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[16] Crystal sructures: **1a** (tetragonal isomorph)**:** $C_{24}H_{24}N_2O_4$, $M_r = 404.47$, yellow crystals from MeOH, m.p. 171 – 172 °C; crystal dimensions: $0.50 \times 0.13 \times 0.13$ mm; tetragonal, space group $I4_1cd$ (No. 110), $a = 18.831(3)$, $b = 18.831(3)$, $c = 11.639(2)$ Å, $\alpha = 90°$, $V = 4127.3(12)$ Å^3, $Z = 8$, $\rho_{calcd} = 1.302$ g cm^{-3}; $F(000) = 1712$; $\mu_{Cu} = 7.3$ cm^{-1}; $\theta_{max} = 74.97°$; 4372 determined, 2058 independent, 1918 observed reflexions with ($F_o^2 > 2\sigma F_o^2$); $R1 = 0.0260$, $wR2 = 0.0714$, GOF (F^2) = 1.083 for 137 parameters, residual electron density 0.026/ – 0.092 e Å^{-3}. **1b** (monoclinic isomorph): $C_{24}H_{24}N_2O_4$, $M_r = 404.47$, yellow crystals from MeOH, m.p. 171 – 172 °C; crystal dimensions: $0.20 \times 0.20 \times 0.09$ mm; monoclinic, space group $P2_1/n$ (No. 14), $a = 7.943(1)$, $b = 10.499(1)$, $c = 12.7930(1)$ Å, $\alpha = 90$, $\beta = 107.00(1)°$, $V = 1020.24(19)$ Å^3, $Z = 2$, $\rho_{calcd} = 1.317$ g cm^{-3}; $F(000) = 428$; $\mu_{Cu} = 7.3$ cm^{-1}; $\theta_{max} = 75°$; 2200 determined, 2017 independent, 1662 observed reflections ($F_o^2 > 2\sigma F_o^2$); $R1 = 0.0358$, $wR2 = 0.1183$, GOF (F^2) = 1.004 for 139 parameters, residual electron density 0.215/ – 0.132 e Å^{-3}. $(\mathbf{1} \cdot PF_6)_2$: $(C_{24}H_{24}N_2O_4F_6P)_2$, $M/2 = 549.43$, bluish-black needles, decomp. > 250 °C; crystal dimensions: $0.25 \times 0.08 \times 0.03$ mm; triclinic, space group $P\bar{1}$ (No. 2), $a = 8.36(1)$, $b = 11.220(1)$, $c = 13.067(1)$ Å, $\alpha = 111.78(1)$, $\beta = 96.23(1)$, $\gamma = 90.56(1)°$; $V = 1129.8(8)$ Å^3, $Z = 2$, $\rho_{calcd} = 1.444$ g cm^{-3}; $F(000) = 510$; $\mu_{Mo} = 1.021$ cm^{-1}; $\theta_{max} = 59.99$; 3304 determined 3304 independent, 1813 observed refections ($F_o^2 > 2\sigma F_o^2$); $R1 = 0.0496$, $wR2 = 0.1234$, GOF (F^2) = 0.908 for 339 parameters, residual electron density 0.297/ – 0.216 e Å^{-3}. The crystallographic data (excludung structure factors for the structures reported in this paper have been deposited with the Crystallographic Data Centre as supplementary publication nos CCDC-102195 (**1a**), -102194 (**1b**), and -102196 $(\mathbf{1} \times PF_6)_2$. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail deposit@ccdc.cam.ac.uk).

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Topological Links between Duplex DNA and a Circular DNA Single Strand**

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DNA is well known to adopt various topological (and pseudotopological) structures like knots, catenanes, Borromean rings, and pseudorotaxanes.[1] It has long been recognized that DNA topology plays a crucial role in such fundamental biological phenomena as DNA supercoiling and topoisomerization.[2] Another reason for the considerable interest in higher order DNA topology structures stems from the realization that DNA topological and pseudotopological forms may provide stable and sequence-specific targeting of DNA. Accordingly, highly localized DNA detection and precise spatial positioning of various ligands on the DNA scaffold become possible. This may lead to new applications in molecular biotechnology, in gene therapy, and in the emerging field of DNA nanotechnology.[1d, 3]

In this connection, one of the promising DNA pseudotopological constructions is the DNA padlock, consisting of a long single-stranded (ss) DNA molecule forming a pseudorotaxane with a short cyclic oligodeoxyribonucleotide (cODN).[4] Another interesting pseudorotaxane-type structure, the sliding clamp, contains a short cODN threaded on double-stranded (ds) DNA.[1c] Notwithstanding the value of the indicated pseudotopological structures for DNA labeling, note that in these constructions the cODN tag is allowed to slide along the target for considerable distances, compromising the precision of spatial positioning of the label.

We have assembled a new supramolecular structure, a linked DNA pseudorotaxane, in which part of a cODN appears to be threaded sequence specifically between complementary strands of dsDNA (see Scheme 1 and Figure 1a). Our structure forms a true topological link as long as the

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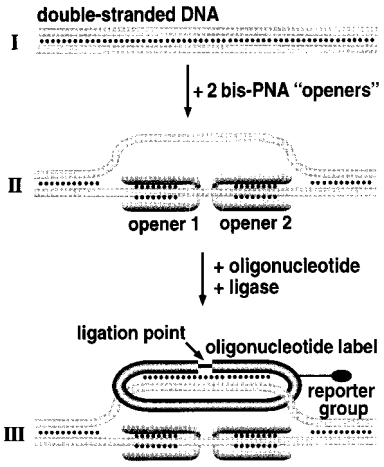
participating dsDNA macromolecule retains its native duplex conformation. In this case, the cODN tag will remain correctly positioned along the double helix during various possible postassembly manipulations. Scheme 1 shows the successive steps of our assembly. First of all, we open dsDNA at two closely located sites using a pair of peptide nucleic acid (PNA) openers.[5, 6] The PNA openers are bound to one of the two DNA strands, leaving the opposite strand displaced and thus accessible for hybridization with an ODN.[5a]



Scheme 1. Major steps to assemble topologically linked pseudorotaxane and catenane (**III**) on dsDNA (**I**). First, the target sequence within dsDNA (linear or ccDNA) is opened at two closely located sites by strand invasion of a pair of PNA clamps. As a result, structure **II** forms, in which one of two dsDNA strands becomes accessible for hybridization with an ODN. The ODN may contain one or more reporter groups, and it is designed in such a way that its termini are complementary to the exposed DNA target and are in juxtaposition upon hybridization. After hybridization, the ODN is circularized by enzymatic ligation.

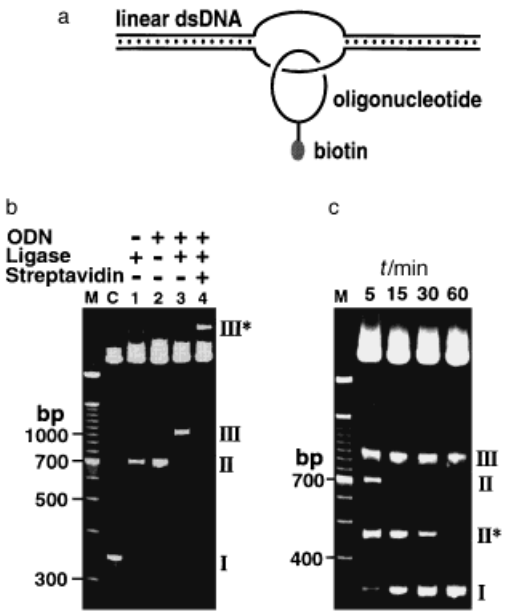


Figure 1. a) Schematic representation (not in scale) of the pseudorotaxane **III** obtained using a linear 340 bp long dsDNA target fragment. As a reporter group, a biotinylated segment was incorporated into the backbone of the oligonucleotide. b) Specific formation of structure **III** (see Scheme 1) as demonstrated by EMSA (lanes 3 and 4; see the experimental section for details). Lane C is for the fragment alone (**I**); here and below, lane M is for a 100 bp long marker (Gibco BRL). Lanes 1 and 2 correspond to control experiments. In lane 4, streptavidin was added to the resulting product before electrophoresis. Bands coinciding with 700 base pairs correspond to DNA complex **II** with both PNA openers. c) EMSA analysis of the stability of structure **III**. A mixture of **II** and **III** was heated in the ligation buffer at 65 °C for the indicated periods of time. While PNA openers gradually dissociate from **II** to yield intermediate complex **II*** (dsDNA target + one PNA opener) and the initial dsDNA target **I**, the amount of pseudorotaxane **III** remains virtually unchanged.

Like in the case of the padlock assembly,[4a] we design a linear ODN which contains an unrelated sequence flanked by two stretches that are eight to ten nucleotides long and complementary to the displaced DNA strand (see the Experimental Section). After hybridization to the target, both ends of the linear ODN appear in juxtaposition and are covalently joined by DNA ligase. The resulting cODN forms almost two turns of a double helix with the displaced DNA strand and is therefore topologically linked with one of the two DNA strands. Consequently, the resulting cODN–dsDNA complex can only be separated by cleavage of the DNA backbone or by complete separation of the DNA complementary strands. Thus, a topological label resembling an earring was introduced sequence-specifically into dsDNA (see Figures 1a and 2a).

In our initial experiments we used a linear 340 base pair (bp) long dsDNA fragment carrying the target site, and analyzed the resulting complex by electrophoretic mobility shift assay (EMSA). Figure 1b (lane 3) demonstrates the formation of a specific product **III** (migrating as slow as a 1050 bp long DNA fragment) only in the presence of both T4 DNA ligase and the ODN.[7] Our topological label carried a biotin molecule, and after streptavidin was added, specific extra retardation of **III** resulting in **III*** was observed due to the complexation of biotin to streptavidin (Figure 1b, lane 4). If **III** were a linked pseudorotaxane, we would expect it to be stable at elevated temperatures at which regular ODN–DNA duplexes are unstable. To check this, we heated **III** together with PNA–DNA complex **II** for one hour at 65 °C. During this period the PNA openers, which form very stable complexes with DNA,[6] completely dissociated from DNA, while the amount of **III** remained unchanged (Figure 1c).

Only binding of all three specific ligands (two PNA openers and the ODN probe) to the dsDNA target resulted in product formation. Practically zero tolerance of the structure formation to mismatches between cODN and dsDNA target could be expected. Indeed, mismatches in the openers' binding sites can be readily discriminated kinetically at the PNA binding step.[8] Moreover, such mismatches will affect the supramolecular assembly twice, during dsDNA opening and at the step of ODN hybridization. As a result, they have to be extremely unfavorable. One can expect a mismatch to be more tolerable when it is located between the binding sites of the PNA openers. In this case, however, the mismatch is located either directly at or very close to the ligation point, so that the weakening of the ODN–DNA complex together with the intrinsic fidelity of the ligation enzyme[9] should prevent circularization of the ODN. In accordance with our expectation, we were unable to assemble a linked pseudorotaxane

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on a dsDNA fragment carrying a single mismatch in the sequence between the two PNA binding sites (data not shown; for the mismatch position, see the Experimental Section).

We then assembled a DNA catenane with the sequence-specifically locked junction using closed-circular (cc) dsDNA plasmid as a target (Figure 2). Note that the sliding-clamp approach cannot be used for topological labeling of ccDNA

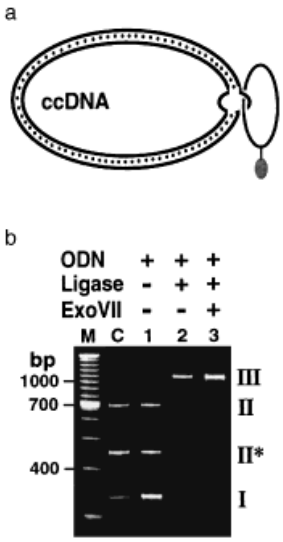

Figure 2. a) Schematic representation (not in scale) of the catenated structure obtained using the ccDNA target. b) After formation of the catenated complex with the ccDNA target, the sample was digested with *Pvu*II endonuclease to perform the EMSA analysis. Complex **III** was obtained in virtually quantitative yield (lane 2). To prove circularization of the oligonucleotide, in this experiment the product was incubated with ten units of ExoVII for 1 h at 37 °C (lane 3).[10] Lane C is a control in which the ODN was preincubated with ten units of ExoVII for 1 h at 37 °C before a ligation reaction, analogous to the reaction shown in lane 2 was performed. In this control as well as in case of control without ligase (lane 1), several bands corresponding to structures **I**, **II***, and **II** are observed due to partial dissociation of PNA oligomers from complex **II** during the enzymatic digestion.

since it requires accessible ends of the duplex DNA template.[1c] To analyze the product by EMSA, it was digested with a restriction enzyme to generate a linear 340 bp long dsDNA fragment. Complex **III** with a characteristically reduced electrophoretic mobility similar to that in Figure 1 was observed (Figure 2b, lane 2). Compound **III** was insensitive to digestion by exonuclease VII (ExoVII, Figure 2b, lane 3).[10] At the same time, noncircularized ODN was degraded by ExoVII (Figure 2b, lane C), providing convincing evidence for circularization of the ODN probe in this case.

Thus, all data are in agreement with the proposed formation of linked pseudorotaxane and catenane yielding sequence-specific earring labels immovably connected to the dsDNA target. The described DNA topological structures are substantially different from previously constructed pseudorotaxanes.[1c, 4a] We have assembled a true topological link between dsDNA and cODN in such a manner that the precisely positioned label has to occupy a strictly fixed site along the double helix. Together with remarkable sequence specificity of the structure formation, this feature should be useful for highly localized detection of various marker sequences within genomes. In addition, the PNA-directed assembly described here introduces PNA oligomers into the repertoire of DNA nanotechnological tools.

Experimental Section

By cloning of the appropriate oligonucleotide pairs into the *Bam*HI site of pUC19, target plasmid containing the sequence 5′-GATC_4T_2C-$GA_2C_2T_2CT_2$TGATC-3′/3′-GATC**$A_3GA_2G_2T_2C$$GA_2G_4A$**TC-5′ (the ODN binding site is italicized, and the PNA binding sites are in boldface) was obtained. The single mismatched target contained an A – T instead of the underlined G – C base pair between the PNA binding sites. The following PNAs were used as openers: H-TC_4T_2C-eg1-$(Lys)_2$-eg1-JT_2J_4T-Lys-NH_2 and H-Lys_2-$T_3JT_2J_2$-$(eg1)_3$-$C_2T_2CT_3$-Lys-NH_2 (Lys denotes lysine, J is for pseudoisocytosine, and eg1 is for 8-amino-3,6-dioxaoctanoic acid as a linker unit). Both PNAs were synthesized as previously described,[11] and purified by reverse-phase HPLC. Their identity was confirmed by MALDI-TOF mass spectrometry analysis. 5′-Phosphorylated ODN, 5′-$GA_2G_4AT_3GT_3C$-$T_2AXT_2GT_3AT_3A_2GA_2G_2T_2C$-3′ (obtained from Operon; X denotes a biotinylated reporter segment (Ibioteg) incorporated into the backbone) was used for circularization. The end stretches that are eight to ten nucleotide long and complementary to the DNA target are italicized.

To assemble linked pseudorotaxane **III**, plasmid DNA was first digested with *Pvu*II endonuclease resulting in two fragments of 2367 and 340 base pairs in length, the latter being target fragment **I**. The digested DNA was then dephosphorylated by alkaline phosphatase (CIP, New England BioLabs). In a typical experiment, PNA – DNA complex **II** was obtained by addition of 10 μL of 0.1 M sodium phosphate buffer (pH 6.8), 63 μL of H_2O, and 10 μL each of 20 μM PNA solutions to 7 μL of digested, dephosphorylated plasmid DNA (7 μg, 4 pmol), and incubation for 4 h at 37 °C. Nonbound PNAs were then removed by gel filtration (Sephadex G-50, Sigma). To a 10-μL aliquot of sample containing **II** (≈0.7 μg total DNA) 2 μL of 10 × ligation buffer (Fermentas), 5 μL of H_2O, 2 μL of 4 μM ODN, and 1 μL of T4 DNA ligase (Fermentas, 30 units μL^{-1}) were added, and sequentially incubated for 2 h at 16 °C, 15 min at 45 °C, and 2 h at 16 °C. Treatment with exonucleaseVII (USB) was performed in 1 × ligation buffer by addition of 1 μL of exoVII (10 units), and incubation for 1 h at 37 °C. Before electrophoresis, samples were desalted by gel filtration, extracted with phenol/chloroform, precipitated with ethanol, and redissolved in 10 μL of TE buffer (10 mM tris(hydroxymethyl)aminomethane (Tris), 0.1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.4). Complex **III*** was obtained by incubation of 10 μL of **III**-containing sample with 2 μL of 1 mM streptavidin for 1 h at 37 °C.

To assemble the linked catenane, supercoiled plasmid DNA was used. The formation of catenated complex was performed analogous to that of **III**. In this case, the samples were cut with *Pvu*II endonuclease after ligation reaction.

The EMSA analysis was performed in non-denaturing 10% polyacrylamide gels using TBE buffer (90 mM Tris, 90 mM boric acid, 2 mM EDTA, pH 8.0). The electrophoresis was run at 250 V and 20 mA for 4 h at room temperature. The gels were stained with ethidium bromide and scanned with a CCD camera using the IS-1000 digital imaging system (Alpha Innotech Corporation).

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[7] The optimal yield of linked product **III** is about 70 – 80 % after a single ligation step. The major factor affecting the yield is the ODN concentration: the yield of **III** decreases significantly when the ODN concentration is too low (< 0.1 μM) or too high (> 1 μM). Still, under optimal conditions 20 to 30 % of the dsDNA molecules remain topologically unlinked with cODN. A second round of ligation, which is performed after dissociation at elevated temperatures of ODNs bound to and yet unlinked with dsDNA, results in virtually quantitative yield of linked products.

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[10] ExoVII is an exodeoxyribonuclease that digests ssDNA from both the 3′ and 5′ ends. The cODN is not a substrate for this enzyme. ExoVII has been previously used to prove the formation of the padlock.[4a]

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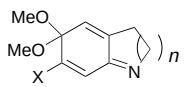
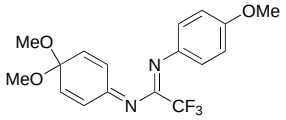
Direct Synthesis of *N*-Arylquinone Imine Acetals and Quinol Imines from Acetals**

M. Carmen Carreño,* Juan M. Cuerva, María Ribagorda, and Antonio M. Echavarren*

Although quinone imines and diimines have a long history in chemistry,[1] their synthetic potential has not been extensively exploited.[2, 3] The most general method for the preparation of these derivatives relies on the chemical oxidation of *p*-methoxyanilides or *p*-phenylenediamides[3a, b] with cerium(IV) salts[4] or $Pb(AcO)_4$.[5] However, the presence of an imide (*N*-acyl or *N*-arylsulfonyl group) is required to prevent decomposition of the oxidation product. Although N-acylated quinone imine acetals[6] are easily available by the anodic oxidation of substituted anilides,[7] the electrochemical oxidation of *p*-aminophenols[8] only allows for the in situ formation of simple quinone imines, which cannot be isolated in pure form due to their lack of stability under the oxidation conditions. The few examples of *N*-alkylquinone imines described to date refer to the labile derivatives of type **I**.[9] Recently, compound **II**, a new type of N-protected quinone imine acetal, has been isolated by oxidation of the trifluoromethylamidine precursor.[10]



I : X = MeO, Br; *n* = 1, 2



II

In connection with a project directed towards the total synthesis of pyridoacridine alkaloids based on cycloaddition chemistry,[11] we examined the condensation of quinone acetals with anilines. Surprisingly, we found that acetals react with anilines to yield the corresponding imines in the absence of any added catalyst.

As shown in Table 1, bisacetal **1**[12] reacted with anilines at 36 – 80 °C to give derivatives **4** [Eq. (1)]. The reactions were carried out without especial precautions in the presence of air. Interestingly, while *p*-methoxyaniline (**3b**) failed to react with **1** (entry 1), less nucleophilic *p*-nitroaniline (**3c**) reacted cleanly to give quinone imine monoacetal **4c** (entry 2). Reaction of **1** with *o*-aminocinnamaldehyde *N*,*N*-dimethylhydrazone[13] was carried out in the absence of solvent to give **4g** (entry 3). Bisacetal **2**[12] was more reactive than **1** and led

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