Syntheses of Organic Molecule–DNA Hybrid Structures

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ABSTRACT Investigation of robust and efficient pathways to build DNA–organic molecule hybrid structures is fundamentally important to realize controlled placement of single molecules for potential applications, such as single-molecule electronic devices. Herein, we report a systematic investigation of synthetic processes for preparing organic molecule–DNA building blocks and their subsequent elongation to generate precise micrometer-sized structures. Specifically, optimal cross-coupling routes were identified to enable chemical linkages between three different organic molecules, namely, polyethylene glycol (PEG), poly(*p*-phenylene ethynylene) (PPE), and benzene-tricarboxylate, with single-stranded (ss) DNA. The resulting DNA–organic molecule hybrid building blocks were purified and characterized by both denaturing gel electrophoresis and electrospray ionization mass spectrometry (ESI-MS). The building blocks were subsequently elongated through both the DNA hybridization and ligation processes to prepare micrometer-sized double-stranded (ds) DNA–organic molecule hybrid structures. The described synthetic procedures should facilitate future syntheses of various hybrid DNA-based organic molecular structures.

KEYWORDS: single-molecule electronics · DNA–organic molecule–DNA hybrid structures · bioconjugation of DNA · DNA extension

DNA has many important applications, such as the screening of organic reactions as novel molecular reporters,[1,2] sorting carbon nanotubes,[3,4] and as templates for construction of new nanostructures, including inorganic, organic, and metallic nanostructures.[5–14] The above applications are enabled by several remarkable properties from DNAs, namely, highly specific molecular recognition from base pairing, tailorable end-functional moieties, and a relatively rigid negatively charged backbone that could electrostatically absorb positively charged nanoparticles, molecules, or metal cations for templated growth of nanostructures.[15,16] Although the persistence length of dsDNAs is about 50 nm at physiological salt conditions,[17] they are fully stretched on metal or glass surface by air–water interface techniques, such as fluidic flow,[11] air flow,[12] droplet translation,[18] and spin-coater.[19] The DNA molecules are aligned on a surface of substrates through the air–water interface methods as the interactions of dsDNA with the surfaces raise the fraction of dsDNA molecules in aqueous solution. Thus, the portions of dsDNA molecules attach on the favorable surfaces when the remaining part of the dsDNA molecules is to be stretched by the air–water interface. Moreover, the favorable interactions between dsDNA and the surface of substrates not only minimize the conformational change of the stretched dsDNA but also prevent the detachment of the dsDNA from the surfaces.[13]

Recently, DNA–organic molecule hybrid structures have been touted as promising molecular-scale building blocks.[20,21] For instance, DNA–polypyrrole hybrids have led to the development of a new type of electrochemical DNA sensors.[20] Additionally, a DNA–organic molecule hybrid structure allowed locating and characterizing a single organic fluorophore.[22] However, the synthesis of DNA–organic molecule hybrids is challenging due to their overall poor solubility in organic solvents, low reactivities of the end-functional groups owing to steric hindrance for long DNA, and complex electrostatic interactions. Therefore, fundamental investigation of synthetic approaches for conjugation of DNA with organic molecules is required to better understand and expand DNA-based applications.

Various synthetic approaches for single-stranded DNA (ssDNA)–organic molecule hybrid oligomers have been previously reported, with DNA-templated cross-coupling reactions[23] or DNA synthesizer based on phosphoramidite chemistry,[24–26] in which the functionalized organic molecule was introduced into the ssDNA *via* one of its bases or *via* an end-functional group. However, the directionality of the ssDNA sequences obtained is only limited to either 5′→3′ (5′-ssDNA-3′-organic molecule-5′-ssDNA-3′) or 3′→5′ (3′-ssDNA-5′-organic molecule-3′-ssDNA-5′), which limits their further usage for DNA extension toward both directions. In contrast, conventional

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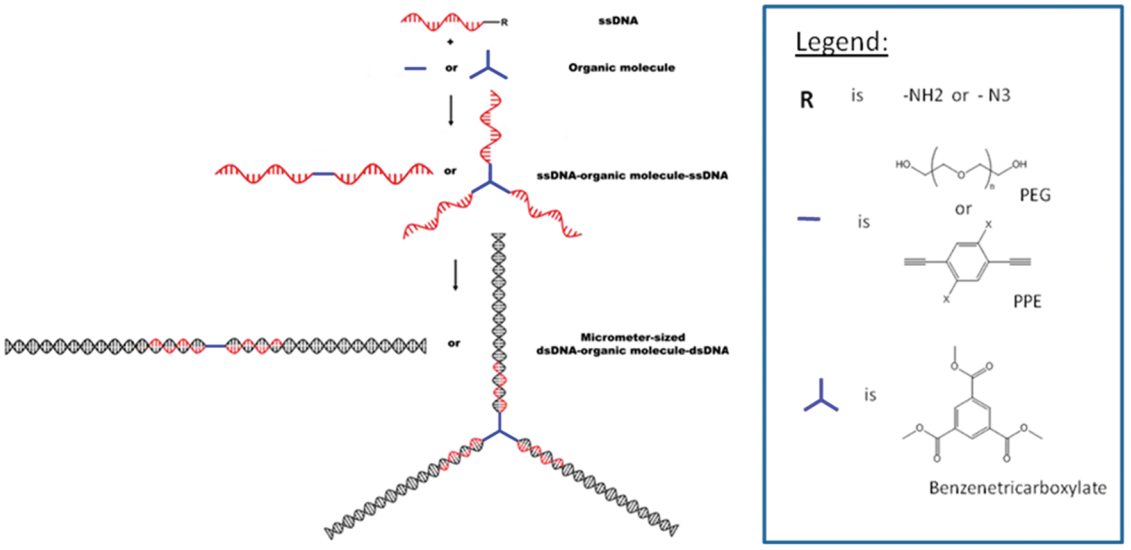


Scheme 1. Representative illustration of building organic molecule/micrometer-sized DNA multiblock structures.

cross-coupling reaction allows the choice of the direction of each tethered ssDNA sequence and thus provides a more versatile approach.[22,27−29]

For instance, the ssDNA−organic molecule−ssDNA building block can consist of four distinct possibilities: (*i*) 5′-ssDNA-3′-organic molecule-3′-ssDNA-5′, (*ii*) 5′-ssDNA-3′-organic molecule-5′-ssDNA-3′, (*iii*) 3′-ssDNA-5′-organic molecule-5′-ssDNA-3′, and (*iv*) 3′-ssDNA-5′-organic molecule-3′-ssDNA-5′. A versatile synthetic method that allows rapid access to all of the above possibilities is a key factor to facilitate elongation of the tethered DNA with common DNA elongation techniques such as hybridization[22] or polymerase chain reaction (PCR).[27] Therefore, it is necessary to identify the most efficient cross-coupling reactions to synthesize ssDNA−organic molecule−ssDNA building blocks with our desired DNA directionality.

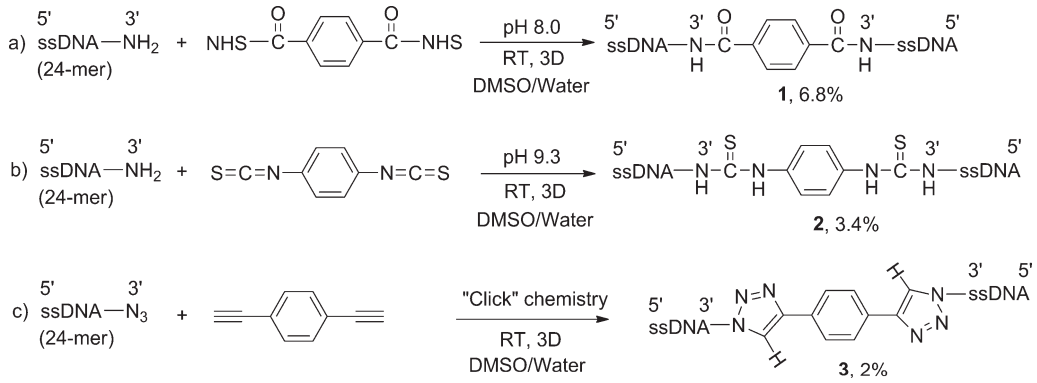
DNA elongation has been recently utilized to assemble complex and novel DNA-based nanostructures, which include various two-dimensional and three-dimensional structures, as well as organic molecule−micrometer-sized dsDNA hybrid structures.[22,27,30−33] Moreover, as a "bottom-up" technique, DNA elongation (especially, DNA hybridization) has been applied to construction of nanoelectronic devices utilizing DNA as the template for metallization. Braun and co-workers reported that two metal electrodes having a ~14 μm gap were electrically connected using both DNA hybridization and ligation processes, with subsequent metallization of the micrometer-sized dsDNA structure[11] as well as DNA-assisted carbon nanotube field-effect transistors.[34] Furthermore, Woolley and co-workers demonstrated DNA-templated "tribranched" nanostructures and their DNA metallization.[35] Collectively, these pioneering works in utilizing DNA as a template for electrode contacts with nanostructures have paved the way toward DNA-based molecular electronics.

Despite its great promise, the above-described approaches for making electrical contacts to a single organic molecule do not allow controlled assembly of precise organic molecule−DNA structures, which is a crucial precursor for DNA-based molecular electronics. Herein, we report the investigation of several cross-coupling reactions for the synthesis of various organic molecule−ssDNA building blocks and their subsequent elongation processes to generate both dibranched and tribranched organic molecule−dsDNA nanostructures (Scheme 1).

RESULTS AND DISCUSSION

Synthesis of Organic Molecule−ssDNA Building Blocks. To investigate the reactivity of functionalized DNA with organic molecules to synthesize organic molecule−ssDNA building blocks, we focused our initial investigations on three commonly used cross-coupling reactions, namely, amide-coupling reaction, isothiourea bond formation, and "click" chemistry (Scheme 2). The goal of this initial study was to determine which cross-coupling reaction is capable of providing the highest yield for our desired product. To this end, we first examined the reactions of 3′-amine- or 3′-azide-terminated 24-mer ssDNA with 1,4-difunctionalized benzene derivatives. The resulting products were then purified through denaturing gel electrophoresis and further characterized by ESI-MS.

Primary amine moieties have been commonly used in both bioconjugation and organic reactions due to their high reactivity and the ability to form stable covalent bonds (*e.g.*, amide and isothiourea).[36,37] Thus, we employed the amide-coupling reaction and isothiourea bond formation with NHS succinimidyl ester and isothiocyanate, respectively. The conditions used here were based on those reported in literature which previously provided optimal yield for each reaction.



Scheme 2. Reactivity of several cross-coupling reactions to synthesize organic molecule−bis(ssDNA) triblock oligomers; ODN = oligodeoxynucleotide and NHS = *N*-hydroxysuccinimide.

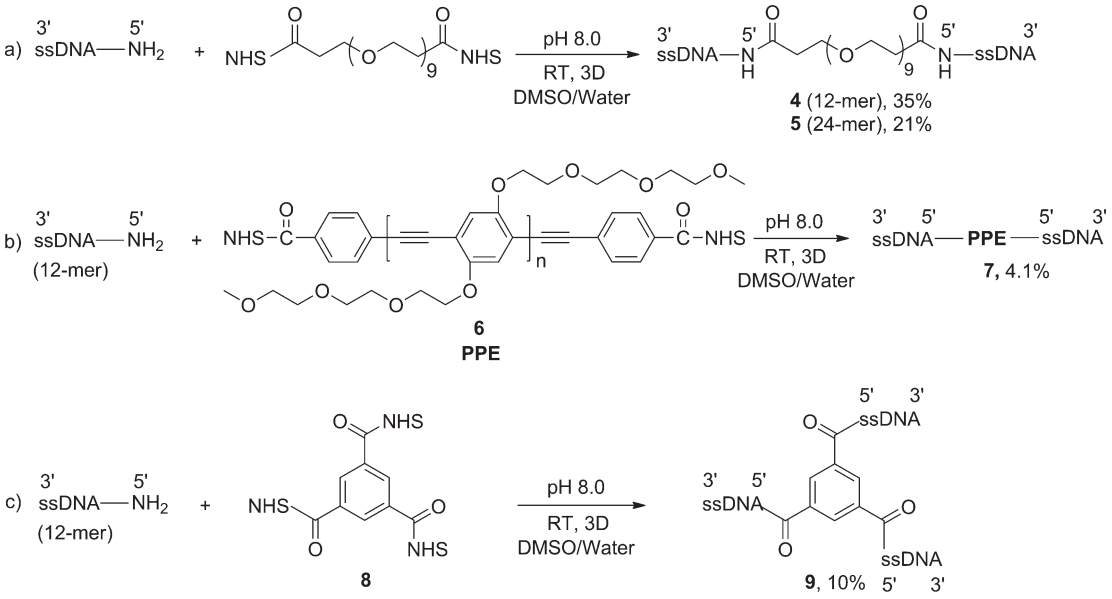
Using the amide-coupling reaction, 1,4-disuccinimidyl terephthalate was conjugated with an 5′-aminated 24-mer ssDNA at pH 8.0.[27] This condition afforded **1** in 6.8% yield with an observed mass value at the expected 15416.0 Da (calculated mass value: 15416.1 Da). The isothiourea bond formation was achieved by cross-coupling of 5′-aminated 24-mer ssDNA with 1,4-phenylene diisothiocyanate at pH 9.3. This condition afforded **2** in 3.4% yield with an observed mass value at 15477.3 Da (calculated mass value: 15478.3 Da).

Last, the commonly employed "click" chemistry was also investigated because of its reportedly high tolerance of other functional groups and excess of either nucleophilic or electrophilic impurities during its reaction.[38,39] For the "click" chemistry, 5′-azide-terminated 24-mer ssDNA (6 mol in 6 μL water) reacted with 1,4-diethynylbenzene (1.8 nmol) in presence of tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA, 1.2 nmol) and copper(I) iodide (0.5 nmol). Compound **3** was obtained in 2% yield after purification with a broad ESI-MS spectrum around 15600 Da (calculated mass value: 15606.2 Da).

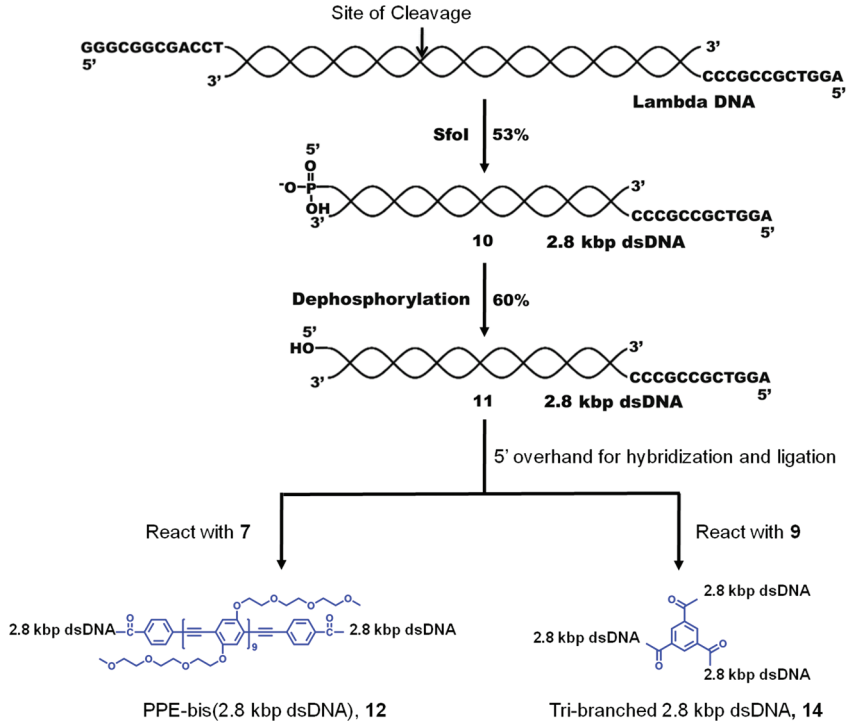
From our initial studies, we observed that the conventional cross-coupling reactions gave relatively low reaction yields. We hypothesized that the low reactivity of the cross-coupling reactions arises from both electrostatic repulsion and steric hindrance of the ssDNA, as well as poor solubility in aqueous solution once ssDNA is attached to the organic molecule. Additionally, we observed that the reaction of ssDNA with phenyl substrates is less efficient, presumably due to steric hindrance, as compared to linear alkane substrates. This hypothesis was substantiated by our observation that the linear substrate, disuccinimidyl suberate, afforded a much higher isolated yield *via* the amide-coupling reaction than the phenyl-based 1,4-disuccinimidyl terephthalate substrate.[27]

PEG molecules are readily available in different molecular weights. They have previously been used as spacers to define precise gap distance between the electrodes for the seed-mediated growth of gold nanorods.[40] In this instance, we have utilized commercially available bis-dPEG$_9$ NHS ester in the amide-coupling reaction to selectively afford PEG$_9$-bis-12-mer ssDNA **4** in 35% yield with observed mass value at 9026.0 Da (calculated mass value: 9025.9 Da) (Scheme 3). It was noted that, when the above reaction was repeated with a 24-mer ssDNA, PEG$_9$-bis-24-mer ssDNA **5** was obtained in a low yield, ~21% (observed mass value at 15763.1 Da; calculated mass value: 15764.3 Da). Hence, the reactivity of the amide-coupling reaction was affected by the size of the oligonucleotides. We believe that, due to both its reduced charges and steric hindrance, the 12-mer ssDNA gave a higher yield than 24-mer ssDNA (35 and 21%, respectively) (Scheme 3). In addition, we observed an increase in the reaction yield using the PEG moiety compared to the phenyl-based moiety. We attributed this to the better solubility of PEG in the DMSO/water mixture, as well as the flexible backbone of bis-dPEG$_9$ NHS ester. This PEG derivative gave 40% higher yield compared to bis(sulfosuccinimidyl) suberate (BS^3), which was used as a substrate having a good solubility in the DMSO/water mixture in our previous report.[27] We again attribute this to the flexible PEG backbone, which allows the end-functional groups to be more accessible for the reaction.

Poly(*p*-phenylene ethynylene) (PPE) derivatives are widely used as molecular wires in molecular electronics due to their conjugated structure and rigidity.[41,42] The synthetic methodologies for PPE derivatives, containing various side chains and end-capping functional groups, have been well-established.[43,44] Here, we employed PEG moieties as side chains to increase the solubility of PPE in the DMSO/water mixture needed for the subsequent amide-coupling reaction. Bis-NHS end-functionalized PPE (NHS-PPE) bearing triethylene glycol moieties as side chains was synthesized according to previously reported procedures (see Supporting Information). After polymerization, the product was purified by preparative gel permeation chromatography (GPC) to remove the unreacted end-capping molecules. The NHS end-capped PPE **6** was further characterized by ^{1}H NMR, FT-IR, and GPC. The GPC profile against polystyrene standards showed a number



Scheme 3. Amide-coupling reactions to form polymer−bis(ssDNA) triblock oligomers and an organic molecule−tris(ssDNA) multiblock oligomer; PPE = poly(*p*-phenylene ethynylene).



Scheme 4. Preparation of the micrometer-sized DNA fragment from λ-DNA using a restriction enzyme and a phosphatase and DNA hybridization/ligation for building micrometer-sized organic molecule−DNA hybrid structures.

averaged molecular weight (M_n) of ~3500 Da and a polydispersity index (PDI) of 1.87. The presence of the NHS end-functional groups in **6** was confirmed by both NMR (2.96 ppm)[27] and FT-IR (1770 and 1742 cm^{-1}).[45] Next, NHS-PPE **6** was conjugated to a 12-mer ssDNA using the amide-coupling reaction conditions described earlier[22,27] to yield the ssDNA−PPE−ssDNA building block **7** with a 4.1% isolated yield (Scheme 3). The ESI-MS molecular weight for **7** was found to be 12 818 Da, which is in good agreement with the calculated mass of 12 120 Da. The calculated mass value was from the sum of the molecular weight of two 12-mer ssDNAs and GPC-based molecular weight of polymer **6**. The undesired product, the monosubstituted ssDNA−PPE, was also observed in denaturing gel electrophoresis and ESI-MS (see Supporting Information).

In addition to linear building blocks, a tribranched building block was also synthesized by amide coupling

of **8** to a 3′-aminated ssDNA. The resulting mixture was analyzed by 15% denaturing TBE-urea gel electrophoresis. After gel extraction and purification through a desalting column, the tribranched building block **9** was obtained in 10% yield with an observed mass value at 12978.5 Da (calculated mass value: 12977.6 Da). Additionally, **9** was observed to appear at the 48-mer band position on the gel, although it is only a 36-mer (see Supporting Information). This may be that tribranched building block **9** has a slower electrophoretic mobility relative to linear organic molecule/DNA oligomers of similar molecular weights due to different conformations.[25,46]

Synthesis of Micrometer Length dsDNA−Organic Molecule−dsDNA Structures. The length of the DNA segment can be effectively elongated to several micrometers through DNA hybridization. For future placement of a single organic molecule, a long dsDNA will allow easy visualization to locate it on a substrate surface. Furthermore, the length of a micrometer-sized structure is comparable to the feature size that can be fabricated utilizing standard laboratory photolithographic techniques for making contacts with microscopic electrodes. Thus, nanoscale devices may subsequently be probed by a regular probe station. Hence, we extend the length of the short ssDNA to construct micrometer-sized dsDNA−organic molecule−dsDNA structures.

Micrometer-sized dsDNA extended from the ssDNA−organic molecule building blocks can be readily prepared from DNA hybridization, followed by ligation. For the DNA hybridization/ligation technique, a micrometer-sized dsDNA, containing both a cohesive end and a blunt end, is required. Thus, we used a commercially available λ-DNA (48.5 kbp dsDNA) to obtain various lengths *via* restriction enzymes. Among the numerous potential restriction enzymes, *SfoI* provided a 2.8 kbp dsDNA fragment (contour length: ~1 μm), bearing both a 5′ blunt end at one end and a 12-base 5′ overhang (5′-AGGTCGCCGCCC-3′ sequences) at the other end from λ-DNA (Scheme 4). The 12-mer cohesive end was generated by the incubation of the λ-DNA at 65 °C for 5−10 min.[47]

Dephosphorylation of the 2.8 kbp dsDNA with alkaline phosphatase (CIP) was performed to generate both a 5′ blunt end and a dephosphorylated λ-DNA fragment **11** (Scheme 4). It is noted that the dephosphorylation step is necessary to prevent further ligation of the 5′ blunt end, thus averting an undesired 5.6 kbp self-ligated dimer structure.[22] DNA ligation step is critical to avoid potential dehybridization arising from the nicks of hybridized structures. Therefore, the remaining 12-mer overhang of 2.8 kbp dsDNA **11** was only subjected to elongate the ssDNA−organic molecule−ssDNA building blocks through the DNA hybridization/ligation. From our previous result, we found that *E. coli* DNA ligase provided an efficient approach to ligate the hybridized DNA structures, while the DNA ligase

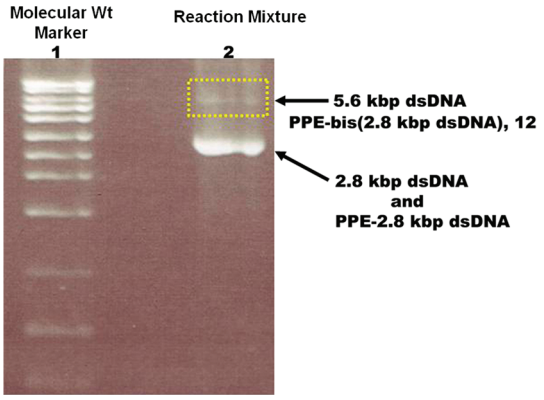


Figure 1. Agarose gel electrophoresis image after the hybridization/ligation of PPE-bis(ssDNA) triblock oligomer 7 with the 2.8 kbp dsDNA 11. Lane 1: DNA molecular weight ladder. Lane 2: the reaction mixture. In lane 2, the top weak band in the yellow box indicates a desired product, PPE-bis (2.8 kbp dsDNA) 12, and the bottom band shows undesired products, unreacted 2.8 kbp dsDNA and PPE-2.8 kbp dsDNA.

prevented the possible formation of a dimer structure stemming from self-ligation of its blunt-ended micrometer-sized DNA fragments.[22,48] Hence, with *E. coli* DNA ligase, we successfully proceeded to synthesize the PEG-bis (2.8 kbp dsDNA) structure (see Supporting Information).

In addition to *E. coli* DNA ligase, we also investigated the use of the 9°N DNA ligase to synthesize **12**. 9°N DNA ligase is active at high temperature (45−90 °C),[49] whereas *E. coli* DNA ligase is deactivated at 65 °C within 10 min.[48] Therefore, both DNA hybridization and ligation processes can be achieved *in situ* using 9°N DNA ligase. Furthermore, 9°N DNA ligase will not ligate short 4-mer overhangs which are typical of restriction enzyme digested products; instead, it will efficiently ligate 12-mer overhangs.[49] Using 9°N DNA ligase, we conducted the DNA hybridization/ligation of ssDNA−PPE−ssDNA **7** with the 2.8 kbp dsDNA **11**, as shown in Scheme 4. After the reaction, the resulting mixture was analyzed using 1% agarose gel electrophoresis.

In Figure 1, the top band in lane 2 is our desired product, PPE-bis (2.8 kbp dsDNA) **12** because this band appears near the 6 kbp dsDNA from the molecular weight marker in lane 1, whereas the bottom band is from both the 2.8 kbp DNA and PPE-2.8 kbp dsDNA adducts. In our previous study, we found that this DNA hybridization/ligation yielded a primary undesired product, which is the dye molecule−micrometer-sized dsDNA.[22] We also performed two different control experiments to monitor the self-ligation process of the phosphorylated 2.8 kbp dsDNA **10** and the dephosphorylated 2.8 kbp dsDNA **11**. In both control experiments, self-ligation products in **10** and **11** were not observed (see Supporting Information). On the basis of these results, we confirmed that **12** is being successfully synthesized from **6** *via* both DNA hybridization and ligation processes with 9°N DNA ligase.

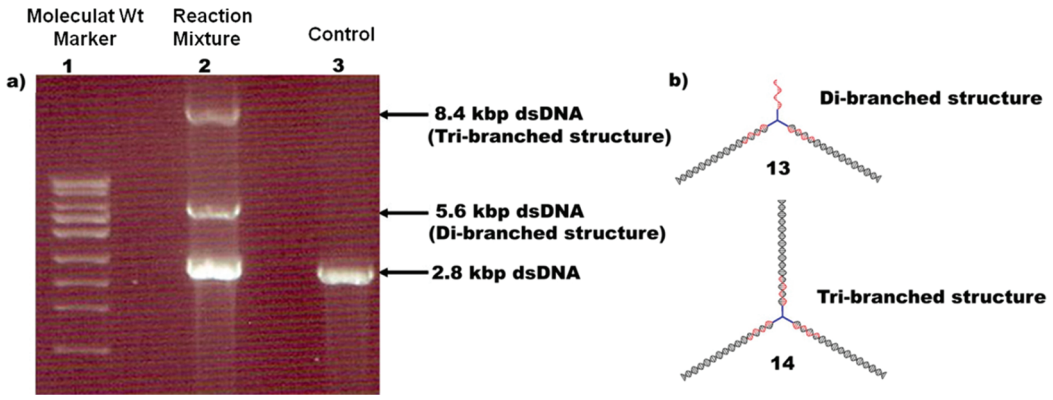


Figure 2. (a) Agarose gel electrophoresis image after the hybridization/ligation of multiblock oligomer 9 with the 2.8 kbp dsDNA 11. (b) Schematic structures of an organic molecule−bis(2.8 kbp dsDNA) dibranched structure 13 and organic molecule−tris(2.8 kbp dsDNA) tribranched structure 14. (Lane 1, 1 kbp DNA molecular weight ladder; lane 2, the reaction mixture; lane 3, control experiment. In lane 2, the top band indicates an organic molecule−tris(2.8 kbp dsDNA) tribranched structure 14; the middle band demonstrates an organic molecule−bis(2.8 kbp dsDNA) dibranched structure 13; and the bottom band shows unreacted a 2.8 kbp dsDNA and an organic molecule−2.8 kbp dsDNA diblock structure.)

In addition to **4**, the tribranched **9** was also extended with **11** by 9°N DNA ligase, *E. coli* DNA ligase, and T4 DNA ligase (Scheme 4). We first used 9°N DNA ligase to construct the tribranched compound **14**, but upon analysis with agarose gel electrophoresis, we instead observed the formation of the dibranched **13**. Alternatively, using *E. coli* DNA ligase as the ligation reagent afforded a very faint band of **14**, but the dibranched **13** was again observed to be the dominant product after gel analysis. Finally, upon the usage of T4 DNA ligase, the reaction yield of **14** improved noticeably.[50] Agarose gel electrophoresis showed the desired product being generated after both DNA hybridization and ligation processes of **9** with T4 DNA ligase.

As shown in Figure 2, lane 2 showed three bands. The top band is assigned to the organic molecule−tribranched dsDNA structure **14** because the molecular weight of tribranched dsDNA structure **14** is higher than the 10 kbp dsDNA in the DNA molecular weight ladder (Figure 2, top band in lane 1). We attribute that the electrophoretic mobility of the tribranched dsDNA structure **14** is slower than that of a linear DNA structure with a similar molecular weight to the shape effect, previously reported for other structures.[25,46] The middle band is assigned to the organic molecule−dibranched dsDNA structure **13**, and the bottom band comprises a mixture of the unreacted 2.8 kbp dsDNA and organic molecule−2.8 kbp dsDNA adducts. As a control experiment, **9** was omitted and only the 2.8 kbp dsDNA **11** was used to carry out the hybridization/ligation; as a result, we observed only 2.8 kbp dsDNA (lane 3, Figure 2). We thus concluded that T4 DNA ligase demonstrates the highest level of ligation activity for our targeted reactions, compared with 9°N DNA ligase and *E. coli* DNA ligase.

CONCLUSION

In summary, three cross-coupling reactions were used to prepare DNA−organic molecule−DNA building blocks. We observed that the amide-coupling reaction afforded these building blocks in the highest yield. Furthermore, we found that the amide-coupling reaction yield was increased when a flexible substrate was used together with a shorter ssDNA. We obtained the ssDNA−organic molecule−ssDNA building block with a yield of as high as 35% using 12-mer ssDNA with a PEG building block. In addition, we report an effective protocol to extend the organic molecule−ssDNA building blocks by hybridization with micrometer-sized dsDNA fragments. After DNA ligation, micrometer-sized DNA−organic molecule structures were successfully synthesized. The development of the synthetic approaches will facilitate future construction of two-terminal and three-terminal DNA-based nanoscale electronic devices.

EXPERIMENTAL PROCEDURES

General Protocols for the Synthesis of Organic Molecule−ssDNA Building Blocks (1−3). 5′-Functionalized ssDNA (12 nmol, 6 μL of 50 mM buffer solution) was added to a DMSO solution (20 μL) with the desired organic molecule (3.6 nmol) at room temperature and incubated for 3 days. To the resulting mixture was added a "Blue Juice" gel loading buffer (30% glycerol with 0.2% bromophenol blue, Invitrogen) and was loaded onto a denaturing 15% TBE (tris-borate-EDTA)−urea gel. Electrophoresis was then carried out at 180 V for 1 h in TBE buffer to allow separation of the excess ssDNA and the molecule−ssDNA product. The resulting gel was imaged under a UV excitation at 260 nm. The desired product was excised from the gel on a TLC (thin layer chromatography) as a template for visualization under a UV lamp at 260 nm. The product was extracted using a 0.2 M NaCl (aq) solution for 12 h with agitation, followed by further purification to remove the inorganic salts with a desalting column (NAP-25 column). The yield of organic molecule−ssDNA product was

measured by a NanoDrop 1000 UV spectrophotometer (Thermo Scientific).[51]

5′-Azide-Terminated ssDNA. 5′-Amine-terminated 24-mer ssDNA (12 nmol in 6 μL of 50 mM borate pH 8.0 buffer solution) was added to *N*-succinimidyl 3-azidopropionate (0.12 μmol) in DMSO (20 μL) and left to react overnight at room temperature.[52] After passing the resulting mixture through a NAP-25 desalting column, the desired product 5′-azide-terminated 24-mer ssDNA was obtained in 75% yield determined using a NanoDrop 1000 UV spectrophotometer (observed mass value = 7738.9 Da, calculated mass value = 7740 Da).

1,3,5-Benzenetricarboxylic Acid NHS Ester, 8.[53]. *N*-Hydroxysuccinimide (780 mg, 6.78 mmol) was added to 1,3,5-benznentricarbonyl trichloride (0.5 g, 1.88 mmol) in DMF (3 mL) at room temperature. The mixture was stirred at room temperature for 30 min and left to react at 80 °C overnight under argon. The reaction mixture was then cooled to room temperature, washed with saturated ammonium chloride solution (50 mL), and extracted with methylene chloride (30 mL). The organic layer was dried over anhydrous magnesium sulfate ($MgSO_4$), filtered, and concentrated *in vacuo*. Following recrystallization with a mixture of CH_3CN and hexane, compound **7** was obtained as a white solid in 30% yield. ^1H NMR (400 MHz, $CDCl_3$): δ 9.14 (s, 3H), 2.94 (s, 12H). ESI-MS: 501.0 (calcd 501.0).

Organic Molecule−Tribranched ssDNA Building Block, 9. **7** (3.0 nmol in 20 μL of DMSO) was added to a solution containing 12-mer ssDNA (12 nmol in 6 μL of 50 mM borate pH 8.0 buffer) with 5′ phosphate and 3′ amine termini at room temperature for 3 days. The desired tribranched building block **9** was obtained in 10% yield determined with a NanoDrop 1000 UV spectrophotometer after purification using a desalting column.

Preparation of 2.8 kbp dsDNA, 11. λ-Phase DNA (50 μg, 1.55 pmol) was added to the solution of *SfoI* restriction enzyme (5 μL, 50 units) in the supplied NEBuffer 4 (500 μL, 1×) at room temperature. The mixture was incubated at 37 °C for 1 h[54] and then incubated at 65 °C for 10 min, subsequently. The resulting product **10** was obtained in 53% after 1% agarose gel electrophoresis and then gel extraction. The 2.8 bkp dsDNA **10** (1.45 μg, 0.78 pmol) was added to the solution of alkaline phosphotase, calf intestinal (CIP) (0.2 μL, 2 unit), in the supplied NEBuffer 3 (150 μL) at room temperature.[55] The mixture was incubated at 37 °C for 1 h, and then 2.8 kbp dsDNA **11** was obtained in 80% isolated yield after 1% agarose gel electrophoresis and gel extraction.

PPE−Bis(2.8 kbp dsDNA) Triblock Structure, 12. ssDNA−PPE−ssDNA triblock oligomer **6** (2.7 ng, 0.21 pmol) and 9°N DNA ligase (1 μL) were added to the 2.8 kbp dsDNA **11** (1 μg, 0.54 pmol) in DNA ligase buffer (15 μL) at room temperature. The mixture was incubated at 68 °C for 10 min, followed by slow cooling to room temperature. The product was analyzed by a 1% agarose gel electrophoresis.

Organic Molecule−Tris(2.8 kbp dsDNA) Tribranched Nanostructure, 14. Tribranched oligomer **9** (2.8 ng, 0.21 pmol) and T4 DNA ligase (0.8 μL) were added to the 2.8 kbp dsDNA fragment **11** (1.4 μg, 0.76 pmol) in its supplied buffer solution (15 μL) that was added at room temperature. The mixture was incubated at 68 °C for 10 min, followed by slowly cooling to room temperature, and then the product was confirmed by a 1% agarose gel electrophoresis.

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Supporting Information Available: Detailed synthetic procedures for PPE-NHS and ESI-MS spectra, and gel images of PAGE and agarose gel electrophoresis for organic molecule−DNA building blocks and structures. This material is available free of charge *via* the Internet at http://pubs.acs.org.

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