Significant enantiomeric discrimination accompanies this covalent binding. The circular dichroism of the supernatant, the unbound fraction, is shown in Figure 2. Optically enriched $Ru(phen)_2Cl_2$ solutions have not been obtained previously by using more conventional methods.[11] The magnitude of the rotation in the ultraviolet region is approximately 5 times larger than that seen earlier for $(phen)_3Ru^{2+}$ solutions at comparable levels of intercalative binding. Hence the degree of chiral selectivity for this covalent adduct appears substantially greater than for $(phen)_3Ru^{2+}$.[12] The absolute configuration may be assigned by derivatization to the tris(phenanthroline)ruthenium(II) species.[14] Consistent with simple exciton theory, we assign the CD in the ultraviolet region given in Figure 2 as that for the Δ isomer. In contrast to earlier observations concerning $(phen)_3Ru^{2+}$ binding to DNA, here it is Λ-$(phen)_2Ru^{2+}$ that binds preferentially to the B-DNA helix.

The enantiomeric discrimination of the bis(phenanthroline)-ruthenium complex in binding to B-DNA must differ from the tris(phenanthroline) cation not only in degree but also in the structural basis for the stereoselectivity. Ruthenium(II) complexes have a high affinity for the heterocyclic bases of DNA.[16] A likely site of metalation would be the N-7 of guanine, which is readily accessible in the major groove. Initial intercalation is probable; immediate hypochromic changes in the ruthenium charge transfer band accompany the addition of DNA. However, additional spectroscopic changes become evident on a time scale that correlates to the covalent binding given in Figure 1. Model building shows that from an initially intercalated position the Λ isomer is well oriented for covalent binding to base positions above and below. The Δ isomer cannot be similarly aligned for covalent binding, since the other nonstacked phenanthroline ligand is considerably crowded by the right-handed helical column (base and sugar phosphate groups). A bifunctional coordination oriented by initial intercalation could account for the high stereoselectivity we observe. It is interesting that for intercalation by $(phen)_3Ru^{2+}$ the Δ isomer, which has the same helical screw sense as the right-handed B-DNA, is preferred, while here metalation of base positions seems to require the Λ configuration, that is a structure *complementary* to the B-DNA helix.

The stereoselective covalent binding of $Ru(phen)_2Cl_2$ to DNA could have significant biological consequences. The neutral $Ru(phen)_2Cl_2$ may be considered an octahedral analogue for *cis*-$Pt(NH_3)_2Cl_2$.[17] Recent reports of antitumor activities and toxicities of various ruthenium complexes,[1,18] the possible similarities between $Ru(phen)_2Cl_2$ and *cis*-DDP in interactions with DNA, and the striking stereoselectivity that we observe all suggest a potential chemotherapeutic application of chiral bis(amine)-ruthenium(II) complexes.

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Conversion of a Cationic Alkene Hydride Complex of Tungsten into a Complex Containing a Terminal Alkylidene Ligand

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The formation of bridging alkylidene ligands from alkene ligands has been reported recently in three binuclear transition-metal complexes,[1] and isomerization of terminal alkylidene ligands to alkenes by a 1,2-hydride shift is well established, particularly for electrophilic transition-metal complexes,[2] but there have been no examples to date of the formation of a terminal alkylidene ligand from an alkene. This is a potentially important process which could be involved in the activation of metathesis catalysts,[3] and we now wish to report the first example of such a reaction by a route that apparently involves alkene insertion into a cis tungsten–hydride bond.

Treatment of the alkene complex $[W(\eta\text{-}C_5H_5)_2(C_2H_4)H]PF_6$[4] (**1**-$PF_6$) with I_2 in polar organic solvents such as acetone, acetonitrile, or tetrahydrofuran (THF) results in formation of the ethylidene complex $[W(\eta\text{-}C_5H_5)_2(CHCH_3)I]^+$ ($\mathbf{2}^+$) (eq 1). Pure

$$[W(\eta\text{-}C_5H_5)_2(C_2H_4)H]^+ + I_2 \rightarrow [W(\eta\text{-}C_5H_5)_2(CHCH_3)I]^+ + HI \quad (1)$$

salts of $\mathbf{2}^+$ can only be isolated under carefully controlled conditions, since the I^- generated tends to complex I_2,[5] leading to mixed PF_6^-/I_3^- salts which cannot be readily purified. Pure **2**-PF_6 was, however, obtained by adding 2.4 molar equiv of 0.073 M I_2 in THF dropwise to a suspension of **1**-PF_6 in THF (0.90 mmol in 150 mL). After 3.5 h the filtered red solution was concentrated to dryness and the black crystals obtained were washed with THF. Extraction with acetonitrile gave a green solution from which microcrystalline green **2**-PF_6[6] (55% yield) was precipitated by slow addition of diethyl ether.

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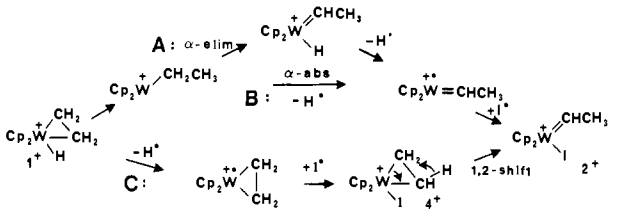
Scheme I. Possible Pathways for the Conversion of the Alkene Hydride Complex $[W(\eta\text{-}C_5H_5)_2(C_2H_4)H]^+$ into the Ethylidene Complex $[W(\eta\text{-}C_5H_5)_2(CHCH_3)I]^+$ (Cp = η-C_5H_5)



The ethylidene ligand in $\mathbf{2}^+$ was primarily identified by its distinctive ^1H and ^{13}C NMR spectra[6] (particularly the characteristically low-field α-H and α-C resonances), which also established (intensity of ^{183}W satellites) that the ethylidene is terminal. The distinct chemical shifts of the two cyclopentadienyl ligands are consistent with the ethylidene ligand adopting the expected perpendicular orientation characteristic of closely related tantallocene alkylidene complexes,[7] which allows a π-symmetry interaction between tungsten and carbon. Chemical characterization of $\mathbf{2}^+$ included reversible deprotonation (eq 2) to the vinyl

$$[W(\eta\text{-}C_5H_5)_2(CHCH_3)I]PF_6 \underset{HPF_6/THF}{\overset{Me_2CO/Al_2O_3}{\rightleftharpoons}} [W(\eta\text{-}C_5H_5)_2(CH{=}CH_2)I] \quad (2)$$

complex $[W(\eta\text{-}C_5H_5)_2(CH{=}CH_2)I]$[8] (**3**): deprotonation accompanied passage of an acetone solution of **2**-PF_6 through an alumina column, and **3** was recrystallized as green-black needles (70%) from toluene.

Although cationic alkylidene complexes of tungstenocene are well established as reactive intermediates,[2d,9] **2**-PF_6 is the first to be isolated. Solutions of **2**-PF_6 are surprisingly stable and can be exposed to moist air for a week without significant decomposition (^1H NMR). The ethylidene ligand shows no tendency to insert into the W–I bond, nor to isomerize to ethylene, and it may be that π-donation from the halide significantly stabilizes the complex.

Three possible pathways for conversion of the alkene in $\mathbf{1}^+$ into the alkylidene in $\mathbf{2}^+$ are outlined in Scheme I.[10] An obvious distinction between path C, which involves the reverse of the known alkylidene to alkene isomerization,[2] and both path A and path B is that in C the hydride initially on the metal is not in the product, while in paths A and B, in which alkene insertion into the metal hydride is followed by α-elimination or direct α-hydrogen abstraction from the intermediate alkyl cation, the hydride initially on the metal finishes in the β-position within the alkylidene ligand. This led us to attempt to prepare $[W(\eta\text{-}C_5H_5)_2(C_2H_4)D]PF_6$, a potential substrate for labeling experiments, by reacting $[W(\eta\text{-}C_5H_5)_2(C_2H_4)]^+$ in toluene with an equivalent of 0.4 M DPF_6/D_2O, but ^1H NMR studies in acetone-d_6 of the resulting precipitate showed that the deuterium in $\mathbf{1}^+$-d_1 exchanged rapidly in solution into the alkene ligand (ca. 10 min). This observation demonstrated that the alkene insertion required if the reaction proceeds by path A or B does occur, and is rapid and reversible, but suggested that conversion of $\mathbf{1}^+$-d_1 to $\mathbf{2}^+$ was unlikely to be mechanistically informative.

Remarkably, however, treatment of scrambled $\mathbf{1}^+$-d_1 with I_2 in acetone resulted in formation of $\mathbf{2}^+$-d_1 labeled exclusively and quantitatively at the β-position.[12] Formation of β-$\mathbf{2}^+$-d_1 could be consistent with path A or B if reversible insertion in $\mathbf{1}^+$ does not scramble the two carbons, suggesting that the alkene in $\mathbf{1}^+$ may not rotate. This seemed unlikely given the singlet resonance for the C_2H_4 in ^1H NMR spectra of $\mathbf{1}^+$[4,13] but examination of ^{13}C NMR spectra showed that, as in the case of the isoelectronic Ta complex,[13] alkene rotation cannot be observed in $\mathbf{1}^+$. Solutions of $\mathbf{1}^+$-d_0 exhibit two alkene resonances, and, even after 18 h in acetone-d_6, the lower field alkene resonance of $\mathbf{1}^+$-d_1 is identical with that of $\mathbf{1}^+$-d_0. The higher field resonance of $\mathbf{1}^+$-d_1, however, consists of a singlet (D on W) superimposed on 1:1:1 triplet (D on C; J_{CD} = 24 Hz, δ +0.13 isotope shift).

Conversion of scrambled $\mathbf{1}^+$-d_1 into β-$\mathbf{2}^+$-d_1 with concomitant formation of no more than 2.4% $\mathbf{2}^+$-d_0[12] could only be consistent with the conversion occurring via path C if hydrogen abstraction from the metal were much slower than the reversible alkene insertion and there was a H/D kinetic isotope effect of >20 for abstraction. This seems unlikely, suggesting that the reaction proceeds by one of the insertion pathways.

As a further probe of the possibility of direct alkene to alkylidene isomerization by path C, we attempted to prepare the putative intermediate $[W(\eta\text{-}C_5H_5)_2(C_2H_4)I]^+$ ($\mathbf{4}^+$). Reaction of $[W(\eta\text{-}C_5H_5)_2(C_2H_4)]$[4] in CH_3CN with an I^+/collidine complex[14] resulted in formation of C_2H_4 (70% by GC) and $[W(\eta\text{-}C_5H_5)_2(NCCH_3)I]PF_6$ (**5**-PF_6,[15] 55% yield). Formation of $\mathbf{5}^+$ and C_2H_4 suggests (eq 3) that $\mathbf{4}^+$ had indeed been formed but that the

$$[W(\eta\text{-}C_5H_5)_2(C_2H_4)] \xrightarrow{I^+/collidine} [W(\eta\text{-}C_5H_5)_2(C_2H_4)I]^+ \xrightarrow{CH_3CN} [W(\eta\text{-}C_5H_5)_2(NCCH_3)I]^+ + C_2H_4 \quad (3)$$

ethylene ligand dissociates from $\mathbf{4}^+$ rather than isomerizing into an ethylidene ligand. Since the reaction conditions are similar to those obtaining when $\mathbf{1}^+$ is converted into $\mathbf{2}^+$ by reaction with I_2 in CH_3CN, we conclude that $\mathbf{4}^+$ is probably not an intermediate on the way from $\mathbf{1}^+$ to $\mathbf{2}^+$.

An insertion pathway for the conversion of $\mathbf{1}^+$ into $\mathbf{2}^+$ is favored both by the labeling study and the sequence in eq 3, but the data

(6) ^1H NMR (300 MHz, acetone-d_6) δ 14.86 (q, J = 8.2 Hz, 1, CH), 6.56 (s, 5, C_5H_5), 6.48 (s, 5, C_5H_5), 3.52 (d, J = 8.1 Hz, 3, CH_3); $^{13}C\{^1H\}$ NMR (75.5 MHz, acetone-d_6) δ 311.3 (s, d in gated spectra with J_{CH} = 142 Hz, satellites J_{WH} = 97 Hz, CH), 103.8 (s, C_5H_5), 103.3 (s, C_5H_5), 44.2 (s, CH_3). Anal. Calcd for $C_{12}H_{14}F_6IPW$: C, 23.48; H, 2.30; I, 20.67. Found (Galbraith; sample prepared from **1**-PF_6): C, 24.01; H, 2.25; I, 20.43. Found (Schwarzkopf; sample prepared from **3** as in eq 2): C, 23.54; H, 2.49; I, 20.20.

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(8) IR (Nujol mull, selected) 1562 m (vinyl C=C) cm^{-1}; ^1H NMR (270 MHz, acetone-d_6) δ 8.24 (dd, J = 11.4 and 18.3 Hz, satellites J_{WH} = 3.3 Hz, 1, WCH), 5.92 (dd, J = 3.2 and 11.5 Hz, 1, H_{trans}), 5.56 (dd, J = 3.2 and 18.3 Hz, 1, H_{cis}), 5.12 (s, 10, C_5H_5); ^{13}C NMR (67.8 MHz, acetone-d_6, shifts from $^{13}C\{^1H\}$ spectra and J from spectra with gated decoupling) δ 134.0 (d, J = 130 Hz, WCH), 132.8 (t, J = 150 Hz, CH_2), 90.8 (d quin, J = 183 and 6 Hz, C_5H_5); mass spectrum, parent ion at m/z 468 (^{184}W). Anal. Calcd for $C_{12}H_{13}IW$: C, 30,80; H, 2.80. Found (Schwarzkopf): C, 30.69; H, 2.79.

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(12) ^1H NMR (80 MHz, acetone-d_6) δ 14.8 (t, J = 8.2 Hz, 1, CH), 6.6 (s, 5, C_5H_5), 6.5 (s, 5, C_5H_5), 3.5 (dt, J = 8.2 Hz, $J_{D\text{-}H}$ = 2.4 Hz, 2, CH_2D). Integration of the δ 14.8 peak established that there could not be more than 5% α-$\mathbf{2}^+$-d_1 present, and the relative heights of the components of the δ 3.5 multiplet established that there could not be more than 2.4% $\mathbf{2}^+$-d_0 present.

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(15) More conveniently prepared by reacting $[W(\eta\text{-}C_5H_5)_2(CH_3)I]$[11a] with HPF_6 in CH_3CN: IR (Nujol mull, selected) 2322 vw, 2290 vw (N≡C) cm^{-1}; ^1H NMR (80 MHz, acetone-d_6) δ 5.95 (s, 10, C_6H_5), 2.99 (s, 3, CH_3); $^{13}C\{^1H\}$ NMR (75.5 MHz, acetone-d_6) δ 96.0 (s, C_5H_5), 4.9 (s, CH_3). Anal. Calcd for $C_{12}H_{13}F_6INPW$: C, 22.99; H, 2.09; I, 20.24. Found (Schwarzkopf): C, 23.07; H, 2.28; I, 19.58.

do not distinguish at this point between path A and path B. Since α-elimination is, however, well established to be a facile reaction in cationic 16-electron tungstenocene alkyls,[9a,b,c,g] we would propose that path A is the more probable, and this would imply, as speculated previously,[3] that alkene hydride insertion followed by α-elimination can provide a facile route from alkenes to alkylidenes and may therefore be involved in the initiation of metathesis by some transition-metal catalysts.

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Isotopic-Label-Directed Observation of the Nuclear Overhauser Effect in Poorly Resolved Proton NMR Spectra

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Proton nuclear magnetic resonance spectroscopy (^{1}H NMR) is a powerful tool for the determination of structure in chemical and biological systems through observation of the nuclear Overhauser effect (NOE).[1-3] The NOE has a r^{-6} dependence on internuclear distance and is commonly manifested as a change in population among spacially proximate protons. The NOE can be used to assign ^{1}H resonances to specific protons in tRNAs and DNA oligomers and to identify local conformation in proteins.[4,5] Unfortunately the lack of resolution in the ^{1}H NMR spectrum for most large nucleic acids and proteins often precludes unambiguous observation of individual NOEs for overlapping signals.

We describe an extremely simple technique for observing the NOE from a specific proton in a complex spectrum. Incorporation of a stable isotope (^{13}C, ^{15}N) at heteroatomic sites produces a large scalar coupling to a directly bonded proton. Application of on-resonance heteronuclear decoupling collapses the scalar coupling, and the ^{1}H signal appears as a singlet.[6,7] The usual INDOR experiment uses on- and off-resonance heteronuclear decoupling during acquisition of the ^{1}H signal to determine heteronuclear chemical shifts. When heteronuclear decoupling and preirradiation at the nominal ^{1}H frequency are applied simultaneously, the resonance for the proton can be saturated. In contrast, if on-resonance ^{1}H saturation is applied in the same way but the frequency of the heteronuclear decoupler is displaced, the ^{1}H signal from the directly bonded proton is not saturated as the frequencies of the signal are shifted by $\pm J/2$. The peaks from other protons that have ^{1}H chemical shifts coincident with the decoupled H-X proton are equally saturated in both cases. Subtraction of the two spectra, with on- and off-resonance heteronuclear decoupling during the period of proton preirradiation, produces a difference spectrum that displays only the NOE from the proton bonded to the uncommon isotope. The NOE from nearby or overlapping proton lines is canceled in the subtraction.

We first demonstrate the experiment for a small molecule, [1,3-$^{15}N_2$]uracil. Signals from the two imino protons, at N3 and N1, appear at 11.08 and 10.90 ppm in the 500-MHz spectrum. However, as shown in Figure 1a, the two doublets ($^1J_{NH}$ = 95 Hz) overlap. The resonance for the proton at N1 can be assigned because its splitting is decoupled at the expected ^{15}N chemical shift of 130.6 ppm relative to ammonia for N1, as seen in Figure 1b.[6] The isotopic-label-directed NOE experiment, given in Figure 1c, shows the expected result: the control, with off-resonance ^{15}N decoupling, produces a doublet while simultaneous on-resonance ^{15}N and ^{1}H irradiation saturates the central component (seen in the INDOR experiment of Figure 1b). Subtraction removes the signal from the proton at N3, which is only slightly perturbed by the ^{15}N rf. Saturation of H1 produces a positive NOE of 4% to the proton at C6, while no NOE is observed to the proton at C5.

In tRNAs, introduction of the ^{15}N label, on the N3 or N1 of uridine or guanosine, respectively, provides a handle to sort out the poorly resolved proton signals.[7,8] The 500-MHz ^{1}H spectrum for *E. Coli* tRNA^{Gln} (Figure 2a) has a composite two-proton signal at 13.8 ppm.[9] Upon preirradiation there are two NOEs, at 7.37 and 8.25 ppm. The NOE at 8.25 ppm disappears in a tRNA sample biosynthetically deuterated at the purine C8 position, showing that it and one of the two 13.8 ppm proton resonances come from one of the two reverse-Hoogsteen base pairs T54A58 or sU8A14.[10,11] Other evidence strongly suggests that it is from T54A58, and this is consistent with the ^{15}N shifts for the nitrogens to which the 13.8 ppm protons are bonded, which are 163.4 and 160.5 ppm as determined by a 2D forbidden echo experiment.[6,8,11] As shown in Figure 2c, application of ^{1}H preirradiation combined with ^{15}N decoupling at these distinct nitrogen frequencies permits the selective observation of the NOE from the two protons. Irradiation at 13.80 and 163.4 ppm produces an NOE at 7.37 ppm. Similar saturation at 13.80 and 160.5 ppm gives rise to an NOE at 8.25 ppm. This triple correlation of nitrogen, imino proton, and ring carbon proton resonances unambiguously assigns the T54 ^{15}N3 to the 160.5 ppm resonance, consistent with observations in other tRNAs that N3 of T54 in the invariant T54A58 base pair resonates at slightly higher field than the uracil N3 of standard Watson-Crick pairs.[6-8] For future studies it permits shifts of the proton and ^{15}N resonances of this base pair to be observed separately from those of other overlapping peaks, as a function of solution conditions.

This methodology should be applicable to a variety of chemical and biological problems. As illustrated for *E. coli* tRNA^{Gln}, the method would allow the NOE in suitably labeled RNA or DNA to be observed, even in congested regions of the spectrum where the resonances from GC and AU base pairs overlap. A similar advantage may be obtained in large proteins, where aromatic and amide protons both generate peaks between 7 and 9 ppm. Regiospecific incorporation of a ^{13}C or ^{15}N isotope will permit the NOE from the Cα and amide protons, for example, to be observed for a specific amino acid. Saturation transfer experiments on complex mixtures of labeled compounds could be performed in this way, to elucidate chemical or metabolic rate constants. Finally, the technique is of great value in studies of the interaction of small molecules with large biopolymers. The ^{1}H chemical shifts for the protons of interest in the small compound usually fall on resonances from protein, and selective saturation of only the protons of the small molecule is not possible. The methodology we describe offers a convenient solution to the problem. We have

(1) Abbreviations used: NOE, nuclear Overhauser effect; tRNA, transfer ribonucleic acid; DNA, deoxytribonucleic acid; rf, radio frequency, 2D, two dimensional.

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