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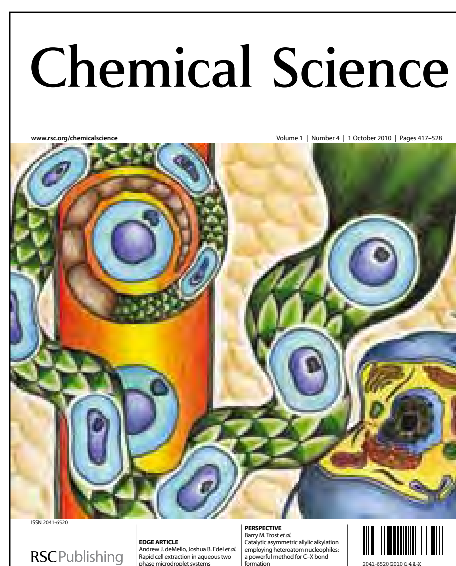

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EDGE ARTICLE

Facile one-step solid-phase synthesis of multitopic organic-DNA hybrids via "Click" chemistry†

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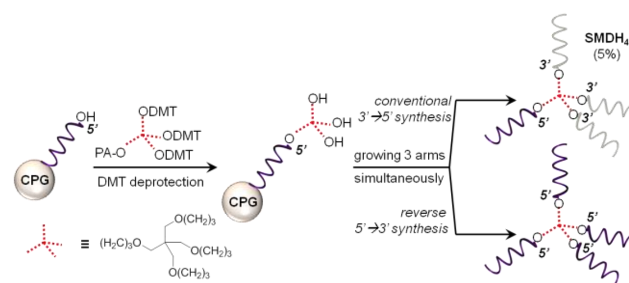
A broad range of synthetically challenging-to-access small molecule-DNA hybrids can be readily synthesized in "one pot" and in high yields by coupling multi-azide cores to alkyne-modified DNAs on a solid support using click chemistry. The multi-functional products can be obtained in pure forms and on large scales (1 μmol) in a facile fashion. In addition, the distribution of the products can be controlled by changing the concentration of the azide core in solution and the strand-loading density on the solid-supports.

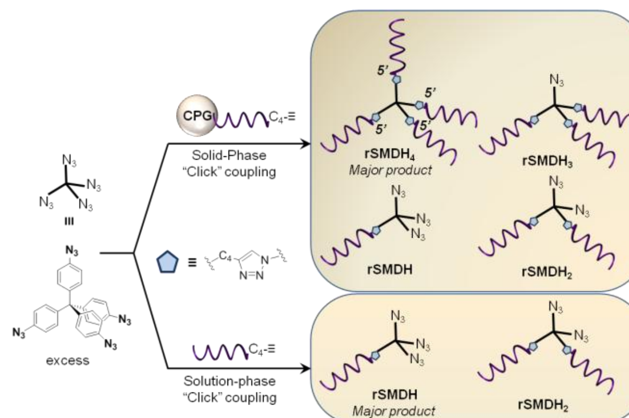
Introduction

In modern materials chemistry, the remarkable recognition properties of DNA, whether in its native molecular form or when conjugated with nanoparticles,[1] have often been utilized to direct the formation of 2D[2] and 3D[3] structures. Multiple DNA strands have also been attached to metal complexes,[4] organic molecules,[5] and polymer[6] cores to endow these structures with both functionality and directionality. The resulting hybrid building blocks can either be used directly in biosensing[6-7] and biomedicine[8] or in the assembly of higher-order structures through programmable interactions with other oligonucleotide-conjugates.[9] For organic-DNA hybrids in particular, a branching point is often introduced using a small-molecule "core" containing multiple protected alcohols and a phosphoramidite (PA) moiety, through which a DNA strand grown on standard controlled pore glass (CPG) beads can be attached (Scheme 1, first step).[10] Deprotection of the alcohol groups on the core and subsequent growth of multiple DNA arms in a concurrent fashion (Scheme 1, second step) via conventional 3′→5′ or reverse 5′→3′ synthesis then affords the desired building blocks. When a hybrid with identical 5′ connections between the arms and the core is desired, a low-yielding and costly reverse DNA synthesis must be used in the second step.[11] Nevertheless, all syntheses using this divergent approach, independent of directionality, still suffer from low yields and tedious purifications due to the increased probability of failures that comes with the simultaneous growth of multiple arms in the second stage of DNA synthesis. In addition, it is hampered by the synthetic challenge of making organic cores that contain only one PA moiety among multiple protected alcohol groups.[11]

Herein, we report an efficient, large-scale one-pot solid-phase synthesis of rigid small molecule-DNA hybrids (rSMDHs) containing one (rSMDH), two ($rSMDH_2$), three ($rSMDH_3$) and four ($rSMDH_4$) DNA strands that were easily isolated and purified using reversed-phase (RP) high-performance liquid chromatography (HPLC). All DNA strands are attached to the core at their 5′ end, affording highly desirable symmetric building blocks. The synthesis of these DNA hybrids was achieved by



Scheme 1 Schematic illustration of the divergent synthesis of $SMDH_4$ starting from a commercially available branching phosphoramidite (PA).[10] In our hands and with DNA strands that are longer than 12 base pairs, this synthesis routinely gives ~5% yields when DNA synthesis is carried out in the conventional 3′→5′ direction for all arms. For ease of visualization, the DNA single-strands are drawn as helices.



Scheme 2 Schematic illustration of the stark contrast in rSMDH product distribution obtained from copper(I)-catalyzed click reaction in solid (top) and solution (bottom) phases

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copper(I)-catalyzed click coupling of an excess of a tetraazide functionalized organic core to alkyne-modified DNA strands anchored to the surface of CPG beads (Scheme 2).

Surprisingly, the $rSMDH_4$ was the major product in this solid-phase synthesis, in stark contrast to the rSMDH major product obtained in a solution-phase reaction under the same conditions. We partly attribute this difference to the much higher local concentrations (i.e., surface density) of the terminal alkynes on the solid-support compared to concentrations that can be achieved in solution (see further discussion below). Given the tetrahedral nature of our tetraazide core and its excess in solution, the prominent presence of $rSMDH_4$ is also quite remarkable (one would have expect more $rSMDH_2$ and $rSMDH_3$ products).

Taking advantage of the multitopic coupling preference, we were able to tune the $rSMDH_n$ product distribution by employing CPG beads with different loadings (26-127 μmol/g of preloaded bases on the surface where DNA strands are grown). In addition, the product distribution can be controlled by changing the total azide concentration in the reaction mixture. We also demonstrated that this method can be successfully utilized to synthesize several small molecule-DNA hybrids with a broad range of DNA lengths (6-18 base pairs (bp)) and sequences, in large scale, and with different types of azide-containing organic cores.

Results and discussion

We begin our solid-phase synthesis by growing a short sequence of single-stranded (ss) DNA (3′-TTCCTT) on a solid support (500 Å CPG beads with an initial base loading of 43 μmol/g) via conventional 3′-phosphoramidite chemistry. The 5′-terminus of this strand was then modified with a hexynyl (C_4-acetylene) moiety. A portion of these beads was deprotected to afford a pure alkyne-DNA synthon (3′-TTCCTT-C_4-acetylene, see the analytical RP-HPLC trace (Fig. 1A) for its purity, 70% isolated yield) to be used for the solution-phase synthesis; the remaining portion was kept for the solid-phase reaction. Both the isolated and CPG-bound alkyne-DNAs were then reacted with an excess of *tetrakis*(4-azidophenyl) methane[12] (tetraazide core, 30 equiv per alkyne-DNA) under the same click-coupling conditions (see Electronic Supporting Information (ESI†) for details). Analytical RP-HPLC analysis showed that the solution-phase reaction mostly yielded rSMDH (Fig. 1B), as expected, while the solid-phase reaction afforded $rSMDH_4$ as the major product (Fig. 1C). Strikingly, as we decreased the concentration of the tetraazide core in the solid-phase reaction by tenfold (1.1 mM, 3 equiv per alkyne-DNA, still in excess), even higher percentages of the $rSMDH_4$ product (Fig. 1D) can be obtained, accentuating the difference in reactivity between the tetraazides still in solution and the remaining unreacted azide groups on cores bound to the surface of the beads.

MALDI-ToF mass spectrometric (MS) analysis of the collected RP-HPLC fractions in the solid-phase reaction (Fig. 1C, see also Fig. S1 in ESI†) revealed the fraction that eluted at 15.8 min (Figs. 1C and 2A) to be the $rSMDH_4$ product obtained with a 32 % isolated yield (m/z = 8048.9 g/mol observed, Fig. 2A inset; 8057.7 g/mol calcd.). The fraction that eluted at 12.5 minutes (Fig. 2B) is the starting alkyne-DNA (3′-TTCCTT-C_4-acetylene, m/z = 1891.5 g/mol observed, Fig. 2B inset; 1893.3 g/mol calcd.). The peaks that eluted at 20.1, 27.2, and 40.2 min (Fig. 1C) are assigned to $rSMDH_3$, $rSMDH_2$, and rSMDH, respectively (see Figs. S2-S4 in the ESI†for the RP-HPLC traces of the purified products and the inset in each of these figures for their MALDI-ToF spectra). The large separations between these fractions in the HPLC traces highlight an advantageous feature of the DNA-hybrids: each product elutes separately and can be purified in a facile manner. We attribute this to an increased exposure of the hydrophobic core to the C_{18} stationary phase of the RP-HPLC column with increasing numbers of unreacted azides. Facile workup is another advantage of the solid-phase reaction as it requires simple washing of the beads to remove excess reagents, and is applicable to both small- and large-scale reactions.

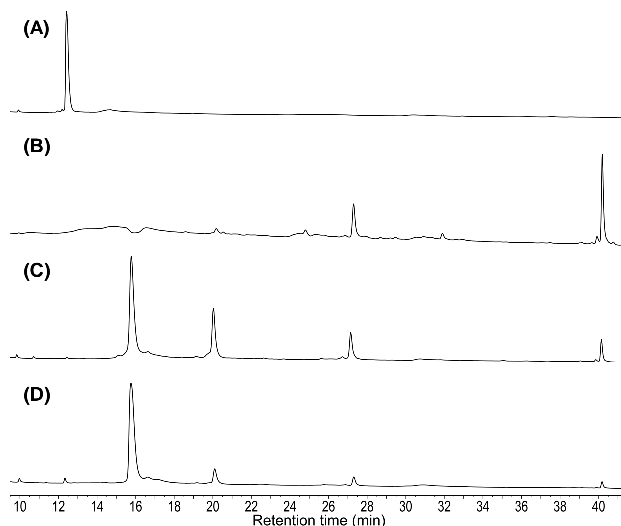

Fig. 1 Analytical RP-HPLC traces of: (A) Alkyne-DNA (3′-TTCCTT-C_4-acetylene). (B) The crude reaction mixture from the solution-phase reaction between the tetraazide core (30 equiv) and the alkyne-DNA. (C) The crude reaction mixture from the solid-phase reaction using a high concentration of the tetraazide core (10.8 mM, 30 equiv) and the CPG-bound alkyne-DNA. (D) The crude reaction mixture for the same solid-phase reaction described in C but with a lower concentration of the core (1.1 mM, 3 equiv).

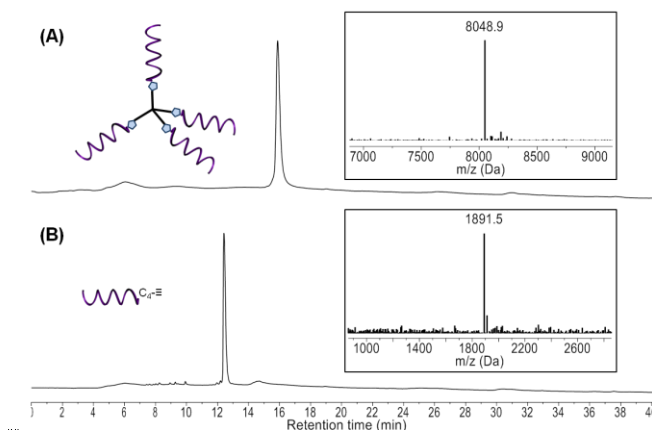

Fig. 2 Analytical RP-HPLC trace of: (A) purified $rSMDH_4$ product and (B) alkyne-modified DNA (3′-TTCCTT-C_4-acetylene). Inset: the MALDI-ToF spectrum for each product.

In contrast, purification of the solution-phase reaction required injection of the full reaction mixture containing many

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components (DMF:H_2O (10:1 v/v), excess tetraazide core, TBTA, $CuSO_4 \cdot 5H_2O$, and L-ascorbic acid; see ESI†for more details) into the RP-HPLC column, which can cause damage to the stationary phase. In addition, this type of purification would necessitate injecting a large amount of analyte (> 10 mL) into the semi-prep column for large-scale (1 μmol) reactions. We note that attempts to improve the solution-phase workup by lyophilization, centrifugation, or desalting of the reaction mixture prior to HPLC purification all caused damage to (or loss of) the products.

Remarkably, the solid-phase strategy can easily be extended to couple the tetraazide core with alkyne-DNA strands of different lengths (7-18 bp) and compositions (Table 1) on a standard large-scale (1 μmol) DNA synthesis (20-40 mg of CPG beads). These couplings were successfully carried out with 5 mM final tetraazide concentrations, affording mostly $rSMDH_4$ and $rSMDH_3$, which were isolated using RP-HPLC in good yields (20-32% for $rSMDH_4$ and 4-8% for $rSMDH_3$; isolated yield calculation was based on the initial 1 μmol CPG loading) and characterized using MALDI-ToF MS (see Figs. S5-S16 in the ESI†). Since our DNA synthesizer typically provides 70-40% yields for the synthesis of 7-18 bp sequences, respectively, the actual yields based on the fully synthesized alkyne-DNA strands are >50% for all $rSMDH_4$ products.

Table 1 The alkyne-DNA sequences (7-18 bp) on CPG beads (1 μmole scale) that were used in click reactions with the tetraazide core.

Entry	Alkyne-DNA sequences on CPG beads[a]
1	CPG-3′-TTTCCTT-C_4-acetylene
2	CPG-3′-AAGGAAA-C_4-acetylene
3	CPG-3′-TTTCCTTTTT-C_4-acetylene
4	CPG-3′-AACAATTATACTCAGCAA-C_4-acetylene

[a]1000 Å dA-CPG (38 μmole/g) and dT-CPG (26 μmole/g) beads were used for the DNA synthesis.

The advantages of our convergent approach can be clearly seen in Fig. 3. The analytical RP-HPLC trace of the crude reaction mixture from the divergent synthesis of $SMDH_4$ (Scheme 1) shows significant amounts of failure strands (Fig. 3A) that must be identified and separated from the DMT-protected $SMDH_4$ product. Once this product is isolated, it must be deprotected in an extra step and the total isolated yield of the desired $SMDH_4$ product (Fig. 3B) is only 5% . On the other hand, the HPLC trace of the crude reaction mixture obtained by our convergent click chemistry approach clearly shows the desired $SMDH_4$ product as a major species (Fig. 3C). The amount of failed products is minimal here; other side products are actually $SMDH_n$ species of interest. This allows for facile separation of products, avoids deprotection steps, and affords 20% isolated yield of the desired $SMDH_4$ product (Fig. 3D).

To further understand the role of loading of alkyne-DNA on the surface of the beads and concentration of the tetraazide core in solution, solid-phase coupling reactions with both low (1.1 mM) and high (10.8 mM) concentrations of the tetraazide core were carried out using several different CPG beads with varying pore sizes (500-2000 Å) and loadings (26-127 μmole) but with the same alkyne-DNA sequence (TTCCTT). As shown in Table 2, the distributions of $rSMDH_4$, $rSMDH_3$, $rSMDH_2$, and rSMDH products illustrate several trends: 1) Higher loadings of alkyne -DNA on the CPG beads greatly favour the formation of $rSMDH_4$ product (Table 2, cf. entries 1, 2 vs 3, 4 and entries 5, 6 vs 7, 8). 2) High concentrations of the tetraazide core (10.8 mM) decrease the amount of $rSMDH_4$ product and increase the formation of $rSMDH_3$, $rSMDH_2$, and rSMDH products (Table 2, cf. entries 1, 3, 5, 7, 9 vs 2, 4, 6, 8, 10, respectively). 3) Smaller pores slightly favor the formation of $rSMDH_3$, $rSMDH_2$, and rSMDH products, especially at high tetraazide concentrations (Table 2, cf. entry 2 vs 6). 4) Increasing the pore size gives more unreacted alkyne-DNA (Table 2, cf. entry 1 vs 5 vs 9) but higher tetraazide concentrations can compensate and allow for complete reaction of the starting alkyne-DNA in certain cases (Table 2, entries 2 and 6). Interestingly, the 1000 Å hybrid-CPG, which has been designed to give high loading and homogenous distributions of strands on the bead's surface,[13] yields mostly $rSMDH_4$ product

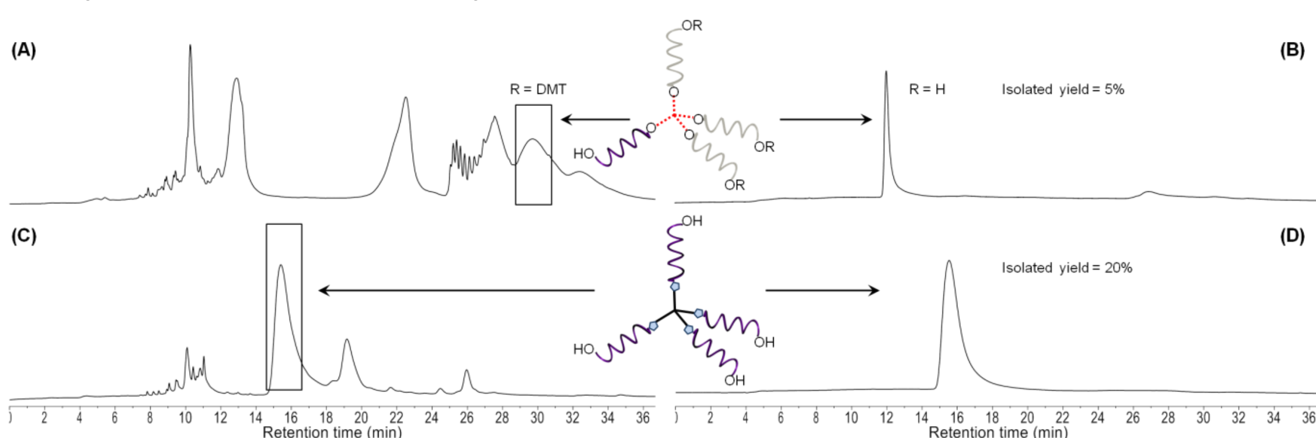

Fig. 3 Comparative evaluation of the $SMDH_4$ products comprising 18 bp arms obtained from the divergent synthesis of the hybrids and the convergent click-based synthesis presented herein. (A) The analytical RP-HPLC trace of the reaction mixture obtained from the divergent synthesis using phosphoramidite chemistry (Scheme 1, top path) showing numerous impurities that resulted from failures in DNA synthesis. These failed impurities must be identified by mass spectrometry before they can be separated from the DMT-protected product peak, which still requires deprotection (treatment with 80% acetic acid). (B) The analytical RP-HPLC trace of the desired $SMDH_4$ product peak after deprotection of the isolated DMT-protected fraction in (A), with only a 5% isolated yield. (C) The analytical RP-HPLC trace of the reaction mixture obtained from our convergent click-chemistry approach (Scheme 2, top) showing the desired $SMDH_4$ product as a major species in addition to other $SMDH_n$ species of interest. (D) The analytical RP-HPLC traces of the purified $SMDH_4$ product in (C) with a 20% isolated yield.

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Table 2 Product distribution for the click reactions between various CPG beads containing 6 bp alkyne-DNA (TTCCTT) and the tetraazide core at both low and high concentration

Entry	CPG bead pore size (loading)	Core conc. (mM)[a]	$rSMDH_4$ (%)[b]	$rSMDH_3$ (%)[b]	$rSMDH_2$ (%)[a]	rSMDH (%)[b]	Alkyne-DNA (%)[b]
1	500 Å	1.1	72.4	9.5	6.3	7.2	4.6
2	(43 μmole/g)	10.8	42.3	24.4	15.6	17.7	0.0
3	500 Å	1.1	88.8	11.2	0.0	0.0	0.0
4	(127 μmole/g)	10.8	64.0	23.1	7.0	5.9	0.0
5	1000 Å	1.1	69.3	6.3	4.5	6.1	13.8
6	(26 μmole/g)	10.8	59.1	18.1	11.1	11.6	0.0
7	*Hybrid-1000 Å*	1.1	94.4	5.6	0.0	0.0	0.0
8	*(74 μmole/g)*[c]	10.8	75.1	14.4	5.5	5.0	0.0
9	2000 Å	1.1	67.0	7.0	5.6	1.1	19.4
10	(34 μmole/g)	10.8	55.9	17.3	9.3	6.5	11.1

[a]Final tetraazide core concentration in DMF. [b]Percentages of the product distribution were based on normalized integration values (see section S3 in the ESI†) of the analytical RP-HPLC peaks at 12.5 min (alkyne-DNA), 15.8 min ($rSMDH_4$), 20.1 min ($rSMDH_3$), 27.2 min ($rSMDH_2$), and 40.2 min (rSMDH). For isolated yields, please see section S4 in the ESI†. [c]Polystyrene-coated Hybrid CPG beads containing evenly distributed DNA strands on the surface with higher loadings.

(94%) when reacted at low tetraazide concentrations (1.1 mM) (Table 2, entry 7) and this does not change significantly when the reaction is performed at high tetraazide concentrations (Table 2, entry 8).

Based on the results described above, it is clear that the loading density of alkyne-DNA strands on the CPG surface and the tetraazide core concentration in the solution dictate the distribution of the products in two distinct steps. In the first "nucleation" step, the reaction is governed by the solution concentration of the tetraazide core, which reacts with the surface-bound copper-activated alkyne-DNA and forms the first covalent triazole linkage. This keeps the organic core bound to the surface of the CPG but with a high degree of freedom due to the highly flexible nature of the ssDNA tether. In the second step, the reaction of the other alkyne-DNA strands in the vicinity with the surface-bound remaining azides occurs much more quickly than that with free tetraazide in solution due to the high local "concentration" of alkyne-DNA, dictated by the density of packing of the preloaded nucleosides. Several other factors facilitate the full conjugation of the seemingly geometrically-restricted tetraazide core (see Figure S17 in the ESI†): 1) The large difference in length scales between the ssDNA and core (10:1); 2) The high flexibility of the ssDNA strands on the solid support; and 3) The relatively "high" curvature of the CPG pores and DNA density. Together, all of these factors allow the arms to reach the core easily and favor the formation of the multiply substituted products.. In addition, densely loaded surfaces, where strands are closely packed, would yield a higher proportion of $rSMDH_4$ than sparsely loaded surfaces.

To further support our two-reaction-regime hypothesis, we employed a flexible, linear 1,6-diazidohexane[14] (diaazide) core that can only yield products with one (fSMDH, f = flexible) or two ($fSMDH_2$) DNA strands in the coupling reaction.[15] Since this flexible core is more soluble in DMF compared to the rigid tetraazide core, which saturates at ~10 mM in DMF, solid-phase coupling reactions over two orders-of-magnitude in core concentration (1.1, 10.6, and 106.1 mM) can be tested. Analytical RP-HPLC traces of these reaction mixtures showed various distributions of two peaks (Figs. 4A-C and Fig. S18 in the ESI†), which were separated and identified using MALDI-ToF MS as $fSMDH_2$ (peak at 13.8 min, see Fig. S19 in the ESI†) and fSMDH (peak at 18 minutes, see Fig. S20 in the ESI†). Increasing the diazide concentration consistently produces increasing amounts of the fSMDH product (i.e., forming less $fSMDH_2$; 1.1, 10.6, and 106.1 mM of diazide afforded 91, 72, and 33% of $fSMDH_2$ product, respectively, Figs. 4A-C) but not to the exclusive formation of the mono-substituted product expected in the corresponding solution-phase reactions. This is consistent with the hypothesis that the second regime of our two-regime reaction is much faster than the first. Even a two orders of magnitude change in azide concentration in solution (400 equiv of azide per alkyne-DNA) still results in a significant amount of $fSMDH_2$ (33%, Fig. 4C).

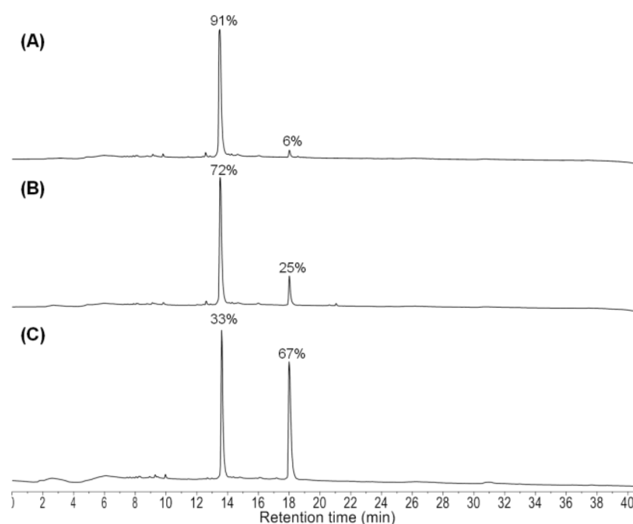

Fig. 4. Analytical RP-HPLC traces of solid-phase reaction on CPG beads (500 Å, 43 μmole/g) containing 6 bp alkyne-DNA (3′-TTCCTT-C_4-acetylene) coupled to a diazide core (1,6-diazidohexane) of three different concentrations: (A) 1.1 mM, 4 equiv/alkyne; (B) 10.6 mM, 40 equiv/alkyne; (C) 106.1 mM, 400 equiv/alkyne. Percentage yields of the products were calculated using the normalized integration values of the peaks at 13.8 min ($fSMDH_2$) and 18 min (fSMDH).

In the divergent synthesis of rSMDHs (Scheme 1) where one starts with a multiply protected, mono-phosphoramidite core, a product with two different types of DNA strands (in 1:3 ratio) can

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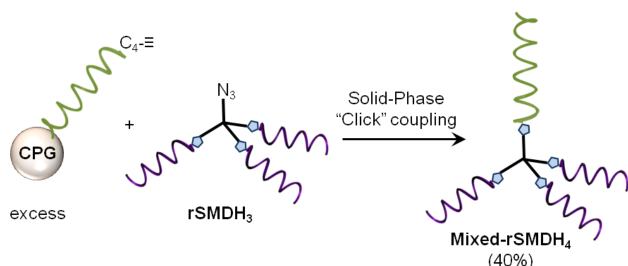
be obtained in a divergent fashion. However, as mentioned in the introduction to this manuscript, such syntheses often afford low yields with difficult purifications due to the higher number of potential failure sites that accompany the simultaneous synthesis of three strands. As reported herein, our solid-phase strategy can afford the same product in a much more facile manner: the tri-substituted product shown in the solid-phase synthesis in Scheme 2 can be isolated and used in an iterative coupling with a different DNA strand, essentially in the reverse order of the divergent synthesis. As a demonstration, an azide-substituted $rSMDH_3$ product (obtained from solid-phase click reaction of Table 1, entry 3; see also Fig. S13 in the ESI†) containing three 10-bp DNA (3′-TTTCCTTTTT-C_4-acetylene) was isolated and reacted with an 18 bp alkyne-DNA on CPG beads (Scheme 3, see ESI† for experimental details). This reaction afforded the desired mixed-$rSMDH_4$ product with three 10 bp and one 18 bp DNA strands (40% yield based on the starting azide), which was purified using RP-HPLC (Fig. S21 in the ESI†) and characterized by MALDI-ToF MS (Fig. S22 in the ESI†). Indeed, this is a greatly improved alternative to syntheses that start with commercially available branching phosphoramidites,[10] which give low yields (~5% in our hands for DNA strands with more than 12 bp) only after tedious purifications.



Scheme 3 A schematic representation of the synthesis of mixed-$rSMDH_4$ consisting of three identical 10 bp strands and one different 18 bp DNA strand. The click coupling was carried out between an 18 bp alkyne-DNA on CPG beads and a $rSMDH_3$ with an azide group and three identical 10 bp strands surrounding a tetraphenyl organic core.

Conclusions

Several features distinguish this work. First, the methodology reported herein constitutes a straightforward, versatile, and robust solid-phase synthesis of an important class of well-defined DNA-hybrids containing multiple DNA strands. The convergent nature of this methodology provides product yields that exceed what is possible via reported divergent hybrid synthesis strategies. Second, the ability to selectively make and purify hybrids, each with a different number of oligonucleotides, will be important for creating building blocks with tunable valency, based upon the number and orientation of the oligonucleotides, that can be used in the DNA-guided synthesis of nanostructures and materials. For example, $SMDH_n$ have been used to construct caged structures,[11] polygons,[16] fibers,[5d] and porous solids[5g] We are confident that ordered 2D and 3D nanostructures such as spheres, rods, and sheets can be prepared using the appropriate combinations of SMDHs. Third, the distributions of products can be easily controlled, and hybrids can be made that contain free azides, which can be utilized subsequently to make multifunctional building blocks. Finally, not only can this synthetic method be scaled up to µmol quantities using commercial DNA synthesizers, but the surface-based click-chemistry also allows for simple and facile product isolation in good yields.

We envision that the convergent, local-concentration-driven methodology reported here can be applied to the synthesis of other DNA hybrids, including those with cores that are linear or planar in nature. Other coupling chemistries can also be used as long as the reactions are high-yielding and the starting materials do not degrade during the reaction time. Taken together, these advances significantly broaden the library of DNA-hybrids available for materials synthesis.

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† Electronic Supplementary Information (ESI) available: Experimental details for the solution- and solid-phase synthesis and purification of SMDHs; analytical and semi-preparative RP-HPLC traces, MALDI-ToF spectra, and a PAGE-gel image of SMDHs. See DOI: 10.1039/b000000x/

‡ These authors contributed equally.

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