Chemistry of Natural Compounds, Vol. 37, No. 1, 2001

SYNTHESIS OF GLYCOSIDES OF A MURAMOYLDIPEPTIDE WITH CHROMONE AGLYCONES

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UDC 547.963.057

The synthesis of β-(2-methyl-3-phenylchromonyl-7)- and β-[2-methyl-3-(3,4-trimethylenedioxy) phenylchromonyl-7]glycosides of the methyl ester of N-acetylmuramoyl-L-alanyl-D-isoglutamine, new derivatives of a muramoyldipeptide with chromone aglycones, is described. The starting arylglycosides of N-acetylglucosamine are prepared by glycosylation of 7-hydroxychromone derivatives with the peracetate of α-glucosamine chloride catalyzed by a crown ether. The synthesized β-aryl-4,6-O-isopropylidene-N-acetylmuramic acids are condensed with the dipeptide and deprotected to give the desired glycopeptides.

Key words: methyl ester of O-[(2-methyl-3-phenylchromonyl-7)-2-acetamido-2-deoxy-β-D-glucopyranosidyl-3]-D-lactoyl-L-alanyl-D-isoglutamine, methyl ester of O-{[-2-methyl-3-(3,4-trimethylenedioxy)phenylchromonyl-7]-2-acetamido-2-deoxy-β-D-glucopyranosidyl-3}-D-lactoyl-L-alanyl-D-isoglutamine, synthesis.

The high antitumor activity of the conjugate of the methyl ester of N-acetylmuramoyl-L-valyl-D-isoglutamine with ubiquinone Q derivatives created great interest in synthetic combinations of muramoyldipeptide (MDP) with various biologically active compounds [1]. Derivatives of benzo- and naphthoquinones [2], phenolic compounds [3], acridine [4], and salicylic and nicotinic acids [5] are widely used as modifiers. In most instances the primary hydroxyl of MDP is modified. The β-glycosides of MDP with aliphatic, aromatic, and phenolic aglycones that we synthesized possessed high immunostimulatory activity [6]. Therefore, it seemed promising to modify the compounds with MDP at the β-O-glycoside bond.

In continuation of the studies of the relationship between the MDP glycoside structures and their biological activity and in order to find new promising immunomodulators, we synthesized β-(2-methyl-3-phenylchromonyl-7)- and β-[2-methyl-3-(3,4-trimethylenedioxy)phenylchromonyl-7] glycosides of the methyl ester of N-acetylmuramoyl-L-alanyl-D-isoglutamine, new glycoside MDP derivatives with chromone aglycones. We chose 7-hydroxychromone derivatives as the aglycone because of their wide spectrum of biological activities [7].

The starting peracetyl glycosides of N-acetylglucosamine (**2a** and -**b**) were prepared from α-D-chloroglucosamine (**1**) by solid-liquid phase-transfer catalysis. The 7-hydroxy-2-methyl-3-phenyl- and 7-hydroxy-2-methyl-3-(3,4-trimethylenedioxy) phenylchromones [8] were glycosylated by an equimolar amount of glycosylating agent at room temperature in acetonitrile in the presence of anhydrous K_2CO_3 and 15-crown-5 (20 mol%). The products (**2a** and -**b**) were isolated by crystallization in yields of 66 and 76%, respectively. The PMR spectra of **2a** and -**b** contain signals of the framework protons, three O-acetyls, and the acetamide of the hydrocarbon (for assignments, see Experimental). Signals of the aglycone protons were identified, in particular, singlets of methyls at 2.31 and 2.30 ppm and multiplets of 8 and 6 aromatic protons at 6.88-7.91 and 6.87-7.85 ppm, respectively. This confirms the structures. The β-configuration of the glycoside bond is consistent with the presence of doublets of the anomeric protons at 5.18 and 5.21 ppm with spin—spin coupling constant (SSCC) 8.5 Hz.

Compounds **2a** and -**b** were deacetylated according to Zemplen. The β-glycol in the resulting triols **3a** and -**b** was closed by 2,2-dimethoxypropane to give the isopropylidene protection. The structure of acetals **4a** and -**b** was confirmed by PMR spectroscopy. The hydrocarbon portions of the spectra contain characteristic signals: two singlets of methyl protons of the

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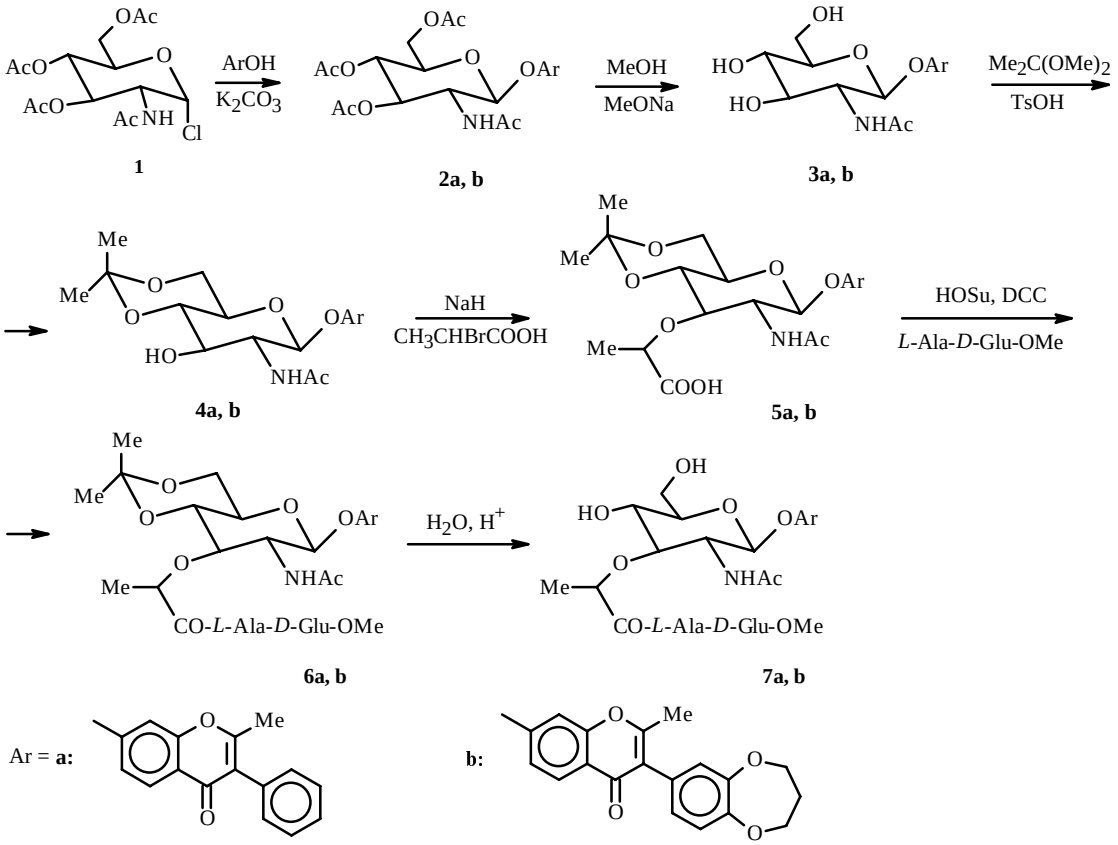
Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 35-38, January-February, 2001. Original article submitted February 22, 2001.

0009-3130/01/3701-0039$25.00

isopropylidene group at 1.46-1.51 ppm, a 3H singlet of the N-acetyls at 2.14 and 2.12 ppm, a broad doublet of the C(3)-OH groups at 4.50 and 4.60 ppm, and a doublet of the glycoside protons at 4.55 and 4.66 ppm, respectively.



1 **2a, b** **3a, b** **4a, b** **5a, b** **6a, b** **7a, b**

Alkylation of the free hydroxyl in derivatives **4a** and -**b** with NaH and (S)-2-bromopropanoic acid was performed in dioxane and gave (R)-N-acetylmuramic acids **5a** and -**b**. Successive condensation of the acids with the methyl ester of L-alanyl-D-isoglutamine using N-hydroxysuccinimide and acid hydrolysis of the isopropylidene protection gave the desired glycopeptides **7a** and -**b**. The presence in the PMR spectra of **7a** and -**b** of a singlet of the methyl-ester protons (δ 3.58 ppm), two multiplets of isoglutamine β-methylenes (δ 1.76 and 1.75 and 2.02 and 2.00 ppm), a triplet of isoglutamine γ-methylene group (δ 2.30 ppm), and two doublets of alanine and lactyl methyls (δ 1.27 and 1.29 ppm) confirm that the lactylpeptide was incorporated. Removal of the acteyl protection was confirmed by the presence of signals for protons of the two hydroxyls: a doublet at 5.41 and 5.44 ppm [C(4)-OH] and a broad triplet at 4.67 ppm [C(6)-OH].

EXPERIMENTAL

Melting points were determined on a PTP apparatus; optical rotation at 20-22°C, on a Polamat-A polarimeter. ^{1}H NMR spectra were obtained on a Varian VXR-300 (300 MHz) spectrometer with TMS internal standard. Chemical shifts are given in ppm (δ-scale). TLC was performed on Sorbfil-AFV-UV plates (Sorbpolymer, Russia). Spots were developed with H_2SO_4 (5%) in ethanol with heating to 200-300°C. Solvent systems were: 1) chloroform—ethylacetate-propanol-2 (25:5:1), 2) chloroform—propanol-2 (3:1), 3) chloroform—propanol-2 (17:3), 4) chloroform—propanol-2 (15:1). Column chromatography used Merck 240-400 mesh silica gel.

(2-Methyl-3-phenylchromonyl-7)-2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside (2a). A solution of 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranosyl chloride (**1**, 1,7 g, 4.65 mmol) [9] in acetonitrile (20 mL) was treated with 7-hydroxy-2-methyl-3-phenylchromone (1.17 g, 4.65 mmol), finely ground anhydrous K_2CO_3 (640 mg, 4.65 mmol), and 15-crown-5 (185 μL, 0.93 mmol) and stirred at room temperature until the glycosylating agent completely disappeared (TLC monitoring in system 1). The precipitate was filtered off. The filtrate was evaporated. The solid was dissolved in $CHCl_3$

(50 mL) and washed with KOH (1 N, 20 mL) and water (2×20 mL). The organic layer was separated, dried with anhydrous Na_2SO_4, and evaporated. The solid was crystallized from propanol-2 to give **2a** (2.0 g, 66%), mp 194-196°C, $[\alpha]_{546}$ +2° (*c* 1.0, CH_2Cl_2). PMR (CD_2Cl_2, δ, ppm, J/Hz): 1.95, 2.04, 2.06, 2.08 (12H, NAc and 3 OAc, s), 2.31 (3H, s, Me), 3.75 (1H, ddd, $J_{5,6a}$ = 2.5, $J_{5,6b}$ = 5.5, H-5), 4.12 (1H, ddd, $J_{2,3}$ = 10, H-2), 4.17 and 4.24 (2H, dd, $J_{6a,6b}$ = 12, H-6a, H-6b), 5.06 (1H, dd, $J_{4,5}$ = 9.5, H-4), 5.18 (1H, d, $J_{1,2}$ = 8.5, H-1), 5.22 (1H, dd, $J_{3,4}$ = 9.5, H-3), 6.30 (1H, d, $J_{2,NH}$ = 8.5, NH), 6.88-6.96 (2H, m, Ar-H), 7.32-7.52 (5H, m, Ph), 7.89 (1H, d, Ar-H).

[2-Methyl-3-(3,4-trimethylenedioxy)phenylchromonyl-7]-2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside (**2b**) was synthesized analogously from α-chloride **1** (2.0 g, 5.48 mmol) and 7-hydroxy-2-methyl-3-(3,4-trimethylenedioxy) phenylchromone (1.7 g, 5.48 mmol): yield 2.4 g (76%), mp 233-235°C (dec.), $[\alpha]_{546}$ +10° (*c* 1.0, CH_2Cl_2).

PMR (CD_2Cl_2, δ, ppm, J/Hz): 1.96, 2.03, 2.05, 2.08 (12H, s, NAc and 3OAc), 2.20 (2H, m, $OCH_2\mathbf{CH_2}CH_2O$), 2.30 (3H, s, Me), 3.80 (1H, ddd, $J_{5,6a}$ = 2.5, $J_{5,6b}$ = 5.5, H-5), 4.15 (1H, ddd, $J_{2,3}$ = 10.5, H-2), 4.21 and 4.30 (2H, dd, $J_{6a,6b}$ = 12, H-6a, H-6b), 4.25 (4H, t, 2 OCH_2), 5.07 (1H, dd, $J_{4,5}$ = 9.5, H-4), 5.21 (1H, d, $J_{1,2}$ = 8.5, H-1), 5.22 (1H, dd, $J_{3,4}$ = 9.5 Hz, H-3), 6.43 (1H, d, $J_{2,NH}$ = 9, NH), 6.87-6.92 (4H, m, Ar-H), 7.06 and 7.84 (2H, d, Ar-H).

(2-Methyl-3-phenylchromonyl-7)-3-acetamido-2-deoxy-β-D-glucopyranoside (3a). A solution of acetate **2a** (1.7 g, 2.9 mmol) in dry methanol (50 mL) was treated with NaOMe (0.5 mL, 0.1 N) in methanol. The precipitate that formed on standing was filtered off and washed with cold methanol. The mother liquor was neutralized with cation exchanger KU-2 (H^+). The resin was filtered off. The filtrate was evaporated. Addition of ether produced an additional amount of crystals. Yield of compound **3a**, 1.15 g (86%), mp 163-165°C, $[\alpha]_{546}$ -10° (*c* 1.0, DMF).

Acetate **2b** (2.4 g, 3.7 mmol) was similarly deacetylated to give [2-methyl-3-(3,4-trimethylenedioxy)phenylchromonyl-7]-2-acetamido-2-deoxy-β-D-glucopyranoside (**3b**, 1.75 g, 90%), mp 148-150°C, $[\alpha]_{546}$ -15° (*c* 1.0, DMF).

(2-Methyl-3-phenylchromonyl-7)-2-acetamido-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranoside (4a). A suspension of substance **3a** (0.70 g, 1.54 mmol) and TsOH (15 mg) in 2,2-dimethoxypropane (5 mL) was heated with stirring until boiling and treated with THF (2 mL) until completely dissolved. The reaction mixture was cooled after 1 h (TLC monitoring in system 3), neutralized with pyridine, and evaporated. The precipitate was purified by column chromatography (eluent CH_2Cl_2 → CH_2Cl_2—propanol-2, 50:1) to give **4a** (0.47 g, 62%), mp 152-154°C, $[\alpha]_{546}$ -73° (*c* 1.0, CH_2Cl_2). PMR ($CDCl_3$, δ, ppm, J/Hz): 1.50 and 1.51 (6H, s, Me_2C), 2.14 (3H, s, NAc), 2.44 (3H, s, Me), 2.99 (1H, ddd, $J_{5,6a}$ = 2.5, $J_{5,6b}$ = 5.5, H-5), 3.28 (1H, dd, $J_{4,5}$ = 9.5, H-4), 3.57 (1H, dd, $J_{3,4}$ = 9.5, H-3), 3.74 (2H, m, H-6a, NH), 3.87 (1H, ddd, $J_{2,3}$ = 10, H-2), 4.00 (1H, dd, $J_{6a,6b}$ = 12, H-6b), 4.49 (1H, br. d, C-3-OH), 4.55 (1H, d, $J_{1,2}$ = 8.5, H-1), 6.49 (1H, d, Ar-H), 6.77 (1H, dd, Ar-H), 7.40-7.58 (6H, m, Ar-H).

The method described above was used to produce from triol **3b** (0.89 g, 1.57 mmol) [2-methyl-3-(3,4-trimethylenedioxy)phenylchromonyl-7]-2-acetamido-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranoside (**4b**, 0.7 g, 73%), mp 155-157°C, $[\alpha]_{546}$ -58° (*c* 0.5, CH_2Cl_2). PMR ($CDCl_3$, δ, ppm, J/Hz): 1.46 and 1.51 (6H, s, Me_2C), 2.12 (3H, NAc, s), 2.23 (2H, m, $OCH_2\mathbf{CH_2}CH_2O$), 2.40 (3H, s, Me), 3.24 (2H, m, H-5, H-6a), 3.60 (1H, dd, $J_{4,5}$ = 9.5, H-4), 3.75 (1H, dd, $J_{3,4}$ = 9.5, H-3), 3.87 (1H, ddd, $J_{2,3}$ = 10, H-2), 3.94 (1H, dd, $J_{5,6b}$ = 5, $J_{6a,6b}$ = 12, H-6b), 4.27 and 4.38 (4H, m, 2 OCH_2), 4.60 (1H, br. d, C-3-OH), 4.66 (1H, d, $J_{1,2}$ = 8, H-1), 6.77 (1H, d, $J_{2,NH}$ = 9, NH), 6.52 and 7.04 (2H, s, Ar-H), 6.97, 7.14, and 7.53 (3H, d, Ar-H), 7.73 (1H, m, Ar-H).

Methyl Ester of O-[(2-Methyl-3-phenylchromonyl-7)-2-acetamido-2-deoxy-β-D-glucopyranosidyl-3]-D-lactyl-L-alanyl-D-isoglutamine (7a). A suspension of compound **4a** (0.33 g, 0.67 mmol) in dry dioxane (20 mL) was stirred and treated in portions with NaH (4 eq.). The reaction mixture was heated to 65°C, held at that temperature for 1 h, treated with 2-S-bromopropionic acid (120 μL, 1.34 mmol), held at 65°C for 3 h, and cooled. The excess of NaH was decomposed with ethanol. The mixture was concentrated, poured into cold water (50 mL), and acidified with HCl (2 N) until the pH was 3-4. The muramic acid was extracted with $CHCl_3$ (3×20 mL). The extract was dried over anhydrous Na_2SO_4 and evaporated. The solid was crystallized by adding ether. Yield of **5a**, 0.35 g (93%).

A solution of acid **5a** (340 mg, 0.60 mmol) in dry dioxane (10 mL) was stirred and treated with N-hydroxysuccinimide (76 mg, 0.66 mmol) and DCC (135 mg, 0.66 mmol). After 3 h the precipitate of dicyclohexylurea was filtered off and washed with solvent. The filtrate was treated with the trifluoroacetate of the methyl ester of L-alanyl-D-isoglutamine [prepared by treating the corresponding Boc-derivative with trifluoroacetic acid (218 mg, 0.66 mmol) and evaporating to dryness] and triethylamine until the pH was 8. After the reaction was complete (TLC monitoring in system 4), the reaction mixture was concentrated. The precipitate was dissolved in $CHCl_3$ (30 mL). The organic layer was washed with water (2×10 mL), dried over anhydrous Na_2SO_4, and evaporated. Addition of ether precipitated **6a** (310 mg, 66%).

The alkylidene derivative **6a** (270 mg, 0.35 mmol) was dissolved with heating on a boiling-water bath in acetic acid (3 mL, 80%) and held at that temperature for 5 min (TLC monitoring in system 2). The solution was evaporated to dryness. The solid was co-evaporated with toluene. Column chromatography (eluent $CHCl_3$ → $CHCl_3$—propanol-2, 3:1) gave glycopeptide **7a** (130 mg, 51%), amorphous powder, $[\alpha]_{546}$ +23° (*c* 1.0, $CHCl_3$—ethanol, 2:1). PMR (DMSO-d_6, δ, ppm, J/Hz): 1.27 and 1.29 (6H, d, 2 **Me**CH), 1.76 and 2.02 (2H, m, β-CH_2-iGln), 1.80 (3H, s, NAc), 2.27 (3H, s, Me), 2.30 (2H, t, γ-CH_2-iGln), 3.58 (3H, s, COOMe), 4.67 (1H, br. t, C-6-OH), 5.21 (1H, d, $J_{1,2}$ = 8, H-1), 5.41 (1H, d, C-4-OH), 7.04 (1H, dd, Ar-H), 7.18 and 7.97 (2H, d, Ar-H), 7.27-7.44 (5H, m, Ar-H), 7.08 and 7.33 (2H, s, $CONH_2$-iGln), 7.50, 7.94, and 8.08 (3H, d, 3NH).

The method described above was used to synthesize from acetal **4b** (220 mg, 0.46 mmol) the methyl ester of O-{[2-methyl-3-(3,4-trimethylenedioxy)phenylchromonyl-7]-2-acetamido-2-deoxy-β-D-glucopyranosidyl-3}-D-lactoyl-L-alanyl-D-isoglutamine (**7b**, 85 mg), amorphous powder, $[\alpha]_{546}$ +22° (*c* 0.67, $CHCl_3$—ethanol, 2:1). PMR (DMSO-d_6, δ, ppm, J/Hz): 1.27 and 1.29 (6H, d, 2 **Me**CH), 1.75 and 2.00 (2H, m, β-CH_2-iGln), 1.80 (3H, s, NAc), 2.13 (2H, m, OCH_2**CH_2**CH_2O), 2.28 (3H, s, Me), 2.30 (2H, t, γ-CH_2-iGln), 3.58 (3H, s, COOMe), 4.67 (1H, br. t, C-6-OH), 5.20 (1H, d, $J_{1,2}$ = 8, H-1), 5.44 (1H, d, C-4-OH), 6.89 and 7.00-7.17 (5H, m, Ar-H), 7.98 (1H, d, Ar-H), 7.10 and 7.35 (2H, s, $CONH_2$-iGln), 7.50, 7.98, and 8.09 (3H, d, 3 NH).

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