A Ni(Salen)-Biotin Conjugate for Rapid Isolation of Accessible DNA

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The oxidation and coordination chemistry of transition metal ions offer substantial opportunities for selective recognition and modification of nucleic acids.[1] In particular, certain nickel complexes have demonstrated alternative abilities to oxidize and couple with highly accessible guanine residues in RNA and DNA after addition of O_2 and sulfite[2,3] or peracids such as monoperoxysulfate or monoperoxyphthalate.[4,5] Adducts formed between guanine and nickel complexes based on a salen ligand (ethylene-*N*-*N*′-bis(salicyaldimine)) strongly inhibit polynucleotide elongation catalyzed by reverse transcriptase and DNA polymerase and consequently allow for sensitive detection through primer extension assays.[5,6]

The salen ligand serves two functions by activating the redox chemistry of the bound Ni(II) and generating a ligand-centered radical for addition to guanine.[5,7] Direct coordination between a transient Ni(III) intermediate and accessible N7 sites on guanine appears to enhance the specificity.[8] Previous investigations have focused on the water-soluble salen complex **1**, NiTMAPES (Figure 1), but a wide range of useful derivatives can be envisioned including a salen-peptide hybrid.[3,5,9] These nickel salens appear unique in their ability to couple with their targets rather than promote direct strand scission as common to the salen complexes of Mn, Co, and Cu.[5b]

Although footprinting well-defined polynucleotides with NiTMAPES may be routine with piperidine cleavage or primer extension, obtaining the actual products of coupling can be an arduous task. As the heterogeneity of the system increases, even footprinting becomes difficult. This type of problem is common in molecular biology, and its solution often relies on a broad range of techniques based on biotin. Labeling, detecting, and purifying any biotinylated species regardless of its complexity is made possible by the extraordinary affinity of biotin for avidin and streptavidin.[10,11] The properties of a combined Ni(salen)-biotin conjugate should then have the potential to diagnosis and isolate genomic sequences that contain guanine residues in unusual and accessible structures. This communication describes the first synthesis of such a conjugate and its initial characterization with a model oligonucleotide.

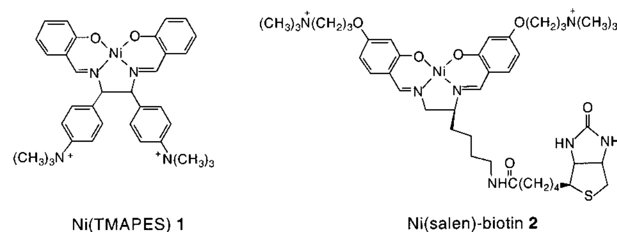

Figure 1. Ni(salen) probes for nucleic acid structure.

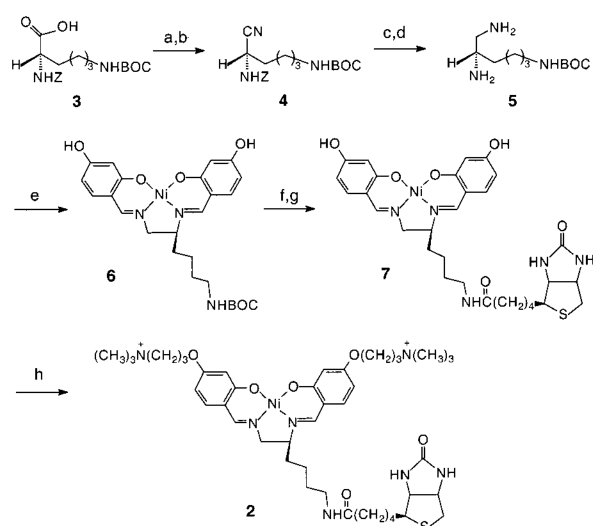

Figure 2. Synthesis of a Ni(salen)-biotin conjugate with solubility in water. Reagents and conditions: (a) Et_3N, ClCOOEt, NH_3, THF, 10 °C to room temperature, 56%. (b) $(CF_3CO)_2O$, Et_3N, THF, N_2, 0° C, 87%. (c) H_2, Ra−Ni, 6.8 atm, NH_3, MeOH, 3 days, 72%. (d) H_2, Pd/C, MeOH, room temperature, overnight, 82%. (e) 2,4-dihydroxybenzaldehyde, Ni-$(OAc)_2$, EtOH, reflux, N_2, 3 h, 78%. (f) TFA, CH_2Cl_2, room temperature, 0.5 h, 86%. (g) BNHS, Et_3N, DMF, 4° C, 3 days, 22%. (h) $Br(CH_2)_3N$-$(CH_3)_3Br$, DMF, room temperature, 3 days, 80%.

Early synthetic targets were designed in direct analogy to TMAPES and were expected to support a range of strategies for biotinylation. However, the instability and low reactivity of the essential intermediates precluded this scheme. These problems were avoided in a subsequent approach that relied on a triamine first developed by the laboratory of Bailly for use in construction of Cu(salen) derivatives.[12] N^{α}-Z-N^{ϵ}-BOC-L-lysine **3** was converted to its nitrile derivative **4** as described previously (Figure 2).[12] A subsequent procedure requiring hydrogenation under high pressure was avoided by use of two sequential hydrogenations in the presence of Raney nickel and then Pd/C to yield the diamine **5** (see Supporting Information for experimental details). The BOC-protected salen derivative **6** was formed by condensation of this diamine under N_2 with 2 equiv of 2,4-dihydroxybenzaldehyde in the presence of $Ni(OAc)_2$. Deprotection by standard conditions (dry TFA/CH_2Cl_2) and coupling with the commercially available

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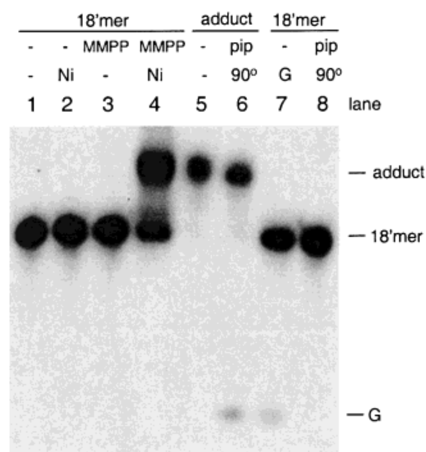

Figure 3. Phosphorimage of a denaturing polyacrylamide gel (20%, 7 M urea) used to identify the high molecular weight conjugate formed between DNA and Ni(salen)-biotin. The 5′-[^{32}P] labeled oligodeoxynucleotide (18′mer), 5′-d(AAAATATCAGATCTAAAA) (12 μM, 6 nCi), in 10 mM sodium phosphate pH 7 (lane 1) was alternatively incubated with Ni(salen)-biotin **2** (50 μM, lane 2), MMPP (120 μM, lane 3), and their combination (lane 4). Adduct formation was repeated on a preparative scale, and the product was isolated from a monomeric avidin affinity column for mass spectrometry. This material was also 5′-[^{32}P] labeled and analyzed before (lane 5) and after piperidine treatment (0.2 M, 90° C, 30 min, lane 6). A standard G-lane was generated by dimethyl sulfate as described by Maxam and Gilbert (lane 7).[14] As a control, the parent oligonucleotide was also treated with piperidine (0.2 M, 90° C, 30 min, lane 8).

N-hydroxysuccinimide ester of biotin (BNHS) produced the neutral and water-insoluble derivative **7**. The obligatory solubility in water was achieved through alkylation of the two free phenolic oxygens with 3-bromopropyltrimethylammonium bromide.[13] The desired perchlorate salt of **2** was precipitated by addition of $KClO_4$ and recrystallized from MeOH/Et_2O. Data from electrospray mass spectroscopy as well as ^{1}H and ^{13}C NMR were consistent with the desired product.

Incubation of a model oligonucleotide (18′mer, one central G) with the Ni(salen)-biotin conjugate **2** (50 μM) or the magnesium salt of monoperoxyphthalic acid (MMPP) (120 μM) in 10 mM sodium phosphate pH 7 generated no apparent products as detected by denaturing polyacrylamide gel electrophoresis (Figure 3, lanes 2 and 3). However, their combined presence produced a high molecular weight DNA adduct in approximately 60% yield (Figure 3, lane 4). This yield decreased to 5% when the ionic strength of the mixture was increased by addition of 100 mM NaCl. In either case, reaction remained specific for the sole guanine residue as indicated below. Biotinylated DNA generated on a preparative scale sufficient for mass spectrometry was conveniently separated from its parent oligonucleotide with a monomeric avidin matrix (Pierce) and desalted with reverse-phase (C-18) chromatography (Figure 3, lane 5). These same procedures can also be expected to function equivalently on an analytical scale. Treatment of the isolated adduct with hot piperidine was subsequently investigated since this condition had previously induced a diagnostic strand scission of adducts formed by NiTMAPES **1**.[5] The product formed by conjugate **2** demonstrated similar lability in the presence of hot piperidine. The resulting oligonucleotide fragment comigrated with the guanine standard produced by Maxam−Gilbert sequencing (Figure 3, lanes 6 and 7, respectively).[14] As expected, the parent oligonucleotide exhibited no sensitivity to piperidine (Figure 3, lane 8).

Composition of the isolated adduct was confirmed by mass spectral analysis. Electrospray mass spectrometry identified a major species of mass 6377 that is consistent with the oligonucleotide + Ni(salen) + oxygen. The additional oxygen was anticipated from MMPP-dependent oxidation of the biotin sulfide. Incubation of the unconjugated biotin under conditions analogous to the DNA experiments above led to formation of biotin α-sulfoxide as indicated by comparison to a standard produced by known reaction with H_2O_2.[15] This oxidation did not significantly interfere with avidin affinity chromatography since the α-sulfoxide product retains high affinity for avidin in contrast to the β-sulfoxide derivative.[16]

The ability of the nickel complex to deliver a biotin tag to accessible guanine residues should allow for future isolation and characterization of noncanonical structures in complex systems such as chromatin. Few alternatives are currently available that share both a dependable conformational specificity and an easy assimilation into the wide ranging and powerful techniques based on biotin recognition.

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Supporting Information Available: Synthesis and characterization of **4**−**7** and ESI-MS data for oligonucleotide adduct (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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