SYNTHESIS OF ADENOSINE MET-ENKAPHALIN CONJUGATE WITH EXPECTED BIOLOGICAL ACTIVITY

Jaafar S. Shia[*] and Kawkab Y. Saour[**]
[*]General Inspector Office, Ministry of Health, Iraq.
[**]Department of Pharmaceutical Chemistry, College of Pharmacy, University of Baghdad, Baghdad-Iraq.

Abstract

In an effort for designing analogue with improved analgesic activity(opioid like activity) or may posses added biological activities as antimetabolite with antineoplastic antibacterial and antiviral, etc., coupled bi natural metabolites (nucleoside peptides) are proposed in this study to synthesize 1′-deoxy-1′-(6-amino-9-purinyl)-2′,3′-o-isopropylidene-B-D-ribfuranuronic acid amide with Tyr-Gly-Gly-Phe-Met-oH. In the hope that such combination have efficient transporting system across membrane, natural penetration inside the cell and nucleus, represent an essential criteria for specifically inducing the analagesic, antiviral, anticancer and also help for exploring other expected biological activities. The analogue was synthesized by applying the conventional solution method and the coupling between peptide and nucleoside adenosine was carried out through amide linkage.Confirmity of the synthetic procedure was achieved by applying different physico-chemical analyses including, thin layer chromatography (T.L.C) and melting point (M.P.), infrared spectroscopy (IR),elemental analysis(CHN analysis), optical rotation, amino acid analysis and NMR.

Keyword: Adenosine, met-Enkaphaline analogue.

Introduction

Opioid is a chemical substance that has a morphine-like action in the body[(1)]. The main use is for pain relief[(2)]. These agents work by binding to opioid receptors, which are found principally in the central nervous system and the gastrointestinal tract[(3)]. The centeral Analgesic activity is mediated by opiate receptors located in the CNS. Five major categories of opioid receptors are known: mu (μ), kappa (κ), sigma (σ), delta (δ), and epsilon (ε). Narcotic[(4)]. [met]-enkephalin is widely distributed in the CNS; [met]-enkephalin is a product of the proenkephalin gene, and acts through μ and δ-opioid receptors. [leu]-enkephalin, also a product of the proenkephalin gene, acts through δ-opioid receptors. The discovery of enkephalins (Hughes 1975) created a new era in which peptides emerged as a novel class of potent analgesics. Soon after their isolation and characterization was reported[(6)], the search for other endogenous peptides possessing opioid activity was further intensified and several of them could be isolated from different mammalian tissues and fluids within a short span of time[(7)]. Almost simultaneously, structure-activity relationship studies on these pentapeptides were undertaken in a number of laboratories, primarily with the object of getting a synthetic peptide that could be utilized as a pain reliever in clinical practice[(8-9)]. Adenosine is a nucleoside composed of a molecule of adenine attached to a ribose sugar molecule (ribofuranose) moiety via a β-N_9-glycosidic bond[(10)]. Adenosine plays an important role in biochemical processes, such as energy transfer-as adenosine triphosphate (ATP) and adenosine diphosphate (ADP) as well as in signal transduction as cyclic adenosine monophosphate, cAMP. It is also an inhibitory neurotransmitter, believed to play a role in promoting sleep and suppressing arousal, with levels increasing with each hour an organism is awake[(11)]. Because of its simple structure there has been an enomrous amount of interest in development of analogues as medicinal agents. Thousands opioid analogues have been synthesized and studied in an attempt to evaluate their structure activity relationship[(12)]. Peptide nucleic acids are oligonucleotide mimics characterised by high chemical and enzymatic stability, high specificity and affinity toward complementary DNA/RNA.

Their solubility in aqueous environment and their ability to cross cell membranes is limited and this will reduce their performance *in vivo* applications. To improve solubility, increase affinity and specificity of binding and to control recognition between nucleic acids, several analogues bearing modifications on the nucleobase, nucleobase-backbone linker and on the backbone were synthesised. This paper describes the synthesis and characterization of Peptide nucleic acid analogues which could be tested in vitro and invivo for ther potential as analgesic, anticancer, antibacterial and antiviral (13), etc.

Materials and Equipments

All solvent and material used were of analar type and used without further purification. the adenosine supplied from sigma Aldrich-Germanay, All amino acids and derivativeswere supplied from sigma Aldrich-Germanay, Fluka-Germanay, Merck-Germanay and BDH-England, Boc-tyrosine. Boc-phenylalanine; were used fully protective supplied.

Melting points were determined by capillary method on Thomas Hoover apparatus (England) and ascending thin layer chromatography was run on Kieslgel GF254 type (60) Merck,for checking the purity of the prepared compounds as well as monitoring the reaction progress. Spots were revealed by reactivity with iodine vapour or irradiation with U.V.light or by ninhydrine[14] spraying reagent 2% in absolute ethanol chromatograms were eluted by the following systems:

A.	Chloroform	Methanol	
	8	3	
B.	Chloroform	methanol	benzene
	8	2	1
C.	Butanol	Acetic Acid	Water
	2	1	1

The final analogue was purfied by using gel filtration on sephadex LH2O column eluted with 0.1 N acetic acid. IR were recorded as KBr film on Shimazu spectrophotometer (Japane). CHN analysis has been done using, H′NMR (resolution at 300 MHz in deteriuarated dimethyl sulfoxide using Tetramethyl silane as astandard showed the following characteristic chemical shift represented in ppm as follows in Table (1). metyl ester was used to protect carboxyl moiety in peptide synthesis.

Methods

Conventional solution method was applied as a coupling method between the protected amino acid for peptide bond formation using Dicyclohexyl carbodiimide DCC [coupling reagent]][15] in the presence of 1-hydroxy benzotriazole [HOBT][16]. The methods as follows*:*

Synthesis of 2′,3′-o-isopropylidene adenosine(comp. 1) scheme.1

(58.4g, 0.308 mol) p-Toluine sulphonic acid with exclusion of moisture was added to a magnetically stirred suspension of adenosine (1) (8.0 g, 30.0 mmole) in anhydrous acetone (1600ml). All solid dissolved immediately after two hours at 25 ^{o}C, The pale yellow solution was added to a vigorously stirred solution of NaHCO3 (53.6 g, 0.64 mmole) in ice and water (640.0 ml). The mixture was evaporated under reduced pressure and the residual solid was collected. Acetone (1000 ml) was added to collected solid, in separated fractions (100 ml). After each addition the mixture was stirred for 30 minutes and left to decant. The acetone layers were collected and concentrated to (20 ml), which up on cooling at 2 ^{o}C overnight gave white crystals.

Yield = 87%, m.p = 219-220 ^{o}C, IR (O –H) is (3741.65 cm^{-1}) broad and (3325-3147.61 cm^{-1}) for asymmetric and symmetric stretching of (N–H) group.(1666.38 cm^{-1}) stretchingfor (C=N) group. (110.92-1072.35 cm^{-1}) stretching or bending for (C–O) group, (2931.60 cm^{-1}) stretching for (C–H) group. (1380 cm^{-1}) stretching for isopropyl group.

Elemental analysis for C13H17N5O4. Calculated C, 50.815; H,5.58; N,22.79; O, 20.83. Found C, 51.10; H, 5.96; N, 23.030;O, 21.010.

Synthesis of 2′,3′-o-isopropylidene adenosine-5′-carboxylica cid (comp. 2) scheme (1)

Compound (1) (4.12 g, 13.4 mmol) was dissolved in warm water (1400ml), the solution was cooled to room temperature, then under steady stirring, KOH (2.24 g, 40 mmol) was added, followed by KMNO4 (8.58, 54 mmol), which was added over a period of 2 hours. The reaction mixture was stirred for 3days at (18 - 20 ^{o}C). Excess KMnO4 was destroyed with H2O2, then filtered to remove MnO2, and the solution was evaporated under vacuum to 150ml, The PH of the solution was adjusted to 4-6 with 10 % HCL at 0 ^{o}C where compound (2) was precipitated out and filtered. Washed with 5% NaHCO3, purification was achieved by re-crystallization from 20 ml methanol: chloroform: ethyl acetate (1:1:2) mixture to obtain acolorless crystalline mass Yield = 86-6% m.p = 275 ^{o}C reported melting point = 276 ^{o}C[17]. Rfc = 0.6,

The IR spectrum show the following absorption bands (3448.49 cm^{-1}) asymmetric stretching for (NH2), (1670-1620 cm^{-1}) stretching for (C=O), (1218.93 cm^{-1}) stretching for (C–O–C).

Elemental analysis for C13H15N5O5. Calculated C, 48.60; H, 4.71; N,21.80; O,24.90. Found : C, 49.01; H, 4.98; N, 22.50; O, 25.70.

Synthesis of 1′-deoxy-1′-(6-ethyloxycarbonylamino-9-purinyl)-2′,3′-o-isopropylidene-B-D-ribofuranuronic acid[18]. (comp.3) scheme(1).

Compound (2) (2 g, 6.2 mmol) was dissolved in water (6 ml), acetone (2 ml), and 2N sodium hydroxide (3 ml). The solution was placed in a (100 ml) three necked flask with a mechanical stirrer and two dropping funnels. The container was chilled in ice bath, ethylchloroformat (0.673 g, 6.2 mmol) and 4N sodium hydroxide (3 ml) were added simultaneously from the dropping funnels to the Vigorously stirred solution over period of 30 minutes. The mixture was stirred for an additional 90 minutes. At the end of the reaction the mixture was extracted with ether. The aqueous layer was isolated and poured into a beaker containing a mixture of crushed ice and concentrated hydrochloric acid (1ml). An oily residue was separated washed with water, dried in an oven at (50-60 ^{o}C) overnight and recrystalizatied from a mixture of acetone: petroleum ether (1:1) to obtain Caramel crystalline shape Yield = 75%, m.p = 224-226 ^{o}C, Rfc = 0.8. The IR spectrum show the following absorption bands 1720 cm^{-1}, 1643.24 cm^{-1} for an ester and amide respectively.

Elemental analysis for C16H19N5O7. Calculated C, 48.85; H, 4.87; N, 17.80; O, 28.47. Found C, 49.47; H, 5.03; N, 18.35; O, 28.97 H′NMR Shown in Table (1).

Synthesis of Glycine-Glycine Methyl Ester[19]. (Gly - Gly - Ome) directly from Gly-Gly (comp.4)

To Gly-Gly (1.17 g, 8 mmol) suspended in methanol (20 ml)was cooled down to -15°C, thionyl chloride (6.19ml,8mmol) was added drop wise with keeping temperature below –10 °C The reaction mixture was kept at 40 °C for 3 hours, followed by refluxing for 3 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, and the residue was collected and recrystallized from methanol-diethyl ether to obtain white crystalline shape Yield = 96%, m.p = 132-134 ^{o}C, RfB = 0.42. The IR spectrum show the following absorption bands 1751.24 cm^{-1}, 1681.81 cm^{-1} for an ester and amide respectively and 1257 cm^{-1} for C–O of the ester.

Synthesis of N-t-Butyloxy Carbonyl Tyrosin-GlycineGlycine-Methyl Ester. (Boc-Tyr-Gly-GIy-OMe, comp.5)

To a stirred solution of BOC–Tyr (1.12 gm, 4 mMol) in DMF (5 ml), NMM (mMole, 0.25 ml) was added with stirring for 10 minutes. Then (Compound 4) (0.585 gm, 4mMol) previously dissolved in DMF (5ml) was also added, and the mixture was cooled down to –10 °C. HOBT (0.54 gm, 4 mMol) and DCCI (0.82 gm, 4 mMole) were added with swirling. Stirring was continued for 7 hours at 0°C and then at room temperature for 7 days. N,N–Dicyclohexyl urea (DCU) was filtered, and washed with ethyl acetate. The filtrate 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 filtrate washed twice with 5% sodium bicarbonate solution, 0.1 N 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 to obtain white crystals, Yield = 85%, m.p = 105-107 ^{o}C, RfA = 0.84. The IR spectrum shows the following absorption bands 1750 cm^{-1}, 1666 cm^{-1} for an ester and amide respectively and 1242 cm^{-1} for C–O of the ester.

Synthesis of N-t-ButyloxyCarbonyl Tyrosine-GlycineGlycine (BOC-Tyr-Gly-Gly), (comp.6)

To a stirred solution of compound 1.5 (0.563 gm, 2 mMol) in dioxin : water (5:1) at 18 °C 1N NaOH (1.2 mMol) was added drop wise 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 equivalent amount of 1 N HCl (1.2 mMol, 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 re-crystallized from ethyl acetate-ether to obtain white crystals, Yield = 80%, m.p. = 137-139 ^{o}C, RfA = 0.78 . The IR spectrum show the following absorption bands disappearance of ester (C=O) absorption at (1750 cm^{-1}) with appearance of (1720cm^{-1}) of carboxylic acid.

Synthesis of methionine methyl ester. (met-ome, comp.7)

A suspension of methionine (0.5968gm, 4mmol) in methanol (15 ml)was cooled down to –15 °C, thionyl chloride (3.1ml,4mmol) was added drop wise with keeping temperature below –10 °C The reaction mixture was worked up as in compound (4) to obtaine white needle crystals shape ,Yield = 96%, m.p =148 –150 ^{o}C, RFA = 0.81. The IR absorption bands show as follow 3286 cm^{-1} for primary amine, 1743 cm^{-1} for (C=O) and 1234 cm^{-1} for (C–O) stretching of ester.

Synthesis of Boc-phenylalanine-methionine methyl ester. (Boc-Ph-met-ome, comp.8)

To a stirred solution of BOC–phenylalanine. (1.592 gm 6 mMol) in DMF (5ml), NMM (0.659ml, 6mMol) was added with stirring for 10 minutes. Then compound (1.7) (0.979 gm, 6 mMol) dissolved in DMF (5 ml) was also added and the mixture was cooled down to –10 °C. HOBT (1.62 gm, 6 mMol) and DCCI (1.17 gm, 6 mMol) were added with swirling and the same procedure for preparation of compound (5) has been done to give compound (8) white crystals needle shape, Yield = 78%, m.p. = 75-78 ^{o}C, RFA =0.95. The IR absorption bands show as follow (1751 cm^{-1}), (1689cm^{-1}), (1650cm^{-1}) for an ester and amide I (C=O), amide II(N-H) respectively and 1249 cm^{-1}, 1172 cm^{-1} for (C–O) stretching of ester.

Synthesis of phenylalanine-methionine methyl ester. (Ph-met-ome), (compound.9)

To compound 1.8(2.5 gm, 6 mMol) in dichloromethane (3 ml), 90% solution of TFA in anisole (2 ml) was added drop wise with continuous 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 off white powder , Yield = 75% , m.p. = 65-68 ^{o}C, RfA = 0.87 .The IR absorption bands show as follow (N–H) stretch at3340 cm^{-1}, (C=O) stretch of ester at 1751 cm^{-1}, of amide band I at 1666 cm^{-1} and of amide II at1550 , (C=C) aromatic at1519 cm^{-1}, 1434 cm^{-1}, (C–H) stretching at 3070 cm^{-1} of aromatic,2939 cm^{-1} stretching of CH3, (C–O) stretching at 1203 cm^{-1}, 1134 cm^{-1}, 1026 cm^{-1}.

Synthesis of BOC- Tyrosne-Glycne-Glycine-phenylalanine-methionin methyl ester. (Boc-met-enkaphaline-ome),(comp.10)

To a stirred solution of BOC–Tyrosine-Glycine-Glycine. (0.679 gm 2 mMol) in DMF (5ml), NMM (0.213 ml, 2 mMol) was added with stirring for 10 minutes. Then compound (1.9) (0.620 gm 2 mMol) dissolved in DMF (5 ml) was also added and the mixture was cooled down to –10 °C. HOBT (0.540 gm, 2 mMol) and DCCI (0.390 gm, 2 mMol) were added with swirling and the same procedure

for preparation of compound (5) has been done to give compound (10), white yellowished crystals Yield = 85%, m.p = 97-99 ^{o}C, RfA = 0.91. The IR absorption bands show as follow absorption bands: (O–H) (3630cm^{-1}) broad and (3325 cm^{-1}) for stretching of (–H) group.(1658 cm^{-1}), (1710) for amide I and ester stretching respectively of (C=O) group. (1242 cm^{-1} 1172 cm^{-1}, 1018 cm^{-1}) stretching for (C–O) group, (2931.60 cm^{-1}) stretching for (C–H), 1512, 1450, stretching aromatic of (C=C). Elemental analysis for C33H45N5O9S. Calculated C, 57.04; H, 6.43; N, 10.39; O, 21.37; S, 4.76. Found C, 58.21; H, 6.79, N, 10.54; O, 21.39; S, 4.93.

Synthesis of Tyrosne-Glycne-Glycine-phenylalanine-methionin methyl ester. (H2N-met-enkaphaline-ome, (comp.11$^{(24)}$)

To compound (10) (1.377 gm, 2 mMol) in dichloromethane (5 ml), 90% solution of TFA in anisole(3ml) was added drop wise with continuous stirring at 0 °C and the reaction continued as in compound (9) to obtained white yellowish crystals Yield = 75%, m.p = 106-109 ^{o}C, RfA = 0.5. The IR absorption bands show as follow absorption bands(O-H)stretch at 3749 cm^{-1} (N–H) stretch at3325 cm^{-1} , (C–H) stretch at2931 cm^{-1} , (C=O) stretch of ester at 1880 cm^{-1}, of amide band I at 1674 cm^{-1} and of amide II at1512 cm^{-1}, (C=C) aromatic at 1512 cm^{-1}, 1450 cm^{-1} (C–H) bending at 1388 cm^{-1}, (C–O) stertching at 1242 cm^{-1}, 1180 cm^{-1}, 1026 cm^{-1}.

Elemental analysis for C28H37N5O7S. C, 57.22; H, 6.35; N, 11.92; O, 19.06;S, 5.46. Found C, 57.75; H, 6.410; N, 12.380; O, 19.540; S, 4.930.

Synthesis of 1′-deoxy-1′-(6-ethyloxycarbonylamino-9-purinyl)-2′,3′-o-isopropylidene-B-D-ribofuranuronic acid amide with Tyrosine-Glycine-Glycine-phenylalanine-methionin methyl ester. (met-enkaphaline). (comp.12) scheme(3).

To a stirred solution of comp.1. 3 (1.89 gm 2 mMol) in DMF (5 ml), NMM (0.22 ml, 2 mMol) was added with stirring for 10 minutes. Then compound (1.11) (1.175 gm 4 mMol) dissolved in DMF (5 ml) was also added and the mixture was cooled down to –10 °C. HOBT (0.540 gm, 2 mMol) and DCCI (0.390 gm, 2 mMol) were added with swirling, the reaction mixture was treated as in compound (5) to gave as white yellowish crystals Yield = 83%, m.p = 152-155 ^{o}C, RfA = 0.72. The IR absorption bands show as follow absorption bands (O–H)stretch at 3600-3420 cm^{-1} (N–H) stretch at 3332 cm^{-1}, (C–H) stretch at2931 cm^{-1}, 2854 cm^{-1}, (C=O) stretch of ester at 1750 cm^{-1}, of amide band I at 1666 cm^{-1} and of amide II at1627 cm^{-1}, (C=C) aromatic at 1519 cm^{-1},1442 cm^{-1} (C–H) bending at 1350cm^{-1}, (C–O) stretching at 1218 cm^{-1}, 1180 cm^{-1}, 1026 cm^{-1}.

Elemental analysis for C44H54N10O13S Calculated C, 54.88; H, 5.65, N, 14.54; O, 21.60; S, 3.33. Found C, 55.39; H, 5.77; N, 14.96; O, 22.02, S, 3.49

Optical rotation $[\alpha]^{25}D = +44^{o}$.

Synthesis of 1′-deoxy-1′-(amino-9-purinyl) - B-D-ribofuranuronic acid amide with Tyrosine-Glycine-Glycine- phenylalanine-methionin methyl ester(comp13).

Compound (12), (1.06 gm, 1.1 mmol) was dissolved in formic acid (4 ml), the reaction mixture was stirred for 20 hours at room temperature, when the excess of formic acid was removed by evaporation under residual pressure, a thick oily residue was obtained which solidified up on, standing. The solid material was re-crystallized from ether to gave compound (13) as white crystals Yield = 80%, m.p = 168-172 ^{o}C, RfA = 0.85. The IR absorption bands show as follow absorption bands Disappearance of isopropyl group (1380 cm^{-1}), appear (3325 cm^{-1}) broad stretching for primary amine (N–H) and (O–H).

Elemental analysis for C38H46N10O11S Calculated C, 53.64; H, 5.45; N, 16.46; O, 20.68; S, 3.7. Found C, 54.01; H, 5.65; N, 16.73; O, 21.11; S, 4.07

Optical rotation$[\alpha]^{25}D = +45^{o}$.

Symthesis of 1′-deoxy-1′-(amino-9-purinyl) - B-D-ribfuranuronic acid amide with Tyrosne-Glycne-Glycine- phenylalanine-methionin-OH(comp.14)

To a stirred solution of compound 1.13 (0.85 gm, 1 mMol) in dioxane : water (5:1) at 18 ^{o}C 1N NaOH (1.2 mMol, 1.2 ml) was added

dropwise over a period of 30 minutes. The reaction was allowed to proceed as in compound (6) to obtain white crystal Yield = 70%, m.p = 165-167 ^{o}C, RfA = 0.76. The IR absorption bands show as follow absorption bands IR spectrum revealed the disappearance of (1750 cm^{-1}) for ester and the appearance of (1710 cm^{-1}) for carboxylic acid.

Elemental analysis for C37H44N10O11S Calculated C, 53.10; H, 5.30; N, 16.74; O, 21.03;S, 3.83. Found C, 53.64; H, 5.47; N, 17.090; O, 21.490; S, 4.123.

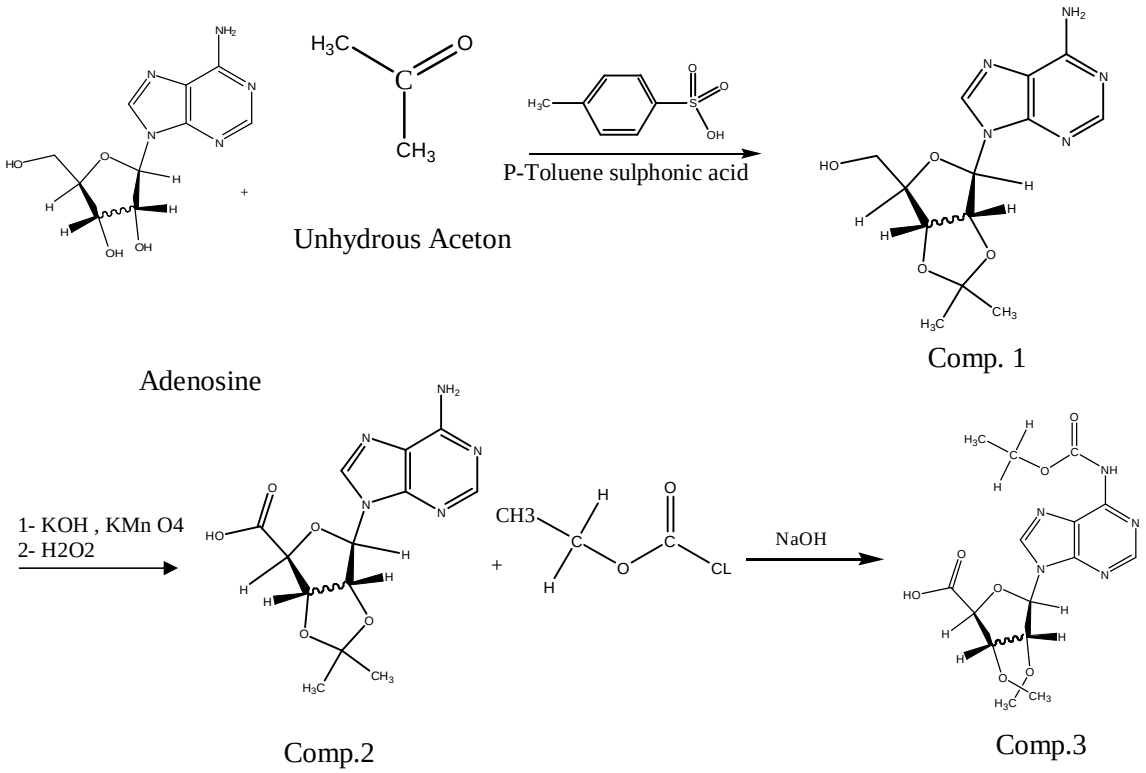
Results and Discussion

The methodology that has been followed to synthesize proposed analogue seems to be convenient concerning protection, deprotection, coupling methodology recrystalization processe, purification by gell filtration as indicated from data obtained by applying different physico-chemical techniques as mansioned earlier and shown in the method like M.P, TLC, and IR, Elemental analysis (CHN) and optical rotation in, and Amino acid analysise (AAA) of Boc-Tyr-Gly-Gly-phe-Met-ome,have been applied to add conformity to the success of the synthetic procedure. Here we would like to focus attention on formic acid as shown to be practically effective hydrogen donor for rapid removal of peptide benzyl and benzyloxycarbony protecting groups by catalytic transfer by hydrogenation[22], also it has been shown that formic acid in methanol causes rapid removal of N-benzyl or benzyl oxycarbonyl protecting group in a good yield 91%[23]. Accordantly formic acid was used in this study for deprotection of the ethyl chloroformat on N^6 group of compound (3) and for deprotecte of 2′,3′-isoprpyl. So, the synthetic approaches according to the step wise manner of deprotection synthesis proved to be effective for the success of the synthetic of homogeneous analogues as indicated from analytical techniques M.P., TLC., optical rotation, CHN, IR, and NMR. *for 2′,3′-o-isopropylidene adenosine(comp.1)* protection 2′,3′ hydroxylgroup of sugar by convertion to the cyclic isopropyl (1,3 dioxan) to prevent their participation in the reaction, the mechanism of comp.1 is shown in scheme.1 *for the 2′,3′-o-isopropylidene adenosine-5'-carboxylica cid (comp.2)*

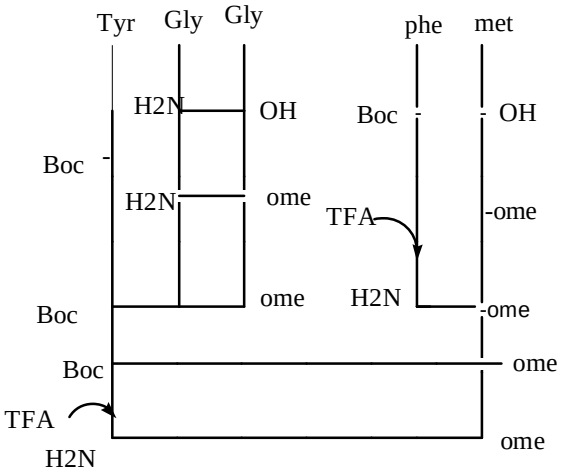
The oxidation of the 5′-hydroxyl of compound (1) by using KMnO4 in alkaline medium to prepare 5′-carboxylic acid derivative[20] compound (2), is shown in scheme.1. Compound (2) was used in preparation of the amide derivative later compound (13). *for 1′-deoxy-1′-(6-ethyloxycarbonylamino-9-purinyl)-2′,3′-o-isopropylidene-B-D-ribofuranuronicacid.(3)* N^6 Protection of compound (3) was achieved by using ethyl chloroformat in alkalin medium to prevent their participation in the reaction (6-amino group in amid linkage formation). Amino acid analysis (AAA) of protected methionin-enkaphalin-ome (Boc-Tyr-Gly-Gly-ph-met-ome) as shown:

Tyr	Gly- Gly	Phe	Methionine
0.92	2.06	1.03	1.10

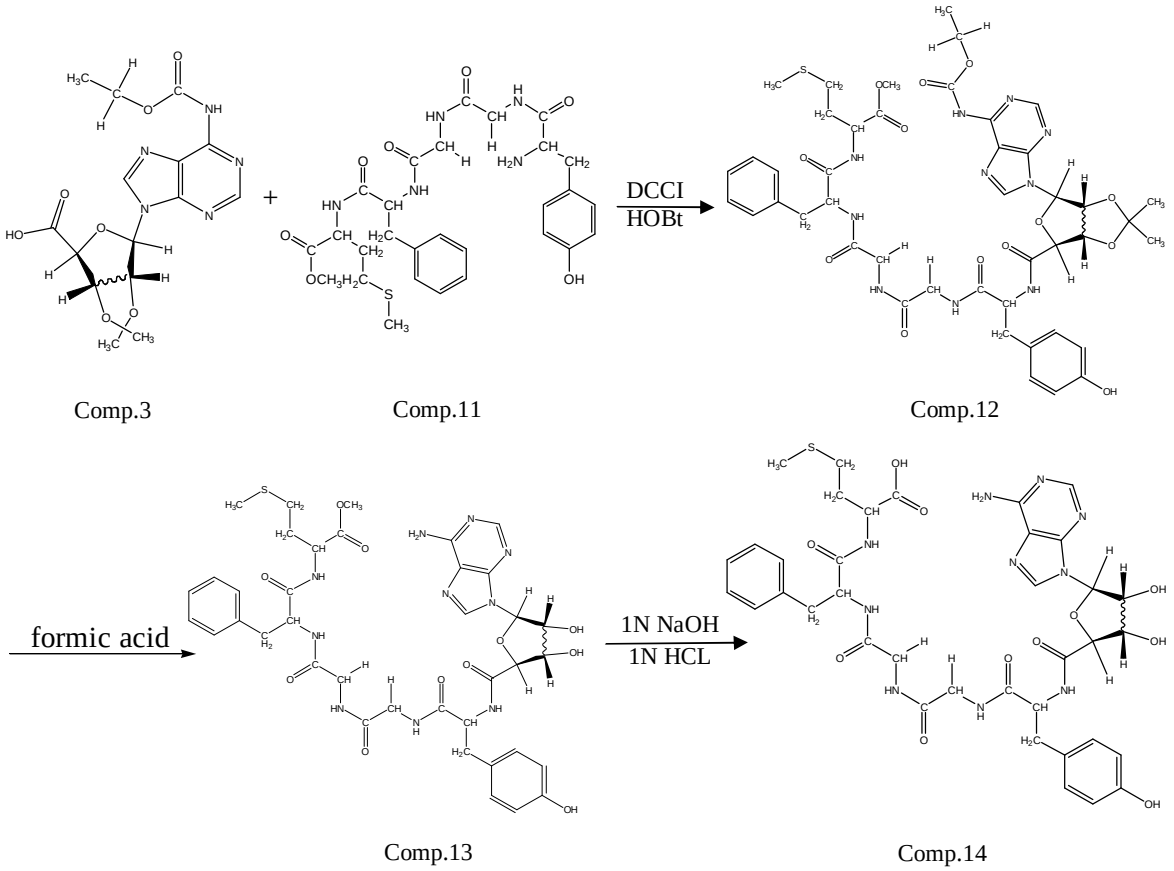
Finally, we hope that the incorporation of such structure modification which might inhibit metabolic activation of enkaphaline or enhance ther receptor binding affinity lead to an analogue that shows high order of potency after systemic administration.



Scheme (1) Synthesis of compound 1, 2 and 3.



Scheme (2) Synthesis of Met-enkaphaline methyl ester.

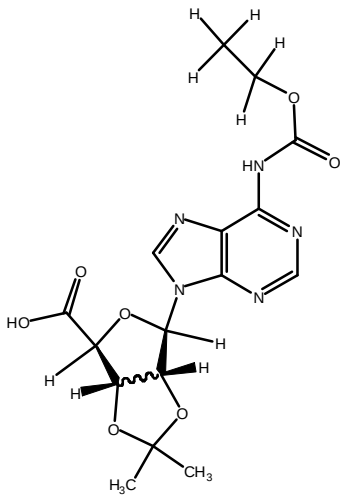


Comp.3 Comp.11 Comp.12

Comp.13 Comp.14

Scheme (3) Coupling Adenosine with met – enkaphaline by amide linkage.

H′NMR comp. 1.3 showed the following characteristic chemical shift represented in ppm as follows in Table (1).



Chemical shift	*characteristics*
1.9	Singlet for each CH3
3.36	1 H for $H^{5'}$
3.43–3.48	Multiplied, doublet $H^{5'}$
4.37	Doublet for $H^{4'}$
3.39	doublet $H^{3'}$
3.39	doublet for $H^{2'}$
7.9	singlet 1 H forNH
6.06	double 1 H for H
δH = 8.12	singlet C–H of purine
δH = 8.7	Singlet C–H of purine
δH = 11.2	Singlet COOH
δH = 3.6	Singlet for CH2

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الخلاصة

في محاولة لتصميم مماثلات للانكيفالينات ذات فعالية مسكنة عالية اضافة الى احتمالية امتلاكها فعاليات بايولوجية اخرى كمضادات ايضية لعلاج بعض انواع السرطان والبكتريا والفايروسات وقد اعتمت هذه الدراسة والتي تتضمن تخليق مركب ذات طبيعة بايلوجية مزدوجة (بيبتيد,نيكليوسايد) من خلال التأصر الاميدي بينهما.

1′-deoxy-1′-(6-amino-9-purinyl)-2′,3′-o-isopropylidene-B-D-ribfuranuronic acid amide with Tyr-Gly-Gly-Phe-Met-oH

من المومل ان مثل هذه المركبات قد تنتقل بشكل كفوء عبر الاغشية او تخترق الخلية والنواة بشكل طبيعي وهذه تعتبر مواصفات اساسية لاظهار الفعالية البايلوجية المحتملة كمسكنات او مضادات للسرطان والبكتريا والفايروسات وقد تساعد ايضا في الكشف عن فعاليا ت بايلوجيةاخرى تم تخليق المماثل (analogue) باستخدام طرق المحلول التقليدي ثم التوصل الى الخواص المميزة للمركب المحضر وبرهنة صحة التحضير من خلال التقنيات الفيزياوية كيمياوية مثل كروماتوغرافيا الطبقة الرقيقة (TLC)، ودرجة الإنصهار (m.p)، والتحليل الطيفي بالأشعة تحت الحمراء (IR)، وتناوب البصرية (optical rotation)، وتحليل العناصر (CHN analysis) وتحليل الأحماض الأمينية. (amino acid analysis) ومطياف الرنين النووي المغناطيسي (NMR).