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Direct Assignment of the Absolute Configuration of a Distinct Class of Deoxyribonucleoside Cyclic *N*-Acylphosphoramidites at Phosphorus by M-GOESY Nuclear Magnetic Resonance Spectroscopy

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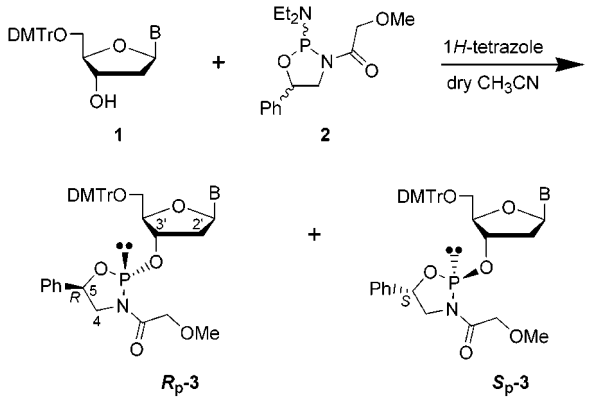
Phosphorothioated oligodeoxyribonucleotides (PS-ODNs) with undefined *P*-chirality continue to be extensively investigated as potential therapeutic agents against cancer and infectious diseases.[1] However, stereodefined S_P-PS-ODNs have been synthesized[2] and have demonstrated superior stability to nucleases found in human serum than either R_P-PS-ODNs or *P*-diastereomeric PS-ODNs.[2e] In this regard, deoxyribonucleoside cyclic *N*-acylphosphoramidites have recently been reported as a new class of monomers for the stereocontrolled synthesis of selected PS-ODNs.[3] Unlike conventional deoxyribonucleoside phosphoramidites, which are activated by weak acids,[4] these cyclic *N*-acylphosphoramidites readily react with base-activated nucleophiles[3] in a manner similar to that of deoxyribonucleoside oxathiaphospholane[2a,b] or indolooxazaphosphorine[2c] derivatives.

Deoxyribonucleoside cyclic *N*-acylphosphoramidites are typically prepared from suitably protected deoxyribonucleosides (**1**) and a cyclic *N*-acylphosphoramidite, such as **2**, in the presence of 1*H*-tetrazole in dry acetonitrile (Scheme 1).[3] Purification of the reaction products by silica gel chromatography affords two diastereomeric cyclic *N*-acylphosphoramidites (***R*$_P$-3** and ***S*$_P$-3**) as a mixture of rotamers.[5] Condensation of diastereomerically pure **3** with the 5′-hydroxyl function of a nucleoside/nucleotide in the presence of 1,1,3,3-tetramethylguanidine presumably proceeds according to a S_N2 type mechanism leading to complete inversion of configuration at P(III).[3]

Considering that the *P*-stereochemistry of any given dinucleoside phosphorothioate can be unambiguously determined,[6] it is possible to deduce the absolute configuration at P(III) of the diastereomerically pure deoxyribonucleoside cyclic *N*-acylphosphoramidite that was used to prepare the dinucleotide *assuming that the base-assisted condensation reaction proceeds through a single nucleophilic substitution event*. Thus, a direct method for assigning the absolute configuration of deoxyribonucleoside cyclic *N*-acylphosphoramidites at P(III) would be desirable to confirm or reject the possibility of any other mechanisms operating during the phosphitylation reaction.

2D-NMR techniques, such as COSY, NOESY, and ROESY, have been used to determine the absolute configuration at phosphorus of thymidine-3′-*O*-methanephosphonothiolate derivatives,[7] and that of dinucleoside phosphoramidate,[8] phosphorothioate,[9] methylphosphonate,[10] and boranophosphate[11] derivatives. 2D-NOESY and ROESY experiments are, however, limited in terms of relatively low sensitivity and narrow ranges of measurable internuclear distances. Conversely, 1D-measurements of the NOE offer an attractive solution to sensitivity limitations encountered in 2D-NOE experiments. By eliminating data collection in the first time domain, a large number of scans may be acquired during this period of time to produce a much higher signal-to-noise ratio. The sensitivity of NOE measurements may also be improved by the use of selective nuclei excitation methods. In this context, M-GOESY[12] and similar techniques described elsewhere[13] selectively excite one targeted nucleus in a molecule, and only those NOE signals generated from nuclei that show through-space dipolar couplings to the excited nucleus can be detected. Internuclear distances of up to 5 Å can reliably be measured with these methods.[12] We now wish to report the application of M-GOESY as a straightforward and sensitive method for assigning the absolute configuration at phosphorus of deoxyribonucleoside cyclic *N*-acylphosphoramidites structurally related to **3**.

Scheme 1



DMTr = 4,4′-dimethoxytrityl; B = 6-*N*-benzoyladenin-9-yl

Computer-modeling **3** revealed a proximal (2.55 Å) through-space interaction between benzylic *H*-5 and sugar *H*-2″ [14] in the case of ***S*$_P$-3** (Scheme 2).[15] Since this specific interaction is too distant (5.85 Å) in ***R*$_P$-3** to be reliably detected by M-GOESY, the NOE signal resulting from the close proximity of benzylic *H*-5 and sugar *H*-2″ should therefore be unequivocally diagnostic in assessing the S_P configuration of diastereomerically pure **3**.

To validate computer-modeling predictions, M-GOESY NMR spectra were recorded for both ***S*$_p$-3** and ***R*$_p$-3**, and the relevant resonances are shown in Figure 1.[16] The large signal at 2.7 ppm corresponds to the selectively excited *H*-2″ resonance in both spectra. The other NOE signals are generated from nuclei near *H*-2″, including those of *H*-2′, *H*-4′, *H*-3′, and *H*-1′ at 3.0, 4.3, 5.1, and 6.5 ppm, respectively. These signals are observed in both ***R*$_P$-3** and ***S*$_P$-3**. Consistent with computer-assisted modeling predictions (vide supra), the diagnostic NOE signal at 5.5 ppm is only detected in ***S*$_P$-3** and reflects the close proximity (3.60 Å) of benzylic *H*-5 and sugar *H*-2″.

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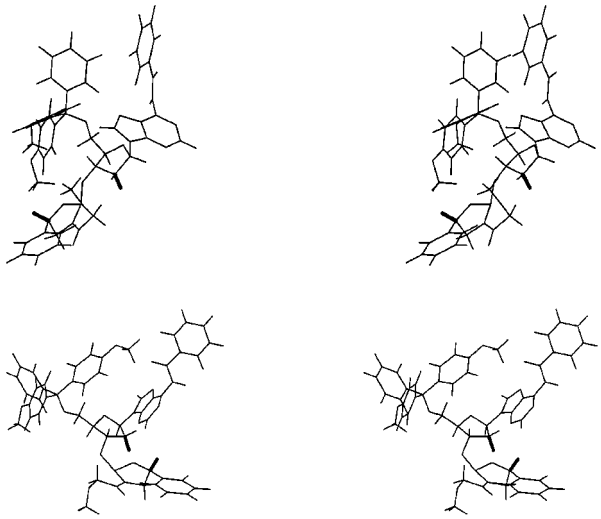
Scheme 2. Stereoview of Energy-Minimized R_P-**3** (top set) and S_P-**3** (bottom set) Displaying the Internuclear Distance between Benzylic *H*-5 and Sugar *H*-2″ in Each *P*-Diastereomer[a]



[a] Benzylic *H*-5 and sugar *H*-2″ are shown as thicker lines in each set.

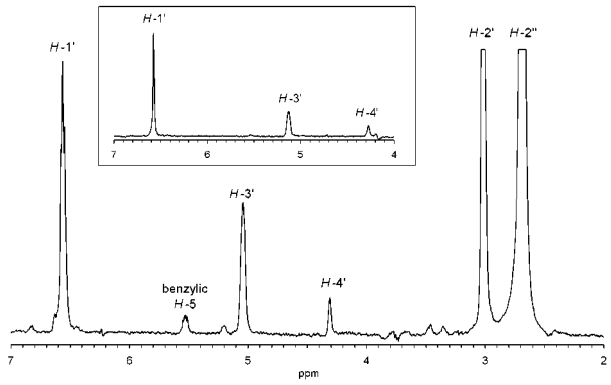


Figure 1. M-GOESY NMR spectra of S_P-**3** and R_P-**3** (inset).

Table 1. Determination of Internuclear Distances on the Basis of Diagnostic M-GOESY NOE Signals Generated from S_P-**3** and R_P-**3**

	S_P-3		R_P-3	
	calcd[a]	found[b]	calcd[a]	found[b]
H-1′	2.30	2.23	2.33	2.30
H-2′	1.76[c]	1.76	1.76[c]	1.75
H-3′	2.93	2.53	2.99	2.51
H-4′	3.28	3.46	2.64	2.69
benzylic *H*-5	2.55	3.60	5.85	none

[a] Internuclear distances (in Å) measured relative to *H*-2″ from energy-minimized models of S_P-**3** and R_P-**3** (see Scheme 2 and ref 15). [b] Internuclear distances (in Å) measured relative to the selectively excited *H*-2″ NOE signal. [c] The distance of 1.76 Å is used as a reference when computing any internuclear distances relative to the selectively excited *H*-2″ NOE signal.

Internuclear distances calculated for S_p-**3** and R_p-**3** with M-GOESY data have been determined,[17] and compared with those measured from the energy-minimized structures displayed in Scheme 2. Table 1 shows that both sets of internuclear distances are consistent with each other and thus stress the validity of computer-modeling calculations in determining such distances.

In summary, M-GOESY NMR spectroscopy is a convenient, rapid, and unambiguous method for determining the *P*-chirality of representative deoxyribonucleoside cyclic *N*-acylphosphoramidites through accurate measurements of internuclear distances between diagnostic nuclei. The method unequivocally confirms that the condensation of deoxyribonucleoside cyclic *N*-acylphosphoramidites with base-activated nucleosidic 5′-hydroxyls proceeds via a single S_N2 nucleophilic substitution event, which is consistent with the *P*-stereochemistry of related dinucleoside phosphorothioates. The application of M-GOESY NMR spectroscopy to deciphering the absolute *P*-configuration of deoxyribonucleoside cyclic *N*-acylphosphoramidites analogous to **3** is currently under investigation. Results of such investigations will be reported in due course.

Supporting Information Available: Detailed preparation of **2** and **3**; 500 MHz ^{1}H NMR spectra of S_P-**3** and R_P-**3**, and tabulated spectral data; 500 MHz HOHAHA, ROESY, and M-GOESY NMR spectra of S_P-**3** and R_P-**3** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(5) Although the four expected diastereomers of **3** were detected in the crude reaction mixture, only R_P-**3** and S_P-**3** could be isolated in substantial amounts by silica gel chromatography. The stereochemistry of benzylic *H*-5 has been corroborated through an alternate synthesis of R_P-**3** and S_P-**3** with enantiomerically pure 2-amino-1-phenylethanol, and comparing chromatographic and NMR properties. Synthesis and characterization of R_P-**3** and S_P-**3** are reported in the Supporting Information.
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(14) *H*-2″ is assigned according to: Wood, D. J.; Hruska, F. E.; Ogilvie, K. K. *Can. J. Chem.* **1974**, *52*, 3353−3366.
(15) Computer modeling experiments were performed in the CHARMm force field with Quanta (v. 98). Structures were relaxed by initial minimization, and short simulations (100 ps) were performed in a vacuum at 300 K. Trajectories of lowest energy were selected and subjected to the Adopted Basis Newton−Raphson minimization method to achieve a 10^{-4} kcal/mol energy gradient.
(16) 500 MHz M-GOESY spectra of R_P-**3** and S_P-**3** were run in $CDCl_3$ at −30 °C. The full spectrum of R_P-**3** is presented in the Supporting Information.
(17) Examples of internuclear distance calculations with M-GOESY data are provided elsewhere (see ref 12).
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