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Novel 3′,5′-Cyclic Nucleotide Analogue: Adenosine 3′,5′-Cyclic Boranomonophosphate

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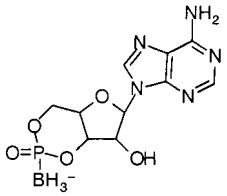
ABSTRACT



cyclic AMPB, **4c**

A general procedure for the first synthesis of a 3′,5′-cyclic boranomonophosphate was established. Specifically, adenosine 3′,5′-cyclic boranomonophosphosphate (cyclic AMPB, 4c), a *P*-borane (BH_3) analogue of adenosine 3′,5′-cyclic monophosphate (cAMP), was synthesized via a phosphite approach in good yield. The method is also applicable for syntheses of natural cAMP and its phosphorothioate analogue. The two diasteromers of cyclic AMPB 4c were separated, and their chemical structures were established via spectroscopic methods.

3′,5′-Cyclic adenosine monophosphate (cAMP) is ubiquitous in all forms of life and vital in the regulation of various biological processes.[1] For example, cAMP is an ancient hunger signal and a second messenger in the action of many hormones; it is central to the coordinated control of glycogen synthesis and breakdown, and stimulates protein kinases and the transcription of several inducible catabolic operons.[1] To better understand biological processes involving cAMP, various modified analogues have been prepared as enzyme substrates or inhibitors.[1−4] Among cAMP analogues, the N^6,2′-*O*-dibutyryl cyclic AMP[2] and cyclic AMP phosphorothioate (cyclic AMPS)[4] are most commonly used. However, N^6,2′-*O*-dibutyryl cyclic AMP releases butyric acid on hydrolysis, which can cause a number of reactions unrelated to the action of cyclic AMP that might not be evident by applying butyric acid to the system as a control.[2,4d] Cyclic AMPS has many special properties such as high nuclease resistance, but its relativly poor lipophilicity[4g] and loss of sulfur during incubation[4c] greatly limit its applications. Boronated nucleotides,[5] in which one of two nonbridging oxygen atoms of phosphate is replaced by a borane group (BH_3), might be useful because they are more lipophilic and nuclease resistant than the normal congener and phosphorothioates.[5f] Here we report the first synthesis of a boronated cyclic nucleotide analogue, specifically adenosine 3′,5′-cyclic boranomonophosphate (cyclic AMPB).

Commonly, nucleoside 3′,5′-cyclic phosphates are prepared from the nucleoside monophosphate through intramolecular

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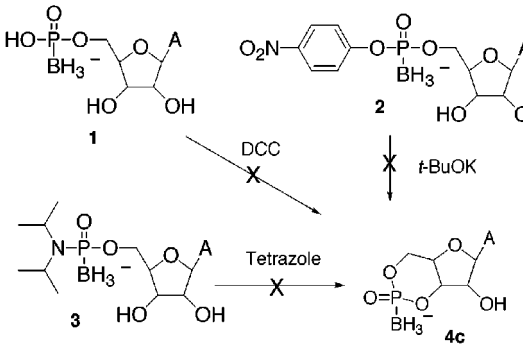
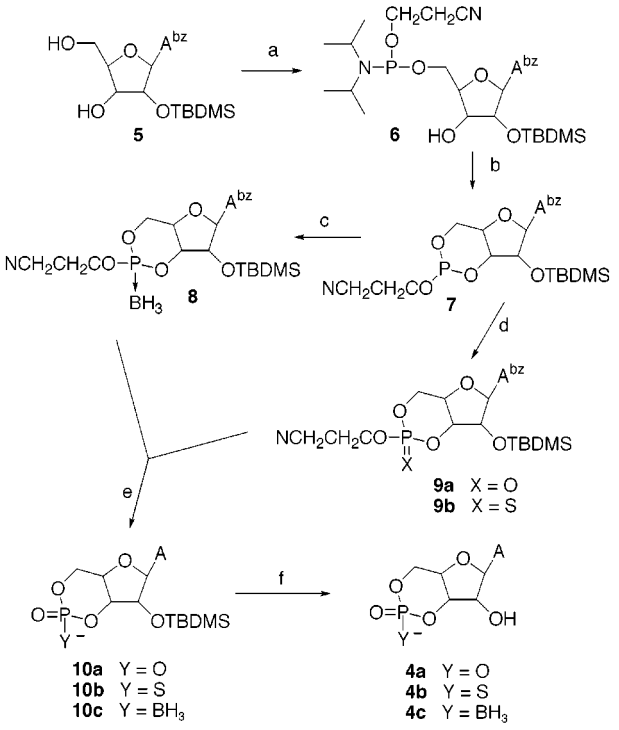
dehydration or from a nucleoside monophosphate derivative via intramolecular cyclization.[6,7] For example, treatment of 5′-AMP with 1,3-dicyclohexylcarbodiimide (DCC)[6] or reaction of 5′-AMP *p*-nitrophenyl ester with t-BuOK[7a] provides convenient methods to synthesize normal 3′,5′-cyclic AMP. Yet, neither the treatment of adenosine 5′-boranomonophosphate (5′-AMPB) **1** with DCC in dilute pyridine solution at room temperature, nor treatment of adenosine 5′-boranomonophosphate *p*-nitrophenyl ester (5′-AMPB-NPE) **2** under strongly basic anhydrous conditions (t-BuOK), yielded compound **4c**. Further, adenosine 5′-boranomonophosphate *p*-diisopropylamidate[8] (5′-AMPB-DIPA) **3** treated with tetrazole (4 equiv) in DMF at 55 °C also failed to give the desired product 3′,5′-cyclic AMPB **4c**. After several unsuccessful attempts to prepare the cyclic boronated phosphodiester compounds by direct methods in Scheme 1, we were forced to explore a synthetic method that involved the phosphite approach in Scheme 2.

Scheme 1



The general procedure for the synthesis of cyclic AMPB is outlined in Scheme 2. Phosphitylation of N^4-benzoyl-2′-*O*-(*tert*-butyldimethylsilyl)adenosine **5** in DMF by 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite $(i\text{-Pr}_2\text{N})_2\text{P(OCH}_2\text{CH}_2\text{CN})$ with diisopropylamine hydrotetrazolide as catalyst gave **6**. After 1 h, tetrazole was added. Without stirring, the intramolecular cyclization occurred to yield intermediate **7** after 3 h. Without purification, intermediate

Scheme 2. Synthesis of Adenosine 3′,5′-Cyclic *P*-Boranomonophosphate[a]



[a] (a) $[(i\text{-Pr})_2\text{N}]_2\text{POCH}_2\text{CH}_2\text{CN}$/diisopropylamine hydrotetrazolide/DMF; (b) tetrazole; (c) $(\text{Et})(i\text{-Pr})_2\text{N:BH}_3$ or $\text{Me}_2\text{S:BH}_3$; (d) from **7** to **9a**, I_2/H_2O/pyridine, from **7** to **9b**, sulfur (S_8); (e) NH_4OH/CH_3OH; (f) Bu_4NF.

7 was treated either with excess (4 equiv) borane−*N*,*N*-diisopropylethylamine complex (DIPEA−BH_3) with stirring for 2 h at room temperature or with 1 M borane−dimethyl sulfide in CH_2Cl_2 at 0 °C with stirring for 10 min to give the borane−phosphite intermediate **8.** After evaporation and extraction with ethyl acetate and water, the organic layer was concentrated and treated with a mixture of ammonium hydroxide and methanol (v:v = 1:1) to give compound **10c**. Without purification, **10c** was treated with 1 M tetrabutylammonium fluoride to give cyclic AMPB **4c**. Compound **4c** was purified by ion-exchange chromatography on a column packed with QA-52 cellulose (HCO_3^-) eluted with a linear gradient of 0.005 and 0.2 M ammonium bicarbonate buffer, pH 9.6. Compound **4c** was obtained in 48% overall yield (from **5** to **4c**) and identified by ^{31}P NMR (typical broad peaks at 92 and 97 ppm), ^{1}H NMR spectroscopy, and FAB$^-$-MS [M^- (*m*/*e*) $C_{10}H_{14}O_5N_5PB$, calcd 326.07, found, 326.08]. Successful separation of the two diastereomers (Rp and Sp) of **4c** was achieved by reverse-phase HPLC; chemical structures were established via spectroscopic methods. The two diastereomers of **4c** have considerably different ^{31}P chemical shifts: 92 ppm (br) for cyclic AMPB isomer I (first eluted diastereomer, **4c-I**), and a broad tetramer from 95.7 to 98.2 ppm for cyclic AMPB isomer II (second eluted diastereomer, **4c-II**). The chemical shift difference arises because the BH_3 group can assume an equitorial or axial position in the 3′,5′-cyclic six-membered ring of cyclic

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AMPB. The spectra are broad because both ^{11}B (spin 3/2) and ^{10}B (spin 5/2) contribute to the spectra.[5d]

The method described in this paper is also applicable for synthesis of normal adenosine 3′,5′-cyclic monophosphate **4a** and adenosine 3′,5′-cyclic phosphorothioate **4b**. Upon oxidation of intermediate **7** with iodine and sulfur, compounds **9a** and **9b** were obtained, respectively. After removing protecting groups, 3′,5′-cAMP **4a** (55% overall yield from **5**) and 3′,5′-cyclic AMPS **4b** (52% overall yield from **5**) were obtained.

Our current synthetic approach (Scheme 2) is based on a cyclic phosphite triester **7** intermediate, prepared by the intramolecular cyclization of a 5′-phosphoramidite **6**. In this step, a competitive side-reaction can be intermolecular coupling to form diadenosine *p*-cyanoethyl phosphite; however, under appropriate conditions (such as low temperature, low concentration of reactant, and without stirring), the desired intramolecular cyclization was the predominant reaction. By boronating, oxidizing, or sulfurizing intermediate **7**, followed by deprotection, the 3′,5′-cyclic AMPB **4c**, normal 3′,5′-cyclic AMP **4a**, or 3′,5′-cyclic AMPS **4b** were obtained, respectively (Scheme 2). This method could also be useful for the synthesis of base-modified nucleoside 3′,5′-cyclic monophosphates since the conditions avoid strong basic or other harsh reaction conditions previously used.[6,7]

The highly lipophilic (less-polar) boron analogue of cAMP should be useful in many ways. (i) Boron-containing compounds facilitate transport of organic molecules through membranes.[9] The isoelectronic substitution of borane for one of the nonbridging oxygens in phosphate diesters should impart an increase in lipophilicity and change in polarity relative to cAMP. The borane should enhance the hydrophobicity of cyclic AMPB and may enable the compound to penetrate the plasma membrane and enter cells. (ii) The BH_3 group in a boronated cyclic nucleoside monophosphate is expected to neither form classical hydrogen bonds nor coordinate metal ions[10] as well as oxygen in the parental cyclic NMP. Like cyclic AMPS, the two diastereomers of cyclic AMPB are anticipated to have different substrate properties toward transferases and hydrolases and should be useful for investigating the roles of phosphate and metal ions in biological processes and elucidating the stereochemical and metal requirements of the enzymatic reactions involving cAMP. (iii) Finally, boronated nucleotides may offer a unique advantage over other modified congeners because they could be used for boron neutron capture therapy (BNCT),[11] a radiation therapy that can selectively destroy cells which have preferentially taken up boron.

To summarize, we have synthesized a new type of modified 3′,5′-cyclic AMP in which one of the two nonbridging oxygen atoms of a phosphodiester group has been replaced with a borane group. The boronated 3′,5′-cyclic AMPB **4c** is stable under a broad range of pH (3−11) conditions and is expected to be highly resistant to enzymatic cleavage. The borane may impart a greater membrane permeability than cAMP. The increased lipophilicity and nuclease resistance, in conjunction with the potential utility as a carrier of ^{10}B in boron neutron capture therapy (BNCT) for the treatment of cancer, make cyclic AMPB analogues promising candidates for therapeutic applications. The two diastereomers of boronated cyclic NMPB should be very useful for investigating biochemical processes involving the normal cyclic NMP.

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Supporting Information Available: Experimental procedures and ^{31}P NMR, ^{1}H NMR, MS (FAB^-), and HRMS (FAB^-) spectra for compound **4c**, cyclic AMPB isomer I (**4c-I**), and cyclic AMPB isomer II (**4c-II**). This material is available free of charge via the Internet at http://pubs.acs.org

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