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 2α,3β-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 3β-OH group to the sugar moiety, the cardenolides calotropin (4) and their related cardenolides (5–10) are connected by the 2α- and 3β-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 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.
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.\(^7\)\(^\text{21}\) 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 acetylation\(^22\) or epimerization\(^9,10,15,23\) of the hydroxyl group, but also its oxidation.\(^10,11,14,15\) Moreover, an attachment of a thiazolidine\(^11,14,15\) 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.\(^25\)\(^\text{26}\) In lower doses, extracts of these plants exhibit a wide range of biological activities including anti-inflammatory,\(^27\)\(^\text{30}\) analgesic,\(^31\)\(^\text{32}\) anti-microbial\(^27,33\) or wound-healing properties.\(^34,35\) In the last years, cytotoxic and antitumor effects of cardenolides glycosides have been reported more frequently.\(^36\)\(^\text{42}\) 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 century\(^8\) and are reported to have similar cardiac properties to well-established cardenolides, 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 moiety at C-19 allowing later on the generation of the aldehyde as well as the installation of the 2α,3β-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-
Fieser via enolisation followed by an acyl group migration presumably from diastereoselectivities. An X-ray crystal structure was obtained of the cyclic intermediate I that has been reported first by the second generation route. (a) Ac₂O, pyridine, r.t., 16 h, 98%; (b) NaBH₄, MeOH, −20 °C → 0 °C, 3 h, 98%; (c) TBSCI, imidazole, DMF, r.t., 16 h, 80% or BuNO—NHCCl₂, TIOH, 1,4-dioxane, r.t., 16 h, 82%; (d) KOH, MeOH, Δ, 5 h; (e) NMO, cat. TPAP, CH₂Cl₂, 0 °C → r.t., 16 h or DMP, CH₂Cl₂, 0 °C → r.t., 16 h; 90% (17-TBS)/79% (17-Bn) over two steps; (f) HClO₄, Ac₂O, EtOAc, r.t. 3 h, 69% (16-Ac)/77% (16-Bn); (g) m-CPBA, CH₂Cl₂, 0 °C → 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) PrOH, K₂CO₃, H₂O, r.t., 16 h, 58% (20-Ac)/48% (20-Bn); (j) NaBH₄, MeOH, −20 °C → 0 °C, 3 h, 76% (14-Ac)/82% (14-Bn); TBS = tert-butyldimethylsilyl; Bn = benzyl.

Scheme 3 Synthesis of the precursor 14-Ac and 14-Bn according to the first generation route. (a) Ac₂O, pyridine, r.t., 16 h, 98%; (b) NaBH₄, MeOH, −20 °C → 0 °C, 3 h, 98%; (c) TBSCI, imidazole, DMF, r.t., 16 h, 80% or BuNO—NHCCl₂, TIOH, 1,4-dioxane, r.t., 16 h, 82%; (d) KOH, MeOH, Δ, 5 h; (e) NMO, cat. TPAP, CH₂Cl₂, 0 °C → r.t., 16 h or DMP, CH₂Cl₂, 0 °C → r.t., 16 h; 90% (17-TBS)/79% (17-Bn) over two steps; (f) HClO₄, Ac₂O, EtOAc, r.t. 3 h, 69% (16-Ac)/77% (16-Bn); (g) m-CPBA, CH₂Cl₂, 0 °C → 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) PrOH, K₂CO₃, H₂O, r.t., 16 h, 58% (20-Ac)/48% (20-Bn); (j) NaBH₄, MeOH, −20 °C → 0 °C, 3 h, 76% (14-Ac)/82% (14-Bn); TBS = tert-butyldimethylsilyl; Bn = benzyl.

Scheme 4 Synthesis of the 2(3)-cis-diol 21 according to the second generation route. (a) p-TsCl, pyridine, r.t., 16 h, 92%; (b) LiBr, Li₂CO₃, DMF, 175 °C, 7 h, 95%; (c) α-AD-mix, MeSO₂NH₂, BuOH/H₂O, r.t., 12 h, 61% (23); (d) β-AD-mix, MeSO₂NH₂, BuOH/H₂O, r.t., 7 d, 41%; (e) m-CPBA, CH₂Cl₂, r.t., 14 h, 75%; (f) 2 m H₂SO₄, THF, r.t., 7 d, 54%; (h) HOCH₂CH₂OH, p-TsOH, HCl(OMe)₂, CH₂Cl₂, r.t., 16 h, 88%; DOS = diversity orientated synthesis.

As previously reported for comparable examples, the treatment with catalytic amounts of OsO₄ and NMO as stoichiometric oxidant favors in our case the formation of the unde-
sired 2α,3α-cis-diol 22, whereas the corresponding 2β,3β-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 immediately formed ester using AgOAc \(^{52,53}\), Tl(OAc)\(_4\) \(^{54}\) 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. \(^{36,57}\) While the AD-mix \(\beta\) introduces the hydroxyl groups at the sterically more accessible \(\alpha\)-side to build the 2α,3α-cis-diol 22, the use of AD-mix \(\alpha\) mainly forms the desired, but sterically more demanding 2β,3β-cis-diol 21. Despite the reported accelerated effect of methanesulfonamide, \(^{58}\) long reaction times of 7 days were required with commercially available AD-mix \(\alpha\). While the extra addition of OsO\(_4\) or K\(_2\)[OsO\(_2\)(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 2β,3β-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 2β,3α-trans-diol was obtained. Once more, the reagents attack occurred from the sterically less hindered \(\alpha\)-side releasing the 2α,3α-epoxide 24 with 75% yield. The epoxide was opened by simple acidic treatment to afford with its diaxial 2β,3α-substituents the least stable 2,3-diol.

Even though 2α,3β-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).

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 \(\alpha\)-hydroxyl group at C-2 by a Rubottom oxidation. This was accomplished by the conversion of 25 to the corresponding TMS-enoI 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 androst-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 2α,3β-trans-diol 27 which shows the same configuration at C-2 and C-3 as calotropigenin (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 2α,3β-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 2α,3α-cis-diol 22 over the 2β,3β-cis-diol 21 since the latter owns an axial hydroxyl group at C-2 which conflicts with the 19-methyl group while in case of the other diastereomer the hydroxyl group at C-2 is equatorially orientated reducing the overall transannular strain.

With 2β,3β-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 2β,3β-cis-diol 21 to the later developed reaction conditions of the C-H oxidation reaction. Although full conversion of the 2β,3β-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 pankrease 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 2β,3β-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 2β,3β-diol 14 in yields higher than 77%. For comparison, we
conducted a kinetically controlled acetylation of 2β,3β-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 2β,3β-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 hypoiodite reaction (Fig. 3). By using lead tetraacetate and iodine in overstoichiometric amounts, androstane67–69 and cholestane70–72 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 hypoiodite reaction using 3-acetylated 2β,3β-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 31 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 diastereotropic 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 hypoiodite 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 hypoiodite 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
Evaluation of the optimal reaction conditions for the radical (sp^3)C–H oxidation of C-19

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

\textsuperscript{a}AgOAc, acetone, r.t., overnight; cy = cyclohexane. \textsuperscript{b}c = 10 mm. \textsuperscript{c}c = 30 mm.

**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 H\textsubscript{2}O\textsubscript{2} was added. The mixture was stirred for 10 min at r.t. before MeSO\textsubscript{2}NH\textsubscript{2} (0.40 equiv.) was added. The yellow suspension was stirred vigorously for 7 d at r.t. and was...
then quenched with saturated aqueous Na₂S₂O₃ solution. After extraction with EtOAc (3×) the combined organic phases were washed consecutively with 2 m aqueous solution of KOH, H₂O and brine. The organic phases were dried over Na₂SO₄, filtered and the solvent was removed. The residue was purified by flash column chromatography on silica gel (cHex/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 I₂ (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 2β-acetoxy-3β-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 Na₂S₂O₃ solution. After phase separation the aqueous phase was extracted with CH₂Cl₂ (3×) and the combined organic extracts were washed with brine and dried over Na₂SO₄. After filtration and evaporation the obtained crude product was purified by flash column chromatography on silica gel (cHex/EtOAc) to afford the 2β,19-epoxy-5α-androstane derivative as colorless solid.

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

A. The 2β,19-epoxy-5α-androstane derivative, the Zn(II) salt (1.5 equiv.–3.5 equiv.) and Ac₂O 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 2β,19-epoxy-5α-androstane derivative was dissolved in Ac₂O and cooled to –30 °C. Then BF₃·OEt₂ (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 NaHCO₃ and stirred until the gas formation stopped. After phase separation, the aqueous phase was extracted with EtOAc (3×). The combined organic phases were washed with H₂O and brine, then dried over Na₂SO₄ and filtered. Solvent removal gave the crude product which was purified by flash column chromatography on silica gel (CHX/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 absorption corrections were applied. For 0.16 × 0.10 × 0.02 mm, triclinic, space group P2₁ (No. 4), a = 11.0162(3), b = 6.7430(2), c = 12.6811(3), β = 103.877(2), V = 914.49(4) Å³, Z = 2, μ = 1.273 Mg m⁻³, μ(Cu-Kα) = 0.684 mm⁻¹, F(000) = 374, 2θmax = 144.6°, 14 546 reflections, of which 3541 were independent (Rint = 0.044), 232 parameters, 3 restraints, R1 = 0.052 (for 2917 I > 2σ(I)), wR2 = 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, C₁₉H₂₅O₅, Mₚ = 350.48, crystal size 0.22 × 0.16 × 0.04 mm, monoclinic, space group P2₁ (No. 4), a = 11.0162(3), b = 6.7430(2), c = 12.6811(3), β = 103.877(2), V = 914.49(4) Å³, Z = 2, μ = 1.273 Mg m⁻³, μ(Cu-Kα) = 0.684 mm⁻¹, F(000) = 374, 2θmax = 144.6°, 14 546 reflections, of which 3541 were independent (Rint = 0.044), 232 parameters, 3 restraints, R1 = 0.052 (for 2917 I > 2σ(I)), wR2 = 0.114 (all data), S = 1.05, largest diff. peak/hole = 0.270/−0.290 e Å⁻³, x = 0.0(2).

27 colourless crystals, C₁₉H₃₀O₅H₂O, Mₚ = 324.44, crystal size 0.28 × 0.14 × 0.06 mm, orthorhombic, space group P2₁2₁2₁ (No. 19), a = 6.3125(4), b = 13.3434(8), c = 10.8575(12), V = 1756.83(18) Å³, Z = 4, μ = 1.227 Mg m⁻³, μ(Cu-Kα) = 0.671 mm⁻¹, F(000) = 712, 2θmax = 144.4°, 17 174 reflections, of which 3469 were independent (Rint = 0.025), 320 parameters, 5 restraints, R1 = 0.032 (for 3439 I > 2σ(I)), wR2 = 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, C₁₉H₂₅O₄, Mₚ = 348.46, crystal size 0.22 × 0.20 × 0.06 mm, monoclinic, space group P2₁ (No. 4), a = 9.8746(3), b = 7.6958(2), c = 12.2229(4), V = 928.14(5) Å³, Z = 2, μ = 1.247 Mg m⁻³, μ(Cu-Kα) = 0.673 mm⁻¹, F(000) = 370, 2θmax = 144.6°, 12 308 reflections, of which 1455 were independent (Rint = 0.030), 230 parameters, 2 restraints, R1 = 0.029 (for 3588 I > 2σ(I)), wR2 = 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, C₁₃H₂₋O₅, Mₚ = 437.58, crystal size 0.20 × 0.16 × 0.08 mm, monoclinic, space group P2₁ (No. 4), a = 9.6663(3), b = 7.5343(2), c = 16.3096(5), V = 1179.31(6) Å³, Z = 2, μ = 1.235 Mg m⁻³, μ(Cu-Kα) = 0.636 mm⁻¹, F(000) = 476, 2θmax = 144.4°, 17 473 reflections, of which 4591 were independent (Rint = 0.024), 290 parameters, 1 restraint, R1 = 0.031 (for 4482 I > 2σ(I)), wR2 = 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, C₁₃H₂₄O₅, Mₚ = 382.52, crystal size 0.50 × 0.35 × 0.25 mm, orthorhombic, space group P2₁2₁2₁ (No. 19), a = 7.7219(3), b = 13.4724(4), c = 20.1539(7), V = 2096.67(5) Å³, Z = 4, μ = 1.243 Mg m⁻³, μ(Cu-Kα) = 0.689 mm⁻¹, F(000) = 856, 2θmax = 144.2°, 20 103 reflections, of which 4139 were independent (Rint = 0.025), 260 parameters, 1 restraint, R1 = 0.028 (for 4106 I > 2σ(I)), wR2 = 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, C₁₃H₂₄O₅, Mₚ = 380.50, crystal size 0.15 × 0.03 × 0.01 mm, monoclinic, space group P2₁ (No. 4), a = 7.1015(3), b = 25.1141(8), c = 11.8745(4), V = 2117.98(13) Å³, Z = 4, μ = 1.225 Mg m⁻³, μ(Cu-Kα) = 0.682 mm⁻¹, F(000) = 848, 2θmax = 114.4°, 12 893 reflections, of which 7180 were independent (Rint = 0.047), 40 parameters, 1 restraint, R1 = 0.064 (for 5010 I > 2σ(I)), wR2 = 0.147 (all data), S = 1.02, largest diff. peak/hole = 0.178/−0.173 e Å⁻³, x = 0.4(3).
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 2α,3β-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 2β,3β-cis-diol was accomplished, but also two more diastereomeric 2,3-diols became accessible. The corresponding 2α,3β-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 scaffold, a remote intramolecular radical C-H oxidation of C-19 with DIB and I2 [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-triacetoxyl 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.

Conflicts of interest

In accordance with our policy on Conflicts of interest please ensure that a conflicts of interest statement is included in your manuscript here. Please note that this statement is required for all submitted manuscripts. If no conflicts exist, please state that “There are no conflicts to declare.”

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