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Development and Design of the tertiary amino effect reaction for DNA-encoded library synthesis‡

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The generation of novel chemical leads for clinical development is a constant challenge in the pharmaceutical industry. The synthesis of DNA-encoded libraries (DELs) has emerged as a powerful method for lead generation. We report the development of the tertiary amino effect reaction on DNA-tethered substrates. A variety of ortho-dialkylaminoaryl aldehydes undergo a cascade reaction involving a Knoevenagel condensation, a [1,5]-hydride shift, and a Mannich cyclization to give diversely substituted spirocycles. NMR analysis of substrates bearing an enriched double-^{13}C label confirmed product formation. The net formation of two carbon-carbon bonds adds to the few examples of carbon-carbon forming reactions performed in presence of DNA-encoding systems.

DNA encoded libraries (DELs) have become a powerful tool for the generation of lead compounds.[1] By associating the identity of each building block with a corresponding DNA tag, it is possible to encode the each building block used in a split-pool synthesis (Figure 1). To date, a variety of DELs have been interrogated against proteins of interest resulting in low micromolar inhibitors of disease targets.[2] DELs have thus become an important component in the lead discovery process.[3] At least two clinical candidates targeting soluble epoxide hydrolase[4] and autotaxin[5] have come to fruition based on DEL hits.

While the results from DEL selections have been impressive, the majority of these libraries rely on a few reactions relative to the richness of organic chemistry to generate chemical diversity,[6] including amide bond formation,[7] Suzuki coupling,[8] reductive amination,[9] S_NAr substitution,[10] and click chemistries.[11] There are few carbon-carbon bond-forming reactions reported for DEL synthesis.[12] The need for diverse, rich lead-like structures

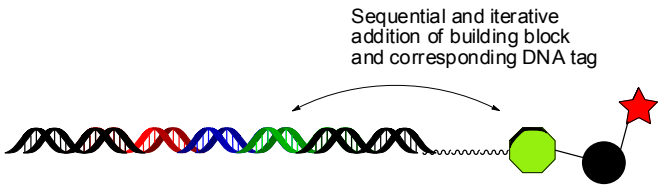

Figure 1. DNA-encoded small molecule library concept

continues to drive the development of new chemistries conducted in concert with DNA. Reactions applicable to DEL synthesis must be conducted in an aqueous environment, have wide substrate scope, and be operationally simple. Herein, we report the exploration and development of chemistry of the tertiary amino effect reaction (T-reaction) for application to a DEL format. The T-reaction enables the rapid assembly of stereochemical complexity by bringing together an ortho-dialkylaminoaryl aldehyde with an activated methylene (Figure 2).[13]

Recently this transformation was utilized in the synthesis of a novel DNA-gyrase inhibitor, ETX0914 (**5**) (Figure 3), now in Phase II clinical trials for the treatment of gonorrhea.[14] This fact is an

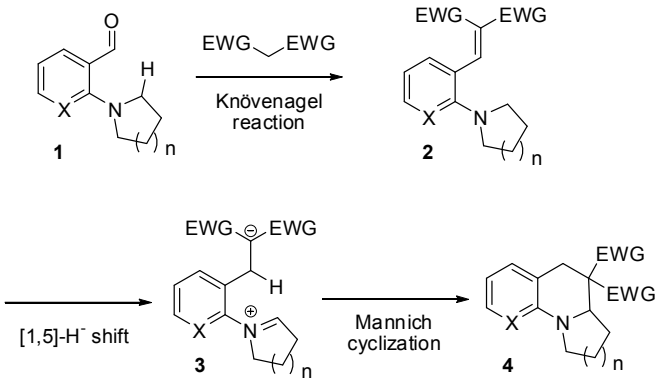

Figure 2. The T-reaction: tertiary amino effect reaction.

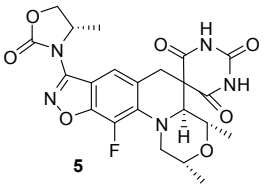

Figure 3. Structure of ETX0914.

indication of the drug-like potential of such structures. The simple and relatively mild reaction conditions boded well for on-DNA work, as at the outset it was known that the reaction cascade initiated by a Knoevenagel condensation proceeds well in water. The facile and irreversible subsequent [1,5]-hydride shift and Mannich cyclization to the 6-membered ring drives the reaction forward, even if the initial Knoevenagel condensation were reversible. The precursor *ortho*-dialkylaminoaryl aldehyde for the T-reaction can be made by S_NAr displacement of *ortho*-fluoroaryl aldehydes with secondary amines. Given the large number of commercially available S_NAr substrate aldehydes, secondary amines and cyclic activated methylenes, the resultant

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library could yield millions of novel, diversely substituted spirocycles. This water-compatible transformation along with the Diels-Alder reaction[15] is only one of a few multi C-C bond formation reactions reported for on-DNA synthesis.

Our investigation began with a model system shown in Scheme 1. A benzyl amine appended oligonucleotide was treated with excess 2-fluoro-3-formylpyridine under S_NAr conditions to yield cleanly aldehyde **6**. Initial attempts to conduct the T-reaction with barbituric acid failed to give appreciable product. A full screen of solvent conditions revealed that 50% dioxane / water as solvent led to good conversions, and a mild organic base, such as diisopropylethylamine, greatly accelerated the reaction. With these modifications, spirocycle **7** cleanly formed in 30 minutes at 60 °C. Notably, these optimized conditions differed from those typically used for the T-reaction.

A small scope study showed the reaction was tolerant to a variety of amines attached to DNA (Table 1). Electronic factors on the benzylic amine did not affect conversion and less activated alkyl amines were tolerated (compounds **10**, **11**, **13**). While these results gave us confidence in the scope of the reaction, two outstanding issues remained. First, the regiochemistry of the reaction was

Scheme 1. Spirocycle formation via T-reaction on-DNA.

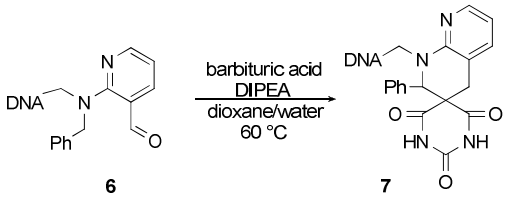
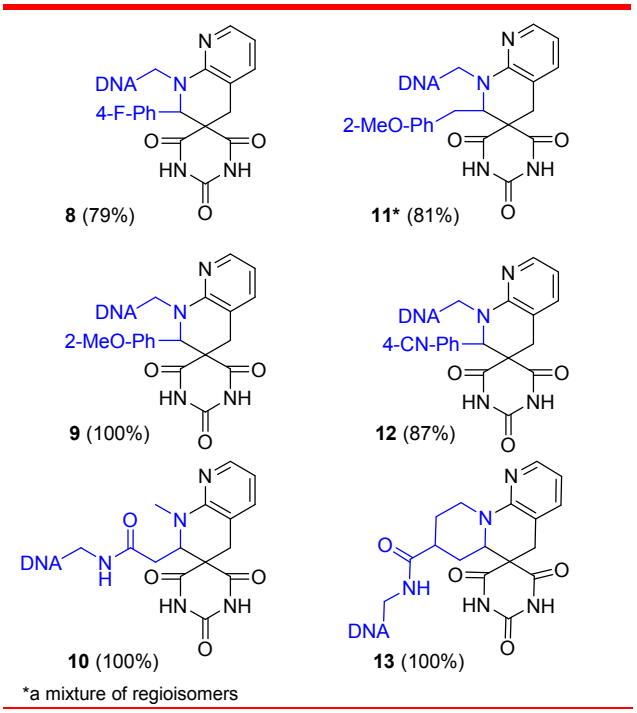


Table 1. Preliminary scope of the T-reaction.



undetermined. Based on literature precedent, it was expected that the kinetics of hydride transfer should proceed as benzylic > alkyl > methyl, though determining the actual selectivity on-DNA is *a priori* impractical.[16] Second, since there is no net mass change between the Knoevenagel adduct and the final spirocycle (Figure 2), whether the [1,5]-H$^-$ shift and subsequent Mannich cyclization occurred on-DNA cannot be determined from the LC-MS trace alone. LC-MS analytical techniques are limited whenever a reaction sequence occurs that generates new bonds and structural complexity but does not involve a change in mass. A general solution to on-DNA reaction analyses, orthogonal to MS, would enhance the validation of DEL transformation.

While successful control experiments were conducted to address the two issues above (e.g., addition of thiophenol into the possible Knoevenagel adduct and reaction with barbituric acid in the absence of a suitable situated hydride donor methylene moiety), they did not confirm the product's structure. We envisioned that the incorporation of isotopically labeled substrates would enable NMR correlation with structure as an orthogonal analytical confirmation of the T-reaction as depicted in Scheme 2. The T-reaction between ^{13}C enriched benzylic substrate **14** and ^{13}C enriched barbituric acid **15** would generate a mixture of spirocycles **16** and **17**. The carbon spectrum of the mixture should yield a pair of doublets and a pair of singlets at the appropriate chemical shifts. The presence of the doublets would unambiguously prove the formation of two new C-C bonds and the desired spirocycle, while the ratio between doublets and singlets would indicate the regioselectivity of the reaction.

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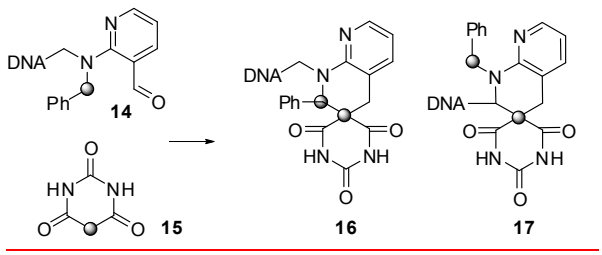


Scheme 2. ^{13}C labeled substrates for structure elucidation.

The required ^{13}C labeled substrates were prepared (details in Supporting Information) and the T-reaction was performed under standard, optimized conditions. Excess **15** was removed by ethanol precipitation of the DNA-conjugated product and spin filtering the re-suspended DNA in aqueous solution through a high molecular weight membrane filter. The ^{13}C spectrum, acquired in D_2O, indicated the predicted doublets and singlets, observed in 9:1 ratio (Figure 4). A ^{13}C DEPT spectrum showed the expected doublet at δ 63.8 and inverted the singlet at δ 42.3 delineating the former and latter as the benzylic carbons of **16** and **17**, respectively. Within the limits of resolution, no resonances were seen that would correlate to the intermediate Knoevenagel adduct. These results demonstrated that the T-reaction took place on-DNA and established our protocol for eventual DEL synthesis. Importantly, the use of isotopically enriched substrates provided an orthogonal method to mass spectrometry for detection of DNA conjugated reaction products at sub-micromole quantities of material. This method s hould have wide application in other DEL chemistry development projects.

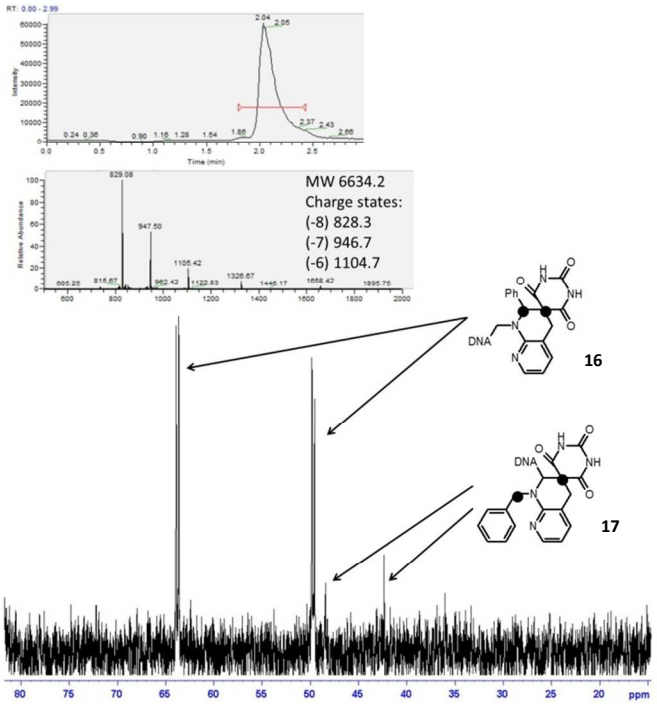
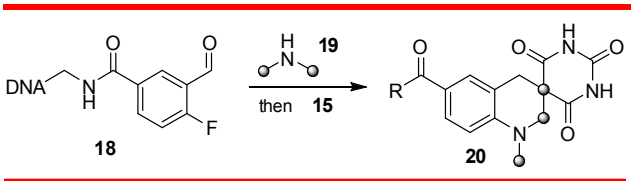


Figure 4. Characterization of on-DNA T-reaction products by LC, MS and ^{13}C spectroscopy



Scheme 3. Labeled 2° amine for the T-reaction.

At this point, we wanted to demonstrate the on-DNA reaction with a markedly less reactive substrate, namely that with a dimethylamine substituent (Scheme 3). We attached 4-fluoro-3-formyl benzoic acid onto DNA and displaced the fluoride with commercially available bis-^{13}C dimethylamine **19** (Scheme 3). Performing the T-reaction with the previously utilized ^{13}C-labeled barbituric acid **15** presents a much more stringent challenge given the lower propensity of methyl groups to serve as hydride donors in the T-reaction. Indeed, the standard conditions 60 °C did not give any desired product. But when the reaction temperature was increased to 80 °C, the LCMS revealed clean conversion to the desired product mass and the ^{13}C spectrum showed the expected pair of doublets and the methyl singlet (see Supporting Information).

Having now shown conclusive data for the key carbon-carbon bond forming events of the T-reaction, we began to explore the reactant scope of this transformation. The adaptation of a reaction to a DEL format requires the investigation of each diversity input's reactivity. To that end, a variety of secondary amine substrates were reacted with barbituric acid at 80°C on-DNA (Table 2). Almost all cases gave high conversion to the desired spirocycle as determined by LCMS. Unsurprisingly, even upon prolonged heating, substrate **25** gave no reaction; this is not unexpected given the highly strained azetidine iminium intermediate that is required for product formation. That only starting material was observed suggests that the rate limiting [1,5]-hydride shift drove the reaction forward from the Knoevenagel adduct in equilibrium with the starting aldehyde. Heterocyclic and structurally complex substrates (**27**, **29**, **31)** performed equally well as simple amines such as piperidine (**21**) and pyrrolidine (**22**). Although only symmetrical amines are presented in Table 2, the use of differentially substituted secondary amines would also be tolerated, providing a mixture of regioisomers (as for the Table 1 compounds) that would need to be deconvoluted upon identification of a high affinity library member.

We then examined the third component of the reaction scheme, that of the activated methylene using an *ortho*-piperdinyl benzaldehyde precursor (Table 3). To this end, **13** was treated sequentially with piperidine and a variety of activated methylenes. The reaction

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tolerated activated cyclic ketones, amides and esters. Various remote functionalities, such as a thiocarbonyl (entry **33**) or ester (entry **38**), did not hinder the reaction. The reactivity of cyclic reactants was higher than acylic reactants (entry **42**). Acyclic malonates and acetoacetates (not shown) were unreactive under these reaction conditions. Hence, high fidelity for the on-DNA T-reaction is only expected with the smaller set of commercially available activated methylenes in a cyclic dicarbonyl framework. Nonetheless, the modular nature of the 3-component reaction scheme would still allow for the generation of millions of diverse compounds. Further elaboration of the T-reaction products would also be possible for Boc-protected substrates such as **24**, or utilizing a hydrolysis/decarboxylation/amidation sequence of Meldrum's acid product **41**.[17]

Table 2. Scope of the amine reactivity (% indicates the purity of product as observed by LCMS trace)

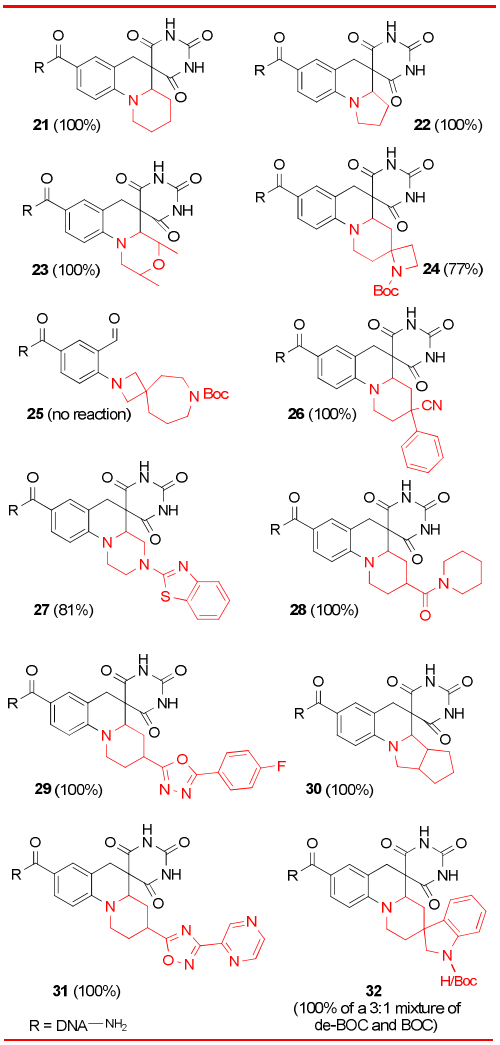

Table 3. Scope of the activated metheylene reactivity (% indicates the purity of product as observed by LCMS trace).

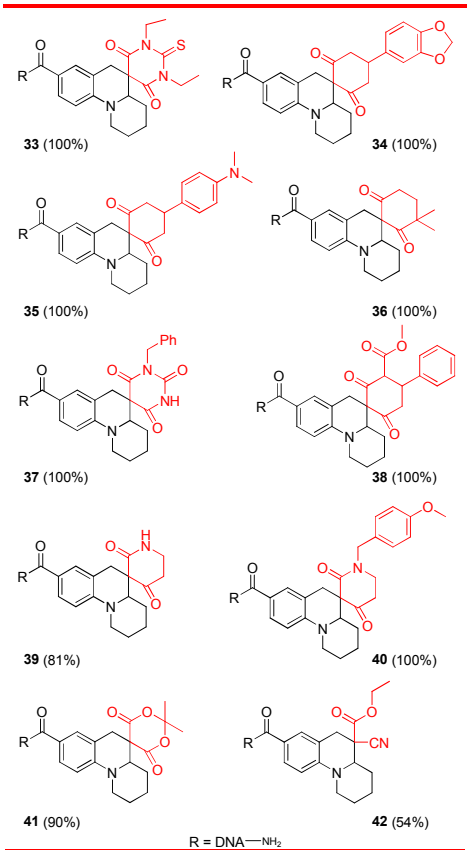

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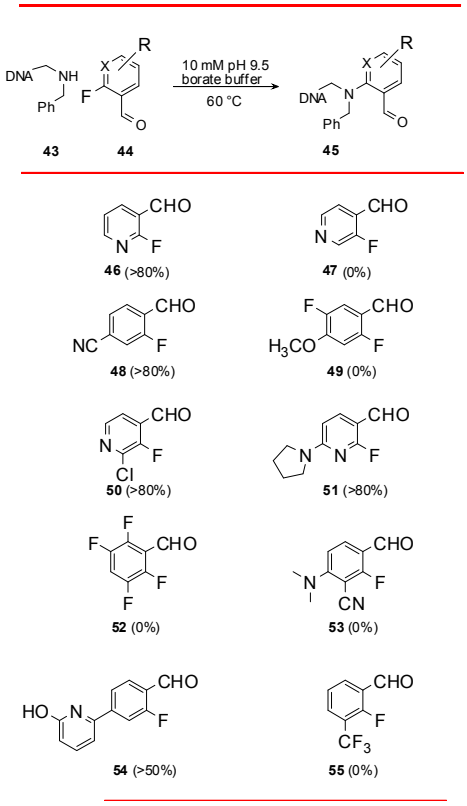
Table 4. Scope of reactivity for the SnAr reaction with amine-appended DNA (% indicates the purity of product as observed by LCMS trace).



Finally, the S_NAr reactivity of flouroaromatic aldehyde diversity reagents was explored. Whereas good yields were obtained with fluoropyridyl carboxaldehydes, certain substitutions on these rings systems or other 2-fluorobenzaldehyde analogues resulted in failed reactions. The survey of this building block highlights the need to verify the reactivity for the creation of a high quality DEL. Overall, 2-fluorobenzaldehydes are best suited for the S_NAr if there is minimal steric hindrance to the fluoride displacement and electronic enhancement by incorporation of electron withdrawing groups otherwise. In line with the relative reactivity, fluoropyridyl carboxaldehydes performed better for the displacement chemistry.

Based on the observed reactivity results and the availability of such building blocks availability, a library of 13.6 million compounds was enumerated based on Scheme 1 and profiled. To optimize the library design based on the computed physical chemical properties, all reagents appearing more than 60% of the time in compounds having the following properties were

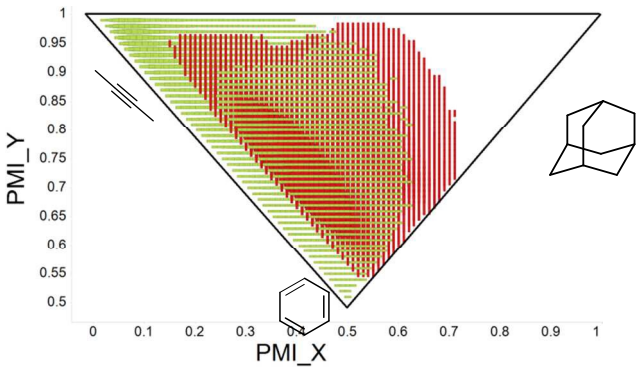


Figure 5. Principal moment of inertia density plot for the AstraZeneca collection (horizontal lines in green) and the T-reaction library (vertical lines in red). The corners of the PMI are dominated by structures presenting different types of shape (e.g. rod-, circular-, sphere-like). The dot sizes are proportional to the number of compounds. For purposes of clarity, only dots associated to at least 50 compounds are depicted.

excluded: molecular weight (MW) >550, or cLogP >4 or polar surface area (PSA) >120 Å^2. The result was a net reduction of library size to 6.4 million compounds and this was compared to the AstraZeneca compound collection. A principle moment of inertia (PMI) density plot comparing these 6.4 million designed library with the AstraZeneca compound collection is presented at Figure 5.[18] The PMI density plot can be used as a proxy to illustrate the overall diversity of molecular shapes within a collection. From Figure 5, one observes that the T-reaction compound library covers a large area of the PMI triangle and more importantly, the library occupies zones in the right side of the plot (more sphere-like structure) that are not well populated by the AstraZeneca collection.

Conclusions

In conclusion, we have demonstrated the feasibility of a T-reaction-based scheme for DNA-encoded library synthesis. This chemistry adds to a currently modest arsenal of multi-carbon bonding forming cascade reactions compatible with DNA-encoding

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systems. The reaction shows wide substrate tolerance and is operationally simple. A novel and broadly applicable ^{13}C NMR-based analytical method for elucidating on-DNA reactions was developed to show unambiguously the formation of the desired product. We expect that the use of labelled substrates will enhance the continuing development of other new chemistries for DEL synthesis. Further, this is an instructive example of the general process for adapting chemistry to a DEL-compatible format.

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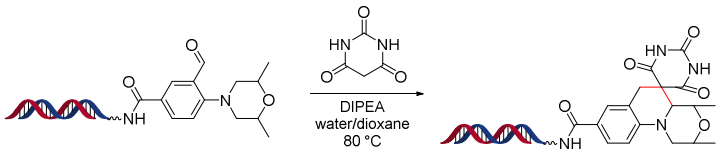

The tertiary amino effect reaction was explored and developed for application to DNA-encoded library synthesis.

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ABSTRACT: The generation of novel chemical leads for clinical development is a constant challenge in the pharmaceutical industry. The synthesis of DNA-encoded libraries (DELs) has emerged as a powerful method for lead generation. We report the development of the tertiary amino effect reaction tethered to DNA. A variety of *ortho*-dialkylaminoaryl aldehdyes undergo the T-reaction sequence: Knövenagel condensation, [1,5]-hydride shift, and Mannich cyclization to give stereochemically rich spirocycles. NMR analysis of substrates bearing an enriched double-^{13}C label confirmed product formation. This is the first example of a cascade reaction developed for and applied toward DELs. Importantly, the net formation of two carbon-carbon bonds adds to the few examples of carbon-carbon forming reactions on DNA.

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