

Thus, 1,3,5-trimethylbenzene fails to react with sterically inaccessible aromatic C–H bonds but prefers activation of less hindered saturated C–H bonds. The only precedent for such selectivity of mesitylene oxidative addition to a platinum atom is the d^{10} transient $[\text{Pt}(\text{Cy}_2\text{PC}_2\text{H}_4\text{PCy}_2)]$.^[13,14] At the same time, formation of mixtures of isomeric aryl- and benzylplatinum(II) complexes as products of methylarene activation with platinum(II) complexes is known.^[15]

Thus, the influence of $\text{X}=\text{H}$ versus $\text{X}=\text{Me}$ in transient $[\text{LPtX}]^+$ is controlled by both the thermodynamics and kinetics of the reductive elimination of the resulting $[\text{LPtH(R)X}]^+$. When $\text{X}=\text{Me}$, methane elimination is accessible, but when $\text{X}=\text{H}$, H_2 elimination is energetically inaccessible, resulting in a Pt^{IV} product and only a single alkane C–H bond cleaved. In short, the number of methyl groups in the $[\text{LPt}(\text{Me})_n\text{H}_{3-n}]^+$ precursor dictates the number of alkane substrate C–H bonds that can be altered. This controllable production of alkane-derived $[\text{Pt}^{\text{IV}}(\text{alkyl})]$ or $[\text{Pt}^{\text{II}}(\text{olefin})]$ complexes may prove valuable in subsequent efforts to derivatize and effect net production of functionalized organic products from alkanes.

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Directing Otherwise Incompatible Reactions in a Single Solution by Using DNA-Templated Organic Synthesis**

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General methods for translating amplifiable information carriers such as DNA into synthetic molecules may enable the evolution of non-natural molecules through iterated cycles of translation, selection, and amplification that are currently available only to proteins and nucleic acids. During the process of developing such a method, we recently discovered that DNA templates can sequence-specifically direct a broad range of chemical reactions without any apparent structural requirements.^[1,2] The generality of DNA-templated synthesis together with appropriate linker and purification strategies enabled the first multistep small-molecule syntheses programmed by DNA templates,^[3] which raised the possibility of using this approach to generate synthetic small-molecule libraries of useful complexity.

DNA-templated synthesis^[4–24] can generate products individually linked to oligonucleotides that both encode and direct their syntheses.^[1–3] This feature may enable reaction modes useful for library construction that are not available through current synthetic approaches. Present synthesis methodology, for example, cannot differentiate functional groups of similar reactivity on different molecules within the same solution even though such differentiation would enable diversification to take place without the effort or constraints associated with spatial separation. Here we report that DNA oligonucleotides can simultaneously direct several different types of synthetic reactions within the same solution, even though the reactants involved would be cross-reactive and therefore incompatible under traditional synthesis conditions. Our findings represent a new mode of reaction made possible by DNA-templated synthesis and may enable the one-pot diversification of synthetic library precursors into products of multiple reaction types.

The ability of DNA templates to mediate diversification by using different types of reaction without spatial separation was first tested by preparing three oligonucleotide templates of different DNA sequences (**1a–3a**) functionalized at their 5'-ends with maleimide groups and three oligonucleotide reagents (**4a–6a**) functionalized at their 3'-ends with an amine, thiol, or nitroalkane group, respectively. The DNA sequences of the three reagents each contained a different 10-base annealing region that was complementary to ten bases

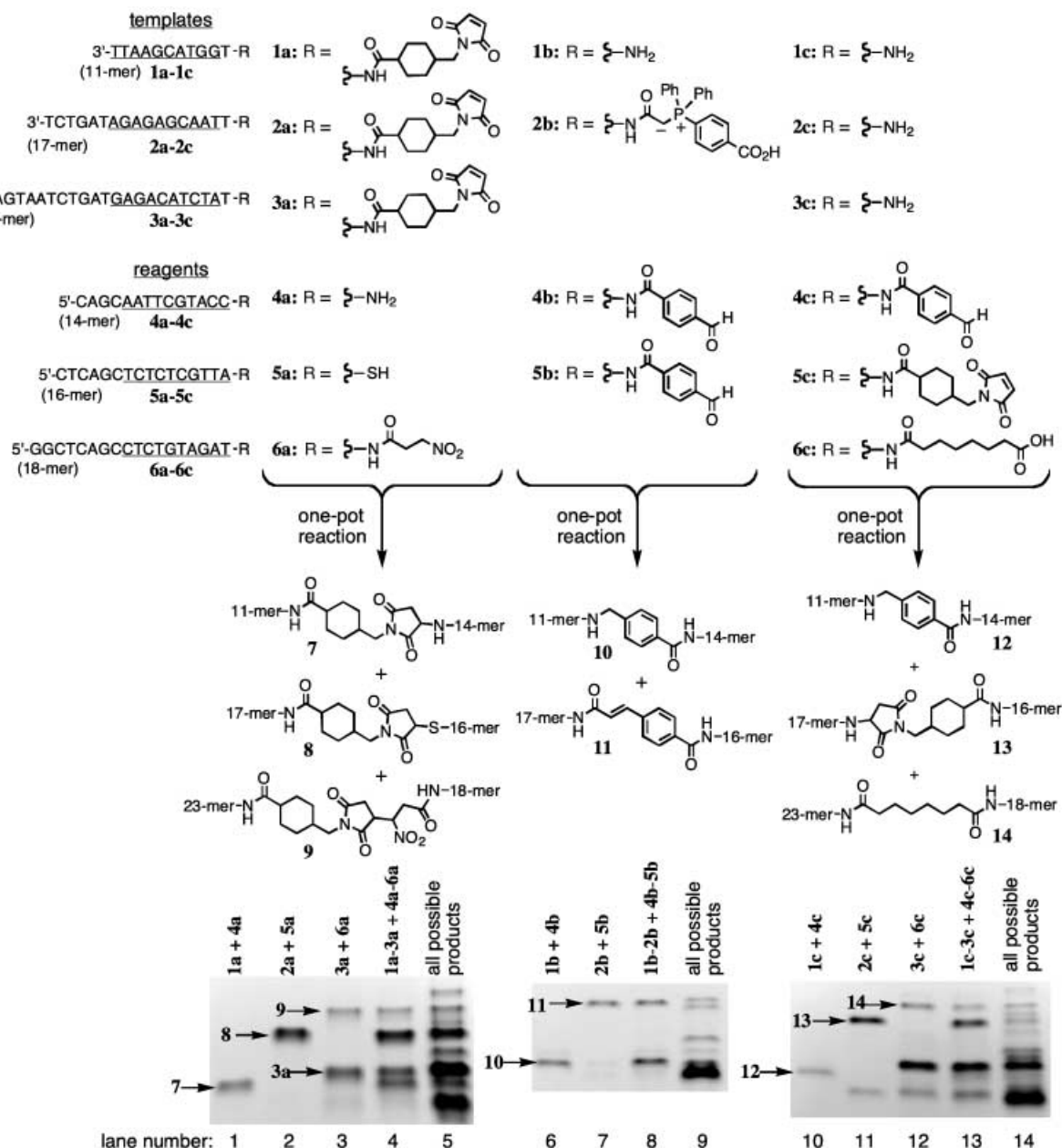
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near the 5'-end of each of the templates. Combining **1a** with **4a**, **2a** with **5a**, or **3a** with **6a** in three separate vessels at pH 8.0 resulted in the expected^[1,2] DNA-templated amine conjugate addition, thiol conjugate addition, or nitro-Michael addition products **7–9** (Scheme 1).

To distinguish the nine possible reaction products that could be generated upon combining **1a–6a**, we varied the lengths of the template oligonucleotides as 11, 17, or 23 bases and the lengths of the reagent oligonucleotides as 14, 16, or 18 bases in length. Differences in oligonucleotide length were achieved using extensions distal from the reactive groups and did not significantly affect the efficiency of the DNA-templated reactions. This design enabled all nine possible

reaction products (linked to 25, 27, 29, 31, 33, 35, 37, 39, or 41 bases of DNA) to be distinguished by denaturing polyacrylamide gel electrophoresis. A solution containing all three templates (**1a–3a**) was combined with a solution containing all three reagents (**4a–6a**) at pH 8.0. The resulting reaction exclusively generated the three desired products **7**, **8**, and **9** with lengths of 25, 33, and 41 bases, respectively, and indicates that only the three reactions corresponding to the complementary template–reagent pairs took place (Scheme 1). Formation of the other six possible reaction products was not detected by densitometry (<5% reaction). In contrast, individually reacting templates and reagents containing the same, rather than different, 10-base annealing regions enabled



Scheme 1. Subjecting maleimides, aldehydes, or amines to multiple DNA-templated reaction types in a single solution. The reactants shown were combined either pairwise (lanes 1–3, 6–7, or 10–12) or in one pot (lanes 4, 8, and 13) under the conditions described in the text and the resulting products were analyzed by denaturing gel electrophoresis. Annealing regions of oligonucleotides are underlined. To generate all the possible products (lanes 5, 9, and 14), which mimics a traditional reaction mode, templates and reagents analogous to those shown in the scheme but containing DNA sequences capable of annealing to any reagent or template, respectively, were reacted in pairs and combined.

the formation of all possible products (Scheme 1, lane 5). This result demonstrates the ability of DNA-templated synthesis to direct the selective one-pot transformation of a single functional group into three distinct types of products (in this example, maleimide into secondary amine, thioether, or α -branched nitroalkane).

To test the ability of this diversification mode to support one-pot reactions requiring non-DNA-linked accessory reagents, we conducted an analogous experiment with two aldehyde-linked reagents of either 14 or 16 bases in length (**4b** or **5b**, respectively) and a complementary 11-base amine-linked template (**1b**) or a 17-base phosphorane-linked template (**2b**). Combining **1b** and **4b** at pH 8.0 in the presence of 3 mM NaBH₃CN resulted in the DNA-templated reductive amination product **10**, while **2b** and **5b** under the same conditions generated Wittig olefination product **11** (Scheme 1). Mixing all four reactants together in one pot resulted in an identical product distribution as the combined individual Wittig olefination or reductive amination reactions (Scheme 1). No reaction between amine **1b** and aldehyde **5b** or between phosphorane **2b** and aldehyde **4b** was detected (Scheme 1, lane 8 versus 9).

We further explored the generality of this approach by including multiple reaction types that required different accessory reagents. Three amine-linked templates (**1c–3c**) of length 11, 17, or 23 bases were combined with a reagent linked to an aldehyde, carboxylic acid, or maleimide group (**4c–6c**) 14, 16, or 18 bases in length, respectively, at pH 8.0 in the presence of 3 mM NaBH₃CN, 10 mM 1-(3-dimethyl-amino-propyl)-3-ethylcarbodiimide (EDC), and 7.5 mM *N*-hydroxysulfosuccinimide (sulfo-NHS). The reactions containing all six reactants afforded the same three reductive amination, amine acylation, and conjugate addition products (**12–14**) that were generated from the individual reactions containing one template and one reagent and did not produce detectable quantities of the six possible undesired products arising from non-DNA-templated reactions (Scheme 1, lanes 10–14). Collectively, these results indicate that DNA-templated synthesis can direct simultaneous reactions between several mutually cross-reactive groups in a single pot to yield only the sequence-programmed subset of many possible products.

The above three examples each diversified a single functional group (maleimide, aldehyde, or amine) into products of different reaction types. A more general format for the one-pot diversification of a DNA-templated synthetic library into products of multiple reaction types would involve the simultaneous reaction of different functional groups linked to both reagents and templates. To examine this possibility we prepared six DNA-linked nucleophile templates (**15–20**) and six DNA-linked electrophile reagents (**21–25**) collectively encompassing all of the functional groups used in the above three examples (amine, aldehyde, maleimide, carboxylic acid, nitroalkane, phosphorane, and thiol). These twelve DNA-linked reactants could, in theory, undergo simultaneous amine conjugate addition, thiol conjugate addition, nitro-Michael addition, reductive amination, amine acylation, and Wittig olefination in the same pot, although the apparent second-order rate constants of these six reactions vary more than tenfold.^[1,2]

Determining the outcome of combining all twelve reagents and templates in a single pot by using oligonucleotides of varying lengths is difficult because of the large number (at least 28) of possible products that could be generated. Instead, we varied the length of the reagents as 15, 20, 25, 30, 35, or 40 bases but fixed the length of the templates at 11 bases (Scheme 2). Each of the six complementary template–reagent pairs when treated separately at pH 8.0 in the presence of 3 mM NaBH₃CN, 10 mM EDC, and 7.5 mM sulfo-NHS generated the expected amine conjugate addition, thiol conjugate addition, nitro-Michael addition, reductive amination, amine acylation, or Wittig olefination products (Scheme 2). Reaction yields were at least half of those previously reported^[1,2] for the corresponding individual reactions, despite having to compromise between differing optimal reaction conditions. Templates **15–20** were also prepared in a 3'-biotinylated form. The biotinylated templates demonstrated reactivities indistinguishable from those of their non-biotinylated counterparts (Scheme 2).

Six separate reactions each containing twelve reactants were then performed at pH 8.0 in the presence of 3 mM NaBH₃CN, 10 mM EDC, and 7.5 mM sulfo-NHS (Scheme 3). Each reaction contained a different biotinylated template (biotinylated **15–20**) together with five non-biotinylated templates (from **15–20**) and six reagents (**21–25**). These reactions were initiated by combining a solution containing six templates with a solution containing six reagents. The products that arose from each biotinylated template were captured with streptavidin-coated magnetic beads and identified by denaturing gel electrophoresis. Since the six reagents in each reaction contained oligonucleotides of unique lengths, the formation of any reaction products involving the biotinylated templates and any of the reagents could be detected. In all six cases, the biotinylated template formed only the single product programmed by its DNA sequence (Scheme 3) despite the possibility of forming up to five other products in each reaction. Taken together, these findings indicate that reactions of significantly different rates requiring a variety of non-DNA-linked accessory reagents can be directed by DNA-templated synthesis in the same solution, even when both templates and reagents contain several different cross-reactive functional groups. The ability of DNA templates to direct multiple reactions at concentrations that exclude nontemplated reactions from proceeding at appreciable rates mimics, in a single solution, a spatially separated set of reactions.

Compared to the use of traditional synthetic methods, the generation of libraries of small molecules by DNA-templated synthesis is limited by several factors including the need to prepare DNA-linked reagents; the restriction of aqueous, DNA-compatible chemistries; and the reliance on characterization methods such as mass spectrometry and electrophoresis that are appropriate for reactions performed on a molecular biology scale (pg to μ g). On the other hand, DNA-templated synthesis allows the direct *in vitro* selection (as opposed to screening) and amplification of synthetic molecules with desired properties, may enable the preparation of synthetic libraries of unprecedented diversity, and requires only minute quantities of material for selection and identification of active library members.^[1] In addition, the above

work demonstrates that potentially useful modes of reactivity not possible using current synthetic methods can be achieved in a DNA-templated format. In this case, we have shown that six different types of reactions can be performed simultaneously in one solution, provided that the required non-DNA-linked accessory reagents are compatible (in the above work, NaBH_3CN , EDC, and sulfo-NHS). This reaction mode may enable the diversification of synthetic small-molecule libraries using different reaction types^[25–29] in a single solution in addition to the more common diversification approach of using different building blocks in a given reaction. The use of this and other novel modes of reactivity enabled by DNA-templated synthesis is the subject of ongoing studies in our laboratory.

Experimental Section

Oligonucleotides were synthesized by using standard automated solid-phase techniques. Modified phosphoramidites and controlled-pore glass supports were obtained from Glen Research (Sterling, VA, USA). Unless otherwise noted, functionalized templates and reagents were synthesized by treating 5'- $\text{H}_2\text{N}(\text{CH}_2\text{O})_2$ -terminated oligonucleotides (for templates) or 3'- $\text{OPO}_3\text{-CH}_2\text{CH}(\text{CH}_2\text{OH})(\text{CH}_2)_4\text{NH}_2$ -terminated oligonucleotides (for reagents) in a 9:1 mixture of aqueous 200 mM pH 7.2 sodium phosphate buffer and DMF containing the appropriate *N*-hydroxysuccinimide ester (2 mg mL⁻¹, Pierce, Rockford, IL, USA) at 25°C. For the aldehyde and nitroalkane-linked oligonucleotides (**4b**, **4c**, **5b**, **6a**, **17**, **24**, and **26**) the NHS esters were generated by combining the appropriate carboxylic acid (900 mM in DMF) with equal volumes of dicyclohexylcarbodiimide (900 mM in DMF) and NHS (900 mM in DMF) for 90 min. Phosphorane-linked oligonucleotides (**2b** and **20**) were prepared by reaction of the appropriate amino-terminated oligonucleotide with a DMF solution (20 mg mL⁻¹, 0.1 volumes) of the NHS ester of iodoacetic acid (SIA, Pierce) in pH 7.2 buffer for 90 min as above, followed by addition of a solution (20 mg mL⁻¹, 0.1 volumes) of 4-diphenylphosphanylbenzoic acid in DMF. Thiol-linked template **16** was synthesized by treating the appropriate oligonucleotide with ethylene glycol bis(succinimidylsuccinate) (EGS, Pierce) for 15 minutes, followed by addition of 2-aminoethanethiol (300 mM, 0.1 volumes). Reagent **5a** was synthesized using 3'- $\text{OPO}_3\text{-(CH}_2)_2\text{SS(CH}_2)_3\text{ODMT}$ -functionalized controlled-pore glass (CPG) support and reduced prior to use according to the manufacturer's protocol. Linkers between DNA oligonucleotides and the functional groups in **1a–6c** are as follows: **1b** and **1c**: DNA-5'- NH_2 ; **1a**, **2a–2c**, **3a**, and **3c**: DNA-5'- $\text{O}(\text{CH}_2)_2\text{O}(\text{CH}_2)_2\text{-NH-}$; **5a**: DNA-3'- $\text{O}(\text{CH}_2)_3\text{SH}$; **4a–4c**, **5b**, **5c**, **6a**, and **6c**: DNA-3'- $\text{O-CH}_2\text{CH}(\text{CH}_2\text{OH})(\text{CH}_2)_4\text{NH-}$. The oligonucleotide sequences used to generate all possible products in Scheme 1 (lanes 5, 9, and 14), with annealing regions underlined, are: R-TATCTACAGAG-3' (**1a–1c**); R-TATCTACAGAGTAGTCT-3' (**2a–2c**); R-TATCTACAGAGTAGTCTAATGAC-3' (**3a–3c**); 5'-CAGCCTCTGTAGAT-R (**4a–4c**); 5'-CTCAGCCTCTGTAGAT-R (**5a–5c**); 5'-GGCTCAGCCTCTGTAGAT-R (**6a–6c**). Functionalized templates and reagents were purified by gel filtration (Sephadex G-25) followed by reverse-phase HPLC (0.1M triethylammonium acetate/acetonitrile gradient). Representative functionalized templates and reagents were further characterized by MALDI mass spectrometry.

3'-biotinylated oligonucleotides were prepared by using biotin-TEG CPG (Glen Research). Products arising from the biotinylated templates were purified by mixing with streptavidin-linked magnetic beads (1.05 equiv, Roche), washing twice with 4M guanidinium hydrochloride, and eluting with aqueous 10 mM tris(hydroxymethyl)aminomethane (Tris) pH 7.6 with 1 mM biotin at 80°C.

All reactions were performed by dissolving reagents and templates in separate vessels in pure water before combining them into a solution of 50 mM aqueous *N*-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid (TAPS buffer), pH 8.0, 250 mM NaCl at 25°C for 16 h with DNA-linked reactants at 60 nM (Scheme 1) or at 12.5 nM (Scheme 2 and Scheme 3). NaBH_3CN , EDC, and sulfo-NHS were present when appropriate as described in the text. Products were analyzed by denaturing

polyacrylamide gel electrophoresis using ethidium bromide staining and UV transillumination. Differences in charge states, attached functional groups, and partial secondary structure resulted in modest variations in gel mobility for different functionalized oligonucleotides of the same length (see Schemes 1, 2, and 3).

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