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Tetrahedron Letters
Tetrahedron Letters 48 (2007) 7614–7617

Synthesis of leucine-enkephalin analogs containing α-amino squaric acid

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Received 23 July 2007; revised 28 August 2007; accepted 30 August 2007
Available online 1 September 2007

Abstract—Novel Leu-enkephalin analogs **10a–c** in which the Tyr[1], Gly[2], or Gly[3] residue of Leu-enkephalin **9** was replaced with α-amino squaric acid analog Sq-Tyr **8b** or Sq-Gly **8a** were synthesized starting from **14** or **18**. Conformational analysis of **10a–c** together with its model compound **26** and their opioid receptor binding activity were evaluated.

1. Introduction

Recently, a number of α-amino acid analogs (AAA) in which the carboxylic acid group of α-amino acid **1** is replaced with other acidic functional groups, for example, sulfinic acid **2**,[1] sulfonic acid **3**,[2] phosphinic acid **4**,[3] phosphonic acid **5**,[3i,4,5] boronic acid **6**,[6] and tetrazole **7**,[7] have been developed (Fig. 1). Incorporation of these AAAs into biologically active peptides has received significant attention from bioorganic and medicinal chemists due to their important roles as enzyme inhibitors and haptens that generate catalytic antibodies.[8] We have recently reported the synthesis of a new AAA **8** bearing a 4-hydroxy-2,3-dioxocyclobut-1-enyl (Sq) group (α-amino squaric acid, α-Asq) (Fig. 1).[9] The Sq group is characterized by its planar structure, acidic OH group, and electron deficient property, and widely employed as a potent carboxylic acid surrogate in medicinal chemistry.[10,11] We now report the synthesis of novel Sq-Gly- or Sq-Tyr-containing Leu-enkephalin (Leu-Enk) analogs **10a–c** bearing α-Asqs **8a**,**b** at the N-terminal or inside the peptide chain.

2. Results and discussion

We began our program with the synthesis of [Sq-Tyr[1]]-Leu-Enk **10a** (Scheme 1). To this end, the coupling precursor **14** was prepared using the Sq group-containing aminomalonate equivalent **11** (see Scheme 1).[9] The alkylation reaction of **11** with bromide **12** in the presence of Bu_4NI and K_2CO_3 gave **13** in 74% yield. Due to the strong electron-withdrawing properties of the Sq group, it was expected that the carboxylic acid group in **13** would be removed by mild decarboxylation reaction conditions via the corresponding carboxylate.[9] As expected, *t*-butyl ester **13** underwent the spontaneous decarboxylation reaction by treatment with TFA that produced **14**. The coupling reaction of **14** with H_2N–Gly-Gly-Phe-Leu-*O*-*t*-Bu was conducted in the presence of Et_3N followed by the removal of the protecting groups with HBr/AcOH to give Leu-enkephalin analog **10a** in high yield.

Next, we examined the synthesis of Leu-encephalin analogs **10b**,**c** possessing Sq-Gly inside the peptide chain. Our initial plan involved the use of Cbz-HN-Sq-Gly-*Oi*-Pr **15** as the coupling precursor, Eq. 1. However, the *N*-Cbz group could not be removed under the catalytic hydrogenation conditions since the Sq group significantly poisoned the catalyst activity, Eq. 1.[12] Attempts to synthesize other possible, such as *N*-Boc or *N*-Fmoc, Sq-Gly derivatives **17** could not be prepared by the

Keywords: Squaric acid; Isostere; Enkephalin; Peptide; Amino acid.
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doi:10.1016/j.tetlet.2007.08.103

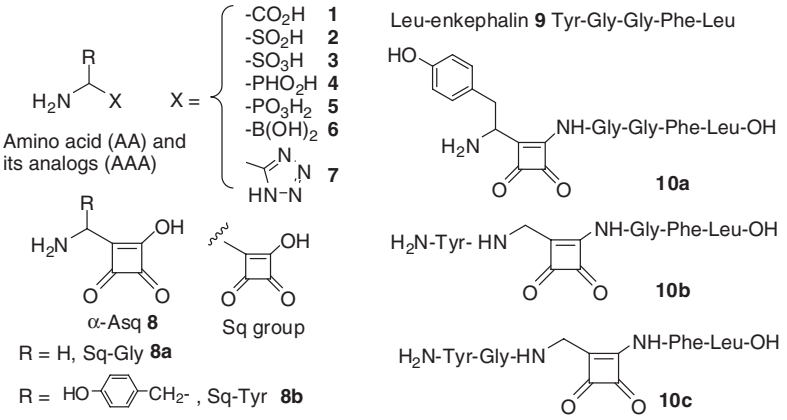
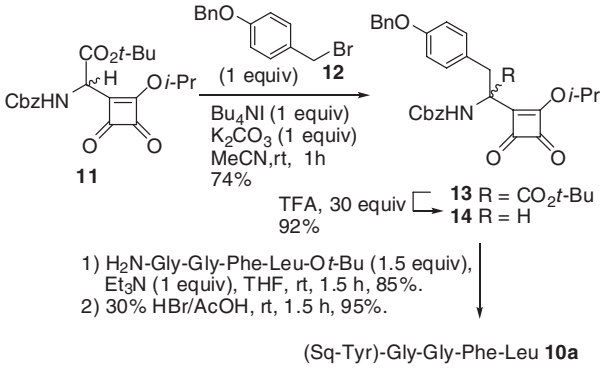
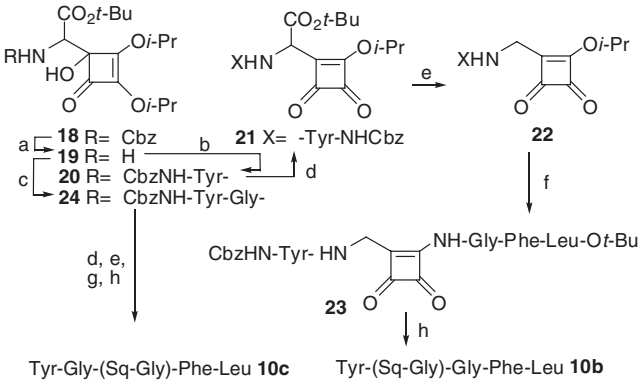


Figure 1. Structures of amino acid mimics Leu-Enkephalin analogs containing the α-Asqs.

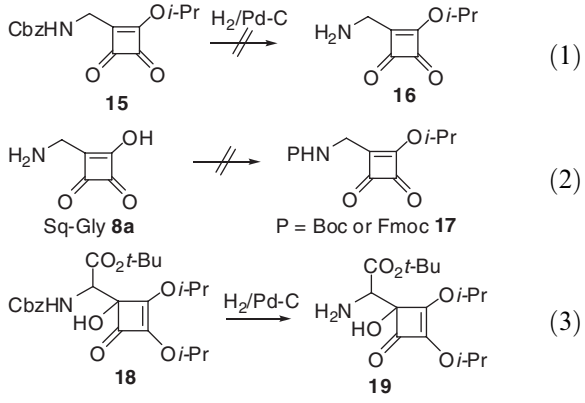


Scheme 1. Synthesis of **10a**.



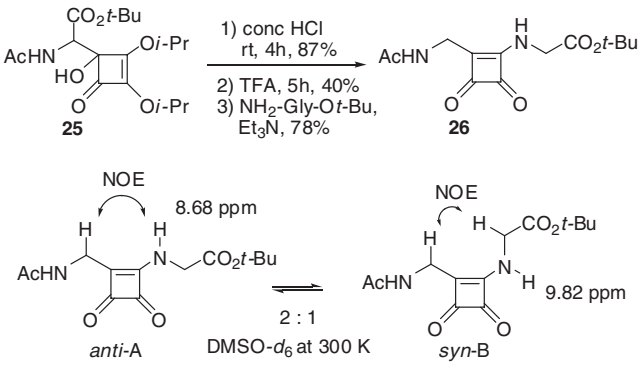
Scheme 2. Reagents and conditions: (a) Pd–C (20% w/w), H_2, MeOH, rt, 2 h; (b) CbzNH-Tyr–OH (1.1 equiv), DEPBT, (2.2 equiv), THF, rt, 48 h, two steps, 78%; (c) CbzNH-Tyr-Gly–OH (1.1 equiv), DEPBT, (1.2 equiv), THF, rt, 48 h, two steps, 76%; (d) concd HCl, (0.9 equiv), CH_2Cl_2, rt, 3 h, 74%; (e) TFA (30 equiv), CH_2Cl_2, rt, 12 h, 74%; (f) H_2N–Gly-Phe-Leu-*O*-*t*-Bu (1.5 equiv), Et_3N (1 equiv), THF, rt, 1.5 h, 91%; (g) H_2N–Phe-Leu-*O*-*t*-Bu (1.5 equiv), Et_3N (1 equiv), THF, rt, 1.5 h, 91%; (h) 30% HBr/AcOH, rt, 1.5 h, 95–98%. Synthesis of **10b** and **10c**.

introduction of protecting groups to Sq-Gly **8a** or using the dianion enolate method, Eq. 2. Therefore, we turned our attention to hydroxybutenone **18**[9b,12] as an alternative coupling precursor, Eq. 3 since **18** underwent elimination of the Cbz group under H_2/Pd–C conditions to generate the free amine **19**, in contrast to the case of the Sq group-containing **16**, Eq. 1.



Along this line, **18** was converted to the corresponding amine **19**, which, upon treatment with Cbz-Tyr–OH in the presence of DEPBT,[13] afforded the coupling product **20** in 78% yield (Scheme 2). This was converted to dipeptide analog **22** via **21** by the following sequence of reactions: (1) conversion to cyclobutenedione **21** by treatment with a small amount of concd HCl, (2) removal of the *t*-butyl ester by TFA, and (3) spontaneous decarboxylation of the corresponding carboxylate. Dipeptide **22** was coupled with H_2N–Gly-Phe-Leu-*O*-*t*-Bu in the presence of Et_3N to give a protected pentapeptide analog **23**. Removal of the protecting group of **23** with HBr/AcOH furnished [Sq-Gly^2]-Leu-Enk **10b**. In a similar manner, [Sq-Gly^3]-Leu-Enk **10c** was synthesized from **18** via **24** in good yield.

The binding affinities of **10a**–**c** for the μ-, δ-, and κ-opioid receptors were investigated. But these analogs did not show any significant binding affinities.[14] To gain insights into the structure and biological activity relationship of **10a**–**c**, the conformational analysis of the α-Asq containing dipeptide analog **26** as a model of **10a**–**c** was examined (see Scheme 3). Dipeptide **26**, prepared from *N*-acetyl hydroxycyclobutenone **25**, provides certain information about the local conformation around the Asq moiety. The ^{1}H NMR data and NOE experiments of **26** in DMSO-d_6 indicated that **26** consisted of a 2:1



Scheme 3. Synthesis of **26** and its conformational analysis.

mixture of rotamers A (*anti*-A) and B (*syn*-B). The formation of a mixture of rotamers was also observed in **10a**–**c** and other Sq-amide derivatives as previously reported.[15] The major rotamer was assigned to the *anti*-form A by the NOE experiments and by the comparison of the shift values of the amide protons of *syn*-B (9.82 ppm) and *anti*-A (8.68 ppm). The lower shift of the amide proton of *syn*-B was attributed to the paramagnetic influence of the carbonyl group in the Sq group. Measurement of the temperature coefficient values[16] ($-d\delta/dT$, ppm/K) of each amide proton of **26** in DMSO-d_6 under variable temperatures showed large values ($-d\delta/dT > 0.0045$) for each amide proton. The results suggested that the solvent-shielded or intramolecular hydrogen bonding amide protons are not involved in this molecule. Enkephalin analogs **10a**–**c** were subjected to the same NMR studies. These results were quite similar to those of the model peptide **26** with respect to the formation of the rotamers in DMSO-d_6 and large temperature coefficient values of the amide protons ($-d\delta/dT > 0.0045$), indicating that the synthetic Asq analogs **10a**–**c** do not have a preferential binding conformation to the opioid receptors in which the β-turn conformation is proposed to be one of the crucial factors for the receptor binding.

In summary, we have established a synthetic route to access the α-Asq-containing enkephalins and evaluated their binding activities to opioid receptors. To the best of our knowledge, this is the first example incorporating α-Asq into peptide. Peptide analogs **10a**–**c** were found to be stable under acidic conditions, HPLC purification conditions, and NMR measurements in DMSO and D_2O. In contrast, peptide analogs embedding aminophosphate **2** and aminosulfate **3** are known to be liable to hydrolytic cleavage.[17] The chemically stable features of **10a**–**c** are advantageous for further application to the synthesis of other α-Asq-containing peptides.

Acknowledgements

This study was supported by a grant from the Research for the Future Program (99L01204) and Grants-in-Aid (16201045, 16073214, and 18510191) for Scientific Research from the Japan Society for the Promotion of Science (JSPS) and the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.08.103.

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