PNA C-C^+ *i*-motif: Superior stability of PNA TC_8Tetraplexes compared to DNA TC_8 tetraplexes

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Experimental Section:

General. Reagents for PNA monomers synthesis, were purchased from Lancaster, UK. DMF was dried by vacuum distillation over P_2O_5, THF refluxed over sodium, pyridine refluxed over KOH then CaH, CH_2Cl_2 refluxed over CaH and CH_3CN refluxed over CaH then distilled under anhydrous conditions. TLC were done using pre-coated silica gel plates 1.0554 DC-Alufolien 20 x 20 cm Kieselgel 60 F_{254} from Merck. . ^1H NMR (200 MHz) and ^{13}C NMR were recorded in solvent $CDCl_3$ and values are quoted in δ ppm. Mass spectra were recorded using Finnigan MAT and MALDI-TOF using ABI mass spectrometer.

General protocol for solid phase synthesis of PNA.

Synthesis of PNA was carried out using BOC-β-alanine derivatized Merrifield resin (Pharmecia) (0.15 mmol/g substitution). The synthesis cycle was as follows: deprotection: 50% TFA in DCM (15 min), wash with DCM, DMF and DCM , neutralize (5% DIEA in DCM), wash DCM, DMF and DCM), coupling (**4b**/HOBT/HBTU/DIEA in DMF, 4 eq, 1.5 h) capping (10% Ac_2O/Pyridine in DCM), wash (DCM, DMF and DCM). Deprotection and amide coupling reactions were monitored by Kaiser's test. The purity of PNA was ascertained on an analytical RP C18 column.

Table 1. Oligomers for the study of *i-motif* of PNA

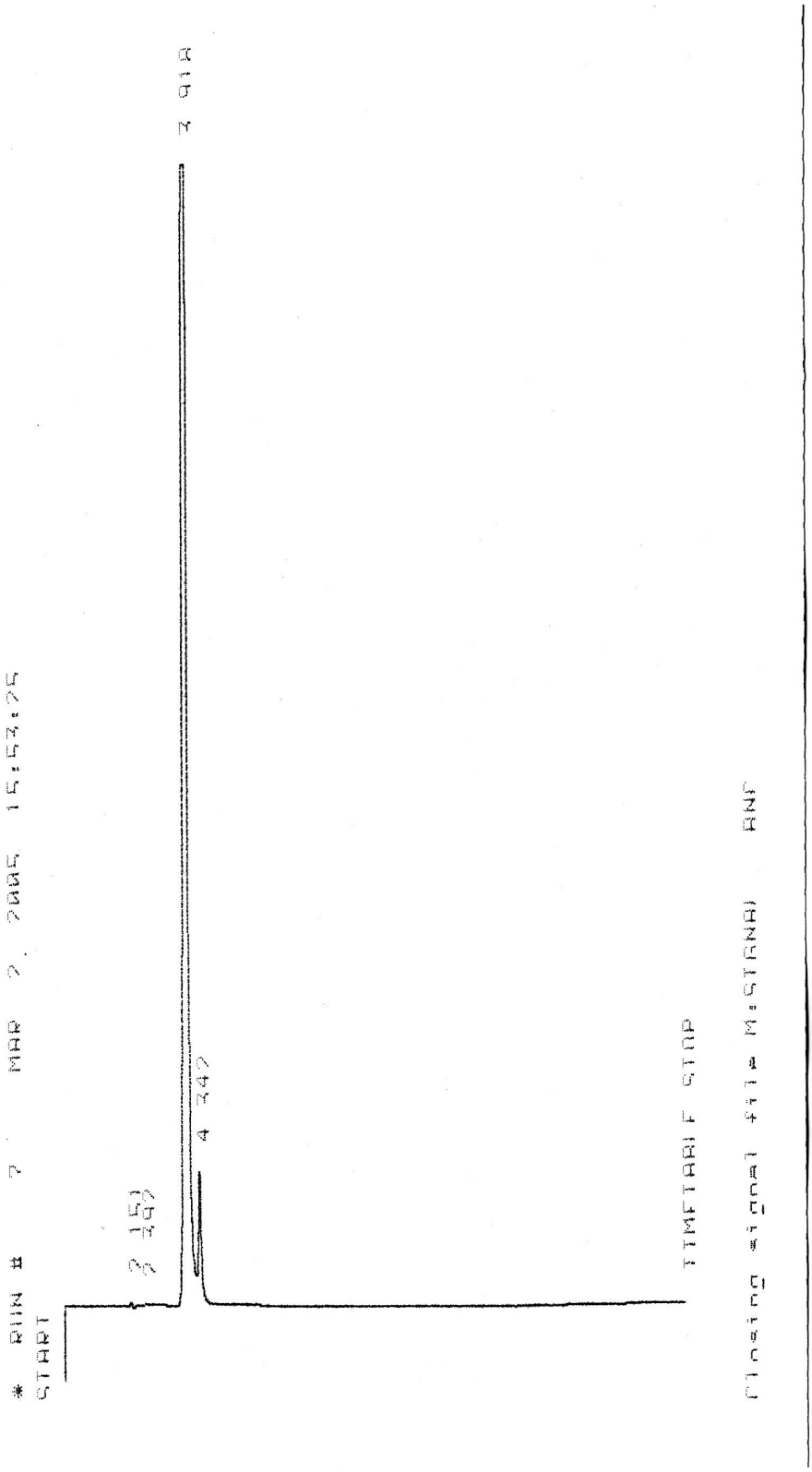
PNA	Sequences of PNA	Molecular Formula	Molecular weight (Calculated)
1	H_2N-T-C-C-βala-COOH	$C_{34}H_{49}N_{15}O_{12}$	859.86
2	H_2N-T-C-C-C-C-βala-COOH	$C_{44}H_{62}N_{20}O_{15}$	1111.11
3	H_2N-T-C-C-C-C-C-βala-COOH	$C_{54}H_{75}N_{25}O_{18}$	1360.36
4	HN-Lys-T-C-C-C-C-C-C-C-C-C-$CONH_2$	$C_{99}H_{137}N_{47}O_{30}$	2463.3

Table 2. DNA was synthesized using standard procedure on automated synthesizer.

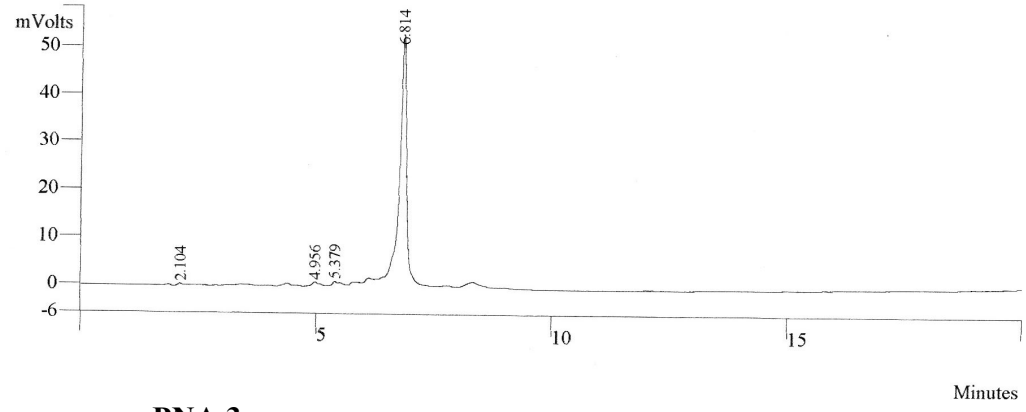
5	d(TCCCC)
6	d(TCCCCCCCC)

Figure 1.HPLC of PNAs **1-4**

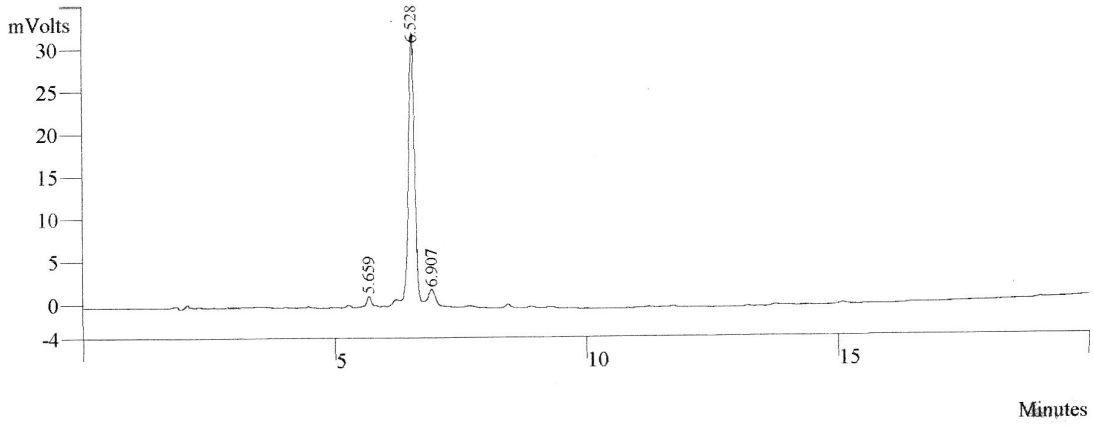
PNA 1

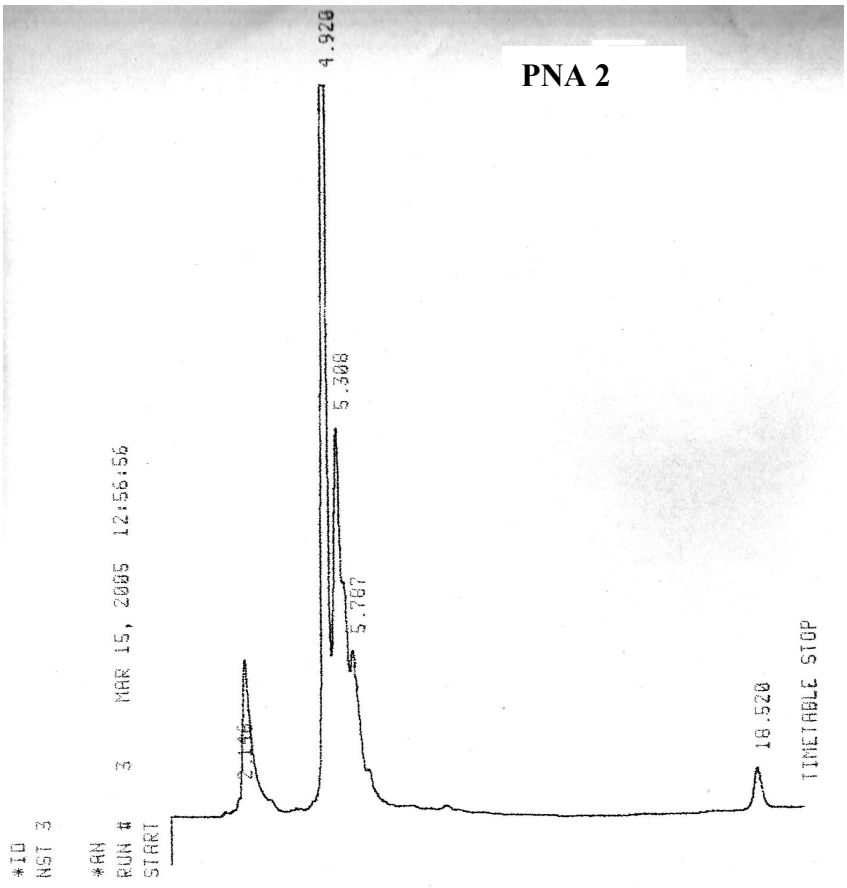


PNA 4



PNA 3

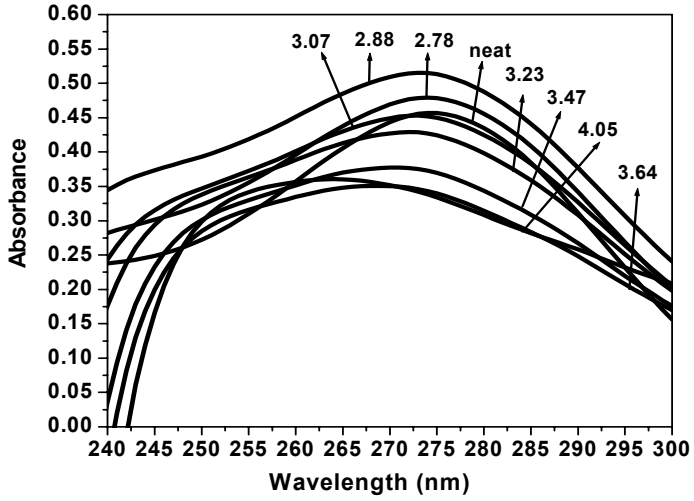




UV-T_m experiments.

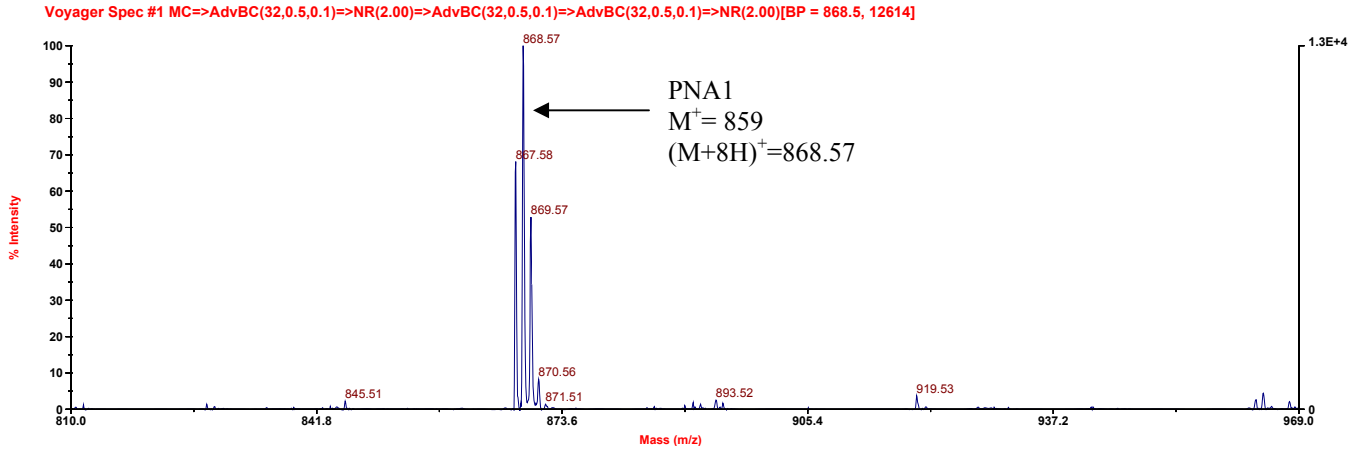
UV melting experiments were performed on Lambda-35 UV Spectrometer (Perkin-Elmer) equipped with a thermal melt system, PTP-6 Peltier Temperature Programmer with water circulator Thermohake K20. The sample for Tm measurement was prepared by mixing calculated amount of stock oligonucleotide and PNA solutions together in 2 mL of sodium phosphate buffer (pH 7.1). The samples 2 mL were transferred to quartz cell and sealed with Teflon stopper after degassing with nitrogen gas for 15 min and equilibrated at the starting temperature for at least 30 min. The OD at 260 nm was recorded in steps from 10-85 ^{o}C with temperature increment of 0.2 ^{o}C/min. The results were normalized and analysis of data was performed on using Origin 5.0 (Microsoft Corp.).

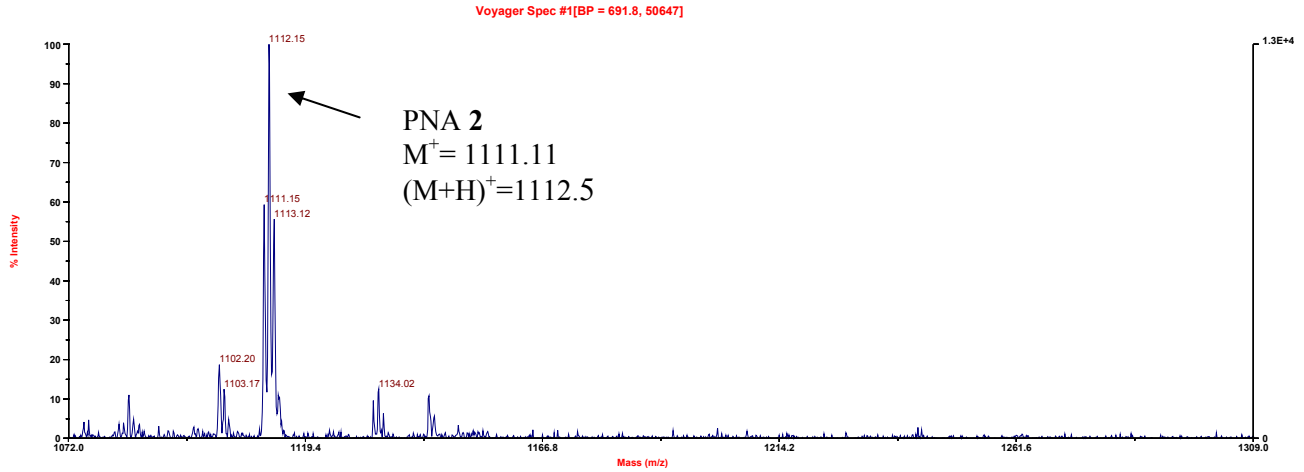
The UV study of the i-motif formation by PNA was done in acidic pH by monitoring at 295 nm. 100mM sodium acetate buffer was used for pH 3.0-5.0, while 10mM potassium phosphate buffer with KCl for pH 5.9-7.0. Figures 3 and 4 show the first derivative UV-melting curves for different PNAs at different pH. Tables 3 and 4 are the Tms computed from the UV data. The ITC results are shown in Figure 5.

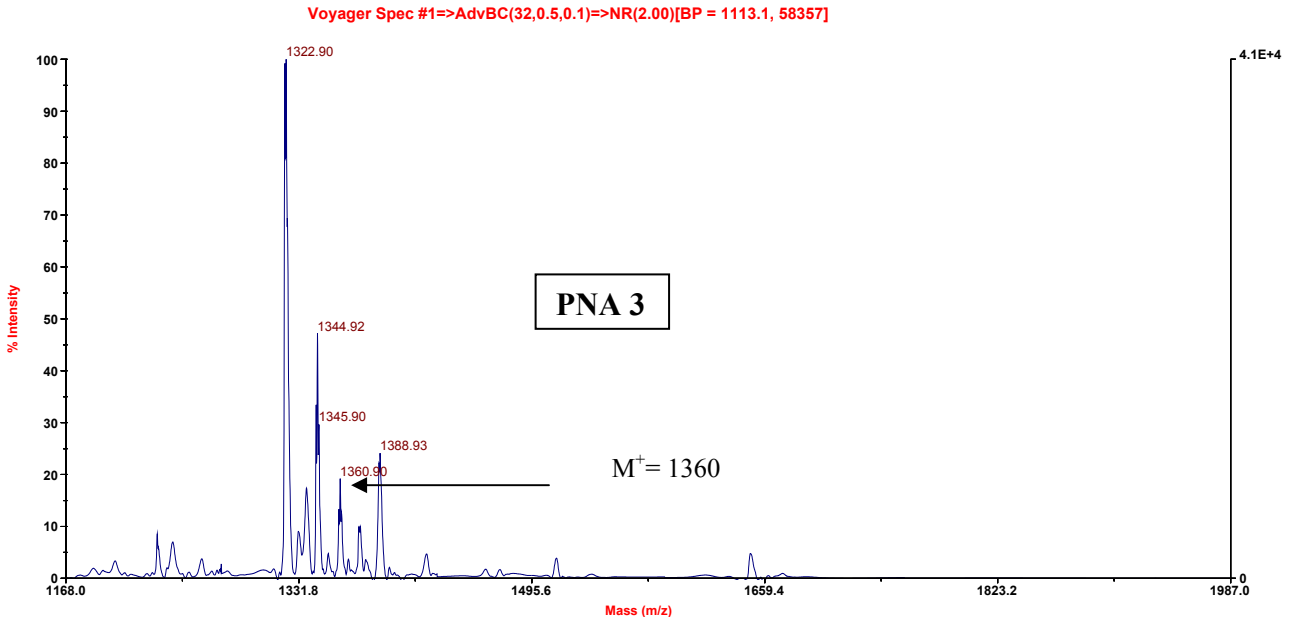


UV-spectra of TC_8 PNA **4** at different pHs

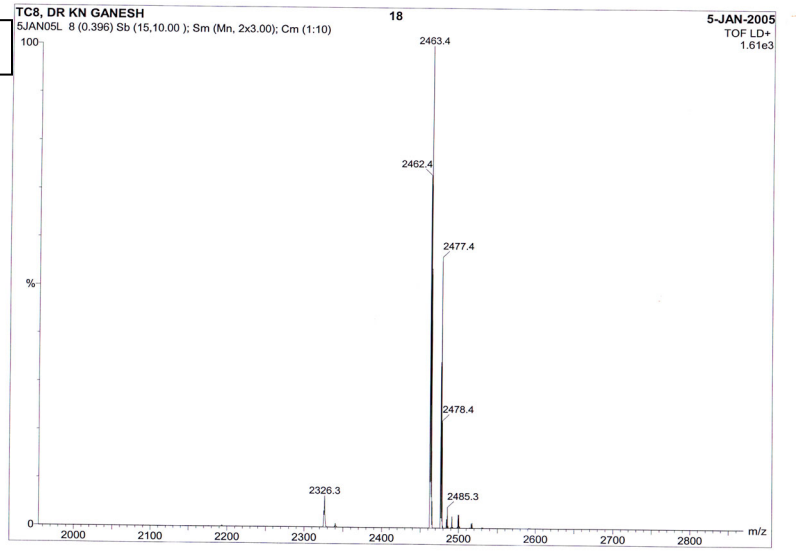
Figure 2. Mass spectra of PNAs 1-

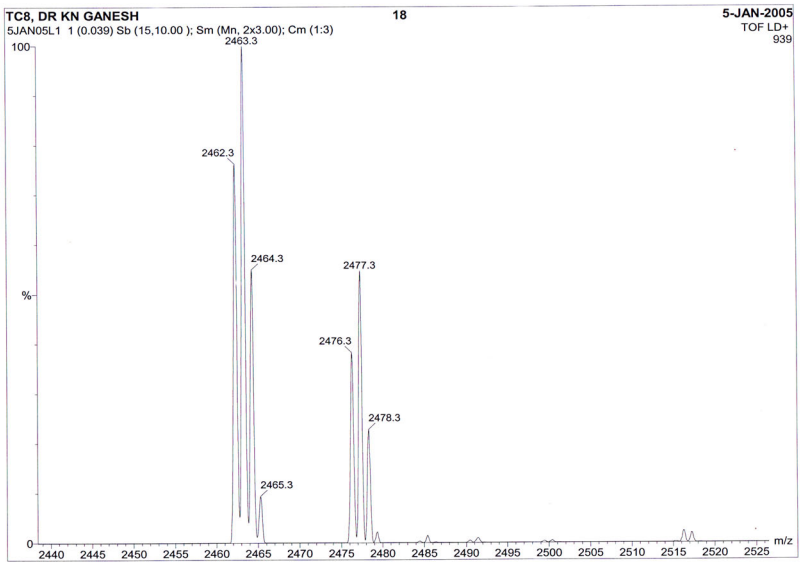






PNA 4


TC8, DR KN GANESH
5JAN05L 8 (0.396) Sb (15,10.00); Sm (Mn, 2x3.00); Cm (1:10)
18
5-JAN-2005
TOF LD+
1.61e3
100
%
0
2463.4
2462.4
2477.4
2478.4
2326.3
2485.3
2000
2100
2200
2300
2400
2500
2600
2700
2800
m/z


TC8, DR KN GANESH
5JAN05L1 1 (0.039) Sb (15,10.00); Sm (Mn, 2x3.00); Cm (1:3)
18
5-JAN-2005
TOF LD+
939
100
%
0
2463.3
2462.3
2464.3
2477.3
2476.3
2478.3
2465.3
2440
2445
2450
2455
2460
2465
2470
2475
2480
2485
2490
2495
2500
2505
2510
2515
2520
2525
m/z

UV-Tm curves

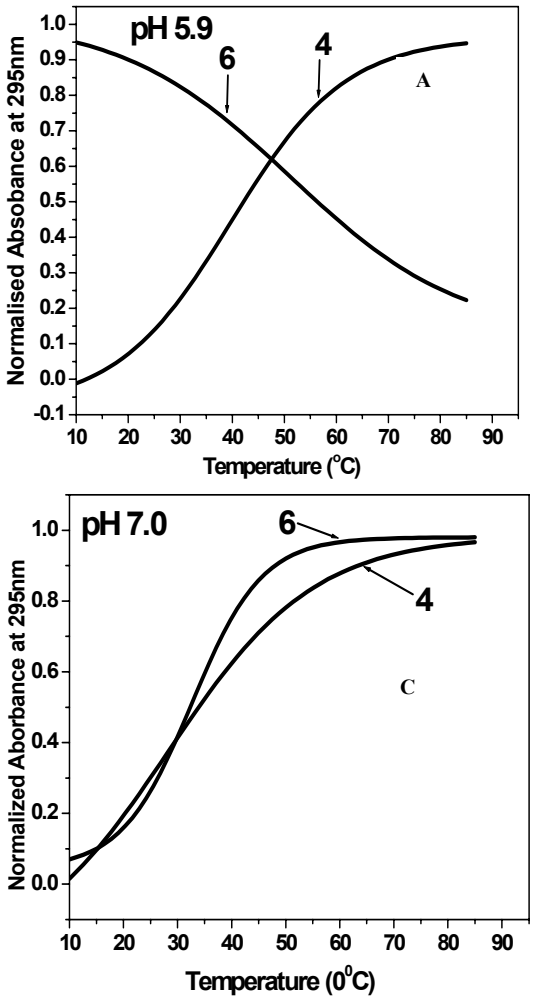


Figure 3. UV-melting profiles of PNA (**4**,)/DNA (**6**) at wavelength 295nm in 100mM sodium acetate buffer in acidic pH. Plots D, E and F arerepresented in pH 5.9, 6..5 and 7.0 respectively.

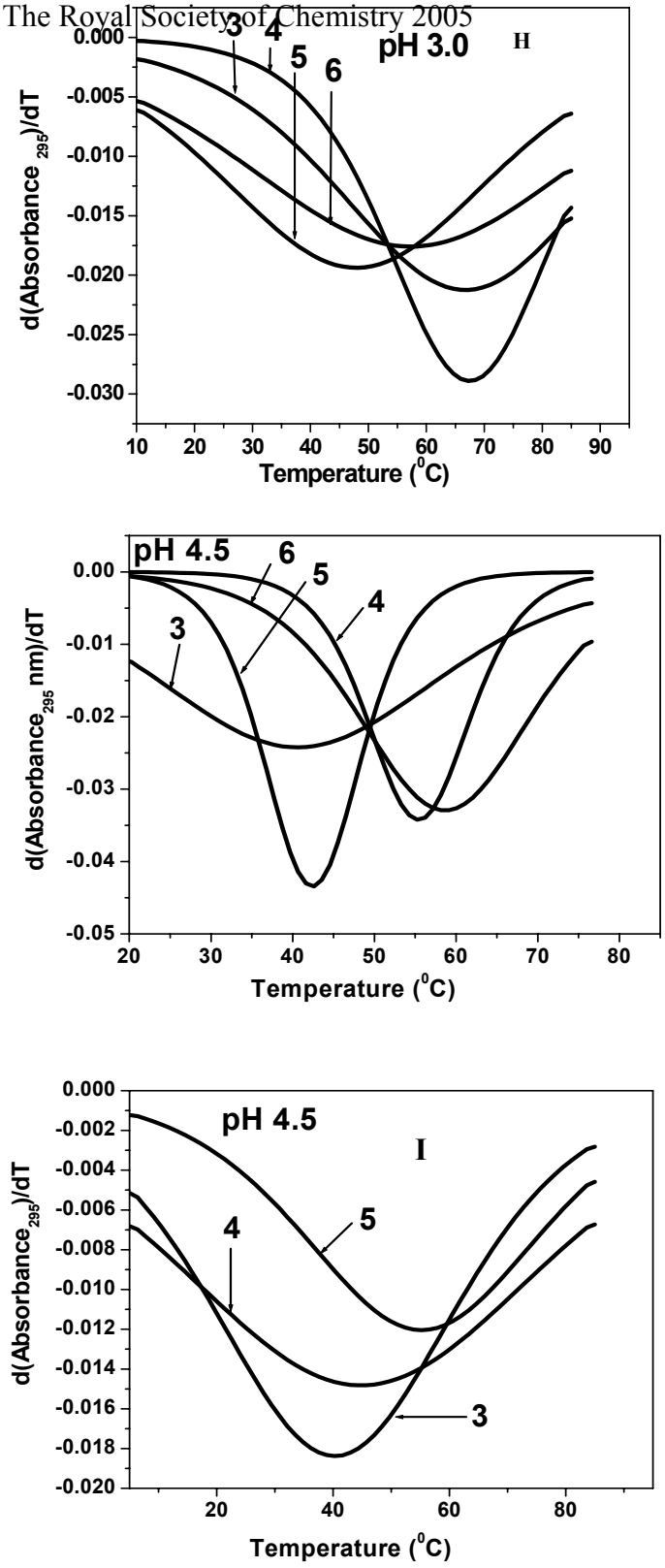


Figure 4. Derivative UV absorbance (295)-temperature profiles, A. PNA/DNA**13** hybrids and B. PNA:poly rA hybrids a. **8** b.**9** c.**10** d.**11**

Table 2. Tm of TC_n in PNA and DNA

PNA/ DNA	Tm at different pH (^{0}C) *	
	3.0	**4.5**
3	67.3	40.0
4	67.4	55.0
5	47.5	43.0
6	58.4	58.7

Table 3. Length dependent Tm of TC_n, in PNA/ DNA

PNA/DNA	Tm at different pH 5.0 (^{0}C) *
1	Not formed
2	Not formed
3	40.5
4	46.0
6	55.7