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Original Scientific Article

Synthesis and Biological Activity of Mannose Conjugates with 1-Adamantamine and Ferrocene Amines

Rosana Ribić,[a] Monika Kovačević,[b] Vesna Petrović-Peroković,[a] Ita Gruić-Sovulj,[a] Vladimir Rapić,[b,*] and Srđanka Tomić[a,*]

[a]*Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102A, HR-10000 Zagreb, Croatia*
[b]*Department of Chemistry and Biochemistry, Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, HR-10000 Zagreb, Croatia*

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Abstract. Pure α- and β-anomers of *O*-mannosyl conjugates with 1-adamantamine and ferrocene amines were prepared. The sugar moiety in these glycoconjugates is connected to the amine by a chiral linker (methyl (*R*)-3-hydroxy-2-methyl propanoate and/or methyl (*S*)-3-hydroxy-2-methyl propanoate). The α-D-mannopyranosides with adamantane and ferrocene aglycon parts were tested using the hemagglutination assay (inhibition of the agglutination of guinea pig erythrocytes by type 1 fimbriated *E. coli* HB101 (pPKl4)). All glycoconjugates showed inhibitory potencies in the mM range.

Keywords: O-Mannopyranosides, 1-Adamantamine, Ferrocene Amines, Hemagglutination

INTRODUCTION

Research in the field of glycobiology has shown that molecular recognition involving carbohydrates and cell-surface proteins (lectins) is of essential importance in many biological processes.[1,2]

Among other biological processes, carbohydrate-protein interactions play important roles in the cell and microbial adhesion. Adhesion of pathogenic organisms to host tissues is the initiation of the majority of infectious diseases. It is often mediated by lectins present on the surface of the infectious organism that combines with complementary sugars on the host surface.[3] One type of receptor-ligand interaction is that of the most common family of bacterial adhesins, mannose-specific type 1 fimbriae.[4] In *Escherichia coli*, mannose-specific adhesion is mediated by the FimH adhesin at the tip of type 1 fimbriae. FimH has two domains, the mannose-binding lectin domain and the fimbria-incorporating pilin domain which are connected by an interdomain linker peptide chain.[5,6]

It is well known that many lectins in addition to the carbohydrate binding sites, possess hydrophobic binding sites, in particular around the site at which the aglycon will be localized upon carbohydrate-lectin complexation. Several studies were reported with large numbers of α-D-mannopyranosides with high-affinity ligands, some of them of significant inhibitory potency for *E. coli* type 1 adhesin. Almost all recent communications have been focused on glycoconjugate dendrimers which were found to bind with high affinity to FimH and prevent hemagglutination of red blood cells mediated by cross-linking of surface epitopes containing mannose.[4,7–10] However, multivalent ligands are predicted to be poorly permeable to the GI tract and probably not amenable for oral dosing because of their large molecular weight and high polarity. Of all the reported monovalent FimH mannoside antagonists long chain alkyl and arylmannosides were shown to display the highest affinity possibly due to increased hydrophobic interactions within the binding pocket.[11] Arylmannoside inhibitors of hemagglutination were first reported many years ago[12,13] but very limited structure-activity relationship studies followed.[12,14] A recent study shows the importance of monovalent aromatic FimH mannoside antagonists designed for the clinical treatment of urinary tract infections.[15]

In view of previous reports it seems of interest to explore the influence of monovalent FimH mannoside antagonists with structurally different aglycons on the resulting binding capacities. Therefore, we decided to study mannose conjugates with 1-adamantamine and ferrocene amines in order to compare the influence of bulky adamantane and aromatic ferrocene moieties on the inhibitory potencies aimed to preventing the adhesion of *E. coli* to erythrocytes. Both aglycons are

* Authors to whom correspondence should be addressed. (E-mail: vrapić@pbf.hr and stomic@chem.pmf.hr)

hydrophobic and therefore when conjugated to mannose, potentially able to interact with lectin's hydrophobic binding sites.

In continuation of our interest and research of monosaccharide conjugates,[16] their enzymatic transformations[17–20] and synthesis,[21,22] especially of those with biological activity[23] we now report the synthesis of series of mannose conjugates with 1-adamantamine and ferrocene amines. The newly synthesized compounds were subsequently submitted to the hemagglutination assay in order to explore their inhibitory potency towards type 1 fimbriated *E. coli*. Several drugs containing the adamantane moiety are currently commercially available. They are mostly used in the prevention of viral diseases and as drugs in the treatment of Parkinson disease.[24] Among them 1-Adamantamine (amantadine) plays an important role since it inhibits the reproduction of influenza viruses.[24,25] 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.[24] On the other hand conjugates of ferrocene with various biomolecules, *e.g.* amino acids, peptides, proteins, DNA, RNA, PNA and carbohydrates play an important role in bioorganometallic chemistry. Stability of the ferrocenyl moiety in aqueous aerobic media and its favourable electrochemical properties make the derived bioorganometallics suitable for biological application: bioanalysis, enzymology, biomimesis, catalysis, bioprobes, genosensors, novel therapeuticals, etc.[26–31] Up to now only a few studies were devoted to ferrocene-carbohydrate conjugates.

EXPERIMENTAL

General Remarks

Starting compound 2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl trichloroacetimidate (**1α**) was prepared as described previously.[32] Ferrocenylamine and methyl 1'-aminoferrocene-1-carboxylic acid were synthesized in the form of *N*-Boc protected derivatives (FcNHBoc, [33] Boc-Fca-Ome[34]) by Curtius rearrangement of Fc-CON_3 and MeOOC-Fn-CON_3 in *tert*-butyl alcohol. Ferrocenylmethylamine was freshly prepared by reduction of ferrocenecarboxamide with $LiAlH_4$/THF.[35] 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) of adamantane conjugates 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. Preparative TLC of ferrocene containing compounds was performed on silica gel using the mixture of diethyl ether/petroleum ether (φ = 0.67) as eluents. 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.

Hemagglutination Test

Bacteria: pPK14 plasmid, bearing genes for *Escherichia coli* type 1 fimbriae[36] was a generous gift from Dr. Per Klemm, Department of Systems Biology, Technical University of Denmark. The plasmid was electroporated into *E. coli* HB101 strain competent cells (defective in type 1 fimbriae production) followed by selection of the transformed cells (HB101 (pPKl4)) on LB/amp plates. A single colony was transferred into 500 mL of LB/amp media and bacterial culture was grown overnight at 37 °C (about 15 h). *E. coli* HB101 (pPKl4) cells were harvested by centrifugation (2000 rpm, 15 min at 4 °C), washed twice with PBS buffer (8 g NaCl, 0.2 g KCl, 1.44 g $Na_2HPO_4\times2H_2O$ and 0.2 g KH_2PO_4 were dissolved in distilled water up to 1 L total volume, pH = 7.0) and resuspended in the same buffer in approximate concentration of 10 mg mL^{-1} wet weight.[4] Produced *E. coli* HB101 (pPKl4) cells having expressed type 1 fimbriae were kept at 4 °C (no longer than two days) prior to the usage in hemagglutination assay. Guinea pig blood (5 mL) was freshly isolated and stabilized by 3.8 % sodium citrate (1 mL). After centrifugation (2000 rpm, 10 min) packed erythrocytes (4 mL) were obtained. The sediment of erythrocytes was carefully suspended in PBS (4 mL) and this procedure of centrifugation and washing of the sediment was repeated twice. Packed erythrocytes (4 mL) were suspended in PBS (76 mL) and stored at 4 °C. Hemagglutination tests were performed in V-shaped 96 well microtiter plates (Nunc). Sugar derivatives were suspended in distilled deionized water and serially diluted solutions (10 μL) were mixed with bacteria suspension (10 μL) in wells. After 10 min guinea pig erythrocytes (10 μL) were added and erythrocyte agglutination was read after 10 min at room temperature.

Synthesis of O-*Mannosylated Derivatives of (*R*)- and (*S*)-Methyl 3-hydroxy-2-methylpropanoates (**2α, 2β, 3α and 3β**)*

General Procedure. (*R*)-(+)- or (*S*)-(–)-Methyl 3-hydroxy-2-methylpropanoate (151.07 μL, 1.37 mmol, 1.6 eq.) was suspended in dry CH_2Cl_2 (2 mL) at 0 °C under N_2 and $BF_3\times Et_2O$ was added (277.52 μL, 2.19 mmol, 1 eq.). Glycosyl trichloroacetimidate **1α** (1.5 g, 2.19 mmol) was suspended in dry CH_2Cl_2 (2 mL) and then added dropwise within 15 min after which the reaction mixture was stirred for the additional 2.5 h and moni-

tored by TLC (diethyl ether/petroleum ether, φ = 0.50). Iced 1 M $NaHCO_3$ was added and the resulting mixture extracted with diethyl ether. Organic layers were washed with water and dried over Na_2SO_4. After filtration, the organic layer was concentrated *in vacuo* and the residue was purified by column chromatography on silica gel (diethyl ether/petroleum ether, φ = 0.50). The products were isolated as anomeric pale yellow oil mixtures **2α**, **2β** (1.023 g, 73 %) and **3α**, **3β** (953 mg, 68 %).

The anomeric mixtures **2α**, **2β** and **3α**, **3β** were then rechromatographied to isolate pure anomers of each derivative. All analytical data for **2α** and **2β** were identical to those desribed in our previous paper.[23]

*(*S*)-Methyl 3-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyloxy)-2-methylpropanoate (**3α**).* $[\alpha]_D$ +34.8º (*c* 1.32, $CHCl_3$); R_f = 0.37 (diethyl ether/petroleum ether, φ = 0.50). ^{1}H NMR ($CDCl_3$) δ / ppm: 7.37–7.16 (m, 20H, H–Ar), 4.86 (d, $J_{1,2}$ = 1.6 Hz, 1H, H-1), 4.85 (d, J_{gem} = 10.72 Hz, 1H, CH_2Ph), 4.75–4.52 (m, 6H, 3 CH_2Ph), 4.51 (d, J_{gem} = 10.99 Hz, 1H, CH_2Ph), 3.98 (app t, J = 9.43 Hz, J = 9.43 Hz, 1H, H-4), 3.82 (dd, $J_{2,3}$ = 2.91 Hz, $J_{3,4}$ = 9.39 Hz, 1H, H-3), 3.87–3.83 (m, 1H, H-2), 3.78 (dd, $J_{5,6a}$ = 5.40 Hz, $J_{6a,6b}$ = 11.02 Hz, 1H, H-6a), 3.73–3.70 (m, 3H, OCH_2, H-6b), 3.61 (s, 3H, OCH_3), 3.45–3.42 (m, 1H, H-5), 2.73–2.67 (m, 1H, CH), 1.13 (d, J = 7.10 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ / ppm: 174.69 (C=O), 138.52, 138.45, 138.42, 138.34 (*C*–Ar), 128.35–127.26 (*CH*–Ar), 97.88 (C-1), 80.01, 74.80, 74.73, 71.99 (C-2–5), 74.85, 73.26, 72.55, 72.16 (CH_2Ph), 69.20 (C-6), 68.95 (OCH_2), 51.64 (OCH_3), 39.65 (CH), 13.80 (CH_3).

Anal. Calcd. mass fraction of elements, *w* / %, for $C_{39}H_{44}O_8$ are: C 73.10, H 6.92, found: C 73.01, H 6.86.

*(*S*)-Methyl 3-(2,3,4,6-tetra-*O*-benzyl-β-D-manno-pyranosyloxy)-2-methylpropanoate (**3β**).* $[\alpha]_D$ –43.2º (*c* 0.44, $CHCl_3$); R_f = 0.30 (diethyl ether/petroleum ether, φ = 0.50). ^{1}H NMR ($CDCl_3$) δ / ppm: 7.45–7.15 (m, 20H, H–Ar), 4.94 (d, J_{gem} = 12.56 Hz, 1H, CH_2Ph), 4.90 (d, J_{gem} = 10.85 Hz, 1H, CH_2Ph), 4.80 (d, J_{gem} = 12.51 Hz, 1H, CH_2Ph), 4.66–4.37 (m, 4H, 2 CH_2Ph), 4.52 (d, J_{gem} = 10.79 Hz, 1H, CH_2Ph), 4.39 (s, 1H, H-1), 4.03–3.98 (m, 1H, H-4), 3.89–3.82 (m, 2H, H-2, H-3), 3.79–3.72 (m, 2H, OCH_2), 3.68 (s, 3H, OCH_3), 3.67–3.61 (m, 1H, H-6a), 3.50–3.41 (m, 2H, H-5, H-6b), 2.91–2.80 (m, 1H, CH), 1.18 (d, J = 7.18 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ / ppm: 175.44 (C=O), 138.62, 138.37, 138.26, 137.08 (4 *C*–Ar), 128.38–127.35 (*CH*–Ar), 102.00 (C1), 82.13, 75.89, 74.77, 73.18 (C-2–5), 75.13, 73.63, 73.42, 71.76 (4 CH_2Ph), 71.31 (C-6), 69.55 (OCH_2), 51.73 (OCH_3), 40.27 (CH), 13.96 (CH_3).

Anal. Calcd. mass fraction of elements, *w* / %, for $C_{39}H_{44}O_8$ are: C 73.10, H 6.92, found: C 72.96, H 6.88.

Synthesis of O*-Mannosylated Propionic Acids (**4α**, **4β**, **5α** and **5β**)*

General Procedure. To a solution of pure anomer of each ester derivative **2** and **3** (100 mg, 0.156 mmol) in dioxane (1 mL) 10 equivalents of 1 M NaOH was added and the reaction mixture was stirred for 24 h at 40 °C and monitored by TLC (C_6H_6/EtOAc, φ = 0.67). Reaction mixtures were then neutralized with 0.5 M HCl (pH = 3.5) and saturated brine was added. Mixtures were extracted with diethyl ether and organic layers dried over Na_2SO_4. After filtration, the organic layer was concentrated *in vacuo* and the residues purified by column chromatography on silica gel ($CHCl_3$/MeOH, φ = 0.90).

All the NMR data for (*R*)-3-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyloxy)-2-methylpropanoic acid **4α** (91.9 mg, 94 %) and (*R*)-3-(2,3,4,6-tetra-*O*-benzyl-β-D-mannopyranosyloxy)-2-methylpropanoic acid **4β** (81.1 mg, 83 %) are identical to those previously reported.[23]

*(*S*)-3-(2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyloxy)-2-methylpropanoic acid (**5α**).* Pale yellow oil (83.1 mg, 85 %); $[\alpha]_D$ +16.9º (*c* 0.42, $CHCl_3$); R_f = 0.59 ($CHCl_3$/methanol, φ = 0.90). ^{1}H NMR ($CDCl_3$) δ / ppm: 7.36–7.16 (m, 20H, H–Ar), 4.89 (d, $J_{1,2}$ = 1.59 Hz, 1H, H-1), 4.88 (d, J_{gem} = 11.07 Hz, 1H, CH_2Ph), 4.74–4.54 (m, 6H, 3 CH_2Ph), 4.50 (d, J_{gem} = 10.83 Hz, 1H, CH_2Ph), 3.96 (app t, J = 9.25 Hz, J = 9.26 Hz, 1H, H-4), 3.88 (dd, $J_{2,3}$ = 2.57 Hz, $J_{3,4}$ = 9.29 Hz, 1H, H-3), 3.87–3.84 (m, 1H, H-2), 3.78–3.73 (m, 4H, OCH_2, H-6a, H-6b), 3.66–3.62 (m, 1H, H-5), 3.08–3.03 (m, 1H, CH), 1.29 (d, J = 7.06 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ / ppm: 179.00 (C=O), 138.45, 138.39, 138.36, 138.32 (4 *C*–Ar), 128.49–127.36 (*CH*–Ar), 98.03 (C-1), 79.99, 74.83, 74.81, 72.04 (C-2–5), 75.13, 73.33, 72.67, 72.25 (4 CH_2Ph), 69.18 (C-6), 68.84 (OCH_2), 39.59 (CH), 13.70 (CH_3).

Anal. Calcd. mass fraction of elements, *w* / %, for $C_{38}H_{42}O_8$ are: C 72.82, H 6.75, found: C 72.70, H 6.63.

*(*S*)-3-(2,3,4,6-Tetra-*O*-benzyl-β-D-mannopyranosyloxy)-2-methylpropanoic acid (**5β**).* Pale yellow oil (75.3 mg, 77 %); $[\alpha]_D$ –41.1º (*c* 0.56, $CHCl_3$); R_f = 0.49 ($CHCl_3$/methanol, φ = 0.90). ^{1}H NMR ($CDCl_3$) δ / ppm: 7.43–7.15 (m, 20H, H–Ar), 4.93 (d, J_{gem} = 12.55 Hz, 1H, CH_2Ph), 4.88 (d, J_{gem} = 10.98 Hz, 1H, CH_2Ph), 4.80 (d, J_{gem} = 12.47 Hz, 1H, CH_2Ph), 4.51 (d, J_{gem} = 10.67 Hz, 1H, CH_2Ph), 4.40 (s, 1H, H-1), 4.64–4.38 (m, 4H, 2 CH_2Ph), 4.02–3.97 (m, 1H, H-4), 3.89–3.82 (m, 2H, H-2, H-3), 3.81–3.63 (m, 3H, H-6a, OCH_2), 3.50–3.43 (m, 2H, H-5, H-6b), 2.91–2.80 (m, 1H, CH), 1.20 (d, J = 7.09 Hz, 3H, CH_3).^{13}C NMR ($CDCl_3$) δ / ppm: 179.41 (C=O), 138.51, 138.19, 138.19, 138.04 (4 *C*–Ar), 128.40–127.39 (*CH*–Ar), 101.95 (C-1), 82.11, 75.77, 74.71, 73.29 (C-2–5), 75.12, 73.75, 73.42, 71.63 (4 CH_2Ph), 71.39 (C-6), 69.50 (OCH_2), 40.28 (CH), 13.75 (CH_3).

Anal. Calcd. mass fraction of elements, *w* / %, for $C_{38}H_{42}O_8$ are: C 72.82, H 6.75, found: C 72.64, H 6.59.

*Synthesis of AMA Mannopyranosides (**7α**, **7β**, **8α** and **8β**)*

General Procedure. To a solution of pure anomer of each carboxylic acid derivative **7**–**10** (100 mg, 0.16

mmol) in dry CH_2Cl_2 at 0 °C EDC×HCl (25.4 mg, 0.208 mmol, 1.3 eq.) previously mixed with Et_3N (28.8 μL) was added. The mixtures were then stirred for 0.5 h at the same temperature. HOBt (21.6 mg, 0.16 mmol, 1 eq.) was added next and the mixtures were left stirring for additional 2 h. During that time the temperature was allowed to gradually warm up to room temperature. AMA×HCl (150.1 mg, 0.8 mmol) previously mixed with Et_3N (110.9 μL) was added next and the reactions were left stirring overnight. After the neutralization with 0.5 M HCl (pH = 3.5) the mixtures were extracted with CH_2Cl_2, combined organic layers washed with saturated $NaHCO_3$ and dried over Na_2SO_4. After filtration, the organic layers were concentrated *in vacuo* and the residues purified by column chromatography on silica gel (C_6H_6/EtOAc, φ = 0.67).

*(*R*)*-N-*(Adamantan-1-yl)-3-(2,3,4,6-tetra*-O-*benzyl-α-D-mannopyranosyloxy)-2-methylpropanamide (**7α**).* Pale yellow oil (69.3 mg, 57 %); $[\alpha]_D$ −7.8º (*c* 1.03, $CHCl_3$); R_f = 0.44 (C_6H_6/EtOAc, φ = 0.67). ^{1}H NMR ($CDCl_3$) δ / ppm: 7.36–7.16 (m, 20H, H–Ar), 5.62 (s, 1H, N–H), 4.90 (d, J_{gem} = 10.87 Hz, 1H, *CH*$_2$Ph), 4.88 (s, 1H, H-1), 4.75–4.54 (m, 6H, 3 *CH*$_2$Ph), 4.51 (d, J_{gem} = 11.04 Hz, 1H, *CH*$_2$Ph), 3.91–3.64 (m, 7H, H-2, H-3, H-4, H-6a, H-6b, OCH_2), 3.54–3.49 (m, 1H, H-5), 2.53–2.42 (m, 1H, CH), 1.99 (s, 3H, 3 *CH*-AMA), 1.88 (s, 6H, 3 *CH*$_2$–AMA), 1.60 (s, 6H, 3 *CH*$_2$–AMA), 0.97 (d, *J* = 6.94 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ / ppm: 173.64 (C=O), 138.54, 138.38, 138.17, 138.05 (4 C–Ar), 128.47–127.31 (*CH*–Ar), 99.54 (C-1), 80.23, 75.08, 74.57, 71.93 (C-2–5), 74.84, 73.58, 72.59, 72.20 (4 *CH*$_2$Ph), 71.99 (C-6), 69.90 (OCH_2), 51.62 (C–N), 42.09 (CH), 41.54 (*CH*$_2$–AMA), 36.30 (*CH*$_2$–AMA), 29.37 (*CH*-AMA), 14.16 (CH_3) ppm.

Anal. Calcd. mass fraction of elements, *w* / %, for $C_{48}H_{57}NO_7$ are: C 75.86, H 7.56, N 1.84, found: C 75.74, H 7.52; N,1.94.

(R*)*-N-*(Adamantan-1-yl)-3-(2,3,4,6-tetra*-O-*benzyl-β-D-mannopyranosyloxy)-2-methylpropanamide (**7β**).* Pale yellow oil (66.9 mg, 55 %); $[\alpha]_D$ −59.4º (*c* 0.32, $CHCl_3$); R_f = 0.37 (C_6H_6/EtOAc, φ = 0.67). ^{1}H NMR ($CDCl_3$) δ / ppm: 7.46–7.17 (m, 20H, H–Ar), 5.80 (s, 1H, N–H), 4.95 (d, J_{gem} = 12.46 Hz, 1H, *CH*$_2$Ph), 4.90 (d, J_{gem} = 11.01 Hz, 1H, CH_2Ph), 4.83 (d, J_{gem} = 12.34 Hz, 1H, CH_2Ph), 4.55 (d, J_{gem} = 11.78 Hz, 1H, *CH*$_2$Ph), 4.65–4.47 (m, 4H, 2 *CH*$_2$Ph), 4.39 (s, 1H, H-1), 4.06–4.00 (m, 1H, H-4), 3.93–3.87 (m, 2H, H-2, H-3), 3.85–3.71 (m, 2H, OCH_2), 3.53–3.42 (m, 3H, H-5, H-6a, H-6b), 2.55–2.44 (m, 1H, CH), 1.97 (s, 3H, 3 *CH*-AMA), 1.93 (s, 6H, 3 CH_2–AMA), 1.59 (s, 6H, 3 *CH*$_2$–AMA), 1.16 (d, *J* = 7.08 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ / ppm: 173.02 (C=O), 138.64, 138.29, 138.25, 138.08 (4 *C*–Ar), 128.44–127.34 (*CH*–Ar), 101.34 (C-1), 82.22, 75.86, 74.82, 74.02 (C-2–5), 75.13, 73.99, 73.47, 71.81 (4 *CH*$_2$Ph), 71.60 (C-6), 69.62 (OCH_2), 51.62 (C–N), 41.57 (CH), 41.43 (*CH*$_2$–AMA), 36.33 (*CH*$_2$–AMA), 29.39 (*CH*-AMA), 14.25 (CH_3).

Anal. Calcd. mass fraction of elements, *w* / %, for $C_{48}H_{57}NO_7$ are: C 75.86, H 7.56, N 1.84, found: C 75.76, H 7.50, N 1.89.

(S*)*-N-*(Adamantan-1-yl)-3-(2,3,4,6-tetra*-O-*benzyl-α-D-mannopyranosyloxy)-2-methylpropanamide (**8α**).* Pale yellow oil (69.3 mg, 57 %); $[\alpha]_D$ +11.9º (*c* 0.42, $CHCl_3$); R_f = 0.46 (C_6H_6/EtOAc, φ = 0.67). ^{1}H NMR ($CDCl_3$) δ / ppm: 7.38–7.14 (m, 20H, H–Ar), 5.32 (s, 1H, N–H), 4.90–4.50 (m, 8H, 4 *CH*$_2$Ph), 4.87 (s, 1H, H-1), 4.01 (app t, *J* = 9.32 Hz, *J* = 9.40 Hz, 1H, H-4), 3.84 (dd, $J_{2,3}$ = 2.69 Hz, $J_{3,4}$ = 9.36 Hz, 1H, H-3), 3.80–3.70 (m, 5H, OCH_2, H-2, H-6a, H-6b), 3.33–3.30 (m, 1H, H-5), 2.36–2.31 (m, 1H, CH), 1.95 (s, 3H, 3 *CH*-AMA), 1.90 (s, 6H, 3 *CH*$_2$–AMA), 1.58 (s, 6H, 3 *CH*$_2$–AMA), 1.05 (d, *J* = 6.88 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ / ppm: 173.10 (C=O), 138.71, 138.49, 138.39, 138.28 (4 *C*–Ar), 128.33–127.32 (*CH*–Ar), 97.84 (C-1), 80.18, 74.85, 74.59, 71.91 (C-2–5), 74.82, 73.28, 72.52, 72.22 (4 *CH*$_2$Ph), 70.01 (C-6), 69.07 (OCH_2), 51.62 (C–N), 41.79 (CH), 41.67 (*CH*$_2$–AMA), 36.32 (*CH*$_2$–AMA), 29.38 (*CH*-AMA), 14.12 (CH_3).

Anal. Calcd. mass fraction of elements, *w* / %, for $C_{48}H_{57}NO_7$ are: C 75.86, H 7.56, N 1.84, found: C 75.75, H 7.59, N 1.89.

*(*S*)*-N-*(Adamantan-1-yl)-3-(2,3,4,6-tetra*-O-*benzyl-β-D-mannopyranosyloxy)-2-methylpropanamide (**8β**).* Pale yellow oil (68.1 mg, 56 %); $[\alpha]_D$ −23.0º (*c* 0.43, $CHCl_3$); R_f = 0.31 (C_6H_6/EtOAc, φ = 0.67). ^{1}H NMR ($CDCl_3$) δ / ppm: 7.46–7.17 (m, 20H, H–Ar), 5.80 (s, 1H, N–H), 4.95 (d, J_{gem} = 12.47 Hz, 1H, *CH*$_2$Ph), 4.90 (d, J_{gem} = 11.00 Hz, 1H, *CH*$_2$Ph), 4.83 (d, J_{gem} = 12.34 Hz, 1H, *CH*$_2$Ph), 4.55 (d, J_{gem} = 11.78 Hz, 1H, *CH*$_2$Ph), 4.65–4.47 (m, 4H, 2 *CH*$_2$Ph), 4.39 (s, 1H, H-1), 4.06–4.00 (m, 1H, H-4), 3.93–3.87 (m, 2H, H-2, H-3), 3.83–3.70 (m, 2H, OCH_2), 3.53–3.42 (m, 3H, H-5, H-6a, H-6b), 2.55–2.44 (m, 1H, CH), 1.97 (s, 3H, 3 *CH*–AMA), 1.92 (s, 6H, 3 *CH*$_2$–AMA), 1.59 (s, 6H, 3 *CH*$_2$–AMA), 1.16 (d, *J* = 7.08 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ / ppm: 173.02 (C=O), 138.64, 138.30, 138.25, 138.08 (4 *C*–Ar), 128.44–127.34 (*CH*–Ar), 101.34 (C-1), 82.22, 75.86, 74.82, 74.02 (C-2–5), 75.13, 73.99, 73.47, 71.80 (4 *CH*$_2$Ph), 71.60 (C-6), 69.62 (OCH_2), 51.62 (C–N), 41.57 (CH), 41.43 (*CH*$_2$–AMA), 36.32 (*CH*$_2$–AMA), 29.38 (*CH*-AMA), 14.25 (CH_3).

Anal. Calcd. mass fraction of elements, *w* / %, for $C_{48}H_{57}NO_7$ are: C 75.86, H 7.56, N 1.84, found: C 75.76, H 7.55, N 1.94.

*Synthesis of Ferrocene Mannopyranosides (**11α**, **11β**, **12 α**, **12 β**, **13 α** and **13 β**)*

General Procedure. A suspension of 1.5 mmol of FcNHBoc or MeOOCFcNHBoc in ethyl acetate was cooled to 0 °C and treated with gaseous HCl for 2 h. After stirring at room temperature for 4 h, mixture was evaporated *in vacuo* to dryness to leave yellow solid hydrochloride. This salt was treated with Et_3N in dry

CH_2Cl_2 (pH ≈ 8) and added to solution of 1 mmol of mannopyranoside carboxylic acids **4α**, **β** activated in a similar way as described in general procedure for the-preparation of AMA glycoconjugates. After stirring for 72 h at room temperature, the mixture was washed thrice with saturated solution of $NaHCO_3$, 10 % aqueous solution of citric acid and H_2O, then dried over Na_2SO_4 and evaporated *in vacuo*. The residue was purified by TLC on silica gel (CH_2Cl_2/EtOAc, φ = 0.83) giving anomeric mixtures which were rechromographed using diethyl ether/petroleum ether (φ = 0.67) to leave pure anomers as orange/yellow oils (**11α**, **11β**, **12α** and **12β**). In a similar way free $FcCH_2NH_2$ (obtained by reduction of $FcCONH_2$ by $LiAlH_4$ in THF) was coupled with mannopyranosides **4α**, **β** giving after TLC purification conjugates **13α** and **13β**.

(R*)*-N-*Ferrocenyl-3-(2,3,4,6-tetra*-O-*benzyl-α-D-manno-pyranosyloxy)-2-methylpropanamide (**11α**)*. Orange oil (57.3 mg, 59 %); $[\alpha]_D$ +28.9º (*c* 2.7, $CHCl_3$); R_f = 0.64 (diethyl ether/petroleum ether, φ = 0.67). ^{1}H NMR ($CDCl_3$) δ / ppm: 7.69 (s, 1H, N–H), 7.32–7.25 (m, 20H, H–Ar), 4.89–4.60 (m, 7H, CH_2Ph), 4.57 (s, 2H, *H*Fc), 4.22 (d, J_{gem} = 10.81 Hz, 1H, CH_2Ph), 4.35 (br s, 1H, H-1), 4.09 (s, 5H, *H*Fc), 4.03 (d, $J_{1,2}$ = 1.77 Hz, 1H, H-2), 3.99 (d, $J_{3,4}$ = 9.33 Hz, 1H, H-4), 3.92 (d, $J_{2,3}$ = 2.46 Hz, 1 H. H-3), 3.88–3.83 (m, 2H, OCH_2), 3.77–3.72 (m, 2H, H-6a, H-6b), 3.47–3.42 (m, 1H, H-5), 2.81–2.76 (m, 1H, CH), 0.99 (d, *J* = 6.72 Hz, 3H, CH_3) ^{13}C NMR ($CDCl_3$) δ / ppm: 173.22 (C=O), 138.31, 138.28, 138.20, 137.39 (4 *C*–Ar), 128.51–127.63 (*CH*–Ar), 101.68 (C-1), 94.74 (C-1, Fc), 79.80, 75.60, 75.50, 72.01 (C-2–5), 74.57, 74.41, 74.04, 73.04 (4 CH_2Ph), 72.51 (C-6), 69.19, 64.51, 63.91, 61.11, 60.95 (*C*Fc), 70.82 (OCH_2), 42.25 (CH), 14.51 (CH_3).

Anal. Calcd. mass fraction of elements, *w* / %, for $C_{48}H_{51}NO_7Fe$ are: C 71.2, H 6.35, N 1.73, found: C 71.35, H 6.38, N 1.76.

*(*R*)*-N-*Ferrocenyl-3-(2,3,4,6-tetra*-O-*benzyl-β-D-manno-pyranosyloxy)-2-methyl-propanamide (**11β**)*. Orange oil (24.9 mg, 77 %); $[\alpha]_D$ –27.5º (*c* 0.8, $CHCl_3$); R_f = 0.51 (diethyl ether/petroleum ether, φ = 0.67). ^{1}H NMR ($CDCl_3$) δ / ppm: 7.68 (s, 1H, N–H), 7.46–7.17 (m, 20H, H–Ar), 4.94–4.55 (m, 6H, CH_2Ph), 4.57 (s, 2H, HFc), 4.53 (s, 2H, *H*Fc), 4.52–4.49 (m, 2H, CH_2Ph), 4.43 (s, 1H, H-1), 4.08 (s, 5H, *H*Fc), 3.94 (d, $J_{2,3}$ = 2.55 Hz, 1H, H-2), 3.90–3.87 (m, 2H, H-3, H-4), 3.78–3.72 (m, 2H, OCH_2), 3.61–3.58 (m, 2H, H-6a, H-6b), 3.52–3.48 (m, 1H, H-5), 2.69–2.66 (m, 1H, CH), 1.20 (d, *J* = 7.33 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ / ppm: 172.14 (C=O), 138.47, 138.32, 138.22, 138.05 (4 *C*–Ar), 128.43–127.53 *CH*–Ar), 100.89 (C-1), 95.21 (C-1, Fc), 82.26, 75.80, 74.88, 74.15 (C-2–5), 75.17, 74.09, 73.37, 71.92 (4 CH_2Ph), 71.27 (C-6), 69.22, 64.44, 64.39, 61.48, 61.38 (*C*Fc), 69.65 (OCH_2), 40.97 (CH), 13.97 (CH_3).

Anal. Calcd. mass fraction of elements, *w* / %, for $C_{48}H_{51}NO_7Fe$ are: C 71.2, H 6.35, N 1.73, found: C 71.37, H 6.37, N 1.75.

*(*R*)*-N-*1'-Methoxycarbonylferrocenyl-3-(2,3,4,6-tetra*-O-*benzyl-α-D-manno-pyranosyloxy)-2-methylpropan-amide (**12α**)*. Orange oil (101.9 mg, 98 %); $[\alpha]_D$ –1.4º (*c* 2.1, $CHCl_3$); R_f = 0.61 (diethyl ether/petroleum ether, φ = 0.67). ^{1}H NMR ($CDCl_3$) δ / ppm: 7.65 (s, 1H, N–H), 7.33–7.17 (m, 20H, H–Ar), 4.95–4.88 (m, 3H, CH_2Ph), 4.69 (s, 2H, *H*Fn), 4.62–4.57 (m, 3H, CH_2Ph), 4.54 (s, 2H, HFn), 4.51–4.49 (m, 2H, CH_2Ph), 4.46 (s, 1H, H-1), 4.29 (s, 2H, *H*Fn), 4.07 (app t, *J* = 8.58 Hz, *J* = 9.00 Hz, 1H, H-4), 3.96 (d, $J_{2,3}$ = 2.67 Hz, 1H, H-3), 3.93 (s, 1H, H-2), 3.90 (s, 2H, *H*Fn), 3.87–3.79 (m, 2H, OCH_2), 3.78–3.76 (m, 2H, H-6a, H-6b), 3.75 (s, 3H, $COCH_3$), 3.65–3.59 (m, 1H, H-5), 2.74–2.67 (m, 1H, CH), 1.25 (d, *J* = 7.62 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ / ppm: 172.31 (C=O), 171.69 (*C*OCH_3), 138.54, 138.28, 138.22, 138.07 (4 *C*–Ar), 128.41–127.36 (*CH*–Ar), 100.94 (C-1), 95.96 (C-1, Fn), 82.25, 75.81, 74.84, 74.20 (C-2–5), 75.14, 74.09, 73.36, 71.87 (4 CH_2Ph), 71.26 (C-6), 72.60, 70.98, 66.24, 66.06, 62.76, 62.72 (*C*Fn), 71.51 (C-1', Fn), 69.59 (OCH_2), 51.54 (CO*C*H_3), 40.96 (CH), 13.81 (CH_3).

Anal. Calcd. mass fraction of elements, *w* / %, for $C_{50}H_{53}NO_9Fe$ are: C 69.20, H 6.16, N 1.61, found: C 69.09, H 6.25, N 1.62.

*(*R*)*-N-*1'-Methoxycarbonylferrocenyl-3-(2,3,4,6-tetra*-O-*benzyl-β-D-manno-pyranosyloxy)-2-methylpropan-amide (**12β**)*. Orange oil (25.1 mg, 72 %); $[\alpha]_D$ +16.3º (*c* 0.8, $CHCl_3$); R_f = 0.78 (diethyl ether/petroleum ether, φ = 0.67). ^{1}H NMR ($CDCl_3$) δ / ppm: 7.53 (s, 1H, N–H), 7.29–7.20 (m, 20H, H–Ar), 4.90–4.57 (m, 5H, CH_2Ph), 4.85 (d, J_{gem} = 10.73 Hz, 1H, CH_2Ph), 4.70–4.69 (m, 2H, *H*Fn), 4.62 (d, J_{gem} = 11.58 Hz, 1H, CH_2Ph), 4.58 (s, 2H, *H*Fn), 4.49 (d, J_{gem} = 11.16 Hz, 1H, CH_2Ph), 4.46 (d, $J_{1,2}$ = 1.38 Hz, 1H, H-1), 4.28 (s, 2H, *H*Fn), 3.97 (d, $J_{3,4}$ = 9.06 Hz, 1H, H-4), 3.94 (pt, 2H, *H*Fn), 3.92–3.91 (m, 1H, H-3), 3.89–3.84 (m, 3H, H-2, OCH_2), 3.81–3.78 (m, 2H, H-6a, H-6b), 3.74 (s, 3H, COCH_3), 3.53–3.50 (m, 1H, H-5), 2.75–2.73 (m, 1H, CH), 1.03 (d, *J* = 6.96 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ / ppm: 172.77 (C=O), 171.42 (*C*OCH_3), 137.77, 137.68, 137.06, 137.04 (4 C–Ar), 127.99–127.07 (*CH*–Ar), 100.51 (C-1), 95.11 (C-1, Fn), 79.21, 74.81, 72.60, 72.33 (C-2–5), 74.17, 73.41, 72.90, 72.41 (4 CH_2Ph), 71.83 (C-6), 71.53, 70.62, 70.31, 66.05, 65.59, 62.08, 61.9 (*C*Fn), 70.99 (C-1', Fn), 70.01 (OCH_2), 51.05 (CO*C*H_3), 41.61 (CH), 13.93 (CH_3).

Anal. Calcd. mass fraction of elements, *w* / %, for $C_{50}H_{53}NO_9Fe$ (M_r = 867.83)·½ CH_2Cl_2 are: C 66.63, H 5.98, N 1.54, found: C 66.65, H 6.02, N 1.56.

(R)-N-*Ferrocenylmethyl-3-(2,3,4,6-tetra*-O-*benzyl-α-D-mannopyranosyloxy)-2-methylpropanamide* (***13α***): Yellow oil (76.1 mg, 77 %); $[\alpha]_D$ –27.5º (*c* 2.0, $CHCl_3$); R_f = 0.70 (diethyl ether/petroleum ether, φ = 0.67).

^{1}H NMR ($CDCl_3$) δ / ppm: 7.37–7.27 (m, 20H, H–Ar), 6.12 (app t, J = 4.79 Hz, J = 4.65 Hz, 1H, N–H), 4.87–4.45 (m, 8H, CH_2Ph), 4.48 (s, 1H, H-1), 4.20–4.13 (m, 2H, *H*Fc), 4.08 (s, 5H, *H*Fc), 4.06 (s, 2H, *H*Fc), 4.01 (d, $J_{2,3}$ = 2.37 Hz, 1H, H-3), 3.94–3.93 (m, 2H, CH_2Fc), 3.80–3.76 (m, 4H, H-4, H-2, OCH_2), 3.72–3.68 (m, 2H, H-6a, H-6b), 3.55–3.50 (m, 1H, H-5), 2.60–2.53 (m, 1H, CH), 1.01 (d, J = 7.02 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ / ppm: 173.40 (C=O), 137.99, 137.95, 137.83, 137.52 (4 *C*–Ar), 127.86–127.07 (*CH*–Ar), 99.34 (C-1), 83.85 (C-1, Fc), 79.70, 74.67, 74.44, 71.42 (C-2–5), 74.47, 72.99, 72.23, 71.71 (4 CH_2Ph), 71.67 (C-6), 68.10, 68.08, 68.04, 67.75, 67.52 (*C*Fc), 69.42 (OCH_2), 40.91 (CH), 38.49 (CH_2Fc), 13.84 (CH_3).

Anal. Calcd. mass fraction of elements, *w* / %, for $C_{49}H_{53}NO_7Fe$ are: C 71.44, H 6.48, N 1.7, found: C 71.54, H 6.52, N 1.73.

*(*R*)*-N-*Ferrocenylmethyl-3-(2,3,4,6-tetra-*O*–benzyl-β-D-manno-pyranosyloxy)-2-methylpropanamide (**13β**):* Yellow oil (20.4 mg, 62 %); $[\alpha]_D$ –25.5º (*c* 1.0, $CHCl_3$); R_f = 0.57 (diethyl ether/petroleum ether, φ = 0.67). ^{1}H NMR ($CDCl_3$) δ / ppm: 7.42–7.16 (m, 20H, H–Ar), 6.29 (app t, J = 4.75 Hz, J = 4.26 Hz, 1H, N–H), 4.88–4.50 (m, 7H, CH_2Ph), 4.76 (d, J_{gem} = 12.21 Hz, 1H, CH_2Ph), 4.36 (s, 1H, H-1), 4.11 (s, 2H, *H*Fc), 4.08 (s, 5H, *H*Fc), 4.06 (s, 2H, *H*Fc), 4.04–4.02 (m, 3H, H-4, CH_2Fc), 3.85 (d, $J_{2,3}$ = 2.73 Hz, 1H, H-3), 3.82–3.74 (m, 3H, H-2, OCH_2), 3.65–3.55 (m, 2H, H-6a, H-6b), 3.50–3.46 (m, 1H, H-5), 2.64–2.57 (m, 1H, CH), 1.20 (d, J = 7.11 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ / ppm: 172.88 (C=O), 138.21, 137.86, 137.76, 137.61 (4 *C*–Ar), 128.05–126.99 (*CH*–Ar), 100.68 (C-1), 84.31 (C-1, Fc), 81.73, 75.32, 74.34, 73.70 (C-2–5), 74.61, 73.59, 72.93, 71.19 (4 CH_2Ph), 70.85 (C-6), 68.05, 68.00, 67.93, 67.55, 67.46 (*C*Fc), 69.16 (OCH_2), 40.53 (CH), 38.27 (CH_2Fc), 13.92 (CH_3).

Anal. Calcd. mass fraction of elements, *w* / %, for $C_{49}H_{53}NO_7Fe$ are: C 71.44, H 6.48, N 1.7, found: C 71.56, H 6.54, N 1.68.

Debenzylation of AMA and Ferrocene Conjugates

General Procedure. To a solution of pure anomer of each AMA **7** and **8** (100 mg, 0.132 mmol) and Fc conjugate **11**–**13** (100 mg) in CH_2Cl_2 (5 mL) 50 mg of 10 % Pd/C and 20 mL of CH_3OH was added. The mixtures were subjected to hydrogen atmosphere under 4 bars at room temperature and stirred for 24 h. Then they were filtered over a celite bed and the filtrates concentrated *in vacuo*. The residues were purified by column chromatography on silica-gel (CH_3CN/H_2O, φ = 0.83) and the compounds **9**, **10**, and **14**–**16** were obtained.

(R)-N-*(Adamantan-1-yl)-3-α-D-mannopyranosyloxy-2-methylpropanamide (**9α**).* Pale yellow crude foam (49.4 mg, 94 %); $[\alpha]_D$ +17.2º (*c* 0.64, CH_3OH); R_f = 0.58 (acetonitrile/H_2O, φ = 0.83). 1H NMR (CD_3OD): δ / ppm = 7.30 (s, 1H, N–H), 4.71 (d, $J_{1,2}$ = 1.47 Hz, 1H, H-1), 3.85–3.80 (m, 1H, H-3), 3.83 (dd, $J_{6b,5}$ = 1.84 Hz, $J_{6a,6b}$ = 11.68 Hz, H-6b), 3.76 (dd, $J_{1,2}$ = 1.62 Hz, $J_{2,3}$ = 3.02 Hz, 1H, H-2), 3.72–3.45 (m, 6H, H-3, H-4, H-5, H-6a, OCH_2), 2.65–2.53 (m, 1H, CH), 2.02 (br s, 9H, 3 *CH*-AMA, 3 CH_2–AMA), 1.71 (s, 6H, 3 CH_2–AMA), 1.01 (d, J = 6.94 Hz, 3H, CH_3). ^{13}C NMR (CD_3OD): δ / ppm = 176.73 (C=O), 102.28 (C-1), 74.72, 72.67, 72.13, 68.62 (C-2–5), 71.55 (C-6), 62.94 (OCH_2), 52.86 (C–N), 42.82 (CH), 42.44 (CH_2–AMA), 37.56 (CH_2–AMA), 30.97 (*CH*-AMA), 14.64 (CH_3).

Anal. Calcd. mass fraction of elements, *w* / %, for $C_{20}H_{33}NO_7$ are: C 60.13, H 8.33, N 3.51, found: C 59.94, H 8.23, N 3.65.

*(*R*)*-N-*(Adamantan-1-yl)-3-β-D-mannopyranosyloxy-2-methylpropanamide (**9β**).* Pale yellow crude foam (46.3 mg, 88 %); $[\alpha]_D$ –45.7º (*c* 0.70, CH_3OH); R_f = 0.53 (acetonitrile/H_2O, φ = 0.83). ^{1}H NMR (CD_3OD): δ / ppm = 7.26 (s, 1H, N–H), 4.20 (d, $J_{1,2}$ = 0.63 Hz, 1H, H-1), 3.96–3.93 (m, 1H, H-4), 3.87 (dd, $J_{6a,5}$ = 2.34 Hz, $J_{6a,6b}$ = 11.78 Hz, 1H, H-6a), 3.84 (d, $J_{2,3}$ = 3.06 Hz, 1H, H-2), 3.71 (dd, $J_{6b,5}$ = 6.02 Hz, $J_{6a,6b}$ = 11.79 Hz, 1H, H-6b), 3.56–3.51 (m, 2H, OCH_2), 3.45 (dd, $J_{2,3}$ = 3.22 Hz, *J*3, 4 = 9.39 Hz, 1H, H-3), 3.22–3.19 (m, 1H, H-5), 2.63–2.57 (m, 1H, CH), 2.04 (s, 3H, 3 *CH*-AMA), 2.03 (s, 6H, 3 CH_2–AMA), 1.72 (s, 6H, 3 CH_2–AMA), 1.07 (d, J = 6.96 Hz, 3H, CH_3). ^{13}C NMR (CD_3OD): δ / ppm = 176.64 (C=O), 101.29 (C-1), 78.35, 75.29, 72.48, 68.65 (C-2–5), 72.29 (C-6), 62.92 (OCH_2), 52.93 (C–N), 42.43 (CH_2–AMA), 42.36 (CH), 37.57 (CH_2–AMA), 30.98 (*CH*–AMA), 14.79 (CH_3).

Anal. Calcd. mass fraction of elements, *w* / %, for $C_{20}H_{33}NO_7$ are: C 60.13, H 8.33, N 3.51, found: C 60.02, H 8.28, N 3.63.

*(*S*)*-N-*(Adamantan-1-yl)-3-α-D-mannopyranosyloxy-2-methylpropanamide (**10α**).* Pale yellow crude foam (47.3 mg, 90 %); $[\alpha]_D$ +46.6º (*c* 0.88, CH_3OH); R_f = 0.61 (acetonitrile/H_2O, φ = 0.83). ^{1}H NMR (CD_3OD): δ / ppm = 7.27 (s, 1H, N–H), 4.74 (d, $J_{1,2}$ = 1.46 Hz, 1H, H-1), 3.83 (dd, $J_{6b,5}$ = 2.38 Hz, $J_{6a,6b}$ = 11.74 Hz, 1H, H-6b), 3.80 (app t, J = 9.26 Hz, J = 9.27 Hz, 1H, H-4), 3.77 (dd, $J_{1,2}$ = 1.67 Hz, $J_{2,3}$ = 3.25 Hz, 1H, H-2), 3.67 (dd, $J_{2,3}$ = 3.30 Hz, $J_{3,4}$ = 9.40 Hz, 1H, H-3), 3.72–3.61 (m, 3H, OCH_2, H-6a), 3.55–3.52 (m, 1H, H-5), 2.62–2.57 (m, 1H, CH), 2.03 (br s, 9H, 3 *CH*-AMA, 3 CH_2–AMA), 1.72 (s, 6H, 3 CH_2–AMA), 1.04 (d, J = 6.93 Hz, 3H, CH_3). ^{13}C NMR (CD_3OD): δ / ppm = 176.53 (C=O), 101.38 (C-1), 74.44, 72.77, 72.24, 68.52 (C-2–5), 70.71 (C-6), 62.87 (OCH_2), 52.90 (C–N), 42.50 (CH_2–AMA), 42.41 (CH), 37.57 (CH_2–AMA), 30.97 (*CH*–AMA), 14.69 (CH_3).

Anal. Calcd. mass fraction of elements, *w* / %, for $C_{20}H_{33}NO_7$ are: C 60.13, H 8.33, N 3.51, found: C 60.00, H 8.21, N 3.69.

*(*S*)*-N-*(Adamantan-1-yl)-3-β-D-mannopyranosyloxy-2-methylpropanamide (**10β**).* Pale yellow crude foam (45.7 mg, 87 %); $[\alpha]_D$ –9.0º (*c* 0.512, CH_3OH); R_f =

0.59 (acetonitrile/H_2O, φ = 0.83). ^{1}H NMR (CD_3OD): δ / ppm = 7.25 (s, 1H, N–H), 4.48 (d, $J_{1,2}$ = 0.74 Hz, 1H, H-1), 3.88 (dd, $J_{6a,5}$ = 2.36 Hz, $J_{6a,6b}$ = 11.75 Hz, 1H, H-6a), 3.83 (d, $J_{2,3}$ = 3.17 Hz, 1H, H-2), 3.80–3.77 (m, 1H, H-4), 3.71 (dd, $J_{6b,5}$ = 5.91 Hz, $J_{6a,6b}$ = 11.76 Hz, 1H, H-6b), 3.64–3.53 (m, 2H, OCH_2), 3.43 (dd, $J_{2,3}$ = 3.22 Hz, $J_{3,4}$ = 9.39 Hz, 1H, H-3), 3.22–3.19 (m, 1H, H-5), 2.63–2.57 (m, 1H, CH), 2.04 (s, 3H, 3 *CH*-AMA), 2.03 (s, 6H, 3 CH_2–AMA), 1.71 (s, 6H, 3 CH_2–AMA), 1.02 (d, J = 7.00 Hz, 3H, CH_3). ^{13}C NMR (CD_3OD): δ / ppm = 176.89 (C=O), 102.29 (C-1), 78.34, 75.32, 72.53, 68.62 (C-2–5), 73.14 (C-6), 62.88 (OCH_2), 52.92 (C–N), 42.96 (CH), 42.42 (CH_2–AMA), 37.58 (CH_2–AMA), 30.98 (*CH*-AMA), 14.45 (CH_3).

Anal. Calcd. mass fraction of elements, *w* / %, for $C_{20}H_{33}NO_7$ are: C 60.13, H 8.33, N 3.51, found: C 60.05, H 8.20, N 3.65.

*(*R*)*-N-*Ferrocenyl-3-α-D-mannopyranosyloxy-2-methylpropanamide (**14α**)*. Yellow solid (51.0 mg, 91 %); $[\alpha]_D$ +33º (*c* 0.6, $CH_3OH/CHCl_3$ (φ = 0.50)); R_f = 0.41 (acetonitrile/H_2O, φ = 0.83). ^{1}H NMR (DMSO-d6): δ / ppm = 9.29 (s, 1H, N–H), 4.64 (d, $J_{1,2}$ = 0.72 Hz, 1H, H-1), 4.60 (s, 2H, *H*Fc), 4.53 (s, 2H, *H*Fc), 4.18 (d, $J_{2,3}$ = 3.86 Hz, 1H, H-2), 4.08 (s, 5H, *H*Fc), 3.97–3.92 (m, 2H, H-3, H-4), 3.63–3.56 (m, 2H, OCH_2), 3.56–3.48 (m, 2H, H-6a, H-6b), 3.41–3.40 (m, 1H, H-5), 2.67–2.61 (m, 1H, CH), 0.99 (d, J = 6.52 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d6): δ / ppm = 172.88 (C=O), 101.09 (C-1), 95.94 (C-1, Fc), 74.41, 71.44, 70.67, 70.57 (C-2–5), 69.95 (C-6), 69.27, 67.43, 64.26, 64.05, 61.04 (*C*Fc), 61.71 (OCH_2), 45.44 (CH), 14.33 (CH_3).

Anal. Calcd. mass fraction of elements, *w* / %, for $C_{20}H_{27}NO_7Fe$ are: C 53.47, H 6.06, N 3.12, found: C 53.58, H 6.03, N 3.10.

*(*R*)*-N-*Ferrocenyl-3-β-D-mannopyranosyloxy-2-methylpropanamide (**14β**)*. Yellow solid (48.8 mg, 87 %); $[\alpha]_D$ –4.4º (*c* 0.46, $CH_3OH/CHCl_3$, φ = 0.50); R_f = 0.34 (acetonitrile/H_2O (φ = 0.83)). ^{1}H NMR (DMSO-d6): δ / ppm = 9.25 (s, 1H, N–H), 4.74 (d, $J_{1,2}$ = 0.90 Hz, 1H, H-1), 4.59 (s, 2H, *H*Fc), 4.44 (s, 2H, *H*Fc), 4.24 (d, $J_{2,3}$ = 3.84 Hz, 1H, H-2), 4.10 (s, 5H, *H*Fc), 3.93–3.90 (m, 1H, H-4), 3.89 (d, $J_{3,4}$ = 8.58 Hz, 1H, H-3), 3.67–3.66 (m, 2H, OCH_2), 3.44–3.43 (m, 2H, H-6a, H-6b), 3.30–3.29 (m, 1H, H-5), 2.66–2.65 (m, 1H, CH), 1.05 (d, J = 6.60 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d6): δ / ppm = 172.24 (C=O), 99.81 (C-1), 95.46 (C-1, Fc), 77.59, 73.69, 70.44, 70.38 (C-2–5), 70.26 (C-6), 68.77, 67.14, 63.60, 60.64, 60.57 (*C*Fc), 61.37 (OCH_2), 45.00 (CH), 14.45 (CH_3).

Anal. Calcd. mass fraction of elements, *w* / %, for $C_{20}H_{27}NO_7Fe$ are: C 53.47, H 6.06, N 3.12, found: C 53.37, H 6.11, N 3.08.

*(*R*)*-N-*1'-Methoxycarbonylferrocenyl-3-α-D-manno-pyranosyloxy-2-methyl-propanamide (**15α**):* Orange solid (48.7 mg, 83 %); $[\alpha]_D$ +172º (*c* 0.9, $CH_3OH/CHCl_3$, φ = 0.50); R_f = 0.45 (acetonitrile/H_2O, φ = 0.83). ^{1}H NMR (DMSO-d6): δ / ppm = 7.54 (s, 1H, N–H), 4.68 (s, 2H, *H*Fc), 4.67 (s, 1H, H-1), 4.52 (s, 2H, *H*Fc), 4.39 (s, 2H, *H*Fc), 4.35 (s, 1H, H-4), 4.06–3.91 (m, 2H, H-3, H-2), 3.78 (s, 2H, *H*Fc), 3.68–3.49 (m, 4H, OCH_2, H-6a, H-6b), 3.59 (s, 3H, $COCH_3$), 3.03–2.93 (m, 1H, H-5), 2.76–2.66 (m, 1H, CH), 1.18 (d, J = 6.70 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d6): δ / ppm = 172.98 (C=O), 168.24 ($COCH_3$), 100.09 (C-1), 97.14 (C-1, Fn), 77.50, 73.97, 73.62, 70.90 (C-2–5), 70.62 (C-6), 70.43, 70.11, 67.07, 66.90 (*C*Fn), 69.98 (C-1', Fn), 69.42 (OCH_2), 51.44 ($COCH_3$), 41.38 (CH), 13.19 (CH_3).

Anal. Calcd. mass fraction of elements, *w* / %, for $C_{22}H_{29}NO_9Fe$ are: C 52.09, H 5.76, N 2.76, found: C 52.17, H 5.71, N 2.81.

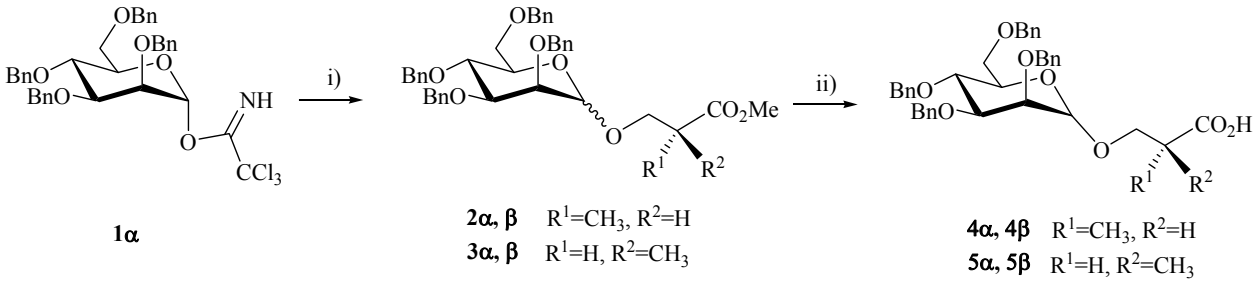
*(*R*)*-N-*1'-Methoxycarbonylferrocenyl-3-β-D-manno-pyranosyloxy-2-methylpropanamide (**15β**)*. Orange solid (43.1 mg, 74 %); $[\alpha]_D$ +13.3º (*c* 0.9, CH_3OH); R_f =0.40 (DCM/CH_3OH, φ = 0.90). ^{1}H NMR (DMSO-d6): δ / ppm = 7.45 (s, 1H, N–H), 4.77 (s, 1H, H-1), 4.75 (s, 2H, HFc), 4.45 (s, 2H, *H*Fc), 4.14 (d, $J_{3,4}$ = 9.35 Hz, 1H, H-4), 4.05 (s, 2H, *H*Fc), 3.90–3.87 (m, 4H, H-2, H-3, OCH_2), 3.76 (s, 3H, $COCH_3$), 3.67 (s, 2H, *H*Fc), 3.38–3.34 (m, 3 H H-6a, H-6b), 3.18–3.08 (m, 1H, H-5), 2.79–2.69 (m, 1H, CH), 1.16 (d, J = 6.17 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d6): δ = 173.03 (C=O), 169.99 ($COCH_3$), 100.14 (C-1), 91.87 (C-1, Fn), 77.47, 73.97, 73.92, 70.87 (C-2–5), 69.54 (C-6), 70.19, 70.09, 66.89, 66.71 (CFn), 69.76 (C-1', Fn), 69.12 (OCH_2), 51.33 ($COCH_3$), 35.09 (CH), 14.58 (CH_3).

Anal. Calcd. mass fraction of elements, *w* / %, for $C_{22}H_{29}NO_9Fe$ are: C 52.09, H 5.76, N 2.76, found: C 52.20, H 5.81, N 2.78.

*(*R*)*-N-*Ferrocenylmethyl-3-α-D-mannopyranosyloxy-2-methylpropanamide (**16α**)*. Yellow solid (49.2 mg, 88 %); $[\alpha]_D$ +20.6º (*c* 0.5, CH_3OH); R_f = 0.51 (acetonitrile/H_2O (φ = 0.83)). ^{1}H NMR (DMSO-d6): δ / ppm = 8.01 (s, 1H, N–H), 4.49 (s, 1H, H-1), 4.15 (s, 2H, *H*Fc), 4.14 (s, 5H, *H*Fc), 4.13 (s, 2H, *H*Fc), 4.06–4.02 (m, 4H, H-2, H-3, OCH_2), 3.97–3.96(m, 3H, H-4, CH_2Fc), 3.78–3.75 (m, 1H, H-5), 3.61–3.52 (m, 2H, H-6a, H-6b), 2.62–2.57 (m, 1H, CH), 1.04 (d, J = 7.01 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d6): δ / ppm = 173.13 (C=O), 97.76 (C-1), 86.32 (C-1, Fc), 78.37, 75.52, 74.06, 71.18 (C-2–5), 72.22 (C-6), 68.36, 67.53, 67.44, 67.14, 67.04 (CFc), 69.22 (OCH_2), 39.95 (CH), 37.29 (CH_2Fc), 14.62 (CH_3).

Anal. Calcd. mass fraction of elements, *w* / %, for $C_{21}H_{29}NO_7Fe$ are: C 54.44, H 6.31, N 3.02, found: C 54.56, H 6.29, N 3.03.

*(*R*)*-N-*Ferrocenylmethyl-3-β-D-mannopyranosyloxy-2-methylpropanamide (**16β**):* Yellow solid (51.0 mg, 91 %); $[\alpha]_D$ –8.8º (*c* 0.4, $CH_3OH/CHCl_3$, φ = 0.50); R_f = 0.45 (acetonitrile/H_2O, φ = 0.83). ^{1}H NMR (DMSO-d6): δ / ppm = 8.00 (app t, J = 5.70 Hz, J = 5.95 Hz, 1H, N–H), 4.39 (s, 1H, H-1), 4.25–4.22 (m, 1H, H-3), 4.17–4.16 (m, 2H, *H*Fc), 4.15 (s, 5H, *H*Fc), 4.14 (s, 2H, *H*Fc),



Scheme 1. i) (*R*)-methyl 3-hydroxy-2-methyl propionate or (*S*)-methyl 3-hydroxy-2-methyl propionate, $BF_3{\times}Et_2O/CH_2Cl_2$, 0 ºC; ii) 1. – separation of α- and β- anomers by column chromatography on silica gel, diethyl ether/petroleum ether (φ = 0.50), 2. – 1 M NaOH, dioxan, 40 ºC.

4.10 (d, $J_{2,3}$ = 2.34 Hz, 1H, H-2), 4.07 (s, 2H, CH_2Fc), 4.05–4.03 (m, 1H, H-4), 4.02–4.01 (m, 2H, OCH_2), 3.95–3.91 (m, 2H, H-6a, H-6b), 3.86–3.83 (m, 1H, H-5), 2.64–2.58 (m, 1H, CH), 1.01 (d, J = 6.89 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d6): δ / ppm = 173.08 (C=O), 99.97 (C-1), 86.18 (C-1, Fc), 77.54, 73.93, 73.66, 70.41 (C-2–5), 70.53 (C-6), 68.32, 67.66, 67.64, 67.18, 67.12 (CFc), 69.72 (OCH_2), 39.58 (CH), 37.39 (CH_2Fc), 14.61 (CH_3).

Anal. Calcd. mass fraction of elements, *w* / %, for $C_{21}H_{29}NO_7Fe$ are: C 54.44, H 6.31, N 3.02, found: C 54.59, H 6.27, N 2.99.

RESULTS AND DISCUSSION

Synthesis of Glycoconjugates

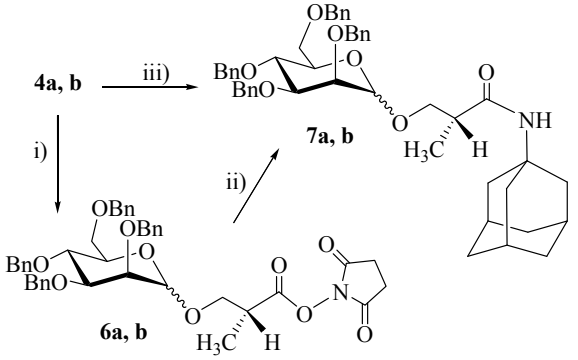
The synthesis of *O*-mannosyl conjugates of 1-adamantamine (AMA) using *O*-glycosidically bonded chiral linkers is reported. Linkers of (*R*)- and (*S*)- configuration were used on purpose in order to study the influence of linkers configuration on the inhibitory potency of conjugates in the hemagglutination test.

Scheme 1 shows the reaction sequence starting from α-D-mannopyranosyl trichloroacetimidate **1α** to the key intermediates **4** and **5** used for the synthesis of AMA (**9**, **10**) and Fc (**14**–**16**) glycoconjugates. Trichloroacetimidate **1α** was prepared as an active intermediate in the base-treated (NaH) reactions of *O*-benzylated mannose with trichloroacetonitrile at 0 °C in dichloromethane.[32] *O*-Mannosylation of (*R*)- and (*S*)-methyl 3-hydroxy-2-methylpropanoates was promoted by the catalytic amount of boron trifluoride diethyl etherate ($BF_3{\times}Et_2O$) in dichloromethane at 0 °C. These trichloroacetimidate-mediated *O*-glycosylations gave anomeric mixtures of the corresponding *O*-glycosides **2**, **3** (68–73 %). α/β Ratios for *O*-mannosides **2** and **3** were found to be approximately 2:1. Since the non-participating protecting group was at the C-2 position, S_N2-type reaction could occur leading to β-anomers. Such inversion was also assisted by the use of the weak Lewis acid catalyst at lower temperatures. On the other hand, α-mannosides are thermodynamically more stable glycosylation products because of the strong anomeric effect. α,β-Glycosides **2** and **3** were readily separated by column chromatography on silica gel using diethyl ether/petroleum ether (φ = 0.50) as the eluent of choice giving pure anomers **2α**, **3α** and **2β**, **3β** in moderate overall yields.

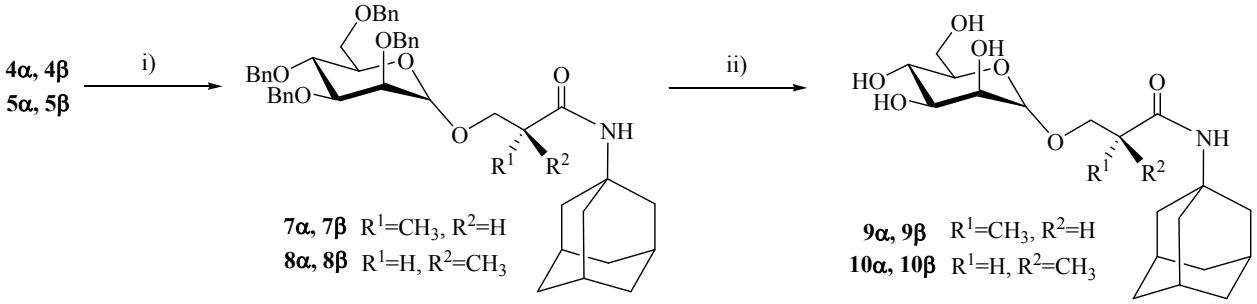
The hydrolysis of methyl esters of each anomer **2** and **3** was performed by saponification using 1 M sodium hydroxide in dioxane leading to 77–94 % of acids **4** and **5** (Scheme 1).

The next step was the activation of the free carboxyl group and condensation with AMA. To achieve the most efficient procedure several methods were explored (Sheme 2).

The first approach was the synthesis of an active *N*-hydroxysuccinimide ester (HOSu) **6** as a good acylating agent for the reaction with AMA.[23] Condensation of AMA with the ester **6** gave the glycoconjugate **7** in 83 % yield (Scheme 2). The pentafluorophenyl ester was also prepared, but the condensation with AMA unfortunately failed. Second strategy included carbodiimide-mediated synthesis: *in situ* activation of carboxyl carbon and amide bond formation using



Scheme 2. i) EDC×HCl, HOSu, CH_2Cl_2, rt, (78%); ii) AMA×HCl/Et_3N, rt, (83 %); iii) DCC/HOBt/AMA×HCl/Et_3N, rt, (78 %) or DCC/DMAP/AMA×HCl/*N*-ethylmorpholine, rt, (67 %).



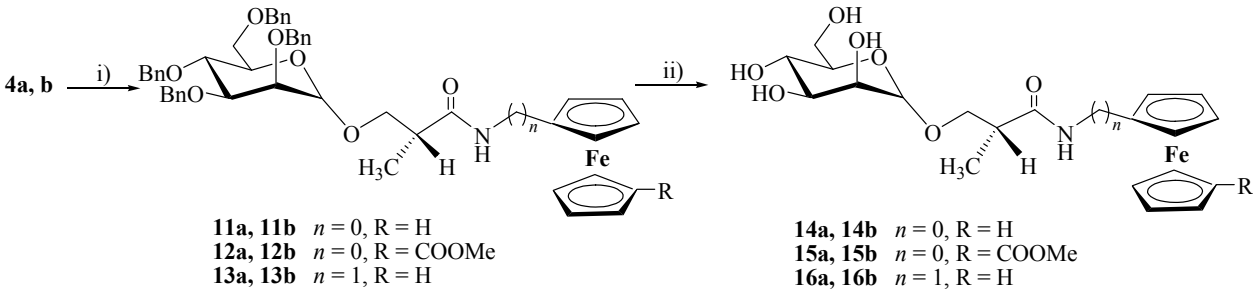
Scheme 3. i) 1. – EDC×HCl/ Et_3N/CH_2Cl_2, 0 ºC, 2. – HOBt, 0 ºC, 3. – AMA×HCl/Et_3N, rt; ii) H_2 (4 bar)/(Pd/C (10 %))/MeOH, rt.

DCC/HOBt,[37] DCC/DMAP[38] or EDC×HCl/HOBt gave in all cases the desired product **7** (Scheme 2). In this context the activation by EDC×HCl was the best method because the by-product (water-soluble urea derivative) can be more easily removed. Since the first approach includes two-step synthesis, less demanding EDC-mediated coupling was chosen for amide bond formation. Glycoconjugates **7** and **8** were obtained in good yields (55–66 %) in just one step starting from pure α- or β-anomers of acids **4** and **5** as shown in Scheme 3.

Debenzylation of compounds **7** and **8** gave the desired AMA conjugates **9** and **10** (87–94 %). Benzyl deprotection was performed by classic hydrogenolysis with 10 % Pd on charcoal in methanol.

Preliminary hemagglutination tests with AMA conjugates showed that the linker configuration does not influence the results. Therefore, further syntheses of ferrocene glycoconjugates were performed by using the linker of only one configuration (*R*). We chose three representative types of ferrocene derivatives (which are usually preserved as *N*-Boc protected derivatives): (i) ferrocenylamine ($FcNH_2$), (ii) methyl 1'-amino-ferrocene-1-carboxylate (MeOOCFcNHBoc) whose heteroannular ester subsistent of the flexible metallocene core could act as an additional site for binding to lectins,[4] and (iii) ferrocenylmethylamine ($FcCH_2NH_2$) which elongates the aliphatic linker in mannose-ferrocene bioconjugates increasing the number of possible conformations of these molecules.

In the ferrocene chemistry (because of the sensitivity of the metallocene core) the general methods for the preparation of amides from carboxylic acids are performed either *via* carbonyl chlorides or by EDC/HOBt method. In our recent papers in the field of ferrocene containing oligopeptides (especially compounds of the type X-$(AA)_n$-Fca-$(AA)_m$-COOMe; *m*, *n* = 0,1,2…; X = Ac, Boc, Bn)[33,39–43] the very successful method was the latter. Therefore, this procedure was applied for the synthesis of mannosides **11**–**13**. Contrary to the previously described method for the preparation of AMA glycosides (Scheme 1) the separation step of anomeric products was performed following the reaction of the mannoside acids mixture **4α**,**β** with ferrocene amines (the ferrocene compounds are yellow to orange coloured which simplifies their chromatographic purification). Thus, after hydrolysis of anomeric mixtures of esters **2α**,**β** (Scheme 1) acids **4α**,**β** were obtained in 90 % yields. Activation of **4α**,**β** was performed by using EDC/HOBt method. Conjugation with free ferrocene amines (obtained by *in situ* deprotection of their *N*-Boc derivatives) produced the bioorganometallics **11α**,**β**–**13α**,**β** as shown in Scheme 4. Their TLC separation on silica gel using diethyl ether/petroleum ether (φ = 0.67) as eluent gave analytically pure α- (59–98 %) and β- anomers (62–77 %) which were hydrogenolyzed (analogously to the procedure used in AMA preparations) to debenzylated conjugates **14**–**16** in almost quantitative yields.



Scheme 3. i) 1. – EDC×HCl/Et_3N/CH_2Cl_2, 0 ºC, 2. – HOBt, 0 ºC, 3. – FcNHBoc and MeOOCFcNHBoc/EtOAc/HCl (g)/Et_3N or $FcCH_2NH_2/CH_2Cl_2$, rt, 4. – TLC separation on silica gel, diethyl ether/petroleum ether (φ = 0.67); ii) H_2 (4 bar)/(Pd/C (10%))/(MeOH/CH_2Cl_2 (φ = 0.50)), rt.

Table 1. Inhibition of the hemagglutination of guinea pig erythrocytes by type 1 fimbriated *E. coli* HB101 (pPKl4)

Compound tested	IT[(a)] / mM	RIT[(b)]
methyl α-D-glucopyranoside[(c)]	no inhibition	-
methyl α-D-mannopyranoside	18	1
9α	6	3
10α	6	3
14α	2.5	7
15α	2.5	7
16α	2.5	7

[(a)] Inhibition titer is average value from three independent tests.
[(b)] Relative inhibition titer based on methyl α-D-mannopyranoside as standard.
[(c)] In 20 mM concentration.

Inhibition Hemagglutination Tests of α-D-Mannopyranosides

The influence of different aglycon moieties (adamantane and ferrocene) on inhibitory potencies of mannoside conjugates was explored, in order to reveal binding potencies toward mannose specific lectin.

The results of our testings are in accord with previously reported studies of specificity of type 1 fimbriae)[7] which showed that mannosyl moieties should be of α-configuration for good binding to the lectin. Therefore, compounds **9**, **10**, **14**–**16** that showed inhibition in hemagglutination tests were of α-configuration and their activities were calculated relative to α-D-mannopyranoside. Hydrophobic moieties in inhibitor molecules add to the overall affinity toward the specific lectin. This may also be due to the binding to subsites adjacent to the mannose-specific binding pocket. The tested compounds incorporate hydrophobic adamantane and ferrocene subunits in non-carbohydrate parts. To the best of our knowledge, they are the first examples of adamantane and ferrocene containing mannosides used in inhibition hemagglutination tests with type 1 fimbriated *E. coli* HB101 (pPKl4). The results of these assays are listed in Table 1. They are semi-quantitative and higly reproducible. The inhibition titer (IT) denotes the lowest concentration at which agglutination of guinea pig erythroytes by *E. coli* is no longer visible. Methyl α-D-glucopyranoside was used as the control compound and non-specific binding did not occur, as expected. All the compounds showed inhibitory potencies in mM range. Ferrocene mannosides have 2.4 times lower IT value than adamantyl derivatives.

These results can be explained by the pronounced aromatic character of the ferrocene core, which is obviously much stronger bound to lectines than the aliphatic adamantane subunit. From the literature it is known that the potent inhibitor of type 1 fimbriae mediated adhesion, *p*-nitrophenyl α-D-mannoside (pNPMan), binds approximately 80 times better than methyl α-D-mannopyranoside.[13] Apparently, planar aromatic moieties of inhibitor molecules contribute more to the binding than the three-dimensional metallocene part which, on the other hand, is superior to the aliphatic adamantane moiety.

CONCLUSION

In conclusion, we have reported the synthesis of novel mannose conjugates with 1-adamantamine and ferrocene amines. Mannosides prepared in this work are first compounds with adamantane and ferrocene in the aglycon part used in inhibition hemagglutination tests with type 1 fimbriated *E. coli* HB101 (pPKl4). They exhibit inhibitory potencies in the mM range. It was shown that ferrocene α-D-mannosides (**14α**–**16α**) were better inhibitors of *E. coli* adhesion than their aliphatic AMA analogues (**9α**, **10α**). This is most probably due to the aromatic character of the ferrocene core and comparable with the very active aralkyl α-D-mannosides.[13] Furthermore, it can be concluded that neither the absolute configuration of the linkers part nor structural changes introduced in ferrocene conjugates, influence the inhibitory potencies of tested compounds.

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SAŽETAK

Sinteza i biološka aktivnost konjugata manoze s 1-adamantaminom i ferocenskim aminima

Rosana Ribić,[a] Monika Kovačević,[b] Vesna Petrović Peroković,[a] Ita Gruić-Sovulj,[a] Vladimir Rapić[b] i Srđanka Tomić[a]

[a]*Kemijski odsjek, Prirodoslovno-matematički fakultet, Sveučilište u Zagrebu, Horvatovac 102A, 10000 Zagreb, Hrvatska*

[b]*Zavod za kemiju i biokemiju, Prehrambeno-biotehnološki fakultet,Sveučilište u Zagrebu, Pierottijeva 6, 10000 Zagreb, Hrvatska*

Pripravljeni su čisti α- i β-anomeri *O*-manozilnih konjugata 1-adamantamina i ferocenskih amina. Šećerni i aminski dijelovi molekula međusobno su povezani kiralnom poveznicom (metil-(*R*)-3-hidroksi-2-metil propanoatom i/ili metil-(*S*)-3-hidroksi-2-metil propanoatom). α-D-manopiranozidni derivati s adamantanskim ili ferocenskim aglikonima biološki su testirani hemaglutinacijskim testom (inhibicijom aglutinacije eritrocita zamorčića s ispoljenim fimbrijama tipa 1 bakterije 1 *E. coli* HB101 (pPKl4)). Svi su glikokonjugati pokazali inhibitorna svojstva u mM rasponu.