Johnson, C. K. (1976). *ORTEP*II. Report ORNL-5138. Oak Ridge National Laboratory, Tennessee, USA.
Lim, W. A., Richards, F. M. & Fox, R. O. (1994). *Nature (London)*, **372**, 375–379.
Magaard, V. W., Sanchez, R. M., Bean, J. W. & Moore, M. L. (1993). *Tetrahedron Lett.* **34**, 381–384.
Molecular Structure Corporation (1988). *MSC/AFC Diffractometer Control Software*. MSC, 3200 Research Forest Drive, The Woodlands, TX 77381, USA.
Molecular Structure Corporation (1989). *TEXSAN. Single Crystal Structure Analysis Software*. Version 5.0. MSC, 3200 Research Forest Drive, The Woodlands, TX 77381, USA.
Okonogi, T., Tsutsumi, S., Shibahara, S., Ohuchi, S., Ichimaru, Y., Egawa, T., Patchett, A. A. & Christensen, B. G. (1993). US Patent 8 006 105.
Rich, D. H. (1990). *Comprehensive Medicinal Chemistry*, edited by C. Hansch, P. G. Sammes & J. B. Taylor, Vol. 2, pp 391–441. Oxford: Pergamon Press.
Tsutsumi, S., Okonogi, T., Shibahara, S., Ohuchi, S., Hatsushiba, E., Patchett, A. A. & Christensen, B. G. (1994). *J. Med. Chem.* **37**, 3492–3502.
Tsutsumi, S., Okonogi, T., Takeuchi, Y. & Kodama, Y. (1995). *Acta Cryst.* C**51**, 1923–1925.
Welches, W. R., Brosnihan, K. B. & Ferrario, C. M. (1993). *Life Sci.* **52**, 1461–1480.
Wiley, R. A. & Rich, D. H. (1993). *Med. Res. Rev.* **13**, 327–384.
Zachariasen, W. H. (1967). *Acta Cryst.* **23**, 558-564.

Acta Cryst. (1995). C**51**, 1927–1929

Prolyl Endopeptidase Inhibitors. III. A Peptidyl α-Keto Benzothiazole Derivative

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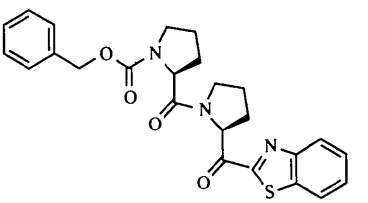
Abstract

In the title compound, benzyl 2-[2-(2-benzothiazoloyl)-pyrrolidinoyl]pyrrolidine-1-carboxylate, $C_{25}H_{25}N_3O_4S$, both the N-terminal urethane bond and the C-terminal amide bond are *trans*. The dipeptide inhibitor is semi-extended and shows coplanarity between the ketone carbonyl group and the benzothiazole ring, with the carbonyl O atom *cis* with respect to the ring S atom.

Comment

We are interested in the structures of peptidyl α-keto heterocyclic inhibitors of prolyl endopeptidase (PEP) (Tsutsumi *et al.*, 1994). We reported previously the structure of an α-keto thiazole inhibitor (Tsutsumi, Okonogi, Takeuchi & Kodama, 1995*b*). We report here the structure of an α-keto benzothiazole inhibitor, (I). This compound is as potent an inhibitor as the α-keto thiazole inhibitor.



(I)

The title dipeptide, with $\varphi_1 = -65\,(2)$, $\psi_1 = 150\,(1)$, $\varphi_2 = -65\,(1)$ and $\psi_2 = 155\,(1)°$ (IUPAC–IUB Commission on Biochemical Nomenclature, 1971), has a polyproline II conformation. Both the N-terminal urethane bond and the C-terminal amide bond are *trans* [O1—C12—N3—C11 173 (1) and C11—C10—N2—C9 176 (1)°]. In the two peptide inhibitors involving an N-terminal indolecarboxylic acid residue (Tsutsumi, Okonogi, Takeuchi & Kodama, 1994*a*,*b*), the corresponding bonds are *cis* and *trans*. Thus, the difference in the conformation appears to depend on the prolyl residue (Magaard, Sanchez, Bean & Moore, 1993) and not on the N-terminal moieties. The ketone carbonyl group and the benzothiazole ring are coplanar [O2—C8—C7—S1 −1 (2) and N1—C7—C8—O2 −180 (1)°]. The carbonyl O atom of the ketone group is *cis* with respect to the ring S atom.

The conformational relationship between the carbonyl O atom O2 and atom N1 of the heterocycle is *trans*, as in the α-keto thiazole inhibitor (Tsutsumi, Okonogi, Takeuchi & Kodama, 1994*b*). We believe that this conformation of peptidyl α-keto heterocyclic inhibitors is necessary in order to stabilize the hemiketal adduct of the active site.

A second aim of our work is to produce a drug with improved duration of *in vivo* action. The title compound and the α-keto thiazole derivative were orally

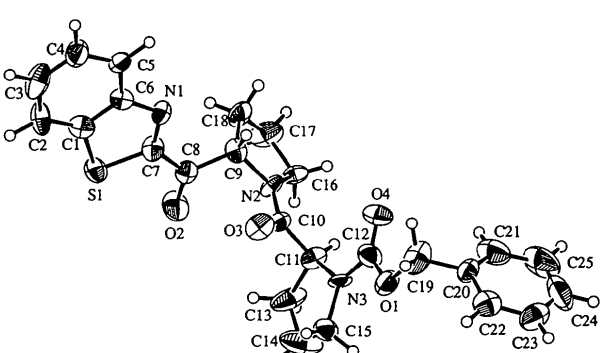


Fig. 1. The molecular structure of the title compound with the crystallographic numbering scheme (*ORTEP*II; Johnson, 1976). Displacement ellipsoids are shown at the 50% probability level and H atoms are drawn as spheres of arbitrary size.

active and were potent brain PEP inhibitors. The α-keto ester inhibitor produced some inhibition of kidney PEP after oral administration but none of the brain enzyme, presumably as a consequence of keto ester metabolism and poor brain penetration. Details of the results will be reported elsewhere (Dawson, 1994).

Experimental

The title compound was synthesized according to the method of Okonogi *et al.* (1993). Single crystals were grown from an ethyl acetate solution.

Crystal data

$C_{25}H_{25}N_3O_4S$
$M_r = 463.55$
Monoclinic
$P2_1$
$a = 10.226\,(1)$ Å
$b = 9.365\,(1)$ Å
$c = 12.073\,(2)$ Å
$\beta = 95.58\,(1)°$
$V = 1150.6\,(2)$ Å^3
$Z = 2$
$D_x = 1.338$ Mg m^{-3}

Cu $K\alpha$ radiation
$\lambda = 1.5418$ Å
Cell parameters from 25 reflections
$\theta = 10.17$–$19.21°$
$\mu = 1.5149$ mm^{-1}
$T = 23.0$ K
Needle
0.1 × 0.1 × 0.03 mm
Colorless

Data collection

AFC-5*R* diffractometer
ω scans with profile analysis
Absorption correction: none
1935 measured reflections
1840 independent reflections
1834 observed reflections [$I > 0$]
$R_{int} = 5.37$

$\theta_{max} = 60°$
$h = -11 \rightarrow 11$
$k = 0 \rightarrow 10$
$l = 0 \rightarrow 13$
3 standard reflections monitored every 150 reflections
intensity decay: 2.30%

Refinement

Refinement on F
$R = 0.2202$
$wR = 0.0735$
$S = 1.121$
1834 reflections
297 parameters
H-atom parameters not refined
$w = 1/\sigma^2(F_o)$
$(\Delta/\sigma)_{max} = 0.80$

$\Delta\rho_{max} = 0.69$ e Å^{-3}
$\Delta\rho_{min} = -1.25$ e Å^{-3}
Extinction correction: type 2 Gaussian isotropic (Zachariasen, 1967)
Extinction coefficient: 5.66×10^{-7}
Atomic scattering factors from *International Tables for X-ray Crystallography* (1974, Vol. IV)

Table 1. *Fractional atomic coordinates and equivalent isotropic displacement parameters* (Å^2)

$U_{eq} = (1/3)\Sigma_i\Sigma_j U_{ij} a_i^* a_j^* \mathbf{a}_i.\mathbf{a}_j.$

	x	y	z	U_{eq}
S1	0.4693 (4)	0.0233	0.1541 (3)	0.063 (1)
O1	1.0101 (10)	−0.418 (1)	0.6819 (8)	0.073 (4)
O2	0.559 (1)	0.075 (1)	0.3908 (9)	0.089 (4)
O3	0.7394 (8)	−0.194 (1)	0.4716 (7)	0.066 (3)
O4	0.7928 (9)	−0.398 (1)	0.7018 (8)	0.076 (4)
N1	0.3911 (9)	−0.206 (1)	0.2449 (8)	0.046 (3)
N2	0.5784 (9)	−0.114 (1)	0.5691 (7)	0.044 (3)
N3	0.9125 (8)	−0.206 (1)	0.6660 (8)	0.049 (3)
C1	0.391 (1)	−0.104 (2)	0.066 (1)	0.057 (5)
C2	0.366 (2)	−0.106 (2)	−0.047 (1)	0.071 (5)
C3	0.300 (2)	−0.220 (2)	−0.099 (1)	0.085 (6)
C4	0.262 (2)	−0.329 (2)	−0.032 (1)	0.074 (6)
C5	0.293 (1)	−0.336 (2)	0.081 (1)	0.056 (5)
C6	0.356 (1)	−0.219 (2)	0.131 (1)	0.047 (4)
C7	0.451 (1)	−0.088 (2)	0.267 (1)	0.053 (4)
C8	0.503 (1)	−0.039 (2)	0.377 (1)	0.053 (5)
C9	0.479 (1)	−0.137 (1)	0.474 (1)	0.051 (4)
C10	0.703 (1)	−0.146 (1)	0.557 (1)	0.048 (4)
C11	0.800 (1)	−0.110 (1)	0.656 (1)	0.052 (4)
C12	0.894 (2)	−0.344 (2)	0.688 (1)	0.065 (6)
C13	0.862 (1)	0.034 (2)	0.641 (1)	0.097 (6)
C14	1.004 (2)	0.012 (2)	0.643 (2)	0.130 (8)
C15	1.032 (1)	−0.141 (2)	0.637 (1)	0.068 (5)
C16	0.521 (1)	−0.062 (1)	0.670 (1)	0.055 (4)
C17	0.381 (1)	−0.028 (2)	0.627 (1)	0.087 (7)
C18	0.351 (1)	−0.102 (2)	0.523 (1)	0.073 (5)
C19	1.003 (2)	−0.575 (2)	0.695 (2)	0.094 (7)
C20	1.064 (1)	−0.624 (2)	0.806 (1)	0.058 (5)
C21	1.003 (2)	−0.613 (2)	0.893 (2)	0.101 (7)
C22	1.189 (2)	−0.680 (2)	0.812 (1)	0.072 (6)
C23	1.248 (2)	−0.725 (2)	0.916 (2)	0.091 (7)
C24	1.174 (2)	−0.719 (3)	1.004 (2)	0.122 (9)
C25	1.054 (2)	−0.659 (3)	1.000 (2)	0.13 (1)

The intensities were extremely weak due to the very small size of the crystal and hence the value of R is high. Refinement using reflections with $I > 3\sigma(I)$ led to a better value of R, but with an unacceptably low observation-to-parameter ratio. This refinement, however, yielded geometrical parameters very close to those of the determination reported here, but with greater e.s.d.'s. H atoms were placed in calculated positions and were not refined.

Data collection: *MSC/AFC Diffractometer Control Software* (Molecular Structure Corporation, 1988). Cell refinement: *MSC/AFC Diffractometer Control Software*. Data reduction: *TEXSAN PROCESS* (Molecular Structure Corporation, 1989). Program(s) used to solve structure: *MITHRIL* (Gilmore, 1984). Program(s) used to refine structure: *TEXSAN LS*. Molecular graphics: *ORTEP*II (Johnson, 1976). Software used to prepare material for publication: *TEXSAN FINISH*.

Lists of structure factors, anisotropic displacement parameters, H-atom coordinates and complete geometry have been deposited with the IUCr (Reference: VJ1014). Copies may be obtained through The Managing Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

References

Dawson, G. R. (1994). Personal communication.

Gilmore, C. J. (1984). *J. Appl. Cryst.* **17**, 42–46.

IUPAC–IUB Commission on Biochemical Nomenclature (1971). *Biochem. Biophys. Acta.* **229**, 1–17.

Johnson, C. K. (1976). *ORTEP*II. Report ORNL-5138. Oak Ridge National Laboratory, Tennessee, USA.

Magaard, V. W., Sanchez, R. M., Bean, J. W. & Moore, M. L. (1993). *Tetrahedron Lett.* **34**, 381–384.

Molecular Structure Corporation (1988). *MSC/AFC Diffractometer Control Software*. MSC, 3200 Research Forest Drive, The Woodlands, TX 77381, USA.

Molecular Structure Corporation (1989). *TEXSAN. Single Crystal Structure Analysis Software*. Version 5.0. MSC, 3200 Research Forest Drive, The Woodlands, TX 77381, USA.

Okonogi, T., Tsutsumi, S., Shibahara, S., Ohuchi, S., Ichimaru, Y., Egawa, T., Patchett, A. A. & Christensen, B. G. (1993). US Patent 8 006 105.

Tsutsumi, S., Okonogi, T., Shibahara, S., Ohuchi, S., Hatsushiba, E., Patchett, A. A. & Christensen, B. G. (1994). *J. Med. Chem.* **37**, 3492–3502.
Tsutsumi, S., Okonogi, T., Takeuchi, Y. & Kodama, Y. (1995*a*). *Acta Cryst.* C**51**, 1923–1925.
Tsutsumi, S., Okonogi, T., Takeuchi, Y. & Kodama, Y. (1995*b*). *Acta Cryst.* C**51**, 1925–1927.
Zachariasen, W. H. (1967). *Acta Cryst.* **23**, 558-564.

Acta Cryst. (1995). C**51**, 1929–1932

Two Methylated Ribonucleosides: 3-Methyluridine and 1-Methylinosine

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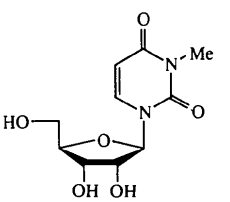
Abstract

3-Methyluridine, $C_{10}H_{14}N_2O_6$, (1), and 1-methylinosine, $C_{11}H_{14}N_4O_5$, (2), adopt conformations generally consistent with those of published ribonucleoside and ribonucleotide crystal structures. (1) has a C(2′)-*endo* ribofuranose pucker (Altona–Sundaralingam pseudorotation angle P = 175.5°); the glycosidic conformation is *anti* (χ_{CN} = −133.1°). (2) has two molecules in the asymmetric unit of which both are C(2′)-*endo* (P = 159.8, 156.9°) with *syn* glycosidic conformations (χ_{CN} = 67.2, 53.6°).

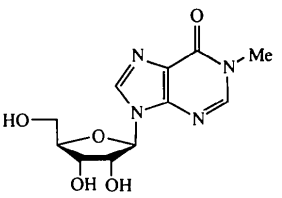
Comment

Nucleoside analogues in which functional groups are replaced by H atoms or are modified by alkylation provide a means of probing mechanisms of molecular recognition involving nucleic acids. Changes in affinity for regulatory proteins that result from systematic removal or hindrance of potentially interacting groups in the target nucleic acid have been used to identify intermolecular contacts in DNA– and RNA–protein complexes (Iwai, Pritchard, Mann, Karn & Gait, 1992). This strategy has also been applied to describe at the molecular level the catalytic activity of ribozymes (Bratty, Chartrand, Ferbeyre & Cedergren, 1993). Structural comparability between these synthetic analogues and their unmodified counterparts is critical to the correct interpretation of experimental results and begins at the nucleoside level.

N-Methylated nucleosides are also natural products; they can result from the action of various chemical carcinogens and mutagens, but many examples are normal components of undamaged DNA and especially RNA. The two ribonucleosides whose structures are described here are minor constituents of transfer RNA. 3-Methyluridine, (1), was detected *inter alia* in human and yeast tRNAs (0.03 and 0.01 mol%, respectively) (Hall, 1971), and 1-methylinosine, (2), has been shown to occur in yeast tRNA (0.05 mol%) (Holley *et al.*, 1965). More specifically, the latter nucleoside is located 3′- to the anticodon of alanine tRNA in yeast and *T. utilis* (Takemura, Ogawa & Nakazawa, 1973).



(1)



(2)

In the case of compounds (1) and (2), the key structural parameters of glycosidic torsion angle (χ_{CN}) and ribofuranose ring pucker are within the ranges typical of ribonucleosides (Saenger, 1984). A survey of purine and pyrimidine ribofuranosides in the Cambridge Structural Database (CSD; Allen *et al.*, 1991) shows in each case glycosidic torsion angles clustered about the values corresponding to *syn* and *anti*. The preference generally for *anti* conformations is less marked in the purine series (69 of 90 for purines; 84 of 94 for pyrimidines); since geometry about the five-membered ring is less sterically demanding, the clash between atoms of the sugar moiety and N(3) of purine is reduced compared to that with O(2) in pyrimidine nucleosides (Haschemeyer & Rich, 1967). Published structures of C(1′)-substituted ribofuranoses are almost equally distributed between two populations corresponding to C(2′)-*endo* and C(3′)-*endo* conformations. C(3′)-*endo* ribofuranose is, however, a characteristic of double-helical RNA (Saenger, 1984).

3-Methyluridine, (1), has an *anti* glycosidic conformation (χ_{CN} = −133.1°) and an unsymmetrical C(2′)-*endo*–C(3′)-*exo* twist ($^2T_3$) described by an Altona–Sundaralingam pseudorotation angle (Altona & Sundaralingam, 1972) of P = 175.5°. All published structures of uridine are also *anti* but have sugar conformations within the C(3′)-*endo* envelope: $^3T_2$, P = 3.7, 14.0° (CSD Refcode: BEURID10), and $^3T_4$, P = 24.9° (CSD Refcode: GIDZIC10).

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