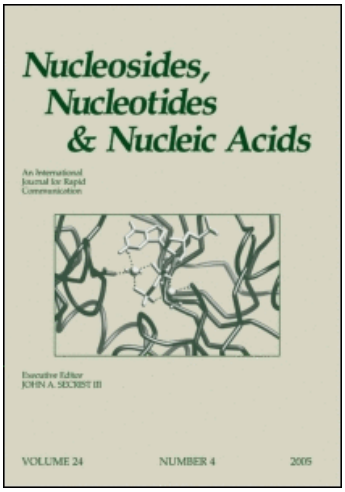

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A New Synthesis of Symmetrical P^1,P^2-Dinucleoside-5'-pyrophosphates

Kyung Bo Kim[a]; Edward J. Behrman[a]
[a] Department of Biochemistry, The Ohio State University, Columbus, Ohio, USA

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A NEW SYNTHESIS OF SYMMETRICAL P^1,P^2-DINUCLEOSIDE-5'-PYROPHOSPHATES

Kyung Bo Kim and Edward J. Behrman*
Department of Biochemistry
The Ohio State University
Columbus, Ohio 43210, USA

ABSTRACT: Treatment of nucleoside 5'-monophosphates with common acylating reagents gave moderate to good yields of the title compounds.

Symmetrical dinucleoside 5'-pyrophosphates were first synthesized more than forty years ago by Todd's group and by Khorana.[1,2] A number of improved routes have since been published.[3-5] We report here yet another approach of considerable convenience and reasonable yield, and it is worth noting that these compounds appeared as major products in experiments designed merely to derivatize the hydroxyl groups of the nucleotide starting materials.

We have synthesized the symmetrical 5'-dinucleoside pyrophosphates (Fig. 1) by reaction of TMP, CMP, UMP and AMP with a variety of common acylating reagents (Table 1). The nucleotides were converted to their tetrabutylammonium salts and then treated with the reagent in anhydrous DMF in the presence of pyridine. Products were purified by gel filtration on Sephadex G-10.

Characterization was carried out by nmr, electrospray mass spectroscopy, and elemental analysis. ^{31}P-nmr was particularly useful. The products showed a characteristic singlet around -11 ppm in contrast to the starting materials whose shift was about -3.5 ppm. Electrospray mass spectroscopy in the negative ion mode gave strong peaks for the molecular ions and can be recommended in addition to MALDI[6] for pyrophosphates.

Among the reagents examined (Table 1), tosyl chloride (p-toluene sulfonyl chloride) and p-nitrophenyl chloroformate gave the highest yields.

We briefly examined the reaction under the same conditions with fructose-1,6-diphosphate and p-nitrophenyl chloroformate. Purification of the product by paper electrophoresis gave a material with the correct molecular weight for the expected intramolecular pyrophosphate.

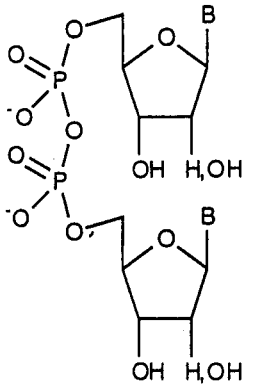

Figure.1

EXPERIMENTAL

TLC was performed on Silica Gel 60F254 (Aldrich) on aluminum plates with UV detection. Column chromatographies were performed on Dowex 50W (50X8-200, Sigma) and Sephadex G-10 (Pharmacia Fine Chemicals) using 18 X 1.8 cm and 44 X 3 cm columns, respectively. UV absorption of eluates was monitored by a Single Path Monitor UV-1 (at 280 nm) and a Linear 1200 (Pharmacia Fine Chemicals) recorder. Ion electrospray mass spectra were obtained using a PE SCIEX API 300 triple quadrupole mass spectrometer. Microanalyses were performed by Quantitative Technologies, Inc., Whitehouse, N.J.. ^{1}H NMR spectra were recorded at 500.1 MHz (Bruker AM-500). ^{13}C NMR spectra were recorded at 125.8 MHz (Bruker AM-500) and ^{31}P spectra at 121.5 MHz (Bruker MSL-300). All reagents were reagent grade and were used without further purification. Yields (Table 1) were calculated from the integrated absorbancies at 280 nm using the appropriate values for the molar extinction coefficients. Errors are estimated at ± 5%. The isolated yield for entry 3 is 81% in reasonable agreement with the yield (93%) prior to isolation.

P^1,P^2-Diuridine-5'-pyrophosphate

The bis(tetrabutylammonium) salt of UMP (109 mg, 0.14 mmoles) was dissolved in 5 ml of dry DMF. To this solution was added 0.5 ml of dry pyridine and <1 mg of 4-dimethylaminopyridine. Then, p-nitrophenyl chloroformate (70 mg, 0.35 mmoles, 2.5 equiv.) was added with stirring under nitrogen. The solution turned yellow and then colorless. Stirring was continued for 24 hr. at 23°C. Water (2 ml) was then added and the resulting precipitate removed by filtration. The soluble materials were separated on a Sephadex G-10 column. The first peak collected by elution with water was lyophilized to give 140 mg of a colorless solid. This tetrabutylammonium salt of the product was converted to the sodium salt on a Dowex 50 column in the sodium form. Lyophilization gave 85 mg (81%) of the disodium salt tetrahydrate.

^{1}H NMR (D_2O): δ 7.81 (d, H-6, J=8.1 Hz) (H-6 for UMP is at δ 8.01). ^{13}C NMR (D_2O, external dioxane reference): 166.99 (C-4), 152.62 (C-2), 142.51 (C-6), 103.55

Table 1 Reaction of reagents with nucleoside monophosphates

	REAGENT	PRODUCT	YIELD (%)	^{31}P NMR (δ)	M.S.
UMP	Benzoyl chloride	Diuridine pyrophosphate	75	-10.90 (s)	Disodium salt (M, 674.3): 673.2 (M-1), 651.0 (M-Na), 629.0 (M+1-2Na)
	Bis-(p-nitrophenyl)carbonate		80		
	p-Nitrophenyl chloroformate		93		
	Tosyl chloride		90		
CMP	Benzoyl chloride	Dicytidine pyrophosphate	50		Monosodium salt (M, 650.4): 650.6 (M), 673.1 (M+Na), 695.0 (M+2Na-H)
	Bis-(p-nitrophenyl)carbonate		45		
	p-Nitrophenyl chloroformate		50		
	Tosyl chloride		55		
TMP	Benzoyl chloride	Dithymidine pyrophosphate	75	-10.97 (s)	Free acid (M, 626.38): 649.0 (M+Na), 671.2 (M+Na-H), 693.0 (M+2Na-2H)
	Bis-(p-nitrophenyl) carbonate		80		
	p-Nitrophenyl chloroformate		90		
	Tosyl chloride		90		
AMP	Benzoyl chloride	Monobenzoylated diadenosine pyrophosphate	50		781.2, 803.2, 825.0
	Bis-(p-nitrophenyl) carbonate	Diadenosine pyrophosphate	40	-10.62 (s)	Free acid (M, 678.2): 677.2 (M-1), 699.2 (M-1+Na), 721 (M-2+2Na)
	p-Nitrophenyl chloroformate		60		
	Tosyl chloride	Monotosylated diadenosine pyrophosphate	55		831.4, 853.0, 875.0

Table 2 ^{1}H NMR (300 MHz) of the Pyrophosphates

UMP: δ 7.81 (d H-6, J=8.09 Hz) 5.86-5.83 (m, H-5,1') 4.25-4.07 (m, H-2', 3', 4', 5')

CMP: δ 8.02 (d, H-6, J=8.4 Hz) 6.09 (d, H-5) 5.77 (d, H-1', J=2.7 Hz) 4.20-4.13 (m, H-2', 3', 4') 4.07-3.88 (m, H-5')

TMP: δ 7.54 (s, H-6) 6.13 (t, H-1', J=6.75 Hz) 4.43 (m, H-3') 4.05-3.90 (m, H-4',5') 2.17 (t, H-2', J=5.55 Hz) 1.74 (s, -C$\underline{H}_3$)

AMP: δ 8.17 (br. s H-8) 8.02 (br. s H-2) 5.84-5.80 (m, H-1') 4.6-4.0 (m, H-2', 3', 4', 5')

solvent: D_2O
reference: HDO δ 4.65

(C-5), 89.26 (C-1'), 84.05 (C-4'), 74.67 (C-2'), 70.51 (C-3'), 65.80 (C-5'), ^{31}P NMR (D_2O, H_3PO_4 external reference) δ-10.90 (singlet). M.S. (negative ion electrospray, Calcd. for $C_{18}H_{22}N_4O_{17}P_2Na_2$=674.3): 673.2 (M-1), 651.0 (M-Na), 629.0 (M+1-2Na), 586.0 (M+1-2Na-CONH), 517.0 (M+1-2Na-uracil). Anal.: Calcd. for $C_{18}H_{20}O_{17}N_4P_2Na_2 \cdot 4H_2O$: C, 29.05; H, 3.79; N, 7.53. Found: C, 28.83; H, 3.55; N, 7.25. Reproductions of ^{1}H, ^{13}C, and ^{31}P nmr spectra and of a mass spectrum can be found in ref. 7.

Reactions with bis(p-nitrophenyl)carbonate were carried out under the same conditions. Reactions with benzoyl chloride and tosyl chloride did not require DMAP but otherwise used the same ratio of reactants. Reactions with the other nucleotides were run identically as those with UMP. The ms and ^{31}P nmr data are given in Table 1 and the ^{1}H nmr data in Table 2.

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