Steroid Synthesis

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]



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 \mathbf{D} , (Scheme 1), which contains, in principle, the functionalities required for ouabagenin

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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 $\mathbf{2}^{[11,12]}$ in the presence of Cs₂CO₃ at 0°C followed by decarboxylation afforded tricycle 4 with an overall yield of 78%. Reduction of the resulting aldehyde with Li(Et₃CO)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 ß 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 NaBH₄ 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 crystallog-raphy.^[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(Et₃CO)₃AlH, THF, -78°C, 89%; d) PMBOC(NH)CCl₃, La(OTf)₃ (0.1 equiv), Et₂O, 0°C, 83%; e) KHMDS, THF, 63°C, 83%; f) K₂CO₃, THF, MeOH, 98%; g) NaBH₄, EtOH, -78°C, 97%; h) DDQ, 4 Å M.S., CH_2Cl_2 , 84%; j) TBSOTf, Et₃N, CH_2Cl_2 , 90%; j) DDQ, 2,6-di-*tert*-butyl-4-methylpyridine, MeCN, 100%; k) NaBH₄, EtOH, THF, -30°C, 96%; l) *m*CPBA, NaHCO₃, CH_2Cl_2 , 85%; m) MsCl, py, CH_2Cl_2 , 98%; n) LiBH₄, DME, RT, 24 h then reflux 8 h, then silica, 58%; o) Ac₂O, py, DMAP, RT, 24 h, 90%; p) TBAF, THF, 0°C \rightarrow 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) Ac₂O, 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 (KHCO₃ solution) of the resulting glyoxalate resulted in the somewhat less stable hydroxy ketone **18**. Reduction of **18** using NaBH₄ in MeOH followed by NaIO₄-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, silvl 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 rhodiumcatalyzed methylenation (Scheme 4).^[23] After dihydroxylation of the olefin moiety in 20 with OsO4 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-

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Scheme 3. Degradation of natural ouabain. Reagents and conditions: a) Conc. HCl, acetone, 0°C, 10 days, 70%; b) Ac₂O, py, DMF, 50°C, 48 h, 83%; c) 1 N HCl/MeOH (1:4), 36 h, 90%; d) Ac₂O, py, CH₂Cl₂, 0°C \rightarrow RT, 6 h, 89%; e) *p*-anisoyl chloride, py, DMAP, CH₂Cl₂, 40°C, 18 h, 76%; f) O₃, CH₂Cl₂, -78°C, 2 h, then Ph₃P, RT, 15 h; g) KHCO₃, MeOH:H₂O (1:1), RT, 3 h; h) NaBH₄, MeOH, 0°C, 30 min, 51% over 3 steps; i) NalO₄, EtOH/H₂O (95:5), RT, 1 h, 82%; j) NaBH₄, MeOH, 0°C, 30 min, 88%; k) TBDPSCl, imidazole, CH₂Cl₂, 0°C \rightarrow RT, 4 h, 90%. DMF = N,N-dimethylformamide.



Scheme 4. Completion of the synthesis of ouabagenin (1b). Reagents and conditions: a) TBAF, THF, $0^{\circ}C \rightarrow RT$, 2 h, 90%; b) Dess–Martin periodinane, CH₂Cl₂, 0°C, 40 min, 75%; c) [(PPh₃)₃RhCl], PPh₃, *i*PrOH, TMSCHN₂, THF, 16 h, 67%; d) OsO₄, NMO, acetone/H₂O (95:5), 6 h, 82%; e) *n*Bu₂SnO, benzene, reflux, 12 h; f) NBS, CHCl₃, 10 min, 73% over 2 steps; g) Ph₃PCCO, TEA, benzene, RT, 12 h, 68%; h) 0.5 N Na₂CO₃, 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 Na₂CO₃, 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 Na₂CO₃, MeOH, 2 h, RT, 88%. Ac = ace-tyl, 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 CH₂Cl₂ 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 longawaited 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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