ISSN 1068-1620, Russian Journal of Bioorganic Chemistry, 2010, Vol. 36, No. 5, pp. 657–662. © Pleiades Publishing, Ltd., 2010.
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Synthesis of Positively Charged Galactose-Bearing Surfactants

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Received January 1, 2010; in final form, March 31, 2010

Abstract—An approach to the synthesis of cationic carbohydrate surfactants with potential antimicrobial and transfecting activities is proposed.

Key words: antimicrobial activity, cationic surfactants, galactose, Fukuyama reaction, transfection

DOI: 10.1134/S1068162010050146

INTRODUCTION

Positively charged surfactants are widely used as antiseptics and disinfectants [1]. As surface-active compounds, they interact with cell membrane phospholipids and damage membrane structures, causing cell lysis [2].[2] When added to bacterial cells or pathogen fungi, cationic surfactants, such as dioctadecyldimethylammonium bromide (DDAB) and hexadecyltrimethylammonium bromide (CTAB), reverse the cell membrane charge from negative to positive and, thus, bring about cell death [3]. It was assumed that the introduction of carbohydrate residues in the structure of cationic surfactants could improve their biocompatibility with cells and tissues [4].

Due to their accessibility and low price, cationic surfactants are also used in gene therapy as delivery systems of therapeutic nucleic acids to eukaryotic cells [5, 6]. Cationic surfactants protect nucleic acids from enzymatic degradation by forming complexes with them and support their transfer via cell membranes, thus initiating endocytosis [7]. A large number of surfactants serve for the insertion of genetic constructs in mammalian [5, 8, 9] and plant [10] cells. Particularly, DDAB and dioleoyl dimethylammonium chloride (DODAC) were used as mediators for the delivery of plasmid DNA and antisense oligonucleotides in in vitro [11–17] and in vivo [18, 19] experiments, as well as in studies of the mechanism of DNA–cationic lipid complex formation [20–22]. The replacement of two DDAB methyl groups by 2-hydroxyethyl groups increased the transfection efficacy as compared with both DDAB and the commercial transfection agent lipofectamine (Lipofectamine, Invitrogen) [23]. The introduction into the cationic surfactant structure of four hydroxyl groups within arabinose or xylose (in the acyclic form) doubled the transfection activity as compared with surfactants containing two hydroxyl groups [24].

The in vivo increased efficacy of nucleic acid delivery could be achieved if transport systems were to bear specific ligands providing highly affine binding to specific receptors of target cells. It is known that hepatocyte membranes contain surface-exposed glycoprotein receptors that allow the cell to recognize, immobilize, and swallow molecules bearing galactose residues [25]. This property was used for the directed delivery of nucleic acids into hepatocytes using galactose-containing cationic surfactants [26, 27]. Thus, the search for biodegraded amphiphils among carbohydrate-containing surfactants would provide an extension of the spectrum of compounds with potential antimicrobial activity and the development of transport systems for the directed delivery of nucleic acids to the target cells.

RESULTS AND DISCUSSION

Earlier, we synthesized a series of cationic carbohydrate amphiphils differing in the position of the positively charged group and the spacer between the carbohydrate unit and the hydrophobic domain [28, 29]. As a continuation of these studies, we report herein the synthesis of cationic carbohydrate surfactants with potential biological activity.

We used a tetradecyl substituent as a hydrophobic domain and an ammonium fragment as a cationic domain for the synthesis of carbohydrate surfactants. Carbohydrate residues (galactose and lactose) were introduced in the surfactant molecule using a hexamethylene spacer. Earlier, for the synthesis of similar compounds, an approach was proposed based on the coupling of a trichloroacetamidate glucose derivative with 2-bromoethanol followed by the quaternization of various tertiary amines with the resulting 2-bromoethylglucoside [30]. Although this method is attractive due to the small number of stages, its major disadvantage is the lack

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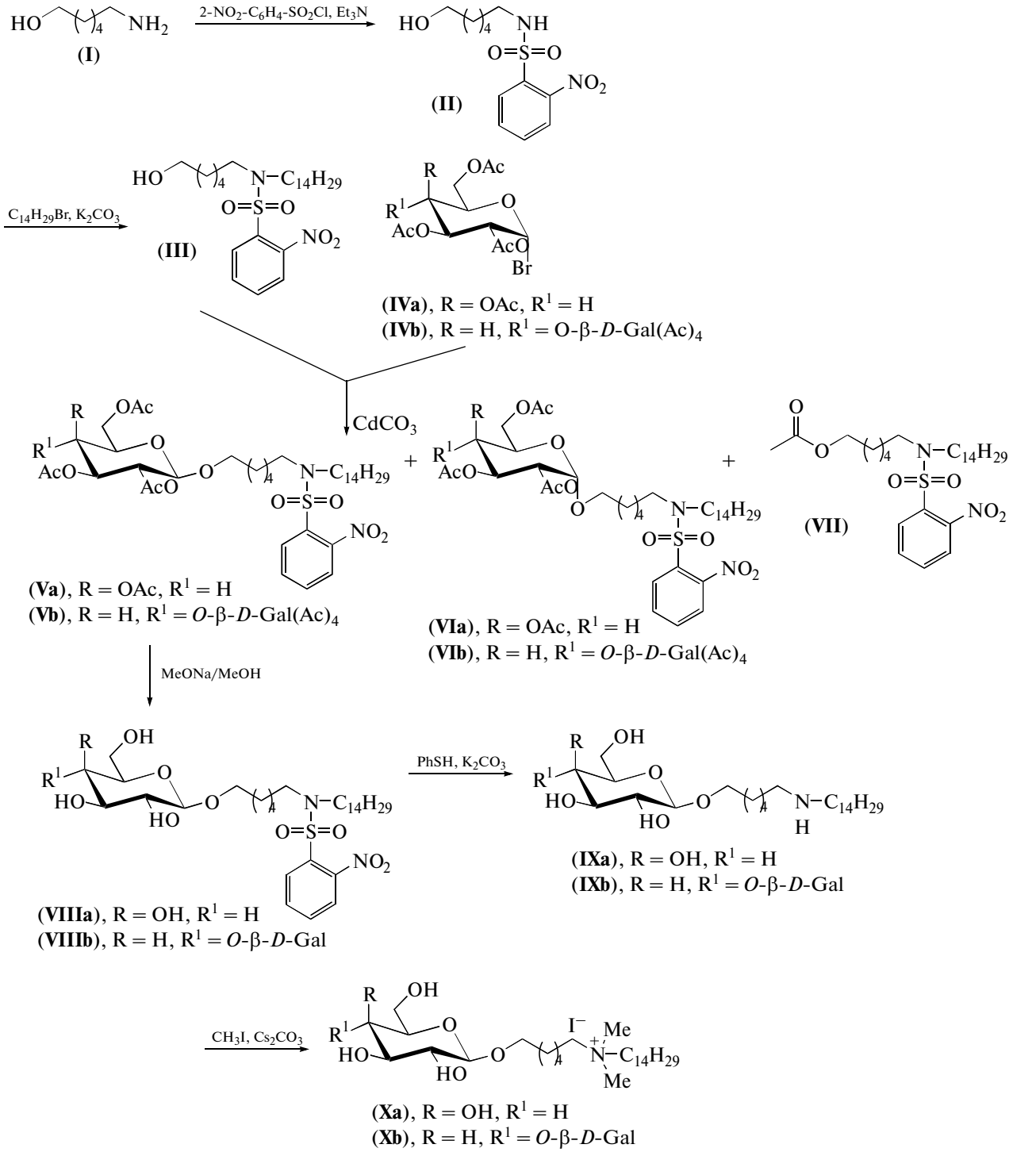
[2] Abbreviations: DDAB, dioctadecyldimethylammonium bromide; CTAB, hexadecyltrimethylammonium bromide; DODAC, dioleoyl dimethylammonium chloride.

of a universal character because of the limited choice of starting materials. A more universal synthetic scheme was described quite recently, which provided length variations of the spacer joining a positively charged group and a carbohydrate residue [27]. However, the total yield of the finished products was only 3%. We proposed another approach based on the Fukuyama reaction [31, 32], namely, the interaction of 2-nitrobenzenesulfonyl amides with alkyl halogenides followed by the removal of the 2-nitrobenzenesulfonyl group. The resulting secondary amines can be easily subjected to quaternization with various alkyl halogenides at the next stages.



Synthesis of cationic glycosurfactants.

The starting compound was 6-aminohexanol (**I**), whose amino group served as a "procationic" center of the future positively charged lipid, whereas its hydroxyl group was used for the addition of carbohydrate residues. The reaction of 6-aminohexanol (**I**) with an excess of 2-nitrobenzenesulfonyl chloride in the presence of triethylamine resulted in sulfonyl amide (**II**). Alkylation under the conditions of the Fukuyama reaction with tetradecyl bromide in the presence of potassium carbonate led to sulfonyl amide (**III**) in a yield of 87%.

The key stage of the synthesis was the glycosylation of compound (**III**) with 2,3,4,6-tetra-*O*-acetyl-α-*D*-galactosyl- (**IVa**) or 2,2',3,3',4',6,6'-hepta-*O*-acetyl-α-*D*-lactosyl bromide (**IVb**) according to the modified Koenigs–Knorr method in a Soxhlet apparatus [33] with cadmium carbonate as a promoter [33]. As glycosyl donors, we used very accessible and highly reactive acetyl bromosugars. The analysis of the reaction mixture by TLC demonstrated the formation of a complex mixture, which agreed with that earlier described in [34]. Along with the major glycosylation products, β-glycosides (**Va**) and (**Vb**), the reaction mixture contained α-anomers (**VIa**) and (**VIb**), 2-hydroxyglycosides, and acetate (**VII**).

For the minimization of the losses of the β-glycosides, the deacetylation of 2-hydroxyglycosides was performed in the reaction mixture. After column chromatography, 1,2-*trans*-glycosides (**Va**) and (**Vb**) were obtained in 62 and 49% yields, respectively. Their structures were confirmed by ^{1}H NMR spectroscopy. For example, the resonance of the anomeric proton of β-glycoside (**Va**) at 4.38 ppm and the coupling constant of 8.1 Hz implied the β-configuration of the glycoside bond. In the ^{1}H NMR spectrum of β-glycoside (**Vb**), resonances of protons at the anomeric centers were observed at 4.37 and 4.42 ppm with the coupling constants $J_{1,2}$ of 8.1 and 7.8 Hz, respectively. α-Anomers of galactoside (**VIa**) and lactoside (**VIb**) were isolated in 6% and 12% yields respectively.

At the final stage, compounds (**Va**) and (**Vb**) were *N*-desulfonylated and deacetylated and the cationic head was completed. At first, acetyl groups were removed because the preserving of the UV-detected group in the lipid molecule facilitated the purification of intermediate compounds. Deacetylation with sodium methylate in methanol resulted in 64 and 68% of products (**VIIIa**) and (**VIIIb**), respectively. The subsequent deprotection of the amino group with thiophenol in the presence of potassium carbonate gave 76 and 68% of secondary amines (**IXa**) and (**IXb**), respectively. Compounds (**IXa**) and (**IXb**) were purified by column reverse-phase chromatography and their structures were characterized by NMR and mass spectrometry. The cationic head was completed by the treatment of secondary amines (**IXa**) and (**IXb**) with methyl iodide in methylethylketone at 70°C. Cationic surfactants (**Xa**) and (**Xb**) were isolated by column chromatography on silica gel in 64 and 67% yields, respectively. Their structures were confirmed by physicochemical methods.

To summarize, we proposed a preparative method for the synthesis of carbohydrate-bearing cationic surfactants based on the Fukuyama reaction with the goal of studying their antimicrobial activity and their potential application for the preparation of transfecting agents. Although the total yield of compounds (**Xa**) and (**Xb**) was 10–13%, it can be increased by the optimization of separate stages. This approach can be used for the development of cationic amphiphils with a more complex structure, for example, comprising diglyceride and sterol residues.

EXPERIMENTAL

Distilled solvents of domestic production were used. Tetradecyl bromide, 6-aminohexanol, thiophenol, and DMF were purchased from Merck; 2-nitrobenzenesulfonyl chloride was from Aldrich. TLC was carried out on Silicagel 60 F_{254} plates (Merck) and HP TLC RP-18 F_{254S} plates (Merck). The compounds were visualized with a $Ce(SO_4)^{2-}$ phosphomolybdic acid solution followed by heating to 100°C [35]; the Dragendorff's reagent [36] or UV irradiation (254 nm) were used. For TLC, the following systems were used: 1.5 : 1 toluene–ethyl acetate (A); 2.2 : 1 petroleum ether–ethyl acetate (B); 2.5 : 1 benzene–ethyl acetate (C); 1 : 3 toluene–acetone (D); 2: 10 : 1.5 toluene–acetone–water (E); 10 : 1 methanol–water (F*); and 50 : 1 methanol–25% ammonia (G*) (* for reverse-phase silica gel). Column chromatography was performed on Kieselgel 60 (40–63 μm, Merck) and LiChroprep® RP-18 (Merck). ^{1}H NMR spectra were registered on a pulse Fourier Bruker MSL-200 or Bruker MSL-400 spectrometers in $CDCl_3$ or a $CDCl_3$–CD_3OD mixture with Me_4Si as the internal standard. Chemical shifts (δ) are given in ppm; *J*, in Hz. Mass spectra were registered on a time-of-flight Bruker Ultraflex mass spectrometer (Germany) using the method of laser–desorption ionization. Melting points were measured on a Boetius microtable (Germany). Optical rotation was measured on a photoelectric Digytor Jasco, model DIP 360, spectropolarimeter (Japan). 2,3,4,6-Tetra-*O*-acetyl-α-D-galactosyl bromide (**IVa**) or 2,2',3,3',4',6,6'-hepta-*O*-acetyl-α-*D*-lactosyl bromide (**IVb**) were obtained as described in [37, 38].

***N*-(6-Hydroxyhexyl)-2-nitrobenzenesulfonyl amide (II).** 2-Nitrobenzenesulfonyl chloride (2.65 g, 11.96 mmol) was added to a solution of 6-aminohexanol (**I**) (1.17 g, 9.98 mmol), anhydrous triethylamine (3.5 ml), and molecular sieves of 4 Å in anhydrous dichloromethane (20 ml) cooled to 0°C and the mixture was stirred for 40 h at 20°C. Molecular sieves were filtered off via Celite® 545 and washed with dichloromethane. The solvent was evaporated in a vacuum and the residue was chromatographed on silica gel eluted with 100 : 1 chloroform–methanol to give

2.41 g (80%) of compound (**II**); mp 77–78°C, R_f 0.38 (A). Found, %: C 47.74; H 6.18; N 9.17. $C_{12}H_{18}N_2O_5S$. Calc., %: C 47.67; H 6.00; N 9.27. ^{1}H NMR (200 MHz): 1.25–1.65 (8 H, m, $HNCH_2(C\underline{H}_2)_4CH_2OH$), 3.05–3.10 (2 H, t, *J* 6.4, $C\underline{H}_2NH$), 3.61 (2 H, t, *J* 6.4,$C\underline{H}_2OH$), 5.26–5.41 (1 H, m, NH), 7.71–7.80 (2 H, m), 7.83–7.90 (1 H, m), and 8.08–8.18 (1 H, m, Ar).

***N*-(6-Hydroxyhexyl)-*N*-tetradecyl-2-nitrobenzenesulfonyl amide (III).** Tetradecyl bromide (0.91 ml, 3.06 mmol) was added to a mixture of compound (**II**) (0.5 g, 1.65 mmol) and potassium carbonate (0.57 g, 4.12 mmol) in DMF (10 ml) at 60°C with intense stirring. In 2.5 h, the reaction mixture was diluted with water (30 ml) and extracted with dichloromethane (3 $Na_2SO_4 \times 20$ ml). The extract was washed with water (20 ml), dried with Na_2SO_4, filtered, and evaporated. The residue was chromatographed on silica gel eluted with a 5 : 1 petroleum ether–ethyl acetate mixture to give compound (**III**) (0.72 g, 87%); R_f 0.61 (B). Found, %: C 92.85; H 9.19; N 5.52; S 6.09. $C_{26}H_{46}N_2O_5S$. Calc., %: C 92.62; H 9.30; N 5.62; S 6.43. ^{1}H NMR (200 MHz): 0.88 (3 H, t, *J* 6.8, $(CH_2)_{13}C\underline{H}_3$), 1.17–1.38 (26 H, m, $(CH_2)_2$, $(CH_2)_{11}$), 1.47–1.66 (6 H, m, $OCH_2\underline{H}_2$, 2 $NCH_2C\underline{H}_2$), 3.22–3.33 (4 H, m, CH_2NCH_2), 3.62 (2 H, t, *J* 6.8, $C\underline{H}_2OH$), 7.58–7.73 (3 H, m) and 7.97–8.05 (1 H, m, Ar).

***N*-[6-(2,3,4,6-Tetra-*O*-acetyl-β-*D*-galactopyranosyloxy)hexyl]-*N*-tetradecyl-2-nitrobenzenesulfonyl amide (Va).** A mixture of compound (**III**) (0.4 g, 0.8 mmol) and calcined cadmium carbonate (0.28 g, 1.6 mmol) in anhydrous toluene (50 ml) was refluxed in a Soxhlet apparatus in the presence of calcined granulated silica gel. After 3 toluene passes via silica gel, 2,3,4,6-tetra-*O*-acetyl-α-*D*-galactosyl bromide (**IVa**) (0.66 g, 1.6 mmol) was added in portions for 30 min and the mixture was refluxed for 3.5 h. The reaction mixture was filtered through Celite® 545 and the solvent was removed in a vacuum. The residue was dried in the vacuum of an oil pump and diluted in anhydrous pyridine (2 ml). Acetic anhydride (0.5 ml) was added, and the mixture was kept for 4 h at 20–23°C and evaporated in a vacuum with toluene. The residue was diluted in chloroform, washed with 3% HCl (3 × 5 ml) and water to pH 7.0, and evaporated. The residue was chromatographed on silica gel eluted with an 8 : 1 toluene–ethyl acetate mixture to give 0.41 g of compound (**Va**) (62%) as yellow oil; $[\alpha]_D^{20}$ –1.5 (*c* 1, $CHCl_3$), R_f 0.6 (C). Found, %: C 57.41; H 7.80; N 3.29; S 3.99. $C_{40}H_{64}N_2O_{14}S$. Calc., %: C 57.95; H 7.78; N 3.38; S 3.87. Mass, *m/z*: 851.538 $[M + Na]^+$, 867.529 $[M + Na]^+$; ^{1}H NMR (400 MHz): 0.81 (3H, t, *J* 6.8, $(CH_2)_{13}C\underline{H}_3$), 1.12–1.28 (26 H, m, $(CH_2)_{11}$, $(CH_2)_2$), 1.37–1.53 (6 H, m, $OCH_2C\underline{H}_2$, 2 $NCH_2C\underline{H}_2$), 1.92 (3 H, s), 1.98 (6 H, s) and 2.08 (3 H, s, 4 $OCOCH_3$), 3.19 (4 H, dt, *J* 7.2, 8.1, 2 $C\underline{H}_2N$), 3.38 (1 H, dt, *J* 6.9, 9.7, $OCH\underline{H}_a$), 3.79 (1 H, dt, *J* 6.2, 9.7, $OCHH_b$), 3.81–3.86 (1 H, m, H-5 Gal), 4.06 (1 H, dd, *J* 6.9, 11.2, H_a-6 Gal), 4.15 (1 H, dd, *J* 6.5, 11.2, H_b-6 Gal), 4.38 (1 H, d, *J* 8.1, H-1 Gal), 4.95 (1 H, dd, *J* 3.4, 10.3, H-3 Gal), 5.13 (1 H, dd, *J* 8.1, 10.3, H-2 Gal), 5.31 (1 H, dd, *J* 3.4, 0.9, H-4 Gal), 7.53–7.64 (3 H, m), and 7.92–7.96 (1 H, m, Ar).

***N*-[6-(2,3,4,6-Tetra-*O*-acetyl-α-*D*-galactopyranosyloxy)hexyl]-*N*-tetradecyl-2-nitrobenzenesulfonyl amide (VIa),** 39.5 mg (6%). Found, %: C 57.53; H 7.85; N 3.27; S 4.01. $C_{40}H_{64}N_2O_{14}S$. Calc., %: C 57.95; H 7.78; N 3.38; S 3.87. ^{1}H NMR (400 MHz): 0.82 (3 H, t, *J* 6.8, $(CH_2)_{13}C\underline{H}_3$), 1.13–1.30 (26 H, m, $(CH_2)_{11}$, $(CH_2)_2$), 1.39–1.56 (6 H, m, $OCH_2C\underline{H}_2$, 2 $NCH_2C\underline{H}_2$), 1.93 (3 H, s), 1.98 (3 H, s), 2.01 (3 H, s) and 2.08 (3 H, s, 4 $OCOCH_3$), 3.20 (4 H, dt, *J* 7.5, 2 $C\underline{H}_2N$), 3.32 (1 H, dt, *J* 6.4, 9.7, $OCH\underline{H}_a$), 3.59 (1 H, dt, 76.4, 9.7, $OCHH_b$), 4.00–4.04 (2 H, m, H-6 Gal), 4.14 (1 H, ddd, *J* 1.3, 5.9, 7.3, H-5 Gal), 5.01–5.08 (2 H, m, H-1, H-2 Gal), 5.27 (1 H, dd, *J* 3.3, 10.3, H-3 Gal), 5.38 (1 H, dd, *J* 3.3, 1.3, H-4 Gal), 7.53–7.65 (3 H, m), and 7.92–7.99 (1H, m, Ar).

***N*-(6-Acetyloxyhexyl)-*N*-tetradecyl-2-nitrobenzenesulfonyl amide (VII),** 69.0 mg (16%); ^{1}H NMR (200 MHz): 0.87 (3 H, t, *J* 6.8, $(CH_2)_{13}C\underline{H}_3$), 1.15–1.35 (26 H, m, $(CH_2)_2$, $(CH_2)_{11}$), 1.42–1.65 (6 H, mm, $OCH_2C\underline{H}_2$, 2 $NCH_2C\underline{H}_2$), 2.02 (3 H, s, $COCH_3$), 3.18–3.31 (4 H, m, 2 CH_2N), 4.00 (2 H, t, *J* 6.5, $C\underline{H}_2OH$), 7.55–7.71 (3 H, m), and 7.94–8.03 (1 H, m, Ar).

***N*-[6-(2,2',3,3',4',6,6,-Hepta-*O*-acetyl-β-lactosyloxy)hexyl]-*N*-tetradecyl-2-nitrobenzenesulfonyl amide (Vb)** was obtained as described for amide (**Va**) from compound (**III**) (0.43 g, 0.86 mmol), cadmium carbonate (0.3 g, 1.73 mmol), and 2,2',3,3',4',6,6'-hepta-*O*-acetyl-α-*D*-lactosyl bromide (**IVb**) (1.21 g, 1.73 mmol). The following compounds were isolated by column chromatography on silica gel (3 : 1 toluene–ethyl acetate): diamide (**Vb**) (0.47 g, 49%) as a yellowish oil; $[\alpha]_D^{20}$ –2.5 (*c* 1, $CHCl_3$), R_f 0.47 (A). Found, %: C 55.60; H 7.25; N 2.40. $C_{52}H_{80}N_2O_{22}S$. Calc., %: C 55.90; H 7.22; N 2.51. ^{1}H NMR (400 MHz): 0.81 (3H, t, *J* 6.8, $(CH_2)_{13}C\underline{H}_3$), 1.11–1.25 (26 H, m, $(CH_2)_{11}$, $(CH_2)_2$), 1.38–1.50 (6 H, m, $OCH_2C\underline{H}_2$, 2 $NCH_2C\underline{H}_2$), 1.89 (3 H, s), 1.96 (3 H, s), 1.98 (6 H, s), 2.00 (3 H, s), 2.05 (3 H, s), 2.09 (3 H, s, 7 $OCOCH_3$), 3.15–3.22 (4 H, m, 2 CH_2N), 3.36 (1 H, dt, *J* 6.5, 9.7, $OCH\underline{H}_a$), 3.50–3.56 (1 H, m, H-5 Lac), 3.69–3.76 (2 H, m, $OCH\underline{H}_b$, H-4 Lac), 3.78–3.83 (1 H, m, H-5' Lac), 3.97–4.10 (3 H, m, H_a-6, $H_{a,b}$-6' Lac), 4.37 (1 H, d, *J* 8.1, H-1 Lac), 4.42 (1 H, d, *J* 7.8, H-1' Lac), 4.40–4.44 (1 H, m, H_b-6 Lac), 4.81 (1 H, dd, *J* 8.1, 9.3, H-2 Lac), 4.88 (1 H, dd, *J* 3.4, 10.3, H-3' Lac), 5.04 (1 H, dd, *J* 7.8, 10.3, H-2' Lac), 5.12 (1 H, t, *J* 9.3, H-3 Lac), 5.27 (1 H, d, *J* 3.4, H-4' Lac), 7.52–7.63 (3 H, m), and 7.91–7.96 (1 H, m, Ar).

***N*-[6-(2,2',3,3',4',6,6'-Hepta-*O*-acetyl-β-lactosyloxy)hexyl]-*N*-tetradecyl-2-nitrobenzenesulfonyl amide (VIb)**, yield 115.0 mg (12%); ^{1}H NMR

(400 MHz): 0.81 (3H,t, *J* 6.8, $(CH_2)_{13}C\underline{H}_3$), 1.12–1.31 (26 H, m, $(CH_2)_{11}$, $(CH_2)_2$), 1.39–1.56 (6 H, m, $OCH_2C\underline{H}_2$, 2 $NCH_2C\underline{H}_2$), 1.91 (3 H, s), 1.97 (3 H, s), 2.00 (3 H, s), 2.04 (3 H, s), 2.08 (3 H, s), 2.09 (3 H, s), 2.10 (3 H, s, 7 $OCOCH_3$), 3.14–3.25 (4 H, m, 2 CH_2N), 3.38 (1 H, dt, *J* 6.5, 9.7, $OCH\underline{H}_a$), 3.50–3.56 (1 H, m, H-5 Lac), 3.63 (1 H, dt, *J* 6.5, 9.7, $OCH\underline{H}_b$), 3.71–3.65 (1 H, m, H-4 Lac), 3.82–4.30 (6 H, m, H-5, H-5', H-6, H-6' Lac), 4.52 (1 H, d, *J* 7.8, H-1' Lac), 4.79 (1 H, dd, *J* 4.1, 10.5, H-2 Lac), 4.89–4.98 (2 H, m, H-1, H-3' Lac), 5.13 (1 H, dd, *J* 7.9, 10.5, H-2' Lac), 5.31 (1 H, dd, *J* 3.4, 1.1, H-4' Lac), 5.30 (1 H, t, *J* 10.5, H-3 Lac), 7.51–7.65 (3 H, m), and 7.90–7.98 (1 H, m, Ar);

***N*-(6-Acetoxyhexyl)-*N*-tetradecyl-2-nitrobenzenesulfonyl amide (VII)**, yield 83.6 mg (18%). $_1$H NMR (200 MHz): 0.87 (3 H, t, *J* 6.8, $(CH_2)_{13}C\underline{H}_3$), 1.15–1.35 (26 H, m, $(CH_2)_2$, $(CH_2)_{11}$), 1.42–1.65 (6 H, m, $OCH_2C\underline{H}_2$, 2 $NCH_2C\underline{H}_2$), 2.02 (3 H, s, $COCH_3$), 3.18–3.31 (4 H, m, 2 CH_2N), 4.00 (2 H, t, *J* 6.5, $C\underline{H}_2OH$), 7.55–7.71 (3 H, m) and 7.94–8.03 (1 H, m, Ar).

***N*-[6-β-*D*-galactopyranosyloxy)hexyl]-*N*-tetradecyl-2-nitrobenzenesulfonyl amide (VIIIa).** A freshly prepared solution of 1N sodium methylate (1 ml) was added to a solution of compound (**Va**) (0.41 g, 0.49 mmol) in methanol (4 ml) and the mixture was kept for 30 min at 23°C. The reaction mixture was neutralized with cation-exchange resin Amberlite IR-120 (H^+), filtered, and evaporated. The residue was chromatographed on a silica gel column eluted with a 1 : 1 toluene–acetone mixture to give 0.208 g of compound (**VIIIa**) (64%) as a yellow oil; $[\alpha]_D^{20}$ −1.0° (*c* 1, $CHC1_3$), R_f 0.4 (E). Mass, *m/z*: 683.450 $[M + Na]^+$, 699.424 $[M + K]^+$; ^{1}H NMR (400 MHz): 0.81 (3 H, t, *J* 6.8, $(CH_2)_{13}C\underline{H}_3$), 1.10–1.29 (26 H, m, $(CH_2)_{11}$, $(CH_2)_2$), 1.36–1.66 (6 H, m, $OCH_2C\underline{H}_2$, 2 $NCH_2C\underline{H}_2$), 3.15–3.24 (H, m, $2CH_2N$), 3.40–3.62 (4 H, m, H-2, H-3, H-5 Gal, $OCH\underline{H}_a$), 3.74–3.90 (3 H, m, 2 H-6 Gal, $OCH\underline{H}_b$), 3.98–4.04 (1 H, m, H-4 Gal), 4.17 (1 H, d, *J* 7.2, H-1 Gal), 7.53–7.65 (3 H, m), and 7.90–7.95 (1 H, m, Ar).

***N*-[6-β-lactosyloxy)hexyl]-*N*-tetradecyl-2-nitrobenzenesulfonyl amide (VIIIb)** was obtained similarly to compound (**VIIIa**) from (**Vb**) (0.18 g, 0.16 mmol) and 1 N sodium methylate in methanol (1 ml). Compound (**VIIIb**) (0.09 g, 68%) was isolated by chromatography on LiChroprep® RP-18 eluted with a 10 : 1 methanol–water mixture. $[\alpha]_D^{20}$ −1.5° (*c* 1, $CHC1_3$), R_f 0.45 (F). Found, %: C 54.58; H 8.31; N 3.07. $C_{38}H_{66}N_2O_{15}S \times 1/2\ H_{20}$. Calc., %: C 54.86; H 8.12; N 3.37. ^{1}H NMR (400 MHz): 0.80 (3 H, t, *J* 6.7, $(CH_2)_{13}C\underline{H}_3$), 1.12–1.29 (26 H, m, $(CH_2)_{11}$, $(CH_2)_2$), 1.38–1.57 (6 H, m, $OCH_2C\underline{H}_2$, 2 NCH_2CH_2), 3.15–3.23 (4 H, m, 2 CH_2N), 3.24–3.85 (14 H, m, Lac protons, OCH_2), 4.21 (1 H, d, *J* 7.8, H-1 Lac), 4.29 (1 H, d, *J* 7.5, H-1' Lac), 7.53–7.67 (3 H, m) and 7.89–7.93 (1 H, m, Ar).

6-(*N*-Tetradecylamino)hexyl-β-*D*-galactopyranoside (IXa). Potassium carbonate (0.25 g, 1.8 mmol) and thiophenol (0.18 ml, 1.8 mmol) were added to a solution of compound (**VIIIa**) (0.12 g, 0.18 mmol) in DMF (10 ml) under stirring. In 2 h, the mixture was filtered through Celite® 545 and the solvent was evaporated in a vacuum. The residue was chromatographed on a LiChroprep® RP-18 column eluted with a 5 : 1 methanol–5% ammonia mixture to give 0.066 g (77%) of compound (**IXa**), $[\alpha]_D^{20}$ +12.7° (*c* 1, 1 : 1 $CHC1_3$–CH_3OH), R_f 0.5 (D). Mass, *m/z*: 475.769 $[M]^+$ 513.817 $[M + K]^+$; ^{1}H NMR (400 MHz): 0.80 (3 H, t, *J* 6.8, $(CH_2)_{13}C\underline{H}_3$), 1.15–1.35 (26 H, m, $(CH_2)_{11}$, $(CH_2)_2$), 1.39–1.50 (4 H, m, 2 $NCH_2C\underline{H}_2$), 1.51–1.60 (2 H, m, $OCH_2C\underline{H}_2$), 2.52–2.58 (4 H, m, 2 NCH_2), 3.07–3.19 (3 H, m, H-2, H-3, H-5 Gal), 3.42 (1 H, dt, *J* 6.5, 9.7, $OCH\underline{H}_a$), 3.67 (2 H, d, *J* 6.2, 2 H-6 Gal), 3.76 (1 H, m, H-4 Gal), 3.80 (1 H, dt, *J* 6.5, 9.7, $OCH\underline{H}_b$), 4.20(1 H, d, *J* 7.8, H-1 Gal).

6-(*N*-Tetradecylamino)hexyl-β-lactopyranoside (IXb) was obtained similarly to compound (**IXa**) from sulfonyl amide (**VIIIb**) (0.22 g 0.267 mmol), potassium carbonate (0.41 g, 3.0 mmol), and thiophenol (0.4 ml, 3.0 mmol). Compound (**IXb**) (0.115 g, 68%) was isolated by chromatography on a LiChroprep RP-18 column eluted with 5 : 100 : 1 chloroform–methanol–5% ammonia, $[\alpha]_D^{20}$ −1.2° (*c* 1, CH_3OH), R_f 0.45 (G). Mass, *m/z*: 676.524 $[M + K]^+$; ^{1}H NMR (400 MHz): 0.80 (3 H, t, *J* 6.9, $(CH_2)_{13}C\underline{H}_3$), 1.17–1.35 (26 H, m, $(CH_2)_{11}$, $(CH_2)_2$), 1.36–1.58 (6 H, m, 2 $NCH_2C\underline{H}_2$, $OCH_2C\underline{H}_2$), 2.42–2.48 (4 H, m, 2 NCH_2), 3.14–3.82 (14 H, m, Lac protons, OCH_2), 4.19 (1 H, d, *J* 7.8, H-1 Lac), 4.29 (1 H, d, *J* 7.5, H-1' Lac).

***N*-[6-β-*D*-galactopyranosyloxy)hexyl]-*N,N*-dimethyl-*N*-tetradecylammonium iodide (Xa).** Cesium carbonate (0.055 g, 0.17 mmol) and CH_3I (1 ml) were added to a solution of compound (**IXa**) (0.046 g, 0.097 mmol) in methylethylketone (1.5 ml) and the mixture was stirred for 3 h at 70°C. The reaction mixture was filtered off, the solvent was evaporated in a vacuum, and the residue was chromatographed on a LiChroprep® RP-18 column eluted with 5 : 1 methanol–5% ammonia to give 0.039 g (64%) of compound (**Xa**), $[\alpha]_D^{20}$ +14.2° (*c* 1, CH_3OH), R_f 0.8 (D). Mass, *m/z*: 504.416 $[M - I]^+$. ^{1}H NMR (400 MHz): 0.82 (3 H, t, *J* 6.8, $(CH_2)_{13}C\underline{H}_3$), 1.19–1.48 (26 H, m, $(CH_2)_{11}$, $(CH_2)_2$), 1.55–1.68 (m, 2 H, $OCH_2C\underline{H}_2$), 1.69–1.74 (m, 4 H, 2 $NCH_2C\underline{H}_2$), 3.01 (6 H, s, $N(CH_3)_2$), 3.20–3.59 (8 H, m, 2 N^+CH_2, H-2, H-3, H-5 Gal, $OCH\underline{H}_a$), 3.65–3.69 (2 H, m, 2 H-6 Gal), 3.78 (1 H, m, H-4 Gal), 3.84 (1 H, dt, *J* 6.5, 9.9, $OCH\underline{H}_b$), 4.15 (1 H, d, *J* 7.2, H-1 Gal).

***N*-[6-β-Lactopyranosyloxy)hexyl]-*N,N*-dimethyl-*N*-tetradecylammonium iodide (Xb)** was obtained similarly to compound (**Xa**) from lactoside (**IXb**) (0.059 g,

0.092 mmol), cesium carbonate (0.163 g, 0.50 mmol), and CH_3I (1 ml). Compound (**Xb**) (0.049 g, 67%) was isolated by chromatography on a LiChroprep® RP-18 column eluted with a 5 : : 1 methanol–water mixture; R_f 0. 5 (E). Mass, *m/z*: 666.536 $[M - I]^+$. ^{1}H NMR (400 MHz): 0.81 (3 H, t, *J* 6.9, $(CH_2)_{13}CH_3$), 1.18–1.39 (26 H, m, $(CH_2)_{11}$, $(CH_2)_2$), 1.49–1.79 (6 H, m, 2 $NCH_2C\underline{H}_2$, $OCH_2C\underline{H}_2$), 3.05 (6 H, s, $N(CH_3)_2$), 3.10–3.87 (18 H, m, Lac protons, OCH22, 2 N^+CH_2), 4.15 (1 H, d, *J* 7.8, H-1 Lac), 4.27 (1 H, d, *J* 7.5, H-1' Lac).

ACKNOWLEDGMENTS

The work was supported by the Russian Foundation for Basic Research, project no. 07-03-00632a, and federal program "Scientific and Teaching Personnel of Innovative Russia" (contract no. P715).

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