Supporting Information

for

N-Alkyl derivatives of diosgenyl 2-amino-2-deoxy-β-D-glucopyranoside; synthesis and antimicrobial activity

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Experimental details for the preparation of compounds 2b, 2c, 4a–d, 5a–d, 6a–d, 8–17, corresponding characterization data and information on the way of determination of minimum inhibitory concentration.

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Experimental

General methods

Solvents and chemical reagents were purchased and used without further purification. Melting points are uncorrected. The IR spectra were recorded as Nujol mulls with a Bruker IFS 66 spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or a mixture of $CDCl_3$:CD_3OD (1:1, v/v) with internal Me_4Si on a Varian Mercury 400 MHz instrument (400.49/100.70 MHz). Positive-ion mode MALDI-TOF mass spectra were obtained using a Bruker Biflex III spectrometer with 4-cyano-4-hydroxycinnamic acid or 2,5-dihydroxy-benzoic acid matrixes. The optical rotations were determined at rt on a Perkin-Elmer polarimeter in a 1-dm tube at the D line of sodium using $CHCl_3$ or a mixture of $CHCl_3$:CH_3OH (1:1, v/v) as the solvents. Elemental analyses were performed on a Carlo Erba EA 1108 analyzer. Thin-layer chromatography (TLC) was performed on aluminium plates coated with E. Merck Kieselgel 60 F_{254} using the following eluent systems (v/v): A, 2:1 toluene : AcOEt; B, 10:1 $CHCl_3$: Et_2O; C, 4:1 CCl_4 : acetone; D, 7:1 toluene : AcOEt; E, 6:1 toluene : AcOEt, F, 5:1 toluene : AcOEt; G, 4:1 $CHCl_3$: MeOH; H, 2:1 CCl_4 : acetone; I, 7:1 $CHCl_3$: MeOH; J, 5:1 $CHCl_3$: MeOH. Column chromatography was performed on MN Kieselgel 60 (<0.8 mm) with one of the above listed eluent systems. For the detection of compounds the dry plates were sprayed with a 5% aqueous sulfuric acid solution and then heated at 150 °C.

3,4,6-Tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-gluco-pyranosyl bromide (**2b**)

To a solution of **1b** [1] (0.6 g, 1.15 mmol) in glacial acetic acid (2 mL) and acetic anhydride (0.4 mL), a 45% solution of HBr in acetic acid (3 mL) was added. The mixture was stirred at rt. After 1 h the mixture was diluted with $CHCl_3$ (40 mL), washed with H_2O (2 x 10 mL), satd

aq $NaHCO_3$ (2 x 10 mL) and again H_2O (20 mL). Then it was dried over $MgSO_4$ and concentrated. This afforded **2b** (0.6 g, white foam, 96%): $[\alpha]_D^{20}$ 135; R_f 0.60 (solvent A); IR: ν 3345 (N-H), 1751 (C=O/Ac and C=O/Troc), 1538 cm^{-1} (N-H, C-N); ^1H NMR (400 MHz, $CDCl_3$): in accordance with literature data [2]; ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.18, 170.71, 169.43 (3 x C=O/Ac), 154.23 (C=O/Troc), 91.19 (C-1), 74.94 (CH_2/Troc), 72.88 (C-5), 70.87 (C-3), 67.03 (C-4), 61.15 (C-6), 55.80 (C-2), 20.86, 20.79, 20.74 (3 x CH_3); MALDI-TOF-MS: *m/z* 544.2 $(M+H)^+$.

3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-α,β-D-glucopyranosyl bromide (**2c**)

To a solution of **1c** [1] (0.58 g, 1.2 mmol) in glacial acetic acid (2 mL) and acetic anhydride (0.55 mL), a 45% solution of HBr in acetic acid (2 mL) was added. The mixture was stirred at rt. After 1 h the mixture was diluted with $CHCl_3$ (40 mL), washed with H_2O (2 x 10 mL), satd aq $NaHCO_3$ (2 x 10 mL) and again H_2O (20 mL). Then it was dried over $MgSO_4$ and concentrated. This afforded **2c** (0.6 g, white foam, 98%): R_f 0.5 (solvent A); ^1H NMR (400 MHz, $CDCl_3$) (α): δ 7.88 (m, 2 H, Phth), 7.78 (m, 2 H, Phth), 6.67 (dd, 1 H, $J_{2,3}$ 11.6, $J_{3,4}$ 9.2 Hz, H-3), 6.58 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1), 5.17 (t, 1 H, $J_{4,5} = J_{3,4}$ 9.2 Hz, H-4), 4.70 (dd, 1 H, $J_{2,3}$ 11.6, $J_{1,2}$ 3.2 Hz, H-2), 4.35 (m, 2 H, H-5, H-6), 4.18 (dd, 1 H, $J_{5,6'}$ 2.4, $J_{6,6'}$ 12.4 Hz, H-6'); (β): δ 7.88 (m, 2 H, Phth), 7.78 (m, 2 H, Phth), 6.42 (d, 1 H, $J_{1,2}$ 9.6 Hz, H-1), 5.77 (dd, 1 H, $J_{2,3}$ 10.4, $J_{3,4}$ 9.2 Hz, H-3), 5.27 (dd, 1 H, $J_{3,4}$ 9.2, $J_{4,5}$ 10.0 Hz, H-4), 4.63 (dd, 1 H, $J_{1,2}$ 9.6, $J_{2,3}$ 10.4 Hz, H-2), 4.34 (dd, 1 H, $J_{5,6}$ 4.8, $J_{6,6'}$ 12.4 Hz, H-6), 4.21 (dd, 1 H, $J_{5,6'}$ 2.4, $J_{6,6'}$ 12.4 Hz, H-6'), 3.98 (m, 1 H, H-5); (α+β): 2.14, 2.12, 2.04, 2.09, 1.91, 1.87 (6 s, 18 H, 6 x OAc); ^{13}C NMR (100 MHz, $CDCl_3$) (α): δ 87.37 (C-1), 72.61 (C-5), 69.00 (C-4), 67.75 (C-3), 61.15 (C-6), 56.40 (C-2); (β): δ 77.55 (C-1), 76.91 (C-5), 70.75 (C-3), 68.29 (C-4), 61.85 (C-6), 58.29 (C-2); (α+β): δ 170.77, 170.65, 170.12, 170.08, 169.45, 169.30 (10 x C=O), 134.75, 124.03 (Ph), 20.89, 20.88, 20.83, 20.71, 20.51 (6 x CH_3).

3,4,6-Tri-*O*-acetyl-2-deoxy-2-trifluoroacetamido-α-D-glucopyranose (**4a**)

Ethylenediamine (0.088 mL, 1.35 mmol) was added to a solution of **1a** (0.5 g, 1.13 mmol) in tetrahydrofuran (28 mL) and glacial acetic acid (88 μL, 1.56 mmol). After stirring for 15 h H_2O (15 mL) was added to the reaction mixture. Then it was extracted with CH_2Cl_2 (3 x 30 mL). The organic extracts were combined, washed with 3% aq HCl, satd aq $NaHCO_3$ (2 x 30 mL) and H_2O (20 mL), dried over $MgSO_4$ and concentrated. This gave **4a** (0.44 g, 97%): mp 165-168 ^oC, lit.[3] mp 174 ^oC; $[\alpha]_D^{20}$ +19 (*c* 0.5, $CHCl_3$), lit.[3] $[\alpha]_D^{20}$ +20 (*c* 0.5, $CHCl_3$); R_f 0.3 (solvent A); spectroscopic analysis in accordance with literature data [3]; Anal. Calcd for $C_{14}H_{18}O_9NF_3$: C, 41.90; H, 4.52; N, 3.49. Found: C, 42.06; H, 4.58; N, 3.44.

3,4,6-Tri-*O*-acetyl-2-deoxy-2,2,2-trichloroethoxycarbonylamino-D-glucopyranose (**4b**)

This was synthesized analogously to **4a**. The following amounts of the substrates were used: **1b** (0.8 g, 1.53 mmol), ethylenediamine (0.12 mL, 1.84 mmol), tetrahydrofuran (39 mL) and glacial acetic acid (0.12 mL, 2.13 mmol). The crude white product (0.73 g) was crystallized from AcOEt/hexane to afford **4b** (0.62 g, white powder, 84%): R_f 0.32 (α) and 0.17 (β) (solvent A); ^{1}H NMR (400 MHz, $CDCl_3$) (α): δ 5.45 (d, 1 H, NH), 5.34 (dd, 1 H, $J_{2,3}$ 9.6, $J_{3,4}$ 10.4 Hz, H-3), 5.32 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1), 5.13 (dd, 1 H, $J_{4,5}$ 9.6, $J_{3,4}$ 10.4 Hz, H-4), 4.80 (d, 1 H, CH_ATroc), 4.64 (d, 1 H, CH_BTroc), 4.26-4.22 (m, 2 H, H-5, H-6'), 4.15 (dd, 1 H, $J_{5,6}$ 4.0, $J_{6,6'}$ 14.0, H-6), 4.05 (dt, 1 H, $J_{1,2}$ 3.2 Hz, $J_{2,3} = J_{2,NH}$ 9.6 Hz, H-2), 3.63 (d, 1 H, OH), 2.11, 2.05, 2.02 (3 s, 9 H, 3 x OAc); ^{13}C NMR (100 MHz, $CDCl_3$) (α): δ 171.27, 170.11, 169.71 (3 x ester C=O), 154.47 (Troc C=O), 92.02 (C-1), 74.82 (Troc CH_2), 70.95 (C-3), 68.51 (C-4), 67.95 (C-5), 62.25 (C-6), 54.38 (C-2), 20.99, 20.92, 20.85 (3 x acetyl CH_3); Anal. Calcd for $C_{15}H_{20}O_{10}NCl_3$: C, 37.48; H, 4.19; N, 2.91. Found: C, 37.45; H, 4.20; N, 2.90.

3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (**4c**)

This was synthesized analogously to **4b**. The following amounts of the substrates were used: **1c** (0.27 g, 1.53 mmol), ethylenediamine (0.12 mL, 1.84 mmol), tetrahydrofuran (39 mL) and glacial acetic acid (0.12 mL, 2.13 mmol). This afforded **4c** (0.23 g, 90%): mp 170-172 ^{o}C; $[\alpha]_D^{20}$ +67 (*c* 0.5, $CHCl_3$); R_f 0.28 (solvent A); IR: ν 3522 (O-H), 1745, 1785 (C=O/OAc), 1713 cm^{-1} (C=O/Phth); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 (m, 2 H, Phth), 7.75 (m, 2 H, Phth), 5.86 (dd, 1 H, $J_{2,3}$ 10.4, $J_{3,4}$ 9.2 Hz, H-3), 5.66 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 5.20 (dd, 1 H, $J_{3,4}$ 9.2, $J_{4,5}$ 10.0 Hz, H-4), 4.31 (dd, 1 H, $J_{5,6}$ 4.8, $J_{6,6'}$ 12.4 Hz, H-6), 4.29 (dd, 1 H, $J_{1,2}$ 8.4, $J_{2,3}$ 10.4 Hz, H-2), 4.21 (dd, 1 H, $J_{5,6'}$ 2.4, $J_{6,6'}$ 12.4 Hz, H-6'), 3.96 (m, 1 H, H-5), 3.66 (s, OH), 2.13, 2.06, 1.88 (3 s, 9 H, 3 x OAc); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.06, 170.34, 169.79 (5 x C=O), 134.59, 123.88 (Ph), 92.84 (C-1), 72.24 (C-5), 70.70 (C-3), 69.10 (C-4), 62.27 (C-6), 56.24 (C-2), 20.98, 20.85, 20.67 (3 x acetyl CH_3); Anal. Calcd for $C_{20}H_{21}O_{10}N$: C, 55.17; H, 4.86; N, 3.22. Found: C, 55.43; H, 5.19; N, 3.04.

Tri-*O*-acetyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranose (**4d**)

Procedure a: This was synthesized analogously to **4a**. The following amounts of the substrates were used: **1d** (0.3 g, 0.49 mmol), ethylenediamine (0.038 mL, 0.59 mmol), tetrahydrofuran (13 mL) and glacial acetic acid (38 μL, 0.68 mmol). Column chromathography (solvent B) gave **4d** (0.12 g, white powder, 36%): mp 189 ^{o}C; $[\alpha]_D^{20}$ +69 (c 0.5 $CHCl_3$); R_f 0.32 (solvent B); IR: ν 3521 (O-H), 1750, 1785 (C=O/OAc), 1722 cm^{-1} (C=O/TCP); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.77 (dd, 1 H, $J_{2,3}$ 10.4, $J_{3,4}$ 9.2 Hz, H-3), 5.63 (d, 1 H, $J_{1,2}$ 8,4 Hz, H-1), 5.20 (dd, 1H, $J_{3,4}$ 9.2, $J_{4,5}$ 10.0 Hz, H-4), 4.26 (dd, 1 H, $J_{1,2}$ 8.4, $J_{2,3}$ 10.4 Hz, H-2), 4.30 (dd, 1 H, $J_{5,6}$ 4.8, $J_{6,6'}$ 12.4 Hz, H-6), 4.20 (dd, 1 H, $J_{5,6'}$ 2.4, $J_{6,6'}$ 12.4 Hz, H-6'), 3.90 (m, 1 H, H-5), 3.28 (d, OH), 2.13, 2.05, 1.91 (3 s, 9 H, 3 x OAc); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.95, 170.72, 169.64 (5 x C=O), 130.29 (Ph), 92.50 (C-1), 72.36 (C-5),

70.79 (C-3), 68.74 (C-4), 62.11 (C-6), 56.89 (C-2), 21.01, 20.83, 20.74 (3 x acetyl CH_3); Anal. Calcd for $C_{20}H_{17}O_{10}NCl_4$: C, 41.91; H, 2.99; N, 2.44. Found: C, 41.93; H, 2.94; N, 2.45.

Procedure b: A solution of **2d** (0.8 g, 1.3 mmol) in acetone (8 mL), protected from light, was stirred at rt for 5 min. Then a suspension of Ag_2CO_3 (0.65 mmol) in H_2O (4 mL) was added and stirring was continued for 2 h. The reaction was monitored by TLC (solvent C). After filtration of the silver salt, the mixture was diluted with AcOEt (50 mL) and washed with H_2O (20 mL), satd aq NaCl (20 mL), satd aq $NaHCO_3$ (2 x 20 mL) and H_2O (20 mL), dried over $MgSO_4$ and concentrated. Silica gel column chromatography (solvent D) of the crude product afforded **4d** (0.54 g, white powder, 73%).

Procedure c: A solution of **3d** (0.58 g, 0.98 mmol) in acetone (8 mL), protected from **l**ight was stirred at rt for 5 min. Then a suspension of Ag_2CO_3 (1.1 mmol) in H_2O (4 mL) was added and stirring was continued for 2 h. The reaction was monitored by TLC (solvent C). After filtration of the silver salt, the mixture was diluted with AcOEt (50 mL) and washed with H_2O (20 mL), satd aq NaCl (20 mL), satd aq $NaHCO_3$ (2 x 20 mL) and H_2O (20 mL), dried over $MgSO_4$ and concentrated. This gave a pale foam, which was crystallized from Et_2O/petroleum ether to afford **4d**. Additional portion of **4d** was obtained after column chromatography of the residue (solvent D) (0.40 g, 72%).

N-Protected 3,4,6-tri-*O*-acetyl-2-deoxy-2-amino-D-glucopyranosyl (*N*-phenyl)trifluoro-acetimidates **5a–d**

General procedure:

The respective *N*-protected 3,4,6-tri-*O*-acetyl-D-glucosamines **4a-d** (1 mmol) were dissolved in anhydrous CH_2Cl_2 (3 mL). Then, K_2CO_3 (2 mmol) and (*N*-phenyl)-2,2,2-trifluoroacetimidoyl chloride (2 mmol) were added. The mixture was stirred at rt and monitored by TLC (solvent A). After stirring for 6-48 h the mixture was diluted with $CHCl_3$ (80 mL), filtered and

concentrated. The residue was chromatographed on silica gel.

3,4,6-Tri-*O*-acetyl-2-deoxy-2-trifluoroacetamido-α,β-D-glucopyranosyl (*N*-phenyl)trifluoroacetimidate (**5a**)

Reaction of **4a** (0.44 g, 1.1 mmol) with (*N*-phenyl)-2,2,2-trifluoroacetimidoyl chloride (0.46 g, 2.2 mmol), K_2CO_3 (0.30 g, 0.22 mmol) in CH_2Cl_2 (3 mL), followed by column chromatography (solvent B) gave first **5aβ** (0.32 g, syrup, 46%): R_f 0.6 (solvent A); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38 (d, 1 H, NH), 7.30 (t, 2 H, NPh), 7.13 (t, 1 H, NPh), 6.75 (d, 2 H, NPh), 5.95 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 5.43 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 9.6 Hz, H-3), 5.13 (dd, 1 H, $J_{3,4}$ 9.6, $J_{4,5}$ 9.2 Hz, H-4), 4.44 (m, 1 H, H-2), 4.27 (dd, 1 H, $J_{5,6}$ 5.2, $J_{6,6'}$ 12.4 Hz, H-6), 4.19 (d, 1 H, $J_{6,6'}$ 12.4 Hz, H-6'), 3.91 (m, 1 H, H-5), 2.07, 2.03, 1.96 (3 s, 9 H, 3 x OAc); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.93, 170.87, 169.50, 157.57 (C=O), 142.67 (O-C=N), 129.17, 125.06, 119.13 (NPh), 94.23 (C-1), 73.13 (C-5), 71.93 (C-3), 68.30 (C-4), 61.94 (C-6), 53.42 (C-2), 20.82, 20.54, 20. 51 (acetyl CH_3).

Eluted second was **5aα** (0.28 g, syrup, 45%): R_f 0.47 (solvent A); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32 (t, 2H, NPh), 7.15 (t, 1H, NPh), 6.89 (bd, 1H, NH), 6.80 (d, 2H, NPh), 6.42 (bs, 1 H, H-1), 5.37 (dd, 1 H, $J_{2,3}$ 10.4, $J_{3,4}$ 9.6 Hz, H-3), 5.26 (t, 1 H, $J_{4,5} = J_{3,4}$ 9.6 Hz, H-4), 4.47 (m, 1 H, H-2), 4.29 (dd, 1 H, $J_{5,6}$ 4.0, $J_{6,6'}$ 12.8 Hz, H-6), 4.12 (d, 1 H, $J_{6,6'}$ 12.8 Hz, H6'), 4.08 (m, 1 H, H-5), 2.11, 2.07 (2 s, 9 H, 3 x OAc); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.48, 168.88, 168.13, (C=O), 142.64 (O-C=N), 119.35, 125.26, 129.15 (NPh), 92.13 (C-1), 70.40 (C-3), 70.19 (C-5), 67.02 (C-4), 61.45 (C-6), 52.55 (C-2), 20.85, 20.67 (acetyl CH_3).

3,4,6-Tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α,β-D-glucopyranosyl (*N*-phenyl)trifluoroacetimidate (**5b**)

Reaction of **4b** (0.25 g, 0.52 mmol), K_2CO_3 (0.14 g, 1.04 mmol) and (*N*-phenyl)-2,2,2-trifluoroacetimidol chloride (0.22 g, 1.04 mmol) in CH_2Cl_2 (3 mL), followed by column

chromatography (solvent B) gave first **5bβ** (0.17 g, syrup, 49%): R_f 0.68 (solvent A); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31 (t, 2 H, NPh), 7.13 (t, 1 H, NPh), 6.82 (d, 2 H, NPh), 5.92 (bs, 1 H, H-1), 5.38 (d, 1 H, NH), 5.30 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 9.6 Hz, H-3), 5.14 (t, 1H, $J_{3,4} = J_{4,5}$ 9.6 Hz, H-4), 4.77 (d, 1 H, CH_2Troc), 4.72 (d, 1 H, CH_2Troc), 4.28 (dd, 1 H, $J_{5,6}$ 4.0, $J_{6,6'}$ 12.4 Hz, H-6), 4.14 (d, 1 H, $J_{6,6'}$ 12.4 Hz, H-6'), 3.98 (m, 1 H, H-2), 3.78 (m, 1 H, H-5), 2.08, 2.05, 2.03 (3 s, 9 H, 3 x OAc); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.90, 170.83, 169.56, 154.11 (C=O), 143.13 (O-C=N), 129.08, 124.90, 119.40 (NPh), 94.84 (C-1), 74.81 (CH_2Troc), 73.05 (C-5), 71.75 (C-3), 68.14 (C-4), 61.81 (C-6), 55.46 (C-2), 20.90, 20.79, 20.77 (acetyl CH_3).

Eluted second was **5bα** (0.15 g, syrup, 44%): R_f 0.60 (solvent A); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31 (t, 2 H, NPh), 7.14 (t, 1H, NPh), 6.80 (d, 2 H, NPh), 6.36 (bs, 1 H, H-1), 5.35 (d, 1 H, NH), 5.33 (dd, 1 H, $J_{3,2}$ 11.2, $J_{3,4}$ 10.4 Hz, H-3), 5.22 (dd, 1 H, $J_{3,4}$ 10.4, $J_{4,5}$ 9.6 Hz, H-4), 4.82 (d, 1 H, CH_2Troc), 4.67 (d, 1 H, CH_2Troc), 4.26 (m, 2 H, H-2, H-6), 4.12 (d, 1 H, $J_{6,6'}$ 11.2 Hz, H-6'), 4.03 (m, 1 H, H-5), 2.10, 2.07, 2.06 (3 s, 9 H, 3 x OAc); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.32, 170.76, 169.48, 154.43 (C=O), 142.96 (O-C=N), 129.12, 125.08, 119.44 (NPh), 93.96 (C-1), 74.94 (CH_2Troc), 70.30, 67.72 (C-3, C-4, C-5), 61.69 (C-6), 53.81 (C-2), 20.87, 20.76 (acetyl CH_3).

3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl (*N*-phenyl)trifluoro-acetimidate (**5c**)

Reaction of **4c** (0.19 g, 0.43 mmol), K_2CO_3 (0.12 g, 0.85 mmol) and (*N*-phenyl)-2,2,2-trifluoroacetimidol chloride (0.09 g, 0.43 mmol) in CH_2Cl_2 (3 mL) gave **5c** (0.22 g, syrup, 85%): R_f 0,57 (solvent A); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87 (m, 2 H, Phth), 7.77 (m, 2 H, Phth), 7.26 (t, 2 H, NPh), 7.09 (t, 1 H, NPh), 6.72 (d, 2 H, NPh), 6.54 (d, 1 H, $J_{1,2}$ 9.6 Hz, H-1), 5.81 (m, 1 H, H-3), 5.23 (t, 1 H, $J_{3,4}$ 9.6, $J_{4,5}$ 9.6 Hz, H-4), 4.57 (t, 1 H, $J_{1,2} = J_{2,3}$ 9.6 Hz, H-2), 4.31 (dd, 1 H, $J_{5,6}$ 4.0, $J_{6,6'}$ 12.4 Hz, H-6), 4.14 (d, 1 H, $J_{6,6'}$ 12.4 Hz, H-6'), 3.82 (m, 1 H,

H-5), 2.10, 2.03, 1.88 (3 s, 9 H, 3 x OAc); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.86, 170.28, 169.58 (C=O), 143.00 (O-C=N), 128.91, 124.81, 119.39 (NPh), 134.76, 124.04 (Ph), 92.88 (C-1), 72.92 (C-5), 70.58 (C-3), 68.39 (C-4), 61.61 (C-6), 53.84 (C-2), 20.92, 20.80, 20.63 (acetyl CH_3).

3,4,6-Tri-*O*-acetyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranosyl (*N*-phenyl)trifluoroacetimidate (**5d**)

Reaction of **4d** (0.2 g, 0.35 mmol), K_2CO_3 (0.097 g, 0.70 mmol) and (*N*-phenyl)-2,2,2-trifluoroacetimidol chloride (0.15 g, 0.70 mmol) in CH_2Cl_2 (2 mL), followed by column chromatography (solvent D) gave **5d** (0.18 g, syrup, 68%): R_f 0,63 (solvent A); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.27 (t, 2 H, NPh), 7.09 (t, 1 H, NPh), 6.76 (d, 2 H, NPh), 6.50 (bs, 1 H, H-1), 5.76 (dd, 1 H, $J_{2,3}$ 9.6, $J_{3,4}$ 10.0 Hz, H-3), 5.25 (dd, 1 H, $J_{3,4}$ 10.0, $J_{4,5}$ 9.2 Hz, H-4), 4.56 (m, 1 H, H-2), 4.32 (dd, 1 H, $J_{5,6}$ 4.4, $J_{6,6'}$ 11.6 Hz, H-6), 4.14 (d, 1 H, $J_{6,6'}$ 11.6 Hz, H-6), 3.90 (m, 1 H, H-5), 2.11, 2.04, 1.92 (3 s, 9 H, 3 x OAc); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.83, 170.74, 169.50 (C=O), 141.08 (O-C=N), 130.43 (Ph), 128.94, 125.01, 119.50 (NPh), 92.71 (C-1), 72.98 (C-5), 70.80 (C-3), 68.09 (C-4), 61.58 (C-6), 54.60 (C-2), 20.91, 20.78, 20.67 (acetyl CH_3).

Diosgenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoroacetamido-β-D-glucopyranoside (**6a**)

*From **5a***: A mixture of **5a** (0.38 g, 0.66 mmol), diosgenin (0.19 g, 0.45 mmol) and 4 Å molecular sieves (1.5 g) in anhyd CH_2Cl_2 (20 mL) was stirred at rt under N_2 for 30 min. Then TMSOTf (30 μL) was added and stirring at rt was continued. After 20 h the mixture was neutralized by Et_3N, diluted with $CHCl_3$ (50 mL), filtered over the gel layer (MN Kieselgel 60) and washed with H_2O (2 x 10 mL), satd aq $NaHCO_3$ (2 x 10 mL) and again H_2O (10 mL). Then it was dried over $MgSO_4$, filtered and concentrated. Silica gel column chromatography

(solvent E) of the crude product afforded **6a** as a white solid (0.31 g, 85%); results of all analyses are in agreement with those previously reported for the reaction with bromide [4].

Diosgenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2,2,2-trichloroethoxycarbonylamino-β-D-gluco-pyranoside (**6b**)

*From **2b***: A mixture of diosgenin (0.13 g, 0.31 mmol), AgOTf (0.2 g, 0.72 mmol) and 4Å molecular sieves (1,2 g) in anhydr mixture of Et_2O (10 mL) and CH_2Cl_2 (6 mL) was stirred at rt under N_2 for 10 min. Then a solution of **2b** (0.27 g, 0.50 mmol) in CH_2Cl_2 (8 mL) was allowed to drip on to the reaction mixture. The end of reaction was verified (TLC, solvent A) after stirring for 20 h at rt. Then the mixture was neutralized with Et_3N, diluted with $CHCl_3$, filtered over the gel layer (MN Kieselgel 60), and concentrated. Column chromatography (solvent F) of the crude product afforded **6b** as a white solid (0.27 g, 98%); results of all analyses are in agreement with those previously reported for the reaction with chloride [1].

*From **5b***: A mixture of **5b** (0.26 g, 0.4 mmol), diosgenin (0.11 g, 0.27 mmol) and 4 Å molecular sieves (1 g) in anhyd CH_2Cl_2 (15 mL) was stirred at rt under N_2 for 30 min. Then TMSOTf (10 μL) was added and stirring at rt was continued. After 20 h the mixture was neutralized by Et_3N, diluted with $CHCl_3$ (50 mL), filtered over the gel layer (MN Kieselgel 60) and washed with H_2O (2 x 10 mL), satd aq $NaHCO_3$ (2 x 10 mL) and again H_2O (10 mL). Then it was dried over $MgSO_4$, filtered and concentrated. Silica gel column chromatography (solvent E) of the crude product afforded **6b** as a white solid (0.19 g, 81%); results of all analyses are in agreement with previously reported for the reaction with chloride [1].

Diosgenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**6c**)

*From **2c***: A mixture of diosgenin (0.28 g, 0.67 mmol) and 4Å molecular sieves (2.0 g) in anhyd CH_2Cl_2 (20 mL) was stirred at rt under N_2 for 10 min. Then AgOTf (0.45 g, 1.63

mmol) and *s*-collidine (0.35 mL, 2.65 mmol) were added and stirring was continued for **10** min. Then a solution of **2c** (0.52 g, 1.12 mmol) in CH_2Cl_2 (10 mL) was allowed to drip on to the reaction mixture. After stirring for 20 h at rt, the mixture was filtered and concentrated. Precipitation with MeOH and silica gel column chromatography of the filtrate (solvent F) afforded **6c** as a white solid (0.5 g, 90%); results of all analyses are in agreement with previously reported for the reaction with chloride [1].

*From **5c***: A mixture of **5c** (0.22 g, 0.37 mmol), diosgenin (0.10 g, 0.24 mmol), and 4Å molecular sieves (0.8 g) in anhyd CH_2Cl_2 (10 mL) was stirred at room temperature under N_2, for 30 min. Then TMSOTf (10 μL) was added and stirring was continued. After 20 h, the mixture was neutralized by Et_3N, diluted with $CHCl_3$ (50 mL), filtered over the gel layer (MN Kieselgel 60) and washed with H_2O (2 x 10 mL), satd aq $NaHCO_3$ (2 x 10 mL) and again H_2O (10 mL). Then it was dried over $MgSO_4$ and concentrated. Precipitation with MeOH and silica gel column chromatography (solvent F) of the filtrate afforded **6c** as a white solid (0.17 g, 83%): results of all analyses are in agreement with previously reported for the reaction with chloride [1].

Diosgenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranoside (**6d**)

*From **5d***: A mixture of **5d** (0.16 g, 0.22 mmol), diosgenin (0.066 g, 0.16 mmol), and 4Å molecular sieves (0.5 g) in anhyd CH_2Cl_2 (10 mL) was stirred at room temperature under N_2, for 30 min. Then TMSOTf (10 μL) was added and stirring was continued. After 20 h, the mixture was neutralized by Et_3N, diluted with $CHCl_3$ (50 mL), filtered over the gel layer (MN Kieselgel 60) and washed with H_2O (2 x 10 mL), satd aq $NaHCO_3$ (2 x 10 mL) and again H_2O (10 mL). Then it was dried over $MgSO_4$ and concentrated. Addition of MeOH caused the

precipitation of **6d** as a white solid (0.08 g, 52%): results of all analyses are in agreement with previously reported for the reaction with bromide [4].

General procedure for *N*-alkylation of **7**

The appropriate amount of diosgenyl 2-amino-2-deoxy-β-D-glucopyranoside (**7**) [1] was dissolved in $CHCl_3$/MeOH (1:1, v/v) and 1.2-fold excess of suitable aldehyde was added. The mixture was stirred for 0.5 h at rt. Then 2-fold excess of $NaBH_3CN$ was added and stirring was continued for 1-3 hours. The end of reaction was detected by TLC (solvents G-J). Then the mixtures were evaporated to dryness. The residue was chromatographed on silica gel.

Diosgenyl 2-deoxy-2-dimethylamino-β-D-glucopyranoside (**8**)

Reaction of **7** (0.1 g, 0.17 mmol) with formaldehyde (5.8 µl, 0.2 mmol) and $NaBH_3CN$ (21.4 mg, 0.34 mmol) in $CHCl_3$/MeOH (8 ml) followed by column chromatography (solvent G) gave **8** (49 mg, 78%): mp 235 ^oC; $[\alpha]_D^{20}$ -56^o (*c* 0.5, $CHCl_3$/MeOH, 1:1); R_f 0,46 (solvent G); ^1H NMR (400 MHz): δ 4.70 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 3.83 (dd, 1 H, $J_{5,6'}$ 2.9, $J_{6,6'}$ 12.0 Hz, H-6'), 3.70 (dd, 1 H, $J_{5,6}$ 5.4, $J_{6,6'}$ 12.0 Hz, H-6), 3.46 (dd, 1 H, $J_{2,3}$ 8.8, $J_{3,4}$ 10.2 Hz, H-3), 3.37 (dd, $J_{3,4}$ 10.2, $J_{4,5}$ 9.3 Hz, H-4), 3.33 (m, 1 H, H-5), 2.52 (s, 6 H, CH_3), 2.38 (dd, 1 H, $J_{1,2}$ 8.3, $J_{2,3}$ 8.8 Hz, H-2); diosgenyl protons: 5.32 (d, C_6-H), 4.40 (dd, C_{16}-H), 3.61 (m, C_{26}-H_e), 3.32 (t, C_{26}-H_a), 2.31 and 2.00 (m, C_4-2H), 1.20 (s, $CH_{3(19)}$), 0.98 (d, $CH_{3(21)}$), 0.80 (s, $CH_{3(18)}$), 0.79 (d, $CH_{3(27)}$); ^{13}C NMR (100 MHz): δ 98.86 (C-1), 78.28 (C-3), 77.23 (C-5), 75.76 (C-4), 66.51 (C-6), 61.80 (C-2), 49.90 (CH_3); diosgenyl carbons: 140.07 (C-5), 121.42 (C-6), 109.28 (C-22), 80.71 (C-16), 77.55 (C-3), 71.00 (C-26), 61.49 (C-17), 56.21 (C-14), 40.95 (C-20), 39.95 (C-13), 39.40 (C-12), 38.96 (C-4), 36.86 (C-1), 36.52 (C-10), 20.48 (C-11), 18.77 (C-19), 16.38 (C-27), 15.69 (C-18), 13.75 (C-21); MALDI-TOF-MS: *m/z* 627.00 $(M+ Na)^+$.

Diosgenyl 2-deoxy-2-ethylamino- (**9**) and -2-diethylamino-β-D-glucopyranoside (**10**)

Reaction of **7** (60 mg, 0.1 mmol) with acetaldehyde (9.2 µl, 0.12 mmol) and $NaBH_3CN$ (12.6 mg, 0.2 mmol) in $CHCl_3$/MeOH (5 ml) followed by column chromatography (solvent H) gave two products. The first was **9** (23.8 mg, 38%): mp 194 °C; $[\alpha]^D_{20}$ -43° (*c* 0.5, $CHCl_3$/MeOH); R_f 0,47 (solvent G); ^{1}H NMR (400 MHz): δ 4.46 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 3.83 (dd, 1 H, $J_{5,6'}$ 2.9, $J_{6,6'}$ 12.2 Hz, H-6'), 3.71 (dd, 1 H, $J_{5,6}$ 4.9, $J_{6,6'}$ 12.2 Hz, H-6), 3.38-3.28 (m, 2 H, H-3, H-4), 3.25 (m, H-5), 3.30 (m, 1 H, NCH_a), 2.80 (m, 1 H, NCH_b), 2.45 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 8,8 Hz, H-2), 1.14 (t, 3 H, CH_3); diosgenyl protons: 5.36 (d, C_6-H), 4.40 (dd, C_{16}-H), 3.58 (m, C_{26}-H_e), 3.45 (m, C_{26}-H_a), 2.40 and 2.24 (m, C_4-2H), 1,15 (s, $CH_{3(19)}$), 0.96 (d, $CH_{3(21)}$), 0.80 (s, $CH_{3(18)}$), 0.79 (d, $CH_{3(27)}$); ^{13}C NMR (100 MHz): δ 98.41 (C-1), 79.21 (C-5), 76.71 (C-3), 72.81 (C-4), 61.28 (C-6), 50.33 (C-2), 42.56 (NCH_2), 11.76 (CH_3); diosgenyl carbons: 140.24 (C-5), 122.09 (C-6), 109.67 (C-22), 81.11 (C-16), 72.81 (C-3), 70.84 (C-26), 61.96 (C-17), 56.62 (C-14), 41.77 (C-20), 40.36 (C-13), 39.79 (C-12), 38.62 (C-4), 37.24 (C-1), 36.88 (C-10), 20.90 (C-11), 19.08 (C-19), 16.72 (C-27), 16.03 (C-18), 14.09 (C-21); MALDI-TOF-MS: *m/z* 627.00 $(M+ Na)^+$.

The second was **10** (19.4 mg, 49%): mp 196 °C; $[\alpha]_D^{20}$ -39° (*c* 0.5, $CHCl_3$/MeOH); R_f 0,85 (solvent G); ^{1}H NMR (400 MHz): δ 4.72 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 3.83 (dd, 1 H, $J_{5,6'}$ 2.9, $J_{6,6'}$ 11.7 Hz, H-6'), 3.72 (dd, 1 H, $J_{5,6}$ 4.9, $J_{6,6'}$ 11.7 Hz, H-6), 3.44 (m, 1 H, H-4), 3.34 (m, 1 H, H-3), 3.26 (m, 1 H, H-5), 2.84 (m, 4 H, 2 x NCH_a, 2 x NCH_b), 2.62 (dd, 1 H, $J_{1,2}$ 8.4, $J_{2,3}$ 9,6 Hz, H-2), 1.10 (t, 6 H, 2 x CH_3); diosgenyl protons: 5.38 (d, C_6-H), 4.40 (dd, C_{16}-H), 3.58 (m, C_{26}-H_e), 2.38 and 2.20 (2 x m, C_4-2H), 0.98 (d, $CH_{3(21)}$), 0.80 (s, $CH_{3\ (18)}$), 0.79 (d, $CH_{3\ (27)}$); ^{13}C NMR (100 MHz): δ 99.47 (C-1), 78.46 (C-3), 77.23 (C-5), 75.87 (C-4), 66.51 (C-2), 64.62 (C-6), 49.89 (NCH_2), 14.31 (CH_3); diosgenyl carbons: 140.09 (C-5), 121.39 (C-6), 109.28 (C-22), 80.71 (C-16), 78.46 (C-3), 71.32 (C-26), 61.51 (C-17), 56.21 (C-14), 41.33 (C-20), 39.95 (C-13), 39.39 (C-12), 38.91 (C-4), 36.85 (C-1), 36.51 (C-10), 20.48 (C-11), 18.79 (C-19), 16.39 (C-27), 15.70 (C-18), 13.75 (C-21), MALDI-TOF-MS: *m/z* 655.00

$(M+Na)^+$.

Diosgenyl 2-deoxy-2-propylamino- (**11**) and -2-dipropylamino-β-D-glucopyranoside (**12**)

Reaction of **7** (200 mg, 0.35 mmol) with propionaldehyde (30 µl, 0.42 mmol) and $NaBH_3CN$ (43.8 mg, 0.69 mmol) in $CHCl_3$/MeOH (15 ml) followed by column chromatography (solvent I) gave two products. The first was **11** (103 mg, 48%): mp 180 °C; $[\alpha]^D_{20}$ -47° (*c* 0,5 $CHCl_3$/MeOH); R_f 0,34 (solvent I); ^{1}H NMR (400 MHz): δ 4.44 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 3.85 (dd, 1 H, $J_{5,6'}$ 3.2, $J_{6,6'}$ 11.8 Hz, H-6'), 3.75 (dd, 1 H, $J_{5,6}$ 5.0, $J_{6,6'}$ 11.8 Hz, H-6), 3.47 (t, 1 H, $J_{3,4}$ 9.2, $J_{4,5}$ 9.6 Hz, H-4), 3.35 (t, 1 H, $J_{2,3}$ 9.8, $J_{3,4}$ 9.2 Hz, H-3), 3.28 (m, 1 H, H-5), 2.91 (m, 1 H, NCH_a), 2.70 (m, 1 H, NCH_b), 2.42 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.6 Hz, H-2), 1.54 (m, 2 H, CH_2), 0.96 (t, 3 H, CH_3); diosgenyl protons: 5.38 (d, C_6-H), 4.40 (dd, C_{16}-H), 3.59 (m, C_{26}-H_e), 2.31 and 2.00 (m, C_4-2H), 1.05 (s, $CH_{3(19)}$), 0.81 (s, $CH_{3(18)}$), 0.78 (d, $CH_{3(27)}$); ^{13}C NMR (100 MHz): δ 101.87 (C-1), 78.84 (C-5), 75.84 (C-3), 74.94, (C-4), 62.92 (C-6), 61.82 (C-2), 49.88 (NCH_2), 22.64 (CH_2), 10.93 (CH_3); diosgenyl carbons: 140.12 (C-5), 121.38 (C-6), 109.27 (C-22), 80.71 (C-16), 70.56 (C-26), 61.51 (C-17), 56.21 (C-14), 41.34 (C-20), 39.95 (C-13), 39.41 (C-12), 38.52 (C-4), 36.90 (C-1), 36.51 (C-10), 20.49 (C-11), 18.79 (C-19), 16.41 (C-27), 15.71 (C-18), 13.77 (C-21), MALDI-TOF-MS: *m/z* 619.00 $(M+ H)^+$.

The second was **12** (58 mg, 45%): mp 171 °C; $[\alpha]^D_{20}$ -40° (c 0.5, $CHCl_3$/MeOH); R_f 0,60 (solvent I); ^{1}H NMR (400 MHz): δ 4.38 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 3.68 (t, 1 H, $J_{3,4}$ 9.2, $J_{4,5}$ 9.6 Hz, H-4), 3.46 (dd, 1 H, $J_{5,6}$ 4.8, $J_{6,6'}$ 12.4 Hz, H-6), 3.50-3.35 (m, 3 H, H-3, H-5 and H-6'), 2.50 (dd, 1 H, $J_{1,2}$ 8.4 $J_{2,3}$ 10.4 Hz, H-2), 2.32 (m, 2 H, 2 x NCH_a), 2.18 (m, 2 H, 2 x NCH_b), 1.60 (m, 4 H, 2 x CH_2), 1.08 (t, 6 H, 2 x CH_3); diosgenyl protons: 5.37 (d, C_6-H), 4.40 (dd, C_{16}-H), 3.61 (m, C_{26}-H_e), 3.32 (t, C_{26}-H_a), 2.00 (m, C_4-2H), 0.97 (d, $CH_{3(21)}$), 0.80 (s, $CH_{3(18)}$), 0.79 (d, $CH_{3(27)}$); ^{13}C NMR (100 MHz): δ 103.32 (C-1), 79.42, 75.84, 74.99, (C-3, C-4, C-5),

62.33 (C-6), 61.51 (C-2), 50.29 (NCH_2), 32.07, 22.68 (CH_2), 11.92 (CH_3); diosgenyl carbons: 140.38 (C-5), 121.49 (C-6), 109.52 (C-22), 81.02 (C-16), 70.26 (C-26), 61.51 (C-17), 56.70 (C-14), 41.83 (C-20), 39.96 (C-13), 39.52 (C-12), 39.12 (C-4), 37.39 (C-1), 37.06 (C-10), 21.06 (C-11), 19.61 (C-19), 17.36 (C-27), 16.52 (C-18), 14.76 (C-21); MALDI-TOF-MS: *m/z* 661.00 $(M+ H)^+$.

Diosgenyl 2-deoxy-2-dibutylamino-β-D-glucopyranoside (**13**)

Reaction of **7** (170 mg, 0.3 mmol) with butyraldehyde (32.4 µl, 0.36 mmol) and $NaBH_3CN$ (37.7 mg, 0.58 mmol) in $CHCl_3$/MeOH (10 ml) followed by column chromatography (solvent J) yielded **13** (51 mg, 42%): R_f 0,61 (solvent J); ^{1}H NMR (400 MHz): δ 4.42 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 3.82 (dd, 1 H, $J_{5,6'}$ 2.9, $J_{6,6'}$ 11.6 Hz, H-6'), 3.76 (dd, 1 H, $J_{5,6}$ 4.8, $J_{6,6'}$ 11.6 Hz, H-6), 3.51 (m, 1 H, H-4), 3.42 (m, 1 H, H-3), 3.30 (m, 1 H, H-5), 2.42 (m, 4 H, NCH_a, NCH_b), 2.40 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.6 Hz, H-2), 1.60 (m, 4 H, 2 x CH_2), 1.35 (m, 4 H, 2 x CH_2), 0.92 (t, 6 H, 2 x CH_3); diosgenyl protons: 5.36 (d, C_6-H), 4.40 (dd, C_{16}-H), 3.40 (m, C_{26}-H_e), 2.32 and 2.00 (m, C_4-2H), 1.02 (s, $CH_{3(19)}$), 0.98 (d, $CH_{3(21)}$), 0.80 (s, $CH_{3(18)}$), 0.78 (d, $CH_{3(27)}$); MALDI-TOF-MS: *m/z* 690.00 $(M+ H)^+$.

Diosgenyl 2-deoxy-2-diisobutylamino-β-D-glucopyranoside (**14**)

Reaction of **7** (170 mg, 0.3 mmol) with isovaleric aldehyde (38.76 µl, 0.36 mmol) and $NaBH_3CN$ (37.7 mg, 0.58 mmol) in $CHCl_3$/MeOH (7ml) followed by column chromatography (solvent I) yielded **14** (48.6 mg, 38%): R_f 0,56 (solvent I); ^{1}H NMR (400 MHz): δ 4.50 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 3.86 (dd, 1 H, $J_{5,6'}$ 2.9, $J_{6,6'}$ 11.8 Hz, H-6'), 3.80 (dd, 1 H, $J_{5,6}$ 4.9, $J_{6,6'}$ 11.8 Hz, H-6), 3.56 (m, 1 H, H-4), 3.48 (m, 1 H, H-3), 3.36 (m, 1 H, H-5), 2.92 (m, 4 H, 2 x NCH_a, 2 x NCH_b), 2.46 (m, 1 H, H-2), 1.79 (m, 4 H, 2 x CH_2), 1.30 (m, 2 H, 2 x CH), 0.96 (d, 12 H, 4 x CH_3); diosgenyl protons: 5.32 (m, C_6-H), 4.30 (dd, C_{16}-H), 3.59 (m, C_{26}-H_e), 3.32 (m, C_{26}-H_a), 2.30 and 2.00 (m, C_4-2H), 1.20 (s, $CH_{3\,(19)}$), 0.98 (d, $CH_{3\,(21)}$), 0.80

(s,$CH_{3\,(18)}$), 0.76 (d, $CH_{3\,(27)}$); MALDI-TOF-MS: *m/z* 717.00 $(M+H)^+$.

Diosgenyl 2-deoxy-2-pentylamino-β-D-glucopyranoside (**15**)

Reaction of **7** (170 mg, 0.3 mmol) with valeric aldehyde (38.3 µl, 0.36 mmol) and $NaBH_3CN$ (37.7 mg, 0.58 mmol) in $CHCl_3$/MeOH (10ml) followed by column chromatography (solvent J) gave **15** (40 mg, 21%): R_f 0,56 (solvent J); IR: *v* 3373-3473 (O-H and N-H), 2847- 2932 (C-H), 1593 cm^{-1} (N-H); ^{1}H NMR (400 MHz): δ 4.55 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 3.83 (dd, 1 H, $J_{5,6'}$ 2.8, $J_{6,6'}$ 12.0 Hz, H-6'), 3.70 (dd, 1 H, $J_{5,6}$ 4.8, $J_{6,6'}$ 12.0 Hz, H-6), 3.45 (m, 1 H, H-4), 3.36 (m, 1 H, H-3), 3.24 (ddd, 1 H, $J_{5,6'}$ 2.8, $J_{5,6}$ 4.8, $J_{4,5}$ 9.6 Hz, H-5), 3.50 (m, 1 H, H-2), 2.20 (m, 2 H, NCH_a, NCH_b), 1.59 (m, 2 H, CH_2), 1.20 (m, 4 H, 2 x CH_2), 1.00 (t, 3 H, CH_3); diosgenyl protons: 5.32 (m, C_6-H), 4.30 (dd, C_{16}-H), 3.59 (m, C_{26}-H_e), 3.32 (m, C_{26}-H_a), 2.30 and 2.00 (m, C_4-2H), 1.20 (s, $CH_{3(19)}$), 0.98 (d, $CH_{3(21)}$), 0.80 (s,$CH_{3(18)}$), 0.76 (d, $CH_{3(27)}$); MALDI-TOF-MS: *m/z* 647.00 $(M+H)^+$.

Diosgenyl 2-deoxy-2-hexylamino- (**16**) and -2-dihexylamino-β-D-glucopyranoside (**17**)

Reaction of **7** (100 mg, 0.17 mmol) with hexanoic aldehyde (25.5 µl, 0.21 mmol) and $NaBH_3CN$ (21.4 mg, 0.33 mmol) in $CHCl_3$/MeOH (8 ml) followed by column chromatography (solvent I) yielded two compounds. The first was **16** (60 mg, 52%): mp 175 ^{o}C, R_f 0,40 (solvent I); ^{1}H NMR (400 MHz): δ 4.69 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 3.89 (dd, 1 H, $J_{5,6'}$ 2.4, $J_{6,6'}$ 11.6 Hz, H-6'), 3.76 (dd, 1 H, $J_{5,6}$ 5.2, $J_{6,6'}$ 11.6 Hz, H-6), 3.59- 3.41 (m, 2 H, H-4, H-5), 3.38 (dd, 1 H, $J_{2,3}$ 10.4, $J_{3,4}$ 9.2 Hz, H-3), 2.65 (m, 2 H, NCH_a, NCH_b), 2.51 (dd, 1 H, $J_{1,2}$ 8.4, $J_{2,3}$ 10.4 Hz, H-2), 1.60 (m, 2 H, CH_2), 1.30 (m, 6 H, 3 x CH_2), 0.88 (t, 3 H, CH_3); diosgenyl protons: 5.36 (d, C_6-H), 4.40 (dd, C_{16}-H), 3.64 (m, C_{26}-H_e), 2.32 and 2.00 (m, C_4-2H), 1.20 (s, $CH_{3(19)}$), 0.97 (d, $CH_{3(21)}$), 0.79 (s,$CH_{3(18)}$), 0.78 (d, $CH_{3(27)}$); MALDI-TOF-MS: *m/z* 659.94 $(M)^+$, 661.00 $(M+H)^+$.

The second was **17** (16,9 mg, 22%): mp 180 ^{o}C, R_f 0,53 (solvent I); ^{1}H NMR (400 MHz): δ 4.50 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 3.86 (dd, 1 H, $J_{5,6'}$ 3.0, $J_{6,6'}$ 11.6 Hz, H-6'), 3.80 (dd, 1 H, $J_{5,6}$ 4.4, $J_{6,6'}$ 11.6 Hz, H-6), 3.56 (m, 1 H, H-4), 3.36 (m, 1 H, H-5), 3.48 (dd, 1 H, $J_{2,3}$ 9.6, $J_{3,4}$ 10.8 Hz, H-3), 2.92 (m, 4 H, 2 x NCH_a, 2 x NCH_b), 2.46 (dd, 1 H, $J_{1,2}$ 8.4, $J_{2,3}$ 9.6 Hz, H-2), 1.48 (m, 4 H, 2 x CH_2), 1.30 (m, 12 H, 6 x CH_2), 0.89 (t, 6 H, 2 x CH_3); diosgenyl protons: 5.36 (d, C_6-H), 4.40 (dd, C_{16}-H), 3.58 (m, C_{26}-H_e), 3.38 (m, C_{26}-H_a), 2.28 and 2.00 (m, C_4-2H), 1.02 (s, $CH_{3\ (19)}$), 0.98 (d, $CH_{3\ (21)}$), 0.79 (s,$CH_{3(18)}$), 0.78 (d, $CH_{3(27)}$); MALDI-TOFMS: *m/z* 744.0 $(M)^+$, 745.00 $(M+ H)^+$.

Determination of minimum inhibitory concentration

The following microbial strains: *Aspergillus niger* ATCC 16404, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231, *Candida tropicalis* PCM 2681, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13882, *Proteus mirabilis* PCM 543, *Proteus vulgaris* ATCC 13315, *Pseudomonas aeruginosa* ATCC 9027, *Rhodococcus equi* ATCC 6939, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* PCM 2118 were purchased from the Polish Academy of Sciences (Wroclaw, Poland). Reference strains of bacteria were inoculated in Mueller-Hinton II broth (MHB II) (*Sigma-Aldrich, Germany*) 24 h before performing the test and incubated at 37 °C with 150 rpm shaking. Fungal isolates were inoculated in *Sabouraud* 2% glucose broth (*Carl Roth GmbH, Germany*) and cultured for 48 h at 25 °C with 150 rpm shaking before the minimum inhibitory concentration was tested.

Minimum inhibitory concentration (MIC) was determined using a serial dilution method according to the guidelines of Clinical and Laboratory Standards Institute (CLSI). Mueller MHB II and initial inoculums of 5×10^5 CFU/ml were used for tested bacterial strains. For the tested fungi, *Sabouraud* glucose 2% broth and initial inoculums 10^3 CFU/ml were applied.

The microbes were inoculated to polystyrene 96-well plates *(Becton Dickinson)* and exposed to graded concentrations of saponins (range 0.5 – 1024 mg/L). The compounds were dissolved in 10% DMSO in a phosphate buffer. The tests containing bacteria were incubated for 18 h at 37˚C, while the ones with fungi for 48h at 25˚C. MIC was taken as the lowest concentration of the compound at which a noticeable growth was inhibited. The experiments were performed in triplicate on three different days.

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