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Synthetic Studies on Amipurimycin: Total Synthesis of a Thymine Nucleoside Analogue

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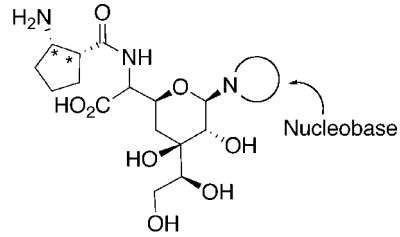

Amipurimycin: Nucleobase = 2-Aminopurine
'**Thymine Amipurimycin**': Nucleobase = Thymine

Amipurimycin, a member of the complex peptidyl nucleoside family of antibiotics, is a *Streptomyces*-derived potent antifungal agent. The mechanism of action of amipurimycin, however, remains undetermined. Additionally, there are no reports on the total synthesis or structure−activity relationships (SAR) of this potentially useful bioactive compound. In a study aimed at the total synthesis and SAR studies of this natural product, the present research reports the development of a synthetic route to the central pyranosyl amino acid core of amipurimycin and its further elaboration, culminating in the synthesis of a unique thymine analogue. Utilizing a D-serine-derived dihydroaminopyrone as a strategic building block, the synthesis involves de novo construction of the fully functionalized C-3-branched carbohydrate amino acid core, followed by glycosidic attachment of thymine at C-1, and peptidic linking of the C-6 amine with the 1,2-aminocyclopentane carboxylic acid side chain.

Introduction

The complex peptidyl nucleoside antibiotic amipurimycin (**1**, Figure 1) was isolated from *Streptomyces novoguineensis* sp. nov., a new strain of *Streptomyces* that was discovered in soil samples collected from New Guinea during the 1970s.[1] Amipurimycin displays impressive antifungal activity against several phytopathogenic fungi, such as *Pyricularia oryzae*, *Alternaria kikuchiana*, and *Helminthosporium sigmoideum* var. *irregulare*.[1] Thus, in field tests, amipurimycin showed excellent controlling and curative effect against blast disease of rice plants. In limited toxicity studies, no toxicity to killifish was observed on exposure to amipurimycin (10 ppm) after the first 2 days; however, all the fish tested died on the third day. In mice and rats, the LD_{50} values of amipurimycin were found to be in the ranges 1−5 mg/kg (intravenous), and 10−30 mg/kg (oral), respectively.[1] The mechanism of antifungal action of amipurimycin, however, remains unknown.

The proposed amipurimycin structure was determined with the help of extensive spectroscopic and chemical degradation studies. Thus, amipurimycin was found to be made up of an unusual C-3′ branched pyranose amino acid, appended to an *N*-terminal amino acid residue at C-6′, and a glycosidic purine nucleobase (Figure 1).[2]

However, the stereochemistry at C-6′ and the absolute configurations at C-2″/C-3″ for the *cis*-aminocyclopentane carboxylic acid derived side chain have not yet been ascertained. Interestingly, the amipurimycin aminocyclopentane carboxylic acid side chain fragment, also known as cispentacin, is itself an antibiotic.[3] Isolated from *Streptomyces setonii*, the enantiopure natural *cis*-(1*R*,2*S*)-cispentacin was found to show good antifungal activity against *Candida albicans*.[3] Although a few

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(1) (a) Iwasa, T.; Kishi, T.; Matsuura, K.; Wakae, O. *J. Antibiot.* **1977**, *30*, 1–10. (b) Harada, S.; Kishi, T. *J. Antibiot.* **1977**, *30*, 11–16.

(2) Goto, T.; Toya, Y.; Ohgi, T.; Kondo, T. *Tetrahedron Lett.* **1982**, *23*, 1271–1274.

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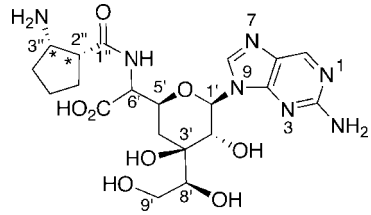


FIGURE 1. Antifungal antibiotic amipurimycin.

syntheses of the various structural fragments of amipurimycin have been reported,[4,5] the total synthesis or structure−activity relationship (SAR) studies of this natural product are yet to be accomplished.

The demonstrated antifungal activity, challenging and partially unresolved structural features, an as yet undetermined mechanism of action, and limited synthetic or medicinal chemical studies render amipurimycin an attractive target for exploration as a new antifungal agent of potential utility. Accordingly, in continuation of our interest in the peptide nucleoside antibiotics,[6] we initiated a total synthesis of amipurimycin. The preliminary results of our studies are described herein.

Results and Discussion

In the few reported syntheses of the amipurimycin structural fragments, the studies were mostly focused on construction of the hexopyranoside amino acid core of this natural product.[4,5] The majority of the above methods employed carbohydrate starting materials to construct the required C-3 (carbohydrate numbering) branched pyranose framework, followed by subsequent functional group manipulations to introduce the C-6 side chain amino acid functionality. Thus, in independent studies, the research groups of Czernecki, Rauter, and Yoshimura have utilized D-glucose as a starting material in their synthetic efforts on amipurimycin.[4] In contrast, in an interesting noncarbohydrate-based approach, a stereocontrolled cyclocondensation between a serine-derived homochiral oxazolidine aldehyde (Garner's aldehyde) and an electron-rich polyoxygenated diene was utilized by Garner to assemble the branched sugar amino acid component of amipurimycin.[5]

It is evident from the available literature that, utilization of carbohydrate starting materials in the synthesis of the sugar cores of amipurimycin and other related peptidyl nucleoside antibiotics has considerable merit when it can be applied in an efficient manner.[7] However, when the target end product incorporates unusual residues or unnatural stereochemistry, the carbohydrate-based approach often suffers from lengthy reaction sequences and necessitates extensive protection−deprotection of functional groups. In the present research, we therefore explored an alternative synthetic strategy involving stereoselective de novo construction of the nucleoside amino acid core of these targets, starting from a structurally simpler and more flexible noncarbohydrate synthon.

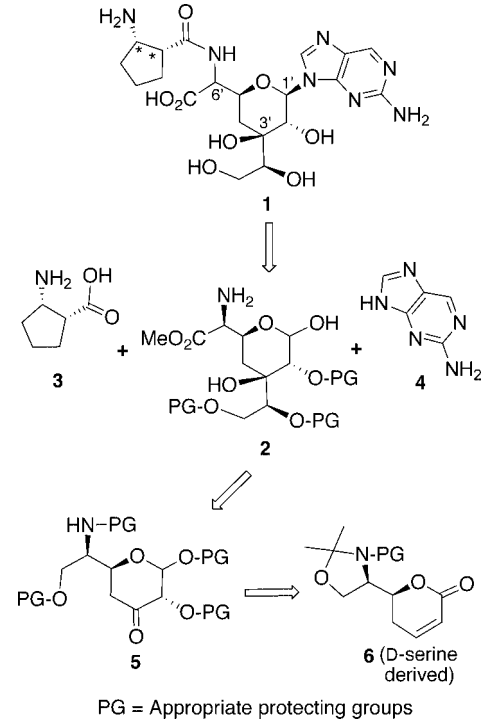


FIGURE 2. Retrosynthetic strategy and approach.

In our retrosynthetic strategy, we envisaged initial construction of a fully functionalized pyranosyl amino acid core **2** (Figure 2), as present in amipurimycin, to be followed by its glycosidic attachment at C-1 with the appropriate nucleobase, and peptidic linking of the C-6 amine with cispentacin. For the sake of simplicity, in our present synthesis, we decided to install an *S*-configured natural amino acid functionality at C-6, the same stereochemical configuration that is present in the other members of the peptidyl nucleoside family of antibiotics.[7] Similarly, the naturally occurring and commercially available enantiopure (1*R*,2*S*)-cispentacin was selected as the side chain amino acid donor. The pivotal C-3 branched polyoxygenated carbohydrate amino acid fragment **2** can be synthesized by appropriate functional group transformations of the strategically functionalized pyrone **5**, which in turn can be obtained from the enantiopure dihydropyran-2-one **6**. Starting from D-serine, an efficient synthetic route to the enantiopure aminopyrone **6** has already been developed during our previously reported studies on complex peptidyl nucleoside antibiotics.[6a]

As and when desired, appropriate modifications of the above synthetic strategy can also lead to the other possible antipodes at the above stereocenters.[8]

(3) (a) Iwamoto, T.; Tsujii, E.; Ezaki, M.; Fujie, A.; Hashimoto, S.; Okuhara, M.; Kohsaka, M.; Imanaka, H.; Kawabata, K. *J. Antibiot.* **1990**, *43*, 1–7. (b) Kawabata, K.; Inamoto, Y.; Sakane, K.; Iwamoto, T.; Hashimoto, S. *J. Antibiot.* **1990**, *43*, 513–518.

(4) (a) Hara, K.; Fujimoto, H.; Sato, K. I.; Hashimoto, H.; Yoshimura, J. *Carbohydr. Res.* **1987**, *159*, 65–79. (b) Rauter, A. P.; Fernandes, A. C.; Czernecki, S.; Valery, J.-M. *J. Org. Chem.* **1996**, *61*, 3594–3598. (c) Czernecki, S.; Valery, J.-M.; Wilkens, R. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 1347–1351. (d) Czernecki, S.; Franco, S.; Valery, J.-M. *J. Org. Chem.* **1997**, *62*, 4845–4847.

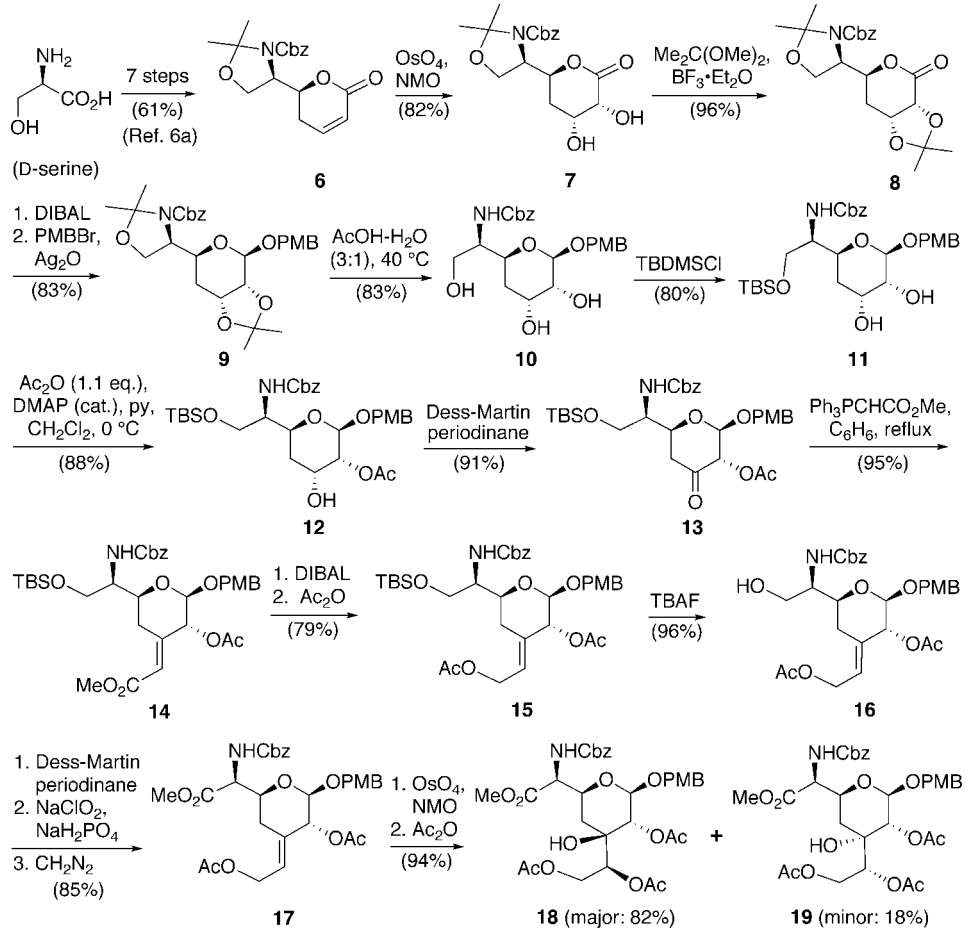
(5) Garner, P.; Yoo, J. U.; Sarabu, R.; Kennedy, V. O.; Youngs, W. J. *Tetrahedron* **1998**, *54*, 9303–9316.

(6) For recent reports, see: (a) Bhaket, P.; Stauffer, C. S.; Datta, A. *J. Org. Chem.* **2004**, *69*, 8594–8601. (b) Khalaf, J. K.; Datta, A. *J. Org. Chem.* **2005**, *70*, 6937–6940. (c) Stauffer, C. S.; Bhaket, P.; Fothergill, A. W.; Rinaldi, M. G.; Datta, A. *J. Org. Chem.* **2007**, *72*, 9991–9997.

(7) For reviews on complex peptidyl nucleoside antibiotics, see: (a) Garner, P. Synthetic approaches to complex nucleoside antibiotics. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier: Amsterdam, The Netherlands, 1988; Stereoselective synthesis (Part A), Vol. 1, pp 397−435. (b) Isono, K. *Pharmacol. Ther.* **1991**, *52*, 269–286. (c) Knapp, S. *Chem. Rev.* **1995**, *95*, 1859–1876. (d) Zhang, D.; Miller, M. J. Curr. Pharm. Des. 1999*5*, 73–99, and references therein.

(8) For example, starting from L-serine, the key *syn*-1,2-amino alcohol fragment as required for the synthesis of the corresponding C6 antipode of the lactone **6** can be easily obtained following a previously reported protocol from our group: Ravi Kumar, J. S.; Datta, A. *Tetrahedron Lett.* **1999**, *40*, 1381–1384.

SCHEME 1

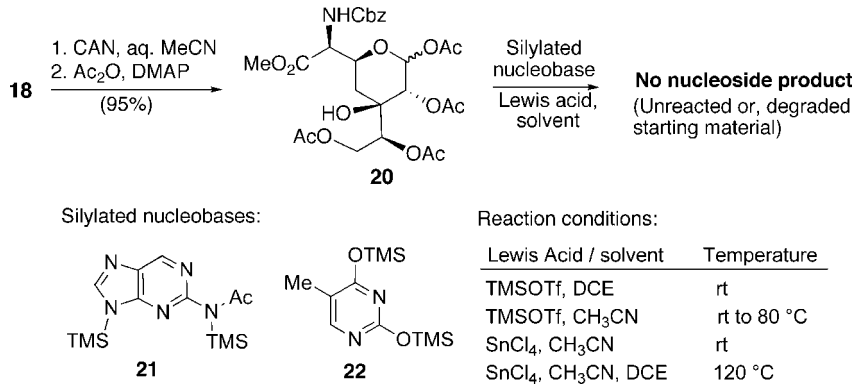


In accordance with the above strategy, and employing a recently developed protocol from our laboratory,[6a] readily available D-serine was converted to the corresponding strategically functionalized aminobutenolide **6** (Scheme 1) in good overall yield (61%, seven steps). Subsequent osmium tetroxide-catalyzed dihyroxylation of **6** under reported conditions resulted in the known diol **7** with high stereocontrol.[6a] Acetonide protection of the diol functionality provided the corresponding fully protected pyrone derivative **8** in near-quantitative yield. DIBAL mediated partial reduction of the lactone **8** to the corresponding lactol, followed by anomeric *O*-alkylation with 4-methoxybenzyl bromide (PMB-Br) yielded the β-glycosidic derivative **9** as the only product. The highly stereoselective formation of the β-anomer can be attributed to the sterically hindered C-2/C-3 substituents influencing the product formation from the less hindered face. One-pot hydrolytic cleavage of both of the acetonide protecting groups generated the trihydroxy derivative **10**. In the ^{1}H NMR spectrum of **10**, the characteristic trans-coupling constant as observed for the anomeric proton ($J_{1,2}$ = 8.0 Hz) helped confirm the assigned C-1 stereochemistry. Selective silyl protection of the resulting primary hydroxyl group led to the C-2/C-3 free diol **11** in good yield. Our next goal was selective C-3 oxidation of the above diol **11** toward construction of the branched polyhydroxylated C-3 side chain as present in amipurimycin. Accordingly, exploiting the differential reactivity profile between the C-2 and C-3 hydroxyl functionalities, reaction of the diol **11** with 1.1 equiv of Ac_2O under carefully controlled conditions resulted in the corresponding monoacetylated deivative **12**. Expectedly, in the above reaction, the more reactive equatorial C-2 hydroxyl group underwent preferential reaction over the axial hydroxyl group at C-3.[9] The free hydroxyl group at C-3 was then converted to the ketone **13**. Subsequent Wittig olefination of **13** with (carbomethoxymethylene)triphenylphosphorane resulted in the selective formation of the corresponding *E*-olefin **14**. High-resolution NMR studies (^{1}H, ^{13}C, COSY, NOE) confirmed the olefin geometry and the assigned structure of **14**. As supported by literature precedence,[4b] the trans-selectivity in the above *E*-alkylidene formation is probably attributable to the steric influence of the neighboring C-2 acetoxy substituent.

Reduction of the two ester functionalities of **14**, in the presence of excess DIBAL, followed by acetylation of the resulting diol with acetic anhydride formed the diacetate derivative **15** in good overall yield. With the aim to form the C-6 amino acid functionality, the silyl protecting group of **15** was removed to unmask the primary alcohol **16**. Subsequent oxidation of **16** to the corresponding carboxylic acid and its esterification under standard conditions provided the methyl ester derivative **17**. Toward the desired target, the next requirement was installation of the C-3 /C-3′ *syn*-diol moiety. In their studies on amipurimycin, both Rauter and Garner research groups have observed that, in the osmium tetroxide-catalyzed dihydroxylation of alkylidene pyrans structurally very similar to **17**, the bulky oxidizing reagent approaches the exocyclic olefin from the less hindered β-face, resulting in stereoselective formation

(9) For similar examples, see: (a) Bhatt, R. K.; Chauhan, K.; Wheelan, P.; Falck, J. R.; Murphy, R. C. *J. Am. Chem. Soc.* **1994**, *116*, 5050–5056. (b) Reference 6b above.

SCHEME 2



of the product diol.[4b,5] Accordingly, employing the above protocol, cis-dihydroxylation of the olefinic moiety of the alkylidene pyran derivative **17**, with a catalytic quantity of osmium tetroxide, followed by acetylation (to facilitate chromatographic separation) of the crude diol resulted in the isolation of the corresponding acetates **18** and **19**, with the expected product **18** being the major diastereoisomer (**18**:**19** = 82:18). In accordance with the aforementioned literature precedence, formation of the major diastereoisomer **18** is probably a result of the sterically more favorable approach of the oxidant from the top face of the olefin. Our subsequent attempts to further improve the selectivity in the above dihydroxylation reaction, via employment of the Sharpless asymmetric dihydroxylation (SAD) protocol,[10] however, failed to produce any desired improvement. The failure of SAD in the present substrate is most probably due to the steric crowding around the trisubstituted exocyclic olefin **17** and its unfavorable interactions with the bulky chiral catalyst.

With the completely functionalized carbohydrate amino acid core of amipurimycin in hand, incorporation of the purine nucleobase toward formation of the desired nucleoside fragment was initiated. Thus, standard deprotection of the PMB protecting group of **18**, followed by acetylation of the resulting crude lactol yielded the corresponding acetate **20** as an anomeric mixture of products (Scheme 2). Employing the Vorbrüggen conditions,[11] the nucleoside-forming reaction between the glycosyl donor **20** and the bis-TMS-2-(*N*-acetylamino)purine **21**[12] was next undertaken. Disappointingly, multiple attempts, involving different combinations of Lewis acid activators/solvents and various reaction conditions (Scheme 2), failed to provide the desired nucleoside, instead resulting in either the recovery or extensive degradation (under more rigorous conditions) of the starting material.

Investigating the possibility of the above nucleoside formation with a simpler pyrimidine nucleobase, reaction of **20** with commercially available bis-TMS-thymine (**22**) was then attempted. Once again the attempted reactions failed to form the desired product. In their studies on amipurimycin, Czernecki and co-workers did report the introduction of a 2-aminopurine nucleoside to a model glycosyl amino acid donor.[4d] However, the above study was carried out on a structurally simplified substrate lacking the branched chain at C-3 of the pyranosyl carbohydrate core.

Being unable to achieve *N*-glycosidation of the pyranoside **20** under the traditional Vorbrüggen conditions, we decided to explore alternative methods toward activation of the above glycosyl donor and study its nucleoside-forming reaction. Formation of activated glycosyl donors via the intermediacy of highly reactive anomeric trichloroacetimidates (Schmidt's trichloroacetimidate protocol)[13] is among the most commonly used methods in contemporary *O*-glycosidic bond-forming reactions. Surprisingly, the employment of this method in the synthesis of nucleosides remains relatively infrequent.[11] With the intent to investigate the trichloroacetimidate protocol in our desired nucleoside synthesis, the anomeric PMB-ether **18** was subjected to standard CAN deprotection, followed by reaction of the resulting crude lactol with trichloroacetonitrile, forming the expected trichloroacetimidate derivative **23** (Scheme 3) in high overall yield. This compound was, however, found to undergo decomposition if left in the chromatographic column for an extended period of time, or on prolonged storage. Therefore, after rapid purification, compound **23** was used immediately for the next reaction. Unfortunately, repeated attempts to couple the trichloroacetimidate **23** with the bis-silylated 2-aminopurine derivative **21** failed to result in the desired nucleoside formation. Gratifyingly, reaction of **23** with bis-(TMS)thymine (**30**), in the presence of TMSOTF as the Lewis acidic activator, finally led to the highly stereoselective formation of the corresponding thymine amipurimycin nucleoside **24**, albeit in a modest yield (Scheme 3). The characteristic coupling constant of the anomeric proton ($J_{1',2'} = 9.6$ Hz)[2] of a downstream product derived from **24** (compound **27**, vide infra) helped confirm the H-1′/H-2′ trans-relationship and the assigned C-1′ stereochemistry of the nucleoside derivative **24**. The anomeric stereoselectivity in the above nucleoside-forming reaction can be rationalized by invoking neighboring C-2′-acetoxy-assisted stabilization of the oxonium ion intermediate and consequent blocking of the α-face, directing the approach of the incoming nucleobase from the opposite β-face.

Subsequently, peptidic attachment of the cispentacin side chain was accomplished by hydrogenolysis of the *N*-Cbz

(10) For recent reviews on Sharpless Asymmetric Dihydroxylation, see: (a) Johnson, R. A.; Sharpless, K. B. Catalytic asymmetric dihydroxylation-discovery and development. In *Catalytic Asymmetric Synthesis*, 2nd ed.; Ojima, I., Ed.; Wiley-VCH: New York, 2000; pp 357−398. (b) Kolb, H. C.; Sharpless, K. B. Asymmetric Dihydroxylation. In *Transition Metals for Organic Synthesis*, 2nd ed.; Beller, M., Bolm, C., Eds.; Wiley-VCH: Weinheim, Germany, 2004; Vol. 2, pp 275−298. (c) Zaitsev, A. B.; Adolfsson, H. Synthesis2006, *172*, 5–1756, and references therein.

(11) Vorbrüggen, H.; Ruh-Pohlenz, C. Org. React. 2000, *55*. 1–630, and references therein.

(12) Garner, P.; Yoo, J. K.; Sarabu, R. *Tetrahedron* **1992**, *21*, 4259–4270.

(13) (a) Schmidt, R. R.; Michel, J. *J. Carbohydr. Chem.* **1985**, *4*, 141–169. (b) Schmidt, R. R. The anomeric O-alkylation and the trichloroacetimidate method—versatile strategies for glycoside bond formation. *Front. Nat. Prod. Res.* **1996**, *1* (Modern Methods in Carbohydrate Synthesis), 20–54. (c) Schmidt, R. R.; Jung, K.-H. Oligosaccharide Synthesis with Trichloroacetimidates. In *Preparative Carbohydrate Chemistry*; Hanessian, S., Ed.; Marcel Dekker Inc.: New York, 1997; pp 283−312, and references therein.

SCHEME 3

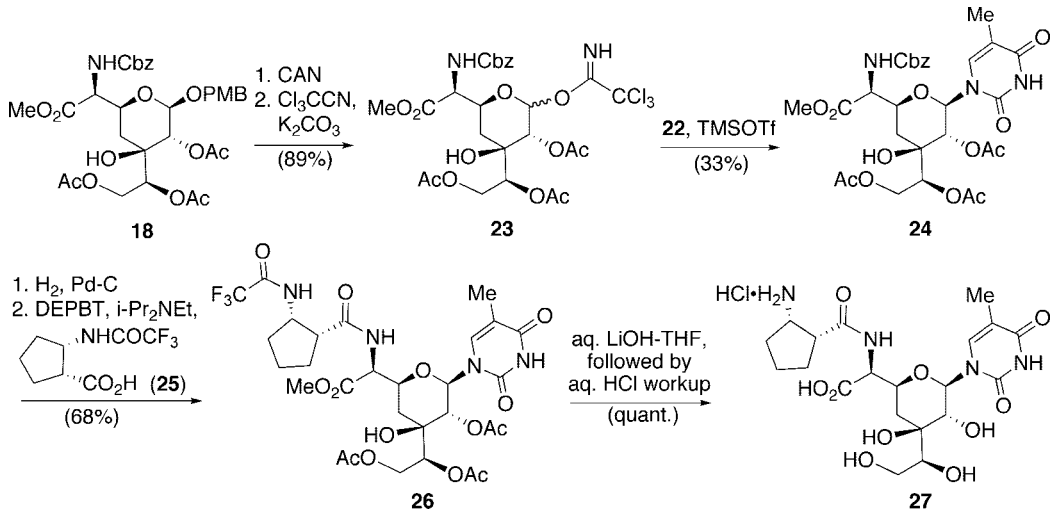


TABLE 1. Comparison of Selected ^{1}H and ^{13}C NMR Chemical Shifts of Amipurimycin and the Thymine Amipurimycin Analogue 27

δ (ppm)	amipurimycin (**1**) (in D_2O)[2]	thymine amipurimycin (**27**) (in D_2O)
H-6′	4.38(J=4Hz)	4.52(J=3.7Hz)
C-6′	58.5	56.1
H-2″	2.94	3.08
C-2″	45.1	45.3
H-3″	3.72	3.73
C-3″	53.8	53.5
H-1′	5.80(J=9Hz)	5.92(J=9.6Hz)
C-1′	80.5	81.7

protecting group of **24** to unmask the free amine, which was then coupled to (1*R*,2*S*)-*N*-trifluoroacetamido cispentacin derivative **25**[4d] to obtain the fully protected peptidyl nucleoside structural core **26**. Global removal of the protecting groups via alkaline hydrolysis completed the total synthesis of the unique thymine amipurimycin analogue **27**. The structural assignment and stereochemistry of the final product and its precursors were confirmed on the basis of their spectral/analytical data and literature precedence, wherever applicable.

Except for the nucleobase component, overlapping similarity between the remaining structural features of natural amipurimycin (**1**) and the thymine amipurimycin analogue **27** prompted us to compare the NMR spectral data of the above compounds. Comparison of the above data, related to the as yet undetermined C-6′, C-2″, and C-3″ stereocenters of amipurimycin (**1**) and the corresponding known stereocenters of the synthetic analogue **27**, led to some interesting observations (Table 1).

As is evident, the proton and carbon chemical shift values associated with the C-6′ α-amino acid stereocenter in amipurimycin (**1**),[2] as well as in the synthetic thymine analogue **27**, are very similar (Table 1). Additionally, the vicinal coupling constant ($J_{5',6'}$) values for H-6′ in both the above compounds are also strikingly close. The above data indicate that the stereochemical configuration of the as yet unassigned C-6′ stereocenter in natural amipurimycin is probably the same (*S*) as in the presently synthesized D-serine-derived analogue **27**. Along similar lines, the high conformity as seen between the respective proton and carbon chemical shift values of the side chain cispentacin stereocenters (2″ and 3″) in compounds **1** and **27** suggests that the hitherto undetermined C-2″ and C-3″ stereocenters in natural amipurimycin are probably in a (2″*R*,3″*S*)-configuration, similar to the enantiopure (1*R*,2*S*)-cispentacin-derived synthetic analogue **27**.

In summary, utilizing a D-serine-derived enantiopure dihydropyranone intermediate as a chiral platform, a robust and efficient synthetic route to the complete glycosyl amino acid core of amipurimycin has been developed. In terms of stereocontrolled formation of new chiral centers and overall product yield, the present method compares well with the previously reported syntheses of the C-3′-branched carbohydrate structural core of amipurimycin. Although our attempts to form the 2-amino purine containing nucleoside segment as present in the natural product were unsuccessful, employing the trichloroacetimidate glycosidation protocol, we could incorporate a pyrimidine nucleobase to the above glycosyl core, thereby accomplishing the first total synthesis of a fully functionalized thymine analogue of amipurimycin. Employing an alternative strategy of introduction of the aminopurine nucleobase on a more simple, early stage carbohydrate precursor (prior to C-3 branching), in future studies, we plan to elaborate the present method toward eventual completion of the total synthesis of amipurimycin, as well as for SAR-targeted syntheses and biological evaluation of strategically modified derivatives thereof.

Experimental Section

(*R*)-Benzyl 4-((3a*R*,6*S*,7a*R*)-2,2-Dimethyl-4-oxotetrahydro-3a*H*-[1,3]-dioxolo[4,5-*c*]pyran-6-yl)-2,2-dimethyloxazolidine-3-carboxylate (8). To a stirring solution of the diol **7**[6a] (4.29 g, 11.8 mmol) in acetone (30 mL) and 2,2-dimethoxypropane (15 mL, 117.5 mmol) at room temperature was added $BF_3 \cdot OEt_2$ (0.1 mL, 0.7 mmol) dropwise. After being stirred at ambient temperature for 3 h, the reaction was quenched with triethylamine (1 mL). The solvent was removed under reduced pressure and the resulting oil was purified by flash chromatography (hexanes/EtOAc = 8:2 to 6:4) to afford the diacetonide derivative **8** as a viscous liquid (4.57 g, 96%): $[\alpha]_D$ 88.4 (*c* 1.00, $CHCl_3$); IR (NaCl) 1752, 1701 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$; mixture of rotamers) δ 1.28 and 1.35 (2 s, 3H), 1.39−1.63 (m, 9H), 1.93−2.14 (m, 2H), 3.94 and 3.97 (2 d, J = 5.1 Hz, 1H), 4.03−4.22 (m, 2H), 4.52−4.68 (m, 3H), 5.09−5.23 (m, 2H), 7.36−7.42 (m, 5H); ^{13}C NMR (125 MHz, $CHCl_3$; mixture of rotamers) δ 23.0, 23.9, 24.0, 24.5, 25.8, 26.0, 27.0, 27.8, 29.7, 31.8, 58.9, 59.7, 64.7, 65.3, 67.2, 67.7, 71.7, 71.8, 73.0, 74.2, 74.4, 94.5, 95.0, 110.8, 128.1, 128.5, 128.7, 135.8, 152.3, 153.9, 167.2, 167.4; HRMS calcd for $C_{21}H_{28}NO_7$ *m/z* (M + H)$^+$ 406.1866, found 406.1876.

(*R*)-Benzyl 4-((3a*R*,4*R*,6*S*,7a*R*)-4-(4-Methoxybenzyloxy)-2,2-dimethyltetrahydro-3a*H*-[1,3]dioxolo[4,5-*c*]pyran-6-yl)-2,2-dimethyloxazolidine-3-carboxylate (9). Step 1: The lactone **8** (4.57 g, 11.28 mmol) was dissolved in anhydrous CH_2Cl_2 (60 mL) and cooled to −78 °C. To this stirring solution was added DIBAL-H (1.0 M in toluene, 13.5 mL, 13.5 mmol) dropwise. The reaction was stirred at −78 °C for 1 h and then quenched by careful addition of MeOH (1.0 mL). The reaction mixture was brought to rt and diluted with EtOAc (80 mL), then saturated aq sodium potassium tartrate solution (80 mL) was added. The resulting mixture was stirred until two clear layers were seen. The layers were separated, and the aqueous layer was extracted with EtOAc (3 × 25 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The crude lactol (4.72 g) thus obtained was carried on to the next step without further purification.

Step 2: The lactol (4.7 g, 11.6 mmol) as obtained above was dissolved in anhydrous CH_2Cl_2 (80 mL). To this stirring solution at room temperature was added tetrabutylammonium iodide (5.14 g, 13.9 mmol) and freshly prepared Ag_2O (4.0 g, 17.4 mmol), followed by *p*-methoxybenzyl bromide (1.8 mL, 12.8 mmol). The resulting brown mixture was stirred for 2.5 h, then the solids were filtered off and washed with CH_2Cl_2 (100 mL). The combined filtrate was concentrated, redissolved in CH_2Cl_2 (40 mL), and washed with 10% sodium thiosulfate (1 × 10 mL), then the aqueous layer was back extracted with CH_2Cl_2 (1 × 10 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under vacuum, then the residue was purified by flash chromatography (hexanes/EtOAc = 8:2) to yield the PMB-glycoside **9** as a colorless oil (4.94 g, 83% over two steps): $[\alpha]_D$ −18.2 (*c* 0.75, $CHCl_3$); IR (NaCl) 1703 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$; mixture of rotamers) δ 1.34−1.40 (4 s, 6H), 1.60 and 1.67 (2 s, 6H), 1.76−1.90 (m, 1H), 2.03 (br s, 1H), 3.81 (s, 3H), 3.87−4.07 (m, 3H), 4.09−4.19 (m, 2H), 4.34 and 4.47 (2 br s, 1H), 4.53−4.60 (m, 2H), 4.79 (t, *J* = 12.1 Hz, 1H), 5.13−5.18 (m, 2H), 6.88 (d, *J* = 8.2 Hz, 2H), 7.32−7.40 (m, 2H), 7.38 (br s, 5H); ^{13}C NMR (125 MHz, $CDCl_3$; mixture of rotamers) δ 23.2, 24.6, 25.4, 25.5, 26.7, 27.4, 27.5, 27.7, 29.6, 29.7, 29.9, 55.3, 60.1, 61.0, 64.8, 65.2, 66.9, 67.4, 69.4, 69.6, 69.9, 71.8, 72.0, 74.7, 74.8, 94.3, 94.7, 99.6, 109.0, 113.8, 128.1, 128.2, 128.3, 128.6, 129.4, 129.5, 136.0, 152.5, 153.8, 159.3; HRMS calcd for $C_{29}H_{38}NO_8$ *m*/*z* $(M + H)^+$ 528.2598, found 528.2568.

Benzyl (*R*)-1-((2*S*,4*R*,5*R*,6*R*)-4,5-Dihydroxy-6-(4-methoxybenzyloxy)tetrahydro-2*H*-pyran-2-yl)-2-hydroxyethylcarbamate (10). The diacetonide **9** (1.4 g, 2.7 mmol) was dissolved in acetic acid/water (30 mL, 3:1) and heated at 40 °C for 12 h. Excess solvent was removed under reduced pressure, and the residue was purified by flash chromatography ($CHCl_3$/MeOH = 97:3 to 95:5) to afford the triol **10** as a white solid (1.07 g, 83%): mp 117−119 °C; $[\alpha]_D$ −44.4 (*c* 0.90, MeOH); IR (Teflon film) 3506, 3325, 1683 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 1.61 (t, *J* = 13.9 Hz, 1H), 1.87 (d, *J* = 14.1 Hz, 1H), 3.66−3.74 (m, 4H), 3.76 (s, 3H), 3.82−3.93 (m, 1H), 4.08 (br s, 1H), 4.57 (d, *J* = 11.4 Hz, 1H), 4.67 (d, *J* = 8.0 Hz, 1H), 4.76 (d, *J* = 11.0 Hz, 1H), 5.07 and 5.12 (2 d, *J* = 6.3 Hz, 2H), 6.87 (d, *J* = 8.0 Hz, 2H), 7.28−7.38 (m, 7H); ^{13}C NMR (125 MHz, CD_3OD) δ 36.1, 55.8, 57.8, 62.4, 67.7, 69.2, 70.9, 71.6, 72.8, 101.2, 114.8, 129.0, 129.1, 129.6, 131.0, 131.4, 138.5, 158.9, 161.0; HRMS calcd for $C_{23}H_{30}NO_8$ *m*/*z* $(M + H)^+$ 448.1971, found 448.1977.

Benzyl (*R*)-2-(*tert*-Butyldimethylsilyloxy)-1-((2*S*,4*R*,5*R*,6*R*)-4,5-dihydroxy-6-(4-methoxybenzyloxy)tetrahydro-2*H*-pyran-2-yl)ethylcarbamate (11). To a stirred solution of the triol **10** (0.37 g, 0.82 mmol) in anhydrous DMF (6 mL), DMAP (25 mg, catalytic), and imidazole (0.12 g, 1.8 mmol) was added TBDMSCl (0.15 g, 0.98 mmol). The resulting solution was heated at 70 °C for 12 h. After cooling to room temperature, the reaction was quenched by addition of H_2O (10 mL), followed by dilution of the mixture by ether (20 mL), and stirred for 5 min. The two layers were separated and the aqueous layer was extracted with ether (3 × 10 mL). The combined organic extracts were washed with brine (1 × 20 mL), dried with Na_2SO_4, and concentrated under vacuum, and the residue was purified by flash chromatography (EtOAc/hexanes = 3:2) to afford the diol **11** as a white solid (0.368 g, 80%): mp 97−99 °C; $[\alpha]_D$ −35.5 (*c* 0.20, $CHCl_3$); IR (NaCl) 3442, 1717, 1701 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.06 (s, 6H), 0.82 (s, 9H), 1.64 (t, *J* = 12.1 Hz, 1H), 1.95 (d, *J* = 14.5 Hz, 1H), 2.45 and 2.48 (2 s, 2H, exchangeable with D_2O), 3.35 (br s, 1H), 3.59 (dd, *J* = 3.4 and 9.9 Hz, 1H), 3.73 (br s, 4H), 3.87−3.95 (m, 2H), 4.11 (br s, 1H), 4.41 (d, *J* = 11.2 Hz, 1H), 4.62 (d, *J* = 7.9, 1H), 4.77 (d, *J* = 11.2, 1H), 5.04 (s, 2H), 5.07 (d, *J* = 9.4 Hz, 1H), 6.82 (d, *J* = 8.6 Hz, 2H), 7.21 (d, *J* = 8.5 Hz, 2H), 7.24−7.27 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.2, −5.1, 18.5, 26.1, 34.1, 55.4, 55.5, 61.6, 67.0, 67.3, 69.2, 71.1, 71.9, 100.1, 114.2, 128.3, 128.4, 128.7, 129.6, 129.9, 136.7, 156.4, 159.7; HRMS calcd for $C_{29}H_{43}NO_8SiNa$ *m*/*z* $(M + Na)^+$ 584.2656, found 584.2655.

(2*R*,3*R*,4*R*,6*S*)-3-Acetoxy-4-hydroxy-2-(4-methoxybenzyloxy)-6-((*R*)-8,8,9,9-tetramethyl-3-oxo-1-phenyl-2,7-dioxa-4-aza-8-siladecan-5-yl)tetrahydro-2*H*-pyran (12). The diol **11** (1.35 g, 2.4 mmol) was dissolved in anhydrous CH_2Cl_2 (20 mL) and cooled to 0 °C. To this solution was added anhydrous pyridine (0.3 mL, 3.6 mmol) and DMAP (25 mg, catalytic), followed by Ac_2O (0.25 mL, 2.6 mmol). The reaction mixture was stirred at 0 °C for 1 h and then quenched with addition of ice-cooled water (5 mL). The two layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (1 × 10 mL) and brine (1 × 10 mL), dried with Na_2SO_4, and concentrated. The residue was purified by flash chromatography (hexanes/EtOAc = 3:2) to provide the monoacetylated product **12** as a foamy solid (1.26 g, 88%): mp 108−110 °C; $[\alpha]_D$ −52.3 (*c* 1.1, $CHCl_3$); IR (NaCl) 3442, 1724 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.09 and 0.10 (2 s, 6H), 0.92 (s, 9H), 1.82 (t, *J* = 12.1 Hz, 1H), 2.03 (d, *J* = 11.2 Hz, 1H), 2.10 (s, 4H, 1H exchangeable with D_2O), 3.67 (dd, *J* = 3.4 and 9.9 Hz, 1H), 3.79 (m, 1H), 3.82 (s, 3H), 4.02 (t, *J* = 9.4 Hz, 2H), 4.28 (br s, 1H), 4.53 (d, *J* = 11.8 Hz, 1H), 4.82 (d, *J* = 11.6 Hz, 2H), 4.87 (d, *J* = 8.2 Hz, 1H), 5.12−5.16 (m, 3H), 6.88 (d, *J* = 8.7, 2H), 7.24 (d, *J* = 8.5 Hz, 2H), 7.33−7.39 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.4, −5.3, 18.3, 21.0, 25.9, 34.2, 55.1, 55.2, 61.4, 66.6, 66.9, 68.8, 70.4, 73.0, 97.6, 113.8, 128.1, 128.2, 128.5, 129.1, 129.7, 136.5, 156.1, 159.3, 169.4; HRMS calcd for $C_{31}H_{45}NO_9SiNa$ *m*/*z* $(M + Na)^+$ 626.2761, found 626.2759.

(2*R*,3*S*,6*S*)-3-Acetoxy-2-(4-methoxybenzyloxy)-6-((*R*)-8,8,9,9-tetramethyl-3-oxo-1-phenyl-2,7-dioxa-4-aza-8-siladecan-5-yl)tetrahydro-2*H*-pyran-4-one (13). To an ice-cooled anhydrous CH_2Cl_2 solution (30 mL) of the alcohol **12** (1.2 g, 2.0 mmol) was added Dess−Martin periodinane (15% in CH_2Cl_2, 8 mL, 2.8 mmol) dropwise. The resulting solution was stirred at 0 °C for 0.5 h, the ice bath was removed, and the reaction was allowed to attain rt. After being stirred for another 2.5 h, the reaction was quenched by addition of saturated aq $NaHCO_3$ (20 mL) and solid sodium thiosulfate (3.0 g) followed by its dilution with EtOAc (50 mL). After allowing the mixture to stir until clear separation of the organic layer, the two layers were separated, and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic extracts were washed sequentially with saturated aqueous $NaHCO_3$ (1 × 20 mL) and brine (1 × 20 mL), dried (Na_2SO_4), and concentrated under vacuum. The residue was purified by flash chromatography to afford the ketone **13** as a viscous oil (1.09 g, 91%): $[\alpha]_D$ −61.6 (*c* 1.28, $CHCl_3$); IR (NaCl) 1753, 1731 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.07 (s, 6H), 0.89 (s, 9H), 2.14 (s, 3H), 2.61−2.76 (m, 2H), 3.66−3.70 (m, 2H), 3.80 (s, 3H), 3.91−3.98 (m, 1H), 4.02 (d, *J* = 9.9 Hz, 1H), 4.58 (d, *J* = 11.6 Hz, 1H), 4.67 (d, *J* = 8.2 Hz, 1H), 4.84 (d, *J* = 11.6, 1H), 5.11−5.14 (m, 4H), 6.88 (d, *J* = 8.6 Hz, 2H), 7.24 (d, *J* = 8.6 Hz, 2H), 7.35 (br s, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.5, −5.4, 18.2, 20.5, 25.8, 44.0, 55.2, 55.3, 61.2, 67.2, 70.3, 71.0, 77.9, 100.6, 113.9, 128.2, 128.4, 128.6, 128.7, 129.4, 136.1, 156.0, 159.5, 169.4, 199.1; HRMS calcd for $C_{31}H_{43}NO_9SiNa$ *m*/*z* $(M + Na)^+$ 624.2605, found 624.2587.

Benzyl (*R*)-2-(*tert*-Butyldimethylsilyloxy)-1-((2*S*,5*R*,6*R*,*E*)-5-hydroxy-4-((*E*)-methoxycarbonylidene)-6-(4-methoxybenzyloxy)-tetrahydro-2*H*-pyran-2-yl)ethylcarbamate (14). To a stirred solution of the ketone **13** (0.186 g, 0.31 mmol) in anhydrous benzene (6 mL) was added (carbomethoxymethylene)triphenylphosphorane (0.22 g, 0.62 mmol). The resulting solution was refluxed at 80 °C for 12 h and then the solvent was removed under vacuum. The resulting residue was purified by flash chromatography (hexanes/EtOAc = 4:1) to yield the *E*-alkylidene ester **14** as a viscous oil (0.197 g, 95%): $[\alpha]_D$ −56.3 (*c* 0.50, $CHCl_3$); IR (NaCl) 1751, 1722 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.09 (s, 6H), 0.91 (s, 9H), 2.14 (s, 3H), 2.17−2.24 (m, 1H), 3.64 (br t, J = 9.5 Hz, 1H), 3.69 (br s, 4H), 3.81 (s, 3H), 3.89−3.96 (m, 1H), 4.00 (d, 10.0 Hz, 1H), 4.14 (d, J = 14.2, 1H), 4.40 (d, J = 7.7 Hz, 1H), 4.55 (d, J = 11.8 Hz, 1H), 4.81 (d, J = 11.8 Hz, 1H), 5.15 and 5.21 (2 d, J = 12.2 Hz, 3H), 5.33 (d, J = 7.5 Hz, 1H), 5.77 (s, 1H), 6.88 (d, J = 8.8 Hz, 2H), 7.23 (d, J = 8.6 Hz, 2H), 7.34−7.39 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.4, −5.3, 18.3, 20.8, 25.9, 31.7, 51.3, 55.3, 61.4, 66.9, 70.3, 72.9, 73.0, 77.3, 101.4, 113.2, 113.8, 128.0, 128.1, 128.5, 129.2, 136.5, 151.4, 156.1, 159.3, 166.1, 169.2; HRMS calcd for $C_{34}H_{48}NO_{10}Si$ *m*/*z* $(M + H)^+$ 658.3047, found 658.3051.

Benzyl (*R*)-2-(*tert*-Butyldimethylsilyloxy)-1-((2*S*,5*R*,6*R*,*E*)-5-acetoxy-4-(2-acetoxyethylidene)-6-(4-methoxybenzyloxy)tetrahydro-2*H*-pyran-2-yl)ethylcarbamate (15). Step 1: The alkylidene ester **14** (1.47 g, 2.2 mmol) was dissolved in anhydrous CH_2Cl_2 (15 mL) and cooled to −40 °C. To this stirring solution was added DIBAL-H (1.0 M in toluene, 7.0 mL, 7.0 mmol) dropwise. The reaction was stirred at −40 °C for 1 h and upon completion of the reaction (TLC monitoring often necessitated an additional 1.0 equiv of DIBAL-H to be added) was quenched by careful addition of MeOH (2.0 mL). The reaction mixture was brought to rt and diluted with EtOAc (50 mL), then saturated aqueous sodium potassium tartrate (25 mL) was added. The resulting mixture was stirred until two clear layers were seen. The two layers were separated, and the aqueous layer was extracted with EtOAc (3 × 30 mL). The combined extracts were washed with brine, dried (Na_2SO_4), and concentrated under vacuum. The residue thus obtained (1.12 g) was directly used for the next reaction without any further purification.

Step 2: The crude diol (1.12 g, 1.87 mmol) as obtained from above was dissolved in anhydrous CH_2Cl_2 (15 mL) and treated with pyridine (0.8 mL, 9.3 mmol), DMAP (20 mg, catalytic), and Ac_2O (0.9 mL, 9.3 mmol). The resulting mixture was stirred at rt for 2 h followed by quenching of the reaction by addition of ice-cooled water (10 mL). The two layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (1 × 5 mL) and brine (1 × 5 mL), dried with Na_2SO_4, and concentrated under vacuum. The crude product obtained was purified by flash chromatography (hexanes/EtOAc = 7:3) affording the diacetate **15** as a low-melting solid (1.16 g, 79% over 2 steps): $[\alpha]_D$ −47.4 (*c* 0.50, $CHCl_3$); IR (NaCl) 1740 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.01 (s, 6H), 0.83 (s, 9H), 1.97 (s, 3H), 2.04 (br s, 4H), 2.74 (d, J = 13.0 Hz, 1H), 3.37 (t, J = 9.4 Hz, 1H), 3.6 (dd, J = 3.3 and 10.0 Hz, 1H), 3.73 (s, 3H), 3.77 (br s, 1H), 3.92 (d, J = 9.9 Hz, 1H), 4.28 (d, J = 7.7 Hz, 1H), 4.39−4.46 (m, 2H), 4.55−4.59 (m, 1H), 4.71 (d, J = 11.9 Hz, 1H), 5.04−5.17 (m, 4H), 5.39 (t, J = 7.0 Hz, 1H), 6.8 (d, J = 8.6 Hz, 2H), 7.14 (d, J = 8.5, 2H), 7.25−7.31 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.3, −5.2, 18.5, 21.1, 21.2, 26.1, 31.3, 55.5, 56.0, 60.2, 61.5, 67.1, 70.4, 73.0, 73.3, 101.6, 114.0, 117.3, 128.3, 128.4, 128.8, 129.3, 129.6, 136.5, 136.7, 156.4, 159.5, 169.6, 170.0; HRMS calcd for $C_{35}H_{50}NO_{10}Si$ *m*/*z* $(M + H)^+$ 672.3204, found 672.3201.

Benzyl (*R*)-2-Hydroxy-1-((2*S*,5*R*,6*R*,*E*)-5-acetoxy-4-(2-acetoxyethylidene)-6-(4-methoxybenzyloxy)tetrahydro-2*H*-pyran-2-yl)-ethylcarbamate (16). To a stirred solution of **15** (0.99 g, 1.48 mmol) in anhydrous THF (10 mL) at 0 °C was added TBAF (1 M in THF, 2.0 mL, 2 mmol). The reaction was stirred for 3 h at 0 °C, and then quenched with saturated aqueous ammonium chloride solution (10 mL). After the mixture was diluted with EtOAc (25 mL), the two layers were separated, and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic extracts were washed with brine (1 × 25 mL), dried with Na_2SO_4, and concentrated under vacuum. The crude residue was purified by flash chromatography (EtOAc/hexanes = 3:2) to afford the alcohol **16** as a foamy solid (0.786 g, 96%): mp = 94−96 °C; $[\alpha]_D$ −44.40 (*c* 1.0, $CHCl_3$); IR (NaCl) 3350, 1736 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.06 (s, 3H), 2.11 (s, 1H), 2.15 (s, 3H), 2.40 (br s, 1H, exchangeable with D_2O), 2.80 (d, J = 12.4 Hz, 1H), 3.55−3.65 (m, 1H), 3.69−3.77 (m, 1H), 3.79 (s, 4H), 3.98 (d, J = 10.7 Hz, 1H), 4.33 (d, J = 7.8 Hz, 1H), 4.49−4.74 (m, 4H), 5.15 (s, 2H), 5.24 (d, J = 7.5 Hz, 1H), 5.48−5.54 (m, 2H), 6.87 (d, J = 8.4 Hz, 2H), 7.22 (d, J = 8.4 Hz, 2H), 7.34−7.40 (m, 5H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 20.9, 21.0, 31.1, 54.6, 55.3, 59.9, 61.4, 67.0, 70.7, 72.6, 75.6, 101.6, 113.9, 117.4, 128.2, 128.3, 128.6, 129.2, 129.4, 135.8, 136.3, 156.3, 159.4, 169.5, 170.9; HRMS calcd for $C_{29}H_{35}NO_{10}Na$ *m*/*z* $(M + Na)^+$ 580.2158, found 580.2050.

(*S*)-Methyl 2-(Benzyloxycarbonylamino)-2-((2*S*,5*R*,6*R*,*E*)-5-(acetoxy)-4-(2-(acetoxyethylidene)-6-(4-methoxybenzyloxy)tetrahydro-2*H*-pyran-2-yl)ethanoate (17). To an ice-cooled anhydrous CH_2Cl_2 solution (10 mL) of the alcohol **16** (0.73 g, 1.3 mmol) was added Dess−Martin periodinane (15% in CH_2Cl_2, 5.0 mL, 1.8 mmol) dropwise. The reaction was stirred at 0 °C for 0.5 h, the ice-bath was removed, and the reaction was allowed to attain rt. After being stirred for another 2.5 h, the reaction was diluted with EtOAc (30 mL) and quenched by the addition of saturated aq $NaHCO_3$ (10 mL) solution and solid sodium thiosulfate (2.0 g). After the mixture was stirred until the organic layer was mostly clear, the two layers were separated, and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic extracts were washed sequentially with saturated aqueous $NaHCO_3$ (1 × 20 mL) and brine (1 × 20 mL), dried (Na_2SO_4), and concentrated under vacuum. The crude aldehyde thus obtained (∼0.7 g) was carried on to the next reaction without further purification.

Step 2: The crude aldehyde (0.7 g, 1.26 mmol) was dissolved in *tert*-butyl alcohol (50 mL) and 2-methyl-2-butene (2 M in THF, 33 mL, 66 mmol). To this stirring mixture was slowly added a solution of sodium chlorite (1.23 g, 13.6 mmol) and sodium dihydrogen phosphate (1.26 g, 10.5 mmol) dissolved in water (20 mL). The biphasic reaction was stirred vigorously at room temperature for 30 min. The two layers were separated, and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated to afford the crude acid as colorless oil (∼0.8 g), which was taken on to the next step without any further purification.

Step 3: [**Caution**: Diazomethane is an explosive and a highly toxic gas. Explosions may occur if the substance is dry and undiluted. All operations involving diazomethane should be carried out in an efficient fume hood following appropriate precaution.] To an ice-cooled biphasic solution of KOH (2 g in 8 mL of water) and ether (20 mL) was added *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG, 1 g) in one lot. The organic layer turned bright yellow. The ethereal layer was decanted into an ice-cooled Erlenmeyer flask containing KOH pellets. The aqueous layer was washed with ether (3 × 15 mL), and the ethereal layers were combined. The diazomethane (CH_2N_2) thus prepared was added to a stirred solution of the crude acid (0.8 g in 10 mL of ether) as obtained from step 2 above, and stirring was continued at room temperature for 30 min. Excess CH_2N_2 was removed by bubbling nitrogen into the reaction mixture for 15 min, followed by removal of solvent under vacuum to yield the crude product. Purification by flash chromatography (hexanes/EtOAc = 3:2) provided the methyl ester **17** as an oil (0.7 g, 85% over 3 steps): $[\alpha]_D$ −29.62 (*c* 1.0, $CHCl_3$); IR (NaCl) 3367, 1736 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.04 (s, 3H), 2.12 (s, 3H), 2.32 (t, J = 13.0 Hz, 1H), 2.80 (d, J = 12.5 Hz, 1H), 3.63 (br d, J = 11.7 Hz, 1H), 3.78 (s, 3H), 3.79 (s, 3H), 4.31 (d, J = 7.6 Hz, 1H), 4.48−4.56 (m, 3H), 4.60−4.66 (m, 1H), 4.70 (d, J = 15.0 Hz, 1H), 5.14 (s, 2H), 5.19 (d, J = 7.4 Hz, 1H), 5.49

(t, J = 6.9 Hz, 1H), 5.62 (d, J = 8.5 Hz, 1H), 6.85 (d, J = 8.4 Hz, 2H), 7.19 (d, J = 8.6 Hz, 2H), 7.32−7.38 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.8, 21.0, 30.7, 52.7, 55.2, 56.9, 59.8, 67.3, 69.9, 72.4, 75.1, 100.8, 113.8, 117.9, 128.2, 128.3, 128.6, 129.1, 129.4, 135.4, 136.0, 155.7, 159.3, 169.5, 169.7, 170.8; HRMS calcd for $C_{30}H_{35}NO_{11}Na$ m/z $(M + Na)^+$ 608.2108, found 608.2096.

Dihydroxylation and Acetylation of 17 To Form the Triacetates 18 and 19. Step 1: The allylic acetate **17** (0.66 g, 1.12 mmol) was dissolved in acetone/water (10 mL, 4:1) and cooled to 0 °C. To this stirring solution was added NMO·H_2O (0.38 g, 2.8 mmol), followed by OsO_4 (5% in toluene, 0.4 mL, 0.08 mmol). The resulting solution was stirred at 0 °C for 6 h. After completion of the reaction (TLC monitoring), 10% aqueous Na_2SO_3 (5 mL) was added to the reaction mixture along with EtOAc (15 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (3 × 5 mL). The combined organic extracts were dried with Na_sSO_4 and concentrated under vacuum. The crude diastereomeric mixture of product diols (0.63 g) was taken on to the next reaction without any further purification.

Step 2: The mixture of diols (0.64 g, 1.03 mmol) as obtained from the above reaction was dissolved in anhydrous CH_2Cl_2 (10 mL), and to this solution was added sequentially anhydrous pyridine (0.14 mL, 1.7 mmol), DMAP (25 mg, catalytic), and Ac_2O (0.15 mL, 1.5 mmol). The reaction was stirred at rt for 2 h and then quenched with ice-cooled water (5 mL). The two layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (1 × 10 mL) and brine (1 × 10 mL), dried with Na_2SO_4, and concentrated under vacuum. The mixture of diastereomeric products was separated by flash chromatography ($CHCl_3$/EtOAc = 9:1 to 4:1) to afford the corresponding triacetates **18** (major) and **19** (minor) (ca. 4:1 ratio) in 94% overall yield.

(*S*)-1-((2*R*,3*R*,4*R*,6*S*)-6-((*S*)-1-(Benzyloxycarbonylamino)-2-methoxy-2-oxoethyl)-3-(ethanoyloxy)-4-hydroxy-2-(4-methoxybenzyloxy)-tetrahydro-2*H*-pyran-4-yl)ethane-1,2-diyl diethanoate (18). Major isomer: foamy solid (0.57 g, 77%), mp = 44−46 °C; $[\alpha]_D$ −45.2 (*c* 1.95, $CHCl_3$); IR (NaCl) 3350, 1744 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.03, 2.04 and 2.07 (3 s, 9H), 2.01−2.22 (m, 2H), 3.59 (br s, 1H, exchangeable with D_2O), 3.81 and 3.82 (2 s, 6H), 4.08 and 4.11 (dd, J = 8.3 and 12.2 Hz, 1H), 4.18−4.25 (m, 1H), 4.45 (d, J = 11.4 Hz, 1H), 4.53 (dd, J = 2.6 and 12.2 Hz, 1H), 4.65−4.70 (m, 1H), 4.74 (d, J = 11.4 Hz, 1H), 4.84 (d, J = 2.8 Hz, 1H), 4.90 (d, J = 2.7 Hz, 1H), 5.15 (br s, 2H), 5.23 (dd, J = 1.8 and 8.2 Hz, 1H), 5.62 (d, J = 8.7 Hz, 1H), 6.87 (d, J = 8.6 Hz, 2H), 7.23 (d, J = 8.6 Hz, 2H), 7.28−7.47 (m, 5H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 20.8, 21.0, 33.5, 52.7, 55.3, 57.2, 62.6, 67.4, 70.2, 70.4, 71.1, 71.7, 72.3, 98.2, 113.9, 128.3, 128.4, 128.6, 129.6, 135.9, 156.0, 159.5, 169.7, 170.0, 170.3, 171.0; HRMS calcd for $C_{32}H_{39}NO_{14}Na$ m/z $(M + Na)^+$ 684.2268, found 684.2236.

(*R*)-1-((2*R*,3*R*,4*S*,6*S*)-6-((*S*)-1-(Benzyloxycarbonylamino)-2-methoxy-2-oxoethyl)-3-(ethanoyloxy)-4-hydroxy-2-(4-methoxybenzyloxy)tetrahydro-2*H*-pyran-4-yl)ethane-1,2-diyl Diethanoate (19). Minor isomer: foamy solid (0.126 g, 17%), mp 56−58 °C; $[\alpha]_D$ −14.79 (*c* 1.9, $CHCl_3$); IR (NaCl) 3354, 1744 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.89−1.99 (m, 2H), 2.05 (s, 3H), 2.12 (s, 6H), 2.50 (s, 1H, exchangeable with D_2O), 3.80 (s, 6H), 4.02−4.13 (m, 2H), 4.36 (d, J = 9.7 Hz, 1H), 4.46−4.52 (m, 2H), 4.70−4.73 (m, 2H), 4.90 (d, J = 7.6 Hz, 1H), 5.09 (dd, J = 2.8 and 7.5 Hz, 1H), 5.18 (s, 2H), 5.58 (d, J = 8.8 Hz, 1H), 6.86 (d, J = 8.3 Hz, 2H), 7.18 (d, J = 8.4 Hz, 2H), 7.39 (br s, 5H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 20.7, 20.9, 21.0, 33.6, 52.7, 55.3, 56.9, 61.8, 67.3, 70.5, 71.4, 71.6, 73.3, 73.9, 98.3, 113.8, 128.3, 128.4, 128.6, 129.2, 129.3, 136.0, 155.8, 159.3, 169.5, 169.7, 170.6, 171.1; HRMS calcd for $C_{32}H_{40}NO_{14}$ m/z $(M + H)^+$ 662.2449, found 662.2459.

(1*R*)-1-((3*R*,4*S*,6*S*)-6-((*S*)-1-(Benzyloxycarbonylamino)-2-methoxy-2-oxoethyl)-2,3-bis(ethanoyloxy)-4-hydroxytetrahydro-2*H*-pyran-4-yl)ethane-1,2-diyl Diethanoate (20). Step 1: To a stirring solution of the PMB-glycoside **18** (0.125 g, 0.19 mmol) in acetonitrile/water (5 mL, 9:1) was added ceric ammonium nitrate (0.32 g, 0.59 mmol). The reaction was stirred at rt for 1.5 h and then poured into a separatory funnel and diluted with EtOAc (10 mL). The organic layer was washed sequentially with water (5 mL), saturated aqueous $NaHSO_3$ (5 mL), and saturated aqueous $NaHCO_3$ (5 mL). The organic layer was dried with Na_2SO_4 and concentrated. The crude lactol (0.1 g) thus obtained was taken on to the next reaction without further purification.

Step 2: The above lactol (0.104 g, 0.19 mmol) was dissolved in anhydrous CH_2Cl_2 (2 mL), and to this solution was added anhydrous pyridine (0.04 mL, 0.5 mmol), DMAP (10 mg, catalytic), followed by Ac_2O (0.03 mL, 0.32 mmol). The reaction was stirred at rt for 2 h and then quenched with addition of ice-cooled water (5 mL). The two layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (1 × 10 mL) and brine (1 × 10 mL), dried with Na_2SO_4, and concentrated under vacuum. The residue was purified by flash chromatography (hexanes/EtOAc = 2:3) to yield the anomeric mixture of the triacetate **20** as a semisolid (0.1 g, 95% over 2 steps): ^{1}H NMR (400 MHz, $CDCl_3$; mixture of anomers) δ 1.93−2.22 (m, 14H), 3.75 and 3.78 (2 s, 3H), 3.91−4.27 (m, 2H), 4.38 (br d, J = 9.3 Hz, 1H), 4.85−5.30 (m, 6H), 5.61−5.69 (m, 1H), 5.95 and 6.44 (2 s, 1H), 7.33−7.42 (m, 5H); ^{13}C NMR (125.7 MHz, $CDCl_3$; mixture of anomers) δ 20.5, 20.7, 20.8, 20.9, 21.0, 22.4, 26.2, 31.2, 31.6, 52.6, 52.7, 56.6, 63.2, 63.5, 63.8, 67.3, 67.7, 69.1, 69.6, 69.9, 70.1, 70.8, 71.8, 72.7, 79.4, 80.2, 89.2, 90.0, 128,1, 128.2, 128.3, 128.6, 128.8, 128.9, 136.0, 155.8, 168.7, 168.9, 169.0, 169.2, 169.3, 169.5, 169.7, 169.8, 170.7, 170.9; HRMS calcd for $C_{26}H_{33}NO_{14}Na$ m/z $(M + Na)^+$ 606.1799, found 606.1755.

(*S*)-1-((3*R*,4*R*,6*S*)-6-((*S*)-1-(Benzyloxycarbonylamino)-2-methoxy-2-oxoethyl)-3-(ethanoyloxy)-4-hydroxy-2-(2,2,2-trichloro-1-iminoethoxy)tetrahydro-2*H*-pyran-4-yl)ethane-1,2-diyl Diethanoate (23). Step 1: To a stirring, room temperature solution of the PMB-glycoside **18** (0.28 g, 0.42 mmol) in acetonitrile/water (10 mL, 9:1) was added ceric ammonium nitrate (0.65 g, 1.19 mmol). After being stirred for 1.5 h the reaction mixture was poured into a separatory funnel and diluted with EtOAc (20 mL). The organic layer was washed sequentially with water (10 mL), saturated aqueous $NaHSO_3$ (5 mL), and saturated aqueous $NaHCO_3$ (5 mL). The organic layer was dried with Na_2SO_4 and concentrated. The crude lactol (0.22 g) was taken on to the next reaction without further purification.

Step 2: The above crude lactol (0.22 g, 0.41 mmol) was dissolved in anhydrous CH_2Cl_2 (8 mL), and to this solution was added anhydrous K_2CO_3 (0.2 g, 1.45 mmol), followed by dropwise addition of trichloroacetonitrile (0.4 mL, 4.0 mmol). The reaction was stirred at room temperature for 12 h and then quenched with water (5 mL). The two layers were separated and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (1 × 10 mL), dried (Na_2SO_4), and concentrated. The residue was quickly purified by flash chromatography (EtOAc/hexanes = 1:1) to afford the trichloroacetimidate **23** as a viscous liquid (0.256 g, 89% over 2 steps). The compound was found to be unstable and was used immediately after purification: IR (NaCl) 3336, 1745, 1677 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$; mixture of anomers) δ 2.05, 2.06, 2.07 and 2.10 (4 s, 9H), 2.14−2.30 (m, 2H), 3.73 and 3.76 (2 s, 3H), 4.05−4.43 (m, 4H), 4.92−5.17 (m, 4H), 5.41 (s, 1H), 5.61−5.70 (m, 1H), 6.08 and 6.58 (2 s, 1H), 7.37 (br s, 5H), 8.58 and 8.68 (2 s, 1H); ^{13}C NMR (125.7 MHz, $CDCl_3$; mixture of anomers) δ 20.7, 20.8, 29.7, 52.8, 55.3, 62.1, 62.2, 65.7, 67.8, 72.1, 72.9, 90.8, 92.2, 95.1, 128.3, 128.4, 128.5, 128.6, 135.6, 156.4, 160.0, 169.5, 170.4, 170.7; HRMS calcd for $C_{26}H_{31}Cl_3N_2O_{13}Na$ m/z $(M + Na)^+$ 707.0789, found 707.0741.

(*S*)-1-((2*R*,3*R*,4*R*,6*S*)-6-((*S*)-1-(Benzyloxycarbonylamino)-2-methoxy-2-oxoethyl)-3-(ethanoyloxy)-4-hydroxy-2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydro-2*H*-pyran-4-yl)ethane-1,2-diyl diethanoate (24). To a solution of trichloroace-

timidate **24** (0.163 g, 0.24 mmol) in anhydrous 1,2-dichloroethane (8 mL) was added commercially available bis(trimethylsilyl)thymine (**22**) (0.32 g, 1.2 mmol), followed by freshly distilled TMSOTf (0.17 mL, 0.96 mmol). The reaction was stirred at rt for 1 h, and then saturated aqueous $NaHCO_3$ (5 mL) solution was added to quench the reaction. The precipitated excess nucleobase was filtered off and the white solid was washed with $CHCl_3$ (3 × 5 mL). The combined filtrate was transferred to a separating funnel and the two layers were separated. The aqueous layer was extracted with $CHCl_3$ (3 × 5 mL) and combined organic extracts were dried with Na_2SO_4 and concentrated under vacuum. The residue was purified by flash chromatography (EtOAc/hexanes = 7:3 to 9:1) to afford the thymine nucleoside **24** as a white solid (0.051 g, 33%): mp 107−109 °C; $[\alpha]_D$ −6.2 (*c* 1.35, $CHCl_3$); IR (NaCl) 3296, 1744, 1697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.88 (s, 3H), 2.05, 2.06, and 2.10 (3 s, 9H), 2.16−2.29 (m, 2H), 3.80 (s, 3H), 4.07 (s, 1H, exchangeable with D_2O), 4.19 (dd, J = 7.7 and 11.9 Hz, 1H), 4.25 (br s, 1H), 4.55 (dd, J = 2.7 and 12.0 Hz, 1H), 4.61 (d, J = 6.6 Hz, 1H), 5.05 (d, J = 5.2 Hz, 1H), 5.14 (s, 2H), 5.41 (d, J = 4.7 Hz, 1H), 5.80 (d, J = 4.4 Hz, 1H), 6.02 (br s, 1H), 7.27 (s, 1H), 7.36 (br s, 5H), 8.92 (s, 1H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 12.6, 20.7, 20.8, 20.9, 33.2, 53.0, 57.2, 62.6, 67.4, 72.1, 72.7, 72.8, 77.3, 111.4, 128.2, 128.4, 128.6, 135.9, 136.1, 150.1, 155.9, 163.4, 169.7, 169.8, 169.9, 170.9; HRMS calcd for $C_{29}H_{36}N_3O_{14}$ *m*/*z* $(M + H)^+$ 650.2219, found 650.2179.

(*S*)-1-((2*R*,3*R*,4*R*,6*S*)-3-(Ethanoyloxy)-4-hydroxy-6-((S)-2-methoxy-2-oxo-1-((1*R*,2*S*)-2-(2,2,2-trifluoroethanamido)cyclopentanecarboxamido)ethyl)-2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydro-2*H*-pyran-4-yl)ethane-1,2-diyl Diethanoate (26). Step 1: To a room temperature solution of the nucleoside **31** (0.034 g, 0.05 mmol) in anhydrous EtOAc (3 mL) was added 10% palladium on activated carbon (0.035 g), then the mixture was stirred under an H_2 atmosphere for 2 h. The reaction mixture was filtered through Celite, and the residue was washed with EtOAc and MeOH. The filtrate was concentrated to yield the free amine as a white solid (0.27 g), which was taken on to the next step without further purification.

Step 2: To a stirring solution of *N*-TFA-(1*R*,2*S*)-cispentacin **25**[4d] (0.02 g, 0.079 mmol) in anhydrous THF (2 mL) were added *N*,*N*-diisopropylethylamine (22 μL, 0.13 mmol) and DEPBT (0.039 g, 0.13 mmol). The reaction was stirred at rt for 30 min, and then the amine (0.027 g, 0.05 mmol), obtained from step 1 above, and dissolved in anhydrous THF (2 mL), was added to the preactivated acid. The reaction mixture was stirred at rt for 12 h, and then was poured into a separatory funnel and diluted with EtOAc (5 mL). The organic layer was washed successively with 5% $NaHSO_4$ (1 × 5 mL), brine (1 × 5 mL), and 10% Na_2CO_3 (1 × 5 mL). The organic layer was dried (Na_2SO_4) then concentrated, and the residue was purified by flash chromatography (EtOAc/hexanes = 4:1 to 9:1) to afford the amide **26** as a white solid (0.025 g, 68%): mp 128−130 °C; $[\alpha]_D$ −23.31 (*c* 0.8, $CHCl_3$); IR (NaCl) 3275, 1740, 1707, 1654 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.60−1.67 (m, 1H), 1.78−1.86 (m, 5H), 1.91−1.99 (m, 2H), 2.02−2.10 (m, 11H), 2.15−2.20 (m, 1H), 2.30−2.38 (m, 1H), 3.02−3.11 (m, 1H), 3.88 (s, 3H), 4.03−4.10 (m, 1H), 4.13 (2 d, J = 6.6 and 11.9 Hz, 1H), 4.53 (br s, 1H), 4.57 (dd, J = 3.3 and 12.1 Hz, 1H), 4.80 (d, J = 7.7 Hz, 1H), 4.99 (br s, 1H), 5.42 (d, J = 4.6 Hz, 1H), 5.47 (br s, 1H), 7.17 (s, 1H), 7.82 (br s, 1H), 8.64 (br s, 1H), 9.23 (br s, 1H); ^{13}C NMR (201 MHz, $CDCl_3$) δ 12.6, 20.7, 21.0, 21.1, 22.9, 29.2, 29.9, 32.7, 46.5, 53.1, 53.6, 55.4, 62.6, 71.8, 73.2, 74.4, 111.8, 114.0, 115.4, 116.8, 118.2, 150.4, 156.7, 156.8, 157.0, 157.2, 163.4, 169.7, 170.0, 170.3, 171.3, 174.3; HRMS calcd for $C_{29}H_{38}F_3N_4O_{14}$ *m*/*z* $(M + H)^+$ 723.2337, found 723.2335.

"Thymine Amipurimycin" Hydrochloride (27). To an ice-cooled solution of the protected nucleoside **26** (0.016 g, 0.02 mmol) in anhydrous THF (2.4 mL) was added a solution of $LiOH \cdot H_2O$ (0.007 g, 0.18 mmol) in water (0.6 mL). After being stirred at 0 °C for 5 h the reaction was carefully acidified to pH 3 with 10% aq HCl at 0 °C. The solvent was removed under high vacuum and the residue was triturated with diethyl ether (3 × 2 mL) to afford "thymine amipurimycin" **27** as a white semisolid (0.011 g, quantitative): $[\alpha]_D$ 6.3 (*c* 0.55, H_2O); IR (NaCl) 3393, 1705, 1684, 1647 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ 1.70−2.00 (m, 8H), 2.11−2.17 (m, 3H), 3.08 (q, J = 6.5 Hz, 1H), 3.71 and 3.74 (2 d, J = 6.8 and 11.8 Hz, 1H), 3.78−3.89 (m, 3H), 4.34−4.39 (m, 2H), 4.52 (d, J = 3.7 Hz, 1H), 5.92 (d, J = 9.6 Hz, 1H), 7.57 (s, 1H); ^{13}C NMR (201 MHz, D_2O) δ 11.5, 21.4, 28.2, 30.2, 36.1, 45.3, 53.5, 56.1, 61.6, 72.4, 72.6, 72.8, 76.7, 81.7, 112.1, 137.2, 152.2, 166.2, 172.2, 174.8; HRMS calcd for $C_{20}H_{31}N_4O_{10}$ (free amine) *m*/*z* $(M + H)^+$ 487.2040, found 487.2015.

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Supporting Information Available: General experimental details and copies of NMR spectra (^1H and ^{13}C) of all the new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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