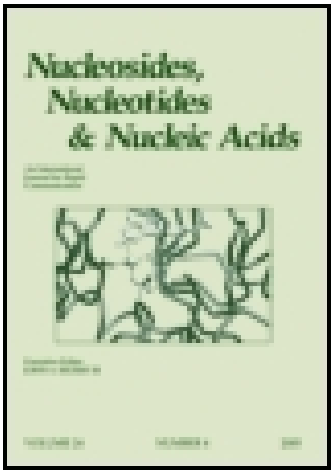

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Toward a Catalytic Site in DNA: Polyaza Crown Ether as Non-Nucleosidic Building Blocks in DNA Conjugates

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TOWARD A CATALYTIC SITE IN DNA: POLYAZA CROWN ETHER AS NON-NUCLEOSIDIC BUILDING BLOCKS IN DNA CONJUGATES

Ulla Jakobsen, Katja Rohr, and Stefan Vogel □ *Nucleic Acid Center, Department of Chemistry, University of Southern Denmark, Odense M, Denmark*

□ *A number of functionalized polyaza crown ether building blocks have been incorporated into DNA-conjugates as catalytic Cu^{2+} binding sites. The effect of the DNA-conjugate catalyst on the stereochemical outcome of a Cu^{2+}-catalyzed Diels-Alder reaction will be presented.*

Keywords Polyaza crown ether; catalysis; DNA-conjugates

INTRODUCTION

Due to its architecture, DNA is an ideal scaffold for the construction of hybrid materials including catalytic systems (e.g., DNAzymes).[1–7] Feringa et al. recently introduced a new modular assembled DNA-based catalyst.[11] The catalytic Cu^{2+}binding site intercalates into dsDNA due to their polyaromatic nature. The Cu^{2+} binding site is thereby brought into proximity of the chiral environment of the dsDNA, which in turn leads to transfer of chirality from the dsDNA to the reaction products with an *ee* of up to 99% for the major (*endo*) isomer. We report in this communication another approach based on covalent incorporation of catalytic polyaza crown ether based Cu^{2+} binding sites into ssDNA.[12]

RESULTS AND DISCUSSION

The synthetic route toward the desired building blocks (monomer **W**, **X**, and **Y**) is very flexible and can be easily adapted for a series of macrocyclic building blocks for automated DNA synthesis based on the phosphoramidite approach (Scheme 1).[8,12] Macrocycle **8** served as universal building block for the *N*-functionalization by acylation (**W**) or reductive amination (**X**, **Y**).

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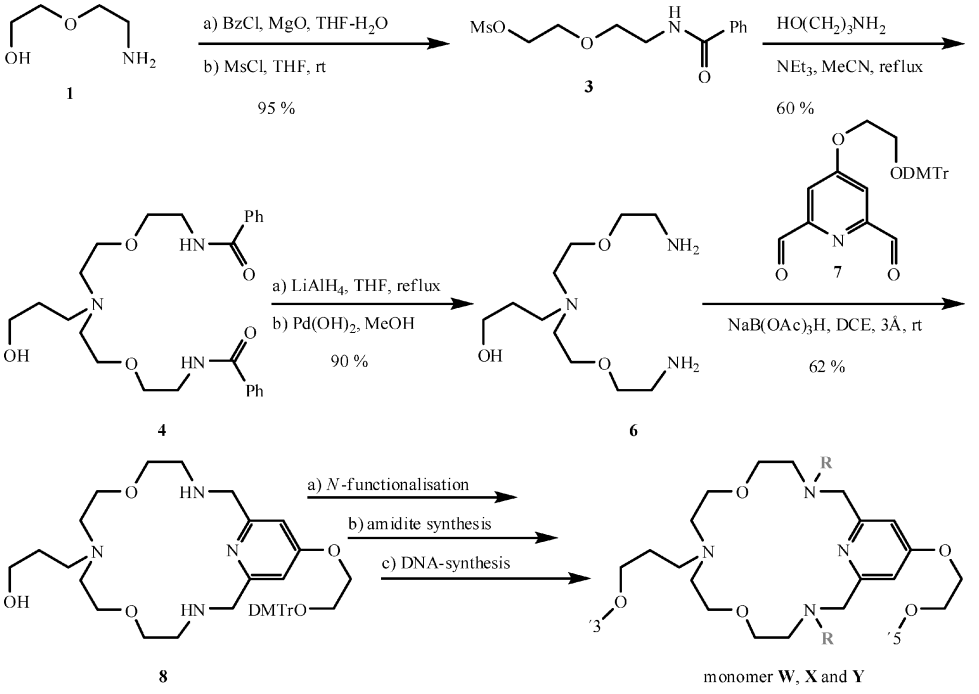


SCHEME 1 Synthesis of polyaza crown ether phosphoramidite building blocks and monomer substitution patterns: **W**: R = H, **X**: R = $C_{16}H_{33}$ (palmityl), **Y**: R = $CH_2C_5H_4N$ (2-pyridyl).

Synthesis of ONs (oligonucleotides) was performed in 0.2 μmol scale on an automated DNA synthesizer using the phosphoramidite approach. Incorporation of monomers **W**, **X**, and **Y** opposite to a complementary 17-mer ss-DNA sequence resulted in a destabilization (-10–12°C, Table 1, entries 2–4). This can be explained by the steric distorsion of the corresponding dsDNA (**W**, **X**, and **Y** are ~2 × larger than A, G, C, and T). Juxtapositioned incorporations of **W**, **X**, and **Y** are well tolerated (+8°C, for **X**, Table 1, entry 5) despite the steric demand and non-nucleosidic structure of the monomers. The remarkable increase in thermal stability displayed by modification **X** is likely due to undisturbed interstrand base distances in the duplex and additional strong hydrophobic forces (C_{16}-palmityl chains). The chosen reaction (Scheme 2) has been used for comparison with the system reported by Feringa et al.[11]

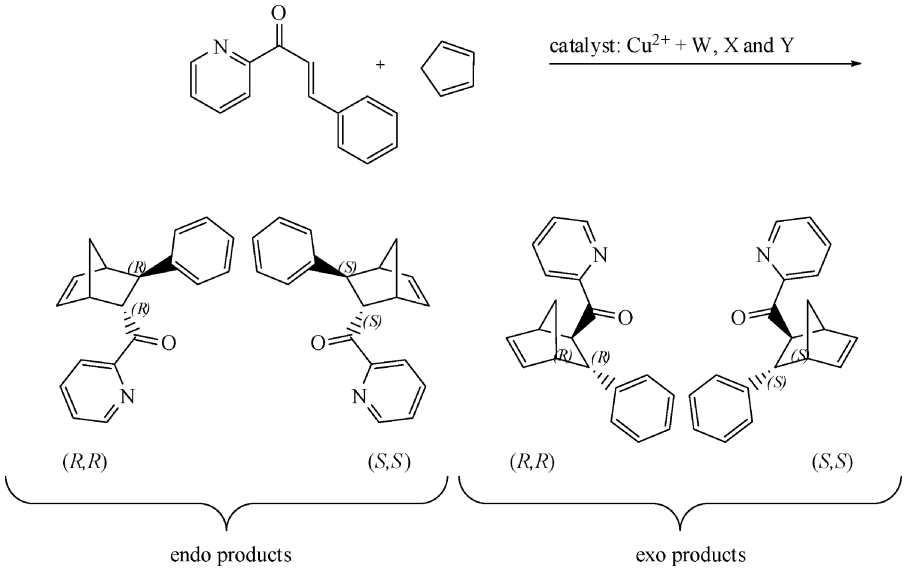
The 2,6-diamino-pyridine unit in the core of **8** is binding Cu^{2+} efficiently accompanied by the appearance of a characteristic UV band at 668 nm. UV-titration with increasing conc. of Cu^{2+} revealed a 1:1 stochiometry of the complex.

The initially performed catalytic reactions have shown that all DNA-conjugates are able to catalyse the Diels-Alder reaction but only one of the DNA-conjugates displayed a very weak asymmetric induction (10% *ee*,

TABLE 1 Influence of monomer W-Y on thermal DNA duplex stability

Entry	Sequence	T_m [°C][a]
1	5′-TGT-GGA-AGA-AGT-TGG-TG	
	3′-ACA-CCT-TCT-TCA-ACC-AC	56.0
2	5′-TGT-GGA-AGA-AGT-TGG-TG	
	3′-ACA-CCT-TC**W**-TCA-ACC-AC	44.0
3	5′-TGT-GGA-AGA-AGT-TGG-TG	
	3′-ACA-CCT-TC**X**-TCA-ACC-AC	44.5
4	5′-TGT-GGA-AGA-AGT-TGG-TG	
	3′-ACA-CCT-TC**Y**-TCA-ACC-AC	45.5
5	5′-TGT-GGA-AG**X**-AGT-TGG-TG	
	3′-ACA-CCT-TC**X**-TCA-ACC-AC	64.0

[a] 10 mM sodium phosphate, 100 mM NaCl, 0.1 mM EDTA, adjusted to pH 7.0, concentration of 1 μM for each DNA strand.



SCHEME 2 Cu^{2+} catalyzed Diels-Alder reaction: DNA-conjugates **W**, **X**, and **Y** were used as catalysts.

Figure 1) under the conditions used by Feringa et al. (measured values of 9 and 11% have been averaged to 10%). We have not investigated the influence of the juxtapositioned base (e.g., T, G, C, instead of A) due to prohibitive amounts of ONs needed for further experiments. From our initial results and the putative structure of the catalytic center we assume that the reaction center is not close enough to the chiral environment to induce a stronger asymmetric induction, the use of a considerably smaller catalytic center is therefore the next step towards improved asymmetric induction.

A flexible synthetic strategy toward functionalized polyaza crown ether amidites as well as an efficient incorporation of the respective building

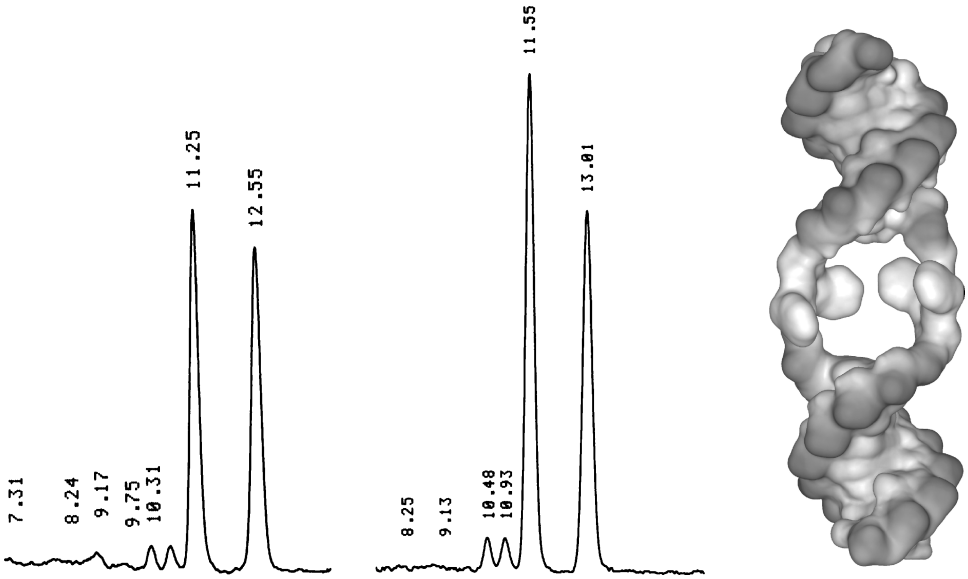


FIGURE 1 HPLC traces for a) racemic mixture, b) reaction catalyzed with dsDNA (Table 1, entry 3) with 10% *ee* (no effect on *ee* for **W** and **Y**), c) schematic model of the catalytic center embedded in a 17-mer dsDNA.

blocks into DNA sequences has been achieved. The results from the catalyzed Diels-Alder reactions have shown that a more rigid catalytic center in close proximity to the helical DNA regions is required to achieve significant asymmetric induction.

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