Tetrahedron Letters No. 24, pp 1913 - 1916, 1975. Pergamon Press. Printed in Great Britain.

2-(N,N-DIMETHYLAMINO)-4-NITROPHENYL PHOSPHATE AND ITS USE IN THE SELECTIVE PHOSPHORYLATION OF UNPROTECTED NUCLEOSIDE.

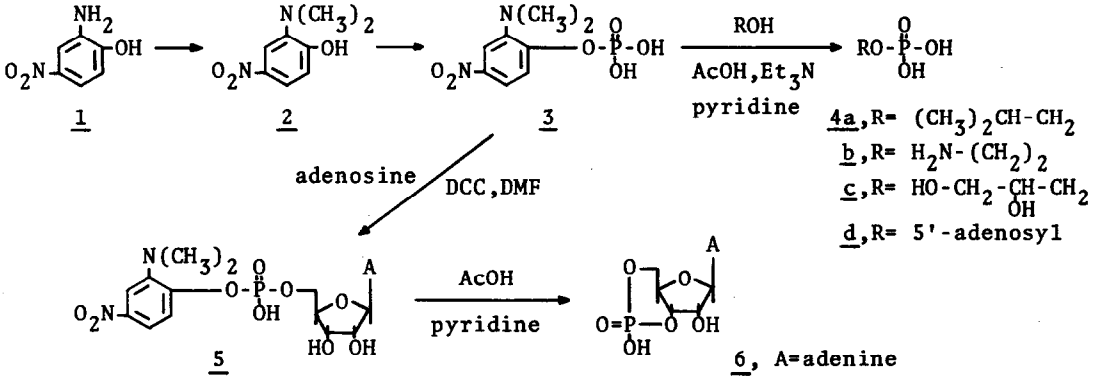
Yoshihiko Taguchi and Yoshitaka Mushika
Research Laboratory of Applied Biochemistry, Tanabe Seiyaku Co., Ltd.,
16-89, Kashima-3-chome, Yodogawa-ku, Osaka 532, Japan

(Received in Japan 21 April 1975; received in UK for publication 25 April 1975)

A number of reagents have been developed for the phosphorylation of nucleosides and other compounds of biological interest. Among them are several reagents[1-7] which have been employed for the selective phosphorylation of the 5'-OH of unprotected nucleosides but they have the disadvantages of requiring excess reagent for driving the reaction to completion.

In this communication, we report on the preparation of 2-(N,N-dimethylamino)-4-nitrophenyl dihydrogen phosphate (3) as a new phosphorylating reagent which could be used in the selective phosphorylation of the 5'-OH of unprotected nucleosides whithout unfavorable interaction with the amino group of the reactant. The reagent 3 was prepared from 2-amino-4-nitrophenol via 3 steps. 2-Amino-4-nitrophenol (0.1 mol) was methylated with methyl iodide (0.25 mol) in acetone (100 ml) in the presence of triethylamine (0.15 mol) under reflux for 4 hr to give 2-(N,N-dimethylamino)-4-nitrophenol (2) as the hydrochloride in 58% yield. The phosphorylation of 2 (0.15 mol) was carried out by refluxing with 10 equiv. of phosphoryl chloride in the presence of KCl (1.5 g). After removal of excess phosphoryl chloride by evaporation, the viscous residue was poured into a mixture of ice water (500 g)—pyridine (16 g), and the solution was allowed to stand at room temperature for 30 min. The solution was passed through a column (2.2 × 50 cm) of Amberlite IR-45 resin (OH^- form). Conc. NH_4OH (4 ml) was added to the combined eluates, and this solution was passed again through a column of Dowex 50 (NH_4^+ form, 1.4 × 45 cm). Concentration of the eluate gave the monoammonium salt of 3 (34 g, 81%) as pale yellow prisms; mp 171-172.5°.

The phosphorylating reactions with this reagent were successfully carried out in pyridine in the presence of acidic catalyst, such as AcOH, CF_3COOH, $BF_3 \cdot Et_2O$, which evidently enhanced the reactivity of the reagent by protonation.[8]



In a typical run a solution of 3 monotriethylammonium salt[9] (0.01 mol), the alcohol (0.01 mol), AcOH (0.03 mol), and triethylamine (0.01 mol) in anhydrous pyridine was refluxed for 3 hr. The concentrated reaction mixture was dissolved in H_2O (50 ml) and the solution was passed through a column (1.0 × 50 cm) of Dowex 50 (H^+ form). The eluate was neutralized with aniline. Concentration of the solution and recrystallization of the residue from 95% EtOH gave the corresponding alkyl dihydrogen phosphates monoanilinium salts[10] in good yields. In the case of 2-aminoethyl dihydrogen phosphate,[11] the isolation was achieved merely by neutralization with barium hydroxide and subsequent concentration of the aqueous solution after removal of insoluble substance. Interestingly, the reaction with glycerin afforded glycerol-1-phosphate[12] selectively and this prompted us to investigate the selective phosphorylation of the 5'-OH of unprotected nucleoside. A solution of adenosine (0.01 mol), the monotriethylammonium salt of 3 (0.01 mol), AcOH (0.03 mol), and triethylamine (0.01 mol) in anhydrous pyridine (30 ml) was refluxed for 3 hr and concentrated under reduced pressure. The residue was washed with acetone and dissolved in water (50 ml). The aqueous solution was passed through a column (1.0 × 30 cm) of Amberlite IRC-50 resin (H^+ form). The eluate was concentrated to dryness under reduced pressure at a temperature below 40°. Crystallization of the residue from

aqueous acetone gave adenosine 5'-phosphate in 77% yield. The formation of the other phosphorylated product was not detected with the worked up mixture on ppc. These results suggested that the nucleoside-5' 2-(N,N-dimethylamino)-4-nitrophenylphosphate, which might be obtained by the DCC(dicyclohexylcarbodiimide) coupling reaction of the nucleoside with 3, would afford the 3',5'-cyclic phosphate. Thus, adenosine (10 mmol) was subjected to the DCC (10 mmol) coupling reaction with 3 (5 mmol) in DMF (150 ml). The mixture was allowed to stand at room temperature for 24 hr and concentrated to dryness under reduced pressure. The residue was dissolved in anhydrous pyridine (1000 ml) containing AcOH (0.3 g). After removal of insoluble dicyclohexylurea by filtration, the filtrate was added dropwise to the boiling anhydrous pyridine (1000 ml) during 3 hr with stirring and the mixture was further refluxed for 3 hr. The concentrated mixture under reduced pressure was dissolved in H_2O (300 ml) and the precipitates were removed by filtration. The filtrate was extracted with EtOAc to remove the 2-(N,N-dimethylamino)-4-nitrophenol formed. The aqueous solution thus obtained was worked up and chromatographed on the DEAE cellulose column as described in the literature,[13] the eluates being followed spectrophotometrically at 260 nm. The nucleotide fractions were combined and concentrated

Phosphorylation Products

Compd. 4[a] & 6	Yield (%)	MP. (°C)	Rf[b]
Isobutylphosphate	86	154-156	0.53[c]
2-Aminoethylphosphate	79	234-235	0.12[d]
Glycerol-1-phosphate[f]	88		0.25[e]
Adenosine 5'-phosphate	77	195-199(dec.)	0.16[d]
Adenosine 3',5'-cyclic phosphate	64	229-232(dec.)	0.41[d]

a) The compounds were identified with authentic samples. b) Paper chromatography was carried out by the descending technique using Toyo Roshi No.51A paper. The solvent systems used were solvent A,isopropanol-conc. NH_4OH-H_2O(7:1:2); solvent B,1-propanol-conc.NH_4OH-H_2O(6:3:1); solvent C, isopropanol-5N NH_4OH(2:1). c) solvent B. d) solvent A. e) solvent C. f) This compound was isolated as disodium salt.

to dryness under reduced pressure at a temperature below 40°. Treatment of the residue with 50% aqueous EtOH (10 ml, pH 2.0) gave crystalline adenosine 3',5'-cyclic phosphate (1.11 g, 64%).

Acknowledgement: We wish to express our thanks to professor Y. Tamura for his helpful suggestions. Thanks are extended to Drs. T. Takayanagi, I. Chibata, M. Miyoshi and I. Inoue, for their encouragement.

References and footnotes:

1) G. M. Tener, J. Amer. Chem. Soc., 83, 159 (1961).
2) K. L. Agarwal and M. M. Dhar, Experientia, 21, 432 (1965).
3) A. Franke, K. H. Scheit and F. Eckstein, Chem. Ber., 101, 2998 (1968).
4) K-I. Imai, S. Fujii, K. Takanohashi, Y. Furukawa, T. Masuda and M. Honjo, J. Org. Chem., 34, 1547 (1969).
5) M. Yoshikawa, T. Kato and T. Takenishi, Bull. Chem. Soc. Japan, 42, 3505 (1969).
6) O. Mitsunobu, K. Kato and J. Kimura, J. Amer. Chem. Soc., 91, 6510 (1969).
7) H. Takaku and Y. Shimada, Tetrahedron Lett., 1974, 1279.
8) T. Hata, Y. Mushika and T. Mukaiyama, J. Amer. Chem. Soc., 91, 4532 (1969).
9) The monotriethylammonium salt was prepared by adding triethylamine in the aqueous solution of the monoammonium salt followed by coevaporation with anhydrous pyridine.
10) T. Obata and T. Mukaiyama, J. Org. Chem., 32, 1063 (1967).
11) E. Cherbuliez and J. Rabinowitz, Helv. Chim. Acta, 39, 1455 (1956).
12) T. Ukita, K. Nagasawa and M. Irie, Chem. Pharm. Bull. (Tokyo), 5, 127 (1957)
13) M. Smith, G. I. Drummond and H. G. Khorana, J. Amer. Chem. Soc., 83, 698 (1961).