are short-lived species and react chemically with DNA. ESR methods clearly indicate that OH radicals can attack at both the C-5 and C-6 sites of the thymine ring. Hydrated electrons react with pyrimidine to form ions which may then undergo protonation. H atoms seem to add preferentially at C-6 and there is a difference between the H atom adduct and the electron adduct spectra indicating a different site of attack for the two short-lived species.[19] The pyrimidine radicals obtained are then able to undergo electron transfer through a dismutation reaction. Solvolytic substitution of the pyrimidic ions and H atom exchange give rise to the final radiation products II to VII.

We believe that the method described herein may be extended to the other DNA bases.[19,20]

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On the Configuration of the *tert*-Butyl Radical[1]

Sir:

The configuration[2] of the *tert*-butyl radical has been the subject of considerable debate.[3-15] The controversy has focused on the magnitude of the EPR ^{13}C hyperfine splitting of the central atom (a[13]C),[2-8,10] the interpretation of photoelectron spectroscopic data,[9,15] and the results of ab initio calculations.[4,13,16]

Most recently the debate has rested on the temperature dependence of a[13]C.[3,4,7,11,12,14] This parameter provides the best available measure of the C_α hybridization and hence of the configuration of the radical. If *tert*-butyl has $\theta_{min} = 0°$, where θ[18] is the angle between the plane of the methyl carbon atoms and a C–C bond, then a[13]C should increase monotonically with increasing temperature. However, a[13]C has been found to decrease with increasing temperature, possibly reaching a minimum at ~275 K.[3,4,7] Two independent theoretical studies[11,12] accounted for these results in terms of rapid pyramidal inversion of the radical, governed by a double-minimum potential. It was concluded that $\theta_{min} \approx 19°$ and that there is a small barrier (~600 cal/mol) to inversion. Since this theoretical work was made to fit EPR data in matrices and since it was suggested that medium effects could have caused the observed anomalies,[6,13,14] we decided to measure a[13]C in solution over the widest possible range of temperatures. Under these conditions the spectral lines are sharp, isotropic, and free from influence by a crystalline lattice.

The radical was generated from $(CH_3)_3$[13]CBr (90 atom % [13]C) by standard methods[19] in either propane or isooctane as solvent. The spectral parameters were the same in both solvents (at the same temperature) and were not influenced by the method of radical generation. At each temperature the field positions and microwave frequency were recorded for 10 to 15 of the "second-order" [20] lines. The spectral parameters were computed using an exact solution of the isotropic Hamiltonian. An iterative least-squares procedure[21] was then applied which adjusted the parameters so as to obtain the best fit to all of the measured lines. Standard deviations were approximately ±0.04 G (a[13]C), ±0.02 G (a[H]), and ±0.00001 (g).[22] The ^{13}C splittings are plotted in Figure 1 and all of the data are available as supplementary material.

Our data are substantially different from those obtained in the solid state,[3,4,7] although we confirm the basic trend observed. Most notably the change in a[13]C is almost an order of magnitude smaller in solution and there is a *well-defined* minimum at 220 K. The latter suggests that there is a small energy barrier (V_0) for the inversion of the radical. A "classical" analysis[12] gives $V_0 = 2.330\ T_{min} = 510$ cal/mol.
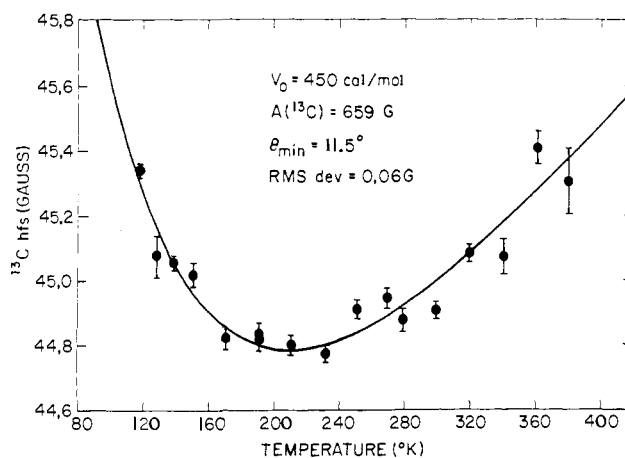

Figure 1. Change with temperature of [13]C$_\alpha$ hfs for $(CH_3)_3$[13]C· in hydrocarbon solution.

Table I. Least-Squares Fitting of $a^{13}C(T)$ to Eq 2

V_0, cal	A_0, G	θ_{min}, deg	root-mean-square deviation
350	998	4.1	0.16
400	904	6.2	0.14
450	659	11.5	0.06
475	727	10.0	0.07
500	779	8.9	0.09
550	874	6.9	0.14
650	1004	3.9	0.18

It is possible to develop further conclusions using a combination of "classical" theory and INDO molecular orbital calculations. The latter provides a relationship between $a^{13}C$ and θ. However, we caution that the inherent weakness of INDO in accommodating vibrational behavior[24] is necessarily introduced by this approach.

We treated the data[23] using a simple double-minimum potential function[25] to represent the vibrational energy (V) (eq 1), where ξ is equal to θ/θ_{min}.

$$V(\xi) = V_0(\xi^4 - 2\xi^2) \tag{1}$$

According to the "classical" theory of the double-minimum potential[12] the temperature dependence of $a^{13}C$ is given by

$$a^{13}C(T) = A_0[\rho_0 + \rho_2\theta_{min}{}^2 F_2(T,V_0) + \rho_4\theta_{min}{}^4 F_4(T,V_0)] \tag{2}$$

where

$$F_n(T,V_0) = \frac{\int_0^\infty \xi^n \exp[-V_0(\xi^4 - 2\xi^2)/RT]d\xi}{\int_0^\infty \exp[-V_0(\xi^4 - 2\xi^2)/RT]d\xi} \tag{3}$$

and

$$\rho(\theta) = \rho_0 + \rho_2\theta^2 + \rho_4\theta^4 \tag{4}$$

A_0 is the atomic scale factor which relates the spin density at the central carbon, $\rho(\theta)$, to $a^{13}C$. The INDO method[24] was used to compute $\rho(\theta)$, and for $\theta \le 30°$ an excellent fit was obtained for eq 4 when $\rho_0 = 0.04151$, $\rho_2 = 0.84552$, and $\rho_4 = -1.16246$ (for θ in radians).

We choose to fit eq 2 to the experimental data using different values of V_0 in the range $250 \le V_0 \le 1000$ cal/mol. For each value of V_0 both A_0 and θ_{min} were treated as independent variables and were adjusted so as to obtain the best least-squares fit to the experimental data. The best solutions for several values of V_0 are shown in Table I. The smallest rms deviation (0.06 G) was obtained for $V_0 = 450$ cal/mol,[26] $\theta_{min} = 11.5°$, and $A_0 = 659$ G. The computed curve and the experimental data are shown in Figure 1.

In view of the limitations of the INDO technique and the experimental error, it is impossible to place precise limits of reliability on these numbers. However, the solution for V_0 shows reasonably good convergence and is the most reliable quantity derived in this work. The well-defined minimum in the $a^{13}C$ vs. T curve implies that there must be *at least* three vibrational levels below the barrier.[12] Since the first two will be nearly degenerate (even–odd doubling), this places an upper limit of $\sim$150 cm^{-1} for the vibrational frequency of the umbrella inversion mode. This value is not entirely inconsistent with the trend observed by infrared[27] for the out-of-plane bending frequency of $CH_3\cdot$ (612), $CH_3CH_2\cdot$ (541), and $(CH_3)_2CH\cdot$ (375 cm^{-1}).

The solution for A_0 and θ_{min} was rather soft. The value of 659 G for A_0 (or $\sim$800 G based on the "classical" barrier height of 510 cal/mol) is smaller than the theoretically cal-

culated values of 1130[28] and 1191 G.[29] However, it is in reasonable agreement with the value of 820 G normally used in the INDO parameterization.[30] Apparent inconsistencies in the relationships between A_0, ρ, and $a^{13}C$ are often observed and have been explained in terms of "lack of orbital following".[31]

While our data strongly suggest that a barrier exists for the inversion of *tert*-butyl, it is *possible* that such a barrier *may have been induced by the solvent*.[32,33] As the radical undergoes out-of-plane vibrations, it acquires a dipole moment which will interact with permanent or induced dipoles on the solvent molecules. This will stabilize bent structures. For any given solvent, the interaction energy will be proportional to θ^2 and will increase with increasing solvent density. This could result in a double-minimum potential for a radical which otherwise would not show a barrier to inversion. However, preliminary calculations indicate that the magnitude of the induced V_0 is only of the order of 20–80 cal/mol.

The frequency for the umbrella inversion motion of *tert*-butyl is undoubtedly quite low and it may well be similar to the methyl torsion frequency, in which case these two motions will be coupled. We are unable to evaluate whether such a motional coupling could be responsible for the unusual temperature dependence of $a^{13}C$.

Studies of the effects of solvents on the hydrogen hyperfine interaction[23] are in progress and will be reported in a full paper.

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(25) The general form of this double-minimum potential function is

$$V(\theta) = A\theta^4 - B\theta^2 \ (A, B > 0)$$

Equation 1 is obtained by substituting $A = V_0/(\theta_{min})$,[4] $B = 2 V_0/(\theta_{min})$,[2] and $\xi = \theta/\theta_{min}$. This transformation to the dimensionless coordinate, ξ, obviously leads to a significant simplification.

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Peptide Formation in the Presence of a Metal Ion Protecting Group. Pentaammine Cobalt(III)–Peptide Complexes

Sir:

The use of metal ions to activate and/or protect amino acid derivatives for peptide formation has been demonstrated in a number of studies using cobalt(III), copper(II), and platinum(II) complexes.[1-8] The work on cobalt(III) which showed the most promise centered around the use of cobalt(III) to *protect* the N terminal of an amino acid ester by coordination and to *activate* the ester carbonyl toward nucleophilic attack by coordination to the same tripositive cobalt(III) center This dual function of the metal ion (i.e., to protect and to activate) appeared promising at first, but has found little application in peptide synthesis presumably because the other methods used for the activation of amino acid derivatives (especially the C-terminal activation using carbodiimides and other active esters) are easier to carry out.

In this communication we present the first example of a synthetic scheme where a kinetically inert metal ion, $[(NH_3)_5Co^{III}-]$, functions as a carboxylate protecting group and directs peptide formation. The type of synthesis reported herein is an example where a designed peptide ligand is synthesized in a stepwise fashion in the vicinity of a metal center.

The kinetic inertness of the $[(NH_3)_5Co^{III}-]$ moiety and its resistance to hydrolysis in strong acids[9] makes it one of the few classes of coordination compounds that can remain in tact under the conditions used in peptide synthesis.[10] Furthermore, the $[(NH_3)_5Co^{III}-]$ moiety selectively binds to the C terminal of an amino acid (acting like an inorganic ester), thus allowing one to employ the known stepwise peptide synthetic techniques.[10] Herein we report our results on the aforementioned scheme for the synthesis of cobalt(III) dipeptide complexes and the progress of our work on the extension of the same methods for the synthesis of cobalt(III) complexes with longer peptide chains.

Cobalt(III) pentaammine–amino acid complexes have been synthesized by a number of investigators.[11-13] The synthesis of these complexes usually required heating $[(NH_3)_5$-$Co(OH_2)]^{3+}$ with an amino acid at >60 °C for a number of hours. A dipeptide, under the conditions required to prepare the cobalt(III)–amino acid complexes, is prone to undergo hydrolysis and therefore cobalt(III)–peptide complexes could not be prepared by the above methods.

A versatile synthetic route for the preparation of cobalt(III) peptide complexes can only be achieved using mild conditions (ca. room temperature). We have succeeded in using two procedures for the synthesis of cobalt(III)–amino acid derivatives and –peptide complexes under ambient conditions. The first procedure involves the reaction of $[(NH_3)_5Co-(OH)]^{2+14}$ with a *tert*-butoxycarbonyl-(Boc-) amino acid (or Boc peptide) active ester. This procedure can be used to introduce cobalt(III) at the C terminal of a N-protected amino acid or peptide. In this work this method was used to prepare only the compound $[Boc-Gly-Co(NH_3)_5](ClO_4)_2$ (Table I).

The second method which involves peptide formation in the presence of cobalt(III) is the subject of the remaining part of this communication. This procedure involves stepwise peptide formation starting with the $[amino\ acid-Co^{III}(NH_3)_5]$ com-

Table I

complex	IR, $\nu_{peptide\ C=O}$, cm^{-1}	UV–visible, $\lambda_{max}(\epsilon)$, nm (M^{-1} cm^{-1})	anal., % C calcd	C found	H calcd	H found	N calcd	N found
[Gly-Co(NH$_3$)$_5$](BF$_4$)$_3$		502 (64) 350 (5)	5.01	5.47	4.20	4.21	17.52	17.68
[Pro-Co(NH$_3$)$_5$](BF$_4$)$_3$		499	11.56	12.03	4.66	4.64	16.17	15.34
[(Boc-Gly)Co(NH$_3$)$_5$](BF$_4$)$_2$	1740^a	505	16.26	16.86	5.26	5.51	16.25	16.81
[Gly-Gly-Co(NH$_3$)$_5$](BF$_4$)$_3$	1698	503 (77) 348 (65)	8.95	9.30	4.32	4.25	16.27	18.10
[Phe-Gly-Co(NH$_3$)$_5$](BF$_4$)$_3$^{b}	1685	503 (70) 348 (sh) (58)	21.08	20.17	4.66	4.74	15.64	14.84
[Pro-Pro-Co(NH$_3$)$_5$](BF$_4$)$_3$·2H$_2$O	1728	502 (77) 348 (70)	17.93	17.93	5.42	4.68	14.64	14.43

^{a} $\nu_{C=O}$. ^{b} Reference 16.