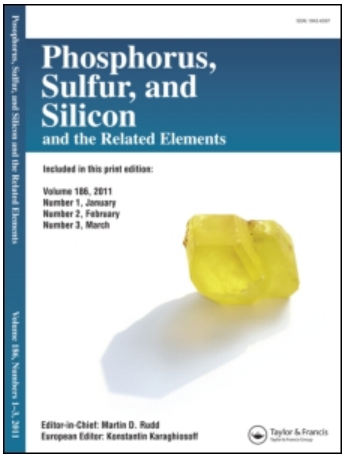

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SYNTHESIS OF PHOSPHONO PHTHALIMIDO-DESMURAMYLDIPEPTIDE ANALOGS

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SYNTHESIS OF PHOSPHONO PHTHALIMIDO-DESMURAMYLDIPEPTIDE ANALOGS

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Orthogonally protected Abu(P) (benzyl (2*R,S*)-4-diethylphosphonyl-2-phthalimido butanoate **4**) has been synthesized. It has been used as a key intermediate for the synthesis of two new phthalimido-desmuramyldipeptide analogs containing diethylphosphnate moiety at the position of ω-carboxylic group of Glu.

Keywords: muramyldipeptide; phosphonate; peptidylphosphonate

INTRODUCTION

N-Acetymuramyl-L-alanyl-D-isoglutamine (Muramyldipeptide, MDP) has been identified as the smallest bacterial cell wall fragment capable of influencing immune system[1]. Being one of the most potent immunostimulants, MDP has attracted interest of medicinal chemists, and a lot of its analogs has been synthesized in order to improve pharmacodynamic and pharmacokinetic properties[2,3]. It is known that *N*-acetyl glucosamine part of the molecule is not essential for immunomodulating activity and our group has replaced it by phthalimido or adamantyl substituted aminoethoxyacetic acids giving immunological active acyclic MDP analogues like LK 423[3,4]. Such phthalimido- and adamantyl-desmuramyldipeptides retain dipeptide fragment (L-Ala-D-iGln) and the specific position of the

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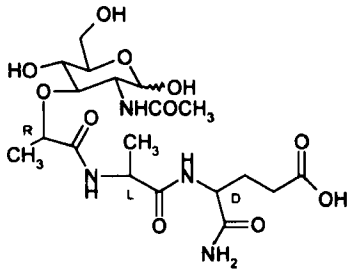
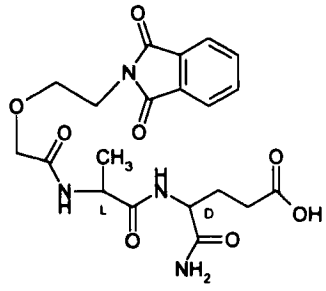
carbonyl group of N-acetylmuramic acid part of MDP, which was hypothesized to be important for immunological activity[5].

Modification of the dipeptide fragment represents another possibility for design and synthesis of new potential immunomodulators. To obtain more SAR data, we have modified the peptide backbone of phthalimido and adamantyl substituted MDP analogs using various phosphorus species. Tetrahedral phosphorus compounds like phosphonates, phosphinates or phosphonamidates have been used as peptide bond (bio)isosteres for the design and synthesis of antibacterials, enzyme inhibitors and production of abzymes[6]. We have replaced the peptide bond at the end of acyclic side chain by phosphonamidate ethyl ester and by phosphinamide moiety[7,8]. The peptide bond between Ala and Glu part of the molecule has been replaced by phosphonamidate methyl ester[7,8]. We are presenting the synthesis of new phthalimido-desmuramyldipeptide analogs, where the ω-carboxylic group of Glu is replaced by diethyl phosphonate isostere.



MDP

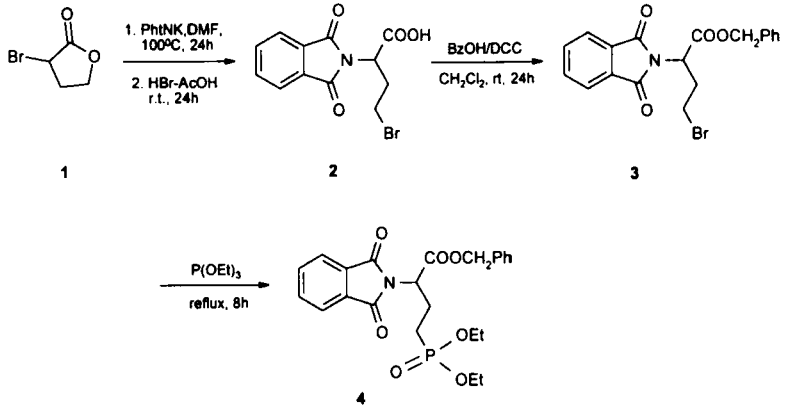


LK 423

RESULTS AND DISCUSSION

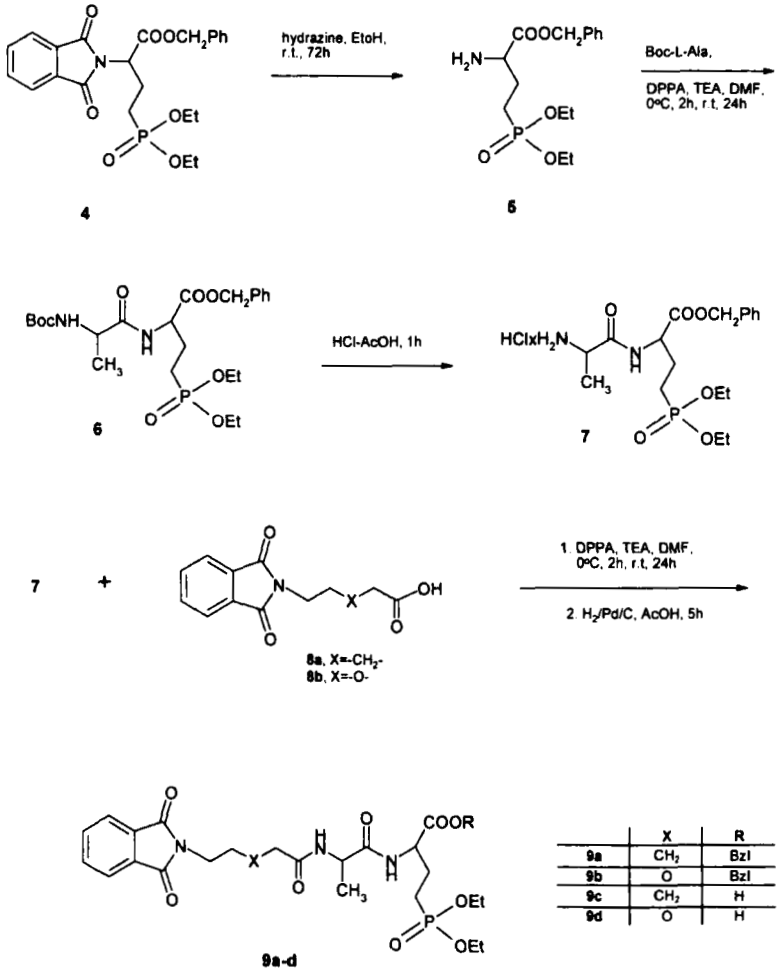
For the preparation of MDP analogs with terminal phosphono group orthogonally protected Abu(P) (2-amino-4-phosphonobutanoic acid) was required. Phosphorus analogs of glutamic acid like Abu(P) and derivatives are interesting as biologically active substances and as building blocks for phosphonopeptide as hydrolysis stable isosteres of serine phosphopeptides. A number of syntheses of both chiral and racemic Abu(P) and its variously protected derivatives have been reported[9]. For the synthesis of

benzyl (2*R*,*S*)-4-diethylphosphonyl-2-phthalimidobutanoate **4** the procedure of Logusch was modified[10] (Scheme 1). Potassium phthalimide was treated with 2-bromo-4-butyrolactone **1** and the product was treated with 40% HBr-AcOH resulting in bromoacid **2**. After esterification with benzyl alcohol using DCC/DMAP benzyl 4-bromo-2-phtalimido butyrate **3** was obtained and used in Michaelis-Arbuzov reaction with $P(OEt)_3$. The new product **4** is orthogonally protected racemic Abu(P) where the amino group was protected by the phthalimido group, the carboxylic acid was masked as benzyl ester and the phosphonic acid was protected as diethyl phosphonate. The compound **4** was used for the synthesis of Abu(PO_3Et_2) containing pseudopeptides as MDP analogs.



SCHEME 1

The phthalimide *N*-protecting group of **4** was removed with hydrazine hydrate under standard conditions (Scheme 2). The resulting crude free amine **5** was coupled with Boc-L-Ala in the presence of DPPA/Et_3N giving Boc-L-Ala-DL-Abu(PO_3Et_2)-OBzL **6**. Boc group was removed with HCl-AcOH to give hydrochloride **7**, which was further coupled with 5-phthalimidopentanoic acid **8a** or 2-(2-phthalimido)-ethoxyacetic acid **8b** giving compounds **9a-b**, respectively. These compounds were finaly catalytically hydrogenated over 10% Pd/C and the products **9c-d** were obtained. The compounds **6, 7** and **9a-d** were isolated as mixtures of two diastereoisomers as indicated by the phosphonate signals in their ^{31}P NMR spectra.



	X	R
9a	CH_2	Bzl
9b	O	Bzl
9c	CH_2	H
9d	O	H

SCHEME 2

The compounds **9c** and **9d** are new phthalimido-MDP analogs where ω-carboxylic acid of glutamic acid has been replaced by diethyl phosphonate moiety. The phosphonate moiety can impart some useful properties to such MDP analogs. The lipophilicity is increased and the tetrahedral configuration can influence the binding of peptidylphosphonate to the receptor. The products are expected to have immunomodulating activity. The

immunological tests are currently underway and will be published elsewhere.

EXPERIMENTAL

Materials and Methods

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. Elemental analyses were performed at Faculty of Chemistry and Chemical Engineering, University of Ljubljana, on a Perkin-Elmer elemental analyzer 240 C. Mass spectra were obtained on an Autospec Q, VG-Analytical mass spectrometer using FAB ionization. NMR spectra were obtained on a Bruker Avance DPX 300 instrument. ^{1}H NMR was done at 300.13 MHz with tetramethylsilane as an internal standard and ^{31}P NMR was done at 121 MHz using H_3PO_4 as an external standard.

4-Bromo-2-phthalimidobutyric acid 2

2-Bromo-4-butyrolactone **1** (9 g, 55 mmol) and potassium phthalimide (10 g, 54 mmol) in DMF (30 ml) were stirred overnight at 100 °C. The reaction mixture was poured on ice, the precipitate was filtered off and crystallized from water-acetone. The product was dissolved in 40% HBr/AcOH (30 mL) and stirred overnight at ambient temperature. After evaporation of the solvent the crude product was disolved in Et_2O and precipitated with hexane. Yield: 60% (two steps); m.p.: 119–121 °C (lit. m.p.: 121–123 °C)[10].

Benzyl (2*R*,*S*)-4-bromo-2-phthalimidobutanoate 3

To the solution of bromoacid **2** (10 g, 32 mmol) and benzyl alcohol (3.6 mL, 35.2 mmol) in dichloromethane were added DCC (7.26 g, 35.2 mmol) and DMAP (390 mg, 3.2 mmol) and the mixture was stirred for 24 h. The precipitated dicyclohexylurea was filtered off, the filtrate was successively washed with 10% citric acid (2 × 30 mL), water (20 mL),

10% $NaHCO_3$(2 × 30 mL), water (20 mL) and brine (30 mL), dried ($MgSO_4$), and evaporated in vacuo. The residue was purified on silica column using EtOAc:hexane (1:1) as an eluent. Pure **3** was obtained as an oil[9]. Yield: 83%.

Benzyl (2*R,S*)-4-diethylphosphonyl-2-phthalimidobutanoate 4

Compound **3** (4 g, 10 mmol) was refluxed in triethylphosphite (3.43 mL, 20 mmol) for 10 h. The excess triethylphosphite was removed by short path distillation under vacuum. The residue was purified by column chromatography (EtOAc:i-PrOH, 15:1) to give pure **4** as an oil.

Yield: 61%; FAB MS: m/z = 460 (MH^+); ^1H NMR (DMSO-d_6) δ (ppm) 1.15 – 1.25 (m, 6H, XH_3), 1.70 – 1.90 (m, 2H, β – CH_2), 2.25 – 2.40 (m, 2H, χ-CH_2), 3.90 – 4.05 (m, 4H, CH_2CH_3), 5.05 (dd, J = 9.8 Hz, J = 5.3 Hz, CH), 5.16 (AB system, J_{AB} = 12.6 Hz, 2H, CH_2Ph), 7.20 – 7.35 (m, 5H, Ph), 7.82 – 7.95 (m, 4H, Phth); ^{31}P NMR (DMSO-d_6) δ (ppm) 31.2; Anal. calc. for $C_{23}H_{26}NO_7P$ (459.14): C 60.11, H 5.71, N 3.05; found: C 59.63, H 5.94, N 3.04%.

Benzyl (2*R,S*)-4-diethylphosphonyl-2-((N-(t-butyloxycarbonyl)-L-alanyl)-amino)-butanoate 6

A mixture of phosphonate **4** (0.918g, 2 mmol) and hydrazine hydrate (0.1 mL, 2.2 mmol) in EtOH was stirred at room temperature for 72 hr. After the filtration the filtrate was concentrated to give crude **5** as a semi-solid pure enough for further synthesis. Compound **5** and Boc-L-Ala were dissolved in dry DMF and triethylamine (0.61 mL, 4.4 mmol) and diphenylphosphorylazide (0.47 mL, 2.2 mmol) were added at 0 °C. After stirring for 1 h at 0 °C, stirring was continued for 24 h at room temperature. EtOAc (50 mL) was added and the solution was extracted subsequently with 10% citric acid (10 mL), water (10 mL), 10% $NaHCO_3$ (10 mL), water (10 mL) and brine (10 mL). The organic phase was dried (anhydrous $MgSO_4$) and the solvent removed under reduced pressure. The residue was purified by column chromatography (EtOAc:i-PrOH, 15:1) giving **6** as an oil.

Yield: 80.8%; FAB MS: m/z = 501 (MH^+); ^1H NMR (DMSO-d_6) δ (ppm) 1.10 – 1.25 (m, 9H, $CHCH_3$ and $2CH_2CH_3$), 1.30 – 1.42 (m, 9H, $C(CH_3)_3$), 1.60 – 2.00 (m, 4H, β-CH_2 and χ-CH_2), 3.87 – 4.03 (m, 5H, CH and $2CH_2CH_3$), 4.32 – 4.44 (m, 1H, CH), 5.12 (AB system,

J_{AB} = 12.6 Hz, 2H, CH_2Ph), 6.86 (6.91*) (d, J = 7.15 Hz, 1H, NH), 7.27 – 7.40 (m, 5H, Ph), 8.19 (8.22*) (d, J = 7.5 Hz, 1H, NH); (* two sets of signals due to diastereoisomers); ^{31}P NMR (DMSO-d_6) δ (ppm) 31.88, 31.97; Anal. calc. for $C_{23}H_{37}N_2O_8P$ (500.23): C 55.17, H 7.45, N 5.60; found: C 54.96, H 7.43, N 5.59%; $[\alpha]_D^{20}$=-15.21 (c = 0.38, MeOH).

Benzyl (2*R,S*)-4-diethylphosphonyl-2-((L-alanyl)-amino)-butanoate hydrochloride 7

Compound **6** (1.0g, 2 mmol) was dissolved in glacial acetic acid and treated with HCl gas for 2 h. The solvent was evaporated under reduced pressure and the desired product **7** was obtained as a white foam.

Yield: 97%; FAB MS: m/z = 401 (MH^+); ^1H NMR (DMSO-d_6) δ (ppm) 1.15 - 1.25 (m, 6H, $2CH_2CH_3$), 1.35 (dd, J = 7.3 Hz, J = 16.2 Hz, 3H, $CHCH_3$), 1.66 – 2.03 (m, 4H, β-CH_2 and χ-CH_2), 3.85 – 4.30 (m, 5H, CH and $2CH_2CH_3$), 4.38 – 4.51 (m, 1H, CH), 5.15 (AB system, J_{AB} = 12.6 Hz, 2H, CH_2Ph), 7.25 and 7.45 (m, 6H, Ph and NH), 8.30 (br s, 3H, NH_3^+), 8.95 (9.05*) (d, J = 7.5 Hz, 1H, NH); (* two sets of signals due to diastereoisomers); ^{31}P NMR (DMSO-d_6) δ (ppm) 31.91.

This material was used in the next reaction step without further purification.

Synthesis of Protected Phosphonopeptides 9a, b, General Procedure

To a stirred solution of 5-phthalimidopentanoic acid **8a** or 2-(2-phthalimido)-ethoxyacetic acid **8b** (2 mmol) and benzyl (2R, S)-4-diethylphosphonyl-2-((L-alanyl)-amino)-butanoate hydrochloride **7** (0.837, 2 mmol) in dry DMF, diphenylphosphorylazide (0.47 mL, 2.2 mmol) and triethylamine (0.61 mL, 4.4 mmol) were added at 0 °C. After stirring for 1 h at this temp., stirring was continued for 24 h at room temp. EtOAc (50 mL) was added and the solution was extracted subsequently with 10% citric acid (10 mL), water (10 mL), 10% $NaHCO_3$(10 mL), water (10 mL) and brine (10 mL). The organic phase was dried (anhydrous $MgSO_4$) and the solvent removed under reduced pressure.

Benzyl (2R,S)-4-diethylphosphonyl-2-(N-(5-phthalimidopentanoyl)-L-alanyl)-amino)-butanoate 9a

Prepared from **8a** and purified by column chromatograpy (EtOAc:i-PrOH, 15:1 vol.). Yield: 67%; m.p.: 64 – 66 °C; FAB MS: m/z = 630 (MH^+); ^1H

NMR (DMSO-d_6) δ (ppm) 1.12 – 1.24 (m, 9H, CHC**H**$_3$ and 2CH_2C**H**$_3$), 1.41 – 1.61 (m, 4H, CH_2CH_2), 1.65 – 2.00 (m, 4H, β-CH_2 and χ-CH_2), 2.08 – 2.18 (m, 2H, CH_2CO), 3.56 (t, J = 6.5 Hz, 2H, CH_2N), 3.85 – 4.00 (m, 4H, 2C**H**$_2$CH$_3$), 4.22 – 4.40 (m, 2H, 2CH), 5.10 (AB system, J_{AB} = 12.6 Hz, 2H, C**H**$_2$Ph), 7.27 – 7.41 (m, 5H, Ph), 7.80 – 7.90 (m, 4H, Phth), 7.93 (7.95*) (d, J = 7.3 Hz, 1H, NH), 8.29 (8.30*) (d, J = 7.5 Hz, 1H, NH); (*two sets of signals due to diastereoisomers); ^{31}P NMR (DMSO-d_6) δ (ppm) 31.87, 31.91; Anal. calc. for $C_{31}H_{40}N_3O_9P$ (629.25): C 59.12, H 6.41, N 6.68; found: C 58.84, H 6.61, N 6.78%; $[\alpha]_D^{20}$=-20.25 (c = 0.39, MeOH).

Benzyl (2R, S)-4-diethylphosphonyl-2-((N-(2-(2-phthalimidoethoxy)-acetyl)-L-alanyl)-amino)-butanoate 9b

Prepared from **8b** and purified by column chromatograpy (EtOAc:i-PrOH, 9:1 vol.). Yield: 72%; m.p.: 48 – 50 °C; FAB MS: m/z = 632 (MH^+); ^{1}H NMR (DMSO-d_6) δ (ppm) 1.15 – 1.25 (m, 9H, CHC**H**$_3$ and 2CH_2C**H**$_3$), 1.65 – 1.95 (m, 4H, β-CH_2 and χ-CH_2), 3.68 (t, J = 5.3 Hz, 3H, NC**H**$_2$CH$_2$O), 3.80 (t, J = 5.3 Hz, 3H, NCH$_2$C**H**$_2$O), 3.88 (s, 2H, OCH_2CO), 3.89 – 4.02 (m, 4H, 2C**H**$_2$CH$_3$), 5.12 (AB system, J_{AB} = 12.6 Hz, 2H, C**H**$_2$Ph), 7.30 – 7.40 (m, 5H, Ph), 7.57 (7.60*) (d, J = 7.5 Hz, 1H, NH), 7.80 – 7.90 (m, 4H, Phth), 8.38 (8.42*) (d, J = 7.5 Hz, 1H, NH); (* two sets of signals due to diastereoisomers); ^{31}P NMR (DMSO-d_6) δ (ppm) 31.78, 31.82; Anal. calc. for $C_{30}H_{38}N_3O_{10}P$ (631.23) × H_2O: C 55.45, H 6.21, N 6.47; found: C 55.20, H 6.28, N 6.52%; $[\alpha]_D^{20}$=-8.37 (c = 0.49, MeOH).

Hydrogenolytic Cleavage of Benzyl Protecting Groups – General Procedure

Compounds **9a-b** (1mmol) were dissolved in glacial acetic acid and hydrogenated for 6 h over 10% Pd/C (50–100 mg) at room temp. and normal pressure. After filtration, the solvent was removed in vacuo.

(2R,S)-4-diethylphosphonyl-2-((N-(5-phthalimidopentanoyl)-L-alanyl)-amino)-butanoic acid 9c

Prepared from **9a**. Yield: 97%; m.p.: 48 – 52 °C; FAB MS: m/z = 540 (MH^+); ^{1}H NMR (DMSO-d_6) δ (ppm) 1.14 – 1.25 (m, 9H, CHC**H**$_3$ and

$2CH_2CH_3$). 1.41 – 1.61 (m, 4H, CH_2CH_2), 1.65 – 2.00 (m, 4H, β-CH_2 and χ-CH_2), 2.09 – 2.19 (m, 2H, CH_2CO), 3.56 (t, J = 6.5 Hz, 2H, CH_2N), 3.89 – 4.03 (m, 4H, $2CH_2CH_3$), 4.18 – 4.35 (m, 2H, 2CH), 7.82 – 7.90 (m, 4H, Phth), 7.93 (7.96*) (d, J = 7.3 Hz, 1H, NH), 8.08 (8.09*) (d, J = 7.5 Hz, 1H, NH); (* two sets of signals due to diastereoisomers); ^{31}P NMR (DMSO-d_6) δ (ppm) 32.17, 32.11; Anal. calc. for $C_{24}H_{34}N_3O_9P$ (539.20): C 53.41, H 6.35, N 7.79; found: C 52.96, H 6.48, N 7.49%; $[\alpha]_D^{20}$=-16.47 (c = 0.46, MeOH).

(2R,S)-4-diethylphosphonyl-2-((N-(2-(2-phthalimidoethoxy)-acetyl)-L-alanyl)-amino)-butanoic acid 9d

Prepared from **9b**. Yield: 96%; hygroscopic foam; FAB MS: m/z = 542 (MH^+); ^1H NMR (DMSO-d_6) δ (ppm) 1.15 – 1.25 (m, 9H, $CHCH_3$ and $2CH_2CH_3$), 1.60 – 1.90 (m, 4H, β-CH_2 and χ-CH_2), 3.68 (t, J = 5.3 Hz, 3H, NCH_2CH_2O), 3.80 (t, J = 5.3 Hz, 3H, NCH_2CH_2O), 3.88 (s, 2H, OCH_2CO), 3.92 – 4.04 (m, 4H, $2CH_2CH_3$), 4.16 – 4.25 (m, 1H, CH), 4.26 – 4.38 (m, 1H, CH), 7.57 (7.60*) (d, J = 7.5 Hz, 1H, NH), 7.80 – 7.93 (m, 4H, Phth), 8.18 (8.22*) (d, J = 7.5 Hz, 1H, NH); (* two sets of signals due to diastereoisomers); ^{31}P NMR (DMSO-d_6) δ (ppm) 32.11, 32.02; Anal. calc. for $C_{23}H_{32}N_3O_{10}P$ (541.19) × $0.5H_2O$: C 50.18, H 6.04, N 7.63; found: C 49.89, H 5.81, N 7.42%; $[\alpha]_D^{20}$=-2.08 (c = 0.38, MeOH).

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