Complexes between Nucleotide Base and Amino Acid. III. Crystal Structure of 5-Bromocytosine:Phthaloyl-DL-glutamic Acid Complex Hemihydrate

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5-Bromocytosine and phthaloyl-DL-glutamic acid co-crystallize from their aqueous solution in space group $P\bar{1}$, with dimensions of $a=10.578(2)$, $b=19.640$ (4), $c=9.890$ (3) Å, $\alpha=88.13$ (2), $\beta=107.86$ (3), $\gamma=104.26$ (1)°. Two molecular complexes and one water molecule are in an asymmetric unit. The structure was solved by the heavy atom method and refined by a block-diagonal least-squares method. The 5-bromocytosine molecule binds with γ-carboxyl group of the amino acid through NH···O and OH···O hydrogen bonds. In spite of largely different molecular environment in the crystal from that of 5-bromocytosine:*N*-tosyl-L-glutamic acid complex, the mode of interaction between the nucleotide base and the amino acid is the same. Thus, it is suggested that the present geometry is a typical binding mode between cytosine and acidic side group of amino acid.

The present structure determination is a part of the serial studies on specific binding patterns of nucleotide base-amino acid interactions.

As reported in Part II on the 5-bromocytosine:*N*-tosyl-L-glutamic acid complex (BCTG),[1] we have found a specific hydrogen bond of cytosine with γ-carboxyl group of glutamic acid, not with α-carboxyl group. In cytidine:*N*-benzyloxycarbonyl-L-glutamic acid complex dihydrate,[2] on the other hand, cytosine binds with α-carboxyl group of glutamic acid. As α-carboxyl group is used for peptide bond formation in protein except for its C-terminal, the hydrogen bonds between cytosine and γ-carboxyl group may represent a more general mode of interaction. So, it must be examined whether the interaction mode found in BCTG complex is preserved or not, when the molecular environment in a crystal is altered. We have investigated this by the structure analysis of the title complex which has a different *N*-protective group of glutamic acid from BCTG.

Experimental

Plate crystals were obtained from an aqueous solution containing equimolar quantities of the two components. The complex formation was confirmed by IR spectroscopy and an elementary analysis of these crystals. Found: C, 43.12; H, 3.39; N, 12.28%. Calcd for $C_4H_4N_3OBr\cdot C_{13}H_{11}NO_6\cdot 1/2H_2O$: C, 42.87; H, 3.39; N, 11.77%. Diffraction pattern of the crystal indicated the crystal system to be triclinic. The unit-cell dimensions were determined by least-squares calculations from 89 reflexions recorded on equator Weissenberg photographs about the three different axes, calibration being made with superposed silicon lines ($a=5.43075$ Å). These results are listed in Table 1.

Table 1. Crystal data

5-Bromocytosine: phthaloyl-DL-glutamic acid hemihydrate
$C_4H_4N_3OBr\cdot C_{13}H_{11}NO_6\cdot \frac{1}{2}H_2O$
F.W.=476.2
Crystal system: triclinic
Space group: $P\bar{1}$
$a=10.578$ (2) Å $b=19.640$ (4) Å $c=9.890$ (3) Å
$\alpha=88.13$ (2)° $\beta=107.86$ (3)° $\gamma=104.26$ (1)°
$U=1893.3$ (7) Å^3 $Z=4$
$D_x=1.671$ g cm^{-3} $D_m=1.69$ g cm^{-3}
μ (Cu$K\alpha$)=38.11 cm^{-1}

Diffraction data were collected on equi-inclination Weissenberg photographs (0*kl*-7*kl* and *hk*0-*hk*7) using Ni-filtered Cu$K\alpha$ radiation and the intensities were measured by a TV densitometer.[3] The crystals used for the a- and c-axis rotations were $0.1\times0.2\times0.3$ mm and $0.07\times0.19\times0.36$ mm in size, respectively. Corrections were made for Lorentz and polarization effects and spot size, but not for absorption. A total of 7649 independent reflexions was obtained, of which zero-reflexions numbered 3908. The $N(z)$ test[4] indicated the crystal to be centro-symmetric; this is reasonable because the amino acid derivatives is racemic, thus the space group being decided to be $P\bar{1}$. The density, measured by flotation in a $CHBr_3$–CCl_4 mixture, indicated that there are two molecular complexes and one water molecule in an asymmetric unit.

Structure Determination and Refinement

The structure was solved by the heavy atom method, and refined by a block-diagonal least-squares method, the minimized function being $\sum w\Delta F^2$ where $\Delta F=|F_o|-|F_c|$. In this refinement, the zero-reflexions were assumed to have the value of $|F_o|_{min}$ (=4.31). The zero-reflexions for which $|F_c|$ values were smaller than $|F_o|_{min}$ were omitted in each cycle of refinement. The weight function used was as follows: $w=\exp(-as^2-bt^2-cst-ds-et-f)$ for $|F_o|>|F_o|_{min}$, where $s=|F_o|$ and $t=\sin\theta/\lambda$; $w=1/\langle\Delta F^2\rangle$ for $|F_o|=|F_o|_{min}$. The coefficients, *a*—*f*, were evaluated by least-squares at each cycle of the structure refinement so that $\langle w\Delta F^2\rangle=1$. The refinement was terminated when the maximum shift of parameters was less than 0.04σ and 0.06σ for positional and thermal parameters, respectively. At the final cycle, the coefficients of weight function were 0.00006012, 23.72, −0.05158, 0.04557, −21.35, and 5.304, respectively, and weight for $|F_o|_{min}$ was 0.0763. The final value of R is 0.13 ($R_w=0.11$). For reflexions of $|F_o|>3\sigma$ ($\sigma=1/\sqrt{w}$), R is 0.078. The comparison between observed and calculated structure factors is given in Table 2.[5] Atomic scattering factors used were taken from "International Tables for X-Ray Crystallography."[6] The final positional and thermal parameters are given in Table 3.

TABLE 3. FINAL POSITIONAL AND THERMAL PARAMETERS

Standard deviations are given in parentheses. The temperature factor has the form $\exp[-(\beta_{11}h^2+\beta_{22}k^2+\beta_{33}l^2+\beta_{12}hk+\beta_{13}hl+\beta_{23}kl)]$. All parameters have been multiplied by 10^4.

Atom	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
N(1A)	−5030(7)	732(3)	6337(8)	85(7)	26(2)	162(11)	31(6)	84(14)	48(7)
C(2A)	−4185(8)	332(4)	7115(9)	80(8)	20(2)	139(11)	14(6)	61(15)	21(7)
N(3A)	−3009(6)	337(3)	6883(8)	77(6)	24(2)	134(9)	18(5)	68(13)	30(6)
C(4A)	−2623(7)	753(4)	5895(9)	80(8)	23(2)	129(11)	24(6)	58(15)	20(7)
C(5A)	−3498(8)	1153(4)	5043(8)	90(8)	24(2)	100(10)	18(6)	54(14)	25(7)
C(6A)	−4695(8)	1125(4)	5285(10)	100(9)	26(2)	131(11)	30(7)	49(16)	27(8)
O(2A)	−4612(5)	−54(3)	8042(7)	90(6)	28(2)	159(8)	29(5)	101(12)	52(6)
N(4A)	−1408(7)	777(4)	5741(9)	89(7)	34(2)	174(11)	39(7)	106(15)	54(8)
Br(5A)	−2979(1)	1711(0)	3616(1)	124(1)	27(0)	123(1)	27(1)	91(2)	32(1)
N(1B)	2738(7)	−278(3)	8361(8)	91(7)	26(2)	148(10)	33(6)	98(14)	39(7)
C(2B)	1906(8)	120(4)	7563(9)	98(8)	20(2)	140(11)	29(6)	78(16)	34(8)
N(3B)	631(6)	26(3)	7603(8)	86(7)	24(2)	145(9)	35(6)	82(13)	49(7)
C(4B)	147(8)	−469(4)	8427(9)	88(8)	27(2)	126(11)	24(7)	87(16)	28(8)
C(5B)	992(8)	−896(4)	9241(9)	95(8)	42(2)	107(10)	32(7)	42(15)	19(7)
C(6B)	2277(8)	−790(4)	9184(9)	102(9)	24(2)	131(11)	30(7)	75(16)	27(8)
O(2B)	2412(6)	574(3)	6810(7)	111(6)	27(2)	179(9)	38(5)	129(13)	76(6)
N(4B)	−1124(7)	−543(4)	8446(9)	97(8)	33(2)	185(12)	46(7)	121(16)	62(8)
Br(5B)	324(1)	−1569(0)	10424(1)	116(1)	30(0)	165(1)	33(1)	117(2)	58(1)
O(3A)	−6109(7)	−4305(3)	8387(8)	138(8)	26(2)	171(10)	−3(6)	120(14)	−6(6)
O(4A)	−4113(7)	−3543(3)	8568(9)	111(7)	32(2)	222(12)	10(6)	136(15)	−4(8)
C(7A)	−5336(8)	−3740(4)	8744(9)	94(8)	27(2)	111(10)	19(7)	57(15)	23(8)
C(8A)	−5644(8)	−3147(4)	9482(9)	77(8)	27(2)	129(11)	7(7)	69(15)	21(8)
C(9A)	−4460(9)	−2769(4)	10761(10)	116(10)	24(2)	129(11)	15(7)	39(17)	12(8)
C(10A)	−3510(9)	−2135(5)	10386(12)	98(9)	30(3)	192(15)	20(8)	51(19)	34(10)
C(11A)	−4175(8)	−1537(4)	9923(10)	83(8)	26(2)	153(12)	25(7)	44(16)	19(8)
O(5A)	−3493(7)	−1074(4)	9235(10)	117(7)	35(2)	287(14)	52(6)	188(17)	88(9)
O(6A)	−5212(7)	−1475(4)	10126(9)	109(7)	41(2)	251(13)	60(7)	138(16)	53(9)
N(5A)	−6893(7)	−3428(3)	9846(8)	94(7)	20(2)	137(9)	22(5)	77(13)	40(6)
C(12A)	−8141(9)	−3236(4)	9241(10)	108(9)	24(2)	134(11)	29(7)	78(17)	28(8)
C(13A)	−7027(8)	−3963(4)	10794(9)	95(8)	22(2)	127(11)	22(7)	57(15)	21(8)
O(7A)	−8303(7)	−2807(4)	8333(8)	128(8)	39(2)	176(10)	54(7)	99(14)	94(8)
O(8A)	−6090(6)	−4208(3)	11459(7)	108(6)	26(2)	157(9)	40(5)	83(12)	60(6)
C(14A)	−9106(8)	−3649(4)	9944(8)	96(8)	24(2)	101(10)	21(7)	60(15)	18(7)
C(15A)	−10423(9)	−3643(5)	9801(9)	100(9)	33(3)	111(11)	30(8)	48(16)	14(8)
C(16A)	−11105(9)	−4105(5)	10616(10)	109(10)	37(3)	128(12)	38(8)	70(17)	9(9)
C(17A)	−10458(9)	−4549(5)	11532(9)	102(9)	32(3)	111(11)	23(8)	64(16)	4(8)
C(18A)	−9101(8)	−4552(4)	11675(9)	106(9)	25(2)	99(10)	15(7)	65(15)	10(7)
C(19A)	−8448(8)	−4097(4)	10854(9)	92(8)	22(2)	114(10)	24(6)	68(15)	11(7)
O(3B)	3892(6)	4443(3)	5505(8)	110(7)	35(2)	169(10)	−15(6)	110(14)	8(7)
O(4B)	2956(6)	4386(3)	7247(7)	110(7)	31(2)	136(8)	9(5)	88(12)	−2(6)
C(7B)	3077(7)	4145(4)	6086(8)	77(7)	26(2)	94(9)	18(6)	25(13)	14(7)
C(8B)	2148(7)	3414(4)	5626(9)	74(7)	25(2)	128(10)	33(6)	79(14)	42(7)
C(9B)	2112(8)	3148(4)	4179(9)	103(9)	23(2)	115(10)	6(7)	63(16)	21(7)
C(10B)	1299(9)	2384(4)	3827(10)	115(10)	24(2)	134(11)	26(7)	49(17)	19(8)
C(11B)	1970(8)	1894(4)	4817(10)	92(8)	25(2)	163(13)	23(7)	113(17)	37(8)
O(5B)	1096(6)	1303(3)	4943(8)	95(6)	26(2)	209(11)	14(5)	111(13)	51(7)
O(6B)	3177(6)	2014(3)	5451(10)	70(6)	31(2)	294(14)	26(5)	53(15)	50(8)
N(5B)	780(6)	3392(3)	5681(7)	79(6)	21(2)	118(8)	23(5)	69(12)	33(6)
C(12B)	113(8)	2932(4)	6508(9)	89(8)	19(2)	121(10)	7(6)	76(15)	18(7)
C(13B)	1(8)	3838(4)	4945(9)	93(8)	24(2)	125(11)	34(7)	48(15)	23(7)
O(7B)	594(6)	2505(3)	7264(7)	131(7)	31(2)	180(10)	51(6)	147(14)	74(7)
O(8B)	373(7)	4296(3)	4203(8)	140(8)	34(2)	179(10)	57(6)	119(15)	72(7)
C(14B)	−1251(7)	3087(4)	6217(9)	72(7)	26(2)	135(11)	1(6)	75(15)	−17(8)
C(15B)	−2345(10)	2769(5)	6711(11)	108(10)	39(3)	156(14)	−13(9)	125(20)	−49(10)
C(16B)	−3528(10)	3040(7)	6202(13)	91(10)	54(4)	188(16)	33(10)	85(20)	−52(13)
C(17B)	−3562(10)	3576(7)	5297(12)	90(10)	61(5)	168(16)	54(11)	45(20)	−6(14)
C(18B)	−2459(10)	3906(6)	4833(12)	92(10)	52(4)	174(15)	62(10)	25(19)	−7(12)
C(19B)	−1307(8)	3631(4)	5289(9)	80(8)	28(2)	121(11)	32(7)	25(15)	8(8)
O(9)	3512(6)	4493(3)	2602(7)	127(7)	29(2)	147(8)	37(6)	76(13)	22(6)

Results and Discussions

Bond lengths and angles of the two independent molecules are listed in Table 4. The values for corresponding bonds are in good agreement, and their averages are given in Fig. 1. The effects of bromine substitution on the bond lengths and angles of pyrimidine ring is not so large; somewhat longer C(2)–O(2) bond and shorter C(2)–N(3) bond are observed as compared with the authentic values of neutral cytosine,[7] but these should be attributable partly to the effects of strong hydrogen bonds involving O(2) (Table 5), which are also observed in BCTG complex.[1] The crystallographically independent molecules are shown in Fig. 2.

The molecules are arranged in layers parallel to the (021) plane (Fig. 3). As shown in Fig. 4, four α-carboxyl groups and two water molecules constitute a 14-membered ring around the center of symmetry at 1/2, 1/2, 1/2 by hydrogen bonds, O(4A)H···O(9), O(9)H···O

Table 4. Bond lengths and angles of the two independent molecules
Standard deviations are given in parentheses.

Bond lengths (l/Å)	Molecule A	Molecule B		Molecule A	Molecule B
N(1)–C(2)	1.38(1)	1.37(1)	C(2)–N(3)	1.33(1)	1.33(1)
N(3)–C(4)	1.35(1)	1.35(1)	C(4)–C(5)	1.42(1)	1.43(1)
C(5)–C(6)	1.35(1)	1.34(1)	C(6)–N(1)	1.36(1)	1.36(1)
C(2)–O(2)	1.29(1)	1.27(1)	C(4)–N(4)	1.33(1)	1.32(1)
C(5)–Br(5)	1.891(8)	1.887(8)			
C(7)–O(3)	1.19(1)	1.20(1)	C(7)–O(4)	1.32(1)	1.31(1)
C(7)–C(8)	1.54(1)	1.52(1)	C(8)–N(5)	1.45(1)	1.46(1)
C(8)–C(9)	1.53(1)	1.53(1)	C(9)–C(10)	1.51(1)	1.53(1)
C(10)–C(11)	1.50(1)	1.50(1)	C(11)–O(5)	1.32(1)	1.32(1)
C(11)–O(6)	1.21(1)	1.20(1)			
N(5)–C(12)	1.41(1)	1.41(1)	N(5)–C(13)	1.40(1)	1.39(1)
C(12)–C(14)	1.48(1)	1.49(1)	C(13)–C(19)	1.48(1)	1.48(1)
C(14)–C(19)	1.39(1)	1.39(1)	C(14)–C(15)	1.36(1)	1.39(1)
C(15)–C(16)	1.41(1)	1.42(2)	C(16)–C(17)	1.38(1)	1.36(2)
C(17)–C(18)	1.40(1)	1.39(2)	C(18)–C(19)	1.39(1)	1.40(1)
C(12)–O(7)	1.21(1)	1.21(1)	C(13)–O(8)	1.21(1)	1.21(1)

Bond angles (ϕ/°)	Molecule A	Molecule B		Molecule A	Molecule B
C(2)–N(1)–C(6)	120.4(7)	121.4(7)	C(8)–N(5)–C(12)	125.4(7)	124.1(7)
N(1)–C(2)–O(2)	116.5(7)	117.2(8)	C(8)–N(5)–C(13)	123.3(7)	123.5(7)
N(1)–C(2)–N(3)	120.9(7)	120.4(8)	C(12)–N(5)–C(13)	111.2(7)	112.4(7)
O(2)–C(2)–N(3)	122.6(8)	122.5(8)	N(5)–C(12)–C(14)	106.0(7)	105.4(7)
C(2)–N(3)–C(4)	119.5(7)	119.8(7)	N(5)–C(12)–O(7)	123.7(8)	124.8(8)
N(3)–C(4)–C(5)	120.6(7)	120.5(8)	C(14)–C(12)–O(7)	130.3(8)	129.8(8)
N(3)–C(4)–N(4)	118.2(8)	117.9(8)	N(5)–C(13)–C(19)	106.1(7)	105.5(7)
C(5)–C(4)–N(4)	121.2(8)	121.7(8)	N(5)–C(13)–O(8)	123.3(8)	124.8(8)
C(4)–C(5)–C(6)	118.4(8)	118.6(8)	C(19)–C(13)–O(8)	130.4(8)	129.7(8)
C(4)–C(5)–Br(5)	120.4(6)	120.1(6)	C(12)–C(14)–C(15)	130.3(8)	129.9(8)
C(6)–C(5)–Br(5)	121.2(6)	121.2(6)	C(12)–C(14)–C(19)	108.1(7)	107.8(7)
C(5)–C(6)–N(1)	120.1(8)	119.3(8)	C(15)–C(14)–C(19)	121.7(8)	122.3(8)
			C(13)–C(19)–C(14)	108.4(7)	108.9(7)
O(3)–C(7)–O(4)	124.6(8)	124.2(8)	C(13)–C(19)–C(18)	130.2(8)	129.7(9)
O(3)–C(7)–C(8)	123.9(8)	123.7(8)	C(14)–C(19)–C(18)	121.4(8)	121.4(9)
O(4)–C(7)–C(8)	111.4(7)	111.9(7)	C(14)–C(15)–C(16)	117.7(8)	115.6(9)
C(7)–C(8)–N(5)	108.8(7)	109.8(6)	C(15)–C(16)–C(17)	121.2(9)	121.2(11)
C(7)–C(8)–C(9)	114.8(7)	113.8(7)	C(16)–C(17)–C(18)	120.9(9)	123.2(11)
N(5)–C(8)–C(9)	112.1(7)	111.7(7)	C(17)–C(18)–C(19)	117.2(8)	116.1(10)
C(8)–C(9)–C(10)	114.0(8)	112.3(7)			
C(9)–C(10)–C(11)	112.8(8)	111.8(8)			
C(10)–(11)–O(5)	111.8(8)	113.1(8)			
C(10)–C(11)–O(6)	125.5(9)	124.7(9)			
O(5)–C(11)–O(6)	122.8(8)	122.2(9)			

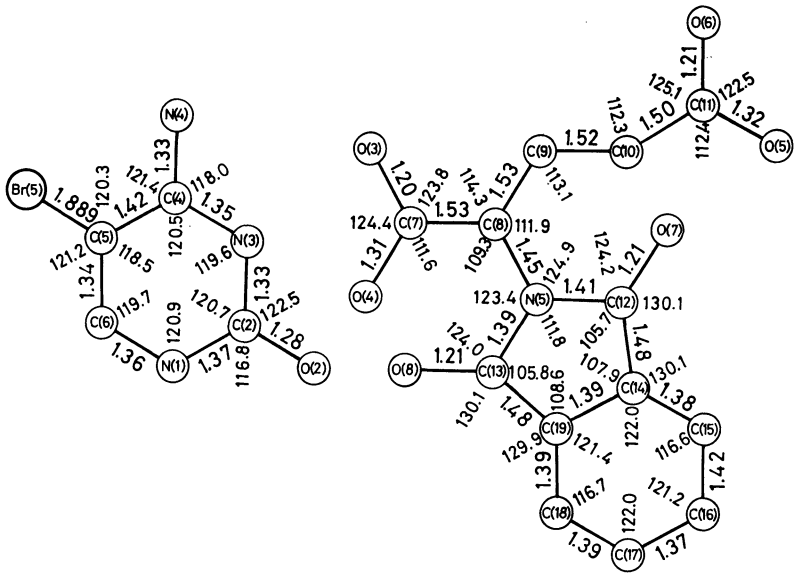

Fig. 1. The average bond lengths (l/Å) and angles (ϕ/°) of the two independent 5-bromocytosine and phthaloylglutamic acid molecules.

Table 5. Distances and angles of hydrogen bonds
(CH···O contacts are also listed in this table.)

Distance (l/Å)					
N(1A)···O(2B)[a]	2.83	N(1B)···O(2A)[b]	2.84	N(4A)···N(3B)[c]	3.03
N(4B)···N(3A)[c]	3.00	N(4A)···O(5B)[c]	2.93	N(4B)···O(5A)[c]	2.80
O(5A)···O(2A)[c]	2.64	O(5B)···O(2B)[c]	2.58	O(4B)···O(3A)[d]	2.67
O(9)···O(3B)[e]	2.78	O(4A)···O(9)[e]	2.53	O(9)···O(8A)[f]	2.75
C(6A)···O(6B)[a]	3.21	C(6B)···O(6A)[b]	3.14		
Angle (ϕ/°)					
N(1A)···O(2B)[a]–C(2B)[a]	126.1	C(2A)–N(1A)···O(2B)[a]	117.2	C(6A)–N(1A)···O(2B)[a]	122.0
N(1B)···O(2A)[b]–C(2A)[b]	125.5	C(2B)–N(1B)···O(2A)[b]	116.6	C(6B)–N(1B)···O(2A)[b]	121.8
N(4A)···N(3B)[c]–C(2B)[c]	123.7	N(4A)···N(3B)[c]–C(4B)[c]	116.3	C(4A)–N(4A)···N(3B)[c]	124.0
N(4B)···N(3A)[c]–C(2A)[c]	123.4	N(4B)···N(3A)[c]–C(4A)[c]	117.0	C(4B)–N(4B)···N(3A)[c]	125.3
N(4A)···O(5B)[c]–C(11B)[c]	141.8	N(4A)···O(5B)[c]···O(2B)[c]	92.0	C(4A)–N(4A)···O(5B)[c]	159.3
N(4B)···O(5A)[c]–C(11A)[c]	149.6	N(4B)···O(5A)[c]···O(2A)[c]	92.8	C(4B)–N(4B)···O(5A)[c]	158.0
O(5A)···O(2A)[c]–C(2A)[c]	122.1	O(5A)···N(4B)[c]···N(3A)[c]	76.7	O(5A)···O(2A)[c]···N(1B)[a]	109.1
C(11A)–O(5A)···O(2A)[c]	117.5	O(5B)···O(2B)[c]–C(2B)[c]	126.2	O(5B)···N(4A)[c]···N(3B)[c]	75.2
O(5B)···O(2B)[c]···N(1A)[b]	106.1	C(11B)–O(5B)···O(2B)[c]	108.4	O(4B)···O(3A)[d]–C(7A)[d]	158.0
C(7B)–O(4B)···O(3A)[d]	128.2	O(9)···O(3B)[c]–C(7B)[c]	127.4	C(7A)–O(4A)···O(9)[e]	113.4
O(9)···O(8A)[f]–C(13A)[f]	122.8	C(6A)···O(6B)[a]–C(11B)[a]	127.9	N(1A)–C(6A)··· O(6B)[a]	88.2
C(5A)–C(6A)···O(6B)[a]	145.2	C(6B)···O(6A)[b]–C(11A)[b]	147.0	N(1B)–C(6B)···O(6A)[b]	99.7
C(5B)–C(6B)···O(6A)[b]	140.6				

a: at $-1+x, y, z$. b: at $1+x, y, z$. c: at x, y, z. d: at $1+x, 1+y, z$. e: at $-x, -y, 1-z$. f: at $1+x, 1+y, -1+z$.

(3B), and O(4B)H···O(3A). Distance of O(4A)··· O(9) is fairly shorter than those of the other OH···O hydrogen bonds (Table 5). Similar short O···O distances are also observed in oxalic acid dihydrate (2.512 Å)[8] and pyromellitic acid dihydrate (2.549 Å).[9] Furthermore, this ring is connected with those in the neighboring unit-cells by the hydrogen bond between water molecule and O(8) atom of phthaloyl group in the A molecule, to complete a three-dimensional network. The structure is further stabilized by the overlapping of the phthaloyl groups; the dihedral angle between the mean planes of the two independent phthaloyl groups is 5.1° and the spacing is about 3.4 Å.

The hydrogen bonding arrangements in the layer is illustrated in Fig. 5, relevant distances and angles being listed in Table 5. The 5-bromocytosine molecules are linked by the hydrogen bonds, N(1A)H···O(2B), N (1B)H···O(2A), N(4A)H···N(3B), and N(4B)H···N (3A), to form a ribbon elongated along [100] direction. To the ribbon, γ-carboxyl group of a glutamic acid is bound through hydrogen bonds in which O(5) atom is a donor to O(2) of 5-bromocytosine and at the same time an acceptor from N(4) of the neighboring base. In addition, O(6) seems to make a CH···O type interaction with C(6) of the base A′. The other crystallographically independent glutamic acid also interacts to the ribbon

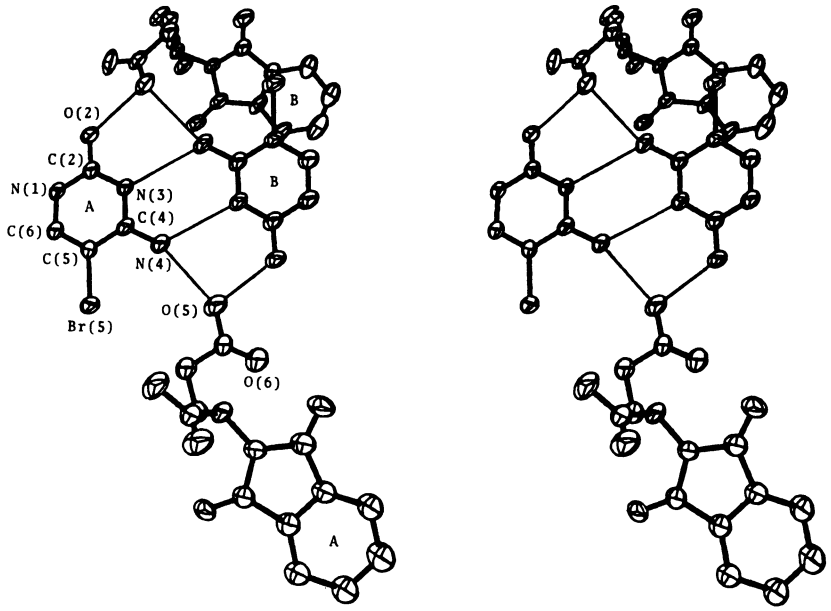

Fig. 2. A stereoscopic view of 5-bromocytosine: phthaloylglutamic acid. Thermal ellipsoids are drawn at the 50% probability level.

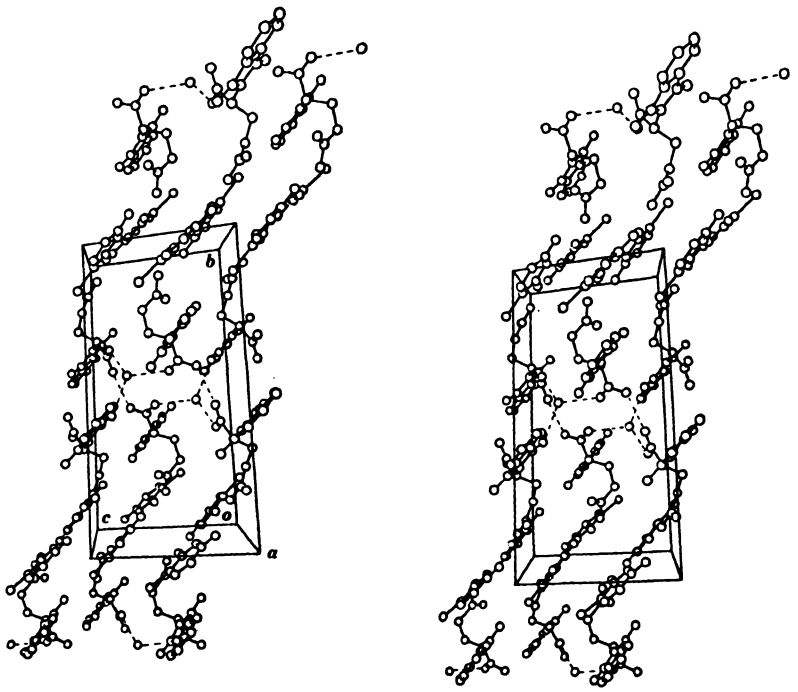

Fig. 3. A stereoscopic view of the crystal structure.

in the same way. It is interesting to note that amino acids bound to one cytosine's ribbon are the same optical isomers.

As seen from the hydrogen bond scheme described above, the N(3) atom of cytosine is not protonated; the C(2)–N(3)–C(4) bond angle of cytosine 119.6° is close to unprotonated form,[7] and the distances and angles of α- and γ-carboxyl groups of glutamic acid correspond to those of undissociated acid.

Each pyrimidine ring is planar within 0.02 Å and each bromine atom also lies in the plane. The dihedral angle between these mean planes is 11.5°.

The conformations of two glutamic acids A and B are different to each other mainly in torsions around the C(7)–C(8) and C(8)–C(9) bonds, as shown in Table 6. In the molecule A, the C(8)–N(5) bond is nearly parallel to α-carboxyl plane and the torsion angle of C(7)–C(8)–C(9)–C(10) is a nearly right angle, while in the molecule B, the C(8)–C(9) bond is parallel to α-carboxyl plane and C(7), C(8), C(9), and C(10) are nearly coplanar.

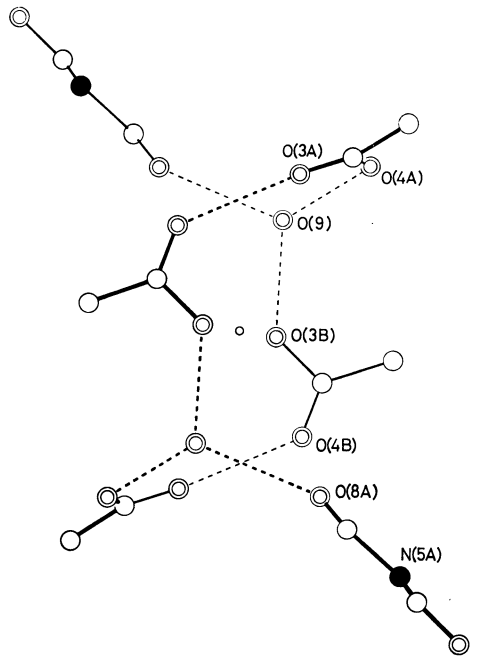

Fig. 4. The hydrogen bonding network around the center of symmetry at 1/2, 1/2, 1/2 viewed along the a axis.

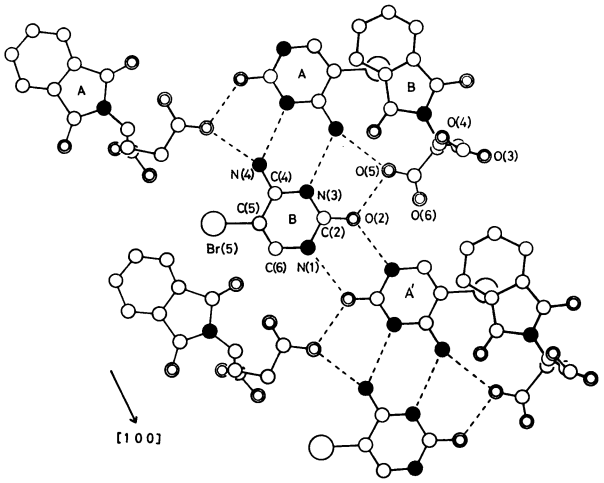

Fig. 5. A projection of one layer of 5-bromocytosine: phthaloyl-DL-glutamic acid onto the (021) plane. This illustrates the hydrogen bonding arrangement in the complex. The crystallographically independent molecules are labeled as A and B.

The side chains of glutamic acids are folded by the rotations around C(9)–C(10) bonds, in contrast to the extended form found in BCTG complex.[1]

As described above, the present crystal structure is different from that of BCTG in a mode of interlayer interaction, in hydrogen bonds of the α-carboxyl groups, and in the conformations of glutamic acids. In spite of these differences, binding scheme between 5-bromocytosine and γ-carboxyl group of glutamic acid is the same as that of BCTG, suggesting that this binding is a typical mode between 5-bromocytosine and the acidic amino acid.

The other type of complex formation between cytosine and glutamic acid has been reported for cytidine: *N*-benzyloxycarbonyl-L-glutamic acid complex dihydrate,[2] in which cytosine moiety interacts with dissociated α-carboxyl group. Such a difference may be attri-

Table 6. Torsion angles of the two independent glutamic acid

	Molecule A	Molecule B
O(3)–C(7)–C(8)–N(5)	8.8°	−138.3°
O(4)–C(7)–C(8)–N(5)	−171.1	46.4
C(7)–C(8)–N(5)–C(12)	−113.0	−121.4
C(7)–C(8)–N(5)–C(13)	62.8	56.9
C(9)–C(8)–N(5)–C(12)	119.0	111.4
C(9)–C(8)–N(5)–C(13)	−65.2	−70.3
O(3)–C(7)–C(8)–C(9)	135.0	−12.3
O(4)–C(7)–C(8)–C(9)	−44.6	172.4
N(5)–C(8)–C(9)–C(10)	−144.8	−61.3
C(7)–C(8)–C(9)–C(10)	90.4	173.7
C(8)–C(9)–C(10)–C(11)	69.0	−65.5
C(9)–C(10)–C(11)–O(5)	−162.4	154.3
C(9)–C(10)–C(11)–O(6)	16.9	−25.4

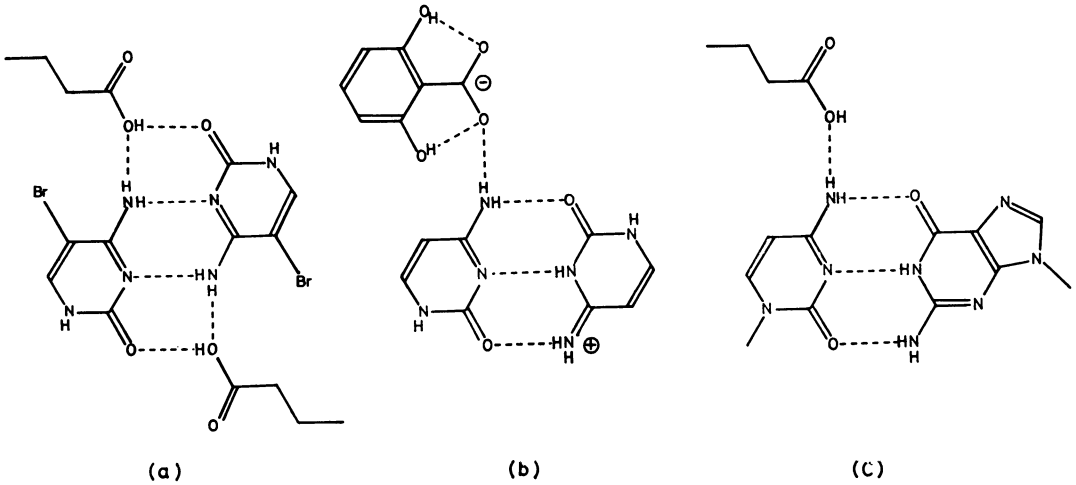

Fig. 6. The hydrogen bond scheme of (a) present and BCTG complexes, (b) cytosine: resorcylic acid 2: 1 complex monohydrate, and (c) a model of interaction between acidic side group of amino acid and the Watson-Crick G: C pair.

buted to the bromine substitution at C(5) of cytosine. Bromine at C(5) decreases the basicity of cytosine, so that a protonation to N(3) is inhibited; pK_a of 5-bromocytosine is 3.04 while those of cytosine and cytidine are 4.61 and 4.1,[10] respectively. In cytidine:*N*-benzyloxycarbonyl-L-glutamic acid, cytosine base is protonated and then hydrogen-bonded with stronger acid, α-carboxyl group of glutamic acid, while in the present and BCTG complexes, the protonation of the base is inhibited so that it becomes possible to interact with milder γ-carboxyl group. This argument is supported by the recent structure analysis of cytosine:phthaloyl-DL-glutamic acid complex,[11] in which cytosine binds with α-carboxyl group of glutamic acid.

Schematic drawing of complex molecules found in the present and BCTG complexes in Fig. 6(a) shows that the mode of three parallel hydrogen bonds of cytosine resembles the triple hydrogen bond in the Watson-Crick guanine:cytosine pair. A similar feature is also found in cytosine: resorcylic acid 2: 1 complex monohydrate[12] (Fig. 6(b)), in which the triple hydrogen bond is formed between neutral and protonated cytosines; furthermore, dissociated carboxyl group of resorcylic acid binds through a hydrogen bond to cytosine in the same geometry as those in the present and BCTG complexes. Thus, if the base on the right side in Fig. 6 (a) and (b) is replaced by guanine as in Fig. 6(c), it might provide a model of interaction between paired cytosine and acidic side group of amino acid. Recently, some model building[13,14] concerning mutual recognition between double helical nucleic acid and protein have been reported, but the present model is the first one based on the observed interaction between nucleotide base and amino acid in the crystal. It is known that elongation factors T_u and G, and initiation factor F_2 in protein synthesis are all acidic protein. Therefore, the present structure might be effected if some acidic side groups of these factors would interact with tRNA, mRNA, and rRNA.

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