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First Total Synthesis of Hunanamycin A

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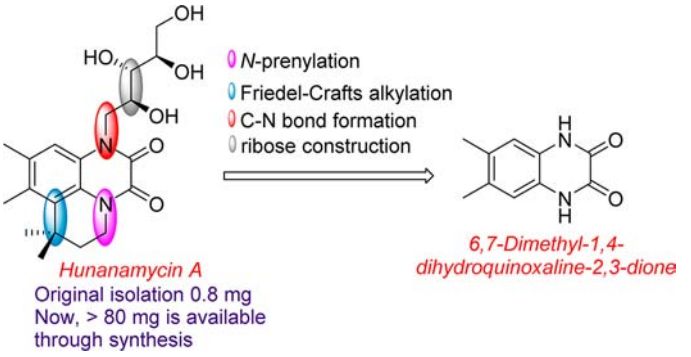
ABSTRACT



The first total synthesis of hunanamycin A, an antibiotic natural product with a pyrido[1,2,3-*de*]quinoxaline-2,3-dione core from a marine-derived *Bacillus hunanensis*, is disclosed. The present effort provides access to sufficient amounts of scarce hunanamycin A for further biological evaluation and confirmation of the assigned absolute configuration. In addition, four new analogues of the natural product are reported.

Very recently, the title compound hunanamycin A (**1**), a natural product, was isolated from a marine-derived *Bacillus hunanensis* by MacMillan et al.[1] The gross structure and stereochemical assignments (Figure 1) of the compound **1** were determined using extensive spectroscopic data analysis and published in this journal.[1] As the structure of hunanamycin A was related to riboflavin degradation products, the MacMillan group screened compound **1** for antimicrobial activity against bacterial strains that lacked riboflavin transport mechanisms. The results showed a minimum inhibitory concentration (MIC) of 12.4 μM against *Salmonella enterica* suggesting it is an inhibitor of riboflavin synthase (*ribB*).[1] Riboflavin synthase is an enzyme that catalyzes the final step in the biosynthesis of riboflavin.[2] In disease models of *Salmonella* infection, knockout of the riboflavin synthase was found to be lethal to the pathogen.[3] The *ribB* has shown to be an attractive antibiotic target, as humans lack this enzyme.[4] Significant efforts from Cushman's group along these lines are worth highlighting.[4] *Salmonella enterica*, the most pathogenic species of *Salmonella*, causes foodborne illness and accounted for considerable damage to humans in both developed and developing countries. For instance, in the United States it was estimated that *Salmonella* ranks number one, accounting for 35% of total foodborne illnesses in the country. About 3000 deaths in the United States are recorded annually owing to foodborne illnesses.[5] Therefore, any step toward addressing these problems are rewarding and satisfying. Another interesting aspect of the hunanamycin A molecule is that it does not violate the Lipinski rule of five.[6]

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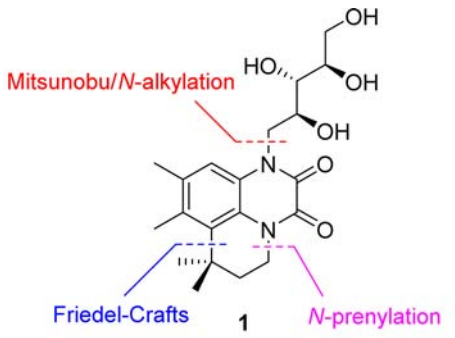
In view of the above, we became interested in (i) the total synthesis of the target compound **1** in sufficient quantity, (ii) preparation of a focused library of molecules around this novel scaffold toward lead optimization, and (iii) confirmation of the assigned structure including the absolute configuration.



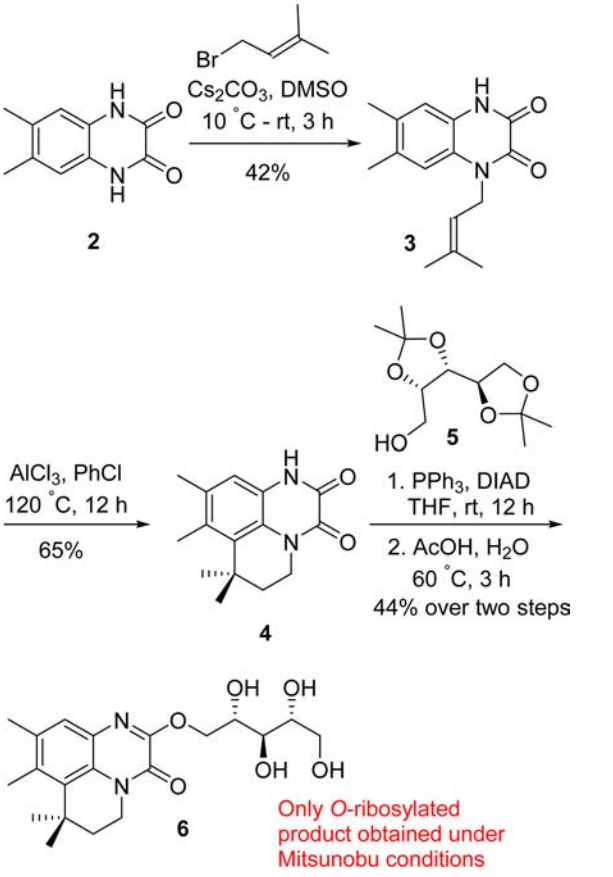
Lipinski rule of five	Calculated parameters for 1
mol wt < 500	mol wt = 392
cLogP < 5	cLogP = 0.86
H-bond donors < 5	H-bond donors = 4
H-bond acceptors < 10	H-bond acceptors = 8

Figure 1. Structure of hunanamycin A, calculated Lipinski parameters, and key disconnections for the planned synthesis.

The key disconnections of hunanamycin A (**1**) are shown in Figure 1. We mainly relied on a central dihydroquinoxaline-2,3-dione moiety, which was readily prepared in multigram quantities, starting from 4,5-dimethyl-1,2-phenylenediamine. Initially, building of the tricyclic aglycon and attachment of a sugar moiety to the aglycon were planned, which makes the sequence amenable to prepare several analogues of **1** using various sugars or sugar mimics.

Our synthesis began with the monoalkylation of **2**[7] using prenyl bromide and Cs_2CO_3 base. We had to make a few attempts to arrive at the optimized conditions for the selective preparation of monoalkylated product **3** (Scheme 1).[8] The intramolecular Friedel–Crafts alkylation was carried out using $AlCl_3$ in chlorobenzene solvent to obtain tricylic aglycon **4**.[9,10] At this stage, the C–N bond formation with known ribose sugar derivative **5**,[11] using

Scheme 1. Initial Attempts toward Synthesis of **1**



Mitsunobu conditions[12] on tricyclic core **4** followed by deprotection, would have provided the target natural product. Instead, we have isolated the compound **6** as the sole product from this exercise. Careful examination of the ^{13}C NMR spectrum suggested the structure as an *O*-ribosylated product.

To overcome the problem of *O*-alkylation, base-mediated alkylations were attempted, using various options as shown in Scheme 2. However, none of these efforts were successful in obtaining the desired *N*-ribosylated product. These unsuccessful results prompted us to explore base-mediated *N*-alkylation with more reactive allyl halides. Accordingly, compound **4** reacted with allyl bromide in the presence of NaH in DMF to give compound **7** in good yield (Scheme 2). It is noteworthy to mention that no *O*-alkylation was observed under these conditions. Dihydroxylation of the double bond present in **7**, using a catalytic amount of OsO_4 and *N*-methyl morpholine oxide (NMO), produced the diol **8** in 68% yield. The ^1H and ^{13}C NMR spectra of compounds **1**, **6**, and **8** were compared, and it was found that the spectra of **1** and **8** were close. Significant differences were observed in the case of the ^{13}C spectra of compound **6** with respect to **1** and **8**. The characteristic peaks around δ 47 and 117 in the

(7) Oxtoby, N. S.; Blake, A. J.; Champness, N. R.; Wilson, C. *Chem.—Eur. J.* **2005**, *11*, 4643–4654.

(8) Small amount (~5%) of dialkylated product also isolated from the reaction. See the Supporting Information for experimental details.

(9) Sakami, S.; Kawai, K.; Maeda, M.; Aoki, T.; Fujii, H.; Ohno, H.; Ito, T.; Saitoh, A.; Nakao, K.; Izumimoto, N.; Matsuura, H.; Endo, T.; Ueno, S.; Natsume, K.; Nagase, H. *Biorg. Med. Chem.* **2008**, *16*, 7956–7967.

(10) **Hazard!** A related Friedel–Crafts reaction was carried out in nitromethane solvent in our lab which resulted in an explosion. Therefore, additional care must be taken while handling mixtures of $AlCl_3$ and nitromethane, as it is potentially hazardous.

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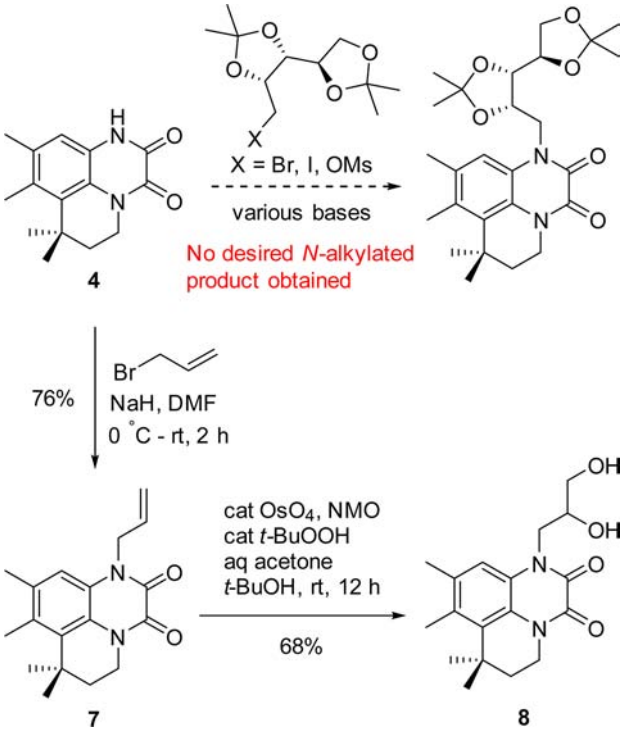
Scheme 2. Efforts toward *N*-ribosylation/*N*-alkylation



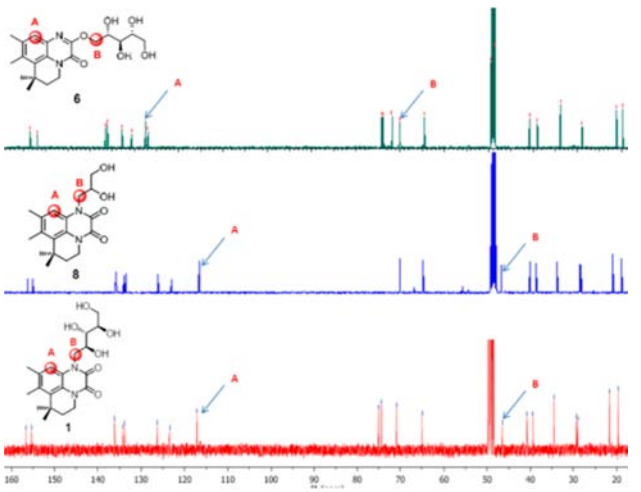
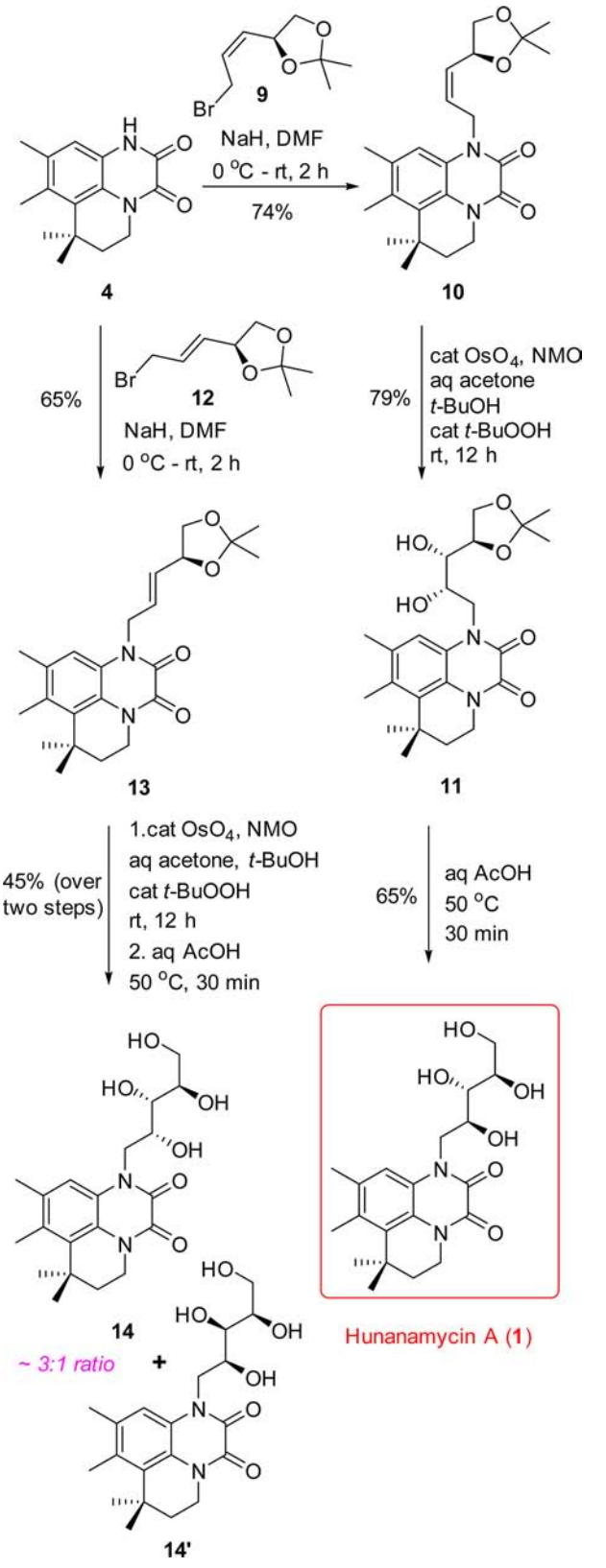


Figure 2. Comparison of ^{13}C spectra of compounds **1**, **6**, and **8** with the key differentiator peaks are highlighted.

^{13}C NMR spectra of **1** and **8** compared to that of compound **6** were deshielded significantly (by >10 ppm; see ^{13}C spectra of three compounds in Figure 2). This observation confirmed that compound **6** is an *O*-alkylated product.

To complete the total synthesis of hunanamycin A in a highly stereoselective manner, an alternate strategy was adopted and the details are shown in Scheme 3. We have considered Kishi's well established model in redesigning

Scheme 3. Synthesis of Hunanamycin A and Its Analogue



the strategy.[13] According to Kishi's empirical formulation, "The relative stereochemistry between the pre-existing hydroxyl or alkoxyl group and the adjacent newly introduced hydroxyl

group of the major product in all cases is *erythro*."[13] The *N*-alkylation of the tricyclic compound **4**, with known bromide **9** (prepared from D-mannitol),[14] was achieved using NaH in DMF to produce the desired compound **10** in 74% yield. Highly diastereoselective dihydroxylation (OsO_4/NMO) of *Z*-olefin **10** resulted in compound **11**, which on deprotection (aq AcOH, 50 °C) furnished the target compound **1** in good yields. All the spectral data of the synthesized hunanamycin A (**1**) were compared with those of the natural one[1] and found to be identical. The absolute stereochemistry of the sugar moiety was confirmed (as predicted by MacMillan group)[1] by comparing the optical rotation values of synthetic and isolated hunanamycin A (**1**) samples. It is worth mentioning that we have prepared a sufficient quantity of the natural hunanamycin A (> 100-fold) for the first time. Toward the preparation of analogues of compound **1**, the aglycon **4** was treated with known *E*-olefin **12**[15] under similar conditions, as stated above, resulting in compound **13**. Dihydroxylation of **13** followed by deprotection of acetonide furnished the analogues **14** and **14′** as a mixture of diastereomers.[16] In the case of *E*-olefin, we have observed low selectivity (~3:1 ratio) compared to that of *Z*-olefin. This observation is consistent with the results of literature precedence.[13]

In summary, we have accomplished the first synthesis of an antibacterial natural product, hunanamycin A and analogues with variation at the sugar moiety, using simple and scalable chemistry. Now, the natural product is available in sufficient quantities for further biological profiling, and the developed route can be used for the synthesis of novel analogues, identification, and optimization of riboflavin synthase inhibitors based on this novel scaffold. Ultimately, the present exercise can be considered as a prelude to the invention of drugs for the treatment of human food poisoning infections, mainly caused by *Salmonella* bacteria. Hunanamycin A is a perfect druggable candidate for lead optimization in drug discovery, as it complies with the Lipinski rule of five. Further optimization of reactions, focused library synthesis, and SAR activities are the subject of future direction of this work.

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Supporting Information Available. Experimental details and copies of NMR spectra of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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(16) We could not separate the diastereomeric mixture of **14** and **14′** based on our initial attempts which needs further trials. The tentative stereostructures for **14** (major) and **14′** (minor) are assigned based on Kishi's model as drawn in Scheme 3.