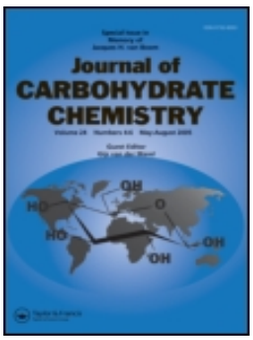

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Synthesis of stigmasteryl (β1→4)-oligoglucosides

Katharina Kettelhoit and Daniel B. Werz

Institut für Organische Chemie, Technische Universität Braunschweig, Braunschweig, Germany

ABSTRACT

Stigmasteryl (β1→4)-oligoglucosides were prepared with cellobiose, cellotriose, and cellotetraose as glycan chains. For the preparation of the peracetylated oligoglucosyl donors anomeric acetate was deprotected and the respective hemiacetals were converted into trichloroacetimidates. Glycosylation with stigmasterol yielded both α- and β-anomers because during the treatment with Lewis acid the 2-OAc is cleaved to some extent; thus, with the emerging hydroxyl group neighboring group participation does not take place. Due to their different number of hydroxyl groups (0 *vs.* 1) separation of the two products proved to be facile. Saponification led to the desired stigmasteryl glucosides.

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Glycosylation; neighboring group participation; peracetylated glycans; saponins; stigmasterol

Introduction

Saponins represent a class of naturally occurring high molecular weight glycosides with triterpenes or steroids as aglycone. They are found in numerous plants like soybeans, chick peas, oats, and lentils where they are believed to play a significant physiological role (Fig. 1). Many saponin-containing plants are applied in natural medicine due to their broad therapeutic effects.[1] Saponins are considered to make up the major active compounds of ginseng. Due to their amphiphilic nature consisting of the polar glycan unit and the apolar steroid scaffold, special physicochemical and biological properties arise. Several of these compounds are known to have hemolytic properties or are even toxic to cold-blooded animals.[2] But also in terms of self-assembly interesting features are expected because of the interplay of polar and apolar parts in the molecules.

A special class of very simple saponins with stigmasterol as steroid scaffold and 1,4-linked oligoglucoses of different chain length was observed in rice (*Oryza sativa*). Both anomeric configurations (α and β), with β being the major isomer, were found at the stereocenter at the reducing end linking the glycan to the aglycon.[5] Because of the extreme importance of rice for the nutrition of mankind, it is worth to further investigate the potential bioactivity of these compounds. Since only miniscule amounts of the respective stigmasteryl oligoglucosides are accessible by

CONTACT Daniel B. Werz d.werz@tu-braunschweig.de Institut für Organische Chemie, Technische Universität Braunschweig, 38106 Braunschweig, Germany.

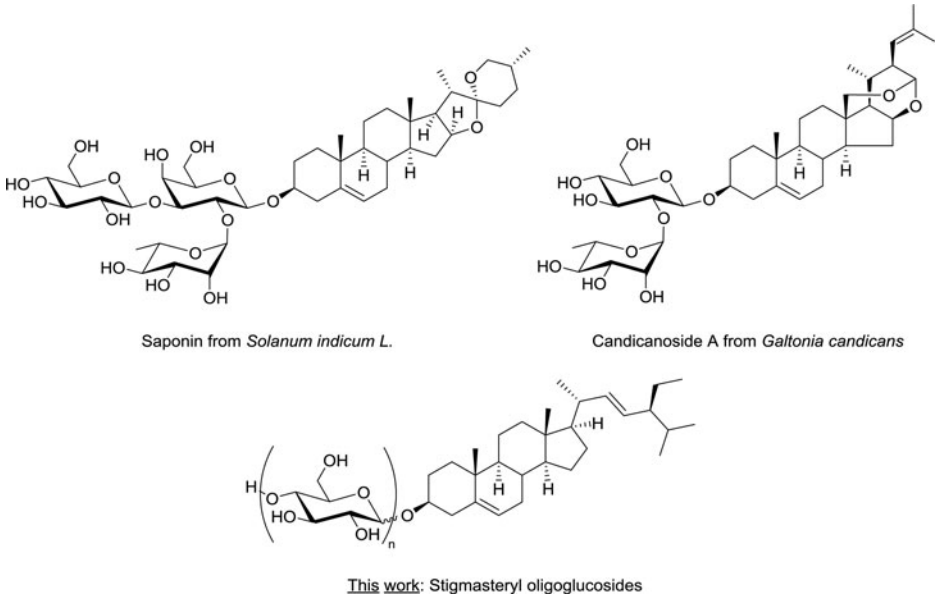


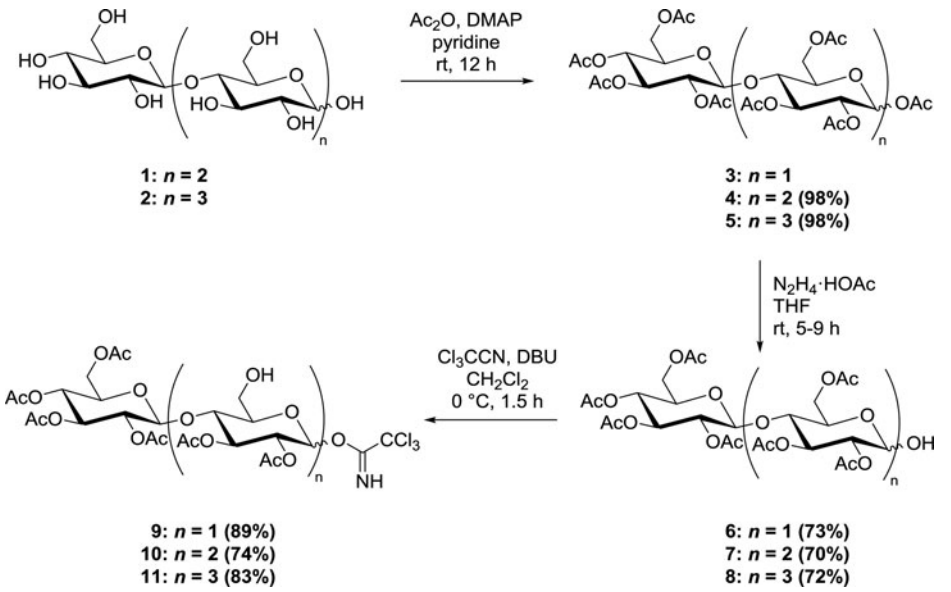
Figure 1. Examples of literature-known saponins and our target compounds.[3,4]

isolation from the plant chemical synthesis is the way to go.[3,6] Herein, we report a general approach for the synthesis of stigmasteryl ($\beta 1 \rightarrow 4$)-oligoglucosides leading to both, α- and β-linkages at the reducing end of the glycan chain.

Results and discussion

Our approach to access both, α- and β-linked stigmasteryl derivatives starts with commercially available cellobiose, cellotriose, and cellotetraose. The naked oligosaccharides were converted quantitatively to the peracetylated congeners by using acetic anhydride in pyridine. Anomeric deprotection was achieved by using hydrazine acetate in tetrahydrofuran (THF) at room temperature.[7] The respective hemiacetals were converted into the oligoglucosyl trichloroacetimidates in good yields (Sch. 1).[8]

Commonly, the acetate group in position 2 of glucose secures β-stereochemistry of the emerging glycosidic bond because of neighboring group participation. However, very recently it was reported that an acetate group in this position is rather labile and might be cleaved when the glycosyl donor gets activated. Therefore, many groups use other protecting groups than acetate in this position to ensure the desired 1,2-*trans*-linkage.[9] However, we intended the fact that the acetate might be cleaved can be turned into an advantage since the emerging hydroxyl group in position 2 might lead to α-stereochemistry. Furthermore, we assumed that the hydroxyl group of the stigmasterol reveals a much higher nucleophilicity leading to a faster reaction of the glycosyl donor rather than a reaction of the glycosyl donor with the 2-hydroxyl of the oligoglycan chain.



Scheme 1. Preparation of oligoglucosyl trichloroacetimidates **9**–**11**.

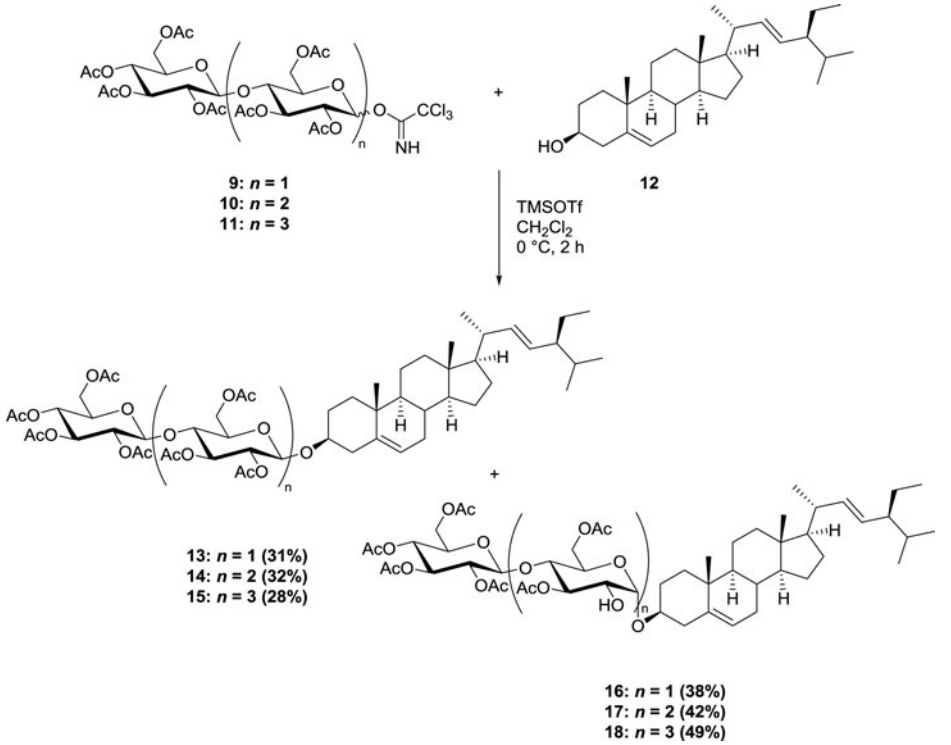
Indeed, glycosylation of **9**–**11** with stigmasterol (**12**) utilizing catalytic amount of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as promotor afforded the expected β-glycosides **13**–**15** together with respective α-linked congeners **16**–**18**, both in moderate yield (Sch. 2).

Since the α-products were deacetylated in position 2 during the course of the reaction, the products revealing different polarity were easily separable by column chromatography. As we expected deacetylation had occurred to some extent in 2-position of the glucose unit adjacent to the aglycone. Thus, the α-glucoside was formed as result of the non-participating hydroxyl group. A mechanism for this deacetylation was postulated by Pakulski for similar substrates.[10] Upon activation with the promotor, acyloxonium ion **19** is formed, followed by orthoester formation with the hydroxyl as nucleophile. Rearrangement of the orthoester then releases deacetylated oxocarbenium ion **21** and ester **22** (Sch. 3). In our protocol, the nucleophile stigmasterol (**12**) was utilized in excess, i.e., **21** was able to undergo the glycosylation reaction affording preferentially the α-product. The acetylated nucleophile **22** with R = stigmasteryl was once isolated in 54% yield to support the postulated deacetylation mechanism.

Finally, all conjugates were subjected to saponification with NaOMe providing six different saponins in good to excellent yields (Sch. 4).

Conclusion

In summary, we have developed an efficient method for the preparation of stigmasterol saponins containing $\beta(1\rightarrow4)$-linked oligo-D-glucans of different length. The glycosylation using peracetylated glucosyl trichloroacetimidates was designed

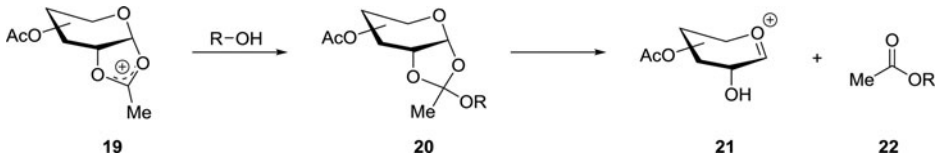


Scheme 2. Glycosylation of stigmasterol (**12**) with oligoglucosyl trichloroacetimidates **9**–**11**.

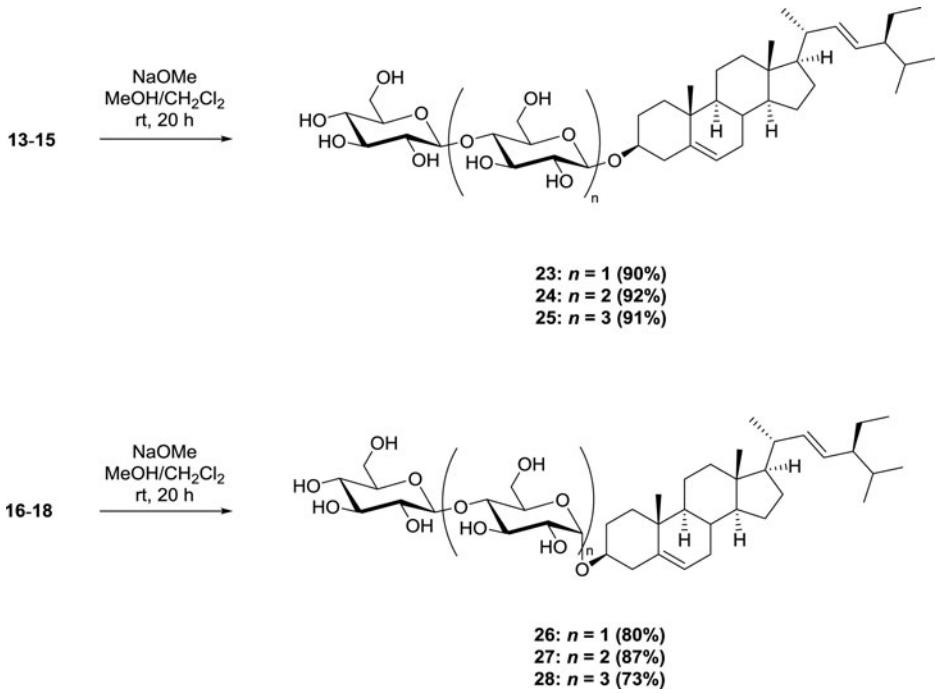
in such a way that both, the α- and β-anomer, were obtained. During the course of the reaction partial deacetylation in position 2 adjacent to the trichloroacetimidate took place resulting in a free hydroxyl group. This non-participating group accounts for the α-anomer. Since the different anomers possess a different number of hydroxyl groups (0 *vs.* 1), a separation by column chromatography was facile. Saponification furnished the respective saponins.

Experimental

Solvents were dried by standard procedures and distilled before use. All reactions were carried out in oven-dried glassware, septum-capped under atmospheric pressure of argon. Commercially available compounds were used without further purification unless otherwise stated. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a 300, 400, or 600 MHz instrument using the residual signals from



Scheme 3. Deacetylation in position 2 due to putative orthoester formation.



Scheme 4. Saponification with NaOMe in MeOH to afford the target compounds **23**–**28**.

tetramethylsilane (TMS) $\delta = 0.00$ ppm as internal references for ^{1}H and ^{13}C chemical shifts, respectively. No integration was performed in case of α/β mixtures. Electrospray Ionization High-Resolution Mass Spectrometry (ESI-HRMS) was carried out on a Fourier Transform Ion Cyclotron Resonance (FTICR) instrument. IR spectra were measured on an Attenuated Total Reflection (ATR) spectrometer. D-Cellobiose octaacetate (**3**) was purchased from Alfa Aesar.

General procedure for the acetylation

To a solution of the respective saccharide in dry pyridine and acetic anhydride (3:2, 0.01 M) was added one crystal of 4-Dimethylaminopyridine (DMAP). After stirring for 12 h at ambient temperature, the reaction was stopped by the addition of sat. aq. $NaHCO_3$-solution. The aqueous phase was extracted with EtOAc (2×), and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2, *n*-pentane/EtOAc).

General procedure for the anomeric deprotection

To a solution of the peracetylated sugar (1.00 equiv) in dry THF (0.04 M) was added hydrazine acetate (1.20 equiv). After stirring for 5–9 h at ambient temperature, EtOAc and H_2O were added. The organic layer was washed with brine and

sat. aq. $NaHCO_3$-solution, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2, *n*-pentane/EtOAc).

General procedure for the preparation of the trichloroacetimidate

A solution of the reducing sugar (1.00 equiv) in dry CH_2Cl_2 (0.08 M) was cooled to 0°C, and trichloroacetonitrile (20.0 equiv) and 1,8-Diazabicycloundec-7-ene (DBU) (15.0 mol%) were added dropwise. The reaction mixture was stirred at 0 °C for 1.5 h, and concentrated *in vacuo* at 30 °C. The residue was filtered through a plug of silica (*n*-pentane/EtOAc, 1% NEt_3) and directly used for the next step.

General procedure for the glycosylation

The respective trichloroacetimidate (1.00 equiv) and stigmasterol (2.00 equiv) were azeotroped with toluene (3×) and dried in high vacuum for 1 h. Dry CH_2Cl_2 (0.1 M) was added and the solution was cooled to 0 °C. TMSOTf (15.0 mol%) was added dropwise and the reaction mixture was stirred for 2 h at 0 °C. The reaction was stopped by the addition of pyridine, the solvents were removed *in vacuo*, and the residue was purified by column chromatography (SiO_2, *n*-pentane/EtOAc).

General procedure for the saponification

To a solution of the protected glycosteroid in CH_3OH and CH_2Cl_2 (3:1, 0.01 M) was added NaOMe (5.4 M in methanol) until pH > 12. The solution was stirred at ambient temperature for 20 h. The solution was neutralized with Amberlite®, diluted with dimethyl sulfoxide (DMSO), filtered, and the solvents were removed *in vacuo*. Dialysis and lyophilization afforded the desired products as white powders.

D-Cellotriose undecaacetate (4)

Acetylation of cellotriose (**1**) (200 mg, 0.396 mmol, 1.00 equiv) was performed according to the general procedure in pyridine (24 mL) and acetic anhydride (16 mL). Purification by column chromatography (SiO_2, *n*-pentane/EtOAc 1:1.5) afforded the peracetylated product **3** (375 mg, 0.388 mmol, 98%) as a pale yellow solid as a mixture of anomers. The spectral data were in accordance with those reported in the literature.[11]

D-Cellotetraose tetradecaacetate (5)

Acetylation of cellotetraose (**2**) (200 mg, 0.300 mmol, 1.00 equiv) was performed according to the general procedure in pyridine (18 mL) and acetic anhydride (12 mL). Purification by column chromatography (SiO_2, *n*-pentane/EtOAc 1:1.5 → 1:3) afforded the peracetylated product **5** (369 mg, 0.294 mmol, 98%) as a pale yellow

solid as a mixture of anomers. The spectral data were in accordance with those reported in the literature.[11]

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-D-glucopyranoside (6)

Anomeric deprotection of D-cellobiose octaacetate (**3**) (2.00 g, 2.95 mmol, 1.00 equiv) was performed according to the general procedure with hydrazine acetate (326 mg, 3.54 mmol, 1.20 equiv) in THF (60 mL) for 5 h. Purification by column chromatography (SiO_2, *n*-pentane/EtOAc 1:1.5 → 1:3) afforded the desired product **6** (1.37 g, 2.15 mmol, 73%) as a white solid as a mixture of anomers. The spectral data were in accordance with those reported in the literature.[12]

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-D-glucopyranoside (7)

Anomeric deprotection of D-cellotriose undecaacetate (**4**) (375 mg, 0.388 mmol, 1.00 equiv) was performed according to the general procedure with hydrazine acetate (42.8 mg, 0.465 mmol, 1.20 equiv) in THF (8.0 mL) for 6 h. Purification by column chromatography (SiO_2, *n*-pentane/EtOAc 1:1.5 → 1:3) afforded the desired product **7** (251 mg, 0.271 mmol, 70%) as a white solid as a mixture of anomers. TLC: $R_f = 0.2$ (SiO_2, *n*-hexane/EtOAc 1:1.5). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.98 (s), 1.99 (s), 2.00 (s), 2.00 (s), 2.01 (s), 2.02 (s), 2.04 (s), 2.07 (s), 2.09 (s), 2.13 (s), 2.14 (s), 2.14 (s), 3.19–3.28 (m), 3.54–3.69 (m), 3.69–3.84 (m), 3.96–4.23 (m), 4.30–4.43 (m), 4.42–4.58 (m), 4.71 (t, J = 7.9 Hz), 4.74–4.96 (m), 5.00–5.17 (m), 5.21 (t, J = 9.3 Hz), 5.37 (t, J = 3.6 Hz), 5.43–5.56 (m). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 20.5, 20.5, 20.6, 20.6, 20.7, 20.7, 20.8, 20.9, 20.9, 61.5, 61.6, 62.1, 67.7, 68.3, 68.2, 71.3, 71.6, 71.7, 71.7, 71.8, 72.1, 72.7, 72.7, 72.9, 73.0, 73.5, 76.1, 76.6, 77.2, 90.0, 100.4, 100.5, 100.7, 169.1, 169.2, 169.3, 169.6, 169.7, 169.8, 170.2, 170.2, 170.2, 170.3, 170.4, 170.5, 170.9. IR (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 3,479, 2,959, 1,738, 1,367, 1,212, 1,032, 903, 599. HRMS (ESI): m/z calcd. for $C_{38}H_{52}O_{26}Na^+$ $[M+Na]^+$ 947.2639; found 947.2636.

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-D-glucopyranoside (8)

Anomeric deprotection of D-cellotetraose tetradecaacetate (**5**) (346 mg, 0.275 mmol, 1.00 equiv) was performed according to the general procedure with hydrazine acetate (30.5 mg, 0.331 mmol, 1.20 equiv) in THF (7.0 mL) for 9 h. Purification by column chromatography (SiO_2, *n*-pentane/EtOAc 1:1.5 → 1:3) afforded the desired product **8** (239 mg, 0.197 mmol, 72%) as a white solid as a mixture of anomers.

TLC: $R_f = 0.2$ (SiO_2, *n*-hexane/EtOAc 1:3). ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) = 1.94–1.98 (m), 1.98 (s), 1.98–2.00 (m), 2.01 (s), 2.02 (s), 2.02 (s), 2.04 (s), 2.06 (s), 2.05–2.09 (m), 2.09 (s), 2.13 (s), 2.14 (s), 2.15 (s), 3.53–3.60 (m), 3.61–3.67 (m), 3.69–3.80 (m), 3.85 (dd, J = 6.7, 1.1 Hz), 4.03 (dd, J = 12.9, 2.1 Hz), 4.06–4.13 (m), 4.14–4.20 (m), 4.36 (dd, J = 12.5, 4.3 Hz), 4.38–4.56 (m), 4.68–4.74 (m), 4.76–4.79 (m), 4.79–4.88 (m), 4.90 (ddd, J = 9.1, 7.9, 1.1 Hz), 5.03–5.09 (m), 5.09–5.16 (m), 5.17–5.24 (m), 5.34–5.40 (m), 5.49 (t, J = 9.7 Hz). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) = 19.0, 20.4, 20.5, 20.5, 20.6, 20.6, 20.7, 20.7, 20.8, 20.8, 20.9, 20.9, 21.0, 27.6, 61.4, 61.3, 61.8, 62.1, 67.7, 68.2, 69.1, 70.6, 71.2, 71.5, 71.6, 71.7, 71.8, 71.9, 72.0, 72.5, 72.6, 72.6, 72.7, 72.7, 72.8, 73.0, 73.4, 76.0, 76.0, 76.1, 76.1, 76.5, 90.0, 95.3, 100.3, 100.5, 100.5, 100.8, 169.1, 169.2, 169.2, 169.3, 169.3, 169.6, 169.7, 169.8, 170.1, 170.2, 170.2, 170.2, 170.4, 170.4, 170.5, 170.9, 171.3. IR (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 3,472, 2,961, 1,738, 1,367, 1,212, 1,032, 902, 600. HRMS (ESI): m/z calcd. for $C_{50}H_{68}O_{34}Na^+$ $[M+Na]^+$ 1235.3484; found 1235.3490.

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-D-glucopyranosyl trichloroacetimidate (9)

Trichloroacetimidate **9** was synthesized according to the general procedure using compound **6** (1.31 g, 2.06 mmol, 1.00 equiv), trichloroacetonitrile (5.94 g, 4.13 mL, 41.1 mmol, 20.0 equiv), and DBU (47.0 mg, 46.2 µL, 0.309 mmol, 15.0 mol%) in CH_2Cl_2 (25 mL). Filtration through silica (SiO_2, *n*-pentane/EtOAc 1:1.5, 1.0% NEt_3) afforded the desired product **9** (1.43 g, 1.83 mmol, 89%) as a yellow foam.

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-D-glucopyranosyl trichloroacetimidate (10)

Trichloroacetimidate **10** was synthesized according to the general procedure using compound **7** (245 mg, 0.265 mmol, 1.00 equiv), trichloroacetonitrile (765 mg, 531 µL, 5.30 mmol, 20.0 equiv), and DBU (6.04 mg, 5.94 µL, 0.0397 mmol, 15.0 mol%) in CH_2Cl_2 (3.3 mL). Filtration through silica (*n*-pentane/EtOAc 1:1.5, 1.0% NEt_3) afforded the desired product **10** (210 mg, 1.83 mmol, 74%) as a yellow foam.

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-D-glucopyranosyl trichloroacetimidate (11)

Trichloroacetimidate **11** was synthesized according to the general procedure using compound **8** (237 mg, 0.196 mmol, 1.00 equiv), trichloroacetonitrile (565 mg,

392 µL, 3.91 mmol, 20.0 equiv), and DBU (4.48 mg, 4.40 µL, 0.0294 mmol, 15.0 mol%) in CH_2Cl_2 (2.5 mL). Filtration through silica (*n*-pentane/EtOAc 1:1.5 → 1:4, 1.0% NEt_3) afforded the desired product **11** (222 mg, 1.83 mmol, 83%) as a beige foam.

Stigmasteryl 2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-D-glucopyranosides 13 and 16

Glycosylation was performed according to the general procedure using compound **9** (466 mg, 0.596 mmol, 1.00 equiv), stigmasterol (443 mg, 1.07 mmol, 1.80 equiv), and TMSOTf (19.9 mg, 16.2 µL, 0.0894 mmol, 15.0 mol%) in CH_2Cl_2 (60 mL). Purification by column chromatography (SiO_2, *n*-pentane/EtOAc 3:1 → 1:1) afforded β-glycoside **13** (191 mg, 0.185 mmol, 31%) and 2-deacetylated α-glycoside **16** (224 mg, 0.226 mmol, 38%) as pale yellow solids. Analytical data of the β-glycoside **13**: TLC: $R_f = 0.3$ (SiO_2, *n*-hexane/EtOAc 1:1). ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) = 0.69 (s, 3H), 0.77–0.82 (m, 7H), 0.84 (d, J = 6.4 Hz, 3H), 0.87–0.95 (m, 2H), 0.98 (s, 3H), 0.99–1.10 (m, 6H), 1.11–1.22 (m, 3H), 1.21–1.31 (m, 2H), 1.38–1.60 (m, 10H), 1.65–1.75 (m, 1H), 1.81–1.91 (m, 2H), 1.94–2.07 (m, 3H), 1.98 (s, 3H), 2.01 (s, 3H), 2.03 (s, 3H), 2.04 (s, 3H), 2.09 (s, 3H), 2.11 (s, 3H), 2.13–2.27 (m, 1H), 3.39–3.50 (m, 1H), 3.57 (ddd, J = 10.0, 5.2, 2.1 Hz, 1H), 3.66 (ddd, J = 10.0, 4.5, 2.3 Hz, 1H), 3.75 (dd, J = 9.9, 9.1 Hz, 1H), 4.04 (dd, J = 12.4, 2.3 Hz, 1H), 4.09 (dd, J = 11.9, 5.2 Hz, 1H), 4.37 (dd, J = 12.5, 4.5, 1H), 4.48 (dd, J = 12.4, 2.5 Hz, 1H), 4.50 (d, J = 7.9 Hz, 1H), 4.54 (d, J = 8.0 Hz, 1H), 4.87 (dd, J = 9.7, 8.0 Hz, 1H), 4.92 (dd, J = 9.4, 7.9 Hz, 1H), 5.01 (dd, J = 15.1, 8.8 Hz, 1H), 5.06 (t, J = 9.7 Hz, 1H), 5.11–5.20 (m, 3H), 5.34 (dt, J = 5.7, 2.0 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) = 12.0, 12.3, 19.0, 19.4, 20.6, 20.6, 20.7, 20.7, 20.9, 21.0, 21.1, 21.2, 24.3, 24.4, 25.4, 28.9, 29.4, 31.8, 31.9, 31.9, 36.7, 37.2, 38.9, 39.6, 40.5, 42.2, 50.1, 51.2, 55.9, 56.8, 61.6, 62.0, 67.8, 71.6, 71.7, 71.9, 72.6, 72.9, 76.6, 80.1, 99.5, 100.7, 122.1, 129.2, 138.3, 140.3, 169.0, 169.3, 169.8, 170.2, 170.3, 170.5. IR (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 2,960, 2,870, 1,742, 1,446, 1,365, 1,222, 1,040, 906, 759, 564. HRMS (ESI): m/z calcd. for $C_{55}H_{82}O_{18}Na^+$ $[M+Na]^+$ 1053.5393; found 1053.5398. Analytical data of the α-glycoside **16**: TLC: $R_f = 0.2$ (SiO_2, *n*-hexane/EtOAc 1:1). ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) = 0.70 (s, 3H), 0.77–0.83 (m, 6H), 0.85 (d, J = 6.4 Hz, 3H), 0.88–0.96 (m, 1H), 0.96–1.09 (m, 9H), 1.10–1.22 (m, 3H), 1.22–1.31 (m, 1H), 1.38–1.59 (m, 9H), 1.65–1.75 (m, 1H), 1.83–1.90 (m, 2H), 1.94–1.98 (m, 1H), 1.97–2.00 (m, 5H), 2.01 (s, 3H), 2.01–2.06 (m, 1H), 2.05 (s, 3H), 2.09 (s, 3H), 2.10 (s, 3H), 2.11 (s, 3H), 2.31–2.41 (m, 2H), 3.43–3.50 (m, 1H), 3.53 (ddd, J = 11.9, 9.8, 4.0 Hz, 1H), 3.59–3.64 (m, 1H), 3.66 (ddd, J = 9.8, 4.1, 2.3 Hz, 1H), 3.99 (ddd, J = 10.1, 5.4, 2.1 Hz, 1H), 4.05 (dd, J = 12.4, 2.4 Hz, 1H), 4.12 (dd, J = 11.9, 5.4 Hz, 1H), 4.39 (dd, J = 12.4, 4.1 , 1H), 4.43 (dd, J = 11.9, 2.1 Hz, 1H), 4.52 (d, J = 7.9 Hz, 1H), 4.94 (dd, J = 9.2, 7.9 Hz, 1H), 4.98 (d, J = 4.1 Hz, 1H), 5.02 (dd, J = 15.1, 8.8 Hz, 1H), 5.07–5.22 (m, 4H), 5.36 (d, J = 5.5 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) = 12.0, 12.2, 19.0, 19.3, 20.5, 20.6, 20.7, 20.9,

21.0, 21.1, 21.2, 24.3, 25.4, 28.1, 28.9, 31.8, 31.9, 31.9, 36.6, 36.9, 39.6, 40.1, 40.5, 42.2, 50.1, 51.2, 55.9, 56.8, 61.6, 62.2, 67.7, 68.5, 71.0, 71.7, 71.8, 73.0, 73.2, 76.7, 79.4, 97.0, 100.8, 122.3, 129.2, 138.3, 140.2, 169.2, 169.2, 170.2, 170.4, 170.5, 170.7. IR (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 3,480, 2,955, 2,871, 1,741, 1,458, 1,368, 1,223, 1,031, 906, 738, 599. IR (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 3,480, 2,955, 2,871, 1,741, 1,458, 1,368, 1,223, 1,031, 906, 738, 599. HRMS (ESI): *m*/*z* calcd. for $C_{53}H_{80}O_{17}Na^+$ $[M+Na]^+$ 1011.5288; found 1011.5285.

Stigmasteryl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl -β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-D-glucopyranosides 14 and 17

Glycosylation was performed according to the general procedure using compound **10** (203 mg, 0.190 mmol, 1.00 equiv), stigmasterol (157 mg, 0.380 mmol, 2.00 equiv), and TMSOTf (6.33 mg, 5.16 µL, 0.0285 mmol, 15.0 mol%) in CH_2Cl_2 (19 mL). Purification by column chromatography (SiO_2, *n*-pentane/EtOAc 2:1 → 1:1.5) afforded *β*-glycoside **14** (80.1 mg, 0.0607 mmol, 32%) and 2-deacetylated *α*-glycoside **17** (101 mg, 0.0791 mmol, 42%) as pale yellow solids. Analytical data of the *β*-glycoside **14**: TLC: R_f = 0.5 (SiO_2, *n*-hexane/EtOAc 1:1.5). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 0.69 (s, 3H), 0.75–0.87 (m, 10H), 0.87–0.95 (m, 2H), 0.98 (s, 3H), 1.02 (d, *J* = 6.6 Hz, 3H), 1.03–1.12 (m, 1H), 1.09–1.32 (m, 6H), 1.37–1.59 (m, 10H), 1.61–1.78 (m, 1H), 1.77–1.92 (m, 2H), 1.98 (s, 3H), 1.98 (s, 3H), 1.99 (s, 3H), 2.01 (s, 3H), 2.02 (s, 3H), 2.03 (s, 3H), 2.03 (s, 3H), 2.09 (s, 3H), 2.11 (s, 3H), 2.14 (s, 3H), 2.16–2.25 (m, 2H), 3.36–3.51 (m, 1H), 3.52–3.68 (m, 3H), 3.75 (td, *J* = 9.4, 6.6 Hz, 2H), 3.98–4.17 (m, 3H), 4.29–4.46 (m, 2H), 4.44–4.57 (m, 4H), 4.80–4.96 (m, 3H), 4.97–5.21 (m, 6H), 5.34 (d, *J* = 5.0 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 12.0, 12.2, 20.5, 20.5, 20.5, 20.6, 20.7, 20.7, 20.8, 20.9, 21.0, 21.1, 21.2, 24.3, 25.4, 25.9, 28.9, 29.4, 29.7, 30.2, 31.8, 31.9, 31.9, 33.5, 36.7, 37.2, 38.9, 39.6, 40.5, 42.2, 50.2, 51.2, 56.0, 56.8, 61.5, 61.9, 61.9, 62.2, 67.7, 71.6, 71.6, 71.8, 72.0, 72.5, 72.6, 72.7, 72.9, 76.2, 76.6, 77.2, 80.1, 99.5, 100.5, 100.8, 122.1, 129.3, 140.3, 169.1, 169.3, 169.5, 169.7, 169.8, 170.2, 170.3, 170.5. IR (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 2,955, 2,936, 2,869, 1,743, 1,436, 1,367, 1,216, 1,216, 1,034, 904, 600. HRMS (ESI) *m*/*z* calcd. for $C_{67}H_{98}O_{26}Na^+$ $[M+Na]^+$ 1341.6239; found 1341.6237. Analytical data of the *α*-glycoside **17**: TLC: R_f = 0.3 (SiO_2, *n*-hexane/EtOAc 1:1.5). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 0.70 (s, 3H), 0.75–0.89 (m, 9H), 0.86–0.97 (m, 1H), 0.97–1.06 (m, 8H), 1.08–1.33 (m, 5H), 1.33–1.61 (m, 11H), 1.62–1.81 (m, 1H), 1.80–1.93 (m, 2H), 1.90–2.00 (m, 1H), 1.98 (s, 3H), 1.97–2.05 (m, 7H), 2.03 (s, 3H), 2.04 (s, 3H), 2.06 (s, 3H), 2.09 (s, 3H), 2.11 (s, 3H), 2.14 (s, 3H), 2.31–2.40 (m, 2H) 3.39–3.54 (m, 2H), 3.53–3.69 (m, 3H), 3.80 (t, *J* = 9.4 Hz, 1H), 3.91–4.03 (m, 1H), 4.04 (dd, *J* = 13.0, 2.6 Hz, 1H), 4.08–4.18 (m, 2H), 4.30–4.49 (m, 3H), 4.49 (d, *J* = 7.8 Hz, 2H), 4.81–5.01 (m, 3H), 5.00–5.21 (m, 6H), 5.35 (d, *J* = 5.0 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 12.3, 12.5, 19.3, 19.6, 20.8, 20.8, 20.8, 20.9, 21.0, 21.1, 21.2, 21.2, 21.2, 21.3, 21.4, 21.5, 24.6, 25.7, 28.4, 29.2, 32.1, 32.2, 32.2, 36.9, 37.3, 39.9, 40.4, 40.8, 42.5, 50.4, 51.5, 56.2, 57.1, 61.8, 62.5, 62.5, 68.0, 68.8, 71.9, 72.2, 72.3, 72.9, 73.1, 73.2, 73.5, 76.4, 77.5, 79.6, 97.3, 101.0, 101.1,

122.6, 129.6, 138.6, 140.5, 169.4, 169.6, 169.7, 170.1, 170.5, 170.5, 170.6, 170.8, 170.9. IR (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 3,478, 2,956, 2,871, 1,741, 1,435, 1,367, 1,217, 1,032, 903, 599. HRMS (ESI): *m*/*z* calcd. for $C_{65}H_{96}O_{25}Na^+$ $[M+Na]^+$ 1299.6133; found 1299.6130.

Stigmasteryl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-D-glucopyranosides 15 and 18

Glycosylation was performed according to the general procedure using compound **11** (220 mg, 0.162 mmol, 1.00 equiv), stigmasterol (134 mg, 0.324 mmol, 2.00 equiv), and TMSOTf (5.40 mg, 4.30 µL, 0.0243 mmol, 15.0 mol%) in CH_2Cl_2 (16 mL). Purification by column chromatography (SiO_2, *n*-pentane/EtOAc 2:1 → 1:1.5) afforded β-glycoside **15** (72.1 mg, 0.0448 mmol, 28%) and 2-deacetylated α-glycoside **18** (124 mg, 0.0792 mmol, 49%) as pale yellow solids. Analytical data of the β-glycoside **15**: TLC: R_f = 0.4 (SiO_2, *n*-hexane/EtOAc 1:1.5). ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) = 0.69 (s, 3H), 0.77–0.82 (m, 6H), 0.84 (d, *J* = 6.3 Hz, 3H), 0.85–0.96 (m, 2H), 0.97 (s, 3H), 0.97–1.04 (m, 4H), 1.02–1.11 (m, 1H), 1.10–1.22 (m, 3H), 1.22–1.35 (m, 4H), 1.37–1.60 (m, 9H), 1.65–1.75 (m, 1H), 1.80–1.90 (m, 2H), 1.95 (s, 3H), 1.97 (s, 3H), 1.98 (s, 3H), 1.99 (s, 3H), 2.01 (s, 3H), 2.01 (s, 3H), 2.02 (s, 3H), 2.03 (s, 3H), 2.03 (s, 3H), 2.09 (s, 3H), 2.11 (s, 3H), 2.14 (s, 3H), 2.14 (s, 3H), 2.16–2.28 (m, 2H), 3.39–3.48 (m, 1H), 3.53–3.60 (m, 3H), 3.63 (ddd, *J* = 9.9, 4.4, 2.3 Hz, 1H), 3.70–3.78 (m, 3H), 4.03 (dd, *J* = 12.5, 2.2 Hz, 1H), 4.05–4.12 (m, 3H), 4.35 (dd, *J* = 12.5, 4.3 Hz, 1H), 4.40 (dd, *J* = 12.2, 2.0 Hz, 2H), 4.42–4.50 (m, 4H), 4.53 (d, *J* = 8.0 Hz, 1H), 4.80–4.88 (m, 3H), 4.90 (dd, *J* = 9.3, 7.9 Hz, 1H), 4.98–5.18 (m, 7H), 5.34 (d, *J* = 5.5 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.0, 12.2, 18.9, 19.3, 20.4, 20.4, 20.5, 20.5, 20.5, 20.5, 20.6, 20.7, 20.7, 20.9, 21.0, 21.0, 21.2, 24.3, 25.4, 28.9, 29.4, 29.6, 31.8, 31.8, 31.8, 36.6, 37.1, 38.8, 39.6, 40.4, 42.1, 50.1, 51.2, 55.9, 56.8, 61.4, 61.9, 62.0, 62.1, 67.6, 71.5, 71.6, 71.7, 71.8, 72.0, 72.4, 72.5, 72.5, 72.6, 72.6, 72.7, 72.8, 76.0, 76.2, 76.5, 80.0, 99.4, 100.4, 100.5, 100.7, 122.1, 129.2, 138.2, 140.2, 169.0, 169.2, 169.2, 169.3, 169.5, 169.7, 169.8, 170.1, 170.1, 170.3, 170.4. IR (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 2,957, 2,936, 2,869, 1,739, 1,435, 1,369, 1,215, 1,034, 903, 601. HRMS (ESI): *m*/*z* calcd. for $C_{79}H_{114}O_{34}Na^+$ $[M+Na]^+$ 1629.7084; found 1629.7084. Analytical data of the α-glycoside **18**: TLC: R_f = 0.2 (SiO_2, *n*-hexane/EtOAc 1:1.5). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 0.70 (s, 3H), 0.77–0.83 (m, 6H), 0.84 (d, *J* = 6.4 Hz, 3H), 0.88–0.96 (m, 2H), 0.97–1.11 (m, 9H), 1.11–1.22 (m, 3H), 1.21–1.31 (m, 2H), 1.36–1.59 (m, 9H), 1.65–1.75 (m, 2H), 1.82–1.91 (m, 2H), 1.96 (s, 3H), 1.98 (s, 3H), 1.99 (s, 3H), 2.01 (s, 3H), 2.02 (s, 3H), 2.04 (s, 6H), 2.05 (s, 3H), 2.09 (s, 3H), 2.11 (s, 3H), 2.14 (s, 3H), 2.15 (s, 3H), 2.28–2.42 (m, 2H), 3.40–3.49 (m, 1H), 3.51 (ddd, *J* = 11.8, 9.8, 4.0 Hz, 1H), 3.53–3.62 (m, 3H), 3.63 (ddd, *J* = 9.9, 4.3, 2.3 Hz, 1H), 3.72–3.81 (m, 2H), 3.97 (ddd, *J* = 10.0, 5.3, 2.1 Hz, 1H), 4.03 (dd, *J* = 12.5, 2.3 Hz, 1H), 4.06–4.13 (m, 3H), 4.36 (dd, *J* = 12.5, 4.4 Hz, 1H), 4.37–4.45 (m, 3H), 4.45 (d, *J* = 7.8 Hz, 1H), 4.47 (dd, *J* = 7.9, 1.1 Hz, 2H), 4.80–4.85 (m, 1H), 4.83–4.86 (m, 1H), 4.90 (dd, *J* = 9.3, 7.9 Hz, 1H), 4.97 (d, *J* = 4.0 Hz, 1H), 4.99–5.19 (m, 8H),

5.35 (d, $J = 5.7$ Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) = 12.0, 12.2, 18.9, 19.3, 20.4, 20.5, 20.5, 20.5, 20.6, 20.6, 20.6, 20.8, 20.8, 20.8, 20.9, 21.0, 21.1, 21.2, 24.3, 25.4, 28.1, 28.9, 31.8, 31.8, 31.9, 36.6, 36.9, 39.6, 40.0, 40.5, 42.2, 50.1, 51.2, 55.9, 56.8, 61.4, 62.0, 62.1, 62.1, 67.7, 68.5, 71.0, 71.5, 71.7, 71.9, 72.0, 72.6, 72.6, 72.7, 72.7, 72.8, 73.1, 76.0, 76.1, 76.7, 79.3, 96.9, 100.5, 100.6, 100.8, 122.3, 129.2, 138.3, 140.2, 169.1, 169.3, 169.3, 169.4, 169.7, 169.7, 170.1, 170.2, 170.2, 170.4, 170.5, 170.7. IR (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 3,450, 2,956, 2,870, 1,741, 1,435, 1,367, 1,214, 1,031, 903, 600. HRMS (ESI): m/z calcd. for $C_{77}H_{112}O_{33}Na^+$ $[M+Na]^+$ 1587.6978; found 1587.6977.

Stigmasteryl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (23)

Deprotection of **13** (171 mg, 0.166 mmol, 1.00 equiv) was performed according to the general procedure in MeOH (12.5 mL) and CH_2Cl_2 (4.2 mL). Dialysis for 5 d and lyophilization afforded the desired product **23** (110 mg, 0.149 mmol, 90%) as a white powder. ^{1}H NMR (600 MHz, DMSO-d_6): δ = 0.67 (s, 3H), 0.74–0.80 (m, 6H), 0.82 (d, $J = 6.3$ Hz, 3H), 0.85–0.93 (m, 1H), 0.96 (s, 3H), 1.00 (d, $J = 6.6$ Hz, 6H), 1.09–1.20 (m, 3H), 1.20–1.28 (m, 1H), 1.34–1.45 (m, 3H), 1.46–1.57 (m, 6H), 1.59–1.70 (m, 1H), 1.76–1.85 (m, 2H), 1.88–1.97 (m, 2H), 1.98–2.06 (m, 1H), 2.13 (t, $J = 11.8$ Hz, 1H), 2.37 (ddd, $J = 13.6$, 5.0, 2.0 Hz, 1H), 2.93–3.02 (m, 2H), 3.05 (td, $J = 9.2$, 5.4 Hz, 1H), 3.14 (dd, $J = 8.9$, 5.0 Hz, 1H), 3.17–3.22 (m, 1H), 3.23–3.33 (m, 3H), 3.40 (dt, $J = 12.0$, 6.3 Hz, 1H), 3.41–3.50 (m, 1H), 3.55–3.62 (m, 1H), 3.66–3.75 (m, 2H), 4.25 (d, $J = 7.9$ Hz, 1H), 4.29 (d, $J = 7.8$ Hz, 1H), 4.53 (t, $J = 6.0$ Hz, 1H), 4.60 (dd, $J = 5.9$, 4.9, 1H), 4.66 (d, $J = 1.7$ Hz, 1H), 4.99 (d, $J = 5.6$ Hz, 1H), 5.01–5.05 (m, 3H), 5.15 (dd, $J = 15.1$, 8.7 Hz, 1H), 5.24 (d, $J = 4.9$ Hz, 1H), 5.30–5.35 (m, 1H). ^{13}C NMR (151 MHz, DMSO-d_6): δ = 11.8, 12.1, 18.9, 19.1, 20.6, 21.0, 21.1, 23.9, 24.9, 28.5, 29.2, 31.4, 31.4, 31.4, 36.2, 36.8, 38.3, 40.0, 40.0, 41.7, 49.6, 50.6, 55.3, 56.3, 60.5, 61.0, 70.0, 73.1, 73.3, 74.7, 75.0, 76.4, 76.8, 77.2, 80.7, 100.6, 103.2, 121.2, 128.8, 138.0, 140.4. IR (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 3,379, 3,934, 2,901, 2,868, 1,643, 1,460, 1,367, 1,022. Optical rotation: $[\alpha]_D^{25} = -41.1°$ (c 0.98, DMSO). HRMS (ESI): m/z calcd. for $C_{41}H_{68}O_{11}Na^+$ $[M+Na]^+$ 759.4654; found 759.4653.

Stigmasteryl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (24)

Deprotection of **14** (80.0 mg, 0.0606 mmol, 1.00 equiv) was performed according to the general procedure in MeOH (4.5 mL) and CH_2Cl_2 (1.5 mL). Dialysis for 5 d and lyophilization afforded the desired product **24** (50.3 mg, 0.0559 mmol, 92%) as a light grey powder. ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 0.67 (s, 3H), 0.74–0.80 (m, 6H), 0.82 (d, $J = 6.2$ Hz, 3H), 0.89 (td, $J = 11.3$, 4.5 Hz, 2H), 0.96 (s, 3H), 1.00 (d, $J = 6.5$ Hz, 6H), 1.08–1.20 (m, 3H), 1.20–1.29 (m, 2H), 1.34–1.57 (m, 10H), 1.58–1.69 (m, 1H), 1.75–1.86 (m, 2H), 1.86–1.99 (m, 2H), 1.98–2.08 (m, 1H), 2.13 (t, $J = 11.6$ Hz, 1H), 2.33–2.41 (m, 1H), 2.93–3.02 (m, 2H),

3.01–3.10 (m, 2H), 3.12–3.18 (m, 1H), 3.18–3.22 (m, 1H), 3.26–3.33 (m, 3H), 3.37–3.43 (m, 1H), 3.42–3.50 (m, 1H), 3.52–3.62 (m, 2H), 3.67–3.75 (m, 2H), 3.78 (dd, $J = 9.4$, 4.7 Hz, 1H), 4.24 (d, $J = 7.9$ Hz, 1H), 4.29 (d, $J = 7.8$ Hz, 1H), 4.32 (d, $J = 7.9$ Hz, 1H), 4.54 (t, $J = 6.0$ Hz, 1H), 4.57–4.63 (m, 2H), 4.67 (t, $J = 5.6$ Hz, 1H), 4.74 (d, $J = 1.8$ Hz, 1H), 4.97–5.06 (m, 4H), 5.15 (dd, $J = 15.1$, 8.7 Hz, 1H), 5.24 (d, $J = 5.0$ Hz, 1H), 5.32 (s, 1H), 5.41 (d, $J = 4.9$ Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6): δ (ppm) = 11.8, 12.1, 18.9, 19.1, 20.6, 21.0, 21.1, 23.9, 24.9, 28.5, 29.2, 31.3, 31.4, 36.2, 36.8, 38.3, 40.0, 40.0, 41.7, 49.6, 50.6, 55.3, 56.3, 60.3, 60.4, 61.0, 70.0, 73.0, 73.1, 73.2, 74.7, 74.8, 75.0, 76.4, 76.8, 77.2, 80.4, 80.6, 100.6, 102.8, 103.2, 121.2, 128.8, 138.0, 140.4. IR (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 3,373, 2,933, 2,869, 1,644, 1,459, 1,368, 1,159, 1,022, 898, 564. Optical rotation: $[\alpha]_D^{25} = -36.9°$ (c 0.94, DMSO). HRMS (ESI): m/z calcd. for $C_{47}H_{78}O_{16}Na^+$ $[M+Na]^+$ 921.5182; found 921.5185.

Stigmasteryl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (25)

Deprotection of **15** (72.0 mg, 0.0448 mmol, 1.00 equiv) was performed according to the general procedure in MeOH (3.5 mL) and CH_2Cl_2 (1.1 mL). Dialysis for 3 d and lyophilization afforded the desired product **25** (43.1 mg, 0.0406 mmol, 91%) as a white powder. ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 0.67 (s, 3H), 0.73–0.80 (m, 6H), 0.82 (d, $J = 6.2$ Hz, 3H), 0.85–0.93 (m, 1H), 0.93–1.08 (m, 9H), 1.09–1.20 (m, 3H), 1.20–1.30 (m, 3H), 1.34–1.45 (m, 3H), 1.45–1.58 (m, 5H), 1.59–1.71 (m, 1H), 1.75–1.87 (m, 2H), 1.87–1.99 (m, 2H), 1.98–2.07 (m, 1H), 2.09–2.18 (m, 1H), 2.37 (dd, $J = 13.4$, 4.5 Hz, 1H), 2.93–3.02 (m, 2H), 3.02–3.11 (m, 3H), 3.15 (t, $J = 8.9$, 1H), 3.17–3.23 (m, 1H), 3.23–3.35 (m, 7H), 3.39–3.54 (m, 3H), 3.53–3.64 (m, 3H), 3.67–3.76 (m, 2H), 3.79 (d, $J = 11.1$ Hz, 2H), 4.24 (d, $J = 7.8$ Hz, 1H), 4.29 (d, $J = 7.8$ Hz, 1H), 4.32 (dd, $J = 7.8$, 3.9 Hz, 2H), 4.55 (t, $J = 6.0$ Hz, 1H), 4.58–4.65 (m, 2H), 4.66–4.74 (m, 3H), 4.78 (s, 1H), 4.95–5.10 (m, 3H), 5.10–5.21 (m, 2H), 5.28 (s, 1H), 5.30–5.35 (m, 1H), 5.43 (d, $J = 4.9$ Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6): δ (ppm) = 11.9, 12.1, 18.9, 19.1, 20.6, 21.0, 21.1, 23.9, 24.9, 28.5, 29.2, 31.4, 31.4, 36.2, 36.8, 38.3, 40.0, 40.0, 41.7, 49.6, 50.6, 55.3, 56.3, 60.3, 60.3, 60.4, 61.0, 70.0, 73.0, 73.0, 73.1, 73.2, 74.7, 74.7, 74.8, 75.0, 76.4, 76.8, 77.2, 80.3, 80.4, 80.6, 100.6, 102.8, 103.2, 121.2, 128.8, 138.0, 140.4. IR (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 3,361, 2,931, 2,891, 2,869, 1,663, 1,446, 1,368, 1,159, 1,022, 900. Optical rotation $[\alpha]_D^{25} = -40.5°$ (c 0.80, DMSO). HRMS (ESI): m/z calcd. for $C_{53}H_{88}O_{21}Na^+$ $[M+Na]^+$ 1083.5710: found 1083.5712.

Stigmasteryl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-α-D-glucopyranoside (26)

Deprotection of **16** (75.0 mg, 0.0727 mmol, 1.00 equiv) was performed according to the general procedure in MeOH (5.4 mL) and CH_2Cl_2 (1.8 mL). Dialysis for 4

d and lyophilization afforded the desired product **26** (42.7 mg, 0.0579 mmol, 80%) as a white powder. ^{1}H NMR (600 MHz, DMSO-d_6): δ = 0.67 (s, 3H), 0.74–0.80 (m, 6H), 0.82 (d, J = 6.1 Hz, 3H), 0.86–0.93 (m, 1H), 0.96 (s, 3H), 1.00 (d, J = 6.6 Hz, 6H), 1.09–1.20 (m, 3H), 1.20–1.29 (m, 2H), 1.33–1.45 (m, 4H), 1.45–1.58 (m, 5H), 1.59–1.70 (m, 1H), 1.76–1.99 (m, 4H), 1.98–2.08 (m, 1H), 2.20–2.28 (m, 1H), 2.27–2.35 (m, 1H), 2.95–3.03 (m, 1H), 3.03–3.10 (m, 1H), 3.11–3.24 (m, 3H), 3.27–3.33 (m, 1H), 3.41 (dt, J = 11.9, 6.2 Hz, 1H), 3.51 (td, J = 9.1, 8.7, 2.1 Hz, 1H), 3.59 (dd, J = 9.7, 3.5 Hz, 1H), 3.61–3.67 (m, 2H), 3.67–3.73 (m, 1H), 4.22 (d, J = 7.9 Hz, 1H), 4.49 (d, J = 2.4 Hz, 1H), 4.55 (t, J = 6.1 Hz, 1H), 4.58 (t, J = 5.4 Hz, 1H), 4.70 (d, J = 6.6 Hz, 1H), 4.78 (d, J = 3.7 Hz, 1H), 4.99 (d, J = 5.5 Hz, 1H), 5.02 (d, J = 4.9 Hz, 2H), 5.15 (dd, J = 15.1, 8.7 Hz, 1H), 5.21 (d, J = 4.8 Hz, 1H), 5.27–5.33 (m, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ = 11.9, 12.1, 18.9, 19.1, 20.6, 21.0, 21.1, 23.9, 24.9, 27.3, 28.5, 31.4, 31.4, 36.2, 36.6, 40.0, 40.0, 41.7, 49.6, 50.6, 55.4, 56.3, 60.2, 61.0, 70.0, 70.7, 71.5, 71.5, 73.3, 76.4, 76.5, 76.5, 76.5, 76.8, 80.6, 96.6, 103.1, 121.1, 128.8, 138.0, 140.6. IR (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 3,404, 2,932, 2,868, 1,460, 1,369, 1,154, 1,023, 774. Optical rotation: $[\alpha]_D^{25} = 33.9°$(c 1.00, DMSO). HRMS (ESI): m/z calcd. for $C_{41}H_{68}O_{11}Na^+$ $[M+Na]^+$ 759.4654; found 759. 4653.

Stigmasteryl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-α-D-glucopyranoside (27)

Deprotection of **17** (100 mg, 0.0783 mmol, 1.00 equiv) was performed according to the general procedure in MeOH (5.8 mL) and CH_2Cl_2 (2.0 mL). Dialysis for 5 d and lyophilization afforded the desired product **27** (61.3 mg, 0.0682 mmol, 87%) as a white powder. ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 0.67 (s, 3H), 0.73–0.81 (m, 6H), 0.82 (d, J = 6.3 Hz, 3H), 0.85–0.93 (m, 1H), 0.93–1.08 (m, 9H), 1.08–1.20 (m, 3H), 1.19–1.28 (m, 2H), 1.34–1.46 (m, 4H), 1.46–1.57 (m, 5H), 1.59–1.70 (m, 1H), 1.77–1.98 (m, 4H), 2.02 (dd, J = 15.5, 9.1 Hz, 1H), 2.21–2.28 (m, 1H), 2.27–2.34 (m, 1H), 2.96–3.02 (m, 1H), 3.01–3.10 (m, 2H), 3.15 (td, J = 8.8, 5.0 Hz, 1H), 3.18–3.24 (m, 2H), 3.28–3.34 (m, 3H), 3.38–3.43 (m, 2H), 3.52 (td, J = 8.8, 1.9 Hz, 1H), 3.55–3.62 (m, 2H), 3.61–3.67 (m, 2H), 3.67–3.73 (m, 1H), 3.75–3.82 (m, 1H), 4.24 (d, J = 7.9 Hz, 1H), 4.29 (d, J = 7.9 Hz, 1H), 4.42 (d, J = 2.1 Hz, 1H), 4.55 (t, J = 6.0 Hz, 1H), 4.60 (t, J = 5.4 Hz, 1H), 4.66 (t, J = 5.5 Hz, 1H), 4.70 (d, J = 6.7 Hz, 1H), 4.74 (d, J = 1.6 Hz, 1H), 4.78 (d, J = 3.8 Hz, 1H), 4.97–5.06 (m, 3H), 5.15 (dd, J = 15.1, 8.7 Hz, 1H), 5.24 (d, J = 4.9 Hz, 1H), 5.30 (s, 1H), 5.37 (d, J = 4.9 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ (ppm) = 11.9, 12.1, 18.9, 19.1, 20.6, 21.0, 21.1, 23.9, 24.9, 27.3, 28.5, 31.4, 31.4, 36.2, 36.6, 40.0, 40.0, 41.7, 49.6, 50.6, 55.3, 56.3, 60.1, 60.3, 61.0, 70.0, 70.7, 71.5, 71.5, 73.0, 73.2, 74.7, 74.8, 76.5, 76.8, 80.4, 80.6, 96.6, 102.7, 103.2, 121.1, 128.8, 138.0, 140.6. IR (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 3,386, 2,933, 2,869, 1,460, 1,369, 1,161, 1,021, 900, 563. Optical rotation:$[\alpha]_D^{25} = 17.9°$ (c 1.25, DMSO). HRMS (ESI): m/z calcd. for $C_{47}H_{78}O_{16}Na^+$ $[M+Na]^+$ 921.5182; found 921. 5182.

Stigmasteryl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-α-D-glucopyranoside (28)

Deprotection of **18** (124 mg, 0.0792 mmol, 1.00 equiv) was performed according to the general procedure in MeOH (6.0 mL) and CH_2Cl_2 (3.0 mL). Dialysis for 3 d and lyophilization afforded the desired product **28** (61.0 mg, 0.0575 mmol, 73%) as a white powder. ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 0.67 (s, 3H), 0.74–0.80 (m, 6H), 0.82 (d, J = 6.2 Hz, 3H), 0.85–0.93 (m, 1H), 0.93–1.06 (m, 9H), 1.09–1.20 (m, 3H), 1.20–1.30 (m, 2H), 1.33–1.45 (m, 4H), 1.45–1.58 (m, 5H), 1.59–1.71 (m, 1H), 1.75–2.00 (m, 4H), 1.99–2.08 (m, 1H), 2.24 (t, J = 11.6 Hz, 1H), 2.27–2.40 (m, 1H), 2.94–3.02 (m, 1H), 3.02–3.11 (m, 3H), 3.12–3.18 (m, 1H), 3.17–3.24 (m, 2H), 3.27–3.35 (m, 5H), 3.36–3.45 (m, 3H), 3.49–3.56 (m, 1H), 3.55–3.62 (m, 3H), 3.61–3.67 (m, 2H), 3.67–3.73 (m, 1H), 3.79 (dd, J = 10.1, 4.8 Hz, 2H), 4.24 (d, J = 7.9 Hz, 1H), 4.29 (d, J = 7.8 Hz, 1H), 4.32 (d, J = 7.9 Hz, 1H), 4.43 (d, J = 2.2 Hz, 1H), 4.55 (t, J = 6.0 Hz, 1H), 4.60 (t, J = 5.3 Hz, 1H), 4.63–4.69 (m, 3H), 4.70 (d, J = 6.7 Hz, 1H), 4.75 (d, J = 1.7 Hz, 1H), 4.78 (d, J = 3.7 Hz, 1H), 4.97–5.07 (m, 3H), 5.15 (dd, J = 15.1, 8.7 Hz, 1H), 5.24 (d, J = 5.0 Hz, 1H), 5.30 (s, 1H), 5.37 (d, J = 4.9 Hz, 1H), 5.41 (d, J = 5.1 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6): δ (ppm) = 11.9, 12.1, 18.9, 19.1, 20.6, 21.0, 21.1, 23.9, 24.9, 27.3, 28.5, 31.4, 31.4, 36.2, 36.6, 40.0, 41.7, 49.6, 50.6, 55.3, 56.3, 60.2, 60.3, 60.3, 61.0, 70.0, 70.7, 71.5, 71.5, 73.0, 73.0, 73.2, 74.7, 74.8, 76.5, 76.8, 80.3, 80.4, 80.6, 96.6, 102.8, 102.8, 103.2, 121.1, 128.8, 138.0, 140.6. IR (ATR): IR (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 3,389, 2,932, 2,904, 2,869, 1,457, 1,371, 1,160, 1,021, 900, 555. Optical rotation $[\alpha]_D^{25}$ = 31.7° (c 0.80, DMSO). HRMS (ESI): m/z calcd. for $C_{53}H_{88}O_{21}Na^+$ $[M+Na]^+$ 1083.5716; found 1083.5727.

Stigmasteryl acetate 22a

TLC: R_f = 0.8 (SiO_2, n-hexane/EtOAc 1:1.5). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 0.70 (s, 3H), 0.75–0.90 (m, 9H), 0.98–1.07 (m, 8H), 1.10–1.30 (m, 6H), 1.34–1.47 (m, 2H), 1.45–1.61 (m, 7H), 1.63–1.76 (m, 1H), 1.79–1.92 (m, 2H), 1.91–2.11 (m, 6H), 2.27–2.37 (m, 2H), 4.52–4.69 (m, 1H), 5.01 (dd, J = 15.2, 8.5 Hz, 1H), 5.16 (dd, J = 15.2, 8.4 Hz, 1H), 5.34–5.42 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 12.0, 12.3, 19.0, 19.3, 21.0, 21.1, 21.2, 21.4, 24.4, 25.4, 27.8, 28.9, 31.9, 31.9, 36.6, 37.0, 38.1, 39.6, 40.5, 42.2, 50.1, 51.2, 55.9, 56.8, 74.0, 77.2, 122.6, 129.3, 138.3, 139.6, 170.5. HRMS (ESI): m/z calcd. for $C_{31}H_{50}O_2Na^+$ $[M+Na]^+$ 477.3703; found 477.3705.

References

1. (a) Shi, J.; Arunasalam, K.; Yeung, D.; Kakuda, Y.; Mittal, G.; Jiang, Y. Saponins from edible legumes: chemistry, processing, and health benefits. *J. Med. Food* **2004**, *7*, 67–78; (b) Faizal, A.; Geelen, D. Saponins and their role in biological processes in plants. *Phytochem. Rev.* **2013**, *12*, 877–893; (c) Hostettmann, K.; Marston, A. *Saponins*, Cambridge Univ. Press: Cambridge,

1995; (d) Sparg, S.G.; Light, M.E.; van Staden, J. Biological activities and distribution of plant saponins. *J. Ethnopharmacol.* **2004**, *94*, 219–243.

2. Lorent, J.H.; Quetin-Leclercq, J.; Mingeot-Leclercq, M.-P. The amphiphilic nature of saponins and their effects on artificial and biological membranes and potential consequences for red blood and cancer cells. *Org. Biomol. Chem.* **2014**, *12*, 8803–8822.
3. Gu, G.; Du, Y.; Linhardt, R.J. Facile synthesis of saponins containing 2,3-branched oligosaccharides by using partially protected glycosyl donors. *J. Org. Chem.* **2004**, *69*, 5497–5500.
4. Tang, P.; Yu, B. Total synthesis of candicanoside A, a potent antitumor saponin with a rearranged steroid side chain. *Angew. Chem. Int. Ed.* **2007**, *46*, 2527–2530.
5. (a) Fujino, Y.; Ohnishi, M. Novel sterylglycosides: cellotetraosyl sitosterol and cellopentaosyl sitosterol in rice grain. *Proc. Japan Acad. Ser. B* **1979**, *55*, 243–246; (b) Kojima, M.; Ohnishi, M.; Ito, S.; Fujino, Y. Characterization of acylmono-, mono-, di-, tri- and tetraglycosylsterol and saponin in Adzuki bean (Vigna angularis) seeds. *Lipids* **1989**, *24*, 849–853; (c) Nyström, L. *Personal Communication*, 2015.
6. (a) Yu, B.; Zhang, Y.; Tang, P. Carbohydrate chemistry in the total synthesis of saponins. *Eur. J. Org. Chem.* **2007**, 5145–5161; (b) Cmoch, P.; Korda, A.; Rárová, L.; Oklešťková, J.; Strnad, M.; Gwardiak, K.; Karczewski, R.; Pakulski, Z. Synthesis of lupane-type saponins containing an unusual α-D-idopyranoside fragment as potent cytotoxic agents. *Eur. J. Org. Chem.* **2014**, 4089–4098; (c) Schimmel, J.; Passos Eleutério, M.I.; Ritter, G.; Schmidt, R.R. Synthesis of saponins with cholestanol, cholesterol, and friedelanol as aglycones. *Eur. J. Org. Chem.* **2006**, 1701–1721; (d) Du, Y.; Gu, G.; Wei, G.; Hua, Y.; Linhardt, R.J. Synthesis of saponins using partially protected glycosyl donors. *Org. Lett.* **2003**, *5*, 3627–3630; (e) Li, C.-X.; Guo, T.-T.; Wang, P.; Guan, H.-S.; Li, Y.-X. Semi-synthesis of several stigmasterol saponins. *Chin. J. Chem.* **2006**, *24*, 917–922.
7. Kale, R.R.; Clancy, C.M.; Vermillion, R.M.; Johnson, E.A.; Iyer, S.S. Synthesis of soluble multivalent glycoconjugates that target the Hc region of botulinum neurotoxin A. *Bioorg. Med. Chem. Lett*. **2007**, *17*, 2459–2464.
8. Schmidt, R.R.; Michel, J. Facile Synthesis of α- and β-*O*-glycosyl imidates; preparation of glycosides and disaccharides. *Angew. Chem. Int. Ed. Engl.* **1980**, *19*, 731–732.
9. (a) Schütte, O.M.; Ries, A.; Orth, A.; Patalag, L.J.; Römer, W.; Steinem, C.; Werz, D.B. Influence of Gb_3 glycosphingolipids differing in their fatty acid chain on the phase behaviour of solid supported membranes: chemical syntheses and impact of Shiga toxin binding. *Chem. Sci.* **2014**, *5*, 3104–3114; (b) Yao, Q.; Song, J.; Xia, C.; Zhang, W.; Wang, P.G. Chemoenzymatic syntheses of iGb_3 and Gb_3 *Org. Lett.* **2006**, *8*, 911–914.
10. Kuczynska, K.; Pakulski, Z. Synthesis of lupane saponins from acetylated glycosyl donors by acetonitrile directed glycosylation. *Tetrahedron* **2015**, *71*, 2900–2905.
11. Flugge, L.A.; Blank, J.T.; Petillo, P.A. Isolation, modification, and NMR assignments of a series of cellulose oligomers. *J. Am. Chem. Soc.* **1999**, *121*, 7228–7238.
12. Moynihan, H.A.; Hayes, J.A.; Eccles, K.S.; Coles, S.J.; Lawrence, S.E. Hydrogen bonding in crystal forms of primary amide functionalised glucose and cellobiose. *Carbohydr. Res.* **2013**, *374*, 29–39.