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Piv-Proψ[CH$_2$–NH–O]Gly–NH^{i}Pr

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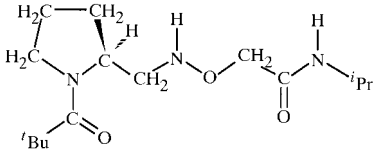
The pseudodipeptide, (*S*)-*N*-isopropyl {[*N*-(pivaloyl)pyrrolidin-2-yl]methylaminooxy}acetamide, $C_{15}H_{29}N_3O_3$, adopts a global extended conformation with the hydroxylamine group in the g^+/g^- structure. The C-terminal amide NH interacts intramolecularly with the hydroxylamine O atom. Both NH bonds of each molecule are hydrogen bonded to the C-terminal amide carbonyl of a neighbouring molecule.

Comment

The so-called reduced peptide group Cα—CH$_2$—NH—Cα is often used to mimic the transient state Cα—C(OH)$_2$—NH—Cα of the peptide group during enzymatic cleavage of the amide bond (Epps *et al.*, 1988; Sawyer, Pals, Mao, Maggiora *et al.*, 1988; Sawyer, Pals, Mao, Staples *et al.*, 1988; Kaltenbronn *et al.*, 1990; Gante, 1994). However, the pK_a of this amide surrogate is about neutrality and may depend on the environment, so that at least a part of the molecule may be protonated into Cα—CH$_2$—$^+$NH$_2$—Cα at the physiological pH (Aumelas *et al.*, 1987; Vanderesse *et al.*, 1998). Contrary to the neutral form, the ionic form is a proton donor capable of strong hydrogen bonds with nucleophiles, that may induce



(I)

particular folded structures (Vanderesse *et al.*, 1998) or interaction modes in intermolecular interactions. We have recently proposed two reduced amide surrogates having the N—N or N—O fragment that decreases the pK_a to such a value that it is not protonated at the physiological pH (Vanderesse *et al.*, 1998; Thévenet *et al.*, 2000). Here we report the crystal molecular structure of a pseudodipeptide containing the methyleneaminoxy link, (I).

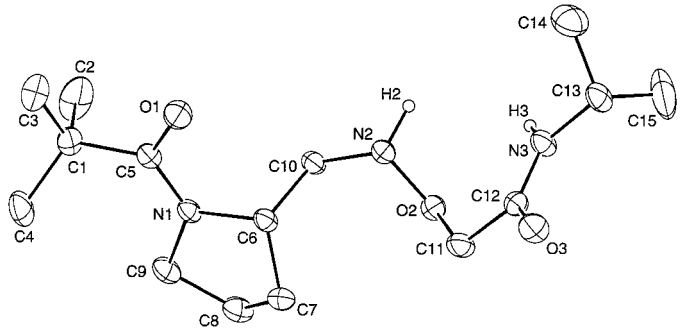


Figure 1
The molecular structure of the title pseudodipeptide with the atom-numbering scheme and 25% probability displacement ellipsoids. H atoms except those of the NH groups have been omitted for clarity.

The three-dimensional structure (Table 1) shows that the C6—C10—N2—O2—C11 hydroxylamine fragment assumes a skew conformation, so that the C6···C11 (Cα···Cα) distance of 3.682 (4) Å is a little bit shorter than the corresponding distance of 3.81 Å for a peptide group (Benedetti, 1977). The molecule assumes a globally extended conformation (Fig. 1) in which the C-terminal NH is intramolecularly hydrogen-like bonded to the hydroxylamine O atom (Table 2), exactly as in chloroform solution (Thévenet *et al.*, 2000). Although it is out of the usual criteria defining hydrogen bonds (Baker & Hubbard, 1984), such a bent interaction has already been encountered in various crystal molecular structures of modified peptides (Toniolo *et al.*, 1989; Aubry *et al.*, 1994; Crisma *et al.*, 1999). Molecules are held in files along the *x* axis by a double interaction involving both NH groups of a given molecule and the C-terminal amide carbonyl of another molecule (Table 2).

Experimental

The pseudodipeptide was obtained by coupling NH$_2$—O—CH$_2$—CO—NH^{i}Pr to the Boc-L-Pro—H aldehyde, with subsequent ^{t}BuCO (Piv) for *tert*-butyloxycarbonyl (Boc) substitution, and reduction of the oxime by NaBH$_3$CN. Commercially available Boc—NH—O—CH$_2$—COOH (382 mg, 2 mmol) and *N*-methylmorpholine (NMM) (0.22 ml, 2 mmol) in tetrahydrofuran (THF) (20 ml) were treated dropwise under stirring with isobutylchloroformate (0.26 ml, 2 mmol) diluted in THF (2 ml) at 255 K, and stirring was maintained for 15 min. Isopropylamine (0.17 ml, 2 mmol) in THF (2 ml) was added dropwise, and the mixture was allowed to reach room temperature and stirred overnight. NMM hydrochloride was filtered off and the solvent was evaporated. Boc—NH—O—CH$_2$—CO—NH^{i}Pr was purified by silica-gel chromatography with ethyl acetate/petroleum ether (60/40, *v*/*v*) as eluent (R_f = 0.58, 348 mg, 1.5 mmol, yield = 75%). The Boc group was quantitatively eliminated with trifluoroacetic acid (TFA) in dichloromethane (DCM) (40/60), and TFA·NH$_2$—O—CH$_2$—CO—NH^{i}Pr was recovered by lyophilization from an aqueous solution. TFA·NH$_2$—O—CH$_2$—CO—NH^{i}Pr (369 mg, 1.5 mmol) in ethanol (20 ml) was treated with NMM (0.16 ml, 1.5 mmol) and coupled to Boc-L-Pro—H (597 mg, 3 mmol), obtained from Boc-L-Pro—OH (Fehrentz & Castro, 1983), in the presence of sodium acetate (492 mg, 6 mmol) and molecular sieves. The mixture was

stirred at room temperature overnight to give Boc-Proψ[CH═N—O]Gly—NH^{i}Pr which was purified by silica-gel chromatography with ethyl acetate/petroleum ether (70/30, *v*/*v*) (R_f = 0.53, 385 mg, 1.23 mmol, yield = 82%). The Boc group was quantitatively eliminated with TFA in DCM (40/60), and the resulting TFA·H—Proψ[CH═N—O]Gly—NH^{i}Pr was obtained by lyophilization from an aqueous solution. TFA·H—Proψ[CH═N—O]Gly—NH^{i}Pr (402 mg, 1.23 mmol) was dissolved in chloroform (20 ml), the solution cooled to 273 K, and diisopropylethylamine (0.42 ml, 2.46 mmol) and pivaloyl chloride (0.23 ml, 1.85 mmol) were successively added dropwise. The mixture was stirred at 273 K for 2 h to give Piv-Proψ[CH═N—O]Gly—NH^{i}Pr which was purified by silica-gel chromatography with ethanol/ethyl acetate/petroleum ether (10/60/30, *v*/*v*/*v*) (R_f = 0.67, 245 mg, 0.82 mmol, yield = 67%). Piv-Proψ[CH═N—O]Gly—NH^{i}Pr (245 mg, 0.82 mmol) was dissolved in methanol (10 ml), and $NaBH_3CN$ (515 mg, 8.2 mmol) was added under stirring at room temperature in eight portions over 96 h while the pH was adjusted to 3 with acetic acid (acid–base indicator: methyl orange). The solution was poured into 5 ml of water saturated with K_2CO_3. The solution was repeatedly extracted with DCM (5 × 5 ml). The organic phases were combined and washed three times with 5% aqueous $NaHCO_3$ and three times with brine. Piv-Proψ[CH$_2$—NH—O]Gly—NH^{i}Pr was purified by silica-gel chromatography with ethanol/ethyl acetate/petroleum ether (10/60/30, *v*/*v*/*v*) as eluent (R_f = 0.45, 125 mg, 0.42 mmol, yield = 51%). Single crystals were obtained by slow evaporation of a DCM solution.

Crystal data

$C_{15}H_{29}N_3O_3$
M_r = 299.41
Orthorhombic, $P2_12_12_1$
a = 5.3860 (5) Å
b = 11.470 (2) Å
c = 27.539 (3) Å
V = 1701.3 (4) Å^3
Z = 4
D_x = 1.169 Mg m^{-3}
Cu $K\alpha$ radiation
Cell parameters from 25 reflections
θ = 9.7–26.2°
μ = 0.658 mm^{-1}
T = 293 (2) K
Prismatic, colourless
0.6 × 0.1 × 0.1 mm

Data collection

Nonius Mach3 diffractometer
$\omega/2\theta$ scans
1877 measured reflections
1877 independent reflections
1702 reflections with $I > 2\sigma(I)$
θ_{max} = 69.69°
$h = 0 \rightarrow 6$
$k = 0 \rightarrow 13$
$l = 0 \rightarrow 33$
2 standard reflections
frequency: 60 min
intensity decay: 3.7%

Refinement

Refinement on F^2
$R[F^2 > 2\sigma(F^2)]$ = 0.054
$wR(F^2)$ = 0.157
S = 1.063
1877 reflections
197 parameters
H atoms treated by a mixture of independent and constrained refinement
$w = 1/[\sigma^2(F_o^2) + (0.1120P)^2 + 0.3040P]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$ = 0.019
$\Delta\rho_{max}$ = 0.28 e Å^{-3}
$\Delta\rho_{min}$ = −0.33 e Å^{-3}
Extinction correction: *SHELXL*97 (Sheldrick, 1997)
Extinction coefficient: 0.0125 (16)

Table 1
Selected torsion angles (°).

C1—C5—N1—C6	177.8 (2)	C10—N2—O2—C11	−125.4 (2)
C5—N1—C6—C10	−77.4 (3)	N2—O2—C11—C12	−73.8 (3)
N1—C6—C10—N2	161.3 (2)	O2—C11—C12—N3	−12.6 (4)
C6—C10—N2—O2	83.8 (3)	C11—C12—N3—C13	−177.7 (3)

Table 2
Hydrogen-bonding geometry (Å, °) (see text for discussoin of first entry).

D—H···A	D—H	H···A	D···A	D—H···A
N3—H3···O3^i	1.02	2.25	3.211 (3)	156
N2—H2···O3^i	1.022	2.29	3.249 (3)	155
N3—H3···O2	1.02	2.37	2.725 (3)	99

Symmetry code: (i) $x-1, y, z$.

The absolute stereochemistry of the title compound was assumed from Boc-L-Pro–OH purchased from Neosystem Corporation (Strasbourg, France). The positions of the H atoms attached to N atoms were located from a difference map and the N—H bond distance was restrained to a value of 1.03 (1) Å (Taylor & Kennard, 1983). H atoms connected to carbon were placed at calculated positions using a riding model. All H atoms had isotropic displacement parameters fixed at 1.3 times that of the parent atom.

Data collection: *CAD*-4 *Software* (Enraf–Nonius, 1989); cell refinement: *CAD*-4 *Software*; data reduction: *maXus* (Mackay *et al.*, 1999); program(s) used to solve structure: *SIR*92 (Altomare *et al.*, 1994); program(s) used to refine structure: *SHELXL*97 (Sheldrick, 1997); molecular graphics: *maXus*.

Supplementary data for this paper are available from the IUCr electronic archives (Reference: GS1085). Services for accessing these data are described at the back of the journal.

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