ORGANIC LETTERS

2009
Vol. 11, No. 22
5298−5301

Total Synthesis of the Putative Structure of Lucentamycin A

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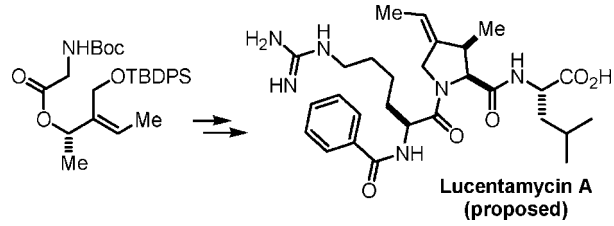
Received September 29, 2009

ABSTRACT



A rapid and stereoselective enolate-Claisen rearrangement provides access to the 4-ethylidene-3-methylproline (Emp) subunit of lucentamycin A. Synthesis of the putative structure of the cytotoxic natural product suggests the need for structural revision.

Marine-derived bacteria are a prolific source of bioactive natural products featuring complex amino acids.[1] In 2007, Fenical and co-workers reported the isolation of lucentamycins A−D, a new family of cytotoxic metabolites from the fermentation broth of *Nocardiopsis lucentensis* (Figure 1).[2] Structural elucidation based on extensive NMR and degradation studies revealed unique tripeptides composed of an N-acylated homoarginine (Har), a C-terminal leucine or tryptophan, and a central 4-ethylidene-3-methylproline (Emp) residue unprecedented in the natural product literature. Of the four closely related structures, lucentamycin A showed potent *in vitro* cytotoxicity against human colon carcinoma cells (HCT-116 IC_{50} = 0.20 μM), and lucentamycin B exibited weaker activity against the same cell line (IC_{50} = 11 μM).

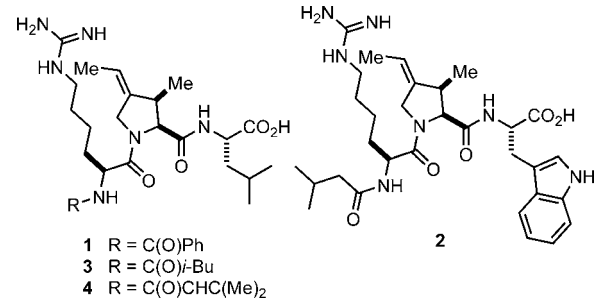


Figure 1. Proposed structures of lucentamycins A (**1**), B (**2**), C (**3**), and D (**4**).

As the first step in our investigation of lucentamycin structure−activity relationships, we targeted **1** for synthesis and sought to devise a versatile strategy for construction of the 4-alkylidene-3-alkylproline subunit common to this family. We were intrigued to find that a derivative of the

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(1) For selected reviews, see: (a) Mauger, A. B. *J. Nat. Prod.* **1996**, *59*, 1205–1211. (b) Fenical, W.; Jensen, P. R. *Nat. Chem. Biol.* **2006**, *2*, 666–673. (c) Jones, A. C.; Gu, L.; Sorrels, C. M.; Sherman, D. H.; Gerwick, W. H. *Curr. Opin. Chem. Biol.* **2009**, *13*, 216–223. (d) Moore, R. E. *J. Indust. Microbiol. Biotechnol.* **1996**, *16*, 134–143. (e) Burja, A. M.; Banaigs, B.; Abou-Mansour, E.; Grant Burgess, J.; Wright, P. C. *Tetrahedron* **2001**, *57*, 9347–9377. (f) Luesch, H.; Harrigan, G. G.; Goetz, G.; Horgen, F. D. *Curr. Med. Chem.* **2002**, *9*, 1791. (g) Ersmark, K.; Del Valle, J. R.; Hanessian, S. *Angew. Chem., Int. Ed.* **2008**, *47*, 1202–1223.

(2) Cho, J. Y.; Williams, P. G.; Kwon, H. C.; Jensen, P. R.; Fenical, W. *J. Nat. Prod.* **2007**, *70*, 1321–1328.

10.1021/ol902251c CCC: $40.75
Published on Web 10/28/2009

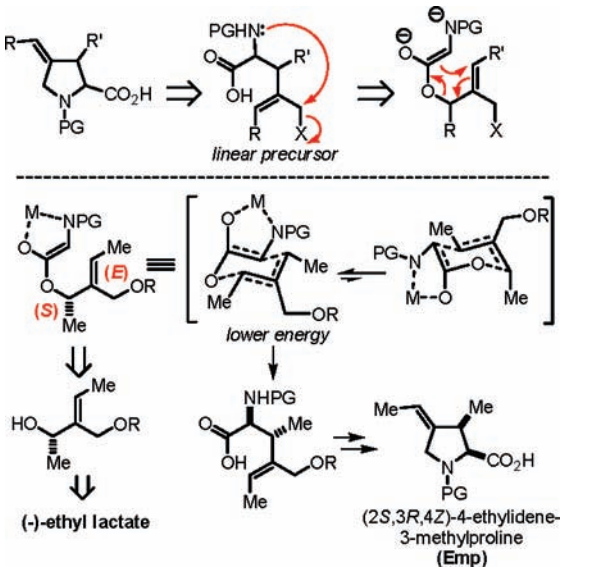


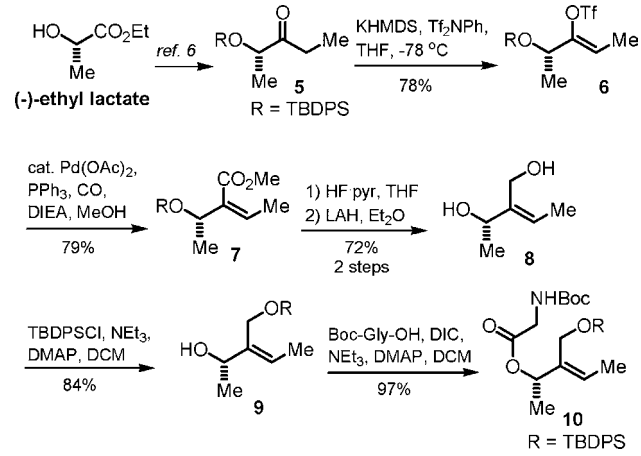
Figure 2. Retrosynthesis of Emp and proposed [3,3] rearrangement transition state.

Emp residue had been prepared by Gais and co-workers as part of a study on the synthetic utility of chiral vinyl aminosulfoxonium salts.[3] Our interest in chimeric prolines prompted us to explore an alternate route employing lactic acid as a chiral progenitor and a stereoselective enolate-Claisen rearrangement as the key step. Here, we report the first total synthesis of **1**, which also calls into question the proposed structure of lucentamycin A.[4]

We envisioned a general approach toward 3,4-disubstituted prolines by way of the linear precursor shown in Figure 2. In turn, this polysubstituted allylglycine substrate could be efficiently accessed via a stereoselective [3,3] sigmatropic rearrangement. On the basis of chair transition state models proposed for similar enolate-Claisen rearrangements,[5] it became apparent that the appropriate allyl ester substrate should possess an *S* configuration and *E* alkene geometry to afford the desired 2*S*,3*R*,4*Z* Emp configuration. The structure of the requisite chiral alcohol was retrosynthetically traced back to (−)-ethyl lactate as a readily available and inexpensive starting material.

Synthesis of the key allyl ester commenced with ketone **5**, conveniently obtained on large scale over three steps according to precedent.[6] Trapping of the potassium enolate

Scheme 1. Synthesis of Glycine Ester **10**



of **5** with *N*-phenyltriflimide afforded the *Z* isomer of vinyl triflate **6** as the major product in 78% yield. Subsequent Heck carboxymethylation in DMF/MeOH led to significant erosion of alkene isomeric purity. We found that the integrity of the *Z* alkene could be largely preserved by using MeOH as the sole solvent. Thus, treatment of **6** with $Pd(OAc)_2$, PPh_3, DIEA, and MeOH under a CO atmosphere gave the expected enoates in a 13:1 ratio and 87% overall yield.[7] After separation by column chromatography, we isolated pure **7** in 79% yield and proceeded with HF-mediated cleavage of the silyl ether and ester reduction to provide allylic diol **8**. Selective TBDPS-protection of the primary alcohol was then followed by condensation with *N*-Boc-glycine to give rearrangement substrate **10**.

We had initially planned to employ Kazmeier's chelation conditions[8] for the ensuing [3,3] sigmatropic shift, as model substrates afforded good yields in our hands. However, we were unable to find precendent for a glycine enolate-Claisen rearrangement carried out on a substrate with the alkene substitution pattern of **10**. The silyloxymethyl substituent did in fact pose an obstacle, as evidenced by low conversions in the presence of LDA, LiHMDS, or KHMDS, with or without $ZnCl_2$. Interestingly, addition of **10** into 2.2 equiv of NaHMDS[9] in THF at −78 °C, followed by warming to rt, gave 63% yield of alcohol **9** and minor amounts of the desired carboxylic acid. Reducing the amount of NaHMDS to 1.1 equiv resulted in near-quantitative recovery of **9**.[10] We reasoned that this unusual ester cleavage is favored over

(3) (a) Tiwari, S. K.; Gais, H. J.; Lindenmaier, A.; Babu, G. S.; Raabe, G.; Reddy, L. R.; Kohler, F.; Gunter, M.; Koep, S.; Iska, V. B. R. *J. Am. Chem. Soc.* **2006**, *128*, 7360–7373. (b) Tiwari, S. K.; Schneider, A.; Koep, S.; Gais, H. J. *Tetrahedron Lett.* **2004**, *45*, 8343–8346.

(4) Although we were unable to find a detailed account of a previous synthesis of **1**, the Lindsley group recently disclosed their efforts towards lucentamycin A at a national ACS meeting: Daniels, N. R.; Lindsley, C. W. *Abstracts of Papers, 235th National Meeting of the American Chemical Society, New Orleans, LA, April 6−10, 2008*; American Chemical Society: Washington, DC, 2008; ORGN 698.

(5) (a) Piero, V.; Hans-Jürgen, H.; Hans, S. *Helv. Chim. Acta* **1975**, *58*, 1293–1309. (b) Bartlett, P. A.; Barstow, J. F. *J. Org. Chem.* **2002**, *47*, 3933–3941. (c) Kazmaier, U. In *The Claisen Rearrangement*; Hiersemmann, M., Nubbemeyer, U., Eds.; Wiley-VCH Verlag Gmbh & Co.: Weinheim, Germany, 2007; pp 233−299.

(6) (a) Ferreró, M.; Galobardes, M.; Martín, R.; Montes, T.; Romea, P.; Rovira, R.; Urpí, F.; Vilarrasa, J. *Synthesis* **2000**, *2000*, 1608–1614. (b) Paterson, I.; Wallance, D. J.; Cowden, C. J. *Synthesis* **1998**, 639–652.

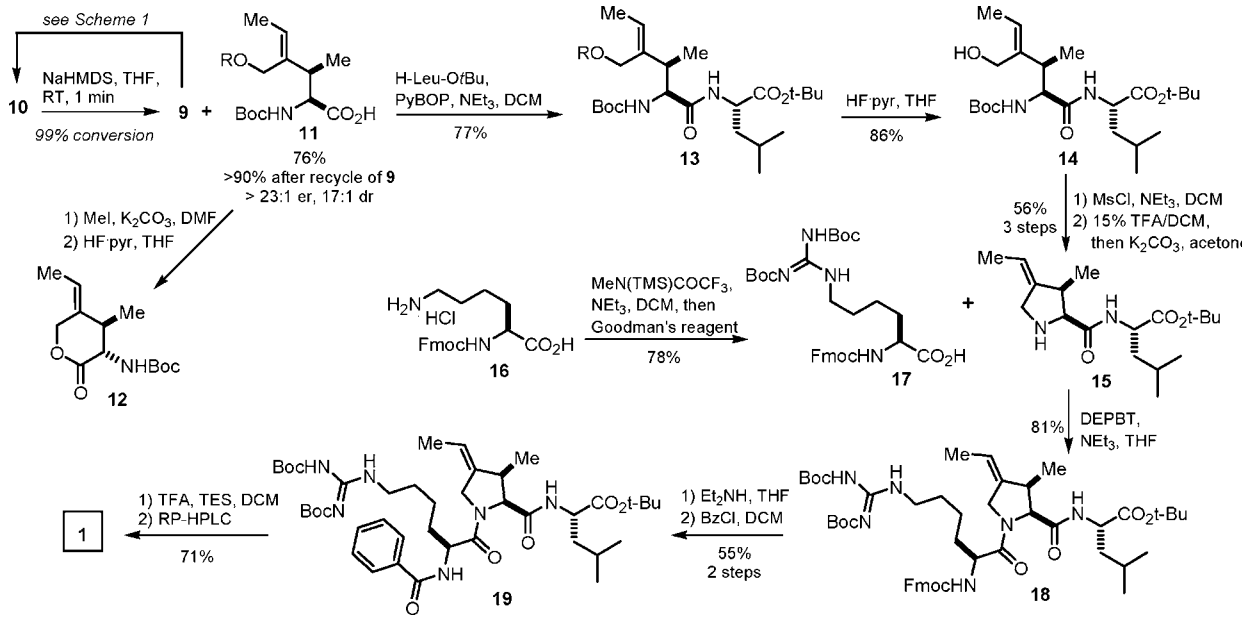
(7) The alkene geometry was confirmed after treatment of **7** with HF and comparison with spectroscopic data for the racemic enoate: Cha, J. H.; Pae, A. M.; Choi, K. I. I.; Cho, Y. S.; Koh, H. Y.; Lee, E. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2079–2081.

(8) (a) Kazmaier, U. *Angew. Chem., Int. Ed.* **1994**, *33*, 998–999. (b) Kazmaier, U.; Krebs, A. *Angew. Chem., Int. Ed.* **1995**, *34*, 2012–2014.

(9) Looper, R. E.; Runnegar, M. T. C.; Williams, R. M. *Tetrahedron* **2006**, *62*, 4549–4562.

(10) Based on pK_a considerations, our results suggest an intramolecular fragmentation induced by attack of the nitrogen anion onto the carbonyl carbon.

Scheme 2. Synthesis of **1**



thermally induced [3,3] sigmatropic rearrangement at low temperatures. Indeed, when **10** was added to a solution of 2.5 equiv of NaHMDS in THF at rt, consumption of the starting material was complete within 1 min, and we obtained **11** in 76% yield, along with 23% of fragmentation product **9** (Scheme 2). On gram-scale, **9** was routinely recycled to provide **11** in >90% overall yield.

With suitable rearrangement conditions in hand, we proceeded to esterify the carboxyl group of **11** and prepare the allylic alcohol for activation and displacement. Unfortunately, treatment of the intermediate methyl ester with HF/pyridine or TBAF/THF led to silyl ether cleavage and rapid cyclization to afford lactone **12**. To circumvent this, we coupled **11** directly to L-leucine *tert*-butyl ester in the presence of PyBOP and NEt_3. Analysis of the crude product by HPLC and identification of minor diastereomers by MS and UV indicated a 59.6:2.8:1 dr, implying >23:1 er (and 17:1 dr) for **11**.[11] Dipeptide **13** was then purified to provide the major isomer and treated with HF/pyridine to unmask the primary alcohol in 86% yield.

Efforts to promote one-pot activation/cyclization to form the pyrrolidine ring were unsuccessful under a variety of conditions (PPh_3, I_2; PPh_3, DIAD; MsCl, pyridine; DAST). Notably, attempted mesylation of **14** afforded a high yield of the allylic chloride,[12] indicating a reluctance toward intramolecular displacement despite the reactivity of the intermediate allylic mesylate. Since carbamate nitrogens have been shown to participate in similar displacements through planar allyl cation mechanisms,[13] we presumed that our desired reaction was formally an attempt at a disfavored 5-*endo-trig* cyclization (S_N1) under these conditions.[14] As an alternative, acidic cleavage of the *N*-Boc group followed directly by treatement with K_2CO_3 in acetone led to rapid S_N2 cyclization to give pyrrolidine **15** in 56% yield over 3 steps.

Figure 3 depicts the key NOE enhancements observed about the pyrrolidine ring of the Emp-Leu subunit **15**. Although the geometry of the alkene could be easily deduced, there was only indirect evidence to suggest a *cis* relationship for H2 and H3. Both protons exhibited correlation to H5α (and not H5*β*), but a direct NOE between H2 and H3 was conspicuously weak relative to other signals. To obtain more

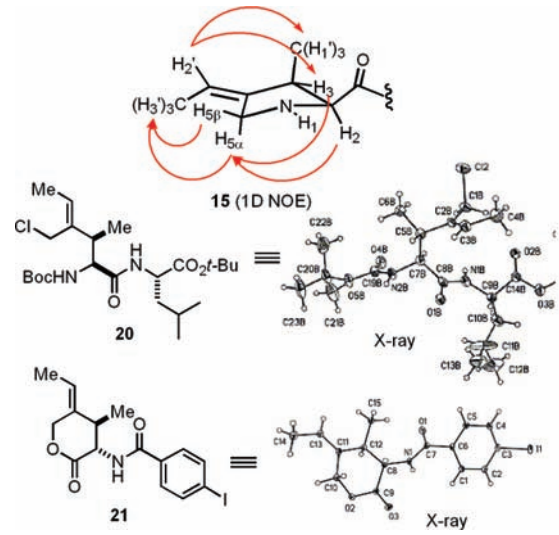


Figure 3. Confirmation of 2*S*,3*R*,4*Z* Emp configuration.

(11) See Supporting Information for details.

(12) For examples of allylic chlorination with methanesulfonyl chloride, see: (a) Artman, G. D.; Grubbs, A. W.; Williams, R. M. *J. Am. Chem. Soc.* **2007**, *129*, 6336–6342. (b) Li, F.; Castle, S. L. *Org. Lett.* **2007**, *9*, 4033–4036.

definitive evidence we carried out crystallization trials on a number of intermediates and derivatives. Suitable diffraction quality crystals were obtained from compounds **20** and **21** (derived from **14** and **12**), and X-ray structures provided unambiguous confirmation of our stereochemical assignments.[11]

The final assembly of **1** employed homoarginine dervative **17**, available in one step from Fmoc-Lys-OH using Goodman's guanidinylation reagent (Scheme 2).[15] Condensation of **17** and **15** in the presence of DEPBT[15] afforded tripeptide **18** in good yield. Fmoc-deprotection of the N-terminus was then carried out by treatment with excess diethylamine, followed by benzoylation.[17] Finally, global deprotection of **19** with 90:5:5 TFA/TES/DCM and subsequent purification by RP-HPLC completed the total synthesis of presumed lucentamycin A.[18]

Although the ^{1}H NMR of **1** and natural lucentamycin A were qualitatively very similar, we noticed a number of chemical shift discrepancies (0.05−0.12 ppm) that could not be attributed to experimental error.[19] Clear differences were also evident upon comparison of ^{13}C NMR spectra, with 6 out of 8 Emp signals exhbiting deviations >0.5 ppm. Serial dilution NMR studies of **1** in DMSO-d_6 ruled out a possible concentration effect, while variable temperature and pH experiments also failed to induce shifts in C−H proton resonances. In addition, the specific rotation we obtained for **1** ($[\alpha]^{25}_D$ −35.2, *c* 0.500 in MeOH) was significantly different from that reported for lucentamycin A ($[\alpha]^{25}_D$ −6.3, *c* 0.175 in MeOH).

Given our structural confirmation of intermediates **20** and **21** and the fact that most signals attributable to the Emp residue in **1** exhbit an NMR discrepancy (in position or splitting) relative to the natural product, we believe that a stereochemical revision is warranted. While the exact nature of this correction is not currently known, key NOE correlations observed for natural lucentamycin A strongly support the 2,3-*cis* substitution and *Z* alkene configuration originally proposed for Emp.[2] It is therefore likely that the *absolute* stereochemistry of the novel proline residue should be revised.

We are currently applying our rearrangement strategy to the synthesis of other polysubstituted prolines and pipecolic acids, as well as to the suspected natural diastereomer of **1**. Results from these studies will be reported in due course.

Acknowledgment. We thank Profs. William Fenical (University of California−San Diego) and Philip Williams (University of Hawaii) for providing raw NMR data for natural lucentamycin A and Prof. Bill Baker (University of South Florida) for helpful discussions. We also gratefully acknowledge Dr. Eileen Duesler (University of New Mexico) for X-ray diffraction studies on compounds **20** and **21**. Funding for this work was provided, in part, by the Moffitt Cancer Center.

Supporting Information Available: Experimental data and copies of NMR spectra for all new compounds; HPLC and UV spectra for **13** and **1**; comparative NMR spectra for **1** and natural lucentamycin A; X-ray diffraction data for compounds **20** and **21**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) (a) Gosselin, F.; Lubell, W. D. *J. Org. Chem.* **2000**, *65*, 2163–2171. (b) Vaswani, R. G.; Chamberlin, A. R. *J. Org. Chem.* **2008**, *73*, 1661–1681.

(14) In contrast to the examples in ref 13, the incipient electrophilic sp_2 center in **14** is formally within the cyclic transition state (*endo*), leading to a strained attack geometry. See: Johnson, C. D. *Acc. Chem. Res.* **2002**, *26*, 476–482.

(15) Feichtinger, K.; Zapf, C.; Sings, H. L.; Goodman, M. *J. Org. Chem.* **1998**, *63*, 3804–3805.

(16) Li, H. T.; Jiang, X. H.; Ye, Y. H.; Fan, C. X.; Romoff, T.; Goodman, M. *Org. Lett.* **1999**, *1*, 91–93.

(17) As expected on the basis of Young's test considerations, attempts to couple **15** directly to *N*-Bz-Har(Boc)$_2$-OH resulted in moderate yields of coupled product and significant racemization of the Har residue.

(18) Semi-preparative RP-HPLC in the presence and absence of formic acid as an aqueous additive gave products with identical spectroscopic properties. We presume that purified **1** exists in the neutral zwitterionic form.

(19) Although an authentic sample of lucentamycin A was unavailable for HPLC co-injection studies, analysis of original NMR data files graciously provided by Prof. Fenical clearly show chemical shift discrepancies. Superimposed NMR expansions are included in the Supporting Information.