Journal of Steroid Biochemistry & Molecular Biology 137 (2013) 345–354

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

# Synthesis of fatty acyl derivatives of 24-epibrassinolide

Vladimir A. Khripach*, Vladimir N. Zhabinskii, Dmitrii V. Tsavlovskii

*Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, Kuprevich str., 5/2, 220141 Minsk, Belarus*

ARTICLE INFO

*Article history:*
Received 30 October 2012
Received in revised form 15 January 2013
Accepted 31 January 2013

*Keywords:*
Brassinosteroids
Epibrassinolide metabolites
Fatty acid esters
Cyclic methylboronates

ABSTRACT

A number of fatty acid (palmitic, myristic and lauric) esters (both 3α- and 3β-isomers) of epibrassinolide has been prepared as reference compounds for metabolic studies. Selective protection of the three of four hydroxyl groups of epibrassinolide was successively performed first as cyclic 22,23-methylboronates and then as 2α-benzyl ethers. α,β-Inversion of C-3 hydroxyl group was achieved through a consecutive oxidation-reduction reactions or by a nucleophilic substitution of the 3α-mesylates. Treatment of the 3α- and 3β-alcohols with palmitic, myristinic or lauric acid chlorides gave the corresponding esters. The hydrolysis of 22,23-methylboronates was performed after their transformation into 2-hydroxy-1,3,2-dioxaborolanes using a cation exchange column with DOWEX 50WX8 in $NH_4^+$ form. Hydrogenolysis of the benzyl ethers catalyzed by palladium yielded the target compounds.

This article is part of a Special Issue entitled 'Synthesis and biological testing of steroid derivatives as inhibitors'.



## 1. Introduction

Since the isolation of the first steroidal plant hormone brassinolide in 1979 [1], a lot of knowledge has been accumulated on the biosynthesis of this class of phytohormones called brassinosteroids [2–5]. Relatively little, however, is known about their metabolic fate. At least in part it is because of poor availability of brassinosteroid metabolites (real or supposed), which could be used as reference compounds in biochemical studies.

Esterification is one of the metabolic processes occurring with brassinosteroids [6] that contribute to their homeostasis. A number of fatty acid conjugates **1a–c** and **2a–c** (Fig. 1) were identified on exogenous application of epicastasterone and epibrassinolide to cell suspension culture of *Ornithopus sativus* [7]. The acyl components for both brassinosteroid conjugate types were found to be palmitic, myristic and lauric acids. Teasterone myristate **3a** [8] and teasterone laurate **3b** [9] were identified in studies of lily cell cultures. These acyl conjugates were supposed to be the reversible teasterone storage forms during the biosynthesis of brassinolide [10]. BAHD acyltransferase-like protein may be involved in transfer of the acyl groups to brassinosteroids in *Arabidopsis* [11].

Since bringing epibrassinolide into agricultural practice as crop-yield-increasing and plant-protecting agent [2,12], we became interested in studying all aspects of this steroidal phytohormone and, namely, in its selective modifications with fatty acids mimicking metabolic pathways. Therefore, the task of the present work was the preparation of epibrassinolide metabolites (including supposed ones), having a fatty acid ester moiety at C-3, for subsequent metabolic studies.

## 2. Experimental

### 2.1. General

Melting points were recorded on a Boetius micro-melting point apparatus and are uncorrected. $^1H$ and $^{13}C$ NMR spectra were obtained using a Bruker AVANCE 500 (Bruker Biospin, Rheinstetten, Germany) spectrometer in $CDCl_3$ (if not stated otherwise) operating at 500 MHz for $^1H$ and 125 MHz for $^{13}C$. $^1H$ chemical shifts were determined relative to the residual solvent peaks ($CHCl_3$, $\delta$ 7.26; $C_5D_5N$, $\delta$ 7.58), and coupling constants are reported in Hz. Carbon spectra were referenced internally to solvent signals, using values $\delta$ 77.00 for $CDCl_3$ and $\delta$ 135.91 for $C_5D_5N$. Mass spectra were performed on a LCQ Fleet mass spectrometer (Thermo Electron Corporation, USA) with an APCI or ESI source. Spectra were collected in a positive ion mode and analyzed by Xcalibur software. Chemicals were purchased from Aldrich and Fluka and used as received unless otherwise noted. All solvents were purified according to standard methods [13]. Reactions were monitored by TLC using aluminium or plastic sheets, silica gel 60 $F_{254}$ precoated (Merck Art. 5715). Column chromatography was carried out on Kieselgel 60 (Merck Art. 7734).

* Corresponding author. Tel.: +375 172 678 647; fax: +375 172 678 647.
*E-mail address:* khripach@iboch.bas-net.by (V.A. Khripach).

0960-0760/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jsbmb.2013.01.016

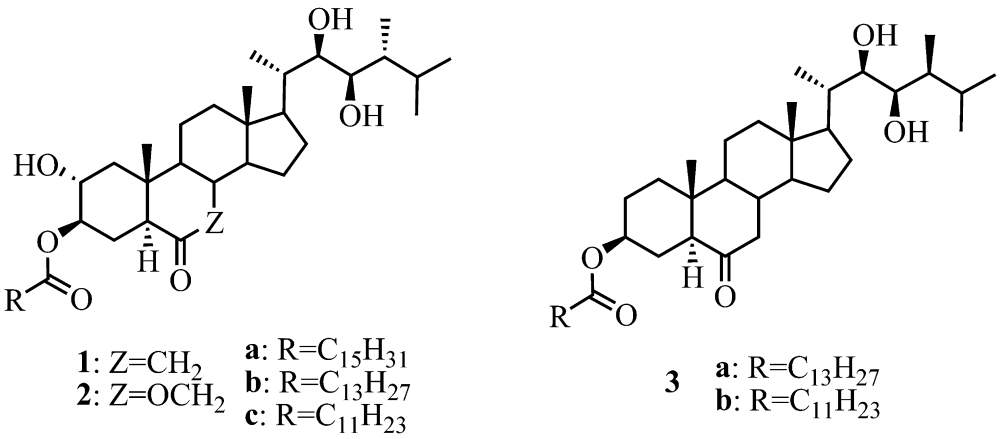



**Fig. 1.** Structures of natural fatty acid derivatives of brassinosteroids: 3β-palmitate- (**1a**), 3β-myristate- (**1b**) and 3β-laurate- (**1c**) of 3,24-bisepicastasterone; 3β-palmitate- (**2a**), 3β-myristate- (**2b**) and 3β-laurate- (**2c**) of 3,24-bisepibrassinolide; 3β-myristate- (**3a**) and 3β-laurate- (**3b**) of teasterone.

### *2.2. Synthesis of compounds*

#### *2.2.1. (22R,23R,24R)-2α-(tert-Butyldimethylsilyl)-3α-palmitoyl-22,23-dihydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one 22,23-methylboronate (**7**)*

A mixture of the silyl ether **6** (150 mg, 0.24 mmol, prepared according to [14]), palmitoyl chloride (158 μL, 142 mg, 0.52 mmol), *N*,*N*-dimethylaminopyridine (32 mg, 0.26 mmol), and pyridine (0.9 mL) was stirred at room temperature overnight. Then solvents were evaporated *in vacuo* and the residue was purified by column chromatography on silica gel (toluene–EtOAc = 20:1 ⇒ 7:1) to afford unreacted starting alcohol **6** (62 mg) and ester **7** (84 mg, 41%, 69% based on reacted starting material) as an oil. $^1$H NMR $\delta$: 0.03 (s, 3H, $CH_3$—Si≡), 0.05 (s, 3H, $CH_3$—Si≡), 0.28 (s, 3H, >B—$CH_3$), 0.69 (d, $J$ = 6.6 Hz, 3H, >$CHCH_3$), 0.70 (s, 3H, C18-H), 0.80–0.94 (m, 21H), 1.21–1.29 (m, 26H, —$OCOCH_2$—$(CH_2)_{13}$—$CH_3$), 2.34 (t, $J$ = 7.4 Hz, 2H, —$OCOCH_2C_{14}H_{29}$), 2.92 (dd, $J$ = 12.2, 4.4 Hz, 1H, C5-H), 3.71 (ddd, $J$ = 11.6, 4.0, 2.7 Hz, 1H, C2-H), 3.77 (dd, $J$ = 8.7, 5.2 Hz, 1H, C23-H), 4.03 (dd, $J$ = 12.3, 9.3 Hz, 1H, 1H, C7-$H_\alpha$), 4.07–4.15 (m, 2H, C7- and C22-H), 5.21 (br.s, 1H, C3-H). $^{13}$C NMR $\delta$: −4.95, −4.86, 9.12, 11.43, 11.57, 14.10, 15.66, 16.43, 18.14, 21.02, 22.20, 22.67, 24.78, 25.17, 25.76, 27.09, 27.72, 29.17, 29.34, 29.40, 29.52, 29.67, 31.90, 34.58, 38.22, 39.13, 39.26, 41.06, 41.95, 42.42, 43.30, 44.36, 51.06, 52.26, 58.33, 67.41, 70.11, 70.52, 81.95, 82.35, 172.88, 175.67. MS ($APCI^+$) $m/z$ (%): 857.4 ($[M+H]^+$, 100).

#### *2.2.2. Desilylation of (22R,23R,24R)-2α-(tert-butyldimethylsilyl)-3α-palmitoyl-22,23-dihydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one 22,23-methylboronate (**7**) (synthesis of compounds (**8**) and (**9**))*

Silyl ether **7** (54 mg, 0.063 mmol) was dissolved in 1 M solution of TBAF in THF (540 μL, 0.54 mmol), the reaction mixture was stirred at room temperature overnight, and then worked up with saturated solution of $NH_4Cl$ (1 mL). Aqueous layer was extracted with EtOAc (3× 1 mL). The organic layer was dried ($Na_2SO_4$) and evaporated. The residue was chromatographed on silica gel (petrol ether-EtOAc = 10:1 ⇒ 7:3) to give compounds **8** and **9**.

*(22R,23R,24R)-3α-Palmitoyl-2α,22,23-trihydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one 22,23-methylboronate **8*** (24 mg, 51%) as an oil. $^1$H NMR $\delta$: 0.27 (s, 3H, >B—$CH_3$), 0.69 (s, 3H, C18-H), 0.69 (d, $J$ = 6.3 Hz, 3H, >$CHCH_3$), 0.83 (d, $J$ = 6.8 Hz, 3H, >$CHCH_3$), 0.85–0.89 (m, 6H, —$CH_3$), 0.91 (d, $J$ = 6.9 Hz, 3H, >$CHCH_3$), 0.96 (s, 3H, C19-H), 1.21–1.29 (m, 26H, —$OCOCH_2$—$(CH_2)_{13}$—$CH_3$), 2.31 (t, $J$ = 7.4 Hz, 2H, —$OCOCH_2C_{14}H_{29}$), 3.16 (dd, $J$ = 12.2, 4.3 Hz, 1H, C5-H), 3.77 (dd, $J$ = 8.7, 5.2 Hz, 1H, C23-H), 4.03–4.15 (m, 4H, C2-, C22- and C7-H), 4.84 (ddd, $J$ = 12.2, 4.3, 2.2 Hz, 1H, C3-H). $^{13}$C NMR $\delta$: 9.11, 11.42, 11.57, 14.10, 15.30, 16.42, 21.02, 22.21, 22.66, 24.75, 24.93, 27.07, 27.73, 29.10, 29.23, 29.33, 29.45, 29.66, 30.82, 31.90, 34.43, 37.74, 38.41, 39.17, 39.25, 40.97, 41.06, 42.40, 44.35, 51.08, 52.23, 58.11, 66.46, 70.40, 71.36, 81.94, 82.36, 172.83, 175.83. MS ($APCI^+$) $m/z$ (%): 743.4 ($[M+H]^+$, 100).

*(22R,23R,24R)-2α-Palmitoyl-3α,22,23-trihydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one 22,23-methylboronate **9*** (10 mg, 21%) as an oil. $^1$H NMR $\delta$: 0.28 (s, 3H, >B—$CH_3$), 0.70 (d, $J$ = 6.6 Hz, 3H, >$CHCH_3$), 0.70 (s, 3H, C18-H), 0.84 (d, $J$ = 6.8 Hz, 3H, >$CHCH_3$), 0.85–0.90 (m, 6H, —$CH_3$), 0.92 (d, $J$ = 6.9 Hz, 3H, >$CHCH_3$), 0.94 (s, 3H, C19-H), 2.39 (t, $J$ = 7.3 Hz, 2H, —$OCOCH_2C_{14}H_{29}$), 2.96 (dd, $J$ = 12.3, 4.3 Hz, 1H, C5-H), 3.78 (dd, $J$ = 8.8, 5.2 Hz, 1H, C23-H), 3.83 (ddd, $J$ = 12.0, 4.0, 2.5 Hz, 1H, C3-H), 4.01–4.16 (m, 3H, C7- and C22-H), 5.23 (br.s, 1H, C2-H). $^{13}$C NMR $\delta$: 9.13, 11.45, 11.58, 14.11, 15.51, 16.44, 21.03, 22.19, 22.68, 24.78, 25.07, 27.10, 27.75, 29.15, 29.35, 29.53, 29.68, 31.91, 34.50, 38.25, 39.14, 39.23, 41.10, 42.05, 42.26, 42.44, 44.37, 51.10, 52.24, 58.41, 67.36, 70.58, 71.28, 81.96, 82.36, 174.57, 175.45. MS ($APCI^+$) $m/z$ (%): 743.2 ($[M+H]^+$, 100).

#### *2.2.3. Benzylation of (22R,23R,24R)-2α,3α,22,23-tetrahydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one 22,23-methylboronate (**5**) (synthesis of compounds (**10–12**))*

*Variant A.* A mixture of diol **5** (146 mg, 0.29 mmol, prepared according to [14]), benzyl bromide (44.2 μL, 0.063 mg, 0.37 mmol) and NaH (11 mg, 0.46 mmol) in dry THF (1.5 mL) was stirred at 0 °C for 2 h. Then it was diluted with saturated $NH_4Cl$ (2 mL) and extracted with EtOAc (3× 5 mL). The combined organic layers were dried ($Na_2SO_4$), and concentrated. The residue was purified by column chromatography on silica gel (toluene–EtOAc = 10:1 ⇒ 1:2) to give dibenzyl compound **12** (14 mg, 7%), monobenzyl compound **11** (10 mg, 6%), monobenzyl compound **10** (22 mg, 13%) and starting diol **5** (94 mg, 64%).

*(22R,23R,24R)-2α,3α-Dibenzyloxy-22,23-dihydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one 22,23-methylboronate **12*** (14 mg, 7%) as an oil. $^1$H NMR $\delta$: 0.28 (s, 3H, >B—$CH_3$), 0.69 (s, 3H, C18-H), 0.70 (d, $J$ = 6.9 Hz, 3H, >$CHCH_3$), 0.84 (d, $J$ = 6.8 Hz, 3H, >$CHCH_3$), 0.88 (s, 3H, C19-H), 0.89 (d, $J$ = 6.8 Hz, 3H, >$CHCH_3$), 0.92 (d, $J$ = 6.9 Hz, 3H, >$CHCH_3$), 3.07 (dd, $J$ = 12.0, 4.5 Hz, 1H, C5-H), 3.43 (ddd, $J$ = 12.2, 4.2, 2.2 Hz, 1H, C2-H), 3.78 (dd, $J$ = 8.8, 5.2 Hz, 1H, C23-H), 3.94 (br.s, 1H, C3-H), 3.99–4.09 (m, 2H, C7-H), 4.13 (d, $J$ = 4.9 Hz, 1H, C22-H), 4.47 (d, $J$ = 11.9 Hz, 1H, —$CH_2$Ph), 4.55 (d, $J$ = 11.9 Hz, 1H, —$CH_2$Ph), 4.65 (d, $J$ = 12.4 Hz, 1H, —$CH_2$Ph), 4.72 (d, $J$ = 12.4 Hz, 1H, —$CH_2$Ph), 7.26–7.41 (m, 10H, Ph). $^{13}$C NMR $\delta$: 9.14, 11.44, 11.49, 11.59, 15.73, 16.47, 21.04, 22.21, 24.78, 27.11, 27.78, 29.53, 38.18, 39.17, 39.25, 40.09, 41.14, 41.99, 42.44, 44.37, 51.03, 52.03, 52.21, 58.10, 70.38, 70.50, 71.44, 71.84, 76.13, 81.96, 82.36,

127.47, 127.52, 127.57, 128.38, 139.04, 176.38. MS (APCI$^+$) *m/z* (%): 685.5 ([M+H]$^+$, 100).

*(22R,23R,24R)-3α-Benzyloxy-2α,22,23-trihydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one 22,23-methylboronate* ***11*** (10 mg, 6%) as an oil. $^1$H NMR $\delta$ 0.28 (s, 3H, >B—$CH_3$), 0.69 (s, 3H, C18-H), 0.70 (d, $J$ = 6.8 Hz, 3H, >$CHCH_3$), 0.84 (d, $J$ = 6.8 Hz, 3H, >$CHCH_3$), 0.88 (d, $J$ = 6.6 Hz, 3H, >$CHCH_3$), 0.91 (d, $J$ = 6.8 Hz, 3H, >$CHCH_3$), 0.92 (s, 3H, C19-H), 2.95 (dd, $J$ = 12.0, 4.5 Hz, 1H, C5-H), 3.62 (ddd, $J$ = 12.3, 4.5, 3.2 Hz, 1H, C2-H), 3.78 (dd, $J$ = 8.8, 5.0 Hz, 1H, C23-H), 4.00 (dd, $J$ = 12.3, 9.3 Hz, 1H, C3-H), 4.05–4.12 (m, 2H, C7-H), 4.13 (d, $J$ = 5.0 Hz, 1H, C22-H), 4.49 (d, 1H d, $J$ = 11.9 Hz, 1H, —$CH_2$Ph), 4.66 (d, $J$ = 11.9 Hz, 1H, —$CH_2$Ph), 7.29–7.42 (m, 5H, Ph). $^{13}$C NMR $\delta$: 9.13, 10.74, 11.44, 11.56, 15.36, 16.45, 21.03, 22.15, 24.78, 27.11, 27.75, 28.31, 38.37, 39.16, 39.27, 41.10, 41.55, 42.43, 43.26, 44.37, 51.06, 52.24, 58.16, 67.83, 70.56, 71.13, 75.72, 81.96, 82.37, 127.60, 127.91, 128.57, 176.14. MS (APCI$^+$) *m/z* (%): 595.7 ([M+H]$^+$, 100).

*(22R,23R,24R)-2α-Benzyloxy-3α,22,23-trihydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one 22,23-methylboronate* ***10*** (22 mg, 13%). Mp 124–126 °C (ether-hexane). $^1$H NMR $\delta$: 0.27 (s, 3H, >B—$CH_3$), 0.69 (s, 3H, C18-H), 0.70 (d, $J$ = 6.2 Hz, 3H, >$CHCH_3$), 0.84 (d, $J$ = 6.8 Hz, 3H, >$CHCH_3$), 0.88 (s, 3H, C19-H), 0.92 (d, $J$ = 6.9 Hz, 3H, >$CHCH_3$), 3.15 (dd, $J$ = 11.9, 4.3 Hz, 1H, C5-H), 3.45 (d, $J$ = 11.0 Hz, 1H, C2-H), 3.78 (dd, $J$ = 8.3, 5.0 Hz, 1H, C23-H), 4.08 (m, 2H, C7-H), 4.13 (d, $J$ = 5.0 Hz, 1H, C22-H), 4.17 (s, 1H, C3-H), 4.55 (d, $J$ = 11.6 Hz, 1H, —$CH_2$Ph), 4.60 (d, $J$ = 11.6 Hz, 1H, —$CH_2$Ph), 7.39–7.28 (m, 5H, Ph). $^{13}$C NMR $\delta$: 9.14, 11.44, 11.60, 15.54, 16.46, 21.03, 22.23, 24.77, 27.11, 27.75, 30.32, 38.13, 38.91, 39.25, 39.28, 41.04, 41.12, 42.44, 44.37, 51.11, 52.22, 58.21, 65.18, 70.36, 70.47, 75.44, 81.96, 82.35, 127.64, 127.93, 128.57, 138.01, 176.20. MS (APCI$^+$) *m/z* (%): 595.2 ([M+H]$^+$, 100).

*Variant B.* A mixture of diol **5** (100 mg, 0.20 mmol), freshly prepared silver oxide (69 mg, 0.30 mmol), and benzyl bromide (47 μL, 0.067 mg, 0.39 mmol) in dry $CH_2Cl_2$ (2 mL) was stirred at room temperature for 4 h. Then it was filtered through a short pad of silica gel followed by washing with $CH_2Cl_2$ (5 mL) and EtOAc (5 mL). After concentration under vacuum, the crude product was separated into four components by column chromatography on silica gel (toluene–EtOAc = 10:1 ⇒ 1:2) to give dibenzyl compound **12** (10 mg, 7%), monobenzyl compound **11** (23 mg, 19%), monobenzyl compound **10** (67 mg, 57%) and starting diol **5** (12 mg, 12%).

*Variant C.* A solution of diol **5** (210 mg, 0.42 mmol) and dibutyl tin oxide (130 mg, 0.52 mmol) in dry toluene (30 mL) was refluxed in a round bottomed flask equipped with a Dean-Stark apparatus for 20 h. Then toluene (20 mL), tetrabutylammonium bromide (80 mg, 0.25 mmol), and benzyl bromide (74 μL, 0.11 mg, 0.62 mmol) were added and reflux was continued for 2 h. After cooling to room temperature, solvents were removed by rotary evaporation. The residue was separated into two components by column chromatography on silica gel (toluene–EtOAc = 10:1 ⇒ 1:2) to give monobenzyl compound **11** (28 mg, 11%) and monobenzyl compound **10** (193 mg, 78%).

### 2.2.4. *(22R,23R,24R)-2α,3α,22,23-Tetrahydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one 22,23-boronate (**13**)*

A solution of methylboronate **5** (98 mg, 0.19 mmol) and trimethylamine *N*-oxide dihydrate (65 mg, 0.59 mmol) in diglyme (8 mL) was stirred under reflux for 45 min. The mixture was cooled to ambient temperature and the solvents were evaporated *in vacuo*. The residue was purified by column chromatography on silica gel ($CHCl_3$–MeOH = 40:1 ⇒ 10:1) to give epibrassinolide **4** (2 mg, 2%) and boronate **13** (95 mg, 96%). Mp 300 °C (ether, decomp). $^1$H NMR ($C_5D_5N$) $\delta$: 0.64 (s, 3H, C18-H), 0.74 (d, $J$ = 6.9 Hz, 3H, >$CHCH_3$), 0.91 (d, $J$ = 6.9 Hz, 6H, >$CHCH_3$), 1.06 (s, 3H, C19-H), 1.10 (d, $J$ = 6.5 Hz, 3H, >$CHCH_3$), 2.15 (d, $J$ = 8.3 Hz, 2H), 2.25 (dq, $J$ = 10.8, 6.9 Hz, 1H), 2.32 (dt, $J$ = 14.8, 4.1 Hz, 1H), 2.53 (t, $J$ = 13.5 Hz, 1H), 3.62 (dd, $J$ = 12.0, 4.2 Hz, 1H, C5-H), 3.96 (dd, $J$ = 9.0, 4.7 Hz, 1H), 4.16–4.01 (m, 3H), 4.30 (d, $J$ = 4.4 Hz, 1H), 4.44 (s, 1H). $^{13}$C NMR ($C_5D_5N$) $\delta$: 9.62, 12.03, 12.27, 16.27, 16.88, 21.51, 22.86, 25.30, 27.88, 28.48, 33.52, 38.94, 40.08, 42.11, 42.28, 42.95, 43.17, 45.24, 51.65, 53.26, 58.66, 68.82, 69.14, 70.63, 81.42, 82.07, 177.02.

### 2.2.5. *(22R,23R,24R)-2α-Benzyloxy-3α-palmitoyloxy-22,23-dihydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one 22,23-methylboronate (**14a**)*

*Variant A.* A mixture of the monobenzyl ether **10** (60 mg, 0.10 mmol), palmytoyl chloride (58 μL, 53 mg, 0.19 mmol), *N,N*-dimethylaminopyridine (3 mg, 0.025 mmol), and pyridine (2 mL) was stirred at room temperature for 40 h. Then solvents were evaporated *in vacuo* and the residue was purified by column chromatography on silica gel (toluene–EtOAc = 20:1 ⇒ 12:1) to afford ester **14a** (73 mg, 87%) as an oil. $^1$H NMR $\delta$: 0.27 (s, 3H, >B—$CH_3$), 0.70 (s, 3H, C18-H), 0.70 (d, $J$ = 6.2 Hz, 3H, >$CHCH_3$), 0.84 (d, $J$ = 6.8 Hz, 3H, >$CHCH_3$), 0.88 (t, $J$ = 6.8 Hz, 3H, —OCO—$(CH_2)_{14}$—$CH_3$), 0.88 (d, $J$ = 6.6 Hz, 3H, >$CHCH_3$), 0.91 (s, 3H, C19-H), 0.92 (d, $J$ = 6.7 Hz, 3H, >$CHCH_3$), 1.21–1.29 (m, 26H, —$OCOCH_2$—$(CH_2)_{13}$—$CH_3$), 2.37 (t, $J$ = 7.4 Hz, 2H, —$OCOCH_2C_{14}H_{29}$), 2.98 (dd, $J$ = 12.2, 4.4 Hz, 1H, C5-H), 3.46 (ddd, $J$ = 12.2, 4.2, 2.7 Hz, 1H, C2-H), 3.78 (dd, $J$ = 8.7, 5.2 Hz, 1H, C23-H), 4.05 (dd, $J$ = 12.4, 9.3 Hz, 1H, C7-$H_\alpha$), 4.10 (br.s, 1H, C7-$H_\beta$), 4.13 (d, $J$ = 5.1 Hz, 1H, C22-H), 4.41 (d, $J$ = 11.3 Hz, 1H, —$CH_2$Ph), 4.71 (d, $J$ = 11.3 Hz, 1H, —$CH_2$Ph), 5.56 (br.s, 1H, C3-H), 7.27–7.36 (m, 5H, Ph). $^{13}$C NMR $\delta$: 9.14, 11.45, 11.59, 14.10, 15.68, 16.47, 21.03, 22.23, 22.68, 24.78, 25.15, 27.12, 27.76, 29.14, 29.35, 29.39, 29.52, 29.69, 31.91, 34.55, 38.19, 39.21, 40.77, 41.12, 42.24, 42.45, 44.38, 51.10, 52.23, 58.50, 66.02, 70.43, 70.55, 73.55, 81.96, 82.34, 127.68, 127.86, 128.39, 138.01, 173.03, 175.57. MS (APCI$^+$) *m/z* (%): 833.5 ([M+H]$^+$, 100).

*Variant B.* A mixture of palmitic acid (181 mg, 0.71 mmol) and DCC (227 mg, 1.1 mmol) in dry $CH_2Cl_2$ (2 mL) was stirred at room temperature for 20 min. Then it was filtered through a cotton plug to remove a small amount of suspended matter and poured into a solution of the monobenzyl ether **10** (168 mg, 0.28 mmol) in dry $CH_2Cl_2$ (3 mL). The mixture was stirred at room temperature for 48 h, and then evaporated till the final volume of 2 mL. The residue was diluted with EtOAc (5 mL) and the resulting mixture was filtered through a silica gel pad, concentrated *in vacuo*, and purified by flash silica gel column chromatography (toluene–EtOAc = 20:1 ⇒ 12:1). Fractions containing the ester **14a** were combined and evaporated, the residue was dissolved in EtOAc (2 mL) and left in refrigerator for 30 min. The mixture was filtered through a Schott filter to remove precipitated contaminants and filtrate was evaporated to give the ester **14a** (226 mg, 89%) as an oil.

### 2.2.6. *(22R,23R,24R)-2α-Benzyloxy-3α-myristoyloxy-22,23-dihydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one 22,23-methylboronate (**14b**)*

The title compound (73 mg) was prepared in 88% yield as an oil from the monobenzyl ether **10** and myristoyl chloride as described above for the preparation of the ester **14a** (variant A). $^1$H NMR $\delta$: 0.27 (s, 3H, >B—$CH_3$), 0.70 (s, 3H, C18-H), 0.70 (d, $J$ = 6.4 Hz, 3H, >$CHCH_3$), 0.84 (d, $J$ = 6.8 Hz, 3H, >$CHCH_3$), 0.88 (t, $J$ = 6.8 Hz, 3H, —OCO—$(CH_2)_{12}$—$CH_3$), 0.88 (d, $J$ = 6.6 Hz, 3H, >$CHCH_3$), 0.91 (s, 3H, C19-H), 0.92 (d, $J$ = 6.8 Hz, 3H, >$CHCH_3$), 1.23–1.27 (m, 22H, —$OCOCH_2$—$(CH_2)_{11}$—$CH_3$), 2.37 (t, $J$ = 7.4 Hz, 2H, —$OCOCH_2C_{12}H_{25}$), 2.99 (dd, $J$ = 12.2, 4.4 Hz, 1H, C5-H), 3.46 (ddd, $J$ = 12.1, 3.9, 2.6 Hz, 1H, C2-H), 3.78 (dd, $J$ = 8.7, 5.2 Hz, 1H, C23-H), 4.05 (dd, $J$ = 12.3, 9.5 Hz, 1H, C7-$H_\alpha$), 4.10 (br.s, 1H, C7-$H_\beta$), 4.13 (d, $J$ = 5.1 Hz, 1H, C22-H), 4.41 (d, $J$ = 11.3 Hz, 1H, —$CH_2$Ph), 4.71 (d, $J$ = 11.3 Hz, 1H, —$CH_2$Ph),

5.56 (br.s, 1H, C3-H), 7.27–7.35 (m, 5H, Ph). $^{13}$C NMR $\delta$: 9.14, 11.45, 11.59, 14.10, 15.68, 16.46, 21.03, 22.22, 22.67, 24.77, 25.15, 27.11, 27.75, 29.14, 29.38, 29.51, 29.67, 31.90, 34.55, 38.19, 39.20, 40.76, 41.12, 42.23, 42.44, 44.37, 51.09, 52.22, 58.49, 66.01, 70.43, 70.55, 73.54, 81.95, 82.33, 127.68, 127.86, 128.39, 138.01, 173.03, 175.57. MS (APCI$^+$) *m/z* (%): 805.4 ([M+H]$^+$, 100).

### 2.2.7. (22R,23R,24R)-2α-Benzyloxy-3α-lauroyloxy-22,23-dihydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one 22,23-methylboronate (**14c**)

The title compound (68 mg) was prepared in 87% yield as an oil from the monobenzyl ether **10** and lauroyl chloride as described above for the preparation of the ester **14a** (variant A). $^1$H NMR $\delta$: 0.27 (s, 3H, >B—$CH_3$), 0.69 (s, 3H, C18-H), 0.70 (d, $J$ = 6.7 Hz, 3H, >$CHCH_3$), 0.84 (d, $J$ = 6.8 Hz, 3H, >$CHCH_3$), 0.88 (t, $J$ = 6.7 Hz, 3H, —OCO—$(CH_2)_{10}$—$CH_3$), 0.88 (d, $J$ = 6.5 Hz, 3H, >$CHCH_3$), 0.91 (d, $J$ = 6.6 Hz, 3H, >$CHCH_3$), 0.91 (s, 3H, C19-H), 1.23–1.27 (m, 18H, —$OCOCH_2$—$(CH_2)_9$—$CH_3$), 2.37 (t, $J$ = 7.4 Hz, 2H, —$OCOCH_2C_{10}H_{21}$), 2.98 (dd, $J$ = 12.2, 4.4 Hz, 1H, C5-H), 3.46 (ddd, $J$ = 12.2, 4.1, 2.7 Hz, 1H, C2-H), 3.78 (dd, $J$ = 8.7, 5.2 Hz, 1H, C23-H), 4.05 (dd, $J$ = 12.3, 9.3 Hz, 1H, C7-H$_\alpha$), 4.10 (br.s, 1H, C7-H$_\beta$), 4.13 (d, $J$ = 5.1 Hz, 1H, C22-H), 4.40 (d, $J$ = 11.3 Hz, 1H, —$CH_2$Ph), 4.71 (d, $J$ = 11.3 Hz, 1H, —$CH_2$Ph), 5.57 (br.s, 1H, C3-H), 7.27–7.35 (m, 5H, Ph). $^{13}$C NMR $\delta$: 9.12, 11.44, 11.58, 14.10, 15.67, 16.44, 21.02, 22.21, 22.66, 24.68, 24.77, 25.14, 27.09, 27.74, 29.04, 29.12, 29.22, 29.32, 29.37, 29.50, 29.57, 29.62, 31.89, 33.81, 34.54, 38.18, 39.18, 40.74, 41.11, 42.22, 42.43, 44.35, 51.07, 52.20, 58.46, 65.99, 70.42, 70.55, 73.53, 81.94, 82.33, 127.67, 127.86, 128.38, 137.97, 173.05, 175.61. MS (APCI$^+$) *m/z* (%): 777.4 ([M+H]$^+$, 100).

### 2.2.8. (22R,23R,24R)-2α-Benzyloxy-3α-palmitoyloxy-22,23-dihydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one (**16a**)

A mixture of **14a** (65 mg, 0.078 mmol) and trimethylamine *N*-oxide dihydrate (27 mg, 0.24 mmol) in diglyme (4 mL) was stirred under reflux for 45 min. The mixture was cooled to 80 °C and evaporated. The residue was purified by column chromatography on silica gel (toluene–EtOAc = 10:1) to give an oily product (56 mg) consisting of a mixture of **15a** and **16a**. It was dissolved in EtOH (4 mL) and passed through a column of Dowex-50W×8, 200–400 mesh, $NH_4^+$ form resin. The column was eluted with a mixture of EtOH-$NH_4OH$ (7:3). The fractions containing the expected product **16a** were combined and concentrated to dryness under reduced pressure. The residue was chromatographed on silica gel (toluene-EtOAc = 5:1 ⇒ 2:1) to give diol **16a** (50 mg, 79%) as an oil. $^1$H NMR $\delta$: 0.70 (s, 3H, C18-H), 0.85 (d, $J$ = 6.2 Hz, 3H, >$CHCH_3$), 0.87 (d, $J$ = 7.5 Hz, 3H, >$CHCH_3$), 0.88 (t, $J$ = 6.8 Hz, 3H, —OCO—$(CH_2)_{14}$—$CH_3$), 0.91 (s, 3H, C19-H), 0.92 (d, $J$ = 7.2 Hz, 3H, >$CHCH_3$), 0.96 (d, $J$ = 6.6 Hz, 3H, >$CHCH_3$), 1.22–1.29 (m, 26H, —$OCOCH_2$—$(CH_2)_{13}$—$CH_3$), 2.37 (t, $J$ = 7.4 Hz, 2H, —$OCOCH_2C_{14}H_{29}$), 2.98 (dd, $J$ = 12.2, 4.4 Hz, 1H, C5-H), 3.41 (t, $J$ = 5.3 Hz, 1H, C23-H), 3.46 (ddd, $J$ = 12.5, 4.1, 2.5 Hz, 1H, C2-H), 3.68 (d, $J$ = 3.7 Hz, 1H, C22-H), 4.05 (dd, $J$ = 12.4, 9.4 Hz, 1H, C7-H$_\alpha$), 4.09–4.16 (m, 1H, C7-H$_\beta$), 4.40 (d, $J$ = 11.3 Hz, 1H, —$CH_2$Ph), 4.71 (d, $J$ = 11.3 Hz, 1H, —$CH_2$Ph), 5.57 (s, 1H, C3-H), 7.27–7.35 (m, 5H, Ph). $^{13}$C NMR $\delta$: 10.86, 11.62, 11.66, 12.40, 14.09, 15.68, 17.31, 22.10, 22.29, 22.68, 24.78, 25.17, 27.08, 27.71, 29.16, 29.35, 29.39, 29.47, 29.52, 29.70, 31.92, 34.57, 38.22, 39.27, 39.53, 40.28, 40.80, 41.52, 42.28, 42.52, 51.25, 52.64, 58.51, 58.57, 66.12, 70.44, 70.58, 72.57, 73.57, 127.67, 127.83, 128.39, 138.09, 173.00, 175.53. MS (APCI$^+$) *m/z* (%): 809.3 ([M+H]$^+$, 100).

### 2.2.9. (22R,23R,24R)-2α-Benzyloxy-3α-myristoyloxy-22,23-dihydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one (**16b**)

The title compound (41 mg) was prepared in 67% yield as an oil from the methylboronate **14b** as described above for the preparation of the diol **16a**. $^1$H NMR $\delta$: 0.70 (s, 3H, C18-H), 0.81–0.94 (m, 18H), 1.22–1.29 (m, 22H, —$OCOCH_2$—$(CH_2)_{11}$—$CH_3$), 2.37 (m, 2H, —$OCOCH_2C_{12}H_{25}$), 2.99 (dd, $J$ = 12.2, 4.3 Hz, 1H, C5-H), 3.41 (t, $J$ = 5.1 Hz, 1H, C23-H), 3.46 (ddd, $J$ = 12.4, 4.2, 2.6 Hz, 1H, C2-H), 3.68 (d, $J$ = 3.5 Hz, 1H, C22-H), 4.01–4.08 (m, 1H, C7-H$_\alpha$), 4.14 (dd, $J$ = 15.4, 5.2 Hz, 1H, C7-H$_\beta$), 4.40 (d, $J$ = 11.3 Hz, 1H, —$CH_2$Ph), 4.71 (d, $J$ = 11.3 Hz, 1H, —$CH_2$Ph), 5.57 (s, 1H, C3-H), 7.27–7.35 (m, 5H, Ph). $^{13}$C NMR $\delta$: 10.82, 11.63, 12.37, 14.12, 15.68, 17.27, 22.11, 22.24, 22.68, 24.74, 25.13, 27.00, 27.68, 29.13, 29.38, 29.51, 29.68, 31.91, 34.53, 38.17, 39.19, 39.45, 40.23, 40.74, 41.40, 42.21, 42.46, 47.04, 51.17, 52.53, 58.47, 65.99, 70.41, 70.56, 72.51, 73.53, 76.34, 127.67, 127.85, 128.38, 173.04, 175.61. MS (APCI$^+$) *m/z* (%): 781.8 ([M+H]$^+$, 100).

### 2.2.10. (22R,23R,24R)-2α-Benzyloxy-3α-lauroyloxy-22,23-dihydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one (**16c**)

The title compound (41 mg) was prepared in 65% yield as an oil from the methylboronate **14c** as described above for the preparation of the diol **16a**. $^1$H NMR $\delta$: 0.70 (s, 3H, C18-H), 0.83–0.93 (m, 12H), 0.91 (s, 3H, C19-H), 0.96 (d, $J$ = 6.6 Hz, 3H, >$CHCH_3$), 1.22–1.29 (m, 18H, —$OCOCH_2$—$(CH_2)_9$—$CH_3$), 2.37 (t, $J$ = 7.5 Hz, 2H, —$OCOCH_2C_9H_{21}$), 2.98 (dd, $J$ = 12.2, 4.4 Hz, 1H, C5-H), 3.40 (t, $J$ = 5.3 Hz, 1H, C23-H), 3.46 (ddd, $J$ = 12.6, 4.2, 2.6 Hz, 1H, C2-H), 3.68 (d, $J$ = 3.6 Hz, 1H, C22-H), 4.05 (dd, $J$ = 12.3, 9.4 Hz, 1H, C7-H$_\alpha$), 4.12 (d, $J$ = 11.7 Hz, 1H, C7-H$_\beta$), 4.40 (d, $J$ = 11.3 Hz, 1H, —$CH_2$Ph), 4.70 (d, $J$ = 11.3 Hz, 1H, —$CH_2$Ph), 5.56 (s, 1H, C3-H), 7.27–7.35 (m, 5H, Ph). $^{13}$C NMR $\delta$: 10.81, 11.62, 12.36, 14.11, 15.67, 17.26, 22.11, 22.23, 22.67, 24.73, 25.13, 27.00, 27.67, 29.12, 29.36, 29.49, 29.62, 31.89, 34.53, 38.17, 39.18, 39.44, 40.23, 40.73, 41.40, 42.22, 42.45, 51.17, 52.52, 58.47, 66.00, 70.40, 70.55, 72.50, 73.52, 76.32, 127.66, 127.84, 128.38, 137.99, 173.04, 175.62. MS (APCI$^+$) *m/z* (%): 753.6 ([M+H]$^+$, 100).

### 2.2.11. (22R,23R,24R)-3α-Palmitoyloxy-2α,22,23-trihydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one (**17a**)

A stirred solution of **16a** (43 mg, 0.053 mmol) in EtOH (5 mL) was hydrogenated over Pd/C (10%, 5 mg) at ambient temperature for 20 h. Then the mixture was filtered through a pad of silica gel and solvent was evaporated. The residue was chromatographed on silica gel (toluene–EtOAc = 3:1 ⇒ 2:1) to give triol **17a** (28 mg, 73%) as an oil. $^1$H NMR $\delta$: 0.71 (s, 3H, C18-H), 0.85 (d, $J$ = 7.0 Hz, 3H, >$CHCH_3$), 0.87 (d, $J$ = 7.0 Hz, 3H, >$CHCH_3$), 0.87 (t, $J$ = 6.9 Hz, 3H, —OCO—$(CH_2)_{14}$—$CH_3$), 0.92 (d, $J$ = 6.9 Hz, 3H, >$CHCH_3$), 0.94 (s, 3H, C19-H), 0.97 (d, $J$ = 6.7 Hz, 3H, >$CHCH_3$), 1.22–1.29 (m, 26H, —$OCOCH_2$—$(CH_2)_{13}$—$CH_3$), 2.38 (t, $J$ = 7.3 Hz, 2H, —$OCOCH_2C_{14}H_{29}$), 2.95 (dd, $J$ = 12.2, 4.4 Hz, 1H, C5-H), 3.41 (t, $J$ = 5.2 Hz, 1H, C23-H), 3.68 (d, $J$ = 3.4 Hz, 1H, C22-H), 3.82 (ddd, $J$ = 12.5, 4.1, 2.8 Hz, 1H, C2-H), 4.00–4.09 (m, 1H, C7-H$_\alpha$), 4.10 (d, $J$ = 12.3 Hz, 1H, C7-H$_\beta$), 5.23 (br.s, 1H, C3-H). $^{13}$C NMR $\delta$: 10.83, 11.62, 12.37, 14.11, 15.51, 17.28, 22.11, 22.21, 22.68, 24.76, 25.06, 27.02, 27.68, 29.15, 29.35, 29.38, 29.52, 29.65, 29.69, 31.91, 34.49, 38.25, 39.15, 39.49, 40.22, 41.41, 42.05, 42.26, 42.47, 51.20, 52.57, 58.41, 67.35, 70.59, 71.27, 72.51, 76.36, 174.53, 175.46. MS (APCI$^+$) *m/z* (%): 719.3 ([M+H]$^+$, 100), 701.4 ([M-$H_2O$+H]$^+$, 21), 683.5 ([M-2$H_2O$+H]$^+$, 17), 463.3 ([M-$C_{15}H_{31}CO_2$H+H]$^+$, 25), 445.4 ([M-$C_{15}H_{31}CO_2$H-$H_2O$+H]$^+$, 32), 427.5 ([M-$C_{15}H_{31}CO_2$H-2$H_2O$+H]$^+$, 39).

*2.2.12. (22R,23R,24R)-3α-Myristoyloxy-2α,22,23-trihydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one (**17b**)*

The title compound (34 mg) was prepared in 97% yield as an oil from the benzyl ether **16b** as described above for the preparation of the triol **17a**. $^1$H NMR $\delta$: 0.71 (s, 3H, C18-H), 0.84 (d, $J$ = 7.0 Hz, 3H, >CHCH$_3$), 0.87 (d, $J$ = 7.5 Hz, 3H, >CHCH$_3$), 0.87 (t, $J$ = 7.2 Hz, 3H, —OCO—(CH$_2$)$_{12}$—CH$_3$), 0.92 (d, $J$ = 6.9 Hz, 3H, >CHCH$_3$), 0.93 (s, 3H, C19-H), 0.96 (d, $J$ = 6.7 Hz, 3H, >CHCH$_3$), 1.22–1.29 (m, 22H, —OCOCH$_2$—(CH$_2$)$_{11}$—CH$_3$), 2.38 (td, $J$ = 7.4, 1.1 Hz, 2H, —OCOCH$_2$C$_{12}$H$_{29}$), 2.95 (dd, $J$ = 12.2, 4.3 Hz, 1H, C5-H), 3.40 (dd, $J$ = 9.7, 5.1 Hz, 1H, C23-H), 3.68 (d, $J$ = 4.9 Hz, 1H, C22-H), 3.81 (dd, $J$ = 17, 9.6 Hz, 1H, C2-H), 4.04 (dd, $J$ = 12.4, 9.4 Hz, 1H, C7-H$_\alpha$), 4.12 (d, $J$ = 12.4 Hz, 1H, C7-H$_\beta$), 5.22 (s, 1H, C3-H). $^{13}$C NMR $\delta$: 10.81, 11.61, 12.36, 14.11, 15.50, 17.26, 22.11, 22.20, 22.67, 24.74, 25.04, 26.99, 27.67, 29.13, 29.34, 29.51, 29.64, 31.90, 34.48, 38.23, 39.13, 39.47, 40.22, 41.39, 42.03, 42.22, 42.45, 51.17, 52.54, 58.38, 67.32, 70.58, 71.24, 72.48, 76.33, 174.54, 175.50. MS (APCI$^+$) $m/z$ (%): 691.5 ([M+H]$^+$, 100), 673.6 ([M-H$_2$O+H]$^+$, 20), 655.6 ([M-2H$_2$O+H]$^+$, 13), 463.4 ([M-C$_{13}$H$_{27}$CO$_2$H+H]$^+$, 7), 445.9 ([M-C$_{13}$H$_{27}$CO$_2$H-H$_2$O+H]$^+$, 32), 427.6 ([M-C$_{13}$H$_{27}$CO$_2$H-2H$_2$O+H]$^+$, 20).

*2.2.13. (22R,23R,24R)-3α-Lauroyloxy-2α,22,23-trihydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one (**17c**)*

The title compound (38 mg) was prepared in 91% yield as an oil from the benzyl ether **16c** as described above for the preparation of the triol **17a**. $^1$H NMR $\delta$: 0.70 (s, 3H, C18-H), 0.84 (d, $J$ = 7.0 Hz, 3H, >CHCH$_3$), 0.86 (d, $J$ = 7.3 Hz, 3H, >CHCH$_3$), 0.87 (t, $J$ = 7.3 Hz, 3H, —OCO—(CH$_2$)$_{12}$—CH$_3$), 0.92 (d, $J$ = 6.9 Hz, 3H, >CHCH$_3$), 0.93 (s, 3H, C19-H), 0.96 (d, $J$ = 6.6 Hz, 3H, >CHCH$_3$), 1.22–1.29 (m, 18H, —OCOCH$_2$—(CH$_2$)$_9$—CH$_3$), 2.38 (t, $J$ = 7.4 Hz, 2H, —OCOCH$_2$C$_{12}$H$_{29}$), 2.95 (dd, $J$ = 12.2, 4.2 Hz, 1H, C5-H), 3.40 (t, $J$ = 5.2 Hz, 1H, C23-H), 3.68 (d, $J$ = 3.7 Hz, 1H, C22-H), 3.82 (d, $J$ = 11.7 Hz, 1H, C2-H), 4.01–4.08 (m, 1H, C7-H$_\alpha$), 4.12 (d, $J$ = 12.3 Hz, 1H, C7-H$_\beta$), 5.23 (s, 1H, C3-H). $^{13}$C NMR $\delta$: 10.81, 11.61, 12.36, 14.11, 15.50, 17.26, 22.11, 22.20, 22.67, 24.74, 25.04, 26.99, 27.67, 29.13, 29.33, 29.51, 29.63, 31.89, 34.48, 38.23, 39.13, 39.47, 40.22, 41.39, 42.03, 42.22, 42.45, 51.17, 52.55, 58.39, 67.32, 70.58, 71.24, 72.49, 76.33, 174.54, 175.49. MS (APCI$^+$) $m/z$ (%): 663.5 ([M+H]$^+$, 100), 645.7 ([M-H$_2$O+H]$^+$, 12), 627.7 ([M-2H$_2$O+H]$^+$, 10), 463.9 ([M-C$_{11}$H$_{23}$CO$_2$H+H]$^+$, 12), 445.8 ([M-C$_{11}$H$_{23}$CO$_2$H-H$_2$O+H]$^+$, 8), 427.5 ([M-C$_{11}$H$_{23}$CO$_2$H-2H$_2$O+H]$^+$, 10).

*2.2.14. (22R,23R,24R)-2α-Benzyloxy-3α-methanesulfonyloxy-22,23-dihydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one 22,23-methylboronate (**18**)*

A mixture of the alcohol **10** (200 mg, 0.34 mmol) and MsCl (0.1 mL, 148 mg, 1.2 mmol) in pyridine (4 mL) was stirred at ambient temperature for 1 h. Then it was diluted with water (12 mL) and the formed suspension was stirred for 30 min. The precipitate was filtered, washed with water (8 mL) and dried under reduced pressure. The resulting material was dissolved in a mixture of EtOAc-hexane (1:10, 8 mL) and placed in the refrigerator for 2 h. The precipitated crystals were filtered, washed on the filter with hexane (4 mL), and dried at room temperature to afford mesylate **18** (176 mg, 78%). Mp 193–195 °C (EtOAc-hexane). $^1$H NMR $\delta$: 0.28 (s, 3H, >B—CH$_3$), 0.70 (s, 3H, C18-H), 0.70 (d, $J$ = 6.7 Hz, 3H, >CHCH$_3$), 0.84 (d, $J$ = 6.8 Hz, 3H, >CHCH$_3$), 0.88 (d, $J$ = 6.7 Hz, 3H, >CHCH$_3$), 0.90 (s, 3H, C19-H), 0.92 (d, $J$ = 6.9 Hz, 3H, >CHCH$_3$), 3.04 (s, 3H, Ms), 3.11 (dd, $J$ = 11.9, 4.8 Hz, 1H, C5-H), 3.48 (ddd, $J$ = 12.3, 4.1, 2.2 Hz, 1H, C2-H), 3.77 (dd, $J$ = 8.8, 5.2 Hz, 1H, C23-H), 4.06–4.12 (m, 2H, C7-H), 4.14 (d, $J$ = 4.9 Hz, 1H, C22-H), 4.14 (s, $J$ = 4.9 Hz, 1H, C3-H), 4.54 (d, $J$ = 11.3 Hz, 1H, —CH$_2$Ph), 4.68 (d, $J$ = 11.3 Hz, 1H, —CH$_2$Ph), 7.39–7.28 (m, 5H, Ph). $^{13}$C NMR $\delta$: 9.12, 11.45, 11.58, 15.74, 16.42, 21.02, 22.20, 24.75, 27.07, 27.74, 30.72, 38.16, 38.93, 39.15, 39.69, 41.11, 41.89, 42.41, 44.35, 50.98, 52.18, 57.83, 70.44, 70.97, 73.46, 77.95, 81.95, 82.33, 127.85, 128.07, 128.58, 137.32, 175.09. MS (APCI$^+$) $m/z$ (%): 673.2 ([M+H]$^+$, 100), 577.3 ([M-MsOH+H]$^+$, 19).

*2.2.15. (22R,23R,24R)-2α-Benzyloxy-22,23-dihydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-3,6-one (**19**)*

A mixture of alcohol **10** (51 mg, 0.086 mmol), pyridinium dichromate (80 mg, 0.213 mmol) and $CH_2Cl_2$ (4 mL) was stirred at room temperature overnight. The mixture was transferred directly to silica gel column for purification. Eluting with toluene–EtOAc (10:1) afforded ketone **19** (50 mg, 98%) as an oil. $^1$H NMR $\delta$: 0.27 (s, 3H, >B—CH$_3$), 0.69 (d, $J$ = 6.9 Hz, 3H, >CHCH$_3$), 0.71 (s, 3H, C18-H), 0.84 (d, $J$ = 6.8 Hz, 3H, >CHCH$_3$), 0.88 (d, $J$ = 6.6 Hz, 3H, >CHCH$_3$), 0.91 (d, $J$ = 6.9 Hz, 3H, >CHCH$_3$), 1.12 (s, 3H, C19-H), 3.27 (dd, $J$ = 12.4, 5.1 Hz, 1H, C5-H), 3.77 (dd, $J$ = 8.8, 5.2 Hz, 1H, C23-H), 4.01 (dd, $J$ = 12.7, 9.5 Hz, 1H, C7-H), 4.12 (m, 3H, C2-, C7- and C22-H), 4.46 (d, $J$ = 11.5 Hz, 1H, —CH$_2$Ph), 4.88 (d, $J$ = 11.5 Hz, 1H, —CH$_2$Ph), 7.24–7.35 (m, 5H, Ph). $^{13}$C NMR $\delta$: 9.12, 11.43, 11.61, 15.81, 16.43, 21.02, 23.18, 24.72, 27.08, 27.70, 38.62, 39.11, 39.21, 39.89, 41.09, 42.40, 44.34, 48.02, 49.49, 51.01, 52.13, 57.39, 70.57, 72.22, 81.91, 82.28, 127.93, 128.49, 137.56, 173.59, 208.25.

*2.2.16. (22R,23R,24R)-2α-Benzyloxy-3β,22,23-trihydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one 22,23-methylboronate (**20**)*

*Variant A.* A mixture of mesylate **18** (160 mg, 0.24 mmol) and $NaNO_2$ (95 mg, 1.38 mmol) in DMF (4 mL) was stirred at 100 °C for 7 h. Then it was cooled to room temperature and poured into 1% NaCl (25 mL). The resulting mixture was extracted with EtOAc (3× 15 mL). The organic layer was dried ($Na_2SO_4$) and evaporated. The residue was chromatographed on silica gel (toluene–EtOAc = 10:1 ⇒ 1:5) to give alcohol **20** (52 mg, 36%) as an oil. $^1$H NMR $\delta$: 0.27 (s, 3H, >B—CH$_3$), 0.69 (s, 3H, C18-H), 0.69 (d, $J$ = 6.5 Hz, 3H, >CHCH$_3$), 0.84 (d, $J$ = 6.8 Hz, 3H, >CHCH$_3$), 0.88 (d, $J$ = 6.6 Hz, 3H, >CHCH$_3$), 0.91 (d, $J$ = 7.0 Hz, 3H, >CHCH$_3$), 0.93 (s, 3H, C19-H), 2.89 (dd, $J$ = 12.2, 4.5 Hz, 1H, C5-H), 3.29 (ddd, $J$ = 12.5, 9.1, 4.1 Hz, 1H, C2- or C3-H), 3.50 (ddd, $J$ = 12.5, 8.8, 4.5 Hz, 1H, C3- or C2-H), 3.77 (dd, $J$ = 8.6, 5.2 Hz, 1H, C23-H), 4.01 (dd, $J$ = 12.5, 9.3 Hz, 1H, C7-H$_\alpha$), 4.10 (d, $J$ = 12.0 Hz, 1H, C7-H$_\beta$), 4.13 (d, $J$ = 4.9 Hz, 1H, C22-H), 4.53 (d, $J$ = 11.4 Hz, 1H, —CH$_2$Ph), 4.64 (d, $J$ = 11.4 Hz, 1H, —CH$_2$Ph), 7.28–7.39 (m, 5H, Ph). $^{13}$C NMR $\delta$: 9.11, 11.42, 11.57, 16.00, 16.41, 21.02, 22.80, 24.73, 27.06, 27.72, 32.02, 38.77, 39.15, 39.25, 41.07, 42.38, 43.55, 44.34, 46.09, 51.06, 52.19, 58.30, 70.41, 71.71, 72.52, 79.51, 81.93, 82.33, 127.84, 127.91, 128.54, 138.17, 174.82.

*Variant B.* To a cooled to −15 °C solution of ketone **19** (49 mg, 0.083 mmol) in EtOAc–MeOH (1:1), $NaBH_4$ (4.5 mg, 0.12 mmol) was added under stirring. The cooling bath was removed, and the reaction mixture was allowed to warm to ambient temperature. Then it was filtered through a short pad of silica gel and rinsed with EtOAc (12 mL). The filtrate was evaporated, and the residue was chromatographed on silica gel (toluene–EtOAc = 10:1 ⇒ 3:1) to give:

a) alcohol **20** (18 mg, 37%).
b) alcohol **10** (30 mg, 61%).

*2.2.17. (22R,23R,24R)-2α-Benzyloxy-3β-palmitoyloxy-22,23-dihydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one 22,23-methylboronate (**21a**)*

A mixture of the alcohol **20** (52 mg, 0.087 mmol), palmytoyl chloride (53 μL, 48 mg, 0.17 mmol), and pyridine (1 mL) was stirred

at room temperature for 5 h. Then solvents were evaporated *in vacuo* and the residue was purified by column chromatography on silica gel (toluene–EtOAc = 10:1 ⇒ 8:1) to afford ester **21a** (49 mg, 67%) as an oil. $^1$H NMR $\delta$: 0.27 (s, 3H, >B—$CH_3$), 0.69 (s, 3H, C18-H), 0.69 (d, $J$ = 6.7 Hz, 3H, >$CHCH_3$), 0.84 (d, $J$ = 6.8 Hz, 3H, >$CHCH_3$), 0.88 (t, $J$ = 6.8 Hz, 3H, —OCO—$(CH_2)_{14}$—$CH_3$), 0.89 (d, $J$ = 6.6 Hz, 3H, >$CHCH_3$), 0.91 (d, $J$ = 6.6 Hz, 3H, >$CHCH_3$), 0.92 (s, 3H, C19-H), 1.21–1.29 (m, 26H, —$OCOCH_2$—$(CH_2)_{13}$—$CH_3$), 2.96 (dd, $J$ = 11.1, 5.8 Hz, 1H, C5-H), 3.49 (ddd, $J$ = 11.7, 9.8, 4.3 Hz, 1H, C2-H), 3.77 (dd, $J$ = 8.8, 5.2 Hz, 1H, C23-H), 4.01 (dd, $J$ = 12.4, 9.4 Hz, 1H, C7-$H_\alpha$), 4.07–4.15 (m, 2H, C7- and C22-H), 4.60 (d, $J$ = 11.8 Hz, 1H, —$CH_2$Ph), 4.65 (d, $J$ = 11.8 Hz, 1H, —$CH_2$Ph), 4.76 (td, $J$ = 10.1, 5.9 Hz, 1H, C3-H), 7.27–7.36 (m, 5H, Ph). $^{13}$C NMR $\delta$: 9.11, 11.43, 11.59, 14.12, 15.78, 16.41, 21.03, 22.68, 24.72, 24.94, 27.06, 27.71, 29.05, 29.15, 29.28, 29.35, 29.43, 29.64, 29.67, 30.11, 31.91, 33.70, 34.63, 38.42, 39.15, 41.10, 42.39, 44.34, 44.99, 45.92, 51.01, 52.14, 58.18, 70.52, 72.47, 74.92, 75.74, 81.92, 82.32, 127.44, 127.61, 128.34, 138.47, 173.32, 174.71. MS (APCI$^+$) $m/z$ (%): 833.4 ([M+H]$^+$, 100), 725.4 ([M-BnOH+H]$^+$, 24), 577.4 ([M-$C_{15}H_{31}CO_2$H+H]$^+$, 13).

### 2.2.18. (22R,23R,24R)-2α-Benzyloxy-3β-myristoyloxy-22,23-dihydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one 22,23-methylboronate (**21b**)

The title compound (24 mg) was prepared in 74% yield as an oil from the alcohol **20** and myristoyl chloride as described above for the preparation of the ester **21a**. $^1$H NMR $\delta$: 0.27 (s, 3H, >B—$CH_3$), 0.68 (s, 3H, C18-H), 0.69 (d, $J$ = 6.6 Hz, 3H, >$CHCH_3$), 0.83 (d, $J$ = 6.8 Hz, 3H, >$CHCH_3$), 0.87 (t, $J$ = 6.9 Hz, 3H, —OCO—$(CH_2)_{14}$—$CH_3$), 0.88 (d, $J$ = 6.6 Hz, 3H, >$CHCH_3$), 0.91 (d, $J$ = 6.6 Hz, 3H, >$CHCH_3$), 0.91 (s, 3H, C19-H), 1.21–1.29 (m, 22H, —$OCOCH_2$—$(CH_2)_{11}$—$CH_3$), 2.96 (dd, $J$ = 10.7, 6.2 Hz, 1H, C5-H), 3.49 (ddd, $J$ = 11.9, 9.7, 4.3 Hz, 1H, C2-H), 3.77 (dd, $J$ = 8.7, 5.2 Hz, 1H, C23-H), 4.01 (dd, $J$ = 12.2, 9.7 Hz, 1H, C7-$H_\alpha$), 4.06–4.17 (m, 2H, C7- and C22-H), 4.60 (d, $J$ = 11.8 Hz, 1H, —$CH_2$Ph), 4.65 (d, $J$ = 11.8 Hz, 1H, —$CH_2$Ph), 4.76 (td, $J$ = 10.0, 5.9 Hz, 1H, C3-H), 7.27–7.36 (m, 5H, Ph). $^{13}$C NMR $\delta$: 9.10, 11.42, 11.58, 14.10, 15.77, 16.40, 21.01, 22.66, 22.72, 24.72, 24.92, 27.05, 27.70, 29.14, 29.26, 29.32, 29.41, 29.62, 30.10, 31.89, 34.62, 38.41, 39.14, 41.08, 42.38, 44.33, 44.97, 45.91, 51.00, 52.13, 58.16, 70.50, 72.46, 74.91, 75.74, 81.91, 82.31, 121.09, 127.43, 127.60, 128.33, 129.61, 138.46, 150.78, 173.30, 174.69. MS (APCI$^+$) $m/z$ (%): 805.3 ([M+H]$^+$, 100).

### 2.2.19. (22R,23R,24R)-2α-Benzyloxy-3β-lauroyloxy-22,23-dihydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one 22,23-methylboronate (**21c**)

The title compound (40 mg) was prepared in 61% yield as an oil from the alcohol **20** and lauroyl chloride as described above for the preparation of the ester **21a**. $^1$H NMR $\delta$: 0.27 (s, 3H, >B—$CH_3$), 0.69 (s, 3H, C18-H), 0.69 (d, $J$ = 6.6 Hz, 3H, >$CHCH_3$), 0.83 (d, $J$ = 6.8 Hz, 3H, >$CHCH_3$), 0.87 (t, $J$ = 6.9 Hz, 3H, —OCO—$(CH_2)_{14}$—$CH_3$), 0.88 (d, $J$ = 6.5 Hz, 3H, >$CHCH_3$), 0.91 (d, $J$ = 6.0 Hz, 3H, >$CHCH_3$), 0.92 (s, 3H, C19-H), 1.21–1.29 (m, 18H, —$OCOCH_2$—$(CH_2)_9$—$CH_3$), 2.96 (dd, $J$ = 11.0, 5.8 Hz, 1H, C5-H), 3.49 (td, $J$ = 11.6, 4.3 Hz, 1H, C2-H), 3.77 (dd, $J$ = 8.8, 5.2 Hz, 1H, C23-H), 4.01 (dd, $J$ = 12.4, 9.4 Hz, 1H, C7-$H_\alpha$), 4.07–4.15 (m, 2H, C7- and C22-H), 4.60 (d, $J$ = 11.8 Hz, 1H, —$CH_2$Ph), 4.65 (d, $J$ = 11.8 Hz, 1H, —$CH_2$Ph), 4.76 (td, $J$ = 10.0, 6.0 Hz, 1H, C3-H), 7.27–7.36 (m, 5H, Ph). $^{13}$C NMR $\delta$: 9.11, 11.43, 11.59, 14.12, 15.79, 16.41, 21.03, 22.67, 22.73, 24.73, 24.94, 27.06, 27.72, 29.05, 29.15, 29.28, 29.32, 29.42, 29.59, 30.12, 31.89, 33.67, 34.63, 38.43, 39.15, 41.10, 42.39, 44.34, 44.99, 45.92, 51.02, 52.14, 58.18, 70.52, 72.48, 74.93, 75.74, 81.93, 82.32, 127.44, 127.62, 128.35, 138.46, 173.33, 174.71. MS (APCI$^+$) $m/z$ (%): 777.4 ([M+H]$^+$, 100), 577.4 ([M-$C_{11}H_{23}CO_2$H+H]$^+$, 10).

### 2.2.20. (22R,23R,24R)-2α-Benzyloxy-3β-palmitoyloxy-22,23-dihydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one (**22a**)

The title compound (25 mg) was prepared in 59% yield as an oil from the methylboronate **21a** as described above for the preparation of the diol **16a**. $^1$H NMR $\delta$: 0.69 (s, 3H, C18-H), 0.84 (d, $J$ = 7.1 Hz, 3H, >$CHCH_3$), 0.86 (d, $J$ = 6.9 Hz, 3H, >$CHCH_3$), 0.87 (t, $J$ = 6.8 Hz, 3H, —OCO—$(CH_2)_{14}$—$CH_3$), 0.91 (s, 3H, C19-H), 0.91 (d, $J$ = 7.2 Hz, 3H, >$CHCH_3$), 0.96 (d, $J$ = 6.6 Hz, 3H, >$CHCH_3$), 1.22–1.29 (m, 26H, —$OCOCH_2$—$(CH_2)_{13}$—$CH_3$), 2.96 (dd, $J$ = 11.0, 5.7 Hz, 1H, C5-H), 3.40 (t, $J$ = 5.2 Hz, 1H, C23-H), 3.49 (ddd, $J$ = 11.8, 9.7, 4.2 Hz, 1H, C2-H), 3.67 (d, $J$ = 3.4 Hz, 1H, C22-H), 4.02 (dd, $J$ = 12.4, 9.5 Hz, 1H, C7-$H_\alpha$), 4.07–4.15 (m, 2H, C7- and C22-H), 4.60 (d, $J$ = 11.8 Hz, 1H, —$CH_2$Ph), 4.64 (d, $J$ = 11.8 Hz, 1H, —$CH_2$Ph), 4.76 (dt, $J$ = 16.1, 7.9 Hz, 1H, C3-H), 7.27–7.35 (m, 5H, Ph). $^{13}$C NMR $\delta$: 10.80, 11.62, 12.35, 14.12, 15.77, 17.24, 22.11, 22.67, 24.69, 24.93, 26.96, 27.64, 29.14, 29.27, 29.34, 29.42, 29.64, 29.67, 30.09, 31.90, 34.62, 38.40, 39.13, 39.42, 40.20, 41.34, 42.40, 44.95, 45.90, 51.10, 52.44, 58.16, 70.52, 72.45, 74.92, 75.71, 76.29, 127.43, 127.61, 128.34, 138.44, 173.34, 174.74. MS (APCI$^+$) $m/z$ (%): 809.7 ([M+H]$^+$, 100), 553.2 ([M-$C_{15}H_{31}CO_2$H+H]$^+$, 7).

### 2.2.21. (22R,23R,24R)-2α-Benzyloxy-3β-myristoyloxy-22,23-dihydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one (**22b**)

The title compound (11 mg) was prepared in 62% yield as an oil from the methylboronate **21b** as described above for the preparation of the diol **16a**. $^1$H NMR $\delta$: 0.70 (s, 3H, C18-H), 0.84 (d, $J$ = 7.0 Hz, 3H, >$CHCH_3$), 0.87 (d, $J$ = 6.8 Hz, 3H, >$CHCH_3$), 0.87 (t, $J$ = 6.8 Hz, 3H, —OCO—$(CH_2)_{12}$—$CH_3$), 0.91 (s, 3H, C19-H), 0.92 (d, $J$ = 6.5 Hz, 3H, >$CHCH_3$), 0.96 (d, $J$ = 6.6 Hz, 3H, >$CHCH_3$), 1.22–1.29 (m, 22H, —$OCOCH_2$—$(CH_2)_{11}$—$CH_3$), 2.34 (t, $J$ = 7.5 Hz, 2H, —$OCOCH_2C_{12}H_{25}$), 2.96 (dd, $J$ = 11.1, 5.6 Hz, 1H, C5-H), 3.38–3.42 (m, 1H, C23-H), 3.44–3.53 (m, 1H, C2-H), 3.68 (d, $J$ = 3.4 Hz, 1H, C22-H), 3.97–4.06 (m, 1H, C7-$H_\alpha$), 4.11 (d, $J$ = 12.4 Hz, 1H, C7-$H_\beta$), 4.60 (d, $J$ = 11.8 Hz, 1H, —$CH_2$Ph), 4.64 (d, $J$ = 11.8 Hz, 1H, —$CH_2$Ph), 4.71–4.80 (m, 1H, C3-H), 7.27–7.35 (m, 5H, Ph). $^{13}$C NMR $\delta$: 10.85, 11.66, 12.39, 14.09, 15.78, 17.29, 22.10, 22.67, 22.80, 24.74, 24.96, 27.05, 27.67, 29.17, 29.28, 29.33, 29.43, 29.64, 30.17, 31.91, 34.65, 38.45, 39.23, 39.52, 40.27, 41.50, 42.48, 45.03, 46.00, 51.19, 52.58, 58.27, 70.55, 72.45, 72.55, 74.95, 75.81, 76.35, 127.45, 127.61, 128.35, 138.54, 173.31, 174.66. MS (APCI$^+$) $m/z$ (%): 781.5 ([M+H]$^+$, 100).

### 2.2.22. (22R,23R,24R)-2α-Benzyloxy-3β-lauroyloxy-22,23-dihydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one (**22c**)

The title compound (18 mg) was prepared in 62% yield as an oil from the methylboronate **21c** as described above for the preparation of the diol **16a**. $^1$H NMR $\delta$: 0.69 (s, 3H, C18-H), 0.79–0.99 (m, 18H), 1.22–1.29 (m, 18H, —$OCOCH_2$—$(CH_2)_9$—$CH_3$), 2.96 (dd, $J$ = 10.9, 5.6 Hz, 1H, C5-H), 3.40 (t, $J$ = 5.1 Hz, 1H, C23-H), 3.44–3.53 (m, 1H, C2-H), 3.67 (d, $J$ = 3.4 Hz, 1H, C22-H), 3.97–4.15 (m, 2H, C7-H), 4.60 (d, $J$ = 11.8 Hz, 1H, —$CH_2$Ph), 4.64 (d, $J$ = 11.8 Hz, 1H, —$CH_2$Ph), 4.76 (td, $J$ = 9.8, 6.1 Hz, 1H, C3-H), 7.27–7.35 (m, 5H, Ph). $^{13}$C NMR $\delta$: 10.81, 11.62, 12.36, 14.11, 15.77, 17.24, 22.11, 22.66, 22.75, 24.70, 24.93, 26.97, 27.64, 29.14, 29.27, 29.31, 29.42, 29.59, 30.10, 31.89, 34.62, 38.41, 39.15, 39.44, 40.22, 41.37, 42.42, 44.97, 45.92, 51.12, 52.47, 58.18, 70.52, 72.45, 72.48, 74.92, 75.73, 76.30, 127.43, 127.61, 128.34, 138.46, 173.33, 174.72. MS (APCI$^+$) $m/z$ (%): 753.4 ([M+H]$^+$, 100).

### 2.2.23. (22R,23R,24R)-3β-Palmitoyloxy-2α,22,23-trihydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one (**2a**)

The title compound (14 mg) was prepared in 63% yield from the benzyl ether **22a** as described above for the preparation of the triol

**17a**. Mp 196–198 °C (EtOH). Its $^1$H NMR spectrum matched that described in the literature [7]. $^{13}$C NMR $\delta$: 10.85, 11.65, 12.40, 14.10, 15.87, 17.29, 22.11, 22.68, 22.77, 24.74, 24.96, 27.05, 27.68, 29.12, 29.24, 29.35, 29.44, 29.60, 29.68, 31.92, 34.52, 38.47, 39.18, 39.51, 40.26, 41.46, 42.48, 46.29, 46.91, 51.19, 52.58, 58.30, 68.90, 70.62, 72.55, 76.37, 174.25, 174.57. MS (APCI$^+$) *m/z* (%): 719.3 ([M+H]$^+$, 91), 701.4 ([M-$H_2O$+H]$^+$, 23), 683.5 ([M-2$H_2O$+H]$^+$, 15), 463.3 ([M-$C_{15}H_{31}CO_2$H+H]$^+$, 100), 445.6 ([M-$C_{15}H_{31}CO_2$H-$H_2O$+H]$^+$, 43), 427.2 ([M-$C_{15}H_{31}CO_2$H-2$H_2O$+H]$^+$, 15).

*2.2.24. (22R,23R,24R)-3β-Myristoyloxy-2α,22,23-trihydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one (**2b**)*

The title compound (5 mg) was prepared in 63% yield from the benzyl ether **22b** as described above for the preparation of the triol **17a**. Mp 203–205 °C (EtOH). Its $^1$H NMR spectrum matched that described in the literature [7]. $^{13}$C NMR $\delta$: 10.83, 11.64, 12.38, 14.11, 15.86, 17.27, 22.11, 22.68, 22.75, 24.73, 24.94, 27.02, 27.67, 29.11, 29.24, 29.34, 29.43, 29.63, 31.91, 34.50, 38.46, 39.15, 39.48, 40.24, 41.41, 42.45, 46.25, 46.86, 51.16, 52.53, 58.26, 68.87, 70.61, 72.52, 174.27, 174.61. MS (ESI$^+$) *m/z* (%): 691.0 ([M+H]$^+$, 100), 673.3 ([M-$H_2O$+H]$^+$, 18), 655.3 ([M-2$H_2O$+H]$^+$, 7), 463.1 ([M-$C_{13}H_{27}CO_2$H+H]$^+$, 56), 445.3 ([M-$C_{13}H_{27}CO_2$H-$H_2O$+H]$^+$, 15), 427.4 ([M-$C_{13}H_{27}CO_2$H-2$H_2O$+H]$^+$, 10).

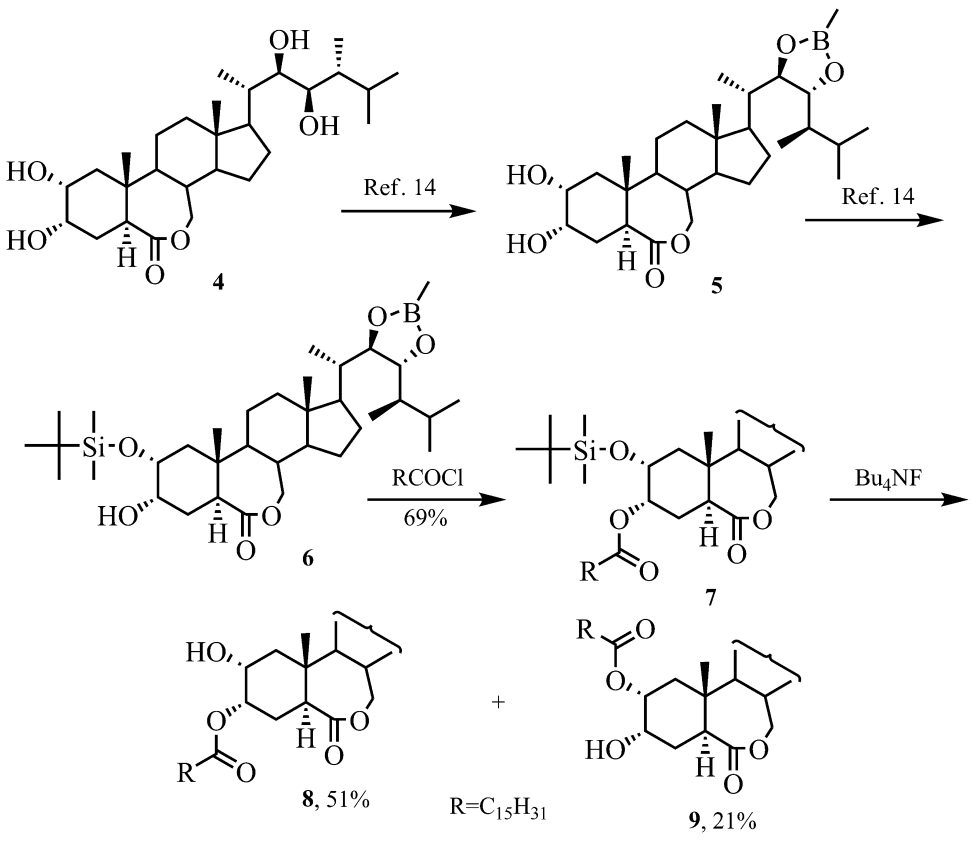
*2.2.25. (22R,23R,24R)-3β-Lauroyloxy-2α,22,23-trihydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one (**2c**)*

The title compound (7 mg) was prepared in 57% yield from the benzyl ether **22c** as described above for the preparation of the triol **17a**. Mp 213–215 °C (EtOH). Its $^1$H NMR spectrum matched that described in the literature [7]. $^{13}$C NMR $\delta$: 10.85, 11.64, 12.38, 14.10, 15.86, 17.31, 22.14, 22.67, 22.75, 24.73, 24.94, 26.99, 27.66, 29.11, 29.23, 29.31, 29.42, 29.58, 29.67, 31.89, 34.50, 38.45, 39.14, 39.47, 40.13, 41.32, 42.45, 46.24, 46.87, 51.15, 52.50, 58.25, 68.86, 70.60, 72.58, 174.26, 174.60.

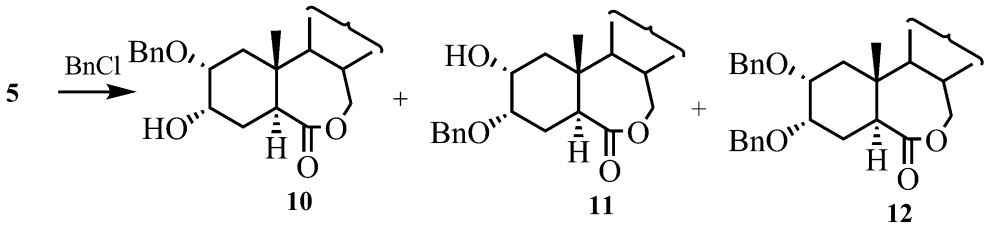
## 3. Results and discussion

The preparation of the desired compounds (**2a–c**, **17a–c**) required the protection of three of the four hydroxyl groups in epibrassinolide molecule **4**. A solution to this problem was proposed by the authors previously [14]. The side chain diol function was protected as 22,23-methylboronate **5** followed by protection of the equatorial 2α-hydroxyl group as TBS-ether to give 3α-alcohol **6** (Scheme 1). However, attempts to use this compound for the preparation of 3-acyl derivatives proved to be somewhat disappointing. Acylation of **6** wih palmitoyl chloride in the presence of DMAP proceeded smoothly to give ester **7**, but subsequent desilylation was accompanied by 1,2-acyl migration with formation of a mixture of 2α- and 3α-esters **8** and **9** in 51% and 21% yield, respectively. The location of an acyl group at C-3 in **8** and at C-2 in **9** was deduced from the values of coupling constants of the corresponding signals. In **9** it appeared as broadened singlet at $\delta$ 5.23. Consequently, this proton was in equatorial orientation, indicating the location of the acyl group at axial C-3 position. The large coupling constant (*J* 12.2 Hz) of signal at $\delta$ 4.84 in $^1$H NMR spectrum of **8** supported the axial orientation of this proton, thus confirming a C-2 equatorial position of the acyl group.

Difficulties in removing the TBS group encouraged the search for an alternative protecting group that could be removed under conditions avoiding acyl migration. Protection of 2α-OH in **5** as the ether **10** (Scheme 2) seemed to be a good choice due to easiness of the benzyl group removal by hydrogenolysis. Although the axial hydroxyl at C-3 is more sterically encumbered than the equatorial one at C-2, the first attempts to prepare the monobenzyl ether



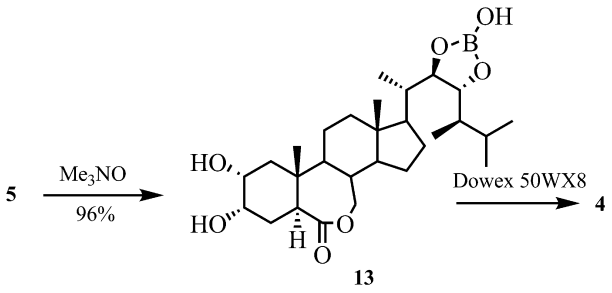

**Scheme 1.**




**Scheme 2.**

**10** were discouraging. Benzylation of **5** with benzyl bromide and sodium hydride [15] gave only 13% of the desired product **10** along with 7% of dibenzyl ether **12**, 6% of monobenzyl ether **11** and 64% of the starting material. Better results (57% of **10**) were obtained using benzyl bromide and silver oxide [16]. Ultimately the best result (78 and 11% yields of the monobenzyl ethers **10** and **11**, respectively) was obtained by the use of dibutyl tin oxide [17].

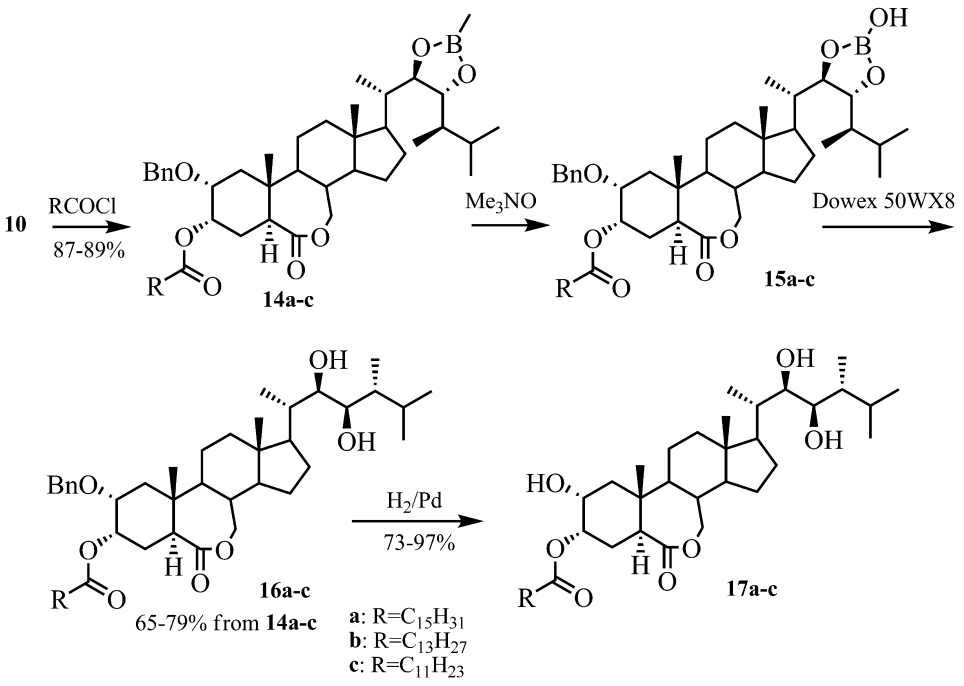
Another problem, which had to be solved, was connected with the removal of methylboronate protective group. This reaction requires basic conditions [14] that are not compatible with the acyl group at C-3. Attempts to remove 22,23-methylboronate using the exchange reaction with pinacol [18] (based on the assumption that pinacolate ester would be more stable) failed. The solution of the problem was found to be initial oxidation of methylboronate. The methodology was first tested on the ester **5**. Its treatment with trimethylamine *N*-oxide gave the corresponding 2-hydroxy-1,3,2-dioxaborolane **13** (Scheme 3) which proved to be less resistant to hydrolysis. Because of this reaction reversibility, the hydrolysis was carried out using a cation exchange column with DOWEX 50W×8 in $NH_4^+$ form. The oxidation of **5** was accompanied by the formation of a small amount of epibrassinolide **4** that is why the two-step deprotection was carried out with other compounds without isolation of intermediate 2-hydroxy-1,3,2-dioxaborolanes.



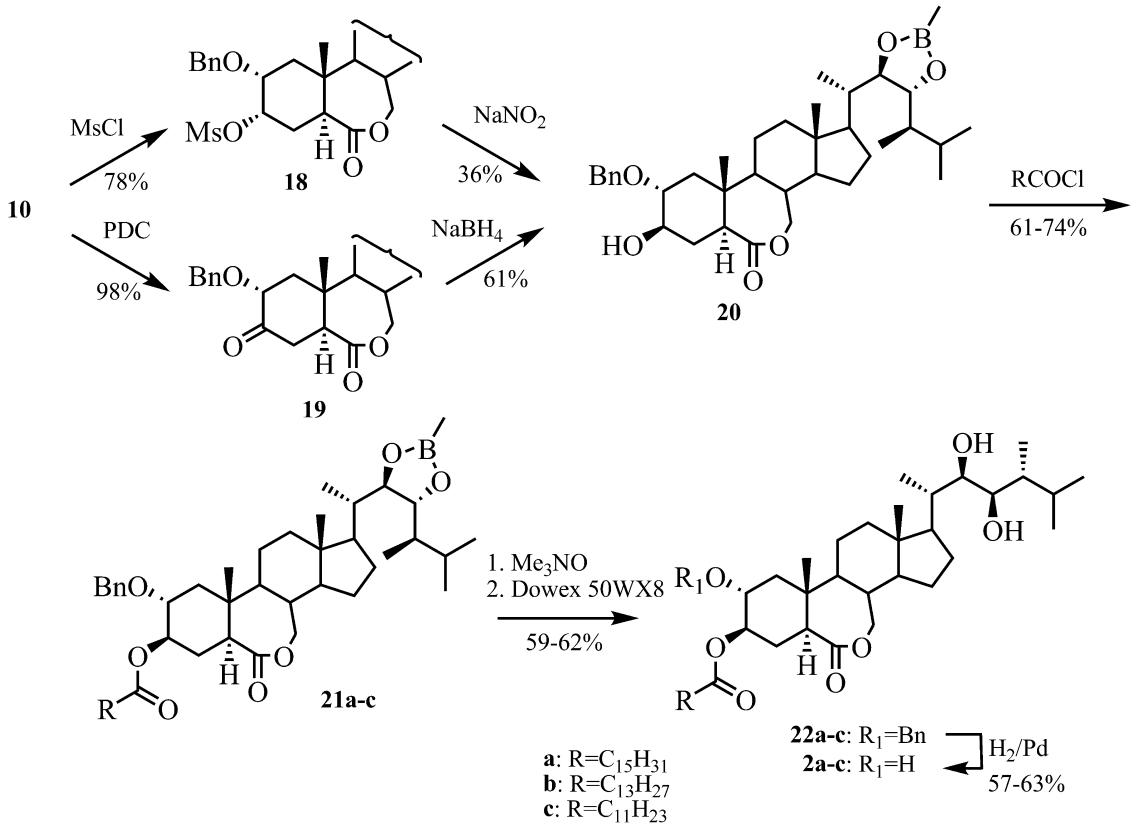

**Scheme 3.**

With these findings in hand, the preparation of 3α-acyl derivatives of epibrassinolide **17a–c** was straightforward enough as outlined in Scheme 4. Treatment of the alcohol **10** with palmitic, myristinic or lauric acid chlorides gave the corresponding esters **14a–c**. In addition, for comparison purposes, the ester **14a** was prepared by the reaction of palmitic acid and DCC. The yield was a little bit higher than with acid chlorides, but troublesome formation and removal of urea by-products have made this method less attractive. Acylation resulted in a downfield signal shift of the methine proton ($\delta$ 5.56–5.57) adjacent to the acyl group in the $^1$H NMR spectra of the esters **14a–c** in comparison with that of the starting material **10**. This signal appeared as a broad singlet suggesting an equatorial orientation of the methine proton, and hence a C-3 axial position of the acyl group. It is also an experimental proof of the benzyl group location at C-2 in **10** and **14a–c**.

The ester groups at C-3 left unreacted in the methylboronate deprotection sequence involving oxidation of **14a–c** followed by




**Scheme 4.**




**Scheme 5.**

hydrolysis of 2-hydroxy-1,3,2-dioxaborolanes **15a–c**. Hydrogenolysis of the benzyl ethers **16a–c** catalyzed by palladium on carbon yielded compounds **17a–c**. Investigation of their $^1$H NMR spectra showed no signs of acyl migration in this reaction.

Synthesis of 3β-acyl derivatives of epibrassinolide **2a–c** implied inversion of configuration at C-3. Two variants of this transformation were compared. The first one was based on mesylation of the alcohol **10** followed by nucleophilic substitution of the mesylate group in **18** to introduce a β-hydroxyl at C-3 (Scheme 5). The overall yield of this transformation was only 28%, mainly due to a low yield at the substitution step. Better results were obtained with the oxidation of alcohol **10** followed by the hydride reduction of the ketone **19**. The desired 3β-alcohol **20** was isolated in 37% yield along with 61% of 3α-isomer **10** which could be recycled again.

## 4. Conclusion

In summary, we have prepared and characterized six fatty acid (palmitic, myristic and lauric) esters of epibrassinolide as reference compounds for biochemical studies. Three of them (3β-derivatives **2a–c**) have been already identified in cell suspension culture of *Ornithopus sativus* as epibrassinolide metabolites [7], and three others (3α-esters **17a–c**) are new compounds and potential candidates for that role.

## Acknowledgments

The author wishes to thank Dr. Alaksiej Hurski for his help in conducting the experiment. We are grateful also to the Belorussian Foundation for Fundamental Research for financial support (Project X12P-169).

## References

[1] M.D. Grove, G.F. Spencer, W.K. Rohwedder, N. Mandava, J.F. Worley, J.D. Warthen, G.L. Steffens, J.L. Flippen-Anderson, J.C. Cook, Brassinolide, a plant growth-promoting steroid isolated from *Brassica napus* pollen, Nature 281 (5728) (1979) 216–217.
[2] V.A. Khripach, V.N. Zhabinskii, A. de Groot, Brassinosteroids. A New Class of Plant Hormones, Academic Press, San Diego, 1999.
[3] S. Choe, Brassinosteroid biosynthesis and inactivation, Physiologia Plantarum 126 (4) (2006) 539–548.
[4] S. Choe, Brassinosteroid Biosynthesis and Metabolism, in: P.J. Davies (Ed.), Plant Hormones. Biosynthesis, Signal Transduction, Action!, Springer, Dordrecht, 2010, pp. 156–178.
[5] L. Hategan, B. Godza, M. Szekeres, Regulation of brassinosteroid metabolism, in: S. Hayat, A. Ahmad (Eds.), Brassinosteroids: A Class of Plant Hormone, Springer, Dordrecht, 2011, pp. 57–81.
[6] A. Bajguz, Metabolism of brassinosteroids in plants, Plant Physiology and Biochemistry 45 (2) (2007) 95–107.
[7] A. Kolbe, B. Schneider, A. Porzel, J. Schmidt, G. Adam, Acyl-conjugated metabolites of brassinosteroids in cell-suspension cultures of *Ornithopus sativus*, Phytochemistry 38 (3) (1995) 633–636.
[8] S. Asakawa, H. Abe, Y. Kyokawa, S. Nakamura, M. Natsume, Teasterone 3-myristate: a new type of brassinosteroid derivative in *Lilium longiflorum* anthers, Bioscience, Biotechnology, and Biochemistry 58 (1) (1994) 219–220.
[9] S. Asakawa, H. Abe, N. Nishikawa, M. Natsume, M. Koshioka, Purification and identification of new acyl-conjugated teasterones in lily pollen, Bioscience, Biotechnology, and Biochemistry 60 (9) (1996) 1416–1420.
[10] K. Soeno, S. Asakawa, M. Natsume, H. Abe, Reversible conversion between teasterone and its ester conjugates in lily cell cultures, Journal of Pesticide Science 25 (2) (2000) 117–122.
[11] H. Roh, C.W. Jeong, S. Fujioka, Y.K. Kim, S. Lee, J.H. Ahn, Y.D. Choi, J.S. Lee, Genetic evidence for the reduction of brassinosteroid levels by a BAHD acyltransferase-like protein in *Arabidopsis*, Plant Physiology 159 (2012) 696–709.
[12] V.A. Khripach, V.N. Zhabinskii, N.B. Khripach, New practical aspects of brassinosteroids and results of their ten-year agricultural use in Russia and Belarus, in: S. Hayat, A. Ahmad (Eds.), Brassinosteroids, Kluwer Academic Publishers, Dordrecht, 2003, pp. 189–230.
[13] W.L.E. Armarego, C.L.L. Chai, Purification of Laboratory Chemicals, Butterworth-Heinemann, 2009.
[14] V.A. Khripach, V.N. Zhabinskii, Y.Y. Zhiburtovich, G.V. Ivanova, O.V. Konstantinova, D.V. Tsavlovskii, S. Lorenz, B. Schneider, Preparation and synthetic

application of partially protected brassinosteroids, Steroids 75 (1) (2010) 27–33.
[15] P.G.M. Wuts, T.W. Greene, Green's Protective Groups in Organic Synthesis, John Wiley and Sons, Inc., New Jersey, 2007.
[16] L. van Hijfte, R.D. Little, Intramolecular 1, 3-diyl trapping reactions. A formal total synthesis of (±)-coriolin, Journal of Organic Chemistry 50 (1985) 3940–3942.
[17] E.A. Masha, L.T.A. Kantora, S.C. Wallera, Monobenzylation of 1,n-diols *via* dibutylstannylene intermediates, Synthetic Communications 27 (3) (1997) 507–514.
[18] H. Kusama, R. Hara, S. Kawahara, T. Nishimori, H. Kashima, N. Nakamura, K. Morihira, I. Kuwajima, Enantioselective total synthesis of (−)-taxol, Journal of the American Chemical Society 122 (2000) 3811–3820.