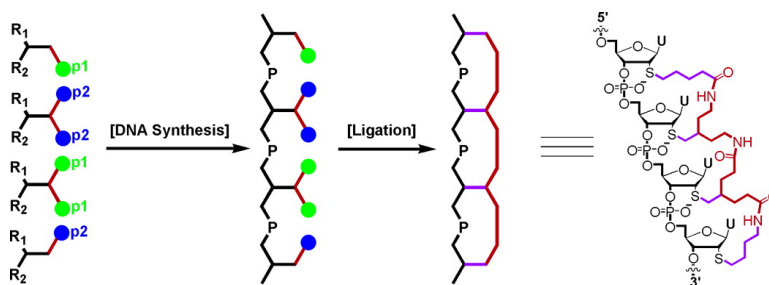## Communication

Nylon/DNA: Single-Stranded DNA with a Covalently Stitched Nylon Lining

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Nylon/DNA: Single-Stranded DNA with a Covalently Stitched Nylon Lining

Lei Zhu, Philip S. Lukeman, James W. Canary,* and Nadrian C. Seeman*

Department of Chemistry, New York University, New York, New York 10003

Received March 16, 2003; E-mail: james.canary@nyu.edu; ned.seeman@nyu.edu

During the past half-century the known functions of nucleic acids have expanded from genetic information carriers and messengers to include catalysis and regulation of a number of cellular processes.[1] In addition, many nucleic acid-based structures have been developed with medicinal applications, catalytic properties, and prebiotic chemistry implications.[1] Notable examples are antisense agents[2], e.g., peptide nucleic acid (PNA),[3] deoxynucleic guanidine (DNG),[4] and locked DNA (LNA).[5] DNAzymes have been developed with functionalized nucleotidyl groups to enhance catalytic abilities.[6] TNA, $(3',2')$-α-L-threose nucleic acid, has been suggested as an evolutionary progenitor of RNA or DNA or both.[7] We wish to develop new nucleic acid-based materials to expand the applications and scope of DNA nanotechnology.[8] A number of topological targets, objects, devices, and two-dimensional (2D) arrays have been prepared from conventional DNA molecules with defined sequences and unusual structural motifs.[9] Analogous DNA/organic polymer conjugates of these structures offer practical interest. For example, DNA 2D arrays[10] may serve as platforms, to assemble molecular electronic devices with nanometer precision, or as templates to synthesize non-DNA polymeric 2D networks that would enjoy the stability and other favorable properties of organic materials. Single-stranded DNA has been used to direct polymerization of DNA oligos with unnatural linkages.[11,19] Our goal would be to harness the full power of DNA nanotechnology, which depends on both secondary and tertiary DNA structural motifs, to assemble organic materials with unique structures. Our approach also entails regiospecific chemistry between non-DNA entities.

Here, we report the first nucleic acid-based structure in which a DNA backbone has been covalently linked to an organic polymer, nylon. The synthesis was accomplished in three stages: preparation of $2'$-β-substituted phosphoramidites, synthesis of oligonucleotides (ODNs) with appended amine and carboxylate groups, and coupling of the pendent groups to form oligopeptide strands covalently linked at each base pair to give a nylon/DNA ladder polymer (Figure 1). The strategy is general and could be used to generate a variety of nylon-based materials, or to direct the assembly of other organic polymers.

Initial synthetic protocols attempted $2'$-OH alkylation of a protected ribonucleoside, but this approach was inefficient for hindered electrophiles.[12] However, $2'$-deoxy-$2'$-mercaptouridine[13] was alkylated with **1a** and **1b** to afford $2'$-S-alkylated nucleosides exclusively (Scheme 1). Tritylation and phosphitylation of **2b** afforded the modified phosphoramidite **4b**. Two extra steps were taken to replace the stable phthalimidyl groups in **3a** with DNA synthesizer-friendly trifluoroacetyl groups.[14] Phosphitylation of the resulting nucleoside **3e** afforded amino-modified phosphoramidite **4e**. Monoamino and carboxyl-modified phosphoramidites **4d** and **4f** were prepared by similar methods. The respective nucleotidyl groups are shown in deprotected form in Figure 2.

Modified phosphoramidites were incorporated into 16-mer ODNs through conventional ODN synthesis. The sequences are shown in Table 1. Methanolic NaOH was used to deprotect and remove the
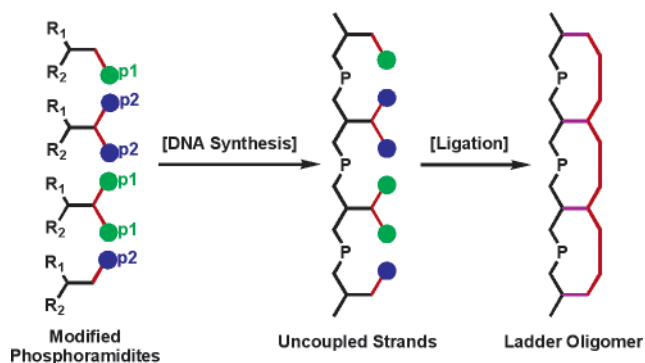

Figure 1. Schematic illustration of a ladder oligomer synthesis. $\mathbf{R_1}$: $-$DMTr; $\mathbf{R_2}$: $-$P(NiPr$_2$)(OCH$_2$CH$_2$CN); green and blue dots are carboxyl and amino groups, respectively; **p1** and **p2** are their respective protecting groups. Black vertical chains with alternate "P" represent a nucleic acid backbone; the red vertical chain represents the nylon backbone; the violet horizontal lines (right) represent "rungs" on the ladder oligomer.

Scheme 1. Synthesis of $2'$-Deoxy-$2'$-alkylthiouridine Phosphoramidites[a]
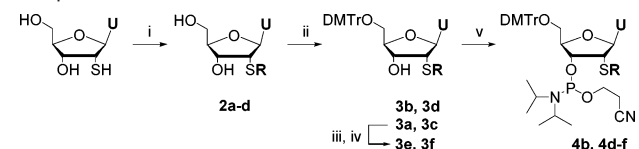
[a] Reagents and conditions: (i) RI (**1a**, **1b**) or RBr (**1c**, **1d**), DIEA/CH$_3$CN, rt, 10 h; (ii) DMTrCl/pyridine, rt, 2 h; (iii) NH$_2$NH$_2$/CH$_3$OH, reflux, 3 h; (iv) CF$_3$CO$_2$Et/CH$_3$OH, rt 16 h; (v) ClP(NiPr$_2$)(OCH$_2$CH$_2$CN)/CH$_2$Cl$_2$, TEA, DMAP, rt, 30 min. (**a**: $\mathbf{R} = $ CH$_2$CH(CH$_2$CH$_2$NPhth)$_2$; **b**: $\mathbf{R} = $ CH$_2$CH(CH$_2$CH$_2$CO$_2$Bn)$_2$; **c**: $\mathbf{R} = $ (CH$_2$)$_4$NPhth; **d**: $\mathbf{R} = $ (CH$_2$)$_4$CO$_2$Et; **e**: $\mathbf{R} = $ CH$_2$CH(CH$_2$CH$_2$NHCOCF$_3$)$_2$; **f**: $\mathbf{R} = $ (CH$_2$)$_4$NHCOCF$_3$).
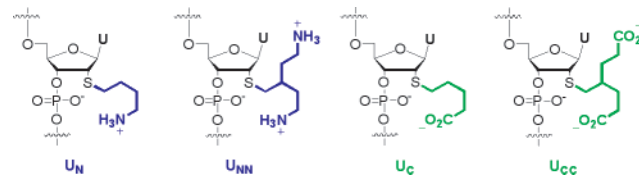

Figure 2. Structures of $2'$-modified nucleotidyl units.

strands from the CPG support. The conventional concentrated ammonium hydroxide treatment could not be used due to aminolysis between NH$_3$ and the ester moieties.[15] Deprotection with prevention of the Michael addition between acrylonitrile and deprotected amines[16] was accomplished by including 10% piperidine in methanolic NaOH. To prevent acetate ions (from hydrolysis of the $5'$-acetyl groups of the capped failure strands) from competing as alternative coupling partners, they were eliminated by triple ethanol precipitation prior to being subjected to amide-coupling conditions. The concurrent deprotection of amino and carboxyl groups and the removal of ODNs from CPG support was therefore achieved with this customized protocol. The ODNs were characterized by MALDI-TOF mass spectrometry[17] (Table 1).

Table 1. Sequences and MALDI-TOF MS Characterization of Synthetic ODNs

ODN No.	ODN Sequence	m/z [M-1]⁻			
		Calcd.	Found	Coupled Calcd.	Coupled Found
1	5'-(dT)$_6$U$_N$U$_C$(dT)$_8$	5011.4	5011.4	**1C** - 4993.4	**1C** - 4993.7
2	5'-(dT)$_3$U$_N$U$_C$(dT)$_2$U$_N$U$_C$(dT)$_2$U$_N$U$_C$(dT)$_3$	5426.0	5427.0	**2C** - 5372.0	**2C** - 5371.4
3	5'-(dT)$_7$U$_N$U$_{CC}$U$_N$(dT)$_6$	5172.7	5172.4	**3C** - 5136.6	**3C** - 5136.2
4	5'-(dT)$_7$U$_C$U$_{NN}$U$_C$(dT)$_6$	5172.7	5172.8	**4C** - 5136.6	**4C** - 5136.9
5	5'-(dT)$_6$U$_C$U$_{NN}$U$_{CC}$U$_N$(dT)$_6$	5333.9	5333.6	**5C** - 5279.8	**5C** - 5280.3
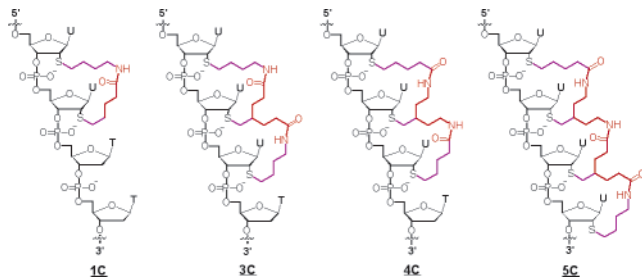
Figure 3. Chemical structures of DNA/nylon conjugates.
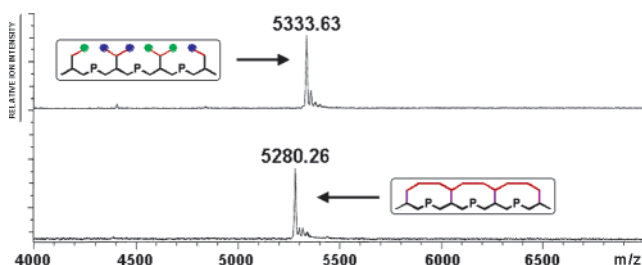
Figure 4. MALDI-TOF MS of **5** (a), and the coupled product **5C** (b).

ODN **1** was first subjected to amide-coupling conditions. Both condensing agents DMT-MM[18] and EDC[19] proved effective in promoting the intrastrand amide-forming reaction between U$_N$ and U$_C$ under various buffer conditions. DMT-MM was preferred, as it did not leave residual covalent adducts. The yield of the coupling reaction was estimated by MALDI-TOF analysis.[20] The yield of ODN **1C** from **1** was estimated to be more than 95%, whereas the yield of **2C** with three amide bonds was 78%, which also put the single amide-bond forming yield over 95%.[21] A control coupling reaction using an ODN with regular T residues replacing U$_C$ in **2** showed no mass loss.

Isomers **3** and **4** were treated under coupling conditions to afford ODNs with two amide bonds closing two fused 21-member rings containing both a phosphodiester backbone and the newly formed aliphatic carboxamide structure, as characterized by MALDI-TOF MS (Table 1). The condensation of ODN **5** yielded three amide bonds to form **5c** (Figures 3 and 4) with three fused 21-member rings.[22] Structures of several of the product strands are indicated in Figure 3. The linear polyamide backbone is essentially Nylon-5,7.

The foregoing results should be extensible using additional bases to more complex systems in which the self-assembling properties of DNA can be exploited. Aside from obvious applications in the antisense and gene therapy areas, we anticipate significant utility in nanotechnology.

Supporting Information Available: Synthesis and characterization of compounds **2**−**4** and all the ODNs (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) These estimates are lower limits, as small amounts of sodium in the spectra obscure and artificially inflate the starting material peak.
(22) In **5C**, a less likely topological isomer is possible under these reaction conditions. Control experiments were performed to study the distance dependence of the coupling reaction between amino and carboxyl groups separated by a spacer. Strands 5'-(dT)$_x$U$_C$(dT)$_n$U$_N$(dT)$_y$ ($x + n + y = 14$, $n = 0, 1, 2, 3, 6, 10$) were subjected to amide-bond promoting conditions. It was found that the coupling yield was highly dependent upon the length of the spacer (dT)$_n$. When $n \geq 2$, the yield was less than 50%; when $n = 6$ or 10, coupled products were barely detectable. Therefore, the amide bonds were biased to form between amino and carboxyl groups on adjacent nucleotidyl residues.

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