molbank

MDPI

Short Note

Sodium *N*-(3,5-Bis(ethoxycarbonyl)-2,6-dimethyl-1,4-dihydropyridine-4-carbonyl)-L-methioninate

Egils Bisenieks [1], Janis Poikans [1], Aiva Plotniece [1], Eiva Bernotiene [2], Wei-Bor Tsai [3] and Arkadij Sobolev [1,*]

1 Latvian Institute of Organic Synthesis, Aizkraukles 21, Riga LV-1006, Latvia; egils.bisenieks@osi.lv (E.B.); jpasts@inbox.lv (J.P.); aiva@osi.lv (A.P.)
2 State Research Institute Centre for Innovative Medicine, Santariskiu 5, LT-08406 Vilnius, Lithuania; eiva.bernotiene@imcentras.lt
3 Department of Chemical Engineering, National Taiwan University, No.1, Sec. 4, Roosevelt Rd., Taipei 10617, Taiwan; weibortsai@ntu.edu.tw
* Correspondence: arkady@osi.lv; Tel.: +371-67014928

Received: 19 May 2020; Accepted: 11 July 2020; Published: 14 July 2020

Abstract: The development of the methods for amide bond formation is important for various uses in the laboratory and industrial applications. The compounds combined in their structures 1,4-dihydroisonicotinic acids and amino acids linked with an amide bond can be considered as "privileged structures" due to their broad range of biological activities. Herein, the formation of amide bond between 1,4-dihydroisonicotinic acid and L-methionine is reported. The coupling of L-methionine with pentafluorophenyl active ester of 1,4-dihydroisonicotinic acid appears to be a convenient and effective method for amide bond formation. Sodium *N*-(3,5-bis(ethoxycarbonyl)-2,6-dimethyl-1,4-dihydropyridine-4-carbonyl)-L-methioninate has been successfully synthesized via a procedure where the key step is amide formation from 5-diethyl 4-(perfluorophenyl) 2,6-dimethyl-1,4-dihydropyridine-3,4,5-tricarboxylate and L-methionine. Sodium salt formation was performed to improve physicochemical properties, such as solubility of the L-methionine-derived 1,4-dihydroisonicotinamide. The obtained target compound was fully characterized by UV, IR, ^{1}H NMR, ^{13}C NMR, MS, and microanalysis.

Keywords: 1,4-dihydropyridine; 1,4-dihydroisonicotinic acid; L-methionine

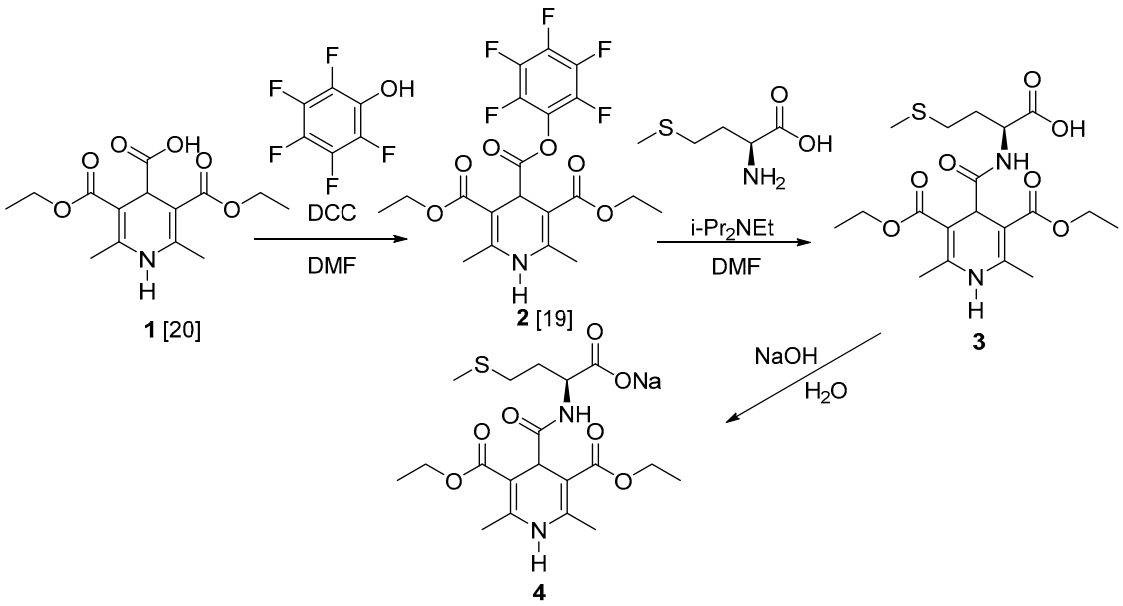
1. Introduction

Many representatives from the 1,4-dihydropyridine (1,4-DHP) class belong to the important class of calcium antagonists which act on the L-type Ca^{2+} channels [1,2]. Currently, interest is growing toward the activities that are not related with calcium channel blocking one. For example, the amino acid-containing 1,4-DHP–disodium salt of 2-(2,6-dimethyl-3,5-diethoxycarbonyl-1,4-dihydropyridine-4-carboxamido) glutaric acid (glutapyrone), in contrast with classical 1,4-DHPs, lacks calcium antagonistic activity and exhibits a range of biological activities such as neuromodulatory and neuroregulatory [3–5]. The compounds of this type remain relatively unexplored in respect to biological activities. Biological properties of 1,4-dihydroisonicotinic acid derivatives modified with taurine [6,7], γ-aminobutyric acid (GABA) [8], and β-alanine [7] were also studied by several research groups. It has been also demonstrated that taurine and L-proline promote and maintain the chondrogenesis [9,10]. In addition to the involvement of amino acids in protein synthesis, they may have protective functions on cells as efficient osmolytes. The group of 1,4-DHPs, having in their structures chemically bound amino acids, may be used also as chondrogenesis stimulating molecules. Synthetically the derivatizations of 1,4-dihydroisonicotinic acids can be approached by several methodologies [7,11,12]. Activation of the carboxylic acid groups is among the most essential

measures which have to be done in any peptide synthesis protocol, where one of the options is use of pentafluorophenol [13], Pentafluorophenyl esters are quite hydrophobic and relatively stable against hydrolysis in aqueous media, therefore this group is frequently used for coupling reactions with minimum side reactions [13,14]. Recently, 4-carbamoyl-1,4-dihydropyridines were used for direct carbamoylation of (hetero)aryl bromides under photoredox-nickel dual catalysis [15]. At present, a series of new 1,4-dihydroisonicotinic acids modified with various amino acids are being developed for further studies of their biological properties. Herein, we report previously unpublished synthesis of 3,5-bis(ethoxycarbonyl)-2,6-dimethyl-1,4-dihydropyridine-4-carboxylic acid with the L-methionine moiety as a substituent on carbonyl at position 4. Selection of L-methionine can be justified by its antioxidative properties [16], and also the hepatoprotective [17] and radioprotective properties [18] of its derivatives.

2. Results and Discussion

The target product was obtained by procedure where the key step is amide formation from 5-diethyl 4-(perfluorophenyl) 2,6-dimethyl-1,4-dihydropyridine-3,4,5-tricarboxylate (**2**) [19] and L-methionine (Scheme 1). First, the activation of carboxylic group of 1,4-dihydroisonicotinic acid **1** [20] was performed with pentafluorophenol in the presence of dicyclohexylcarbodiimide (DCC) in DMF according to the previously elaborated methodology [19]. The exchange of perfluorophenyl substituent with L-methionine to produce amide bond was performed in DMF in the presence of diisopropylethylamine to 48 h give L-methionine-derived 1,4-dihydroisonicotinamide **3** with 62% yield. The corresponding sodium salt **4** was obtained by the treatment of 1,4-dihydroisonicotinamide **3** with NaOH in 80% yield (Scheme 1). Salt formation was performed to improve physicochemical properties, such as solubility of L-methionine-derived 1,4-dihydroisonicotinamide **3**.



Scheme 1. Synthesis of sodium *N*-(3,5-bis(ethoxycarbonyl)-2,6-dimethyl-1,4-dihydropyridine-4-carbonyl)-L-methioninate (**4**).

The structures of the obtained compounds **3** and **4** are confirmed by NMR data (spectra are included in the Supplementary Materials). The ^{1}H NMR spectra demonstrated the characteristic singlets of 2,6-methyl groups as two individual peaks with chemical shifts at 2.22 and 2.20 ppm for compound **4** and one singlet of the same 2,6-methyl groups with a chemical shift at 2.21 ppm for compound **3**, while the signals of the methyl groups of the ester moieties at 3,5 positions of 1,4-dihydroisonicotinic acid derivatives as two signals with chemical shifts 1.23 and 1.19 ppm for

compound **4** and one signal as multiplet with the maximum of chemical shift around 1.21 ppm for compound **3**.

3. Materials and Methods

All reagents were purchased from (Geel, Belgium), Sigma-Aldrich/Merck KGaA (Darmstadt, Germany), or Alfa Aesar (Lancashire, UK) and used without further purification. TLC was performed on silica gel 60 F254 aluminium sheets 20 × 20 cm (Merck KGaA). Melting points were recorded on an OptiMelt digital melting point apparatus (Stanford Research Systems, Sunnyvale, CA, USA) and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded at 400 MHz (^{1}H) and 100 MHz (^{13}C) operating frequencies with a Bruker Avance Neo 400 MHz (Bruker Biospin Gmbh, Rheinstetten, Germany). Chemical shifts of the hydrogen and carbon atoms are presented in parts per million (ppm) and referred to the residual signals of partially deuterated DMSO-d_6 (δ: 2.50) solvent for ^{1}H NMR spectra and DMSO-d_6 (δ: 39.5) solvent for ^{13}C NMR, respectively. Coupling constants, *J*, were reported in hertz (Hz). Low-resolution mass spectra (MS) were determined on an Acquity UPLC system (Waters, Milford, MA, USA) connected to a Waters SQ Detector-2 operating in the ESI positive ion mode on a Waters Acquity UPLC BEH C18 column (1.7 μm, 2.1 × 50 mm, using gradient elution with acetonitrile (0.01% formic acid) in water (0.01% formic acid). Infrared spectra were recorded with a Prestige-21 FTIR spectrometer (Shimadzu, Kyoto, Japan). Elemental analyses were determined on an Elemental Combustion System ECS 4010 (Costech International S.p.A., Milano, Italy) at Laboratory of Chromatography of Latvian Institute of Organic Synthesis.

3.1. N-(3,5-Bis(ethoxycarbonyl)-2,6-dimethyl-1,4-dihydropyridine-4-carbonyl)-L-methionine (**3**)

To a stirred solution of 3,5-diethyl 4-(perfluorophenyl) 2,6-dimethyl1,4-dihydropyridine-3,4,5-tricarboxylate (**2**, 5.56 g, 12 mmol) in dry DMF at 0 °C diisopropylethylamine (2.1 mL, 12 mmol) and L-methionine (2.1 g, 14 mmol, as a powder) were added, after which a resulting suspension was stirred for 48 h at room temperature. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in ethyl acetate, washed with a solution of $NaHSO_4$ (5%) and water, then evaporated and dried in vacuum to give a pale yellow crystalline product (3.18 g, 62%) with mp 130–140 °C (decomposition). IR ν_{max} (KBr) 3397, 3297, 3226, 2978, 2924, 1718, 1705, 1659. UV λmax (lg ε): 233 (4.25), 358 (3.79) nm (water). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.78 (br. s, 1H), 8.84 (s, 1H), 7.05 (d, *J* = 7.9 Hz, 1H), 4.41 (s, 1H), 4.29–4.24 (m, 1H), 4.11–4.06 (m, 4H), 2.39–2.32 (m, 2H), 2.21 (s, 6H), 2.05–1.94 (m, 1H), 2.00 (s, 3H), 1.87–1.78 (m, 1H), 1.23–1.19 (m, 6H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 173.0, 172.4, 167.0, 166.9, 147.1, 147.0, 97.2, 97.1, 59.4, 50.8, 40.7, 40.2, 31.2, 29.2, 18.4, 18.3, 14.6, 14.3, 14.2. MS (ESI$^+$) *m*/*z* 429 ([M + H]$^+$, 100). Anal. calc. for $C_{19}H_{28}N_2NaO_7S$: C, 53.26; H, 6.59; N, 6.54; found: 53.10; H, 6.94; N, 6.88.

3.2. Sodium N-(3,5-Bis(ethoxycarbonyl)-2,6-dimethyl-1,4-dihydropyridine-4-carbonyl)-L-methioninate (**4**)

To a stirred suspension of (3,5-bis(ethoxycarbonyl)-2,6-dimethyl-1,4-dihydropyridine-4-carbonyl)-L-methionine (**3**, 4.28 g, 10 mmol) in distilled water (10 mL) a solution of NaOH (1 N, 10 mmol) was added at room temperature, and the mixture was stirred until the starting material dissolved (5 min), after which a clear solution was evaporated under reduced pressure. The residue was crystallized from ethanol to give a pale yellow crystalline product (3.6 g, 80%) with mp 130–140 °C (decomposition). IR ν_{max} (KBr) 3289, 3227, 3096, 2982, 2916, 3080, 1704, 1675, 1608. UV λmax (lg ε): 231 (4.22), 359 (3.77) nm (water), ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.05 (s, 1H), 7.12 (d, *J* = 5.9 Hz, 1H), 4.39 (s, 1H), 4.09–4.04 (m, 4H), 3.67 (q, *J* = 5.2 Hz, 1H), 2.29–2.17 (m, 2H), 2.22 (s, 3H), 2.20 (s, 3H), 1.94 (s, 3H), 1.92–1.84 (m, 1H), 1.79–1.69 (m, 1H), 1.23 (t, *J* = 7.1 Hz, 3H), 1.19 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 173.2, 171.2, 166.9, 166.8, 146.9, 146.5, 97.9, 97.4, 59.3, 59.2, 53.7, 40.7, 40.0, 32.3, 29.2, 18.3, 18.2, 14.6, 14.2. MS (ESI$^+$) *m*/*z* 429 ([(M − Na) + 2H]$^+$, 100); 451 ([M + H]$^+$, 5). Anal. calc. for $C_{19}H_{27}N_2O_7SNa$: C, 50.66; H, 6.04; N, 6.22; found: 50.16; H, 6.00; N, 6.20.

Supplementary Materials: ^{1}H NMR: ^{13}C NMR, IR, and LC-MS spectra of compounds **3** and **4** are available online.

Author Contributions: E.B. (Eiva Bernotiene) and W.-B.T. prepared and revised the manuscript; E.B. (Egils Bisenieks) and J.P. synthesized compounds **1** and **2** and analyzed spectral data; A.P. and A.S. synthesized compounds **3** and **4**, and recorded and analyzed spectral data. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the project entitled as "Development of injectable biomimetic hydrogels for engineering of cartilage tissue" financed by Mutual Funds Taiwan–Latvia–Lithuania, grant number LV-LT-TW/2020/1.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Sharma, V.K.; Singh, S.K. Synthesis, utility and medicinal importance of 1,2- & 1,4-dihydropyridines. *RSC Adv.* **2017**, *7*, 2682–2732. [CrossRef]
2. Godfraind, T. Calcium channel blockers in cardiovascular pharmacotherapy. *J. Cardiovasc. Pharmacol. Ther.* **2014**, *19*, 501–515. [CrossRef] [PubMed]
3. Misane, I.; Klusa, V.; Dambrova, M.; Germane, S.; Duburs, G.; Bisenieks, E.; Rimondini, R.; Ögren, S.O. "Atypical" neuromodulatory profile of glutapyrone, a representative of a novel "class" of amino acid-containing dipeptide-mimicking 1,4-dihydropyridine (DHP) compounds: In vitro and in vivo studies. *Eur. Neuropsychopharmacol.* **1998**, *8*, 329–347. [CrossRef]
4. Klusa, V. Atypical 1,4-dihydropyridine derivatives, an approach to neuroprotection and memory enhancement. *Pharmacol. Res.* **2016**, *113*, 754–759. [CrossRef] [PubMed]
5. Ošiņa, K.; Rostoka, E.; Sokolovska, J.; Paramonova, N.; Bisenieks, E.; Duburs, G.; Sjakste, N.; Sjakste, T. 1,4-Dihydropyridine derivatives without Ca^{2+}-antagonist activity up-regulate Psma6 mRNA expression in kidneys of intact and diabetic rats. *Cell Biochem. Funct.* **2016**, *34*, 3–6. [CrossRef]
6. Dzirkale, Z.; Pupure, J.; Rumaks, J.; Svirskis, S.; Vanina, M.; Mezhapuke, R.; Sile, V.; Fernandes, M.A.; Duburs, G.; Klusa, V. Comparative study of taurine and tauropyrone: GABA receptor binding, mitochondrial processes and behaviour. *J. Pharm. Pharmacol.* **2011**, *63*, 230–237. [CrossRef] [PubMed]
7. Borovic, S.; Tirzitis, G.; Tirzite, D.; Cipak, A.; Khoschsorur, G.A.; Waeg, G.; Tatzber, F.; Scukanec-Spoljar, M.; Zarkovic, N. Bioactive 1,4-dihydroisonicotinic acid derivatives prevent oxidative damage of liver cells. *Eur. J. Pharmacol.* **2006**, *537*, 12–19. [CrossRef] [PubMed]
8. Pilipenko, V.; Narbute, K.; Amara, I.; Trovato, A.; Scuto, M.; Pupure, J.; Jansone, B.; Poikans, J.; Bisenieks, E.; Klusa, V.; et al. GABA-containing compound gammapyrone protects against brain impairments in Alzheimer's disease model male rats and prevents mitochondrial dysfunction in cell culture. *J. Neurosci. Res.* **2019**, *97*, 708–726. [CrossRef] [PubMed]
9. Yao, X.; Huang, H.; Li, Z.; Liu, X.; Fan, W.; Wang, X.; Sun, X.; Zhu, J.; Zhou, H.; Wei, H. Taurine Promotes the Cartilaginous Differentiation of Human Umbilical Cord-Derived Mesenchymal Stem Cells In Vitro. *Neurochem. Res.* **2017**, *42*, 2344–2353. [CrossRef] [PubMed]
10. Shoulders, M.D.; Raines, R.T. Collagen Structure and Stability. *Annu. Rev. Biochem.* **2009**, *78*, 929–958. [CrossRef] [PubMed]
11. Sobolev, A.; Franssen, M.C.R.; Poikans, J.; Duburs, G.; de Groot, A. Enantioselective lipase-catalysed kinetic resolution of acyloxymethyl and ethoxycarbonylmethyl esters of 1,4-dihydroisonicotinic acid derivatives. *Tetrahedron Asymmetry* **2002**, *13*, 2389–2397. [CrossRef]
12. Sobolev, A.; Franssen, M.C.R.; Duburs, G.; De Groot, A.E. Chemoenzymatic synthesis of enantiopure 1,4-dihydropyridine derivatives. *Biocatal. Biotransformation* **2004**, *22*. [CrossRef]
13. Ramapanicker, R.; Baig, N.B.R.; De, K.; Chandrasekaran, S. One-pot protection and activation of amino acids using pentafluorophenyl carbonates. *J. Pept. Sci.* **2009**, *15*, 849–855. [CrossRef] [PubMed]
14. Hermanson, G.T. The Reactions of Bioconjugation. In *Bioconjugate Techniques*; Elsevier: Amsterdam, The Netherlands, 2013; pp. 229–258.
15. Alandini, N.; Buzzetti, L.; Favi, G.; Schulte, T.; Candish, L.; Collins, K.D.; Melchiorre, P. Amide Synthesis by Nickel/Photoredox-Catalyzed Direct Carbamoylation of (Hetero)Aryl Bromides. *Angew. Chem. Int. Ed.* **2020**, *59*, 5248–5253. [CrossRef] [PubMed]

16. Zeitz, J.O.; Kaltenböck, S.; Most, E.; Eder, K. Effects of L-methionine on performance, gut morphology and antioxidant status in gut and liver of piglets in relation to DL-methionine. *J. Anim. Physiol. Anim. Nutr.* **2019**, *103*, 242–250. [CrossRef] [PubMed]
17. Wunsch, E.; Raszeja-Wyszomirska, J.; Barbier, O.; Milkiewicz, M.; Krawczyk, M.; Milkiewicz, P. Effect of *S*-adenosyl-L-methionine on liver biochemistry and quality of life in patients with primary biliary cholangitis treated with ursodeoxycholic acid. A prospective, open label pilot study. *J. Gastrointest. Liver Dis.* **2018**, *27*, 273–279. [CrossRef] [PubMed]
18. Li, J.; Cai, Y.; Chen, W.; Liu, X.; Lin, L.; Feng, X. Highly enantioselective fluorination of unprotected 3-substituted oxindoles: One-step synthesis of BMS 204352 (MaxiPost). *J. Org. Chem.* **2012**, *77*, 9148–9155. [CrossRef] [PubMed]
19. Poikans, J.; Tirzitis, G.; Bisenieks, E.; Uldrikis, J.; Gurevich, V.S.; Mikhailova, I.A.; Duburs, G. The derivatives of 2,6-dimethyl-1,4-dihydroisonicotinic acid and their antiplatelet properties. *Eur. J. Med. Chem.* **1994**, *29*, 325–328. [CrossRef]
20. Dubur, G.Y.; Uldrikis, Y.R. Preparation of 3,5-diethoxycarbonyl-2,6-dimethyl-1,4-dihydroisonicotinic acid and 3,5-diacetyl-2,6-dimethyl-1,4-dihydroisonicotinic acid and their salts. *Chem. Heterocycl. Compd.* **1972**, *5*, 762–763. [CrossRef]