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Cocrystals of 5-fluorocytosine. II. Coformers with variable hydrogen-bonding sites

Maya Tutughamiarso and Ernst Egert*

Institut für Organische Chemie und Chemische Biologie, Goethe-Universität Frankfurt, Max-von-Laue-Str. 7, 60438 Frankfurt am Main, Germany

Correspondence e-mail: egert@chemie.uni-frankfurt.de

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Two flexible molecules, biuret and 6-acetamidouracil, were cocrystallized with 5-fluorocytosine to study their conformational preferences. In the cocrystal with 5-fluorocytosine (I), biuret exhibits the same conformation as in its hydrate. In contrast, 6-acetamidouracil can adopt two main conformations depending on its crystal environment: in crystal (II) the *trans* form characterized by an intramolecular hydrogen bond is observed, while in the cocrystal with 5-fluorocytosine (III), the complementary binding induces the *cis* form. Three cocrystals of 6-methylisocytosine demonstrate that complementary binding enables the crystallization of a specific tautomer. In the cocrystals with 5-fluorocytosine, (IV*a*) and (IV*b*), only the 3*H* tautomer of 6-methylisocytosine is present, whereas in the cocrystal with 6-aminoisocytosine, (V), the 1*H* tautomeric form is adopted. The complexes observed in the cocrystals are stabilized by three hydrogen bonds similar to those constituting the Watson–Crick C·G base pair.

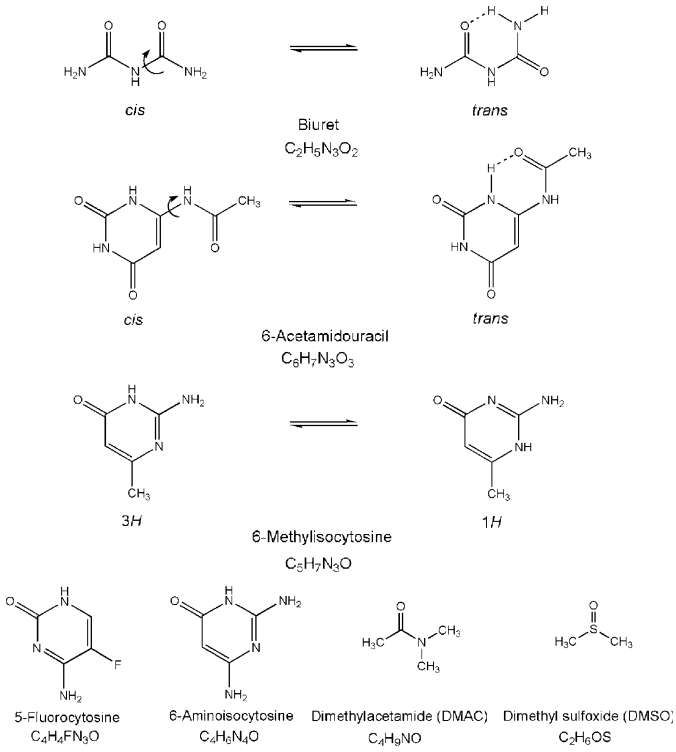
1. Introduction

Molecular recognition is a central topic in drug research and development. It describes the specific binding of molecules, controlled by non-covalent interactions, in particular ionic and hydrogen bonds as well as hydrophobic and van der Waals interactions. Focusing on recognition by hydrogen bonding, the drug and its receptor should possess complementary hydrogen-bonding sites. Since both are usually flexible, their specific binding is often accompanied by conformational changes. Similar structural alterations are also observed in protein–protein, DNA–protein and RNA–protein complexes (Pabo & Sauer, 1984; Johnson, 1993; Draper, 1995; Williamson, 2000) as well as in substrate–enzyme complexes (Hammes, 2002). In such supramolecular complexes, all groups capable of forming hydrogen bonds strive to be involved in the intermolecular interactions; hence conformations characterized by intramolecular hydrogen bonds are rarely observed at the binding site (Böhm & Klebe, 1996).

Another factor with a significant impact on molecular recognition is tautomerism. Due to the migration of H atoms, a tautomeric rearrangement results in different molecular shapes and hydrogen-bonding properties. Since nominal resolutions typical of X-ray structures of macromolecules do not allow for a reliable location of H atoms, the preferred tautomeric form at the binding site can only be estimated by the analysis of hydrogen-bonding interactions. A study of the Cambridge Structural Database (CSD; Allen, 2002) may provide information about the tautomeric preference of small molecules in crystal structures, but in the solid state less stable tautomers, which may possibly show the best interaction during the drug binding, might just be absent. Although the

majority of drugs containing heteroaromatic systems can exist in diverse tautomeric forms, there is little known about tautomerism in drug research.

After having investigated cocrystals of the antifungal drug 5-fluorocytosine (5-FC) with coformers containing fixed hydrogen-bonding sites (Part I; Tutughamiarso *et al.*, 2012), we now present cocrystals with compounds containing variable hydrogen-bonding sites in order to study the influence of complementary binding on the conformational and the tautomeric equilibrium. Thus, we examined the compounds biuret, 6-acetamidouracil and 6-methylisocytosine. Owing to their rotatable C—N bond, biuret and 6-acetamidouracil show potential conformational flexibility. Since one main conformer of each of these molecules exhibits a hydrogen-bonding site complementary to the 5-FC molecule, we were interested if this conformer is observed in the cocrystal. 6-Methylisocytosine can adopt the 1*H* or the 3*H* tautomeric form. In order to investigate whether one specific tautomer can selectively be crystallized, 6-methylisocytosine was cocrystallized with 5-FC and 6-aminoisocytosine, respectively.



(I) 5-Fluorocytosine · Biuret · DMSO
(II) 6-Acetamidouracil
(III) 5-Fluorocytosine · 6-Acetamidouracil · DMSO
(IV*a*) 5-Fluorocytosine · 6-Methylisocytosine
(IV*b*) 5-Fluorocytosine · 6-Methylisocytosine · H_2O
(V) 6-Aminoisocytosine · 6-Methylisocytosine · 2 DMAC

Scheme 1

2. Experimental

2.1. Sample preparation

Almost all reagents are commercially available and were used without further purification. Single crystals of (I) were obtained by cocrystallization of 5-FC (2.1 mg, 0.016 mmol) and biuret (3.9 mg, 0.038 mmol) in dimethyl sulfoxide (DMSO, 150 µL) at 323 K; because of the higher solubility of biuret, 1:1 mixtures yielded only crystals or pseudopolymorphs of 5-FC. 6-Acetamidouracil was prepared from 6-aminouracil (5.0 g, 0.039 mol) by reaction with acetic anhydride (10.4 ml, 0.110 mol) in 1,4-dioxane (100 ml). After refluxing at 383 K for 24 h, the mixture was cooled to room temperature and the solvent was removed, whereupon a white solid precipitated (4.8 g, 72%). Crystals of (II) were obtained by recrystallization of 6-acetamidouracil (3.7 mg, 0.022 mmol) from DMSO (200 µL) at room temperature, while solvent evaporation experiments from a mixture of 5-FC (1.9 mg, 0.015 mmol) and 6-acetamidouracil (1.2 mg, 0.007 mmol) in DMSO (250 µL) at 323 K yielded crystals of (III). Crystals of (IV*a*) and (IV*b*) were obtained during attempts to cocrystallize 5-FC [2.2 mg, 0.017 mmol (IV*a*) and 2.3 mg, 0.018 mmol (IV*b*)] and 6-methylisocytosine [1.0 mg, 0.008 mmol (IV*a*) and 2.1 mg, 0.017 mmol (IV*b*)] in dimethylacetamide [DMAC, 400 µL (IV*a*) and 300 µL (IV*b*)] at 323 K. Cocrystallization of 6-methylisocytosine (2.3 mg, 0.018 mmol) and 6-aminoisocytosine (2.7 mg, 0.021 mmol) from DMAC (300 µL) at 323 K yielded crystals of (V). None of the solvents used in the experiments were water-free.

2.2. Crystal structure determination

The X-ray diffraction data were collected on a Stoe IPDS II two-circle diffractometer using monochromatic Mo $K\alpha$ radiation (λ = 0.71073 Å) at 173 K. Data reduction and cell refinement were carried out with *X-AREA* (Stoe & Cie, 2001). All structures were solved by direct methods (*SHELXS*97; Sheldrick, 2008) and refined by full-matrix least-squares techniques (*SHELXL*97; Sheldrick, 2008).[1]

The H atoms, except of those bonded to disordered solvent atoms, were initially located by difference Fourier synthesis. Subsequently, H atoms bonded to C atoms were refined using a riding model, with methyl C—H = 0.98 Å and aromatic C—H = 0.95 Å, and with U_{iso}(H) = 1.5U_{eq}(C) for methyl H or 1.2 U_{eq}(C) for aromatic H atoms. In (III) and (IV*a*) H atoms bonded to N atoms were refined isotropically. In the other structures their isotropic displacement parameters were smaller than the equivalent isotropic displacement parameter of the respective N atom [U_{iso}(H) < U_{eq}(N)]; thus they were coupled to those of the N atoms with U_{iso}(H) = 1.2U_{eq}(N). Additionally, the N—H distances, except for (IV*a*), for N3 and N1′ in (IV*b*) as well as for N1, N21 and N21′ in (V) were restrained to 0.88 (2) Å. For the water molecule in (IV*b*) the following restraints were applied during refinement: O—H = 0.88 (2) and H···H = 1.44 (4) Å, with U_{iso}(H) = 1.2U_{eq}(O). The methyl groups of 6-acetamidouracil [in (II) and (III)], 6-methylisocytosine [in (IV*a*), (IV*b*) and (V)] and the ordered dimethylacetamide [in (V)] were allowed to rotate about their local threefold axes.

[1] Supplementary data for this paper are available from the IUCr electronic archives (Reference: GP5053). Services for accessing these data are described at the back of the journal.

Table 1
Experimental details.

Experiments were carried out at 173 K with Mo $K\alpha$ radiation using a Stoe IPDS II two-circle diffractometer. H atoms were treated by a mixture of independent and constrained refinement.

	(I)	(II)	(III)
Crystal data			
Chemical formula	$C_4H_4FN_3O\cdot C_2H_5N_3O_2\cdot C_2H_6OS$	$C_6H_7N_3O_3$	$C_4H_4FN_3O\cdot C_6H_7N_3O_3\cdot C_2H_6OS$
M_r	310.32	169.15	376.38
Crystal system, space group	Triclinic, $P\overline{1}$	Monoclinic, $P2_1/c$	Monoclinic, $P2_1/c$
a, b, c (Å)	8.4065 (17), 8.9045 (18), 10.6441 (19)	11.4303 (12), 7.1695 (8), 8.9753 (11)	9.4478 (9), 23.7171 (16), 7.2635 (7)
α, β, γ (°)	111.163 (15), 96.337 (15), 105.703 (16)	90, 102.194 (9), 90	90, 96.700 (8), 90
V (Å^3)	695.8 (2)	718.93 (14)	1616.4 (2)
Z	2	4	4
μ (mm^{-1})	0.27	0.13	0.25
Crystal size (mm)	0.60 × 0.30 × 0.20	0.20 × 0.10 × 0.10	0.40 × 0.20 × 0.20
Data collection			
Absorption correction	Multi-scan *MULABS* (Blessing, 1995; Spek, 2009)	–	Multi-scan *MULABS* (Blessing, 1995; Spek, 2009)
T_{min}, T_{max}	0.856, 0.948	–	0.906, 0.952
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	4530, 2617, 1669	3983, 1337, 773	15 378, 3031, 2410
R_{int}	0.144	0.187	0.116
$(\sin\theta/\lambda)_{max}$ (Å^{-1})	0.611	0.607	0.611
Refinement			
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.094, 0.282, 0.97	0.061, 0.160, 0.85	0.045, 0.112, 1.05
No. of reflections	2617	1337	3031
No. of parameters	208	119	257
No. of restraints	8	3	3
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.62, −0.85	0.28, −0.27	0.35, −0.34

	(IV*a*)	(IV*b*)	(V)
Crystal data			
Chemical formula	$C_4H_4FN_3O\cdot C_5H_7N_3O$	$C_4H_4FN_3O\cdot C_5H_7N_3O\cdot H_2O$	$C_5H_7N_3O\cdot C_4H_6N_4O\cdot 2C_4H_9NO$
M_r	254.24	272.25	425.51
Crystal system, space group	Triclinic, $P\overline{1}$	Triclinic, $P\overline{1}$	Triclinic, $P\overline{1}$
a, b, c (Å)	4.5285 (7), 10.1491 (17), 12.626 (2)	5.1193 (8), 9.3392 (15), 12.4862 (19)	6.9824 (11), 9.1224 (14), 17.651 (3)
α, β, γ (°)	75.967 (13), 89.785 (13), 82.918 (13)	97.103 (12), 93.662 (13), 95.766 (13)	88.329 (12), 89.875 (12), 75.513 (12)
V (Å^3)	558.47 (16)	587.63 (16)	1088.1 (3)
Z	2	2	2
μ (mm^{-1})	0.12	0.13	0.10
Crystal size (mm)	0.30 × 0.30 × 0.30	0.40 × 0.40 × 0.20	0.40 × 0.20 × 0.20
Data collection			
Absorption correction	–	–	–
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	4506, 2099, 1675	8436, 2070, 1124	8046, 3820, 2241
R_{int}	0.113	0.181	0.085
$(\sin\theta/\lambda)_{max}$ (Å^{-1})	0.608	0.595	0.595
Refinement			
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.063, 0.175, 0.96	0.050, 0.122, 0.78	0.076, 0.234, 1.05
No. of reflections	2099	2070	3820
No. of parameters	189	197	308
No. of restraints	0	7	25
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.32, −0.31	0.21, −0.30	0.29, −0.29

Computer programs used: *X-AREA* (Stoe & Cie, 2001), *SHELXS*97, *SHELXL*97 (Sheldrick, 2008), *Mercury* (Macrae *et al.*, 2008), *XP* (Sheldrick, 2008).

In (III) the S atom of the dimethyl sulfoxide molecule is disordered over two positions, with a site-occupation factor of 0.944 (3) for the major occupied site; for the minor occupied site, similarity restraints were applied to the 1,2-distances and the S atom was refined isotropically.

In (V) the carbonyl C and the N atom of the dimethylacetamide molecule *A* are disordered over two positions, with a site-occupation factor of 0.75 (1) for the major occupied orientation. For the minor occupied orientation these atoms were refined isotropically. The following restraints were applied during the refinement of this solvent molecule: $O{=}C_{carbonyl}$ = 1.26 (1), $C_{carbonyl}{-}N$ = 1.29 (1), $C_{carbonyl}{-}C_{methyl}$ = 1.52 (1), $N{-}C_{methyl}$ = 1.49 (1), $O\cdots N$ = 2.22 (2), $O\cdots C_{methyl}$ = 2.43 (2), $N\cdots C_{methyl}$ = 2.40 (2), $C_{carbonyl}\cdots C_{methyl}$ = 2.42 (2) and $C_{methyl}\cdots C_{methyl}$ = 2.54 (2) Å.

For structural drawings the following programs were used: *Mercury* (Version 2.3; Macrae *et al.*, 2008) and *XP* (Sheldrick, 2008). Selected crystal data and experimental details are summarized in Table 1.

2.3. *Ab initio* calculations

Starting molecular geometries were generated either from crystal data or from molecular sketches using *Avogadro* (*Avogadro*: an open-source molecular builder and visualization tool. Version 1.0.3. http://avogadro.openmolecules.net/). *Ab initio* energy calculations were performed with geometry optimization and dispersion correction using *GAUSSIAN* (Frisch *et al.*, 2004) at the B3LYP-D/SVP level.

3. Influence of specific binding on the conformation

Depending on their molecular conformation, biuret and 6-acetamidouracil can undergo various hydrogen-bonding interactions. The two main conformers of biuret show different binding-site arrangements: in the *cis* form, the NH_2 and NH groups present a donor–donor–donor (*DDD*; *D*: hydrogen-bond donor) site, while in its *trans* form the intramolecular hydrogen bond between an amino H and a carbonyl O atom stabilizes a donor–donor–acceptor (*DDA*; *A*: hydrogen-bond acceptor) site. In addition to the fixed acceptor–donor–acceptor (*ADA*) site of the six-membered ring, 6-acetamidouracil also shows a variable hydrogen-bonding site with the participation of its side chain: a *DDA* site is present if the two adjacent NH groups point to the same direction (*cis* form), whereas an *ADA* site is formed if they are oriented in opposite directions (*trans* form). Both the *trans* conformation of biuret and the *cis* conformation of 6-acetamidouracil are complementary to the *AAD* site of 5-FC so that supramolecular complexes characterized by an *AAD*/*DDA* pattern can be formed. This was verified by the cocrystal solvates (I) and (III).

3.1. Cocrystal with biuret

Cocrystal (I), which was grown from DMSO solution, contains one 5-FC, one biuret and one dimethyl sulfoxide molecule in the asymmetric unit, with the solvent molecule accepting an N—H···O hydrogen bond from 5-FC (Fig. 1). The biuret molecule adopts the *trans* form, which is complementary to 5-FC; the N1 and C4 atoms are in a synperiplanar conformation [torsion angle = −6.5 (8)°] stabilized by an intramolecular N—H···O hydrogen bond. The 5-FC and the biuret molecule are held together by two N—H···O hydrogen bonds and a central N—H···N hydrogen bond with a dihedral angle of 9.6 (2)° enclosed between the planes through the non-H atoms of both molecules. In addition to the three desired hydrogen-bonding interactions, the 5-FC molecule is linked to another biuret molecule by two N—H···O hydrogen bonds

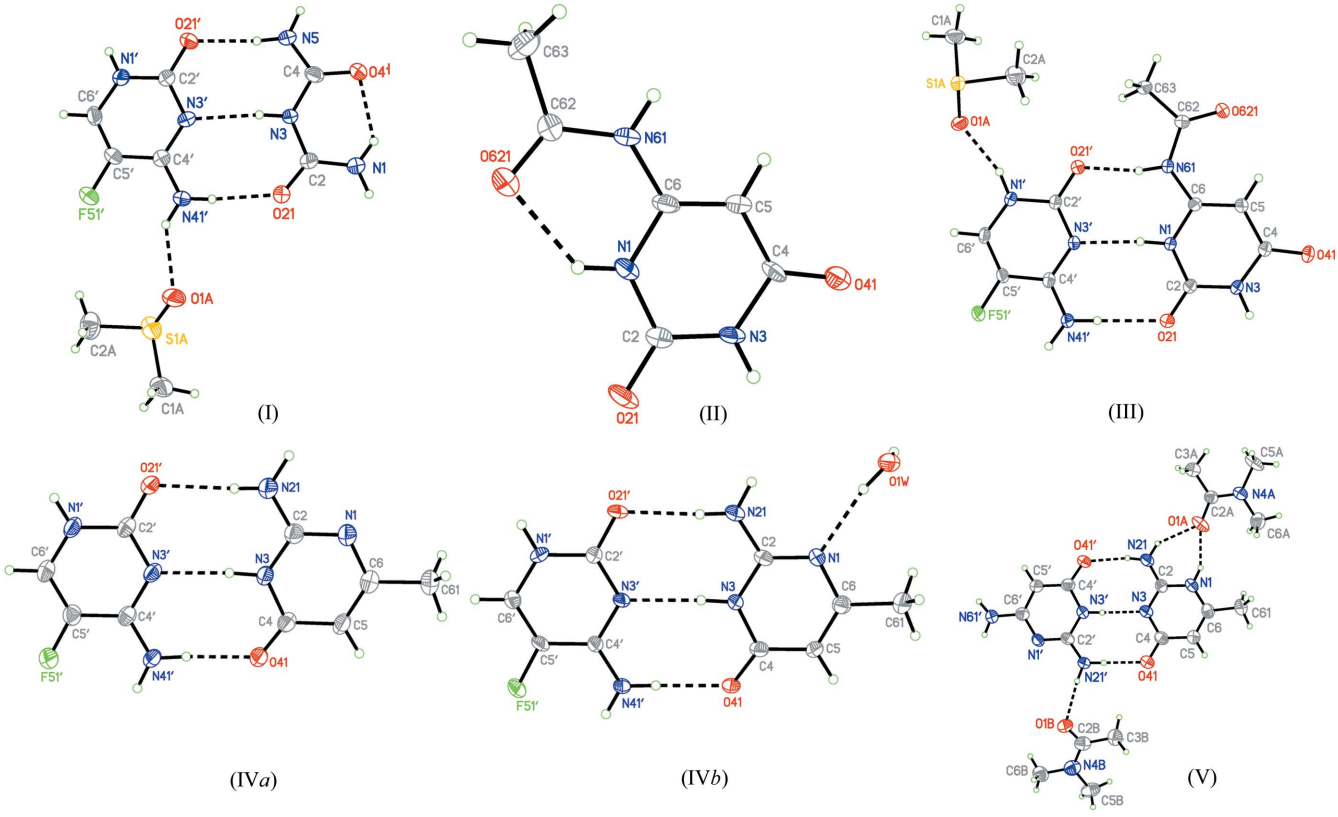


Figure 1
The asymmetric units and numbering schemes of (I)–(V). Displacement ellipsoids are drawn at the 50% probability level and H atoms are shown as small spheres of arbitrary radii. Hydrogen bonds are shown as a dashed line. The dimethyl sulfoxide molecule in (III) and one of the dimethylacetamide molecules (molecule *A*) in (V) are disordered; only the major occupied sites of these molecules are shown.

Table 2
Hydrogen-bond geometry (Å, °) for (I).

D—H···A	D—H (Å)	H···A (Å)	D···A (Å)	D—H···A (°)
N1′—H1′···O21^{i}	0.87 (2)	2.01 (2)	2.870 (5)	172 (5)
N41′—H42···O21	0.89 (2)	2.04 (3)	2.872 (5)	156 (6)
N41′—H41···O1*A*	0.87 (2)	2.06 (4)	2.845 (5)	149 (5)
N1—H12···O21′^{ii}	0.89 (2)	1.96 (3)	2.820 (5)	162 (5)
N1—H11···O41	0.88 (2)	2.06 (5)	2.694 (5)	128 (5)
N3—H3···N3′	0.88 (2)	2.09 (2)	2.972 (5)	174 (5)
N5—H51···O41^{iii}	0.88 (2)	2.12 (3)	2.982 (5)	166 (6)
N5—H52···O21′	0.87 (2)	2.01 (3)	2.834 (6)	156 (6)

Symmetry codes: (i) $x+1, y, z$; (ii) $x-1, y, z$; (iii) $-x+1, -y, -z+2$.

Table 3
Hydrogen-bond geometry (Å, °) for (II).

D—H···A	D—H (Å)	H···A (Å)	D···A (Å)	D—H···A (°)
N1—H1···O621	0.885 (19)	1.96 (3)	2.645 (4)	133 (4)
N3—H3···O41^{i}	0.880 (19)	1.89 (2)	2.759 (4)	171 (4)
N61—H61···O21^{ii}	0.867 (19)	1.94 (2)	2.799 (4)	170 (4)

Symmetry codes: (i) $-x+1, y-\frac{1}{2}, -z+\frac{3}{2}$; (ii) $x, y+1, z$.

with an $R_2^2(8)$ pattern (Bernstein *et al.*, 1995; Fig. 2, Table 2). Furthermore, two biuret molecules are also connected by an $R_2^2(8)$ interaction involving two N—H···O hydrogen bonds. This extended hydrogen-bonding network leads to ribbons parallel to (021) with the participation of the solvent molecules.

3.2. Cocrystal with 6-acetamidouracil

Recrystallization of 6-acetamidouracil from DMSO yielded a solvent-free structure (II). The 6-acetamidouracil molecule adopts the *trans* form: the N1 and C62 atoms as well as the C6 and O621 atoms are arranged in synperiplanar conformations [torsion angles: N1—C6—N61—C62 = −2.9 (5)° and C6—N61—C62—O621 = 2.0 (6)°; Fig. 1]. There is an intramolecular N—H···O hydrogen bond closing (as in biuret) a six-membered ring, and the r.m.s. deviation of 0.015 Å for all non-H atoms indicates that the molecule is planar. In the crystal

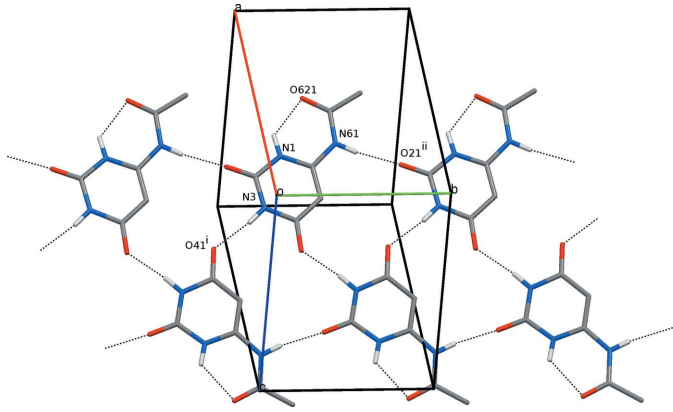


Figure 3
Partial packing diagram for (II). Dashed lines indicate hydrogen bonds. Only the H atoms involved in hydrogen bonding are shown. Symmetry codes: (i) $-x+1, y-\frac{1}{2}, -z+\frac{3}{2}$; (ii) $x, y+1, z$.

packing, the 6-acetamidouracil molecules are connected to ribbons, which run along the *b* axis and are stabilized by repeated $R_3^3(14)$ trimer interactions involving three single N—H···O hydrogen bonds (Fig. 3, Table 3).

Attempts to cocrystallize 5-FC and 6-acetamidouracil from DMSO yielded the cocrystal solvate (III) with the S atom of the solvent molecule disordered over two positions (Fig. 1). The 6-acetamidouracil molecule is essentially planar (r.m.s. deviation = 0.039 Å for all non-H atoms) and, in contrast to (II), adopts the *cis* form, which is complementary to the 5-FC molecule. Now the C62 atom is antiperiplanar to the N1 atom, while the C6 and O621 atoms are still synperiplanar to each other [torsion angles: N1—C6—N61—C62 = 175.31 (17)° and C6—N61—C62—O621 = 2.3 (3)°]. 5-FC and 6-acetamidouracil are connected by three hydrogen bonds forming a heterodimer with an *AAD*/*DDA* pattern; a dihedral angle of 6.9 (1)° is enclosed between the planes through the non-H atoms of both molecules. In the crystal packing, N—H···O hydrogen bonds link the heterodimers into chains, which are further bridged to layers parallel to (102) by N—H···O hydrogen bonds between the solvent molecules and both cocrystal components (Fig. 4, Table 4).

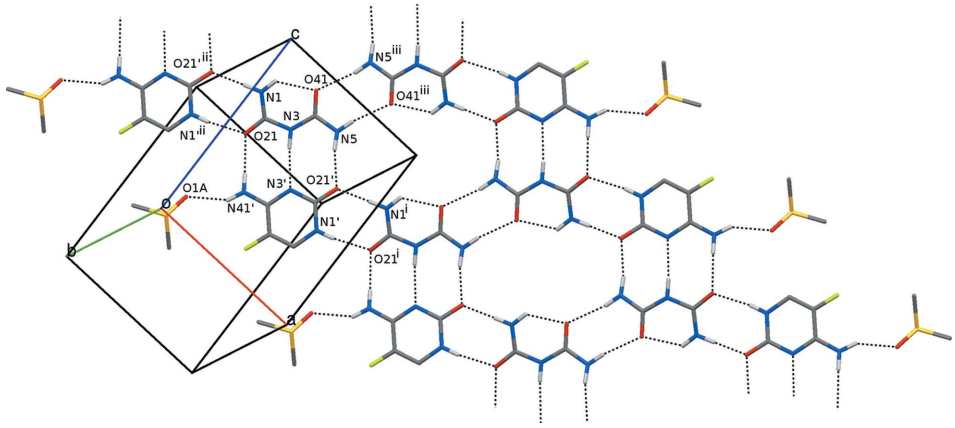


Figure 2
Partial packing diagram for (I). Dashed lines indicate hydrogen bonds. Only the H atoms involved in hydrogen bonding are shown. Symmetry codes: (i) $x+1, y, z$; (ii) $x-1, y, z$; (iii) $-x+1, -y, -z+2$.

4. Influence of specific binding on the tautomeric equilibrium

6-Methylisocytosine can exist in two main tautomeric forms: 1*H* and 3*H*. These tautomers show different arrangements of hydrogen-bond donor and acceptor groups: the 1*H* tautomer exhibits an *AAD* hydrogen-bonding site, the 3*H* tautomer a *DDA* hydrogen-bonding site. In addition, a *DD* (1*H*) or a *DA* (3*H*) site is available.

A previous tautomeric study indicated that (in contrast to 6-

Table 4
Hydrogen-bond geometry (Å, °) for (III).

D—H···A	D—H (Å)	H···A (Å)	D···A (Å)	D—H···A (°)
N1—H1···N3′	0.90 (3)	1.99 (3)	2.883 (2)	173 (3)
N3—H3···O1A^{i}	0.89 (3)	2.06 (3)	2.938 (2)	168 (2)
N61—H61···O21′	0.88 (3)	1.87 (3)	2.736 (2)	168 (2)
N1′—H1′···O1A	0.92 (3)	1.92 (3)	2.799 (2)	160 (3)
N41′—H41C···O621^{ii}	0.94 (3)	2.01 (3)	2.944 (2)	177 (3)
N41′—H41D···O21	0.89 (3)	2.08 (3)	2.966 (2)	175 (2)

Symmetry codes: (i) $-x+1, y-\frac{1}{2}, -z+\frac{3}{2}$; (ii) $x-1, -y+\frac{1}{2}, z+\frac{1}{2}$.

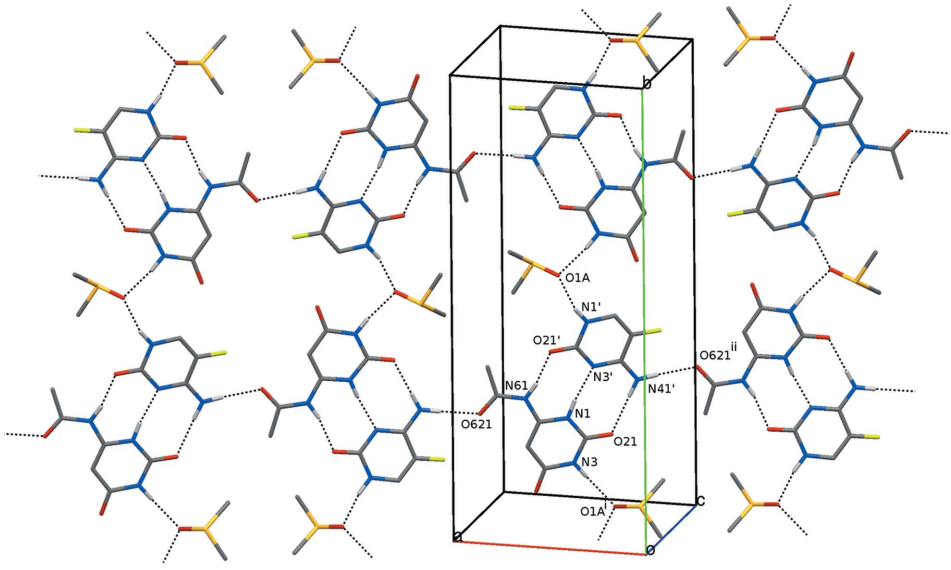


Figure 4
Partial packing diagram for (III). Dashed lines indicate hydrogen bonds. Only the major occupied sites of the disordered solvent molecules and the H atoms involved in hydrogen bonding are shown. Symmetry codes: (i) $-x+1, y-\frac{1}{2}, -z+\frac{3}{2}$; (ii) $x-1, -y+\frac{1}{2}, z+\frac{1}{2}$.

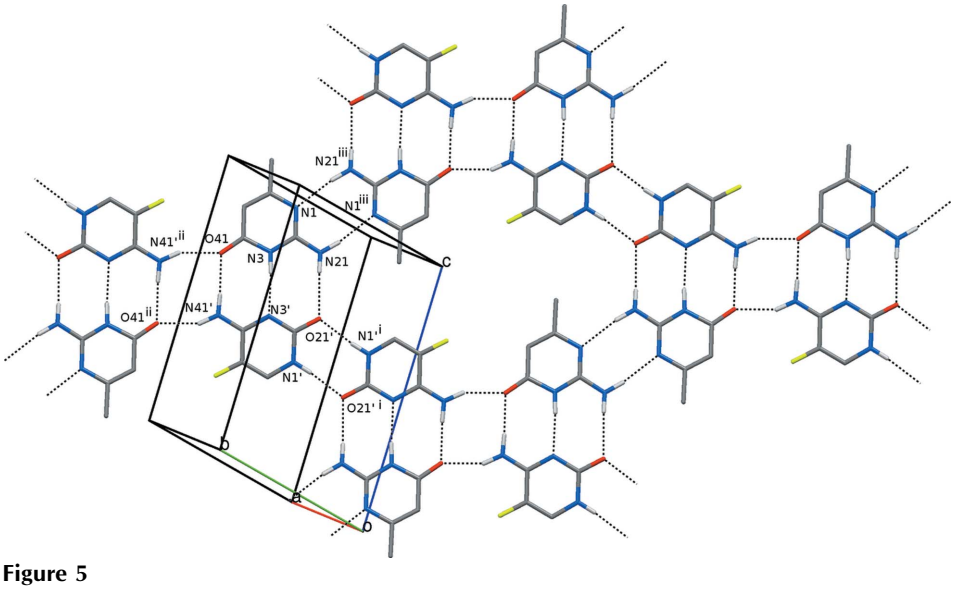


Figure 5
Partial packing diagram for (IVa). Dashed lines indicate hydrogen bonds. Only the H atoms involved in hydrogen bonding are shown. Symmetry codes: (i) $-x, -y+1, -z+1$; (ii) $-x+2, -y+2, -z+1$; (iii) $-x+1, -y+1, -z+2$.

aminoisocytosine, see below) 6-methylisocytosine can crystallize as the 1*H* or 3*H* tautomer, or as a 1:1 mixture of both tautomers (Gerhardt *et al.*, 2011). Therefore, in order to crystallize one tautomeric form selectively, a complementary coformer allowing for *AAD*/*DDA* heterodimer formation could be very useful; 5-FC (*AAD*) and 6-aminoisocytosine (*DDA*) meet this condition. Indeed cocrystallization experiments with 5-FC and 6-methylisocytosine yielded a solvent-free cocrystal (IVa) and a cocrystal hydrate (IVb) in which 6-methylisocytosine adopts the 3*H* tautomeric form. On the other hand, cocrystallization of 6-aminoisocytosine and 6-methylisocytosine gave cocrystal (V); here 6-methylisocytosine crystallized as the 1*H* tautomer.

4.1. Cocrystals with the (3*H*) tautomeric form of 6-methylisocytosine

In the solvent-free cocrystal (IVa) the 5-FC and the 6-methylisocytosine molecules are planar (r.m.s. deviations for all non-H atoms = 0.015 and 0.020 Å, respectively) with a dihedral angle of 10.7 (1)° between the molecular planes (Fig. 1). Two N—H···O hydrogen bonds and one N—H···N hydrogen bond connect the two molecules to the desired *AAD*/*DDA* heterodimer. Furthermore, two heterodimers are linked to a tetramer by four N—H···O hydrogen bonds with an $R_4^2(8)$ motif (Fig. 5, Table 5). The packing shows layers parallel to $(\bar{1}21)$ containing circular arrangements of four tetramers, stabilized by $R_2^2(8)$ interactions with either two N—H···O hydrogen bonds between the 5-FC molecules or two N—H···N hydrogen bonds between the 6-methylisocytosine molecules. This crystal packing resembles that of the 5-FC–6-aminoisocytosine–dimethylformamide (2:2:1) cocrystal [structure (Vc) in Part I; Tutughamiarso *et al.*, 2012].

In the hydrate (IVb) the 5-FC and the 6-methylisocytosine molecules are also planar (r.m.s. deviations for all non-H atoms = 0.004 and 0.009 Å, respectively). As in (IVa), both molecules are connected to a heterodimer by three hydrogen bonds, with a

Table 5
Hydrogen-bond geometry (Å, °) for (IV*a*).

D—H···*A*	*D*—H (Å)	H···*A* (Å)	*D*···*A* (Å)	*D*—H···*A* (°)
N1′—H1′···O21′^{i}	0.88 (3)	1.90 (3)	2.775 (2)	172 (3)
N41′—H411···O41^{ii}	0.93 (3)	1.94 (3)	2.810 (2)	154 (2)
N41′—H412···O41	0.90 (3)	1.93 (3)	2.828 (2)	176 (3)
N3—H3···N3′	0.93 (3)	2.00 (3)	2.930 (2)	176 (2)
N21—H21*A*···N1^{iii}	0.98 (3)	2.12 (3)	3.090 (3)	171 (2)
N21—H21*B*···O21′	0.88 (3)	2.17 (3)	3.045 (2)	176 (3)

Symmetry codes: (i) $-x, -y+1, -z+1$; (ii) $-x+2, -y+2, -z+1$; (iii) $-x+1, -y+1, -z+2$.

Table 6
Hydrogen-bond geometry (Å, °) for (IV*b*).

D—H···*A*	*D*—H (Å)	H···*A* (Å)	*D*···*A* (Å)	*D*—H···*A* (°)
N3—H3···N3′	0.87 (3)	2.06 (3)	2.921 (3)	177 (3)
N21—H21*A*···O21′^{i}	0.871 (18)	2.09 (3)	2.831 (3)	143 (3)
N21—H21*B*···O21′	0.910 (18)	2.043 (19)	2.952 (4)	177 (3)
N1′—H1′···O1*W*^{i}	0.88 (3)	1.96 (3)	2.829 (3)	170 (3)
N41′—H41*A*···O41	0.924 (18)	1.922 (19)	2.845 (4)	178 (3)
N41′—H41*B*···O41^{ii}	0.893 (18)	2.02 (2)	2.807 (3)	146 (3)
O1*W*—H1*W*···O21′^{iii}	0.898 (18)	1.913 (19)	2.803 (3)	171 (3)
O1*W*—H2*W*···N1	0.891 (18)	2.013 (19)	2.896 (3)	171 (3)

Symmetry codes: (i) $-x+1, -y, -z+1$; (ii) $-x+1, -y+1, -z+2$; (iii) $-x, -y, -z+1$.

dihedral angle of 8.8 (1)° between the planes through each component (Fig. 1). In the packing of (IV*b*), however, the heterodimers are connected to ribbons parallel to $(1\bar{2}2)$ by two alternating groups of four N—H···O interactions with an $R_4^2(8)$ pattern (Fig. 6, Table 6). The solvent water molecules participate in three hydrogen bonds: the N—H···O_w and the O_w—H_w···N interactions further connect the heterodimers within a ribbon, while the O_w—H_w···O hydrogen bonds bridge adjacent ribbons to a three-dimensional network. The crystal packing of (IV*b*) is similar to that of the 5-FC–6-aminoisocytosine–water (1:1:1) cocrystal [structure (V*a*) in Part I; Tutughamiarso *et al.*, 2012].

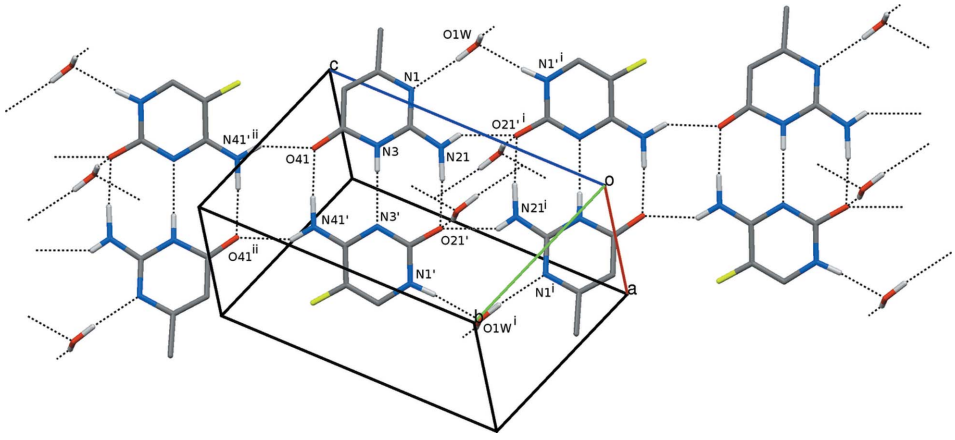


Figure 6
Partial packing diagram for (IV*b*). Dashed lines indicate hydrogen bonds. Only the H atoms involved in hydrogen bonding are shown. Symmetry codes: (i) $-x+1, -y, -z+1$; (ii) $-x+1, -y+1, -z+2$.

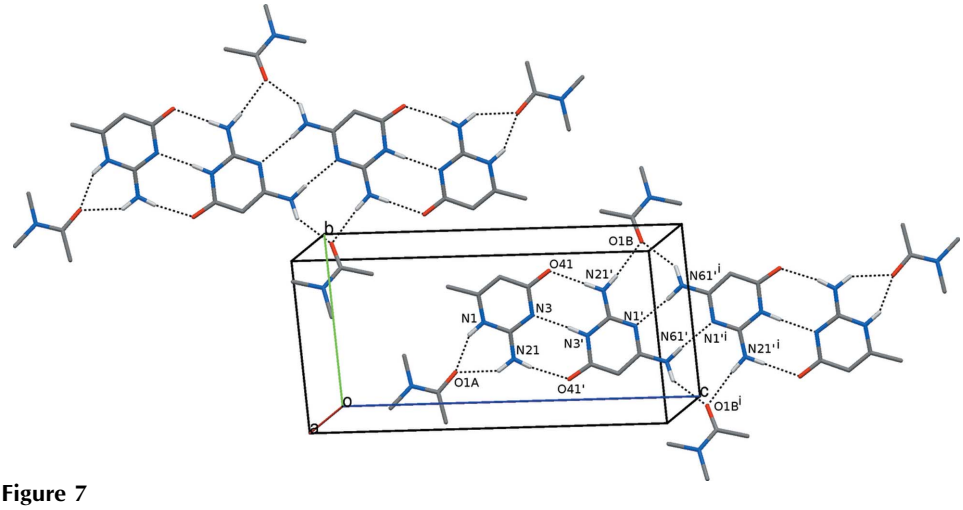


Figure 7
Partial packing diagram for (V). Dashed lines indicate hydrogen bonds. Only the major occupied sites of the disordered solvent molecules and the H atoms involved in hydrogen bonding are shown. Symmetry code: (i) $-x+1, -y+1, -z+2$.

4.2. Cocrystal with the (1*H*) tautomeric form of 6-methylisocytosine

The asymmetric unit of cocrystal (V) consists of one 6-aminoisocytosine, one 6-methylisocytosine and two DMAC molecules (Fig. 1). 6-Aminoisocytosine – in the 3*H* form (as usual) – and 6-methylisocytosine – in the 1*H* form – are complementary to each other; thus the desired *AAD*/*DDA* heterodimer is observed. One DMAC molecule is N—H···O hydrogen-bonded to 6-aminoisocytosine, while the other one is linked to 6-methylisocytosine by two N—H···O hydrogen bonds with an $R_2^1(6)$ motif. The latter solvent molecule is disordered over two sites with all non-H atoms of these two sites in a common plane (r.m.s. deviation = 0.019 Å). In the crystal packing, N—H···N hydrogen bonds with an $R_2^2(8)$ pattern connect two symmetry-equivalent heterodimers to a tetramer, which is further stabilized by DMAC molecules through two N—H···O hydrogen bonds. The packing of (V) shows layers parallel to (211) consisting of discrete arrange-

Table 7
Hydrogen-bond geometry (Å, °) for (V).

D—H···A	D—H (Å)	H···A (Å)	D···A (Å)	D—H···A (°)
N21′—H21B···O1B	0.88 (5)	2.10 (5)	2.981 (5)	174 (4)
N21′—H21A···O41	0.90 (5)	1.94 (5)	2.836 (5)	176 (4)
N3′—H3′···N3	0.89 (2)	1.96 (2)	2.839 (4)	170 (4)
N61′—H61B···N1′^{i}	0.89 (2)	2.15 (2)	3.019 (5)	165 (5)
N61′—H61A···O1B^{i}	0.90 (2)	2.11 (3)	2.893 (5)	145 (4)
N1—H1···O1A	0.86 (5)	1.97 (5)	2.770 (5)	154 (4)
N21—H21D···O41′	0.88 (5)	1.98 (5)	2.832 (5)	164 (5)
N21—H21C···O1A	0.89 (5)	2.13 (5)	2.885 (5)	143 (4)

Symmetry code: (i) $-x+1, -y+1, -z+2$.

ments of these tetramers (Fig. 7, Table 7).

5. Discussion

In the absence of strong environmental effects, molecular conformations with an intramolecular hydrogen bond – as in the *trans* form of biuret and 6-acetamidouracil – are especially stable (Böhm & Klebe, 1996). According to a CSD search (Cambridge Structural Database, Version 5.33 of November 2011, plus two updates; Allen, 2002) for acyclic biuret fragments, 15 out of 17 entries (88%) show conformations with intramolecular hydrogen bonds (the charges of the two outer biuret N atoms were defined to be neutral and only the H atom of the central N atom was added in the search query). In the biuret hydrate (CSD refcode BIUHYD; Hughes *et al.*, 1961) and in the cyanuric acid–biuret cocrystal (refcode JOLSAE; Stainton *et al.*, 1991), the biuret molecule also crystallized in the *trans* form. However, in metal complexes [refcodes: BIURSR (Haddad & Gentile, 1975), BIUZNC (Nardelli *et al.*, 1963), FUJYEO (Haddad, 1987), BIZJUQ and BIZKAX (Harrison, 2008)], in which biuret acts as a bidentate ligand, it prefers the *cis* form in spite of the repulsion between the two adjacent carbonyl O atoms. In cocrystal (I) biuret adopts the *trans* conformation and is connected to 5-FC by three hydrogen bonds. Since the *trans* form shows a hydrogen-bonding site complementary to 5-FC and is apparently more stable than the *cis* form, the formation of the desired heterodimer in (I) is straightforward.

In this study both principal conformations of 6-acetamidouracil were obtained: in the solvent-free structure (II) the *trans* form is observed, while in the cocrystal with 5-FC (III) the *cis* conformation is adopted. Similar to biuret, the *trans* form of 6-acetamidouracil is stabilized by an intramolecular N—H···O hydrogen bond; hence its formation in (II) is expected. Although the *cis* form of 6-acetamidouracil has a higher energy than the *trans* form (ΔE = 23.9 kJ mol^{-1} according to *ab initio* calculations), it exhibits a hydrogen-bonding site complementary to 5-FC and therefore allows the formation of the desired *AAD*/*DDA* heterodimer in cocrystal (III). Obviously the energy release during complex formation is large enough to compensate for the energy difference between the molecular conformations.

Ab initio energy calculations were also undertaken to determine the relative stabilities between the 1*H* and the 3*H* tautomeric forms of 6-methylisocytosine and 6-aminoisocytosine. In both cases the 3*H* tautomer is significantly more stable than the 1*H* tautomer (ΔE = 44.1 and 71.7 kJ mol^{-1} for 6-methylisocytosine and 6-aminoisocytosine, respectively). The large energy difference between the two tautomers of 6-aminoisocytosine may be caused by the repulsion of the three amino H atoms presenting a *DDD* hydrogen-bonding site. This might explain why our attempts to selectively crystallize its 1*H* form were not successful. Thus, 6-aminoisocytosine can be regarded as a compound with fixed hydrogen-bonding sites [*cf.* structures (V*a*)–(V*d*) in Part I; Tutughamiarso *et al.*, 2012]. Despite the calculated energy difference of 44.1 kJ mol^{-1}, both tautomeric forms of 6-methylisocytosine were selectively crystallized: in cocrystals with 5-FC, (IV*a*) and (IV*b*), it exists as a 3*H* tautomer, while in the cocrystal with 6-aminoisocytosine, (V), the 1*H* tautomer is observed. In the presence of a suitable coformer, 6-methylisocytosine apparently adopts the tautomeric form with a complementary binding site, which enables the formation of the desired *AAD*/*DDA* heterodimer.

6. Conclusion

We have presented five crystal structures of supramolecular complexes; each is held together by three hydrogen bonds similar to those constituting the Watson–Crick C·G base pair. Cocrystallization attempts with 5-FC and the two conformationally flexible molecules biuret and 6-acetamidouracil yielded the desired complexes (I) and (III). In both cocrystals, each coformer adopts the conformation which allows the formation of an *AAD*/*DDA* heterodimer: biuret exhibits the energetically favoured *trans* form, but 6-acetamidouracil exhibits the less stable *cis* form. The three complexes of 6-methylisocytosine demonstrate that one tautomer can be selectively crystallized by the formation of a heterodimer: in the cocrystals with 5-FC, (IV*a*) and (IV*b*), only the 3*H* tautomeric form is present, whereas in the cocrystal with 6-aminoisocytosine, (V), 6-methylisocytosine exists in the 1*H* tautomeric form. Obviously the specific binding is well able to shift the conformational or the tautomeric equilibrium, if the energy gained during complex formation is larger than the energy difference between the conformations or the tautomers.

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