

Synthesis and Selection of a Library of Macrocycles Generated by DNA-Templated Organic Synthesis

Despite the efforts of pharmaceutical companies to streamline the identification of lead drug compounds through combinatorial processes, natural products continue to provide an extremely useful role in drug design. In fact, during the period 1981–2002, natural products comprised 35–40% of approved drugs.¹ Of these natural products, macrocycles present a useful, albeit, challenging scaffold for drug development. Macrocycles have medium to large ring systems (i.e. greater than 8 atoms) and usually a rigid conformation.^{1,2} The benefit of these large cyclic backbones is a reduction of entropic loss relative to enthalpic gain upon binding to the target.

Efforts to synthesize libraries of these macrocycle compounds have led to the development of sophisticated combinatorial approaches using solid-phase supports.³ However, extensive investigation is often required to provide general reaction conditions for all substrates.⁴ The large number of products typically produced in combinatorial synthesis also requires the use of encoding strategies to identify, or tag, the reaction sequence leading to the candidate product.⁵ These encoding strategies, however, can influence reaction conditions and minor products can be signaled out by other members of the library.⁴ As a result of these limitations, there exists a need to develop a combinatorial approach that can support reactions to structurally complex compounds, be directly assayed, and provide a means to amplify the signal of products. **DNA-templated organic synthesis (DTS) can provide the answer to these challenges of library compilation, providing scientists a technique for direct translation, selection, amplification, and diversification of structurally complex compounds, such as macrocycles.**

DNA-templated synthesis is a process in which two complementary DNA sequences carry reactants that chemically couple to one another when the sequences hybridize. DTS has been found to support a wide variety of reactions, including amide bond formation, additions to α,β -unsaturated systems, and Wittig olefinations.^{6,7} Reactions between complementary templates have substantially higher reaction rates than those bearing mismatches while distances between reactants have relatively little effect on reaction rate.⁶ This precedence allows for the synthesis, or translation, of a number of synthetic compounds at the same time in a one-pot procedure.⁶ DTS, therefore, provides a means to encode reactants in a library and a support to direct synthesis. Since these reactions occur in an aqueous environment, products are able to be directly assayed for a target protein without further manipulations.⁸

DTS provides a powerful technique to synthesize complex ligands. Encoding tandem regions of an oligonucleotide presents an efficient opportunity to carryout multistep syntheses.⁹ This technique extends upon established solid-phase chemistries by providing a unique method of purification based on the affinities of proteins for certain molecular moieties. The final proof-of-principle for the use of DTS was the multistep synthesis of a 65-member macrocyclic library from which one macrocycle was selected by a target protein over the other 64 macrocycles.¹⁰

These findings collectively identify DNA-templated organic synthesis as a powerful technique for generation of macrocycles libraries. The development of DTS has led to the establishment of the company Ensemble Discovery, which utilized this technology to discover drug candidates for TNF receptor and BCL-XL.

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