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Paramagnetic ^{19}F NMR and Electrospray Ionization Mass Spectrometric Studies of Substituted Pyridine Complexes of Chromium(III): Models for Potential Use of ^{19}F NMR to Probe Cr(III)-Nucleotide Interaction¹

Nicholas R. Rhodes, Ken Belmore, Carolyn J. Cassady, and John B. Vincent*

Department of Chemistry and Coalition for Biomolecular Products, The University of Alabama, Tuscaloosa, AL 35487-0336, USA

Abstract

The synthesis and characterization of chromium basic carboxylate complexes, $[\text{Cr}_3(\text{O}_2\text{CR})_6\text{L}_3]^+$, containing trifluoroacetate, 3-fluoropyridine, 3-trifluoromethylpyridine, and 4-trifluoromethylpyridine are described. The substituted pyridine ligands are used as models of DNA bases to determine whether ^{19}F NMR would be a potentially useful probe of the binding of Cr^{3+} to DNA. The ^{19}F NMR resonances of the coordinated ligands, while broadened by delocalization of unpaired electron density from the $S=3/2$ chromic centers, are readily discernable, and the contact shifts are of sufficient magnitude that the signals from coordinated and free ligands can easily be differentiated. Thus, ^{19}F NMR appears to be a potentially useful probe of the binding of Cr^{3+} to DNA containing F-labeled bases. Additionally, electrospray MS is shown to be a convenient method to establish the identity of chromium basic carboxylate assemblies.

Keywords

Chromium(III); Paramagnetic NMR; Fluoropyridine; Trifluoromethylpyridine; Nucleotide

1. Introduction

The reduction of the carcinogen and mutagen chromate by cellular reductants results in the trapping of Cr(III) inside cells; a portion of this chromium is in the form of Cr–DNA adducts [1]. The significance of these adducts in the carcinogenic and mutagenic potential of chromate is currently highly debated [2]. At low concentrations, Cr(III) may bind preferentially to guanine-rich segments of DNA [3] and appears to bind primarily to guanine bases through N-7 and to PO_2 groups of the backbone of DNA as shown by difference FTIR spectra [4]. A more direct and sensitive spectroscopic probe of Cr(III)–guanine complexes and other potential Cr–DNA adducts would be highly desirable.

¹Dedicated to Prof. George Christou on the event of his 60th birthday.

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*Corresponding Author. Tel: +1 205 348 9203, fax: +1 205 348 9104, jvincent@bama.ua.edu (J. B. Vincent).

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Cr(III) complexes of guanosine monophosphate (GMP), diphosphate (GDP), and triphosphate (GTP) have been prepared previously [5–13]; however, they were generally characterized by elemental analysis, ultraviolet visible spectroscopy, infrared spectroscopy, and other techniques that provided little if any detailed structural information. They were not characterized by X-ray crystallography. ^{31}P NMR studies on GDP and GTP complexes have been reported [14]. Yet, because solutions of Cr(III) sources with large nucleotide to Cr ratios were examined, only broadening and relatively small chemical shift changes of free ligand signals were observed; ^{31}P signals from the Cr(III) complexes were not observed.

To interpret paramagnetic ^1H NMR spectra unambiguously, complexes whose three-dimensional structures are known are desirable. Consequently, basic chromic acetate assemblies, $[\text{Cr}_3\text{O}(\text{O}_2\text{CCH}_3)_6\text{L}_3]^+$ [15], have been utilized by this laboratory. The structures of several members of this family have been established by X-ray diffraction [16]; L can be a variety of monodentate ligands such as water or pyridine, and the paramagnetic ^1H and ^{13}C NMR spectra of several members have been assigned [16–18]. The monodentate ligands, being bound *trans* to the central triply bridging oxo ligand, are reasonably labile, presenting an available site for ligand exchange with heterocyclic ligands. For example, pyridine and imidazole derivatives can readily be prepared by exchange for terminal aqua ligands. Yet, ^1H and ^2H NMR studies of the imidazole derivatives revealed that these NMR techniques were unlikely to serve as useful probes of Cr-binding to DNA [19]. The only non-exchangeable hydrogen of guanine is bound to C-8, located between the nitrogens of the imidazole ring. Using imidazole derivatives as models, this hydrogen corresponds to the 2-position hydrogen of imidazole. The NMR studies of Cr(III) assemblies with imidazole terminal ligands revealed that the NMR resonance of this hydrogen resides in a region unlikely to be separated from other NMR resonances and that its signal was extremely broad ($\sim 1.4 \times 10^3$ Hz) [19]. Thus, the signal is unlikely to be detected at any reasonable concentration of DNA. However, the use of fluoro-substituted nucleotides and paramagnetic F NMR may yield a technique for probing the binding of Cr(III) to oligonucleotides and potentially DNA substituted with artificial F-labeled nucleotides. Herein are described attempts to synthesize basic acetate Cr(III) assemblies with fluoro- and trifluoromethyl-substituted pyridines as models of nucleotides and characterization of the complexes by paramagnetic ^{19}F NMR.

2. Experimental

2.1 Synthesis

All manipulations were performed under aerobic conditions, and all chemicals were used as received unless otherwise noted. Deionized water was utilized throughout. $[\text{Cr}_3\text{O}(\text{O}_2\text{CCH}_2\text{CH}_3)_6(\text{H}_2\text{O})_3]\text{NO}_3 \cdot \text{H}_2\text{O}$ was prepared by the method of Earnshaw, et al. [20]. $[\text{Cr}_3\text{O}(\text{O}_2\text{CCF}_3)_6(\text{H}_2\text{O})_3]\text{NO}_3 \cdot \text{H}_2\text{O}$ was prepared by the method of Glazunova, et al. [21].

2.2.1. General preparation of $[\text{Cr}_3\text{O}(\text{O}_2\text{CCH}_2\text{CH}_3)_6(\text{L})_3]\text{NO}_3$ —

$[\text{Cr}_3\text{O}(\text{O}_2\text{CCH}_2\text{CH}_3)_6(\text{H}_2\text{O})_3]\text{NO}_3 \cdot \text{H}_2\text{O}$ (0.25 g, 0.35 mmol) and ligand, L, (0.25 mL) were dissolved in 1 mL EtOH. Ligands, L, were pyridine (py), 3-fluoropyridine (3-F-py), 3-trifluoromethylpyridine (3-CF₃-py), and 4-trifluoromethylpyridine (4-CF₃-py). The solution was heated in a boiling water bath with stirring until the color changed from green to dark brown and allowed to cool. A gray solid was isolated by filtration and dried under vacuum. Ultraviolet/visible spectra in acetonitrile: λ , nm (ϵ , $\text{M}^{-1}\text{cm}^{-1}$): $[\text{Cr}_3\text{O}(\text{O}_2\text{CCF}_3)_6(\text{H}_2\text{O})_3]\text{NO}_3$: 354(sh), 447(34.2), 597(28.3), 679(11.2); $[\text{Cr}_3\text{O}(\text{O}_2\text{Cet})_6(3\text{-F-py})_3]\text{NO}_3$: 348(sh), 360(sh), 432(52.4), 566(35.2), 677(7.7); $[\text{Cr}_3\text{O}(\text{O}_2\text{Cet})_6(3\text{-CF}_3\text{-py})_3]\text{NO}_3$: 348(sh), 359(sh), 433(86.2), 561(29.6), 679(7.1); $[\text{Cr}_3\text{O}(\text{O}_2\text{Cet})_6(4\text{-CF}_3\text{-py})_3]\text{NO}_3$: 348(sh), 360(sh), 430(55.9), 569(38.1), 679(10.7). IR: cm^{-1} (intensity): $[\text{Cr}_3\text{O}(\text{O}_2\text{Cet})_6(3\text{-F-py})_3]\text{NO}_3$: 1617(vs), 1445(w), 1377(m), 1304(m), 1258(m), 1237(vs), 1189(s), 1099(s), 1081(w),

1055(m), 1017(s), 903(vs), 846(m), 822(w), 810(s), 696(vs), 647(m); $[\text{Cr}_3\text{O}(\text{O}_2\text{CEt})_6(3\text{-CF}_3\text{-py})_3]\text{NO}_3$: 1614(vs), 1473(s), 1442(s), 1333(m), 1306(w), 1136(m), 1089(m), 1052(vs), 938(w), 832(w), 812(w), 750(w), 705(m), 656(w); $[\text{Cr}_3\text{O}(\text{O}_2\text{CEt})_6(4\text{-CF}_3\text{-py})_3]\text{NO}_3$: 1615(vs), 1477(s), 1440(m), 1356(m), 1321(vs), 1215(m), 1181(m), 1145(s), 1089(m), 1061(s), 1029(m), 846(w), 837(m), 678(s); $[\text{Cr}_3\text{O}(\text{O}_2\text{CCF}_3)_6(\text{H}_2\text{O})_3]\text{NO}_3$: 1701(vs), 1604(w), 1496(vs), 1366(vs), 1241(m), 1201(vs), 1165(m), 1039(m), 868(w), 789(vs), 735(vs), 661(s), 622(w).

2.2 Electrospray Ionization/Quadrupole Ion Trap (ESI/QIT) Mass spectrometry (MS)

ESI mass spectra were obtained on a Bruker (Billerica, MA, USA) HCT Ultra high capacity ion trap mass spectrometer. The samples were dissolved in acetonitrile and diluted in a 1:1 solution of acetonitrile and water before analysis. The ESI source uses a drying gas of nitrogen, which was heated to 300 °C. Collision-induced dissociation (CID) was performed in the ion trap using helium as the collision gas. Collision energies were in the low energy range from 0–100 eV.

2.3 NMR Spectroscopy

^1H and ^{19}F NMR spectra were obtained using a Bruker AV-360 spectrometer at circa 23°C. Chemical shifts are on the δ scale (shifts downfield are positive) using solvent or free ligand protio- or fluoro-component signals as references. ^{19}F chemical shifts are referenced against Freon-11.

2.3 Other methods

UV/visible spectra were obtained using a Hewlett Packard 8453 spectrophotometer. IR spectra were collected with a Jasco FT/IR-4100 as mineral oil mulls or KBr disks.

3. Results and discussion

3.1 Synthesis

The central $[\text{Cr}_3\text{O}(\text{O}_2\text{CR})_6]^+$ triangle of basic carboxylate type assemblies (Figure 1) provides an excellent platform on which to add monodentate ligands. This triangular core possesses an available coordination site *trans* to the oxo group on each Cr(III) center. The Cr–O(oxo) bond is short and strong such that bonds from ligands *trans* to the oxo groups are unusually labile for chromium(III) complexes. The terminal ligands are usually water molecules, but the water can readily be substituted with pyridine, imidazole, or other nitrogen heterocyclic molecules, for example [15–19]. Notably, imidazole and benzimidazole complexes, $[\text{Cr}_3\text{O}(\text{O}_2\text{CR})_6\text{L}_3]^+$ where L=imidazole or benzimidazole and R is an alkyl group, can readily be prepared in this manner in good yield; however, they have limited solubility in common NMR solvents [19]. Similar complexes starting from the benzoate analogue $[\text{Cr}_3\text{O}(\text{O}_2\text{CPh})_6(\text{H}_2\text{O})_3]^+$ can readily be prepared in good yield in the same manner; however, they are even less soluble. Unfortunately, guanine and other DNA bases and other potential N-heterocyclic ligands which more closely approximate guanine than imidazole or benzimidazole (purine, xanthine, and hypoxanthine) do not readily form isolable chromium complexes of the desired type [19]. Given the solubility problems with the trinuclear imidazole and benzimidazole complexes, pyridine was chosen as the heterocyclic terminal ligands to base these studies on.

The nitrate salts of the cations $[\text{Cr}_3\text{O}(\text{O}_2\text{CEt})_6\text{L}_3]^+$ where L=pyridine, 3-fluoropyridine, 3-trifluoromethylpyridine, and 4-trifluoromethylpyridine were all readily prepared by adding an excess of the pyridine to an ethanolic solution of $[\text{Cr}_3\text{O}(\text{O}_2\text{CEt})_6(\text{H}_2\text{O})_3]^+$ followed by gentle heating to produce a color change from green to brown; the products, $[\text{Cr}_3\text{O}(\text{O}_2\text{CCH}_2\text{CH}_3)_6(\text{L})_3]\text{NO}_3$ where L=pyridine, 3-fluoropyridine, 3-

trifluoromethylpyridine, and 4-trifluoromethylpyridine, precipitated upon cooling. This procedure had previously been shown to lead to substitution of the terminal water ligands of the acetate analogue $[\text{Cr}_3\text{O}(\text{O}_2\text{CMe})_6(\text{H}_2\text{O})_3]^+$ with pyridine and 3-methylpyridine [22].

The synthesis of the derivatives with 2-fluoropyridine, 2-trifluoromethylpyridine, and 2,4,6-trifluoropyridine was unsuccessful using this method. Unsuccessful efforts to synthesize the compounds also included the use of higher boiling solvents than ethanol or the use of the potential pyridine-based ligands as solvents. The highly electronegative ligands in the 2-position apparently reduce the Lewis basicity of the pyridine nitrogen such that these pyridine derivatives will not bind to the chromic centers. The successful preparation of chromium basic carboxylate complexes containing 2-methylpyridine [16] eliminates a steric problem from preventing binding, at least for 2-fluoropyridine.

3.2 Ultraviolet/visible Spectroscopy

The visible spectra of pseudo-octahedral chromium(III) complexes are expected to display two dominant bands corresponding to the $^4\text{A}_{2g} \rightarrow ^4\text{T}_{2g}$ and $^4\text{A}_{2g} \rightarrow ^4\text{T}_{1g}(\text{F})$ transitions. The chromium(III) basic carboxylates are not exceptions with these bands at approximately 560–600 nm and 430–450 nm, respectively [18]. The complexes with substituted pyridine terminal ligands display these two bands in their visible spectra at 561–569 nm and 430–433 nm, respectively. The complex with bridging trifluoroacetate ligands displays these bands at 447 and 597 nm. A third spin-allowed d-d transition is expected in the ultraviolet region for pseudo-octahedral chromium(III) complexes but cannot be resolved from the ligand π -transitions. A series of weaker transitions at lower energy corresponding to formally spin-forbidden transitions for pseudo-octahedral chromium(III) complexes result in weak bands normally between approximately 660 and 730; in the antiferromagnetically coupled basic carboxylates, the transitions gain some allowed character and increase in intensity. These most intense of these are observed for all the complexes described in this work between 675 and 680 nm with the increased molar absorptivity expected for basic carboxylate assemblies. This provides evidence that these complexes retain their structure in solution, as also indicated by mass spectral studies (vide infra).

3.3 ESI MS

ESI MS provides a gentle method of transferring pre-existing ions from the solution to the gas phase and of generating gas-phase ions. Basic chromium carboxylates have been examined previously by ESI MS [23–25]. Under “soft” low voltage conditions, the molecular ion is the most intense feature in the spectra, although minor features corresponding to loss of terminal ligands or exchange of terminal ligands with solvent molecules can be observed [23–25]. Because increasing the voltage at the first skimmer electrode results in more collisions of species of interest with solvent molecules within the ion source, higher voltages result in loss of more of the terminal ligands. The voltage can be increased until the core $[\text{Cr}_3\text{O}(\text{O}_2\text{CR})_6]^+$ cation gives rise to the most intense feature in the spectrum [23]. Fragmentation of the molecular ion can be generated using collision-induced dissociation at sufficient energy [23].

The results of ESI MS experiments on the nitrate salts of $[\text{Cr}_3\text{O}(\text{O}_2\text{Cet})_6(\text{py})_3]^+$ and the newly prepared $[\text{Cr}_3\text{O}(\text{O}_2\text{Cet})_6(3\text{-F-py})_3]^+$, $[\text{Cr}_3\text{O}(\text{O}_2\text{Cet})_6(3\text{-CF}_3\text{-py})_3]^+$, and $[\text{Cr}_3\text{O}(\text{O}_2\text{Cet})_6(4\text{-CF}_3\text{-py})_3]^+$ revealed that the spectra were dominated by the signals from the molecular ions although signals from loss of one or two terminal ligands could also be observed (Table 1). [Broad, low intensity signals from the addition of water to some fragments of the molecular ions can also be observed (data not shown).] The identity of the cations, and thus their successful synthesis, can readily be confirmed by comparison of the isotopic distribution patterns of the molecular cation signals (Figures 2, S1–S3). CID ESI

MS generally was found to simply lead to loss of terminal ligands, which provided additional confirmation of the structures.. For example, the CID ESI MS of the parent ion of $[\text{Cr}_3\text{O}(\text{O}_2\text{Cet})_6(\text{py})_3]^+$ (m/z 847.2) gave a spectrum with the most intense signal at m/z 768.1, corresponding to $[\text{Cr}_3\text{O}(\text{O}_2\text{Cet})_6(\text{py})_2]^+$. The successful synthesis of $[\text{Cr}_3\text{O}(\text{O}_2\text{CCF}_3)_6(\text{H}_2\text{O})_3]^+$ was also confirmed by the isotopic distribution of its molecular ion signals (Figure S3).

3.4 NMR Spectroscopy

Cr(III) complexes have historically not been characterized by NMR studies as the chromic ion has an electronic spin of $3/2$. For such a d^3 ion, zero field splittings are small with the result that the electronic spin-lattice relaxation time, T_1 , is very long; this gives poorly resolved NMR spectra. Thus, NMR spectra of Cr(III) complexes display greatly broadened and shifted resonances, particularly for the atoms of aromatic ligands where the half-occupied t_{2g} orbitals are oriented at the pi clouds of the ligands. Thus, knowledge of the structure of the complex is often required to interpret the spectra, rather than the reverse. Yet, paramagnetic ^1H , ^2H , and ^{13}C NMR have been demonstrated to be of utility in characterizing Cr(III) carboxylate assemblies [16–18].

The trinuclear oxo-centered carboxylate assemblies in this study possess the “basic carboxylate” type structure, where the three metal centers and the μ_3 -oxo are coplanar. Each pair of chromic ions is bridged by two carboxylate ligand. Coordination about each octahedral chromic ion is completed by a terminal ligands, either water or a pyridine based ligand. Thus, assuming free rotation about the Cr-N(pyridine) or Cr-O(water) bonds, each trinuclear cation has three-fold symmetry so that all the chromium centers and similarly the terminal ligands are equivalent; this simplifies interpretation of the NMR spectra. Also, the t_{2g} electronic configuration of the chromic centers is magnetically isotropic or nearly so dipolar shifts should be negligible; hence, isotropic shifts should be essentially only contact in origin, also simplifying interpretation of the NMR spectra. The chromium centers in these trinuclear assemblies are antiferromagnetically coupled, resulting in a reduction of the magnetic moment per Cr(III) ion (e.g., μ_{Cr} for $[\text{Cr}_3\text{O}(\text{O}_2\text{CMe})_6(\text{py})_3]^+$ is $3.14 \mu_{\text{B}}$ vs. $\sim 3.87 \mu_{\text{B}}$ for mononuclear species); thus, the paramagnetic shifts and broadening of the resonances should be slightly reduced in comparison to NMR spectra of mononuclear Cr(III) complexes.

3.3.1 $[\text{Cr}_3\text{O}(\text{O}_2\text{CCF}_3)_6(\text{H}_2\text{O})_3]^+$ —For bridging carboxylate ligands where the metals and carboxylates are co-planar, two mechanisms for spin delocalization are expected: σ and π , with the π -pathway expected to predominate for atoms more removed from the metal center [26]. For chromic ions, only the σ -pathway is expected to be significant, as observed previously for basic chromic carboxylates [16]. The ^{19}F NMR spectrum of $[\text{Cr}_3\text{O}(\text{O}_2\text{CCF}_3)_6(\text{H}_2\text{O})_3]^+$ (Figure 3) reveals one prominent signal at -70.8 ppm, shifted appreciably downfield (by ~ 5 ppm) from the signal arising from a trace quantity of the uncoordinated acid. The contact shift (~ 5 ppm) is smaller in magnitude to that of the corresponding methyl hydrogen in $[\text{Cr}_3\text{O}(\text{O}_2\text{CCH}_3)_6(\text{H}_2\text{O})_3]^+$ compared to free acetic acid of $\sim +34$ ppm (table 2). This suggests the mechanism of spin delocalization is not identical in both cations; the degree of hyperconjugation in the trifluoroacetate must be considerably greater than for acetate. The signal from the paramagnetic cation is dramatically broadened compared to that of the free acid (width at half height >200 times broader for the former), although both are broadened by the presence of the paramagnet in solution. This broadening of the ^{19}F NMR signals is in accord with that of the ^1H NMR signals of the acetate analogue; in this latter case, the paramagnetically broadened signal would be most difficult to detect in dilute solutions [16].

3.3.2 $[\text{Cr}_3\text{O}(\text{O}_2\text{CEt})_6(3\text{-F-py})_3]^+$ —The ^{19}F NMR spectrum of $[\text{Cr}_3\text{O}(\text{O}_2\text{CEt})_6(3\text{-F-py})_3]^+$ (Figure 4) reveals one prominent signal at -150 ppm, shifted appreciably upfield (by ~ -21 ppm) from the signal arising from a trace quantity of the uncoordinated pyridine ligand. (The detection of trace quantities of free ligands is common in the NMR spectra of Cr(III) complexes. While the height of the signals at first glance might appear to suggest a significant amount of free ligand may be present, integration of the sharp signals compared to those of the broad signals from the coordinated ligands (to the extent they can be compared with such different relaxation times) indicates the amounts are not significant. For the pyridine ligands, the trace of ligand may arise from impurities in the sample and/or a very small degree of dissociation in solution.) The contact shift (~ -21 ppm) is greater in magnitude and opposite in sign to that of the corresponding pyridine hydrogen in $[\text{Cr}_3\text{O}(\text{O}_2\text{CMe})_6(\text{py})_3]^+$ (table 2). Thus, the contact shift mechanism must be significantly different for the pyridine ring F versus H. Previous ^{19}F NMR studies with fluoroaromatic ligands have observed similar differences between the shifts of fluorine and hydrogen atoms in equivalent positions [27]; these changes have been attributed to participation by the fluorine in conjugation in the fluoroaromatics, a mechanism not available to the corresponding H atom. The magnitude of the contact shift makes the signal readily discernable from the free ligand. Additionally, the coordinated ligand resonance is broadened by a factor of approximately five-fold compared to that of the free ligand. Despite the broadening, the signal is readily observed. Thus, between the contact shift and paramagnetic broadening, the coordinated ligand signal can readily be observed and distinguished from that of the free ligand, making F-substitution in the equivalent position of nucleotides potentially interesting probes of chromium-coordination to oligonucleotides.

Substitution of a hydrogen with fluorine has little effect on the chemical shifts of the hydrogen resonances in the compound's ^1H NMR spectrum (Table 3). For pyridine ligands of chromium basic carboxylate complexes, the 2-position hydrogens or deuterons have signals at -40 to -45 ppm, the 3-position hydrogen resonances are at $+10$ – $+11$ ppm, and the 4-position resonances are at approximately -14 ppm [16]. The chemical shifts of all the proton signals of the 3-F-py complex are very similar to those of the pyridine complex; the 4-position is found at -12.1 ppm and the 2- and 6-positions at approximately -44.1 ppm. The 2-position and 6-position resonances are broad and overlap; ^2H NMR is normally required to resolve these resonances [16]. The 5-H position hydrogen signal at $\sim +8$ is shifted slightly compared to those of unsubstituted pyridine complexes; this chemical shift of this signal is difficult to determine precisely as it is overlain by the signals from the hydrogens of traces of free 3-F-py.

3.3.3 $[\text{Cr}_3\text{O}(\text{O}_2\text{CEt})_6(3\text{-CF}_3\text{-py})_3]^+$ and $[\text{Cr}_3\text{O}(\text{O}_2\text{CEt})_6(4\text{-CF}_3\text{-py})_3]^+$ —The ^{19}F NMR spectra of $[\text{Cr}_3\text{O}(\text{O}_2\text{CEt})_6(3\text{-CF}_3\text{-py})_3]^+$ (Figure 5) and $[\text{Cr}_3\text{O}(\text{O}_2\text{CEt})_6(4\text{-CF}_3\text{-py})_3]^+$ (Figure 6) each reveal one prominent signal at -73 ppm and -44 ppm, respectively, shifted appreciably (upfield by ~ -8 ppm and downfield by ~ 24 ppm, respectively) from the signals arising from trace quantities of the uncoordinated pyridine ligands. The contact shifts (Table 2) are essentially identical to those of the equivalent hydrogen in the non-fluorinated analogues, indicating an identical mechanism for the delocalization of the unpaired electron density. Similar effects have been observed previously for aromatic ligands with CF_3 substituents [28,29]. The coordinated signals are broadened (based on width at half height) compared to the free ligand signals by approximately 1/3 and 2-fold, respectively. CF_3 substitution at equivalent positions of nucleotides could also provide interesting probes for chromium binding to oligonucleotides. However, the larger shift and greater broadening suggest from a spectroscopic perspective that the 4-position substitution might generate better probes. Again, the replacement of a hydrogen on the pyridine ring by a trifluoromethyl group has little effect on the proton resonances in the compounds' ^1H NMR spectra, as seen in the spectrum of the 3-F-py complex (Table 3).

4. Conclusion

^{19}F NMR has been used previously by inorganic chemists to probe the structure of paramagnetic metal centers in proteins [30–32], although the technique has not been applied to chromium. Chromium basic carboxylate complexes provide a useful scaffold to attach a variety of N-containing heterocyclic ligands as models of the bases of DNA, including fluorine- or trifluoromethyl-substituted pyridine molecules. The ^{19}F NMR spectra of the trinuclear chromic complexes of 3-fluoropyridine, 3-trifluoromethylpyridine, and 4-trifluoromethylpyridine possess readily discernible resonances from the coordinated ligands, although broadened by delocalization of electron density from the chromic centers onto the ligands, and the contact shifts are of sufficient magnitude that the signals from coordinated and free ligands can easily be differentiated. As these antiferromagnetically coupled trinuclear complexes give rise to contact shifts of sufficient magnitude, the probably mononuclear sites of Cr(III) bound to DNA should give rise to slightly greater contact shifts and broadening and thus even more readily differentiated signals. Thus, ^{19}F NMR appears to be a potentially useful probe of the binding of Cr(III) to DNA containing F-labeled bases. Electrospray MS has been shown as a convenient method to establish the identity of chromium basic carboxylate assemblies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version.

Chromium basic carboxylate complexes containing trifluoroacetate, 3-fluoropyridine, 3-trifluoromethylpyridine, and 4-trifluoromethylpyridine ligands are used as models of DNA bases to demonstrate that ^{19}F NMR could be a potentially useful probe of the binding of Cr^{3+} to DNA containing F-labeled bases. Additionally, electrospray MS is shown to be a convenient method to establish the identity of chromium basic carboxylate assemblies.

Figure 1.
Structure of basic carboxylate cations of the general type $[\text{Cr}_3\text{O}(\text{O}_2\text{CR})_6(\text{L})_3]^+$.

Figure 2. Calculated (black bars) and experimental (gray bars) isotopic distributions for the molecular ion in the ESI MS spectrum of $[\text{Cr}_3\text{O}(\text{O}_2\text{CCH}_2\text{CH}_3)_6(\text{py})_3]^+$.

Figure 3.
 ^{19}F NMR of $[\text{Cr}_3\text{O}(\text{O}_2\text{CCF}_3)_6(\text{H}_2\text{O})_3]^+$ in acetonitrile. * - free HO_2CCF_3 .

Figure 4.
 ^{19}F NMR of $[\text{Cr}_3\text{O}(\text{O}_2\text{CEt})_6(3\text{-F-py})_3]^+$ in acetonitrile. * - free 3-F-py.

Figure 5.
 ^{19}F NMR of $[\text{Cr}_3\text{O}(\text{O}_2\text{CCH}_2\text{CH}_3)_6(3\text{-CF}_3\text{-py})_3]^+$ in acetonitrile. * - free 3- CF_3 -py.

Figure 6.
 ^{19}F NMR of $[\text{Cr}_3\text{O}(\text{O}_2\text{CCH}_2\text{CH}_3)_6(4\text{-CF}_3\text{-py})_3]^+$ in acetonitrile. * - free 4- $\text{CF}_3\text{-py}$.

Table 1

ESI MS data for trinuclear Cr(III) assemblies.

a) [Cr₃O(O₂CEt)₆(py)₃]⁺		
<u>Assignment</u>	<u>m/z</u>	<u>Relative Intensity</u>
[Cr ₃ O(O ₂ CEt) ₆ (py) ₃] ⁺	847.2	100%
[Cr ₃ O(O ₂ CEt) ₆ (py) ₂] ⁺	768.1	4%

b) [Cr₃O(O₂CEt)₆(3-F-py)₃]⁺		
<u>Assignment</u>	<u>m/z</u>	<u>Relative Intensity</u>
[Cr ₃ O(O ₂ CEt) ₆ (3-F-py) ₃] ⁺	901.1	100%
[Cr ₃ O(O ₂ CEt) ₆ (3-F-py) ₂] ⁺	804.1	53%
[Cr ₃ O(O ₂ CEt) ₆ (3-F-py)] ⁺	707.0	2%

c) [Cr₃O(O₂CEt)₆(3-CF₃-py)₃]⁺		
<u>Assignment</u>	<u>m/z</u>	<u>Relative Intensity</u>
[Cr ₃ O(O ₂ CEt) ₆ (3-CF ₃ -py) ₃] ⁺	1051.1	100%
[Cr ₃ O(O ₂ CEt) ₆ (3-CF ₃ -py) ₂] ⁺	904.2	19%

d) [Cr₃O(O₂CEt)₆(4-CF₃-py)₃]⁺		
<u>Assignment</u>	<u>m/z</u>	<u>Relative Intensity</u>
[Cr ₃ O(O ₂ CEt) ₆ (4-CF ₃ -py) ₃] ⁺	1051.1	100%
[Cr ₃ O(O ₂ CEt) ₆ (4-CF ₃ -py) ₂] ⁺	904.2	12%

e) [Cr₃O(O₂CCF₃)₆(H₂O)₃]⁺		
<u>Assignment</u>	<u>m/z</u>	<u>Relative Intensity</u>
[Cr ₃ O(O ₂ CCF ₃) ₆ (H ₂ O) ₃] ⁺	903.7	100%

Table 2

Chemical shifts and contact shifts for trinuclear chromium(III) complexes. Nucleus of interest is in bold and italics.

¹⁹ F				
<u>Cation</u>	–	<u>Free Ligand</u>	<u>Contact Shift</u>	<u>Ref.</u>
[Cr ₃ O(O ₂ CCF ₃) ₆ (H ₂ O) ₃] ⁺	–70.8	–76.2	+5.4	This work
[Cr ₃ O(O ₂ CEt) ₆ (3- F -py) ₃] ⁺	–150	–129	–21	This work
[Cr ₃ O(O ₂ CEt) ₆ (3- CF ₃ -py) ₃] ⁺	–73	–65	–8	This work
[Cr ₃ O(O ₂ CEt) ₆ (4- CF ₃ -py) ₂] ⁺	–44	–68	+24	This work
¹ H				
<u>Cation</u>	–	<u>Free Ligand</u>	<u>Contact Shift</u>	<u>Ref.</u>
[Cr ₃ O(O ₂ CCH ₃) ₆ (H ₂ O) ₃] ⁺	+36.2	+2.1	+34.1	16
[Cr ₃ O(O ₂ CMe) ₆ (py) ₃] ⁺ (py 3- H)	+11.2	+7.1	+4.1	16
[Cr ₃ O(O ₂ Me) ₆ (3- CH ₃ -py) ₃] ⁺	–6.4	~+2	~–8	16
[Cr ₃ O(O ₂ Me) ₆ (4- CH ₃ -py) ₃] ⁺	–26.6	~+2	~+24	16

Table 3Chemical shifts of ^1H NMR spectra of trinuclear chromium complexes.

Species	H	Chemical shift (ppm)
$[\text{Cr}_3\text{O}(\text{O}_2\text{CEt})_6(3\text{-F-py})_3]^+$	$\text{O}_2\text{CCH}_2\text{CH}_3$	+48.2
	$\text{O}_2\text{CCH}_2\text{CH}_3$	+0.33
	4-H	-12.1
	py 5-H	~+8.0
	2-H, 6-H	-44.1
$[\text{Cr}_3\text{O}(\text{O}_2\text{CEt})_6(3\text{-CF}_3\text{-py})_3]^+$	$\text{O}_2\text{CCH}_2\text{CH}_3$	+50.1
	$\text{O}_2\text{CCH}_2\text{CH}_3$	+0.44
	4-H	-12.0
	py 5-H	+12.0
	2-H, 6-H	-42.6
$[\text{Cr}_3\text{O}(\text{O}_2\text{CEt})_6(4\text{-CF}_3\text{-py})_3]^+$	$\text{O}_2\text{CCH}_2\text{CH}_3$	+48.1
	$\text{O}_2\text{CCH}_2\text{CH}_3$	+0.60
	py 3-H	+13.5
	2-H, 6-H	-42.8