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3′-Bromo Analogues of Pyrimidine Nucleosides as a New Class of Potent Inhibitors of *Mycobacterium tuberculosis*

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Tuberculosis (TB) is a major health problem worldwide. We herein report a new class of pyrimidine nucleosides as potent inhibitors of *Mycobacterium tuberculosis* (*M. tuberculosis*). Various 2′- or 3′-halogeno derivatives of pyrimidine nucleosides containing uracil, 5-fluorouracil, and thymine bases were synthesized and evaluated for antimycobacterial activities. Among the compounds tested, 3′-bromo-3′-deoxy-arabinofuranosylthymine (**33**) was the most effective antituberculosis agent in the in vitro assays against wild-type *M. tuberculosis* strain (H37Ra) ($MIC_{50} = 1\ \mu g/mL$) as well as drug-resistant (H37Rv) (rifampicin-resistant and isoniazid-resistant) strains of *M. tuberculosis* ($MIC_{50} = 1-2\ \mu g/mL$). Compound **33** also inhibited intracellular *M. tuberculosis* in a human monocytic cell line infected with H37Ra, demonstrating higher activity against intramacrophagic mycobacteria (80% reduction at 10 μg/mL concentration) than extracellular mycobacteria (75% reduction at 10 μg/mL concentration). In contrast, pyrimidine nucleosides possessing 5-fluorouracil base were weak inhibitors of *M. tuberculosis*. No cytotoxicity was found up to the highest concentration of compounds tested ($CC_{50} > 100-200\ \mu g/mL$) against a human cell line. Overall, these encouraging results substantiate the potential of this new class of compounds as promising antituberculosis agents.

Introduction

Mycobacterium tuberculosis (*M. tuberculosis*),[a] the causative agent of tuberculosis (TB), is one of the most serious infections among immunocompromised and/or immunocompetent individuals and is the second leading cause of mortality worldwide. According to the World Health Organization (WHO), *M. tuberculosis* has infected approximately 2 billion people globally.[1]

TB is a contagious disease that spreads through aerosol. TB and human immunodeficiency virus (HIV) have formed a new and deadly combination. There is a resurgence in the incidence of TB in developed and developing countries with high rates of HIV−TB coinfection. The increase in the incidence of TB is strongly associated with the prevalence of HIV infection.[2] *M. tuberculosis* poses a significant challenge to the clinical management of HIV-infected patients and is often responsible for their death.[3] For both TB and HIV, misdiagnosis and noncompliance with treatment regimens further compound the problem and facilitate the development of drug resistance.[2,4,5]

The emergence of new TB strains that are not susceptible to a number of available drugs, that is, multidrug-resistant tuberculosis (MDR-TB), has further complicated the management of this disease.[5−7] Around 50 million people have been infected with MDR-TB, which is defined as resistant to at least two first-line drugs, isoniazid and rifampicin, and requires the use of second-line drugs. An outbreak of recently recognized "extensively drug-resistant tuberculosis" or XDR-TB, threatens the TB control globally. XDR-TB is MDR-TB that is also resistant to three or more of the six classes of second-line drugs.[8,9] A 100% correlation has been observed in XDR-TB with HIV coinfection and mortality.[8]

The current course of therapy with first-line TB drugs is more than 40 years old. Because of the global health problems associated with TB, the increasing rate of MDR-TB, and the high rate of coinfection with HIV, the discovery and development of potent new anti-TB agents, without cross-resistance with current antimycobacterial agents, are urgently needed.

After transmission into an individual, *M. tuberculosis* lodges in pulmonary alveoli, where it is engulfed by macrophages, and multiplies and survives within these macrophages. One major challenge in TB drug development is the difficulty in identifying new compounds with activity against intracellular mycobacteria. Newly discovered molecules active against *M. tuberculosis* should be able to inhibit both extracellular and intracellular mycobacterial replication.

The complete genome sequence of *M. tuberculosis* has been deciphered,[10] facilitating the identification of novel targets for developing inhibitors of the replication of this organism. A number of enzymes involved in purine and pyrimidine metabolism differ significantly between mammals and mycobacteria and could be used as targets for antituberculosis drug design, especially by nucleoside and nucleotide analogues.[11−13] *M. tuberculosis*-encoded thymidine monophosphate kinase (TMPKmt) has been put forward as one of the mycobacterial targets. It has high affinity for deoxythymidine and could also

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[a]Abbreviations: TB, tuberculosis; MDR-TB, multidrug-resistant tuberculosis; XDR-TB, extensively drug-resistant tuberculosis; *M. tuberculosis*, *Mycobacterium tuberculosis*; MABA, microplate Alamar blue assay; CFU, colony-forming unit; GI, growth index; MIC, minimum inhibitory concentration; CC, cytotoxic concentration.

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phosphorylate thymidine to TMP for *M. tuberculosis*.[12] Therefore, the design of pyrimidine nucleosides capable of interfering with nucleic acid biosynthetic pathways of mycobacteria is an excellent approach for the investigation of new classes of antimycobacterial agents.

In our earlier studies, we investigated various series of 2′-, 3′-, and 5-substituted deoxy and dideoxy pyrimidine nucleoside analogues, where nucleosides containing C-5-alkynyl moieties exhibited potent activity against replicating mycobacterial cultures.[14−19] In our continuing efforts to investigate new anti-TB agents, our group has discovered a new class of 3′-bromo-substituted pyrimidine nucleosides. In these studies, we note that 3′-bromo-3′-deoxy-arabinofuranosyl thymine (**33**) in particular displays the most potent inhibition of wild-type *M. tuberculosis* (H37Ra), drug-resistant *M. tuberculosis* (H37Rv, rifampicin- or isoniazid-resistant strains), as well as *M. tuberculosis* (H37Ra) harbored intracellularly in a human monocyte cell line. The new class of compounds investigated did not show cytotoxicity on the viability and proliferation of a human cell line. These promising results offer an opportunity for further exploration of this new class of analogues as antimycobacterial agents.

Chemistry

5′-*O*-Tritylation of thymidine (**1**) and 5-fluoro-2′-deoxyuridine (**2**) followed by mesylation at the 3′-position afforded the respective 5′-*O*-trityl-3′-*O*-mesyl derivatives (**3** and **4**) in 90 and 87% yield, respectively. The compound **3** upon treatment with lithium bromide (LiBr) or lithium chloride (LiCl) in anhydrous acetonitrile at reflux temperature for 24 and 80 h, respectively, yielded 5′-*O*-trityl-3′-bromothymidine (**5**) (51%) and 5′-*O*-trityl-3′-chlorothymidine (**6**) (50%), which were then converted to the target deblocked counterparts, **7** (36%) and **8** (57%), respectively, using 80% aqueous acetic acid. However, when compound **4** was reacted with LiBr under similar reaction conditions, three detritylated products **9**−**11** (due to release of HBr in situ because of trapped traces of moisture) were isolated in 24, 18, and 1% yields, respectively (Scheme 1). The *erythro* 3′-bromo compound **9** could have been formed via double Walden inversion[20] where attack of bromide ion occurred from the α-face, whereas the *threo* isomer **10** was formed by direct S_N2 displacement of 3′-methanesulfonyl group by bromide ion from the β-face of the carbohydrate moiety (Scheme 1). In contrast, a similar reaction of compound **4** with LiCl in acetonitrile under the same conditions provided only a *threo* chloride compound **12** (Scheme 2). The sole formation of *threo* chloride **12** (apart from detritylation of starting material) can be explained by facile attack of the chloride ion from the β-face, due to the smaller size in comparison to the bromide ion. However, apart from the steric factors, some electronic effects of the C_5-fluorine atom also seem to play an important role, since the results differ from the 5-CH_3 analogue (**3**) under the same conditions (Scheme 1).

In an attempt to synthesize the desired 5-fluoro-3′-chloro-2′,3′-dideoxyuridine (**20**) with a chloride in the *erythro* configuration, the 5′-*O*-tritylated 5-fluoro-2′-deoxyuridine (**13**) was allowed to react with triphenylphosphine and carbon tetrachloride (CCl_4) in anhydrous dimethylformamide (DMF) at room temperature for 24 h. However, in this reaction, we obtained a compound with chloride in the *threo* configuration (**15**) along with the formation of O^2,3′-cyclonucleoside **16** (Scheme 3). Compound **16** failed to convert to the target compound **19** or a simultaneously detritylated product **20** under the reaction conditions (Scheme 3).

In our next attempt for the synthesis of target compound **20**, compound **13** was treated with methyltriphenoxyphosphonium iodide in dry pyridine at room temperature whereby the formation of intermediate **17** and removal of phenoxyphosphonium ion led to the O^2,3′-cyclonucleoside **18** in 57% yield. Compound **18** was then treated with pyridine hydrochloride in DMF at room temperature, where chloride ion attacked from the less-hindered α-face, yielding **19** in 34% yield with overall retention of configuration (Scheme 3). Compound **19** was deblocked with 80% aqueous acetic acid at 90 °C to afford the required compound **20** in 64% yield. 1-(3-Iodo-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine (**22**), 1-(3-iodo-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-5-fluorouracil (**25**), and 1-(3-iodo-2,3-dideoxy-β-D-*threo*-pentofuranosyl)-5-fluorouracil (**26**) were synthesized by the reaction of sodium iodide (NaI) with respective nucleosides **3** and **4** in dry 1,2-dimethoxyethane (DME) at reflux temperature followed by detritylation of obtained compounds using 80% acetic acid to provide **22**, **25**, and **26** in 37, 45, and 1% yield, respectively (Scheme 4).

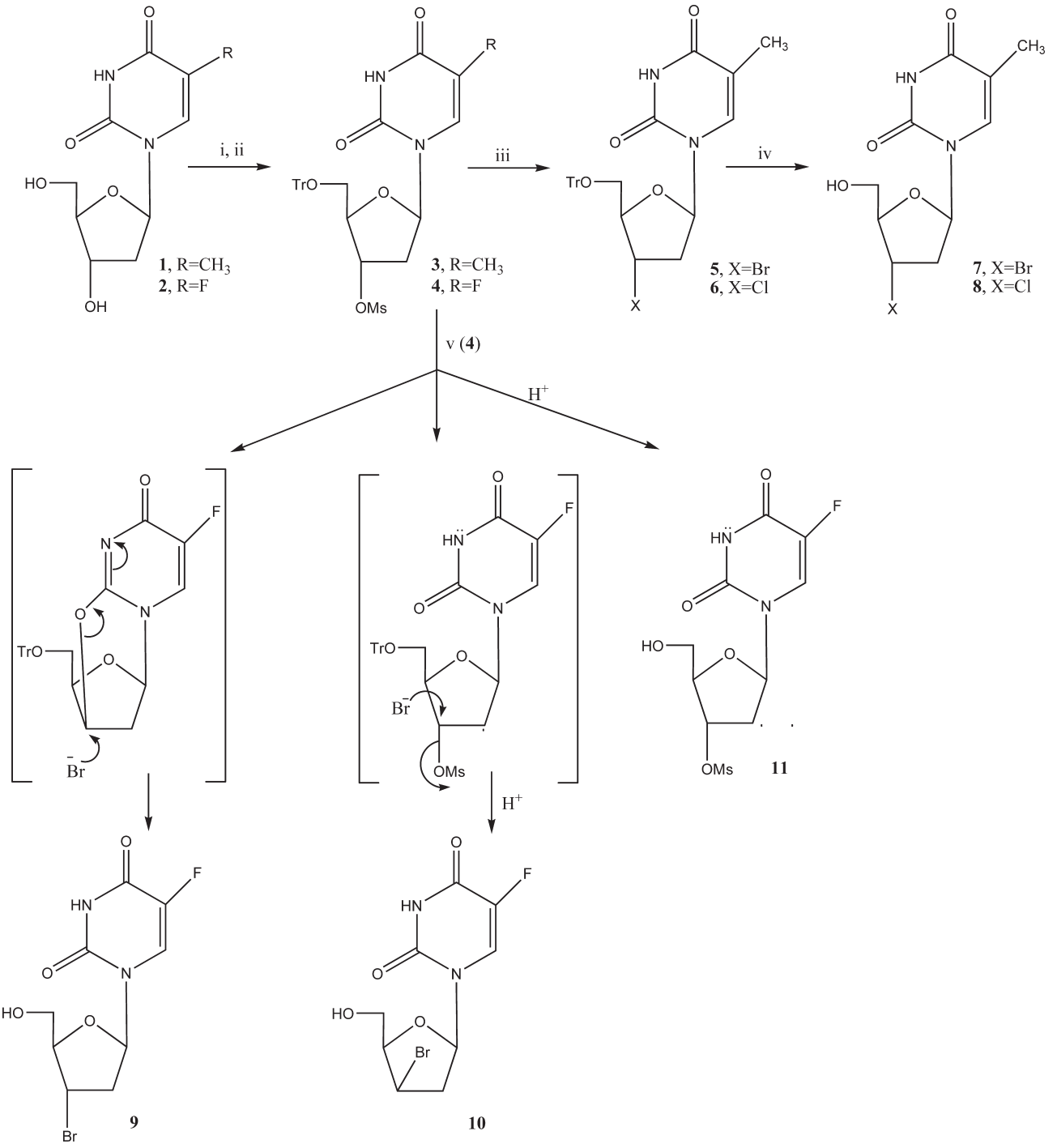
The stereochemistry of the compounds **7**−**10**, **12**, **20**, **22**, **25**, and **26** was assigned on the basis of NMR spectra, which are in agreement with well-established assignments,[21] that is, (i) C-2′ and C-2″ protons in the compounds with the *erythro* configuration have either very close chemical shifts or overlapping signals, while the same protons with the *threo* configuration have chemical shifts generally separated from each other by 0.5−1 ppm, and (ii) in the *erythro* configuration, the C-1′ proton appears as triplet, while in *threo*-, this proton appears as a quartet (or double doublets).[21,22]

To prepare the target compounds **32**−**34** and **35**−**37**, the free hydroxyl functions of uridine (**27a**), 5-methyluridine (**27b**), and 5-fluorouridine (**27c**) were protected using trityl chloride followed by reaction with mesyl chloride to yield compounds **28a**−**c**, which were converted to corresponding 2′,3′-*lyxo*-epoxide derivatives **29a**−**c**, using 1 N NaOH in 50% (v/v) aqueous acetone at room temperature. The compounds **29a**−**c** were then treated with ammonium bromide (NH_4Br) in absolute ethanol to yield their respective bromohydrins **30a**−**c**, in addition to the bromohydrins **31a**−**c** (Scheme 5). In these reactions, bromide ion attacked exclusively from the α-face either at C-2′ or at C-3′ due to the hindered *lyxo* configuration of the epoxides. The predominant formation of the 3′-deoxy-3′-halo-arabino compounds **30a**−**c** as major products indicates that the attack of bromide ion is more facile at C-3′ instead of C-2′. This may be due to (i) the favorable conformation of the sugar rings facilitating attack at C-3′ of halide ion predominantly and (ii) the closer proximity of C-2′ to the electron-withdrawing anomeric center, which makes the epoxide opening facile at C-3′, with an oxygen atom intact at C-2′.[23] The compounds **30a**−**c** and **31a**−**c** were deprotected using 80% aqueous (v/v) acetic acid to afford the target compounds **32**−**34** and **35**−**37**, respectively (Scheme 5).

Results and Discussion

Pyrimidine nucleoside analogues **7**−**10**, **12**, **20**, **22**, **25**, **26**, and **32**−**37** were first evaluated for their activity against the multiplication of wild-type *M. tuberculosis* strain H37Ra by the microplate alamar blue assay (MABA)[24] at 1−100 μg/mL concentrations. Rifampicin, isoniazid, and cycloserine were used as reference drugs. The compounds that exhibited promising inhibition against the wild-type strain were further

Scheme 1[a]

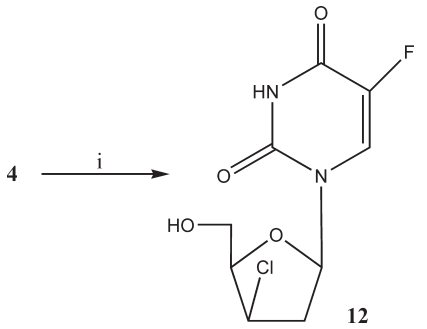


[a] Reagents and conditions: (i) Trityl chloride, 4-(dimethylamino)pyridine, anhydrous pyridine, 80 °C, 5 h. (ii) Methanesulfonyl chloride, 0−5 °C. (iii) LiBr or LiCl (appropriately) 24 and 80 h days, respectively, anhydrous CH_3CN, reflux. (iv) 80% AcOH, 90 °C, 30 min. (v) LiBr, anhydrous CH_3CN, reflux, 22 h.

evaluated against drug-resistant strains of *M. tuberculosis* H37Rv by the BACTEC assay.[25] Isoniazid-resistant and rifampicin-resistant strains of *M. tuberculosis* were used in these studies. The antimycobacterial effect of potent compounds was also determined against intracellular mycobacteria in a human monocytic cell-line (THP-1) infected with *M. tuberculosis* strain H37Ra. Concentrations of 25, 10, and 2 μg/mL were tested using the colony-forming units (CFU) assay.[26] The results obtained for anti-TB activity of the compounds **7**−**10**, **12**, **20**, **22**, **25**, **26**, and **32**−**37** along with reference first-line and second-line anti-TB drugs are summarized in Table 1. The antimycobacterial activity data for 3′-fluoro-2′,3′-dideoxythymidine (**38**) and 3′,5-difluoro-2′,3′-dideoxyuridine (**39**) previously reported by us[14] are also included in Table 1 for comparison. The compounds investigated here for their antimycobacterial effects are divided into four different categories based on the positions of the halogen substituent in the carbohydrate moiety: (1) pyrimidine analogues possessing a halogen atom in the 3′-*erythro* configuration (**7**−**9**, **20**, **22**, and **25**), (2) pyrimidine analogues possessing a halogen atom

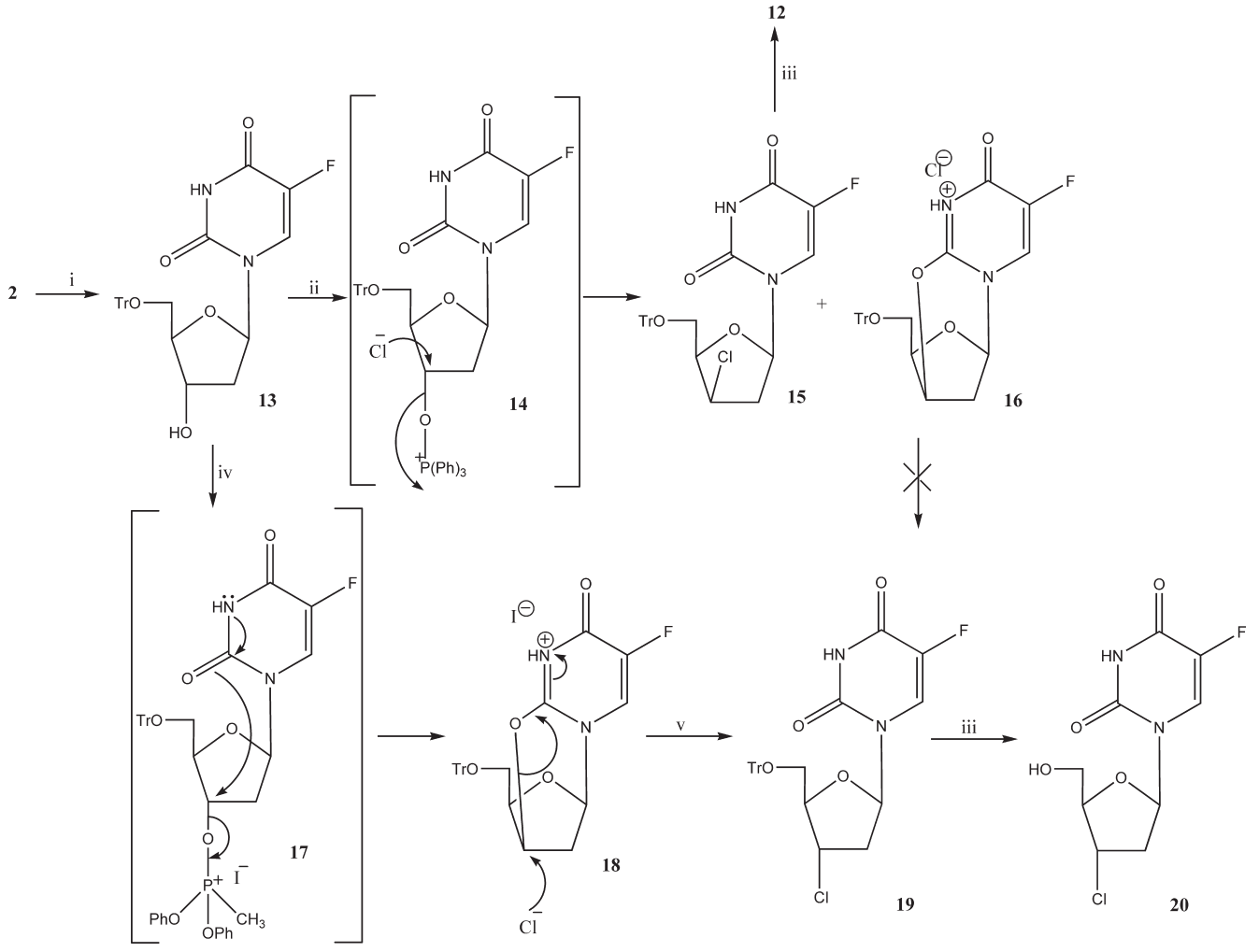
in the 3′-*threo* configuration (**10**, **12**, and **26**), (3) 3′-bromo-3′-deoxy-arabinofuranosyl analogues (**32**−**34**), and (4) 2′-bromo-2′-deoxy-xylofuranosyl compounds (**35**−**37**). Among the 3′-halogeno-2′,3′-dideoxythymidine compounds (**7**, **8**, **22**, and **38**) described in this paper, only 3′-bromo-2′,3′-dideoxythymidine [**7**, minimum inhibitory concentration $(MIC)_{50}$ = 5−10 μg/mL] and 3′-chloro-2′,3′-dideoxythymidine (**8**, MIC_{50} = 50 μg/mL) exhibited appreciable inhibition of *M. tuberculosis* (H37Ra). However, it is interesting to note that the chloro analogue **8** provided some inhibition of *M. tuberculosis*, whereas the activity of the bromo derivative **7** was significantly improved as compared to the corresponding 3′-fluoro (**38**) compound, which showed no activity in earlier studies.[14] Thus, the relative antimycobacterial activity order in the 3′-halogeno-2′,3′-dideoxythymidine series was found to be Br > Cl > F═I. Replacement of the methyl group at the C-5 position of compounds **7**, **8**, and **22** by a fluoro substituent provided compounds **9**, **20**, and **25** with reduced anti-TB activity; the 3′-bromo analogue (**9**) showed 50% inhibition at 50 μg/mL, whereas 3′-chloro (**20**) and 3′-iodo (**25**) analogues exhibited 40 and 32% inhibition at 100 μg/mL, respectively. The 3′-fluoro derivative (**39**) in this series of compounds also showed no inhibition of *M. tuberculosis*, similar to the 2′-3′-dideoxythymidine series (compound **38**).[14] Therefore, a similar pattern of relative activity order was observed viz. Br > Cl > F < I among compounds **9**, **20**, **25**, and **39** except the 3′-iodo derivative (**25**). These results indicate that the bromo substituent contributed more to anti-TB activity than the chloro, fluoro, and iodo groups at the C-3′ position. Interestingly, inversion of the halogen atoms in the "up" or threo configuration at the C-3′ carbon atom in the pyrimidine nucleoside analogues **10**, **12** (MIC_{50} = 25−50 μg/mL), and **26** proved to be more effective in comparison to their 3′-halogenated

Scheme 2[a]



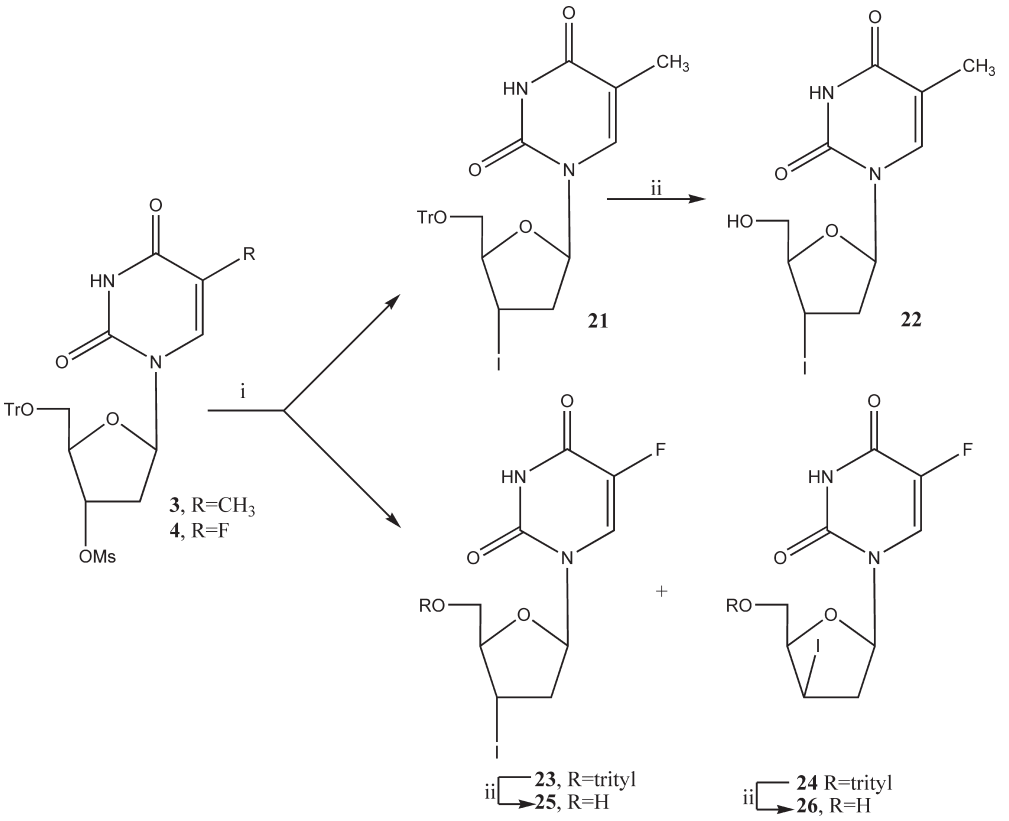
[a] Reagents and conditions: (i) LiCl, anhydrous CH_3CN, reflux, 24 h.

Scheme 3[a]



[a] Reagents and conditions: (i) Trityl chloride, 4-(dimethylamino)pyridine, anhydrous pyridine, 80 °C, 5 h. (ii) Triphenylphosphine, CCl_4, anhydrous DMF, room temperature, 24 h. (iii) 80% aqueous AcOH, 90 °C, 30 min. (iv) Methyltriphenoxyphosphonium iodide, anhydrous pyridine, room temperature, 1 h. (v) Pyridine hydrochloride, anhydrous DMF, room temperature, 8 days.

Scheme 4[a]



[a] Reagents and conditions: (i) NaI, anhydrous DME, reflux, 20 h. (ii) 80% aqueous AcOH, 90 °C, 30 min.

"down" or *erythro* isomers (**9**, **20**, and **25**, respectively). However, compounds **10** and **12** were less inhibitory as compared to the 3′-bromo-2′,3′-dideoxythymidine (**7**). It would be worthwhile to investigate antimycobacterial activity of *threo* analogues of **7** in our next study.

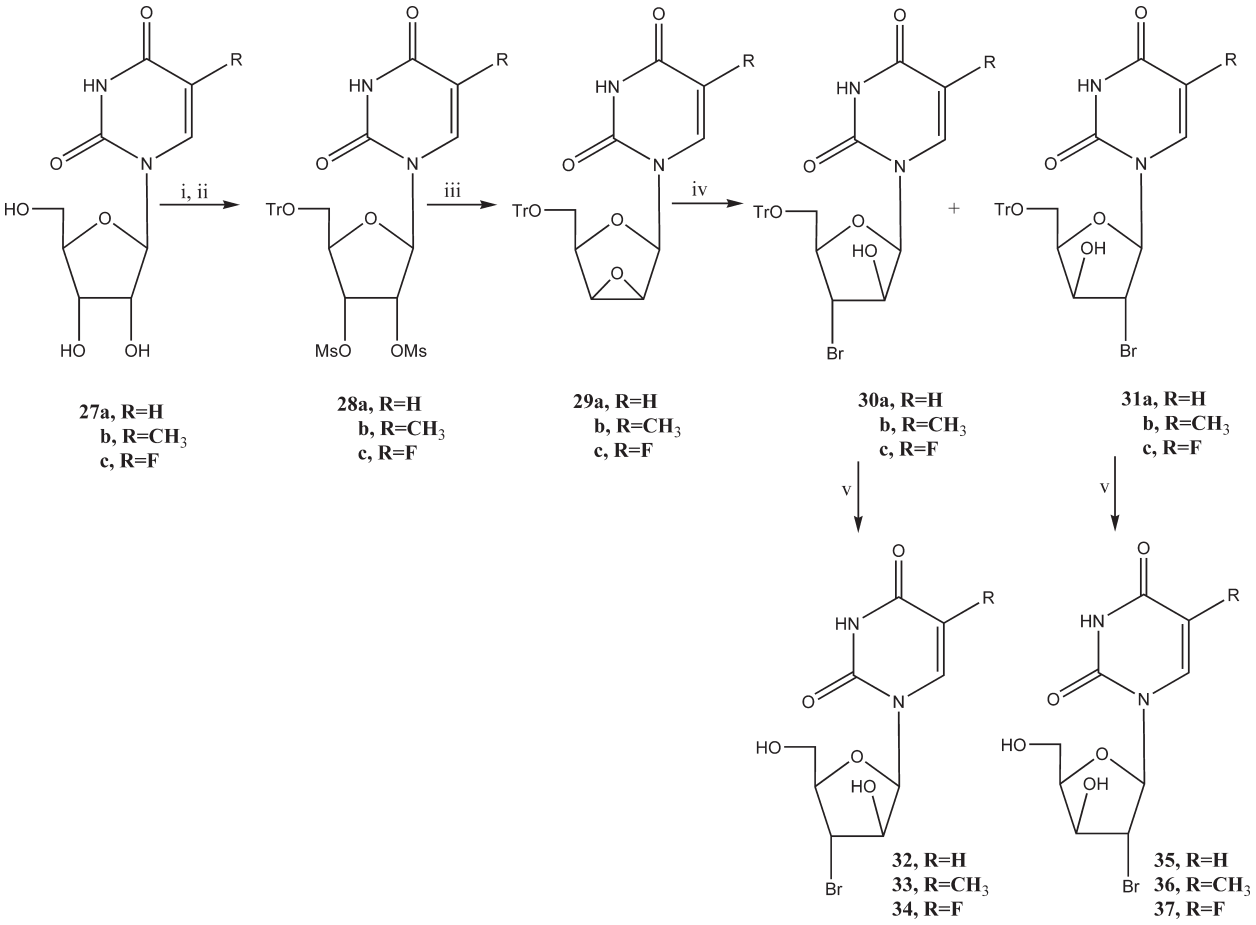
Among the 3′-bromo-3′-deoxy-arabinofuranosyl analogues (**32**−**34**) tested, 3′-bromo-3′-deoxy-arabinofuranosylthymine (**33**) (MIC_{50} = 1 μg/mL) showed the best antimycobacterial activity against H37Ra. Intriguingly, compound **33** had activity significantly higher than the 3′-bromo compound **7** (MIC_{50} = 5−10 μg/mL), as well as a second-line anti-TB drug cycloserine (MIC_{50} = 5 μg/mL) (Table 1). Similarly, in this series of compounds, the 5-fluoro derivative (**34**) also had improved activity (MIC_{50} = 25 μg/mL) over compound **9** (MIC_{50} = 50 μg/mL), suggesting that incorporation of a hydroxyl substituent at the C-2′ position in the arabino configuration contributes to antimycobacterial activity.

Of the 2′-bromo-2′-deoxy-*xylo* compounds (**35**−**37**) tested, only thymine analogue **36** exhibited modest activity against H37Ra replication (MIC_{50} = 25 μg/mL), which is significantly reduced as compared to the corresponding 3′-bromo derivatives **33** and **7**, suggesting that bromine at the C-3′ position is an influential factor in the carbohydrate portion. Also, in the 2′-bromo class of compounds, the uracil analogue (**35**) and 5-fluorouracil analogue (**37**) were found to be inactive, suggesting that a methyl group at the C-5 position is an important determinant for the anti-TB activity. Interestingly, the same observation was made with other compounds (**7**, **8**, and **33**) investigated in this report.

In our next studies, the most promising compounds emerging from this work (**7** and **33**) were examined for their potency against rifampicin- and isoniazid-resistant strains (H37Rv) of *M. tuberculosis* (Table 1). Encouragingly, we found that both of the drug-resistant strains were susceptible to these compounds at concentrations similar to those for drug-sensitive *M. tuberculosis* strain H37Ra, suggesting that the identified pyrimidine nucleoside compounds are not cross-resistant to the currently available first-line antituberculosis drugs. There was no inhibition of the drug-resistant strains by the respective drugs rifampicin or isoniazid at 2 μg/mL.

Compounds **7** and **33** were also assayed for their activity toward intracellular *M. tuberculosis*. In the human monocytic cell line (THP-1) infected with H37Ra, both compounds significantly inhibited intracellular mycobacterial growth at the two concentrations tested (10 and 25 μg/mL). Biological results reported in Table 1 indicate that both **7** and **33** exhibited a concentration-dependent inhibition of intramacrophagic mycobacterial growth. In this assay, compound **7** provided 50% reduction in the CFU at 10 μg/mL, whereas compound **33** showed 80% reduction in the CFU counts at 10 μg/mL. These data of **7** and **33** correlate well with their respective MICs against extracellular mycobacteria. In addition, we were surprised to note that the intracellular antimycobacterial activity exhibited by both compounds **7** and **33** was similar or improved with respect to their inhibition of extracellular mycobacterial replication. The potent activity shown by compounds **7** and **33** against intracellular mycobacteria suggests that they have the ability to inhibit mycobacteria harbored within macrophages in infected individuals. *M. tuberculosis* is an intracellular pathogen, which resides, propagates, and hides within macrophages. Most people with latent *M. tuberculosis* infection, but without an active disease, contain this pathogen intracellularly for years albeit in

Scheme 5[a]



[a] Reagents and conditions: (i) Trityl chloride, 4-(dimethylamino)pyridine, anhydrous pyridine 80 °C, 5 h. (ii) Methanesulfonyl chloride, anhydrous pyridine, 0−5 °C, 24 h. (iii) NaOH (1 N) in acetone:water (1:1, v/v), room temperature, 24 h. (iv) Ammonium bromide, absolute ethanol, reflux, 20 h. (v) 80% aqueous AcOH, 90 °C, 30 min.

nonreplicating form. Although our experiments were performed with human monocytes infected with actively replicating mycobacteria, the concentration-dependent inhibition of intracellular *M. tuberculosis* by both compounds, in particular **33**, is a very significant observation.

The XTT and ^{3}H incorporation assays were performed to evaluate the toxicity of investigated compounds (**7**−**10**, **12**, **20**, **22**, **25**, **26**, and **32**−**37**) in vitro against a human hepatoma cell line (Huh7). No toxicity was observed up to the highest concentration tested, 100−200 μg/mL [cytotoxic concentration $(CC)_{50}$ > 100−200 μg/mL].

The precise mechanism of action of the compounds inhibiting mycobacterial multiplication in this study is not yet clear. It is possible that the active nucleoside analogues, after their metabolic conversion to phosphorylated forms by mycobacterial kinases, may be selectively inhibiting DNA and/or RNA synthesis, by acting as substrates and/or inhibitors of metabolic enzymes of DNA/RNA synthesis.

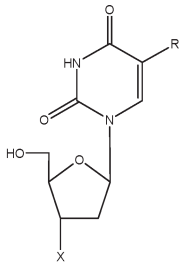
In conclusion, a new class of pyrimidine nucleosides with promising antituberculosis activity and low or no cytotoxicity was identified. Compounds **7** and **33** emerged as the most active analogues and exhibited activity toward both drug-sensitive and drug-resistant strains of *M. tuberculosis* and had the ability to inhibit intracellular growth of *M. tuberculosis*, providing further evidence that this class of compounds has immense promise. The best antimycobacterial agent identified in this study, 3′-bromo-3′-deoxy-arabinofuranosylthymine (**33**), was found to be more potent than cycloserine, a second-line drug, and could serve as a useful lead compound for the development of a much needed new antituberculosis agent.

Experimental Section

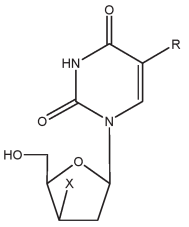
Melting points were determined with a Buchi capillary apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were determined for samples in Me_2SO-d_6, CD_3OD, or $CDCl_3$ on a Bruker AM 300 spectrometer using Me_4Si (TMS) as an internal standard. ^{13}C NMR (*J* modulated spin echo) spectra were determined for selected compounds where methyl and methyne carbon resonances appear as positive peaks and where methylene and quaternary carbon resonances appear as negative peaks. Chemical shifts are given in ppm relative to TMS. The assignment of all exchangeable protons (OH, NH) was confirmed by the addition of D_2O. All of the final compounds had >95% purity, determined by microanalysis. Microanalysis results were within ±0.4% of theoretical values for all elements listed, unless otherwise indicated. Silica gel column chromatography was carried out using Merck 7734 silica gel (100−200 μM particle size). Thin-layer chromatography (TLC) was performed with Machery-Nagel Alugram SiL G/uv silica gel slides (20 μM thickness). Thymidine (**1**), 5-fluoro-2′-deoxyuridine (**2**), uridine (**27a**), 5-methyluridine (**27b**), and 5-fluorouridine (**27c**) were purchased from Aldrich.

1-(2-Deoxy-3-*O*-methanesulfonyl-5-*O*-trityl-*β*-D-ribopentofuranosyl)thymine (3).[20] A mixture of thymidine **1** (1.0 g, 4.13 mmol), trityl chloride (1.95 g, 7.0 mmol), and 4-(dimethylamino)-pyridine (0.01 g, 0.80 mmol) in anhydrous pyridine was heated

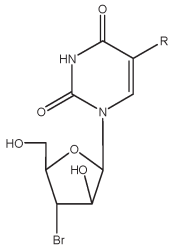
Table 1. In Vitro Antimycobacterial Activity of Pyrimidine Nucleosides against Wild-Type and Rifampicin- and Isoniazid-Resistant Strains of *M. tuberculosis* and Intracellular *M. tuberculosis*



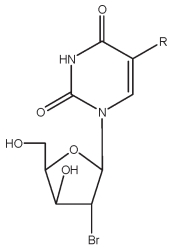
7, 8, 9, 20, 22, 25, 38, 39



10, 12, 26



32, 33, 34



35, 36, 37

			antimycobacterial activity (*M. tuberculosis*)				
			wild-type (H37Ra)		rifampicin-resistant (H37Rv)	isoniazid-resistant (H37Rv)	intracellular (H37Ra)
compd	R	X	% inhibition (concn μg/mL)[a]	MIC_{50}[b] (μg/mL)	% inhibition (concn μg/mL)[a]	% inhibition (conc. μg/mL) [a]	% reduction (concn μg/mL)[c]
7	CH_3	Br	100 (100), 82 (50), 50 (5−10)	5−10	50 (5−10)	75 (25), 50 (10)	90 (25), 50 (10)
8	CH_3	Cl	68 (100), 50 (50)	50	ND[d]	ND	ND
9	F	Br	80 (100), 50 (50)	50	ND	ND	ND
10	F	Br	100 (100), 63 (50), 40 (25)	25−50	ND	ND	ND
12	F	Cl	90 (100), 60 (50), 40 (25)	25−50	ND	ND	ND
20	F	Cl	40 (100)		ND	ND	ND
22	CH_3	I	0 (100)		ND	ND	ND
25	F	I	32 (100)		ND	ND	ND
26	F	I	40 (100)		ND	ND	ND
32	H		24 (100)		ND	ND	ND
33	CH_3		100 (100), 50 (100), 75 (10), 50 (1)	1	70 (10), 50 (1−2)	75 (10), 50 (2)	> 90 (25), 80 (10)
34	F		91 (100), 50 (25)	25	ND	ND	ND
35	H		0 (100)		ND	ND	ND
36	CH_3		80 (100), 60 (50), 50 (25)	25	ND	ND	ND
37	F		0 (100)		ND	ND	ND
38[e]	CH_3	F	0 (100)		ND	ND	ND
39[f]	F	F	0 (100)		ND	ND	ND
isoniazid			100 (1)	< 1	100 (1)	0 (2)	> 98 (2.5)
rifampicin			100 (0.5−1)	< 0.5	0 (2)	100 (0.5−1)	> 95 (2.5)
cycloserine			100 (50), 80 (25), 50 (5)	5	ND	ND	ND

[a] The antimycobacterial activity was determined at concentrations of 100, 50, 25, 10, 5, and 1 μg/mL. [b] Concentration of compounds exhibiting 50% inhibition in mycobacterial growth. [c] % reduction of the number of surviving bacteria in the human monocyte cell line (THP-1) with respect to the untreated control. [d] ND = not determined. [e] 3′-Fluoro-2′,3′-dideoxythymidine.[14] [f] 3′,5-Difluoro-2′,3′-dideoxyuridine.[14]

at 80 °C for 5 h. The reaction was cooled to 0 °C, methanesulfonyl chloride (0.89 g, 7.8 mmol) was added dropwise, and the reaction mixture was stirred at 0 °C for 1 h. Then, the reaction mixture was kept in the refrigerator for 24 h. After the addition of H_2O (1 mL), the solvent was evaporated, and the resulting oil was diluted with chloroform (100 mL), washed with H_2O (2 × 25 mL), dried (Na_2SO_4), and evaporated in vacuo. The residue was purified by silica gel column chromatography using $CHCl_3$/MeOH (97:3, v/v) as the eluent to yield **3** (2.1 g, 90%) as a white solid; mp 120−122 °C. ^{1}H NMR ($CDCl_3$): δ 1.47 (s, 3H, CH_3), 2.43−2.53 (m, 1H, H-2′), 2.65−2.72 (m, 1H, H-2″), 3.03 (s, 3H, CH_3SO_2), 3.50 (m, 2H, H-5′), 4.32 (m, 1H, H-4′), 5.41 (m, 1H, H-3′), 6.43 (m, 1H, H-1′), 7.29−7.41 (m, 15H 5′-*O*-trityl),7.54 (s, 1H, H-6), 8.52 (br s, 1H, NH).

1-(2-Deoxy-3-*O*-methanesulfonyl-5-*O*-trityl-β-D-ribopentofuranosyl)-5-fluorouracil (4). This compound was synthesized in 87% yield as a solid using a published procedure[20] and used directly in the next reaction. ^{1}H NMR ($CDCl_3$): δ 2.35−2.47 (m, 1H, H-2′) and 2.70−2.80 (m, 1H, H-2″), 3.05 (s, 3H, CH_3SO_2), 3.52 (m, 2H, H-5′), 4.36 (m, 1H, H-4′), 5.36 (m, 1H, H-3′), 6.32 (m, 1H, H-1′), 7.21−7.49 (m, 15H, 5′-*O*-trityl), 7.78 (d, $J_{6,F}$ = 6.10 Hz, 1H, H-6), 8.71 (br s, 1H, NH).

1-(3-Bromo-2, 3-dideoxy-5-*O*-trityl-β-D-*erythro*-pentofuranosyl)thymine (5). A mixture of **3** (0.10 g, 0.18 mmol) and LiBr (0.20 g, 2.30 mmol) in anhydrous acetonitrile (20 mL) was refluxed for 24 h. The solvent was removed in vacuo, and the residue was purified on a silica gel column using EtOAc/hexane (20:80; v/v) as the eluent to yield **5** (0.05 g, 51%) as a syrup. ^{1}H NMR (DMSO-d_6): δ 1.57 (s, 3H, CH_3), 2.59−2.85 (m, 2H, H-2′), 3.27 (m, 2H, H-5′), 4.24 (m, 1H, H-4′), 4.77 (dd, J = 14.65 and 7.32 Hz, 1H, H-3′), 6.22 Hz (dd, J = 7.32 and 4.88 Hz, 1H, H-1′), 7.25−7.49 (m, 15H, 5′-*O*-trityl), 7.55 (s, 1H, H-6), 11.42 (s, 1H, NH).

1-(3-Chloro-2,3-dideoxy-5-*O*-trityl-β-D-*erythro*-pentofuranosyl)thymine (6). For the synthesis of this compound, a similar procedure was followed as described for compound **5** using LiCl instead of LiBr. It was obtained in 50% yield as a syrup. ^{1}H NMR ($CDCl_3$): δ 1.46 (s, 3H, CH_3), 2.66 (m, 2H, H-2′), 3.47 (m, 2H, H-5′), 4.24 (m, 1H, H-4′), 4.61 (dd, J = 9.76 and 4.88 Hz, 1H, H-3′), 6.38 (t, J = 6.10 Hz, 1H, H-1′), 7.23−7.50 (m, 15H, 5′-*O*-trityl), 7.61 (s, 1H, H-6), 8.19 (s, 1H, NH).

1-(3-Bromo-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine (7). A solution of **5** (0.05 g, 0.09 mmol) in 80% aqueous acetic acid (10 mL; v/v) was heated at 90 °C for 30 min. The solvent was evaporated in vacuo. The residue so obtained was purified on a silica gel column using MeOH/$CHCl_3$ (4:96; v/v) as the eluent to yield **7** (0.010 g, 36%) as a white solid; mp 150−152 °C, dec. ^{1}H NMR (DMSO-d_6): δ 1.77 (s, 3H, CH_3), 2.60 (m, 2H, H-2′), 3.65

(m, 2H, H-5′), 4.19 (m, 1H, H-4′), 4.63 (dd, J = 12.2 and 6.10 Hz, 1H, H-3′), 5.26 (t, J = 4.88 Hz, 1H, 5′-OH), 6.23 (t, J = 6.71 Hz, 1H, H-1′), 7.70 (d, J = 1.22 Hz, 1H, H-6), 11.36 (s, 1H, NH). Anal. ($C_{10}H_{13}BrN_2O_4$) C, H, N.

1-(3-Chloro-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine (8). Detritylation of compound **6** using the same procedure as described above provided the title compound. This was obtained in 57% yield as a white solid. The ^{1}H NMR spectrum obtained for **8** was identical to that reported previously;[21] mp 180−182 °C. ^{1}H NMR (DMSO-d_6): δ 1.77 (s, 3H, CH_3), 2.60 (m, 2H, H-2′), 3.64 (m, 2H, H-5′), 4.05 (m, 1H, H-4′), 4.64 (dd, J = 9.16 and 4.27 Hz, 1H, H-3′), 5.26 (br s, 1H, 5′−OH), 6.23 (t, J = 6.71 Hz, 1H, H-1′), 7.69 (s, 1H, H-6), 11.36 (s, 1H, NH). Anal. ($C_{10}H_{13}ClN_2O_4$) C, H, N.

1-(3-Bromo-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-5-fluorouracil (9), 1-(3-Bromo-2,3-dideoxy-β-D-*threo*-pentofuranosyl)-5-fluorouracil (10), and 1-(3-*O*-Methanesulfonyl-2-deoxy-β-D-*erythro*-pentofuranosyl)-5-fluorouracil (11). A mixture of **4** (1.05 g, 1.85 mmol) and LiBr (2.09 g, 24.10 mmol) in CH_3CN was refluxed for 22 h. The solvent was removed in vacuo. The residue was purified on a silica gel column using MeOH/$CHCl_3$ (6.5:93.5; v/v) as the eluent to yield **9** (0.135 g, 24%) as a white solid; mp 144−146 °C, dec. ^{1}H NMR (DMSO-d_6): δ 2.52−2.73 (m, 2H, H-2′), 3.67 (m, 2H, H-5′), 4.20 (m, 1H, H-4′), 4.59 (dd, J = 13.42 and 6.71 Hz, 1H, H-3′), 5.40 (t, J = 4.88, 1H, 5′−OH), 6.15 (t, J = 4.88 Hz, 1H, H-1′), 8.24 (d, $J_{6,F}$ = 7.32 Hz, 1H, H-6), 11.88 (d, $J_{NH,F}$ = 4.88 Hz, 1H, NH). Anal. ($C_9H_{10}BrFN_2O_4$) C, H, N.

Subsequent elution with MeOH/$CHCl_3$ (7:93; v/v) afforded **11** as an impure substance, which was purified on preparative TLC using MeOH/CH_2Cl_2 (7:93; v/v) to yield **11** (0.008 g, 1%) as a syrup. ^{1}H NMR (DMSO-d_6): δ 2.39−2.55 (m, 2H, H-2′), 3.29 (s, 3H, CH_3SO_2), 3.64 (m, 2H, H-5′), 4.18 (m, 1H, H-4′), 5.26 (m, 1H, H-3′), 5.42 (br s, 1H, 5′-OH), 6.16 (t, J = 6.70 Hz, 1H, H-1′), 8.17 (d, $J_{6,F}$ = 7.32 Hz, 1H, H-6), 11.55 (br s, 1H, NH). Anal. ($C_{10}H_{13}FN_2O_7S$) C, H, N.

Further elution with MeOH/$CHCl_3$ (7:93; v/v) provided impure **10**, which was purified on preparative TLC using MeOH/CH_2Cl_2 (7:93; v/v) as the eluent to yield **10** (0.10 g, 18%) as a syrup. ^{1}H NMR (DMSO-d_6): δ 2.45 and 3.11 (2 m, 2H, H-2′), 3.70 (m, 2H, H-5′), 4.02 (m, J = 10.38 and 4.88 Hz, 1H, H-4′), 4.79 (m, 1H, H-3′), 5.11 (t, J = 4.88 Hz, 1H, 5′-OH), 6.01 (ddd, J = 5.49 Hz, 4.88 and 1.83 Hz, 1H, H-1′), 8.01 (d, $J_{6,F}$ = 6.71 Hz, 1H, H-6), 11.92 (s, 1H, NH). Anal. ($C_9H_{10}BrFN_2O_4$) C, H, N.

1-(3-Chloro-2,3-dideoxy-β-D-*threo*-pentofuranosyl)-5-fluorouracil (12). Compound **4** (1.0 g, 1.76 mmol) was reacted with LiCl (1.50 g, 35.4 mmol) in anhydrous acetonitrile in a similar fashion as described for **5** to yield **12**. This was obtained in 11% yield as a semisolid. ^{1}H NMR (DMSO-d_6): δ 2.31 (m, J = 15.26 and 1.83 Hz, 1H, H-2′), 2.94−3.05 (m, 1H, H-2″), 3.73 (m, 2H, H-5′), 4.15 (m, 1H, H-4′), 4.77 (m, 1H, H-3′), 5.05 (t, J = 5.49 Hz, 1H, 5′-OH), 6.01 (ddd, J = 7.93 Hz, 3.05 and 1.83 Hz, 1H, H-1′), 7.91 (d, $J_{6,F}$ = 7.32 Hz, 1H, H-6), 11.91 (s, 1H, NH). Anal. ($C_9H_{10}ClFN_2O_4$) C, H, N.

1-(3-Chloro-2,3-dideoxy-β-D-*threo*-pentofuranosyl)-5-fluorouracil (12) from 15. Detritylation of **15** using 80% acetic acid at 90 °C for 30 min provided compound **12** in 79% yield as a white solid. The physical data were identical in all respects with that of **12** obtained in the previous reaction.

1-(2-Deoxy-5-*O*-trityl-β-D-*erythro*-pentofuranosyl)-5-fluorouracil (13). A mixture of 5-fluoro-2′deoxyuridine (**2**, 0.50 g, 2.0 mmol), trityl chloride (0.91 g, 3.26 mmol), and 4-(dimethylamino)pyridine (0.03 g, 0.24 mmol) in anhydrous pyridine was heated at 80 °C for 5 h. Pyridine was removed in vacuo. The residue was diluted with water (20 mL) and extracted with ethyl acetate (2 × 50 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue so obtained was purified on a silica gel column using MeOH/$CHCl_3$ (3:97, v/v) as the eluent to yield **13** (0.80 g, 81%) as a syrup. ^{1}H NMR (DMSO-d_6): δ 2.08−2.29 (m, 2H, H-2′), 3.11 (dd, J = 10.99 and 3.05 Hz, 1H, H-5′), 3.27 (dd, J = 10.38 and 5.49 Hz, 1H, H-5″), 3.87 (m, 1H, H-4′), 4.27 (m, 1H, H-3′), 5.33 (d, J = 4.88 Hz, 1H, 3′-OH), 6.13 (dd, J = 8.55 and 6.71 Hz, 1H, H-1′), 7.21−7.47 (m, 15 H, 5′-*O*-trityl), 7.87 (d, $J_{6,F}$ = 6.71 Hz, 1H, H-6), 11.86 (s, 1H, NH).

1-(3-Chloro-2,3-dideoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)-5-fluorouracil (15) and 5′-*O*-Trityl-O^2,3′-cyclo-5-fluoro-2′-deoxyuridinium Chloride (16). A mixture of **13** (2.7 g, 11.8 mmol), triphenylphosphine (1.45 g, 5.53 mmol), and CCl_4 (1.7 g, 11.05 mmol) in dry DMF (10 mL) was kept at room temperature for 24 h, diluted with methanol, and evaporated to dryness. The residue was chromatographed on silica gel using EtOAc/CH_2Cl_2 (30:70, v/v) as the eluent to yield **15** (1.6 g, 57%) as a syrup. This product was used in the next step.

Subsequent elution from the column using MeOH/$CHCl_3$ (10:90, v/v) as the eluent yielded **16** (0.11 g, 4%) as a syrup. ^{1}H NMR (DMSO-d_6): δ 2.50 and 2.65 (2 m, 2H, H-2′), 3.14 (d, $J_{4',5'}$ = 6.10 Hz, 2H, H-5′), 4.46 (m, 1H, H-4′), 5.38 (m, 1H, H-3′), 5.90 (d, J = 3.05 Hz, 1H, H-1′), 7.22−7.44 (m, 15H, 5′-*O*-trityl), 8.17 (d, $J_{6,F}$ = 4.27 Hz, 1H, H-6). Anal. ($C_{28}H_{24}ClFN_2O_4$) C, H, N.

5′-*O*-Trityl-O^2,3′-cyclo-5-fluoro-2′-deoxyuridinium Iodide (18). Methyltriphenoxyphosphonium iodide (0.185, 0.41 mmol) was added to **13** (0.10 g, 0.20 mmol) in dry pyridine (5 mL), and the reaction mixture was stirred at room temperature for 1 h. After the pyridine was removed in vacuo, ethyl acetate was added, washed with 5% (w/v) aqueous sodium thiosulfate solution (10 mL) followed by water (2 × 10 mL), and dried over Na_2SO_4. The organic phase was concentrated and triiturated with ether to afford **18** (0.070 g, 57%) as a syrup. ^{1}H NMR (DMSO-d_6): δ 2.52 and 2.65 (2 m, 2H, H-2′), 3.10 (d, $J_{4',5'}$ = 7.32 Hz, 2H, H-5′), 4.46 (m, 1H, H-4′), 5.37 (m, 1H, H-3′), 5.89 (d, J = 3.05 Hz, 1H, H-1′), 7.16−7.45 (m, 15H, 5′-*O*-trityl), 8.15 (d, $J_{6,F}$ = 5.49 Hz, 1H, H-6). Anal. ($C_{28}H_{24}FIN_2O_4$) C, H, N.

1-(3-Chloro-2,3-dideoxy-5-*O*-trityl-β-D-*erythro*-pentofuranosyl)-5-fluorouracil (19). Pyridine hydrochloride (2.44 g, 21.1 mmol) was added to **18** (1.05 g, 1.75 mmol) in dry DMF (5 mL), and the reaction mixture was stirred at room temperature for 8 days. DMF was removed in vacuo. Ethyl acetate (50 mL) was added, and the ethyl acetate layer was washed with water (5 mL), dried over Na_2SO_4, and concentrated in vacuo. The resulting residue was purified on a silica gel column using EtOAc/hexane (40:60, v/v) as the eluent to yield **19** (0.30 g, 34%) as a syrup. ^{1}H NMR (DMSO-d_6): δ 2.53 and 2.72 (2 m, 2H, H-2′), 3.30 (m, 2H, H-5′), 4.11 (m, 1H, H-4′), 4.72 (dd, J = 13.43 and 6.71 Hz, 1H, H-3′), 6.17 (m, 1H, H-1′), 7.20−7.45 (m, 15 H, 5′-*O*-trityl), 7.97 (d, $J_{6,F}$ = 6.71 Hz, 1H, H-6), 11.92 (d, $J_{NH,F}$ = 3.66 Hz, 1H, NH).

1-(3-Chloro-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-5-fluorouracil (20). The title compound was obtained in 64% yield as a white solid after detritylation of **19** using the same procedure as described for compound **7**; mp 152−154 °C. ^{1}H NMR (DMSO-d_6): δ 2.52 (m, 2H, H-2′), 3.65 (m, 2H, H-5′), 4.05 (dd, J = 7.93 and 3.05 Hz, 1H, H-4′), 4.60 (dd, J = 11.60 and 5.49 Hz, 1H, H-3′), 5.37 (t, J = 4.88 Hz, 1H, 5′-OH), 6.17 (td, J = 6.10, Hz and 1.22 Hz, 1H, H-1′), 8.20 (d, $J_{6,F}$ = 7.32 Hz, 1H, H-6), 11.88 (s, 1H, NH). Anal. ($C_9H_{10}ClFN_2O_4$) C, H, N.

1-(3-Iodo-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine (22). This compound was synthesized according to the published procedure,[20] in 37% yield as a solid. The physical data for **22** were identical to those reported.[20]

1-(3-Iodo-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-5-fluorouracil (25) and 1-(3-Iodo-2,3-dideoxy-β-D-*threo*-pentofuranosyl)-5-fluorouracil (26). A dried mixture of **4** (2.3 g, 4.06 mmol) and NaI (10.89 g, 72.65 mmol) in dry DME (50 mL) was refluxed for 20 h. The resulting brown mixture was cooled and filtered, and the filtrate was evaporated to a solid. The solid was dissolved in methylene chloride (50 mL) and washed with 5% sodium thiosulfate solution (2 × 50 mL). The organic layer was finally washed with water (25 mL), dried over Na_2SO_4, and concentrated to give the crude product. Aqueous 80% AcOH (50 mL; v/v) was added to the crude product, and the reaction mixture was heated at 90 °C for 30 min. The solvent was removed in vacuo, and the

crude product thus obtained was purified on a silica gel column using EtOAc/hexane (60:40; v/v) as the eluent to give **25** (0.65 g, 45%) as a semisolid. ^{1}H NMR (DMSO-d_6): δ 2.63−2.70 (m, 2H, H-2′), 3.72 (m, 2H, H-5′), 4.15−4.21 (m, 1H, H-4′), 4.31 (dd, J = 16.78 and 8.24 Hz, 1H, H-3′), 5.41 (t, J = 5.18 Hz, 1H, 5′-OH), 5.99−6.03 (m, 1H, H-1′), 8.30 (d, $J_{6,F}$ = 7.63 Hz, 1H, H-6), 11.82 (d, $J_{NH,F}$ = 3.97 Hz, 1H, NH). Anal. ($C_9H_{10}FIN_2O_4$) C, H, N.

Further elution with EtOAc/Hexane (90:10; v/v) provided impure **26**, which was purified on preparative TLC using MeOH/$CHCl_3$ (10:90; v/v) as the eluent to yield **26** (0.040 g, 1%) as a syrup. ^{1}H NMR (DMSO-d_6): δ 2.50−2.56 (m, 1H, H-2′), 3.00−3.14, (m, 1H, H-2″), 3.56−3.74 (m, 3H, H-4′, H-5′), 4.58 (dd, J = 10.99 and 4.88 Hz, 1H, H-3′), 5.17 (br s, 1H, 5′-OH), 5.95 (m, 1H, H-1′), 8.16 (d, $J_{6,F}$ = 7.32 Hz, 1H, H-6), 11.92 (d, $J_{NH,F}$ = 4.88 Hz, 1H, NH). Anal. ($C_9H_{10}FIN_2O_4$) C, H, N.

1-(2,3-Anhydro-5-*O*-trityl-β-D-lyxofuranosyl)uracil (29a). Compound **28a**[27] (1.6 g, 2.5 mmol) was dissolved in 1 N NaOH in acetone−water (1:1, 36 mL). The resulting yellow solution was kept at room temperature for 18 h and then neutralized with 1 N HCl. The resulting precipitate was filtered. The residue was diluted with $CHCl_3$ (50 mL), washed with water (2 × 10 mL), dried (Na_2SO_4), and concentrated in vacuo to give **29a** (1.0 g, 86%) as a semisolid. ^{1}H NMR (DMSO-d_6): δ 3.20 (m, 2H, H-5′), 4.11 (m, 2H, H-2′, H-3′), 4.26 (m, 1H, H-4′), 5.62 (d, J = 7.93 Hz, 1H, H-5), 6.11 (s, 1H, H-1′), 7.22−7.44 (m, 15H, 5′-*O*-trityl), 7.51 (d, J = 7.93 Hz, 1H, H-6), 11.45 (s, 1H, NH).

1-(2,3-Anhydro-5-*O*-trityl-β-D-lyxofuranosyl)thymine (29b). This compound was synthesized in 99% yield as a white solid from **28b**[28] using the same procedure as mentioned above from **29a**; mp 130−132 °C. ^{1}H NMR (DMSO-d_6): δ 1.64 (s, 3H, CH_3), 3.22 (m, 2H, H-5′), 4.07 (m, 2H, H-2′, H-3′), 4.23 (t, J = 4.88 and 5.49 Hz, 1H, H-4′), 6.10 (s, 1H, H-1′), 7.22−7.50 (m, 16H, H-6 and 5′-*O*-trityl), 11.45 (s, 1H, NH).

1-(2,3-Anhydro-5-*O*-trityl-β-D-lyxofuranosyl)-5-fluorouracil (29c). The title compound was obtained in 91% yield as a syrup from **28c**[29] using the method described for compound **29a**. ^{1}H NMR (DMSO-d_6): δ 3.15−3.27 (m, 2H, H-5′), 4.10 (s, 2H, H-2′, H-3′), 4.26 (m, 1H, H-4′), 6.12 (d, J = 1.83 Hz, 1H, H-1′), 7.24−7.48 (m, 15H, 5′-*O*-trityl), 7.61 (d, $J_{6,5F}$ = 6.7 Hz, 1H, H-6), 12.00 (s, 1H, NH).

1-(3-Bromo-3-deoxy-5-*O*-trityl-β-D-arabinofuranosyl)uracil (30a) and 1-(2-Bromo-2-deoxy-5-*O*-trityl-β-D-xylofuranosyl)uracil (31a). A mixture of **29a** (0.50 g, 1.07 mmol) and NH_4Br (0.62 g, 6.33 mmol) in absolute ethanol (20 mL) was refluxed for 20 h. Ethanol was removed in vacuo, and the residue was purified by silica gel column chromatography using MeOH/$CHCl_3$ (2:98, v/v) as the eluent to yield **31a** (0.08 g, 14%) as a syrup. ^{1}H NMR (DMSO-d_6): δ 3.20−3.30 (m, 1H, H-5′), 3.35−3.97 (m, 1H, H-5″), 4.22−4.37 and 4.47−4.57 (2 m, 3H, H-2′, H-3′, H-4′), 5.49 (dd, J = 7.84 Hz, 2.44 and 1.8 Hz, 1H, H-5), 6.03 (d, J = 3.57 Hz, 1H, H-1′), 6.13 (d, J = 1.8 Hz, 1H, 3′−OH), 7.22−7.47 (m, 16H, H-6, 5′-*O*-trityl), 11.41 (s, 1H, NH).

Subsequent elution using $CHCl_3$/MeOH (98:2, v/v) yielded **30a** (0.26 g, 44%) as a syrup. ^{1}H NMR (DMSO-d_6): δ 3.25−3.45 (m, 2H, H-5′), 4.15−4.35 (2 m, 2H, H-3′, H-4′), 4.54 (d, J = 6.10 Hz, 1H, H-2′), 5.33 (dd, J = 8.54 Hz, 2.44 and 1.83 Hz, 1H, H-5), 6.15 (d, J = 6.10 Hz, 1H, H-1′), 6.27 (d, J = 6.10 Hz, 1H, 2′-OH), 7.22−7.45 (m, 15H, 5′-*O*-trityl), 7.60 (d, J = 8.5 Hz, 1H, H-6), 11.35 (s, 1H, NH).

1-(3-Bromo-3-deoxy-5-*O*-trityl-β-D-arabinofuranosyl)thymine (30b), 1-(3-Bromo-3-deoxy-5-*O*-trityl-β-D-arabinofuranosyl)-5-fluorouracil (30c), 1-(2-Bromo-2-deoxy-5-*O*-trityl-β-D-xylofuranosyl)thymine (31b), and 1-(2-Bromo-2-deoxy-5-*O*-trityl-β-D-xylofuranosyl)-5-fluorouracil (31c). For the synthesis of these compounds, the same method was used as described above for compounds **30a** and **31a**. Compounds **30b,c** and **31b,c** were obtained as syrups in 40, 26, 12, and 7% yield, respectively, and were used directly in the subsequent deprotection reactions.

1-(3-Bromo-3-deoxy-β-D-arabinofuranosyl)uracil (32). Detritylation of **30a** using 80% aqueous acetic acid (v/v) at 90 °C for 30 min afforded **32** in 70% yield as a white solid; mp 184−185 °C. ^{1}H NMR (DMSO-d_6): δ 3.66 (m, 2H, H-5′), 4.07 (m, 1H, H-4′), 4.15 (pseudo triplet, J = 7.93 and 6.71 Hz, 1H, H-3′), 4.53 (q, J = 6.10 Hz,1H, H-2′), 5.27 (br s, 1H, 5′-OH), 5.58 (dd, J = 8.54 and 1.83 Hz, 1H, H-5), 6.13 (d, J = 6.10 Hz, 1H, H-1′), 6.24 (d, J = 5.49 Hz, 1H, 2′-OH), 7.70 (d, J = 7.93 Hz, 1H, H-6), 11.31 (s, 1H, NH). Anal. ($C_9H_{11}BrN_2O_5$) C, H, N.

1-(3-Bromo-3-deoxy-β-D-arabinofuranosyl)thymine (33). Detritylation of **30b** using the procedure described above for **32** afforded compound **33** in 81% yield as a white solid; mp 206−210 °C, dec. ^{1}H NMR (DMSO-d_6): δ 1.76 (s, 3H, CH_3), 3.69 (m, 2H, H-5′), 4.05 (m, 1H, H-4′), 4.19 (pseudo triplet, J = 8.54 and 7.32 Hz, 1H, H-3′), 4.55 (q, J = 13.43 Hz, 6.71 Hz, 1H, H-2′), 5.32 (t, J = 4.88 Hz, 1H, 5′-OH), 6.12 (d, J = 6.10 Hz, 1H, H-1′), 6.19 (d, J = 6.10 Hz, 1H, 2′-OH), 7.61 (s, 1H, H-6), 11.30 (s, 1H, NH). Anal. ($C_{10}H_{13}BrN_2O_5$) C, H, N.

1-(3-Bromo-3-deoxy-β-D-arabinofuranosyl)-5-fluorouracil (34). Detritylation of **30c** using the procedure described above for **32** provided compound **34** in 58% yield as a white solid; mp 190−192 °C. ^{1}H NMR (DMSO-d_6): δ 3.69 (m, 2H, H-5′), 4.01−4.05 (m, 1H, H-4′), 4.15 (pseudo triplet, J = 8.50 and 7.30 Hz, 1H, H-3′), 4.56 (q, J = 7.32 Hz, 1H, H-2′), 5.49 (t, J = 5.44 Hz 1H, 5′-OH), 6.08 (dd, J = 6.10 and 1.83 Hz, 1H, H-1′), 6.25 (d, J = 6.10 Hz, 1H, 2′-OH), 8.11 (d, $J_{6,F}$ = 7.32 Hz, 1H, H-6), 11.87 (d, $J_{NH,F}$ = 4.27 Hz, 1H, NH). Anal. ($C_9H_{10}BrFN_2O_5$) C, H, N.

1-(2-Bromo-2-deoxy-β-D-xylofuranosyl)uracil (35). This compound was obtained in 67% yield as a syrup after detritylation of **31a** using the same procedure described above. ^{1}H NMR (DMSO-d_6): δ 3.65−3.77 (m, 2H, H-5′), 4.26−4.39 (m, 3H, H-2′, H-3′, H-4′), 4.89 (t, J = 4.88 Hz, 1H, 5′-OH), 5.64 (dd, J = 8.54 and 1.83 Hz, 1H, H-5), 6.04 (d, J = 3.05 Hz, 1H, H-1′), 6.09 (d, J = 1.83 Hz, 1H, 3′-OH), 7.72 (d, J = 7.93 Hz, 1H, H-6), 11.39 (s, 1H, NH). Anal. ($C_9H_{11}BrN_2O_5$) C, H, N.

1-(2-Bromo-2-deoxy-β-D-xylofuranosyl)thymine (36). This compound was obtained after detritylation of **31b** using the procedure described above for compound **32**, in 75% yield, as a syrup. ^{1}H NMR (DMSO-d_6): δ 1.76 (s, 3H, 5-CH_3), 3.70 (m, 2H, H-5′), 4.26 and 4.35 (2 m, 3H, H-2′, H-3′, H-4′), 4.89 (t, J = 4.88 Hz, 1H, 5′-OH), 6.04 (d, J = 3.66 Hz, 1H, H-1′), 6.12, (d, J = 3.05 Hz, 1H, 3′-OH), 7.60 (s, 1H, H-6), 11.42 (s, 1H, NH). Anal. ($C_{10}H_{13}BrN_2O_5$) C, H, N.

1-(2-Bromo-2-deoxy-β-D-xylofuranosyl)-5-fluorouracil (37). Detritylation of **31c** by the process used above provided **37**, in 58% yield, as a syrup. ^{1}H NMR (DMSO-d_6): δ 3.73 (m, 2H, H-5′), 4.30 and 4.40 (2 m, 3H, H-2′, H-3′ and H-4′), 4.94 (t, J = 6.10 Hz, 1H, 5′-OH), 6.09 (d, J = 1.83 Hz, 1H, H-1′) 6.14 (d, J = 3.66 Hz, 1H, 3′-OH), 7.96 (d, $J_{6,5F}$ = 7.32 Hz, 1H, H-6), 12.00 (d, $J_{NH,F}$ = 4.28 Hz, 1H, NH). Anal. ($C_9H_{10}BrFN_2O_5$) C, H, N.

In Vitro Antimycobacterial Activity Assay. *M. tuberculosis* (H37Ra) was obtained from the American Type Culture Collection (Rockville, MD). The antimycobacterial activity was determined using the MABA.[24] Test compounds were dissolved in DMSO at 10 mg/mL, and subsequent dilutions were performed in 7H9GC (Difco Laboratories, Detroit, MI) medium in 96-well plates. For these experiments, each compound was tested at 100, 50, 25 10, 5, and 1 μg/mL in triplicate. The experiments were repeated two times, and the mean percent inhibition is reported in Table 1. The standard deviations were within 10% of the mean value. Frozen mycobacterial inocula were diluted in medium 7H9GC and added to each well at final concentration of 2.5 × 10^5 CFU/mL. Sixteen control wells consisted of eight with bacteria alone (B) and eight with medium alone (M). Plates were incubated for 6 days, and then, 20 μL of 10× alamar blue and 12.5 μL of 20% Tween 80 were added to one M and one B well. Wells were observed for an additional 24−48 h for visual color change from blue to pink and read by spectrophotometer (at excitation 530 nm/525 nm and emission 590 nm/535 nm) to determine OD values. If the B well became pink by 24 h (indicating growth), reagent was added to the entire plate. If the B well remained blue, additional M and B wells were tested

daily until bacterial growth could be visualized by color change. After the addition of the reagent to the plate, cultures were incubated for 24 h, and plates were observed visually for color change and also read by spectrophotometer. Visual MIC was defined as the lowest concentration of a compound that prevented a color change from blue to pink. The percent inhibition was calculated as (test well-M bkg./B well-M bkg.) × 100. The lowest drug concentration affecting an inhibition of ~50% was considered as the MIC_{50}. Rifampicin, isoniazid, and cycloserine were used as positive controls. As negative controls, DMSO (2, 1, 0.2, and 0.02 μL) was added to the B well at concentrations similar to those of compound wells; M wells served as negative controls. In most of the experiments, the M wells gave an OD of 3000−4000, and the B wells had OD values ranging between 60000 and 100000.

Antimycobacterial Activity against Drug-Resistant Strains of *M. tuberculosis*. The activity of compounds **7** and **33** was determined against rifampicin-resistant *M. tuberculosis* H37Rv (ATCC 35838, resistant to rifampicin at 2 μg/mL) and isoniazid-resistant *M. tuberculosis* H37Rv (ATCC 35822, resistant to isoniazid at 2 μg/mL) using a radiometric BACTEC assay.[25] This assay detects the metabolism of ^{14}C-labeled palmitic acid, where evolving $^{14}CO_2$ is captured and counted as a measure of mycobacterial growth and metabolism. The growing inoculum ($2.5-5.0 \times 10^5$ CFU/vial) was diluted in a BACTEC vial containing radiometric 7H12 (BACTEC 12B) medium and incubated at 37 °C. Two-fold dilutions of test compounds were delivered to the inoculum-containing BACTEC vials. Negative control vials consisted of media with bacteria inoculum, medium with bacteria inoculum at 1:100, and medium alone. As reference drugs, rifampicin and isoniazid were used at their MIC_{90} concentrations. All of the vials were incubated at 37 °C, and the growth index (GI) was determined in a BACTEC 460 instrument until the GI of the 1:100 inoculum controls reached 30. Vials were read daily, and a change in GI (ΔGI) was recorded for each compound. The percent inhibition was defined as (GI of test sample/GI of control) × 100. For the no drug control, the ΔGI continued to increase and was much higher than the 1:100 inoculum control. The BACTEC assay was preferred with the resistant strain, because the method provides a safe, enclosed, and biocontained method to monitor the kinetics of drug inhibition.

Intracellular Antimycobacterial Activity in Human Monocytic Cell Line Infected with *M. tuberculosis* (H37Ra). Human monocytic cell line (THP-1) was cultured at 2×10^5 cells/well in a 24-well tissue culture plate with acid-washed, sterilized glass coverslips. A 100 nM concentration of PMA (phorbol myristate acetate) was added to each well, and the plates were incubated for 3 days. At this time, ~10^7 CFU of *M. tuberculosis* was added to each well and allowed to infect the macrophages for 3 h. Nonphagocytosed mycobacteria were removed with the supernatant, and the coverslips were moved to new 24-well plates. Test compounds were added in triplicate to the infected cell culture in concentrations of 25, 10, 5, or 1 μg/mL along with control wells of 2.5 μg/mL rifampicin or isoniazid and DMSO/media and incubated for 4 days. After the contents of each well were lysed, aliquots of lysates were transferred to 7H11 plates in serial dilution. The growing colonies were counted at 2−3 weeks. The experiment was repeated twice, and the percent reduction in CFUs was calculated.

In Vitro Cytotoxicity Assay. Human hepatoma cell line (Huh-7) was used to determine the effect of test compounds on human cell cytotoxicity using XTT and ^3H-thymidine assays. Cell viability was measured using the cell proliferation kit II (XTT; Roche), as per the manufacturer's instructions. Briefly, a 96-well plate was seeded with Huh-7 cells at a density of 2×10^4 cells per well. Cells were allowed to attach for 6−8 h when the medium was replaced with medium containing compounds at concentrations of 200, 100, 50, 10, and 1 μg/mL. DMSO was also included as the control. Plates were incubated for 2 days at 37 °C. The color reaction involved adding 50 μL XTT reagents per well and incubating for 4 h at 37 °C. Plates were read on an enzyme-linked immunosorbent assay plate reader (Abs 450−500 nm). For the ^3H-Tdr incorporation assay, Huh-7 cells were plated at 1×10^4 cells/well in 96-well flat bottom plates. Medium containing compounds at concentrations of 200, 100, 50, 10, and 1 μg/mL was added to the plates in triplicate. DMSO was also included as the control. Plates were incubated for 2 days at 37 °C. The wells were pulsed with 0.5 μCi/well [^3H]-thymidine (Amersham) for 12−18 h. After this, the plates were harvested on filter papers (Perkin-Elmer) using a 96-well plate harvester (Tomtech MACH III M). The levels of [^3H]-thymidine incorporated into the DNA of proliferating cells were counted in a Microbeta Trilux liquid scintillation counter (Perkin-Elmer).

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