JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

COMMUNICATION

pubs.acs.org/JACS

Nucleic Acid-Templated Energy Transfer Leading to a Photorelease Reaction and its Application to a System Displaying a Nonlinear Response

Manuel Röthlingshöfer, Katarzyna Gorska, and Nicolas Winssinger*

Institut de Science et Ingénierie Supramoléculaires (ISIS − UMR 7006), Université de Strasbourg − CNRS, 8 allée Gaspard Monge, F67000 Strasbourg, France

Supporting Information

ABSTRACT: The photocleavage of a nitrobenzyl-type linker (NPPOC) at 405 nm wavelength was enabled by nucleic acid-templated energy transfer from a sensitizer (thioxanthenone) to the linker. This strategy was used to release profluorescent rhodamine, which facilitated monitoring of the reaction via fluorescence measurement in a nonoverlapping window with the sensitizer/photocleavage reaction. The rate acceleration of the templated reaction was greater than 20-fold over the background reaction. The templated reaction was used in conjunction with strand displacement to design four-component systems that responded to an analyte (DNA). Programming a specific hierarchical relationship among the four components enabled the design of a system that responded first positively and then negatively to increasing levels of an analyte.

The programmability of nucleic acid hybridization makes it an appealing platform for the design of systems[1] responding to stimuli or leading to nanoscopic assemblies.[2] Applications of stimuli responses that have attracted significant attention include nucleic acid sensing[3] and logic gate operations that are leveraged on strand displacement (molecular beacons) or templated reactions.[4] To date, the repertoire of templated reactions that have been successfully used in sensing and logic gate operations is limited to a few reaction types reflecting the following stringent requirements:[5−9] the reaction must be compatible with an aqueous environment and the diversity of functionalities present on nucleic acids; it must ideally take place rapidly when the effective concentration of the reactants is increased through the hybridization process; it must operate at a temperature compatible with the hybridization process; and preferably, it should be bio-orthogonal for further implementation in biological settings.

Photolabile groups offer a unique orthogonality profile that has been harnessed to uncage bioactive molecules or reporters.[10] Most photolabile groups require UV light to achieve the cleavage using one-photon excitation. It was recently shown that the triplet sensitizer thioxanthenone significantly increases the rate of deprotection of the photolabile 2-(2-nitrophenyl)propyloxycarbonyl (NPPOC) protecting group by intramolecular energy transfer.[11] The fact that some nitrobenzyl-type groups have very poor photocleavage efficiencies with light above 300 nm while this sensitizer has an absorption maximum close to 400 nm led us to speculate that the photocleavage of such a linker could be carried out in a templated fashion at 400 nm. The close contact resulting from hybridization of two nucleic acid strands derivatized respectively with a photolabile linker and a sensitizer (Figure 1) should enable the energy transfer leading to photocleavage.

To monitor the progress of the photocleavage, we reasoned that this system could be used to release a profluorescent molecule. To this end, rhodamine was selected because it has an absorption/emission profile (λ_{ex} = 490 nm, λ_{em} = 530 nm) that is red-shifted relative to the sensitizer and photolabile linker. Furthermore, it is known that conversion of its anilines to amides, carbonates, ureas, or combinations thereof abrogates its fluorescence which can be restored upon recovery of a single aniline.[12] On the basis of our prior work[8] in templated reactions using peptide nucleic acid (PNA),[13] we opted for this nucleic acid platform in this study. The 4-carboxy-NPPOC group in **2** was chosen because it has an absorption maximum at 230 nm and, unlike the 3,4-dihydroxynitrobenzyl-type linker, very poor photolytic efficiency above 300 nm[14] (see Scheme S1 in the Supporting Information for synthetic details for **1** and **2**).

With the two components for templated photoreactions in hand, we next investigated the requirements of duplex stability to achieve an efficient energy transfer between the sensitizer and the photolabile linker. To this end, a 7-mer PNA derivatized with the photolabile linker−rhodamine adduct was irradiated in the presence of the sensitizer coupled to complementary PNA ranging in length from a 3-mer to a 7-mer. The irradiation was performed with a light-emitting diode (LED) having an emission maximum at 405 nm (no emission below 370 nm or above 440 nm). As controls, the same experiments were performed in the absence of sensitizer or with the sensitizer simply derivatized with a poly(ethylene glycol) (PEG) to enhance its water solubility. As shown in Figure 2, when a solution containing only the photolabile PNA (PL, 250 nM) was irradiated, only a very small increase in fluorescence was observed, indicating that the photolabile linker is essentially stable at this wavelength. In the presence of the water-soluble sensitizer derivatized with a PEG (1 equiv) rather than PNA, a comparable low level of photocleavage was observed. However, in the presence of the fully matching complementary 7-mer PNA−sensitizer adduct (S, 1 equiv), a dramatic acceleration in the photocleavage was observed. Taking the slope of the reaction for the first 60 min as an approximation of the first-order kinetics, a greater than 20-fold rate acceleration for the perfect-match complementary PNA−sensitizer adduct was observed. On the basis of the level

Received: September 14, 2011
Published: October 17, 2011

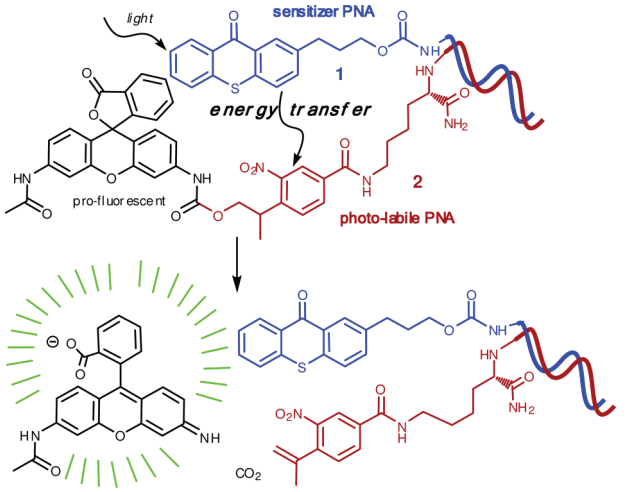

Figure 1. Schematic representation of a sensitized photoreaction based on hybridization of oligonucleotides.

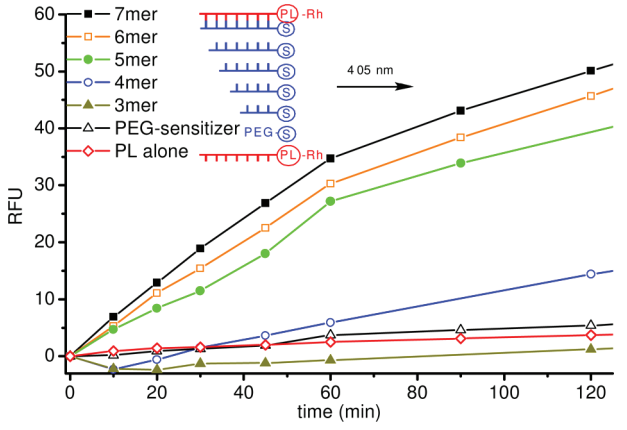

Figure 2. Kinetics of photorelease using sensitizer−PNA adducts with different PNA lengths, a PEG−sensitizer adduct, or no sensitizer (250 nM). LED: 405 nm, 100 mW. Buffer: 10 mM PBS (pH 7.4), 154 mM NaCl, 25 mM $MgCl_2$, 0.05% Tween. PL: Lys(PL)-TG*AC*TA*C-Arg [written from C to N; stars denote guanidine-based PNA (GPNA) residues[15]]. Data points are averages of two experiments. The *y* axis shows relative fluorescence units (RFU) measured (λ_{ex} = 490 nm, λ_{em} = 530 nm).

of fluorescence, the reaction was estimated to have proceeded to 80% conversion after 2 h. The 6-mer and 5-mer showed slightly attenuated rates, while a significant drop in the rates was observed for shorter probes. The 3-mer PNA sensitizer adduct afforded a reaction rate comparable to the sensitizer alone, while the 4-mer PNA sensitizer afforded a rate intermediate between background rate and that for the 5-mer PNA adduct. Taken together, the data strongly support the fact that the rate acceleration observed with the complementary sensitizer PNAs is the product of energy transfer enabled by the preorganization of the supramolecular assembly and that a 5-mer PNA is required to achieve sufficient duplex stability under these conditions. It is interesting to note that no apparent differences in performance were observed when the reaction was run under a nitrogen atmosphere or exposed to air. It is known that photoexcitation of thioxanthenone leads to a triplet excited state that can react with oxygen. Indeed, when the lamp power was increased to 1 W

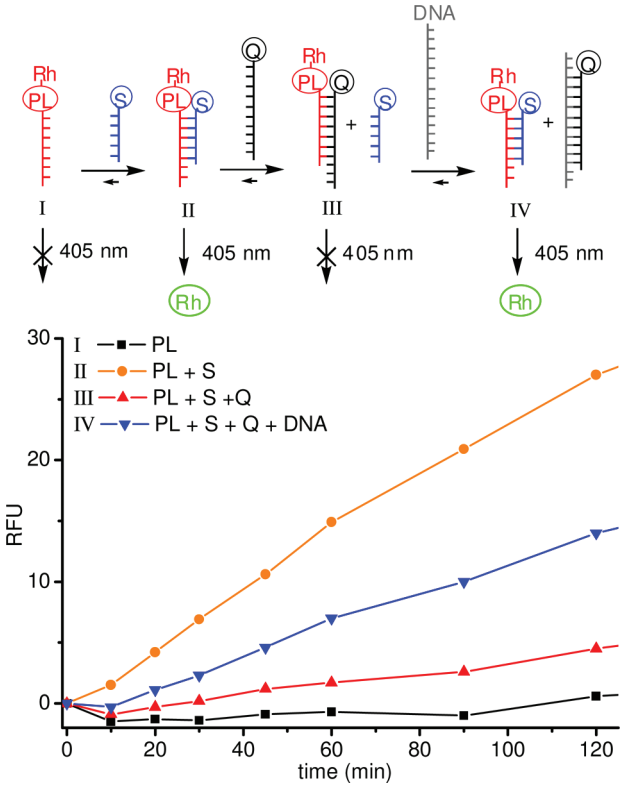

Figure 3. Kinetics of photorelease using 166 nM PL [Lys(PL)-TG*AC*TA*C-Arg], 166 nM S (Arg-AG*TCA*-S), and 250 nM Q (Arg-G*TTG*TAG*TCA*-Q) at 375 nM. DNA: (TCAACATCAGT-CTGATAAGCTA). LED: 405 nm, 100 mW. Buffer: 10 mM PBS (pH 7.4), 154 mM NaCl, 25 mM $MgCl_2$, 0.05% Tween. Data points are averages of two experiments.

(reactions were carried out at 0.1 W), the sensitizer−PEG adduct was fully oxidized after 60 min. However, under the same conditions, the sensitizer−PNA adducts showed less than 10% oxidation, suggesting the presence of an alternative energy dissipation mechanism. MALDI analysis of preliminary reactions (templated photocleavage of aniline rather than rhodamine) indicated a clean conversion from the starting material to the product without any degradation byproduct.

We next investigated whether this templated photorelease reaction could be combined with strand displacement to add a switching-off element. To avoid any background reaction, the third strand was modified with a *p*-(*p*-dimethylaminophenylazo) benzoic acid (dabcyl) quencher (Q). Thus, in the presence of this third nucleic acid strand competing with the sensitizer for the PNA−photolabile adduct, the reaction could be switched off (Figure 3). Furthermore, the system could be pushed one step further with a fourth strand competing for the quenching nucleic acid strand in order to restore the photocleavage. The hierarchical relationship among the interactions (duplex stability) could be programmed using the lengths of the individual nucleic acid strands. As a starting point, the shortest sensitizer−PNA adduct (5-mer) that proved efficacious in the templated reaction with the 7-mer photolabile linker−PNA adduct was chosen. A 10-mer PNA quencher adduct fully complementary to the photolabile linker−PNA adduct should sequester the strand from its interaction with the sensitizer. Next, a DNA strand complementary to the 10-mer PNA was used to out-compete the quenching strand

dx.doi.org/10.1021/ja2086504 |*J. Am. Chem. Soc.* 2011, 133, 18110–18113

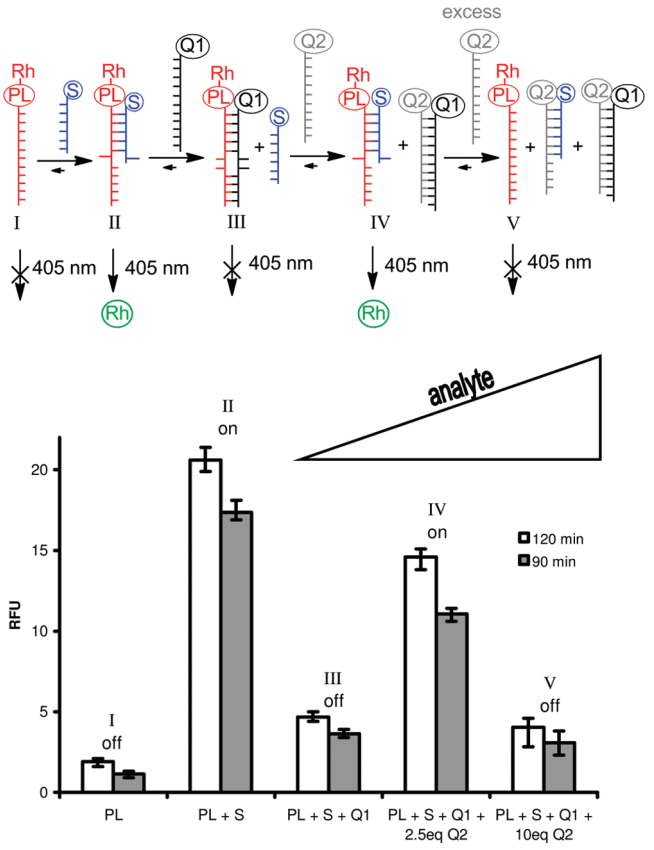

Figure 4. Fluorescence measurements of photoreaction after 90 and 120 min with different amounts (0−2.5 μM) of 10-mer Q2 (Lys(Q)-TG*AC*TA*CA*AC*-Arg) under irradiation with an LED (405 nm, 100 mW) using 250 nM PL [Lys(PL)-TG*AC*TC*GA*AC*T-Arg], 250 nM S (Arg-TAG*TCA*-S), and 375 nM Q1 (Arg-AG*TTG*TAG*TCA*-Q). Buffer: 10 mM PBS (pH 7.4), 154 mM NaCl, 25 mM $MgCl_2$, 0.05% Tween. Data points are averages of three experiments, and error bars represent standard deviations.

(Q) and restore the interaction between the sensitizer (S) and the photolabile linker strands (PL). On the basis of an end-point measurement at 120 min (Figure 3), the photorelease of the fluorophore could be diminished by 6-fold in the presence of the quencher strand. This reduced cleavage supports the efficacy of the strand displacement mechanism. While this is not a complete inhibition of the photorelease, it reflects the dynamic competition between two perfect-match PNAs containing five and seven nucleotides. Addition of the DNA strand complementary to the quencher restored the photocleavage to half of its original efficacy. Again, while this is not as efficient as the photocleavage of the system with just two components (sensitizer and photolabile adduct), the reduced efficiency is the result of complex equilibria. Nevertheless, the system's response was directly proportional to an analyte (DNA) up to saturation at 2.5 molar equiv (Figure S2).

We next asked whether we could design a system that would respond positively to an analyte up to a given concentration and then negatively to further increases in concentration. To this end, a four component system (Figure 4) was designed to respond to a fourth nucleic acid strand derivatized with a quencher (Q2) complementary to the first quencher strand (Q1) and the sensitizer strand (S). The first quencher strand (Q1) was also complementary to the photolabile linker strand (PL) with the

Table 1. Truth Tables for Logic Gates[a]

input strand	binary inputs			
	AND Gate (PL/Q1)			
S	0	1	0	1
Q2 (2.5 equiv)	0	0	1	1
output	0	0	0	1
	XNOR Gate (PL/S)			
Q1	0	1	0	1
Q2 (2.5 equiv)	0	0	1	1
output	1	0	0	1
	NOR Gate (PL/S)			
Q1	0	1	0	1
Q2 (10 equiv)	0	0	1	1
output	1	0	0	0
	NOT Gate (PL/S)			
Q1	0	1		
output	1	0		

[a] Binary inputs and outputs of possible logic gates. Conditions: irradiation with an LED (405 nm, 100 mW) using 250 nM PL [Lys(PL)-TG*AC*TC*GA*AC*T-Arg], 250 nM S (Arg-TAG*TCA*-S), 375 nM Q1 (Arg-AG*TTG*TAG*TCA*-Q), and 625 nM (2.5 equiv) or 2500 nM (10 equiv) Q2 (Lys(Q)-TG*AC*TA*CA*AC*-Arg). Buffer: 10 mM PBS (pH 7.4), 154 mM NaCl, 25 mM $MgCl_2$, 0.05% Tween.

following hierarchy of interaction: PL−S < PL−Q1; PL−Q1 < Q2−Q1; Q2−S > PL−S; Q2−Q1 > Q2−S. To achieve this hierarchy of interaction, two mismatches were introduced in the interaction of the PL strand with Q1 whereas the interaction of Q1 and Q2 was fully matched. End-point measurements taken after 90 or 120 min showed that indeed, in the absence of analyte (Q2), the system afforded a low level of photocleavage; at 2.5 molar equiv of analyte, the system afforded maximal photorelease (3-fold enhancement), and increasing the concentration of analyte to 10 equiv retuned the level of photocleavage to its original state in the absence of analyte.

The unique behavior of this four-component system makes it amenable to four different logic gates (Table 1).[16] An AND gate is created using the photolabile strand (PL) and second quencher (Q2) with the sensitizer (S) and the first quencher (Q1) as inputs. An XNOR gate is created using the PL and S strands as the gate with Q1 and Q2 at 2.5 molar equiv as input. A NOR gate is created in the same manner as the XNOR gate but using 10 molar equiv of Q2 as input. A NOT gate is created using the PL and S strands as the gate and Q1 as the input. On the basis of the well-established technologies for immobilizing nucleic acids in microarray formats, this scheme could be used for logic gates in a parallel mode.[17]

To the best of our knowledge, this is the first example of nucleic acid-templated photorelease enabled by energy transfer from a sensitizer to a photolabile linker. This system should be applicable to the release of a broad variety of functional molecules beyond fluorophores and thus represents an important addition to the arsenal of templated reactions. Furthermore, the reaction can be spatially and temporally controlled with light. The system presented herein offers a simple fluorescent readout

of the reaction that can be used in networks. The ability to engineer different hierarchical relationships among the components of the network can lead to a nonlinear response, as was illustrated with a system that was tuned on then off in response to increasing levels of input.

■ ASSOCIATED CONTENT

Supporting Information. Synthesis, experimental details, characterization, and sequences of all PNAs used. This material is available free of charge via the Internet at http://pubs.acs.org/.

■ AUTHOR INFORMATION

Corresponding Author
winssinger@unistra.fr

■ ACKNOWLEDGMENT

This work was supported by a grant from the European Research Council (ERC 201749). The Institut Universitaire de France (IUF) and Boehringer Ingelheim Fonds (fellowship to K.G.) are gratefully acknowledged.

■ REFERENCES

(1) Lehn, J. M. *Science* **2002**, *295*, 2400. Otto, S.; Ludlow, R. F. *Chem. Soc. Rev.* **2008**, *37*, 101.

(2) Seeman, N. C. *Nature* **2003**, *421*, 427. Feldkamp, U.; Niemeyer, C. M. *Angew. Chem., Int. Ed.* **2006**, *45*, 1856. Rothemund, P. W. K. *Nature* **2006**, *440*, 297. Lo, P. K.; Metera, K. L.; Sleiman, H. F. *Curr. Opin. Chem. Biol.* **2010**, *14*, 597. Silverman, S. K. *Angew. Chem., Int. Ed.* **2010**, *49*, 7180. Ura, Y.; Beierle, J. M.; Leman, L. J.; Orgel, L. E.; Ghadiri, M. R. *Science* **2009**, *325*, 73. Brudno, Y.; Birnbaum, M. E.; Kleiner, R. E.; Liu, D. R. *Nat. Chem. Biol.* **2010**, *6*, 148. Pianowski, Z.; Winssinger, N. *Chem. Soc. Rev.* **2008**, *37*, 1330. For examples of a peptide-based network, see:Ashkenasy, G.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2004**, *126*, 11140. Dadon, Z.; Samiappan, M.; Safranchik, E. Y.; Ashkenasy, G. *Chem.—Eur. J.* **2010**, *16*, 12096.

(3) Tyagi, S.; Kramer, F. R. *Nat. Biotechnol.* **1996**, *14*, 303. Silverman, A. P.; Kool, E. T. *Chem. Rev.* **2006**, *106*, 3775. Jacobsen, M. F.; Clo, E.; Mokhir, A.; Gothelf, K. V. *ChemMedChem* **2007**, *2*, 793.

(4) Benenson, Y.; Paz-Elizur, T.; Adar, R.; Keinan, E.; Livneh, Z.; Shapiro, E. *Nature* **2001**, *414*, 430. Benenson, Y.; Gil, B.; Ben-Dor, U.; Adar, R.; Shapiro, E. *Nature* **2004**, *429*, 423. Qian, L.; Winfree, E. *Science* **2011**, *332*, 1196.

(5) For examples of nucleophilic reactions, see: Ma, Z.; Taylor, J. S. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 11159. Xu, Y. Z.; Karalkar, N. B.; Kool, E. T. *Nat. Biotechnol.* **2001**, *19*, 148. Ficht, S.; Mattes, A.; Seitz, O. *J. Am. Chem. Soc.* **2004**, *126*, 9970. Boll, I.; Kramer, R.; Brunner, J.; Mokhir, A. *J. Am. Chem. Soc.* **2005**, *127*, 7849. Meguellati, K.; Koripelly, G.; Ladame, S. *Angew. Chem., Int. Ed.* **2010**, *49*, 2738.

(6) For examples of Staudinger-based reactions, see: Cai, J.; Li, X.; Yue, X.; Taylor, J. S. *J. Am. Chem. Soc.* **2004**, *126*, 16324. Franzini, R. M.; Kool, E. T. *J. Am. Chem. Soc.* **2009**, *131*, 16021. Furukawa, K.; Abe, H.; Hibino, K.; Sako, Y.; Tsuneda, S.; Ito, Y. *Bioconjugate Chem.* **2009**, *20*, 1026.

(7) For further examples of Staudinger-based reactions, see: Pianowski, Z.; Gorska, K.; Oswald, L.; Merten, C. A.; Winssinger, N. *J. Am. Chem. Soc.* **2009**, *131*, 6492. Gorska, K.; Manicardi, A.; Barluenga, S.; Winssinger, N. *Chem. Commun.* **2011**, *47*, 4364. Gorska, K.; Keklikoglou, I.; Tschulena, U.; Winssinger, N. *Chem. Sci.* **2011**, *2*, 1969.

(8) For examples using charge transfer, see: Boon, E. M.; Ceres, D. M.; Drummond, T. G.; Hill, M. G.; Barton, J. K. *Nat. Biotechnol.* **2000**, *18*, 1096. Augustyn, K. E.; Merino, E. J.; Barton, J. K. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 18907.

(9) For an example of singlet oxygen generation, see: Ogilby, P. R.; Clo, E.; Snyder, J. W.; Voigt, N. V.; Gothelf, K. V. *J. Am. Chem. Soc.* **2006**, *128*, 4200.

(10) Lee, H. M.; Larson, D. R.; Lawrence, D. S. *ACS Chem. Biol.* **2009**, *4*, 409.

(11) Wöll, D.; Smirnova, J.; Pfleiderer, W.; Steiner, U. E. *Angew. Chem., Int. Ed.* **2006**, *45*, 2975. Wöll, D.; Smirnova, J.; Galetskaya, M.; Prykota, T.; Buhler, J.; Stengele, K. P.; Pfleiderer, W.; Steiner, U. E. *Chem.—Eur. J.* **2008**, *14*, 6490.

(12) Leytus, S. P.; Melhado, L. L.; Mangel, W. F. *Biochem. J.* **1983**, *209*, 299. Lavis, L. D.; Chao, T. Y.; Raines, R. T. *ACS Chem. Biol.* **2006**, *1*, 252.

(13) Nielsen, P. E. *Mol. Biotechnol.* **2004**, *26*, 233. Egholm, M.; Buchardt, O.; Christensen, L.; Behrens, C.; Freier, S. M.; Driver, D. A.; Berg, R. H.; Kim, S. K.; Norden, B.; Nielsen, P. E. *Nature* **1993**, *365*, 566.

(14) Holmes, C. P.; Jones, D. G. *J. Org. Chem.* **1995**, *60*, 2318.

(15) Zhou, P.; Wang, M.; Du, L.; Fisher, G. W.; Waggoner, A.; Ly, D. H. *J. Am. Chem. Soc.* **2003**, *125*, 6878.

(16) Saghatelian, A.; Volcker, N. H.; Guckian, K. M.; Lin, V. S.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2003**, *125*, 346.

(17) Frezza, B. M.; Cockroft, S. L.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2007**, *129*, 14875.