DOI 10.1007/s10600-018-2574-x
Chemistry of Natural Compounds, Vol. 54, No. 6, November, 2018

SYNTHESIS OF α-CYCLOOCTYL- AND α-CYCLOPENTADECYLGLYCOSIDES OF *N*-ACETYLMURAMYL-L-ALANYL-D-ISOGLUTAMINE

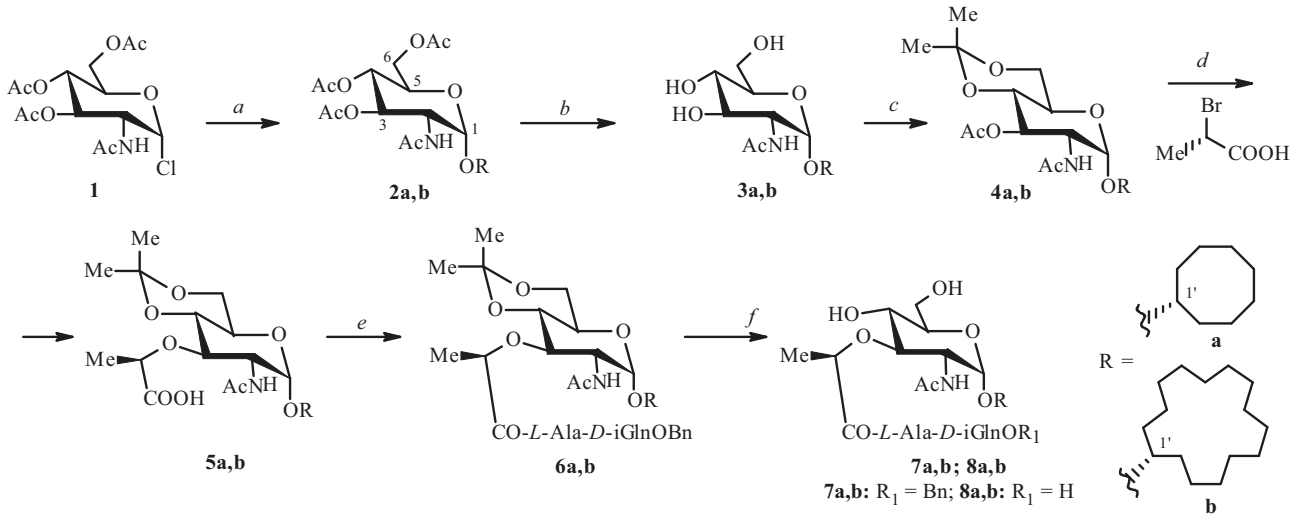
A. E. Zemlyakov,* V. V. Tsikalov, and V. N. Tsikalova

N-Acetylmuramyl-L-alanyl-D-isoglutamine α-cyclooctyl- and α-cyclopentadecylglycosides were synthesized. The starting peracetylated α-N-glucosaminides were synthesized by reacting the cycloalkanols with peracetyl α-D-glucosaminyl chloride in the presence of Hg(II) iodide in CH_3NO_2 with heating or by using $ZnCl_2$/tetrabutylammonium bromide in CH_2Cl_2 at room temperature. Sequential deacetylation, isopropyl protection, and alkylation by (S)-2-bromopropanoic acid gave α-cycloalkyl-4,6-O-isopropylidene-N-acetyl-D-muramic acids, condensation of which with the benzyl ester of L-Ala-D-iGln using the HOSu/DCC method and deprotection afforded the target glycopeptides.

Keywords: *N*-acetylglucosamine glycosides, glucosaminides muramyl dipeptide, muramyl-dipeptide glycosides.

Studies of the influence of the configuration of the anomeric center in *N*-acetylmuramyl-L-alanyl-D-isoglutamine (muramyl dipeptide, MDP) glycosides on the immunostimulating activity found that the α-anomers of glycosides with amphiphilic aglycons (α- and β-butyl-, α- and β-heptyl-, α- and β-cyclohexyl-) exhibited lower induction in *in vitro* and *in vivo* experiments [1, 2]. Earlier, MDP β-methyl- and β-benzylglycosides were reported to have higher adjuvant activity than the corresponding α-isomers [3, 4]. Conversely, more lipophilic α- and β-dodecyl- or α- and β-cyclododecyl-MDP did not show statistically significant differences for stimulation of anti-infection resistance of mice to *Staphylococcus aureus* [5, 6].

The set of compounds was expanded by synthesizing two new anomeric MDP derivatives in this series, i.e., α-cyclooctyl- and α-cyclopentadecyl-MDP (**8a**,**b**). Syntheses of the corresponding MDP β-glycosides were described in a preceding article [7].



a. ROH; *b*. MeONa, MeOH; *c*. $Me_2C(OMe)_2$, TsOH; *d*. NaH; *e*. 1. HOSu, DCC, 2. TFA·*L*-Ala-*D*-iGlnOBn, Et_3N; *f*. 1. H_2O, H^+, 2. H_2 (Pd/C)

V. I. Vernadsky Crimean Federal University, 4 Prosp. Acad. Vernadskii, Simferopol′, 295007, Russian Federation, e-mail: alex_z56@mail.ru. Translated from *Khimiya Prirodnykh Soedinenii*, No. 6, November–December, 2018, pp. 968–971. Original article submitted June 5, 2018.

TABLE 1. Characteristic PMR Resonances of **6a**,**b**, **7a**,**b**, and **8a**,**b** (DMSO-d_6, δ, ppm, J/Hz)*

Atom	**6a**	**6b**	**7a**	**7b**	**8a**	**8b**
R: $(CH_2)_n$	1.46 m, 1.65 m	1.29 m, 1.47 m	1.46 m, 1.65 m	1.30 m, 1.51 m	1.47 m, 1.66 m	1.29 m, 1.52 m
MurNAc–NAc	1.80 s	1.81 s	1.78 s	1.78 s	1.78 s	1.78 s
H-1	4.85 (d, J = 4)	4.85 (d, J = 3)	4.79 (d, J = 3)	4.79 (d, J = 3)	4.79 (d, J = 3.5)	4.80 (d, J = 3)
NH	7.87 (d, J = 9)	7.98 (d, J = 9)	7.95 (d, J = 8)	7.94 (d, J = 8)	7.97 (d, J = 8)	7.96 (d, J = 8)
CMe_2	1.32 s, 1.46 m	1.33 s, 1.47 s	–	–	–	–
C-4-OH	–	–	5.26 (d, J = 6.5)	5.23 (d, J = 6.5)	5.28 br.d	5.30 br.d
C-6-OH	–	–	4.53 (t, J = 6)	4.43 (t, J = 6)	4.55 (t, J = 6)	4.52 br.d
$\underline{CH_3}CHCO$	1.21 (d, J = 6.5), 1.24 (d, J = 7.5)	1.23 (d, J = 7.5), 1.25 (d, J = 7.5),	1.21 (d, J = 7), 1.24 (d, J = 7.5)	1.23 (d, J = 7), 1.26 (d, J = 7)	1.22 (d, J = 7), 1.25 (d, J = 7.5)	1.21 (d, J = 7), 1.24 (d, J = 7.5)
Ala: NH	7.46 (d, J = 7)	7.51 (d, J = 7)	7.62 (d, J = 7.5)	7.63 (d, J = 7)	7.63 (d, J = 6.5)	7.61 (d, J = 6)
iGln: γ-CH_2	2.36 (t, J = 8)	2.35 (t, J = 7)	2.36 (t, J = 7)	2.35 (t, J = 8)	2.21 (t, J = 7.5)	2.20 (t, J = 7.5)
β-CH_2	1.80 m, 2.01 m	1.79 m, 2.01 m	1.79 m, 1.99 m	1.80 m, 2.01 m	1.75 m, 1.94 m	1.75 m, 1.94 m
$CONH_2$	7.01 s, 7.29 s	7.07 s, 7.28 s	7.08 s, 7.29 s	7.01 s, 7.26 s	7.07 s, 7.29 s	7.05 s, 7.27 s
CO_2CH_2Ph	5.08 s, 7.35 m	5.08 s, 7.36 m	5.08 s, 7.36 m	5.07 s, 7.35 m	–	–
NH	8.07 (d, J = 8)	8.17 (d, J = 8.5)	8.16 (d, J = 9)	8.17 (d, J = 8.5)	8.15 (d, J = 8)	8.15 (d, J = 8)

*Operating frequency 300 MHz; for **6b** and **7b**, 400 MHz.

Reactions of alcohols with peracetyl α-D-glucosaminyl chloride (**1**) at ~100°C in CH_3NO_2 in the presence of Hg(II) chloride is a simple route to α-D-glucosaminides [8, 9] and could produce α-cyclooctyl- and α-cyclopentadecylglycosides **2a**,**b** in 49 and 46% yields, respectively.

Alternatively, the literature method [10] that carried out the reaction at room temperature in CH_2Cl_2 using $ZnCl_2$ and Bu_4NBr as activators could increase the yield to 51% only for **2b**.

PMR spectra of **2a**,**b** contained resonances for the carbohydrate protons and multiplets for the aglycon methylene protons at 1.33–1.80 ppm and a quintet for the methine proton at δ 3.77 and 3.65 ppm, respectively. The spin–spin coupling constant of 4 Hz for the anomeric proton was consistent with a 1,2-*cis*-glycoside bond in **2a**,**b**.

The glycopeptides were synthesized from glycosides **2a**,**b** using the classical scheme. The β-glycol group in triols **3a**,**b**, which were prepared by Zemplen deacetylation of **2a**,**b**, was blocked using 2,2-dimethoxypropane. The C-3 hydroxyl in acetals **4a**,**b** was converted to the alcoholate and alkylated by (*S*)-2-bromopropanoic acid. Protected D-muramic acids **5a**,**b** were condensed with L-alanyl-D-isoglutamine benzyl ester using the activated ester method. PMR spectra of glucopeptides **6a**,**b** had proton resonances for the carbohydrate and characteristic resonances for the peptide protons (Table 1).

Glycopeptides **6a**,**b** were deprotected stepwise. The acetal was hydrolyzed with heating in AcOH (70%). The benzyl esters in the isoglutamine of diols **7a**,**b** were removed by catalytic hydrogenolysis. PMR spectra of diols **7a**,**b** and final glycopeptides **8a**,**b** were consistent with the structures of the compounds (Table 1).

EXPERIMENTAL

Melting points were determined on a PTP apparatus. Optical rotation at 20–22°C was measured on a Polamat-A polarimeter (λ 546 nm). PMR spectra were taken with TMS internal standard on Varian VXR-300 (300 MHz) and Mercury 400 spectrometers (400 MHz). TLC used Sorbfil-AFV-UF plates (Sorbpolimer, Russia). Compounds were detected by H_2SO_4 solution (5%) in EtOH with heating to 200–300°C. The solvent systems were C_6H_6–*i*-PrOH (10:1, 1); $CHCl_3$–*i*-PrOH (15:1, 2; 5:1, 3; 3:1, 4); and *n*-BuOH–H_2O–AcOH (3:3:1, 5). Column chromatography (CC) used silica gel 60 (63–200 μm, Merck). Cyclooctanol and cyclopentadecanol were prepared via $LiAlH_4$ reduction of cyclooctanone and cyclopentadecanone (Alfa Aesar). The constants of the alcohols agreed with handbook data.

Cyclooctyl-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranoside (2a). *Version 1.* A solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (**1**, 2.50 g, 6.84 mmol) [11] in anhydrous CH_3NO_2 (50 mL) was treated with Hg(II) iodide (3.59 g, 7.9 mmol), molecular sieves (400 mg, 0.3 nm), and cyclooctanol (1.31 g, 10.26 mmol). The mixture was stirred at ~100°C (bath temperature) until the glycosyl donor disappeared (TLC monitoring using systems 1 and 2) and then heated for another 2 h. The molecular sieves and salts were filtered off. The filtrate was

evaporated. The resulting solid was dissolved in $CHCl_3$ (100 mL) and washed with saturated sodium thiosulfate solution (2 × 2 mL) and H_2O (20 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The solid was purified by CC (gradient elution, $C_6H_6 \rightarrow C_6H_6$–*i*-PrOH, 100:1→50:1). Yield of glycoside **2a**, 1.53 g (49%); oily compound, $[\alpha]_{546}$+92° (*c* 1.0, $CHCl_3$). ^{1}H NMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.45–1.80 (14H, m, 7 CH_2), 1.95 (3H, s, NAc), 2.03, 2.04, 2.10 (3H each, s, OAc), 3.77 (1H, qt, H-1′), 4.03 (1H, ddd, J = 9.5, 2.5, 5, H-5), 4.10 (1H, dd, J = 2.5, 12.5, H-6a), 4.22 (1H, dd, J = 5, 12.5, H-6b), 4.31 (1H, m, H-2), 4.93 (1H, d, J = 4, H-1), 5.10 (1H, dd, J = 9.5, 9.5, H-4), 5.20 (1H, dd, J = 10.5, 9.5), 5.63 (1H, d, J = 9.5, NH).

Cyclopentadecyl-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranoside (**2b**) was prepared analogously, yield 1.75 g (46%); mp 112–114°C, $[\alpha]_{546}$ +104° (*c* 1.0, $CHCl_3$). ^{1}H NMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.33, 1.48–1.62 (28H, m, 14 CH_2), 1.95 (3H, s, NAc), 2.03, 2.04, 2.10 (3H each, s, OAc), 3.65 (1H, qt, H-1′), 4.04 (1H, ddd, J = 9.5, 2.5, 5, H-5), 4.07 (1H, dd, J = 2.5, 12.5, H-6a), 4.24 (1H, dd, J = 5, 12.5, H-6b), 4.32 (1H, m, H-2), 4.94 (1H, d, J = 4, H-1), 5.10 (1H, dd, J = 9.5, 9.5, H-4), 5.20 (1H, dd, J= 10, 9.5, H-3), 5.64 (1H, d, J = 9.5, NH).

Version 2. A reaction mixture consisting of α-D-glucopyranosyl chloride (**1**, 2.50 g, 6.84 mmol), anhydrous $ZnCl_2$ (0.93 g, 6.84 mmol), cyclooctanol (875 mg, 6.84 mmol), Bu_4NBr (2.20 g, 6.83 mmol), and anhydrous CH_2Cl_2 (30 mL) was held for 48 h at room temperature, diluted with CH_2Cl_2 (20 mL), and washed with H_2O (5 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The solid was purified analogously to version 1 to afford **2a** (1.53 g, 49%). Glycoside **2b** (1.94 g, 51%) was also synthesized analogously.

Cyclooctyl-2-acetamido-2-deoxy-α-D-glucopyranoside (3a). Acetate **2a** (1.50 g, 3.3 mmol) was dissolved in anhydrous MeOH (30 mL) and treated with NaOMe in MeOH (0.5 mL, 0.1 M). When the reaction was finished (TLC monitoring using systems 1 and 2), the solution was neutralized by KU-2 cation exchanger (H^+). The resin was rinsed with MeOH. The filtrate was evaporated to afford **3a** (1.0 g, 92%), mp 109–111°C, $[\alpha]_{546}$ +168° (*c* 1.0, EtOH).

Cyclopentadecyl-2-acetamido-2-deoxy-α-D-glucopyranoside (**3b**, 1.3 g, 88%) was prepared analogously; mp 212–218°C, $[\alpha]_{546}$ +131° (*c* 1.0, EtOH).

Cyclooctyl-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranoside (4a). A suspension of **3a** (0.95 g, 2.87 mmol) in anhydrous THF (20 mL) was stirred, heated to 50–55°C, treated with 2,2-dimethoxypropane (1.0 mL) and anhydrous *p*-toluenesulfonic acid (10 mg), cooled after 1 h (TLC monitoring using system 3), neutralized with Py (~50 μL), and evaporated. The solid was purified by CC (gradient elution, C_6H_6–*i*-PrOH, 50:1→10:1). Yield of **4a**, 0.85 g (80%); glassy compound, $[\alpha]_{546}$ +102° (*c* 1.0, $CHCl_3$).

Cyclopentadecyl-2-acetamido-4,6-*O*-isopropylidene-α-D-glucopyranoside (**4b**, 1.25 g, 91%) was prepared analogously; glassy compound, $[\alpha]_{546}$ +83° (*c* 1.0, $CHCl_3$).

Benzyl Ester of *O*-(Cyclooctyl-2-acetamido-2,3-dideoxy-4,6-*O*-isopropylidene-α-D-glucopyranosid-3-yl)-D-lactyl-L-alanyl-D-isoglutamine (6a). A suspension of **4a** (740 mg, 1.99 mmol) in anhydrous dioxane (20 mL) was stirred, treated in portions with a suspension of NaH (320 mg, 7.96 mmol, 60%), heated to 95°C, held at that temperature for 1 h, cooled to 65°C, treated with (*S*)-2-bromopropanoic acid (0.27 mL, 3.00 mmol), held at 65°C for 3 h, and cooled. The excess of NaH was decomposed by EtOH. The mixture was concentrated, poured into cold H_2O (50 mL), and acidified with HCl (2 M) to pH 3–4. Muramic acid was extracted with $CHCl_3$ (3 × 30 mL). The extract was dried over anhydrous Na_2SO_4 and evaporated.

The resulting partially protected muramic acid **5a** (800 mg, 1.8 mmol, 90%) was used without further purification by dissolving in anhydrous THF (10 mL), stirring, and treating with *N*-hydroxysuccinimide (HOSu, 250 mg, 2.16 mmol) and *N*,*N*′-dicyclohexylcarbodiimide (DCC, 445 mg, 2.16 mmol). The precipitate of dicyclohexylurea was filtered off after 3 h and rinsed with solvent. The filtrate was treated with L-alanyl-D-isoglutamine benzyl ester trifluoroacetate [prepared by treating the corresponding Boc-derivative with trifluoroacetic acid (730 mg, 1.79 mmol) followed by evaporating to dryness] and Et_3N to pH 8. When the reaction was finished (TLC monitoring using system 3), the mixture was evaporated. The solid was dissolved in $CHCl_3$ (70 mL). The solution was washed with HCl (25 mL, 1 M), saturated $NaHCO_3$ solution (25 mL), and H_2O (25 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The solid was purified by CC (gradient elution, $CHCl_3$–*i*-PrOH, 50:1→10:1) to afford **6a** (1.15 g, 87%), mp 68–72°C, $[\alpha]_{546}$ +54° (*c* 1.0, $CHCl_3$). Table 1 lists the PMR spectral data.

The benzyl ester of *O*-(cyclopentadecyl-2-acetamido-2,3-dideoxy-4,6-*O*-isopropylidene-α-D-glucopyranosid-3-yl)-D-lactyl-L-alanyl-D-isoglutamine (**6b**, 560 mg, 60%) was synthesized analogously, amorphous compound, $[\alpha]_{546}$ +80° (*c* 0.6, $CHCl_3$). Table 1 lists the PMR spectral data.

Benzyl Ester of *O*-(Cyclooctyl-2-acetamido-2,3-dideoxy-α-D-glucopyranosid-3-yl)-D-lactyl-L-alanyl-D-isoglutamine (7a). Glycopeptide **6a** (650 mg, 0.89 mmol) was dissolved with heating on a boiling-water bath in AcOH (10 mL, 70%), held at that temperature for 15 min (TLC monitoring using systems 3 and 4), and evaporated to dryness. The solid was co-evaporated with toluene and purified by CC (gradient elution, $CHCl_3$–*i*-PrOH, 50:1→5:1) to afford **7a** (270 mg, 44%), mp 115–119°C, $[\alpha]_{546}$ +96° (*c* 1.0, EtOH). Table 1 lists the PMR spectral data.

The benzyl ester of *O*-(cyclopentadecyl-2-acetamido-2,3-dideoxy-α-D-glucopyranosid-3-yl)-D-lactyl-L-alanyl-D-isoglutamine (**7b**) was prepared analogously; mp 177–179°C, $[\alpha]_{546}$ +63° (*c* 1.0, EtOH). Table 1 lists the PMR spectral data.

***O*-(Cyclooctyl-2-acetamido-2,3-dideoxy-α-D-glucopyranosid-3-yl)-D-lactyl-L-alanyl-D-isoglutamine (8a).** Benzyl ester **7a** (260 mg, 0.38 mmol) was dissolved in THF–H_2O (30 mL, 9:1) and hydrogenated over Pd/C (50 mg, 10%) at room temperature for 4 h (TLC monitoring using systems 4 and 5). The catalyst was filtered off and rinsed with the solvent mixture (5 mL). The filtrate was evaporated to dryness. Addition of Et_2O precipitated amorphous **8a** (140 mg, 61%), $[\alpha]_{546}$ +90° (*c* 1.0, EtOH). Table 1 lists the PMR spectral data.

***O*-(Cyclopentadecyl-2-acetamido-2,3-dideoxy-α-D-glucopyranosid-3-yl)-D-lactyl-L-alanyl-D-isoglutamine** (**8b**) was synthesized analogously; amorphous compound, $[\alpha]_{546}$ +75° (*c* 1.0, EtOH). Table 1 lists the PMR spectral data.

REFERENCES

1. Yu. L. Krivorutchenko, I. B. Andronovskaja, J. Hinkula, Yu. S. Krivoshein, E. Ljung-dahl-Stahle, S. S. Pertel, V. I. Grishkovets, A. E. Zemlyakov, and B. Wahren, *Vaccine*, **15**, 1479 (1997).
2. A. E. Zemlyakov, V. V. Tsikalov, O. V. Kalyuzhin, V. O. Kur′yanov, and V. Ya. Chirva, *Bioorg. Khim.*, **29**, 316 (2003).
3. Y. Nagai, K. Akiyama, S. Kotani, Y. Watanabe, T. Shimono, T. Shiba, and S. Kusumoto, *Cell. Immunol.*, **35**, 168 (1978).
4. I. Azuma, H. Okumura, I. Saiki, M. Kiso, A. Hasegawa, Y. Tanio, and Y. Yamamura, *Infect. Immun.*, **33**, 834 (1981).
5. A. E. Zemlyakov, V. N. Tsikalova, V. V. Tsikalov, V. Ya. Chirva, E. L. Mulik, and O. V. Kalyuzhin, *Bioorg. Khim.*, **32**, 424 (2006).
6. O. V. Kalyuzhin, A. E. Zemlyakov, N. G. Kalina, E. L. Mulik, F. N. Kuzovlev, and O. V. Makarova, *Byull. Eksp. Biol. Med.*, **145**, 561 (2008).
7. A. E. Zemlyakov, V. N. Tsikalova, and V. V. Tsikalov, *Chem. Nat. Compd.*, **53**, 929 (2017).
8. A. E. Zemlyakov, V. O. Kur′yanov, E. A. Sidorova, and V. Ya. Chirva, *Bioorg. Khim.*, **24**, 623 (1998).
9. A. E. Zemlyakov, V. N. Tsikalova, S. A. Zemlyakov, and V. Ya. Chirva, *Uch. Zap. Tavrich. Nats. Univ. im. V. I. Vernadskogo, Ser. Biol. Khim.*, **23**, 225 (2010).
10. E. R. Kumar, H.-S. Byun, S. Wang, and R. Bittman, *Tetrahedron Lett.*, **35**, 505 (1994).
11. D. Horton, *Methods in Carbohydrate Chemistry*, Vol. 6, Academic, New York, 1972.