Synthesis of Pacidamycin Analogues via an Ugi-Multicomponent Reaction

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Abbreviations

α,β-diaminobutylic acid (DABA), 2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), phospho-MurNAc-pentapeptide transferase (MraY), *N*-methylmorpholine (NMM), structure-activity relationship (SAR), Ugi-four component reaction (U-4CR)

Abstract

The second-generation synthesis of 3'-hydroxypacidamycin D (**2**) has been accomplished via an Ugi-four component reaction at a late stage of the synthesis. This approach provided ready access to a range of analogues including diastereomers of the diaminobutylic acid residue and hybrid-type analogues of mureidomycins. Biological evaluations of these analogues indicated that the stereochemistry at the diaminobutylic acid residue has a crucial impact on both the MraY biochemical inhibition and whole-cell antibacterial activity.

The pacidamycins (Figure 1, **1**),[1] first isolated from *Streptomyces coeruleorubidus* AB 1183F-64 in 1989, are members of a class of uridylpeptide antibiotics,[2] that are composed of the mureidomycins,[3] the napsamycins,[4] and the recently identified sansanmycins.[5] Sharing a characteristic structural feature, namely a 3′-deoxyuridine which is attached to a tetrapeptide moiety by an enamide linkage. These compounds exhibit selective antibacterial activity against *Pseudomonas aeruginosa*, a common nosocomial pathogen that is intrinsically resistant to a variety of drugs currently used in the clinic. Consequently, immuno-compromised patients are at greater risk of infection. Moreover, the increased rate of acquired multidrug resistance in *P. aeruginosa* complicates anti-Pseudomonas chemotherapy, thereby making the development of novel antibacterial agents that are active against multi-drug resistance *P. aeruginosa* all the more urgent.[6-8] These uridylpeptide antibiotics are sub-nanomolar inhibitors of their biological target, the phospho-MurNAc-pentapeptide transferase (MraY),[2,9] which is responsible for the formation of lipid I in the peptidoglycan biosynthesis pathway.[2,10] Inhibition of the MraY by these antibiotics causes a malfunction of the peptidoglycan production in a manner different from β-lactams such as the carbapenems. Since MraY is an essential enzyme in bacteria[2] and a novel target, uridylpeptide antibiotics are expected to be highly useful anti-Pseudomonal agents. Intrigued by the promising

biological activity, several groups have conducted structure-activity relationship studies.[11-15]

We have recently accomplished the total synthesis of pacidamycin D (**1**) and its 3′-hydroxyl analogue **2**, which features a stereoselective construction of the *Z*-oxy-acyl enamide architecture by a copper-catalyzed C-N cross-coupling.[16] These compounds exhibited strong MraY inhibitory activity (IC_{50} = 22 nM for **1**, 42 nM for **2**) and potent antibacterial activity against *P. aeruginosa* (MIC 8-64 μg/mL). The existence of the hydroxyl group at the 3′-position of the uridine moiety does not have any impact on either MraY inhibition or antibacterial activity. This first generation strategy, however, is a linear synthesis, where the vinyl iodide derivative of uridine was coupled with the tetrapeptide carboxamide moiety. Nonetheless, from a medicinal point of view, the development of a convergent synthetic strategy that would provide analogues more efficiently is preferable. A multi-component reaction would be ideally suited for structure-activity relationship (SAR) studies since it allows for diversification of accessible analogs simply by altering each component. We have successfully applied an Ugi-four component reaction (U-4CR) in pursuing the SAR of the muraymycins, which are antibacterial nucleoside natural products.[17] Herein we describe the second-generation synthesis of **2** via a U-4CR in order to establish a convergent

synthetic strategy appropriate for the SAR study of uridylpeptide antibiotics. The biological evaluation of several analogues is also described.

Our second-generation retrosynthetic analysis of **2** is summarized in Scheme 1. We planned to install the *N*-terminal amino acid residue **3** on **4** in the final stage of the synthesis considering the initial efforts to prepare **2** as described later. We retrosynthetically divided **4** into the urea dipeptide **5**, 2,4-dimethoxybenzylamine **6**, the 2-*N*-methylaminopropionaldehyde derivative **7**, and the α,β-unsaturated isonitrile derivative of uridine **8** for the U-4CR. This strategy allowed us to construct **4** with the non-proteinogenic amino acid α,β-diaminobutylic acid (DABA) residue, with linkages to the urea dipeptide and uridine moieties at the *C*- and *N*-termini. Moreover, the U-4CR strategy has the advantage of efficiently furnishing diastereomers that are useful for the SAR. Considering the nature of the multi-component assemblage, this strategy allows easy access to diversified analogues.

The aldehyde unit **7** was prepared as shown in Scheme 2. Namely, *N*-formyl-L-alanine **9** was reduced by $LiAlH_4$, and the resulting *N*-methylaminoalcohol was selectively protected by a Boc group to provide **10** in 98% yield over 2 steps. The primary hydroxyl group of **10** was oxidized by Dess-Martin periodinane to give **7** in 84% yield. The isonitrile **8** was prepared from the *Z*-enamide **12** by the

copper-catalyzed C-N cross-coupling of the *Z*-vinyl iodide **11**[16] and formamide as describe in Scheme 3. The iodide **11** was reacted with formamide using 0.2 equiv. of CuI, 0.4 equiv. of $MeNHCH_2CH_2NHMe$, Cs_2CO_3 in THF at 70 °C to give the desired *Z*-enamide **12**, which was obtained selectively in 90% yield. The corresponding *E*-isomer was not observed at all during the course of the reaction. Dehydration of **12** was achieved by a treatment with triphosgene and Et_3N in CH_2Cl_2 at −78 °C to provide the isonitrile **8** in 88% yield.

With **7** and **8** in hand, we then examined the assemblage of the four components by the U-4CR (Scheme 4). The assemblage proceeded simply by mixing **5**, **6**, **7**, and **8** in EtOH at room temperature for 48 h, and the desired **13** and its diastereomer at the newly formed stereogenic center were obtained in acceptable yields (33% for **13** and 30% for **14**). These were separated by silica gel column chromatography. Initially we planned to use an aldehyde of the dipeptide derivative such as **21**, which was prepared by reduction of the Weinreb amide derivative of the dipeptide **20**, as the aldehyde component in the U-4CR assemblage (Scheme 5). However, it turned out that the resulting aldehyde **21** easily cyclized to form a rather stable cyclic aminal **22**, and the U-4CR did not proceed at all. Therefore we turned our attention to the stepwise synthetic route toward the target compounds as shown in Scheme 4 in order to avoid the undesired cyclization.

The final assemblage of the remaining amino acid and global deprotection were then investigated. Upon selective removal of the Boc group of **13** (10% TFA, CH_2Cl_2, 0 °C), the liberated secondary amine of **15** was condensed with *N*-Boc-L-Ala **3** (HATU, *N*-methylmorpholine (NMM), CH_2Cl_2) to give the fully protected 3′-hydroxyl pacidamycin D analogue **17** in 79% yield over 2 steps. Finally removal of all the protecting groups (BCl_3, CH_2Cl_2, −78 °C, $5HF{\cdot}NEt_3$, 22% over 2 steps) successfully afforded **2**. The analytical data for **2** in this second generation synthesis were identical to those of the material previously synthesized.[16a] The deprotection step gave poor yield of the desired **2** because BCl_3 treatment resulted in decomposition of the acid sensitive enamide moiety as well as migration of the 2,4-dimethoxy group to the indole moiety of the Trp residue. Similar problems were also observed for the formation of **19**, **27**, **30**, and **31**, which were described later. The epimer at the DABA residue **19** was also prepared from **14** in a manner similar to that of the synthesis of **2**.

The mureidomycins (Figure 2)[3] were first isolated from *Streptomyces flavidoviridens* SANK 60486 and belong to the same class of uridylpeptide antibiotics. However, their amino acids differ from those of the pacidamycins. Mureidomycin C, which has the dipeptide (Gly-L-*m*-Tyr) at the *N*-terminus, showed the most potent

antibacterial activity against strains of *P. aeruginosa* among the uridylpeptide antibiotics with MICs ranging from 0.1 to 1.56 μg/mL.[3b]

We therefore planned to prepare an analogue **27a**, which is a hybrid-type of pacidamycin D and mureidomycin C, by introducing the Gly-L-*m*-Tyr moiety into the intermediates **15** and **16** (Scheme 6). The analogue **27b**, where the L-*m*-Tyr residue at the *N*-terminus was replaced by L-Tyr, was designed in order to determine the consequences of placing of the hydroxyl group in this position. The glycine analogue **27c** was also prepared. These analogues **27a-c**, as well as **30**, the epimer of **27a** at the DABA residue, were prepared by the same strategy as that for the synthesis of **2** and **19** by using the dipeptide **23**, **24**,[18] or Boc-Gly-OH (**25**). Of particular note was the coupling of the amine derived from **16** and the dipeptide **23**, where extensive racemization occurred to give predominantly the epimer **29** (22% for **28** vs. 66% for **29**). Deprotection of **29** gave **31**, the diastereomer at the L-*m*-Tyr residue of **30**.

The inhibitory activity of the analogues **2**, **19**, **27a-c**, **30**, and **31** on the purified MraY enzyme (*Staphylococcus aureus*) was next examined by fluorescence based MraY assay using UDP-MurNAc-dansylpentapeptide, where the formation of dansylated lipid I was monitored by fluorescence enhancement (excitation at 355 nm, emission at 535 nm),[19] and the results are summarized in Table 1. It turned out that the epimer **19** was much

weaker than the MraY inhibitors **1** or **2** with an IC_{50} value of 4000 nM. The stereochemistry at the DABA residue was very important for enzyme inhibitory activity. This was also true for the analogues **30** and **31**, which possess the same stereochemistry at the DABA residue (IC_{50} 650 and 8900 nM, respectively). Analogue **27a** showed similar inhibitory activity (IC_{50} 22 nM) to that of **1** and **2**, and introducing the Gly-L-*m*-Tyr moiety at the *N*-terminus did not improve but instead retained the activity. However, moving the hydroxyl group to the *para*-position at the *N*-terminal phenyl ring resulted in a large decrease in inhibitory activity with an IC_{50} value of 4000 nM for **27b**. Therefore the position of the hydroxyl group on the phenyl ring plays a crucial role. Analogue **27c** showed a slight decrease in MraY inhibitory activity. The antibacterial activity of **2**, **19**, **27a-c**, **30**, and **31** was then evaluated against a range of clinically isolated *P. aeruginosa* strains (Table 2).[20] The overall antibacterial activity was well correlated to the MraY inhibitory activity. Thus, **27a** showed a similar anti-Pseudomonal activity to that of **1** and **2**, whereas **19**, **27b**, **27c**, **30,** and **31** exhibited no activity up to 32 μg/mL. The exception was the Gly analogue **27c**, which showed no anti-Pseudomonal activity in spite of potent MraY inhibitory activity (Table 1, IC_{50} 65 nM).

In summary, the second-generation synthesis of **2** has been accomplished via a U-4CR at a late stage of the synthesis. This approach provided ready access to a range of analogues including the diastereomers of the DABA residue and hybrid-type analogues of the mureidomycins. Biological evaluations of these analogues indicated that the stereochemistry at the DABA residue has a significant impact on both the MraY biochemical inhibition and whole-cell antibacterial activity.

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18. The dipeptides **23** and **24** were prepared by peptide coupling of either L-Tyr-OBn or L-*m*-Tyr-OBn with **25**, protection of the phenol with the TBS group, and hydrogenolysis of the Bn group.
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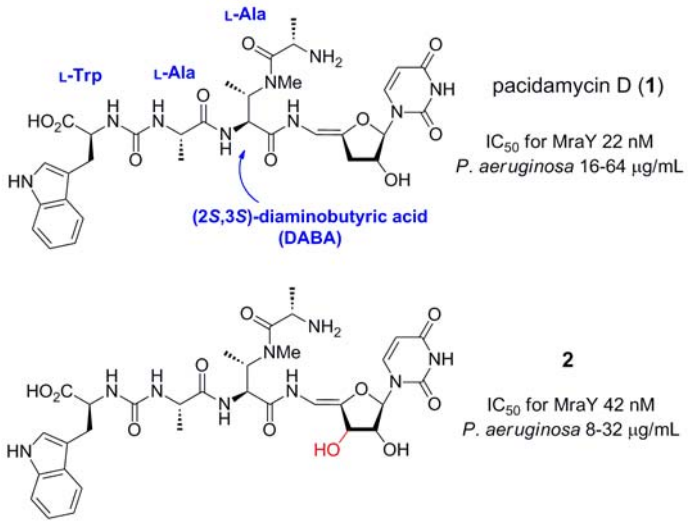


Figure 1. Structure of Pacidamycin D and Its 3'-Hydroxy analogue 2

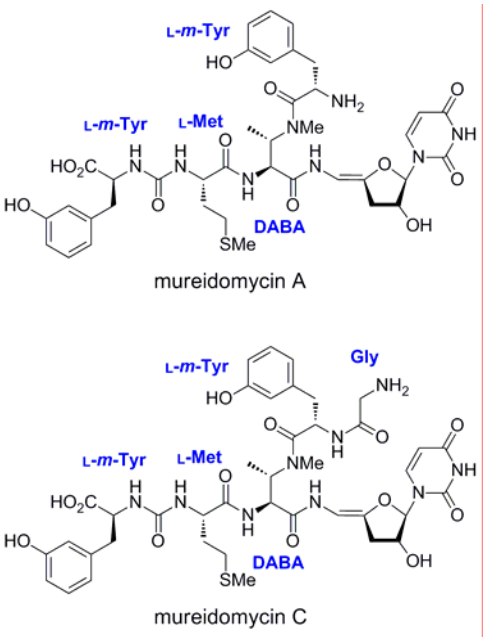
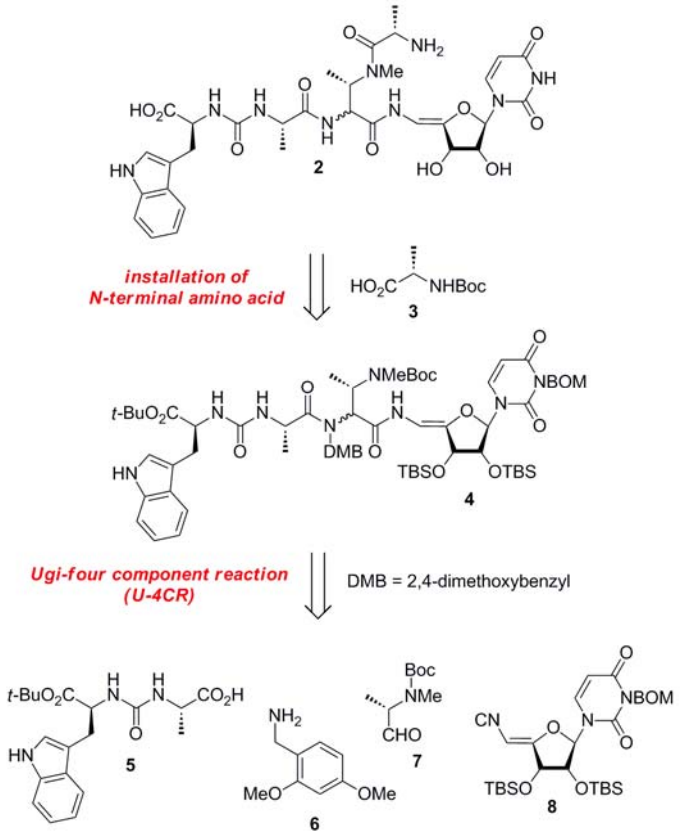
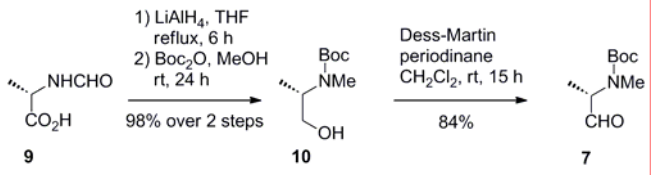


Figure 2. Structure of Mureidomycins

Scheme 1. Retrosynthetic Analysis of 2 Disconnected by Ugi-Four Component Reaction

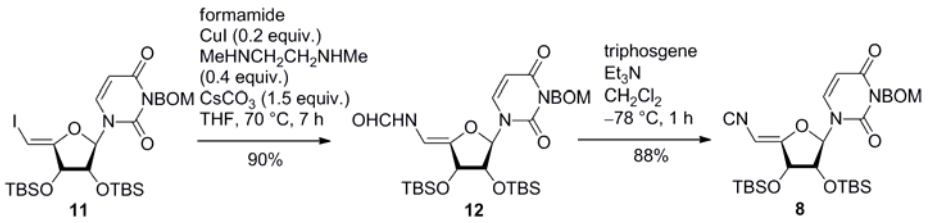


Scheme 2. Preparation of Aldehyde Unit 7

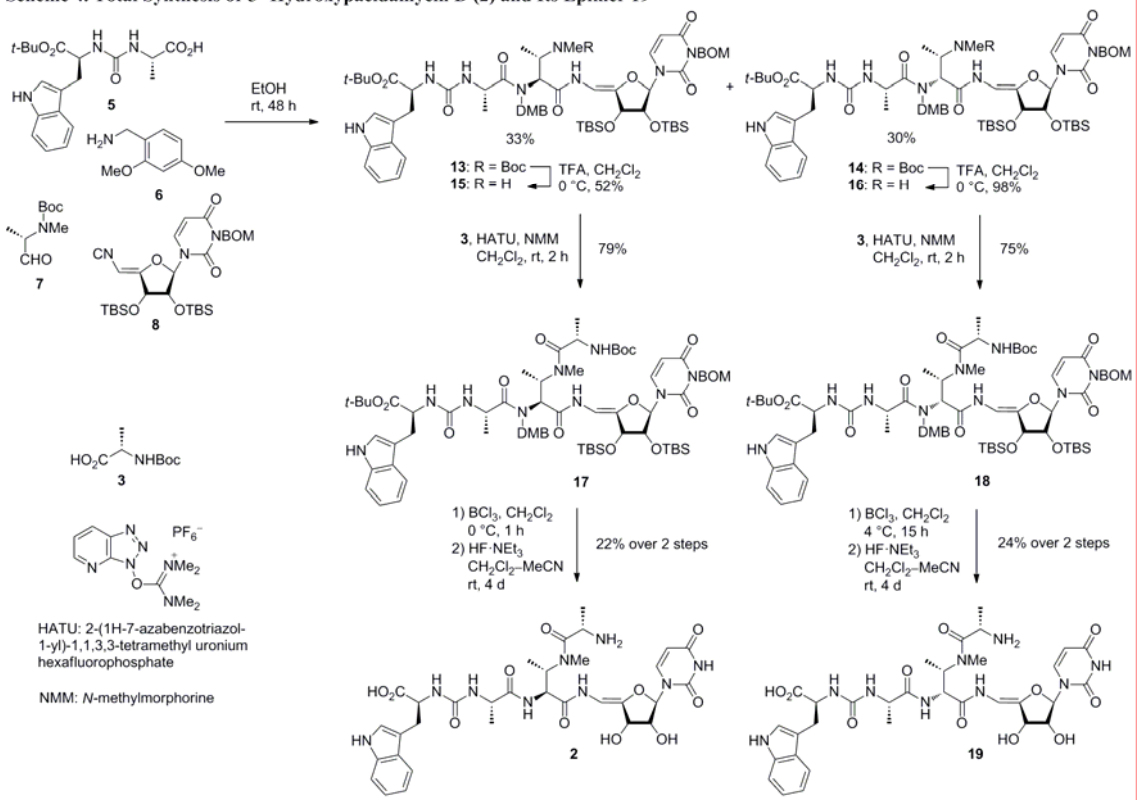


9 **10** **7**

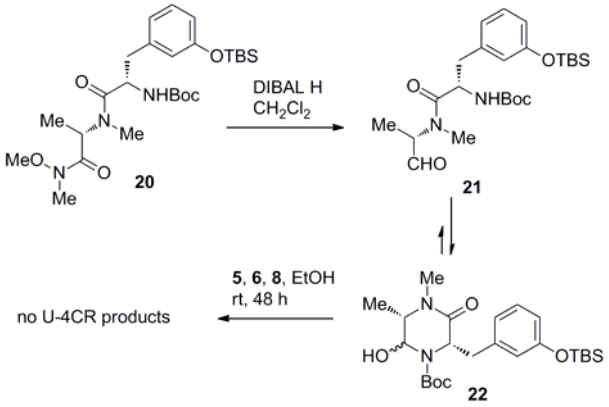
Scheme 3. Preparation of Isonitrile Unit 8



Scheme 4. Total Synthesis of 3'-Hydroxypacidamycin D (2) and Its Epimer 19



Scheme 5. Attempt to Use Dipeptide Aldehyde 21 in U-4CR



Scheme 6. Synthesis of Analogues 27a-c, 30 and 31

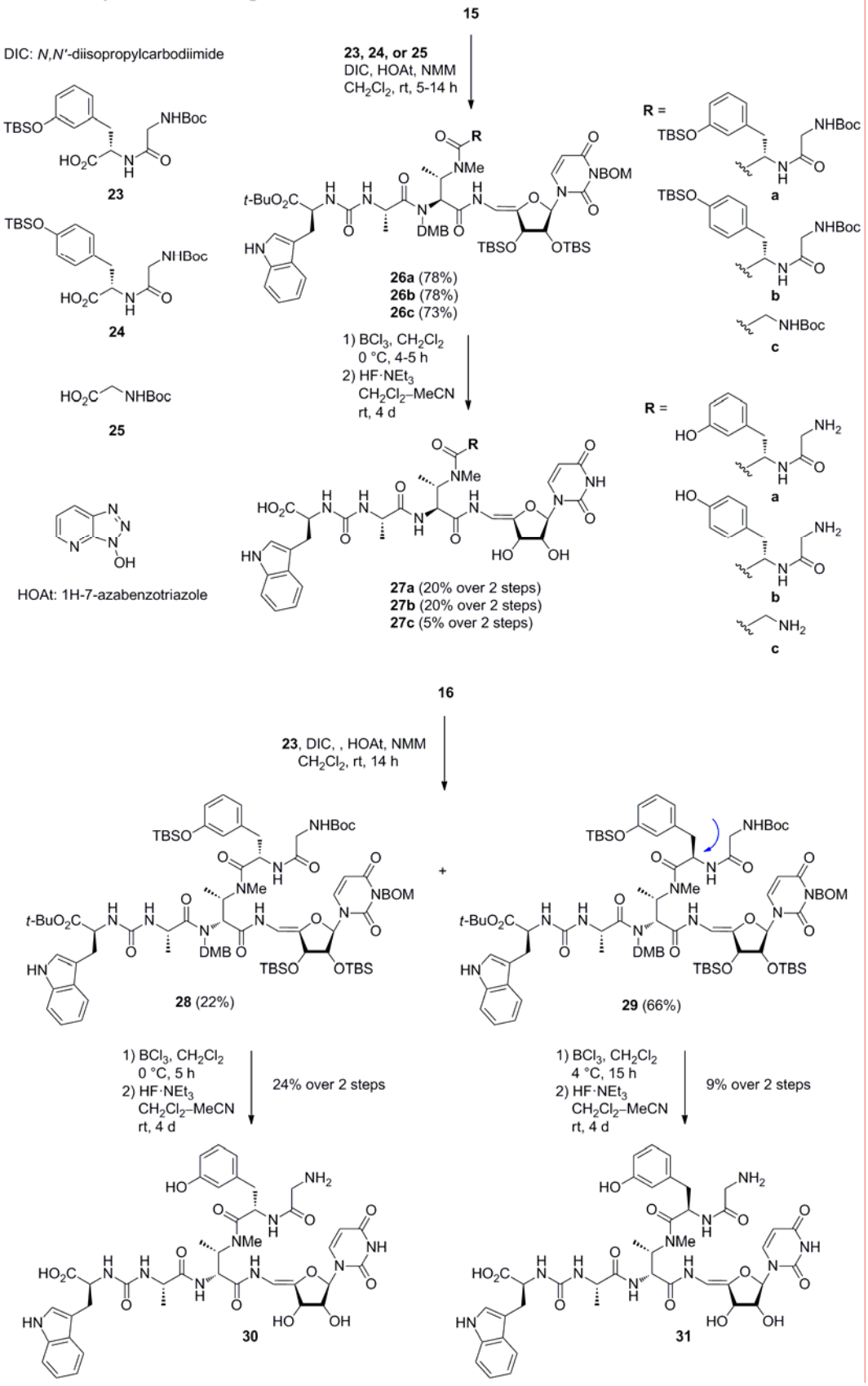


Table 1. MraY Inhibitory Activity of 3'-Hydroxypacidamycin Analogues

	1	**2**	**19**	**27a**	**27b**	**27c**	**30**	**31**
IC_{50} (nM)[a]	22	42	4000	22	4000	65	650	8900

[a]The inhibitory activity of compounds on the purified MraY enzyme (*Staphylococcus aureus*) was then examined by fluorescence based MraY assay using UDP-MurNAc-dansylpentapeptide, where the formation of dansylated lipid I was monitored by fluorescence enhancement (excitation at 355 nm, emission at 535 nm).

Table 2. Anti-Pseudomonal Activity of 3'-Hydroxypacidamycin Analogues

compound	MIC (μg/mL)[a]			
	P. aeruginosa PAO1	*P. aeruginosa* YY165 (ΔmexB)	*P. aeruginosa* ATCC 25619	*P. aeruginosa* SR 27156
1	64	16	16	16
2	32	8	16	16
19	>32	>32	>32	>32
27a	>32	16	32	16
27b	>32	>32	>32	>32
27c	>32	>32	>32	>32
30	>32	>32	>32	>32
31	>32	>32	>32	>32

[a]MICs were determined by a microdilution broth method as recommended by the NCCLS with cation-adjusted Mueller-Hinton broth (CA-MHB). Serial two-fold dilutions of each compound were made in appropriate broth, and the plates were inoculated with 5 x 10^4 CFU of each strain in a volume of 0.1 mL. Plates were incubated at 35 °C for 20 h and then MICs were scored.

Captions

Figure 1. Structure of Pacidamycin D and Its 3'-Hydroxy analogue **2**

Figure 2. Structure of the Mureidomycins

Scheme 1. Retrosynthetic Analysis of **2** Disconnected by an Ugi-Four Component Reaction

Scheme 2. Preparation of the Aldehyde Unit **7**

Scheme 3. Preparation of the Isonitrile Unit **8**

Scheme 4. Total Synthesis of 3'-Hydroxypacidamycin D (**2**) and Its Epimer **19**

Scheme 5. Attempt to Use the Dipeptide Aldehyde **21** in the U-4CR

Scheme 6. Synthesis of Analogues **27a-c**, **30** and **31**

Table 1. MraY Inhibitory Activity of 3'-Hydroxypacidamycin Analogues

Table 2. Anti-Pseudomonal Activity of 3'-Hydroxypacidamycin Analogues