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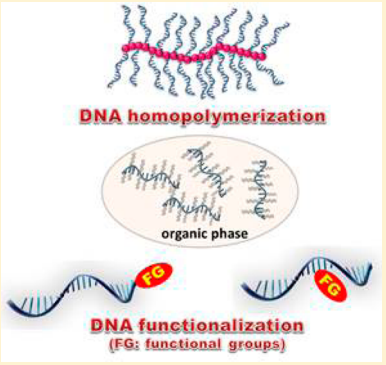
Nucleic Acid Chemistry in the Organic Phase: From Functionalized Oligonucleotides to DNA Side Chain Polymers

Kai Liu,[†] Lifei Zheng,[†] Qing Liu, Jan Willem de Vries, Jennifer Y. Gerasimov, and Andreas Herrmann*

Zernike Institute for Advanced Materials, Nijenborgh 4, 9747 AG Groningen, The Netherlands

Supporting Information

ABSTRACT: DNA-incorporating hydrophobic moieties can be synthesized by either solid-phase or solution-phase coupling. On a solid support the DNA is protected, and hydrophobic units are usually attached employing phosphoramidite chemistry involving a DNA synthesizer. On the other hand, solution coupling in aqueous medium results in low yields due to the solvent incompatibility of DNA and hydrophobic compounds. Hence, the development of a general coupling method for producing amphiphilic DNA conjugates with high yield in solution remains a major challenge. Here, we report an organic-phase coupling strategy for nucleic acid modification and polymerization by introducing a hydrophobic DNA−surfactant complex as a reactive scaffold. A remarkable range of amphiphile−DNA structures (DNA−pyrene, DNA−triphenylphosphine, DNA−hydrocarbon, and DNA block copolymers) and a series of new brush-type DNA side-chain homopolymers with high DNA grafting density are produced efficiently. We believe that this method is an important breakthrough in developing a generalized approach to synthesizing functional DNA molecules for self-assembly and related technological applications.



INTRODUCTION

The combination of the sequence addressability of DNA and the diversity of functional groups that may be introduced at various sites of the molecule (nucleobase, sugar, or phosphodiester backbone) by conventional synthesis[1−6] has led to the widespread implementation of DNA in diverse applications.[7−12] Conjugates of DNA with organic molecules,[13−16] polymers,[17−20] metal coordination complexes,[21] and nanoparticles[22,23] have served as important tools in the development of new biohybrid materials and reagents that are designed for use in template-directed synthetic chemistry,[24,25] catalysis,[26,27] biomimetics,[28] magnetics,[29] (opto)-electronics,[30−32] diagnostics,[33−35] biomedicine,[36−38] and therapeutics.[39,40]

Presently, solid-phase synthesis and solution-phase coupling are the two methodologies one can employ to chemically modify the natural DNA scaffold. Solid-phase synthesis of functionalized DNA most often relies on a commercially available automated DNA synthesizer. Terminal functionalization or the introduction of non-natural nucleotides can be easily integrated into the automated synthesis protocol mostly relying on phosphoramidite chemistry. Alternatively, postsynthetic modification may be carried out on the solid support outside of the synthesizer. In this case, not only phosphoramidite chemistry is useful for functionalization but also other transformations are suited for DNA modification including amide formation, Michael addition, or Huisgen cycloaddition.[2,3] However, all the approaches on the solid phase have the following general limitations: (I) Yields are usually lower compared to reactions in solution due to the heterogeneous character of the reaction. (II) Special care needs to be taken in solvent selection if a polymer support is employed for DNA synthesis due to solvent-dependent swelling properties of cross-linked polymer resins. (III) Finally, the new products or introduced functional groups need to be stable against the harsh basic deprotection conditions used for removing the protective groups on the nucleic acid scaffold. Thus, modification strategies of DNA based on solid-phase synthesis remain prohibitive for some well-established coupling reactions and chemical functionalities. The absence of a general methodology to introduce various functional moieties renders DNA modification by full solid-phase synthesis a realistic approach only when significant effort can be dedicated to overcoming the many synthetic challenges involved.

Solution-phase DNA modification in the aqueous environment has proven to be highly versatile and efficient in coupling hydrophilic molecules at various DNA positions. However, the synthesis of amphiphilic DNA hybrid materials containing hydrophobic functional moieties is less efficient due to the difficulty of finding solvents that accommodate both extreme lipophiles and hydrophilic DNA strands.[2−6] To overcome this incompatibility, we employ a method of solubilizing DNA in organic solvents by exchanging the counterions, which are present along the charged DNA backbone, with quaternary ammonium surfactants. In doing so, we neutralize the charge on the DNA and provide a hydrophobic coating that can shuttle DNA into the organic phase.[41−44]

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Inspired by previous work that utilizes DNA−surfactant complexes for DNA-templated reactions and DNA−lipid conjugation,[45,46] here, we demonstrate that the DNA−surfactant complex can be much more broadly applied as a simple, generic strategy for overcoming incompatibilities in solubility in the production of functionalized DNA molecules. First, the prepared DNA−surfactant complex permits the terminal acylation of 3′-amine-modified oligonucleotides (ODNs) by a series of hydrophobic NHS esters, including pyrene (PY), triphenylphosphine (TPP), hydrocarbon (HC), poly(propylene oxide) (PPO), polyisoprene (PI), and polystyrene (PS). We also establish the general nature of our approach by introducing site-specific modifications at nucleobases within a fully deprotected DNA molecule by the palladium-catalyzed Sonogashira−Hagihara coupling reaction, which was reported to be unsuccessful in the past.[47,48] Finally, we synthesize DNA side chain homopolymers by ring-opening metathesis polymerization (ROMP), a new type of DNA polymer architecture similar to brush-type poly(peptide nucleic acid).[49] While statistical copolymers exhibiting DNA side chains are known,[20,33,50−52] to the best of our knowledge, such homopolymer DNA brushes have not been realized yet.

RESULTS AND DISCUSSION

Organic-Phase Synthesis of Amphiphile Conjugates at the DNA Terminus. A general scheme of the pathway taken to functionalize a terminal reactive amine group is presented in Figure 1. First, DNA−surfactant complexes were

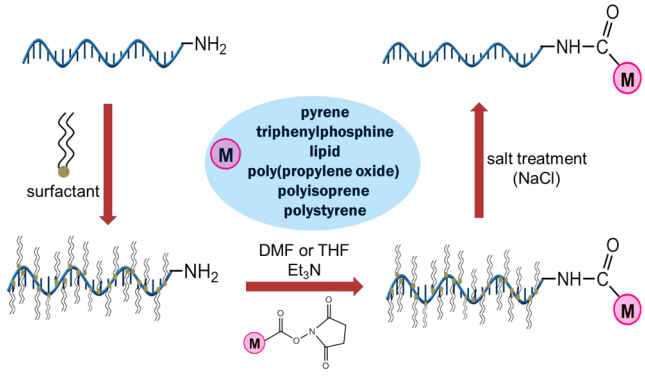

Figure 1. Scheme of the organic-phase synthesis of functional DNA, nucleic acid amphiphiles, and DNA block copolymers employing activated esters. Amine-modified DNA is precipitated out of the aqueous phase through the complexation with cationic surfactants. The insoluble complex is then extracted and redissolved in an organic solvent, in the present case, DMF or THF. The activated ester form of the hydrophobic unit, which is prepared separately, is then coupled to the terminal amine of the DNA in the homogeneous organic phase. Finally, DNA conjugates are obtained after a mold sodium chloride treatment to remove surfactant.

prepared by electrostatic complexation of 3′-amino-modified oligonucleotides (6mer, 14mer, and 22mer) with the cationic surfactant didodecyldimethylammonium bromide (DDAB) in the aqueous phase. Complex formation leads to the precipitation of the DNA from the aqueous solution and allows the recovery of the surfactant-coated DNA by centrifugation. Following lyophylization, the DNA−DDAB complex is soluble in organic solvents such as DMF, DMSO, THF, and $CHCl_3$. Separately, activated ester derivatives of hydrophobic small molecules and polymers were prepared by the reaction of a carboxylate group with *N*-hydroxysuccinimide (NHS).[53] Here, carboxylic acid functionalized PY, TPP, HC, PPO, PI, and PS are selected as representative hydrophobic moieties for the NHS ester formation in either DMF or THF. The homogeneous amine acylation reaction between DNA−DDAB and the hydrophobic moiety is carried out in an organic solvent, after which the products are recovered by means of highly concentrated salt solution treatment to release the DNA from the surfactant shell. The as-prepared samples were investigated by denaturing polyacrylamide gel electrophoresis (PAGE), reverse-phase high-performance liquid chromatography (HPLC), and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (see Table 1 and Supporting Information for details).

Coupling efficiencies from the reaction of ODN (14mer and 22mer) with PY were determined by HPLC to be above 70% (Figure S2, Supporting Information). The UV and visible light spectra of the purified ODN−PY solutions show two absorption bands below 400 nm, which are consistent with DNA (∼260 nm) and pyrene (∼350 nm) (Figure S3, Supporting Information).[54] MALDI-TOF MS analysis verified the formation of ODN−PY conjugates, which are characterized by an amide bond (Figure S4, Supporting Information). A control synthesis carried out with 14mer ODN lacking the terminal amine yielded only pristine ODN (Figure S5, Supporting Information), indicating that the amine groups of nucleobases do not react with NHS ester of PY under the same experimental conditions. Thus, it was confirmed that our designed synthetic approach is simple and effective in coupling hydrophobic molecules at terminal ODN functionalities without byproduct formation. Another hydrophobic molecule, TPP, was coupled with 14mer and 22mer ODN by the same amine acylation in absolute DMF, which efficiently generated amide products of ODN−TPP (coupling efficiency, 70% for 14mer and 72% for 22mer), as determined by HPLC and MALDI-TOF MS (Figures S6 and S7, Supporting Information). These results demonstrate that the length of the ODN does not significantly influence the coupling efficiency between the ODN and the hydrophobic small molecules. It should be noted that two products, including ODN−TPP and the oxidized conjugate, were obtained. The oxidation of ODN−TPP occurs during the postsynthetic handling, the HPLC purification step, and was observed previously as well.[27] Besides the aromatic molecules, which can be effectively conjugated with the ODN by our organic-phase conjugation method, long-chain aliphatic compounds were also successfully coupled to the ODN terminus. In the synthesis of ODN−HC(C22), THF was used as the organic solvent because HC(C22) is insoluble in DMF, while ODN−DDAB also dissolves in THF very well, confirming the universality of the DNA−surfactant complex as the coupling scaffold in our synthetic strategy. After analysis by HPLC and MALDI-TOF MS (Figures S8 and S9, Supporting Information), moderate coupling efficiencies for 14mer ODN−HC (33%) and 22mer ODN−HC (27%) were obtained. These values are lower than for ODN−PY and ODN−TPP conjugates, which probably can be ascribed to interference from van der Waals interactions between alkyl chains of DDAB and the HC component. High affinity of the ODN complex with HC could hinder the approach of the NHS ester to the amine-modified ODN terminus, thus decreasing the coupling yield of the final product.

This approach has also shown to be effective in coupling the ODN to hydrophobic polymers, including PPO, PI, and PS,

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Table 1. Summary of Coupling Efficiencies of ODN Conjugates by Means of NHS Ester Amine Acylation in Organic Solvent[a]

	(PY Mw=288)	(TPP Mw=306)	(HC Mw=340)	(PPO Mn=2500)	(PI Mn=3500)	(PS Mn=900)
Solvent	DMF	DMF	THF	DMF	THF	DMF
6merODN	n.c.	n.c.	n.c.	55%	42%	47%
14merODN	74%	70% (92%)	33%	36%	46%	23%
22merODN	70%	72%	27%	32%	43%	22%

[a]n.c. represents not conducted; coupling efficiencies of ODN−PY, ODN−TPP, and ODN−HC were determined by integrating the HPLC eluting peak intensities measured at an absorption wavelength of 260 nm; coupling efficiencies of ODN−PPO, ODN−PI, and ODN−PS were calculated according to the integration of PAGE lane intensity by ImageJ software; coupling yield presented in parentheses was obtained by the acyl chloride reaction.

highlighting the large range of polarity that reactants may exhibit. Three amine-terminated oligonucleotides of different lengths (6mer, 14mer, and 22mer) were conjugated to the activated ester form of each polymer. Our first attempt was to synthesize the ODN-*b*-PPO(2.5k) block copolymer in DMF following the procedure outlined in Figure 1. PAGE analysis of the reaction mixtures of the ODN and PPO components (Figure S10, Supporting Information) showed two distinct bands, which suggest that at least two types of ODN with different molecular weights are present. The rapidly migrating band is assigned to the unreacted ODN due to the similarity of its mobility to pristine ODN. The second band exhibits slower mobility and is assigned to the ODN−PPO conjugate. ODN−PPO conjugation efficiencies were monitored by denaturing PAGE, which revealed 55% yield for 6merODN-*b*-PPO, 36% for 14merODN-*b*-PPO, and 32% for 22merODN-*b*-PPO. Using the same procedure, we were also able to conjugate PI(3.5k) and PS(900) to 6mer, 14mer, and 22mer ODN. The ODN−PI and ODN−PS block copolymers were generated efficiently with similar yields to ODN-*b*-PPO (Table 1), as analyzed by PAGE (Figures S11 and S12, Supporting Information). To further prove the generality of the approach, telechelic PS(800) terminated with carboxylic acid at both ends was coupled to 14mer and 22mer ODN. Diblock ODN-*b*-PS and triblock ODN-*b*-PS-*b*-ODN were obtained simultaneously by one-flask reaction with moderate coupling yields (Figure S13a, Supporting Information). Furthermore, polymers with higher molecular weight PI(5.1k) and PS(4.7k) were efficiently grafted with 14mer ODN and generated the conjugated products, 14merODN-*b*-PI and 14merODN-*b*-PS, in 40% and 35% yields, respectively (Figure S13b and 13c, Supporting Information). As additional structural proof, the molecular weight of some ODN−polymer conjugates was obtained by MALDI-TOF MS (Figures S14−S16, Supporting Information), further confirming the formation of ODN hybrid copolymers by amide formation in the organic phase. Moreover, the separation of purified diblock and triblock amphiphile ODN conjugates is clearly observed by 20% denaturing PAGE (Figure 2). It indicated the change of electrophoretic mobility of the ODN conjugates due to the appended different hydrophobic groups, further confirming the formed ODN amphiphiles. The present coupling efficiencies of ODN to large hydrophobic polymer moieties were at least comparable to or even higher than those from solid-phase and other heterogeneous solution grafting approaches.[17−20,55−57] Our strategy,

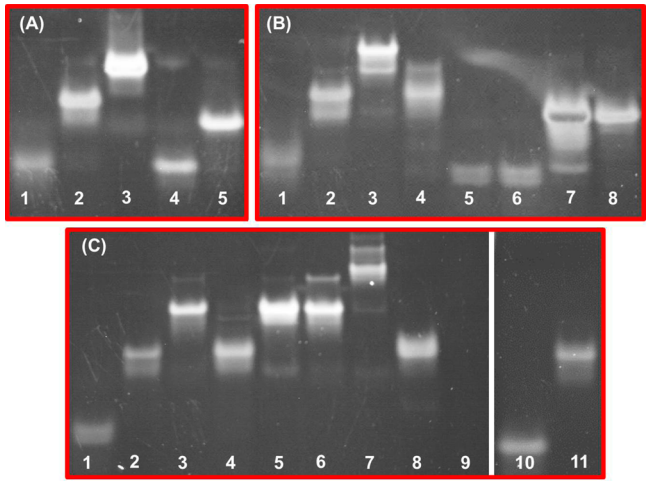

Figure 2. Denaturing polyacrylamide gel electrophoresis (20%, stained by SYBER Gold) analysis of the purified ODN conjugates and ODN block copolymers obtained by amide bond formation. (A) The purified ODN-*b*-PPO block copolymers and ODN−PY conjugates. 1, 6merODN-*b*-PPO(2.5k); 2, 14merODN-*b*-PPO(2.5k); 3, 22merODN-*b*-PPO(2.5k); 4, 14merODN−PY; 5, 22merODN−PY. (B) The purified ODN-*b*-PI block copolymers, ODN−TPP and ODN−HC conjugates. 1, 6merODN-*b*-PI(3.5k); 2, 14merODN-*b*-PI(3.5k); 3, 22merODN-*b*-PI(3.5k); 4, 14merODN-*b*-PI(5.1k); 5, 14merODN−TPP; 6, 14merODN−HC; 7, 22merODN−TPP; 8, 22merODN−HC. (C) The purified ODN−PS diblock and triblock copolymers. 1, 6merODN-*b*-PS(900); 2, 14merODN-*b*-PS(900); 3, 22merODN-*b*-PS(900); 4, 14merODN-*b*-PS(800); 5, 14merODN-*b*-PS(800)-*b*-14merODN; 6, 22merODN-*b*-PS(800); 7, 22merODN-*b*-PS(800)-*b*-22merODN; 8, 14merODN-*b*-PS(4.7k); 9, 6merODN (electrophoretic mobility was too high to be captured on the gel); 10, 14mer ODN; 11, 22merODN. The number in brackets indicates the number-average molecular weight Mn of the synthetic polymer.

however, requires neither the purchase and maintenance of an in-house DNA synthesizer nor the use of sophisticated and expensive reagents, greatly reducing the investment cost of attempting problematic syntheses via the absolute organic-phase coupling method. Therefore, the DNA−surfactant complex could offer a new platform to functionalize DNA with various previously inaccessible hydrophobic adducts in a facile and efficient way.

We additionally developed an alternative method of synthesizing amphiphile−ODN conjugates by inducing amide

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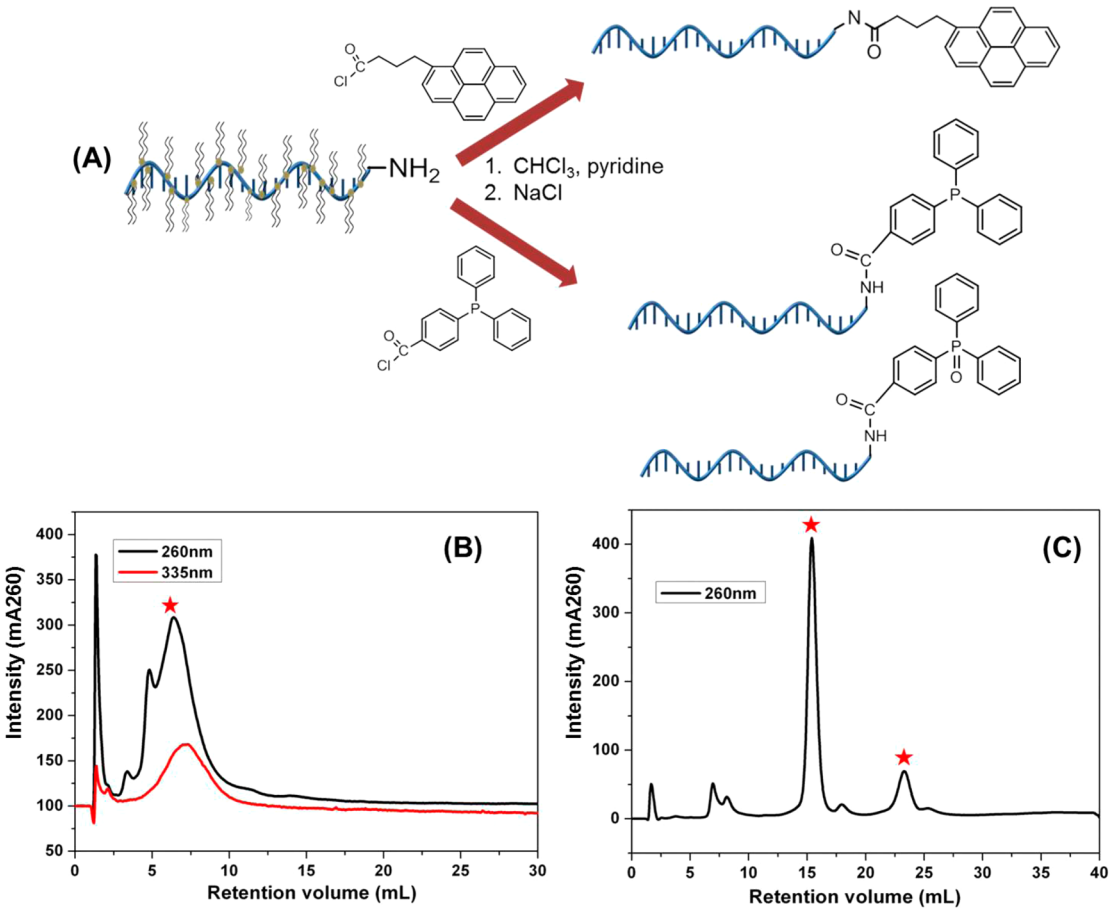

Figure 3. Preparation and purification of amphiphile ODN conjugates (ODN–PY and ODN–TPP) by means of the acyl chloride reaction in anhydrous $CHCl_3$. (A) Schematic of the synthesis route of ODN–PY and ODN–TPP. (B) Reverse-phase HPLC analysis of the crude product of the 18mer self-complementary ODN–PY conjugate (sequence: 5′-CGATCGATTTATCGATCG-3′). The presence of the DNA was monitored by the absorbance at 260 nm (mA260). Only one elution peak (marked with a red star) corresponds to the generated ODN–PY, indicating an almost quantitative transformation. Due to the self-complementary nature of the ODN sequence, hairpin, dimer, and multimer duplexes by different hybridization paths could be formed. These structures presumably lead to the broad peak observed in reverse-phase HPLC. (C) Reverse-phase HPLC analysis of the 14merODN–TPP conjugate suggests a coupling efficiency of 92%. ODN–TPP and oxidized TPP–ODN are the actual products (marked with red star) due to TPP oxidation during the purification process outside of an inert atmosphere.

bond formation through the reaction of acyl chloride-containing hydrophobic molecules and amine-terminated ODN. It is well-known that a carboxylate group can be transferred to the very active acyl chloride by thionyl chloride treatment at room temperature.[58] Here, PY and TPP are selected as the model molecules to demonstrate that 3′-amino-modified oligonucleotide can be attached to the hydrophobic molecules via their acyl chlorides (Figure 3A). First, PY acyl chlorides were coupled with ODN–DDAB complexes in anhydrous $CHCl_3$. HPLC characterization revealed that self-complementary 18mer ODN–PY was produced with high coupling efficiency (above 90%, Figure 3B), which is much higher than non-self-complementary ODN–PY (20%, Figure S17, Supporting Information). This may indicate that DNA structure can influence the PY conjugation efficiency in acyl chloride reactions. Then, TPP acyl chloride was also used for the formation of ODN conjugates. 14mer ODN–TPP conjugates (including oxidized TPP–ODN) were produced—with observed coupling efficiencies around 92% (Figures 3C). The acyl chloride reaction produces significantly higher coupling efficiencies than what was observed with NHS coupling (Table 1), presumably due to the higher reactivity of acyl chloride. In addition, the molecular weights of the above samples measured by MALDI-TOF MS (Figures S18 and S19, Supporting Information) are in good agreement with the calculated values, further confirming the synthesis of conjugated products. As a comparison, we conducted a coupling experiment between TPP acyl chloride and the ODN that lacks a terminal amine group. We observed no generation of ODN–TPP conjugates, suggesting that the reactivity of amine groups of the bases is not enough to couple with acyl chloride (Figure S20, Supporting Information). This result further confirms that the primary terminal amine exclusively reacts to form the conjugates at the chosen condition. Therefore, one attractive facet of the developed method is that contamination with byproducts from nucleobase conjugates may be avoided completely. We have found that this method is less suitable for the synthesis of DNA-block copolymers due to the inactivation of the acyl chloride over the long reaction times required (24 h). In the conjugation of small, hydrophobic molecules to DNA, however, the acyl chloride method may produce higher conversions than the NHS method.

Site-Specific Functionalization of a Nonstandard DNA Base in the Organic Phase. Postsynthetic modification strategies have thus far mostly relied on DNA immobilized on a solid support to overcome the differences of solubility in the

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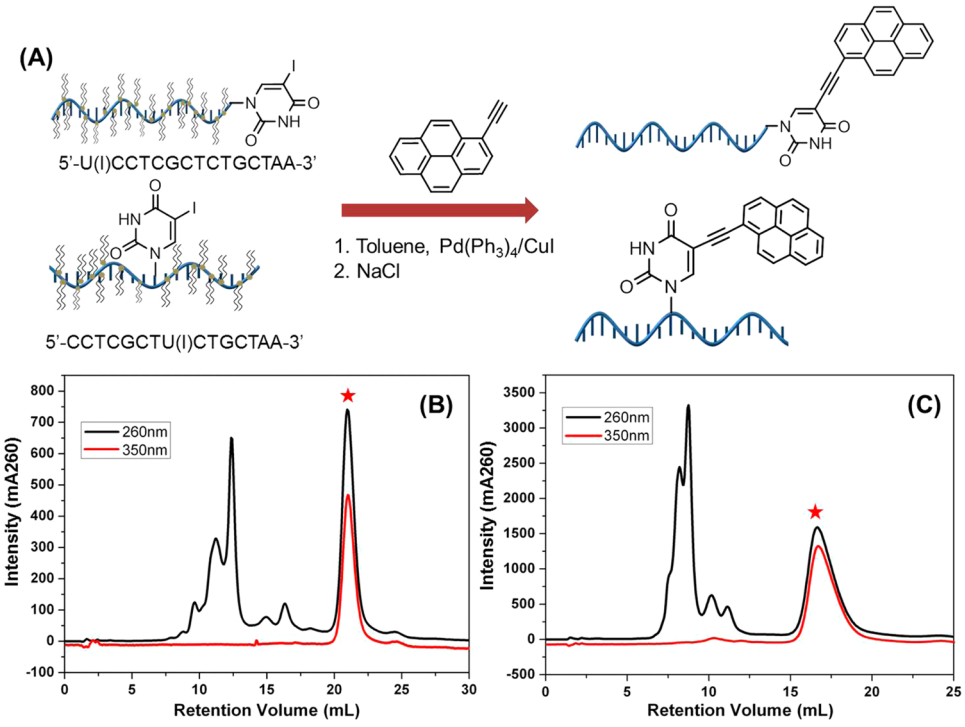

Figure 4. Site-specific functionalization of nucleobases by Pd-catalyzed Sonogashira–Hagihara reaction in DMF/Et_3N. (A) Schematic of the route for the modification of 5I-dU nucleobase at terminus (U_T) or located in the middle (U_M). Reverse-phase HPLC analysis of U_TODN–PY (B) and U_MODN–PY (C) crude products. The presence of the DNA was monitored by the absorbance at 260 nm (mA260). The product elution peaks (marked with a red star) where both the pyrene absorption at 350 nm and the DNA absorption (260 nm) are detected represent the ODN–PY conjugates obtained with 55% (U_TODN–PY) and 45% (U_MODN–PY) coupling efficiencies.

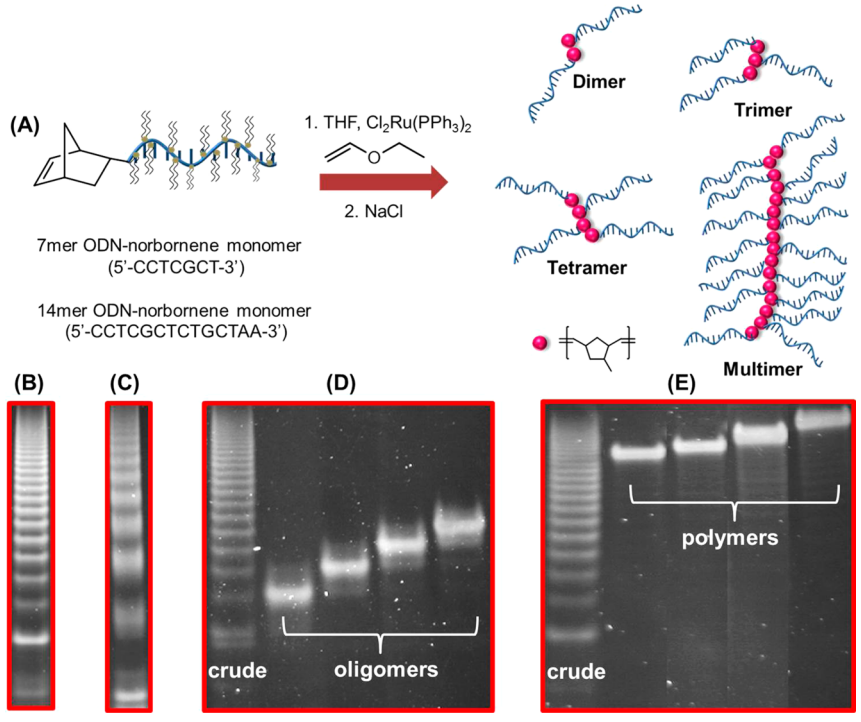

Figure 5. Series of DNA side-chain homopolymers obtained from the ODN–norbornene (NBODN) macromonomer. (A) Schematic of route for 7merNBODN–DDAB and 14merNBODN–DDAB polymerized in absolute THF by ring-opening metathesis polymerization. Denaturing polyacrylamide gel electrophoresis (PAGE) analyses of the polymerized 7merNBODN (B) and 14merNBODN–DDAB (C) crude products indicate the formation of DNA brushes. PAGE purification of the 7merNBODN brush oligomers (D) and polymers (E), where each band corresponds to a single side-chain DNA brush with specific polymerization degree.

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synthesis of amphiphilic structures. For instance, postsynthetic Sonogashira reactions on an activated nucleotide that is positioned in the middle or at the end of the sequence have been realized by treating the protected on-column oligonucleotides with a reaction mixture. However, the application of such a reaction on the same sequence while being cleaved from the solid support was reported to be unsuccessful.[47,48] To address this challenge, we have developed a strategy for the site-specific modification on nucleobases within a fully deprotected sequence by using the DNA−surfactant complex as a reaction scaffold.

As shown in Figure 4, two 15-mer oligonucleotides, with the sequences U_TODN: 5′-U(I)CCTCGCTCTGCTAA-3′ and U_MODN: 5′-CCTCGCTU(I)CTGCTAA-3′, were synthesized by standard phosphoramidite chemistry. 5′-Dimethoxytrityl-5-iodo-2′-deoxyuridine 3′-[(2-cyanoethyl)-(*N*,*N*-diisopropyl)] phosphoramidite (5I-dU phosphoramidite) was used to introduce a 5I-dU residue as the terminal and internal residue of the two oligonucleotides, which renders the oligonucleotides active for further modification by the Sonogashira−Hagihara reaction.[47] The synthesized U_TODN and U_MODN were confirmed by MALDI-TOF MS (Figure S21, Supporting Information). However, it is necessary to point out that both of the samples contained the corresponding deiodinated side products, which could not be separated completely by HPLC. As a model coupling reagent, 1-ethynylpyrene (PY) was synthesized in accordance with methods reported in the literature.[59] Following the procedure for Sonogashira reaction (Figure 4A), DNA−surfactant complexes were first prepared and lyophilized and then, under argon atmosphere, were allowed to react with PY in the presence of $Pd(P(Ph)_3)_4$ and CuI in DMF/Et_3N at 60 °C. The final crude products, after cation exchange with sodium chloride, were purified and analyzed by reverse-phase HPLC (Figure 4B and 4C). The ODN−PY conjugates are attributed to the elution peak that simultaneously exhibits not only the DNA absorption at 260 nm but also the typical pyrene absorption at 350 nm. Transformation efficiencies, derived from the HPLC chromatograms, are calculated to be 55% for U_TODN−pyrene and 45% for U_MODN−pyrene, respectively. It is noteworthy, however, that the actual coupling efficiencies would be higher if the nonreactive deiodinated U_TODN and U_MODN components were extracted from the starting material. The purified U_TODN−pyrene and U_MODN−pyrene conjugates were characterized by MALDI-TOF MS (Figure S22, Supporting Information), confirming the formation of the desired products. These results showed that fully deprotected oligonucleotides containing a convertible 5I-dU nucleotide base can be effectively and precisely functionalized by Sonogashira−Hagihara reaction on the scaffold provided by the lipophilic DNA−surfactant complex.

Organic-Phase Polymerization of DNA-Norbornene for Side-Chain DNA Homopolymers. Due to the exceptional tolerance toward a variety of functional groups, ROMP provides distinct advantages over other polymerization systems for the construction of brush-type DNA/polymer hybrid materials.[33,52] Here, our novel strategy was applied for the generation of a new type of DNA brushes, i.e., DNA side-chain homopolymers (Figure 5A). Two 5′-norbornene-modified DNA macromonomers (7merNBODN: 5′-CCTCGCT-3′ and 14merNBODN: 5′-CCTCGCTCTGCTAA-3′) were synthesized and characterized by MALDI-TOF MS (Figure S23, Supporting Information), after which the NBODNs were complexed with DDAB by electrostatic interaction as described above.

We first optimized the ROMP reaction conditions for the 7merNBODN−DDAB macromonomer. The homopolymerization was carried out using the Grubbs catalysts (first or second generation) in organic solvents (THF or $CHCl_3$). The premade monomer complex and catalyst solutions were separately purged under argon for 30 min each and then mixed together. Polymerization was allowed to proceed for 3 h at room temperature, after which it was terminated by adding ethyl vinyl ether. After cation exchange employing sodium chloride solution, the crude products were directly subjected to denaturing PAGE analysis (Figure S24, Supporting Information). The reaction products exhibit multiple distinct bands that form a ladder-like pattern, indicating the formation of DNA species with higher molecular weight. According to the optimization studies, it was found that polymerization carried out with second-generation Grubbs catalyst in THF led to a crude product with the greatest number of distinct bands (Figure 5B). We posit that this approach has been uniquely successful where others were not because the surfactant serves the additional functions of reducing the repulsion force by shielding the negative charges and at the same time generating additional affinity between DNA complexes by hydrophobic interactions. Upon subjecting the other macromonomer (14merNBODN) to the ROMP reaction under the optimized conditions, polymerization is also observed. However, fewer bands were observed by PAGE (Figure 5C), indicating a lower degree of polymerization for the bulkier monomer complex. This result is likely observed due to the steric effects that hinder the approach of norbornene units to the bulky monomer complex. As an important parameter of the polymers, the PDIs of the two brushes were then calculated (see the Supporting Information for details). It was found that both syntheses showed rather narrow polydispersities with a value around 1.2, which suggests that initiation is faster than propagation. The slower propagation, again, can be explained by the steric hindrance of the macromonomers.

Subsequently, the crude DNA brushes were separated and purified by PAGE. As indicated from the analytical PAGE (Figure 5B), DNA brushes with different degree of polymerization showed distinct mobilities, and thus they were well separated on the gel. In this respect, the one-step purification was carried out efficiently by preparative PAGE. Each band corresponds to a single side-chain DNA brush with specific polymerization degree and well-defined structure (one ODN per repeating NB), indicating the monodispersity of the purified products. Taking 7merNBODN brushes as an example, the molecular weights of the oligmers (from band 2 to band 5, Figure 5D) are in good agreement with the calculated values of monodisperse DNA brushes with polymerization degrees ranging from 4 to 7 (Figure S25, Supporting Information). Oligmers of 14merNBDNA brushes characterized by MALDI-TOF MS also showed a constant mass increase which is equal to the molecular weight of the macromonomer (Figure S26, Supporting Information). For the DNA side chain homopolymers (Figure 5E), the attempt of characterization by MALDI-TOF MS was unsuccessful. However, their molecular weights and polymerization degree could be reasonably estimated according to the relevant band numbers on the PAGE gel.

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CONCLUSIONS

In this work, a facile synthetic method for nucleic acid functionalization in the organic phase has been developed. Utilizing a DNA−surfactant complex as a versatile and general scaffold for DNA functionalization in the organic phase, we have incorporated hydrophobic modifications at terminal and internal DNA positions. Various functional moieties, from hydrophobic small molecules to polymers, including pyrene, triphenylphosphine, long hydrocarbon chains, poly(propylene oxide), polyisoprene, and polystyrene, have been efficiently and conveniently coupled with DNA. Moreover, the polymerization of norbornene-functionalized DNA−surfactant complex macromonomers allows the fabrication of novel DNA side chain homopolymers. The established strategy has some striking features. First, high yields for amphiphilic DNA conjugates are achieved, relative to existing methods. Second, the coupling reactions are simple, easily controllable, and applicable to a variety of hydrophobic moieties. More importantly, this approach avoids the large instrumental investment of acquiring an automated DNA synthesizer in the laboratory, thus expanding the accessibility of problematic, custom-synthesized DNA conjugates to the level of research groups. As a consequence, this technique paves the way to the synthesis of a wide variety of amphiphilic DNA hybrids for exploring DNA applications in bio- and nanotechnology more broadly.

EXPERIMENTAL SECTION

DNA−Surfactant Complex Preparation. Aqueous solutions (0.5 mM) of 3′-amino modified ODN (6mer, 14mer, 22mer, and a pair of self-complementary 18mers), 5-iodo deoxyuridine-modified ODN (U_TODN and U_MODN), and norbornene (NB) modified ODN (7merNBODN and 14merNBODN) were prepared by dissolving the purified ODN in ultrapure water. In a second solution made from ultrapure water, the concentration of surfactant of didodecyldimethylammonium bromide (DDAB) or cetyltrimethylammonium bromide (CTAB) (CTAB only used for U_MODN complex) was adjusted to 5−10 mM at room temperature. When the ODN and surfactant solutions (~5 mol equiv of surfactant relative to nucleotides of the ODN) were mixed together, the insoluble complex precipitated from the aqueous phase. After centrifugation, the water and unreacted surfactants were removed, and finally, the complexes were lyophilized overnight before further dissolving in organic solvents for coupling with hydrophobic molecules.

NHS Ester Preparation. Small molecules and polymers (0.1 mmol) (1-pyrenebutyric acid, 4-(diphenylphosphino)benzoic acid, α-methoxy-ω-COOH-terminated poly(propylene glycol), and carboxy-terminated polystyrene) were first dissolved in 1.0 mL of DMF, respectively. Then *N*-hydroxysuccinimide (NHS, 0.12 mmol) was added into the above DMF solutions with stirring. After 5 min, 0.5 mL of DMF containing *N*,*N*′-dicyclohexylcarbodiimide (DCC, 0.12 mmol) was added dropwise to the reaction solution. The final mixture was stirred at room temperature under argon, and white precipitate of dicyclohexylurea (DCU) appeared. After purification, the obtained products were used for the coupling experiments with DNA−surfactant complexes. NHS esters of behenic acid and carboxy-terminated polyisoprene were prepared following the same procedures except THF as the organic solvent.

Acyl Chloride Preparation. An amount of 1.0 mL of $CHCl_3$ solution containing 1-pyrenebutyric acid and 4-(diphenylphosphino)-benzoic acid (0.1 mmol) were prepared, respectively. To the solution, 1.0 mL of thionyl chloride ($SOCl_2$, 13 mmol) was added dropwise. The mixtures were stirred at room temperature under argon. After 5 h, $SOCl_2$ was evaporated, and the formed acyl chloride was used for the coupling experiments with DNA−surfactant complexes.

Synthesis of Amphiphile DNA Conjugates by Amide Bond Formation. The prepared ODN−DDAB complexes (1.0 μmol) were dissolved in 200 μL of DMF. Then, the prepared NHS esters (15.0 μmol) of small molecules (1-pyrenebutyric acid and 4-(diphenylphosphino)benzoic acid) and polymers (poly(propylene glycol) and polystyrene) in 500 μL of DMF and triethylamine (Et_3N, 1.4 mmol, 200 μL) were added to ODN−DDAB solutions. The final mixtures were stirred for 24 h at room temperature under argon. After that, saturated NaCl aqueous solution (150 μL) was added into the above organic solutions and stirred for another 3 h. After evaporation of DMF, 500 μL of Milli-Q water was added, and the mixtures were filtered to separate the unreacted esters of hydrophobic molecules and polymers. Then the desalting process was carried out using a centrifugal concentrator (Vivaspin). Finally, the crude products were purified before further characterization. The coupling between ODN−DDAB and the NHS ester of behenic acid or polyisoprene was carried out using the same procedures, except using THF as the organic solvent. For the synthesis of ODN triblock polystyrene, 4 μmol of ODN−DDAB complexes in 300 μL of DMF was used to react with the NHS ester of α,ω-dicarboxy-terminated polystyrene (2 μmol in 100 μL of DMF). Furthermore, the acyl chloride of 1-pyrenebutyric acid and 4-(diphenylphosphino)benzoic acid was reacted with ODN−DDAB complexes in $CHCl_3$ with argon protection for 3−5 h following the above protocol.

Purification and Coupling Efficiency Calculation of Amphiphile DNA Conjugates. Reverse-phase HPLC was used to purify the DNA conjugated small molecules (1-pyrenebutyric acid, 4-(diphenylphosphino)benzoic acid, and behenic acid). HPLC information: RESOURCE RPC column (1 mL), 0−100% B gradient, flow rate 2 mL/min, 0.1 M triethylammonium acetate buffer containing 2.5% acetonitrile as buffer A, 0.1 M triethylammonium acetate buffer containing 65% acetonitrile as buffer B. Coupling efficiencies of the above conjugates were determined by integrating the eluting peak intensities of 260 nm. Denaturing PAGE (20%) was used to analyze crude products of the 14mer and 22mer ODN block copolymer with SYBER gold staining. Coupling efficiencies were calculated according to the integration of PAGE lane intensity by ImageJ software. Preparative PAGE (8%) was used to purify 6mer, 14mer, and 22mer ODN block copolymers. Coupling efficiencies of 6mer ODN block copolymers were determined by analysis of the preparative PAGE (8%).

Modification of the Nucleotide Base by Sonogashira−Hagihara Reaction. To a round-bottom flask, the U_TODN−DDAB (U_MODN−CTAB) complex (0.5 μmol), pyrene-1-alkyne (10.0 μmol), [$Pd(PPh_3)_4$] (4.6 μmol), and CuI (9.0 μmol) were added. After purging with argon for 30 min, 2.0 mL of DMF/Et_3N (v/v = 1/1) was added as solvent to the flask, and the reaction was allowed to proceed for 12 h at 60 °C. Then, saturated NaCl aqueous solution was added to the organic mixture, which was stirred for another 3 h. After desalting by a centrifugal concentrator, the crude products were purified by reverse-phase HPLC (as illustrated above) and characterized by MALDI-TOF MS.

Polymerization of ODN−Norbornene by Ring-Opening Metathesis Polymerization. The monomer complex solution (3.5 μmol in 1 mL of THF) and stock solution of Grubbs' catalyst (second generation, 2.8 μmol in 2 mL of THF) were separately purged under argon for 30 min. Polymerization was initiated by mixing 100 μL of the catalyst solution (4% mol) with the monomer solution. Polymerization was allowed to proceed for 3 h at room temperature and was terminated by the addition of ethyl vinyl ether (100 μL). After salt treatment, the crude products were directly subjected to denaturing PAGE (20%) analysis and preparative PAGE (8%) purification.

ASSOCIATED CONTENT

Supporting Information

Full details regarding the used materials, characterization, and additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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AUTHOR INFORMATION

Corresponding Author

a.herrmann@rug.nl

Author Contributions

†These authors contributed equally to this work.

Notes

The authors declare no competing financial interest.

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