BROMINE DERIVATIVES OF RIBONUCLEOSIDE 3':5'-CYCLOPHOSPHATES

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UDC 547.853:547.857

Halogen-substituted purine and pyrimidine nucleotides and nucleosides are widely used for modifying the corresponding compounds. These compounds are used in the study of the mechanisms of enzymatic reactions [1], mutational processes [2], and antiviral chemotherapy [3], and in the performance of the reaction of reductive dehalogenation [4] for the introduction of a tritium label.

Halogen-substituted nucleoside 3':5'-cyclophosphates can be synthesized by the direct bromination of the corresponding compounds [5] or the cyclization of a 5'-phosphate group in a dilute solution of a nucleotide can be performed under the action of dicylcohexylcarbodiimide (DCC) [6, 7]. We have used a method proposed by Symons [6] for the synthesis of micro amounts of ^{32}P-labeled nucleoside 3':5'-cyclophosphates and have somewhat modified it for application to the synthesis of bromine-substituted nucleoside 3':5'-cyclophosphates, starting from the corresponding bromine-substituted ribonucleoside 5'-monophosphate. In view of the fact that 8-bromo-GMP is very sparingly soluble in organic solvents (especially DMSO and pyridine), for the cyclization of the 5'-phosphate group we took the corresponding N-benzoyl derivative, which was obtained by the method of Khorana et al. [8]. We used ion-exchange chromatography to isolate and purify the compounds obtained. The R_f values of the chromatographically pure compounds were determined, their UV absorption and circular dichroism (CD) spectra were recorded, and their coefficients of millimolar extinction ε were also found (Table 1).

TABLE 1. Chromatographic Mobilities of Brominated Nucleoside 3':5'-Cyclophosphates (R_f Values × 100)

Compound	Cellulose F (TLC) Solvent system				PEI-cellulose (TLC)	Whatman 3 mm
	1	2	3	4	0,75 M KH_2PO_4 pH 3,5	4
8-Bromo-A (3':5')>p	95	23	50	66	46	62
8-Bromo-G (3':5')>p	5	4	61	52	34	50
5-Bromo-U (3':5')>p	25	40	46	56	68	42
5-Bromo-C (3':5')>p	21	37	42	55	74	47

System: 1) butanol—acetic acid—water (4 : 1 : 5), 2) butanol—acetic acid—water (5 : 2 : 3), 3) ethanol—1M ammonium acetate pH 7.5 (5 : 2), 4) n-propanol—ammonia (concentrated)—water (55 : 20 : 25).

TABLE 2. UV Spectral Characteristics of the Brominated Nucleoside 3':5'-Cyclophosphates

Compound	Medium	λ_{max}, nm	$\varepsilon_{max} \times 10^{-3}$	λ_{min}, nm	Spectral ratios		
					250	280	290
8-Bromoadenosine 3':5'-cyclophosphate	0,01 N HCl	262	16,9	230	0,69	0,42	0,12
	H_2O	263	16,5	229	0,68	0,41	0,09
	0,01N NaOH	263	16,5	231	0,64	0,43	0,11
8-Bromoguanosine 3':5'-cyclophosphate	0,01 N HCl	263	15,3	226	0,78	0,79	0,55
	H_2O	263	15,3	227	0,77	0,80	0,56
	0,01 N NaOH	270	13,3	233	0,75	0,97	0,66
5-Bromouridine 3':5'-cyclophosphate	0,01 N HCl	280	8,0	244	0 50	1,88	1,46
	H_2O	267	8,0	235	0,70	1,54	1,26
	0,01N NaOH	277	5,7	252	0,86	1,50	0,96
5-Bromocytidine 3':5'-cyclophosphate	0,01 N HCl	296	10,0	257	1,09	2,61	3,45
	H_2O	288	7,5	263	1,23	1,59	1,70
	0,01 N NaOH	288	7,5	263	1,22	1,58	1,72

Institute of Molecular Genetics, Academy of Sciences of the USSR, Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 208-213, March-April, 1979. Original article submitted December 12, 1978.

 0009-3130/79/1502-0176$07.50

The UV absorption spectra were recorded on a Unicam SP 800 spectrophotometer (Table 2). The CD spectra were recorded on a Cary-60 spectropolarimeter with a 6001 attachment for measuring circular dichroism. The measurements were performed in a 1 cm cell at a temperature of 27°C and a recording time constant of 10 sec. The slit program was set at a constant resolution of 15 Å over the whole range of wavelengths (210-300 mm). The CD spectra of purine ribonucleoside 3':5'-cyclophosphates are shown in Fig. 1a, b and those of the pyrimidine analogs in Fig. 1c, d, and the values of the Cotton effects (CEs) are given in Table 3.

For the 8-bromo-substituted purine nucleoside 3':5'-cyclophosphates we recorded three CEs corresponding to $\pi-\pi^*$ transitions in the B_{2u}, B_{1u} (mainly for 8-bromo-G (3':5') > p), and E_{1ua} localized in the 265-285, 232-242, and 200-222 nm regions, respectively (Fig. 1). All the curves are characterized by positive CEs in the B_{2u} spectral region over a wide pH range and in methanol. From the facts given by Rogers and Ulbricht [9], it may be concluded that the compounds investigated have a conformation close to the syn conformation in solution.

In the CD spectra of the 5-bromine-substituted pyrimidine nucleoside 3':5'-cyclophosphates (Fig. 2) we recorded three CEs in each case corresponding to $\pi-\pi^*$ transitions in the B_{2u}, B_{1u}, and E_{1ua} bands (267-295, 236-246, and 210-230 nm bands, respectively). In the CD spectra of 5-bromo-C (3':5') > p the B_{1u} band was masked by the stronger B_{2u} and E_{1u} bands and was not recorded. All the spectra have a well-defined positive CE in the B_{2u} band and, according to information given by Rogers and Ulbricht [10], they correspond to the anti conformation over a wide pH range in methanol.

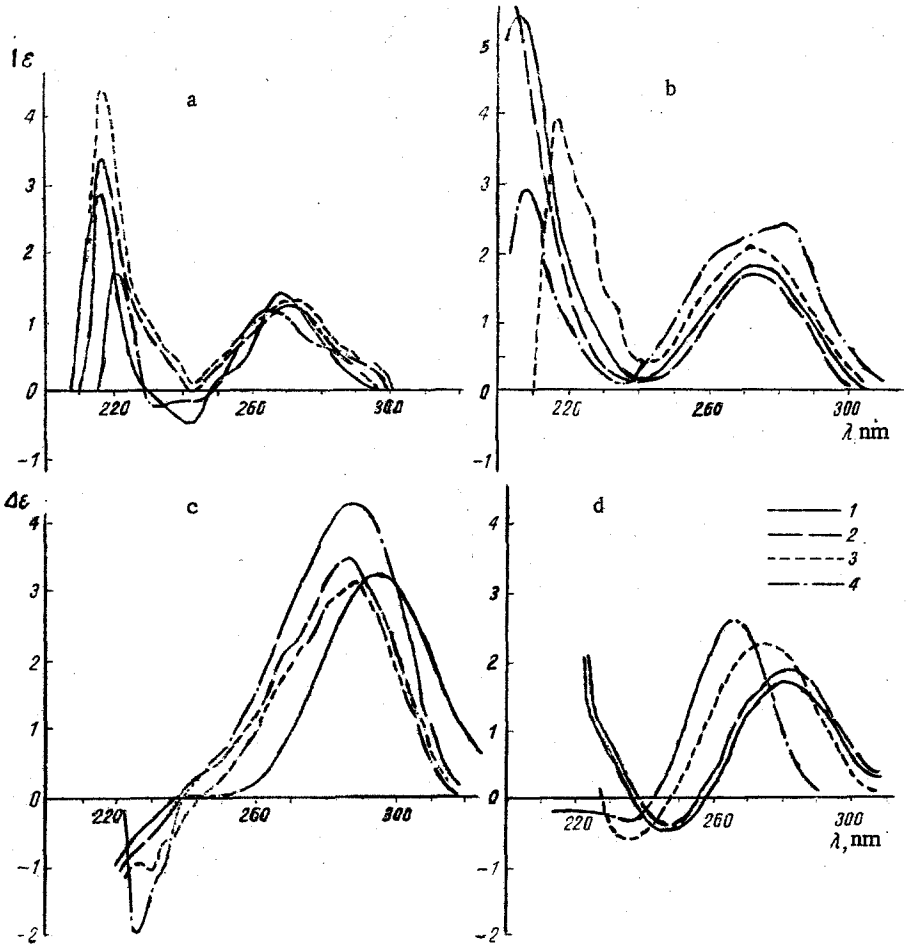

Fig. 1. CD spectra of 8-bromo-A (3':5') > p (a), 8-bromo-G (3': 5') > p (b), 5-bromo-C (3':5') > p (c), and 5-bromo-U (3': 5') > p (d): 1) pH 2; 2) pH 7; 3) pH 12; 4) methanol.

TABLE 3. Values of the Cotton Effects in the Circular Dichroism Spectra of Bromine-Substituted Ribonucleoside 3':5'-Cyclophosphates

Compound	Medium	Value of the CE		
		E_{1ua} λ, nm (Δε)	B_{1u} λ, nm (Δε)	B_{2u} λ, nm (Δε)
8-Bromoadenosine 3':5'-cyclophosphate	pH 2	217,5 (3.1)		270 (1.40)
	pH 7	217.5 (3.4)		272 (1,27)
	pH 12	218 (4,4)		272 (1,27)
	Methanol	222 (1,8)	245 (—0,15)	265 (1,2)
8-Bromoguanosine 3':5'-cyclophosphate	pH 2	200—210 (>4)	242 (0,20)	273 (1,75)
	pH 7	200—210 (>4)	243 (0,20)	273 (1,75)
	pH 12	217,5 (3,90)	245 (0,35)	272 (2,05)
	Methanol	209 (2,85)	232 (0,20)	282 (2,37)
5-Bromocytidine 3':5'-cyclophosphate	pH 2	210—220 (?)	Positive shoulder	295 (3,30)
	pH 7			
	pH 12	210—220 (?)	Positive shoulder	285 (3,45)
	Methanol	230 (—1,1)		287,5 (3,15)
		225 (—2,00)		287,5 (4,30)
5-Bromouridine 3':5'-cyclophosphate	pH 2	200—210 (?)	246 (—0,41)	280 (1,73)
	pH 7	200—210 (?)	246 (—0,44)	280 (1,87)
	pH 12	210—220 (?)	235 (—0,60)	278 (2,25)
	Methanol		236 (—0,35)	267 (2,60)

As can be seen from Table 3, the accuracy of the determination of the E_{1ua} CE is low in a number of cases. This is connected with the fairly high absorption of the compounds investigated in this region, and also with the fact that in this recording region the inherent noise of the instrument is comparable with the value of the CE.

EXPERIMENTAL

8-Bromoadenosine 3':5'-Cyclophosphate (8-Bromo-A (3':5') > p). A solution of 72.4 mg (0.17 mmole) of 8-bromo-AMP in the form of the free acid in 10 ml of water was lyophilized at 25°C. The dry residue was dissolved in 2 ml of DMSO, and then 20 ml of anhydrous pyridine and 3.4 ml (1.36 mmole) of a 0.4 M solution of DCC in pyridine were added and the mixture was boiled for 15 min. Then it was cooled and the pyridine was distilled off in vacuum in a rotary evaporator. After this, water (3 × 15 ml) was distilled off from the residue at a bath temperature of 40-45°C. Chromatographic purification led to 40.3 mg of 8-bromo-A (3':5') > p (yield 56%).

8-Bromoguanosine 3':5'-Cyclophosphate (8-Bromo-G (3':5') > p). The pyridine salt of N-benzoyl-8-bromo-GMP (0.24 mmole) was dissolved in 25 ml of dry pyridine containing N-cyclohexyl-N'-[2-(4-methylmorpholinio)-ethyl]carbodiimide p-toluenesulfonate ($C_{14}H_{26}N_3O \cdot C_7H_7O_3S$; 102 mg, 0.24 mmole). The solution obtained was poured dropwise over two hours into a boiling solution of DCC (206 mg, 1 mmole) in pyridine (30 ml). After the end of the addition, the mixture was boiled for another hour. Then it was evaporated to dryness in a rotary evaporator and the residue was extracted with water (3 × 10 ml). The extract was evaporated to dryness and the resinous mass so obtained was dissolved in 95% ethanol (7 ml) and concentrated ammonia (12 ml). The solution was transferred to a tube, which was sealed. The tube was heated in the boiling water bath for two hours. The reaction mixture was evaporated to dryness, and the residue was dissolved in 100 ml of water and extracted with ether. The desired product was isolated from the aqueous solution by column ion-exchange chromatography. This gave 51 mg of 8-bromo-G (3':5') > p (yield 50%) in the form of a noncrystalline mass.

5-Bromouridine 3':5'-Cyclophosphate (5-Bromo-U (3':5') > p). 5-Bromo-UMP (140 mg; 0.43 mmole) was lyophilized at 25°C and dissolved in 1 ml of DMSO, and then 40 ml of anhydrous pyridine and 9.5 ml (3.8 mmole) of a 0.05 M solution of DCC in pyridine were added and the mixture was boiled for 30 min. After the elimination of the pyridine, 44.5 mg of 5-bromo-U (3':5;) > p was isolated by chromatography (yield 30%).

5-Bromocytidine 3':5'-Cyclophosphate (5-Bromo-C (3':5')>p). A solution of 123 mg (0.31 mmole) of lyophilized 5-bromo-CMP in 1 ml of DMSO was treated with 40 ml of anhydrous pyridine and 8 ml (3.1 mmole) of 0.4 M DCC in pyridine. The reaction mixture was boiled for 30 min. After the pyridine had been driven off, chromatographic purification led to the isolation of 87 mg of 5-bromo-C (3':5') > p (yield 74%).

Ion-Exchange Chromatography. To separate the nucleoside material from the other components, the mixture after reaction was passed through a column of Dowex 1 × 8 in the formate form (25 × 1.2 cm). The column was washed with water (100 ml). We used a 1.0 N solution of formic acid for the elution of 8-bromo-A (3':5') > p, 0.4 N ammonium formate in 0.8 N formic acid for 8-bromo-G (3':5') > p, 0.5 N HCOOH for 5-bromo-C (3':5') > p, and 4 N HCOOH for 5-bromo-U (3':5') > p.

The eluates containing the nucleotides were concentrated and evaporated to dryness. The final purification was performed on a column of DEAE-cellulose in the bicarbonate form (30.0 ×1.2 cm). Elution was performed with a linear concentration gradient (0.5-0.2 M) of triethylammonium bicarbonate buffer (pH 8.6, 1000 ml). The fractions containing the compound synthesized were combined and evaporated to dryness. Residues of the triethylammonium bicarbonate buffer were eliminated by repeated (7-10 times) distillation with water to dryness (5-ml portions).

Paper Chromatography and Thin-Layer Chromatography. We used ascending chromatography on plates of cellulose F (Merk, layer thickness 0.1 mm) ascending chromatography on PEI-cellulose (Merk, layer thickness 0.1 mm), and descending chromatography on Whatman 3 MM paper. The results are given in Table 1.

SUMMARY

Bromine-substituted purine and pyrimidine ribonucleoside 3':5'-cyclophosphates have been synthesized. R_f values and coefficients of millimolar extinction have been determined, and UV absorption and circular dichroism spectra have been recorded. It has been concluded that 8-bromine-substituted purine ribonucleoside 3':5'-cyclophosphates have in solution a conformation close to the syn form, and 5-bromine-substituted pyrimidine ribonucleoside 3':5'-cyclophosphates have the anti conformation over a wide pH range and in methanol.

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