Table II. Equilibrium Data for Migratory Insertion Provesses

L	T, °C	K_+/K_0	10^2K_+, M^{-1}	K_0, M^{-1}
$(i\text{-PrO})_3P$	0[a]	4×10^{12}	5.2 ± 0.6	1.3×10^{-10}
Ph_3P	0[b]	2×10^{11}	0.23 ± 0.02	1×10^{-10}
	−12[b]		0.78 ± 0.22	
	−22.5[a]		2.4 ± 0.3	

[a] [AN] = 3–30 mM in acetone/0.10 M TBAP, [Fe] = 1.0 mM. [b] [AN] = 10–100 mM, [Fe] = 3.0 mM.

scan rate is competitive with the alkyl–acyl (**3** → **4**) equilibration rate. For example, a *decrease* in the scan rate for **1a** or **1b** *increased* the relative cathodic peak current for wave II and *decreased* the peak current of the acyl cathodic wave III (Figure 1). Increasing the temperature had the same effect. It must be noted that the shape of the cathodic wave II does not conform to Cottrell behavior. Rather the current decay between waves II and III is substantially slower and depends on the concentration of AN, implying coupled homogeneous kinetics.

The utilization of the recently developed technique of reverse pulse voltammetry[9] has permitted the direct determination of K_+ for the phosphite, **1a**, and phosphine, **1b**, derivatives. In this method, the potential at a planar Pt electrode, dipped into an acetone solution of AN and **1a** or **1b**, was stepped for 8 s to 150 mV above $(E_a + E_c)/2$ for the alkyl couple. This was sufficient time to oxidize the complex near the electrode surface and for the alkyl–acyl equilibrium to be established.[10] A pulsed scan toward negative potentials gave two clearly defined waves at potentials corresponding to the reduction of the alkyl and acyl cation radicals. On the basis of the assumption that the diffusion coefficients of the alkyl and acyl cation radicals are the same, the plateau currents were used to determine the concentration ratios and thus the equilibrium constants, K_+, for a range of AN concentrations. The data in Table II show that the K_+ values at 0 °C parallel the electron-withdrawing power and a decrease in steric requirements of the ancillary ligands.

From the temperature dependence of K_+ for the equilibrium between **3b** and **4b**, $\Delta H°$ and $\Delta S°$ were computed to be −15 ± 2 kcal/mol and −50 ± 10 eu, respectively. A large negative entropy change seems to be characteristic of the carbon monoxide insertion reaction, although thermodynamic information is limited.[11]

The thermodynamic enhancements (K_+/K_0 in Table II) of the migratory insertion that occur on oxidation were readily computed for the alkyl and acyl redox processes via the expression

$$\ln (K_+/K_0) = (nF/2RT)[(E_a + E_c)_{alkyl} - (E_a + E_c)_{acyl}]$$

Data were obtained in acetone for the alkyl complexes and in AN for the acyl complexes. Medium corrections were made by comparison to the ferrocene couple. The ratios of 10^{11}–10^{12} correspond to a free energy promotion of 14–15 kcal/mol (0 °C) for the Fe(III) state over the Fe(II) state for the migratory insertion. While K_0 cannot be determined directly because of its intrinsically low value, the thermodynamic cycle of Scheme I permits its calculation whenever K_+ and electrochemical information are available (Table II).

The changes in the equilibrium positions on oxidation appear to be associated with changes principally in the forward dynamics rather than in the reverse dynamics. Predicated on the scan rates necessary to detect the anodic waves IV for phosphite and phosphine complexes in neat AN (vide supra), we estimate half-lives for the conversion of the neutral acyl to alkyl complexes to be on the order of 10^{-1} s at 0 °C. Hence the forward pseudo-first-order rate constant $k_f°$ under these conditions is estimated to be about 10^{-11} s^{-1}.[12] For the oxidation step no cathodic wave II was observed for either of the phosphorous derivatives at scan rates up to 20 V s^{-1}, thereby placing lower bounds on the observed k_f^+ of 10^2 s^{-1}.

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Registry No. 1a, 83006-06-8; **1b**, 12100-51-5; **1c**, 12080-06-7; **2a**, 83006-07-9; **2b**, 83006-08-0; **2c**, 83006-09-1; **3a**, 83006-10-4; **3b**, 83006-11-5; **3c**, 83006-12-6; **4a**, 83006-13-7; **4b**, 83006-14-8; **4c**, 75778-51-7; Fc, 102-54-5; acetonitrile, 75-05-8.

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(10) A PAR Model 174A polarograph, with an intrinsic pulse width of 57 ms and current sampling during the last 17 ms, was used at a scan rate of 20 mV s^{-1} with 1 s between pulses. The moderately rapid scan rate was necessary to minimize decomposition of the radical species. The success of this method depends on the rapid restoration of the equilibrium between pulses (supported by CV data) and the relatively small current attributable to back reaction and reduction during the pulse (supported by chronoamperometry). A small correction for the latter was made, as well as for complexation of AN.

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(12) This assumes pseudo-first-order behavior for solvent incorporation, and K_0 has been adjusted for [AN].

$^{13}C\{^2H\}$ Insensitive Nuclei Enhanced by Polarization Transfer (INEPT): A New NMR Strategy for Isotopic Labeling Studies

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Isotopic labeling studies are commonly used to elucidate mechanistic and structural features in chemistry and biochemistry, either as a tracer or as a structural probe for a selected environment in a large molecule. One method by which this has been accomplished is by selectively labeling sites of interest with enriched NMR-active nuclei such as ^{13}C[1] or ^2H.[2] In many instances, ^{13}C labeling of selective sites is expensive and difficult to accomplish synthetically. ^2H is generally easier to incorporate in organic molecules, and ^2H-labeled reagents are much less expensive. However, ^2H NMR in isotropic systems has the disadvantage of much smaller chemical shift dispersion.

A new strategy has been developed for studying selected ^{13}C sites in low concentrations that takes advantage of both the ^{13}C chemical shift dispersion and the expediency of ^2H incorporation. In those cases where ^2H labeling of a site is easier, polarization transfer from ^2H to ^{13}C via an INEPT (insensitive nuclei enhanced by polarization transfer) pulse sequence provides the needed selectivity.

The INEPT pulse sequence was designed to tap the reservoir of the larger spin population difference of high γ nuclei (especially of protons) coupled to spins of low γ nuclei.[3,4] The enhancement results from polarization transfer by way of the internuclear scalar coupling (J) between the observed (I) and coupled (S) spins. The sequence for accomplishing this is

$$90°_x(S)-\tau-(180°_x(S),180°(I))-\tau-(90°_y(S),90(I))-\text{acquisition}$$

if coupled spectra are obtained or

$$90°_x(S)-\tau-(180°_x(S),180°(I))-\tau-(90°_y(S),90(I))-\Delta-180(I)-\Delta-(\text{decouple}(S),\text{acquisition})$$

if decoupling is desired, where $\tau = 1/4J$, and $\Delta = 3/8J$ for triplets.

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Figure 1. ^{13}C NMR spectra of 40% dioxane in benzene-d_6. Both spectra were obtained from four transients using a 5000-Hz spectral window, 0.4-s acquisition time, and 3.5-Hz exponential weighting of the free induction decay: (a) the "normal" coupled ^{13}C spectrum obtained with a 90° pulse and repetition time of 30 s; (b) the $^{13}C\{^2H\}$ INEPT spectrum obtained with a repetition time of 2.8 s and τ = 0.0104 s.

Since one-bond $^{13}C-^1H$ coupling constants are similar but yet significantly different from long-range couplings, this technique is selective for carbons with directly bonded protons. Similar arguments apply for deuterium.[2] Due to the low natural abundance of ^2H (0.015%) polarization transfer with the INEPT pulse sequence can be used, and selectivity can be achieved by synthetically deuterating only those carbons of interest, as can be seen in Figure 1. The one pulse, fully coupled "normal" (Figure 1a) and $^{13}C\{^2H\}$ INEPT (Figure 1b) spectra of 40% dioxane in benzene-d_6 are shown. In Figure 1b, the protonated carbon resonances of dioxane are greatly attenuated. A −1:0:1 multiplet is seen for the ^2H coupled ^{13}C triplet as expected.[3,4] Maximum ^{13}C signal intensity for deuterated carbons was obtained by using a delay of $1/4J_{CD}$ (10.4 ms, J_{CD} = 24 Hz).

Although in a single-pulse experiment the polarization transfer of the ^2H spin population differences to ^{13}C does not enhance the signal of coupled ^{13}C nuclei (it should in theory diminish since $\gamma_{^2H}/\gamma_{^{13}C} = {}^3/_5$ and there is a loss of the nuclear Overhauser enhancement), the faster pulsing rate in a multipulse experiment allowed by the shorter T_1 relaxation time of the deuterium maintains the obtainable signal-to-noise ratio of the analogous proton-decoupled spectrum. More importantly, by greatly attenuating the protonated carbon resonances from the spectrum, the resonances of interest are clearly delineated without interference from protonated solvent or other unlabeled carbon resonances.

The spectra of 1-phenylethanol (1-PE) in chloroform (Figure 2) illustrate this point. The spectrum shown in Figure 2a is of

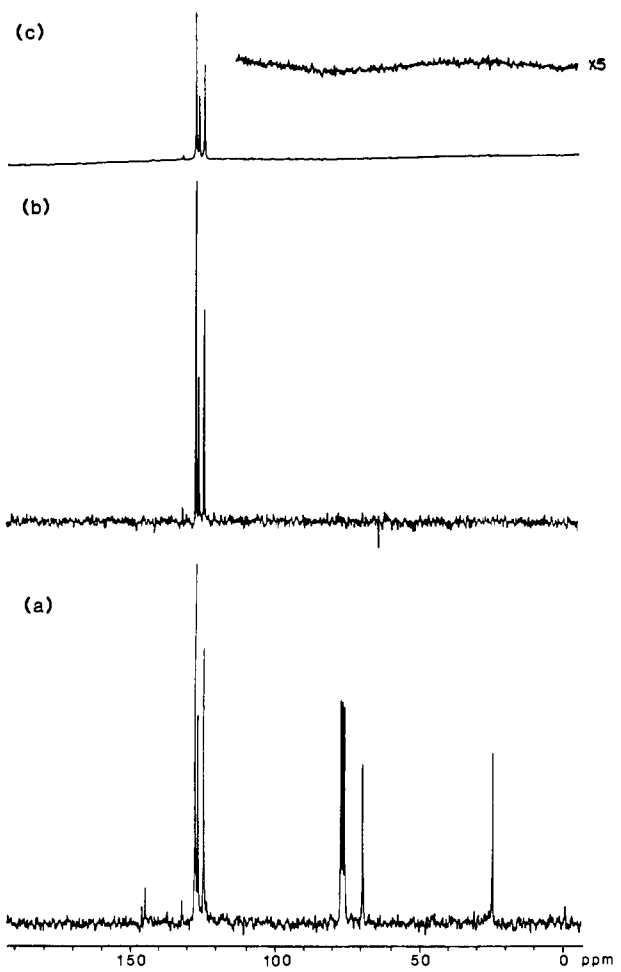

Figure 2. ^{13}C NMR spectra of 1-phenylethanol (1-PE) in chloroform, obtained by using a spectral window of 5000 Hz, acquisition time of 0.4 s, and an exponential weighting of 3.5 Hz: (a) normal proton-decoupled spectrum of 5% 1-PE in $CDCl_3$, obtained with a total experiment time of 4 min, 40 transients, repetition time 6 s, 90° pulse; (b) $^{13}C\{^2H\}$ INEPT spectrum of 1-PE-d_5 (deuterated in the aromatic positions) in $CHCl_3$ obtained with a total experimental time of 4 min, 240 transients, and 1-s repetition time; (c) same as b but with 1% 1-PE-d_5 in $CHCl_3$, 20 000 transients, and 1.4-s repetition time.

5% 1-PE in $CDCl_3$, while the spectrum in Figure 2b is of a 5% solution of 1-PE-d_5 in $CHCl_3$. Comparable signal to noise is obtained in a similar experimental time (4 min) since a larger number of transients could be obtained in the $^{13}C\{^2H\}$ polarization transfer experiment. At the same time the resonances of the protonated solvent are effectively eliminated. This elimination arises from a number of factors. The $^{13}C-^1H$ coupling is not suppressed, the NOE of protonated carbons is suppressed, and these carbons also have relatively long relaxation times compared to ^2H and are therefore saturated. An extended accumulation (20 000 transients) on a 1% solution of 1-PE in $CHCl_3$ was obtained to test the extent of this selectivity. This spectrum is shown in Figure 2c. No solvent resonance is detected, despite the greater than 100-fold molar excess of solvent. Note also that each of the deuterated positions is clearly distinguished in Figures 2b and 2c, whereas the aryl resonances in the ^2H and ^1H spectra not shown are broad singlets.

The $^{13}C\{^2H\}$ INEPT sequence was implemented on a Varian XL-200 by inserting the ^2H transmitter board (normally in the lock channel) into the decoupler channel. The signal was routed through the broad-band observe coil, which is double tuned for the lock frequency. Construction of a narrow band reject filter to attenuate the ^2H input at 30.7 MHz relative to the ^{13}C observe signal at 50.3 MHz was required. The standard INEPT pulse sequence program supplied with the instrument software was used. Maximum ^{13}C signal intensity was obtained by using a delay of

$1/4J_{CD}$ between 90 and 180° pulses.

While other workers have extended the INEPT technique to include observed nuclei with spins greater than half[5] and polarization transfer from nuclei with $I = {}^1/_2$ other than ^1H,[6] we believe this is the first report of the adaptation of the polarization transfer from deuterium.

While utilization for ^{13}C observation is described, this experiment is expected to be especially useful for nuclei such as ^{15}N, since polarization transfer will result in signal enhancement and eliminates problems associated with the negative NOE of ^{15}N. Furthermore, in most instances protons attached to nitrogen are generally exchangeable, allowing facile monitoring of ^2H exchange.

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The Antitumor Drug *cis*-$[Pt(NH_3)_2Cl_2]$ Forms an Intrastrand d(GpG) Cross-Link upon Reaction with $[d(ApGpGpCpCpT)]_2$

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The primary target responsible for the cytotoxicity of the antitumor drug *cis*-$[Pt(NH_3)_2Cl_2]$ (*cis*-DDP) is believed to be DNA.[1,2] It is therefore of interest to determine the stereochemistry of *cis*-DDP adducts of DNA. Spectroscopic studies of the binding of *cis*-DDP to mono-[3] and dinucleotides[4] reveal the heterocyclic nitrogen atoms of the purine and pyrimidine bases to be the most favorable DNA binding sites, with N7 of guanine being the most preferred kinetically.[5] Using nucleases, we showed previously that *cis*-DDP binds to regions of DNA rich in $(dG)_n{\cdot}(dC)_n$ ($n \geq 2$) sequences, a result consistent with the formation of intrastrand cross-links between nearest neighbor guanine or cytosine bases.[6] This conclusion was recently supported by ^1H NMR studies of di-[4] and tetranucleotides[7] containing such sequences. Intrastrand cross-linking of two guanines separated by one or more bases has also been demonstrated.[8] In the present investigation we have examined the reaction of *cis*-$[Pt(NH_3)_2Cl_2]$ with the self-complementary deoxyribohexanucleoside pentaphosphate, $[d(ApGpGpCpCpT)]_2$. As shown here, the isolated product contains an intrastrand *cis*-diammineplatinum(II)–d(GpG) cross-link, confirming at the hexanucleotide level the lesion believed to be significant on DNA. The results are of potential value in elucidating the mechanism of action of platinum antitumor drugs.

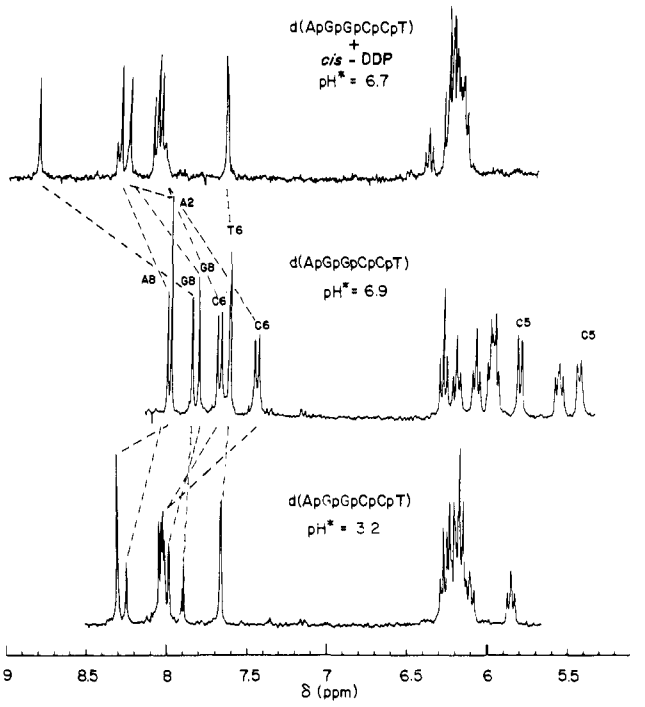

Figure 1. Downfield regions of 300-MHz ^1H NMR spectra (9.0–5.0 ppm) of $[d(ApGpGpCpCpT)]_2$ (3.5 mM duplex, 35 °C, no buffer) and its *cis*-DDP adduct (2.5 mM strand, 70 °C [see caption to Figure 2], no buffer). The central guanine H8 and cytosine H6 resonances are tentatively assigned as the more downfield peaks. The symbol pH* signifies uncorrected meter readings for samples in D_2O.[18]

Deoxyribohexanucleoside pentaphosphate was synthesized by a previously reported solid-phase procedure.[9,10] *cis*-DDP (3.33 mM) was allowed to react with 0.75 equiv of 10^{-3} M $[d(ApGpGpCpCpT)]_2$ (K^+ form) in the dark at 37 °C for 144 h in unbuffered aqueous solution, pH 6.0–6.7. ^1H NMR studies[11] showed that the unmodified oligonucleotide retains considerable duplex structure under these conditions. The mixture was separated by reverse-phase high-performance liquid chromatography.[12] One main product and unreacted starting material accounted for >95% of the total optical density at 260 nm. The retention time of the platinated oligonucleotide was shorter than

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(11) Sequential melting of the deoxyribohexanucleoside pentaphosphate was monitored by observing the G-C amino protons (^1H NMR, Redfield water supression technique) involved in duplex H-bonding as a function of temperature as well as the transition midpoints of the temperature-dependent chemical shifts of the nonexchangeable base protons (3.5 mM duplex, 5 mM phosphate, 1 mM EDTA). The latter experiment showed the transition midpoint to be 55 ± 4 °C for the GpGpCpC core and a much lower transition midpoint (~30 °C) for the A-T base pairs. The duplex-to-strand transition was found to be noncooperative, a result confirmed by temperature-dependent UV and CD studies. Thus at 37 °C, partial duplex structure is still present. Full details will be reported at a later date.
(12) Waters μBondapak C-18 column, 30 × 0.78 cm; dual pump system, pump A, 0.1 M NH_4OAc, pH 6.5; pump B, 0.1 M NH_4OAc/CH_3CN (1:1), pH 6.5; primary linear gradient, 5–60% B, 43 min, 2 mL/min; secondary linear gradient, 15–35% B, 30 min, 2 mL/min. Under these conditions the oligonucleotide would be expected to be in single stranded form.

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