

## **Synthesis of New Conjugates of MDP and nor-MDP with Retro-Tuftsins derivatives as Potential Immunomodulators**

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Synthesis of new conjugates of muramyl dipeptide (MDP) and nor-muramyl dipeptide (nor-MDP) with retro-tuftsins derivatives is presented. The corresponding protected retro-tuftsins derivatives were also synthesized. The synthesis of a greater number of conjugates will enable structure-activity relationship studies.

**Key words:** muramyl dipeptide, MDP, nor-muramyl dipeptide, nor-MDP, retro-tuftsins derivatives, tuftsins derivatives, synthesis, immunomodulators

Muramyl dipeptide (MurNAc-L-Ala-D-isoGln; MDP) is the minimal bioactive structure of bacterial peptidoglycan (PGN) [1]. Among the many biological effects of MDP and its derivatives, the most promising for immunotherapy are: adjuvancity, stimulation of nonspecific resistance against bacterial, viral or parasitic infections, protection against tumors and somnogenicity. Numerous reviews on usefulness of muramyl peptides in immunotherapy have been published [2-8]. The another immunomodulator is tuftsins, a natural tetrapeptide of sequence H-Thr-Lys-Pro-Arg-OH (TKPR), present in the blood of humans and other mammals, capable of stimulating certain white blood cells (monocytes, macrophages, and neutrophils) [9-11]. Tuftsins exerts a wide spectrum of biological activities, notably enhances phagocytosis, immune response, bactericidal, tumoricidal and antifungal activities. One of

tuftsin derivatives is retro-tuftsin H-Arg-Pro-Lys-Thr-OH (RPKT) [12-15]. Because the data concerning the phagocytosis-stimulating activity of retro-tuftsin were inconsistent [14,15], we became interested in this derivative and its conjugates with muramyl peptides. Up to now, we have synthesized 12 conjugates of MDP and nor-MDP with tuftsin **1a-f** or retro-tuftsin **2a-f** (Figure 1) [16-18]. The idea behind preparation of these conjugates was based on the assumption that the activity of the product would combine the biological activities of both tuftsin or retro-tuftsin and MDP, or hopefully even enhance their individual effectiveness as immunostimulants. The immunostimulatory potency of the synthesized compounds was investigated at Medical University of Gdańsk, Poland. The results of experiments involving conjugates **1a-f**, showed their high ability to stimulate of TNF $\alpha$  (tumour necrosis factor  $\alpha$ ) and IL6 (interleukin 6) secretion by monocytes and lymphocytes, thus pointing to these compounds usefulness as potential therapeutic agents (mainly **1a** and **1c**). The observed effects were probably mediated by the induction of oxidative burst by the examined compounds [16]. The second group of conjugates **2a-f** differs from the first one, because it contains retro-tuftsin instead of native tuftsin [17]. A comparison of biological activity of native tuftsin and its retro analogue revealed that retro-tuftsin displayed biological activity similar to native tetrapeptide [18]. However, retro-tuftsin seems to be more stable and resistant to degradation, as we observed in the viability tests performed on subpopulation of white blood cells [18]. Therefore, we decided to synthesize the conjugates containing retro-tuftsin **2a-f**. Their immunomodulatory properties were assessed by using *in vitro* cultures of human subpopulations of white blood cells (peripheral blood mononuclear cells, peripheral blood lymphocytes, monocytes). We examined the viability of blood cells incubated with examined conjugates, as well as their ability to stimulate secretion of cytokines (TNF $\alpha$ , IL-6) and cytotoxic activity of NK (Natural Killer) cells. Two compounds **2a** and **2b** turned out to



exhibit greater activity in tests applied than both parent substances which suggests their usefulness as potential drugs [18].

Continuing our program of syntheses of MDP and nor-MDP conjugates as potential immunomodulators, we designed novel conjugates of MDP or nor-MDP with retro-tuftsins derivatives containing isopeptide bond between  $\epsilon$ -amino group of lysine and carboxylic group of simple amino acids such as alanine, glycine, leucine and valine. In current studies, tuftsins analogues containing isopeptide bond showed increased chemical resistance and activity in relation to tuftsins. The introduction of the additional residue at  $\epsilon$ -amino group of lysine by -NHCO- formation caused that isopeptide bond became stronger than peptide bond in central chain [19-22]. Finally, the combined use of muramyl peptides with other immunomodulators, e.g. such as tuftsins or other chemotherapeutics is promising in the therapy of different infections, autoimmunological diseases and anticancer therapy. Results of biological tests of these compounds will be reported in the future.

## RESULTS AND DISCUSSION

1-Benzyl-MDP **3a** and 1-benzyl-nor-MDP **3b** described in previous papers [23-25] were used for synthesis of conjugates with retro-tuftsins derivatives (H-Arg-Pro-Lys(Y)-Thr-OMe, Y=Ala,Gly,Leu,Val). Acylation of the Arg amino group of partially protected pentapeptides **5a-d** by MDP **3a** or nor-MDP **3b** was performed using the mixed anhydride method with isobutyl chloroformate and *N*-methyilmorpholine (NMM) in dry DMF (Table 1, Scheme 1). The protected conjugates **6a-j** were isolated and purified with a preparative TLC. The identities of the protected products were confirmed by high resolution  $^1\text{H-NMR}$  (500 MHz, COSY, TOCSY, ROESY, gHSQC, gHMBC) spectroscopy. The final products were hydrogenated with  $\text{H}_2/\text{Pd/C}$  in 50% methanol- acetic acid and purified with preparative TLC with C as a solvent system. The identities of the conjugates **7a-j** were confirmed by TLC



qualitative amino acid analysis, and elemental analyses. The protected pentapeptides **4a-d** were synthesized by the conventional chemical procedure using also mixed anhydride method (Scheme 2). Starting from HClxThr-OMe and Boc-Lys(Z)-OH gave dipeptide Boc-Lys(Z)-Thr-OMe. Removal of the Z-protecting groups in Boc-Lys(Z)-Thr-OMe by catalytic hydrogenation  $H_2/Pd/C$  and then coupling with Z-Y-OH (Y=Ala,Gly,Leu,Val) gave tripeptides: Boc-Lys(ZY)-Thr-OMe. Next the *tert*-butoxycarbonyl (Boc) group was removed by treatment with TFA and the obtained peptides were coupled to Boc-Pro-OH and Boc-Arg(NO<sub>2</sub>)-OH. The pentapeptides **4a-d** were isolated by column chromatography and purified with preparative TLC on silica gel. The amino acid composition of the protected pentapeptides **4a-d** was confirmed by the elemental analysis, the analysis of <sup>1</sup>H and <sup>13</sup>C-NMR (500 MHz) spectra and by the TLC qualitative amino acid analysis. Finally, from peptides **4a-d**, the Boc group was removed by treatment with TFA to give trifluoroacetate **5a-d**, which was used for synthesis of conjugates **7a-j** (Scheme 1).

## EXPERIMENTAL

Melting points (uncorrected) were determined on the Kofler-block apparatus. <sup>1</sup>H-NMR spectra were measured in DMSO or CDCl<sub>3</sub> solutions with a Varian 500 and 200 NMR spectrometers. Preparative column chromatography was performed on silica gel (Kieselgel 60, 100-200 mesh) in solvent systems specified in the text. All chemicals and solvents were of reagent grade and were used without further purification. The reactions were monitored by TLC on Merck F<sub>254</sub> silica gel precoated plates. The following solvent systems (by vol.) were used for TLC, radial and column chromatography development: (A) n-BuOH-pyridine-AcOH-H<sub>2</sub>O (60:45:4:30), (B) 2-propanol-H<sub>2</sub>O-AcOH (100:50:3), (C) n-BuOH-AcOH-H<sub>2</sub>O (4:2:2), (D) n-BuOH-AcOH-H<sub>2</sub>O (2:1:1), (E) CHCl<sub>3</sub>-MeOH (4:1), (F) CHCl<sub>3</sub>-MeOH (9:1). All synthesized protected peptides were homogeneous on TLC. Qualitative amino acid

analyses of the hydrolyzates of the compounds was performed by TLC. Detected by: UV and ninhydrin. The optical rotation values were measured on a Rudolph Research automatic polarimeter Autopol II.

In the following Glc stands for glucosamine and H stands for residue of the lactic or acetic acid. 1-*O*-Benzyl-*N*-acetyl-muramyl-L-amino acid-D-isoglutamine **3a** and 1-*O*-benzyl-*N*-acetyl-nor-muramyl-L-amino acid-D-isoglutamine **3b** were prepared in our laboratory [24,25]. The procedure for the synthesis of compounds **4a**, **4d** has been published previously [26].

**Boc-Arg(NO<sub>2</sub>)-Pro-Lys(ZGly)-Thr-OMe (4b).** To a stirred solution of Pro-Lys(ZGly)-Thr-OMe trifluoroacetate (0.840 g, 1.267 mmole) in anhydrous DMF (4 ml) cooled to -15 °C, NMM (0.14 ml, 1.267 mmole) and isobutyl chloroformate (0.16 ml, 1.267 mmole) were added, followed after 15 min. by an addition of cool solution of Boc-Arg(NO<sub>2</sub>)-OH (0.404 g, 1.267 mmole) in anhydrous DMF (2 ml) and the stirring was continued for 4 h in -15 °C and then for 24 h at room temperature. After evaporation of the solvent, the product was dissolved in ethyl acetate and washed with the following cooled liquids: solution of hydrochloric acid (5%), water, solution of potassium hydrogencarbonate (0.5 N), water and saturated sodium chloride solution, and then dried with magnesium sulfate. After evaporation of ethyl acetate in a vacuum rotary evaporator the raw product was purified using column chromatography (Silica Gel 60 230-400 mesh ASTM, 0.040-0.063 mm) in solvent F and preparative TLC to obtain compound **4b** (Table 2). <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>) δ: 1.05 (d, *J* = 6.4 Hz, 3H, T-CH<sub>3</sub>), 1.34 (m, 2H, K-γCH<sub>2</sub>), 1.39 (m, 2H, K-δCH<sub>2</sub>), 1.36 (s, 9H, *t*-Bu), 1.49 (m, 1H, R-βCH), 1.53 (m, 1H, K-βCH), 1.57(m, 2H, R-γCH<sub>2</sub>), 1.61 (m, 1H, R-βCH), 1.67 (m, 1H, K-βCH), 1.82 (m, 1H, P-βCH), 1.59 (m, 2H, R-γCH<sub>2</sub>), 1.90 (m, 2H, P-γCH), 2.02 (m, 1H, P-βCH), 3.05 (m, 2H, K-εCH<sub>2</sub>), 3.13 (m, 2H, R-δCH<sub>2</sub>), 3.56 (m, 1H, P-δCH), 3.60 (m, 2H, G-αCH), 3.62 (m, 1H, P-δCH), 3.63 (s, 3H, COOCH<sub>3</sub>), 4.12 (ddq, *J* = 3.2 Hz, *J* = 5.5 Hz, *J* = 6.4 Hz, 1H, T-βCH), 4.16 (m, 1H, R-αCH), 4.28 (dd, *J* = 3.2 Hz, *J* = 8.3 Hz, 1H, T-αCH), 4.30 (m, 1H, K-αCH),

4.39 (dd,  $J = 3.7$  Hz,  $J = 8.1$  Hz, 1H, P- $\alpha$ CH), 5.00 (d,  $J = 5.5$  Hz, 1H, T-OH), 5.04 (s, 2H, Z-CH<sub>2</sub>), 6.95 (d,  $J = 7.7$  Hz, 1H, R- $\alpha$ NH), 7.3-7.38 (m, 5H, Ph), 7.40 (t,  $J = 6.3$  Hz, 1H, G- $\alpha$ NH), 7.78 (d,  $J = 8.3$  Hz, 1H, T- $\alpha$ NH), 7.83 (t,  $J = 5.5$  Hz, 1H, K- $\epsilon$ NH), 8.02 (d,  $J = 7.9$  Hz, 1H, K- $\alpha$ NH), 8.49 (bs, 1H, R- $\delta$ NH). <sup>13</sup>C NMR (125.7 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 20.0 (T-CH<sub>3</sub>), 22.6 (K- $\gamma$ CH), 24.5 (P and R- $\gamma$ CH<sub>2</sub>), 28.2 (Boc-CH<sub>3</sub>), 28.0 (R- $\beta$ CH<sub>2</sub>), 28.7 (K- $\delta$ CH), 29.0 (P- $\beta$ CH<sub>2</sub>), 31.4 (K- $\beta$ CH), 38.4 (K- $\epsilon$ CH<sub>2</sub>), 40.5 (R- $\delta$ CH<sub>2</sub>), 43.5 (G- $\alpha$ CH), 46.7 (P- $\delta$ CH<sub>2</sub>), 51.8 (T-OCH<sub>3</sub>), 52.3 (K- $\alpha$ CH), 51.7 (R- $\alpha$ CH), 57.6 (T- $\alpha$ CH), 59.1 (P- $\alpha$ CH), 65.4 (Z-CH<sub>2</sub>), 66.2 (T- $\beta$ CH), 78.0 (C(CH<sub>3</sub>)<sub>3</sub>), 127.7, 127.8, 128.3, 137.1 (Ph), 155.4 (Boc-CO), 156.5 (Z-CO), 159.3 (C=NNO<sub>2</sub>), 168.8, 170.5, 171.0, 171.5, 172.2 (CO). Anal. Calcd. for C<sub>37</sub>H<sub>58</sub>N<sub>10</sub>O<sub>13</sub>: C, 52.23; H, 6.87; N, 16.46. Found: C, 52.12; H, 6.91; N, 16.39.

**Boc-Arg(NO<sub>2</sub>)-Pro-Lys(ZLeu)-Thr-OMe (4c).** To a stirred solution of Pro-Lys(ZLeu)-Thr-OMe trifluoroacetate (0.912 g, 1.267 mmole) in anhydrous DMF (4 ml) cooled to -15 °C, NMM (0.14 ml, 1.267 mmole) and isobutyl chloroformate (0.16 ml, 1.267 mmole) were added, followed after 15 min. by an addition of cool solution of Boc-Arg(NO<sub>2</sub>)-OH (0.404 g, 1.267 mmole) in anhydrous DMF (2 ml) and the stirring was continued for 4 h in -15 °C and then for 24 h at room temperature. After evaporation of the solvent, the product was dissolved in ethyl acetate and washed with the following cooled liquids: solution of hydrochloric acid (5%), water, solution of potassium hydrogencarbonate (0.5 N), water and saturated sodium chloride solution, and then dried with magnesium sulfate. After evaporation of ethyl acetate in a vacuum rotary evaporator the raw product was purified using column chromatography (Silica Gel 60 230-400 mesh ASTM, 0.040-0.063 mm) in solvent F and preparative TLC to obtain compound **4c** (Table 2). <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>)  $\delta$ : 0.84 (d,  $J = 6.6$  Hz, 3H, L-CH<sub>3</sub>), 0.85 (d,  $J = 6.6$  Hz, 3H, L-CH<sub>3</sub>), 1.04 (d,  $J = 6.3$  Hz, 3H, T-CH<sub>3</sub>), 1.32 (m, 2H, K- $\gamma$ CH<sub>2</sub>), 1.37 (m, 2H, K- $\delta$ CH<sub>2</sub>), 1.37 (s, 9H, *t*-Bu), 1.40 (m, 2H, L- $\beta$ CH<sub>2</sub>), 1.48 (m, 1H, R- $\beta$ CH), 1.52 (m, 1H, K- $\beta$ CH), 1.56 (m, 2H, R- $\gamma$ CH<sub>2</sub>), 1.57 (m, 2H, L- $\gamma$ CH), 1.62 (m, 1H, R- $\beta$ CH), 1.68 (m, 1H,

K-βCH), 1.82 (m, 1H, P-βCH), 1.88 (m, 2H, P-γCH), 2.01 (m, 1H, P-βCH), 3.02 (m, 2H, K-εCH<sub>2</sub>), 3.13 (m, 2H, R-δCH<sub>2</sub>), 3.54 (m, 1H, P-δCH), 3.62 (m, 1H, P-δCH), 3.62 (s, 3H, COOCH<sub>3</sub>), 3.99 (dt, *J* = 5.2 Hz, *J* = 9.0 Hz, 1H, L-αCH), 4.12 (m, 1H, T-βCH), 4.15 (m, 1H, R-αCH), 4.29 (dd, *J* = 3.4 Hz, *J* = 8.5 Hz, 1H, T-αCH), 4.28 (m, 1H, K-αCH), 4.38 (dd, *J* = 4.0 Hz, *J* = 8.3 Hz, 1H, P-αCH), 5.01 (d, *J* = 5.7 Hz, 1H, T-OH), 5.03 (m, 2H, Z-CH<sub>2</sub>), 7.3-7.37 (m, 5H, Ph), 6.93 (d, *J* = 7.3 Hz, 1H, R-NH), 7.35 (bs, 1H, L-αNH), 7.79 (d, *J* = 8.5 Hz, 1H, T-αNH), 7.90 (t, *J* = 5.5 Hz, 1H, K-εNH), 8.03 (d, *J* = 7.8 Hz, 1H, K-αNH), 8.50 (bs, 1H, R-δNH). <sup>13</sup>C NMR (125.7 MHz, DMSO-d<sub>6</sub>) δ: 20.0 (T-CH<sub>3</sub>), 21.5 and 22.9 (L-δCH<sub>2</sub>), 22.5 (K-γCH<sub>2</sub>), 24.2 (P-γCH<sub>2</sub>), 24.4 (L and R-γCH<sub>2</sub>), 28.0 (R-βCH), 28.2 (Boc-CH<sub>3</sub>), 28.8 (K-δCH<sub>2</sub>), 28.9 (P-δCH<sub>2</sub>), 31.3 (K-βCH<sub>2</sub>), 38.4 (K-εCH<sub>2</sub>), 40.4 (R-δCH<sub>2</sub>), 40.9 (L-βCH<sub>2</sub>), 46.6 (P-δCH<sub>2</sub>), 51.6 (R-αCH), 51.8 (T-OCH<sub>3</sub>), 52.4 (K-αCH), 53.1 (L-αCH), 57.6 (T-αCH), 59.1 (P-αCH), 65.3 (Z-CH<sub>2</sub>), 66.2 (T-βCH), 78.0 (C(CH<sub>3</sub>)<sub>3</sub>), 127.6, 127.7, 128.3, 137.0 (Ph), 155.4 (Boc-CO), 155.8 (Z-CO), 159.3 (C=NNO<sub>2</sub>), 170.4, 170.9, 171.4, 172.0, 172.1 (CO). Anal. Calcd. for C<sub>41</sub>H<sub>66</sub>N<sub>10</sub>O<sub>13</sub>: C, 54.29; H, 7.33; N, 15.44. Found: C, 54.22; H, 7.28; N, 15.37.

**General procedures for the syntheses of conjugates 7a-j.** To a stirred solution of MDP **3a** or nor-MDP **3b** derivatives (0.1108 mmol) in anhydrous DMF (1 ml) cooled to -15 °C, NMM (0.1108 mmol) and isobutyl chloroformate (0.1108 mmol) were added, followed after 15 min. by the addition of cooled solution of Arg(NO<sub>2</sub>)-Pro-Lys(ZY)-Thr-OMe trifluoroacetate (0.1293 mmol) and NMM (0.1293 mmol) in anhydrous DMF (0.5 ml) and the stirring was continued for 4 h in -15 °C and then for 24 h at room temperature. After evaporation of the solvent, the reaction mixture was purified using radial chromatography and preparative TLC in solvent E or F to obtain compounds **6a-j**. Protected compounds **6a-j** gave satisfactory elemental analysis and NMR (500 MHz, COSY, TOCSY, ROESY, gHSQC,

gHMBC) spectroscopy. I present below the NMR spectra of the four compounds **6a**, **6c**, **6g** and **6i**.

**1-O-Bn-nor-Mur(NAc)-Ala-D-Glu(Arg(NO<sub>2</sub>)-Pro-Lys(ZLeu)-Thr-OMe)-NH<sub>2</sub> (6a).**

<sup>1</sup>H-NMR (DMSO) δ: 0.82-0.84 (dd  $J = 6.5$  Hz, 6H, L-CH<sub>3</sub>), 1.02 (d,  $J = 6.3$  Hz, 3H, T-CH<sub>3</sub>), 1.20 (d,  $J = 7.3$  Hz, 3H, A-CH<sub>3</sub>), 1.30 (m, 2H, K-γCH<sub>2</sub>), 1.37 (m, 4H, L-βCH<sub>2</sub>, K-δCH<sub>2</sub>), 1.49-1.52 (m, 4H, K-βCH, R-βCH, R-γCH<sub>2</sub>), 1.58 (m, 2H, L-γCH), 1.63-1.65 (m, 3H, R-βCH, K-βCH, Q-βCH), 1.80 (m, 1H, P-βCH), 1.86 (s, 3H, Glc-CH<sub>3</sub>CON), 1.87 (m, 2H, P-γCH<sub>2</sub>), 1.97 (m, 1H, Q-βCH), 2.04 (m, 1H, P-βCH), 2.12 (m, 2H, Q-γCH<sub>2</sub>), 2.98 (m, 2H, K-εCH<sub>2</sub>), 3.12 (m, 2H, R-δCH<sub>2</sub>), 3.38 (dt,  $J = 6.2$  Hz,  $J = 8.9$  Hz, 1H, Glc4-CH), 3.50 (m, 2H, Glc3-CH, Glc5-CH), 3.54 (m, 1H, Glc6-CH), 3.54 (m, 1H, P-δCH), 3.62 (m, 1H, P-δCH), 3.60 (s, 3H, T-COOCH<sub>3</sub>), 3.65 (m, 1H, Glc6-CH), 3.84 (ddd,  $J = 3.4$  Hz,  $J = 8.7$  Hz,  $J = 10.4$  Hz, 1H, Glc2-CH), 3.96 (m, 1H, L-αCH), 4.09 (d,  $J = 16.1$  Hz, 1H, H-CH), 4.11 (m, 2H, T-βCH, Q-αCH), 4.29 (m, 1H, T-αCH), 4.20 (d,  $J = 16.0$  Hz, 1H, H-CH), 4.25 (m, 1H, K-αCH), 4.32 (m, 1H, A-αCH), 4.35 (dd,  $J = 3.7$  Hz,  $J = 8.2$  Hz, 1H, P-αCH), 4.45 (d,  $J = 12.3$  Hz, 1H, Glc-CH<sub>2</sub>φ), 4.45 (m, 1H, R-αCH), 4.66 (t,  $J = 5.5$  Hz, 1H, Glc6-OH), 4.67 (d,  $J = 12.3$  Hz, 1H, Glc-CH<sub>2</sub>φ), 4.72 (d,  $J = 3.6$  Hz, 1H, Glc1-CH), 5.02 (d,  $J = 5.6$  Hz, 1H, T-OH), 5.0 (s, 2H, L-ZCH<sub>2</sub>), 5.78 (d,  $J = 6.3$  Hz, 1H, Glc4-OH), 6.95 (bs, 2H, R-NH<sub>2</sub>), 7.06 and 7.26 (s,s, 1H, 1H, Q-CONH<sub>2</sub>), 7.19 (t,  $J = 5.3$  Hz, 1H, K-εNH), 7.26-7.37 (m, 5H, φ), 7.36 (bs, 1H, L-αNH), 7.82 (d,  $J = 8.3$  Hz, 1H, T-NH), 7.96 (d,  $J = 7.7$  Hz, 1H, K-αNH), 8.04 (d,  $J = 6.6$  Hz, 1H, R-αNH), 8.09 (d,  $J = 6.7$  Hz, 1H, A-αNH), 8.17 (d,  $J = 8.4$  Hz, 1H, Glc-NH), 8.23 (d,  $J = 8.2$  Hz, 1H, Q-NH), 8.52 (bs, 1H, R-δNH).

**1-O-Bn-nor-Mur(NAc)-Ala-D-Glu(Arg(NO<sub>2</sub>)-Pro-Lys(ZVal)-Thr-OMe)-NH<sub>2</sub> (6c).**

<sup>1</sup>H-NMR (DMSO) δ: 0.84 and 0.86 (dd,  $J = 6.5$  Hz, 6H, V-CH<sub>3</sub>), 1.05 (d,  $J = 6.4$  Hz, 3H, T-CH<sub>3</sub>), 1.24 (d,  $J = 6.7$  Hz, 3H, A-CH<sub>3</sub>), 1.32 (m, 2H, K-γCH<sub>2</sub>), 1.40 (m, 2H, K-δCH<sub>2</sub>), 1.50





(m, 1H, R-βCH), 1.52 (m, 1H, K-βCH), 1.54 (m, 2H, R-γCH<sub>2</sub>), 1.68 (m, 1H, K-βCH), 1.67 (m, 1H, R-βCH), 1.84 (s, 3H, Glc-CH<sub>3</sub>CON), 1.83 (m, 1H, P-βCH), 1.86 (m, 2H, P-γCH<sub>2</sub>), 1.92 (m, 1H, V-βCH), 1.95 (m, 1H, Q-βCH), 2.03 (m, 1H, P-βCH), 2.12 (m, 2H, Q-γCH<sub>2</sub>), 2.98 (m, 1H, K-εCH<sub>2</sub>), 3.08 (m, 1H, K-εCH<sub>2</sub>), 3.13 (m, 2H, R-δCH<sub>2</sub>), 3.40 (m, 1H, Glc4-CH), 3.46 (m, 1H, Glc5-CH), 3.53 (m, 2H, Glc3-CH, Glc6-CH), 3.53 (m, 1H, Glc6-CH), 3.56 (m, 1H, P-δCH), 3.64 (m, 1H, P-δCH), 3.63 (s, 3H, T-COOCH<sub>3</sub>), 3.67 (m, 1H, Glc6-CH), 3.78 (dd,  $J = 7.4$  Hz,  $J = 8.5$  Hz, 1H, V-αCH), 3.86 (ddd,  $J = 3.6$  Hz,  $J = 8.3$  Hz,  $J = 10.6$  Hz, 1H, Glc2-CH), 4.09 (d,  $J = 16.3$  Hz, 1H, H-CH), 4.12 (m, 2H, T-βCH, Q-αCH), 4.27 (m, 1H, T-αCH), 4.21 (d,  $J = 16.3$  Hz, 1H, H-CH), 4.29 (m, 1H, K-αCH), 4.31 (m, 1H, A-αCH), 4.37 (dd,  $J = 3.7$  Hz,  $J = 8.1$  Hz, 1H, P-αCH), 4.44 (d,  $J = 12.3$  Hz, 1H, Glc-CH<sub>2</sub>φ), 4.48 (m, 1H, R-αCH), 4.65 (t,  $J = 5.5$  Hz, 1H, Glc6-OH), 4.68 (d,  $J = 12.3$  Hz, 1H, Glc-CH<sub>2</sub>φ), 4.71 (d,  $J = 3.6$  Hz, 1H, Glc1-CH), 5.0 (m, 1H, T-OH), 5.02 (s, 2H, V-ZCH<sub>2</sub>), 5.81 (d,  $J = 5.7$  Hz, 1H, G4-OH), 6.95 (bs, 2H, R-NH<sub>2</sub>), 7.08 and 7.29 (s,s, 1H, 1H, Q-CONH<sub>2</sub>), 7.22 (d,  $J = 8.5$  Hz, 1H, V-NH), 7.92 (t,  $J = 5.3$  Hz, 1H, K-εNH), 7.26-7.41 (m, 10H, φ), 7.79 (d,  $J = 8.1$  Hz, 1H, T-NH), 8.00 (d,  $J = 7.6$  Hz, 1H, K-αNH), 8.11 (d,  $J = 7.6$  Hz, 1H, R-αNH), 8.06 (d,  $J = 6.6$  Hz, 1H, A-αNH), 8.15 (d,  $J = 8.3$  Hz, 1H, Glc-NH), 8.18 (d,  $J = 8.2$  Hz, 1H, Q-NH), 8.49 (bs, 1H, R-δNH). <sup>13</sup>C NMR (125.7 MHz, DMSO-d<sub>6</sub>) δ: 19.0 (A-CH<sub>3</sub>), 19.2; 20.0 (V-CH<sub>3</sub>), 20.9 (T-CH<sub>3</sub>), 23.5 (AcN-CH<sub>3</sub>), 23.5 (K-γCH<sub>2</sub>), 25.4 (R-γCH<sub>2</sub>), 25.4 (P-δCH<sub>2</sub>), 28.5 (Q-βCH), 28.0 (R-βCH), 29.6 (K-εCH<sub>2</sub>), 30.0 (P-βCH<sub>2</sub>), 31.0 (V-βCH<sub>2</sub>), 32.4 (Q-γCH<sub>2</sub>), 32.3 (K-βCH<sub>2</sub>), 39.2 (K-δCH<sub>2</sub>), 41.1 (R-δCH<sub>2</sub>), 47.8 (P-γCH<sub>2</sub>), 48.9 (A-αCH), 50.6 (R-αCH), 52.6 (T-OCH<sub>3</sub>), 52.9 (Q-αCH), 53.1 (K-αCH), 53.4 (Glc2), 58.3 (T-αCH), 61.2 (V-αCH), 60.0 (P-αCH), 61.2 (Glc6), 66.2 (Z-CH<sub>2</sub>), 66.9 (T-βCH), 68.6 (Bn-CH<sub>2</sub>), 70.5 (Glc4), 71.4 (H-CH), 73.8 (Glc5), 81.5 (Glc3), 127-130 (Ph), 156.9 (Z-CO), 159.3 (C=NNO<sub>2</sub>), 96.9 (Glc1), 170.2, 170.9, 171.7, 172.9 (CO).

**1-O-Bn-Mur(NAc)-Val-D-Glu(Arg(NO<sub>2</sub>)-Pro-Lys(ZVal)-Thr-OMe)-NH<sub>2</sub> (6g).** <sup>1</sup>H-NMR (DMSO) δ: 0.81 (dd, *J* = 6.6 Hz, 6H, V-CH<sub>3</sub>), 1.03 (d, *J* = 6.3 Hz, 3H, T-CH<sub>3</sub>), 1.26 (d, *J* = 6.7 Hz, 3H, H<sub>3</sub>-CH<sub>3</sub>), 1.31 (m, 2H, K-γCH<sub>2</sub>), 1.37 (m, 2H, K-δCH<sub>2</sub>), 1.49 (m, 1H, R-βCH), 1.51 (m, 4H, K-βCH, R-γCH<sub>2</sub>), 1.65 (m, 1H, R-βCH), 1.66 (m, 2H, K-βCH, Q-βCH), 1.79 (s, 3H, Glc-CH<sub>3</sub>CON), 1.81 (m, 1H, P-βCH), 1.82 (m, 1H, P-γCH<sub>2</sub>), 1.89 (m, 1H, P-γCH<sub>2</sub>), 1.91 (m, 1H, V-βCH), 1.95 (m, 1H, Q-βCH), 1.99 (m, 1H, P-βCH), 2.11 (m, 2H, Q-γCH<sub>2</sub>), 2.97 (m, 2H, K-εCH<sub>2</sub>), 3.12 (m, 2H, R-δCH<sub>2</sub>), 3.31 (m, 1H, Glc4-CH), 3.49-3.63 (m, 5H, Glc3-CH, Glc5-CH, Glc6-CH, P-δCH<sub>2</sub>), 3.62 (s, 3H, T-COOCH<sub>3</sub>), 3.65 (m, 1H, Glc6-CH), 3.72 (m, 1H, Glc2-CH), 3.77 (t, *J* = 8.0 Hz, 1H, V2-αCH), 4.12 (m, 1H, T-βCH), 4.16 (m, 1H, Q-αCH), 4.25 (m, 1H, T-αCH), 4.27 (t, *J* = 7.0 Hz, 1H, V1-αCH), 4.28 (m, 1H, K-αCH), 4.36 (dd, *J* = 3.9 Hz, *J* = 7.8 Hz, 1H, P-αCH), 4.45 (d, *J* = 12.6 Hz, 1H, Glc-CH<sub>2</sub>φ), 4.46 (m, 1H, R-αCH), 4.46 (m, 1H, H<sub>2</sub>-CH), 4.60 (t, *J* = 4.8 Hz, 1H, Glc6-OH), 4.68 (d, *J* = 12.6 Hz, 1H, Glc-CH<sub>2</sub>φ), 4.82 (d, *J* = 3.3 Hz, 1H, G1-CH), 5.02 (d, *J* = 5.3 Hz, 1H, T-OH), 5.04 (s, 2H, V-ZCH<sub>2</sub>), 5.36 (d, *J* = 7.0 Hz, 1H, Glc4-OH), 6.86 (bs, 2H, R-NH<sub>2</sub>), 7.02 and 7.35 (bs, 2H, Q-CONH<sub>2</sub>), 7.23 (d, *J* = 8.4 Hz, 1H, V2-NH), 7.27-7.40 (m, 5H, φ), 7.61 (d, *J* = 8.2 Hz, 1H, V1-NH), 7.8 (d, *J* = 5.3 Hz, 1H, T-NH), 7.92 (t, *J* = 5.3 Hz, 1H, K-εNH), 8.01 (d, *J* = 7.7 Hz, 1H, K-αNH), 8.12 (d, *J* = 8.2 Hz, 1H, R-αNH), 8.27 (d, *J* = 7.2 Hz, 1H, Glc-NH), 8.32 (d, *J* = 7.9 Hz, 1H, Q-NH), 8.50 (bs, 1H, R-δNH). <sup>13</sup>C NMR (125.7 MHz, DMSO-d<sub>6</sub>) δ: 18.5; 32.0 (V1-CH<sub>3</sub>), 19.0; 31.0 (V2-CH<sub>3</sub>), 19.5 (H-CH<sub>3</sub>), 20.5 (T-CH<sub>3</sub>), 23.0 (AcN-CH<sub>3</sub>), 23.5 (K-γCH<sub>2</sub>), 24.5 (R-γCH<sub>2</sub>), 24.5 (P-γCH<sub>2</sub>), 28.5 (Q-βCH), 28.5 (R-βCH), 29.5 (K-εCH<sub>2</sub>), 29.5 (P-βCH<sub>2</sub>), 31.0 (V-βCH<sub>2</sub>), 32.0 (K-βCH<sub>2</sub>), 32.4 (Q-γCH<sub>2</sub>), 39.0 (K-δCH<sub>2</sub>), 41.0 (R-δCH<sub>2</sub>), 47.5 (P-δCH<sub>2</sub>), 50.5 (R-αCH), 52.5 (T-OCH<sub>3</sub>), 52.9 (Q-αCH), 53.0 (K-αCH), 54.0 (Glc2), 58.0 (T-αCH), 58.0 (V1-αCH), 60.0 (P-αCH), 61.0 (V2-αCH), 61.0 (Glc6), 66.0 (Z-CH<sub>2</sub>), 67.0 (T-

$\beta$ CH), 68.5 (Bn-CH<sub>2</sub>), 70.0 (Glc4), 74.0 (Glc5), 76.0 (H-CH), 78.5 (Glc3), 127-130 (Ph), 157.0 (Z-CO), 159.2 (C=NNO<sub>2</sub>), 96.4 (Glc1), 170.0, 171.5, 172.0, 172.5, 173.0, 174.0 (CO).

**1-O-Bn-Mur(NAc)-Ala-D-Glu(Arg(NO<sub>2</sub>)-Pro-Lys(ZGly)-Thr-OMe)-NH<sub>2</sub> (6i).** <sup>1</sup>H-NMR (DMSO)  $\delta$ : 1.04 (d,  $J = 6.3$  Hz, 3H, T-CH<sub>3</sub>), 1.22 (d,  $J = 6.6$  Hz, 3H, A-CH<sub>3</sub>), 1.23 (d,  $J = 6.7$  Hz, 3H, H<sub>3</sub>-CH<sub>3</sub>), 1.27 (m, 2H, K- $\gamma$ CH<sub>2</sub>), 1.40 (m, 2H, K- $\delta$ CH<sub>2</sub>), 1.48-150 (m, 4H, K- $\beta$ CH, R- $\beta$ CH, R- $\gamma$ CH<sub>2</sub>), 1.61 (m, 3H, R- $\beta$ CH, K- $\beta$ CH, Q- $\beta$ CH), 1.80 (s, 3H, Glc-CH<sub>3</sub>CON), 1.83 (m, 2H, P- $\gamma$ CH<sub>2</sub>), 1.89 (m, 2H, P- $\beta$ CH), 1.91 (m, 1H, Q- $\beta$ CH), 2.06 (m, 1H, P- $\beta$ CH), 2.16 (m, 2H, Q- $\gamma$ CH<sub>2</sub>), 2.96 (m, 2H, K- $\epsilon$ CH<sub>2</sub>), 3.14 (m, 2H, R- $\delta$ CH<sub>2</sub>), 3.46 (m, 1H, Glc4-CH), 3.50 (m, 2H, Glc3-CH, Glc5-CH), 3.51 (m, 1H, Glc6-CH), 3.54 (m, 1H, P- $\delta$ CH), 3.60 (m, 1H, P- $\delta$ CH), 3.60 (s, 3H, T-COOCH<sub>3</sub>), 3.62 (m, 2H, G- $\alpha$ CH), 3.66 (m, 1H, Glc6-CH), 3.83 (m, 1H, Glc2-CH), 4.10 (m, 2H, T- $\beta$ CH, Q- $\alpha$ CH), 4.27 (m, 1H, T- $\alpha$ CH), 4.29 (m, 1H, K- $\alpha$ CH), 4.33 (m, 1H, A- $\alpha$ CH), 4.36 (dd,  $J = 3.7$  Hz,  $J = 7.9$  Hz, 1H, P- $\alpha$ CH), 4.41 (d,  $J = 12.5$  Hz, 1H, Glc-CH<sub>2</sub> $\phi$ ), 4.44 (m, 1H, R- $\alpha$ CH), 4.55 (t,  $J = 5.7$  Hz, 1H, Glc6-OH), 4.63 (d,  $J = 12.5$  Hz, 1H, G-CH<sub>2</sub> $\phi$ ), 4.71 (d,  $J = 3.3$  Hz, 1H, Glc1-CH), 5.01 (d,  $J = 5.4$  Hz, 1H, T-OH), 5.03 (s, 2H, G-ZCH<sub>2</sub>), 5.32 (d,  $J = 6.4$  Hz, 1H, Glc4-OH), 6.91 (bs, 2H, R-NH<sub>2</sub>), 7.05 and 7.24 (s,s, 1H, 1H, Q-CONH<sub>2</sub>), 7.78 (t,  $J = 5.3$  Hz, 1H, K- $\epsilon$ NH), 7.24-7.40 (m, 5H,  $\phi$ ), 7.42 (t,  $J = 6.2$  Hz, 1H, G- $\alpha$ NH), 7.56 (d,  $J = 7.2$  Hz, 1H, T-NH), 7.83 (d,  $J = 8.4$  Hz, 1H, K- $\alpha$ NH), 7.99 (d,  $J = 7.6$  Hz, 1H, R- $\alpha$ NH), 8.13 (d,  $J = 8.1$  Hz, 1H, Glc-NH), 8.19 (d,  $J = 8.2$  Hz, 1H, Q-NH), 8.47 (bs, 1H, R- $\delta$ NH).

The final products **7a-j** were hydrogenolysed in 50 % methanolic acetic acid containing palladium black, purified with preparative TLC in solvent C and were lyophilized to give hygroscopic solids.

**nor-Mur(NAc)-Ala-D-Glu(Arg-Pro-Lys(Leu)-Thr-OMe)-NH<sub>2</sub> (7a).** Yield 38 %; amino acid analysis (6 M, 110 °C, 20 h): Ala, Arg, Glu, Lys, Leu, Pro, Thr. Anal. Calcd. for

(C<sub>48</sub>H<sub>87</sub>N<sub>13</sub>O<sub>17</sub> x 2CH<sub>3</sub>COOH x H<sub>2</sub>O): C, 49.71; H, 7.78; N, 14.49. Found: C, 49.86; H, 7.87; N, 14.41.

**nor-Mur(NAc)-Ala-D-Glu(Arg-Pro-Lys(Ala)-Thr-OMe)-NH<sub>2</sub> (7b).** Yield 42 %; amino acid analysis (6 M, 110 °C, 20 h): Ala, Arg, Glu, Lys, Pro, Thr. Anal. Calcd. for (C<sub>45</sub>H<sub>81</sub>N<sub>13</sub>O<sub>17</sub> x 2CH<sub>3</sub>COOH x H<sub>2</sub>O): C, 48.47; H, 7.55; N, 15.00. Found: C, 48.55; H, 7.67; N, 14.85.

**nor-Mur(NAc)-Ala-D-Glu(Arg-Pro-Lys(Val)-Thr-OMe)-NH<sub>2</sub> (7c).** Yield 39 %; amino acid analysis (6 M, 110 °C, 20 h): Ala, Arg, Glu, Lys, Pro, Thr, Val. Anal. Calcd. for (C<sub>47</sub>H<sub>85</sub>N<sub>13</sub>O<sub>17</sub> x 2CH<sub>3</sub>COOH x H<sub>2</sub>O): C, 49.30; H, 7.71; N, 14.66. Found: C, 49.56; H, 7.86; N, 14.57.

**nor-Mur(NAc)-Pro-D-Glu(Arg-Pro-Lys(Val)-Thr-OMe)-NH<sub>2</sub> (7d).** Yield 36 %; amino acid analysis (6 M, 110 °C, 20 h): Ala, Arg, Glu, Lys, Pro, Thr, Val. Anal. Calcd. for (C<sub>49</sub>H<sub>87</sub>N<sub>13</sub>O<sub>17</sub> x 2CH<sub>3</sub>COOH x H<sub>2</sub>O): C, 50.19; H, 7.71; N, 14.36. Found: C, 50.35; H, 7.80; N, 14.43.

**nor-Mur(NAc)-Pro-D-Glu(Arg-Pro-Lys(Leu)-Thr-OMe)-NH<sub>2</sub> (7e).** Yield 35 %; amino acid analysis (6 M, 110 °C, 20 h): Ala, Arg, Glu, Lys, Leu, Pro, Thr. Anal. Calcd. for (C<sub>50</sub>H<sub>89</sub>N<sub>13</sub>O<sub>17</sub> x 2CH<sub>3</sub>COOH x H<sub>2</sub>O): C, 50.57; H, 7.78; N, 14.20. Found: C, 50.65; H, 7.82; N, 14.12.

**Mur(NAc)-Val-D-Glu(Arg-Pro-Lys(Ala)-Thr-OMe)-NH<sub>2</sub> (7f).** Yield 40 %; amino acid analysis (6 M, 110 °C, 20 h): Ala, Arg, Glu, Lys, Pro, Thr. Anal. Calcd. for (C<sub>48</sub>H<sub>87</sub>N<sub>13</sub>O<sub>17</sub> x 2CH<sub>3</sub>COOH x H<sub>2</sub>O): C, 49.71; H, 7.78; N, 14.49. Found: C, 49.82; H, 7.81; N, 14.56.

**Mur(NAc)-Val-D-Glu(Arg-Pro-Lys(Val)-Thr-OMe)-NH<sub>2</sub> (7g).** Yield 38 %; amino acid analysis (6 M, 110 °C, 20 h): Ala, Arg, Glu, Lys, Pro, Thr, Val. Anal. Calcd. for (C<sub>50</sub>H<sub>91</sub>N<sub>13</sub>O<sub>17</sub> x 2CH<sub>3</sub>COOH x H<sub>2</sub>O): C, 50.49; H, 7.93; N, 14.18. Found: C, 50.55; H, 8.02; N, 14.07.



**Mur(NAc)-Val-D-Glu(Arg-Pro-Lys(Gly)-Thr-OMe)-NH<sub>2</sub> (7h).** Yield 36 %; amino acid analysis (6 M, 110 °C, 20 h): Ala, Arg, Glu, Gly, Lys, Pro, Thr. Anal. Calcd. for (C<sub>47</sub>H<sub>85</sub>N<sub>13</sub>O<sub>17</sub> x 2CH<sub>3</sub>COOH x H<sub>2</sub>O): C, 49.30; H, 7.71; N, 14.66. Found: C, 49.46; H, 7.77; N, 14.57.

**Mur(NAc)-Ala-D-Glu(Arg-Pro-Lys(Gly)-Thr-OMe)-NH<sub>2</sub> (7i).** Yield 41 %; amino acid analysis (6 M, 110 °C, 20 h): Ala, Arg, Glu, Gly, Lys, Pro, Thr. Anal. Calcd. for (C<sub>45</sub>H<sub>81</sub>N<sub>13</sub>O<sub>17</sub> x 2CH<sub>3</sub>COOH x H<sub>2</sub>O): C, 48.47; H, 7.55; N, 15.00. Found: C, 48.58; H, 7.64; N, 14.87.

**Mur(NAc)-Ala-D-Glu(Arg-Pro-Lys(Val)-Thr-OMe)-NH<sub>2</sub> (7j).** Yield 42 %; amino acid analysis (6 M, 110 °C, 20 h): Ala, Arg, Glu, Lys, Pro, Thr, Val. Anal. Calcd. for (C<sub>48</sub>H<sub>87</sub>N<sub>13</sub>O<sub>17</sub> x 2CH<sub>3</sub>COOH x H<sub>2</sub>O): C, 49.71; H, 7.78; N, 14.49. Found: C, 49.85; H, 7.86; N, 14.38.

#### Acknowledgments

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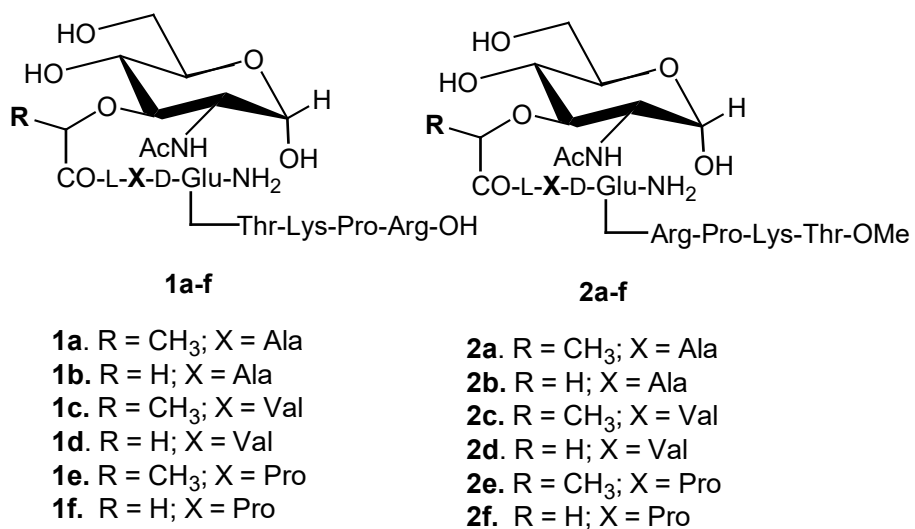
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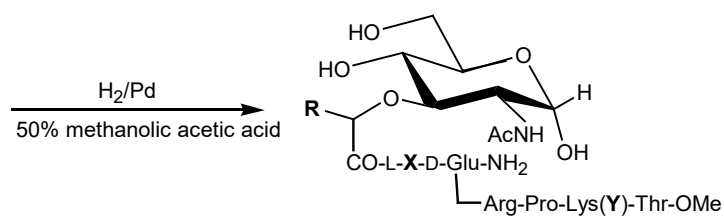
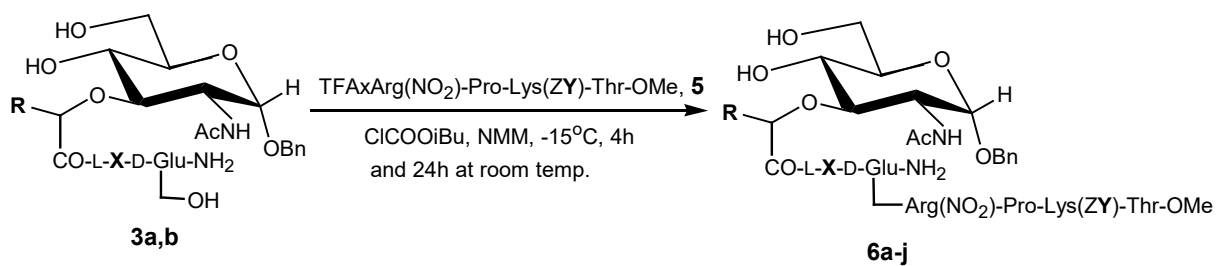
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**Figure 1.** Synthesized conjugates of MDP or nor-MDP with tuftsin **1a-f** and retro-tuftsin **2a-f** [16-18].



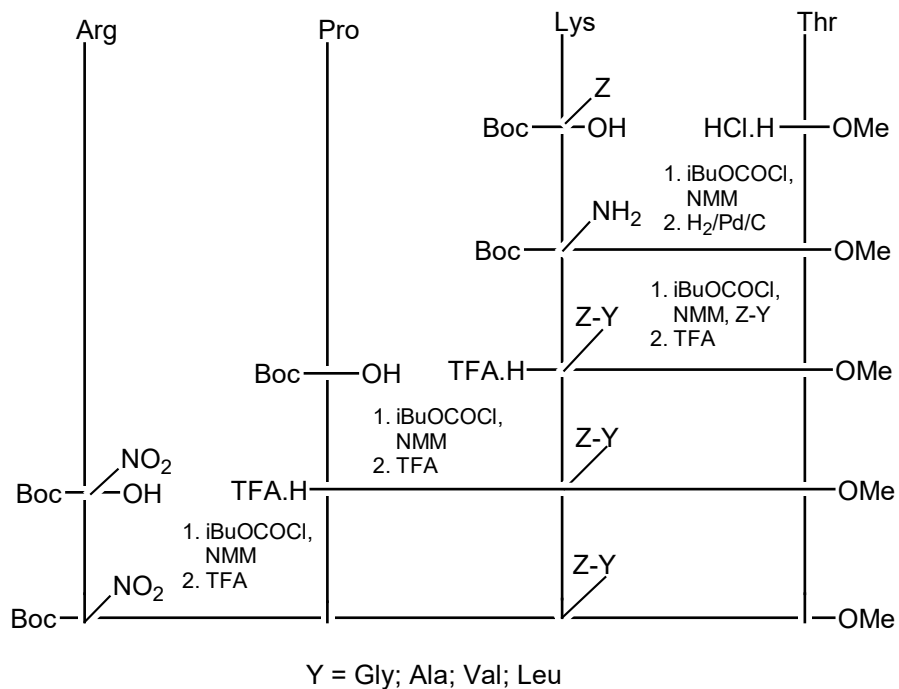
### Scheme 1



**Y** = Gly, Ala, Val, Leu  
**X** = Ala, Val, Pro  
**R** = CH<sub>3</sub> (**3a**), H (**3b**)

**5a:** TFAxArg(NO<sub>2</sub>)-Pro-Lys(ZAla)-Thr-OMe  
**5b:** TFAxArg(NO<sub>2</sub>)-Pro-Lys(ZGly)-Thr-OMe  
**5c:** TFAxArg(NO<sub>2</sub>)-Pro-Lys(ZLeu)-Thr-OMe  
**5d:** TFAxArg(NO<sub>2</sub>)-Pro-Lys(ZVal)-Thr-OMe

Comp.	R	X	Y
<b>7a</b>	H	Ala	Leu
<b>7b</b>	H	Ala	Ala
<b>7c</b>	H	Ala	Val
<b>7d</b>	H	Pro	Val
<b>7e</b>	H	Pro	Leu
<b>7f</b>	CH <sub>3</sub>	Val	Ala
<b>7g</b>	CH <sub>3</sub>	Val	Val
<b>7h</b>	CH <sub>3</sub>	Val	Gly
<b>7i</b>	CH <sub>3</sub>	Ala	Gly
<b>7j</b>	CH <sub>3</sub>	Ala	Val



**Scheme 2.** Preparation of Boc-Arg(NO<sub>2</sub>)-Pro-Lys(ZY)-Thr-OMe **4a-d**.

**Table 1.** Protected Compounds **6a-j**.

Comp. <sup>a</sup>	R	X	Y	Molecular formula	Yield (%)	M.p. (°C)
<b>6a</b>	H	Ala	Leu	C <sub>61</sub> H <sub>92</sub> N <sub>14</sub> O <sub>21</sub> (1357.46)	40	139-143
<b>6b</b>	H	Ala	Ala	C <sub>58</sub> H <sub>86</sub> N <sub>14</sub> O <sub>21</sub> (1315.39)	38	114-118
<b>6c</b>	H	Ala	Val	C <sub>60</sub> H <sub>90</sub> N <sub>14</sub> O <sub>21</sub> (1343.44)	36	123-127
<b>6d</b>	H	Pro	Val	C <sub>62</sub> H <sub>92</sub> N <sub>14</sub> O <sub>21</sub> (1369.50)	35	133-137
<b>6e</b>	H	Pro	Leu	C <sub>63</sub> H <sub>94</sub> N <sub>14</sub> O <sub>21</sub> (1383.50)	33	179-184
<b>6f</b>	CH <sub>3</sub>	Val	Ala	C <sub>61</sub> H <sub>92</sub> N <sub>14</sub> O <sub>21</sub> (1357.46)	35	104-106
<b>6g</b>	CH <sub>3</sub>	Val	Val	C <sub>63</sub> H <sub>96</sub> N <sub>14</sub> O <sub>21</sub> (1385.53)	34	131-134
<b>6h</b>	CH <sub>3</sub>	Val	Gly	C <sub>60</sub> H <sub>90</sub> N <sub>14</sub> O <sub>21</sub> (1342.64)	32	132-136
<b>6i</b>	CH <sub>3</sub>	Ala	Gly	C <sub>58</sub> H <sub>86</sub> N <sub>14</sub> O <sub>21</sub> (1315.39)	34	118-124
<b>6j</b>	CH <sub>3</sub>	Ala	Val	C <sub>61</sub> H <sub>92</sub> N <sub>14</sub> O <sub>21</sub> (1357.47)	38	125-128

<sup>a</sup> <sup>1</sup>H NMR spectra of all compounds were in compliance with the expected ones.

**Table 2.** Retro-tuftsins derivatives **4b**, **4c**, **5b** and **5c**.

Compounds <sup>a</sup>	Molecular formula	M.p. (°C)	Yield (%)	[α] <sub>589</sub> MeOH (c 1.0)
Boc-Arg(NO <sub>2</sub> )-Pro-Lys(ZGly)- Thr-OMe; <b>4b</b>	C <sub>37</sub> H <sub>58</sub> N <sub>10</sub> O <sub>13</sub> (850.92)	97-100	65	-30°
H-Arg(NO <sub>2</sub> )-Pro-Lys(ZGly)- Thr-OMe trifluoroacetate; <b>5b</b>	C <sub>34</sub> H <sub>51</sub> N <sub>10</sub> O <sub>13</sub> F <sub>3</sub> (864.82)	oil	88	-
Boc-Arg(NO <sub>2</sub> )-Pro-Lys(ZLeu)- Thr-OMe; <b>4c</b>	C <sub>41</sub> H <sub>66</sub> N <sub>10</sub> O <sub>13</sub> (907.02)	102-106	68	-40°
H-Arg(NO <sub>2</sub> )-Pro-Lys(ZLeu)- Thr-OMe trifluoroacetate; <b>5c</b>	C <sub>38</sub> H <sub>59</sub> N <sub>10</sub> O <sub>13</sub> F <sub>3</sub> (920.93)	oil	92	-

<sup>a</sup> <sup>1</sup>H NMR spectra of all compounds were in compliance with the expected ones.