DNA-TEMPLATED CHEMICAL REACTIONS

Reported by Jennifer M. Heemstra February 24, 2003

INTRODUCTION

Nature has produced the most selective and efficient catalysts known, often in the form of enzymes having well defined structures to direct substrate recognition and subsequent catalysis of chemical transformations.[1] These capabilities have inspired multiple research efforts focused on the development of supramolecular assemblies that mimic enzymes by serving as templates for chemical reactions. Pederson was among the first to demonstrate non-enzymatic reaction templation by his use of a potassium ion to direct the cyclization of benzo[18]crown-6 (Figure 1).[2] With the development of more complex templation motifs, the definition of templation has emerged as the use of a supramolecular assembly to organize reactants in a specific spatial arrangement, favoring formation of a single product where the possibility to form more than one product exists.[3] One motif that has recently received increased attention due to its versatility in templating chemical reactions is single stranded DNA.

Figure 1. Templated ring closure of benzo[18]crown-6.

Since the discovery by Watson and Crick nearly 50 years ago that DNA exists as a double helix stabilized by complementary base-pair interactions (Figure 2),[4] the role of base-pairing in the synthesis of new DNA strands through the process of replication has become clear.[5] This knowledge in turn led Mullis and coworkers to make a critical technological advancement with their use of base-pair complementarity to enable *in vitro* amplification of DNA via the polymerase chain reaction (PCR).[6] Given the ability of a strand of DNA to template the polymerase-catalyzed synthesis of its complementary strand, researchers have recognized the potential of DNA to serve as a template for a myriad of different non-enzymatic chemical reactions. This review will discuss the development of DNA as a reaction template from early efforts aimed at native DNA ligation to its recent application in multistep small-molecule synthesis.

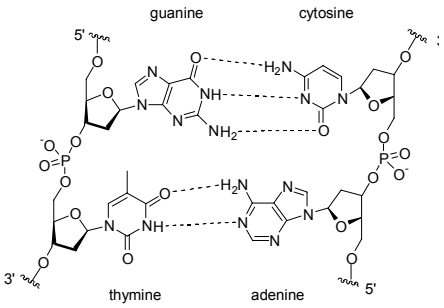


Figure 2. The structure of DNA.

DNA-TEMPLATED LIGATION REACTIONS

The first non-enzymatic DNA-templated reaction was reported by Naylor and coworkers in 1966, using polyadenosine as a template for the ligation of two thymidine hexanucleotides.[7] However, the low yield of 5% after 4 days rendered the chemistry of little practical use. The next significant

advance in this field did not come until 1984, when Orgel and coworkers successfully synthesized dGGCGG using a dCCGCC template and activated guanosine and cytidine monomers.[8] Unfortunately, this methodology was also hampered by poor reaction efficiency as a modest 17% yield was achieved and numerous byproducts from untemplated reactions were observed in the product mixture. In 1986, von Kiedrowski and coworkers proposed a potential solution to this problem with their report of the first autocatalyzed DNA-templated ligation reaction. Self-complementary hexanucleotide **1** was used to template the ligation of trinucleotides **2** and **3**. Because the resulting ligation product was identical to the original template, upon dissociation of duplex **4** two template molecules become available to participate in the subsequent catalytic cycle (Figure 3).[9] While the yield for this reaction was only 12% and catalytic turnover of the template was minimal due to the stability of the native DNA duplex, the design of a catalytically active template laid the conceptual foundation for much of the work that later followed in this field.

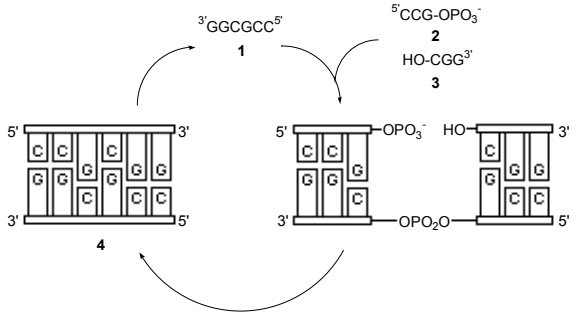


Figure 3. Autocatalyzed ligation of self-complementary DNA template.

Ligation of Modified DNA Oligonucleotides

Though conceptually significant, early attempts at DNA-templated ligation by Naylor, Orgel, and von Kiedrowski were burdened by product inhibition, low yields, and strict limitations on both the length and sequence of DNA strands that could be synthesized. Since the time of these initial reports, numerous research efforts have focused on circumventing these limitations to develop DNA-templated ligation reactions of practical use.

The discovery that backbone-modified nucleotides could be incorporated into DNA strands without significantly hindering the binding affinity between the modified strand and its complementary DNA strand lead Lynn and coworkers to develop a DNA-templated ligation strategy utilizing the imine condensation reaction.[10] To apply the imine condensation reaction to DNA ligation, reagent oligonucleotides **5** and **6** were synthesized bearing a 5'-amino or 3'-aldehyde functionality, respectively. The catalytic cycle for the templated ligation of **5** and **6** is shown in Figure 4. Upon combination of the reagent oligonucleotides with complementary template **7**, association occurred, followed by condensation to give associated imine ligation product **8**. Due to the lability of the imine bond, $NaBH_3CN$ was used as an internal reducing agent to trap the product as amine complex **9**. Dissociation of the two strands proceeded with an equilibrium constant of 1.2×10^2, releasing the product from the template and initiating a new catalytic cycle. Lynn hypothesized that the predisposition of the

backbone-modified DNA duplex to dissociate results from collapse of the aminoethyl group into the hydrophobic core of the double helix. This collapse moves the neighboring phosphate groups into closer proximity, destabilizing hydrogen bonding and disrupting π-stacking interactions between the nucleotide bases.[11] Thus, a high level of reaction efficiency can be achieved as the template is turned over in the catalytic cycle of the reaction.

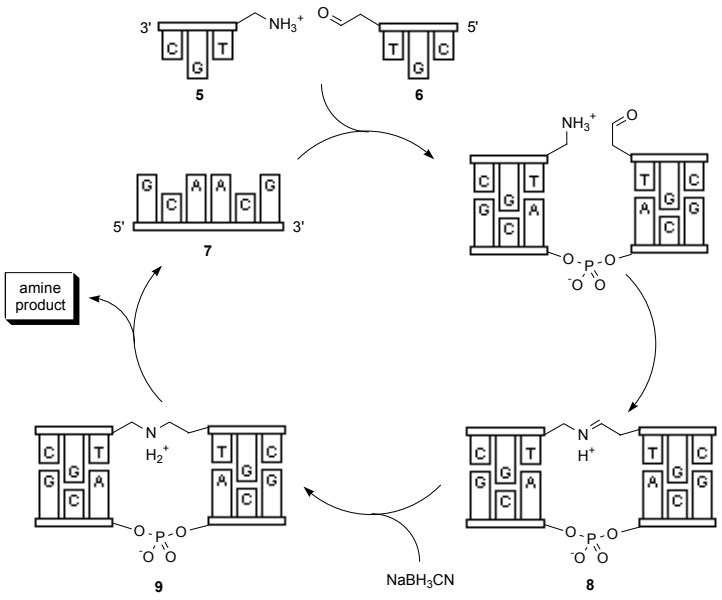
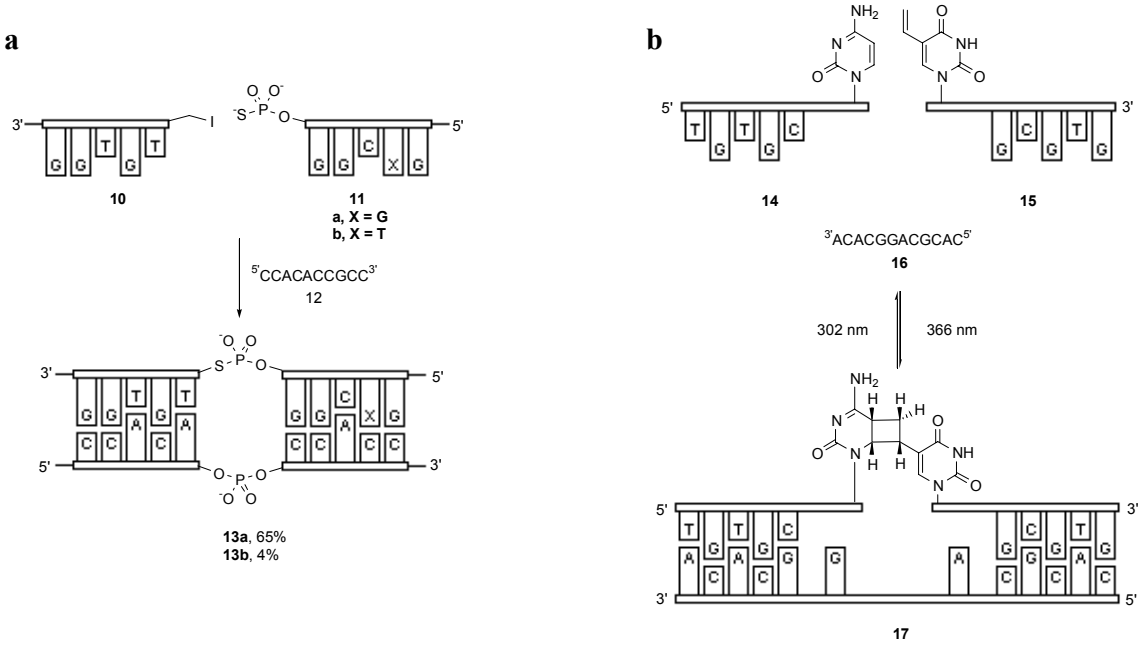


Figure 4. Catalytic cycle of DNA-templated ligation via reductive amination.

Nucleophilic substitution reactions can also be applied to DNA-templated ligation, as demonstrated by Kool and coworkers. Reaction of 5'-iodothymidine oligonucleotide **10** with oligonucleotide **11a** bearing a 3'-phosphorothioate anion in the presence of DNA template **12** yielded ligated product **13** (Scheme 1a).[12] Upon introduction of a single base-pair mismatch between **11b** and **12**, the reaction rate decreased 2000-fold relative to that for the correctly matched substrates. Kool subsequently went on to demonstrate the practical use of this high template fidelity for the detection of single point mutations in DNA.

Taking a different approach, Saito and coworkers used photoligation in the DNA-templated synthesis of oligonucleotides. Upon irradiation of 3'-cytosine **14** and 5'-vinyldeoxyuridine **15** with 366 nm light in the presence of DNA template **16**, ligated complex **17** was formed. Irradiation of the product with 302 nm light effected the reverse reaction, regenerating the starting materials (Scheme 1b).[13] The use of this methodology to perform multiple concomitant ligation reactions was also demonstrated by irradiation of five hexanucleotides in the presence of a complementary DNA template to produce the desired 30-mer oligonucleotide product. With this work, Saito and coworkers have demonstrated that photoligation of modified nucleotides is a viable method for DNA-templated ligation reactions, and that this method possesses the added benefit over previously presented backbone ligation methods in that the reaction is easily reversible.

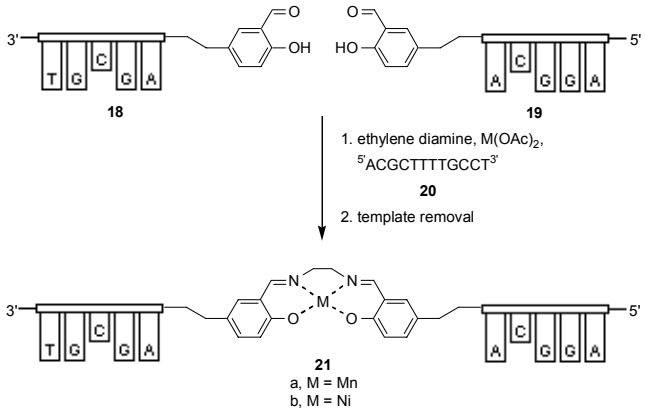
Scheme 1. (a) DNA-Templated ligation via nucleophilic substitution. (b) DNA-Templated photoligation via [2 + 2] cycloaddition.



DNA-TEMPLATED ORGANOMETALLIC COMPLEX FORMATION

The assembly of an organometallic complex within a DNA strand by Sheppard and coworkers represented a significant conceptual advancement in the field of DNA-templated synthesis. Whereas previous methodologies had focused on the use of DNA as a template for ligation of modified oligonucleotides, Sheppard designed a ligation reaction in which the product was a DNA-metallosalen complex capable of acting as a catalyst in subsequent reactions. To synthesize the metallosalen complex, 5'-salicylaldehyde **18** and 3'-salicylaldehyde **19** were reacted with complementary template **20**, ethylenediamine, and either $Mn(OAc)_2$ or $Ni(OAc)_2$. Upon removal of the template, the desired Mn (**21a**) or Ni (**21b**) DNA-metallosalen complex was obtained (Scheme 2). The metal ion was found to play a key role in templating complex formation, as a 65% yield was obtained for **21a**, but only a 4% yield of unmetallated DNA-salen was obtained when $Mn(OAc)_2$ was omitted from the reaction mixture. Using this chemistry, Sheppard has demonstrated the DNA-templated synthesis of

Scheme 2. Templated synthesis of DNA-metallosalen.



new organometallic reagents with potential applications in targeted nucleic acid cleavage, biosensors, and catalysis.[14]

DNA-TEMPLATED SMALL MOLECULE SYNTHESIS

Perhaps the greatest conceptual advancement in the field of DNA-templated synthesis was made by Liu and coworkers with their recent use of single-stranded DNA as a carrier of reagents and substrates for small molecule synthesis. In this methodology, a DNA template is covalently attached to each small-molecule substrate. Each reagent is also equipped with a strand of DNA. Upon annealing of the reagent DNA to its complementary template, the reagent and substrate are brought in close proximity, promoting the desired reaction. Since the time of their initial report,[15] Liu and coworkers have developed this methodology to encompass a broad range of chemical transformations, and have demonstrated its potential for generating combinatorial libraries in solution and directing multistep small-molecule syntheses.

Reaction Scope of DNA-Bound Reagents

Liu and coworkers first introduced their methodology with the use of DNA templates to direct conjugate addition and S_N2 reactions. The substrate generality of these reactions was demonstrated by successful nucleophilic additions of DNA-bound thiol reagent **22** to DNA-bound electrophiles **24-30** as shown in Figure 5. Also, DNA-bound amine reagent **23** was shown to function as a suitable nucleophile for conjugate addition to electrophiles **29** and **30**.[15] To verify the role of DNA in templating these reactions, Liu introduced base pair mismatches between the reagents and their templates. In each case when the reagent and template were mismatched, a dramatic decrease in reaction rate was observed, indicating the importance of the DNA-binding interaction in templating the reaction.

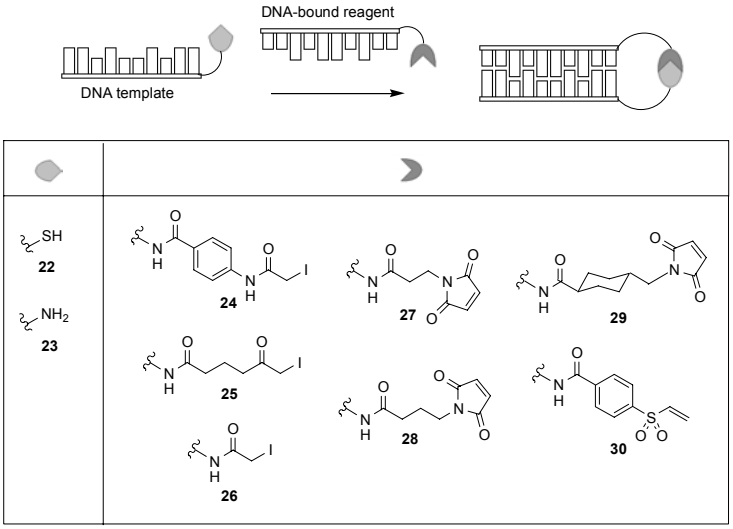


Figure 5. Reagents employed in DNA-templated conjugate addition and nucleophilic substitution reactions.

After demonstrating the ability of DNA templates to direct conjugate addition and S_N2 reactions, Liu and coworkers expanded the scope of their methodology to encompass a broader range of synthetic transformations. Moving beyond the previously studied bimolecular reactions, reductive amination and peptide coupling reactions requiring the addition of external reagents were shown to be compatible with

DNA-templating. Also, carbon-carbon bond forming reactions were explored owing to their significant synthetic utility, and successful templation of nitro-aldol, nitro-Michael, Wittig olefination, and 1,3-dipolar cycloaddition reactions was achieved. Finally, the ability of DNA to template organometallic reactions was demonstrated by carrying out a series of Pd-catalyzed Heck reactions.[16] With access to a sizeable arsenal of DNA-templated reactions, Liu and coworkers were then poised to explore potential applications of their methodology.

"Virtually" Segregated Reactions in Solution

The synthesis of combinatorial libraries has proven to be a powerful tool in the discovery of small molecules with unique properties, primarily owing to the potential for generating an exponentially greater number of different structures than could be synthesized individually.[17] However, from this ability to generate a large number of products in one reaction arises the challenge of deconvoluting the reaction mixture to determine which structures possess the desired properties. With the introduction of spatially segregated libraries, this challenge was partially met by physically separating each of the individual reactions in the library. Unfortunately, this separation reintroduced many of the practical limitations that combinatorial chemistry was designed to overcome, so the demand for new deconvolution techniques persisted. Liu and coworkers addressed this demand with their use of DNA-templated synthesis to create "virtually" segregated libraries having the benefits of spatial segregation but the potential to carry out all of the reactions in one solution.

Spatially segregating each reaction in a combinatorial library provides the ability to deliver the desired reagents to each substrate, and with this, to keep a record of the presumed structure generated in each reaction. This record makes deconvolution unnecessary once a product with the desired properties is identified. Liu and coworkers showed that DNA-templating allows these benefits to be achieved in solution, eliminating the inconveniences of physically separating each reaction. To demonstrate this concept, they synthesized a three-member combinatorial library by reaction of three DNA-bound nucleophile reagents with three DNA-bound electrophile substrates.[18] The DNA sequence of each reagent was synthesized to be complementary to that of the intended substrate, allowing for precise delivery of each reagent in the library. The delivery of each reagent to its target substrate was successful, as only the desired three out of the possible nine products were formed. Also, a record was kept of the DNA sequence attached to each reagent and substrate, so if a product had been found to possess a desired property, the presumed structure of the molecule could be elucidated by PCR amplification and subsequent sequencing of the attached DNA templates, making deconvolution unnecessary. This work has demonstrated the power of DNA-templation for precisely directing

simultaneous reactions in solution, allowing for the synthesis of "virtually" segregated combinatorial libraries.

DNA-Templated Multistep Small-Molecule Synthesis

In the course of their investigations, Liu and coworkers discovered that many of the DNA-templated reactions were tolerant to incorporation of additional base pairs between the substrate and reagent molecules. They hypothesized that this distance independence was observed when the rate of the reaction was significantly higher than the rate of annealing between the two DNA strands. In these cases, any decrease in the reaction rate caused by the intervening base pairs would go unnoticed so long as the reaction remained faster than the overall rate-limiting step of DNA annealing.[15] This discovery that DNA-templated reactions were possible even when the reagent and substrate were separated by long stretches of intervening base pairs opened the door for the design of templates programmed to carry out multistep small-molecule syntheses.

To demonstrate the feasibility of this new methodology, they performed two different multistep syntheses each using a single template to direct three sequential chemical transformations. The reaction cycle for these syntheses is highlighted in Figure 6.[19] Annealing of the first reagent to the template predisposes it for reaction with the substrate. The reagent is then cleaved from its coding DNA strand and immobilized on avidin beads. The product can then be isolated from the reaction mixture, at which point it is ready to bind the next reagent, commencing a new reaction cycle. This method was applied to the synthesis of an unnatural tripeptide by three subsequent amide bond forming reactions and the synthesis of a novel small molecule through a sequence of amide coupling, Wittig olefination, and conjugate addition reactions. The development of templates programmed to perform multistep syntheses allows for the generation of small molecules with higher levels of complexity and diversity than were attainable with the previously available DNA-templation methods.

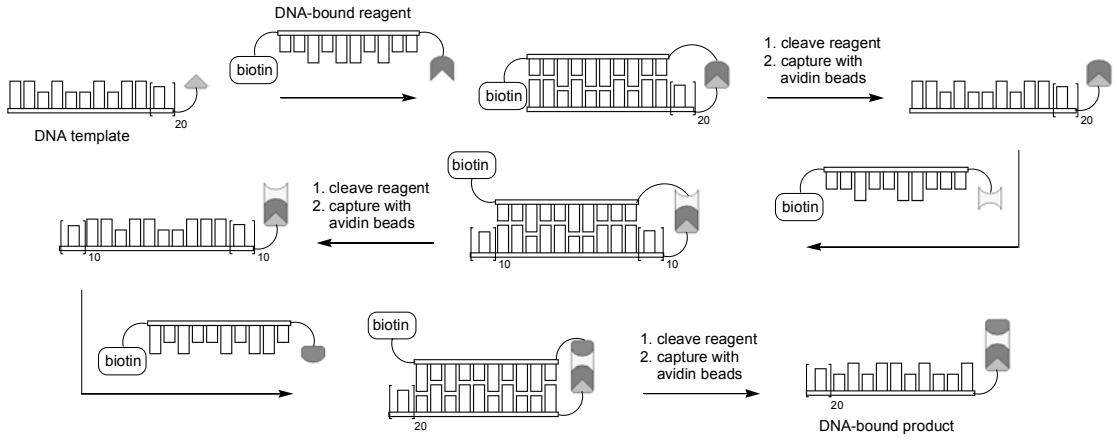


Figure 6. DNA-Templated multistep small-molecule synthesis.

CONCLUSION

The reliability with which complementary DNA strands are able to recognize and bind to one another makes DNA a useful template for chemical transformations. Until recently, however, the scope of this methodology was limited to DNA ligation reactions. The first use of DNA as a reaction template for small-molecule synthesis by Liu and coworkers represented a major conceptual breakthrough, and upon further development of the templation motif, its potential applications in generating "virtually" segregated combinatorial libraries in solution and directing multistep small-molecule synthesis were realized. As enzymes were the primary inspiration for supramolecular templation, it is not surprising that nature has also inspired a versatile and efficient templation motif.

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