Supplementary Data

Site specific replacements of a single loop nucleoside with a dibenzyl linker may switch the activity of TBA from anticoagulant to antiproliferative

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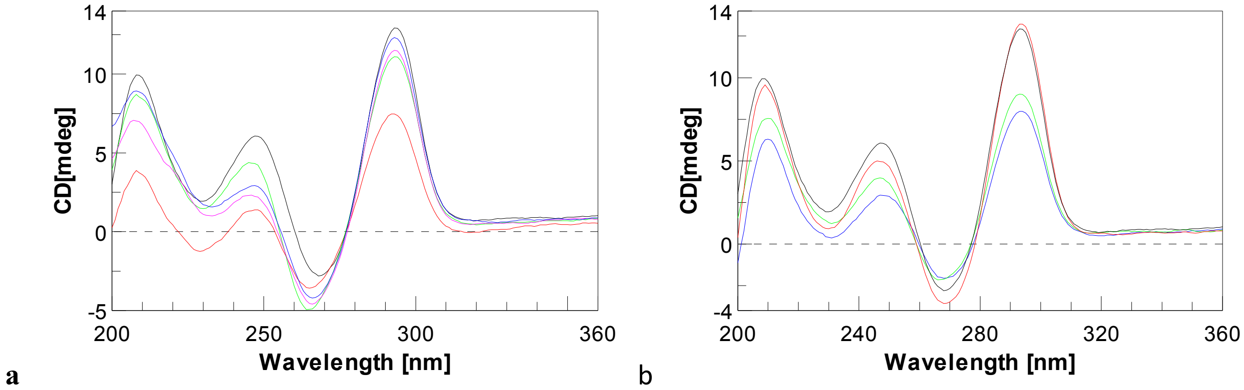
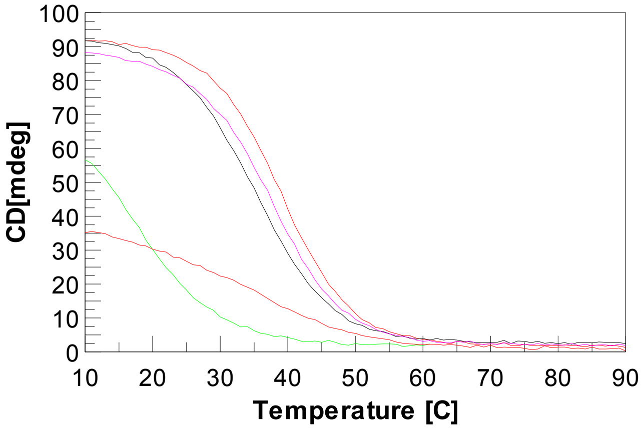


Figure 1S. CD profile of TBA and TBA-based sequences in K^+ buffer. Spectra were collected at 10 °C, using [ON] of 2.0×10^{-5} M. **A**. Lines: black TBA; red TBA-bs8; green TBA-bs9; fuchsia TBA-bs4; blue TBA-bs13. **B.** Lines: black TBA; blue TBA-bs3; green TBA-bs7; red TBA-bs12.

a



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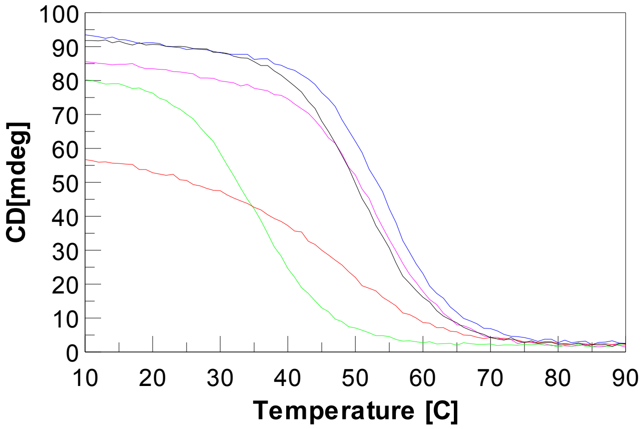
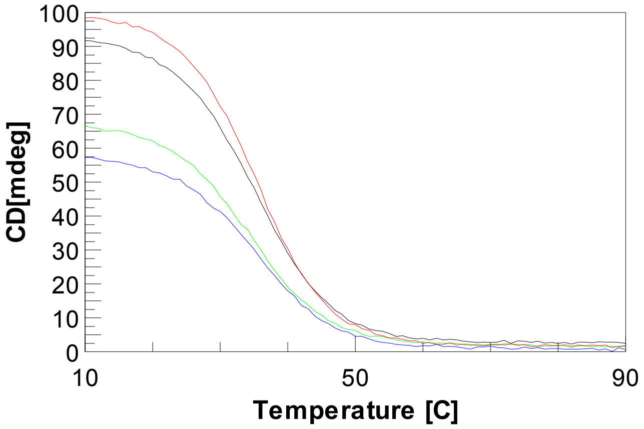
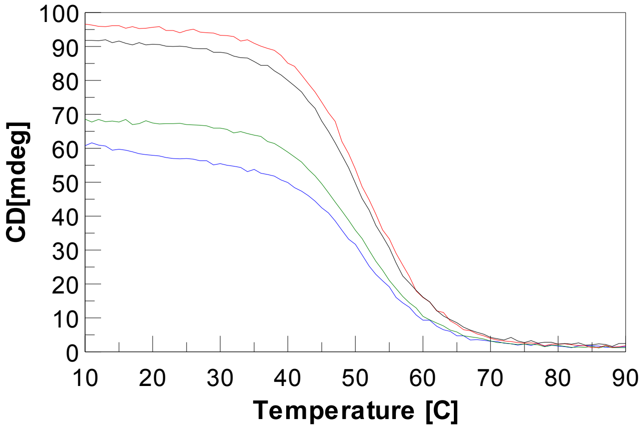


Figure 2Sa-b. CD melting profiles of TBA and TBA-based sequences. Fixed wavelength 295 nm. [ON] of 2.0×10^{-5} M. Cell length 0.5 cm. Temperature scan speed 0.5 °C/min. **a.** PBS. **b**. K^+ buffer. Lines: black TBA; red TBA-bs8; green TBA-bs9; fuchsia TBA-bs4; blue TBA-bs13.

c

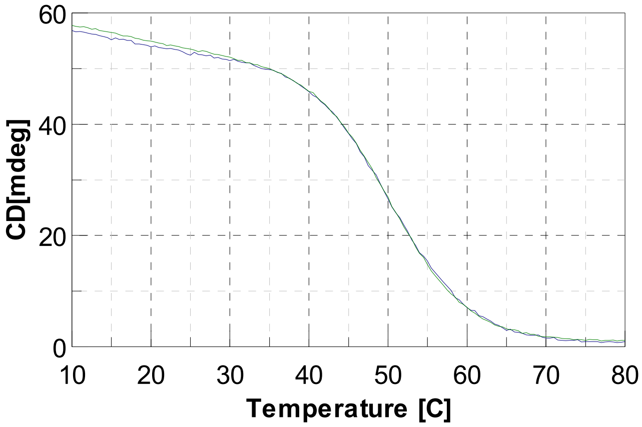


d

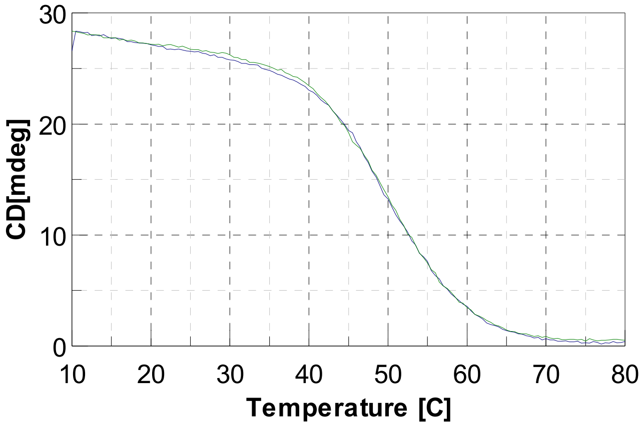


Figures 2Sc-d. CD melting profiles of TBA and TBA-based sequences. Fixed wavelength 295 nm. [ON] of 2.0×10^{-5} M. Cell length 0.5 cm, vol 1400 μL. Temperature scan speed 0.5 °C/min. **c.** PBS. **d**. K^+ buffer. Lines: black TBA; blue TBA-bs3; green TBA-bs7; red TBA-bs12.

a1



a2



a3

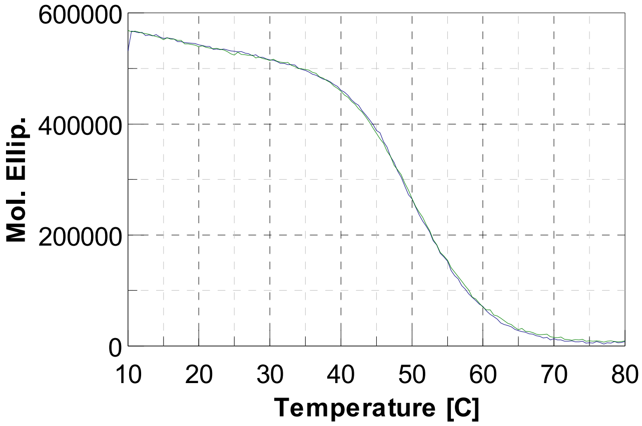
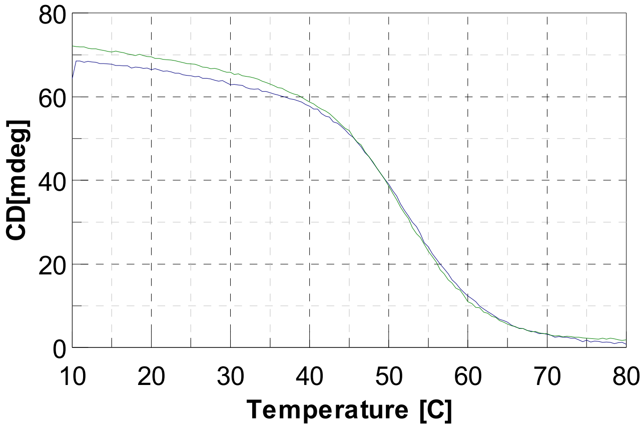
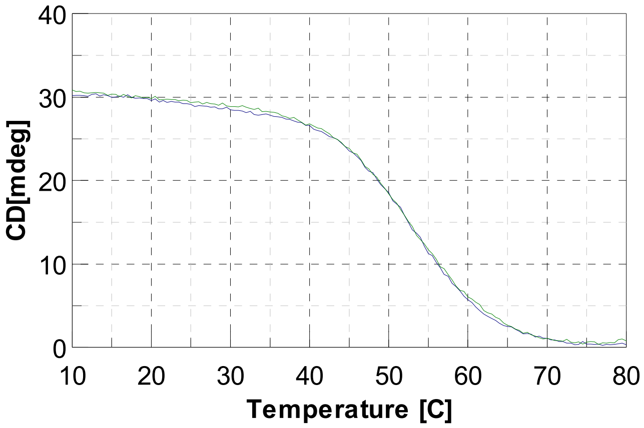


Figure 3Sa. **a1.** Melting-annealing curve of TBA-bs3 at 1.0×10^{-4} M, cell length 0.1 cm, vol 400 μL. **a2.** Annealing-melting curve of TBA-bs3 at 8.3×10^{-6} M, cell length 0.5 cm, vol 1200 μL. Fixed wavelength 295 nm, temperature scan speed 0.2 °C/min. **a3.** Superimposition of melting curves reported in **a1** and **a2** expressed as molecular ellipticity.

b1



b2



b3

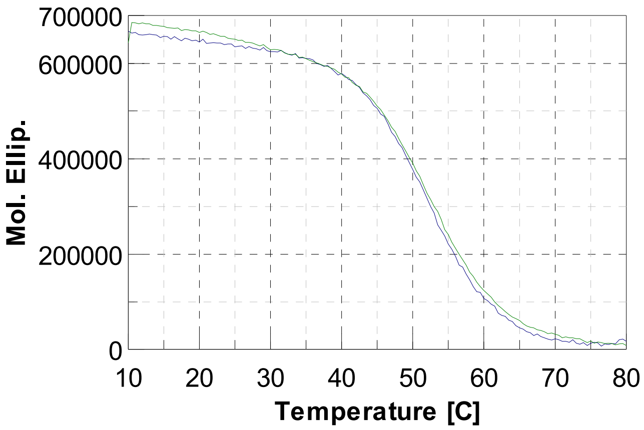
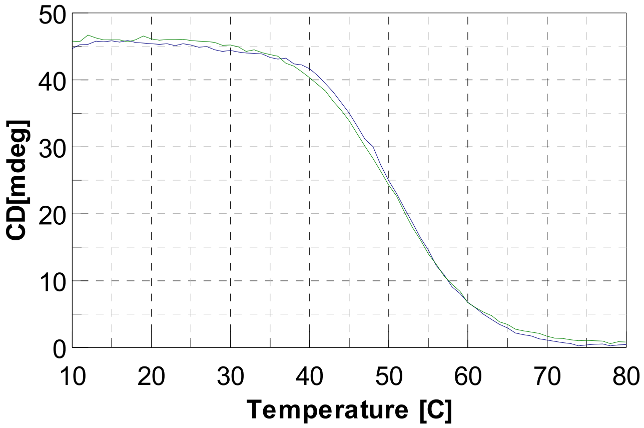
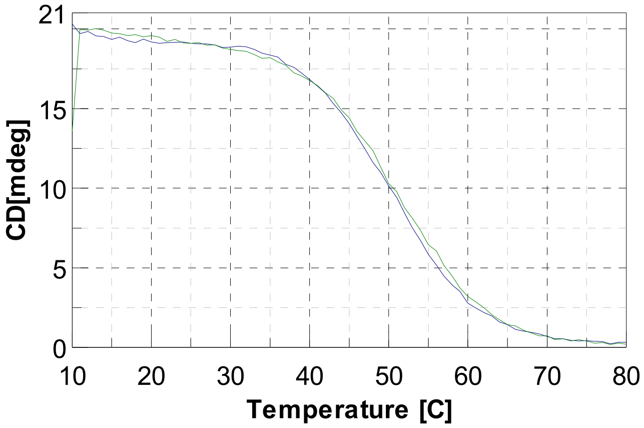


Figure 3Sb. b1. Melting-annealing curve of TBA-bs4 at 1.0×10^{-4} M, cell length 0.1 cm, vol 400 μL. **b2.** Annealing-melting curve of TBA-bs4 at 8.3×10^{-6} M, cell length 0.5 cm, vol 1200 μL. Fixed wavelength 295 nm, temperature scan speed 0.2 °C/min. **b3.** Superimposition of melting curves reported in **b1** and **b2** expressed as molecular ellipticity.

c1



c2



c3

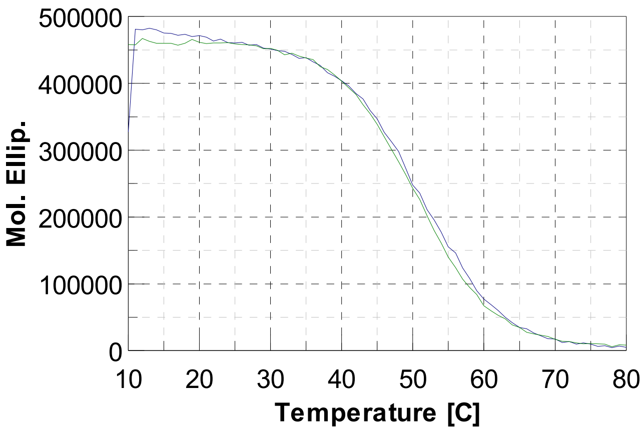
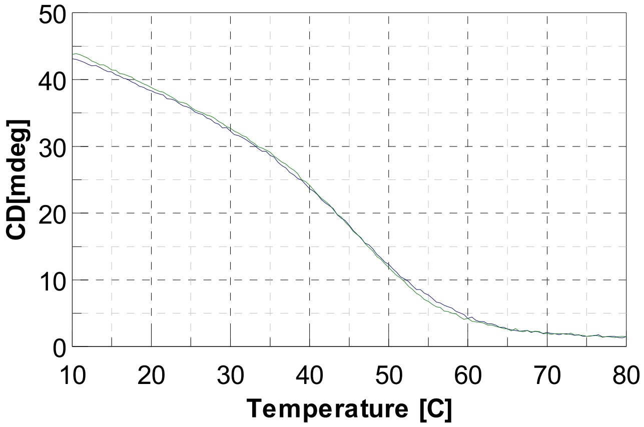
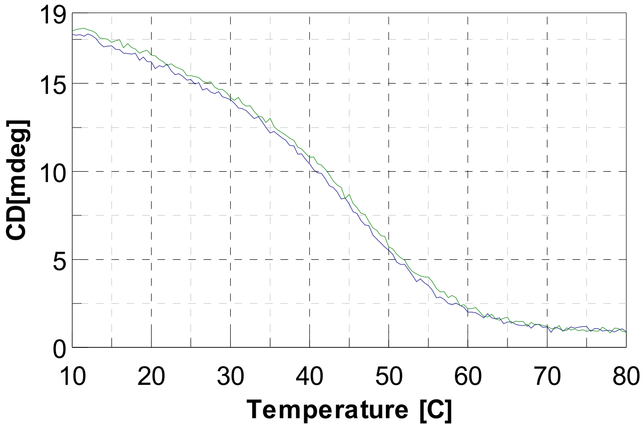


Figure 3Sc c1. Melting-annealing curve of TBA-bs7 at 1.0×10^{-4} M, cell length 0.1 cm, vol 400 µL. **c2.** Annealing-melting curve of TBA-bs7 at 8.3×10^{-6} M, cell length 0.5 cm, vol 1200 µL. Fixed wavelength 295 nm, temperature scan speed 0.2 °C/min. **c3.** Superimposition of melting curves reported in **c1** and **c2** expressed as molecular ellipticity.

d1



d2



d3

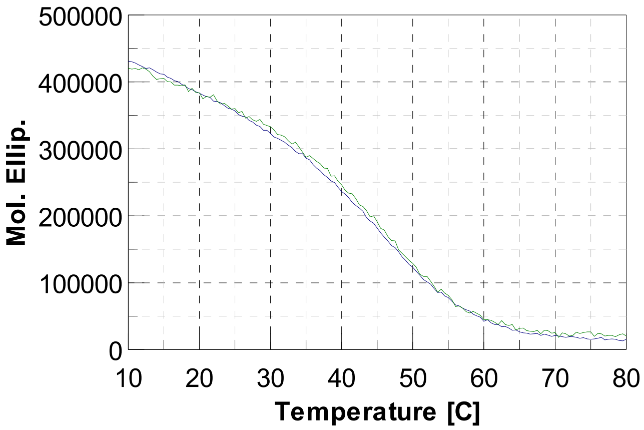
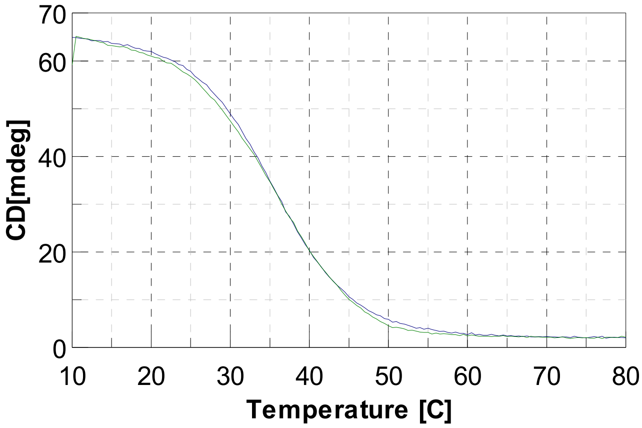
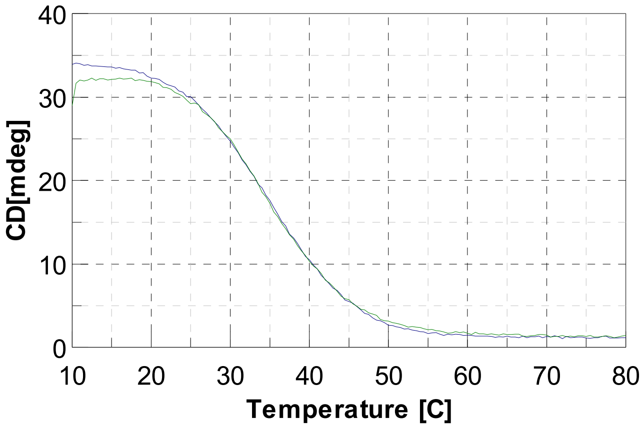


Figure 3Sd. d1. Melting-annealing curve of TBA-bs8 at 1.0×10^{-4} M, cell length 0.1 cm, vol 400 µL. **d2.** Annealing-melting curve of TBA-bs8 at 8.3×10^{-6} M, cell length 0.5 cm, vol 1200 µL. Fixed wavelength 295 nm, temperature scan speed 0.2 °C/min. **d3.** Superimposition of melting curves reported in **d1** and **d2** expressed as molecular ellipticity.

e1



e2



e3

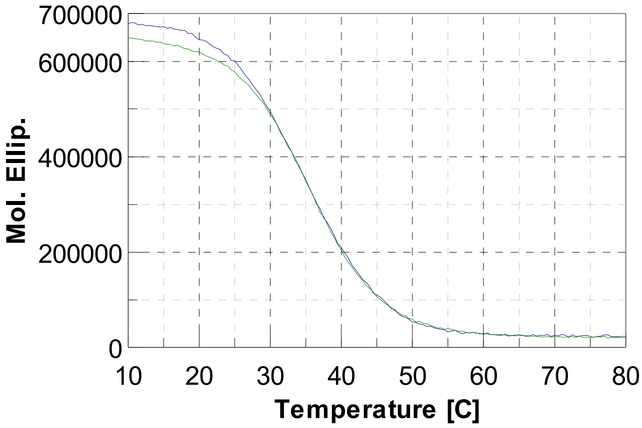
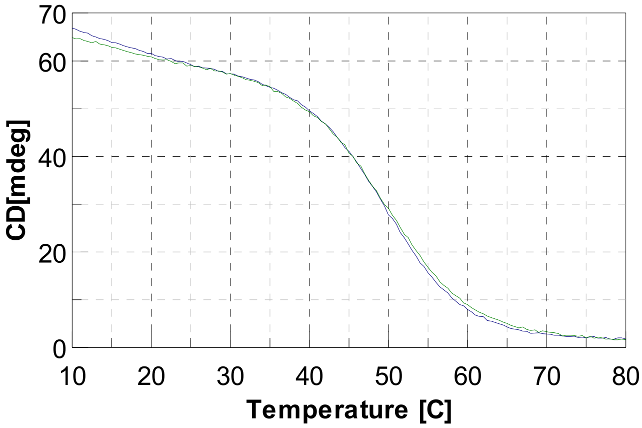
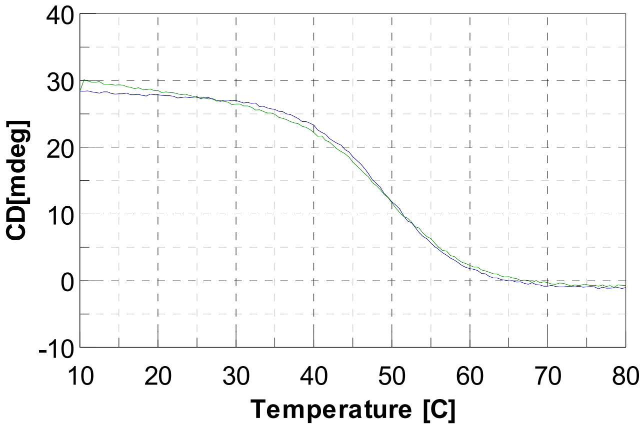


Figure 3Se. e1. Melting-annealing curve of TBA-bs9 at 1.0×10^{-4} M, cell length 0.1 cm, vol 400 μL. **e2.** Annealing-melting curve of TBA-bs9 at 8.3×10^{-6} M, cell length 0.5 cm, vol 1200 μL. Fixed wavelength 295 nm, temperature scan speed 0.2 °C/min. **e3.** Superimposition of melting curves reported in **e1** and **e2** expressed as molecular ellipticity.

f1



f2



f3

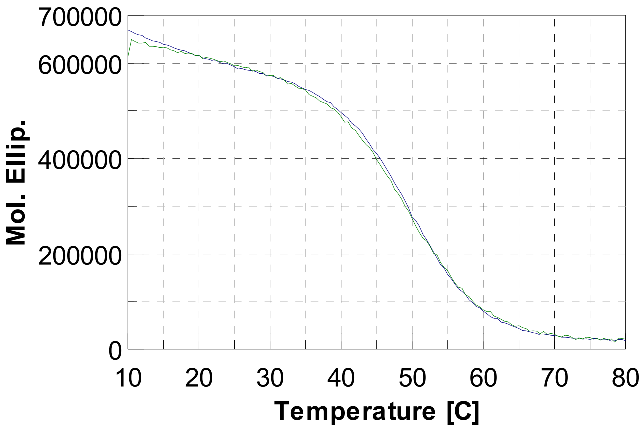
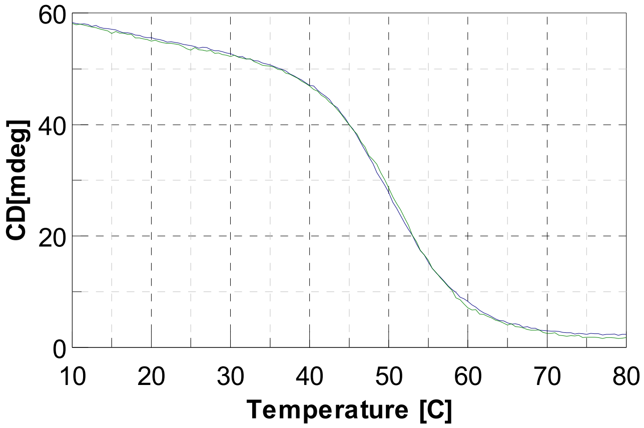
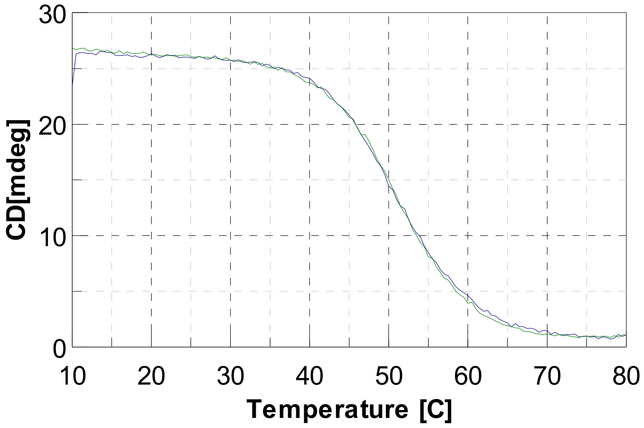


Figure 3Sf. f1. Melting-annealing curve of TBA-bs12 at 1.0×10^{-4} M, cell length 0.1 cm, vol 400 μL. **f2.** Annealing-melting curve of TBA-bs12 at 8.3×10^{-6} M, cell length 0.5 cm, vol 1200 μL. Fixed wavelength 295 nm, temperature scan speed 0.2 °C/min. **f3.** Superimposition of melting curves reported in **f1** and **f2** expressed as molecular ellipticity.

g1



g2



g3

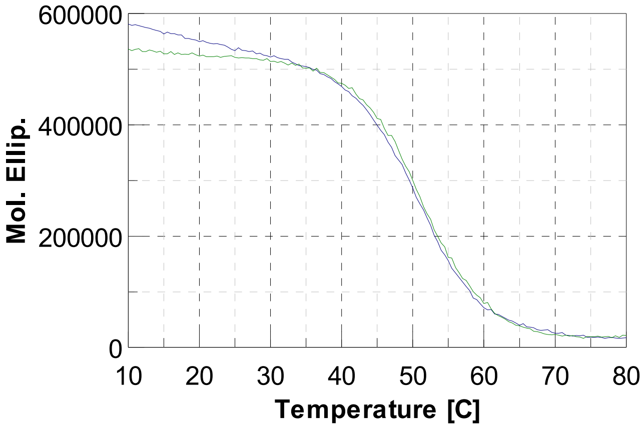


Figure 3Sg. g1. Melting-annealing curve of TBA-bs13 at 1.0×10^{-4} M, cell length 0.1 cm, vol 400 µL. **g2.** Annealing-melting curve of TBA-bs13 at 8.3×10^{-6} M, cell length 0.5 cm, vol 1200 µL. Fixed wavelength 295 nm, temperature scan speed 0.2 °C/min. **g3.** Superimposition of melting curves reported in **g1** and **g2** expressed as molecular ellipticity.

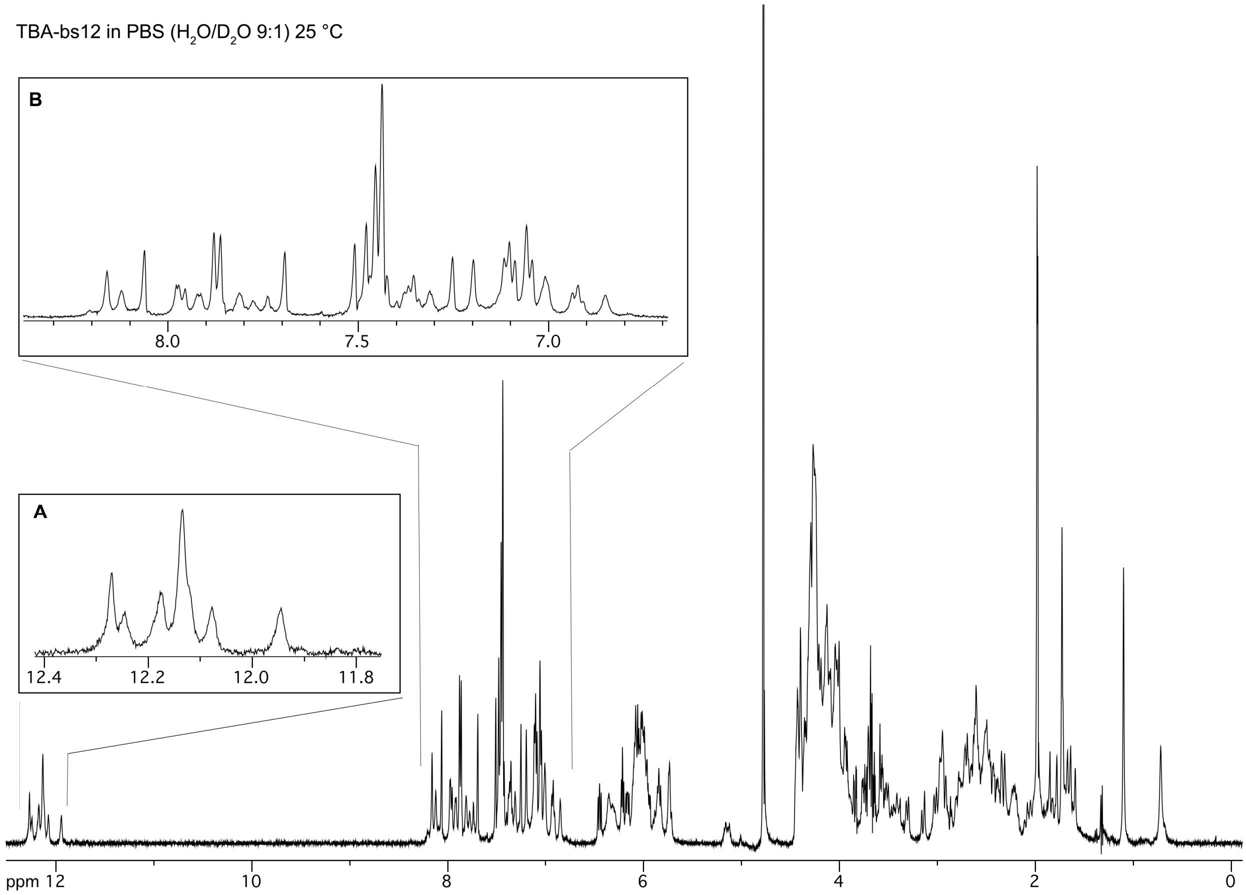


Figure 4Sa. [1]H NMR spectrum of TBA-bs12 in PBS (H_2O/D_2O 9:1) recorded at 25 °C. A) Expansion of the imino protons region; B) Expansion of aromatic protons region.

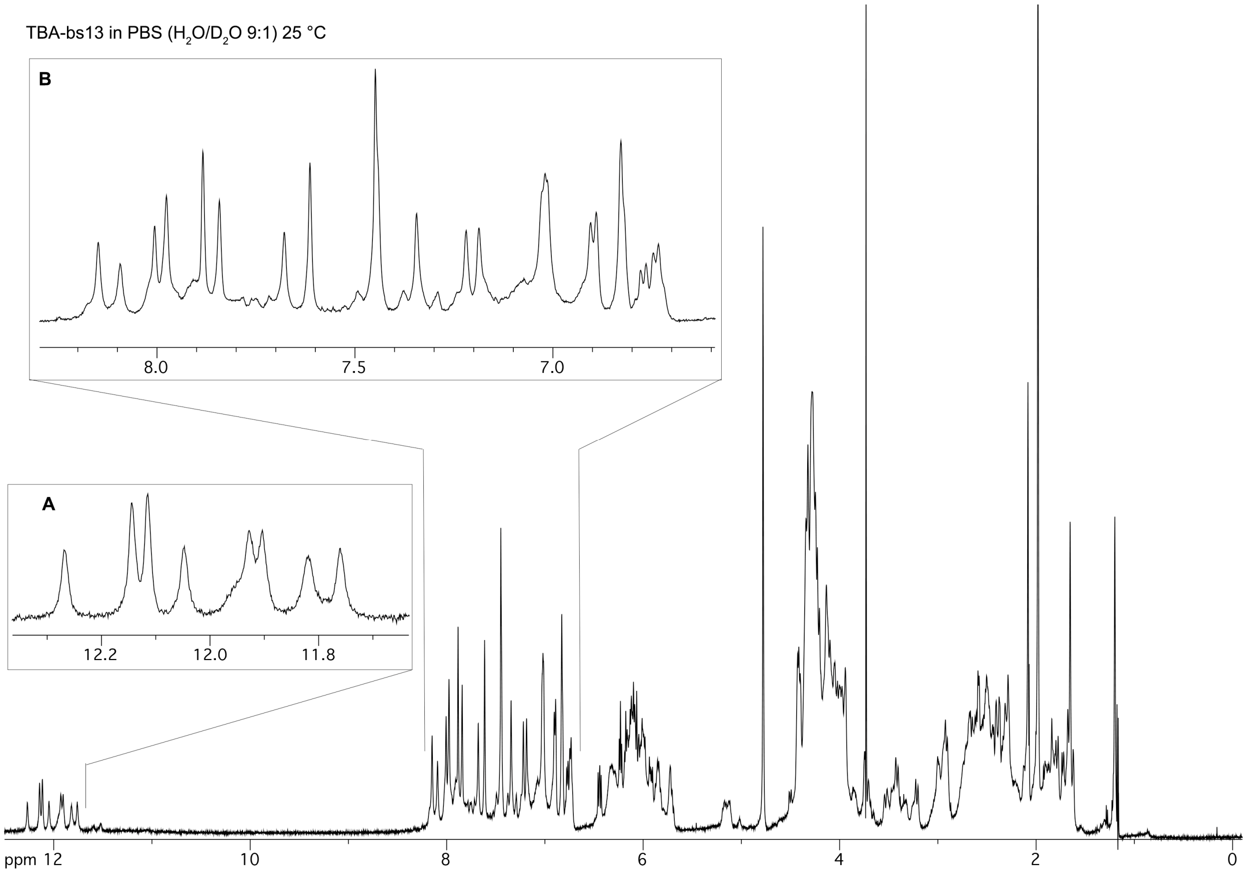


Figure 4Sb. [1]H NMR spectrum of TBA-bs13 in PBS (H_2O/D_2O 9:1) recorded at 25 °C. A) Expansion of the imino protons region; B) Expansion of aromatic protons region.

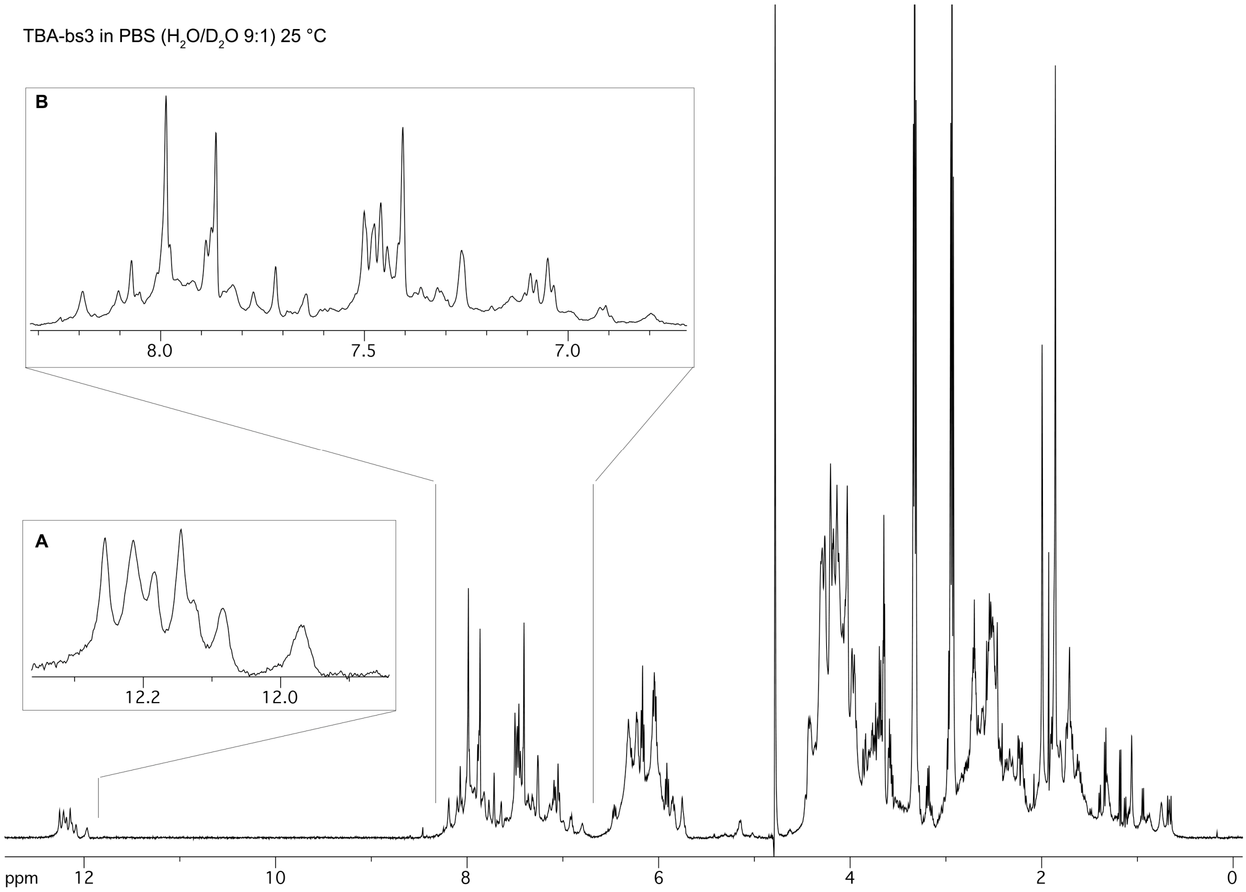


Figure 4Sc. ^{1}H NMR spectrum of TBA-bs3 in PBS (H_2O/D_2O 9:1) recorded at 25 °C. A) Expansion of the imino protons region; B) Expansion of aromatic protons region.

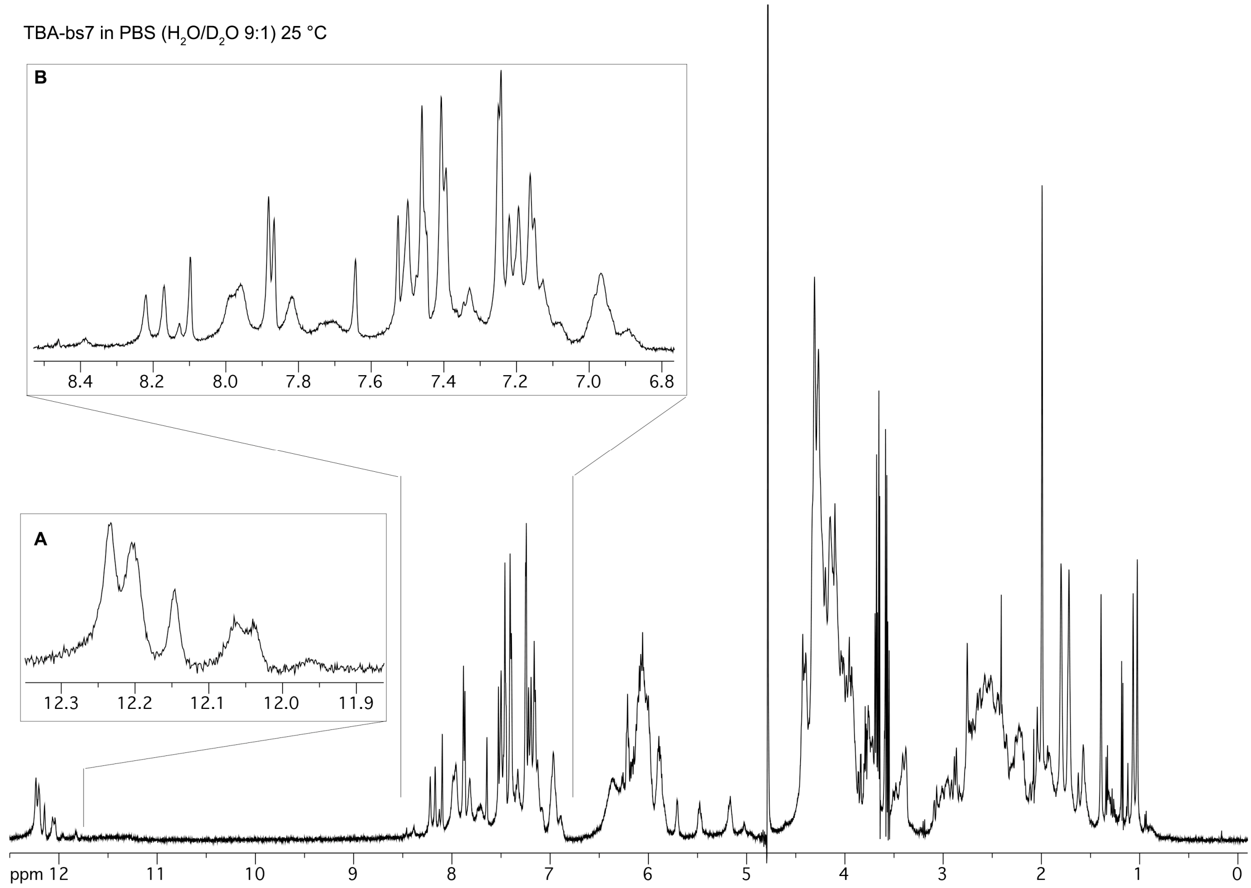


Figure 4Sd. [1]H NMR spectrum of TBA-bs7 in PBS (H_2O/D_2O 9:1) recorded at 25 °C. A) Expansion of the imino protons region; B) Expansion of aromatic protons region.

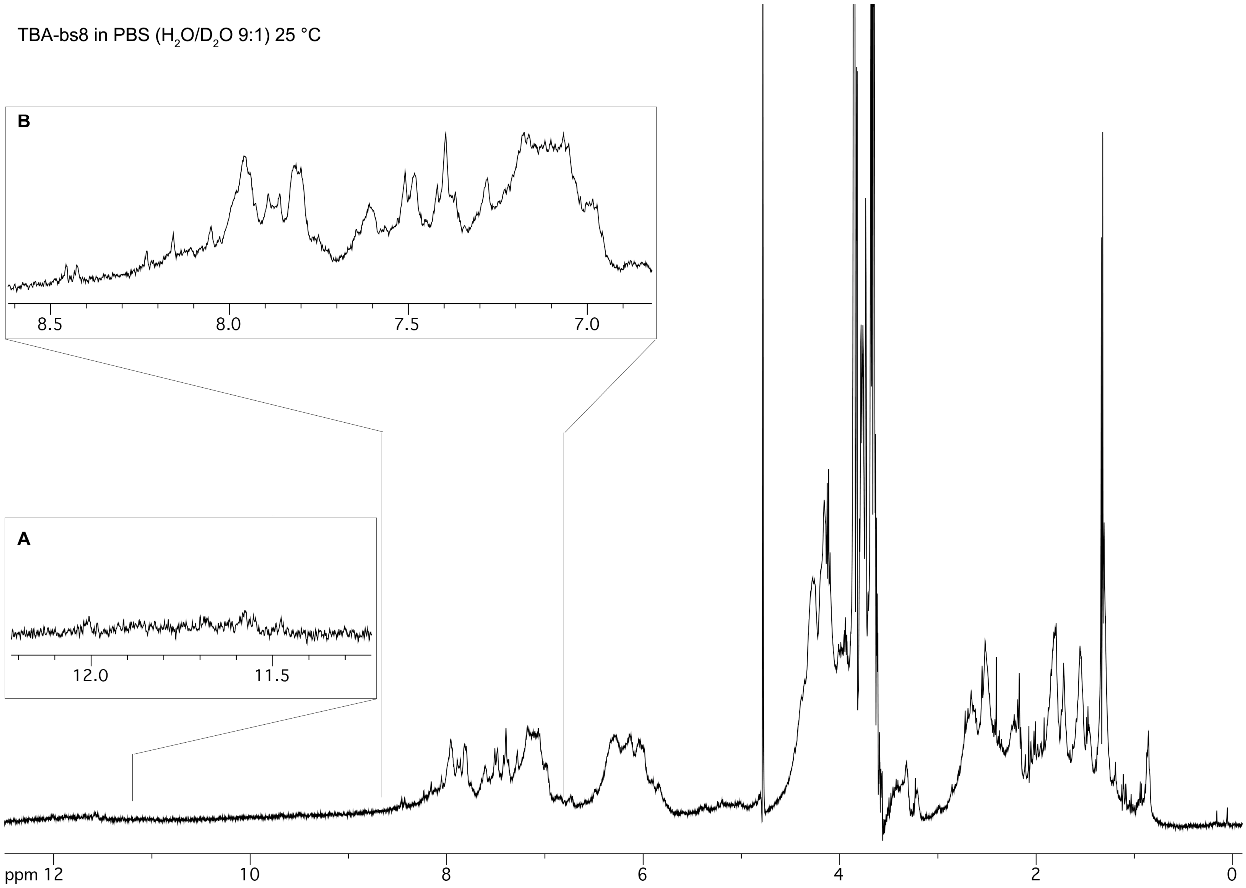


Figure 4Se. ^{1}H NMR spectrum of TBA-bs8 in PBS (H_2O/D_2O 9:1) recorded at 25 °C. A) Expansion of the imino protons region; B) Expansion of aromatic protons region.

Table 1S. ^{1}H NMR assignments for TBA-bs12 at 10 °C (δ in ppm).

X12	a	b	c	d	e	f	g	h	i	l	
Nucl	H_6/H_8	$H_{1'}$	$H_{2'}$	$H_{2''}$	$H_{3'}$	$H_{4'}$	$H_{5'}/H_{5''}$	CH_3	H_3/H_1	NH2i	NH2e
G_1	7,42	6,09	2,99	2,96	4,92	4,41	4,05/4,14	-	12,10	9,41	
G_2	8,20	6,02	3,04	2,34	5,16	4,42	4,23	-	12,16		
T_3	7,89	6,20	2,24	2,59	4,91	4,29		1,98	-	-	-
T_4	7,20	6,07	2,04	2,66	4,89	4,28	3,93	1,07	-	-	-
G_5	7,45	6,03	3,37	2,89	4,86	4,41	4,29	-	12,29	9,46	6,79
G_6	7,71	5,94	2,80	2,60	5,11	4,46	4,25	-	12,19	10,19	6,68
T_7	7,92	6,48	2,51	2,61	4,86	4,43	4,24/4,28	1,97	-	-	-
G_8	7,48	5,75	1,97	2,32	4,79	3,99		-	-	-	-
T_9	7,26	5,83	1,98	2,42	4,63	3,75	3,56/3,03	1,80	-	-	-
G_{10}	7,42	6,01	3,67	2,79	4,92	4,25		-	11,92	7,26	5,83
G_{11}	7,43	6,09	2,97	1,99	4,91	4,28		-	12,09		
X_{12}	**6,91**	**7,10**	**7,03**	**7,35**	**7,44**	**7,47**	**2,95**	**2,33**	**3,28**	**3,17**	**-**
T_{13}	7,46	6,07	2,00	2,73	4,89	4,30	3,94	0,62	-	-	-
G_{14}	7,50	6,10	3,52	2,96	4,92	4,45	4,37	-	12,14	9,8	6,79
G_{15}	8,09	6,16	2,71	2,45	4,80	4,27		-	12,34	9,41	

Table 2S. Δδ in ppm between ^{1}H NMR assignments of TBA-bs12 and TBA* (10 °C).

Nucl	H6/H8	H1'	H2'	H2''	H3'	H4'	H5'/H5''	CH3	H3/H1
G1	0,00	0,01	-0,03	0,00	-0,07	0,01	0,02/0,02	-	-0,02
G2	0,00	0,01	0,00	0,00	0,01	0,01	0,00	-	0,02
T3	-0,01	0,01	0,02	0,03	0,00	0,00		0,00	-
T4	0,02	0,00	-0,02	0,00	0,01	0,08	0,01	0,04	-
G5	0,00	-0,01	-0,01	-0,02	-0,01	0,00	−0,01	-	0,07
G6	0,01	-0,01	0,01	-0,01	-0,01	0,01	0,02/0,02	-	0,01
T7	-0,01	-0,01	0,00	0,00	0,00	0,00	0,00/0,00	-0,01	-
G8	0,01	-0,02	-0,01	0,00	0,02	0,01		-	-
T9	0,00	0,01	0,02	0,01	0,01	-0,01	0,02/0,01	0,01	-
G10	-0,04	-0,05	-0,06	**-0,14**	0,00	-0,03		-	0,00
G11	**-0,80**	0,07	-0,01	**-0,34**	**-0,23**	**-0,11**		-	0,02
T13	**0,21**	-0,04	-0,08	0,01	-0,02	0,08	0,02	**-0,36**	-
G14	0,02	0,02	0,01	0,00	0,00	0,03	−0,00	-	0,03
G15	0,01	0,00	0,02	0,01	0,00	0,02		-	0,03

Δδ ≥ ± 0.10 ppm are in bold. *Chemical shift values for TBA were taken from ref. 41

Table 3S. ^{1}H NMR assignments for TBA-bs13 at 10 °C (δ in ppm).

X13											
	a	b	c	d	e	f	g	h	i	l	
Nucl	H_6/H_8	$H_{1'}$	$H_{2'}$	$H_{2''}$	$H_{3'}$	$H_{4'}$	$H_{5'}/H_{5''}$	CH_3	H_3/H_1	NH2i	NH2e
G_1	7,43	6,09	3,01	3,01	4,99	4,41	4,05/4,13	–	12,08		
G_2	8,18	6,10	3,05	2,36	5,16	4,44	4,29	–	12,10	10,58	
T_3	7,88	6,22	2,30	2,61	4,95	4,31	4,27	1,98	–		
T_4	7,18	6,08	2,07	2,67	4,90	4,42	3,96	1,18	–		
G_5	7,46	6,05	3,44	2,92	4,89	4,28		–	12,30	9,48	6,76
G_6	7,69	5,91	2,76	2,59	5,11	4,45		–	11,98	9,99	6,54
T_7	7,92	6,47	2,48	2,59	4,84	4,43	4,24/4,28	1,98	–		
G_8	7,45	5,72	1,98	2,30	4,74	3,90	3,98/4,06	–	–		
T_9	7,22	5,82	1,90	2,37	4,60	3,79	2,91/3,52	1,72	–		
G_{10}	7,33	6,01	3,71	2,93	4,90		4,13	–	11,78		
G_{11}	8,14	5,84	2,95	2,52	5,13	4,36		–	11,88		
T_{12}	8,01	6,16	2,11	2,30	4,87	4,33	4,51/4,71	2,09	–		
X_{13}	**6,75**	**6,70**	**6,90**	**6,81**	**6,81**	**7,02**	**1,85/2,40**	**1,73/2,29**	**3,19/3,85**	**3,42/3,95**	
G_{14}	7,62	6,13	3,40	2,93	4,91	4,71	4,32/4,51	–	11,78	9,77	
G_{15}	8,04	6,13	2,68	2,42	4,81	4,28	4,13/4,21	–	12,20		

Table 4S. Δδ in ppm between [1]H NMR assignments of TBA-bs13 and TBA* (10 °C).

Nucl	H_6/H_8	$H_{1'}$	$H_{2'}$	$H_{2''}$	$H_{3'}$	$H_{4'}$	$H_{5'}/H_{5''}$	CH_3	H_3/H_1
G_1	0,01	0,01	0,05	0,05	0,00	0,01	0,02-0,01	-	0,00
G_2	-0,02	0,07	0,01	0,02	0,01	0,03	0,06	-	-0,04
T_3	-0,02	-0,03	0,08	0,05	0,04	0,02	0,03	0,00	-
T_4	0,00	0,01	0,01	0,01	0,02	0,22	0,04	**0,15**	-
G_5	0,01	0,01	0,06	0,01	0,02	−0,13		-	0,08
G_6	-0,01	-0,04	-0,03	-0,02	-0,01	0,00		-	**-0,20**
T_7	-0,01	0,02	-0,03	-0,02	-0,02	0,00	0,00-0,00	0,00	-
G_8	-0,02	-0,05	0,00	-0,02	-0,03	-0,08	−0,03/−0,04	-	-
T_9	-0,04	0,00	-0,06	-0,04	-0,02	0,03	**−0,10**/−0,03	-0,07	-
G_{10}	**-0,13**	-0,05	-0,01	0,00	-0,02		-0,01	-	**-0,14**
G_{11}	-0,09	**-0,18**	-0,03	**0,19**	-0,01	-0,03		-	**-0,19**
T_{12}	**0,11**	-0,03	-0,09	**-0,28**	-0,04	0,04	**0,27/0,39**	**0,11**	
G_{14}	**0,14**	0,05	**-0,11**	-0,03	-0,01	**0,29**	0,02/**0,14**	-	**-0,39**
G_{15}	-0,04	-0,03	-0,01	-0,02	0,01	0,03	0,01/0,03	-	**-0,11**

Δδ ≥ ± 0.10 ppm are in bold. *Chemical shift values for TBA were taken from ref. 41

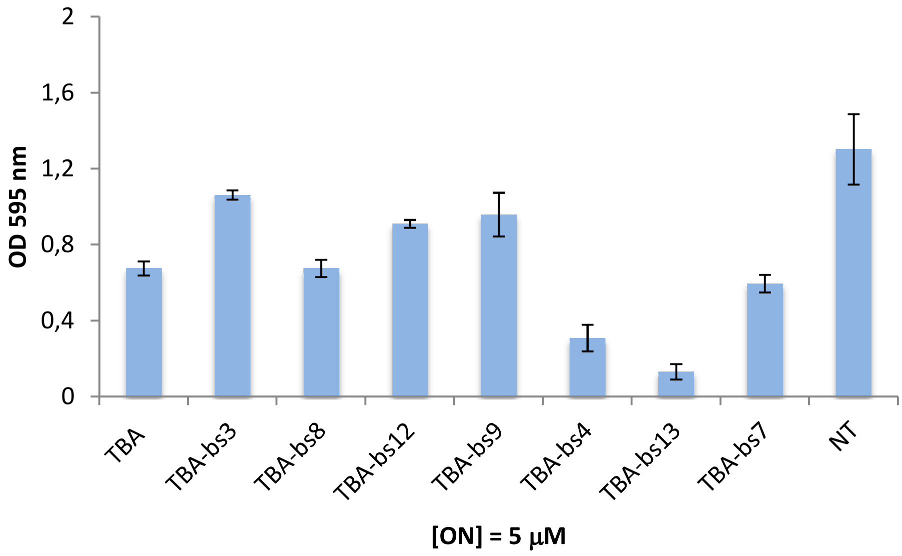


Figure 5S. Antiproliferative activity on HeLa cervical carcinoma. Cells were treated with 5 μM oligonucleotide, annealed in K^+ buffer (see experimental section). Cell viability was assayed 7 days after addition of oligonucleotide using the MTT assay. A pool of three different set of experiments (each repeated three times) were performed and the data expressed as the mean ±standard deviation.

. **Table 5S**. Fibrinogen clotting time calculated from UV scattering curves.

[ON]	20 nM	40nM	100 nM
TBA-bs3	72,5±0,5	130,5±23,5	359,0±10
TBA-bs4	29,0±0,5	29,0±1	30,5±1,5
TBA-bs7	102,0±1	219,0±15	424,5±6,5
TBA-bs8	55,0±1	71,5±14,5	107,0±4
TBA-bs9	80,5±2	110,5±22,5	194,0±4
TBA-bs12	218,0±20	337,0±30,5	651,3±49
TBA-bs13	29,8±1	25,5±3	26,6±2
TBA	134,5±10,5	227,3±4,0	427,5±25
CONTROL	26,8±1,0	25,9±0,9	24,0±0,8
VEHICLE	25,8±1,0	24,2±1,0	23,7±2,0

The curves were obtained measuring, as a function of the time, the UV scattering caused by fibrin polymerization. 1.0 mL of fibrinogen (2 mg/1 mL) in PBS solution was incubated for 1 min with 10 μL of each ON, to reach the final concentration reported in the table, and clot formation was triggered by addition of 1.0 NIH of human thrombin. The basal curves were registered starting the polymerization reaction in absence of any inhibitor. The effect of dilution was valued registering the polymerization reaction after the addition of PBS alone (10 μL). Wavelength was fixed at 380 nm. Each value was determinate in triplicate, and the average values and the standard errors were reported.

Table 6S. PT values (s) measured for TBA and its analogues at 2 µM. PT (s) of control and vehicle (PBS) are 13.3 ± 0.24 s and 13.2 ± 0.22 s respectively.

ODN	PT (20 µM)	PT (2µM)
TBA	**49.8 ± 1.28**	**23.1 ± 0.80**
TBA-bs3	43.6 ± 2.15	22.7 ± 1.00
TBA-bs4	14.1 ± 0.25	[a]n.t.
TBA-bs7	56.3 ± 0.97	24.2 ± 0.65
TBA-bs8	25.8 ± 0.30	[a]n.t.
TBA-bs9	24.8 ± 0.80	[a]n.t.
TBA-bs12	52.0 ± 1.73	24.9 ± 2.40
TBA-bs13	12.5 ± 0.55	[a]n.t.

Table 7S. Data of absorbance at 595 nm measured in concentration-dependent response MTT assay for TBA-bs13.

[TBA-bs13] μM	**n1**	**n2**	**n3**	**average**	**w/o**	**Standard deviation**
50	0.165	0.157	0.167	0.163	0.09833333	0.0052915
10	0.161	0.156	0.161	0.15933333	0.09466667	0.00288675
5	0.869	0.997	0.798	0.888	0.82333333	0.10085138
1	0.988	1.299	1.001	1.096	1.03133333	0.17592328
0.5	0.919	0.968	0.793	0.89333333	0.82866667	0.0902792
0.1	1.145	1.082	1.025	1.084	1.01933333	0.06002499
0.01	1.887	1.711	1.693	1.76366667	1.699	0.10718831
0.001	1.637	1.81	1.753	1.73333333	1.66866667	0.08816084
NT	1.158	1.511	1.433	1.36733333	1.30266667	0.18543552
bottom	0.059	0.066	0.069	0.06466667	0	0.0051316

Table 8S. Data of absorbance at 595 nm measured in concentration-dependent response MTT assay for TBA.

[TBA] μM	n1	n2	n3	average	w/o	standard deviation
50	0.161	0.303	0.208	0.224	0.15933333	0.07233948
10	0.73	0.633	0.831	0.73133333	0.66666667	0.09900673
5	0.801	0.768	0.822	0.797	0.73233333	0.02722132
1	1.495	1.281	1.321	1.36566667	1.301	0.11377756
0.5	1.284	1.297	1.453	1.34466667	1.28	0.09404432
0.1	1.717	1.647	1.736	1.7	1.63533333	0.04687217
0.01	1.622	1.681	1.89	1.731	1.66633333	0.14082258
0.001	1.778	1.729	1.74	1.749	1.68433333	0.02570992
NT	1.158	1.511	1.433	1.36733333	1.30266667	0.18543552
bottom	0.059	0.066	0.069	0.06466667	0	0.0051316