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A locked derivative of 8-aza-7-deaza-adenosine

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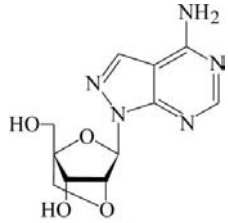
The title compound [systematic name: (1*S*,3*S*,4*R*,7*S*)-3-(4-amino-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-1-hydroxymethyl-2,5-dioxabicyclo[2.2.1]heptan-7-ol], $C_{11}H_{13}N_5O_4$, belongs to a family of nucleosides with modifications in both the sugar and nucleobase moieties: these modifications are known to increase the thermodynamic stability of DNA and RNA duplexes. There are two symmetry-independent molecules in the asymmetric unit that differ significantly in conformation, and both exhibit a high-*anti* conformation about the N-glycosidic bond, with χ torsion angles of −85.4 (3) and −87.4 (3)°. The sugar C atom attached to the nucleobase N atom is −0.201 (4) and 0.209 (4) Å from the 8-aza-7-deaza-adenine skeleton plane in the two molecules. The molecules are assembled into layers *via* hydrogen bonds and π–π stacking interactions between the modified nucleobases.

Comment

Modifications influencing the properties of native nucleic acids can be introduced into the nucleobase, the sugar ring or the phosphodiester backbone. The introduction of the 2′-*C*,4′-*C*-oxomethylene linker into the nucleotide sugar moiety is known to 'lock' chemically the pentofuranose ring in the 3′-*endo* conformation, which is ideal for recognition of RNA and predominates in the *A*-type helical duplexes (Koshkin *et al.*, 1998; Obika *et al.*, 1998; Kaur *et al.*, 2007). Oligonucleotides incorporating these bicyclic analogues are termed 'locked nucleic acids' (LNAs) (Koshkin *et al.*, 1998). LNAs show unprecedented thermodynamic stability when hybridized with complementary DNA or RNA strands, and this substantially increases the melting point temperature when compared to unmodified duplexes (Koshkin *et al.*, 1998; Obika *et al.*, 1997; Kierzek *et al.*, 2006; Pasternak *et al.*, 2007). Owing to their many unusual properties, LNAs are expected to have an impact in many areas of medicine and biotechnology (Petersen & Wengel, 2003; Kaur *et al.*, 2007).



(VII)

Increased stability of oligonucleotide duplexes can also be achieved by nucleobase modifications. For example, oligonucleotides containing 8-aza-7-deaza-2′-deoxyadenosine (the numbering scheme used is as for the purine skeleton) form duplexes with slightly increased stability compared with those containing unmodified adenine (Seela & Kaiser, 1988). This stability can be further enhanced when C7-modified 8-aza-7-deazaadenines are incorporated into oligonucleotide strands (Seela & Zulauf, 1999). 8-Aza-7-deazaadenine is considered to be one of the universal bases due to similar thermodynamic stability when interacting with all four natural nucleobases (Seela & Debelak, 2000, 2001).

In this paper, we report the synthesis (Fig. 1) and crystal structure of the title compound, (VII), a locked derivative of 8-aza-7-deazaadenosine and a sugar- and nucleobase-modified nucleoside derivative. Compound (VII) was prepared using a silyl method (Vorbrüggen & Krolikiewicz, 1975) with 8-aza-7-deazaadenine and a precursor of the LNA derivative of ribose used for the condensation reaction. The reaction yielded two products, with the major product (60%) transformed into (VII) using standard procedures (Koshkin *et al.*, 1998). Compound (VII) was identified as the major product: the minor product, presumably the N^8-isomer, was not isolated.

Molecules of (VII), comprising a rigid sugar moiety locked in the 3′-*endo* conformation, can be considered as having two conformational degrees of freedom (O—H bonds not considered). The two torsions are: (i) the angle χ (O4′—C1′—N9—C4) at the N-glycosidic bond and (ii) the angle γ (C3′—C4′—C5′—O5′) at the bond between the hydroxymethylene group and the pentofuranose ring [for nomenclature of torsion angles, see IUPAC–IUB Joint Commission on Biochemical Nomenclature (1983)]. There are two symmetry-independent molecules (*A* and *B*) in the asymmetric unit of (VII) (Fig. 2) and for both molecules the χ angle, which describes the orientation of the base relative to the sugar residue (*syn*/*anti*), has a value which lies in the high-*anti* conformation range [−85.4 (3) and −87.4 (3)° for molecules *A* and *B*, respectively], whereas in the adenine analogue, the nucleobase is in the *anti* orientation (χ = −163.8°; Morita *et al.*, 2003). The high-*anti* conformation of (VII) along with the C3′-*endo* 'locked' sugar moiety conformation leads to intramolecular C—H···N interactions and, in molecule *A*, a H3′···N8 contact of 2.35 Å is present; in molecule *B*, atom N8 forms contacts with H2′ and H3′ (2.59 and 2.46 Å, respectively). From previous studies, conformations about the glycosidic bond in locked purine nucleosides can be either *anti* or high-*anti*:

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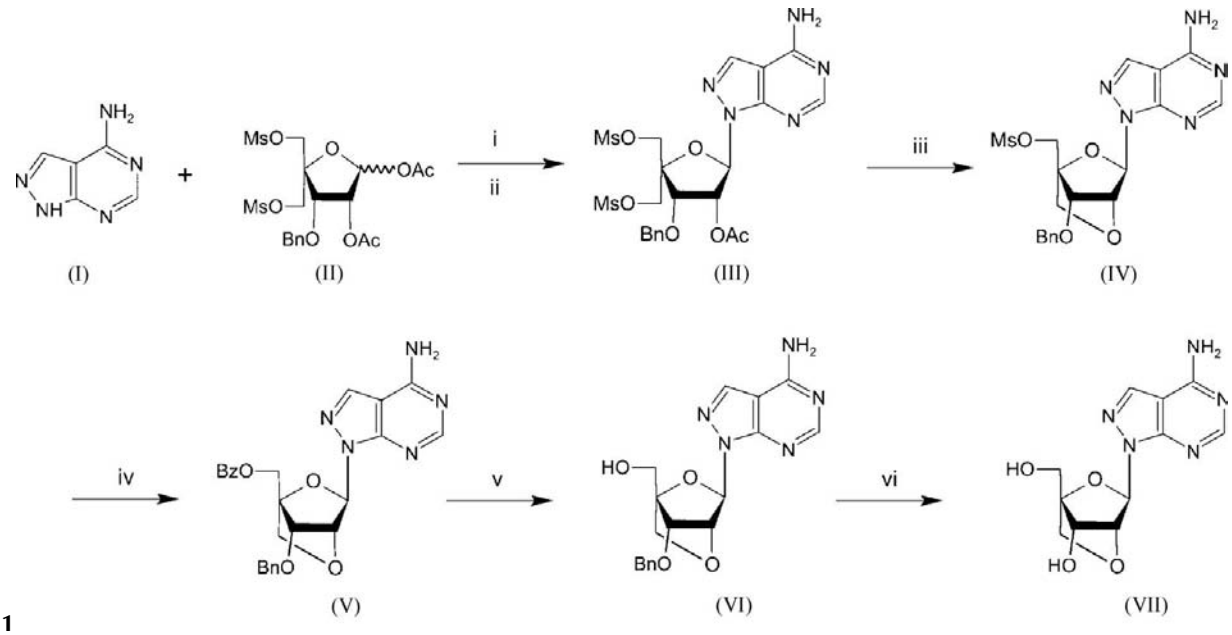

Figure 1
The synthesis of (VII). Notes: (i) hexamethylenedisilazane (HMDS), $(NH_4)_2SO_4$, 4 h, 403 K; (ii) trimethylsilyl trifluoromethanesulfonate (TMSOTf), CH_3CN, 48 h, 353 K; (iii) $LiOH{\cdot}H_2O$, THF, H_2O, 4 h, room temperature; (iv) BzOLi, DMF, 18 h, 353 K; (v) NH_4OH, pyridine, 24 h, 328 K; (vi) HCO_2NH_4, Pd/C, MeOH, 2 h, 333 K. Bn is benzyl ($PhCH_2$–) and Ms is methylsulfonyl ($MeSO_2$–).

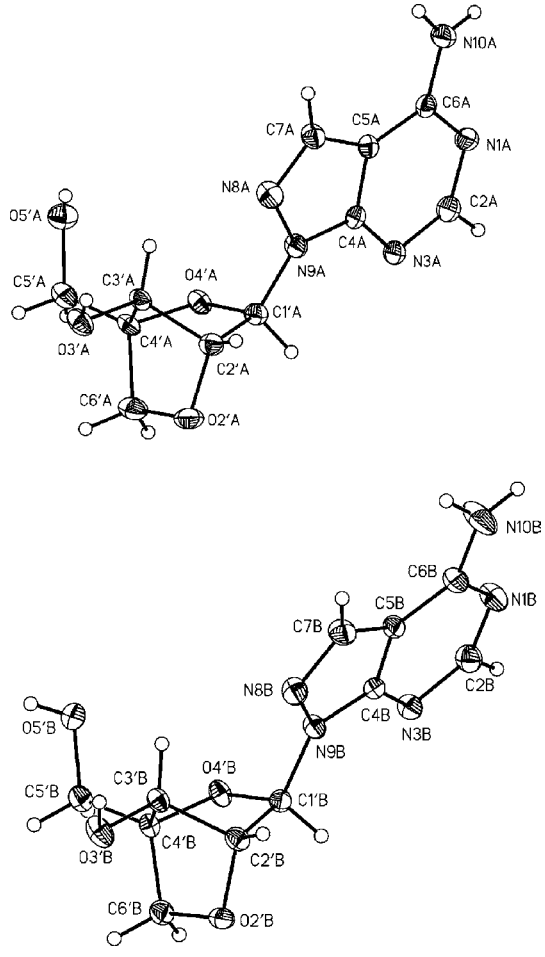

Figure 2
The molecular structure of (VII), showing the two symmetry-independent molecules, *viz.* *A* (top) and *B* (bottom), with displacement ellipsoids drawn at the 30% probability level.

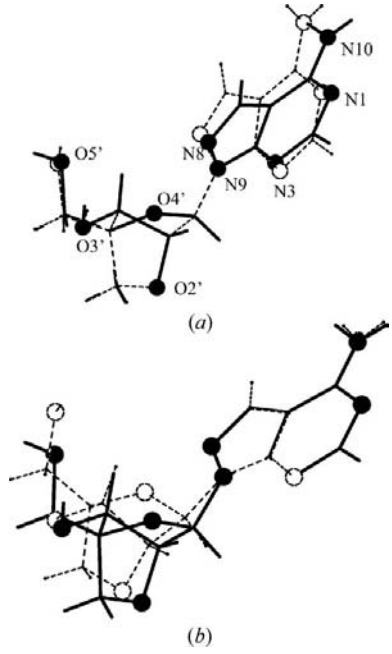

Figure 3
Superposition of the two symmetry-independent molecules of (VII), showing (*a*) the superposition in which 2,5-dioxabicyclo[2.2.1]heptane moieties were fitted and (*b*) the superposition in which 8-aza-7-deazaadenine fragments were fitted. Molecule *A* is shown with dashed lines and molecule *B* is shown with full lines. O and N atoms are represented as spheres.

structural evidence of the guanine analogue of (VII) (Rosenbohm *et al.*, 2004) has two molecules adopting the high-*anti* conformation (χ = −75.5 and −78.2°) and two cases with an *anti* conformation [χ = −174.5 and −177.8° (Z' = 4)]. Similarly, in 8-aza-7-deazaadenine nucleosides, both *anti* and high-*anti* conformations are preferred, with a sugar residue showing a large diversity of puckering types (Sprang *et al.*,

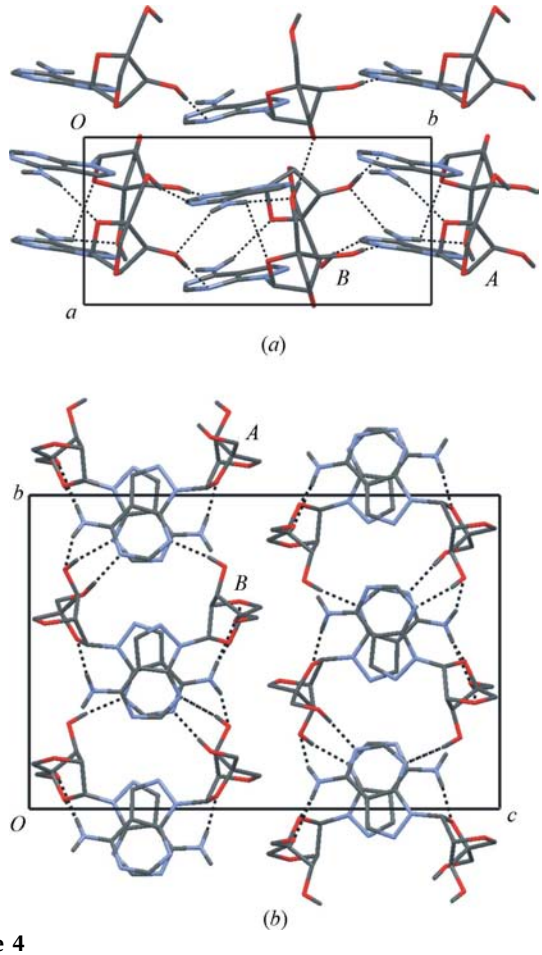

Figure 4
The crystal packing of (VII), showing (*a*) the (001) layer assembly *via* hydrogen bonds and nucleobase stacking interactions and (*b*) projection of the crystal packing along the 8-aza-7-deazaadenine π-stacks. Hydrogen bonds are shown with dashed lines. For clarity, only H atoms of O—H and N—H groups are shown.

1978; Seela, Becher *et al.*, 1999; Seela, Zulauf *et al.*, 1999; Seela *et al.*, 2000, 2005; Lin *et al.*, 2005). In both molecules of (VII), the conformation around the C4′—C5′ bond is *gauche–gauche* [+*sc* from torsion angles of γ; 56.2 (3) and 49.0 (3)°].

The nucleobase is nearly planar (as expected), with an r.m.s. deviation of the nine ring atoms from the best plane of less than 0.015 Å. The sugar C1′ atom is displaced from the heteroplane by −0.201 (4) and 0.209 (4) Å for molecules *A* and *B*, respectively, in opposite directions from the nucleobase plane. Consequently, the *A* and *B* molecules comprise two rigid fragments having similar torsion angle χ and angle γ but showing significant differences in their three-dimensional shape (Fig. 3). Thus, the reported crystal structure shows that configurational changes at the nucleobase N9 atom can be an additional factor influencing the stereochemistry.

In the crystal structure, molecules assemble into (001) layers *via* hydrogen bonds and π–π stacking interactions (Fig. 4). From eight N—H and O—H proton donors of molecules *A* and *B*, one N—H group is not involved in hydrogen bonding and six are involved as N—H···O and O—H···N hydrogen bonds (*i.e.* interactions between the locked sugar and modified nucleobase) (Table 1). The numerous and strong interactions, supported by head-to-head stacking interactions between slightly inclined [5.32 (3)°] 8-aza-7-deazaadenine moieties of the two symmetry-independent molecules, assemble (VII) into a one-dimensional polymeric structure extended along the *b* axis (Fig. 3*a*). This rod-type structure is generated by operation of a twofold screw axis parallel to *b* and by translation along *b*. In turn, the shortest hydrogen bond, *viz*. between O5′*B*—H and O2′*B*, links two *B* molecules belonging to adjacent rods and which are related by unit translation along *a*. This interaction, supported additionally by π–π stacking interactions between nucleobases, leads to the formation of a two-dimensional assembly with 8-aza-7-deazaadenine π-stacks (inner) and sugar moieties (outer) (Figs. 4*a* and 4*b*). Thus, the crystal structure of (VII) is stabilized mainly by interactions between the modified nucleobase and the locked sugar residue, and π–π stacking interactions between the nucleobase units.

Experimental

8-Aza-7-deazaadenine was prepared according to literature procedures (Robins, 1956) and the pentafuranose precursor according to the procedure of Pfundheller & Lomholt (2002). The chemical synthesis of (VII) was performed by a silyl method (Vorbrüggen & Krolikiewicz, 1975), where 8-aza-7-deazaadenine and the precursor of the LNA derivative of ribose were used for the condensation reaction. This condensation leads to two products: in addition to the N^9-substituted nucleoside derivative (III) (major product), the N^8-substituted nucleoside (minor product) was presumably also obtained. The major product of condensation (*ca* 60% of the reaction mixture) was transformed into (VII) according to standard procedures (Koshkin *et al.*, 1998). Details of the synthetic procedures yielding (VII) are shown in the scheme and in the supplementary CIF. Crystals suitable for X-ray analysis in the form of colourless very thin plates were obtained by recrystallization from methanol. ^{1}H NMR (Bruker AVANCEII spectrometer 400.13 MHz, CD_3OD, 298 K): δ 8.19 (1H, *s*, H2), 8.12 (1H, *s*, H7), 6.24 (1H, *s*, H1′), 5.10 (1H, *s*, H2′), 4.37 (1H, *s*, H3′), 4.11 and 3.89 (2H, 2 × *d*, H6′/H6″), 3.89 (2H, *s*, H5′, H5″). ^{13}C NMR (100.61 MHz, CD_3OD, 298 K): δ 159.86 (C6), 157.14 (C2), 155.19 (C4), 134.81 (C7), 101.82 (C5), 88.93 (C4′), 85.91 (C1′), 82.12 (C2′), 73.81 (C3′), 73.11 (C6′), 59.43 (C5′).

Crystal data

$C_{11}H_{13}N_5O_4$
M_r = 279.26
Orthorhombic, $P2_12_12_1$
a = 7.1097 (2) Å
b = 14.7372 (5) Å
c = 22.0732 (7) Å
V = 2312.77 (13) Å^3
Z = 8
Mo $K\alpha$ radiation
μ = 0.13 mm^{-1}
T = 293 (2) K
0.4 × 0.2 × 0.01 mm

Data collection

Kuma KM-4-CCD κ-geometry diffractometer
18884 measured reflections
2346 independent reflections
1723 reflections with $I > 2\sigma(I)$
R_{int} = 0.041

Refinement

$R[F^2 > 2\sigma(F^2)]$ = 0.027
$wR(F^2)$ = 0.056
S = 0.97
2346 reflections
392 parameters
1 restraint
H atoms treated by a mixture of independent and constrained refinement
$\Delta\rho_{max}$ = 0.13 e Å^{-3}
$\Delta\rho_{min}$ = −0.19 e Å^{-3}

Table 1
Hydrogen-bond geometry (Å, °).

D—H···A	D—H	H···A	D···A	D—H···A
N10A—H1AN···O3′A^{i}	0.88 (3)	2.45 (3)	3.229 (4)	148 (3)
N10A—H2AN···O5′B^{ii}	0.86 (3)	2.12 (3)	2.980 (4)	179 (3)
O3′A—H3AO···N3B^{iii}	0.86 (3)	2.19 (3)	3.035 (3)	169 (3)
O5′A—H5AO···N1A^{ii}	0.93 (2)	1.96 (2)	2.869 (3)	166 (3)
N10B—H2BN···O4′A^{i}	0.91 (4)	2.40 (4)	3.292 (4)	165 (4)
O3′B—H3BO···N3A	0.89 (3)	1.98 (3)	2.861 (3)	171 (3)
O5′B—H5BO···O2′B^{iv}	0.77 (4)	2.00 (4)	2.734 (3)	162 (4)

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

The absolute configuration of (VII) was assigned from the known chirality of the starting material. In the absence of significant anomalous scattering, Friedel pairs were merged before the final refinement. All H atoms were located in electron-density difference maps. However, for the structure refinement, carbon-bound H atoms were placed in calculated positions, with C—H = 0.93–0.98 Å, and treated as riding on their parent atoms, with U_{iso}(H) = 1.2U_{eq}(C). Positional and isotropic displacement parameters of the H atoms of N—H and O—H groups were fully refined, except for the O5′A—H5AO bond where the O—H distance was restrained to 0.90 (3) Å.

Data collection: *CrysAlis CCD* (Oxford Diffraction, 2007); cell refinement: *CrysAlis CCD*; data reduction: *CrysAlis RED* (Oxford Diffraction, 2007); program(s) used to solve structure: *SHELXS97* (Sheldrick, 2008); program(s) used to refine structure: *SHELXL97* (Sheldrick, 2008); molecular graphics: *Stereochemical Workstation Operation Manual* (Siemens, 1989) and *Mercury* (Macrae *et al.*, 2006); software used to prepare material for publication: *SHELXL97*.

Supplementary data for this paper are available from the IUCr electronic archives (Reference: GG3163). Services for accessing these data are described at the back of the journal.

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