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# Solid-state $^{13}\text{C}$ CPMAS spectra of rapidly equilibrating carbocations

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O) by oxidation with  $I_2-H_2O$  (Scheme I). The latter step can be performed with either  $[^{17}O]-$  or  $[^{18}O]H_2O$  to obtain specifically labeled phosphate moieties **3** ( $Y = ^{17}O$  or  $^{18}O$ ),<sup>12</sup> as exemplified by compounds **6** and **5** in Table I. Reaction of **2** with either sulfur ( $S_8$ ) in 2,6-lutidine (0.4 M, 15 min, 60 °C) or KSeCN in  $CH_3CN$  (0.1 M, 1 h, 60 °C) gives **3** with either  $Y = S$  or Se, respectively. The PS moiety in **3** survived a relatively large number of subsequent cycles of synthesis, as demonstrated by isolation of T-[(pT)<sub>48</sub>]<sub>PS</sub>T (**41**). The variety of products listed in Table I indicated that "thiolation" can be achieved regardless of the nature of the bases in **2** and the value of  $n$ .

Although use of an ~10-fold molar excess of ~1:1 ( $R_p$ )- and ( $S_p$ )-**1a** could lead to highly diastereoselective formation of PS linkages, this was not found, even with diastereomerically enriched **1a**, which was attributed to epimerization at phosphorus in the tetrazoyl intermediate **1b**. In any event, most of the diastereomeric oligonucleotides (up to ~5 mg) were separable with more or less ease, depending on chain length, the position of the PS moiety in a given sequence, and the number of PS centers. A two-stage chromatographic procedure was used, starting with elution on a reverse-phase ( $C_{18}$ ) HPLC column to separate "5'-dimethoxytrityl" (DMT) product(s) from relatively fast-eluting fully deprotected failure sequences and 5'-DMT oxo byproduct (5-10% of the desired oligomer). The second stage of isolation involved detritylation<sup>11c</sup> and HPLC of the resultant 5'-HO-DNA. The presence of PS linkages was established by <sup>31</sup>P NMR spectroscopy, in selected cases, and in all cases by treatment with either snake venom phosphodiesterase (SVPDE, type II) or nuclease P1 followed, if necessary, by addition of alkaline phosphatase and then HPLC identification of the undigested dinucleoside phosphorothioate. The action of SVPDE, which is  $R_p$  selective,<sup>13</sup> was not sufficient for the assignment of absolute configuration at phosphorus in oligonucleotides having two adjacent PS centers, as this 3'-exonuclease did not hydrolyze  $R_pS_p$  and  $S_pS_p$  diastereomers. Parallel digestions with SVPDE and nuclease P1, which is  $S_p$  selective,<sup>4</sup> were therefore used to obtain complementary results that revealed the absolute configurations in question.

The self-complementary octamers **47** and **48** were tested as substrates for EcoRI endonuclease, which cleaves between G and A in duplex DNA having the sequence GAATTC.<sup>14</sup> Interestingly, it was found that neither of the two separated diastereomers of **47** was a good substrate for this enzyme, relative to oxo-**47**<sup>15</sup> and that ( $R_p$ )-**48** but not ( $S_p$ )-**48** was cleaved. The resistance of **47** toward hydrolysis by EcoRI implies that it may be possible to selectively "protect" restriction sites. Since the diastereomers of **48** represent modifications of internucleotide linkages with retention of ionic character, it appears that the recognition process may not involve a purely Coulombic interaction between the protein and phosphate groups that are not at the site of cleavage;<sup>14</sup> however, further structural and biochemical studies are now in progress to clarify this point.

The presently described procedures, which may be performed manually<sup>16,17</sup> and can be applied to <sup>35</sup>S labeling, have been used to prepare longer mono- and polyphosphorothioate analogues of oligonucleotides for studies that will be reported elsewhere.

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## Solid-State <sup>13</sup>C CPMAS Spectra of Rapidly Equilibrating Carbocations

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We report studies of the variable-temperature solid-state CPMAS <sup>13</sup>C NMR spectra of four carbocations (**1-4**), all of which

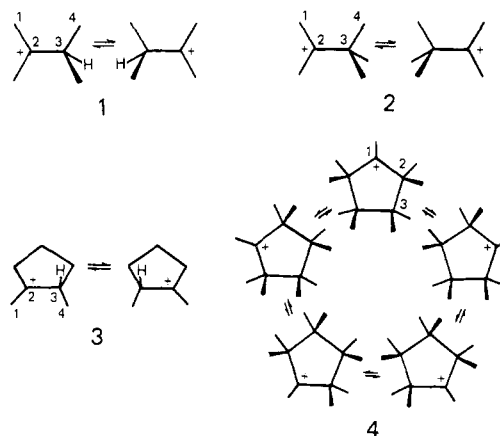


exhibit average spectra in solution owing to rapid degenerate rearrangements at all accessible temperatures.<sup>1</sup> In contrast, the solid-state CPMAS spectra show resonances expected for static classical ions at low temperatures. At higher temperatures (about 200 K), the spectra show these classical ions undergoing rapid degenerate rearrangement. Table I lists the chemical shift data observed at the upper and lower temperatures of this solid-state investigation,<sup>2</sup> together with comparisons of liquid-state values. The most interesting feature, however, is the appearance of the spectra at intermediate temperatures.

If the dynamics of these ions in the solid conformed to solution behavior, one would expect line broadening and coalescence as the system passes from the slow to the fast exchange regime. The spectra of the 2,3-dimethyl-2-butyl cation (**1**) in solid  $SbF_5$  (Figure 1) do not show this. Instead, the spectra at temperatures between 193 and 128 K show *both* static and rapidly equilibrating classical ions in apparent coexistence, with decreasing amounts of static ion as the temperature increases. The spectral patterns found for the 2,3,3-trimethyl-2-butyl cation (**2**), 1,2-dimethylcyclopentyl cation (**3**), and cyclopentyl cation (**4**) are similar, although some evidence of line broadening and coalescence is observed, especially in the cases of **3** and **4**.

Cycling the sample temperature indicates that the relative populations of static and dynamic forms can be reproduced. Hence, the spectral observations do not appear to be due to transient phenomena; rather, they indicate an equilibrium property of the system at a given temperature. By changing the solid matrix

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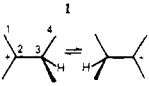
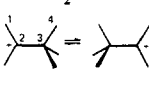
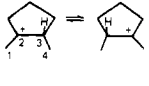
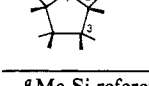
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Table I. Spectral Data for Ions 1-4

Ion	Temp, K (°C)	State	Chemical Shift, PPM <sup>a</sup>			
			1	2	3	4
	193 (-80)	solid	31	200	200	31
	203 (-70)	soln <sup>b</sup>	32	198	198	32
	128 (-145)	solid	40	335	59	17
	predicted low temp spectrum <sup>c</sup>		43	336	59	22
	193 (-80)	solid	31	195	195	31
	193 (-80)	soln <sup>c</sup>	33	197	197	33
	165 (-108)	solid	41	330	56	--
	predicted low temp spectrum <sup>c</sup>		41	337	58	29
	199 (-74)	solid	23	200	200	23
	203 (-70)	soln <sup>b</sup>	23	203	203	23
	128 (-145)	solid	33	329	75	12
	213 (-60)	solid	99	99	99	--
	193 (-80)	soln <sup>d</sup>	99	99	99	--
	70 (-203)	solid	320	71	28	--
	predicted low temp spectrum <sup>d</sup>		300	65	28	--

<sup>a</sup> Me<sub>4</sub>Si reference, external for solids; chemical shifts of solid samples are believed to be reliable to  $\pm 3$  ppm. <sup>b</sup> Reference 12. <sup>c</sup> Reference 4a. <sup>d</sup> Reference 13.

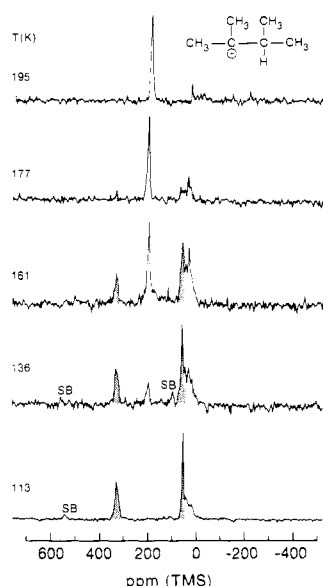


Figure 1. Temperature dependence of <sup>13</sup>C CPMAS spectra of the 2,3-dimethyl-2-butyl cation (1) (enriched with <sup>13</sup>C at C2 and C3) showing the coexistence of static and rapidly equilibrating cations. The shading is used to highlight the resonances of the charged (335 ppm) and methyne (59 ppm) carbons in the static ion. Spectra were obtained using both <sup>1</sup>H and <sup>19</sup>F decoupling.

from SbF<sub>5</sub> to a mixture of magic acid (HSO<sub>3</sub>F and SbF<sub>5</sub>) plus some SO<sub>2</sub>ClF, the relative populations of coexisting static and dynamic ions can be altered significantly at a given temperature. This indicates that the phenomenon is a function of the matrix (or counterion) as well as of the structure of the carbocation.

Explicit note should be made of the high-temperature CPMAS spectrum of the 2,3,3-trimethyl-2-butyl cation (2), which shows only two carbon resonances at 195 and 31 ppm (Me<sub>4</sub>Si) and no evidence for a methyl peak that would be expected in a bridged structure. Thus, one can discount the notion that the spectra of 2 in the solid (and by inference 1 and 3) are interpretable as bridged or nonclassical ions.

The low-temperature spectra represent a clear demonstration of the classical structure of these rapidly equilibrating ions. These results support earlier conclusions based on chemical shift

analyses,<sup>4</sup> isotopic perturbation studies,<sup>5</sup> and line-broadening studies<sup>1</sup> conducted in the solution state. The observation of coexisting static and rapidly equilibrating forms of these carbocations at intermediate temperatures requires further comment. Two points should be noted. First, spectra of ions 1-4 in their "frozen" state are observed in the solid at temperatures well above solution temperature limits; solution-state spectra at the same temperatures would show rapidly equilibrating ions. It seems reasonable that a solid lattice would, in general, inhibit a degenerate rearrangement, thus increasing the temperature at which most ions will appear "frozen out".<sup>3</sup> Second, all carbocations that have been examined in solid SbF<sub>5</sub> show resonances that are much broader than normally observed in CPMAS spectral studies. This line broadening has been attributed to a distribution of sites within the amorphous solid.<sup>3,6</sup> Correspondingly, we assume that different sites in solid SbF<sub>5</sub> would also impose differing constraints on the rate of degenerate rearrangement with the result that the assembly of ions in the solid might exhibit a range of rates of rearrangement. Given this, one can simulate the spectral behavior of the 2,3-dimethyl-2-butyl cation by assuming a broad distribution of rates and a temperature dependence of the distribution function.<sup>7</sup> Similar spectral behavior has been observed for the hydrogen bond tautomerism in crystalline naphthazarin and has been interpreted as a second-order phase transition.<sup>6,8</sup> Although we have no experimental data that unequivocally distinguishes between the multisite and phase-transition models, the inhomogeneous nature of the SbF<sub>5</sub> matrix<sup>9</sup> and concomitant evidence for site distribution in the <sup>13</sup>C line widths lead us to favor the multisite model.<sup>10</sup>

Independent of the manner in which these results are modeled, they do suggest that the solid-state effect on the rate of degenerate rearrangements of carbocations is a variable one, particularly in a lattice as poorly defined as solid SbF<sub>5</sub>. Furthermore, unlike the situation in liquids, it may not be possible to specify a rate constant in such a solid, since a distribution of rates may be found at any one temperature.

It is important to ask why this kind of effect was not observed in previously reported kinetic studies of the 6,2,1-hydride shift in the norbornyl cation.<sup>11</sup> We suggest that the importance of the solid-state effect will be a marked function of the symmetry and shape of the ion. Ions approaching spherical symmetry could, by virtue of their internal rotations, or by the template that they present to the lattice, be much less affected by differences in the lattice sites with the net effect that their solid-state dynamics appear much more liquidlike. The progression toward liquidlike behavior observed on going from ion 1 to 2 to 3 to the norbornyl cation seems consistent with this view.

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**Registry No.** 1, 17603-18-8; 2, 17603-19-9; 3, 26378-06-3; 4, 25076-72-6; SbF<sub>5</sub>, 7783-70-2; SO<sub>2</sub>ClF, 13637-84-8; magic acid, 23854-38-8.

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