

Total Synthesis of Pacidamycin D by Cu(I)-Catalyzed Oxy Enamide Formation

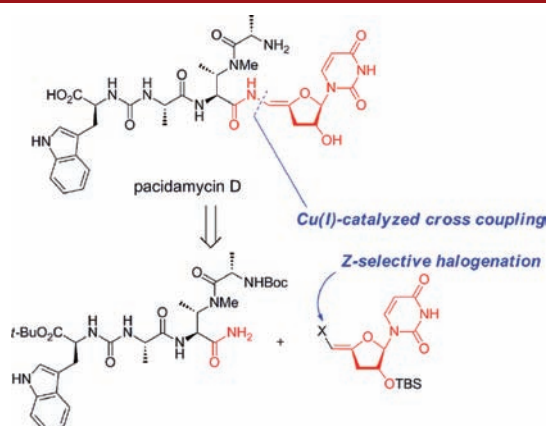
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ABSTRACT



The first total synthesis of pacidamycin D, which is expected to be a good candidate as an antibacterial agent against *P. aeruginosa*, is described. The key elements of our approach feature an efficient and stereocontrolled construction of the Z-oxyvinyl iodide and copper-catalyzed cross-coupling with the tetrapeptide carboxamide.

Uridylpeptide antibiotics are nucleoside natural products sharing a common structural feature, namely, a 3'-deoxyuridine with an enamide linkage at the 5'-position that is attached to a tetrapeptide moiety via a central α,β -diaminobutyric acid that connects the N-terminal amino acid, the ureadipeptide, and the 3'-deoxyuridine moieties (Figure 1).^{1,2} Among the class of uridylpeptide antibiotics, the pacidamycins (**1**),³ isolated from the fermentation

broth of the *Streptomyces coeruleorubiduns* strain, showed potent and selective antibacterial activity against strains of *Pseudomonas* (MIC 1.5–12.5 $\mu\text{g/mL}$). The biological target of the pacidamycins is believed to be phospho-MurNAc-pentapeptide transferase (MraY),^{4,5} which is responsible for the formation of lipid I in the peptidoglycan biosynthesis pathway.^{6–9} Since MraY is an essential enzyme in bacteria,^{1,2} it is a potential target for the

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development of general antibacterial agents. Consequently, uridylypeptide antibiotics which have a novel mode of action are expected to be good candidates as antibacterial agents effective against *P. aeruginosa*.

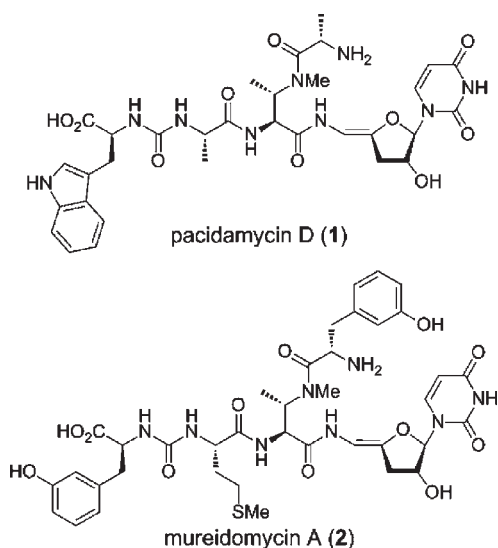
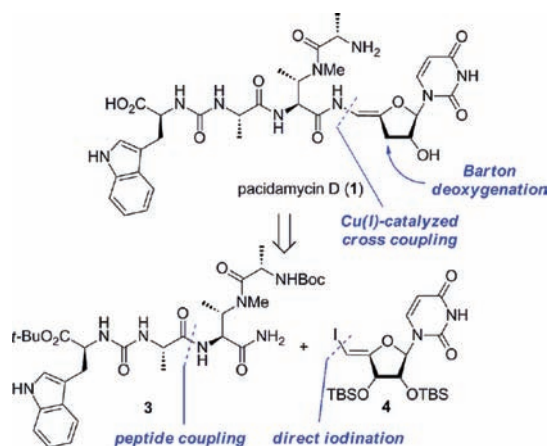


Figure 1. Structure of uridylypeptide natural products.

Despite extensive efforts to prepare analogues of the uridylypeptide antibiotics, including **1**,^{10–16} no total synthesis has yet been accomplished. The difficulty in the chemical synthesis **1** involves the *Z*-oxyenamide moiety, which is chemically labile and therefore a challenging chemical structure to construct. Moreover, analogues having the enamide functionality have been prepared only by semisynthesis from natural sources¹⁷ and by biosynthesis.¹⁸ Herein we describe the first total synthesis of pacidamycin D (**1**). Scheme 1 highlights the key elements of our retrosynthetic approach to the synthesis of **1**, which features an efficient and stereocontrolled construction of the *Z*-oxyvinyl iodide **4** and a copper-catalyzed cross-coupling¹⁹ of the iodide **4** with the highly functionalized

Scheme 1. Retrosynthetic Analysis of Pacidamycin D



tetrapeptide carboxamide **3**. The tetrapeptide carboxamide **3** contains a number of potentially reactive functional groups that render selective synthetic modification difficult. We first planned to remove the allylic 3'-hydroxyl group at the uridine moiety by Barton deoxygenation after the cross-coupling.

Preparation of the tetrapeptide is described in Scheme 2. The carboxylic acid **5**²⁰ and the pentafluorophenyl (Pfp) ester of the unsymmetrical urea **7**²¹ were prepared as previously described. Deprotection of the Boc group of **5** and the subsequent condensation of the liberated amine **6** with **7** gave the tripeptide **8**. *N*–*O* Bond breakage was achieved by catalytic hydrogenation, and the resulting secondary amine **9** (quant. over three steps from **5**) was further reacted with the Pfp ester of *N*-Boc-L-Ala **10** to afford the tetrapeptide carboxylic acid **11** in 69% yield. Finally, the carboxyl group of **11** was converted to the carboxamide (HATU, NH₄Cl, NMM, DMF) to give **3** in 82% yield.

The *Z*-oxyvinyl iodide **4** was prepared as shown in Scheme 3. After protecting group manipulation of the uridine derivative **12**²² (BOMCl, DBU, DMF, 99%, TFA–THF–H₂O, 0 °C, 83%), the primary alcohol of **14** was converted to the iodide (I₂, PPh₃, pyridine, dioxane, 99%). Elimination of HI from **15** was promoted by DBU to afford the *exo*-olefin **16**²³ in 93% yield. Previously, vinyl halide derivatives of nucleoside were generally prepared from an *exo*-olefin derivative by a rather lengthy conversion, where the terminal hydrogen atom was substituted sequentially with a phenylthio, a tributylstannyl, and an

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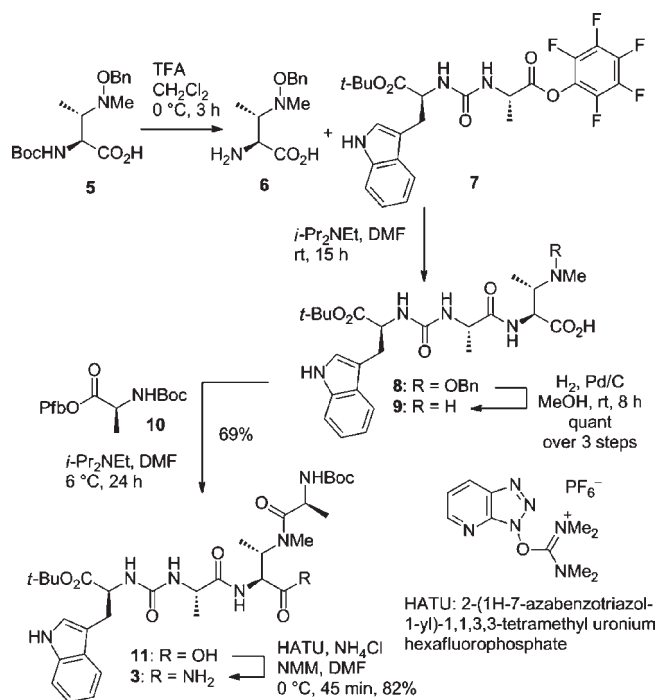
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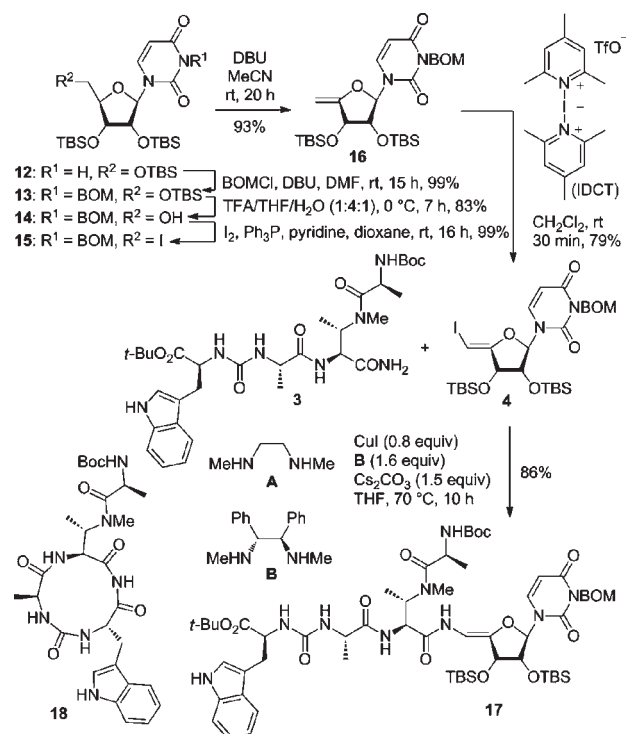
Scheme 2. Preparation of the Tetrapeptide Carboxamide **3**



iodo group.²⁴ Extensive efforts to obtain **4** directly from **16** revealed that the use of the iodonium dicollidinium triflate^{25,26} (IDCT) was indeed effective. The desired *Z*-vinyl iodide **4** was obtained in 79% yield as the sole product when **16** was treated with 1.0 equiv of IDCT in CH₂Cl₂ at room temperature. The geometry of the olefin was confirmed by a 500 MHz NOE experiment in CDCl₃, where the correlation to H-3' was observed upon irradiation at H-5' (7.2%).

Then, the key coupling of **4** with the tetrapeptide carboxamide **3** was investigated. First, the iodide **4** was reacted with **3** under the following conditions: 0.2 equiv of CuI, 0.4 equiv of MeNHCH₂CH₂NHMe (**A**), Cs₂CO₃, THF, 70 °C.^{27,28} However, a large amount of the iodide remained unreacted, and only a trace amount of the desired **17** was obtained. On the other hand, the tetrapeptide **3** was consumed, and cyclic products such as **18** were obtained from the reaction mixture indicated by MS analysis although not fully confirmed. In general, the copper-mediated C–N cross-coupling reaction proceeds through initial formation of the nitrogen–copper complex followed by an oxidative insertion into the halide and then reductive elimination.²⁹ It is presumed that if the oxidative insertion is slow, the nitrogen atom, activated by formation of the carboxamide–copper(I) complex, reacts

Scheme 3. Initial Attempt to Synthesize **1**



with the *tert*-Bu ester at the *C*-terminus to form the cyclic product **18**. In order to suppress the approach of the nitrogen atom to the *tert*-Bu ester, we increased the size of the ligand coordinating to the copper atom using ligands such as **B**. As expected, the use of the ligand resulted in an increased yield (32%). The yield of **17** was improved up to 86% by increasing the catalyst loading (0.8 equiv). Of note is the highly selective reaction at the *N*-unsubstituted carboxamide moiety in spite of the presence of a number of potential reactive sites, including the primary amide, the carbamate, and the urea groups.

Next, a selective deoxygenation of the allylic 3'-hydroxyl group on the model cyclic thiocarbonate **20**³⁰ was then investigated (Scheme 4). Thus, TBS groups of **19** were removed (TBAF, THF, 99%), and the resulting diol was reacted with phenyl chlorothionocarbonate to afford the cyclic thiocarbonate **20** in 75% yield. However, exposure of **20** to either Bu₃SnH and AIBN in toluene at reflux or Bu₃SnH and V-70³¹ in CH₂Cl₂ at room temperature led to a complex mixture of products, and the desired deoxygenated compound **21** was not isolated.

Since the model study in Scheme 4 suggested that the late stage deoxygenation of the 3'-hydroxyl group may be difficult, the total synthesis of **1** was pursued with the 3'-deoxyvinyl iodide **27** (Scheme 5). As in the synthesis of **4**,

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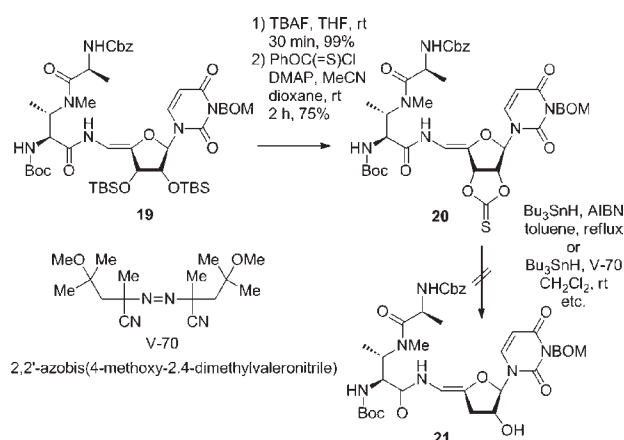
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(30) The model compound **19** was prepared in 89% yield in a similar manner to the synthesis of **17** from **4** and the corresponding dipeptide carboxamide.

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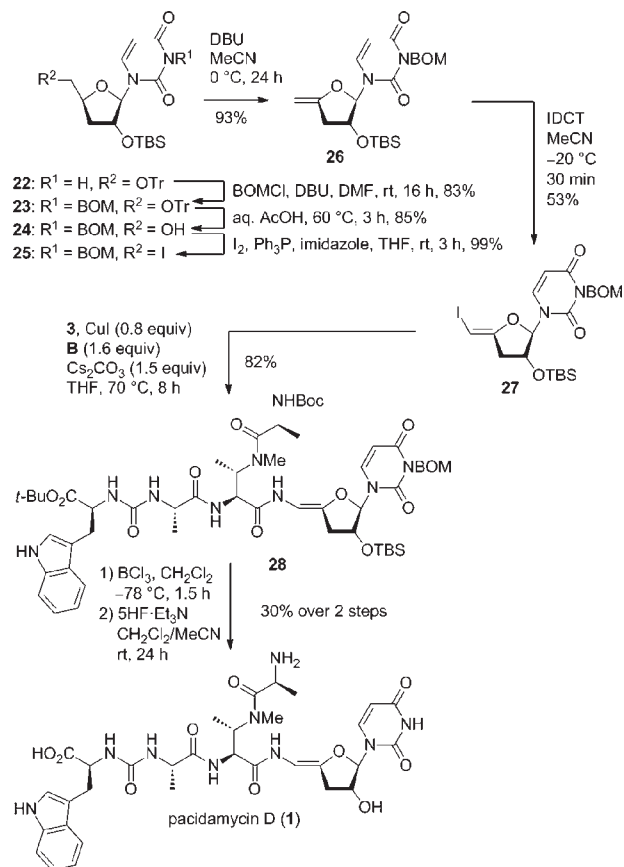
Scheme 4. Attempt of Deoxygenation with Model Compound **20**



the *exo*-olefin **26**, which was obtained from **22**,² was treated with IDTC in CH_2Cl_2 . However a significant amount of *E-exo*-olefin (10% yield) and *endo*-olefin (39%) were also produced in addition to the desired *Z-exo*-olefin **27** (28%). The observed decrease in selectivity could be attributed to the absence of the substituted hydroxyl group at the 3'-position. The yield of **27** was improved up to 53% by conducting the reaction in MeCN at -20°C although the effect of solvent on the selectivity remains unclear. The iodide **27** and the tetrapeptide **3** were coupled using the optimized conditions (0.8 equiv of CuI, 1.6 equiv of ligand **B**, Cs_2CO_3 , THF, 70°C) to afford the fully protected pacidamycin D **28** in 82% yield. Finally, deprotection of the BOM, Cbz, and *tert*-Bu groups (BCl_3 , CH_2Cl_2 , -78°C) and the TBS group ($5\text{HF}\cdot\text{NEt}_3$, 30% over two steps) successfully afforded pacidamycin D (**1**). Analytical data for the synthetic compound were in good agreement with those reported for the natural material.^{3d} Preliminary biological evaluation indicated that **1** showed potent inhibitory activity (IC_{50} 22 nM) against isolated *MraY* from *S. aureus* and antibacterial activity selectively against a range of *P. aeruginosa* strains (MIC 16 $\mu\text{g}/\text{mL}$ for *P. aeruginosa* ATCC 25619 and *P. aeruginosa* SR 27156 and 64 $\mu\text{g}/\text{mL}$ for *P. aeruginosa* PAO1, respectively).

In conclusion, the first total synthesis of pacidamycin D (**1**) has been accomplished. By virtue of the assemblage, via cross-coupling, of the *Z*-oxyvinylhalide **27** and the tetrapeptide **3** at a late stage in the synthesis, and despite the challenges this imposes because of the inherent lability with

Scheme 5. Total Synthesis of **1**



potential epimerization, this approach provided ready access to a range of uridylypeptide antibiotics and their analogs simply by altering the tetrapeptide moiety. Results of further studies will be forthcoming.

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Supporting Information Available. Full experimental procedures and characterization data for all new compounds are available. This material is available free of charge via the Internet at <http://pubs.acs.org>.