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Synthesis of nucleoside 3′,5′-cyclic boranophosphorothioate, a new type of cyclic nucleotide†

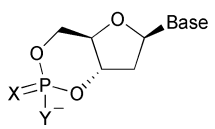
Ping Li and Barbara Ramsay Shaw*

Paul M.Gross Chemical Laboratory, Department of Chemistry, Duke University, Durham, North Carolina, 27708-0346, USA. E-mail: brs@chem.duke.edu; Fax: 1-919-6601618; Tel: 1-919-6601551

Received (in Cambridge, UK) 26th July 2002, Accepted 3rd August 2002
First published as an Advance Article on the web 4th November 2002

The first examples of a borane-containing doubly *P*-modified chiral cyclic nucleoside monophosphate (cNMP), *e.g.*, thymidine and 5-fluoro-2′-deoxyuridine 3′,5′-cyclic boranophosphorothioates, have been synthesized; these cNMP analogues with increased lipophilicity could be potential anticancer prodrugs and useful probes for mechanistic studies.

Nucleoside 3′,5′-cyclic monophosphates (cNMPs) **1** (Fig. 1), such as cAMP and cGMP, are important regulators of biochemical processes.[1] For example, cAMP acts as a mediator of hormone action and modulator of enzymatic activity. To better understand these processes, various modified cNMPs have been prepared to probe the stereochemical and mechanistic aspects of phosphoryl- and nucleotidyl transfer reactions and their inhibition.[2] However, to the best of our knowledge, only the 3′,5′-cyclic nucleoside phosphorothioate (cNMPS) **2** and phosphorodithioate ($cNMPS_2$) **3** (Fig. 1) have been well studied

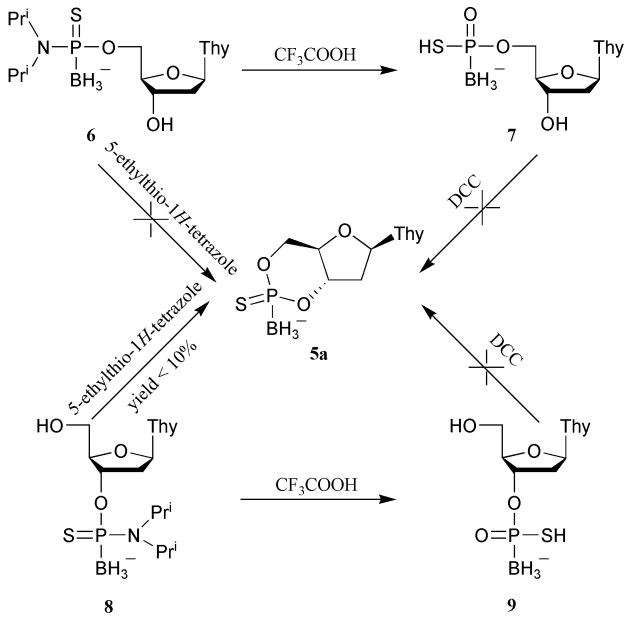


1 X = O, Y = O: cNMP
2 X = O, Y = S: cNMPS
3 X = S, Y = S: $cNMPS_2$
4 X = O, Y = BH_3: cNMPB
5 X = S, Y = BH_3: cNMPBS

Fig. 1 cNMP, its analogues and their abbreviations.

as *P*-modified analogues of the cNMPs.[2a–c,3] More analogues could be useful in order to fully understand processes involving cNMPs.[2b] By structurally combining the phosphorothioate and boranophosphate, our group recently reported the synthesis of dithymidine boranophosphorothioate and thymidine 5′-[α-*P*-borano, α-*P*-thio]triphosphate,[4] wherein the two nonbridging phosphoryl oxygen atoms were replaced with a sulfur atom and borane group (BH_3). The excellent lipophilicity for dithymidine boranophosphorothioate relative to natural dithymidine phosphate makes this modification a convenient basis for creation of possible anticancer prodrugs.[4a] For example, 5-fluoro-2′-deoxyuridine monophosphate (FdUMP) is a well-known cancer inhibitor that inhibits thymidylate synthase, a target enzyme for the control of cell growth. However, the high polarity of FdUMP makes it difficult to penetrate the cell membrane. Several prodrugs based on the delivery of FdUMP have been synthesized and studied *in vitro*, but few appear to be efficient.[5] By virtue of replacing two non-bridging phosphoryl oxygens in cNMP with a more lipophilic sulfur atom and borane group, we propose to synthesize the first class of doubly-modified nucleoside 3′,5′-cyclic boranophosphorothioate (cNMPBS) compounds **5**. Based on the studies of other cyclic monophosphate analogues[6] and boranophosphates,[7] the cNMPBS may more readily penetrate into cells and be hydrolyzed to the corresponding nucleoside 5′-monophosphate.

Typically, nucleoside 3′,5′-cyclic monophosphates can be prepared from the nucleoside monophosphates or their derivatives *via* intramolecular dehydration and/or cyclization.[8] For example, treatment of nucleoside 5′-monophosphate with dicyclohexylcarbodiimide (DCC) provides a convenient approach to synthesize normal cNMP.[8a–c] However, in our case, this method failed to give the desired compound **5a** from thymidine 5′- or 3′-boranophosphorothioate (**7** or **9**)[4c] (Scheme 1). Reaction of thymidine 5′-boranophosphorothioate *P*-diisopropylamidate **6** with two equiv. of 5-ethylthio-1*H*-tetrazole[9] was also unsuccessful. A similar intramolecular reaction of thymidine 3′-boranophosphorothioate *P*-diisopropylamidate **8**[10] gave compound **5a**, but in less than 10% yield.[11] The steric hinderance of a primary and secondary alcohol may account for the reactivity difference between compounds **6** and **8**.[12]

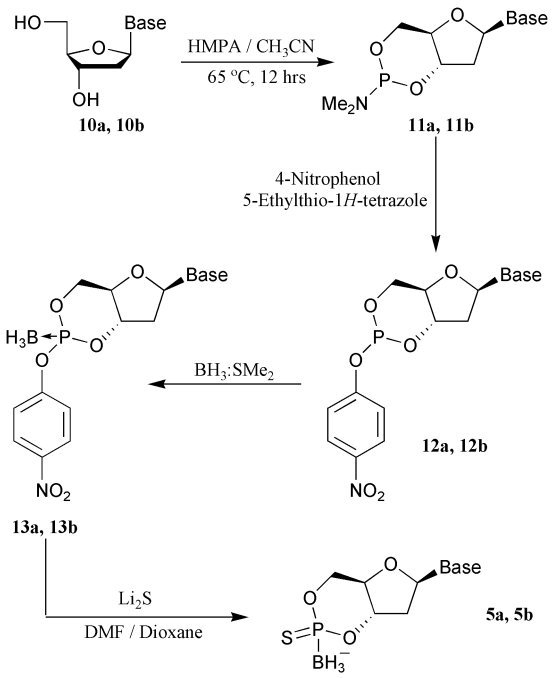


Scheme 1 Possible approaches for synthesizing cTMPBS **5a**.

Since the direct cyclization of boranophosphorothioates was unsuitable, we decided to explore the possibility of introducing the modification into a nucleoside 3′,5′-cyclophosphite. We developed the procedure here for the one-pot synthesis of cNMPBS **5** (Scheme 2) based on a modification of the methods reported by our group for the synthesis of 3′,5′-cyclic boranomonophosphate (cNMPB) **4**[4,13a] and by Eckstein for the synthesis of dithiotriphosphates.[13b] One crucial step in the synthesis of cNMPBS **5** is the formation of phosphoramidite **11** by the reaction of nucleoside **10** with a bis-functional phosphoramide, such as hexamethylphosphoramide (HMPA),[14] bis(diisopropylamino)chlorophosphine, and diisopropylphosphoramidous dichloride. By using HMPA with thorough deoxygenation of the solvent (acetonitrile) and slowly increasing the reaction temperature to 65 °C, we were able to obtain a relatively high yield (> 95%) of compound **11**. Phosphoramidite **11** was then transformed to phosphite triester **12** by reaction with 4-nitrophenol in the presence of 5-ethylthio-1*H*-tetrazole. Upon *in situ* boronation, compound **13**[15] was formed and exhibited a broad ^{31}P NMR peak centered at 107 ppm, which is character-

† Electronic supplementary information (ESI) available: Experimental details and spectral data. See http://www.rsc.org/suppdata/cc/b2/b207350a/

DOI: 10.1039/b207350a



a: Base = Thymine
b: Base = 5-Fluorouracil

Scheme 2 Synthesis of cNMPBS **5**.

istic of boranophosphite.[16] Of several boronating reagents tried, borane–dimethyl sulfide gave the best yield after a 4 h treatment. Introduction of a sulfur atom at this stage is another key point in our synthesis. After evaporation of the solvent, boranophosphite **13** could be directly and quantitatively converted to cNMPBS **5** *via* Li_2S substitution in DMF/dioxane without the addition of 18-crown-6 used in the synthesis of thymidine 5′-[α-*P*-borano, α-*P*-thio]triphosphate.[4a]

Nucleoside 3′,5′-cyclic boranophosphorothioate **5a** and **5b** are the only known *P*-disubstituted chiral cyclic monophosphate with a negative charge. The borano-, thio-disubstitution will change the electron distribution over the phosphate, as well as the interactions between cyclic monophosphate and metal ions.[7b,17] These properties could make cNMPBS **5** useful in elucidating the stereochemical course and role of metal ions in phosphoryl and nucleotidyl transfer reactions.

In partitioning experiments, the partition coefficients, defined as the ratio of concentration in octan-1-ol to that in water, were 1.7×10^{-2}, 2.2×10^{-2}, 5.1×10^{-5} and 7.6×10^{-5} for cTMPBS **5a**, cFdUMPBS **5b**, cTMP and cFdUMP, respectively. Thus, 3′,5′-cyclic boranophosphorothioates cTMPBS **5a** and cFdUMPBS **5b** were 340- and 290-fold more lipophilic than parental cTMP and cFdUMP, accordingly. A good lipophilicity in combination with the negative charge and good solubility in water could make 3′,5′-cyclic nucleoside boranophosphorothioates promising as prodrug reagents.

In summary, we have synthesized a new type of doubly *P*-modified chiral cyclic NMP, *e.g.*, cNMPBS **5a**,**b**,† by a one-pot reaction in 65–70% overall yield after isolation. The two *P*-diastereoisomers of cNMPBS were separated by RP-HPLC. The increased lipophilicity and the putatively different substrate properties imparted by the borane group[7] make the cNMPBS a potential anticancer prodrug and a new probe for mechanistic studies on cNMP.

We thank Drs Zinaida Sergueeva, Dmitri Sergueev and Mikhail Dobrikov for helpful discussion and critical comments. This work was supported by NIH grant RO1-AI-52061 to B. R. S.

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