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COMMUNICATIONS

Published on Web 02/16/2006

# Enantioselective Synthesis of Pentacycloanammoxic Acid

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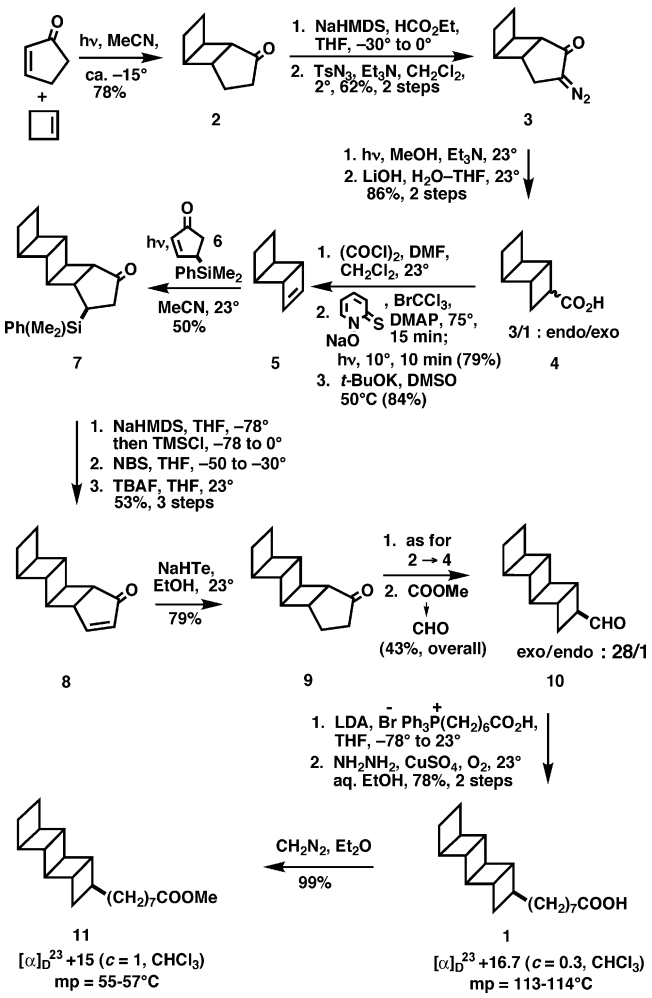
The anaerobic microbe *Candidatus Brocadia anammoxidans* utilizes nitrite ion and ammonia as an energy source (by conversion to $N_2$ and $H_2O$) in a compartment that is enclosed in an unusually dense membrane. The principal lipid components of the membrane (ca. 90%) are derivatives of a highly unusual $C_{20}$ fatty acid, **1** (Scheme 1), or mirror image.[1,2] It has been found that this rigid

***Scheme 1*** [a]




[a] Temperatures in °C.

lipid forms a tight membrane which is thought to protect the organism from the toxic intermediates (e.g., $HONH_2$ and $H_2NNH_2$) involved in the energy-yielding production of $N_2$ and $H_2O$. The unusual ladderane structure of **1** and also its scarcity (because of difficulties in culturing *C. B. anammoxidans* and admixture with many other lipids) pose a challenge to synthetic chemistry. There are no clues as to possible synthetic pathways to **1** since the mode of biosynthesis of this very strained molecule is quite mysterious. As has been pointed out recently, the pentacyclic ladderane core of **1** is unstable relative to two molecules of (*E*)-1,3,5-hexatriene as reference by nearly 20 kcal/mol ($\Delta\Delta H_f$).[3] We estimate from data in the literature[4] that the strain energy of **1** is, at a minimum, ca. 75 kcal/mol, approximately 3 times that of cyclobutane. The first synthesis of **1** in racemic form, which was reported recently from this laboratory,[3] confirmed the assignment of the structure. The absolute configuration of naturally produced **1** is still unknown. An indication of the difficulty of culturing *C. B. anammoxidans* comes from the fact that insufficient **1** is available even for the determination of optical rotation or absolute configuration.[5] The scarcity of naturally produced **1** and the fundamental questions regarding its absolute configuration and pathway of biosynthesis provided the motivation to develop a new synthesis that was (1) enantioselective, (2) unambiguous with regard to absolute configuration, and (3) capable of producing as much **1** as required for biochemical and biophysical investigations. The pathway of that synthesis is summarized in Scheme 1.

Irradiation of a mixture of cyclobutene and 2-cyclopentenone (2 equiv) in dry $CH_3CN$ with a medium pressure mercury lamp (Hanovia, 450 W) at −30 to −5 °C for 20−24 h produced, after removal of solvent and flash chromatography on silica gel (sg), the *exo* 1:1-adduct **2** in 78% yield (scale: 30 g of **2**). The tricyclic ketone **2** was transformed into the α-diazoketone **3** by α-formylation of the sodio enolate of **2** in tetrahydrofuran and Regitz diazo transfer by reaction of the resulting α-hydroxymethylene derivative with 4-toluenesulfonyl azide ($TsN_3$) in $CH_2Cl_2$. Irradiation of a methanolic solution of **3** (Hanovia, 450 W ultraviolet lamp) at 23 °C produced a 3:1 mixture of *endo* and *exo* methyl esters, which were saponified to the corresponding acids, **4**. The acids **4** were converted via the corresponding acid chlorides to *N*-hydroxy-2-thiopyridone (Barton) esters, which without isolation were irradiated in $BrCCl_3$ at 10 °C for 10 min to effect replacement of the COOH group by bromine by a radical-chain process. The resulting cyclobutyl bromide derivative was transformed into the tricyclic olefin **5** by dehydrobromination at 50 °C with KO*t*-Bu in dimethyl sulfoxide, under reduced pressure (9−10 mm Hg) to transfer **5** from the reaction mixture to a −78 °C cold trap as formed.

Ultraviolet irradiation (Hanovia 450 W lamp) of a solution of the tricyclic olefin **5** with (*R*)-4-dimethylphenylsilyl-2-cyclopentenone (**6**) (molar ratio 1.25:1) in $CH_3CN$ solution under $N_2$ at 25 °C for 12 h produced after sg chromatography (92:8 hexanes:EtOAc) 50% yield of the *exo*-silyl adduct **7** along with ca. 7% of the *endo*-silyl diastereomer. (A simple synthesis of **6** is outlined in Scheme 2.) Treatment of **7** with 2 equiv of sodium hexamethyldisilazane in THF at −78 °C followed by 4 equiv of $Me_3SiCl$ (TMSCl) generated the silyl enol ether which upon exposure to *N*-bromosuccinimide in THF at −30 to −50 °C gave the α-bromo derivative of the pentacyclic ketone **7**. Reaction of this α-bromo-*β*-silyl ketone with tetra-*n*-butylammonium fluoride (TBAF) in THF at 23 °C provided, after sg column chromatography, the crystalline pentacyclic α,*β*-enone **8**, which could be reduced to the saturated ketone **9** using sodium hydrogen telluride in EtOH at 23 °C.[7] The structure of **8** was confirmed by single-crystal X-ray diffraction


10.1021/ja058370g CCC: $33.50 

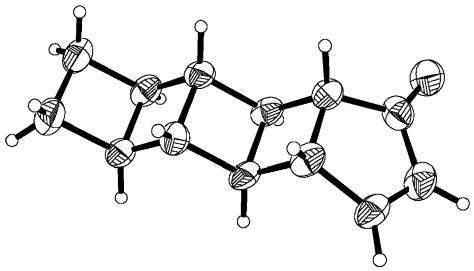
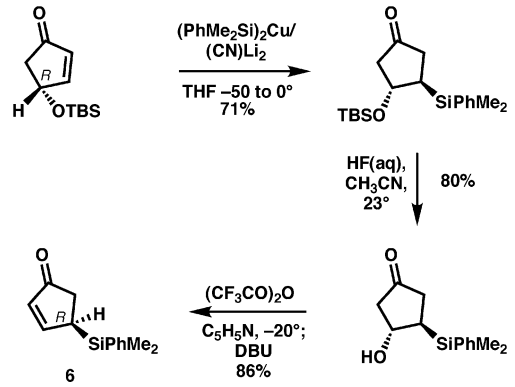


***Figure 1.*** ORTEP representation of the X-ray structure of **8**.

***Scheme 2***




analysis (see Figure 1). The application of the octant rule to ketone **9**, $[\alpha]^{23}_D$ +407 ($c$ = 0.35, $CHCl_3$), allows unambiguous assignment of the absolute configuration shown, which is that expected from the known absolute configuration of α,β-enone **6** that led to the *exo*-silyl photoadduct **7**.

Racemic **9** was readily prepared by photoaddition of 2-cyclopentenone to the achiral tricyclic olefin **5**. The (+)- and (−)-enantiomers of **9** were obtained from this racemic mixture by HPLC separation on a CHIRALPAK AD column (Chiral Technologies, Inc.).[8]

The (+)-ketone **9** was converted to the *exo* aldehyde **10** by the following sequence: (1) α-diazoketone formation by the Regitz method (as above for **2** → **3**), (2) photoinduced Wolff ring contraction in methanol to form the pentacyclic ladderane methyl esters (*exo* + *endo*), (3) *i*-$Bu_2AlH$ reduction−Swern oxidation sequence[3] to a mixture of the corresponding *exo*−*endo* aldehyde mixture, and (4) equilibration of the mixture to the *exo* aldehyde **10** (as a 28:1 *exo*−*endo* mixture) using a 0.06 M solution in $Et_3N$ at 23 °C for 6 days (80% yield for isomerization; 43% overall). The chiral *exo* aldehyde **10** was then transformed into the chiral acid **1** by a combination Wittig reaction−diimide reduction process as previously described for (±)-pentacycloanammoxic acid.[3] Esterification of **3** afforded the chiral methyl ester **11**. Both chiral **1** and **11** made from the (+)-ketone **9** were dextrorotatory. We are currently awaiting a reference sample of naturally produced pentacycloanammoxic acid to establish its absolute configuration.

The synthesis outlined in Scheme 1 was greatly facilitated by the development of a convenient and practical process for preparing cyclobutene on a molar scale in laboratory glassware. The starting material was cyclopropyl carbinol, a compound that has been prepared industrially by the sequence 1,3-butadiene monoepoxide → 2,3-dihydrofuran → cyclopropanecarboxaldehyde (Δ, $Al_2O_3$) → cyclopropyl carbinol ($NaBH_4$).[9] Cyclopropyl carbinol was converted to the corresponding mesylate ($CH_3SO_2Cl$, $Et_3N$, $CH_2Cl_2$, −20 to 0 °C, 97−99% yield). Treatment of the mesylate with 0.06 equiv of $BF_3{\cdot}Et_2O$ in $CH_2Cl_2$ at 22 °C for 12 h gave in quantitative yield a mixture of cyclobutyl mesylate and but-3-enyl mesylate (ratio ca. 11:1). The latter was removed from the mixture by oxidation with $KMnO_4$ in aqueous acetone to provide, after extractive workup, cyclobutyl mesylate in 81% overall yield. Heating cyclobutyl mesylate with KO*t*-Bu in DMSO at 65 °C[10] provided a distillate of pure cyclobutene (60%).

The synthesis of the (*R*)-α,β-enone **6** started with readily available (*R*)-4-*tert*-butyldimethylsilyloxy-2-cyclopentenone[11] (Scheme 2) in three steps: (1) conjugate addition under steric control of the 2:1 dimethylphenylsilyllithium:CuCN reagent, (2) desilylation, and (3) dehydration.

These studies are being continued to gain further information on the absolute configuration and biosynthesis of **1**. $^1H$ NMR studies on the thermal stability of the methyl ester of **1** in deuterated chlorobenzene have revealed a half-life of only ca. 1 h at 140 °C. From this result, it is clear despite the uniqueness of the anammoxic lipid it may not have left a signature in geological sediments.

With regard to the question of the biosynthesis of **1**, the perspectives of synthetic chemistry may prove helpful. Although the original synthesis of (±)-**1**[3] and the new synthesis outlined herein have relied heavily on photochemical reactions, it is doubtful that photochemical processes are involved in the biosynthesis of **1** since the environment of *C. B. anammoxidans* is dark and anaerobic. If the biosynthesis were to occur by a cascade-type polycyclization, it would have to be novel in terms of the chemistry used because of the unfavorable energetics and the paucity of the known chemical reactions of this type. One possible candidate as substrate for such a cascade polycyclization pathway would be the allenic $C_{20}$ fatty acid 9,10,12,16,18,19-docosahexaenoic acid.[12] In any case, unraveling the biosynthetic mechanism is fully as challenging as the chemical synthesis.

Finally, it should be noted that the highly selective photoreaction **5** + **6** → **7** represents a useful and general solution to the long-standing problem of creating an enantioselective version of [2 + 2]-photocycloaddition. The use of the bulky silyl group in **6** was essential to success; TBSO was ineffective.

**Supporting Information Available:** Experimental procedures and characterization data for the process shown in Schemes 1 and 2 (PDF). X-ray crystallographic date for **8** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(5) Personal communication from Dr. Jaap Damsté.

(6) For a small-scale preparation of the 1,5-diene corresponding to **5**, see: Avram, M.; Dinulescu, I. G.; Marica, E.; Mateescu, G.; Sliam, E.; Nenitzescu, C. D. *Chem. Ber.* **1964**, *97*, 382−389. A search of the literature revealed no reliable alternative route for the synthesis of **5**.

(7) For method, see: Yamashito, M.; Kato, Y.; Suemitsu, R. *Chem. Lett.* **1980**, 847−848.

(8) The separation was conducted with a preparative CHIRALPAK AD column using 99.5:0.5 hexanes:*i*-PrOH at 23 °C with a flow rate of 5 mL/min and UV detection at 306 nm. The retention time for (+)-**9** was 46 min, 52 s and that for the enantiomer was 66 min, 17 s.

(9) Liang, S.; Price, T. W. U.S. Patent 5,633,410, May 27, 1997 to Eastman Chemical Co.

(10) See: Salaün, J.; Fadel, A. *Organic Syntheses*; Wiley & Sons: New York, 1990; Collect. Vol. VII, pp 117−120.

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(12) A reductive polycyclization of this allenic substrate to **1** (with addition of 2 H) would be thermodynamically favorable.


JA058370G