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COMMUNICATIONS

Published on Web 04/01/2004

Translation of DNA into Synthetic *N*-Acyloxazolidines

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DNA-templated organic synthesis (DTS)[1,2] can (i) mediate diverse synthetic reactions to generate structures unrelated to the DNA backbone;[1a,b] (ii) direct the multistep synthesis of small molecules and polymers;[1c,g] (iii) enable new modes of controlling synthetic reactivity that cannot be realized using traditional synthesis methods;[1d] and (iv) allow the translation, in vitro selection,[1h] and amplification of libraries of DNA oligonucleotides encoding synthetic small molecules.[1a,h] Despite these advances, the synthetic capabilities of DTS are still in their infancy as compared with conventional organic synthesis. Indeed, only two DNA-templated multistep small-molecule syntheses have been described to date, comprising a tripeptide and a branched thioether.[1c] The development of increasingly sophisticated multistep DNA-templated syntheses is critical to maximize the functional potential of molecules discovered through DTS and selection-based[1h] methods.

Small heterocycles are a common structural element of many natural and synthetic molecules with desirable biological or chemical activities.[3] Oxazolidines and *N*-acyloxazolidines are prominent examples of heterocycles found in bioactive natural products.[3a−d] These structures are also of special interest for compound library generation because they can be constructed modularly from aldehydes, amino alcohols, and acyl donors.[3a] Here, we describe efficient multistep DNA-templated syntheses of monocyclic and bicyclic *N*-acyloxazolidines using new DNA-templated reactions and linker strategies together with recently developed[1b,e] second-generation template architectures. These structures represent the most complex synthetic small molecules to date translated from DNA sequences.

We designed DNA-templated syntheses of two types of *N*-acyloxazolidines. The first target structure (**1a**) consists of an oxazolidine core functionalized at C2, N3, and C5 and conjugated to its corresponding DNA template at C4 (Figure 1, top left). To increase the complexity and structural rigidity of this target, we also designed the DNA-templated synthesis of a bicyclic *N*-acyloxazolidine (**1b**) incorporating a macrocyclic linkage between the C2 and N3 substituents (Figure 1, top right). For the synthesis of both target structures, oxazolidine formation was a key step. We therefore began by optimizing DNA-templated oxazolidine formation from the combination of template-linked amino alcohols and aldehydes conjugated to complementary 10-base DNA oligonucleotides. Oxazolidine formation proceeded efficiently in a DNA-templated format at an optimized pH of 7.5. The resulting oxazolidines were stable while linked to hybridized template and reagent oligonucleotides that offset the entropic cost of oxazolidine formation. Once cleaved from the reagent oligonucleotide, however, the oxazolidines were unstable to mild aqueous conditions required for their purification. As is expected on the basis of their predominance among oxazolidine-containing natural products,[3] *N*-acyloxazolidines, in contrast, were found to be very stable under a wide range of conditions.

The recently described T architecture for DTS[1e] enables two DNA-templated reactions to take place on one template in a single step. This architecture, in principle, could solve the problem of oxazolidine instability by enabling oxazolidine *N*-acylation to proceed while the oxazolidines remained stabilized by template-reagent hybridization. We also envisioned that the careful use of biotin affinity capture and elution strategies[1c] would enable desired template-linked *N*-acyloxazolidines to emerge from multistep syntheses with high purity.

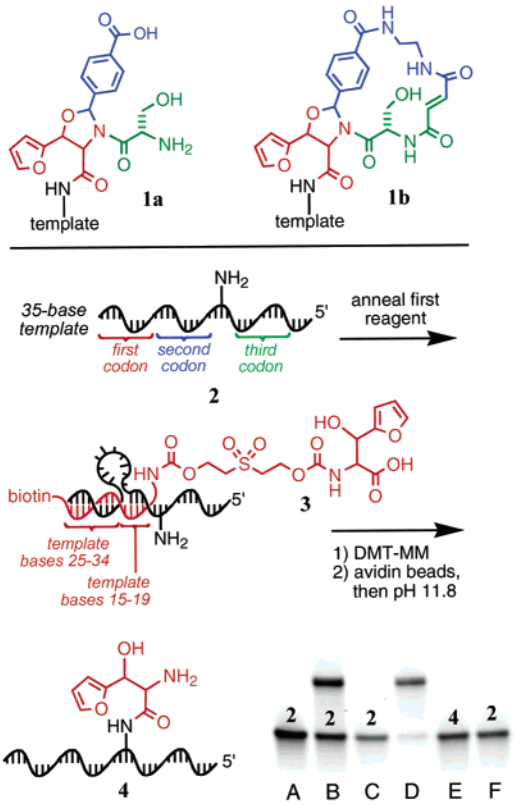

Figure 1. Top: *N*-Acyloxazolidine targets (**1a** and **1b**) for multistep DTS. Bottom: Denaturing PAGE analysis of step 1 toward **1a** and **1b**. Lane A, **2** only; lane B, reaction containing **2** (60 nM), **3** (120 nM), and DMT-MM (50 mM); lane C, species from lane B not captured by avidin-linked beads (supernatant); lane D, species from lane B captured by avidin-linked beads (pellet); lane E, lane D material after base-induced linker cleavage; lane F, identical to lane B but using a sequence-mismatched variant of reagent **3** instead of **3**. The upper band is the amide product prior to sulfone linker cleavage. Reagent oligonucleotides are not visible.

We integrated and implemented the above strategies to achieve the efficient DNA-templated synthesis of both **1a** and **1b**, each using two steps comprising three DNA-templated reactions. The first reaction of both syntheses consisted of DNA-templated amine acylation[1b] between a primary amine-linked template (**2**) and a DNA-linked amino alcohol reagent (**3**). As compared with a simple long-distance DNA-templated amine acylation,[1a] yields of desired product were roughly doubled (to 51%) when the recently developed Ω template architecture[1e] was used to further increase the effective

10.1021/ja049666+ CCC: $27.50

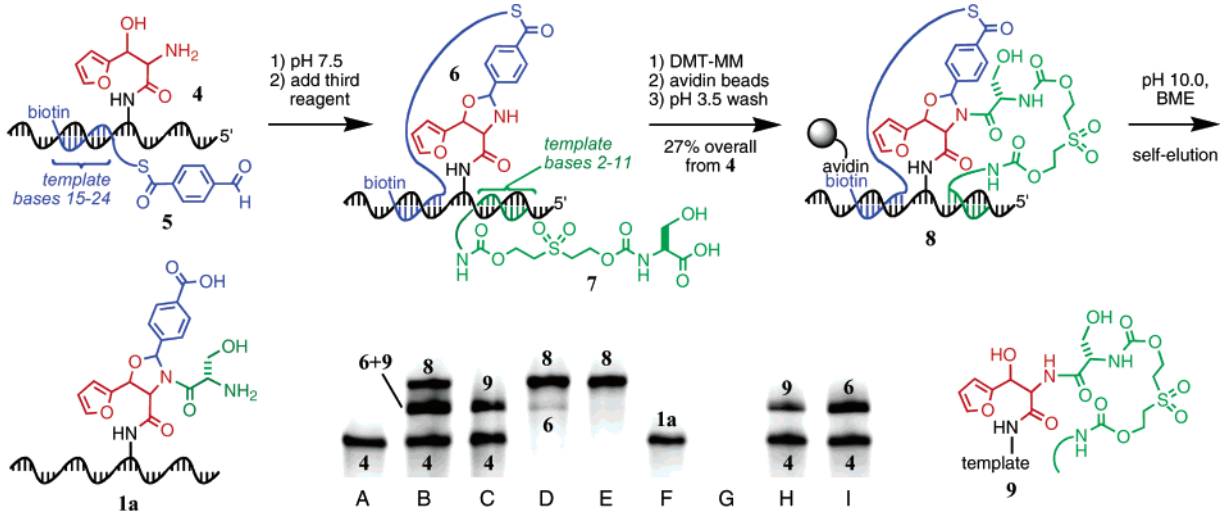

Figure 2. Completion of the DNA-templated synthesis and purification of **1a**. Denaturing PAGE lane A, **4** only (Figure 1); lane B, **4** (60 nM) treated with **5** (120 nM) at pH 7.5, then with **7** (120 nM) and DMT-MM (50 mM); lane C, the species from lane B not captured by avidin-linked beads (supernatant); lane D, the biotinylated species from lane B captured by avidin-linked beads (pellet); lane E, avidin bead-bound species from lane D following washing with pH 3.5 buffer to hydrolyze and remove unacylated oxazolidine (**6**); lane F, material from lane E self-eluting from avidin beads after thioester and sulfone linker cleavage induced by pH 10 buffer containing 10 mM β-mercaptoethanol; lane G, material remaining bound to beads after linker cleavage; lanes H and I, identical to lane B but using sequence-mismatched variants of **5** or **7**, respectively. Reagents **5** and **7** are not visible.

molarity of the reactive groups (Figure 1, bottom). The optimized reaction was therefore directed both by the 10 bases near the 3′ end of the template oligonucleotide (bases 25−34) as well as by the five bases adjacent to the reactive template amine (bases 15−19), which together complement the 15-base oligonucleotide linked to the amino alcohol. Template and reagent concentrations were 60 and 120 nM, respectively, precluding significant intermolecular (nontemplated) reaction.[1a] The sequence-specificity and templated mechanism of this reaction were confirmed by the absence of observable product when three point mutations were introduced into the reagent oligonucleotide sequence (Figure 1, lane F). Desired template-linked amino alcohol (**4**) was purified by capture with streptavidin-linked beads followed by base-induced sulfone linker cleavage[1c] and was further characterized by MALDI-TOF mass spectrometry (expected mass = 11 064.5; observed mass = 10 173.6 ± 11).

Using the T template architecture, both oxazolidine formation and oxazolidine acylation toward **1a** were executed in a single step (Figure 2). Amino alcohol-linked template **4** was combined with a benzaldehyde-linked biotinylated reagent (**5**, designed to anneal to template bases 15−24) at pH 7.5 to effect oxazolidine formation. Without isolation of the oxazolidine intermediate (**6**), a nonbiotinylated acylation reagent (**7**, designed to anneal to template bases 2−11) together with coupling agent DMT-MM was added to the solution, effecting *N*-acylation and stabilization of the oxazolidine ring to generate *N*-acyloxazolidine **8** (Figure 2). Treatment of **4** with sequence-mismatched variants of **5** or **7** did not generate detectable *N*-acyloxazolidine (Figure 2, lanes H and I), indicating that both DNA-templated reactions proceed sequence-specifically.

The use of the T template architecture to effect two DNA-templated reactions in a single step makes the purification of the *N*-acyloxazolidine intermediate (**8**) an unusual challenge. At least six species could be present in the crude step 2 reaction mixture: unreacted **4**, unreacted **5**, unreacted **7**, unacylated oxazolidine (**6**), misacylated amino alcohol (**9**), and the desired *N*-acyloxazolidine intermediate (**8**) (Figure 2). Partial purification was achieved by capturing the biotinylated species (**5**, **6**, and **8**) using streptavidin-linked magnetic beads, and then washing the beads with pH 3.5 buffer to hydrolyze and remove unacylated oxazolidine (**6**) (Figure 2, lanes C−E). The remaining immobilized **5** and **8** were treated with pH 10 buffer containing 10 mM β-mercaptoethanol to cleave both the thioester and the sulfone linkers. The biotin group in **8** is lost as a consequence of thioester linker cleavage, causing the desired *N*-acyloxazolidine **1a** to self-elute from the beads (Figure 2, lanes F and G) while the biotinylated oligonucleotide from **5** and **8** remains bound. To remove any contaminating 10-mer reagent oligonucleotide from sulfone linker cleavage of **8**, the final **1a** material was further purified by denaturing PAGE. MALDI-TOF mass spectrometric analysis of the final product was consistent with its expected structure (expected mass = 11 283.3; observed mass = 11 286.8 ± 11). *N*-Acyloxazolidine **1a** was synthesized in 3−6% overall yield from **2** through three DNA-templated reactions, three linker cleavages, and three purification steps.

To achieve the synthesis of bicyclic *N*-acyloxazolidine **1b**, a nonbiotinylated aldehyde reagent (**10**) containing a 1,2-diol linker was used in place of **5**, while a biotinylated oxazolidine acylating reagent (**11**) containing a phosphonium linker replaced **7**. The DNA-templated synthesis of oxazolidine **12** and *N*-acyloxazolidine intermediate **13** starting from amino alcohol **4** proceeded sequence-specifically using the T template architecture as described above (Figure 3, lanes A−D, I, and J). The partial purification of **13** (together with unreacted **11** and misacylated amino alcohol **14**) was achieved using streptavidin-linked magnetic beads (Figure 3, lanes C and D) to remove **4**, **10**, and **12**. The diol linker in immobilized **13** was cleaved with $NaIO_4$ in acidic buffer to afford aldehyde **15** (Figure 3, lane F). Under these conditions, the phosphonium linker remains protonated and premature macrocyclization does not take place appreciably (Figure 3, lane E), enabling the 10-mer reagent oligonucleotide generated during diol cleavage to be washed away. Efficient Wittig macrocyclization and concomitant loss of the biotin group was then induced by treatment with pH 8.5 buffer, causing the desired macrobicyclic *N*-acyloxazolidine (**1b**) to self-elute from the immobilized streptavidin in pure form while undesired **11** and **14** remain immobilized (Figure 3, lanes G and H). Bicyclic *N*-acyloxazolidine product **1b** was formed in 3−7% overall yield from **2** after three DNA-templated reactions, three linker cleavages, two purification steps, and a macrocyclization (expected mass = 11 405.8; observed mass = 11 398.9 ± 11). Starting with 1 nmol of **2**, sufficient **1a** and **1b** can be generated by the above syntheses for many in vitro selections.[1h]

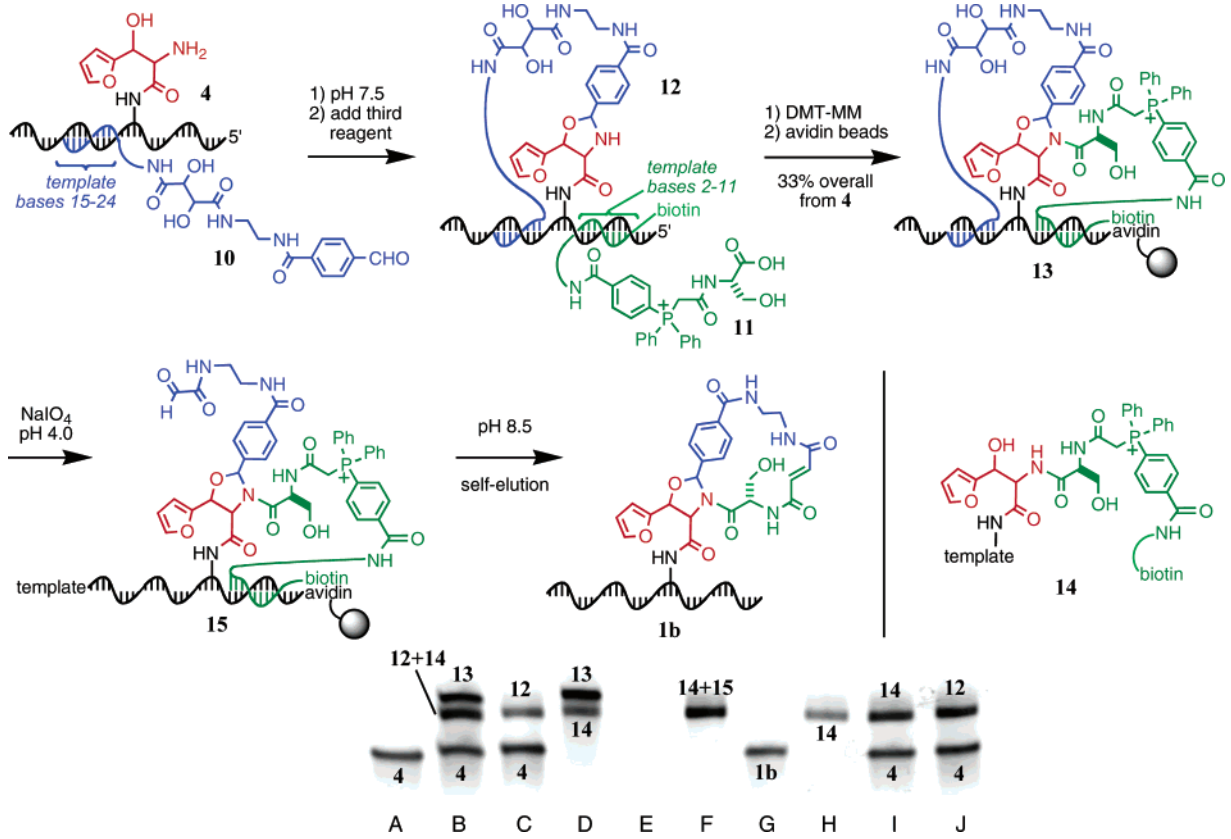

Figure 3. Completion of the DNA-templated synthesis and purification of **1b**. Denaturing PAGE lane A, **4** only (Figure 1); lane B, **4** (60 nM) treated with **10** (120 nM) at pH 7.5, then with **11** (120 nM) and DMT-MM (50 mM); lane C, the species from lane B not captured by avidin-linked beads (supernatant); lane D, the biotinylated species from lane B captured by avidin-linked beads (pellet); lane E, species from lane D released from avidin beads upon washing with $NaIO_4$ in pH 4.0 buffer to induce diol linker cleavage; lane F, avidin bead-bound species from lane D after $NaIO_4$ treatment; lane G, material from lane F self-eluting from avidin beads upon macrocyclization induced by pH 8.5 buffer; lane H, material remaining bound to beads after pH 8.5 treatment; lanes I and J, identical to lane B but using sequence-mismatched variants of **10** or **11**, respectively. Reagents **10** and **11** are not visible.

The *N*-acyloxazolidines generated in this work, while still modest in size as compared with many targets of traditional organic synthesis, represent the most complex small-molecule structures to date assembled in a DNA-templated manner. These multistep syntheses are also the first to take advantage of second-generation template architectures (Ω and T)[1e] that were crucial to their completion. In addition, these studies add oxazolidine formation and thioester linker cleavage to the growing repertoire[1] of DNA-templated chemistries and linker manipulation strategies. In contrast with previously described products of multistep DTS,[1c] the products created in this work present three building blocks in a branched arrangement about a rigid and cyclic, rather than flexible and linear, core structure. This functionally dense arrangement together with the commercial availability of amino alcohols, aldehydes, and carboxylic acids compatible with the schemes in Figures 2 and 3 suggest that libraries of DNA-templated *N*-acyloxazolidines may be promising sources of functional small molecules. Efforts to translate, select, and amplify libraries of DNA templates encoding functional monocyclic and bicyclic *N*-acyloxazolidines are underway in our laboratories.

Acknowledgment. We are grateful to the NIH/NIGMS (R01 GM065862), the Arnold and Mabel Beckman Foundation, the Searle Scholars Program (00-C-101), and the Office of Naval Research (N00014-03-1-0749) for support. Z.J.G. is a Bristol-Myers Squibb graduate research fellow; B.N.T. is an NSF graduate research fellow.

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

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JA049666+