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N^8-(2′-*O*-Methylribofuranosyl)-8-aza-7-deazaadenine monohydrate

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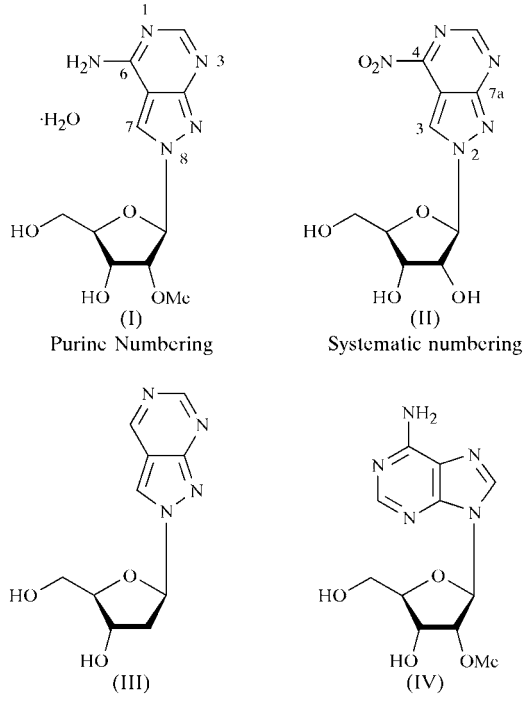
In the title compound, 4-amino-2-(2-*O*-methyl-β-D-ribofuranosyl)-2*H*-pyrazolo[3,4-*d*]pyrimidine monohydrate, $C_{11}H_{15}N_5O_4\cdot H_2O$, the conformation of the N-glycosylic bond is *syn* [χ = 20.1 (2)°]. The ribofuranose moiety shows a C3′-*endo* ($^3T_2$) sugar puckering (N-type sugar), and the conformation at the exocyclic C4′—C5′ bond is −*ap* (*trans*). The nucleobases are stacked head-to-head. The three-dimensional packing of the crystal structure is stabilized by hydrogen bonds between the 2′-*O*-methylribonucleosides and the solvent molecules.

Comment

Considerable efforts have been focused on the study of nucleobase analogues that fulfil the concepts of an ideal universal base. A universal base should be capable of replacing any of the four naturally occurring bases without discrimination and significant destabilization of the DNA duplex. Such bases would be of great use in both mutagenesis and recombinant DNA experiments (Loakes, 2001). The N^8-2′-deoxyribonucleosides of 8-aza-7-deazaadenine and 2-amino-8-aza-7-deazaadenine show the properties of universal nucleosides because of their ambiguous base pairing when incorporated into oligonucleotide duplexes opposite to the four canonical DNA constituents (Seela & Debelak, 2000; Seela *et al.*, 2000; He & Seela, 2002, 2003).

The 2′-*O*-methyl modification of ribonucleosides is a natural variety of the canonical RNA constituents and is part of the minor components found in tRNA. Synthetically obtained 2′-*O*-methyloligoribonucleotides are of great use in antisense technology because of their enhanced RNase and DNase resistance, and the increased thermal stability of their duplexes and triplexes (Sproat *et al.*, 1989; Oberhauser & Wagner, 1992; Goodchild, 1992). In order to design an artificial nucleoside that combines the concept of a universal base and the properties of a highly enzymatically resistant DNA/RNA building block into one molecule, 4-amino-2-(2-*O*-methyl-β-D-ribofuranosyl)-2*H*-pyrazolo[3,4-*d*]pyrimidine monohydrate [N^8-(2′-*O*-methylribofuranosyl)-8-aza-7-deazaadenine monohydrate; purine numbering is used throughout the paper], (I), was synthesized (Leonard *et al.*, 2006).



The single-crystal X-ray structure of (I) is described here. The three-dimensional structure is shown in Fig. 1 and selected geometric parameters are summarized in Table 1.

For the canonical ribonucleosides, the orientation of the base relative to the sugar (*syn*/*anti*) is defined by the torsion angle χ (O4′—C1′—N9—C4) (purine numbering; IUPAC–

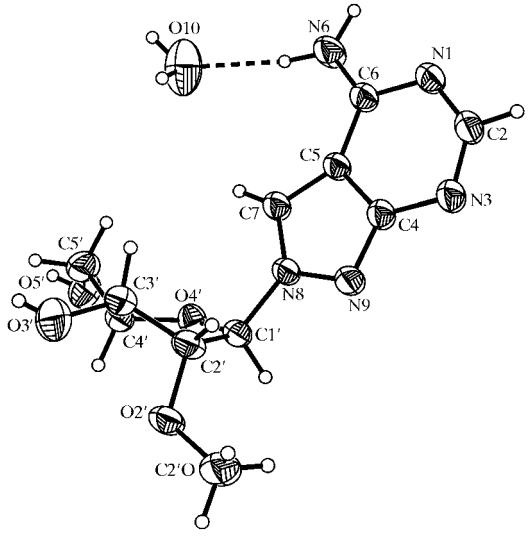


Figure 1
A perspective view of (I), showing the atomic numbering scheme. Displacement ellipsoids of non-H atoms are drawn at the 50% probability level and H atoms are shown as small spheres of arbitrary size.

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IUB Joint Commission on Biochemical Nomenclature, 1983). For (I), this nomenclature is, however, not suitable. According to Seela & Debelak (2000), the conformation of the N-glycosylic bond is defined as O4′—C1′—N8—C7. The nucleoside is in the *anti* conformation when the distance between atoms H1′ and H7 is at a minimum and *syn* when at a maximum. In the crystal structure of (I), the torsion angle of the glycosylic bond is χ = 20.1 (2)°, confirming *syn* conformation. Similar values were observed for the crystal structures of 4-nitro-2-(β-D-ribofuranosyl)-2*H*-indazole, (II) (type 2; Seela *et al.*, 2004), and 2-(2-deoxy-β-D-*erythro*-furanosyl)-2*H*-pyrazolo[3,4-*d*]pyrimidine, (III) (He *et al.*, 2002). The length of the N-glycosylic bond is 1.472 (2) Å in (I), which is almost identical to that of (II) (type 2; Seela *et al.*, 2004).

Owing to the stabilizing effect of the hydroxy group in the 2′-position of the ribose ring, the predominant conformation observed for the ribofuranosyl moiety is the north conformation (C3′-*endo*). In contrast, both the C2′-*endo* (south) conformation (Hingerty *et al.*, 1977) and the C3′-*endo* (north) conformation (Prusiner & Sundaralingam, 1976) have been reported for 2′-*O*-methylated ribonucleosides. The 2-*O*-methylribofuranose moiety of (I) exhibits a pseudorotation phase angle, *P*, of 14.3 (1)° and an amplitude, τ_m, of 39.6 (1)° (Rao *et al.*, 1981), indicating that the sugar is in the N conformation. The C3′-*endo* ($^3T_2$) sugar puckering of (I) is consistent with the conformation found for unmodified ribonucleosides. This type of sugar conformation has also been reported for (II) (type 2; Seela *et al.*, 2004) and 2′-*O*-methyladenosine, (IV) (molecule *A*; Prusiner & Sundaralingam, 1976).

The conformation of the exocyclic C4′—C5′ bond is *−ap* (*trans*) with a C3′—C4′—C5′—O5′ torsion angle of γ = −177.23 (13)°. A similar value has also been found for the structures of (II) [type 2; γ = −176.1 (5)°; Seela *et al.*, 2004], whereas a *+sc* (*+gauche*) (γ = 48.7° for molecule *A*) and *+ap* (*trans*) (γ = 179.3° for molecule *B*) conformation about the C4′—C5′ bond has been observed for (IV).

Another parameter of interest is the conformation of the methoxy group. In (I), the methoxy group adopts a *+sc* conformation [C1′—C2′—O2′—C2′O = 85.3 (2)°]. In contrast, torsion angles with a *+ac* conformation have been found for the crystal structure of (IV) (Prusiner & Sundaralingam, 1976).

The heterocyclic ring system of (I) is nearly planar, the r.m.s. deviation of the ring atoms from the N1/C2/N3/C4/C5/C6/C7/N8/N9 least-squares plane being 0.0087 Å, with a maximum deviation of −0.017 (2) Å for atom C5. The exocyclic groups lie on different sides of the plane. Atom N6 of the amine group is situated 0.037 (3) Å above and atom C1′ of the sugar moiety lies 0.015 (2) Å below this plane. Compound (I) forms a 1:1 complex with water, which is stabilized by hydrogen bonds.

In the crystalline state, the ribonucleoside molecules form a layered three-dimensional network consisting of alternating layers (Fig. 2), with the base moieties stacked head-to-head. The water molecules are situated between the ribonucleotide layers and connect the 2′-*O*-methylribonucleosides of adjacent layers *via* hydrogen bonding (Table 2 and Fig. 2). The water molecules act as hydrogen-bond acceptors (O10), forming bifurcated hydrogen bonds with the H3′—O3′ and N6′—H6 groups, and as donors (H10). Furthermore, intermolecular hydrogen bonds are formed between neighbouring nucleobases (N6—H6*A*···N1^{i}) and the sugar moieties of the adjacent molecules (O5′—H5′···N3^{iii}) (Table 2).

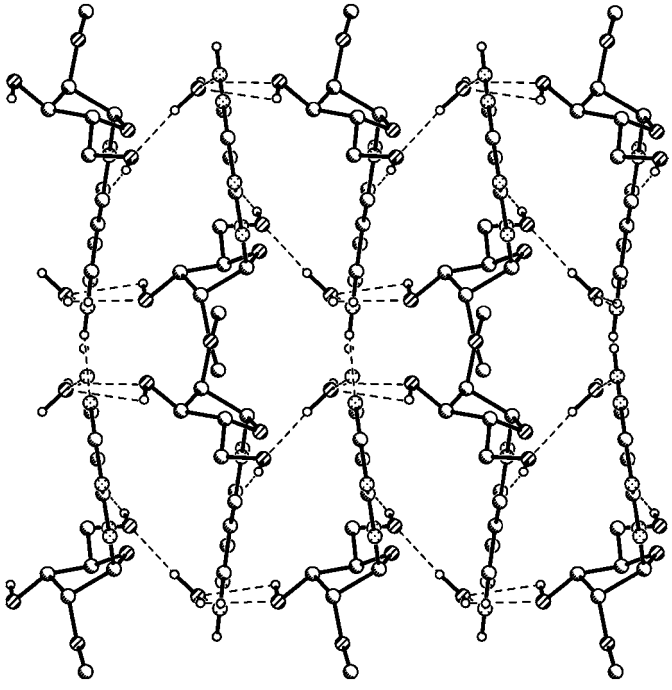

Figure 2
The two-dimensional crystal packing of (I), viewed down the *c* axis (*a* axis up), showing the layered structure of the crystal. Intermolecular hydrogen bonds are indicated by dashed lines.

Experimental

The benzoyl-protected derivative of (I) was synthesized in nitromethane with BF_3 etherate as catalyst. The glycosylation of 4-amino-1*H*-pyrazolo[3,4-*d*]pyrimidine (Robins, 1956) with 2-*O*-methyl-1,3,5-tri-*O*-benzoyl-α-D-ribofuranose (Chavis *et al.*, 1982) furnished two regioisomers, *viz.* 4-amino-1-(2-*O*-methyl-3,5-di-*O*-benzoyl-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine, (V), and 4-amino-2-(2-*O*-methyl-3,5-di-*O*-benzoyl-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]-pyrimidine, (VI). The regioisomer (VI) (650 mg, 1.3 mmol) was deprotected in methanol saturated with ammonia (60 ml) at room temperature overnight. Flash chromatography (silica gel; column 10 × 4 cm; CH_2Cl_2/CH_3OH 4:1) furnished (I) as a colourless foam (240 mg, 66%). Crystallization from a methanol–water mixture gave the title compound (m.p. 422–424 K). TLC (CH_2Cl_2/CH_3OH 4:1): R_F = 0.5. UV (MeOH): 238 (6400). ^{1}H NMR (DMSO-d_6): δ 8.57 (*s*, 1H, H–C6), 8.15 (*s*, 1H, H–C3), 7.71 (*br s*, 2H, NH_2), 6.04 (*d*, 1H, *J* = 3.7 Hz, H–C1′), 5.25 (*d*, 1H, *J* = 5.9 Hz, HO–C3′), 4.94 (*t*, 1H, *J* = 5.5 Hz, HO–C5′), 4.30 (*dd*, 1H, J_1 = 10.5 Hz, J_2 = 5.2 Hz, H–C3′), 4.17 (*t*, 1H, *J* = 4.2 Hz, H–C2′), 4.00 (*dd*, 1H, J_1 = 9.0 Hz, J_2 = 4.7 Hz, H–C4′), 3.70–3.49 (*m*, 2H, H–C5′), 3.38 (*s*, 3H, OCH_3).

Crystal data

$C_{11}H_{15}N_5O_4\cdot H_2O$
M_r = 299.30
Monoclinic, $C2$
a = 17.263 (2) Å
b = 7.5673 (7) Å
c = 10.4757 (8) Å
β = 93.128 (8)°
V = 1366.4 (2) Å^3
Z = 4
D_x = 1.455 Mg m^{-3}
Mo $K\alpha$ radiation
Cell parameters from 42 reflections
θ = 4.8–12.5°
μ = 0.12 mm^{-1}
T = 293 (2) K
Block, colourless
0.54 × 0.4 × 0.3 mm

Data collection

Bruker $P4$ diffractometer
$2\theta/\omega$ scans
2836 measured reflections
2318 independent reflections
2225 reflections with $I > 2\sigma(I)$
R_{int} = 0.018
θ_{max} = 31.0°
$h = -1 \rightarrow 24$
$k = -10 \rightarrow 1$
$l = -15 \rightarrow 15$
3 standard reflections
every 97 reflections
intensity decay: none

Refinement

Refinement on F^2
$R[F^2 > 2\sigma(F^2)]$ = 0.036
$wR(F^2)$ = 0.108
S = 1.00
2318 reflections
201 parameters
H atoms treated by a mixture of independent and constrained refinement
$w = 1/[\sigma^2(F_o^2) + (0.0802P)^2 + 0.1324P]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max} < 0.001$
$\Delta\rho_{max}$ = 0.29 e Å^{-3}
$\Delta\rho_{min}$ = −0.16 e Å^{-3}

Table 1
Selected geometric parameters (Å, °).

N8—N9	1.3555 (15)	C2′—O2′	1.4086 (17)
N8—C1′	1.4718 (17)	O2′—C2′O	1.420 (2)
N6—C6—N1	118.58 (13)	C7—N8—C1′	128.26 (12)
N6—C6—C5	123.34 (13)	N9—N8—C1′	116.86 (11)
C7—N8—N9	114.85 (11)	C2′—O2′—C2′O	113.74 (13)
C2—N1—C6—N6	−178.91 (18)	N9—N8—C1′—C2′	80.13 (17)
C7—C5—C6—N6	0.6 (3)	C1′—C2′—O2′—C2′O	85.3 (2)
C7—N8—C1′—O4′	20.1 (2)	O4′—C4′—C5′—O5′	65.55 (16)
N9—N8—C1′—O4′	−161.81 (14)	C3′—C4′—C5′—O5′	−177.23 (13)
C7—N8—C1′—C2′	−97.96 (19)		

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

D—H···A	D—H	H···A	D···A	D—H···A
N6—H6A···N1^{i}	0.86	2.12	2.964 (2)	169
N6—H6B···O10^{ii}	0.86	2.10	2.927 (2)	161
O3′—H3′···O10	0.81 (1)	2.38 (5)	2.724 (3)	107 (4)
O5′—H5′···N3^{iii}	0.82	1.95	2.756 (2)	170
O10—H10A···O10^{iv}	0.96 (1)	2.61 (4)	3.125 (5)	115 (3)
O10—H10B···O5′^{v}	0.97 (4)	1.90 (4)	2.852 (3)	169 (4)

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

In the absence of suitable anomalous scattering, Friedel equivalents could not be used to determine the absolute structure. Refinement of the Flack (1983) parameter led to inconclusive values (Flack & Bernardinelli, 2000) for this parameter [0.6 (9)]. Therefore, Friedel equivalents (282) were merged before the final refinements. The known configuration of the parent molecule was used to define the enantiomer employed in the refined model. All H atoms were initially found in a difference Fourier synthesis. In order to maximize the data/parameter ratio, the H atoms were placed in geometrically idealized positions (C—H = 0.93–0.98 Å, O—H = 0.82 Å and N—H = 0.86 Å) and constrained to ride on their parent atoms with U_{iso}(H) values of $1.2U_{eq}$(C,N) and $1.5U_{eq}$(O). DFIX constraints were used for O—H distances involving atoms O3′ and O10.

Data collection: *XSCANS* (Siemens, 1996); cell refinement: *XSCANS*; data reduction: *SHELXTL* (Sheldrick, 1997); program(s) used to solve structure: *SHELXTL*; program(s) used to refine structure: *SHELXTL*; molecular graphics: *SHELXTL*; software used to prepare material for publication: *SHELXTL* and *PLATON* (Spek, 1999).

Supplementary data for this paper are available from the IUCr electronic archives (Reference: TR1128). Services for accessing these data are described at the back of the journal.

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