HCl (1M). The white precipitate formed is collected by filtration and dried under reduced pressure; the final compound is obtained as a white solid (581 mg, 2.05 mmol, chemical yield = 44 %). **^1H NMR** (300 MHz, DMSO, 298K) δ (ppm): 8.14 (s, 1H); 6.91 (s, 2H); 4.03 (t, 2H, ^3J = 7.20 Hz); 2.19 (t, 2H, ^3J = 7.20 Hz); 1.77 (m, 2H, ^3J = 7.35 Hz); 1.51 (m, 2H, ^3J = 7.50 Hz); 1.25 (m, 2H); **$^{13}C\{^1H\}$ NMR** (75 MHz, DMSO, 298 K) δ (ppm): 159.7 (C-NH_2); 154.0; 149.2 (C-Cl); 143.2 (C-H); 48.3 (-CH_2-); 33.5 (-CH_2-); 28.7 (-CH_2-); 25.5 (-CH_2-); 23.9 (-CH_2-); **MS** (MALDI-TOF): m/z = 283.706 $[M^{35}Cl]^{+\cdot}$ (100%); 285.708 $[M^{37}Cl]^{+\cdot}$ (30%); 305.710 $[M+Na]^+$ (12%).

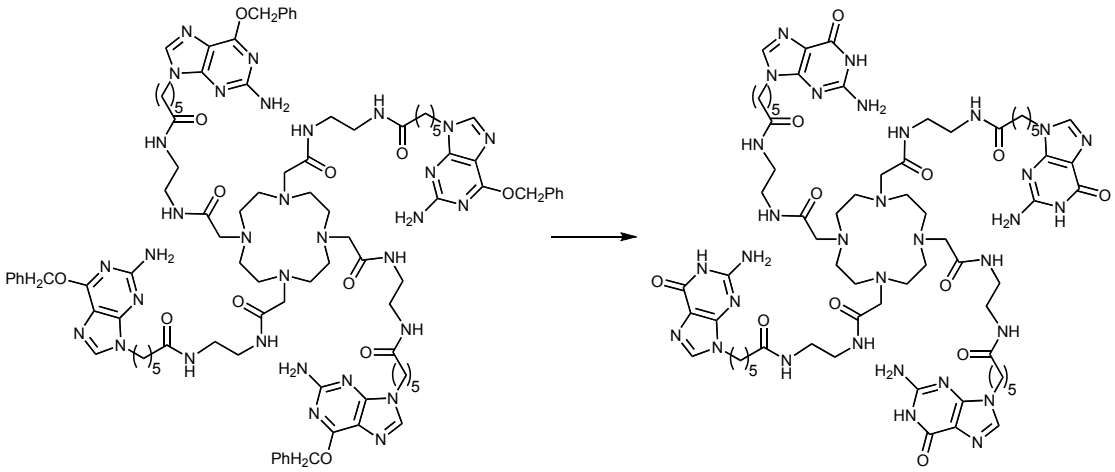


To a solution of the starting material (571.0 mg, 2.01 mmol, 1.0 eq) in benzyl alcohol (30 mL) are added K_2CO_3 (306.0 mg, 2.21 mmol, 1.1 eq) and DABCO (45 mg, 0.40 mmol, 0.2 eq). The mixture is stirred overnight at 85°C. The sluggish orange residue is solved in water (50 mL) and the resulting aqueous phase is extracted with ethyl acetate (40 mL); the aqueous phase is acidified at pH = 2 by a solution of HCl (1M). The white precipitate formed is

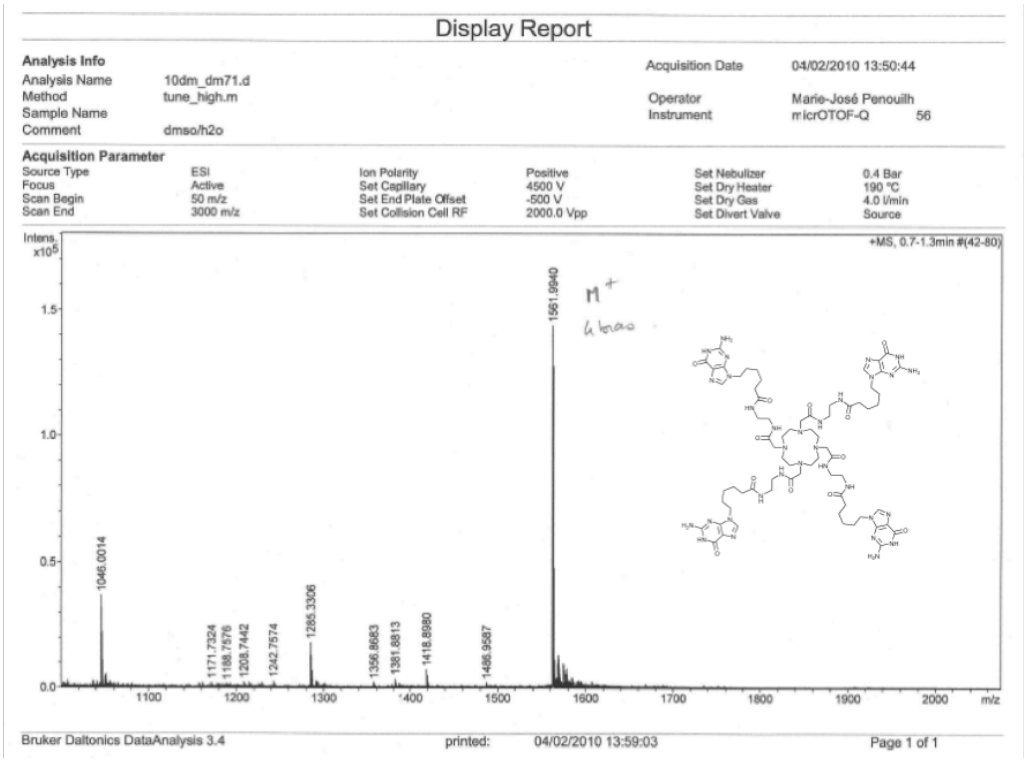
extracted by ethyl acetate (2 x 30 mL) and the resulting solution is concentrated under reduced pressure. The final product is obtained as a white solid (350 mg, 0.98 mmol, chemical yield = 49 %). **^{1}H NMR** (300 MHz, DMSO, 298K) δ (ppm): 7.93 (s, 1H); 7.55 - 7.19 (m, 5H); 6.50 (s, 2H); 5.50 (s, 2H); 4.00 (t, 2H, ^{3}J = 7.05 Hz); 2.20 (t, 2H, ^{3}J = 7.20 Hz); 1.75 (m, 2H, ^{3}J = 7.28 Hz); 1.52 (m, 2H, ^{3}J = 7.50 Hz); 1.24 (m, 2H); **^{13}C{^{1}H} NMR** (75 MHz, DMSO, 298 K) δ (ppm): 174.4 (C=O); 159.9 (C-OBn); 159.7 (C-NH_2); 154.3; 142.5 (C-H); 126.4; 126.6; 128.0; 128.4; 128.5; 136.6; 113.4; 62.9 (-CH_2-Ph); 46.5 (-CH_2-); 34.0 (-CH_2-); 29.3 (-CH_2-); 26.4 (-CH_2-); 24.4 (-CH_2-); **MS** (MALDI-TOF) : m/z = 355.799 $[M]^{+\cdot}$; 377.810 $[M+Na]^+$ (8%); 393.758 $[M+K]^+$ (16%).



To a solution of the starting material (52.5 mg, 0.09 mmol, 1.0 eq) in DMF (7 mL) is added the protected purine (185.1 mg, 0.55 mmol, 6.0 eq). HOBt (74.6 mg, 0.55 mmol, 6.0 eq) and EDCI (105.8 mg, 0.55 mmol, 6.0 eq) are thus added and the resulting mixture is stirred at 20°C for 4 days. After concentration under reduced pressure the yellow oily residue is taken up in water (20 mL) and concentrated under reduced pressure (3 times). The oily residue is solved in a saturated solution of $NaHCO_3$ (10mL) that leads to the formation of a precipitate; the liquid phase is removed and ethyl acetate is added (10 mL) thus maximizing the precipitation of the final product. The formed white precipitate is collected by filtration and dried under reduced pressure. The final compound is obtained as a pale yellow solid (166.3 mg, 0.09 mmol, chemical yield = 95%). **MS** (ESI): m/z = 981.03144 $[M+K]^{2+}$; **MS** (MALDI-TOF): m/z = 1945.175 $[M+Na]^+$ (3%); 981.074 $[M+H+K]^{2+}$ (100%).

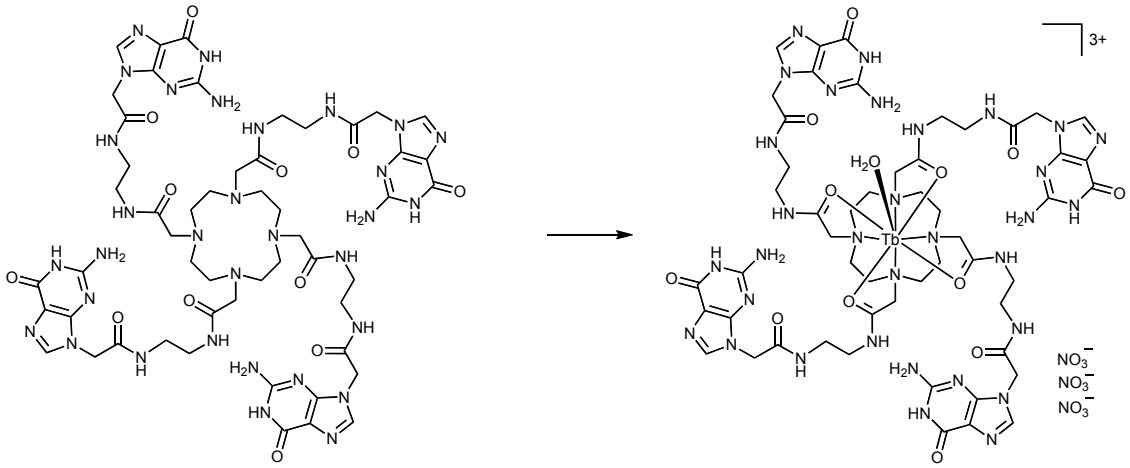


Protected **DOTASQ-C_5** (105.0 mg, 0.05 mmol) is taken up in a solution of MeOH saturated by HCl (3 mL). The mixture is stirred at 20°C for 1 hour. The final product is precipitated by addition of diethyl ether (50 mL). **DOTASQ-C_5** is obtained as a white solid (85.0 mg, 0.05 mmol, chemical yield = 100%). **MS** (ESI): m/z = 1561.29940 $[M+H]^+$ (100%).

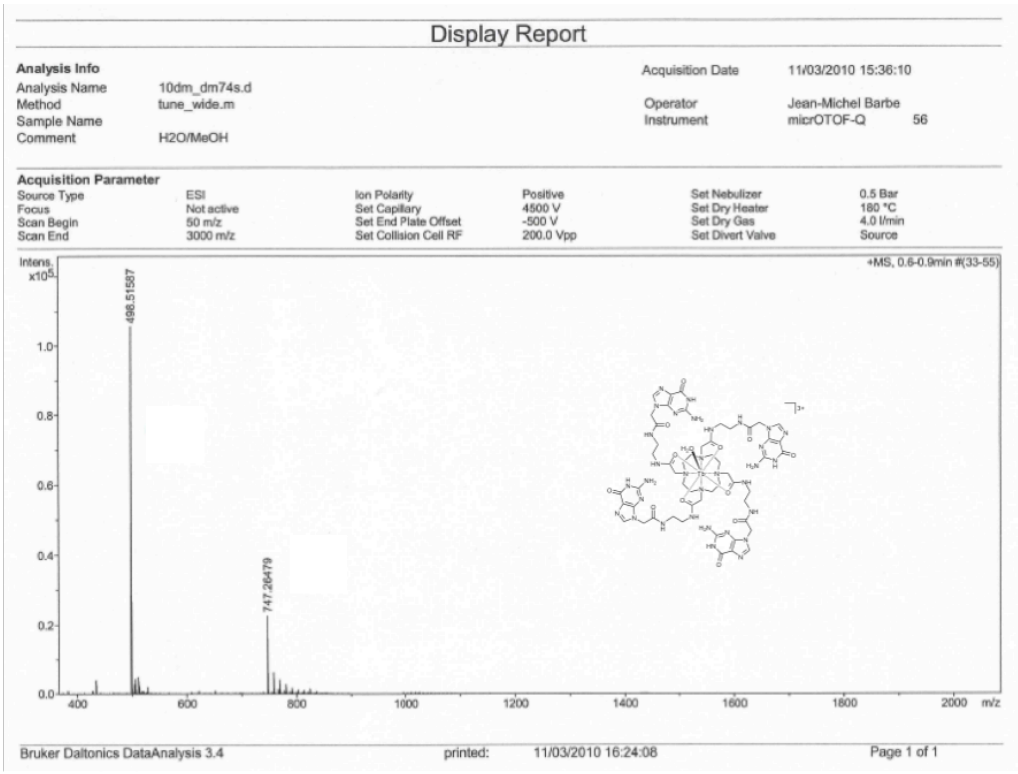


Part 3. Synthesis of Tb.DOTASQs

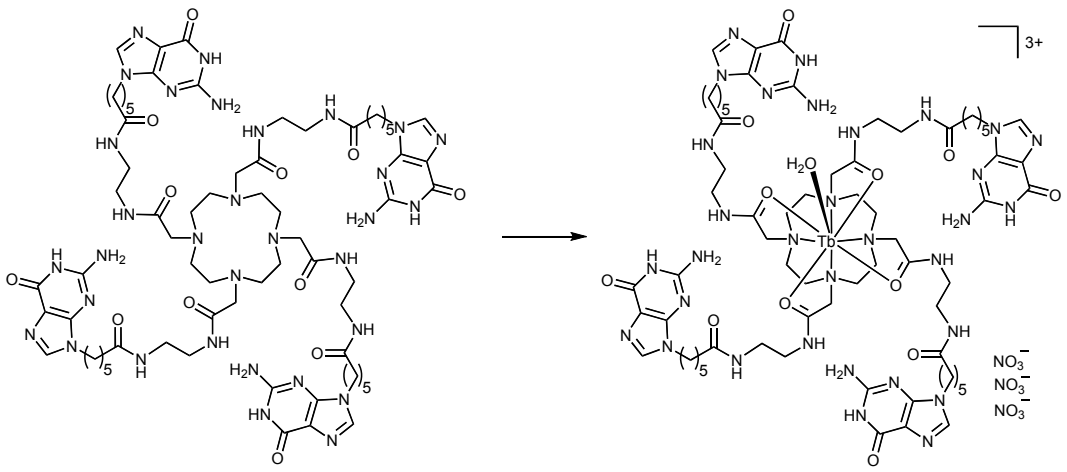
a) Tb.DOTASQ-C_1



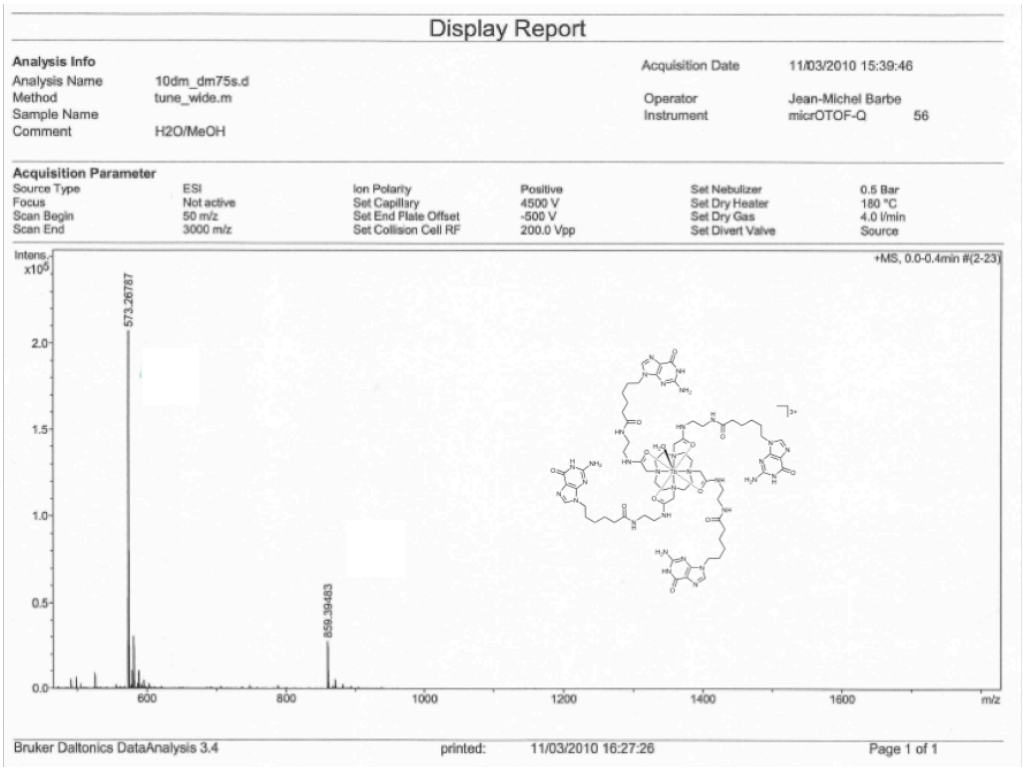
To a solution of **DOTASQ-*C*$_1$** (37.7 mg, 0.03 mmol, 1.0 eq) in a mixture of MeOH/lithium cacodylate buffer 20mM pH=7.2 (1/1, 2 mL) is added $Tb(NO_3)_3.6H_2O$ (13.4 mg, 0.03 mmol, 1.0 eq). The mixture is stirred at 55°C for 3 days. After cooling to 20°C, the final product is precipitated by addition of acetone (6 mL); the solid is collected by filtration and washed with acetone (2 x 6 mL). After being dried under reduced pressure, **Tb.DOTASQ-*C*$_1$** is obtained as a white solid (30.9 mg, 0.02 mmol, chemical yield = 72%). **MS** (ESI): m/z = 498.516 $[M]^{3+}$ (100%); 747.264 $[M]^{2+}$ (22%).



b) Tb.DOTASQ-C_5



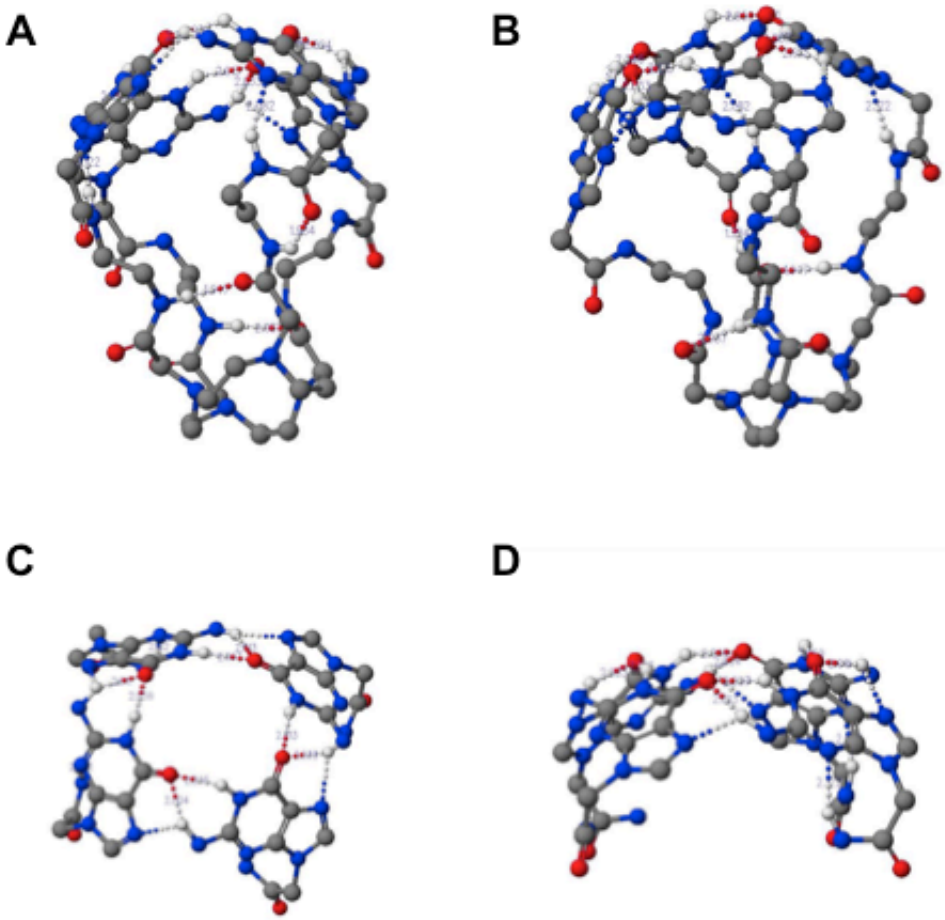
To a solution of **DOTASQ-C_5** (12.2 mg, 0.07 mmol, 1.0 eq) in a mixture of MeOH/lithium cacodylate buffer 20mM pH=7.2 (1/1, 2 mL) is added $Tb(NO_3)_3.6H_2O$ (3.9 mg, 0.08 mmol, 1.1 eq). The mixture is stirred at 55°C for 3 days. After cooling to 20°C, the final product is precipitated by addition of acetone (6 mL); the solid is collected by filtration and washed with acetone (2 x 6 mL). After being dried under reduced pressure, **Tb.DOTASQ-C_1** is obtained as a white solid (10.0 mg, 0.06 mmol, chemical yield = 75%). **MS** (ESI): m/z = 573.267 $[M]^{3+}$ (100%); 859.394 $[M]^{2+}$ (13%).



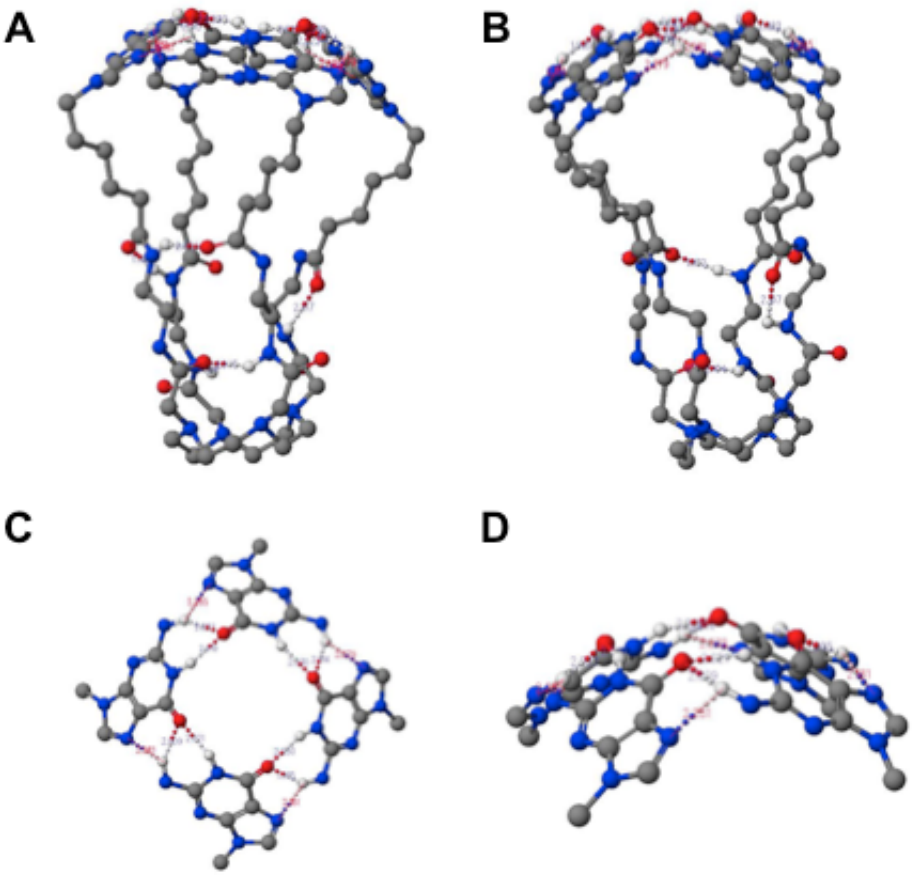
Part 4. Molecular Modeling

Initial estimates for the geometries were obtained by the 'optimization geometry' program of CAChe (CAChe Worksystem Pro Version 7.5.0.85, © 2000-2006 Fujitsu Ltd, © 1989-2000 Oxford Molecular Ltd), followed by full optimization of all geometrical variables (bond lengths, bond angles and dihedral angles), without any symmetry constraint, using the molecular mechanics MM3 force field or the semi-empirical AM1 and PM5 methods of CAChe (CAChe Worksystem Pro Version 7.5.0.85, © 2000-2006 Fujitsu Ltd, © 1989-2000 Oxford Molecular Ltd).

As depicted on both figures below, the tetrad of DOTASQs is possibly bent, because, unlike what is observed within a quadruplex structure, there are no additional tetrads on the top that make it flat; additionally, DOTASQs adopt an oblong 3D structures thanks to the formation of H-bonds between the DOTA cycle and the amide group of the arms.



Molecular modeling of DOTASQ-C1: two side-views (**A**,**B**) of the whole molecule and detailed representation of the G-tetrad (top-view (**C**) and side-view (**D**)). Calculated H-bonds appear as dotted lines.



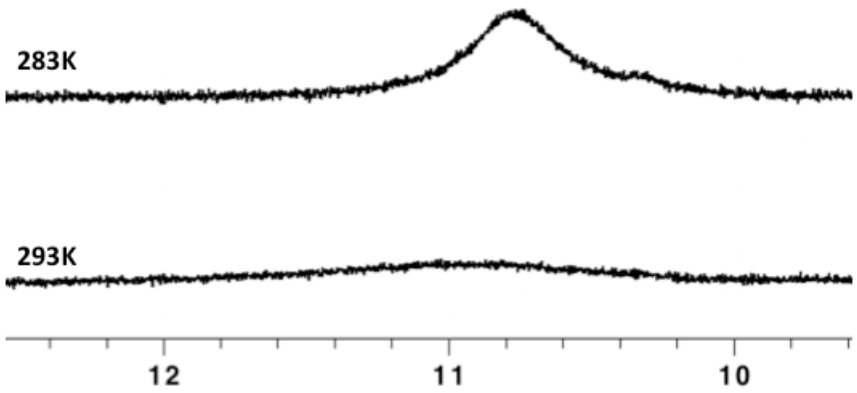
Molecular modeling of DOTASQ-C5: two side-views (**A**,**B**) of the whole molecule and detailed representation of the G-tetrad (top-view (**C**) and side-view (**D**)). Calculated H-bonds appear as dotted lines.

As depicted on both figures above, the tetrad of DOTASQ-C5 is still bent but slightly less than that of DOTASQ-C1: this can be explained by the fact that C5 arms are longer than C1 and consequently offer more sterical freedom to the G-quartet. Additionally, an additional H-bond can be seen between the DOTA cycle and the amide group of the arms (3 vs 4 for DOTASQ-C1 and DOTASQ-C5 respectively).

Part 5. Conformational studies of DOTASQ by NMR

a) NMR study of DOTASQ-C5 alone:

3.2mM of DOTASQ-C5 was dissolved in 20mM Kpi buffer plus 70mM KCl, pH 7.0. Two spectra were recorded, at 20°C (lower panel) and at 10°C (upper panel); as depicted in the figure below, DOTASQ-C5 alone does not massively adopt the closed conformation at room temperature (293K), as judged by the absence of the imino proton in the ~11ppm region (no imino signals means that the exchange of this proton is fast, thus implying that it is not involved in a H-bond network); however, when performed at lower temperature (283K), this signal appears thus demonstrating that DOTASQ-C5 is able to adopt the desired closed conformation.



Parts of NMR spectra of a DOTASQ solution (3.2mM in 20mM Kpi buffer plus 70mM KCl, pH 7.0) at 293 and 283K.

b) NMR study of DOTASQ-C5 in presence of a quadruplex-DNA:

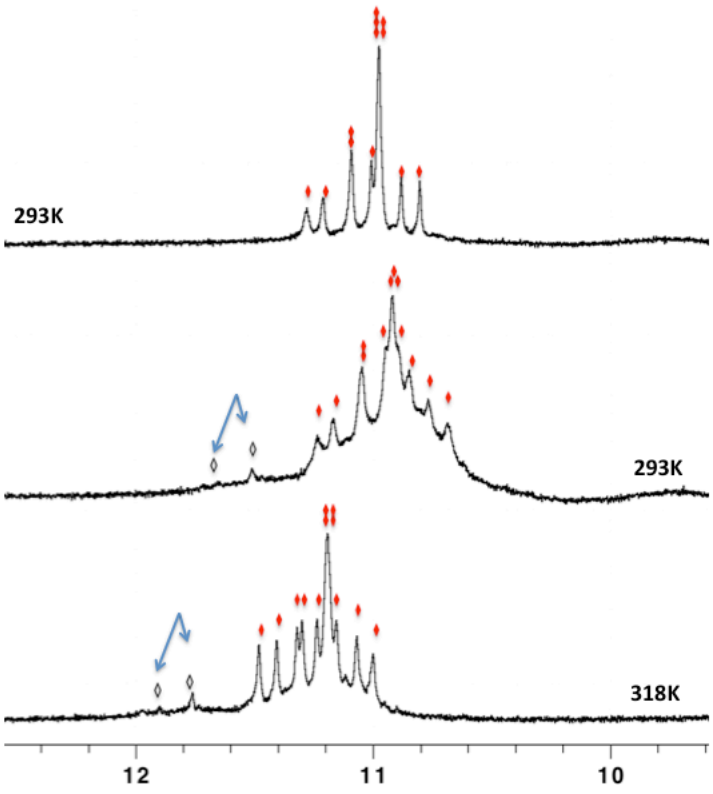
We performed NMR experiments in presence of a quadruplex-DNA of sequence GGGTGGGTTGGGTGGG, since this sequence leads to a relatively 'simple' NMR spectrum:

i- we firstly recorded the NMR spectrum of the quadruplex-DNA alone: to this end, 0.2mM of the $G_3TG_3T_2G_3TG_3$ oligonucleotide (in 4mM Kpi buffer plus 14mM KCl, pH 7.0) was annealed by heating the corresponding solution at 93°C for 5min followed by a slow cooling to 20°C. As depicted in the figure below (upper panel), the obtained NMR spectrum is of good quality, and the 12 imino peaks that correspond to the imino groups of the 12 constitutive guanines can easily be seen (red diamonds).

ii- we thus performed the NMR spectrum of the quadruplex-DNA (0.2mM) in presence of 1 equiv. of DOTASQ-C5 (0.2mM) in 4mM Kpi buffer plus 14mM KCl, pH 7.0:

to this end, the solution is prepared and annealed by heating at 93°C for 5min followed by a rapid cooling to 4°C. As depicted in the figure below (middle panel), the obtained NMR spectrum indicates that an interaction takes place between the ligand and the quadruplex-DNA (testified by the broadening/shifting of the imino peaks). Interestingly, two new weak peaks appear in the ~11.9ppm region (blue arrows), which belong to the DOTASQ spectrum, thus indicating that DOTASQ interacts with its DNA target under its folded form.

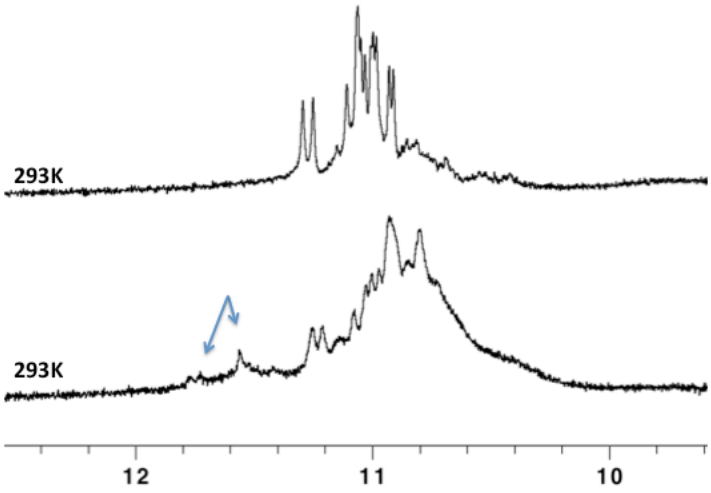
iii- finally, a spectrum of this solution was recorded at 45°C (318K): as depicted below (lower panel), the imino peaks corresponding to the quadruplex structure shift and sharpen (reminiscently of what is usually observed for quadruplex NMR) and very interestingly the two DOTASQ's peaks are stil visible. However, this experiment tends to demonstrate that DOTASQ is folded at ~20%.



Parts of NMR spectra of the $G_3TG_3T_2G_3TG_3$ oligonucleotide alone (upper panel), of the 1:1 DOTASQ/quadruplex complex at 20°C (middle panel) and at 45°C (lower panel) in 4mM Kpi buffer plus 14mM KCl, pH 7.0.

Finally, to confirm these results, we performed similar experiments with another quadruplex-forming oligonucleotide, $G_3TG_3T_3G_3TG_3$, which differs from the previous one by the nature of the central loop (TTT *vs* TT). To this end, 0.2mM of the $G_3TG_3T_3G_3TG_3$ oligonucleotide (in 20mM Kpi buffer plus 70mM KCl, pH 7.0) was annealed by heating the corresponding

solution at 93°C for 5min followed by a slow cooling to 20°C. The same protocol was used to prepare the 1:1 DOTASQ/quadruplex complex. As depicted in the figure below, DOTASQ interacts efficiently with this quadruplex-DNA since the spectrum obtained for the 1:1 DOTASQ/quadruplex complex (lower panel) shows broadened and shifted peaks as compared to the spectrum obtained for the quadruplex-DNA alone (upper panel). Once more, the presence of two new peaks that appear in the ~11.8ppm region, which belong to the DOTASQ spectrum, indicates that DOTASQ interacts with its DNA target under its folded form. In the present case, DOTASQ appears to be folded at ~50%



Parts of NMR spectra of the $G_3TG_3T_3G_3TG_3$ oligonucleotide alone (upper panel), of the 1:1 DOTASQ/quadruplex complex (lower panel) at 20°C in 20mM Kpi buffer plus 70mM KCl, pH 7.0.

Part 6. FRET-melting results of DOTASQs and Tb.DOTASQs

a) FRET-melting protocol:

Labelled oligonucleotides are purchased from Eurogentec (Belgium); after an initial dilution at 100μM concentration in purified water, further dilutions are carried out in the relevant buffer. FRET assay is performed as a high-throughput screen in a 96-well format, with FxxxT (FAM-xxx-Tamra (see Table below), with FAM: 6-carboxyfluorescein and Tamra: 6-carboxy-tetramehtylrhodamine). Fluorescence melting curves were determined with a Stratagene Mx3005P real-time PCR machine, using a total reaction volume of 25μL, with 0.2μM of tagged oligonucleotide in a buffer containing 10mM lithium cacodylate pH 7.2 plus 100mM NaCl (for the "Na^+-conditions"), 10mM KCl/90mM LiCl (for the "K^+-conditions" for F21T) or 1mM KCl/99mM LiCl (for the "K^+-conditions" for F21T/FmycT/Fkit2T/Fkras35B1T studies). After a first equilibration step at 25°C during 5 minutes, a stepwise increase of 1°C every minute for 71 cycles to reach 95°C was performed and measurements were made after each "cycle" with excitation at 492nm and detection at 516nm. The melting of the G-quadruplex was monitored alone or in the presence of various concentrations of compounds and/or of double-stranded competitor ds26 (5'-$CA_2TCG_2ATCGA_2T_2CGATCCGAT_2G$-3'). Final analysis of the data was carried out using Excel and Kaleida graph software. Emission of FAM was normalized between 0 and 1, and $T_{1/2}$ was defined as the temperature for which the normalized emission is 0.5. $\Delta T_{1/2}$ values are mean of 2 to 4 experiments ± standard deviation.

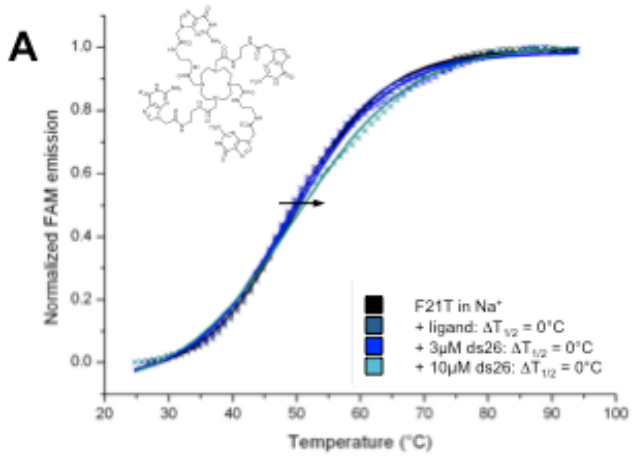
Name	Bases	5'	Sequence	3'
F21T	21	Fam	**GGG** TTA **GGG** TTA **GGG** TTA **GGG**	Tamra
FmycT	22	Fam	TTGA **GGG** T **GGG** TA **GGG** T **GGG** TAA	Tamra
FKit2T	20	Fam	**GGG** C **GGG** CGCGA **GGG** AG **GGG**	Tamra
FKras35B1T	35	Fam	A **GGG** CGGTGT **GGG** AAGA **GGG** AAGA **GGGGG** AGGCAG	Tamra

NB: guanines involved in G-quartet are highlighted in bold

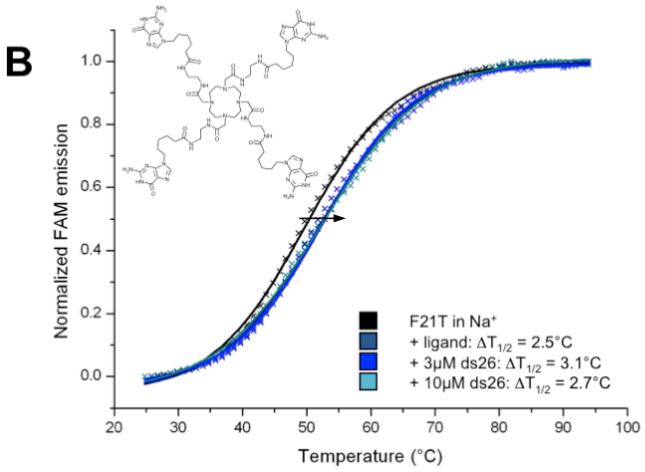
b) FRET-melting experiments with F21T and ds26 as competitor

b1 – in Na^+-rich conditions

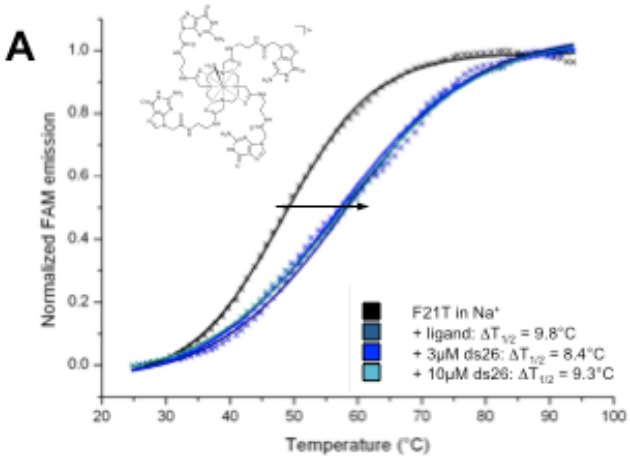
- DOTASQ-C1



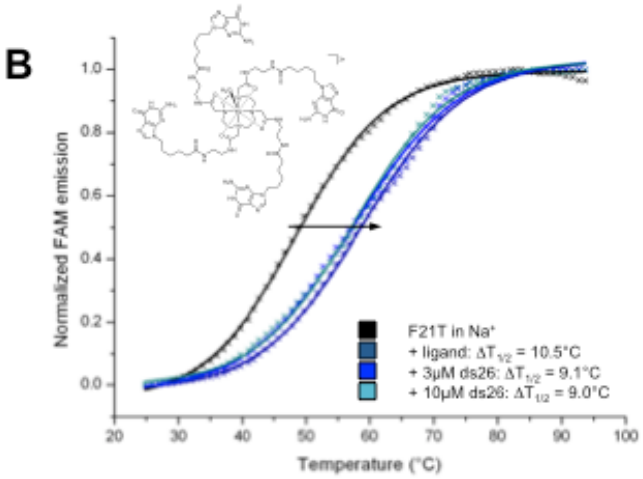
- DOTASQ-C5



- Tb.DOTASQ-C1

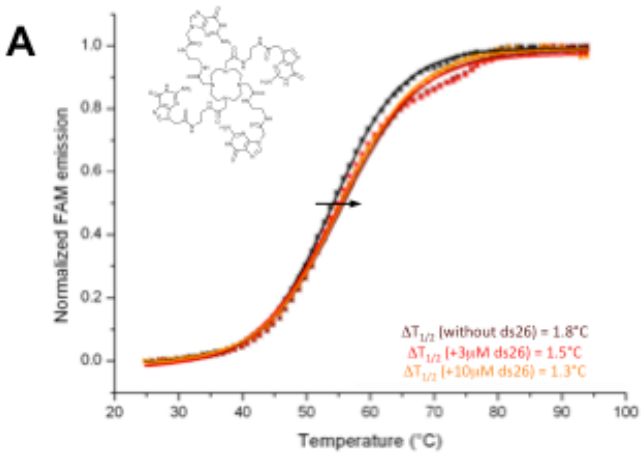


- Tb.DOTASQ-C5

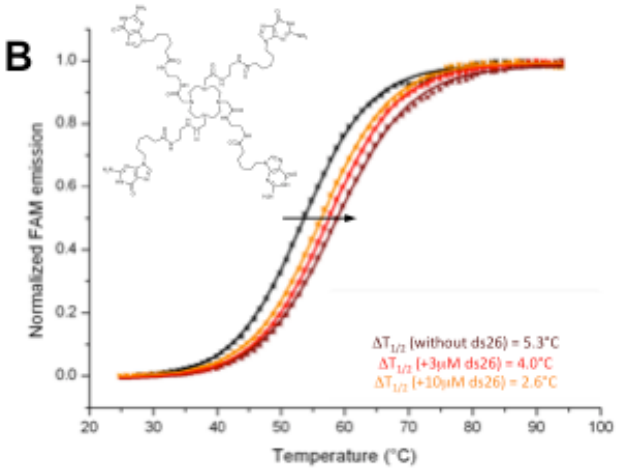


b2 – in K^+-rich conditions

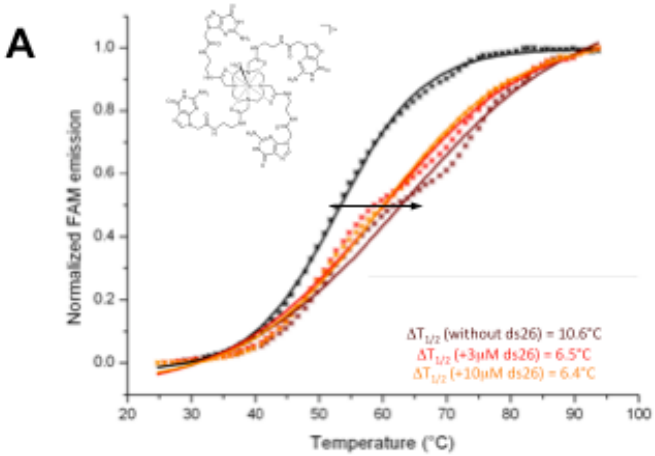
- DOTASQ-C1



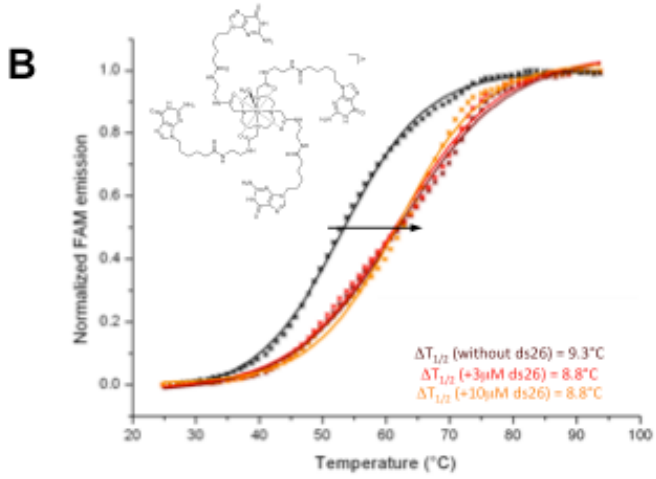
- DOTASQ-C5



- Tb.DOTASQ-C1



- Tb.DOTASQ-C5



b3 – Summary

G-quadruplex stabilization (quantified by $\Delta T_{1/2}$ values) and selectivity (quantified by S values)[a] evaluated by FRET-melting assay

Quadruplex stabilization ($\Delta T_{1/2}$ values, °C)					
DOTASQ-		*-C1*	*-C5*	Tb.-*C1*	Tb.-*C5*
Na^+ [b]	no ds26	0	2.5	9.8	10.5
	3μM	0	3.1	8.4	9.1
	10μM	0	2.7	9.3	9.0
K^+ [b]	no ds26	1.8	5.3	10.6	9.3
	3μM	1.5	4.0	6.5	8.8
	10μM	1.3	2.6	6.4	8.8

Quadruplex selectivity (*S* values) [a]				
	Na^+ [b]		K^+ [b]	
[ds26]:	3μM	10μM	3μM	10μM
Tb.DOTASQ-*C1*	0.86	0.94	0.61	0.60
Tb.DOTASQ-*C5*	0.87	0.86	0.95	0.95

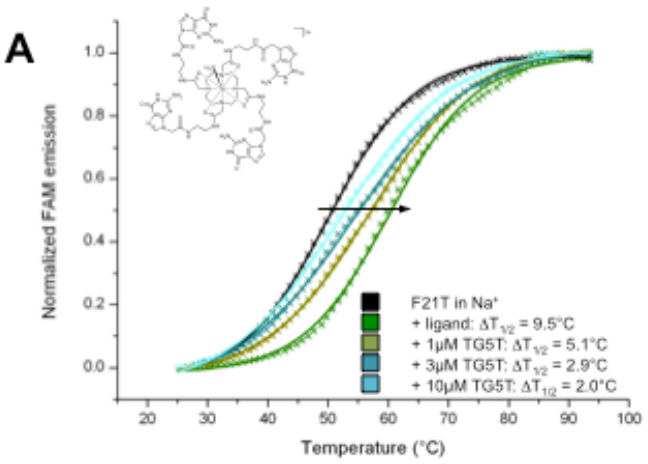
[a] The quadruplex selectivity of ligands is quantified by S values, defined as S = [$\Delta T_{1/2}$ (+3 or 10μM ds26)] / [$\Delta T_{1/2}$ (without ds26)], so, with S→1 for selective ligands;

[b] "Na^+" and "K^+" stand for Na^+-rich and K^+-rich conditions respectively, *i.e.* lithium cacodylate (pH 7.2) 10mM plus 100mM NaCl (for Na^+) or 10mM KCl/90mM LiCl (for K^+).

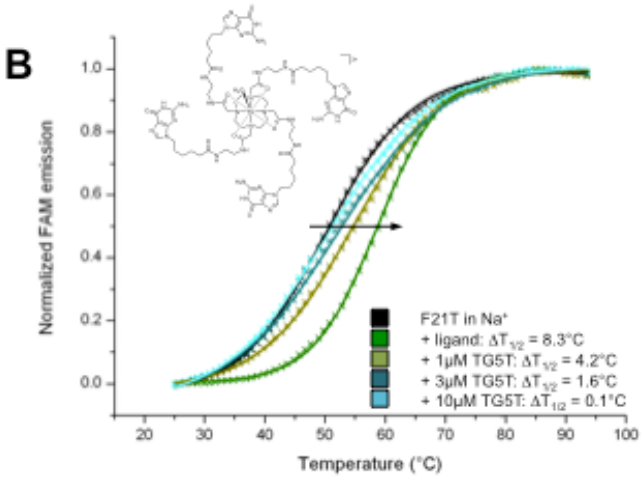
c) FRET-melting experiments with F21T and $[TG5T]_4$ as competitor

c1 – in Na^+-rich conditions

- Tb.DOTASQ-C1

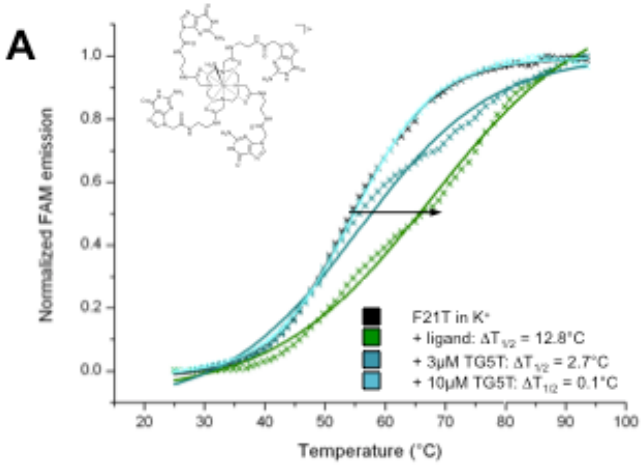


- Tb.DOTASQ-C5

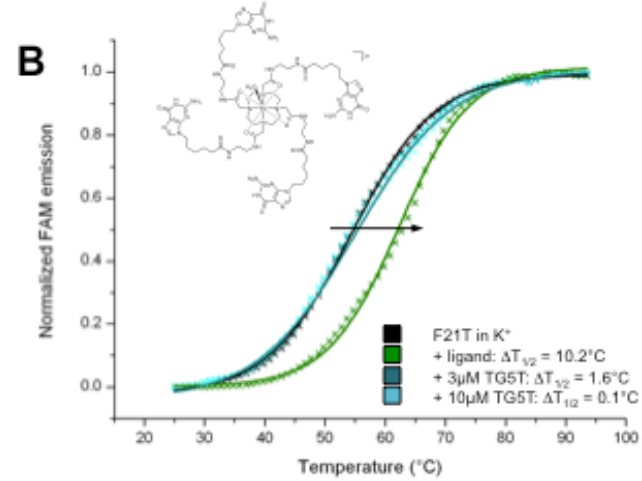


c2 – in K^+-rich conditions

- Tb.DOTASQ-C1



- Tb.DOTASQ-C5



c3 – Summary

G-quadruplex stabilization (quantified by $\Delta T_{1/2}$ values) and selectivity (quantified by S values)[a] evaluated by FRET-melting assay

Quadruplex stabilization ($\Delta T_{1/2}$ values, °C)			
		Tb.DOTASQ-*C1*	Tb.DOTASQ-*C5*
Na^+ [b]	no TG5T	9.5	8.3
	3μM	2.9	1.6
	10μM	2.0	0.1
K^+ [b]	no TG5T	12.8	10.2
	3μM	2.7	1.6
	10μM	0.1	0.1

Quadruplex selectivity (*S* values)				
	Na^+		K^+	
[TG5T]:	3μM	10μM	3μM	10μM
Tb.DOTASQ-*C1*	0.30	0.21	0.21	0.01
Tb.DOTASQ-*C5*	0.19	0.01	0.15	0.01

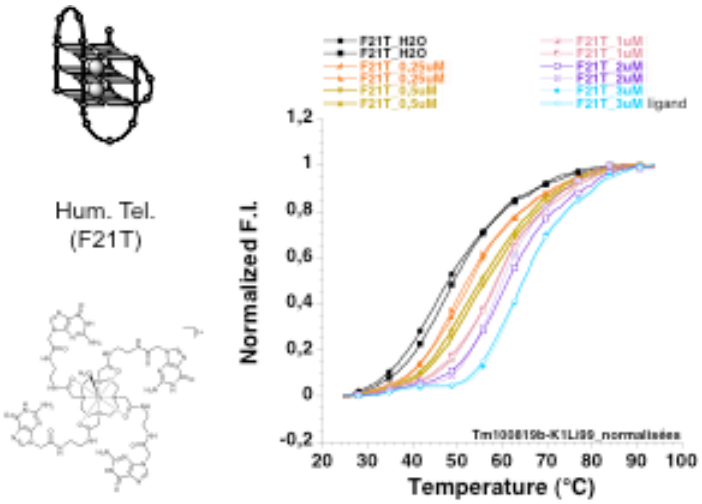
[a] The quadruplex selectivity of ligands is quantified by S values, defined as S = [$\Delta T_{1/2}$ (+3 or 10μM TG5T)] / [$\Delta T_{1/2}$ (without TG5T)], so, with S→1 for selective ligands;

[b] "Na^+" and "K^+" stand for Na^+-rich and K^+-rich conditions respectively, *i.e.* lithium cacodylate (pH 7.2) 10mM plus 100mM NaCl (for Na^+) or 10mM KCl/90mM LiCl (for K^+).

d) FRET-melting experiments with other QFOs

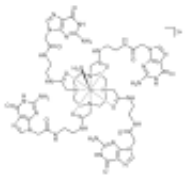
d1 - Tb.DOTASQ-*C1*, in K^+-rich conditions

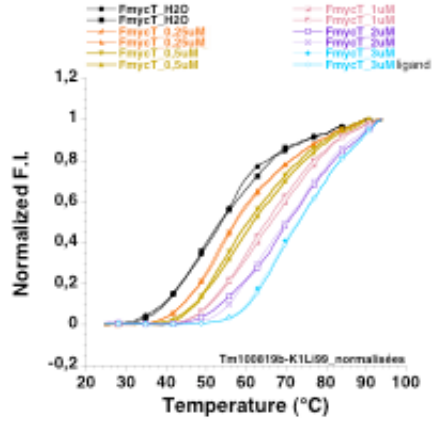
- F21T



- FmycT

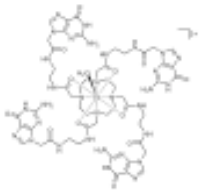
c-myc
(FmycT)

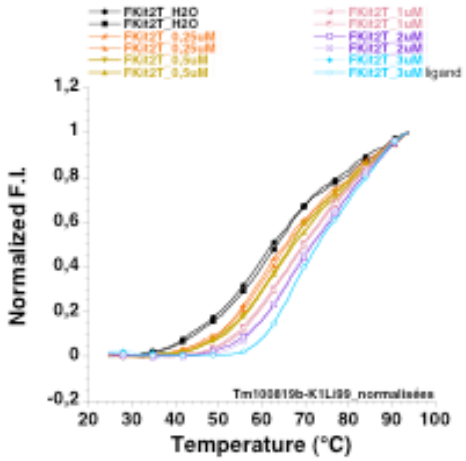




- Fkit2T

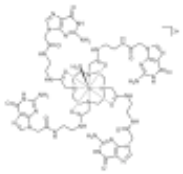
c-kit
(Fkit2T)

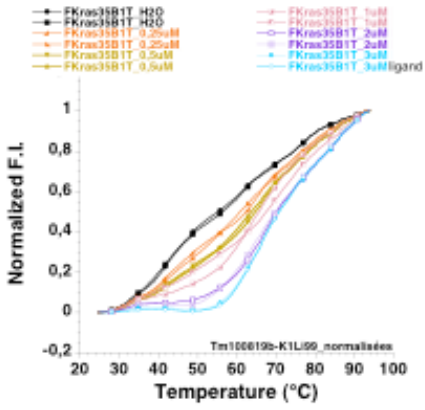




- FKras35B1T

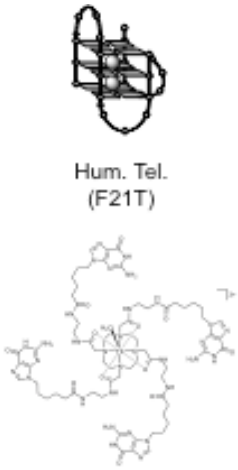
K-ras
(FKras35B1T)

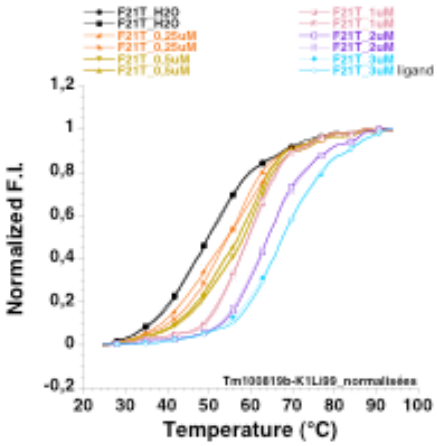




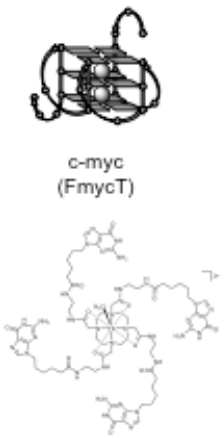
d1 - Tb.DOTASQ-*C5*, in K^+-rich conditions

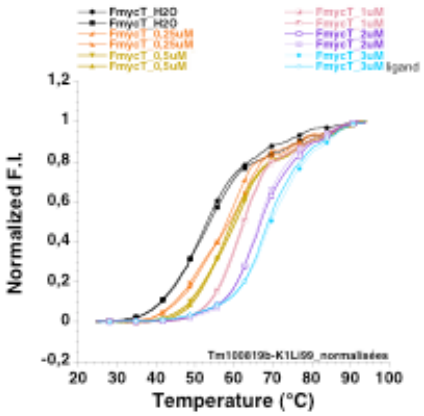
- F21T



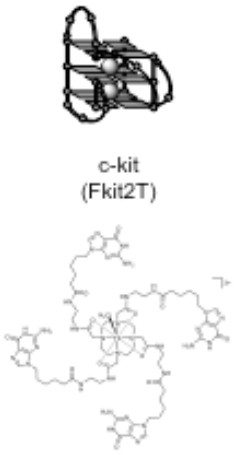


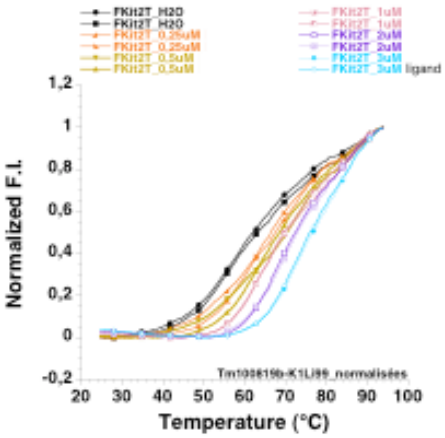
- FmycT





- Fkit2T





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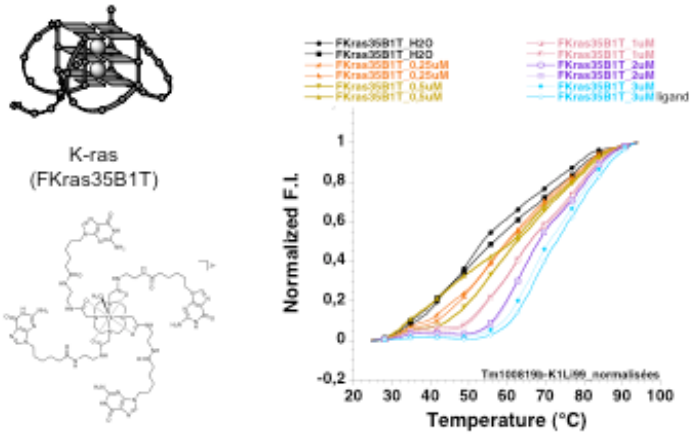
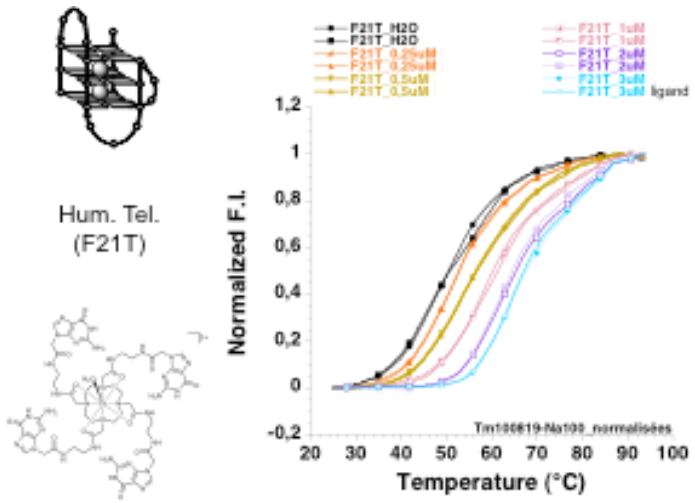
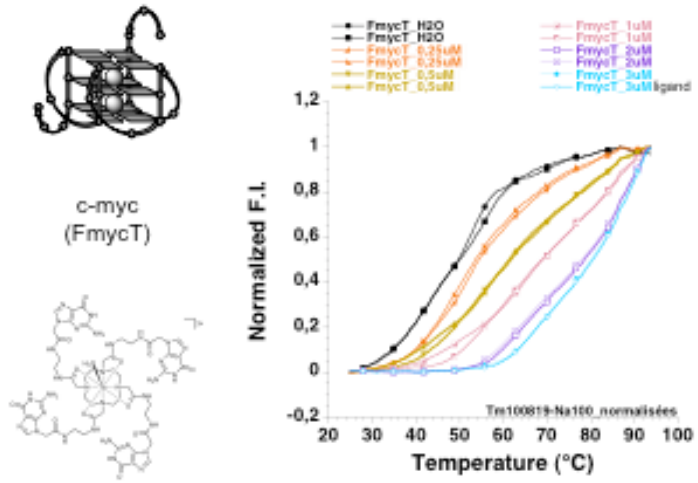


d1 - Tb.DOTASQ-*C1*, in Na^+-rich conditions

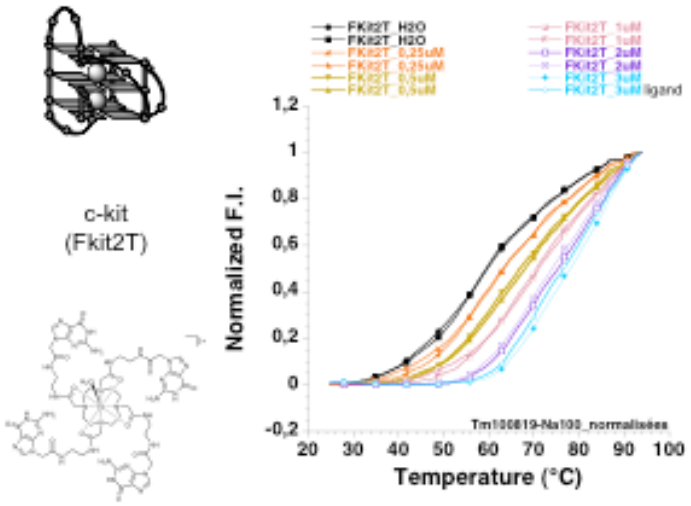
- F21T



- FmycT



- Fkit2T



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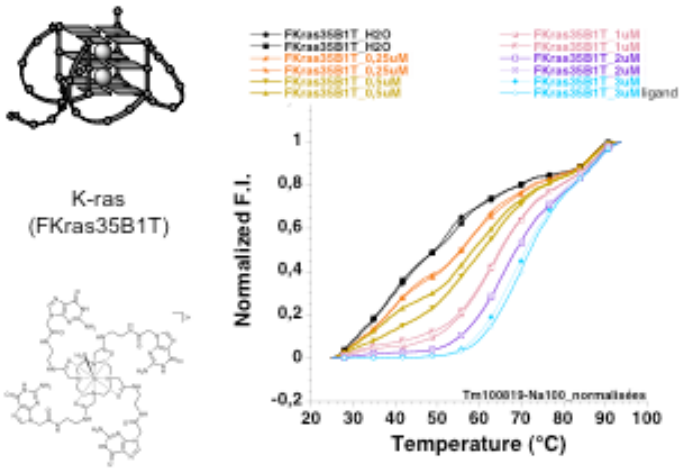
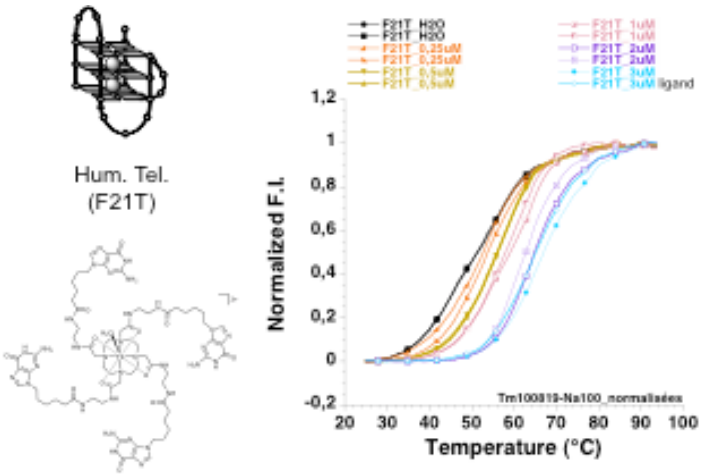
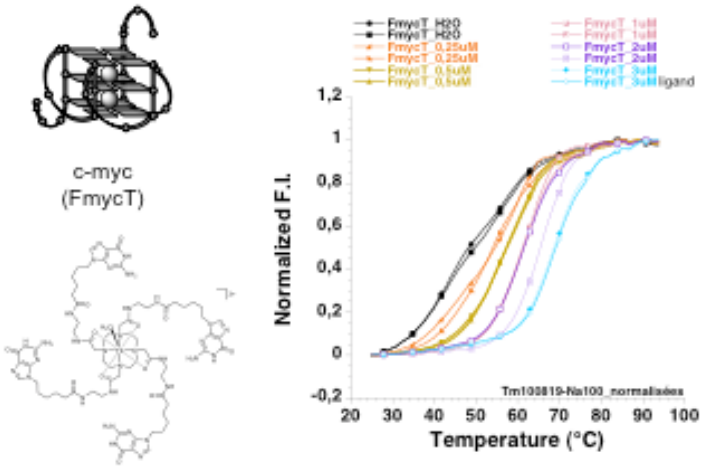


d1 - Tb.DOTASQ-*C5*, in Na^+-rich conditions

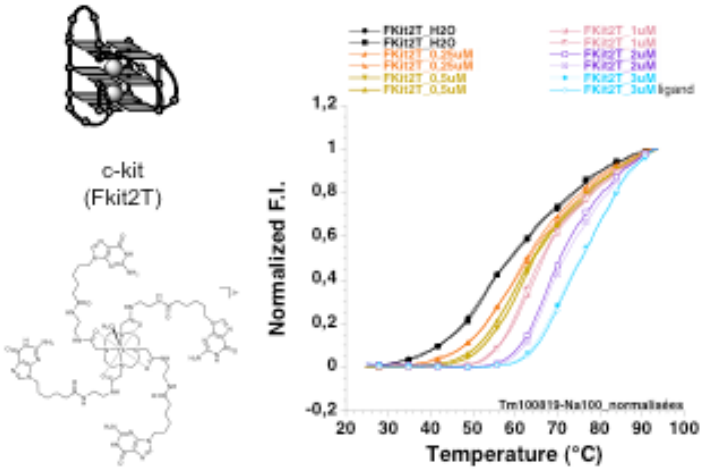
- F21T



- FmycT



- Fkit2T



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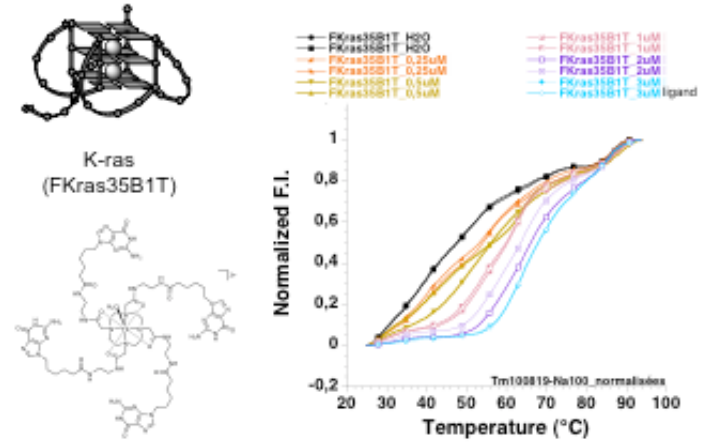
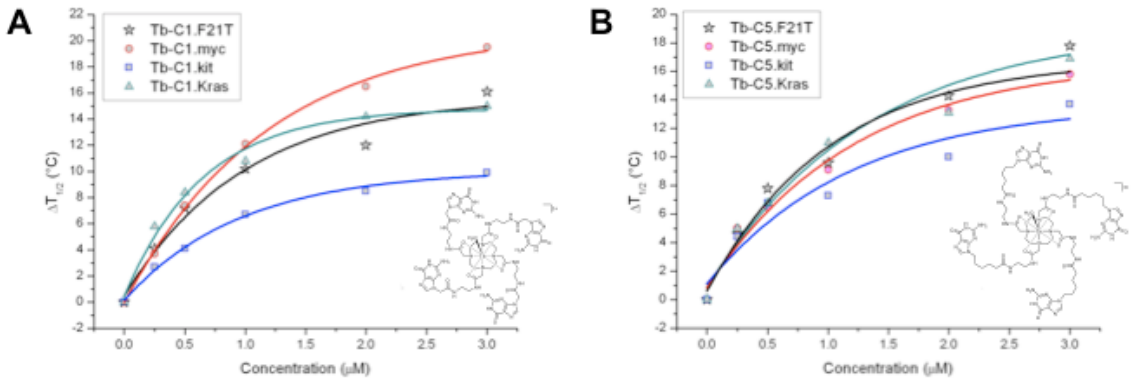
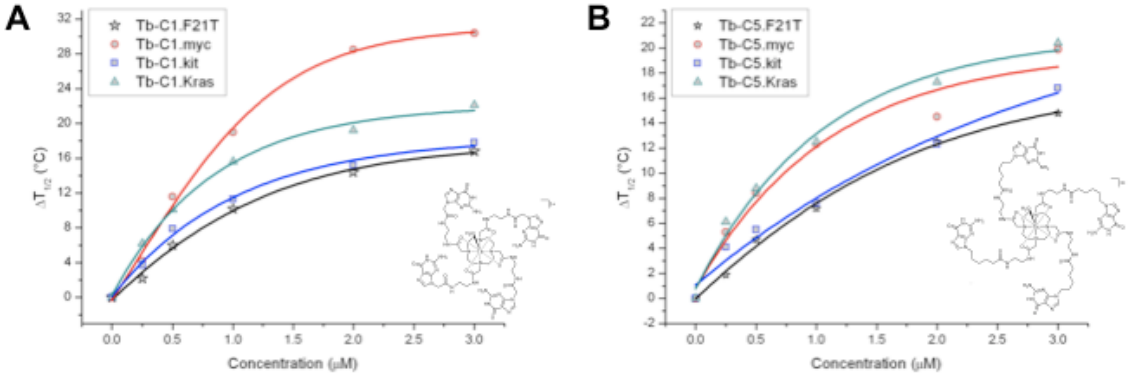


d5 - Summary, in K^+-rich conditions



d6 - Summary, in Na^+-rich conditions



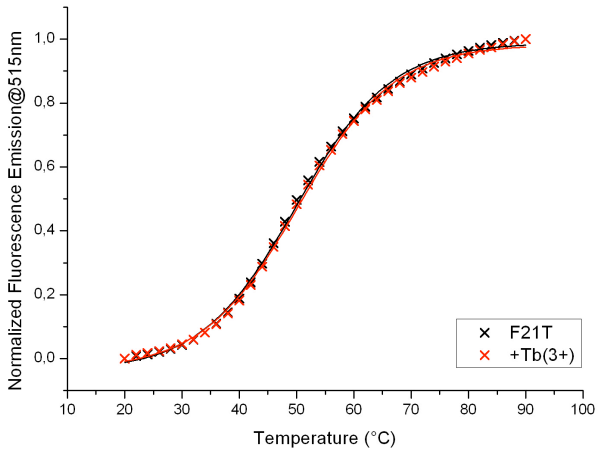
d7 - Summary, in both conditions

		K^+ conditions				Na^+ conditions			
	Conc.	Hum. Tel.	c-myc	c-kit	K-ras	Hum. Tel.	c-myc	c-kit	K-ras
Tb. DOTASQ -C1	0	0	0	0	0	0	0	0	0
	0.25	4.1	3.7	2.7	5.8	2.2	4.2	3.7	6.2
	0.5	7.2	7.4	4.1	8.4	6.0	11.6	7.9	10.1
	1	10.2	12.1	6.7	10.8	10.2	19.0	11.3	15.6
	2	12.0	16.5	8.5	14.2	14.4	28.5	15.2	19.2
	3	16.1	19.5	9.9	15.0	16.8	30.4	17.8	22.1
Tb. DOTASQ -C5	0	0	0	0	0	0	0	0	0
	0.25	4.6	5.0	4.4	5.0	1.9	5.3	4.1	6.1
	0.5	7.8	6.5	6.8	6.8	4.7	8.4	5.5	8.8
	1	9.6	9.1	7.3	11.0	7.2	12.3	7.6	12.5
	2	14.3	13.3	10.0	13.1	12.5	14.5	12.3	17.3
	3	17.8	15.8	13.7	16.9	14.8	19.9	16.8	20.4

Concentrations are expressed in μM, stabilizations ($\Delta T_{1/2}$) in °C.

e) Control experiment: FRET-melting experiments with F21T and Tb^{3+}

FRET assay is performed as a 1mL quartz cuvette, with F21T; fluorescence melting curves were determined with a JASCO FP-6500 Spectrofluorimeter, using a total reaction volume with 0.2μM of tagged oligonucleotide in a buffer containing 10mM lithium cacodylate pH 7.2 plus 100mM NaCl (for the "Na^+-conditions") in absence or presence of 1μM (5 equiv.) of $Tb(NO_3)_3.6H_2O$. Final analysis of the data was carried out using OriginPro8 graph software. Emission of FAM was normalized between 0 and 1, and $T_{1/2}$ was defined as the temperature for which the normalized emission is 0.5. $\Delta T_{1/2}$ values are mean of 3 experiments.

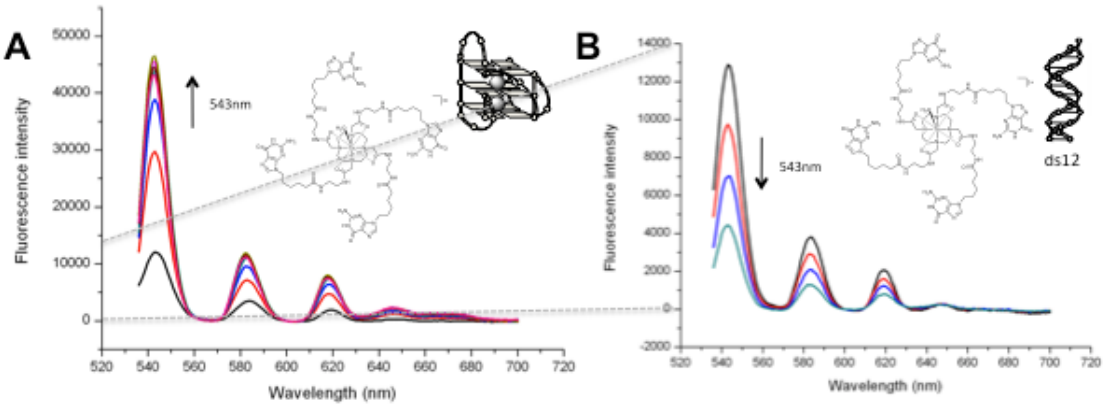


As seen above, there are no differences between experiments carried out in absence (black lines) or presence (red line) of an excess of Tb^{3+} ions.

Part 7. Preliminary luminescent titrations

Preliminary luminescence titrations have been performed; these titrations are done in non-optimal conditions since Tb.DOTASQ complexes are devoid of 'sensitizing antenna'; consequently, we have to irradiate the guanine bases of the DOTASQ (λ_{ex} = 245nm) that therefore transfer their energy to the terbium ion, implying that DNA will also absorb part of incoming energy (known as "attenuation effect" or "screening effect").

The presence of the terbium makes the adoption of the 'closed' conformation easier; thus conformation may sterically lock the complex (formation of the G4 plane) in such a way that the movements of the water molecule apically bound to the terbium are restricted. This particular relaxivity is responsible (among other) for the luminescent response (less movement gives higher luminescence). Titrations have thus been performed with Tb.DOTASQ-C5 and both a quadruplex- and a duplex-DNA: c-kit and ds12 have been selected since they are comprised of a comparable nucleobases (21 *vs* 24 bases for c-kit and ds12 respectively), implying a comparable "screening effect" from the DNA. The addition of increasing amounts of DNA (up to 2 equiv.) results in an enhanced luminescence response with c-kit while ds12 leads to a decrease of the response; the interpretation is 2-fold: the closed conformation is strongly reinforced in the presence of the quadruplex, and the Tb.DOTASQ is in a close vicinity of the DNA that itself plays the role of a second 'sensitizer': both facts result in a higher luminescence. In the case of ds12, the closed conformation makes the Tb.DOTASQ complex unable to interact with duplex-DNA (high selectivity determined by FRET-melting, *vide supra*), the complex is consequently free in solution (the duplex DNA does not act as a second 'sensitizer') and the observed decreased luminescent originates in the screening effect. These experiments, even if very preliminary, demonstrates univocally that Tb.DOTASQ-C5 interacts closely only with quadruplex-DNA.



Increasing concentrations (from 0 to 2 equiv.) of DNA (c-kit (**A**) and ds12 (**B**)) added to 10μM of Tb.DOTASQ-C5 in Caco.K (10mM Caco.Li + 10mM KCl + 90mM LiCl, pH 7.2) at 20°C (λ_{ex} = 245nm).