Agricultural and Biological Chemistry

ISSN: 0002-1369 (Print) (Online) Journal homepage: http://www.tandfonline.com/loi/tbbb19

Demethylthiolating Agents for Ribonucleoside 5'-S-Methyl Phosphorothiolates

Masayoshi Eto, Hideo Maeda, Masayoshi Iio & Hirohisa Omura

To cite this article: Masayoshi Eto, Hideo Maeda, Masayoshi Iio & Hirohisa Omura (1984) Demethylthiolating Agents for Ribonucleoside 5'-S-Methyl Phosphorothiolates, Agricultural and Biological Chemistry, 48:9, 2355-2360, DOI: 10.1080/00021369.1984.10866482

To link to this article: http://dx.doi.org/10.1080/00021369.1984.10866482

Published online: 09 Sep 2014.

Agric. Biol. Chem., **48** (9), 2355～2360, 1984

Demethylthiolating Agents for Ribonucleoside 5′-*S*-Methyl Phosphorothiolates†

Masayoshi ETO, Hideo MAEDA,*1 Masayoshi IIO*2 and Hirohisa OMURA*3,††

*Himeji Women's College, *1Kao Soap Co., Ltd., *2Kumanoto Women's University, and *3Faculty of Agriculture, Kyushu University, Fukuoka 812, Japan*

Received March 19, 1984

Several oxidizing agents were examined for their ability to demethylthiolate adenosine- and cytidine 5′-*S*-methyl phosphorothiolates.

Iodine dissolved in an aqueous potassium iodide solution or in dimethyl sulfoxide (DMSO) was the most effective demethylthiolating agent of those tested in the present study, rapidly giving the demethylthiolated products in quantitative yields. The iodine–DMSO solution demethylthiolated the ribonucleoside 5′-*S*-methyl phosphorothiolates to give ribonucleoside 5′-monophosphates even under anhydrous conditions, DMSO acting as an oxygen donor in this reaction.

Hydrogen peroxide has high demethylthiolating ability in spite of its low reaction rate. Isoamyl nitrite, an effective demethylthiolating agent for *O*-alkyl *S*-methyl phosphorothiolates, was not effective for the demethylthiolation of ribonucleoside 5′-*S*-methyl phosphorothiolates, because the unprotected amino groups of the *S*-methyl nucleotides were attacked by the reagent to give deaminated products. *N*-Chlorosuccinimide had no effect on the demethylthiolation of *S*-methyl phosphorothiolates.

Our previous papers[1～13] described that 2-methylthio-4*H*-1,3,2-benzodioxaphosphorin 2-oxide (MTBO) is a unique phosphorylating agent with two characteristic protecting and activating groups, *o*-hydroxybenzyl and methylthio groups, on the phosphoryl center. The former group is readily removable by the action of an amine used as the catalyst, while the latter is acid- and oxidation-labile. Acid-hydrolytic removal of the latter group is inappropriate for the purpose because acid treatment may also degrade some other acid-sensitive parts of the compounds and is limited only to the preparation of phosphorus monoesters. The oxidation procedure is more suitable for demethylthiolation than is hydrolysis, because the reactive site is limited to the sulfur atom of the methylthio group.

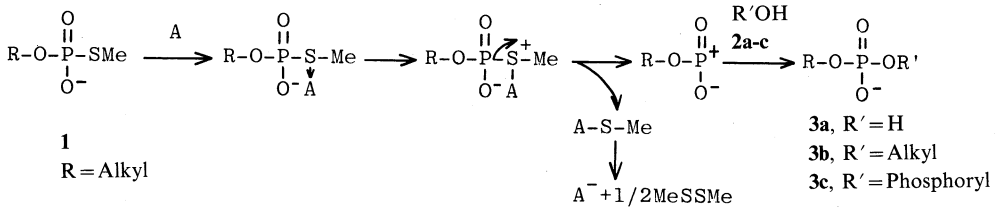
Scheme I shows the mechanism of demethylthiolation of *O*-alkyl *S*-methyl phosphorothiolate (**1**) by an oxidizing agent (**A**), in which the agent **A** binds at first to the sulfur of **1** to withdraw its electrons, activating the phosphorus center to give phosphorus monoesters (**3a**) and diesters (**3b**), and anhydrides (**3c**) in the presence of nucleophiles (**2**) such as water (**2a**), alcohols (**2b**) and phosphate anions (**2c**).

Ribonucleoside 5′-*S*-methyl phosphorothiolates (**5**) produced from ribonucleoside (**4**) and MTBO (Scheme II) were successfully used as intermediates for the synthesis of a variety of biochemically important nucleotides and nucleotide anhydrides such as cyclic 3′,5′-AMP, ATP, and UDPG.[4,7]

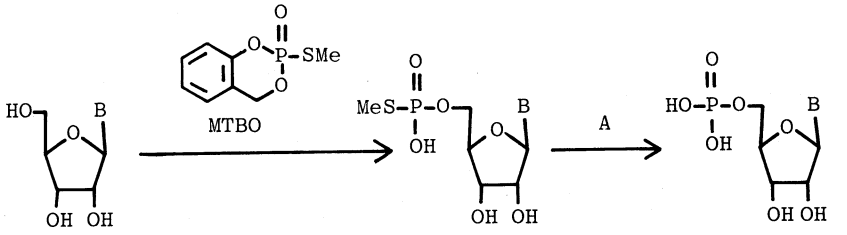
The present paper describes the results of the examination of several oxidizing agents for their ability to demethylthiolate ribonucleoside 5′-*S*-methyl phosphorothiolates (**5**) to give the 5′-monophosphates (**6**) (Scheme II). Iodine–dimethyl sulfoxide (I_2–DMSO) and

† A New Phosphorylating Agent, 2-Methylthio-4*H*-1,3,2-benzodioxaphosphorin 2-Oxide. Part X. For Part IX see ref. 13.

†† Reprint requests should be addressed to this author.



SCHEME I. Demethylthiolation of *O*-Alkyl *S*-Methyl Phosphorothiolate (**1**) by Oxidizing Agent (**A**) and the Resulting Phosphorylation.



d, B=Adenine; e, B=Cytosine; f, B=Guanine; g, B=Uracil

SCHEME II. Synthesis of Ribonucleoside 5′-*S*-Methyl Phosphorothiolates (**5**) and Ribonucleoside 5′-Monophosphates (**6**) from Ribonucleosides (**4**).

aqueous iodine potassium iodide solution (I_2–KI–H_2O) were found to be the most effective.

EXPERIMENTAL

General methods.

Materials. Reagent grade dimethyl sulfoxide (DMSO) was dried over calcium hydride, distilled and stored over Linde-type 4A molecular sieves. All other materials were of reagent grade and were used without further purification, unless otherwise stated. Adenosine- and cytidine 5′-*S*-methyl phosphorothiolates (**5d** and **5e**) were prepared from the corresponding nucleosides (**4d** and **4e**) and MTBO as previously reported.[4]

Paper electrophoresis was carried out with Toyo Roshi No. 51 paper and 0.05 M sodium borate buffer (pH 9.2) or 0.1 M triethylammonium bicarbonate (pH 7.5) at 700 V (35 cm) for 1 hr. Nucleotidic compounds were detected by viewing under a UV lamp; phosphorus-containing compounds, by a molybdate–perchloride spray;[16] and sulfur-containing compounds, by a palladium chloride spray.[17]

Spectroscopic measurements. UV absorbance was measured with a Hitachi Perkin Elmer 139 UV-VIS spectrometer. UV spectra were recorded on a Shimadzu MPS-50 spectrometer.

Measurement of the demethylthiolating ability of oxidizing agents

Solutions of demethylthiolating agents (A-solutions).

i) 0.5 N iodine–acetone solution

ii) 0.5 N aqueous iodine–potassium iodide (0.6 M) solution (I_2–KI–H_2O)

iii) 0.5 N iodine–DMSO solution (I_2–DMSO)

iv) isoamyl nitrite–acetone solution: (a) 0.5 M solution and (b) 2.5 M solution.

v) hydrogen peroxide solution: (a) 0.5 M aqueous solution and (b) 2.5 M aqueous solution.

vi) 0.5 M N-chlorosuccinimide-acetone solution

Solutions of ribonucleoside 5′-S-methyl phosphorothiolates (**5**) (*B-solutions*). Sodium cytidine 5′-*S*-methyl phosphorothiolate (**5e**) (407 mg, 1 mmol) was dissolved in water and diluted to 10 ml. Sodium adenosine 5′-*S*-methyl phosphorothiolate (**5d**) (431 mg, 1 mmol) was dissolved in water and diluted to 10 ml. The sodium salt of **5d** (0.1 mmol) was dissolved in DMSO (1 ml). Each 1 ml of these solutions contained 0.1 mmol of the materials.

The reagent to stop demethylthiolation (*C-solution*). Sodium thiosulfate was used as the reagent to stop demethylthiolation, and sodium bicarbonate was used to neutralize the resulting acid such as hydroiodic acid which might hydrolyze the demethylthiolated product.

A mixture of sodium thiosulfate·$5H_2O$ (8.29 g) and sodium bicarbonate (1.9 g) was dissolved in water and

diluted to 200 ml. Each 1 ml of this solution contained 0.167 mmol of sodium thiosulfate and 0.113 mmol of sodium bicarbonate.

Procedure. To the B-solution (1 ml) (**5**, 0.1 mmol) was added the A-solution (0.5 ml) (demethylthiolating agent, 0.25 meq.) with rapid mixing. The mixture was allowed to stand at 30°C. Small parts (0.1 ml each) of the reaction mixture were withdrawn for analysis and injected into the C-solution (0.3 ml, 0.05 meq.) at room temperature at time intervals of 5, 10, 20 and 40 min, and 1, 1.5, 2, 4, 12, 16 and 24 hr. The use of 2.5 M solution (5 times as much as usual) of A-solution in the case of isoamyl nitrite (iv (b)) or hydrogen peroxide (v (b)) required 1.5 ml of C-solution (5 times as much as usual). The color of the reaction mixture due to iodine disappeared rapidly during mixing on its injection into the C-solution. The mixtures were subjected to paper electrophoresis. The resulting spots corresponding to unchanged **5** and the demethylthiolated product (**6**) on the electrophorogram were each cut out of the paper, cut up, and extracted with water (5 ml) at 37°C for 1 day. The yield of the product **6** was estimated as the percentage of absorbance of the product (**6**) to the total absorbance of **5** and **6** at $\lambda_{260\,nm}$ (**5e** and **6e**) or at $\lambda_{273\,nm}$ (**5e** and **6e**).

RESULTS

Measurement of the demethylthiolating ability of oxidizing agents

Ribonucleoside 5′-*S*-methyl phosphorothiolates (**5**) were used as the substrates to measure the demethylthiolating ability of the oxidizing agents, because they can be easily determined by measuring UV absorption and the results can be applied directly to the synthesis of 5′-nucleotides (**6**) as shown in Scheme II. Two nucleotides, adenosine- and cytidine 5′-*S*-methyl phosphorothiolates (**5d** and **5e**) with different UV absorption bands, were used for this purpose to avoid confusion due to the by-products having UV absorption bands near $\lambda_{260\,nm}$. The yields of the demethylthiolated products could be estimated with ease by spectrophotometric comparison of the products, 5′-monophosphates (**6**), with unchanged 5′-*S*-methyl phosphorothiolates (**5**) after extraction from paper electrophorograms of the reaction mixture. The great difference in the electrophoretic mobility between the mono- (**6**) and diesters (**5**) (see Fig. 1) made this estimation exact.

Aqueous iodine solution

Since iodine is the mildest halogen oxidant and water is the most convenient OH donor,[14] aqueous iodine solutions were examined for their ability to demethylthiolate ribonucleoside 5′-*S*-methyl phosphorothiolates (**5**). Acetone[14] or aqueous potassium iodide solution[15] was used to dissolve the iodine in water.

An aqueous iodine–acetone solution (I_2–acetone–H_2O) demethylthiolated adenosine-

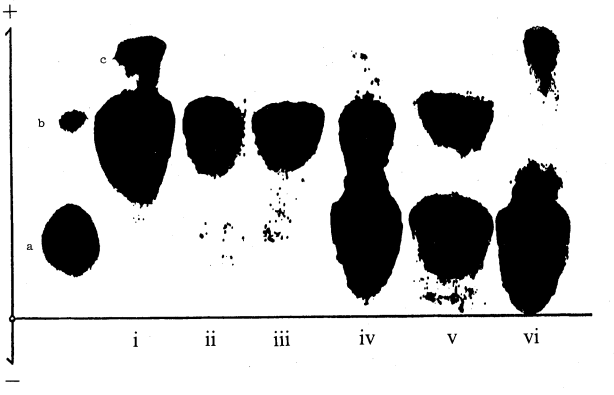


FIG. 1. Paper Electrophorograms (0.1 M Triethylammonium Bicarbonate) of Cytidine 5′-*S*-Methyl Phosphorothiolate (**5e**) after Treatment with Oxidizing Agents.

a, **5e**; b, cydidine 5′-monophosphate (**6e**); c, by-product; i, treated with I_2–acetone–H_2O for 40 min; ii, I_2–KI–H_2O for 5 min; iii, I_2–DMSO for 5 min; iv, isoamyl nitrite for 24 hr, v, H_2O_2 for 16 hr; vi, *N*-chlorosuccinimide for 16 hr.

and cytidine 5′-*S*-methyl phosphorothiolates (**5d** and **5e**) to give the corresponding 5′-monophosphates (**6d** and **6e**) in quantitative yields at 30°C within 1 hr, as shown in Fig. 2. I_2–acetone–H_2O was effective in the demethylthiolation, but has the disadvantage that it produces a considerable amount of a by-product (λ_{max} 258 nm), possibly derived from acetone, as shown in Fig. 1.

An aqueous iodine-potassium iodide solution (I_2–KI–H_2O) demethylthiolated both **5d** and **5e** quantiatively at 30°C within 5 min, as shown in Fig. 2. No formation of by-products having any UV absorption bands near 260 nm was observed on the paper electrophorogram of the reaction mixture, as shown in Fig. 1. This solution was concluded to be a very effective demethylthiolating agent for **5**.

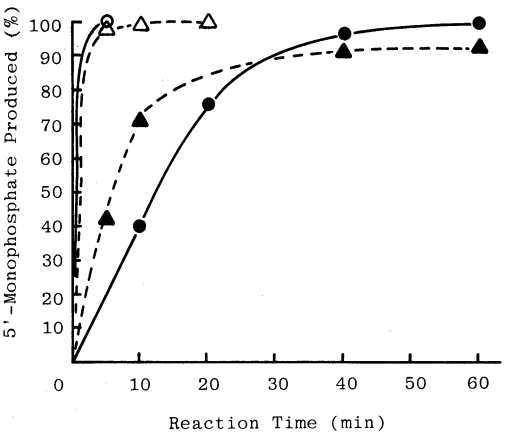


FIG. 2. Demethylthiolating Effects of Oxidizing Agents Containing Iodine.

○—○, **5d** (I_2–DMSO), **5e** (I_2–DMSO), **5e** (I_2–KI–H_2O); △------△, **5d** (I_2–KI–H_2O); ▲------▲, **5d** (I_2–acetone–H_2O); ●——●, **5e** (I_2–acetone–H_2O).

Iodine–dimethyl sulfoxide solution (I_2–DMSO)

In the course of our examination for the optimum reaction conditions to synthesize 3′,5′-cyclic nucleotides,[4] guanosine 5′-*S*-methyl phosphorothiolate (**5f**) dissolved in anhydrous DMSO was not converted to the cyclic phosphate, but to the 5′-monophosphate (**6f**) quantitatively by iodine-oxidation for 5 min.[19] On the other hand, **5f** was so stable in DMSO itself that the 5′-monophosphate (**6f**) was not produced, unless iodine was present, even after keeping at room temperature for 1 day. These preliminary findings led us to examine I_2–DMSO for its ability to demethylthiolate **5**.

The sodium salt of **5d** dissolved in anhydrous DMSO was used as the substrate, while an aqueous solution of the sodium salt of **5e** was used as the substrate because of its low solubility in anhydrous DMSO.

Figures 2 and 1 show that I_2–DMSO was a very effective demethylthiolating agent for **5** to give the corresponding 5′-monophosphates (**6**) in quantitative yields within 5 min at 30°C without forming any by-products.

Isoamyl nitrite

In our previous paper[3] isoamyl nitrite was used as an effective demethylthiolating agent for *O*-alkyl *S*-methyl phosphorothiolates (**1**). The demethylthiolating effect of the nitrite on **5** was examined.

Isoamyl nitrite in an aqueous acetone solution has a poor ability to demethylthiolate **5** as shown in Figs. 3 and 1: when **5e** was treated with the isoamyl nitrite solution for 24 hr, a ribonucleoside 5′-monophosphate which gave the same UV spectrum (λ_{max} 260 nm) as that of uridine 5′-monophosphate (**6g**) was produced in a 15% yield, as shown in Fig. 3. This fact shows that isoamyl nitrite acted not only to demethylthiolate but also to deaminate the amino groups of the nucleotide **5e**.

Despite the use of 5 times the amount of isoamyl nitrite compared with that used in the case of **5e**, **5d** was demethylthiolated by isoamyl nitrite to give the product in a very poor yield, as shown in Fig. 3.

Hydrogen peroxide

Since hydrogen peroxide appeared a convenient oxidizing agent for ready isolation of the demethylthiolated product (**6**), owing to the simplicity of the decomposition products of the oxidant (oxygen and water), it was also examined as a demethylthiolating agent for **5**.

Hydrogen peroxide oxidized **5e** very slowly

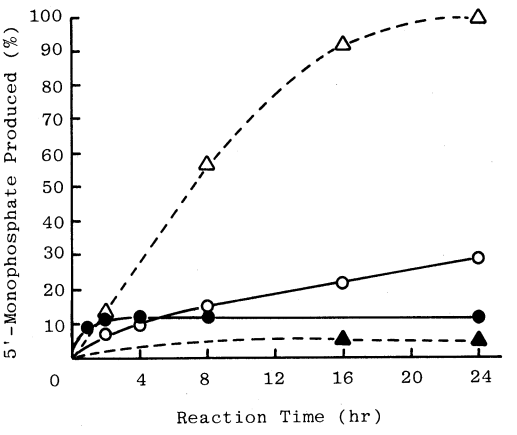


FIG. 3. Demethylthiolating Effects of Hydrogen Peroxide (H_2O_2) and Isoamyl Nitrite.
△------△, **5d** ($H_2O_2 \times 5$); ○——○, **5e** (H_2O_2); ●——●, **5e** (isoamyl nitrite); ▲------▲, **5d** (isoamyl nitrite × 5).

to give the product in a 28% yield (Fig. 3), whose UV spectrum (λ_{max} 273 nm) was identical with that of cytidine 5′-monophosphate (**6e**), indicating no decomposition of the base moiety of the product. The use of 5 times the amount of the oxidant compared with that used in the case of **5e** increased the yield of the demethylthiolated product of **5d** (**6d**) up to 100% within 24 hr as shown in Fig. 3.

N-Chlorosuccinimide

Examination of *N*-chlorosuccinimide showed that it had no appreciable effect as a demethylthiolating agent (Fig. 1).

DISCUSSION

S-Methyl phosphorothiolates can act as phosphorylating agents by activating the phosphorus center by means of demethylthiolation with an oxidant. The examination of the demethylthiolating effects of several oxidizing agents by the use of ribonucleoside 5′-*S*-methyl phosphorothiolates (**5**) as the substrates indicated that iodine was the most effective demethylthiolating agent: I_2–acetone–H_2O was effective to demethylthiolate, but produced a UV absorptive by-product. I_2–KI–H_2O and I_2–DMSO solutions rapidly gave the demethylthiolated products (**6**) in quantitative yields and the reaction mixtures were clean.

The more rapid demethylthiolation rates in I_2–KI–H_2O than in I_2–acetone–H_2O might be due to the difference in polarity between the KI solution and acetone.

I_2–DMSO is more advantageous than I_2–KI–H_2O for the following reasons: 1) I_2–DMSO is a good oxidizing agent to give the phosphorus monoesters (**6**), even under non-aqueous conditions, while the I_2–KI–H_2O solution may hydrolyze the products by the action of highly acidic hydroiodic acid resulting from the reduction of I_2 in water. To avoid possible hydrolysis, a buffer solution may be required,[15] which makes the reaction mixture more complicated. 2) I_2–DMSO may be applicable to the demethylthiolation of lipophilic compounds.

The reason why I_2–DMSO demethylthiolates the 5′-*S*-methyl phosphorothiolates (**5**) to give the 5′-monophosphates (**6**) even under anhydrous conditions is that DMSO attacks at the phosphorus center to give oxygen.

The I_2–DMSO solution is very effective for the synthesis of phosphorus monoesters from the *S*-alkyl phosphorothiolates. Its application to the synthesis of 5′-ribonucleotides will be published in our following papers.

Kinoshita *et al.*[18] have reported that I_2–DMSO is useful as an oxidizing agent for thiocarbamates.

We found that isoayml nitrite was an effective demethylthiolating agent for *O*-alkyl *S*-methyl phosphorothiolates (**1**).[3] It has, however, a poor demethylthiolating effect for ribounucleoside 5′-*S*-methyl phosphorothiolates (**5**), because it reacted with the amino groups of the base moiety.

Hydrogen peroxide takes a much longer time (24 hr) to demethylthiolate **5** than do the iodine solutions, while the reaction mixture leaves no salt, and the isolation of the products (**6**) is easy. *N*-Chlorosuccinimide has no demethylthiolating effect.

Consequently, iodine emerges as the best reagent for the demethylthiolation of ribonucleoside 5′-*S*-methyl phosphorothiolates (**5**),

and its efficacy was fully demonstrated in the synthesis of a variety of nucleotides under different conditions.[4,7]

Acknowledgment. We wish to express our hearty thanks to Professor Morifusa Eto of Kyushu University for his helpful suggestions.

REFERENCES

1) M. Eto, M. Sasaki, M. Iio, M.-Y. Eto and H. Ohkawa, *Tetrahedron Lett.*, 4263 (1971).
2) M. Sasaki, H. Ohkawa and M. Eto, *J. Fac. Agric., Kyushu Univ.*, **17**, 173 (1973).
3) M. Iio, M.-Y. Eto, M. Eto and H. Omura, *Agric. Biol. Chem.*, **37**, 115 (1973).
4) M.-Y. Eto, M. Iio, Y. Kobayashi, H. Omura and M. Eto, *Agric. Biol. Chem.*, **38**, 2081 (1974).
5) M. Iio, Y. Kobayashi, M.-Y. Eto, K. Yano, H. Omura and M. Eto, *J. Fac. Agric., Kyushu Univ.*, **19**, 73 (1975).
6) K. Shinohara, M.-Y. Eto, Y.-K. Tseng, K. J. Joo, Y. Tomita and H. Omura, *Nippon Nôgeikagaku Kaishi*, **49**, 223 (1975).
7) M. Iio, K. Yano, M.-Y. Eto, H. Omura and M. Eto, *Agric. Biol. Chem.*, **41**, 155 (1977).
8) M.-Y. Eto, Doctoral Dissertation, Kyushu University (1975).
9) M.-Y. Eto, M. Iio, H. Omura and M. Eto, *Nucleic Acids Research*, Special publication No. 2, 1976, s65.
10) M.-Y. Eto, M. Iio, H. Omura and M. Eto, *J. Fac. Agric., Kyushu Univ.*, **22**, 25 (1977).
11) M.-Y. Eto, M. Iio, H. Maeda and H. Omura, *Agric. Biol. Chem.*, **42**, 299 (1978).
12) M.-Y. Eto, M. Iio, H. Omura and M. Eto, *Agric. Biol. Chem.*, **42**, 199 (1978).
13) M.-Y. Eto, M. Iio, H. Hanai and H. Omura, *Himeji Tanki Daigaku Kenkyū Hōkoku*, **No. 26**, 129 (1981).
14) A. F. Cook, M. J. Holmann and A. L. Nussbaum, *J. Am. Chem. Soc.*, **91**, 1522 (1969).
15) M. S. Poonian, E. F. Nowoswiat and A. L. Nussbaum, *J. Am. Chem. Soc.*, **94**, 3992 (1972).
16) C. S. Haines and F. A. Isherwood, *Nature*, **164**, 1107 (1949).
17) H. Niessen, H. Tietz and H. Frehese, *J. Chromatogr.*, **9**, 111 (1962).
18) Y. Kinoshita, S. Kubota, S. Hashimoto and H. Ishikawa, *Agric. Biol. Chem.*, **37**, 701 (1973).
19) M.-Y. Eto, unpublished observation.