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# Precursor of a β-lactamase inhibitor: allyl (4*S*,8*S*,9*R*)-10-[(*E*)-ethylidene]-4-methoxy-11-oxo-1-azatricyclo-[7.2.0.0$^{3,8}$]undec-2-ene-2-carboxylate

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The molecular structure of the title tricyclic compound, $C_{17}H_{21}NO_4$, which is the immediate precursor of a potent synthetic inhibitor {Lek157: sodium (8*S*,9*R*)-10-[(*E*)-ethylidene]-4-methoxy-11-oxo-1-azatricyclo[7.2.0.0$^{3,8}$]undec-2-ene-2-carboxylate} with remarkable potency, provides experimental evidence for the previously modelled relative position of the fused cyclohexyl ring and the carbonyl group of the β-lactam ring, which takes part in the formation of the initial tetrahedral acyl–enzyme complex. In this hydrophobic molecule, the overall geometry is influenced by C—H···O intramolecular hydrogen bonds [3.046 (4) and 3.538 (6) Å, with corresponding normalized H···O distances of 2.30 and 2.46 Å], whereas the molecules are interconnected through intermolecular C—H···O hydrogen bonds [3.335 (4)–3.575 (5) Å].

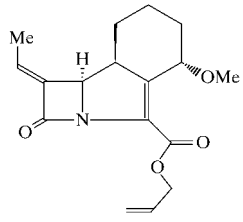
## Comment

Because of their effectiveness and safety, β-lactam antibiotics play a central role in the treatment of bacterial infections. However, the clinical use of these drugs has been compromised due to hydrolytic cleavage of the β-lactam ring by bacterial β-lactamases, which renders them inactive. In particular, the clinically used inhibitors which are employed to block lactamase activity and to preserve the antibacterial activity of the antibiotic are ineffective in bacterial strains bearing class C β-lactamases (Massova & Mobashery, 1997). Thus, the development of inhibitors of class C β-lactamases is an active area of antimicrobial research (Frère *et al.*, 1999).

As part of our ongoing research program concerned with the structure-based design of β-lactamase inhibitors, we have designed and synthesized a series of potent tricyclic carbapenems (Čopar *et al.*, 1998), which have been shown to exhibit very useful biological activity as inhibitors of class A, class C, and also class D β-lactamases (Vilar *et al.*, 2001). Our design procedure introduces a hydrophobic cyclohexane ring at the C3—C4 position of the penem, which should block the access of water molecules to the tetrahedral acyl–enzyme complex formed in the first step of the enzyme-inhibitor interaction process, thus stabilizing the tetrahedral intermediate and preventing the deacylation step (Heinze-Krauss *et al.*, 1998; Čopar *et al.*, 2002).

Molecular-modelling studies (Šolmajer & Čopar, 1998) were performed to analyse the complexes of the designed compounds with the enzyme target, based on the reported crystal structure of class C β-lactamase from *Enterobacter cloacae* P99 (Lobkovsky *et al.*, 1993). The present structural study was carried out to establish unequivocally the stereochemistry of the allyl precursor of the inhibitor Lek157 {sodium (8*S*,9*R*)-10-[(*E*)-ethylidene]-4-methoxy-11-oxo-1-azatricyclo[7.2.0.0$^{3,8}$]undec-2-ene-2-carboxylate}, which can be obtained in high yields (94%) from the title precursor by a single-step deallylation. The absolute structure was assigned to agree with the chirality known by chemical means (Rossi *et al.*, 1995).

The crystal structure of the title compound, (I) (Fig. 1), clearly shows the *cis* stereochemistry (8*S*,9*R*) of the azatricyclo[7.2.0.0$^{3,8}$]undec-2-ene ring system and demonstrates the spatial position of the 4-methoxy substituent with respect to the carbonyl group of the β-lactam ring, which takes part in the tetrahedral intermediate formed by a covalent interaction with Ser 64 of the class C enzymes or Ser 70 of the class A β-lactamases.




(I)

The structure of allyl Lek157 also confirms our initial hypothesis from modelling that the 4-methoxy substituent is suitably positioned in Lek157 to form a hydrogen bond with the side-chain amide of Asn 152 in the complex with *Enterobacter cloacae* P99 β-lactamase.

A precheck using the Cambridge Structural Database (Release 5.2.1 of April 2001; Allen & Kennard, 1993) gave no results for β-lactam-like compounds with a tricyclic structure.

The molecule of (I) with the atomic numbering scheme is depicted in Fig. 1. Selected geometric parameters are presented in Table 1. The bond lengths and angles are normal and in agreement with the values for related compounds, for example, 2-benzyloxycarbonyl-3-methoxycarbonyl-4-methyl-7-oxo-1-azabicyclo[3.2.0]heptane (Martel *et al.*, 1997). The bond lengths of C2—C3 [1.341 (3) Å], C10—C20 [1.332 (4) Å] and C16—C17 [1.280 (6) Å] suggest double-bond character (*International Tables for Crystallography*, Vol. C, 1995).

The most interesting feature of the structure of (I) is the system of intra- and intermolecular C—H···O hydrogen

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bonds (Taylor & Kennard, 1982; Steiner, 1997), which govern the shape of the molecule. With regard to the C—H···O hydrogen bonding, which is relatively weak but important, the significance of the H···*Y* distances rather than the *X*···*Y* distances (hydrogen-bond type *X*—H···*Y*) was first clearly shown in a study of N—H···O=C hydrogen bonds by Taylor & Kennard (1983). It was also believed for a long time that the van der Waals sum cut-off definition of the hydrogen bond requires that the H···*Y* and *X*···*Y* distances must be smaller than the sum of the van der Waals radii. For C—H···O interactions, the H···O separation was taken as 2.6–2.7 Å. At this distance, the interaction was assumed to switch from a 'hydrogen bond' to a 'van der Waals'-type interaction. However, this definition was recently declared faulty, the argument being that the electrostatic field of dipoles does not terminate sharply at any cut-off distance. Longer H···O distances of up to 3.2 Å and angular cut-off angles greater than 90° (Steiner, 2002; Steiner & Saenger, 1992) were therefore suggested. An additional but necessary criterion for hydrogen bonding is a positive directionality preference, that is, linear C—H···O angles are statistically favoured over bent ones. However, the degree of directionality decreases with the polarity of the C—H group, namely –C≡C—H > –C=$CH_2$ > –$CH_3$. Even in C—H···O contacts involving methyl groups, there is a preference for linearity, whereas this is quite contrary to the van der Waals contacts of methyl groups (Steiner & Desiraju, 1998). Additionally, hydrogen bonds are directional also at the acceptor side. In the case of $R_2$C=O as an acceptor, the H···O=C angles are expected to be spread around a value of 120° (Steiner, 2002, and references therein). Furthermore, the common opinion is that the H···O distances obtained from X-ray diffraction data are not worthy of mention because the C—H distances are less than those obtained from a more precise neutron diffraction study. However, this difference was found to be systematic and could well be accounted for with normalized C—H values (Taylor & Kennard, 1983) and the corresponding C—H···O angles could be calculated from X-ray data (Nardelli, 1983, 1995).

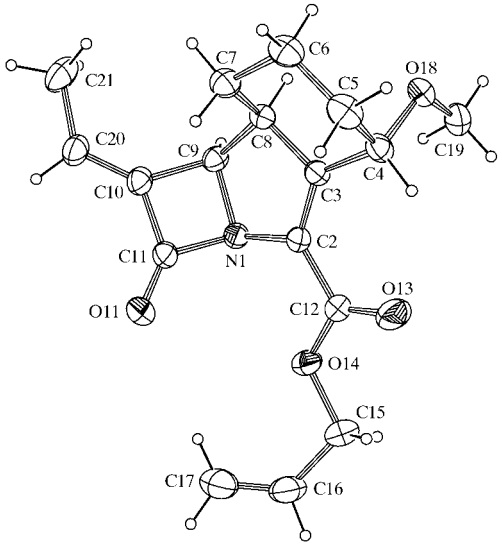


**Figure 1**
*ORTEP*II (Johnson, 1971) view of the title molecule with the atomic numbering. Displacement ellipsoids are drawn at the 30% probability level. H atoms are displayed as small circles of arbitrary size.

Using all these findings, the shape of the title molecules can be explained as being the result of various C—H···O interactions. The allyl carboxylate moiety is curled in such a way that the terminal C17—H17*A* group forms a long contact to the carboxyl O11 atom of the β-lactam group, and also to atom O14 of the carboxyl group (see Table 2 for details of C—H···O interactions). This bond could be classified as a three-centre C—H···O bond (Steiner, 2002). There is another short intermolecular bond, C4—H4···O13 [C···O 3.046 (4) Å], with a normalized H4···O13 distance of 2.30 Å, which forces atoms C2, C3, C4, C12 and O13 to adopt a planar arrangement. The β-lactam and five-membered rings are nearly planar, the maximum deviations from the planes being 0.035 (1) and 0.066 (1) Å, respectively, with a corresponding dihedral angle of 53.7 (1)°. The cyclohexyl ring attached at the C3—C8 bond adopts a normal chair conformation. There are three intermolecular C—H···O interactions, with C···O distances ranging from 3.507 (5) to 3.575 (5) Å and normalized H···O distances ranging from 2.46 to 2.69 Å. The corresponding angles at the H···O—C acceptor site are H15*A*···O11$^{i}$=C11$^{i}$ 141°, H20···O18$^{ii}$—C4$^{ii}$ 134° and H21*B*···O13$^{iii}$=C12$^{iii}$ 175° [symmetry codes: (i) $x+\frac{1}{2}, -y+\frac{3}{2}, -z$; (ii) $1-x, y+\frac{1}{2}, -z+\frac{1}{2}$; (iii) $-x+\frac{3}{2}, 1-y, z+\frac{1}{2}$]. The molecules are interlinked in a columnar arrangement along the shorter *a* axis through C—H···O hydrogen bonds.

## Experimental

The synthesis and bioactivity characterization of Lek157 have been reported previously (Čopar *et al.*, 1998; Vilar *et al.*, 2001). Single crystals of the allyl precursor of Lek157 were obtained with difficulty by slow evaporation from a methanol solution at 289 K. The crystals exhibited bad scattering properties.

*Crystal data*

$C_{17}H_{21}NO_4$
$M_r$ = 303.35
Orthorhombic, $P2_12_12_1$
*a* = 7.6810 (2) Å
*b* = 14.4610 (3) Å
*c* = 14.6190 (4) Å
*V* = 1623.80 (7) Å$^3$
*Z* = 4
$D_x$ = 1.241 Mg m$^{-3}$
$D_m$ = 1.22 (5) Mg m$^{-3}$
$D_m$ measured by flotation in chloroform/benzene
Mo *Kα* radiation
Cell parameters from 1815 reflections
θ = 3–27.2°
μ = 0.09 mm$^{-1}$
*T* = 293 (2) K
Plate, colourless
0.40 × 0.30 × 0.20 mm

*Data collection*

Nonius KappaCCD diffractometer
ω scans
3538 measured reflections
2008 independent reflections
1710 reflections with $I > 2\sigma(I)$
$R_{int}$ = 0.016
$\theta_{max}$ = 27.2°
*h* = −9 → 9
*k* = −18 → 18
*l* = −18 → 18

*Refinement*

Refinement on $F^2$
$R[F^2 > 2\sigma(F^2)]$ = 0.043
$wR(F^2)$ = 0.127
*S* = 1.06
2008 reflections
200 parameters
H-atom parameters constrained
$w = 1/[\sigma^2(F_o^2) + (0.0691P)^2 + 0.1661P]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$ = 0.001
$\Delta\rho_{max}$ = 0.14 e Å$^{-3}$
$\Delta\rho_{min}$ = −0.12 e Å$^{-3}$
Extinction correction: *SHELXL*97
Extinction coefficient: 0.122 (17)

**Table 1**
Selected geometric parameters (Å, °).

| | | | |
|---|---|---|---|
| N1—C11 | 1.432 (3) | C8—C9 | 1.541 (3) |
| N1—C2 | 1.434 (3) | C9—C10 | 1.512 (4) |
| N1—C9 | 1.509 (3) | C10—C11 | 1.486 (4) |
| C2—C3 | 1.341 (3) | C11—O11 | 1.196 (3) |
| C3—C8 | 1.506 (3) | | |
| | | | |
| C11—N1—C2 | 124.1 (2) | N1—C9—C10 | 87.3 (2) |
| C11—N1—C9 | 91.5 (2) | N1—C9—C8 | 105.2 (2) |
| C2—N1—C9 | 107.7 (2) | C10—C9—C8 | 122.3 (2) |
| C3—C2—N1 | 110.8 (2) | C11—C10—C9 | 89.4 (2) |
| C2—C3—C4 | 130.4 (2) | O11—C11—N1 | 131.0 (3) |
| C2—C3—C8 | 112.2 (2) | O11—C11—C10 | 137.7 (3) |
| C3—C8—C9 | 102.7 (3) | N1—C11—C10 | 91.3 (2) |
| | | | |
| C8—C3—C4—C5 | 55.2 (3) | C5—C6—C7—C8 | −56.3 (3) |
| C3—C4—C5—C6 | −51.8 (3) | C4—C3—C8—C7 | −57.8 (3) |
| C4—C5—C6—C7 | 55.7 (3) | C6—C7—C8—C3 | 55.0 (3) |

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

| *D*—H···*A* | *D*—H | H···*A* | *D*···*A* | *D*—H···*A* |
|---|---|---|---|---|
| C4—H4···O13 | 1.08 | 2.30 | 3.046 (4) | 124 |
| C17—H17*A*···O11 | 1.08 | 2.46 | 3.538 (6) | 178 |
| C17—H17*A*···O14 | 1.08 | 2.46 | 2.780 (5) | 96 |
| C15—H15*A*···O11$^{i}$ | 1.08 | 2.69 | 3.575 (5) | 139 |
| C20—H20···O18$^{ii}$ | 1.08 | 2.58 | 3.335 (4) | 127 |
| C21—H21*B*···O13$^{iii}$ | 1.08 | 2.46 | 3.507 (5) | 163 |

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

Space group $P2_12_12_1$ was deduced from the systematic absences and intensity statistics. All H atoms were found in a difference electron-density map and were placed in calculated positions (C—H = 0.93–0.98 Å), with $U_{iso}$ values taken as 1.2 (1.5 for methyl) times the $U_{eq}$ values of the parent atoms. The H atoms of the C21 methyl group were found to be disordered over two positions and option *AFIX* 123 of *SHELXL*97 (Sheldrick, 1997) was used. There were no suitable anomalous scatterers for Mo $K\alpha$ radiation and therefore the determination of the absolute configuration was not possible from the X-ray data. The absolute configuration was assigned to agree with the chirality determined by chemical means and the Friedel diffraction data were merged accordingly.

Data collection: *COLLECT* (Nonius, 1999); cell refinement: *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997); data reduction: *DENZO* and *SCALEPACK*; program(s) used to solve structure: *SHELXS*86 (Sheldrick, 1985); program(s) used to refine structure: *SHELXL*97 (Sheldrick, 1997); molecular graphics: *ORTEP*II (Johnson, 1971), *PLUTON* (Spek, 1991), *PLATON* (Spek, 1998; Farrugia, 2000) and *ORTEP*-3 (Farrugia, 1999*b*); software used to prepare material for publication: *SHELXL*97, *PARST* (Nardelli, 1983, 1995) and *WinGX* (Farrugia, 1999*a*).

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Supplementary data for this paper are available from the IUCr electronic archives (Reference: NA1571). Services for accessing these data are described at the back of the journal.

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