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Novel and convergent synthesis of modified glycosphingolipids, galactosyl-5-aza-sphinganines, by a diversity-oriented method

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ABSTRACT

The stereocontrolled synthesis of β-galactosyl-5-aza-sphinganines was accomplished with high efficiency by a novel and convergent strategy. The truncated β-galactosphinganine **5** was easily acylated with different long chains. Moreover, the simple conversion of thiazole to formyl enabled 5-aza-alkylchains to be introduced via an amino-reduction reaction. The overall yield for the synthesis of β-galacto-5-aza-sphinganines **9** and **13** was about 30–35% starting from **5**. Preliminary results concerning the β-transgalactosylation of a truncated sphinganine catalyzed by galactosidases are also presented and discussed.

1. Introduction

β-Galactosyl ceramides (β-GalCer), an important class of glycolipids are abundant in the myelin sheath where they are constituted by a large variety of galactosphingolipids differing in chain length, with or without alkene structures and having hydroxyl groups either in the main chain or in the acyl chain.[1] Since the building of myelin requires an interaction between cerebroside and cerebroside sulfate,[2] the lack of synthesis of these compounds is responsible for many neurodegenerative pathologies.[3] Alterations in the metabolism of sphingolipids or glycosphingolipids are mainly disorders of the degradation of these compounds caused by defects in the system of lysosomal sphingolipid degradation, with subsequent accumulation of non-degradable storage material in organs.[4] β-GalCer is also known to act as an alternative ligand for the HIV-1 glycoprotein gp120 responsible for the entry of the virus into epithelial cells.[5,6] In addition, β-GalCer derivatives could act as possible ligands for the adhesion of *Helicobacter pylori* to cells in the gastric system.[7] While glycosphingolipids are present in animals, glycophytosphingolipids are mainly found in plants and sometimes in bacteria and marine organisms. Unlike sphingolipids, phytosphingolipids have a saturated lipidic chain (generally C_{18}). These kinds of GalCer, which are constituents of cell membranes, also play important biological roles. For example, the α-galactosyl form of D-*ribo*-phytosphingosine (known as KRN7000), extracted from a marine sponge, exhibits potent immunostimulatory properties, being able to activate natural killer T (NKT) cells to produce cytokines.[8]

Due to the biological significance of β-GalCer derivatives, many strategies have been proposed for their synthesis[9–12] and the need for a large library of derivatives has arisen. However, many reported syntheses suffer from a lack of flexibility to access various chain lengths or functionalized chains.[13] Thus, recent methods have involved in their first steps, the coupling of an activated glycoside moiety with truncated precursors of sphingoids acting as acceptors. Then, the truncated galactosylceramide precursor thus obtained is (i) *N*-acetylated and (ii) alkylated, or vice versa. Among the acceptors designed for this purpose, olefinic ones have been extensively used (this is the case, for example, of the truncated D-*ribo*-sphingosine, (2*S*,3*S*,4*R*)-2-aminohex-5-ene-1,3,4-triol) because they enable long alkene chains to be introduced via olefinic cross-metathesis with a high *E*-stereoselectivity.[14–22] Numerous methods are available to synthesize truncated sphingoids, generally starting from natural chiral sources (mainly carbohydrates and amino-acids)[5,23–31] or from asymmetric C–C bond formation.[10,32–34] Using this later approach, a very efficient diastereoselective strategy described by Dondoni et al. afforded optically pure sphingoid precursors bearing a thiazole ring.[35,36] Since the thiazole is a 'masked' aldehyde group,[37,38] this strategy allows the introduction of an olefinic long chain via Wittig reactions. Unfortunately, the relatively low yields and stereoselectivity of the Wittig reaction have limited the application of this method. However, this approach was used for the synthesis of D-*erythro*-C_{20}-sphingosine and of D-*ribo*-C_{18}-phytosphingosine.[39] Curiously, this powerful strategy used for the preparation of ceramides has not been applied to the synthesis of

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glycoceramides. With the aim of extending the library of available β-GalCer derivatives, we report here the synthesis of diversely substituted β-galactosyl-5-aza-sphinganines using the approach developed by Dondoni. The choice of β-galactosyl-5-aza-sphinganines as target compounds was motivated by the following reasons:

(i) their greater water solubility compared to the parent β-galactosyl sphinganines.
(ii) an easier synthesis than that of β-galactosyl sphingosides since these compounds are devoid of a 4-hydroxy group.

(iii) recent work has shown that the presence of the 4-hydroxy group is not absolutely necessary to obtain galactoceramides able to stimulate iNKT cells.[40]

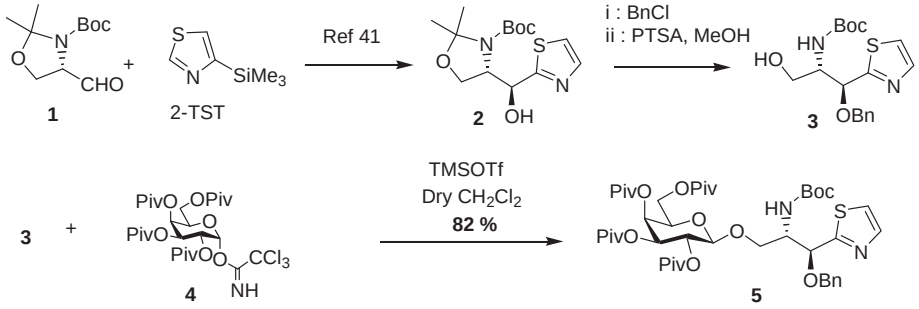
2. Results and discussion

The synthetic pathways and the β-galactosyl-5-aza-sphinganines prepared are described in Schemes 1–3.

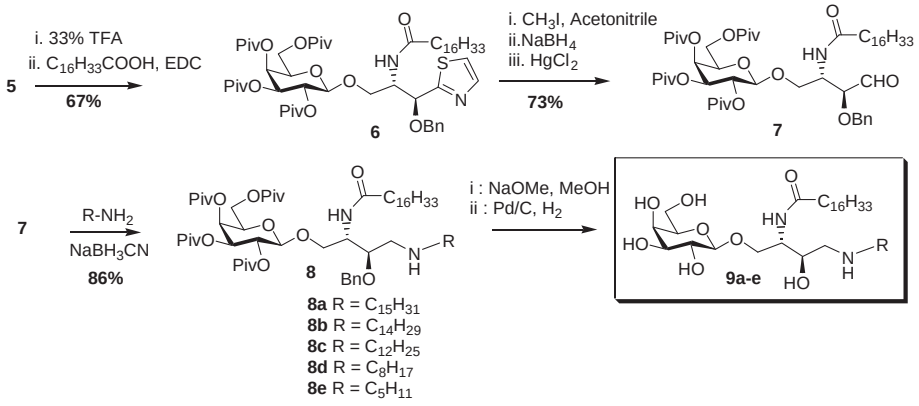
2-(Trimethylsilyl)thiazole (2-TST) was prepared according to a well known procedure starting from commercially available 2-bromothiazole via ^{n}BuLi lithiation and trimethylsilylation.[41] Then, the highly diastereoselective condensation of 2-TST with Garner's aldehyde **1** afforded the *O*-,*N*-protected (2*S*,3*S*)-2-amino-3-thiazolylpropan-1,3-diol **2** (de=0.92), the later being easily purified by crystallization.[35] Next, the 2-hydroxyl group was benzylated and further cleavage of the *O*-,*N*-acetonide in the presence of catalytic amounts of PTSA in methanol afforded **3**. This compound was used as an acceptor in the coupling reaction with 2,3,4,6-tetra-*O*-pivaloyl-α-D-galactopyranosyl trichloroacetimidate **4**.[42] Using TMSOTf as a catalyst in dry CH_2Cl_2, the target (2*S*,3*S*)-3-*O*-benzyl-2-*tert*-butyloxycarbonylamino-1-*O*-(2,3,4,6-tetra-*O*-pivaloyl-β-D-galactopyranosyl)-3-(thiazol-2-yl)-1,3-propanediol **5** was obtained in good yield (82%) with high stereoselectivity since the presence of the α-anomer was not observed on the ^{1}H and ^{13}C NMR spectra. Starting from **5**, we developed two methods to obtain β-galactosyl-5-aza-sphinganines. The first one, shown in Scheme 2 enabled the heptadecanoyl chain to be introduced after Boc deprotection and the reaction of heptadecanoïc acid with the amino group in the presence of EDC.

The (2*S*,3*S*)-3-*O*-benzyl-2-heptadenanoylamino-1-*O*-(2,3,4,6-tetra-*O*-pivaloyl-β-D-galactopyranosyl)-3-(thiazol-2-yl)-1,3-propanediol **6** thus obtained in good yields was then submitted to a thiazolyl to formyl transformation.[43] The amination reduction reaction carried out on the formyl group of (2*S*,3*S*)-2-*O*-benzyl-3-heptadecanoylamino-4-*O*-(2,3,4,6-tetra-*O*-pivaloyl-β-D-galactopyranosyl)-butanal **7** in the presence of diverse primary aliphatic amines (hexanamine to pentadecanamine) in reductive medium afforded the corresponding (2*S*,3*R*)-5-aza-3-*O*-benzyl-2-heptadecanoylamino-1-*O*-(2,3,4,6-tetra-*O*-pivaloyl-β-D-galactopyranosyl)-1,3-alcanediols **8** in very good conditions. Then, the deprotection of **8** was achieved by the removal of the pivaloyl groups (NaOMe/MeOH) followed by the Pd/C catalyzed hydrogenation to cleave the benzyl group. Previous attempts to reverse the order of the two operations were unsuccessful, probably due to the bulkiness of compounds **8**. Even when carried out in the correct order, the reduction still remained a slow rate reaction (see Experimental section). Nevertheless, the deprotection of **8** afforded (2*S*,3*R*)-5-aza-2-heptadecanoylamino-1-*O*-(β-D-galactopyranosyl)-1,3-alcanediols **9** in nearly quantitative yields.

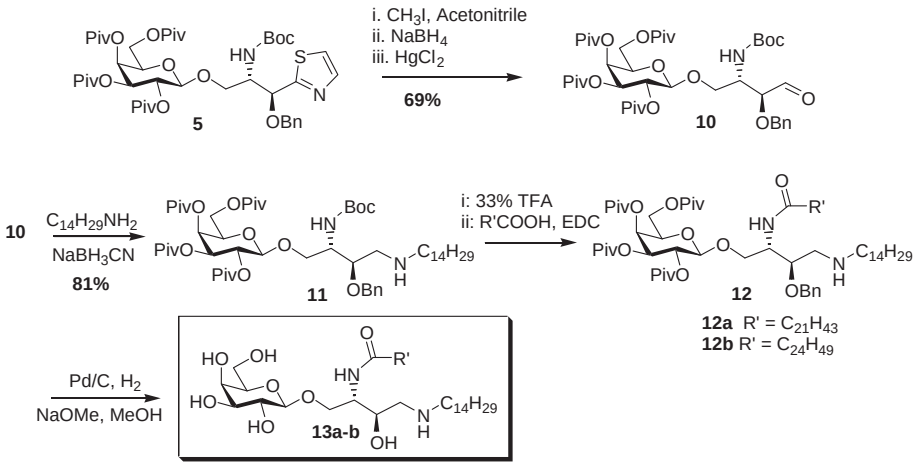
Starting from **5**, we also developed a second strategy, which consisted of i: transforming the thiazole into formyl followed by the subsequent amination–reduction introduction of the fatty chain and ii: acylation of the amino group with different chain lengths (see Scheme 3). Whichever method was used, the overall yields (around



Scheme 1. Synthesis of truncated β-D-galactosylsphinganine **5**.



Scheme 2. Synthesis of β-D-galactosyl-5-aza-ceramides **9** via the introduction of (i) the acyl chain and (ii) the 5-aza-alkyl chain.

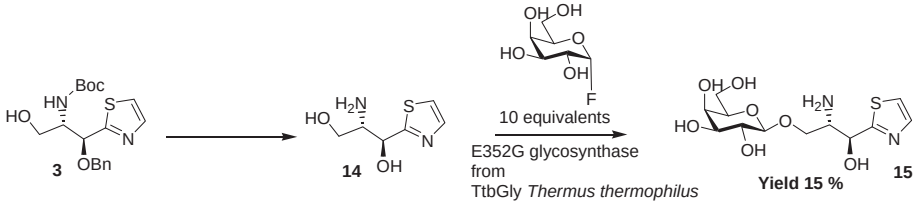


Scheme 3. Synthesis of β-D-galactosyl-5-aza-ceramides **9** via the introduction of (i) the 5-aza-alkyl chain and (ii) the acyl chain.

32–37% starting from **3**) for compounds **9** and **12** remained almost identical.

Our results clearly indicate the power of Dondoni's approach to prepare truncated sphingoids. Obviously, one of the limitations of the method still remains in the stereoselectivity of the glycosyl/truncated sphingoid coupling. Although the NMR spectra of compounds **5** revealed the absence of the α-anomer, the presence of small amounts (<0.1%) of the latter cannot be excluded. This is an important point to consider for therapeutic applications of galactosylceramide since in some cases α-anomers were 1000-fold more active than the corresponding β-ones. Thus, traces of α-anomers and not the major acceptors in transglycosylation reactions catalyzed by β-galactosidases (see Scheme 4). We have tested this idea by using the mutant E382G glycosynthase from TtβGly *Thermus thermophilus* β-galactosidase prepared in our laboratory,[47] in the presence of α-galactosyl fluoride as a donor. The (2*S*,3*S*)-2-amino-1-*O*-(β-D-galactopyranosyl)-3-(thiazol-2-yl)-1,3-propanediol **15** was thus obtained as a single regioisomer with a disappointing 15% yield, despite the use of a 10-fold amount of α-galactosyl fluoride (see Scheme 4).

Moreover, prohibitive quantities of the enzyme (see Experimental section) had to be used in order to compete with the spontaneous hydrolysis of the α-galactosyl fluoride. In our efforts to



Scheme 4. Attempts to synthesize unprotected truncated β-D-galactosylsphinganine **15** via glycosynthase catalysis.

β-anomers could be responsible for the activity. From this point of view, only enzymatic reactions can provide absolute stereoselectivity. Clearly, hypothetical α-anomers present in compounds **9** for instance could be removed by hydrolysis catalyzed by an α-galactoceramidase. We shall perform this experiment in the near future after testing the possible therapeutic activity of our β-galactosyl-5-aza-sphinganines. A most promising strategy would be to use the transglycosylation power of β-galactoceramidases to build the β-glycosidic bond with complete stereoselectivity. Moreover, this 'in water' strategy offers the possibility of reducing the number of steps in organic solvents. While retaining glycoceramidases were able to accept some simple alcohols (like ethanol, for example), these glycosyl hydrolases could not transfer ceramides due to their insolubility in water. Meanwhile, Withers et al. recently showed that glycosynthases created from *Rhodococcus* sp. *endo*-glycoceramidase (EGCII) were able to transfer D-*erythro*-sphingosine to G_{M3} oligosaccharyl α-fluoride with high yields.[44] Unfortunately, due to the high lipid chain specificity of this enzyme, these glycosynthases did not enable a library of glycoceramides to be synthesized. For example, the replacement of D-*erythro*-sphingosine by phytosphingosine resulted in a 10,000-fold decrease of the k_{cat}/K_m value.[45] However, a directed evolution approach applied to the E351S glycosynthase led to variants that were able to utilize phytosphingosine at improved rates.[46] A more general enzymatic approach could involve using truncated sphingoids, like 2-amino-3-thiazolylpropan-1,3-diol **14** as provide glycosidases possessing high transglycosylation power, we have recently developed new directed evolution strategies leading to mutant glycosidases, called 'transglycosidases' since they are devoid of their original hydrolytic activity.[48,49] This method will be applied to generate an enzyme capable of catalyzing this target reaction more efficiently.

In summary, we have shown that the use of truncated thiazolylsphinganines, prepared according to Dondoni's procedure provides a general and flexible approach to the synthesis of diversely substituted β-galactosyl-5-aza-sphinganines. The acyl chain can be extended to the desired length as well as the 5-aza-alkyl chain via an amino-reduction reaction. The amino group present in the alkyl chain of these new β-D-GalCer should improve their solubility in water. Some promising developments of the method using β-galactosidases as catalysts in the glycosylation step have also been presented.

3. Experimental

3.1. General

All reactions were monitored by thin-layer chromatography (TLC) on silica gel 60 F_{254} with detection by fluorescence and/or by charring followed by immersion in a 10% ethanolic solution of sulfuric acid. An orcinol dip, prepared by the careful addition of sulfuric acid (20 mL) to

an ice-cold solution of 3,5-dihydroxytoluene (360 mg) in ethanol (250 mL) and water (10 mL), was used to detect deprotected compounds by charring. All reagents and solvents were dried prior to use according to standard methods. Commercial reagents were used without further purification unless otherwise stated. Flash chromatography was performed with silica gel 230–400. Optical rotation was measured at the sodium D-line at room temperature. ^{1}H and ^{13}C NMR spectra were recorded on a spectrometer (Bruker) at 400 MHz and 500 MHz. Assignment of ^{1}H and ^{13}C spectra was achieved by means of conventional 2D experiments (COSY, HMQC, HOHAHA, and TOCSY). In the case of β-glycolipid, ^{1}H NMR parameters were denoted as H for the galactosyl moiety and H′ for sphinganine. For ^{13}C NMR assignment, similar primes were used.

The thiazolyl compound **2** was prepared according to Dondoni's procedure.[35,36]

3.2. (2*S*,3*S*)-3-*O*-Benzyl-2-*tert*-butyloxycarbonylamino-3-(thiazol-2-yl)-1,3-propanediol 3

A catalytic amount of *p*-toluenesulfonic acid (150 mg, 0.87 mmol) was added to a solution of acetonide **2** (1.75 g, 4.32 mmol) in methanol (20 mL) and the reaction mixture was stirred at room temperature for 3 h. Then, methanol was removed at low temperature under reduced pressure. The residue was dissolved in ether, and the organic layer was washed with aq $NaHCO_3$ (5%), brine, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified on silica gel column with ethyl acetate in hexane (1:2) as an eluent. Pure compound **3** (1.42 g, 90%, 3.89 mmol) was thus obtained as a white solid. Mp 122 °C. $[\alpha]_D^{20}$+36° (*c* 1.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82 (d, 1H, *J*=3.24 Hz, thiazole-*H*), 7.39 (m, 6H), 5.19 (br s, 1H, N*H*), 5.01 (d, 1H, *J*=5.32 Hz, H-3), 4.78 (d, 1H, *J*=11.6 Hz, $C_6H_5CH_2$), 4.62 (d, 1H, *J*=11.6 Hz, $C_6H_5CH_2$), 4.16 (m, 1H, H-2), 3.96 (dd, 1H, *J*=3.4 Hz, 1-H_a), 3.69 (dd, 1H, *J*=4.5 Hz, 1-H_b), 1.41 (s, 9H, 3× CH_3). ^{13}C NMR (400 MHz, $CDCl_3$): δ 170.8 (*C*O of Boc), 155.7 (C-3), 142.9, 137.1, 128.8, 128.3, 119.9, 79.7, 72.9, 62.3, 55.2 ($C(CH_3)_3$), 28.5 ($C(CH_3)_3$). Elemental analysis: calcd for $C_{18}H_{24}N_2O_4S$ (364.14): C 59.32, H 6.64, N 7.69, S 8.80; found C 59.13, H 6.57, N 7.58, S 8.91.

3.3. (2*S*,3*S*)-3-*O*-Benzyl-2-*tert*-butyloxycarbonylamino-1-*O*-(2,3,4,6-tetra-*O*-pivaloyl-β-D-galactopyranosyl)-3-(thiazol-2-yl)-1,3-propanediol 5

To a solution of 2,3,4,6-tetra-*O*-pivaloyl-β-D-galactopyranose (2.8 g, 5.42 mmol) in dry CH_2Cl_2 (30 mL), CCl_3CN (2.7 mL, 27.0 mmol), and DBU (405 μL, 2.71 mmol) were added. The mixture was stirred at room temperature for 25 min and the solvents were evaporated. The residue was loaded on a flash silica gel column and eluted with *n*-hexane/EtOAc (15:1) to afford pure trichloroacetamidate **4** (2.26 g, 63%). Then, a mixture of acceptor **3** (500 mg, 1.37 mmol), donor **4** (2.26 g, 3.42 mmol) and activated 4 Å molecular sieves (1 g) in dry CH_2Cl_2 (50 mL) was stirred under nitrogen atmosphere for 1 h. TMSOTf (61 μL, 0.342 mmol) was added and the mixture was stirred for 3 h at −15 °C. When TLC (*n*-hexane/EtOAc, 4:1) showed complete consumption of the acceptor, the mixture was filtered through a pad of Celite® and the filtrate was diluted with CH_2Cl_2 (20 mL) and washed successively with aq $NaHCO_3$ (2×30 mL) and brine (30 mL). The organic layer was separated, dried (Na_2SO_4), and evaporated to give a syrup. The crude product was purified by flash chromatography using *n*-hexane/EtOAc (6:1) to afford pure **5** (350 mg, 0.405, 82%) as a colorless foam. $[\alpha]_D^{25}$ +66° (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.75, 7.38 (2d, 2H, *J*=3.12 Hz, Thiazole-H), 7.31 (m, 5H, C_6H_5), 5.79 (d, 1H, *J*=2.9 Hz, H-4), 5.09 (dd, 1H, *J*=7.4, 10.4 Hz, H-2), 5.07 (dd, 1H, *J*=2.9, 10.4 Hz, H-3), 4.86 (d, 1H, *J*=8.0 Hz, H′-3), 4.62, 4.52 (2d, 2H, *J*=11.4 Hz, $CH_2C_6H_5$), 4.49 (d, 1H, *J*=8.0 Hz, H-1), 4.38 (m, 1H, H′-2), 4.22 (m, 2H, H-6a, H-5), 3.98 (m, 2H, H-6b, H′-1_{ab}), 3.51 (dd, 1H, *J*=3.7, 8.53 Hz, H-6b), 1.33 (s, 9H, (CO)OC(CH_3)$_3$), 1.24, 1.20, 1.18, 1.06 (4s, 36H, 4× COC(CH_3)$_3$). ^{13}C NMR (400 MHz, $CDCl_3$): δ 177.9 (2), 177.8, 177.3 (4× *C*OC(CH_3)$_3$),176.0, 163.7 ((*C*O)OC(CH_3)$_3$), 137.2, 129.3, 128.4 (2), 128.3, 128.0, 120.2, 119.7, 101.1 (C-1), 80.13, 78.05, 77.2 (C′-2), 72.9 ($CH_2C_6H_5$), 71.4 (C-5), 71.0 (C-3), 70.8 (C-2), 69.0 (C′-1), 66.6 (C-4), 61.0 (C-6), 59.6 (C′-2), 54.4, 41.1, 39.0, 38.9, 38.8, 38.7, 28.1, 27.1, 27.0 (3), 24.8. Elemental analysis: calcd for $C_{44}H_{66}N_2O_{13}S$ (862.42): C, 61.23; H, 7.71; N, 3.25; S, 3.72; found C, 61.31; H, 7.68; N, 3.30; S, 3.65.

3.4. (2*S*,3*S*)-3-*O*-Benzyl-2-heptadecanoylamino-1-*O*-(2,3,4,6-tetra-*O*-pivaloyl-β-D-galactopyranosyl)-3-(thiazol-2-yl)-1,3-propanediol 6

Compound **5** (350 mg, 0.405 mmol) and 50% TFA in CH_2Cl_2 (10 mL) were stirred for 3 h. When TLC showed complete conversion of the starting material, the solution was diluted with dichloromethane (25 mL) and washed successively with ice-cold aq $NaHCO_3$ (2×25 mL) and brine (25 mL). The organic layer was separated, dried over $MgSO_4$, and evaporated under vacuum. The crude amine resulting from the Boc deprotection was used in the next step without any further purification.

To a solution of the crude amine and heptadecanoic acid (220 mg, 0.810 mmol) in dry DMF (10 mL), was added EDC (107 μL, 0.607 mmol) at 0 °C under a dry nitrogen atmosphere. The reaction mixture was kept at 0 °C for 30 min and stirred overnight at room temperature. After removal of the solvent, the residue was dissolved in dichloromethane (30 mL) and washed with brine (2×15 mL). The organic layer was separated, dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was purified by flash chromatography using *n*-hexane/EtOAc (2:1) to afford pure **6** (315 g, 75%) as a white solid. Mp 81 °C. $[\alpha]_D^{20}$ +5.6 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.73, 7.38 (2d, 2H, *J*=3.12 Hz, Thiazole-H), 7.34 (m, 5H, C_6H_5), 5.42 (d, 1H, *J*=2.9 Hz, H-4), 5.22 (dd, 1H, *J*=7.7, 10.4 Hz, H-2), 5.13 (dd, 1H, *J*=3.2, 10.4 Hz, H-3), 4.99 (d, 1H, *J*=8.4 Hz, H′-3), 4.63, 4.59 (2d, 2H, *J*=11.4 Hz, $CH_2C_6H_5$), 4.56 (d, 1H, *J*=7.7 Hz, H-1), 4.51 (m, 1H, H′-2), 4.33 (dd, 1H, *J*=3.1, 9.4 Hz, H′-1a), 4.1 (dd, 1H, *J*=6.5, 10,8 Hz, H-6a), 4.05 (dd, 1H, *J*=7.3, 10.8 Hz, H-6b), 3.97 (m, 1H, H-5), 3.67 (dd, 1H, *J*=3.5, 9.5 Hz, H′-1b), 2.02 (m, 2H, $COCH_2$), 1.43 (m, 2H, $COCH_2CH_2$), 1.27 (m, 35H), 1.18, 1.17, 1.13 (3s, 27H, 3× COC(CH_3)$_3$), 0.89 (t, 3H, CH_3). ^{13}C NMR (400 MHz, $CDCl_3$): δ 177.9, 177.4, 176.9 (2) (4× *C*OC(CH_3)$_3$), 172.74 (*C*O$C_{16}H_{33}$), 171.2 (C′-3), 141.7, 137.7, 128.6 (2), 128.1 (3), 120.2, 101.3 (C-1), 77.3 (C′-1), 73.0 ($CH_2C_6H_5$), 71.2 (C-5), 71.0 (C-3), 69.2 (C-2), 67.8 (C′-3), 66.7 (C-4), 61.1 (C-6), 53.0 (C′-2), 36.7 ($COCH_2$), 25.5 ($COCH_2CH_2$), 29.4, 27.1, 14.2 (CH_3). Elemental analysis: calcd for $C_{56}H_{90}N_2O_{12}S$ (1015.38): C 66.24, H 8.93, N 2.76, S 3.16; found C 66.31, H 9.08, N 2.74, S 3.16.

3.5. Thiazolyl to formyl conversion: synthesis of (2*S*,3*S*)-2-*O*-benzyl-3-heptadecanoylamino-4-*O*-(2,3,4,6-tetra-*O*-pivaloyl-β-D-galactopyranosyl)-butan-1-al 7

To a solution of compound **6** (300 mg, 0.296 mmol) in dry CH_3CN (5 mL), MeI (184 μL, 2.963 mmol) was added and the solution was refluxed for 12 h. When TLC showed complete conversion of the starting material, the solvents were evaporated and dried over vacuum. Next, the methylated thiazolyl ammonium iodide was redissolved in dry methanol (5 mL) and $NaBH_4$ (22 mg, 0.592 mmol) was added at 0 °C. The mixture was kept for 5 min at this temperature, warmed to room temperature and stirred for 30 min. Then, methanol was evaporated and the crude product was dissolved in 20 mL of CH_3CN/H_2O (8:2). After the introduction of $HgCl_2$ (66 mg, 0.246 mmol), the mixture was stirred at room temperature for 1 h. Then, the solvents were evaporated and the residue was portioned between EtOAC (30 mL) and water (30 mL). The

organic layer was separated, washed with brine (2×30 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified on a silica gel column using *n*-hexane/EtOAc (3:1) to afford pure aldehyde **7** (207 mg, 73%) as a colorless oil. $[\alpha]_D^{20}$ +27° (*c* 1.5, CH_2Cl_2). ^1H NMR ($CDCl_3$, 400 MHz): δ 9.56 (1H, CHO), 7.35 (m, 5H, C_6H_5), 5.79 (d, 1H, *J*=8.4 Hz, N*H*), 5.42 (d, 1H, *J*=3.12 Hz, H-4), 5.19 (dd, 1H, *J*=7.55, 10.47 Hz, H-2), 5.13 (dd, 1H, *J*=3.14, 10.47 Hz, H-3), 4.65, 4.59 (2d, 2H, *J*=11.43 Hz, C$H_2C_6H_5$), 4.54 (d, 1H, *J*=7.52 Hz, H-1), 4.46 (m, 1H, H′-3), 4.14 (m, 2H, H-5, H′-2), 4.05, 3.96 (dd, 2H, *J*=7.14, 10.89 Hz, H-6ab), 3.85 (dd, 1H, *J*=3.09, 7.9 Hz, H′-4a), 3.64 (dd, 1H, *J*=4.38, 9.59 Hz, H′-4b), 2.09 (t, 2H, COCH_2), 1.58 (m, 2H, $COCH_2CH_2$), 1.28 (m, 36H), 1.19, 1.14 (3s, 27H, 3× COC(CH_3)$_3$), 0.90 (t, 3H, CH_3). ^{13}C NMR (400 MHz, $CDCl_3$): δ 200.5 (*C*HO), 177.7, 177.1, 176.8, 176.7 (4× *C*OC(CH_3)$_3$), 172.8 ($COCH_2CH_2$), 136.9, 128.6 (2), 128.3, 128.1 (2) (C_6H_5), 101.0 (C-1), 82.4 (C′-2), 77.2, 73.5 ($CH_2C_6H_5$), 71.2 (C-3), 70.6 (C-2), 69.0, 66.8, 66.5, 60.9, 48.6, 39.0, 38.8, 38.7, 38.6, 36.5, 31.8, 29.6, 29.4, 29.3, 27.1, 27.0, 25.3, 22.6, 14.0 (*C*H_3). Elemental analysis: calcd for $C_{54}H_{89}NO_{13}$ (960.28): C 67.54, H 9.34, N 1.46; found C 66.99, H 9.08, N 2.74.

3.6. General procedure for reductive amination. Synthesis of (2*S*,3*R*)-5-aza-3-*O*-benzyl-2-heptadecanoylamino-1-*O*-(2,3,4,6-tetra-*O*-pivaloyl-β-D-galactopyrano-syl)-1,3-alcanediols 8a–e

A solution of aldehyde **7** (120 mg, 0.125 mmol) and alcanamine [for example, pentadecanamine (56 mg, 0.250 mmol) in MeOH (5 mL)] was stirred at room temperature for 2 h. When TLC showed complete formation of the intermediate imine, $NaBH_3CN$ (11 mg, 0.1873 mmol) was added at the same temperature and the reaction mixture was stirred for an additional period of 2 h at room temperature. Then, MeOH was evaporated under vacuum and 10% HCl (10 mL) was added to the residue. The aqueous solution was stirred for 0.5 h at room temperature and aq Na_2CO_3 (ca. 2.5 g in 10 mL H_2O) was added under cooling to make the solution alkaline. The aqueous solution was extracted with CH_2Cl_2 (30 mL×2), and the combined organic layers were washed with brine (15 mL), dried over Na_2SO_4, and concentrated. The crude product was purified on a silica gel column with *n*-hexane/AcOEt (1:1) to afford pure **8a** (127 mg, 87%). The same procedure was used for the synthesis of **8b**–**e** (yields, **8b** 81%, **8c** 79%, **8d** 85%, **8e** 88%).

*3.6.1. Compound **8a**.* $[\alpha]_D^{20}$ +3.7 (*c* 1.2, $CHCl_3$). ^1H NMR ($CDCl_3$, 500 MHz): δ 7.48–7.36 (m, 6H, C6*H*5, N*H*), 5.41 (d, 1H, *J*=3.3 Hz, H-4), 5.20 (dd, 1H, *J*=7.6, 10.5 Hz, H-2), 5.12 (dd, 1H, *J*=3.3, 10.5 Hz, H-3), 4.70 (d, 1H, *J*=11.5 Hz, C$H_2C_6H_5$), 4.57 (d, 1H, *J*=7.6 Hz, H-1), 4.56 (d, 1H, *J*=11.5 Hz, C$H_2C_6H_5$), 4.39 (m, 1H, H′-2), 4.07 (m, 4H), 3.70 (m, 1H), 3.58 (dd, 1H, *J*=5.9, 9.9 Hz, H′-1), 2.90 and 2.81 (2dd, 2H, *J*=4.09, 12.8 Hz), 2.58 (m, 2H), 2.13 (t, 3H, COCH_2), 1.60 (m, 4H), 1.47 (m, 2H), 1.27 (m, 61H), 1.17 (2), 1.13 (3s, 27H, 3× COC(CH_3)$_3$), 0.89 (t, 6H, 2× CH_3). ^{13}C NMR (500 MHz, $CDCl_3$): δ 177.7, 177.1, 176.7 (2) (4× *C*OC(CH_3)$_3$), 173.3 ($COCH_2CH_2$), 138.2, 128.4 (2), 127.9 (2), 127.8 (C_6H_5) 101.4 (C-1), 77.2, 75.6, 72.0, 71.1, 70.8, 69.1, 68.6, 66.6, 60.9, 50.6, 50.1, 39.0, 38.8, 38.7, 38.6, 36.9, 31.9, 29.6, 29.5, 29.4, 29.3, 29.3, 27.1, 27.0, 25.7, 22.6, 14.0. Elemental analysis: calcd for $C_{69}H_{122}N_2O_{12}$ (1171.71): C 70.73, H 10.49, N 2.39; found C 69.34, H 10.44, N 2.59.

*3.6.2. Compound **8b**.* $[\alpha]_D^{20}$ +2.1° (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$, 500 MHz): δ 7.46 (br s, 1H, NH), 7.34 (m, 5H, C6*H*5), 5.39 (d, 1H, *J*=3.2 Hz, H-4), 5.18 (dd, 1H, *J*=7.8, 10.5 Hz, H-2), 5.10 (dd, 1H, *J*=3.2, 10.5 Hz, H-3), 4.68 (d, 1H, *J*=11.6 Hz, C$H_2C_6H_5$), 4.55 (d, 1H, *J*=7.8 Hz, H-1), 4.53 (m, 2H, C$H_2C_6H_5$, H′-2), 4.39 (m, 1H, H-5), 4.04 (m, 4H), 3.71 (m, 1H), 3.56 (dd, 1H, *J*=5.9, 9.6 Hz, H′-1), 2.89, 2.79 (2dd, 2H, *J*=3.94, 12.7 Hz), 2.57 (m, 2H), 2.10 (t, 3H, COCH_2), 1.58 (m, 4H), 1.45 (m, 2H), 1.24 (m, 64H), 1.15, 1.15, 1.11 (3s, 27H, 3× COC(CH_3)$_3$), 0.87 (t, 6H, 2× CH_3). ^{13}C NMR (500 MHz, $CDCl_3$): δ 177.8, 177.2, 176.8, 176.7 (4× *C*OC(CH_3)$_3$), 173.3 ($COCH_2CH_2$), 138.2, 128.4 (2), 127.9 (2), 127.8 (C_6H_5), 101.5 (C-1), 77.2, 75.7, 72.0, 71.1, 70.8, 69.1, 66.6, 61.0, 50.7, 50.1 39.0, 38.8, 38.7, 38.6, 36.9, 31.9, 29.7, 29.6, 29.3, 28.3, 27.1, 25.7, 22.6, 14.0. Elemental analysis: calcd for $C_{68}H_{120}N_2O_{12}$ (1157.68): C 70.55, H 10.45, N 2.42; found C 70.42, H 10.29, N 2.35.

*3.6.3. Compound **8c**.* $[\alpha]_D^{20}$ +6.1 (*c* 1.8, $CHCl_3$). ^1H NMR ($CDCl_3$, 500 MHz): δ 7.38–7.34 (m, 6H, C6*H*5, NH), 5.41 (d, 1H, *J*=3.3, Hz, H-4), 5.19 (dd, 1H, *J*=7.6, 10.5 Hz, H-2), 5.12 (dd, 1H, *J*=3.3, 10.5 Hz, H-3), 4.70 (d, 1H, *J*=11.5 Hz, C$H_2C_6H_5$), 4.57 (d, 1H, *J*=7.6 Hz, H-1), 4.56 (dd, 1H, *J*=11.5 Hz, C$H_2C_6H_5$), 4.38 (m, 1H, H′-2), 4.05 (m, 4H), 3.71 (m, 1H), 3.58 (dd, 1H, *J*=5.8, 9.9 Hz, H′-1), 2.90 and 2.80 (2dd, 2H, *J*=4.0, 12.8 Hz), 2.59 (m, 2H), 2.13 (t, 3H, COCH_2), 1.6 (m, 4H), 1.46 (m, 2H), 1.26 (m, 54H), 1.17 (2), 1.12 (3s, 27H, 3× COC(CH_3)$_3$), 0.89 (t, 6H, 2× CH_3). ^{13}C NMR (500 MHz, $CDCl_3$): δ 177.7, 177.1, 176.8, 176.7 (4× *C*OC(CH_3)$_3$), 173.4 ($COCH_2CH_2$), 138.1, 128.4 (2), 127.9 (2), 127.8 (C_6H_5), 101.5 (C-1), 77.2, 75.7, 72.1, 71.1, 70.8, 69.1, 68.6, 66.6, 60.9, 50.6, 50.1, 39.0, 38.8, 38.7, 38.6, 36.9, 31.9, 29.7, 29.5 (2), 29.4, 29.3, 27.1(2), 27.0 (2), 25.6, 22.6, 14.0. Elemental analysis: calcd for $C_{66}H_{116}N_2O_{12}$ (1129.63): C 70.17, H 10.35, N 2.48; found C 69.83, H 10.40, N 2.73.

*3.6.4. Compound **8d**.* $[\alpha]_D^{20}$ +4.1 (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ 7.44 (br s, 1H, NH), 7.31 (m, 5H, C6*H*5), 5.37 (d, 1H, *J*=3.2 Hz, H-4), 5.16 (dd, 1H, *J*=7.8, 10.5 Hz, H-2), 5.08 (dd, 1H, *J*=3.2, 10.5 Hz, H-3), 4.66 (d, 1H, *J*=11.5 Hz, C$H_2C_6H_5$), 4.54 (d, 1H, *J*=7.8 Hz, H-1), 4.50 (m, 2H, C$H_2C_6H_5$, H′-2), 4.36 (m, 1H, H-5), 4.01 (m, 4H), 3.68 (m, 1H), 3.54 (dd, 1H, *J*=5.9, 9.6 Hz, H′-1), 2.87, 2.77 (2dd, 2H, *J*=3.8, 12.7 Hz), 2.55 (m, 2H), 2.08 (t, 2H, COCH_2), 1.56 (m, 2H), 1.43 (m, 2H), 1.22 (m, 42H), 1.14, 1.13, 1.09 (3s, 27H, 3× COC(CH_3)$_3$), 0.85 (t, 6H, 2× CH_3). ^{13}C NMR (400 MHz, $CDCl_3$): δ 177.9, 177.3, 176.9 (2) (4× *C*OC(CH_3)$_3$), 173.4 ($COCH_2$), 138.4, 128.6 (2), 128.1 (2), 127.9 (C_6H_5) 101.6 (C-1), 75.9, 75.7, 72.2, 71.3, 71.0, 69.3, 68.8, 66.8, 61.2, 50.8, 50.3, 39.2, 38.9 (2), 37.1, 32.0 (2), 29.8, 29.7, 29.5, 29.4, 27.4, 27.3, 27.2, 25.8, 22.8, 14.2. Elemental analysis: calcd for $C_{62}H_{108}N_2O_{12}$ (1073.52): C 69.37, H 10.14, N 2.61; found C 69.39, H 10.09, N 2.55.

*3.6.5. Compound **8e**.* $[\alpha]_D^{20}$ +3.8 (*c* 0.8, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ 7.36 (m, 6H, C6*H*5, N*H*), 5.41 (d, 1H, *J*=3.2 Hz, H-4), 5.19 (dd, 1H, *J*=7.5, 10.4 Hz, H-2), 5.13 (dd, 1H, *J*=3.2, 10.4 Hz, H-3), 4.69 (d, 1H, *J*=11.5 Hz, C$H_2C_6H_5$), 4.60 (d, 1H, *J*=11.5 Hz, C$H_2C_6H_5$), 4.57 (d, 1H, *J*=7.4 Hz, H-1), 4.3 (m, 1H, H′-2), 4.09 (m, 4H), 3.73 (m, 1H), 3.60 (dd, 1H, *J*=5.4, 9.8 Hz, H′-1), 2.94 and 2.79 (2dd, 2H, *J*=3.9, 12.7 Hz), 2.7–2.59 (m, 2H), 2.14 (t, 2H, COCH_2), 1.56 (m, 4H), 1.26 (m, 38H), 1.17, 1.13 (2s, 18H, 2× COC(CH_3)$_3$), 0.89 (t, 6H, 2× CH_3). ^{13}C NMR (400 MHz, $CDCl_3$): δ 177.7, 177.1, 176.9, 176.6 (4× *C*OC(CH_3)$_3$), 173.9 ($COCH_2$), 137.9, 128.5 (2), 128.1 (2), 127.9 (C_6H_5) 101.3 (C-1), 77.2, 75.2, 72.3, 71.1, 70.7, 69.2, 68.2, 66.6, 60.9, 50.5, 49.9, 39.0, 38.8, 38.7, 38.6, 36.7, 31.9, 29.6 (3), 29.5, 29.3 (3), 27.1 (2), 27.0, 25.6, 22.6, 22.4, 14.0, 13.9. Elemental analysis: calcd for $C_{59}H_{102}N_2O_{12}$ (1031.44): C 68.70, H 9.97, N 2.72; found C 67.34, H 9.93, N 3.30.

3.7. Synthesis of (2*S*,3*R*)-5-aza-2-heptadecanoylamino-1-*O*-(β-D-galactopyranosyl)-1,3-alcanediols 9a–e

To a solution of **8a** (112 mg, 0.095 mmol) in MeOH (10 mL), NaOMe (50 mg) was added and the suspension was stirred at room temperature for 24 h. At this time, TLC (1:2 *n*-hexane/EtOAc) showed complete conversion of the starting material. Solvents were evaporated in vacuo and co-evaporated with toluene. The resulting syrup (55 mg, 0.4 mmol) was dissolved in CH_3OH and 20% $Pd(OH)_2$/C (50 mg) was added. The reaction mixture was stirred at room temperature under a positive pressure of hydrogen for 12 h.

The reaction mixture filtered through a Celite® bed and evaporation to dryness afforded the pure **9a** (65 mg, 65%) as a white foam.

*3.7.1. Compound **9a**.* $[\alpha]_D^{25}$ +21.3 (*c* 1.4, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz):δ 4.41 (m), 3.94 (m, 2H), 3.59–3.37 (5H), 2.75 (m, 2H), 2.19 (m, 2H), 1.48 (m, 2H), 1.18 (m), 0.80 (t, 6H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 172.0, 101.0, 78.0, 76.0, 74.5, 72.0, 71.5, 69.5, 61.5, 48.5, 39.0, 32.0, 30.0, 27.0, 26.5, 23.5, 14.0. HRMS calcd for $C_{42}H_{88}N_3O_8$ $(M+NH_4)^+$: 763.1250; found *m*/*z* 763.1241. Elemental analysis: calcd for $C_{42}H_{84}N_2O_8$ (744): C 67.74, H 11.29, N 3.76; found C 67.58, H 11, 10 N 3.87.

*3.7.2. Compound **9c**.* $[\alpha]_D^{20}$ +19.4 (*c* 1.2, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ 4.2 (m), 3.9 (m), 3.63–3.5 (m), 2.52–2.21 (m), 1.90 (m), 1.2, 1.02–0.89 (m), 0.77 (t). ^{13}C NMR (400 MHz, $CDCl_3$): δ 169.5, 104.0, 75.0, 74.5, 73.0, 71.0 (2), 70.0 (2), 61.0, 49.0, 40.0, 35.0, 32.0, 30.0, 28.0, 27.5, 22.0, 14.0. HRMS calcd for $C_{39}H_{82}N_3O_8$ $(M+NH_4)^+$: 720.5758; found *m*/*z* 720.5749. Elemental analysis: calcd for $C_{39}H_{78}N_2O_8$ (702): C 66.67, H 11.11, N 3.99; found C 67.01, H 10.85, N 3.81.

Compounds **9b**, **9d**, and **9e** gave ^1H and ^{13}C spectra similar to those of **9a** and **9c**.

*3.7.3. Compound **9b**.* $[\alpha]_D^{25}$ +24.3 (*c* 1.2, $CHCl_3$), elemental analysis: calcd for $C_{41}H_{82}N_2O_8$ (730): C 67.40, H 11.23, N 3.84; found C 67.11, H 10.95, N 3.91.

*3.7.4. Compound **9d**.* $[\alpha]_D^{25}$ +20.9 (*c* 1.1, $CHCl_3$), elemental analysis: calcd for $C_{35}H_{70}N_2O_8$ (646): C 65.02, H 10.84, N 4.33; found C 64.85, H 10.95, N 4.17.

*3.7.5. Compound **9e**.* $[\alpha]_D^{25}$ +18.6 (*c* 0.9, $CHCl_3$), elemental analysis: calcd for $C_{32}H_{64}N_2O_8$ (604): C 63.58, H 10.60, N 4.64; found C 63.41, H 10.95, N 4.58.

3.8. Thiazolyl to formyl conversion: synthesis of (2*S*,3*S*)-2-*O*-benzyl-3-*tert*-butyloxycarbonylamino-4-*O*-(2,3,4,6-tetra-*O*-pivaloyl-β-D-galactopyranosyl)-2,4-butan-1-al 10

To a solution of compound **5** (700 mg, 0.81 mmol) in dry CH_3CN (10 mL), MeI (504 μL, 8.11 mmol) was added and the solution was refluxed for 12 h. When TLC showed complete conversion of the starting material, the solvents were evaporated under vacuum. The residue was dissolved in dry methanol (10 mL) and $NaBH_4$ (61 mg, 1.62 mmol) was added at 0 °C. After 5 min reaction at 0 °C, the mixture was warmed to room temperature and stirred for 30 min. Then, MeOH was evaporated and the crude product was dissolved in 20 mL of CH_3CN/H_2O (8:2). Next, $HgCl_2$ (263 mg, 0.97 mmol) was added and the mixture was stirred at room temperature for an additional 1 h period. The solvents were evaporated and the residue was portioned between EtOAC (50 mL) and water (50 mL). The organic layer was separated, washed with brine (2×35 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified on a silica gel column using *n*-hexane/EtOAc (5:1) to afford pure **10** (537 mg, 82%) as a colorless oil. $[\alpha]_D^{20}$ +16.4 (*c* 1.5, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ 9.61 (d, 1H, *J*=2.94 Hz, C*H*O), 7.35 (m, 5H, C_6H_5), 5.42 (d, 1H, *J*=3.42 Hz, H-4), 5.22 (dd, 1H, *J*=7.8, 10.5 Hz, H-2), 5.13 (dd, 1H, *J*=3.3, 10.4 Hz), 4.89 (d, 1H, *J*=8.6 Hz, H′2), 4.65 and 4.60 (2d, 2H, *J*=11.2 Hz, $CH_2C_6H_5$), 4.55 (d, 1H, *J*=7.81 Hz, H-1), 4.21 (m, 1H, H-5), 4.13 (m, 1H, H′-3), 4.06 (dd, 1H, *J*=7.1, 11.0 Hz, H-6b), 3.98 (m, 1H, H-6a), 3.83 (dd, 1H, *J*=2.9, 8.2 Hz, H′-4a), 3.6 (dd, 1H, *J*=3.9, 9.4 Hz, H′-4b), 1.4 (s, 9H, OC(CH_3)$_3$), 1.26, 1.19, 1.18, 1.13 (4s, 36H, COC(CH_3)$_3$). ^{13}C NMR (400 MHz, $CDCl_3$): δ 200.6 (*C*HO), 177.8, 177.2, 176.9, 176.8 (4× *C*OC(CH_3)$_3$), 155.0, 136.9, 128.5 (2C), 128.2 (2C), 101.0 (C-1), 82.4, 77.2, 73.6, 71.1, 70.7, 68.8, 67.3, 66.6, 61.0, 41.0, 28.2, 27.14, 27.10, 27.06, 27.05. Elemental analysis: calcd for $C_{42}H_{65}NO_{14}$ (807.96): C 62.43, H 8.11, N 1.72; found C 62.39, H 7.54, N 1.59.

3.9. Synthesis of (2*S*,3*S*)-5-aza-3-*O*-benzyl-2-*tert*-butyloxycarbonylamino-1-*O*-(2,3,4,6-tetra-*O*-pivaloyl-β-D-galactopyranosyl)-1,3-nonadecananediol 11

A solution of aldehyde **10** (535 mg, 0.662 mmol) and tetradecanamine (282 mg, 1.32 mmol) in MeOH (10 mL) was stirred at room temperature for 2 h. When TLC showed complete formation of the intermediate imine, $NaBH_3CN$ (62 mg, 0.993 mmol) was added at the same temperature and the reaction mixture was stirred for an additional 2 h at room temperature. Then, MeOH was evaporated under vacuum and 10% HCl (20 mL) was added to the residue. The aqueous solution was stirred for 0.5 h at room temperature and aq Na_2CO_3 (ca. 2.5 g in 50 mL H_2O) was added under cooling to make the solution alkaline. The aqueous layer was extracted with CH_2Cl_2 (500 mL×2), and the combined organic layers were washed with brine (35 mL), dried over Na_2SO_4, and concentrated. The crude product was purified on a silica gel column with *n*-hexane/AcOEt (1:1) as an eluent to afford pure **11** (565 mg, 85%). $[\alpha]_D^{20}$ −3.9 (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ 7.35 (m, 5H, C_6H_5), 5.47 (d, 1H, *J*=8.1 Hz, N*H*), 5.41 (d, 1H, *J*=3.2 Hz, H-4), 5.24 (dd, 1H, *J*=7.6, 10.5 Hz, H-2), 5.13 (dd, 1H, *J*=3.2, 10.5 Hz, H-3), 4.7, 4.6 (2d, 2H, *J*=11.2 Hz, $CH_2C_6H_5$), 4.56 (d, 1H, *J*=7.9 Hz, H-1), 4.2 (m, 1H, H′-2), 4.0 (m, 4H, H-6ab, H′1a), 3.67 (m, H-5), 3.35 (dd, 1H, *J*=4.2, 9.4 Hz, H′-1b), 2.8 (m, 2H), 2.6 (m, 2H), 1.4 (s, 9H, (CO)OC(CH_3)$_3$), 1.27 (m, 27H), 1.23, 1.20, 1.18, 1.13 (4s, 27H, 4× COC(CH_3)$_3$). ^{13}C NMR (400 MHz, $CDCl_3$): δ 177.7 (2), 177.2, 176.8, 176.7, 138.2, 101.1 (C-1), 77.2, 72.8, 71.0, 70.8, 69.0, 68.5, 61.0, 50.0, 39.0, 38.8, 38.7 (2), 31.9, 29.6 (5), 29.5, 29.3, 28.3 (3), 27.1 (12), 22.66, 14.0. Elemental analysis: calcd for $C_{56}H_{96}N_2O_{13}$ (1005.36): C 66.90, H 9.62, N 2.79; found C 66.83, H 9.61, N 2.79.

3.10. Synthesis of (2*S*,3*R*)-5-aza-3-*O*-benzyl-2-alcanoylamino-1-*O*-(2,3,4,6-tetra-*O*-pivaloyl-β-D-galactopyranosyl)-1,3-nonadecananediol 12

*3.10.1. Compound **12a** (alcanoylamino group=—CO—$C_{21}H_{43}$).* A solution of compound **11** (250 mg, 0.248 mmol) and 50% TFA in CH_2Cl_2 (10 mL) was stirred for 3 h. When TLC showed complete conversion of the starting material, the solution was diluted with dichloromethane (25 mL) and washed successively with ice-cold $NaHCO_3$ (2×50 mL) and brine (50 mL). Then, the organic layer was separated, dried over $MgSO_4$, and evaporated under vacuum. The crude amine produced was used for the next step without any further purification.

To a solution of the crude amine and docosanoic acid (170 mg, 0.248 mmol) in dry DMF (10 mL) was added EDC (55 μL, 0.298 mmol) at 0 °C under a nitrogen atmosphere. After 30 min, the reaction mixture was warmed to 15 °C and stirred overnight. The solvents were evaporated and the residue was dissolved in dichloromethane (30 mL) and washed with brine (25 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography using *n*-hexane/EtOAc (2:1) to afford pure **12a** (250 mg, 82%) as a colorless oil. $[\alpha]_D^{20}$ +5.8° (*c* 1.3, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ 7.5 (d, 1H, *J*=8.5 Hz, NH), 7.35 (m, 5H, C6*H*5), 5.40 (d, 1H, *J*=3.2 Hz, H-4), 5.20 (dd, 1H, *J*=7.7, 10.5 Hz, H-2), 5.12 (dd, 1H, *J*=3.2, 10.5 Hz, H-3), 4.7 (d, 1H, *J*=11.6 Hz, $CH_2C_6H_5$), 4.57 (d, 1H, *J*=7.5 Hz, H-1), 4.5 (m, 2H, $CH_2C_6H_5$, H′-2), 4.4 (m, 1H, H-5), 4.06 (m, 4H), 3.7 (m, 1H), 3.58 (dd, 1H, *J*=6.1, 9.4 Hz), 2.81, 2.79 (2dd, 2H, *J*=3.91, 12.5 Hz), 2.56 (m, 2H), 2.12 (t, 3H, COCH_2), 1.59 (m, 2H), 1.45 (m, 4H), 1.26 (m, 75H), 1.17, 1.16, 1.12 (3s, 27H, 3× (CO)OC(CH_3)$_3$), 0.89 (t, 6H, 2× CH_3). ^{13}C NMR (400 MHz, $CDCl_3$): δ 177.7 (2), 177.1, 176.7 (2), (4× *C*OC(CH_3)$_3$), 173.3 (*C*OCH$_2$CH$_2$), 138.2, 128.4 (2), 127.9 (2), 127.7

(C_6H_5) 101.4 (C-1), 77.2, 75.7, 72.0, 71.1, 70.8, 69.1, 68.6, 66.6, 61.0, 50.7, 39.0, 38.8, 38.7 (2), 31.9, 29.7 (5), 29.6, 29.3, 28.3 (3), 27.1 (12), 22.6, 14.0. Elemental analysis: calcd for $C_{73}H_{130}N_2O_{12}$ (1227.81): C 71.41, H 10.67, N 2.28; found C 71.21, H 10.60, N 2.20.

3.10.2. Compound ***12b*** *(alcanoylamino group=—CO—$C_{24}H_{49}$).* Same experimental procedure as for **12a** (yield 81%). $[\alpha]_D^{20}$ +7.1° (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.5 (d, 1H, *J*=8.5 Hz, NH), 7.35 (m, 5H, C6*H*5), 5.40 (d, 1H, *J*=3.2 Hz, H-4), 5.20 (dd, 1H, *J*=7.7, 10.5 Hz, H-2), 5.12 (dd, 1H, *J*=3.2, 10.5 Hz, H-3), 4.7 (d, 1H, *J*=11.6 Hz, C*H*$_2$C$_6$H$_5$), 4.57 (d, 1H, *J*=7.5 Hz, H-1), 4.5 (m, 2H, C*H*$_2$C$_6$H$_5$, H′-2), 4.4 (m, 1H, H-5), 4.06 (m, 4H), 3.7 (m, 1H), 3.58 (dd, 1H, *J*=6.1, 9.4 Hz), 2.81, 2.79 (2dd, 2H, *J*=3.91, 12.5 Hz), 2.56 (m, 2H), 2.12 (t, 3H, COC*H*$_2$), 1.59 (m, 2H), 1.45 (m, 4H), 1.26 (m, 75H), 1.17, 1.16, 1.12 [3s, 27H, 3× (CO)OC(C*H*$_3$)$_3$], 0.89 (t, 6H, 2× CH_3). ^{13}C NMR (400 MHz, $CDCl_3$): δ 177.7 (2), 177.1, 176.7 (2), (4× *C*OC(CH$_3$)$_3$), 173.3 (*C*OCH$_2$CH$_2$), 138.2, 128.4 (2), 127.9 (2), 127.7 (C_6H_5) 101.4 (C-1), 77.2, 75.7, 72.0, 71.1, 70.8, 69.1, 68.6, 66.6, 61.0, 50.7, 39.0, 38.8, 38.7 (2), 31.9, 29.7 (5), 29.6, 29.3, 28.3 (3), 27.1 (12), 22.6, 14.0. Elemental analysis: calcd for $C_{76}H_{136}N_2O_{12}$ (1269.89): C 71.88, H 10.79, N 2.21; found C 71.90, H 10.65, N 2.23.

3.11. Synthesis of (2*S*,3*R*)-2-alcanoylamino-5-aza-1-*O*-β-D-galactopyranosyl-1,3-nonadecanediols 13a—b

To a solution of **12a** (95 mg, 0.077 mmol) in MeOH (10 mL), NaOMe (50 mg) was added and the suspension was stirred at room temperature for 24 h. At this time, TLC (1:2 *n*-hexane/EtOAc) showed complete conversion of the starting material. Solvent was evaporated in vacuo and co-evaporated with toluene. The resulting syrup (55 mg, 0.4 mmol) was dissolved in CH_3OH and $Pd(OH)_2$/C (20%, 50 mg) was added. The reaction mixture was stirred at room temperature under a positive pressure of hydrogen for 12 h. It was then filtered through a Celite® bed and evaporated to dryness to afford the pure target (2*S*,3*S*)-5-aza-2-docosanoylamino-1-*O*-(β-D-galactopyranosyl)-1,3-nonadecanediol (**13a**) (45 mg, 72%) as a white foam.

$[\alpha]_D^{25}$ +27.7 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.67 (d, 1H, H-1), 4.40 (m), 4.09—4.07 (m), 3.95 (m, 1H, H-4), 3.68—3.35 (m), 2.75 (m), 2.19 (m), 1.49, 1.35—11.18 (m), 0.80 (t). ^{13}C NMR (400 MHz, $CDCl_3$): δ 174.0 (CO—NH), 101.5 (C-1), 75.0 (C-5), 74.5, 73.5 (C-3), 71.0 (C-2), 70.0 (C-4 and C′-1), 61.0 (C-6), 49.0, 40.0, 35.0, 32.0, 31.0, 27.0, 27.5, 22.5, 14.0. HRMS calcd for $C_{46}H_{96}N_3O_8$ $(M+NH_4)^+$: 819.2313; found *m*/*z* 819.2307.

3.11.1. Compound ***13b****.* $[\alpha]_D^{25}$ +25.1 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.66 (d, 1H, H-1), 4.40 (m), 4.09—4.06 (m), 3.96 (m, 1H, H-4), 3.68—3.35 (m), 2.75 (m), 2.19 (m), 1.49, 1.35—11.18 (m), 0.80 (t). ^{13}C NMR (400 MHz, $CDCl_3$): δ 174.1 (CO—NH), 101.7 (C-1), 75.2 (C-5), 74.5, 73.6 (C-3), 71.0 (C-2), 70.0 (C-4 and C′-1), 61.1 (C-6), 49.0, 40.0, 35.0, 32.0, 31.0, 27.0, 27.5, 22.5, 14.0. HRMS calcd. For $C_{49}H_{102}N_3O_8$ $(M+NH_4)^+$: 861.2673; found *m*/*z* 861.2662.

3.12. Synthesis of (2*S*,3*S*)-2-amino-3-(thiazol-2-yl)-1,3-propanediol 14

Compound **2** (1 g, 3.18 mmol) was dissolved in a mixture of dioxan (10 mL) and 1 N HCl (5 mL), refluxed for 1 h, and cooled to 25 °C. A solution of 2 N NaOH was added until pH 10 was reached. The mixture was extracted with CH_2Cl_2 (3×250 mL). The organic layers were washed with brine (250 mL), dried over $MgSO_4$, and concentrated in vacuo. After silica-gel column chromatography (CH_2Cl_2/MeOH 4:1), compound **14** was obtained as a pale yellow liquid (512 mg, 91%). $[\alpha]_D^{20}$ +51° (*c* 1.3, MeOH). ^{1}H NMR (400 MHz, D_2O): δ 8.27, 8.23 (2d, 2H, *J*=3.88 Hz, thiazolyl-*H*), 5.89 (d, 1H, *J*=3.24 Hz, OH), 4.07 (m, 1H, H-3), 3.95 (m, 1H, 1-H_a), 3.82 (m, 2H, H-2 and 1-H_b). ^{13}C NMR (400 MHz, D_2O): δ 177.2, 140.3, 125.5 (thiazolyl), 56.4 (C-3), 52.8 (C-1), 40.3 (C-2). Elemental analysis: calcd for $C_6H_{10}N_2O_2S$ (174.04): C 41.36, H 5.79, N 16.08, S 18.40; found C 41.12, H 5.23, N 15.4, S 18.21.

3.13. Enzymatic synthesis of (2*S*,3*S*)-2-amino-1-*O*-(β-D-galactopyranosyl)-3-(thiazol-2-yl)-1,3-propanediol 15

α-D-Galactosyl fluoride (83 mg, 0.46 mmol) and (1*S*,2*S*)-2-amino-3-(thiazol-2-yl)-1,3-propanediol **14** (91 mg, 0.27 mmol) were dissolved in phosphate buffer (50 mmol/L, pH 7.0, 8 mL). Then, E338G glycosynthase solution[47] (3 mL), was added. The reaction was allowed to proceed at 55 °C for 12 h. At this time, the Gal-F had completely disappeared. After elimination of the solvent under diminished pressure, the crude mixture was dissolved in D_2O and the yield (15%) was determined by ^{1}H NMR spectroscopy. At this stage, we did not perform any purification for compound **15**. Nevertheless, NMR parameters were extracted from the mixture using TOCSY NMR experiments.

^{1}H NMR (D_2O, 400 MHz): δ 8.26, 8.21 (2d, 2H, *J*=3.9 Hz, thiazolyl-*H*),4.60 (d, 1H, *J*=7.8 Hz, H-1), 4.22—4.17 (m, 2H, H′-3 and H′-1_a), 4.01 (dd, 1H, H′-1_b), 3.95 (m, 1H, H-4), 3.60—3.90 (6H, H-2, H-3, H-5, H-6_{ab}, H′-2).

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Supplementary data

^{1}H and ^{13}C spectra of compounds **3**—**14** (only some examples of galactosyl ceramides **8**, **9**, **12**, and **13** are given). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2011.05.054.

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