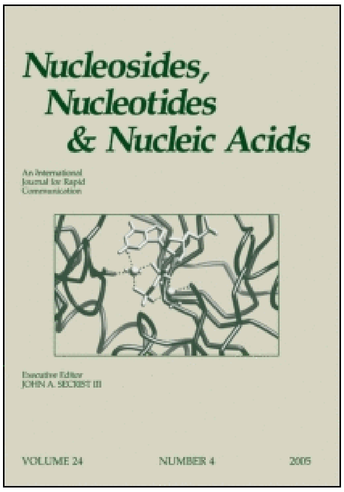

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James M. Hewitt[a]; William C. Lenhart[a]; Richard N. Moore[ab]; Ashis K. Saha[c]; Alexander L. Weis[c]
[a] Eastman Kodak Company, Rochester, NY [b] Chemagnetics, Fort Collins, CO. [c] Sterling Research Group, Sterling Drug Inc., Malvern, PA

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STRUCTURAL DETERMINATION OF SILICON-CONTAINING OLIGONUCLEOTIDES BY ^{1}H-^{29}Si LONG-RANGE HETERONUCLEAR MULTIPLE QUANTUM CORRELATION NMR SPECTROSCOPY

James M. Hewitt,[†*] William C. Lenhart,[†] Richard N. Moore,[†B]
Ashis K. Saha,[≠*] Alexander L. Weis[≠]

[†] *Eastman Kodak Company, 1999 Lake Avenue*
Rochester, NY 14650-2132, and
[≠]*Sterling Research Group, Sterling Drug Inc., 25 Great Valley Parkway,*
Malvern, PA 19355.

Abstract: The technique of ^{1}H-^{29}Si Long-Range Heteronuclear Multiple Quantum Correlation NMR Spectroscopy was used to determine the structure of silicon- containing oligonucleotides. Trimers which contained silicon instead of phosphorus as part of the oligonucleotide link were synthesized through a synthetic route that required minimal hydroxyl protection. The resulting trimer could have one of two possible structures. Through the use of ^{1}H-^{29}Si HMQC NMR spectroscopy, it was possible to link the 3′-hydroxymethine proton of one sugar to the 5′-hydroxymethylene proton of an adjacent sugar by correlation to the same silicon atom, thus elucidating the final structure.

The recent development of indirect detection NMR techniques has had a dramatic impact on the ability to assign complex molecules.[1-6] Much of the attention has focused on the structure elucidation of proteins using ^{1}H-^{13}C[2,3] and ^{1}H-^{15}N[3-6] heteronuclear multiple quantum correlation experiments. However, these methods also provide powerful tools for more traditional structure determination of organic compounds.[7] We report the application of this methodology to the silicon nucleus to resolve a complex structural question.

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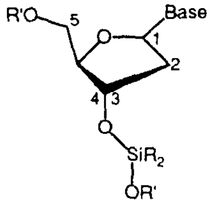
[*] Authors to whom correspondence should be addressed.

[B] Current address: Chemagnetics, Fort Collins, CO.

The silicon nucleus has similar properties to the carbon nucleus.[8,9]. The combination of low natural abundance (4%), similar sensitivity, long relaxation times, and negative nuclear Overhauser enhancement upon proton irradiation lead to long acquisition times for direct observation. Hence, the ^{29}Si-INEPT experiment[10,11] is often preferred to obtain one-dimensional silicon spectra with an increase in sensitivity and a decrease in the required delay times. However, this method is still limited by the inherent insensitivity of the silicon nucleus.

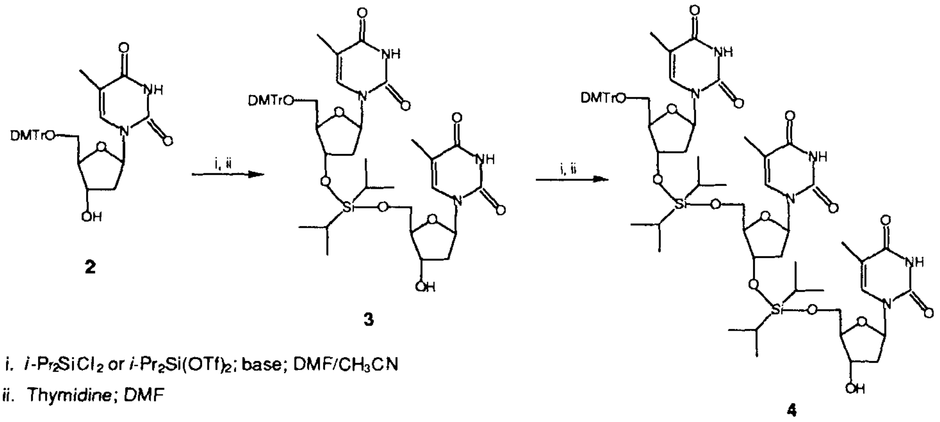
The need to develop the ^{1}H-^{29}Si Long-Range Heteronuclear Multiple Quantum Correlation experiment arose from the structural study of compounds which have the general structure **1**. Synthetic oligonucleotides which contain silicon instead of phosphorus as part of the internucleotide link were introduced recently by Ogilvie et al.[12,13] In the course of our investigation of silicon-containing oligonucleotides, we designed a synthetic route which required minimal hydroxyl group protection (Scheme I).



R = alkyl substituent
R' = additional units

1

Scheme I

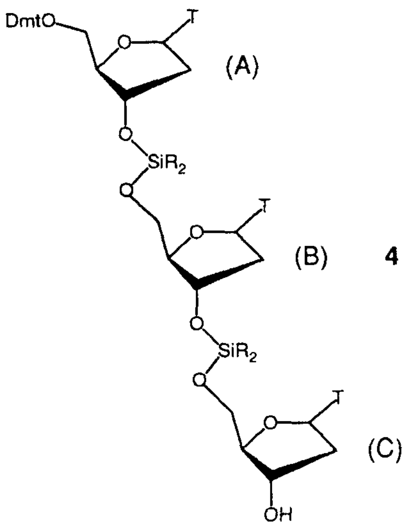


i. i-Pr$_2$SiCl$_2$ or i-Pr$_2$Si(OTf)$_2$; base; DMF/CH$_3$CN

ii. *Thymidine; DMF*

This procedure allowed a facile production of gram quantities of trimer **4** and other related oligomers, as well as adaptation to an automated solid support based synthesis of Si-linked oligonucleotide analogs.[14]

For complete structural elucidation, it was important to establish the 5'-3' nature of the oligomer. The data obtained from proton spectra (COSY,[15] TOCSY[16,18]) confirmed that a single compound was present which contained three intact thymidine sugar rings. In addition, chemical shift information also indicated that one 5'-hydroxymethylene group was dimethoxytritylated. However, this data did not distinguish between structure **4** and **5**, or other structures in which the linkage position to the silicon atoms had been reversed. Since the conformation of this molecule was unknown, this eliminated the potential for using through-space experiments (ROESY,[19] NOESY) for structural assignment.



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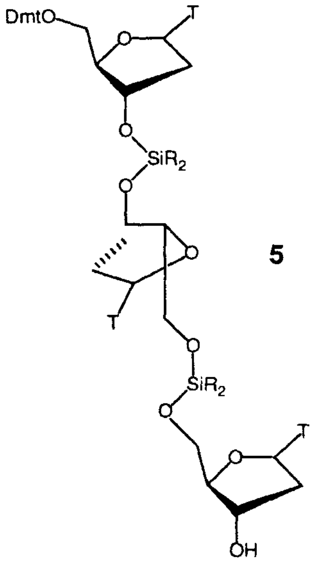

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However, it became apparent that an experiment which would link the 3'-hydroxymethine proton of one sugar to the 5'-hydroxymethylene protons of an adjacent sugar by correlation to the same silicon atom would provide the evidence needed to elucidate the structure. It was required that the experiment have sufficient resolution in the proton frequency domain to resolve the 5'-hydroxymethylene region, with high sensitivity since only limited amounts of oligonucleotides are usually available. Thus, the Indirect Detection methodology was ideally suited for this study since a gain in sensitivity by a factor of approximately ten[20] could be expected over a ^{29}Si-^{1}H normal heteronuclear shift correlation[21] and would produce a spectrum highly resolved in the proton frequency domain.

Since no proton is directly attached to either of the silicon atoms in the molecule, an HMQC pulse sequence[22] was used which utilized delay times based on a coupling constant of 3 Hz. Although the three-bond proton-silicon coupling constant (CHR-O-Si) was expected to be between 1 and 10 Hz,[23] the use of a coupling constant less than 3 Hz would cause an unnecessary loss of signal due to an increase in the required delay times.[24] The results of the Long Range HMQC experiment are shown in Figure 1.

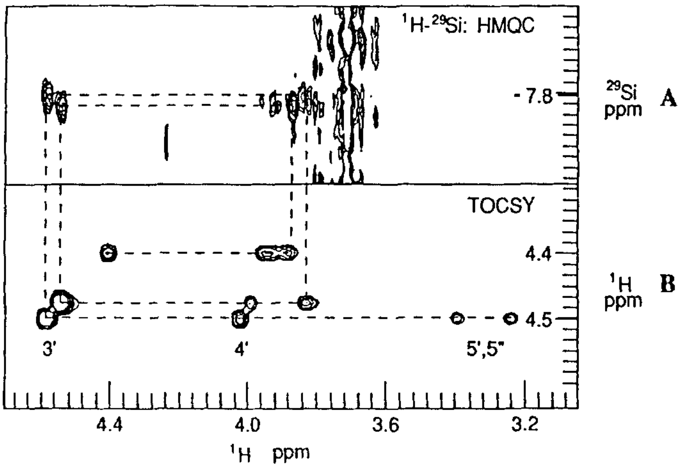

FIGURE 1. (A) Two-dimensional ^{1}H-^{29}Si Long-Range HMQC spectrum acquired with the use of a $J_{H\text{-}Si}$ value of 3.0 Hz and an acquisition time of 0.3 s to minimize heating effects from ^{29}Si WALTZ decoupling. This experiment [1024(t_1) x 2880(t_2)] was collected using a sweep width of 4803.4 Hz in the proton frequency domain, a sweep width of 22899.4 Hz in the silicon frequency domain, a proton 90° pulse of 10.5 µs, and a silicon 90° pulse of 28.0 µs. (B) Two-dimensional ^{1}H-TOCSY spectrum acquired with an MLEV-17 mixing period of 100 ms and a 2.0 ms trim pulse before and after the MLEV-17 sequence. This experiment [512(t_1) x 1024(t_2)] was collected using a sweep width of 4803.4 Hz, a hard proton 90° pulse of 10.5 µs, and a soft 90° pulse of 27.0 µs. All NMR spectra were acquired on a Varian VXR 500S at 30°C with the use of a 5 mm Broadband Indirect Detection Probe on a solution of 12.0 mg of **4** dissolved in 0.7 mL of $CDCl_3$ (99.95%). All spectra were processed using FTNMR on a VAX 8600 computer.

These data clearly show that two of the H3' sugar protons have a direct correlation to separate silicon atoms, the existence of two silicon chemical shifts having been demonstrated by a ^{29}Si-INEPT spectrum.[25] These data confirm that structure **4** is the correct structure. In addition, the 5'-methylene region shows that each silicon also has a direct correlation to the 5'-methylene protons. This is also consistent with the proposed structure. In combination with the TOCSY data the complete assignment can be made. Indeed, 5'-methylene protons on the **A** sugar ring of structure **4** can be correlated to the H3' proton on the same sugar ring with the TOCSY experiment. The H3' proton on the **A** ring can then be correlated to the 5'-methylene protons of the adjacent **B** ring using the HMQC experiment. This pattern of connectivity can be traced down to the final H3' sugar proton of the **C** sugar ring.

In summary, we have shown how this method can be used to identify the structure of a novel compound which contains multiple silicons. Further work will extend the application to various silicon-containing materials and other structural problems which contain low-abundance nuclei.

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Abbreviations

HMQC Heteronuclear Multiple Quantum Correlation; TOCSY Total Correlated Spectroscopy; ROESY Rotating Frame Overhauser Spectroscopy; NOESY Laboratory Frame Overhauser Spectroscopy; COSY Homonuclear Correlated Spectroscopy. INEPT Insensitive Nuclei Enhanced by Polarization Transfer.

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25. A ^{29}Si-INEPT experiment on 12 mg of compound **3** was acquired on a Bruker AM 500 NMR spectrometer at 30°C using a 5 mm broadband probe operating at 99.363 MHz. An INEPTRD.AU microprogram was used with delay times of 50 ms and 10 ms for D2 and D3 respectively. The silicon 90° pulse was 9.5 μs, proton 90° was 16.0 μs, the spectral width was 25000 Hz, and with the acquisition of 8192 data points a WALTZ-16 decoupling sequence was used. Fourier transformation of the data was performed after exponential multiplication was applied with a line broadening of 10.0 Hz. Two silicon signals were obtained from compound **4** at -7.2 and -7.8 ppm referenced to the silicon signal of TMS.

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