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Progress toward the Total Synthesis of Lucentamycin A: Total Synthesis and Biological Evaluation of 8-*epi*-Lucentamycin A

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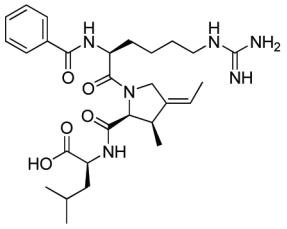

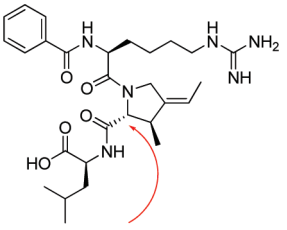

Synthetic efforts toward the cytotoxic peptides lucentamycins A−D are described that resulted in the total synthesis and biological evaluation of 8-*epi*-lucentamycin A in 15 steps with 2.2% overall yield. The key *epi*-nonproteogenic 3-methyl-4-ethylideneproline was synthesized via a titanium-mediated cycloisomerization reaction.

Recently, Fenical and co-workers reported on the isolation and characterization of four novel 3-methyl-4-ethylideneproline-containing peptides, lucentamycins A−D, from the fermentation broth of a marine-derived actinomycete identified as *Nocardiopsis lucentensis* (Figure 1).[1] Importantly, lucentamycins A and B displayed significant in vitro cytotoxicity, IC_{50} values of 0.2 and 11 μM, respectively, against HCT-116 human colon carcinoma.[1] On the basis of the biological activity of lucentamycin A and the broad spectrum of biological activity (antibiotic, antifungal, anticancer) of other agents derived from *Nocardiopsis*,[1−4] a total synthesis campaign targeting lucentamycin A to provide sufficient material for biological evaluation seemed warranted.[5] Moreover, the lucentamycins were attractive as a target for our Program in the synthesis of unnatural analogues coupled with biological evaluation and target elucidation.[6−10]

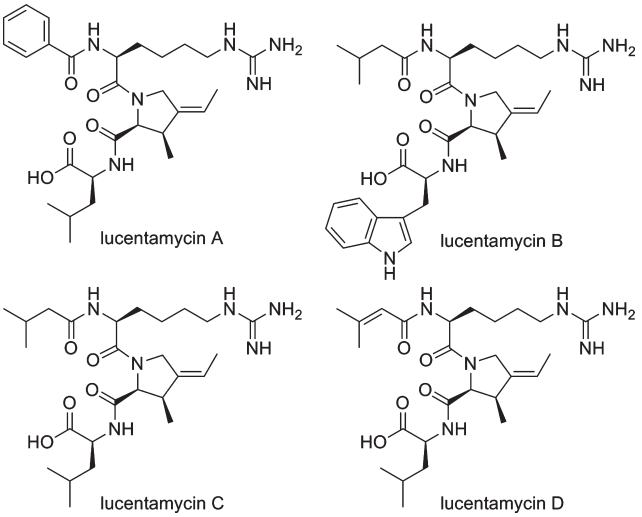

FIGURE 1. Structures of lucentamycin A−D.

The retrosynthesis of lucentamycin A (**1**) involved cleavage of the two amide bonds of the nonproteogenic 3-methyl-4-ethylideneproline nucleus to afford L-leucine *tert*-butyl ester **2**, the functionalized lysine **3**, and unnatural proline **4** (Scheme 1). The functionalized lysine was envisioned to arise by acylation and guanidation of L-lysine methyl ester **5**. The key nonproteogenic 3-methyl-4-ethylideneproline **6** would be accessed through chiral vinyl aminosulfoxonium salt chemistry as reported by Gais, for which a single X-ray crystal of **6** was disclosed.[11]

While synthetic effort was focused on the synthesis of **6**, we initiated a model study en route to an unnatural congener **16** of lucentamycin A, wherein the nonproteogenic 3-methyl-4-ethylideneproline **6** was replaced with natural L-proline (Scheme 2) to evaluate potential racemization problems and to develop structure−activity relationships for the cytotoxicity of **1** against HCT-116 cells. Racemization at C16 was not unexpected as the α-amino group of the lysine was acylated with a benzoyl group, well documented to form a Leuch anhydride intermediate, and notorious for promoting racemization as the "enol" form is aromatic.[12] In the event, Fmoc-protected L-proline **7** was coupled under HATU conditions with leucine *tert*-butyl ester **2** to provide the dipeptide

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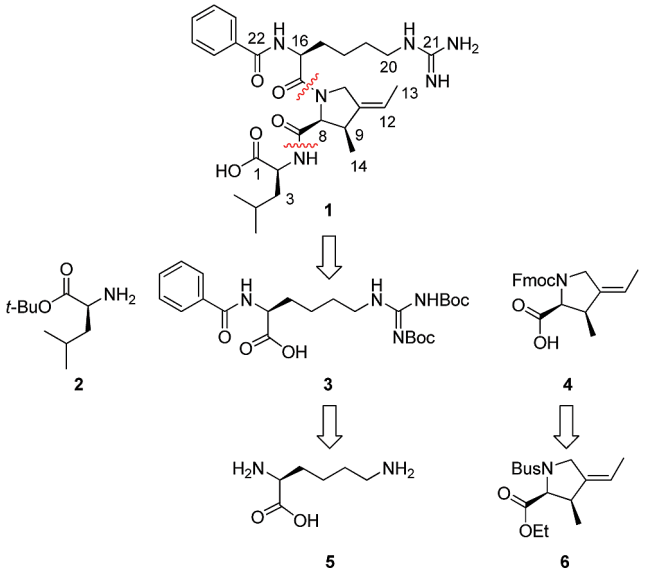
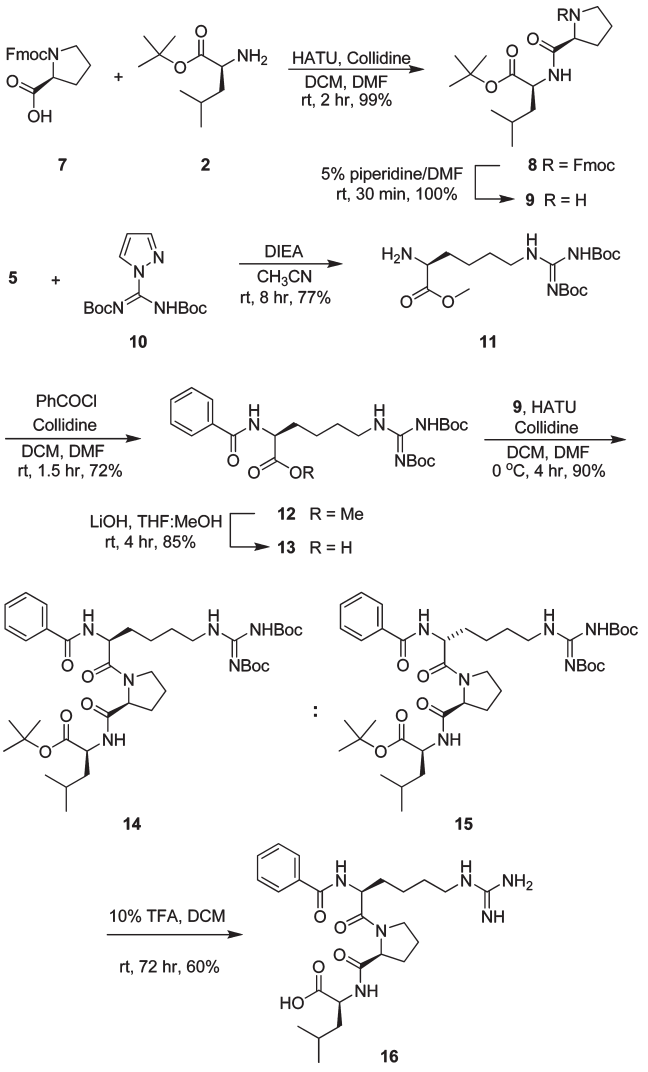
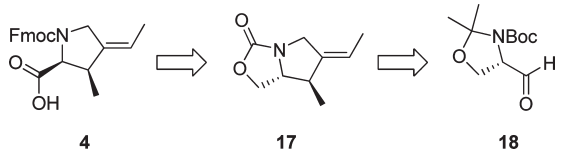
SCHEME 1. Retrosynthesis of Lucentamycin A (1)



SCHEME 2. Lucentamycin A Model Study To Deliver 16



SCHEME 3. Revised Retrosynthesis of 4



8 in 99% yield. Fmoc deprotection with 5% piperidine in DMF delivered the dipeptide **9** in quantitative yield. L-Lysine methyl ester **5** was treated with **10** to provide the bis-*N*-Boc-protected arginine derivative **11** in 77% yield.[13] Acylation with benzoyl chloride and hydrolysis employing LiOH afforded **13** in 62% yield over the two steps. The coupling of **9** to **13** justified the model study, as our initial EDCI/HOBt/collidine coupling conditions generated >90% chemical yield, but a 39:61 ratio of the desired **14** to the epimerized **15**. The degree of racemization was determined by analytical LCMS and confirmed by ^{1}H NMR. At this point, we evaluated a variety of coupling reagents, additives, and solvent/temperature conditions. Ultimately, the conditions of HATU and collidine without HOAt proved optimal, generating a 92:8 ratio of **14**:**15** in yields exceeding 90% and readily separable by column chromatography. A final global deprotection with 10% TFA in DCM afforded the unnatural analogue **16** of lucentamycin A in 60% yield.

Attention now turned to the construction of the key nonproteogenic 3-methyl-4-ethylideneproline **4**. Application of the Gais protocol proved arduous, with difficult *E*/*Z* mixtures at multiple points along the 11-step sequence, which ultimately result in complex chromatographic separations where even sophisticated reverse phase systems failed to deliver key intermediates in yields satisfactory for carrying forward en route to a total synthesis of **1**. At this point, we revised our retrosynthesis for **4**, and we envisioned access to **4** by a titanium-mediated cycloisomerization reaction[14] (Scheme 3) to afford **17**, which would be derived from Garner's aldehyde **18**. Key to the success of this route would be the ability to epimerize the α-carbon; however, this route would also enable the synthesis of the C8 epimer of lucentamycin A, 8-*epi*-lucentamycin A, and further construct SAR.

In the event, Garner's aldehyde **18** smoothly undergoes a Wittig reaction providing **19** in 83% yield.[15] Deprotection with *p*-TsOH in MeOH affords **20**, which is then converted to the corresponding oxazolidin-2-one **21** in 83% yield for the two steps. Deprotonation with KHMDS and alkylation with mesylate **22** delivers **23**. A titanium-mediated cycloisomerization reaction delivers bicycle **17**, which sets the methyl stereocenter and the relative regiochemistry of the 3-methyl-4-ethylideneproline nucleus in 91% yield.[14] Detailed NOE studies and historical accounts confirmed the stereochemical assignment.[16] Opening of the cyclic carbamate with methoxide generates **24**, which is oxidized to the aldehyde **25** in 98% yield. Finally, a buffered bleach oxidation of the aldehyde leads to **26**, the epimer of the key nonproteogenic 3-methyl-4-ethylideneproline **4**, in 60% yield. A double deprotonation/kinetic quench with AcOH failed to provide the desired **27**

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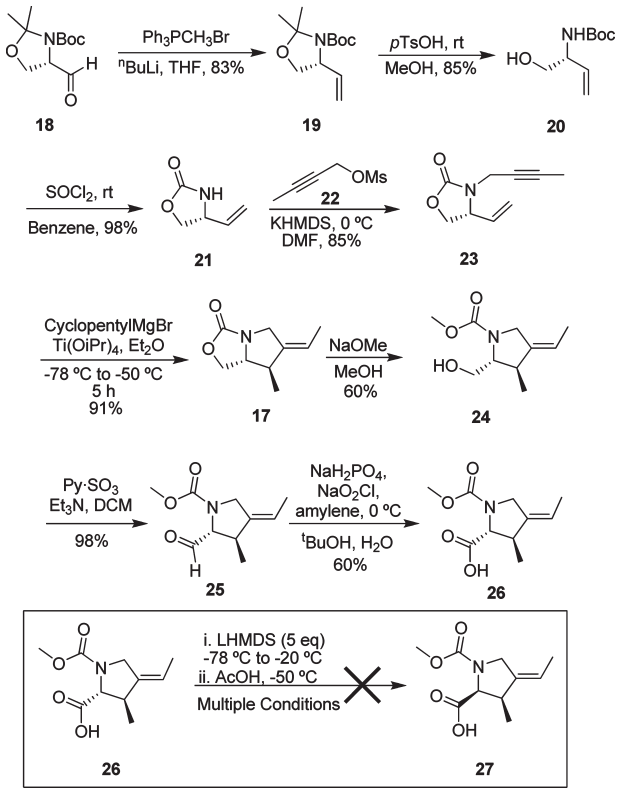
(16) See the Supporting Information for full details.

SCHEME 4. Synthesis of an Epimeric Non-Proteogenic 3-Methyl-4-ethylideneproline 26 and Attempts To Epimerize To Deliver 27

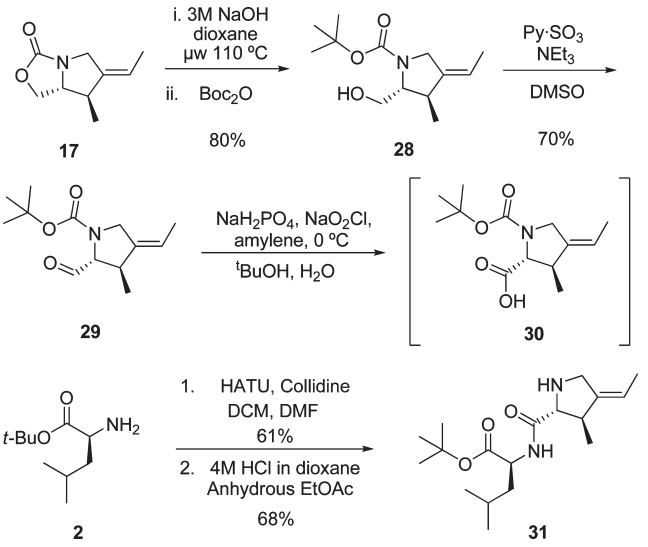


(Scheme 4), affording only starting material **26** with no evidence of any epimerization. Alternative approaches to epimerize **25**, **26**, and esters of **26** met with similar unproductive results.[17] Thus, our strategy adjusted to target the synthesis and biological evaluation of 8-*epi*-lucentamycin A employing **26**. However, removal of the methyl carbamate in **26** proved equally challenging, and we were unable to affect this key transformation under a variety of reaction conditions.

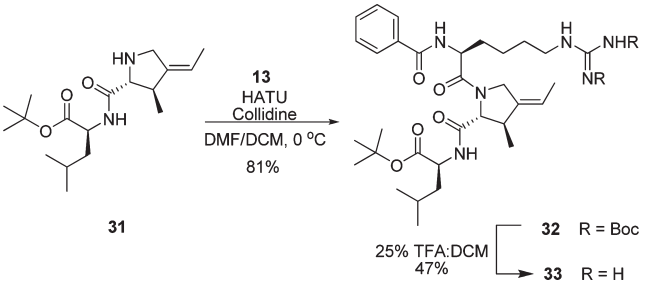
Therefore, we again modified our approach for the total synthesis of 8-*epi*-lucentamycin A. As shown in Scheme 5, bicycle **17** was treated in a single pot with 3.0 M NaOH under microwave irradiation, followed by an in situ protection of the secondary amine to deliver Boc-protected pyrrolidine **28** in 80% yield.[18] Alcohol **28** smoothly underwent oxidation to the corresponding aldehyde **29** in 70% yield. Repetition of the buffered bleach oxidation of **29** provides **30**, the epimer of the key nonproteogenic **4**. The reaction to deliver acid **30** proceeded cleanly, therefore crude material (>95% pure) was carried forward into the coupling step. Thus, crude **30** was directly coupled to L-leucine *tert*-butyl ester **2** under HATU conditions (61% for two steps), followed by a chemoselective deprotection of the Boc group under anhydrous acidic conditions to deliver **31** in 68% yield.[19]

Dipeptide **31** and **13** were treated with our optimal coupling system of HATU and collidine in DMF/DCM at 0 °C

SCHEME 5. Synthesis of the Key Non-Proteogenic 3-Methyl-4-ethylideneproline 30 and Southern Dipeptide Fragment 31



SCHEME 6. Total Synthesis of 8-*epi*-Lucentamycin A (33)



to deliver **32** in 81% yield, as a 92:8 ratio of diastereomers. After separation of the racemized material, a global deprotection employing 25% TFA in DCM provided 8-*epi*-lucentamycin A **33** in an unoptimized 47% yield (Scheme 6). NMR data of the epimeric lucentamycin A **33** agreed well with the natural product **1**, with the expected exceptions due to the epimerization of C8.

Lucentamycin A (**1**) displayed significant in vitro cytotoxicity, IC_{50} value of 0.2 μM, against HCT-116 human colon carcinoma. With 8-*epi*-lucentamycin A (**33**) in hand, we evaluated its affect on an HCT-116 cell line in order to determine if the stereochemistry of the nonproteogenic 3-methyl-4-ethylideneproline nucelus was critical for biological activity. Thus, a standard 48 h cell viability assay was performed with **16** and **33** at six concentrations (0, 0.025, 0.1, 0.4, 2.0, and 10 μM) relative to podophyllotoxin as a positive control.[20] The control performed as expected, providing an IC_{50} value of 0.03 μM. However, neither unnatural, epimeric Lucentamycin A (**33**) nor **16** had an effect on HCT-116 cell viability up to 10 μM. The cytotoxicity data generated with both **33** and **16** suggest that the stereochemistry at C8, and the topology afforded by the natural product, is essential for potent in vitro cytotoxicity (IC_{50} = 0.2 μM) of lucentamycin A (**1**) against HCT-116 cells.

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Thus, synthetic efforts toward the lucentamycins A−D, **1**−**4**, have been reported culminating in the total synthesis of 8-*epi*-lucentamycin A (**33**). The synthesis features a titanium-mediated cycloisomerization reaction to construct the key, epimeric nonproteogenic 3-methyl-4-ethylideneproline **30**. The convergent synthetic route afforded 8-*epi*-lucentamycin A **33** in 15 steps, with a 10 step longest linear sequence, and an overall yield of 2.2%. Biological evaluation of 8-*epi*-lucentamycin A (**33**) and another unnatural congener **16** indicated that both were inactive relative to natural **1**, with an IC_{50} value of $>10\ \mu M$ in a HCT-116 human carcinoma cell line. Interestingly, these studies suggest that the natural configuration of the nonproteogenic 3-methyl-4-ethylidene-proline **4** is essential for bioactivity. Future efforts are focused on developing chemistry to access the natural, nonproteogenic **4** and the total synthesis and biological evaluation of lucentamycin A (**1**), as there are not sufficient quantities of **1** for biological evaluation.

Experimental Section

(*S*)-*tert*-Butyl 2-((2*R*,3*R*,*Z*)-4-Ethylidene-3-methylpyrrolidine-2-carboxamido)-4-methylpentanoate (31). Boc-protected **31** (50.8 mg, 0.12 mmol) was added to a small vial and placed under argon. Anhydrous EtOAc (0.45 mL) and anhydrous HCl in dioxane (4 M, 0.15 mL, 0.6 mmol) were then added and the reaction was stirred for 1 h. Additional anhydrous EtOAc (0.45 mL) and anhydrous HCl in dioxane (4 M, 0.15 mL, 0.6 mmol) were then added at 1, 2, and 4 h total reaction time for a total of four injections of each of EtOAc and HCl in dioxane. NaOH (1 N) was added to neutralize the reaction. The aqueous layer was separated and extracted with EtOAc (3 × 5 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (1:0 to 19:1 DCM:MeOH) to yield the product as an oil (26.2 mg, 0.081 mmol) in 67.5% yield. $[\alpha]^{20}_D$ 29.7 (*c* 0.2, $CHCl_3$); R_f 0.52 (9:1, DCM:MeOH); IR (thin film) 3331, 2960, 2929, 2870, 1735, 1673, 1509, 1368, 1151 cm^{-1}; ^1H NMR (600.1 MHz, $CDCl_3$) δ (ppm) 7.58 (d, J = 8.5 Hz, 1H), 5.24 (m, 1H), 4.47 (td, J = 8.9, 5.0 Hz, 1H), 3.64 (s, 2H), 3.25 (d, J = 7.5 Hz, 1H), 2.63 (m, 1H), 2.36 (br s, 1H), 1.62 (m, 1H), 1.56 (d, J = 6.5 Hz, 3H), 1.52 (m, 1H), 1.43 (s, 9H), 1.19 (d, J = 6.8 Hz, 3H), 0.93 (d, J = 6.2 Hz, 6H); ^{13}C NMR (150.9 MHz, $CDCl_3$) δ (ppm) 173.1, 172.1, 143.8, 114.3, 81.5, 68.3, 50.8, 47.8, 42.7, 41.7, 27.9, 25.0, 22.9, 21.9, 17.7, 14.5; HRMS (TOF, ES+) $C_{18}H_{33}N_2O_3$ $[M + H]^+$ calcd 325.2491, found 325.2491.

(*S*)-*tert*-Butyl 2-((2*R*,3*R*,4*Z*)-1-((*S*)-2-Benzamido-6-(2,3-bis(*tert*-butoxycarbonyl)guanidino)hexanoyl)-4-ethylidene-3-methylpyrrolidine-2-carboxamido)-4-methylpentanoate (32). 31 (23.7 mg, 0.073 mmol) and **13** (36.0 mg, 0.073 mmol), in anhydrous DCM (0.3 mL each), were added to a flame-dried flask under argon via syringe. Anhydrous DMF (0.3 mL) was added and the solution was cooled to 0 °C. *O*-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N*,*N*-tetramethyluronium hexafluorophosphate (41.7 mg, 0.111 mmol) was added immediately followed by collidine (10.6 μL, 0.080 mmol). The reaction was stirred at 0 °C for 6 h and then diluted with DCM (4 mL) and washed with H_2O (3 × 2 mL) and filtered through a phase separator. The crude mixture was concentrated in vacuo and purified by flash chromatography (1:0 to 1:1 Hex:EtOAc) to yield the product as a crusty foam (47.0 mg, 0.059 mmol) in 80.5% yield. $[\alpha]^{20}_D$ 23.9 (*c* 0.2, $CHCl_3$); IR (thin film) 3330, 2964, 2929, 2929, 1722, 1639, 1154, 1135 cm^{-1}; ^1H NMR (600.1 MHz, $CDCl_3$) δ (ppm) 11.47 (br s, 1H), 8.35 (br s, 1H), 7.82 (m, 2H), 7.48 (t, J = 7.4 Hz, 1H), 7.40 (t, J = 7.7 Hz, 2H), 7.11 (d, J = 8.5 Hz, 1H), 6.94 (br s, 1H), 5.44 (m, 1H), 4.83 (q, J = 6.8 Hz, 1H), 4.49 (d, J = 14.3 Hz, 1H), 4.42 (m, 1H), 4.37 (d, J = 1.4 Hz, 1H), 4.30 (d, J = 14.3 Hz, 1H), 3.43 (m, 2H), 3.14 (q, J = 7.0 Hz, 1H), 1.93 (m, 1H), 1.86 (m, 1H), 1.67 (m, 2H), 1.62 (d, J = 6.8 Hz, 3H), 1.59 (m, 1H), 1.52 (m, 4H), 1.48 (s, 9H), 1.47 (s, 9H), 1.36 (s, 9H), 1.16 (d, J = 7.2 Hz, 3H), 0.82 (d, J = 6.1 Hz, 3H), 0.70 (d, J = 6.1 Hz, 3H); ^{13}C NMR (150.9 MHz, $CDCl_3$) δ (ppm) 171.81, 171.76, 169.8, 167.5, 163.3, 156.1, 153.2, 139.0, 133.3, 131.8, 128.5, 127.3, 118.1, 83.2, 81.2, 79.5, 66.6, 51.7, 51.2, 47.8, 41.2, 40.9, 40.6, 31.7, 28.9, 28.2, 28.0, 27.9, 24.6, 22.9, 22.6, 21.8, 21.2, 14.7; HRMS (TOF, ES+) $C_{42}H_{67}N_6O_9$ $[M + H]^+$ calcd 799.4970, found 799.4970.

8-*epi*-Lucentamycin A (33). 32 (38.3 mg, 0.048 mmol) was dissolved in anhydrous DCM (0.38 mL) and cooled to 0 °C. TFA (0.12 mL) was added dropwise and the solution was allowed to warm to room temperature over 3 h and stirred overnight under an argon atmosphere. MeOH was added to the reaction mixture, which was then concentrated in vacuo. More MeOH was added and the reaction was concentrated again. Because of the high polarity of the compound, the product was purified by reverse phase preparatory HPLC to yield the product as a white solid (12.3 mg, 0.023 mmol) in 47.3% yield. $[\alpha]^{20}_D$ 28.5 (*c* 0.2, MeOH); ^1H NMR (600.1 MHz, DMSO-d_6) δ (ppm) 10.19 (m, 1H), 8.63 (d, J = 7.1 Hz, 1H), 8.22 (d, J = 9.0 Hz, 1H), 7.90 (m, 2H), 7.51 (t, J = 7.3 Hz, 1H), 7.44 (t, J = 7.6 Hz, 2H), 6.95 (m, 2H), 5.26 (m, 1H), 4.69 (d, J = 1.7 Hz, 1H), 4.45 (m, 1H), 4.16 (d, J = 16.2 Hz, 1H), 4.00 (m, 1H), 3.89 (d, J = 16.5 Hz, 1H), 3.32 (br s, 2H), 3.05 (m, 1H), 2.93 (m, 1H), 2.62 (m, 1H), 1.65 (m, 1H), 1.55 (m, 7H), 1.47 (m, 1H), 1.39 (m, 1H), 1.32 (m, 2H), 1.16 (d, J = 7.0 Hz, 3H), 0.86 (d, J = 6.1 Hz, 3H), 0.80 (d, J = 6.1 Hz, 3H); ^{13}C NMR (150.9 MHz, DMSO-d_6) δ (ppm) 177.0, 171.3, 170.5, 166.3, 157.6, 140.4, 133.8, 131.3, 128.1, 127.6, 115.0, 66.5, 52.3, 51.4, 47.9, 45.1, 41.3, 40.6, 40.0, 30.1, 28.1, 24.9, 23.2, 21.6, 21.2, 14.3; HRMS (TOF, ES+) $C_{28}H_{43}N_6O_5$ $[M + H]^+$ calcd 543.3295, found 543.3295.

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Supporting Information Available: Experimental procedures, characterization data, and ^1H and ^{13}C NMR spectra for all new compounds, **8**−**33**. This material is available free of charge via the Internet at http://pubs.acs.org.