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# An approach to the synthesis and attachment of scillabiose to steroids

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ABSTRACT

Hellebrin and transvaalin are two naturally occurring saponins with biological activity. In the present paper, we describe a high yielding route to the synthesis and coupling of their shared glycone, scillabiose, to a model steroid. A convergent coupling strategy utilizing a scillabiose-based glycosyl donor was devised for the glycosylation. This convergent approach is appealing due to its high efficiency and simple deprotection procedure and may find further use in total synthesis of naturally occurring saponins and related compounds sharing the same glycone. Due to the widespread occurrence of this glycone in nature, the complete NMR spectroscopic characterization of all compounds prepared herein is provided as reference material. In addition, glycosylations were performed with the monosaccharide constituents of scillabiose, thereby providing a limited series of glycosylated steroids for potential future evaluation of the effects of the glycone on the overall biological activity.



## 1. Introduction

Hellebrin and transvaalin are bufenolides, a subclass of saponins, and are well known for their pharmacological activities. Hellebrin has been isolated from *Helleborus niger* and is notorious for its potent cytotoxic activity [1]. In addition, hellebrin has been found to display T-cell suppressive effects and has found further use as an immunoregulatory molecule [2]. Transvaalin, also called scillaren, has been isolated from *Urginea sanguinea* and has been extensively used as a constituent of herbal medicines to purify blood, remove abdominal pain and backache and as an abortifacient [3]. Transvaalin is also known for its poisonous effect on livestock [4].

As shown in Fig. 1, the carbohydrate parts, scillabiose (4-*O*-(β-D-glucopyranosyl)-α-L-rhamnopyranoside), of these two saponins are identical. The scillabiose moiety has also been found in *Scilla maritime* as the glycone part of glucoscilliphaeoside [5]. In addition to the presence in these saponins, a closely related glycon containing glucuronic acid instead of glucose has been found in molecules isolated from *Acrosiphonia centralis*, *ulva lactuca* and *klebsiella* [6].

It is well known that the biological interactions displayed by biomolecules depend on both their aglycone and glycone structures, as exemplified by the complete lack of activity of the erythromycine and daunomycin antitumor agents when the carbohydrate part is modified or removed [7]. Due to the occurrence of scillabiose and closely related compounds in several naturally occurring biomolecules and pharmaceutically active species, we became interested in the synthesis of this glycone. In the present paper, we describe a high yielding, convergent strategy for the synthesis of the scillabiose glycone and its monosaccharide constituents, as well as their coupling reaction to a model steroid. Being widely distributed in nature, the scillabiose moiety is relevant from both the synthetic and analytical points of view. The synthetic methodology presented herein should thus be of interest for several areas of research. Importantly, for reference purposes, we also describe here the fully characterized $^{1}H$ and $^{13}C$ NMR spectra of all of the building blocks as well as the glycosylated steroids.

## 2. Experimental

Reaction solvents were dried and distilled prior to use when necessary. All reactions containing moisture- or air-sensitive reagents were carried out under argon atmosphere. The NMR spectra were recorded with a Bruker Avance spectrometer operating at 600.13 MHz ($^{1}H$: 600.13 MHz, $^{13}C$: 150.90 MHz). The probe temperature during the experiments was kept at 25 °C unless indicated otherwise. All products were fully characterized by utilization of $^{1}H$, 1D-TOCSY and $^{13}C$ 1D-NMR techniques in combination with DQF-COSY, NOESY, HSQC and HMBC 2D-NMR techniques by using pulse sequences provided by the manufacturer. Chemical shifts are expressed on the $\delta$ scale (in ppm) using TMS (tetramethylsilane), residual chloroform, acetone, $H_2O$ or methanol as internal

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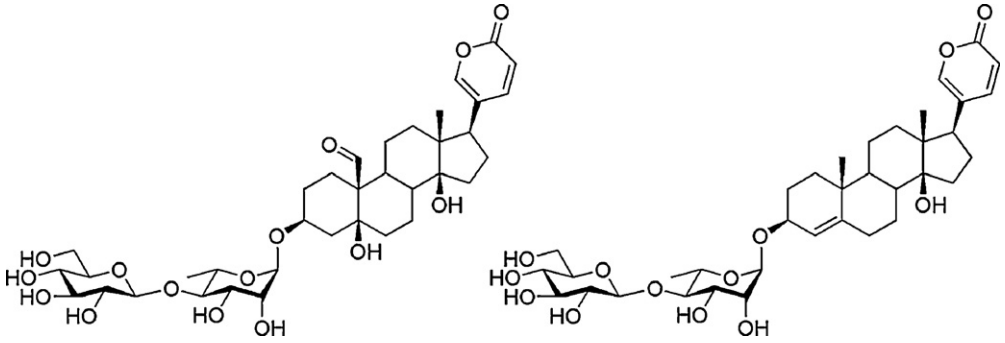


**Fig. 1.** Structures containing the scillabiose glycon: hellebrin (left) and transvaalin (right).

standards. Coupling constants are given in Hz and provided only once when first encountered. Coupling patterns are given as s (singlet), d (doublet), t (triplet) etc. The following indexes are used to distinguish between protons on the same carbon: eq (equatorial) and ax (axial). The computational analysis of the $^1$H NMR of all compounds was performed by use of PERCH NMR software with starting values and spectral parameters obtained from the various NMR techniques applied [8]. HRMS were recorded using a Bruker Micro Q-TOF with ESI (electrospray ionization) operated in positive mode. Optical rotations were measured at 23 °C with a Perkin Elmer 241 polarimeter equipped with Na-lamp (589 nm) and are reported in units of $10^{-1}$ degree cm$^2$ g$^{-1}$. TLC was performed on aluminium sheets precoated with silica gel 60 $F_{254}$ (Merck). Flash chromatography was carried out on silica gel 60 (0.040–0.060 mm, Merck). Spots were visualized by UV followed by charring with 1:10 $H_2SO_4$/MeOH and heating.

### 2.1. General procedure for glycosylation

To a solution containing the corresponding acceptor (1 equiv.) and pre-activated 4 Å MS in dry $CH_2Cl_2$ (1.6 ml/0.1 mmol substrate) was added TMSOTf (0.2 equiv.) at −20 °C. The reaction mixture was stirred for 10 min and the corresponding donor (1.4 equiv.) dissolved in dry $CH_2Cl_2$ (1.7 ml/0.1 mmol substrate) was added dropwise to the solution. The resulting mixture was stirred for 1.5–2 h, brought to rt, diluted with $CH_2Cl_2$ (20 ml) and washed with sat. $NaHCO_3$-solution (20 ml). The organic phase was dried over $Na_2SO_4$, filtered and concentrated. The crude product was purified by column chromatography (hexane–EtOAc, 4:1) to give the corresponding glycosteroid.

### 2.2. General procedure for deprotection

To a solution containing the protected glycosteroid (1 equiv.) in dry MeOH or dry MeOH:THF mixture (2:1) (6.3 ml/0.1 mmol substrate) was added NaOMe (2 equiv.) and the resulting mixture was stirred for 3–20 h at rt, neutralized with DOWEX 50 H$^+$-form, filtered and concentrated. The crude product was purified by column chromatography ($CH_2Cl_2$ → $CH_2Cl_2$–MeOH 3:1) to yield the deprotected glycosteroid.

### 2.3. Phenyl 2,3-O-isopropylidene-4-O-(2′,3′,4′,6′-tetra-O-benzoyl-β-D-glycopyranosyl)-1-thio-α-L-rhamnopyranoside (**5**)

Synthesized from **4** (91 mg, 0.3 mmol) and **2** (295 mg, 0.4 mmol) according to the general procedure for glycosylation providing **5** as a white foam (290 mg, 85%). $R_f$ = 0.41 (hexane–EtOAc 2:1); $[\alpha]_D$ −73.3 (c 0.2, $CHCl_3$). $^1$H NMR (600.13 MHz, $CDCl_3$): $\delta$ 8.04–7.23 (m, 25 H, arom. *H*), 5.94 (dd, 1 H, $J_{3',4'} = 9.5$ Hz, $J_{3',2'} = 9.8$ Hz, H-3′), 5.66 (dd, 1 H, $J_{4',5'} = 10.0$ Hz, H-4′), 5.66 (d, 1 H, $J_{1,2} = 0.9$ Hz, H-1), 5.52 (dd, 1 H, $J_{2',1'} = 8.0$ Hz, H-2′), 5.34 (d, 1 H, H-1′), 4.66 (dd, 1 H, $J_{6'a,5'} = 3.1$ Hz, $J_{6'a,6'b} = -12.1$ Hz, H-6′a), 4.48 (dd, 1 H, $J_{6'b,5'} = 5.7$ Hz, H-6′b), 4.20 (dd, 1 H, $J_{2,3} = 5.6$ Hz, H-2), 4.15 (ddd, 1 H, H-5′), 4.04 (dq, $J_{5,6} = 6.2$, $J_{5,4} = 10.0$ Hz, H-5), 4.03 (dd, 1 H, $J_{3,4} = 7.5$ Hz, H-3), 3.67 (dd, 1 H, H-4), 1.48 and 1.26 (each s, each 3 H, $O_2C(CH_3)_2$), 1.22 (d, 3 H, H-6) ppm.

$^{13}$C NMR (150.9 MHz, $CDCl_3$): $\delta$ 166.1 (6′-OCOPh), 165.8 (3′-OCOPh), 165.3 (2′-OCOPh, 4′-OCOPh), 133.5–127.7 (arom. *C*), 109.5 ($O_2C(CH_3)_2$), 100.5 (C-1′), 83.6 (C-1), 81.0 (C-4), 77.7 (C-3), 76.6 (C-2), 73.1 (C-3′), 72.3 (C-5′), 72.1 (C-2′), 69.9 (C-4′), 65.5 (C-5), 63.2 (C-6′), 27.9 and 26.3 ($O_2C(CH_3)_2$), 17.3 (C-6) ppm.

HRMS: calcd. for $C_{49}H_{46}O_{13}SNa$ [M+Na]$^+$ 897.2557; found 897.2554.

### 2.4. Phenyl 4-O-(2′,3′,4′,6′-tetra-O-benzoyl-β-D-glycopyranosyl)-1-thio-α-L-rhamnopyranoside (**6**)

A solution containing **5** (300 mg, 0.34 mmol) in 80% AcOH (5 ml) was stirred at 80 °C for 5 h, brought to rt and concentrated. The crude product was purified by column chromatography with hexane–EtOAc 2:3 as eluent to yield **6** as a colorless oil (210 mg, 95%). $R_f$ = 0.28 (hexane–EtOAc 1:1); $[\alpha]_D$ −125.0 (c 0.1, $CHCl_3$). $^1$H NMR (600.13 MHz, $CDCl_3$): $\delta$ 8.04–7.20 (m, 25 H, arom. *H*), 5.95 (dd, 1 H, $J_{3',4'} = 9.5$ Hz, $J_{3',2'} = 9.8$ Hz, H-3′), 5.69 (dd, 1 H, $J_{4',5'} = 9.9$ Hz, H-4′), 5.56 (dd, 1 H, $J_{2',1'} = 7.9$ Hz, H-2′), 5.42 (d, 1 H, $J_{1,2} = 1.4$ Hz, H-1), 5.29 (d, 1 H, H-1′), 4.70 (dd, 1 H, $J_{6'a,5'} = 3.1$ Hz, $J_{6'a,6'b} = -12.1$ Hz, H-6′a), 4.46 (dd, 1 H, $J_{6'b,5'} = 5.3$ Hz, H-6′b), 4.18 (ddd, 1 H, H-5′), 4.18 (dq, $J_{5,6} = 6.2$, $J_{5,4} = 9.6$ Hz, H-5), 4.06 (dd, 1 H, $J_{2,3} = 3.4$ Hz, H-2), 3.81 (dd, 1 H, $J_{3,4} = 9.3$ Hz, H-3), 3.74 (dd, 1 H, H-4), 1.26 (d, 3 H, H-6) ppm.

$^{13}$C NMR (150.9 MHz, $CDCl_3$): $\delta$ 166.2 (6′-OCOPh), 165.9 (3′-OCOPh), 165.4 (4′-OCOPh), 165.3 (2′-OCOPh), 133.9–127.5 (arom. *C*), 101.0 (C-1′), 87.3 (C-1), 81.4 (C-4), 73.1 (C-3′), 72.6 (C-2), 72.4 (C-2′), 72.3 (C-5′), 71.4 (C-3), 69.7 (C-4′), 67.5 (C-5), 62.9 (C-6′), 17.5 (C-6) ppm.

HRMS: calcd. for $C_{46}H_{42}O_{13}SNa$ [M+Na]$^+$ 857.2544; found 857.2215.

### 2.5. Phenyl 2,3-di-O-benzoyl-4-O-(2′,3′,4′,6′-tetra-O-benzoyl-β-D-glycopyranosyl)-1-thio-α-L-rhamnopyranoside (**7**)

**6** (207 mg, 0.25 mmol) was dissolved in a mixture of pyridine (3 ml) and BzCl (1.5 ml) and allowed to stir until TLC indicated complete disappearance of starting material. The reaction was quenched after 4 h with MeOH, concentrated, dissolved in $CH_2Cl_2$ (40 ml) and washed with water (2× 20 ml) and brine (20 ml). The organic phase was dried over anhydrous $Na_2SO_4$, filtered and con-

centrated. The crude product was purified by flash chromatography with hexane–EtOAc (1:0 → 4:1) as eluent to give **7** as a slightly yellowish foam (230 mg, 89%). $R_f$ = 0.60 (hexane–EtOAc 1:1); $[\alpha]_D$ −35.0 (c 0.2, $CHCl_3$). $^1$H NMR (600.13 MHz, $CDCl_3$): $\delta$ 8.07–7.05 (m, 35 H, arom. *H*), 5.84 (dd, 1 H, $J_{2,1}$ = 1.7, $J_{2,3}$ = 3.3 Hz, H-2), 5.80 (dd, 1 H, $J_{3',4'}$ = 9.6 Hz, $J_{3',2'}$ = 9.9 Hz, H-3′), 5.67 (dd, 1 H, $J_{4',5'}$ = 9.9 Hz, H-4′), 5.55 (dd, 1 H, $J_{2',1'}$ = 7.9 Hz, H-2′), 5.55 (d, 1 H, H-1), 5.55 (dd, 1 H, $J_{3,4}$ = 9.7 Hz, H-3), 5.15 (d, 1 H, H-1′), 4.80 (dd, 1 H, $J_{6'a,5'}$ = 3.2 Hz, $J_{6'a,6'b}$ = −12.1 Hz, H-6′a), 4.48 (dd, 1 H, $J_{6'b,5'}$ = 5.3 Hz, H-6′b), 4.48 (dq, $J_{5,6}$ = 6.2 Hz, $J_{5,4}$ = 9.4 Hz, H-5), 4.25 (ddd, 1 H, H-5′), 4.14 (dd, 1 H, H-4), 1.48 (d, 3 H, H-6) ppm.

$^{13}$C NMR (150.9 MHz, $CDCl_3$): $\delta$ 166.1 (6′-*O*COPh), 165.7 (3′-*O*COPh), 165.2 (4′-*O*COPh), 165.1 (2′-*O*COPh, 2-*O*COPh), 164.8 (3-*O*COPh), 137.8–125.3 (arom. *C*), 101.3 (C-1′), 85.7 (C-1), 77.6 (C-4), 72.8 (C-3′), 72.5 (C-3), 72.0 (C-5′, C-2), 71.8 (C-2′), 69-7 (C-4′), 68.5 (C-5), 62.9 (C-6′), 17.9 (C-6) ppm.

HRMS: calcd. for $C_{60}H_{50}O_{15}SNa$ $[M+Na]^+$ 1065.2768; found 1065.2763.

### 2.6. 2,3-Di-*O*-benzoyl-4-*O*-(2′,3′,4′,6′-tetra-*O*-benzoyl-β-D-glycopyranosyl)-α-L-rhamnopyranosose trichloroacetimidate (**3**)

To a solution containing **7** (157 mg, 0.15 mmol) in acetone:$H_2O$ (10:1, 4.4 ml) was added NBS (40 mg, 1.5 equiv.) at 0 °C. The reaction was brought to rt and stirring was continued for 1 h. This process was repeated twice after which TLC indicated the reaction to be complete. The reaction was quenched with sat. $NaHCO_3$-solution (15 ml), diluted with $CH_2Cl_2$ (30 ml) and washed with brine (20 ml). The organic phase was dried over anhydrous $Na_2SO_4$, filtered and concentrated. The crude product was purified by column chromatography with Hexane–EtOAc 4:1 as eluent to yield 2,3-di-*O*-benzoyl-4-*O*-(2′,3′,4′,6′-tetra-*O*-benzoyl-β-D-glycopyranosyl)-α-L-rhamnopyranosose as a white foam (131 mg, 92%). 2,3-Di-*O*-benzoyl-4-*O*-(2′,3′,4′,6′-tetra-*O*-benzoyl-β-D-glycopyranosyl)-α-L-rhamnopyranosose (97 mg, 0.10 mmol) was dissolved in $CH_2Cl_2$ (2 ml) and DBU (3 μl, 0.1 equiv.) and $Cl_3CCN$ (25 μl, 2.4 equiv.) was added at 0 °C. The reaction mixture was stirred for 1.5 h, brought to rt, diluted with $CH_2Cl_2$ (30 ml) and washed with brine (20 ml). The organic phase was dried over anhydrous $Na_2SO_4$, filtered and concentrated. The crude product was purified by column chromatography with Hex:EtOAc:$Et_3N$ 3:1:0.01 as eluent to give **3** as a white foam (86 mg, 77%). $R_f$ = 0.64 (hexane–EtOAc 1:1); $[\alpha]_D$ +25.2 (c 0.2, $CHCl_3$). $^1$H NMR (600.13 MHz, $CDCl_3$): $\delta$ 8.71 (s, 1 H, OCN*H*$CCl_3$), 8.06–7.05 (m, 30 H, arom. *H*), 6.33 (d, 1 H, $J_{1,2}$ = 2.0 Hz, H-1), 5.76 (dd, 1 H, $J_{2,3}$ = 3.4 Hz, H-2), 5.75 (dd, 1 H, $J_{3',4'}$ = 9.5 Hz, $J_{3',2'}$ = 9.9 Hz, H-3′), 5.62 (dd, 1 H, $J_{4',5'}$ = 9.9 Hz, H-4′), 5.52 (dd, 1 H, $J_{3,4}$ = 9.6 Hz, H-3), 5.49 (dd, 1 H, $J_{2',1'}$ = 7.9 Hz, H-2′), 5.12 (d, 1 H, H-1′), 4.78 (dd, 1 H, $J_{6'a,5'}$ = 3.2 Hz, $J_{6'a6'b}$ = −12.0 Hz, H-6′a), 4.45 (dd, 1 H, $J_{6'b,5'}$ = 5.4 Hz, H-6′b), 4.21 (ddd, 1 H, H-5′), 4.18 (dq, $J_{5,6}$ = 6.2, $J_{5,4}$ = 9.6 Hz, H-5), 4.12 (dd, 1 H, H-4), 1.49 (d, 3 H, H-6) ppm.

HRMS: calcd. for $C_{56}H_{46}O_{16}Cl_3Na$ $[M+Na]^+$ 1116.1780; found 1116.1755.

### 2.7. 3β-(α-L-Rhamnopyranosyloxy)-androst-5-en-17-one (**10**)

Compound **10** was synthesized from **8** (32 mg, 0.12 mmol) and **1** (100 mg, 0.16 mmol) according to the general procedure for glycosylation. Pure product was, however, not obtained and the deprotection step was carried out according to the general procedure for deprotection providing **10** as a white solid (40 mg, 89% over two steps). $R_f$ = 0.23 (EtOAc). $^1$H NMR (600.13 MHz, $CD_3OD$ and $CDCl_3$): $\delta$ 5.39 (ddd, 1 H, $J_{6,4ax}$ = −1.9, $J_{6,7ax}$ = 2.0, $J_{6,7eq}$ = 5.2 Hz, H-6), 4.88 (d, 1 H, $J_{1',2'}$ = 1.7 Hz, H-1′), 3.82 (dd, 1 H, $J_{2',3'}$ = 3.4 Hz, H-2′), 3.71 (dd, 1 H, $J_{3',4'}$ = 9.4 Hz, H-3′), 3.69 (dq, 1 H, $J_{5',6'}$ = 6.2 Hz, $J_{5',4'}$ = 9.5 Hz, H-5′), 3.50 (dddd, 1 H, $J_{3,2eq}$ = 4.4, $J_{3,4eq}$ = 4.8, $J_{3,2ax}$ = 11.4, $J_{3,4ax}$ = 11.4 Hz, H-3), 3.39 (dd, 1 H, H-4′), 2.48 (ddd, 1 H, $J_{16eq,15eq}$ = 0.4, $J_{16eq,15ax}$ = 8.8, $J_{16eq,16ax}$ = −19.5 Hz, H-16eq), 2.38 (ddd, 1 H, $J_{4eq,2ax}$ = −2.1, $J_{4eq,4ax}$ = −13.1 Hz, H-4eq), 2.20 (ddddd, 1 H, $J_{4ax,7ax}$ = 3.2, $J_{4ax,7eq}$ = 3.2 Hz, H-4ax), 2.13 (dddd, 1 H, $J_{7eq,8}$ = 5.4, $J_{7eq,7ax}$ = −17.4 Hz, H-7eq), 2.11 (ddd, 1 H, $J_{16ax,15eq}$ = 8.9, $J_{16ax,15ax}$ = 9.3 Hz, H-16ax), 1.98 (dddd, 1 H, $J_{15eq,14}$ = 5.8, $J_{15eq,15ax}$ = −12.5 Hz, H-15eq), 1.88 (ddd, 1 H, $J_{1eq,2eq}$ = 3.3, $J_{1eq,2ax}$ = 3.7, $J_{1eq,1ax}$ = −13.5 Hz, H-1eq), 1.87 (ddd, 1 H, $J_{2eq,1ax}$ = 3.8, $J_{2eq,2ax}$ = −12.6 Hz, H-2eq), 1.84 (ddd, 1 H, $J_{12eq,11eq}$ = 2.8, $J_{12eq,11ax}$ = 4.3, $J_{12eq,12ax}$ = −13.5 Hz, H-12eq), 1.70 (dddd, $J_{11eq,12ax}$ = 4.2, $J_{11eq,9}$ = 5.3, $J_{11eq,11ax}$ = −13.7 Hz, H-11eq), 1.69 (dddd, 1 H, $J_{8,7ax}$ = 10.3, $J_{8,9}$ = 10.9, $J_{8,14}$ = 11.2 Hz, H-8), 1.68 (ddd, 1 H, H-7ax), 1.59 (ddddd, 1 H, $J_{2ax,1ax}$ = 14.0 Hz, H-2ax), 1.58 (dddd, 1 H, $J_{15ax,14}$ = 12.7 Hz, H-15ax), 1.51 (dddd, 1 H, $J_{11ax,9}$ = 12.3, $J_{11ax,12ax}$ = 13.8 Hz, H-11ax), 1.32 (ddd, 1 H, H-14), 1.28 (d, 1 H, H-6′), 1.29 (ddd, 1 H, H-12ax), 1.11 (ddd, 1 H, H-1ax), 1.05 (s, 3 H, H-19), 1.03 (ddd, 1 H, H-9), 0.91 (s, 3 H, H-18) ppm.

$^{13}$C NMR (150.9 MHz, $CD_3OD$ and $CDCl_3$): $\delta$ 222.8 (C-17), 140.4 (C-5), 121.0 (C-6), 97.9 (C-1′), 76.2 (C-3), 72.8 (C-4′), 71.2 (C-3′), 72.1 (C-2′), 68.1 (C-5′), 51.6 (C-14), 50.1 (C-9), 47.6 (C-13), 38.2 (C-4), 37.1 (C-1), 36.7 (C-10), 35.7 (C-16), 31.3 (C-8), 31.2 (C-12), 30.6 (C-7), 29.1 (C-2), 21.7 (C-15), 20.1 (C-11), 19.1 (C-19), 17.1 (C-6′), 12.3 (C-18) ppm.

HRMS: calcd. for $C_{25}H_{38}O_6Na$ $[M+Na]^+$ 457.2566; found 457.2549.

### 2.8. 3β-(β-D-Glucopyranosyloxy)-androst-5-en-17-one (**12**)

Synthesized from **8** (37 mg, 0.13 mmol) and **2** (133 mg, 0.18 mmol) according to the general procedure for glycosylation. Pure product was, however, not obtained and the deprotection step was carried out according to the general procedure for deprotection providing **12** as a white powder (41 mg, 78% over two steps). $R_f$ = 0.60 (MeOH–$CH_2Cl_2$ 1:5). $^1$H NMR (600.13 MHz, $CD_3OD$ and $CDCl_3$): $\delta$ 5.42 (ddd, 1 H, $J_{6,4ax}$ = −1.6, $J_{6,7ax}$ = 2.5, $J_{6,7eq}$ = 5.3 Hz, H-6), 4.40 (d, 1 H, $J_{1',2'}$ = 7.8 Hz, H-1′), 3.86 (dd, 1 H, $J_{6'a,5'}$ = 2.6 Hz, $J_{6'a,6'b}$ = −11.9 Hz, H-6′a), 3.71 (dd, 1 H, $J_{6'b,5'}$ = 5.5 Hz, H-6′b), 3.62 (dddd, 1 H, $J_{3,2eq}$ = 4.4, $J_{3,4eq}$ = 4.7, $J_{3,4ax}$ = 11.3, $J_{3,2ax}$ = 11.6 Hz, H-3), 3.40 (dd, 1 H, $J_{3',4'}$ = 8.9 Hz, $J_{3',2'}$ = 9.3 Hz, H-3′), 3.35 (dd, 1 H, $J_{4',5'}$ = 9.8 Hz, H-4′), 3.29 (ddd, 1 H, H-5′), 3.21 (dd, 1 H, H-2′), 2.48 (ddd, 1 H, $J_{16eq,15eq}$ = 1.1, $J_{16eq,15ax}$ = 8.9, $J_{16eq,16ax}$ = −19.6 Hz, H-16eq), 2.46 (ddd, 1 H, $J_{4eq,2ax}$ = −2.4, $J_{4eq,4ax}$ = −13.2 Hz, H-4eq), 2.30 (dddddd, 1 H, $J_{4ax,2eq}$ = −2.4, $J_{4ax,7ax}$ = 2.6, $J_{4ax,7eq}$ = 2.7 Hz, H-4ax), 2.14 (dddd, 1 H, $J_{7eq,8}$ = 5.0, $J_{7eq,7ax}$ = −17.3 Hz, H-7eq), 2.11 (ddd, 1 H, $J_{16ax,15eq}$ = 8.9, $J_{16ax,15ax}$ = 9.4 Hz, H-16ax), 1.99 (dddd, 1 H, $J_{15eq,14}$ = 5.9, $J_{15eq,15ax}$ = −12.4 Hz, H-15eq), 1.96 (ddddd, $J_{2eq,1eq}$ = 2.4, $J_{2eq,1ax}$ = 3.8, $J_{2eq,2ax}$ = −12.9 Hz, H-2eq), 1.90 (ddd, 1 H, $J_{1eq,2ax}$ = 3.6, $J_{1eq,1ax}$ = −13.4 Hz, H-1eq), 1.82 (ddd, 1H, $J_{12eq,11eq}$ = 2.6, $J_{12eq,11ax}$ = 4.2, $J_{12eq,12ax}$ = −12.8 Hz, H-12eq), 1.71 (dddd, 1 H, $J_{8,7ax}$ = 10.9, $J_{8,14}$ = 10.9, $J_{8,9}$ = 11.1 Hz, H-8), 1.70 (dddd, $J_{11eq,12ax}$ = 4.1, $J_{11eq,9}$ = 4.8, $J_{11eq,11ax}$ = −13.9 Hz, H-11eq), 1.68 (dddd, 1 H, H-7ax), 1.64 (ddddd, 1 H, $J_{2ax,1ax}$ = 13.9 Hz, H-2ax), 1.60 (dddd, 1 H, $J_{15ax,14}$ = 12.8 Hz, H-15ax), 1.53 (dddd, 1 H, $J_{11ax,12ax}$ = 12.5, $J_{11ax,9}$ = 13.0 Hz, H-11ax), 1.34 (ddd, 1 H, H-14), 1.29 (ddd, 1 H, H-12ax), 1.11 (ddd, 1 H, H-1ax), 1.07 (s, 3 H, H-19), 1.03 (ddd, 1 H, H-9), 0.91 (s, 3 H, H-18) ppm.

$^{13}$C NMR (150.9 MHz, $CD_3OD$ and $CDCl_3$): $\delta$ 222.4 (C-17), 140.2 (C-5), 120.5 (C-6), 100.7 (C-1′), 78.1 (C-3), 76.1 (C-3′), 75.8 (C-5′), 73.1 (C-2′), 69.8 (C-4′), 61.1 (C-6′), 51.2 (C-14), 49.8 (C-9), 47.1 (C-13), 38.0 (C-4), 36.7 (C-1), 36.2 (C-10), 35.2 (C-16), 30.9 (C-8), 30.8 (C-12), 30.2 (C-7), 28.9 (C-2), 21.2 (C-15), 19.7 (C-11), 18.4 (C-19), 12.6 (C-18) ppm.

HRMS: calcd. for $C_{25}H_{38}O_7Na$ $[M+Na]^+$ 473.2515; found 473.2513.

*2.9. 3β-(2′,3′-Di-O-benzoyl-4′-(2″,3″,4″,6″-tetra-O-benzoyl-β-D-glucopyranosyl)-α-L-rhamnopyranosyloxy)-androst-5-en-17-one (**13**)*

Synthesized from **3** (17 mg, 0.06 mmol) and **8** (82 mg, 0.07 mmol) according to the general procedure for glycosylation providing **13** as a white foam (82 mg, 88%). $R_f$ = 0.48 (hexane–EtOAc 1:1); $[\alpha]_D$ +15.0 (c 0.2, $CHCl_3$). $^1$H NMR (600.13 MHz, $CDCl_3$): $\delta$ 8.06–7.07 (m, 30 H, arom. *H*), 5.74 (dd, 1 H, $J_{3'',4''}$ = 9.7 Hz, $J_{3'',2''}$ = 9.8 Hz, H-3″), 5.63 (dd, 1 H, $J_{4'',5''}$ = 9.9 Hz, H-4″), 5.49 (dd, 1 H, $J_{3',2'}$ = 3.4 Hz, $J_{3',4'}$ = 9.7 Hz, H-3′), 5.49 (dd, 1 H, $J_{2',1'}$ = 1.5 Hz, H-2′), 5.49 (dd, 1 H, $J_{2'',1''}$ = 7.9 Hz, H-2″), 5.34 (ddd, 1 H, $J_{6,4ax}$ = −1.8, $J_{6,7ax}$ = 2.1, $J_{6,7eq}$ = 5.2 Hz, H-6), 5.08 (d, 1 H, H-1″), 5.01 (d, 1 H, H-1′), 4.78 (dd, 1 H, $J_{6''a,5''}$ = 3.3 Hz, $J_{6''a,6''b}$ = −12.0 Hz, H-6″a), 4.46 (dd, 1 H, $J_{6''b,5''}$ = 5.3 Hz, H-6″b), 4.21 (ddd, 1 H, H-5″), 4.06 (dq, $J_{5',6'}$ = 6.2 Hz, $J_{5',4'}$ = 9.5 Hz, H-5′), 3.49 (dddd, 1 H, $J_{3,2eq}$ = 4.5, $J_{3,4eq}$ = 4.8, $J_{3,4ax}$ = 11.3, $J_{3,2ax}$ = 11.5 Hz, H-3), 2.46 (ddd, 1 H, $J_{16eq,15eq}$ = 1.0, $J_{16eq,15ax}$ = 8.9, $J_{16eq,16ax}$ = −19.3 Hz, H-16eq), 2.35 (ddd, 1 H, $J_{4eq,2ax}$ = −2.3, $J_{4eq,4ax}$ = −13.3 Hz, H-4eq), 2.27 (dddddd, 1 H, $J_{4ax,2eq}$ = −1.1, $J_{4ax,7eq}$ = 2.7, $J_{4ax,7ax}$ = 3.3 Hz, H-4ax), 2.10 (dddd, 1 H, $J_{7eq,8}$ = 5.0, $J_{7eq,7ax}$ = −16.8 Hz, H-7eq), 2.08 (ddd, 1 H, $J_{16ax,15eq}$ = 8.9, $J_{16ax,15ax}$ = 9.3 Hz, H-16ax), 1.94 (dddd, 1 H, $J_{15eq,14}$ = 5.7, $J_{15eq,15ax}$ = −12.4 Hz, H-15eq), 1.93 (dddd, 1 H, $J_{2eq,1ax}$ = 3.9, $J_{2eq,2ax}$ = −12.2 Hz, H-2eq), 1.88 (ddd, 1 H, $J_{1eq,2eq}$ = 3.3, $J_{1eq,2ax}$ = 3.7, $J_{1eq,1ax}$ = −13.8 Hz, H-1eq), 1.86 (ddd, 1 H, $J_{12eq,11eq}$ = 2.7, $J_{12eq,11ax}$ = 4.3, $J_{12eq,12ax}$ = −13.1 Hz, H-12eq), 1.69 (dddd, $J_{11eq,12ax}$ = 4.1, $J_{11eq,9}$ = 4.5, $J_{11eq,11ax}$ = −13.8 Hz, H-11eq), 1.67 (ddddd, 1 H, $J_{2ax,1ax}$ = 13.4 Hz, H-2ax), 1.66 (dddd, 1 H, $J_{8,7ax}$ = 10.2, $J_{8,14}$ = 10.8, $J_{8,9}$ = 11.0 Hz, H-8), 1.62 (dddd, 1 H, H-7ax), 1.54 (dddd, 1 H, $J_{15ax,14}$ = 12.9 Hz, H-15ax), 1.50 (dddd, 1 H, $J_{11ax,9}$ = 11.0, $J_{11ax,12ax}$ = 13.4 Hz, H-11ax), 1.42 (d, 1 H, H-6′), 1.29 (ddd, 1 H, H-12ax), 1.28 (ddd, 1 H, H-14), 1.08 (ddd, 1 H, H-1ax), 1.05 (s, 3 H, H-19), 1.00 (ddd, 1 H, H-9), 0.89 (s, 3 H, H-18) ppm.

$^{13}$C NMR (150.9 MHz, $CDCl_3$): $\delta$ 221.2 (C-17), 166.1 (6″-OCOPh), 165.7 (3″-OCOPh), 165.4 (2′-OCOPh), 165.2 (4″-OCOPh, 2″-OCOPh), 164.8 (3′-OCOPh), 140.7 (C-5), 133.5–128.1 (arom. *C*), 121.1 (C-6), 101.4 (C-1″), 95.9 (C-1′), 77.9 (C-3), 77.8 (C-4′), 72.8 (C-3″), 72.3 (C-3′), 71.9 (C-5″), 71.8 (C-2″), 71.2 (C-2′), 69.8 (C-4″), 67.0 (C-5′), 63.0 (C-6″), 51.8 (C-14), 50.2 (C-9), 47.6 (C-13), 38.5 (C-4), 37.2 (C-1), 36.9 (C-10), 35.9 (C-16), 31.5 (C-8), 31.4 (C-12), 30.8 (C-7), 29.3 (C-2), 21.9 (C-15), 20.3 (C-11), 19.4 (C-19), 18.0 (C-6′), 13.5 (C-18) ppm.

HRMS: calcd. for $C_{73}H_{72}O_{17}Na$ $[M+Na]^+$ 1243.4667; found 1243.4642.

*2.10. 3β-(4′-(β-D-Glucopyranosyl)-α-L-rhamnopyranosyloxy)-androst-5-en-17-one (**14**)*

Synthesized from **13** (55 mg, 0.05 mmol) according to the general procedure for deprotection providing **14** as a white solid (24 mg, 90%). $R_f$ = 0.35 (MeOH–$CH_2Cl_2$ 1:5); $[\alpha]_D$ −32.1 (c 1.0, MeOH). $^1$H NMR (600.13 MHz, $CD_3OD$): $\delta$ 5.42 (ddd, 1 H, $J_{6,4ax}$ = −1.8, $J_{6,7ax}$ = 2.2, $J_{6,7eq}$ = 5.2 Hz, H-6), 4.82 (d, 1 H, $J_{1',2'}$ = 1.8 Hz, H-1′), 4.58 (d, 1 H, $J_{1'',2''}$ = 7.8 Hz, H-1″), 3.87 (dd, 1 H, $J_{3',2'}$ = 3.3 Hz, $J_{3',4'}$ = 9.7 Hz, H-3′), 4.84 (dd, 1 H, $J_{6''a,5''}$ = 2.4 Hz, $J_{6''a,6''b}$ = −11.9 Hz, H-6″a), 3.77 (dd, 1 H, H-2′), 3.70 (dq, $J_{5',6'}$ = 6.4 Hz, $J_{5',4'}$ = 9.5 Hz, H-5′), 3.69 (dd, 1 H, $J_{6''b,5''}$ = 5.3 Hz, H-6″b), 3.60 (dd, 1 H, H-4′), 3.44 (dddd, 1 H, $J_{3,2eq}$ = 4.4, $J_{3,4eq}$ = 4.7, $J_{3,2ax}$ = 11.2, $J_{3,4ax}$ = 11.6 Hz, H-3), 3.36 (dd, 1 H, $J_{3'',2''}$ = 9.2 Hz, $J_{3'',4''}$ = 9.6 Hz, H-3″), 3.30 (dd, 1 H, $J_{4'',5''}$ = 9.0 Hz, H-4″), 3.26 (ddd, 1 H, H-5″), 3.21 (dd, 1 H, H-2″), 2.45 (ddd, 1 H, $J_{16eq,15eq}$ = 0.4, $J_{16eq,15ax}$ = 8.9, $J_{16eq,16ax}$ = −19.5 Hz, H-16eq), 2.38 (ddd, 1 H, $J_{4eq,2ax}$ = −2.3, $J_{4eq,4ax}$ = −13.1 Hz, H-4eq), 2.19 (dddddd, 1 H, $J_{4ax,2eq}$ = −2.2, $J_{4ax,7eq}$ = 2.9, $J_{4ax,7ax}$ = 3.2 Hz, H-4ax), 2.13 (dddd, 1 H, $J_{7eq,8}$ = 4.7, $J_{7eq,7ax}$ = −16.6 Hz, H-7eq), 2.08 (ddd, 1 H, $J_{16ax,15eq}$ = 9.0, $J_{16ax,15ax}$ = 9.4 Hz, H-16ax), 1.97 (dddd, 1 H, $J_{15eq,14}$ = 5.8, $J_{15eq,15ax}$ = −12.5 Hz, H-15eq), 1.91 (ddd, 1 H, $J_{1eq,2eq}$ = 3.4, $J_{1eq,2ax}$ = 3.8, $J_{1eq,1ax}$ = −13.4 Hz, H-1eq), 1.85 (dddd, 1 H, $J_{2eq,1ax}$ = 3.8, $J_{2eq,2ax}$ = −12.7 Hz, H-2eq), 1.79 (ddd, 1 H, $J_{12eq,11eq}$ = 2.6, $J_{12eq,11ax}$ = 4.2, $J_{12eq,12ax}$ = −12.7 Hz, H-12eq), 1.70 (dddd, 1 H, $J_{8,7ax}$ = 10.5, $J_{8,14}$ = 11.1, $J_{8,9}$ = 11.2 Hz, H-8), 1.70 (dddd, $J_{11eq,12ax}$ = 4.3, $J_{11eq,9}$ = 5.0, $J_{11eq,11ax}$ = −13.4 Hz, H-11eq), 1.68 (dddd, 1 H, H-7ax), 1.59 (dddd, 1 H, $J_{15ax,14}$ = 12.7 Hz, H-15ax), 1.58 (ddddd, 1 H, $J_{2ax,1ax}$ = 13.9 Hz, H-2ax), 1.54 (dddd, 1 H, $J_{11ax,9}$ = 12.6, $J_{11ax,12ax}$ = 13.5 Hz, H-11ax), 1.34 (ddd, 1 H, H-14), 1.31 (d, 1 H, H-6′), 1.27 (ddd, 1 H, H-12ax), 1.11 (ddd, 1 H, H-1ax), 1.06 (s, 3 H, H-19), 1.04 (ddd, 1 H, H-9), 0.89 (s, 3 H, H-18) ppm.

$^{13}$C NMR (150.9 MHz, $CD_3OD$): $\delta$ 223.8 (C-17), 141.9 (C-5), 122.4 (C-6), 105.7 (C-1″), 99.6 (C-1′), 83.6 (C-4′), 78.2 (C-3″), 78.0 (C-5″), 77.9 (C-3), 76.1 (C-2″), 72.6 (C-2′), 72.4 (C-3′), 71.5 (C-4″), 68.5 (C-5′), 62.7 (C-6″), 53.0 (C-14), 51.8 (C-9), 48.8 (C-13), 39.5 (C-4), 38.5 (C-1), 38.0 (C-10), 36.7 (C-16), 32.8 (C-8), 32.7 (C-12), 31.9 (C-7), 30.6 (C-2), 22.8 (C-15), 21.5 (C-11), 19.8 (C-19), 18.1 (C-6′), 13.9 (C-18) ppm.

HRMS: calcd. for $C_{31}H_{48}O_{11}Na$ $[M+Na]^+$ 619.3094; found 619.3080.

## 3. Results and discussion

### 3.1. Synthesis

Previously, at our laboratories, we have completed a thorough investigation on finding an optimal glycosyl donor for glycosylation of steroids. After screening of several donors, containing both electron withdrawing and electron donating protective groups, promoters and reaction conditions, benzoylated imidate donors were identified as superior in terms of efficiency and minimal waste generation [9]. Additional advantages of the imidate donors are the simple and rapid methods applied for their preparation [10]. Glycosylation of steroids has received significant attention in recent years as exemplified by several papers dealing with this subject [11]. While several donors have been successfully applied for glycosylation of sapogenins earlier, the high efficiencies displayed by benzoylated imidate donors led us to implement them in the present study [12]. In previous work, we have reported the improved synthesis of donors **1** and **2** according to a modified literature procedure [9,13]. In the present study, these procedures were applied to provide donors **1** and **2** in 60% overall yield over three steps, starting from the corresponding unprotected sugars [13a] (Fig. 2).

While the synthesis of the methyl glycoside of scillabiose has been published earlier [14], glycosylations utilizing scillabiose based donors have appeared in one report only [15]. In the previous work, however, the thioglycoside utilized resulted in an α:β mixture of the product. Overall, in the synthesis of glycosylated biomolecules, minimization of the number of synthetic steps incorporating the aglycone is often preferred. This is mainly to ensure that the reactive centers in the aglycone moiety remain untouched. Therefore, wherever possible, glycosylations by convergent routes are preferred over linear ones [16]. Furthermore, protective groups should essentially be removable in one step and under mild conditions in order to avoid multiple deprotection sequences which may affect the sensitive functional groups. With these prerequisites in mind, we devised an approach to the preparation of a scillabiose glycosyl donor that could be coupled to a biomolecule by a convergent route and contained only one type of protective groups removable in a single reaction step. The synthesis of the donors **7** and **3** is illusrated in Scheme 1.

In more detail, the present strategy was based on the preparation of a thiophenyl rhamnoside acceptor. The thiophenyl

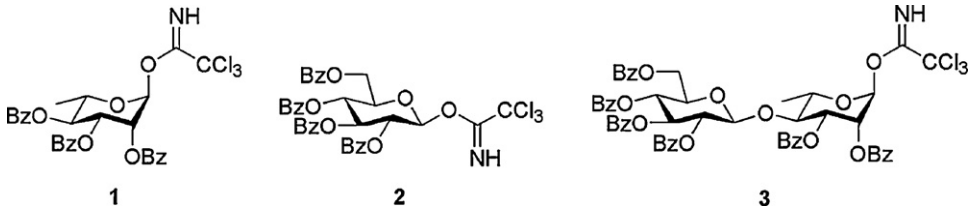
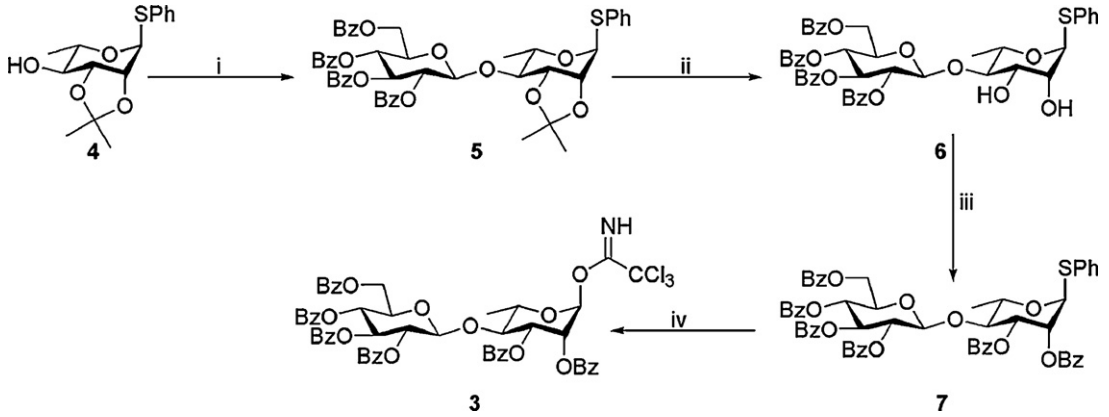
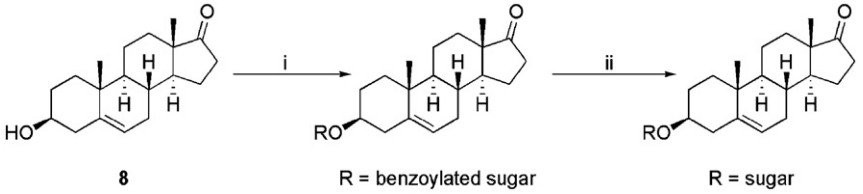


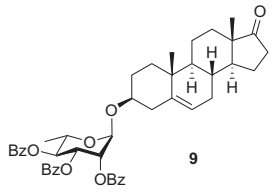
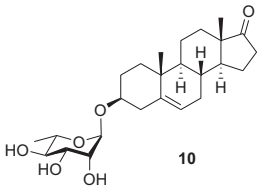
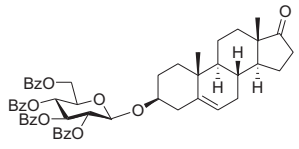
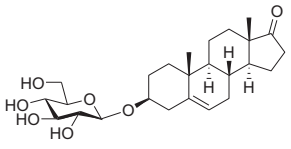
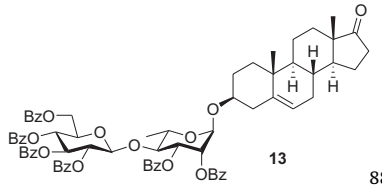
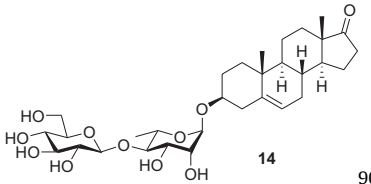
**Fig. 2.** Structures of the benzoylated imidate donors **1**–**3**.



**Scheme 1.** Synthesis of donor **3**: (i) **2**, TMSOTf, DCM, 85%; (ii) 80% AcOH, 80 °C, 96%; (iii) BzCl, pyridine, 89%; (iv) (1) NBS, Acetone:$H_2O$ 10:1, 92%; (2) DBU, $Cl_3CCN$, $CH_2Cl_2$, 77%.

**Table 1**
A summary of the performed glycosylations and deprotections: (i) TMSOTf, corresponding donor, −20 °C, $CH_2Cl_2$; (ii) NaOMe, MeOH:THF 1:1.



| Entry | Glycosyl acceptor | Glycosyl donor | Product | Glycosylation yield[a] | Deprotected product | Deprotection yield[a] |
|---|---|---|---|---|---|---|
| 1 | **8** | **1** |  **9** | Not determined[b] |  **10** | 89 (over two steps) |
| 2 | **8** | **2** |  **11** | Not determined[b] |  **12** | 78 (over two steps) |
| 3 | **8** | **3** |  **13** | 88 |  **14** | 90 |

[a] Isolated yields.
[b] Due to purification difficulties the deprotection was carried out on a mixture of product and starting material.

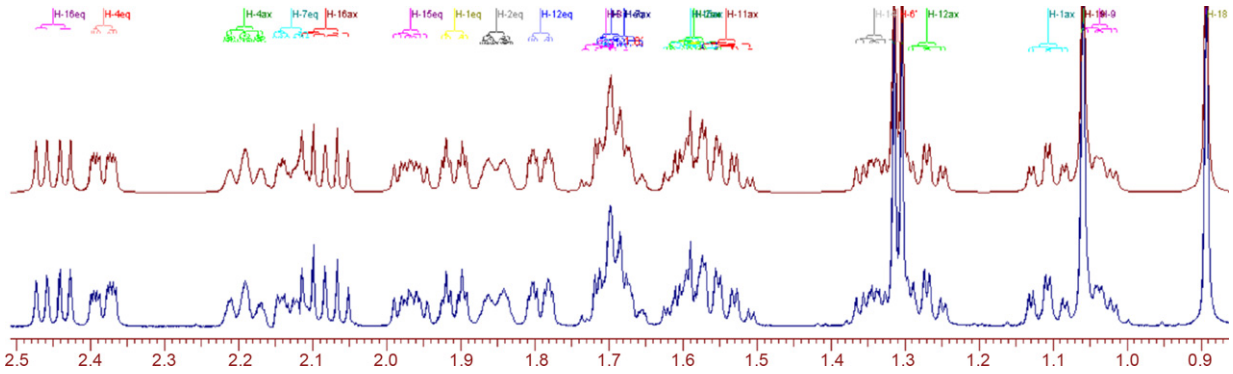



**Fig. 3.** Spectral simulation of the 2.52–0.85 ppm region of the $^1$H NMR spectrum of **14** using PERCH NMR software; top: simulated spectrum, bottom: observed spectrum.

functionality has been shown to be stable under various reaction conditions and, furthermore, it can be selectively activated in the presence of other functionalities and under mild conditions, thereby making it an excellent anomeric protective group [17]. In order to minimize the protective group manipulations needed, the *cis* C-2 and C-3 hydroxyl groups were protected in one step as an isopropylidene acetal [18]. Conveniently, the hydroxyl group at C-4 remains untouched thus directly providing acceptor **4**. In the previous synthesis by Bebault and Dutton, the isopropylidation was followed by an acetylation/deacetylation sequence at the C-4 hydroxyl group [14]. These two steps are, however, not necessary for preparation of acceptor **4** as also shown in a more recent report [19]. In short, the synthesis of acceptor **4** commenced by slightly modified literature procedures involving $BF_3{\cdot}OEt_2$ promoted glycosylation of peracetylated L-rhamnose with thiophenol [20], followed by deacetylation under Zemplén conditions [21] and, as the final step, formation of the 2,3-*O*-isopropylidene acetal in 61% overall yield. Glycosylation of acceptor **4** with donor **2** using TMSOTf as promoter provided the disaccharide **5** in 85% isolated yield [9,12,16a]. While compound **5** could in principle be used as a donor molecule as such, in previous work a similar donor was shown to yield a mixture of products with poor selectivity [15]. In addition, removal of the 2,3-*O*-isopropylidene acetal requires harsh conditions which may affect sensitive functionalities in biomolecules [22]. Furthermore, in contrast to ester protective groups at C-2, the isopropylidene acetal does not enhance α-selectivity in the glycosylation. For improving the desired properties, in particular neighboring group participation, displayed by ester protective groups, the isopropylidene acetal was cleaved under acidic conditions (80% AcOH) at 80 °C providing **6** in 96% yield. The newly formed hydroxyl groups were benzoylated thus providing the thiodonor **7** in 89% yield. In our previous work, the best results in glycosidation of steroid alcohols were obtained by TMSOTf promoted activation of an imidate donor [9]. Consequently, in the present study, the scillabiose thiodonor was converted to an imidate donor. The thiophenyl group was activated with NBS, hydrolyzed [23] and the newly formed hemiacetal converted to the corresponding imidate with DBU and trichloroacetonitrile [10], thus providing donor **3** in 71% isolated yield over two steps (Table 1).

With donors **1**, **2**, **3** and **7** at hand, the focus was shifted to the glycosylation study. 3β-Hydroxy-androst-5-en-17-one (**8**) was used as a model substrate in the glycosylation reaction. This steroid carries identical stereochemistry (at C-3, C-8, C-9, C-13 and C-14) to the aglycones of transvaalin and hellebrin and was therefore selected as a suitable model [1,3]. As mentioned earlier, TMSOTf promoted glycosylation of steroids with imidate donors was, in our previous study, the most efficient methodology [9]. In order to explore the suitability of this strategy to the present study, we first evaluated the monosaccharide donors **1** and **2**. With both of these donors glycosylations proceeded smoothly and, after deprotection under Zemplén conditions [21], the unprotected glycosteroids **10** and **12** were isolated in high yields (77–89% over two steps). Next, we explored the possibility of utilizing donor **7** in the glycosidation. The use of this donor would be desirable as it shortens the reaction sequence by two steps. Due to the presence of alkene functionality in steroid **8**, the commonly employed activation procedure using NIS/TfOH was not feasible [17]. Instead, activation of the thioglycoside using the methodology of Crich was employed [24]. The key features of this method are based on low temperature activation of a thioglycoside with BSP, TTBP and $Tf_2O$, thereby generating a reacting triflate in situ. By use of this activation protocol, moderate conversion (~50%) was obtained. In order to evaluate and compare the reactivity of the scillabiose donors **7** and **3**, the imidate donor was tested next. When the glycosylation reaction was performed using donor **3** and following the standard activation protocol, the glycosylated steroid could be isolated in 88% yield. One step debenzoylation under Zemplén conditions provided the deprotected glycosteroid **14** in 90% yield. THF was required as co-solvent in the deprotection due to the amphiphilicity of this molecule in the deprotected state. With the final products being amphiphilic ($R_f$ values of ~0.3–0.7 in MeOH:$CH_2Cl_2$ 1:5), they were also amenable to purification by column chromatography thus enabling a simple method for removal of the methyl benzoate formed during the final deprotection step.

### 3.2. Characterization by NMR spectroscopy

Steroids and glycosteroids commonly exhibit complicated NMR spectra. Thus, special attention was focused on the NMR spectroscopic characterization of the compounds prepared in present work. While some reports on complete NMR spectroscopic characterization of this class of compounds exist, the spectral data is of importance as reference material, providing also valuable structural information on the solution phase properties of the compounds. The NMR spectroscopic assignation is exemplified by the characterization of compound **14** with all other compounds being characterized in an analogous fashion.

The glycone part of **14** was characterized first starting with the well separated anomeric protons of rhamnose (d at 4.82 ppm) and glucose (d at 4.58 ppm). Since some of the signals from these two sugars were overlapping in the $^1$H NMR-spectrum (especially the region between 3.90 and 3.65 ppm), the anomeric signals where targeted by selective excitation (400 ms spinlock time) utilizing the 1D-TOCSY experiment [25]. By use of this measurement technique, these two spin-systems could be easily separated thus providing a convenient way of identifying the signals corresponding to each sugar unit. By use of COSY and HSQC, all signals from the glycone part were identified next in both $^1$H and $^{13}$C NMR-spectra. From the HMBC spectrum the correlation between H-1′ and C-3 (77.9 ppm)

and H-3 (3.44 ppm) and C-1′ (99.6 ppm) was clearly visible, thus providing a starting point for the characterization of the aglycone. By use of COSY, both of the H-2 and H-4 protons were identified. The corresponding signals in the $^{13}$C NMR spectra were easily identified by use of HSQC. Next, the spectral assignment was continued from the well separated signals of H-6 (ddd at 5.42 ppm), C-6 (122.4 ppm) and C-5 (141.9 ppm). From the HMBC correlation of C-5, H-19 (s at 1.06 ppm) and H-1eq (ddd at 1.91 ppm) were identified. By use of HSQC, the corresponding carbon signals were solved. From the COSY spectra the correlations between H-6 and both of the H-7 protons were visible. Furthermore, the HMBC correlation between H-6 and C-10 was used to completely solve the A-ring of the steroid. The HMBC correlation of H-19–C-9 and H-6–C-8 enabled the complete characterization of the B-ring. For solving the C- and D-rings, the carbonyl carbon of C-17 (223.8 ppm) and the methyl group connected to C-13 were selected as starting points. From the HMBC correlations of C-17, both of the H-15 protons could be found. From the HMBC correlations of H-18 it was possible to identify C-12, C-13 and C-14 carbon atoms. By use of COSY and HSQC, the C- and D-rings could now be completely solved. Although assignations of this type can be considered straightforward or routine, obtaining accurate coupling constants from such systems is extremely difficult. This is mainly due to the severe overlap of signals in the steroid region. We have in previous work described the complete assignation of a β-1,2-linked mannotetraose by use of the NMR simulation software PERCH [8c]. Also in the present work this program was utilized for complete assignment of the proton spectra of the compounds prepared, exemplified by the high field region of the $^{1}$H NMR spectrum of **14** shown in Fig. 3. The standard NMR spectroscopic techniques ($^{1}$H, $^{13}$C, 1D-TOCSY, COSY, HSQC and HMBC), in combination with the simulation software, thus enabled the complete assignation of all glycosteroids synthesized in the present work, being also applicable to the characterization of other types of molecules in the future.

## 4. Conclusions

To conclude, a viable approach to the synthesis and coupling of the glycone of transvaalin and hellebrin to a model steroid has been developed. This convergent approach is appealing due to its high efficiency and simple deprotection and may find further use in the total synthesis of naturally occurring saponins and related compounds sharing the same glycone [1,3,5,6]. Due to the wide distribution of this glycone in nature, the complete NMR spectroscopic characterization of all compounds prepared here have been provided as reference material. In addition, glycosylations were performed with the monosaccharide constituents of scillabiose, thereby providing a limited series of glycosylated steroids for potential future evaluation of the effects of the glycone structure on the overall biological activity. All compounds reported here will in future work be subjected to screening for cytotoxic activity.

## Supporting information

The $^{1}$H and $^{13}$C NMR spectra of all new compounds are available on the www or from the author.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.steroids.2011.02.010.

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