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Systematic replacement of amides by 1,4-disubstituted[1,2,3] triazoles in Leu-enkephalin and the impact on the delta opioid receptor activity

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ABSTRACT

Using Cu(I)-catalyzed azide–alkyne cycloaddition in a mixed classical organic phase and solid phase peptide synthesis approach, we synthesized four analogs of Leu-enkephalin to systematically replace amides by 1,4-disubstituted[1,2,3]triazoles. The peptidomimetics obtained were characterized by competitive binding, contractility assays and ERK1/2 phosphorylation. The present study reveals that the analog bearing a triazole between Phe and Leu retains some potency, more than all the others, suggesting that the hydrogen bond acceptor capacity of the last amide of Leu-enkephalin is essential for the biological activity of the peptide.

Although Leu-enkephalin possesses poor selectivity (1 to 5×) for DOPr over the Mu opioid receptor (MOPr), it is considered to be an endogenous DOPr ligand.[1] Due to the rapid degradation by enzymes and the poor hydrophilic character of Leu-enkephalin, a lot of research activity has been done to design and synthesize peptidomimetics of this peptide.

Since the discovery of the Cu(I)-catalyzed azide–alkyne cycloaddition known as 'click chemistry',[2] various applications of this robust reaction were published.[3] In the field of peptidomimetics, the use of [1,2,3]triazole as an amide surrogate has been applied to create locked mimetics of both trans[4] and cis[5] amides producing biologically active peptidomimetics. To a certain extend, the molecular properties of the triazole and the amide groups are similar[6] (Fig. 1).

Both functions have hydrogen bond acceptor properties and a similar dipolar moment.[7] The triazole was rarely proved to be a weak hydrogen bond donor.[8] Incorporation of triazoles as amide mimics substantially stretches the resulting peptidomimetics by ~1.2 Å. In several peptidomimetics this extension induced no significant change to the biological activity.[4,5] Since the amide bonds in endogenous peptides are the primary site targeted by degrading enzymes, their replacement by triazole groups represents an efficient way to design analogs with enhanced biological half-life.[7]

This study presents a series of Leu-enkephalin (Tyr-Gly-Gly-Phe-Leu) analogs obtained by systematic replacement of amides by triazole groups (Fig. 2). The analogs were tested to determine their biological activity at the delta opioid receptor (DOPr). It has been hypothesized that selective DOPr agonists could lead to the development of improved chronic pain medication,[9,10] emphasizing the interest of this biological target.

In previous studies, we have shown that the systematic replacement of amides in Leu-enkephalin by *E*-alkenes,[11] esters[12] and N-Methyl amides[12] can reveal important information regarding the biological role of each amide in relevant analogs of Leu-enkephalin and can therefore increase our understanding of the non-covalent interactions between DOPr and its endogenous ligands. To our knowledge the use of a triazole as a dipeptide isostere has never been applied to the field of opioid peptidomimetics, although some tetrazoles mimicking cis-amides have already been reported.[5d]

We used a mixed approach combining solid-phase synthesis with classical organic synthesis for the preparation of Leu-enkephalin analogs bearing a triazole. First, the azide moiety was prepared in solution using known procedures[13] (Scheme 1). Triflyl azide

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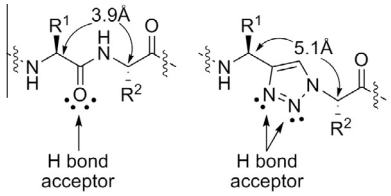


Figure 1. Comparison of the *s*-trans amide and the 1,4-disubstituted[1,2,3]triazole dipeptide isostere.

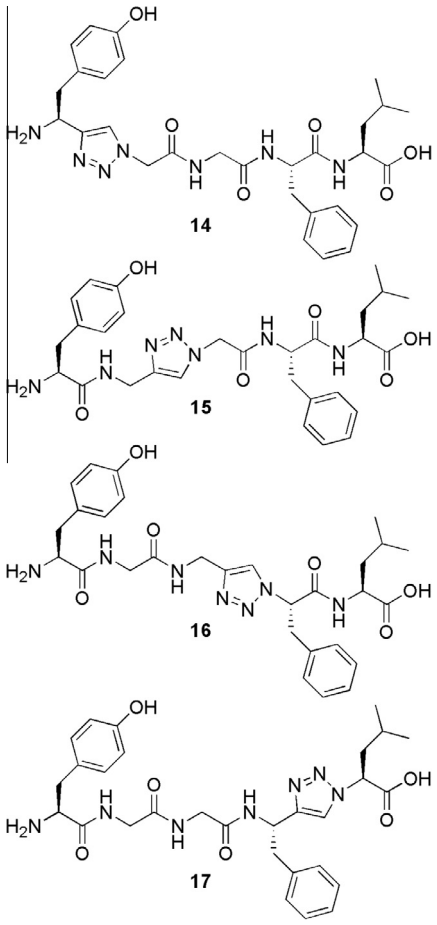
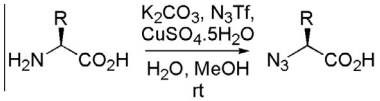


Figure 2. 1,4-Disubstituted[1,2,3]triazole analogs of Leu-enkephalin.

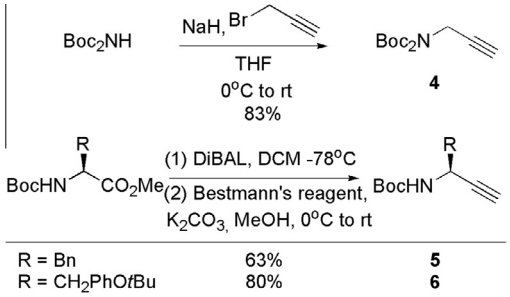
(N_3Tf) was prepared first and then added to the amino acids to give the azido acids **1**–**3**.

Two different strategies were taken for the preparation of the alkyne moieties (Scheme 2). Alkyne **4** was obtained in one alkylation step from commercial di-*t*Bu-imino-dicarboxylate.[14] Alkynes **5** and **6** were obtained by partial reduction, with DIBAL, of protected amino esters to yield the corresponding aldehydes, followed by Seyferth–Gilbert homologation using Bestmann's reagent.[5b] It is worth noting that no epimerization of the chiral center was observed during this step.



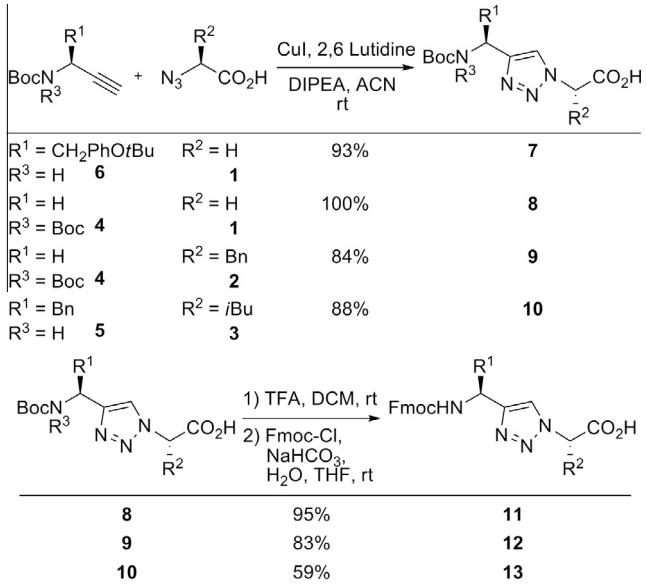
R = H	89%	**1**
R = Bn	98%	**2**
R = *i*Bu	64%	**3**

Scheme 1. Synthesis of the azide moiety.



R = Bn	63%	**5**
R = $CH_2PhOtBu$	80%	**6**

Scheme 2. Synthesis of the alkyne moiety.



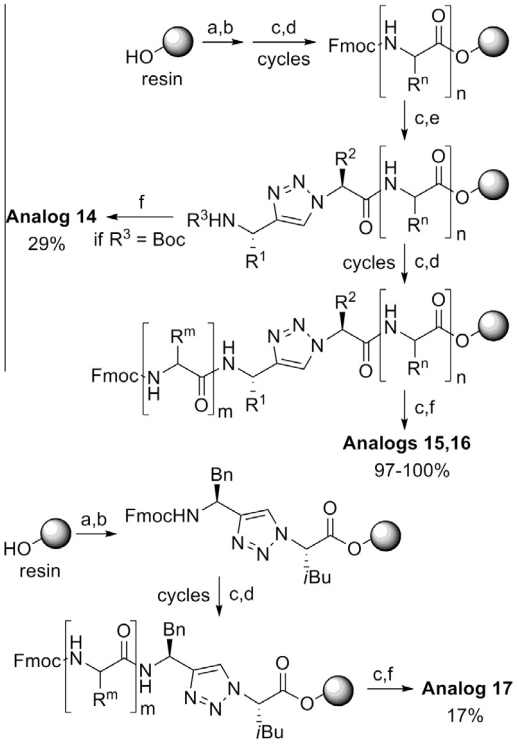
R^1 = $CH_2PhOtBu$, R^3 = H **6**	R^2 = H **1**	93%	**7**
R^1 = H, R^3 = Boc **4**	R^2 = H **1**	100%	**8**
R^1 = H, R^3 = Boc **4**	R^2 = Bn **2**	84%	**9**
R^1 = Bn, R^3 = H **5**	R^2 = *i*Bu **3**	88%	**10**
8		95%	**11**
9		83%	**12**
10		59%	**13**

Scheme 3. Synthesis of the triazoles and Fmoc protection.

The azides **1**–**3** and the alkynes **4**–**6** were cross-coupled in Cu(I)-catalyzed azide–alkyne cycloaddition reactions (Scheme 3).[15] The resulting Boc protected building block **7** was used in solid phase peptide synthesis as such without further modification due to its *N*-terminal position in the Leu-enkephalin mimetic **14** (Fig. 2). For compounds **8**–**10**, the Boc protective group was cleaved and a Fmoc protection was introduced instead to give Fmoc protected[11] building blocks **11**–**13** (Scheme 3). The synthesis of each building block was achieved in good overall yields from their corresponding amino-alkyne and azide–acid precursors.

Afterward, the building blocks **7**, **11**–**13** were used in solid phase peptide synthesis with Fmoc methodology either on TentaGel S PHB resin to generate the mimetic **14** or on Wang resin to produce the analogs **15**–**17** (Scheme 4). The loading onto the resin, the benzoyl capping, the Fmoc deprotection, the HATU normal peptide coupling and the resin cleavage were all carried out following standard protocols.[11,16] Whereas, the couplings of triazole building blocks **7**, **11**–**13** were done using DIC and HOBt. These base free conditions are known to minimize racemization of triazole dipeptide isosteres.[15] After cleavage, the crude peptides were purified on preparative reverse phase HPLC and all fractions over 95% in purity were combined. Analogs **14**–**17** (Fig. 2) were obtained in yields ranging from 17% to 100%.

In order to evaluate the ability of each compound to bind DOPr, we performed competitive binding assays using GH3/DOPr cell membrane preparations. As shown in Table 1, the systematic replacement of amides by triazoles did not produce peptidomimetics with high retention of the parent peptide biological activity. Among all compounds tested, analog **17** showed the highest



Scheme 4. Solid phase synthesis of the Leu-enkephalin analogs: Reagents and conditions: (a) Fmoc-Leu or **13**, 2,6-diClBzCl, Pyr, DMF; (b) BzCl, Pyr, DMF; (c) Pip/DMF (1:1); (d) Fmoc-AA (3 eq), HATU (3 eq), NMM (6 eq), DMF; (e) **7** or **11** or **12** (5 eq), DIC (5 eq), HOBt (5 eq), DMF, DCM; (f) TFA/TIPS/H_2O (38:1:1).

Table 1
Affinities and potencies of 1,4-disubstituted[1,2,3]triazole analogs of Leu-enkephalin

Compound	K_i (nM)[a]	EC_{50} (nM)[b]
14	>1000	>1500
15	>1000	>1500
16	460 ± 250	>1500
17	89 ± 12	830 ± 66

[a] The binding affinity (K_i) of each compound was determined by its ability to inhibit the binding of [^{3}H]-deltorphin II (competitive binding), a selective DOPr agonist, to GH3/DOPr cell membrane extracts. K_i values are the means ± SEM of three to four separate experiments each performed in triplicate.

[b] Potency (EC_{50}) of each compound was determined by evaluating their ability to inhibit the electric-field induced contractions of the mouse vas deferens. EC_{50} values are the means ± SEM of three separate experiments.

affinity (K_i = 89 nM) for DOPr (Table 1). At this fourth amide position, we previously showed that introducing an *E*-alkene induced an even larger decrease in the affinity,[11] while the replacement by the hydrogen bond acceptors ester[12] and *N*-Methyl amide[12] generated peptidomimetics almost as potent as Leu-enkephalin itself (K_i = 6.3 nM).[11] The analog **17** also displayed a weak potency to inhibit electrical field-induced mouse vas deferens contraction (Table 1) and to induce ERK1/2 phosphorylation (Fig. S1). Together, our findings suggest that in the analog **17** the triazole is not only holding peptide segments in place but is also involved in biologically relevant interactions, possibly as a hydrogen bond acceptor. The ~1.2 Å extension (Fig. 1) induced by the triazole incorporation might be involved in the significant loss of affinity.

In this study we presented a mixed synthetic approach combining solid-phase synthesis and classical organic synthesis to obtain peptidomimetics with medium to high overall yields. Although active peptidomimetics were obtained by triazole replacements,[4,5] our study shows that this heterocycle is in no ways a viable bioisostere for any of the four amides of Leu-enkephalin, as clearly evidenced by the decreased activity of the corresponding analogs **14**–**17**. Those findings should be taken in consideration before choosing triazoles as peptide bond surrogates, since their electronics are noticeably different as well as their geometries. The strategy to introduce triazole dipeptide isosteres in different parts of a peptide sequence could be applied to other endogenous ligands.

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Supplementary data

Supplementary data (pharmacological characterization, experimental procedures and spectral data) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.08.020.

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