Nucleic Acid Template-Directed Assembly of Metallosalen−DNA Conjugates

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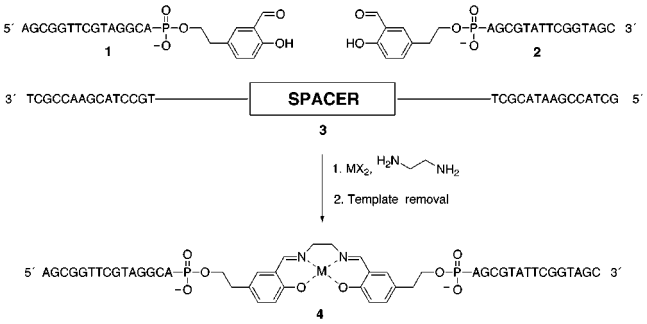
Watson−Crick base pairing organizes DNA duplex formation necessary for genetic information storage in biological systems. DNA and RNA templates also direct the specific binding of nucleotide substrates during diverse enzyme-catalyzed reactions in replication, transcription, and DNA repair pathways. Recently, nucleic acid recognition properties have been extended to non-biological systems, where DNA base pairing has been used to drive the template-directed chemical ligation of oligonucleotides,[1] and the assembly of nanostructures and novel materials.[2]

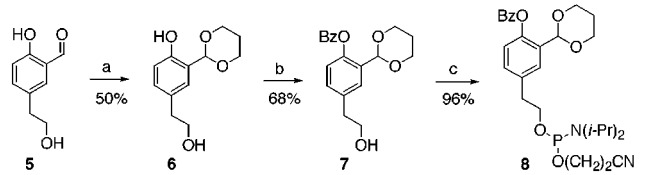
We have been interested in expanding the versatility of nucleic acid base pairing for the addressable synthesis of bioconjugates in aqueous solution. Metallosalen−DNA (**4**, Scheme 1) represents an ideal system to demonstrate the concept of nucleic acid template-directed molecular synthesis. Salens, which are constructed from two salicylaldehydes and a diamine, serve as ligands for a broad range of metal ions. Many metallosalens are compatible with aqueous conditions[3] and have demonstrated utility as DNA cleavage reagents[4] and versatile catalysts for enantioselective transformations.[3a,5] Template-directed synthesis of metallosalen−DNA conjugates offers a unique approach to a new class of metal−DNA hybrids. Metal−DNA conjugates previously have been employed as probes of DNA structure and electron transfer,[6] "chemical nucleases" for targeted nucleic acid cleavage,[7] and scaffolds for metal-mediated base pairing motifs.[8] Thus, metallosalen−DNA may offer a new bioconjugate platform for DNA-organized materials, nucleic acid cleavage and detection strategies, and in vitro evolution of novel ribozymes and deoxyribozymes.[9]

Scheme 1. Template-Directed Metallosalen−DNA Assembly[a]



[a] **3a** (DNA), SPACER = TT or **3b** (RNA), SPACER = UU.

Scheme 2[a]



[a] Key: (a) $HO(CH_2)_3OH$, acidic alumina, $PhCH_3$, reflux; (b) NaOH, BzCl, THF; (c) $[(i\text{-}Pr)_2N]_2POCH_2CH_2CN$, $(i\text{-}Pr)_2NH_2{\cdot}CHN_4$, CH_2Cl_2.

Our approach to template-directed synthesis of metallosalen−DNA is illustrated in Scheme 1. The DNA−metallosalen building blocks consist of two DNA oligonucleotides modified, at either the 3′ or 5′ end, with salicylaldehyde moieties (**1** and **2**). The modified strands are aligned on a complementary nucleic acid template (**3**), bringing the salicylaldehyde groups into proximity in a duplex. The metallosalen conjugate then is assembled by addition of an appropriate metal and diamine. Herein we report the efficient DNA and RNA template-directed synthesis and characterization of purified metallosalen−DNA conjugates.

A salicylaldehyde phosphoramidite (**8**, Scheme 2) was synthesized as a precursor to salicylaldehyde−DNA conjugates **1** and **2**, necessary for metallosalen−DNA assembly. The protecting groups for **8**, including a benzoate ester for the phenol and a 1,3-dioxane for the aldehyde, were chosen for their compatibility with DNA synthesis and postsynthetic deprotection. Starting from known salicylaldehyde derivative **5**,[10] dioxane **6** was prepared by alumina-catalyzed acetalization. Direct benzoylation of **6** with benzoyl chloride afforded **7**, which was converted to phosphoramidite **8** by standard methods.[11]

Oligonucleotide **2** was synthesized by DNA phosphoramidite chemistry (3′-to-5′), using **8** in the final coupling step. Oligonucleotide **1**, bearing a 3′-terminal salicylaldehyde, was produced by 5′-to-3′ DNA synthesis using commercial nucleoside 5′-phosphoramidites. Standard DNA deprotection with concentrated ammonia removed the phenolic benzoate group, and subsequent

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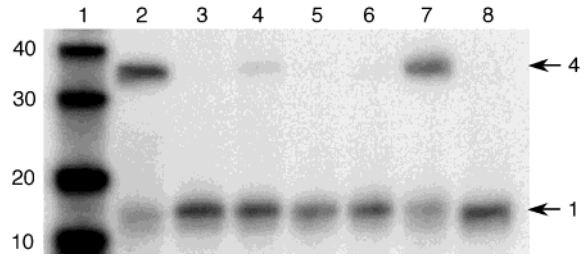

Figure 1. Gel electrophoresis assay of metallosalen−DNA assembly. (Lane 1) 10 bp marker. (Lanes 2−6) Mn assembly reactions at pH 8.0 for 1 h. (Lane 2) All components: $Mn(OAc)_2$, **1**, **2**, and **3b**, EN. (Lane 3) −EN. (Lane 4) $-Mn(OAc)_2$. (Lane 5) −**2**. (Lane 6) −**3b**. (Lane 7) Ni(II) assembly reaction with all components at pH 6.5 for 24 h. (Lane 8). Labeled **1**. All reactions included 10 mM buffer (HEPES for Mn and MES for Ni) and 150 mM NaCl.

incubation in 15 mM NaOAc/HOAc buffer (pH 4.0) for 2 h at 37 °C cleaved the dioxane group to the aldehyde. Oligonucleotides **1** and **2** were purified by denaturing polyacrylamide gel electrophoresis (PAGE) or reverse-phase HPLC (RP-HPLC), and their identities were confirmed by matrix-assisted laser desorption time-of-flight (MALDI-TOF) mass spectrometry (for **1**: $[M]^-$ calcd, 4860.17; found, 4860.86; for **2**: $[M]^-$ calcd, 4835.15; found, 4835.37).

The DNA template-directed assembly of a Mn−metallosalen−DNA conjugate (**Mn-4)** is demonstrated in Figure 1. Assembly reactions were monitored by gel electrophoresis using radiolabeled **1** strand as a tracer; metallosalen−DNA formation was reflected in a shift of the labeled strand to lower gel mobility upon conjugation. When DNA **1** and **2** were annealed to template **3a** and incubated for 1 h at 37 °C in the presence of 100 μM ethylenediamine (EN) and 400 μM $Mn(OAc)_2$, a new DNA complex, corresponding to **Mn-4**, was produced in ∼65% yield (Lane 2). Removal of assembly components EN (lane 3) or strand **2** (lane 5) prevented complex formation. In the absence of manganese (lane 4), ∼4% maximum yield of unmetalated **4** was observed. Most importantly, DNA template **3a** (lane 6) was required for the assembly reaction. While the templated reaction was complete in 1 h, untemplated complex formation was detectable only after 8 h. Taken together, the results in Figure 1 demonstrate that metallosalen−DNA formation is DNA template- and metal ion-dependent.[12] Two additional contributors to **Mn-4** assembly efficiency were the identity of the template spacer units and the pH of the reaction. Two nucleotide residues (TT) provided an optimal spacer for **Mn-4** conjugate formation, consistent with our molecular modeling of DNA−metallosalen duplexes. Optimal yields of **Mn-4** assembly were observed at pH 8 but decreased with increasing pH as predicted by metal speciation data and the limited solubility of manganese ions with increasing pH.[13]

Purified metallosalen−DNA conjugate **Mn-4** was characterized by two complementary methods: MALDI-TOF MS and nucleoside composition analysis. Preparative synthesis of metallosalen−DNA (∼4 nmol) was performed using a complementary RNA

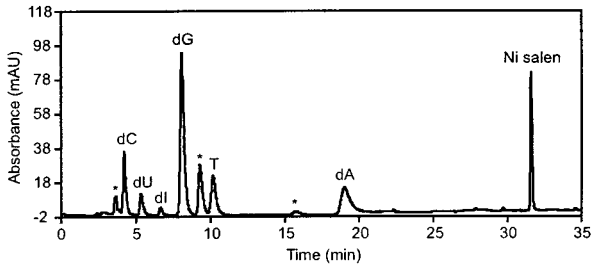

Figure 2. RP-HPLC analysis of **Ni-4** nucleoside/Ni−salen enzymatic digestion products. dU, deoxyuridine (internal standard). dI, deoxyinosine. *, peak present in the digestion blank.

template, **3b**.[14] Following **Mn-4** assembly with **3b**, selective RNase H digestion of the RNA template strand of the DNA/RNA hybrid facilitated the purification of **Mn-4**. After isolation, **Mn-4** was stable in buffered aqueous solutions for several days at 25 °C. MALDI-TOF characterization of purified **Mn-4** provided the mass expected for metalated **Mn-4** ($[M]^-$ calcd, 9773.35; found, 9772.83). To verify the base and salen composition, **Mn-4** was subjected to nuclease digestion and quantitative RP-HPLC analysis.[15] The component nucleosides and salen ligand were present in the ratios expected for **Mn-4**.[16]

Metallosalen−DNA conjugates containing Ni(II) (**Ni-4**) were assembled by an analogous template-directed strategy. **Ni-4** was synthesized using 2 μM each of **1**, **2**, and **3a** (or **3b**), 300 μM $Ni(OAc)_2$, and 150 μM EN at pH 6.5. **Ni-4** assembly proceeded in 74% yield at 37 °C in 24 h (Figure 1, lane 7).[17] MALDI-TOF MS characterization ($[M]^-$ of **Ni-4** calcd, 9777.06; found, 9777.64) verified the identity of the metal−DNA conjugate. **Ni-4** was further characterized by enzymatic digestion to deoxynucleosides and Ni−salen, which were present in the correct ratios for **4** as shown by quantitative RP-HPLC analysis in Figure 2.

We have demonstrated the efficient nucleic acid template-directed synthesis and characterization of a new class of metal−DNA conjugates, metallosalen−DNA. The chemical diversity of metallosalen−DNA assembly processes and the applications of these metal−DNA hybrids currently are under investigation. Nucleic acid template-directed molecular synthesis offers a powerful approach for the addressable synthesis of new DNA bioconjugates, which may offer significant potential for applications including targeted nucleic acid cleavage, biosensors, and catalysis.

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Supporting Information Available: Experimental procedures, spectroscopic and analytical data for compounds **1**−**8**, HPLC and MALDI-TOF data for compounds **1**, **2**, and **4**, base composition data for **4** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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