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2-[*N*-(*tert*-Butoxycarbonyl)tyrosyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid nitromethane solvate

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Key indicators

Single-crystal X-ray study
T = 223 K
Mean σ(C–C) = 0.009 Å
Disorder in solvent or counterion
R factor = 0.075
wR factor = 0.195
Data-to-parameter ratio = 6.9

For details of how these key indicators were automatically derived from the article, see http://journals.iucr.org/e.

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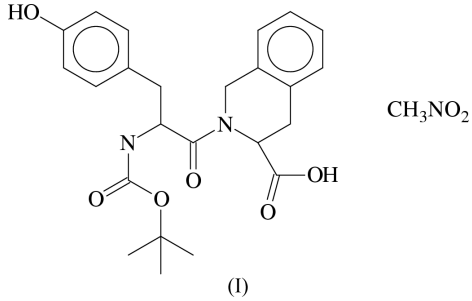
The title compound, $C_{24}H_{28}N_2O_6\cdot0.825CH_3NO_2$, is a member of the TIPP (*i.e.* Try–Tic–Phe–Phe) family of opioid ligands. The asymmetric unit contains one peptide molecule and one nitromethane molecule. Unlike other members of this familiy, this dipeptide has an extended conformation [*i.e.* $\varphi_1 = -103.6\ (6)^\circ$, $\omega_1 = 168.1\ (4)^\circ$, $\psi_1 = 152.4\ (5)^\circ$]. This conformation was further examined with the program *CHEM3D*. No significant energy difference was found between the energy-minimized conformation and a more tightly folded model conformation.

Comment

There are at least four main opioid receptors (μ, δ, κ and σ), some of which also have distinct sub-types (Walker *et al.*, 1990; Schiller, 1991). Most natural opioid peptides have a low receptor-site selectivity (Hruby & Gehrig, 1989). Diversity of the opioid receptors and low receptor-site selectivity of the natural opioid peptides have complicated the interpretation of binding and structural studies of these molecules.

The structural requirements for receptor-site selectivity and activity of the opioid peptides has been attributed to the composition and conformation of the peptide ligand and the net charge of the ligand (Hruby & Gehrig, 1989; Schiller, 1984; Temussi *et al.*, 1989; Rapaka, 1986; Schwyzer, 1986). Of these structural parameters, the relative location and orientation of the aromatic side chains and the relationship of the N-terminal nitrogen to the phenolic oxygen have been identified as critical elements for biological activity (Hansen & Morgan, 1984). To overcome the limitations imposed by studies of natural



(I)

opioids, several systematic approaches for the rational design of potent and selective analogues of the endogenous opioids have been developed. One approach places a tetrahydroisoquinoline-3-carboxylic acid (Tic) residue in the second position in order to constrain the conformation of the peptide backbone, which in turn constrains some of the structural parameters relevant to selectivity and activity. Peptides with the initial sequence Tyr–Tic–*X* are δ-selective antagonists. The tetrapeptide Tyr–Tic–Phe–Phe is one of the most potent and

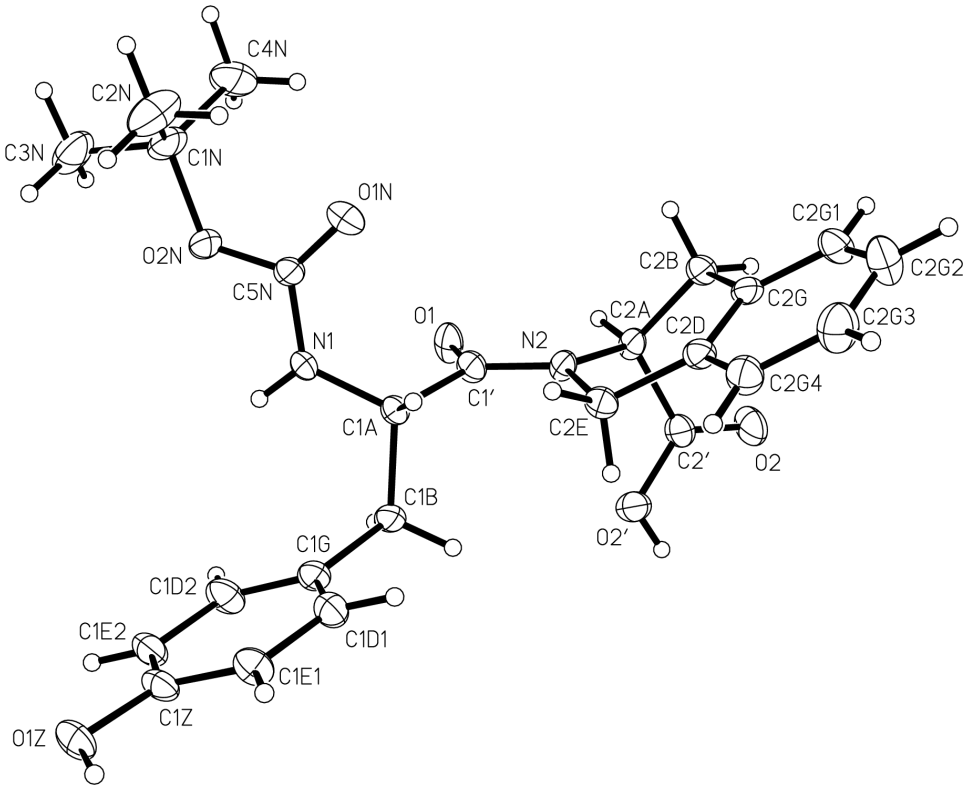


Figure 1
View of (I) showing the labeling of all non-H atoms in the dipeptide. The nitromethane molecule, which does not interact with the peptide, has been omitted for clarity. Displacement elipsoids are shown at 30% probability level; H atoms are drawn as small circles of arbitrary radii.

selective δ-antagonists known (Schiller *et al.*, 1993). Antagonist activity has even been observed in the dipeptide Tyr–Tic–NH_2 (Temussi *et al.*, 1994).

To better understand the structural elements required for selectivity and potency, detailed structural studies have been completed on many of the natural opioids and their synthetic analogues (Deschamps *et al.*, 1996). As part of this effort, the structure of boc–Tyr–Tic, (I), where boc is *tert*-butoxycarbonyl, was determined by X-ray diffraction.

There are three intermolecular hydrogen bonds (Table 2) which link the dipeptide in an infinite three-dimensional network. Each of the hydroxyls and the N-terminal nitrogen form hydrogen bonds to carbonyl-O atoms in neighboring molecules. The nitromethane solvent does not form any hydrogen bonds. Its closest approach to the dipeptide is at van der Waals separation.

The torsion angles that define the conformation of this peptide are reported in Table 1. The conformation of this dipeptide is more open than that of the tetrapeptide TIPP (Flippen-Anderson *et al.*, 1994) or any other Tic containing peptide reported to date. This could be due, in part, to the presence of the bulky boc moiety.

Molecular modeling was used to increase our understanding of the conformational differences between boc–Tyr–Tic and TIPP. In TIPP, ω_1 (C1*A*—C1′—N2—C2*A*) is *cis*, but in boc–Tyr–Tic, ω_1 is *trans*. The closely related agonists Tyr–D-Tic–Phe–Phe (D-TIPP) and Tyr–D-Tic (Flippen-Anderson *et al.*, 1997; Deschamps *et al.*, 1997) also have a *trans* conformation at ω1, but are more tightly folded than boc–Tyr–Tic. These differences in folding result in variations in the the separation of the aromatic rings. In boc–Tyr–Tic, the distance between the rings (reported as the distance between the centroids of the rings) is 8.4 (1) Å. In the other Tic containing peptides, the rings are closer together, with separations ranging from 6.6–6.7 Å in D-TIPP, to 5.93 Å in TIPP, and to 3.9–4.1 Å in the D-Tic dipeptides (Deschamps *et al.*, 1998).

Using TIPP (the only L-Tic containing peptide whose solid-state X-ray structure has been reported) as a template, an alternate conformation for boc–Tyr–Tic was constructed. Using MIDAS, the torsion angles in boc–Tyr–Tic were changed to more closely resemble those in TIPP. A comparison of the steric energy (after energy minimization) of the observed X-ray structure and this 'rotated' model reveals only a 1.8 kcal difference. This small difference can not account for the rather large conformational differences between TIPP and boc–Tyr–Tic. Thus, in solution, boc–Tyr–Tic could have a conformation like that of TIPP which could account for the observed antagonist activity of the dipeptide Tyr–Tic–NH_2 (Temussi *et al.*, 1994). In the rotated conformation, the distance between the rings is 4.4 Å before energy minimization and 4.7 Å after minimization. These distances are longer than the separation of the aromatic rings observed in the D-Tic dipeptides and shorter than the distance observed in TIPP. Since a conformation similar to that observed in TIPP (*i.e.* the rotated model) is not energetically unfavored and since the unblocked dipeptides have biologic activity similar to longer

peptides of the same series, the differences in the conformation of TIPP and boc–Tyr–Tic are most likely due to the presence of the boc moiety.

Experimental

Crystallization: the dipeptide, boc-tyrosyl-tetrahydroisoquinoline-3-carboxylic acid, was obtained from Research Triangle Institute (RTI). Crystals were grown by evaporation from methanol–nitromethane (2:1). Attempts to produce higher quality crystals out of aqueous solutions were not successful. Modeling: using *MIDAS* (Ferrin *et al.*, 1988), the torsion angles of the dipeptide were rotated until a good match with residues 1 and 2 of Tyr–Tic–Phe–Phe (TIPP; Flippen-Anderson *et al.*, 1994) was achieved. The steric energy of the energy-minimized X-ray structure and the 'rotated' model were then calculated using *MM2* parameters as implemented in the program *CHEM3D* plus (Version 3.1; Cambridge Scientific Computing, Inc., Cambridge, MA 02139, USA). Several missing torsional parameters were approximated by the substitution of a similar atom into the atomic sequence. The values for the optimal bond lengths for the atoms in the ring systems were taken from the median values listed for those ring systems in International Tables for Crystallography, Vol. C.

Crystal data

$C_{24}H_{28}N_2O_6{\cdot}0.825CH_3NO_2$
M_r = 490.85
Orthorhombic, $P2_12_12_1$
a = 11.464 (3) Å
b = 14.827 (2) Å
c = 16.444 (2) Å
V = 2795.1 (9) Å^3
Z = 4
D_x = 1.166 Mg m^{-3}
Cu $K\alpha$ radiation
Cell parameters from 35 reflections
θ = 8.0–55.0°
μ = 0.73 mm^{-1}
T = 223 (2) K
Prism, clear colourless
0.52 × 0.50 × 0.46 mm

Data collection

Siemens $P4$ diffractometer
$2\theta/\omega$ scans
Absorption correction: analytical (*XPREP*; Siemens, 1994)
T_{min} = 0.723, T_{max} = 0.807
2366 measured reflections
2249 independent reflections
2080 reflections with $I > 2\sigma(I)$
R_{int} = 0.049
θ_{max} = 56.5°
$h = 0 \rightarrow 12$
$k = 0 \rightarrow 16$
$l = -2 \rightarrow 17$
3 standard reflections
every 97 reflections
intensity decay: 6.8%

Refinement

Refinement on F^2
$R[F^2 > 2\sigma(F^2)]$ = 0.075
$wR(F^2)$ = 0.195
S = 1.09
2249 reflections
326 parameters
H-atom parameters constrained
$w = 1/[\sigma^2(F_o^2) + (0.1436P)^2 + 1.0149P]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$ = 0.002
$\Delta\rho_{max}$ = 0.42 e Å^{-3}
$\Delta\rho_{min}$ = −0.36 e Å^{-3}

Table 1
Selected torsion angles (°).

N1—C1*A*—C1′—N2	152.4 (5)	C2*A*—C2*B*—C2*G*—C2*G*1	160.8 (5)
C1′—N2—C2*A*—C2′	−103.6 (6)	C2*A*—C2*B*—C2*G*—C2*D*	−21.9 (7)
N1—C1*A*—C1*B*—C1*G*	−61.6 (6)	C2*B*—C2*G*—C2*D*—C2*E*	0.4 (8)
C1*A*—C1′—N2—C2*A*	168.1 (4)	C2*G*—C2*D*—C2*E*—N2	−6.5 (8)
C1*A*—C1*B*—C1*G*—C1*D*1	−77.6 (7)	C2*D*—C2*E*—N2—C2*A*	36.2 (6)
C1*A*—C1*B*—C1*G*—C1*D*2	101.5 (6)	C2*E*—N2—C2*A*—C2*B*	−58.4 (6)
N2—C2*A*—C2*B*—C2*G*	48.8 (6)		

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

D—H···A	D—H	H···A	D···A	D—H···A
O1Z—H1*Z*···O1N^{i}	0.82	1.87	2.682 (6)	173
O2′—H2′···O1^{ii}	0.82	1.84	2.637 (6)	163
N1—H1N···O2^{iii}	0.86	2.241	2.901 (6)	133

Symmetry codes: (i) $\frac{5}{2} - x, 2 - y, \frac{1}{2} + z$; (ii) $\frac{1}{2} + x, \frac{3}{2} - y, -z$; (iii) $x - \frac{1}{2}, \frac{3}{2} - y, -z$.

Despite low-temperature data collection, the crystals suffered almost a 7% loss of intensity during data collection. This, in combination with restrictions caused by the low-temperature device itself, limited the data collection range. The solvent (nitromethane) does not interact with any other molecule and it is likely that solvent was lost from the crystal during data collection. The loss of solvent during data collection would explain both the partial occupancy of the solvent and the final R value of aproximately 7% even though $R_{internal}$ and R_{sigma} are 4.9 and 3.8, respectivly. The nitromethane molecule also has larger displacement parameters than the peptide. The correct configuration was set by comparison to TIPP, a related peptide whose absolute configuration is known.

Data collection: *XSCANS* (Bruker, 1994); cell refinement: *XSCANS*; data reduction: *XPREP* (Bruker, 1994); program(s) used to solve structure: *SHELXS* (Sheldrick, 1990); program(s) used to refine structure: *SHELXTL* (Sheldrick, 1997); molecular graphics: *SHELXTL*; software used to prepare material for publication: *SHELXTL*.

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