DNA-Templated Coupling

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Bridging One Helical Turn in Double-Stranded DNA by Templated Dimerization of Molecular Rods**

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A variety of molecular building blocks with promising electronic,[1] optical,[2] and mechanical[3] properties can be prepared. However, it is very difficult to assemble and couple such structures into complex functional devices by traditional synthetic methods. The ability to assemble molecular building blocks into large monodisperse macromolecular structures is therefore a major challenge for modern chemistry and nanoscience.[4] Within the area of DNA nanotechnology, a large number of impressive nanostructures formed by DNA self-assembly have been reported during the past 15 years.[5] The next challenge in this field is to apply these DNA structures as platforms for functional devices.[5,6] One important goal would be to build macromolecular organic structures on the top of such DNA platforms; however, new approaches must be developed if we are to succeed in this task.

Several examples of the DNA-programmed assembly of organic building blocks, such as molecular beacons[7] or other organic compounds,[8] have been reported, but as the assembled organic building blocks are not covalently linked, the assembled structures are geometrically less well defined. Nevertheless, it has been demonstrated in several examples of DNA-directed synthesis that the covalent coupling of small organic compounds can be controlled by attached oligonucleotides.[9]

To the best of our knowledge, the only examples of the parallel assembly and coupling of more complex organic building blocks to form organic oligomers were reported by us.[10] This method was efficient for the DNA-directed coupling of up to four potentially conducting modules; however, it was not suitable for preparing higher-order structures owing to intramolecular cyclization.[10]

Herein, we report the synthesis of a new type of oligonucleotide-functionalized rod-type modules and the exploration of a fundamentally new strategy for their DNA-programmed coupling. The concept of this strategy is to arrange these molecular rods on a DNA template and couple the modules to bridge 10 base pairs (bps) along the axis of the DNA helix (Figure 1a). For this purpose, molecular rods are conjugated to a 10-mer oligonucleotide, and two of these conjugates are arranged on a DNA template with two repeated 10-mer regions complementary to the sequence of the oligonucleotide attached to the molecular rod. The modules are placed on approximately the same side of the DNA helix, one helical turn apart. In analogy to our previous studies,[10] the modules are functionalized at each terminal position with salicylaldehyde moieties. An activated ester is positioned at the center of the rod, which is applied for conjugation to an amino-modified oligonucleotide. Finally,

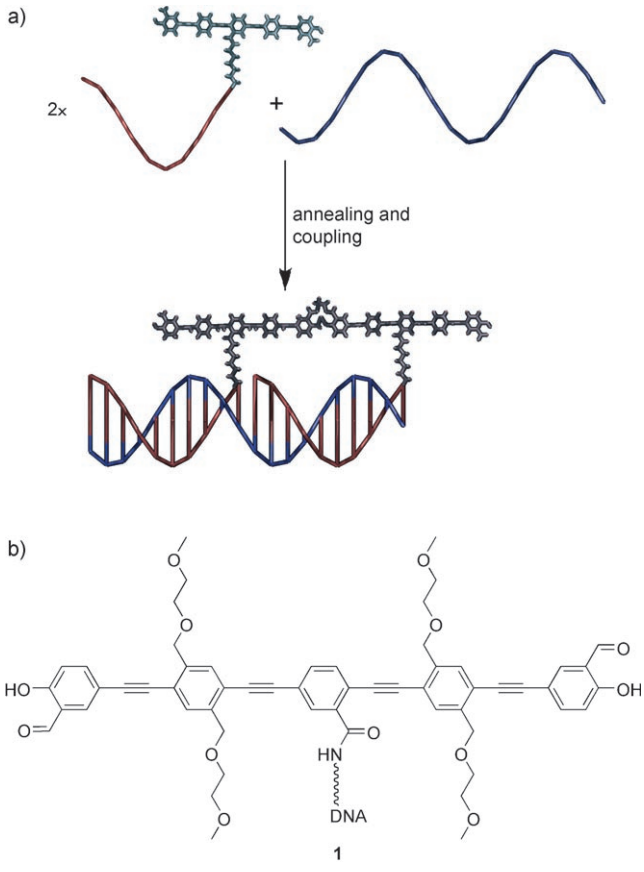

Figure 1. a) Strategy for building a covalent bridge over one helical turn in double-stranded DNA (dsDNA). b) Structure of the molecular-rod-type building block conjugated to DNA.

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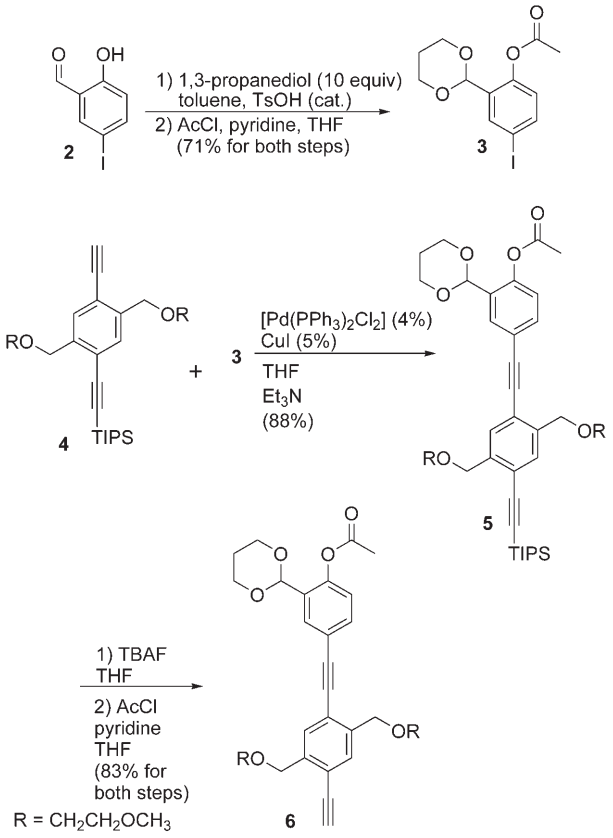
we demonstrate the DNA-templated formation of a dimer by the use of this strategy.

A number of specific requirements must be fulfilled in the organic module for the realization of the strategy outlined above. We chose an oligo(phenylene ethynylene) backbone to give the module a certain rigidity and avoid intramolecular cyclization (Figure 1b). The oligo(phenylene ethynylene) backbone was modified with hydrophilic methoxyethoxymethylene side chains to increase its solubility in both organic solvents and aqueous buffer solutions. The module **1** has a length of approximately 3.3 nm, which is increased to the desired distance for bridging one helical turn in DNA by the ethylenediamine bridge. For the formation of a metal–salen complex between two modules, the terminal positions of the module were functionalized with salicylaldehyde groups, which were protected prior to oligonucleotide conjugation. Finally, the organic module was functionalized with an activated ester group for conjugation to an oligonucleotide strand that had been modified at one terminus with an amino group.

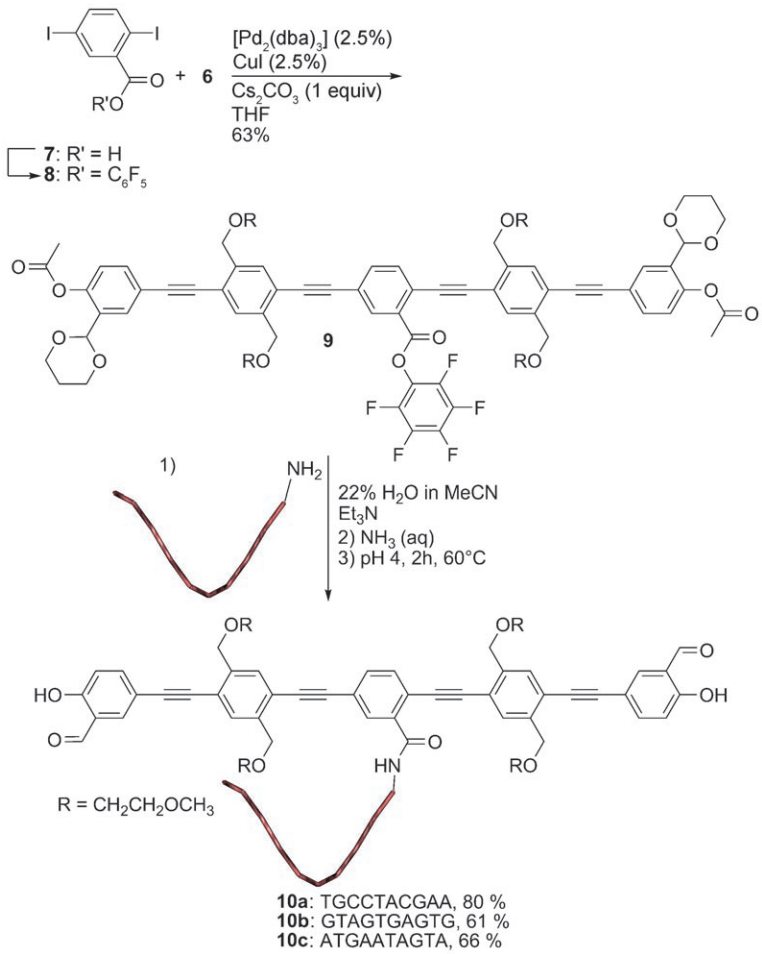
The introduction of salicylaldehyde groups at the terminal positions of the module required acetal protection of the aldehyde functionality and ester protection of the phenol group (Scheme 1). Thus, 4-iodosalicylaldehyde (**2**) was converted into **3** by standard protection procedures in 71% yield. The building block **4** was prepared by a previously reported procedure[11] and coupled in a Sonogashira reaction[12] to the protected 4-iodosalicylaldehyde **3**. Subsequent deprotection of the TIPS-protected acetylene **5** with TBAF was accompanied by partial hydrolysis of the phenol ester. Therefore, the crude product was treated with acetyl chloride and pyridine to reinstall the acetyl protecting group before the product **6** was isolated.

The central part of the module was prepared from **7**, which was converted into the activated pentafluorophenyl ester **8** (Scheme 2). The module was assembled in its full length by coupling two equivalents of compound **6** to the central unit **8**. The reaction proceeded without hydrolysis of the activated ester, and the product could be purified by flash chromatography on silica gel.

The module **9** is surprisingly stable towards hydrolysis and reacts readily with amino-modified oligonucleotides in a solvent mixture of 22% water in acetonitrile in the presence of triethylamine (Scheme 2). The reactions proceeded almost quantitatively, and the phenol and aldehyde groups were



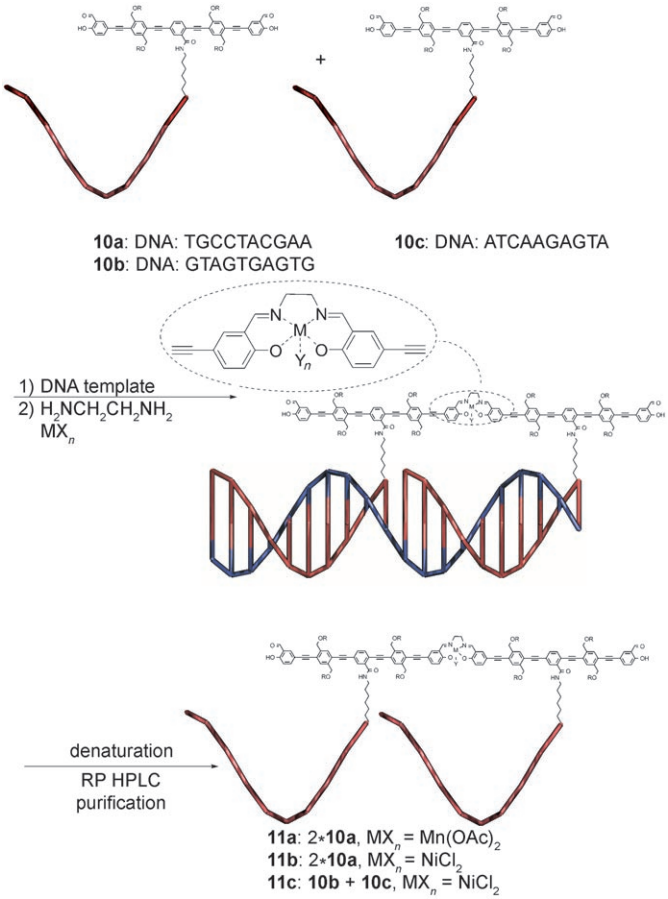
Scheme 1. Synthesis of the terminal moiety **6**. TBAF = tetrabutylammonium fluoride, TIPS = triisopropylsilyl, Ts = *p*-toluenesulfonyl.



Scheme 2. Synthesis of **10a–c** by formation of the module **9**, coupling to an amino-modified oligonucleotide, and subsequent deprotection of the salicylaldehyde groups. dba = dibenzylideneacetone.

deprotected in a one-pot reaction. The excess organic reactant **9** was removed by precipitation, and the resulting deprotected conjugates **10a–c** were purified by preparative reversed-phase (RP) HPLC and characterized by UV/Vis spectroscopy and MALDI-TOF MS. The HPLC retention times suggest that conjugates **10a–c** are very hydrophobic relative to nonmodified DNA strands (see the Supporting Information).

With the conjugates **10** in hand, we proceeded to test the templated coupling between two modules along the axis of a short dsDNA helix (Scheme 3). First, the homocoupling was



Scheme 3. DNA-templated dimerization of two molecular rods by metal–salen formation.

tested by using a 20-mer template with two repeated 10-mer regions complementary to the sequence of **10a**. The template (1 μM) and **10a** (2 μM) were annealed by slowly cooling the mixture from 90 °C to room temperature over two hours. Subsequently, the coupling was performed by adding ethylenediamine and $Mn(OAc)_2$. The dimer was isolated by RP HPLC and analyzed by UV/Vis spectroscopy and MALDI-TOF MS, which verified the formation of the dimer **11a**. HPLC purification was performed at 60 °C, at which temperature the DNA duplex denatures, whereas the Mn–salen complex is not degraded. The dimer **11a** was isolated in approximately 10% yield, as determined by the relative intensity of the UV absorption in the HPLC chromatogram. The formation of the Ni–salen complex is more efficient: The dimer **11b** was formed in 25% yield when $NiCl_2$ was used in place of $Mn(OAc)_2$. A heterodimer was obtained from two different sequences, **10b** and **10c**, which were annealed to a complementary DNA strand and then treated with ethylenediamine and $NiCl_2$ to give **11c** in 13% yield. No homodimers were observed in this reaction.

We believe that there are several reasons for the low yields observed for these reactions. In all cases, the starting materials were consumed, as shown by the HPLC chromatograms. The salicylaldehyde groups at the terminal positions of the starting materials and the dimer products can react with ethylenediamine and in turn form metal complexes with Mn or Ni to give a plethora of side products. Furthermore, the coupled modules are highly hydrophobic, and material might be lost as a result of aggregation or adsorption to glassware and other equipment, such as HPLC columns.

We repeated the three coupling reactions in Scheme 3 in the absence of a template to verify the directional effect of the template. In all cases, the desired product was formed in less than 2% yield. We also attempted to prepare a trimer by the application of **10a** and a 30-mer template with three repeated 10-mer regions. Disappearance of the starting material was observed; however, we were unable to identify the product by MALDI-TOF MS.

During the formation of the manganese–salen complex, Mn^{II} is oxidized in situ to Mn^{III}, which allows for the coordination of a third anionic ligand such as acteate or chloride from the buffer outside the salen plane. Hence, when analyzing the Mn–salen product **11a** by MALDI-TOF mass spectrometry, it is most often observed that another anion from the buffer or from the matrix is exchanged with the acetate of chloride anion at the metal center.

In summary, we have presented the synthesis of a 3.3 nm long oligo(phenylene ethynylene) building block, which was applied in the DNA-templated formation of an metal–salen-linked dimer along the axis of the DNA strand. This strategy for the programmed assembly and coupling of conjugated and potentially conducting molecules has the advantage over our previously reported strategy[10] that only one conjugate is required for the formation of a dimer or any potential higher-order structures. In future studies we aim to prepare a similar module that is more hydrophilic, and we hope to then be able to extend this approach to the formation of higher-order products, such as trimers and potentially much larger structures.

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