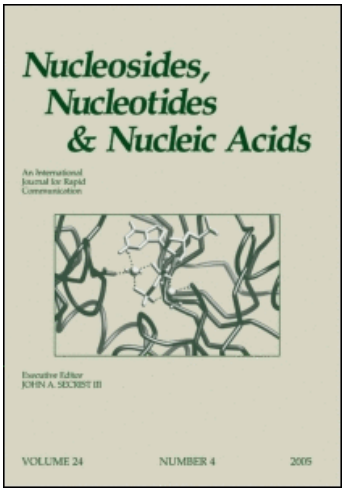

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THE SYNTHESIS OF DITHYMIDINE BORANOPHOSPHATE BY THE OXATHIAPHOSPHOLANE APPROACH

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ABSTRACT

The application of the oxathiaphospholane approach for the synthesis of dithymidine boranphospate was evaluated. It was shown, that although the nucleoside-3′-*O*-oxathiaphospholane-borane complexes **2** or **6** could not be chromatographically separated into diastereomerically pure species due to their apparent instability to moisture, they can be successfully applied to the non-stereocontrolled formation of internucleotide boranophosphate bond by reaction with 5′-OH-nucleoside in the presence of DBU. Attempts to apply the related dithiaphospholane approach for the preparation of dithymidine boranophosphorothioate were unsuccessful.

INTRODUCTION

Boranophosphates, containing negatively charged borane moiety (BH_3^-) in place of one of the non-bridging oxygen atoms, constitute a new backbone-modified class of oligonucleotides[1]. The boranophosphate group is isoelectronic to the natural phosphodiester group and isosteric to the methylphosphonate modification. Introduction of a boranophosphate moiety

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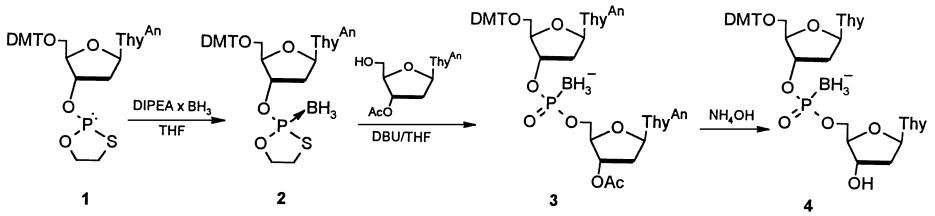
retains the ability of oligonucleotides to form reasonably stable complexes with the complementary DNA or RNA[2–4] and to activate RNase H[4,5], and leads to significant increase in resistance of oligonucleotides towards nucleolytic degradation[2,3] thus making them promising candidates for therapeutic applications including antisense/antigene approach or boron neutron capture therapy (BNCT)[6].

Although the first chemical synthesis of oligo(thymidine boranophosphate)s was performed by phosphoramidite approach[1] it soon became obvious that much better results could be obtained by H-phosphonate methodology with simultaneous boronation of *pre*-synthesized oligo(thymidine-H-phosphonate) after its trimethylsilylation[3,4,7]. These syntheses provide more or less random mixtures of diastereomers due to the chirality of each internucleotide boranophosphate centre. Full stereocontrol was only observed during enzymatic primer template-directed synthesis of boranophosphate oligonucleotides from diastereomerically pure R_P-nucleoside α-boranotriphosphates, which were found to be good substrates for a number of DNA polymerases[2,8]. For S_P dithymidine boranophosphate a stereoselective synthesis was reported by Jin and Just[9,10] using (*S*)-3-hydroxy-4-(2-indolyl)butyronitrile as a chiral auxiliary. These authors were also able to separate into individual diastereomers appropriately protected dithymidyl-β-cyanoethylphosphite-borane complexes and transform them into diastereomerically pure dithymidine boranophosphates.

RESULTS AND DISCUSSION

Since the oxathiaphospholane approach proved to be a method of choice for a stereocontrolled synthesis of oligo(nucleoside phosphorothioate)s[11–13], it was of interest to check its applicability to the synthesis of oligo(nucleoside boranophosphate)s. Thus, freshly prepared 3-*N*-anisoyl-5′-*O*-dimethoxytritylthymidine-3′-*O*-1,3,2-oxathiaphospholane (**1**)[14] (0.1 mmol) was reacted with 6 equivalents of borane-diisopropylethylamine (DIPEA) complex (10.5 μL, Aldrich) in THF (5 mL, freshly distilled over CaH_2). The reaction was followed by ^{31}P NMR (Bruker AC 200, 85% H_3PO_4 as a standard) and after 2 h at rt a complete disappearance of signals corresponding to the substrate was observed with the formation of a broad signal at δ 164.4 ppm (half-line width 1.9 ppm) which was assigned to oxathiaphospholane-borane complex **2** (Scheme 1)[15].

The attempts to isolate complex **2** by column chromatography in order to separate P-chiral diastereomers were unsuccessful. The complex **2** appeared to be quite reactive and decomposed readily in the presence of moisture. Therefore, no separation of the crude product **2** was attempted. Thus, the THF solution of **2** prepared from 1 mmol of 1 and 6 mmol of BH_3-DIPEA was reacted with 1 mmol of 3-*N*-anisoyl-3′-*O*-acetylthymidine in the



Scheme 1.

presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 1 mmol). After 2 h at rt the ^{31}P NMR control (CD_3CN added), showed complete disappearance of the signal of **2** and the appearance of a new broad signal at δ 93.7 ppm which was assigned to the formation of protected dithymidine boranophosphate **3**[16] (see Scheme 1). The compound **3** was isolated by silica gel column chromatography in 97% yield and was characterized by ^{31}P NMR (CD_3CN, δ 91.6, 93.5 ppm, broad dublet)[16], ^{11}B NMR (CD_3CN, δ −36.6 ppm, broad signal, half-line width 2.2 ppm, BF_3xEt_2O as a standard)[16], and by FAB MS (m/z 1155.9; calcd MW 1155.94). A sample of **3** was deprotected at the 3′-position with 30% aq. ammonia (48 h, rt), and purified by RP HPLC (ODS Hypersil 5 μ column, linear gradient of CH_3CN in 0.1 M TEAB, 1.5%/min). The substance collected at R_T 32.7 min (main peak) was identified by FAB MS as 5′-*O*-DMT-dithymidine boranophosphate **4** (m/z 845.6, calcd MW 845.62). Although compound **4** was supposed to be obtained as a mixture of diastereomers due to phosphorus chirality, only a single peak was observed by HPLC under the conditions employed.

The same reaction was performed under the conditions of solid-phase synthesis, using LCA CPG support loaded with 5′-*O*-dimethoxytritylthymidine *via* a sarcosinylated[17] linker. Thus, the support (1 μmole) was placed in a DNA synthesis column (Applied Biosystems) attached to a syringe, and was treated with 3% trichloroacetic acid in dichloromethane in order to remove the 5′-DMT group. After washing (CH_2Cl_2, CH_3CN) and drying (argon flush) the THF solution of **2**[18], premixed with DBU, was applied to the column and reacted with the support bound nucleoside for a given period of time (see Table 1). Then, the column was subjected to washing (THF, CH_3CN) and drying (argon), and the product was cleaved from the support by overnight treatment with 30% aq. ammonia. The solution was evaporated and analyzed by RP HPLC (conditions as above). The yield of 5′-*O*-DMT-dithymidine boranophosphate **4** under the conditions of solid phase synthesis was calculated from integrated chromatograms taking into account the intensity of the peak of **4** (R_T 32.7 min) and the peak of unreacted thymidine cleaved from the support (R_T 8.7 min). The results of syntheses performed under various experimental conditions are listed in Table 1.

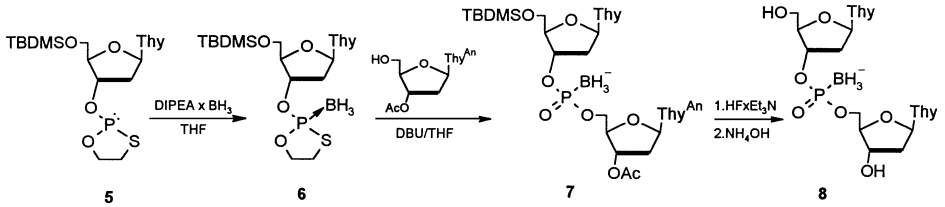
Table 1. The Synthesis of Dithymidine Boranophosphate on the Solid Support (1 μmol)

Oxathiaphospholane Complex **2** (Molar Equivalents)	DBU (Molar Equivalents)	Reaction Time (min)	Yield of 4 (%)
6	6	5	18
12	12	5	55
24	24	5	58
30	30	5	68
30	30	10	68
50	50	5	58
30	100	5	65
30	200	5	58
30	30	10	35[a]

[a] With CH_3CN instead of THF as a solvent.

It was found, that the best yield of **4** (68%) was obtained when THF was used as a solvent, with a 30-fold molar excess of both **2** and DBU with respect to support bound nucleoside, and the reaction time restricted to 5 min. Further increase in the concentration of the reagents or prolongation of the reaction time did not lead to any improvement of the yield of condensation. A change of the solvent to acetonitrile resulted in a substantial decrease of the yield of **4**.

These experiments proved that the internucleotide boranophosphate bond is formed under the conditions of oxathiaphospholane synthesis. Since acidic deprotection of the 5′-hydroxyl function in **4** would inevitably lead to the degradation of boranophosphate by its reaction with dimethoxytrityl cation[4], further experiments aimed at the preparation of the fully deprotected dithymidine boranophosphate were performed with the substrate protected at the 5′-hydroxyl with a *t*-butyldimethylsilyl (TBDMS) group. Thus, the 5′-*O*-TBDMS-thymidine-3′-*O*-l,3,2-oxathiaphospholane (**5**)[19] was reacted in THF with 10 molar equivalents of borane-DIPEA complex. After 2 h at rt the ^{31}P NMR inspection (CD_3CN added) showed the formation of oxathiaphospholane-borane complex **6** as a broad signal at δ 164.0 ppm (Scheme 2). The complex **6** appeared to be too unstable to be purified by flash column chromatography, so the THF solution prepared as above was used for further experiments without purification. Thus, the THF solution of **6**, prepared from 1 mmol of **5** and 10 mmol of BH_3-DIPEA, was reacted with 0.9 mmol of 3-*N*-anisoyl-3′-*O*-acetylthymidine in the presence of DBU (3 mmol). The formation of protected dinucleoside boranophosphate **7** was evidenced by ^{31}P NMR analysis (CD_3CN added, broad signal at δ 93.2 ppm)[16]. The compound **7** was purified by silica gel column chromatography (CH_2Cl_2-MeOH as an



Scheme 2.

eluent) in 60% yield, and was characterized by ^{31}P NMR (CD_3CN, δ 91.2 ppm, broad signal)[16], and by FAB MS (m/z 833.5; calcd MW 833.70). A sample of **7** was incubated with 1 M $HFxEt_3N$ (THF, 48 h, rt) followed by treatment with 30% aq. ammonia (48 h, rt). The fully deprotected dithymidine boranophosphate **8** was isolated by RP HPLC (Econosil 5 μ column, linear gradient of CH_3CN in 0.1 M TEAB, 1.5%/min) at R_T 10.2 min. The unsymmetrical appearance of the HPLC peak of **8** (shoulder) was related to the fact that boranophosphate **8** was supposed to be formed as a mixture of diastereomers. However no separation of the peaks related to diastereomers was achieved under the conditions employed. The structure of compound **8** isolated as above was confirmed by FAB MS (m/z 543.2; calcd MW 543.25).

The above results on the application of the oxathiaphospholane approach to the preparation of dinucleoside boranophosphate prompted us to check the possibility of the synthesis of dinucleoside boranophosphorothioate[20] by the dithiaphospholane approach[21]. Thus, 5′-*O*-TBDMS-thymidine-3′-*O*-l,3,2-dithiaphospholane (**9**)[22] was reacted with 10 molar equivalents of borane-DIPEA complex in THF solution. After 2 h at rt the ^{31}P NMR inspection (CD_3CN added) showed the presence of the signal of **9** and the appearance of a broad signal at δ 173 ppm which could be assigned to the corresponding dithiaphospholane-borane complex (**10**). The integration of the spectrum showed the ratio **9**:**10** to be *ca* 1:1. The attempts to shift the equilibrium towards the formation of a higher proportion of **10** by prolongation of the time of experiment or by increasing of the excess of borane-DIPEA complex failed. Similarly, the isolation of pure **10** by flash column chromatography was not possible. In this situation it was attempted to react the crude mixture prepared as above with 3-*N*-anisoyl-3′-*O*-acetylthymidine in the presence of DBU (10 molar equivalents). Although in the obtained mixture the ^{31}P NMR analysis showed the presence of some amounts (*ca* 30%) of the broad signal at δ 166 ppm which could be attributed to the presence of corresponding dithymidine boranophosphorothioate[20], isolation attempts by column chromatography were not successful, and further attempts of dinucleoside boranophosphorothioates by this approach were abandoned.

CONCLUSIONS

The results presented in this paper indicate that the formation of internucleotide boranophosphate bond can be accomplished under the conditions of oxathiahospholane synthesis both in solution and on the solid support, although the yields for the solid support synthesis are far from being satisfactory. Unfortunately, due to the apparent instability of oxathiaphospholane-borane complexes **2** and **6**, their chromatographic separation into diastereomerically pure species was not possible, so we could not pursue the matter of the stereospecificity of ring-opening condensation. An attempt to apply the dithiaphospholane approach for the preparation of dithymidine boranophosphorothioate was not successful.

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14. **1** Was Prepared From 3-*N*-Anisoyl-5′-*O*-DMT-Thymidine and 2-*N,N*-Diisopropylamino-1,3,2-Oxathiaphospholane[11] in CH_2Cl_2 in the Presence of Tetrazole

and was Isolated by Flash Column Chromatography (Silica Gel, Benzene-$CHCl_3$ as Eluent) in 60% Yield. ^{31}P NMR (CD_3CN) δ 171.05, 172.90 ppm. The 3-*N*-Anisoyl Protection of Thymine was Employed to Prevent the Reductive Degradation of the Base by the Borane Complex[4].

15. Similar Results were Obtained when THF-BH_3 or Me_2S-BH_3 Complexes were used Instead of DIPEA-BH_3 for the Reaction with **1**.
16. For D_2O Solutions of Unprotected Dithymidine Boranophosphates Jin and Just[9] Reported Following NMR Chemical Shift Data: ^{31}P NMR, δ 93.51, 93.76 ppm (Broad Signals, for S_p and R_p Diastereomers, Respectively); ^{11}B NMR, δ −41.64, 41.29 ppm (Broad Signals, for S_p and R_p Diastereomers, Respectively).
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18. For each Solid Support Experiment a THF Solution of **2** was Freshly Prepared in a Separated Flask from a Given Amount of **1** (see Table 1) and 6 Molar Equivalents of BH_3-DIPEA (2 h at rt, total volume 140 μL). Before Applying to the Column the Solution of **2** was Treated with DBU (Amounts are Given in Table 1). In this Series of Experiments the Thymidine Derivative Without 3-*N*-anisoyl Protection was used.
19. **5** was Prepared from 5′-*O*-TBDMS-thymidine and 2-*N,N*-diisopropylamino-1,3,2-oxathiaphospholane[11] in CH_2Cl_2 in the Presence of Tetrazole and was Isolated by Flash Column Chromatography (Silica Gel, $CHCl_3$-MeOH as Eluent) in 78% Yield. ^{31}P NMR (C_6D_6) δ 172.20, 173.64 ppm.
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22. **9** was Prepared from 5′-*O*-TBDMS-thymidine and 2-*N,N*-diisopropylamino-1,3,2-dithiaphospholane[21] in CH_2Cl_2 in the Presence of Tetrazole, and was Isolated by Flash Column Chromatography (Silica Gel, $CHCl_3$-MeOH as Eluent) in 93% Yield. ^{31}P NMR (CD_3CN) δ 150.95 ppm.

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