

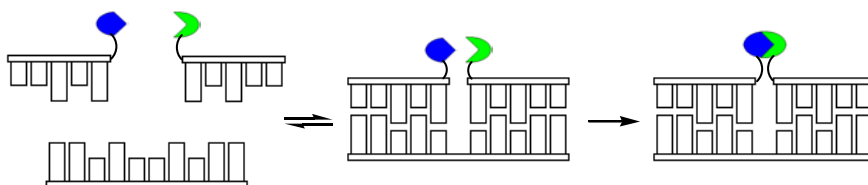
DNA-Templated Chemical Reactions

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ABSTRACT



DNA serves as an efficient template for a broad range of chemical transformations. Early attempts at DNA-templated ligation were plagued by uniformly low yields. Later, this obstacle was overcome through catalytic use of the DNA template. More recently, DNA has been used to direct the assembly of organometallic complexes and synthesis of a diverse collection of small molecules.

Since the discovery by Watson and Crick over 50 years ago that DNA exists as a double helix stabilized by complementary base-pair interactions,¹ the pivotal role of base-pairing in the synthesis of new DNA strands through replication has become clear.² This knowledge in turn inspired the use of base-pair complementarity by Mullis and coworkers to enable *in vitro* amplification of DNA via the polymerase chain reaction (PCR).³ The ability of a strand of DNA to template the polymerase-catalyzed synthesis of its complementary strand has also motivated researchers to explore the potential of DNA to serve as a template for a myriad of 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.

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.⁴ However, a low yield of 5% rendered the chemistry of little practical use. Nearly two decades passed without another significant advancement in this field until 1984, when Orgel and coworkers successfully synthesized dGGCGG using a dCCGCC template and activated guanosine and cytidine monomers.⁵ 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 these problems with their report of the first autocatalyzed DNA-templated ligation reaction. A self-complementary hexanucleotide was used to template the ligation of two trinucleotides. Because the resulting ligation product was identical to the original template, upon dissociation of the template-product duplex, two template molecules became available to participate in the subsequent catalytic cycle.⁶ While the yield for this reaction was only 12% and catalytic turnover of the template was minimal due to the stability of the native

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DNA duplex, the design of a catalytically active template laid the conceptual foundation for much of the work that later followed in this field.

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 have only a minor impact on binding affinity lead Lynn and coworkers to develop a DNA-templated ligation strategy utilizing the imine condensation reaction.⁷ To apply the imine condensation reaction to DNA ligation, reagent oligonucleotides **1** and **2** were synthesized bearing a 5'-amino or 3'-aldehyde functionality, respectively. The catalytic cycle for the templated ligation of **1** and **2** is shown in Figure 1. Upon combination of the reagent oligonucleotides with complementary template **3**, association occurs, followed by condensation to give associated imine ligation product **4**. Due to the lability of the imine bond, NaBH₃CN was used as an internal reducing agent to trap the product as amine complex **5**. 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.⁸ Thus, a high level of reaction efficiency is achieved as the template is turned over in the catalytic cycle of the reaction.

Taking a different approach, Saito and coworkers used photoligation for the DNA-templated synthesis of oligonucleotides. Upon irradiation of 3'-cytosine **6** and 5'-vinyldeoxyuridine **7** with 366 nm light in the presence of DNA template **8**, ligated complex **9** was formed. Irradiation of the product with 302 nm light effected the reverse reaction, regenerating the starting materials (Figure 2).⁹ The use of this methodology to perform multiple concomitant ligation reactions was 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 demonstrated that photoligation of modified nucleotides is a viable method for DNA-templated ligation

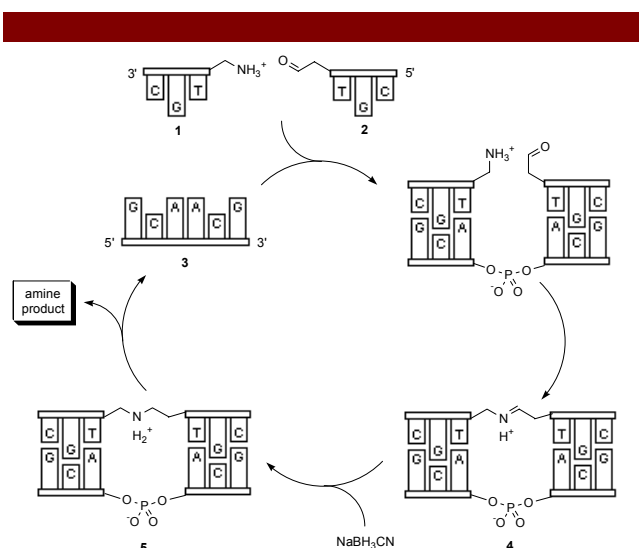


Figure 1. Catalytic cycle of DNA-templated ligation via reductive amination.

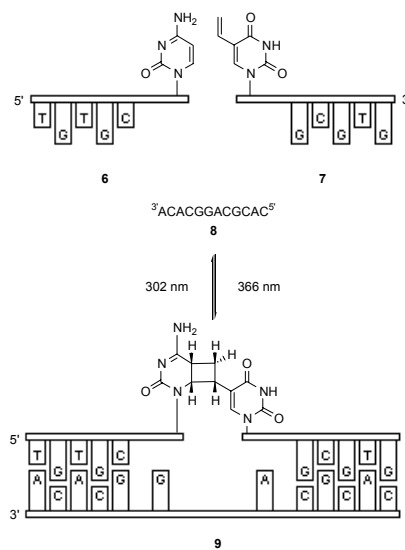


Figure 2. DNA-Templated photoligation via [2 + 2] cyclo-addition.

reactions, and that this method possesses the added benefit of reversibility.

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 focused on the use of DNA as a template for ligation of modified oligonucleotides, Sheppard designed a ligation reaction that produces a DNA-metallosalen complex

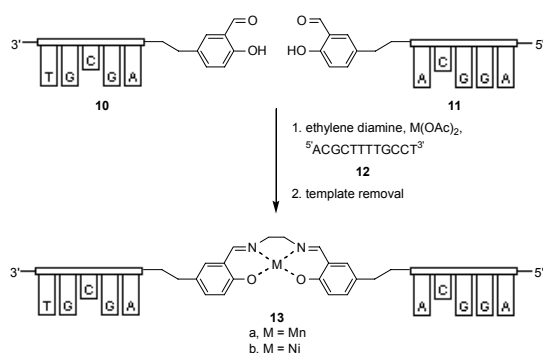
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capable of acting as a catalyst in subsequent reactions. To synthesize the metallosalen complex, 5'-salicylaldehyde **10** and 3'-salicylaldehyde **11** were reacted with complementary template **12**, ethylenediamine, and either $\text{Mn}(\text{OAc})_2$ or $\text{Ni}(\text{OAc})_2$. Upon removal of the template, the desired Mn (**13a**) or Ni (**13b**) DNA-metallosalen complex was obtained (Scheme 1). The metal ion was found to play a key role in templating complex formation, as a 65% yield was obtained for **13a**, but only a 4% yield of unmetallated DNA-salen was obtained when $\text{Mn}(\text{OAc})_2$ was omitted from the reaction mixture. Potential applications of the DNA-based organometallic reagents generated using this methodology include targeted nucleic acid cleavage, biosensors, and catalysis.¹⁰

Scheme 1. Templated synthesis of DNA-metallosalen.



DNA-Templated Small Molecule Synthesis

Perhaps the greatest conceptual advancement in the field of DNA-templated synthesis was achieved 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 constrained within close proximity, increasing the effective molarity and thus promoting the desired reaction. Since the time of their initial report,¹¹ Liu and coworkers have expanded this methodology to encompass a broad range of chemical transformations, and have demonstrated its potential for directing the synthesis of complex small molecules through multistep processes.

Liu and coworkers first introduced their methodology with the use of DNA templates to direct conjugate addition and $\text{S}_{\text{N}}2$ reactions. The substrate generality of these reactions was demonstrated by successful nucleophilic additions of DNA-bound thiol reagent **14** to DNA-bound electrophiles **16-22** as shown in Figure 3. Also, DNA-bound amine reagent **15** was shown to function as a

suitable nucleophile for conjugate addition to electrophiles **21** and **22**.¹¹ To verify the role of DNA in templating these reactions, base pair mismatches were introduced between the reagent and template sequences. A dramatic decrease in reaction rate was observed with the introduction of a single base pair mismatch, signifying the importance of the DNA-binding interaction in templating the reaction.

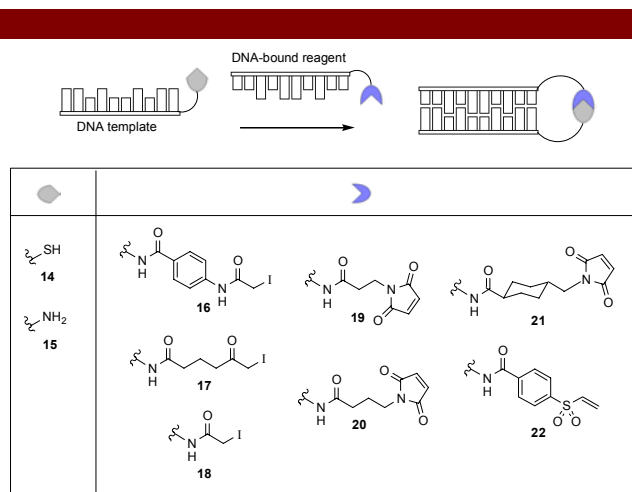


Figure 3. Reagents employed in DNA-templated conjugate addition and nucleophilic substitution reactions.

After demonstrating the ability of DNA templates to direct conjugate addition and $\text{S}_{\text{N}}2$ 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 templating 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 with a series of Pd-catalyzed Heck reactions.¹² Having gained access to a sizeable arsenal of DNA-templated reactions, Liu and coworkers were poised to explore the potential applications of their methodology.

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,

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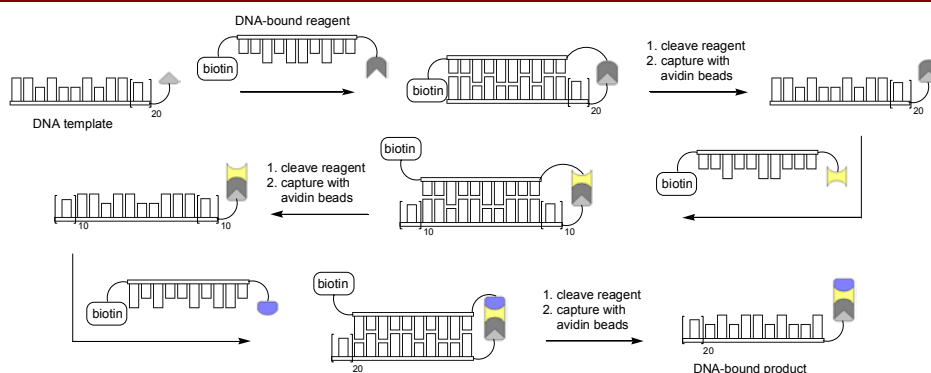


Figure 4. DNA-Templated multistep small-molecule synthesis.

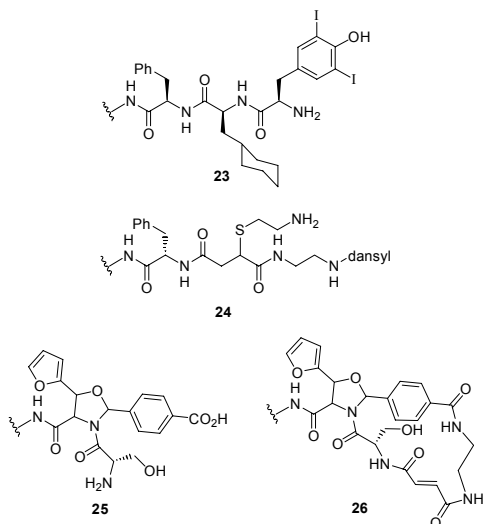
any decrease in the reaction rate caused by the intervening base pairs would go unnoticed so long as the reaction remained faster than the overall rate-limiting step of DNA annealing.¹¹ This discovery that DNA-templated reactions were possible even when the reagent and substrate were separated by long 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 methodology, two different multistep syntheses were performed each using a single template to direct three sequential chemical transformations. The reaction cycle for these syntheses is highlighted in Figure 4.¹³ Annealing of the first reagent to the template promotes reaction with the substrate. The reagent is then cleaved from its coding DNA strand and immobilized on avidin beads. After isolation of the product from the reaction mixture, the second reagent binds to the template, commencing a new reaction cycle. This method was applied to the synthesis of unnatural tripeptide **23** through three subsequent amide bond forming reactions and the synthesis of **24** through a

sequence of amide coupling, Wittig olefination, and conjugate addition reactions. In later work, Liu and coworkers developed novel template architectures¹⁴ and explored additional chemical transformations allowing for the synthesis of mono- and bicyclic *N*-acyloxazolidines **25** and **26**.¹⁵ Although only four small molecule products are reported, this work collectively demonstrates the potential for generation of diverse combinatorial libraries given the large number of commercially available reagent precursors.

Summary and Future Outlook

The high fidelity with which complementary DNA strands bind to one another makes DNA a useful template for chemical transformations. Until recently, however, the scope of this methodology was limited primarily to DNA ligation reactions. The use of DNA as a reaction template for small-molecule syntheses by Liu and coworkers represented a major conceptual breakthrough, and further development of the templation motif allowed its potential applications in directing multistep small-molecule synthesis to be realized. The half-century that has passed since Watson and Crick first elucidated the structure of the double helix has witnessed the development of multiple strategies for using DNA as a reaction template. And, given the high concentration of advances within the previous four years, it is nearly certain that researchers have only begun to realize the potential of DNA to efficiently and selectively direct chemical reactions.



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