Synthesis of a Pseudo Tetrasaccharide Mimic of Ganglioside GM1

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The pseudo tetrasaccharide **2** was designed to mimic ganglioside GM1, the membrane receptor of both the cholera toxin and of the heat-labile toxin of *E. coli*. Compound **2** retains the Gal and Neu5Ac recognition determinant of GM1 and uses as the scaffold element a new, conformationally restricted cyclohexanediol (DCCHD **3**), with the same relative and absolute configuration of natural galactose. The diol **3** was enantioselectively synthesized by an asymmetric Diels–Alder reaction, followed by dihydroxylation of the resulting cyclohexene. Glycosylation of **3** with the sialyl donor **17** and the Galβ(1-3)GalNAc donor **15**, followed by removal of the protecting groups, completed the synthesis of **2**.

Introduction

The cholera toxin (CT) and other related enterotoxins, like the heat-labile toxin of *E. Coli* (LT), are recognized in the epithelial cells of the host by the pentasaccharide portion of the ganglioside GM1 **1** (Figure 1).[1] In the course of a program, directed toward the design and synthesis of artificial cholera toxin binders,[2] the pseudo tetrasaccharide **2** (Figure 1) was designed as a promising substitute for the natural receptor. Saccharides **1** and **2** share the presence of a galactose (Gal) and a sialic acid (Neu5Ac) units at the oligosaccharide non-reducing end. X-ray crystallography of the GM1:CT complex has shown that these residues interact directly with the toxin.[3] However, in **2** the reducing end of the ganglioside has been substitued by the conformationally restricted dicarboxycyclohexanediol (DCCHD) **3** (Figure 1). The use of cyclohexanediols as core sugar mimetics is an attractive strategy in order to simplify the molecular structure of bioactive oligosaccharides, and produce functional analogues which may be easier to synthesize and of increased metabolic stability. This approach has been used with success to design sialyl LewisX mimics by replacing an *N*-acetylglucosamine unit with (*R,R*)-*trans*-1,2-cyclohexanediol.[4] In order to replace a 3,4-disubstituted Gal residue, as it is found in the GM1 oligosaccharide core region, a *cis*-cyclohexanediol is required, which must be enantiomerically pure and conformationally stable in order to distinguish unequivocally between the axial and the equatorial substituents. The diol **3**, which features the same absolute configuration of natural galactose, and largely exists in a single chair conformation, appears to be a likely candidate. Indeed, DCCHD **3** was calculated by molecular mechanics to behave as an adequate replacement for the core Gal unit of GM1 and to place the binding determinants Gal and Neu5Ac of **2** in the appropriate position for protein recognition.[2b] In this paper we describe the synthesis of **2**.

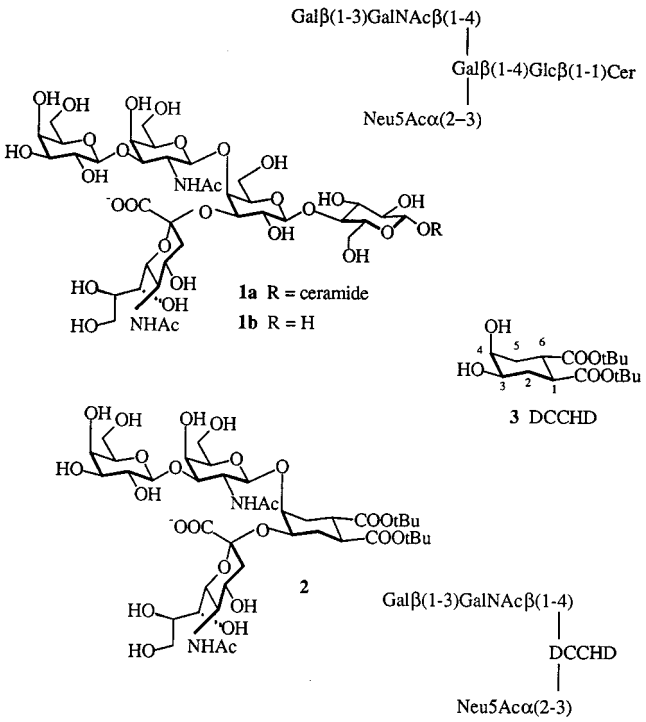

Figure 1. Ganglioside GM1 **1**, and its designed mimic **2**

The retrosynthetic analysis is shown in Scheme 1. The diol **3**, which surprisingly has never been described in the literature, can be synthesized by an enantioselective Diels–Alder reaction between a chiral fumarate equivalent and butadiene, followed by double-bond dihydroxylation. From **3**, the pseudo tetrasaccharide could be assembled by reaction of the axial hydroxy group with an appropriate donor of Galβ(1-3)GalNAc, followed by sialylation of **4** (see Scheme 1, Route a). Alternatively, sialylation of the equa-

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torial hydroxy group followed by reaction of **5** with the Galβ(1-3)GalNAc donor could be planned (see Scheme 1, Route b). Both strategies have been successfully employed in the chemical synthesis of GM1,[5] although leaving the sialylation to the last step, as in Route a, was found to be more problematic.[5b] Route a was initially followed, and allowed the definition of optimal conditions for the selective formation of the β-glycosidic linkage between GalNAc and DCCHD. However, the sialylation of **4** was found to be impractical, and the target molecule was eventually synthesized following Route b.

Results and Discussion

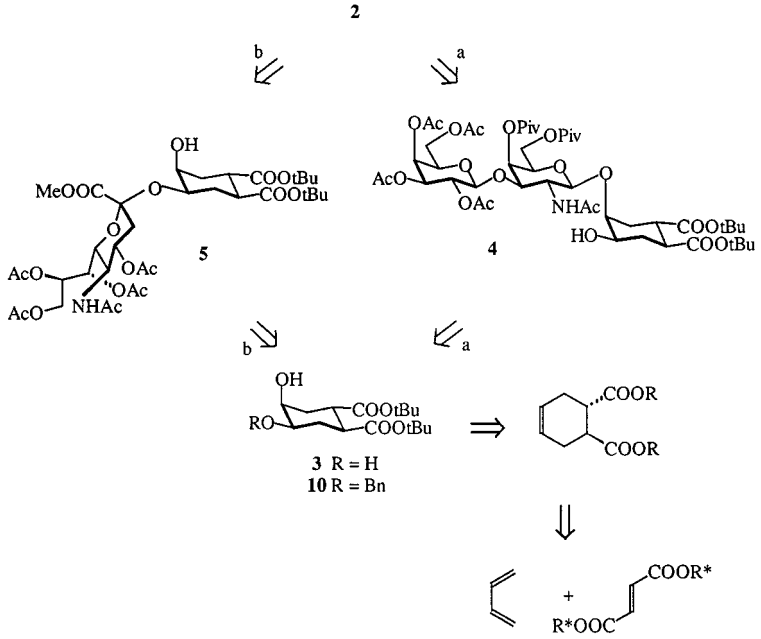
Enantioselective Synthesis of DCCHD 3

Although many methods for the enantioselective Diels−Alder reaction have been introduced in recent years,[6] few of them were reported to be effective for the cycloaddition of butadiene to fumarates.[7] Among them, Helmchen's methodology[7c] was chosen, which involves the low-temperature $TiCl_4$-promoted reaction of butadiene with the commercially available bis[ethyl (*S*)-lactate]fumaric ester **6** (Scheme 2). In the present study the desired (*S*,*S*)-dicarboxycyclohexene **7** was obtained as a 6:1 mixture with the (*R*,*R*) isomer. The ratios were determined by ^{13}C NMR of the crude reaction mixture. The crude product was hydrolyzed with LiOH in $MeOH/H_2O$, and the acid **8** was isolated in an overall yield of 69% by flash chromatography.[8] At this stage, the (*S*,*S*) enantiomer of the acid was obtained in an enantiopure form by crystallization of its quinine salt, following a published procedure.[9] Finally, the di-*tert*-butyl ester **9** was synthesized in a yield of 73%, by reaction of (*S*,*S*)-**8** with dimethylformamide di-*tert*-butyl acetal in refluxing benzene.[10] Dihydroxylation of **9** with catalytic amounts of $OsCl_3$ and Me_3NO in acetone/water[11] gave the diol **3** quantitatively. This was selectively protected as a benzyl ether at the equatorial hydroxy group by sequential reaction with Bu_2SnO and benzyl bromide/Bu_4NI in benzene,[12] to give **10** in a yield of 99%.

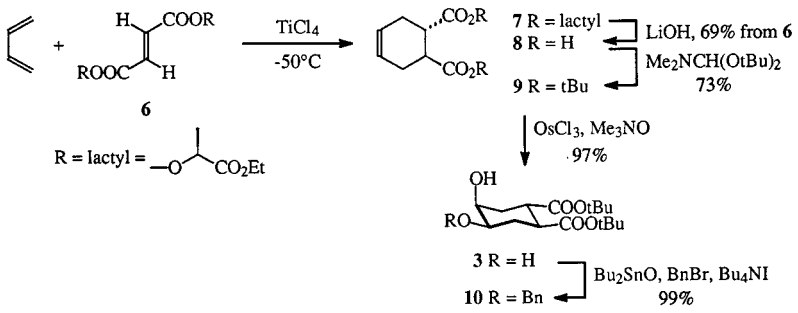
Synthesis of the Galβ(1-3)GalNAc Donor 15

2-Acetamido sugars are not usually regarded as valuable glycosyl donors.[13] However, if the 2-acetamidotrichloroacetimidate **15** (Scheme 3) could be used as the Galβ-(1-3)GalNAc donor, it would straightforwardly introduce the desired 2-acetamido functionality in the reaction product, thus avoiding further manipulation of the oligosaccharide, and possibly compensating for low yields in the glycosylation reaction. Furthermore, an easy entry to **15** is ensured by the elegant procedure reported by Russo and coworkers,[14] which allows a multigram-scale synthesis of the protected GalNAc derivative **11** in 5 steps and an overall yield of 30% from pentaacetylglucosamine. The above reasons led to an investigation of the donor ability of **15** (vide infra).

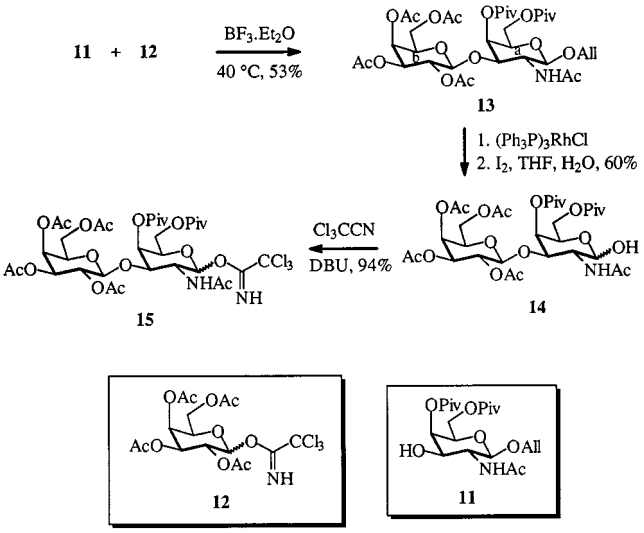
For the synthesis of **15**, starting from **11**, galactosylation with the trichloroacetimidate **12**[15] was attempted. This reaction proved to be sluggish at room temp., with both TMSOTf or $BF_3 \cdot Et_2O$ as promoters. Raising the reaction temp. to 40°C in the presence of a catalytic amount of TMSOTf resulted in the loss of β selectivity. Finally, stereoselective β-glycosylation of **11** with **12** was achieved with 1 mol-equiv. of $BF_3 \cdot Et_2O$ at 40°C and afforded **13** in a yield of 53%.[16] Deallylation of the anomeric oxygen atom[17] gave **14** in an overall yield of 60%. Finally, the trichloroacetimidate **15** was obtained from **14** by the standard procedure[15] in an almost quantitative yield, after silica gel chromatography.



Scheme 1. Retrosynthesis of **2**



Scheme 2. Enantioselective synthesis of **3**

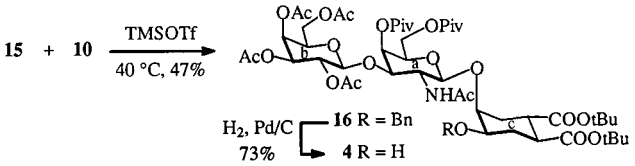


Scheme 3. Synthesis of the Galβ(1-3)GalNAc donor **15**

Synthesis of 2

Route a

As mentioned above, 2-acetamido sugars tend to be poor glycosyl donors. Rapid intramolecular reaction in the presence of Lewis acids results in the formation of oxazolines, which react very sluggishly with glycosyl acceptors. Considering the low reactivity of axial hydroxy groups, such as the 4-OH of DCCHD (Figure 1), difficulties can be anticipated in the formation of the Galβ(1-3)GalNAc−DCCHD bond. Thus, the conditions for this bond formation were initially studied with the benzyl ether **10** as the glycosyl acceptor (Scheme 4). After some experimentation, optimal conditions were found with 2.5 mol-equiv. of acceptor, and adding a catalytic amount of TMSOTf to a refluxing CH_2Cl_2 solution of the glycosylation partners **10** and **15**. The pseudotrisaccharide **16** (Scheme 4) was obtained in a yield of 47%, and the excess acceptor was recovered by flash chromatography. The structure of compound **16** was assigned with the help of H,H-COSY and HETCOR spectra. The GalNAc β-anomeric configuration could be readily derived from the $J_{1,2}$ coupling constant of 9 ± 1 Hz.



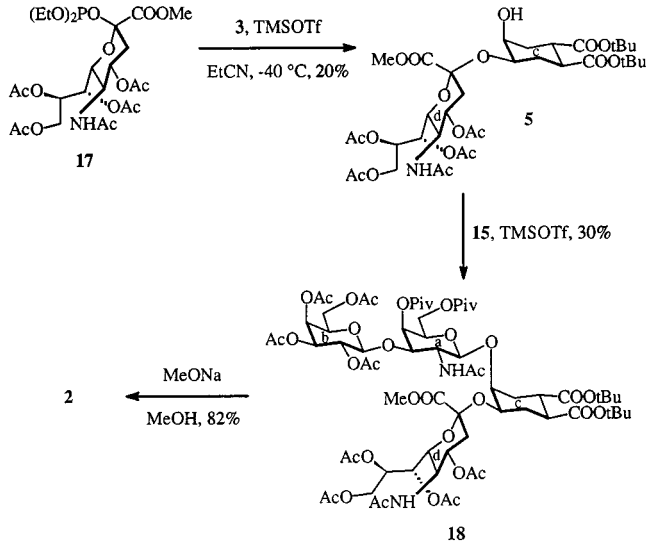
Scheme 4. Route a

Hydrogenolysis of the benzyl ether in **16** gave the alcohol **4** which was subjected to sialylation with phosphite-activated sialic acid donors. However, probably due to the presence of the 2-acetamido group,[5a] **4** turned out not to be a suitable sialyl acceptor in these reactions. Having shown that **15** can perform as a reasonable Galβ(1-3)GalNAc donor, it appeared that Route a could conveniently be abandoned in favor of Route b.

Route b

If the α-sialylation reaction is to be carried out first, advantage can be taken of the markedly different reactivity of equatorial and axial hydroxy groups in this type of reaction. Neu5Ac donors are known to be very sensitive to the steric hindrance of the acceptor, typically permitting regioselective sialylation of 3,4-unprotected galactose.[18] Indeed, sialylation of the unprotected diol **3** could be performed regioselectively at the equatorial position with the phosphite **17**[19][20] (Scheme 5). The reaction was carried out with TMSOTf as a promoter in EtCN at −40°C.[19] The sialyl derivative **5** was isolated in a yield of 20% as a 7:1 α/β mixture, and 60% of non-reacted **3** was recovered. The structure of **5** was assigned as follows. The sialylation site was determined by ^{13}C NMR on the basis of the 4.3 ppm downfield shift of the DCCHD 3-C signal. The diastereomeric α/β ratios were also measured by ^{13}C NMR, exploiting the relative intensity of the anomeric C-2 atoms of the sialic acid moiety at δ = 98.0 (α anomer) and δ = 99.0 (β anomer). The α configuration was assigned to the major isomer by measuring the $J_{C\text{-}1,3\text{-}Hax}$ coupling constant of Neu5Ac.[21] The α anomer gave a doublet at δ = 168.5, with a $J_{C\text{-}1,3\text{-}Hax}$ coupling constant of 6.9 Hz, whereas a

$J_{C\text{-}1,3\text{-}Hax}$ coupling constant of 3.4 Hz was found for the β isomer at δ = 167.9.



Scheme 5. Route b; synthesis of **2**

The desired α anomer could be further purified by flash chromatography (see Experimental Section) and submitted to glycosylation by the Galβ(1-3)GalNAc donor **15** (Scheme 5). An excess of donor (2.3 mol-equiv.) was used, otherwise the reaction conditions were the same as employed for the reaction between **15** and the acceptor **10** (catalytic TMSOTf, refluxing CH_2Cl_2). Thus, the pseudotetrasaccharide **18**, exhibiting the expected coupling constants for the anomeric protons ($J_{1a,2a}$ = 8.7 Hz, $J_{1b,2b}$ = 7.7 Hz), was isolated in a yield of 30%. Finally, MeONa/MeOH hydrolysis gave the target pseudo GM1 **2**, which was fully characterized by 500-MHz 1D- and 2D-NMR spectroscopy.

ELISA and TLC overlay assays showed that **2** is as potent as the GM1 oligosaccharide **1b** in inhibiting the formation of the CT:GM1 complex.[2b]

Experimental Section

General: Solvents: Pyridine and DMF were dried over 4-Å molecular sieves. The other dry solvents were distilled under nitrogen (from liquid nitrogen) shortly before use. THF and benzene were distilled from Na; CH_2Cl_2, MeOH, TEA, DIPEA, and EtCN from CaH_2. − Flash chromatography: Silica gel (Kieselgel 60, 230−400 mesh). − M.p.s: Büchi 535 apparatus, uncorrected values. − MS: VG 7070 EQ-HF. − NMR: Bruker AC-200, AC-300, and AMX-500 (200 MHz, 300 MHz, and 500 MHz for ^1H; 50.3 MHz, 75.4 MHz and 125.7 MHz for ^{13}C), for ^{13}C spectra only selected signals are reported, for spectra in $CDCl_3$ TMS as internal standard, for spectra in CD_3OD δ_H = 3.3, for spectra in [D_6]DMSO δ_H = 2.5, for spectra in C_6D_6 δ_H = 7.2. − IR: Perkin−Elmer FTIR 1600 spectrometer. − Optical rotations: Perkin−Elmer 241 at 589 nm, using 1-mL cells. − Elemental analysis: Perkin−Elmer 240. − Starting materials: **11**,[14] **12**,[15] **17**.[19] For spectral assignments the DCCHD moiety was numbered as shown in Figure 1.

(1*S*,2*S*)-Cyclohex-4-ene-1,2-dicarboxylic Acid (8): The fumarate **6** (2.6 mL, 9.5 mmol) was dissolved in 60 mL of dry CH_2Cl_2, the solution cooled at −50°C and 1 M $TiCl_4$ in CH_2Cl_2 (13.3 mL, 13.3 mmol) was added under N_2. Butadiene was added in small portions over 6 h to the stirred mixture. After 24 h at −50°C, the reaction was quenched with phosphate buffer and the solution filtered through a Celite pad. The organic phase was washed with phosphate buffer, then dried with Na_2SO_4, and concentrated in vacuo to give 3.2 g of crude **7** contaminated by non-reacted **6**. The crude reaction product was dissolved in 3:1 MeOH/H_2O (57 mL) and LiOH (5.1 g) was added. After 24 h at room temp., the mixture was concentrated at reduced pressure to about half the initial volume, 6 N HCl was added to pH = 1, and the resulting solution extracted with AcOEt. The residue was purified by flash chromatography (hexane/AcOEt/AcOH, 2:8:0.5) to give 1.1 g of **8** (69%). − $[\alpha]_D^{20}$ = +100 (*c* = 2.7, EtOH). − (*S*,*S*)-**8** was obtained in enantiomerically pure form by crystallization of its quinine salt,[9] m.p. 145−147°C. − $[\alpha]_D^{20}$ = +150 (*c* = 2.7, EtOH) {ref.[9] $[\alpha]_D^{20}$ = +160 (*c* = 2.7, EtOH)}. − ^1H NMR (200 MHz, $CDCl_3$): δ = 2.1−2.7 (m, 4 H), 2.85 (m, 2 H), 5.75 (d, 2 H, *J* = 3 Hz), 9.6 (br. s, 2 H). − ^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 28.5, 42.1, 125.5, 181.5. − IR ($CHCl_3$): $\tilde{\nu}$ = 3300−2900 cm^{-1} (OH), 1714 (C=O). − $C_8H_{10}O_4$ (170.2): calcd. C 56.47, H 5.92; found C 56.75, H 6.03.

DCCHD 3: A solution of **8** (761 mg, 4.47 mmol) and (*t*BuO)$_2$-CHNMe$_2$ (6.6 mL, 27.8 mmol) in dry benzene was refluxed under N_2 overnight. Water was added, the organic phase washed with satd. $NaHCO_3$ and brine, dried with Na_2SO_4, and concentrated in vacuo. The crude di-*tert*-butyl ester **9** (822 mg, 2.9 mmol, 73% from **8**) was dissolved in 14 mL of 4:1 acetone/water. $OsCl_3$ (77 mg, 0.26 mmol) and $Me_3NO \cdot H_2O$ (648 mg, 5.8 mmol) were added, and the solution was stirred at room temp. overnight, before quenching with a satd. Na_2SO_3 solution. After stirring for 15 min, the mixture was extracted with AcOEt. The organic phase was dried with Na_2SO_4 and concentrated in vacuo. The crude product was purified by flash chromatography (hexane/AcOEt, 1:1), to give 916 mg of **3** (97%), m.p. 98−100°C. − $[\alpha]_D^{20}$ = +28.4 (*c* = 1.2, EtOH). − ^1H NMR (200 MHz, $CDCl_3$): δ = 1.45 [s, 18 H, $COC(CH_3)_3$], 1.5−1.68 (m, 1 H, 5_{ax}-H), 1.7−1.9 (m, 1 H, 2_{ax}-H), 2.0 (ddd, J_{gem} = 13 Hz, $J_{2eq,1}$ = $J_{2eq,3}$ = 4 Hz, 1 H, 2_{eq}-H), 2.18 (ddd, J_{gem} = 14 Hz, $J_{5eq,6}$ = $J_{5eq,4}$ = 4.5 Hz, 1 H, 5_{eq}-H), 2.3 (br. s, 2 H, O*H*), 2.55 (ddd, $J_{1,6}$ = 13 Hz, $J_{1,2eq}$ = 4 Hz, $J_{1,2ax}$ = 10 Hz, 1 H, 1-H), 2.86 (ddd, $J_{6,1}$ = 13 Hz, $J_{6,5eq}$ = 4.5 Hz, $J_{6,5ax}$ = 10 Hz, 1 H, 6-H), 3.63−3.75 (m, 1 H, 3-H), 3.95−4.2 (m, 1 H, 4-H). − ^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 27.8, 30.3, 33.0, 39.3, 44.0, 67.9, 70.3, 80.6, 80.7, 173.3, 174.3. − IR ($CHCl_3$): $\tilde{\nu}$ = 3500−3200 cm^{-1} (OH), 1720 (C=O). − $C_{16}H_{28}O_6$ (316.4): calcd. C 60.74, H 8.92; found C 60.53, H 8.84.

Benzyl Ether 10: A solution of the diol **3** (735 mg, 2.4 mmol) and Bu_2SnO (596 mg, 2.4 mmol) in dry benzene (13 mL) was refluxed under N_2 and 4-Å molecular sieves interposed between the flask and the reflux condenser. After 6 h, the solution was concentrated to half its volume, before adding Bu_4NI (880 mg, 2.4 mmol) and benzyl bromide (0.595 mL, 5 mmol). The resulting solution was refluxed under N_2 for 3 h, then the solvent evaporated, and the crude product purified by flash chromatography (hexane/AcOEt, 8:2) to yield **10** (970 mg, 99%), m.p. 108−110°C. − $[\alpha]_D^{20}$ = +15 (*c* = 1, EtOH). − ^1H NMR (200 MHz, $CDCl_3$): δ = 1.3−1.7 (m, 1 H, 5_{ax}-H), 1.45 [s, 18 H, $OC(CH_3)_3$], 1.70−1.92 (m, 1 H, 2_{ax}-H), 2.1 (ddd, J_{gem} = 14 Hz, $J_{2eq,1}$ = $J_{2eq,3}$ = 4.6 Hz, 1 H, 2_{eq}-H), 2.20−2.35 (m, 1 H, 5_{eq}-H), 2.40−2.58 (m, 1 H, 1-H), 2.84−3.04 (m, 1 H, 6-H), 3.38−3.54 (m, 1 H, 3-H), 4.10−4.20 (m, 1 H, 4-H), 4.60 (m, 2 H, CH_2Ph), 7.35 (s, 5 H, aromatic H). − ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 28.3, 28.6, 33.3, 39.9, 44.9, 66.5, 71.0, 78.0, 80.9, 81.1, 128.2, 128.5, 129.1, 173.4, 174.8. − IR ($CHCl_3$):

$\tilde{\nu}$ = 3550 cm^{-1} (OH), 1710 (C=O), 1600 (C=C). − $C_{23}H_{34}O_6$ (406.5): calcd. C 67.96, H 8.43; found C 68.11, H 8.21.

Allyl *O*-(2,3,4,6-Tetraacetyl-β-D-galactopyranosyl)-(1−3)-2-acetamido-2-deoxy-2,3-di-*O*-pivaloyl-β-D-galactopyranoside (13): $BF_3 \cdot Et_2O$ (0.07 mL, 0.58 mmol) was added to a refluxing solution of **11**[14] (226 mg, 0.52 mmol) under N_2 in dry CH_2Cl_2 (2 mL), then a solution of **12**[15] (285 mg, 0.58 mmol) in dry CH_2Cl_2 (2.3 mL) was added dropwise. After 2 h, a few drops of Et_3N were added (to pH = 6−7) and the mixture concentrated to dryness. Flash chromatography (hexane/AcOEt, 2:8) gave **13** in a yield of 53%. − $[\alpha]_D^{20}$ = +23 (*c* = 1.07, $CHCl_3$). − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.22, 1.25 [2 s, 18 H, COC(CH_3)$_3$], 1.98, 2.0, 2.05, 2.08, 2.14 (5 s, 15 H, COCH_3), 3.16−3.32 (m, 1 H, 2a-H), 3.81−3.96 (m, 2 H, 5a-H, 5b-H), 3.98−4.24 (m, 5 H, 6a-H, 6′a-H, 6b-H, 6′b-H, −*H*CH−CH=CH_2), 4.33 (dd, J_{gem} = 13 Hz, J_{vic} = 5.5 Hz, 1 H, −HC*H*−CH=CH_2), 4.61 (d, $J_{1,2}$ = 7.5 Hz, 1 H, 1b-H), 4.74 (dd, $J_{3,2}$ = 10.5 Hz, $J_{3,4}$ = 4 Hz, 1 H, 3a-H), 4.96 (dd, $J_{3,2}$ = 10 Hz, $J_{3,4}$ = 4 Hz, 1 H, 3b-H), 5.03−5.16 (m, 1 H, 2b-H), 5.08 (d, $J_{1,2}$ = 8.5 Hz, 1 H, 1a-H), 5.18−5.38 (m, 2 H, −CH=CH_2), 5.32 (dd, $J_{4,3}$ = 4 Hz, $J_{4,5}$ < 1 Hz, 1 H, 4b-H), 5.41 (dd, $J_{4,3}$ = 4 Hz, $J_{4,5}$ < 1 Hz, 1 H, 4a-H), 5.7 (d, $J_{NH,2}$ = 7 Hz, 1 H, N*H*), 5.77−5.98 (m, 1 H, −C*H*=CH_2). − ^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 20.6, 23.6, 27.0, 38.6, 55.6, 61.0, 62.5, 66.7, 68.5, 69.1, 70.2, 70.5, 70.8, 71.5, 97.9, 100.9, 118.0, 133.6. − $C_{35}H_{53}NO_{17}$ (759.8): calcd. C 55.33, H 7.03, N 1.84; found C 55.05, H 7.11, N 1.71.

(2,3,4,6-Tetraacetyl-β-D-galactopyranosyl)-(1−3)-2-acetamido-2-deoxy-2,3-di-*O*-pivaloyl-α/β-D-galactopyranoside (14): DABCO (9 mg, 0.08 mmol) and $(Ph_3P)_3RhCl$ (22 mg, 0.024 mmol) were added to a solution of **13** (300 mg, 0.39 mmol) in 9:1 EtOH/H_2O (4 mL). The mixture was refluxed for 5 h, then concentrated to dryness. The residue was filtered through a short silica gel column (hexane/AcOEt, 3:7) and the crude product (225 mg) was dissolved in 4:1 THF/H_2O (3 mL). I_2 (149 mg, 0.59 mmol) was added at room temp., the solution stirred for 30 min, H_2O added, and the mixture extracted with $CHCl_3$. The organic phase was washed with 5% $Na_2S_2O_5$ (2 ×), dried with Na_2SO_4, and concentrated in vacuo. The crude product was purified by flash chromatography (AcOEt/$CHCl_3$/MeOH, 9:1:0.2) to yield **14** (165 mg, 59%) as an α/β mixture. − $[\alpha]_D^{20}$ = +38.7 (*c* = 1.14, $CHCl_3$) (α anomer). − ^{1}H NMR (300 MHz, $CDCl_3$): δ = (α anomer) 1.2, 1.23 [2 s, 18 H, COC(CH_3)$_3$], 1.75 (s, 3 H, COCH_3), 2.0 (s, 3 H, COCH_3), 2.05 (s, 6 H, COCH_3), 2.16 (s, 3 H, COCH_3), 3.1 (br. s, 1 H, O*H*), 3.87 (ddd, $J_{5,6}$ = $J_{5,6'}$ = 6 Hz, $J_{5,4}$ < 1 Hz, 1 H, 5b-H), 3.92−4.1 (m, 3 H, 3a-H, 6a-H, 6′a-H), 4.13 (m, 2 H, 6b-H, 6′b-H), 4.33 (ddd, $J_{5,6}$ = $J_{5,6'}$ = 6 Hz, $J_{5,4}$ < 1 Hz, 1 H, 5a-H), 4.42 (m, 1 H, 2a-H), 4.61 (d, $J_{1,2}$ = 8 Hz, 1 H, 1b-H), 4.95 (dd, $J_{3,2}$ = 10 Hz, $J_{3,4}$ = 4 Hz, 1 H, 3b-H), 5.1 (dd, $J_{2,3}$ = 10 Hz, $J_{2,1}$ = 8 Hz, 1 H, 2b-H), 5.35 (dd, $J_{4,3}$ = 4 Hz, $J_{4,5}$ < 1 Hz, 1 H, 4b-H), 5.37 (br. s, 1 H, 1a-H), 5.4 (dd, $J_{4,3}$ = 3.5 Hz, $J_{4,5}$ < 1 Hz, 1 H, 4a-H), 5.7 (d, $J_{NH,2}$ = 8 Hz, 1 H, N*H*). − ^{13}C NMR (50.3 MHz, $CDCl_3$): δ (α anomer) = 20.5, 23.1, 26.9, 39.6, 39.9, 49.4, 61.0, 62.0, 66.7, 67.0, 68.5, 68.7, 70.5, 70.9, 73.0, 91.9, 94.0, 169.5, 170.3, 176.7, 178.1. − $C_{32}H_{49}NO_{17}$ (719.7): calcd. C 53.40, H 6.86, N 1.95; found C 52.62, H 6.77, N 1.67.

Trichloroacetimidate 15: Cl_3CCN (0.1 mL, 1 mmol) and DBU (0.005 mL, 0.033 mmol) were added to a solution of **14** (132 mg, 0.18 mmol) in dry CH_2Cl_2 (1.8 mL). After 30 min at room temp. under N_2, the reaction mixture was concentrated in vacuo and the residue filtered through a short silica gel column (hexane/AcOEt, 2:8) to give **15** (148 mg, 95%). − ^{1}H NMR (200 MHz, $CDCl_3$): δ (α anomer) = 1.15 [s, 9 H COC(CH_3)$_3$], 1.25 [s, 9 H, COC(CH_3)$_3$], 1.98 (s, 6 H, COCH_3), 2.07 (s, 3 H, COCH_3), 2.1 (s, 3 H, COCH_3), 2.2 (s, 3 H, COCH_3), 3.9−4.4 (m, 7 H, 3a-H, 5a-H, 6a-H, 6′a-H, 5b-H, 6b-H, 6′b-H), 4.6 (m, 1 H, 2a-H), 4.72 (d, $J_{1,2}$ = 7.5 Hz, 1 H, 1b-H), 5.0 (dd, $J_{3,4}$ = 3 Hz, $J_{3,2}$ = 10 Hz, 1 H, 3b-H), 5.2 (dd, $J_{2,1}$ = 7.5 Hz, $J_{2,3}$ = 10 Hz, 1 H, 2b-H), 5.4 (dd, $J_{4,3}$ = 3 Hz, $J_{4,5}$ < 1 Hz, 1 H, 4b-H), 5.47 (dd, $J_{4,3}$ = 2.5 Hz, $J_{4,5}$ < 1 Hz, 1 H, 4a-H), 5.72 (d, $J_{NH,2}$ = 7.4 Hz, 1 H, N*H*Ac), 6.55 (d, $J_{1,2}$ = 3 Hz, 1 H, 1a-H), 8.74 (s, 1 H, N*H*=C).

Synthesis of 16: TMSOTf (0.013 mL, 0.07 mmol) was added to a refluxing solution of **15** (100 mg, 0.116 mmol) and **10** (118 mg, 0.29 mmol) in dry CH_2Cl_2 under N_2 After 1 h, a few drops of Et_3N were added and the solvent evaporated. The product **16** (60 mg, 47%) was isolated by flash chromatography (AcOEt/hexane, 55:45), which also allowed recovery of the excess of **10** (77 mg, 0.19 mmol). − $[\alpha]_D^{20}$ = +11.9 (*c* = 1.02, $CHCl_3$). − ^{1}H NMR (300 MHz, C_6D_6): δ = 1.25 (m, 1 H, $5c_{ax}$-H), 1.27 [s, 9 H, COC(CH_3)$_3$], 1.3 [s, 9 H, COC(CH_3)$_3$], 1.4 [s, 9 H, OC(CH_3)$_3$], 1.45 [s, 9 H, OC(CH_3)$_3$], 1.62, 1.65, 1.72, 1.74, 1.79 (5 s, 15 H, COCH_3), 2.09 (m, 1 H, $2c_{ax}$-H), 2.25 (ddd, J_{gem} = 12 Hz, $J_{2eq,1}$ = $J_{2eq,3}$ = 4 Hz, 1 H, $2c_{eq}$-H), 2.43 (ddd, J_{gem} = 14 Hz, $J_{5eq,6}$ = $J_{5eq,4}$ = 4 Hz, 1 H, $5c_{eq}$-H), 2.78 (ddd, $J_{1,6}$ = $J_{1,2ax}$ = 12 Hz, $J_{1,2eq}$ = 4 Hz, 1 H, 1c-H), 3.05 (ddd, $J_{3,2ax}$ = 12 Hz, $J_{3,2eq}$ = 4 Hz, $J_{3,4}$ = 2 Hz, 1 H, 3c-H), 3.25 (m, 2 H, 2a-H, 6c-H), 3.32 (ddd, $J_{5,6}$ = $J_{5,6'}$ = 6.5 Hz, $J_{5,4}$ < 1 Hz, 1 H, 5a-H), 3.71 (ddd, $J_{5,6}$ = $J_{5,6'}$ = 6.5 Hz, $J_{5,4}$ < 1 Hz, 1 H, 5b-H), 4.05−4.4 [m, 8 H, H-H COSY: 4.1 (1 H, 4c-H), 4.15 (2 H, 6a-H, 6′a-H), 4.20 (1 H, 6b-H), 4.30 (1 H, 6′b-H) 4.32 (2 H, CH_2Ph), 4.35 (d, $J_{1,2}$ = 8 Hz, 1 H, 1b-H)], 4.75 (dd, $J_{3,2}$ = 11 Hz, $J_{3,4}$ = 4 Hz, 1 H, 3a-H), 4.8 (d, $J_{NH,2}$ = 7 Hz, 1 H, N*H*), 5.08 (dd, $J_{3,2}$ = 11 Hz, $J_{3,4}$ = 3 Hz, 1 H, 3b-H), 5.35−5.5 [m, 4 H, H-H COSY: 5.36 (1 H, 2b-H), 5.4 (d, $J_{1,2}$ = 9 ± 1 Hz, 1 H, 1a-H), 5.4 (1 H, 4a-H), 5.41 (1 H, 4b-H)], 7.2−7.4 (m, 5 H, aromatic H). − ^{13}C NMR (75.4 MHz, C_6D_6): δ = 20.9, 24.2, 28.0, 28.7, 29.7, 34.5, 41.0, 45.5, 56.6, 61.7, 63.4, 67.8, 69.9, 70.2, 71.3, 71.5, 72.1, 72.5, 73.5, 76.8, 79.9, 80.7, 80.9, 100.5, 102.4. − $C_{55}H_{81}NO_{22}$ (1108.2), calcd. C 59.61, H 7.37, N 1.27; found C 59.89, H 7.31, N 1.34.

Synthesis of 4: 10% Pd/C was added to a solution of **16** (61 mg, 0.055 mmol) in MeOH (3 mL) and the resulting mixture stirred for 1 h at room temp. under H_2. The catalyst was filtered off, the solvent evaporated, and the crude product purified by flash chromatography ($CHCl_3$/acetone/MeOH, 85:15:0.2) to yield **4** (40 mg, 73%). − $[\alpha]_D^{20}$ = +33 (*c* = 1.55, $CHCl_3$). − ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.2 [s, 9 H, COC(CH_3)$_3$], 1.3 [s, 9 H, COC(CH_3)$_3$], 1.42 [s, 9 H, OC(CH_3)$_3$], 1.47 [s, 9 H, OC(CH_3)$_3$], 1.5 (m, 1 H, $2c_{ax}$-H), 1.63 (m, 1 H, $5c_{ax}$-H), 1.86 (br. s, 1 H, O*H*), 1.96 (s, 3 H, COCH_3), 1.98 (s, 3 H, COCH_3), 2.0 (m, 1 H, $5c_{eq}$-H), 2.06 (s, 6 H, COCH_3), 2.14 (s, 3 H, COCH_3), 2.33 (ddd, J_{gem} = 13 Hz, $J_{2eq,1}$ = $J_{2eq,3}$ = 3.5 Hz, 1 H, $2c_{eq}$-H), 2.54 (ddd, $J_{6,1}$ = $J_{6,5ax}$ = 11 Hz, $J_{6,5eq}$ = 3.3 Hz, 1 H, 6c-H), 2.83 (ddd, $J_{1,6}$ = $J_{1,2ax}$ = 11 Hz, $J_{1,2eq}$ = 3.5 Hz, 1 H, 1c-H), 3.42 (ddd, $J_{2,1}$ = 8.7 Hz, $J_{2,3}$ = 11 Hz, $J_{2,NH}$ = 6.4 Hz, 1 H, 2a-H), 3.58 (m, 1 H, 4c-H), 3.7−4.1 [m, 7 H, H-H COSY: 3.83 (2 H, 5a-H, 5b-H), 3.94 (2 H, 6a-H, 6b-H), 3.95 (1 H, 4c-H), 4.08 (2 H, 6′a-H, 6′b-H)], 4.38 (dd, $J_{3,2}$ = 11 Hz, $J_{3,4}$ = 3.2 Hz, 1 H, 3a-H), 4.6 (d, $J_{1,2}$ = 7.4 Hz, 1 H, 1b-H), 4.98 (dd, $J_{3,2}$ = 10 Hz, $J_{3,4}$ = 3.5 Hz, 1 H, 3b-H), 5.1 (dd, $J_{2,1}$ = 7.4 Hz, $J_{2,3}$ = 10 Hz, 1 H, 2b-H), 5.3 (d, $J_{1,2}$ = 8.7 Hz, 1 H, 1a-H), 5.35 (dd, $J_{4,3}$ = 3.5 Hz, $J_{4,5}$ < 1 Hz, 1 H, 4b-H), 5.4 (dd, $J_{4,3}$ = 3.2 Hz, $J_{4,5}$ < 1 Hz, 1 H, 4a-H), 5.86 (d, $J_{NH,2}$ = 6.4 Hz, 1 H, N*H*). − ^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 20.4, 20.5, 20.6, 20.7, 23.5, 26.9, 27.1, 27.8, 31.5, 33.0, 39.7, 44.1, 55.3, 60.9, 62.2, 66.8, 68.2, 69.1, 70.5, 70.6, 70.7, 71.7, 75.8, 76.7, 100.1, 100.8. − $C_{48}H_{75}NO_{22}$ (1018.1): calcd. C 56.63, H 7.43, N 1.38; found C 56.48, H 7.65, N 1.21.

3-Sialyl-DCCHD 5: A solution of the sialyl phosphite **17**[19] (296 mg, 0.48 mmol) in dry EtCN (0.75 mL) was added dropwise

to a solution of **3** (151 mg, 0.48 mmol) and TMSOTf (0.02 mL, 0.11 mmol) in dry EtCN (0.8 mL) at −40°C under N_2. After 3 h the reaction was neutralized with Et_3N, the solvent evaporated, and the residue purified by flash chromatography (hexane/acetone/MeOH, 6:4:0.1) to give **5** as a 7:1 α/β mixture contaminated by the Neu5Ac glycal. A second flash chromatography ($CHCl_3$/acetone, from 8:2 to 7:3) yielded the α anomer (76 mg, 20%). − $[\alpha]_D^{20}$ = −7.6 (*c* = 1.6, $CHCl_3$). − ^{1}H NMR (300 MHz, C_6D_6): δ = 1.36, 1.42 [2 s, 18 H, OC(CH_3)$_3$], 1.55, 1.58 (2 s, 6 H, COCH_3), 1.70 (m, 1 H, 5c_{ax}-H), 1.82 (s, 3 H, COCH_3), 1.92 (s, 3 H, COCH_3), 2.0 (m, 1 H, 3d_{ax}-H), 2.02 (s, 3 H, COCH_3), 2.22 (ddd, J_{gem} = $J_{2ax,3}$ = $J_{2ax,1}$ = 12.5 Hz, 1 H, 2c_{ax}-H), 2.35−2.5 [m, 2 H, H-H COSY: 2.38 (1 H, 2c_{eq}-H), 2.42 (1 H, 5c_{eq}-H)], 2.62 (dd, J_{gem} = 12.5 Hz, $J_{3eq,4}$ = 4.5 Hz, 1 H, 3d_{eq}-H), 3.12 (ddd, $J_{1,6}$ = $J_{1,2ax}$ = 12.5 Hz, $J_{1,2eq}$ = 4 Hz, 1 H, 1c-H), 3.32 (s, 3 H, OCH_3), 3.37 (m, 1 H, 6c-H), 3.95 (m, 1 H, 4c-H), 4.0−4.55 [m, 5 H, H-H COSY: 4.05 (dd, $J_{6,5}$ = 11 Hz, $J_{6,7}$ = 3 Hz, 1 H, 6d-H), 4.12 (d, $J_{NH,5}$ = 10 Hz, 1 H, N*H*), 4.3 (1 H, 3c-H), 4.4 (2 H, 5d-H, 9d-H)], 4.65 (dd, $J_{9',9}$ = 13 Hz, $J_{9',8}$ = 3 Hz, 1 H, 9′d-H), 4.81 (ddd, $J_{4,3ax}$ = $J_{4,5}$ = 11 Hz, $J_{4,3eq}$ = 4.5 Hz, 1 H, 4d-H), 5.48 (dd, $J_{7,8}$ = 8 Hz, $J_{7,6}$ = 3 Hz, 1 H, 7d-H), 5.80 (ddd, $J_{8,7}$ = $J_{8,9}$ = 8 Hz, $J_{8,9'}$ = 3 Hz, 1 H, 8d-H). − ^{13}C NMR (75.4 MHz, $CDCl_3$) δ = 20.4, 20.6, 21.1, 22.9, 28.0, 30.1, 33.6, 38.2, 39.6, 44.6, 49.0, 52.4, 62.7, 67.4, 67.6, 69.3, 70.0, 73.4, 74.6, 80.6, 98.0 (C-2d), 168.5 (1d-C, $J_{C\text{-}1,3\text{-}Hax}$ = 6.9 Hz). − $C_{36}H_{55}NO_{18}$ (789.8): calcd. C 54.75, H 7.02, N 1.77; found C 54.96, H 6.81, N 1.93.

Synthesis of 18: TMSOTf (0.007 mL, 0.043 mmol) was added to a refluxing solution of **5** (57 mg, 0.072 mmol) and **15** (146 mg, 0.17 mmol) in dry CH_2Cl_2 (0.4 mL) under N_2. After 5 h, the mixture was neutralized with Et_3N and the solvent evaporated. The product **18** (32 mg, 30%) was isolated by flash chromatography ($CHCl_3$/acetone, 7:3) and further purified by a second chromatography (toluene/acetone, 6:4). − $[\alpha]_D^{20}$ = +4.8 (*c* = 0.8, $CHCl_3$). − ^{1}H NMR (500 MHz, C_6D_6): δ = 1.25 [s, 9 H, COC(CH_3)$_3$], 1.32 [s, 9 H, COC(CH_3)$_3$], 1.38 [s, 9 H, OC(CH_3)$_3$], 1.47 [s, 9 H, OC(CH_3)$_3$], 1.59 (s, 3 H, COCH_3), 1.63 (s, 3 H, COCH_3), 1.67 (s, 3 H, COCH_3), 1.70 (s, 6 H, COCH_3), 1.74 (s, 3 H, COCH_3), 1.75−5.85 [48 H, H-H COSY: 1.80 (m, 1 H, 5c_{ax}-H), 1.83 (s, 3 H, COCH_3), 1.91 (s, 6 H, COCH_3), 2.08 (s, 3 H, COCH_3), 2.1 (m, 1 H, 3d_{ax}-H), 2.2 (m, 1 H, 2c_{ax}-H), 2.45 (ddd, J_{gem} = 12 Hz, $J_{2eq,1}$ = $J_{2eq,3}$ = 4 Hz, 1 H, 2c_{eq}-H), 2.52 (ddd, J_{gem} = 13.5 Hz, $J_{5eq,6}$ = $J_{5eq,4}$ = 4 Hz, 1 H, 5c_{eq}-H), 3.08 (dd, J_{gem} = 13.5 Hz, $J_{3eq,4}$ = 4.5 Hz, 1 H, 3d_{eq}-H), 3.22 (m, 1 H 1c-H), 3.26 (m, 1 H, 6c-H), 3.30 (m, 1 H, 2a-H), 3.38 (ddd, $J_{5,6}$ = $J_{5,6'}$ = 6.5 Hz, $J_{5,4}$ < 1 Hz, 1 H, 5b-H), 3.65 (s, 3 H, OCH_3), 3.98 (ddd, $J_{5,6}$ = $J_{5,6'}$ = 7 Hz, $J_{5,4}$ < 1 Hz, 1 H, 5a-H), 4.02 (dd, $J_{6,7}$ = 2.2 Hz, $J_{6,5}$ = 10.7 Hz, 1 H, 6d-H), 4.05 (m, 1 H, 4c-H), 4.20 (m, 2 H, 6b-H, 6′b-H), 4.25 (m, 1 H, N*H*d), 4.30 (m, 2 H, 6a-H, 6′a-H), 4.35 (m, 1 H, 3c-H), 4.40 (m, 1 H, 9d-H), 4.42 (m, 1 H, 5d-H), 4.55 (d, $J_{1,2}$ = 7.7 Hz, 1 H, 1b-H), 4.65 (dd, J_{gem} = 12.5 Hz, $J_{9',8}$ = 2.5 Hz, 1 H, 9′d-H), 4.92 (ddd, $J_{4,3eq}$ = 4.5 Hz, *J* = 10 Hz, *J* = 11.6 Hz, 1 H, 4d-H), 5.05 (dd, $J_{3,4}$ = 3.8 Hz, $J_{3,2}$ = 11 Hz, 1 H, 3a-H), 5.12 (dd, $J_{3,4}$ = 3.5 Hz, $J_{3,2}$ = 11 Hz, 1 H, 3b-H), 5.20 (br. s, 1 H, N*H*a), 5.40 (d, $J_{1,2}$ = 8.7 Hz, 1 H, 1a-H), 5.42 (dd, $J_{2,1}$ = 7.7 Hz, $J_{2,3}$ = 11 Hz, 1 H, 2b-H), 5.45 (dd, $J_{4,3}$ = 3.5 Hz, $J_{4,5}$ < 1 Hz, 1 H, 4b-H), 5.48 (dd, $J_{7,6}$ = 2.2 Hz, $J_{7,8}$ = 7.7 Hz, 1 H, 7d-H), 5.58 (dd, $J_{4,3}$ = 3.8 Hz, $J_{4,5}$ < 1 Hz, 1 H, 4a-H), 5.80 (ddd, $J_{8,9}$ = 2.5 Hz, $J_{8,9'}$ = 6.6 Hz, $J_{8,7}$ = 7.7 Hz, 1 H, 8d-H)]. − ^{13}C NMR (125.7 MHz, C_6D_6): δ = 27.3, 27.4, 28.0, 28.1, 30.0, 31.3, 38.6, 39.2, 40.4, 44.7, 49.4, 52.5, 55.8, 60.9, 62.4, 62.8, 67.1, 67.6, 69.3, 69.6, 69.8, 70.6, 71.5, 73.3, 74.3, 75.8, 80.0, 80.2, 99.3, 100.3, 101.8, 145.9, 149.4, 149.5, 149.6, 168.7, 169.5, 169.7, 170.0, 170.2, 173.5, 173.9, 176.7. − $C_{68}H_{102}N_2O_{34}$ (1491.6): calcd. C 54.76, H 6.89, N 1.88; found C 54.57, H 6.81, N 2.24. − MS (FAB); *m*/*z*: 1512 [M^+ + Na].

Pseudo GM1 2: 1 M MeONa in MeOH (0.2 mL) was added to a solution of **18** (44 mg, 0.03 mmol) in dry MeOH (3 mL). After stirring for 24 h at room temp. under N_2, H_2O (0.6 mL) was added to the reaction mixture. The solution was stirred for additional 12 h, then Amberlite IR 120 (H^+ form) was added and the solvent evaporated. The product **2** (24 mg, 82%) was isolated by flash chromatography ($CHCl_3$/MeOH/H_2O, 60:35:5). − $[\alpha]_D^{20}$ = +8.6 (*c* = 0.35, MeOH). − ^{1}H NMR (500 MHz, D_2O): δ = 1.50, 1.53 [2 s, 18 H, OC(CH_3)], 1.65−4.97 [37 H, H-H COSY: 1.69 (m, 1 H, 5c_{ax}-H), 1.72 (m, 1 H, 2c_{ax}-H), 1.85 (dd, J_{gem} = $J_{3ax,4}$ = 12 Hz, 1 H, 3d_{ax}-H), 2.09 (m, 1 H, 2c_{eq}-H), 2.07, 2.10 (2 s, 6 H, COCH_3), 2.26 (ddd, J_{gem} = 14 Hz, $J_{5eq,6}$ = $J_{5eq,4}$ = 4 Hz, 1 H, 5c_{eq}-H), 2.66 (ddd, $J_{1,6}$ = $J_{1,2ax}$ = 12 Hz, $J_{1,2eq}$ = 4 Hz, 1 H, 1c-H), 2.76 (dd, J_{gem} = 12 Hz, $J_{3eq,4}$ = 5 Hz, 1 H, 3d_{eq}-H), 2.79 (ddd, $J_{6,1}$ = $J_{6,5ax}$ = 12 Hz, $J_{6,5eq}$ = 4 Hz, 1 H, 6c-H), 3.56−3.62 (m, 2 H, 2b-H, 6d-H), 3.69 (m, 1 H, 7d-H), 3.7 (m, 1 H, 3b-H), 3.75 (m, 2 H, 5b-H, 9d-H), 3.78 (m, 1 H, 5a-H), 3.80 (m, 1 H, 4d-H), 3.83 (m, 3 H, 6a-H, 6b-H, 6′b-H), 3.88 (m, 3 H, 6′a-H, 5d-H, 8d-H), 3.92 (m, 1 H, 3a-H), 3.93 (m, 1 H, 9′d-H), 3.98 (dd, $J_{4,3}$ = 3 Hz, $J_{4,5}$ < 1 Hz, 1 H, 4b-H), 4.11 (dd, $J_{2,1}$ = 8 Hz, $J_{2,3}$ = 10.5 Hz, 1 H, 2a-H), 4.12 (m, 1 H, 3c-H), 4.21 (m, 1 H, 4c-H), 4.24 (dd, $J_{4,3}$ = 3 Hz, $J_{4,5}$ < 1 Hz, 1 H, 4a-H), 4.57 (d, $J_{1,2}$ = 7.5 Hz, 1 H, 1b-H), 4.96 (d, $J_{1,2}$ = 8 Hz, 1 H, 1a-H)]. − ^{13}C NMR (125.7 MHz, D_2O): δ = 29.3, 32.5, 38.2, 39.4, 44.1, 51.0, 51.1, 60.5, 62.0, 67.6, 68.2, 68.4, 70.2, 71.6, 72.1, 72.4, 72.6, 74.3, 74.7, 74.8, 79.8, 82.3, 82.4, 101.7, 104.5. − $C_{41}H_{68}N_2O_{24}$ (973.0): calcd. C 50.61, H 7.04, N 2.88; found C 50.74, H 6.85, N 2.71.

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