Bulgarian Chemical Communications, Special Issue E, (pp. 31 – 34) 2017

Synthesis and characterization of new endomorphin analogs with N-terminal phosphonate

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Received October 10, 2016; Revised January 18, 2017

Endomorphins are small endogenous neuropeptides that are produced by the body and act to reduce pain. They are tetrapeptides with the highest known affinity and selectivity for the μ-opioid receptor. This report refers to the synthesis and characterization of novel endomorphin analogues containing phosphonate moiety. The new endomorphins with N-terminal phosphonate were prepared using solid phase peptide synthesis by Fmoc chemistry. The phosphonate moiety was incorporated by modification of Kabachnik-Fields reaction. The crude neuropeptides were purified on a reversed-phase high-performance liquid chromatography and the molecular weights were determined, using electrospray ionization mass-spectrometry, and also determining of the specific angles of optical rotation.

Keywords: Opioid peptides; Endomorphin analogues; Peptide synthesis

INTRODUCTION

The numerous endogenous opioid peptides (endomorphins, enkephalins, nociceptin, etc.) and the exogenous opioids (such as morphine) exert their effects through the activation of receptors belonging to four main types: μ, δ, κ and ε. The endomorphins are a group of endogenous opioid peptides consisting of endomorphin-1 (H-Tyr-Pro-Trp-Phe-NH_2) and endomorphin-2 (H-Tyr-Pro-Phe-Phe-NH_2). They are tetrapeptides with the highest known affinity and selectivity for the μ-opioid receptor. Since chronic pain is notoriously difficult to treat using currently available therapeutics, the development of analgesics represents a major pharmaceutical objective. Endogenous opioids are intensively and extensively studied in search for a new powerful analgesic, lacking the adverse effects of most alkaloid opioids. Some of the greatest achievements in medicine in theoretical and in clinical aspect are connected with the research on pain and especially on the development of analgesic drugs [1-6].

Phosphonopeptides are phosphorus analogues of naturally occurring peptides containing a tetrahedral phosphorus atom. Their importance is obvious from the fact that they have been widely used as enzyme inhibitors and, as happens in catalytic antibody research, because they can be considered as stable mimetics of tetrahedral transition states in ester and amide hydrolysis and formation [7-10]. To date, several efficient synthetic routes have been developed for synthesis of phosphonopeptides and phosphinopeptides, containing C-terminal α-aminoalkylphosphinic acids [11-10]. As part of our research, the synthesis, the characterization and the biological activity of new series of small peptides with aminophosphonates moiety as NOP receptor ligands, have previously been described [13-14].

Herein, we report the synthesis and characterization of novel endomorphin analogues containing phosphonate moiety. The new endomorphins with N-terminal phosphonate were prepared using SPPS by Fmoc (9-fluorenylmethoxy-carbonyl) chemistry. The phosphonate moiety was incorporated by modification of Kabachnik-Fields reaction. All of the newly synthesized peptide was C-terminal amides. It has been determined that the peptides with C-terminal amide group are more resistant to enzyme degradation and that their conformation suits better the interaction with μ-opioid receptors [15,16].

RESULTS AND DISCUSSION

The new endomorphin analogues with N-terminal phosphonate shown in Table 1 were prepared with good yield by solid phase synthesis using TBTU, an efficient peptide-coupling reagent. Rink-amide resin was used as a solid-phase carrier. All coupling reactions were performed, using for amino acid/TBTU/HOBt/DIEA/resin a molar ratio of 3/2.9/3/6/1. A 20%-piperidine solution in N,N-dimethylformamide (DMF) was used to remove the Fmoc group at every step. The phosphonate moiety was incorporated by modification of Kabachnik-Fields reaction. The peptidyl-resin with deprotected amino group, as amino-component, was treated with paraformaldehyde or benzaldehyde, as

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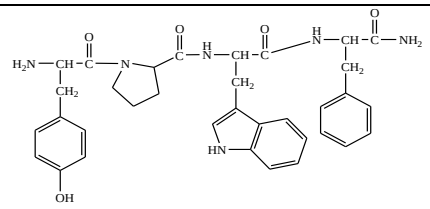
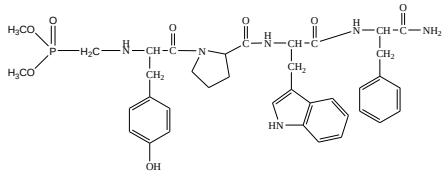
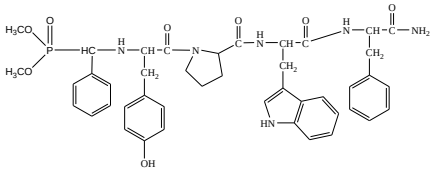
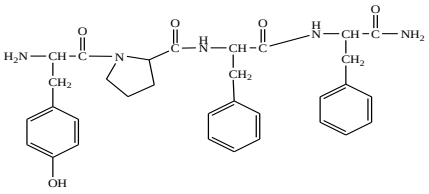
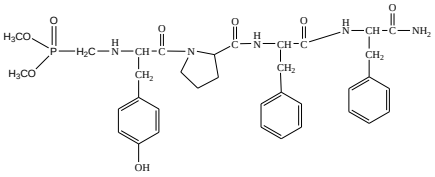
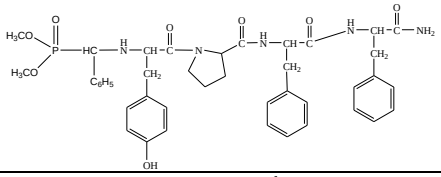
carbonyl component, in the presence of triethylamine in methanol and dimethyl hydrogen-H-phosphonate. With both the aliphatic and aromatic aldehyde the reaction conditions were the same. The reaction mixtures, monitored by Kaiser test, were stirred at 65-70°C for 7 h and gave the expected endomorphin analogues containing phosphonate moiety **1-6**.

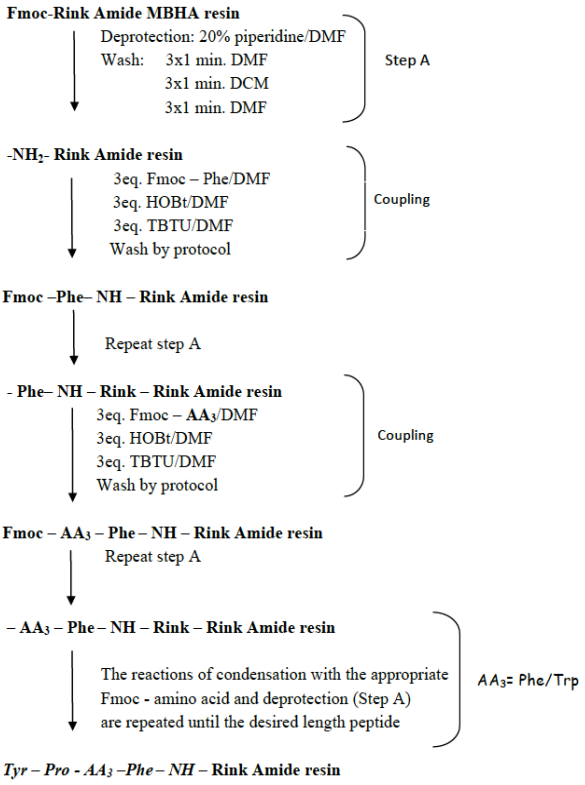
The coupling and deprotection reactions were checked by the Kaiser test. The cleavage of the synthesized peptide from the resin was done, using a mixture of 95 % trifluoroacetic acid (TFA), 2.5 % triisopropylsilan (TIS) and 2.5 % water.

After filtration of the exhausted resin, the solvent was concentrated in vacuum and the residue triturated with cool ether. The purity of the peptides was checked by RP-HPLC, column: SymmetryShield™ RP-18, 3.5μ, (50 x 4.6mm), flow: 1 ml/min, H_2O (0.1 % TFA)/CH_3CN (0.1 % TFA), gradient 0→100 % (15 min) and 100 % (4 min). The crude peptides were purified using semi-preparative HPLC, column XBridge™ Prep C18 10μm (10 x 250mm), flow: 5ml/min, H_2O (0.1 % TFA)/CH_3CN (0.1 % TFA), gradient 20→70 % (20 min). The ESI mass spectra were recorded with a platform II quadrupole mass spectrometer fitted with an electrospray source. Optical rotations were measured with a Perkin Elmer 341 polarimeter.

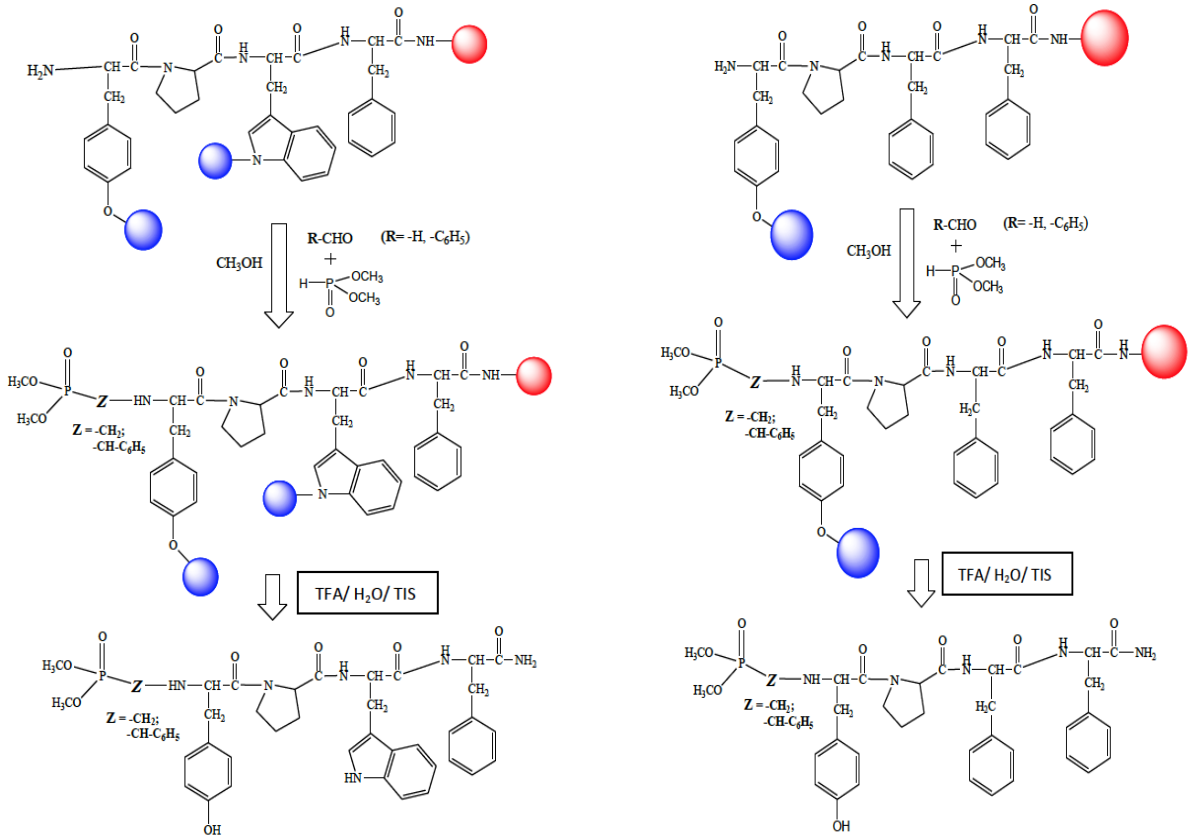
Table 1. Analytical data of synthetic peptides.

№	Peptides	$[\alpha]_D$[a] (°)	[b] $t_{R,\,min}$	[c] ESI MS: $(MH)^+$ calculated	found
1		-29	6.17	610.3	611.2
2		-26	7.12	732.3	733.7
3		-28	7.53	808.8	809.6
4		-31	4.58	571.6	572.2
5		-27	6.20	693.7	694.7
6		-32	7.05	769.8	770.3

[a] Optical rotation in methanol (c = 1) at 20 °C; [b] t_R is the retention time determined by HPLC; [c] The mass ion (MH^+) was obtained by electrospray mass spectrometry.



Scheme 1. Solid Phase Peptide Synthesis of endomorphin analogues.



Scheme 2. SPPS of endomorphin-1 and endomorphin-2 analogues.

The synthetic routes for preparation of new endomorphin analogues are shown in Scheme 1 and Scheme 2.

CONCLUSION

We describe the synthesis and characterization of novel N-modified analogues of endomorphin-1 and endomorphin-2 with phosphonate moiety. The newly synthesized compounds were obtained by solid-phase peptide synthesis - Fmoc-strategy. The phosphonate moiety was incorporated by modification of Kabachnik-Fields reaction. The neuropeptides were purified on an RP-HPLC and the molecular weights were determined, using ES-MS, and also determining of the specific angles of optical rotation. The biological trials are in the progress.

Acknowledgements: *We gratefully acknowledge the financial support by University of Chemical Technology and Metallurgy (UCTM), Sofia, Bulgaria, and the Scientific Research Center at UCTM - contract 11607/2016.*

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СИНТЕЗ И ОХАРАКТЕРИЗИРАНЕ НА НОВИ *N*-МОДИФИЦИРАНИ АНАЛОЗИ НА ЕНДОМОРФИНИТЕ С ФОСФОНАТЕН ОСТАТЪК

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Постъпила на 07 октомври, 2016 г.; Коригирана на 18 януари, 2017 г.

(Резюме)

Ендоморфините са малки ендогенни опиоидни пептиди, които се произвеждат от организма и действат за намаляване на болката. Те са тетрапептиди с най-висок афинитет и селективност към μ-опиоидния рецептор. В статията ние описваме синтеза и охарактеризирането на нови ендоморфинови аналози, съдържащи фосфонатна група. Новите *N*-модифицирани аналози на ендоморфините, с фосфонатен остатък бяха получени с помощта на твърдофазен пептиден синтез. Фосфонатният остатък беше въведен чрез модификация на реакцията на Кабачник-Филдс. Невропептидите бяха пречистени с помощта на обратно-фазова високоефективна течна хроматография, бяха определени и молекулните им маси, използвайки електроспрей йонизационна масспектрометрия, а също така и специфичните ъгли на оптично въртене.