ORGANIC LETTERS

2011
Vol. 13, No. 19
5240–5243

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

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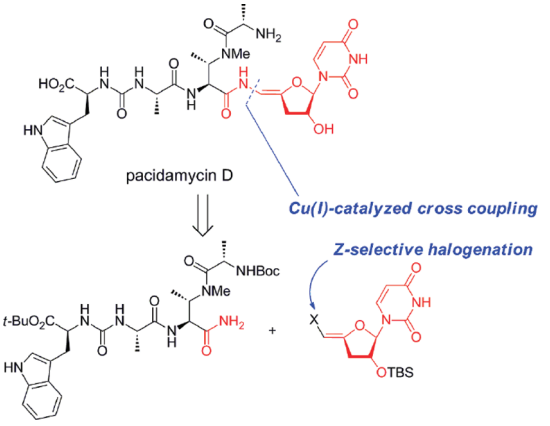
Received August 5, 2011

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 μg/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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10.1021/ol202124b
Published on Web 09/08/2011

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

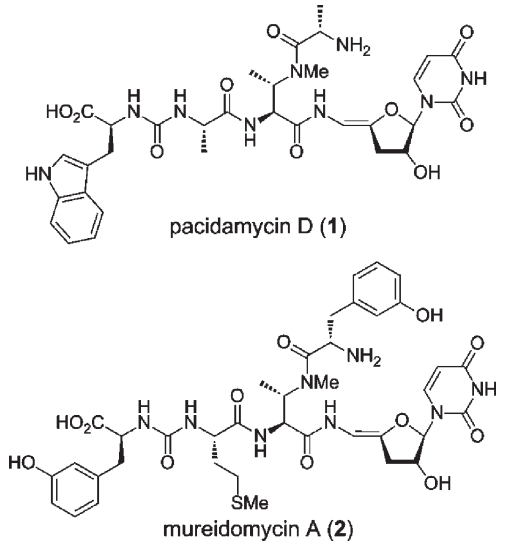
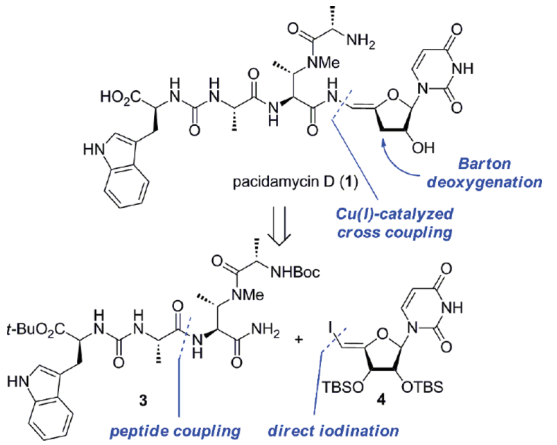


Figure 1. Structure of uridylpeptide natural products.

Despite extensive efforts to prepare analogues of the uridylpeptide 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 sources[17] 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-coupling[19] 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, NH_4Cl, NMM, DMF) to give **3** in 82% yield.

The *Z*-oxyvinyl ioide **4** was prepared as shown in Scheme 3. After protecting group manipulation of the uridine derivative **12**[22] (BOMCl, DBU, DMF, 99%, TFA–THF–H_2O, 0 °C, 83%), the primary alcohol of **14** was converted to the iodide (I_2, PPh_3, 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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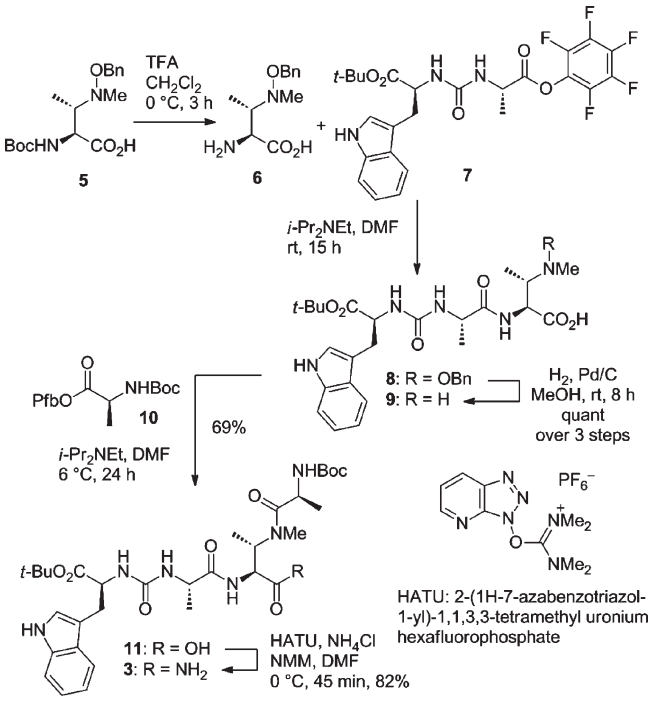
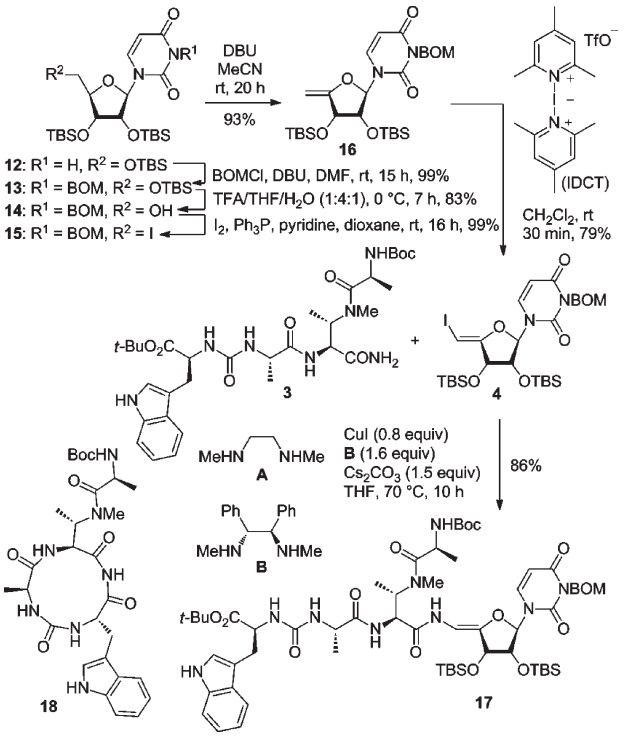
Scheme 2. Preparation of the Tetrapeptide Carboxamide **3**



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



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 CH_2Cl_2 at room temperature. The geometry of the olefin was confirmed by a 500 MHz NOE experiment in $CDCl_3$, 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 $MeNHCH_2CH_2NHMe$ (**A**), Cs_2CO_3, 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 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 Bu_3SnH and AIBN in toluene at reflux or Bu_3SnH and V-70[31] in CH_2Cl_2 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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(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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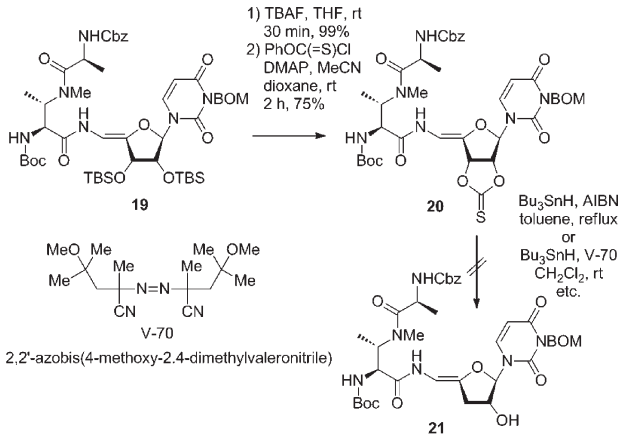
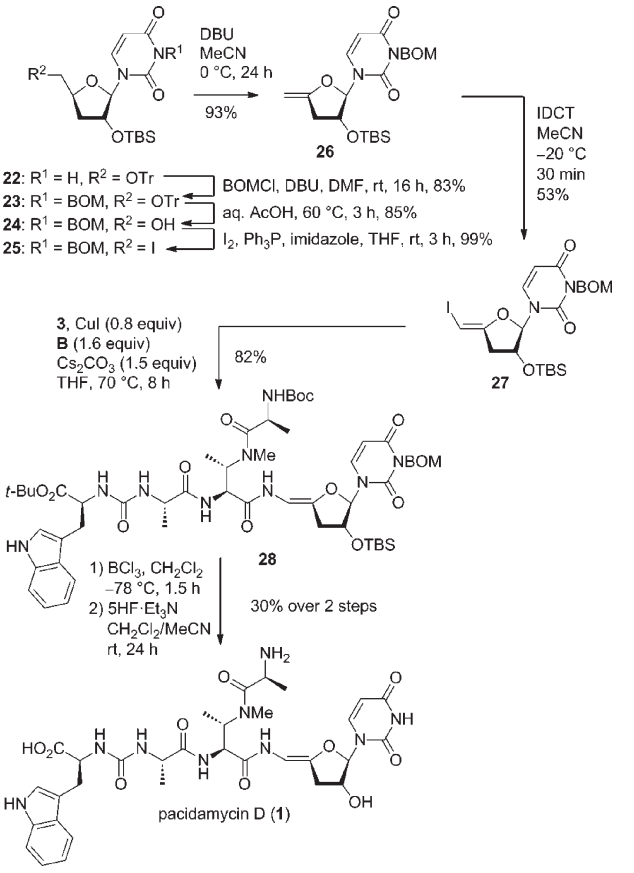
Scheme 4. Attempt of Deoxygenation with Model Compound **20**



Scheme 5. Total Synthesis of **1**



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 ($5HF \cdot NEt_3$, 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 μg/mL for *P. aeruginosa* ATCC 25619 and *P. aeruginosa* SR 27156 and 64 μg/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 potential epimerization, this approach provided ready access to a range of uridylpeptide antibiotics and their analogs simply by altering the tetrapeptide moiety. Results of further studies will be forthcoming.

Acknowledgment. We thank Mr. Kouichi Uotani (Medicinal Research Laboratories, Shionogi & Co., Ltd.) for evaluating the antibacterial activities and Ms. Fumiyo Takahashi (Shionogi Innovation Center for Drug Discovery, Shionogi & Co., Ltd) for evaluating the MraY inhibitory activity.

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.