Phosphorus Research Bulletin Vol. 36 (2020) pp. 029-035

PHOSPHONYLATION OF 5'-DEOXY-5-FLUOROURIDINE AND 1-β-D-ARABINOFRANOSYLCTYOSINE WITH DISODIUM DIPHOSPHONATE

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Keywords: Phosphonylation; 5'-deoxy-5-fluorouridine; 1-β-D-Arabinofranosylcytosine; Disodium diphosphonate; HPLC; multinuclear NMR

Phosphonoylations of 5'-deoxy-5-fluorouridine (5'-DFUR) and 1-β-D-arabinofranosylcytosine (Ara C) in aqueous solution were successfully performed using disodium diphosphonate (DP, Na₂P₂O₇H₂). The former reaction resulted in the synthesis of both 3'-phosphonylethyl-5'-DFUR and 2'-phosphonyl-5'-DFUR, with an overall yield of 100%. The reaction of 1-β-D-arabinofranosylcytosine (Ara C) with DP gave 5'-phosphonyl-Ara C, 3'-phosphonyl-Ara C and 2'-phosphonyl-Ara C in a total yield of more than 35%. The optimal condition for the phosphorylation of either 5'-DFUR or Ara C with DP was determined to be a 1:10 ratio of 5'-DFUR or Ara C to DP and a solution pH of 11. The phosphorylated products of 5'-DFUR and Ara C were stable at 10 ºC and 25 ºC, respectively, over the pH range of 7 to 9. A proposed mechanism for the reactions of 5'-DFUR and Ara C with DP was developed.

(Received Nov 10, 2020; Accepted Nov 30, 2020)

INTRODUCTION

Biological importance and practical significance of phosphate esters and their analogues are reflected to the numerous practical applications in many fields.¹⁻³ The synthesis of nucleotide analogues and the evaluation of their biological activities have been researched with the aim of producing new and useful compounds.⁴⁻⁵ Our group has previously reported the successful phosphorylation of nucleosides and nucleotides using inorganic phosphorylating agents such as cyclo-triphosphate⁶⁻⁷ and monooimido-cyclo-triphosphate.⁸ Phosphorylation with these agents can be achieved by a one-pot reaction in aqueous solution without side reactions. Our work demonstrated that the product yield associated with these reactions was high under strongly basic condition, such as pH 12, but no reaction was observed at all in weakly acidic and neutral solutions. The products themselves were extremely stable at pH 12, although they were found to decompose below pH 7.

Blaser and Worms reported that another inorganic reagent, disodium diphosphonate (Na₂P₂O₇H₂), reacts with several nucleophilic, such as monophosphate and fluoride, to form new compounds.⁹,¹⁰ As shown in Fig. 1, DP is phosphonate, in which phosphorus has an oxidation state of +3, and thus this compound contains the P(III)-H bond, which is not found in the monophosphate. Yamamoto et al. previously studied that adenosine 5'-monophosphate was phosphorylated by DP to give an analogue of adenosine 5'-diphosphate (5'-ADP).¹¹ Later, they have reported the phosphorylation of nucleoside 5'-diphosphate with DP in order to form H-phosphate analogues of nucleotide 5'-triphosphate (5'-NTP).¹² More recently, our group studied the reactions of nucleoside with DP.¹³ In the reaction of inosine with DP, 2'-phosphonylinosine and 3'-phosphonylinosine were synthesized with the total yield of more than 95%. We have also successfully introduced the phosphate group in a total yield of 40% at pH 7.

The anti-cancer drugs fluorouracil (5-FU) and 1-β-D-arabinofranosylcytosine (cytarabine or Ara C), for example, have been applied to the treatment of many kinds of cancer, but result in severe side effects.¹⁴,¹⁵ Therefore, the chemical modification of 5-FU and Ara C has been attempted for improving their absorption and reducing side effects.¹⁶⁻¹⁸ 5'-Deoxy-5-fluorouridine (doxifluridine, 5'-DFUR), for instance, which is a prodrug of 5-FU, has been applied to malignant tumors.

In the present work, we investigated the reactions of 5'-DFUR and Ara C with DP in aqueous solution with the aim of synthesizing new phosphate derivatives of these compounds. We further examined the stability of the resulting products in both weakly acidic and neutral solutions, and examined the reactivity of 2'- and 3'-OH groups of the β-D-ribofuranosyl unit.
RESULTS AND DISCUSSION

Reaction of 5'-DFUR with DP

The nucleosides examined in this study are shown in Fig. 1. Phosphorylation was carried out according to our previously reported method, and Fig. 2 presents representative HPLC chromatograms of a reaction mixture, in which peaks attributed to the phosphorylated compounds are evident at retention times of 40 and 45 min. Peak areas were used to calculate product yields, and the yields of products 1 and 2 were found to increase with prolonged reaction times, reaching maximum values, 57% and 50% after 14 d and 7 d, respectively.

\[ \text{Sodium diphosphonate (DP)} \]

\[ \text{Doxifluridine (5'-DFUR)} \]

\[ \text{Cytarabine (Ara C)} \]

FIGURE 1 Structures of sodium diphosphonate (DP), doxifluridine (5'-DFUR), and cytarabine (Ara C).

31P and 1H NMR spectra were acquired to assist the identification of products 1 and 2. Figure 3 shows the 1H-31P heteronuclear multiple bond correlation (HMBC) NMR spectrum of a mixture of 1 and 2. The 1H decoupled 31P NMR spectrum (x axis of Fig. 3) exhibits characteristic peaks at 6.62 and 6.36 ppm, attributed to the monophosphate esters of 5'-DFUR. On the basis of the chemical shifts of reference samples, the two singlets at 4.24 ppm and -3.93 ppm are assigned to phosphonate (P(III)) and diphosphonate (DP), respectively. The spectra show two correlations, one between the 31P signal at 6.62 ppm and the 1H signal at 4.23 ppm, and the other between 31P at 6.36 ppm and 1H at 4.58 ppm. The signal at 4.23 ppm was assigned to the H-3 of 1, and the signal at 4.58 ppm was assigned to the H-2 of 2, based on the 1H-1H COSY spectrum. These results demonstrate that 5'-DFUR reacts with DP to form both 3'-phosphonyl-5'-DFUR (1) and 2'-phosphonyl-5'-DFUR (2).

\[ \text{FIGURE 2 HPLC profiles for the reaction solution of 5'-DFUR (0.1 M) and DP (0.5 M) at pH 11 and 10 °C} \]

\[ \text{FIGURE 3 1H-31P 2D HMBC NMR spectra of 1 and 2. 5'-DFUR : DP} = 0.1 \text{ M : 1.0 M, pH 11, and 10 °C, after 2d.} \]

Table 1 summarizes the individual and overall yields of 1 and 2 obtained in the reaction of 5'-DFUR with DP under various conditions. At a 5'-DFUR to DP molar ratio of 1:10 (0.1 M:1.0 M) and 10 °C, the total yields of products 1 and 2 were 100, 99, 95 and 34% at pH 12, 11, 9 and 7, respectively. Based on the data in Table 1, it appears that the reaction is more efficient at either pH 11.5 or 12. However, as discussed later, products 1 and 2 are very unstable at pH 11.5 and 12. Therefore pH 11 is preferable pH. At a molar ratio of 1:5 and pH 11, the total yields of products 1 and 2 were 95, 89, and 77% at 10, 25 and 40 °C, whereas at pH 11 and 10 °C and using molar ratios of 1:10, 1:5 (0.1 M : 0.5 M), 1:1 (0.1 M:0.1 M) and 2:1 (0.1 M:0.05 M), the total yields were 99, 95, 79 and 17%, respectively. Taking into account of both yield and reaction time, the most appropriate condition for the phosphorylation of 5'-DFUR with DP are pH 11, 10 °C and a 5'-DFUR to DP molar ratio of 1:10. Under this condition, the total yield of 1 and 2 was 99%. This yield remained constant for 11 d, after which the products gradually decomposed to 5'-DFUR and phosphonate (P(III)).

In the reaction of 5'-DFUR with P(III) 2'- and 3'-OH groups of the β-D-ribofuranosyl unit on 5'-DFUR were phosphorylated with a yield of 95%. The reactivity of 5'-DFUR with DP as observed in this study is therefore almost the same as that of 5'-DFUR with P(III).
Phosphorylation of Ara C with DP

HPLC chromatograms were obtained for the reaction mixture of Ara C (0.1 M) and DP (1.0 M) incubated at pH 12 and 25 °C, in which a peak attributed to the phosphorylated product appeared at a retention time of about 10 min, together with a second peak assigned to unreacted Ara C. Although the chromatogram exhibited a single peak as the reaction product, $^{31}$P NMR spectrum (Fig. 4) show the presence of three phosphonate esters, 3, 4 and 5, which evidently could not be separated by HPLC. Using an Ara C to DP molar ratio of 1:10 (0.1 M:1.0 M) together with a pH of 12 and a temperature of 25 °C, the combined yield of 3, 4 and 5 was 39% after 1 d, although the yield decreased to 5% after 10 d.

$^{31}$P and $^1$H NMR spectra were acquired of the isolated products of 3, 4 and 5, as shown in Fig. 4, in which the $^1$H-$^{31}$P HMBC NMR spectrum of a mixture of 3, 4 and 5 is presented. The $^1$H decoupled $^{31}$P NMR spectrum (x axis of Fig. 4) shows characteristic peaks at 7.68, 6.16 and 5.51 ppm, presumed to result from the monophosphate esters$^{20}$ of Ara C. The correlations were observed between $^{31}$P at 7.68 ppm and $^1$H at 4.03 ppm, and between $^{31}$P at 7.68 ppm and $^1$H at 3.96 ppm. The signals at 4.03 and 3.96 ppm were assigned to the H-5' of 3 and the H-5' of 3 based on the $^1$H-$^1$H COSY spectrum. Correlations between $^{31}$P at 6.16 ppm and $^1$H at 4.42 ppm and between $^{31}$P at 5.51 ppm and $^1$H at 4.54 ppm were also seen. The signals at 4.42 and 4.54 ppm were assigned to the H-3' of 4 and the H-2' of 5 based on the $^1$H-$^1$H COSY spectrum. These results show that Ara C reacts with DP to form 5'-phosphonyl-Ara C (3), 3'-phosphonyl-Ara C (4) and 2'-phosphonyl-Ara C (5). The main product was determined to be 5'-phosphonyl-Ara C (3) based on a comparison of

the intensities of the $^{31}$P signals at 7.68, 6.16 and 5.51 ppm in the $^1$H decoupled $^{31}$P NMR spectrum.

Table 2 summarizes the total yields of 3, 4 and 5 obtained from the reaction of Ara C with DP under various conditions. At an Ara C to DP molar ratio of 1:10 (0.1 M:1.0 M) and 25 °C, the combined yields of products 3, 4 and 5 were 39, 35, 34, 26, 9 and 6% at pH values of 12, 11, 10, 9, 7 and 5, respectively. From these data, the reaction appears to progress more readily at pH 12 as compared to pH 11, but the phosphorylated products were unstable at pH 12. Therefore, pH 11 was considered to be more suitable. Overall, the optimal condition for the phosphorylation of Ara C with DP consists of pH 11, 25 °C and a 1:10 molar ratio between Ara C and DP, which results in total yields of 3, 4 and 5 of 35%. It is noteworthy that, in the phosphorylation of

![FIGURE 4 $^1$H-$^{31}$P 2D HMBC NMR spectra of 3, 4, and 5. Ara C : DP = 0.1 M : 1.0 M, pH 12, and 25 °C, after 3h.](image-url)
5'-DFUR by DP, the 2'- and 3'-OH groups of the β-D-ribofuranosyl unit on the 5'-DFUR were phosphorylated with a combined yield of 100%, whereas the phosphorylation of Ara C by DP resulted in a total yield of only 35%. The reactivity of 5'-DFUR with DP is therefore substantially higher than that of Ara C with DP.

**Reactions of 5'-DFUR and Ara C with DP**

The reactions of 5'-DFUR and Ara C with DP are summarized by Scheme 1. It is known that DP is readily attacked by nucleophilic reagents such as ammonia, fluoride and glucose 1-monophosphate, and thus, in the reaction presented in this work, the lone electron pair on the hydroxyl group of the β-D-furanosyl unit acts as a nucleophile to attack a phosphorus atom on DP.

Previous studies have reported that hydroxyl groups at the C-5' position of nucleosides and nucleotides do not react with P_m, whereas C-2' and C-3' groups which are positioned cis to one another do react to form monophosphate esters. The reaction of 5'-DFUR with P_m initially forms the triphosphate derivative of 5'-DFUR, which immediately hydrolyzes to form both 3'-monophospho-5'-DFUR and 2'-monophospho-5'-DFUR via a 2',3'-cyclic monophosphate derivative. In the phosphorylation of 5'-DFUR by DP, 5'-DFUR reacts with DP to form both 3'-phosphoryl-5'-DFUR (1) and 2'-phosphoryl-5'-DFUR (2), with the 3'-phosphoryl ester as the majority product.

Conversely, Ara C, in which the hydroxyl groups at C-2' and C-3' are trans to one another, reacts with P_m to form triphosphate esters (5'-triphospho-Ara C, 3'-triphospho-Ara C and 2'-triphospho-Ara C). In a similar manner, the phosphorylation of Ara C by DP also occurs at the 2', 3' and 5'-OH groups of the β-D-arabinofuranosyl unit on the Ara C. In this reaction, the order of reactivity of these groups was found to be 5'-OH > 3'-OH > 2'-OH, and thus the reactivity of the β-D-furanosyl unit strongly depends on the configuration of the hydroxyl group at the C-2' position.

**FIGURE 5** The effect of pH on the phosphorylated 5'-DFUR (1 + 2) synthesized under the reaction condition of 5'-DFUR : DP = 0.1 M : 1.0 M and 10 °C. ○ : pH 12, ♦ : pH 11.5, ■ : pH 11, △ : pH 9, □ : pH 7, X : pH 5

**TABLE 2** Yields of phosphorylated Ara C by DP

| Conc. (M) | Temp. (°C) | Time (d) | Total Yield (%) |
|----------|------------|----------|-----------------|
| AraC DP  | pH         |          |                 |
| 0.1      | 1.0        | 12       | 10              | 13               | 35   |
|          |            | 12       | 25              | 1                | 39   |
|          |            | 12       | 40              | 0.01             | 33   |
|          |            | 10       | 25              | 8                | 35   |
|          |            | 10       | 25              | 8                | 34   |
|          |            | 9        | 25              | 8                | 26   |
|          |            | 5        | 25              | 6                | 9    |
|          |            | 5        | 25              | 10               | 6    |
| 0.05     | 0.5        | 11       | 10              | 13               | 37   |
|          |            | 11       | 25              | 2                | 31   |
| 0.05     | 1.0        | 11       | 10              | 6                | 31   |

Scheme 1 Reaction scheme of 5'-DFUR and Ara C with DP

**Stability of phosphorylated 5'-DFUR and Ara C**

Figure 5 summarizes the effects of pH on the phosphorylated products 1 and 2. The products were initially synthesized using reaction conditions of 10 °C and a 5'-DFUR:DP molar ratio of 1:10. It is evident from these plots that, at pH 11, 11.5 and 12, the phosphorylated products rapidly decompose, whereas at pH 7 and 9 the products were stable even after 30 days of ageing. At pH 5, the phosphorylation reaction did not take place. Based on these results, products 1 and 2 are considered to be stable over the pH range 7 to 9.
yield is decreased to 5% after 10 days. At pH 11, the products were stable up to 13 days, after which they began to gradually decompose, and at pH 7 and 9 the products were stable even after 30 days. Therefore, we conclude that the phosphonlated products 3, 4 and 5 are also stable over the pH range of 7 to 9.

FIGURE 6 The effect of pH on the phosphonlated Ara C (3 + 4 + 5) synthesized under the reaction condition of Ara C : DP = 0.1 M : 1.0 M and 25 °C. ●: pH 12, ▲: pH 11, △: pH 9, ■: pH 7

CONCLUSION

The reaction of 5'-DFUR with DP was successful in synthesizing both 3'-phosphonyl-5'-DFUR (1) and 2'-phosphonyl-5'-DFUR (2), with a combined yield of approximately 100%, via phosphorylation at the 2'-OH or 3'-OH of the 5'-deoxy-β-D-ribofuranose unit. In the reaction of Ara C with DP, 5'-phosphonanyl-Ara C (3), 3'-phosphonanyl-Ara C (4) and 2'-phosphonyl-Ara C (5) were synthesized in a total yield of more than 35%. The phosphonlated products were stable in neutral and weakly alkaline solutions. These results suggest the possibility of synthesizing novel anionic derivatives of 5'-DFUR and Ara C containing phosphonyl groups, as well as the phosphorylation of nucleosides and nucleotides by DP.

EXPERIMENTAL

Materials and methods

Materials

Sodium diphostonate (Na3P2O5, DP) was prepared according to a method previously reported in the literature.11,12 Ara C, 5'-DFUR and sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) were purchased from Tokyo Kasei Co., Ltd., Wako Chemical Industries, Ltd. and Sigma. Unless otherwise stated, all other reagents were Guaranteed Reagent grade from Wako Chemical Industries.

NMR measurements

1H NMR spectra were acquired using a Varian INOVA-500 spectrometer (Palo Alto, California, USA). Samples were dissolved in D2O (99.9%) and DSS was used as an external standard. 31P NMR spectra with and without broad band 1H-decoupling and 1H-31P 2D HMBC spectra were obtained on a Varian INOVA-500 spectrometer, using 85% H3PO4 as an external standard.

HPLC measurements

HPLC analysis was performed on a JASCO PU-2080i system (Tokyo, Japan), using a 150x6.0 mm i.d. column packed with polystyrene-based anion exchange resin (TSK gel, SAX, 5 µm, TOSOH, Japan) maintained at 40 °C. Isocratic elution using a 0.2 M KCl solution was employed at a flow rate of 1.0 ml/min with UV detection at 271 nm. The system control, data collection, and data analysis were carried by JASCO-ChromNAV system (Version 1.18.03, JASCO, Tokyo, Japan).

MS measurements

Electrospray ionization mass spectrometry (ESI-MS) and liquid chromatography mass spectrometry (LC-MS) were performed using a Thermo Fisher Scientific ExactiVe™ mass spectrometer (Thermo Fisher Scientific, Commonwealth of Massachusetts, USA) operating in the negative-ion mode.

Synthetic Procedure

To produce 1 and 2, DP (1.8994 g, 1.0 M) and 5'-DFUR (0.2462 g, 0.1 M) were dissolved in H2O (20 ml) at 10 °C and the solution was adjusted to the desired pH by adding 6 M NaOH or 5 M HCl solution. Different reagent ratios and temperatures were also applied as required. The mixture had been allowed to react for the desired length of time. The yields of products 1 and 2 were determined by HPLC analysis. Products 1 and 2 were separated from the reaction mixture by anion-exchange chromatography using a 2x80 cm column filled with Dowex 1-X2 resin (100–200 mesh, chloride form). Prior to eluting the products, residual 5'-DFUR was removed by passing 300 ml distilled water through the column, followed by 630 ml of 0.3 M aqueous KCl to remove phosphate and diphostonate. Finally, the products were stripped off using 0.6 M aqueous KCl solution, and was collected in 30 ml fractions. The fractions over the volume range of 0 to 120 ml, during in which the products eluted, were analyzed by HPLC. Each fraction was evaporated to a volume of approximately 2 ml and then further concentrated by freeze-drying under vacuum at -113 °C.

A portion of the synthesized product was dissolved in D2O for HPLC, 1H NMR and 31P NMR
measurements. The ESI-MS spectrum of 1 and 2 exhibited a peak at m/z 309.02950, corresponding to the molecular ions of 3'-phosphoryl-5'-DFUR (1) and 2'-phosphoryl-5'-DFUR (2).

Products 3, 4, and 5 were synthesized by dissolving Ara C (0.1216 g, 0.1 M) and DP (0.9497 g, 1.0 M) in H2O (10 ml) at 40 °C, and adjusting the solution to the desired pH was performed by adding 6 M NaOH or 5 M HCl solution. Different reagent ratios and temperatures were also applied as required. After the desired reaction time, the yields of 3, 4, and 5 were established by HPLC analysis, and then the solution was evaporated to dryness. The separation of 3, 4, and 5 was accomplished by column chromatography on a 3×45 cm column filled with Wacosil 40C18 resin (30–50 μm, spherical form), using a 10:90 (v/v) methanol/H2O mobile phase. Fractions of 10 ml were collected during the elution process, and those fractions obtained during the volume range of 130 to 140 ml (during which the products were obtained) were analyzed via HPLC. Then, each fraction was evaporated to about 2 ml in volume, and then further concentrated via freeze-drying as mentioned above.

A portion of the product thus obtained was dissolved in D2O for HPLC. 1H NMR and 31P NMR measurements. The ESI-MS spectrum of 3, 4, and 5 showed a peak at m/z 306.05014 corresponding to the molecular ions of 5'-phosphoryl-Ara C (3), 3'-phosphoryl-Ara C (4), and 2'-phosphoryl-Ara C (5).

3'-Phosphoryl-5'‐DFUR (1): 1H NMR (D2O) δ 7.50 (1H, d, J1,′P = 6.0 Hz, H-6), 6.71 (1H, d, JPH = 649 Hz, P-H), 5.78 (1H, d, J1,′2 = 3.5 Hz, H-1′), 4.27 (1H, dd, J1,′2 = 3.5 Hz, J2,′3 = 5.1 Hz, H-2′), 4.23 (1H, m, J2,′3 = 5.1 Hz, J3,′4 = 5.0 Hz, JPH = 10.2 Hz, H-3′), 4.15 (1H, m, J3,′4 = 5.0 Hz, J4,′5 = 6.5 Hz, H-4′), 1.28 (3H, d, J4,′5 = 6.5 Hz, H-5′). 31P NMR (D2O) δ 6.62 (1P, dd, JPH,P,H = 9.7 Hz, JPH = 648 Hz, P-H), ESI-MS m/z 309.0295 (Calcd for [C9H10O4N2P]+: 309.03662).

2'-Phosphoryl-5'-DFUR (2): 1H NMR (D2O) δ 7.48 (1H, d, J1,′H = 6.0 Hz, H-6), 6.68 (1H, d, JPH = 650 Hz, P-H), 5.77 (1H, d, J1,′2 = 5.0 Hz, H-1′), 4.58 (1H, m, J1,′2 = 5.0 Hz, J2,′3 = 5.0 Hz, JPH = 10.0 Hz, H-2′), 3.90 (1H, dd, J2,′3 = 5.0 Hz, J3,′4 = 6.0 Hz, H-3′), 3.99 (1H, m, J3,′4 = 6.0 Hz, J4,′5 = 6.5 Hz, H-4′), 1.28 (3H, d, J4,′5 = 6.5 Hz, H-5′). 31P NMR (D2O) δ 6.36 (1P, dd, JPH,P,H = 10.4 Hz, JPH = 650 Hz, P-H), ESI-MS m/z 309.0295 (Calcd for [C9H10O4N2P]+: 309.03662).

5'-Phosphoryl-Ara C (3): 1H NMR (D2O) δ 7.69 (1H, d, J5,′H = 8.0 Hz, H-6), 6.65 (1H, d, JPH = 640 Hz, P-H), 6.08 (1H, d, J1,′2 = 5.5 Hz, H-1′), 5.92 (1H, d, J5,′K = 8.0 Hz, H-5), 4.30 (1H, dd, J1,′2 = 5.5 Hz, J2,′3 = 4.0 Hz, H-2′), 4.04 (1H, m, J3,′4 = 4.0 Hz, J4,′5 = 5.0 Hz, H-3′), 3.98 (1H, m, J4,′5 = 4.0 Hz, J5,′S = 3.5 Hz, J5,′S = 5.5 Hz, H-4′), 4.03 (1H, m, J5,′S = 3.5 Hz, J5,′S = 12.5 Hz JPH,S = 9.5 Hz, H-5′). 31P NMR (D2O) δ 7.68 (1P, dt, JPH,P,H = 9.5 Hz, JPH,S = 9.5 Hz, JPH = 640 Hz, P-H), ESI-MS m/z 306.05014 (Calcd for [C9H11O4N2P]+: 306.05694).

3'-Phosphoryl-Ara C (4): 1H NMR (D2O) δ 7.67 (1H, d, J5,′H = 7.5 Hz, H-6), 6.72 (1H, d, JPH = 646 Hz, P-H), 6.06 (1H, d, J1,′2 = 4.5 Hz, H-1′), 5.89 (1H, d, J5,′K = 7.5 Hz, H-5), 4.39 (1H, dd, J1,′2 = 4.5 Hz, J2,′3 = 4.0 Hz, H-2′), 4.42 (1H, m, J2,′3 = 4.0 Hz, J3,′4 = 5.0 Hz, JPH = 9.5 Hz, H-3′), 4.15 (1H, m, J3,′4 = 5.0 Hz, J5,′S = 5.5 Hz, H-4′), 3.79 (1H, dd, J3,′4 = 3.5 Hz, J5,′S = 12.5 Hz, H-5′), 3.71 (1H, dd, J5,′S = 5.5 Hz, J5,′S = 12.5 Hz, H-5′). 31P NMR (D2O) δ 6.16 (1P, dd, JPH,P,H = 9.5 Hz, JPH = 646 Hz, P-H), ESI-MS m/z 306.05014 (Calcd for [C9H11O4N2P]+: 306.05694).

2'-Phosphoryl-Ara C (5): 1H NMR (D2O) δ 7.62 (1H, d, J5,′H = 8.0 Hz, H-6), 6.32 (1H, d, JPH = 645 Hz, P-H), 6.14 (1H, d, J1,′2 = 4.0 Hz, H-1′), 5.90 (1H, d, J5,′K = 8.0 Hz, H-5), 4.54 (1H, m, J1,′2 = 4.0 Hz, J2,′3 = 4.0 Hz, JPH = 11.5 Hz, H-2′), 4.15 (1H, m, J3,′4 = 4.0 Hz, J3,′4 = 4.0 Hz, H-3′), 4.03 (1H, m, J4,′5 = 4.0 Hz, J5,′S = 3.5 Hz, J5,′S = 5.5 Hz, H-4′), 3.75 (1H, dd, J3,′4 = 3.5 Hz, J5,′S = 12.5 Hz, H-5′), 3.69 (1H, dd, J5,′S = 5.5 Hz, J5,′S = 12.5 Hz, H-5′). 31P NMR (D2O) δ 5.51 (1P, dd, JPH,P,H = 11.5 Hz, JPH = 644 Hz, P-H), ESI-MS m/z 306.05014 (Calcd for [C9H11O4N2P]+: 306.05694).

ACKNOWLEDGEMENTS

The authors thank associate professor C. Tode of Kobe Pharmaceutical University for the measurements of 31P, 31P-1H HMBC, 1H-1H COSY, 1H-1H TOCSY 2D NMR spectra. This work was partially supported by the Promotion and Mutual Aid Corporation for Private School of Japan.

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