SYNTHESIS OF A NOVEL CONFORMATIONAL RESTRICTED AMINO ACID FOR POTENTIAL USE IN PEPTIDE CHEMISTRY

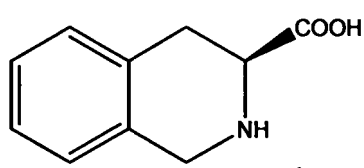
Wei-wei WANG , Ting YU , Chao WANG *

Department of Medicinal Chemistry, School of Pharmaceutical Sciences,
Peking University, Beijing 100191, China
e-mail: chaowang@bjmu.edu.cn

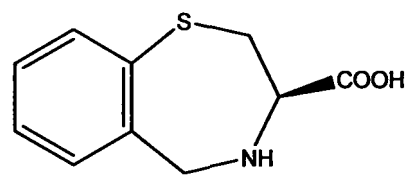
Abstract : In this context, a novel amino acid, (R)-2,3,4,5-tetrahydro-benzo-[1,4]-thiazepine-3- carboxylic acid (2), was synthesized using L-Cysteine and 2-fluoro-benzaldehyde as starting materials to undergo five-step reactions. The desired compound may be used in peptide chemistry as a potent conformational restricted amino acid.

Introduction

Many of the small peptide hormones and neurotransmitters are structurally flexible molecules and may assume a number of different conformations of comparatively low energy depending on the environment. The inherent flexibility of the natural peptide may also be the reason for their generally lack of selectivity towards different receptor types or subtypes, since conformational adaptation to different receptor topographies is possible. In view of this situation, the incorporation of conformational constraints into peptides appears attractive, not only because meaningful conformational studies aimed at determining bioactive conformations become possible, but also because compounds with improved receptor selectivity may result from such efforts.



1



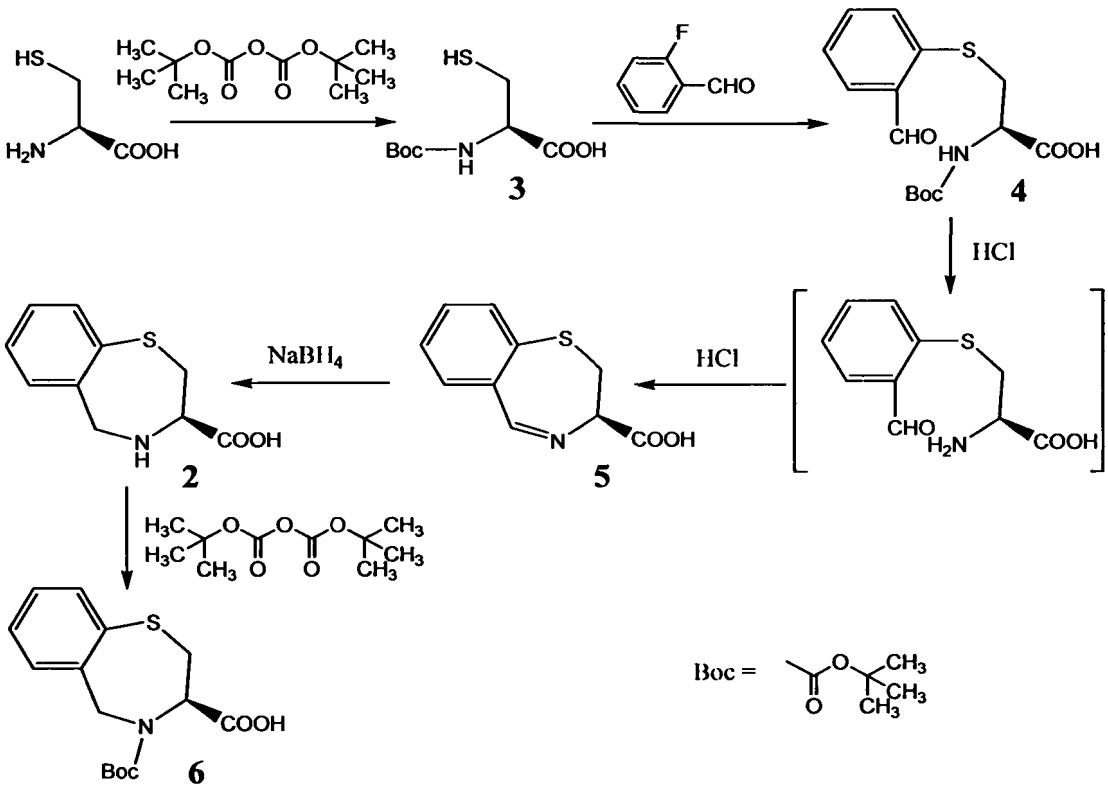
2

Fig 1. Chemical diagrams of (R)-1,2,3,4-tetrahydroisoquinoline-3-carboxy acid (1) and (R)-2,3,4,5-tetrahydro-benzo-[1,4]-thiazepine-3-carboxylic acid (2)

Constrained derivatives of phenylalanine are often incorporated into peptides to investigate the conformational requirements of receptors, and 1,2,3,4-tetrahydroisoquinoline-3-carboxy acid (1) also play an important role as building blocks for the stabilization of short peptides in a well-defined conformation[1]. 1,2,3,4-tetrahydroisoquinoline-3-carboxy

acid can be synthesized from phenylalanine reacted with formaldehyde in one step through Pictet-Spengler condensation, but the similar product with the seven-membered ring could not be prepared using homophenylalanine under the same condition[2].

The similar experiment in our lab for synthesis of Benzo-fuzed heteroheptacyclic derivatives was also unsuccessful because the seven-membered ring could not been closed by Pictet-Spengler condensation. So we carried out mentioned approach in this paper for ring formation using L-Cysteine and 2-fluoro-benzaldehyde as starting materials to synthesize the title compound.



Scheme 1. Synthetic pathway for preparation of 2 and its protected derivative (6)

Results and Discussions

The structures of synthesized were proved by spectroscopic methods and it is worth to mention in two appearance. At first, the presence of the zwitter-ionic form in the structure of 2 was deduced from careful analysis of ^{1}H-NMR data. The signal of two N-H protons at 9.99ppm was observed and in the meanwhile the signal of carboxyl proton was not found in the spectrum. The second, compound 6 is formed as a mixture of cis- and trans- isomers because amido bond in the structure has partial double bond character with a rotational barrier. So, some typical signals in compound 6, such as tert-butyl coming from Boc moiety show two single peak in ^{1}H-NMR spectrum. The similar results can be observed in other literatures[3,4].

The variation in the degree of conformational restriction in structure can have a profound effect on receptor affinity and selectivity. In addition, the structure of desired compound with benzo-fuzed seven-membered moiety in this paper is different from that conformational constrained amino acids derivatived from phenylalanine in published literature[5]. The major difference is the position of the aromatic ring and the distance from benzene ring to C^{α} in the amino acid residue. This moderate structure changes may possibly result better effect than the six member ring structure

in some phenylalanine-containing peptides applications especially for developing of the peptide receptor antagonists.

Experimental

Melting points were measured using a microscope hot-stage apparatus and are uncorrected. Spectroscopic data were obtained on the instruments listed: ^{1}H NMR (VXR-300); MS (ZQ-2000). Elemental analyses were performed on a PE-2400 elemental analyzer. All reactions were monitored by TLC using precoated plates of silica gel 60 F 254 (Merck).

N-Boc-L-Cysteine (3):

It was obtained as an oil in 80% yield according to the method of literature [6].

N-Boc-S-(2-formyl-phenyl)-L-Cysteine (4):

To a solution of compound 3 (7.50 g, 33.9 mmol) in DMF (15 ml), 2-Fluoro-benzaldehyde (9.87 g, 84.4 mmol) and Sodium carbonate (10.63 g, 77 mmol) were added. The reaction mixture was stirred under argon at 60□ for 36hr. The solution was evaporated in vacuo to dryness, and dilute solution of $KHSO_4$ was added to pH 1-2. The aqueous phase was extracted with ethyl acetate (3×50 ml). The combined organic layers were washed twice with water and dried over anhydrous Na_2SO_4. After elimination of the solvent at reduced pressure, the residue was purified by silica gel column chromatography (AcOEt-petroleum ether-AcOH 1:3:0.2), and the obtained colorless powder was triturated with petroleum ether to give 5.60 g (49%) of the title compound, m.p. 111-113□. ESI-MS: m/e 348$[M+Na]^+$, 364$[M+K]^+$, 226$[M+H-Boc]^+$; $[\alpha]_D$ = -13.8°(c 1.0, MeOH); ^{1}H-NMR ($CDCl_3$) δ(ppm): 1.44(s, 9H, $(-CH_3)_3$), 3.14(dd, J=13.8, 5.2Hz, 1H, -S-CH_2-), 3.40(dd, J=13.9, 5.2Hz, 1H, -S-CH_2-), 4.63(br, 1H, -N-CH-COO), 7.28-7.59(m, 4H, H-Ar), 7.88(d, J=7.7Hz, 1H, NH), 10.46(s, 1H, -CHO).

(R)-2, 3-dihydro-benzo-[1, 4]-thiazepine-3-carboxylic acid (5):

Compound 4 (1.0 g, 3.07 mmol) was dissolved in 10 ml of HCl/AcOEt (4N), and the solution was stirred at room temperature for 8 h. The precipitate was collected on a filter, washed with ethyl ether to yield 0.715g (100%) of the title compound as a yellow powder. ESI-MS: m/e 208 $(M+H)^+$. It was used directly without further purification for next stage preparation.

(R)-2, 3, 4, 5-tetrahydro-benzo-[1, 4]-thiazepine-3-carboxylic acid (2):

A solution of compound 5 (2.0 g, 9.7 mmol) in methanol (30 ml) was cooled in an ice-water bath and sodium borohydride (2.0 g) was added with stirring in 20 portions during 2 days. The solution was evaporated in vacuo, and residue was dissolved in ethyl acetate. The solution was acidification with acetic acid to pH 2-3. The precipitate was eliminated by filtration. The filtrate was evaporated in vacuo to driness, and the residue was purified by crystallization from methanol-ethyl ether to yield 1.57 (77.7%) of the title compound as a white crystal. m.p. 232□(decomposed). ESI-MS: m/e: 210$[M+H]^+$; $[\alpha]_D$ = -4.4°(c 0.5, methanol); ^{1}H-NMR(DMSO-d_6), δ(ppm): 3.22(br, 1H, S-CH_2-), 3.39(dd, J=13.9Hz, J=7.0Hz, 1H, S-CH_2-), 4.39(d, 1H, J=14.4Hz, -CH_2-N), 4.55(br, 1H, N-CH-COO), 4.58(d, J=14.4Hz, 1H, -CH_2-N), 7.36-7.58(m, 4H, H-Ar), 9.99(br, 2H, $^+NH_2$).

(R)-N-Boc-2, 3-dihydro-5-hydro-benzo-[1, 4]-thiazepine-3-carboxylic acid (6):

To a solution of compound 2 (1.276 g, 6.1 mmol) in water/1,4-dioxane (1:1, 16 ml), sodium hydrogen carbonate (0.513 g, 6.1 mmol) and di-tert-butyl dicarbonate (1.331 g, 6.1 mmol) were added. The reaction mixture was stirred under argon at room temperature for 10 h. The solution was evaporated in vacuo to dryness, and then the dilute solution of $KHSO_4$ was added to pH 2-3. The aqueous phase was extracted with ethyl acetate (3×50 ml). The combined organic layers were washed twice with water and dried over anhydrous Na_2SO_4. After elimination of the solvent at reduced

pressure, the residue was purified by silica gel column chromatography ($CHCl_3$-CH_3OH-AcOH 1:3:0.2), and the obtained colorless powder was triturated with petroleum ether to give 0.974 g (51.6%) of the title compound. m.p. 190-192□. ESI-MS: m/e: 310$[M+H]^+$, 332$[M+Na]^+$, 210$[M+H\text{-}Boc]^+$, $[\alpha]_D$ = -0.9°(c 1.0, MeOH); ^{1}H-NMR(DMSO-d_6), δ(ppm): 1.34, 1.36(2s, 9H, $(-CH_3)_3$), 2.94, 3.04(2d, J=15.5Hz, 1H, -S-CH_2-), 3.55, 3.61(2d, J=15.5Hz, 1H, -S-CH_2-), 4.46,4.51(2d, J=15.0Hz, 1H, -N-CH_2-), 4.61,4.73(2d, J=15.0Hz, 1H, N-CH_2-), 5.00,5.17 (2br ,1H, N-CH-COO), 7.22-7.42 (m, 4H, H–Ar), 13.21(s, 1H, -COOH); ^{13}C-NMR(DMSO-d_6), δ(ppm): 28.40,($(-CH_3)_3$), 34.68, 34.99(-S-CH_2-), 48.01, 48.88(-N-CH_2-), 58.70,60.58(N-CH-COO), 80.22,80.26(-O-C-$(CH_3)_3$), 127.48, 127.83, 128.02, 130.60, 130.86, 131.38(C-Ar), 154.61, 155.16 (N-C=O), 171.64(-COOH); Anal. calcd. for $C_{15}H_{19}O_4SN$: C, 58.13; H, 6.27; N, 4.53; Found: C, 58.23; H, 6.19; N, 4.53.

References:

1. V. J. Hruby, P. M. Balse, Curr. Med. Chem. 7, 945 (2000)
2. J. C. Martins, K. V. Rompaey, G. Wittmann, J. Org. Chem. 66, 2884 (2001)
3. R. L. E. Furlan, E. Mata, O. A. Mascaretti, Thtrahedron, 54, 3023 (1998)
4. R. K. Dieter, S. J. Li, J. Org. Chem. 62(22), 7726 (1997)
5. S. E. Gibson, N. Guillo, S. B. Kalindjian and M. J. Tozer, Med. Chem. Lett. 7, 1289 (1997)
6. B. Pierluigi, A. Marco, B. Daniela, Heterocycles. 16, 1529 (1993)

Received on January 15, 2009.