

Development of a Novel Family of NSAIDs: Tripeptides

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In order to discover new useful antiinflammatory agent, many new compounds are continually being synthesized and tested.¹⁻⁵ A certain peptide which contains an anisolated glutamic acid has been reported to show considerable biological activities.⁶

Discover of Potential Antiinflammatory Tripeptide

In various animal models of tissue injury, corticotropine-releasing factor (CRF) and related peptides inhibit swelling, edema and loss of protein from the vascular compartment.⁷ The smaller peptide segments of CRF might retain antiinflammatory activity. Undecapeptides, D-Leu-Ala-Thr-D-Tyr-Arg-Lys-Leu-Leu-Glu-Ile-D-Leu-NH₂ and D-Ala-His-Ser-D-Asn-Arg-Lys-Leu-Leu-Glu-Ile-D-Leu-NH₂ were found to have activity in heat-induced rat's hind paw edema test. Undecapeptides whose glutamic acid residue were substituted with anisolated glutamic acid showed more increased overall potency.

D-Leu-Ala-Thr-D-Tyr-Arg-Lys-Leu-Leu-Glu*(P)-Ile-D-Leu-NH₂

D-Ala-His-Ser-D-Asn-Arg-Lys-Leu-Leu-Glu*(P)-Ile-D-Leu-NH₂

[Glu* : 2-amino-5-(methoxyphenyl)-5-oxopentanoic acid]

The anisole derivatives come from the Fridel-Crafts acylation reaction which occurs during hydrogen fluoride cleavage of glutamyl containing natural peptides. The two peptides among several undecapeptides reduced edema with ED₅₀ values of 0.004 and 0.005 mg/kg i.v., respectively which shown a potent activity. If this peptides will be developed as an antiinflammatory agent, the derivatives could be a novel family of NSAIDs (Nonsteroidal antiinflammatory drugs).

Design of Target Compound

Thus, we were interested in the synthesis of the analogues with anisolated glutamic acid derivatives. Then we had a goal to synthesize tripeptide 1a and 1b in sequence, NH₂-Met-Glu*-Ile-OMe as shown in Fig 1.

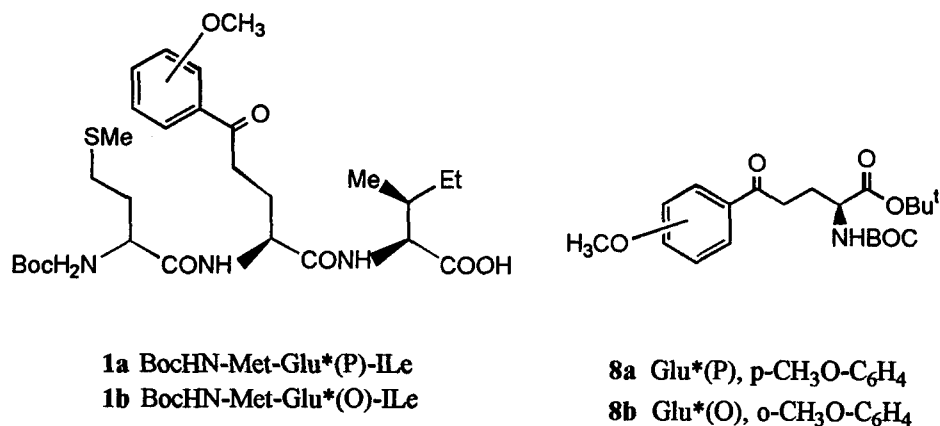


Fig 1

Chemistry of Synthesized Tripeptides

1. Synthesis of α -t-butyl anisoylated glutamates

The BOC α -t-butyl anisoylated glutamate **8** were synthesized from L-glutamic acid. First, the solution of L-glutamic acid and benzyl alcohol in benzene was refluxed with catalysis of p-toluene sulphonic acid monohydrate and azeotropic removal of water. The dibenzylester **2** was briefly isolated.

Benzyl ester has an unique place in peptide synthesis for the reversible protection of side chain carboxyl groups. This ester is relatively stable to the mildly acidic and basic conditions of peptide synthesis, but can be easily removed at the end of the synthesis by strongly acidic or reductive cleavage.⁸

Using the same methodology of Gmeiner,⁹ the conversion of **2** to **3** was carried out with cupric carbonate monobasic. After the reaction, H₂S was passed through the reaction mixture to cleave the copper complex with monobenzylester in 91% yield. The monobenzylester p-toluene sulphonate salt **3** was soluble and could be separated by filtration. In case of using 4 equivalent of cupric carbonate monobasic, the yield was lower than that of using 5 equivalent.

And, the amino group of **3** was protected as a N-BOC glutamate **4** with (BOC)₂O, triethylamine in THF and H₂O. The protection of the free acid as a t-butyl ester was performed with new isourea of BDDC (O-t-butyl-N,N'-di-2,2-dimethyl-1,3-dioxolane-4-methyl carbodiimide) that was synthesized by method of Gibson.¹⁰ The t-butyl ester group of amino acid is useful carboxyl-protecting group in peptide synthesis because it is cleaved readily by acids.^{11,14} In a preliminary paper, we described a procedure for converting free amino acids to their t-butyl esters by reaction with the same isourea.¹² In order to find the optimal condition, an effective amount of t-butylisourea, solvent, time and temperature have been changed. As use of toluene, the yield of **5** was highest of THF, dichloromethane and DMF. As

the reaction time was longer, the yield was also decreased. The most effective amount of *t*-butylisourea was 3.5 equivalent to the reactant. In case of 2.5 equivalent, the starting material was still included in the reaction mixture. A temperature of the reaction mixture was proper below 80°C. As the temperature was increased upper 95°C, the side reaction was bigger than it at 80°C.

Table 1. Optimal Condition of Substitution Anisole Moiety

Exp#	Nucleophile (Morality)	Solvent	Temperature/Reaction °C/Time(h)	Yield(%) of 8
1	<i>p</i> -MeOPhLi (0.26 M)	THF	-40°C /1h	37%
2	<i>p</i> -MeOPhLi (0.26 M)	Ether	-20°C/40min	35%
3	<i>p</i> -MeOPhLi (0.26 M)	THF/Ether(1/6)	-20°C/40min	52%
4	<i>p</i> -MeOPhLi (0.64 M)	THF/Ether(1/6)	-20°C/1h	71%
5	<i>o</i> -MeOPhLi (0.61 M)	THF/Ether(1/6)	-20°C/1h	72%
6	<i>p</i> -MeOPhLi (0.86 M)	THF/Ether(1/6)	-20°C/1h	80%

Then, the benzyl ester was cleaved by hydrogenolysis to give β -acid 6.¹³ Catalytic transfer hydrogenation has been shown to be a useful procedure for the removal of benzyl and benzyloxycarbonyl protecting groups in peptide synthesis.¹⁴ The ester 5 and 10% Pd/C in methanol were shaken with hydrogen at 40 psi for 2h. As using hydrogen balloon, the TLC indicated that the reaction was completed for 15h. So, the compound 6 in methanol for long time was slowly converted with a methylester by the side reaction.

The ester 6 was converted to isoxazolidine 7 by treating with *N*-methylmorpholine and isobutyl chloroformate in THF then with a mixture of triethylamine and isoxazolidine hydrochloride in DMF. Applying the procedure of Cupps, the isoxazolidine hydrochloride was prepared.¹⁵ All these reactions were carried at -15°C.

The ketone 8 was prepared from isoxazolidine 7 and *p*-methoxyphenyllithium in 35-71% yield. The best condition has been investigated to improve the yield as shown in Table 1. Using THF and ether, the yields were always poor. Using THF and ether (1:6) and the more concentrated *p*-methoxyphenyllithium we could increase them a little (about 52%). When we used the faster quenching method by 1M Na₂HPO₄ solution at the lower temperature(-20°C), the yield was increased by 71%. For the reaction, using the more concentrated *p*-methoxyphenyllithium solution was very important.

The organometallic reagent *p*-methoxyphenyllithium was prepared from 4-bromoanisole and *n*-butyllithium in THF. The *p*-methoxyphenyllithium was titrated by diphenylacetic acid.

2. Protection of ketoamino acid

For the synthesis of tripeptide **1a,b**, the ketone **8a,b** have to be converted to the free acids in order to couple with isoleucine methyl ester using dry HCl or TFA and the resulting free amines should be protected again before coupling. Using TFA, **8** could be the free acids **19** but protection of the amino group in **19** unexpectedly gave the cyclized imines **20** instead of expected protection of the amines. To avoid this cyclization problem, the ketoaminoester **8a,b** were protected as dithioketals **26a,b** for the synthesis of the tripeptides. Deprotection of the dithiolane could be accomplished under mild conditions which do not oxidize CH₃S substituent of L-methionine later in peptide synthesis.

Due to the poor yields of direct protection of **8a,b** with 1,2-ethanedithiol, **8a,b** were protected as the ketals **24a,b** with 2-methoxy 1,3-dioxane then the ketals were treated with 1,2-ethanedithiol in the presence of BF₃-Et₂O to give the thioketals **26a,b** and Boc cleaved products **25a,b**. In case of *p*-methoxy substituent, **24a** was isolated in 76% yield from the reaction of ketone **8a** with 2-methoxy 1,3-dioxane. Treatment of the ketal **24a** with 1,2-ethanedithiol in the presence of BF₃-Et₂O (80 mol%) gave the thioketal **26a** in 49% yield and the Boc cleaved product **25a** in 43 % yield. While increase of the amount of BF₃-Et₂O (100 mol%) produced the Boc cleaved product **25a** more, reduction to 50 mol% gave less amount of **25a** but gave the unreacted starting material back. For the synthesis of ortho substituent compounds, the ketone **8b** was subjected to the dioxolane to give **24b** in 45% yield and starting material (30%). Such a low yield of this reaction might be addressed to the susceptibility of the product during chromatographic purification. Therefore, crude **24b** which was obtained from the ketone **8b** without purification was reacted with 1,2-ethanedithiol in the presence of BF₃-Et₂O (80 mol%) to give the thioketal **26b** in 41% yield and the Boc cleaved product **26b** in 29% yield from the ketone **8b** (two steps). However, the mixture of **26** and **25** was treated with HCl dissolved in EtOAc to give the salt **27** in 85% yield for para methoxy compound and in 86% yield for ortho methoxy product. Protection of amino group in **27** using di-*tert*-butyl dicarbonate was subjected to give the ketoamino acids **28a,b** in 91-95% yield.

3. Peptide coupling to tripeptide

A solution of **28a,b** and methylimidazole in a mixed solvent of THF and nitromethane (1:1) was added to the cloudy CBMIT solution in nitromethane at 0°C. After 10 min stirring, a solution of isoleucine methyl ester and *N*-methylmorphine in THF was added. The resulting reaction mixture was stirred for 2h at rt to give **29a** in 72% yield and 61% yield for the dipeptide **29b**. The cleavage of the Boc group in the dipeptide **29a** was accomplished in 93% yield by using a solution of HCl in EtOAc. The same condition was subjected to give **30b** in 85% yield.

For the synthesis of tripeptides, dipeptides **30a,b** was coupled with

Boc-Met using our CBMIT peptide coupling reagent. To a freshly made solution of CBMIT in nitromethane at 0°C was added a solution of Boc-Met in a mixed solvent of THF and nitromethane. After 10min stirring a solution of **30** and N-methylmorphine in a mixed solvent of THF and DMF was added. The reaction mixture was stirred for 2h at rt. It was purified by flash column chromatography to give the tripeptide **31** in 56% yield for para and 71% for ortho compound,

To get desired tripeptide **1a,b**, compounds **31a,b** should be converted to the ketones under mild condition and subsequently followed by hydrolysis of the ester functional group to obtain the free acids. Deprotection of the dithiolane in tripeptide **31** was studied by slow addition of the methanol solution of $\text{Hg}(\text{ClO}_4)_2 \cdot 3\text{H}_2\text{O}$ (200 mol%) to the solution of tripeptide **31** in CHCl_3 at rt. The reaction mixture was stirred for 1h at rt then 10% K_2CO_3 was added until pH of the solution was 9. The tripeptide **32** was obtained in 85% yield after normal work up and purification by chromatography. The possible dimethyl acetal tripeptide was not detected significantly even in case of using Hunig base and this was confirmed carefully by ^1H , ^{13}C NMR and combustion analysis. It is also noticeable that Boc cleaved tripeptide **20a** was obtained as reaction time went longer and/or different work up procedure (the reaction mixture being basified with 10% K_2CO_3 aqueous solution before filtration of mercury residue gave **32a** otherwise it was obtained as a mixture of **32a** and **33a**).

Hydrolysis of the tripeptide **33a,b** were accomplished by treatment of the esters with LiOH. To a solution of **32a,b** in 1,4-dioxane was added a solution of LiOH in H_2O , which the ratio of solvents is 1:1. The reaction was completed within 45min at rt to give the free acid **1a** in 96% yield and **1b** in 93% yield respectively. The amount of LiOH used in this reaction was 1600mol% at first, gradually cut down to 400mol% without any deduction of the yield.

Conclusion remarks

In order to discover new useful antiinflammatory agents, a new kind of tripeptide which may retain potentially antiinflammatory activity, was synthesized.

N-BOC-tert-butyl anisole glutamate **8a,b** was synthesized from L-glutamic acid through protection of functional group. **8a,b** was coupled with isoleucine methyl ester for the synthesis of dipeptides **30a,b**, followed to form final tripeptide **1a,b** with Boc-Met.

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