Stereochemical Elucidation and Total Synthesis of Dihydropacidamycin D, a Semisynthetic Pacidamycin

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Abstract: Hydrogenation of the C(4′) exocyclic olefin of the pacidamycins has been shown to produce a series of semisynthetic compounds, the dihydropacidamycins, with antimicrobial activity similar to that of the natural products. Elucidation of stereochemistry in the pacidamycins has been completed through a campaign of natural product degradation experiments in combination with the total synthesis of the lowest-molecular weight dihydropacidamycin, dihydropacidamycin D. The stereochemical identities of the tryptophan and two alanine residues contained in pacidamycin D have been shown to be of the natural (*S*) configuration, and the unique 3-methylamino-2-aminobutyric acid contained in this series of antibiotics has been shown to be of the (2*S*,3*S*) configuration. Finally, the stereochemistry obtained by hydrogenation of the C(4′)−C(5′) exocyclic olefin has been shown to be (*R*) at the C(4′) nucleoside site.

Introduction

The increasing occurrence of multiresistant bacterial infections in both nosocomial and community settings has prompted the need for new antibiotic therapies.[1−7] Strategies to this end have included modification of existing classes of antibiotics, use of agents to potentiate existing antibiotics against resistant organisms, and the identification of new classes of antibiotics that have unexploited modes of action and thus are not cross-resistant with old classes.[8,9] Three structurally related families of natural products, the pacidamycins,[10−12] mureidomycins,[13−17] and napsamycins[18] (also known as uridyl-peptide antibiotics, or UPAs, see Table 1), presented themselves as attractive starting points for the development of a new class of antibacterial drugs for three reasons. First, both the pacidamycins and mureidomycins have demonstrated promising bioavailability and mureidomycin C has shown in vivo efficacy.[11,15] Second, these antibiotics have an unexploited mode of action; the mureidomycins have been shown to inhibit translocase (transferase or MraY),[19−21] an essential enzyme in peptidoglycan biosynthesis[22,23] in most Gram-positive and Gram-negative organisms and mycobacteria. Translocase is not the target of any agents in current clinical use. Consequently, strains of *Pseudomonas aeruginosa* that are resistant to *β*-lactam and fluoroquinolone antibiotics remain sensitive to mureidomycin.[24] Third, unlike other translocase inhibitors such as tunicamycin,[21] which also inhibits the formation of the pentasaccharide core found in mammalian N-linked glycoproteins, the pacidamycins and mureidomycins are specific for their bacterial targets. Consistent with this, mureidomycins A and C have been shown to have low cytotoxicity.[25]

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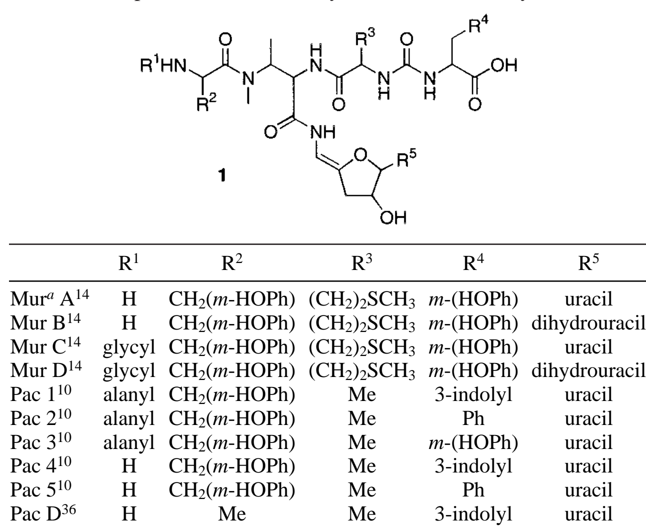
Table 1. Representative Pacidamycins and Mureidomycins



	R^1	R^2	R^3	R^4	R^5
Mur[a] A[14]	H	CH_2(*m*-HOPh)	$(CH_2)_2SCH_3$	*m*-(HOPh)	uracil
Mur B[14]	H	CH_2(*m*-HOPh)	$(CH_2)_2SCH_3$	*m*-(HOPh)	dihydrouracil
Mur C[14]	glycyl	CH_2(*m*-HOPh)	$(CH_2)_2SCH_3$	*m*-(HOPh)	uracil
Mur D[14]	glycyl	CH_2(*m*-HOPh)	$(CH_2)_2SCH_3$	*m*-(HOPh)	dihydrouracil
Pac 1[10]	alanyl	CH_2(*m*-HOPh)	Me	3-indolyl	uracil
Pac 2[10]	alanyl	CH_2(*m*-HOPh)	Me	Ph	uracil
Pac 3[10]	alanyl	CH_2(*m*-HOPh)	Me	*m*-(HOPh)	uracil
Pac 4[10]	H	CH_2(*m*-HOPh)	Me	3-indolyl	uracil
Pac 5[10]	H	CH_2(*m*-HOPh)	Me	Ph	uracil
Pac D[36]	H	Me	Me	3-indolyl	uracil

[a] Mureidomycin = Mur; Pacidamycin = Pac

Interestingly, these antibiotics possess selective activity against *P. aeruginosa*,[24] an organism that is among the most refractory to current therapies. While a selective anti-pseudomonas agent is attractive from an academic standpoint, a limited spectrum is a liability in the clinic where broad-spectrum empiric therapy is often necessary. Members of the mureidomycin family have been shown to be potent inhibitors of not only the translocase of *Escherichia coli*[20] but also of *Staphylococcus aureus*,[26] suggesting that a broad-spectrum agent based on the UPA structure is possible. The pacidamycins and mureidomycins suffer from the additional limitation that *P. aeruginosa* develops resistance at a high frequency ($10^{-5}-10^{-6}$).[11,15] Due to the limited spectrum and frequency of resistance, efforts by pharmaceutical companies in this area have been sparse.[27−35]

We hypothesized that the problems of resistance and spectrum were a function of uptake (or lack thereof) into bacterial cells and that modulation of the physical properties of this class of antibiotic might allow for improved uptake, thus achieving a broader-spectrum agent of reduced resistance frequency. To this end, we required a synthetic protocol that provided access to diverse UPA analogues. Before a program to synthesize diverse UPAs could be executed, the stereochemistry at each of the asymmetric centers needed to be established. This publication describes the stereochemical elucidation of the UPAs and total synthesis of a semisynthetic derivative of a recently isolated pacidamycin, pacidamycin D.[36]

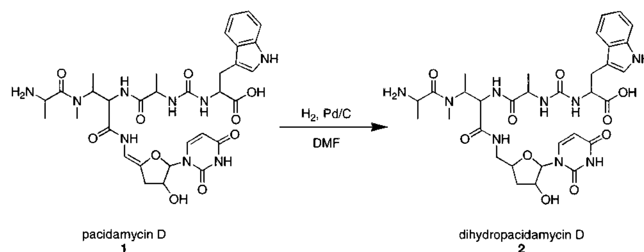


Figure 1. Hydrogenation of pacidamycin D.

Results and Discussion

Hydrogenation of Pacidamycins. Early in the course of our work, it became apparent that the unusual C(4′)-exocyclic olefin would prove problematic from the point of view of chemical stability and synthetic accessibility. If hydrogenation of the 4′-exocyclic olefin could be shown to produce active compounds, then dihydropacidamycins would be superior to pacidamycins as medicinal targets. Hydrogenation of pacidamycins 1, 4, and D was performed in DMF over 10% Pd/C at 1 atm (Figure 1). Typically, at least 24 h were required for the complete consumption of starting material. The product corresponding to the reduced 4′-exocyclic olefin (dihydropacidamycin) formed in all cases, along with an additional product having both the 4′-exocyclic olefin and the uracil double bond reduced (tetrahydropacidamycin). Further hydrogenation of pacidamycin D gave tetrahydropacidamycin D, uncontaminated with any dihydro compound. All of these reduced pacidamycins remained comparably active to the parent compounds against wild-type *P. aeruginosa*. These results justified a program directed toward the total synthesis of dihydropacidamycin-like compounds.

Since dihydropacidamycin D (**2**) was the least structurally complex of the semisynthetic dihydropacidamycins, it was chosen as our target for total synthesis. Before embarking on a total synthesis of **2**, it was first necessary to determine the stereochemistry at several chiral centers in the molecule. The assumption was made that the configuration of each corresponding chiral center was constant throughout the pacidamycins and mureidomycins; degradative studies were performed on pacidamycins 1, 4, and 5 since they were available in larger amounts than pacidamycin D. The *C*-terminal amino acid residue of pacidamycin 1 could be removed employing carboxypeptidase A, indicating that this residue was likely of the natural (*S*) configuration. Further, the product of this enzymatic hydrolysis was subjected to coupling with both (*S*)- and (*R*)-phenylalanine isocyanates. The semisynthetic compounds resulting from the coupling with the (*S*) amino acid isocyanate produced a compound with anti-pseudomonal activity, while the (*R*)-derived material was inactive.[37] Therefore, the *C*-terminal amino acid was assigned the natural (*S*) configuration. Our objective was to establish the remaining centers through both analysis of degraded pacidamycins and the comparison of totally synthetic dihydropacidamycin D with semisynthetic dihydropacidamycin D.

Isolation and Stereochemical Determination of 3-Methylamino-2-aminobutyric Acid. A sample of pacidamycin 5 (obtained by fermentation)[12,36] was hydrolyzed according to a modified literature procedure reported for the mureidomycins.[14] The 3-methylamino-2-aminobutyric acid (DABA) was isolated by size-exclusion chromatography. A literature report regarding the related 3-amino-2-aminobutyric acid suggested that in an

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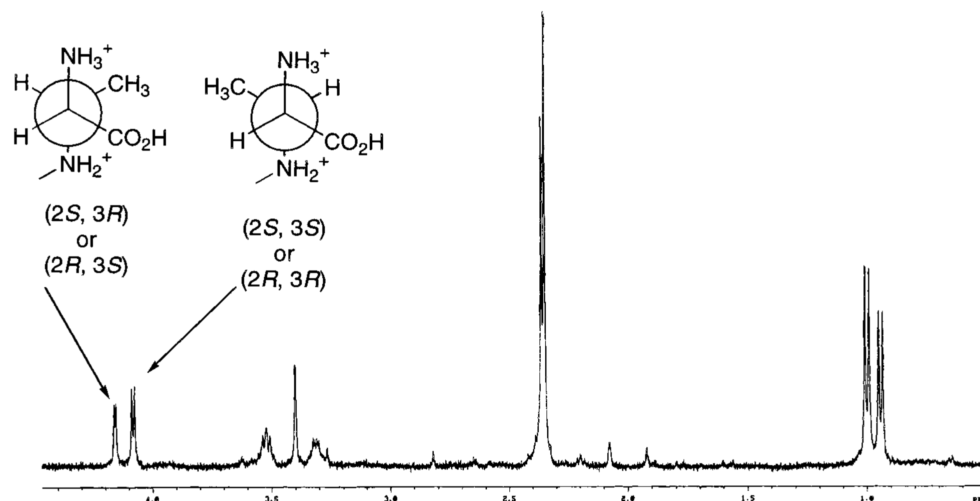


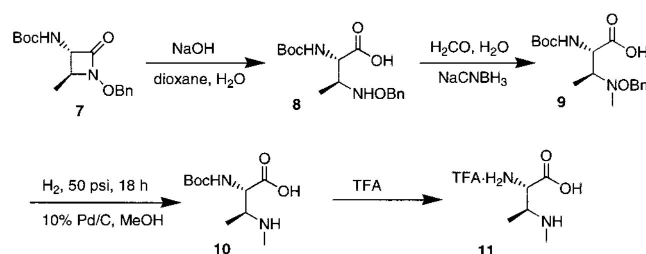
Figure 2. ^{1}H NMR of 3-methylamino-2-aminobutyric acid obtained from the natural products.

acidic medium, the ammonium moieties would adopt an *anti* orientation to minimize electrostatic repulsion.[38] Accordingly, a ^{1}H NMR spectrum was obtained in 2 N DCl in D_2O, revealing two isomeric diaminobutyrates. J_{H2-H3} for the major isomer was 4.4 Hz. The minor component, which is likely a diastereomer of the major component, had $J_{H2-H3} = 2.8$ Hz. The major isomer was surmised to be that present in the pacidamycins since resubjection of the mixture to the hydrolysis conditions caused equilibration of the two components to a ~1:1 ratio. As an *anti* orientation of amino groups in the (2*S*,3*S*) or (2*R*,3*R*) leads to an *anti* orientation of hydrogens, expected to have a larger coupling constant, a tentative sterochemical assignment was made that the diaminobutyrate of the pacidamycins was either (2*S*,3*S*) or (2*R*,3*R*) (see Figure 2). This relative stereochemical assignment was confirmed by addition of a totally synthetic sample (vide infra, synthesis) of (2*S*,3*S*)-DABA to an NMR sample of the DABA isolated from pacidamycin degradation; enhancement of the major component was observed.

The absolute configuration of this residue was determined by HPLC, employing a chiral mobile phase.[39] Pacidamycin 4 was hydrolyzed, and the resultant mixture was shown to contain (2*S*,3*S*)-, but not (2*R*,3*R*)-DABA by comparison with authentic synthetic samples of both (vide infra). This assignment was further corroborated by the observations that totally synthetic dihydropacidamycins containing the (2*S*,3*S*)-DABA had anti-pseudomonal activity, while only inactive compounds were obtained from the (2*R*,3*R*)-DABA.

Stereospecific Synthesis of (2*S*,3*S*)- and (2*R*,3*R*)-3-Methylamino-2-aminobutyric Acid. Both enantiomers of azetidinone **7** were prepared according to literature procedure starting from the appropriate enantiomer of Boc-protected threonine.[40,41] The azetidinone ring was hydrolyzed using NaOH, providing compound **8**, and the *N*-methyl group was introduced by reductive amination with formaldehyde (see Scheme 1), affording **9**. The benzyloxy group was removed by hydrogenolysis to provide compound **10**. A sample of **10** was further deprotected by removal of the Boc group, and the resulting 3-methylamino-2-aminobutyric acid demonstrated an identical ^{1}H NMR spectrum when mixed together with the sample isolated from the natural product, thus confirming our initial relative stereochemical assignment. At no point in the reaction sequence was there any evidence (TLC or NMR) for the production of diasteromeric products resulting from epimerization of the α-carbon.

Scheme 1



Synthesis of 5′-Amino-3′,5′-dideoxyuridine Derivatives 18 and 26. Since the stereochemistry at C(4′) of dihydropacidamycin D was unknown, we set out to synthesize both compounds **18** and **26** for incorporation into totally synthetic dihydropacidamycins. While this contribution was in preparation, an alternate synthetic method for preparation of compound **18** appeared in the literature.[42] Our synthesis of **18** began with **12**, obtained according to literature procedure (see Scheme 2).[43,44] The free hydroxyl group was activated for radical reduction using thiocarbonyldiimidazole. Reduction was performed employing azacyclohexylcarbonitrile and tributyltin hydride as reducing agent, providing compound **13**.[45] The 5′-silyl ether was then selectively removed using 80% acetic acid in H_2O to afford compound **14**. Compound **14** was treated with *p*-tosyl chloride, and the resulting tosylate **15** was converted to azide **16**. Compound **16** afforded the desired nucleoside **18** upon deprotection with TBAF and reduction with 1,3-propanedithiol. The azide was reduced only after the silyl ether had been removed since it was found that reduction of the azide in the presence of the TBS-protected alcohol led to a product that decomposed on standing. We chose propanedithiol as our reductant since use of hydrogenation led to the desired product

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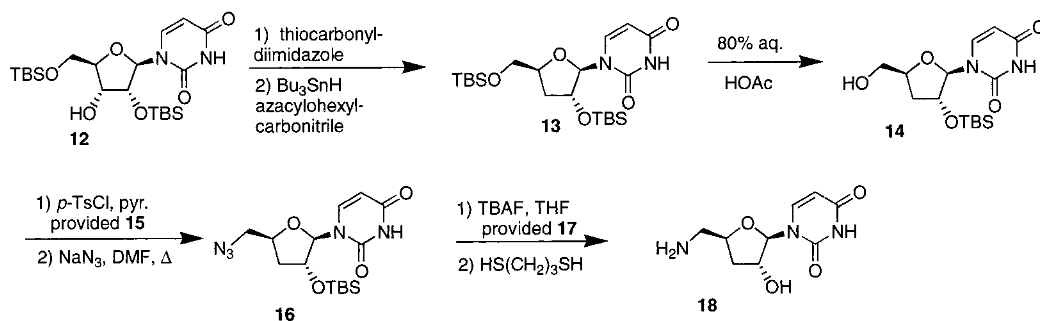
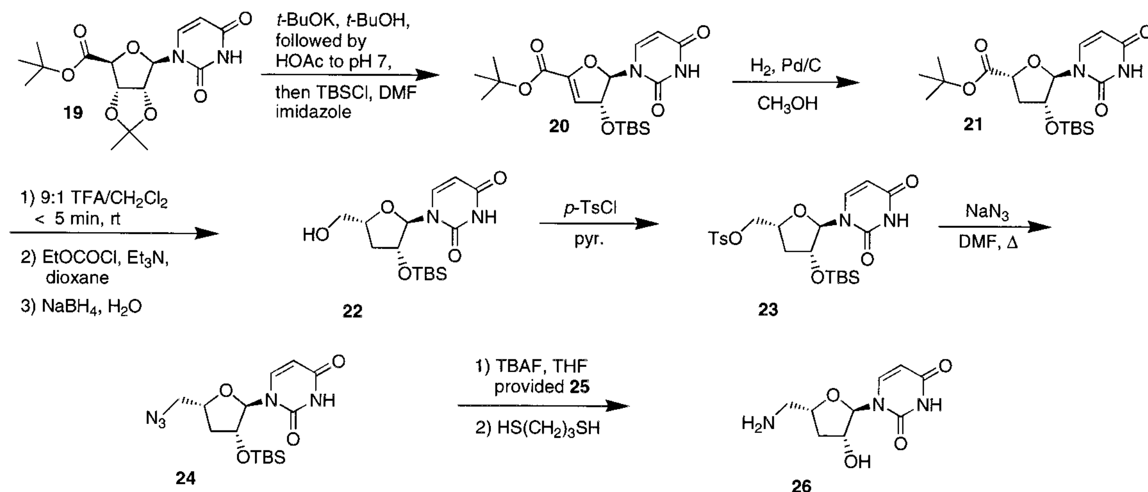
Scheme 2



Scheme 3



contaminated with its dihydronucleoside analogue. Furthermore, all byproducts of this reduction were methylene chloride soluble, while the product remained in the aqueous phase during extraction.

The synthesis of **26** began with **19**, also prepared according to literature procedure.[46] Correction of the stereochemistry at C(4′) was addressed concomitantly with deoxygenation at the 3′ site in an initial base-mediated elimination step (see Scheme 3), modified from the method of Nagpal and Horwitz;[47] the optimized sequence involved trapping the resulting crude free alcohol as its *tert*-butyldimethylsilyl ether **20**. The desired stereochemistry, (*R*), at the C(4′) center was installed by a hydrogenation directed to the β face of the molecule by the bulky silyl ether-protecting group. The desired stereoisomer was obtained in a 9:1 ratio relative to the undesired, which was removed by recrystallization of **21**. Brief treatment with 9/1 $TFA:CH_2Cl_2$ was found to be optimal for selectively deprotecting the carboxylic acid over the silylated alcohol; the free carboxylic acid resulting from these deprotection conditions was subjected to a two-step activation−reduction sequence, affording alcohol **22**. Comparison of isomers **22** and **14** provided unequivocal proof that we had installed the desired (*R*) stereochemistry at C(4′) since **22** and **14** were spectroscopically distinct, and **14** was known to be of the (*S*) configuration at C(4′). Nitrogen functionality was introduced by tosylation of **22** followed by azide displacement, yielding compound **24**. The silyl ether was removed under standard conditions, and finally the azide was reduced using neat 1,3-propanedithiol, affording analytically pure compound **26**, after an extractive purification.

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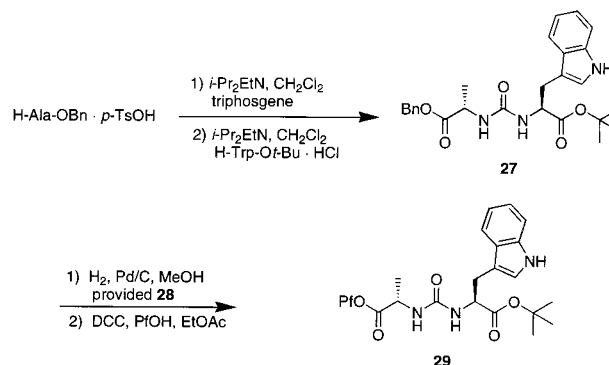
Scheme 4



Total Synthesis of Dihydropacidamycin D (2). Given our earlier stereochemical deductions, only three asymmetic centers remained to be assigned: the R^2 and R^3 amino acid residues (see Table 1), and C(4′) of the ribose. Fortunately, both stereoisomers of the two amino acid components were commercially available with the appropriate protecting groups, and both C(4′) stereoisomers (**18** and **26**) were available via the synthetic methods described above. Thus, we set out to prepare all eight (2^3) possible isomers and to determine the stereochemistry of dihydropacidamycin D through comparison of biological activity and physical properties of these isomers with semisynthetic **2**. Our synthetic protocol is exemplified with L-amino acids and nucleoside **26** since these are the components that provided a totally synthetic product with physical and biological properties identical to that of semisynthetic **2**.

The urea portion of the molecule was prepared according to the method of Majer and Randad.[48] H-Ala-OBn and H-Trp-

Scheme 5

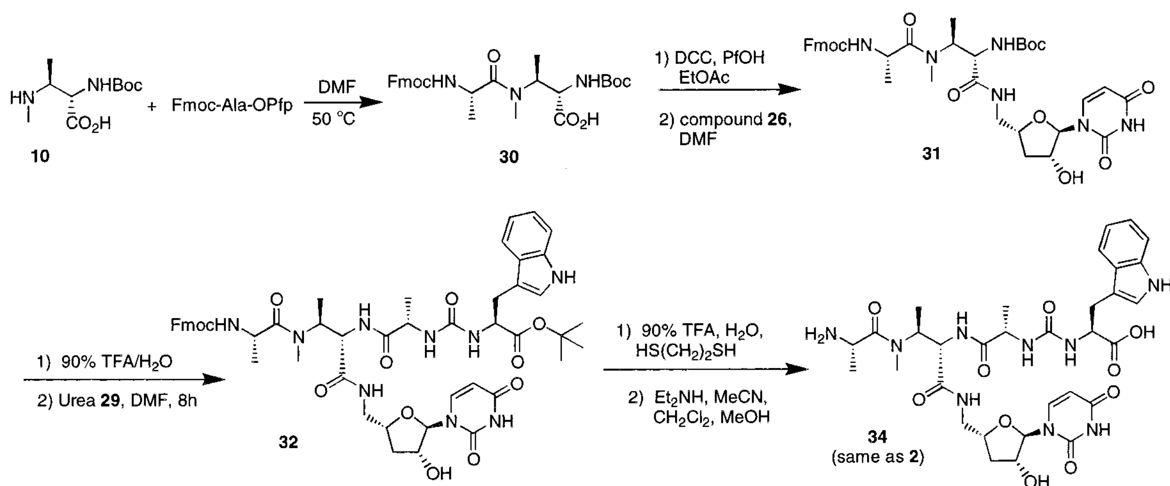


Table 2. Minimum Inhibitory Concentrations (MICs) for Key Compounds

strain	description	minimum inhibitory concentrations (μg/mL)[49]			
		Pac[a] 4	Pac D	**2**	**34**
P. aeruginosa PAM1020[50]	wild-type	32	64	64	32
P. aeruginosa PAM1154[50]	PAM1020 *oprM::Hg*[51]	4	4	8	4

O*t*Bu were condensed with triphosgene, and the resulting unsymmetrical urea **27** was debenzylated by hydrogenation at 1 atm. The liberated carboxylic acid was converted to the pentafluorophenyl ester **29** under standard conditions (see Scheme 4).

Boc-protected DABA (**10**) was coupled to the pentafluorophenyl ester of Fmoc-L-alanine (Scheme 5). The dipeptide **30** was activated as its pentafluorophenyl ester and coupled with aminonucleoside **26**, providing compound **31**. The C-terminal portion of the molecule was appended by removal of the Boc group followed by coupling with **29**, affording **32**. Deprotection and subsequent HPLC purification provided **34**, a compound whose ^{1}H NMR spectra and HPLC retention time were identical to those of compound **2**. The remaining seven stereoisomers were synthesized by similar methods and their biological activities measured. As final proof that **34** was indeed the stereoisomer corresponding to semisynthetic **2**, only **34** displayed significant activity against wild-type *P. aeruginosa*, equivalent to that measured for the semisynthetic compound **2** (see Table 2).

Conclusions

In conclusion, the identity of the stereocenters of the pacidamycins has been elucidated; all proteinogenic amino acids in the pacidamycins are of the natural, (*S*) configuration, and the DABA residue is of the (2*S*,3*S*) configuration. Hydrogenation of the pacidamycins results in biologically active compounds containing the (*R*) configuration at C(4′). A straightforward method is reported for the total synthesis of these C(4′)−C(5′) saturated pacidamycins. This allows synthesis of structurally varied pacidamycins based on adaptation of the methodology reported herein to include other amino acid residues, thereby facilitating the search for a broad-spectrum agent of this type. The biological activities of a diverse synthetic set of dihydropacidamycins will be reported in due course.

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Supporting Information Available: All experimental procedures and compound characterization; reproductions of the ^{1}H and ^{13}C NMR spectra of totally synthetic dihydropacidamycin D (**34**) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA003292C

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