Crystal and Molecular Structures of Carbon-Bridged Pyrimidine Cyclonucleosides. Substrate Analogs of Ribonuclease A[1)]

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The crystal and molecular structures of carbon-bridged 6,5′-cyclo-5′-deoxy-4-thiouridine (6,5′-Cs^{4}U), 6,5′-cyclo-5′-deoxy-2′,3′-*O*-isopropylideneuridine (6,5′-CiU) and 6,6′-cyclo-5′,6′-dideoxy-allofuranosyluracil (6,6′-CU) have been determined by X-ray diffraction. The molecular conformations of 6,5′-Cs^{4}U and 6,5′-CiU are very similar; the conformation about the glycosidic bond is *anti* (*low* region), the torsion angle O(4′)–C(1′)–N(1)–C(2) being −150.0° for 6,5′-Cs^{4}U and −145.5° for 6,5′-CiU, and the sugar puckering being both O(4′)-*exo*. On the other hand, 6,6′-CU takes the glycosidic torsion angle of −116.9(4)° (*middle anti* region) and the sugar conformation of C(4′)-*endo*. The cyclization causes little alteration in the geometry of the base moiety. 6,5′-Cs^{4}U and 6,5′-CiU exhibit the similar base–base interactions between adjacent molecules, although their molecular packings are quite different; the 4-thiouracil or uracil moiety interacts with adjacent base moieties through hydrogen bonding and stacking interactions. In 6,6′-CU, cyclonucleosides were connected by hydrogen bondings between the hydroxyl and sugar ring oxygen atoms and between the hydroxyl groups and the base nitrogen and oxygen atoms. As the 2′,3′-cyclic phosphates of these carbon-bridged cyclonucleosides are hydrolyzed by ribonuclease A, it is suggested that the conformers found in these cyclonucleosides are recognized by the enzyme.

Keywords carbon-bridged cyclonucleoside; nucleoside conformation; X-ray analysis; enzyme recognition model

In general, nucleosides take a flexible conformation about the glycosidic and exocyclic bonds and sugar puckering. On the other hand, cyclonucleosides with covalent bonds connecting the base and sugar are conformationally rigid, in particular about the glycosidic bond, and serve as useful model compounds for interpretation of muclear magnetic resonance (NMR) and circular dichroism (CD) spectroscopic data on conformationally flexible nucleosides.[2,3)] From the crystal structure determinations of several β-pyrimidine cyclonucleosides,[4–8)] interesting information about structural characteristics related to the cyclization sites has been obtained. In the present work, the crystal and molecular structures of carbon-bridged cyclonucleosides cyclized directly or through a carbon atom between the C(6) and C(5′) atoms were determined by X-ray diffraction analysis. It is known that the 2′,3′-cyclic phosphates of these carbon-bridged cyclonucleosides are hydrolyzed by ribonuclease A, although the rates of hydrolysis were slower than those of the natural substrates, uridine 2′,3′-cyclic phosphate and 4-thiouridine 2′,3′-cyclic phosphate.[9)] Therefore, these X-ray results might be useful for understanding the recognition and the hydrolysis of pyrimidine nucleoside 2′,3′-cyclic phosphates by the enzyme.

Experimental

Three cyclonucleosides were synthesized as described in the previous papers.[9)] The crystals were obtained by slow evaporation from aqueous solution for 6,5′-Cs^{4}U and 6,5′-CiU and from aqueous methanol solution for 6,6′-CU. Crystal data, experimental conditions and refinement procedures are summarized in Table I. The crystal of 6,5′-CiU was isomorphous to the already solved structure of 6(*R*),5′-cyclo-5′-deoxy-2′,3′-*O*-isopropylidene-5,6-dihydrouridine (6,5′-CihU)[2)] with unit-cell dimensions $a=11.220$ (2), $b=6.393$ (1), $c=18.963$ (3) Å and $\beta=107.98$ (1)° and space group $C2$, and therefore the solution of the structure was obtained by application of the isomorphous method. The structures of 6,5′-Cs^{4}U and 6,6′-CU were solved by direct methods using the program MULTAN 78.[10)] Each structure was refined by a full-matrix least-squares procedure with anisotropic temperature factors for non-hydrogen atoms. All the hydrogen atoms in the three cyclonucleosides, except those attached to O(2′) and O(3′) of 6,5′-Cs^{4}U, were located from difference Fourier maps. The final positional and thermal parameters for all atoms of the three cyclonucleosides are listed in Tables II to IV. All numerical calculations

TABLE I. Crystal Data, Experimental Conditions and Refinement Details

	6,5′-Cs^{4}U	6,5′-CiU	6,6′-CU
Chemical formula	$C_9H_{10}N_2O_4S$	$C_{12}H_{14}N_2O_5$	$C_{10}H_{12}N_2O_5$
M_r	242.2	266.3	240.2
Cell parameters			
a (Å)	6.759 (1)	11.388 (1)	7.159 (1)
b (Å)		6.252 (1)	8.244 (1)
c (Å)	38.430 (4)	18.523 (2)	9.342 (2)
β (°)		107.11 (1)	112.18 (2)
V (Å)	1520.2 (3)	1260.4 (2)	510.6 (1)
Space group	$P3_221$	$C2$	$P2_1$
Z	6	4	2
$F(000)$	756	560	252
μ (CuK_α) (mm^{-1})	2.84	0.89	1.04
D_x (Mg m^{-3})	1.588	1.403	1.562
Crystal color	Light yellow	Colorless	Colorless
Shape	Plate	Plate	Prism
Size (mm)	0.5×0.2×0.05	0.6×0.2×0.07	0.35×0.3×0.25
Diffractometer	Rigaku AFC-5		
Radiation	Graphite-monochromated CuK_α		
Scan mode	ω–2θ scan		
Number of reflections	25	20	20
2θ range (°) used for measuring lattice parameters	$23°<2\theta<30°$	$30°<2\theta<44°$	$30°<2\theta<41°$
Max. $\sin\theta/\lambda$ (Å^{-1})	0.58	0.58	0.58
Number of unique reflections	1039	1119	891
Number of observed reflections	808	997	868
Criterion for observed reflections	$F_o>2\sigma(F_o)$	$F_o>\sigma(F_o)$	$F_o>\sigma(F_o)$
Atomic scattering factors	International Tables for X-Ray Crystallography (1974)		
R (wR)	0.078 (0.080)	0.065 (0.058)	0.042 (0.054)
S	3.88	1.34	1.28
Weighting scheme	1.0	1.0	$[\sigma^2(F_o)+0.014F_o]^{-1}$
Max. Δ/σ	0.15	0.25	0.18

TABLE II. Atomic Coordinates and Isotropic Thermal Parameters (Å^2) for 6,5′-Cs^{4}U

Atom	x	y	z	B_{eq}/B_{iso}
S(4)	0.5868 (5)	0.3682 (5)	0.2089 (1)	3.5 (1)
O(2)	0.9025 (15)	0.9777 (12)	0.1215 (1)	3.8 (3)
O(4′)	0.5480 (17)	0.5698 (18)	0.0432 (2)	4.9 (4)
O(2′)	1.0403 (17)	0.8593 (18)	0.0217 (2)	5.2 (4)
O(3′)	0.8645 (24)	0.4319 (23)	−0.0005 (2)	7.8 (6)
N(1)	0.6904 (15)	0.6168 (15)	0.1000 (2)	2.5 (3)
N(3)	0.7399 (15)	0.6791 (15)	0.1597 (2)	2.5 (3)
C(2)	0.7881 (18)	0.7717 (19)	0.1271 (2)	2.6 (4)
C(4)	0.6215 (18)	0.4475 (16)	0.1674 (2)	2.3 (4)
C(5)	0.5337 (19)	0.2990 (20)	0.1386 (2)	3.1 (4)
C(6)	0.5687 (20)	0.3806 (19)	0.1061 (2)	3.2 (4)
C(1′)	0.7407 (24)	0.7153 (25)	0.0645 (2)	3.6 (5)
C(2′)	0.9329 (27)	0.6951 (30)	0.0488 (3)	4.8 (7)
C(3′)	0.8114 (37)	0.4412 (35)	0.0351 (3)	6.4 (9)
C(4′)	0.5588 (32)	0.3581 (28)	0.0402 (3)	5.5 (7)
C(5′)	0.4711 (27)	0.2319 (25)	0.0745 (3)	5.1 (5)
H(3)	0.800 (24)	0.781 (23)	0.178 (3)	3.5
H(5)	0.458 (23)	0.121 (24)	0.143 (3)	3.5
H(1′)	0.761 (24)	0.882 (23)	0.065 (3)	3.5
H(2′)	1.047 (25)	0.705 (26)	0.065 (3)	3.5
H(3′)	0.886 (26)	0.373 (26)	0.048 (3)	3.5
H(4′)	0.457 (22)	0.274 (23)	0.012 (3)	3.5
H(5′a)	0.302 (24)	0.161 (25)	0.075 (3)	3.5
H(5′b)	0.472 (23)	0.078 (24)	0.080 (3)	3.5

TABLE III. Atomic Coordinates and Isotropic Thermal Parameters (Å^2) for 6,5′-CiU

Atom	x	y	z	B_{eq}/B_{iso}
O(2)	0.9365 (3)	0.2474 (0)	−0.0967 (2)	4.4 (1)
O(4)	0.7195 (4)	0.2530 (11)	0.0729 (2)	4.2 (1)
O(4′)	0.6424 (4)	0.3894 (10)	−0.2640 (3)	5.2 (2)
O(2′)	0.7575 (4)	0.0036 (13)	−0.3120 (2)	6.8 (2)
O(3′)	0.5542 (5)	0.0398 (15)	−0.3692 (3)	7.8 (2)
N(1)	0.7300 (4)	0.2638 (11)	−0.1410 (2)	3.1 (1)
N(3)	0.8261 (4)	0.2557 (12)	−0.0132 (2)	3.3 (1)
C(2)	0.8374 (5)	0.2510 (14)	−0.0844 (3)	3.4 (2)
C(4)	0.7172 (5)	0.2532 (14)	0.0064 (3)	3.2 (1)
C(5)	0.6094 (5)	0.2572 (15)	−0.0571 (3)	3.7 (2)
C(6)	0.6174 (5)	0.2637 (14)	−0.1277 (3)	3.4 (2)
C(1′)	0.7366 (5)	0.2536 (17)	−0.2188 (3)	4.1 (2)
C(2′)	0.7057 (5)	0.0348 (15)	−0.2515 (3)	4.5 (2)
C(3′)	0.5638 (6)	0.0441 (17)	−0.2909 (4)	5.2 (2)
C(4′)	0.5318 (5)	0.2687 (19)	−0.2707 (3)	5.2 (2)
C(7′)	0.6637 (7)	−0.0319 (17)	−0.3814 (3)	5.7 (3)
C(5′)	0.5050 (6)	0.2861 (18)	−0.1956 (3)	5.3 (2)
C(8′)	0.6912 (9)	0.1015 (18)	−0.4423 (5)	7.7 (4)
C(9′)	0.6585 (9)	−0.2663 (24)	−0.4007 (5)	8.4 (4)
H(3)	0.909 (6)	0.258 (2)	0.032 (4)	4.3
H(5)	0.525 (6)	0.253 (2)	−0.049 (4)	4.3
H(1′)	0.828 (6)	0.304 (1)	−0.216 (4)	4.3
H(2′)	0.745 (6)	−0.093 (1)	−0.206 (4)	4.3
H(3′)	0.502 (6)	−0.076 (1)	−0.276 (4)	4.3
H(4′)	0.450 (6)	0.326 (1)	−0.313 (4)	4.3
H(5′a)	0.440 (6)	0.167 (1)	−0.193 (4)	4.3
H(5′b)	0.461 (6)	0.436 (1)	−0.193 (4)	4.3
H(8′a)	0.788 (6)	0.063 (1)	−0.444 (4)	4.3
H(8′b)	0.700 (6)	0.253 (2)	−0.424 (4)	4.3
H(8′c)	0.629 (6)	0.091 (1)	−0.495 (4)	4.3
H(9′a)	0.588 (6)	−0.297 (1)	−0.457 (4)	4.3
H(9′b)	0.648 (6)	−0.355 (1)	−0.360 (4)	4.3
H(9′c)	0.753 (6)	−0.316 (1)	−0.409 (4)	4.3

were carried out on a ACOS 850 computer at the Crystallographic Research Center, Institute for Protein Research, Osaka University, using the programs of the Universal Crystallographic Computing System-Osaka (1979)[11] and ORFLS.[12]

TABLE IV. Atomic Coordinates and Isotropic Thermal Parameters (Å^2) for 6,6′-CU

Atom	x	y	z	B_{eq}/B_{iso}
N(1)	0.7796 (4)	0.7824 (4)	1.0408 (3)	2.1 (1)
C(2)	0.8701 (6)	0.7356 (7)	0.9397 (5)	3.3 (1)
N(3)	0.7451 (5)	0.7409 (7)	0.7849 (4)	3.9 (1)
C(4)	0.5459 (6)	0.7845 (6)	0.7229 (4)	3.0 (1)
C(5)	0.4651 (6)	0.8268 (6)	0.8342 (5)	2.6 (1)
C(6)	0.5785 (5)	0.8289 (5)	0.9880 (4)	2.2 (1)
O(2)	1.0416 (4)	0.6887 (7)	0.9793 (4)	6.0 (1)
O(4)	0.4525 (4)	0.7794 (6)	0.5816 (3)	4.4 (1)
C(1′)	0.9087 (5)	0.7681 (5)	1.2068 (4)	2.1 (1)
C(2′)	0.9378 (5)	0.9279 (5)	1.2991 (4)	2.2 (1)
C(3′)	0.8281 (6)	0.8971 (5)	1.4124 (4)	2.4 (1)
C(4′)	0.7099 (6)	0.7430 (5)	1.3505 (4)	2.4 (1)
C(5′)	0.4989 (6)	0.7737 (6)	1.2319 (4)	2.6 (1)
C(6′)	0.4890 (5)	0.8872 (6)	1.1001 (4)	2.5 (1)
O(4′)	0.8282 (4)	0.6554 (0)	1.2805 (3)	2.4 (1)
O(2′)	1.1446 (4)	0.9665 (4)	1.3740 (3)	2.8 (1)
O(3′)	0.9699 (4)	0.8651 (4)	1.5652 (3)	3.0 (1)
H(3)	0.809 (7)	0.703 (7)	0.720 (6)	2.1 (11)
H(5)	0.317 (7)	0.854 (8)	0.799 (5)	1.9 (10)
H(1′)	1.039 (7)	0.728 (7)	1.203 (5)	2.0 (11)
H(2′)	0.892 (8)	1.041 (8)	1.256 (6)	2.8 (13)
H(3′)	0.731 (7)	0.988 (7)	1.415 (5)	1.7 (10)
H(4′)	0.700 (7)	0.691 (7)	1.441 (6)	2.5 (12)
H(5′a)	0.414 (7)	0.820 (7)	1.289 (5)	1.8 (10)
H(5′b)	0.435 (7)	0.664 (7)	1.190 (5)	2.4 (12)
H(6′a)	0.567 (7)	0.996 (7)	1.150 (6)	2.4 (12)
H(6′b)	0.333 (7)	0.912 (7)	1.036 (5)	1.7 (10)
H(O2′)	1.214 (7)	0.896 (7)	1.447 (5)	2.0 (11)
H(O3′)	1.029 (7)	0.965 (7)	1.616 (5)	2.1 (11)

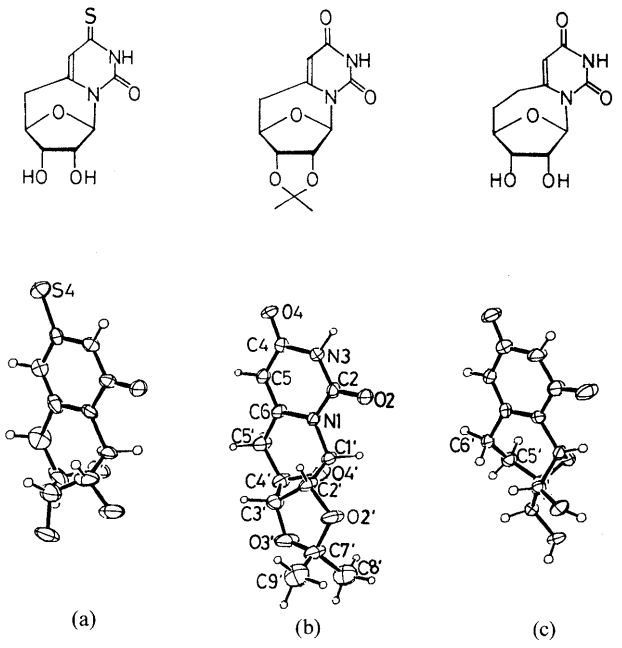

Fig. 1. Molecular Conformations of Carbon-Bridged Pyrimidine Cyclonucleosides: (a) 6,5′-Cs^{4}U, (b) 6,5′-CiU and (c) 6,6′-CU

Results and Discussion

Molecular Conformation and Dimensions ORTEP[13] drawings showing the overall molecular conformations of 6,5′-Cs^{4}U, 6,5′-CiU and 6,6′-CU are given in Fig. 1. The relevant torsion angles are listed in Table V.

TABLE V. Selected Torsion Angles (°)

		6,5′-Cs⁴U	6,5′-CiU	6,6′-CU
χ	C(2)–N(1)–C(1′)–O(4′)	−150.0 (10)	−145.5 (6)	−116.9 (4)
	C(6)–N(1)–C(1′)–O(4′)	35.4 (15)	41.1 (8)	59.1 (5)
τ_0	C(4′)–O(4′)–C(1′)–C(2′)	46.9 (14)	44.8 (7)	29.9 (4)
τ_1	O(4′)–C(1′)–C(2′)–C(3′)	−32.9 (15)	−24.7 (7)	−9.8 (4)
τ_2	C(1′)–C(2′)–C(3′)–C(4′)	7.6 (17)	−3.2 (7)	−12.3 (4)
τ_3	C(2′)–C(3′)–C(4′)–O(4′)	19.5 (17)	29.7 (7)	29.9 (4)
τ_4	C(3′)–C(4′)–O(4′)–C(1′)	−41.4 (15)	−46.7 (7)	−38.4 (4)

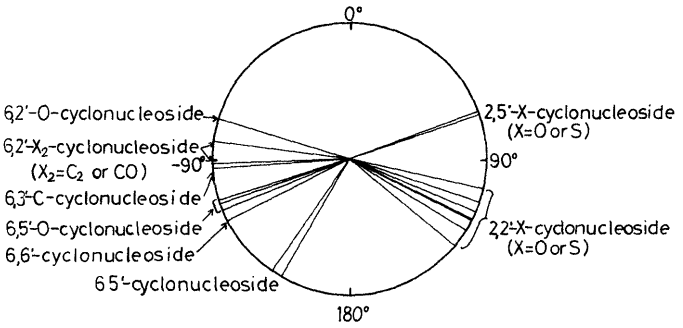

Fig. 2. Glycosidic Torsion Angles (°) for Pyrimidine Cyclonucleosides[4-8]

Values were calculated using crystallographic data obtained from the Cambridge Crystallographic Database.

The molecular conformations of 6,5′-Cs⁴U and 6,5′-CiU are very similar, irrespective of whether the ribofuranose group is cyclized at the O(2′) and O(3′) atoms or not. On the other hand, 6,6′-CU takes a somewhat different conformation from them, because of the difference of the sugar–base cyclization system. The glycosidic torsion angle [χ: O(4′)–C(1′)–N(1)–C(2)] is −150.0° for 6,5′-Cs⁴U and −145.5° for 6,5′-CiU, and both are in the somewhat *low anti* region, while 6,6′-CU is in the typical *middle anti* conformation with the torsion angle of −116.9°. The X-ray data on pyrimidine cyclonucleosides revealed the relation of the glycosidic torsion angle with the cyclization site between base and sugar, as shown in Fig. 2. It seems that the range of the glycosidic torsion angles in 2,2′-cyclonucleosides with the same cyclization site is about 30° and is unexpectedly wide.

The six-membered fused ring formed by the cyclization in 6,5′-Cs⁴U or 6,5′-CiU assumes a half-chair conformation with the O(4′) atom being displaced by 0.63 (2) Å for 6,5′-Cs⁴U or 0.634 (9) Å for 6,5′-CiU from the plane through the remaining five atoms. The newly formed seven-membered ring of 6,6′-CU is a chair form. Model building studies suggested that cyclonucleosides of the type C(5′)–X–C(base) (X=C, O or S), such as 6,6′-CU, may adopt either of two conformations: the boat form with atom X positioned above sugar O(4′), or the chair form with atom X over the center of the sugar ring. As the boat form would lead to stereochemically unfavorable short contacts between atoms O(4′) and X, only the chair form is observed in the five cyclonucleosides reported.

The sugar ring takes an unusual O(4′)-*exo* (6,5′-Cs⁴U and 6,5′-CiU) or C(4′)-*endo* (6,6′-CU) conformation. The pseudorotational parameters are $P=279°$ and $\tau_m=46°$ for 6,5′-Cs⁴U, $P=266°$ and $\tau_m=48°$ for 6,5′-CiU and $P=251°$ and $\tau_m=37°$ for 6,6′-CU. The higher τ_m values of 6,5′-Cs⁴U and 6,5′-CiU than the mean value (38.7°)[14] indicate that the sugar ring puckers more steeply. This remarkable sugar conformation is also found in the crystal structures of 6,5′-

TABLE VI. Bond Distances (Å) for Non-hydrogen Atoms

	6,5′-Cs⁴U	6,5′-CiU	6,6′-CU
N(1)–C(2)	1.388 (14)	1.358 (8)	1.386 (8)
C(2)–N(3)	1.366 (14)	1.363 (9)	1.384 (6)
N(3)–C(4)	1.388 (14)	1.392 (9)	1.369 (6)
C(4)–C)5)	1.413 (15)	1.429 (10)	1.412 (6)
C(5)–C(6)	1.336 (16)	1.339 (10)	1.358 (6)
C(6)–N(1)	1.402 (15)	1.375 (8)	1.388 (5)
C(2)–O(2)	1.228 (13)	1.215 (7)	1.205 (6)
C(4)–S(4)[O(4)]	1.660 (11)	1.223 (8)	1.235 (6)
N(1)–C(1′)	1.482 (16)	1.468 (9)	1.481 (5)
C(1′)–C(2′)	1.499 (21)	1.495 (10)	1.545 (6)
C(2′)–C(3′)	1.577 (26)	1.566 (11)	1.558 (6)
C(3′)–C(4′)	1.520 (26)	1.525 (11)	1.515 (6)
C(4′)–O(4′)	1.473 (20)	1.442 (10)	1.444 (4)
O(4′)–C(1′)	1.432 (17)	1.430 (9)	1.403 (4)
C(2′)–O(2′)	1.426 (20)	1.426 (9)	1.414 (5)
C(3′)–O(3′)	1.424 (23)	1.423 (10)	1.430 (5)
C(4′)–C(5′)	1.518 (23)	1.513 (11)	1.519 (6)
C(5′)[C(6′)]–C(6)	1.504 (19)	1.514 (10)	1.498 (6)
C(5′)–C(6′)	—	—	1.527 (6)
O(2′)–C(7′)	—	1.426 (10)	—
O(3′)–C(7′)	—	1.405 (11)	—
C(7′)–C(8′)	—	1.508 (13)	—
C(7′)–C(9′)	—	1.506 (14)	—

TABLE VII. Bond Angles (°) for Non-hydrogen Atoms

	6,5′-Cs⁴U	6,5′-CiU	6,6′-CU
C(6)–N(1)–C(2)	121.3 (9)	122.6 (5)	121.6 (3)
N(1)–C(2)–N(3)	115.4 (9)	115.2 (6)	114.7 (4)
C(2)–N(3)–C(4)	125.7 (9)	126.7 (6)	127.6 (4)
N(3)–C(4)–C(5)	115.7 (9)	113.6 (6)	113.8 (4)
C(4)–C(5)–C(6)	121.0 (10)	121.1 (6)	122.4 (4)
C(5)–C(6)–N(1)	120.5 (10)	120.6 (6)	119.9 (4)
N(1)–C(2)–O(2)	121.0 (10)	122.1 (6)	124.3 (4)
N(3)–C(2)–O(2)	123.6 (10)	122.6 (6)	120.9 (5)
N(3)–C(4)–S(4)[O(4)]	118.5 (8)	120.4 (6)	120.1 (4)
C(5)–C(4)–S(4)[O(4)]	125.7 (8)	125.9 (6)	126.1 (4)
C(5)–C(6)–C(5′)[C(6′)]	123.4 (11)	122.0 (6)	120.0 (3)
N(1)–C(6)–C(5′)[C(6′)]	115.9 (10)	117.2 (6)	120.1 (4)
C(6)–N(1)–C(1′)	122.5 (10)	119.7 (5)	123.2 (3)
C(2)–N(1)–C(1′)	115.9 (9)	117.4 (5)	115.1 (3)
N(1)–C(1′)–O(1′)	106.9 (10)	107.6 (5)	110.9 (3)
O(4′)–C(1′)–C(2′)	103.9 (11)	105.1 (6)	106.5 (3)
N(1)–C(1′)–C(2′)	110.2 (11)	111.3 (6)	114.6 (3)
C(1′)–C(2′)–C(3′)	103.3 (13)	104.0 (6)	104.1 (3)
C(1′)–C(2′)–O(2′)	110.5 (13)	109.9 (6)	111.3 (3)
C(3′)–C(2′)–O(2′)	112.9 (14)	104.3 (6)	113.7 (3)
C(2′)–C(3′)–C(4′)	103.7 (15)	102.1 (6)	103.3 (3)
C(2′)–C(3′)–O(3′)	111.3 (15)	103.5 (6)	111.0 (3)
C(4′)–C(3′)–O(3′)	111.7 (15)	108.4 (6)	108.0 (3)
C(3′)–C(4′)–O(4′)	104.1 (14)	103.7 (6)	104.7 (3)
C(5′)–C(4′)–O(4′)	106.4 (13)	106.9 (6)	110.2 (3)
C(3′)–C(4′)–C(5′)	116.8 (14)	114.8 (7)	113.4 (3)
C(1′)–O(4′)–C(4′)	104.0 (11)	103.0 (5)	108.1 (3)
C(4′)–C(5′)–C(6)[C(6′)]	114.1 (13)	114.0 (6)	114.9 (3)
C(5′)–C(6′)–C(6)	—	—	117.7 (3)
C(2′)–O(2′)–C(7′)	—	110.9 (6)	—
C(3′)–O(3′)–C(7′)	—	111.3 (6)	—
O(2′)–C(7′)–O(3′)	—	105.7 (6)	—
O(2′)–C(7′)–C(8′)	—	108.8 (7)	—
O(2′)–C(7′)–C(9′)	—	109.4 (7)	—
O(3′)–C(7′)–C(8′)	—	109.5 (7)	—
O(3′)–C(7′)–C(9′)	—	111.9 (8)	—
C(8′)–C(7′)–C(9′)	—	111.3 (8)	—

CihU ($P=267°$, $\tau_m=47°$)[2] and 8,5′-cycloadenosine (8,5′-CA; $P=289°$, $\tau_m=48°$).[15]

The bond distances and angles are presented in Tables VI and VII, respectively. Corresponding bond distances and angles in the base moieties of the cyclonucleosides generally agree within 3σ. The cyclization between C(6) and C(5′) causes little alteration in the bond distances or angles of the 4-thiouracil or uracil moiety. The X-ray structures of other 6-cyclonucleosides with C(6) in the base connecting to the sugar moiety also indicate that little influence of cyclization at the C(6) position on the bond distances and angles is observed in the pyrimidine base moiety.[6-8] In the sugar moiety, the bond angles between the three cyclonucleosides are significantly different. For example, C(3′)–C(2′)–O(2′) and C(2′)–C(3′)–O(3′) are smaller for 6,5′-CiU than 6,5′-Cs^{4}U and 6,6′-CU, because of an extra ring cyclization at O(2′) and O(3′) in 6,5′-CiU. The C(1′)–O(4′)–C(4′) bond angles of 104.0° for 6,5′-Cs^{4}U and 103.0° for 6,5′-CiU are smaller than those of 6,6′-CU (108.2°) and normal nucleosides (109.6°),[16] and this is probably due to the O(4′)-*exo* conformation with high values of τ_m, as pointed out by Westhof and Sundaralingam.[14]

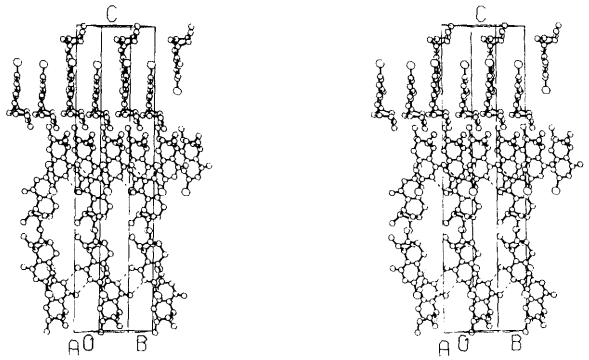

Fig. 3. Stereoscopic Drawing of the Crystal Structure for 6,5′-Cs^{4}U
Dashed lines indicate the hydrogen bonds.

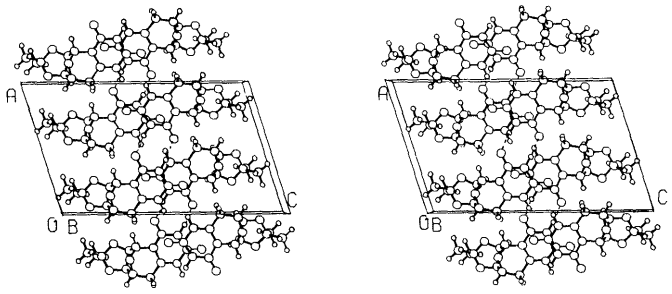

Fig. 4. Stereoscopic Drawing of the Crystal Structure for 6,5′-CiU along the *b* Axis
Dashed lines indicate the hydrogen bonds.

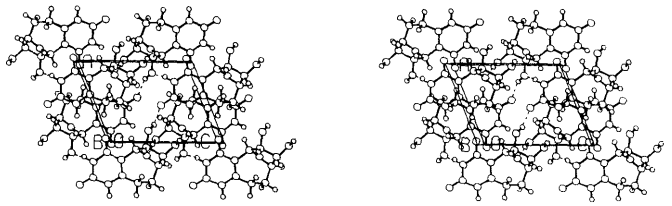

Fig. 5. Stereoscopic Drawing of the Crystal Structure for 6,6′-CU along the *b* Axis
Dashed lines indicate the hydrogen bonds.

Hydrogen Bonding and Molecular Packing The molecular packings in the crystals of the three nucleosides are shown in Figs. 3 to 5.

Although the crystals of 6,5′-Cs^{4}U and 6,5′-CiU are different in the crystal system and space group, the intermolecular base–base interactions are very similar to each other. The 4-thiouracil and uracil moieties in 6,5′-Cs^{4}U and 6,5′-CiU commonly form dimers with two hydrogen bonds between adjacent molecules related by 2-fold rotational symmetry [N(3)–H···O(2); 2.838 (12) Å for 6,5′-Cs^{4}U and 2.867 (6) Å for 6,5′-CiU]. Furthermore, stacking interactions occur between the bases related by the 2-fold screw axis. In both cases, the major overlappings are found between the polar substituents [C(4)–S(4) and C(4)–O(4) groups for 6,5′-Cs^{4}U and 6,5′-CiU, respectively] and pyrimidine rings, as shown in Figs. 3 and 4. Such overlappings are also frequently observed between pyrimidine bases.[17] In 6,5′-Cs^{4}U, two kinds of hydrogen bonds between the hydroxyl groups of sugar moieties are also found; one between the O(2′) hydroxyl groups and the other between O(2′) and O(3′) hydroxyl groups. These hydrogen bonds form a two-dimensional network extending over the planes parallel to the *ab* plane as shown in Fig. 6. In this case, flip–flop type hydrogen bonds could be formed and so the two hydrogen atoms attached to O(2′) and O(3′) are disordered. On the other hand, in the crystal of 6,6′-CU, neither base pairing nor stacking is observed. The molecules are connected by three kinds of hydrogen bonds employing all three hydrogen atoms bonded to oxygens and nitrogen [O(2′)–H···O(4); 2.785 (5) Å, O(3′)–H···O(4′); 2.880 (3) Å and N(3)–H···O(2′); 2.968 (5) Å].

Enzyme-Bound Conformations of Nucleotide Substrates It is well known that the catalytic reaction of ribonuclease (RNase) A involves a two-step mechanism: (1) reversible transphosphorylation to form an intermediate oligonucleotide ending in a pyrimidine 2′,3′-cyclic nucleotide, (2) hydrolysis of the intermediate cyclic nucleotide to produce oligonucleotide having a 3′-terminal pyrimidine nucleotide.[18] The crystal structures of RNase A, RNase S (a related enzyme) and their complexes with several nucleotides have been reported by several workers.[19-21] Of these, the crystalline complexes between RNase A and 2′-cytidylic acid[20] and between RNase S and uridylyl-3′,5′-methyleneadenosine (UpcA), cytidylyl-2′,5′-adenosine, 3′-cytidylic acid and 3′-uridylic acid[21] provide information on the binding mode of phosphate to the enzyme and the recognition of the pyrimidine base by the enzyme in the inhibitor–enzyme interaction. However, because enzyme–substrate analog complexes suitable as models of the hydrolysis of

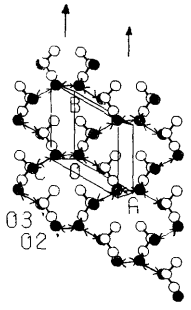

Fig. 6. Flip–Flop Hydrogen Bonds of 6,5′-Cs^{4}U along the *c* Axis
Filled circles indicate the O(2′) and O(3′) atoms.

TABLE VIII. Conformational Parameters for Pyrimidine 2′,3′-Cyclic Nucleotides and Related Nucleosides

Compound	χ (°)		P (°)	τ_m (°)	2′,3′-Cyclic ring	Class
i-Br^{5}U[a]	−164.9	*low anti*	211 (^4_3T)	21	*exo*	1
i-U[b]	−170.0	*low anti*	216 (^4_3T)	24	*exo*	
2′,3′-cUMP(S)[c]	−176.1	*low anti*	261 ($_0E$)	23	*exo*	2
i-C1U[d]	−123.5	*middle anti*	38 (^3_4T)	29	*exo*	3
2′,3′-cCMP[e]	−118.4	*middle anti*	173 (^2E)	29	*exo*	4
mmU[f]	−120.5	*middle anti*	163 (^2E)	23	*exo*	
2′,3′-cCMP·Na(B)[g]	65.8	*syn*	Planar	2	*endo*	5
i,Ac-U[h]	73.6	*syn*	−19 ($_2E$)	17	*exo*	6
i,tosyl-U[i]	67.9	*syn*	17 (^3E)	21	*exo*	7
i-CN^{6}U[j]	70.0	*syn*	51 ($_4E$)	30	*exo*	8
2′,3′-cCMP·Na(B)[g]	61.1	*syn*	81 (^0E)	35	*endo*	9
h-U[k]	60.5	*syn*	107 (^0_1T)	27	*exo*	10

a) 2′,3′-*O*-Isopropylidene-5-bromouridine,[22] *b*) 2′,3′-*O*-isopropylideneuridine,[23] *c*) triethylammonium salt of uridine 2′,3′-cyclic thiophosphonate,[24] *d*) 5′-acetyl-7,7-dichloro-2′,3′-isopropylidene-3-methylcyclothymidine,[25] *e*) cytidine 2′,3′-cyclic phosphate,[26] *f*) 2′,3′-*O*-methoxymethyleneuridine,[27] *g*) sodium salt of cytidine 2′,3′-cyclic phosphate,[28] *h*) 5′-*O*-acetyl-2′,3′-*O*-isopropylideneuridine,[29] *i*) 5′-*O*-tosyl-2′,3′-*O*-isopropylideneuridine,[30] *j*) 2′,3′-*O*-isopropylidene-6-cyanouridine,[31] *k*) 2′,3′-*O*-cyclohexylidene-4′-*C*-(2-methyl-2-propenyl)uridine.[32]

pyrimidine 2′,3′-cyclic nucleotide have not been reported, the conformation of the pyrimidine nucleoside 2′,3′-cyclic phosphate in the hydrolysis state is not yet known. In this study, we examined the stereochemistry of the substrate with the aim of understanding the hydrolysis of pyrimidine 2′,3′-cyclic phosphate by RNase A.

The conformations about the glycosidic bond and the sugar and 2′,3′-cyclic rings for natural 2′,3′-cyclic nucleotides and related nucleosides having an additional five-membered ring *cis* to the C(2′)–C(3′) of ribose are summarized in Table VIII. This table indicates conformational flexibilities. In particular, the preference of *syn* conformation about the glycosidic bond is remarkably higher than that in unmodified pyrimidine nucleosides and nucleotides, and the puckering of the sugar ring is also variable, in contrast to the normal sugar puckering of C(2′)-*endo* type or C(3′)-*endo* type. The overall molecular conformations can be classified into ten types as noted in Table VIII. Among them, only four kinds of conformational types have been observed in the pyrimidine 2′,3′-cyclic nucleotides. However, 2′,3′-cyclic nucleotides probably could also take the other six types, because of the presence of the similar ring system with the bridging of the vicinal *cis* oriented O(2′) and O(3′) hydroxyls. Therefore, we constructed ten molecular models of 2′,3′-cyclic nucleotides with P–O bond length of 1.61 Å and C(2′)–O(2′)–P angle of 113° (obtained from the nucleoside 2′,3′-cyclic monophosphates).[26]

Which of them is the preferred conformation for recognition or hydrolysis by RNase A ? Since the 2′,3′-cyclic monophosphates of 6-alkyluridine which prefer the *syn* conformation rather than the *anti* one are very poor substrates,[9] six models with the *syn* conformation must be excluded. The 2′,3′-cyclic monophosphates of 6,5′-Cs^{4}U, 6,5′-cyclo-5′-deoxyuridine (6,5′-CU) and 6,6′-CU are hydrolyzed by RNase A to give the respective 3′-phosphates, although the rates of hydrolysis are slower than those of the natural substrates.[9] Among them, 6,5′-cyclonucleoside 2′,3′-cyclic monophosphates are better substrates than 6,6′-

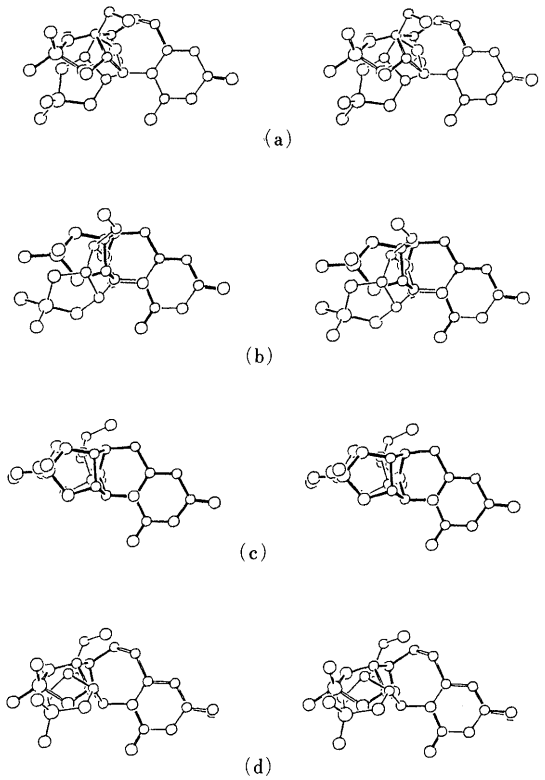

Fig. 7. Four Stereo Overlays of 6,5′-(Open Lines) or/and 6,6′-Cyclic Nucleotide (Solid Lines) and Models with Typical Conformations for Pyrimidine 2′,3′-Cyclic Nucleotides

Models (thin lines): (a) χ = −165°, C(4′)-*endo*, C(3′)-*exo* conformation, (b) χ = −176°, O(1′)-*exo* conformation, (c) χ = −123°, C(3′)-*endo*, C(4′)-*exo* conformation, (d) χ = −118°, C(2′)-*endo* conformation.

cyclonucleoside 2′,3′-cyclic monophosphate. Futhermore, all their hydrolyzed products, 6,5′- and 6,6′-cyclonucleoside 3′-monophosphates, are strong inhibitors of RNase A in the hydrolysis of natural substrates.[9] These results indicate that 6,5′- and 6,6′-cyclonucleotides are both recognized by the enzyme. In the case of 6,5′- or 6,6′- cyclonucleoside 2′,3′-cyclic monophosphate, very little conformational change is possible during the enzyme reaction, and its conformation could be constructed using the molecular dimensions of 6,5′- or 6,6′-cyclonucleoside. In Fig. 7, 6,5′- or/and 6,6′-cyclonucleotide models are superimposed on four models with *anti* conformations. When the bases are brought into coincidence, the phosphate groups are very close to each other in the cases of 6,5′-cyclonucleotide and the *middle anti*-C(3′)-*endo* model [Fig. 7(c)] and of 6,6′-cyclonucleotide and the *middle anti*-C(2′)-*endo* model [Fig. 7(d)]. These considerations suggest that 2′,3′-cyclic pyrimidine nucleotides with the *middle anti* conformation are recognized by the RNases A and the *middle anti*, C(3′)-*endo* model is more favorable in the enzyme reaction that the *middle anti*, C(2′)-*endo* model. However, 6,5′- or 6,6′-cyclonucleoside 2′,3′-cyclic phosphate was cleaved slowly compared to the normal substrates. As previously pointed out,[9] the reason may be that the hydrolyzates bind as strongly as the substrates to the enzyme after the cleavage, since very little conformational change is possible between the substrates and hydrolyzates, due to the rigid cyclic structure. The remaining *low anti* models with O(4′)-*exo* and C(4′)-*endo* have significantly different positions of

the phosphate group from those of 6,5′-cyclonucleotides and thus they may be poorly recognized by the enzyme.

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