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Synthesis of Clionamine B, an Autophagy Stimulating Aminosteroid Isolated from the Sponge *Cliona celata*

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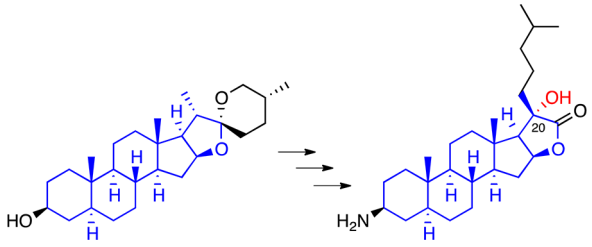
ABSTRACT



Clionamine B (2), an aminosteroid isolated from the marine sponge *Cliona celata*, has been synthesized starting from the plant sapogenin tigogenin (5). A key step in the synsthesis is the stereoselective introduction of the C-20 α-hydroxyl substituent via oxidation of a γ-lactone enolate with molecular oxygen. Synthetic clionamine B (2) strongly stimulated autophagy in human breast cancer MCF-7 cells.

Autophagy is a cellular process that captures organelles and proteins in a double membrane vesicle called an autophagosome and delivers them to the lysosome for degradation and recyling of the amino acids, monosaccharides, and lipids.[1] Formation of autophagosomes from nascent double membrane fragments called phagophores, fusion of autophagosomes with the lysosome, and the subsequent degradation and recycling of their organelle and protein cargo is a complex multistep process that is under the control of more than 35 autophagy-related (Atg) proteins.[2] The basal level of autophagy in cells plays a quality control role in cellular homeostasis by eliminating old and damaged organelles and proteins that are essential cellular components but are no longer functioning reliably. During starvation, autophagy (Greek for "self-eating") is upregulated to provide energy for the nutrient deprived cell from degraded self-components. Autophagy is also an important mechanism used by cells to clear pathogenic viruses[3] and bacteria[4] and deal with microenvironment stresses such as exposure to reactive oxygen species.[5] Defective autophagy has been implicated in many diseases such as neuronal degeneration and cancer, and it is thought to play a role in aging. Pharmacological stimulation or inhibition of autophagy have both been proposed as approaches to treating a variety of diseases[6] and promoting healthy aging.[7]

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(1) (a) Oczypok, E. A.; Oury, T. D.; Chu, C. T. *Am. J. Pathol.* **2013**, *182*, 612–622. (b) Codogno, P.; Mehrpour, M.; Proikas-Cezanne, T. *Nat. Rev. Mol. Cell. Biol.* **2012**, *13*, 7–12.

(2) (a) Yang, Z.; Klionsky, D. J. *Curr. Opin. Cell. Biol.* **2010**, *22*, 124–131. and (b) Subramani, S.; Malhotra, V. *EMBO rep.* **2013**, *14*, 143–151.

(3) Yordy, B.; Tai, M. C.; Hayashi, K.; Arojo, O.; Iwasaki, A. *Intl. Immunol.* **2012**, *25*, 1–10.

(4) (a) Mostowy, S. *Cell. Microbiol.* **2013**, *15*, 395–402. (b) Campoy, E.; Colombo, M. I. *Biochim. Biophys. Acta* **2009**, *1793*, 1465–1477. (c) Songane, M.; Kleinnijenhuis, J.; Netea, M. G.; van Creval, R. *Tuberculosis* **2012**, *92*, 388–396.

(5) Jones, S. A.; Mills, K. H. G.; Harris, J. *Immunol. Cell. Biol.* **2013**, *91*, 250–258.

(6) (a) Rubinsztein, D. C.; Cogogno, P.; Levine, B. *Nat. Rev. Drug Discovery* **2012**, *11*, 709–730. (b) Floto, R. A.; Sarkar, S.; Perlstein, E. O.; Kampmann, B.; Schreiber, S. L.; Rubinsztein, D. C. *Autophagy* **2007**, *3*, 620–622.

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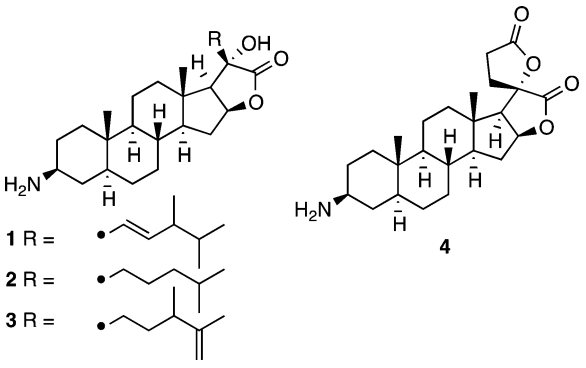
Although basic knowledge about the role of autophagy in cell biology and disease progression is rapidly increasing, many specific details about the roles of individual Atg proteins remain unknown.[2] It has been proposed that selective small molecule modulators of Atg protein function that work in a cellular context would be extremely useful chemical genetics/cell biology tools for the further study of autophagy.[8] Despite the recognized need for chemical tools to study autophagy, the number of effective compounds available for this purpose is extremely limited.



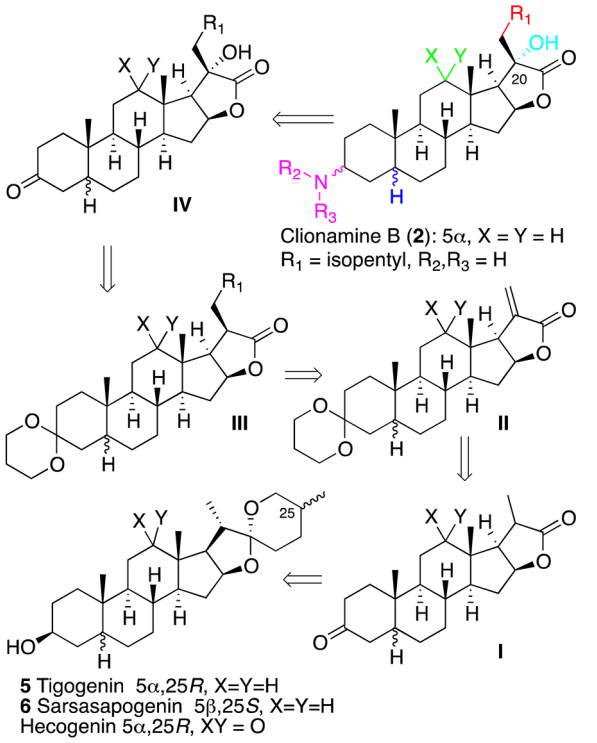
Prompted by the need for novel small molecule modulators of autophagy as chemical tools and drug leads,[9] a library of marine organism crude extracts were screened in a cell-based high content assay designed to find both stimulators and inhibitors[10] of autophagy. A MeOH extract of the sponge *Cliona celata* collected on the Wild Coast of South Africa showed promising autophagy stimulation in the screen. Assay-guided fractionation of the extract revealed that the amino steroids clionamines A (**1**) to D (**4**) were responsible for the desired biological activity.[11] Clionamine A (**1**), the major component in the extract, strongly stimulated autophagy in human breast cancer MCF-7 cells in the screening assay.

The clionamines contain structural features not previously encountered in naturally occurring steroids. Foremost among these is the combination of an E ring γ-lactone and C-20 hydroxylation found in all of the analogues and the spirobislactone side chain found in clionamine D (**4**). The clionamines are also 3β-amino steroids, a structural variation that has precedent in natural steroids, but is rare.

Only single digit milligram quantities of most of the clionamines were available from the sponge extracts. This severely limited the range of biological evaluation that could be carried out on the compounds. In order to solve the supply issue for the clionamines and to facilitate the generation of photoaffinity probes for molecular target identification and provide analogues for SAR studies, we undertook a total synthesis of clionamine B (**2**), one of the least abundant analogues in the sponge extract. Since the ultimate goal was to be able to generate sufficient quantities of a natural clionamine or a more potent analogue for in vivo studies in animal models, the synthesis was designed to use cheap starting materials and reagents.

The retrosynthetic analysis for a practical general synthesis of clionamines is shown in Scheme 1. Steroidal saponins are common metabolites of plants and their aglycones (sapogenins) have been extensively used as starting materials for the production of steroidal hormone drugs.[12] As a consequence, sapogenins are cheap reagents that are readily available in kg quantities. Tigogenin (**5**) and sarsasapogenin (**6**) (Scheme 1) are two of the most easily accessed sapogenins, but variants with substituents at C-12 such as hecogenin are also available. Methodology for the degradation of sapogenin side chains to give the E ring γ-lactone found in the clionamines has been known for decades,[12] but continues to be refined.[13]

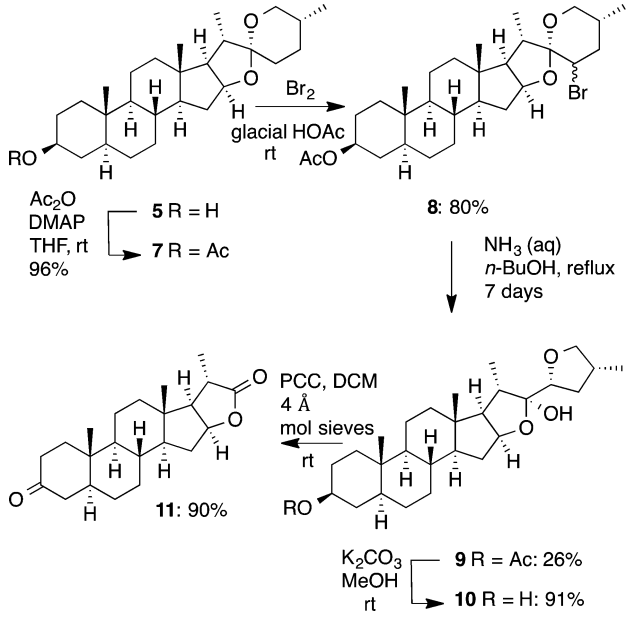
Scheme 1. Retrosynthetic Analysis for a Clionamine Synthesis



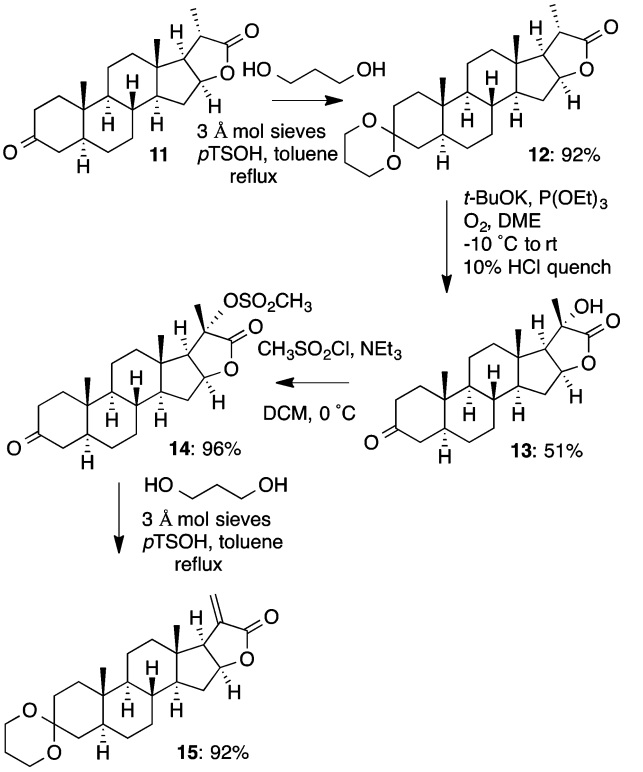
As proposed in Scheme 1, the last step in the clionamine B synthesis would be a standard reductive amination of ketone intermediate **IV**, which would come from C-20 hydroxylation of intermediate **III**. Intermediate **III** could be prepared via conjugate addition of R_1 to the α-methylene lactone **II**, which could arise from dehydrogenation of the α-methyl-γ-lactone in the sapogenin degradation product **I**. Degradation

(7) Pallauf, K.; Rimbach, G. *Ageing Res. Rev.* **2013**, *12*, 237–252.
(8) Rubinsztein, D. C.; Gestwicki, J. E.; Murphy, L. O.; Klionsky, D. J. *Nat. Rev. Drug Discovery* **2007**, *6*, 304–312.
(9) Lam, K. K. Y.; Zheng, X.; Forestieri, R.; Balgi, A. D.; Nodwell, M.; Vollett, S.; Anderson, H. J.; Andersen, R. J.; Av-Gay, Y.; Roberge, M. *PLoS Pathogens* **2012**, *8*, e10022691.
(10) Carr, G.; Williams, D. E.; Díaz-Marrero, A. R.; Patrick, B. O.; Bottriell, H.; Balgi, A. D.; Donohue, E.; Roberge, M.; Andersen, R. J. *J. Nat. Prod.* **2010**, *73*, 422–427.
(11) Keyzers, R. A.; Daoust, J.; Davies-Coleman, M. T.; Van Soest, R.; Balgi, A.; Donohue, E.; Roberge, M.; Andersen, R. J. *Org. Lett.* **2008**, *10*, 2959–2962.
(12) (a) Marker, R. E.; Rohrmann, E. *J. Am. Chem. Soc.* **1940**, *62*, 518–520. (b) *Ibid.* 898–900. (c) Marker, R. E.; Krueger, J. *J. Am. Chem. Soc.* **1940**, *62*, 3349–3350. (d) Djerassi, C. *Steroids* **1992**, *57*, 631–641.
(13) Anulewicz-Ostrowska, R.; Jastrzebska, I.; Morzycki, J. W.; Wójcik, J. *J. Org. Chem.* **2002**, *67*, 6916–6924.

Scheme 2. Degradation of the Side Chain of Tigogenin (**5**)



Scheme 3. Preparation of the E Ring α-Methylene Lactone



of the sapogenin starting material to give **I** would follow literature procedures.[12,13] The commercial availability of a variety of sapogenin starting materials [e.g., tigogenein (**5**) and sarsasapogenin (**6**)] would allow for the synthesis of a library of analogues (varying in the colored functionalities) for SAR optimization studies. Variations in R_1, R_2, and R_3 would facilitate the incorporation of the photophore and alkyne or azide Click chemistry components required to make probes for cellular protein target identification.

The synthesis of clionamine B (**2**) started with acetylation of tigogenin (**5**) to give the acetate **7** in nearly quantitative yield as shown in Scheme 2.[13] Treatment of **7** with Br_2 in glacial acetic acid led to bromination at C-23 on the dihydropyran ring adjacent to the spiro ketal. A mixture of the axial and equatorial brominated products **8** was obtained in 80% combined yield. It is well documented that when the methyl substituent at C-25 is axial, as in sarsasapogenin (**6**), bromination gives almost exclusively the equatorial bromine at C-23 due to steric interactions with the methyl, but when the C-25 methyl substituent is equatorial, as in tigogenin (**5**), bromination gives a mixture of epimeric bromides at C-23 as we observed for **8**.[13]

Treatment of the epimeric mixture of bromides **8** with aqueous ammonia in *n*-butanol at reflux for 7 days led to solvolysis and rearrangement to give the desired product **9** in a modest 26% yield.[13] The low yield was anticipated because it was known that the C-23 axial bromides do not effectively rearrange to give C-22 hemiketals such as **9** under these conditions. Even though the transformation of **8** to **9** proceeded in low yield, the ready availability of **5** and the ability to carry out the steps from **5** to **9** on multigram scales using cheap reagents meant that these first steps were still a viable entry point to a practical clionamine synthesis.

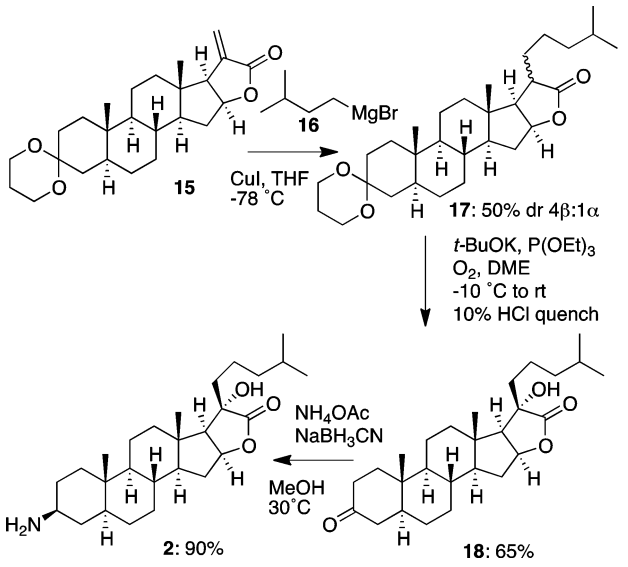
Hydrolysis of the acetate **9** with K_2CO_3 in MeOH gave the alcohol **10** in 91% yield. Oxidative degradation of the hemiketal **10** was carried out by treatment with PCC in DCM at rt in the presence of 4 Å molecular sieves to give the ketone **11**, containing the E ring γ-lactone found in the clionamines, in 90% yield.[13]

Installation of the C-20 hydroxyl group was expected to be a key transformation in the synthesis of clionamine B (**2**). Therefore, we decided to explore methodology for carrying out C-20 hydroxylation on ketone **11** before continuing the further elaboration of the side chain carbon skeleton. Our plan for C-20 hydoxylation involved oxidation of the γ-lactone enolate. To set the stage for this transformation, the C-3 ketone in **11** was protected as the cyclic ketal **12** in 92% yield as shown in Scheme 3 to prevent unwanted base catalyzed condensation and ketone α-oxidation side reactions. Formation of the enolate of lactone **12** with *t*-BuOK in DME at −10 °C in the presence of a stream of oxygen gas and triethyl phosphite[14] followed by a 10% HCl workup successfully installed the desired hydroxyl group at C-20 with the α-orientation found in the clionamines and removed the cyclic ketal at C-3 to give **13** in 51% yield.

With the C-20 hydroxyl group in hand, we turned our attention to dehydration as a route to an α-methylene lactone intermediate. Mesylation of **13** gave the sulfonate ester **14** in 96% yield.[15] In anticipation of using a strong base to promote elimination of the mesylate to give the

(14) Coburn, C. E.; Anderson, D. K.; Swenton, J. S. *J. Org. Chem.* **1983**, *48*, 1455–1461.
(15) Crossland, R. K.; Servis, K. L. *J. Org. Chem.* **1970**, *35*, 3195–3196.

Scheme 4. Final Steps in the Synthesis of Clionamine B (**2**)



conjugated alkene,[16] the C-3 ketone was again protected as a cyclic ketal. Fortuitously, during the ketalization reaction on **14**, the mesylate also eliminated to give the desired α-methylene lactone **15** in 92% yield (Scheme 3).[17]

The isopentyl Grignard reagent **16** was reacted with excess CuI, and the resulting organocuprate intermediate[18] was added to the α-methylene lactone **15** to give **17** as a 4:1 mixture of β and α epimers in 50% overall yield (Scheme 4). Hydroxylation of the mixture of C-20 epimers **17** using the same enolate oxidation conditions developed for lactone **11** gave the C-20 hydroxy derivative **18** as a single epimer having the clionamine configuration in 65% yield. The approach of molecular oxygen to the β-face of C-20 is completely blocked by steric interaction with Me-18. Reductive amination of **18** using ammonium acetate and sodium cyanoborohydride gave clionamine B (**2**) in 90% yield.[19] The proton NMR spectrum recorded for synthetic clionamine B (**2**) at 600 MHz in MeOD was identical to the spectrum of natural clionamine B (**2**) recorded in the same solvent (Supporting Information).

The lack of an adequate and sustainable supply of compound is most often a limiting factor in the preclinical biological evaluation and eventual clinical development of

(16) Chiao, W.-B.; Saunders, W. H. *J. Org. Chem.* **1980**, *45*, 1319–1320.

(17) Bolster, M. G.; Jansen, B. J. M.; de Groot, A. *Tetrahedron* **2001**, *57*, 5663–5679.

(18) House, H. O.; Respess, W. L.; Whitesides, G. M. *J. Org. Chem.* **1966**, *31*, 3128–3141.

(19) Borch, R. F.; Bernstein, M. D.; Durst, H. D. *J. Am. Chem. Soc.* **1971**, *93*, 2897–2904.

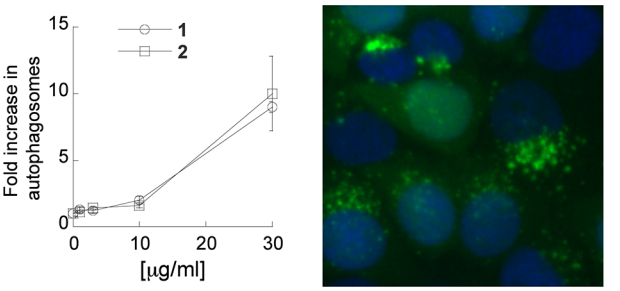


Figure 1. Autophagy stimulation by clionamines A (**1**) and B (**2**). MCF-7 cells expressing the autophagy marker EGFP-LC3 were incubated for 4 h with **1** or **2**, and autophagosomes (green puncta) were measured (Supporting Information). Cell nuclei are in blue.[9,11].

bioactive marine invertebrate secondary metabolites. Clionamines are novel amino steroids isolated in small quantities from a marine sponge.[11] They stimulate autophagy at low μM concentrations, making them potentially interesting drug leads for a variety of human diseases.[6]

Herein, we describe a total synthesis of clionamine B (**2**) that starts with a readily available and low cost starting material tigogenin (**5**) and makes use of simple reagents. A key transformation in the synthesis is the stereoselective installation of the α-hydroxyl substituent at C-20 that uses molecular oxygen as the oxidant. The synthetic route is flexible in design and scalable and should, therefore, be able to provide natural clionamines and unnatural analogues in sufficient quantities for further biological evaluation. The synthesis of clionamine B (**2**) represents the first synthesis of a natural clionamine. It has confirmed the proposed structure of **2** and allowed us to show for the first time that **2** strongly stimulates autophagy, with similar potency to clionamine A (**1**)[11] (Figure 1). Synthesis of additional clionamines and unnatural analogues is ongoing, and their autophagy-stimulating and potential therapeutic properties will be reported elsewhere.

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Supporting Information Available. ^{1}H and ^{13}C NMR spectra of synthetic intermediates and clionamine B (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.