

Total Synthesis and Structural Confirmation of the Marine Natural Product Dysynosin A: A Novel Inhibitor of Thrombin and Factor VIIa

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Received June 7, 2002

The marine natural product dysynosin A **1**¹ is a new member of serine protease inhibitors generally known as the aeruginosins (Figure 1).² It exhibits activity against thrombin, an essential enzyme in the blood coagulation cascade,³ and Factor VIIa which is involved in blood vessel damage in complex with tissue factor (TF).⁴ The structure of dysynosin A was determined by detailed NMR studies and its absolute stereochemistry deduced from an X-ray structure of a complex with thrombin.¹ Dysynosin A possesses unique structural and functional features that distinguish it among the aeruginosins. Noteworthy is the presence of a 5S,6R-dihydroxy octahydroindole carboxylic acid, an unusual guanidine as part of a pyrroline ring,^{2d,5} and a sulfate group. Bonjoch,⁶ Wipf,⁷ and their respective groups have independently reported the total synthesis and stereochemical revision of aeruginosin 298-A utilizing L-tyrosine as a starting material.

We report herein the total synthesis of dysynosin A utilizing a carbon construct strategy that generates subunits originating from L-glutamic acid, butyrolactone, D-leucine, and D-mannitol as shown in Figure 1. The synthesis of the enantiopure octahydroindole carboxylic acid⁸ capitalized on the prospects of a ring-closure metathesis reaction⁹ from a chiron derived from L-pyrroglutamic acid, and subsequent stereoselective epoxidation and epoxide opening. To this end we had to secure methodology that introduced two C-allylic appendages with a *syn*-disposition at C-4 and C-5 of L-proline as shown in Scheme 1. The (4S)-allyl analogues **2** and **3** have been previously synthesized by stereoselective enolate alkylation of the corresponding L-glutamate esters.¹⁰ Conversion of **2** and **3** into the corresponding methyl L-pyrroglutamates,¹¹ selective reduction, and acetylation afforded the expected hemiaminal derivatives **4** and **5**. The introduction of a *syn*-allyl group at C-5 via *N*-acyl iminium ion chemistry¹² proved to be challenging. After extensive variations of solvents, the nature of Lewis acids, and *N*-substituents,¹³ allylation of **5** could be realized with a 5.5:1 *all-syn/anti* selectivity with allyl tributylstannane in the presence of BF₃·Et₂O in *toluene* to afford **7**, easily separable from the minor diastereomer by chromatography. Allylation of **4** under the same conditions led to a 1:2 ratio of *syn/anti* isomers of **6**.

Olefin metathesis of **6** and **7** using the original and elegant Grubbs method⁹ led to the carbocyclization products **8** and **9**, respectively, in excellent yields. Epoxidation with *m*-CPBA proved to be highly selective, affording the epoxides **10** and **11** in each case, presumably as a result of an attack from the more accessible convex face of the bicyclic system. When treated with aqueous TFA, each epoxide led to the enantiopure intermediates **12** and **13**, respectively, whose structures were unequivocally proven by single-crystal X-ray analysis. For reasons of functional group compatibility, the synthesis was continued with **13**, which was transformed to

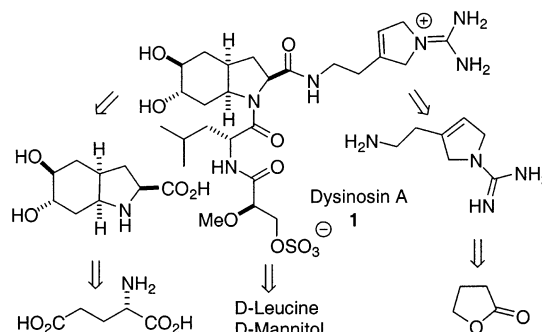
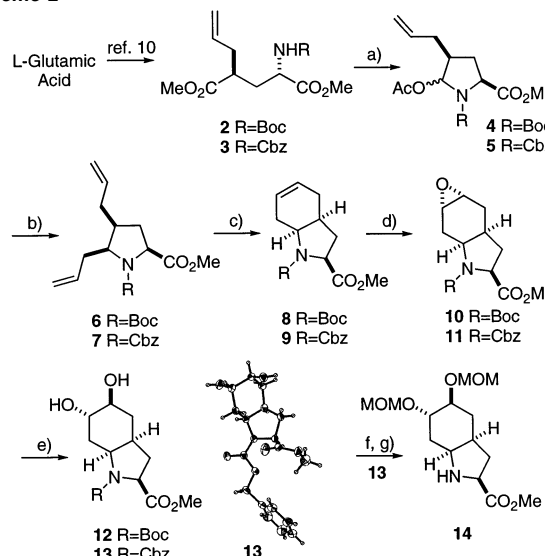


Figure 1. Disconnection of dysynosin A to subunits and chirons.

Scheme 1^a

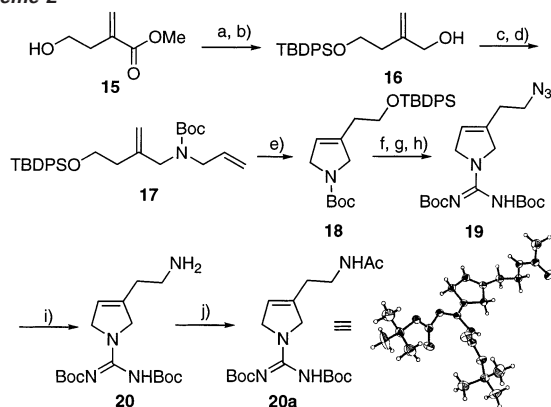


^a Reagents and conditions: (a) 1. TFA, CH₂Cl₂; 2. NaHCO₃; 3. Δ, toluene; 4. LiHMDS, CbzCl, THF -78 °C; 5. LiHBEt₃, THF -78 °C; 6. Ac₂O, DMAP, CH₂Cl₂; overall 85%. (b) BF₃·OEt₂, allyl tributylstannane, toluene -78 °C (*syn/anti* 5.5:1); overall 83%. (c) Ru benzyldiene(Cy₃P)₂Cl₂ 1 mol %, CH₂Cl₂; 99%. (d) *m*-CPBA, CH₂Cl₂. (e) TFA (0.2 equiv), THF/H₂O (1/1); 75–79% (2 steps). (f) MOMCl, (Pr)₂NEt, CH₂Cl₂; 98%. (g) Pd/C 20%, H₂, MeOH; 95%.

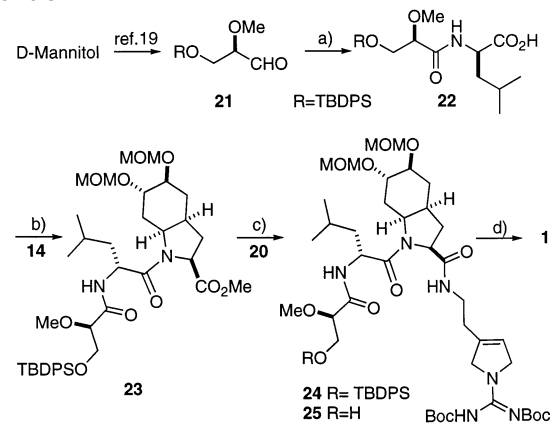
the bis-MOM ether **14**. The highly site-selective *trans*-diaxial acid-catalyzed opening could be due in part to the shielding effect of the pseudodiaxial¹⁴ carbomethoxy group on the concave face of the bicyclic motif, as evidenced by X-ray analysis (Scheme 1).

The synthesis of the Δ-3 pyrroline unit¹⁵ shown in Scheme 2, started with the hydroxy ester **15** readily available from butyrolactone.¹⁶ Reduction of the ester function gave the allylic alcohol **16**,¹⁷ which was further transformed to the diolefin **17** in high overall yield. The versatility of the Grubbs metathesis reaction⁹ and its tolerance of functional groups was evidenced by the

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Scheme 2^a

^a Reagents and conditions: (a) TBDPSCl, imidazole, DMF; 90%. (b) DIBAL-H, CH₂Cl₂; 90%. (c) MsCl, Et₃N, CH₂Cl₂; then allylamine; 84%. (d) Boc₂O, Et₃N, CH₂Cl₂; quant. (e) Ru benzylidene(Cy₃P)₂Cl₂ 10 mol %, CH₂Cl₂; 90%. (f) TBAF, THF; 92%. (g) PPh₃, DEAD, (PhO)₂P(O)N₃, THF; 82%. (h) TFA, CH₂Cl₂; then Et₃N, Goodman's reagent; 86%. (i) PPh₃, H₂O, THF then AcOH; 72%. (j) Ac₂O, Et₃N, MeOH; 90%.

Scheme 3^a

^a Reagents and conditions: (a) 1. NaClO₂, 2-methylbut-2-ene, NaH₂PO₄, *t*-BuOH; 2. TFA-D-Leu-Bn, EDC, HOBT, CH₂Cl₂; 3. Pd/C 10%, H₂, MeOH; overall 76%. (b) 14, BopCl, (iPr)₂NEt, MeCN; 63%. (c) 1. LiOH, THF/MeOH; 2. 20, EDC, HOBT, CH₂Cl₂; overall 92%. (d) 1. TBAF, THF; 2. Py-SO₃, Bu₂SnO, CH₂Cl₂; 6 h; 3. TFA, CH₂Cl₂; 6 h, prep. HPLC; 34% overall.

successful cyclization of **17** to the pyrroline **18** in 90% yield.¹⁸ A series of well-precedented transformations gave **20** which was definitively characterized by single-crystal X-ray analysis of the corresponding *N*-acetyl derivative **20a**.

The acyclic peptide chain **22** was prepared as shown in Scheme 3 from D-leucine and 2-*O*-methyl-D-glyceraldehyde easily available from D-mannitol.¹⁹ Peptide coupling between **14** and **22** afforded **23** which was hydrolyzed to the free acid. A second peptide coupling with **20** proceeded in good overall yield to give **24**, which was desilylated to the alcohol **25**. Treatment of **25** with pyridine-SO₃ complex in dichloromethane in the presence of a catalytic quantity of dibutyltin oxide²⁰ afforded the corresponding sulfate ester. Hydrolysis of the *N*-Boc group with TFA in dichloromethane, followed by isolation of the crude product and purification by reverse phase HPLC afforded dysinosin A as a white solid, identical in all respects to the natural product (HPLC, ¹H, ¹³C NMR, FAB and electrospray mass spectrometry).

The total synthesis of dysinosin A by an enantioselective route provides definitive evidence for its structural and configurational identity. It also represents the first total synthesis of a hitherto unknown member of the aeruginosin family of marine antithrombin natural products, with inhibitory activity against Factor VIIa.

Acknowledgment. Dedicated to Professor Robert H. Grubbs for his seminal work in olefin metathesis. We thank NSERCC for generous financial support from AstraZeneca (Mölnådal Sweden) through the Medicinal Chemistry Chair Program. We appreciate the enthusiastic support given by Dr. David Rees and Dr. Kenneth Granberg (AstraZeneca). We also thank Elaine Fournelle for HPLC analyses and Dr. Michel Simard for X-ray analyses. M.T. acknowledges scholarships from NSERC and FCAR.

Supporting Information Available: Experimental procedures of key reactions, NMR, and X-ray and other data (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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JA0208153