ORGANIC LETTERS

2000
Vol. 2, No. 19
3019−3022

One-Step Construction of the Pentacyclic Skeleton of Saframycin A from a "Trimer" of α-Amino Aldehydes

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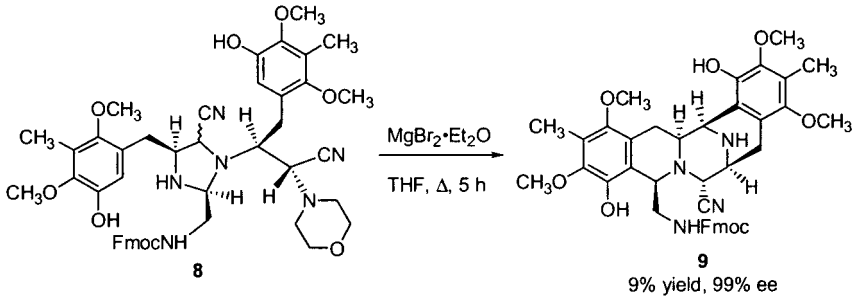
Received July 17, 2000

ABSTRACT



The entire skeleton of the saframycin antitumor antibiotics is assembled in one remarkable transformation (8 → 9) from an *N*-linked oligomer of three α-amino aldehyde components, a reaction pathway that may parallel the biosynthetic route to the saframycins.

Biosynthetic studies have shown that the potent antitumor alkaloid saframycin A (**1**) can be assembled from glycine, alanine, and two molecules of tyrosine.[1,2] Five methionine-derived methyl groups and exogenous hydrogen cyanide provide all remaining carbon atoms. The path that connects these precursors to the pentacyclic skeleton of **1** is not known, nor has any detailed proposal been presented. Gross features of the connectivity have been established on the basis of isotopic incorporation studies (Figure 1).[1] When the α-amino acid precursors are mapped onto structure **1** it is evident that the carboxyl groups of the glycine residue and both tyrosine residues have undergone reduction during biosynthesis and rest in the final product at an oxidation state equivalent to that of an aldehyde group (indicated by the open circles in structure **1**).[3] Researchers at Ciba-Geigy have recently isolated and cloned the genes involved in the biosynthesis of the closely related antibiotic saframycin Mx1.[4] On the basis of the DNA sequence analysis it was proposed that the genes isolated code for two multifunctional (nonribosomal) peptide synthetases and a third protein with *O*-methyl transferase activity. Each synthetase was proposed to contain two amino acid activating domains and, unprecedented among nonribosomal peptide synthetases, a putative reductase activity. It was proposed that saframycin A is derived from an oligopeptide precursor, but the stepwise process by which this might occur was not defined.[4]

We recently developed a short (eight-step) and enantioselective synthetic route to **1** by the directed condensation of compounds **2** and **3** (*N*- and *C*-protected versions,

(1) (a) Mikami, Y.; Takahashi, K.; Yazawa, K.; Arai, T.; Namikoshi, M.; Iwasaki, S.; Okuda, S. *J. Biol. Chem.* **1985**, *260*, 344−348. (b) Arai, T.; Yazawa, K.; Takahashi, K.; Maeda, A.; Mikami, Y. *Antimicrob. Agents Chemother.* **1985**, *28*, 5−11.

(2) Reviews of the saframycins: (a) Arai, T.; Kubo, A. In *The Alkaloids*; Brossi, A., Ed.; Academic Press: New York, 1983; Vol. 21, Chapter 3. (b) Remers, W. A. In *The Chemistry of Antitumor Antibiotics*; Wiley-Interscience: New York, 1988; Vol. 2, Chapter 3.

(3) This assignment of oxidation states is made from the perspective of synthetic equivalency, where the cyano and benzoquinone substituents are treated as heteroatom equivalents.

(4) (a) Pospiech, A.; Cluzel, B.; Bietenhader, J.; Schupp, T. *Microbiology* **1995**, *141*, 1793−1803. (b) Pospiech, A.; Bietenhader, J.; Schupp, T. *Microbiology* **1996**, *142*, 741−746.

10.1021/ol0063398 CCC: $19.00
Published on Web 08/23/2000

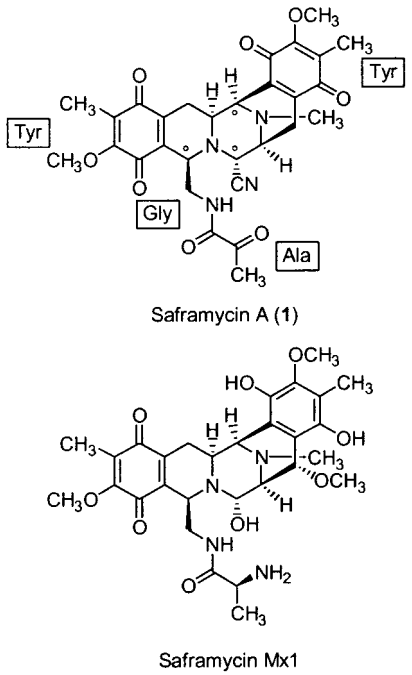


Figure 1. Saframycins A and Mx1, identification of amino acid precursors and acid to aldehyde oxidation state changes (°).

respectively, of the same complex α-amino aldehyde), and *N*-Fmoc glycinal (**4**, see Figure 2).[5,6] Over the course of five

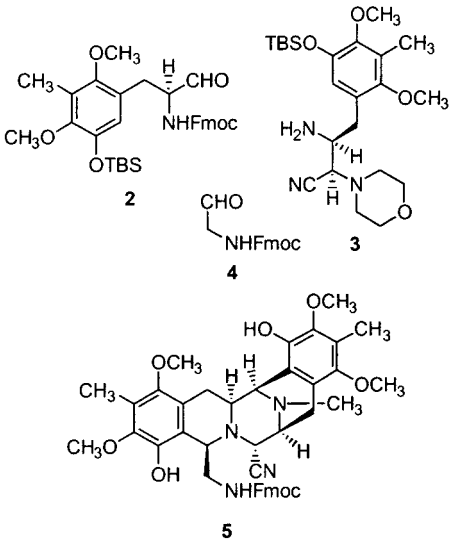


Figure 2. Precursors in the synthesis of saframycin A (**1**).

steps these components were linked in stepwise fashion, in a sequence involving two Pictet−Spengler cyclization reactions and an intramolecular Strecker reaction, to form the pentacyclic saframycin A precursor **5**.[5] Here, we show that the entire saframycin skeleton can be assembled in one remarkable transformation from an *N*-linked oligomer of the three α-amino aldehyde components **2**, **3**, and **4**, a reaction that suggests for the first time a viable pathway linking **1** with an oligopeptide precursor and, therefore, a possible biosynthetic route.

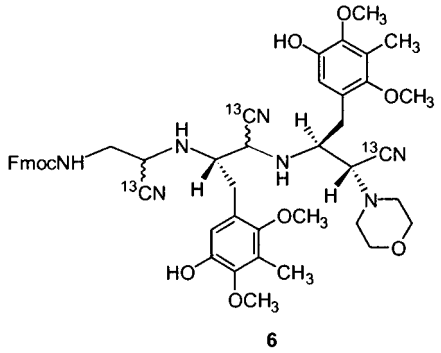


Figure 3. A proposed trimeric α-amino aldehyde precursor to **1**.

The specific oligomer that was targeted initially was the trimeric amino nitrile **6**, in which **2**, **3**, and **4** are linked by sequential Strecker reactions. The amino nitrile groups serve to covalently join the three α-amino aldehyde components and were proposed to function later as precursors to electrophilic imine or iminium intermediates that would mediate the three cyclization reactions leading to the saframycin skeleton.[7] We had previously demonstrated that α-amino aldehydes can be coupled using the Strecker reaction without epimerization of the α-stereocenter.[8] Because amino nitrile formation was anticipated to form two diastereomeric products in each case (of no consequence in later C−C bond-forming reactions), we elected to use ^{13}C-labeled cyanide in the synthesis to facilitate ^{13}C-NMR analysis of the products. Also, we began the sequence with a single diastereomer of the *C*-protected α-amino aldehyde component **3**, bearing a ^{13}C-label on the cyano group.

The order of introduction of α-amino aldehyde components was **3** + **2**, and then **4**, representing *C*- to *N*-terminus directionality in the synthesis. Mixing **3** (1 equiv, 92% ee,

(5) Myers, A. G.; Kung, D. W. *J. Am. Chem. Soc.* **1999**, *121*, 10828−10829.

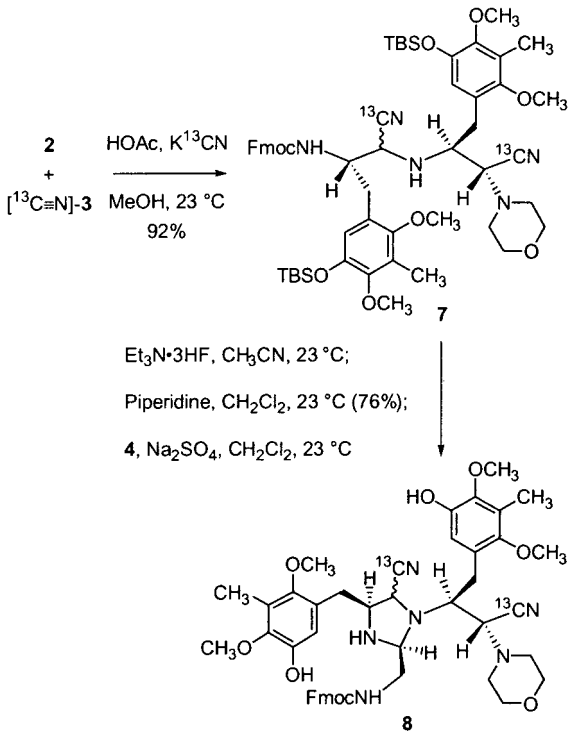
(6) Other syntheses of saframycins: (±)-saframycin B: (a) Fukuyama, T.; Sachleben, R. A. *J. Am. Chem. Soc.* **1982**, *104*, 4957−4958. (b) Kubo, A.; Saito, N.; Yamato, H.; Masubuchi, K.; Nakamura, M. *J. Org. Chem.* **1988**, *53*, 4295−4310. (±)-saframcyin A: (c) Fukuyama, T.; Yang, L.; Ajeck, K. L.; Sachleben, R. A. *J. Am. Chem. Soc.* **1990**, *112*, 3712−3713. (−)-saframycin A: (d) Martinez, E. J.; Corey, E. J. *Org. Lett.* **1999**, *1*, 75−77. See also: (e) Zhou, B.; Edmondson, S.; Padron, J.; Danishefsky, S. J. *Tetrahedron Lett.* **2000**, *41*, 2039−2042. (f) Zhou, B.; Guo, J.; Danishefsky, S. J. *Tetrahedron Lett.* **2000**, *41*, 2043−2046.

(7) For selected examples of the use of amino nitriles as imine/iminium ion precursors in biomimetic systems, see: (a) Overman, L. E.; Jacobsen, E. J. *Tetrahedron Lett.* **1982**, *23*, 2741−2744. (b) Ksander, G.; Bold, G.; Lattman, R.; Lehmann, C.; Fruh, T.; Xiang, Y.-B.; Inomata, K.; Buser, H.-P.; Schreiber, J.; Zass, E.; Eschenmoser, A. *Helv. Chim. Acta* **1987**, *70*, 1115−1172. (c) Bonin, M.; Grierson, D. S.; Royer, J.; Husson, H.-P. *Org. Synth.* **1992**, *70*, 54−59.

(8) Myers, A. G.; Kung, D. W.; Zhong, B.; Movassaghi, M.; Kwon, S. *J. Am. Chem. Soc.* **1999**, *121*, 8401−8402.

^{13}C-labeled cyano group) and its *N*-protected α-amino aldehyde counterpart **2** (1.05 equiv, 96% ee) in dichloromethane with suspended sodium sulfate led to formation of the corresponding imine, cleanly and without α-epimerization, as previously demonstrated.[5] In this instance, however, the imine was captured by Strecker reaction with hydrogen cyanide in methanol at 23 °C (1.6 equiv of acetic acid, 1.5 equiv of K^{13}CN, Scheme 1), whereas in the prior route the imine was cyclized by warming (35 °C) in the presence of lithium bromide.[5] The expected α-amino nitriles **7** (1.1:1 mixture of diastereomers) were obtained in 92% yield after isolation by flash column chromatography. Sequential removal of the silyl ethers (triethylamine trihydrofluoride, 2.5 equiv, CH_3CN, 23 °C) and the *N*-Fmoc group (30% piperidine−CH_2Cl_2, 23 °C, 76%, two steps) of **7** afforded the fully deprotected "dimer" for coupling with the third component, *N*-Fmoc glycinal (**4**). Attempted Strecker coupling of these components was complicated by internal cyclization of the glycinaldimine intermediate. Recognizing that such a process provided an aminal product that was functionally equivalent to the trimeric α-amino nitrile originally targeted, the condensation reaction was optimized to form this product (compound **8**, Scheme 1). Thus, addition of **4** (1.1 equiv) to a solution of the deprotected dimeric α-amino aldehyde (1 equiv) in dichloromethane at 23 °C led to smooth condensation in the absence of hydrogen cyanide to afford a product formulated as the cyclic aminals **8**. These products were not stable to chromatography on silica gel, but ^{1}H- and ^{13}C-NMR analysis showed that they had been formed cleanly (~90% combined yield). Only two diastereomers were detected spectroscopically, and these were present in the same ratio as the starting material **7**, suggesting that the cyclic aminal had been formed with a single stereochemistry, tentatively assigned as shown in Scheme 1.

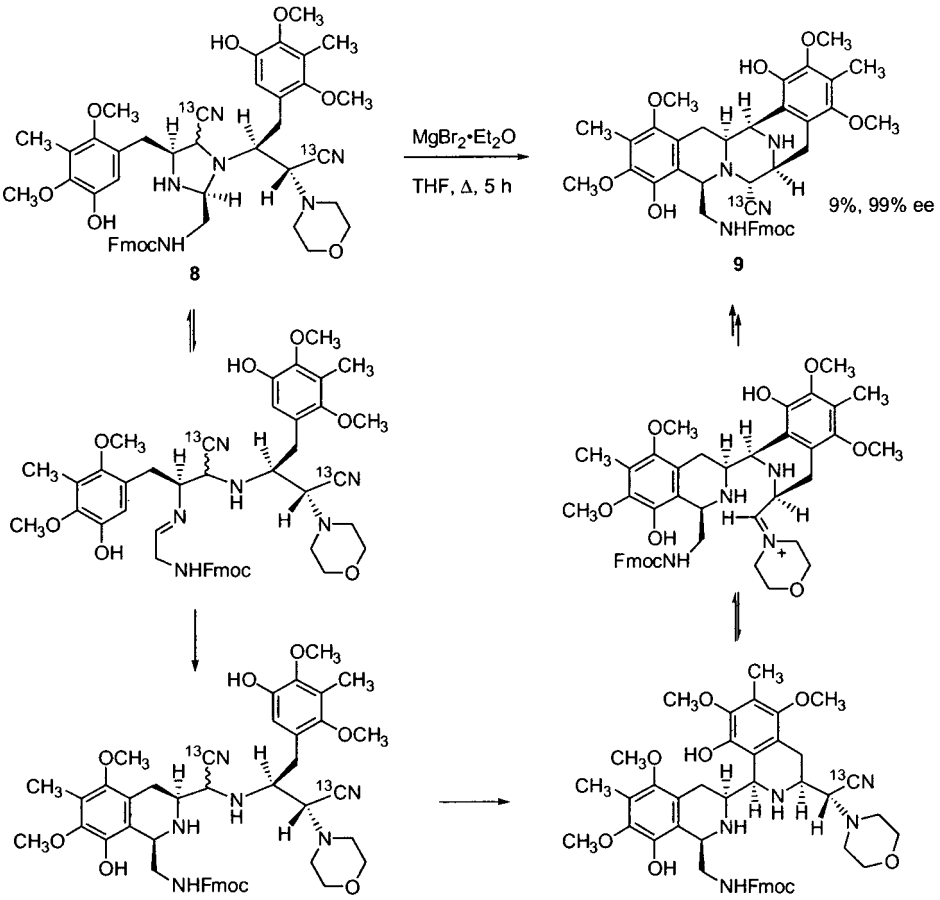
Scheme 1. Synthesis of an *N*-Linked Trimeric α-Amino Aldehyde Precursor to the Saframycin Skeleton



Several experiments were conducted to investigate the proposed rearrangement of **8** to the pentacyclic skeleton of the saframycins (**9**). Treatment of **8** with Lewis acid catalysts typically led to highly complex and difficultly characterized reaction mixtures, an outcome that was not surprising given the many reaction manifolds potentially available to this tris α-amino aldehyde equivalent. However, by sequential treatment of **8** with the Lewis acids lithium bromide (dimethoxyethane, reflux) and then zinc chloride (trifluoroethanol−THF, 23 °C), and assisted by the fact that **8** was fortuitously well separated chromatographically from all other reaction products, it was possible to isolate the desired pentacyclic saframycin A precursor **9** from the reaction mixture in pure form (4%). With further experimentation, conditions were found to bring about the transformation of **8** to **9** in one step and in higher yield (Scheme 2); heating a solution of **8** in tetrahydrofuran at reflux in the presence of magnesium bromide etherate (20 equiv) afforded **9** in 8.4 and 9.0% yield in two separate experiments. Importantly, *N*-acylation of **9** with the enantiomeric Mosher acid chlorides followed by HPLC analysis of the amide products established that **9** had been formed without racemization (**9** was of 99% ee). *N*-Methylation of **9** with formalin and sodium triacetoxyborohydride in acetonitrile afforded the pentacyclic saframycin A precursor **5**, identical with an authentic sample prepared by the earlier synthetic route (^{1}H NMR, IR, TLC, and HPLC analysis), except for the anticipated spectroscopic differences attributed to the ^{13}C-label. Intermediate **5** can be transformed into saframycin A in three steps (50% yield).[5]

The one-step conversion of the *N*-linked oligomer **8** to the pentacyclic intermediate **9** involves an exceptional number of individual steps. Three cyclization reactions occur, and three of the five stereocenters of saframycin A are established in this step. In theory, each of the five stereogenic centers of the precursor **5** is epimerizable under the reaction conditions. A single epimerization event may divert the course of reaction from **9**. In that product which is formed, the α-amino aldehyde-derived α-centers are preserved. Many viable sequences can be envisioned to transform **8** into **9**; the pathway shown in Scheme 2 is proposed as that which actually occurs. In background studies, we have found that aminals have a greater propensity to form imine or iminium ion intermediates under mildly acidic conditions than secondary amino nitriles which, in turn, are more labile than tertiary amino nitriles.[8] For this reason, we propose that cleavage of the aminal occurs first, followed by trapping of the resultant imine by Pictet−Spengler cyclization, as depicted in Scheme 2. Subsequent ionization of the secondary amino nitrile is proposed to initiate a second Pictet−Spengler cyclization. Finally, ionization of the tertiary amino nitrile group leads to internal Strecker reaction to form the pentacyclic product **9**. It is interesting to note that the ordering of the two Pictet−Spengler reactions in this

Scheme 2. Proposed Pathway for the Transformation of the *N*-Linked Oligomer **8** to the Pentacyclic Saframycin A Precursor **9**



proposed sequence is opposite to that of our earlier stepwise condensation route.[5] Both Pictet−Spengler cyclizations are believed to proceed with cis selectivity, as observed in the earlier stepwise route, but stereochemical ratios cannot be assigned in this experiment.

Given the number of steps involved and the many alternative reaction pathways available to the intermediates of Scheme 2, the transformation of **8** to **9** is remarkable. Although the efficiency of the sequence does not rival that of our earlier route (it is, however, one step shorter), the primary significance of the transformation is not as a process improvement, rather, it is the demonstration that an oligo α-amino aldehyde is a viable synthetic precursor to **1**, even in the absence of an enzymic catalyst. In light of the implication that **1** is biosynthesized from an oligopeptide precursor,[4] the transformation of **8** to **9** is particularly significant, for it validates the connectivity implicit in such a proposal. Moreover, it provides the first detailed stepwise pathway that has been proposed to link an oligopeptide with **1**. Although many alternative sequences of reduction and cyclization reactions can be imagined, the idea that the three carboxylate carbons of the oligopeptide precursor are reduced prior to any cyclization event is now clearly shown to be one viable pathway, inasmuch as spontaneous cyclization to the saframycin skeleton has been demonstrated.

Acknowledgment. Financial support from the National Institutes of Health is gratefully acknowledged. D.W.K. is grateful to the National Science Foundation for predoctoral fellowship support.

OL0063398