Chemistry of Natural Compounds, Vol. 49, No. 6, January, 2014 [Russian original No. 6, November–December, 2013]

SYNTHESIS OF MURAMYL DIPEPTIDE ISOFLAVONE GLYCOSIDE

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UDC 547.963.057

The peracetate of 7-(2-acetamido-2-deoxy-β-D-glucopyranosyloxy)-2-methyl-6-propyl-3′,4′-trimethylenedioxyisoflavone was synthesized under phase-transfer catalysis conditions by quaternary ammonium salts at room temperature and with heating to 50°C in K_2CO_3 solution (0.18 M):$CHCl_3$. The corresponding glycoside of N*-acetylmuramyl-L-alanyl-D-isoglutamine (MDP) was synthesized from the peracetate.*

Keywords: *O*-glycosides of isoflavone, glycosides of *N*-acetylglucosamine, phase-transfer catalysis, muramyl dipeptide, muramyl dipeptide glycosides.

We reported earlier on the synthesis of *β*-(2-methylisoflavon-7-yl)- and *β*-(2-methyl-3′,4′-trimethylenedioxyisoflavon-7-yl)glycosides of the methyl ester of *N*-acetylmuramyl-L-alanyl-D-isoglutamine (muramyl dipeptide, MDP) [1]. Interferon-generating activity was found for MDP *β*-(2-methylisoflavon-7-yl)glycoside in *in vivo* tests [2]. In continuation of these investigations, we synthesized a new chromone glycoside of MDP, i.e., *β*-(2-methyl-6-propyl-3′,4′-trimethylenedioxyisoflavon-7-yl)-MDP.

A study of the glycosylation of 7-hydroxyisoflavone derivatives showed good yields of the 7-*O*-*β*-D-glucosaminides in a phase-transfer (PT) system (solid K_2CO_3:CH_3CN) with crown-ether catalysis [3, 4] and in a system of K_2CO_3 solution:$CHCl_3$ using quaternary ammonium salts (QAS) [4].

We studied the influence of the QAS structure using the reaction of 7-hydroxy-2-methyl-6-propyl-3′,4′-trimethylenedioxyisoflavone (**2**) with a two-fold excess of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*α*-D-glucopyranosylchloride (**1**) and PT catalysis in the system K_2CO_3 (0.18 M):$CHCl_3$ as an example. The reactions were carried out at room temperature and with heating to 50°C. It was found (Fig. 1) that use of a weakly hydrophobic catalyst (Et_4NBr) was ineffective whereas the best results for glycosylation of 7-hydroxyisoflavone (**2**) by highly hydrophobic QAS were obtained for Bu_4NBr. In all instances, conducting the reaction at room temperature increased the conversion time of *α*-chloride **1**. This was accompanied by extensive decomposition of the glycosyl donor and reduction of the yield of target glycoside **3**.

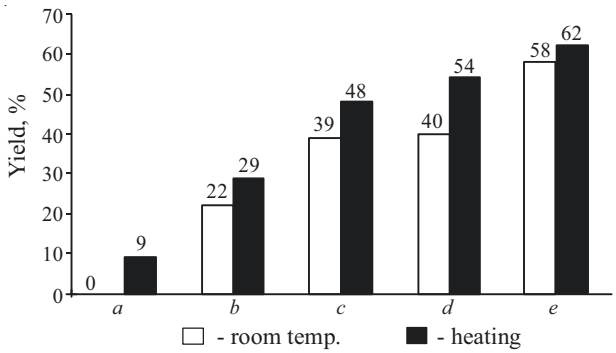

Fig. 1. Yields of glycoside **3** using various PT catalysts and temperatures (*a*, Et_4NBr; *b*, Et_3BnNCl; *c*, $C_{18}H_{37}Me_3NCl$; *d*, Bu_4NI; *e*, Bu_4NBr).

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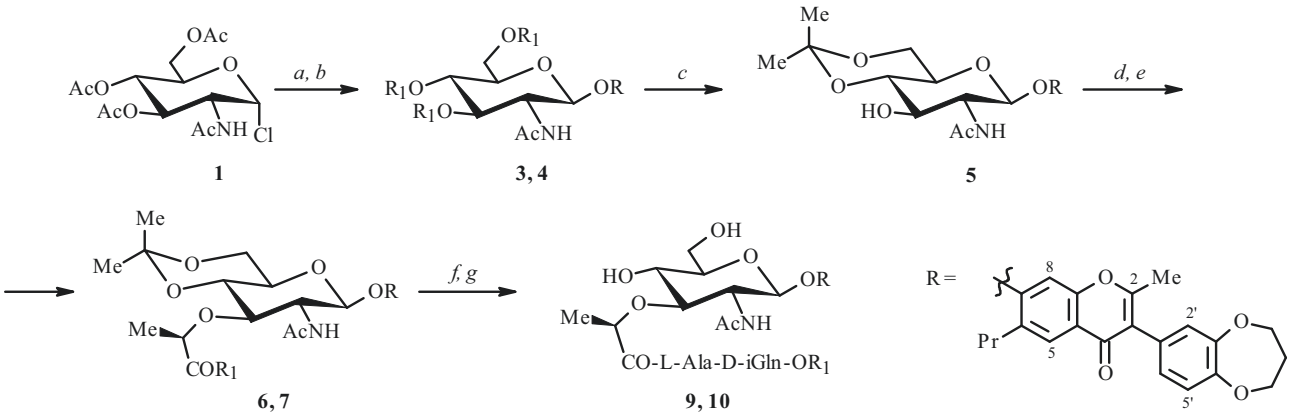
TABLE 1. Characteristic PMR Resonances for **3**, **7**, and **8** (DMSO-d_6, δ, ppm, J/Hz)

H atom	**3**	**7**	**8**
Me-2	2.26 s	2.28 s	2.27 s
H-5	7.77 s	7.74 s	7.74 s
H-8	7.31 s	7.31 s	7.21 s
Pr-6	0.87 t, 1.48 m, 2.13 t	0.87 t, 1.48 m, 2.13 t	0.87 t, 1.48 m, 2.12 t
H-2′	6.89 (d, J = 2.0)	6.88 br.d	6.87 br.d
H-5′	7.01 (d, J = 8.8)	7.01 (d, J = 8.8)	7.00 (d, J = 7.6)
H-6′	6.84 (dd, J = 8.4; J = 2.0)	6.84 (dd, J = 8.0; J = 1.6)	6.84 (dd, J = 8.4; J = 1.6)
MurNAc:H-1	5.43 (d, J = 8.4)	5.29 (d, J = 8.0)	5.10 (d, J = 8.0)
NAc	1.80 s	1.80 s	1.78 s
NH	8.18 (d, $J_{NH,2'}$ = 9.6)	8.17 (d, $J_{NH,2'}$ = 9.2)	8.22 (d, $J_{NH,2'}$ = 8.0)
Me_2C		1.36 s, 1.51 s	–
$\underline{CH_3}CHCO$		1.25 d	1.24 m
Ala: $\underline{CH_3}CH$		1.25 d	1.24 m
NH		7.36 m	7.53 d
iGln: γ-CH_2		2.36 t	2.37 t
β-CH_2		1.81 m, 2.01 m	1.81 m, 2.01 m
$CONH_2$		7.14 s, 7.36 m	7.13 s, 7.40 s
NH		8.07 d	8.02 d
$COOCH_2Ph$		5.08 s, 7.36 m	5.08 s, 7.36 m



3: R_1 = Ac; **4, 10:** R_1 = H; **6:** R_1 = OH; **7:** R_1 = L-Ala-D-iGln-OBn; **9:** R_1 = Bn

a. ROH (**2**), K_2CO_3-H_2O/$CHCl_3$, $(R_2)_4NX$; *b*. MeOH, MeONa; *c*. $Me_2C(OMe)_2$, TsOH; *d*. NaH, MeCHBrCOOH, *e*. HOSu, DCC, L-Ala-D-iGln-OBn; *f*. H_2O, AcOH, t°C; *g*. H_2/Pd

The structure of **3** was confirmed by comparison with an authentic sample [4] and by PMR spectral data (Table 1).

The 4,6-diol sugar group was blocked by acetal protection through the reaction of deacetylated derivative **4** [4] with 2,2-dimethoxypropane. The C3-hydroxyl in **5** was treated with NaH and (*S*)-2-bromopropanoic acid under conditions facilitating an S_N2 mechanism. The catalyst for the alkylation reaction was 15-crown-5.

The resulting glycosylmuramic acid **6** was activated by *N*-hydroxysuccinimide and *N*,*N*′-dicyclohexylcarbodiimide and condensed with the benzyl ester of L-alanyl-D-isoglutamine. Protected glycopeptide **7** was isolated in 94% yield by column chromatography.

The PMR spectrum of **7** contained characteristic resonances corresponding to the isoflavone protons, the carbohydrate residue, and the dipeptide (Table 1). Two-step deprotection of **7** included acid hydrolysis of the isopropylidene group and removal of the benzyl ester in isoglutamine derivative **8** by catalytic hydrogenolysis. This gave the target glycoside of MDP **9**.

EXPERIMENTAL

Melting points were determined on a PTP apparatus; optical rotation at 20–22°C, on a Polamat A polarimeter (λ 546 nm). PMR spectra were taken in DMSO-d_6 with TMS internal standard on a Varian Mercury 400 spectrometer

(400 MHz). TLC was carried out on Sorbfil-AFV-UV plates (Sorbpolimer, Russia). Compounds were detected using H_2SO_4 (5%) in EtOH with heating to 200–300°C and UV (254 nm). We used solvent systems C_6H_6–propan-2-ol (10:1, 1), $CHCl_3$–propan-2-ol (10:1, 2), $CHCl_3$–propan-2-ol–AcOH (5:5:1, 3); Et_4NBr, $C_{18}H_{37}Me_3NCl$, Bu_4NI, Bu_4NBr (Merck), and benzyltriethylammonium chloride (Et_3BnNCl) [5].

(2-Methyl-6-propyl-3′,4′-trimethylenedioxyisoflavon-7-yl)-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (3). General Glycosylation Method. A mixture of $CHCl_3$ (7.5 mL) and K_2CO_3 solution (7.5 mL, 0.18 M) was treated with **1** (250 mg, 0.68 mmol) [6], **2** (124 mg, 0.34 mmol), and Et_4NBr (71 mg, 0.34 mmol) and stirred at room temperature (A) or at 50°C (B) until the glycosyl donor disappeared (TLC monitoring using systems 1 and 2). When the reaction was finished, the mixture was treated with $CHCl_3$ (15 mL). The organic layer was separated, washed with H_2O (5 mL), dried over anhydrous Na_2SO_4, and evaporated. The residue was crystallized from MeOH (5 mL) to afford **3** (yields in Fig. 1), mp 235–237°C, $[\alpha]_{546}$ +52° (*c* 1.0, $CHCl_3$). Table 1 lists the PMR spectrum.

(2-Methyl-6-propyl-3′,4′-trimethylenedioxyisoflavon-7-yl)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (5). A solution of **4** (130 mg, 0.23 mmol) [4] in THF (10 mL) was heated to 60°C and treated with 2,2-dimethoxypropane (0.12 mL, 0.92 mmol) and *p*-toluenesulfonic acid (15 mg). After 1 h (TLC monitoring using system 2), the mixture was treated with Py (0.05 mL) and evaporated. Column chromatography (gradient elution with C_6H_6–propan-2-ol, 50:1→10:1) isolated **5** (50 mg, 36%), $[\alpha]_{546}$ +20° (*c* 1.0, $CHCl_3$).

Benzyl Ester of *O*-[(2-Methyl-6-propyl-3′,4′-trimethylenedioxyisoflavon-7-yl)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosid-3-yl]-D-lactoyl-L-alanyl-D-isoglutamine (7). A solution of **5** (50 mg, 0.08 mmol) in anhydrous dioxane (10 mL) was stirred, treated in portions with NaH (8 mg, 0.32 mmol), heated (water bath ~95°C) for 1 h (TLC monitoring using system 2), cooled to 65°C, treated with 15-crown-5 (0.05 mL, 0.25 mmol) and after 1 h with (*S*)-2-bromopropanoic acid (11 μL, 0.12 mmol), held at 65°C for another 1.5 h, and cooled. The excess of NaH was decomposed with H_2O. The mixture was evaporated, dissolved in H_2O (20 mL), and acidified with H_2SO_4 (0.1 N) until the pH was 3–4. Muramic acid was extracted with $CHCl_3$ (3 × 10 mL). The $CHCl_3$ layer was washed once with H_2O (5 mL), dried over anhydrous Na_2SO_4, and evaporated. The residue was co-evaporated with benzene.

Acid **6** (56 mg, 0.08 mmol) in THF (10 mL) was stirred and treated with *N*-hydroxysuccinimide (18 mg, 0.16 mmol) and *N*,*N*′-dicyclohexylcarbodiimide (33 mg, 0.16 mmol). After 27 h (TLC monitoring using system 2), the precipitate of *N*,*N*′-dicyclohexylurea was filtered off and washed with solvent (3 mL). The filtrate was treated with *N*-deprotected dipeptide [obtained by treatment of the benzyl ester of Boc-L-alanyl-D-isoglutamine (34 mg, 0.08 mmol) with trifluoroacetic acid (100 μL) with subsequent evaporation to dryness] and Et_3N until the pH was 7–8. When the reaction was finished (TLC monitoring using system 2), the precipitate was filtered off. The filtrate was evaporated. The residue was dissolved in $CHCl_3$ (20 mL) and washed once with HCl solution (5 mL, 0.01 N), saturated $NaHCO_3$ solution (5 mL), and H_2O (5 mL). The $CHCl_3$ layer was dried over anhydrous Na_2SO_4 and evaporated. The residue was co-evaporated with benzene to afford **7** (75 mg, 94%), mp 175–180°C, $[\alpha]_{546}$ +21° (*c* 1.0, $CHCl_3$). Table 1 lists the PMR spectrum.

Benzyl Ester of *O*-[(2-Methyl-6-propyl-3′,4′-trimethylenedioxyisoflavon-7-yl)-2-acetamido-2-deoxy-β-D-glucopyranosid-3-yl]-D-lactoyl-L-alanyl-D-isoglutamine (8). Protected glycopeptide **7** (65 mg, 0.067 mmol) was dissolved in AcOH (5 mL, 60%) with heating on a boiling-water bath and held for 20 min (TLC monitoring using systems 2 and 3). The reaction mixture was evaporated. The residue was co-evaporated with benzene and crystallized from Et_2O to afford **8** (50 mg, 81%), mp 167–171°C, $[\alpha]_{546}$ +8° (*c* 1.0, $CHCl_3$). Table 1 lists the PMR spectrum.

***O*-[(2-Methyl-6-propyl-3′,4′-trimethylenedioxyisoflavon-7-yl)-2-acetamido-2-deoxy-β-D-glucopyranosid-3-yl]-D-lactoyl-L-alanyl-D-isoglutamine (9).** Benzyl ester **8** (40 mg, 0.04 mmol) was dissolved in THF–H_2O (5 mL, 9:1) and subjected to hydrogenolysis over Pd/C (20 mg, 10%) at room temperature for 2 h. When the reaction was finished (TLC monitoring using system 3), the catalyst was filtered off and washed with the solvent mixture (3 mL). The filtrate was evaporated. The residue was co-evaporated with benzene. The resulting product was ground with Et_2O to afford **9** (15 mg, 42%), $[\alpha]_{546}$ –10° (*c* 1.0, EtOH).

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