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COMMUNICATION

The Remarkable UV Light Invulnerability of Thymine GNA Dinucleotides

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We herein demonstrate the UV resistance of glycol nucleic acid (GNA) dinucleotides. This resistance sustains the hypothesis of GNA as nucleic acid prebiotic ancestor on early Earth, a time of intense solar UV light. Such photorobustness, due to absence of intrastrand base stacking, could offer opportunity for nanodevices development requiring challenging UV conditions.

Glycol nucleic acid (GNA) is a nucleic acid analogue that exhibits the structurally simplest and minimal phosphate backbone capable of high duplex stability.^{1,2} The GNA backbone is made of a succession of α -propylene glycol phosphodiester units affording (*S*) or (*R*)-GNA enantiomers (Fig. 1). The facile chemical access to these polymers combined to their remarkable hybridization properties offer unprecedented perspectives in structural and functional nucleic acid-based nanotechnology.³ In the medicine field, GNA has also been used to probe the folding topology of G quadruplexes and to improve the therapeutic index of siRNA.^{4,5}

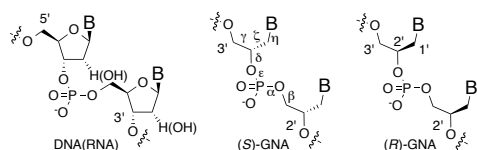


Fig. 1 Structures of DNA, RNA and (*S*)- and (*R*)-enantiomers of GNA.

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Finally, the accommodation of some polymerases with the glycol motif makes this artificial polymer highly attractive for the evolution of functional GNA by *in vitro* selection.⁶ The structural resemblance of GNA and nucleic acid, and the possible genetic information transfer between these two species have led to propose GNA as a predecessor of contemporary nucleic acids.^{1,6a,7} In early earth when life formed, surface UV radiation level was much higher than at present.⁸ Nevertheless, UV light is a well known nucleic acid damaging agent due to photon absorption by nucleobases followed by cytotoxic and mutagenic photoproduct (PP) formation.⁹ Consequently, nucleic acids had to face these harsh UV conditions to avoid deleterious consequences for the cell. Meanwhile, the UV vulnerability of nucleic acids is considered to have played a key role in shaping the adaptation and evolution of life through PP induced mutations.¹⁰ Therefore, suggestion of GNA as a nucleic acid ancestor rises the crucial question of its UV photoreactivity.

To address this question, we studied the photochemical behaviour of GNA dinucleotides when exposed to far UV light. Considering that 1) UV-induced DNA damage arises mostly between adjacent pyrimidine residues, dithymine sites being the most reactive ones, and results in three types of dipyrimidine PPs: cyclobutane pyrimidine dimers (CPDs), (6-4) and Dewar¹¹ 2) UV-induced pyrimidine hydrate formation in DNA is a minor process with respect to dipyrimidine PPs formation,¹² and that 3) thymidyl-(3',5')-thymidine (TpT, **1**) is sufficient to mimic the photoreactivity of the same sequence in DNA,¹³ we synthesized GNA analogues of **1** and studied their photochemical behaviour, anticipating **1** to be a good model compound to study the photochemistry of GNA. Due to the easiness of the synthetic protocol, we synthesized all four diastereomers, the two homochiral enantiomers (*S,S*)-GNA TT **2** and (*R,R*)-GNA TT **3**, as well as the heterochiral enantiomers (*S,R*)-GNA TT **4** and (*R,S*)-GNA TT **5** (Fig. 2) in view of future experiments. Therefore, this study gave us the opportunity to synthesize for the first time heterochiral GNAs then to

evaluate the photochemical behavior of homo- and heterochiral GNAs.

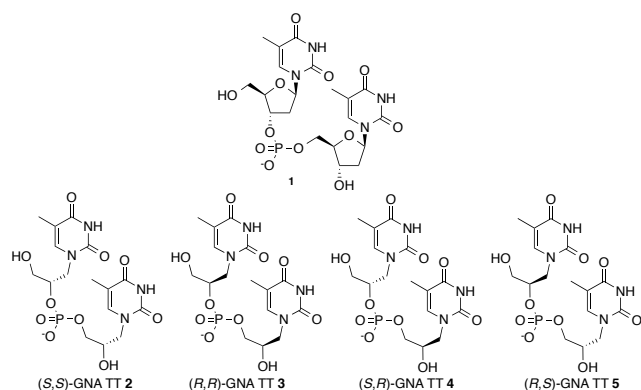
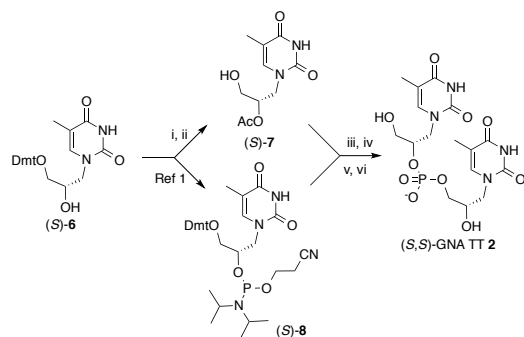


Fig. 2 Structure of TpT **1** and of its four GNA surrogates **2-5**.

The synthesis of **2-5** made use of the known tritylated thymine glycol nucleosides (*R*)-**6** and (*S*)-**6**¹ (Scheme 1). For example, to prepare **2**, a one step acetylation detritylation procedure of (*S*)-**6** afforded (*S*)-**7** in 83% yield. Phosphoramidite (*S*)-**8**,¹ synthesized from (*S*)-**6** was condensed to primary alcohol (*S*)-**7** in acetonitrile in the presence of imidazolium triflate and the resulting phosphite triester was oxidized with *t*-butylhydroperoxide. Concentrated ammonia followed by aqueous acetic acid treatment of the phosphate triester afforded **2** that was purified by RP HPLC and isolated in 2% yield (four steps).



Scheme 1. Synthesis of GNA TTs. Shown is the route leading to (*S,S*)-GNA TT **2**. Reaction conditions: (i) Ac₂O, pyridine; (ii) TFA, CH₂Cl₂; (iii) Imidazolium triflate, CH₃CN; (iv) *t*-BuOOH in nonane; (v) conc. NH₄OH; (vi) 80% aqueous AcOH.

Aqueous solutions (2 mM) of (*S,S*)-GNA TT **2**, (*R,S*)-GNA TT **5** and TpT **1** as a standard were individually exposed to 254 nm radiation. UV monitoring of irradiation reactions of **2** and **5** revealed no significant change in UV absorbance around 270 nm and 330 nm after 3 h indicating that the thymine chromophore remained intact. In contrast, a loss of ~ 50% in 270 nm absorbance of **1** attested that approximately half of the thymine residues had reacted (Fig. S25, ESI[†]), in addition, appearance of an absorption near 330 nm attested of the formation of the (6-4) PP¹⁴ (Fig. S25, ESI[†]). Photoresistance of

2 and **5** was confirmed by ¹H NMR analysis of the crude photoreaction mixture (Fig. S22-S23, ESI[†]) that remained unchanged. No shielded methyl signals near 1.5 ppm corresponding to the saturation of the C5-C6 double bonds of CPD were observed¹⁵ as observed for TpT **1** (Fig. S24, ESI[†]). Similarly, no H6 and methyl signals characteristic of the (6-4) PP structure¹⁴ appeared.

Photodimerization efficiency of two thymine moieties linked by a sugar phosphate backbone highly depends on their stacking ability.¹⁶ In addition, only favourable base-stacked geometries will induce photoproducts formation.¹⁷ Therefore, we investigated the stacking properties of homochiral (*S,S*)-**2** and of heterochiral (*R,S*)-**5** by circular dichroism (CD) to establish its possible involvement in the photostability of GNA dinucleotides **2** and **5** (Figure 3). The CD spectra of enantiomeric (*S*)- and (*R*)-thymine GNA **9** as well as those of **3** and **4** were recorded for comparison.

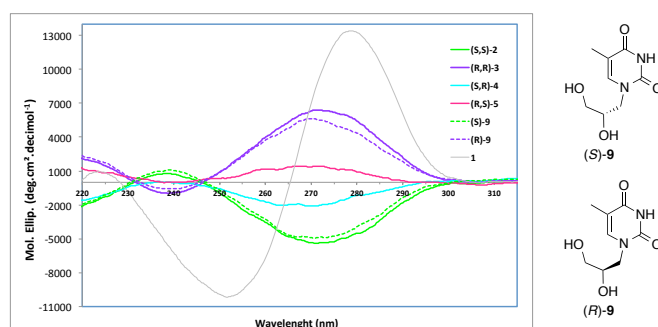


Fig. 3 CD spectra of **2-5** and **9** at 10°C. The CD spectrum of TpT (**1**) at 10°C is shown for reference.

The negative long-wavelength CD band of homodimer (*S,S*)-**2** is consistent with an (*S*)-GNA oligonucleotide structure.^{1,2b,6a} The limited CD amplitude difference between **2** and its constituent monomer (*S*)-**9** indicated that the CD signal of **2** is roughly the exclusive result of the chiral centers of its glycol phosphate backbone. (*R,S*) Dimer **5** displayed a small positive CD signal around 270 nm (Fig. 3) whose significance was nevertheless attested by the mirror image CD spectrum of the (*S,R*) enantiomer **4**. In the absence of base interaction, no CD signals should be expected for heterochiral diGNAs. Therefore, the CD signals of **4** and **5**, even low, attested of a weak but detectable interbase interaction. The shifts of the maximal CD bands of **2** and **5** with respect to **1** ($\lambda \sim 280$ nm for **1** versus $\lambda \sim 272$ nm for **2** or ~ 268 for **5**) indicated that the geometry pattern of this interaction in **2** and **5** is different from that of TpT (**1**) (Fig. 3). Hence, the small CD signal of **2** and **5** could be due to either a very low population of CD responsive stacked species or a population of stacked species devoid of intense CD signal,¹⁸ two hypotheses that, at this stage were impossible to dissociate. Consequently, we performed the conformational analysis of **2** and **5** in solution by NMR (see SI for details).

In the solid state and in simulated solution, the major conformational characteristic of homo GNA duplexes is an almost complete absence of intrastrand base-base overlapping which is the predominant mode of base interaction in DNA.^{19,20}

In solution, homo single strand (ss) GNA is preorganized in a conformation similar to that of the individual strands of homo GNA duplexes.^{2b} Therefore, conservation of the large sliding between adjacent bases in ssGNA can be hypothesized. This unusual interbase stacking mode, if present in homo diGNA **2**, could explain its UV hardness in solution. Single GNA nucleosides in solution^{2b} and some GNA nucleoside residues within GNA duplexes in the solid state^{19,20} and in simulated solution²⁰ adopt the same conformation (η , ζ and γ (Fig. 1) in the g- domain). Our study indicated that, in solution, both GNA nucleoside units of **2** also prefer this conformation. This result experimentally confirms the previously simulated gauche conformational preference of γ in a duplex GNA strand in solution.²⁰ Regarding the phosphodiester torsion angles δ , ϵ and β (Figure 1) of **2**, both of them adopted the same orientation as that observed in solid state GNA duplexes.^{19,20} Torsion angle α was shown to be in a gauche orientation instead of the anti geometry found in solid state GNA duplexes.^{19,20} Such favoured g-/g- geometry of ϵ/α is consistent with the calculated global energy minimum of diethylphosphate in solution.²¹ Independently on a possible uncertainty on the geometry of α , it can be concluded that the two thymine residues of **2** are not suitably positioned to photoreact. Concerning the conformation adopted by the hetero diGNA **5**, all torsion angles were determined to be similar to those of **2** considering the antipodal chirality of the 3'-end residue. In **5**, the two thymine residues were also not properly arranged for UV reaction. This conformational study indicates that the predominant conformation adopted by **2** and **5** corresponds to a state in which the thymine residues are not stacked and the trace CD-detected stacked population corresponds to a very minority stacked conformation that is not suitable to be photoreactive. The lack of thymine stacking in **2** and **5** is fully consistent with their UV robustness.

This study also indicates that the strong preorganization reported for the ssGNA backbone^{2b} already occurs at the GNA TT level. Associated to the conformational analogy between ssGNA and duplex GNA strands,^{2b,19,20} and to the conformational dependence of PP formation,^{16e,17,22} this likely suggests that the photochemical behaviour of GNA TT mimics the one of the TT sequence in ssGNA and in duplex GNA. In addition, our results obtained using the most photoreactive sequence indicates high photostability of short ssGNA. However, more tests should be performed to definitively rule out the possibility of formation of C or U photohydrates and PPs in GNA, and to confirm that ssGNA with mixed sequences are indeed photostable.

In conclusion, the replacement of the five-carbon cyclic sugar of nucleic acids by the acyclic chiral three-carbon glycerol moiety in GNA which preserves Watson-Crick base pairing and helicity property is expected to preclude intra-strand photoproduct formation which is an hallmark of modern nucleic acids. We have demonstrated that the photoresistance of GNA TT in aqueous solution is due to the low overlap interaction between adjacent thymine residues. Consequently, GNA may have played a role in the prebiotic emergence of nucleic acids through assembling nucleobases

while preserving their remarkably high UV photostability²³ leading to a more robust genetic information storage than do current nucleic acids. However, they may not have participated in the evolution of life due to their impossibility to elicit mutagenic photodamage. Interestingly, the UV stability of GNAs highly contrasts with the UV vulnerability of PNA TT which also possess an acyclic backbone.²⁴ In this later case, the aminoethylglycine backbone is compatible with intra-strand base stacking overlaps similar to those present in DNA.²⁵ To our knowledge, GNA would constitute the first UV resistant polymer among promising xeno nucleic acids reported so far. Such distinctive property is highly appealing for challenging nucleic acid application conditions that requires UV light and structural integrity.²⁶ The ability of GNAs to form nanoshape structures^{3a} combined to its unique UV stability represent a formidable opportunity for the potential development of photometalized nano structures and phototriggered nanodevices under non-destructive UV conditions.^{24,25}

Conflicts of interest

There are no conflicts to declare.

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