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Studies on carbohydrates. Part 33: Synthesis of spacer-armed 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-α-D-mannosides and their dimers

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Abstract

The mixture of 3,6-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-azido-2-deoxy-D-glucopyranosyl 1-acetates or 1-trichloroacetates and the corresponding mannose type glycosyl donors reacted with the spacer arms di- and triethylene glycol, in dichloromethane solution with $BF_3 \cdot OEt_2$ and TMSOTf as promotors at room temperature to give highly selective products. Only the mannose type products were obtained.

1. Introduction

Laminin, an important glycoprotein on the basement membrane, could inhibit the attachment, migration, invasiveness, and proliferation of cancer cells in vitro, and could prevent lung and liver metastasis of tumors in vivo.[1,2] The repeating unit of laminin carbohydrate moiety, *N*-acetyllactosamine, might play an important role in the prevention of tumor metastasis.[3] In our attempts to prepare the divalent lactosamine with a spacer arm of diethylene glycol or triethylene glycol as potential tumor metastasis inhibitors, lactosamine or its precursors needed to be prepared first.

The most common method for the synthesis of 2-amino-2-deoxy sugars is that an azide group is introduced to the C-2 of the acetyl glycal **1**[4–6] by addition with sodium azide and cerium nitrate, after which the azide group can be readily reduced to amine. Usually in the procedure of azide-addition of the lactal there could be three possible products **2**, **3** and **4**, namely, glucose type and mannose type, respectively, according to the different azide-addition directions to the C-2 of lactal **1**. Arnap et al.[6]

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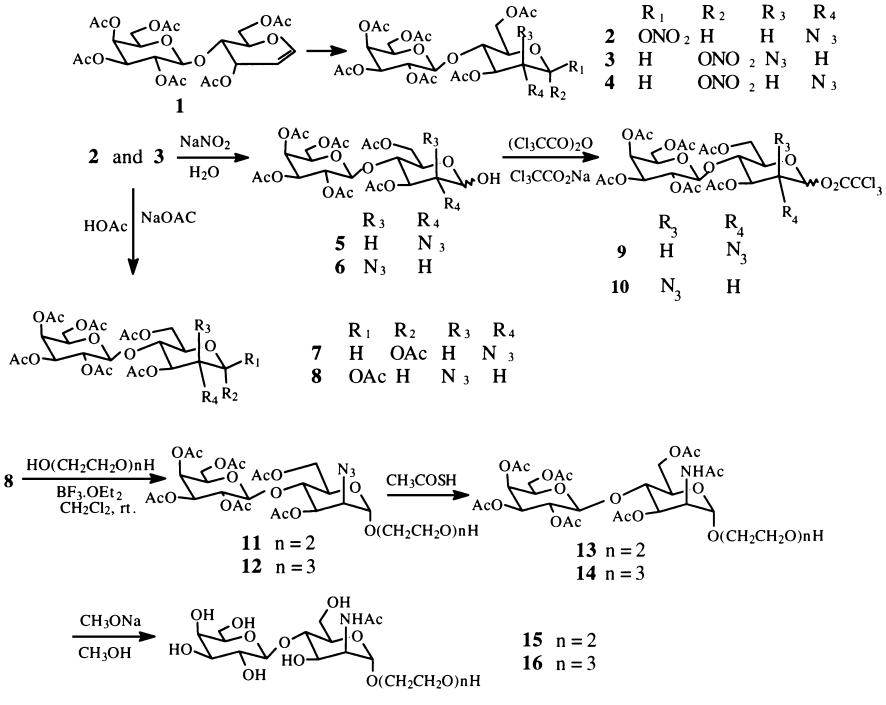
reported that compounds **2** and **3** were obtained in the ratio of 8:1 and were impossible to separate by chromatography. In our experiment, however, compounds **2** and **3** were obtained in almost equivalent amounts (~4:3, determined from NMR spectra). Furthermore, the subsequent glycosylation of the spacer arms rendering acetoxy or trichloroacetoxy groups as leaving group (**7** and **8** or **9** and **10**) gave an interesting result which will be reported as follows.

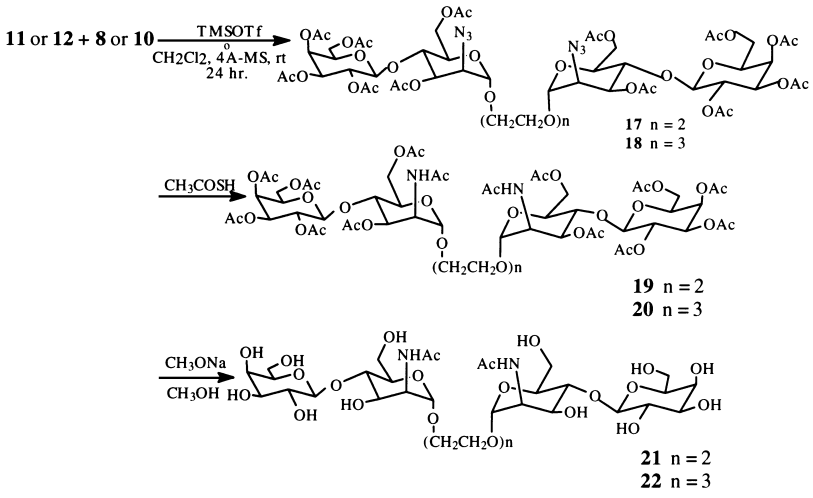
2. Results and discussion

Hexa-*O*-acetyl-D-lactal **1**[7] was treated with cerium(IV) ammonium nitrate and sodium azide[6] to give the azidonitrates **2**, **3** and **4** (Scheme 1). The mixture of **2** and **3** (~4:3 according to ^{1}H NMR) was separated from **4** by chromatography and then treated with anhydrous sodium acetate in glacial acetic acid at 100°C for 1 h to give glycosyl donors **7** and **8**[4] (~1.2:1 according to ^{1}H NMR). When the mixture of **7** and **8** was reacted with the spacer arms in the dichloromethane solution at room temperature with $BF_3 \cdot OEt_2$ as the promoter only mannose type glycosyl donor **8** converted to spacer-armed derivatives **11** or **12** in overall yields of 78 and 73%, respectively, exclusively as α-anomers. The physical data of the subsequently obtained spacer-armed **11** and **12** confirmed the unique mannose type products: the coupling constants $^3J_{H1,2}$<1.8 Hz, $^1J_{C-H}$>170 Hz.[8] The unreacted glucose type donors **7** could be recovered from the reaction mixture. No reaction occurred when the recovered pure **7** was treated with the same procedure as described above. The mixture of **7** and **8** reacted with **11** or **12** to afford mannose type symmetric dimers **17** or **18** (rt CH_2Cl_2, TMSOTf as promoter, 24–48 h) in 79 or 77% yield, respectively (Scheme 2).



Scheme 1.

The trichloroacetoxy group, a new leaving group, has been used successfully in our laboratory to prepare many glycosides and oligosaccharides.[9–13] We tried again to use the glycosyl trichloroacetate



Scheme 2.

donors in the glycosylation of **11** or **12** for the preparation of **17** or **18**. The mixture of **2** and **3** was first treated with $NaNO_2$ and H_2O in dioxane at 80°C for 10 h to give a mixture of **5** and **6**[14] in the yield of 90% which was then treated with trichloroacetic anhydride and anhydrous CCl_3COONa in CH_2Cl_2 at 40°C for 30 min to afford **9** and **10**[10] in the yield of 97%. When the mixture of 1-trichloroacetate **9** and **10** was reacted with **11** or **12** (rt CH_2Cl_2, TMSOTf as promoter, 5–10 h) the same results were achieved, namely mannose-type products **17** or **18** were obtained selectively in good yield (>75%) but the reactivity of **10** was higher than **8** (because the reactions were faster than when using **8** as donor). This fact showed that the trichloroacetoxy group was a good leaving group in the synthesis of mannose type dimers **17** or **18** and mannose type 1-trichloroacetate **10** was more active than its glucose type isomer **9**. This work is currently underway and further results will be reported in due course.

The above experimental results indicated that the reaction activities of the glucose type glycosyl donors and the corresponding mannose ones are obviously different, which could lead to a method for the separation of the two isomeric disaccharides from the azidonitration of the lactal.

Reduction of the azido group in **11**, **12**, **17** and **18** involved reductive *N*-acetylation of the azido group using thioacetic acid.[15] Treatment of **11**, **12**, **17** and **18** with thioacetic acid for 30 h at room temperature gave the corresponding 2-acetamido-2-deoxy derivatives **13**, **14**, **19** and **20** in the yield of 80, 72, 67, 58%, respectively, which were then deprotected to give the target compounds **15**, **16**, **21** and **22**.

Compounds **15**, **16**, **21** and **22** and their glucose type analogs will be used in the studies on anti-metastasis and structure–activity relationships.

3. Experimental

3.1. General methods

Optical rotation was recorded using an Optical Activity AA-10R type polarimeter. NMR spectra were recorded with a Bruker ARX-400 type spectrometer, $CDCl_3$, CD_3OD and D_2O as solvents. Purity of products was assured by TLC on Silica Gel GF_{254}. Column chromatography was performed on Silica Gel H_{60}. Elemental analyses were performed on Perkin–Elmer 240C instrument.

3.2. *Azidonitration of hexa-O-acetyl-D-lactal* **1**

Compound **1** (20 g, 35.7 mmol) in acetonitrile (170 ml) was added to a mixture of sodium azide (3.25 g) and cerium(IV) ammonium nitrate (45 g). The mixture was stirred for 16 h at −15°C under nitrogen. Diethyl ether (400 ml) and water (250 ml) were added and the mixture was shaken. The organic phase was washed with water and concentrated to dryness. The residue was purified on silica gel with petroleum ether (60–90°C):ethyl acetate (3:2) to give a mixture (11.4 g, 48%) of **2** and **3** (~4:3 according to ^{1}H NMR; 5.58 ppm, $J_{1,2}$=8.5 Hz, 6.15 ppm, $J_{1,2}$=3.5 Hz, attributed to the anomeric protons of **2** and **3**, respectively) and **4** (3.3 g, 14%).

3.3. *1,3,6-Tri-O-acetyl-2-azido-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-glucopyranose* **7** *and 1,3,6-tri-O-acetyl-2-azido-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-D-mannopyranose* **8**

A mixture of **2** and **3** (10 g) and anhydrous NaOAc (2.4 g) in glacial acetic acid (40 ml) was heated to 100°C for 1 h. The reaction mixture was diluted with CH_2Cl_2 (250 ml) and the mixture was washed with water and saturated aqueous $NaHCO_3$, then dried and concentrated and the residue was purified by column of silica gel (petroleum ether (60–90°C):ethyl acetate, 3:2) to give a mixture (8 g, 80%) of **7** and **8** (~1.2:1 according to ^{1}H NMR; 6.25 ppm, $J_{1,2}$=3.64 Hz, 6.06 ppm, $J_{1,2}$=2.28 Hz, attributed to the anomeric protons of **7** and **8**, respectively).

3.4. *3,6-Di-O-acetyl-2-azido-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-D-glucopyranose* **5** *and 3,6-di-O-acetyl-2-azido-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-D-mannopyranose* **6**

A mixture of **2** and **3** (3 g) was dissolved in dioxane (50 ml), then water (15 ml) and $NaNO_2$ (4 g) were added, and the mixture was heated for 10 h at 80°C with stirring, then concentrated and 50 ml $CHCl_3$ was added. The organic layer was washed with water, dried and concentrated. The crude product was purified by chromatography (petroleum ether (60–90°C):ethyl acetate, 1:1) to give the mixture (2.4 g) of **5** and **6** (~2:1 according to ^{1}H NMR) as a colorless syrup in the yield of 85%.

3.5. *3,6-Di-O-acetyl-2-azido-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-D-glucopyranose 1-trichloroacetate* **9** *and 3,6-di-O-acetyl-2-azido-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-D-mannopyranose 1-trichloroacetate* **10**

The mixture of **5** and **6** (300 mg) was dissolved in anhydrous CH_2Cl_2 (6 ml), then trichloroacetic anhydride (1 ml) and anhydrous CCl_3COONa (70 mg) were added. The solution was boiled under reflux for 1 h, cooled and the sodium trichloroacetate was filtered off. The filtrate was washed with water and aqueous $NaHCO_3$, dried and concentrated to give a syrup of **9** and **10** (360 mg, 97%).

3.6. *5-Hydroxy-3-oxa-pentyl 3,6-di-O-acetyl-2-azido-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-mannopyranoside* **11**

$BF_3 \cdot OEt_2$ was added to a solution of **7** and **8** (~1.2:1, 3 g, 4.5 mmol) and diethylene glycol (1 ml) in dry CH_2Cl_2 (10 ml), the mixture was stirred at room temperature for 20 h, then diluted with CH_2Cl_2 (10 ml) and washed with water and saturated aqueous $NaHCO_3$, dried over anhydrous

Na_2SO_4 and evaporated under reduced pressure. The resulting oily brown residue was purified by flash chromatography with petroleum ether (60–90°C):ethyl acetate (2:5) as eluent, 1.14 g pure product **11** was obtained as a colorless syrup in the yield of 78%. $[\alpha]_D$ +55.0 (*c* 1.31, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 5.41 (dd, 1H, H-3), 5.37 (dd, 1H, H-4′), 5.13 (dd, 1H, H-2′), 4.97 (dd, 1H, H-3′), 4.85 (d, 1H, $J_{1,2}$=1.5 Hz, H-1), 4.53 (d, 1H, $J_{1',2'}$=7.9 Hz, H-1′), 4.44–4.07 (m, 4H, H-6a,6b, H-6a′,6b′), 4.03 (dd, 1H, H-2), 3.97–3.92 (m, 3H, H-4,5, H-5′), 3.79–3.59 (m, 8H, -CH_2O-), 2.17–1.98 (6s, 18H, 6×OAc); ^{13}C NMR ($CDCl_3$) δ: 170.61–169.39 (6C, C=O), 101.40 (C-1′), 97.82 (C-1), 74.22 (C-4), 72.59 (-CH_2O-), 71.13 (C-3), 71.02 (C-3′), 70.66 (C-5), 69.89 (-CH_2O-), 69.16 (C-5′), 69.13 (C-2′), 67.18 (-CH_2O-), 66.77 (C-4′), 62.30 (C-6), 61.77 (-CH_2O-), 61.48 (C-2), 61.09 (C-6′), 20.89–20.53 (6C, 6×*C*H_3CO-). Anal. calcd for $C_{28}H_{41}N_3O_{18}$: C, 47.53; H, 5.84; N, 5.94. Found: C, 47.27; H, 5.67; N, 5.49.

*3.7. 8-Hydroxy-3,6-dioxa-octyl-3,6-diacetyl-2-azido-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galacto-pyranosyl)-α-*D*-mannopyranoside* ***12***

Compound **12** was prepared as described for the preparation of **11** and the crude product was purified by chromatography with petroleum ether (60–90°C):ethyl acetate (2:7) as eluent. A colorless syrup was obtained in 73% yield. $[\alpha]_D$ +41.4 (*c* 0.58, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 5.38 (dd, 1H, H-3), 5.34 (dd, 1H, H-4′), 5.11 (dd, 1H, H-2′), 4.95 (dd, 1H, H-3′), 4.84 (d, 1H, $J_{1,2}$=1.84 Hz, H-1), 4.51 (d, 1H, $J_{1',2'}$=7.92 Hz, H-1′), 4.41–4.04 (m, 4H, H-6a,6b, H-6a′,6b′), 4.02 (dd, 1H, H-2), 3.95–3.88 (m 3H, H-4,5, H-5′), 3.71–3.56 (m, 12H, -CH_2O-), 2.14–1.94 (6s, 18H, 6×OAc); ^{13}C NMR ($CDCl_3$) δ: 170.54–169.30 (6C, C=O), 101.31 (C-1′), 97.94 (C-1), 74.12 (C-4), 72.50 (-CH_2O-), 71.09 (C-3), 70.99 (C-3′), 70.69 (-CH_2O-), 70.59 (C-5), 70.26 (-CH_2O-), 69.93 (-CH_2O-), 69.11 (C-5′), 69.04 (C-2′), 67.13 (-CH_2O-), 66.71 (C-4′), 62.16 (C-6), 61.68 (-CH_2O-), 61.45 (C-2), 61.02 (C-6′), 20.81–20.45 (6C, 6×*C*H_3CO-). Anal. calcd for $C_{30}H_{45}N_3O_{19}$: C, 47.94; H, 6.03; N, 5.59. Found: C, 47.69; H, 6.28; N, 5.30.

*3.8. 3-Oxa-pentyl 1,1′-di-*O*-di-[3,6-di-*O*-acetyl-2-azido-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-β-*D*-galacto-pyranosyl)-α-*D*-mannoside] dimer* ***17***

Method 1: a mixture of **7** and **8** (~1.2:1, 1.54 g, 2.33 mmol) and **11** (0.707 g, 1 mmol) in dry CH_2Cl_2 (10 ml) in the presence of powered molecular sieve 4A was cooled to −20°C and TMSOTf (0.2 ml) was added dropwise with stirring. The mixture was kept for 1 h at −20–0°C and 24 h at room temperature, then filtered and the filtrate was washed with aqueous sodium bicarbonate and water, dried (Na_2SO_4) and concentrated. The residue was subjected to chromatography on silica gel (petroleum ether (60–90°C):ethyl acetate, 4:5) to give **17** as a colorless syrup (1.03 g, 79%). Method 2: the same as for method 1 but the mixture of **9** and **10** was used as the donor and the reaction time was 10 h. The yield of **17** was 78%. $[\alpha]_D$ +55.0 (*c* 1.31, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 5.41 (dd, 1H, H-3), 5.36 (dd, 1H, H-4′), 5.12 (dd, 1H, H-2′), 4.99 (dd, 1H, H-3′), 4.86 (d, 1H, $J_{1,2}$=1.64 Hz, H-1), 4.54 (d, 1H, $J_{1',2'}$=7.92 Hz, 1H, H-1′), 4.42–4.07 (m, 4H, H-6a,6b, H-6a′,6b′), 4.05 (dd, 1H, H-2), 3.99–3.89 (m, 3H, H-4,5, H-5′), 3.74–3.65 (m, 4H, -CH_2O-), 2.17–1.97 (6s, 18H, 6×OAc); ^{13}C NMR ($CDCl_3$) δ: 170.43–169.15 (6C, C=O), 101.29 (C-1′), 97.80 (C-1), 74.13 (C-4), 71.00 (C-3), 70.96 (C-3′), 70.56 (C-5′), 69.90 (-CH_2O-), 69.07 (C-2′), 69.06 (C-5), 67.00 (-CH_2O-), 66.72 (C-4′), 62.17 (C-6), 61.39 (C-2), 61.03 (C-6′), 20.75–20.40 (6C, 6×*C*H_3CO-). Anal. calcd for $C_{52}H_{72}N_6O_{33}$: C, 47.49; H, 5.56; N, 6.12. Found: C, 47.76; H, 5.62; N, 6.18.

3.9. 3,6-Dioxa-octyl 1,1′-di-O-di-[3,6-di-O-acetyl-2-azido-2-deoxy-4-O-(2,3,4,6-tetra-O-β-D-galacto-pyranosyl)-α-D-mannoside] dimer **18**

Compound **18** was prepared as described for the preparation of **17**. The crude product was purified by chromatography with petroleum ether (60–90°C):ethyl acetate (2:3) and a colorless syrup was obtained in the yield of 77% for method 1 and 75% for method 2. $[\alpha]_D$ +44.0 (*c* 1.00, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 5.39 (dd, 1H, H-3), 5.37 (dd, 1H, H-4′), 5.13 (dd, 1H, H-2′), 4.97 (dd, 1H, H-3′), 4.87 (d, 1H, $J_{1,2}$=1.80 Hz, H-1), 4.53 (d, 1H, $J_{1',2'}$=7.92 Hz, H-1′), 4.42–4.07 (m, 4H, H-6a,6b, H-6a′,6b′), 4.03 (dd, 1H, H-2), 3.97–3.91 (m, 3H, H-4,5, H-5′), 3.78–3.63 (m, 6H, -CH_2O-), 2.18–1.99 (6s, 18H, 6×OAc); ^{13}C NMR ($CDCl_3$) δ: 170.44–169.15 (6C, C=O), 101.29 (C-1′), 97.89 (C-1), 74.09 (C-4), 71.07 (C-3), 70.97 (C-3′), 70.84 (-CH_2O-), 70.57 (C-5′), 69.93 (-CH_2O-), 69.08 (C-2′), 68.98 (C-5), 67.19 (-CH_2O), 66.70 (C-4′), 62.16 (C-6), 61.21 (C-2), 61.00 (C-6′), 20.79–20.43 (6C, 6×*C*H_3CO-). Anal. calcd for $C_{54}H_{76}N_6O_{34}$: C, 47.93; H, 5.66; N, 6.21. Found: C, 47.83; H, 5.74; N, 5.82.

3.10. 5-Hydroxy-3-oxa-pentyl 3,6-di-O-acetyl-2-acetamido-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-mannopyranoside **13**

A solution of **11** (400 mg) in thioacetic acid (5 ml) was stirred at room temperature for 24 h, then concentrated. The residue was eluted from a column of silica gel with chloroform:methanol (50:1) to give **13** as a colorless syrup (327 mg, 80%). $[\alpha]_D$ +32.1 (*c* 1.12, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 5.99 (d, 1H, -NH-), 5.35 (dd, 1H, H-4′), 5.31 (dd, 1H, H-3), 5.13 (dd, 1H, H-2′), 4.99 (dd, 1H, H-3′), 4.80 (d, 1H, $J_{1,2}$=1.52 Hz, H-1), 4.61 (dd, 1H, H-2), 4.58 (d, 1H, $J_{1',2'}$=7.96 Hz, H-1′), 4.32–4.06 (m, 4H, H-6a,6b, H-6a′,6b′), 4.03–4.01 (m, 1H, H-5), 3.90 (m, 1H, H-5′), 3.77 (m, 1H, H-4), 3.74–3.59 (m, 8H, -CH_2O-), 2.16–1.98 (7s, 21H, 6×OAc, C*H*$_3$CONH-); ^{13}C NMR ($CDCl_3$) δ: 173.12–169.30 (7C, -$COCH_3$, -CONH-), 100.97 (C-1′), 98.80 (C-1), 74.49 (C-4), 72.75 (-CH_2O-), 70.86 (C-3′), 70.35 (C-3), 69.95 (-CH_2O-), 69.88 (C-5′), 69.17 (C-2′), 68.53 (C-5), 67.29 (-CH_2O-), 66.58 (C-4′), 62.68 (C-6), 61.59 (C-6′), 60.80 (-CH_2O-), 50.04 (C-2), 23.20–20.46 (7C, 6×OAc, *C*H_3CONH-). Anal. calcd for $C_{30}H_{45}NO_{19}$: C, 49.79; H, 6.27; N, 1.94. Found: C, 49.34; H, 6.14; N, 1.56.

3.11. 8-Hydroxy-3,6-dioxa-octyl 3,6-di-O-acetyl-2-acetamido-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-mannopyranoside **14**

Compound **14** was prepared as described for the preparation of **13**. The crude product was purified by chromatography with chloroform:methanol (40:1) and a colorless syrup was obtained in the yield of 72%. $[\alpha]_D$ +43.1 (*c* 1.02, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 5.93 (d, 1H, -NH-), 5.34 (d, 1H, H-4′), 5.31 (d, 1H, H-3), 5.14 (dd, 1H, H-2′), 4.98 (dd, 1H, H-3′), 4.83 (d, 1H, $J_{1,2}$=1.50 Hz, H-1), 4.62 (dd, 1H, H-2), 4.59 (d, 1H, $J_{1',2'}$=7.96 Hz, H-1′), 4.36–4.04 (m, 4H, H-6a,6b, H-6a′,6b′), 4.03–3.99 (m, 1H, H-5), 3.89 (m, 1H, H-5′), 3.76 (m, 1H, H-4), 3.74–3.59 (m, 12H, -CH_2O-), 2.16–1.98 (7s, 21H, CH_3CO-, C*H*$_3$CONH-); ^{13}C NMR ($CDCl_3$) δ: 170.49–169.24 (7C, -*C*OCH_3, -NHCO-), 100.92 (C-1′), 98.81 (C-1), 74.49 (C-4), 72.44 (-CH_2O-), 70.89 (C-3′), 70.65 (-CH_2O-), 70.38 (C-3), 70.23 (-CH_2O-), 70.10 (-CH_2O-), 70.08 (C-5′), 69.18 (C-2′), 68.45 (C-5), 67.04 (-CH_2O-), 66.57 (C-4′), 62.61 (C-6), 61.64 (-CH_2O-), 60.80 (C-6′), 49.97 (C-2), 23.21–20.45 (7C, *C*H_3CO-, *C*H_3CONH-). Anal. calcd for $C_{32}H_{49}NO_{20}$: C, 50.06; H, 6.43; N, 1.82. Found: C, 49.81; H, 6.51; N, 1.96.

3.12. 3-Oxa-pentyl 1,1′-di-O-di-[3,6-di-O-acetyl-2-acetamido-2-deoxy-4-O-(2,3,4,6-tetra-O-β-D-galactopyranosyl)-α-D-mannoside] dimer **19**

A solution of **17** (300 mg) in thioacetic acid (5 ml) was stirred for 36 h at room temperature, then concentrated. The residue was eluted from a column of silica gel with chloroform:methanol (40:1) to give **19** (206 mg, 67%) as a colorless syrup. $[\alpha]_D$ +43.6 (*c* 1.01, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 6.20 (d, 1H, -NH-), 5.35 (d, 1H, H-4′), 5.29 (dd, 1H, H-3), 5.14 (dd, 1H, H-2′), 4.99 (dd, 1H, H-3′), 4.79 (d, 1H, $J_{1,2}$=1.56 Hz, H-1), 4.60 (dd, 1H, H-2), 4.59 (d, 1H, $J_{1',2'}$=8.00 Hz, H-1′), 4.34–4.02 (m, 4H, H-6a,6b, H-6a′,6b′), 3.98 (m, 1H, H-5), 3.90 (m, 1H, H-5′), 3.76 (m, 1H, H-4), 3.74–3.63 (m, 4H, -CH_2O-), 2.18–1.98 (7s, 21H, 6OAc, -NHCOCH_3); ^{13}C NMR ($CDCl_3$) δ: 172.99–169.20 (7C, -*C*OCH_3, -NH*C*OCH_3), 101.01 (C-1′), 98.84 (C-1), 74.68 (C-4), 70.87 (C-3′), 70.33 (C-3), 70.32 (C-5), 70.03 (-CH_2O-), 69.13 (C-2′), 68.48 (C-5′), 67.17 (-CH_2O-), 66.59 (C-4′), 62.68 (C-6), 60.77 (C-6′), 49.79 (C-2), 29.18–20.45 (7C, *C*H_3CO-, *C*H_3CONH-). Anal. calcd for $C_{56}H_{80}N_2O_{35}$: C, 50.15; H, 6.01; N, 2.09. Found: C, 50.08; H, 6.15; N, 1.83.

3.13. 3,6-Dioxa-octyl 1,1′-di-O-di-[3,6-di-O-acetyl-2-acetamido-2-deoxy-4-O-(2,3,4,6-tetra-O-β-D-galactopyranosyl)-α-D-mannoside] dimer **20**

Compound **20** was prepared as described for the preparation of **19**. The crude product was purified by chromatography with chloroform:methanol (35:1) and a colorless syrup was obtained in the yield of 58%. $[\alpha]_D$ +36.9 (*c* 1.01, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 5.84 (d, 1H, -NH-), 5.34 (d, 1H, H-4′), 5.30 (dd, 1H, H-3), 5.13 (dd, 1H, H-2′), 4.98 (dd, 1H, H-3′), 4.84 (d, 1H, $J_{1,2}$=1.51 Hz, H-1), 4.59 (m, 1H, H-2), 4.58 (d, 1H, $J_{1',2'}$=7.72 Hz, H-1′), 4.34–4.04 (m, 4H, H-6a,6b, H-6a′,6b), 4.01 (m, 1H, H-5), 3.89 (m, 1H, H-5′), 3.76 (m, 1H, H-4), 3.71–3.64 (m, 6H, -CH_2O-), 2.18–1.98 (7s, 21H, 6×OAc, C*H*$_3$CONH-); ^{13}C NMR ($CDCl_3$) δ: 170.48–69.18 (7C, -*C*OCH_3, -NH*C*OCH_3), 100.95 (C-1′), 98.85 (C-1), 74.53 (C-4), 70.90 (C-3′), 70.61 (-CH_2O-), 70.36 (C-3), 70.22 (C-5), 69.85 (-CH_2O-), 69.16 (C-2′), 68.32 (C-5′), 67.38 (-CH_2O-), 66.57 (C-4′), 62.68 (C-6), 60.76 (C-6′), 49.87 (C-2), 29.21–20.47 (7C, *C*H_3CO-, *C*H_3CONH-). Anal. calcd for $C_{58}H_{84}N_2O_{36}$: C, 50.29; H, 6.11; N, 2.02. Found: C, 50.54; H, 6.39; N, 1.77.

3.14. 5-Hydroxy-3-oxa-pentyl 2-acetamido-2-deoxy-4-O-β-D-galactopyranosyl-α-D-mannopyranoside **15**

A catalytic amount of sodium was added to a solution of compound **13** (100 mg) in methanol (4 ml). The mixture was stirred at room temperature for 12 h, then neutralized with H^+ cation–exchange resin. The solution was filtered and concentrated and the residue was dissolved in 10 ml water and freeze-dried to give **15** as a white solid (62 mg, 95%). $[\alpha]_D$ +30.1 (*c* 1.86, CH_3OH). ^1H NMR (CD_3OD) δ: 4.78 (s, 1H, H-1), 4.35 (d, 1H, $J_{1',2'}$=7.44 Hz, H-1′), 4.33 (dd, 1H, H-2), 4.03 (dd, 1H, H-3), 3.93–3.85 (m, 2H, H-6a,6b), 3.82–3.55 (m, 14H, H-4′, H-4, -CH_2O-, H-5′, H-5, H-6a′,6b′), 3.53 (d, 1H, H-2′), 3.50 (dd, 1H, H-3′), 2.01 (s, 3H, -NHCOC*H*$_3$); ^{13}C NMR (CD_3OD) δ: 174.04 (-NHCO-), 105.11 (C-1′), 100.38 (C-1), 77.85 (C-4), 77.27 (C-5′), 74.78 (C-3′), 73.75 (-CH_2O-), 72.55 (C-2′), 72.50 (C-5), 71.30 (-CH_2O-), 70.44 (C-4′), 69.12 (C-3), 68.17 (-CH_2O-), 62.74 (-CH_2O-), 62.29 (C-6′), 61.76 (C-6), 53.87 (C-2), 22.64 (*C*H_3CONH-). Anal. calcd for $C_{18}H_{33}NO_{13}$: C, 45.86; H, 7.06; N, 2.97. Found: C, 45.54; H, 7.04; N, 2.88.

3.15. 8-Hydroxy-3,6-dioxa-octyl 2-acetamido-2-deoxy-4-O-β-D-galactopyranosyl-α-D-mannopyranoside ***16***

Compound **16** was prepared as described for the preparation of **15**. The yield was 97%. $[\alpha]_D$ +32.3 (*c* 1.61, CH_3OH). ^1H NMR (CD_3OD) δ: 4.77 (s, 1H, H1), 4.35 (d, 1H, $J_{1',2'}$=7.48 Hz, H-1′), 4.33 (dd, 1H, H-2), 4.03 (dd, H, H-3), 4.06–3.84 (m, 2H, H-6a,6b), 3.82–3.56 (m, 18H, H-4, H4′, -CH_2O-, H-5, H-5′, H-6a′,6b′), 3.55 (dd, 1H, H-2′), 3.50 (dd, 1H, H-3′), 2.00 (s, 3H, CH_3CONH-); ^{13}C NMR (CD_3OD) δ: 173.96 (-NHCO-), 105.14 (C-1′), 100.39 (C-1), 77.82 (C-4), 77.29 (C-5′), 74.81 (C-3′), 73.71 (-CH_2O-), 72.53 (C-2′), 72.52 (C-5), 71.73 (-CH_2O-), 71.45 (-CH_2O-), 71.33 (-CH_2O-), 70.45 (C-4′), 69.14 (C-3), 68.09 (-CH_2O-), 62.74 (-CH_2O-), 62.28 (C-6′), 61.75 (C-6), 53.85 (C-2), 22.65 (*C*H_3CONH-).

3.16. 3-Oxa-pentyl 1,1′-di-O-di-(2-acetamido-2-azido-2-deoxy-4-O-β-D-galactopyranosyl-α-D-mannoside) dimer ***21***

Compound **21** was prepared as described for the preparation of **15**. The yield was 97%. $[\alpha]_D$ +37.5 (*c* 0.96, CH_3OH). ^1H NMR (CD_3OD) δ: 4.78 (s, 1H, H-1), 4.35 (d, 1H, $J_{1',2'}$=7.76 Hz, H-1′), 4.33 (m, 1H, H-2), 4.03 (dd, 1H, H-3), 3.96–3.89 (m, 2H, H-6a,6b), 3.87–3.59 (m, 10H, H-4, H-4′, -CH_2O-, H-5, H-5′, H-6a′,6b′), 3.58 (m, 1H, H-2′), 3.50 (dd, 1H, H-3′), 2.01 (s, 3H, -NHCOCH_3); ^{13}C NMR (CD_3OD) δ: 174.03 (-NHCO-), 105.17 (C-1′), 100.49 (C-1), 77.83 (C-4), 77.29 (C-5′), 74.82 (C-3′), 72.53 (C-5), 72.46 (C-2′), 71.38 (-CH_2O-), 70.50 (C-4′), 69.15 (C-3), 68.31 (-CH_2O-), 62.77 (C-6′), 61.70 (C-6), 53.85 (C-2), 22.69 (*C*H_3CONH-).

3.17. 3,6-Dioxa-octyl 1,1′-di-O-di-(2-acetamido-2-deoxy-4-O-β-D-galactopyranosyl-α-D-mannoside) dimer ***22***

Compound **22** was prepared as described for the preparation of **15**. The yield was 98%. $[\alpha]_D$ +35.7 (*c* 1.12, CH_3OH). ^1H NMR (CD_3OD) δ: 4.78 (d, 1H, $J_{1,2}$=1.08 Hz, H-1), 4.36 (d, 1H, $J_{1',2'}$=7.40 Hz, H-1′), 4.34 (m, 1H, H-2), 4.05 (dd, 1H, H-3), 3.97–3.86 (m, 2H, H-6a,6b), 3.84–3.57 (m, 12H, H-4, H-4′, -CH_2O-, H-5, H-5′, H-6a′,6b′), 3.56 (m, 1H, H-2′), 3.51 (dd, 1H, H-3′), 2.01 (s, 3H, -NHCOCH_3); ^{13}C NMR (CD_3OD) δ: 174.06 (-NHCO-), 105.13 (C-1′), 100.34 (C-1), 77.82 (C-4), 77.27 (C-5′), 74.84 (C-3′), 72.53 (C-5), 72.51 (C-2′), 71.71 (-CH_2O-), 71.35 (-CH_2O-), 70.47 (C-4′), 69.18 (C-3), 68.12 (-CH_2O-), 62.75 (C-6′), 61.74 (C-6), 53.85 (C-2), 22.66 (*C*H_3CONH-).

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