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A Short Synthesis of Clionamine D

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Herein we describe a scalable four-step preparation of α-methylene-γ-lactone **3** from steroidal sapogenin. This method allowed a facile synthesis of clionamine D, a natural aminosteroid with potent autophagy bioactivity and unprecedented chemical structure.

Keywords clionamine D, α-methylene-γ-lactone, singlet oxygen, natural product, synthesis

Introduction

Clionamines A—D are a family of natural steroidal alkaloids with potent autophagy bioactivity and unprecedented chemical structures (Figure 1).[1] Synthesis of clionamine B has been achieved by the Andersen group[2] and clionamine D by our group.[3] Both groups took steroidal α-methylene-γ-lactones as key intermediates and developed their synthetic methods. During the course of our synthesis, we learned that there is still no effective method for preparing α-methylene-γ-lactone. Traditional methods, such as the one we developed during our synthesis, prepared γ-lactone (such as **4**) from steroidal sapogenin, and then introduced the methylene unit via dehydration or other procedures. We therefore decided to develop a more concise method that requires fewer chemical operations. Herein, we report a short synthesis of this intermediate, which enabled a synthesis of clionamine D.

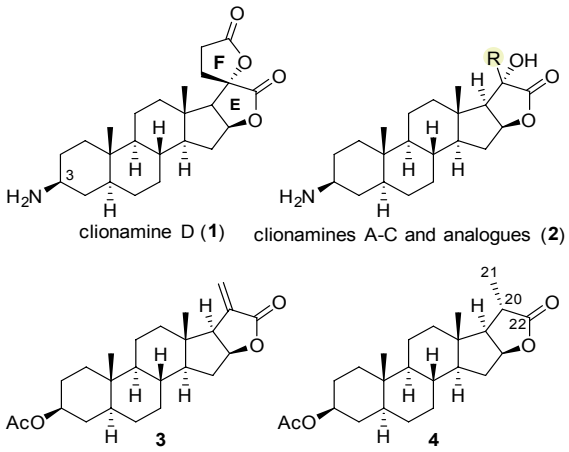

Figure 1 Chemical structures of clinamines **1**/**2** and key intermediates **3**/**4**.

Experimental

Compounds 5 and 8 To a suspension of oxone (19 g, 30.9 mmol) and $NaHCO_3$ (5.5 g) in acetone/water (150 mL/150 mL) at 0 ℃ was added a solution of *pseudo*-tigogenin (15.0 g, 30 mmol) in acetone (100 mL) slowly over 30 min. The mixture was stirred at 0 ℃ for 8 h and quenched with an aqueous solution of Na_2SO_3. Acetone was removed and the residue was extracted with ethyl acetate three times. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and purified via flash column chromatography on silica gel to give **5** (10.1 g, 64%) and **8** (3.68 g, 23%), both as white solid. Compound **5**: m.p. 105—107 ℃; $[\alpha]_D^{27}$ +5.6 (*c* 0.76, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.88 (td, J=7.3, 3.8 Hz, 1H), 4.70—4.63 (m, 1H), 4.27 (t, J=7.4 Hz, 1H), 3.90 (d, J=6.2 Hz, 2H), 2.40 (brs, 1H), 2.17—2.10 (m, 1H), 2.04 (s, 3H), 2.01 (s, 3H), 1.96 (d, J=6.3 Hz, 1H), 1.49 (s, 3H), 0.92 (d, J=6.7 Hz, 3H), 0.82 (s, 3H), 0.77 (s, 3H), 0.69—0.60 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.1, 170.5, 162.6, 91.1, 83.9, 73.5, 68.9, 66.4, 56.5, 53.9, 44.5, 40.3, 39.0, 36.6, 35.4, 34.4, 33.9, 33.2, 32.9, 31.8, 28.5, 28.3, 27.3, 21.3, 21.0, 20.9, 20.3, 16.9, 13.3, 12.1; IR ν: 3458, 2937, 1736, 1365, 1245, 1033, 736 cm^{-1}; ESI-MS *m/z*: 539.3 $[M+Na]^+$; HRMS(ESI) *m/z*: $[M+H]^+$ calcd for $C_{31}H_{48}O_6$ 517.3529, found 517.3519. Compound **8**: m.p. 122—123 ℃; $[\alpha]_D^{27}$ –3.7 (*c* 0.44, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.71—4.61 (m, 2H), 4.41 (s, 1H), 3.93 (d, J=6.26 Hz, 2H), 3.76 (br s, 1H), 2.79—2.59 (m, 2H), 2.29—2.19 (m, 1H), 2.06 (s, 3H), 2.00 (s, 3H), 1.52 (s, 3H), 0.98 (s, 3H), 0.95 (d, J=6.6 Hz, 3H), 0.80

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(s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 214.3, 171.3, 170.8, 81.7, 73.7, 73.6, 69.0, 59.7, 54.4, 54.3, 44.7, 42.5, 39.1, 37.1, 36.8, 35.6, 34.7, 34.5, 34.0, 32.2, 31.8, 28.5, 27.5, 27.0, 21.5, 21.0, 20.8, 16.9, 15.2, 12.3; IR ν: 3503, 2975, 1732, 1388, 1262, 1228, 1028 cm^{-1}; ESI-MS *m/z*: 557.1 $[M+Na]^+$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{31}H_{50}O_7$ 535.3629, found 535.3624.

Converting **8** to **5**: To a solution of **8** (1.06 g, 2 mmol) in dry dioxane (20 mL) was added pyridinium *p*-toluenesulfonate (PPTS, 348 mg, 2.2 mmol) at ambient temperature. The mixture was stirred for 6 h, concentrated under reduced pressure, and purified via flash column chromatography on silica gel to give **5** (798 mg, 78%) as a white solid.

Compound 9 A solution of enol ether **5** (517 mg, 1.0 mmol) and *meso*-tetraphenylporphine (TPP, 4 mg) in dichloromethane (20 mL) at 0 ℃ was bubbled with O_2 under filament lamp's illumination for 10 h. The solvent was removed and the residue was purified via flash column chromatography on silica gel to give lactone **9** (307 mg, 76%) as a white solid. The reaction on a 10 g scale required 24 h and gave **9** in 75% yield. m.p. 239—240 ℃; $[\alpha]_D^{27}$ –44.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.07 (td, *J*=7.8, 4.1 Hz, 1H), 4.73—4.63 (m, 1H), 2.23 (dt, *J*=14.2, 7.3 Hz, 1H), 2.02 (s, 3H), 1.57 (s, 3H), 0.83 (s, 3H), 0.79 (s, 3H), 0.68 (td, *J*=11.1, 3.7 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 178.2, 170.9, 82.9, 77.5, 77.2, 76.8, 75.0, 73.7, 62.9, 55.8, 53.9, 44.6, 40.6, 38.7, 36.8, 35.6, 34.6, 34.0, 31.9, 31.8, 28.4, 27.5, 21.6, 20.3, 19.7, 13.9, 12.3; IR ν: 3450, 2941, 2853, 1754, 1450, 1247, 1022, 1980 cm^{-1}; ESI-MS: 405.0 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{24}H_{36}O_5$ 405.2636, found 405.2631.

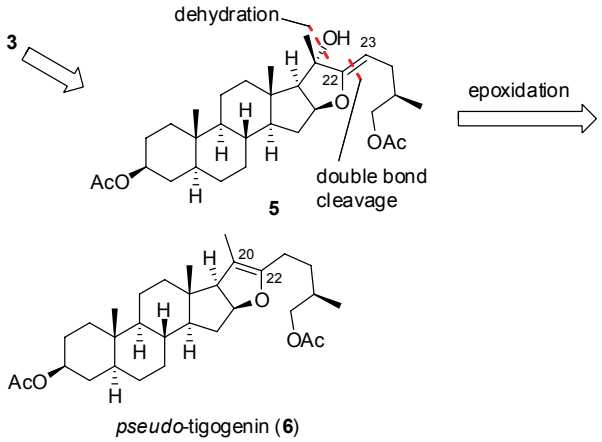
Compound 10 To a solution of compound **3** (950 mg, 2.5 mmol) in HOAc (100 mL) were added $Mn(OAc)_3 \cdot 2H_2O$ (2.34 g, 8.7 mmol) and KOAc (6.8 g, 70 mmol). The resultant solution was stirred and refluxed for 1.5 h and TLC analysis showed complete disappearance of **3**. The solvent was evaporated under reduced pressure and the residue was dissolved with ethyl acetate (200 mL) and water (100 mL). The resultant solution was added saturated aqueous $NaHCO_3$ to adjust pH value to 7 and extracted with ethyl acetate (100 mL) twice. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, PE/EA: 8∶1—3/1) to furnish **10** (1.02 g, 92%) as a white solid. m.p. 243—244 ℃; $[\alpha]_D^{27}$ –73.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.04—5.12 (m, 1H), 4.62—4.74 (m, 1H), 2.93—3.04 (m, 1H), 2.53—2.74 (m, 2H), 2.38—2.49 (m, 1H), 2.25—2.34 (m, 2H), 2.01 (s, 3H), 0.82 (s, 3H), 0.80 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.6, 174.2, 170.8, 86.3, 83.7, 77.5, 77.2, 76.8, 73.5, 60.3, 55.3, 53.9, 44.6, 41.3, 39.1, 36.7, 35.6, 34.6, 34.0, 32.1, 31.9, 28.3, 28.2, 27.5, 24.8, 21.6, 20.4, 14.3, 12.3; IR ν: 2934, 2854, 1798, 1773, 1726, 1246 cm^{-1}; ESI-MS *m/z*: 467.0 $[M+Na]^+$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{26}H_{36}O_6$ 445.2585, found 445.2579.

Clionamine D (1) as CF_3CO_2H salt To a solution of ketone (80 mg, 0.20 mmol) in methanol (10 mL) was added 4 Å MS (90 mg), $NaBH_3CN$ (10 mg, 0.16 mmol) and NH_4OAc (162 mg, 2.1 mmol). The resultant solution was stirred for 6 h at 30 ℃. Concentrated HCl (2 mL) was added and the resultant solution was stirred for 30 min, filtered through a pad of celite, and evaporated under reduced pressure. The residue was purified by reverse phase column chromatography (C_{18}-reverse phase silica gel, CH_3CN/H_2O 9/1 to 3/1) to give clionamine D as HCl salt (72 mg, 0.16 mmol, 82%). The HCl salt was dissolved in MeOH (3 mL), neutralized with saturated $NaHCO_3$ solution, and extracted with ethyl acetate. The combined organic layers were concentrated under reduced pressure, and the residue was dissolved in MeOH (5 mL) and treated with CF_3CO_2H (13 μL, 19 mg, 0.16 mmol). The solvent was removed and the residue was purified by reverse phase column chromatography (C_{18}-reverse phase silica gel, CH_3CN/H_2O 9/1 to 3/1) to give clionamine D as CF_3CO_2H salt for analysis. Clionamine D (**1**) as CF_3CO_2H salt: $[\alpha]_D^{20}$ –41 (*c* 1.00, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD) δ: 5.08 (td, *J*=7.4, 4.1 Hz, 1H), 3.10 (tt, *J*=11.9, 4.4 Hz, 1H), 2.86 (dt, *J*=18.3, 9.3 Hz, 1H), 2.71—2.50 (m, 3H), 2.47 (d, *J*=6.4 Hz, 1H), 2.33 (dt, *J*=14.2, 7.4 Hz, 1H), 2.01—1.99 (m, 1H), 1.75—1.71 (m, 1H), 0.88 (s, 3H), 0.82 (s, 3H).

Results and Discussion

pseudo-Sapogenins are important intermediates and their preparations and transformations have been extensively studied.[4] It has been reported that oxidation of *pseudo*-sapogenins could move the C(20)-C(22) double bond of enol ether to C(22)-C(23) position and introduce a C20α-OH (Scheme 1).[5] We reasoned **5** enol ether could be our starting point to prepare α-methylene-γ-lactone **3**. Therefore, initial study was the synthesis of enol **5**.

Scheme 1 A simple synthetic analysis



As depicted in Scheme 2, *pseudo*-tigogenin (**6**) was prepared by heating tigogenin (**7**) in refluxing acetic

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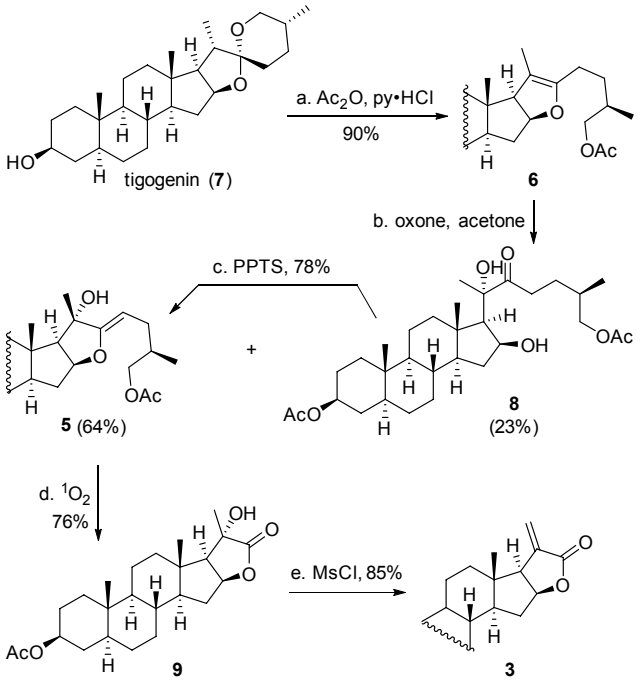
anhydrous in the presence of pyridinium chloride. Oxidation of **6** with dimethyldioxirane (DMDO) generated *in situ* afforded the desired exocyclic enol ether **5** in 64% yield, along with the ring-opening product **8** in 23%. Upon being treated with PPTS in dioxane, ketone **8** was effortlessly transformed to **5** in good yield, thus improving the overall yield of **5**. The reaction was performed on a 15 g scale.

Scheme 2 Preparation of α-methylene-γ-lactone **3**



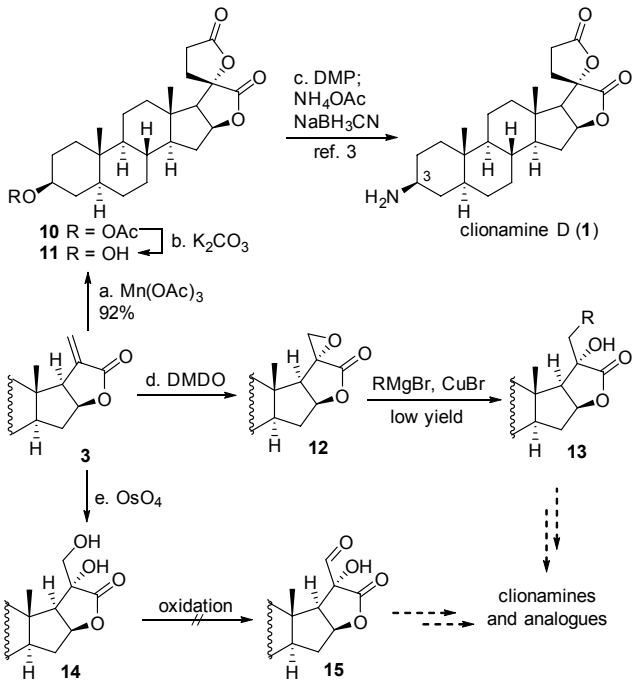
Coditions and reagents: (a) Ac_2O, PPTS, reflux, 6 h, 90%; (b) oxone, $NaHCO_3$, acetone, water, 0 ℃, 8 h, **5**, 64%, **8**, 23%; (c) PPTS, dioxane, r.t., 6 h, 78%; (d) O_2, TPP, sunlamp, CH_2Cl_2, 0 ℃, 10 h, 76%; (e) MsCl, Et_3N, PhMe, reflux, 2 h, 85%.

We then tried to cleave the exocyclic double bond of **5** to establish the required lactone. We considered the [2+2]/retro-[2+2] process with singlet oxygen an attractive solution.[6] The reaction of **5** with singlet oxygen (O_2, 0.6 mol% *meso*-tetraphenylporphine, sunlamp, CH_2Cl_2, 0 ℃) smoothly delivered the desired lactone **9** in 74%—78% yield on a 10 g scale. The C(20)-OH on **9** was eliminated upon treating with MsCl in refluxing toluene, providing **3** in 82% yield. The whole process was easily carried out on a multigram scale.

With **3** in hand, we then employed a $Mn(OAc)_3$-mediated radical [3+2]-cycloaddition to build the spirobislactone unit,[3] obtaining **10** in high yield (Scheme 3). Methanolysis of **10**, Dess–Martin oxidation of **11**, and reductive amination of the resulting C3-ketone gave clionamine D in good yield.

Efficient synthesis of the natural clionamines A—C and their analogues from **3** demanded convenient strategy for introducing the requisite side chains and C20α-OHs. The 1,4-addition/oxidation process explored by the Andersen group succeeded but only gave the products in moderate yields (50% for 1,4-addition, 60% for oxidation).[2] Epoxidation and dihydroxylation of the double bond on **3** introduced the C20α-OHs and provided suitable functional groups at C21 for carbon-chain elongation, therefore, were investigated preliminarily in our group. Oxidations of **3** with DMDO[7] and OsO_4 provided **12** and **14**, respectively, in high yields. Disappointedly, we found that treating **12** with various alkylmetallic reagents was inefficient (less than 30% yield, presumably due to the hindrance of the epoxide) and that oxidation of **14** failed to give aldehyde **15** but cleaved the diol unit.

Scheme 3 Synthesis of clionamine D



Coditions and reagents: (a) $Mn(OAc)_3$•$2H_2O$, KOAc, HOAc, reflux, 1.5 h, 92%; (b) K_2CO_3, MeOH, r.t., 5 h, 97%; (c) Dess-Martin periodinane, CH_2Cl_2, r.t., 5 h, 91%; NH_4OAc, $NaBH_3CN$, 4 Å MS, MeOH, 30 ℃, 6 h, 82%; (d) DMDO, CH_2Cl_2, acetone, 0 ℃, 10 h, 97%; (e) OsO_4, NMO, *t*-BuOH, THF, water, r.t., 2 h, 99%.

Conclusions

We have developed a new and efficient method for preparing α-methylene-γ-lactone **3** from tigogenin and realized an eight-step synthesis of clionamine D. The use of singlet oxygen to break the double bond is the key to the success of this method. Currently we are working on expanding the substrate scope of this method and exploring more possibilities of using **12** and **14** in the syntheses of the natural clionamines and their analogues.

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