Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting information

Achiral, acyclic nucleic acids; Synthesis and biophysical studies of a possible prebiotic polymer

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Table of contents-

Template assisted, enzymatic, primer extension assay of DEANA triphosphate:

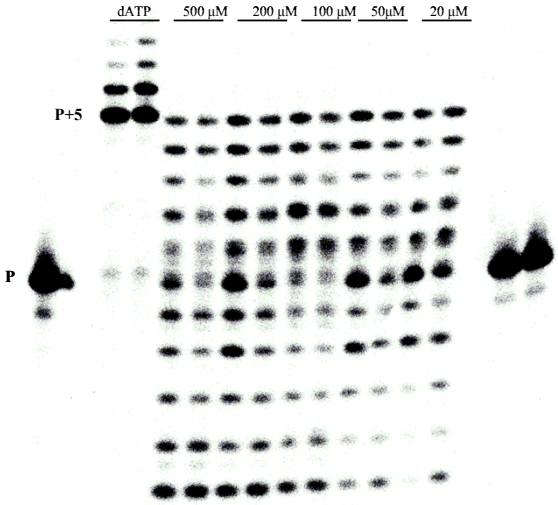

Figure SI-1: Autoradiograms of 20% denaturing PAGE in a primer extension assay of ^{33}P-labeled primer (P) annealed to the template (T) with, 10 µM of dATP and different concentrations of DEANA triphosphate and 0.005U/µl of TherminatorTM DNA polymerase at 75 °C. Aliquots were taken at 60 and 120 minutes respectively.

In the first set of experiment (Figure SI-1) different concentrations (20µM-500µM) of our DEANA triphosphate (Figure 6A) were incubated with ^{33}P labeled primer (P) annealed to the template (T). The results were encouraging as we could see primer extension by 5 residues when incubated for 60 to 120 minutes, though at the same time we also observed degradation of the primer in the autoradiogram.

A control assay was done with 10µM of dATP. For confirming the incorporation of the modified nucleotide, a mass analysis of the extended primer was attempted. However, we were not able to obtain the calculated mass for the extended sequence.

Since pyrophosphate induced DNA degradation is described,[1,2] we suspected the involvement of the inorganic pyrophosphate (PPi) in the degradation of our primer (P) and/or the template (T). Previous reports on Therminator^{TM} DNA polymerase catalyzed primer extension of GNA triphosphates have been done with[3] and without[4] added pyrophosphatases, and such anomaly has not been reported.

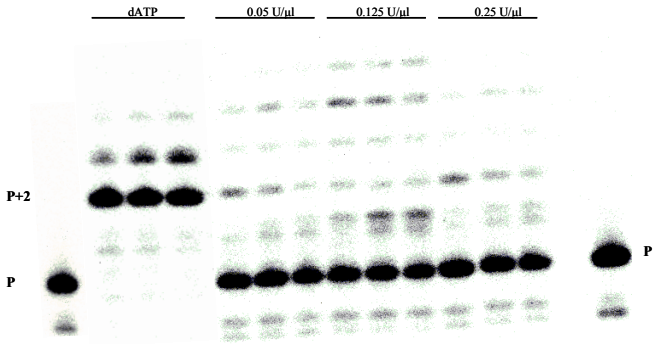

Figure SI-2: Pyrophosphatase assay- Autoradiograms of 20% denaturing PAGE in a primer extension assay of 33P-labeled primer (P) annealed to template (T) with 500µM of DEANA triphosphate; 0.005 U/µl of Therminator^{TM} DNA polymerase and different concentrations of pyrophosphatase (TIPP). Aliquots were taken at 30, 60 and 120 minutes. Control assay was performed using 10µM of dATP and no TIPP was added.

Another set of experiment hence was done in the presence of pyrophosphatase (TIPP).The results (Figure SI-2) showed that extensive degradation of the labelled primer (P) was halted by the use of pyrophosphatase (TIPP). At the same time, the assay also revealed that our acyclic nucleotide analogue is not incorporated into a DNA chain. Again, in the case of dATP no pyrophosphate induced degradation is seen. The pyrophosphate induced degradation of the template (T) was also inhibited by the use of pyrophosphatase (TIPP, data not shown). Though we have not been able to detect the formation of cyclic phosphate in the high resolution mass of our acyclic triphosphate, we suspect that our acyclic analogue is more prone to decomposition via nucleophilic attack of hydroxyl function of the second arm owing to flexibility (liberating pyrophosphate), a phenomenon analogous to the well documented and extensively studied isomerization/transesterification/degradation of RNA via formation of 2', 3' cyclic phosphate[5] or degradation at high temperature conditions of the assay.

General experimental procedures

Oligonucleotide synthesis

Oligonucleotide assembly was performed on an ExpediteTM DNA synthesiser (Applied Biosystems) by using the phosphoramidite approach. The standard DNA assembly protocol was used, except for 10 min coupling time for unnatural amidites using 5-

(Ethylthio)tetrazole as the coupling agent. The oligomers were deprotected and cleaved off from the solid support by treatment with concentrated aqueous ammonia (55 °C, 16 h). After gel filtration on a NAP-10® column (Sephadex G25-DNA grade; Pharmacia) with water as eluent, the crude product was analyzed on a Mono-Q® HR 5/5 anion exchange column, after which purification was achieved on a Mono-Q® HR 10/10 column (Pharmacia) with the following gradient system (A=10 mM TrisHCl pH 7.4, 10 mM $NaClO_4$, 15% CH_3CN; B=10 mM TrisHCl pH 7.4, 600 mM $NaClO_4$, 15% CH_3CN; gradient used dependent on the oligo; flow rate 2 ml/min). The low-pressure liquid chromatography system consisted of a Merck-Hitachi L 6200 A intelligent pump, a Mono Q® -HR 10/10 column (Pharmacia), an Uvicord SII 2138 UV detector (Pharmacia-LKB) and a recorder. The product containing fraction was desalted on a NAP-10® column and lyophilized. Oligonucleotides were characterized and their purity was checked by HPLC/MS on a capillary chromatograph (CapLC, Waters, Milford, MA). Flow rate was 5μL/min.

Mass spectrometry

Exact mass measurement spectra were acquired on a quadrupole orthogonal acceleration time-of-flight mass spectrometer (Synapt G2 HDMS, Waters, Milford, MA). Samples were infused at 3uL/min and spectra were obtained in positive (or: negative) ionization mode with a resolution of 15000 (FWHM) using leucine encephalin as lock mass. Deconvoluted electrospray ionization mass spectrometric analysis was used to obtain mass for the oligonucleotides.

T_m measurement

Oligomers were dissolved in 0.1 M NaCl, 0.02 M potassium phosphate, pH 7.5, 0.1 mM EDTA. The concentration was determined by measuring the absorbance in MilliQ water at 260 nm at 80 °C and assuming the acyclic nucleoside analogues to have the same extinction coefficients in the denatured state as the natural nucleosides. The oligo concentration was 8 μM for palindromic Dickerson Drew sequence (Figure SI-3, SI-4, SI-5, and SI-6) and 4 μM for singly modified TEANA sequence (Figure-SI-6). Melting curves were determined with a Varian Cary 300 BIO spectrophotometer. Cuvettes were

maintained at constant temperature by means of water circulation through the cuvette holder. The temperature of the solution was measured with a thermistor directly Immersed in the cuvette. Temperature control and data acquisition were done automatically with an IBM-compatible computer using Cary WinUV thermal application software. The samples were heated at a rate of 0.2 °C/min starting at 10 °C up to 80 °C and cooling again at the same speed. Melting temperatures were determined by plotting the first derivative of the absorbance versus temperature curve and are the average of two runs. Up and down curves in general showed identical T_m values.

Primer extension assay

DNA oligonucleotides were purchased from Eurogentec. The concentrations were determined with a Cary-300-Bio UV Spectrophotometer (Varian). The lyophilized oligonucleotides were dissolved in diethylpyrocarbonate (DEPC)-treated water and stored at −20 °C. The primer (P, see Figure 6B in the text) oligonucleotides were 5′-labelled with [γ-33P] ATP (PerkinElmer) by using T4 polynucleotide kinase (New England Biolabs) according to standard procedures. Labeled oligonucleotides were further purified on Illustra Microspin G-25 columns (GE Healthcare). DNA polymerase reactions: End-labeled primer was annealed to its template by combining primer and template (molar ratio 1:2) and heating the mixture (70°C, 10 min), followed by slow cooling to room temperature over a period of 2 h. The Therminator™ DNA polymerase catalyzed primer extension assay and pyrophosphatase assay were done as described in the text (Figures SI-1 and SI-2). The polymerase reactions were quenched by the addition of loading buffer (10 μL, formamide (90%), bromophenol blue (0.05 %), xylene cyanol (0.05 %) and ethylenediaminetetraacetic acid (EDTA, 50 mM). Samples were heated (75°C, 5 min) prior to analysis by electrophoresis (2.5 h, 2000 V) on a 20% PAGE in the presence of a Tris·borate (100 mM, pH 8.3) and EDTA (2.5 mM). Products were visualised by phosphor imaging.

T_m graphs

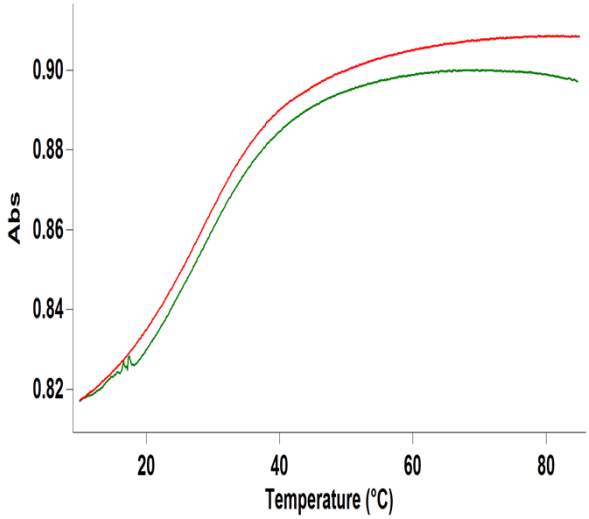

Figure SI-3: Melting profile of ON-3 (d(CG**cgaattcg**CG)$_2$ at 260nm; Heating curve (red), cooling curve (green); Change in absorbance measured at 260 nm in NaCl (0.1M) buffer with KH_2PO_4 (20 mM, pH 7.5) and EDTA (0.1 mM) at the concentration of 8 μM for the self-complementary Dickerson Drew dodecamer.

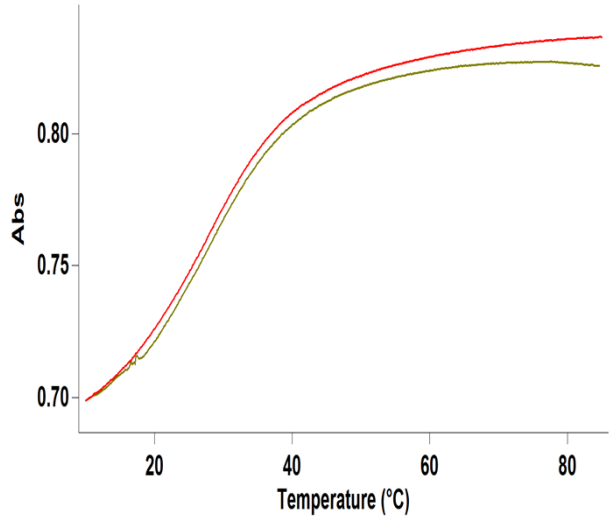

Figure SI-4: Melting profile of ON-3 (d(CG**cgaattcg**CG)$_2$ at 270nm; Heating curve (red), cooling curve (green); Change in absorbance measured at 260 nm in NaCl (0.1M) buffer with KH_2PO_4 (20 mM, pH 7.5) and EDTA (0.1 mM) at the concentration of 8 μM for the self-complementary Dickerson Drew dodecamer

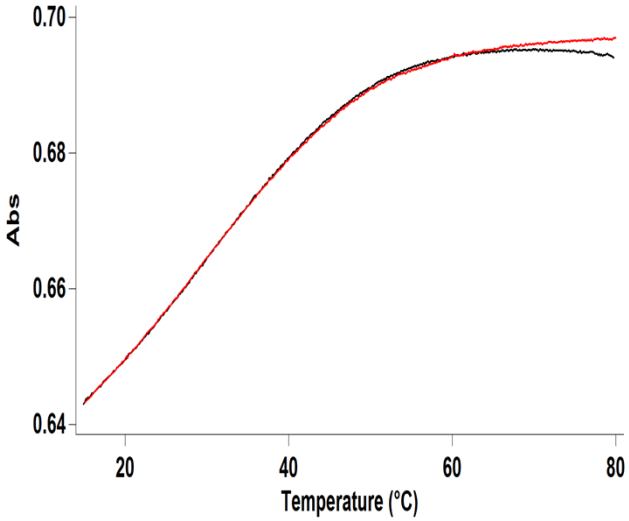

Figure SI-5: Melting profile of ON-4 $[CH_3O(CH_2CH_2O)_2$**gcgcgcgcg**$C]_2$ at 260nm; Heating curve (red), cooling curve (green); Change in absorbance measured at 260 nm in NaCl (0.1M) buffer with KH_2PO_4 (20 mM, pH 7.5) and EDTA (0.1 mM) at the concentration of 8 µM of self complementary ON-4

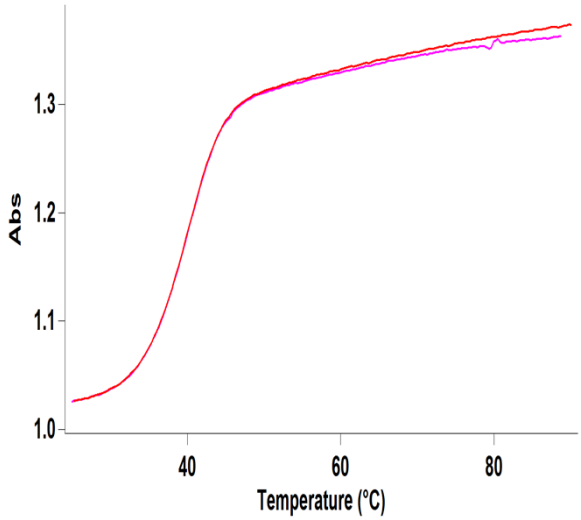

Figure SI-6: Melting profile of 5'-d(CTTC**a**TTTTTTCTTC)-3':3'-d(GAAGTAAAAAAGAAG)-5'at 260nm Containing single TEANA modification (**a**); Heating curve (red), cooling curve (pink); Change in absorbance measured at 260 nm in NaCl (0.1M) buffer with KH_2PO4 (20 mM, pH 7.5) and EDTA (0.1 mM) at the concentration of 4 µM for each strand.

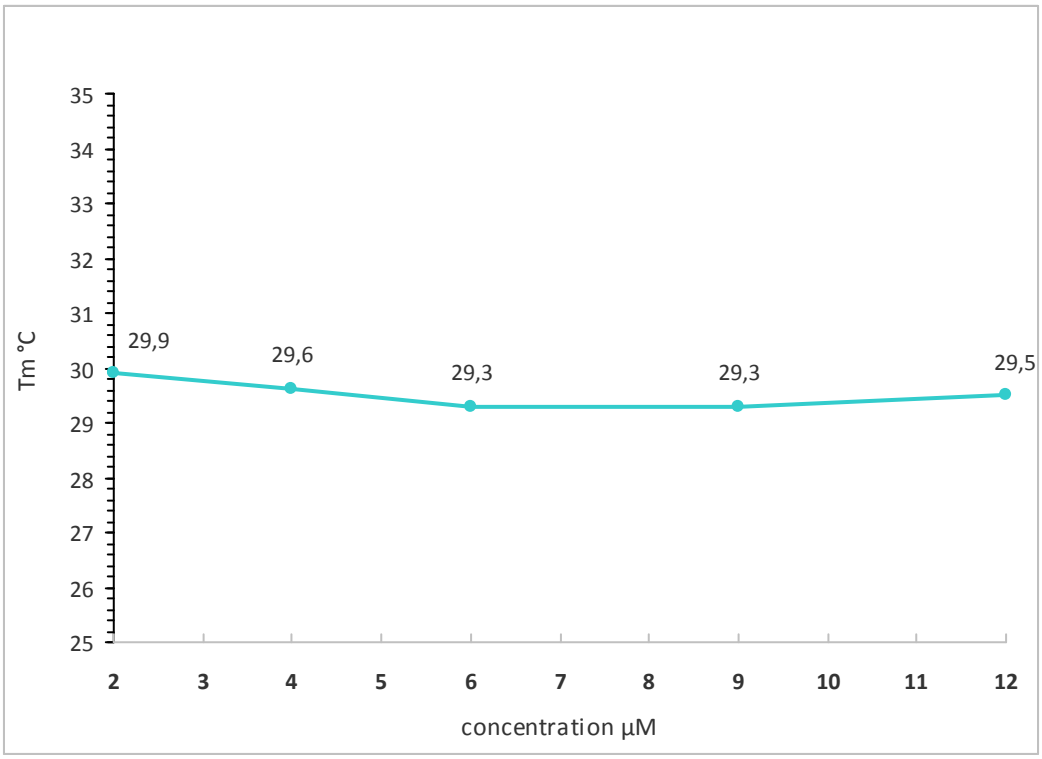

Figure SI-7: Concentration dependent T_m profile of ON-4 measured at 260 nm in NaCl (0.1M) buffer with KH_2PO4 (20 mM, pH 7.5) and EDTA (0.1 mM) at the concentration of 2,4,6,8,12 µM.

DEANA 10-mer hairpin model

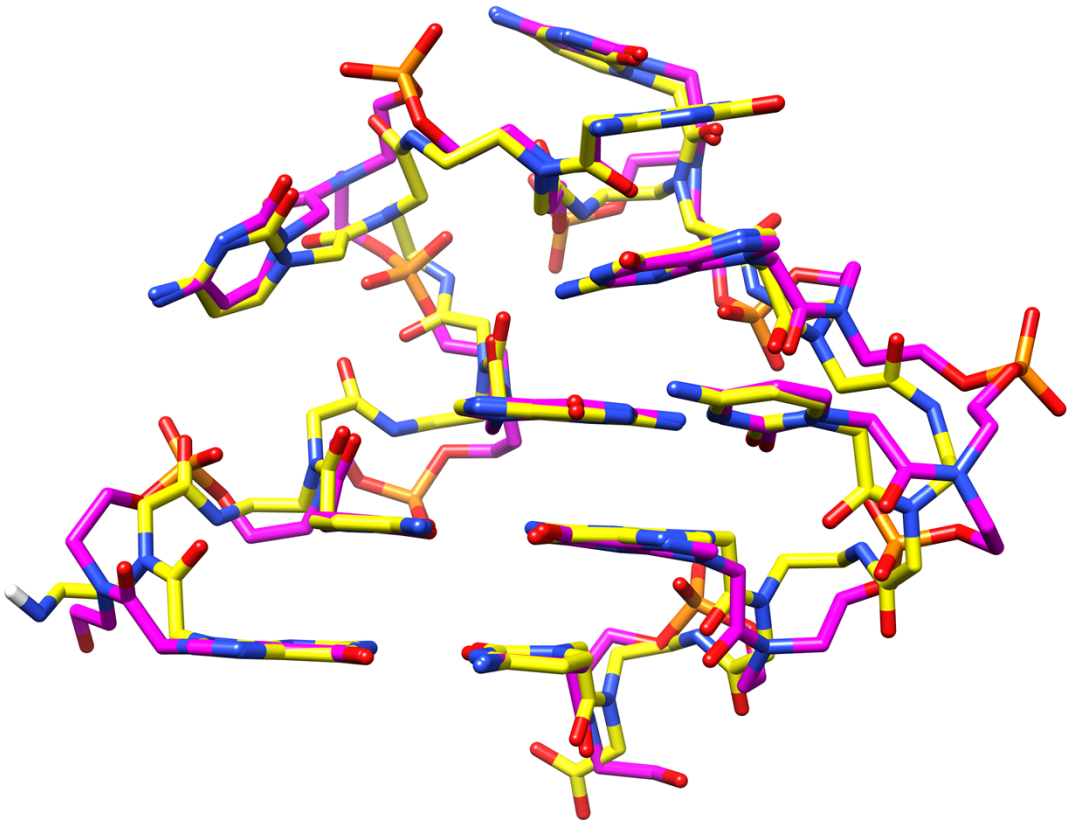

Figure SI-8: Superposition of the static DEANA 10-mer hairpin model (gcgcgcgcgc) onto the PNA hairpin, constructed using a PNA double helix from the 3MBS pdb entry.[6] Pink carbons are of DEANA residues and yellow carbons from PNA residues.

References:

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(6) Yeh, J. I.; Pohl, E.; Truan, D.; He, W.; Sheldrick, G. M.; Du, S.; Achim, C. *Chem. - Eur. J.* **2010**, *16*, 11867-11875.