Instrumental Achievements

Crystal Structure of the Dipeptide Cyclo(glycyl-L-glutamine)

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The 31-residue polypeptide β-endorphin is a neurotransmitter that produces opioid-like effects on the central nervous system.[1] Cleavage of the β-endorphin C-terminal in the brain and pituitary gland yields the biologically active dipeptide glycyl-L-glutamine (Gly-L-Gln, β-endorphin 30-31), which inhibits β-endorphin-induced cardiorespiratory depression.[2,3] The stereospecificity of this effect is evident, since Gly-D-Gln has recently been reported to be inactive in this respect.[3] On the other hand, the compound cyclo(Gly-L-Gln) has similar biological activity to that of Gly-L-Gln.[4] As part of a study aimed at elucidating the factors responsible for CNS activity, we report here the crystal structure of the active compound cyclo(Gly-L-Gln), shown in Fig. 1. Preliminary results were reported earlier.[5]

The compound was purchased from Bachem Bioscience Inc., King of Prussia, PA, USA as the anhydrous form of the title stereoisomer. The absolute configuration was therefore known and was maintained in the structural analyses. Single crystals were obtained by recrystallization of the anhydrous material from acetic acid/ethanol solution. Intensity data (including Friedel opposites) were obtained by a combination of 1.8° φ and ω scans which yielded > 99% data completeness. The structure was solved by direct methods and refined by full-matrix least-squares on F^2, with all non-H atoms thermally anisotropic. All H atoms were located from difference Fourier maps. Those attached to N or O atoms were refined with a distance restraint (O–H, N–H 1.000 Å, σ = 0.005 Å) while all others were placed in idealized positions in a riding model. The weighting scheme was $w = 1/[(\sigma^2(F_o^2) + (0.0534P)^2 + 0.0429P]$, where $P = (F_o^2 + 2F_c^2)/3$.

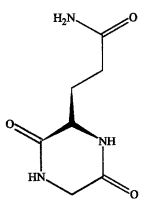

Fig. 1 Chemical structure of cyclo(glycyl-L-glutamine).

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Crystal and refinement details appear in Table 1. Positional and thermal parameters are listed in Table 2.

Figure 2 shows the molecular structure and conformation. These can be described with reference to the two essentially planar units, the diketopiperazine ring and the glutamine side chain. For the ring, the largest deviation from the mean plane is 0.033(1)Å for C2, and for the extended side chain including the five non-H atoms, the largest deviation from the mean plane is 0.018 Å for C10. The dihedral angle between these mean

Table 1 Crystal and experimental data

Formula: $C_7H_{11}N_3O_3$
Formula weight = 185.19
Crystal system: monoclinic
Space group: $P2_1$
Z = 2
a = 4.510(1)Å
b = 18.919(2)Å
c = 5.087(1)Å
β = 99.375(5)°
V = 428.3(1)Å^3
D_x = 1.436 g cm^{-3}
D_m = 1.44(1)g cm^{-3} (by flotation in aq. KI solution)
μ(Mo K$_\alpha$) = 0.114 mm^{-1}
T = 295 K
Color: colorless
F(0 0 0) = 196
Dimensions = 0.30 × 0.20 × 0.16 mm
Radiation: Mo K$_\alpha$ (λ = 0.71069 Å)
$2\theta_{max}$ = 61.0°
R = 0.039 (on F)
Rw = 0.104 (on I)
$(\Delta/\sigma)_{max}$final cycle = < 0.001
$(\Delta\rho)_{max}$ = 0.20 eÅ^{-3}
$(\Delta\rho)_{min}$ = -0.18 eÅ^{-3}
No. of reflections measured = 4374
No. of reflections used = 2365
No. of parameters = 131
Goodness-of-fit = 1.045
Measurement: Nonius Kappa CCD
Program system: DENZO-SMN
Structure determination: SHELXS-97
Refinement: full-matrix: SHELXL-97

Table 2 Atomic coordinates and equivalent temperature factors (Å^2)

Atom	*x*	*y*	*z*	U_{eq}
N1	0.3069(3)	0.61216(8)	-0.1343(3)	0.0331(4)
C2	0.5452(4)	0.56825(10)	0.0043(4)	0.0367(5)
C3	0.7080(4)	0.59976(8)	0.2580(3)	0.0297(4)
N4	0.6176(3)	0.66093(7)	0.3401(3)	0.0287(4)
C5	0.3709(4)	0.70525(9)	0.2089(3)	0.0264(4)
C6	0.2159(3)	0.67339(9)	-0.0519(3)	0.0300(5)
O7	0.9271(3)	0.56737(7)	0.3819(3)	0.0463(4)
O8	0.0016(3)	0.70641(8)	-0.1801(2)	0.0479(4)
C9	0.4794(4)	0.78012(10)	0.1644(3)	0.0316(5)
C10	0.5722(4)	0.81998(9)	0.4244(3)	0.0355(5)
C11	0.7296(4)	0.88919(10)	0.3909(3)	0.0356(5)
N12	0.8256(5)	0.92377(10)	0.6167(3)	0.0569(6)
O13	0.7729(4)	0.91106(9)	0.1749(3)	0.0605(6)

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

Table 3 Selected bond distances (Å), angles (˚) and torsion angles (˚) for cyclo(Gly-L-Gln)

N1 - C2	1.448(2)			O8 - C6	1.243(2)		
N1 - C6	1.320(2)			O7 - C3	1.243(2)		
C2 - C3	1.500(3)			O13 - C11	1.219(2)		
N4 - C3	1.317(2)			C9 - C10	1.521(2)		
N4 - C5	1.464(2)			C10 - C11	1.512(3)		
C5 - C6	1.520(2)			N12 - C11	1.331(2)		
C5 - C9	1.527(3)						
C2 -N1 -C6	126.41(15)			N1 -C2 -C3	114.29(15)		
N1 -C6 -C5	120.18(14)			C5 -C9 -C10	112.36(13)		
N4 -C5 -C6	112.21(14)			C9 -C10 -C11	113.52(13)		
C3 -N4 -C5	127.58(14)			N12 -C11 -C10	114.89(15)		
N4 -C3 -C2	119.23(15)			O13 -C11 -N12	122.24(19)		
C2 -N1 -C6 -C5	1.7(2)			C5 -C9 -C10 -C11	170.13(15)		
C5 -N4 -C3 -C2	-1.2(3)			C9 -C10 -C11 -N12	-177.11(17)		
N4 -C5 -C9 -C10	-67.46(18)						

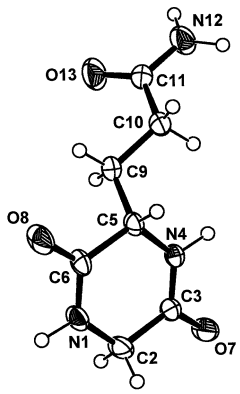

Fig. 2 Structure and conformation of cyclo(glycyl-L-glutamine) with atom labeling. Thermal ellipsoids are drawn at the 50% probability level.

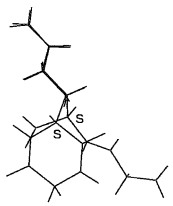

Fig. 3 Overlay of Gly-L-Gln and cyclo(Gly-L-Gln).

planes is 69.4(1)˚. In contrast to Gly-L-Gln[6] where the peptide unit is *trans* and strictly planar, the two *cis*-dipeptide groups in the title compound deviate slightly from planarity with endocyclic ω torsion angles of –1.2(3)˚ and 1.7(2)˚. These reflect some strain introduced by closure of two *cis*-peptide bonds in forming the ring.[7] From Fig. 2, the glutamine side chain is seen to be extended, in contrast to the folded conformation found in Gly-L-Gln.[6] Selected molecular parameters appear in Table 3. An intricate three-dimensional network of hydrogen bonds maintains the crystal structure. Infinite chains of hydrogen-bonded diketopiperazine rings propagate parallel to the (101) crystal planes. Each chain contains molecules related by diagonal translation only (*e.g. x*, *y*, *z* and 1+*x*, *y*, 1+*z*). The sets of 2_1-related hydrogen-bonded rings are cross-linked by hydrogen bonding effected by the amide group of the glutamine side chain. The relevant N12–H··O hydrogen bonds propagating in planes parallel to the (100) crystal planes can be seen in the region $y = 1/2$.

On the question of structure and activity, Fig. 3 shows a molecular overlay of cyclo(Gly-L-Gln) on Gly-L-Gln, with superposition of the glutamine amide residues as the common feature. These compounds show some degree of molecular complementarity, which may be a contributing factor to their common activity. However, firm conclusions await further molecular modeling and analysis of solution NMR data.

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