Synthesis of a novel brassinosteroid type with an annelated dioxonane side chain

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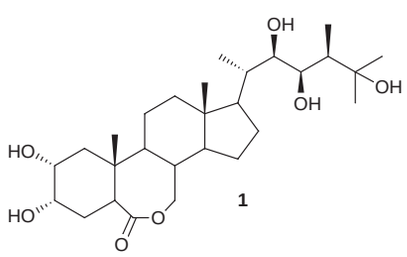
In studies directed towards the synthesis of 25-hydroxybrassinolide **1** we found, that upon reaction of intermediate **7** with trimethylaluminium–*n*-butyllithium an alkylating fission of the epoxide ring and rearrangement of the tetrahydropyranyl unit takes place to afford after deprotection the new dioxonane-annelated brassinosteroid **9**.

Introduction

Brassinosteroids are a new class of steroidal phytohormones with high growth promoting and antistress activity. Since the discovery of brassinolide in 1979 more than 40 native brassinosteroids have been isolated and characterized from a large variety of plants.[1] In cell suspension cultures of *Lycopersicum esculentum* 24-epibrassinolide was converted to 25-β-D-glucopyranosyloxy-24-epibrassinolide which afforded upon hydrolysis the pentahydroxylated 25-hydroxy-24-epibrassinolide.[2] Such a hydroxylation at C-25 plays an important role also for other steroidal hormones, especially in the ecdysone and vitamin D metabolite series. In our efforts to synthesize 25-hydroxybrassinolide **1** *via* side-chain construction[3] we observed an unusual rearrangement leading to a new type of brassinosteroid analog with a dioxonane annelated side-chain moiety.

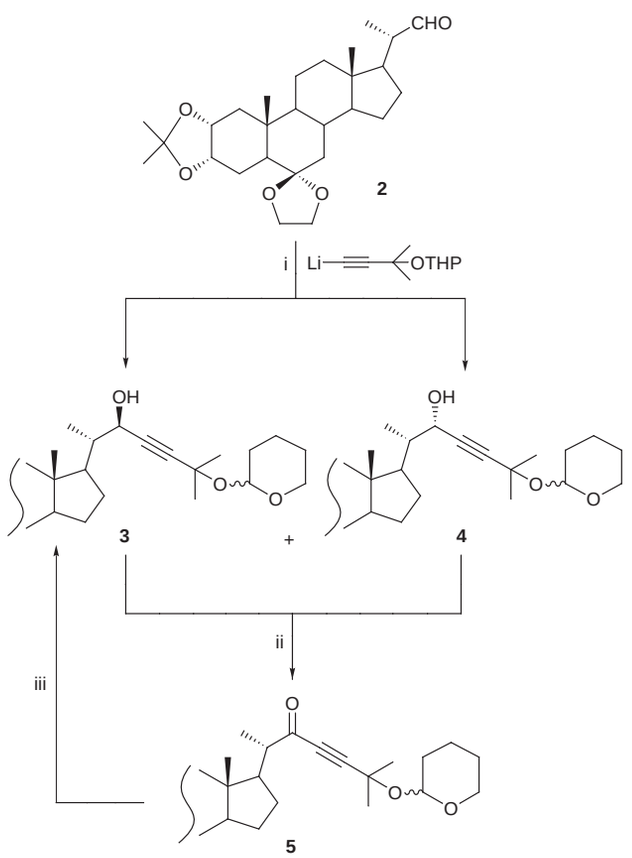


Results and discussion

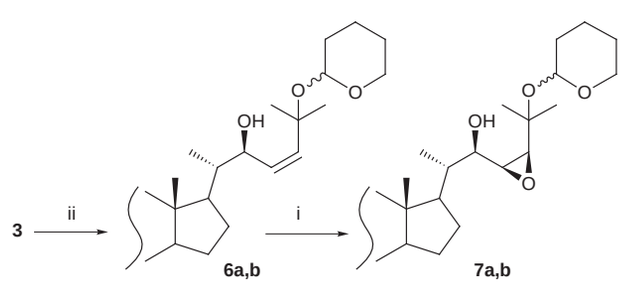
Synthesis

As a starting compound the protected 22-aldehyde **2** was used, which is available in eight steps from stigmasterol by functionalization of the A/B-ring system followed by side-chain degradation.[4] Coupling of **2** with the lithium salt of 3-tetrahydropyran-2-yloxy-3-methylbut-1-yne[5] as the side-chain synthon afforded, similar to that described for the synthesis of ecdysone[5,6] and brassinosteroids,[7,8] a 1 : 1 mixture of the (22*R*)- and (22*S*)-hydroxylated diastereomers **3** and **4**, separable by HPLC.[9] Both compounds show some split ^{13}C NMR signals assigned to the side-chain ($\Delta\delta$ less than 0.1) due to the asymmetric nature of C-1′ in the tetrahydropyranyl ring (Table 1). Oxidation[6] of **3** + **4** with pyridinium chlorochromate to ketone **5** followed by asymmetric reduction with (*R*)-alpine-borane[6] gave the desired (20*R*)-hydroxylated epimer **3** with excellent stereoselectivity (Scheme 1).

Partial hydrogenation[8] of **3** over P2-nickel catalyst in the presence of ethylenediamine afforded two 22-hydroxy-(23*Z*)-enes **6a**,**b** with identical MS-data, which are separable by chromatography over silica gel (Scheme 2).



Scheme 1 *Reagents and conditions*: i, *n*-BuLi, 3-tetrahydropyran-2-yloxy-3-methylbut-2-yne, THF, −78 °C; ii, pyridinium chlorochromate, CH_2Cl_2; iii, (R)-alpine-borane, THF.



Scheme 2 *Reagents and conditions*: i, $NaBH_4$, $Ni(OAc)_2{\cdot}4H_2O$, $H_2N(CH_2)_2NH_2$, H_2, EtOH; ii, MCPBA, Na_2HPO_4, CH_2Cl_2.

Table 1 ^{1}H [a] and ^{13}C NMR data of compounds **3**, **4** and **9** (in $CDCl_3$)

	3		**4**		**9**	
	δ_H (*J*/Hz)	δ_C	δ_H (*J*/Hz)	δ_C	δ_H (*J*/Hz)	δ_C
1	1.91/1.06	42.7	1.92/1.05	42.7	1.75/1.55	40.2
2	4.10 ddd (10.8/6.8/5.1)	72.9	4.10 m	72.9	3.76 ddd (11.6/4.5/2.9)	68.2
3	4.27 m	72.8	4.28 m	72.8	4.05 ddd (2.9/2.9/2.9)	68.3
4	2.15/1.80	22.0	2.15/1.80	22.0	1.91/1.71	26.3
5	1.80	45.5	1.80	45.5	2.69 dd (12.6/3.0)	50.7
6	—	109.7	—	109.6	—	212.2
7	1.75/1.02	41.0	1.75/1.02	41.0	2.30 dd (13.2/4.6) 2.01 dd (13.2/13.2)	46.7
8	1.51	32.9	1.51	32.9	1.77	37.7
9	0.78 ddd (12.1/10.8/4.1)	52.9	0.77	53.0	1.40	53.6
10	—	38.0	—	38.0	—	42.5
11	1.55/1.30	20.7	1.54/1.31	20.7	1.66/1.32	21.2
12	1.95/1.17	39.4	2.00/1.16	39.6	2.02/1.31	39.3
13	—	42.4	—	42.7	—	42.8
14	1.09	55.7	1.04	55.6	1.33	56.5
15	1.60/1.09	24.1	1.58/1.09	24.3	1.56/1.09	23.8
16	1.89/1.35	27.4	1.80/1.30	27.3	1.99/1.21	27.9
17	1.47	51.7	1.24	53.1	1.69	52.4
18	0.68 s	12.0	0.69 s	12.2	0.66 s	11.7
19	0.84 s	13.3	0.84 s	13.4	0.76 s	13.5
20	1.59	42.1	1.74	42.0	1.51	36.9
21	1.10 d (6.8)	13.2	1.04 d (6.7)	12.7	0.93 d (6.7)	12.0
22	4.48 br s	65.4	4.45 d (3.6)	65.4	3.41 d (8.8)	74.0
23	—	85.0	—	82.4	3.33 dd (8.8/8.8)	71.1
24	—	87.7	—	88.6	3.22 s	87.1
25	—	71.0	—	71.2	—	76.8
26 [b]	1.52 s	29.9	1.53 s	29.9	1.32 s	18.6
27 [b]	1.48 s	30.5	1.49 s	30.6	1.15 s	28.7
Isopr.1	—	107.6	—	107.6	—	—
Isopr.2 [c]	1.48 s	28.6	1.47 s	28.6	—	—
Isopr.3 [c]	1.33 s	26.5	1.33 s	26.6	—	—
Ethyl.1 [d]	3.95/3.90	65.5	3.95/3.90	65.5	—	—
Ethyl.2 [d]	3.90/3.75	64.2	3.91/3.74	64.2	—	—
1′	5.03 m	96.1	5.02 m	96.3	4.23 ddq (14.9/3.1/6.1)	69.1
2′	1.70/1.53	32.0	1.69/1.52	32.0	1.52/1.52	38.0
3′	1.84/1.52	20.5	1.84/1.52	20.6	1.73/1.64	23.6
4′	1.52/1.52	25.3	1.52/1.52	25.3	1.84/1.53	28.9
5′	3.95/3.49	63.3	3.95/3.48	63.5	4.03 m/3.41 m	76.4
1′-Me					1.11 d (6.1)	24.9

[a] ^{1}H chemical shifts without multiplet specification are chemical shifts of HSQC correlation peaks. [b] Diastereotopic methyl groups 26/27 are not assigned. [c] Methyl groups of the isopropylidene dioxy group may be reversed. [d] Methylene groups of the ethylene dioxy group may be reversed.

Table 2 Selected ^{1}H NMR data of compounds **5**–**8** (in $CDCl_3$)

	δ_H (*J*/Hz)					
	5	**6a**	**6b**	**7a**	**7b**	**8**
16	1.78/1.26	1.98/1.34	1.95/1.37	1.89/1.27	1.87/1.39	1.97/1.19
17	1.63	1.59	1.55	1.60	1.57	1.63
18	0.70	0.67	0.67	0.68	0.68	0.66 s
19	0.84	0.83	0.84	0.84	0.84	0.84 s
20	2.53 m	1.41	1.47	1.54	1.64 m	1.50
21	1.22 d (6.6)	0.99 d (6.7)	0.96 d (6.6)	1.08 d (6.5)	1.07 d (6.5)	0.92 d (6.6)
22	—	4.73 br d (7.5) [4.95 d (7.2)] [a]	4.70 br s (Δ_2^1 = 8.5) [4.89 br s (Δ_2^1 = 12.0)] [a]	3.99 br d (6.1)	4.15 br d (6.2)	3.40 br d (8.8)
23	—	5.56 dd (12.2/7.5) [5.70 dd (12.3/7.2)] [a]	5.46 [5.52 dd (12.5/6.1)] [a]	2.97 dd (6.1/4.3)	2.99 dd (6.2/4.4)	3.32 dd (8.8/8.8)
24	—	5.43 dd (12.2/0.8) [5.33 dd (12.3/1.2)] [a]	5.46 [5.36 dd (12.5/1.4)] [a]	2.91 d (4.3)	2.89 d (4.4)	3.23 s
26 [b]	1.59 s	1.40 s	1.42 s	1.42 s	1.45 s	1.32 s
27 [b]	1.55 s	1.38 s	1.41 s	1.33 s	1.36 s	1.15 s
1′	5.00 m	4.76 m	4.83 m	4.86 m	4.82 m	4.23 m
2′	1.72/1.54	1.70/1.55	1.72/1.56	1.67/1.51	1.69/1.49	1.52/1.52
3′	1.85/1.54	1.85/1.54	1.85/1.53	1.81/1.51	1.81/1.53	1.74/1.63
4′	1.54/1.54	1.51/1.51	1.52/1.52	1.50/1.50	1.48/1.48	1.84/1.53
5′	3.95/3.50	3.96/3.47	3.94/3.47	3.95/3.46	3.90/3.45	4.02 m/3.43 m
1′-Me	—	—	—	—	—	1.11 d (6.2)

[a] In C_6D_6. [b] Diastereotopic methyl groups 26/27 are not assigned.

Table 3 Selected ^{13}C NMR data of compounds **5**–**8** (in $CDCl_3$)

	δ_C					
	5	**6a**	**6b**	**7a**	**7b**	**8**
16	27.1	27.8	27.5	27.5	27.3	28.1
17	51.9	52.5	52.5	52.2	52.4	52.6
18	12.3	11.9	11.9	11.9	11.9	11.7
19	13.3	13.4	13.3	13.3	13.3	13.4
20	51.9	40.6	41.2	39.8	39.5	37.0
21	16.2	12.4	12.3	12.5	12.5	12.0
22	191.6	68.2	69.4	68.4	68.9	74.2
23	81.7	134.5	133.6	60.1	60.8	71.1
24	94.9	135.6	136.7	64.5	64.3	87.2
25	70.8	76.6	77.1	74.7	74.1	76.7
26[a]	29.2	29.5	29.0	26.0	25.0	18.6
27[a]	29.6	29.4	28.5	24.0	25.8	28.7
1′	96.4	95.4	94.8	94.6	95.5	69.1
2′	31.8	32.5	31.9	32.0	31.6	38.0
3′	20.3	20.7	20.4	20.6	20.9	23.7
4′	25.2	25.1	25.2	25.1	25.2	28.9
5′	63.4	63.9	63.2	63.4	63.7	76.4
1′-Me	—	—	—	—	—	24.9

[a] Diastereotopic methyl groups 26/27 are not assigned.

The vicinal coupling constant $J_{H\text{-}23/H\text{-}24}$ of 12.2 Hz found for **6a** indicates the *Z* configuration of the double bond. For the more polar **6b**, H-23 and H-24 are accidentally isochronous in $CDCl_3$ solution and thus no coupling constant can be determined. However, upon solution of **6b** in C_6D_6, H-23 (δ 5.52 dd 12.5/6.1 Hz) and H-24 (δ 5.36 dd 12.5/1.4 Hz) show a vicinal coupling constant of 12.5 Hz. This clearly proves that the C-23/C-24 double bond is *Z* configurated in **6b**, too. Therefore, **6a** and **6b** must differ only in the configuration at C-1′ of the tetrahydropyranyl ring, which is reflected also in small differences of some ^1H- and ^{13}C-NMR signals of the side-chain moiety (Tables 2 and 3).

Subsequent epoxidation[8] of the separated C-1′† isomers **6a** and **6b** with MCPBA–Na_2HPO_4 afforded the epoxides **7a** and **7b** as the major products[8,10] (Scheme 2).

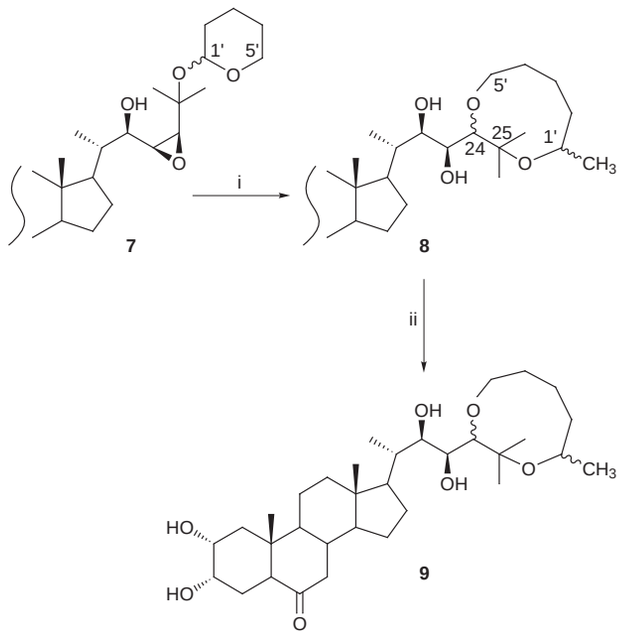
Comparison of **7a** and **7b**, obtained from **6a** or **6b**, respectively, shows that both products have identical HPLC retention times but the side-chain atoms have different ^1H and ^{13}C NMR chemical shifts (Tables 2 and 3).

Upon reaction[8] of **7a** with trimethylaluminium and *n*-butyllithium in dry cyclohexane–*n*-hexane at −78 °C, instead of the expected opening of the epoxide on methylation, surprisingly a new alkylating rearrangement involving the tetrahydropyranyl system was observed to give compound **8** [*m*/*z* 648 (M^+)] with a nine-membered ring system (Scheme 3).

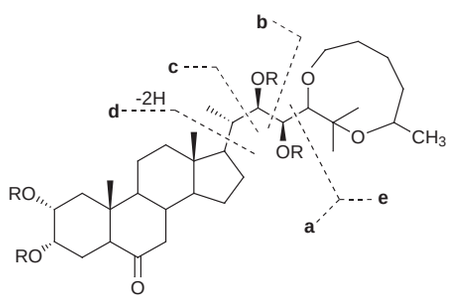
Under the same conditions, reaction of epimer **7b** did not give **8**, but a complex mixture of fission products of the side-chain. These results indicate that the direction of the reaction is controlled by the configuration at the C-1′ of the tetrahydropyranyl function. Upon treatment of **8** with 80% acetic acid the deprotected new dioxonane **9** was obtained.

The molecular weight of compound **9** is indicated both by the positive and negative ion electrospray (ES) mass spectra (*m*/*z* 565 $[M + H]^+$ and *m*/*z* 623 $[M + OAc]^-$), respectively. The EI mass spectral fragmentation of the trimethylsilyl ether **9a** is mainly characterized by α-cleavage between C-23/C-24 leading to the complementary key ion **a** (*m*/*z* 681) and **e** (*m*/*z* 171) and C22/C-23 (ion **b** at *m*/*z* 579). The structure of **9** is also supported by the EIMS of the methylboronate **9b** displaying a molecular ion M^+ at *m*/*z* 612. Furthermore, key ions at *m*/*z* 441 (**a**), 357 (**c**) and 327 (**d**) appear (Fig. 1).

† The numbering system used in this paper to describe carbon positions in compounds **6**–**9** and in NMR data has been employed for ease of comparison. The locants in the names of steroids comply with IUPAC nomenclature.



Scheme 3 *Reagents and conditions*: i, $AlMe_3$, *n*-BuLi, *n*-hexane, cyclohexane; ii, AcOH (80%), 50 °C.



9: R=H **9a**: R=TMS **9b**: R,R=B(CH_3)

Fig. 1 Mass spectral fragmentation pattern of the dioxonane-annelated brassinosteroids.

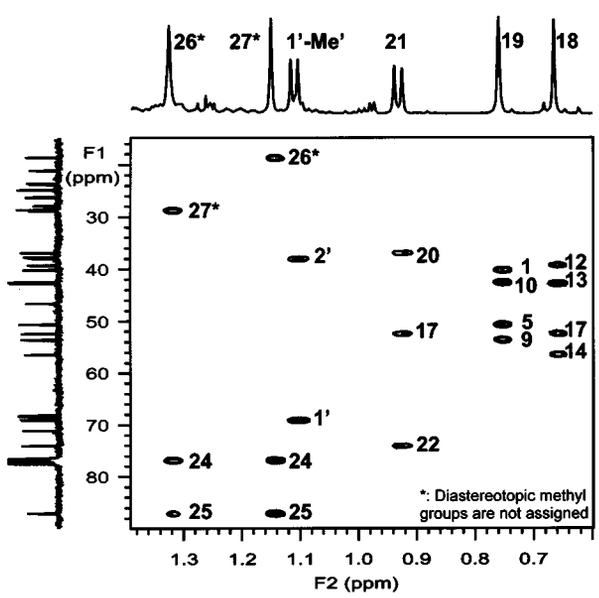


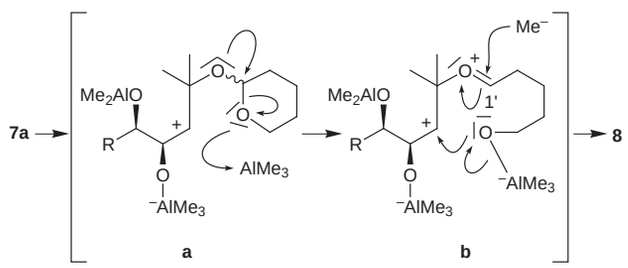
Fig. 2 ^1H NMR methyl group part of HMBC spectrum of **9**.

Combined use of one- and two-dimensional NMR experiments (including DQFCOSY, HSQC and HMBC) resulted in an unambiguous assignment of all ^1H and ^{13}C NMR signals (Table 1). The proton signal of 1′-Me shows a COSY crosspeak with H-1′ as well as HMBC correlation peaks with C-1′ and C-2′ (Fig. 2). Moreover, H-24 shows a HMBC correlation with

C-5′. These findings unequivocally prove the dioxonane structure in **9**. Interestingly, the OH group attached to C-23 shows a coupling constant of 8.8 Hz with H-23, whereas OH-22 appears as a broad singlet. This suggests a partial steric fixation of the dioxonane ring *via* an intramolecular hydrogen bond to the OH group at C-23. Efforts to obtain suitable crystals for X-ray analysis failed, therefore the configuration at C-24 and C-1′ remains uncertain.

Conclusions

In conclusion, reaction of epoxide **7a** with trimethylaluminium and *n*-butyllithium afforded a novel type of brassinosteroid with a 24(25)-annelated dioxonane ring. The formation of compound **8** can be explained *via* epoxy ring opening to **a**, followed by endocyclic cleavage of the C(1′)–O-bond of the tetrahydropyran ring to give the intermediate **b**, which undergoes ring closure under simultaneous methylation at C-1′ to afford the dioxonane **8** (Scheme 4).



Scheme 4 Proposed reaction sequence for the formation of dioxonane **8**.

It is of special interest that only one of the C-1′ isomers exhibits the appropriate steric arrangement required for this reaction, probably due to a different spacial fixation of the tetrahydropyran ring *via* an intramolecular hydrogen bond. Similar endocyclic cleavage of glycosidic acetals under alkylation with Me_3Al have been described very recently by Olsson *et al*.[11]

Experimental

Materials and methods

Solvents were partially dried prior to use over metallic sodium. All commercial reagents were used without further purification. 3-Tetrahydropyran-2-yloxy-3-methylbut-1-yne was prepared as previously reported.[5]

For flash column chromatography we used Merck silica gel 60 (particle size 0.040–0.063 mm, 230–400 mesh ASTM). Melting points (uncorrected) were determind on a Boetius heating table. Optical rotation indices $[\alpha]_D$ were measured at room temperature (rt) on a Jasco Digital Polarimeter DIP-1000 and $[\alpha]_D$ values are given in units of 10 cm^{-1} deg cm^2 g^{-1}. For C,H analyses a LECO CHNS-239 was used. EI mass spectra were recorded on an AMD 402 (AMD Intrecta GmbH) spectrometer (70 eV). IR spectra were obtained by using a Bruker IFS 28. ^{13}C and APT NMR spectra were recorded on a VARIAN GEMINI 300 spectrometer at 75.50 MHz. The deuterated solvent $CDCl_3$ was used as an internal reference (δ 77.0). ^1H and 2D (DQFCOSY, NOESY, GHSQC, GHMBC) NMR spectra were recorded on a VARIAN UNITY 500 spectrometer at 499.83 MHz. TMS was used as an internal reference.

(22*R*)-2α,3α-Isopropylidenedioxy-6,6-ethylenedioxy-22-hydroxy-25-(tetrahydropyran-2-yloxy)-5α-cholest-23-yne (3) and its (22*S*)-isomer (4)

To a solution of *n*-BuLi (1.6 M in n-hexane, 11.2 ml, 17.9 mmol, 4 equiv.) in dry and cooled (−25 °C) THF (20 ml), 3-tetrahydropyran-2-yloxy-3-methylbut-1-yne (3.38 g, 20.16 mmol, 4.5 equiv.) was added with a syringe at −60 °C under an argon atmosphere. This mixture was stirred and warmed up at room temperature for 30 min and then cooled to −78 °C. Then a solution of the 22-aldehyde **2** (2.0 g, 4.48 mmol) in dry THF (30 ml) was added at −78 °C and stirring was continued at −78 °C for 1 h. The cooling bath was then removed and the excess reagent was destroyed with sat. aq. NH_4Cl below −20 °C. The solution was raised to room temperature and diluted with EtOAc. Work up (EtOAc for extraction) gave a crude product, which was chromatographed with *n*-hexane–EtOAc (85:15) to give a 1:1 mixture of **3** + **4** (2.34 g, 85%). Analytical HPLC: Merck, LiChrospher 100 RP 18, 5 μm, 125 × 4 mm, MeCN:H_2O = 85:15, 1 ml min^{-1}, 210 nm, retention time **3**: 7.36 min, **4**: 5.18 min. Separation by HPLC: Merck, LiChrospher 100 RP 18, 10 μm, 250 × 10 mm, MeCN:H_2O = 90:10, 3 ml min^{-1}, 210 nm. Compounds **3** and **4** were obtained as colourless oils; $[\alpha]_D$ compound **3** +44.5 (*c* 1.1, MeOH), compound **4** +42.7 (*c* 1.2, MeOH); ν_{max}(nujol)/cm^{-1} 3420 (OH); *m*/*z* (rel. int.) = 614 (M^+, 3), 599 (30), 530 (12), 515 (66), 418 (25), 403 (12), 360 (39), 319 (82), 239 (22), 99 (38), 85 (100) (Found for compound **3**: C, 72.22; H, 9.24 and for compound **4**: C, 72.32; H, 9.25. $C_{37}H_{58}O_7$ requires C, 72.28; H, 9.51%). ^1H and ^{13}C NMR data are displayed in Table 1.

2α,3α-Isopropylidenedioxy-6,6-ethylenedioxy-25-(tetrahydropyran-2-yloxy)-5α-cholest-23-yn-22-one (5)

To a solution of **3** + **4** (2 g, 3.25 mmol) in 100 ml dry CH_2Cl_2 (over molecular sieves) pyridinium chlorochromate (1.12 g, 5.2 mmol, 1.6 equiv.) was added. The suspension was stirred for 20 h at room temperature. Silica gel was added and CH_2Cl_2 was evaporated under reduced pressure and the residue chromatographed with *n*-hexane–EtOAc (85:15) to afford amorphous **5** (1.6 g, 81%); $[\alpha]_D$ +25.6 (*c* 1.0, MeOH); ν_{max}(nujol)/cm^{-1} 2245 (C≡C), 1677 (C=O); λ_{max}/nm (lg ε) = 231 (0.48); *m*/*z* (rel. int.) = 612 (M^+, 4), 597 (22), 528 (19), 513 (78), 417 (38), 401 (16), 317 (100), 239 (41), 99 (57), 85 (37) (Found: C, 72.26; H, 9.03. $C_{37}H_{56}O_7$ requires C, 72.52; H, 9.21%). ^1H and ^{13}C NMR data are displayed in Tables 2 and 3.

(22*R*)-2α,3α-Isopropylidenedioxy-6,6-ethylenedioxy-22-hydroxy-25-(tetrahydropyran-1′-yloxy)-5α-cholest-23-yne 3

The 22-ketone **5** (1.5 g, 2.44 mmol) was dissolved in dry THF (30 ml) and *R*-alpine-borane (0.5 M in THF, 11.2 ml, 5.6 mmol, 2.3 equiv.) was added under an argon atmosphere. This mixture was stirred at room temperature for three days and then chromatographed to give only **3** (1.3 g, 86%).

(22*R*,23*Z*)-2α,3α-Isopropylidenedioxy-6,6-ethylenedioxy-22-hydroxy-25-(tetrahydropyran-2-yloxy)-5α-cholest-23-ene (2-epimers 6a,b)

P2-nickel catalyst (1 equiv.) was prepared under a N_2 atmosphere from 1 M $NaBH_4$ in EtOH (1.95 ml, 1 equiv.) and Ni(OAc)$_2$·4H_2O (485 mg, 1 equiv.) in 96% EtOH (10 ml). After replacing N_2 by H_2 and addition of ethylenediamine (260 μl, 2 equiv.) a solution of **3** (1.2 g, 1.95 mmol) in 96% EtOH was added and stirred overnight at room temperature. The mixture was then diluted with ether (30 ml) and filtrated. The filtrate was concentrated *in vacuo* and the residue chromatographed over silica gel. Elution with *n*-hexane and increasing amounts of EtOAc gave with *n*-hexane–EtOAc (85:15) firstly **6a**, followed by the more polar **6b** (overall yield 1.1 g, 91%) which are only different in the stereochemistry at C-1′ of the THP-ether. Compounds **6a** and **6b** were obtained as white crystals (from $CHCl_3$), mp **6a**: 71–72 °C, **6b**: 160–162 °C; $[\alpha]_D$ compound **6a** +25.7 (*c* 1.1, MeOH), compound **6b** +65.5 (*c* 1.1, MeOH); ν_{max}(nujol)/cm^{-1} **6a**: 3418 (OH), **6b**: 3500 (OH); *m*/*z* (rel. int.) = 616 (M^+, 3), 601 (26), 514 (40), 418 (51), 360 (100), 303 (13), 239 (10), 99 (22), 97 (63), 85 (58) (Found for compound

6a: C, 71.76; H, 9.72 and for compound **6b**: C, 71.87; H, 9.47. $C_{37}H_{60}O_7$ requires C, 72.04; H, 9.80%). ^{1}H and ^{13}C NMR data are displayed in Tables 2 and 3.

(22*R*,23*S*,24*R*)-2α,3α-Isopropylidenedioxy-6,6-ethylenedioxy-22-hydroxy-23,24-epoxy-25-(tetrahydropyran-2-yloxy)-5α-cholestane (2-epimers 7a,b)

To a solution of **6a** or **6b** (0.5 g, 0.81 mmol) in CH_2Cl_2 (20 ml), anhydrous powdered Na_2HPO_4 (345 mg, 2.43 mmol, 3 equiv.) and MCPBA (*ca*. 85%, 1.78 mmol, 362 mg, 2.2 equiv.) was added. The mixture was stirred at 5 °C for 20 h and stopped with NaOH (0.5 M, 15 ml). Work-up with CH_2Cl_2 for extraction and chromatography with *n*-hexane–EtOAc (80 : 20) afforded **7a** (405 mg, 79%) or **7b** (431 mg, 84%), respectively. Recrystallization from $CHCl_3$–MeOH gave needles, mp **7a**: 186–187 °C, **7b**: 162–163 °C; $[a]_D$ compound **7a** +10.0 (*c* 1.1, MeOH), compound **7b** +45.7 (*c* 1.3, MeOH; ν_{max}(nujol)/cm^{-1} 3487 (OH); *m*/*z* (rel. int.) = 632 (M^+, 2), 617 (6), 548 (7), 533 (23), 489 (7), 418 (8), 360 (20), 337 (42), 319 (24), 239 (21), 99 (50), 85 (100) (Found for compound **7a**: C, 69.89; H, 9.28 and for compound **7b**: C, 69.97; H, 9.36. $C_{37}H_{60}O_8$ requires C, 70.22; H, 9.56%). ^{1}H and ^{13}C NMR data are displayed in Tables 2 and 3.

(22*R*,23*S*)-2α,3α-Isopropylidenedioxy-6,6-ethylenedioxy-22-hydroxy-24,25-hexane-1,5-diyldioxy-5α-cholestane (8)

The epoxide **7a** (390 mg, 0.62 mmol) was dissolved in dry cyclohexane (70 ml) and *n*-hexane (70 ml). The stirred mixture was cooled at −78 °C under an argon atmosphere. Me_3Al (2 M in *n*-hexane, 2.81 ml, 5.62 mmol, 10 equiv.) and *n*-BuLi (1.6 M in *n*-hexane, 0.71 ml, 1.13 mmol, 2 equiv.) was added through a cannula. The mixture was stirred for 1 h at −78 °C and allowed to warm to room temperature and left to stand for 3–5 days. The mixture was then cooled to −40 °C and the reaction was stopped by addition of 1 M HCl (15 ml). After extraction with EtOAc, the organic layer was dried over Na_2SO_4 and concentrated. The residue was chromatographed on silica gel with *n*-hexane–EtOAc (70 : 30) to give amorphous **8** (172 mg, 50%), $[a]_D$ +20.0 (*c* 1.0, MeOH); *m*/*z* (rel. int.) = 648 (M^+, 2), 633 (7), 533 (12), 418 (30), 389 (32), 360 (79), 337 (30), 239 (29), 99 (71), 85 (100). ^{1}H and ^{13}C NMR data are displayed in Tables 2 and 3.

(22*R*,23*S*)-2α,3α,22,23-Tetrahydroxy-24,25-hexane-1,5-diyldioxy-5α-cholestan-6-one (9)

For deprotection **8** (150 mg, 0.23 mmol) was dissolved in 80% AcOH (20 ml) and stirred for 1 h at 50 °C. The solution was then neutralized with aq. K_2CO_3 and extracted with $CHCl_3$. After drying over Na_2SO_4 the solvent was removed under reduced pressure and chromatographed with *n*-hexane–EtOAc (50 : 50) to give the pure product **9** (119 mg, 92%).

Upon recrystallization from $CHCl_3$–MeOH, white crystals were obtained, mp 201–203 °C; $[a]_D$ +2.7 (*c* 1.0, MeOH); ν_{max}(nujol)/cm^{-1} 3564 (OH), 3374 (OH), 1703 (C=O). Compound **9** Positive Ion ES-MS *m*/*z* (rel. int.) = 565 ($[M+H]^+$, 70), 506 (22), 488 (25), 465 (13), 447 (42), 364 ([**b** + H], 100). Negative Ion ES-MS: *m*/*z* 623 ($[M + OAc]^-$). Trimethylsilyl ether **9a**. EIMS *m*/*z* (rel. int.) = 681 (**a**, $[M - 171]^+$, 1.0), 579 (**b**, 1.8), 491 (1.4), 489 ($[\mathbf{b} - TMSiOH]^+$, 1.2), 447 (**d**, 1.0), 399 ($[\mathbf{b} - 2TMSiOH]^+$, 1.5), 389 (3.1), 359 (0.8), 327 (1.0), 309 ($[\mathbf{b} - 3TMSiOH]^+$, 1.7), 300 (4.7), 299 (2.5), 215 (3.0), 204 (1.6), 171 (**e**, 2.1), 157 (1.8), 73 (100), 59 ($(CH_3)_2OH^+$, 39). Methylboronate **9b**. EIMS *m*/*z* (rel. int.) = 612 (M^+, 0.1), 554 ($[M - C_3H_6O]^+$, 2.0), 454 ($[M - C_3H_6O - C_6H_{12}O]^+$, 3.2), 441 (**a**, 0.6), 357 (**c**, 1.3), 327 (**d**, 1.6), 287 (1.0), 197 (14), 113 ($[C_6H_{13}O]^+$, 68), 59 ($(CH_3)_2OH^+$, 100) (Found: C, 70.23; H, 9.78. $C_{33}H_{56}O_7$ requires C, 70.18; H, 9.99%). ^{1}H and ^{13}C NMR data are displayed in Table 1.

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