Nickel-Based Probes of Nucleic Acid Structure Bind to Guanine N7 but Do Not Perturb a Dynamic Equilibrium of Extrahelical Guanine Residues

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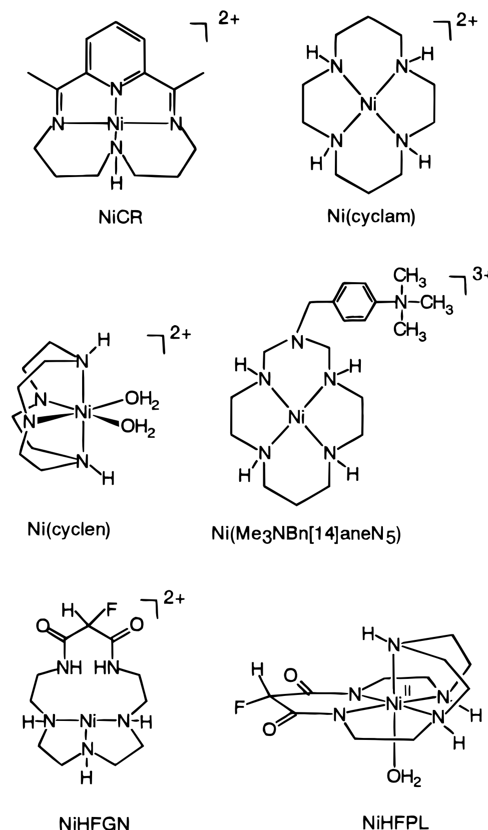
Abstract: Physical and chemical data are presented to resolve two important aspects of the mechanism and application of nucleic acid probes based on macrocyclic complexes of nickel. Direct coordination between nickel and guanine N7 had previously been suggested by the probes' sensitivity to the structural environment surrounding this nucleotide ligand. The first evidence for such recognition has now been obtained by a diagnostic ability of certain nickel complexes to convert poly(dG-dC) from a B- to Z-helix. Interaction between these complexes and 5′-GMP was also monitored by ^{1}H NMR and found to be characteristic of ligation between guanine N7 and nickel. Despite this mode of binding, the specificity of guanine oxidation reflects native conformations of DNA that are independent of nickel. To demonstrate this, the relative reactivity of each guanine forming an equilibrium of extrahelical bulges was shown here to mimic the distribution of species determined by previous NMR studies in the absence of nickel. Accordingly, nickel reagents may now be applied with confidence when a number of dynamic features of nucleic acid structure are examined.

Introduction

Chemical and enzymatic modification remains the most expedient method of identifying polynucleotide structure.[1] Conformation-specific reagents are readily available, broadly applicable, and easily employed. While crystallographic methods provide the greatest resolution, results from covalent modification have led to impressive structural details when combined with phylogenetic[2] and computational[3] techniques as illustrated by the recent elaboration of group I introns.[4] Such investigations require the use of a complementary set of reagents to detect solvent exposure of individual regions of the nucleobases and phosphoribose backbone. Ideally, data from a range of reagents may resolve any potential ambiguities intrinsic to a particular type or mechanism of modification. A number of valuable reagents such as hydroxyl radical, DNase, and RNase T1 yield direct cleavage of the backbone which is very convenient but may lead to changes in the native conformation during analysis. Similarly, reagents that associate with their targets prior to modification may also perturb the native conformation. The most pervasive concern with all of these structural probes is their ability to identify only the most reactive species. This may or may not correspond to the predominant species in solution. Although the guanine-specific reagent NiCR[5] appears to bind to its target, the reaction profile still accurately reflects the equilibrium distribution of guanine bulges in a well characterized oligodeoxynucleotide model as described below.



Previous investigations with tRNA^{Phe} provided a dramatic correlation between the solvent and electrostatic environment of guanine N7 and oxidation of this base in the presence of

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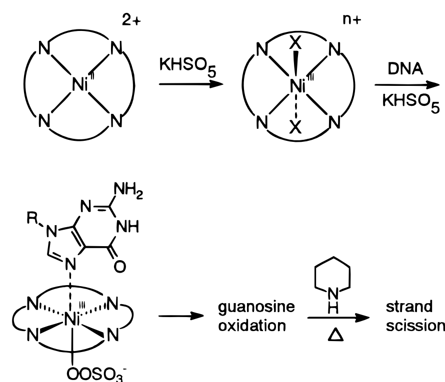
Scheme 1



NiCR and a peracid.[6] From these results and the work of others,[7] we postulated that a key intermediate containing a direct guanine N7−Ni^{III} bond was formed during reaction (Scheme 1).[5] The laboratory of Taillandier had previously demonstrated that Ni^{2+} salts preferentially coordinate to guanine N7[8] by infrared spectroscopy, but no analogous study had been attempted with the rather sterically encumbered macrocyclic complexes of nickel. The initial four-coordinate, square planar compounds such as NiCR and its derivatives were not expected to bind since they exhibited little propensity for acquiring axial ligands.[9] The transient Ni^{III} intermediates produced by an oxidant, however, should have high affinity for guanine N7 since these species strongly favor additional coordination by axial ligation.[9] Ni^{II} complexes should also exhibit an affinity for guanine N7 when their coordination properties mimic those of Ni^{III}CR. Such interactions with DNA have now been observed by a diagnostic conversion of poly(dG-dC) from a B- to Z-helical form, a technique previously used to detect equivalent interactions of simple metal salts including Ni^{2+}, Mn^{2+}, and Co^{2+}.[8,10] Direct association between nickel complexes and guanine have additionally been characterized by the paramagnetic effect of nickel on the ^{1}H NMR of 5′-GMP and by the oxidative selectivity of NiCR for a dynamic population of extrahelical guanine residues.

Results and Discussion

Recognition of Guanine N7 in DNA by Nickel Complexes. Guanine N7 is the preferred binding site for numerous transition metals,[7b] but often steric constraints limit direct coordination of this site within the major groove of B-helical DNA. Conversion of DNA from a B- to Z-helix relocates G N7 to the exterior of the helix and facilitates metal binding.[10] Consequently, metal ions such as Ni^{2+} promote formation of Z-DNA by stabilizing this structure relative to B-DNA.[8] Since the square planar complexes Ni^{II}CR and Ni^{II}(cyclam) have little tendency to coordinate additional ligands, these complexes were not expected to convert poly(dG-dC) from a B- to Z-form by interaction with G N7. However, such a conversion would still be possible if these complexes acted like Mg^{2+} to decrease the electrostatic repulsion within DNA or like $Co(NH_3)_6{}^{3+}$ to decrease this repulsion and supply extensive hydrogen bonding.[11]

Table 1. Certain Metal Salts and Complexes Induce Conversion of Poly(dG-dC) from a B- to Z-Helical Form As Indicated by $[\theta]_{255}$ and $[\theta]_{295}$[a]

species promoting convsn	transn midpt (μM)	inactive species	no convsn at limiting concn[b] (μM)
$NiCl_2$	63	$Ni^{II}CR(ClO_4)_2$	>96
$Ni^{III}(cyclam)Cl_2ClO_4$	25	$Ni^{II}(cyclam)(ClO_4)_2$	>190
$Ni^{II}HFGN(OAc)_2$	31	$Ni^{II}(Me_3NBn[14]\text{-}aneN_5)(ClO_4)_3$	>100
Ni^{II}HFPL	34	$Ni^{II}(cyclen)(H_2O)_2\text{-}(ClO_4)_2$	>300

[a] The molar ellipicity of poly(dG-dC) (100 μM of nucleotides) at 25 °C in 1 mM potassium phosphate pH 7 was measured as a function of metal concentration. Midpoint values are the average of three independent investigations, and the standard error is less than 10% for each determination. [b] Analysis above the indicated concentration was limited by precipitation.

Neither Ni^{II}CR nor Ni^{II}(cyclam) induced a B- to Z-helical transition as detected by circular dichroism (CD) under a range of conditions that were sufficient for a $NiCl_2$-dependent conversion (Table 1). The transient nature of the proposed intermediate, Ni^{III}CR, precluded its characterization with DNA, but Ni^{II}(cyclam), another square planar complex that oxidizes guanine residues with the same conformational specificity as NiCR,[9] formed a Ni^{III} octahedral derivative that was stable enough to examine.[12,13] This intermediate converted poly(dG-dC) from a B- to a Z-helix (Figure 1A) with greater efficiency than $NiCl_2$ (Table 1), and therefore, the macrocyclic ligand did not prevent direct guanine N7−nickel ligation.

The presence of a kinetically accessible coordination site within a nickel complex best predicted its ability to stabilize Z-DNA. In addition to $NiCl_2$ and Ni^{III}(cyclam), the macrocyclic complex Ni^{II}HFGN[14] (Figure 1B) and Ni^{II}HFPL[14] also induced formation of this alternative structure of DNA (Table 1). However, this is not a general property of octahedral geometry, net charge, or H-bonding of the complex. Ni^{II}(cyclen) forms an octahedral complex by coordinating two water molecules in a cis orientation that only slowly exchange with other ligands,[15] and hence, Ni^{II}(cyclen) was unable to stabilize Z-DNA (Table

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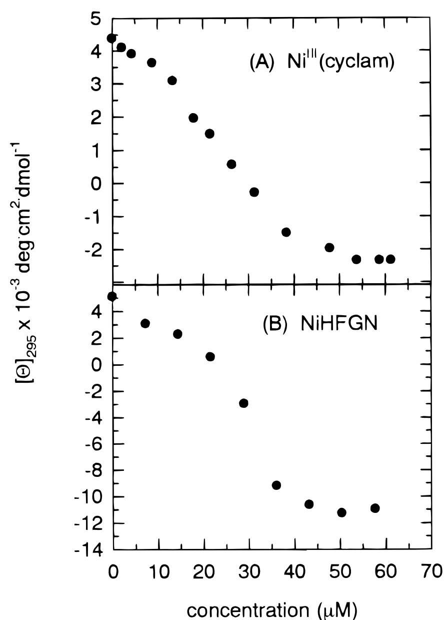

Figure 1. Sample titrations of poly(dG-dC) with (A) Ni^{III}(cyclam) and (B) Ni^{II}HFGN to monitor a B-to-Z-helical transition as indicated by molar ellipicity at 295 nm.

1). Although an increase in the formal charge of nickel may enhance its binding to DNA,[9] the B-to-Z transition is promoted by Ni^{III}(cyclam), Ni^{II}HFGN, and Ni^{II}HFPL with approximately equal efficiency. Similarly, addition of a cationic side chain to a cyclam-like macrocycle did not bestow the resulting nickel complex, Ni^{II}Me$_3$NBn[14]aneN$_5$, with an ability to effect the B-to-Z transition even though this new complex had a net charge of +3. Finally, both Ni^{II}(cyclam) and Ni^{III}(cyclam) provide nearly the same H-bonding capacity, but only the Ni^{III} derivative with a high affinity for axial ligands stabilized the Z-structure. Conformation-specific modification achieved by the active nickel complexes thus appears to rely on the dual function of the macrocyclic ligands to support recognition of accessible G N7 as well as to promote the redox activity of nickel.

Recognition of N7 in Purine Mononucleotides by Nickel Complexes. ^{1}H NMR was used to gain further evidence for direct interaction between nickel complexes and guanine. Octahedral nickel complexes are paramagnetic and perturb the chemical shift and relaxation of adjacent protons. These effects are distance dependent and were qualitatively used in this study to locate the nickel−nucleotide coordination. The ribose protons H2′−H5′ of 5′-GMP showed only a modest enhancement in relaxation as evident by a slight broadening of their resonances under increasing concentrations of NiHFPL (Figure 2). Additional broadening was detected for proton H1′. Most strikingly, the purine H8 signal was extremely sensitive to NiHFPL and exhibited significant relaxation and shifting in the presence of this complex. These results suggest that H8 and to a lesser degree H1′ are in close proximity to the paramagnetic nickel due to direct ligation between G N7 and NiHFPL. This model complex thus mimics the coordination of guanine and simple metal salts of Ni^{2+}.[7]

The general affinity for purine N7 derives from its basicity,[16] and consequently, purines with weakly basic N7 positions should bind nickel with lower efficiency. Signal broadening by NiHFPL within a series of purines demonstrates this trend (Figure 3). Guanine H8 is most readily perturbed by titration

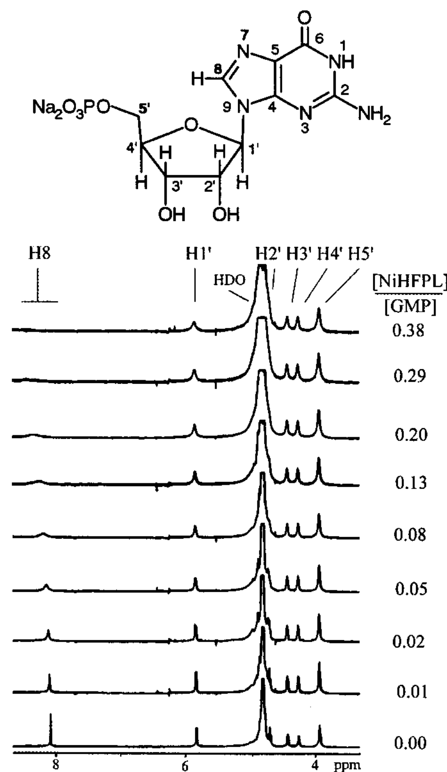

Figure 2. ^{1}H NMR spectra of 5′-GMP (50 mM, 25° C) were sequentially recorded after each addition of NiHFPL.

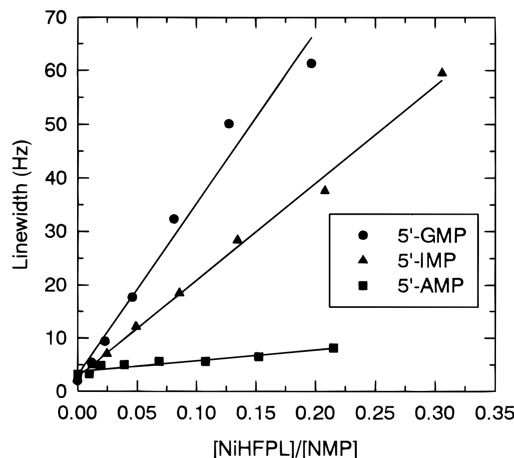

Figure 3. Effect of NiHFPL on the ^{1}H NMR resonance of H8 in various purine nucleotides (50 mM). The lines represent linear least-squares fits of the data.

with NiHFPL whereas inosine H8 and adenine H8 are successively less affected under equivalent conditions. This is consistent with the relative decrease in N7 basicity from guanine to inosine and inosine to adenine.[16]

Signal broadening of G H8 also helped to characterize association between 5′-GMP and the series of nickel derivatives used in the CD studies above. Square planar Ni^{II} complexes such as Ni^{II}CR and Ni^{II}(cyclam) displayed a negligible ability to interact with G N7 and affect the resonances of H8 as expected for species with minimal propensity to expand their coordination and become paramagnetic (Figure 4). These nickel complexes were also unable to promote conversion of poly(dG-dC) from a B- to Z-helix (Table 1). Ni^{III}(cyclam), Ni^{II}HFGN, Ni^{II}HFPL, and Ni^{2+} all maintain at least one exchangeable coordination site for binding to G N7, and all produce paramagnetic relaxation of G H8 (Figure 4). The relative

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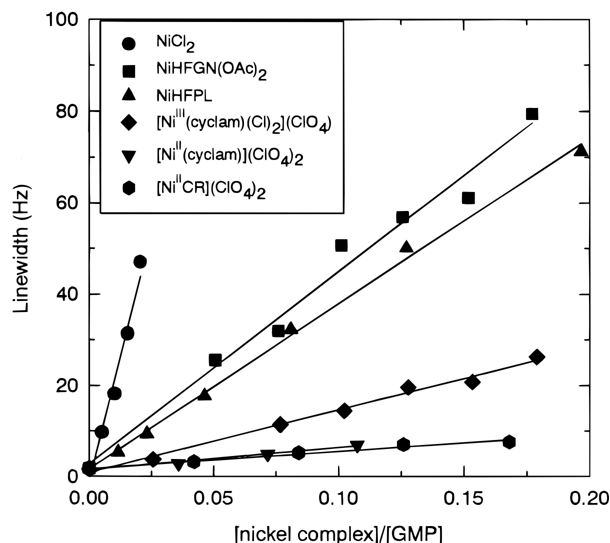
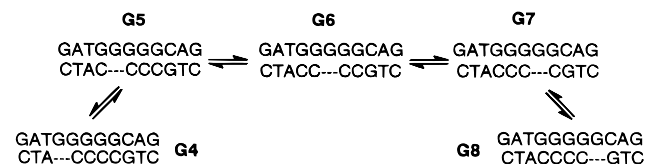


Figure 4. Effect of various nickel complexes on the line width of H8 in 5′-GMP (50 mM). The lines represent linear least-squares fits of the data.

Scheme 2



activity of these species, Ni^{2+} > Ni^{II}HFPL ≈ Ni^{II}HFGN > Ni^{III}-(cyclam), with 5′-GMP, however, does not parallel their ability to promote formation of Z-DNA. This latter process depends on coordination to G N7 and subsequent steric destabilization of B-DNA. When this transition is not possible, the steric constraints within the major groove of B-DNA likely restrict recognition and modification of guanine by most nickel complexes including the Ni^{III} intermediate illustrated in Scheme 1. Still, some conformational change might result from interaction between this intermediate and its nucleic acid target.

Specificity of Guanine Oxidation Reflects the Native Conformation of DNA. While the reagent NiCR had not appeared to affect polynucleotide folding, previous analysis had primarily focused on nucleotide sequences that formed unique and stable structures in solution.[5b] The low concentration of NiCR required for guanine oxidation (∼3 μM) might have avoided unanticipated perturbations of nucleic acid structure, although this reagent was never particularly suspect. The resting state of NiCR has little propensity for binding to guanine (Table 1, Figure 4). Instead, the transient Ni^{III} intermediate proposed to bind accessible G residues could shift the distribution of targets during oxidation. Similar ambiguities could arise if only a subset of Ni^{III}−DNA complexes were capable of adopting the necessary geometry for reaction. Either event would generate chemical modification data that misrepresent the native equilibrium of structures.

NiCR successfully characterized a dynamic population of extrahelical guanine residues despite the potential problems associated with direct probe−target interaction. The model system chosen for study readily forms an equilibrium of five structures differing in their unpaired guanine (Scheme 2) and has been the subject of prior NMR characterization by Woodson and Crothers.[17] Bulge migration was found to be fast relative to the NMR time scale, and the fractional concentration of each

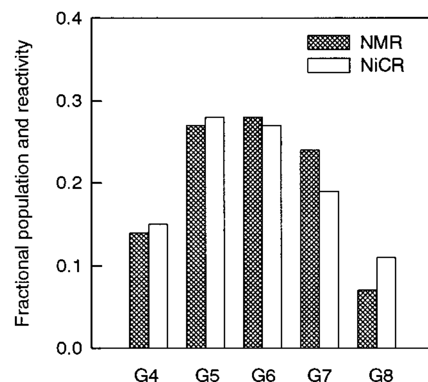


Figure 5. Fractional population of each extrahelical G structure as indicated by NMR[17] and modification by NiCR. The relative reactivity at each site was calculated with respect to the total reactivity of the central G_5-track and represents the average of nine determinations (standard error of less than 1.5%). Inspection of the NMR data published previously suggests an error of less than 5%.

species was determined by the observed chemical shift and T_1 relaxation of imino resonances[17] (Figure 5).

Reaction of this system with NiCR indicated partial exposure of the central G_5-track and full exposure of the 3′-terminal guanine. Most interestingly, the relative reactivity within the G_5-track mimicked the propensity of each guanine to form a bulged structure (G5 ≈ G6 > G7 > G4 > G8, Figure 5) as determined by NMR. DNA modification was not influenced by the ionization potentials of individual guanines which might have favored oxidation at the 5′-terminus of the guanine track stacked within a helix[18] or a bulge.[19] Finally, the pattern of reactivity illustrated in Figure 5 was not intrinsic to the central guanines since these residues were uniformly inert or reactive in the respective presence or absence of a fully complementary oligodeoxynucleotide.

Conclusion

The conformational specificity expressed by NiCR and a peracid originally implicated an intimate association between target nucleotides and the ultimate nickel-bound oxidant.[20] Formation of a transient G N7−Ni^{III}CR bond was also consistent with the ligand requirements for reaction[9] and the reagent's sensitivity to solvent exposure and the electrostatic environment of guanine N7.[6] This type of coordination has now been demonstrated more directly by the ability of certain macrocyclic complexes of nickel to promote formation of Z-DNA. The apparent ligation between nickel and G N7 further correlates with the paramagnetic effects of this metal on the ^{1}H NMR of G H8. Fortunately, association between the reactive nickel intermediate and accessible guanines does not detectably alter nucleic acid structure, and consequently the relative reactivity of each target may accurately identify the native distribution of conformers. Previous data based on NiCR modification of similar G-bulges in DNA[21] and RNA[22] may now also be regarded as diagnostic of their structural equilibrium. Conse-

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quently, NiCR and related probes should find considerable application in defining subtle features within dynamic structures.

Experimental Section

General Instrumentation and Methods. Circular dichroism (CD) spectra were measured with a Jasco J-20A spectropolarimeter at room temperature. NMR spectra were recorded on GE QE-300 MHz and Bruker AC-250 MHz spectrometers. UV−vis spectra were obtained on Perkin-Elmer λ-5 and Hewlett-Packard 8452A diode array spectrophotometers. Electrochemical data (cyclic voltammetry) were determined with a Bioanalytic Systems 100B electrochemical analyzer equipped with a glassy carbon or platinum working electrode, a platinum auxiliary electrode, and a Ag/AgCl reference electrode (all from Bioanalytic Systems).

Materials. Nucleotides and poly(dG-dC) were purchased from Sigma Chemical Co. and used without further purification. Tetraazamacrocyclic metal complexes, NiCR$(ClO_4)_2$,[23] Ni^{II}(cyclam)$(ClO_4)_2$,[24] Ni^{III}(cyclam)$(Cl)_2(ClO_4)$,[24] and Ni(cyclen)$(OH_2)_2](ClO_4)_2$,[25] were synthesized and precipitated as their perchlorate salts by Dr. James G. Muller. NiHFGN$(OAc)_2$ was prepared by Dr. Chien-Chung Cheng and converted to NiHFPL immediately before use by addition of 2.1 equiv of NaOH (or NaOD).[26] Nickel chloride, sodium chloride, potassium phosphate, and magnesium monoperoxyphthalic acid were used as received. All aqueous solutions were prepared using purified water (Nanopure, Sybron/Barnsted).

[3-(4-(Dimethylamino)benzyl)-1,3,5,8,12-pentaazacyclotetradecane]-nickel(II)Diperchlorate. A solution of Ni[*N*,*N*-di(2-aminoethyl)-1,3-propanediamine)]Cl_2 (1 mmol) and an equimolar amount of 4-(dimethylamino)benzylamine in methanol (5 mL) was stirred and warmed to 60 °C under nitrogen.[27,28] A solution of 37% aqueous formaldehyde (5 equiv) and triethylamine (2 mL) in methanol (1 mL) was added to the reaction mixture dropwise over a period of 2 h. The reaction was heated to 60−70 °C and stirred under nitrogen for 24 h. The resulting mixture was cooled to room temperature, filtered to eliminate the apple-green nickel hydroxide precipitate, and evaporated to remove methanol and trace amounts of water. Saturated $LiClO_4$ in methanol was slowly added to precipitate the nickel complex as a yellow-orange perchlorate salt (yield 10−30%): ^{1}H NMR (CD_3NO_2) δ 2.91 (m, 6H, CH_3), 3.80 (s, 2H, CH_2), 6.74 (d, 2H, Ph), 7.13 (d, 2H, Ph); λ_{max} (CH_3CN) = 446 nm; $E_{1/2}$ ($Ni^{III/II}$, CH_3CN) = 455 mV (ΔE_P = 72 mV).

[3-(4-(Trimethylammonio)benzyl)-1,3,5,8,12-pentaazacyclotetradecane]nickel(II) Triperchlorate [$Ni^{II}(Me_3NBn[14]aneN_5)(ClO_4)_3$]. A solution of the intermediate above (50 mg) and iodomethane (2 mL) in acetonitrile (5 mL) was sealed and stirred at room temperature for 24 h. The reaction mixture was filtered to obtain an orange solid product. The counterions of this product were exchanged to chlorides by dissolving the solid in water and passing the resulting solution through Dowex-1 anion-exchange resin (chloride form). Saturated $LiClO_4$ in methanol was then added to the eluant to precipitate the nickel complex as the yellow triperchlorate salt: ^{1}H NMR (CD_3NO_2) δ 3.70 (s, 9H, CH_3), 4.05 (d, 2H, CH_2), 7.62 (d, 2H, Ph), 7.82 (d, 2H, Ph); λ_{max} (H_2O) = 448 nm; $E_{1/2}$ ($Ni^{III/II}$, H_2O) = 519 mV (ΔE_P = 167 mV).

NMR Measurements. Nucleotides were dissolved in D_2O (99.9%) and lyophilized to dryness twice and then stored as 0.1 M stock solutions in D_2O. Each nickel complex was directly dissolved in D_2O just prior to use. Samples for NMR analysis (500 μL) contained 50 mM nucleotide and varying concentrations of nickel complexes. Line broadening of ^{1}H signals was evaluated by plotting the line width (Hz) at half-height ($\omega_{1/2}$) vs the molar ratio of nickel complex to nucleotide.

Oligodeoxynucleotide Oxidation by NiCR. Oligodeoxynucleotides containing the central -G_5- (3 μM, 5′-^{32}P-labeled) and the -C_4- (9 μM) were annealed in potassium phosphate (10 mM, pH 7) and NaCl (100 mM) by heating to 90 °C for 10 min followed by slow cooling to ambient temperature (3 h). Guanine oxidation was initiated by addition of NiCR (3 μM) and magnesium monoperoxyphthalic acid (100 μM) at 25 °C and quenched with Na_2SO_3 (0.8 mM) after 30 min. Samples were then treated with piperidine, characterized by electrophoresis and autoradiography, and quantified by densitometry under standard conditions.[20]

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Supporting Information Available: Autoradiogram of DNA fragments produced by reaction of NiCR (1 page, print/PDF). See any current masthead page for ordering information and Web access instructions.

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