SYNTHESIS AND X-RAY DIFFRACTION ANALYSIS OF THE TETRAZOLE PEPTIDE ANALOGUE PRO-LEUψ[CN_4]GLY-NH_2

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Received January 14th, 1988
Accepted March 16th, 1988

Dedicated to the memory of Dr Karel Bláha.

The synthesis of an analogue of the neuropharmacologically active peptide Pro-Leu-Gly-NH_2 in which the Leu-Gly peptide bond has been replaced with a tetrazole moiety was carried out. The molecular and crystal structure of the tetrazole analogue Pro-Leuψ[CN_4]Gly-NH_2 was determined by X-ray diffraction and a comparison was made with the published X-ray structure of Pro-Leu-Gly-NH_2. The tetrazole annular system turns out to be a good conformationally--restricted replacement for the *cis*-peptide bond in terms of bond lengths, bond angles and the ω torsion angle. The molecule was found to be folded at the -Leuψ[CN_4]Gly- sequence, but it did not form the intramolecular N—H···O=C hydrogen bond characteristic of the type VIa β-bend conformation. In contrast to Pro-Leu-Gly-NH_2, Pro-Leuψ[CN_4]Gly-NH_2 was found to be unable to enhance the binding of dopamine receptor agonists to the dopamine receptor.

Peptide bond surrogates have seen increasing use in recent years in the design and synthesis of analogues of bioactive peptides[1]. We have been interested in the tripeptide Pro-Leu-Gly-NH_2 (PLG) since it has been shown to enhance the binding of dopamine receptor agonists to central dopamine receptors[2] and to prevent, as well as reverse, neuroleptic drug induced supersensitivity of dopamine receptors[3,4]. In an attempt to determine whether the Leu-Gly amide bond of PLG needs to be in a *cis* or *trans* conformation when it modulates dopamine receptors we set out to synthesize analogues of PLG that would mimic either the *cis* or *trans* Leu-Gly amide bond. One of the analogues of PLG previously synthesized by us was Pro-Leuψ-[CH=CH]Gly-NH_2 (ref.[5]) wherein the amide bond has been replaced with a trans olefinic moiety[6].

Although the corresponding *cis* olefin would be a good mimic for the *cis* amide

bond, the synthesis of such peptide analogues has proved extremely difficult because of the propensity of the *cis*-β,γ-unsaturated carbonyl system to isomerize to the *trans*-α,β-unsaturated carbonyl system even under the relatively mild conditions of peptide synthesis[7]. As an alternative to the *cis*-olefinic peptide analogues Marshall[8] has proposed using the tetrazole ring system to mimic a *cis* amide bond. We recently have reported on the synthesis and chemical properties of tetrazole peptide analogues[9]. In the present paper we wish to report the synthesis of a tetrazole analogue of PLG, Pro-Leuψ[CN_4]Gly-NH_2 (*I*). Furthermore, with the aim of characterizing the geometrical and conformational properties of the tetrazole peptide bond surrogate we also describe the molecular and crystal structure of *I* as determined by X-ray diffraction analysis and compare it with the published structure of PLG (ref.[10]).

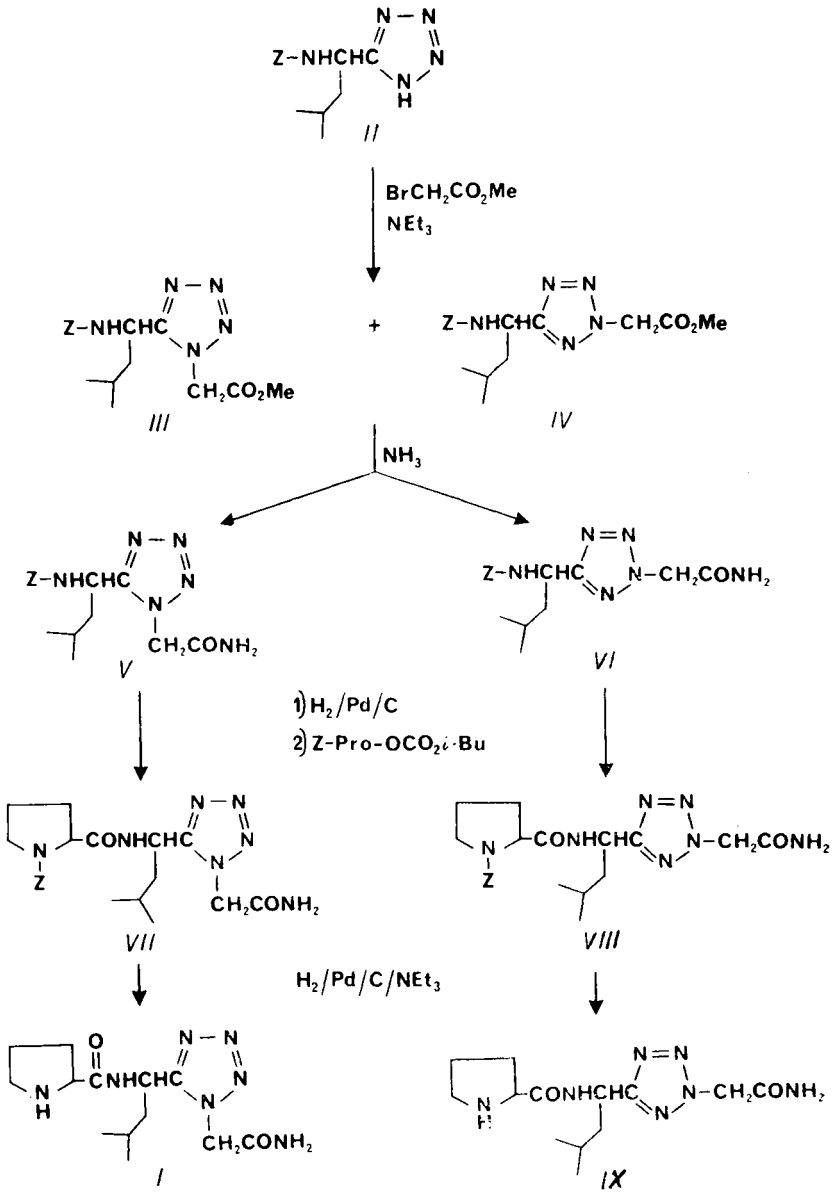
RESULTS AND DISCUSSION

Synthesis. The synthetic route that was used to synthesize the Leu-Gly tetrazole analogue of PLG, compound *I*, is shown in Scheme 1. Alkylation of the known tetrazole *II* (ref.[11]) with methyl bromoacetate in the presence of Et_3N provided a mixture of 1- and 2-tetrazolylacetic acid methyl esters, *III* and *IV*, respectively, in a 1 : 1 ratio as determined by the integration of the ester methyl resonances in the ^1H NMR spectrum. Although *III* and *IV* could be readily separated from one another on TLC, we found it more convenient to react the mixture of esters with methanolic ammonia to give the corresponding isomeric tetrazolylacetamides, *V* and *VI*. These two were readily separated from one another by fractional recrystallization.

The structures of the regioisomeric tetrazolylacetamides were determined by ^1H and ^{13}C NMR. In previous ^1H NMR studies[12,13] it had been shown that the carboxymethylene signal for 1-tetrazolylacetic acids appears further upfield than the carboxymethylene signal in the 2-tetrazolylacetic acids, while in ^{13}C NMR studies of 1- and 2-methyltetrazoles[14,15] it had been shown that the methyl and C-5 carbon atoms of 1-methyltetrazole are more shielded than the corresponding carbon atoms of 2-methyltetrazole. Based on the above observations the tetrazolylacetamide that was first to crystallize out of the mixture of *V* and *VI* was designated as the 1,5-tetrazolylacetamide *V* since it had its carboxymethylene ^1H signal at 5·25 ppm and its carboxymethylene and C-5 carbon resonances at 48·5 and 156·8 ppm, respectively. The tetrazolylacetamide that crystallized out second had a higher R_F value on TLC and had its carboxymethylene ^1H signal at 5·36 ppm and its carboxymethylene and C-5 carbon resonances at 54·2 and 165·5 ppm. respectively. This assignment was verified by the X-ray diffraction analysis described below.

The benzyloxycarbonyl protecting group of *V* was removed by catalytic hydrogenolysis to afford the corresponding free amine which was immediately coupled to the mixed anhydride between Z-Pro-OH and isobutylchloroformate to give the tripeptide analogue *VII*. Catalytic hydrogenolysis of *VII* in the presence of Et_3N

(ref.[16]) provided the desired tetrazole analogue of PLG, compound *I*. The regioisomeric 2,5-tetrazolylacetamide *VI* was converted to the tripeptide analogue *IX* using this same general procedure.



SCHEME 1

X-Ray Crystallography. The crystallographic data for *I* are shown in Table I while in Table II the positional parameters for the non-hydrogen atoms are given. Bond lengths (Table III) and bond angles (Table IV) of Pro-Leuψ[CN_4]Gly-NH_2 (*I*, Fig. 1) agree well with those shown by amide[17] and peptide[18] units, 1,5-disubstituted tetrazolyl groups[19,20], and Pro[21–26] and Leu[26–28] derivatives. In particular, with regard to the tetrazolyl group: (i) The N(3)—N(4) bond, 1·290 (19) Å, is significantly shorter than the N(2)—N(3) and N(4)—N(5) bonds, 1·400 (17) and 1·386 (19) Å, respectively. This is a clear indication of its double bond character. (ii) The N(3)—N(4)—N(5) endocyclic bond angle, 114·8 (12)°, is markedly wider than the N(2)—N(3)—N(4) and N(4)—N(5)—C(3) bond angles, 102·0 (12)° and 102·8 (12)°. (iii) The two exocyclic bond angles, C(2)—N(2)—C(3) and N(2)—C(3)—C(4), 133·7 (12)° and 126·0 (13)°, respectively, are much wider than expected.

The pyrrolidine ring of the prolyl residue has a conformation described as C_s (envelope) symmetry, the ring-puckering parameters[29] being q_2 = 0·663(29) Å and ϕ_2 = 145·1(24)° and the ring torsion angles[22] θ [C(11)—N(7)—C(10)—C(13)], χ_1 [N(7)—C(10)—C(13)—C(12)], χ_2 [C(11)—C(12)—C(13)—C(10)], χ_3 [N(7)—C(11)—C(12)—C(13)], and χ_4 [C(10)—N(7)—C(11)—C(12)] being 32·8 (17), −14·1 (20), −9·8 (24), 30·6 (23), and −39·4 (19)°, respectively (Table V).

The tetrazole ring is essentially planar, the ring-puckering amplitude q_2 being 0·003 (12) Å and the ring torsion angles N(3)—N(2)—C(3)—N(5), C(3)—N(2)—N(3)—N(4), N(2)—N(3)—N(4)—N(5), N(3)—N(4)—N(5)—C(3), and N(4)—N(5)—C(3)—N(2) being 0·3 (16), −0·1 (15), −0·1 (15), 0·3 (16), and −0·3 (16)°, respectively. The dihedral angle between normals to the average planes of the pyrrolidine and tetrazole rings is 81·5 (18)°.

TABLE I

Crystallographic data for Pro-Leuψ[CN_4]Gly-NH_2(*I*)

Molecular formula	$C_{13}H_{23}N_7O_2$
Molecular weight	189·27
Crystal system	orthorhombic
Space group	$P2_12_12_1$
Cell parameters[a]	
a(Å)	31·044(3)
b(Å)	9·827(1)
c(Å)	5·368(1)
V(Å^3)	1637·6(4)
D_c(g/cm^{-3})	1·253
Z	4

[a] 1 Å = 10^{-10} m.

The leucyl side chain takes the common $g^-(t, g^-)$ conformation[26-28], with the χ^1[N(6)—C(4)—C(5)—C(6)], $\chi^{2,1}$[C(4)—C(5)—C(6)—C(8)], and $\chi^{2,2}$[C(4)—C(5)—C(6)—C(7)] torsion angles being −69·6 (15), 159·7 (15), and −82·7 (18)°, respectively. The N-terminal prolyl residue is found in the unusual C_5 conformation [ψ torsion angle N(6)—C(9)—C(10)—N(7) = −16·1 (19)°][30] stabilized by a (peptide) N—H···N (amino) intramolecular hydrogen bond[31,32]. The N(6)···N(7) separation is 2·732 (17) Å and the N(6)—H···N(7) separation is 2·183 (20) Å[33,34]. The peptide torsion angle ω at the Pro-Leu junction, C(4)—N(6)—C(9)—C(10), is in the usual *trans* conformation[18], −171·9 (12)°.

The molecule is folded at the Leuψ[CN_4]Gly- sequence, the sets of ϕ, ψ torsion angles for the Leu[C(9)—N(6)—C(4)—C(3) and N(2)—C(3)—C(4)—N(6)] and Gly[C(3)—N(2)—C(2)—C(1) and N(1)—C(1)—C(2)—N(2)] residues being −120·4 (14), 46·8 (18) and −88·8 (19), −20·1 (18)°, respectively. The pseudo-ω torsion angle at the Leu-Gly junction, C(2)—N(2)—C(3)—C(4), is forced by the tetrazole annular structure to be *cis*[8], 7·2 (25)°. The Leu ϕ, ψ torsion angles are significantly removed from those typical of the $i + 1$ residue in either the type-VIa (−60, 120°)

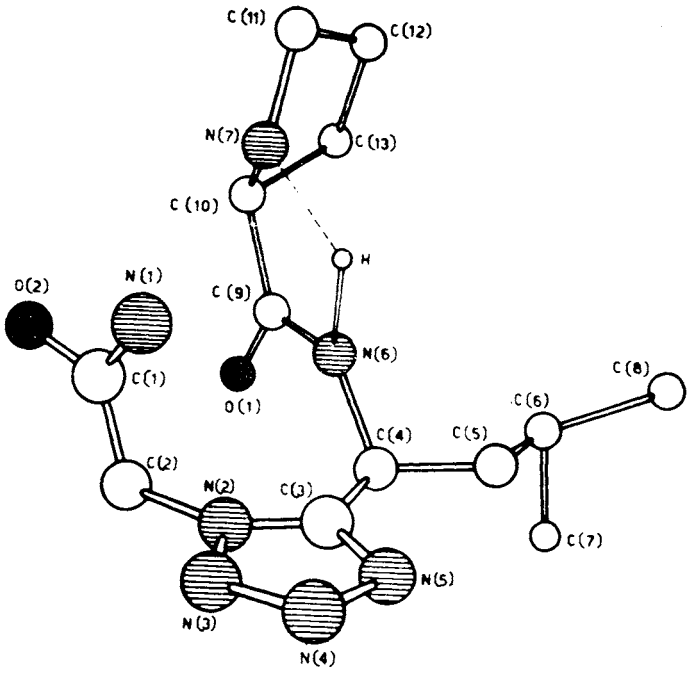


FIG. 1

Molecular structure of Pro-Leuψ[CN_4]Gly-NH_2 (*I*) with numbering of the atoms. The intramolecular hydrogen bond is shown as a dashed line

CONCLUSIONS

In the published X-ray diffraction structure of Pro-Leu-Gly-NH_2 (ref.[10]) this tripeptide amide adopts a regular type-II β-bend conformation[35-39] where the Leu ϕ, ψ torsion angles are $-61{\cdot}2$, $127{\cdot}8°$ and the Gly ϕ torsion angle is $71{\cdot}8°$. A relatively long (Gly) N—H···O=C (Pro) intramolecular hydrogen bond, 3·04 Å, stabilizes this folded conformation. The Pro-Leu and Leu-Gly ω torsion angles are *trans* and the Pro ψ torsion angle, which gives the orientation of the pyrrolidine ring relative to the rest of the molecule, is $152{\cdot}9°$.

The incorporation of a tetrazolyl moiety at the Leu-Gly junction of the PLG molecule not only forces the pseudo-peptide bond to be *cis*, as expected, but it also alters markedly the preferred conformation of all three amino acid residues. The Pro ψ torsion angle is $-16{\cdot}1\,(19)°$, about 180° removed from the parent tripeptide. The Leu ϕ, ψ torsion angles are both significantly changed to $-120{\cdot}4(14)$ and $46{\cdot}8(18°)$, respectively. The Gly conformation is still observed in the helical region [$\phi = -88{\cdot}8(19)°$, $\psi = -20{\cdot}1(18)°$], but its screw-sense is reversed. As a consequence, the tetrazole tripeptide analogue, although folded at the Leu-Gly sequence, does not appear to adopt any β-bend conformation with a central *cis*-peptide bond (type-VIa or VIb β-bend[35,36]). The main reason for this observation should be found in the unusual Leu ϕ, ψ torsion angles.

Fig. 3 compares the geometrical properties of the *cis*-peptide bond, obtained from a recent statistical analysis of published peptide X-ray diffraction structures[18] with those of the tetrazole peptide bond surrogate. It is evident that the tetrazole ring

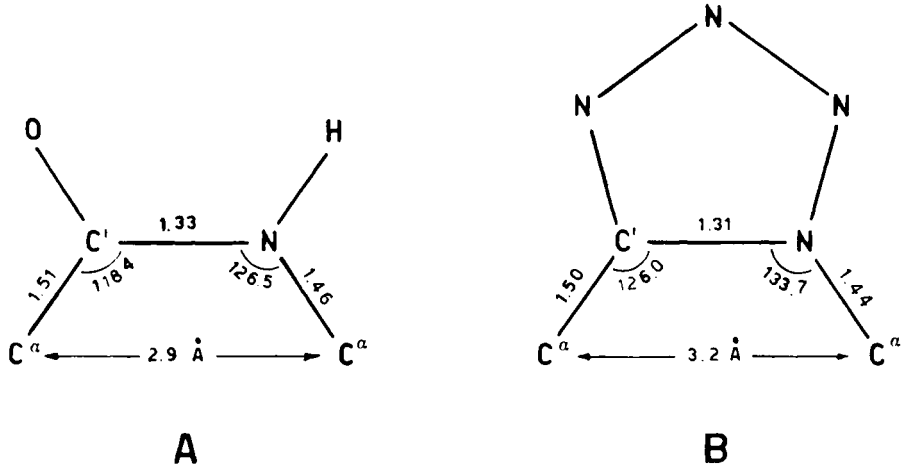


FIG. 3

Comparison of the geometrical parameters of *cis*-amide (A) and tetrazole (B) peptides

represents a good conformationally-restricted mimic with bond lengths and bond angles reasonably close to those of the *cis*-peptide conformer. In the tetrazole-containing peptide the critical $C^{\alpha}\cdots C^{\alpha}$ separation (3·2 Å) is only 0·3 Å longer than that typical of a *cis*-peptide bond, 2·9 Å (in the *trans*-peptide conformer this separation is as large as 3·8 Å). This small variation is mainly due to the wider bond angles at the C′ and N atoms as a consequence of the increased steric bulk of the heterocyclic moiety.

Although a preliminary study suggested that the *trans* olefinic PLG analog, Pro-Leuψ[CH=CH]Gly-NH_2, was capable of enhancing the binding of ADTN to dopamine receptors[6], more extensive and statistically meaningful studies have shown this not to be the case. Since the tetrazole analogue of PLG, compound *I*, is also inactive, it becomes impossible to say in the case of PLG whether the Leu-Gly amide bond is in a *cis* or *trans* conformation when PLG modulates the dopamine receptor by using the olefinic and tetrazole peptide bond surrogates. Rather these results suggest that the Leu-Gly amide bond itself may be required for the dopamine receptor modulating activity of PLG.

EXPERIMENTAL

Melting points were determined on a Thomas–Hoover Unimelt apparatus and are uncorrected. Specific rotations were measured with a Rudolph Autopol III polarimeter. ^{1}H NMR spectra were obtained on either a Jeol FX-90 MHz or a Nicolet 300 MHz NMR spectrometer. ^{13}C NMR spectra were obtained on the Jeol FX-90 instrument at 22·5 MHz. Elemental analyses were performed by M–H–W Laboratories, Phoenix, AZ. Thin-layer chromatography (TLC) was carried out on Analtech 250 μm silica gel GF uniplates. Visualization was done with either UV, I_2, or ninhydrin.

5-[1-(*S*)-(Benzyloxycarbonylamino)-3-methylbutyl]-1- and
2-tetrazolylacetic Acid Methyl Ester (*III* and *IV*)

To a solution of 5-[1-(*S*)-(benzyloxycarbonylamino)-3-methybutyl]tetrazole (*II*) (ref.[11]) (3·85 g, 13·3 mmol) in 20 ml of dry acetone Et_3N (1·86 ml, 13·3 mmol) and methyl bromoacetate (2·04 g, 13·3 mmol) were added at 0°C. The reaction mixture was stirred at 0°C for 5 min, and then at room temperature for 8 h. The solvent was evaporated and the residue was partitioned between EtOAc (150 ml) and 10% HCl (50 ml). The organic layer was washed with 1M-$NaHCO_3$ (50 ml), water (50 ml), and saturated NaCl aqueous solution and then dried over $MgSO_4$. Evaporation of the EtOAc under reduced pressure gave an oil. Medium pressure chromatography on silica gel (2·5 × 100 cm) eluting with EtOAc–CH_2Cl_2 (1 : 20) gave 4·42 g (92%) of a mixture of *III* and *IV*. The R_F values of *III* and *IV* in the solvent system EtOAc–hexane (1 : 2) were 0·46 and 0·54, respectively. This mixture was not purified further but used directly in the next reaction. One of the column fractions containing pure *III* was analyzed by NMR. ^{1}H NMR (90 MHz, $CDCl_3$): 0·96 d, 6 H (2 CH_3), J = 6·2 Hz). 1·40–1·92 m, 3 H (CH_2CH), 3·80 s, 3 H (OCH_3), 5·11 s, 2 H (OCH_2). 5·20–5·46 m, 1 H (NCH), 5·38 s, 2 H (CH_2CO), 7·33 s, 5 H (Ph). ^{13}C NMR ($CDCl_3$): 21·76 and 22·16 (2 CH_3), 24·35 (CH), 43·94 (CH_2), 46·24 (NCH), 52·64 (OCH_3), 52·80 (CH_2CO), 66·53 (OCH_2), 127·65, 128·00, 136·29 (Ph), 155·44 (Z C=O), 165·08 (CN_4), 167·82 ($COOCH_3$).

5-[1-(*S*)-(Benzyloxycarbonylamino)-3-methylbutyl]-1- and -2-tetrazolylacetamide (*V* and *VI*)

A mixture (4·17 g, 11·5 mmol) of the tetrazolylacetic acid methyl esters *III* and *IV* was treated with 120 ml of methanolic ammonia solution. The resulting reaction mixture was stirred at room temperature overnight. Evaporation of the MeOH and excess NH_3 gave a solid mixture of the amides. Fractional recrystallization of this mixture from EtOAc gave first 1·51 g (38%) of the 1,5-tetrazolylacetamide *V*: m.p. 189–190°C; $[\alpha]_D^{23}$ +22·7° (*c* 1·1, MeOH); TLC: $R_F = 0{\cdot}59$ ($CHCl_3$–MeOH, 10 : 1); ^{1}H NMR (90 MHz, $(CD_3)_2SO$: 0·72–1·00 m, 6 H (2 CH_3), 1·40–2·10 m, 3 H (CH_2CH), 4·70–5·08 m, 1 H (NCH), 5·00 s, 2 H (OCH_2), 5·25 s, 2 H (CH_2CO), 7·32 s, 5 H (Ph), 7·51 br s, 1 H (*cis* $CONH_2$), 7·85 br s, 1 H (*trans* $CONH_2$), 8·30 (br d, 1 H, NH J = 8·1 Hz). ^{13}C NMR $(CD_3)_2SO$: 21·00, 22·39 (2 CH_3), 23·79 (CH), 40·77 (CH_2), 43·07 (NCH), 48·49 (CH_2CO), 65·58 (OCH_2), 127·49, 127·27, 128·00, 136·45 (Ph), 155·69 (Z C=O), 156·80 (CN_4), 165·90 (Gly C=O). For $C_{16}H_{22}N_6O_3$ (346·4) calculated: 55·48% C, 6·40% H, 24·26% N; found: 55·65% C, 6·40% H, 24·46% N.

Recrystallization of the residue from the mother liquor using EtOAc–Et_2O afforded 1·66 g (42%) of the 2,5-tetrazolylacetamide *VI*: m.p. 130–132°C: $[\alpha]_D^{23}$ −52·5° (*c* 1·02, MeOH); TLC: $R_F = 0{\cdot}54$ ($CHCl_3$–MeOH, 10 : 1); ^{1}H NMR (90 MHz, $(CD_3)_2SO$: 0·90 d, 6 H, (2 CH_3), J = 4·8 Hz) 1·28–2·04 m, 3 H (CH_2CH), 5·03 and 4·76–5·08 s over m, 3 H (OCH_2 and NCH), 5·36 s, 2 H (CH_2CO), 7·35 s, 5 H (Ph), 7·49 s, 1 H (*cis* $CONH_2$), 7·82 s, 1 H (*trans* $CONH_2$), 7·95 br d, 1 H (NH, J = 8·8 Hz). ^{13}C NMR $(CD_3)_2SO$: 21·35, 22·31 (2 CH_3), 23·93 (CH), 42·40 (CH_2), 45·56 (NCH), 54·24 (CH_2CO), 65·26 (OCH_2), 127·26, 127·43, 127·99, 136·82 (Ph), 155·46 (Z C=O), 165·54 (CN_4), 167·35 (Gly C=O). For $C_{16}H_{22}N_6O_3$ (346·4) calculated: 55·48% C, 6·40% H, 24·26% N; found: 55·38% C, 6·50% H, 24·39% N.

5-[1-(*S*)-(Benzyloxycarbonyl-L-prolylamino)-3-methylbutyl]-1-tetrazolylacetamide (*VII*)

Compound *V* (815 mg, 2·35 mmol) was suspended in 8 ml of MeOH. To this solution conc. HCl (0·21 ml, 2·52 mmol) and 10% Pd/C (80 mg) were added. Hydrogenolysis was performed on a Parr shaker at 276 kPa of H_2 for 2 h. The reaction mixture was filtered through a pad of Celite. The filtrate was stripped of solvent to give a quantitative yield of the amine hydrochloride salt. This material was coupled to Z-Pro-OH without further purification in the following manner.

Z-L-Pro-OH (587 mg, 2·35 mmol) and N-methylmorpholine (0·26 ml, 2·35 mmol) were mixed in 20 ml of THF. The solution was cooled to −15°C and isobutylchloroformate (321 mg, 2·35 mmol) was added. The resulting turbid mixture was stirred at −15°C to −20°C for 5 min and then treated with a solution of the above amine hydrochloride salt and Et_3N (0·33 ml, 2·35 mmol) in water (3 ml). The reaction mixture was stirred at −15° to −10°C for 1 h and then allowed to slowly warm up to room temperature. The solvent was evaporated from the reaction mixture and the residue that was obtained was partitioned between EtOAc (150 ml) and 10% citric acid (50 ml). The organic layer was washed with 1M-$NaHCO_3$ (50 ml), water (50 ml), and saturated NaCl aqueous solution (100 ml), and then dried over $MgSO_4$. Evaporation of the EtOAc gave a white solid that was recrystallized from EtOAc to afford 902 mg (86%) of *VII*: m.p. 161–163°C; $[\alpha]_D^{23}$ −46·3° (*c* 1·02, MeOH); TLC: $R_F = 0{\cdot}49$ ($CHCl_3$–MeOH, 10 : 1). The ^{1}H NMR spectrum of *VII* showed a mixture of *trans* and *cis* isomers in a 10 : 7 ratio due to isomerism about the Z-Pro carbamate bond. ^{1}H NMR (300 MHz, $(CD_3)_2SO$: 0·71 and 0·79 d, 6 H (J = 5·9 and 6·0 Hz, *trans* isomer, 2 CH_3), 0·84 and 0·91 d, 6 H (J = 5·5 and 5·6, *cis* isomer 2 CH_3), 1·50–1·80 and 1·85–2·16 m, 7 H ($CHCH_2$, Pro γCH_2 and βCH_2), 3·32–3·48 m, 2 H (Pro δCH_2), 4·15 dd (J = 3·5, 8·4 Hz, *cis* isomer Pro αCH), 4·22 dd (J = 2·7, 8·5 Hz, *trans* isomer Pro αCH), 4·88 d, 1 H (J = 13·1 Hz, OCH_2), 5·00–5·15 d over m, 2 H (OCH_2 and NCH), 5·15 d, 1 H (J = 17·0 Hz, $CHCONH_2$), 5·25 d, 1 H (J = 17·0 Hz, $CHCONH_2$), 7·20–7·40 m,

5 H (Ph), 7·52 br s, 1 H (*cis* $CONH_2$), 7·88 br s, 1 H (*trans* $CONH_2$), 8·68 br d (J = 7·9 Hz, *cis* isomer NH), 8·76 br d (J = 7·9 Hz, *trans* isomer NH). For $C_{21}H_{29}N_7O_4$ (443·5) calculated: 56·87% C, 6·59% H, 22·11% N; found: 56·91% C, 6·61% H, 22·16% N.

5-[1-(*S*)-(L-Prolylamino)-3-methylbutyl]-1-tetrazolylacetamide (*I*)

Protected tripeptide tetrazole *VII* (500 mg, 1·13 mmol), Et_3N (1 ml), and 10% Pd/C (50 mg) were mixed in 5 ml of MeOH. Hydrogenolysis was carried out on a Parr shaker (H_2 at 276 kPa) for 2 h. The catalyst was removed by filtration through a pad of Celite and the filtrate was evaporated. The residue was crystallized from MeOH–Et_2O to give 279 mg of fine needles of *I*: m.p. 188–191°C; $[\alpha]_D^{23}$ −26·7° (*c* 1·06, MeOH); TLC: R_F = 0·68 (1-propanol–NH_4OH, 5 : 1). ^{1}H NMR (300 MHz, $(CD_3)_2SO$: 0·84 d (J = 6·2 Hz, CH_3), 0·90 d (J = 6·3 Hz, CH_3), 1·45 to 2·15 m, 7 H (CH_2CH, Pro γCH_2 and βCH_2), 2·72–2·88 m, 2 H (Pro δCH_2), 3·49 dd (J = 4·8, 8·7 Hz, Pro αCH), 5·05–5·15 m, 1 H (CHN), 5·23 s, 2 H (CH_2CONH_2), 7·52 br s, 1 H (*cis* $CONH_2$), 7·87 br s, 1 H (*trans* $CONH_2$), 8·43 br d (J = 8·5 Hz, NH). ^{13}C NMR $(CD_3)_2SO$: 21·06, 22·43 (2 CH_3), 23·94 (CH), 25·33 (Pro γC), 29·89 (Pro βC), 40·27 (CH_2), 40·88 (NCH), 46·34 (Pro δC), 48·49 (CH_2CONH_2), 59·77 (Pro αC), 156·38 (CN_4), 165·94 ($CONH_2$), 174·43 (Pro C=O). FAB MS: m/z 308 $(M - H]^-$, 310 $[MH]^+$. For $C_{13}H_{22}N_7O_2$ (309·4) calculated: 50·47% C, 7·49% H, 31·70% N; found: 50·50% C, 7·73% H, 31·48% N.

5-[1-(*S*)-(Benzyloxycarbonyl-L-prolylamino)-3-methylbutyl]-2-tetrazolylacetamide (*VIII*)

The same procedure that was used to synthesize *VII* was used for the synthesis of *VIII*. Crystallization of the crude product obtained from EtOAc–Et_2O provided *VIII* in a 84% yield. M.p. 114–116·5°C; $[\alpha]_D^{23}$ −103·7° (*c* 1·11, MeOH); TLC: R_F = 0·44 ($CHCl_3$–MeOH, 10 : 1). ^{1}H NMR (300 MHz, $(CD_3)_2SO$): 0·78 d (J = 6·8 Hz, *trans* isomer CH_3), 0·80 d (J = 7·4 Hz, *trans* isomer CH_3), 0·88 d (J = 5·5 Hz, *cis* isomer CH_3), 0·91 d (J = 5·6 Hz, *cis* isomer CH_3), 1·44–1·88 m, 6 H (CH_2CH, Pro γCH_2 and βCH), 2·00–2·20 m, 1 H (Pro βCH), 3·28–3·50 m, 2 H (Pro δCH_2), 4·22–4·33 m, 1 H (Pro αCH), 4·93 d, 1 H (J = 13·1 Hz, OCH_2), 5·02–5·13 m (OCH_2 and NCH of *cis* isomer), 5·20 dd (J = 8·8, 14·5 Hz, *trans* isomer NCH), 5·34 s (*trans* isomer CH_2CONH_2), 5·36 s (*cis* isomer CH_2CONH_2), 7·28–7·40 m, 5 H (Ph), 7·49 (*cis* $CONH_2$), 7·81 (*trans* $CONH_2$), 8·52 br d (J = 8·4 Hz, *cis* isomer NH), 8·57 br d (J = 8·7 Hz, *trans* isomer NH). ^{13}C NMR, $(CD_3)_2SO$: 21·34, 22·26 (2 CH_3), 22·89 (Pro γC), 23·78 (CH), 30·28 (Pro βC), 42·47 (CH_2), 43·01 (NCH), 46·56 (Pro δC), 54·17 (CH_2CONH_2), 59·13 (Pro αC), 65·55 (OCH_2), 126·82, 127·23, 127·90, 136·79 (Ph), 153·67 (Z C=O), 165·45 (CN_4), 167·04 ($CONH_2$), 171·35 (Pro C=O). For $C_{21}H_{29}N_7O_4$ (443·5) calculated 56·87% C, 6·59% H, 22·11% N; found 57·03% C, 6·67% H, 22·13% N.

5-[1-(*S*)-(L-Prolylamino)-3-methylbutyl]-2-tetrazolylacetamide Hydrochloride (*IX*)

Tripeptide *VIII* was deprotected using the same method as that described above for compound *I*. The free amine was obtained as an oil. The material was treated with excess ethereal HCl (10 ml). The ether and excess HCl were removed under reduced pressure and the residue was dried over NaOH in vacuo. Crystallization of this material from MeOH–Et_2O gave *IX* in a 66% yield: m.p. 184–187°C; $[\alpha]_D^{23}$ −100·0° (*c* 1·0, MeOH); TLC: R_F = 0·64 (1-propanol–NH_4OH, 5 : 1). ^{1}H NMR (300 MHz, $(CD_3)_2SO$): 0·91 d, 3 H (J = 6·5 Hz, CH_3), 0·93 d, 3 H (J = 6·8 Hz, CH_3), 1·60–1·95 m, 6 H (CH_2CH, Pro γCH_2 and βCH), 2·20–2·38 m, 1 H (Pro βCH), 3·13–3·28 m, 2 H (Pro δCH_2), 4·20–4·30 m (Pro αCH), 5·24 dd, 1 H (J = 8·9, 14·3 Hz, CHN), 5·40 s, 2 H (CH_2CONH_2), 7·52 br s, 1 H (*cis* $CONH_2$), 7·97 br s, 1 H (*trans* $CONH_2$), 8·56 br s, 1 H

(Pro NH), 9·25 br d, 1 H (NH), 10·14 br s, 1 H (Pro NH). ^{13}C NMR $(CD_3)_2SO$: 21·35, 22·21 (2 CH_3), 23·06 (Pro γC), 23·84 (CH), 29·20 (Pro βC), 42·05 (CH_2), 43·76 (NCH), 45·27 (Pro δC), 54·29 (CH_2CONH_2), 58·43 (Pro αC), 165·41 (CN_4), 166·48 ($CONH_2$), 167·45 (Pro C=O). FAB MS: *m/z* 310 $[MH]^+$. For $C_{13}H_{23}N_7O_2Cl$ (345·8) calculated: 45·15% C, 6·99% H, 28·35% N; found: 45·43% C, 7·00% H, 28·24% N.

X-Ray Diffraction Measurements

Colorless crystals (0·1 × 0·25 × 0·8 mm) of Pro-Leuψ[CN_4]Gly-NH_2 (*I*) were obtained from a methanol–diethyl ether solution by slow evaporation. The unit cell dimensions were obtained from 25 reflections in the Θ range 8—12°. Intensities were collected on a Philips PW 1100-four circle diffractometer operating in the ω scan mode (scan width 1·5° and scan speed 0·03 s^{-1}) with graphite-monochromatized MoKα radiation (λ = 0·71069 Å). During data collection three standard reflections were measured every 180 min to check stability of the crystal and the electronics. Intensities were corrected for Lorentz and polarization effects. No absorption correction was applied. Unique reflections (1 725) up to 2Θ = 50° were measured, of which 794 had intensities greater than 3σ(*I*). *F*(000) = 668; μ = 0·55 cm^{-1}.

The structure was solved by direct methods using MULTAN 80 (ref.[43]) and refined by block-diagonal least squares with anisotropic thermal parameters for all non-hydrogen atoms (*w* = 1). All hydrogen atoms were found on the difference Fourier map and refined isotropically. All calculations were performed on the IBM 370/158 computer of the University of Padova, using SHELX-76 (ref.[44]). The final *R* value for the 794 observed reflections with $I > 3\sigma(I)$ was 0·087. *S* = 1·8. The max. and min. heights in the final difference Fourier synthesis were 0·30 and −0·40 e. . $Å^{-3}$, respectively. The $(\Delta/\sigma)_{max}$ in the final refinement cycle for the non-hydrogen atoms was 1·09.*

This work was supported by a grant to R.L.J. from the National Institutes of Health (NS 20036)

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* Supplementary materials containing anisotropic thermal parameters, hydrogen-atom parameters, and structure factor tables can be obtained on request from the Padova laboratory.

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