CROATICA CHEMICA ACTA
CCACAA, ISSN 0011-1643, e-ISSN 1334-417X
*Croat. Chem. Acta* **84** (2) (2011) 233–244.
CCA-3470

*Original Scientific Article*

# Synthesis and Biological Evaluation of New Mannose Derived Immunomodulating Adamantyltripeptides†

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RECEIVED FEBRUARY 25, 2011; REVISED MAY 15, 2011; ACCEPTED MAY 31, 2011

*Abstract.* A novel class of mannosylated adamantyltripeptides, general formula of which is α-Man-$OCH_2CH(CH_3)CO$-AdGly-L-Ala-D-*iso*Gln, were prepared, characterized, and their possible immunomodulatory properties investigated in some preliminary tests *in vivo*. All newly synthesized glycopeptides comprise in their structure the adamantylglycyl moiety linked to the dipeptide L-Ala-D-*iso*Gln, as well as mannose. Adjuvant activity of mannosylated adamant-2-yl tripeptides was tested in the mouse model using ovalbumin as an antigen and in comparison to the peptidoglycan monomer (PGM, β-D-GlcNAc-(1→4)-D-MurNAc-L-Ala-D-*iso*Gln-*meso*DAP(εNH$_2$)-D-Ala-D-Ala). (doi: 10.5562/cca1827)

*Keywords:* *O*-mannopyranosides, adamantyltripeptides, peptidoglycan monomer, immunomodulation

## INTRODUCTION

Peptidoglycans, the essential and unique components of bacterial cell walls, possess diverse biological activities that depend upon the size and/or structure of peptidoglycan fragments. They are generally built of large polysaccharide chains and short peptide units. Natural and synthetic peptidoglycans as well as their fragments of different molecular mass exhibit remarkable biological activites. It was established that they in particular affect the immune system of mammalian hosts.[1,2] Smaller size peptidoglycans as well as some peptidoglycan fragments of well defined structures, the best known of which are muramyl peptides, had been extensively studied as possible adjuvants for human and animal vaccines.[3,4,5] Muramyl peptides are considered to be structural fragments of the monomeric peptidoglycans (*e.g.* PGM, **1**, GlcNAc-MurNac-L-Ala-D-*iso*Gln-*meso*DAP(εNH$_2$)-D-Ala-D-Ala, Figure 1) of bacterial cell walls, and MDP (muramyl dipeptide; *N*-acetyl-muramyl-L-alanyl-D-*iso*glutamine) is known as the smallest synthetic adjuvant molecule capable of replacing whole *Mycobacteria* in Freund's adjuvant.[5]

Several hundred chemically defined MDP analogs and derivatives were synthesized, in order to modulate the properties of the parent molecule. Up to now our research in the field of potential adjuvants was directed towards desmuramyl peptides in which the *N*-acetylmuramic portion of MDP was replaced with the lypophilic adamantylglycine. Thus, previously synthesized diastereoisomers of admant-1-yl tripeptides **2a**, **2b**[6] (Figure 1) and adamant-2-yl tripeptides **3a**, **3b**[7] were tested and shown to differ in biological activity in several model systems.[6–10]

Compounds containing adamantyl residues are known to exhibit various biological activities. They are mostly used in the prevention of viral diseases, and in the treatment of Parkinson disease and depression.[11–13] Some glycoconjugates of adamantane are also used as drugs. The most often used one is gludantan, the adamantamine conjugate of glucuronic acid, which is used as an antiviral agent as well as a drug in the treatment of Parkinson disease and depression.[11] It is also known that introducing an adamantyl moiety into substances with known biological activity improves their pharmacological properties mostly due to its pronounced lipophilic character.[12,13] Furthermore, the role of adamantane conjugates with mannose in the process of preventing microbial adhesion to host cells was recently discussed as well.[14] Therefore, it seems to be of interest to further explore the influence of the adamantyl moiety on biological properties of the dipeptide building block of MDP, with emphasis on its adjuvant activity.

† This article belongs to the Special Issue *Chemistry of Living Systems* devoted to the intersection of chemistry with life.
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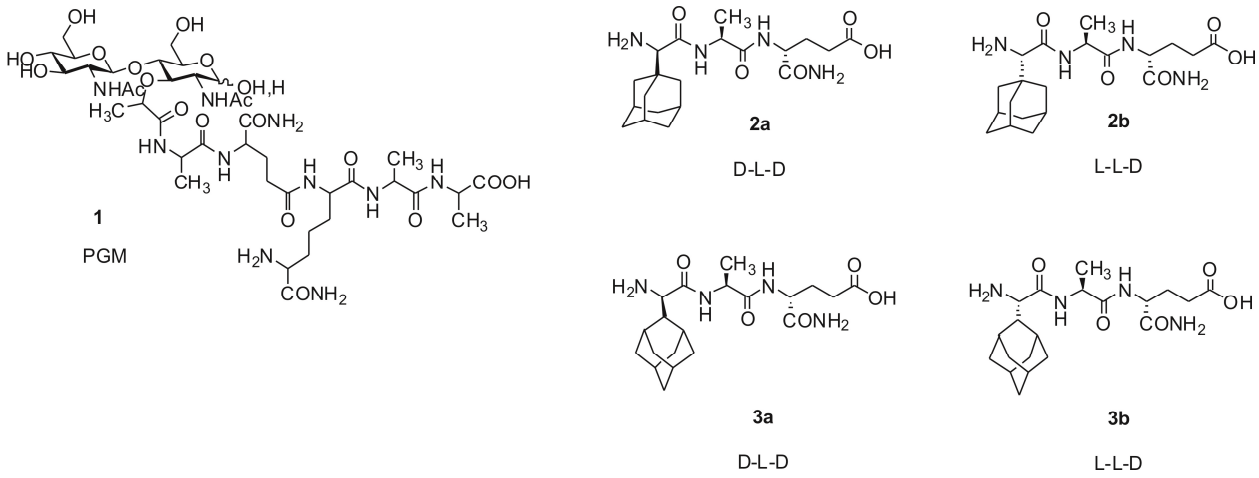



**Figure 1.** Structures of PGM (**1**), adamant-1-yl tripeptides (**2a**, **2b)** and adamant-2-yl tripeptides (**3a**, **3b)**.

In continuation of our interest and research of monosaccharide conjugates,[15] their enzymatic transformations[16–19] and synthesis,[20,21] especially of those with biological activity[8,22] we now report the synthesis of mannose conjugates with admant-1-yl tripeptides **2** and adamant-2-yl tripeptides **3** containing the L-alanyl-D-*iso*glutamine dipeptide sequence as in PGM and MDP. Furthermore, the study was designed to investigate how mannosylation of adamantyl tripeptides through a chiral spacer of defined configuration (*R* or *S*) might affect the immunostimulating activity in mice. Mannose receptors (MR) present on the cell surface of different macrophages and dendritic cells are considered to be pattern-recognition receptors binding compounds comprising mannose (Man), *N*-acetylglucosamine (GlcNAc) and fucose. Therefore, they are responsible for the binding, among others, of mannosylated antigens or relevant biologically active molecules containing mannose, thus affecting the immune reactions. Previous reports on conjugates of mannosylated proteins with MDP showed enhanced immunostimulating and antitumor properties in comparison to conjugates without mannose.[23,24] A previous report was also given on the effects of mannosylated PGM on immune reactions in mice and the susceptibility of these derivatives to the enzyme *N*-acetylmuramyl-L-alanine amidase.[22]

Our current study in regard to biological activity of novel mannosylated adamantyltripeptides should provide information on their adjuvant effect on humoral IgG immune response in mice. Ovalbumin was used as a customary antigen for studying the adjuvant effect. The comparison of induced anti-OVA IgG levels was carried out quantitatively and the subclasses of IgG, IgG1 and IgG2a, as indicator of Th1 and Th2 type of immune response were also determined.

## EXPERIMENTAL

### General Remarks

Bovine serum albumin (BSA), Tween 20, monoclonal anti-chicken egg albumin (clone OVA-14, mouse IgG1 isotype), avidin-peroxidase and *o*-phenylenediamine dihydrochloride (OPD) were from Sigma. Ovalbumin (OVA) was purchased from Serva. Horseradish peroxidase conjugated goat anti-mouse IgG (HRP-anti-mouse IgG) was from Bio-Rad Laboratories. Biotin-conjugated rat anti-mouse IgG1 and anti-mouse IgG2a monoclonal antibodies and streptavidin-peroxidase were purchased from PharMingen, Becton Dickinson. Chemicals for buffers and solutions were from Kemika, unless stated otherwise. Peptidoglycan monomer was prepared in PLIVA, Chemical and Pharmaceutical Works (Zagreb, Croatia), according to the previously described procedure.[25] Racemic (adamant-2-yl)glycine[26] was prepared at the Institute of Immunology (Zagreb, Croatia), as described earlier. Racemic (adamant-1-yl)glycine hydrochloride was prepared as previously described.[27] Most of the chemical reagents used in syntheses were obtained from Fluka and Aldrich Corp. All solvents were purified using standard procedures. Column cromatography (solvents and proportions are given in the text) was performed on Merck silica gel 60 (size 70−230 mesh ASTM) and TLC monitoring on Fluka silica gel (60 F 254) plates (0.25 mm). Visualization was effected mostly by use of UV light and/or by charring with $H_2SO_4$. Optical rotations were measured at room temperature using the Schmidt + Haensch Polartronic NH8. NMR spectra were recorded using Bruker Avance spectrometer at 300 or 600 MHz. C, H and N analyses were provided by the Analytical Services Laboratory of Ruđer Bošković Institute, Zagreb. Mass spectra were recorded with Waters MS − Quattro micro instrument.

Chromatographic separations were carried out using the Waters HPLC System equipped with 2996 PDA detector and Empower software (Milford, MA, USA). A LiChrosorb RP-18 column, 244 mm × 4 mm, 5 μm, and a LiChrospher guard column 100 RP-18 (5 μm) were used (Merck, Darmstadt, Germany). Analyses were run at a flow rate of 1.0 mL/min at room temperature and the eluate was monitored at 200 nm. The gradient solvent system used was made of acetonitrile containing 0.035 % TFA and water containing 0.05 % TFA.[28] The percentage of acetonitrile at 0, 15 and 20 min was 10, 30 and 10, respectively and a running time was 25 min. Acetonitrile and trifluoroacetic acid (TFA) were of HPLC – grade from Merck (Darmstadt, Germany). A daily supply of water was obtained from Millipore Simplicity – Personal ultra pure water system (Bedford, MA, USA). The percentage of each peak in the respective chromatograms was calculated by the integration of the UV response (peak area).

Characterization was performed on the mixture of diasteromers. Whenever distinguishable, values given are for one isomer with those of the second one listed in square brackets.

## Synthesis

*General Procedure for 9-Fluorenylmethyloxycarbonyl Protection of Adamantylglycine*

L,D-(Adamant-1-yl)glycine or L,D-(adamant-2-yl)glycine (350 mg, 1.67 mmol) was suspended in a 2:1 mixture of dioxane/$H_2O$ (12 mL). 9-Fluorenylmethyloxycarbonyl chloride (518 mg, 2.0 mmol, 1.2 equiv) and $Na_2CO_3$ (531 mg, 3 equiv) were added. The reaction mixture was stirred 1 h at 0 ºC and then overnight at room temperature. The mixture was acidified (pH 3) with saturated $KHSO_4$ (aq), and extracted twice with AcOEt. The organic layers were washed with water and dried over $Na_2SO_4$.

*9-Fluorenylmethyloxycarbonyl-L,D-(adamant-1-yl)glycine (**5**):* White solid foam (684 mg, 95 %); $R_f$ = 0.62 ($CH_3CN/H_2O$ = 5:1). $^1$H NMR (MeOH-$d_4$) $\delta$ / ppm: 7.77 (d, 2H, $J$ = 7.4 Hz, $CH_{arom}$; Fmoc), 7.64 (d, 2H, $J$ = 7.4 Hz, $CH_{arom}$; Fmoc), 7.53 (t, 2H, $J$ = 7.2 Hz, $CH_{arom}$; Fmoc), 7.28 (t, 2H, $J$ = 7.3 Hz, $CH_{arom}$; Fmoc), 4.01 (t, 1H, $J$ = 6.8 Hz, CH; Fmoc), 3.87 (d, 2H, $J$ = 11.2 Hz, $CH_2$; Fmoc), 3.82 (s, 1H, CH-α), 1.95 (br s, 3H, Ad), 1.73−1.64 (m, 12H, Ad); $^{13}$C NMR (MeOH-$d_4$) $\delta$ / ppm: 158.52 (CO; Fmoc), 146.30, 145.45, 145.17, 142.63 ($C_{arom}$; Fmoc), 128.78, 128.43, 128.16, 128.15, 127.94, 126.14, 120.94, 120.78 ($CH_{arom}$; Fmoc), 65.88 ($CH_2$; Fmoc), 51.66 (CH-α), 48.59 (CH; Fmoc), 39.99, 37.99 ($CH_2$; Ad), 29.95 (CH; Ad).

*9-Fluorenylmethyloxycarbonyl-L,D-(adamant-2-yl)glycine (**6**):* White solid foam (684 mg, 95 %); $R_f$ = 0.62 ($CH_3CN/H_2O$ = 5:1), $^1$H NMR ($CDCl_3$) $\delta$ / ppm: 7.81−7.25 (m, 9H, $CH_{arom}$; Fmoc, NH), 4.59 (br s, 1H, OH), 4.39 (m, 2H, $CH_2$; Fmoc), 4.12 (d, 1H, $J$ = 7.0 Hz, CH-α), 4.06 (t, 1H, $J$ = 5.9 Hz, CH; Fmoc), 2.04−1.51 (m, 15H, 5CH, $5CH_2$; Ad); $^{13}$C NMR ($CDCl_3$) $\delta$ / ppm: 143.90, 143.70, 143.65, 143.54 ($C_{arom}$; Fmoc), 127.57, 126.99, 125.05, 119.86 ($CH_{arom}$; Fmoc), 65.18 ($CH_2$; Fmoc), 50.31 (CH-α), 47.12 (CH; Fmoc), 38.55, 37.90, 31.69, 31.59 ($CH_2$; Ad), 29.51, 28.13, 27.75, 27.50 (CH; Ad). ESI-MS: calcd. for $C_{27}H_{29}NO_4$: 431.52; found $[M+H]^+$ at *m/z* 432.30.

*General Procedure for Synthesis of Adamantyltripeptides*

To a solution of Fmoc-AdGly **5** or **6** (562 mg, 1.30 mmol) in dry DCM (10 mL) at 0 °C EDCxHCl (300 mg, 1.56 mmol, 1.2 equiv) and HOBt (176 mg, 1.30 mmol, 1 equiv) were added. The mixture was stirred for 0.5 h at the same temperature and then added to a solution of L-alanyl-D-*iso*glutamine *tert*-butyl ester **4** (355 mg, 1.30 mmol, 1 equiv) in dioxane (5 mL). Subsequently *N*-ethylmorpholine (329 μL, 2.60 mmol, 2 equiv) was added to the reaction mixture. The mixture was stirred for 1 h at 0 °C and then for 48 h at room temperature. The solution was diluted with AcOEt and washed with water. The organic layer was dried over $MgSO_4$ and after filtration, evaporated. The residue was purified by column chromatography on silica gel (AcOEt). White solid foam was afforded as a mixture of diastereomers (only one spot by TLC).

*9-Fluorenylmethyloxycarbonyl-L,D-(adamant-1-yl)glycyl-L-alanyl-D-*iso*glutamine* tert*-butyl ester (7):* 723 mg (81 %); $R_f$ = 0.43 (AcOEt); $^1$H NMR ($CDCl_3$) $\delta$ / ppm: 7.78−7.26 (m, $CH_{arom}$; Fmoc), 6.93 (br s, NH), 6.80 (br s, NH), 6.08−5.57 (m, NH), 4.52−3.90 (m; 3CH-α; *iso*Gln, Ala, AdGly and CH, $CH_2$; Fmoc), 2.52−2.25 (m, $CH_2$-β, $CH_2$-γ; *iso*Gln), 1.96 (br s, 3CH; Ad), 1.65−1.44 (m, $6CH_2$; Ad), 1.41 (s, $3CH_3$; *t*-Bu), [1.39 (s, $3CH_3$; *t*-Bu)], 1.40 (d, $J$ = 7.1 Hz, $CH_3$; Ala), [1.39 (d, $J$ = 7.1 Hz, $CH_3$; Ala)]; $^{13}$C NMR ($CDCl_3$) $\delta$ / ppm: 173.81, 173.75, 172.67, 172.58, 172.55, 172.37, 170.30, 170.26 (C=O), 156.75, 156.64 (CO; Fmoc), 144.35, 143.88, 143.61, 141.47, 141.24 ($C_{arom}$; Fmoc), 127.65, 127.52, 127.02, 125.10, 125.05, 124.69, 120.00, 119.93, 119.90 ($CH_{arom}$; Fmoc), 80.92, 80.87 (C; *t*-Bu), 66.98, 65.07 ($CH_2$; Fmoc), 63.44, 63.36 (CH; AdGly), 52.35, 52.10 (CH; *iso*Gln), 50.33, 47.14 CH; Fmoc), 49.23, 48.91 (CH; Ala), 38.67, 38.54, 36.64, 36.54, 36.20, 31.67 ($CH_2$; γ-*iso*Gln, $CH_2$; Ad), 28.16, 27.99 (CH; Ad, $CH_3$; *t*-Bu), 27.69, 27.37 ($CH_2$; β-*iso*Gln), 18.25, 18.13 ($CH_3$; Ala). ESI-MS: calcd. for $C_{39}H_{50}N_4O_7$: 686.84; found $[M+H]^+$ at *m/z* 687.40.

*9-Fluorenylmethyloxycarbonyl-L,D-(adamant-2-yl)glycyl-L-alanyl-D-*iso*glutamine* tert*-butyl ester (8):* (661 mg, 74 %); $R_f$ = 0.45 (AcOEt); $^1$H NMR ($CDCl_3$) $\delta$ / ppm: 7.86-7.22 (m, $CH_{arom}$; Fmoc), 6.80 (br s, NH), 6.73 (br s, NH), 6.26 (br s, NH), 6.07 (br s, NH), 5.89 (d, $J$ = 8.1 Hz, NH), 5.83 (d, $J$ = 9.0 Hz, NH), 4.65−4.11

(m; 3CH-α; *iso*Gln, Ala, AdGly and CH, $CH_2$; Fmoc), 2.44−1.53 (m, $CH_2$-β, $CH_2$-γ; *iso*Gln and 5CH, $5CH_2$; Ad), 1.41 (s, $3CH_3$; *t*-Bu), [1.39 (s, $3CH_3$; *t*-Bu)], 1.35 (d, $J$ = 7.1 Hz, $CH_3$; Ala), [1.33 (d, $J$ = 6.7 Hz, $CH_3$; Ala)]; $^{13}$C NMR ($CDCl_3$) $\delta$ / ppm: 173.82, 173.80, 172.55, 172.48, 172.38, 172.29, 172.27 (C=O), 156.65, 156.53 (CO; Fmoc), 143.92, 143.80, 143.62, 143.58, 141.22, 141.20 ($C_{arom}$; Fmoc), 127.66, 127.62, 127.03, 125.13, 125.06, 119.92, 119.90 ($CH_{arom}$; Fmoc), 80.88, 80.75 (C; *t*-Bu), 67.05, 66.94 ($CH_2$; Fmoc), 56.06, 52.04 (CH; AdGly), 49.25, 48.87 (CH; *iso*Gln), 52.37, 47.06 CH; Fmoc), 46.57, 46.43 (CH; Ala), 38.65, 38.52, 37.85, 31.77 ($CH_2$; γ-*iso*Gln, $CH_2$; Ad), 29.40, 29.23, 27.87, 27.77, 27.50 (CH; Ad), 28.01, 28.00 ($CH_3$; *t*-Bu), 27.65, 27.17 ($CH_2$; β-*iso*Gln), 18.07, 17.97 ($CH_3$; Ala). ESI-MS: calcd. for $C_{39}H_{50}N_4O_7$: 686.84; found $[M+H]^+$ at *m/z* 687.40.

*General Procedure for* N-*Deprotection of 9-Fluorenylmethyloxycarbonyl Adamantyltripeptides*

To a solution of **7** or **8** (500 mg, 0.73 mmol) in dry THF (5 mL) under $N_2$ 1-octanthiol (1.26 mL, 7.3 mmol, 10 equiv) and DBU (35.8 μL, 0.24 mmol, 0.33 equiv) were added. The reaction mixture was stirred at room temperature for 16 h and monitored by TLC ($CH_3CN/H_2O$ 5:1). The mixture was then triturated with diethyl ether and a white solid residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 10:1→1:1).

*L,D-(Adamant-1-yl)glycyl-L-alanyl-D-*iso*glutamine* tert-*butyl ester (**9**):* 292 mg (86 %); $R_f$ = 0.59 ($CH_3CN/H_2O$ 5:1). $^1$H NMR (MeOH-$d_4$) $\delta$ / ppm: 7.90 (s, OH), 7.78 (d, $J$ = 7.5 Hz, NH), 7.65 (dd, $J$ = 7.9 Hz, $J$ = 0.8 Hz, NH), 7.36 (t, $J$ = 7.5 Hz, NH), 7.29 (dt, $J$ = 6.9 Hz, $J$ = 1.1 Hz, NH), 4.35−4.29 (m; 2CH-α; *iso*Gln, Ala), 3.10 (s, CH-α; AdGly), [3.09 (s, CH-α; AdGly)], 2.35−2.32 (m, $CH_2$-γ; *iso*Gln ), 2.24−2.18 (m, CH-β; *iso*Gln), 2.00 (br s, 3CH; Ad), 1.94−1.82 (m, CH-β'; *iso*Gln), 1.78−1.55 (m, $6CH_2$; Ad), 1.45 (s, $3CH_3$; *t*-Bu), [1.44 (s, $3CH_3$; *t*-Bu)], 1.40 (d, $J$ = 7.1 Hz, $CH_3$; Ala), [1.39 (d, $J$ = 7.1 Hz, $CH_3$; Ala)]. $^{13}$C NMR (MeOH-$d_4$) $\delta$ / ppm: 176.43, 176.37, 175.38, 175.06, 173.86, 173.81, 173.23, 173.12 (C=O), 81.90, 81.88 (C; *t*-Bu), 64.71, 64.63 (CH; AdGly), 53.75, 53.61 (CH; *iso*Gln), 51.22, 50.84 (CH; Ala), 39.58, 39.43, 37.83, 36.81, 36.57, 32.76 ($CH_2$; γ-*iso*Gln, $CH_2$; Ad), 29.81, 28.39 (CH; Ad, $CH_3$; *t*-Bu), 28.21, 28.07 ($CH_2$; β-*iso*Gln), 17.80, 17.58 ($CH_3$; Ala). ESI-MS: calcd. for $C_{24}H_{40}N_4O_5$: 464.60; found $[M+H]^+$ at *m/z* 465.30.

*L,D-(Adamant-2-yl)glycyl-L-alanyl-D-*iso*glutamine* tert-*butyl ester (**10**):* 275 mg (81 %); $R_f$ = 0.57 ($CH_3CN/H_2O$ 5:1); $^1$H NMR (MeOH-$d_4$) $\delta$ / ppm: 7.90 (s, OH), 7.80−7.24 (m, NH), 4.37−4.27 (m; 2CH-α; *iso*Gln, Ala), 3.74 (d, $J$ = 9.1 Hz, CH-α; AdGly), [3.70 (d, $J$ = 9.0 Hz, CH-α; AdGly)], [3.61 (q, $J$ = 7.1 Hz, CH-α; Ala)], 2.36−2.28 (m, $CH_2$-γ; *iso*Gln), 2.25−2.09 (m, $CH_2$-β; *iso*Gln), 2.04−1.51 (m, 5CH, $5CH_2$; Ad), 1.45 (s, $3CH_3$; *t*-Bu), [1.44 (s, $3CH_3$; *t*-Bu)], 1.39 (d, $J$ = 7.1 Hz, $CH_3$; Ala), [1.37 (d, $J$ = 7.1 Hz, $CH_3$; Ala) ]; $^{13}$C NMR (MeOH-$d_4$) $\delta$ / ppm: 176.39, 176.26, 175.38, 175.30, 173.82, 173.80 (C=O), 81.91, 81.88 (C; *t*-Bu), 53.71, 53.53 (CH; AdGly), 51.05, 50.75 (CH; *iso*Gln), 49.50, 48.88 (CH; Ala), 39.86, 39.75, 39.10, 39.02, 32.97, 32.89, 32.75, 32.70, 32.57, 32.53 ($CH_2$; γ-*iso*Gln, $CH_2$; Ad), 31.11, 30.71, 29.37, 29.33, 29.18, 29.14, 28.95 (CH; Ad), 28.39 ($CH_3$; *t*-Bu), 28.32, 28.11 ($CH_2$; β-*iso*Gln), 17.72, 17.60 ($CH_3$; Ala). ESI-MS: calcd. for $C_{24}H_{40}N_4O_5$: 464.60; found $[M+H]^+$ at *m/z* 465.30.

*General Procedure for Condensation of Mannose and Adamantlytripeptides*

To a solution of mannosylated carboxylic acid **11** or **12** (322 mg, 0.52 mmol) in dry DCM (10 mL) at 0 °C EDCxHCl (119 mg, 0.63 mmol, 1.2 equiv) and HOBt (70 mg, 0.52 mmol, 1 equiv) were added. The mixture was stirred for 0.5 h at the same temperature and then added to a solution of tripeptide **9** or **10** (200 mg, 0.43 mmol) in dioxane (5 mL). $Et_3N$ (144 μL, 1.04 mmol, 2 equiv) was added next to the reaction mixture. The mixture was stirred for 1 h at 0 °C and then for 48 h at room temperature. The solution was diluted with AcOEt and washed with water. The organic layer was dried over $Na_2SO_4$ and after filtration, evaporated. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 10:1).

N-*[(R)-3-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyloxy)-2-methylpropanoyl]-L,D-(adamant-1-yl)glycyl-L-alanyl-D-*iso*glutamine* tert-*butyl ester (**13**):* White solid foam (342 mg, 74 %); $R_f$ = 0.58 ($CHCl_3$/MeOH = 10:1); $^1$H NMR (MeOH-$d_4$) $\delta$ / ppm: 8.47 (d, $J$ = 7.4 Hz, NH), 8.42 (d, $J$ = 6.5 Hz, NH), 7.95 (pt, $J$ = 10.4 Hz, $J$ = 7.2 Hz, NH), 7.89 (d, $J$ = 7.8 Hz, NH), 7.86 (d, $J$ = 8.0 Hz, NH), 7.37−7.13 (m, $H_{arom}$), 4.90 (s, H-1), 4.81−4.41 (m, CH; Bn, CH-α; AdGly), [4.76 (d, $J_{gem}$ = 10.8 Hz, CH; Bn)], 4.30−4.11 (m, 2CH-α; Ala, *iso*Gln), 3.91−3.27 (H-2, H-3, H-4, H-5, H-6a, H-6b, $OCH_2$), 2.72−2.61 (m, CH), 2.18−1.42 (m, $CH_2$-β, $CH_2$-γ; *iso*Gln, 3CH, $6CH_2$; Ad), 1.37 (s, $3CH_3$; *t*-Bu), [1.36 (s, $3CH_3$; *t*-Bu)], 1.22 (d, $J$ = 5.4 Hz, $CH_3$; Ala), [1.18 (d, $J$ = 6.8 Hz, $CH_3$; Ala)], 0.98 (d, $J$ = 6.8 Hz, $CH_3$), [0.90 (d, $J$ = 6.9 Hz, $CH_3$)]; $^{13}$C NMR (MeOH-$d_4$) $\delta$ / ppm: 183.51, 183.13, 182.62, 182.56, 181.52, 181.10, 180.61 (C=O), 148.19, 148.12, 148.06, 148.00, 147.92, 147.89 ($C_{arom}$), 137.81−135.93 ($CH_{arom}$), 106.85, 106.61 (C-1), 89.26, 88.98, 84.10, 84.00, 83.94, 83.89, 80.87, 80.79 (C-2, C-3, C-4, C-5), 89.12, 83.68, 81.89, 81.33, 81.07, 80.94, 80.54, 80.17, 79.28, 78.55 (C-6, $CH_2$; Bn), 75.89, 72.42 ($OCH_2$), 64.13, 62.97 (CH; AdGly), 61.37, 60.93 (CH; *iso*Gln), 58.46, 57.45 (CH; Ala), 54.92, 54.70 (CH); 47.94, 47.77, 47.66, 47.20, 47.09, 40.82, 40.76 ($CH_2$; γ-*iso*Gln, $CH_2$; Ad, C; Ad), 36.70, 36.47 ($CH_2$; β-*iso*Gln), 37.25 ($CH_3$; *t*-Bu), 38.27, 37.85, 36.88, 36.56 (CH; Ad), 27.41, 27.09 ($CH_3$; Ala), 24.07, 23.81 ($CH_3$); ESI-MS:

calcd. for $C_{62}H_{81}N_4O_{12}$: 1073.32; found $[M+H]^+$ at *m*/*z* 1073.50.

N-*[(*S*)-3-(2,3,4,6-tetra*-O-*benzyl-α-D-mannopyranosyl-oxy)-2-methylpropanoyl]-L,D-(adamant-1-yl)glycyl-L-alanyl-D-isoglutamine* tert-*butyl ester (**14**)*: White solid foam (333 mg, 72 %); $R_f$ = 0.50 ($CHCl_3$/MeOH = 10:1); $^1$H NMR (MeOH-$d_4$) $\delta$ / ppm: 8.47 (d, *J* = 7.0 Hz, NH), 8.32 (s, NH), 7.96 (d, *J* = 7.7 Hz, NH), 7.92 (d, *J* = 8.8 Hz, NH), 7.79 (d, *J* = 8.2 Hz, NH), 7.57 (d, *J* = 8.0 Hz, NH), 7.37−7.09 (m, $H_{arom}$), 4.94 (d, *J* = 1.1 Hz, H-1), [4.92 (s, H-1)], 4.77−4.47 (m, $CH_2$ Bn, CH-α; AdGly), 4.24 (q, *J* = 7.2 Hz, CH-α; Ala), 4.20 (q, *J* = 7.0 Hz, CH-α; Ala), 4.16 (dd, *J* = 6.1 Hz, *J* = 5.0 Hz, CH-α; *iso*Gln), 4.14 (dd, *J* = 5.9 Hz, *J* = 5.0 Hz, CH-α; *iso*Gln), 3.82−3.55 (m, H-2, H-3, H-4, H-6a, H-6b, $OCH_2$), 3.46−3.44 (m, H-5), [3.25−3.21 (m, H-5)], 2.76−2.65 (m, CH), 2.18−1.53 (m, $CH_2$-β, $CH_2$-γ; *iso*Gln, 3CH, 6$CH_2$; Ad), 1.38 (s, 3$CH_3$; *t*-Bu), [1.37 (s, 3$CH_3$; *t*-Bu)], 1.09 (d, *J* = 7.2 Hz, $CH_3$; Ala), [1.06 (d, *J* = 7.0 Hz, $CH_3$; Ala)], 0.96 (d, J = 6.9 Hz, $CH_3$), [0.90 (d, J = 6.9 Hz, $CH_3$)]; $^{13}$C NMR (MeOH-$d_4$) $\delta$ / ppm: 183.49, 182.80, 182.49, 181.50, 181.41, 181.05, 180.94, 180.80 (C=O), 148.08, 148.04, 147.99, 147.94, 147.92 ($C_{arom}$), 137.76−136.71 ($CH_{arom}$), 106.39, 105.88 (C-1), 89.52, 89.27, 84.17, 84.13, 83.97, 83.9180.76, 80.56 (C-2, C-3, C-4, C-5), 89.17, 83.77, 83.56, 81.83, 81.76, 81.29, 81.25, 80.56, 80.37, 78.46, 78.37, 75.87 (C-6, $CH_2$; Bn, $OCH_2$), 63.67, 62.53 (CH; AdGly), 61.15, 60.76 (CH; *iso*Gln), 58.41, 57.65 (CH; Ala), 55.11, 54.88 (CH), 47.92, 47.80, 47.56, 47.17, 46.95 ($CH_2$; Ad, $CH_2$; γ-*iso*Gln), 40.78, 40.61 ($CH_2$; β-*iso*Gln), 37.24 ($CH_3$; *t*-Bu), 38.29, 37.96, 37.00, 36.87, 36.47 (CH; Ad), 27.38, 27.12 ($CH_3$; Ala), 24.36, 23.79 ($CH_3$); ESI-MS: calcd. for $C_{62}H_{81}N_4O_{12}$: 1073.32; found $[M+H]^+$ at *m*/*z* 1074.51.

N-*[(*R*)-3-(2,3,4,6-tetra*-O-*benzyl-α-D-mannopyranosyl-oxy)-2-methylpropanoyl]-L,D-(adamant-2-yl)glycyl-L-alanyl-D-*isoglutamine* tert-*butyl ester (**15**)*: White solid foam (351 mg, 76 %); $R_f$ = 0.56 ($CHCl_3$/MeOH = 10:1); $^1$H NMR (MeOH-$d_4$) $\delta$ / ppm: 8.47 (d, *J* = 7.7 Hz, NH), 8.42 (d, *J* = 6.2 Hz, NH), 8.32 (s, NH), 7.98−7.82 (m, NH), 7.78 (d, *J* = 8.2 Hz, NH), 7.37−7.19 (m, $H_{arom}$), 4.89 (s, H-1), [4.76 (d, $J_{gem}$ = 11.0 Hz, CH; Bn)], 4.67−4.40 (m, $CH_2$ Bn, CH-α; AdGly), 4.29−4.25 (m, 2CH-α; Ala, *iso*Gln), 3.90−3.27 (m, H-2, H-3, H-4, H-5, H-6a, H-6b, $OCH_2$), 2.69−2.62 (m, CH), 2.20−1.42 (m, $CH_2$-β, $CH_2$-γ; *iso*Gln, 5CH, 5$CH_2$; Ad), 1.37 (s, 3$CH_3$; *t*-Bu), [1.35 (s, 3$CH_3$; *t*-Bu)], 1.22 (d, *J* = 6.5 Hz, $CH_3$; Ala), [1.17 (d, *J* = 7.0 Hz, $CH_3$; Ala)], 0.97 (d, *J* = 6.8 Hz, $CH_3$), [0.99 (d, *J* = 6.8 Hz, $CH_3$)]; $^{13}$C NMR (MeOH-$d_4$) $\delta$ / ppm: 183.49, 183.10, 182.59, 182.54, 181.51, 181.47, 181.08, 180.60 (C=O), 148.18, 148.12, 148.05, 147.99, 147.97, 147.92, 147.90, 147.88 ($C_{arom}$), 137.80−135.92 ($CH_{arom}$), 106.84, 106.60 (C-1); 88.98, 84.09, 84.00, 83.94, 83.88, 80.85, 80.77 (C-2, C-3, C-4, C-5), 89.18, 83.66, 81.87, 81.31, 81.06, 80.93, 80.54, 80.16, 78.58, 78.64 (C-6, $CH_2$; Bn), 75.87, 72.41 ($OCH_2$), 64.11, 62.95 (CH; AdGly), 61.35, 60.91 (CH; *iso*Gln), 58.44, 57.45 (CH; Ala), 54.89, 54.69 (CH), 47.92, 47.74, 47.66, 47.06, 40.81, 40.75 ($CH_2$; Ad, $CH_2$; γ-*iso*Gln), 36.67, 36.46 ($CH_2$; β-*iso*Gln), 37.22 ($CH_3$; *t*-Bu), 38.27, 37.05, 36.87, 36.54 (CH; Ad), 27.41, 27.09 ($CH_3$; Ala), 24.07, 23.80 ($CH_3$); ESI-MS: calcd. for $C_{62}H_{81}N_4O_{12}$: 1073.32; found $[M+H]^+$ at *m*/*z* 1073.50.

N-*[(*S*)-3-(2,3,4,6-tetra*-O-*benzyl-α-D-mannopyranosyl-oxy)-2-methylpropanoyl]-L,D-(adamant-2-yl)glycyl-L-alanyl-D-*isoglutamine* tert-*butyl ester (**16**)*: White solid foam (355 mg, 77 %); $R_f$ = 0.62 ($CHCl_3$/MeOH = 10:1). $^1$H NMR (MeOH-$d_4$) $\delta$ / ppm: 7.93 (d, *J* = 6.7 Hz, NH), 7.85 (pt, *J* = 7.2 Hz, *J* = 8.3 Hz, NH), 7.47 (d, *J* = 7.0 Hz, NH), 7.38−7.15 (m, $H_{arom}$), 4.89 (s, H-1), [4.86 (s, H-1)], 4.81−4.47 (m, $CH_2$ Bn, CH-α; AdGly ), [4.52 (d, $J_{gem}$ = 11.4 Hz, CH; Bn)], [4.51 (d, $J_{gem}$ = 10.8 Hz, CH; Bn)], [4.37 (q, *J* = 7.1 Hz, CH-α; Ala)], 4.34−4.30 (m, CH-α; *iso*Gln), [4.25 (q, *J* = 7.1 Hz, CH-α; Ala)], 3.97−3.28 (m, H-2, H-3, H-4, H-5, H-6a, H-6b, $OCH_2$), 2.75−2.65 (m, CH), 2.30 (pt, *J* = 7.0 Hz, *J* = 8.2 Hz, $CH_2$-γ; *iso*Gln), 2.23 (pt, *J* = 8.1 Hz, *J* = 7.4 Hz, $CH_2$-γ; *iso*Gln), 2.18−1.51 (m, $CH_2$-β; *iso*Gln, 5CH, 5$CH_2$; Ad), 1.424 (s, 3$CH_3$; *t*-Bu), [1.420 (s, 3$CH_3$; *t*-Bu)], 1.18 (d, *J* = 7.1 Hz, $CH_3$; Ala), [1.11 (d, *J* = 7.1 Hz, $CH_3$; Ala)], 1.08 (d, *J* = 7.0 Hz, $CH_3$), [1.03 (d, *J* = 7.0 Hz, $CH_3$)]. $^{13}$C NMR (MeOH-$d_4$) $\delta$ / ppm: 177.68, 177.60, 177.04, 176.40, 175.94, 174.74, 174.59, 174.33, 174.16, 173.71 (C=O), 140.04, 140.00, 139.94, 139.92, 139.81, 139.77, 139.71, 139.67 ($C_{arom}$), 129.43−128.57 ($CH_{arom}$), 99.29, 98.97 (C-1), 82.08, 81.66, 76.50, 76.44, 76.05, 75.92, 73.30, 73.24 (C-2, C-3, C-4, C-5), 76.16, 75.90, 74.44, 74.34, 73.78, 73.71, 73.17, 72.98, 70.91, 70.79, 70.20, 68.16 (C-6, $CH_2$; Bn, $OCH_2$), 56.72, 55.28 (CH; AdGly), 54.08, 53.41 (CH; *iso*Gln), 50.95, 50.16 (CH; Ala), 47.26, 46.60 (CH), 39.84, 39.81, 39.79, 39.59, 39.13, 38.91, 32.87, 32.65 ($CH_2$; Ad, $CH_2$; γ-*iso*Gln), 28.60, 28.30 ($CH_2$; β-*iso*Gln), 28.45, 28.42 ($CH_3$; *t*-Bu), 30.78, 30.46, 29.66, 29.55, 29.42, 29.33, 29.14, 28.91 (CH; Ad), 17.70, 17.63 ($CH_3$; Ala), 14.96, 14.83 ($CH_3$). ESI-MS: calcd. for $C_{62}H_{81}N_4O_{12}$: 1073.32; found $[M+H]^+$ at *m*/*z* 1073.50.

*General Procedure for Hydrolysis of* tert-*Butyl Esters*

Compounds **13**−**16** (300 mg; 0.28 mmol) were dissolved in $CF_3COOH/H_2O$ (3.3 mL; 95:5, v/v). Reaction mixtures were stirred at room temperature for 2 h and then concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel ($CHCl_3$/MeOH = 5:1) and compounds **17**−**20** were obtained as white solid foams.

N-*[(*R*)-3-(2,3,4,6-tetra*-O-*benzyl-α-D-mannopyranosyl-oxy)-2-methylpropanoyl]-L,D-(adamant-1-yl)glycyl-L-alanyl-D-*isoglutamine *(**17**)*: (185 mg, 65%), $R_f$ = 0.47 ($CHCl_3$/MeOH = 3:1); $^1$H NMR ($CDCl_3$) $\delta$ / ppm: 7.52−7.15 (m, $H_{arom}$), 5.04 (s, H-1), [4.95 (s, H-1)],

4.89−4.28 (m, CH-α; AdGly, Ala, *iso*Gln, CH; Bn), [4.84 (d, $J_{gem}$ = 10.7 Hz, CH; Bn)], [4.49 (d, $J_{gem}$ = 11.5 Hz, CH; Bn)], 4.13−3.44 (m, H-2, H-3, H-4, H-5, H-6a, H-6b, $OCH_2$), 2.81−2.76 (m, CH), 2.38−2.01 (m, $CH_2$-β, $CH_2$-γ; *iso*Gln), 1.93 (s, CH; Ad), 1.68−1.56 (m, $CH_2$; Ad), 1.26 (d, *J* = 4.9 Hz, $CH_3$; Ala), [1.21 (d, *J* = 4.7 Hz, $CH_3$; Ala)], 1.16 (d, *J* = 6.6 Hz, $CH_3$), [1.07 (d, *J* = 6.6 Hz, $CH_3$)]; $^{13}$C NMR ($CDCl_3$) *δ* / ppm: 174.87, 173.88, 172.66, 172.33, 172.22, 169.85, 168.60 (C=O), 138.49, 138.41, 138.31, 138.21 ($C_{arom}$), 128.24−127.37 ($CH_{arom}$), 98.13, 97.89 (C-1), 80.39, 80.17, 79.89, 74.68, 74.58, 72.05 (C-2, C-3, C-4, C-5), 80.57, 75.10, 75.02, 73.23, 72.58, 71.91, 69.20, 69.09, 68.94, 68.91 (C-6, $CH_2$; Bn, $OCH_2$), 61.10, 57.29 (CH; AdGly), 51.81, (CH; *iso*Gln), 48.91, 48.72 (CH; Ala), 38.85, 38.64, 36.70, 36.43, 31.54, 29.62, 25.50, 25.38 ($CH_2$; β-*iso*Gln, $CH_2$; γ-*iso*Gln, $CH_2$; Ad, C; Ad), 28.07, 27.97 (CH, CH; Ad), 14.41, 13.49 ($CH_3$, $CH_3$; Ala). ESI-MS: calcd. for $C_{58}H_{72}N_4O_{12}$: 1017.21; found $[M+H]^+$ at *m*/*z* 1017.50.

N-*[(*S*)-3-(2,3,4,6-tetra*-O-*benzyl-α-D-mannopyranosyl-oxy)-2-methylpropanoyl]-L,D-(adamant-1-yl)glycyl-L-alanyl-D*-iso*glutamine* (**18**)*:* 134 mg (47 %), $R_f$ = 0.48 ($CHCl_3$/MeOH = 3:1); $^1$H NMR (MeOH-$d_4$) *δ* / ppm: 8.30 (d, *J* = 6.3 Hz, NH), 8.21 (d, *J* = 6.9 Hz, NH), 7.89 (d, *J* = 9.0 Hz, NH), 7.84 (d, *J* = 7.9 Hz, NH), 7.71 (d, *J* = 9.2 Hz, NH), 7.39−7.03 (m, $H_{arom}$), 4.97 (s, H-1), [4.93 (s, H-1)], 4.75−4.41 (m, $CH_2$; Bn, CH-α; AdGly), 4.30−4.11 (m, 2CH-α; Ala, *iso*Gln), 4.03−3.35 (m, H-2, H-3, H-4, H-5, H-6a, H-6b, $OCH_2$), 2.93−2.84 (m, CH), 2.06 (t, *J* = 7.0 Hz, $CH_2$-γ; *iso*Gln), 1.89−1.46 ($CH_2$-β; *iso*Gln, CH, $CH_2$; Ad), 1.19 (d, *J* = 7.0 Hz, $CH_3$; Ala), [1.08 (d, *J* = 7.0 Hz, $CH_3$; Ala)], 1.00 (d, *J* = 6.8 Hz, $CH_3$), [0.91 (d, *J* = 6.8 Hz, $CH_3$)]; $^{13}$C NMR (MeOH-$d_4$) *δ* / ppm: 183.46, 183.34, 182.80, 182.56, 182.42, 182.21, 181.54, 181.42, 180.98, 180.82 (C=O), 148.44, 148.33, 148.09 ($C_{arom}$), 138.24−136.74 ($CH_{arom}$), 106.42, 105.88 (C-1), 89.54, 89.27, 84.14, 83.97, 80.57, 80.19, 76.19 (C-2, C-3, C-4, C-5), 81.83, 81.77, 81.29, 80.45, 80.39, 78.38, 78.26, 75.89, 72.42 (C-6, $CH_2$; Bn, $OCH_2$), 63.59, 62.55 (CH; AdGly), 61.24, 60.86 (CH; *iso*Gln), 58.44, 57.68 (CH; Ala), 55.16, 55.01 (CH), 47.94, 47.86, 47.52, 47.20, 46.96, 40.87, 40.69, 39.65, 39.49 ($CH_2$; Ad, $CH_2$; β-*iso*Gln, $CH_2$; γ-*iso*Gln), 38.32, 37.96, 36.94, 36.60, 36.50 (CH; Ad), 27.41, 27.13 ($CH_3$; Ala), 24.40, 23.79 ($CH_3$); ESI-MS: calcd. for $C_{58}H_{72}N_4O_{12}$: 1017.21; found $[M+H]^+$ at *m*/*z* 1017.40.

N-*[(*R*)-3-(2,3,4,6-tetra*-O-*benzyl-α-D-mannopyranosyl-oxy)-2-methylpropanoyl]-L,D-(adamant-2-yl)glycyl-L-alanyl-D*-iso*glutamine* (**19**)*:* 151 mg (53 %), $R_f$ = 0.47 ($CHCl_3$/MeOH = 3:1); $^1$H NMR (MeOH-$d_4$) *δ* / ppm: 8.52 (d, *J* = 7.5 Hz, NH), 8.44 (d, *J* = 5.5 Hz, NH), 8.06 (d, *J* = 7.8 Hz, NH), 8.02 (d, *J* = 7.6 Hz, NH), 7.95 (d, *J* = 8.1 Hz, NH), 7.82 (d, *J* = 7.2 Hz, NH), 7.75 (d, *J* = 6.2 Hz, NH), 7.41−7.17 (m, $H_{arom}$), 4.91 (d, *J* = 1.6 Hz, H-1), [4.90 (d, *J* = 1.3 Hz, H-1)], 4.75−4.46 (m, $CH_2$; Bn, CH-α; AdGly), 4.41−4.32 (m, 2CH-α; Ala, *iso*Gln,), [4.25 (q, *J* = 6.3 Hz, CH-α; Ala)], 4.08−3.54 (m, H-2, H-3, H-4, H-5, H-6a, H-6b, $OCH_2$), 2.74−2.67 (m, CH), 2.23−2.16 (m, CH-γ; *iso*Gln), 2.07−1.57 (m, $CH_2$-β and CH-γ; *iso*Gln, CH, $CH_2$; Ad), 1.36 (d, *J* = 7.1 Hz, $CH_3$; Ala), [1.32 (d, *J* = 7.2 Hz, $CH_3$; Ala)], 1.07 (d, *J* = 6.9 Hz, $CH_3$), [1.00 (d, *J* = 7.0 Hz, $CH_3$)]; $^{13}$C NMR (MeOH-$d_4$) *δ* / ppm: 177.63, 177.52, 177.38, 176.51, 176.34, 176.24, 174.89, 174.80, 174.03, 173.93 (C=O), 140.04, 139.90, 139.87, 139.80, 139.68, 139.62, 139.58 ($C_{arom}$), 129.43−128.62 ($CH_{arom}$), 100.11, 99.79 (C-1), 81.69, 81.48, 76.48, 76.18, 76.10, 73.39, 73.36 (C-2, C-3, C-4, C-5), 76.05, 75.99, 74.56, 74.52, 73.84, 73.52, 73.30, 73.06, 71.89, 71.23, 70.60, 65.28 (C-6, $CH_2$; Bn, $OCH_2$), 54.25, 53.73 (CH; AdGly), 50.13, 50.02 (CH; *iso*Gln), 47.16, 46.71 (CH; Ala), 42.11, 41.95 (CH), 39.86, 39.78, 39.68, 39.02, 32.83, 32.71, 32.42, 31.34 ($CH_2$; γ-*iso*Gln, $CH_2$; Ad), 28.26, 28.20 ($CH_2$; β-*iso*Gln), 30.75, 30.39, 29.71, 29.36, 29.07 (CH; Ad), 17.73, 17.50 ($CH_3$; Ala), 14.76, 14.53 ($CH_3$); ESI-MS: calcd. for $C_{58}H_{72}N_4O_{12}$: 1017.21; found $[M+H]^+$ at *m*/*z* 1017.50.

N-*[(*S*)-3-(2,3,4,6-tetra*-O-*benzyl-α-D-mannopyranosyl-oxy)-2-methylpropanoyl]-L,D-(adamant-2-yl)glycyl-L-alanyl-D*-iso*glutamine* (**20**)*:* 154 mg (54 %), $R_f$ = 0.50 ($CHCl_3$/MeOH = 3:1); $^1$H NMR (MeOH-$d_4$) *δ* / ppm: 8.02 (d, *J* = 8.3 Hz, NH), 7.96 (d, *J* = 8.1 Hz, NH), 7.83 (d, *J* = 7.7 Hz, NH), 7.75 (pt, *J* = 6.9 Hz, *J* = 9.3 Hz, NH), 7.57 (d, *J* = 7.4 Hz, NH), 7.52−7.04 (m, $H_{arom}$), 5.21 (d, *J* = 1.7 Hz, H-1), [5.17 (s, H-1)], 5.03−4.34 (m, $CH_2$; Bn, 2CH-α; AdGly, Ala), [4.69(d, $J_{gem}$ = 11.1 Hz, CH; Bn)], [4.65 (d, $J_{gem}$= 12.0 Hz, CH; Bn)], 4.27 (q, *J* = 6.4 Hz, CH-α*;* Ala), 4.12−4.00 (m, CH-α*; iso*Gln,), 3.95−3.31 (m, H-2, H-3, H-4, H-5, H-6a, H-6b, $OCH_2$), 2.74−2.65 (m, CH), 2.24−2.17 (m, CH-γ; *iso*Gln), 2.07−1.57 (m, $CH_2$-β, CH-γ; *iso*Gln, CH, $CH_2$; Ad), 1.33 (d, *J* = 6.6 Hz, $CH_3$; Ala), [1.32 (d, *J* = 7.2 Hz, $CH_3$; Ala)], 1.02 (d, *J* = 6.7 Hz, $CH_3$), [1.00 (d, *J* = 6.9 Hz, $CH_3$)]; $^{13}$C NMR (MeOH-$d_4$) *δ* / ppm: 177.58, 177.38, 176.30, 176.16, 174.82, 174.74, 174.22, 173.90 (C=O), 139.99, 139.84, 139.77, 139.65, 139.61, 139.56, 139.54 ($C_{arom}$), 129.41−127.98 ($CH_{arom}$), 100.07, 99.76 (C-1), 81.86, 81.45, 76.46, 76.16, 76.08, 73.36 (C-2, C-3, C-4, C-5), 76.02, 75.96, 75.91, 74.53, 74.48, 73.81, 73.81, 73.51, 73.27, 73.18, 73.03 (C-6, $CH_2$; Bn, $OCH_2$), 53.70, 53.54 (CH; 2-AdGly), 52.22, 51.05 (CH; *iso*Gln), 47.10, 46.68 (CH; Ala), 42.07, 42.03 (CH), 39.84, 39.74, 39.65, 38.99, 32.80, 32.68 ($CH_2$; Ad, $CH_2$; γ-*iso*Gln), 30.71, 30.37, 29.68, 29.32, 29.03 (CH; Ad), 28.26, 28.17 ($CH_2$; β-*iso*Gln), 17.74, 17.52 ($CH_3$; Ala), 14.77, 14.54 ($CH_3$); ESI-MS: calcd. for $C_{58}H_{72}N_4O_{12}$: 1017.21; found $[M+H]^+$ at *m*/*z* 1017.48.

*General Procedure for* O-*debenzylation*

To a solution of compounds **17**−**20** (100 mg, 0.132 mmol) in 3:1 mixture of $CHCl_3$/MeOH (5 mL) 50 mg of

10 % Pd/C and 20 mL of 50% EtOH in water were added. The mixtures were subjected to hydrogen atmosphere under 4 bars at room temperature and stirred for 24 h. Then catalysts were filtrated and filtrates concentrated under reduced pressure. The residues were purified by column chromatography on silica-gel ($CHCl_3$/MeOH = 1:1) and compounds **21**−**24** were obtained.

N-*[(*R*)-3-(α-D-mannopyranosyloxy)-2-methylpropanoyl]-L,D-(adamant-1-yl)glycyl-L-alanyl-D-*iso*glutamine* ***(21)****:* 66 mg (76 %); $R_f$ = 0.31 ($CHCl_3$/MeOH = 1:1); $^1$H NMR ($D_2O$/dioxan) $\delta$ / ppm: 8.34 (s, OH), 7.86 (d, $J$ = 7.2 Hz, NH), 7.64 (d, $J$ = 7.2 Hz, NH), 7.58 (d, $J$ = 7.9 Hz, NH), 7.41−7.31 (m, NH), 5.10 (d, $J$ = 1.4 Hz, H-1), [4.82 (d, $J$ = 1.4 Hz, H-1)], 4.64 (br s, CH-α; AdGly), 4.30−4.09 (m, CH-α; Ala, *iso*Gln), [4.27 (q, $J$ = 7.1 Hz, CH-α; Ala)], [4.19 (dd, $J$ = 4.4 Hz, $J$ = 7.7 Hz, CH-α; *iso*Gln)], 4.04−3.42 (m, H-2, H-3, H-4, H-5, H-$6_a$, H-6b, $OCH_2$), 2.85−2.75 (m, 1H, CH), 2.22−2.00 (m, $CH_2$-β, $CH_2$-γ; *iso*Gln), [2.18 (q, 1H, $J$ = 6.9 Hz, $CH_2$-β; *iso*Gln)], 1.91 (br s, CH; Ad), 1.66−1.52 (m, $CH_2$; Ad), 1.33 (d, $J$ = 6.9 Hz, $CH_3$; Ala), [1.32 (d, $J$ = 7.0 Hz, $CH_3$; Ala)], 1.06 (d, $J$ = 7.2 Hz, $CH_3$), [0.98 (d, $J$ = 6.9 Hz, $CH_3$)]; $^{13}$C NMR ($D_2O$/dioxan) $\delta$ / ppm: 177.97, 177.79, 176.79, 176.33, 175.69, 174.81, 171.54 (C=O), 100.16, 100.05 (C-1), 76.09, 72.97, 72.60, 72.32, 71.15, 70.59, 70.15, 69.90 (C-2, C-3, C-4, C-5), 69.44 (C-6), 66.83, 66.77 (CH; AdGly), 60.89, 60.84 ($OCH_2$), 53.43, 53.35 (CH; *iso*Gln), 49.87, 48.80 (CH; Ala), 40.61, 40.52 (CH), 38.62, 38.26, 36.02, 35.72, 33.77, 33.57 ($CH_2$; γ-*iso*Gln, $CH_2$; Ad, C; Ad), 28.04, 28.00 (CH; Ad), 27.70, 27.61 ($CH_2$; β-*iso*Gln), 16.73, 16.19 ($CH_3$; Ala), 12.90, 12.83 ($CH_3$); ESI-MS: calcd. for $C_{30}H_{48}N_4O_{12}$: 656.72; found $[M+H]^+$ at *m*/*z* 657.40.

N-*[(*S*)-3-(α-D-mannopyranosyloxy)-2-methylpropanoyl]-L,D-(adamant-1-yl)glycyl-L-alanyl-D-*iso*glutamine* ***(22)****:* 44 mg (50 %); $R_f$ = 0.30 ($CHCl_3$/MeOH = 1:1); $^1$H NMR ($D_2O$/dioxan) $\delta$ / ppm: 8.08 (d, $J$ = 7.7 Hz, NH), 7.71 (s, NH), 7.55 (d, $J$ = 8.7 Hz, NH), 7.47-7.44 (m, NH), 7.21 (s, NH), 7.12 (s, NH), 7.03 (s, NH), 5.16 (s, H-1), [4.89 (s, H-1)], 4.50−3.98 (m, 3CH-α; AdGly, Ala, *iso*Gln), [4.33 (br s, CH-α; AdGly)], 3.92−3.33 (m, H-2, H-$6_a$, $OCH_2$, H-3, H-4, H-6b, H-5), 2.88−2.19 (m, CH, $CH_2$-γ and $CH_2$-β; *iso*Gln), 1.99−154 (m, Ad), 1.40 (d, $J$ = 5.6 Hz, $CH_3$; Ala), [1.38 (d, $J$ = 6.2 Hz, $CH_3$; Ala)], 1.11 (d, $J$ = 6.6 Hz, $CH_3$), [1.04 (d, $J$ = 6.4 Hz, $CH_3$)]; $^{13}$C NMR ($D_2O$/dioxan) $\delta$ / ppm: 179.20, 173.58, 173.50, 173.43, 172.31 (C=O), 93.93, 93.54 (C-1), 76.01, 72.93, 72.30, 71.14, 70.61, 70.15, 70.00, 66.80 (C-2, C-3, C-4, C-5), 62.49, 60.89, 60.77 (C-6, $OCH_2$), 49.64, 49.30 (CH; AdGly), 46.50, 46.45 (CH; *iso*Gln), 44.60, 44.07 (CH; Ala), 40.22, 39.89 (CH), 38.32, 38.23, 38.21, 35.96, 29.89, 29.85 ($CH_2$; β-*iso*Gln, $CH_2$; γ-*iso*Gln, $CH_2$; Ad), 27.89, 27.87, 27.84 (CH; Ad), 18.43, 18.42 ($CH_3$; Ala), 16.51, 16.49 ($CH_3$); ESI-MS: calcd. for $C_{30}H_{48}N_4O_{12}$: 656.72; found $[M+H]^+$ at *m*/*z* 657.50.

N-*[(*R*)-3-(α-D-mannopyranosyloxy)-2-methylpropanoyl]-L,D-(adamant-2-yl)glycyl-L-alanyl-D-*iso*glutamine* ***(23)****:* 42 mg (48 %); $R_f$ = 0.29 ($CHCl_3$/MeOH = 1:1). $^1$H NMR ($D_2O$/dioxan) $\delta$ / ppm: 8.44 (s, OH), 8.06 (d, $J$ = 7.8 Hz, NH), 7.87 (d, $J$ = 7.5 Hz, NH), 7.71 (d, $J$ = 7.5 Hz, NH), 7.64 (pt, $J$ = 7.7 Hz, $J$ = 7.4 Hz, NH), 7.44−7.32 (m, NH) 5.16 (d, $J$ = 1.0 Hz, H-1), [4.88 (s, H-1)], 4.76 (d, $J$ = 6.7 Hz, CH-α; AdGly), [4.64−4.63 (m, CH-α; AdGly)], 4.40 (q, $J$ = 7.1 Hz, CH-α; Ala), [4.34 (q, $J$ = 7.1 Hz, CH-α; Ala)], 4.25 (dd, $J$ = 5.0 Hz, $J$ = 6.6 Hz, CH-α; *iso*Gln ), [4.16−4.14 (m, CH-α; *iso*Gln)], 3.93−3.45 (m, H-2, H-$6_a$, $OCH_2$, H-3, H-4, H-6b, H-5), 2.78−2.71 (m, CH), 2.65−2.60 (m, CH), 2.26−1.60 (m, $CH_2$-γ and $CH_2$-β; *iso*Gln, Ad), 1.41−1.37 (m, $CH_3$; Ala), 1.09−1.02 (m, $CH_3$); $^{13}$C NMR ($D_2O$/dioxan) $\delta$ / ppm: 177.60, 177.58, 176.12, 174.83, 174.56, 173.90, 173.79, 173.55 (C=O), 100.08, 100.06 (C-1), 72.63, 72.55, 70.60, 70. 57, 70.48, 70.46, 66.76, 66.71 (C-2, C-3, C-4, C-5), 69.32, 69.30 (C-6); 60.87 ($OCH_2$), 55.09, 53.04 (CH; AdGly), 49.55, 49.36 (CH; *iso*Gln), 45.37, 45.34 (CH; Ala), 40.74, 40.71 (CH), 38.05, 37.83, 37.20, 31.25, 31.18, 31.13, 31.02 ($CH_2$; γ-*iso*Gln, $CH_2$; Ad), 29.01, 28.97, 27.91, 27.39, 27.12 (CH; Ad), 26.76 ($CH_2$; β-*iso*Gln), 17.07, 16.76 ($CH_3$; Ala), 13.36, 12.78 ($CH_3$). ESI-MS: calcd. for $C_{30}H_{48}N_4O_{12}$: 656.72; found $[M+H]^+$ at *m*/*z* 657.30.

N-*[(*S*)-3-(α-D-mannopyranosyloxy)-2-methylpropanoyl]-L,D-(adamant-2-yl)glycyl-alanyl-D-*iso*glutamine* ***(24)****:* 59 mg (68 %); $R_f$ = 0.34 ($CHCl_3$/MeOH = 1:1); $^1$H NMR ($D_2O$/dioxan) $\delta$ / ppm: 8.44 (s, OH), 7.87 (d, $J$ = 7.7 Hz, NH), 7.71 (pt, $J$ = 7.4 Hz, $J$ = 7.5 Hz, NH), 7.63 (pt, $J$ = 7.5 Hz, $J$ = 7.3 Hz, NH), 7.43−7.38 (m, NH), 5.16 (s, H-1), [4.88 (s, H-1)], 4.64 (d, $J$ = 6.5 Hz, CH-α; AdGly), [4.59 (d, $J$ = 5.6 Hz, CH-α; AdGly)], 4.36 (q, $J$ = 7.5 Hz, CH-α; Ala), [4.32 (q, $J$ = 7.1 Hz, CH-α; Ala)], 4.24 (dd, $J$ = 4.7 Hz, $J$ = 7.0 Hz, CH-α; *iso*Gln ), [4.20−4.11 (m, CH-α; *iso*Gln)], 3.97−3.33 (m, H-2, H-$6_a$, $OCH_2$, H-3, H-4, H-6b, H-5), 2.78−2.73 (m, CH), 2.63−2.59 (m, CH), 2.25−1.56 (m, $CH_2$-γ and $CH_2$-β; *iso*Gln, Ad), 1.39−1.36 (m, $CH_3$; Ala), 1.06−1.01 (m, $CH_3$). $^{13}$C NMR ($D_2O$/dioxan) $\delta$ / ppm: 177.97, 177.93, 177.40, 177.23, 176.08, 174.65, 173.87, 173.56 (C=O), 99.34, 99.28 (C-1), 72.62, 72.33, 70.63, 70.55, 70.17, 70.02, 66.80 (C-2, C-3, C-4, C-5), 68.91, 68.88 (C-6), 60.91, 60.82 ($OCH_2$), 53.19, 53.00 (CH; AdGly), 49.87, 49.82 (CH; *iso*Gln), 45.55, 45.52 (CH; Ala), 40.22, 39.89 (CH), 38.09, 38.05, 37.98, 37.86, 37.23, 31.28, 31.26, 31.15, 31.08 ($CH_2$; γ-*iso*Gln, $CH_2$; Ad), 28.97, 28.82, 28.72, 27.87, 27.74, 27.41, 27.16, 27.11, 27.15 (CH; Ad), 27.26, 27.06 ($CH_2$; β-*iso*Gln), 16.24, 16.21 ($CH_3$; Ala), 13.28, 13.05 ($CH_3$). ESI-MS: calcd. for $C_{30}H_{48}N_4O_{12}$: 656.72; found $[M+H]^+$ at *m*/*z* 657.30.

### Experiments *in vivo*

Experiments *in vivo* were performed on CBA (H-$2^k$) inbread mice strains. All mice used were females 2 to 2.5 months old. Commercial food and water were provided *ad libitum*. During the experiments animals were kept at the animal facility at the Institute of Immunology and all experiments were performed according to the Croatian Law on Animal Welfare (The Official Gazette "NN" 135/06).

Experimental groups of five mice were immunized and boosted two times subcutaneously (s.c.) into the tail base at 21-days intervals. Mice were anesthetised prior to blood collection on 7$^{th}$ day after the second booster. Sera were collected, decomplemented at 56 °C for 30 minutes and stored at −20 °C until tested.

The dose of OVA (antigen) was 10 μg per mouse. The dose of PGM and adamantyltripeptides was 200 μg per mouse. OVA and tested substances were dissolved in saline and the injection volume in all experimental groups was 0.1 mL per mouse.

### Enzyme immunoassays for qualitative and quantitative determination of OVA-specific IgG (anti-OVA IgG) in mice sera

Enzyme immunoassays (ELISA) were performed on flat-bottomed high binding microtitre plates (Costar, USA) according to previously described procedures.[22,29] The relative quantities of anti-OVA IgG were determined by parallel line assay comparing each serum to monoclonal anti-chicken egg albumin, declared as standard preparation, to which 20,000 arbitrary units per milliliter (AU/mL) were voluntarily assigned.[30]

### Enzyme immunoassays for qualitative and quantitative determination of OVA-specific IgG subtypes anti-OVA IgG1 and anti-OVA IgG2a respectively, in mice sera

ELISA for determination of anti-OVA IgG1 and anti-OVA IgG2a were performed as described previously.[31] The relative quantities of antibody subtypes were determined by parallel line assay using appropriate standard preparation. The monoclonal anti-OVA IgG1 was a standard for relative quantification of anti-OVA IgG1 to which 400,000 AU/mL was assigned, while polyclonal mouse serum containing high levels of anti-OVA IgG2a was used as a standard for relative quantification of IgG2a specific antibodies with voluntarily assigned 20,000 AU/mL. The ratio of anti-OVA IgG1 and anti-OVA IgG2a (IgG1/IgG2a) was used as indication of the Th1/Th2-bias of induced immune response.

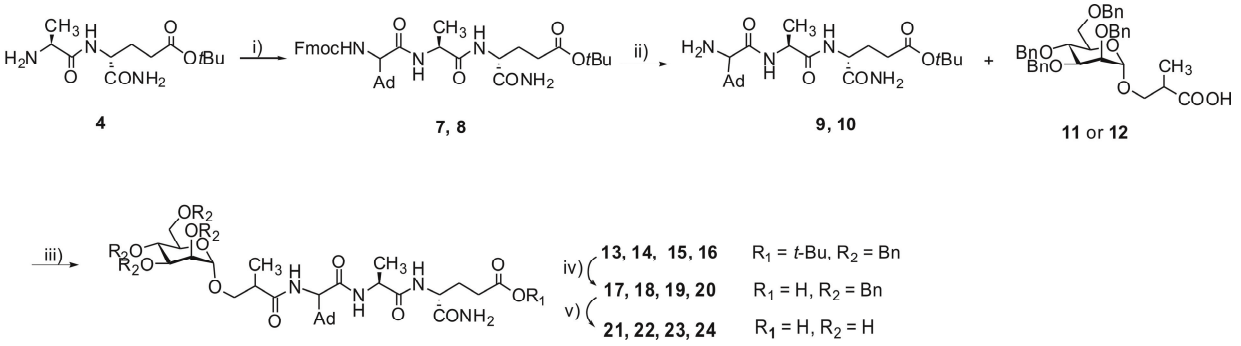
### Statistics

Statistical analyses were performed using Statistica 6.0 for Windows, StatSoft Inc. The significant difference between experimental groups was evaluated by Kruskal-Wallis ANOVA, followed by multiple Mann-Whitney U-nonparametric tests. A probability values less than 0.05 ($p < 0.05$) were considered significant.

## RESULTS AND DISCUSSION

Immunomodulating characteristics of natural peptidoglycan molecules are well known,[2,32] and some of these compounds were considered for use as adjuvants for human and animal vaccines. Smaller, synthetic molecules, such as muramyldipeptides and tripeptides were prepared and their immunomodulating properties investigated as well. These investigations led to a number of structure-activity studies which include synthetic modifications, possibilities of which are numerous since the molecules are multifunctional, and both saccharide and peptide parts may be modified. Synthetic modifications described so far were mostly on smaller molecules since the protection and deprotection of multifunctional compounds with groups of similar activity represent a continuous synthetic problem. Thus, several synthetically modified MDP, syntheses of which were patented, have already been included in clinical trials.[33] Despite numerous synthetic problems encountered in transformations of larger molecules several successful attempts to synthetically modify PGM monomer **1** as a parent compound were made. The previous reports describe the synthesis of PGM modified with Boc-tyrosine,[34] adamant-1-yl,[35] as well as with mannopyranosyl[22] residues. This was possible because the preferential conformation of PGM in aqueous and dimethylsulfoxide solutions showed that the amino group of *meso*-diaminopimelic acid is exposed and can therefore be manipulated without the interference of other reactive groups.[36]

In the present work a novel class of mannosylated adamantyl tripeptides, general formula of which is α-Man-$OCH_2CH(CH_3)CO$-AdGly-L-Ala-D-*iso*Gln, were prepared, characterized, and their possible immunomodulatory properties investigated in some preliminary tests *in vivo*. All newly synthesized glycopeptides comprise in their structure the adamantylglycyl moiety linked to the dipeptide L-Ala-D-*iso*Gln, characteristic of the peptide portion of natural peptidoglycans and synthetic muramyldipeptides. They also comprise mannose, an important sugar whose receptors on immunocompetent cells (such as macrophages and dendritic cells) are considered to be pattern-recognition receptors biding compounds comprising mannose, *N*-acetylglucosamine and fucose. Previous investigations showed that adamant-1-yl tripeptides **2a**, **2b**[6] (Figure 1) and adamant-2-yl tripeptides **3a**, **3b**[7] can be prepared as pure diastereoisomers and they were tested and shown to differ in biological activity in several model systems.[6–10] Synthesis of mannosylated adamantyltripeptides reported in




**Scheme 1.** i) EDCxHCl, HOBtx$H_2O$, *N*-ethylmorpholine, Fmoc-(adamant-1-yl)Gly **5** or Fmoc-(adamant-2-yl)Gly **6**, dioxane:$CH_2Cl_2$=1:1; ii) DBU, 1-octanthiol, THF; iii) EDCxHCl, HOBtx$H_2O$, $Et_3N$, dioxane:$CH_2Cl_2$ = 1:1; iv) TFA; v) $H_2$, 10 % Pd/C, 50 % EtOH.

this work led to compounds with fixed anomeric configuration on the sugar ring (α), fixed chirality of the spacer (*R* or *S*) linking the mannose moiety to the rest of these complex molecules, but in the final step we were not able to separate diastereoisomers formed due to the isomeric structures of the tripeptide portion of these novel molecules. Nevertheless, preliminary investigation of their adjuvant (immunostimulating) activity *in vivo* was conducted in order to obtain results indicating possible differences in their immunostimulating activities as a result of the introduced mannose and chiral spacer parts. Positive results obtained make further investigations worthwhile especially in view of the significance of mannose molecules on the cell surfaces, and further attempts to synthesize pure diasteroisomers are in progress.

The glycopeptides **21**–**24** were obtained in a sequence of steps (Scheme 1). The amino group of racemic adamant-1-yl or -2-yl glycine was protected with 9-fluorenylmethyloxycarbonyl (Fmoc) to give protected compounds **5** and **6**. Condensation of **5** and **6** with *tert*-butyl ester of L-Ala-D-*iso*Gln **4**[6] followed, to produce completely protected tripeptides **7** and **8**, respectively. The base labile Fmoc protecting group was them removed using catalytic amounts of dia-za(1,3)bicyclo[5.4.0]undecane (DBU) and 1-octanthiol as a scavenger to trap the liberated dibenzofulvene. *Tert*-butyl esters of adamantyltripeptides **9** and **10** were obtained and coupled to perbenzylated α-mannosyloxy-2-methylpropionic acid of *R* (**11**) or *S* (**12**) configuration. As previously described the acids **11** and **12** were prepared in a sequence of steps.[14,22] Commercially available methyl (*R*) or (*S*)-3-hydroxy-2-methylpropio-nate was *O*-mannosylated using benzyl protected mannosyl trichloroacetimidate as a good glycosyl donor and catalytic amounts of Lewis acids as reaction promotors. *O*-Glycosylations gave anomeric mixtures of the corresponding *O*-glycosides in different ratios. The obtained α,β-glycosides were readily separated by column chromatography on silica gel giving pure anomers in moderate overall yields. The hydrolysis of methyl esters of α-anomers was performed by saponification leading to acids **11** and **12**.

Completely protected mannosylated adamantyltripeptides **13**–**16** were obtained in good yields using EDC/HOBt coupling method. Acid hydrolysis of *tert*-butyl protecting group led to compounds **17**–**20**. Attempts to separate diastereoisomers were unsuccessful and compounds **17** ((*R*)α-Man-$OCH_2CH(CH_3)CO$-(Ad-1-

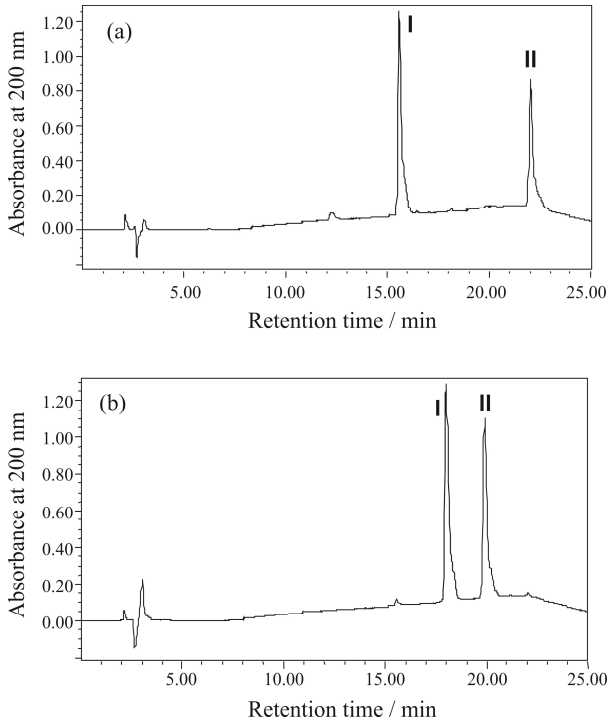



**Figure 2.** HPLC chromatogram of mannosylated adamantyltripeptides diastereoisomeric mixtures: a) **23**, *R*-spacer; peak **I** L-L-D of **3b** (*t* = 15.6 min), peak **II** D-L-D of **3a** (*t* = 22.0 min); and b) **24**, *S*-spacer; peak **I** L-L-D of **3b** (*t* = 17.9 min), peak **II** D-L-D of **3a** (*t* = 19.9 min); in both cases the ratio of peak **I** : peak **II** was 6 : 4.

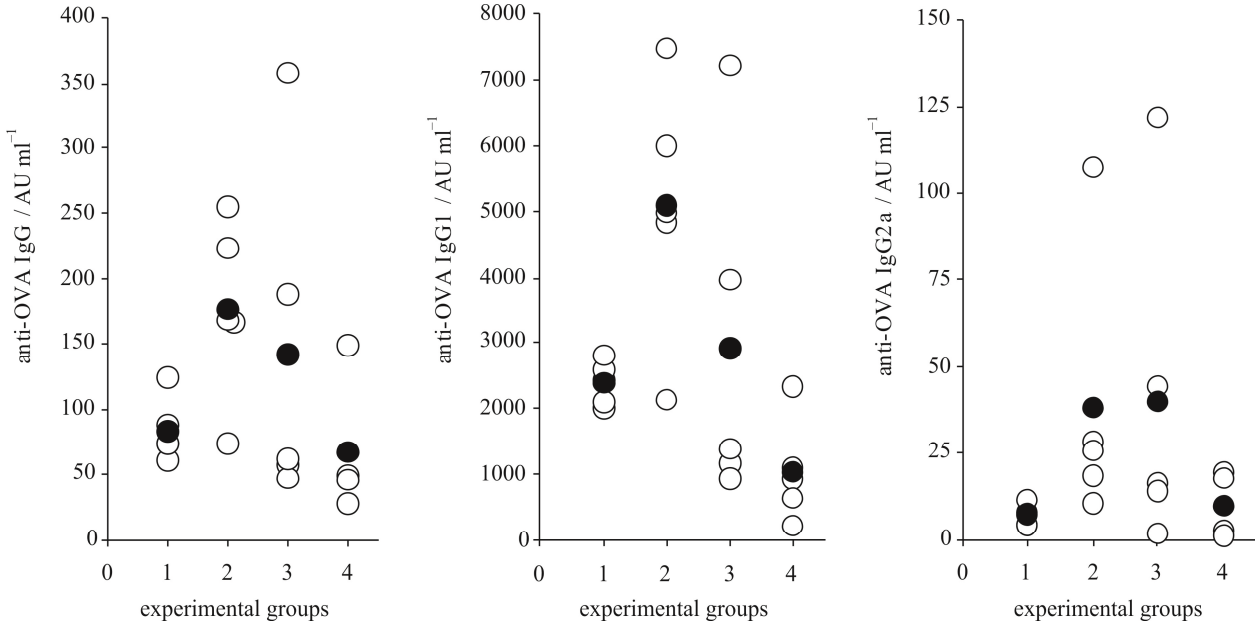



**Figure 3.** The effect of mannosylated adamantyltripeptides **23** and **24** on the production of anti-OVA IgG (a) and its subtypes anti-OVA IgG1 (b) and anti-OVA IgG2a (c), respectively, in CBA (H-2$^k$) mice. Mice were immunized with OVA as an antigen and sera were analyzed after second booster. Experimental groups: 1-OVA alone; 2-OVA+**1**; 3-OVA+**23**; 4-OVA+**24**. • denotes group mean value, o denotes each serum separately.

yl)Gly-L-Ala-D-*iso*Gln), **18** ((*S*)α-Man-OCH$_2$CH(CH$_3$)-CO-(Ad-1-yl)Gly-L-Ala-D-*iso*Gln),**19** ((*R*) α-Man-OCH$_2$CH(CH$_3$)CO-(Ad-2-yl)Gly-L-Ala-D-*iso*Gln) and **20** ((*S*) α-Man-OCH$_2$CH(CH$_3$)CO-(Ad-2-yl)Gly-L-Ala-D-*iso*Gln) were obtained as mixtures of diastereoisomers. Catalytic hydrogenation of **17**–**20** gave the completely deprotected compounds **21**–**24**. Diasteroisomeric mixtures were not separated but compounds **23** (spacer of *R* configuration) and **24** (spacer of *S* configuration) were obtained as mixtures of diastereoisomers with identical ratios (4:6) of tripeptides of D-L-D and L-L-D configuration (Figure 2). Compounds **23** and **24** were then further tested *in vivo* for their immunostimulating activity.

The adjuvant activity of mannosylated adamantyltripeptides **23** and **24** in comparison to PGM **1** was studied. PGM was established as a reference for adjuvant activity in a previously well-defined mouse model *in vivo*, proven suitable for comparison of the observed effects.[37,38] Ovalbumin was used as an antigen for studying the adjuvant effect of mannosylated adamantyltripeptides in CBA (H-2$^k$) mouse strain.

The comparison of induced anti-OVA IgG levels was carried out quantitatively and the subclasses of IgG, IgG1 and IgG2a, as indicator of Th1 or Th2 type of immune response were also determined (Figure 3).

In general, no significant enhancement in total anti-OVA IgG antibody production was observed when mannosylated adamantyltripeptides **23** and **24** were applied, in comparison to no adjuvant (OVA alone) treated group, although glycoconjugate **23** elicited better immune response than OVA alone. In comparison to the control, PGM-injected group, the better immunostimulating effect of tested compounds was not observed either. However, the measured quantity of anti-OVA IgG antibodies was markedly higher when compound **23** was applied in comparison to **24.**

Similar results were obtained when anti-OVA IgG1 and anti-OVA IgG2a were measured. Thus, for manosylated adamantyltripeptide **23** the response was again a little higher than for **24**. Basically, it was the same pattern as for anti-OVA IgG antibodies.

From IgG1/IgG2a ratio, calculated for each serum (obtained after the second booster), it could be observed that neither of tested glycoconjugates significantly shifted the IgG1/IgG2a ratio in comparison to no adju-

**Table 1.** The ratio of anti-OVA IgG1 and anti-OVA IgG2a (IgG1/IgG2a) in CBA (H-2$^k$) mice. For each mouse serum, obtained after the second booster, $\log_{10}$ IgG1/IgG2a was calculated and the result for each experimental group ($n$ = 5) is presented as average ± standard deviation (SD)

| Experimental groups | Average of $\log_{10}$ IgG1/IgG2a ± SD |
|---|---|
| OVA | 2.55 ± 0.2 |
| OVA + **1** | 2.23 ± 0.4 |
| OVA + **23** | 2.05 ± 0.6 |
| OVA + **24** | 2.06 ± 0.8 |

vant treated group of animals (Table 1). Accordingly, the tested mannosylated adamantyltripeptides **23** and **24** did not stimulate immunomodulatory activity towards more pronounced Th1 or Th2 type of immune response in comparison to antigen alone.

## CONCLUSION

In the present work a novel class of mannosylated adamantyl tripeptides, general formula of which is α-Man-$OCH_2CH(CH_3)$CO-AdGly-L-Ala-D-*iso*Gln, were prepared and characterized. All newly synthesized glycopeptides comprise in their structure the adamantylglycyl moiety linked to the dipeptide L-Ala-D-*iso*Gln, characteristic of the peptide portion of natural peptidoglycans and synthetic muramyldipeptides. They also comprise mannose, an important sugar whose receptors on immunocompetent cells are considered to be pattern-recognition receptors biding compounds comprising mannose or some other specific saccharides.

The immunostimulating activity of mannosylated adamant-2-yl tripeptide connected with a spacer of *R*-configuration **23** was compared to its analogue **24** connected by a spacer of *S*-configuration. It was observed that glycoconjugates **23** exhibited higher activity than **24** in testing of IgG, IgG1 and IG2a levels. The obtained results indicate that the chirality of a spacer plays a significant role. Since it is not possible to introduce mannose directly into the tripeptide molecules due to sterical reasons the direct influence of mannose on activity of the whole molecule, excluding additional chirality in the spacer, can only be determined in further testing using compounds with achiral spacers. The results reported so far gave no evidence that novel mannosylated tripeptides **23** and **24** bind to mannose receptors. They do not though exclude the possible influence of mannose since several previous reports suggested that binding to MR was associated with decreased immune response.[39,40] Therefore, further research on this topic is required.

*Acknowledgements.* We wish to thank the Ministry of Science, Education and Sports of the Republic of Croatia for support of this work (projects 119-1191344-3121 and 021-0212432-2431).

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