Synthesis and crystal structure of *cis*-4-azido-L-proline methyl ester hydrochloride

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The key precursor for the synthesis of novel influenza neuraminidase inhibitors, *cis*-4-azido-L-proline methyl ester hydrochloride ($C_6H_{11}ClN_4O_2$, *Mr* = 206.63), was prepared as white needle-shaped crystals and its structure was elucidated by single-crystal X-ray diffraction. In the crystal structure, molecules are linked through intermolecular hydrogen bonds, forming layers perpendicular to the *bc* plane.

Keywords: pyrrolidine, synthesis, crystal structure, hydrogen bonds

The collagen triple helix is a unique structural motif found in proteins, with a characteristic repeat of Gly-X-Y, where X and Y are often L-proline (Pro, **1**) and *trans*-4-hydroxy-L-proline (Hyp, **2**) residues, respectively.[1] Hyp is produced by hydroxylation of its precursor Pro *in situ* after protein synthesis in the body. It permits the sharp twisting of the collagen helix and helps provide stability to the triple helical structure of collagen. However, the mechanism by which stabilisation is achieved needs further discussion.[2,3]

These two cyclic amino acids have been used to good effect in the design of peptides and peptidomimetics with defined conformation.[4,5] In previous work from our laboratory, Hyp had been used to prepare a series of pyrrolidine derivatives as inhibitors targeting matrix metalloproteinase (MMP),[6-10] inducible nitric oxide synthase (iNOS)[11] and influenza neuraminidase (NA).[12]

The present work is part of our research involving a series of novel pyrrolidine derivatives which can be more potent NA inhibitors. *cis*-4-Azido-L-proline methyl ester hydrochloride, a key intermediate that can be modified further, were prepared *via* the sequence of esterification, Boc-protection, mesylation, azidation and removal of protecting group by using enantiomerically pure *trans*-4-hydroxy-L-proline as the starting materials (Scheme 1). The preparation and application of the title compound were involved in many references and patents.[13-20] However, the cleavage of the Boc-protecting group of **6** with trifluroacetic acid (TFA) gave the product as a yellow oil, which is not convenient to store or handle.[13,19,20] Two groups removed Boc using HCl in ethyl acetate or dixoane with quantitive yield, but no spectral data were provided.[14,18] Here, the synthetic steps leading to **6** and **7** were improved and all the products were obtained as crystals from suitable solvent except for compound **6**. The crystal structure of **7** is also reported.

Experimental

The starting material **2** is a white crystalline powder with m.p. 274–275 °C (dec.), $[\alpha]^{20}_D$ –75.1° (*c* 4, H_2O), purchased from Jinzhou Jirong Amino Acid Co. Ltd, China. Unless otherwise specified, other materials were purchased from commercial suppliers. Anhydrous reactions were carried out in oven-dried glassware under a nitrogen atmosphere. All reactions were monitored by TLC on 25.4 × 76.2 mm silica gel plates (GF-254) and visualised with iodine vapour. Specific rotation were determined on a GYROMAT-HP high-precision digital automatic polarimeter (Kernchen, Germany). Melting points were determined on an electrothermal melting point apparatus and are uncorrected. ^{1}H NMR spectra were determined on a Bruker Avance 300 spectrometer in D_2O or $CDCl_3$ using TMS as an internal standard. IR spectra were measured on a nicolet nexus 470 FT-IR spectrometer using smear KBr crystal or KBr plate. ESI-MS were determined on an API 4000 spectrometer. Elemental analysis for compounds were performed using an elementar vario EL III CN analyser (Germany).

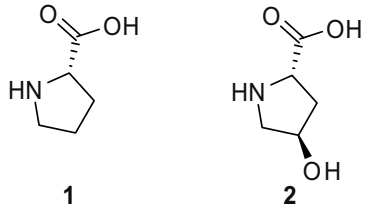


Fig. 1 Chemical structures of L-proline (Pro) and *trans*-4-hydroxy-L-proline (Hyp).

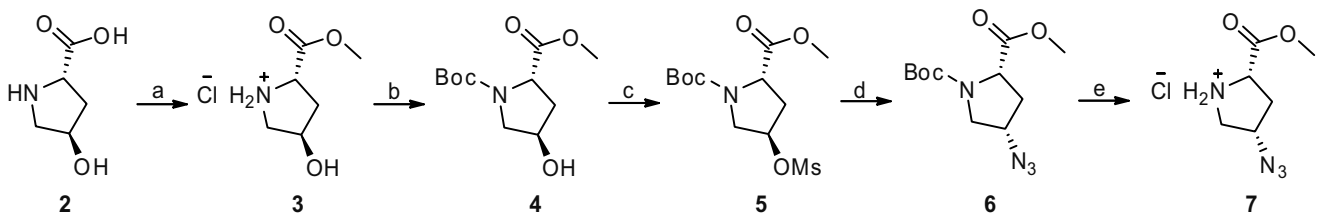
Synthetic procedure

Synthesis of trans-*4-hydroxy-L-proline methyl ester hydrochloride* **(3)**: Prepared from compound **2** as described by Mckillop *et al*.[21] White needles (79.50%); $[\alpha]^{20}_D$ –25.3°(*c* 4, H_2O); m.p. 160–164 °C (lit.[22] m.p. 156–160 °C).

Synthesis of N-Boc-trans-*4-hydroxy-L-proline methyl ester* **(4)**: Prepared from compound **3** as described by Abraham *et al*.[13] to give a colourless, viscous oil. This oil could be crystallised after standing at room temperature (100.0%). $[\alpha]^{25}_D$ –75.9°(*c* 1, CH_3OH); m.p. 93–94 °C [Ref.[13] $[\alpha]^{28}_D$ –75.2°(*c* 1, CH_3OH), 97–98 °C].

Synthesis of N-Boc-trans-*4-methanesulfonyloxy-L-proline methyl ester* **(5)**: Prepared from compound **4** as described by Abraham *et al*.[13] as white granular particle (87.11%): $[\alpha]^{25}_D$ –54.2°(*c* 2, $CHCl_3$); m.p. 85–86 °C (lit.[13] $[\alpha]^{25}_D$ –52.3° (*c* 1.6, $CHCl_3$), 85–86 °C).

Synthesis of N-Boc-cis-*4-azido-L-proline methyl ester* **(6)**: Mesylate **5** (26.80 g, 82.88 mmol, 323.36 g mol^{-1}) and sodium azido (NaN_3, 10.78 g, 165.82 mmol, 65.01 g mol^{-1}, 2 equiv.) were stirred



Scheme 1 Synthesis of the key precursor **7**. Reagents and conditions: (a) Acetyl chloride, CH_3OH; (b) $(Boc)_2O$, TEA, DCM; (c) MsCl, Pyr, DCM, 0 °C; (d) NaN_3, DMF, 55 °C; (e) HCl/EtOAc.

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in dry DMF (30 mL) at 55–60 °C overnight. The reaction mixture was cooled to room temperature and then partitioned between ice water and EtOAc (100 mL). The organic layer was washed with brine, dried with anhydrous $MgSO_4$, and evaporated to give a pale yellow oil (24.12 g, crude >100%), which was suitable for the next step. $[\alpha]^{25}_D$ –35.6°(*c* 3, $CHCl_3$) [lit.[13] $[\alpha]^{25}_D$ –36.6° (*c* 2.8, $CHCl_3$)]; ^{1}H NMR ($CDCl_3$) δ 1.42, 1.48 [s, 9H, C(CH_3)$_3$]; 2.14–2.21, 2.40–2.54 (m, 2H, 3-CH_2), 3.76 (s, 3H, OCH_3), 3.44–3.52, 3.67–3.74 (m, 2H, 5-H), 4.14–4.19 (m, 1H, 2-H), 4.31–4.35, 4.41–4.45 (m, 1H, 4-H); IR (KBr, cm^{-1}): 2978, 2105, 1703, 1399, 1262, 1204; ESI-MS, *m/z* Calcd for $C_{11}H_{18}N_4O_4$ $[M + H]^+$ 271.1. Found: 271.1

Synthesis of cis-*4-azido-L-proline methyl ester hydrochloride* **(7)**: Azide **6** (24.12 g, 89.24 mmol, 270.29 g mol^{-1}) was dissolved in EtOAc (20 mL), cooled in ice, and then treated with 3 N HCl/EtOAc (100 mL). After the mixture was stirred for 30 min, the solvent was filtrated, the precipitate washed with EtOAc and then dried under high vacuum to give the final compound as a white solid that could be crystallised from ethanol as white needle-shaped crystals (12.93 g, 70.12%). $[\alpha]^{25}_D$ 33.1°(*c* 1, H_2O); m.p. 153–155 °C; ^{1}H NMR ($CDCl_3$) δ 1.75 (br s, 1H, 1-NH), 2.48–2.53, 2.64–2.72 (m, 2H, 3-CH_2), 3.57–3.74, (m, 2H, 5-CH_2), 3.90 (s, 3H, OCH_3), 4.47 (m, 1H, 2-H), 4.70–4.72 (m, 1H, 4-H); IR (KBr, cm^{-1}): 3339, 2945, 2876, 2105, 1681, 1434, 1324, 1258; ESI-MS *m/z* Calcd for $C_6H_{10}N_4O_2$ $[M + H]^+$ 171.2. Found: 171.3; Anal. Calcd for $C_6H_{11}ClN_4O_2$ (206.63), C, 34.88; H, 5.37; N, 27.11; Found: C, 34.86; H, 5.39; N, 27.13%.

Table 1 Crystal data and refinement parameters

Molecular formula	$C_6H_{11}N_4O_2Cl$
Molecular weight	206.64
Temperature (K)	298(2)
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
a/Å	5.335(1)
b/Å	12.218(2)
c/Å	15.169(3)
V/Å^3	988.8(3)
Z	4
D_{calc}(g cm^{-3})	1.388
Crystal size (mm)	0.20 × 0.20 × 0.15
Crystal colour	Colourless
Reflections collected/unique	4319/2412 [R_{int} = 0.0179]
Index ranges (*h,k,l*)	–7,6; –17,10; –10,20
Theta ranges for data collection	2.14–30.17
Absorption coefficient (cm^{-1})	0.363
F(000)	432
No. of observed data, $I>2\sigma(I)$	2412
No. of variables	126
No. of restraints	0
Goodness of fit on F^2	1.062
R_1,wR_2[$I\geq2\sigma(I)$][a]	0.0375, 0.0885
R_1,wR_2 (all data)[a]	0.0472, 0.0953
Largest diff peak and hole	0.157, –0.164
CCDC deposit no.	731637

[a] $R_1 = \Sigma||F_O|-|F_C||/\Sigma|F_O|$, $wR_2 = [\Sigma w(F_O^2-F_C^2)^2/\Sigma w(F_O^2)^2]^{1/2}$, $w = 1/[\sigma^2(F_O^2) + (0.0459P)^2 + 0.0437P]$ where $P = (F_O^2 + 2F_C^2)/3$.

Table 2 Selected bond lengths (Å) and angles (°) for the title compound

N1–C4	1.498(3)	C4–N1–C1	108.62(15)
N1–C1	1.500(2)	C3–C2–C1	103.60(17)
N2–C2	1.492(3)	N2–C2–C3	112.1(2)
N2–N3	1.219(3)	N3–N2–C2	114.7(2)
N3–N4	1.110(3)	N4–N3–N2	173.0(3)
O1–C5	1.198(2)	O1–C5–O2	125.56(19)
O2–C5	1.326(2)	O1–C5–C4	123.92(17)
O2–C6	1.441(3)	O2–C5–C4	110.50(17)

X-ray diffraction analysis

Crystals suitable for single crystal X-ray diffraction were grown from an ethanol solution. The data were collected on a Bruker SMART CCD area-detector diffractometer at room temperature using graphite-monochromated MoKα radiation (λ = 0.71073Å) by ω scan mode within the angular range 2.14–30.17. The details of the crystallographic data are given in Table 1. The structure was solved by direct methods and all of the non-H atoms were refined against F^2 by full-matrix least-squares methods using the SHELXTL program.[23] The amino group hydrogen atoms were located from a difference Fourier map and refined with isotropic temperature factors. All other H atoms were placed in geometrically idealised positions and constrained to ride on their parent atoms. No absorption correction was applied. CCDC 731637 contains the supplementary crystallographic data for this paper. The data can be obtained free of charge from the Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request.cif.

Discussion

Figure 2 gives a perspective view of the molecular structure of the title compound with the atomic labelling system. Selected bond lengths and angles are listed in Table 2. The skeleton of the title compound consists of a pyrrolidinium ring, a methoxycarbonyl group and an azide group. All the bond lengths and angles are within normal ranges. The 2-methoxycarbonyl group and 4-azido group are at the same side of the pyrrolidinium ring. The torsion angles of N2–C2–C3–C4,C2–C3–C4–C5 and C2–N2–N3–N4 are 75.2(2)°, –84.5(2)° and –170(3)°, respectively. In the crystal structure, N1, Cl(*x,y,z*) and

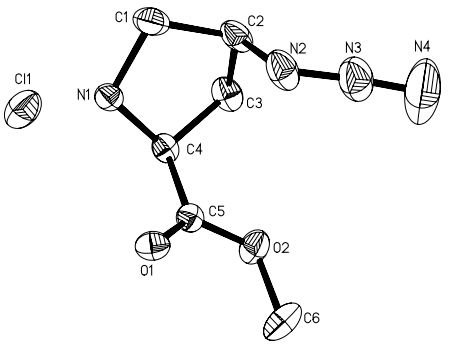

Fig. 2 The molecular structure with the atom-numbering scheme. Displacement ellipsoids are drawn at 30% probability level.

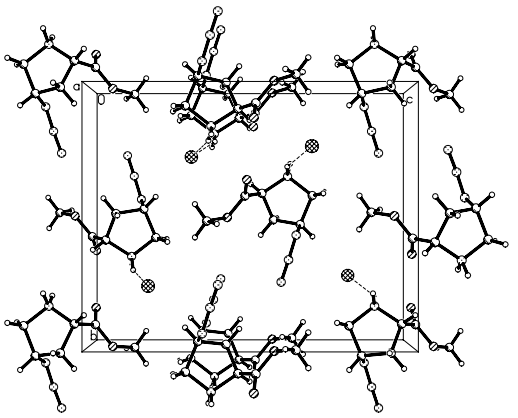

Fig. 3 Packing diagram. Hydrogen bonds are shown as dashed lines.

Table 3 Geometrical parameters for hydrogen bonds for the title compound

Hydrogen bonds	D–H(Å)	H…A(Å)	D…A(Å)	D–H…A(°)
N1–HN1A…O1	0.86(2)	2.34(2)	2.746(2)	109.0(16)
N1–HN1A…Cl1	0.86(2)	2.39(2)	3.17(2)	150.3(18)
N1–HN1B…Cl1[a]	0.97(3)	2.21(3)	3.11(2)	154(2)

[a] *X* + 1,*y*,*z*.

Cl(x + 1,y,z) form strong intermolecular N–H…Cl hydrogen bonds (Table 3). The serial hydrogen bonds form layers perpendicular to *bc* plane (Fig. 3), which is vital for the stability of the crystal structure.

Conclusions

In summary, using enantiomerically pure *trans*-4-hydroxy-L-proline as the starting material, we prepared *cis*-4-azido-L-proline methyl ester hydrochloride as white needle-shaped crystals with total yield about 50%, whose chemical structure was confirmed by ^{1}H NMR, IR, ESI-MS and elemental analysis. All the reactions were processed under mild conditions and the final compound should be optically pure deduced from the determination of specific rotation and elucidation of its crystal structure. This key precursor could be used for the synthesis of novel influenza neuraminidase inhibitors, and many other pharmaceutical intermediates.

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