Specific Effects of 4-Morpholine-N,N'-dicyclohexylcarboxamidine as a Catalyst for the Alkylation and Phosphorylation of Ribonucleosides by a Two-step Phosphorylating Agent, MTBO: Its Application to the Synthesis of Cytokinin-like Nucleotides

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An examination of amines as catalysts for the first-step phosphorylation of nucleosides by a two-step phosphorylating agent, 2-methylthio-4H-1,3,2-benzodioxaphosphorin 2-oxide (MTBO, 2), revealed that, in the presence of 4-morpholine-7N,N'-dicyclohexylcarboxamidine (MDC, 3) as the catalyst, MTBO phosphorylates and o-hydroxybenzylates adenosine borate complex (1) to give 1-(o-hydroxybenzyl)adenosine 5'-S'-methyl phosphorothiolate (5), with subsequent Dimroth rearrangement to give N^6-(o-hydroxybenzyl)adenosine 5'-S'-methyl phosphorothiolate (6).

The guanosine borate complex (7) gave 1-(o-hydroxybenzyl)guanosine 5'-S'-methyl phosphorothiolate (8) under the same conditions.

MTBO phosphorylated N^6-benzyladenosine and N^6-allyladenosine borate complexes (9a and b) in the presence of n-propylamine (11) to give their 5'-S'-methyl phosphorothiolates (10a and b), respectively.

Thus, the 5'-S'-methyl phosphorothiolates of cytokinin-like nucleosides were synthesized by the use of different amine catalysts from both intact and N^6-substituted nucleosides.

2-Methylthio-4H-1,3,2-benzodioxaphosphorin 2-oxide (MTBO, 2) is an active phosphorylating agent,\textsuperscript{1,4–18} which was primarily designed to increase the chemical reactivity of biologically active saligenin cyclic phosphate esters,\textsuperscript{2,3} and is useful for synthetic phosphorylation of alcohols,\textsuperscript{1,4,5} nucleosides\textsuperscript{6} and enediol derivatives.\textsuperscript{8}

MTBO has two protective groups of different nature in the structure, \textit{i.e.}, the methylthio and o-hydroxybenzyl (OHB) groups. These protective groups give MTBO a two-step phosphorylating property: the first step is methylthio-phosphorylation by the benzodioxaphosphorin ring fission of MTBO and subsequent deprotection of the OHB group by an amine catalyst, and the second step is phosphorylation by activating the phosphorus center through removal of the methylthio group in the presence of an oxidizing agent.\textsuperscript{5,9,15,16,19}

These properties of MTBO provide a method to synthesize several biochemically important compounds such as 3',5'-cyclic nucleotides,\textsuperscript{6} 2',3'-cyclic nucleotides,\textsuperscript{7,14} ribonucleoside 5'-S'-methyl phosphorothiolates,\textsuperscript{6} triose reductone 2-phosphate\textsuperscript{8} and ATP.\textsuperscript{9}

Amine catalysts highly promote the phosphorylation of nucleosides by MTBO, greatly influencing the reaction rates and yields of the phosphorylated products.\textsuperscript{13} In addition, amine catalysts play another role in the methylthiophosphorylation of nucleosides by

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\text{To whom inquiries about the paper should be addressed.} 
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serving as an acceptor of the OHB group released from MTBO. However, the greater the ability of an amine to accept an OHB group, the more readily a side reaction proceeds by direct attack of the amine on the methylene carbon of MTBO, resulting in its decomposition. We searched for amine catalysts which would have a diminished ability to accept the OHB group but have a high ability to promote methylthiophosphorylation by MTBO.

The present paper describes the examination of base catalysts which resulted in the finding of a unique catalyst, 4-morpholine-N,N'-dicyclohexylcarboxamidine (MDC, 3), which promoted the phosphorylating ability of MTBO, but had little ability to accept the OHB group produced from MTBO. A cytokinin-like nucleotide, N6-(o-hydroxybenzyl)adenosine 5'-S-methyl phosphorothiolate (N6-OHBA-5'-SMP, 6) was synthesized from an adenosine borate complex (1) with MTBO and MDC.

Preliminary short communications on parts of this investigation have already been published.13)

Results

Unusual catalytic effect of MDC and the synthesis of N6-(o-hydroxybenzyl)adenosine 5'-S-methyl phosphorothiolate (N6-OHBA-5'-SMP, 6)

Our observations while examining the catalytic effect of amines on the methylthiophosphorylation of ribonucleoside by MTBO suggested that the catalytic activity was related to the basic nature of the amine and its ability to solubilize the product, ribonucleoside 5'-S-methyl phosphorothiolate. Amines such as triethylamine and tri-n-butylamine, whose salts of the product were less soluble in dimethylformamide (DMF) solvent, had a much lower catalytic activity than other amines like cyclohexylamine, whose salts of the product were readily soluble in DMF.

The guanidine MDC (3) was examined for its catalytic activity, because it is basic and has a great ability to solubilize nucleotides as its salts. The adenosine borate complex (1) was allowed to react with MTBO (2) at 0°C for 2 days in the presence of MDC (3), according to the method for examining the catalytic activity of amines. The paper electrophorogram of the reaction mixture showed the exclusive formation of an unusual product which had a lower migration rate on the paper electrophorogram than adenosine 5'-S-methyl phosphorothiolate (5'-ASMP; the usual product from the use of primary or secondary amine catalysts), but still gave a positive test result for the methylthio group.

In a preparative scale for the reaction system, adenosine borate complex (1) was allowed to react with MTBO at 0°C for 2 days in the presence of MDC. The product (6) was deborated by co-distillation with methanol, and isolated in a 50% yield as the white powder sodium salt by DEAE-cellulose column chromatography and subsequent passing through a Dowex 50W column (sodium form). The elemental analysis data of this compound were consistent with those for the calculated value of N6-OHBA-5'-SMP (6). The existence of an OHB group was also confirmed by 1H- and 13C-NMR spectroscopy of compound 6.

UV spectroscopy of the product confirmed the position of the OHB group on the base moiety of adenosine. The UV absorption bands of the product (6) had the same features as N6-methyladenosine and N6-benzyladenosine; their UV absorption maxima exhibit a bathochromic shift with a hyperchromic effect at each pH compared with that of adenosine. The same feature was observed in the case of N6-benzyladenosine 5'-S-methyl phosphorothiolate (N6-BA-5'-SMP, 10a) and N6-allyladenosine 5'-S-methyl phosphorothiolate (N6-AA-5'-SMP, 10b), as shown in Table I. These results show that the product was N6-OHBA-5'SMP (6).

Synthesis of 1-(o-hydroxybenzyl)guanosine 5'-S-methyl phosphorothiolate (1-OHBG-5'-SMP, 8)

When the guanosine borate complex (7) was used as a starting material for the same synthesis.
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Table I. UV Absorption of the Nucleotides and Related Compounds

<table>
<thead>
<tr>
<th>Nucleotide and Nucleoside</th>
<th>( \lambda_{\text{max}}(\text{H}_2\text{O}) \text{ nm (e)} ) @ pH 2</th>
<th>( \lambda_{\text{max}}(\text{H}_2\text{O}) \text{ nm (e)} ) @ pH 7</th>
<th>( \lambda_{\text{max}}(\text{H}_2\text{O}) \text{ nm (e)} ) @ pH 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>( N^\circ)-OHBA-5'-SMP (6)</td>
<td>265 (21,600)</td>
<td>268 (22,000)</td>
<td>268 (22,200)</td>
</tr>
<tr>
<td>( N^\circ)-BA-5'-SMP (10a)</td>
<td>265 (20,700)</td>
<td>268 (21,000)</td>
<td>268 (20,200)</td>
</tr>
<tr>
<td>( N^\circ)-AA-5'-SMP (10b)</td>
<td>264 (20,500)</td>
<td>266 (21,400)</td>
<td>266 (20,000)</td>
</tr>
<tr>
<td>1-OHBA-5'-SMP (5)</td>
<td>260 (21,000)</td>
<td>260 (21,000)</td>
<td>260 (21,000)</td>
</tr>
<tr>
<td>Adenosine</td>
<td>257 (14,600)*</td>
<td>260 (14,900)*</td>
<td>259 (15,400)*</td>
</tr>
<tr>
<td>1-Methyladenosine</td>
<td>257 (13,700)*</td>
<td>257 (14,600)*</td>
<td>257 (14,600)*</td>
</tr>
<tr>
<td>1-Benzyladenosine hydrobromide</td>
<td>259 (13,800)*</td>
<td>259 (13,700)</td>
<td>260 (13,900)*</td>
</tr>
<tr>
<td>( N^6)-Methyladenosine</td>
<td>262 (16,600)*</td>
<td>266 (15,900)</td>
<td>266 (13,900)*</td>
</tr>
<tr>
<td>( N^6)-Benzyladenosine</td>
<td>265 (20,300)*</td>
<td>269 (20,600)</td>
<td>269 (20,900)*</td>
</tr>
<tr>
<td>I-OHBG-5'-SMP (8)</td>
<td>258 (14,000)</td>
<td>256 (16,700)</td>
<td>256 (15,200)</td>
</tr>
<tr>
<td>Guanosine</td>
<td>256 (12,300)*</td>
<td>253 (13,600)*</td>
<td>256-266 (11,300)*</td>
</tr>
<tr>
<td>1-Methylguanosine 3'(2')-phosphate</td>
<td>258 (9,400)*</td>
<td>255 (10,200)*</td>
<td>258 (10,700)*</td>
</tr>
<tr>
<td>( N^2)-Methylguanosine 3'(2')-phosphate</td>
<td>259 (14,300)*</td>
<td>253 (15,800)*</td>
<td>258 (13,400)*</td>
</tr>
<tr>
<td>7-Methylguanosine</td>
<td>256 (13,300)*</td>
<td>275 (9,650)*</td>
<td>265 (12,200)*</td>
</tr>
</tbody>
</table>

* pH 1,  \( b \) pH 0.7,  \( c \) pH 6,  \( d \) water,  \( e \) pH 11,  \( f \) pH 13,  \( g \) pH 11.3.

Increasing the reaction temperature from 0° to 40°C greatly increased the solubility of MDC in DMF and the reaction rate. Methanol was used as the solution to stop the methylthiophosphorylation of the nucleosides by MTBO, as described in ref. 13. The cyclohexylamine-methanol solution was also used as the solution to simultaneously stop both the methylthiophosphorylation and \( \alpha \)-hydroxybenzylation of the nucleosides by MTBO because of the high ability of cyclohexylamine to accept the OHB group.

Optimization of the conditions for paper electrophoresis of the reaction mixture with adenosine resulted in complete separation among the components, using Toyo-Roshi No. 526 filter paper and 0.1M boric acid–tri-
Table II. *Rm* and *Rf* Values for the Nucleotides and Related Compounds

<table>
<thead>
<tr>
<th>Solvent</th>
<th><em>Rm</em></th>
<th><em>Rf</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 9.5</td>
<td>pH 9.0</td>
</tr>
<tr>
<td>5'-AMP</td>
<td>1.0b</td>
<td>1.0b</td>
</tr>
<tr>
<td>5'-ASMP</td>
<td>0.73b</td>
<td>0.73b</td>
</tr>
<tr>
<td>N'6-OHBA-5'-SMP (6)</td>
<td>0.57b</td>
<td>0.58b</td>
</tr>
<tr>
<td>1-OHBA-5'-SMP (5)</td>
<td>0.46b</td>
<td>0.39b</td>
</tr>
<tr>
<td>Adenosine</td>
<td>0.33b</td>
<td>0.31b</td>
</tr>
<tr>
<td>5'-GMP</td>
<td>1.0c</td>
<td>1.0c</td>
</tr>
<tr>
<td>5'-GSMP</td>
<td>0.73c</td>
<td>0.75c</td>
</tr>
<tr>
<td>1-OHBG-5'-SMP (8)</td>
<td>0.52c</td>
<td>0.58c</td>
</tr>
<tr>
<td>Guanosine</td>
<td>0.39c</td>
<td>0.38c</td>
</tr>
</tbody>
</table>

* Paper electrophoresis using boric acid-triethylamine buffer gave the *Rm* values of all the nucleotides and related compounds listed in this table as their borate complexes.

b Ratio of electrophoretic mobility of the nucleotides and their related compounds to that of adenosine 5'-monophosphate (5'-AMP).

c that to guanosine 5'-monophosphate (5'-GMP).

The methanol solution of the reaction mixture with adenosine gave three products, a new major product (5), and small amounts of 6 and adenosine 5'-S-methyl phosphorothiolate (5'-ASMP) on the electrophorogram at pH 9.5. On the other hand, the spots for the two components, 5 and 1, and 5 and 6, overlapped at pH 9.0 and 10.4, respectively (Table II).

Product 5 just after being extracted from the paper electrophorogram had the same UV absorption band of $\lambda_{max} = 260 \text{ nm}$ at pH 2, 7 and 12. This feature is the same as those of 1-methyladenosine and 1-benzyladenosine hydrobromide, as shown in Table I.

Incubating 5 in the pH 12 buffer solution at room temperature for one day changed its UV absorption $\lambda_{max}$ from 260 to 268 nm, which is the same as that of N'6-OHBA-5'-SMP (6). Alternatively, the cyclohexylamine–methanol solution of the reaction mixture gave no 5, but did produce major product 6 and a small amount of 5'-ASMP on the paper electrophorogram.

These results demonstrate that product 5 was 1-(o-hydroxybenzyl)adenosine 5'-S-methyl phosphorothiolate (1-OHBA-5'-SMP), which readily changed into product 6, N'6-OHBA-5'-SMP, under strongly alkaline conditions such as those in the pH 12 buffer solution and in the methanol solution containing an excess amount of cyclohexylamine.

With guanosine, the best separation of reaction mixture components by paper elec-
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trophoresis was obtained with the No. 526 paper and the 0.1 M boric acid–triethylamine solution (pH 9.0; Table II). The reaction mixture with guanosine gave a major product, 1-OHBG-5'-SMP (8), and a small amount of guanosine 5'-S-methyl phosphorothiolate (5'-GSMP) on the paper electrophorogram at pH 9.0. On the other hand, the spots for two components, 8 and 7, overlapped at pH 10.4 (Table II).

Methylthiophosphorylation and o-hydroxybenzylation of the nucleoside borate complexes (1 and 7) by MTBO occurred almost simultaneously, as shown in Figs. 1 and 2.

The selectivity (B/P) for o-hydroxybenzylation (B) to total phosphorylation (P) of the nucleosides by MTBO in the presence of MDC was very high, being about 88 and 87% with adenosine and guanosine, respectively, after the completion of the reactions.

Phosphorylation of N°-benzyladenosine and N°-allyladenosine

MTBO phosphorylated N°-substituted adenosines such as N°-benzyladenosine (9a) and N°-allyladenosine (9b) and produced the 5'-S-methyl phosphorothiolates (10a and b) in 84 and 86% yields, respectively, in the presence of n-propylamine (11: Scheme 1).

Discussion

Mechanism for the phosphorylation and o-
hydroxybenzylation of nucleosides by MTBO in the presence of MDC

When the 5'-OH group of adenosine borate complex (1) was activated by MDC (3), the OH group attacked MTBO (2) and produced adenosine 5'-O-(o-hydroxybenzyl) S-methyl phosphorothiolate (4). Then, the 1-nitrogen atom of the adenine ring accepted the OHB group and produced N6-OHBA-5'-SMP (6), as shown in Scheme 2.

In the case of the guanosine borate complex (7), 1-OHBG-5'-SMP (8) was produced under the same conditions, as shown in Scheme 3.
These results demonstrated that MDC was a unique catalyst that effectively activated alcohol for methylthiophosphorylation by MTBO, but that did not accept the OHB group produced from MTBO, and that the property as a base catalyst for alcohol activation completely differed from that for OHB group acceptance.

The relationships between the structure and catalytic activity of analogs of MDC showed that the unique property of MDC as a catalyst resulted at least partially from its bulky structure, which will be discussed in detail in future papers.

Cytokinin-like nucleotides

Cytokinins, a group of plant hormones, are derivatives of adenine with lipophilic side chains at the 6-amino position. These hormones play a major role in plant cell division and differentiation. N\(^{6}\)-(o-Hydroxybenzyl)-adenosine has been isolated as a naturally occurring cytokinin from leaves of Populus robusta by Horgan et al. The reaction of 6-chloropurineriboside with o-hydroxybenzylamine was used for the synthesis of N\(^{6}\)-(o-hydroxybenzyl)adenosine.

We developed a new method that simultaneously phosphorylated and alkylated nucleosides and produced o-hydroxybenzynucleoside 5'-S-methyl phosphorothiolates (Schemes 2 and 3). A phosphorylated cytokinin, N\(^{6}\)-OHBA-5'-SMP (6), was synthesized from the adenosine borate complex (1) with MTBO in the presence of MDC. Guanosine produced 1-OHBG-5'-SMP under the same conditions.

When N\(^{6}\)-substituted adenosines, e.g., N\(^{6}\)-benzyladenosine or N\(^{6}\)-allyladenosine, were used as the starting material, and a primary amine was used as the catalyst, 5'-S-methyl phosphorothiolates were produced in a high yield (Scheme 1).

This type of alkylation is also biologically important in connection with the aging reaction of saligenin cyclic phosphate.

Experimental

General methods. All the nucleosides were dried in vacuo
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at 95°C for more than 5 hr. Reagent grade dimethylformamide (DMF) was distilled and dried over Linde 4A molecular sieve. 4-Morpholine-N,N'-dicyclohexylcarboxamide (MDC) was prepared by the method described in reference 28, and MTBO was prepared from saligenin by previously described.5,12

Paper electrophoresis was carried out at 700 V (35 cm) on Toyo-Roshi No. 51 filter paper with a 0.05 m sodium borate buffer solution (pH 9.2) for 1 hr, or on No. 526 paper with buffer solutions of 0.1 m boric acid-triethylamine (pH 9.0), 0.1 m boric acid-0.05 m triethylamine (pH 9.5) and 0.1 m boric acid-0.1 m triethylamine (pH 10.4) for 1.5 hr.

Paper chromatography was carried out by the ascending technique on Toyo Roshi No. 51 paper. The following solvent systems were used: A, isopropanol-28% ammonia-water (7 : 1 : 2); B, isopropanol-28% ammonia-0.1 m boric acid (7 : 1 : 2); C, n-butanol-acetic acid-water (5 : 2 : 3); D, ethanol-1 m ammonium acetate (5 : 2); E, isopropanol-1% ammonium sulfate (2 : 1). The Rm and Rf values of the nucleotides and their related compounds are listed in Table II.

The nucleotides and their related compounds were detected by viewing under an ultraviolet lamp, and the sulfur-containing compounds by palladium chloride spraying.29

1H- and 14C-NMR spectra were recorded on a Hitachi Perkin Elmer R-20 (60 MHz for 1H-NMR) or a JEOL FX-100 (100 MHz for 1H-NMR and 25.05 MHz for 13C-NMR) instrument. Chemical shifts are expressed in parts per million down field from DSS as an internal standard.

UV spectra were recorded on a Shimadzu MPS-50 or a UV-200 spectrometer. UV absorption maxima data for the nucleotides and their related compounds were listed in Table I.

The following buffer solutions were used as solvents for measuring UV absorption at the final concentrations: pH 2, a 0.05 m KCl-0.13 m HCl aqueous solution; pH 7, a 0.1 m sodium phosphate buffer solution; and pH 12, a 0.05 m KCl-0.012 m NaOH aqueous solution.

An examination of catalytic activity of MDC was carried out by using the procedure described in ref. 13.

N°-(o-Hydroxybenzyl)adenosine 5'-S-methyl phosphorothioate (N°-OHBA-5'-SMP, 6). A mixture of adenosine (5.345 g, 20 mmol), metabolic acid (964 mg, 22 mmol) and DMF (120 ml) was dissolved by gentle heating to 120°C with stirring. Benzene (40 ml) was added to the solution, which was then concentrated to about 80 ml under reduced pressure. MTBO (12.96 g, 60 mmol) was added to the concentrate, which was next cooled to 0°C. MDC (100 mmol) dissolved in DMF (80 ml) by heating was added to this concentrated solution, and the mixture was incubated at 0°C for 2 days. The mixture was then concentrated to dryness in vacuo, and the residue was treated with a mixture of chloroform (200 ml), water (500 ml) and n-propylamine (16 ml). The chloroform layer was extracted with an aqueous n-propylamine solution, and the aqueous layers were combined and evaporated to dryness under reduced pressure. The residue was dissolved in methanol (2 l) and distilled. After removing the metabolic acid by methanol distillation, the residue was dissolved in water. One-fourth part of the solution was applied to a DEAE-cellulose column (bicarbonate form, 6 x 40 cm). After washing with water (10 l), elution was carried out with a linear gradient of 0.01 m triethylammonium bicarbonate (TEABC) (pH 7.5, 5 l) in the mixing vessel and 0.1 m TEABC (5 l) in the reservoir. Fractions of 400 ml were collected, and those fractions containing the product (from No. 7 to No. 16, A254 unit = 61,800, 56.2% yield) were combined and evaporated to dryness. After removing the residual TEABC by repeated co-evaporation with water, the residue was dissolved in water and converted into the sodium salt by passing it through a Dowex 50W column (sodium form). The eluate was evaporated to dryness, ethanol (50 ml) was added to the residue, and the solution dried by co-evaporation. The resulting white solid was dissolved in methanol (20 ml), ethyl ether (180 ml) was added, and a white solid precipitated. Recrystallization of the resulting white solid from methanol (20 ml) and ethyl ether (180 ml) was repeated three more times to remove saligenin bis-(S-methyl phosphorothioate),6 a decomposition product of MTBO. The solid was dried in vacuo to give 1.39 g (49.6%) of N°-OHBA-5'-SMP sodium salt.

Anal. Found: C, 42.61; H, 5.17; N, 12.42. Calcd. for C18H17N5O7PSNa·1/2(C2H5)2O H2O: C, 42.86; H, 5.04; N, 12.50%.

NMR δH (D2O): 2.33 (3H, doublet, J = 15.1 Hz, P-S-CH3), 4.0 (2H, singlet, Ph-CH2), 4.2-5.05 (6H, H2, H3, H4, H5 and HOD), 6.12 (1H, doublet, J = 5.5 Hz, H6), 6.9-7.6 (4H, Ph-H), 8.21 (1H, singlet, H2), 8.30 (1H, singlet, H4).

NMR δC (D2O): 14.91, 15.14 (SCH3), 42.34 (Ph-CH2), 64.17 (5'-C), 73.15 (3'-C), 76.46 (2'-C), 88.17 (4'-C), 91.24 (1'-C), 121.88 (5'-C), 122.88 (3'-C), 126.70 (5'-C), 130.98 (6'-C, 4'-C), 131.57, 131.80 (1'-C), 142.37 (8'-C), 149.90 (4'-C), 151.89, 152.30 (2'-C), 154.83 (2-C), 156.76 (6'-C). 1-(o-Hydroxybenzyl)guanosine 5'-S-methyl phosphorothioate (1-OHGB-5'-SMP, 8). A similar procedure as that used for the synthesis of N°-OHBA-5'-SMP (6) was used for the synthesis of compound 1-OHGB-5'-SMP (8), except for minor differences. Guanosine (20 mmol) was used and benzene was distilled off without concentrating the reaction mixture. After methanol distillation, the mixture was applied to the DEAE-cellulose column (7.5 x 68 cm). Elution was carried out with a linear gradient, using water (1 l) in the mixing vessel and 0.1 m TEABC (10 l) in the reservoir, and 400-ml fractions were collected. A peak containing the UV-absorbing compound (A254 unit = 189,700, 56.8% yield) was eluted in fraction Nos. 21-28. The sodium salt of 1-OHGB-5'-SMP was isolated as a white solid (5.68 g, 50.9% as)
Characterization of the intermediate products by paper electrophoresis. A mixture of a nucleoside (adenosine or guanosine; 1 mmol), metabolic acid (1.1 mmol) and DMF (10 ml) was heated at 120°C. Co-evaporation of the mixture with benzene (3 ml) under reduced pressure was repeated three times to remove traces of water. MDC (3.6 mmol) was added to the mixture and dissolved by heating at 50°C. MTBO (1.2 mmol) was added to the mixture at 0°C and kept at 40°C for 8 hr. Aliquots (0.1 ml) of the reaction mixture were withdrawn and injected into a methanol (1 ml) at room temperature after 10, 20, 30, 40, 50 and 60 min, and 2, 4 and 8 hr. Each solution was subjected to paper electrophoresis.

The spots on the paper electrophorogram were cut out and extracted with water while mixing. Immediately after mixing the extracted solutions with a pH 2, 7 or 12 buffer unit=91,800, 85.8% yield) was eluted in fraction Nos. 16-24. The sodium salt of N⁶-BA-S'-MMP (10a) was obtained as a white solid (1.62 g, in 60.6% yield).

A single peak (A₂₅₀nm unit=88,200, 84.0% yield) was obtained in fraction Nos. 16-24. The sodium salt of N⁶-BA-S'-MMP (10a) was obtained as a white solid (1.62 g, in 60.6% yield).

Anal. Found: C, 42.80; H, 4.80; N, 13.06. Calcd. for C₁₄H₁₉N₅O₆PSNa 0.4(C₂H₅)₂O H₂O: C, 38.47; H, 5.17; N, 14.38%.

NMR δH (D₂O): 2.20 (3H, doublet, J=13.5Hz, Ph-S-CH₃), 4.1-4.46 (2H, Ph-CH₂), 4.46-4.9 (4H, H₂, H₃, H₄, and H₅), 6.22 (1H, doublet, J=4.5 Hz, H₁), 7.23 (5H, singlet, Ph-H), 8.26 (1H, singlet, H₂) and 8.47 (1H, singlet, H₃). This spectrum shows the presence of a benzyl group and adenosine 5'-S-methyl phosphorothiolate.

N⁶-Allyladenosine 5'-S-methyl phosphorothiolate (N⁶- AA-S'-MMP, 10b). N⁶-Allyladenosine (5 mmol) was used as the starting material, and N⁶-AA-S'-MMP (10b) was synthesized as described before.

DEAE-cellulose column (7.5 x 34 cm) chromatography was carried out by linear gradient elution with 0.02 M TEABC (5 l) in the reservoir. Four-hundred-ml fractions were collected, and a sharp peak containing N⁶-AA-S'-MMP (A₂₅₀nm unit=91,800, 85.8% yield) was eluted in fraction Nos. 7-11. The sodium salt of N⁶-AA-S'-MMP was obtained as a white solid (1.679 g, 68.9% yield) as described before.

Anal. Found: C, 38.53; H, 5.14; N, 14.18. Calcd. for C₁₄H₁₉N₅O₆PSNa 0.4(C₂H₅)₂O H₂O: C, 38.47; H, 5.17; N, 14.38%.

NMR δH (D₂O): 2.21 (3H, doublet, J=14 Hz, P-S-CH₃), 4.05-4.45 (4H, H₁, N-C₉H₅=CH₂), 4.45-5.03 (5H, H₂, H₃, H₄, and H₅), 5.1-5.55 (2H, CH=CH₂), 5.8-6.35 (1H, CH₂=CH=CH₂), 6.22 (1H, doublet, J=5 Hz, H₁), 8.21 (1H, singlet, H₂), 8.49 (1H, singlet, H₃). This spectrum shows the presence of an allyl group and adenosine 5'-S-methyl phosphorothiolate.

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References
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21) For example, see J. P. Helgeson, Science, 161, 974 (1968).