Synthetic Studies on Phosphorylating Reagent. IV. A Novel Synthesis of Nucleoside-3′,5′ Cyclic Phosphates by the Use of 2-(*N*,*N*-Dimethylamino)-4-nitrophenyl Phosphate

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A novel method for the synthesis of nucleoside-3′,5′ cyclic phosphate has been investigated. The intermediates, nucleoside-5′ 2-(*N*,*N*-dimethylamino)-4-nitrophenyl phosphates (**3a—d**), were prepared in good yields by the condensation of unprotected nucleosides with 2-(*N*,*N*-dimethylamino)-4-nitrophenyl phosphate (**2**), using dicyclohexylcarbodiimide (DCC) in dimethylformamide (DMF). Treatment of the intermediates **3a—d** with acetic acid in boiling pyridine under high dilution condition gave nucleoside-3′,5′ cyclic phosphates in fairly good yields and small amount of other nucleotides, whereas nucleoside-2′,3′ cyclic phosphate was obtained by the reaction in DMF at 100 °C. An attempt at the direct synthesis of the cyclic nucleotide by refluxing the reaction mixture of unprotected nucleoside with DCC and **2** in pyridine was also successful to afford the similar results as in the stepwise procedure.

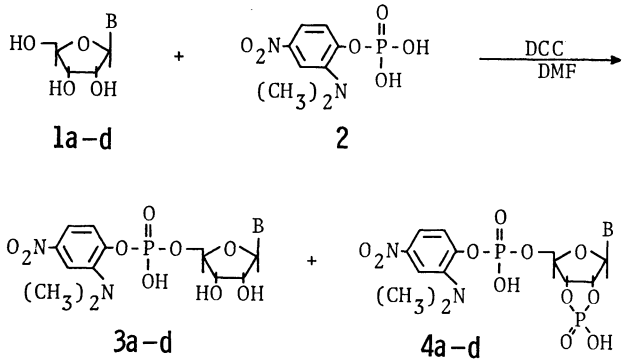
Adenosine-3′,5′ cyclic phosphate has been recognized as an intracellular second messenger of hormone action, and some of the various biological properties attributed to adenosine-3′,5′ cyclic phosphate can be explained by the stimulation of these protein kinases.

A number of procedures[1–4] have been applied to the synthesis of nucleoside-3′,5′ cyclic phosphates. Lipkin *et al.*[1] obtained adenosine-3′,5′ cyclic phosphate by the barium hydroxide-catalyzed degradation of adenosine-5′ triphosphate. Intramolecular cyclization of nucleoside-5′ phosphate was carried out by Smith *et al.*[2] using dicyclohexylcarbodiimide (DCC) in pyridine under high dilution condition. Borden and Smith[3] reported the synthesis of nucleoside-3′,5′ cyclic phosphate from nucleoside-5′ 4-nitrophenyl phosphates with potassium *t*-butoxide in dimethyl sulfoxide. Recently, Mukaiyama and Hashimoto[4] obtained nucleoside-3′,5′ cyclic phosphate in a similar manner to the method of Smith *et al.* except using triphenylphosphine and 2,2′-dipyridyl disulfide as condensation reagents.

In previous papers,[5–7] we reported that a new phosphorylating reagent, 2-(*N*,*N*-dimethylamino)-4-nitrophenyl phosphate (**2**), which has an activatable protecting group, reacted with various alcohols and amino alcohols to afford the corresponding alkyl dihydrogen phosphates and amino alkyl dihydrogen phosphates, respectively. The acid catalyzed phosphorylations of unprotected nucleosides with just one equiv. of this reagent **2** gave the corresponding nucleoside-5′ phosphates selectively.

In view of our success in the direct synthesis of nucleoside-5′ phosphate from unprotected nucleoside, it was considered that the DCC coupling reaction of unprotected nucleoside with one molar equivalent of **2** would give nucloside-5′ 2-(*N*,*N*-dimethylamino)-4-nitrophenyl phosphate (**3a—d**) selectively, probably because of the bulkiness of the reagent, and subsequently its intramolecular phosphorylation would afford nucleoside-3′,5′ cyclic phosphate. In this communication, we describe a new synthetic method of nucleoside-3′,5′ cyclic phosphate by the use of **2**.

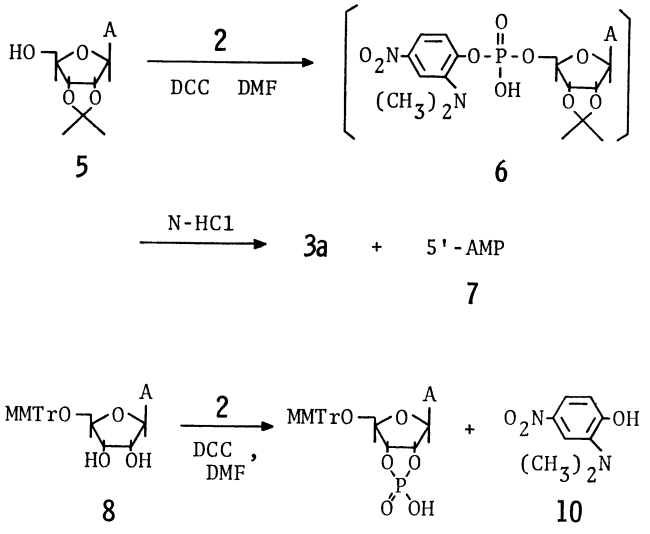
Initial attempt was carried out with adenosine. One mmole of adenosine in dimethylformamide (DMF) was allowed to react with the pyridinium salt of **2** (1.5 mmol) and DCC (2 mmol) at room temperature. After the usual work-up, the column chromatography of the reaction products using DEAE Sephadex afforded adenosine-5′ 2-(*N*,*N*-dimethylamino)-4-nitrophenyl phosphate (**3a**) in 79% yield and adenosine-2′,3′ cyclic phosphate-5′ 2-(*N*,*N*-dimethylamino)-4-nitrophenyl phosphate (**4a**) in 12% yield. The former product could be crystallized as calcium salt, though a good elemental analysis was not obtained. The NMR spectrum in D_2O showed the presence of the 2-(*N*,*N*-dimethylamino)-4-nitrophenyl group and the 5′-methylene protons at 4.32 ppm, which usually appears at lower field than those of the original nucleoside upon 5′-*O*-phosphorylation.[8] Comparison of the mobility in paper electrophoresis (PEP) with those of the analogous nucleotides (Table 4) also supported the assigned structure. The latter product was crystallized as lithium salt pentahydrate, mp 242—243 °C. The NMR spectrum in D_2O exhibited the 2′,3′-protons at 5.16—5.65 ppm and the 4′,5′-protons at 4.23—4.67, showing again downfield shift upon phosphorylation. In addition, the structures of both compounds were ascertained by the consideration of the following chemical reactions. Treatment of 2′,3′-*O*-isopropyl-



a, B=adenine, **b**, B=guanine
c, B=uracil, **d**, B=cytosine

Scheme 1.

ideneadenosine (**5**) with the triethylammonium salt of **2** and DCC under the same conditions gave a sole nucleotide, 2',3'-*O*-isopropylideneadenosine-5' 2-(*N*,*N*-dimethylamino)-4-nitrophenyl phosphate (**6**), having R_f value of 0.81 in Solvent A on paper partition chromatography (PPC). Subsequent deblocking with 1M-hydrochloric acid afforded a mixture of **3a** and adenosine-5' phosphate (**7**), which were characterized by PPC and PEP. On the other hand, when 5'-monomethoxytrityladenosine (**8**) was allowed to react with the triethylammonium salt of **2** and DCC under the same conditions, the liberation of a significant amount of 2-(*N*,*N*-dimethylamino)-4-nitrophenol (**10**) was observed, showing the occurrence of intramolecular cyclization after the phosphorylation of **8** at either the 2'- or 3'-hydroxyl group. In fact, usual work-up and DEAE Sephadex column chromatography afforded 5'-monomethoxytrityladenosine-2',3' cyclic phosphate



Scheme 2.

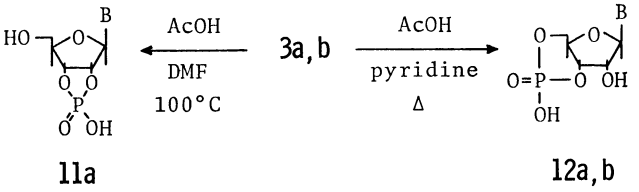
(**9**) quantitatively. This ready formation of the 2',3'-cyclic phosphate linkage under mild conditions contrasts with the conversion of nucleoside-2'(3') 4-nitrophenyl phosphate into the corresponding 2',3'-cyclic nucleotide under rather vigorous conditions,[3,9] and also with the studies of Agarwal and Dhar.[10] Condensation reactions of other nucleosides underwent similarly to give the corresponding nucleotides, **3b—d** and **4b—d** (Table 1).

Table 1. Products in the reaction of nucleosides with **2**

Nucleoside	Products (Yield %)[a]		
	3	**4**	Recovered nucleoside
Adenosine	79	12	9
Guanosine	99	0[b]	1
Uridine	49	7[b]	44
Cytidine	62	6[b]	32

a) Yields were estimated from the total optical densities at their absorption maxima corresponding to the each products. b) The yields of these products were calculated from the additive optical densities of corresponding **1** and **2**.

DMF was found to be a suitable solvent for this reaction, whereas the use of pyridine resulted in the recovery of the unreacted nucleoside. Next, the intramolecular phosphorylation of **3** to nucleoside-3',5' cyclic phosphate was attempted in pyridine under high dilution condition. The triethylammonium salt of **3** was so soluble in anhydrous pyridine that the use of 4-morpholine-*N*,*N*'-dicyclohexylcarboxamidine was unnecessary. The cyclization was carried out by the dropwise addition of a solution of **3** and acetic acid in anhydrous pyridine to a large amount of boiling pyridine that is enough to make high dilution condition. In this reaction, acetic acid would serve as a proton catalyst, converting the dimethylamino group to the electron-withdrawing group. Thus, when **3a** was treated under these conditions, adenosine-3',5' cyclic phosphate (**12a**) was obtained in 61% yield, the other porducts being adenosine-5' phosphate, adenylyl-(5'→5')-adenosine, and dephosphorylated adenosine. These products were separated by linear gradient chromatography with DEAE Sephadex and identified with the authentic samples by the criteria of PPC and PEP. Similar result was obtained in the cyclization reaction with guanosine-5' 2-(*N*,*N*-dimethylamino)-4-nitrophenyl phosphate (**3b**) (Table 2). Further attempt was made to improve this method



Scheme. 3.

Table 2. Products composition in the cyclization of **3a,b** in pyridine

Products from **3a**	Yield %[a]	Products from **3b**	Yield %[a]
12a	61	**12b**	52
5'-AMP (**7**)	8	5'-GMP	3
A_pA (5'→5')	13	G_pG (5'→5')	22
1a	12	**1b**	21

a) Yields were estimated from the total optical densities at their absorption maxima corresponding to those of the authentic samples.

without isolation of the intermediate **3**. In a typical run, the reaction of one equivalent amount of **2** and two equivalents of adenosine with DCC was carried out in DMF at room temperature. Treatment of this reaction mixture in DMF at 100 °C under high dilution condition afforded a major product with the same PEP mobility as adenosine-(2',3' and 3',5') cyclic phosphates (**11a** and **12a**). The yield of this material was estimated spectrophotometrically to be 75%. On mechanistic consideration, it seemed likely that this product was adenosine-3',5' cyclic phosphate (**12a**). However, this proved not to be the case: that the product was indeed adenosine-2',3' cyclic phosphate (**11a**) followed from its hydrolysis properties. When the product was treated with Dowex-50 (H^+ form) in water, it was readily converted into a mixture of adenosine-(2' and

Table 3. Products composition in the direct synthesis of **12a—d**

Compd from **1a**	Yield %[a]	Compd from **1b**	Yield %[a]	Compd from **1c**	Yield %[a]	Compd from **1d**	Yield %[a]
12a	64	**12b**	51	**12c**	58	**12d**	32
5′-AMP	12	5′-GMP	6	5′-UMP	17	5′-CMP	11
A_pA (5′→5′)	12	G_pG (5′→5′)	11	U_pU (5′→5′)	2	C_pC (5′→5′)	2
1a	111	**1b**	128	**1c**	120	**1d**	128

a) Yields were based on the phosphorylating agent **2**, and estimated from the total optical densities at the absorption maxima corresponding to those of the authentic samples.

3′) phosphates. No adenosine-5′ phosphate could be detected in the products. Reese and Khwaja[11] have observed the same phenomenon in the intramolecular phosphorylation of adenosine-5′ 2-hydroxyphenyl phosphate in pyridine, and proposed the pyridine-promoted dephosphorylation, followed by the phosphorylation of the nucleoside thus produced, as a possible mechanism. However, this would not be the case with ours, because the present reaction was run in DMF and the reagent has no participating *O*-hydroxyl group. The mechanism of this reaction remains as a matter of further investigation.

Finally, the cyclization reaction proceeded in the desired direction by high dilution treatment in boiling pyridine. Thus, after concentration of the reaction mixture, the original solvent was replaced by pyridine, and the solution was added dropwise to the boiling pyridine in the presence of acetic acid under high dilution condition. The reaction mixture was worked up as described in the Experimental section and subjected to DEAE Sephadex column chromatography with a linear gradient of triethylammonium bicarbonate to give product compositions similar to those in the stepwise procedure. All the compounds listed in the Table 3 were identified with authentic samples by PPC and PEP, and the yields were estimated spectrophotomotrically.

This reagent **2** with its high solubility and selectivity would provide a useful tool for the preparations of nucleoside-3′,5′ cyclic phosphate and nucleoside-2′,3′ cyclic phosphate.

Experimental

General Methods. Reagent grade pyridine was used after being dried over calcium hydride or Linde 4-A Molecular Sieves for several days. Solutions were concentrated to dryness at 30 to 40 °C using either a rotary evaporator or, when anhydrous conditions were required, an oil pump equipped with Dry Ice trap.

Paper chromatography was carried out using the descending technique on Toyo Roshi No. 51A paper. The solvent systems used were: isopropyl alchohol–concentrated ammonia–water (7:1:2) (Solvent A); *n*-butyl alcohol–acetic acid–water (5:2:3) (Solvent B). Paper electrophoresis was performed at pH 7.5 (0.05 M triethylammonium bicarbonate, 900 V/40 cm). Chromatographic and electrophoretic mobilities are recorded in Table 4. Melting points are uncorrected and were determined on a Yamato apparatus MP-21. The NMR spectra were determined on a Hitachi Perkin-Elmer R-20A instrument (DSS). IR spectra were determined on a Shimadzu IR-27G spectrometer, and UV spectra, on a Hitachi EPS-3T spectrometer.

*Adenosine-5′ 2-(*N,N*-Dimethylamino)-4-nitrophenyl phosphate (3a)* Adenosine (0.27 g, 1 mmol) and the pyridinium salt of **2** (1.5 mmol) were treated with DCC (0.41 g, 2 mmol) in DMF (50 ml) for 24 hr at room temperature. The reaction mixture was concentrated to dryness and water (500 ml) was added. After the precipitate was removed by filtration, the filtrate was applied to a 1.8×60 cm column of DEAE Sephadex (HCO_3^-) which was eluted with a linear gradient of triethylammonium bicarbonate (4 l., 0.0005—0.1 M), and the elution was followed spectrophotometrically at 260 nm. A major peak (22460 OD units, 79%) of electrophoretically and chromatographically homogeneous adenosine-5′ 2-(*N*,*N*-dimethylamino)-4-nitrophenyl phosphate (**3a**) was first eluted and a small peak of adenosine-2′,3′ cyclic phosphate-5′ 2-(*N*, *N*-dimethylamino)-4-nitrophenyl phosphate (**4a**) (3410 OD units, 12%) was followed. The pooled peak of **3a** was evaporated to dryness and coevaporated several times with methanol. The residue was dissolved in water (10 ml), and a stoichiometric amount of calcium hydroxide was added. The solution was concentrated to dryness and the residue was crystallized from aqueous ethanol to give **3a** calcium salt, in pale yellow powder; mp 187—190 °C (decomp.); $\lambda_{max}^{H_2O}$ (pH 6.85 and 12.3) 259 nm, 292 (sh); (pH 1.55) 259; NMR (D_2O) 2.73 (s, 6, N-Me_2), 4.15—5.07 (m, 5, $C_2'H+C_3'H+C_4'H+C_5'H_2$), 5.97 (d, J=5 Hz, 1, $C_1'H$), 7.10—7.64 (m, 3, Ph), 8.03 and 8.16 ppm (2 s, 2, C_2H and C_8H). The fractions containing **4a** were evaporated to dryness and the residue was dissolved with methanol. The solution was treated with lithium chloride, and precipitation with acetone gave pale yellow powder; mp 242—243 °C (decomp.); $\lambda_{max}^{H_2O}$ (pH 6.85 and 12.3) 259 (28400) nm, 292 (sh) (10800); (pH 1.55) 259 (24200); NMR (D_2O) 2.74 (s, 6, N-Me_2), 4.23—4.68 (m, 3, $C_4'H+C_5'H_2$), 5.16—5.65 (m, 2, $C_2'H+C_3'H$), 6.27 (d, J=3.2 Hz, 1, $C_1'H$), 7.06—7.71 (m, 3, Ph), 8.09 and 8.23 ppm (2 s, 2, C_2H and C_8H). Found: C, 32.07; H, 4.43; N, 14.52%. Calcd for $C_{18}H_{19}N_7O_{11}P_2Li_2 \cdot 5H_2O$: C, 32.30; H, 4.52; N, 14.63%.

*Guanosine-5′ 2-(*N,N*-Dimethylamino)-4-nitrophenyl Phosphate (3b).* Guanosine (0.28 g, 1 mmol) and the pyridinium salt of **2** (1.5 mmol) were treated with DCC (0.41 g, 2 mmol) in DMF (50 ml) at room temperature for 24 hr. The reaction mixture was concentrated to dryness and water (500 ml) was added. After the precipitates was removed by filtration, the filtrate was applied to a 1.8×60 cm column of DEAE Sephadex (HCO_3^-), which was eluted with linear gradient of triethylammonium bicarbonate (4 l., 0.005—0.1 M). A major peak (27580 OD units, 99%) of electrophoretically and chromatographically homogeneous guanosine-5′ 2-(*N*,*N*-dimethylamino)-4-nitrophenyl phosphate (**3b**) was eluted. The by-product **4b** was not detected in the products. The pooled peak of **3b** was evaporated to dryness and coevaporated several times with methanol. The residue was dissolved in water (10 ml) and a stoichiometric amount of calcium hydroxide was added. The solution was concentrated to dryness and the residue was crystallized from aqueous ethanol to give **3b** calcium salt, in pale yellow powder; mp 193—196 °C (decomp.); $\lambda_{max}^{H_2O}$ (pH 6.85) 254.5 nm; (pH 1.55) 258.5, 269 (sh); (pH 12.3)

Table 4. Paper chromatography and paper electrophoresis of nucleotides

Compound	R_f values		Electrophoretic mobility[a]
	Sovlent A	Solvent B	
Adenosine-5′ phosphate	0.07	0.16	1.00
Adenosine-2′(3′)phosphate	0.13		1.00
Adenosine-3′,5′ cyclic phosphate	0.35	0.31	0.66
Adenosine-2′,3′ cyclic phosphate	0.41		0.66
Adenosine-5′ 2-(*N*,*N*-dimethylamino)-4-nitrophenyl phosphate	0.62		0.49
Adenosine-2′,3′ cyclic phosphate-5′ 2-(*N*,*N*-dimethylamino)-4-nitrophenyl phosphate	0.50		0.83
P^1,P^2-Diadenosine-5′-pyrophosphate	0.10	0.07	0.79
Adenylyl-(5′→5′)-adenosine	0.18		0.51
Adenylyl-(3′→5′)-adenosine	0.14		0.51
Adenosine	0.52		0.09
5′-Monomethoxytrityladenosine	0.74		0
5′-Monomethoxytrityladenosine-2′,3′ cyclic phosphate	0.72		0.63
2′,3′-*O*-isopropylideneadenosine	0.88		0
2′,3′-*O*-isopropylideneadenosine-5′ 2-(*N*,*N*-dimethylamino)-4-nitrophenyl phosphate	0.81		0.48
Guanosine-5′ phosphate	0.03	0.20	1.00
Guanosine-2′(3′) phosphate	0.07		1.00
Guanosine-3′,5′ cyclic phosphate	0.25	0.26	0.68
Guanosine-2′,3′ cyclic phosphate	0.24		0.68
Guanosine-5′ 2-(*N*,*N*-dimethylamino)-4-nitrophenyl phosphate	0.52		0.42
Guanosine-2′,3′ cyclic phosphate-5′ 2-(*N*,*N*-dimethylamino)-dimethylamino)-4-nitrophenyl phosphate	0.27		0.70
Guanylyl-(5′→5′)-guanosine	0.04		0.53
Guanosine	0.28		0.15
Uridine-5′ phosphate	0.07	0.24	1.00
Uridine-2′(3′) phosphate	0.12		1.00
Uridine-3′,5′ cyclic phosphate	0.35	0.31	0.65
Uridine-2′,3′ cyclic phosphate	0.34		0.67
Uridine-5′ 2-(*N*,*N*-dimethylamino)-4-nitrophenyl phosphate	0.65		0.46
Uridine-2′,3′ cyclic phosphate-5′ 2-(*N*,*N*-dimethylamino)-4-nitrophenyl phosphate	0.44		0.72
Uridylyl-(5′→5′)-uridine	0.14		0.64
Uridine	0.49		0.17
Cytidine-5′ phosphate	0.09	0.08	1.00
Cytidine-2′(3′) phosphate	0.14	0.04	1.00
Cytidine-3′,5′ cyclic phosphate	0.38	0.32	0.63
Cytidine-2′,3′ cyclic phosphate	0.41		0.63
Cytidine-5′ 2-(*N*,*N*-dimethylamino)-4-nitrophenyl phosphate	0.64		0.44
Cytidine-2′,3′ cyclic phosphate-5′ 2-(*N*,*N*-dimethylamino)-4-nitrophenyl phosphate	0.52		0.71
Cytidylyl-(5′→5′)-cytidine	0.18		0.56
Cytidine	0.56		0.09
2-(*N*,*N*-Dimethylamino)-4-nitrophenol	0.80		0.70[b]
2-(*N*,*N*-Dimethylamino)-4-nitrophenyl phosphate	0.39		1.37[b]

a) The mobilities are relative to those of the parent nucleoside-5′ phosphate. b) The mobilities are relative to adenosine-5′ phosphate.

257; NMR (D_2O) 2.68 (s, 6, N-Me_2), 4.05—4.48 (m, 5, $C_2'H+C_3'H+C_4'H+C_5'H_2$), 5.74 (d, J=4.5 Hz, 1, $C_1'H$), 7.02—7.66 (m, 3, Ph), and 7.76 ppm (s, 1, C_8H).

*Uridine-5′ 2-(*N,N*-Dimethylamino)-4-nitrophenyl Phosphate (**3c**).* The procedure described for the preparation of guanosine-5′ 2-(*N*,*N*-dimethylamino)-4-nitrophenyl phosphate was applied. Uridine-5′ 2-(*N*,*N*-dimethylamino)-4-nitrophenyl phosphate (**3c**) calcium salt (11900 OD units, 49%) was obtained by crystallization from aqueous ethanol; mp> 260 °C; $\lambda_{max}^{H_2O}$ (pH 6.85) 258.5 nm; (pH 1.55) 268; (pH 12.3) 257; NMR (D_2O) 2.82 (s, 6, N-Me_2), 4.19—4.40 (m, 5, $C_2'H+C_3'H+C_4'H+C_5'H_2$), 5.70 (d, J=8.2 Hz, 1, C_5H), 5.88. (d, J=3 Hz, 1, $C_1'H$) and 7.35—7.93 ppm (m, 4, Ph+C_6H).

*Cytidine-5′ 2-(*N,N*-Dimethylamino)-4-nitrophenyl Phosphate (**3d**).* The procedure described for the preparation of guanosine-5′ 2-(*N*,*N*-dimethylamino)-4-nitrophenyl phosphate was applied. Cytidine-5′ 2-(*N*,*N*-dimethylamino)-4-nitrophenyl phosphate (**3d**) calcium salt (16300 OD units, 62%) was obtained by the crystallization from aqueous ethanol; mp>260 °C; $\lambda_{max}^{H_2O}$ (pH 6.85) 261 nm; (pH 1.55) 280; (pH 12.3) 253; NMR (D_2O) 2.87 (s, 6, N-Me_2), 4.14—4.35 (m, 5, $C_2'H+C_3'H+C_4'H+C_5'H_2$), 5.86 (d, J=7.5 Hz, 1, C_5H), 5.91 (d, J=3 Hz, 1, $C_1'H$), and 7.73—7.98 ppm (m, 4, Ph+C_6H).

Adenosine-3′,5′ Cyclic Phosphate (12a) from 3a. The triethylammonium salt of **3a** (0.2 mmol) was dissolved in pyridine (20 ml) containing acetic acid (12 mg, 0.2 mmol), and the solution was added dropwise to a boiling solution of pyridine (20 ml) over a 3-hr period. Heating was continued for further 3 hr and then the solution was concentrated to dryness. The residue was suspended in a mixture of water (10 ml) and ethyl acetate (20 ml). The aqueous layer was adjusted to pH 2 with 1M-hydrochloric acid. The solution was applied to a column of DEAE Sephadex (1.2×20 cm, HCO_3^-), which was eluted with a linear gradient of triethylammonium bicarbonate (4 l., 0.005—0.1 M) (fraction size, 20 g). Fractions containing **12a** (fractions 68—85) were concentrated to dryness. The residue was dissolved in 50% aqueous ethanol and adjusted to pH 2 with 10% hydrochloric acid. The precipitate was collected by filtration and washed with ethanol to give adenosine-3′,5′ cyclic phosphate monohydrate (43 mg, 61%), in white powder; $\lambda_{max}^{H_2O}$ (pH 2.0) 256 nm (ε 14500); (pH 7.0) 258 (14650); NMR [$D_2O+(NH_4)_2CO_3$] 4.23—5.25 (m, 5, $C_2'H+C_3'H+C_4'H+C_5'H_2$), 6.08 (s, 1, $C_1'H$), 8.09 and 8.15 ppm (2s, 2, C_2H and C_8H); ν_{max}^{nujol} 1690 (C=N), 1233 (P=O), 1202, 1081 cm^{-1} (P–O–C). This product was identified with an authentic specimen.

Guanosine-3′,5′ Cyclic Phosphate (12b) from 3b. The reaction was carried out as described for the preparation of **12a**. Guanosine-3′,5′ cyclic phosphate (1420 OD units, 55%) was isolated after the ion-exchange chromatography. This product was identified with an authentic specimen on PPC, PEP, and UV spectra; $\lambda_{max}^{H_2O}$ (pH 1.0) 256.5 nm (ε 11350); (pH 7.0) 254 (12950).

Adenosine-3′,5′ Cyclic Phosphate (12a) from Adenosine. Adenosine (2.7 g, 10 mmol) and the monotriethylammonium salt of **2** (5 mmol) were treated with DCC (2.05 g, 10 mmol) in DMF at room temperature for 24 hr. The reaction mixture was then concentrated to dryness. The residue was dissolved in pyridine (1 l) containing acetic acid (0.3 g, 5 mmol), and the insoluble dicyclohexylurea was removed by filtration. The solution was added dropwise to a boiling solution of pyridine (1 l) over a 3-hr period. Heating was continued for further 3 hr and then the solution was concentrated to dryness. The residue was suspended in a mixture of water (1 l) and ethyl acetate (500 ml). The aqueous layer was adjusted to pH 2 with 1M-hydrochloric acid. The solution was applied to a column of DEAE Sephadex (HCO_3^-, 1.5×60 cm). Elution was carried out using linear gradient with 0.005 M triethylammonium bicarbonate (2 l) in the mixing chamber and 0.1 M triethylammonium bicarbonate (2 l) in the reservoir. Fractions (20 g) were collected; adenosine-3′,5′ cyclic phosphate appeared in fractions 74—108. The combined fractions was concentrated to dryness and the solid residue was taken up in water (50 ml). The aqueous solution was evaporated to dryness to remove any residual triethylammonium bicarbonate. The residue was dissolved in 50% aqueous ethanol (10 ml) and adjusted to pH 2 with 1M-hydrochloric acid. The precipitates were filtered and washed with ethanol to give adenosine-3′,5′ cyclic phosphate (**12a**) monohydrate (1.11 g, 64%) as white powder. This product was identified with an authentic specimen.

Guanosine-3′,5′ Cyclic Phosphate (12b) from Guanosine. Guanosine (2.83 g, 10 mmol) and the monotriethylammonium salt of **2** (5 mmol) were treated with DCC (2.05 g, 10 mmol) in DMF as in the preparation of adenosine-3′,5′ cyclic phosphate. After usual work-up and ion-exchange chromatography, guanosine-3′,5′ cyclic phosphate (**12b**) (35400 OD units, 55%) was obtained in fractions 68 to 95. The product was homogeneous and identical with authentic guanosine-3′,5′ cyclic phosphate on PPC in Solvents A and B and on PEP.

Uridine-3′,5′ Cyclic Phosphate (12c) from Uridine. Uridine (2.44 g, 10 mmol) and the monotriethylammonium salt of **2** (5 mmol) were treated with DCC (2.05 g, 10 mmol) in DMF as in the preparation of adenosine-3′,5′ cyclic phosphate. After usual work-up and ion-exchange chromatography, uridine-3′,5′ cyclic phosphate (28600 OD units, 58%) was obtained in fractions 45 to 68. The nucleotide was homogeneous on PPC in Solvents A and B and on PEP.

Cytidine-3′,5′ Cyclic Phosphate (12d) from Cytidine. The procedure described for the preparation of adenosine-3′,5′ cyclic phosphate was applied. Cytidine-3′,5′ cyclic phosphate (**12d**) (14900 OD units, 32%) was obtained in fractions 45 to 72. The product was identified with an authentic specimen.

Adenosine-2′,3′ Cyclic Phosphate (11a) from Adenosine. Adenosine (5.4 g, 20mmol) and the monotriethylammonium salt of **2** (10 mmol) were treated with DCC (4.1 g, 20 mmol) in DMF (300 ml) at room temperature for 40 hr. To the reaction mixture, acetic acid 0.6 g (10 mmol) was added. The solution was added dropwise to a solution of DMF (30 ml) at 100 °C over a 3-hr period. Heating was continued for further 1 hr and the solution was concentrated to dryness. The residue was suspended in a mixture of water (1 l) and ethyl acetate (500 ml). The aqueous layer was applied to a column of DEAE Sephadex (HCO_3^-, 3.8×60 cm). Elution was carried out using linear gradient with 0.005 M triethylammonium bicarbonate (4 l) in the mixing chamber and 0.1 M triethylammonium bicarbonate (4 l) in the reservoir. The elution of nucleotides was followed spectrophotometrically at 260 nm. The fractions containing the major peak were combined and concentrated to dryness to give adenosine-2′,3′ cyclic phosphate (**11a**) monotriethylammonium salt (105140 OD units, 75.1%). This product was identified with an authentic specimen on PPC, PEP, and UV spectra.

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