SYNTHESIS OF ESTRONE-OPIOID PEPTIDE ANALOGUE WITH EXPECTED ANALGESIC ACTIVITY

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Abstract

The search for new peptides to be used as analgesics in place of morphine has been mainly directed to develop peptide analogues to have higher biological stability and receptor selectivity.

Therefore extensive researches have been carried out on naturally occurring and synthetic opiates in order to enhance the analgesic potency, so we intended to prepare opioiod peptide analogue linked to estrone which may increase the analgesic effect of this compound. Steric hindrance clearly will be produced by estrone may affect the enzymatic activity on the synthesized analogue and this in turn may enhance the bioavailability of the analogue itself, meanwhile estrone may affect physicochemical properties of the analogue by increasing its lipophilicity and so facilitate its passage through the biological barriers, also estrone may affect receptor binding selectivity of this analogue and decrease the side effect of the original opioid peptide.

The designed analogue is **(estrone-3-O-acyl-tyr-gly-gly-phe-met-OH)**, where this opioid peptide synthesized following the conventional solution method, then linked to estrone by amide linkage and it was characterized using: thin layer chromatography (TLC), melting point, infrared spectroscopy (IR), elemental microanalysis(CHN), optical rotation, amino acid analysis, and hydrogen-nuclear magnetic resonance(^{1}H-NMR).

Keywords: estrone, opioid peptide, analgesia.

Introduction

The endogenous opioid peptides have been studied extensively since their discovery aiming to develop effective drugs for the treatment of pain in humans[1].

The opioid peptides are significantly implicated in antinociceptive processes. Their action is modulated by opioid receptors, widely distributed in the CNS[2]. They belong to the class of G-protein coupled receptors, and divided into three types, mu (μ), kappa (κ) and delta (δ)[3].

One of the major drawbacks of using natural opioid peptides as analgesics is their poor receptor specificity that has been attributed to a number of factors, primarily to the chemical structure and conformation[4].

Beside a partial receptor selectivity, a second important limitation to the use of native peptides as pharmacological tools is their rapid enzymatic degradation[5].

One of the remaining problems preventing the use of opioid peptides as drugs is poor bioavailability, which is due to the low penetration across various biological barriers, especially blood-brain barrier (BBB)[6], which act as a barrier to unnecessary substances, and admits vital nutrients for proper function of the centeral nervous system (CNS) and possess many peptidases enzymes such as aminopeptidase, and arylamidase[7].

Several strategies to manipulate BBB transport processes have been developed including lipidization, chemical modifications of the N-terminal end, coupling of transport with post BBB metabolism and formation of potent neuroactive peptides, use of chimeric peptides in which a non-transportable peptide is chemically linked to a transportable peptide, and binding of circulating peptides to apolipoproteins[8].

Endogenous peptides generally have a short lifetime *in vivo* due to enzymatic biodegradation, and consequently have poor bioavailability in tissues. Therefore, it has been a challenge to improve the stability of an enkephalin while still maintaining its potency and selectivity[9].

The pharmacology of opioid peptids was greatly stimulated by Kosterlitz's discovery of two endogenous pentapeptides Met-enkephalin

and Leu-enkephalin in 1975, where the pharmacological profile of Met-enkephalin Fig.(1) is similar to that of morphine, attempts to utilize this peptide as a therapeutic agent have been hindered by its polarity and metabolic lability[10].

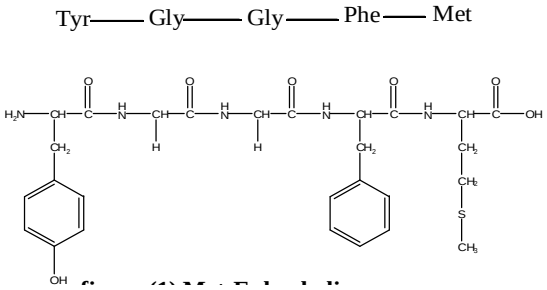


Fig.(1) : Met-Enkephalin.

Estrone Fig.(2) is steroid hormone have estrogenic activity synthesized by the action of aromatase enzymes on testosterone in the ovaries and placenta, and exert its pharmacological activity on the estrogen receptor (ER) which is member of nuclear receptors found intracellulary, and there are of two subtypes (α and β)[11]; Have important role in the menstrual cycle, development of secondary sex characteristics, several metabolic (e.g. anabolic action), and prevent osteoporosis[12].

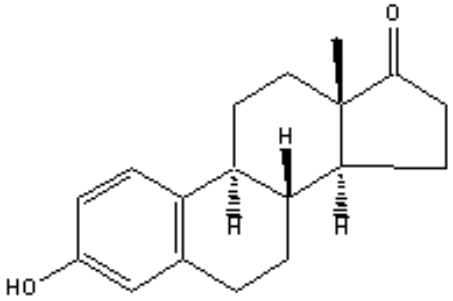


Fig.(2) : Estrone.

As endogenous bioactive substances peptide and steroids play important roles in the normal physiology or disease processes of mammalian systems[13].

The linkers of the steroids and peptides may simulate the permissive action, where the effects of peptides enhanced by steroids through increasing their receptor numbers[14], for example daily administration of 25 mg of estradiol benzoate to adult female rats for 9 days led to an approximately three fold increase in number of pituitary thyrotropin-releasing hormone (TRH) binding site[15], estrogen treatment in vivo lead to an increase sensitivity of the thyroid-stimulating hormone (TSH) contents[16], the interaction between opioid peptides and steroids were observed e.g. kyotorphin (Tyr-Arg-OH) which is isolated from bovine brain with morphine like analgesic effects, its analgesic activity and duration of analgesia significantly enhanced when linked to steroids (e.g., estrone, and hydrocortisone)[13].

In addition to their analgesic effects, endogenous peptides are involved in the regulation of many disorders like stress response[17], of several behavioral and emotional effects, such as dependence, alcohol and other drugs consumption[18]. They are also effective in a large number of physiological functions: gastrointestinal, cardiovascular, respiration and immunological responses, pregnancy, etc [19].

Materials and Methods

Estrone, L-amino acids and Boc-protected L-amino acids are purchased from fluka AG/Switzerland and all solvents are of analar type without need further purification; Melting points determined by Thomas Hoover apparatus England by capillary method, and ascending thin layer chromatography TLC was run on Keisl gel GF 254 type60 Merk, W. Germany to check the progression of reaction and purity. IR spectra were recorded in KBr film FTIR in college of science, university of Baghdad. Elemental microanalysis done by using Carloarba elemental analyzer, amino acids analysis Hitachi amino acid analyzer Model 6-3800, optical rotation performed with Perken Elmer apparatus, and H^1-NMR resolution in deuteriurated dimethyl solfoxide using tetramethyl silane as a standard done by using Bruker spectrophotometer 300 MHz, all done at Cleveland clinical Foundation Learner Research Institute-France.

The final analogue purified by using gel filtration on sephodex LH-20 column eluted with 0.1N acetic acid done in college of pharmacy, university of Baghdad.

Table (1)
Solvents Systems Used in the TLC.

A	Chloroform	Methanol	
	8	2	
$B^{(20)}$	chloroform	Ether	
	97	3	
$C^{(20)}$	benzene	Ethyl acetate	Acetic acid
	90	10	0.5

The protective pentapeptide was prepared by use of Stepwise elongation, in which the amino acids are connected step-by-step via solution method, where dicyclohexyl-carbodiimide (DCC) was used as coupling agent and 1-hydroxybenzotriazole (HOBt) was used as coupling agent and prevents racemization in the peptide formation[21].

Also we use fragment condensation when synthesis long peptides in smaller sections, were later coupled to form the overall peptide[22].

It is desirable to have a range of protecting groups available that can be selectively orthogonally removed, two protecting groups are said to be orthogonal protection when they are removed by totally different classes of reagents (e.g. one by acid and the other by base), the butyloxycarbonyl (BOC) group a widely used protective group that is generally removed from the N-terminal by using moderate-strong acids e.g. triflouro acetic acid (TFA) and is stable under the basic conditions, while the C- terminal protected by methyl ester which removed by using strong base e.g. NaOH and is stable under the moderate-strong acidic conditions [19].

The O-alkylation of estrone with ethyl bromoacetate and sodium ethoxide provided ethyl estrone-3-oxymethylenecarboxylated which treated with 1M KOH and 1M HCL respectively to obtain estrone-3-oxymethylene carboxylic acid which coupled with NH–Tyr-Gly–Gly–phe-met–OMe ester by amide bond in the presence of DCC and HOBt to offer C-terminal protective pentapeptide-estrone, which saponified and acidified by using 1N NaOH, and 1N HCL to give the goal analogue[20,21].

Synthesis

Synthesis of (Gly–OMe ester) (Comp. 1)

A suspension of glycine (8mmol, 600 mg) in absolute methanol, was cooled down to –15 °C then thionyl chloride was added dropwise (8mmol, 0.63 ml). (the temperature should be keep below –10 °C).

The reaction mixture was left at 40°C for three hours, then refluxed for three hours and left at room temperature overnight.

The solvent was evaporated to dryness in vacuum, redissolved in methanol and evaporated. This process was repeated several times to ensure complete removal of excess thionyl chloride.

The residue was collected and recrystallized from methanol-ether[23].

Yield percent, physical appearance, melting point, R_f value, and IR interpretation were listed in Table (2).

Synthesis of (BOC–Tyr–Gly–OMe ester). (Comp. 2)

To a stirred solution of BOC-Tyr (5 mmol, 1.4 g) in DMF, NMM (5mmol, 0.55ml) was added with stirring for 10 minutes. Then (5 mmol, 445 mg) of (Comp. 1) previously dissolved in DMF was also added, and the mixture was cooled down to (–10 °C). HBT (10 mmol, 1.35 g) and DCC (5 mmol, 1.03 g) were added with stirring, which was continued for 2 days at 0 °C and then at room temperature for 5 days. N,N–Dicyclohexyl urea (DCU) was filtered, and washed with ethyl acetate. The filterate was concentrated under vacuum, the residue was mixed with ethyl–acetate, the excess DCU which was still adhesive on the peptide residue was precipitated out and filtered, and the clear filterate washed twice with 5% sodium carbonate solution, 0.1N HCl, once with water, and with saturated sodium chloride solution. The ethyl acetate layer was dried with anhydrous sodium sulphate and evaporated in vacuum.

The resulted product was collected, recrystallized from methanol-ether[23].

Yield percent, physical appearance, melting point, R_f value, and IR interpretation were listed in Table (2).

Synthesis of (Boc-Tyr-Gly-OH). (Comp. 3).

Compound (2) (3 mmol, 1.057 g) was dissolved in minimum volume of dioxane : water (5:1) at 18 °C, 1N NaOH (4.5 mmol, 4.5 ml) was added dropwise over a period of 30 minutes. The reaction was allowed to proceed for additional 3 hours, at a temperature range 18–22 °C, during the course of the reaction the hydrolysis was checked by TLC until the disappearance of the starting material.

The reaction mixture was acidified with 1N HCl (4.5mmol, 4.5ml) in an equivalent amount to that of 1N NaOH and ice-water was added to get a faint yellow precipitate. The peptide was then filtered, dried, and recrystallized from ethyl acetate-ether[23].

Yield percent, physical appearance, melting point, R_f value, and IR interpretation were listed in Table (2).

Synthesis of (BOC–Tyr-Gly –Gly – OMe ester). (Comp.4)

To a stirred solution of (2 mmol, 676 mg) of compound (3) in DMF, NMM (2mmol, 0.22 ml) was added with stirring for 10 minutes, then (2 mmol, 178 mg) of (Comp. 1) previously dissolved in DMF was also added, and the mixture was cooled down to –10 °C. HBT (4 mmol, 540 mg) and DCC (2mmol, 412 mg) were added with stirring, which was continued for 2 days at 0 °C and then at room temperature for 5 days, then continue the work as in the synthesis of compound (2).

Yield percent, physical appearance, melting point, R_f value, and IR interpretation were listed in Table (2).

Synthesis of (BOC–Tyr-Gly –Gly – OH). (Comp. 5)

Compound (4) (1.2 mmol, 491 mg) was dissolved in minimum volume of dioxane : water (5:1) at 18 °C 1N NaOH (1.8 mmol, 1.8 ml) was added dropwise over a period of 30 minutes, the reaction was allowed to proceed for additional 3 hours, at a temperature range 18–22 °C. during the course of the reaction the hydrolysis was checked by TLC until the disappearance of the starting material then continue the work as in the synthesis of compound (3).

Yield percent, physical appearance, melting point, R_f value, and IR interpretation were listed in Table (2).

Synthesis of (Met– OMe ester) (Comp. 6).

A suspension containing (2.5 mmol, 372.5 mg) of methionine in methanol was cooled down to –15 °C, then thionyl chloride (2.5 mmol, 0.2 ml) was added dropwise, then continue the work as in the synthesis of compound (1).

Yield percent, physical appearance, melting point, R_f value, and IR interpretation were listed in Table (2).

Synthesis of (Boc – Phe – Met – OMe ester). (Comp. 7).

To a stirred solution of (2mmol, 326mg) of compound (6) in DMF, NMM (2 mmol, 0.22 ml) was added with stirring for 10 minutes, then (2 mmol, 530 mg) of Boc–Phe, already dissolved in DMF was also added and the mixture was cooled down to –10 °C. HOBt (4 mmol, 540 mg) and DCC (2 mmol, 412 mg) were added with stirring, which was continued for 3days at 0 °C and then at room temperature over 4 days; Then continue the work as in the synthesis of compound (2).

Yield percent, physical appearance, melting point, R_f value, and IR interpretation were listed in Table (2).

Synthesis of (NH_2 – Phe – Met – OMe ester). (Comp. 8).

Compound (7) (1.2 mmol, 493 mg) was dissolved in dichloromethane, then 1.2 ml of 90% solution of TFA in anisole was added dropwise with stirring at 0 °C (the temperature should be kept at this low degree).

The mixture was left for 30 minutes at room temperature, then the completion of the deprotection was checked by TLC.

The solvents were removed under reduced pressure leaving a sticky product which after triturated several times with diethel ether a solid material obtained and was recrystallized from methanol-diethyl ether (3-1)[23].

Yield percent, physical appearance, melting point, R_f value, and IR interpretation were listed in Table (2).

Synthesis of (BOC–Tyr-Gly –Gly –phe-met–OMe ester). (Comp. 9)

To a stirred solution of compound (5) (0.75 mmol, 296.5 mg) in DMF, NMM (0.75 mmol, 0.08 ml) was added with stirring for 10 minutes. Then (0.75mmol, 232mg) of (Comp. 8) previously dissolved in DMF was also added, and the mixture was cooled down to –10°C. HOBt (1.5 mmol, 202.5 mg) and DCC (0.75mmol, 154.5mg) were added with stirring, which was continued for 2days at 0 °C and then at room temperature for 5 days, then the work continue same as synthesis of compound (2).

Yield percent, physical appearance, melting point, R_f value, IR interpretation, elemental microanalysis, amino acids analysis were listed in Tables (2, 3, and 4) respectively.

Synthesis of (NH2–Tyr-Gly –Gly –phe-met–OMe ester). (Comp. 10).

Compound (9) (0.5 mmol, 344 mg) was dissolved in dichloromethane, then 0.5 ml of 90% solution of TFA in anisole was added dropwise with stirring at 0 °C the reaction mixture was stirred for 30 minutes. The N–deprotected pentapeptide was obtained after continue the work as synthesis of compound (8).

Yield percent, physical appearance, melting point, R_f value, IR interpretation, elemental microanalysis, amino acids analysis were listed in Tables (2, 3, and 4) respectively.

Synthesis of ethyl estron-3-O-ylacetate (3-O-carbethoxymethyl estrone) (Comp. 11)

Eestrone (1 mmol, 270 mg) was dissolve in minimum volume of [ethanol: tetrahydrofuran (THF)] (3.5:1) then sodium ethoxide (1.5 mmol; 0.75 ml of 2M in ethanol) and (3 mmol, 500 mg, 0.4 ml) of ethyl bromoacetate were added, the reaction mixture refluxed for 24 hr; An additional portion of sodium ethoxide and ethyl bromoacetate was added and refluxed continued for 2hr and the completion of reaction checked by TLC, the reaction mixture quenched in saturated NaCL, the product isolation by (ethylacetate-THF; $MgSO_4$) after this the solvent evaporated by vacuum and triturate the sticky residue with hexane several times to remove the small amount of contaminated ethyl bromoacetate to get white crystals which recrystalized from acetone-hexane[20].

Yield percent, physical appearance, melting point, R_f value, and IR interpretation were listed in Table (2).

Synthesis of estrone-3-O-ylacetic acid (3-O-Carboxymethylestrone) (comp. 12).

Compound (11) (0.66 mmol, 235 mg) was dissolved in 3.5ml of ethanol and (1 mmol, 1 ml) of a 1 M of potassium hydroxide solution was added, the reaction mixture was stirred overnight at room temperature, then the solvent evaporated, and the residual salt washed with ether. The washed residue was dissolved in water and acidified with 0.1 N of HCL. The product isolation with (ether-THF; $MgSO_4$) to gave crude acid which recrtylized from benzene-ethylacetate[20].

Yield percent, physical appearance, melting point, R_f value, IR interpretation, elemental microanalysis, and ^{1}H-NMR results were listed in Tables (2, 3, and 5) respectively.

Synthesis of estrone-3-O-acyl-tyr-gly-gly-phe-met-O-methyl ester (comp. 13)

To a stirred solution of compound (12) (0.3 mmol, 98.5 mg) in DMF (5ml), NMM (0.33 mmol, 0.04 ml) was added with stirring for 10 minutes. Then (0.3mmol, 176mg) of (Comp. 10) previously dissolved in DMF was also added, and the mixture was cooled down to –10 °C. HOBt (0.6 mmol, 81 mg) and DCC (0.3mmol, 62mg) were added with stirring, which was continued for 2days at 0°C and then at room temperature for 5 days then the work continue as synthesis of compound (2)[13].

Yield percent, physical appearance, melting point, R_f value, IR interpretation, elemental microanalysis, amino acids analysis, and optical activity were listed in Tables (2, 3, and 4) respectively.

Synthesis of estrone-3-O-acyl-tyr-gly-gly-phe-met-OH (comp. 14 final analogue).

Compound (13) (0.2mmol, 180mg) was dissolved in minimum volume of dioxane: water (5:1) at 18 °C 1N NaOH (0.3 mmol, 0.3 ml) was added dropwise over a period of 30 minutes. The reaction was allowed to proceed for additional 3 hours, at a temperature range 18–22 °C. during the course of the reaction the hydrolysis was checked by

TLC until the disappearance of the starting material.

The reaction mixture was acidified with 1N HCl (0.3 mmol, 0.3 ml, in an equivalent amount to that of 1N NaOH) and ice-water was added to get a faint yellow precipitate. The peptide was then filtered, dried, and recrystallized from ethyl acetate-ether[13].

Yield percent, physical appearance, melting point, R_f value, IR interpretation, elemental microanalysis, amino acids analysis, and optical activity were listed in Tables (2, 3, and 4) respectively.

Results and Discussion

The results shown in the tables (2, 3, 4, and 5) indicate that the final analogue was excellently synthesized by the conventional solution method as shown in scheme (1) where we use both stepwise elongation and segment condensation of amino acids sequences to obtain the desired peptide, which then bind to estrone by amide bond to gain the final analogue as shown in scheme (2) that may be increase the analgesic potency and duration of the opioid peptide itself due to permissive action between them[13], also the estrone increase the lipophilicity of the opioid peptide and this will lead more bioavailability, and enzymes resistance.

Table (2)
Yield percent (%), physical appearance, melting points, R_f values, and IR interpretation.

Comp No.	Yield %	Physical Appearance	Found Melting Points °C		Found R_f values	IR interpretation (V cm-1)
			Found	Reported		
1	96	White crystals	173-175	175[24]	0.65[A]	Appearance of C=O stretching of ester at 1751.24 and C-O stretching of ester at 1237.5.
2	75	White needle shape crystals	111-113	106-110[25]	0.94[A]	Presence of C=O stretching of ester at 1751.24, C-O stretching of ester at 1237.5, C=O stretching of urethane at 1689.53,and appearance of C=O stretching of amide at 1643.24.
3	70	White crystals	153-157	155-158[25]	0.72[A]	Disappearance of C=O stretching of ester at1751.24, and appearance of C=O stretching of carboxylic acid at 1700, C=O stretching of urethane at 1650.95, and presence of broad OH stretching at 3325.
4	75	White crystals	110-113	106-110[25]	0.84[A]	Appearance of C=O stretching of ester at 1751, C-O stretching of ester at 1242, C=O stretching of urethane at 1689.3, and C=O stretching of amide at 1666.38.
5	79	White powder	136-138	135-139[25]	0.78[A]	Disappearance of C=O stretching of ester at1751, and appearance of C=O stretching of carboxylic acid at 1720.39, C=O stretching of urethane at 1658, and presence of broad OH stretching at 3332.
6	96	Shinny White needle shape crystals	148-150		0.81[A]	Appearance of C=O stretching of ester at 1743.53 and C-O stretching of ester at 1234.36.

7	78	White needle shape crystals	75-78		0.95[A]	Presence of C=O stretching of ester at 1751.24, C-O stretching of ester at 1249.79, C=O stretching of urethane at 1689.53, and appearance of C=O stretching of amide at 1650.95.
8	72	Off-white powder	65-68		0.87[A]	Presence of C=O stretching of ester at 1751.29, C=O stretching of amide at 1666.38, and appearance primary NH stretching at 3340.48, and disappearance of C=O stretching of urethane at 1689.53.
9	77	Yellowi-sh white crystals	97-99		0.91[A]	Disappearance of primary NH stretching at 3340.48 and appearance of C=O stretching of urethane at 1690, and broad band at 1658.67 for multiple C=O stretching of amide.
10	75	Yellow needle shape crystals	106-109		0.5[A]	Disappearance of C=O stretching of urethane at 1690, broad band at 1647.1 for C=O stretching of amide, presence of C-O stretching of ester at1242.07, and appearance of asymmetric 2 bands of primary NH stretching at 3278.76 and 3325.05.
11	85	White crystals	97-99	98-100[(20)]	0.59[B*]	appearance of C=O stretching of ester at 1765, presence of C=O stretching at1735 for carbonyl group that present in the estrone and disappearance of broad phenolic OH stretching at 3400.
12	70	White crystals	212-214	214-215[(20)]	0.84[A] 0.42[B] 0.38[C**]	Disappearance of C=O stretching of ester at 1765, presence of C=O stretching at 1728.1 for carboxylic acid, and appearance of broad OH stretching at 3301.91.
13	77	Off-white crystals	156-158		0.86[A]	Presence of C=O stretching of ester at 1750, C-O stretching of ester at 1249.79, C=O stretching of amide at 1700, 1658.67 and 1627.81, and secondary NH stretching of amide at 3325.
14	70	Final analogue	147-149		0.65	Disappearance of C=O stretching of ester at1750, and appearance of C=O stretching of carboxylic acid at 1705, and presence of broad OH stretching at 3345.

**** reported 0.58[B(20)]***
***** reported 0.39[C(20)]***

Table (3)
Elemental microanalysis of some intermediates and final analogue.

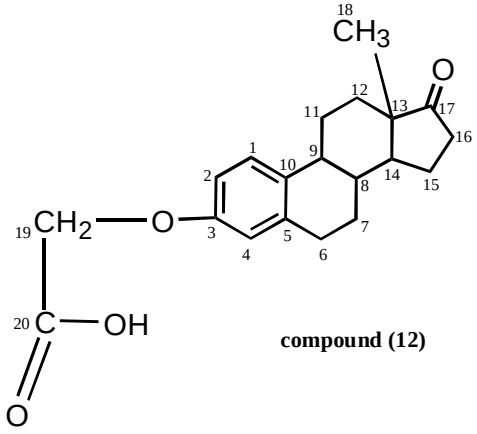
Comp. No	*Chemical formula*	*Calculated/found elemental analysis*				
		C%	**H%**	**N%**	**O%**	**S%**
9	$C_{33}H_{45}N_5O_9S$	57.63 58.21	6.59 6.79	10.18 10.45	20.94 21.39	4.66 4.73
10	$C_{28}H_{37}N_5O_7S$	57.22 57.75	6.35 6.41	11.92 12.38	19.06 19.54	5.46 4.93
12	$C_{20}H_{24}O_4$	73.15 73.61	7.37 7.44	19.49 19.73		
13	$C_{48}H_{59}N_5O_{10}S$	64.19 64.57	6.62 6.77	7.80 8.32	17.82 18.42	3.57 3.90
14 Final analogue	$C_{47}H_{57}N_5O_{10}S$	63.85 64.49	6.50 6.71	7.92 8.37	18.10 18.45	3.63 3.83

Table (4)
Amino acids analysis and optical rotation of some intermediates and final analogue.

Comp. No	*Amino acids*				*Optical rotation* $[\alpha]_{D,}$ *(C=1 in DMF)*
	tyr	*2gly*	*phe*	*Met*	
9	0.92	2.06	1.03	1.10	
13	0.96	2.17	0.98	1.07	+35
14	1.13	2.00	1.09	1.04	+41

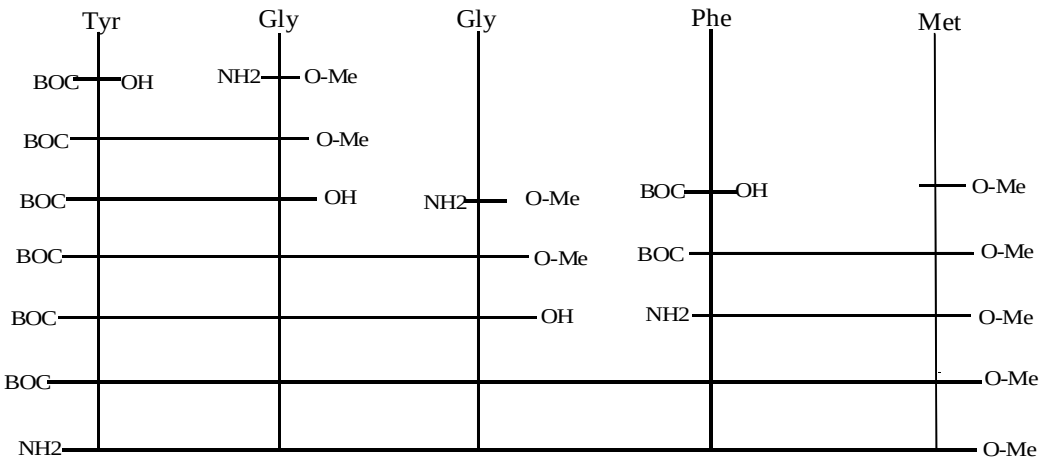
Table (5)

H^1-NMR spectra show the presence of the fallowing characteristic chemical shift for compound (12) as fallows:

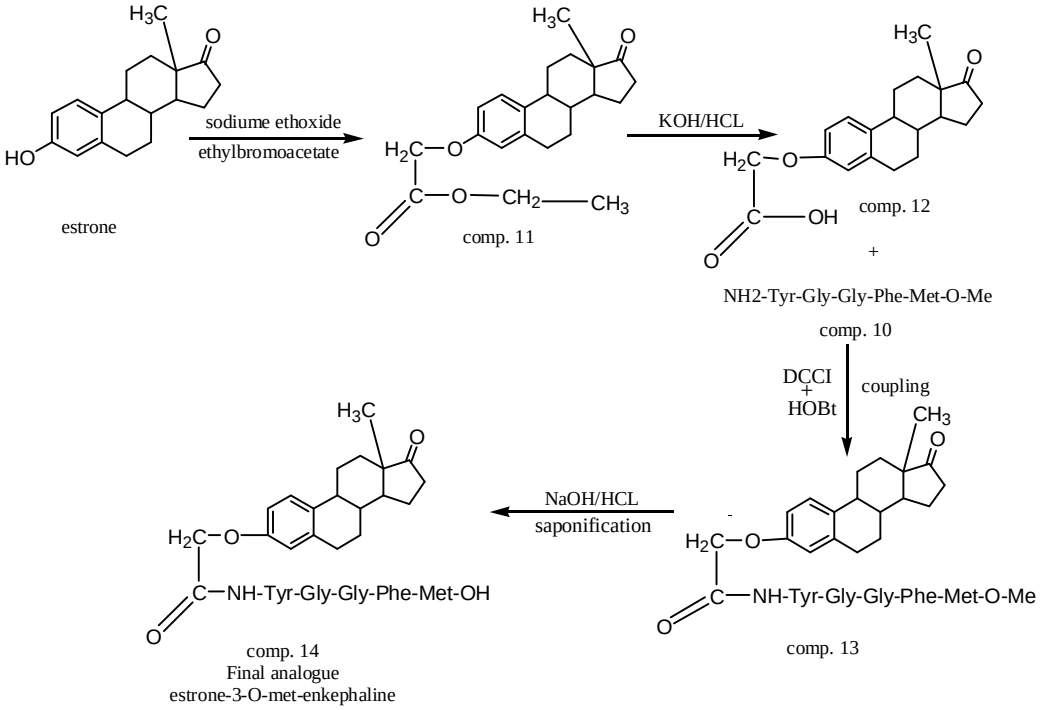


compound (12)

Chemical shift ppm	*Interpretation*
6.84 (doublet aromatic, 1H)	For aromatic C_1
6.4 (doublet aromatic, 1H)	For aromatic C_2
6.45 (singlet aromatic, 1H)	For aromatic C_4
2.9, 2.8 (triplet, 2H)	For C_6
1.68, 1.43 (quartet, 2H)	For C_7
1.57 (pentate, 1H)	For C_8
2.83 (quartet, 1H)	For C_9
1.86, 1.61(quartet, 2H)	For C_{11}
1.76, 1.51(triplet, 2H)	For C_{12}
1.63 (quartet, 1H)	For C_{14}
2.11, 1.86(quartet, 2H)	For C_{15}
2.15, 2.07(triplet, 2H)	For C_{16}
0.92 (singlet, 3H)	For C_{18}
4.63 (singlet, 2H)	For C_{19}
11.2 (broad singlet H, OH)	For carboxylic acid C_{20}



Scheme (1): met-enkephalime-O-me ester (comp.10) synthesis.



Scheme (2): Analogue synthesis.

Conclusion

The estrone-met-enkephaline analogue was successfully synthesized by the conventional solution method and the structure formula of the analogue was consistent with the proposed structure and they proved after get the excellent fallowing techniques: TLC, melting point, IR, CHN, optical rotation, amino acid analysis, and ^{1}H-NMR.

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الخلاصة

البحث عن ببتيدات جديدة لاستخدامها كمسكنات للآلام بدلا من المورفين موجها أساسا لتطوير ببتيدات مماثلة لتمتلك أعلى استقرار أحيائي وأكثر ارتباط انتقائي للمستقبلات. لذلك أجريت بحوث على نطاق واسع تخص الأفيونات المصنعة والطبيعية لزيادة الفعالية المسكنة للألام، لذا فإننا بهدف تحضير ببتيد أفيوني مماثل يرتبط مع الأسترون من أجل زيادة فعالية تسكين الآلام. أن الإعاقة الفراغية التي تنتج عن ارتباط الاسترون يمكن أن تؤثر في فعالية الإنزيمات التي تؤثر على المركب المصنع وهذا بدوره قد يعزز توافره الأحيائي، ومن ناحية أخرى فأن الاسترون قد يؤثر على الخصائص الفيزيوكيمائية للمركب عن طريق زيادة خصائصه ألدهنية وبذلك سيسهل المرور عبر الحواجز الحياتية، كما يمكن للاسترون أن يؤثر على انتقائية الارتباط مع المستقبلات، وتقليل الآثار الجانبية للببتيد الأفيوني.

أن المماثل المخلق في هذه الدراسة هو: **(estrone-3-O-acyl-tyr-gly-gly-phe-met-OH)** وتم تخليقه بأتباع طريقة المحلول التقليدية، وتم ربط الاسترون مع الببتيد الأفيوني من خلال آصرة الأمايد، وتم تمييزه باستخدام: كروماتوكرافيا الطبقة الرقيقة، مقياس درجة الانصهار، مطياف الأشعة دون الحمراء، التحليل الدقيق للعناصر المكونة، تحليل الحامض الأميني، وقياس مطياف الرنين النووي المغناطيسي.