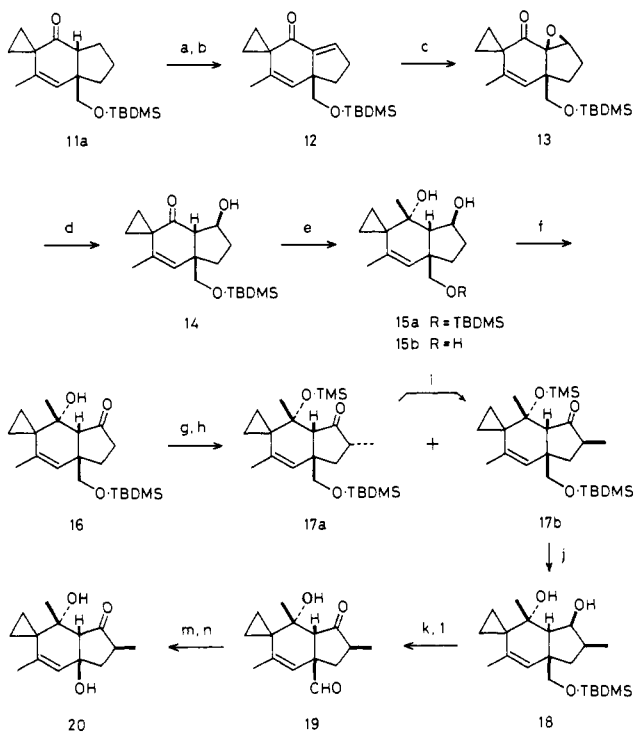
Scheme III[a]



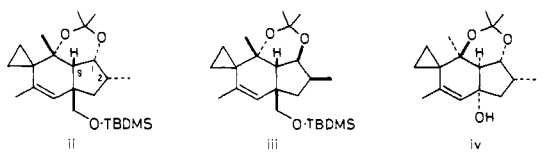
[a](a) LDA, then PhSeCl, THF, −78 °C, 1 h; (b) 30% H_2O_2, pyr, CH_2Cl_2, 23 °C, 40 min; (c) 30% H_2O_2, NaOH, MeOH, 10 °C, 6.5 h; (d) Ca, liquid NH_3/THF (2:1), −78 °C, 2 h; (e) MeMgI, ether, 23 °C, 1.5 h; (f) DMSO, $(COCl)_2$, CH_2Cl_2, −60 °C, 15 min, then Et_3N, −60 °C → 23 °C, 30 min; (g) LDA (10 equiv), then Me_3SiCl, DME, 0 °C, 15 min → 23 °C, 1 h; (h) $PhCH_2NMe_3{\cdot}F$, MeI, molecular sieves 4A, THF, 23 °C, 2 h; (i) *t*-BuOK, *t*-BuOH, 30 °C, 4 h; (j) $LiAlH_4$, ether, 23 °C, 30 min; (k) Bu_4NF, THF, 45 °C, 23 h; (l) DMSO, $(COCl)_2$, CH_2Cl_2, −68 °C, 15 min, then Et_3N, −68 °C → 23 °C, 35 min; (m) O_2, EtOAc, 50 °C, 18 h; (n) PPh_3, ether, 23 °C, 1 h.

Swern oxidation of which furnished ketone **16** (98%). The X-ray crystallographic analysis of racemic triol **15b**[14] obtained by desilylation of racemic **15a**[14] (Bu_4NF, THF) confirmed the assigned stereochemistry of **15a** as indicated. Monomethylation α to the keto group in **16** was executed by the Kuwajima procedure:[15] the enol silyl ether prepared from **16** reacted with MeI in the presence of $PhCH_2NMe_3F$ to give a separable mixture of two diastereomers, **17a** (37%) and **17b** (14%), the former **17a** being converted into the latter **17b** by base treatment (75%). The thermodynamically more stable isomer **17b** was shown to have the desired stereochemistry regarding the secondary methyl group.[16] Transformation of **17b** into aldehyde **19** (95% overall) was effected through the sequence: (1) reduction of the keto group and removal of the TMS group to give **18**; (2) deprotection of the TBDMS group; (3) Swern oxidation.

(14) Mp of racemic **15b**, 146–148 °C. Racemic **15a** was available by an alternative synthetic route starting from α-allyl-δ-valerolactone: Kigoshi, H.; Sawada, A.; Nakayama, Y.; Niwa, H.; Yamada, K., unpublished results.

(15) (a) Kuwajima, I.; Nakamura, E. *J. Am. Chem. Soc.* **1975**, *97*, 3257. (b) Kuwajima, I.; Nakamura, E.; Shimizu, M. *J. Am. Chem. Soc.* **1982**, *104*, 1025.

(16) Stereochemistry of the secondary methyl groups in **17a** and **17b** was established by the ^1H NMR spectral analysis: **17a** and **17b** were converted in two steps (1. $LiAlH_4$; 2. $CH_2{=}C(Me)OMe$, H^+) into conformationally rigid derivatives, ii and iii, respectively, and their coupling constants (ii, $J_{1,2} = J_{1,9} = 4.0$ Hz; iii, $J_{1,2} = J_{1,9} = 9.6$ Hz) were compared with those ($J_{1,2} = J_{1,9} = 9.7$ Hz) of the compound iv derived from natural **1**.



The final phase of the synthesis was oxidative removal of the angular formyl group in **19** to introduce a hydroxyl group at the ring juncture under the conditions mild enough for the unstable product ptaquilosin (**20**) to survive. Thus, the concentrated solution of **19** in EtOAc under the oxygen atmosphere was warmed at 50 °C to afford a hydroperoxide,[17] which was reduced with PPh_3 providing (+)-ptaquilosin (**20**)[18] (37%) as a colorless oil, identical with natural (−)-**2**[18,19] in every respect (^1H NMR, IR, MS, α_D, TLC) except for the sign of specific rotation.

Acknowledgment. We thank Prof. H. Yamamoto and Dr. K. Furuta, Faculty of Engineering, Nagoya University for providing us with the experimental details for the preparation of **4** prior to publication. This work was supported in part by Grant-in-Aids for Encouragement of Young Scientists (No. 60740290 and 62740308 to H.K.) from the Ministry of Education, Science, and Culture, Japan.

Supplementary Material Available: Spectral and physical data for compounds **5** and **7–20** and X-ray crystallographic data for racemic **15b** (12 pages). Ordering information is given on any current masthead page.

(17) This deformylation–oxygenation reaction could also be effected in benzene at 50 °C in the presence of AIBN in less yield.

(18) Synthetic **20**: $[\alpha]_D^{20}$ +232° (*c* 0.17, $CHCl_3$). Natural **2**: $[\alpha]_D^{20}$ −246° (*c* 0.82, $CHCl_3$).

(19) Natural **2** was derived from **1** by chemical means: Kigoshi, H.; Sawada, A.; Imamura, Y.; Niwa, H.; Yamada, K., unpublished results.

DNA Structural Data from a Dynamics Probe. The Dynamic Signatures of Single-Stranded, Hairpin-Looped, and Duplex Forms of DNA Are Distinguishable

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Received September 26, 1988

Efforts to establish structure-function relationships involving nucleic acids have focused attention upon a variety of non-B conformations of DNA, for example, A-,Z-bent, and hairpin-looped conformations.[1] When such features are embedded within B-DNA, as would be the case in vivo, spectroscopic structural assignment is complicated because the region of interest constitutes only a small portion of the macromolecule. The presence of unusual structures within large DNA's is often inferred from differential chemical reactivity;[2] the possibility of dynamic equilibrium among two or more DNA conformations complicates interpretation of such data. Spectroscopic methods which provide information about structural elements which constitute a small portion of the DNA are thus of great interest. EPR spectroscopy has been widely used to monitor local dynamic features of macromolecules; should a correlation of DNA local structure and dynamics exist, the EPR technique in combination with site-specific DNA spin labeling[3] would become a powerful tool in DNA structural studies.

We have previously reported that a nitroxide spin-labeled analogue of thymidine (e.g., **1**, T*) may be incorporated by automated chemical synthesis into deoxyoligonucleotides and that this probe does not significantly perturb the solution B-structure of the duplex form of 5′-d(CGCGAATT*CGCG).[3] EPR studies of this duplex indicated that the spin probe's effective rotational

(1) (a) Weintraub, H. *Cell* **1983**, *32*, 1191. (b) Elgin, S. C. R. *Nature* **1984**, *309*, 213.

(2) Furlong, J. C.; Lilley, D. M. J. *Nucleic Acids Res.* **1986**, *14*, 3995 and references 18–25 cited therein.

(3) Spaltenstein, A.; Robinson, B. H.; Hopkins, P. B. *J. Am. Chem. Soc.* **1988**, *110*, 1299.

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correlation time (τ_r) was close to that predicted for tumbling of the macromolecule in the aqueous environment, demonstrating that any local motion of the spin label independent of the macromolecule occurs on a time scale comparable to or slower than tumbling of the dodecamer duplex. We now report examples in which incorporation of this spin probe into single-stranded (**2**), nonbase-paired loop regions (**3**), and larger double-stranded regions (**5**) of DNA afford readily distinguishable spectra. Comparison of these spectra with simulated spectra of objects with varying rates of isotropic motion reveals a positive correlation of the degree of structural organization with dynamic restraint. These EPR spectra thus constitute useful "dynamic signatures" of DNA structures. Reported here are the dynamic signatures of single-stranded, nonbase-paired looped, and double-stranded regions of DNA.

The spin-labeled DNA's **1–5** shown in Figure 1 were synthesized[3] and purified by sequential polyacrylamide gel electrophoresis and gel filtration on Sephadex G-15.[4] These oligonucleotides were expected to adopt solution structures including single-stranded (**2**), hairpin-looped (**3** and **4**), and duplex (**5**) forms. EPR spectra of the synthetic oligonucleotides were measured in aqueous solution (100 mM NaCl, 10 mM pH 7.0 phosphate buffer, 0.1 mM EDTA, 0°, 0.1 mM in single strands) and are shown in Figure 1 overlaid in each case with a simulated isotropic spectrum[5] which was visually judged to be an acceptable fit.

Incorporation of the monophosphate **1**, which exhibits subnanosecond motion (τ_r ca. 0.1 ns), into single strand **2** slows the probe to τ_r ca. 1 ns. The EPR spectrum of **3**, a spin-labeled hairpin loop,[6] is consistent with a further dynamic constraint (τ_r ca. 3 ns) relative to that of a single strand. Importantly, oligonucleotide **4**, which differs from **3** only in the position of the spin label, affords an EPR signature (τ_r ca. 10 ns) distinct from that of **3** but essentially indistinguishable[7] from that previously observed for the corresponding spin-labeled dodecamer (5′-d(CGCGAATT*CGCG)) lacking the dT_5 loop. That is, the stem-labeled hairpin **4** affords the dynamic signature of a duplex whose dynamics are dominated by overall molecular tumbling, while the EPR spectrum of the hairpin loop region (**3**) is diagnostic of motion more rapid than global tumbling.[8] The EPR spectrum (not shown) of the oligonucleotide 5′-d(CGCGAATT*ACGCG), a base sequence which in unlabeled form has been shown to be a hairpin,[9] is virtually indistinguishable from that of **3**, despite differences in the base composition of the loop, position of the spin label within the loop, and overall molecular size. The EPR spectrum of a spin labeled hairpin is thus distinguishable from that of a single strand yet is still dominated by a motion more rapid than global molecular tumbling.

The EPR spectrum of oligonucleotide **5** (5′-d(GCGAATT*CGCGCGCGC)), which was designed to self assemble as shown into a duplex of indefinite length, is representative of the EPR spectra we have observed for several such self-assembling sequences. This signature is not appreciably altered by enzymatic ligation (T4 DNA ligase) of the 5′-phosphate analogue of **5** to form "sticky ended" multimers composed of single strands up to 96 bases in length, implying that the "gaps" in the unligated self-assembling sequences do not alter the signature of the spin-

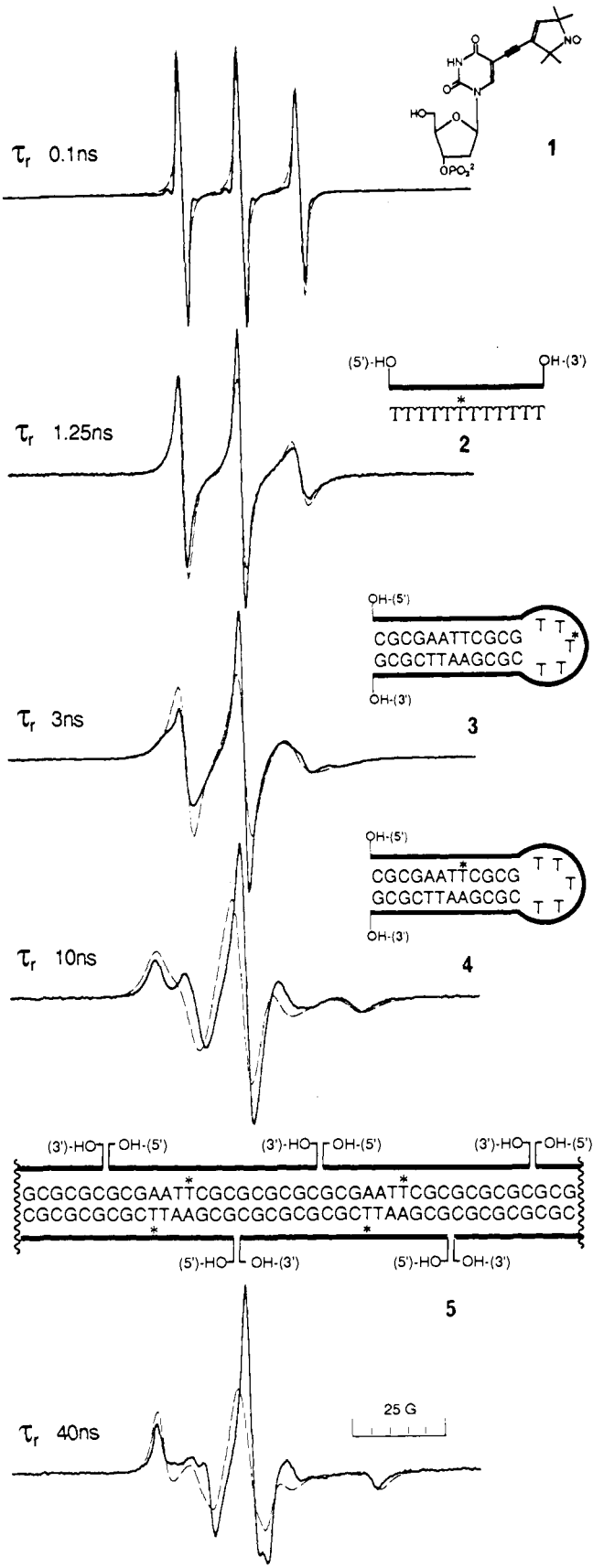

Figure 1. EPR spectra of **1–5** (—) overlaid with a simulated spectrum of an isotropically tumbling object (– –) with the indicated τ_r.

(4) Subsequent to the completion of our preliminary study,[3] we learned that the duration of heating of aqueous solutions of DNA containing spin probe **1** (~90°) correlates with the subnanosecond component in EPR spectra at 0°. We currently believe that this results from thermal depyrimidination of the labeled residue. Size exclusion chromatography on Sephadex virtually eliminates this component.

(5) Spectra were simulated assuming isotropic rotational Brownian diffusion. The following parameters were assumed: $A_{xx} = A_{yy} = 8$ G, $A_{zz} = 32$ G; $g_{xx} = 2.0088$, $g_{yy} = 2.0060$, $g_{zz} = 2.0022$; $T_{2e} = 0.05$ μs.

(6) A concentration dependence of the UV-monitored thermal denaturation profiles of **3** and **4** similar to that observed for 5′-d(CGCGCGTTTTTCGCGCG) (see: Xodo, L. E.; Manzini, G.; Quadrifoglio, F.; van der Marel, G. A.; van Boom, J. H. *Nucleic Acids Res.* **1986**, *14*, 5389) suggests the presence of some higher order structures.

(7) Sharpening of the high and low field features of the EPR spectrum of **4** relative to duplex 5′-d(CGCGAATT*CGCG) is consistent with **4** tumbling slightly more slowly as a result of the dT_5 loop.

(8) (a) Ikuta, S.; Chattopadhyaya, R.; Ito, H.; Dickerson, R. E.; Kearns, D. R. *Biochemistry* **1986**, *25*, 4840. (b) Williamson, J. R.; Boxer, S. G. *Nucleic Acids Res.* **1988**, *16*, 1529.

(9) (a) Miller, M.; Kirchoff, W.; Schwarz, F.; Apella, E.; Chiu, Y. H.; Cohen, J. S.; Sussman, J. L. *Nucleic Acids Res.* **1987**, *15*, 3877. (b) Roy, S.; Weinstein, S.; Borah, B.; Nickol, J.; Apella, E.; Sussman, J. L.; Miller, M.; Shindo, H.; Cohen, J. S. *Biochemistry* **1986**, *25*, 7417.

labeled duplex. As might be anticipated, none of the simulated isotropic spectra provide an especially good fit to the dynamic signature of the distinctly anisotropic[10] long duplex DNA. The spectrum does, however, contain features found in isotropic simulations in the 20–100 ns τ_r range.[11] For the present purposes, the signature of a large DNA duplex is easily distinguished from that of the hairpin and single-stranded forms.[12]

The spectra presented here suggest that spin-labeled DNA in combination with EPR is a simple and powerful tool for the structural evaluation of regions of DNA suspected not to exist in the B form.[13] Large and characteristic differences between duplex and unpaired bases should permit detection of the latter in DNA of any size. Furthermore, the EPR method is not subject to one major problem common to all chemical reactivity probes, that of dynamic equilibration of conformations. In fact, the nanosecond time scale of the EPR method should permit quantitation of such equilibria (e.g., duplex/cruciform equilibria). This spin label method of structure evaluation may also find use as a preliminary characterization of small DNA's whose study by NMR or X-ray diffraction is contemplated. Whether spin probes will reveal distinct signatures for other nucleic acid structures (e.g., A-, Z-, and bent DNA, RNA structures) remains to be found.

Acknowledgment. We thank the National Science Foundation (PCM-82-16762 and DMB-87-06175), National Institutes of Health (GM32681), and the Searle Scholars Program for financial support and E. Hustedt for technical assistance. P.B.H. thanks the National Institutes of Health for a Research Career Development Award (1988–1993) and is a Sloan Fellow (1988–1990).

(10) Barkley, M. D.; Zimm, B. H. *J. Chem. Phys.* **1979**, *70*, 2991.
(11) Both theoretical and experimental studies are underway to understand the observed spectra.
(12) Bobst has reported minor differences in the single- and double-stranded forms of a less rigidly tethered spin-labeled DNA (see: Bobst, A. M.; Kao, S.-C.; Toppin, R. C.; Ireland, J. C.; Thomas, I. E. *J. Mol. Biol.* **1984**, *173*, 63). However, extensive motion of the spin probe independent of the DNA caused the resulting spectra for even long duplex DNA to be dominated by a subnanosecond motion, seriously compromising the sensitivity of such probes to the motional processes characteristic of DNA.
(13) To what extent motion of the nitroxide independent of the attached base has affected the EPR signatures recorded here is unknown. The cited τ_r values should not be assumed to reflect exclusively motion of the pyrimidine heterocycle.

Stereoselective Circumambulatory Methyl Migrations in the Nonamethylcyclopentylium Ion

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Received July 11, 1988

Degenerate 1,2-rearrangements of carbocations are usually very rapid, and dynamic NMR spectroscopy has been used to determine the barriers of such processes.[1] Saunders reported a barrier of 3.1 kcal/mol for the 1,2-H shift in the dimethylcyclopentyl cation **1**.[2] As the barriers for 1,2-H and 1,2-CH_3 migrations are of similar magnitude in the tetramethyl- and pentamethylethyl cation,[3] one can estimate the bridged cation **2a** to be 3–4 kcal/mol less stable than the open cation **2b**.

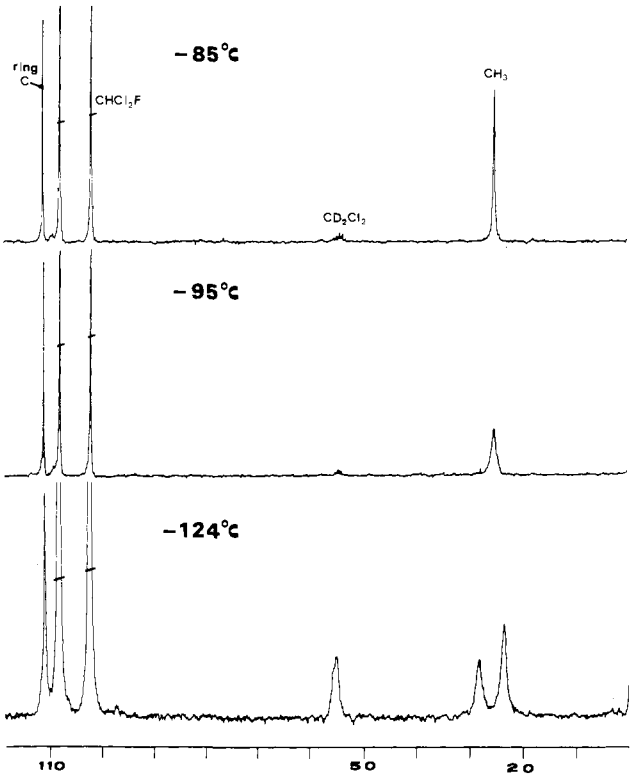
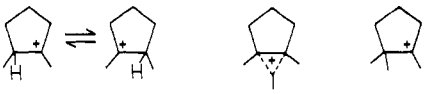


Figure 1. ^{13}C NMR spectra of the nonamethylcyclopentylium ion in $FSO_3H/SbF_5/SO_2ClF/CHCl_2F$.



It should be possible to overcompensate this small energy difference by torsional strain, and we had speculated that the nonamethylcyclopentylium ion **3** might possess a methyl-bridged structure, since the classical structure **3a** incorporates four adjacent quaternary carbon centers.

When nonamethylcyclopentanol **4**,[4a] prepared from octamethylcyclopentanone[4b,c] and CH_3Li, was dissolved in a solution of FSO_3H/SbF_5 in SO_2ClF[5] at −90 °C, a single peak (δ 1.86) was observed in the 200 MHz ^1H NMR spectrum, indicating total scrambling of all methyl groups, analogous to the spectra previously reported for the unsubstituted cyclopentylium ion.[6] Decomposition of **3** with formation of *tert*-butyl cations takes place at $T > -70$ °C. When the solution of **3** in $SO_2ClF/CHCl_2F$ was cooled, the ^1H NMR signal broadened, and, at −137 °C, two resonances at δ 2.00 and 1.63 with relative intensities 5:4 were observed.

(1) Reviews: (a) Saunders, M.; Chandrasekhar, J.; Schleyer, P. v. R. In *Rearrangements in Ground and Excited States*; de Mayo, P., Ed.; Academic Press: New York, 1980; Vol. I, p 1. (b) Siehl, H.-U. *Adv. Phys. Org. Chem.* **1987**, *23*, 63. (c) Olah, G. A.; Surya Prakash, G. K.; Sommer, J. *Superacids*; Wiley: New York, 1985; p 128. (d) Vogel, P. *Carbocation Chemistry*; Elsevier: Amsterdam, 1985; pp 142. (e) Telkowski, L. A.; Saunders, M. In *Dynamic Nuclear Magnetic Resonance Spectroscopy*; Jackman, L. M., Cotton, F. A., Eds.; Academic Press: New York, 1975; p 523. (f) Oki, M. *Applications of Dynamic NMR Spectroscopy to Organic Chemistry*; VCH Verlagsgesellschaft: Weinheim, 1985; p 378. (g) Brown, H. C. (with comments by Schleyer, P. v. R.) *The Nonclassical Ion Problem*; Plenum Press: New York, 1977. (h) Shubin, V. G. *Top. Curr. Chem.* Springer: Berlin, 1984; Vol. 116/117, p 267.
(2) Saunders, M.; Telkowski, L.; Kates, M. R. *J. Am. Chem. Soc.* **1977**, *99*, 8070.
(3) Saunders, M.; Kates, M. R. *J. Am. Chem. Soc.* **1978**, *100*, 7082.
(4) (a) Wehle, D.; Scheuermann, H.-J.; Fitjer, L. *Chem. Ber.* **1986**, *119*, 3127. (b) Fitjer, L.; Wehle, D.; Scheuermann, H.-J. *Chem. Ber.* **1986**, *119*, 1162. (c) Mayr, H.; Koschinsky, R.; Will, E.; Bäuml, E. *J. Org. Chem.* **1987**, *52*, 1342.
(5) Reference 1c, p 42.
(6) (a) Olah, G. A.; Lukas, J. *J. Am. Chem. Soc.* **1968**, *90*, 933. (b) Olah, G. A.; White, A. M. *J. Am. Chem. Soc.* **1969**, *91*, 3954. (c) Myhre, P. C.; Kruger, J. D.; Hammond, B. L.; Lok, S. M.; Yannoni, C. S.; Macho, V.; Limbach, H. H.; Vieth, H. M. *J. Am. Chem. Soc.* **1984**, *106*, 6079.

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