Synthesis of Pacidamycin Analogues via an Ugi-Multicomponent Reaction

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Abbreviations

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

The second-generation synthesis of 3'-hydroxyacidamycin 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), first isolated from *Streptomyces coeruleorubidus* AB 1183F-64 in 1989, are members of a class of uridylpeptide antibiotics, that are composed of the mureidomycins, the napsamycins, and the recently identified sansanmycins. 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. These uridylpeptide antibiotics are sub-nanomolar inhibitors of their biological target, the phospho-MurNAc-pentapeptide transferase (MraY), which is responsible for the formation of lipid I in the peptidoglycan biosynthesis pathway. 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 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.\textsuperscript{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.\textsuperscript{16} These compounds exhibited strong MraY inhibitory activity (IC\textsubscript{50} = 22 nM for 1, 42 nM for 2) and potent antibacterial activity against \textit{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.\textsuperscript{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 LiAlH₄, 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 and formamide as describe in Scheme 3. The iodide 11 was reacted with formamide using 0.2 equiv. of CuI, 0.4 equiv. of MeNHCH₂CH₂NHMe, Cs₂CO₃ 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 Et₃N in CH₂Cl₂ 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, CH₂Cl₂, 0 °C), the liberated secondary amine of 15 was condensed with N-Boc-L-Ala 3 (HATU, N-methylmorpholine (NMM), CH₂Cl₂) to give the fully protected 3'-hydroxyl pacidamycin D analogue 17 in 79% yield over 2 steps. Finally removal of all the protecting groups (BCl₃, CH₂Cl₂, −78 °C, 5HF·NEt₃, 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 BCl₃ 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.\textsuperscript{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-\textit{m}-Tyr moiety into the intermediates 15 and 16 (Scheme 6). The analogue 27b, where the L-\textit{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,\textsuperscript{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-\textit{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),\textsuperscript{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 $\mu$g/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.

References


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


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

Installation of N-terminal amino acid

Ugi-four component reaction (U-4CR)

DMB = 2,4-dimethoxybenzyl
Scheme 2. Preparation of Aldehyde Unit 7

1) LiAlH₄, THF reflux, 6 h
2) Boc₂O, MeOH rt, 24 h

10

98% over 2 steps

84%

Dess-Martin periodinane
CH₂Cl₂, rt, 15 h

10

Roc

NMe

CHO

7
Scheme 3. Preparation of Isonitrile Unit 8

11

<table>
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<th>Step</th>
<th>Reaction Conditions</th>
<th>Product</th>
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</table>

**Chemical Reactions**

- **Isonitrile Unit 8**
  - **Step 1**: Treatment with formamide (0.2 equiv.) and MeHNC\(\text{CH}_2\text{NMe}\) (0.4 equiv.) in THF, 70 °C, 7 h, yield 90%.
  - **Step 2**: Treatment with triphosgene in CH\(_2\text{Cl}_2\) at -78 °C, 1 h, yield 88%.
Scheme 4. Total Synthesis of 3'-Hydroxyacidamycin D (2) and Its Epimer 19

HATU: 2-(1H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
NMMe: N-methylmorpholine
Scheme 5. Attempt to Use Dipeptide Aldehyde 21 in U-4CR

20

\[
\begin{align*}
\text{CHO} & \\
\text{Me} & \\
\text{Me} & \\
\text{NHBoc} & \\
\text{Me} & \\
\text{Me} & \\
\text{OTBS} & \\
\end{align*}
\]

DIBAL-H

\[
\begin{align*}
\text{CHO} & \\
\text{Me} & \\
\text{Me} & \\
\text{NHBoc} & \\
\text{Me} & \\
\text{Me} & \\
\text{OTBS} & \\
\end{align*}
\]

no U-4CR products

5, 6, 8, EtOH

rt, 48 h

21

\[
\begin{align*}
\text{HO} & \\
\text{N} & \\
\text{Boc} & \\
\text{Me} & \\
\text{Me} & \\
\text{Me} & \\
\text{OTBS} & \\
\end{align*}
\]

22
Scheme 6. Synthesis of Analogues 27a-c, 30 and 31

DIC: N,N'-disopropylcarbodiimide

23, 24, or 25
DIC, HOAt, NMM
CH₂Cl₂, rt, 5-14 h

R = TBSO

OH

26a (78%)

26b (78%)

26c (73%)

1) BCl₃, CH₂Cl₂
0 °C, 4-5 h
2) HF·NEt₃,
CH₂Cl₂-MeCN
rt, 4 d

R = TBSO

27a (20% over 2 steps)

27b (20% over 2 steps)

27c (5% over 2 steps)

HOAt: 1H-7-azabenzotriazole

23, DIC, HOAt, NMM
CH₂Cl₂, rt, 14 h

TBSO

HN

28 (22%)

BN

29 (66%)

1) BCl₃, CH₂Cl₂
0 °C, 5 h
2) HF·NEt₃,
CH₂Cl₂-MeCN
rt, 4 d

24% over 2 steps

1) BCl₃, CH₂Cl₂
4 °C, 15 h
2) HF·NEt₃,
CH₂Cl₂-MeCN
rt, 4 d

9% over 2 steps

HN

30

HN

31
### Table 1. MraY Inhibitory Activity of 3'-Hydroxypacidamycin Analogues

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<th></th>
<th>1</th>
<th>2</th>
<th>19</th>
<th>27a</th>
<th>27b</th>
<th>27c</th>
<th>30</th>
<th>31</th>
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<td>22</td>
<td>42</td>
<td>4000</td>
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<td>65</td>
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ᵃThe 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).
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<th>P. aeruginosa ATCC 25619</th>
<th>P. aeruginosa SR 27156</th>
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<td>27a</td>
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* 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 10^6 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