

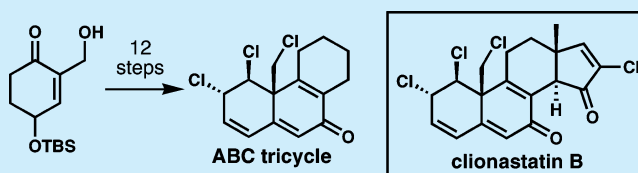
A Synthesis of the ABC Tricyclic Core of the Clionastatins Serves To Corroborate Their Proposed Structures

Samuel S. Tartakoff and Christopher D. Vanderwal*

Department of Chemistry, University of California, 1102 Natural Sciences II, Irvine, California 92697-2025, United States

S Supporting Information

ABSTRACT: A synthesis of the ABC tricyclic ring system of the clionastatins, an unusual pair of highly chlorinated androstane steroids, has been accomplished. This work provides strong support for the original structural proposal. An unexpected substrate-dependent reversal in alkene chlorination diastereoselectivity was critical to success. This approach should be amenable to an eventual enantioselective synthesis of the natural products themselves.



The steroid family of metabolites comprises an enormous number of diverse structures, largely conforming to the tetracyclic 6–6–6–5 ring system. They are among the most studied natural products, with well understood biosynthetic pathways¹ and many successful laboratory syntheses.² Clionastatins A and B (**1** and **2**, Figure 1), isolated from the

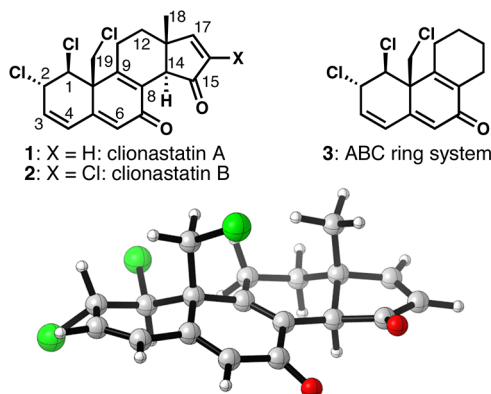


Figure 1. Clionastatins A and B, the ABC tricyclic ring system, and an energy-minimized structure of clionastatin A.

Mediterranean burrowing sponge *Cliona nigricans* in 2004,³ possess several features that distinguish them from any previously isolated steroid. In spite of their well-known androstane steroidal framework, they each have a 3,5,8(9)-16-tetraen-7,15-dione oxidation pattern not found in any other natural or synthetic steroids. Furthermore, they are the first polyhalogenated steroids observed in nature and remain one of the few chlorinated steroids where the chlorine atoms are not integral to chlorohydrins. Fattorusso and co-workers assigned the relative stereochemistry by ROESY correlations but were not able to obtain an X-ray crystal structure from the small amount of amorphous solid isolated. The novel structures of **1** and **2**, their potent cytotoxicity, and our long-standing interest

in the stereoselective synthesis of polychlorinated natural products⁴ led us to undertake a synthesis.

When considering a retrosynthetic approach to the clionastatins, several obvious challenges needed to be addressed. Both the C1 and C19 chlorides are neopentyl, presumably making late-stage introduction by substitution difficult. This issue is further complicated by the fact that the C1 and C2 chlorides are both pseudoequatorial (both in energy-minimized structures, Figure 1, and according to coupling constant data³), and dichlorination reactions of cyclohexenes tend to favor the formation of diaxial products.⁵ Finally, the high degree of unsaturation found in **1** and **2** prevents rapid synthesis by biomimetic π -cyclization cascades⁶ and adds significant strain to the ring system. Of special note is the tetrasubstituted $\Delta^{8,9}$ -enone which, while not unknown, is difficult to install via standard Saegusa–Ito or other similar oxidative conditions.⁷

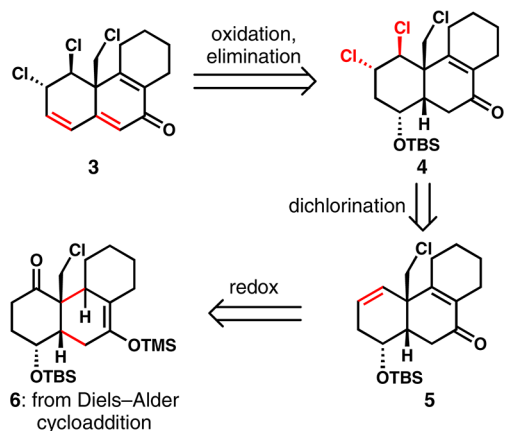
Because of the aforementioned challenges, and also to corroborate the unusual proposed structure, we first decided to target a truncated tricyclic system (**3**, Scheme 1) containing much of the complexity found in the natural products. A late-stage elimination and oxidation of the B-ring enone would take us back to intermediate **4**, the dichlorination product of **5**. Redox manipulations separate **5** from Diels–Alder product **6**, and the precursors of this cycloaddition are simple, readily available materials. This route was particularly attractive because, in addition to addressing several of the expected challenges outlined above, our model diene **9** could later be replaced by an enantioenriched Hajos–Parrish ketone-derived diene, introducing the D-ring with the functionality needed to complete the total syntheses of **1** and **2**.

Known cyclohexenone **7** is made by the Baylis–Hillman reaction of formaldehyde with 4-(*tert*-butyldimethylsilyloxy)-cyclohexenone, which can be obtained enantioselectively.⁸

Received: January 24, 2014

Published: February 26, 2014

Scheme 1. Retrosynthetic Plan for ABC Ring System 3



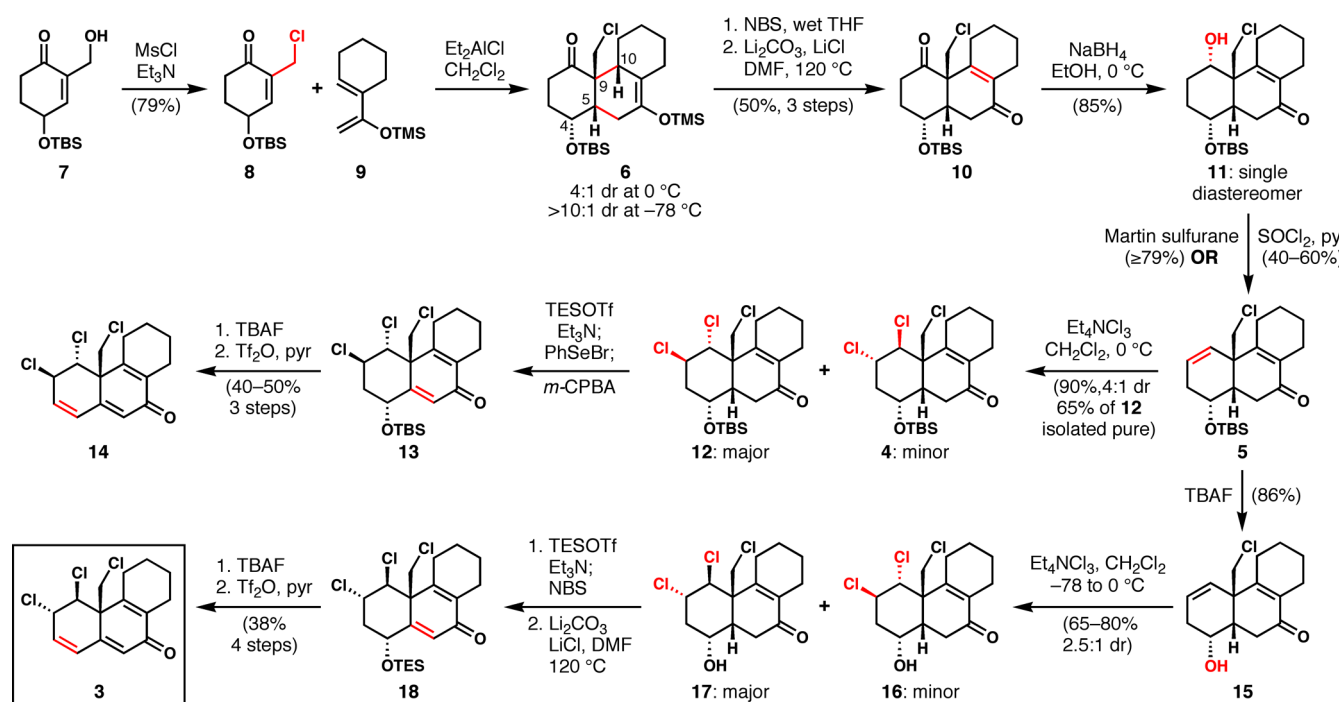
Allylic alcohol **7** was converted to chloride **8** in good yield, and silyoxydiene **9** is a known compound.⁹ Thermal Diels-Alder cycloadditions with α -substituted cyclic enones are known to be challenging,¹⁰ but Corey and co-workers have shown that Lewis acid activation can result in rapid, stereoselective reactions.⁷ Indeed, when diene **9** was added to a mixture of dienophile **8** and 2 equiv of Et_2AlCl , cycloadduct **6** was formed in nearly quantitative yield. The cycloadduct could be obtained in >10:1 diastereoselectivity when the reaction was conducted at -78°C for 12 h; however, these reactions did not reach completion. As a result, the Diels-Alder reaction was usually performed over 15 min at 0°C , resulting in a 4:1 dr, with greater overall yield than that obtained from the lower temperature, more selective protocol. The reaction appears to be entirely *endo*-selective, with the major diastereomer as shown in Scheme 2, and with the minor isomer epimeric at C5, C9, and C10 (using C4 as the point of reference). The observed configuration, which was confirmed by NOE correlations at a later stage, was in

agreement with the observations of Danishefsky,¹¹ namely that systems of this type usually proceed through an *endo* transition state, with the diene approaching from the sterically more hindered face.

Attempts to oxidize the resultant enol ether to the desired enone **10** by a variety of one-step methods failed to give more than modest yields. However, treatment of the Diels-Alder cycloadduct with NBS, followed by heating the crude mixture to reflux in DMF in the presence of LiCl and Li_2CO_3 , generated the desired enone in 50% yield over three steps. This procedure was modified from the published conditions¹² because the use of LiBr instead of LiCl resulted in a mixture of C19 chlorinated and brominated product. Reduction of the A-ring ketone using NaBH_4 afforded alcohol **11** in 85% yield as a single diastereomer, in agreement with the observations of Mechoulam for similar steroidal systems.¹³

Dehydration of the alcohol to form alkene **5** proved problematic because formation of the intermediate triflate using either $\text{Tf}_2\text{O}/\text{Et}_3\text{N}$ or $\text{PhNTf}_2/\text{LDA}$ resulted in complex mixtures, with isolated yields of desired product ranging between 10 and 50%. Attempts to form the mesylate or tosylate under a variety of conditions were unsuccessful under a variety of conditions, presumably because of the sterically encumbered nature of the alcohol. Treatment with Martin sulfurane¹⁴ resulted in efficient and reproducible dehydration, but because of the expense associated with that reagent, SOCl_2 and pyridine were most often used, affording alkene **5** in 40–60% yield.

Dichlorination of **5** with Et_4NCl_3 produced dichloride **12** as the major component of a 4:1 diastereomeric mixture. NOESY experiments indicated the preferential formation of the undesired stereoisomer. Efforts to alter the dichlorination diastereoselectivity by varying concentration, solvent, and temperature were unproductive. Other dichlorinating agents, including PhICl_2 and SO_2Cl_2 , either failed to cause reaction or resulted in a complex mixture of products. Unfortunately, the desired diastereomer could not be isolated cleanly from the

Scheme 2. Synthesis of the Unnatural and Natural Diastereomers of the Clonastatin ABC Ring System (**14** and **3**, Respectively)

dichlorination reaction product mixture (4/12). However, a small amount of the undesired diastereomer could be purified, and conversion of enone 12 to the TES enol ether followed by sequential *in situ* treatment with PhSeBr and *m*-CPBA resulted in dienone 13. Cleavage of the silyl ether of 15 followed by elimination using excess TiF_4 in pyridine/ CH_2Cl_2 produced the C1,C2 epimeric core (14) in 50% yield over three steps.

Not surprisingly, the dichlorination of 5 had led predominantly to pseudodiaxial dichloride 12 and would not suffice to obtain a significant quantity of the desired pseudodiequatorial diastereomer 4. To evaluate dichlorination in a different context, deprotection of 5 was conducted with TBAF to afford the somewhat unstable free alcohol 15. Unexpectedly, this alkene could be dichlorinated with Et_4NCl_3 to afford a mixture favoring the desired diastereomer 17, albeit in moderate yields. Ratios of up to 5:1 in favor of 17 have been observed, but the best compromise with respect to diastereoselectivity and yield arises from the specific conditions shown. The major complication in this reaction is the competitive oxidation to a C4 ketone that apparently facilitates decomposition.

The reason for the dramatic alteration in dichlorination stereoselectivity between substrates 5 and 15 is not clear. We

have ruled out a significant change in conformational preferences because the ^1H NMR resonances attributed to the A-ring protons of these two substrates are virtually superimposable. Analysis of coupling constants in the A-ring (especially $^3J_{4-5} = 3.6$ Hz) suggests that the hydroxy/silyloxy groups preferentially occupy pseudoequatorial positions in the ground state. Furthermore, a pseudoaxial disposition of these groups would enforce a boatlike A-ring conformation, with attendant flagpole interactions between the C3 proton and the angular chloromethyl group. The pseudoequatorial positioning of these groups militates against any explanations based on steric differences for the change in dichlorination selectivity between 5 and 15. It would be premature to comment any further on this phenomenon at this point, especially given the complexity of chlorination mechanisms. Studies to understand this result are ongoing.

The diastereomeric mixture of 16/17 could be carried through a sequence similar to that used to convert 12 to 14, and desired ABC tricyclic model system 3 was obtained uneventfully. Purification to diastereomeric purity at any single stage was difficult, but complete elimination of the undesired stereoisomer was reliably achieved over the course of the four-step sequence.

The relative configurations shown for diastereomeric dichlorination products 12 and 4 are supported by the NOE correlations presented in Figure 2. Access to both diastereomers further secures these assignments. The NOE correlation between protons on C2 and C19 in the final products, 3, which is replaced by a correlation between C1 and C19 in diastereomer 14, further improves our confidence.

^1H NMR spectra of the diastereomeric tricyclic products 14 and 3 were compared with the published NMR spectra for clionastatin B (for simplicity, a spectrum of a mixture is shown in Figure 3; purified spectra for each can be found in the Supporting Information). Clearly, the C1 and C2 protons of the unnatural diastereomer 14 do not correlate well with the authentic spectra, likely owing to their deshielded, equatorial orientation. However, while the splitting pattern for the model system 3 differs slightly from the spectrum of the natural

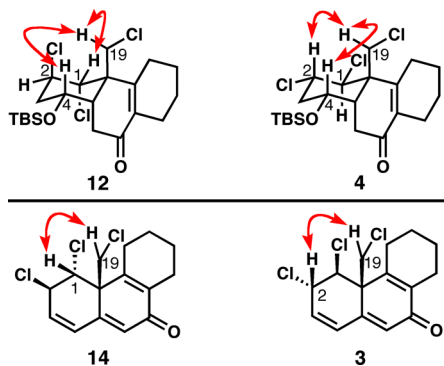


Figure 2. Key NOE correlations that support the relative stereochemistry put forth for compounds 12, 4, 14, and 3.

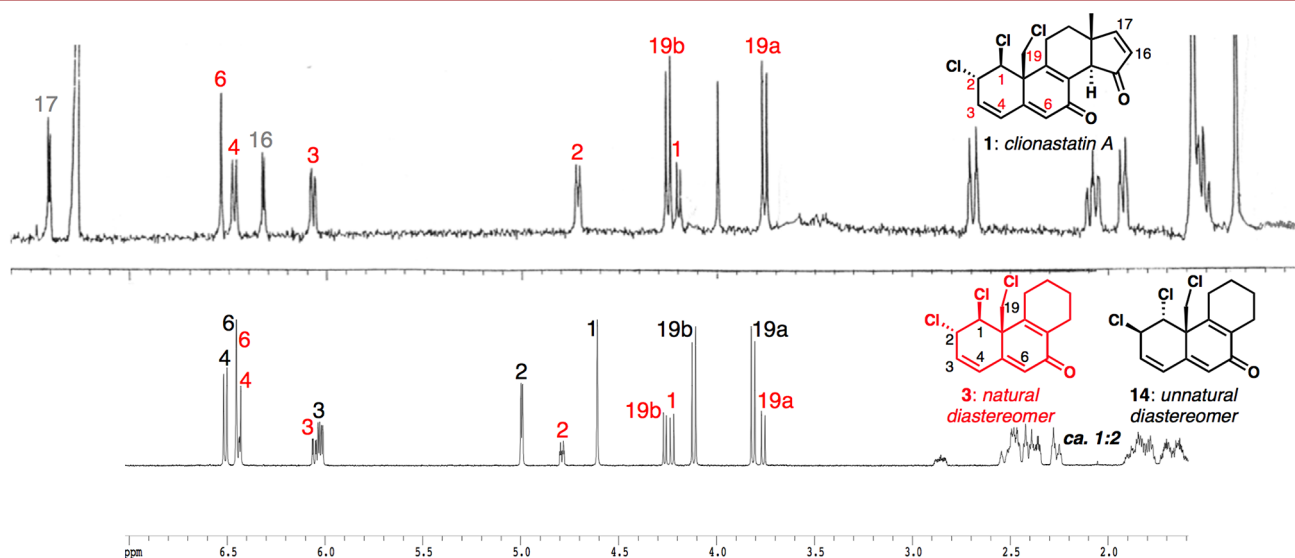


Figure 3. Comparison of ^1H NMR spectra of clionastatin B with a mixture of diastereomeric clionastatin ABC tricycles strongly corroborates the original structural and stereochemical assignment (top spectrum reprinted from the Supporting Information of ref 3. Copyright 2004, American Chemical Society). $^3J_{1,2}(1) = 9.0$ Hz, $^3J_{1,2}(3) = 8.6$ Hz, $^3J_{1,2}(14) = 0$ Hz (singlet).

products, the chemical shifts of the key resonances from the A and B rings match nearly perfectly.

We have synthesized the highly chlorinated ABC tricyclic ring system of clionastatins A and B in 12 steps from known, readily available enone **7**. We have validated the key Diels–Alder disconnection to this highly oxidized and chlorinated steroid and addressed the installation of all three chlorides found in clionastatin A, which lays the groundwork for a convergent synthesis of the natural products themselves. Important aspects of this work include (1) the diastereoselectivity of the Diels–Alder cycloaddition of chiral cyclohexenone **7**, which should ultimately permit an enantioselective synthesis of the clionastatins starting from enantioenriched **7**, (2) the remarkable reversal of dichlorination diastereoselectivity upon cleavage of the silyl ether in **5**, and (3) the confidence gained in Fattorusso's original structural and stereochemical assignments of clionastatins A and B. Clearly, the fortuitous diastereoselective dichlorination reaction of homoallylic alcohol **15** could not have been predicted at the outset, and this work underscores the empirical nature of the chemical synthesis of unusual natural products. Work toward the enantioselective syntheses of **1** and **2** is currently underway and will be reported in the future.

■ ASSOCIATED CONTENT

■ Supporting Information

Experimental procedures and spectral data for all new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: cdv@uci.edu.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the NIH for research support via grant GM-086483 and the NSF for support via a Graduate Research Fellowship to S.S.T. We are grateful to Hung V. Pham (UCLA) for providing the minimized structure of clionastatin A in Figure 1 and to Dr. Allen Hong and Sean Feng (UCI) for assisting in the synthesis of early intermediates.

■ REFERENCES

- (1) For a recent review and good lead reference, see: Nes, W. D. *Chem. Rev.* **2011**, *111*, 6423–6451.
- (2) For some representative reviews, see: (a) Groen, M. B.; Zeelen, F. J. *Recl. Trav. Chim. Pays-Bas* **1986**, *105*, 465–48. (b) Zeelen, F. J. *Nat. Prod. Rep.* **1994**, *11*, 607–612. (c) Hanson, J. R. *Nat. Prod. Rep.* **2010**, *27*, 887–899. The latter reference is the latest in a series of reviews covering the partial synthesis of steroids in medicinal chemistry.
- (3) Fattorusso, E.; Tagliatela-Scafati, O.; Petrucci, F.; Bavestrello, G.; Calcinai, B.; Cerrano, C.; Di Meglio, P.; Ianaro, A. *Org. Lett.* **2004**, *6*, 1633–1635.
- (4) Chung, W.-j.; Vanderwal, C. D. *Acc. Chem. Res.* **2014**, *47*, 718–728.
- (5) Jung, M. E.; Gomez, A. V. *Tetrahedron Lett.* **1993**, *34*, 2891–2894.
- (6) Yoder, R. A.; Johnston, J. N. *Chem. Rev.* **2005**, *105*, 4730–4756.
- (7) Stoltz, B. M.; Kano, T.; Corey, E. J. *J. Am. Chem. Soc.* **2000**, *122*, 9044–9045.

- (8) Arthurs, C. L.; Lingley, K. F.; Piacenti, M.; Stratford, I. J.; Tatic, T.; Whitehead, R. C.; Wind, N. S. *Tetrahedron Lett.* **2008**, *49*, 2410–2413.
- (9) Symmes, C.; Quin, L. D. *J. Org. Chem.* **1976**, *41*, 238–242.
- (10) Charest, M. G.; Siegel, D. R.; Myers, A. G. *J. Am. Chem. Soc.* **2005**, *127*, 8292–8293.
- (11) Jeroncic, L. O.; Cabal, M. P.; Danishefsky, S. J.; Schulte, G. M. *J. Org. Chem.* **1991**, *56*, 387–395.
- (12) Wender, P. A.; Kogen, H.; Lee, H. Y.; Munger, J. D., Jr.; Wilhelm, R. S.; Williams, P. D. *J. Am. Chem. Soc.* **1989**, *111*, 8957–8958.
- (13) Noam, M.; Tamir, I.; Breuer, E.; Mechoulam, R. *Tetrahedron* **1981**, *37*, 597–604.
- (14) Arhart, R. J.; Martin, J. C. *J. Am. Chem. Soc.* **1972**, *94*, 5003–5010.