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Towards the synthesis of calotropin and related cardenolides from 3-epiandrosterone: A-ring related modifications†

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Calotropin and related cardiac glycosides isolated from plants such as *calotropis gigantea* represent an interesting target for biological investigations and are based on a cardiac steroid that is doubly connected to a sugar moiety. This naturally occurring family of cardiac glycosides was not only reported to have similar cardiac properties as the drugs digitoxin and digoxin, but also show cytotoxic activity against several cancer cell lines. Herein, the first synthetic access to these molecules is reported highlighting the required transformations of the A-ring of the steroid when starting from commercially available and inexpensive 3-epiandrosterone. Our strategy is based on a regioselective C–H oxidation of the methyl group at C-17 delivering the 2 α ,3 β -*trans*-diol moiety at the same time and ensuring its connection to the sugar unit.

Introduction

Cardiac glycosides were already known to the ancient Egyptians over 3000 years ago and have been always used in folk medicine.¹ Since the 18th century, cardiac glycosides from the *digitalis* plant typified by digitoxin (1) or digoxin (2) were successfully applied by Withering for the treatment of congestive heart failure.^{2,3} This was later attributed to the so called “cardiotonic effect” which is correlated to an inhibitory interaction with the sodium pump (Na⁺/K⁺-ATPase) leading to an increased intercellular sodium and calcium ion concentration. The inhibition finally results in a more powerful and faster contraction of the cardiomyocytes explaining the successful application of these drugs.^{4,5}

Cardiac glycosides show several characteristic features if compared to other classes of steroids. For example, they possess a tertiary hydroxyl group at C-14 and a β -oriented butenolide substituent at C-17 (Fig. 1).⁶ The A/B and C/D rings of cardiac glycosides are usually both *cis*-fused whereas cardiac glycosides isolated from the milkweed family *Asclepiadaceae*

differ in having a *trans*-junction of the A/B rings.⁷ While digitoxin (1), digoxin (2) and ouabain (3) are attached *via* their 3 β -OH group to the sugar moiety, the cardenolides calotropin (4) and their related cardenolides (5–10) are connected by the 2 α - and 3 β -position to the sugar unit forming a 1,4-dioxane ring.^{8–15} This double linkage of the steroidal pattern to the carbohydrate explains the unusual stability towards acids¹⁶ and reduction.¹⁷

Calotropin (4) and related cardenolides (5–10) bear moreover an aldehyde functionality at C-19^{16,18–20} that distinguishes

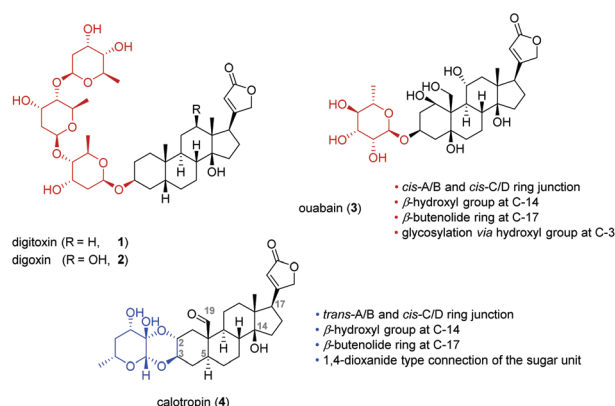


Fig. 1 Different cardiac glycosides and their connection of the steroidal aglycon to the sugar fragment. Calotropin (4) is doubly attached to the sugar moiety forming a 1,4-dioxane ring, while digitoxin (R=H, 1), digoxin (R=OH, 2) and ouabain (3) are linked to the sugar unit *via* the 3-OH group.

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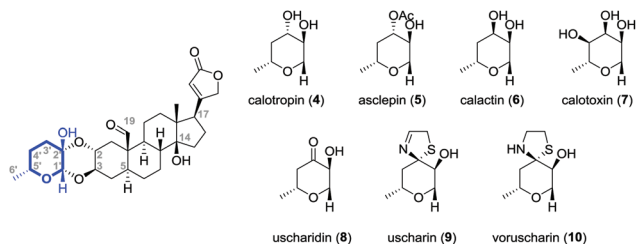


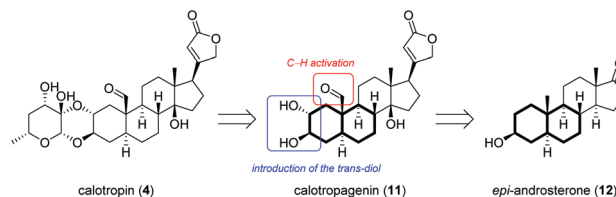
Fig. 2 Calotropin (4) and selected cardenolide glycosides (5–10) isolated from the milkweed family *Asclepiadaceae*.

them from gomphoside derivatives and occurs occasionally in some cardenolide classes, for instance also in *k*-strophanthin. Most cardiac glycosides contain one to four sugar residues attached to the genin inducing both the water solubility and the ability to bind the heart muscle.^{7,21} In the case of the Calotropin family, many different substitution patterns were found at the sugar moiety (Fig. 2). Modifications occur mostly at C-3' including the acetylation²² or epimerization^{9,10,15,23} of the hydroxyl group, but also its oxidation.^{10,11,14,15} Moreover, an attachment of a thiazolidine^{9–11,13,15} or a dihydrothiazolidine^{11,14,15} moiety was discovered at C-3' as a frequent modification of the sugar building block demonstrating the broad diversity of the presented cardenolide class.

While cardiac glycosides were used by the ancient Egyptians and Romans as heart tonic, emetic and diuretic,²⁴ natives in Africa took advantage of their toxic effects by applying *calotropis* plants as arrow poison.^{25,26} In lower doses, extracts of these plants exhibit a wide range of biological activities including anti-inflammatory,^{27–30} analgesic,^{31,32} antimicrobial^{27,33} or wound-healing properties.^{34,35} In the last years, cytotoxic and antitumor effects of cardenolides glycosides have been reported more frequently.^{36–42} The mode of action is still under investigation leading to several conclusions. For instance, Ishibashi and coworkers showed that calotropin (4) and related compounds inhibit the *wnt* signaling pathway in a dose-dependent manner.⁴³ Although these cardenolides have been studied more than one century⁸ and are reported to have similar cardiac properties to well-established cardiac glycosides, they remain less investigated.^{40,44}

Motivated by the interesting biological properties of the cardenolide family, we envisioned a modular semisynthesis from easy available starting materials that enables the modification of the lead structure on all parts of the molecule in order to gain insight into the structure activity relationship. A retrosynthetic analysis divides calotropin (4) into two fragments (Scheme 1), one of which is based on a sugar unit, while the other gives calotropagenin (11) as steroidal building block.

As steroidal precursor, we chose commercially available 3-epiandrosterone (12) possessing already six of the eight required stereogenic centers. Nevertheless a couple of modifications at the A-ring of the steroid are required including the installation of the 2,3-*trans*-diol and the oxidation of C-19. Herein, we wish to report on the introduction of the oxygen



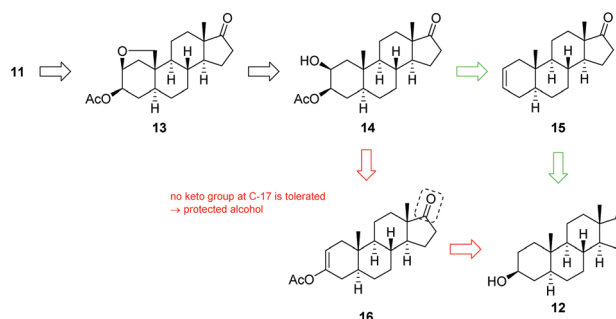
Scheme 1 Retrosynthetic analysis of calotropin (4) gives calotropagenin (11) as steroidal fragment. Highlighted are the desired transformations for the A/B-ring system with 3-epiandrosterone (12) as starting material. This includes the installation of the *trans*-diol at C-2/C-3 and the oxidation to an aldehyde at C-19.

moiety at C-19 allowing later on the generation of the aldehyde as well as the installation of the 2 α ,3 β -diol which allows the attachment to the sugar unit according to Lichtenthaler's glycosylation.⁴⁵

Results and discussion

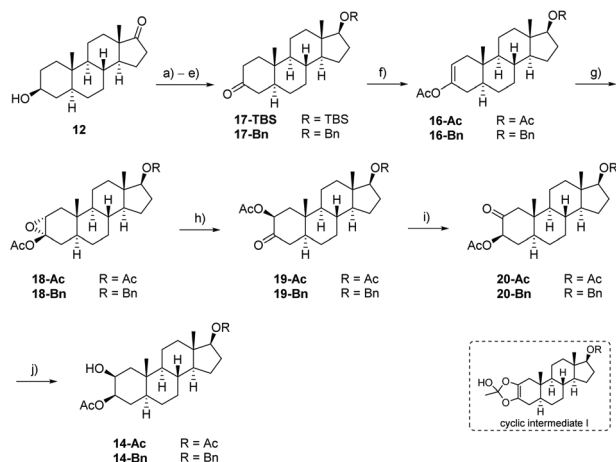
Our approach for the synthesis of calotropin (4) is based on the idea to introduce the 2,3-*trans*-diol and the oxygen functionality at C-19 *via* a remote intramolecular free radical C–H oxidation giving the key intermediate 13. A Lewis-acid promoted ring-opening of the A-ring bridging ether in 13 should deliver the desired *trans*-diol as well as the desired oxygen functionality at C-19. As suitable precursor for the intended remote C–H oxidation, the C-3 acetylated *cis*-diol 14 was chosen (Scheme 2).

The key intermediate 14 was initially synthesized according to typical steroidal transformation leading to the first generation approach (Scheme 2, highlighted in red). This approach is based on the epoxidation and ring-opening of the acetyl enol ether 16. Since this route is not compatible with the keto group at C-17, the latter was reduced and the obtained alcohol was protected with either an acetyl (14-Ac) or a benzyl protect-



Scheme 2 Overview of the two concepts for the installation of the *trans*-diol moiety at C-2/C-3 and the aldehyde at C-19 starting from 3-epiandrosterone (12). While the first generation approach (red) is based on an epoxidation of the acetyl enol ether 16, the second generation approach (green) relies on a diastereoselective Sharpless dihydroxylation of 15 followed by a regioselective acetylation of C-3's hydroxyl group.





Scheme 3 Synthesis of the precursor **14-Ac** and **14-Bn** according to the first generation route. (a) Ac_2O , pyridine, r.t., 16 h, 98%; (b) NaBH_4 , MeOH, $-20^\circ\text{C} \rightarrow 0^\circ\text{C}$, 3 h, 98%; (c) TBSCl, imidazole, DMF, r.t., 16 h, 80% or $\text{BnCO}=\text{NHCCl}_3$, TfOH, 1,4-dioxane, r.t., 16 h, 82%; (d) KOH, MeOH, Δ , 5 h; (e) NMO, cat. TPAP, CH_2Cl_2 , $0^\circ\text{C} \rightarrow \text{r.t.}$, 16 h or DMP, CH_2Cl_2 , $0^\circ\text{C} \rightarrow \text{r.t.}$, 16 h, 90% (**17-TBS**)/79% (**17-Bn**) over two steps; (f) HClO_4 , Ac_2O , EtOAc, r.t., 3 h, 69% (**16-Ac**)/77% (**16-Bn**); (g) *m*-CPBA, CH_2Cl_2 , $0^\circ\text{C} \rightarrow \text{r.t.}$, 16 h, 70% (**18-Ac**)/47% (**18-Bn**); (h) pyridine/toluene (1:10), Δ , 16 h, 86% (**19-Ac**)/75% (**19-Bn**); (i) $i\text{-PrOH}$, K_2CO_3 , H_2O , r.t., 16 h, 58% (**20-Ac**)/48% (**20-Bn**); (j) NaBH_4 , MeOH, $-20^\circ\text{C} \rightarrow 0^\circ\text{C}$, 3 h, 76% (**14-Ac**)/82% (**14-Bn**); TBS = *tert*-butyldimethylsilyl; Bn = benzyl.

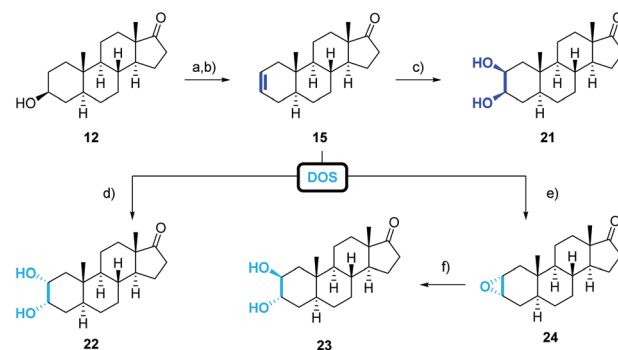
ing group (**14-Bn**). 3-Epiandrosterone (**12**) was therefore transformed to the keto compound **17** bearing (a) a TBS-protected or (b) a benzyl-protected alcohol at C-17 (Scheme 3). Treatment of **17** with Ac_2O and catalytic amounts of HClO_4 produces regioselectively the acetyl enol ether **16** whereby the acidic conditions replace the TBS-group by an acetyl group. After a substrate-induced stereoselective epoxidation from the α -face, the corresponding 2,3-epoxides **18** were opened with pyridine in toluene to give the 3-oxo 2 β -acetate **19** in good yields. Under basic conditions, **19** rearranged to the 2-oxo 3 β -acetate **20** by 1,2-carbonyl transposition. The isomerization proceeds by an enolisation followed by an acyl group migration presumably *via* the cyclic intermediate I that has been reported first by Fieser *et al.*^{46–48} The structure was determined by ^2D NMR experiments and X-ray crystallography (the crystal structure of **20-Ac** can be found in the ESI†) confirming the presumed configuration of the acetoxy group. Side reactions such as the cleavage of the acetyl protecting group at the A-ring or epimerization could not be completely suppressed by modifications in reaction time or the choice of the base and solvent. The reduction of the 2-keto group with NaBH_4 provided the desired precursors **14-Ac** and **14-Bn** in satisfying yields and good diastereoselectivities. An X-ray crystal structure was obtained from **14-Ac** confirming the molecular structure and the configuration at C-2 and C-3 in the solid state. In addition, NOESY experiments in combination with further ^2D NMR experiments verified the *cis*-configuration of the hydroxyl and acetoxy group. The first generation approach including ten classical steroidal transformation steps delivered the precursor with an

acetyl protecting group at C-17 **14-Ac** with 13% overall yield, while the corresponding benzyl protected precursor **14-Bn** was delivered with 6.5% yield. It is noteworthy that this route requires two additional steps including the cleavage of the protecting group and reoxidation of C-17 for being applicable for the synthesis of calotropin since the installation of the butenolide moiety demands a keto functionality at C-17 as it was previously shown by us.⁴⁹

The obtained poor overall yield of the required precursor along with the number of synthetic steps, encouraged us to search for an alternative route maintaining the oxidation state at C-17 (Scheme 2, green route). The novel approach relies on a Sharpless dihydroxylation of androst-2-ene **15** followed by a regioselective acetylation of the C-3 hydroxyl group. With androst-2-ene **15** as key intermediate, the opportunity of a diversity orientated synthesis was given allowing the synthesis of several 2,3-diols (Scheme 4).

Androst-2-ene **15** was prepared from 3-epiandrosterone (**12**) by tosylation followed by an elimination reaction (Scheme 4). From all tested bases comprising inorganic and organic bases such as 2,4,6-collidine or 2,6-lutidine, the combination of LiBr and Li_2CO_3 gave the best result regarding the yield and the regioselectivity. Unfortunately, the direct conversion with *p*-toluenesulfonic acid adsorbed on silica gel⁵⁰ did not afford androst-2-ene **15** in satisfying yield and selectivity. We were also able to obtain single crystals that were suitable for X-ray crystallography (Fig. 5) confirming the stated position of the double bond. The molecular structure shows that due to the sp^2 hybridization the A-ring adapts a half chair conformation. When comparing both structures, the steric strain for the androst-3-ene is higher than for its regioisomer explaining the observed regioselectivity of the elimination favoring the more stable androst-2-ene **15**.

As previously reported for comparable examples,⁵¹ the treatment with catalytic amounts of OsO_4 and NMO as stoichiometric oxidant favors in our case the formation of the unde-



Scheme 4 Synthesis of the 2 β ,3 β -*cis*-diol **21** according to the second generation route. (a) *p*-TsCl, pyridine, r.t., 16 h, 92%; (b) LiBr, Li_2CO_3 , DMF, 175°C , 7 h, 95%; (c) α -AD-mix, MeSO_2NH_2 , $i\text{-BuOH}/\text{H}_2\text{O}$, r.t., 12 h, 61% (**23**); (d) β -AD-mix, MeSO_2NH_2 , $i\text{-BuOH}/\text{H}_2\text{O}$, r.t., 7 d, 41%; (e) *m*-CPBA, CH_2Cl_2 , r.t., 14 h, 75%; (f) 2 *m* H_2SO_4 , THF, r.t., 7 d, 54%; (h) $\text{HOCH}_2\text{CH}_2\text{OH}$, *p*-TsOH, HC(OMe)_3 , CH_2Cl_2 , r.t., 16 h, 88%; DOS = diversity orientated synthesis.

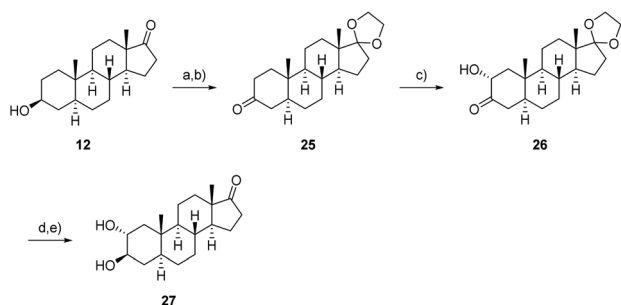


sired $2\alpha,3\alpha$ -*cis*-diol **22**, whereas the corresponding $2\beta,3\beta$ -*cis*-diols are reported to be prepared by the addition of iodine followed by a nucleophilic displacement with acetate in the presence of water and the hydrolysis of the intermediately formed ester using AgOAc,^{52,53} $\text{Ti}(\text{OAc})_3$ ⁵⁴ or CuOAc.⁵⁵ Driven by the idea of a direct conversion of androst-2-ene to the corresponding 2,3-*cis*-diol, we contemplated a Sharpless dihydroxylation adjusting the side of attack by the choice of the ligand.^{56,57} While the AD-mix β introduces the hydroxyl groups at the sterically more accessible α -side to build the $2\alpha,3\alpha$ -*cis*-diol **22**, the use of AD-mix α mainly forms the desired, but sterically more demanding $2\beta,3\beta$ -*cis*-diol **21**. Despite the reported accelerating effect of methanesulfonamide,⁵⁸ long reaction times of 7 days were required with commercially available AD-mix α . While the extra addition of OsO_4 or $\text{K}_2[\text{OsO}_2(\text{OH})_4]$ did not lead to a faster conversion of the starting material, 2.5 equiv. of “self-made” reduces luckily the reaction time to only 12 h even on 0.1 mmol scale (please see ESI† for further information). However, the corresponding 2,3-*cis*-diols were obtained in good yields and selectivities.

Different C-17 derivatives bearing a ketal (**27**) or TBS/Bn protected OH-group (**18-OH**, **18-TBS**, **18-Bn**) at C-17 were synthesized and subjected to the developed reaction conditions generating the corresponding $2\beta,3\beta$ -*cis*-diols (**29** and **30**) in good yields. Unfortunately, the purification *via* column chromatography on silica turned challenging resulting in rather low isolated yields (please see ESI†).

When androst-2-ene **15** was reacted with *m*-CPBA followed by an acid-catalyzed epoxide ring-opening, the $2\beta,3\alpha$ -*trans*-diol was obtained. Once more, the reagents attack occurred from the sterically less hindered α -side releasing the $2\alpha,3\alpha$ -epoxide **24** with 75% yield. The epoxide was opened by simple acidic treatment to afford with its diaxial $2\beta,3\alpha$ -substituents the least stable 2,3-diol.

Even though $2\alpha,3\beta$ -*trans*-diol **27** is not accessible from the precursor **15**, the missing *trans*-diol can be synthesized by a five-step procedure from 3-epiandrosterone (**12**) (Scheme 5).



Scheme 5 Synthesis of the $2\alpha,3\beta$ -*trans*-diol **27** starting from 3-epiandrosterone (**12**). (a) $\text{HOCH}_2\text{CH}_2\text{OH}$, *p*-TsOH, $\text{HC}(\text{OMe})_3$, CH_2Cl_2 , r.t., 16 h, 88%; (b) DMP, CH_2Cl_2 , 0 °C \rightarrow r.t., 16 h, 80% (**25**); (c) I. TMSOTf, NEt_3 , CH_2Cl_2 , 0 °C, 1 h; II. *m*-CPBA, NaHCO_3 , *n*-hexane, 0 °C, 2 h; III. $\text{C}_2\text{H}_2\text{O}_4$, MeOH, r.t., 5 min; 56% (**26**) or III. TBAF, THF, r.t., 10 min; 66% (**26**); (d) NaBH_4 , MeOH, CH_2Cl_2 , $-20 \rightarrow 0$ °C, 4 h, 66% (**33**); (e) *p*-PTSA, acetone, r.t., 3 h, 88% (**27**).

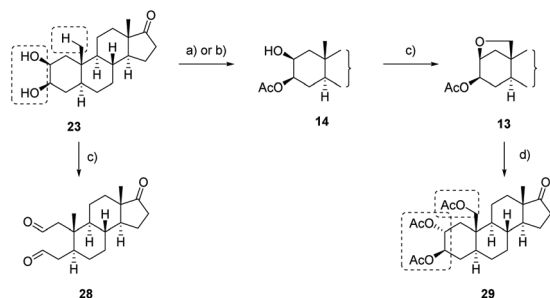
The synthesis starts with the protection of the keto functionality at C-17 followed by the oxidation of the hydroxyl group at C-3 enabling the stereo- and regioselective introduction of an α -hydroxyl group at C-2 by a Rubottom oxidation. This was accomplished by the conversion of **25** to the corresponding TMS-enol ether, its epoxidation with *m*-CPBA and a consecutive ring-opening followed by a silyl migration and finally the cleavage of the silyl group to give **26**. By reducing the reaction time for the cleavage with oxalic acid or by replacing it by TBAF, the 2α -hydroxylated androstan-3-one was formed with improved yields of about 56%, 66% respectively. Diastereoselective reduction from the sterically less hindered side and removal of the acetal protecting group with *p*-PTSA result in the formation of the $2\alpha,3\beta$ -*trans*-diol **27** which shows the same configuration at C-2 and C-3 as calotropagenin (**11**).

We were able to obtain suitable single crystals from the four diastereomeric 2,3-diols which are depicted in Fig. 5. The stated molecular structures and orientation of the substituents could be confirmed. The naturally occurring $2\alpha,3\beta$ -diol represents with its diequatorial substituents the thermodynamically preferred and therefore most stable diastereomer, while the other *trans*-diol possesses two axial hydroxyl groups causing an increased transannular strain. The 1,3-diaxial interaction explain also the preferred formation of the $2\alpha,3\alpha$ -*cis*-diol **22** over the $2\beta,3\beta$ -*cis*-diol **21** since the latter owns an axial hydroxyl group at C-2 which conflicts with the 19-methylgroup while in case of the other diastereomer the hydroxyl group at C-2 is equatorially orientated reducing the overall transannular strain.

With $2\beta,3\beta$ -*cis*-diol **21** in hands, we demonstrated the need of a hydroxyl group protected at C-3 in the directed sp^3 C-H oxidation reaction (Scheme 6). Therefore, we exposed the $2\beta,3\beta$ -*cis*-diol **21** to the later developed reaction conditions of the C-H oxidation reaction. Although full conversion of the $2\beta,3\beta$ -*cis*-diol **21** was observed, no formation of the desired product was detected. Instead, the dialdehyde **28** was isolated in 48% yield generated by glycol cleavage affirming the relevance of the protection group in order to avoid glycol cleavage.

In order to furnish a regioselective protection of the 3-OH group,^{59–62} several lipases comprising different yeast lipases (*C. cylindracea*, *C. rugosa*, *C. antarctica*), a mold lipase (*Mucor miehei*), one porcine pancreas and a lipase extracted from the Gram-negative bacterium *pseudomonas fluorescens*, were evaluated. The esterification reactions were run in toluene or *tert*-butanol as solvent, all at 36 °C using vinyl acetate as acetyl donor whereby the esterification progress was monitored *via* TLC. Three of the tested lipases showed partial or complete conversion in toluene within 2 d: in the case of *C. cylindracea* and *pseudomonas fluorescens* the formation of a unknown byproduct was observed, whereby *C. rugosa* delivered only one product which was determined as the desired 3-acetylated $2\beta,3\beta$ -diol **14**. By lowering the reaction temperature to r.t. and modifying the reaction experimental setup, an upscale on multigram scale was succeeded providing the 3-acetylated $2\beta,3\beta$ -diol **14** in yields higher than 77%. For comparison, we





Scheme 6 Transformation of the 2 β ,3 β -*cis*-diol **21** to 2 α ,3 β -*trans*-19-triacetate **29**. (a) *Candida rugosa*, vinyl acetate, toluene, r.t., 2 d, >77% (**14**) or (b) AcCl (1.05 equiv.), pyridine, 40 h, r.t., 39% (**14**); (c) DIB, I₂, cyclohexane, C₆H₆, 30–39 °C, 80 min, sonication (~), 48% (**28**)/72% (**13**); (d) Zn(OTf)₂, Ac₂O, 45 °C, 16 h \rightarrow 60 °C, 2 h, 68% (**29**).

conducted a kinetically controlled acetylation of 2 β ,3 β -diol **21** with 1.05 equiv. of acetyl chloride in pyridine giving the desired product **14** in only 39% yield.

In order to oxidize the methyl group at C-19, a remote intramolecular free radical C–H oxidation was envisioned exploiting the rigidity of the steroid and the close proximity of the OH-group at C-2 and the C-19 methyl group. The obtained crystal structure of 3-acetylated 2 β ,3 β -diol **14** confirms the stated structure and allows the determination of the distance between the oxygen of the hydroxyl group at C-2 and C-19 or its proton in the solid state to 2.928 Å and 2.278 Å, respectively. In accordance with the literature,^{63–66} this proximity allows the transfer of a radical from the alkoxy group to the methyl group at C-19 *via* a hypiodite reaction (Fig. 3). By using lead tetraacetate and iodine in overstoichiometric amounts,

androstane^{67–69} and cholestane^{70–72} derivatives have been successfully oxidized.

Driven by the idea to replace toxic Pb(OAc)₄ with DIB and encouraged by the fundamental work of Suárez,^{73,74} we initiated our studies by probing various reaction parameters such as the amounts of reagents, the concentration, the solvent, reaction time and temperature for the envisioned hypiodite reaction using 3-acetylated 2 β ,3 β -diol **14** as substrate (Table 1). Preliminary studies revealed problems with reproducibility when a tungsten lamp or LED lamp were deployed. Therefore, we report exclusively on a visible-light free alternative and the usage of a sonication bath to produce the required iodine radicals. In this case, we experienced results that are more consistent and reliable. We noticed the formation of four products including the desired A-ring bridging ether **13**, the iodide **30**, the overoxidized iodide **31** and the overoxidation product **32** (Table 1). The iodides **30** and **31** can be converted to **13** or respectively **32** by a nucleophilic substitution induced by silver acetate in acetone. All products can be differentiated *via* ¹H NMR spectroscopy, whereby the spectra of **30** and **13** show some similarities (Fig. 4). For the iodide **30** the proton at C-2 is shifted downfield, while the proton at C-3 and the more shielded protons of the diastereotopic methylene group at C-16 are shifted upfield compared to the signals of ether **13**.

In the beginning, 1.5 equiv. of (diacetoxyiodo)benzene (DIB) and 1.3 equiv. of iodine were used for the hypiodite reaction and the reaction was conducted at r.t. for 120 min under sonication (entry 1). After column chromatography on silica gel, the desired product **13** was isolated with 34% yield together with 20% reisolated starting material. Therefore, both the amounts of reagents and the reaction time were increased (entries 2 and 3) giving finally the product with synthetically useful 53% yield, although still small amounts of starting material **14** remained. In order to run the reaction at higher concentrations, we added benzene as co-solvent to increase the solubility of **14** and DIB. Keeping the amounts of reagents constant, we screened the reaction times for a higher concentrated solution of **14** (entries 4–6). After 140 min the desired ether **13** was isolated with an improved yield of 69% (entry 6) and no starting material was remained. Instead, the formation of hemiacetal acetate **32** was observed presumably as a result of a second hypiodite reaction due to an excess of reagents. In order to figure out which parameters promote overoxidation, we applied harsher reaction conditions. At higher temperatures for example the ratio of the desired and double oxidized product shifts towards overoxidation (entry 7). Since the amount of applied reagents should have an effect on the ratio of the two products, we kept reaction time at 140 min, but reduced the amounts of reagent. In this case we observed a dramatic drop in yield of the desired ether **13** (entry 8). In summary, increased amounts of reagent or elevated reaction times led to higher and faster conversion of the starting material, but also to an enhanced formation of the hemiacetal acetate **32** (not shown). Fine tuning of all these parameters finally provides the ether **13** with 72% yield (entry 9). Since the

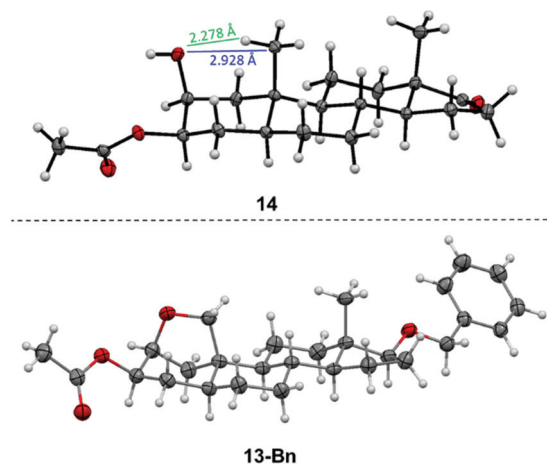
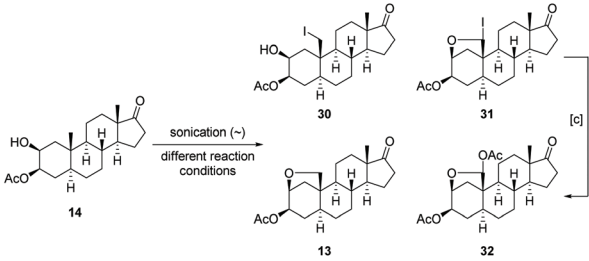


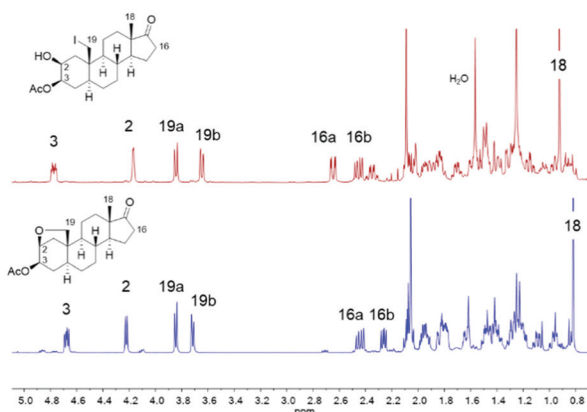
Fig. 3 Molecular structure of **14** and **13-Bn** (displacement parameters are drawn at 50% probability level) including distance measurements from oxygen at C-2 to the hydrogen at C-19 of **14**. Characteristic angles (relative deviations from the standard angle of 111.4°) of **13-Bn**: C1–C2–C3: 109.50(16)° (–1.7%), C2–C3–C4: 110.53(14)° (–0.79%), C3–C4–C5: 113.39(15)° (+1.8%), C4–C5–C10: 111.74(15)° (+0.31%), C5–C10–C1: 106.72(14)° (–4.2%), C10–C1–C2: 100.00(14)° (–10%).



Table 1 Evaluation of the optimal reaction conditions for the radical (sp^3)C–H oxidation of C-19


Entry	DIB [equiv]	I ₂ [equiv]	Reaction conditions ^a	Isolated yield [%] of (x)
1	1.5	1.3	cy ^b , 20–30 °C 120 min	34 (13) 20 (14)
2	2.0	1.5	cy ^b , 20–30 °C 139 min	38 (13) 35 (14)
3	2.2	1.5	cy ^b , 20–30 °C 150 min	53 (13) 16 (14)
4	2.2	1.5	cy/C ₆ H ₆ ^c , 20–30 °C 100 min	39 (13) 18 (30)
5	2.2	1.5	cy/C ₆ H ₆ ^c , 20–30 °C 120 min	39 (13) <18 (30)
6	2.2	1.5	cy/C ₆ H ₆ ^c , 20–30 °C 140 min	69 (13) 21 (32)
7	2.2	1.5	cy/C ₆ H ₆ ^c , 30–50 °C 140 min	50 (13) 30 (32)
8	2.0	1.4	cy/C ₆ H ₆ ^c , 20–25 °C 140 min	<16 (30) 27 (13)
9	2.0	1.2	cy/C ₆ H ₆ ^c , 20–30 °C 200 min	72 (13) 14 (32)
10	4.4	3.0	cy/C ₆ H ₆ ^c , 20–30 °C 140 min	62 (13) 37 (32)
11	4.4	3.0	cy/C ₆ H ₆ ^c , 20–30 °C 200 min	58 (13) 34 (32)

^a AgOAc, acetone, r.t., overnight; cy = cyclohexane. ^b c = 10 mm. ^c c = 30 mm.

**Fig. 4** ¹H NMR spectra (500 MHz, CDCl₃, r.t.) of the iodide **30** (red) and the desired ether **13** (blue).

hemiacetal acetate **32** already possess the correct oxidation state at C-19, we were also interested how its formation can be promoted. By the addition of higher equivalents of reagents

(entry 10) and prolonged reaction times (entry 11), the ratio of the ether **13** and the hemiacetal acetate **32** could be increased in the direction of the latter.

The molecular structure of the C-17 benzyl protected ether derivative **13-Bn** by X-ray crystallography confirms the formation of the bridging ether (Fig. 3) and shows a deformation of the chair geometry on the A-ring induced by the bridging ether. In particular, the angles close to the bridging THF ring differ by up to 10% from the usual angle of 111.4° with values of 106.72(14)° (C5–C10–C1) or 100.00(14)° (C10–C1–C2).

The final step for the synthesis focused on the nucleophilic substitution in order to open the previously constructed bridging THF ring which leads concurrently to an integration of the desired oxygen functionality at C-19. Since the ring-opening requires an activation for instance by a Lewis acid, we selected boron trifluoride diethyl etherate being widely used for the opening of cyclic ethers^{75,76} or the transformation of methyl ethers into acetyl ester.^{77–80} If applied to the C-17 acetyl protected ether **13-Ac** or the C-17 benzyl protected ether **13-Bn**, the same ring-opened product was obtained, both in good yields of greater than 70% (see ESI†). Unfortunately, the selective cleavage of just one ether moiety of the C-17 benzyl protected ether derivative **13-Bn** did not succeed. However, same reaction conditions applied to ether **13** (Table 2, entry 1) did not cleave the cyclic ether. Even if the reaction temperature and/or the amount of BF₃·OEt₂ were increased, mainly starting material was recovered accompanied by small amounts of an unknown byproduct. Hernandez *et al.*⁸¹ reported that a combination of ZnI₂ and Ac₂O can initiate the opening of cyclic ethers. At elevated reaction temperatures of 40 °C, we did observe the formation of the desired product for all three cyclic ethers (compare entries 2 and 3), along with the iodide **34** – in most cases as a byproduct, but occasionally even as the main product. To overcome this drawback further zinc(II) salts were tested (entries 4–7). When Zn(OAc)₂ was added (entry 4), no conversion to the desired product **29** was determined. In case of ZnBr₂, a similar reaction outcome was observed as with ZnI₂ as Lewis acid (entry 5) yielding the undesired bromide **33** as byproduct. Luckily, a Zn(OTf)₂ induced ring-opening reaction gave the desired product **29** in 68% yield (entry 6). Since small amounts of starting material remained, more reagent was applied, but instead of improving the yield, the latter decreased even though full conversion was achieved (entry 7). Noteworthy, the hemiacetal acetate **32** forms under the same reaction conditions the corresponding aldehyde **43** (not shown).

Experimental section

General procedure for the dihydroxylation (GP-1)

The androst-2-ene derivative (1.0 equiv.) was dissolved in *tert*-BuOH and the required commercially AD-mix, dissolved in the same amount of H₂O, was added. The mixture was stirred for 10 min at r.t. before MeSO₂NH₂ (0.40 equiv.) was added. The yellow suspension was stirred vigorously for 7 d at r.t. and was



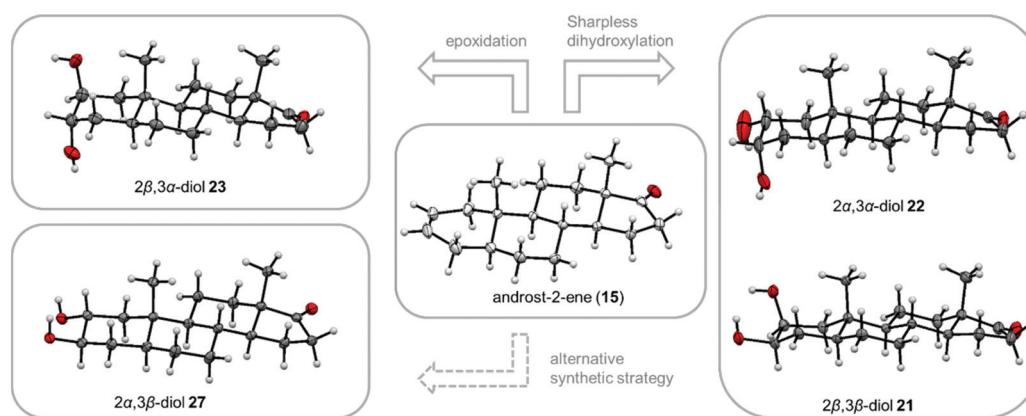


Fig. 5 Overview of the synthetic strategies for the four possible 2,3-diol diastereomers starting from androst-2-ene **15** (middle) and their molecular structures (displacement parameters are drawn at 50% probability level). The *cis*-2,3-diol alcohols **22** and **21** (right) were obtained by a Sharpless dihydroxylation, whereas the *trans*-diol **23** (left) was accessible via an epoxidation followed by ring-opening. **27** (left) was synthesized from 3-epian-drosterone (**12**) via a 5-step procedure.

Table 2 Lewis acid-mediated THF opening to form the 2 α ,3 β ,19-*trans*-triacetate **29**

Entry	Lewis acid	Reaction conditions	Isolated yield [%]
1	20 equiv. $\text{BF}_3 \cdot \text{OEt}_2$	Ac_2O , $-30^\circ\text{C} \rightarrow 0^\circ\text{C}$, 4 h	0 ^a
2	1.4 equiv. ZnI_2	Ac_2O , r.t., 16 h	0 ^a
3	1.5 equiv. ZnI_2	Ac_2O , 40°C , 16 h	20 ^b
4	1.5 equiv. $\text{Zn}(\text{OAc})_2$	Ac_2O , 40°C , 16 h $\rightarrow 60^\circ\text{C}$, 2 h	0 ^a
5	1.5 equiv. ZnBr_2	Ac_2O , 40°C , 16 h $\rightarrow 60^\circ\text{C}$, 2 h	0 ^c
6	1.5 equiv. $\text{Zn}(\text{OTf})_2$	Ac_2O , 45°C , 16 h $\rightarrow 60^\circ\text{C}$, 2 h	68
7	2.0 equiv. $\text{Zn}(\text{OTf})_2$	Ac_2O , 45°C , 16 h $\rightarrow 60^\circ\text{C}$, 2 h	56

^a Mainly starting material was detected accompanied by an unidentified byproduct. ^b The formation of **34** was observed. ^c The formation of **33** was observed.

then quenched with saturated aqueous $\text{Na}_2\text{S}_2\text{O}_3$ solution. After extraction with EtOAc (3 \times) the combined organic phases were washed consecutively with 2 M aqueous solution of KOH , H_2O and brine. The organic phases were dried over Na_2SO_4 , filtered and the solvent was removed. The residue was purified by flash column chromatography on silica gel (*c*Hex/*EtOAc*) to obtain the corresponding *cis*-diol as a colorless powder.

Please note that the reaction times can be reduced when 2.5 equiv. of “self-made” AD-mix were used (please see ESI†).

General procedure for the C–H oxidation via a hypoiodite reaction (GP-2)

A suspension of DIB (1.5 equiv.) and I_2 (1.3 equiv.) in a mixture of cyclohexane and benzene (10:1, 25–30 mm) was degassed by bubbling with argon (15 min) which was followed by the addition of the 2 β -acetoxy-3 β -hydroxyandrostane derivative (1.0 equiv.). The reaction was sonicated at the given temperature and for the given time before it was quenched with

saturated aqueous $\text{Na}_2\text{S}_2\text{O}_3$ solution. After phase separation the aqueous phase was extracted with CH_2Cl_2 (3 \times) and the combined organic extracts were washed with brine and dried over Na_2SO_4 . After filtration and evaporation the obtained crude product was purified by flash column chromatography on silica gel (*c*Hex/*EtOAc*) to afford the 2 β ,19-epoxy-5 α -androstane derivative as colorless solid.

General procedure for the Lewis acid-mediated opening of the THF ring (GP-3)

A. The 2 β ,19-epoxy-5 α -androstane derivative, the $\text{Zn}(\text{II})$ salt (1.5 equiv.–3.5 equiv.) and Ac_2O were heated at 40°C for 16 h. For completion, the mixture was stirred at 60°C for further 2 h and then cooled to r.t.

B. The 2 β ,19-epoxy-5 α -androstane derivative was dissolved in Ac_2O and cooled to -30°C . Then $\text{BF}_3 \cdot \text{OEt}_2$ (3.8 equiv.–10.0 equiv.) was added dropwise and the resulting mixture was stirred for a few hours and warmed to -10°C . Afterwards the



mixture was allowed to warm to r.t. and stirred for another 30 min.

For the work up, the reaction was added to a solution of saturated aqueous NaHCO_3 and stirred until the gas formation stopped. After phase separation, the aqueous phase was extracted with EtOAc (3 \times). The combined organic phases were washed with H_2O and brine, then dried over Na_2SO_4 and filtered. Solvent removal gave the crude product which was purified by flash column chromatography on silica gel ($c\text{Hex}/\text{EtOAc}$).

Crystal structure determinations

The single-crystal X-ray diffraction study was carried out on a Bruker D8 Venture diffractometer with Photon 100 detector PhotonII CPAD (for **27**) at 123(2) K using $\text{Cu-K}\alpha$ radiation ($\lambda = 1.54178$ Å). Direct Methods (SHELXS-97)⁸² or dual space methods (SHELXT for **13-Bn** and **27**)⁸³ were used for structure solution and refinement was carried out using SHELXL-2014 (full-matrix least-squares on F^2).⁸³ Hydrogen atoms were localized by difference electron density determination and refined using a riding model (H(O) free, except **21**). Semi-empirical absorption corrections were applied. For **15**, **14-Ac**, **27** and **20-Ac** an extinction correction were applied. The absolute configuration was determined by refinement of Parsons' x -parameter⁸⁴ or the enantiomer has been assigned by reference to an unchanging chiral center in the synthetic procedure (see cif-files for details). In **21** there seems to be a possible disorder of the OH-groups at C3 and C4 (see cif-files for details).

15. Colorless crystals, $\text{C}_{19}\text{H}_{28}\text{O}$, $M_r = 272.41$, crystal size $0.14 \times 0.20 \times 0.08$ mm, orthorhombic, space group $P2_12_12_1$ (No. 19), $a = 6.4188(2)$ Å, $b = 8.9046(3)$ Å, $c = 26.9419(9)$ Å, $V = 1538.91(9)$ Å³, $Z = 4$, $\rho = 1.175$ Mg m⁻³, $\mu(\text{Cu-K}\alpha) = 0.527$ mm⁻¹, $F(000) = 600$, $2\theta_{\text{max}} = 144.2^\circ$, 13 656 reflections, of which 3011 were independent ($R_{\text{int}} = 0.021$), 182 parameters, $R_1 = 0.031$ (for 2981 $I > 2\sigma(I)$), $wR_2 = 0.079$ (all data), $S = 1.08$, largest diff. peak/hole = $0.287/-0.172$ e Å⁻³. $x = -0.05(5)$.

21. Colorless crystals, $\text{C}_{19}\text{H}_{30}\text{O}_3$, $M_r = 306.43$, crystal size $0.08 \times 0.06 \times 0.02$ mm, monoclinic, space group $P2_1$ (No. 4), $a = 6.0976(2)$ Å, $b = 22.7525(7)$ Å, $c = 6.2997(2)$ Å, $\beta = 108.857(3)^\circ$, $V = 827.08(5)$ Å³, $Z = 2$, $\rho = 1.230$ Mg m⁻³, $\mu(\text{Cu-K}\alpha) = 0.637$ mm⁻¹, $F(000) = 314$, $2\theta_{\text{max}} = 143.8^\circ$, 8766 reflections, of which 3222 were independent ($R_{\text{int}} = 0.042$), 199 parameters, 1 restraint, $R_1 = 0.063$ (for 2741 $I > 2\sigma(I)$), $wR_2 = 0.169$ (all data), $S = 1.04$, largest diff. peak/hole = $1.003/-0.282$ e Å⁻³. $x = -0.2(2)$.

22. Colorless crystals, $\text{C}_{19}\text{H}_{30}\text{O}_3$, $M_r = 306.43$, crystal size $0.16 \times 0.10 \times 0.02$ mm, triclinic, space group $P1$ (No. 1), $a = 7.0823(2)$ Å, $b = 10.2458(3)$ Å, $c = 12.0686(3)$ Å, $\alpha = 73.204(2)^\circ$, $\beta = 89-279(2)^\circ$, $\gamma = 84.178(2)^\circ$, $V = 833.93$ Å³, $Z = 2$, $\rho = 1.220$ Mg m⁻³, $\mu(\text{Cu-K}\alpha) = 0.633$ mm⁻¹, $F(000) = 314$, $2\theta_{\text{max}} = 144.6^\circ$, 15 480 reflections, of which 6149 were independent ($R_{\text{int}} = 0.032$), 399 parameters, 7 restraints, $R_1 = 0.042$ (for 5585 $I > 2\sigma(I)$), $wR_2 = 0.104$ (all data), $S = 1.03$, largest diff. peak/hole = $0.239/-0.179$ e Å⁻³. $x = 0.08(17)$.

23. Colorless crystals, $\text{C}_{19}\text{H}_{30}\text{O}_3$, $M_r = 306.43$, crystal size $0.26 \times 0.24 \times 0.02$ mm, monoclinic, space group $P2_1$ (No. 4), $a = 9.5164(4)$ Å, $b = 7.7586(3)$ Å, $c = 11.9999(5)$ Å, $\beta = 111.938(3)^\circ$,

$V = 822.11(6)$ Å³, $Z = 2$, $\rho = 1.237$ Mg m⁻³, $\mu(\text{Cu-K}\alpha) = 0.642$ mm⁻¹, $F(000) = 314$, $2\theta_{\text{max}} = 144.4^\circ$, 9462 reflections, of which 3203 were independent ($R_{\text{int}} = 0.044$), 205 parameters, 3 restraints, $R_1 = 0.052$ (for 2917 $I > 2\sigma(I)$), $wR_2 = 0.114$ (all data), $S = 1.05$, largest diff. peak/hole = $0.270/-0.290$ e Å⁻³. $x = 0.0(2)$.

33. Colorless crystals, $\text{C}_{21}\text{H}_{34}\text{O}_4$, $M_r = 350.48$, crystal size $0.22 \times 0.16 \times 0.04$ mm, monoclinic, space group $P2_1$ (No. 4), $a = 11.0162(3)$ Å, $b = 6.7430(2)$ Å, $c = 12.6811(3)$ Å, $\beta = 103.877(2)^\circ$, $V = 914.49(4)$ Å³, $Z = 2$, $\rho = 1.273$ Mg m⁻³, $\mu(\text{Cu-K}\alpha) = 0.684$ mm⁻¹, $F(000) = 374$, $2\theta_{\text{max}} = 143.6^\circ$, 14 546 reflections, of which 3541 were independent ($R_{\text{int}} = 0.044$), 232 parameters, 3 restraints, $R_1 = 0.045$ (for 3243 $I > 2\sigma(I)$), $wR_2 = 0.109$ (all data), $S = 1.04$, largest diff. peak/hole = $0.317/-0.191$ e Å⁻³. $x = 0.14(18)$.

27 colourless crystals, $\text{C}_{19}\text{H}_{30}\text{O}_3 \cdot \text{H}_2\text{O}$, $M_r = 324.44$, crystal size $0.28 \times 0.14 \times 0.06$ mm, orthorhombic, space group $P212121$ (No. 19), $a = 6.3125(4)$ Å, $b = 13.3434(8)$ Å, $c = 10.8575(12)$ Å, $V = 1756.83(18)$ Å³, $Z = 4$, $\rho = 1.227$ Mg m⁻³, $\mu(\text{Cu-K}\alpha) = 0.671$ mm⁻¹, $F(000) = 712$, $2\theta_{\text{max}} = 144.4^\circ$, 17 174 reflections, of which 3469 were independent ($R_{\text{int}} = 0.025$), 220 parameters, 5 restraints, $R_1 = 0.032$ (for 3439 $I > 2\sigma(I)$), $wR_2 = 0.087$ (all data), $S = 1.05$, largest diff. peak/hole = $0.302/-0.267$ e Å⁻³. $x = -0.09(4)$.

14. Colorless crystals, $\text{C}_{21}\text{H}_{32}\text{O}_4$, $M_r = 348.46$, crystal size $0.22 \times 0.20 \times 0.06$ mm, monoclinic, space group $P2_1$ (No. 4), $a = 9.8746(3)$ Å, $b = 7.6958(2)$ Å, $c = 12.2229(4)$ Å, $\beta = 92.870(1)^\circ$, $V = 928.14(5)$ Å³, $Z = 2$, $\rho = 1.247$ Mg m⁻³, $\mu(\text{Cu-K}\alpha) = 0.673$ mm⁻¹, $F(000) = 370$, $2\theta_{\text{max}} = 144.6^\circ$, 12 308 reflections, of which 1455 were independent ($R_{\text{int}} = 0.030$), 230 parameters, 2 restraints, $R_1 = 0.029$ (for 3588 $I > 2\sigma(I)$), $wR_2 = 0.076$ (all data), $S = 1.04$, largest diff. peak/hole = $0.187/-0.160$ e Å⁻³. $x = 0.01(6)$.

13-Bn. Colorless crystals, $\text{C}_{28}\text{H}_{37}\text{O}_4$, $M_r = 437.58$, crystal size $0.20 \times 0.16 \times 0.08$ mm, monoclinic, space group $P2_1$ (No. 4), $a = 9.6663(3)$ Å, $b = 7.5343(2)$ Å, $c = 16.3096(5)$ Å, $\beta = 96.857(2)^\circ$, $V = 1179.31(6)$ Å³, $Z = 2$, $\rho = 1.235$ Mg m⁻³, $\mu(\text{Cu-K}\alpha) = 0.636$ mm⁻¹, $F(000) = 476$, $2\theta_{\text{max}} = 144.4^\circ$, 17 473 reflections, of which 4591 were independent ($R_{\text{int}} = 0.024$), 290 parameters, 1 restraint, $R_1 = 0.031$ (for 4482 $I > 2\sigma(I)$), $wR_2 = 0.086$ (all data), $S = 1.05$, largest diff. peak/hole = $0.260/-0.161$ e Å⁻³. $x = 0.13(6)$.

14-Ac. Colorless crystals, $\text{C}_{23}\text{H}_{34}\text{O}_5$, $M_r = 382.52$, crystal size $0.50 \times 0.35 \times 0.25$ mm, orthorhombic, space group $P2_12_12_1$ (No. 19), $a = 7.7219(3)$ Å, $b = 13.4724(4)$ Å, $c = 20.1539(7)$ Å, $V = 2096.67(13)$ Å³, $Z = 4$, $\rho = 1.243$ Mg m⁻³, $\mu(\text{Cu-K}\alpha) = 0.689$ mm⁻¹, $F(000) = 856$, $2\theta_{\text{max}} = 144.2^\circ$, 20 103 reflections, of which 4139 were independent ($R_{\text{int}} = 0.025$), 260 parameters, $R_1 = 0.028$ (for 4106 $I > 2\sigma(I)$), $wR_2 = 0.075$ (all data), $S = 1.07$, largest diff. peak/hole = $0.214/-0.167$ e Å⁻³. $x = -0.02(4)$.

20-Ac. Colorless crystals, $\text{C}_{23}\text{H}_{34}\text{O}_5$, $M_r = 380.50$, crystal size $0.15 \times 0.03 \times 0.01$ mm, monoclinic, space group $P2_1$ (No. 4), $a = 7.1015(3)$ Å, $b = 25.1141(8)$ Å, $c = 11.8745(4)$ Å, $\beta = 92.269(2)^\circ$, $V = 2117.98(13)$ Å³, $Z = 4$, $\rho = 1.225$ Mg m⁻³, $\mu(\text{Cu-K}\alpha) = 0.682$ mm⁻¹, $F(000) = 848$, $2\theta_{\text{max}} = 114.4^\circ$, 12 893 reflections, of which 7180 were independent ($R_{\text{int}} = 0.047$), 40 parameters,



1 restraint, $R_1 = 0.064$ (for 5010 $I > 2\sigma(I)$), $wR_2 = 0.147$ (all data), $S = 1.02$, largest diff. peak/hole = $0.178/-0.173 \text{ e } \text{\AA}^{-3}$. $x = 0.4(3)$.

CCDC 1998175 (27), CCDC 1822521 (15), CCDC 1822522 (21), CCDC 1822523 (22), CCDC 1822524 (23), CCDC 1822525 (33), CCDC 1822526 (14), CCDC 1822527 (13-Bn), CCDC 1822528 (14-Ac), and 1822529 (20-Ac) contain the supplementary crystallographic data for this paper.†

Conclusions

Due to the interesting biological properties of calotropin (4) and related cardenolides, we herein presented a modular synthetic route for these cardenolide aglycones. The presented work enables the regioselective C–H oxidation of the methyl group at C-19 followed by the subsequent introduction of the $2\alpha,3\beta$ -trans-diol moiety in order to enable the connection to the sugar moiety. We faced two synthetic routes for the preparation of the precursor 14 for the remote C–H oxidation starting from commercially available 3-epiandrosterone (12). The first generation route based on traditional steroid chemistry could be replaced by a more convenient and efficient sequence of an asymmetric Sharpless dihydroxylation and a regioselective acetylation. With androst-2-ene as intermediate not only the synthesis of the required $2\beta,3\beta$ -cis-diol was accomplished, but also two more diastereomeric 2,3-diols became accessible. The corresponding $2\alpha,3\beta$ -trans-diol is not accessible from androst-2-ene, but can be accessed from 3-epiandrosterone via a Rubottom oxidation and a consecutive reduction of the 3-keto group. Due to the close proximity of the axial C-2 hydroxyl and C-19 methyl group in the steroidal framework, a remote intramolecular radical C–H oxidation of C-19 with DIB and I_2 (hypoiodite reaction) led to the formation of the A-ring bridging ether which was then opened by a Lewis acid-mediated ether cleavage to give the 2,3,19-triacetoxy derivative 29. The overoxidation product 32, resulting from a double hypoiodite reaction under harsher reaction conditions, can be directly converted to the desired aldehyde functionality at C-19 after acidic treatment.

In summary, we have devised a novel strategy towards all 2,3-diol diastereomers and the introduction of an oxygen functionality at C-19 via a remote C–H oxidation without using any light source or transition metal. The herein presented method should not only allow the synthesis of calotropin (4) and related cardenolides, but should also inspire upcoming synthesis of steroids and cyclic terpenes.

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