J|A|C|S
COMMUNICATIONS

Published on Web 07/21/2007

# Stereocontrolled Synthesis of Dafachronic Acid A, the Ligand for the DAF-12 Nuclear Receptor of *Caenorhabditis elegans*

Simon Giroux and E. J. Corey*

*Department of Chemistry and Chemical Biology, Harvard University, Cambridge, Massachusetts 02138*

Received June 13, 2007; E-mail: corey@chemistry.harvard.edu

The nematode *C. elegans* has become a valuable engine for biological discovery since the early studies of Sydney Brenner,[1] especially for the investigation of developmental and metabolic processes. (The developmental course of each of the 1000 or so somatic cells has already been ascertained.) The progression of *C. elegans* through the various life stages depends on the availability of nutrients. When deprived of food, its metabolism slows and it enters a "dauer" or diapausal state that prolongs its life. Recently, it has been discovered that the loss of function of two genes, daf-2 and daf-9, can extend the life span from 2 weeks to ca. 12 weeks, a finding that attracted even more attention than *C. elegans*' survival of the crash of the Space Shuttle Columbia in 2003.[2] Intensified interest in these genes has led to the discovery that daf-9 codes for the protein (DAF-9) which is a cytochrome P450 enzyme responsible for the biosynthesis of a small molecule that activates another gene, daf-12. Subsequently, Mangelsdorf, Antebi, and their colleagues have deduced a structure for the DAF-12 ligand starting with the hypothesis that it is a sterol that is biosynthesized by DAF-9-mediated oxidation of a precursor sterol.[3,4] Since the natural DAF-12 ligand was only available in trace amounts, insufficient for structural characterization, these workers carried out a series of bioassays of many test sterol derivatives in comparison with the natural ligand. Their findings led them to conclude that a 3-keto group, a $\Delta^7$-olefinic linkage, and a 27-carboxylic function correlated with increased DAF-12 potency and to assign structure **1** to the natural ligand, which they named dafachronic acid.[3,4] We undertook the synthesis of **1** in order to obtain definitive evidence of structure and to make the DAF-12 ligand available for biological investigations, including the study of the genes affected by DAF-12 activation. We describe herein the first synthesis of **1**, for which we propose the slightly modified name dafachronic acid A, since additional members of this hormonal series may emerge.

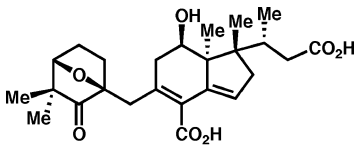
The readily available plant sterol, $\beta$-stigmasterol, was transformed into the known 3,5-cyclosteroid aldehyde **2**[5] by the three-step sequence shown in Scheme 1. Reaction of **2** with the lithium salt of the methyl ester **3**[6] in THF afforded the (*E*,*E*)-diene ester **4** (>20:1 *E*:*Z*) in excellent yield. The (*E*)-$\alpha$,$\beta$-unsaturated acid **5** was prepared from **4** by selective hydrogenation of the $\Delta^{22}$-olefinic linkage followed by saponification (88% from **4**). Further hydrogenation of **5** using $H_2$ and achiral catalysts proceeded non-diastereoselectively to form an inseparable 1:1 mixture of 25-*S* and 25-*R* saturated carboxylic acids.[7] However, homogeneous hydrogenation with 4 mol % of Ru(OAc)$_2$[(*S*)-H$_8$-BINAP] and $H_2$ (1 atm) in MeOH at 50 °C afforded the desired 25-(*S*)-acid **6** with 8:1 diastereoselectivity.[8,9] Recrystallization of this mixture from diisopropyl ether furnished pure **6** (>10:1 by $^{13}$C NMR analysis).[7] Esterification of **6** followed by acetolysis provided the 3$\beta$-acetoxy-$\Delta^5$-steroidal ester **7**. Allylic oxidation of **7** to the $\Delta^5$-7-ketone[10] and catalytic hydrogenation produced the saturated 7-ketone **8**. Reduction of the 7-keto group of **8** and dehydration of the resulting 7$\alpha$-alcohol gave the $\Delta^7$-unsaturated methyl ester **9**, from which dafachronic acid A (**1**) was obtained by the sequence: (1) deacetylation, (2) oxidation of the hydroxyl at C(3), and (3) ester saponification.

We have also developed a second pathway for the stereocontrolled elaboration of the dafachronic A side chain starting from the aldehyde **2** (Scheme 2). Diastereoselective addition (Felkin mode) of vinylmagnesium bromide to **1** in THF at −78 °C followed by trapping of the intermediate alkoxide by propionic anhydride, Et$_3$N, and 4-dimethylaminopyridine afforded selectively the allylic propionate ester **10**. Reaction of **10** with lithium diisopropylamide in THF−HMPA at −78 °C produced an enol silyl ether which without isolation was heated at reflux to effect highly stereoselective Claisen rearrangement.[11] Hydrogenation of the resulting (*E*)-$\beta$,$\gamma$-unsaturated acid **11** ($H_2$, Pd−C, 1 atm, EtOAc) provided the acid **6**, identical in all respects to the product obtained by the route outlined in Scheme 1. Although we have not yet optimized the yields of **6** by the Claisen route via **10** and **11**, it clearly provides a second, viable and completely stereocontrolled route to dafachronic acid A.

Synthetic dafachronic acid A is currently being subjected to detailed biological studies by Drs. Adam Antebi, David Mangelsdorf, and their colleagues. The results of the Antebi laboratory to date show that synthetic **1** can rescue daf-9 mutants at subnanomolar concentrations and is equipotent with the natural DAF-12-ligand. Unfortunately, insufficient natural material is available for spectroscopic comparison at this stage.

The synthesis of **1** reported herein is easily scalable and capable of providing large amounts of this rare nuclear receptor ligand for detailed study since an overall yield of 37% of dafachronic acid from the aldehyde **2** has been reproducibly obtained.

The role of dafachronic acid A in regulating the development of *C. elegans* is reminiscent of the action of the natural product glycinoeclepin A (**12**) on the nematode *Heterodera glycines*, a predator of the soybean plant (and various other beans).[12,13] At concentrations as low as $10^{-12}$ g/mL, glycinoeclepin A, which is produced in and released from the roots of the soybean plant, stimulates the hatching of otherwise dormant eggs of *H. glycines*.



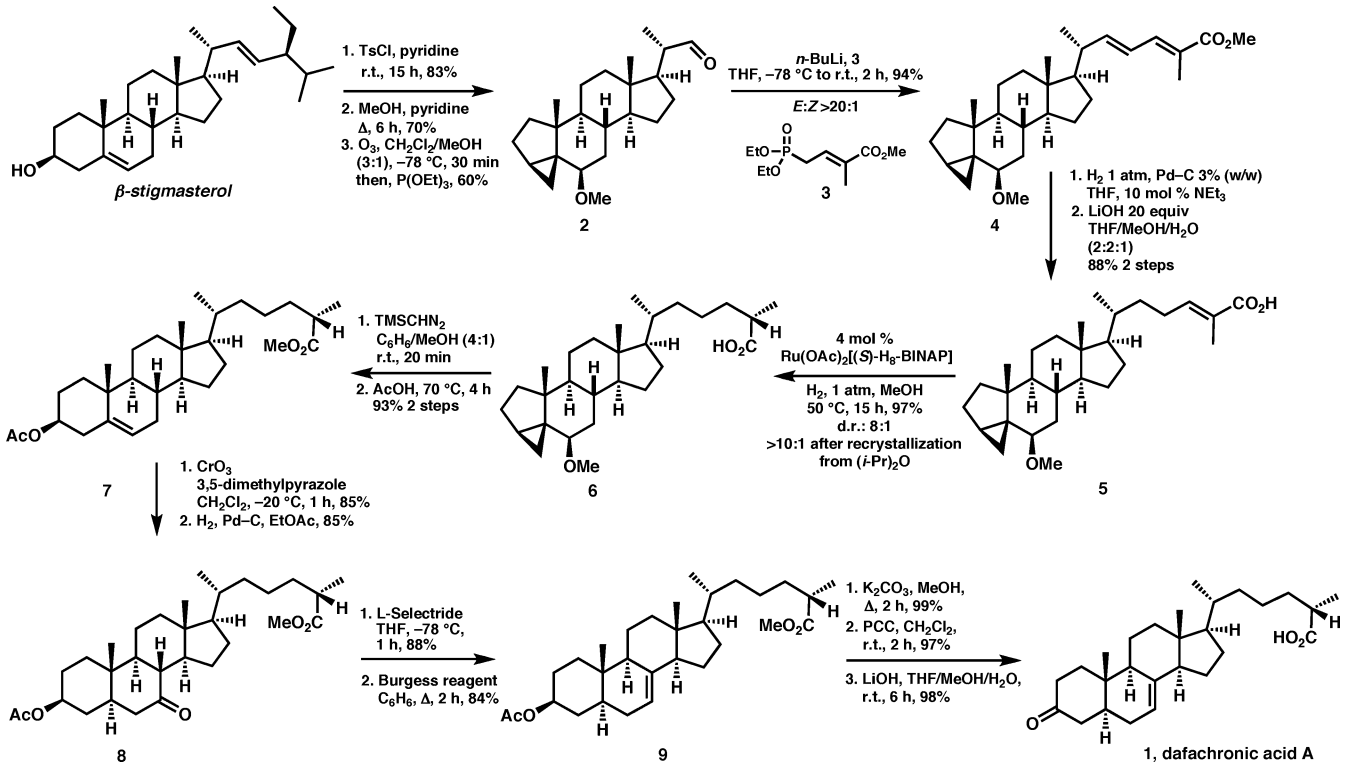

**12**

It is interesting that glycinoeclepin A, a highly oxidized transformation product of the plant triterpene cycloartenol, and dafachronic acid A link nematode development to environmental

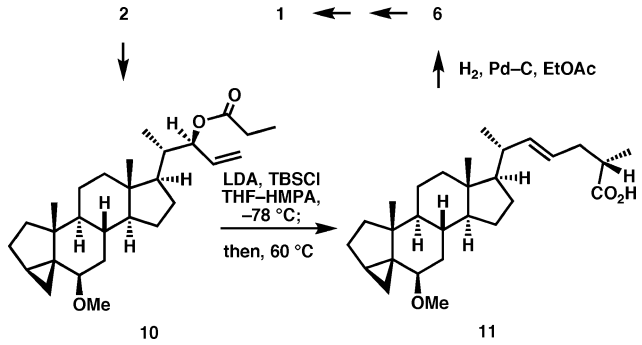


**10.1021/ja074306i CCC: $37.00** 

***Scheme 1***




***Scheme 2***




signals in quite parallel ways. The possibility exists that various other biooxidation products of sterols and triterpenes will be discovered that profoundly influence nematode development through their interaction with nuclear receptors and regulation of genes.

**Acknowledgment.** S.G. is grateful to NSERC for a postdoctoral fellowship. We thank Dr. Adam Antebi for the initial biological results with synthetic **1**.

**Supporting Information Available:** Experimental procedures and characterization data for all reactions and products, including copies of $^{1}H$ and $^{13}C$ NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

## References

(1) Brenner, S. *Nobel Lecture*, http://nobelprize.org/nobel-prizes/medicine/laureates/2002/brenner-lecture.pdf.
(2) (a) Antebi, A. *Science* **2005**, *310*, 1911−1913. (b) Gerisch, B.; Antebi, A. *Development* **2004**, *131*, 1765−1776. (c) Antebi, A. Wormbook; Chalfie, M., Girard, L., Eds. An online resource for *C. elegans*. http://wormbook.org/.
(3) Motola, D. L.; Cummins, C. L.; Rottiers, V.; Sharma, K. K.; Li, T.; Li, Y.; Suino-Powell, K.; Xu, H. E.; Auchus, R. J.; Antebi, A.; Mangelsdorf, D. J. *Cell* **2006**, *124*, 1209−1223.
(4) Gerisch, B.; Rottiers, V.; Li, D.; Motola, D. L.; Cummins, C. L.; Lehrach, H.; Mangelsdorf, D. J.; Antebi, A. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 5014−5019.
(5) (a) Steele, J. A.; Mosettig, E. *J. Org. Chem.* **1963**, *28*, 571−572. (b) Salmond, W. G.; Sobala, M. C. *Tetrahedron Lett.* **1977**, *18*, 1695−1698.
(6) Kitahara, T.; Horiguchi, A.; Mori, K. *Tetrahedron* **1988**, *44*, 4713−4720.
(7) The ratio of C(25) diastereomers was determined by $^{13}C$ NMR analysis using the distinctly separate peaks for the C(25)−Me.
(8) For precedent, see: (a) Ohta, T.; Takaya, H.; Kitamura, M.; Nagai, K.; Noyori, R. *J. Org. Chem.* **1987**, *52*, 3174−3176. (b) Eumura, T.; Zhang, X.; Matsumura, K.; Sayo, N.; Kumobayashi, H.; Ohta, T.; Nozaki, K.; Takaya, H. *J. Org. Chem.* **1996**, *61*, 5510−5516.
(9) The Ru catalyst is available from either the Takasago Co. or Strem Chemicals.
(10) (a) Salmond, W. G.; Barta, M. A.; Havens, J. L. *J. Org. Chem.* **1978**, *43*, 2057−2059. (b) Corey, E. J.; Fleet, G. W. J. *Tetrahedron Lett.* **1973**, *14*, 4425−4428.
(11) The stereochemistry of the Claisen rearrangement follows the course expected for the (*E*)-silyl enol ether rearranging via a chair-formed transition state. See: Ziegler, F. E. *Chem. Rev.* **1988**, *88*, 1423−1452.
(12) (a) Fukuzawa, A.; Furusaki, A.; Ikura, M.; Masamune, T. *J. Chem. Soc., Chem. Commun.* **1985**, 221−222, 748. (b) Masamune, T.; Anetai, M.; Takasugi, M.; Katsui, N. *Nature* **1982**, *297*, 495−496.
(13) Corey, E. J.; Houpis, I. N. *J. Am. Chem. Soc.* **1990**, *112*, 8997−8998.

JA074306I