Synthesis of a novel triphosphate analogue: nucleoside α-*P*-borano, α-*P*-thiotriphosphate

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 Covallis, OR, USA) 22nd June 2000, Accepted 10th August 2000
First published as an Advance Article on the web 11th October 2000

The first boranothiotriphosphate compound, thymidine 5′-[α-*P*-borano, α-*P*-thio]triphosphate, in which borane and sulfur replace the two non-bridging oxygens of the α-phosphate in natural nucleoside triphosphates (NTPs), has been synthesized; some chemical properties of this borane–sulfur disubstituted nucleoside triphosphate analogue have been investigated. The synthetic approach reported here should be applicable for preparation of any α-borano, α-thio modified NTP or deoxy NTP; potential applications are discussed.

Modifications of nucleoside triphosphates (NTPs) [Fig. 1a] have received much attention in searches for potential diagnostic and therapeutic agents and as probes in a multitude of biological processes.[1,2] Of these modified NTPs, only the nucleoside 5′-[α-thio]triphosphates[1] [Fig. 1b] and nucleoside 5′-[α-borano]triphosphates[2] [Fig. 1c] can substitute for normal NTP and be readily incorporated into DNA and RNA by DNA or RNA polymerases.[1,2] Yet once in DNA or RNA, the phosphorothioate[3] and boranophosphate[4] linkages are more resistant to *exo*- and *endo*-nucleases than normal phosphate diesters. By structurally combining the phosphorothioate [S–P=O]$^-$ and boranophosphate [O=P–BH_3]$^-$, we recently reported the first example of a boranothiophosphate moiety [S=P–BH_3]$^-$, dithymidine boranphosphorothioate,[5] which is stable from pH 3 to 11 and is a more nuclease resistant and highly lipophilic phosphodiester analogue of DNA. We expected that the corresponding NTP analogue, nucleoside [α-borano, α-thio]triphosphate (Fig. 1d), wherein one of the two non-bridging oxygen atoms of the α-phosphate group is replaced by a sulfur atom and the other is replaced by a borane group, should be more lipophilic than the parent NTP and have other useful properties. The borano-, thio-disubstitution of nucleoside triphosphate could greatly increase the stability of the molecule against enzymatic cleavage, thus facilitating studies of enzymes which utilize NTPs and enabling studies of the nature of phosphate ester bond formation and cleavage.

Here, we report the first example of a novel boranothiotriphosphate compound, specifically the thymidine 5′-[α-*P*-borano, α-*P*-thio]triphosphate **5**, its synthesis and properties.

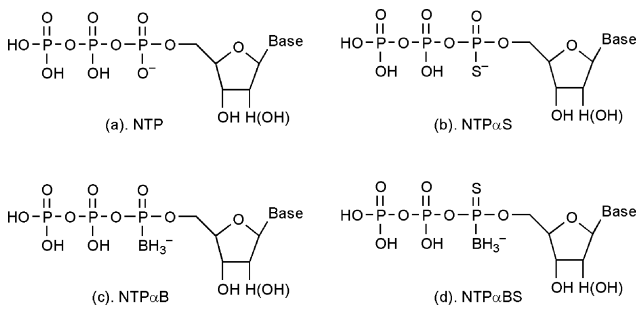
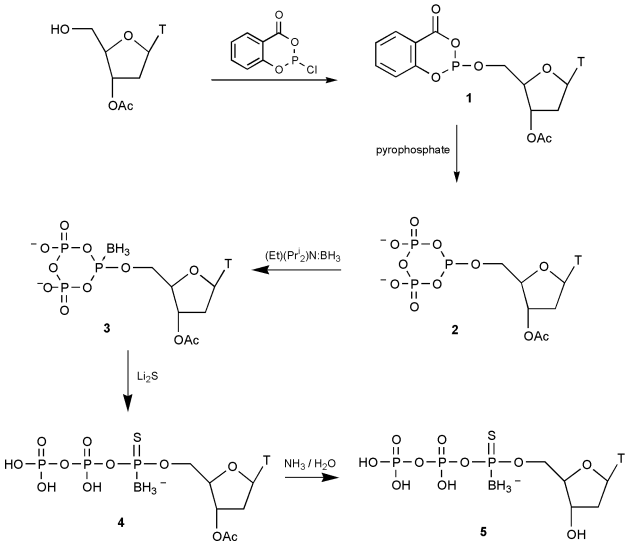


Fig. 1 Nucleoside triphosphate analogue structures and abbreviations. (a) Nucleoside triphosphates (NTP). (b) Nucleoside [α-thio]triphosphates (NTPαS). (c) Nucleoside [α-borano]triphosphates (NTPαB). (d) Nucleoside [α-borano, α-thio]triphosphates (NTPαBS).

The general procedure[6] for the one-pot synthesis of nucleoside 5′-[α-borano, α-thio]triphosphates shown in Scheme 1 is an extension of a method reported by us[2c,d] for the synthesis of boranotriphosphates and by Ludwig and Eckstein[1c] for the synthesis of thiotriphosphates. The overall yield of thymidine 5′-[α-*P*-borano, α-*P*-thio]triphosphate **5** (TTPαBS) was about 26%. The chemical structure of **5** (^{31}P NMR, δ: 153.1 ppm(br) for disubstituted α-*P*) was established *via* spectroscopic methods.[7] Successful separation of the two diastereomers (*R*p and *S*p) of **5** was achieved by reverse-phase HPLC.[7b] The first eluted isomer TTPαBS I (**5a**) and second eluted isomer TTPαBS II (**5b**) were characterized by ^{31}P NMR and ^{1}H NMR.[7c] The method here should be applicable for the synthesis of any deoxy- or ribonucleoside [α-borano, α-thio]triphosphate.

The [S=P–BH_3]$^-$ α-triphosphate is the only known non-bridging-disubstituted chiral α-triphosphate with a negative charge.[8] The borano-, thio-disubstitution in a nucleotidic linkage will result in changes in polarity of the phosphate, as well as its interactions with metal ions.[2f,g] These properties coupled with ready synthesis of isotopic [NTPα^{35}S=P–BH_3] compounds from Li_2S^* (S^* = ^{35}S) could make this modification useful as a probe for nucleotide binding sites in enzymes, for elucidating the stereochemical course and role of metal ions of phosphoryl and nucleotidyl transfer reactions, and for probing whether a non-bridging oxygen is absolutely necessary in these reactions.

To summarize, the synthesis of a new type of doubly modified nucleoside triphosphate analogue, the first [S=P–BH_3]$^-$ triphosphate, opens the possibility of preparing an entirely new and intriguing class of borane–sulfur modified phosphate analogues. Their potential utility as substrates, cofactors, or inhibitors of polymerases and nucleotide binding and metabolizing enzymes, as molecular probes, and as carriers of ^{35}S for radiolabeling and radiation therapy,[9] make the



Scheme 1 Synthesis of thymidine [α-*P*-borano, α-*P*-thio]triphosphate.

DOI: 10.1039/b005059h

nucleoside boranothiotriphosphate a promising candidate for further mechanistic, diagnostic and therapeutic applications.

We thank Drs Vladimir Rait, Dmitri S. Sergueev, Zinaida Sergueeva and Kaizhang He for their encouragement and suggestions. This work was supported by grants 1R01-GM57693-01 from NIH and DE-FG05-97ER62376 from DOE to B. R. S.

Notes and references

1 (*a*) F. Eckstein, *Angew. Chem., Int. Ed. Engl.*, 1983, **22**, 423; (*b*) J. Ludwig and F. Eckstein, *J. Org. Chem.*, 1989, **54**, 631; (*c*) J. Ludwig and F. Eckstein, *J. Org. Chem.*, 1991, **56**, 1777; (*d*) G. Gish and F. Eckstein, *Science*, 1988, **240**, 1520; (*e*) P. Sideras, K. Funa, I. Zalcberg-Quintana, K. G. Xanthopoulos, P. Kisielow and R. Palacios, *Proc. Natl. Acad. Sci. U.S.A.*, 1988, **85**, 218; (*f*) S. Verma and F. Eckstein, *Ann. Rev. Biochem.*, 1998, **67**, 99.

2 (*a*) J. Tomasz, B. R. Shaw, K. Porter, B. F. Spielvogel and A. Sood, *Angew. Chem., Int. Ed. Engl.*, 1992, **31**, 1373; (*b*) K. W. Porter, J. D. Briley and B. R. Shaw, *Nucleic Acids Res.*, 1997, **25**, 1611; (*c*) K. He, A. Hasan, B. Krzyzanowska and B. R. Shaw, *J. Org. Chem.*, 1998, **63**, 5769; (*d*) B. Krzyzanowska, K. He, A. Hasan and B. R. Shaw, *Tetrahedron*, 1998, **54**, 5119; (*e*) K. He, K. W. Porter, A. Hasan, J. D. Briley and B. R. Shaw, *Nucleic Acids Res.*, 1999, **27**, 1788; (*f*) B. R. Shaw, D. Sergueev, K. He, K. Porter, J. Summers, Z. Sergueeva and V. Rait, *Methods Enzymol.*, 1999, **313**, 226; (*g*) J. S. Summers, D. Roe, P. D. Boyle, M. Colvin and B. R. Shaw, *Inorg. Chem.*, 1998, **37**, 4158; (*h*) J.-L Lin, K. He and B. R. Shaw, *Helv. Chim. Acta*, 2000, **83**, 1392.

3 (*a*) P. M. J. Burgers and F. Eckstein, *Biochemistry*, 1979, **18**, 592; (*b*) P. M. J. Burgers and F. Eckstein, *Proc. Natl. Acad. Sci. U.S.A.*, 1978, **75**, 4798.

4 (*a*) A. Sood, B. R. Shaw and B. F. Spielvogel, *J. Am. Chem. Soc.*, 1990, **112**, 9000; (*b*) Y.-Q. Chen, F.-C. Qu and Y.-B. Zhang, *Tetrahedron Lett.*, 1995, **36**, 745; (*c*) D. S. Sergueev and B. R. Shaw, *J. Am. Chem. Soc.*, 1998, **120**, 9417.

5 J.-L. Lin and B. R. Shaw, *Chem. Commun.*, 1999, 1517.

6 The following procedure for the preparation of nucleoside [α-borano, α-thio]triphosphates is representative: 3′-*O*-acetylthymidine (0.5 mmol, 142 mg) was phosphitylated with 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one (0.55 mmol, 112 mg in 0.8 ml anhydrous DMF) at 0 °C for 10 min to yield two diastereomers of **1**. They were identified by the appearance of a doublet around 127 ppm in the ^{31}P NMR spectra. Instead of introducing the borane group at this stage, **1** was treated with tributylammonium pyrophosphate (240 mg in 1.0 ml anhydrous DMF) and 0.15 ml triethylamine at rt for 1 h to form a cyclic intermediate **2**. The upfield shift from around 127 to 107 ppm for trivalent phosphorus P^{III} together with a doublet around −18 ppm for pentavalent phosphorus P^{V} in ^{31}P NMR confirmed the formation of **2**. The borane group was introduced by the reaction of **2** with excess borane–diisopropylethylamine complex (1 ml) at rt for 2 h to afford **3**. The critical step in the synthesis is ring-opening of the cyclic boronated triphosphate **3** by the treatment with 2 eq. lithium sulfide at 55 °C for 1 h to yield **4**, which was converted to compound **5** with NH_3–H_2O–CH_3OH (aq. NH_3–CH_3OH = 1 : 1, v/v) at rt for 4 h. The low boiling point solvents were removed under reduced pressure and the residue was extracted with ethyl acetate (16 ml) and water (10 ml). The organic layer was washed twice with 2 ml water. The water portions were combined and concentrated to 2 ml. The resulting crude mixture was applied to ion-exchange chromatography on a QA-Cellulose (HCO_3^-) column eluted with a linear gradient of 0.005–0.20 M NH_4HCO_3 (pH = 9.56, 700 ml each) and appropriate portions were collected to give **5** as the ammonium salt. Further purification of **5** (one-sixth of total amount) by HPLC using a linear gradient of 50 mM triethylammonium acetate (TEAA, pH 7.0) and CH_3CN (from 0 to 18% in 40 min) gave pure **5**, R_t = 21.4 min (HPLC conditions: Sphereclone 5 μ ODS(2), 10 × 250 mm column; flow rate, 3.0 ml min^{-1}). The desired fraction was dried by lyophilization, and excess salt was removed by repeated lyophilization with deionized water to yield the triethylammonium salt of **5** (13 mg).

7 Characterization and separation of **5** and its two diastereomers (**5a** and **5b**): (*a*) TTP$^{\alpha}$BS (**5**): UV λ_{max} = 267 nm; δ_P (D_2O, 161.9 MHz) 153.1 (br) for α-*P*, −22.4 and −22.7 for β-*P* and −10.0 and −10.1 for γ-*P*; δ_H (D_2O, 400 MHz) 7.62, 7.57 (2s, 2 isomers, 1 H, H6), 6.16 (unresolved, 1 H, H1′), 4.54–4.46 (m, 1 H, H3′), 4.29–4.15 (m, 1 H, H4′), 4.02–3.99 (m, 2 H, H5′), 2.15 (2 H, H2′), 1.78, 1.74 (2s, 3 H, 5-CH_3), 0.92–0.54 (br, 3 H, BH_3); MS (FAB$^-$): *m*/*z* for M^- 495; HRMS (FAB$^-$) calc. for $C_{10}H_{19}O_{12}N_2P_3SB$ M^- 494.9963, found 494.9942; (*b*) *HPLC conditions:* Sphereclone 5 μ ODS(2), 10 × 250 mm column; eluants were 10% MeOH and 90% 50 mM triethylammonium acetate (pH 7.0); flow rate, 3.0 ml min^{-1}. R_t (**5a**) = 24.9 min. R_t (**5b**) = 30.4 min. (*c*) *Two diastereomers: TTP$^{\alpha}$BS I* (**5a**): δ_P (D_2O, 161.9 MHz) 152.3 (br) for α-*P*, −21.20, −21.33, −21.44 and −21.57 for β-*P* and −5.72 and −5.85 for γ-*P*; δ_H (D_2O, 400 MHz) 7.62 (s, 1 H, H6), 6.18 (t, 1 H, *J* = 7.0 Hz, H1′), 4.51–4.48 (m, 1 H, H3′), 4.32–4.28 (m, 1 H, H4′), 4.05–4.01 (m, 2 H, H5′), 2.19–2.15 (m, 2 H, H2′), 1.79 (s, 3 H, 5-CH_3), 0.90–0.30 (br, 3 H, BH_3). *TTP$^{\alpha}$BS II* (**5b**): δ_P (D_2O, 161.9 MHz) 151.3 (br) for α-*P*, −21.28, −21.41, −21.50 and −21.63 for β-*P* and −7.03 and −7.15 for γ-*P*; δ_H (D_2O, 400 MHz) 7.57 (s, 1 H, H6), 6.16 (t, 1 H, *J* = 7.0 Hz, H1′), 4.51 (m, 1 H, H3′), 4.20–4.16 (m, 1 H, H4′), 4.12–4.06 (m, 2 H, H5′), 2.19–2.16 (m, 2 H, H2′), 1.78 (s, 3 H, 5-CH_3), 0.90–0.30 (br, 3 H, BH_3).

8 S/CH_3 phosphate analogues, in which the two non-bridging oxygens in a phosphate are replaced by S and CH_3 moieties, carry no charge; S_2 (dithio) phosphate analogues,[1c] in which the two non-bridging oxygens in a phosphate are replaced by S atoms, are achiral.

9 M. F. Hawthorne, *Angew. Chem., Int. Ed. Engl.*, 1993, **32**, 950.