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The Synthesis of the Sp and Rp Diastereomers of Dithymidine Boranophosphate

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Abstract: The diastereomers of thymidyl-(3'-5')thymidyl O,O-boranophosphate Sp-**4** and Rp-**4** were easily separated and transformed to title compounds Sp-**7** and Rp-**7**. Their configurations were assigned by comparison with literature data.

Boranophosphates are nuclease resistant[1], and may be very useful antisense agents, in particular since their hybrids with mRNA may be substrates for RNase'H. The boranophosphate linkage was first synthesized in dinucleotides by Sood *et al.*[2] Several years later, the separation of the two diastereomers of dithymidine boranomonophosphate using reverse phase HPLC was reported.[3] Recently, a 14-mer oligodeoxynucleotide (ODN) containing a single incorporation of one diastereomer of boranophosphate was synthesized enzymatically. This ODN bound to a complementary DNA molecule with slightly poorer binding affinity relative to an unmodified control.[4] Later, Matteucci and coworkers[5] reported the chemical synthesis of a T_{15} ODN fully linked by boranophosphates. They found that the binding affinity of the diastereomeric mixture of the boranophosphate-linked oligothymidine ODN with complementary RNA and DNA was much poorer than that of the native phosphodiester ODN control. Consequently, diastereomeric mixtures of boranophosphates are unlikely to be useful replacements for phosphate diesters in antisense research. The uses of the diastereomerically pure boranophosphates that are not attainable by enzymatic methods, but potentially from stereocontrolled chemical synthesis, remain an open question.

In this paper, we describe the method of the chemical synthesis of the Sp and Rp diastereomers of dithymidine boranophosphate **7**.

Reaction of commercially available[6] 5'-DMTr-thymidine phosphoramidite **1** with 3'-protected thymidine (**T_5,OH**: R = TBDPS or TBDMS) in the presence of tetrazole resulted in the formation of an intermediate phosphite triester **2**, which was then converted to the dithymidyl boranophosphate cyanoethyl ester **3** by reaction with 3.3 eq. of dimethyl sulfide-borane (Scheme 1). Both reactions can be easily followed by ^{31}P NMR. In the first reaction the amidite peaks at 150.88 and 150.82 ppm were replaced by the new phosphite peaks at 140.74 and 140.31 ppm within the time required for recording the spectrum. In the second reaction the phosphite peaks disappeared within 5-10 min; after a large number of accumulations, a broad peak at 117.61 ppm for the boranophosphate phosphorus was observed.

The boronation step with Me_2S-BH_3 also removes the DMTr protecting group from the 5'-hydroxyl position. Separation of **3** into individual diastereomers was not easy by chromatography method. However, the Sp-**4** and Rp-**4** diastereomers can be easily separated by chromatography after reinstalling the DMTr protecting group at the 5' position in **3**. To get Sp-**4** and Rp-**4** directly from **3** without detritylation, we tried to use 1 or 2 eq. of Me_2S-BH_3. The reaction did not go to completion and a mixture of **3**, Sp-**4** and Rp-**4** was obtained.[7] Each of the diastereomers Sp-**4** and Rp-**4** was separately converted with 3 eq. of TBAF and 24 eq. of HOAc in THF at RT to give Sp-**5** (yield: 65 - 70 %) and Rp-**5** (yield: 65 - 70 %).

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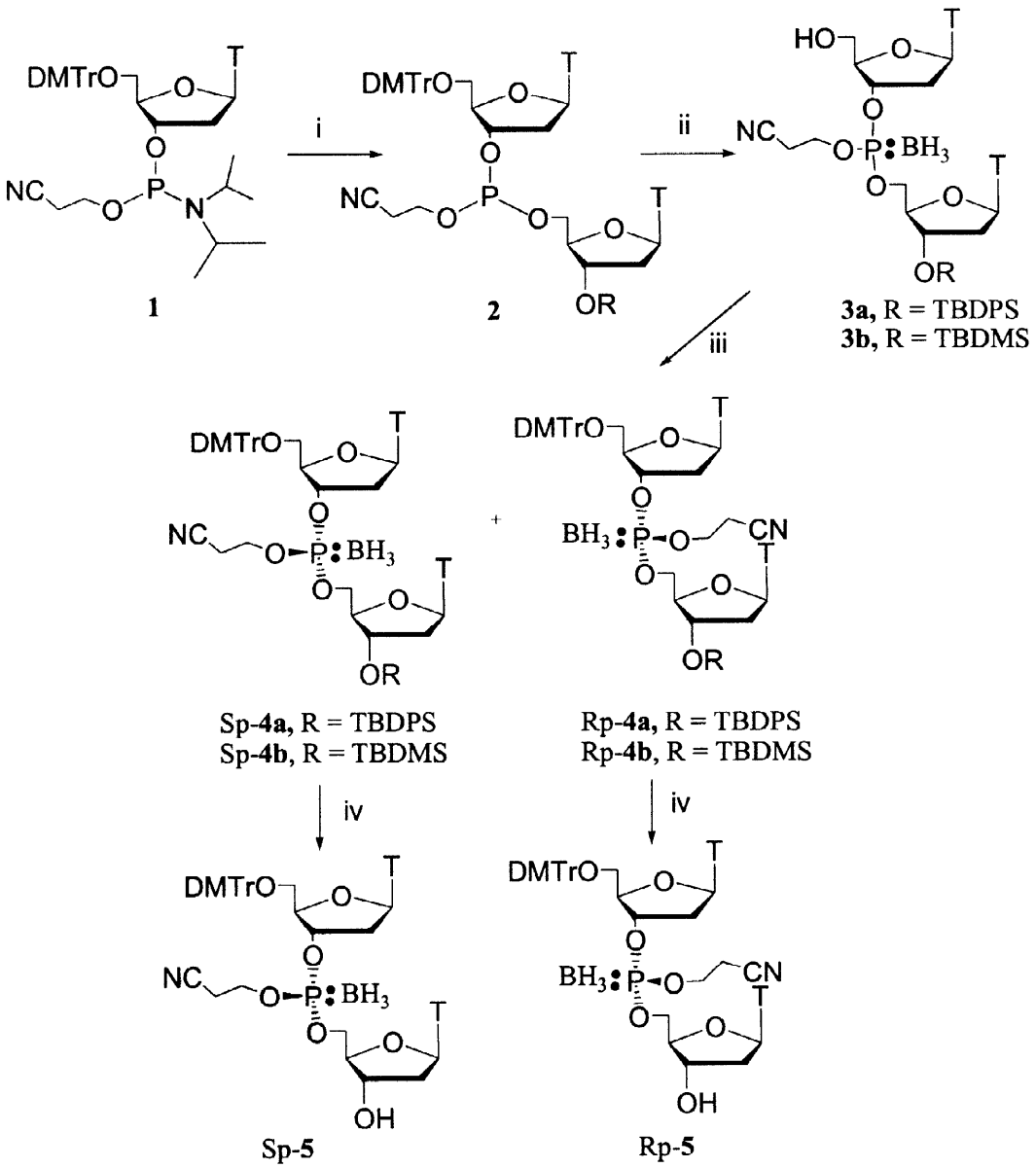
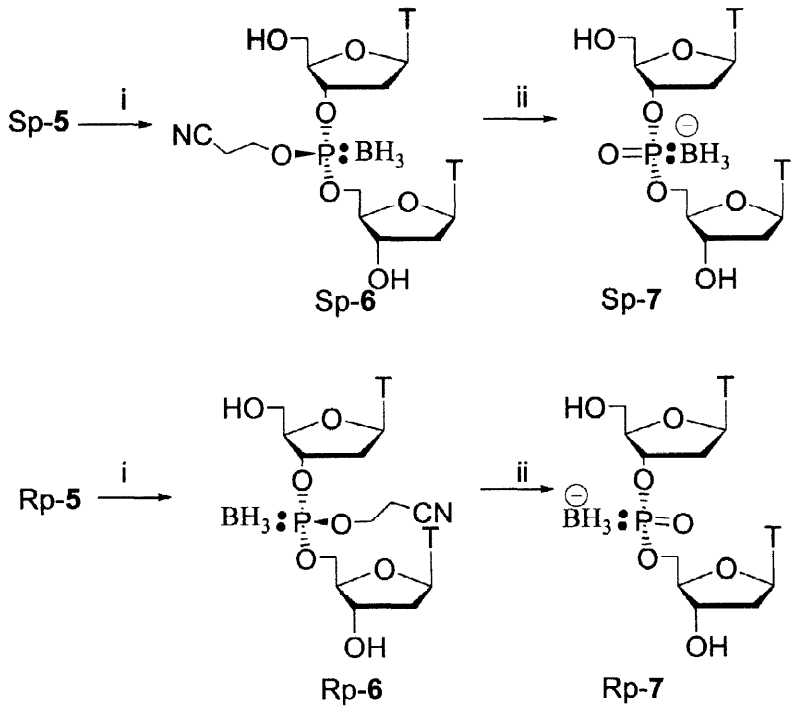
SCHEME 1



i. T_5-OH, tetrazole, CH_3CN, RT; ii. BH_3-Me_2S in 2.0 M THF; iii. DMTrCl, 2.5 eq. pyridine,CH_3CN, RT, then chromatography separation; iv. TBAF 3 eq. /HOAc 24 eq. / THF, RT.

Both Sp-**5** and Rp-**5** gave, after treatment with 70% acetic acid, Sp-**6** (yield: 87%) and Rp-**6** (yield: 90%). Reaction with concentrated NH_4OH at RT gave desired diastereomers Sp-**7** and Rp-**7** in yields of 80% and 75%, respectively (Scheme 2). The structures and configurations of Sp-**7** and Rp-**7** were assigned by comparison with literature data.[3,8] Since the β elimination leading from boranophosphate cyanoethyl ester **6** to the free boranophosphates **7** does not involve any change in stereochemistry at the phosphorus atom, all intermediates have the stereochemistry as depicted as Scheme 1 and Scheme 2.

SCHEME 2



i. 70% HOAc, RT, 2 h; ii. 28% NH_4OH, RT, 2 h.

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REFERENCES AND NOTES

1. Shaw, B. R.; Madison, J.; Sood, A.; Spielvogel, B. F. *Methods in Molecular Biology, Vol. 20: Protocols for Oligonucleotides and Analogs* Edited by: S. Agrawal, copyright, **1993,** Human Press Inc., Totowa, NJ.
2. Sood, A.; Shaw, B. R.; Spielvogel, B. F. *J. Am. Chem. Soc.* **1990**, *112*, 9000 - 9001.
3. Li, H.; Huang, F.; Shaw, B. R. *Bioorganic & Medicinal Chemistry* **1997**, *5*, 787 - 795.
4. Li, H.; Porter, K.; Huang, F.; Shaw, B. R. *Nucleic Acids Res.* **1995**, *23*, 4495 - 4501.
5. Zhang, J.; Terhorst, T.; Matteucci, M. D. *Tetrahedron Lett.* **1997**, *38*, 4957 - 4960,.
6. 5'-DMTr-thymidine phosphoramidite **1** was a gift from Dalton Chemical Laboratories Inc, Toronto.
7. The structures of the diastereomers of Sp-4 (yield ~ 45%, R_f = 0.39 - 0.40, fast eluting in ethyl acetate / hexanes = 2 /1) and Rp-**4** (yield ~ 40%, R_f = 0.25, slow eluting in ethyl acetate / hexanes = 2 /1) were confirmed by ^{1}H, ^{13}C, ^{31}P, and ^{11}B NMR, and MS.

8. Sp-7: ^{31}P NMR (121 MHz, D_2O): δ 93.51 ppm; ^{11}B NMR (96 MHz, D_2O): δ - 41.64 ppm; ^{1}H NMR (500 MHz, D_2O): δ 7.51 (d, J = 1.2 Hz, 1H, ^{5}H-6), 7.48 (d, J = 1.2 Hz, 1H, ^{3}H-6), 6.17 (dd, J = 6.8 Hz, J = 6.8 Hz, 1H, ^{5}H-1'), 6.07 (dd, J = 6.8 Hz, J = 6.8 Hz, 1H, ^{3}H-1'), 4.68 - 4.64 (m, 1H, ^{3}H-3'), 4.43 - 4.41 (m, 1H, ^{5}H-3'), 4.00 - 3.97 (m, 1H, ^{3}H-4', ^{5}H-4'), 3.94 - 3.92 (m, 2H, ^{5}H-5', ^{5}H-5''), 3.69 -3.59 (AB, 2H, ^{3}H-5', ^{3}H-5''), 2.36 - 2.31 (m, 1H, ^{3}H-2'), 2.22 (m, 2H, ^{5}H-2', ^{5}H-2''), 2.20 - 2.16 (m, 1H, ^{3}H-2''), 1.75 (d, J = 1 Hz, 3H, $^{5}CH_3$C-5), 1.71 (d, J = 1 Hz, 3H, $^{3}CH_3$C-5), 0.20 (b, 3H, BH_3); ^{13}C NMR (125.7 MHz, D_2O): δ 166.18 (^{5}C-4), 166.05 (^{3}C-4), 151.43 (^{5}C-2), 151.23(^{3}C-2), 137.07 (^{5}C-6, ^{3}C-6), 111.26 (^{5}C-5), 111.19 (^{3}C-5) , 85.56 (d, J = 4.6 Hz, ^{3}C-4'), 85.03 (d, J = 6.4 Hz, ^{5}C-4'), 84.88 (^{3}C-1'), 84.45 (^{5}C-1'), 72.61 (d, J = 3.0 Hz, ^{3}C-3'), 70.42 (^{5}C-3'), 61.37 (d, J = 4.6 Hz, ^{5}C-5'), 60.55 (^{3}C-5'), 38.41 (^{5}C-2'), 37.60 (^{3}C-2'), 11.50 ($^{5}CH_3$C-5), 11.29 ($^{3}CH_3$C-5); MS (negative electrospray, TEA/H_2O): 543.2 (M^-).
Rp-7: ^{31}P NMR (121 MHz, D_2O): δ 93.76 ppm; ^{11}B NMR (96 MHz, D_2O): δ -41.29 ppm; ^{1}H NMR (500 MHz, D_2O): δ 7.57 (d, J = 1.2 Hz, 1H, ^{5}H-6), 7.51 (d, J = 1.2 Hz, 1H, ^{3}H-6), 6.15 (dd, J = 6.8 Hz, J = 6.8 Hz, 1H, ^{5}H-1'), 6.08 (dd, J = 6.8 Hz, J = 6.8 Hz, 1H, ^{3}H-1'), 4.75 - 4.70 (m, 1H, ^{3}H-3'), 4.43 -4.40 (m, 1H, ^{5}H-3'), 4.02 (m, 1H, ^{3}H-4'), 4.00 (m, 1H, ^{5}H-4'), 3.98 -3.90 (m, 2H, ^{5}H-5', ^{5}H-5''), 3.70 -3.61 (AB, 2H, ^{3}H-5', ^{3}H-5''), 2.38 - 2.34 (m, 1H, ^{3}H-2'), 2.24 -2.17 (m, 3H, ^{5}H-2', ^{5}H-2'', ^{3}H-2''), 1.76 (d, J = 1 Hz, 3H, $^{5}CH_3$C-5), 1.73 (d, J = 1 Hz, 3H, $^{3}CH_3$C-5), 0.20 (b, 3H, BH_3); ^{13}C NMR (125.7 MHz, D_2O): δ 166.14 (^{5}C-4), 166.06 (^{3}C-4), 151.36 (^{5}C-2), 151.24(^{3}C-2), 137.09 (^{5}C-6), 137.04 (^{3}C-6), 111.19 (^{5}C-5), 111.15 (^{3}C-5), 85.55 (d, J = 4.6 Hz, ^{3}C-4'), 85.08 (d, J = 7.3 Hz, ^{5}C-4'), 84.87 (^{3}C-1'), 84.78 (^{5}C-1'), 72.04 (d, J = 4.6 Hz, ^{3}C-3'), 70.48 (^{3}C-3'), 61.44 (d, J = 3.7 Hz, ^{5}C-5'), 60.60 (^{3}C-5'), 38.61 (^{5}C-2'), 37.91 (^{3}C-2'), 11.42 ($^{5}CH_3$C-5), 11.30 ($^{3}CH_3$C-5); MS (negative electrospray, TEA/H_2O): 543.2 (M^-).
^{1}H NMR spectra were assigned by COSY and ^{13}C NMR spectra were assigned by HMQC.