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COMMUNICATIONS

Published on Web 02/27/2008

# Diamine Catalyzed Hemicyanine Dye Formation from Nonfluorescent Precursors through DNA Programmed Chemistry

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DNA templated synthesis has evolved dramatically from its use to study interactions of oligonucleotides,[1] to a method for producing DNA-like structures,[2] to a general approach for compound synthesis and reaction discovery.[3,4] Essentially, DNA programmed chemistry (DPC) is the coupling of organic chemical reactions to a biological recognition event (DNA hybridization). As such, DPC has tremendous potential for biodetection.[5] DPC-based biodetection employs recognition elements such as antibodies, aptamers, or polypeptides to drive DNA hybridization, and a resultant analyte-dependent synthesis of a fluorescent reporter compound. In a previous publication,[6] we demonstrated that DPC can be used to generate a coumarin label from a less fluorescent azidocoumarin by phosphine reduction following similar work by Taylor's lab.[7] However, phosphines are susceptible to oxidation by air and through reaction with disulfides, somewhat limiting their utility for biodetection.

We then turned our attention to hemicyanine dyes which have been widely used as fluorescent probes.[8] Hemicyanine dyes are generally synthesized by aldol-type condensations of heterocyclic quaternary salts bearing an active methyl group with aldehydes under anhydrous conditions. However, recent reports have shown asymmetric aldol condensation can be performed in aqueous media in the presence of a Lewis acid[9] or enamine catalyst[10] with an improved reaction rate. Preliminary screening of catalysts in a model reaction indicated that organocatalyst (*S*)-pyrrolidine methylpyrrolidine,[10c] ((*S*)-PMP)) performed the best under the aqueous conditions chosen (Table S2).

Both end-of-helix (E)[3b]and middle-of-helix (M) DNA architectures were then tested for (*S*)-PMP-mediated DNA-templated hemicyanine formation (Figure 1). For the E-architecture, the reactants were coupled to a pair of complementary DNA strands, such that, upon DNA hybridization, the reactants would be juxtaposed at the end of the helix. Figure 1A shows fluorescence emission spectra of the reaction mixture of the indolinium- and aldehyde-labeled complementary 15-mers, I_DNA4 and A_DNA2, and various control reactions. First, there was no background fluorescence emission for hemicyanine precursors I_DNA4 and A_DNA2 alone (2 and 3 in Figure 1A) as compared to the buffer blank. Second, without the addition of (*S*)-PMP, there was no fluorescent signal generated. Third, increasing the pH alone from 8.4 to 10.0 had no effect on the yield (5 and 6 in Figure 1A). Last, the DPC reaction was sequence-specific (DNA dependent), as no fluorescence signal was generated using DNA strands having three mismatched bases (DNA3) after 140 min, and only a trace amount of fluorescence signal was detected after 16 h (data not shown). For the matched sequences, LC−MS showed a major product peak and a small residual peak of A_DNA2 after 16 h (Figure S3).

In the M architecture, the reactants were coupled to DNAs designed to bind contiguously to a DNA template placing the reactive groups in the middle of the helix upon DNA hybridization. The experimental results showed that product and signal were formed only in the presence of the complementary template DNA1

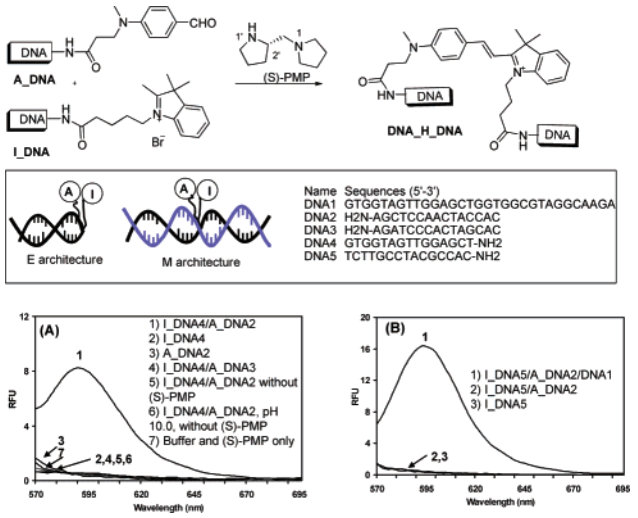



***Figure 1.*** Relative fluorescent intensity of hemicyanine DPC reaction mixtures. DPC reactions were performed using 0.2 $\mu$M reagent strands (and equimolar template for M architecture) at RT for 140 min in 10 mM (*S*)-PMP, 50 mM sodium phosphate (NaPi), 1 M NaCl, pH 8.4 unless otherwise specified. Results for E and M architecture are shown in (A) and (B), respectively.

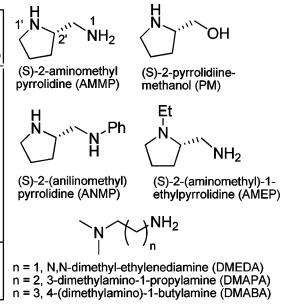
(Figure 1B). The quantum yields and extinction coefficients measured for purified template-free DPC products (DNA2_H_DNA5, H: hemicyanine; $\Phi$: 0.1; $\epsilon_{550}$ ($H_2O$) : 75 000 $cm^{-1}$ $M^{-1}$) produced in the M architecture and DNA2_H_DNA4 produced in the E architecture ($\Phi$: 0.1; $\epsilon_{550}$ ($H_2O$) : 87 000 $cm^{-1}$ $M^{-1}$) were similar, as were the DPC reaction kinetics for the two architectures (Figure S4). However, we noticed that the relative fluorescence intensity of the M reaction mixture (Figure 1B, reaction 1) was approximately twice that of the E reaction mixture (Figure 1A, reaction 1). This 2-fold difference in intensity between E and M architectures might have been due to intercalation and/or restricted rotation of the two heteroaromatic fragments of the hemicyanine within the M architecture. The observed increase in fluorescence of cyanines upon DNA binding through intercalation is a well-known phenomena.[11] When DNA1 template was added back to purified template-free DNA2_H_DNA5 in the presence of salt, the fluorescence intensity was doubled consistent with this hypothesis (Figure S5).

To understand the catalytic role of PMP and its analogues in hemicyanine formation by DPC, we performed a structure−activity study whereby we removed or varied the substitution of the two nitrogen atoms (N1 and N1′) and the chirality at the C2′ position of the ring (Table 1, pyrrolidine analogue). We found that replacing N1 with a hydroxyl group resulted in a low yield (PM). When N1 was a primary amine (AMMP), the catalyst was much more active as when N1 was a secondary or tertiary amine substituted with aryl or alkyl moieties (PMP and ANMP). The poor water solubility of ANMP also contributed to the low product yield. Moreover, whether N1′ of the pyrrolidine ring was substituted or not had little


10.1021/ja0753602 CCC: $40.75 

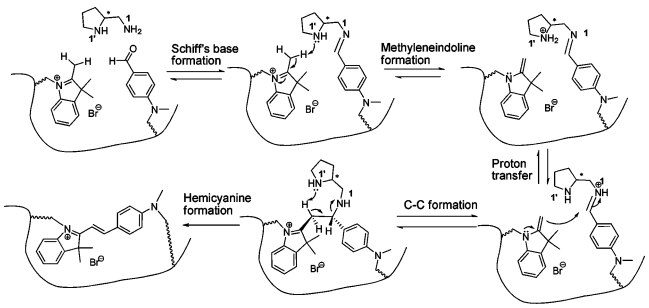
***Table 1.*** Relative Fluorescence of DPC Reaction Mixtures in the Presence of Different Catalysts after 2 h[a]

| | Catalyst | E-architecture RFU[b] | E-architecture Yield[c] (%) | M-architecture RFU[b] | M-architecture Yield[c] (%) |
|---|---|---|---|---|---|
| pyrrolidine analog | None | 0.8 | 0 | 0.8 | 0.1 |
| | (S)-PMP | 20.1 | 6.8 | 46.9 | 5.2 |
| | (R)-PMP | 21.2 | 7.2 | 54.4 | 6.0 |
| | (S)-PM | 3.3 | 1.1 | 3.6 | 0.4 |
| | (S)-AMMP | 78.4 | 26.5 | 213.3 | 23.6 |
| | (R)-ANMP | -0.1 | 0 | 1.3 | 0.1 |
| | (S)-ANMP | 0.2 | 0 | 1.8 | 0.2 |
| | (R)-AMEP | 83.9 | 28.4 | 179.4 | 19.8 |
| | (S)-AMEP | 78.2 | 26.4 | 178.2 | 19.7 |
| acyclic diamine | DMEDA[d] | 134.7 | 45.5 | 370.9 | 41.0 |
| | DMAPA | 80.2 | 27.1 | 216.6 | 24.0 |
| | DMABA | 2.0 | 0.7 | 4.0 | 0.4 |




[a] DPC reactions were performed using 0.2 $\mu$M reagent strands (and equimolar template for M architecture) at RT in 10 mM catalyst, 50 mM NaPi, 150 mM NaCl, pH 8.4. [b] RFUs were recorded using a microplate spectrofluorometer (Molecular Device, GeminiXPS). [c] Product yields after 2 h were calculated from RFUs based on standard curves (Figure S6). [d] In a separate experiment, the $T_{1/2}$ of the DMEDA reaction was determined to be 52 min (Figure S8).

***Scheme 1***




effect on the product yield (AMEP vs AMMP). In a separate experiment, we determined that no DPC products were formed when simple primary amines such as isobutylamine, cyclopentylamine, ethylamine, and isopropylamine were used. Inversion of the chiral center at C2′ had little effect on product yield. Finally, we examined the effect of concentration of (*S*)-PMP and found that increasing the concentration of catalyst increased the reaction rate to a plateau at ∼15 to 20 mM catalyst (Figure S7). Taken together these data suggested that two basic centers were required, that one must be a primary amine, that catalyst concentration had a significant impact, and that catalyst chirality while having some influence on reaction rate was of lesser importance.

This led us to propose a mechanism by which pyrrolidine diamines were facilitating hemicyanine DPC through Schiff's base formation (Scheme 1). The observed requirement for a high catalyst concentration was consistent with the need to establish and maintain a Schiff's base under aqueous conditions throughout the initial reaction sequence in order to localize the catalyst at the reaction site. Further evidence to support this mechanism came from studies of acyclic diamines with different carbon chain lengths (Table 1). Increasing the distance between the primary and tertiary amine centers by a single carbon atom decreased the product yield by a factor of 2 (DMAPA), and extending the distance by two carbon atoms effectively abolished all catalytic effect (DMABA). Addition of $NaCNBH_3$ to DPC reaction mixtures provided direct evidence for the Schiff's base intermediate, as LC−MS showed quantitative formation of the reduced Schiff's base (secondary amine formation) for (*S*)-AMEP, DMEDA, DMAPA, and 60% for DMABA (Figure S9). That no DPC product is formed in the presence of DMABA despite the Schiff's base formation demonstrates the importance of the close proximity of the two nitrogen centers in support of the proposed mechanism.

To date, almost all DPC approaches have relied on the proximity and intrinsic chemical reactivity of components. Exceptions are palladium(0) and copper(I) catalyzed, cross-coupling, and Huisgen reactions, which, although rapid, damage and degrade DNA.[4c] The finding that simple diamines can act in a concerted fashion upon two otherwise unreactive moieties to rapidly catalyze a high yield of product without side reactions illustrates that other methods of control may be used to facilitate DPC reactions.

The DPC-mediated synthesis of hemicyanine dyes from non-fluorogenic precursors was undertaken to allow development of homogeneous fluorescent bioassays. We have found that matrices such as serum that are rich in primary amines and other nucleophiles do not decrease the rate and yield of hemicyanine formation and resulting fluorescent signal (Figure S11). Preliminary results indicate that hemicyanine creation can be used broadly for detection of biomolecules when the chemistry is linked to a variety of recognition elements such as oligonucleotides, aptamers, and antibodies. Moreover, it is likely the functionality of the simple diamine catalysts described here can be engineered into the template and/or reactant strands. Such "all-in-one" designs might allow for hemicyanine-based biodetection in living systems where the introduction of an excess of free diamine would be experimentally difficult and potentially toxic to the cell or organism being studied.

**Acknowledgment.** We thank Scott Harbeson, Nick Terrett, Ben Seigal, and Larry Haff for useful discussions.

**Supporting Information Available:** Experimental details and figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA0753602