Structure confirmation of L-iso-glutamine derivatives

Xun Li and Wen-Fang Xu*

College of Pharmacy, Shandong University, Jinan 250012, P.R. China

L-iso-glutamine derivatives as an APNIs was prepared by the condensation reaction of N-(3,4,5-trimethoxybenzoyl)-glutamic acid anhydride with L-amino acid methyl ester hydrochloride. The structure was confirmed by MS, Elemental and X-ray single crystal diffraction.

Keywords: APN, L-iso-glutamine derivatives, APN inhibitors, X-ray structure

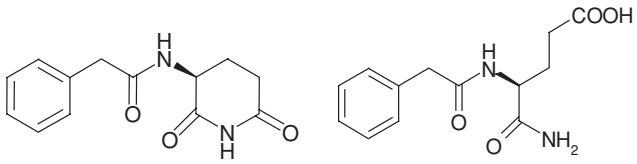
Aminopeptidase N (APN) is a member of membrane-bound Zinc-dependent exopeptidase family involved in the metabolism of angiotensin III in the brain and peripheral organs. The over-expression of APN has been involved in several pathological conditions including cancer,[1] leukemia, diabetic nephropathy,[2] rheumatoid arthritis,[3] angiogenesis[4] and central nervous system diseases such as Alzheimer's disease.[5] This has led to the search for APN inhibitors (APNIs) as potential therapeutic agents. Unfortunately no such effective agent exists at the present time.

We have been interested in the Antineoplaston 10 and its active metabolite N2-phenylacetyl L-iso-glutamine (Scheme 1), which are attractive synthetic APNIs for their low toxicity and side effects.[6]

Previous paper from our laboratory described the synthesis of novel L-iso-glutamine derivatives as potential antitumor agents, some of them showed significant inhibitory activity against APN. [7]

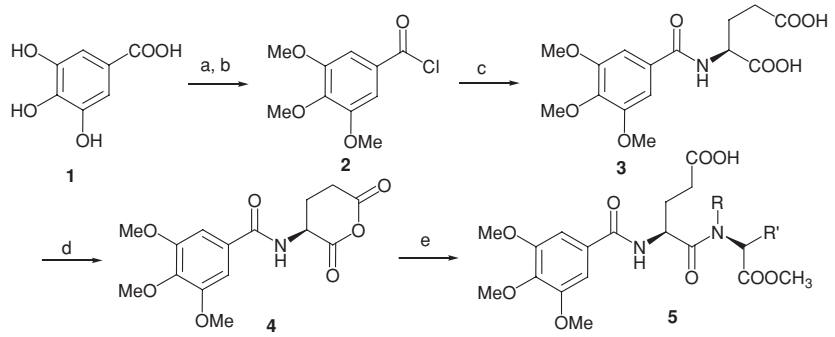
The synthetic route was shown in Scheme 2.

When the asymmetric annular acetic anhydride (**4**) is treated with various L-amino acid methyl esters, the anhydride ring might be opened from two sides (Scheme 3), and two final products L-glutamine (**5**) and/or L-iso-glutamine (**6**) could be obtained theoretically.

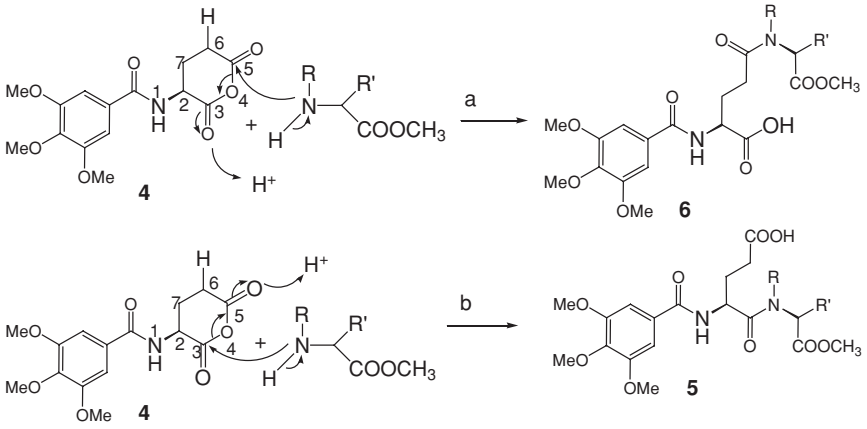


Scheme 1

In fact, only one product (**5**) was isolated from the reaction mixture. Theoretically speaking, whilst 3-C is adjacent to 4-O and 2-NH, while 5-C is adjacent to 4-O and 6-H, thus 2-NH is more electronegative than 6-H, so the electron cloud density of 3-C is lower than 5-C. When the electronegative nitrogen atom of L-amino acid methyl esters attacks the electropositive carbon atom of a carbonyl group, it is inclined to attack 3-C to get compound (**5**). In order to validate this theory, the structure was further confirmed by IR, NMR HMBC spectrum, mass



Scheme 2 Reagents and conditions: (a) Me_2SO_4, NaOH; (b) $SOCl_2$, C_6H_6; (c) L-Glutamic acid, $NaHCO_3$, H_2O, C_6H_6, HCl; (d) Ac_2O, (e) $RCH(NH_2)COOCH_3{\cdot}HCl$,CH_2Cl_2, Et_3N.

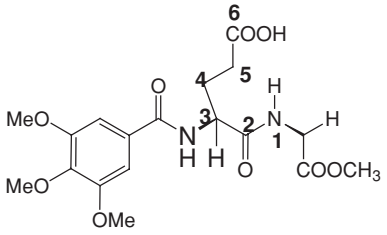


Scheme 3

* Correspondent. E-mail: tjulx2003@yahoo.com.cn

spectrometry (MS) and elemental analysis, and X-ray single crystal diffraction.

Taking compound (4S)-5-(2-methoxy-2-oxoethylamino)-5-oxo-4-(3,4,5-trimethoxy benzamido) pentanoic acid (Scheme 4) as an example, its structure is determined as follows.



Scheme 4 (4S)-5-(2-methoxy-2-oxoethylamino)-5-oxo-4-(3,4,5-trimethoxy benzamido) pentanoic acid.

Experimental

IR, ^1H NMR, ESI-MS, elemental analysis: The IR, ^1H NMR, MS, elemental analysis (Table 1) for the compound are in good agreement with the structure.

HMBC spectrum (500MHz, DMSO-d6): From the HMBC spectrum (Fig 1), there are cross-peaks between N-1 (8.38ppm) and C-2 (171.9ppm), C-2 (C-H) (4.47ppm) and C-2 (171.9ppm), H-5 (2.34ppm) and C-6 (173.9ppm). N-1 (N-H) (8.38ppm) and C-6 (dissociative carboxyl 173ppm) have no correlative cross-peaks. This information indicated that the glycine moiety was attacked at the C-2 position, the dissociative carboxyl group was at the C-6 position route and, route b of Scheme 3 was correct.

The above conclusion could be further explained as Scheme 5, the main correlativity of C-H and C-C are shown by arrows.

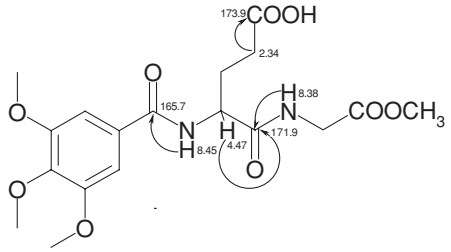
X-ray single crystal diffraction: *Crystallographic data and structure determination*: Compound (4S)-5-(2-methoxy-2-oxoethylamino)-5-oxo-4-(3,4,5-trimethoxybenzamido) pentanoic acid, $C_{18}H_{24}N_2O_9$, Mr 412.39, monoclinic, space group P2 (1)/C, a=27.665(9), b=5.1444(16), c=13.907(4) Å, V=1958.0(11) Å^3, T=298(2)K, Z=4, Dc=1.399Mg/m^3, F(000, 872, monochromated Mo-Kα radiation, λ=0.71073Å, μ= 0.113mm^{-1}. Data were collected using a crystal of size 0.25×0.15 × 0.10 mm on a fine-focus sealed tube in a random orientation. A total of 9819 reflections were collected with 3619 unique ones (R_{int}=0.0410) for 2.23≤θ≤25.50°and –33≤h≤26, –6≤k≤6 and -16≤l≤16.

2497 Reflections with I>2σ(I) were considered as observed and used in the succeeding refinements. There was no crystal decay and no absorption correction was applied.

The structure was solved by direct methods and expanded by using Fourier difference techniques with SHELXL-97. [8] All non-hydrogen atoms were located with successive difference Fourier syntheses. The structure was refined by full-matrix 1least-squares method on F^2 with anisotropic thermal parameters for all non-hydrogen atoms. The hydrogen atoms were added according to theoretical models.

Table 1 IR, ^1H NMR, ESI-MS, elemental analysis data

	(4S)-5-(2-methoxy-2-oxoethylamino)-5-oxo-4-(3,4,5-trimethoxybenzamido)pentanoic acid	
IR (KBr, cm^{-1})	3325.1(NH), 2939.6(CH), 1745.7 and 1714.5 (O=C-NH), 1637.8 (O=C-O), 1584.3 (C=C), 1131.3 (C-O)	
ESI-MS (*m/z*)	411.8[M-H]$^+$	
Elemental analysis	Calculated	Found
	C% (52.42); H% (5.87); N% (6.79)	C% (52.60); H% (5.96); N% (6.69)
^1H NMR (600MHz, DMSO-d_6, δ,ppm)	8.45 (d, 1H, J=7.5Hz, NH), 8.38 (t, 1H, J=5.4Hz, NH), 7.23 (s, 2H, Ar-H), 4.47 (m, 1H, CH), 3.88 (d, 2H, J=5.4Hz, CH_2), 3.83 (s, 6H, 2-OCH_3), 3.69 (s, 3H, OCH_3), 3.62 (s, 3H, $COOCH_3$), 2.34 (t, 2H, J=8.0Hz, CH_2), 2.06, 1.92 (2m, 2H, CH_2).	



Scheme 5

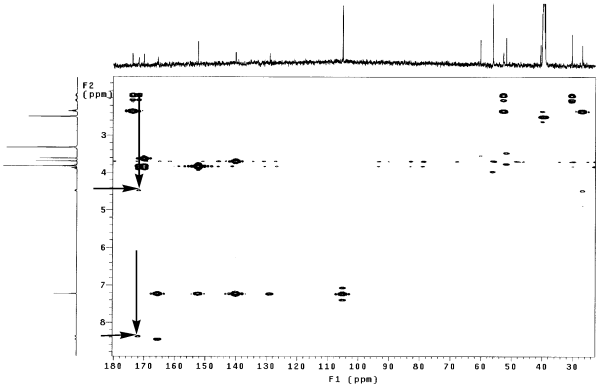


Fig. 1 HMBC Spectrum of compound (4S)-5-(2-methoxy-2-oxoethylamino)-5-oxo-4-(3,4,5-trimethoxybenzamido) pentanoic acid.

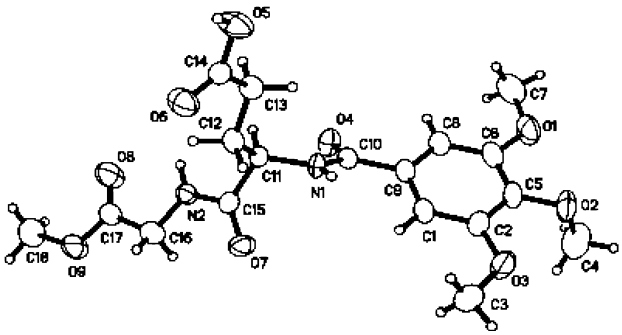


Fig. 2 X-Ray crystal structure of compound (4S)-5-(2-methoxy-2-oxoethylamino)-5-oxo-4-(3,4,5-trimethoxybenzamido) pentanoic acid.

The final refinement R indices were R_1=0.0606 and wR_2=0.1405 (w=I/[σ2($F_0$2) + (0.0676P)2 +0.7151P] where P= ($F_0$2+2 F_c2)/3) and R indices (all data): R_1=0.0895, wR_2= 0.1546. S=1.036.

This work was greatly supported by the National Natural Science Foundation of China (Grant No.20272033).

Received 21 October 2004; accepted 29 November 2004
Paper 04/2831

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