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Note

Syntheses of alkenylated carbohydrate derivatives toward the preparation of monolayers on silicon surfaces

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Abstract—This note describes the synthesis of different alkenylated carbohydrate derivatives suitable for direct attachment to hydrogen-terminated silicon surfaces. The derivatives were alkenylated at the C-1 position, while the remaining hydroxyl groups were protected. The development of such new carbohydrate-based sensing elements opens the access to new classes of biosensors.

Keywords: Mono- and disaccharides; Alkenylation; Hydrosilylation; Receptors; Biosensors

Only very recently the construction of carbohydrate arrays on glass has been reported.[1,2] This allows for optical or spectroscopic detection of specific carbohydrate–protein interactions. Given the essential role of carbohydrates in many biological processes, the development of new carbohydrate sensing elements will likely provide a tremendous stimulus to this field. The application of modified silicon in this area can open up a whole new field of research, as the semiconducting nature of the substrate provides a unique feature in silicon-based biosensing.[3–5] This latter property allows for detection of selective recognition of, for example, antibodies to oligosaccharide receptors via changes in capacitance. An example of this principle can be found in a recent study in which real-time capacitance measurements were performed to monitor DNA hybridization on oligodeoxynucleotide-modified silicon.[6]

In 2003 two papers on the attachment of monosaccharides to crystalline silicon appeared in the literature. One[7] describes the preparation of homogeneous monolayers of allyl α-D-galactopyranoside on the Si(100)–H surface. The authors observed specific adsorption of ricinus communis agglutinin (RCA_{120}) molecules from a contacting solution as well as nonspecific adsorption on the Si–O area. The other paper[8] presents our work on the functionalization of Si(100)–H with well-defined, covalently attached heterogeneous monolayers containing carbohydrates (Fig. 1). The monosaccharides we have chosen in this study do not have properties regarding any specific adsorption, but were used to develop different methods and conditions to produce the first case of well-defined, carbohydrate-substituted monolayers. Two methods were used to attain this aim: a thermal method (refluxing in mesitylene) and an extremely mild photochemical method[9] (irradiation with 447 nm at room temperature).

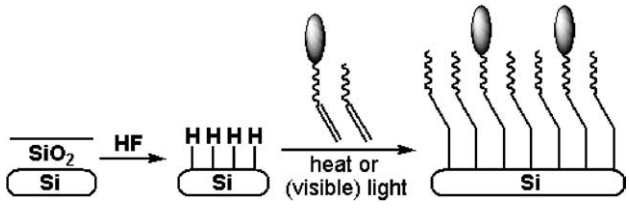

Figure 1. Formation of a well-defined, carbohydrate-substituted monolayer on the silicon surface. The ellipses and wavy bonds represent a protected carbohydrate and alkyl spacers, respectively.

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For the preparation of such well-defined monolayers of carbohydrate derivatives on silicon surfaces, at least two structure properties are required: (a) presence of an ω-alkenyl tail in order to perform the hydrosilylation reaction, and (b) protection of the hydroxyl groups—of both alcohols and acids—in order to prevent the interfering reaction between these groups and the hydrogen-terminated silicon surface.[10–12] In this note we report the synthesis of several monosaccharides and one disaccharide with those properties.

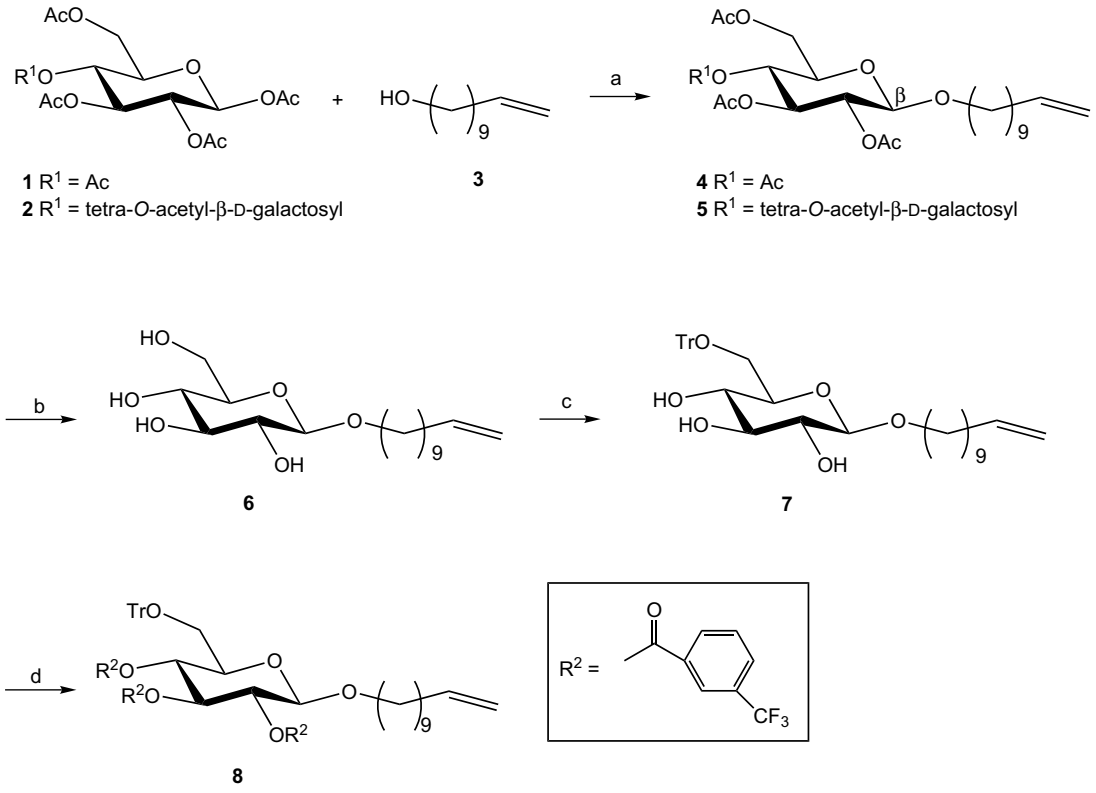
Using $SnCl_4$ as a Lewis acid catalyst,[13] 10-undecen-1-ol (**3**) was attached to penta-*O*-acetyl-β-D-glucopyranose (**1**) resulting in compound **4** (Scheme 1, step a). After purification via column chromatography, compound **4** was directly available for the attachment on the hydrogen-terminated surface. It is obvious that all remaining hydroxyl groups need to be protected in order to avoid random binding of the alkenylated carbohydrate derivatives via either the 1-alkene or hydroxyl functionality.[10]

Shirahata et al. used allyl α-D-galactopyranoside to modify the Si(100)–H surface.[7] Although free hydroxyl groups will also react with this surface, the authors did not discuss the resulting aspecificity of the hydrosilylation. Another relevant difference between allyl α-D-galactopyranoside and the carbohydrate derivatives presented in this Note is the length of the alkenyl chain. Due to the twisted conformation of the bonds closest to the Si surface (Si–C–C–C), a rapidly deteriorating monolayer quality is observed for alkenes with alkyl chains that are shorter than 12 atoms.[14] For this reason the use of 10-undecen-1-ol (the longest ω-alkene-1-ol commercially available) to alkenylate carbohydrates is advocated and applied in the present work.

$SnCl_4$ was also used to attach 10-undecen-1-ol (**3**) to octa-*O*-acetyl-β-lactose (**2**), a commercially available peracetylated disaccharide. The resulting product **5** was attached to crystalline silicon surfaces, and the analysis of the resulting mixed monolayers is in progress.

Apart from the direct attachment to the silicon surface, compound **4** was also applied in the three-step synthesis of compound **8** (Scheme 1, steps b–d, respectively). In the first step the acetyl groups are removed according to the general deacetylation method developed by Zemplén and Pascu.[15] The use of $NaOCH_3$ in MeOH afforded compound **6** in an almost quantitative yield. Using trityl chloride,[16] the primary hydroxyl group at C-6 was tritylated yielding compound **7**. Subsequently, the remaining three free hydroxyl groups were protected with trifluoromethylbenzoyl groups,[16] giving compound **8** in a good yield.

In the synthesis of compound **8** the trityl group was introduced in order to have a handle to synthesize



Scheme 1. Reagents and conditions: (a) $SnCl_4$ in dry CH_2Cl_2, rt, 38–56%; (b) $NaOCH_3$ in MeOH, rt, 96%; (c) Ph_3CCl, DMAP in pyridine, rt, 64%; (d) 3-(trifluoromethyl)benzoylchloride, DMAP in pyridine, rt, 77%.

oligosaccharides. Detritylation[17] would result in a glucose derivative with only one free hydroxyl group at C-6, which can be linked with a glycosyl donor yielding an oligosaccharide. The trifluoromethylbenzoyl protection group was chosen since the fluorine atom proved to be a good probe for XPS studies of monolayers with compound **8**.[8,9]

In the second part of this report we focus on the derivatization of sialic acids, which are a crucial component in oligosaccharides that play a role in cell recognition processes.[16,18,19] Their chemistry frequently requires more subtle procedures for regio- and stereoselective introduction of glycosidic linkages.[16,18,20–22] Sialic acid-containing carbohydrates display a diminished thermal stability, but can in combination with our recently developed photochemical approach nevertheless be linked smoothly to the Si surface.[8,9]

Protection of the hydroxyl groups that are present in commercially available sialic acid (compound **9**) took place in two steps (Scheme 2, steps a and b, respectively). Treatment of **9** with acidified MeOH gave the methyl glycoside **10**.[23] Acetylation of **10** with AcCl[23] acetylates all the hydroxyl groups including the one at C-1. At C-1 this is followed by the in situ formation of the α/β chloride at the anomeric centre yielding **11**.

The glycosylation conditions used in step c of Scheme 2 were obtained from the work of Tomoo et al.[24] The alkenylation was promoted with AgOTf, and according to the NMR spectrum, the crude product was a mixture containing the α:β isomers of compound **12** in a ratio of 3:2. The total yield was 79%.

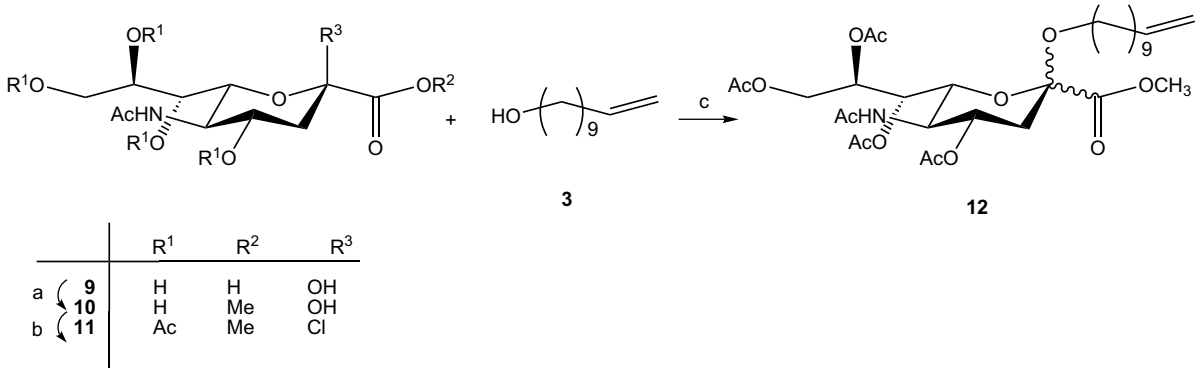
In conclusion, the syntheses of three different protected alkenylated monosaccharides (compounds **4**, **8**, and **12**) and one disaccharide (compound **5**) are reported. The monosaccharide derivatives have been used successfully in the modification of silicon surfaces as described recently in our paper 'Covalently attached saccharides on silicon surfaces'.[8] The development of new 1-alkenyl-derivatized carbohydrate-based sensing elements plays a crucial role in the development of capacitance-based biosensors on silicon. Future work will focus on the derivatization and embedding of larger oligosaccharides.

1. Experimental

1.1. General methods

CH_2Cl_2 and CH_3CN were distilled over CaH_2. Distilled MeOH was dried over 3 Å molecular sieves. Pyridine was dried over KOH. All solvents for extractions and chromatography were distilled. Petroleum ether refers to the bp 40–60 °C fraction. All reactions were carried out under a nitrogen or argon atmosphere with glassware dried at ⩾120 °C. Thin-layer chromatography (TLC) was performed on E. Merck Silica Gel 60F254 plastic sheets, and detection was realized by either of the following methods: charring with a solution of $KMnO_4$ (aq), molybdenum reagents (ammonium molybdate (21 g), ammonium cesium(IV) sulfate (1.8), water (469 mL), and H_2SO_4 (31 mL)), 5% (v/v) sulfuric acid in MeOH and subsequent heating or UV detection. Column chromatography was conducted by elution of a column of E. Merck Kieselgel (230–400 mesh) using eluents as specified below. NMR spectra were recorded on a Bruker AC-E 200 or a Bruker DPX 400 spectrometer in solvents as specified below at room temperature.

Samples of compounds **4**, **5**, **8**, and **12** were subjected to accurate-mass LC–MS on a high-resolution time-of-flight mass spectrometer with lock mass correction.[25] The ESIMS (Q-TOF Ultima, Waters Corporation) was calibrated with 0.05% phosphoric acid in 50% acetonitrile. The carbohydrate derivatives were dissolved and diluted in 75% acetonitrile and subsequently injected at 10 μL/min using a syringe pump (Harvard Apparatus PHD2000). The following settings were applied: desolvation temperature of 250 °C with a nitrogen gas flow of 300 L/h, capillary spray at 2.5 kV, source temperature of 120 °C, cone voltage at 35 eV with



Scheme 2. Reagents and conditions: (a) MeOH, (H^+) resin, rt; (b) AcCl, rt, 48 h, 66%; (c) dry CH_3CN (dark), rt, 1 h; AgOTf, stirred at 35–45 °C for 48 h, 79%.

50 L/h nitrogen gas flow, collision energy ranging from 3 to 10 eV (depending on compound). Ions in the *m/z* range 70–1500 were detected in the centroid mode, using a scan time of 0.9 s and an interscan delay of 0.1 s. In case the mass signal was not detectable or too low for reliable accurate mass measurements, HCO_2H (0.1%) or NH_4OAc (2 mM) was added to enhance the signal. Leucine enkaphalin in 50% acetonitrile plus 2 mM NH_4OAc was used as a lock mass (recorded every 10 s), injected at a flow rate of 10 μL/min using a separate syringe pump. MassLynx software version 4.0 (Waters) was used to control the MS and to calculate accurate masses.

1.2. 10-Undecenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (4)

A solution of penta-*O*-acetyl-β-D-glucopyranose (**1**) (6.59 g, 16.9 mmol), 10-undecen-1-ol (**3**) (7.5 mL, 37.2 mmol), and 4 Å molecular sieves (3.5 g) in CH_2Cl_2 (dry, 30 mL) was stirred at room temperature. Subsequently, $SnCl_4$ (2.0 mL) was added in one portion by syringe. After 6.5 h $NaHCO_3$ (2.5 g) and $MgSO_4$ (2.5 g) were added. The solution was filtered over a Celite layer, and the filtrate was concentrated under reduced pressure. Column chromatography (4:1 petroleum ether–EtOAc) yielded **4** (3.20 g, 38%) as a colorless oil.

TLC R_f 0.44 (1:1 EtOAc–petroleum ether); ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.90–5.69 (m, 1H, –C*H*=CH_2), 5.20 (t, 1H, *J* 9.3 Hz, H-3), 5.07 (t, 1H, *J* 9.5 Hz, H-2), 5.05–4.85 (m, 2H, –CH=*CH*$_2$), 4.47 (d, 1H, *J* 7.9 Hz, H-1), 4.30–4.22 (dd, $J_{6,6'}$ 12.3 Hz, $J_{5,6}$ 4.6 Hz, 1H, H-6), 4.15–4.08 (dd, 1H, $J_{6,6'}$ 12.2 Hz, $J_{5,6'}$ 2.4 Hz, H-6′), 3.91–3.80 (dt, 1H, ^2J 9.6 Hz, ^3J 6.3 Hz, –OCH_a), 3.72–3.63 (m, 1H, H-5), 3.38–3.52 (dt, 1H, ^2J 9.6 Hz, ^3J 6.7 Hz, –OCH_b), 2.07–1.98 (m, 14H, –C*H*$_2$CH=CH_2 + 4 × –OCH_3 at 2.07, 2.03, 2.01, 1.99), 1.70–1.47 (m, 2H, –OCH_2CH_2–), 1.25 (s, 12H, –$(CH_2)_6$–); ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.7, 170.4, 169.4, 169.3 (4 × C=O), 139.2 (–*C*H=CH_2), 114.1 (–CH=*C*H_2), 100.8 (C-1), 72.9 (C-3), 71.7 (C-5), 71.3 (C-2), 70.3 (OCH_2–), 68.5 (C-4), 62.0 (C-6), 33.8 (–*C*H_2CH=CH_2), 29.5, 29.4, 29.4, 29.3, 29.1, 28.9 (6 × –CH_2–), 25.8 (–OCH_2CH_2–), 20.8 (CH_3), 20.7 (3 × CH_3). QTOFMS $[M-H]^-$ 499.2541 (calcd 499.2543; Δ ppm = −0.4); $[M+H]^+$ 501.2711 (calcd 501.2699; Δ ppm = 2.3).

1.3. 10-Undecenyl 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactoside (5)

A solution of octa-*O*-acetyl-β-D-lactose (**2**) (3.00 g, 4.42 mmol), 10-undecen-1-ol (**3**) (1.5 g, 8.84 mmol), and 4 Å molecular sieves (1.0 g) in CH_2Cl_2 (dry, 30 mL) was stirred at room temperature. Subsequently, $SnCl_4$ (0.52 mL) was added in one portion by syringe. The mixture was stirred overnight. Then $NaHCO_3$ (2.5 g), Na_2SO_4 (2 g), and water (~5 mL) were added portionwise. After the formation of gas stopped, the solution was filtered over Hyflo. The precipitate was washed thoroughly with CH_2Cl_2. The combined organic phase was evaporated to give 4.2 g of residue. Column chromatography (gradient of 2:1 petroleum ether–EtOAc to 100% EtOAc) yielded **5** (1.95 g, 56%) as a white solid.

TLC R_f 0.63 (1:1 petroleum ether–EtOAc); ^{1}H NMR (400 MHz, C_6D_6): δ 5.97–5.87(m, 1H, –C*H*=CH_2), 5.61 (dd, 1H, $J_{2',3'}$ 10.5 Hz, $J_{1',2'}$ 7.9 Hz, H-2′), 5.57 (dd, 1H, $J_{3',4'}$ 3.4 Hz, $J_{4',5'}$ 0.8 Hz, H-4′), 5.52 (t, 1H, $J_{2,3} = J_{3,4}$ 9.0 Hz, H-3), 5.34 (dd, 1H, $J_{2,3}$ 9.0 Hz, $J_{1,2}$ 7.5 Hz, H-2), 5.21 (dd, 1H, $J_{2',3'}$ 10.5 Hz, $J_{3',4'}$ 3.4 Hz, H-3′), 5.20–5.09 (m, 2H, –CH=CH_2), 4.64 (dd, 1H, $J_{6a,6b}$ 11.5 Hz, $J_{5,6a}$ 2.0 Hz, H-6a), 4.48 (d, 1H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.38 (d, 1H, $J_{1,2}$ 7.5 Hz, H-1), 4.30–4.19 (m, 3H, H-6b, H-6′a, H-6′b), 3.91 (dt, 1H, ^2J 9.5 Hz, ^3J 6.5 Hz, –OCH_a–), 3.79 (~t, 1H, $J_{4,5}$ 9.5 Hz, $J_{3,4}$ 9.0 Hz, H-4), 3.60–3.57 (m, 1H, H-5′), 3.48 (dt, 1H, ^2J 9.5 Hz, ^3J 6.5 Hz, –OCH_b–), 3.40 (ddd, 1H, $J_{4,5}$ 9.5 Hz, $J_{5,6b}$ 5.5 Hz, $J_{5,6a}$ 2.0 Hz, H-5), 2.17–2.07 (m, 5H, –C*H*$_2$–CH=CH_2, Ac at 2.09), 2.05 (s, 3H, Ac), 1.95 (s, 3H, Ac), 1.86 (s, 3H, Ac), 1.84 (s, 3H, Ac), 1.80 (s, 3H, Ac), 1.70 (s, 3H, Ac), 1.69–1.26 (m, 14H, –$(CH_2)_7$–); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 170.2, 170.0, 169.9, 169.6, 169.4, 169.2 (7 × C=O), 139.4 (–*C*H=CH_2), 114.7 (–CH=*C*H_2), 101.9 (C-1′), 101.0 (C-1), 77.5 (C-4), 74.0 (C-3), 73.0 (C-5), 72.6 (C-2), 71.7 (C-3′), 71.0 (C-5′), 70.0 (OCH_2–), 69.9 (C-2′), 67.1 (C-4′), 62.9 (C-6), 61.1 (C-6′), 34.4 (–*C*H_2–CH=CH_2), 30.1, 30.0, 29.9, 29.8, 29.7, 29.5, 29.4 (–$(CH_2)_7$–), 21.0, 20.7, 20.6, 20.5, 20.4, 20.3, 20.0 (7 × *C*H_3–C(O)–). QTOFMS $[M-H]^-$ 787.3405 (calcd 787.3389; Δ ppm = 2.1); $[M+H]^+$ 789.3572 (calcd 789.3545; Δ ppm = 3.4).

1.4. 10-Undecenyl β-D-glucopyranoside (6)

Compound **4** (2.62 g, 5.23 mmol) was dissolved in MeOH (dry, 30 mL) and freshly prepared 1 M $NaOCH_3$ (150 μL) was added. The solution was stirred at room temperature, and the conversion was followed by TLC (EtOAc). After ~2 h the starting material ($R_f \sim 0.5$) was converted into a more polar compound ($R_f \sim 0.2$). The reaction was quenched by adding Dowex-50 $[H^+]$ resin. After standing overnight, the mixture was filtered, and the filtrate was concentrated, yielding **6** (1.66 g, 96%) as a colorless viscous oil.

TLC R_f ~0 (1:1 petroleum ether–EtOAc); ^{1}H NMR (200 MHz, CD_3OD): δ 5.90–5.70 (m, 1H, –C*H*=CH_2), 5.02–4.88 (m, 2H, –CH=*C*H_2), 4.23 (d, 1H, *J* 7.7 Hz, H-1), 3.95–3.83 (m, 2H), 3.69–4.46 (m, 2H), 3.39–3.03 (m, 6H), 2.05–1.97 (q, 2H, *J* ~ 6.7 Hz, CH_2CH=CH_2), 1.64–1.55 (m, 2H, –OCH_2CH_2–), 1.31 (s, 12H, –$(CH_2)_6$–); ^{13}C NMR (50 MHz, CD_3OD): δ 140.2 (–*C*H=CH_2), 114.7 (–CH=*C*H_2), 104.2 (C-1), 78.1, 77.9, 75.1, 71.6 (C-3, C-5, C-2, and C-4), 70.9 (OCH_2–),

62.7 (C-6), 34.9 (–CH_2CH=CH_2), 30.6, 30.2, 30.1, 29.0, 27.5, 27.1 (6 × –CH_2– and –OCH_2CH_2–).

1.5. 10-Undecenyl 6-*O*-trityl-β-D-glucopyranoside (7)

Compound **6** (1.66 g, 5.01 mmol) was dissolved in pyridine (dry, 19 mL). Trityl chloride (1.67 g, 6.00 mmol) and a catalytic amount of DMAP were added. The mixture was stirred at room temperature. The reaction was followed by TLC (2:1 petroleum ether–EtOAc; R_f product ~0.8). After 24 h the reaction mixture was poured into ice water (ca. 150 mL). The mixture was extracted with CH_2Cl_2 (2 × 60 mL and 2 × 30 mL, respectively). The combined organic layers were washed with $NaHCO_3$ (3 × 50 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated. Traces of pyridine were co-evaporated with toluene (3×). Column chromatography (19:1 CH_2Cl_2–MeOH with one drop of TEA per 100 mL) yielded **7** (1.84 g, 64%).

TLC R_f ~0.3 (19:1 CH_2Cl_2–MeOH); ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.47–7.23 (m, 15H, aromatic H), 5.91–5.70 (m, 1H, –C*H*=CH_2), 5.02–4.90 (m, 2H, –CH=*C*H_2), 4.26 (d, 1H, *J* 7.5 Hz, H-1), 3.55–3.39 (m, 7H), 3.27 (br s, 1H), 3.10 (br s, 1H), 2.76 (br s, 1H), 2.07–1.97 (q, 2H, *J* ~ 6.8 Hz, C*H*$_2$CH=CH_2), 1.72–1.57 (m, 2H, –OCH_2C*H*$_2$–), 1.27 (s, 12H, –$(CH_2)_6$–); ^{13}C NMR (50 MHz, $CDCl_3$): δ 143.5 (–C-6-OC*C*–), 139.2 (–*C*H=CH_2), 128.6, 127.9, 127.1 (aromatic C), 114.1 (–CH=*C*H_2), 102.4 (C-1), 87.1 (C(phenyl)$_3$), 76.2, 73.6, 73.6, 72.2 (C-2, C-3, C-4, and C-5), 70.1 (–OCH_2–), 64.4 (C-6), 33.8 (–*C*H_2CH=CH_2), 29.6, 29.5, 29.4, 29.1, 28.9 (–CH_2–), 26.0 (–OCH_2CH_2–).

1.6. 10-Undecenyl 2,3,4-*O*-(3-trifluoromethylbenzoyl)-6-*O*-trityl-β-D-glucopyranoside (8)

Compound **7** (0.91 g, 1.58 mmol) was dissolved in pyridine (dry, ~10 mL). The solution was stirred at 0 °C, and 3-(trifluoromethyl)benzoylchloride (1.46 mL, 9.66 mmol) and a catalytic amount of DMAP were added. The reaction was followed by TLC. After 44 h the mixture was poured into ice water (~135 mL). The mixture was separated, and the layer was extracted with CH_2Cl_2 (50, 25, and 25 mL, respectively). The combined organic layers were washed successively with satd $NaHCO_3$ (50 mL) and twice with water (50 and 35 mL, respectively). The organic layer was dried over $MgSO_4$. The solution was concentrated under reduced pressure, and traces of pyridine were removed by co-evaporation with small volumes of toluene (4×). Column chromatography (1:1 petroleum ether–CH_2Cl_2) yielded **8** (1.33 g, 77%).

TLC R_f 0.41 (1:1 petroleum ether–CH_2Cl_2); R_f 0.25 (1:1 petroleum ether–EtOAc); ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.15–6.98 (m, 27H, aromatic H), 5.9–5.5 (m, 1H, –C*H*=CH_2), 5.79–5.59 (m, 4H), 4.95–4.74 (m, 3H), 4.76 (d, 1H, ^3J 7.8 Hz, H-1), 3.96–3.92 (dt, 1H, ^2J 9.7 Hz, ^3J 6.3 Hz, –OCH_a–), 3.83–3.78 (m, 1H, H-5), 3.58–3.53 (dt, 1H, ^2J 9.7 Hz, ^3J 6.7 Hz, –OCH_b–), 3.38–3.32 (dd, 1H, $J_{6,6'}$ 10.5 Hz, $J_{5,6}$ 2.2 Hz, H-6), 3.19–3.11 (dd, 1H, $J_{6,6'}$ 10.6 Hz, $J_{5,6'}$ 4.6 Hz, H-6′), 1.98–1.87 (q, 2H, *J* ~ 7.0 Hz, C*H*$_2$CH=CH_2), 1.23–1.13 (m, 12H, –$(CH_2)_6$–); ^{13}C NMR (50 MHz, $CDCl_3$): δ 164.8, 163.9, 163.5 (3 × C=O), 143.4 (–C-6-OC*C*–), 139.2 (–*C*H=CH_2), 132.9, 132.8, *131.5*, *130.7*, *130.2*, 129.9, *129.6*, 129.0, 128.6, 127.7, 127.0, 126.7 (aromatic C atoms, quaternary C atoms in italics), ~120.8 and ~115.4 (2 × 3 signals from quartet, $J_{C,F}$ 273 Hz, CF_3. N.B. Only two signals of each quartet were observed, since the other two overlap with the signals from the aromatic region; the shift of each quartet is about 3 Hz), 114.1 (–CH=*C*H_2), 101.0 (C-1), 86.7 (C(phenyl)$_3$), 74.1, 73.5, 72.7, 69.9 (C-2, C-3, C-4, and C-5), 70.1 (–OCH_2), 62.2 (C-6), 33.8 (–*C*H_2CH=CH_2), 29.5, 29.4, 29.3, 29.1, 28.9 (6 × –CH_2–), 26.0 (–OCH_2CH_2–). QTOFMS $[M-H]^-$ not found (calcd 1089.3624), $[M-H+HCOOH]^-$ 1135.3696 (calcd 1135.3679; Δ ppm = 1.5); $[M+H]^+$ 1091.3823 (calcd 1091.3780; Δ ppm = 3.7).

1.7. Methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-nonulopyranosylchloride)onate (11)

A suspension of compound **9** (1.5 g, 4.85 mmol) in 50 mL of MeOH, containing 1.5 g of Amberlite IR-120 (H^+) resin was stirred overnight at room temperature. After that second portion of 1.5 g of Amberlite IR-120 (H^+) resin was added. After stirring for another 7 h a clear solution was obtained. The resin was filtered off, and the solution was evaporated giving a dark viscous oil. The latter was dissolved in a minimal amount of MeOH, and Et_2O was added to turbidity. Upon standing methyl *N*-acetyl-α-D-neuraminate (**10**) crystallized (1.6 g).

Freshly distilled AcCl (30 mL) was added dropwise to ice-cooled methyl *N*-acetyl-α-D-neuraminate (**10**) (0.8 g, 2.47 mmol) over 15 min. The suspension was allowed to warm to room temperature and was stirred for 48 h. The reaction mixture was evaporated to dryness and co-evaporated a few times with dry toluene at 35 °C. The residue was crystallized from a benzene–hexane–Et_2O mixture to give compound **11** (0.83 g, 66%) as a colorless solid. The ^{1}H NMR and ^{13}C NMR spectra of compound **10** and **11** data were in accordance with those in the literature.[26]

1.8. Methyl (undec-10-enyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulopyranosyl chloride)onate (12)

Compound **11** (0.15 g, 0.294 mmol) was dissolved in dry acetonitrile (5 mL) containing 10-undecen-1-ol (**3**) (0.1 g, 0.588 mmol) and 4 Å molecular sieves (1 g). The mixture

was stirred for 1 h at room temperature in the dark, and then silver triflate (0.15 g, 0.588 mmol) was added in one portion. The mixture was stirred at 35–45 °C for 48 h. After cooling to room temperature, the solids were filtered off through Celite, and washed thoroughly with acetonitrile. The filtrate was diluted with EtOAc (15 mL), washed successively with 5% $NaHCO_3$, satd aq $Na_2S_2O_3$, and finally with brine. The organic layer was dried over Na_2SO_4, and the solvent removed in vacuo at 35 °C. According to the NMR spectrum, the residue was a mixture containing the α:β isomers in a ratio of 3:2. Chromatography (gradient of 1:1 petroleum ether–EtOAc to 100% EtOAc) of the residue on a column of silica gel afforded (in order of elution) β glycoside **12** (0.04 g, 21%), α/β glycoside **12** (0.04, 21%), and α glycoside **12** (0.07 g, 37%).

1.8.1. α-Isomer. TLC R_f 0.45 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.88–5.79 (m, 1H, –C*H*=CH_2), 5.41 (ddd, 1H, $J_{7,8}$ 8.2 Hz, $J_{8,9}$ 5.5 Hz, $J_{8,9'}$ 2.7 Hz, H-8), 5.35 (dd, 1H, $J_{7,8}$ 8.2 Hz, $J_{6,7}$ 1.9 Hz, H-7), 5.15 (d, 1H, $J_{5,NH}$ 9.6 Hz, NH), 5.03–4.93 (m, 2H, –CH=CH_2), 4.86 (ddd, 1H, $J_{3,4}$ 12.3 Hz, $J_{4,5}$ 9.9 Hz, $J_{3',4}$ 4.65 Hz, H-4), 4.33 (dd, 1H, $J_{9,9'}$ 12.4 Hz, $J_{8,9'}$ 2.7 Hz, H-9′), 4.17–4.04 (m, 3H, H-5, H-6, H-9), 3.81 (s, 3H, CH_3O–), 3.77 (dt, 1H, ^2J 9.4 Hz, ^3J 6.4 Hz, –OCH_a–), 3.22 (dt, 1H, ^2J 9.4 Hz, ^3J 6.6 Hz, –OCH_b–), 2.60 (dd, 1H, $J_{3,3'}$ 12.8 Hz, $J_{3',4}$ 4.7 Hz, H-3′), 2.16–1.90 (m, 18H, H-3, –CH_2–CH=CH_2, 5 Ac at 2.16, 2.15, 2.06, 2.04, 1.90), 1.54 (m, 2H, –OCH_2CH_2–), 1.32–1.26 (m, 12H, –$(CH_2)_6$–); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.5, 171.1, 170.60, 170.56, 170.4, 169.0 (6 × C=O), 139.6 (–*C*H=CH_2), 114.5 (–CH=*C*H_2), 99.1 (C-2), 72.8 (C-6), 69.6 (C-4), 69.1 (C-8), 67.8 (C-7), 65.5 (–OCH_2–), 62.7 (C-9), 53.0 (–OCH_3), 50.1 (C-5), 38.5 (C-3), 34.2 (–*C*H_2–CH=CH_2), 30.0 (–OCH_2–*C*H_2–), 29.9, 29.8, 29.7, 29.5, 29.3, 26.3 (–$(CH_2)_6$–), 23.6, 21.5, 21.30, 21.25, 21.2 (5 × CH_3–C(O)–).

1.8.2. β-Isomer. TLC R_f 0.50 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.89–5.79 (m, 1H, –C*H*=CH_2), 5.43–5.41 (m, 1H), 5.31–5.19 (m, 3H), 5.04–4.94 (m, 2H, –CH=CH_2), 4.81 (dd, 1H, $J_{9,9'}$ 12.4 Hz, $J_{8,9'}$ 2.5 Hz, H-9′), 4.19–4.10 (m, 2H), 3.94–3.89 (m, 1H), 3.82 (s, 3H, CH_3O–), 3.47 (dt, 1H, ^2J 9.4 Hz, ^3J 6.4 Hz, –OCH_a–), 3.33 (dt, 1H, ^2J 9.4 Hz, ^3J 6.7 Hz, –OCH_b–), 2.48 (dd, 1H, $J_{3,3'}$ 12.9 Hz, $J_{3',4}$ 5.0 Hz, H-3′), 2.17–1.90 (m, 18H, H-3, –CH_2–CH=CH_2, 5 Ac at 2.17, 2.09, 2.05, 2.04, 1.90), 1.59 (m, 2H, –OCH_2CH_2), 1.34–1.28 (m, 12H, –$(CH_2)_6$–); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.1, 170.7, 170.5, 170.20, 170.18, 167.6 (6 × C=O), 139.2 (–*C*H=CH_2), 114.1 (–CH=*C*H_2), 98.5 (C-2), 72.1 (C-6), 71.7 (C-4), 69.0 (C-8), 68.5 (C-7), 64.2 (–OCH_2–), 62.4 (C-9), 52.6 (–OCH_3), 49.5 (C-5), 37.5 (C-3), 33.8 (–*C*H_2–CH=CH_2), 29.6 (–OCH_2–*C*H_2–), 29.50, 29.45, 29.4, 29.1, 28.9, 26.5 (–$(CH_2)_6$–), 23.6, 21.4, 21.3, 21.2 (5 × *C*H_3–C(O)–).

QTOFMS $[M-H]^-$ 642.3124 (calcd 642.3126; Δ ppm = −0.3); $[M+H]^+$ 644.3311 (calcd 644.3262; Δ ppm = 4.4).

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