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A facile approach to diosgenin and furostan type saponins bearing a 3β-chacotriose moiety

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Dedicated to Professor Derek Horton on the occasion of his 70th birthday

Abstract

Combination of a one-pot coupling technique and the use of benzyl ethers as permanent protecting groups offered a short and simple route to dioscin-type saponins. This strategy in combination with a mild reductive opening procedure of the spiroketal function in diosgenin also offered a convenient approach to bidesmosidic furostan type saponins. $Me_3N{\cdot}BH_3/AlCl_3$ promoted acetal opening of 3-*O*-TBDMS-protected diosgenin gave the 26-OH acceptor **9** into which a benzylated β-glucose moiety was introduced by a S_N2-type imidate coupling. After cleavage of the silyl ether, the 3β-*O*-glucose and the 4-*O*-linked rhamnose of the chacotriose unit were introduced by a NIS/AgOTf-promoted one-pot coupling sequence utilising thioglycoside donors and their different reactivity in different solvents. After removal of a benzoyl group, the same coupling conditions were also used for the coupling of the second 2-*O*-linked rhamnose unit. The target substance was obtained after cleavage of the protecting benzyl ethers under Birch-type conditions, which did not affect the double bond in the steroid skeleton.

Keywords: Saponins; One-pot glycosylation; Reductive acetal opening; Birch-type reduction; Chacotriose; Diosgenin

1. Introduction

The large family of saponins has received considerable attention because of the variety of their promising pharmaceutical properties.[1,2] Since ancient times plant extracts containing saponins have been used in traditional Chinese medicine. Cholestan and spirostan saponins, 3-OH glycosylated steroids (Fig. 1), have been the best-studied representatives of this class of compounds. The discovery of the bidesmosidic furostan saponins (e.g., **1**, Fig. 1), which have an additional β-D-glucopyranosyl substituent at 26-OH, was delayed due to formation of the ring F under acidic isolation conditions, yielding spirostan saponins (e.g., dioscin **3**) as artefacts.[3] However, by screening saponin-rich plants for biologically active substances, new furostan saponins have been found.[4] Recently the isolation of a glycosylated furostan **1** and its cytotoxity against the HL-60 tumour cell line ($IC_{50} = 29$ mM) has been reported.[5] Therefore, dihydrodiosgenin **2**, a dioscin-related substance should be of interest as a mimetic of this labile compound.

Compelled by the heterogeneity of saponins in plants and the difficulties of isolation of homogeneous material, several synthetic routes of spirostan saponins have recently been presented, but only one synthesis of a furostan saponin has been reported.[6] The use of one-pot glycosylation techniques has reduced the number of synthetic steps; however, the protection group strategies are still often a problem (mainly orthogonal ester functions have been used).[7–10] Another drawback has been the rather harsh conditions used when opening the spiroketal function to produce the essential steroid acceptor.[11–14]

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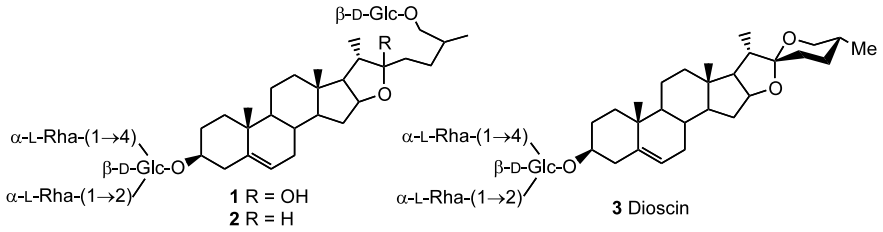


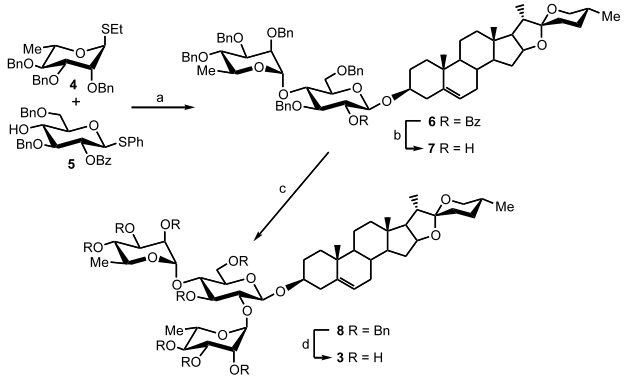
Figure 1. Target saponins **1**–**3**.

In a continuation of previous studies,[15–17] we present here a short and simple approach to dioscin **3** (Fig. 1), a diosgenyl glycoside presenting the main constituents of the saponin family with marked cytotoxic activity ($GI_{50} = 1.12–0.40$ μg/mL)[18] and the furostan saponin **2**. Furthermore, we describe a mild reductive opening of the spiroketal function allowing a wide range of protecting groups in the steroid moiety, giving easy access to the steroid acceptor **9** with differentiation of the 3β and the 26 position.

2. Results and discussion

A common strategy preparing spirostan saponins has been to construct the glycosidic bond between the steroid and a monosaccharide moiety first, before extending the sugar part by standard carbohydrate chemistry.[7–10,19] Still, the yields of the first coupling step have been low to moderate, probably due to the low reactivity of the (most often) used glycosyl donor, ethyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside.[7–10]

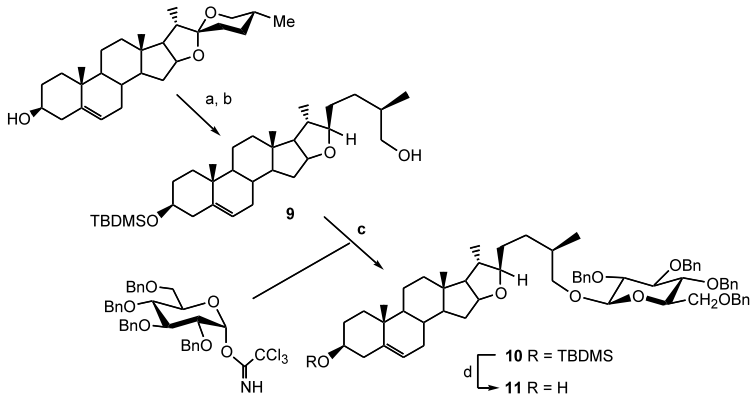
We decided to introduce the 4-*O*-substituent and the β-linkage to the steroid in a one-pot coupling sequence (Scheme 1). The main attraction of this approach was to perform subsequent couplings just by changing of the solvent system, using the difference in glycosylation rates between various solvents.[20] The thioethyl rhamnopyranosyl donor **4**[21] reacted in almost quantitative yield with the thiophenyl glucopyranosyl acceptor (**5**) promoted by NIS/AgOTf in Et_2O. The addition of diosgenin alone to the mixture, as well as together with an additional portion of promoter, did not lead to any further coupling. However, as soon as enough CH_2Cl_2 was present in the mixture, the next glycosylation occurred. The use of mainly benzyl ethers as protecting groups guaranteed an activated glycosyl donor, and, thus, a high yield in the coupling step with the steroid was obtained (→**6**, 85% overall yield). The 2-*O*-benzoyl group, which is essential for selective β-linkage formation, has been known to be difficult to remove if the 3-substituent was an 3-*O*-TBDMS group.[9] However, the use of NaOMe in CH_2Cl_2/MeOH at elevated temperature removed the benzoate in a satisfying yield (→**7**, 71%). The introduction of the second rhamnosyl residue with NIS/AgOTf in Et_2O proceeded smoothly and gave **8** in excellent yield (93%). As planned, the application of Birch-type conditions removed the benzyl protecting groups almost quantitatively (95%), but preserved the double bond in the steroid skeleton, which often is saturated under hydrogenolysis conditions. Thus, dioscin **3** was prepared starting from two, easily prepared monosaccharide units and commercially available diosgenin in a 55% overall yield over four steps.



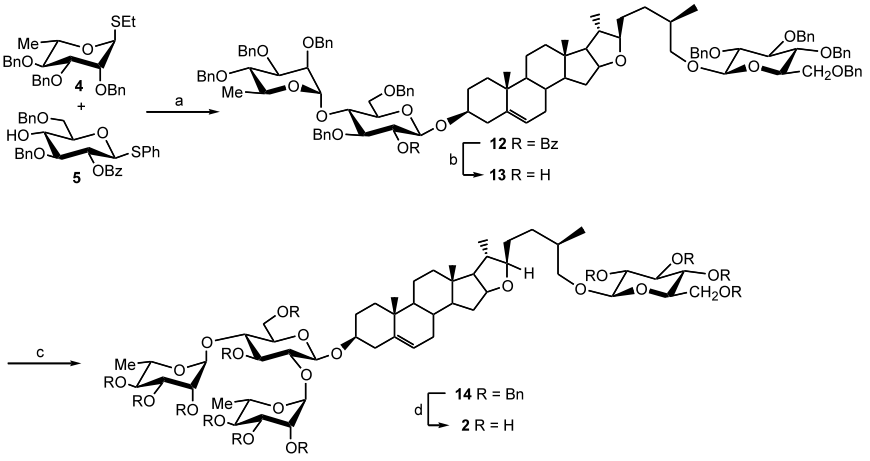
Scheme 1. (a) NIS–AgOTf–Et_2O, rt, 4 Å MS then diosgenin, CH_2Cl_2–Et_2O, 85%; (b) NaOMe, MeOH, CH_2Cl_2, 50 °C, 71%; (c) NIS–AgOTf–CH_2Cl_2, rt, 4 Å MS, 93%; (d) Na/NH_3 (l), 95%.

The combination of this one-pot coupling technique, benzyl protecting groups and a mild reductive opening procedure of the spiroketal function in diosgenin resulted in a short and simple route also to the corresponding furostan saponins. Diosgenin was silylated under standard conditions and transformed into the acceptor **9** in high yield (91%) using the $BH_3 \cdot Me_3N$ complex together with $AlCl_3$ (Scheme 2). This reaction proceeded without any loss of the silyl protecting group, which has been a known side reaction when conducting the reductive opening with either $LiAlH_4$/$AlCl_3$ or $NaCNBH_3$ in HOAc.

During the synthesis of dioscin we had already shown that the selection of the benzyl ether was an appropriate choice as a permanent protecting group despite the presence of the double bond in the steroid. Because no modification has to be done at the 26-*O*-glucoside, a perbenzylated donor was preferable to



Scheme 2. (a) TBDMSOTf, *sym*-collidine, 0 °C, 92%; (b) $BH_3{\cdot}NMe_3$, $AlCl_3$, $-70\,°C \rightarrow -10\,°C$, 91%; (c) $BF_3{\cdot}Et_2O$, CH_2Cl_2, $-30\,°C$, 91%; (d) TBAF, THF, rt, 96%.



Scheme 3. (a) NIS–AgOTf–Et_2O, rt, 4 Å MS, then **11**, DMTST, CH_2Cl_2–Et_2O, 65%; (b) NaOMe, MeOH, CH_2Cl_2, 50 °C, 90%; (c) NIS–AgOTf–CH_2Cl_2, rt, 4 Å MS, 93%; (d) Na/NH_3 (l), 97%.

simplify deprotection. The coupling was accomplished with the perbenzylated α-D-glucopyranosyl imidate[22] and aglycon **9** using a catalytic amount of $BF_3{\cdot}Et_2O$, which formed the desired β-linkage in high yield ($\rightarrow$**10**, 91%) without neighbouring group assistance. Removal of the TBDMS protecting group afforded acceptor **11** (96%), which was subjected to a procedure similar to that described for the preparation of dioscin (Scheme 3). The one-pot glycosylations furnished the saponin derivative **12** in 65% yield. Cleavage of the 2-*O*-benzoyl group ($\rightarrow$**13**, 90%), followed by NIS/AgOTf-promoted coupling with **4** as donor ($\rightarrow$**14**, 72%), and finally a one-step deprotection applying Birch-type conditions, gave the target compound **2** in 38% overall yield over eight steps.

In conclusion, using a combination of a one-pot coupling technique, a mild reductive opening procedure of the spiroketal function in diosgenin and benzyl groups as permanent protecting groups, an effective and high-yielding approach towards diosgenin and furostan type saponins has been developed.

3. Experimental

General methods.—All organic solvents were distilled before use, except Et_2O, which was stored over Na. Organic solutions were dried over $MgSO_4$ before concentration, which was performed under reduced pressure at < 40 °C (bath temperature). NMR spectra were recorded at 300 or 400 MHz (Varian) (^1H) or at 75 or 100 MHz (^{13}C), respectively, in $CDCl_3$ or CD_3OD. Me_4Si was used as the internal standard ($\delta = 0$) for ^1H-spectra. ^{13}C-spectra were referenced to the chloroform signal ($\delta = 77.17$). Silica Gel 60 (E. Merck) (0.040–0.063) was used for flash chromatography. TLC was performed on Silica Gel 60 F_{254} (E. Merck) plates with detection by UV-light and/or charring with 8% sulfuric acid. Column chromatography was performed on silica gel (Matrix Silica Si 60A, 35–70m, Amicon). MALDI TOF mass spectra were recorded on a Bruker Biflex III using PEG 600, PEG 1000 and PEG 1500 as calibration reference and 2′,4′,6′-trihydroxyacetophenone monohydrate (THAP) as matrix.

Diosgenyl 3,6-di-O-*benzyl-2,4-di*-O-*(2,3,4-tri*-O-*benzyl-α*-L-*rhamnopyranosyl)-β*-D-*glucopyranoside* (**8**).—Phenyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-1-thio-β-D-glucopyranoside (**5**) was prepared from phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside,[23] which was benzoylated under standard conditions with benzoyl chloride in pyridine, followed by reductive opening of the 4,6-*O*-benzylidene acetal using $NaCNBH_3$ and HCl in Et_2O. ^{13}C NMR ($CDCl_3$): δ 70.46, 71.91, 72.24, 73.88, 74.86, 78.52, 83.78, 86.55, 127.79–130.23, 132.41, 133.03, 133.30, 133.66, 137.80, 165.19 (Ph*C*O). A solution of ethyl 2,3,4-tri-*O*-benzyl-1-thio-α-L-rhamnopyranoside (**4**,[21] 77 mg, 138 μmol) and phenyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-1-thio-β-D-glucopyranoside (**5**, 66 mg, 138 μmol) in dry Et_2O (2 mL) was stirred with powdered molecular sieves (4 Å) under argon for 1 h when NIS (40 mg, 0.18 mmol) and a catalytic amount AgOTf were added. TLC (18:1 toluene–EtOAc) showed complete conversion into the disaccharide after 45 min. Subsequently, diosgenin (57 mg, 137 μmol), NIS (40 mg, 0.18 mmol) and a catalytic amount of AgOTf or DMTST (dimethyl(methylthio)sulfonium triflate) (117 mg, 0.45 mmol) dissolved in CH_2Cl_2 (8 mL) containing molecular sieves were added. After 30 min the reaction was quenched by addition of Et_3N (50 μL) and the mixture was diluted with CH_2Cl_2 (20 mL), filtered through Celite and concentrated. The residue was applied onto a silica gel column and eluted (toluene (Et_3N) → 20:1 toluene–EtOAc) to give diosgenyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-4-*O*-(2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside (**6**, 150 mg, 117 μmol, 85%): ^1H NMR ($CDCl_3$): δ 0.78–2.20 (m, 39H), 3.30–3.95, 4.38–4.73 (m, 23H), 4.94 (d, 1 H, *J* 11 Hz, 5.09 (s, 1 H), 5.22 (sb, 1H), 5.28 (t, 1H, *J* 8 Hz), 7.00–7.60 (m, 28H), and 8.05 (d, 2H, *J* 9 Hz). ^{13}C NMR: δ 14.8, 16.5, 17.4, 18.1, 19.6, 21.0, 29.0, 29.8, 30.5, 31.6, 32.0, 32.3, 37.0, 37.4, 39.1, 40.0, 41.8, 50.3, 56.7, 62.3, 67.0, 69.0, 72.2, 72.6, 73.7, 74.3, 74.6, 74.9, 75.5, 75.6, 79.6, 79.8, 80.7, 80.9, 81.7, 98.7, 100.1 (C1, C1′), 109.3 (C^{dio}22), 121.5 (C^{dio}6), 125.3, 127.5–130.1, 133.0, 137.6, 138.2, 138.3, 138.6, 138.8 (Ar C), 140.7 (C^{dio}5), and 165.1 (Ph*C*O).

NaOMe (1 M, 0.2 mL) was added to a solution of **6** (125 mg, 98 μmol) in 4:1 MeOH–CH_2Cl_2 (5 mL). The mixture was stirred at 50 °C overnight, at the end of which time the reaction was quenched by addition of CO_2, and the mixture was concentrated and purified by flash chromatography (toluene → 6:1 toluene–EtOAc) to yield diosgenyl 3,6-di-*O*-benzyl-4-*O*-(2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside (**7**, 82 mg, 70 μmol, 71%): ^1H NMR ($CDCl_3$): δ 0.78–2.20 (m, 39H), 3.30–3.60 (m, 9H), 3.72 (m, 2H), 3.82 (dd, 2H, *J* 9 Hz, *J* 3 Hz), 3.98 (m, 1H), 4.38 (d, 1H, *J* 8 Hz), 4.42 (m, 1H), 4.56 (s, 2H), 4.62 (m, 5H), 4.76 (d, 1H, *J* 11 Hz), 4.93 (t, 2H, *J* 8 Hz), 5.05 (s, 1H), 5.38 (m, 1H), and 7.10–7.42 (m, 25H). ^{13}C NMR: δ 14.7, 16.4, 17.3, 18.0, 19.5, 21.0, 28.9, 29.8, 30.4, 31.5, 32.0, 32.2, 37.0, 37.4, 39.0, 39.9, 40.4, 41.7, 50.2, 56.6, 62.2, 67.0, 68.8, 72.2, 72.6, 73.6, 74.8, 75.0, 75.1, 75.4, 75.5, 79.2, 79.8, 80.8, 80.9, 83.0, 98.5, 100.4 (C1, C1′), 109.4 (C^{dio}22), 121.9 (C^{dio}6), 125.4, 127.6–129.2, 138.3, 138.5, 138.7, 138.8, 138.9 (Ar C), and 140.5 (C^{dio}5).

A solution of **7** (80 mg, 68 μmol) and **4** (40 mg, 84 μmol) in dry Et_2O (2 mL) was stirred with powdered molecular sieves (4 Å) under argon for 1 h when NIS (20 mg, 89 μmol) and a catalytic amount AgOTf (dissolved in toluene) were added. After 24 h stirring at room temperature, an additional amount of donor (**7**, 10 mg) and AgOTf solution were added. The reaction was quenched after a further 24 h by addition of Et_3N (50 μL), and the mixture was diluted with CH_2Cl_2 (20 mL), filtered through Celite and concentrated. The residue was applied onto a silica gel column and eluted (toluene (Et_3N) → 18:1 toluene–EtOAc) to give **8** (100 mg, 63 μmol, 93%); $[\alpha]_D$ −31° (*c* 4.3, CH_2Cl_2): ^1H NMR ($CDCl_3$): δ 0.78–2.40 (m, 42H), 3.30–3.85, 4.20–5.00 (m, 36H), 5.26 (m, 2H), and 7.10–7.42 (m, 40H). ^{13}C NMR, δ 14.8, 16.6, 17.4, 18.1, 18.2, 19.5, 21.1, 29.0, 29.9, 30.0, 30.6, 31.7, 32.1, 32.4, 37.1, 37.5, 38.6, 40.0, 40.5, 41.8, 50.3, 56.7, 62.3, 67.0, 68.2, 68.9, 69.1, 72.0, 72.2, 72.7, 73.6, 74.0, 75.0, 75.1, 75.4, 75.7, 76.8, 77.5, 78.8, 79.7, 80.1, 80.6, 80.7, 81.0, 84.1, 98.1 98.6, 100.0 (C1, C1′, C1″), 109.4 (C^{dio}22), 121.5 (C^{dio}6), 126.3, 127.2–129.0, 138.0, 138.1, 138.3, 138.4, 138.6, 138.7, 138.8, 139.1 (Ar C), and 140.6 (C^{dio}5). MALDI TOFMS: Calcd for $C_{101}H_{120}O_{16}$ 1588.86 [M]. Found 1590.04 $[M+H]^+$, 1611.93 $[M+Na]^+$, 1627.94 $[M+K]^+$.

Diosgenyl 2,4-di-O-*(α*-L-*rhamnopyranosyl)-β*-D-*glucopyranoside (dioscin)* (**3**).—Compound **8** (10 mg, 6 μmol) dissolved in dry THF (1 mL) was dropped into liquid ammonia (10 mL). A catalytic amount of sodium was added to the white emulsion, which rapidly turned into a deep blue solution. After 15 min, NH_4Cl (s) was carefully added until the solution turned white again. The liquid ammonia was allowed to evaporate. The dry residue was treated with Et_2O and MeOH and concentrated. The residue was then dissolved in water (5 mL) and freeze-dried. After filtration through a short RP C_{18} column (Sep-Pak), **3** (5 mg, 95%) was obtained, $[\alpha]_D$ −111° (*c* 0.2, MeOH); lit.[9] −113.6°; MALDI TOFMS: Calcd for $C_{45}H_{72}O_{16}$ 868.48 [M]. Found 869.51 $[M+H]^+$, 891.51 $[M+Na]^+$.

*26-Hydroxy-25(*R*)-furost-5-en-3β-ol 3-tertbutyldimethylsilyl ether* (**9**).—A solution of TBDMSOTf (freshly prepared from TBDMSCl (135 mg, 0.9 mmol) and AgOTf (230 mg, 0.9 mmol) in CH_2Cl_2 (1.5 mL) was dropped to a cooled (0 °C) mixture of diosgenin (250 mg, 0.60 mmol) and *sym*-collidine (158 μL, 1.8 mmol) under argon. The mixture was left to attain room temperature, and TBDMSCl (90 mg, 0.6 mmol) was added after 18 h to obtain complete conversion.

After concentration, the crude product was purified by flash chromatography (pentane → 6:1 pentane–EtOAc) to produce diosgenyl 3-*O*-tertbutyldimethylsilyl ether (290 mg, 0.55 mmol, 92%): ^{1}H NMR ($CDCl_3$): δ 0.02 (s, 6H, Me_2Si), 0.78–2.38 (m, 47H), 3.41 (m, 3H), 4.40 (m, 1H), and 5.26 (d, 1H). ^{13}C NMR: δ −4.9 (Me_2Si), 14.7, 16.4, 17.3, 18.4, 19.6, 21.0, 26.1 (^{t}*Bu*Si), 29.0, 30.5, 31.5, 31.6, 32.0, 32.2, 32.3, 36.9, 37.5, 40.0, 40.4, 41.8, 43.0, 50.3, 56.7, 62.3, 67.0, 72.7, 81.0, 109.4 (C^{dio}22), 121.0 (C^{dio}6), and 141.8 (C^{dio}5). MALDI TOFMS: Calcd for $C_{33}H_{56}O_3Si$ 528.40 [M]. Found 529.59 $[M + H]^+$; mp 132–134 °C; lit.[13] mp 134–136 °C.

Diosgenyl 3-*O*-tertbutyldimethylsilyl ether (285 mg, 0.54 mmol) and $BH_3{\cdot}Me_3N$ complex (1.6 g, 22 mmol) were dissolved in dry 6:1 CH_2Cl_2–Et_2O (70 mL). Powdered molecular sieves (4 Å) were added, and the mixture was stirred for 1 h and then cooled (−70 °C). $AlCl_3$ (275 mg) was slowly added into another flask containing Et_2O (10 mL, 0 °C). This mixture was then added dropwise to the above mixture. During 2 h the reaction mixture was allowed to warm up to −10 °C, and it was then poured onto a slurry of ice (ca. 100 mL) and CH_2Cl_2 (100 mL). The organic phase was separated, washed with $NaHCO_3$ and brine and concentrated The residue was applied onto a silica gel column and eluted (toluene (Et_3N) → 6:1 toluene–EtOAc) to give **9** (260 mg, 0.49 mmol, 91%): $[\alpha]_D$ −30° (*c* 0.4, CH_2Cl_2): ^{1}H NMR ($CDCl_3$): δ 0.04 (s, 6H, Me_2Si), 0.80–2.38 (m, 45H), 3.32 (m, 2H), 3.37 (t, 1 H, *J* 11 Hz), 3.46 (m, 2H), 4.30 (m, 1H), and 5.28 (m, 1H). ^{13}C NMR: δ −4.4 (Me_2Si), 16.6, 16.8, 18.4, 19.6, 20.8, 26.1 (^{t}*Bu*Si), 30.2, 30.6, 31.7, 32.1, 32.2, 32.4, 35.9, 36.8, 37.5, 38.0, 39.6, 40.8, 42.9, 50.3, 57.2, 65.2, 68.1, 72.7, 83.4, 90.5, 121.0 (C^{dio}6), 141.7 (C^{dio}5) ppm. MALDI TOFMS: Calcd for $C_{33}H_{58}O_3Si$ 530.41 [M]. Found 553.36 $[M + Na]^+$, 569.30 $[M + K]^+$.

26-O-*(2,3,4,6-Tetra*-O-*benzyl-β*-D-*glucopyranosyl)-3β*-O-*tertbutyldimethylsilyl-25(*R*)-furost-5-en* (**10**).—A solution of **9** (35 mg, 66 μmol) and 2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl trichloracetimidate (100 mg, 145 μmol) in dry CH_2Cl_2 (5 mL) was cooled (−40 °C) and $BF_3{\cdot}Et_2O$ (10 μL of 50 μL $BF_3{\cdot}Et_2O$ in 1 mL of CH_2Cl_2) was added. After 2 h at −30 °C the reaction mixture was concentrated, and the residue was applied onto a silica gel column and eluted (19:1 pentane (Et_3N)–EtOAc → 9:1 pentane–EtOAc) to give **10** (63 mg, 60 μmol, 91%); $[\alpha]_D$ −2.6 (*c* 1.0, CH_2Cl_2): ^{1}H NMR ($CDCl_3$): δ 0.10 (s, 6H, Me_2Si), 0.80–2.38 (m, 45H), 3.33 (m, 1H), 3.42–3.86 (m, 9H), 4.33 (m, 1H), 4.42 (d, 1H, *J* 8 Hz), 4.48–5.06 (m, 8H), 5.37 (m, 1H), and 7.10–7.40 (m, 20H); ^{13}C NMR: δ −4.4 (Me_2Si), 16.6, 17.1, 18.3, 19.1, 19.6, 20.8, 26.1 (^{t}*Bu*Si), 30.7, 31.0, 31.7, 32.1, 32.2, 32.3, 33.9, 36.8, 37.5, 38.0, 39.6, 40.8, 42.9, 50.3, 57.1, 65.3, 69.1, 72.7, 73.6, 74.9, 75.0, 75.1, 75.2, 75.8, 78.1, 82.4, 83.2, 84.8, 90.3, 103.8 (C1), 121.0 (C^{dio}6), 127.6–128.6, 138.2, 138.3, 138.6, 138.8 (Ar C), and 141.6 (C^{dio}5). MALDI TOFMS: Calcd for $C_{67}H_{92}O_8Si$ 1052.66 [M]. Found 1075.66 $[M + Na]^+$, 1091.66 $[M + K]^+$.

26-O-*(2,3,4,6-Tetra*-O-*benzyl-β*-D-*glucopyranosyl)-3β-hydroxy-25(*R*)-furost-5-en* (**11**).—TBAF (tetrabutyl ammonium tetrafluoroborate) (0.4 mL, 1 M in THF) was added to a solution of **10** (260 mg, 0.25 mmol) in freshly distilled THF (5 mL). After 18 h the mixture was concentrated. The crude material was purified by flash chromatography (6:1 pentane–EtOAc → 6:1 toluene–EtOAc) to give **11** (227 mg, 0.24 mmol, 96%): $[\alpha]_D$ −8.5° (*c* 2.0, CH_2Cl_2): ^{1}H NMR ($CDCl_3$): δ 0.80–2.38 (m, 36H), 3.30 (m, 1H), 3.38–3.82 (m, 9H), 4.30 (m, 1H), 4.40 (d, 1H, *J* 8 Hz), 4.44–5.02 (m, 8H), 5.36 (m, 1H), and 7.10–7.40 (m, 20H). ^{13}C NMR: δ 16.6, 17.2, 19.1, 19.6, 20.8, 30.7, 31.1, 31.7, 32.1, 32.4, 33.9, 36.7, 37.4, 38.0, 39.6, 40.8, 42.4, 50.2, 57.1, 65.3, 69.1, 71.8, 73.6, 74.9, 75.0, 75.1, 75.3, 75.8, 78.1, 82.4, 83.3, 84.8, 90.4, 103.9 (C1), 121.6 (C^{dio}6), 127.7–128.5, 138.3, 138.4, 138.7, 138.8 (Ar C), and 140.9 (C^{dio}5). MALDI TOFMS: Calcd for $C_{61}H_{78}O_8$ 938.57 [M]. Found 961.62 $[M + Na]^+$, 977.63 $[M + K]^+$.

26-O-*(2,3,4,6-Tetra*-O-*benzyl-β*-D-*glucopyranosyl)-3β*-O-*[4*-O-*(2,3,4-tri*-O-*benzyl-α*-L-*rhamnopyranosyl)-3,6-di*-O-*benzyl-β*-D-*glucopyranosyl]-25(*R*)-furost-5-en* (**13**).—A solution of **4** (77 mg, 138 μmol) and **5** (66 mg, 138 μmol) in dry Et_2O (2 mL) was stirred with powdered molecular sieves (4 Å) under argon for 1 h at the end of which time NIS (40 mg, 0.18 mmol) and a catalytic amount AgOTf were added. TLC (18:1 toluene–EtOAc) showed complete conversion into the disaccharide after 45 min. Subsequently **11** (130 mg, 138 μmol) and DMTST (117 mg, 0.45 mmol) dissolved in CH_2Cl_2 (8 mL) containing molecular sieves (4 Å) were added. After 30 min the reaction was quenched by addition of Et_3N (50 μL), diluted with CH_2Cl_2 (20 mL), filtered through Celite, and concentrated. The residue was applied onto a silica gel column and eluted (toluene (Et_3N) → 12:1 toluene–EtOAc) to give 26-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-3β-*O*-[4-*O*-(2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl]-25(*R*)-furost-5-en (**12**, 160 mg, 89 μmol, 64%): ^{1}H NMR ($CDCl_3$): δ 0.78–2.20 (m, 36H), 3.30 (m, 1H), 3.40–3.82 (m, 18H), 4.30 (m, 1H), 4.40 (d, 1H, *J* 8 Hz), 4.44–5.02 (m, 19H), 5.10 (d, 1H, *J* 2 Hz), 5.21 (m, 1H), 5.27 (dd, 2H, *J* 8 Hz, *J* 9 Hz), 7.05–7.60 (m, 48H), and 8.04 (d, 2H). ^{13}C NMR: δ 16.6, 17.2, 18.0, 19.1, 19.5, 20.8, 29.7, 30.7, 31.1, 31.7, 32.1, 32.3, 33.9, 36.9, 37.4, 38.1, 39.0, 39.6, 40.8, 50.3, 57.1, 65.3, 69.0, 69.1, 72.2, 72.6, 73.6, 73.7, 74.3, 74.6, 74.9, 75.0, 75.1, 75.3, 75.4, 75.5, 75.6, 75.8, 78.1, 79.5, 79.9, 80.7, 81.7, 82.4, 83.3, 84.9, 90.4, 98.7, 100.1, 103.9 (C1, C1′, C1″, C1‴), 121.6, 127.6–129.8, 130.2, 133.2, 137.7, 138.3, 138.4, 138.6, 138.7, 138.8, 138.9 (Ar C), 140.8, and 165.3 (PhCO).

Compound **12** (70 mg, 39 μmol) was dissolved in 3:1 MeOH–CH_2Cl_2 (4 mL), and NaOMe was added. The mixture was stirred at 50 °C overnight, at the end of which time the reaction was quenched by addition of CO_2, and the mixture was concentrated and purified by flash chromatography (toluene (Et_3N) → 12:1 toluene–EtOAc) to yield **13** (62 mg, 35 μmol, 90%): $[\alpha]_D$ −21° (*c* 0.3, CH_2Cl_2): ^{1}H NMR ($CDCl_3$): δ 0.80–2.40 (m, 40H), 3.30–3.82 (m, 20H), 4.00 (m, 1H), 4.30 (m, 1H), 4.34 (d, 1H, *J* 8 Hz), 4.40 (d, 1H, *J* 8 Hz), 4.52–5.06 (m, 18H), 5.21 (m, 1H), and 7.18–7.46 (m, 45H). ^{13}C NMR: δ 16.6, 17.2, 18.0, 19.1, 19.5, 20.8, 29.8, 30.7, 31.1, 31.7, 32.2, 32.3, 33.9, 37.0, 37.4, 38.1, 39.0, 39.6, 40.8, 50.3, 57.1, 65.3, 68.8, 69.1, 72.2, 72.6, 73.5, 73.6, 74.8, 74.9, 75.0, 75.1, 75.3, 75.4, 75.5, 75.8, 78.1, 79.2, 79.8, 80.8, 82.4, 83.0, 83.3, 84.9, 90.4, 98.5, 100.4, 103.9 (C1, C1′, C1″, C1‴), 122.0, 127.6–129.2, 138.2, 138.3, 138.4, 138.5, 138.6, 138.7, 138.8, 138.9 (Ar C), and 140.5. MALDI TOFMS: Calcd for $C_{108}H_{128}O_{17}$ 1696.92 [M]; 1720.05 $[M + Na]^+$.

*26-*O*-(2,3,4,6-Tetra-*O*-benzyl-β-*D*-glucopyranosyl)-3β-*O*-[2,4-di-*O*-(2,3,4-tri-*O*-benzyl-α-*L*-rhamnopyranosyl)-3,6-di-*O*-benzyl-β-*D*-glucopyranosyl]-25(*R*)-furost-5-en* (**14**).—A solution of **13** (120 mg, 71 μmol) and **4** (40 mg, 84 μmol) in dry Et_2O (2 mL) was stirred with powdered molecular sieves (4 Å) under argon for 1 h at the end of which time NIS (20 mg, 89 μmol) and a catalytic amount AgOTf were added. After 1 h stirring at room temperature, the reaction mixture was quenched by addition of Et_3N (50 μL), diluted with Et_2O (20 mL), filtered through Celite and concentrated. The residue was applied onto a silica gel column and eluted (toluene (Et_3N) → 18:1 toluene–EtOAc) to give **14** (108 mg, 51 μmol, 72%): $[\alpha]_D$ −14° (*c* 3.5, CH_2Cl_2): ^{1}H NMR ($CDCl_3$): δ 0.80–2.45 (m, 43H), 3.30–3.82, 4.20–5.05 (m, 51H), 5.30 (s, 1H), 5.33 (m, 1H), and 7.18–7.40 (m, 60H); ^{13}C NMR: δ 16.6, 17.2, 18.0, 18.1, 19.1, 19.3, 20.8, 29.8, 30.8, 31.1, 31.7, 32.2, 32.4, 33.9, 37.0, 37.4, 38.1, 38.5, 39.6, 40.8, 50.3, 57.1, 65.3, 68.2, 68.9, 69.1, 72.0, 72.2, 72.6, 73.6, 73.7, 74.9, 75.0, 75.1, 75.3, 75.5, 75.7, 75.8, 76.8, 78.1, 78.8, 79.6, 80.1, 80.6, 80.7, 82.4, 84.2, 84.9, 90.4, 98.2 ($J_{C1,H1}$ 175 Hz), 98.7 ($J_{C1,H1}$ 174 Hz), 100.0 ($J_{C1,H1}$ 155 Hz), 103.9 ($J_{C1,H1}$ 162 Hz), 121.7, 127.3–128.5, 138.2, 138.3, 138.4, 138.5, 138.6, 138.7, 138.8, 138.9, 139.0, 139.2 (Ar C), and 140.8. MALDI TOFMS: Calcd for $C_{135}H_{156}O_{21}$ 2113.11 [M]. Found 2136.34 $[M + Na]^+$.

*26-*O*-β-*D*-Glucopyranosyl-3β-*O*-[2,4-di-*O*-(α-*L*-rhamnopyranosyl)-β-*D*-glucopyranosyl]-25(*R*)-furost-5-en* (**2**).—Compound **14** (65 mg, 31 μmol) dissolved in dry EtOH (2 mL) was dropped into liquid ammonia (25 mL). Small pieces of sodium were added to the white emulsion until it turned into a deep blue solution, then the liquid ammonia was allowed to evaporate. The residue was diluted with MeOH (25 mL), neutralised with H^+ ion exchange resin and concentrated. The residue was dissolved in water (5 mL) and freeze dried. After filtration through a short RP C_{18} column (Sep-Pak) **2** (31 mg, 97%) was obtained. ^{1}H NMR (MeOD, 35 °C): δ 0.80–2.06 (m, 41H), 2.29 (m, 1H), 2.44 (m, 1H), 3.18 (dd, 1H, *J* 8 Hz, *J* 9 Hz), 3.21–3.42, 3.43–3.88 (m, 21H), 4.11 (m, 1H), 4.22 (d, 1H, *J* 8 Hz), 4.31 (m, 1H), 4.49 (d, 1H, *J* 8 Hz), 4.83 (d, 1H, *J* 2 Hz), 5.20 (d, 1H, *J* 2 Hz) 5.37 (m, 1H). ^{13}C NMR (25 °C), δ 17.1, 17.4, 18.0, 18.1, 19.4, 20.0, 22.0, 30.9, 31.5, 31.9, 33.1, 33.3, 34.8, 38.2, 38.7, 39.2, 39.6, 40.7, 42.0, 51.8, 58.3, 62.1, 62.9, 66.6, 69.9, 70.8, 71.8, 72.3, 72.6, 73.8, 74.0, 75.3, 76.2, 76.7, 78.0, 78.1, 78.2, 79.3, 79.5, 80.0, 84.8, 91.8, 100.5, 102.5, 103.1, 104.8, 122.8, and 142.0 $[\alpha]_D$ −62° (*c* 1.2, MeOH); MALDI TOFMS: Calcd for $C_{51}H_{84}O_{21}$ 1032.55 [M]; 1055.46 $[M + Na]^+$.

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