spond to the molecular ion of the chlorophyll *a* monohydrate, Chl $a \cdot H_2O^+ \cdot$. It seems unlikely that this ion is the result of adduct formation after ionization of the chlorophyll because postionization adduct ions are generally even electron species.[11a,b] In cases where molecules specifically interact with water, e.g., when crystals contain stoichiometric amounts of water, then $MH_2O^+ \cdot$ ions are seen in FD spectra presumably as a result of ionization of the associated adduct.[11c] The fact that the relative intensity of the ion at *m/z* 910 was less than one third the intensity of the molecule ion (*m/z* 892) and less than one fifth the intensity of the protonated molecule (*m/z* 893) suggests that the coordinate-covalent bond in the hydrate is not unusually strong, as molecules with single bond strengths in excess of 30 kcal/mol routinely survive FD analysis.[11c]

The ion at *m/z* 929 in the hydrate spectrum (Figure 1b) corresponds to the protonated dihydrate of chlorophyll *a*. This ion, being an even electron ion, is probably a postionization adduct ion.

The results reported above support the view that, contrary to assertions in the literature,[1b,8c] it is possible (under admittedly unusual circumstances) to obtain chlorophyll *a* without coordinated water or any additional ligand on the central magnesium atom. The fact that the intensity of the mass spectrum substantially increased as did the intensity of the chlorophyll *a* molecular ion when the chlorophyll was hydrated prior to FD analysis suggests that carbonyl groups can effectively compete with water for coordination to the central magnesium of chlorophyll. The results indicate that the lattice energy of chlorophyll *a* hydrate is lower than that of anhydrous chlorophyll *a* in which the chlorophylls are associated by carbonyl-magnesium coordination.[1]

The anhydrous chlorophyll lattice is held together by magnesium-chlorophyll carbonyl coordination interactions.[1] The hydrated chlorophyll lattice is held together by coordination of water with the central magnesium atom and hydrogen bonding between the water and an adjacent chlorophyll carbonyl function. Since magnesium-oxygen coordination bonds are generally stronger than hydrogen bonds, the increase in intensity of the FD spectrum on hydration of the chlorophyll is to be expected. The anhydrous chlorophyll lattice is an interesting model for antenna chlorophyll in vivo.[1b,5d]

Water serves a role in the organization of the photoreaction center chlorophyll special pair[3b,5a] because it can bind chlorophylls by both magnesium-oxygen coordination and hydrogen bonding to carbonyl groups. The direction of water coordination in an unsymmetrical chlorophyll *a* special pair[3b,5a] would direct formation of a charge-transfer excited state of the special pair (Figure 2)[12] as the first step in photosynthesis. Removal of one electron by the primary electron acceptor in photosynthesis (L in Figure 2) would give the radical-cation dimer which is observed in EPR and ENDOR experiments[1,3] and would have a greater energy economy than a one-electron oxidation of a special pair which had been charge polarized by proton transfer.[8c] This model is compatible with the radical-pair mechanism for the primary light conversion event in photosynthesis.[13] We expect that continued investigation of the desorption behavior of chlorophyll *a* and its hydrates will shed light on this electron-transfer process.

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Natural Abundance ^{13}C NMR Spectroscopy of Double-Stranded DNA

Sir:

Although ^{13}C NMR spectroscopy has already proved extremely useful in studies of biopolymers,[1] including t-RNA's,[2] and single-stranded polynucleotides,[3] no successful study of native double-stranded DNA has been reported.[4] This failure is mainly due to extremely unfavorable ^{13}C spin relaxation parameters (very short T_2's and long T_1's) expected for the long and relatively rigid chains of typical DNA preparations (length >10 000 Å; weight $>2 \times 10^6$ Daltons).

Standard hydrodynamic calculations of rotational correlation times of rigid rod models suggested that the spectrum of short DNA molecules, on the order of 100 nucleotide pairs (NP), could be observed with modern instrumentation. DNA near this size can be obtained from native chromatin digested extensively with micrococcal nuclease. Histone binding to DNA in chromatin restricts access of the nuclease to specific sites. As a result, digestion yields DNA that is mostly near 140 NP in length along with some smaller fragments.[5,6] Unlike sonication, this well-studied method of reducing the size of DNA is very gentle and yields fragments that are almost entirely doubly stranded, with few unpaired nucleotides extending from the ends.[7]

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Table I

	1,[a,b] heat denatured DNA (52 mg/5 mL), 67.9 MHz[f]		2,[a,c] native DNA (117 mg/3.5 mL), 67.9 MHz		3,[a,d] native DNA (171 mg/3.5 mL), 67.9 MHz		4,[a,d] native DNA (87 mg/2.5 mL), 37.7 MHz		5,[a,d,e] ethidium bromide + native DNA (81 mg/2.5 mL), 37.7 MHz	
	ppm	$\Delta\nu_{1/2}$, Hz	ppm	$\Delta\nu_{1/2}$, Hz	ppm	$\Delta\nu_{1/2}$, Hz	ppm	$\Delta\nu_{1/2}$, Hz	ppm	$\Delta\nu_{1/2}$, Hz
					Pentose Carbons					
C-2′	38	114	37.5	243	37.0	196	37.5	150	40.5	338
C-3′	77	59	75.0	245	74.5	295[g]	76.5	165	76.0	
C-5′	66.5	64	64.5	117	64-70[g]		65.0	65	64.0	364
					Base Carbons					
T(Me)			12.0	117	12.0	137	12.5	60	15.0	234
T-5			110.5	176	110.5	114	111.0	120		
G-5			114.5	137	114.5	98	115.0	120		
A-5			116.5	125	116.5	82	117.5	105		

[a] Sample number. [b] Conditions: 90 °C, 0.1 M NaCl (pH 5.62). [c] Conditions: ambient temperature, 0.1 M NaCl (pH 5.62). [d] Conditions: ambient temperature, 42.2 mM Na_2PO_4, 15.6 mM $NaHPO_4$ (pH 7.35). [e] (Ethidium bromide)/(DNA phosphate) = 1:8. [f] ^{13}C frequency. [g] This peak is composed of several lines which may be noise.

Accordingly, for this study we isolated a large quantity of DNA from calf thymus chromatin digested to the limit, when the chromatin precipitates and ~50% of the DNA is rendered acid soluble.[8] Chromatography of this DNA on Bio-gel A0.5m (Bio-Rad Laboratories)[9] yielded fragments ranging from 110 to 170 NP long, with a median length of 140-145 NP.[10] Further treatment with micrococcal nuclease under conditions where it acts almost solely on single-stranded DNA[11] did not affect the electrophoretic mobility of the major band. The single-stranded DNA contents of preparations used for NMR studies were assessed from measurements of hyperchromicity upon thermal denaturation, and also from release of acid-soluble oligonucleotides upon treatment with single-strand specific nuclease S1[12] (Sigma). Results from both methods were in close agreement and indicated single-strand contents of 3-6% for the samples *after* NMR analyses. (The higher value probably is overestimated since a slow, nonspecific nuclease activity was noted in the S1 preparation.) The integrated ^{13}C spectra do not allow for major contributions from single-stranded DNA.

Two superconducting solenoid-based spectrometers were used to acquire ^{13}C NMR spectra at 67.9 and 37.7 MHz. High-field spectra were taken on a Bruker HX-270 and intermediate field spectra on the SEMINOLE spectrometer[13] equipped with a broad-band side-spinner design probe.[14] A sample spectrum of native DNA is shown in Figure 1. Table I summarizes data from five spectra: three of native DNA, one of heat denatured DNA, and one of native DNA containing intercalated ethidium bromide.

Quantitative interpretations of these preliminary spectra were not attempted, but several trends deserve comment. First, the assigned resonances of native DNA are quite close to literature values for mono- and oligonucleotides.[15-17] For example, C-1′ and C-5′ resonances are within 1.5 ppm of the positions reported for deoxyribonucleotides. C-2′ is shielded by ~2.5 ppm, while C-4′ and C-3′ are deshielded by 2-3 ppm relative to the mononucleotide resonances. Changes in shieldings of 0.4-3.4 ppm were observed for the base ring carbons.

The observed line widths for native ~140-NP DNA are also interesting. In general these are factors of 5 to 10 *less* than the line widths predicted from the rotational correlation time of a rigid rod, suggesting that internal torsional or librational motions contribute significantly to the spin relaxation. The nonprotonated ring carbon resonance line widths at each magnetic field (frequency) are nearly comparable with the sugar protonated-carbon line widths, whereas, in ^{13}C spectra of proteins, nonprotonated carbon lines are much narrower

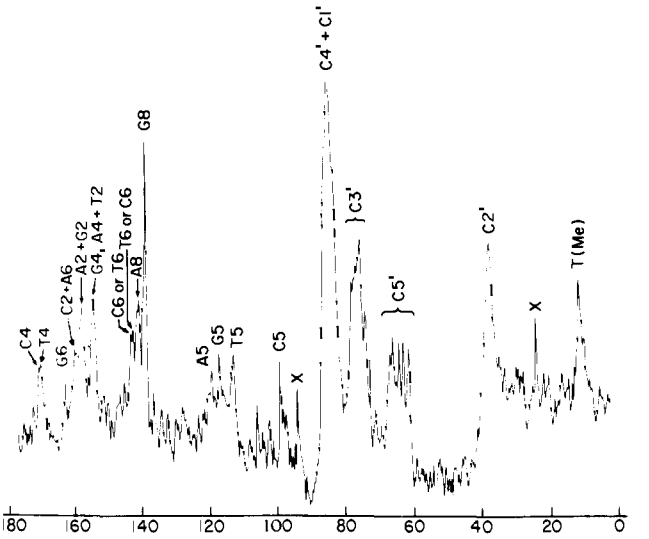

Figure 1. Natural abundance ^{13}C NMR spectrum of native DNA (sample 3 in Table I) at 67.9 MHz; 43 000 scans were accumulated with a cycle repeat time of 1.5 s. Chemical shifts are reported in parts per million relative to an external Me_4Si standard.

than CH lines (lower efficiency dipolar relaxation).[18] The relatively narrow sugar CH and CH_2 lines reflect additional internal motions, especially at C-5′. This flexibility seems restricted in the DNA-ethidium complex. The field dependence of several of the CH and nonprotonated carbon line widths is inverted, with narrower lines observed at the lower frequency. The narrower lines at 37.7 MHz could be due in part to susceptibility inhomogeneity broadening[19] or to the nature of torsional reorientation of C-H vectors along the "rigid" DNA rod (20 Å × ~500 Å). Most likely the larger line widths at higher field contain contributions from chemical-shift dispersion (subunits in different chemical environments). This will be investigated further.

Finally, heat denaturation significantly decreased the line widths observed for this short DNA, as expected from an increase in chain conformational flexibility. In contrast, intercalation of ethidium caused a significant increase in line widths, presumably due to an increase in length and/or a decrease in torsional flexibility. These line-width changes are consistent with established hydrodynamic data.[20]

Natural abundance ^{13}C spectroscopy of ~140-NP native DNA is now practical. Isolation of smaller double-stranded DNA molecules by the same methods (albeit in lesser yields),

combined with foreseeable improvements in probe and instrument design, should greatly improve spectral quality and permit careful examination of structural details of DNA alone and in combination with specific chemical agents.

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(9) The DNA was dissolved in 0.195 M NaCl, 5 mM Na_2EDTA, 10 mM Tris, 1 mM cacodylic acid, and 0.02% NaN_3. This DNA was fractionated on a Bio-Gel A0.5 m column (5 × 100 cm) eluted with the above buffer made 34% in sucrose (flow rate, 112 mL/h).
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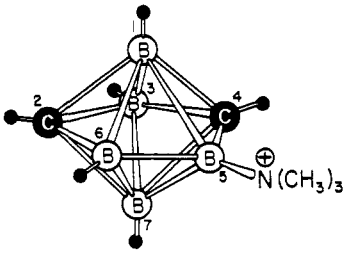
Synthesis of a Nitrogen Derivative of *closo*-2,4-$C_2B_5H_7$

Sir:

Most of the known boron-substituted derivatives of the nonicosahedral *closo*-carboranes, $C_2B_nH_{n+2}$ (n = 3-9), contain either halogen or alkyl attachments, and there are no known compounds containing boron-bonded group 5 substituents.[1,2] Furthermore, it is known that the parent *closo*-2,4-$C_2B_5H_7$ does not react with trimethylamine below the nucleophile decomposition temperature; however, secondary and primary amines cleave this pentagonal bipyramidal cage compound to noncage fragments.[3]

We report here a nitrogen derivative of *closo*-2,4-dicarbaheptaborane, [5-$(CH_3)_3$N-2,4-$C_2B_5H_6$]$^+$ (I), which is



I

prepared from 5-Cl-2,4-$C_2B_5H_6$ and trimethylamine followed by the removal of the halogen in what might be termed a "net two-step displacement" reaction. The first step is the formation of a 1:1 adduct (II) of the two reagents to form $(CH_3)_3N\cdot ClC_2B_5H_6$. The same adduct is formed both in the absence of solvent and also upon employing solvents such as methylene chloride or chloroform. Further, an excess of either reagent, the amine or the chlorocarborane, results in the formation of a 1:1 adduct only, with the excess reagent removed by vacuum distillation. The adduct (II), dissolved in methylene chloride or chloroform, exhibits proton decoupled ^{11}B NMR resonances at δ −19.4 (upfield), +2.3 (downfield), +6.3, +17.4 ppm from $(C_2H_5)_2O\cdot BF_3$ in an area ratio of 2:1:1:1; the undecoupled ^{11}B NMR spectrum shows the low-field peak at δ + 17.4 to be a singlet resonance and the other three resonances to be 1:1 doublets with $J(^{11}BH)$ = 179, ~130, and ~160 Hz from high to low field, respectively. Boron-11 decoupled proton resonances were observed at τ (relative to internal H_2CCl_2, τ 4.67) 2.37 (HC), 4.32 (HC), 5.43 (HB), 6.23 (HB), 6.60 (Me_3N), and 9.64 (HB) in an area ratio of 1:1:0.8:0.8:9:1.6, respectively. (Note that the ^{10}B isotope, ~20% of naturally occurring boron, remains coupled to the terminal boron-attached hydrogens and does not substantially contribute of the measured areas of the boron-11 decoupled proton singlets.)

The addition of BCl_3 to adduct II removes chloride ion to form the quaternary ammonium salt [5-$(CH_3)_3$N-*closo*-2,4-$C_2B_5H_6$]$^+$[BCl_4]$^-$ (I): ^{11}B NMR δ −19.3 ($J(^{11}BH)$ = 190 Hz) for B(1,7), +1.7 ($J(^{11}BH)$ = 156 Hz) for B(6), +6.3 ($J(^{11}BH)$ = 183 Hz) for B(3), +10.3[4] for BCl_4^-, +16.8 ppm for B(5); ^{11}B decoupled ^{1}H NMR τ 3.73 (m) for HC(2 or 4), 4.14 (m) for HC(4 or 2), 5.12 (skewed t, J[H(3), H(2)] ≃ J[H(3), H(4)] = 5 to 7 Hz) for HB(3), 5.79 (1:1 d, J[H(6), H(2)] ≃ 9 Hz) for H(6), 6.59 for $(CH_3)_3$N, 9.32 ppm for HB(1,7). The marked similarity of the ^{11}B NMR of I to both 5-Cl- and 5-CH_3-2,4-$C_2B_5H_6$[5-7] as well as the coupling patterns, particularly in the ^{11}B decoupled ^{1}H NMR,[8] leaves little doubt that the assigned substituted *closo*-carborane structure is correct. In addition, the trimethylphosphine analogue of compound I, prepared in a fashion similar to that for I, shows a ^{31}P-^{11}B(5) coupling of 158 Hz, verified by decoupling experiments. The structure of the initial adduct (II) is more speculative. By analogy to the 1:1 adduct of trimethylamine and 1,6-$C_2B_4H_6$,[3,9,10] adduct II might be expected to be that of a *nido*-C_2B_5 moiety,[11] but the close similarity of ^{11}B NMR resonance positions of the compound to those of 5-X-2,4-$C_2B_5H_6$ (X = Cl, CH_3)[5-7] suggests that II may have retained a substantial closo bipyramidal character. The broadness of the resonances additionally suggests some degree of cage fluxional behavior in which a closo framework equilibrates with the expected nido structure and/or with a [$(CH_3)_3$N-$C_2B_5H_6$]$^+$Cl$^-$ ion pair.

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