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Coupling Across a DNA Helical Turn Yields a Hybrid DNA/Organic Catenane Doubly Tailed with Functional Termini

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Catenanes constructed from organic building blocks have been used in the demonstration of molecular devices such as positional switches, unidirectional motors, and other nanoscale functional materials.[1] In the nucleic acid world, catenanes have long been known; they are found in nature and are common in structural DNA nanotechnology.[2] Designed, all-DNA catenanes have been used as topological labels.[3] Cross-links with organic linkages have been utilized to probe the structures and properties of DNA duplexes, hairpins, and higher order structures, such as t-RNA and ribozymes,[4] as well as to build DNA nanostructures and nanodevices.[5] In this paper, we describe the synthesis of a macrocycle that is prepared by formation of an amide linkage across one full turn of DNA, forming a tailed DNA/organic catenane containing 5′- and 3′-termini that are available for further functionalization.

In prior work, we showed that 2′-pendent amines and carboxylates could be linked to form nylon along the phosphodiester backbone contour.[6] Our inquiry here began when we asked whether longer linkers could be used to attach polymeric components parallel to the helix axis. This target requires bridging approximately 35 Å across a nucleic acid turn, which had not been reported previously (Figure 1A), although there is now a report by Gothelf et al.[7] Tetraethylene glycol was used as a spacer into a carboxylate-functionalized uridine, and undecaethylene glycol was incorporated into an amine-functionalized uridine. The linker distances have not been optimized, although studies with tetraethylene glycol as a spacer for both amine and caboxylate were unsuccessful.

Three 16-mer ODNs **1**, **2**, and **3** were synthesized, where two modified uridines were separated by 8, 9, and 10 unmodified nucleotides, respectively. Cross-turn coupling using a DNA hairpin template gave >97% yields of ODN **1** and **2**, as estimated by MALDI-TOF spectra and denaturing gel electrophoresis, whereas the coupling yield of **3** was about 62% (Table 1, Supporting Information). For further characterization, the coupled product was subjected to complete nuclease digestion followed by analysis to detect linked nucleotides. The detection of a polyethylene glycol (PEG)-amide linked uridine dimer by LCMS corroborated the formation of coupled products (Supporting Information).

These data established the formula of the reaction product but did not distinguish between a newly formed linkage parallel to the helix axis (i.e., across the turn) versus along the phosphodiester backbone contour. However, templation of the reaction by a circular DNA template (Figure 1A) would yield an interlocked complex only if the coupling reaction occurred across the turn. ODNs **1** and **2** paired with a 78-mer DNA circular template were subjected to the same reaction procedure as the hairpin-templated coupling. Almost-quantitive yields of catenanes were produced from the coupling of both **1** and **2**, which was established by denaturing gel analysis. Thus, the linkage across a full turn of DNA helix generated a doubly tailed catenane with special features discussed below. This

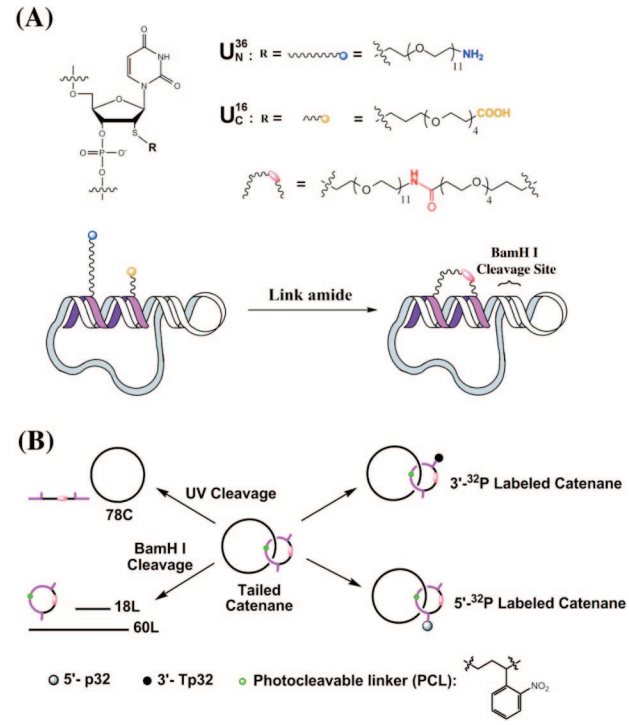

Figure 1. Schematic depiction of (A) templated synthesis of the tailed catenane, (B) cleavage, and labeling of the tailed catenane constructed from strand **4**. The catenane stereoisomer is established by the antiparallel pairing of DNA before closure.

demonstrated that the cross-turn coupling strategy produced a linkage parallel to the DNA helix axis.

To test for the catenated structure, the synthesized catenane was first treated with exonuclease; no dissociation was observed, although the tails of the hybrid macrocycle were degraded. To release the DNA circular template by cleaving the hybrid macrocycle, a catenane was constructed from ODN **4** containing a photocleavable linker (PCL) (Figure 1B). It was then restricted with BamH I, which cleaved the 78-mer DNA circular template as indicated in Figure 1B to produce an 18-mer and a 60-mer and released the PEG-amide-oligonucleotide hybrid circle as shown by its electrophoretic mobility (Figure 2, lane 6). Identical products were obtained from cleavage of the DNA template alone (Figure 2, lane 7) and coupled ODN **4** (lane 8). Upon irradiating the catenane with near-UV light (350 nm), the PCL was cleaved, resulting in linearization of the hybrid macrocycle and release of the template circle. The reaction products appear in Figure 2 (lane 4) and can be compared with intact catenane and the DNA circular template (lanes 5 and 3) and photocleavage products of coupled strand **4** (lane 2).

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Table 1. Oligonucleotides Used in This Study and MALDI-TOF MS Analysis[a]

ODNs	sequence	uncoupled		coupled	
		calcd	found	calcd	found
1	5′-TG $\mathbf{U}_N^{36}$ACGTGCGA$\mathbf{U}_C^{16}$TTCG	5729.2	5729.4	5711.2	5711.3
2	5′-TG $\mathbf{U}_N^{36}$ACGTGCGAT$\mathbf{U}_C^{16}$TCG	5729.2	5728.4	5711.2	5710.5
3	5′-TG $\mathbf{U}_N^{36}$ACGTGCGATT$\mathbf{U}_C^{16}$CG	5729.2	5728.9	5711.2	5711.0, 5728.1
4	5′-TG $\mathbf{U}_N^{36}$ACGTG(*PCL*)CGAT$\mathbf{U}_C^{16}$TCG [b]	5988.4	5988.4	5970.4	5969.1

[a] Cross-turn coupling trials were conducted with a DNA hairpin as template. [b] PCL: Photocleavable linker.

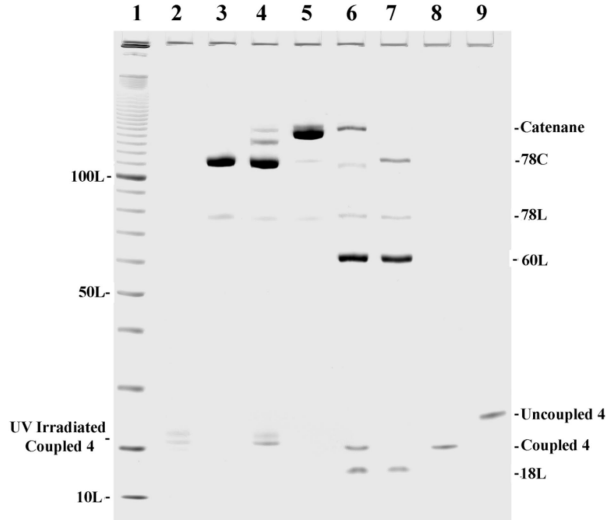

Figure 2. Denaturing gel analysis of the dissociation of the tailed catenane by BamH I digestion and UV cleavage. Lane 1: 10bp DNA ladder marker. Lane 2: UV-cleaved products of coupled strand **4**. Lane 3: 78-mer DNA circle. Lane 4: UV-cleaved products of the tailed catenane. Lane 5: The tailed catenane. Lane 6: BamH I digested products of the tailed catenane. Lane 7: BamH I digested products of 78-mer DNA circle. Lane 8: Coupled strand **4**. Lane 9: Uncoupled strand **4**. **L**: Linear DNA. **C**: Circular DNA.

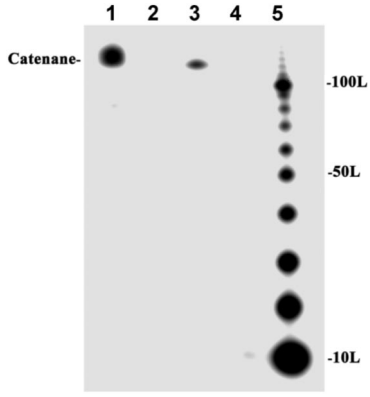

Figure 3. Autoradiogram of a denaturing gel showing both 5′ and 3′ labeling of the tailed catenane. Lane 1: 3′-^{32}P labeling of the tailed catenane. Lane 2: 3′-^{32}P labeling of 78-mer DNA circle. Lane 3: 5′-^{32}P labeling of the tailed catenane. Lane 4: 5′-^{32}P labeling of 78-mer DNA circle. Lane 5: 10bp DNA ladder marker.

The catenane formation reaction may be viewed as a "padlock" function as the oligonucleotide acted as a probe to recognize the target circular DNA, and interlocking was accomplished by the pendent linkers. The 2′-localization of the linkers creates a bibracchial lariat structure,[8] leaving the free 5′- and 3′-ends of the oligonucleotides available to serve as accessible sites for further modification or labeling, which is an advantage over prior approaches that employ 5′- and 3′-terminal linkers.[5a,9] To test the feasibility of postsynthetic modification of these tails, the catenane containing ODN **4** was subjected to 5′- and 3′-^{32}P labeling in two experiments. The generation of 3′-labeled (Figure 3, lane 1) and 5′-labeled product (lane 3) are readily visible in the autoradiogram of the denaturing gel. Thus, this cross-turn coupling strategy may be advantageous for nucleic acid labeling. In principle, nucleic acids of any sequence could be targeted and labeled by this approach.

In conclusion, coupling across a DNA helical turn was achieved utilizing ODNs decorated with 2′-functionalized linkers. The formation of a doubly tailed catenane based on this strategy demonstrates intrastrand cross-linking. The free ends of the catenated PEG-amide-oligonucleotides provide useful handles for postsynthetic modification or labeling.

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Supporting Information Available: Full experimental details including: Syntheses of phosphoramidites, MALDI-TOF MS of ODNs, LCMS analysis of complete nuclease digestion, denaturing gel analyses of catenane synthesis, exonuclease digestion, and dissociation by restriction enzyme treatment. This material is available free of charge via the Internet at http://pubs.acs.org.

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