Glycerol nucleic acid (GNA), which is based upon an acyclic, three-carbon backbone (Figure 1A), is a promising starting point for the construction of an artificial genetic system owing to its ability to form stable, antiparallel duplexes.\textsuperscript{1,2} As proposed by Orgel,\textsuperscript{3} we imagine that a nonenzymatic, template-directed polymerization process mediated replication during the evolution of early genetic systems,\textsuperscript{4,5} in place of enzymatic replication as in the modern genetic system. To recapitulate this process, our group has focused on the chemistry of polymerization facilitated by an imidazole-activated phosphate group and an amino group as the nucleophile (Figure 1B and 1C, highlighted in red).\textsuperscript{5} This approach requires the newly synthesized strand with N\textsuperscript{2} to form stable, Watson–Crick base pairs with itself and with GNA. We also show that GNA oligonucleotides containing N\textsuperscript{2}–P\textsuperscript{3} phosphoramidate linkages can be assembled via a nonenzymatic, template-directed ligation of 3'-imidazole-activated-2'-amino GNA dinucleotides (Figure 1C). These results suggest that npGNA is a potential candidate for a self-replicating system based on phosphoramidate linkages.

Figure 1. GNA-based genetic system. (A) Structures of GNA and npGNA. (B and C) Chemistry of templated synthesis of npGNA using imidazole-activated monomer (B) or dimer (C). The nucleophile and leaving group involved in each transformation are highlighted in red.

npGNA oligomers were synthesized via a solid-phase, oxidative amination coupling method\textsuperscript{7,8} using 2'-amino glycerol nucleoside monomers as the monomers (1t–1g, Scheme 1). Compared with the lengthy synthesis (7–9 steps) of phosphoramidites used in the previously reported amine-exchange method,\textsuperscript{9} 1t–1g can be prepared in 2–3 steps from the readily available 3'-dimethoxytrityl (DMT) protected glycerol nucleosides 2t–2g (Scheme 1).\textsuperscript{7,9} Each synthetic cycle of monomer addition consists of the following steps: detritylation, phosphorylation of the 3'-hydroxyl, hydrolysis, and oxidation followed by coupling as previously described by Gryaznov et al. (Scheme 2) (see Supporting Information for detailed procedures).\textsuperscript{7,8} Controlled-pore glass (CPG) resins premodified with thymidine were used in the synthesis (Scheme 2), which led to an extra thymidine at the 2'-terminus in all npGNA sequences synthesized in this study (Table S1).

This method was validated by the synthesis of dinucleotide model compounds (5t–5g, Figure S1). Only one diastereomer was observed for all 4 dinucleotides in \textsuperscript{1}H and \textsuperscript{31}P NMR spectra, indicating a clean S\textsubscript{N}2 attack by azide leading to 4t–4g. In addition, the dinucleotides had \textsuperscript{31}P chemical shifts of ~7 to 8 ppm (Supporting Information, Figure S1) and underwent acid-catalyzed decomposition, both characteristic of a phosphoramidate linkage. The coupling efficiency of each cycle is ~40% for 1a and 70–80% for 1t, 1c, and 1g. The decreased coupling efficiency compared with phosphoramidate DNA synthesis is thought to be related to the decreased nucleophilicity of the 2'-NH\textsubscript{2} due to the presence of...
sequences (Table 1). The duplex formation was also observed in several other npGNA of each strand was established by a Job plot (Figure S3). Stable npGNA formed a stable duplex (Figure 2). The 1:1 stoichiometry npGNA oligomers. UV and CD experiments were performed on gel- or HPLC-purified and characterized by MALDI-TOF MS (Table S1). All subsequent mide gel electrophoresis or by anion-exchange HPLC (Figure S2) npGNA products were further purified by denaturing polyacryla-
were quantitatively recovered after each coupling step. Crude sufficient for base-pairing studies. The unreacted monomers (Figure 2).

Table 1. Summary of Thermal Denaturation Studies

| sequence              | top:bottom strand | Tm (°C) |
|-----------------------|-------------------|---------|
| 3′-AAA AAA AAA A-2′ | npGNA:npGNA<sup>a</sup> | 34.5(32.5)<sup>b</sup> |
| 2′-TTT TT T TT T T-3′ | GNA:npGNA       | 37.6(37.5)<sup>b</sup> |
|                       | GNA:GNA          | 49.5    |
|                       | DNA:DNA          | 20.1    |
| 3′-CGT ACG ACA T-2′  | npGNA:npGNA<sup>a</sup> | 50.0(52.5)<sup>b</sup> |
| 2′-GCA TGC TGT A-3′  | GNA:npGNA       | 64.1    |
|                       | GNA:GNA          | 74.0    |
|                       | DNA:DNA          | 45.0    |
| 3′-(CGA ATT CG)1-2′  | npGNA:npGNA<sup>a</sup> | 32.0    |
|                       | GNA:GNA          | 40<sup>c</sup> |
|                       | TNA:TNA<sup>d</sup> | 29.8<sup>d</sup> |
|                       | DNA:DNA          | 26<sup>d</sup> |

<sup>a</sup> For A/T<sub>m</sub>. <sup>b</sup> Measured in CD experiments. <sup>c</sup> Reference 1. <sup>d</sup> TNA: α-i-threose nucleic acid, ref 11.

Figure 2. Thermal denaturation curve of a 1:1 mixture of A9 and T10 npGNA compared with A10:T10 duplexes of DNA, GNA, and GNA: npGNA.

Figure 3. Temperature-dependent CD studies on a 1:1 A9:T10 npGNA complex. The temperature range is between 10 and 55 at 5 °C intervals. The inset shows temperature-dependent CD signal change monitored at 273 nm.

Scheme 3. Synthesis of Imidazole-Activated Nucleotides for Templated Polymerization<sup>a</sup>

<sup>a</sup> CDI, carbonyl diimidazole; Im, imidazole; Nvoc, 6-nitroveratryl carbamate.

the 1′-nucleobase substitution. Nevertheless, we were able to isolate several npGNA oligomers (up to 10-mer) in amounts sufficient for base-pairing studies. The unreacted monomers (1t−1g) were quantitatively recovered after each coupling step. Crude npGNA products were further purified by denaturing polyacryl-
were measured by HPLC (Figure S8). The breakdown products of 12a stable than

We envisioned that 13a could be used directly in templated polymerization reactions by generating 14a in situ via photolysis. Because GNA forms a stable duplex with npGNA and can be conveniently synthesized in a large quantity, GNA-(T)<sub>10</sub> was used as the template for studying polymerization of 14a. As shown in Figure 4A, we observed polymerization of 14a by MALDI-TOF MS analysis. The full-length product (10-mer) was detected together with shorter oligomers after photolysis (Figure 4A). In the absence of the GNA template, only the 4-mer and a small amount of 6-mer products were observed with no full-length or 8-mer product (Figure 4B), suggesting that the template is necessary for efficient polymerization of 14a. We also investigated incorporation of 14a into a primer-template complex. Instead of using a GNA or npGNA that of DNA. We found that GNA, GNA:npGNA, and npGNA duplexes share similar CD features (Figure S5), suggesting that all three share a similar conformation in solution.

We then investigated the template-dependent synthesis of npGNA oligomers using 3′-imidazole-activated precursors (Figure 1B). A photolytic protecting group (6-nitroveratryl carbamate or Nvoc)<sup>13</sup> was used to protect the 2′-NH<sub>2</sub> before activation of the 3′-phosphate group with carbonyl diimidazole (10a, Scheme 3A). After photolysis, however, we found that the imidazolide 11a underwent rapid intramolecular cyclization at pH < 13 to yield 12a (Scheme 3A). The rate of cyclization was too fast to be accurately measured by either NMR or HPLC analysis under the conditions for templated polymerization (at 4 °C, pH 8.4). However, we found that an activated dinucleotide (14a), which can be generated by photo-deprotection of its precursor (13a, Scheme 3B), was much more stable than 12a with a half-life of 53.7 h at 4 °C and pH 8.4 as measured by HPLC (Figure S8). The breakdown products of 14a were cyclic and linear dinucleotides with a ratio of ∼3:5:1 (Figure S8).

Templated Polymerization
or with a mismatched template (lane 4), suggesting that polymerization products were significantly reduced without the template (lane 2) and extension products after photolysis (lane 3, arrows). Extension with 14a the primer-template pairing region is not favorable for primer-primer-template complex, we used a well-characterized, 5'-underlined denotes the GNA 10-mer sequence): (A) with the template; (B) without the template. In B, the GNA template was cospotted with the sample underlayered to their expected masses. [M + H]+ for 4-mer: calcd, 1101.733; obsd, 1100.01. For 6-mer: calcd, 1642.349; obsd, 1640.67. For 8-mer: calcd, 2184.443; obsd, 2182.59. For 10-mer: calcd, 2725.798; obsd, 2723.68. For the template: calcd, 3168.994; obsd, 3167.08. (* denotes the n = 1 synthetic byproduct of the template).

In summary, we have demonstrated that npGNA can form stable homo- and heteroduplexes with a conformation similar to that of GNA. In addition, N2'→P3' linkages can be formed by template-dependent polymerization, which suggests that sequence information transfer can be achieved nonenzymatically in a genetic system based on GNA oligonucleotides.

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Supporting Information Available: Procedures for synthesis and characterization of 1t-1g, 5t-5g, and 6a-14a, and solid-phase synthesis of npGNA, HPLC profile of the npGNA-(T)10 crude product, MALDI-TOF analysis of GNA and npGNA oligonucleotides in this study, UV melting curves, and additional CD spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Schlegel, M. K.; Essen, L. O.; Meggers, E. J. Am. Chem. Soc. 2008, 130, 8158–9.
(2) Zhang, L.; Peritz, A.; Meggers, E. J. Am. Chem. Soc. 2005, 127, 4174–5.
(3) Orgel, L. E. Nature 1992, 358, 203–9.
(4) Szostak, J. W.; Bartel, D. P.; Luai, P. L. Nature 2001, 409, 387–90.
(5) Mansy, S. S.; Schrum, J. P.; Krishnamurthy, M.; Tobe, S.; Treco, D. A.; Szostak, J. W. Nature 2008, 454, 122–5.
(6) Zhou, D.; Froeyen, M.; Rozenski, J.; Van Aerschot, A.; Herdewijn, P. Chem. Biodivers. 2007, 4, 740–61. Zhou, D.; Lagoja, I. M.; Rozenski, J.; Busson, R.; Van Aerschot, A.; Herdewijn, P. ChemBioChem 2005, 6, 2298–304.
(7) Chen, J. K.; Schultz, R. G.; Lloyd, D. H.; Gryaznov, S. M. Nucleic Acids Res. 1995, 23, 2661–8.
(8) Gryaznov, S.; Chen, J. K. J. Am. Chem. Soc. 1994, 116, 3143–4.
(9) Zhang, L.; Peritz, A. E.; Carrikk, P. J.; Meggers, E. Synthesis 2005, 4, 645–53.
(10) Schulz, R. G.; Gryaznov, S. M. Nucleic Acids Res. 1996, 24, 2966–73.
(11) Schoning, K.; Scholz, P.; Guntha, S.; Wu, X.; Krishnamurthy, R.; Eschenmoser, A. Science 2000, 290, 1347–51.
(12) Cieplak, P.; Cheatham, T. E.; Kollman, P. A. J. Am. Chem. Soc. 1997, 119, 6722–30. Tereshko, V.; Gryaznov, S.; Egli, M. J. Am. Chem. Soc. 1998, 120, 269–83.
(13) Amit, B.; Zehavi, U.; Patchorn, A. J. Org. Chem. 1974, 39, 192–6.
(14) Stutz, J. A. R.; Richert, C. J. Am. Chem. Soc. 2001, 123, 12718–9.
(15) Hagenbuch, P.; Kervio, E.; Hochgesand, A.; Plutowski, U.; Richert, C. Angew. Chem., Int. Ed. 2008, 44, 6858–92.

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