Communication

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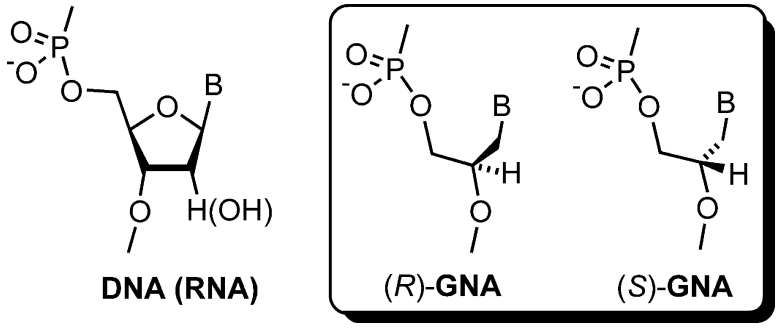

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A Simple Glycol Nucleic Acid

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We here wish to disclose a novel nucleic acid analogue which displays exceptional structural simplicity and atom economy while supporting stable duplex formation. The discovered glycol nucleic acid (GNA) uses the canonical Watson−Crick base pairing scheme combined with an acyclic three-carbon propylene glycol phosphodiester backbone (Figure 1).

Groundbreaking studies of the Eschenmoser group on the chemical etiology of nucleic acid structure have demonstrated that Watson−Crick base pairing can be supported by sugars in the backbone that differ from RNA or DNA.[1−3] Most surprisingly, a nucleic acid derived from a tetrose sugar (TNA) was found to be capable of antiparallel duplex formation and crosspairing with DNA and RNA (Figure 1).[4]

Our laboratory is interested in structurally simplified nucleic acid backbones in order to improve synthetic accessibility of artificial duplexes. Inspired by Eschenmoser's TNA structure, we envisioned that acyclic glycol nucleosides could be synthesized just by regioselective and stereospecific nucleophilic ring opening of a "spring-loaded" epoxide.[5,6]

Accordingly, commercially available (*R*)-(+)- and (*S*)-(−)-glycidol were tritylated to **1** following a standard protocol and successfully ring opened with the unprotected nucleobases thymine and adenine in the presence of substoichiometric amounts of NaH, yielding **2** and **3**, respectively (Figure 2).[7] The thymine derivative was directly transformed into the final phosphoramidite **4**, whereas the adenine derivative was first benzoylated at the exocyclic amino group, followed by the formation of the final phosphoramidite **5** for the automated solid-phase oligonucleotide synthesis. With **4** and **5** in hand, we synthesized seven 18mer oligonucleotides consisting entirely out of either enantiomer of the acyclic glycol nucleotides (Table 1). (*R*)-(+)-glycidol yields (*S*)-GNA, and (*S*)-(−)-glycidol yields (*R*)-GNA (Figure 1).

First, we investigated duplex formation of GNA with temperature-dependent UV spectroscopy at 260 nm. Figure 3 displays the results with (*S*)-GNA. Mixtures (1:1) of two complementary strands, **6**:**7** and **8**:**9**, yield characteristic sigmoidal melting curves with melting points (T_M) of 63 °C each, thus indicating cooperative melting of GNA duplexes. No sigmoidal melting and weaker hyperchromicities were observed with the single strands alone (Figure 3a,b) or with two strands **8**:**10** that are complementary in a parallel fashion (Figure 3c), indicating the requirement for antiparallel duplex formation of GNA. To reassure that the duplex relies on proper Watson−Crick base pairing, we also investigated the UV melting behavior of an 18mer duplex **8**:**11** which contains one T:T mismatch. The stability of this duplex decreases significantly, yielding a T_M of only 55 °C for the single mismatched duplex. Furthermore, a duplex **8**:**12** with two mismatches (one T:T and one A:A) is further destabilized with a T_M of only 44 °C (Figure 3c). Identical results were obtained with the enantiomeric (*R*)-GNA strands.

For subsequent analysis, we investigated duplex formation of GNA with circular dichroism (CD). The CD spectra of both

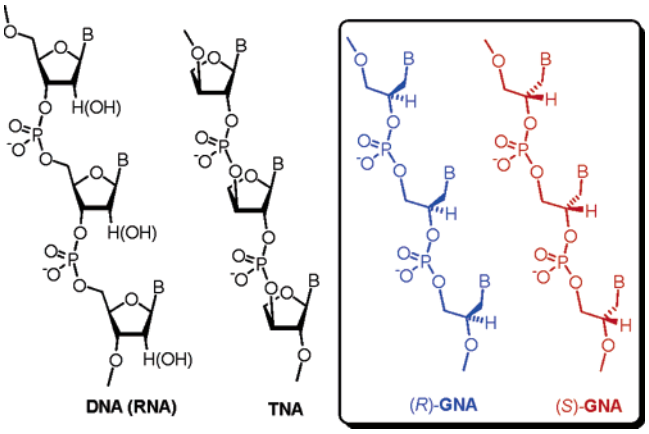

Figure 1. Comparison of the constitutions of DNA, RNA, and Eschenmoser's threofuranosyl nucleic acid (TNA) with our glycol nucleic acid (GNA).

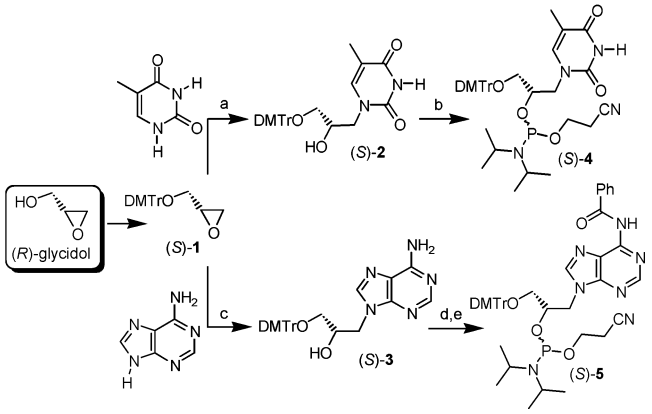

Figure 2. Synthesis of glycol nucleotides for the automated solid-phase oligonucleotide synthesis. Shown is the route from (*R*)-(+)-glycidol leading to (*S*)-GNA: (a) 0.2 equiv of NaH, DMF (49%). (b) (iPr$_2$N)(NCCH$_2$CH$_2$O)-PCl, 3 equiv of (iPr)$_2$NEt (77%). (c) 0.2 equiv of NaH, DMF (51%). (d) TMSCl, PhCOCl, then concentrated NH_3 in H_2O. (e) (iPr$_2$N)(NCCH$_2$CH$_2$O)-PCl, 3 equiv of (iPr)$_2$NEt (52% over both steps).

enantiomers of GNA duplexes **8**:**9** and the individual single strands are shown in Figure 4a and demonstrate strongly increased CD bands at around 205, 220, and 275 nm upon mixing of **8** and **9** in a 1:1 fashion, clearly supporting the formation of a helical duplex. This CD signal is strongest at a 1:1 ratio of **8**:**9**, as shown in the insert of Figure 4a. In addition, the temperature-dependent CD spectra in Figure 4b show that the Cotton effect decreases with increasing temperature, again in agreement with the temperature-dependent melting of a helical GNA duplex. As expected, the enantiomeric (*S*)-GNA and (*R*)-GNA duplexes **8**:**9** yield mirror-imaged CD spectra. Both spectra are very distinguished from a DNA duplex of the same sequence (see Supporting Information).

The thermal stability of the GNA duplex **8**:**9** exceeds the stability of the analogous duplex of DNA (T_M = 40.5 °C) and RNA (T_M =

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Table 1. Synthesized Sequences of 18mer GNA Oligonucleotides

3′-TTTTAAATTTTAATATAT-2′ 2′-AAAATTTAAAATTATATA-3′	**6** **7**	antiparallel duplex (T_M = 63 °C)
3′-TAAAATTTATATTATTAA-2′ 2′-ATTTTAAATATAATAATT-3′	**8** **9**	antiparallel duplex (T_M = 63 °C)
3′-TAAAATTTATATTATTAA-2′ **3′**-ATTTTAAATATAATAATT-2′	**8** **10**	parallel duplex (no T_M)
3′-TAAAA**T**TTTA**T**ATTATTAA-2′ 2′-ATTTTAAAT**T**TAATAATT-3′	**8** **11**	one mismatch (T_M = 55 °C)
3′-TAAA**A**TTTA**T**ATTATTAA-2′ 2′-ATTT**A**AAAT**T**TAATAATT-3′	**8** **12**	two mismatches (T_M = 44 °C)

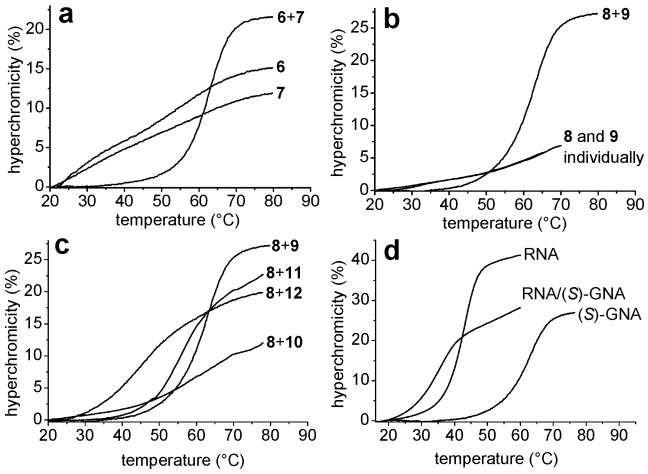

Figure 3. UV melting curves monitored at 260 nm. (a) (*S*)-GNA strands **6** + **7** (2 μM each) and the individual strands **6** (2 μM) and **7** (2 μM). (b) (*S*)-GNA strands **8** + **9** (2 μM each) and the individual strands **8** and **9** (2 μM each). (c) (*S*)-GNA strands (2 μM each) **8** + **9** (leading to an antiparallel duplex), **8** + **10** (parallel duplex), **8** + **11** (antiparallel duplex with one mismatch), and **8** + **12** (antiparallel duplex with two mismatches). (d) Duplexes with the sequence **8** + **9** (2 μM each strand) of RNA (U instead of T), (*S*)-GNA, and RNA/(*S*)-GNA hybrid. Experiments were performed in 10 mM sodium phosphate pH 7.0 with 200 mM NaCl, and 1 mM of EDTA in experiments which include RNA strands. Data for UV melting experiments were collected at 0.2 or 0.5 °C increments with a temperature ramp of 0.5 °C/min.

42.5 °C, U instead of T) by 22.5 and 20.5 °C, respectively. This is astonishing considering the fact that the GNA backbone is completely acyclic. It has been widely assumed that nucleic acid analogues containing a phosphodiester backbone need to be cyclic in order to produce the required conformational preorganization for duplex formation.[8,9] However, the GNA strands **8** and **9** show a significant Cotton effect, suggesting a helical preorganization of the GNA backbone already in the single strand. This conclusion is also supported by the observation that a mixture of glycol nucleosides T and A almost does not give any CD signals, demonstrating that the Cotton effect is not simply the result of the chiral centers in the glycol backbone (Figure 4a).

Finally, we investigated antiparallel crosspairing between (*S*)-GNA, (*R*)-GNA, DNA, and RNA. We observed that (*S*)-GNA and (*R*)-GNA do not undergo antiparallel crosspairing with each other, nor do they form stable antiparallel crosspairs with DNA. On the other hand, only (*S*)-GNA crosspairs with RNA (T_M = 35 °C for RNA **8** with (*S*)-GNA **9**; see Figure 3d). In this respect, it is noteworthy that (*S*)-GNA is directly derived from TNA by eliminating a CH_2O unit from the tetrahydrofuran ring.

In summary, we have described a structurally simple and synthetically easily accessible acyclic glycol nucleic acid which

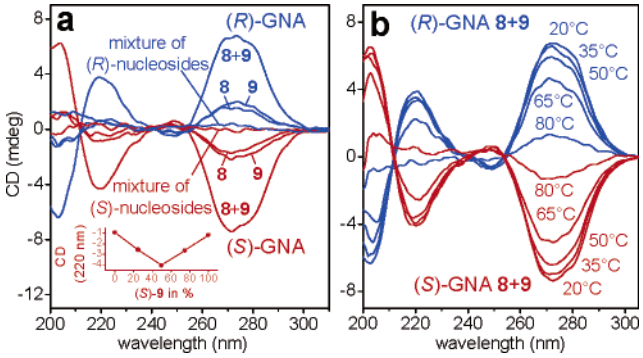

Figure 4. (a) CD spectra of a 1:1 mixture of GNA strands **8** + **9** (4 μM each), the individual strands **8** and **9** (4 μM each), and a mixture of glycol nucleosides T and A (36 μM each) at 25 °C. Insert: CD signal at 220 nm for different ratios of **8** to **9** in which the concentration of single strands **8** and **9** together is 8 μM for each data point. (b) Temperature-dependent CD spectra of a 1:1 mixture (4 μM each) of GNA strands **8** + **9**. Experiments were performed in 10 mM sodium phosphate pH 7.0 with 200 mM NaCl. The color coding is related to Figure 1.

forms highly stable antiparallel helical duplex structures following the Watson−Crick base pairing rules. We believe that these properties will render GNA a very valuable tool in contemporary nucleic acid chemistry. At last, we wish to note that GNA may be the most atom economical solution for a functional nucleic acid backbone. We are therefore tempted to propose that GNA was a potential predecessor of RNA as a genetic material and catalyst for Earth's earliest organisms.[10]

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Supporting Information Available: Experimental procedures for the synthesis of (*S*)-**4**, (*R*)-**4**, (*S*)-**5**, (*R*)-**5**, and their incorporation into oligonucleotides, UV melting curves and CD spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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