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Synthesis of Base-Modified Dihydropacidamycins

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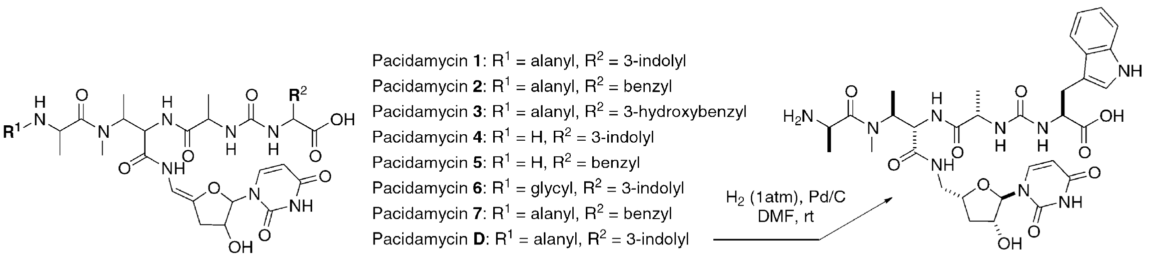
Abstract—We describe in this paper the synthesis of 1,2-di-*O*-acetyl-5-azido-3,5-dideoxy-α,β-L-arabinofuranose, a carbohydrate donor that was used for the synthesis of 1-(5′-amino-3′,5′-dideoxy-α-L-arabinofuranosyl)uracil, the nucleoside found in dihydropacidamycin D. The carbohydrate donor was also used for the synthesis of a set of new nucleosides that were introduced in new dihydropacidamycins. These compounds were tested for biological activity, and the results showed that uracil is the only base recognized by MraY. © 2002 Elsevier Science Ltd. All rights reserved.

The incidence of bacterial resistance to antibiotics continues to increase at an alarming rate.[1] Furthermore, cross-resistance (the simultaneous resistance in a single organism to more than one class of antibacterial agents) is also on the rise. To combat these phenomena, there is a clinical need not merely for variants within existing classes of antimicrobial agents, but also for entirely new classes of antibiotics. The pacidamycins[2] (see Fig. 1) are natural product members of the family of uridyl peptide antibiotics (UPAs) also represented by the mureidomycins[3] and the napsamycins.[4] The UPAs provide an attractive starting point for the development of a novel class of antibacterial drugs, in that they share a bacterial cellular target that has yet to be exploited in the clinical context. This target, MraY, is the enzyme (phospho-*N*-acetylmuramyl-pentapeptide translocase) that catalyzes the first reaction in the membrane-bound cycle of reactions in bacterial peptidoglycan biosynthesis.[5] The pacidamycins are isolated from *Streptomyces coeruleorubidus* and show specific bactericidal activity against *Pseudomonas aeruginosa*.[6] Recently we reported the isolation, purification and characterization of pacidamycin D, the lowest molecular weight pacidamycin.[7] We showed that the compound resulting from reduction of the chemically unstable C(4′)-*exo* double bond retained antibacterial activity similar to that of pacidamycin D. We also assigned the absolute stereochemistry of the eight chiral centers of dihydropacidamycin D (see Fig. 1), and developed an efficient total synthesis.[8]

In the context of our SAR studies on dihydropacidamycin D, we were interested in assessing the importance of the uracil ring of the nucleoside for binding to MraY. We therefore needed the means to prepare a wide variety of base-modified dihydropacidamycins.



Pacidamycin 1: R^1 = alanyl, R^2 = 3-indolyl
Pacidamycin 2: R^1 = alanyl, R^2 = benzyl
Pacidamycin 3: R^1 = alanyl, R^2 = 3-hydroxybenzyl
Pacidamycin 4: R^1 = H, R^2 = 3-indolyl
Pacidamycin 5: R^1 = H, R^2 = benzyl
Pacidamycin 6: R^1 = glycyl, R^2 = 3-indolyl
Pacidamycin 7: R^1 = alanyl, R^2 = benzyl
Pacidamycin D: R^1 = alanyl, R^2 = 3-indolyl

Dihydropacidamycin D (9a)

Figure 1. The pacidamycin family of UPAs. Hydrogenation of pacidamycin D.

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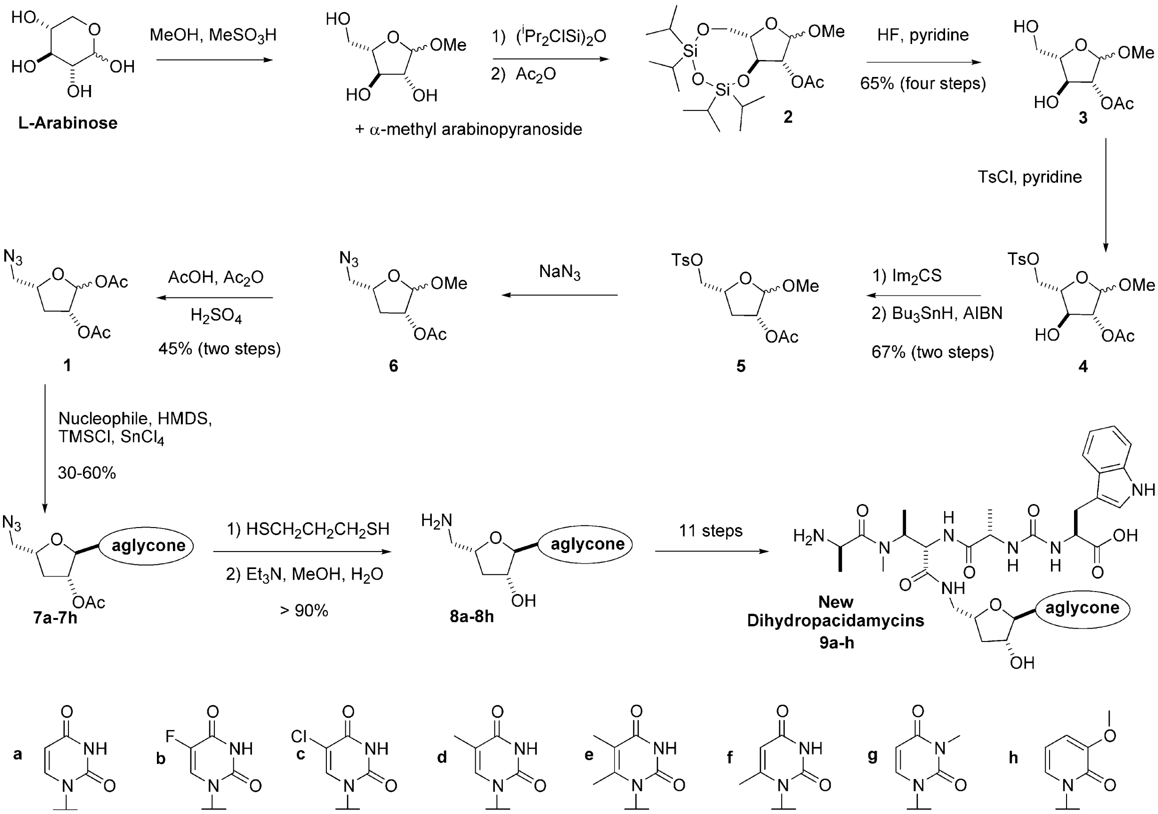

Scheme 1. Synthesis of base-modified dihydropacidamycins.

In order to achieve sufficient nucleoside diversity, we required a carbohydrate intermediate that would allow the introduction of a wide range of *N*- and *O*-aglycones. We also wanted to introduce the aglycone moiety at a late stage in the synthesis in order to minimize sequential chemical steps involved in the synthesis of a new dihydropacidamycin. Most of the functionality present in the molecule had therefore to be present prior to glycosylation. Finally, the synthesis had to be amenable to scale-up to allow many nucleosides to be prepared. The intermediate **1** (see Scheme 1) bears the following functional characteristics: an acetate as activating group at the anomeric position, an acetate as participating group at the 2-position, deoxygenation at the 3-position, 4-(*R*)-stereochemistry, and an amine precursor at the 5-position. The presence of an acetate as a participating group at the 2-position would allow the stereospecific formation of the α-L-arabinofuranoside during the glycosylation reaction. We developed the synthesis of our carbohydrate intermediate **1** from L-arabinose which is the only reasonably priced, commercially available L-pentose with the 4-(*R*) configuration already in place. L-arabinose is converted under kinetic control into a mixture of methyl glycosides using modified literature conditions.[9] They were optimized in order to obtain the best α-furanoside/β-furanoside/α-pyranoside ratio (i.e., 63/27/10). The ratio was determined by integration of the corresponding anomeric proton signals of the ^{1}H NMR spectrum. The crude reaction mixture was used in the next step without further purification. Simultaneous protection of the 3-OH and the 5-OH groups by reaction with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, leaving the 2-OH residue available for acetylation with acetic anhydride, was achieved according to a modified literature procedure.[10] We optimized the workup in order to separate, without purification, the arabinopyranoside from the arabinofuranosides **2**, which were obtained as a 65/35 mixture of α and β anomers per NMR (^{1}H and ^{13}C). Following deprotection by treatment with hydrofluoric acid/pyridine complex, the methylfuranosides **3** were isolated without purification in 65% yield from L-arabinose (95% purity by NMR analysis of the crude residue). The two remaining steps in the synthesis were the deoxygenation at C3 and the displacement of the oxygen at C5 with a nitrogen nucleophile. This was best accomplished by first derivatizing the primary alcohol as the tosylate **4**, then deoxygenating at C3 by radical reduction of a thiocarbamate. The C5 tosylate of **5** was then displaced with sodium azide to give the azides **6**. After submitting **6** to acetolysis reaction conditions, the key intermediate **1** was obtained in 45% yield as a 70/30 mixture of α and β acetates. Preliminary glycosylation experiments showed that both anomers of **1** could react under standard nucleoside formation conditions, and therefore the anomers were not separated.

To validate our approach, the α and β mixture of activated intermediates **1** was first reacted with uracil in the presence of *N*,*N*-hexamethyldisilazane, trimethylsilyl chloride and tin(IV) chloride. The acetate at position 2 participated in the reaction by forming an acetoxonium intermediate blocking the α face of the tetrahydrofuran ring hence directing the attack of the nucleophile solely from the β face. The product, obtained in 50% yield, was nucleoside **7a**, the remainder being unreacted starting material **1**. The acetate at position 2 was hydrolyzed using a mixture of triethylamine, methanol, and water. The 5′-azide was then reduced by reaction with 1,3-propanedithiol to give 1-(5′-amino-3′,5′-dideoxy-α-L-arabinofuranosyl)uracil **8a** (>90% yield over two steps).[11] Compound **8a** was spectroscopically identical to a sample prepared from uridine, used in the total synthesis of dihydropacidamycin D, **9a**.[8]

We then undertook the task of synthesizing a set of new nucleosides using **1** as glycosyl donor. **8b–8h** were obtained in moderate yields (30–60% after flash chromatography on silica gel) as α-nucleosides in the presence of *N,N*-hexamethyldisilazane, trimethylsilyl chloride, and tin(IV) chloride. They were further transformed into the dihydropacidamycins **9b–9h** using published methodology from our laboratories.[8] Their activities were tested and compared to that of dihydropacidamycin D. All of the base-modified dihydropacidamycins showed minimum inhibitory concentrations greater than 512 μg/mL against isolates of *P. aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Pasteurella multocida*. It was demonstrated in a cell-free assay[12] that the lack of in vitro activity was due to loss of enzymatic inhibition, indicating that MraY recognizes solely uracil-containing nucleosides.

In conclusion, we have developed a highly efficient synthesis of 1,2-di-*O*-acetyl-5-azido-3,5-dideoxy-α,β-L-arabinofuranose readily amenable to scale-up (we prepared 3.4 g of **1** from 20 g of L-arabinose). This carbohydrate has been shown to be a good precursor for the synthesis of nucleosides, as exemplified by the synthesis of 1-(5′-amino-3′,5′-dideoxy-α-L-arabinofuranosyl)uracil, a key component for the synthesis of the antibacterial dihydropacidamycin D. A set of base-modified dihydropacidamyins was prepared from **1**; they were devoid of any in vitro activity, stressing the specificity of MraY for uracil-based substrates.

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9. Fletcher, H. J., Jr. In *Methods Carbohydr. Chem.*; 1963; Vol. 2, p 228. To a solution of L-arabinose (40 g, 0.266 mmol) in 1 L of anhydrous methanol at 40 °C, was added methanesulfonic acid (13.8 mL, 0.213 mmol). The reaction mixture was stirred at 40 °C for 40 min before being cooled to room temperature and quenched by addition of sodium hydrogencarbonate (21.5 g, 0.256 mmol) and 5 mL of water. The reaction mixture was evaporated, the residue taken into hot acetone and the precipitate was filtered off. Evaporation of the filtrate gave 42 g of a 63/27/10 mixture of α-furanoside, β-furanoside, and α-pyranoside, respectively.

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11. ^{1}H NMR (400 MHz, CD_3OD) δ 1.8 (ddd, *J* = 13.8, 4.8, 3.8 Hz, 1H), 2.33 (ddd, *J* = 13.8, 8.4, 6.2 Hz, 1H), 2.81 (dd, *J* = 13.2, 6.0 Hz, 1H), 2.90 (dd, *J* = 13.2, 3.6 Hz, 1H), 4.41 (ddd, *J* = 6.0, 3.6, 2.0 Hz, 1H), 4.54–4.59 (m, 1H), 5.68 (d, *J* = 8 Hz, 1H), 5.74 (d, *J* = 2.0 Hz), 7.55 (d, *J* = 8.4 Hz, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ 36.2, 46.3, 76.5, 83.8, 95.7, 102.4, 142.2, 152.4, 166.6.

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