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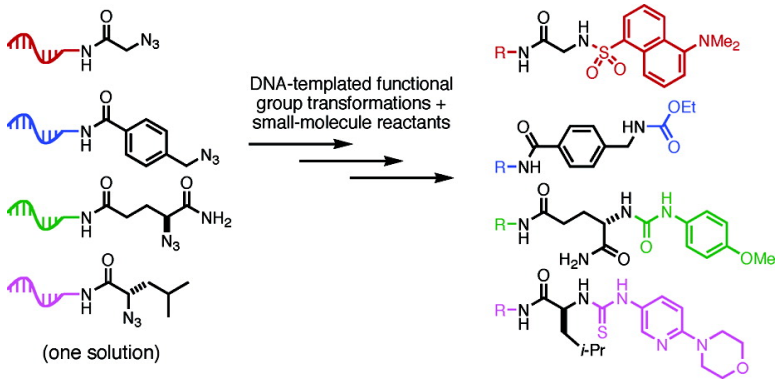

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DNA-Templated Functional Group Transformations Enable Sequence-Programmed Synthesis Using Small-Molecule Reagents

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DNA-templated organic synthesis (DTS)[1] enables modes of controlling reactivity that are not possible in a conventional synthesis format[1d,g] and allows synthetic molecules to be manipulated using translation, selection, and amplification methods previously available only to biological macromolecules.[1a,2] The structures that can be accessed through DTS have been limited predominantly to products of coupling reactions between two DNA-linked reactants.[1i] In some cases, however, reactants are difficult or impossible to tether to a DNA oligonucleotide. The development of strategies that enable non-DNA-linked small-molecule reagents to react in a sequence-programmed manner therefore would significantly expand the synthetic capabilities of DTS.

We hypothesized that DNA-templated functional group transformations[3] of unreactive to reactive groups would enable sequence-specified subpopulations of synthetic molecules to react with small-molecule reagents not tethered to DNA. Here we describe the development of three efficient DNA-templated functional group transformations (azide to amine, azide to thiol, and azide to carboxylic acid) and their use to transform a single-solution mixture of organic azides into sequence-programmed sulfonamide, carbamate, urea, and thiourea products using sulfonyl chloride, chloroformate, isocyanate, and isothiocyanate reactants not linked to DNA.

The Staudinger reaction[4] between a tertiary phosphine and an organic azide to form an iminophosphorane is the basis of recent bioconjugation methodology.[5] In the absence of reactive electrophiles, iminophosphoranes can be readily hydrolyzed to generate primary amine groups.[4b] The chemoselectivity[5a] and efficiency of the Staudinger reaction make this process an ideal starting point for the development of robust DNA-templated functional group transformations.

We reacted a variety of organic azides linked to the 5′ termini of 30-mer DNA oligonucleotide templates with a triphenylphosphine conjugated to the 3′ terminus of a complementary DNA 10-mer (Figure 1a). The resulting iminophosphorane was identified by MALDI-TOF mass spectrometry and found to be unexpectedly stable to hydrolysis especially under acidic conditions, presumably due to formation of a stable HCl salt.[6] Treatment of template-linked azides with DNA-linked phosphine in pH 10 buffer at 25 °C for 0.5 h followed by 37 °C for 12 h, however, resulted in quantitative iminophosphorane hydrolysis to generate the corresponding primary amines.

Unlike DNA-templated coupling reactions,[1] the azide-to-amine transformations could not be monitored directly by denaturing polyacrylamide gel electrophoresis (PAGE) because the starting materials and products are of similar molecular weight. To assay the progress of these reactions, the putative amine products were captured with 20-mer-linked carboxylic acids in the presence of a carbodiimide, or with 20-mer-linked aldehydes in the presence of $NaBH_3CN$. These secondary reagents displace the 10-mer linked phosphine oxide and efficiently couple with primary amines, but not with azides. The resulting amide or secondary amine products

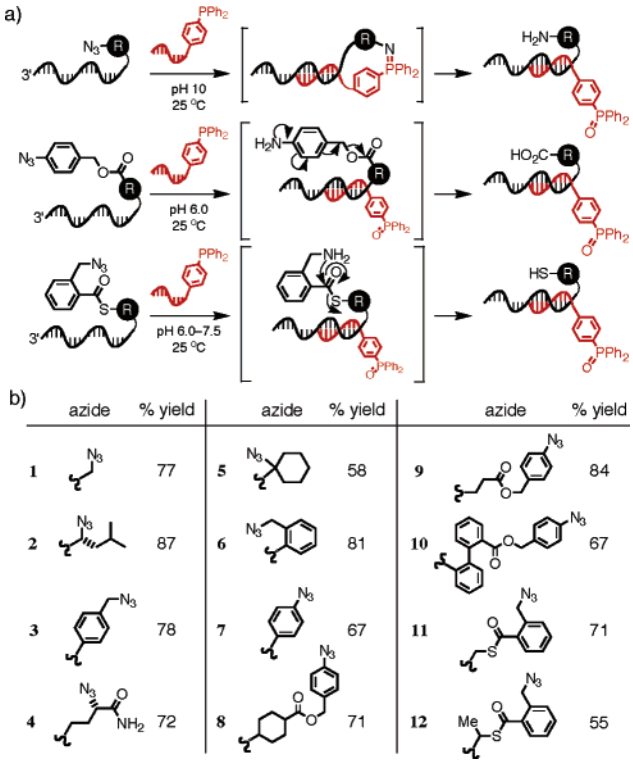

Figure 1. (a) DNA-templated transformation of azides into primary amines, carboxylic acids, and thiols. (b) Substrate scope of DNA-templated reactions. Listed yields represent lower limits (see text). Representative conditions: (**1**−**7**) 60 nM azide, 120 nM phosphine, 0.1 M CAPS pH 10, 0.5 M NaCl; (**8**−**11**) as above, except 0.1 M MES pH 6.0, 1 M NaCl; (**12**) as above, except 0.1 M MOPS pH 7.5, 1 M NaCl.

gain the molecular weight of the 20-mer and can easily be distinguished from starting azides by PAGE. The yields of these secondary products therefore represent lower limits for the efficiency of DNA-templated azide-to-amine transformations.

For the seven azides tested (**1**−**7**), DNA-templated azide reduction proceeded efficiently at pH 10 (Figure 1b). In each case, control reactions in which the phosphine was linked to a non-complementary (mismatched) oligonucleotide did not generate significant amide or secondary amine products, indicating that these DNA-templated azide-to-amine transformations proceed sequence-specifically. Mass spectrometric analysis of azide reduction reactions was consistent in each case with the formation of expected primary amine products (Supporting Information).

We extended the scope of these reactions to effect azide-to carboxylic acid and azide-to-thiol functional group transformations (Figure 1a). In both cases, azide reduction induced spontaneous fragmentation to unmask carboxylic acid[7] or thiol[8] groups. To assess the efficiency of these reactions, DNA-linked amines were used to capture carboxylic acids in the presence of a carbodiimide, while

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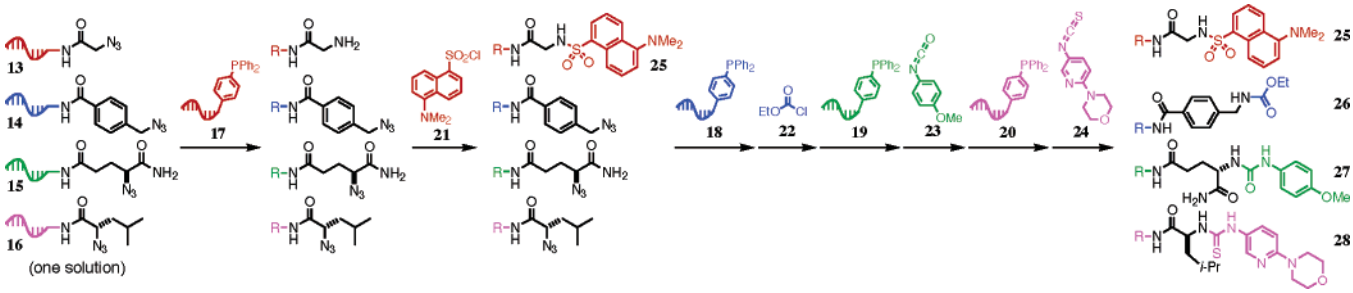

Figure 2. Reaction of a single solution containing four azides with four non-DNA-linked small-molecule electrophiles to generate four sequence-programmed sulfonamide, carbamate, urea, and thiourea products. See Supporting Information for detailed reaction conditions.

DNA-linked alkyl bromides were used to capture thiol products. Denaturing PAGE and mass spectrometric analysis (Supporting Information) indicated that DNA-templated functional group transformations to unmask carboxylic acid and thiol groups also proceeded efficiently and sequence-specifically (Figure 1b, substrates **8**−**12**).

To explore the ability of DNA-templated functional group transformations to enable non-DNA-linked reagents to participate in sequence-programmed synthesis, we prepared four DNA templates (**13**−**16**, Figure 2) each containing a different azide at the 5′ terminus, one of four unique six-base codons, and a biotin group at the 3′ terminus to facilitate template manipulation and purification. Dansyl chloride (**21**), ethyl chloroformate (**22**), 4-methoxy phenyl isocyanate (**23**), and 6-morpholino pyridinyl 3-isothiocyanate **(24**) were chosen as amine-reactive agents that cannot easily be attached to DNA due to their structure or their reactivity with water. When **21**, **22**, **23**, or **24** were added in excess (10 or 20 mM final concentration) in DMF to a template-linked primary amine under basic conditions (pH 9−10), the corresponding sulfonamide, carbamate, urea, or thiourea was efficiently generated (70% yield for **21**, >86% for **22**, **23**, and **24** as evaluated by HPLC analysis; see Supporting Information).

Finally, a single solution mixture containing 100 nM concentrations each of templates **13**−**16** (Figure 2) was combined with DNA-linked phosphine **17** to sequence-specifically transform **13** into the corresponding amine. The reaction mixture was then exposed to 10 mM sulfonyl chloride **21** in 1:1 DMF/50 mM aqueous $NaHCO_3$. If the DNA-templated azide-to-amine transformation proceeded sequence-specifically, only the amine arising from **13** should react with **21** to generate sulfonamide **25**, while **14**−**16** should remain unaltered (Figure 2).

Excess sulfonyl chloride was removed upon ethanol precipitation, and any unreacted amines were removed using *N*-hydroxysuccinimidyl (NHS) ester-linked resin. The resulting solution was similarly combined with **18** followed by **22**, **19** followed by **23**, and **20** followed by **24**. After the final step, any unreacted azides were removed by reduction with 5 mM TCEP followed by capture with NHS ester-linked resin. The final mixture of products was purified by capturing template-linked biotin groups with immobilized streptavidin.

MALDI-TOF mass spectrometry revealed that the final product mixture contains predominantly the four sequence-programmed products (sulfonamide **25**, carbamate **26**, urea **27**, and thiourea **28**) with none of the 12 possible undesired cross-products observed (Supporting Information). UV spectrometry indicated that the final product mixture was generated in 51% overall yield for the four consecutive DNA-templated reduction and small-molecule coupling sequences. These results establish that DNA-templated functional group transformations enable non-DNA-linked small molecules to participate in sequence-programmed reactions. The efficiency of this process also highlights the value of purification and washing strategies made possible when performing organic synthesis on this minute (sub-nmol) scale.

Taken together, the DNA-templated functional group transformations described above expand the synthetic capabilities of DNA-programmed synthesis by addressing the need for reagents to be tethered to DNA oligonucleotides. When the linkage of reagents to DNA is not possible or convenient, these transformations allow such reagents to nevertheless contribute to small-molecule syntheses while preserving the crucial correspondence between DNA sequence and product structure. In addition, by decoupling the DNA-templated step from the coupling reaction, this approach allows bond formation to take place under conditions that do not necessarily support DNA hybridization.

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Supporting Information Available: Experimental details and additional data. This material is available free of charge via the Internet at http://pubs.acs.org.

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