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Synthesis of Dithymidine Boranophosphates via Stereospecific Boronation of *H*-Phosphonate Diesters and Assignment of Their Configuration.

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Abstract: The absolute configurations of the two dithymidine boranophosphate isomers were established by chemical correlation with the *H*-phosphonate isomers. All chemical transformations leading from *H*-phosphonate diesters to boranophosphate diesters were found to be stereospecific with retention of configuration around phosphorus. The data verify our previous assignment of R_p and S_p isomers of dithymidine boranophosphate made on the basis of NMR and enzymatic hydrolysis.

Recently introduced by our group, the oligonucleoside boranophosphates represent a novel type of chiral analog of phosphodiester oligomers in which one of the nonbridging oxygens has been replaced by a borane group ($-BH_3$) [1–4]. These compounds, being isoelectronic and isostructural with methylphosphonates and negatively charged like normal phosphodiester nucleic acids, are now the subject of growing interest of several research groups [5-7] as promising research tools in DNA sequencing [6], in antisense and therapeutic applications, and in boron neutron capture therapy.

It has been known for years that stereochemistry at phosphorus in P-chiral oligonucleotide analogs affects their physicochemical characteristics and biological properties such as lipophilicity, water solubility, affinity to a complementary strand, and resistance to enzymatic digestion. Implicit for understanding these properties is the importance of determination of the absolute configuration of the boranophosphate linkage.

Earlier we studied the configuration of the simplest oligonucleotide molecule – dithymidine boranophosphate. R_p and S_p isomers of the dithymidine boranophosphate were separated on RP HPLC and the absolute configuration at the chiral phosphorus center of the dimers was tentatively assigned by 1D NOE difference experiments and substrate properties in a study with phosphodiesterases [8].

Recently, we reported a new method for the synthesis of nucleoside boranophosphates by boronation of an *H*-phosphonate internucleotidic bond following in situ silylation [3,4]. In this paper we describe a procedure for the assignment of absolute configuration of dithymidine boranophosphate by chemical correlation via *H*-phosphonate intermediates. The method is based on the findings that sulfurization and methylation of dinucleoside *H*-phosphonates occur stereospecifically and that dinucleoside *H*-phosphonate diesters can be conveniently separated into the R_p and S_p diastereomers using silica gel chromatography [9,10].

As outlined in Scheme 1, the boronation procedure involves silylation with bis-trimethylsilylacetamide (BSA) followed by a borane exchange reaction with borane–diisopropylethylamine complex (DIPEA:BH_3). Silylation proceeds with retention of the configuration around the phosphorus atom [10]. The addition of a borane group to Lewis bases proceeds stereospecifically [11], preserving the stereochemistry at the base. The final alkaline hydrolysis of the silyl ester would not change the phosphorus configuration. Therefore, starting with one isomer of *H*-phosphonate diester, the boronation procedure should afford only one isomer of the boranophosphate diester with the same absolute configuration.

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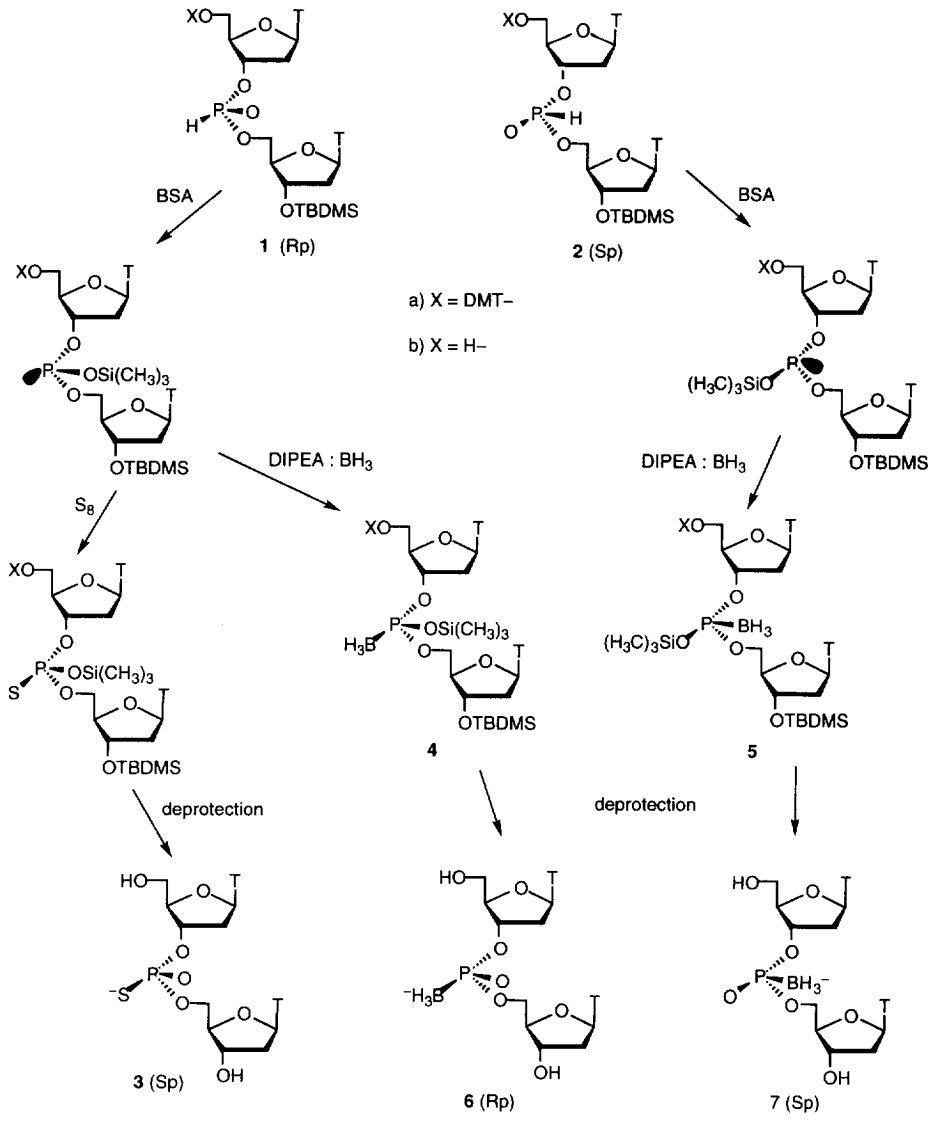

We prepared the individual dithymidine *H*-phosphonate isomers following the procedure described by Seela et al. [10]. The isomers were separated by silica gel HPLC using EtOAc : MeOH : CH_2Cl_2 (40:5:55) instead of EtOAc : AcOH (998:2) used by Seela et al. The faster migrating *H*-phosphonate dimer **1a** (R_t = 11.1 min), which exhibited a ^{31}P-NMR chemical shift at 9.15 ppm, was assigned as the R_p isomer, and the slower migrating one **2a** (R_t = 12.1 min, σ = 10.51 ppm) was assigned as S_p according to the literature data [10]. The diastereomeric purity in the preparations was 98% for the faster eluting isomer and 92% for the slower eluting one (see Fig. 1a,e).

The presence of a 5'-dimethoxytrityl protecting group (DMT) was very helpful for separation of diastereomeric mixture of *H*-phosphonate dimers; however it appears to be inconvenient for a boronation reaction.

We and others reported incompatibility of borano- and trityl groups during acid treatment [2,5b,12]. To avoid side reactions, we decided to remove the DMT protecting group before boronation. Each diastereomer was treated with 2.5 % dichloroacetic acid in CH_2Cl_2 for 15 min and subsequently purified by silica HPLC.

To demonstrate that removal of DMT groups proceeds without epimerization, we prepared dithymidine phosphorothioate **3** from the protected **1a** and unprotected **1b** H-phosphonate R_p stereoisomer. As judged from RP HPLC and ^{31}P-NMR spectra analysis, both reactions after deprotection yielded the same Sp-isomer of dithymidine phosphorothioate **3** (^{31}P NMR chemical shift 57.05 ppm), confirming that the reaction proceeded stereospecifically regardless of the presence of DMT group. The configuration change from R_p (*H*-phosphonates) into S_p (phosphorothioates) is due to the CIP rules [13].

Silylation of the separate dithymidine *H*-phosphonate diastereomers **1b** and **2b** with BSA was stereospecific. The ratios of silylated isomers (98 : 2 for **1b** and 92 : 8 for **2b**, see Fig. 1b,f) were the same as the ratios of *H*-phosphonate isomers. Subsequent in situ boronation with 20 eq. DIPEA:BH_3 resulted in formation of boranophosphate triesters **4b** and **5b**. The ^{31}P-NMR resonances of these species appear as broad peaks (Fig. 1c,g). Due to peak broadening [14] the chemical shifts for isomers were not sufficiently different (σ=104.4 ppm for **4b** and 104.6 ppm for **5b**) to allow the diastereomeric ratio to be determined. Without isolation of intermediates, the compounds obtained were treated with water to remove the silyl group from the boranophosphate, deprotected, and analyzed by RP HPLC (Fig. 2).

The separation of the two diastereomers of dithymidine boranophosphate using reverse phase HPLC was demonstrated earlier in our laboratory [8]. The absolute configurations at the chiral phosphorus center of the dimers were tentatively assigned with the faster eluting diastereomer as S_p and the slower eluting one as the R_p isomer.

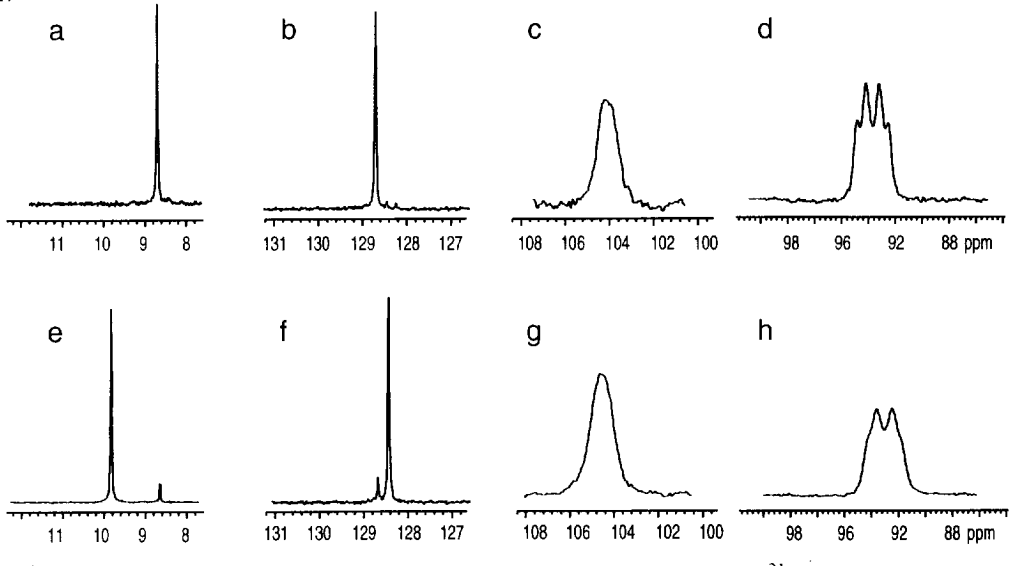

Figure 1. Boronation of individual isomers of dithymidine *H*-phosphonate as monitored by ^{31}P-NMR spectroscopy.
(a) ^{31}P-NMR spectrum of **1b**; (b) ^{31}P-NMR spectrum after presilylation of **1b**; (c) ^{31}P-NMR spectrum of **4b**; (d) ^{31}P-NMR spectrum of **6** after purification.
(e) ^{31}P-NMR spectrum of **2b**; (f) ^{31}P-NMR spectrum after presilylation of **2b**; (g) ^{31}P-NMR spectrum of **5b**; (h) ^{31}P-NMR spectrum of **7** after purification.

The present data (Fig. 2) are consistent with our previous observations [8]. The boranophosphate isomer **6,** obtained from the faster migrating (R_p) isomer of *H*-phosphonate, has a longer retention time on a reversed phase HPLC column (20.3 min for **6** vs. 19.3 min for **7**) and a larger downfield chemical shift in ^{31}P-NMR spectra (93.64 ppm for **6** vs. 93.21 ppm for **7** in D_2O relative to 85% H_3PO_4). The diastereomeric purity of the resulting dithymidine boranophosphates, as judged from HPLC analysis (Fig. 2) and ^{1}H-NMR spectra, was exactly the same as for the original preparations of *H*-phosphonate diesters (Fig. 1a). This agreement makes it possible to say that the boronation reaction via *H*-phosphonate intermediates proceeds stereospecifically with retention of configuration and verifies our previous configuration assignment of dithymidine boranophosphate [8].

During the course of this work another report was published describing the stereoselective synthesis of the S_p isomer of the dithymidine boranophosphate through a chiral indole-oxaphosphorine intermediate [7b]. The observed stereospecificity of the borane exchange reaction and assignment of isomers of boranophosphate dimers are in complete agreement with our conclusions.

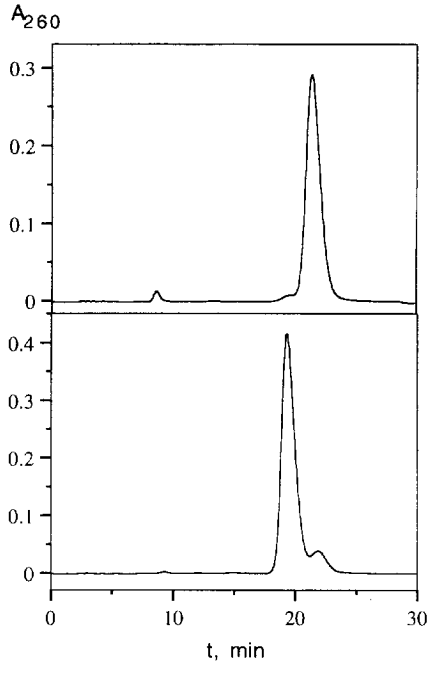

Figure 2. HPLC analysis of the individual isomers of dithymidine boranophosphate **6** (upper) and **7** (lower). Elution on a 3.9 x 300 mm Delta Pak 15 µm column with 10% CH_3CN in 20 mM KH_2PO_4 (pH 4.2).

Optically pure precursors of type **6** and **7** also have potential for use (after suitable selection of protecting groups) in the preparation of stereochemically defined synthons for incorporating chiral internucleotidic boranophosphate linkages into oligonucleotides. This is currently under investigation in our laboratory.

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13. An S_p configuration in phosphorothioate corresponds to an R_p configuration in boranophosphate due to CIP rules (sulfur is the largest atom around the phosphorus center in phosphorothioate while boron is the smallest one in boranophosphate).
14. The breadth of this line is due to quadrupolar relaxation by the ^{11}B nucleus (^{11}B, I=3/2), see Li, H.; Hardin, C.; Shaw, B.R. *J. Am. Chem. Soc.* **1996**, *118*, 6606-6614.