Synthesis of New Lipophilic Phosphonate and Phosphonamidate Analogues of N-Acetylmuramyl-L-alanyl-D-isoglutamine Related to LK 423

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Abstract: A syntheses of three new muramyl dipeptide (MDP) analogues related to LK 423 as potential immunomodulators are presented. The dipeptide part of the lead compound was modified by introducing a phosphonamide isostere instead of the amide bond between L-alanine and D-glutamic acid (or D-isoglutamine), yielding new MDP analogues 5 and 9. Furthermore, the amide bond between L-Ala and D-Glu was replaced by a phosphonate isostere, giving peptidyl phosphonate 14. The scope and limitations of the synthetic strategies employed are discussed.

Keywords: Muramyl dipeptide, analogues, phosphonamidates, phosphonates, immunomodulators.

Introduction

Bacterial cell wall components like proteoglycans, lipopolysaccharides and lipoproteins possess strong immunostimulating activities. Since 1974 N-acetylmuramyl-L-alanyl-D-isoglutamine (muramyl dipeptide, MDP, Figure 1) has been known as the smallest immunologically active fragment of bacterial cell wall peptidoglycan [1]. As MDP is one of the most potent immunostimulants, many of its derivatives and analogues have been synthesized and evaluated biologically in order to obtain new
molecules with improved pharmacological properties [2]. Recently the lipophilic MDP derivative \( N^2 - [N-(acetylmuramyl)] - L - alanyl-D-isoglutaminyl] - N^6 - stearoyl-L-lysine \) (rumortide) was introduced for the treatment of radiotherapy-induced leukopenia [2,3]. While most of the MDP analogs synthesized so far possess an intact dipeptide L-Ala-D-Glu-NH\(_2\) or L-Ala-D-Glu moiety, it has been generally accepted that the N-acetyl-D-glucosamine fragment is not essential for the immunomodulating activity of this class of compounds [4-6]. Replacement of the N-acetylmuramyl moiety with various acyl groups thus represents an important approach in the design and synthesis of new immunologically active MDP analogues, as demonstrated by FK-156 [7], pimelautide [8], 7-oxoacyl-L-alanyl-D-isoglutamines [9], some carbocyclic MDP analogues [10,11], and by the adamantyl-substituted MDP analogue LK 415 [12].

Figure 1

In the search for new lipophilic MDP analogues some phthalimido desmuramyl dipeptides were synthesized whose N-acetylmuramic acid part was replaced by different N-phthaloylated amino acids [13,14]. The most promising compound in this series was LK 423 (Figure 1), which exhibited some interesting immunomodulating activities. It was found to augment the capacity to produce interleukin-10 in the spleen cells of cyclophosphamide-treated mice [15], and it alleviated the dextran sulfate sodium-induced colitis in rodents [16]. LK 423 is thus a candidate substance to be developed as an anti-inflammatory pharmaceutical agent [16]. The compound was also able to stimulate the production of tumor necrosis factor in \textit{in vitro} phorbol 12-myristate 13-acetate and ionomycin-stimulated cultures of human peripheral blood mononuclear cells [17].

Recently we have been interested in the synthesis of new MDP analogues related to LK 423. To obtain more information about structure-activity relationships, we modified the peptide backbone of phthalimido desmuramyl dipeptides by introducing various phosphorus-containing species. We replaced the amide bond at the end of the acyclic side chain by phosphonamidate ethyl ester [18] and by the phosphinamide moiety [19]. We have also replaced the amide bond between Ala and Glu by phosphonamidate methyl ester [19], and the \( \gamma \)-carboxylic group of Glu by diethyl phosphonate isostere [20]. Stimulated by the results of preliminary immunological tests of selected phosphorus MDP analogues [17], we present the syntheses of three new phosphapeptides related to LK 423, whose amide bonds between L-alanyl and D-glutamate moieties are replaced by phosphonamidate and
phosphonate bonds, respectively. In order to increase the lipophilicity of novel desmuramyl dipeptides, the target compounds are in the form of either methyl or benzyl esters.

**Results and Discussion**

The synthesis of the phosphonamidate MDP analogue 5 was carried out from methyl (1R,S)-1-(N-benzyloxycarbonyl)aminoethyl phosphonate (1) [21] according to Scheme 1. Methyl D-isoglutaminate hydrochloride (2) was prepared from D-glutamic acid as described for the corresponding benzyl ester [22]. Monomethyl phosphonate 1 was coupled with compound 2 using diphenylphosphorylazide (DPPA) as a coupling reagent, giving a protected phosphadipeptide 3 in a moderate, but satisfactory yield. We were unable to couple compounds 1 and 2 using the oxalyl chloride method, the most commonly employed method in the synthesis of phosphonamidates [23], probably due to poor solubility of hydrochloride 2 in dichloromethane. The Z protecting group was removed by catalytic hydrogenation in a Parr hydrogenator and the free amine obtained was used immediately in the coupling reaction with 2-(2-phthalimidoethoxy)acetic acid (4) [24], affording the target phosphonamidate 5.

**Scheme 1**

The lipophilic phosphonamidate MDP analogue 9 was prepared according to Scheme 2. The starting compound 1 was hydrogenated over Pd/C to remove the Z protecting group, and the 9-fluorenylmethoxycarbonyl (Fmoc) group was introduced to give methyl (1R,S)-1-(N-(9-fluorenylmethoxycarbonyl)amino)ethyl phosphonate (6) [25]. Monomethyl phosphonate 6 was then coupled with dibenzyl D-glutamate p-toluenesulfonate [26] using the acid chloride method [23] to yield the triple-protected phosphadipeptide 7. After selectively removing the Fmoc protecting group with diethylamine the phosphonamidate 9 was obtained by coupling of the free amine with 5-phthalimido-pentanoic acid 8 [27].
In order to prepare the phosphonamidate muramyl dipeptide analogue 10 that closely resembles LK 423, we wanted to remove the benzyl protecting groups of the D-Glu moiety. However, catalytic hydrogenation of compound 9 over Pd/C in methanol yielded a heterogeneous mixture, from which we were able to isolate only phosphonic acid 11, recently synthesized by us from 5-phthalimidopentanoic acid and phosphonoalanine [28]. It is well known that phosphonamidates are unstable under acidic conditions [29]. To overcome this problem, the use of so called »capped« phosphonamidates (phosphonamidate esters), reported to be stable in acidic aqueous media, was suggested [30]. However, in our hands phosphonamidate methyl ester 9 decomposed even during mild catalytic hydrogenation. We could observe similar decomposition during our efforts to either hydrolytically or acidolytically deprotect closely related phosphonamidate methyl esters, bearing methyl, ethyl or tert-butyl protection on the C-terminal D-Glu residue [19]. Hence, we can conclude that the stability of phosphonamide bond depends strongly on the chemical structure of phosphonamidate ester pseudopeptide under investigation. The target phosphonate MDP analogue 14 was synthesized according to previously described strategies for the assembly of phosphapeptides [31].

Mixed phosphonate 13 was obtained after condensation of monomethyl phosphonate 1 with (2S)-2-hydroxyglutaric acid dimethyl ester (12) [32] in the presence of the BOP reagent [33]. Catalytic hydrogenation in a Parr apparatus furnished the free amine, which was finally acylated with 2-(2-phthalimidoethoxy)acetic acid (4) (Scheme 3).
In all syntheses we used readily available racemic methyl phosphonoalaninate 1 as a starting material. It is well known that during the preparation of either phosphonamidate or phosphonate bond a racemic mixture is formed on new stereogenic centre – phosphorus atom [23]. Hence, all target compounds were syntetized as mixtures of four diastereomers. In Figure 2 all diastereomers of the target phosphonate 14 are presented.

Conclusions

In summary, we present the synthesis of three new phosphorus desmuramylidipeptides related to LK 423. Efforts to separate mixtures of diastereomers and to evaluate each isomer in an in vitro immunological test are underway and will be published elsewhere. The immunological activities of these compounds will provide important information about the effects of the replacement of the planar peptide bond between Ala and Glu moieties with tetrahedral phosphonate and phosphonamidate esters to the activity of the series of phthalimido desmuramylidipeptides.
Experimental

General

All reagents and solvents were of commercial grade and used as such. Melting points were determined on a Reichert hot stage microscope and are uncorrected. Optical rotations were measured on a Perkin-Elmer 1241 MC polarimeter using a 1 dm cell. Elemental C, H, N analyses were performed at the Faculty of Chemistry and Chemical Engineering, University of Ljubljana, on a Perkin-Elmer elemental analyzer 240 C. IR spectra were obtained using a Perkin-Elmer FTIR 1600 instrument from KBr peletted samples. Mass spectra were obtained with a Micromass AutospecQ mass spectrometer using FAB ionization. NMR spectra were obtained on a Bruker Avance DPX 300 instrument. $^1$H-NMR spectra were obtained at 300.13 MHz with tetramethylsilane as an internal standard and $^{31}$P-NMR spectra at 121 MHz using $\text{H}_2\text{PO}_4$ as an external standard.

Methyl N-[[[(1R,S)-1-(N-(benzyloxy carbonyl)amino)ethyl]methoxyphosphinyl]-D-isoglutaminat (3). To a stirred solution of methyl (1R,S)-1-(N-(benzyloxy carbonyl)amino)ethyl phosphonate (1) (3.50 g, 12.8 mmol) and methyl D-isoglutaminat hydrochloride (2) (2.52 g, 12.8 mmol) in dry DMF (40 mL), diphenylphosphorylazide (DPPA) (3.3 mL, 15.4 mmol) and triethylamine (Et$_3$N) (3.92 mL, 28.2 mmol) were added at 0 °C. Stirring was continued for 2 h at 0 °C and overnight at room temperature (r.t.). EtOAc (200 mL) was added and the solution extracted successively with 10% citric acid, H$_2$O, saturated NaHCO$_3$ solution, H$_2$O, and saturated NaCl solution (50 mL each). The organic phase was dried (anh. MgSO$_4$) and evaporated in vacuo. The oily residue was purified by column chromatography on silica gel, eluting with 5:1 CHCl$_3$ – MeOH to give the desired product 3 as a white powder. Yield: 25 %; m.p.: 171-172.5 °C; IR (KBr, cm$^{-1}$) 3412.1, 2947.7, 1740.1, 1692.0, 1665.6, 1537.7, 1440.2, 1324.9, 1253.9, 1212.8, 1106.4, 1045.0, 923.5, 798.6, 668.1; $^1$H-NMR (DMSO-d$_6$) $\delta$ (ppm) = 1.2 (dd, J=7.3 Hz, J=16 Hz, 3H, CH$_3$), 1.70-1.88 (m, 2H, CH$_2$-β-Glu-NH$_2$), 2.28-2.39 (m, 2H, CH$_2$-γ-Glu-NH$_2$), 3.4-3.58 (m, 6H, 2OCH$_3$), 3.60-3.75 (m, 1H, CH), 3.75-3.92 (m, 1H, CH), 4.55-4.85 (m, 1H, PNH), 5.03 (s, 2H, PhCH$_2$), 7.06 (s, 1H, CONH$_2$), 7.30-7.50 (m, 7H, C$_6$H$_5$ + CONH$_2$ + NH); $^{31}$P-NMR (DMSO-d$_6$) $\delta$(ppm) = 31.62, 32.35; $[\alpha]^{20}_D$ =+16.58 (c=0.41, MeOH); FAB-MS m/z 416 (M+H)$^+$; Anal. Calc. For C$_{17}$H$_{25}$N$_3$O$_7$P: C, 49.16; H, 6.31; N, 10.12. Found: C, 48.93; H, 6.39; N, 10.09%.

Methyl N-[[[(1R,S)-1-(N-(2-(2-phthalamidoethoxy)acetyl)amino)ethyl]methoxyphosphinyl]-D-isoglutaminat (5). To a solution of phosphonamidate 3 (0.415 g, 1 mmol) in dry methanol (20 mL) cooled to 0 °C was added 10% Pd/C (80 mg) and a balloon of hydrogen gas. The reaction was warmed to r.t. and stirred overnight. After filtration through a sintered glass funnel, the solvent was removed in vacuo. The colorless oil obtained was pure enough to be used in the next reaction step. To some of this free amine (0.270 g, 0.96 mmol) was added DMF (5 mL), (2-phthalimidoethoxy)acetic acid (4) (0.237 g, 0.96 mmol), DPPA (0.25 mL, 1.15 mmol) and Et$_3$N (0.29 mL, 2.11 mmol) at 0 °C while stirring. The
ice bath was removed after two hours and the reaction was stirred at r.t. overnight. EtOAc (70 mL) was added and the solution was extracted successively with 10% citric acid, H_2O, saturated NaHCO_3 solution, H_2O, and saturated NaCl solution (20 mL each), dried (anhydrous MgSO_4) and finally evaporated in vacuo. The oily residue was purified by column chromatography on silica gel, eluting with 7:1 CHCl_3–MeOH to give the desired product 5 as a white solid. Yield: 81% (two steps); m.p.: 123-127 °C; IR (KBr, cm^{-1}) 3442.4, 1711.8, 1541.4, 1398.9, 1208.0, 1033.2, 797.1, 722.5; ^1H-NMR (DMSO-d_6) δ (ppm) = 1.10-1.25 (m, 3H, CH_3), 1.65-1.95 (m, 2H, CH_2-β-Glu-NH2), 2.23-2.40 (m, 2H, CH_2-γ-iGln), 3.44-3.60 (m, 6H, 2OCH_3), 3.68 (t, J=6 Hz, CH_2O), 3.80 (t, J=6 Hz, NCH_2), 4.08-4.25 (m, 2H, 2CH), 4.64-4.90 (m, 1H, PNH), 7.05 (s, 1H, CONH_2), 7.35 (s, 1H, CONH_2), 7.40-7.60 (m, 1H, NH), 7.80-7.88 (m, 4H, phthaloyl); ^31P-NMR (DMSO-d_6) δ(ppm) = 31.20, 31.35, 32.35; [α]_D^{20} =+15.40 (c=0.37, MeOH); FAB-MS m/z 513 (M+H)^+; Anal. Calc. For C_{21}H_{29}N_4O_9P: C, 49.22; H, 5.70; N, 10.93. Found: C, 48.95; H, 5.80; N, 10.64%.

Methyl (1R,S)-1-(N-(9-fluorenylmethoxycarbonyl)amino)ethyl phosphonate (6). Methyl (1R,S)-1-(N-benzyloxy carbonyl)aminoethyl phosphonate (1) (5.0 g, 18.0 mmol) was dissolved in dry MeOH (100 mL) and cooled to 0 °C. 10% Pd/C (0.5 g) and a balloon of hydrogen gas were added and the reaction mixture was stirred overnight at r.t. After filtration the solvent was removed on a rotary evaporator to give a white solid. Dioxane (70 mL), saturated NaHCO_3 (70 mL) and a solution of 9-fluorenylmethoxycarbonyl chloroformate (6 g, 23.4 mmol) in 50 mL of dioxane were then added and the solution was stirred overnight. The reaction mixture was diluted with water (50 mL) and acidified to pH 1. The precipitate was filtered off and dried in vacuo. Yield: 35% (two steps); lit [27]: 30%.

Dibenzyl N-[(1R,S)-1-(N-(9-fluorenylmethoxycarbonyl)amino)ethyl]methoxy phosphinyl]-D-glutamate (7). To a solution of methyl (1R,S)-1-(N-(9-fluorenylmethoxycarbonyl)amino)ethyl phosphonate (6) (3.0 g, 8.31 mmol) in 30 mL CH_2Cl_2 at 0 °C, DMF (64 µL, 0.83 mmol) and oxalyl chloride (1.43 mL, 16.62 mmol) were added. The solution was stirred at 0 °C for 0.5 h and at r.t. for 1.5 h and the solvent was evaporated in vacuo. The residue was taken up in dry toluene and re-evaporated to remove volatile byproducts. The resulting crude phosphochloridate was dissolved in CH_2Cl_2 (50 mL), cooled to 0 °C and treated with Et_3N (2.89 mL, 20.77 mmol) followed by a solution of dibenzyl D-glutamate p-toluenesulfonate (4.16 g, 8.31 mmol) in CH_2Cl_2. The reaction mixture was stirred overnight at r.t., the solvent was removed in vacuo and the residue was purified by column chromatography on silica gel, using 2:1 EtOAc – hexane as the eluent, giving pure 7 as a white solid. Yield: 86%; m.p.: 78.5-82 °C; IR (KBr, cm^{-1}) 3308.9, 2946.1, 1734.0, 168.8, 1540.1, 1450.7, 1316.4, 1252.7, 1211.6, 1046.1, 797.1, 739.8, 695.4; ^1H-NMR (DMSO-d_6) δ (ppm) = 1.20 (dd, 3H, J=7.3 Hz, J=16 Hz, CH_3), 1.75-1.90 (m, 2H, CH_2-β-Glu), 2.30-2.45 (m, 2H, CH_2-γ-Glu), 3.48 (d, 3H, J=11 Hz, POCH_3), 3.78-3.98 (m, 2H, 2CH), 4.15-4.35 (m, 3H, CH-fluorenyl + CO_2CH_2), 4.90-5.35 (m, 5H, 2CH_2Ph + PNH), 7.20-7.45 (m, 14H, 2C_6H_5 + 4H-fluorenyl), 7.73 (t, 2H-fluorenyl, J=7.2 Hz), 7.88 (d, 2H-fluorenyl, J=7.5 Hz); ^31P-NMR (DMSO-d_6) δ(ppm) = 31.43, 31.47, 31.71, 31.76; [α]_D^{20} =+13.27 (c=0.446, MeOH); FAB-MS
Dibenzyl $N$-[[1(R,S)-1-(N-(5-phthalimidopentanoyl)amino)ethyl]methoxyphosphinyl]-D-glutamate (9).

Phosphonamidate 7 (1.50 g, 2.24 mmol) was dissolved in DMF/CH₂Cl₂ (30:70, 15 mL) and diethylamine (3.49 mL, 33.6 mmol) was added. The reaction was stirred at r.t. for 2 h, the solvent was removed in vacuo and the residue suspended in Et₂O (40 mL). A solution of anhydrous oxalic acid (0.202 g, 2.24 mmol) in Et₂O (20 mL) was slowly poured into the stirred suspension and the mixture was chilled in a refrigerator overnight. The solvent was decanted and the resulting oil dissolved in CHCl₃ (80 mL). The solution was washed with 1M NaOH (20 mL) and brine (20 mL), dried (anh. MgSO₄) and evaporated to give the free amine as a brown oil, which was immediately used in the next reaction step. It was dissolved in dry DMF (7 mL) and 5-phthalimidopentanoic acid 8 (0.549 g, 2.24 mmol), and DPPA (0.6 mL, 2.69 mmol) and Et₃N (0.69 mL, 4.93 mmol) were added at 0 °C while stirring. The stirring was continued at 0 °C for 2 h and at r.t. overnight. EtOAc (100 mL) was added and the solution was extracted successively with 10% citric acid, water, saturated NaHCO₃ solution, water and saturated NaCl solution (20 mL each). The organic phase was dried (anh. Na₂SO₄), the solvent removed under reduced pressure and the residue was purified on a silica gel column using 30:1 EtOAc – MeOH as an eluent, to give pure compound 9 as a white wax. Yield: 48% (two steps); IR (KBr, cm⁻¹) 3303.9, 2941.5, 1710.3, 1642.6, 1532.2, 1532.2, 1398.4, 1209.0, 1039.4, 797.7, 719.4; ¹H-NMR (CDCl₃) δ (ppm) = 1.18-1.39 (m, 3H, CH₃), 1.60-1.80 (m, 4H, CH₂CH₂), 1.95-2.30 (m, 4H, CH₂CO + CH₂-β-Glu), 2.35-2.55 (m, 2H, CH₂-γ-Glu), 3.60-3.75 (m, 5H, NCH₂ + POCH₃), 4.00-4.15 (m, 1H, CH), 4.30-4.55 (m, 1H, CH), 5.05-5.20 (m, 5H, 2CH₂Ph + PNH), 7.20-7.40 (m, 11H, 2C₆H₅ + NH), 7.65-7.72 (m, 2H, phthaloyl), 7.78-7.84 (m, 2H, phthaloyl); ³¹P-NMR (CDCl₃) δ (ppm) = 31.16, 32.44, 32.77; [α]₂₀D =+2.95 (c=0.43, MeOH); FAB-MS m/z 678 (M+H)+; Anal. Calc. For C₃₅H₄₀N₃O₉P₂x0.5H₂O: C, 61.22; H, 6.02; N, 6.12. Found: C, 60.98; H, 5.96; N, 6.21%.

Dimethyl (2S)-2-[[1(R,S)-1-(N-(benzyloxycarbonyl)amino)ethyl]methoxyphosphinoyl]glutarate (13).

To a solution of monophosphonate 1 (0.88 g, 3.22 mmol), dimethyl (S)-2-hydroxyglutarate (12) (0.85 g, 4.83 mmol) and BOP reagent (2.04 g, 4.83 mmol) in DMF (7 mL), diisopropylethylamine (2.30 mL, 12.88 mmol) was added at r.t. under stirring. After 2h, DMF was evaporated under reduced pressure, the residue was dissolved in EtOAc (100 mL) and the solution was washed with a saturated NaHCO₃ solution. (3 x 10 mL) and brine (3 x 10 mL), dried (anh. Na₂SO₄), and the solvent removed under reduced pressure. The crude product was purified on a silica gel column using 2:1 EtOAc – MeOH as an eluent, to give pure compound 13 as a colourless oil. Yield: 84 %; IR (KBr, cm⁻¹) 3258.1, 2955.7, 1736.2, 1533.9, 1440.7, 1232.3, 1044.8, 845.0; ¹H-NMR (CDCl₃) δ (ppm) = 1.36-1.48 (m, 3H, CH₃), 2.15-2.30 (m, 2H, CH₂-β-glutarate), 2.35-2.55 (m, 2H, CH₂-γ-glut.), 3.66-3.90 (m, 9H, 3OCH₃), 4.18-4.42 (m, 1H, CH), 4.90-5.05 (m, 1H, OCH), 5.15 (s, 2H, CH₂Ph), 5.36-5.72 (m, 1H, NH), 7.30-7.45 (m, 5H, C₆H₅); ³¹P-NMR (CDCl₃) δ (ppm) = 27.19, 27.48, 27.88, 28.64; [α]₂₀D =+26.01 (c=0.48, MeOH); FAB-MS
m/z 432 (M+H)+; Anal. Calc. For C_{18}H_{26}NO_{9}P: C, 50.12; H, 6.08; N, 3.25. Found: C, 49.82; H, 6.22; N, 3.19%.

**Dimethyl (2S)-2-[(1R,S)-1-[(N-(2-(2-phthalimidoethoxy)acetyl)amino]ethyl] methoxyphosphinyloxy]-glutarate** (14). Mixed phosphonate 13 (0.64 g, 1.48 mmol) was dissolved in dry MeOH and hydrogenated over 10 % Pd/C at 40 psi for 18 h in a Parr hydrogenator. The catalyst was filtered off, the solvent was evaporated and the resulting free amine was immediately used in the next reaction step. It was dissolved in dry DMF, and 2-(2-phthalimidoethoxy)acetic acid (4) (1.48 mmol) was added followed by DPPA (0.38 mL, 1.78 mmol) and Et$_3$N (0.45 mL, 3.26 mmol) at 0 °C. After stirring for 2 h at this temperature, stirring was continued for 48 h at r.t. EtOAc (80 mL) was added and the solution was extracted successively with 10% citric acid, water, saturated NaHCO$_3$ solution, water and saturated NaCl solution (15 mL each). The organic phase was dried (anh. Na$_2$SO$_4$), the solvent removed under reduced pressure and the residue purified by column chromatography on silica gel using 15:1 CHCl$_3$ – MeOH as an eluent, yielding compound 14 as a white wax. Yield: 68 % (two steps); IR (KBr, cm$^{-1}$) 3484.5, 1713.9, 1649.3, 1541.3, 1394.2, 1152.0, 1014.4, 951.6, 722.3; $^1$H-NMR (CDCl$_3$) $\delta$ (ppm) = 1.44 (dd, 3H, J=7.3, J=16 Hz, CH$_3$), 2.10-2.30 (m, 2H, CH$_2$-β-glut.), 2.35-2.52 (m, 2H, CH$_2$-γ-glut.), 3.66-3.88 (m, 11H, 3OCH$_3$ + NCH$_2$), 3.95-4.04 (m, 4H, CH$_2$O + OCH$_2$CO), 7.10-7.25 (m, 1H, NH), 7.80-7.90 (m, 2H, phthaloyl), 7.85-7.95 (m, 2H, phthaloyl); $^{31}$P-NMR (CDCl$_3$) $\delta$ (ppm) = 27.78, 28.07, 28.98; $[\alpha]_{D}^{20}$+1.71 (c=0.41, MeOH); FAB-MS m/z 529 (M+H)$^+$; Anal. Calc. For C$_{22}$H$_{29}$N$_2$O$_{11}$P: C, 50.00; H, 5.53; N, 5.30. Found: C, 50.35; H, 5.42; N, 5.49%.

**References**

1. Ellouz, F.; Adam, A.; Cirobaru, R.; Lederer, E. Minimal Structural Requirements for Adjuvant Activity of Bacterial Peptidoglycan Derivatives. *Biochem. Biophys. Res. Commun.* 1974, 59, 1317-1325.
2. Azuma, I.; Otani, T. Potentiation of Host Defense Mechanism against Infection by a Cytokine Inducer, an acyl-MDP Derivative, MDP-Lys(L18) (Romurtide) in Mice and Humans. *Med. Res. Rev.* 1994, 14, 401-414.
3. Tsubura, E.; Azuma, I.; Une, T. Muroctasin, a Muramyl Dipeptide Derivative. *Arzneim.-Forsch./Drug Res.* 1988, 38, 951-952.
4. Adam, A.; Lederer, E. Muramyl Peptides: Immunomodulators, Sleep Factors and Vitamins. *Med. Res. Rev.* 1984, 4, 111-152.
5. Baschang, G. Muramylpeptides and Lipopeptides: Studies toward Immunostimulants. *Tetrahedron* 1989, 45, 6331-6360.
6. Lefrancier, P.; Lederer, E. Muramyl-peptides. *Pure Appl. Chem.* 1987, 59, 449-454.
7. Hemmi, K.; Takeno, H.; Okada, S.; Nakaguchi, O.; Kitaura, Y.; Hashimoto, M. Total Synthesis of FK-156 Isolated from a *Streptomyces* as an Immunostimulating Peptide: Application of a Novel Copper Chelate Amino Protection. *J. Am. Chem. Soc.* 1981, 103, 7026-7028.
8. Migliore-Samour, D.; Bouchaudon, J.; Flo'ch, F.; Zerial, A.; Ninet, L.; Werner, G.H.; Jolles, P.A. A Short Lipopeptide, Representative of a New Family of Immunological Ajuvans Devoid of Sugar, Life Sci. 1980, 26, 883-888.

9. Sollner, M.; Pečar, S.; Štalc, A. The Influence of the Lipophilicity of the 7-Oxoacl-L-alanyl-D-isoglutamines on Their Immunorestoration Activity in vivo. Eur. J. Med. Chem. 1996, 31, 927-933.

10. Barton, D.H.R.; Camara, J.; Dalko, P.; Gero, S.D. Synthesis of Biologically Active Carbocyclic Analogues of N-Acetylmuramyl-L-Alanyl-D-isoglutamine (MDP). J. Org. Chem. 1989, 54, 3764-3766.

11. Kikelj, D.; Pečar, S.; Kotnik, V.; Štalc, A.; Wraber-Herzog, B.; Simčič, S.; Ilhan, A.; Klamfer, L.; Povšič, L.; Grahek, R.; Suhadolc, E.; Hočeval, M.; Hönig, H.; Rogi-Kollenprath, R. N-\{trans-2-[[2'-(Acetylamino)cyclohexyl]oxy]acetyl\}-L-alanyl-D-glutamic Acid: A Novel Immunologically Active Carbocyclic Muramyl Dipeptide Analogue. J. Med. Chem. 1998, 41, 530-539.

12. Gobec, S.; Urleb, U.; Simčič, S.; Wraber, B. Synthesis and Modulation of Cytokine Production by Two New Adamantane Substituted Acyclic Desmuramylidipeptide Analogs. Pharmazie, 2001, 56, 523-526.

13. Urleb, U.; Krбавčič, A.; Sollner, M.; Kikelj, D.; Pečar, S. Synthesis of Phthalimido-Desmuramylpeptide Analogues as Potential Immunomodulating Agents. Arch. Pharm. (Weinheim) 1995, 328, 113-117.

14. Danklmaier, J.; Hönig, H. Synthesis of Acyclic Analogues of N-Acetylmuramyl-L-alanyl-D-isoglutamine (MDP). Liebigs Ann. Chem. 1990, 145-150.

15. Ochi, C.; Norisada, N.; Moriguchi, M.; Štalc, A.; Urleb, U.; Muraoka, S. Interleukin-10 Inducing Activity of LK423, a Phthalimido-desmuramyldipeptide Compound. Arzneim.-Forsch./Drug Res. 1999, 49, 72-79.

16. Moriguchi, M.; Urabe, K.; Norisada, N.; Ochi, C.; Štalc, A.; Urleb, U.; Muraoka, S. Therapeutic Effects of LK 423, a Phthalimido-desmuramyldipeptide Compound, on Dextran Sulphate Sodium-Induced Colitis in Rodents Through Restoring Their Interleukin-10 Producing Capacity. Arzneim.-Forsch./Drug Res. 1999, 49, 184-192.

17. Simčič, S.; Wraber, B.; Sollner, M.; Urleb, U.; Gobec, S. Modulation of Tumor Necrosis Factor Production With Desmuramyldipeptide Analogues. Pflug. Arch. Eur. J. Phy. 2000, 440, R64-R66.

18. Urleb, U.; Gobec, S.; Prelog, D. Synthesis and Activity of Phosphono Desmuramyldipeptide Analogs. Lett. Pept. Sci. 1995, 2, 193-197.

19. Gobec, S.; Urleb, U. Synthesis of New Phosphonamidate and Phosphinamide Desmuramyldipeptide Analogas. Lett. Pept. Sci. 1998, 5, 109-114.

20. Gobec, S.; Urleb, U. Synthesis of Phosphono Phthalimido-desmuramyldipeptide Analogs, Phosphorus, Sulfur, Silicon Relat. Elem. 2000, 156, 125-133.

21. Vo-Quang, Y.; Gravey, A.M.; Simonneau, R.; Vo-Quang, L.; Lacoste, A.M.; Le Goffic, F. Towards New Inhibitors of D-Alanine: D-Alanine Ligase: The Synthesis of 3-Amino Butenylphosphonic and Aminophosphonamidic Acids. Tetrahedron Lett. 1987, 28, 6167-6170.
22. Lefrancier, P.; Bricas, E. Synthese de la subunite peptidique du peptidoglycane de la paroi de trois bacteries gram-positif et de peptides de structure analogue. Bull. Soc. Chim. Biol. 1967, 49, 1257-1271.
23. Hirschmann, R.; Yager, K.M.; Taylor, C.M.; Witherington, J.; Sprengeler, P.A.; Phillips, B.W.; Moore, W.; Smith, A.B. Phosphonate Diester and Phosphonamide Synthesis. Coordinate Analysis by $^{31}$P NMR Spectroscopy: Identification of Pyrophosphonate Anhydrides and Highly Reactive Phosphonylammonium Salts. J. Am. Chem. Soc. 1997, 119, 8177-8190.
24. Korošec, E.; Poljšak, D.; Urleb, U. Synthesis of N-Acylamino-ethoxycetic Acid Derivatives. Arch. Pharm. (Weinheim) 1992, 325, 251-252.
25. Campbell, D.; Lentini, D.P. Methods For the Synthesis of Phosphonate Esters, WO Patent 94/06808, 1994.
26. Lefrancier, P.; Choay, J.; Derrien, M.; Lederman, I. Synthesis of N-Acetyl-muramyl-L-alanine-D-isoglutamine, an Adjuvant of Immune-response, and of Some N-Acetyl-muramyl-peptide Analogs. Int. J. Pept. Protein Res. 1977, 9, 249-257.
27. Nefkens, G.H.L.; Tesser, G.I.; Nivard, R.J.F. A Simple Preparation of Phthaloyl Amino Acids Via a Mild Phthaloilation. Rec. Trav. Chim. Pays-Bas 1960, 79, 688-698.
28. Gobec, S.; Urleb, U.; Auger, G.; Blanot, D. Synthesis and Biochemical Evaluation of Some Novel N-Acyl Phosphono- and Phosphinoalanine Derivatives As Potential Inhibitors of the D-Glutamic Acid-Adding Enzyme. Pharmazie 2001, 56, 295-297.
29. Jacobsen, N.E.; Bartlett, P.A. A Phosphonamidate Dipeptide Analogue as an Inhibitor of Carboxypeptidase A. J. Am. Chem. Soc. 1981, 103, 654-657.
30. McLeod, D.A.; Brinkworth, R.I.; Ashley, J.A.; Janda, K.D.; Wirsching, P. Phosphonamidates and Phosphonamidate Esters as HIV-1 Protease Inhibitors. Bioorg. Med. Chem. Lett. 1991, 1, 653-658.
31. Tsukamoto, T.; Haile, W.H.; McGuire, J.J.; Coward, J.K. Mechanism-Based Inhibition of Human Folylpolyglutamate Synthetase: Design, Synthesis, and Biochemical Characterisation of a Phosphapeptide Mimic of a Tetrahedral Intermediate. Arch. Biochem. Biophys. 1998, 355, 109-118.
32. Keck, G.E.; Andrus, M.B.; Romer, D.R. A Useful New Enantiomerically Pure Synthon from Malic Acid: Chelation-Controlled Activation as a Route to Regiselectivity. J. Org. Chem. 1991, 56, 417-420.
33. Campagne, J.-M.; Coste, J.; Jouin, P. (1H-Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium Hexafluorophosphate-mediated and (1H-Benzotriazol-1-yloxy)tripyrrolidinophosphonium Hexafluorophosphate-mediated Activation of Monophosphonate Esters – Synthesis of Mixed Phosphonate Diesters, the Reactivity of the Benzotriazolyl Phosphonic Esters vs the Reactivity of the Benzotriazolyl Carboxylic Esters. J. Org. Chem. 1995, 60, 5214-5223.

Sample availability: Samples of compounds 5, 7, 9 and 14 are available from the authors.

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