Synthesis of α-L-Threofuranosyl Nucleoside Triphosphates (tNTPs)

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ABSTRACT

The α-L-threofuranosyl nucleoside triphosphates of T, G, and D (tTTP, tGTP, and tDTP) were synthesized from the described 2′-O-DMT-protected derivatives using the Eckstein method, while the corresponding C derivative (tCTP) was prepared from the 2′-O-acetyl derivative. The prepared α-L-threofuranosyl nucleoside triphosphates, despite being one carbon shorter than the native 2′-deoxyfuranosyl nucleoside triphosphates, are effective substrates for selected DNA polymerases.

Unnatural (3′ → 2′)-α-L-threose oligonucleotides (TNA oligos) are linked together through their 3′- and 2′-hydroxyls and create a nucleic acid polymer with a backbone consisting of five bonds rather than the six-bond backbone of DNA and RNA. Even with the shorter backbone, TNA oligos were observed to cross-hybridize with complementary DNA, RNA, and TNA itself during a systematic study of alternative carbohydrate-based nucleic acids.1,2a These findings have suggested new questions regarding the prebiotic development of these fundamental biological molecules. The property of intersystem base-paring, together with the structural simplicity of the TNA-based oligomers relative to natural RNA oligomers, suggests that TNA might be a candidate for an evolutionary progenitor of the proposed “RNA world.”2a–c,3a,b Nucleic acids such as the TNAs would have carried out fundamental receptor-ligand binding as well as catalytic reactions in such an early stage in the evolution of life. The ability to select functional TNA molecules in vitro4a–c from...

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(1) Eschenmoser, A. Science 1999, 284, 2118–2124.
(2) (a) Schöning, K.-U.; Scholz, P.; Guntha, S.; Wu, X.; KrishnaMurthy, R.; Eschenmoser, A. Science 2000, 290, 1347–1351. (b) Schöning, K.-U.; Scholz, P.; Wu, X.; Guntha, S.; Delgado, G.; KrishnaMurthy, R.; Eschenmoser, A. Helv. Chim. Acta 2002, 85, 4111–4153. (c) Wu, X.; Guntha, S.; Ferencic, M.; KrishnaMurthy, R.; Eschenmoser, A. Org. Lett. 2002, 4, 1279–1282. (d) Wu, X.; Delgado, G.; KrishnaMurthy, R.; Eschenmoser, A. Org. Lett. 2002, 4, 1283–1286.
(3) (a) Orgel, L. E. Science 2000, 290, 1306–1307. (b) Joyce, G. F. Nature 2002, 418, 214–221. (c) Herdewijn, P. Angew. Chem., Int. Ed. 2001, 40, 2249–2251.
(4) (a) Carothers, J. M.; Oestreich, S. C.; Davis, J. H.; Szostak, J. W. J. Am. Chem. Soc. 2004, 126, 5130–5137. (b) Tuerk, C.; Gold, L. Science 1990, 249, 505–510. (c) Ellington, A. D.; Szostak, J. W. Nature 1990, 346, 818–822. (d) Famulok, M. Curr. Opin. Struct. Biol. 1999, 9, 324–329. (e) Wilson, D. S.; Szostak, J. W. Annu. Rev. Biochem. 1999, 68, 611–647. (f) Breaker, R. R. Chem. Rev. 1997, 97, 371–390.
random pools of TNA sequences would provide some evidence for the plausibility of such a proposal. The ability to select functional TNA molecules requires the availability of the TNA building blocks, the nucleoside-3′-triphosphates (tNTPs), and their ability to function with modern protein-based polymerization catalysts. Recently, we and others have observed that certain DNA polymerases can incorporate tTTP or tUTP to extend a DNA or RNA primer in response to a dA or A template.5a Synthetic studies on these materials include the preparation of the 3′-O-DMT 2′-phosphoramidites necessary for the chemical synthesis of TNA oligomers.1 Here we describe the synthesis of the 3′-tNTP analogues and their substrate activity.

In the described syntheses of the TNA phosphoramidites,2a a DMT group was used as a temporary protecting group for the secondary 3′-OH, but since the remaining 2′-OH is also a secondary hydroxyl, this protection reaction typically resulted in a mixture of regioisomeric products. In most cases, the two DMT isomers (2′- and 3′-) could be chromatographically resolved, but in the case of α-L-theofuranosyl cytosine, the 3′-isomer was preferentially obtained.2b The 3′-DMT isomers were subsequently converted to the corresponding 2-phosphoramidites for use in the synthesis of TNA sequences. Here we explore whether the late-stage intermediate side products, the 2′-DMT isomers (1, Scheme 1), can be used to prepare the 3′-triphosphates (3).

Scheme 1. Synthesis of α-L-Theofuranosyl Nucleoside Triphosphates from 2′-O-DMT Nucleosides

| Structure | Description |
|-----------|-------------|
| 1         | 2′-O-DMT Nucleoside |
| 2         | 3′-DMT derivative of 1 |
| 3         | 3′-triphosphate |

Syntheses of nucleoside-5′-triphosphates using POCl₃ in trimethyl phosphate rely upon the greater reactivity and lesser steric hindrance of the primary 5-hydroxyl. That approach was potentially problematic for the TNA building blocks with two secondary hydroxyls. Additionally, the acid-labile DMT group would be lost during such procedures. Eckstein’s method6 (Scheme 1) can be more generally used, but it requires protection of the nontarget hydroxyl groups.

For three of the tNTPs, this strategy could work using the 2′-DMT-protected TNA derivatives, providing that the acid conditions necessary for DMT removal after completion of the reaction sequence (2 → 3, Scheme 1) do not damage the 3′-triphosphate. Using these late-stage intermediates for T, G, and α-L-theofuranosyl 2,6-diaminopurine (DAP), we first removed the nucleobase protecting groups in 8 M methylamine—ethanol/12 M methylamine—water 1:1:1. We then prepared the desired triphosphates using Eckstein’s method and subsequently removed the 2′-DMT group under mild acidic conditions with no observable cleavage of the triphosphate (3, Scheme 1).

The triphosphate products were purified by anion exchange HPLC chromatography (DEAE Sephadex), and after the fractions containing product were pooled and reduced in volume, desalting was performed using a 20 × 200 mm column of polyvinyl-benzene eluting with aqueous triethylammonium acetate. The products were lyophilized to dryness and stored at −20 ºC.

This strategy failed in the case of tCTP, where the 3′-DMT protected nucleoside (4, Scheme 2) was the major product⁶b and the presence of the 2′-regiosomer was negligible. We therefore prepared the 3′-DMT derivative of the nucleobase-protected α-L-theofuranosyl cytosine and then acylated the 2′-hydroxy group (→ 5). Removal of the DMT group generated the 2′-acetate (6). This derivative was used to prepare the corresponding triphosphate using Eckstein’s method (tTTP has also been prepared⁶b using POCl₃), after which concentrated aqueous ammonia was used to deprotect the nucleobase and generate the final product 8, which could be purified and desalted as described above.

To probe the activity of these derivatives with a selected DNA polymerase, we prepared a primer sequence and a series of five templates. Four of the templates were used for single-nucleotide incorporation studies of each of the four tNTPs, and the fifth template was used with all four tNTPs (Figure 1). To date, the polymerase that most effectively uses the tNTPs as substrates is the thermophilic Thermotoga DNA polymerase, a site-specifically engineered (A485L) exonuclease-deficient form of “9°N” DNA polymerase. Incubation of a radiolabeled DNA primer with each appropriate template

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(5) (a) Chaput, J. C.; Szostak, J. W. J. Am. Chem. Soc. 2003, 125, 9274–9275. (b) Kempeneers, V.; Vastmans, K.; Rozenzki, J.; Hendewijn, P. Nucleic Acids Res. 2003, 31, 6221–6226. (c) Horhota, A.; Zou, K.; Ichida, J. K.; Biao, Y.; McLaughlin, L. W.; Szostak, J. W.; Chaput, J. C. J. Am. Chem. Soc. 2005, submitted.
(6) Ludwig, J.; Eckstein, F. J. Org. Chem. 1989, 54, 631–635.
permitted an examination of single-nucleotide extension reactions using the tNTP substrates. In all cases, the single-elongation reactions were observed to occur essentially quantitatively within 15 min. Although most reactions functioned best in the presence of the divalent metal ions Mg$^{2+}$ and Mn$^{2+}$, tGTP only required the former. In the presence of all four tNTPs, the primer could be fully elongated by eight residues within 120 min.

The procedures described here provide a straightforward approach to the preparation of the 3'-nucleoside triphosphate derivatives of the α-L-threofuranosyl sugars. These building blocks will be important for the study of polymerase activity as well as for the selection of functional TNA sequences.

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**Supporting Information Available:** Experimental procedures and $^1$H and $^{13}$C NMR for compound 5 and 6 and $^{31}$P NMR for 8 and compounds 3. This material is available free of charge via the Internet at http://pubs.acs.org.

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