Steroid Synthesis

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Total Synthesis of Ouabagenin and Ouabain**

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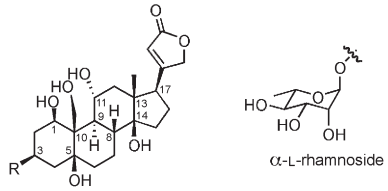
Dedicated to Professor Zdenek Valenta on the occasion of his 80th birthday

Ouabain (**1a**), a cardioactive glycoside isolated from the bark of the African ouabio tree (*Acokanthera ouabio*) by Arnaud in 1888),[1] has received considerable attention since it was discovered that an ouabain-like compound occurs naturally in mammals and acts as an endogenous digitalis as proposed by Szent–Gyorgyi.[2] After some debate, it was established that the endogenous and plant derived ouabain are in fact identical.[3] Ouabagenin (**1b**), the aglycone of ouabain, was isolated for the first time in 1942 by Mannich and Siewert,[4]

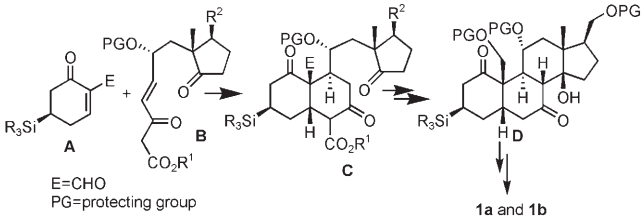


1a: ouabain, R=α-L-rhamnoside
1b: ouabagenin, R=OH

who proposed a structure for the aglycone, and the structure for ouabain, which was later proven to be correct.[5]

Progress towards the construction of these highly oxygenated steroids has been made recently,[6–8] but to date no total synthesis has been reported. Our foray into this field was based on a hypothesis that the polyanionic cyclization (double-Michael addition followed by aldol condensation) methodology developed by our research group[9] would allow facile access to an appropriately functionalized tetracyclic intermediate with the desired A/B *cis*, B/C *trans*, and C/D *cis* ring junctions. Our initial studies[9,10] suggested a promising synthetic route towards 14-β-OH steroidal intermediates, and herein we report the completion of the first total synthesis of ouabagenin (**1b**) and in turn ouabain (**1a**).

Our strategy was based on the initial rapid construction of densely functionalized tetracycle **D**, (Scheme 1), which contains, in principle, the functionalities required for ouabagenin



Scheme 1. Synthetic plan for ouabagenin and ouabain.

(**1b**) and in turn ouabain (**1a**). Tetracycle **D** would be readily available from tricycle **C**, which in turn could be produced from the condensation of chiral building blocks **A** and **B**.

The initial steps of the synthesis, drawn from our previous studies on steroid skeleton synthesis,[9–11] were successful (Scheme 2). Thus, the union of Nazarov substrate **3**[10b] with freshly prepared cyclohexenone **2**[11,12] in the presence of Cs_2CO_3 at 0 °C followed by decarboxylation afforded tricycle **4** with an overall yield of 78 %. Reduction of the resulting aldehyde with $Li(Et_3CO)_3AlH$[13] and then protection of the alcohol as its PMB ether[14] afforded aldol precursor **5** in 74 % yield over two steps The aldol reaction to form the desired tetracycle **6** in 83% yield occurred in the presence of KHMDS. In order to reduce the ketone at C1 to give the desired β stereochemistry, it was necessary to first deprotect the alcohol at C11, by saponification of the acetate, which assisted the subsequent surprisingly facile reduction of the C1 ketone with $NaBH_4$ in EtOH at −78 °C to produce **7** with an overall yield of 95 %.

Oxidation of PMB ether **7** was carried out in a CH_2Cl_2 solution, which was rigorously dried with molecular sieves prior to addition of DDQ, to afford orthoester **8** in 84 % yield.[15,16] From orthoester **8**, formation of a silyl enol ether using excess TBSOTf and oxidation to the enone with DDQ[17] proceeded smoothly to afford the desired enone **9** with an overall yield of 90 %. Reduction of the enone by using sodium borohydride afforded the β-allylic alcohol, which upon exposure to *m*CPBA underwent selective β-face epoxidation to afford epoxide **10** with an overall yield of 81 %. The structure of **10** was ultimately confirmed by X-ray crystallography.[18]

Mesylation of the epoxy alcohol was followed by treatment with excess $LiBH_4$ in DME and subsequent hydrolysis on silica gel, to afford **11**. Thus, hydrogenolysis of the mesylate took place as well as the desired reductive opening of the epoxide. The resulting orthoester intermediate was found to be unstable and was therefore hydrolyzed under mild acidic conditions with silica gel producing mainly the *p*-methoxybenzoate at the C1 position.[19] The overall yield for these operations was 58 %. Attempts to protect the C19 and

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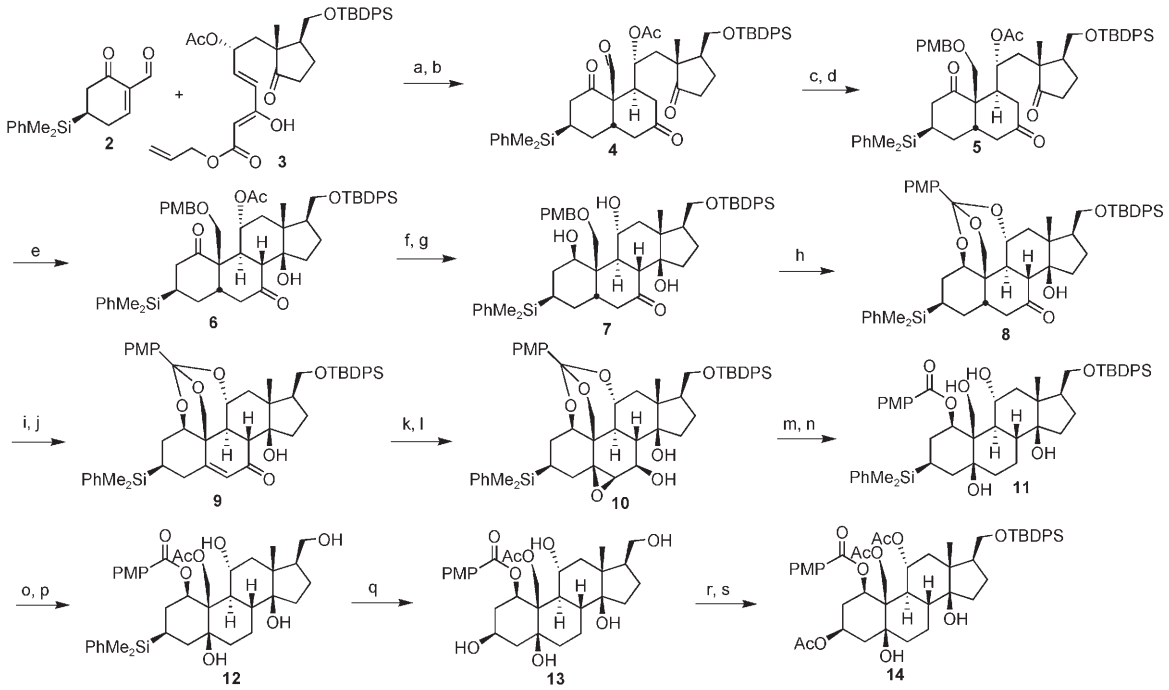


Scheme 2. Synthesis of key fragment **14**. Reagents and conditions: a) Cs_2CO_3, CH_2Cl_2, 0°C, 85%; b) $[Pd(PPh_3)_4]$, morpholine, THF, 92%; c) $Li(Et_3CO)_3AlH$, THF, −78°C, 89%; d) $PMBOC(NH)CCl_3$, $La(OTf)_3$ (0.1 equiv), Et_2O, 0°C, 83%; e) KHMDS, THF, 63°C, 83%; f) K_2CO_3, THF, MeOH, 98%; g) $NaBH_4$, EtOH, −78°C, 97%; h) DDQ, 4 Å M.S., CH_2Cl_2, 84%; i) TBSOTf, Et_3N, CH_2Cl_2, 90%; j) DDQ, 2,6-di-*tert*-butyl-4-methylpyridine, MeCN, 100%; k) $NaBH_4$, EtOH, THF, −30°C, 96%; l) *m*CPBA, $NaHCO_3$, CH_2Cl_2, 85%; m) MsCl, py, CH_2Cl_2, 98%; n) $LiBH_4$, DME, RT, 24 h then reflux 8 h, then silica, 58%; o) Ac_2O, py, DMAP, RT, 24 h, 90%; p) TBAF, THF, 0°C→RT, 6 h, 88%; q) $Hg(OAc)_2$, AcOH/AcOOH (1:1), RT, 4 h, 93%; r) TBDPSCl, imidazole, CH_2Cl_2, 0°C, 4 h, 75%; s) Ac_2O, py, DMAP, CH_2Cl_2, 40°C, 24 h, 65%. DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, DMAP = 4-dimethylamino pyridine, DME = 1,2-dimethoxyethane, KHMDS = potassium bis(trimethylsilyl)amide, *m*CPBA = *m*-chloroperoxybenzoic acid, M.S. = molecular sieves, Ms = methanesulfonyl, PMB = *p*-methoxybenzyl, PMP = *p*-methoxyphenyl, py = pyridine, TBAF = tetra-*n*-butylammonium fluoride, TBDPS = *tert*-butyldiphenylsilyl, TBS = *tert*-butyldimethylsilyl, Tf = trifluoromethanesulfonyl, THF = tetrahydrofuran.

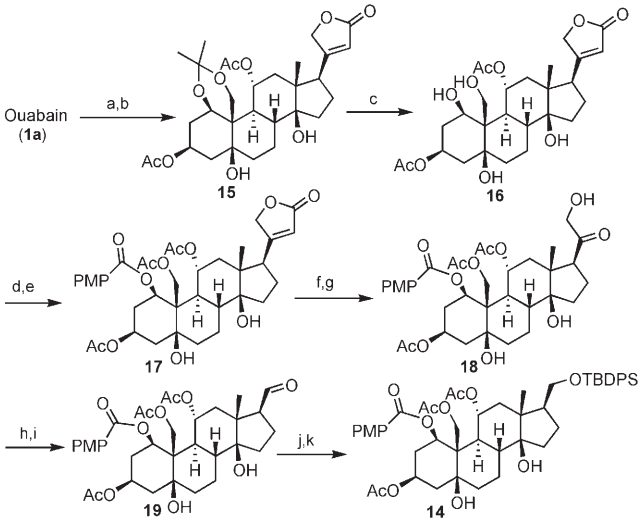
C11 hydroxy groups of **11** as acetates afforded only the C19 acetate. We considered this to be a suitable stage for unmasking the C3 hydroxy group. Removal of the TBDPS group followed by Tamao oxidation[20] of **12** neatly furnished **13** in 93% yield. The primary hydroxy group of **13** was reprotected as TBDPS ether and the secondary hydroxy groups as acetates to give **14**.

At this stage, with all the required stereochemistry installed, we wanted to secure completely unambiguous structure confirmation. For that, we opted for the degradation of natural ouabain (**1a**)[21] to obtain the intermediate **14** and compare it with the synthetic intermediate. Accordingly, the acidic hydrolysis of natural ouabain[4,6e] and selective acetylation[22] of secondary alcohols gave **15** which upon hydrolysis of the acetonide group with HCl in MeOH produced tetraol **16** in 52% yield over three steps (Scheme 3).

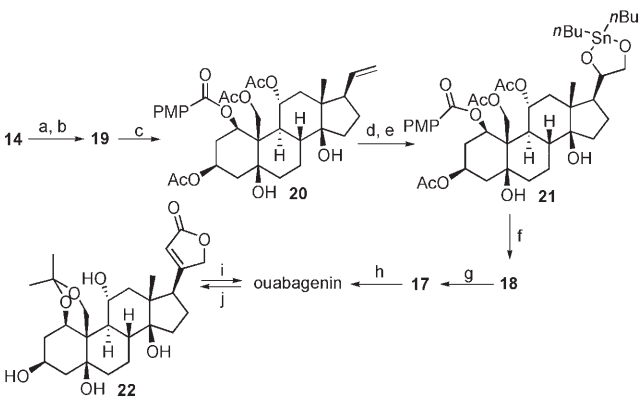
Transformation of the primary hydroxy group of **16** into an acetate and the secondary hydroxy group into *p*-methoxybenzoate gave **17** with an overall yield of 67%. Ozonolysis of **17** followed by mild hydrolysis ($KHCO_3$ solution) of the resulting glyoxalate resulted in the somewhat less stable hydroxy ketone **18**. Reduction of **18** using $NaBH_4$ in MeOH followed by $NaIO_4$-mediated oxidative cleavage of the resulting 1,2-diol produced aldehyde **19**. Again reduction of **17** and protection of the resulting primary hydroxy group as the TBDPS ether cleanly furnished **14** in 37% overall yield. This degradation product was identical to the synthetic material and was used as a relay to complete the total synthesis of ouabagenin (**1b**) and in turn ouabain (**1a**).

Thus, silyl ether cleavage of **14** with TBAF in THF and then oxidation of the resulting primary hydroxy group again gave aldehyde **19**, which was transformed into **20** by rhodium-catalyzed methylenation (Scheme 4).[23] After dihydroxylation of the olefin moiety in **20** with OsO_4 and NMO, the selective oxidation of the secondary hydroxy group to afford **18** was achieved with NBS through the cyclic tin ether **21**.[24] Construction of the butenolide ring to obtain **17** was achieved by exposing hydroxyketone **18** to triphenyl phosphoranylidene ketene[25] and subsequent hydrolysis of the ester groups gave ouabagenin (**1b**). Since literature data needed to confirm the structure is limited,[4,5,26] we decided to obtain a pure sample of the natural product for an unambiguous comparison. Ouabagenin acetonide **22**,[6e,22] which was obtained by degradation of ouabain (**1a**) was hydrolyzed by using conc. HCl in MeOH to provide an authentic sample of ouabagenin (**1b**). For further confirmation of the structure it was then reprotected to give ouabagenin acetonide **22** by using conc. HCl in acetone. The synthetic ouabagenin was identical with the one obtained from degradation.[27]

Completion of the total synthesis of ouabain (**1a**) is described in Scheme 5. Accordingly, we required suitably functionalized coupling partners **25** and **26**. Thus, perbenzo-

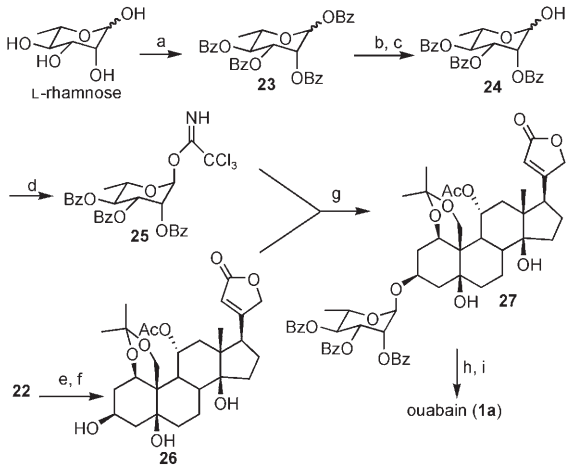


Scheme 3. Degradation of natural ouabain. Reagents and conditions: a) Conc. HCl, acetone, 0 °C, 10 days, 70%; b) Ac_2O, py, DMF, 50 °C, 48 h, 83%; c) 1 N HCl/MeOH (1:4), 36 h, 90%; d) Ac_2O, py, CH_2Cl_2, 0 °C→RT, 6 h, 89%; e) *p*-anisoyl chloride, py, DMAP, CH_2Cl_2, 40 °C, 18 h, 76%; f) O_3, CH_2Cl_2, −78 °C, 2 h, then Ph_3P, RT, 15 h; g) $KHCO_3$, MeOH:H_2O (1:1), RT, 3 h; h) $NaBH_4$, MeOH, 0 °C, 30 min, 51% over 3 steps; i) $NaIO_4$, EtOH/H_2O (95:5), RT, 1 h, 82%; j) $NaBH_4$, MeOH, 0 °C, 30 min, 88%; k) TBDPSCl, imidazole, CH_2Cl_2, 0 °C→RT, 4 h, 90%. DMF = *N*,*N*-dimethylformamide.



Scheme 4. Completion of the synthesis of ouabagenin (**1b**). Reagents and conditions: a) TBAF, THF, 0 °C→RT, 2 h, 90%; b) Dess–Martin periodinane, CH_2Cl_2, 0 °C, 40 min, 75%; c) $[(PPh_3)_3RhCl]$, PPh_3, *i*PrOH, $TMSCHN_2$, THF, 16 h, 67%; d) OsO_4, NMO, acetone/H_2O (95:5), 6 h, 82%; e) nBu_2SnO, benzene, reflux, 12 h; f) NBS, $CHCl_3$, 10 min, 73% over 2 steps; g) Ph_3PCCO, TEA, benzene, RT, 12 h, 68%; h) 0.5 N Na_2CO_3, MeOH, 2 h, RT, 85%; i) Conc. HCl, MeOH, 50 °C, 4 h, 88%; j) Conc. HCl, acetone, RT, 4 h, 80%. NBS = *N*-bromosuccinimide, NMO = 4-methylmorpholine *N*-oxide, TEA = triethylamine, TMS = trimethylsilyl.

ylation of L-rhamnose gave **23**, which upon anomeric bromination using acetyl bromide and MeOH, followed by hydrolysis with Ag_2CO_3 in aqueous acetone, produced lactol **24**. Treatment of **24** with K_2CO_3 and Cl_3CCN exclusively afforded **25**.[28] The aglycone **26** was easily obtained from **22** by diacetylation[22] followed by selective hydrolysis of the C3 acetate group. It was necessary to block the C11-OH group



Scheme 5. Preparation of glycoside partner **25** and completion of the total synthesis of ouabain (**1a**). Reagents and conditions: a) BzCl, py, DMAP, CH_2Cl_2, RT, 82%; b) AcBr, MeOH, CH_2Cl_2, RT; c) Ag_2CO_3, acetone, H_2O, RT, 58% over 2 steps; d) K_2CO_3, Cl_3CCN, CH_2Cl_2, RT, 68%; e) Ac_2O, py, DMF, DMAP, 50 °C, 78%; f) 0.5 N Na_2CO_3, MeOH, 1 h, RT, 70%; g) TMSOTf, 4 Å M.S., CH_2Cl_2, RT, 90%; h) 2 N HCl, MeOH, RT, 2 h, 92%; i) 0.5 N Na_2CO_3, MeOH, 2 h, RT, 88%. Ac = acetyl, Bz = benzoyl.

because our initial glycosylation trials of **22** were not regioselective.

With both building blocks **25** and **26** in hand, our next task was to tether the two parts of the molecule through an acetal bridge. Thus coupling of **25** and **26** was carried out using TMSOTf in CH_2Cl_2 at 0 °C to obtain **27** as the exclusive isomer in 90% yield. Not unprecedented, the pleasing outcome with glycosylation was a result of anchimeric assistance.[28] For the global deprotection, we first treated **27** with mild acidic and then with mild basic conditions (the reverse treatment did not work out to our satisfaction) to furnish ouabain (**1a**) in 80% yield over the last two steps. The synthetic compound was identical with an authentic sample of the natural material.[21,27]

In conclusion, we have successfully completed the long-awaited first total synthesis of ouabagenin (**1b**) and in turn ouabain (**1a**) through a polyanionic cyclization strategy via a key tetracyclic intermediate **14** in 19 steps. This in turn led to the preparation of ouabagenin (**1b**) in eight steps. Finally, ouabagenin (**1b**) was converted into ouabain (**1a**) in six steps.

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