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KNI-272, a highly selective and potent peptidic HIV protease inhibitor

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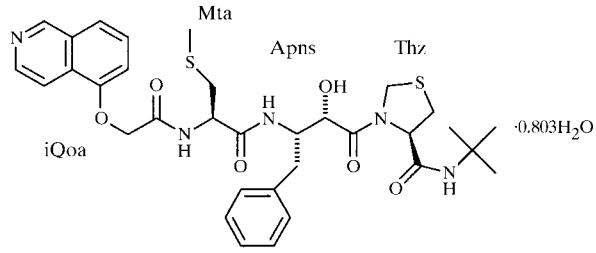
Kynostatin {KNI-272; systematic name: 3-[3-benzyl-2-hydroxy-9-(isoquinolin-5-yloxy)-6-methylsulfanylmethyl-5,8-dioxo-4,7-diazanonanoyl]-*N*-*tert*-butyl-1,3-thiazolane-4-carboxamide}, a highly selective and potent HIV protease inhibitor containing allophenylnorstatin [(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid], has been crystallized as the hydrate, $C_{33}H_{41}N_5O_6S_2\cdot0.803H_2O$, from aqueous hexylene glycol. The observed disorder of the phenyl group in the structure is related to the mode of hydration. The backbone conformation of the molecule is twisted and the overall conformation of the free inhibitor is similar to that observed in its complex with HIV protease.

Comment

Kynostatin (KNI-272), hereinafter (I), a peptide mimic containing an allophenylnorstatin residue [Apns, (2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid; Mimoto *et al.*, 1991], was designed based on the substrate transition state concept for aspartic protease inhibition (Kiso, 1996; Kiso *et al.*, 1999). Compound (I) showed high selectivity and potent inhibitory activity (*K*i = 0.0055 n*M*) for HIV protease (Mimoto *et al.*, 1992), and also displayed good pharmacokinetics and an excellent therapeutic index (Kageyama *et al.*, 1993). Compound (I) is composed of five modules, namely 5-isoquinolyloxy acetic acid (iQoa), methylthioalanine (Mta), Apns, thioproline (Thz) and *tert*-butylamine, as shown in the Scheme. Crystals of (I) were grown from various alcohol solutions, and the crystal structure obtained from aqueous hexylene glycol is reported here.



(I)

The molecular structure of (I) is shown in Fig. 1. The phenyl group of the Apns residue is disordered, with two alternate

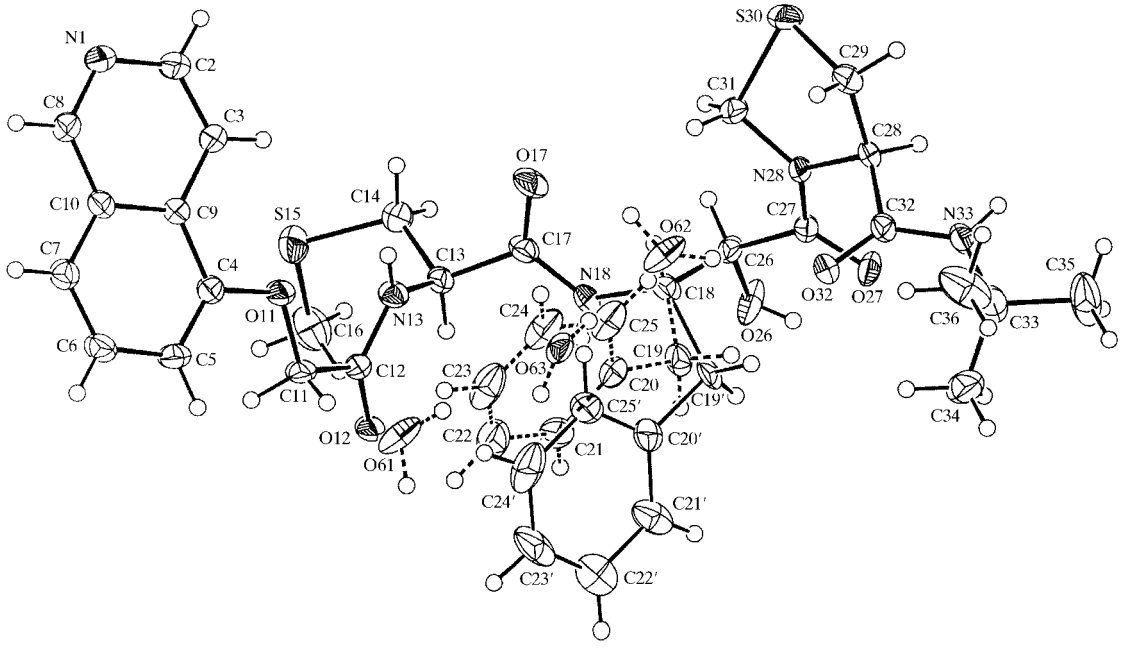


Figure 1
A view of the molecule of (I) with displacement ellipsoids drawn at the 30% probability level. Thin and dotted lines show the two disordered structures of the Apns residue and the water molecules.

orientations. Analysis of the electron-density map (Fig. 2) shows that rotation of the phenyl ring about the C18—C19 and C19—C20 bonds creates space for the binding of three disordered water molecules whose total site occupancy is 0.8. The water molecules are linked to each other by hydrogen bonds [Table 1; O61···O63 3.05 (3) Å and O63···O62 2.67 (3) Å] and also interact with the peptide [O62···O32 2.77 (2) Å, O61···N1 2.932 (12) Å and O62···N1 2.88 (2) Å]. Hydrogen bonds are also formed between the peptides; N18···O27 3.038 (6) Å, N33···O26 2.984 (7) Å and O26···O12 2.837 (6) Å.

The peptide backbone of (I) is twisted (Fig. 2), and the conformation is different from a β-turn or extended (β-sheet) structure. The structural features of (I) seem to be similar to those of the molecule in the complex with HIV protease (Baldwin *et al.*, 1995). Selected torsion angles are listed in Table 2, to compare the backbone structures of the two compounds. The angles involving the atoms of the Mta residue

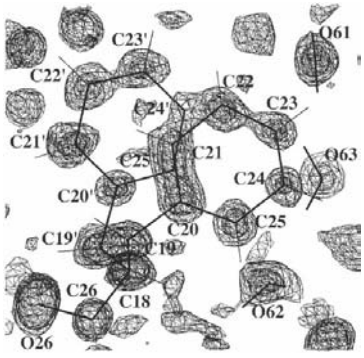


Figure 2
The electron-density map of (I) (Swiss-PdbViewer; Guex & Peitsch, 1997), showing the disordered state of (I) and the water molecules. Contours show the boundaries of 1.0 and 2.5σ. Three water molecules (O61, O62 and O63) bind when the phenyl ring swings into an alternate conformation designated by the primed atoms.

are significantly different in the free and complex structures (*e.g.* N13—C13—C14—S15), and the thiazolidine ring is puckered differently (N28—C28—C29—S30). In contrast, the backbone conformation of the Apns–Thz–Tbu moiety observed in the crystal of (I) is closely related to that observed in the enzyme complex. Furthermore, it is known that the range of observed solution conformations of this fragment is rather limited (Ohno *et al.*, 1996). Thus, it is suggested that the Apns–Thz–^{t}Bu fragment has a relatively rigid conformation, which is nearly optimal for tight interactions between the Apns moiety of (I) and the catalytic centre of the HIV protease active site.

Experimental

Compound (I) was synthesized according to the method of Mimoto *et al.* (1991). Compound (I) (20 mg) was dissolved in hexylene glycol (0.3 ml), and water was added to this solution in several small portions of 0.01 ml. The addition of water was stopped before the solution became opaque. The solution was then sealed in a vial and crystals were grown for 2–5 d at room temperature. A crystal of (I) was mounted on a nylon loop with mother liquor solution (aqueous hexylene glycol) and frozen in a nitrogen stream at 100 K.

Crystal data

$C_{33}H_{41}N_5O_6S_2\cdot0.803H_2O$	$Z = 2$
$M_r = 682.33$	$D_x = 1.281$ Mg m^{-3}
Monoclinic, $P2_1$	Synchrotron radiation
$a = 10.7631$ (4) Å	$\lambda = 0.83600$ Å
$b = 13.1751$ (4) Å	$\mu = 0.20$ mm^{-1}
$c = 12.5623$ (5) Å	$T = 100$ (2) K
$\beta = 96.887$ (2)°	Plate, colourless
$V = 1768.54$ (11) Å^3	0.25 × 0.10 × 0.02 mm

Data collection

Rigaku R-AXIS-IV image-plate detector	$R_{int} = 0.070$
	$\theta_{max} = 31.5°$
Oscillation scans	$h = -13 \rightarrow 13$
5965 measured reflections	$k = -16 \rightarrow 16$
3571 independent reflections	$l = 0 \rightarrow 15$
3321 reflections with $I > 2\sigma(I)$	

Refinement

Refinement on F^2	$w = 1/[\sigma^2(F_o^2) + (0.1047P)^2 + 2.2778P]$ where $P = (F_o^2 + 2F_c^2)/3$
$R[F^2 > 2\sigma(F^2)] = 0.067$	
$wR(F^2) = 0.191$	
$S = 0.99$	$(\Delta/\sigma)_{max} = 0.020$
3571 reflections	$\Delta\rho_{max} = 0.81$ e Å^{-3}
504 parameters	$\Delta\rho_{min} = -0.61$ e Å^{-3}
H-atom parameters constrained	Extinction correction: *SHELXL*97 (Sheldrick, 1997)
	Extinction coefficient: 0.30 (3)

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

D—H···A	D—H	H···A	D···A	D—H···A
N18—H20···O27^{i}	0.86	2.25	3.038 (6)	152
N33—H36···O26^{ii}	0.86	2.14	2.984 (7)	169
O26—H30···O27	0.76	2.27	2.669 (6)	114
O61—H62···O63	0.83	2.22	3.05 (3)	178
O62—H64···O32	0.87	1.92	2.77 (2)	166
O63—H66···O62	0.77	1.90	2.67 (3)	177
O26—H30···O12^{ii}	0.76	2.15	2.837 (6)	153
O61—H61···N1^{iii}	0.89	2.08	2.932 (12)	161
O62—H63···N1^{iv}	0.87	2.17	2.88 (2)	139

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

Intensity data were collected by the oscillation method using synchrotron radiation (SPring-8/BL24XU-A). A total of 60 images covered 180° rotation on the φ axis. Image data were processed using *MOSFLM* (Leslie, 1999) and the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994). High background regions were observed at approximately 3.0 Å resolution and these regions were excluded from the processing. A total of 15 444 reflections were observed from the image data, and these were averaged to 6157 reflections by the *CCP*4 program suite, with $R_{merge} = 0.092$. The H atoms of the peptide were placed in calculated positions and constrained during the refinement, with alkyl and aromatic C—H distances of 0.96–0.97 and 0.93 Å, respectively, and U_{iso}(H) = 1.2–1.4U_{eq}(C) and U_{iso}(H) = 1.0–1.2U_{eq}(C_{methyl}). The H atoms of the water molecules were positioned by considering the hydrogen-bonding networks and were fixed during the refinement. The absolute stereochemistry is known from the known chirality of the starting materials in the synthesis, but it could not be determined from the present data.

Table 2
Selected torsion angles for (I) in the crystal and in the complex with HIV protease (°).

Torsion angle	Crystal	Complex
O11—C11—C12—N13	12.4 (6)	39.4
C11—C12—N13—C13	−171.4 (4)	168.8
C12—N13—C13—C17	−125.3 (5)	−120.1
N13—C13—C14—S15	−57.3 (5)	−161.4
N13—C13—C17—N18	112.7 (5)	71.8
C13—C17—N18—C18	−162.8 (4)	−175.9
C17—N18—C18—C26	−104.8 (6)	−108.2
N18—C18—C19—C20†	−61.0 (12)	−54.1
N18—C18—C19′—C20′†	−41.7 (11)	−54.1
C18—C19—C20—C21	105.2 (17)	98.9
C18—C19—C20—C25	−70.5 (17)	−81.2
C18—C19′—C20′—C21′	139.1 (11)	98.9
C18—C19′—C20′—C25′	−39.2 (17)	−81.2
N18—C18—C26—O26	−65.1 (6)	−74.9
N18—C18—C26—C27	172.3 (4)	165.1
C18—C26—C27—N28	−66.8 (6)	−88.4
O26—C26—C27—O27	−14.2 (7)	−32.5
C26—C27—N28—C28	173.7 (4)	169.1
C27—N28—C28—C32	−65.8 (6)	−64.7
N28—C28—C29—S30	−32.3 (4)	23.6
N28—C28—C32—N33	148.8 (4)	145.6
C28—C32—N33—C33	179.0 (5)	−169.6
C32—N33—C33—C34	53.4 (8)	54.5

† The phenyl group is disordered on two sites.

Data collection: *PROCESS* (Higashi, 1996); cell refinement: *MOSFLM* (Leslie, 1999); data reduction: *MOSFLM*; program(s) used to solve structure: *SHELXS*97 (Sheldrick, 1997); program(s) used to refine structure: *SHELXL*97 (Sheldrick, 1997); molecular graphics: *PLATON* (Spek, 1998); software used to prepare material for publication: *PARST* (Nardelli, 1983).

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Supplementary data for this paper are available from the IUCr electronic archives (Reference: SX1119). Services for accessing these data are described at the back of the journal.

References

Baldwin, E. T., Bhat, T. N., Gulnik, S., Liu, B., Tolpol, I. A., Kiso, Y., Mimoto, T., Mitsuya, H. & Erickson, J. W. (1995). *Structure*, **3**, 581–590.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Guex, N. & Peitsch, M. C. (1997). *Electrophoresis*, **18**, 2714–2723.
Higashi, T. (1996). *PROCESS.* Version 4.0. Rigaku Corporation, Tokyo, Japan.
Kageyama, S., Mimoto, T., Murakawa, Y., Nomizu, M., Ford, H. Jr, Shirasaka, T., Gulnik, S., Erikson, J., Takada, K., Hayashi, H., Broder, S., Kiso, Y. & Mitsuya, H. (1993). *Antimicrob. Agents Chemother.* **37**, 810–817.
Kiso, Y. (1996). *Biopolymers*, **40**, 235–244.
Kiso, Y., Matsumoto, H., Mizumoto, S., Kimura, T., Fujiwara, Y. & Akaji, K. (1999). *Biopolymers*, **54**, 59–68.
Leslie, A. G. W. (1999). *Acta Cryst.* D**55**, 1696–1702.
Mimoto, T., Imai, J., Kisanuki, S., Enomoto, H., Hattori, N., Akaji, K. & Kiso, Y. (1992). *Chem. Pharm. Bull.* **40**, 2251–2253.
Mimoto, T., Imai, J., Tanaka, S., Hattori, N., Takahashi, O., Kisanuki, S., Nagano, Y., Shintani, M., Hayashi, H., Sakikawa, H., Akaji, K. & Kiso, Y. (1991). *Chem. Pharm. Bull.* **39**, 2465–2467.
Nardelli, M. (1983). *Comput. Chem.* **7**, 95–98.
Ohno, Y., Kiso, Y. & Kobayashi, Y. (1996). *Bioorg. Med. Chem.* **4**, 1565–1572.
Sheldrick, G. M. (1997). *SHELXL*97 and *SHELXS*97. University of Göttingen, Germany.
Spek, A. L. (1998). *PLATON.* University of Utrecht, The Netherlands.