This implies that the residues of both homopolymers may have alternating conformations. Thus, each homopolymer might be syndiotactic or have some other alternating form. Alternating forms of polynucleotides have been observed previously but in all cases there has also been an alternating sequence.[9] The NMR results do not discriminate between the wide variety of possible alternating forms nor are they readily interpretable in terms of conformational or other property, such as solvation,[10] which might induce chemical shift differences. However, now that ^{31}P signals can be reliably assigned the information from additional assignments may well lead to an understanding of the origin of the chemical shift dispersion of nucleic acids. The following communication describes the applications of this method to oligonucleotides.[11]

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Registry No. ^{17}O, 13968-48-4; [^{17}O]poly(I)·[^{17}O]poly(C), 88295-88-9; [^{17}O]poly(A)·[^{17}O]poly(U), 88295-92-5.

A General Procedure for Assigning the ^{31}P Spectra of Nucleic Acids

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Although the ^{31}P NMR resonances associated with the phosphodiester groups in the sugar–phosphate backbones of oligonucleotides frequently show sufficient dispersion in chemical shift to permit detection of individual resonances, little quantitative use of the chemical shift data in characterizing conformation and dynamics has been possible due to the difficulty in assigning the resonances to particular nuclei in the backbone. A recently reported solution to this problem is to identify the 3′- and 5′-^1H nuclei coupled to each ^{31}P nucleus either by decoupling experiments[2] or by two-dimensional heteronuclear chemical shift correlation spectroscopy,[3] but both of these techniques require resolved and assigned ^1H NMR spectra. In this communication we describe a general method for the assignment of the resonances in the ^{31}P NMR spectra of oligonucleotides. Our approach is based on labeling the nonesterified phosphoryl oxygens of the backbone with ^{17}O in a sequence-specific manner; the efficient scalar relaxation of the second kind provided to the ^{31}P nucleus by the directly bonded ^{17}O nucleus causes extensive line broadening and a decrease in the apparent intensity of the associated ^{31}P NMR resonance.[4,5] To illustrate this method, we have prepared two samples of the self-complementary tetradeoxynucleoside triphosphate d(CpGpCpG) labeled in each d(CpG) unit with a single atom of ^{17}O and used their spectra to assign the ^{31}P NMR resonances of the oligonucleotide both in the absence and in the presence of the intercalative drug actinomycin D.

A solution phosphotriester method was used for the synthesis of unlabeled d(CpGpCpG) and two samples labeled in each d(CpG) unit with a single atom of ^{17}O.[6,7] This chemistry involves the coupling of a protected d(CpGp) having a 3′-(chlorophenyl phosphate) group with a protected d(CpG) having a free 5′-hydroxyl group; 1-(*p*-tolylsulfonyl)-4-nitroimidazole was used as the coupling reagent. The labeled d(CpGp) and d(CpG) were prepared from [^{17}O]$POCl_3$ by the hydroxybenzotriazole method described by van Boom and co-workers;[8] the isotopic composition of the labeled phosphoryl oxygen was 35.5% ^{16}O, 38.3% ^{17}O, and 26.2% ^{18}O.[9] Following purification of the completely protected tetramers on silica gel and removal of the protecting groups,[10] the samples of d(CpGpCpG) were isolated by ion-exchange chromatography on DEAE cellulose using a triethylammonium bicarbonate gradient as the eluent.[11] The ^1H NMR spectra at 500 MHz were in excellent agreement with literature data[12–14] and demonstrated a very high degree of chemical purity.

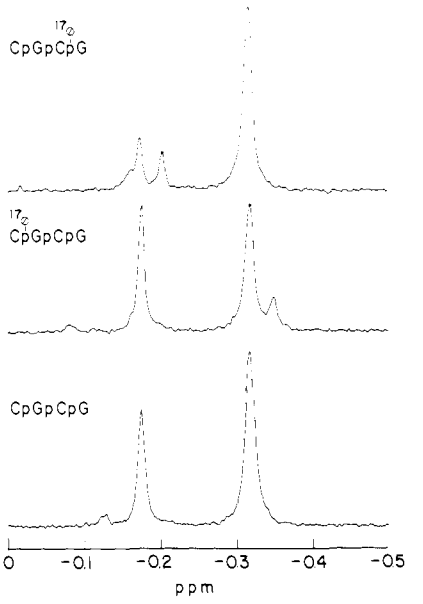

Figure 1. Proton-decoupled ^{31}P NMR spectra at 202.5 MHz and 30 °C of samples of 10 mM d(CpGpCpG) dissolved in D_2O containing 0.1 M sodium cacodylate, pH 7.0, and 2 mM EGTA. The spectra are of the unlabeled sample (bottom), the sample labeled with ^{17}O in the 3′-terminal d(CpG) unit (top), and the sample labeled with ^{17}O in the 5′-terminal d(CpG) unit (middle). Chemical shifts are measured relative to external 85% H_3PO_4.

The ^{31}P NMR spectra of the unlabeled and labeled oligonucleotides were obtained at 202.5 MHz and 30 °C, and these are reproduced in Figure 1. The spectrum of the unlabeled material (bottom spectrum) reveals two resonances in an intensity ratio of 1:2. The spectrum of the material labeled in the 3′-terminal d(CpG) unit (top spectrum) shows that the intensity of the downfield signal is diminished by the degree of ^{17}O enrichment. The remaining signal for this phosphodiester group is composed of two resonances of approximately equal intensity that are separated by 0.030 ppm; these resonances are associated with unlabeled and ^{18}O-labeled oligonucleotides which are present by virtue of the isotopic composition of the $POCl_3$ used to prepare the dimeric units. The spectrum of the material labeled in the

(1) NIH Research Career Development Awardee (CA-00499), 1978–1983. Fellow of the Alfred P. Sloan Foundation, 1981–1983.
(2) Cheng, D. M.; Kan, L.-S.; Miller, P. S.; Leutzinger, E. E.; Ts'o, P. O. *Biopolymers* **1982**, *21*, 697.
(3) Pardi, A.; Walker, R.; Rapoport, H.; Wider, G.; Wüthrich, K. *J. Am. Chem. Soc.* **1983**, *105*, 1652.
(4) Tsai, M. D. *Biochemistry* **1979**, *18*, 1468.
(5) Lowe, G.; Potter, B. V. L.; Sproat, B. S.; Hull, W. E. *J. Chem. Soc., Chem. Commun.* **1979**, 733.
(6) Gough, G. R.; Singleton, C. G.; Weith, H. L.; Gilham, P. T. *Nucleic Acids Res.* **1979**, *6*, 1557.
(7) Gough, G. H.; Collier, K. J.; Weith, H. L.; Gilham, P. T. *Nucleic Acids Res.* **1979**, *7*, 1955.
(8) van der Marel, G.; van Boeckel, C. A. A.; Willis, G.; van Boom, J. H. *Tetrahedron Lett.* **1981**, *22*, 3887.
(9) The isotopic composition was established by reaction of the labeled $POCl_3$ with excess methanol followed by gas chromatography/mass spectroscopy of the resulting labeled trimethyl phosphate.
(10) Gough, G. R.; Brunden, M. J.; Nadeau, J. G.; Gilham, P. T. *Tetrahedron Lett.* **1982**, *23*, 3439.
(11) Because the labeled units were used as diastereomeric mixtures of internucleotide triesters, the deblocked products are racemic at the labeled phosphorus atoms.
(12) Patel, D. J. *Biopolymers* **1976**, *15*, 533.
(13) Uesugi, S.; Shida, T.; Ikehara, M. *Chem. Pharm. Bull.* **1981**, *29*, 3573.
(14) Kan, L.-S., private communication.

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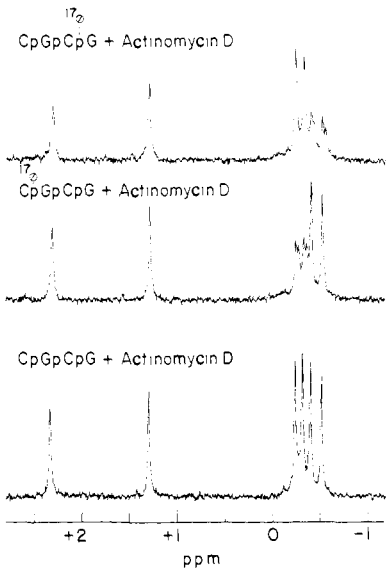

Figure 2. Proton-decoupled ^{31}P NMR spectra at 202.5 MHz and 30 °C of samples of 10 mM d(CpGpCpG) and 5 mM actinomycin D dissolved in D_2O containing 0.1 M sodium cacodylate, pH 7.0, and 2 mM EGTA. The spectra are of the unlabeled sample (bottom), the sample labeled with ^{17}O in the 3′-terminal d(CpG) unit (top), and the sample with ^{17}O in the 5′-terminal d(CpG) unit (middle). Chemical shifts are measured relative to external 85% H_3PO_4.

5′-terminal d(CpG) unit (middle spectrum) demonstrates that the intensity of the upfield resonance is diminished. We note that the naive expectation that the chemical shifts of the external d(CpG) phosphodiester units in the double-helical structure might be similar but different than that of the internal d(GpC) unit is not realized.

The ^{31}P NMR spectra of the unlabeled and labeled oligonucleotides in the presence of actinomycin D (2:1 d(CpGpCpG)–actinomycin D) were also obtained at 202.5 MHz and 30 °C, and these are reproduced in Figure 2. The spectrum of the unlabeled ternary complex (bottom spectrum) is composed of six resonances of equal intensity because all of the ^{31}P nuclei are nonequivalent, and the dissociation of the drug from the complex is slow on the NMR time scale. As expected on the basis of Patel's studies of this complex,[12] two of the resonances are shifted dramatically downfield while the remaining four are found at approximately the same chemical shifts as those of the oligonucleotide in the absence of the drug. The spectra of complexes labeled in the d(CpG) units (top and middle spectra) reveal that the intensities of the upfield resonances are affected by the isotopic labeling, thereby unambiguously establishing that the downfield resonances are associated with the d(GpC) units. These assignments prove Patel's assumption that the phosphodiester groups at the site of intercalation of the drug experience the large downfield changes in chemical shift.[12]

The previously described methods for assigning the ^{31}P NMR resonances of oligonucleotides[2,3] are dependent on the ability to make ^1H NMR assignments. While these spectroscopic methods may be more convenient than the isotopic labeling method described in this communication, it is unlikely that the essential ^1H NMR assignments will always be possible, especially for longer oligonucleotides and oligonucleotides bound to proteins. Thus, our more general isotopic labeling method should permit detailed study of a number of biochemically important problems. Preparation of the required labeled materials can be readily accomplished by any of a variety of procedures now available for the rapid synthesis of oligonucleotides.

The development of general methodology for ^{31}P NMR chemical shift assignments should make ^{31}P NMR a more definitive spectroscopic technique for studying oligonucleotide conformation and dynamics. In addition, sequence-specific ^{17}O labeling of the backbones of oligonucleotides should be useful for ^{17}O NMR studies of dynamics.[15]

The preceding communication describes the application of this method to polynucleotides.[16]

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Registry No. ^{17}O, 13968-48-4; d(CpGpCpG), 58927-25-6; actinomycin D, 50-76-0; [$^{17}O_2$]d(CpGpCpG), 88295-83-4.

(15) Petersheim, M.; Miner, V. W.; Gerlt, J. A.; Prestegard, J. H. *J. Am. Chem. Soc.* **1983**, *105*, 6357.
(16) Joseph, A. P.; Bolton, P. H., preceding paper in this issue.

1*H*-Cycloprop[*b*]anthracene

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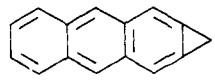
Although cyclopropabenzene[1,2] and both cyclopropanaphthalenes[3,4] have been synthesized and some of their chemical and physical properties investigated, several unsuccessful attempts to prepare parent members of the higher cycloproparenes have been reported.[5-10] We describe here a new approach to the cycloproparenes that we have used to synthesize 1*H*-cycloprop[*b*]anthracene (**1**).[11]



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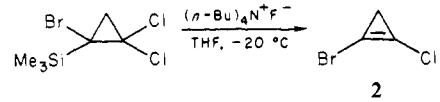
A salient feature of this method is the synthesis[12] of the new reagent 1-bromo-2-chlorocyclopropene (**2**) (eq 1).[13] The



(1)

2

cyclopropene can be generated in high yield at −20 °C, transferred in vacuo and stored in tetrahydrofuran at −20 °C for several days.

(1) Vogel, E.; Grimme, W.; Korte, S. *Tetrahedron Lett.* **1965**, 3625.
(2) Billups, W. E.; Blakeney, A. J.; Chow, W. Y. *Chem. Commun.* **1971**, 1461.
(3) Billups, W. E.; Chow, W. Y. *J. Am. Chem. Soc.* **1973**, *95*, 4099.
(4) Tanimoto, S.; Schafer, R.; Ippen, J.; Vogel, E. *Angew. Chem., Int. Ed. Engl.* **1976**, *158* 613; *Angew. Chem.* **1976**, *88*, 643 (German version).
(5) Davalian, D.; Garratt, P. J. *Tetrahedron Lett.* **1976**, *32*, 2815.
(6) Billups, W. E. *Acc. Chem. Res.* **1978**, *11*, 245.
(7) Davalian, D.; Garratt, P. J.; Koller, W.; Mansuri, M. M. *J. Org. Chem.* **1980**, *45*, 4183.
(8) Halton, B. *Ind. Eng. Chem. Prod. Res. Dev.* **1980**, *19*, 349.
(9) Müller, P.; Rey, M. *Helv. Chim. Acta* **1981**, *64*, 354.
(10) Müller, P.; Rey, M. *Helv. Chim. Acta* **1982**, *65*, 1157.
(11) This name conforms to that used by *Chemical Abstracts*.
(12) Details regarding the synthesis of **2** will be reported elsewhere.
(13) Chan and Massuda have reported the synthesis of 1-bromo- and 1-chlorocyclopropene by treating 1,1-dibromo-2-(trimethylsilyl)cyclopropane and 1,1-dichloro-2-(trimethylsilyl)cyclopropane, respectively, with CsF in diglyme: Chan, T. H.; Massuda, D. *Tetrahedron Lett.* **1975**, 3383. The dichlorocyclopropenes have been prepared in low yield by the reduction of tetrachlorocyclopropene using tri-*n*-butyltin hydride: Breslow, R.; Ryan, G.; Groves, J. T. *J. Am. Chem. Soc.* **1970**, *92*, 988. These workers caution that the dichlorocyclopropenes are explosive and possibly toxic.

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