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

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The marine natural product dysinosin 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 dysinosin A was determined by detailed NMR studies and its absolute stereochemistry deduced from an X-ray structure of a complex with thrombin.[1] Dysinosin A possesses unique structural and functional features that distinguish it among the aeruginosins. Noteworthy is the presence of a 5*S*,6*R*-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 dysinosin 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 acid[8] capitalized on the prospects of a ring-closure metathesis reaction[9] from a chiron derived from L-pyroglutamic 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 (4*S*)-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-pyroglutamates,[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 chemistry[12] proved to be challenging. After extensive variations of solvents, the nature of Lewis acids, and *N*-substitutents,[13] allylation of **5** could be realized with a 5.5:1 all-*syn/anti* selectivity with allyl tributylstannane in the presence of $BF_3.Et_2O$ 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 method[9] 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 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 pseudodiaxial[14] carbomethoxy group on the concave face of the bicyclic motif, as evidenced by X-ray analysis (Scheme 1).

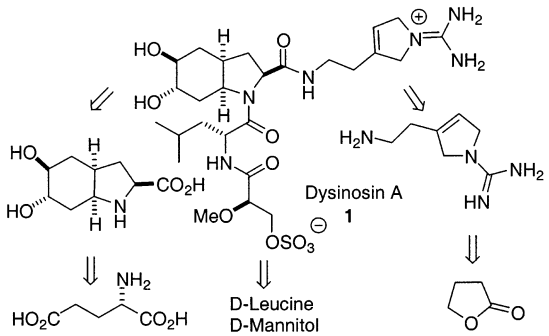
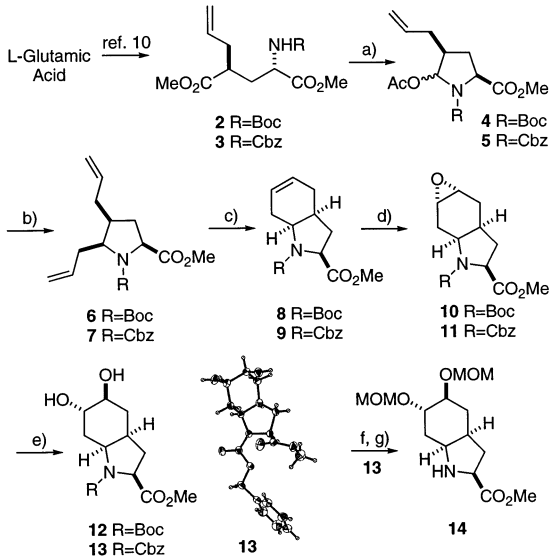


Figure 1. Disconnection of dysinosin A to subunits and chirons.

Scheme 1 [a]



[a] Reagents and conditions: (a) 1. TFA, CH_2Cl_2; 2. $NaHCO_3$; 3. Δ, toluene; 4. LiHMDS, CbzCl, THF −78 °C; 5. $LiHBEt_3$, THF −78 °C; 6. Ac_2O, DMAP, CH_2Cl_2; overall 85%. (b) $BF_3.OEt_2$, allyl tributylstannane, toluene −78 °C (*syn/anti* 5.5:1); overall 83%. (c) Ru benzylidene$(Cy_3P)_2Cl_2$ 1 mol %, CH_2Cl_2; 99%. (d) *m*-CPBA, CH_2Cl_2. (e) TFA (0.2 equiv),THF/H_2O (1/1); 75−79% (2 steps). (f) MOMCl, $(^iPr)_2NEt$, CH_2Cl_2; 98%. (g) Pd/C 20%, H_2,MeOH; 95%.

The synthesis of the Δ-3 pyrroline unit[15] 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 reaction[9] and its tolerance of functional groups was evidenced by the

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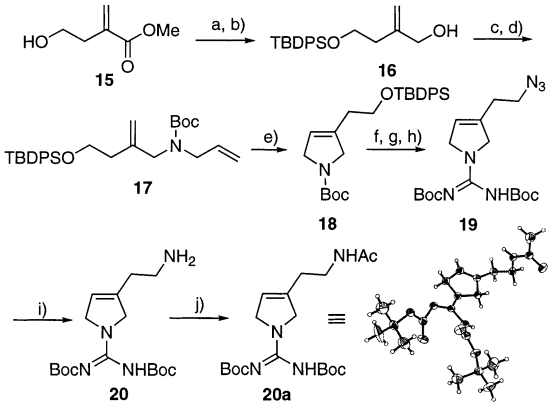
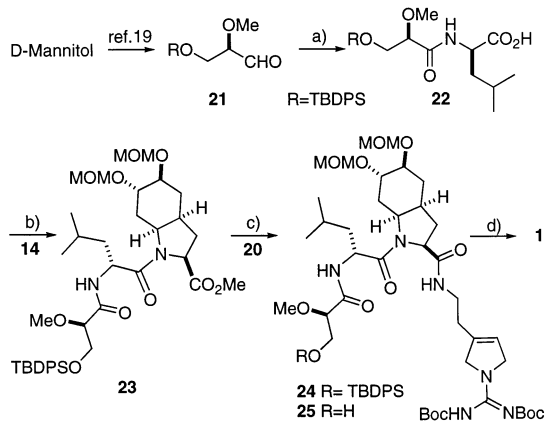
Scheme 2 [a]



[a] Reagents and conditions: (a) TBDPSCl, imidazole, DMF; 90%. (b) DIBAL-H,CH_2Cl_2; 90%. (c) MsCl, Et_3N, CH_2Cl_2; then allylamine; 84%. (d) Boc_2O, Et_3N, CH_2Cl_2; quant. (e) Ru benzylidene$(Cy_3P)_2Cl_2$ 10 mol %, CH_2Cl_2; 90%. (f) TBAF, THF; 92%. (g) PPh_3, DEAD, $(PhO)_2P(O)N_3$, THF; 82%. (h) TFA, CH_2Cl_2; then Et_3N, Goodman's reagent; 86%. (i) PPh_3, H_2O, THF then AcOH; 72%. (j) Ac_2O, Et_3N, MeOH; 90%.

Scheme 3 [a]



[a] Reagents and condtions: (a) 1. $NaClO_2$, 2-methylbut-2-ene, NaH_2PO_4, *t*-BuOH; 2. TFA-D-Leu-Bn, EDC, HOBt, CH_2Cl_2; 3. Pd/C 10%, H_2, MeOH; overall 76%. (b) **14**, BopCl, $(^iPr)_2NEt$, MeCN; 63%. (c) 1. LiOH, THF/MeOH; 2. **20**, EDC, HOBt, CH_2Cl_2; overall 92%. (d) 1. TBAF, THF; 2. Py-SO_3, Bu_2SnO, CH_2Cl_2;, 6 h; 3. TFA, CH_2Cl_2; 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−SO_3 complex in dichloromethane in the presence of a catalytic quantity of dibutyltin oxide[20] 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,^{13}C 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.

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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.

References

(1) Carroll, A. R.; Pierens, G.; Fechner, G.; de Almeida Leone, P.; Ngo, A.; Simpson, M.; Hooper, J. N. A.; Boström, S.-L.; Musil, D.; Quinn, R. J. *J. Am. Chem. Soc.* **2002**, *124*, 13340.

(2) (a) Ishida, K.; Okita, Y.; Matsuda, H.; Okino, T.; Murakami, M. *Tetrahedron* **1999**, *55*, 10971. (b) Steiner, J. R.; Murakami, M.; Tulinsky, A. *J. Am. Chem. Soc.* **1998**, *120*, 597 (c) Sandler, B.; Murakami, M.; Clardy, J. *J. Am. Chem. Soc.* **1998**, *120*, 595. (d) Fujii, K.; Sivonen, K.; Adachi, K.; Noguchi, K.; Shimizu, Y.; Sano, H.; Hirayama, K.; Suzuki, M.; Harada, K. *Tetrahedron Lett.* **1997**, *38*, 5529.

(3) Steinmetzer, T.; Hauptmann, J.; Stürzebecher, J. *Exp. Opin. Invest. Drugs* **2000**, *10*, 845. (b) Sanderson, P. E. J.; Nayler-Olsen, A. M. *Curr. Med. Chem.* **1998**, *5*, 289.

(4) Kalafatis, M.; Egan, J. O.; van't Veer, C.; Cawthern, K.; Mann, K. G. *Curr. Rev. Eukaryotic Gene Expression* **1997**, *7*, 241. (b) Bouma, B. N.; von dem Borne P. A. K.; Meijers, J. C. M. *Thromb. Haemostasis* **1998**, *80*, 24. (c) Mann, K. G. *Thromb. Haemostasis* **1999**, *82*, 165.

(5) Engh, R.; Konetschny-Rapp, S.; Krell, H.-W.; Martin, U.; Tsaklakidis, C., PCT Pat. No. WO97121725; *Chem. Abst.* **1997**, *127*, 12202.

(6) Valls, N.; López-Canet, M.; Vallribera, M.; Bonjoch, J. *J. Am. Chem. Soc.* **2000**, *122*, 11248.

(7) Wipf, P.; Methot, J.-L. *Org. Lett.* **2000**, *2*, 4213.

(8) For the synthesis of similar octahydroindole structures see: (a) Coldham, I.; Crapnell, K. M.; Moseley, J. D.; Rabot, R. *J. Chem. Soc., Perkin Trans I* **2001**, 1758. (b) Belvisi, L.; Colombo, L.; Colombo, M.; DiGiacomo, M.; Manzoni, L.; Vodopirec, B.; Scolastico, C. *Tetrahedron* **2001**, *57*, 6463. (c) Wipf, P.; Maresko, D. A. *Tetrahedron Lett.* **2000**, *41*, 4723. (d) Wipf, P.; Li, W. *J. Org. Chem.* **1999**, *64*, 4576. (e) Wipf, P.; Kim, Y.; Goldstein, D. M. *J. Am. Chem. Soc.* **1995**, *117*, 1606. (f) Bonjoch, J.; Catena, J.; Isabal, E.; Löpez-Canet, M.; Valls, N. *Tetrahedron: Asymmetry* **1996**, *7*, 1899. (g) Harwood, L. M.; Lilley, I. A. *Tetrahedron Lett.* **1993**, *34*, 537. (h) Harwood, L. M.; Kitchen, L. C. *Tetrahedron Lett.* **1993**, *34*, 6603. (i) Waga, T.; Matsui, S.; Saito, S.; Watanable, M.; Kaijiwara, Y.; Shirota, M.; Iijima, M.; Kitabatake, K. *Drug Res.* **1990**, *40*, 407. (j) Henning, R.; Rubach, H. *Tetrahedron Lett.* **1983**, *24*, 5339 and references therein.

(9) For recent reviews, see; (a) Grubbs, R.; Chang, S. *Tetrahedron* **1998**, *54*, 4413. (b) Armstrong, S. K. *J. Chem. Soc., Perkin Trans.* **1998**, 371. (c) Fürstner, A.; Picquet, M.; Bruneau, C.; Dixneuf, P. H. *Chem. Commun.* **1998**, 1315. (d) Schuster, M.; Blechert, S. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2036.

(10) Hanessian, S.; Margarita, R. *Tetrahedron Lett.* **1998**, *39*, 5887.

(11) Li, H.; Sakamoto, T.; Kato, M.; Kikugawa, Y. *Synth. Commun.* **1995**, *25*, 4045.

(12) Speckamp, W.; Moolenaar, M. J. *Tetrahedron* **2000**, *56*, 3817. (b) Hiemstra, H.; Speckamp, N. In *Comprehensive Organic Synthesis*; Trost, B. M., Fleming, I., Heathcock, C. H., Eds.; 1991; Vol. 2, p 1047.

(13) The *C*-allylation of *N*-acyliminium ions derived from 5-alkoxy or 5-acetoxy proline esters varies with the nature of the Lewis acid, the nucleophile, and the solvent, see Supporting Information (a) Chiesa, M. V.; Manzoni, L.; Scolastico, C. *Synlett* **1996**, 441. (b) Hanessian, S.; Margarita, R.; Hall, A.; Parlanti, L. unpublished results; see also ref 12.

(14) See for example, Cox, C.; Lectka, T. *J. Am. Chem. Soc.* **1998**, *120*, 10660.

(15) For the synthesis of Δ-3 pyrrolines, see Wang, X.; Espinosa, J. F.; Gellman, S. H. *J. Am. Chem. Soc.* **2000**, *122*, 4821; see also ref 4, 18.

(16) Grigg, R.; Markandu, J.; Perrior, T.; Surendrakumar, S.; Warnock, W. J. *Tetrahedron* **1992**, *48*, 6929.

(17) Myers, A. G.; Dragovich, P. S.; Kuo, E. Y. *J. Am. Chem. Soc.* **1992**, *114*, 9369. (b) Weigand, S.; Brückner R. *Synthesis* **1996**, 475.

(18) For the synthesis of Δ-3 pyrrolines by ring-closure metathesis, see: (a) Briot, A.; Bujard, M.; Gouverneur, V.; Nolan, S. P.; Mioskowski, C. *Org. Lett.* **2000**, *2*, 1517. (b) Mori, M.; Sakakibara, N.; Kinoshita, A. *J. Org. Chem.* **1998**, *63*, 6082.

(19) Nicolaou, K. C.; Piscopio, A. D.; Bertinato, P.; Chakraborty, T. K.; Minowa, N.; Koide, K. *Chem. Eur. J.* **1995**, *1*, 318.

(20) Lubineau, A.; Lemoine, R. *Tetrahedron Lett.* **1994**, *35*, 8795. (b) Sanders, W. J.; Manning, D. D.; Koeller, K. M.; Kiessling, L. L. *Tetrahedron* **1997**, *53*, 16391. (c) Martinelli, M.; Vaidyanathan, R.; Pawlak, J. M.; Nayyar, N. K.; Dhokte, U. P.; Doecke, C. W.; Zollars, L. M.; Moher, E. D.; Khau, V. V.; Kosmrlj, B. *J. Am. Chem. Soc.* **2002**, *124*, 3578.

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