DNA-Directed Chemistry

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DNA-Programmed Glaser–Eglinton Reactions for the Synthesis of Conjugated Molecular Wires**

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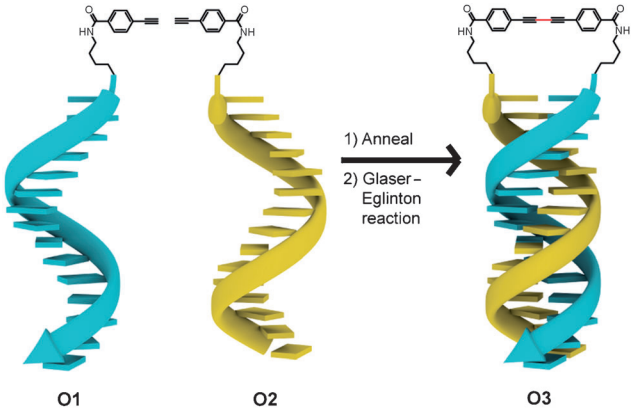
The development of novel molecular fabrication methods may lead to the production of complex functional nanomaterials and devices with discrete and monodisperse structures. Today, a large variety of small organic molecules with potentially useful electronic properties are available through classical synthetic approaches.[1] However, one of the major obstacles for taking advantage of the unique properties of individual molecular components for, for example, electronics is our lacking ability to connect such components to each other and to metallic contacts in a manner that is controllable at the molecular scale.[2] It is very difficult to assemble individual molecular components by top-down methods such as scanning probe microscopy and even more challenging to introduce covalent linkages between the components by these techniques. Herein we report a method to apply DNA-programmed assembly and covalent coupling of oligo(phenylene ethynylene) building blocks to form monodisperse conjugated molecular wires connected by carbon–carbon bonds and with a length of up to 8 nm.

DNA has proven to be a powerful tool for the self-assembly of complex nanostructures and the programmable organization of molecules and materials other than DNA itself.[3] Furthermore, DNA-programmed chemistry offers the opportunity to control chemical reactions between individual molecules and the formation of covalent linkages between molecular building blocks.[4] This approach has been demonstrated by the synthesis of encoded combinatorial libraries of organic molecules applied in the identification of new drug leads.[5] Only few examples of controlled assembly of molecular components into functional nanostructures utilizing DNA have been described.[6] We have previously reported on the DNA-programmed assembly and coupling of molecular wire fragments that consist of oligo(phenylene ethynylene) (OPE) units conjugated to DNA strands in different ways. Our previous chemical coupling strategy was based on the coupling of salicylaldehydes to form metal–salen linkages as was first reported by Czlapinski and Sheppard.[7] However, formation of the metal–salen complex is reversible and the link is labile in aqueous media. In spite of its coplanar ground structure it is structurally flexible. Furthermore, it is questionable if the linkage is electronically conducting.

We have therefore been exploring alternative coupling strategies to irreversibly form linear and conjugated linkages between molecular modules. Inspired by previous endeavors in the synthesis of conductive modules,[8] we speculated that 1,3-diyne linkages would be well suited to meet these demands. The 1,3-diynes can be obtained through the classic Glaser and Eglinton reactions, in which oxidative homocoupling reactions of terminal alkynes are promoted by a Cu^{I} or Cu^{II} source, and an amine base.[9,10] Glaser–Eglinton reactions in water have previously been reported.[11] Encouraged by these findings, we sought to develop a DNA-directed version that could form 1,3-diyne linkages between conjugated molecular building blocks. Unlike the classical Glaser–Eglinton reactions (GE reactions), it is possible to selectively perform heterocouplings of terminal alkynes by using DNA to direct the formation of nonsymmetrical products.

For initial tests, we designed a model system based on a simple end-of-helix architecture (Scheme 1) that consists of two aryl-alkyne modified 18-mer oligonucleotide sequences, **O1** and **O2**. The two oligonucleotide sequences were synthesized from the corresponding amino-modified oligonucleotide sequences using *N*-hydroxysuccinimide(NHS)-ester chemistry (see the Supporting Information). Upon annealing and subsequent GE reaction the ligated product **O3** would be obtained.



Scheme 1. DNA-directed Glaser–Eglinton reaction utilizing the end-of-helix architecture.

To investigate the effects of the pH value, the copper source, and copper ligand, a series of experiments were carried out (Figure 1). Oligonucleotides **O1** and **O2** were

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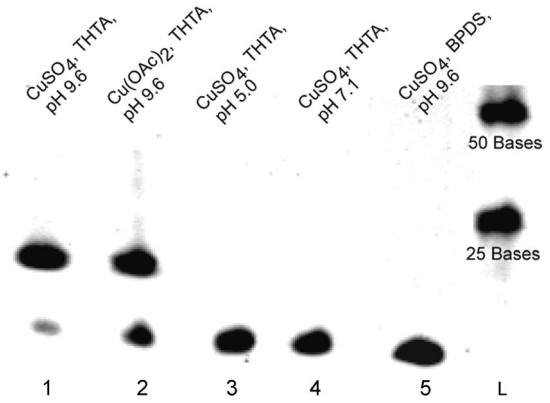


Figure 1. Denaturating PAGE analysis of the DNA-directed Glaser–Eglinton reactions.

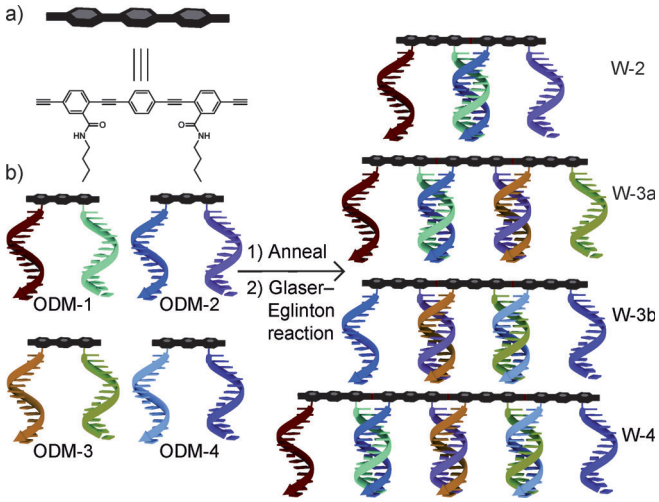


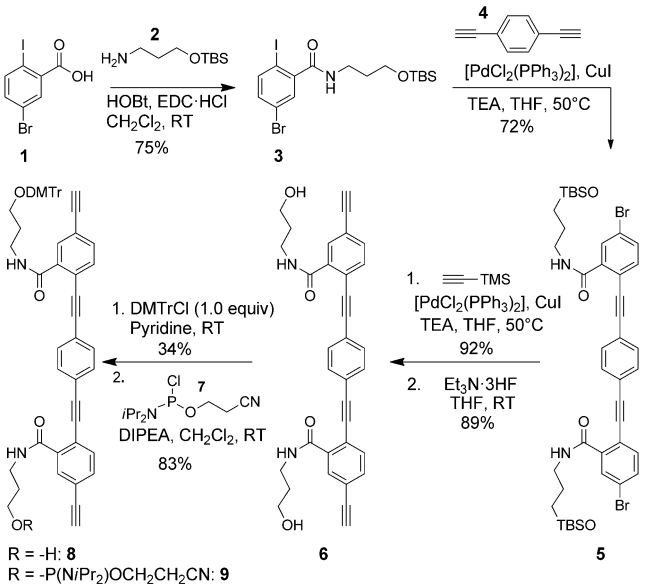
Figure 2. a) Schematic representation of the incorporated organic module. b) Oligomerization of ODM monomers by multiple Glaser–Eglinton reactions.

annealed at three different pH-values: 5.0, 7.1, and 9.6, and a mixture that consists of a copper(II) source ($CuSO_4$ or $Cu(OAc)_2$) and a ligand for copper (THTA[12] or BPDS[13]) was added. It should be noted that in analogy to Cu^I alone, Cu^I–phenanthroline complexes react rapidly with oxygen, and are well documented to generate oxygen species that can damage biomolecules such as DNA.[14] Extensive decomposition of DNA is observed when using Cu^I or Cu^I-phenanthroline. On the other hand, the THTA ligand is known to protect DNA from Cu^I-mediated degradation.[15] Denaturing PAGE analysis of the reactions showed no formation of **O3** for the reactions at acidic or neutral pH values (Figure 1, lanes 3 and 4, respectively); performing the reaction at basic pH led to major product formation (Figure 1, lane 2). These observations are consistent with the fact that both Glaser and Eglinton reactions require the presence of a base, which facilitates the formation of the reactive Cu–acetylide intermediate.[16] Further improvement is possible by switching to $CuSO_4$, which facilitates the almost complete conversion to **O3** (Figure 1, lane 1). The crucial role of the ligand for copper is clearly demonstrated by the absence of product when BPDS was employed (Figure 1, lane 5).

To confirm the identity of the observed product, a reaction mixture corresponding to lane 1 (Figure 1) was subjected to reversed phase (RP-)HPLC analysis, which showed nearly full conversion to a new product in consistency with the denaturing PAGE analysis. Moreover, MALDI-TOF mass analysis of the RP-HPLC purified product gave a mass of 11601 Da, in good agreement with the calculated mass for the GE product **O3** of 11602 Da.

In extension of the DNA-directed GE reaction between two molecules, the next step was to synthesize an OPE molecule that contains two terminal acetylene groups, since this will enable the assembly and coupling of more molecules to form conjugated linear oligomers (Figure 2a). By employing multiple DNA-directed GE reactions, we envisioned that several OPE molecules could be attached at their ends to form one conjugated entity of predetermined length (Figure 2b). These oligomeric products are calculated to span lengths of 4 nm, 6 nm, and 8 nm for the dimer (**W-2**), trimer (**W-3a** or **W-3b**), and tetramer (**W-4**), respectively. The required OPE molecule must be equipped with functionalities for incorporation into DNA.

In our strategy, the target molecule **9** is equipped with 4,4′-dimethoxytrityl (DMTr) and phosphoramidite functionalities for incorporation into a DNA oligonucleotide by automated DNA synthesis (Scheme 2). The synthesis of the organic module starts from 5-bromo-2-iodobenzoic acid **1**. Introduction of a molecular handle was achieved by an amide coupling with *tert*-butyldimethylsilyl(TBS)-protected 3-aminopropanol **2** in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide(EDC)·HCl and 1-hydroxybenzotriazol (HOBt) to give amide **3** in 75% yield. By utilizing the preference for C–I over C–Br bond insertion by the metal in Pd-catalyzed Sonogashira reactions,[17] we were able to react amide **3** selectively with 0.55 equiv of 1,4-diethynylbenzene



Scheme 2. Synthesis of OPE phosphoramidite **9**; TEA = triethylamine.

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(**4**) to give bromide **5** in 72% yield. The introduction of the terminal alkyne functionalities was achieved by a subsequent Sonogashira reaction with ethynyltrimethylsilane leading to **6** in an excellent yield. Global deprotection of the four silyl protection groups was accomplished under mild conditions with $Et_3N{\cdot}3HF$, resulting in **4** in 89% yield. Notably, deprotection using tetra-n-butylammonium fluoride (TBAF) instead failed and led to extensive decomposition. A statistical mono-DMTr protection of the two equivalent alcohol functionalities upon treatment of **4** with 4,4′-dimethoxytrityl chloride (DMTrCl) in pyridine gave a mixture of di-DMTr product, mono-DMTr product **8**, and diol **6**. However, column chromatography provided excellent separation of the product mixture and allowed for recycling of unreacted **6**. The desired mono-protected product **8** could be isolated in 34% yield after one reaction. Treatment of **8** with chloride **7** in dry dichloromethane and *N*,*N*-diisopropylethylamine (DIPEA) gave the desired phosphoramidite **9** in 83% yield. Due to the unstable nature of **9**, all automated DNA synthesis was carried out with freshly prepared samples.

A series of four 30mer oligonucleotides was prepared by automated DNA synthesis. During the synthesis, the phosphoramidite **9** was incorporated into the middle of each of the 30mer oligonucleotides. The synthesis thus gave four oligonucleotide-functionalized diacetylene modules (ODMs) consisting of the organic moiety with two 15mer oligonucleotides flanking its terminal regions (Figure 2). Low yields were observed in the couplings of phosphoramidite **9** and therefore prolonged coupling times and additional capping cycles were introduced to avoid truncated sequence byproducuts. The four ODMs were purified using RP-HPLC and were characterized by MALDI-TOF mass analysis (see the Supporting Information).

Performing the DNA-directed GE reactions between ODM sequences using slightly altered conditions compared to the model system proved beneficial. A mixture of a Cu^{I} and a Cu^{II} source was applied, and the reaction time was increased from 2 to 24 h. As observed by denaturing PAGE analysis (Figure 3a) oligomerization to dimers and trimers proceeds to a high degree (Figure 3a, lanes 2–4). The observed mobility difference between pure **ODM-1** (Figure 3a, lane 1) and the unreacted ODM monomers after GE reactions (Figure 3a, lanes 2–5) is caused by a difference in salt concentrations in the samples. It is possible to form the two unique trimers **W-3a** (lane 3) and **W-3b** (Figure 3a, lane 4) in comparable yields. However, when the number of GE reactions is increased for the formation of the tetramer **W-4**, the yield decreases (Figure 3a, lane 5), and considerable amounts of dimer and trimer products are also observed. Various conditions such as different salt concentrations and temperatures were tested, but in all experiments, the tetramer was formed in unproportional low yields compared to the trimer. We propose that the increased electrostatic repulsion and steric hindrance between the increasing number of DNA oligonucleotides in the assemblies cause the dramatic decrease in yield of the tetramer. For future studies, potential solutions to this obstacle could be to use PNA instead of DNA to avoid charge repulsion or to increase the length of the organic modules.

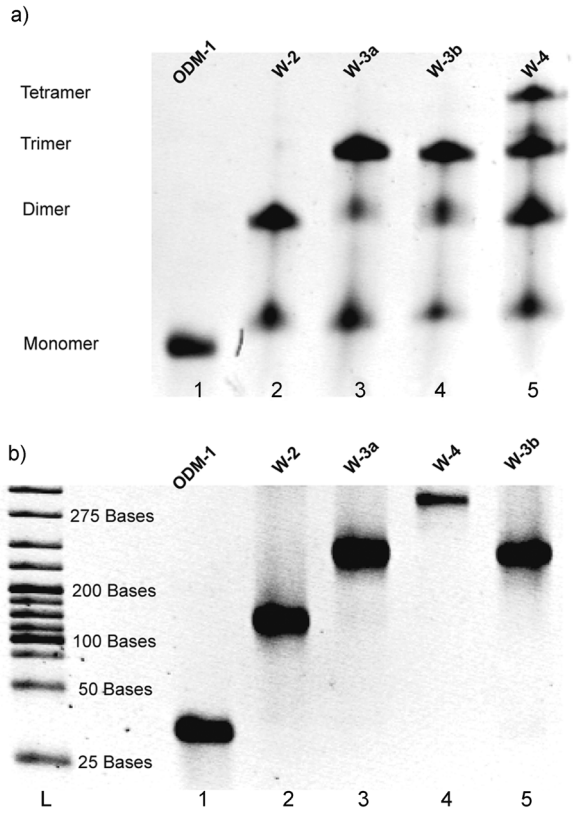

Figure 3. a) Denaturing PAGE analysis of DNA-directed Glaser–Eglinton oligomerization of ODM sequences. b) Denaturing PAGE analysis of purified ODM di-, tri-, and tetramer products.

It is noteworthy that the oligomers have much higher retention than linear oligonucleotides with the same base count, as seen by comparing lane 1–5, Figure 3b, with DNA ladder lane L. This observation may be explained by the more compact organization of the DNA in the ODM wires than in a linear DNA sequence. The oligomeric products of the DNA-directed GE reactions could be isolated by excision of the corresponding gel bands followed by extraction of the oligomers. The products are obtained in high purity as evident from denaturing PAGE analysis (Figure 3b). The identity of the dimer product **W-2a** was further confirmed by MALDI-TOF mass analysis (see the Supporting Information).

In summary, we have demonstrated that DNA-directed GE reactions are feasible and can proceed in high yield. The DNA-directed approach allows for the formation of non-symmetric GE products, which cannot be obtained in the classical intermolecular reaction. Furthermore, we have extended the scope of this novel DNA-directed reaction to the formation of molecular rods based on oligomerization of oligonucleotide-functionalized dialkyne modules (ODMs). This methodology enables the preparation of molecular rods of predetermined lengths ranging from 4 to 8 nm, which are potential conducting nanowires. The single-stranded DNA (ssDNA) sequences at the two termini of the wires may be utilized to specifically position the molecular wires in a more complex DNA structure such as DNA origami.[18,19] The ideal distance between ssDNA sequences extending from the same

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surface of a 2D DNA origami is approximately 6 nm, which corresponds well with the 6 nm length of trimer wire **W-3**. Potentially such short wires positioned on DNA origami could be coupled by further GE reactions and future studies will aim at making long conducting wires on DNA origami. In turn it may be envisioned that such a hybrid system is immobilized between electrodes to provide electronic contact to single molecules.

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