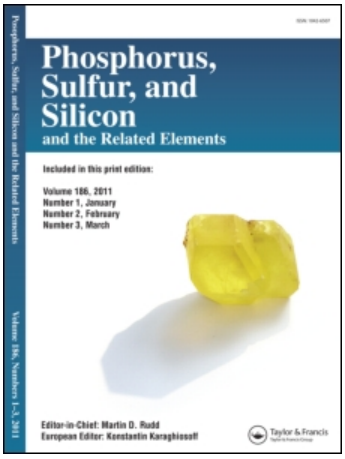

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A NEW AND VERSATILE APPROACH TO THE PREPARATION OF H-PHOSPHONATE ANALOGUES FOR ADP AND ATP

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ABSTRACT Phosphonylation of nucleotides with a new reagent, diphosphonate, was found to be very effective for the preparation of P-O-P bridged H-phosphonate analogues of ADP and ATP.

INTRODUCTION

More recently much interest has been focused on an H-phosphonate approach for the development of new and efficient methods of synthesizing DNA and RNA[1,2], nucleoside-phospholipid conjugates[3a], and internucleotide phosphate analogues[3b] which are widely used as a metabolic regulator[4]. The increasing significance of H-phosphonate approach has also stimulated further research in organic synthesis. However, a reliable and economical route to the synthesis of H-phosphonates involving a P-O-P linkage has, to our knowledge, not yet been published. The H-phosphonates involving a P-O-P linkage become a new class of analogues as nucleoside polyphosphates, the analogues which are important probes for the mechanistic studies on enzymatic reactions as well as organic phosphonates[4] and thiopyrophosphates[5].

We now report a new and versatile method for the preparation of H-phosphonate analogues of ADP(**3a**), ATP(**5a**), and other nucleoside polyphosphates(**3b-g**, **5b-e**) by phosphonylation using a new phosphonylating agent **1**, diphosphonate[6-9].

EXPERIMENTAL

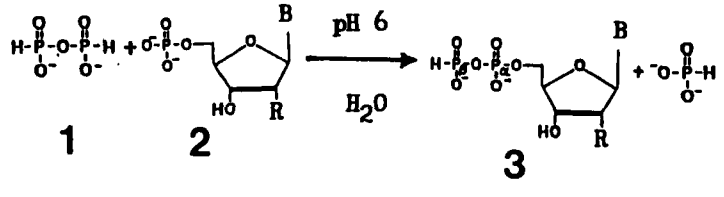
The phosphonylating agent (**1**; disodium diphosphonate, $Na_2P_2H_2O_5$) was

prepared as follows: In an Erlenmeyer flask with a glass stopper, 300 ml acetic anhydride was added to a mixture of 18.5 g of phosphonic acid and 48.6 g of disodium phosphonate. The resulting mixture was agitated for 1 h and allowed to be incubated in an ultrasonic cleaner for 3 h at room temperature to give white precipitate. Reaction time for the dehydration of phosphonic acid and phosphonate was reduced from 7 days without ultrasound to 3 h with ultrasound. The precipitate was washed with acetone, dissolved in 150 ml of water, and recrystallized by addition of 210 ml of ethanol. Recrystallized precipitate was filtered and dried at 50 °C to get about 15 g of **1**. Diphosphonate can be stored unchanged in a screw-cap vial in a desiccator for several years. The purity of **1** was checked by the HPLC[10] to be 97-99 % (as P).

RESULTS AND DISCUSSION

Preparation of H-phosphonate analogues of nucleoside 5'-diphosphates

Aqueous reaction mixture of **2a**(0.1M) and **1**(1.5M) was allowed to react at 60 °C and pH 6. The reaction was monitored by ^{31}P-NMR to show that signals of **1** and **2a** gradually disappeared and new doublets which indicated the formation of a new P-O-P linkage were observed in the high-field region of -4~-10 ppm. Monitoring of this reaction by HPLC[8-10] indicated 70% conversion of **2a** into a P-O-P bridged H-phosphonate analogue **3a** of ADP after 1 h incubation. Other H-phosphonate analogues **3b-g** were also given by use of the same conditions as mentioned. To optimize reaction conditions, yields of **3** were measured varying pH and initial concentration of **1**. The optimal conditions for the preparation of **3** were found to be initial concentration of 1.5M (**1**) and 0.1M (**2**), respectively, pH 6 and 60 °C.



B: Ade, Gua, Ura, Cyt, Hyp, Thy, Ade
R: OH, OH, OH, OH, OH, H, H
a **b** **c** **d** **e** **f** **g**

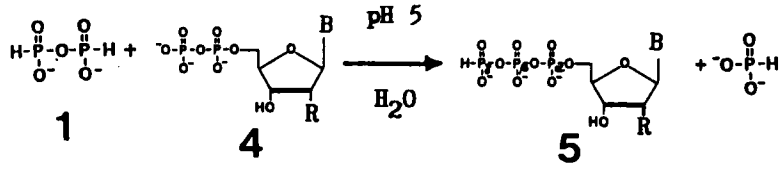
Yields under the optimal conditions and ^{31}P-NMR parameters for **3** were listed in Table I. It can be seen in the Table I that the yields were almost the same as each other. After the purification by column chromatography, each analogue **3** could be isolated in a purity of 90-92%.

TABLE I Yields and ^{31}P-NMR parameters of **3**.

Nucleo-tides	Yield of 3(%)	$\delta(P_\alpha)$	$\delta(P_\beta)$	$^1J_{PH}$ (Hz)	$^2J_{PP}$ (Hz)
2a	70	-9.51	-4.03	667	19.8
2b	56	-7.18	-1.30	665	19.8
2c	66	-9.64	-4.19	666	19.8
2d	73	-9.67	-4.19	665	19.1
2e	66	-9.35	-4.12	665	19.8
2f	68	-9.53	-4.11	665	19.8
2g	67	-10.4	-4.87	665	19.8

Preparation of H-phosphonate analogues of nucleoside 5'-triphosphates

Next, the reaction was applied to the synthesis of an H-phosphonate analogue of nucleoside 5'-triphosphate. Diphosphonate(1.8M) reacted with **4a**(0.1M) at pH 5 and 70 °C in an aqueous solution and the reaction mixture was monitored by ^{31}P-NMR. The spectrum represented that a new triplet which indicated the formation of P-O-P-O-P bond appeared at -21.2 ppm. Kinetic HPLC profile for the reaction showed 56 % conversion of **4a** into **5a** after 20 min incubation. In a similar manner other H-phosphonate analogues **5b–e** were also obtained. The optimal conditions to get the highest yield for **5** were found to be initial concentration of 1.8M (**1**) and 0.1M (**4**), respectively, pH 5 and 50 °C.



1 4 5

B: Ade, Gua, Ura, Cyt, Thy
R: OH, OH, OH, OH, H
a **b** **c** **d** **e**

Yields and ^{31}P-NMR parameters for **5** were compiled in Table II. Yield of **5** was smaller than that of **3** in Table I. The difference in yield between **3** and **5** is attributable to the different negative charges on nucleophiles, **2** and **4**, because more negatively charged **4** is repulsed

and slowed down to higher degree in attacking dianionic **1**. After the purification by column chromatography, each analogue **5** could be isolated in a purity of 85-90%.

TABLE II Yields and ^{31}P-NMR parameters of **5**.

Nucleotides	Yield of 5(%)	$\delta(P_\alpha)$	$\delta(P_\beta)$	$\delta(P_\gamma)$	$^1J_{PH}$ (Hz)	$^2J_{PP}$ (Hz)
4a	56	-10.4	-21.2	-4.10	669	19.0
4b	44	-10.2	-20.8	-3.91	668	19.0
4c	48	-10.2	-20.9	-3.89	668	19.0
4d	56	-10.6	-21.4	-4.27	669	19.0
4e	54	-10.2	-20.8	-3.81	666	18.9

The results clearly demonstrate that the one-step route described above would prove to be very effective for the preparation of new nucleoside H-phosphonates involving P-O-P linkages in a good yield. By using the present method, the H-phosphonate function could be introduced fast and under almost neutral conditions without any side reactions.

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