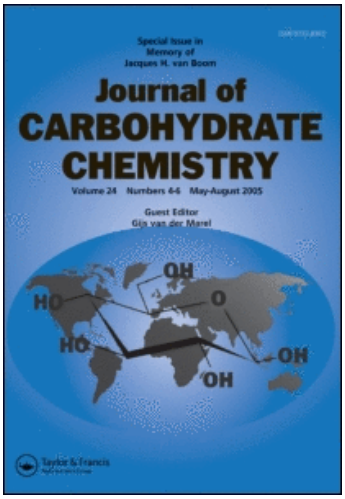

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Azidosphingosine Glycosylation in Glycosphingolipid Synthesis

Peter Zimmermann[a]; René Sommer[a]; Thomas Bär[a]; Richard R. Schmidt[a]
[a] Fakultät Cheraie, universität Konstanz, Konstanz, Germany

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AZIDOSPHINGOSINE GLYCOSYLATION IN GLYCOSPHINGOLIPID SYNTHESIS [1]

Peter Zimmermann, René Bommer, Thomas Bär,
and Richard R. Schmidt *

Fakultät Chemie, Universität Konstanz
Postfach 5560, D-7750 Konstanz, Germany

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ABSTRACT

The 3-O-protected azide derivatives of C_{18}-sphingosine 1A-1C reacted with O-acyl protected trichloroacetimidates of D-glucose, D-galactose, and lactose to afford the corresponding ß-glycosides in high yields. Ortho-ester formation in the case of O-acetyl compound could be avoided by increasing the amount of boron trifluoride·diethyl ether catalyst. Deprotection and azido group reduction provided the psychosines of D-glucose, D-galactose, and lactose (5, 10, and 15), which are versatile intermediates for the attachment of different fatty acid residues. With hexadecanoyl chloride, for instance, the corresponding glycosphingolipids 6, 11, and 16, respectively, were obtained.

INTRODUCTION

The large variety of different glycosphingolipids found in natural sources make it very difficult to isolate the homogeneous oligosaccharide and the ceramide containing material required for biological and pharmacological investigations. Therefore, efficient syntheses of this class of compounds are urgently needed.[2,3] We have been concerned recently with this problem.[4-9]

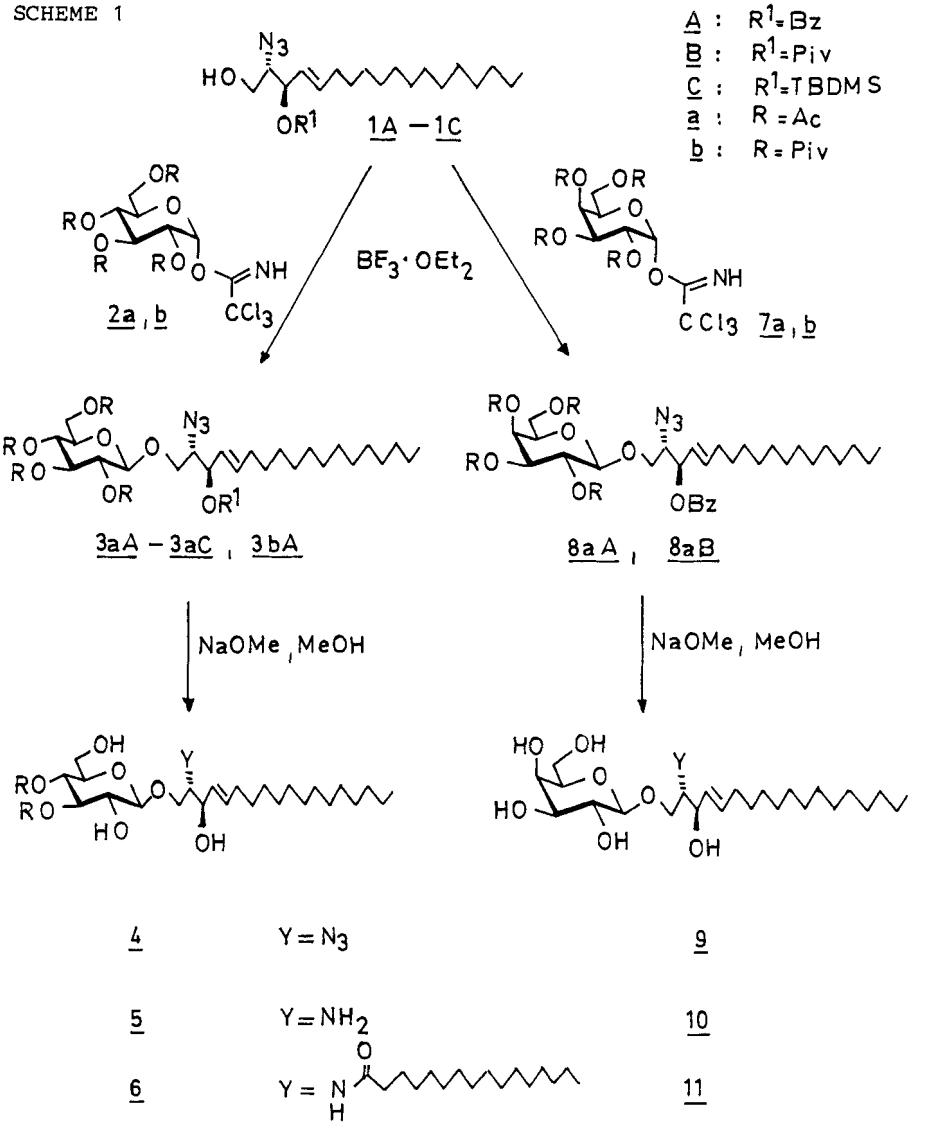
Several rather lengthy syntheses for the required sphingosines have been reported.[10] We have developed, via an erythro-specific aldol reaction of N,O-persilylated glycine with α,ß-unsaturated aldehydes, a two step synthesis of racemic sphingosines.[4] This efficient route provided ceramides very readily. The required racemate resolution and the 3-O-protection could be easily combined.[2,11] The best method for the glyscosylation of these compounds proved to be the trichloroacetimidate me-

thod, which afforded better yields in glycosphingolipid syntheses than syntheses according to the Koenigs-Knorr method or the Helferich-Weis method.[5,12,13] This ceramide glycosylation procedure was also successfully applied by Ogawa et al. in the syntheses of the G_{M3} ganglioside, the asialo-G_{M1} and the asialo-G_{M2} gangliosides.[14] However, the yields in the glycoside bond forming step are far from satisfactory. The reason for this seems to be attachment of the catalyst to the ceramide molecule leading to incomplete glycoside bond formation.[13] We have therefore developed a new and productive entry to this class of compounds, based on a new and efficient sphingosine synthesis.[2,6,7,15] In this new methodology, described in detail here,[16] glycosylation takes place at the sphingosine stage and not, as described above, at the ceramide stage.[2,7,8]

RESULTS AND DISCUSSION

Starting from D-galactose a high yield, five step synthesis of the azido derivative of D-erythro-sphingosine (SCHEME 1, 1, R^1 = H) could be developed[6,7] which had the required latent amino group functionality. Preliminary experiments in direct glycoside bond formation with this azidosphingosine demonstrated an insufficient selectivity for the primary hydroxy group.[18] Therefore, we transformed this compound, via a selective 1-O-tritylation, 3-O-protection, and 1-O-detritylation sequence, into the 3-O-benzoyl, the 3-O-pivaloyl, and the 3-O-tert-butyldimethylsilyl (TBDMS) derivatives 1A-1C, respectively (SCHEME 1). 1-O-Glucosylation of compound 1A with the O-acetylated trichloroacetimidate 2a[19] at room temperature in the presence of 0.3 equivalents of boron trifluoride·diethyl ether as a catalyst afforded the ß-glucopyranoside 3aA in 80% yield. However, thin layer chromatography of the reaction mixture revealed the intermediate formation of an ortho-ester derivative between the O-acetylated glucosyl donor and the sphingosine acceptor. This ortho-ester subsequently rearranged to the product in the presence of the Lewis acid catalyst. The ortho-ester can be isolated when the relative amount of catalyst is reduced. This was demonstrated in the reactions of the sphingosine derivatives 1B and 1C with the trichloroacetimidate 2a in presence of 0.08 equivalents of boron trifluoride·diethyl ether as a catalyst. After ~ 6-8 h a 1:3-mixture of the ß-glucopyranosides 3aB, 3aC and the corresponding ortho-ester derivatives were formed. The products could be separated by chromatography on silica gel and their structures assigned from ^{1}H NMR data: The compounds 3aA-

SCHEME 1



3aC had the H-1 signal at δ ~ 4.5 with the coupling constant $J_{1,2}$ of ~ 8 Hz and the methyl proton signals of all acetyl groups at δ ~ 2.0. The ortho-esters typically had the H-1 signal at δ ~ 5.7 with the $J_{1,2}$ coupling constant of ~ 5 Hz and the methyl protons of the former 2-O-acetyl group at δ ~ 1.7.

The formation of ortho-esters either as intermediates or as products can be discriminated against by O-acyl protecting groups with high steric demand or with high electron delocalization.[20] The advantage of O-pivaloyl protected, glycosyl do-

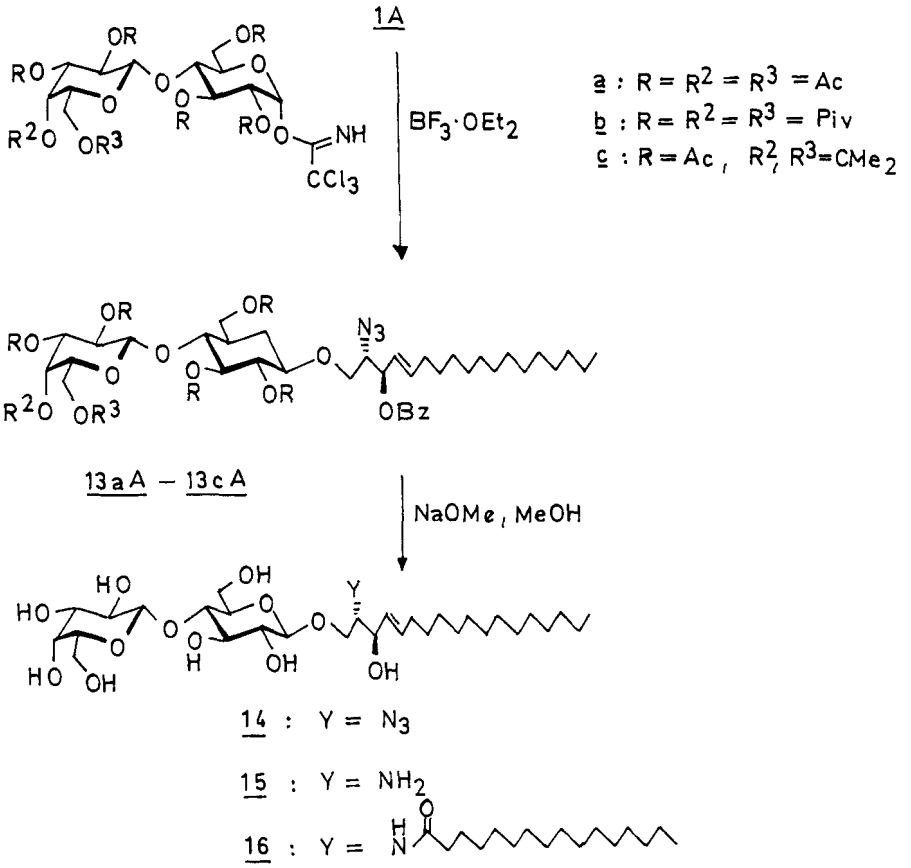
nors in this regard has been demonstrated by Kunz and coworkers.[21] We have previously synthesized the O-pivaloylated trichloroacetimidate 2b[13,22] and found the same behaviour for this glucosyl donor. For instance, with the acceptor 1A, the desired product 3bA was obtained in practically quantitative yield though in a relatively slow reaction.

Compounds 3aA, 3aB, and 3bA were readily transformed by treatment with sodium methoxide/methanol into the unprotected derivative 4 which afforded after azide reduction with hydrogensulfide/pyridine, the ß-D-glucopyranosyl-sphingosine 5 in high yield. This so called "glucopsychosine" is a metabolic product in Gaucher's disease.[23] From the point of view of synthetic strategy this compound is of special importance because by attaching different fatty acids to the amino group, it becomes useful in various glucosyl ceramide syntheses.[8] For instance, hexadecanoylation of 5 in presence of sodium acetate provided the glucosyl ceramide 6, which had properties identical to material obtained via the above mentioned ceramide glucosylation procedure.[5,13,24]

These results could be readily applied to the synthesis of the galactopyranosyl ceramide 11 (SCHEME 1). Thus, from the sphingosine derivative 1A, using the O-acetylated and the O-pivaloylated trichloroacetimidates 7a[19] and 7b as galactosyl donors, the ß-galactopyranosyl sphingosine derivatives 8a and 8b, respectively, were obtained in high yields. Subsequently, applying the same methods for O-acyl group removal, azide reduction, and hexadecanoylation provided the unprotected compound 9, the galactopsychosine 10, and finally the desired product 11, again in high overall yield. Compound 11 was identical with material obtained via the ceramide galactosylation procedure.[5,13]

The importance of lactosyl ceramides as basic structures of many natural glycosphingolipids provided a rationale for applying this new sphingosine glycosylation methodology also to the synthesis of the lactosyl ceramide 16 (SCHEME 2). Thus, with the per-O-acetylated trichloroacetimidate 12a[25] as the lactosyl donor and the sphingosine 1A as the acceptor, reaction afforded, through in situ rearrangement of intermediately formed ortho-ester, the desired lactoside 13aA in 85% yield. Similarly, the 4',6'-O-isopropylidene protected lactosyl trichloroacetimidate 12b provided cleanly the corresponding

SCHEME 2



lactoside 13bA, a compound which might prove useful in further reactions involving extensions of the oligosaccharide moiety. Even using the newly synthesized per-O-pivaloylated trichloroacetimidate 12c as a lactosyl donor produced the desired ß-connected lactoside 13cA in high yield. However, complete O-pivaloyl group removal in this compound (13cA) with sodium methoxide/methanol to provide compound 14 required one week. This extended reaction time indicated that this O-protective group may have limitations in the synthesis of more complex glycosphingolipids. Compound 14 was readily obtained from the O-acetylated derivative 13aA by treatment with sodium methoxide/methanol in a few hours. Transformation of compound 14 into the corresponding lactopsychosine 15 and subsequent hexadecanoylation to provide lactosyl ceramide 16 went smoothly under the standard conditions. Compound 16 again was identical

to material obtained via the ceramide glycosylation procedure.[5,13]

EXPERIMENTAL

General Procedures. Melting points are uncorrected. ^{1}H NMR spectra were recorded in the solvents noted (Me_4Si, 0.00 ppm) with a Bruker "WM 250 Cryospec" and a Jeol "JNM-FX 90 Q". R_F values refer to TLC performed on silica gel (Merck) with the solvent systems noted. Column chromatography was performed under normal pressure with silica gel 60 (Merck, 70-230 mesh ASTM), and under medium pressure with silica gel (Merck, "LiChroprep" Si 60, 15-25 m) with the solvent systems noted. For flash chromatography silica gel 60 (Merck 230-400 mesh ASTM) was used. The solvents for chromatography were distilled. Petroleum ether was taken from bp 35-60 °C. Optical rotations were determined with a Perkin Elmer 241 MC spectrometer.

(2S,3R,4E)-2-Azido-3-benzoyloxy-1-hydroxy-, (2S,3R,4E)-2-Azido-1-hydroxy-3-pivaloyloxy-, and (2S,3R,4E)-2-Azido-3-tert-butyldimethylsilyloxy-1-hydroxy-4-octadecene (1A, 1B, and 1C). These compounds were obtained as described previously.[17]

O-(2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl)trichloroacetimidate (2a). This compound was obtained as described previously.[19]

O-(2,3,4,6-Tetra-O-pivaloyl-α-D-glucopyranosyl)trichloroacetimidate (2b).[13,22] (a) **Synthesis of 2,3,4,6-Tetra-O-pivaloyl-D-glucose.** To a solution of 1,2,3,4,6-penta-O-pivaloyl-D-glucopyranose[13,21] (12 g, 20 mmol) in 40 mL dry N,N-dimethylformamide (40 mL) was added dry hydrazine acetate (2.2 g, 25 mmol) at 50 °C. After 3 h ethyl acetate (160 mL) was added and the mixture was washed with saturated sodium chloride solution (3 x 100 mL). The organic extract was dried with sodium sulfate and then concentrated. The oily residue was chromatographed on silica gel (petroleum ether/ethyl acetate = 4:1, normal pressure): total yield 9.0 g (87 %) of material (TLC R_F 0.45, petroleum ether/ethyl acetate = 4:1), which was immediately used for the synthesis of compound 2b.

(b) 2b: To a solution of 2,3,4,6-tetra-O-pivaloyl-D-glucose (1.3 g, 2 mmol) and trichloroacetonitrile (0.8 mL, 8 mmol) in dry dichloromethane (10 mL) was added sodium hydride (144 mg, 6 mmol) at room temperature. After 1.5 h the reaction mixture was filtered through celite and subsequently chromato-

graphed on silica gel (toluene/acetone = 3:1, normal pressure) to yield 800 mg (60%) of compound 11b as an amorphous powder; $[\alpha]_D^{20}$ = +82.6° (c=3.5, $CHCl_3$); TLC R_F 0.45 (toluene/acetone = 4:1); ^1H NMR (250 MHz, $CDCl_3$) δ 8.68 (s, 1H, NH), 6.57 (d, 1H, H-1; $J_{1,2}$ = 4.4 Hz), 5.67 (dd, 1H, H-4; $J_{3,4}$ = $J_{4,5}$ = 10.5 Hz), 5.23 (dd, 1H, H-3; $J_{2,3}$ = $J_{3,4}$ = 10.5 Hz), 5.17 (dd, 1H, H-2; $J_{1,2}$ = 4.4 Hz, $J_{2,3}$ = 10.5 Hz), 4.15 (m, 3H, H-5, H-6, H-6'); 1.17 (m, 36H, 4 t-Bu).

Anal. Calc. for $C_{28}H_{24}Cl_3NO_{10}$ (661.01): C, 50.88; H, 6.71; N, 2.12. Found: C, 51.00; H, 6.72; N, 2.05.

(2S,3R,4E)-2-Azido-3-benzoyloxy-1-(2,3,4,6-tetra-O-acetyl-ß-D-glucopyranosyloxy)-4-octadecene (3aA). Compound 1A (270 mg, 0.63 mmol) and compound 2a (470 mg, 0.95 mmol) were dissolved in dry hexane (8 mL) and dry dichloromethane (2 mL) and the solution stirred in presence of molecular sieves (4A, 0.1 g) at room temperature for 30 min. Boron trifluoride·diethyl ether in dry dichloromethane (0.1 M solution, 2 mL) was added within 30 min. After 6-8 h TLC (petroleum ether/ethyl acetate = 4:1) indicated the formation of a single product. The reaction mixture was diluted with petroleum ether (30 mL) and then treated with saturated sodium hydrogen carbonate solution (10 mL). The organic extract was dried over magnesium sulfate and then concentrated. The residue was chromatographed on silica gel (petroleum ether/ethyl acetate = 4:1, medium pressure) to yield 380 mg (80%) of compound 3aA as colourless crystals; mp 45 °C from petroleum ether/ethyl acetate = 4:1; $[\alpha]_D^{20}$ = -31.0° (c=2, $CHCl_3$); TLC R_F 0.27 (petroleum ether/ethyl acetate = 4:1); ^1H NMR (250 MHz, $CDCl_3$) δ 8.06-7.43 (m, 5H, C_6H_5, 5.96-5.89, 5.61-5.52 (2m, 3H, -CH(OBz)-CH=CH), 5.23-5.01 (m, 3H, H-2, H-3, H-4), 4.55 (d, 1H, H-1; $J_{1,2}$ = 8.0 Hz), 4.24-4.09 (m, 2H, O-CH_2), 3.96-3.88 (m, 2H, 2 H-6), 3.71-3.67 (m, 1H, H-5), 3.61-3.57 (m, 1H, CH-N_3), 2.09, 2.05, 2.02, 2.01 (4s, 12H, 4CO-CH_3), 1.24 (brs, 24H, 12CH_2), 0.89-0.86 (t, 3H, CH_3).

Anal. Calc. for $C_{39}H_{57}N_3O_{12}$ 1/3 H_2O (765.90): C, 61.64; H, 7.58; N, 5.53. Found: C, 61.18; H, 7.46; N, 5.50

(2S,3R,4E)-2-Azido-3-pivaloyloxy-1-(2,3,4,6-tetra-O-acetyl-ß-D-glucopyranosyloxy)-4-octadecene (3aB). From compounds 2a and 1B the reaction product 3aB was obtained as described for compound 3aA. Only 0.4 mL boron trifluoride·diethyl ether solution in dry dichloromethane (0.1 M) was used as the catalyst. The reaction product was chromatographed on sili-

ca gel (toluene/acetone = 19:1, normal pressure) to yield 22% of compound 3aB as a colorless oil and 61 % of the corresponding ortho-ester derivative.

3aB: TLC R_F 0.12 (toluene/acetone = 40:1); $[\alpha]^{22}$ = 33.6° (c=1, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ 5.90-5.75 (m, 1H, CH=CH-CH_2), 5.43 (dd, 1H, CH=CH-CH_2, J = 15.2 Hz, J = 7.9 Hz), 5.31-4.97 (m, 4H, H-2, H-3, H-4, CH-OPiv.), 4.51 (d, 1H, H-1, $J_{1,2}$ = 7.9 Hz), 4.29-3.47 (m, 6H, 2H-6, H-5, CH-N_3, CH_2-O), 2.15-1.95 (m, 14H, 4 CO-CH_3, CH=CH-CH_2), 1.45-1.12 (m, 31H, 11 CH_2, $COCMe_3$) 0.88 (t, 3H, CH_3).

Anal. Calcd for $C_{37}H_{61}N_3O_{12}$ (739.89): C, 60.06; H, 8.31; N, 5.68. Found: C, 60.01; H, 8.15; N, 5.60

Ortho-ester: $[\alpha]_D^{22}$ 4.6° (c=0.5, $CHCl_3$); TLC R_F 0.18 (toluene/acetone = 40:1); ^1H NMR (250 MHz, $CDCl_3$) δ 5.87-5.77 (m, 1H, CH=CH-CH_2), 5.73 (d, 1H, H-1, $J_{1,2}$ = 5.1 Hz), 5.43 (dd, 1H, CH=CH-CH_2, J = 15.2 Hz, J = 7.9 Hz), 5.32 (dd, 1H, CH-OPiv, J = 7.9 Hz, J = 4.2 Hz), 5.85 (dd, 1H, H-3), 4.93 (dd, 1H, H-4, J = 9.4 Hz, J = 1.8 Hz), 4.39-3.45 (m, 7H, H-2, H-5, 2H-6, CH-N_3, CH_2-O), 2.15-2.00 (m, 11H, 3CO-CH_3, CH=CH-CH_2), 1.71 (s, 3H, O_3CCH_3), 1.45-1.12 (m, 31H, 11 CH_2, $COCMe_3$), 0.88 (t, 3H, CH_3).

Anal. Calcd for $C_{37}H_{61}N_3O_{12}$ (739.89): C, 60.06; H, 8.31; N, 5.68. Found: C, 60.06; H, 8.44; N, 5.74.

(2S,3R,4E)-2-Azido-3-tert-butyldimethylsilyloxy-1-(2,3,4,6-tetra-O-acetyl-ß-D-glucopyranosyloxy)-4-octadecene (3aC). From compounds 2a and 1C the reaction product 3aC was obtained as described for compounds 3aA and 3aB. The reaction product was chromatographed on silica gel (petroleum ether/ethyl acetate = 9:1, normal pressure) to yield 17% of compound 3aC as a colorless oil and 48% of the corresponding ortho-ester derivative.

3aC: TLC R_F (petroleum ether/ethyl acetate = 4:1); $[\alpha]_D^{20}$ = -25.2° (c=2, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ 5.70-5.62 (m, 1H, CH=CH-CH_2), 5.41 (dd, 1H, CH=CH-CH_2, J = 15.2 Hz, J = 7.9 Hz), 5.27-4.98 (m, 3H, H-2, H-3, H-4), 4.53 (d, 1H, H-1), $J_{1,2}$ = 7.9 Hz), 4.30-3.34 (m, 7H, H-5, 2H-6, CH-N_3, CH_2-O, CH-OSi), 2.15-1.99 (m, 14H, 4CO-CH_3, CH=CH-CH_2), 1.45-1.18 (m, 22H, 11 CH_2), 0.95-0.83 (m, 12H, CH_3, Si-$C(CH_3)_3$), 0.08 (s, 3H, $SiCH_3$ 0.03 (s, 3HH, $SiCH_3$).

Anal. Calcd for $C_{38}H_{67}N_3O_{11}Si$ (770.05): C, 59.27; H, 8.77; N, 5.46. Found: C, 59.36; H, 8.85; N, 5.53.

Ortho-ester: TLC R_F 0.41 (petroleum ether/ethyl acetate = 4:1); $[\alpha]_D^{20}$ -2.9° (c=1, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ 5.76 (d, 1H, H-1, $J_{1,2}$ = 5.1 Hz), 5.76-5.61 (m, 1H, CH=C<u>H</u>-CH_2), 5.41 (dd, 1H, C<u>H</u>=CH-CH_2, J = 15.2 Hz, J = 7.9 Hz), 5.20 (dd, 1H, H-3), 4.93 (dd, 1H, H-4, J = 9.4 Hz, J = 1.8 Hz), 4.39-3.39 (m, 8H, H-2, H-5, 2H-6, C<u>H</u>-N_3, CH_2-O, CH-O-Si), 2.15-2.00 (m, 11H, 3CO-CH_3, CH=CH-C<u>H_2</u>), 1.73 (s, 3H, O_3CCH_3), 1.45-1.15 (m, 22H, 11 CH_2), 0.95-0.83 (m, 12H, CH_3, Si-$C(CH_3)_3$), 0.08 (s, 3H, $SiCH_3$), 0.03 (s, 3H, $SiCH_3$).

Anal. Calcd for $C_{38}H_{67}N_3O_{11}Si$ (770.05): C, 59.27; H, 8.77; N, 5.46. Found: C, 59.17; H, 8.76; N, 5.45.

(2S,3R,4E)-2-Azido-2-benzoyloxy-1-(2,3,4,6-tetra-O-pivaloyl-β-D-glucopyranosyloxy)-4-octadecene (3bA). Compound 1A (2 g, 4.6 mmol) and compound 2b (4.6 g, 7.0 mmol) were dissolved in dry dichloromethane (40 mL) and the solution stirred in presence of molecular sieves (4 A, 0.1 g) for 30 min. Boron trifluoride·diethyl ether in dry dichloromethane (0.1 M solution, 2.2 mL) was added in five portions within 30 h. After 48 h the reaction mixture was diluted with petroleum ether (200 mL), filtered, and the filtrate treated with saturated sodium hydrogen carbonate solution (50 mL). The organic extract was dried over sodium sulfate and then concentrated. The residue was chromatographed on silica gel (toluene/acetone = 40:1, normal pressure) to yield 4.0 g (94%) of compound 3bA as a colourless oil: $[\alpha]_D^{20}$ = 16.2° (c=1, $CHCl_3$); TLC R_F 0.57 (toluene/acetone = 40:1); ^1H NMR (250 MHz, $CDCl_3$) δ 8.05, 7.58, 7.45 (3m, 5H, C_6H_5), 5.99-5.83 (m, 1H, CH=C<u>H</u>-CH_2), 5.65-5.45 (m, 2H, C<u>H</u>=CH-CH_2, C<u>H</u>-OBz), 5.37-5.02 (m, 3H, H-2, H-3, H-4), 4.58 (d, 1H, H-1; $J_{1,2}$ = 7.9 Hz), 4.25-3.58 (m, 6H, 2H-6, H-5, CH-N_3, CH_2-O), 2.06 (m, 2H, CH=CH-C<u>H_2</u>), 1.45-1.04 (m, 58H, 12 CH_2, 4 CMe_3), 0.89 (t, 3H, CH_3.

Anal. Calcd for $C_{51}H_{81}N_3O_{12}$ (928.21): C, 65.99; H, 8.79; N, 4.53. Found: C, 66.12; H, 8.67; N, 4.50.

(2S,3R,4E)-2-Azido-1-(β-D-glucopyranosyloxy)-3-hydroxy-4-octadecene (4). **(a) From compound** 3aA. A solution of compound 3aA (3.2 g, 4.3 mmol) in dry dichloromethane (50 mL) was treated with sodium methoxide in dry methanol (0.05 M solution, 8 mL) for 6 h. The mixture was neutralized with an ion exchange resin (Amberlite JR 120, H^+), filtered, and the filtrate concentrated. The residue was chromatographed on silica gel (chlo-

roform/methanol = 17:3, normal pressure): yield 1.9 g (90%) of compound 4 as an amorphous powder, which was used directly in the next step; TLC R_F 0.20 (chloroform/methanol = 9:1); ^1H NMR (250 MHz, DMSO-d_6) δ 5.73-5.58 (m, 1H, CH=C<u>H</u>-CH_2), 5.42 (dd, 1H, C<u>H</u>=CH-CH_2, J = 15.2 Hz, J = 7.9 Hz), 5.25 (d, 1H, OH, J = 4.9 Hz), 4.96-4.87 (m, 2H, OH), 4.49 (dd, 1H, OH, J = J = 5.8 Hz), 4.16-4.04 (m, 2H, H-1, C<u>H</u>N_3), 3.77-2.90 (m, 9H, H-2, H-3, H-4, H-5, 2H-6, CH_2-O, C=CH-C<u>H</u>-OH), 2.08-1.92 (m, 2H, HC=CH-C<u>H</u>$_2$), 1.40-1.12 (m, 22H, 11 CH_2), 0.86 (t, 3H, CH_3).

(b) From compounds 3aB and 3bA: As described above, after 48 h treatment of compounds 3aB and 3bA with sodium methoxide/methanol an 85% and a 78% yield, respectively, of compound 4 was obtained.

(2S,3R,4E)-2-Amino-1-(ß-D-glucopyranosyloxy)-3-hydroxy-4-octadecene (5). A solution of compound 4 (1.65 g, 3.4 mmol) in pyridine/water (1:1, 50 mL) was saturated at room temperature with hydrogen sulfide. After 24 h the reaction mixture was concentrated and purified by chromatography on silica gel (chloroform/methanol = 1:1 and chloroform/methanol/water = 5:4:1, normal pressure) to yield 1.47 g (94%) of compound 5 as an amorphous powder, which was used directly in the next step; TLC R_F 0.64 (chloroform/methanol/water = 5:4:1); ^1H NMR (250 MHz, DMSO-d_6) δ 5.70-5.55 (m, 1H, CH=C<u>H</u>-CH_2), 5.39 (dd, 1H, C<u>H</u>=CH-CH_2, J = 15.2 Hz, J = 7.9 Hz), 5.15-4.85 (m, 2H, NH_2, OH), 4.15-3.99 (m, 2H, H-1, C<u>H</u>-NH_2); 3.75-2.90 (m, 14H, H-2, H-3, H-4, H-5, 2H-6, CH_2-O, C=CH-C<u>H</u>-O, NH_2, OH); 2.03-1.90 (m, 2H, CH=CH-C<u>H</u>$_2$), 1.38-1.07 (m, 22H, 11 CH_2), 0.80 (t, 3H, CH_3).

(2S,3R,4E)-1-(ß-D-Glucopyranosyloxy)-2-hexadecanoylamino-3-hydroxy-4-octadecene (6). A solution of compound 5 (1.47 g, 3.2 mmol) in tetrahydrofuran (50 mL) and an aqueous solution of sodium acetate (50%, 50 mL) were combined and vigorously stirred with hexadecanoyl chloride (0.87 g, 3.2 mmol). After 2 h the mixture was diluted with tetrahydrofuran (350 mL), the organic phase was separated, washed with saturated sodium chloride solution (2 x 50 mL) and concentrated finally under high vacuum (0.01 torr). The residue was chromatographed on silica gel (chloroform/methanol = 9:1, normal pressure) to yield 1.81 g (81%) of compound 6 as an amorphous powder; $[\alpha]_{598}^{20}$ = -9.5 (<u>c</u>=1, pyridine); TLC R_F 0.40 (chloroform/methanol = 17:3); ^1H NMR (250 MHz, DMSO-d_6) δ 7.50 (d, 1H, NH; J = 8.7 Hz), 5.52 (td, 1H, CH=C<u>H</u>-CH_2; J = 13.4 Hz, J = 6.1 Hz), 5.35 (dd, 1H, C<u>H</u>=CH-CH_2; J = 13.4 Hz, J = 6.5 Hz), 5.02 (d, 1H, OH; J = 4.3

Hz), 4.92 (m, 3H, 3 OH), 4.50 (t, 1H, OH; J = 4.9 Hz), 4.09 (d, 1H, H-1; J = 8.2 Hz), 4.00-3.55 (m, 4H), 3.45 (m, 2H), 3.15-2.90 (m, 4H), 2.10-1.90 (m, 4H, CO-CH_2, CH=CH-C$\underline{H}_2$), 1.45 (m, 2H, CH_2), 1.22 (m, 46H, 23 CH_2), 0.88 (m, 6H, 2 CH_3).

O-(2,3,4,6-Tetra-O-acetyl-α-D-galactopyranosyl)trichloroacetimidate (7a). This compound was obtained as described previously.[19]

O-(2,3,4,6-Tetra-O-pivaloyl-α-D-galactopyranosyl)trichloroacetimidate (7b). (a) **Synthesis of 2,3,4,6-Tetra-O-pivaloyl-D-galactose.** As described for the synthesis of 2,3,4,6-tetra-O-pivaloyl-D-glucose from 1,2,3,4,6-penta-O-pivaloyl-D-galactopyranose, this compound was obtained in 85% total yield. (7b): This compound was immediately used for the synthesis of compound 7b by applying the procedure described for compound 2b. Yield 60% of compound 7b as an oil; TLC R_F 0.42 (petroleum ether/ethyl acetate = 9:1); ^1H NMR (250 MHz, $CDCl_3$) δ 8.68 (s, 1H, NH), 6.62 (d, 1H, H-1; $J_{1,2}$ = 3.4 Hz), 5.58-5.39 (m, 3H, H-2, H-3, H-4); 4.50 (dd, 1H, H-5; $J_{5,6}$ = $J_{5,6'}$ = 6.7 Hz), 4.15-4.05 (m, 2H, 2H-6), 1.33-1.10 (m, 36H, 4 CO-CMe_3).

Anal. Calcd for $C_{28}H_{44}Cl_3NO_{10}$ (661.01): C, 50.88; H, 6.71; N, 2.12. Found: C, 51.40; H, 6.76; N, 1.85.

(2S,3R,4E)-2-Azido-3-benzoyloxy-1-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyloxy)-4-octadecene (8aA). Compound 8aA was obtained in 80% yield from compounds 7a and 1A as described for compound 3aA. Compound 8aA was a colorless oil; $[\alpha]_D^{20}$ = -2.6° (c=7, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ 8.07-7.44 (m, 5H, C_6H_5), 5.97-5.90 (m, 1H, CH=C$\underline{H}$-CH_2), 5.62-5.53 (m, 2H, C$\underline{H}$(OBz)-CH=C$\underline{H}$), 5.39 (dd, 1H, H-4; $J_{3,4}$ = 2.4 Hz; $J_{4,5}$ = 1 Hz), 5.27-5.22 (dd, 1H, H-2; $J_{1,2}$ = 8.0 Hz; $J_{2,3}$ = 10 Hz), 5.03-4.99 (dd, 1H, H-3; $J_{2,3}$ = 10 Hz; $J_{3,4}$ = 2.4 Hz), 4.50 (d, 1H, H-1; $J_{1,2}$ = 7.8 Hz), 4.16-4.07 (m, 2H, 2H-6), 4.00-3.89 (m, 3H, H-5, O-C$\underline{H}_2$), 3.60-3.57 (m, 1H, CH-N_3), 2.15, 2.10, 2.02, 1.99 (4s, 12H, 4 $COCH_3$), 1.24 (brs, 24H, 12 CH_2), 0.89-0.86 (t, 3H, CH_3).

Anal. Calcd for $C_{39}H_{57}N_3O_{12}$ 1.5 H_2O (786.92): C, 59,52; H, 7.43; N, 5.40. Found: C, 59.77; H, 7.44; N, 5.50.

(2S,3R,4E)-2-Azido-3-benzoyloxy-1-(2,3,4,6-tetra-O-pivaloyl-β-D-galactopyranosyloxy)-4-octadecene (8bA). This compound was obtained in 96% yield from compounds 7b and 1A as described for compound 3bA. Compound 8bA was a colorless oil; $[a]_D^{20}$ = -2.3° (c=1.2, $CHCl_3$); TLC R_F 0.55 (toluene/acetone = 40:1); ^1H NMR (250 MHz, $CDCl_3$ δ 8.05, 7.58, 7.45 (3m, 5H, C_6H_5), 5.91 (m,

1H, CH=CH-CH_2), 5.63-5.47 (m, 2H, CH=CH-CH_2, CH-OBz), 5.40 (d, 1H, H-4, $J_{3,4}$ = 3,0 Hz), 5.26 (dd, 1H, H-2, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 10 Hz), 5.09 (dd, 1H, H-3, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 3.0 Hz), 4.57 (d, 1H, H-1, $J_{1,2}$ = 7.9 Hz), 4.20-3.62 (m, 6H, H-5, 2H-6, CH_2-O, CH-N_3), 2.12-2.01 (m, 2H, CH=CH_2), 1.45-1.04 (m, 58H, 11 CH_2, 4 CMe_3), 0.89 (t, 3H, CH_3).

Anal. Calcd for $C_{51}H_{81}N_3O_{12}$ (928.21): C 65.99; H, 8.79; N, 4.53. Found: C, 65.83; H, 8.85; N, 4.54.

(2S,3R,4E)-2-Azido-1-(ß-D-galactopyranosyloxy)-3-hydroxy-4-octadecene (9). This compound was obtained in 71% yield from compounds 8aA and 8bA as described for compounds 3aA and 3bA, respectively. Compound 9 was an amorphous powder, which was directly used in the next step; TLC R_F 0.18 (chloroform/methanol = 9:1).

(2S,3R,4E)-2-Amino-1-(ß-D-galactopyranosyloxy)-3-hydroxy-4-octadecene (10). This compound was obtained in 95% yield from compound 9 as described for compound 5. Compound 10 was an amorphous powder, which was used directly in the next step; TLC R_F 0.64 (chloroform/methanol/water = 5:4:1); ^1H NMR (250 MHz, $CDCl_3$) δ 5.70-5.55 (m, 1H, CH=CH-CH_2), 5.39 (dd, 1H, CH=CH-CH_2; J = 15.2 Hz, J = 7.9 Hz), 5.15-4.85 (m, 2H, NH_2, OH), 4.15-3.99 (m, 2H, H-1, CH-NH_2), 3.75-2.90 (m, 14H, H-2, H-3, H-4, H-5, 2H-6, CH_2O, C=CH-CH-O, NH_2, OH), 2.03-1.90 (m, 2H, CH=CH-CH_2), 1.38-1.07 (m, 22H, 11CH_2), 0.80 (t, 3H, CH_3).

(2S,3R,4E)-1-(ß-D-Galactopyranosyloxy)-2-hexadecanoylamino-3-hydroxy-4-octadecene (11). This compound was obtained in 83% yield from compound 10 as described for compound 6. Compound 11 was an amorphous powder; mp 182 °C from chloroform/methanol = 9:1); $[\alpha]_{598}$ = -5.2° (c=1, pyridine); ^1H NMR (250 MHz, DMSO-d_6) ° 7.51 (d, 1H, NH; J = 8.9 Hz), 5.53 (td, 1H, CH=CH-CH_2; J = 15.6 Hz, J = 6.1 Hz), 5.34 (dd, 1H, CH=CH-CH_2; J = 15.6 Hz, J = 7.1 Hz), 4.88 (m, 2H, 2 OH), 4.70 (d, 1H, OH; J = 4.5 Hz), 4.56 (dd, 1H, OH; J = 5.3 Hz, J = 5.3 Hz), 4.36 (d, 1H, OH; J = 6.3 Hz), 4.03 (d, 1H, H-1; J = 7.0 Hz), 3.98 (m, 1H), 3.87 (m, 1H), 3.79 (m, 2H), 3.62 (m, 2H), 3.50 (m, 3H), 3.31 (m, 2H), 2.02 (t, 2H, CO-CH_2; J = 7.5 Hz), 1.92 (m, 2H, CH=CH-CH_2), 1.45 (m, 2H, CH_2), 1.23 (m, 46H, 23 CH_2), 85 (t, 6H, 2 CH_3; J = 6.2 Hz).

Anal. Calcd for $C_{40}H_{77}NO_8 \cdot H_2O$ (718.05): C, 67.75; H, 11.09; N, 1.98. Found: C, 67.43; H, 10.76; N, 2.04.

O-[2,3,6-Tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-ß-D-galactopyranosyl)-α-D-glucopyranosyl]trichoroacetimidate (12a). This compound was obtained as described previously.[25]

O-[2,3,6-Tri-O-pivaloyl-4-O-(2,3,4,6-tetra-O-pivaloyl-ß-D-galactopyranosyl)-α-D-glucopyranosyl]trichloroacetimidate (12b). (a) **1,2,3,6-tetra-O-pivaloyl-4-O-(2,3,4,6-tetra-O-pivaloyl-ß-D-galactopyranosyl)-ß-D-glucopyranose** was obtained as described for the synthesis of 1,2,3,4,6-penta-O-pivaloyl-D-glucose.[2] It was necessary to reflux the reaction mixture for 5 days because of the slow reaction. The product yield was 72%, mp 168-169 °C (from ethanol); TLC R_F 0.38 (petroleum ether/ethyl acetate = 9:1); $[\alpha]_D^{20}$ = +0.5° (c=4, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ 5.69 (d, 1H, H-1, $J_{1,2}$ = 8.2 Hz), 5.40 (d, 1H, H-4'; $J_{3',4'}$ = 2.4 Hz), 5.33-4.95 (m, 4H, H-2, H-2', H-3, H-3'), 4.58-3.62 (m, 8H, H-1', H-4, H-5, H-5', 2H-6, 2H-6'), 1.35-1.00 (m, 72H, 8 CMe_3).

Anal. Calcd for $C_{52}H_{86}O_{19}$ (1015.24): C, 61.52; H, 8.54. Found: C, 61.34; H 8.43.

(b) 2,3,6-Tri-O-pivaloyl-4-O-(2,3,4,6-tetra-O-pivaloyl-ß-D-galactopyranosyl)-D-glucopyranose. This compound was synthesized in 65% yield as described for 2,3,4,6-tetra-O-pivaloyl-D-glucose. It was a colorless oil; TLC R_F 0.35 (petroleum ether/ethyl acetate = 4:1). This product was directly used for the synthesis of compound 12b by using the procedure described for compound 11b.

(c) 11b. This compound was obtained in 52% yield as an amorphous powder; $[\alpha]_D^{20}$ = +39.5° (c=2, chloroform). TLC R_F 0.6 (petroleum ether/ethyl acetate = 8:2), ^1H NMR (250 MHz, $CDCl_3$) δ 8.65 (s, 1H, NH), 6.49 (d, 1H, H-1; J = 3.6 Hz), 5.60 (dd, 1H, H-3; $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz), 5.41 (d, 1H, H-4', $J_{3',4'}$ = 2.5 Hz), 5.16 (dd, 1H, H-2'; J = 10.3 Hz, $J_{1',2'}$ = 7.9 Hz), 5.02-4.93 (m, 2H, H-2, H-3'), 4.58-3.90 (m, 8H, H-1', H-4, H-5, H-5', 2H-6, 2H-6'), 1.40-1.00 (m, 63H, 7 CMe_3).

Anal. Calcd for $C_{49}H_{78}Cl_3NO_{18}$ (1075.51): C, 54.72; H, 7.31; N, 1.30. Found: C, 54.70; H, 7.43; N 1.10.

O-[2,3,6-Tri-O-acetyl-4-O-(2,3-di-O-acetyl-4,6-di-O-isopropylidene-ß-D-galactopyranosyl)-α-D-glucopyranosyl]-trichloroacetimidate (12c). 2,3,6-Tri-O-acetyl-4-O-(2,3-di-O-acetyl-4,6-O-isopropylidene-ß-D-galactopyranosyl)-D-glucopyranose was obtained in 81% yield from 1,2,3,6-Tri-O-acetyl-4-O-

(2,3-di-O-acetyl-4,6-O-isopropylidene-ß-D-galactopyranosyl)-ß-D-glucopyranose[26] by using the same procedure as described for 2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-ß-D-galactopyranosyl)-D-glucopyranose.[25] This compound [TLC R_F 0.2 (toluene/methanol = 8:1)] was directly used for the synthesis of compound 12c by application of the procedure reported for the synthesis of compound 12a. The yield of 12c was 66%; $[\alpha]_D^{20}$ = +80° (c=3.5, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$), δ 8.65 (s, 1H, NH), 6.49 (d, 1H, H-1; $J_{1,2}$ = 3.6 Hz), 5.56 (dd, 1H, H-3; $J_{2,3}$ = $J_{3,4}$ = 10 Hz), 5.22 (dd, 1H, H-2', $J_{2',3'}$ = 10 Hz; $J_{1',2'}$ = 7.9 Hz), 5.09 (dd, 1H, H-2; $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 10 Hz), 4.79 (dd, 1H, H-3'; $J_{2',3'}$ = 10 Hz, $J_{3',4'}$ = 3.6 Hz), 4.57-3.77 (m, 8H, H-1', H-4, H-4', H-5, 2H-6, 2H-6'), 3.32 (s, 1H, H-5'), 2.20-1.95 (m, 15H, 5 $COCH_3$), 1.44 (s, 3H, CH_3), 1.38 (s, 3H, CH_3).

Anal. Calcd for $C_{27}H_{36}Cl_3NO_{15}$ (736.94): C, 44.00; H, 4.92; N, 1.90. Found: C, 44.13; H,5.11; N, 1.59.

(2S,3R,4E)-2-Azido-3-benzoyloxy-1-[2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-ß-D-glucopyranosyl)-ß-D-glucopyranosyloxy]-4-octadecene (13aA). Compound 1A (1.0 g, 2.33 mmol) and compound 12a (2.75 g, 3.66 mmol) were dissolved in dry tetrachloromethane (50 mL) and the solution stirred in the presence of molecular sieves (4A, 0.1 g) for 15 min at room temperature. Boron trifluoride·diethyl ether in dry dichloromethane (0.1 M solution, 1.1 mL) was added within 4 h. The ortho-ester formed rearranged under the reaction conditions to compound 13aA. The reaction mixture was diluted with petroleum ether (150 mL) and then treated with saturated sodium hydrogen carbonate solution (10 mL). The organic extract was dried over magnesium sulfate and then concentrated. The residue was chromatographed on silica gel (petroleum ether/ethyl acetate = 3:2, medium pressure) to yield 2.10 g (85%) of compound 13aA as a colorless oil; $[\alpha]_D^{20}$ = -22.3° (c=3.8, $CHCl_3$); TLC R_F 0.46 (petroleum ether/ethyl acetate = 3:2); ^1H NMR (250 MHz, $CDCl_3$) δ 8.06-7.42 (m, 5H, C_6H_5), 5.96-5.85 (m, 1H, CH=C<u>H</u>-CH_2), 5.65-5.48 (m, 2H, C<u>H</u>(OBz)-C<u>H</u>=CH), 5.35 (d, 1H, H-4'; J = 2.7 Hz), 5.23-5.07 (m, 2H, H-2', H-3), 4.98-4.89 (m, 2H, H-2, H-3'), 4.53-4.45 (m, 3H, H-1, H-1', H-4), 4.12-4.02 (m, 3H, O-CH_2, H-5'), 3.97-3.79 (m, 4H), 3.65-3.54 (m, 2H), 2.20-1.96 (m, 23H, 7 CO-CH_3, CH=CH-C<u>H</u>$_2$), 1.37-1.24 (m, 22H, 11 CH_2), 0.87 (t, 3H, CH_3; J = 6.2 Hz).

Anal. Calcd for $C_{62}H_{73}N_3O_{20}$ (1048.15): C, 58.44; H, 7.02; N, 4.01. Found: C, 58.35; H, 7.08; N, 3.99.

(2S,3R,4E)-2-Azido-2-benzoyloxy-1-[2,3,6-tri-O-pivaloyl-4-O-(2,3,4,6-tetra-O-pivaloyl-ß-D-galactopyranosyl)-ß-D-glucopyranosyloxy]-4-octadecene (13bA). This compound was obtained in 87% yield from compounds 12b and 1A as described for compound 3bA. Compound 13bA was a colorless oil; $[\alpha]_D^{25}$ = -13.8° (c=2, $CHCl_3$); TLC R_F 0.77 (petroleum ether/ethyl acetate = 4:1); ^1H NMR (250 MHz, $CDCl_3$) δ 8.05, 7.58, 7.45 (3m, 5H, C_6H_5), 5.91 (m, 1H, CH=CH-CH_2), 5.65-5.45 (m, 2H, CH=CH-CH_2, CH-OBz), 5.40 (d, 1H, H-4'; $J_{3',4'}$ = 3 Hz), 5.26-4.85 (m, 4H, H-2, H-2', H-3, H-3'), 4.62-3.50 (m, 12H, H-1, H-1', H-4, H-5, H-5', 2H-6, 2H-6', CH-N_3, CH_2-O), 2.12-2.0 (m, 2H, C=CH-CH_2), 1.50-1.03 (m, 85H, 11 CH_2, 7 CMe_3), 0.86 (t, 3H, CH_3).

Anal. Calcd for $C_{72}H_{115}N_3O_{13}$ (1342.59): C, 64.41; H, 8.62; N, 3.13. Found: C, 64.81; H, 8.53; N, 3.08.

(2S,3R,4E)-2-Azido-3-benzoyloxy-1-[2,3,6-Tri-O-acetyl-4-O-(2,3-di-O-acetyl-4,6-O-isopropylidene-ß-D-galactopyranosyl)-ß-D-glucopyranosyloxy]-4-octadecene (13cA). This compound was synthesized in 78% yield by using the procedure described for the synthesis of compound 13aA; $[\alpha]_D^{25}$ = -3,3° (c=1, $CHCl_3$); TLC R_F 0.31 (toluene/acetone = 4:1; ^1H NMR (250 MHz, $CDCl_3$) δ 8.05, 7.58, 7.45 (3m, 5H, C_6H_5), 5.91 (m, 1H, CH=CH-Ch_2), 5.65-5.45 (m, 2H, CH=CH-CH_2, CH-OBz), 5.20 (dd, 1H, H-3; $J_{2,3}$ = $J_{3,4}$ = 10 Hz), 4.95 (dd, 1H, H-2'; $J_{2',3'}$ = 10 Hz, $J_{1',2'}$ = 7.9 Hz), 4.80 (dd, 1H, H-3'; $J_{2',3'}$ = 10 Hz, $J_{3',4'}$ = 3.6 Hz), 4.55-3.50 (m, 13H, H-1, H-1', H-2, H-4, H-4', H-5, 2H-6, 2H-6', CH-N_3, CH_2-O), 2.20-1.95 (m, 17H, 5$COCH_3$, C=CH-CH_2), 1.42 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.35-1.18 (m, 22H, 11CH_2), 0.86 (t, 3H, CH_3).

Anal. Calcd for $C_{50}H_{73}N_3O_{18}$ (1004.13): C, 59.81; H,7.33; N, 4.18. Found: C, 59.60; H, 7.42; N, 4.07.

(2S,3R,4E)-2-Azido-1-[4-O-(ß-D-galactopyranosyl)-ß-D-glucopyranosyloxy]-3-hydroxy-4-octadecene (14). This compound was obtained from compounds 13aA and 13bA as described for compounds 3aA and 3bA, respectively. Compound 14 was obtained in 75% yield as an amorphous powder, which was immediately used in the next step; TLC R_F 0.08 (chloroform/methanol = 9:1).

(2S,3R,4E)-2-Amino-1-[4-O-(ß-D-galactopyranosyl)-ß-D-glucopyranosyloxy]-3-hydroxy-4-octadecene (15). This compound was obtained in 96% yield from compound 14 as described for

compound 5. Compound 15 was an amorphous powder, which was used directly in the next step; TLC R_F 0.71 (chloroform/ methanol/ water = 5:4:1); ^1H NMR (250 MHz, DMSO-d_6) δ 5.62 (m, 1H, CH=CH-CH_2), 5.45 (dd, 1H, CH=CH-CH_2, J = 15.2 Hz; J = 6.7 Hz), 5.12 (d, 1H, OH), 4.82-4.47 (m, 6H, 6OH), 4.25-4.15 (m, 2H) 3.86-2.77 (m, 21H, 2H-1, 2H-2, 2H-3, 2H-4, 2H-5, 4H-6, O-CH_2, CH-NH_2, CH=CH-CH-OH), 2.01-1.95 (m, 2H, CH=CH-CH_2), 1.33-1.20 (m, 22H, 11 CH_2), 0.86 (t, 3H, CH_3).

(2S,3R,4E)-1-[4-O-(ß-D-glucopyranosyl)-ß-D-glucopyranosyloxy]-2-hexadecanoylamino-3-hydroxy-4-octadecene (16). This compound was obtained in 80% yield from compound 15 as described for compound 6. Compound 16 was an amorphous powder; mp 198 °C from chloroform/methanol (17:3); $[\alpha]_{598}^{20}$ = -9.9° (c=0.7, pyridine); TLC R_F 0.20 (chloroform/methanol = 17:3); ^1H NMR (250 MHz, DMSO-d_6): δ 7.52 (d, 1H, NH; J = 9.3 Hz), 5.33 (td, 1H; CH=CH-CH_2; J = 15.6 Hz, J = 6.7 Hz), 5.13 (m, 2H, 2 OH), 4.89 (d, 1H, OH; J = 5.2 Hz), 4.81 (d, 1H, OH; J = 3.1 Hz), 4.68 (m, 2H, 2 OH), 4.58 (m, 1H, OH), 4.53 (d, 1H, OH; J = 5.1 Hz), 5.20 (m, 3H, H-1, H-1', H-4), 4.05-3.0 (m, 15H), 2.0 (m, 4H, CO-CH_2, CH=CH-CH_2), 1.45 (m, 2H, CH_2), 1.25 (m, 46H, 23 CH_2), 0.88 (t, 6H, 2 CH_3; J = 7.2 Hz).

Anal. Calcd for $C_{46}H_{57}NO_{13}\cdot 0.5\ H_2O$ (871.12): C, 63.42; H, 10.18; N, 1.60. Found: C, 63.45; H, 10.08; N, 1.85.

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