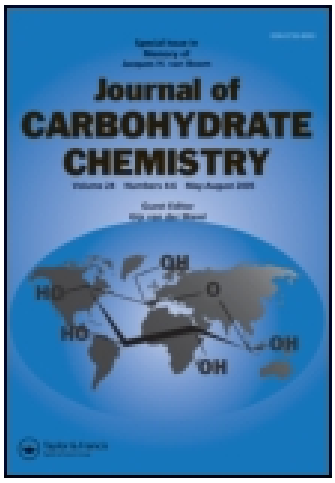


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# SYNTHESIS OF NEW GALACTOSYL AND LACTOSYL CARBAMATE-CONTAINING GLYCOLIPIDS

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## ABSTRACT

An efficient synthesis of mono-, di- and tetrasaccharides linked to a lipid has been developed. Galactose or lactose was covalently coupled to a glycyldioctadecylamide, *via* well-defined chemical steps, in both an α and β anomeric configuration. The multiantennary galactosyl ligands were obtained using 1,3-diamino-2-propanol as a scaffold.

## INTRODUCTION

Gene therapy is a promising and rapidly growing field of medical research directed towards correcting genetic or somatic disorders.[1] One of the major obstacles in gene therapy consists in finding methods which allow for specific and efficient delivery of the therapeutic genes. Besides the viruses, cationic liposomes are attractive systems for use in gene delivery. Non-viral vectors represent safe and efficient gene transfer agents[2] which, unlike viral vectors, do not elicit immune responses.[3] However, the non-viral gene carriers lack cell specificity, thus limiting their *in vivo* application. To solve this problem, cell targeting ligands were introduced to synthetic vectors[4] based on the concept of receptor-mediated endocytosis.[5] Hepatocytes exclusively express large numbers of high affinity cell-surface

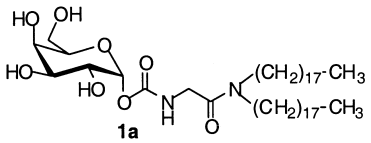
receptors that bind asialoglycoproteins (ASGP-R) and subsequently internalize them to the cell interior.[6] Selective transfection of hepatocytes *via* ASGP-R has been accomplished with a ligand-possessing galactose residue such as asialoorosomucoid,[7] galactosylated proteins or polymer[8] and galactosylated synthetic ligands.[9]

A study of available data agrees with the fact that human hepatocytes have a membrane lectin recognizing glycoproteins terminated by a β-D-galactose residue.[10] Moreover, recent results[11] indicate that the substitution of this β-D-galactosyl residue, coupled to polyethylenimine (PEI) to give a linear tetragalactose Galα$_3$-Galβ$_4$-Galα$_3$-Galβ-PEI, improves the gene transfer into hepatocytes. In the same report, it was also observed that the terminal α-galactosyl residue is easily accessible and recognized by using a galactose-binding RCA$_{120}$ lectin-mediated agglutination.

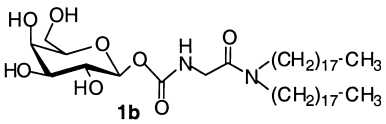
## RESULTS AND DISCUSSION

The aim of the work reported here was to determine if the α or β anomeric configuration of the galactose moiety plays an essential role in the binding with the lectin-mediated gene transfer into hepatocytes. To address this question, we undertook the synthesis of six new glycolipids (**1 a**, **1 b**, **2 a**, **2 b**, **3**, **4**) and subsequently investigated their potentiality for a specific and efficient targeting of hepatocytes.

In this paper, we describe the synthesis of glycolipids having a single α or β galactosyl unit **1 a** or **1 b** (for lactose **2 a**, **2 b)** linked to a glycine nitrogen *via* a carbamate function, with the glycine carboxyl group protected as an *N,N*-dialkylamide. We were interested in the development of a carbamate-linked galactose to insure better stability towards glycosidases.




**1a**




**1b**

The first two glycolipids were prepared according to reaction steps depicted in Scheme 1.

Compound **6** was prepared by reaction between benzyloxylcarbonyl-glycyl-*p*-nitrophenyl ester **5** and dioctadecylamine as reported.[12] Then, **6** was hydrogenolysed for 48 h at atmospheric pressure to remove the benzyloxycarbonyl protective group (*Z*), giving the glycyldioctadecylamide **7**[12] in good yield.

In regard to preparation of the galactosyl unit of the target lipids, galactose **8** was first peracetylated[13] and the pentaacetyl galactose **9** was selectively deprotected at the anomeric position using hydrazine acetate[14] in order to give the 2,3,4,6-tetra-*O*-acetyl-D-galactopyranose **10**, as inseparable mixture of anomers.





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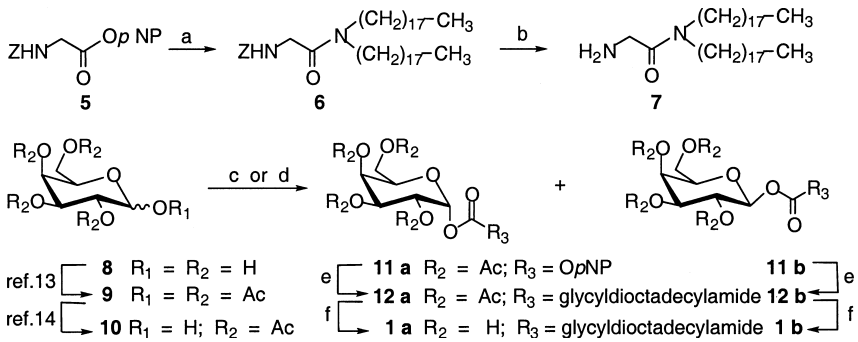
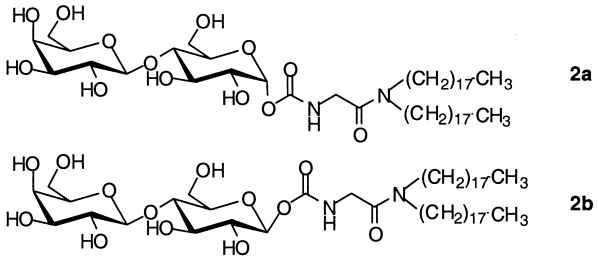



***Scheme 1.*** Reagents and conditions: a) $CH_2Cl_2$, dioctadecylamine, $Et_3N$, 40°C, 45%; b) $CH_2Cl_2$/EtOH (1:1), 10% Pd/C, $H_2$, 85%; c) $CH_2Cl_2$, 4-nitrophenylchloroformate, pyridine, 0°C, 80% (anomeric ratio α/β, 4/1); d) DME, 4-nitrophenylchloroformate, NaH, 65% (anomeric ratio α/β, 1/2); e) $CH_2Cl_2$, glycyldioctadecylamide 7, $Et_3N$, 75% from **11 a,** 70% from **11 b;** f) $CH_3OH$, NaOMe 1M, 75% from **12 a,** 69% from **12 b.** Z = benzyloxycarbonyl, *p*NP = *para*-nitrophenyl.

Crude **10** was then treated with 4-nitrophenylchloroformate under two different conditions. First, with dimethylformamide (DMF) at 0°C in the presence of pyridine, the α-anomer was afforded with a good diastereoselectivity (molar ratio of α/β, 4/1). In order to enhance β-anomer formation, we used the conditions as described by Klotz and Schmidt[15]—1,2-dimethoxyethane (DME) in the presence of sodium hydride at room temperature. As the anomeric diastereocontrol is temperature-dependent when carried out at room temperature, the β-anomer was preferentially obtained (molar ratio of α/β, 1/2). At this stage, the α and β anomers **11 a**, **b** were separated by flash chromatography. The carbamate **12 a** was obtained by reaction between 4-nitrophenyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl carbonate **11 a** and compound **7**. Transesterification of **12 a** with sodium methoxide in methanol led to the desired compound **1 a**. The carbamate **1 b** was prepared by the same procedure from the β-activated glycoside **11b**.




**2a**

**2b**

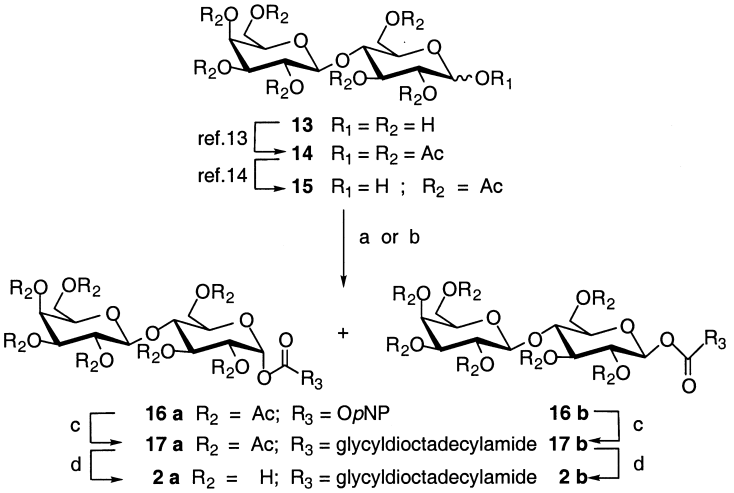
The synthesis of the next two glycolipids **2 a**, **b** was also accomplished by a similar reactional sequence described in Scheme 2, using lactose **13** as starting material.

Preparations of the corresponding carbonate-activated lactose **16 a, b** were performed with low diastereoselectivity using the same conditions as previously

described (DMF, 0°C, pyridine or DME, rt, NaH) since the molar ratios of β/α were 1/2 and 1/1, respectively.

Numerous studies have established that individual protein-sugar interactions are weak (Kd, mM). To overcome this restriction, many processes mediated by oligosaccharide-lectin interactions involve multivalent binding.[16] For example,[17] the specificity for terminal galactose residue, binding to the ASGP-receptor strongly depends on the oligosaccharide structure: mono-, bi- and tetraantennary galactose-terminal oligosaccharides bind with increasing affinity (Kd, from mM to nM). In agreement with these data, indicating that oligomeric structures are required for efficient binding to a galactose receptor, we decided to prepare the bi- and tetraantennary galactosyl lipids **3**, **4** as described in Scheme 3.

The biantennary glycolipid **3** was obtained by linking the glycosyl carbonate **11 a** with the commercially available 1,3-diamino-2-propanol to give the biantennary galactosylated compound **18**. The free alcohol of this molecule was activated with 4- nitrophenylchloroformate before coupling with glycyldioctadecylamide **7** to yield **20**. The last step to give **3** was the removal of the acetyl groups by brief treatment with the Zemplén method. The tetraantennary galactosyl lipid **4** was obtained following a similar reactional sequence involving condensation of a second molecule of 1,3-diamino-2-propanol with the bis-galactosyled carbonate derivative **19** to give first the tetraantennary compound **21** which was converted to the carbonate **22**. Coupling of **22** with compound **7** yielded the corresponding glycolipid **23**, which was then deacetylated under Zemplén conditions to give the final product.




***Scheme 2.*** Reagents and conditions: a) $CH_2Cl_2$, 4-nitrophenylchloroformate, pyridine, 0°C, 72% (anomeric ratio α/β, 2/1); b) DME, 4-nitrophenylchloroformate, NaH, 55% (anomeric ratio α/β, 1/1); c) $CH_2Cl_2$, 7, Et3N, 65% from **16 a,** 60% from **16 b;** d) $CH_3OH$, NaOMe 1M, 70% from **17 a,** 60% from **17 b.**





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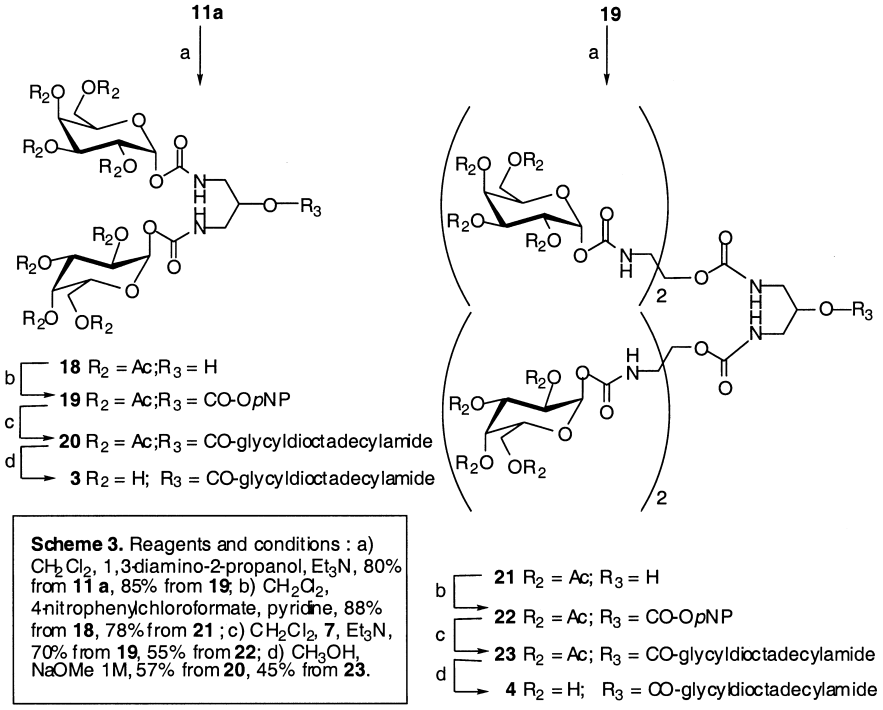


**Scheme 3.** Reagents and conditions : a) $CH_2Cl_2$, 1,3-diamino-2-propanol, $Et_3N$, 80% from **11 a**, 85% from **19**; b) $CH_2Cl_2$, 4-nitrophenylchloroformate, pyridine, 88% from **18**, 78% from **21** ; c) $CH_2Cl_2$, **7**, $Et_3N$, 70% from **19**, 55% from **22**; d) $CH_3OH$, NaOMe 1M, 57% from **20**, 45% from **23**.

*Scheme 3.*

## CONCLUSION

The synthetic procedure described here exploited converting the free anomeric hydroxyl group of an otherwise protected glycopyranosyl residue into the corresponding activated α or β carbonate for coupling with diaminopropanol as a scaffold to provide an entry for the synthesis of a multiantennary compound. In summary, we succeeded in the preparation of artificial glycolipids involving a single α or β galactosyl or lactosyl unit, as well as the synthesis of bi- or tetraantennary-α-galactosyl lipids. These compounds offer the advantage of being fully synthetic, rapidly prepared and easily purified. The lectin recognition and transfection potential of these new galactosyl lipids are under investigation and results regarding this aspect will be forthcoming.

## EXPERIMENTAL

**General Methods.** Thin-layer chromatography (TLC) was performed on silica gel $60F_{254}$ (Merck) and visualized first with light, and second by heating after alcoholic sulfuric or phosphomolybdic acid treatment. Column chromatography was performed on $SiO_2$ (Merck, particle size 0.004–0.063 nm) using the flash

chromatography technique. Optical rotations were determined with a Perkin-Elmer 241 polarimeter (589 nm) at 20°C with a concentration expressed in g/100 mL. For mass spectra, CI ($NH_3$) were recorded with a Nermag R10-10C, FAB with a Jeol MS-700 and MALDI/Tof with a Voyager (Applied Biosystems) using reflector mode and 2,5-dihydroxybenzoic acid (DHB) as the matrix. $^1$H NMR spectra were recorded using a Bruker AC-300 (300 MHz). Chemical shifts are expressed in ppm downfield from internal $Me_4Si$ with notation indicating the multiplicity of the signal. For the NMR assignments, galactose atoms are noted 1–6, those of glucose 1′-6′. Compounds **6**, **7**;[12] **9**, **14**;[13] **10**[14,18] and **15**[19] were prepared as described in the literature.

**4-Nitrophenyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl carbonate (11 a)** and **4-Nitrophenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl carbonate (11 b)**.

*First procedure*: To a cooled solution of **10** (1.26 g, 3.33 mmol) in $CH_2Cl_2$ (50 mL) were successively added 4-nitrophenyl chloroformate (1 g, 5 mmol) and pyridine (0.44 mL, 5 mmol). After stirring for 4 h at 0°C, the crude mixture was extracted with additional $CH_2Cl_2$ (≈150 mL) and the organic layer was washed with water and brine. Evaporation of solvent, followed by flash chromatography (cyclohexane-EtOAc, 2:1) afforded **11 a** as a crystalline compound (1.13 g, 66%); mp 67°C; $[\alpha]_D$ +103 (*c* 1, $CHCl_3$); $^1$H NMR ($CDCl_3$) δ 2.01, 2.05, 2.06, 2.16 (4s, 4 × 3H, OAc), 4.14 (d, 2H, J = 6.6 Hz, H-6a, H-6b), 4.45 (m, 1H, H-5), 5.40 (m, 2H, H-2, H-3), 5.55 (m, 1H, H-4), 6.32 (d, 1H, $J_{1,2}$ = 3 Hz, H-1), 7.40 (d, 2H, J = 10 Hz, Ar), 8.30 (d, 2H, J = 10 Hz, Ar), and **11 b** as a colourless oil (0.28 g, 16%); $[\alpha]_D$ +55 (*c* 1.3, $CHCl_3$), $^1$H NMR ($CDCl_3$) δ 1.99, 2.01, 2.05, 2.16 (4s, 4 × 3H, OAc), 4.15 (m, 3H, H-5, H-6a, H-6b), 5.10 (dd, 1H, $J_{3,2}$ = 4 Hz, $J_{3,4}$ = 10 Hz, H-3), 5.40 (m, 2H, H-2, H-4), 5.60 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1), 7.44 (d, 2H, J = 10 Hz, Ar), 8.33 (d, 2H, J = 10 Hz, Ar).

*Second procedure*: To a solution of **10** (0.52 g, 1.5 mmol) in dry DME (20 mL) at room temperature, NaH (42 mg, 1.65 mmol, 96% suspension in paraffin oil) was added. After 15 min, the 4-nitrophenyl chloroformate (450 mg, 2.25 mmol) was added and stirring was continued for 15 h. The reaction mixture was filtered through silica and the silica was washed with ethyl acetate (50 mL). The clear solution was washed with saturated NaCl (3 × 75 mL), dried with $MgSO_4$, concentrated and then purified by flash chromatography (cyclohexane-ethyl acetate, 2:1); **11 a** (160 mg, 20%) and **11 b** (340 mg, 44%) were obtained, respectively.

***N*-(2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyloxycarbonyl)-L-glycyldioctadecylamide (12 a)**. To a solution of **11 a** (980 mg, 1.9 mmol) in $CH_2Cl_2$ (50 mL) were successively added glycyldioctadecylamide (650 mg, 2.8 mmol) and $Et_3N$ (400 μL, 2.8 mmol). The mixture was stirred at room temperature for 8 h, then concentrated under reduced pressure. Water was added, and the aqueous phase was extracted with ethyl acetate. The combined organic layers were dried, filtered, concentated and purified by flash chromatography (cyclohexane-ethyl acetate, 6:4) to give **12 a** (1360 mg, 75%) as an oil; $[\alpha]_D$ + 2 (*c* 0.8, $CHCl_3$), $^1$H NMR

($CDCl_3$) δ 0.88 (t, 2 × 3H, $CH_3$), 1.20 (br s, 60H, —$CH_2$—), 1.60 (br s, 4H, C$H_2$—$CH_3$), 2.00, 2.04, 2.05, 2.16 (4s, 4 × 3H, OAc), 3.25 (t, 2H, $CH_2$—N), 3.35 (t, 2H, $CH_2$—N), 4.00 (m, 2H, NH—C$H_2$—CO), 4.10 (m, 2H, H-6a, H-6b), 4.35 (t, 1H, H-5), 5.33 (m, 2H, H-2, H-3), 5.50 (m, 1H, H-4), 6.00 (t, 1H, NH), 6.30 (d, 1H, J = 3 Hz, H-1); Positive FAB-MS: *m/z* 953 $(M + H)^+$, 975 $(M + Na)^+$.

***N*-(α-D-Galactopyranosyloxycarbonyl)-L-glycyldioctadecylamide (1 a)**. To a cooled solution of **12 a** (320 mg, 0.34 mmol) in 10 mL of a $CH_2Cl_2$—$CH_3OH$ mixture (2:1), 30 mg of sodium methoxide powder were added. The solution was stirred for 4 h, then neutralized with IRC 50-S Amberlite and filtered. The filtrate was concentrated to an oily residue and purified by chromatography ($CH_2Cl_2$—MeOH, 9:1) to give **1 a** (200 mg, 75%) as a white foam; $[\alpha]_D$ + 55 (*c* 0.2, $CHCl_3$), $^1$H NMR ($CDCl_3$—$CD_3OD$) δ 0.88 (t, 2 × 3H, $CH_3$), 1.20 (br s, 60H, —$CH_2$—), 1.50 (br s, 4H, C$H_2$—$CH_3$), 2.50 (br s, 2H, OH), 3.15 (br s, 2H, $CH_2$—N), 3.30 (br s, 2H, $CH_2$—N), 4.10 (m, 6H, H-2, H-3, H,4, H-5, H-6a, H-6b), 4.95 (br s, 1H, OH), 5.24 (br s, 1H, OH), 6.10 (br s, 1H, H-1); Positive FAB-MS: *m/z* 785 $(M + H)^+$.

Anal. Calcd for $C_{45}H_{88}N_2O_8$: C, 68.83; H, 11.38; N, 3.57. Found: C, 68.99; H, 11.76; N, 3.25.

***N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyloxycarbonyl)-L-glycyldioctadecylamide (12 b)**. Compound **12b** was obtained from **11 b** (870 mg, 1.7 mmol) as described for the preparation of **12 a** and isolated as an oil in 70% yield after flash chromatography (cyclohexane-ethyl acetate, 1:1); $[\alpha]_D$ +32 (*c* 1, $CHCl_3$), $^1$H NMR ($CDCl_3$) δ 0.86 (t, 2 × 3H, $CH_3$), 1.22 (br s, 60H, —$CH_2$—), 1.43 (br s, 4H, C$H_2$—$CH_3$), 1.99, 2.04, 2.05, 2.17 (4s, 4 × 3H, OAc), 3.12 (br t, 2H, $CH_2$—N), 3.30 (br t, 2H, $CH_2$—N), 4.00 (d, 2H, J = 4 Hz, $CH_2$—CO), 4.15 (m, 3H, H-5, H-6a, H-6b), 5.09 (dd, 1H, $J_{3,2}$ = 3.5 Hz, $J_{3,4}$ = 10.3 Hz, H-3), 5.30 (m, 2H, H-2, H-4), 5.60 (d, 1H, J = 8.2 Hz, H-1), 6.05 (m, 1H, NH); Positive FAB-MS: *m/z* 953 $(M + H)^+$.

***N*-(β-D-Galactopyranosyloxycarbonyl)-L-glycyldioctadecylamide (1 b)**. Compound **1b** was prepared in 69% yield by deprotection of **12 b** (950 mg, 1 mmol) as described for **1 a**; $[\alpha]_D$ + 3 (*c* 2.2, $CHCl_3$), $^1$H NMR ($CDCl_3$—$CD_3OD$) δ 0.88 (t, 2 × 3H, $CH_3$), 1.26 (br s, 60H, —$CH_2$—), 1.50 (br s, 4H, C$H_2$—$CH_3$), 2.42 (br s, 2H, OH), 3.15 (br s, 2H, $CH_2$—N), 3.25 (br s, 2H, $CH_2$—N), 3.80 (m, 6H, H-2, H-3, H-4, H-5, H-6a, H-6b), 4.80 (br s, 1H, OH), 5.14 (br s, 2H, OH, NH), 5.42 (br d, 1H, J =7 Hz, H-1), 7.10 (br s, 1H, NH); Positive FAB-MS: *m/z* 785 $(M + H)^+$, 807 $(M + Na)^+$.

Anal. Calcd for $C_{45}H_{88}N_2O_8$: C, 68.83; H, 11.38; N, 3.57. Found: C, 69.23; H, 11.67; N, 3.52.

**4-Nitrophenyl-[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)] carbonate (16 a)** and **4-Nitrophenyl-[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-D-**

**glucopyranosyl)] carbonate (16 b)**. Both compounds were obtained from **15** as described for the preparation of **11 a** and **11 b**.

*By the first procedure*: **16 a** was obtained in 48% yield after purification as white solid; mp 143–144°C; $[\alpha]_D$ +16 (*c* 3.5, $CHCl_3$), $^1H$ NMR ($CDCl_3$) δ 1.98, 2.02, 2.06, 2.07, 2.13, 2.14 (7s, 7 × 3H, OAc), 3.90 (m, 2H, H-5, H-4′), 4.15 (m, 4H, H-6′a, H-5′, H-6a, H-6b), 4.51 (m, 2H, H-1, H-6′b), 5.00 (dd, 1H, $J_{3-2}$ = 10.25 Hz, $J_{3-4}$ = 3.1 Hz, H-3), 5.10 (m, 2H, H-2′, H-2), 5.35 (br d, 1H, H-4′), 5.50 (t, 1H, $J_{3'-4'}$ = 9.2 Hz, H-3′), 6.22 (d, 1H, J = 3.6 Hz, H-1′), 7.45 (d, 2H, J = 8 Hz, Ar), 8.30 (d, 2H, J = 8 Hz, Ar); CI ($NH_3$)-MS: *m/z* 802 $(M + H)^+$, 819 $(M + NH_4)^+$. **16 b** was obtained in 24% yield as an oil after chromatography; $[\alpha]_D$ +52 (*c* 0.4, $CHCl_3$), $^1H$ NMR ($CDCl_3$) δ 1.98, 2.06, 2.08, 2.09, 2.10, 2.14, 2.17 (7s, 7 × 3H, OAc), 3.90 (m, 3 H, H-5, H-5′, H-4′), 4.10 (m, 3H, H-6a, H-6b, H-6′a), 4.50 (m, 2H, H-1, H-6′b), 5.00 (dd, 1H, $J_{3-4}$ = 3.5 Hz, $J_{3-2}$ = 10.4 Hz, H-3), 5.15 (m, 2H, H-2, H-2′), 5.30 (t, 1H, J = 8 Hz, H-3), 5.38 (br d, 1H, J = 3 Hz, H-4), 5.65 (d, 1H, J = 8 Hz, H-1′), 7.45 (d, 2H, J = 8 Hz, Ar), 8.30 (d, 2H, J = 8 Hz, Ar).

*By the second procedure*: Both **16 a** and **16 b** were obtained in 24% yield.

***N*-[(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyloxycarbonyl)]-L-glycyldioctadecylamide (17 a)**. To a solution of **16 a** (300 mg, 0.37 mmol) in dry dichloromethane (20 mL) were added glycyldioctadecylamide (130 mg, 0.55 mmol) and $Et_3N$ (80 μL, 0.56 mmol). The mixture was stirred at rt for 6 h, then concentrated under reduced pressure. Water was added and the aqueous phase was extracted with $CH_2Cl_2$. The combined organic phases were dried over $MgSO_4$, filtered and concentrated. Purification by flash chromatography (cyclohexane-ethyl acetate, 2:1) afforded **17 a** (300 mg, 65%); $[\alpha]_D$ +31 (*c* 1.5, $CHCl_3$), $^1H$ NMR ($CDCl_3$) δ 0.88 (t, 2 × 3H, $CH_3$), 1.22 (br s, 60H, —$CH_2$—), 1.96, 2.03, 2.04, 2.05, 2.06, 2.12, 2.15 (7s, 7 × 3H, OAc), 3.12 (br t, 2H, $CH_2$—N), 3.30 (br t, 2H, $CH_2$—N), 3.85 (m, 3H, H-5, H-5′, H-4′), 4.10 (m, 5H, NH—C$H_2$—CO—, H-6a, H-6b, H-6′a), 4.50 (m, 2H, H-1, H-6′b), 5.00 (m, 2H, H-2, H-3), 5.10 (dd, 1H, $J_{2'-1'}$ = 3 Hz, $J_{2'-3'}$ = 10 Hz, H-2′), 5.35 (m, 1H, H-4), 5.40 (t, 1H, J = 9.8 Hz, H-3′), 6.05 (br t, 1H, NH), 6.17 (d, 1H, J = 3.5 Hz, H-1′); Positive FAB-MS: *m/z* 1241 $(M + H)^+$, 1263 $(M + Na)^+$.

***N*-[(β-D-Galactopyranosyl)-(1→4)-(α-D-glucopyranosyloxy-carbonyl)]-L-glycyldioctadecylamide (2 a)**. To a cold solution of **17 a** (190 mg, 0.15 mmol) in 30 mL of a $CH_2Cl_2$—$CH_3OH$ mixture (2:1), 30 mg of sodium methoxide powder were added. The solution was stirred for 4 h and neutralized with Amberlite IRC-50S. Filtration, followed by solvent evaporation from the filtrate under reduced pressure, afforded a crude residue which was purified by flash chromatography ($CH_2Cl_2$—MeOH, 8:2), giving **2 a** (100 mg, 70%) as a white solid; mp 166–167°C; $[\alpha]_D$ + 9 (*c* 1, $CHCl_3$—MeOH, 1:1), $^1H$ NMR ($CDCl_3$—$CD_3OD$) δ 0.88 (t, 2 × 3H, $CH_3$), 1.20 (m, 60H, —$CH_2$—), 1.50 (br s, 4H, C$H_2$—$CH_3$), 2.50 (br s, 4H, OH), 3.15 (br s, 2H, $CH_2$—N), 3.30 (br s, 2H, $CH_2$—N), 3.90 (m, 9H, NH—C$H_2$—C═O, H-4′, H-5′, H-5, H-6′a, H-6′b, H-6a, H-1), 4.50 (m, 5H, H-2,

H-2′, H-3, H-3′, H-4), 4.90 (br s, 2H, OH), 5.22 (br s, 3H, OH, NH), 5.90 (br s, 1H, H-1); Positive FAB-MS: *m/z* 947 $(M + H)^+$, 969 $(M + Na)^+$.

Anal. Calcd for $C_{51}H_{98}N_2O_{13}$: C, 64.66; H, 10.43; N, 2.96. Found: C, 64.60; H, 10.54; N, 3.09.

***N*-[(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxycarbonyl)]-L-glycyldioctadecylamide (17 b)**. Compound **17b** was obtained from **16 b** as described for the preparation of **17 a** and isolated in 60% yield as an oil after flash chromatography (cyclohexane-ethyl acetate, 6:4); $[\alpha]_D$–3 (*c* 1, $CHCl_3$), $^1H$ NMR ($CDCl_3$) δ 0.88 (t, 2 × 3H, $CH_3$), 1.20 (br s, 60H, —$CH_2$—), 1.97, 2.03, 2.04, 2.05, 2.07, 2.12, 2.16 (7s, 7 × 3H, OAc), 3.10 (br t, 2H, $CH_2$—N), 3.30 (br t, 2H, $CH_2$—N), 3.90 (m, 3H, H-5, H-5′, H-4), 4.00 (d, 2H, J = 3 Hz, NH—C*H*$_2$—C═O), 4.10 (m, 3H, H-6a, H-6b, H-6′a), 4.50 (m, 2H, H-1, H-6′b), 4.95 (dd, 1H, $J_{3-4}$ = 3 Hz, $J_{3-2}$ = 11 Hz, H-3), 5.10 (m, 2H, H-2, H-2′), 5.25 (t, 1H, J = 9 Hz, H-3′), 5.35 (m, 1H, H-4), 5.64 (d, 1H, J = 9 Hz, H-1′), 6.05 (br t, 1H, NH); Positive FAB-MS: *m/z* 1241 $(M + H)^+$.

***N*-[(β-D-Galactopyranosyl)-(1→4)-(β-D-glucopyranosyloxy-carbonyl)]-L-glycyldioctadecylamide (2 b)**. Compound **2 b** was prepared in 60% yield by deprotection of **17 b** as described for **2 a**; $[\alpha]_D$–35 (*c* 1, $CHCl_3$—MeOH, 1:1), $^1H$ NMR ($CDCl_3$—$CD_3OD$) δ 0.88 (t, 2 × 3H, $CH_3$), 1.60 (br s, 60H, —$CH_2$—), 2.50 (br s, 4H, OH), 3.12 (br s, 2H, $CH_2$—N), 3.28 (br s, 2H, $CH_2$—N), 3.80 (m, 8H, H-6′a, H-6′b, H-6a, H-6b, H-5, H-5′, H-4′, H-1), 4.50 (m, 5H, H-3, H-3′, H-2, H-2′, H-4), 4.90 (br s, 2H, OH), 5.22 (br s, 3H, OH, NH), 5.40 (br d, 1H, J = 9 Hz, H-1′), 6.05 (br t, 1H, NH); Positive FAB-MS: *m/z* 947 $(M + H)^+$.

Anal. Calcd for $C_{51}H_{98}N_2O_{13}$: C, 64.66; H, 10.43; N, 2.96. Found: C, 64.89; H, 10.64; N, 3.11.

***N,N′*-(2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyloxycarbonyl)-1,3-diaminopropan-2-ol (18)**. To a solution of **11 a** (3.6 g, 7 mmol) in dry $CH_2Cl_2$ (75 mL) were added 1,3-diaminopropanol (315 mg, 3.5 mmol) and triethylamine (1 mL, 7 mmol). After stirring for 1 h at rt, the reaction mixture was diluted with water and extracted with $CH_2Cl_2$ (2 × 50 mL). The combined organic layers were washed with a saturated NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash chromatography ($CH_2Cl_2$—$CH_3OH$, 95:5) to afford **18** (4.7 g, 80%); $[\alpha]_D$ + 36 (*c* 2.8, $CHCl_3$), $^1H$ NMR ($CDCl_3$) δ 1.97, 1.99, 2.03, 2.15 (4s, 24H, OAc), 3.30 (m, 4H, C*H*$_2$—CH—OH), 3.87 (m, 1H, $CH_2$—C*H*—OH), 4.08 (d, 4H, J = 6 Hz, H-6a, H-6b), 4.34 (t, 2H, J = 7 Hz, H-5), 5.30 (m, 4H, H-2, H-3), 5.47 (m, 2H, H-4), 5.60 (m, 2H, NH), 6.26 (br s, 2H, H-1); Positive FAB-MS: *m/z* 839 $(M + H)^+$, 861 $(M + Na)^+$.

**4-Nitrophenyl-[*N,N′*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyloxy-carbonyl)-1,3-diaminopropyl]-carbonate (19)**. To a solution of 4-nitrophenyl chloroformate (300 mg, 1.5 mmol) in $CH_2Cl_2$ (25 mL) and pyridine (120 μL, 1.5 mmol), a solution of **18** (650 mg, 0.77 mmol) in $CH_2Cl_2$ (25 mL) was gradually

added. The mixture was stirred over night, then concentrated under reduced pressure. After purification by flash chromatography (ethyl acetate-cyclohexane, 2:1), **19** was obtained as an oil (680 mg, 88%); $[\alpha]_D$ + 30 (*c* 1, $CHCl_3$), $^1H$ NMR ($CDCl_3$) δ 1.98, 2.00, 2.03, 2.05, 2.16 (4s, 2(4 × 3H), OAc), 3.45 (m, 2H, C*H*$_2$—CH—O—CO), 3.65 (m, 2H, C*H*$_2$—CH—O—CO), 4.10 (d, 4H, J = 6 Hz, H-6a, H-6b), 4.30 (m, 2H, H-5), 4.85 (m, 1H, $CH_2$—C*H*—O—CO), 5.30 (m, 4H, H-2, H-3), 5.50 (m, 4H, H-5, NH), 6.30 (d, 1H, J = 2.5 Hz, H-1), 6.35 (d, 1H, J = 2.5 Hz, H-1), 7.44 (d, 2H, J = 10 Hz, Ar), 8.30 (d, 2H, J = 10 Hz, Ar); Positive FAB-MS: *m/z* 1004 $(M + H)^+$.

***N,N′*-[(2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyloxycarbonyl)-1,3-diaminopropyloxycarbonyl]-L-glycyldioctadecylamide (20)**. To a solution of **19** (435 mg, 0.43 mmol) in dry $CH_2Cl_2$ (30 mL) were successively added glycyldioctadecylamide (112 mg, 0.47 mmol) and $Et_3N$ (70 μL, 0.49 mmol). The mixture was stirred overnight at rt, then extracted following the classical procedure. Purification by flash chromatography (cyclohexane-ethyl acetate, 1:1) led to compound **20** (495 mg, 70%) as a white foam; $[\alpha]_D$ + 11 (*c* 1.5, $CHCl_3$), $^1H$ NMR ($CDCl_3$) δ 0.88 (t, 2 × 3H, $CH_3$), 1.26 (br s, 60H, —$CH_2$—), 1.60 (m, 4H, C*H*$_2$—$CH_3$), 2.00, 2.04, 2.05, 2.16 (4s, 24H, OAc), 3.15 (m, 2H, C*H*$_2$—CH—O—CO), 3.30 (m, 2H, $CH_2$—N), 3.40 (m, 2H, C*H*$_2$—CH—O—CO), 3.50 (m, 2H, $CH_2$—N), 4.00 (d, 2H, J = 3 Hz, NH—C*H*$_2$—C═O), 4.15 (br d, 4H, H-6a, H-6b), 4.33 (m, 2H, H-5), 4.82 (m, 1H, $CH_2$—C*H*—O—CO), 5.30 (m, 4H, H-2, H-3), 5.50 (m, 4H, NH, H-4), 5.71 (br t, 1H, NH), 6.30 (br s, 2H, H-1); Positive FAB-MS: *m/z* 1443 $(M + H)^+$, 1465 $(M + Na)^+$.

***N,N′*-[(α-D-galactopyranosyloxycarbonyl)-1,3-diaminopropyloxycarbonyl]-L-glycyldioctadecylamide (3)**. To a cold solution of **20** (470 mg, 0.33 mmol) in 50 mL of $CH_2Cl_2$–—MeOH (2:1) were added 140 mg of MeONa. The reaction mixture was stirred for 3 h and neutralized with Amberlite IRC-50S. After filtration, the filtrate was concentrated under reduced pressure to give a solid. Flash chromatography ($CH_2Cl_2$—MeOH, 7:3) gave **3** (208 mg, 57%) as white solid; mp 162–164°C; $[\alpha]_D$ + 21 (*c* 1, $CHCl_3$—$CH_3OH$, 1:1); $^1H$ NMR ($CDCl_3$—$CD_3OD$) δ 0.88 (t, 2 × 3H, $CH_3$), 1.20 (br s, 60H, —$CH_2$—), 1.50 (br s, 4H, C*H*$_2$—$CH_3$), 2.54 (br s, 4H, OH), 3.15 (br s, 4H, $CH_2$—N, C*H*$_2$—CH—O—CO), 3.30 (br s, 4H, $CH_2$—N, C*H*$_2$—CH—O—CO), 3.80 (m, 12H, H-2, H-3, H-4, H-5, H-6a, H-6b), 4.80 (br s, 2H, OH), 5.14 (br s, 2H, OH), 6.10 (br s, 2H, H-1); Positive FAB-MS: *m/z* 1107 $(M + H)^+$.

Anal. Calcd for $C_{56}H_{106}N_4O_{17}$: C, 60.73; H, 9.65; N, 5.06. Found: C, 61.05; H, 9.69; N, 5.17.

***N,N′*-[Bis-*N,N′*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyloxycarbonyl)-1,3-diaminopropyloxycarbonyl]-1,3-diaminopropan-2-ol (21)**. Compound **21** was obtained in 78% yield from **19** by the same protocol used for the preparation of compound **18**; $[\alpha]_D$ + 49 (*c* 2, $CHCl_3$), $^1H$ NMR ($CDCl_3$) δ 2.05 (m, 36H, OAc), 2.20 (br s, 12H, OAc), 3.32 (m, 12H, NH—C*H*$_2$—), 3.80 (m, 1H,





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$CH_2$—C*H*—OH), 4.10 (m, 8H, H-6a, H-6b), 4.30 (m, 4H, H-5), 4.80 (m, 1H, $CH_2$—C*H*—O—C═O), 5.30 (m, 8H, H-2, H-3), 5.65 (m, 4H, H-4), 5.90 (m, 4H, NH), 6.25 (m, 4H, H-1); Positive FAB-MS: *m/z* 1841 $(M + Na)^+$, 1857 $(M + K)^+$.

**4-Nitrophenyl-[*N,N′*-(bis-*N,N′*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyloxycarbonyl)-1,3-diaminopropyloxycarbonyl)-1,3-diaminopropyl]-carbonate (22)**. Compound **22** was prepared in 78% yield as described for **19**; $[\alpha]_D$ +74 (*c* 2, $CHCl_3$), 1H NMR ($CDCl_3$) δ 2.01 (m, 36H, OAc), 2.20 (br s, 12H, OAc), 3.40 (m, 12H, NH—C$H_2$—), 4.10 (m, 8H, H-6a, H-6b), 4.20 (m, 4H, H-5), 4.80 (m, 2H, C*H*—O—CO—NH), 5.07 (m, 1H, CH—O—CO—O), 5.30 (m, 8H, H-2, H-3), 5.48 (m, 4H, H-4), 5.90 (m, 6H, NH), 6.15 (m, 4H, H-1); Positive FAB-MS: *m/z* 1984 $(M + H)^+$, 2006 $(M + Na)^+$.

***N,N′*-[Bis-*N,N′*-((2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyloxycarbonyl)-1,3-diaminopropyloxycarbonyl)-1,3-diaminopropyl]-L-glycyldioctadecylamide (23)**. Compound **23** was obtained from **22** as described for the preparation of **20** and isolated in 55% yield as a white foam; $[\alpha]_D$ + 42 (*c* 3.4, $CHCl_3$), 1H NMR ($CDCl_3$) δ 0.88 (t, 6H, $CH_3$), 1.20 (br s, 60H, —$CH_2$—), 1.60 (br s, 4H, C$H_2$—$CH_3$), 2.05 (m, 36H, OAc), 2.15 (br s, 12H, OAc), 3.40 (m, 14H, NH—C$H_2$—CH—, NH—C$H_2$—CO), 4.10 (m, 8H, H-6a, H-6b), 4.35 (m, 4H, H-5), 4.80 (m, 2H, C*H*—O—CO—NH), 5.01 (m, 1H, CH—O—CO—O), 5.30 (m, 8H, H-2, H-3), 5.47 (m, 4H, H-4), 6.28 (m, 4H, H-1); MALDI/Tof-MS (DHB): *m/z* 2424 $(M + H)^+$, 2446 $(M + Na)^+$.

Anal. Calcd for $C_{110}H_{174}N_8O_{51}$: C, 54.49; H, 7.23; N, 4.62. Found: C, 54.85; H, 7.56; N, 4.84.

***N,N′*-[Bis-*N,N′*-((α-D-galactopyranosyloxycarbonyl)-1,3-diaminopropyloxycarbonyl)-1,3-diaminopropyl]-L-glycyldioctadecylamide (4)**. Compound **4** was prepared by deprotection of compound **23** (450 mg, 0.18 mmol) as described for **3**, after purification by flash chromatography ($CH_2Cl_2$—MeOH, 7:3). Compound **4** (146 mg, 45%) was isolated as a white solid; mp 196–198°C; $[\alpha]_D$ + 66 (*c* 0.5, $CHCl_3$—$CH_3OH$, 1:1); MALDI/Tof-MS (DHB): *m/z* 1773 $(M + Na)^+$, 1789 $(M + K)^+$.

Anal. Calcd for $C_{78}H_{142}N_8O_{35}$: C, 53.47; H, 8.17; N, 6.40. Found: C, 53.37; H, 8.04; N, 6.33.

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