Synthesis and properties of a novel phosphodiester analogue, nucleoside boranophosphorothioate

Jinlai Lin and Barbara Ramsay Shaw*

Paul M. Gross Chemical Laboratory, Department of Chemistry, Duke University, Durham, North Carolina 27708-0346, USA. E-mail: brs@chem.duke.edu

Received (in Corvallis, OR, USA) 8th February 1999, Accepted 28th April 1999

The first boranophosphorothioate [$(RO)_2P(S)(BH_3)^-$] mimic of a phosphodiester compound, dithymidine boranophosphorothioate, has been synthesized; while it is water soluble, this new analogue is more lipophilic and nuclease resistant than natural nucleoside phosphodiesters [$(RO)_2P(O)(O)^-$] and phosphorothioates [$(RO)_2P(S)(O)^-$].

Novel oligonucleotide analogues are currently attracting attention as probes in biochemistry and molecular biology[1] and as possible therapeutic agents against cancer and viral diseases.[2] An impressive variety of these analogues[3] have been developed as potential therapeutic drugs for 'antisense' and 'antigene' targeting of specific genes to modulate their expression.[4] Of these modified oligonucleotides, the nucleoside phosphorothioates[5] and nucleoside boranophosphates[6] (Fig. 1) are among the most promising because they are resistant to nucleases and support RNase H induced cleavage[7] of the complementary messenger RNA. Nearly a dozen phosphorothioate oligonucleotides are now in clinical trials.[8] By structurally combining the phosphorothioate and boranophosphate backbones, we have created a new phosphodiester analogue, the boranophosphorothioate, $[S{=}P{-}BH_3]^-$, wherein the two nonbridging oxygen atoms of a phosphodiester group are replaced with a sulfur atom and borane group.

Here, we report the first example of a novel boranophosphorothioate compound, specifically the dithymidine boranophosphorothioate, its synthesis and properties.

The general procedure for the synthesis of dinucleoside boranophosphorothioates is outlined in Scheme 1. 5′-*O* Fluorenylmethoxycarbonyl (Fmoc)-thymidine[9] was phosphitylated with $(Pr^i_2N)_2PCl$ catalyzed by DMAP to give **1**. Phosphite **1** was treated *in situ* with 3′-*O*-acetylthymidine and tetrazole in DMF to give **2**. To the above mixture, 4-nitrophenol and tetrazole in DMF were added to yield **3**, which was then treated with excess $BH_3{\cdot}SMe_2$ complex to afford the phosphite–borane **4** with ^{31}P NMR signal at δ_P 116.6 (br). Dry **4** was reacted with Li_2S to give **5** (broad ^{31}P NMR signal at δ_P 161.0) which was converted to **6** with conc. NH_4OH–MeOH (1 : 1) at room temperature. The crude mixture was purified by ion-exchange column chromatography on QA-52 (HCO_3^-) cellulose to give **6** as the ammonium salt and isolated by HPLC. The overall yield of dithymidine boranophosphorothioate **6** (T^SpBT) was about 28%. Successful separation of the two diastereomers (R_P and S_P) of **6** was achieved by reverse-phase HPLC. The first eluted isomer T^SpBT I (**6a**) and the second eluted isomer T^SpBT II (**6b**) were characterized by ^{31}P and ^1H NMR.[10] The Li_2S method used above should be applicable to the synthesis of other boranophosphorothioates including $[^{35}S{=}P{-}BH_3]^-$ phosphodiesters.

In oligodeoxynucleotides (ODN), the replacement of a nonbridging oxygen atom in the natural phosphodiester linkage by S^-, Me or BH_3^- imparts resistance to nucleases that cleave DNA.[6f,g,11] For instance, the non-ionic methylphosphonates (Me-ODN) are highly resistant[11c] to phosphodiesterases; the

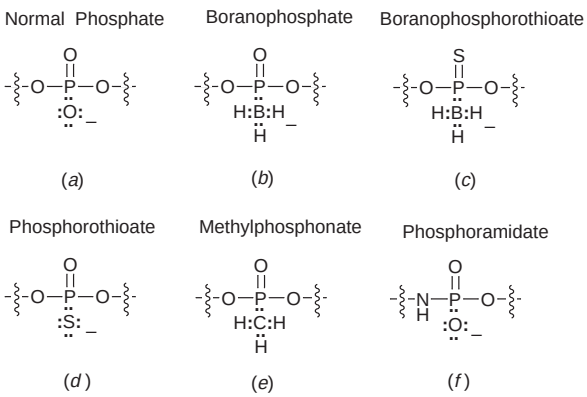
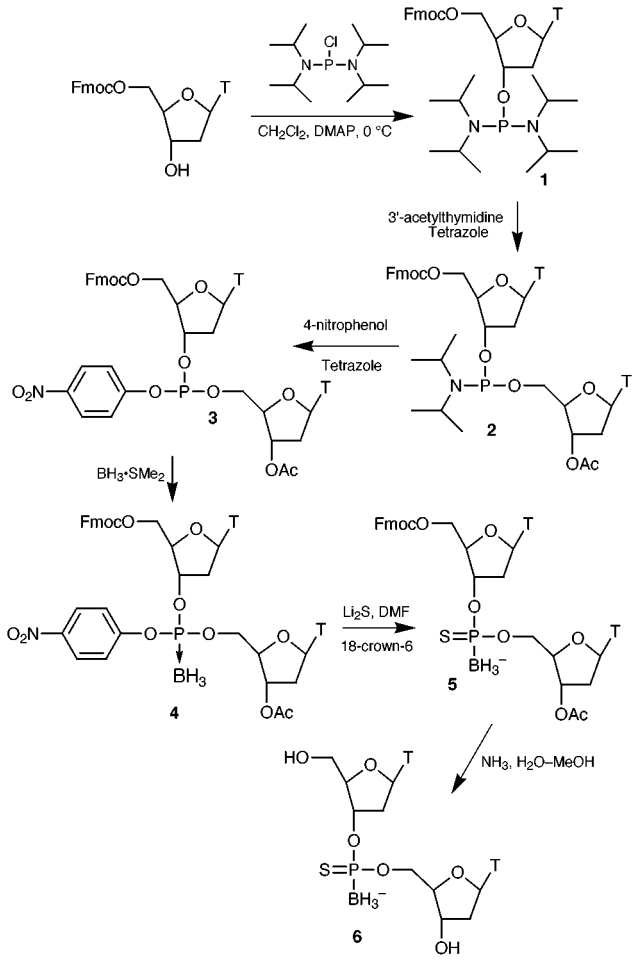


Fig. 1 Structurally and/or electronically similar internucleotide linkages and their abbreviations: (*a*) normal phosphate [O=P–O$^-$], (*b*) boranophosphate [O=P–BH$_3^-$], (*c*) boranophosphorothioate [S=P–BH$_3^-$], (*d*) phosphorothioate [O=P–S$^-$], (*e*) methylphosphonate [O=P–Me], (*f*) phosphoramidate. Placement of the negative charge does not necessarily reflect the actual spatial location of charge in the molecule.



Scheme 1

Table 1 Special properties of $[S{=}P{-}BH_3]^-$ backbone relative to natural and other phosphodiester backbone analogues

Property	$[O{=}P{-}O]^-$	$[O{=}P{-}S]^-$	O=P–Me	$[O{=}P{-}BH_3]^-$	$[S{=}P{-}BH_3]^-$
Nuclease resistance	—	+	++	+	++
Lipophilicity	—	+	+++	+	++
RNase H activity	+	+	—	+	N.D.

anionic phosphorothioates (S^--ODN) can be hydrolyzed by phosphodiesterase I, but with a much lower rate[11d] than O^--ODN. Boranophosphates (BH_3^--ODN) are even more resistant[6g] to certain phosphodiesterases than S^--ODN. This nuclease resistance, together with the ability to form stable duplex structures with DNA or RNA, has led to broad trials of S^-- and Me-ODN as agents for regulating gene expression *in vitro* and *in vivo*.[4] By combining aspects of both the charged $[O{=}P{-}S]^-$ and the non-ionic O=P–Me into a $[S{=}P{-}BH_3]^-$ hybrid phosphodiester linkage, it was anticipated that the resulting compound may exhibit greater nuclease resistance and other unique or potentially useful properties. The BH_3 moiety in $[S{=}P{-}BH_3]^-$ is isoelectronic with oxygen and isosteric with the Me group in O=P–Me, but imparts a negative charge to the backbone, like S^- in $[O{=}P{-}S]^-$. By virtue of the larger volume of the BH_3 group and its lack of lone pair electrons, we expect that $[S{=}P{-}BH_3]^-$ oligonucleotides should be more lipophilic than $[O{=}P{-}S]^-$ oligonucleotides. The new $[S{=}P{-}BH_3]^-$-ODN, which is a hybrid backbone of S^--ODN and BH_3^--ODN, has some special properties summarized in Table 1.

The boranophosphorothioate group is very stable towards basic or acidic hydrolysis. The diastereomers, T^Sp^BT I and T^Sp^BT II, were each dissolved in 100 mM AcOH–NH_4OH pH 3 or pH 11 buffer and incubated for 24 h at 37 °C. No hydrolyzed or degraded products were detected *via* HPLC.

The boranophosphorothioate internucleotide linkage in dimer **6** is quite stable towards cleavage by both snake venom phosphodiesterase (SVPDE) and bovine spleen phosphodiesterase (BSPDE). Under conditions where the natural dithymidine phosphate (TpT) was > 99% cleaved by SVPDE, both T^Sp^BT I and T^Sp^BT II were > 99% stable. Similarly, with BSPDE, while TpT was > 96% cleaved, T^Sp^BT I and T^Sp^BT II were > 98 and 97% stable, respectively.

The $[S{=}P{-}BH_3]^-$ dimers carry a full negative charge and are water soluble, yet are intermediate between normal phosphates and methylphosphonates in lipophilicity. In partitioning experiments,[12] T^Sp^BT was 320- and 18-fold more lipophilic than natural TpT and Tp^BT (dithymidine boranophosphate) accordingly.

The $[S{=}P{-}BH_3]^-$ nucleotidic linkage is the only non-bridging disubstituted chiral phosphodiester with a negative charge. This property coupled with ready synthesis of isotopic $[^{35}S{=}P{-}BH_3]^-$ compounds from Li_2S^* ($S^* = {}^{35}S$) could make this linkage very useful for elucidating the stereochemical course of phosphoryl and nucleotidyl transfer reactions and at the same time probing whether one or two non-bridging oxygens are necessary in these reactions.

To summarize, we have synthesized a totally new type of modified phosphodiester analogue, in which the two non-bridging oxygen atoms of a phosphodiester group have been replaced with a sulfur atom and borane group. The analogue has been placed in a nucleic acid and the resulting dithymidine boranophosphorothioate diastereomers shown to be stable under a broad range of pH conditions and highly resistant to enzymatic cleavage relative to natural DNA. Based on partitioning into octanol, the boranophosphorothioates may exhibit a greater membrane permeability than the O-oligonucleotides, yet maintain nuclease resistance like the methylphosphonates. The novel combination of high lipophilicity, reasonable water solubility and nuclease resistance could be extremely useful for drug design;[13] the $[S{=}P{-}BH_3]^-$ linkage instead of $[O{=}P{-}O]^-$ may enable the compounds to penetrate the plasma membrane and to enter cells.

Thus, synthesis of the first $[S{=}P{-}BH_3]^-$ phosphodiester analogue offers the possibility of preparing an entirely new and intriguing class of compounds, including modified nucleotides and nucleic acids. Their similarity to natural nucleic acids and unique properties such as high lipophilicity and resistance to enzymatic cleavage, in conjunction with their potential utility as molecular probes for the study of stereochemical aspects of enzymatic and nonenzymatic reactions and as carriers of ^{10}B in boron neutron capture therapy (BNCT)[14] for the treatment of cancer, make the $[S{=}P{-}BH_3]^-$ linkage a promising candidate for further mechanistic, diagnostic and therapeutic applications.

We thank Drs Dmitri S. Sergueev, Vladimir Rait and Zinaida Sergueeva and Mr Kaizhang He for their suggestions and help. This work was supported by grants 1R01-GM57693-01 from NIH and DE-FG05-97ER62376 from DOE to B. R. S. and is in partial fulfillment of requirements (J. L.) for a PhD at Duke University.

Notes and references

1 F. Eckstein, *Angew. Chem., Int. Ed. Engl.*, 1983, **22**, 423.
2 *Antisense Therapeutic: Progress and Prospect*, ed. G. L. Trainor, ESCOM Science Publishers, The Netherlands, 1996, pp. 1–85.
3 M. Egli, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 1894; E. Uhlmann and A. Peyman, *Chem. Rev.*, 1990, **90,** 543.
4 S. T. Crooke and C. F. Bennett, *Annu. Rev. Pharmacol. Toxicol.*, 1996, **36**, 107.
5 F. Eckstein, *Annu. Rev. Biochem.*, 1985, **54**, 367.
6 (*a*) A. Sood, B. R. Shaw and B. F. Spielvogel, *J. Am. Chem. Soc.*, 1990, **112**, 9000; (*b*) J. Tomasz, B. R. Shaw, K. Porter, B. F. Spielvogel and A. Sood, *Angew. Chem., Int. Ed. Engl.*, 1992, **31**, 1373; (*c*) B. R. Shaw, J. Madison, A. Sood and B. F. Spielvogel, *Methods Mol. Biol.*, 1993, **20**, 225; (*d*) H. Li, C. Hardin and B. R. Shaw, *J. Am. Chem. Soc.*, 1996, **118**, 6606; (*e*) K. W. Porter, J. D. Briley, B. R. Shaw, *Nucleic Acids Res.*, 1997, **25**, 1611; (*f*) F. Huang, A. Sood, B. F. Spielvogel and B. R. Shaw, *J. Biomol. Struct. Dynam.*, 1993, **10**, a078; (*g*) D. S. Sergueev and B. R. Shaw, *J. Am. Chem. Soc.*, 1998, **120**, 9417.
7 V. Rait and B. R. Shaw, *Antisense Nucleic Acid Drug Dev.*, 1999, **9**, 53; B. R. Shaw, in Papers from the 5th Annual International Conference on Antisense: DNA and RNA Based Therapeutics. IBC, Southborough, MA, 1998, pp. 1–8; RNase H activity was mentioned by Higson *et al.* (A. P. Higson, A. Sierzchala, H. Brummel, Z.-G. Zhao and M. H. Caruthers, *Tetrahedron Lett.*, 1998, **39**, 3899), but no data were shown.
8 S. Agrawal, *Trends Biotechnol.*, 1996, **14**, 376.
9 C. Lehmann, Y.-Z. Xu, C. Christodoulou, Z.-K. Tan and M. J. Gait, *Nucleic Acids Res.*, 1989, **17**, 2379.
10 *Selected data* for **6** $\delta_H(D_2O$, 400 MHz) 7.56, 7.52, 7.50, 7.47 (4s, 1H, H6), 6.18–6.05 (m, 2H, H1′), 4.85–4.72 (m, 1H, H3′), 4.44–4.34 (m, 1H, H3′), 4.00 (m, 2H, H4′), 3.94–3.59 (2m, 4H, H5′), 2.38–2.14 (2m, 4H, H2′), 1.77, 1.75, 1.74, 1.71 (4s, 6H, 5-CH_3), 0.68–—0.44 (br, 3H, BH_3); $\delta_P(D_2O)$ 160.1 (br); λ_{max}/nm 267; *m/z* (FAB$^-$) 559.16 (M^-) [Calc. for $C_{20}H_{29}O_{10}N_4BPS$, 559.1435 (M^-), found, 559.1421]; HPLC conditions: Waters Delta Pak C18-300 Å, 15 μ, 7.8 × 300 mm column; eluants were 18% MeOH and 82% 20 mM KH_2PO_4 (pH 7.0); flow rate, 3.0 ml min^{-1}; t_R (**6a**) = 28.69 min, t_R (**6b**) = 32.52 min.
11 (*a*) P. S. Sarin, S. Agrawal, M. P. Civéira, J. Goodchild, T. Ikeuchi and P. C. Zamecnik, *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 7448; (*b*) D. M. Tidd, *Anticancer Res.*, 1990, **10**, 1169; (*c*) K. L. Agarwal and F. Riftina, *Nucleic Acids Res.*, 1979, **6**, 3009; (*d*) P. M. J. Burgers and F. Eckstein, *Biochemistry*, 1979, **18**, 592.
12 Partition coefficients, defined as the ratio of concentration in octan-1-ol to that in water, were 2.8×10^{-2}, 1.6×10^{-3} and 8.7×10^{-5} for T^Sp^BT (mixture of diastereomers), Tp^BT (dithymidine boranophosphate), and TpT respectively. The partition coefficient of T^Sp^BT was 320 times greater than that of TpT.
13 C. McGuigan, D. Cahard, A. Salgado, E. De Clercq and J. Balzarini, *Antiviral Chem. Chemother.*, 1996, **7**, 31.
14 M. F. Hawthorne, *Angew. Chem., Int. Ed. Engl.*, 1993, **32**, 950.

Communication 9/01157I