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Modulating the Rate of a Native Ligation Coupling between Tripyrrole Derivatives by Using Specific dsDNA Sequences

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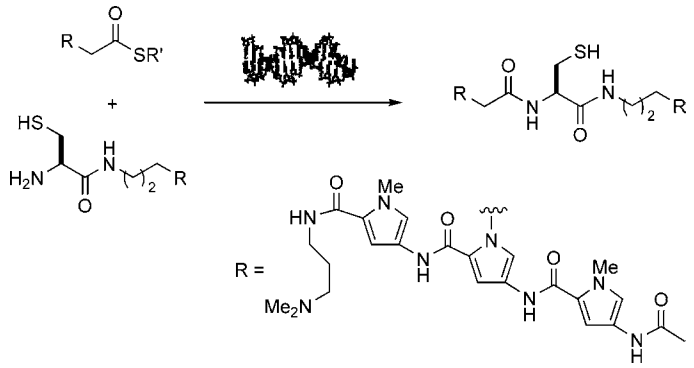
ABSTRACT



The intrinsic recognition code associated with dsDNA allows either accelerating or retarding of a native chemical ligation reaction between tripyrrole ligands. The rate changes most probably stem from the sequence-dependent characteristics of the dsDNA–ligand complexes.

The biosynthesis of nucleic acids and proteins is a highly regulated cellular process that requires an appropriate translation of the information stored in double-stranded DNA (dsDNA).[1] It would be of great interest to use the intrinsic code provided by DNA to control the synthesis of chemical entities other than those occurring in nature.[2] In this context, it has been recently shown that it is possible to use single-stranded DNA sequences to template a variety of synthetically relevant transformations of small organic compounds.[2a,3] The strategy relies on the covalent attachment of the reacting units to appropriate complementary oligonucleotides that, upon hybridization, increase the effective molarity of the reagents and thereby promote their reaction.

The use of double-stranded instead of single-stranded DNA to control chemical reactions is more challenging and much less established. Indeed, to our knowledge, only a couple of examples of dsDNA-promoted chemical transformations have been reported,[4] one based on a triplex recognition strategy[4a] and the other relying on a dipolar cycloaddition ligation between two six-ring hairpin polyamides capable of binding to contiguous DNA sites.[4b] These strategies require the use of stoichiometric amounts of the catalyst as a

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consequence of the high affinity of the product for the DNA template. This limitation might be overcome if the reaction system generates products that bind to the DNA template with moderate affinity and not more strongly than the reactants. We envisaged that the well-known ability of distamycin-related analogues to recognize the minor groove of specific DNA sequences with different affinities and stoichiometries (1:1 or 2:1)[5,6] could provide new, unique opportunities to advance toward such a goal.

Here, we report preliminary results in this area which demonstrate the feasibility of modulating the rate and efficiency of a "native chemical ligation" (NCL)[7] between tripyrrole peptide derivatives, by using specific dsDNA sequences.

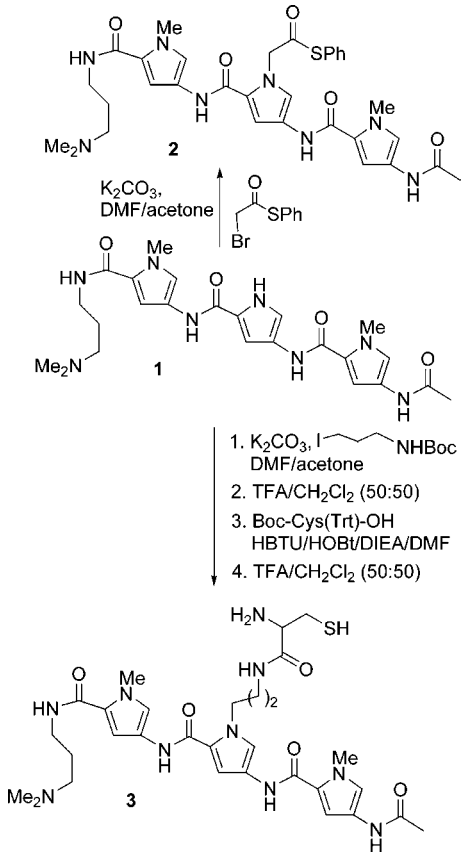
The available structural data on the interaction of distamycin derivatives with DNA suggested that the *N*-methyl groups of the pyrroles, which protrude out of the minor groove, could be appropriate sites to introduce the required reactive moieties.[5,6] Therefore, we focused our efforts on the coupling between tripyrrole derivatives **2** and **3**, compounds in which short alkyl chains containing the reactive thioester and cysteine units are incorporated into the middle pyrrole of the tripeptides. At the outset of the work, we were aware that the similar nature of the DNA recognition unit of these compounds might hamper a rigorous analysis and interpretation of the results, mainly because of the presumable formation of competing homodimeric and heterodimeric complexes in the presence of dsDNA. However, the synthetic accessibility of these compounds, which can be straightforwardly prepared from the same precursor **1** (Scheme 1),[8] advised their use as initial probes to check the feasibility of the concept.

The NCL coupling should proceed through an initial trans thioesterification between the electrophilic partner (thioester **2**) and the nucleophile (cysteine derivative **3**) to give the corresponding intermediate **4**, which would then rearrange to the final amide **5** (Scheme 2).[7]

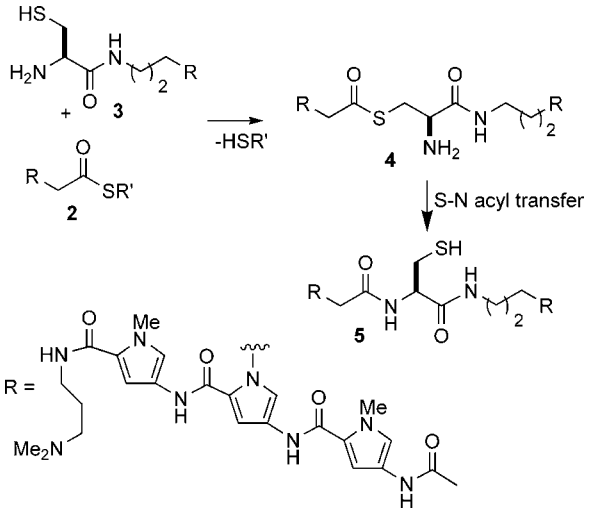
In theory, a dsDNA sequence capable of bringing **2** and **3** into proximity in a 2:1 interaction mode might facilitate the coupling process, whereas perhaps other sequences that could favor the formation of homodimers or other types of unproductive complexes might retard the reaction. Unfortunately, it is difficult to predict which sequences would favor homo- or heterodimeric assemblies.

The ligation reaction between **2** and **3** in the absence of DNA was studied using several types of buffers and in the presence of different concentrations of the reacting partners

Scheme 1



Scheme 2



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(5) For reviews on pyrrole polyamide DNA recognition, see: (a) Dervan, P. B. *Bioorg. Med. Chem.* **2001**, *9*, 2215−2235. (b) Dervan, P. B.; Edelson, B. S. *Curr. Opin. Struct. Biol.* **2003**, *13*, 284−299. (c) Wemmer, D. E. *Biopolymers* **2001**, *52*, 197−211.

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(8) For the synthesis of tripyrrole polyamides containing alkyl side chains other than methyl, see, for instance: Satz, A. L.; Bruice, T. C. *Acc. Chem. Res.* **2002**, *35*, 86−95 and references cited therein.

(see the Supporting Information). The results of these studies showed that the reaction can be accomplished in an aqueous phosphate buffer at pH 7.5, with its efficiency being quite sensitive to the ionic strength of the medium, so that small increases in the [Na^+] lead to higher reaction rates. We also observed that hydrolysis of the thioester is a significant competitive process, although this can be minimized by carrying out the reaction at pH 7.0.

To study the effect of DNA on the coupling, it was convenient to use conditions that induce a slow reaction: 12 mM phosphate buffer containing 120 mM NaCl and 5 mM triscarboxyethylphosphine (TCEP) at pH 7.0 and equimolar amounts of **2** and **3** (0.65 μM each). These conditions induce only a 5% conversion to **5** after 4 h. Remarkably, the addition of hairpin dsDNA **A** (1 equiv) to the reaction medium promoted a substantial acceleration of the coupling process, as can be deduced from the HPLC traces in Figure 1 (see also Figure 2a).

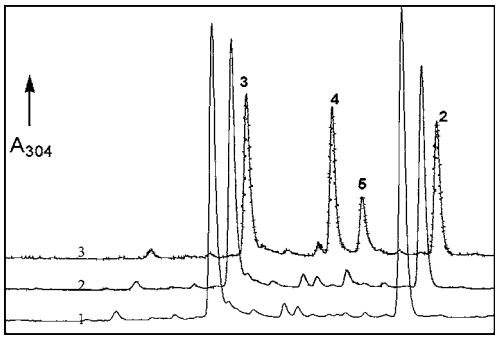

Figure 1. HPLC traces of the reaction between equimolar mixtures of **2** and **3** (0.65 μM each, 12 mM phosphate buffer, 120 mM NaCl, pH 7.0, 5 mM TCEP, 25 °C): (1) initial time; (2) in the absence of DNA after 40 min; (3) in the presence of 1 equiv of ds-oligonucleotide **A** after 40 min. Peaks were assigned by LC-MS.

The reaction in the presence of this dsDNA proceeds near 40 times faster than in its absence (considering initial rates), leading to an approximate conversion of 74% after 4 h (Figure 2a). A 24bp ds-oligonucleotide **B**, which lacks the T-rich hairpin unit, led to the same results as those with ds-oligo **A**. The acceleration is certainly a consequence of the presence of the double helical DNA because a single-stranded DNA containing the same sequence (either chain of duplex **B**) did not affect the reaction rate.

Moreover, in contrast to the reaction in the absence of DNA, in which the thioester intermediate **4** was not detected, in the DNA-promoted couplings this intermediate was clearly observed, a result consistent with a reduced conformational freedom of **4** when sited under the constraints of the DNA groove.[9]

Analysis of the influence of the amount of dsDNA catalyst **A** on the reaction showed that the rate increases on raising the DNA concentration from 0.2 to 1 equiv; however, further addition of the oligonucleotide induces a rate decrease to an extent that, in the presence of 5 equiv, the reaction stops altogether (see the Supporting Information). This result is consistent with a model in which the presence of excess DNA lowers the amount of the productive heterodimeric complex.[10]

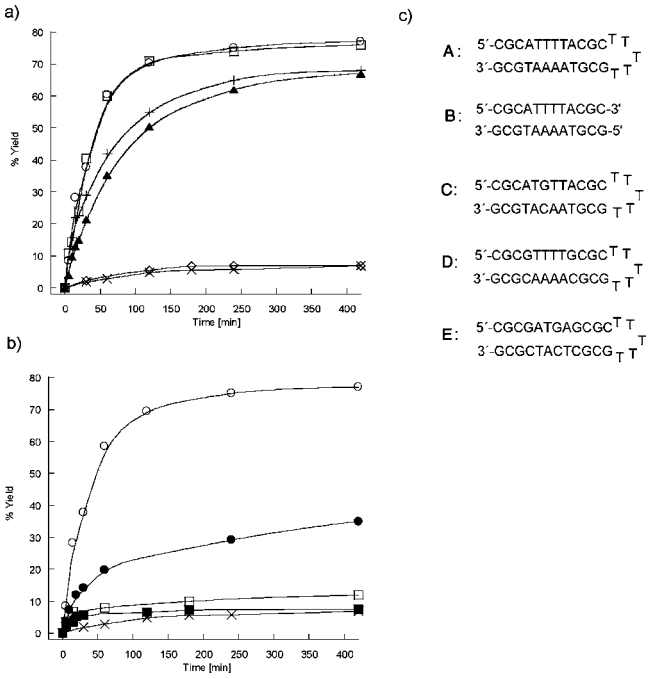

Figure 2. (a) Graphic showing the product (**4** + **5**) formation as a function of time in the reaction between **2** and **3** (0.65 μM each, 12 mM phosphate buffer, 120 mM NaCl, pH 7.0, 5 mM TCEP, 25 °C) in the absence of DNA (x), in the presence of 1 equiv of dsDNA **A** (○), dsDNA **B** (□), dsDNA **A** with 1.5 equiv of **5** (+), or a single chain of DNA **B** (◇) (both strands gave similar results), or in the presence of 0.5 equiv of dsDNA **A** (▲). (b) Graphic showing the product (**4** + **5**) formation as a function of time in the absence of DNA (x) or in the presence of 1 equiv of dsDNA **A** (○), dsDNA **C** (●), dsDNA **D** (□), or dsDNA **E** (■). (c) Sequences of the oligonucleotides used for the study.

Using 0.5 equiv of hairpin **A**, the reaction is slightly slower but leads to an approximate yield of 70% after 24 h, which suggests the existence of some turnover, even considering the background reaction. Interestingly, addition of 1.5 equiv of **5** to the standard reaction mixture leads only to a weak decrease in the reaction rate and efficiency (curve +, Figure 2a), which is in accordance with a relatively modest affinity of this product for dsDNA **A**.[11]

Other dsDNAs that might allow the formation of 2:1 complexes,[6] such as **C**, also accelerate the coupling process, although in this case the reaction was only 20 times faster than that in the absence of DNA. dsDNAs with sequences

(9) A similar effect has been observed previously in native chemical ligations promoted by peptides: (a) Severin, K.; Lee, D. H.; Kennan, A. J.; Ghadiri, M. R. *Nature* **1997**, *389*, 706−709. (b) Kennan, A. J.; Haridas, V.; Severin, K.; Lee, D. H.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2001**, *123*, 1797−1803.

(10) Huc, I.; Pieters, R. J.; Rebek, J. *J. Am. Chem. Soc.* **1994**, *116*, 10296−10297.

(11) Using circular dichroism titrations we have calculated an approximate K_d of 3 μM (see the Supporting Information).

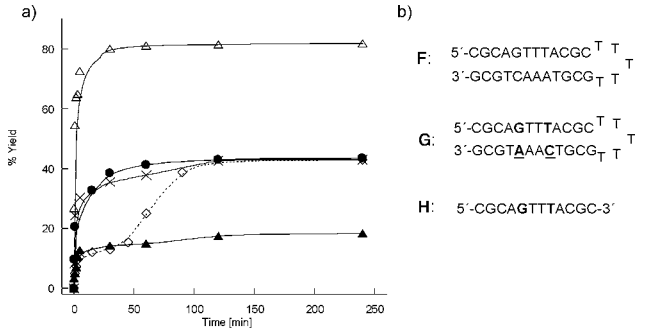

Figure 3. (a) Graphic showing the product (**4** + **5**) formation as a function of time in the reaction between **2** and **3** (10 μM each, 20 mM phosphate buffer, 200 mM NaCl, pH 7.0, 5 mM TCEP, 25 °C) in the absence of DNA (x) or in the presence of 2 equiv of dsDNA **F** (△), mismatched dsDNA **G** (▲), or single-stranded **H** (●). The discontinuous curve represents the reaction progress in the presence of **G** after addition of urea (8 M, 33 min from the beginning) (◇). (b) Sequences of the ds-oligonucleotides used.

known to favor a 1:1 interaction mode with distamycin,[6e] such as **D**, accelerate the reaction by less than 5 times compared to the background and lead to low conversions (10% after 4 h, Figure 2b), as does a noncognate DNA template such as **E**. Therefore, these results confirm that the rate and efficiency of the coupling reaction depend on the dsDNA sequence. The observed differences might arise from the relative amount of productive DNA−heterodimer complexes present in the reaction mixture, but a definitive explanation must wait for further studies.

We also considered the viability of retarding the reaction by using dsDNAs that could particularly favor the formation of unproductive complexes. To this end, we studied the effect of adding 2 equiv of different dsDNAs to a reaction mixture containing 10 μM **2** and **3** in 20 mM phosphate buffer and 200 mM NaCl, conditions in which the intrinsic coupling proceeds relatively fast.[12]

Screening of several ds-oligonucleotides allowed us to discover that the reaction is slower and leads to poorer conversions when carried out in the presence of dsDNA **G**, which features a couple of mismatched base pairs with respect to its parent duplex **F**.[13] Remarkably, addition of excess urea after 30 min from the start restored the coupling progress (Figure 3a).[14] Circular dichroism titration experiments revealed that although either tripyrrole (**2** or **3**) binds dsDNA **F** or **G** as homodimers in a 2:1 mode the stability of the complexes with the mismatched oligo **G** is higher (∼400 times higher for **3** and 260 times higher for **2**; see the Supporting Information), which suggests that the reaction retardation might be related to the capability of this mismatched DNA to trap the reagents in unproductive homodimeric complexes.[15]

In conclusion, we have demonstrated that the rate and efficiency of a native chemical ligation reaction between two tripyrrole units can be altered by using short dsDNAs with specific sequences. The sequence-dependent rate changes are most probably a consequence of the different complexation abilities of the dsDNAs, albeit a definitive explanation must wait for further studies. The fact that the dsDNA-accelerated reaction can also be achieved in the presence of oversto-ichiometric amounts of the product suggests that an appropriate refinement of the system might lead to the discovery of truly catalytic dsDNA-promoted reactions.

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Supporting Information Available: Synthetic procedures, characterization data, experimental data on the different factors that influence the coupling reaction, and circular dichroism titrations. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) In the absence of dsDNA, under these reaction conditions, thioester **2** does also participate in secondary processes and thereby it is almost completely consumed after 1 h.

(13) Circular dichroism analysis indicates that mismatched DNA **G** keeps the double helical structure (see the Supporting Information).

(14) A control experiment demonstrated that the excess of urea does not alter the intrinsic coupling reaction.

(15) UV-damaged dsDNAs with widened minor grooves show high affinity for 2:1 complexes with distamycin A: Inase, A.; Kodama, T. S.; Sharif, J.; Xu, Y.; Ayame, H.; Sugiyama, H.; Iwai, S. *J. Am. Chem. Soc.* **2004**, *126*, 11017−11023.