Synthesis and Crystal Structure of Elastin Sequence (Boc-Val-Pro-OBzl)

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The fragments of elastin sequences of mammalian elastic protein elastins like Val-Pro were synthesized and characterized by X-ray crystallography. The sequence (C₂₂H₃₂N₂O₅) crystallizes in the orthorhombic space group *P*2₂1₂1 with the parameters *a* = 6.2300(5) Å, *b* = 11.3220(17) Å, *c* = 32.329(5) Å, *V* = 2280.4(5) Å³, *Z* = 4. The final *R* factor is 0.694. The structure has both intra and intermolecular hydrogen bonds.

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Elastic protein-based polymers have their origins in repeating sequences of the mammalian elastic protein.¹ The parent polymers, (Val-Pro-Gly-Val-Gly)_{*n*} or poly(Val-Pro-Gly-Val-Gly), (Ala-Pro-Gly-Val-Gly-Val)_{*n*} or poly(Ala-Pro-Gly-Val-Gly-Val), (Val-Pro-Gly-Gly)_{*n*} or poly(Val-Pro-Gly-Gly) and (Val-Pro-Gly-Phe-Gly-Val-Gly-Ala-Gly) or poly(Val-Pro-Gly-Phe-Gly-Val-Gly-Ala-Gly) derive from sequences that occur in all sequenced mammalian elastin protein.^{2,3} These polymers have a number of medical and non-medical applications.³

The synthesis of these elastic protein-based polymers by chemical synthesis has always been a challenge. The key to successful chemical synthesis is to stepwise build up the repeating sequence, taking care to ensure that the composite peptides are pure. Thus, racemization is a problem during the coupling to form Val-Pro and Ala-Pro, and the purification and crystallization of these dipeptides are critical to the optical purity of the pentamer. The absolute configuration of the title compound is known from the chemical evidence. The structure of the synthesized dipeptide was characterized by chemical analysis, optical rotation, ¹H-NMR and ¹³C-NMR. In this context, it is of interest to investigate the crystal structure of Boc-Val-Pro-OBzl (Fig. 1).

To Boc-Val-OH (20 mmol), dissolved in acetonitrile (35 mL) and cooled to 0°C, was added *N*-methylmorpholine (NMM) (2.2 mL, 20 mmol). The solution was cooled to -15°C ± 1°C and isobutylchloroformate (IBCF) (2.6 mL, 20 mmol) was added under stirring while maintaining the temperature at -15°C.

Fig. 1 Schematic diagram of the molecule.

After stirring the reaction mixture for 10 min at this temperature, a pre-cooled solution of HOBt (3.1 g, 20 mmol) in DMF (30 mL) was added. The reaction mixture was stirred for an additional 10 min and a pre-cooled solution of HCl. H-Pro-OBzl (4.85 g, 20 mmol) and NMM (2.2 mL, 20 mmol) in DMF (40 mL) was added slowly. After 20 min, the pH of the solution was adjusted to eight by the addition of NMM and the reaction mixture was stirred overnight at room temperature. Acetonitrile was removed under reduced pressure and the residual solution was poured into about 400 mL of ice-cooled 90% saturated KHCO₃ solution, and this mixture was stirred for 30 min. The precipitate was filtered, washed with water, cold 1 M HCl, water and finally dried. The crude peptide was recrystallized from ether and petroleum ether to obtain 6.58 g of Boc-Val-Pro-OBzl (yield, 90.8%). *R*_f, 0.55; *R*_f, 0.68. [*α*]_D²⁵ (C, 1; MeOH) -10.4, m.p. 81-83°C.

A single crystal of dimensions 0.25 × 0.2 × 0.2 mm was

Table 1 Crystallographic data

Formula: C ₂₂ H ₃₂ N ₂ O ₅	
Formula weight = 404.50	
Crystal system: orthorhombic	
Space group: <i>P</i> 2 ₂ 1 ₂ 1	<i>Z</i> = 4
<i>a</i> = 6.2300(5) Å	
<i>b</i> = 11.3220(17) Å	
<i>c</i> = 32.329(5) Å	
<i>V</i> = 2280.4(5) Å ³	
<i>D</i> _x = 1.178 Mg/m ³	
2 θ _{max} = 64.5° with Mo K α	
No. of reflections used = 1977 [<i>I</i> > 2 σ (<i>I</i>)]	
<i>R</i> ₁ = 0.0694	
($\Delta\rho$) _{max} = 0.000	
($\Delta\rho$) _{max} = 0.251 eÅ ⁻³	
($\Delta\rho$) _{min} = -0.296 eÅ ⁻³	
Measurement: DipLabo Kappa(IP)	
Program system: Denzo and Scalepak	
Structure determination: SHELXS-97	
Refinement: SHELXL-97	

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Table 2 Atomic coordinates and equivalent thermal parameters

Atom	x	y	z	U_{eq}
C1	0.6374(11)	0.1743(9)	-0.02614(15)	0.145(3)
C2	0.2488(9)	0.1799(9)	-0.01430(18)	0.164(4)
C3	0.4715(19)	0.3498(9)	0.0043(2)	0.185(4)
C4	0.4655(7)	0.2208(6)	0.00118(12)	0.0889(19)
O5	0.4781(4)	0.1653(3)	0.04214(8)	0.0835(11)
C6	0.6460(7)	0.1828(6)	0.06731(12)	0.0828(17)
O7	0.8065(6)	0.2373(5)	0.05868(9)	0.1177(18)
N8	0.6125(5)	0.1261(4)	0.10328(9)	0.0780(13)
C9	0.7597(6)	0.1362(5)	0.13815(10)	0.0722(14)
C10	0.8880(7)	0.0230(5)	0.14580(12)	0.0786(16)
C11	1.0382(7)	-0.0013(7)	0.10940(17)	0.109(2)
C12	0.7444(9)	-0.0849(6)	0.15314(14)	0.0963(19)
C13	0.6306(6)	0.1673(5)	0.17611(11)	0.0716(14)
O14	0.4383(4)	0.1424(4)	0.17921(8)	0.0906(13)
N15	0.7333(5)	0.2242(4)	0.20721(8)	0.0734(12)
C16	0.9555(6)	0.2670(6)	0.20742(12)	0.0918(19)
C17	0.9849(8)	0.3116(8)	0.24980(16)	0.129(3)
C18	0.7749(7)	0.3349(6)	0.26828(14)	0.0933(18)
C19	0.6222(6)	0.2485(5)	0.24563(11)	0.0739(15)
C20	0.5846(7)	0.1394(7)	0.26959(13)	0.086(2)
O21	0.6618(7)	0.0442(5)	0.26354(11)	0.1040(15)
O22	0.4535(5)	0.1597(4)	0.30190(9)	0.0965(13)
C23	0.4229(9)	0.0656(7)	0.33128(17)	0.115(2)
C24	0.6124(9)	0.0453(7)	0.35864(14)	0.0898(19)
C25	0.7347(10)	0.1390(7)	0.37235(14)	0.0998(19)
C26	0.9087(11)	0.1181(9)	0.39760(17)	0.122(3)
C27	0.9659(13)	0.0066(12)	0.4099(2)	0.133(4)
C28	0.8433(17)	-0.0838(10)	0.3967(2)	0.143(4)
C29	0.6674(13)	-0.0686(8)	0.37067(18)	0.118(3)

$$U_{eq} = (1/3)\sum_i \sum_j U_{ij}(a_i^* a_j^*)(a_i \cdot a_j).$$

chosen for X-ray diffraction studies. The measurements were made on a DIPLabo Imaging Plate system with graphite monochromated radiation (Mo K_{α}). The structure was solved and refined using the SHELX-97⁵ program. All the non-hydrogen atoms were revealed in the first map. Full-matrix least-squares refinement based on 1977 observed reflections ($I > 2\sigma(I)$) with isotropic temperature factors for all the atoms converged residual to $R = 0.1455$. Refinement of non-hydrogen atoms with anisotropic thermal parameters was started at this stage. After eight cycles of refinement the residuals saturated at $R = 0.0694$ for 1977 observed reflections and 263 parameters. The hydrogen atoms were placed at calculated positions and were not refined. Table 1 gives the details of crystal data, data collection and refinement.

The final positional coordinates with equivalent isotropic temperature factors for all the non-hydrogen atoms are listed in Table 2. The bond lengths and bond angles agree with their standard values. Figure 2 represent the ORTEP diagram of the molecules at 50% probability. The molecules appear stacked and exhibits layer-type polymeric structure when viewed along the b axis. The structure has both intra and intermolecular hydrogen bonds of the type C-H...O. C1-H16A...O7 (3.023(6)Å, 119.21(6)°), C3-H18C...O7 (3.011(11)Å, 119.34(5)°) and C28-H5...O7 (3.308(11)Å, 137.12(9)°), C17-H28B...O21 (3.459(9)Å, 167.14(4)°), C16-H29B...O14 (3.445(5)Å, 148.09(3)°) with symmetry codes (2 - x , -1/2 + y , 1/2 - z), (2 - x , 1/2 + y , 1/2 - z), (1 + x , y , z) respectively. The dihedral angle found between least-squares planes 1(C24 C25 C26 C27 C28 C29) and 2(N15 C16 C17 C18 C19) is 82.9(3)°,

Fig. 2 ORTEP of the molecule at 50% probability.

with a maximum deviation of 0.157(6)Å for C18.

The bond lengths and angles of the peptide link are in conformity with those of similar molecules.⁶ The peptide bond between C13-N15 is 1.355(5)Å and between C13-O14 is 1.235(4)Å. Such values are expected in peptide linkages. The conformation of the dipeptide is identified by the following rotations: the rotations about C13-C9, N8-C9 and C13-N15 are respectively $\psi = -155.13^\circ$, $\phi = 129.22^\circ$ and $\omega = -175.39^\circ$.

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