Programmable One-Pot Multistep Organic Synthesis Using DNA Junctions

Mireya L. McKee,[†] Phillip J. Milnes,[‡] Jonathan Bath,[†] Eugen Stulz,[§] Rachel K. O'Reilly,[‡] and Andrew J. Turberfield*,[†]

[†]Department of Physics, University of Oxford, Clarendon Laboratory, Parks Road, Oxford, OX1 3PU, U.K.
[‡]Department of Chemistry, University of Warwick, Gibbet Hill Road, Coventry, CV4 7AL, U.K.
[§]School of Chemistry, University of Southampton, Highfield, Southampton, SO17 1BJ, U.K.

S *Supporting Information*

ABSTRACT: A system for multistep DNA-templated synthesis is controlled by the sequential formation of DNA junctions. Reactants are attached to DNA adapters which are brought together by hybridization to DNA template strands. This process can be repeated to allow sequence-controlled oligomer synthesis while maintaining a constant reaction environment, independent of oligomer length, at each reaction step. Synthesis can take place in a single pot containing all required reactive monomers. Different oligomers can be synthesized in parallel in the same vessel, and the products of parallel synthesis can be ligated, reducing the number of reaction steps required to produce an oligomer of a given length.

DNA-templated chemistry offers a simple and efficient means to control the synthesis of oligomers. DNA hybridization can trigger the coupling of monomers attached to oligonucleotide adapters by increasing their effective molarity and thus reaction rate; cross-reactions between other monomers, also present in solution but attached to adapters with unrelated nucleotide sequences, are minimized.[1−9] Systems in which each type of monomer is identified by a unique DNA adapter allow multistep synthesis of oligomers using linear templates,[10−13] branched junctions,[14] DNA walkers,[15] or strand-displacement mechanisms.[16,17] It is desirable to be able to program the simultaneous synthesis of different oligomers in a single reaction vessel, allowing for the parallel synthesis of diverse products.[1,3,11,14] One of the challenges in multistep systems is the maintenance of constant reactivity after each reaction step to maximize the potential product length. We have described a system for sequence-controlled oligomer synthesis, based on DNA strand displacement, that does not restrict the number of coupling reactions attempted.[16] Here, we explore a new DNA-templated mechanism that also permits all reactive monomers to be present simultaneously in one pot. Synthesis is controlled by the sequential addition of instruction strands. This confers significant advantages over systems which require sequential addition of reactants. The method also permits programmable parallel synthesis of multiple products.

The system consists of oligonucleotide adapters modified with reactive monomers at either the 5′ or 3′ end. Each type of

DNA adapter has a unique address domain that identifies the monomer that it carries, and one of two short, complementary, universal domains adjacent to the attached monomer. Reactions are initiated and programmed by addition of template strands that carry two address domains that are complementary to those in two specific adapters. Hybridization of the selected adapters to the template holds reactive monomers in close proximity; subsequent hybridization of their universal domains forms a transient three-way junction in which the monomers are positioned in an end-of-helix configuration which favors the coupling reaction[18,19] (Figure 1a). Unpaired thymines, two between the address and universal domains of the adapters and one between the address domains of the template, are included in order to increase the stability of the junction.[20,21] The DNA template has an overhanging single-stranded toehold[22] that allows it to be displaced by addition of a complementary removal strand, generating a double-stranded waste product and releasing the reaction product. Coupling of further subunits is programmed by the addition of further template and remover strands, alternately placing the next monomer in the sequence in the correct position to react with the growing chain and then removing the spent DNA template and adapter. The final product can be separated from the reaction mixture using a biotinylated strand that is complementary to the final adapter: no intermediate purification steps are necessary.

The monomers have aldehyde and ylide functionalities that allow them to be coupled by the Wittig reaction:[12,16] nucleophilic attack of the phosphonium ylide of one monomer on the aldehyde of another detaches the growing chain from its DNA adapter and couples it to the incoming monomer through an olefin linkage. As in the ribosome, this pattern of coordinated cleavage and chain transfer to the incoming unit ensures that each reaction occurs in the same environment, in this case, between the aldehyde of the incoming monomer and the ylide of the previously added unit at the base of the growing chain. Figure 1 shows the initial steps of chain growth, starting with a monofunctional (ylide) initiator and proceeding by addition of bifunctional monomers in a sequence determined by the addition of template and removal strands. The reaction is terminated when a monomer containing only an aldehyde is
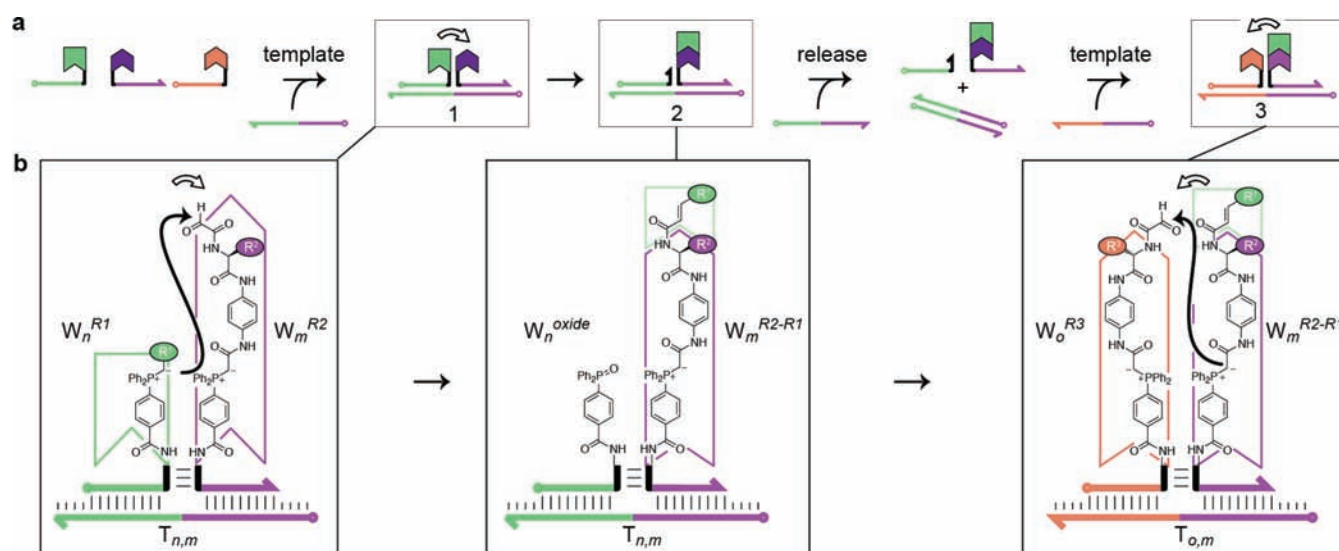
Figure 1. Mechanism for sequence-controlled oligomer synthesis. Reactive monomers are attached to DNA adapters whose nucleotide sequences encode the identities of the monomers. Monomers with both aldehyde and ylide groups are represented by chevrons: the symbol capped at the top represents a monofunctional (ylide) initiator. (a) Addition of a template strand binds together a specific pair of monomers, enabling the coupling reaction (indicated by the white arrow). Sequential addition of template and removal strands allows sequence-controlled oligomer growth. (b) Chemistry of labeled intermediates, with arrows indicating nucleophilic attack of the incoming ylide on the aldehyde of the previously added monomer that results in cleavage and transfer of the growing chain.
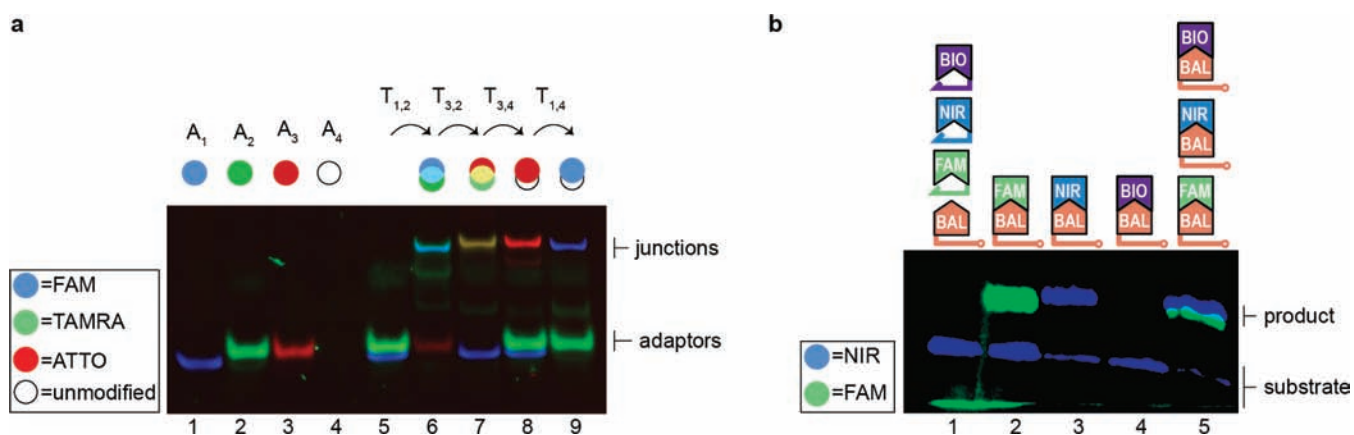

Figure 2. 'Template and release' mechanism allows control of sequential pairwise interactions. (a) Demonstration of template and release mechanism using fluorescently labeled adaptors, analyzed by PAGE. Adapters A_{1-3} were modified with fluorescent groups fluorescein (FAM), tetramethylrhodamine (TAMRA), and ATTO647; A_4 was unmodified. The image of the 20% nondenaturing gel is an overlay of three channels: blue (FAM) 488/530 nm, green (TAMRA) 532/605 nm, and red (ATTO) 635/695 nm. Individual channels are shown in the Supporting Information. (b) Specific single-step reactions, controlled by addition of template strands to a mixture of adapters. The reaction mix contains the start adapter W_4^{BAL} and three stop adapters W_1^{FAM}, W_9^{NIR}, W_3^{BIO} (lane 1). Addition of templates $T_{1,4}$, $T_{9,4}$, and $T_{3,4}$ (lanes 2−4) results in the formation of dimer products BAL-FAM, BAL-NIR, and BAL-BIO respectively. Simultaneous addition of all three templates results in parallel synthesis of all three dimers (lane 5). The image of the 20% denaturing gel is an overlay of two channels: green (FAM) 488/530 nm and blue (NIR) 635/695 nm.

incorporated (not shown). Each monomer incorporates a characteristic functional unit which here consists of an amino acid residue or a fluorophore. The structures of the monomers and their synthesis are described in the Supporting Information (SI). We use symbol A_n to denote DNA adapter sequence n and W_n^R to denote the corresponding adapter carrying a reactive (Wittig) monomer incorporating functional group R.

All reactive monomers can be present in the same solution: they will not react at a significant rate until they are linked by hybridization to the correct template because the universal sequence is too short to promote stable binding between DNA adapters in the absence of a template. The technique can therefore be applied to parallel multistep reactions and has the potential to create complex oligomer libraries in one pot.

The use of template and removal strands to control association between monomers was tested using dummy adapter strands A_1-A_4 coupled to fluorophores (Figure 2a). Nondenaturing polyacrylamide gel electrophoresis (PAGE) shows that there is no interaction between the adapters in solution in the absence of template (Figure 2a, lane 5). Addition of template $T_{1,2}$ generates a band containing both FAM and TAMRA corresponding to the three-strand complex $A_1^{FAM} \bullet A_2^{TAMRA} \bullet T_{1,2}$ (Figure 2a, lane 6). Subsequent addition of the removal instruction $R_{1,2}$ (complementary to $T_{1,2}$) and a second template instruction ($T_{3,2}$) releases A_1^{FAM} and generates a new band corresponding to $A_2^{TAMRA} \bullet A_3^{ATTO} \bullet T_{3,2}$ (Figure 2a, lane 7). Similarly, addition of $R_{3,2}$ and $T_{3,4}$ and then $R_{3,4}$ and $T_{1,4}$ generates junctions $A_2 \bullet A_4^{TAMRA} \bullet T_{2,4}$ and then
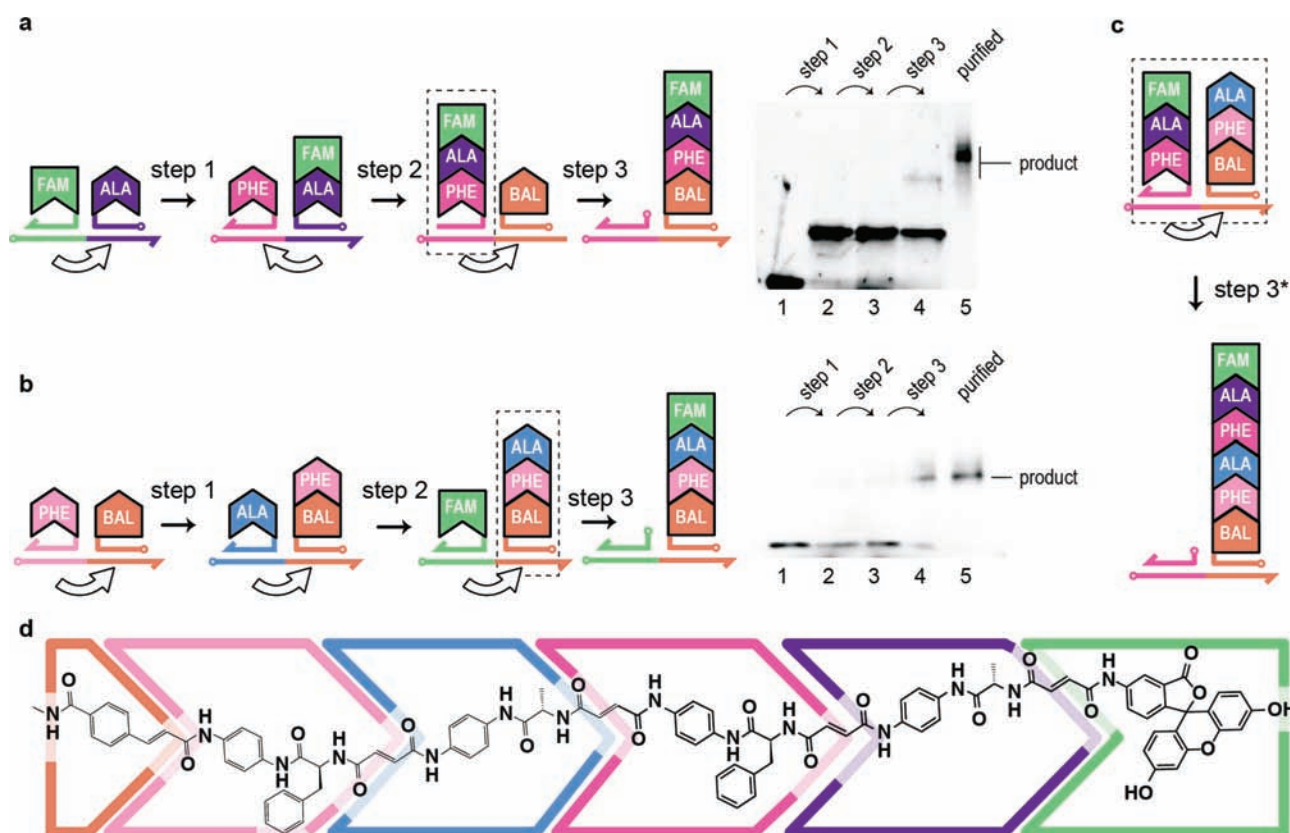
Figure 3. Two mechanisms for the synthesis of oligomers. (a) The alternating-strand mechanism is initiated with a monofunctional ylide monomer (FAM). At each step, the growing chain is transferred to the incoming monomer. The reaction is terminated by transfer to a monofunctional aldehyde monomer (BAL). Adapters W_1^{FAM}, W_2^{ALA}, W_3^{PHE}, and W_4^{BAL} with template/removal pairs ($T_{1,2}/R_{1/2}$, $T_{3,2}/R_{3,2}$, and $T_{3,4}/R_{3,4}$) were used. (b) The same-strand mechanism is initiated with an aldehyde monomer: the growing chain remains on the same adapter throughout the synthesis, and the chain is terminated by addition of an ylide monomer (FAM). Adapters W_4^{BAL}, W_5^{ALA}, W_7^{PHE}, and W_1^{FAM} with template/removal pairs ($T_{7,4}/R_{7,4}$, $T_{5,4}/R_{5,4}$, and $T_{1,4}/R_{1,4}$) were used. In both cases, the mixture of adapters (lane 1), intermediates of stepwise synthesis (lanes 2−4), and purified products (lane 5) were analyzed on a 20% denaturing gel scanned at 488/530 nm em/ex to reveal FAM fluorescence. (c) By coupling products generated by the two mechanisms simultaneously, in the same reaction vessel, the number of steps required to make a given product can be halved. Here, a 6-mer (d) was made in three operations (Supporting Information): the 3-mers boxed with dotted lines in (a) and (b) were produced in parallel and then ligated using template $T_{3,4}$.

$A_1^{FAM} \bullet A_4 \bullet T_{1,4}$, each containing a single fluorophore (Figure 2a, lanes 8 and 9). This demonstrates that it is possible to control the sequence in which specified pairs of adapters are brought together, even when all adapters are always present in the reaction mixture.

This system allows more than one reaction sequence to be programmed simultaneously in a single reaction vessel. Control of parallel reactions is possible because no significant reactions occur in the absence of template strands. Initial experiments were performed using the coupling of monofunctional monomers to form olefin dimers. Three different ylide adapters and one aldehyde adapter were used (Figure 2b): two of the ylides contained fluorescent tags W_1^{FAM} and W_9^{NIR} and the third, W_3^{BIO}, contained a biotin (BIO) group. Transfer of these monomers to the longer aldehyde adapter was monitored using denaturing PAGE. Addition of individual template strands $T_{1,4}$, $T_{9,4}$, or $T_{3,4}$ produced the intended products (Figure 2b, lanes 2−4) whereas simultaneous addition all three template strands together with 3 equiv of W_4^{BAL} (BAL = benzaldehyde) resulted in formation of all three olefin dimers (Figure 2b, lane 5; generation of the nonfluorescent BAL−BIO dimer was confirmed by MS).

Multistep reactions can be programmed with either a monofunctional ylide or an aldehyde monomer as the chain

initiator. When an ylide is used as the initiator (Figure 1b) the growing chain is transferred between successive adapters, alternating between 3′ and 5′ ends (alternating-strand mechanism). With an aldehyde initiator, the oligomer product remains covalently attached to the initial adapter throughout the series of reactions (same-strand mechanism, Figure S5). The same-strand mechanism does not preserve a constant reaction environment (the reactive aldehyde is at the distal end of the growing chain), but it does offer the advantage that the adapter bearing the oligomer can always be unambiguously identified, allowing the use of a single adapter sequence for a monomer that is to be incorporated at more than one site.

Both mechanisms were tested by programming the synthesis of 4-mers (Figure 3). To facilitate analysis by PAGE, adapters with different sizes from 20 to 32 nucleotides were used (see SI for nucleotide sequences). In both syntheses the adapter to which the final product is attached, W_4^{BAL}, is longer than other adapters. The monofunctional ylide adapter W_1^{FAM}, used to initiate the alternating-strand synthesis and to terminate the same-strand synthesis, contains the fluorescent group FAM, allowing reaction progress to be monitored. Transfer between adapters of the fluorescent tag was observed after the first step using the alternating-strand mechanism (Figure 3a, lane 2); using the same-strand mechanism, there was no significant

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dx.doi.org/10.1021/ja2101196 | J. Am. Chem. Soc. 2012, 134, 1446−1449

change in mobility of the fluorescent band until the last step (Figure 3b, lane 4), as expected. The identity of each product was confirmed by mass spectrometry (see SI for MS and yield estimates). Incomplete products but no out-of-sequence monomer additions were observed when the products of each step in the same-strand mechanism were analyzed. The main cause of the formation of truncated products is oxidation of the ylide.[12] Parallel multistep chemistry was also tested by synthesizing two different 4-mers in a single reaction vessel (SI). These results are consistent with the successful operation of both variants of the sequence-controlled synthesis mechanism.

The number of reaction steps required to make an oligomer of a given length can be reduced by synthesizing sections in parallel. This tactic could greatly increase the complexity of product libraries and simplify synthetic schemes. As proof of principle, a 6-mer was synthesized in three reaction steps. Two trimers were synthesized simultaneously in a single vessel, one using the alternating-strand and the other the same-strand mechanism. The two intermediate products were then brought together by a third template strand $(T_{3,4})$ triggering coupling to form the olefin 6-mer (Figure 3c). The resulting products were purified using streptavidin beads and analyzed by PAGE and mass spectrometry. The desired 6-mer, with 5-mer, 4-mer, and dimer truncated products, was observed (SI).

We have demonstrated a method for one-pot, sequence-controlled oligomer synthesis using a DNA-templated reaction mechanism in which all adapters are present but react only when a template instruction strand is introduced. This system has several new and useful characteristics. It allows parallel multistep synthesis in a single reaction vessel. We have demonstrated multistep synthesis using two distinct mechanisms: in the first, the growing chain is transferred between adapters at each step (as in the ribosome); in the second, the growing chain is assembled on a single adapter. Combining the two mechanisms halves the number of operations required to make a product of a given length, potentially allowing access to more complex product libraries. The alternating-strand mechanism maintains a constant reaction environment and therefore constant reactivity, independent of oligomer length, at each reaction step.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental methods and supporting data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

a.turberfield@physics.ox.ac.uk

■ ACKNOWLEDGMENTS

■ REFERENCES

(1) Gartner, Z. J.; Liu, D. R. *J. Am. Chem. Soc.* **2001**, *123*, 6961−6963.

(2) Gartner, Z, J.; Kanan, M. W.; Liu, D. R. *Angew. Chem., Int. Ed.* **2002**, *41*, 1796−1800.

(3) Calderone, C. T.; Puckett, J. W.; Gartner, Z. J.; Liu, D. R. *Angew. Chem., Int. Ed.* **2002**, *41*, 4104−4108.

(4) Li, X.; Liu, D. R. *Angew. Chem., Int. Ed.* **2004**, *43*, 4848−4870.

(5) Chhabra, R.; Sharma, J.; Liu, Y.; Yan, H. *Nano Lett.* **2006**, *6*, 978−983.

(6) Grossmann, T. N.; Seitz, O. *J. Am. Chem. Soc.* **2006**, *128*, 15596−15597.

(7) Jacobsen, M. F.; Cló, E.; Mokhir, A.; Gothelf, K. V. *ChemMedChem* **2007**, *2*, 793−799.

(8) Scheuermann, J.; Dumelin, C. E.; Melkko, S.; Neri, D. *J. Biotechnol.* **2006**, *126*, 568−581.

(9) Ravnsbæk, J. B.; Jacobsen, M. F.; Rosen, C. B.; Voigt, N. B.; Gothelf, K. V. *Angew. Chem., Int. Ed.* **2011**, *50*, 10851−10854.

(10) Gartner, Z. J.; Kanan, M. W.; Liu, D. R. *J. Am. Chem. Soc.* **2002**, *124*, 10304−10306.

(11) Gartner, Z. J.; Tse, B. N.; Grubina, R.; Doyon, J. B.; Snyder, T. M.; Liu, D. R. *Science* **2004**, *305*, 1601−1605.

(12) Snyder, T. M.; Liu, D. R. *Angew. Chem., Int. Ed.* **2005**, *44*, 7379−7382.

(13) Li, X. Y.; Gartner, Z. J.; Tse, B. N.; Liu, D. R. *J. Am. Chem. Soc.* **2004**, *126*, 5090−5092.

(14) Hansen, M. H.; Blakskjaer, P.; Petersen, L. K.; Hansen, T. H.; Hojfeldt, J. W.; Gothelf, K. V.; Hansen, N. J. V. *J. Am. Chem. Soc.* **2009**, *131*, 1322−1327.

(15) He, Y.; Liu, D. R. *Nat. Nanotechnol* **2010**, *5*, 778−782.

(16) McKee, M.; Milnes, P.; Bath, J.; Stulz, E.; Turberfield, A. J.; O'Reilly, R. K. *Angew. Chem., Int. Ed.* **2010**, *49*, 7948−7951.

(17) He, Y.; Liu, D. R. *J. Am. Chem. Soc.* **2011**, *133*, 9972−9975.

(18) McKee, M. L.; Evans, A. C.; Gerrard, S. R.; O'Reilly, R. K.; Turberfield, A. J.; Stulz, E. *Org. Biomol. Chem.* **2011**, *9*, 1661−1666.

(19) Gartner, Z. J.; Grubina, R.; Calderone, C. T.; Liu, D. R. *Angew. Chem., Int. Ed.* **2003**, *42*, 1370−11375.

(20) Leontis, N. B.; Kwok, W.; Newman, S. *Nucleic Acids Res.* **1991**, *19*, 759−766.

(21) Zhong, M.; Michael, S.; Rashes, M. S.; Leontis, N. B.; Kallenbach, N. R. *Biochemistry* **1994**, *33*, 3660−3667.

(22) Yurke, B.; Turberfield, A. J.; Mills, A. P. Jr.; Simmel, F. C.; Neumann, J. L. *Nature* **2000**, *406*, 605−608.