Natural Products Synthesis

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Total Synthesis of Candicanoside A, a Potent Antitumor Saponin with a Rearranged Steroid Side Chain**

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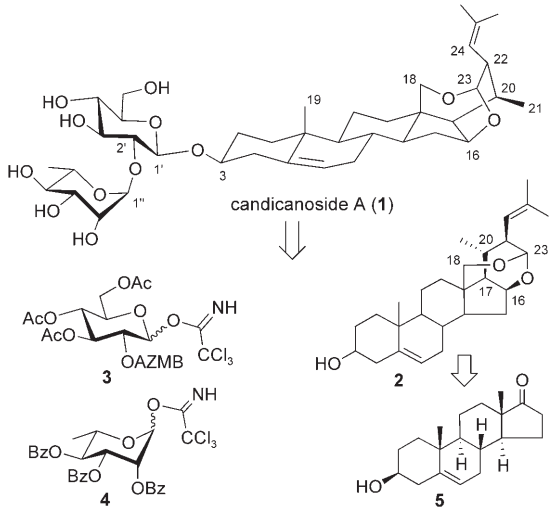
Since the disclosure of the exceptionally potent antitumor activity of OSW saponins,[1] much attention has been devoted to cholestan glycosides of this type. These compounds occur in the bulbs of *Ornithogalum saudersiae* and taxonomically related plants, which are members of a lily family native to southern Africa.[2] Continuous research on these plants led to the discovery of minor cholestan glycosides of a new type with a rearranged steroidal side chain.[3,4] Candicanoside A (**1**), isolated in 2000 from the bulbs of *Galtonia candicans* (0.00076% of the fresh weight), is such a compound.[3] It differs from the other congeners through its unique fused-ring scaffold created by acetal formation between the aldehyde group at C23 and the diol at C16,18. This compound shows potent antitumor activity (e.g., $IC_{50} = 32$ nM against HL-60 human promyelocytic leukemia cells) comparable to that of the clinically applied anticancer agents etoposide and methotrexate, whereas the congener in which C16 and C23 form part of a hemiacetal group was found to be inactive.[4d] More interestingly, candicanoside A displays differential cytotoxicities against various tumor cell lines, and its antitumor profile does not correlate with that of any other antitumor compound.[3] Thus, candicanoside A may have a unique mode of action and the potential to act as a lead for the synthesis of a new type of antitumor agents. Herein we report the first total synthesis of this interesting natural product.

The assembly of the target disaccharide saponin **1** would demand a mild and stereoselective approach to the glycosylation of the cholestan aglycone **2**, which contains two double bonds and an acetal functionality (Scheme 1).[5] Glycosyl imidates with a benzoyl group at the 2-*O*-position have been shown to be ideal donors, as only a catalytic amount of TMSOTf is required to promote their coupling to steroids and triterpenoids. The 2-*O*-benzoyl group controls the construction of the 1,2-*trans* glycosidic bond by neighboring-group participation while minimizing the formation of the corresponding orthoester.[6,7] Therefore, the D-glucosyl and L-rhamnosyl trichloroacetimidates **3** and **4**[8] were selected as precursors to the desired diasaccharide. Selective removal of the 2-*O*-AZMB group in **3** would release a hydroxy group at this position for subsequent coupling with **4**.[7] Finally, the acetyl and benzoyl groups remaining in the resulting disaccharide could be removed under mild basic conditions to provide candicanoside A (**1**).



Scheme 1. Retrosynthetic analysis of candicanoside A. AZMB = 2-(azidomethyl)benzoyl, Bz = benzoyl.

A great synthetic challenge was presented by the novel scaffold of the steroid aglycone **2**. We planned to use the readily available and cheap steroid dehydroisoandrosterone (**5**) as the starting material. The stereoselective introduction of the diol at C16,18, installation of the rearranged side chain at C17, and formation of the acetal at C23 would then be required to provide **2**.

A 20-OH group in the steroids could facilitate the functionalization of the proximal 18-Me group.[9] Therefore, compound **7**, with a hydroxy group at C20(*R*)[10] and 3,5-cyclo-6-methoxy protection of the 3-hydroxy-5,6-ene functionality in the AB ring, was prepared from alkene **6** by hydroboration and oxidation; compound **6** was obtained conveniently from **5** in three steps (62%).[11] Irradiation of **7** in the presence of iodine and DIB in cyclohexane with a 300-W tungsten lamp for approximately 30 min, followed immediately by oxidation with PCC, provided the desired ketone **8** with an iodo substituent at C18 in a satisfactory yield (50%).[9] Hydrolysis of the iodide **8** with silver acetate in aqueous dioxane resulted in the formation of the hemiketal **9** (92%).[12] The reduction of **9** with $NaBH_4$ yielded a pair of C18,20(*R*/*S*) diols, which was subjected directly to selective acetylation to provide the

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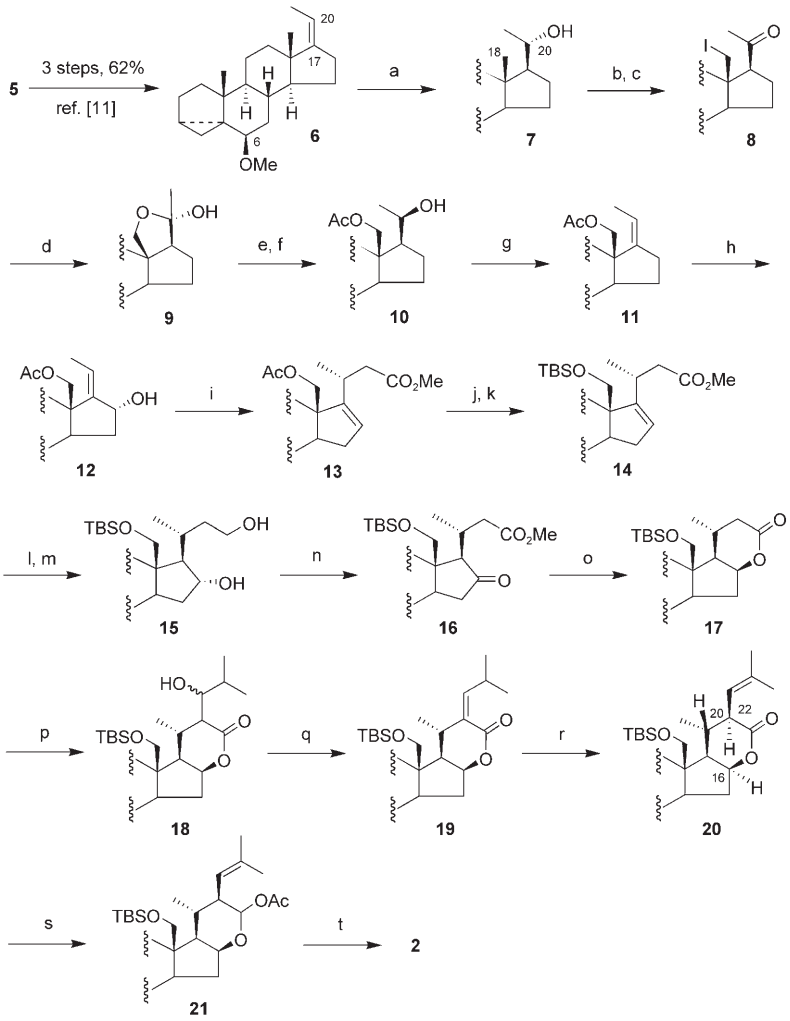
acetoxy alcohol **10** with the *R* configuration at C20 as the readily isolable major product (50% over two steps).[13] Next, the required *Z* alkene at C17–C20 was regenerated by dehydration under mild conditions to give **11** (79%).[14] The stereoselective introduction of the allylic hydroxy group at C16 with selenium dioxide and *tert*-butylhydroperoxide then led to the desired alcohol **12** (82%; Scheme 2).[15]



Scheme 2. Synthesis of the steroid aglycone **2**: a) 9-BBN, THF, room temperature; then H_2O_2, NaOH, room temperature, 96%; b) DIB, I_2, cyclohexane, room temperature, *hν*; c) PCC, NaOAc, CH_2Cl_2, 4-Å MS, room temperature, 50% (two steps); d) AgOAc, 1,4-dioxane/H_2O (9:1), 65°C, 92%; e) $NaBH_4$, CH_2Cl_2/CH_3OH (1:1), room temperature; f) AcCl, pyridine, −20°C, 50% (two steps); g) $POCl_3$, pyridine, −20°C→RT, 79%; h) SeO_2, *t*BuOOH, CH_2Cl_2/H_2O, room temperature, 82%; i) $CH_3C(OCH_3)_3$, propanoic acid (cat.), 140°C, 87%; j) NaOMe, MeOH/THF (1:1), 0°C; k) TBSOTf, 2,6-lutidine, room temperature, 90% (two steps); l) $LiAlH_4$, THF, room temperature; m) BH_3, THF, 0°C; then H_2O_2, NaOH, 0°C, 80% (two steps); n) Swern oxidation; $NaClO_2$, Na_2HPO_4, 2-methyl-2-butene, *t*BuOH, room temperature; then CH_2N_2, ether, room temperature, 87%; o) $LiAlH(OtBu)_3$, THF, room temperature, 81%; p) LDA, THF, −78°C; then isobutyraldehyde, −78°C; q) $POCl_3$, pyridine, −20°C→RT, 50% (two steps); r) *hν* (*λ* = 254 nm), MeOH, room temperature, 90%; s) L-selectride, −78°C; then Ac_2O, DMAP, Et_3N, room temperature, 97%; t) HF (40%), CH_3CN, room temperature, 85%. 9-BBN = 9-borabicyclo[3.3.1]nonane, DIB = (diacetoxyiodo)benzene, DMAP = 4-dimethylaminopyridine, LDA = lithium diisopropylamide, MS = molecular sieves, PCC = pyridinium chlorochromate, TBS = *tert*-butyldimethylsilyl, Tf = trifluoromethanesulfonyl.

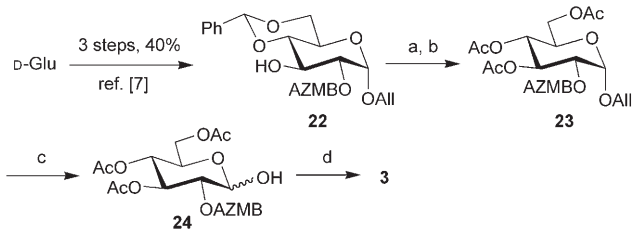
Following the successful installation of the hydroxy groups at C16 and C18, we were only able to introduce the rest of the side chain at C20 through a multistep sequence. Thus, subjection of the allylic alcohol **12** to the standard conditions for Johnson–Claisen rearrangement afforded the ester **13** as a single isomer in 87% yield.[16] The *R* configuration at C20 was confirmed by X-ray diffraction analysis of a later derivative.[17] The acetate group at C18 and ester group at C23 in **13** were found to be detrimental to the hydroboration reaction of the alkene to introduce the hydroxy group at C16. Therefore, the acetyl protecting group was exchanged for a TBS protecting group. The ester group in the resulting compound **14** was reduced to the corresponding alcohol with $LiAlH_4$. Subsequent treatment of the resulting alkene with diborane followed by H_2O_2 afforded the desired 16α,23-dihydroxy compound **15** in a stereoselective manner and 80% yield. Swern oxidation of **15**, followed by further oxidation of the resulting aldehyde to the carboxylic acid with $NaClO_2$[18] and subsequent formation of the corresponding methyl ester with diazomethane provided the keto methyl ester **16** in good yield (87%). $LiAlH(OtBu)_3$ was found to be the best reagent for the selective reduction of the keto group in **16**. The resulting alcohol underwent concomitant cyclization to generate the lactone **17**. The use of other reducing agents, such as $NaBH_4$/$CeCl_3$ and L-selectride, led to considerable amounts of the products of overreduction.

The remaining portion of the side chain was introduced successfully at C22 (in lactone **17**) through an aldol condensation. Thus, the treatment of **17** with LDA in THF, followed by the addition of isobutyraldehyde at −78°C, gave a diastereoisomeric mixture of alcohols **18**, which was subjected directly to dehydration with $POCl_3$ in pyridine to provide the α,β-unsaturated lactone **19** in 50% yield. No improvement in the yield of the aldol reaction was found when KHMDS (potassium hexamethyldisilazide) was used as the base,[19] when HMPA (hexamethyl phosphoramide) was present in the solvent, or with Bu_2BOTf/diisopropylethylamine.[20] Fortunately, the rearrangement of the conjugated double bond in the lactone **19** to give a nonconjugated double bond at C24–25 was promoted by irradiation with UV light[21] to provide **20** as a single isomer at C22 in excellent yield. NOE correlations between 22-H (δ = 2.89 ppm, dd, J = 12.3, 9.8 Hz) and 16-H (δ = 4.75 ppm, m), and between 24-H (δ = 5.06 ppm, d, J = 9.6 Hz) and 20-H (δ = 2.35–2.27 ppm, m) were found, but not between 22-H and 20-H, as evidence for the desired *R* configuration at C22.

The lactone **20** was then reduced with L-selectride; the resulting deprotonated lactol was trapped in situ with acetic anhydride to give the

acetate **21** in 97% yield.[22] Finally, HF (40%) was used to remove the 18-*O*-TBS group in **21**. The desired acetal in the target compound was formed simultaneously, and the hydroxy group at C3 and alkene at C5–C6 were also recovered from the 3,5-cyclo-6-methoxy protection, which had remained intact since the beginning of the synthesis. Thus, the aglycone **2** was elaborated successfully in 23 steps and in 1.5% overall yield from the commercially available steroid dehydroisoandrosterone (**5**).

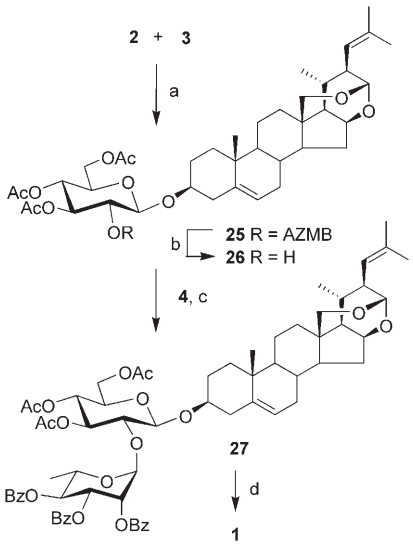
The allyl glucopyranoside **22** with its 2-OH group protected with AZMB had already been prepared in our laboratory.[7] The preparation of the imidate **3** took four more steps: removal of the benzylidene protecting group, acetylation of the hydroxy groups at C3,C4, and C6, cleavage of the allyl group at the anomeric position, and subsequent formation of the trichloroacetimidate (Scheme 3). Thus, the desired glucosyl donor **3** was prepared from glucose in seven steps and 26% overall yield. Another three steps were required for the preparation of the rhamnosyl donor **4** (in 76% yield).[8]



Scheme 3. Synthesis of the glucose donor **3**: a) AcOH (70%), 70°C; b) Ac_2O, Et_3N, DMAP, CH_2Cl_2, room temperature, 86% (two steps); c) $PdCl_2$, CH_2Cl_2/MeOH (1:1), room temperature, 85%; d) $CNCCl_3$, DBU, CH_2Cl_2, room temperature, 90%. DBU = 1,8-diazabicyclo-[5.4.0]undec-7-ene.

As expected, glycosylation of the steroid **2** with the glucosyl imidate **3** in the presence of TfOH (0.2 equiv) afforded the β glycoside **25** in excellent yield (96%; Scheme 4). The AZMB group was then removed selectively in the presence of acetyl groups with PBu_3 to provide **26**. The unmasked hydroxy group in **26** is sterically hindered; nonetheless, coupling with the benzoyl-protected rhamnosyl imidate **4** in the presence of TfOH (0.2 equiv) produced the desired disaccharide **27** in 81% yield (over two steps). Finally, the acetyl and benzoyl groups on the sugar residue in **27** were removed with NaOMe in MeOH/THF at room temperature to furnish the target candicanoside A (**1**) in 90% yield. The analytical data of **1** are in good agreement with those reported in the literature.[3,17]

In summary, candicanoside A (**1**), a steroid disaccharide with a novel fused-ring steroid scaffold, has been synthesized for the first time. The synthesis requires a total of 37 steps from cheap starting materials (dehydroisoandrosterone, D-glucose, and L-rhamnose), with a longest linear sequence of 27 steps and a 1.0% overall yield of **1**. Improvement of the synthesis and, more importantly, studies on the structure–activity relationships and mechanism of action of this novel natural lead compound, which shows potent antitumor activity with a unique differential pattern, become projects for further research.



Scheme 4. Completion of the synthesis of candicanoside A (**1**): a) TfOH (0.2 equiv), CH_2Cl_2, 4-Å MS, room temperature, 96%; b) PBu_3, THF/H_2O, room temperature; c) TfOH (0.2 equiv), toluene, 4-Å MS, room temperature, 81% (two steps); d) NaOMe, THF/MeOH (1:1), room temperature, 90%.

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