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6-Benzylamino-2-(2-hydroxyethylamino)-9-methylpurine-1,7-diium bis(perchlorate) monohydrate

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Key indicators

Single-crystal X-ray study
T = 100 K
Mean σ(C–C) = 0.003 Å
R factor = 0.046
wR factor = 0.120
Data-to-parameter ratio = 12.2

For details of how these key indicators were automatically derived from the article, see http://journals.iucr.org/e.

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The title compound, $C_{15}H_{20}N_6O^{2+}\cdot 2ClO_4^-\cdot H_2O$, belongs to a group of cytokinin-derived compounds. It has been found that some cytokinin derivatives, *e.g.* 2,6,9-trisubstituted purine derivatives, behave as potent inhibitors of cyclin-dependent kinases (CDKs) and show anticancer activity. Olomoucine, *i.e.* the title cation in its neutral form, represents one of the first CDK specific inhibitors that selectively blocks CDK1, CDK2 and CDK5 kinases at micromolar concentrations. The asymmetric unit of the title compound consists of the diprotonated cation, two perchlorate anions and a water molecule of crystallization. The cation contains nearly planar benzene and purine ring systems, the dihedral angle between them being 46.77 (6)°. The crystal structure is stabilized by $N_{purine}—H\cdots O_{water}/O_{hydroxy}$, $N_{amine}—H\cdots O_{water}/O_{hydroxy}$, $O_{water}—H\cdots O_{perchlorate}$ and $O_{hydroxy}—H\cdots O_{perchlorate}$ hydrogen bonds.

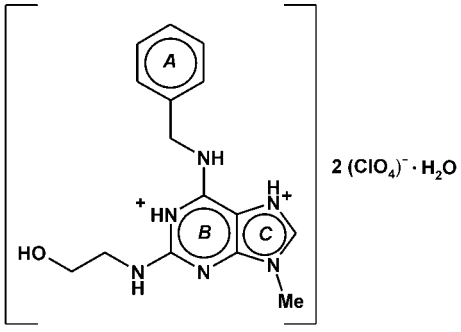
Comment

The frequent dysregulation of cancer cells has stimulated an intensive search for new chemical substances targeting cell division cycle events. The cell cycle is regulated by the timely and spatially coordinated action of cyclin-dependent kinases (CDKs), their positive and negative effectors. Inappropriate expression or mutations of CDKs, their modulators and even their substrates have often been detected in various cancers (Sherr, 1996). Therefore, CDK activity represents one of the most interesting targets for new generations of anticancer drugs. However, CDKs are also involved in other processes, such as apoptosis (CDK1, CDK5), neuronal functions (CDK5, CDK11) and transcription (CDK7, CDK8, CDK9). One can thus expect a large variety of cellular effects, and therefore also applications for CDK inhibitors. Besides oncology, the compounds are studied extensively for their promising influence on neurodegenerative disorders (Alzheimer's and Parkinson's diseases), cardiovascular diseases (restenosis), viral (HIV, Herpes, cytomegalovirus, papillomavirus) and parasitic (*Plasmodium*, *Leishmania*, *Trypanosoma*) infections and also for their potential use in *in vitro* reproduction and cloning (Meijer & Raymond, 2003).

The first compound identified as a specific and selective inhibitor of CDK1 and CDK2, 6-benzylamino-2-(2-hydroxyethylamino)-9-methylpurine, also named olomoucine (Veselý *et al.*, 1994), became a lead compound for the further design of improved drugs, *e.g.* roscovitine and purvalanols (Meijer & Raymond, 2003). The X-ray structure of, among others, the CDK2-olomoucine complex has also been determined (Schulze-Gahmen *et al.*, 1995). Many biological effects of CDK inhibitors in cellular assays have been described, and a significant correlation has been found between inhibition of

CDKs and antiproliferative activity (Vermeulen *et al.*, 2002). In general, cell treatment with relevant concentrations of CDK inhibitors leads to dephosphorylation of the corresponding protein substrates and delay in progression through the cell cycle, to stimulation of *p*53-dependent transcription and subsequent synthesis of $p21^{WAF1}$ (Kotala *et al.*, 2001), to induction of apoptosis in cancer cells both *in vitro* and *in vivo* (McClue *et al.*, 2002).

Among known purine inhibitors, (*R*)-roscovitine (under synonym CYC202) is currently undergoing phase II clinical trials against lung and breast cancers and phase I tests against glomerulonephritis (Meijer & Raymond, 2003). In summary, the antiproliferative and proapoptotic effects of 2,6,9-trisubstituted purines suggest that these drugs have both an important and a promising anticancer potential.



(I)

The asymmetric unit of the title compound, (I), consists of a diprotonated organic cation, two perchlorate anions and a solvent water molecule (Fig. 1 and Table 1). It should be noted that the title cation represents only the fifth structurally characterized analog of 2,6,9-trisubstituted purines involving the 6-benzylaminopurine moiety (Cambridge Structural Database, Version 5.25.2; Allen, 2002) after (*R*)- and (*S*)-6-benzylamino-2-[(1-hydroxymethyl)propylamino]-9-isopropylpurine (Wang *et al.*, 2001), 2,6-diamino-N^2,N^6-dibenzoyl-9-(α-L-*threo*furanosyl)purine (Wu *et al.*, 2002; Schoning *et al.*, 2002), *N*-[(2-azepan-1-yl)-9-isopropyl-9*H*-purin-6-yl]-4-methoxybenzylamine (Trávníček & Zatloukal, 2004) and pentakis[trichloro(6-benzylamino-2-(3-hydroxypropyl)amino)-9-isopropylpurinium]platinum(II) (Trávníček *et al.*, 2003). Moreover, the structure of the cation is also similar to those determined for 6-(4-methoxybenzylamino)-purinium chloride (4MeOBapH; Trávníček *et al.*, 2004), 6-benzylaminopurinium bromide (BapH; Umadevi *et al.*, 2001), 6-(2-chlorobenzylamino)purine (2ClBap; Maloň *et al.*, 2001), 6-(3-chlorobenzylamino)purinium chloride (3ClBapH; Maloň *et al.*, 2001), 6-(4-chlorobenzylamino)purinium perchlorate (4ClBapH; Maloň *et al.*, 2002) and 6-(2-hydroxybenzylamino)purine (2OHBap; Trávníček *et al.*, 1997)

The cation contains nearly planar benzene (*A*), pyrimidine (*B*) and imidazole (*C*) ring systems, with maximum deviations from each plane of 0.006 (3), 0.013 (2) and 0.008 (2) Å for ring *A*, six-membered ring *B* and five-membered ring *C*, respectively (Nardelli, 1995). Atoms forming the purine ring (*B* + *C*) deviate slightly from planarity, the greatest deviation being 0.0149 (19) Å for atom N9. Planes *B* and *C* are nearly coplanar, with a dihedral angle of 0.79 (8)°, whilst the dihedral angles between planes *A* and *B*, and *A* and the purine ring (*B* + *C*) are 46.97 (7) and 46.77 (6)°, respectively. The *Cg*1···*Cg*2, *Cg*1···*Cg*3 and *Cg*2···*Cg*3 distances are 6.3670 (2), 7.0460 (2) and 2.0817 (1) Å, respectively, where *Cg*1, *Cg*2 and *Cg*3 are the centroids of rings *A*, *B* and *C*, respectively. The torsion angles C6—N6—C9—C10, C9—N6—C6—C5 and N6—C9—C10—C15 are 154.8 (2), 171.0 (2) and 61.3 (3)°, respectively.

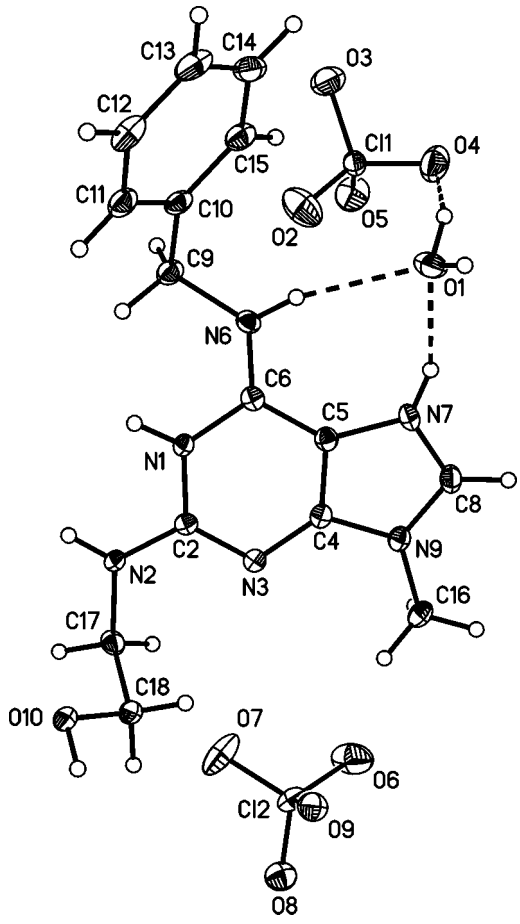


Figure 1
The asymmetric unit of the title compound, showing the hydrogen bonding (dashed lines). Displacement ellipsoids are drawn at the 50% probability level and H atoms are shown as small spheres at arbitrary radii.

The cation is protonated at the N1 and N7 positions of the purine ring, *i.e.* it represents the N1-protonated N7 tautomer. It is evident that changes in protonation and substitution of the purine moiety cause changes in the interatomic parameters within the purine ring, mainly in the C—N—C angles. To date, 31 structures of compounds involving the 6-benzylaminopurine moiety have been deposited with the Cambridge Structural Database (Version 5.25.2; Allen, 2002). The main changes occur at the C2—N3—C4, C8—N7—C5 and C8—N9—C4 angles. A comparison of these interatomic parameters

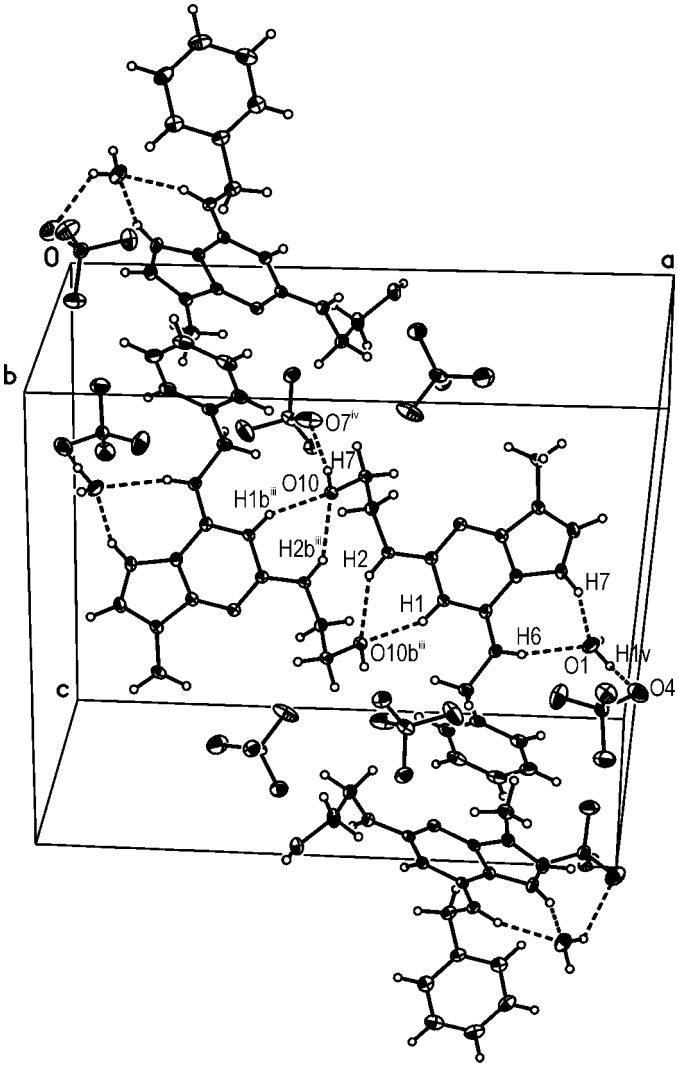


Figure 2
Part of the crystal structure of the title compound, showing the hydrogen bonding (dashed lines). [Symmetry codes: (iii) $1-x, 1-y, 1-z$; (iv) $1-x, y-\frac{1}{2}, \frac{1}{2}-z$.]

for selected cytokinin-derivatives with the same group is given in Table 3.

The positive charge of the cation is compensated by two perchlorate anions. The crystal structure is stabilized by a network of $N_{purine}-H\cdots O_{water}/O_{hydroxy}$, $N_{amine}-H\cdots O_{water}/O_{hydroxy}$, $O_{water}-H\cdots O_{perchlorate}$ and $O_{hydroxy}-H\cdots O_{perchlorate}$ hydrogen bonds, connecting adjacent cation, perchlorate anions and water molecules (Fig. 2 and Table 2).

Experimental

6-Benzylamino-2-(2-hydroxyethylamino)-9-methylpurine (olomoucine) was synthesized by a procedure similar to that described in the literature for the preparation of 2,6,9-trisubstututed purine derivatives (Imbach *et al.*, 1999). Colorless crystals of the title compound suitable for single-crystal X-ray analysis were obtained by recrystallization of olomoucine from 2 *M* $HClO_4$. Elemental analysis (CHN Analyzer Flash EA 1112, ThermoFinnigen), calculated for $C_{15}H_{22}Cl_2N_6O_{10}$: C 34.83, H 4.29, N 16.25%; found: C 34.6, H 4.2, N 16.3%.

Crystal data

$C_{15}H_{20}N_6O^{2+}\cdot 2ClO_4^-\cdot H_2O$	$D_x = 1.584$ Mg m^{-3}
$M_r = 517.29$	Mo $K\alpha$ radiation
Monoclinic, $P2_1/c$	Cell parameters from 12391 reflections
$a = 17.8770$ (7) Å	$\theta = 1.8$–$31.8°$
$b = 7.4072$ (3) Å	$\mu = 0.37$ mm^{-1}
$c = 16.4214$ (6) Å	$T = 100$ (2) K
$\beta = 94.187$ (4)°	Cube, colorless
$V = 2168.69$ (15) Å^3	0.40 × 0.40 × 0.40 mm
$Z = 4$	

Data collection

Oxford Diffraction Xcalibur2 (Sapphire2 CCD) diffractometer	3569 reflections with $I > 2\sigma(I)$
ω scans	$R_{int} = 0.030$
Absorption correction: none	$\theta_{max} = 25.0°$
13724 measured reflections	$h = -21 \rightarrow 21$
3802 independent reflections	$k = -8 \rightarrow 7$
	$l = -19 \rightarrow 19$

Refinement

Refinement on F^2	$w = 1/[\sigma^2(F_o^2) + (0.06P)^2 + 4.28P]$ where $P = (F_o^2 + 2F_c^2)/3$
$R[F^2 > 2\sigma(F^2)] = 0.046$	$(\Delta/\sigma)_{max} = 0.001$
$wR(F^2) = 0.120$	$\Delta\rho_{max} = 0.94$ e Å^{-3}
$S = 1.00$	$\Delta\rho_{min} = -0.63$ e Å^{-3}
3802 reflections	
311 parameters	
H atoms treated by a mixture of independent and constrained refinement	

Table 1
Selected geometric parameters (Å, °).

N1—C6	1.366 (3)	C5—N7	1.387 (3)
N1—C2	1.394 (3)	C5—C6	1.406 (3)
N2—C2	1.329 (3)	N6—C6	1.317 (3)
C2—N3	1.327 (3)	N6—C9	1.472 (3)
N3—C4	1.336 (3)	N7—C8	1.320 (3)
C4—C5	1.378 (3)	C8—N9	1.346 (3)
C4—N9	1.380 (3)		
C6—N1—C2	124.03 (19)	C4—C5—C6	118.8 (2)
N3—C2—N1	123.3 (2)	N7—C5—C6	134.0 (2)
C2—N3—C4	112.54 (19)	N1—C6—C5	113.1 (2)
N3—C4—C5	128.2 (2)	C8—N7—C5	108.10 (19)
N3—C4—N9	125.2 (2)	N7—C8—N9	110.0 (2)
C5—C4—N9	106.61 (19)	C8—N9—C4	108.05 (19)
C4—C5—N7	107.25 (19)		
C9—N6—C6—C5	171.0 (2)	N6—C9—C10—C15	61.3 (3)
C6—N6—C9—C10	154.8 (2)		

Table 2
Hydrogen-bonding geometry (Å, °).

D—H···A	D—H	H···A	D···A	D—H···A
O1—H1W···O3^{i}	0.90 (3)	1.99 (3)	2.865 (3)	163 (4)
O1—H1V···O3^{ii}	0.90 (3)	2.10 (3)	2.885 (3)	146 (4)
O1—H1V···O4	0.90 (3)	2.51 (3)	3.214 (3)	136 (3)
N1—H1···O10^{iii}	0.88	1.98	2.794 (2)	154
N2—H2···O10^{iii}	0.88	2.18	2.942 (2)	145
N6—H6···O1	0.88	2.17	3.024 (3)	162
N7—H7···O1	0.88	1.86	2.688 (3)	156
O10—H10···O7^{iv}	0.936 (10)	1.844 (16)	2.738 (3)	159 (3)

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

Table 3
Comparative bond angles (°) for selected cytokinin-derived compounds containing the 6-benzylaminopurine moiety.

Compound	C2—N3—C4	C8—N7—C5	C8—N9—C4
OlomoucineH_2[a]	112.54 (19)	108.10 (19)	108.05 (19)
NG38[b]	110.77 (10)	103.50 (11)	105.95 (10)
4MeOBapH[c]	117.02 (16)	106.58 (16)	103.01 (15)
BapH[d]	118.2 (7)	107.4 (6)	103.5 (6)
2ClBap[e]	111.32 (14)	103.68 (15)	106.19 (14)
3ClBapH[f]	117.6 (2)	106.8 (2)	102.60 (18)
4ClBapH[g]	113.8 (8)	109.1 (8)	103.7 (8)
	119.7 (8)	104.6 (8)	100.9 (8)
Bap[h]	110.70	103.90	106.41
2OHBap[i]	111.5 (3)	104.1 (3)	106.4 (3)
(*R*)-Roscovitine[j]	110.91 (17)	103.48 (18)	105.55 (17)
	111.56 (16)	103.58 (17)	105.39 (17)
BTAP[k]	110.99 (26)	104.68 (26)	105.35 (24)

Notes: (*a*) this work, where olomoucineH_2 is the title cation; (*b*) Trávníček *et al.* (2004), where NG38 is *N*-[(2-azepan-1-yl)-9-isopropyl-9*H*-purin-6-yl]-4-methoxybenzylamine; (*c*) Trávníček *et al.* (2004), where 4MeOBapH is the 6-(4-methoxybenzylamino)purinium cation; (*d*) Umadevi *et al.* (2001), where BapH is the 6-benzylaminopurinium cation; (*e*) Maloň *et al.* (2001), where 2ClBap is 6-(2-chlorobenzylamino)purine; (*f*) Maloň *et al.* (2001), where 3ClBapH is the 6-(3-chlorobenzylamino)purinium cation; (*g*) Maloň *et al.* (2002), where 4ClBapH is the 6-(4-chlorobenzylamino)purinium cation (the structure consists of two crystallographically independent molecules); (*h*) Raghunathan *et al.* (1983), where Bap is 6-benzylaminopurine; (*i*) Trávníček *et al.* (2004), where 2OHBap is 6-(2-hydroxybenzylamino)purine; (*j*) Wang *et al.* (2001), where (*R*)-roscovitine is (*R*)-6-benzylamino-2-[(1-hydroxymethyl)propylamino]-9-isopropylpurine (the structure consists of two crystallographically independent molecules); (*k*) Wu *et al.* (2002), where BTAP is 2,6-diamino-N^2,N^6-dibenzoyl-9-(α-L-*threo*furanosyl)purine.

H atoms attached to C and N atoms were found in difference Fourier maps, idealized and refined using a riding model, with C—H distances of 0.95 and 0.99 Å and N—H distances of 0.88 Å, and with $U_{iso}(H) = 1.2U_{eq}$(CH, $CH_2$and NH) or $1.5U_{eq}(CH_3)$. All H atoms attached to O atoms were refined isotropically.

Data collection: *CrysAlis CCD* (Oxford Diffraction, 2004); cell refinement: *CrysAlis RED* (Oxford Diffraction, 2004); data reduction: *CrysALis RED*; program(s) used to solve structure: *SHELXS*97 (Sheldrick, 1990); program(s) used to refine structure: *SHELXL*97 (Sheldrick, 1997); molecular graphics: *ORTEP*III (Burnett & Johnson, 1996); software used to prepare material for publication: *SHELXL*97 and *PARST* (Nardelli, 1995).

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