Inhibition of *Mycobacterium tuberculosis, Mycobacterium bovis,* and *Mycobacterium avium* by Novel Dideoxy Nucleosides

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Received April 3, 2007

The prevalence of tuberculosis (TB) and mutidrug-resistant tuberculosis (MDR-TB) has been increasing, leading to serious infections, high mortality, and a global health threat. Here, we report the identification of a novel class of dideoxy nucleosides as potent and selective inhibitors of *Mycobacterium bovis, Mycobacterium tuberculosis,* and drug-resistant *Mycobacterium tuberculosis*. A series of 5-acetylenic derivatives of 2′,3′-dideoxyuridine (**3**−**8**) and 3′-fluoro-2′,3′-dideoxyuridine (**22**−**27**) were synthesized and tested for their antimycobacterial activity against *M. bovis*, *M. tuberculosis*, and *M. avium*. 2′,3′-Dideoxyuridine possessing 5-decynyl, 5-dodecynyl, 5-tridecynyl, and 5-tetradecynyl substituents (**4**−**7**) exhibited the highest antimycobacterial activity against all three mycobacteria. In contrast, in the 3′-fluoro-2′,3′-dideoxyuridine series, a 5-tetradecynyl analogue (**26**) displayed the most potent activity against these mycobacteria. Among other derivatives, 5-bromo-2′,3′-dideoxycytidine (**11**), 5-methyl-2′,3′-dideoxycytidine (**12**), and 5-chloro-4-thio-2′,3′-dideoxyuridine (**19**) exhibited modest inhibition of *M. bovis* and *M. tuberculosis*. In the series of dideoxy derivatives of adenosine, guanosine, and purines, 2-amino-6-mercaptoethyl-9-(2,3-dideoxy-β-D-glycero-pentofuranosyl)purine (**32**) and 2-amino-4-fluoro-7-(2,3-dideoxy-β-D-glyceropentofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (**35**) were the most efficacious against *M. bovis* and *M. tuberculosis*, and *M. avium*, respectively.

Introduction

Tuberculosis (TB[a]) is a contagious respiratory disease transmitted through the air by coughing and sneezing and is caused by several species of the bacterial genus *Mycobacterium*. There was a gradual decline in the number of cases of TB in North America from the early 1950s, but there has been a resurgence since about 1984.[1−3] A number of inter-related factors have contributed to the resurgence of TB including, (i) synergism with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) epidemic, (ii) increased mobility and immigration of people from countries where TB is endemic, (iii) increased poverty and homelessness, (iv) premature dismantling of the health infrastructure for TB treatment, and (v) poor compliance of treatment regimens and their side effects. Consequently, TB was declared a global health emergency by the World Health Organization (WHO) in 1993. The WHO estimates that one-third of the world's population is infected by *Mycobacterium tuberculosis*. Annually TB infects eight million people and causes 2−3 million deaths around the world.[4−6]

Discovery of streptomycin and subsequent introduction of *p*-aminosalicylic acid, isoniazid, and rifampicin led to the optimism that tuberculosis could be effectively treated and controlled. However, new tuberculosis strains are emerging and spreading that are not susceptible to a number of available drugs, i.e., multidrug-resistant tuberculosis (MDR-TB).[4,7,8] A deadly new drug-resistant strain of tuberculosis is on the rise and is classified as extensively drug-resistant tuberculosis (XDR-TB), which is virtually incurable with existing antibiotics.[9] The HIV−AIDS patients infected with XDR-TB have a very quick disease progression and mortality rate of 100%.

Although TB is considered a single disease, it can be caused by several microorganisms. Two groups of mycobacteria *M. tuberculosis* and *M. avium* pose a significant challenge to the clinical management of tuberculosis in HIV-infected patients and are often responsible for their deaths.[10] Bacillus Calmette Guerin (BCG)[11] is an attenuated strain of *M. bovis* that is more than 98% homologous to *M. tuberculosis* and therefore is closely related to *M. tuberculosis*. *M. tuberculosis* is most likely an evolved form of *M. bovis*. *M. bovis* infections in humans have been reported from 4000 to 5000 BC. Interestingly, *M. bovis* infections have re-emerged and are causing TB in humans, particularly those who are HIV-positive. In addition, MDR strains of *M. bovis* have been isolated.[12] In Europe, primary MDR-TB caused by *M. bovis* has been found to be resistant to 11 anti-TB drugs with a mean survival of 44 days in 19 patients.[12] There is an ever-increasing threat of drug-resistant TB appearing as an epidemic in many developing as well as developed countries particularly because no new classes of drugs have been especially developed for the treatment of tuberculosis since 1967.[6]

The development of drug-resistant clinical isolates of mycobacteria makes the investigation of new classes of anti-TB agents a high priority, since new agents that work by mechanisms different from those of current drugs and are not cross-resistant with them are likely the best long-term prospect to augment current therapy, address the resistance crisis, and meet the global health emergency.

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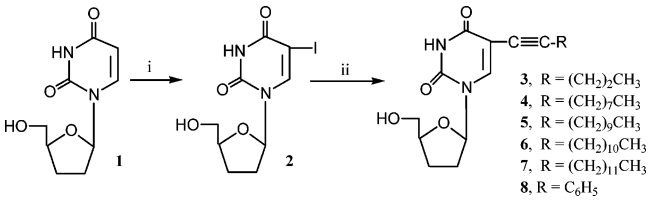
[a] Abbreviations: TB, tuberculosis; MDR-TB, multidrug-resistant tuberculosis; XDR-TB, extensively drug-resistant tuberculosis; *M. bovis, Mycobacterium bovis*; *M. tuberculosis, Mycobacterium tuberculosis*; *M. avium, Mycobacterium avium*; BCG, Bacillus Calmette Guerin; MABA, microplate Alamar blue assay; HFF, human foreskin fibroblast; cmc, critical micellar concentration; CFU, colony forming unit; GI, growth index; *B. subtilis, Bacillus subtilis*; *S. pneumoniae, Streptococcus pneumoniae*; *S. pyogenes, Streptococcus pyogenes*; DDC, 2′,3′-dideoxycytidine; 3-TC, 3′-thiacytidine.

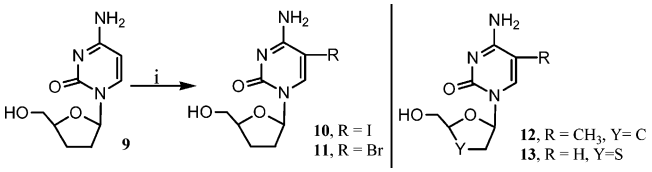
10.1021/jm070391t CCC: $37.00 © 2007 American Chemical Society
Published on Web 08/16/2007

Scheme 1[a]



[a] Reagents: (i) iodine monochloride, NaN_3, CH_3CN, 0−25 °C; (ii) H−C≡C-R, $Pd(PPh_3)_4$, CuI, $(i\text{-Pr})_2$EtN, DMF, room temp.

Scheme 2[a]



[a] Reagents: (i) dry DMF, *N*-iodosuccinimide, (**10**), *N*-bromosuccinimide (**11**), 25 °C, overnight.

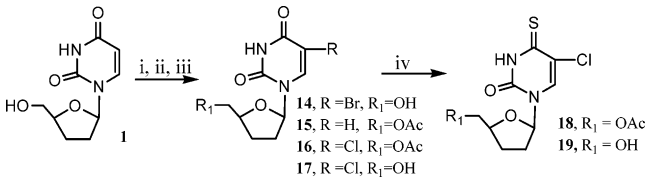
In an effort to discover new and effective chemotherapeutic agents for the treatment of tuberculosis, we recently reported the in vitro antimycobacterial activity of various 5-substituted pyrimidine nucleosides.[13,14] Among a large number of molecules examined, 5-alkynyl derivatives of 2′-deoxyribose nucleosides displayed good inhibition of *M. bovis* and *M. avium* replication in vitro.[13] In order to understand the structural requirements at the sugar moiety for antimycobacterial activity, thus further exploring structure−activity relationships in this series of promising antimycobacterial agents, we have now synthesized and investigated the antituberculosis activity of various 2′,3′-dideoxy nucleosides with halo, alkyl, and alkynyl substituents at the C-5 position of the pyrimidine ring. The results showed that 5-decynyl, 5-dodecynyl, 5-tridecynyl, and 5-tetradecynyl analogues of 2′,3′-dideoxyuridine (**4**−**7**) and 5-tetradecynyl-3′-fluoro-2′,3′-dideoxyuridine (**26**) in particular exhibit marked inhibitory activity against *M. bovis* and *M. tuberculosis* in vitro. In addition, we discovered that the most active compounds were inhibitory against a drug (rifampicin) resistant *M. tuberculosis* strain, suggesting that these agents work by a mechanism of action different from that of rifampicin. In contrast, 2′,3′-dideoxy derivatives of purine nucleosides examined in these studies were mostly devoid of antimycobacterial activity.

Chemistry

The key intermediate, 5-iodo-2′,3′-dideoxyuridine (**2**), required in the coupling reactions for the preparation of target 5-alkynyl derivatives of 2′,3′-dideoxyuridine (**3**−**8**), was synthesized by a mild and efficient method in quantitative yield. Reaction of 2′,3′-dideoxyuridine (**1**) with iodine monochloride and sodium azide in acetonitrile at 0−25 °C yielded **2** in 87% yield. This key synthon, using the procedure we reported earlier,[13] was alkynylated with a series of terminal alkynes in DMF in the presence of Pd, Cu, and diisopropylethylamine to provide the desired compounds **3**−**8** (Scheme 1).

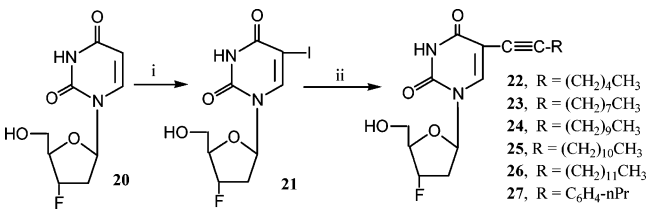
5-Iodo-2′,3′-dideoxycytidine (**10**) and 5-bromo-2′,3′-dideoxycytidine (**11**) were synthesized from 2′,3′-dideoxycytidine (**9**) as described earlier (Scheme 2).[15] 5-Bromo-2′,3′-dideoxyuridine (**14**) was obtained in excellent yield (76%) by the reaction of 2′,3′-dideoxyuridine (**1**) with NBS in DMF (Scheme 3). This reaction provided improved yield over the one reported previously by using bromine in carbon tetrachloride (49% yield).[16] Preparation of the 5-chloro-2′,3′-dideoxyuridine (**17**) and its

Scheme 3[a]



[a] Reagents: (i) *N*-bromosuccinimide, DMF (**14**), pyridine, acetic anhydride, 0 °C (**15**); (ii) *N*-chlorosuccinimide, pyridine, 90 °C (**16**); (iii) NH_3−MeOH, 0 °C (**17**, **19**); (iv) Lawesson's reagent, 1,4-dioxane, reflux (**18**).

Scheme 4[a]



[a] Reagents: (i) iodine monochloride, MeOH, 60 °C; (ii) H−C≡C−R, $Pd(PPh_3)_4$, CuI, $(i\text{-Pr})_2$EtN, DMF, 25 °C.

4-thio derivative (**19**) was carried out using 5′-O-acetylated 2′,3′-dideoxyuridine (**15**) as shown in Scheme 3.

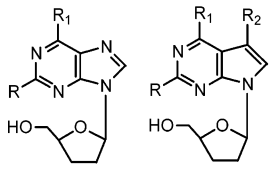
The 5′-O-acetylated derivative (**15**) was conveniently prepared in 97% yield by the reaction of **1** with acetic anhydride in dry pyridine at 0−25 °C. Chlorination of 5′-*O*-acetyl-2′,3′-dideoxyuridine (**15**) with *N*-chlorosuccinimide in pyridine at 90 °C produced **16** in 94% yield after purification by silica gel column chromatography. Deprotection of acetate derivative **16** with methanol saturated with ammonia produced the target 5-chloro-2′,3′-dideoxyuridine (**17**) in quantitative yield (87%) after column chromatography. This method provided a slightly improved yield of compound **17** compared to a previously reported method (75%).[17] Thiation of 5′-*O*-acetyl-2′,3′-dideoxyuridine (**15**) with Lawesson's reagent in 1,4-dioxane heated to reflux provided a 4-thio analogue (**18**) in 84% yield. Reaction of **18** with a solution of saturated ammonia in methanol yielded 5-chloro-4-thio-2′,3′-dideoxyuridine (**19**) in 81% yield after purification by column chromatography.

The synthetic route for the preparation of 5-heptynyl- (**22**), 5-decynyl- (**23**), 5-dodecynyl- (**24**), 5-tridecynyl- (**25**), 5-tetradecynyl- (**26**), and 5-(4-*n*-propylphenylethynyl)- (**27**) 3′-fluoro-2′,3′-dideoxyuridines was adopted after slight modification over the method used for 2′-deoxyuridine analogues.[13] The Pd-catalyzed coupling reaction of respective alkynes and 4-*n*-propylphenylacetylene with 5-iodo-3′-fluoro-2′,3′-dideoxyuridine (**21**)[18] proceeded readily to yield hitherto unknown target compounds **22**−**27** in moderate to excellent yields (Scheme 4).

2′,3′-Dideoxyadenosine (**28**),[19] 2′,3′-dideoxyguanosine (**29**),[20] 2,6-diamino-2′,3′-dideoxypurine ribofuranoside (**30**),[21] 2-amino-6-methoxy-9-(2,3-dideoxy-β-D-glyceropentofuranosyl)purine (**31**),[22] 2-amino-6-mercaptoethyl-9-(2,3-dideoxy-β-D-glyceropentofuranosyl)purine (**32**),[23−25] 4-amino-5-cyano-7-(2,3-dideoxy-β-D-glyceropentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**33**),[22,24] 4-amino-5-carboxamido-7-(2,3-dideoxy-β-D-glyceropentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**34**),[22] and 2-amino-4-fluoro-7-(2,3-dideoxy-β-D-glyceropentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**35**)[26] (Figure 1) were prepared by using published procedures.

Results and Discussion

The antimycobacterial activities of the dideoxypyrimidines (**3**−**19**, **22**−**27**) and dideoxypurines (**28**−**35**) were evaluated against three mycobacteria (*M. bovis* BCG, *M. tuberculosis*



28-32 33-35

Compd	R	R_1	R_2
28	H	NH_2	-
29	NH_2	OH	-
30	NH_2	NH_2	-
31	NH_2	OCH_3	-
32	NH_2	SC_2H_5	-
33	H	NH_2	CN
34	H	NH_2	$CONH_2$
35	NH_2	F	H

Figure 1.

H37Ra, *M. avium* ATCC 25291) using the microplate Alamar blue assay (MABA)[27] at 1−100 μg/mL concentrations. To analyze the antimycobacterial effect of the promising compounds against the drug-resistant mycobacteria, rifampicin-resistant *M. tuberculosis* H37Rv (ATCC 35838) was used. Two drugs in clinical use, rifampicin and clarithromycin, were included in these assays as reference compounds. Examination of antimycobacterial activity was undertaken as described in the Experimental Section. The in vitro test results for dideoxypyrimidine and dideoxypurine analogues are described in Tables 1 and 2 as percent inhibition at various concentrations and minimum concentration bringing 90% inhibition (MIC_{90}). Dideoxypyrimidine nucleosides containing a long carbon chain at the C-5 position, 5-decynyl, 5-dodecynyl, 5-tridecynyl, and 5-tetradecynyl, appear to provide strong activity against both *M. bovis* and *M. tuberculosis*. The 90% inhibition in multiplication of *M. bovis* by the 2′,3′-dideoxyuridine analogues 5-decynyl-2′,3′-dideoxyuridine (**4**), 5-dodecynyl-2′,3′-dideoxyuridine (**5**), 5-tridecynyl-2′,3′-dideoxyuridine (**6**), and 5-tetradecynyl-2′,3′-dideoxyuridine (**7**) was at 1−10 μg/mL (Table 1). The activity of **4** and **5** against *M. tuberculosis* was similar to that against *M. bovis* (MIC_{90} = 1−10 μg/mL range), whereas the activity of **6** and **7** was reduced against *M. tuberculosis* (MIC_{90} = 10−50 μg/mL). The 3′-fluoro-2′,3′-dideoxyuridines containing 5-decynyl (**23**), 5-dodecynyl (**24**), and 5-tridecynyl (**25**) moieties displayed moderate antimycobacterial activity against both *M. bovis* and *M. tuberculosis* (MIC_{90} = 10−50 μg/mL) in comparison to the corresponding 2′,3′-dideoxyuridine derivatives (**4**−**6**). However, it was observed that incorporation of a 3′-fluoro substituent in compound **7**, resulting in compound **26**, provided significantly enhanced biological activities against these mycobacteria [*M. bovis* (MIC_{90} = 1 μg/mL), *M. tuberculosis* (MIC_{90} = 1−2 μg/mL range)] (Table 1). Interestingly, inclusion of the aryl or *p*-alkylaryl ring in the 5-alkynyl side chain of the present dideoxyuridines was detrimental to antimycobacterial activity, as exemplified by 5-(2-phenylethynyl) (**8**) and 5-(4-*n*-propylphenylethynyl) (**27**) analogues that possessed significantly reduced activity (Table 1). However, it is important to note that activity displayed by **27**, which contains a 3′-fluoro-2′,3′-dideoxy sugar moiety, was superior (MIC_{90} = 50−100 μg/mL) to **8** having a 2′,3′-dideoxy moiety (25% inhibition at 100 μg/mL).

Next, we determined the activity of these compounds against *M. avium*. Compounds **4**−**7**, **24**, and **26** exhibited notable inhibitory activity, having MIC_{90} values in the 10−100 μg/mL range. We observed that 2′,3′-dideoxy analogues (**4**−**7**) were more effective than their 3′-fluoro-2′-3′-dideoxy counterparts (**23**−**26**). The most active compounds, 5-dodecynyl- (**5**) and 5-tridecynyl- (**6**) dideoxyuridines, exhibited MIC_{90} values of 10−50 and 10−25 μg/mL, respectively. Importantly, compound **6** showed significant reduction of *M. avium* growth (80% inhibition at 10 μg/mL) compared to the reference drug clarithromycin that showed 90−95% inhibition at 2 μg/mL. Replacement of the 5-alkynyl moiety with hydrogen (**15**), halogens (**10**, **11**, **14**, **16**−**19**), or methyl (**12**) groups did not offer any advantage in the inhibition of *M. avium* growth but rather resulted in a dramatic reduction of activity.

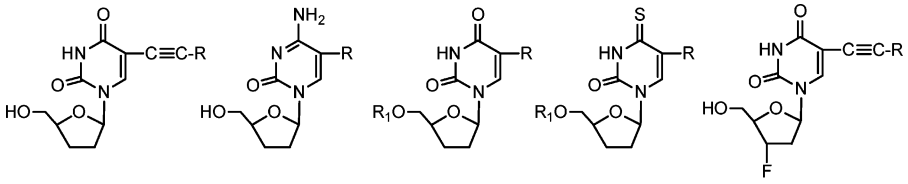
The most promising compounds emerging from this work, **5** and **26**, were selected to determine their antimycobacterial activity against a rifampicin-resistant H37Rv strain of *M. tuberculosis* using the BACTEC assay.[27] Encouragingly, the drug-resistant *M. tuberculosis* strain was susceptible to compounds **5** and **26** (MIC_{90} = 1−2 μg/mL) at concentrations similar to the those of drug-sensitive strains. In this assay, rifampicin showed no activity up to 2 μg/mL, whereas isoniazid provided 100% inhibition at 1 μg/mL. We also determined the effect of **5** in intracellular *M. bovis* (BCG) in human monocytic cell line U937 at 10 and 2 μg/mL concentrations using the colony forming units (CFU) assay.[27] It inhibited intramacrophagic mycobacterial growth at both concentrations (>90% reduction).

Structure−activity relationship (SAR) studies of 5-alkynyl-dideoxypyrimidine nucleosides indicated that substituents at the C-5 of the pyrimidine ring and 3′-position of the carbohydrate moiety greatly influence the activity. Among 5-substituents, a clear SAR can be seen; the dideoxy nucleosides (**4**−**7** and **23**−**26**) containing a longer carbon chain at the C-5 position are potent inhibitors of *M. bovis* and *M. tuberculosis*, demonstrating activity depending on the length of the alkynyl side chains. Antimycobacterial activity of compounds **8** and **27** indicates that restriction of the conformational freedom of the alkyl side chain is less tolerated. It thus appears that in order to confer a substantial antimycobacterial effect, the side chain should be flexible. Alternatively, the reduced activity of **8** and **27** could be due to differences in the shape and size of the side chains that may produce steric hindrance. With regard to *M. avium* activity, it was observed that replacement of a hydrogen atom by a fluorine substituent at the 3′-position of the sugar moiety is detrimental for *M. avium* activity in this series of compounds.

In comparing the antimycobacterial activities of the investigated dideoxy pyrimidine nucleosides with their corresponding 2′-deoxyuridine analogues reported previously,[13] we noted that the activities of the 5-decynyl and 5-dodecynyl analogues of 2′,3′-dideoxyuridine (**4** and **5**) and 3′-fluoro-2′,3′-dideoxyuridine (**23** and **24**) against *M. bovis* and *M. avium* were significantly enhanced compared to their 2′-deoxyribose derivatives [(*M. bovis*, MIC_{90} = 100 and 50 μg/mL, respectively) (*M. avium*, 30% at 100 μg/mL and 75% at 100 μg/mL, respectively)]. The 5-tetradecynyl analogues of 2′,3′-dideoxyuridine (**7**) and 3′-fluoro-2′,3′-dideoxyuridine (**26**) also gained slight improvement in the activity against *M. bovis* and *M. avium* compared to their 2′-deoxyuridine counterparts [(*M. bovis*, MIC_{90} = 10 μg/mL) (*M. avium*, 75% at 100 μg/mL)]. The activities of compounds **4**, **5**, and **26** against *M. tuberculosis* (H37Ra) were also significantly improved compared to the activities of their corresponding 2′-deoxyuridine congeners reported previously[13] (MIC_{90} = 50−100, 50−100, and 25−50 μg/mL, respectively, from unpublished data). These results suggest that conformational features acquired by 5-alkynyl nucleosides containing 2′,3′-dideoxy and 3′-fluoro-2′,3′-dideoxy sugars are preferred over the 2′-deoxyribose carbohydrate portion and that alterations in sugar moieties can lead to improved antimycobacterial potency. Further, dideoxypyrimidine nucleosides are at an advantage because of their better oral bioavailability and plasma half-life compared to 2′-deoxy analogues.

Among the other structural modifications studied, dideoxycytidine compounds, the 2′,3′-dideoxycytidine (**9**), a known anti-HIV agent, and its 5-iodo derivative (**10**) were found to be devoid of antimycobacterial activity whereas related 5-bromo

Table 1. In Vitro Antimycobacterial Activity of Dideoxypyrimidine Nucleosides against *M. bovis*, *M. tuberculosis*, and *M. avium*

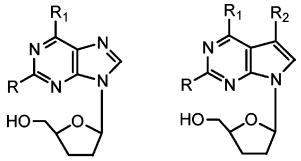


3-8 **9-12** **14-17** **18,19** **22-27**

			antimycobacterial activity					
			M. bovis (BCG)		*M. tuberculosis* (H37Ra)		*M. avium* (ATCC 25291)	
compd	R	R_1	% inhibition (concn, μg/mL)[a]	MIC_{90} [b] (μg/mL)	% inhibition (concn, μg/mL)[a]	MIC_{90} [b] (μg/mL)	% inhibition (concn, μg/mL)[a]	MIC_{90} [b] (μg/mL)
3	$(CH_2)_2CH_3$		50 (100)		50 (100)		0 (100)	
4	$(CH_2)_7CH_3$		100 (100, 50, 10), 50 (1)	1−10	100 (100, 50, 10), 50 (1)	1−10	90 (100), 25 (50,10)	100
5	$(CH_2)_9CH_3$		100 (100, 50, 10), 80−90 (1)	1−2	100 (100, 50, 10), 80−90 (1)	1−2	100 (100, 50), 50 (10)	10−50
6	$(CH_2)_{10}CH_3$		100 (100, 50,10), 60 (1)	1−10	100 (100, 50), 50 (10,1)	10−50	100 (100, 50), 80 (10)	10−25
7	$(CH_2)_{11}CH_3$		100 (100, 50,10), 50 (1)	1−10	100 (100, 50), 50 (10), 25 (1)	10−50	100 (100), 50 (50)	50−100
8	C_6H_5		25 (100, 50)		25 (100, 50)		25 (100, 50, 10)	
9	H (DDC)[c]		0 (100)		0 (100)		0 (100)	
10	I		0 (100)		0 (100)		0 (100)	
11	Br		70 (100), 50 (10)		50 (10)		0 (100)	
12	CH_3		50 (100, 50, 10)		50 (100, 50, 10)		0 (100)	
13	3-TC[d]		0 (100)		0 (100)		0 (100)	
14	Br	H	0 (100)		0 (100)		0 (100)	
15	H	Ac	0 (100)		0 (100)		0 (100)	
16	Cl	Ac	0 (100)		0 (100)		0 (100)	
17	Cl	H	0 (100)		0 (100)		0 (100)	
18	Cl	Ac	100 (100), 70 (50)	50−100	100 (100), 60 (50)	50−100	0 (100)	
19	Cl	H	100 (100), 50 (50)	50−100	100 (100), 50 (50)	50−100	0 (100)	
22	$(CH_2)_4CH_3$		100 (100), 50 (50, 10)	50−100	50 (100)		0 (100)	
23	$(CH_2)_7CH_3$		100 (100, 50), 60 (10)	10−50	100 (100, 50), 25 (10)	10−50	50 (100)	
24	$(CH_2)_9CH_3$		100 (100, 50), 75 (10)	10−50	100 (100, 50), 50 (10)	10−50	90 (100), 40 (50)	100
25	$(CH_2)_{10}CH_3$		100 (100, 50), 75 (10)	10−50	100 (100, 50), 60 (10)	10−50	25 (100)	
26	$(CH_2)_{11}CH_3$		100 (100, 50, 10), 90 (1)	1	100 (100, 50, 10), 80−90 (1)	1−2	90 (100)	100
27			100 (100, 50), 50 (10)	50−100	100 (100, 50), 50 (10)	50−100	25 (100)	
Std1[e]			100 (0.5−1)	0.5−1	100 (0.5−1)	0.5−1	90 (2)	2
Std2[e]			ND[f]	ND[f]	ND[f]	ND[f]	95 (2)	2

[a] Antimycobacterial activity was determined at concentrations 100, 50, 10, and 1 μg/mL. [b] Concentration of compounds exhibiting 90% inhibition in mycobacterial growth. [c] DDC = 2′,3′-dideoxycytidine. [d] 3-TC = 3′-thiacytidine. [e] Positive control drugs. Std 1 = rifampicin. Std2 = clarithromycin. [f] ND = not determined.

Table 2. In Vitro Antimycobacterial Activity of Dideoxypurine Nucleosides against *M. bovis*, *M. tuberculosis*, and *M. avium*



28-32 **33-35**

				antimycobacterial activity					
				M. bovis BCG		*M. tuberculosis* (H37Ra)		*M. avium* (ATCC 25291)	
compd	R	R_1	R_2	% inhibition (concn, μg/mL)[a]	MIC_{90} [d] (μg/mL)	% inhibition (concn, μg/mL)[a]	MIC_{90} [d] (μg/mL)	% inhibition (concn, μg/mL)[a]	MIC_{90} [d] (μg/mL)
28	H	NH_2		0 (100)		0 (100)		0 (100)	
29	NH_2	OH		0 (100)		0 (100)		0 (100)	
30	NH_2	NH_2		50 (100, 50)		25 (100)		0 (100)	
31	NH_2	OCH_3		25 (100, 50)		0 (100)		20 (100, 50)	
32	NH_2	SC_2H_5		100 (100, 50), 40 (10)	10−50	100 (100, 50), 35 (10)	10−50	25 (100,50)	
33	H	NH_2	CN	50 (100), 40 (50)		50 (100), 40 (50)		0 (100)	
34	H	NH_2	$CONH_2$	25 (100, 50)		0 (100)		0 (100)	
35	NH_2	F	H	50 (100, 50), 30 (10)		50 (100), 40 (50), 25 (10)		100 (100, 50), 35 (10)	10−50
Std1[b]				100 (0.5−1)	0.5−1	100 (0.5−1)	0.5−1	90 (2)	2
Std2[b]				ND[c]	ND[c]	ND[c]	ND[c]	95 (2)	2

[a] Antimycobacterial activity was determined at concentrations 100, 50, 10, and 1 μg/mL. [b] Positive control drugs. Std 1 = rifampicin. Std2 = clarithromycin. [c] ND = not determined. [d] Concentration of compounds exhibiting 90% inhibition in mycobacterial growth.

(**11**) and 5-methyl (**12**) analogues showed modest inhibition of *M. bovis* and *M. tuberculosis*. Akin to compound **9**, no activity was detected for another anti-HIV agent, 3′-thiacytidine (**13**), up to 100 μg/mL. We observed that deamination of **11** to **14**

eliminated the potency of the inhibitor completely. Similarly, analogous 5-chlorodideoxyuridines (**16** and **17**) provided no antimycobacterial effect. Interestingly, replacement of the oxygen by a sulfur at the 4-position in compounds **16** and **17** led to a gain in activity for compounds **18** and **19**, which had MIC_{90} values in the range of 50−100 μg/mL (Table 1). Thus, amino and sulfur groups may substitute for oxygen at the 4-position of the base and still retain activity. The gain in activity could possibly be attributed to the increased lipophilicity of compounds **18** and **19**. The presence of an *O*-acetyl protecting group at the 5′-position in compound **18**, however, does not improve the activity much over compound **19** because both **18** and **19** have the same activity profile and **18** is more lipophilic, arguing against the suggestion of the activity being a function of lipophilicity. Alternatively, the improved activities of compounds **18** and **19** could be due to different abilities of these molecules to generate hydrogen bond interactions or protomeric equilibrium on the HN−C═S and NH−C═O moieties, compared to **16** and **17**.

In the series of 2′,3′-dideoxypurine nucleoside analogues (**28**−**35**) investigated here, only 2-amino-6-mercaptoethyl-9-(2,3-dideoxy-β-D-glyceropentofuranosyl)purine (**32**) demonstrated appreciable activity for *M. bovis* and *M. tuberculosis*, having an MIC_{90} in the range of 10−50 μg/mL, whereas 2-amino-4-fluoro-7-(2,3-dideoxy-β-D-glyceropentofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (**35**) showed notable inhibition of *M. avium* (MIC_{90} = 10−50 μg/mL) (Table 2). We observed that a thioalkyl moiety (**32**) is preferred over the amino, hydroxyl, methoxy, or fluoro substituents at the 6-position of the purine ring for activity against *M. bovis* and *M. tuberculosis* because compounds **28**−**31** and **33**−**35** were inactive or weak inhibitors. In contrast, a smaller sized substituent (fluorine) is required at this position for shifting the activity toward *M. avium*, as demonstrated by the activity of compound **35**.

In order to differentiate whether the active compounds **4**−**7** and **23**−**26** are acting as promiscuous inhibitors or specific inhibitors of mycobacteria, their activities were determined in the presence of detergents (Triton X-100 and Tween-20) at concentrations up to their critical micellar concentrations (cmc) (data not shown).[28,29] It was interesting to note that the inhibition profiles of these compounds did not change in the presence of various detergents. These results clearly indicated that compounds **4**−**7** and **23**−**26** are not promiscuous inhibitors whose activities are dependent on their aggregation in biological medium but are specific inhibitors of mycobacterial growth and could be considered as starting points for the design of therapeutic agents designed to treat mycobacterial infections.

The precise mechanism of action of the compounds inhibiting mycobacterial multiplication in this study is not clear yet. The complete genome sequence of *M. tuberculosis* has been deciphered.[30] It encodes many of the enzymes required for DNA and RNA synthesis and for pyrimidine and purine nucleoside biosynthesis. It is possible that active nucleoside analogues, after their metabolic conversion to phosphorylated forms by mycobacterial kinases, may be selectively inhibiting the bacterium's DNA and/or RNA synthesis by acting as substrates and/or inhibitors of metabolic enzymes of DNA/RNA synthesis.

The compounds **3**−**19** and **22**−−**35** were also evaluated for their activities against several Gram-positive and Gram-negative bacterial species. The details of organisms used are provided in the Experimental Section. Only 5-decynyl-2′,3′-dideoxyuridine [**4**, MIC_{100} at 100 μg/mL for *B. subtilis*, *S. pneumonaie*, and *S. pyogenes*] and 5-dodecynyl-2′,3′-dideoxyuridine [**5**, MIC_{100} at 100 μg/mL for *B. subtilis*] exhibited antibacterial activity for selected Gram-positive pathogens. None of the corresponding 3′-fluoro-2′,3′-dideoxyuridine derivatives (**23**−**26**) provided antibacterial activity. These results revealed that dideoxypyrimidine nucleosides **4**, **5**, and **26** exhibiting potent antimycobacterial activity have specificity for *M. bovis* and *M. tuberculosis*.

The MTT test was performed to evaluate the toxicity of promising compounds (**4**−**7**, **23**−**26**) in vitro against Vero cells and human foreskin fibroblast (HFF cells). No toxicity was observed up to the highest concentrations tested (CC_{50} > 100 μg/mL).

Summary

In conclusion, our work demonstrates a new family of inhibitors with potent and selective antimycobacterial properties. We report synthesis and antibacterial activities of various categories of dideoxy nucleoside analogues that have provided insight into the functionalities and their contributions in the inhibition of mycobacterial and selective Gram-positive bacterial growth. The results obtained here are consistent with our previous findings that pyrimidine nucleosides with long-chain alkynyl substituents at the C-5 position are endowed with selective inhibitory activity. In this study 2′,3′-dideoxyuridine analogues possessing 5-decynyl, 5-dodecynyl, 5-tridecynyl, and 5-tetradecynyl substituents were found to be strong inhibitors of *M. bovis* multiplication whereas 5-decynyl- and 5-dodecynyl-2′,3′-dideoxyuridines provided potent antimycobacterial activity against both *M. bovis* and *M. tuberculosis*. The most promising compounds in the present series, 5-dodecynyl-2′,3′-dideoxyuridine (**5**) and 5-tetradecynyl-3′-fluoro-2′,3′-dideoxyuridine (**26**), showed excellent activity and inhibited the growth of *M. bovis* and *M. tuberculosis* with MIC_{90} of 1−2 μg/mL range, which approaches the reference drug rifampicin. It is noteworthy that these dideoxy nucleosides were also active against a drug-resistant strain of *M. tuberculosis*. Further, it was encouraging that the 5-tridecynyl analogue of 2′,3′-dideoxyuridine (**6**) demonstrated significant inhibition of *M. avium* (80% inhibition at 10 μg/mL), an important opportunistic pathogen. Thus, acetylenic dideoxypyrimidine nucleosides emerging from this work constitute an important class of antimycobacterial agents because of potent in vitro activity, low cytotoxicity, and no apparent cross-resistance with a standard TB drug. Studies on lipophilic vs hydrophilic properties of the identified compounds may further enhance their biological activities. These new agents merit further studies to explore them as potential chemotherapeutic agents for tuberculosis and for lead optimization for further design of novel antituberculosis agents. In vivo antimycobacterial activity evaluation of these new agents is underway in our laboratories to explore their clinical potential.

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 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 as an internal standard. The assignment of all exchangeable protons (OH, NH) was confirmed by the addition of the D_2O. 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). 2′,3′-Dideoxycytidine (**9**), 2′,3′-dideoxy-5-methylcytidine (**12**), and (−)-3′-thiacytidine (**13**) were purchased from Sigma. 2′,3′-Dideoxyuridine (**1**) and 3′-fluoro-2′,3′-dideoxyuridine (**20**) were synthesized according to literature procedures.[16,31]

5-Iodo-2′,3′-dideoxyuridine (2). Iodine monochloride (753 mg, 4.65 mmol) was added to a suspension of sodium azide (585 mg, 9.0 mmol) in acetonitrile (25 mL) at ice-bath temperature with stirring. This mixture was stirred for another 5 min, a solution of 2′,3′-dideoxyuridine (**1**) (490 mg, 2.32 mmol) in acetonitrile (60 mL) was added, and the mixture was warmed at 25 °C and stirred for 24 h. The progress of the reaction was monitored by TLC [$CHCl_3$/MeOH (9:1, v/v)]. Removal of the solvent in vacuo (bath temperature of 50 °C) and purification of the residue obtained by elution from silica gel column chromatography using $CHCl_3$/MeOH (92:8, v/v) as the eluent afforded **2** (728 mg, 87%) as a syrup. ^{1}H NMR (DMSO-d_6) δ: 1.85 (m, 2H, H-3′), 2.05 and 2.28 (2m, 2H, H-2′), 3.55 and 3.79 (2m, 2H, H-5′), 4.07 (m, 1H, H-4′), 5.25 (m, 1H, 5′-OH), 5.90 (m, 1H, H-1′), 8.60 (s, 1H, H-6), 11.63 (s, 1H, NH). Anal. ($C_9H_{11}IN_2O_4$) C, H, N.

Preparation of 5-Acetylenic-2′,3′-dideoxynucleosides. A full procedure is provided for 5-pentynyl-2′,3′-dideoxyuridine (**3**). For other analogues, only brief spectroscopic data are presented.

5-Pentynyl-2′,3′-dideoxyuridine (3). Tetrakis(triphenylphosphine)palladium(0) (69 mg, 0.059 mmol), copper(I) iodide (23 mg, 0.118 mmol), diisopropylethylamine (0.20 mL, 1.18 mmol), and 1-pentyne (0.17 mL, 1.77 mmol) were added to a solution of 5-iodo-2′,3′-dideoxyuridine **2** (200 mg, 0.59 mmol) in dry dimethylformamide (30 mL). The reaction mixture was stirred at room temperature overnight under a nitrogen atmosphere (the progress of the reaction was monitored by TLC in MeOH/$CHCl_3$ (1:9, v/v). After the mixture was stirred overnight, 20 drops of 5% disodium salt of EDTA/H_2O were added to the reaction mixture and then concentrated in vacuo. The resulting residue was purified on a silica gel column using $CHCl_3$/MeOH (96:4, v/v) to yield **3** (120 mg, 73%) as a syrup. ^{1}H NMR (DMSO-d_6) δ: 0.96 (t, J = 7.3 Hz, 3H, CH_3), 1.52 (m, 2H, β-CH_2), 1.84 (m, 2H, H-3′), 1.98−2.30 (m, 2H, H-2′), 2.32 (t, J = 7.0 Hz, 2H, α-CH_2), 3.53 and 3.70 (2m, 2H, H-5′), 4.05 (m, 1H, H-4′), 5.13 (t, J = 4.88 Hz, 1H, 5′-OH), 5.89 (dd, J = 3.05, 6.71 Hz, 1H, H-1′), 8.28 (s, 1H, H-6), 11.50 (s, 1H, NH). ^{13}C NMR (CD_3OD) δ: 13.86 (CH_3), 22.21, 23.15, 25.53, (2 × CH_2 and C-2′), 33.81 (C-3′), 63.28 (C-5′), 72.77 (C-β), 83.69 (C-4′), 87.99 (C-1′), 94.71 (C-α), 100.49 (C-5), 144.29 (C-6), 151.19 (C-2), 164.77 (C-4). Anal. ($C_{14}H_{18}N_2O_4$) C, H, N.

5-Decynyl-2′,3′-dideoxyuridine (4). This was obtained as a syrup in 88% yield. ^{1}H NMR (DMSO-d_6) δ: 0.85 (t, J = 7.0 Hz, 3H, CH_3), 1.32 (m, 10H, 5 × CH_2), 1.45 (m, 2H, β-CH_2), 1.88 (m, 2H, H-3′), 1.98−2.30 (m, 2H, H-2′), 2.33 (t, J = 7.0 Hz, 2H, α-CH_2), 3.52 and 3.72 (2m, 2H, H-5′), 4.04 (m, 1H, H-4′), 5.14 (t, J = 4.9 Hz, 1H, 5′-OH), 5.90 (dd, J = 3.05, 6.41 Hz, 1H, H-1′), 8.26 (s, 1H, H-6), 11.52 (s, 1H, NH). ^{13}C NMR (CD_3OD) δ: 14.48 (CH_3), 20.24, 23.72, 25.60, 30.04, 30.11, 30.27, 30.34, 33.02 (7 × CH_2 and C-2′), 33.80 (C-3′), 63.35 (C-5′), 72.64 (C-β), 83.69 (C-4′), 88.00 (C-1′), 94.94 (C-α), 100.55 (C-5), 144.24 (C-6), 151.19 (C-2), 164.79 (C-4). Anal. ($C_{19}H_{28}N_2O_4$) C, H, N.

5-Dodecynyl-2′,3′-dideoxyuridine (5). This compound was obtained as a syrup in 72% yield. ^{1}H NMR (DMSO-d_6) δ: 0.85 (t, J = 7.0 Hz, 3H, CH_3), 1.33 (m, 14H, 7 × CH_2), 1.46 (m, 2H, β-CH_2), 1.85 (m, 2H, H-3′), 2.02 and 2.25 (2m, 2H, H-2′), 2.33 (t, J = 7.0 Hz, 2H, α-CH_2), 3.53 and 3.72 (2m, 2H, H-5′), 4.03 (m, 1H, H-4′), 5.12 (t, 1H, 5′-OH), 5.89 (dd, J = 3.05, 6.41 Hz, 1H, H-1′), 8.25 (s, 1H, H-6), 11.50 (s, 1H, NH). Anal. ($C_{21}H_{32}N_2O_4$) C, H, N.

5-Tridecynyl-2′,3′-dideoxyuridine (6). Compound **7** was obtained as a syrup in 64% yield. ^{1}H NMR (DMSO-d_6) δ: 0.84 (t, 3H, CH_3), 1.35 (m, 16H, 8 × CH_2), 1.47 (m, 2H, β-CH_2), 1.87 (m, 2H, H-3′), 2.04 and 2.22 (2m, 2H, H-2′), 2.35 (t, J = 7.0 Hz, 2H, α-CH_2), 3.55 and 3.72 (2m, 2H, H-5′), 4.04 (m, 1H, H-4′), 5.12 (t, 1H, 5′-OH), 5.90 (dd, J = 3.05, 6.40 Hz, 1H, H-1′), 8.25 (s, 1H, H-6), 11.50 (s, 1H, NH). Anal. ($C_{22}H_{34}N_2O_4$) C, H, N.

5-Tetradecynyl-2′,3′-dideoxyuridine (7). This was obtained as a syrup in 70% yield. ^{1}H NMR (DMSO-d_6) δ: 0.85 (t, 3H, CH_3), 1.32 (m, 18H, 9 × CH_2), 1.46 (m, 2H, β-CH_2), 1.85 (m, 2H, H-3′), 2.02 and 2.25 (2m, 2H, H-2′), 2.33 (t, J = 7.0 Hz, 2H, α-CH_2), 3.52 and 3.73 (2m, 2H, H-5′), 4.03 (m, 1H, H-4′), 5.12 (t, J = 4.88 Hz, 1H, 5′-OH), 5.89 (dd, J = 3.05, 6.41 Hz, 1H, H-1′), 8.25 (s, 1H, H-6), 11.50 (s, 1H, NH). Anal. ($C_{23}H_{36}N_2O_4$) C, H, N.

5-(2-Phenylethynyl)-2′,3′-dideoxyuridine (8). Compound **8** was obtained as a syrup in 81% yield. ^{1}H NMR (DMSO-d_6) δ: 1.89 (m, 2H, H-3′), 2.03 and 2.32 (2m, 2H, H-2′), 3.57 and 3.78 (2m, 2H, H-5′), 4.06 (m, 1H, H-4′), 5.27 (t, 1H, 5′-OH), 5.91 (m, 1H, H-1′), 7.38−7.46 (m, 5H, aromatic), 8.60 (s, 1H, H-6), 11.66 (s, 1H, NH). ^{13}C NMR (CD_3OD) δ: 25.32 (C-2′), 34.0 (C-3′), 63.10 (C-5′), 81.94 (C-β), 83.91 (C-4′), 88.23 (C-1′), 93.62 (C-α), 99.69 (C-5), 124.33, 129.99, 132.35, 144.62 (C-phenyl), 145.25 (C-6), 151.10 (C-2), 164.36 (C-4). Anal. ($C_{17}H_{16}N_2O_4$) C, H, N.

5-Bromo-2′,3′-dideoxyuridine (14). Bromination of the 5-position of 2′,3′-dideoxyuridine (**1**) was performed with 1.1 equiv of *N*-bromosuccinimide in DMF for 6 h at 25 °C to provide **14** in 76% yield. The ^{1}H NMR of this compound was similar to that previously reported.[16]

5′-*O*-Acetyl-2′,3′-dideoxyuridine (15). To an ice-cooled (0 °C) solution of 2′,3′-dideoxyuridine (**1**) (1 g, 4.71 mmol) in anhydrous pyridine (50 mL) was added acetic anhydride (0.54 mL, 5.68 mmol) dropwise. The reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 24 h. Pyridine was removed in vacuo followed by coevaporation with ethanol (2 × 25 mL). The resulting residue was purified on a silica gel column using $CHCl_3$/MeOH (98:2, v/v) to yield **15** (1.17 g, 97.5%) as a syrup. ^{1}H NMR ($CDCl_3$) δ: 1.76−2.14 (br m, 3H, H-2′ and H-3′), 2.12 (s, 3H, −OAc), 2.42−2.53 (m, 1H, H-2′), 4.28−4.35 (m, 3H, H-4′ and H-5′), 5.73 (d, 1H, J = 7.93 Hz, H-5), 6.05 (m, 1H, H-1′), 7.65 (d, 1H, J = 7.93 Hz, H-6), 8.90 (s, 1H, NH). Anal. ($C_{11}H_{14}N_2O_5$) C, H, N.

5′-*O*-Acetyl-5-chloro-2′,3′-dideoxyuridine (16). 5′-*O*-Acetyl-2′,3′-dideoxyuridine **15** (1.15 g, 4.52 mmol) and *N*-chlorosuccinimide (0.79 g, 5.92 mmol) were dissolved in anhydrous pyridine (50 mL). The reaction mixture was heated for 45 min at 90 °C. Pyridine was removed in vacuo followed by coevaporation with ethanol (2 × 25 mL). The resulting residue was purified on a silica gel column using $CHCl_3$/MeOH (98:2, v/v) to yield **16** (1.23 g, 93.8%) as a white solid. Mp 175−177 °C, dec. ^{1}H NMR ($CDCl_3$) δ: 1.79−2.18 (br m, 3H, H-2′ and H-3′), 2.20 (s, 3H, −OAc), 2.45−2.55 (m, 1H, H-2′), 4.34−4.44 (m, 3H, H-4′ and H-5′), 6.05 (m, 1H, H-1′), 8.00 (s, 1H, H-6), 8.85 (s, 1H, NH). Anal. ($C_{11}H_{13}ClN_2O_5$) C, H, N.

5-Chloro-2′,3′-dideoxyuridine (17). This compound was synthesized by deacetylation of **16** (620 mg, 2.15 mmol) using a saturated solution of ammonia in methanol (20 mL) in 86.8% yield. ^{1}H NMR of **17** was similar to that previously reported.[17]

5′-*O*-Acetyl-5-chloro-4-thio-2′,3′-dideoxyuridine (18). To a dried mixture of 5-chloro-5′-*O*-acetyl-2′,3′-dideoxyuridine **16** (600 mg, 2.08 mmol) and Lawesson's reagent (1.17 g, 2.88 mmol) was added dry 1,4-dioxane (30 mL). The reaction mixture was refluxed for 9 h and after cooling concentrated in vacuo. The crude product thus obtained was purified on a silica gel column using $CHCl_3$/MeOH (99.5:0.5, v/v) to yield **18** (530 mg, 83.7%) as a yellow solid. Mp 165−167 °C, dec. ^{1}H NMR ($CDCl_3$) δ: 1.81−2.23 (m, 3H, H-2′ and H-3′), 2.20 (s, 3H, −OAc), 2.44−2.57 (m, 1H, H-2′), 4.34−4.46 (m, 3H, H-4′ and H-5′), 6.00 (m, 1H, H-1′), 8.06 (s, 1H, H-6), 9.90 (s, 1H, NH). Anal. ($C_{11}H_{13}ClN_2O_4S$) C, H, N, S.

5-Chloro-4-thio-2′,3′-dideoxyuridine (19). Compound **18** (480 mg, 1.57 mmol) was treated with a saturated solution of ammonia in methanol at 0 °C for 6 h. The reaction mixture was concentrated in vacuo. The crude product thus obtained was purified on a silica gel column using $CHCl_3$/MeOH (98:2, v/v) to yield **19** (334 mg, 80.7%) as a yellow solid. Mp 66−68 °C. ^{1}H NMR (DMSO-d_6) δ: 1.78−1.89 (m, 2H, H-3′), 2.13−2.49 (m, 2H, H-2′), 3.54 and 3.82 (2m, 2H, H-5′), 4.09 (m, 1H, H-4′), 5.32 (t, 1H, 5′-OH), 5.84 (m, 1H, H-1′), 8.68 (s, 1H, H-6), 13.06 (s, 1H, NH). Anal. ($C_9H_{11}ClN_2O_3S$) C, H, N, S.

Preparation of 5-Acetylenic-3′-fluoro-2′,3′-dideoxynucleosides. A full procedure is provided for 5-heptynyl-3′-fluoro-2′,3′-dideoxyuridine (**22**). For other analogues, only brief spectroscopic data are presented.

5-Heptynyl-3′-fluoro-2′,3′-dideoxyuridine (22). Tetrakis(triphenylphosphine)palladium(0) (49 mg, 0.042 mmol), copper(I) iodide (16 mg, 0.084 mmol), diisopropylethylamine (0.15 mL, 0.84 mmol), and 1-heptyne (0.17 mL, 1.26 mmol) were added to a solution of 5-iodo-3′-fluoro-2′,3′-dideoxyuridine (**21**) (150 mg, 0.42 mmol) in anhydrous dimethylformamide (25 mL). The orange reaction mixture was stirred at room temperature for 20 h in a nitrogen atmosphere (the progress of the reaction was monitored by TLC in MeOH/$CHCl_3$ (3:97, v/v)). After 20 h of stirring, 15 drops of 5% of disodium salt of EDTA/H_2O were added to the reaction mixture and the contents were concentrated in vacuo. The resulting residue was purified on a silica gel column using $CHCl_3$/MeOH (99:1, v/v) as eluent to yield **22** (90 mg, 66%) as a syrup. ^{1}H NMR (DMSO-d_6) δ: 0.87 (t, J = 7.32 Hz, 3H, CH_3), 1.32 (m, 4H, 2 × CH_2), 1.51 (m, 2H, β-CH_2), 2.35 (t, J = 7.0 Hz, 2H, α-CH_2), 2.23 and 2.46 (2m, 2H, H-2′), 3.62 (m, 2H, H-5′), 4.18 (dt, $J_{4',F}$ = 27.5 Hz, 1H, H-4′), 5.24 (t, 1H, 5′-OH), 5.29 (dm, $J_{3',F}$ = 53.1 Hz, 1H, H-3′), 6.16 (dd, $J_{1',2'}$ = 5.50 Hz, $J_{1',2''}$ = 8.85 Hz, 1H, H-1′), 8.07 (s, 1H, H-6), 11.63 (s, 1H, NH). ^{13}C NMR (CD_3OD) δ: 14.33 (CH_3), 20.17, 23.28, 29.44, 32.24 (4 × CH_2), 39.60 (C-2′, $J_{2',F}$ = 20.87 Hz), 62.59 (C-5′, $J_{5',F}$ = 11.0 Hz), 72.39 (C-β), 86.82 (C-1′), 87.18 (C-4′, $J_{4',F}$ = 24.2 Hz), 95.34 (C-α), 95.91 (C-3′, $J_{3',F}$ = 175.80 Hz), 101.67 (C-5), 143.76 (C-6), 151.20 (C-2), 164.47 (C-4). Anal. ($C_{16}H_{21}FN_2O_4$) C, H, N.

5-Decynyl-3′-fluoro-2′,3′-dideoxyuridine (23). This was obtained as a syrup in 78% yield. ^{1}H NMR (DMSO-d_6) δ: 0.85 (t, J = 7.0 Hz, 3H, CH_3), 1.32 (m, 10H, 5 × CH_2), 1.48 (m, 2H, β-CH_2), 2.35 (t, J = 7.0 Hz, 2H, α-CH_2), 2.23 and 2.47 (2m, 2H, H-2′), 3.61 (m, 2H, H-5′), 4.17 (dt, $J_{4',F}$ = 27.5 Hz, 1H, H-4′), 5.24 (m, 1H, 5′-OH), 5.28 (dm, $J_{3',F}$ = 53.70 Hz, 1H, H-3′), 6.16 (dd, $J_{1',2'}$ = 5.50 Hz, $J_{1',2''}$ = 8.85 Hz, 1H, H-1′), 8.07 (s, 1H, H-6), 11.63 (s, 1H, NH). ^{13}C NMR (CD_3OD) δ: 14.45 (CH_3), 20.21, 23.72, 29.75, 30.02, 30.26, 30.34, 33.02 (7 × CH_2), 39.60 (C-2′, $J_{2',F}$ = 20.9 Hz), 62.59 (C-5′, $J_{5',F}$ = 11.0 Hz), 72.41 (C-β), 86.81 (C-1′), 87.18 (C-4′, $J_{4',F}$ = 24.2 Hz), 95.35 (C-α), 95.91 (C-3′, $J_{3',F}$ = 175.80 Hz), 101.67 (C-5), 143.76 (C-6), 151.19 (C-2), 164.48 (C-4). Anal. ($C_{19}H_{27}FN_2O_4$) C, H, N.

5-Dodecynyl-3′-fluoro-2′,3′-dideoxyuridine (24). Compound **24** was obtained as a syrup in 80% yield. ^{1}H NMR (DMSO-d_6) δ: 0.86 (t, J = 7.0 Hz, 3H, CH_3), 1.30 (m, 14H, 7 × CH_2), 1.48 (m, 2H, β-CH_2), 2.35 (t, J = 7.0 Hz, 2H, α-CH_2), 2.25 and 2.47 (2m, 2H, H-2′), 3.62 (m, 2H, H-5′), 4.18 (dt, $J_{4',F}$ = 27.5 Hz, 1H, H-4′), 5.25 (m, 1H, 5′-OH), 5.28 (dm, $J_{3',F}$ = 53.70 Hz, 1H, H-3′), 6.18 (dd, $J_{1',2'}$ = 5.50 Hz, $J_{1',2''}$ = 8.85 Hz, 1H, H-1′), 8.06 (s, 1H, H-6), 11.64 (s, 1H, NH). Anal. ($C_{21}H_{31}FN_2O_4$) C, H, N.

5-Tridecynyl-3′-fluoro-2′,3′-dideoxyuridine (25). This was obtained as a syrup in 76% yield. ^{1}H NMR (DMSO-d_6) δ: 0.85 (t, J = 7.0 Hz, 3H, CH_3), 1.31 (m, 16H, 8 × CH_2), 1.46 (m, 2H, β-CH_2), 2.34 (t, J = 7.33 Hz, 2H, α-CH_2), 2.25 and 2.47 (2m, 2H, H-2′), 3.63 (m, 2H, H-5′), 4.17 (dt, $J_{4',F}$ = 27.16 Hz, 1H, H-4′), 5.25 (t, 1H, 5′-OH), 5.27 (dm, $J_{3',F}$ = 53.4 Hz, 1H, H-3′), 6.17 (m, 1H, H-1′), 8.08 (s, 1H, H-6), 11.64 (s, 1H, NH). ^{13}C NMR (CD_3OD) δ: 14.46 (CH_3), 20.20, 23.75, 29.72, 30.01, 30.27, 30.49, 30.65, 30.75, 33.08 (10 × CH_2), 39.61 (C-2′, $J_{2',F}$ = 19.8 Hz), 62.60 (C-5′, $J_{5',F}$ = 11.0 Hz), 72.41 (C-β), 86.81 (C-1′), 87.18 (C-4′, $J_{4',F}$ = 24.17 Hz), 95.35 (C-α), 95.91 (C-3′, $J_{3',F}$ = 174.70 Hz), 101.67 (C-5), 143.76 (C-6), 151.20 (C-2), 164.47 (C-4). Anal. ($C_{22}H_{33}FN_2O_4$) C, H, N.

5-Tetradecynyl-3′-fluoro-2′,3′-dideoxyuridine (26). Compound **26** was obtained as a syrup in 37% yield. ^{1}H NMR (DMSO-d_6) δ: 0.85 (t, J = 7.0 Hz, 3H, CH_3), 1.33 (m, 18H, 9 × CH_2), 1.47 (m, 2H, β-CH_2), 2.35 (t, J = 7.33 Hz, 2H, α-CH_2), 2.23 and 2.43 (2m, 2H, H-2′), 3.62 (m, 2H, H-5′), 4.17 (dt, $J_{4',F}$ = 26.9 Hz, 1H, H-4′), 5.25 (m, 1H, 5′-OH), 5.27 (dm, $J_{3',F}$ = 54.02 Hz, 1H, H-3′), 6.15 (m, 1H, H-1′), 8.06 (s, 1H, H-6), 11.63 (s, 1H, NH). ^{13}C NMR (CD_3OD) δ: 14.46 (CH_3), 20.21, 23.75, 29.73, 30.01, 30.27, 30.49, 30.65, 30.78, 33.08 (11 × CH_2), 39.61 (C-2′, $J_{2',F}$ = 20.9 Hz), 62.60 (C-5′, $J_{5',F}$ = 11.0 Hz), 72.42 (C-β), 86.82 (C-1′), 87.18 (C-4′, $J_{4',F}$ = 24.17 Hz), 95.35 (C-α), 95.92 (C-3′, $J_{3',F}$ = 175.80 Hz), 101.69 (C-5), 143.76 (C-6), 151.19 (C-2), 164.47 (C-4). Anal. ($C_{23}H_{35}FN_2O_4$) C, H, N.

5-(4-*n*-Propylphenylethynyl)-3′-fluoro-2′,3′-dideoxyuridine (27). This was obtained as a syrup in 32% yield. ^{1}H NMR (DMSO-d_6) δ: 0.88 (t, J = 7.33 Hz, 3H, CH_3), 1.59 (m, 2H, CH_2), 2.39 and 2.47 (2m, 2H, H-2′), 2.57 (t, J = 7.0 Hz, 2H, CH_2), 3.64 (m, 2H, H-5′), 4.19 (dt, $J_{4',F}$ = 26.85 Hz, 1H, H-4′), 5.32 (dm, 1H, H-3′), 5.33 (t, 1H, 5′-OH), 6.21 (m, 1H, H-1′), 7.39−7.21 (m, 4H, Ar−H), 8.30 (s, 1H, H-6), 11.72 (s, 1H, NH). ^{13}C NMR (CD_3OD) δ: 14.04 (CH_3), 25.51, 38.91 (2 × CH_2), 39.76 (C-2′, $J_{2',F}$ = 22.80 Hz), 62.59 (C-5′, $J_{5',F}$ = 11.0 Hz), 80.88 (C-β), 86.97 (C-1′), 87.26 (C-4′, $J_{4',F}$ = 24.17 Hz), 94.19 (C-α), 95.91 (C-3′, $J_{3',F}$ = 175.80 Hz), 101.16 (C-5), 121.41, 129.54, and 132.38 (C-phenyl), 144.37 (C-6), 144.65 (C-phenyl), 151.11 (C-2), 164.09 (C-4). Anal. ($C_{20}H_{21}FN_2O_4$) C, H, N.

In Vitro Antimycobacterial Activity Assay (*M. bovis*, *M. tuberculosis*, and *M. avium*). *M. bovis* (BCG), *M. tuberculosis* (H37Ra), and *M. avium* (ATCC 25291) were obtained from the American Type Culture Collection, Rockville, MD. The antimycobacterial activity was determined using the microplate Alamar blue assay (MABA).[27] Test compounds were dissolved in DMSO at 100× the highest final concentration used, 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, 10, and 1 μg/mL in triplicate. The experiments were repeated two times, and the mean percent inhibition is reported in the table. The most promising compounds were further repeated at 2-fold dilutions to obtain MIC_{90} values. The standard deviations were within 10% of the mean. Frozen mycobacterial inocula were diluted in medium 7H9GC and added to each well at a final concentration of 2.5×10^5 CFU/mL. Control wells consisted of eight with bacteria alone (B) and eight with media 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 a 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. Percent inhibition was calculated as

$$\frac{(\text{test well}) - (\text{M background})}{(\text{B well}) - (\text{M background})} \times 100$$

The lowest drug concentration effecting an inhibition of ∼90% was considered as the MIC_{90}. Similar methodology was used for all (three) mycobacteria strains. Rifampicin and clarithromycin were used as positive controls. As negative controls, DMSO was added to the B well at a concentration similar to that of test 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 60 000 and 100 000.

The antimycobacterial activity of compounds **4**−**7** and **23**−**26** was also determined against *M. bovis* in the presence of various detergents using the MABA assay. For these experiments, titrating amounts of detergents, i.e., Tween-20 and Triton X-100 (Fisher Scientific, critical micellar concentrations (cmc) of 0.05 and 0.3 mM, respectively), at 1.0, 0.1, 0.01, and 0.001 μg/mL cmc (final concentration) were preincubated with test compounds at 100, 50, 10, and 1 μg/mL (final concentration) for 15 min before addition to the *M. bovis* wells in triplicate. Control wells included *M. bovis* cultured with various amounts of both detergents without the test compounds. In the initial experiment, we determined the effect of

various concentrations of these detergents on *M. bovis* growth. Tween-20 had no effect on *M. bovis* growth at all four concentrations, whereas Triton X-100 was toxic at 1 μg/mL cmc. Therefore, our experimental (test) compounds were tested in the presence of nontoxic doses of detergents.

Antimycobacterial Activity against a Drug-Resistant Strain of *M. tuberculosis*. The activities of compounds **5** and **26** were determined against rifampicin-resistant *M. tuberculosis* H37 Rv (ATCC 35838, resistant to rifampicin at 2 μg/mL) using a radiometric-BACTEC assay.[27] 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. The 2-fold diluted test compounds were delivered to the inoculum-containing BACTEC vials. Negative control vials consisted of media with bacteria inoculum alone, media with bacteria inoculum at 1:100, and medium alone. Isoniazid and rifampicin were used at an MIC_{90} concentration as reference drugs. All 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 ($\triangle$GI) was recorded for each compound. Percent inhibition was defined as

$$\frac{\text{GI of test sample}}{\text{GI of control}} \times 100$$

For the no-drug control, the $\triangle$GI 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.

In Vitro Antibacterial Activity Assay. A total of 12 bacterial organisms were used for the determination of the in vitro antibacterial activity of the studied compounds. Bacterial pathogens used in this work were mostly ATCC (American Type Culture Collection) strains *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermis* (ATCC 14990), *Enterococcus faecalis* (ATCC 29212), *Bacillus subtilis* (ATCC 6633), *Streptococcus pneumoniae* (ATCC 49619), *Streptococcus pyogenes* (ATCC 19615), *Salmonella typhimurium* (clinical isolate), *Escherichia coli* (ATCC 25922), *Proteus vulgaris* (ATCC 49132), *Pseudomonas aeruginosa* (ATCC 13048), *Lysteria monocytogenes* (ATCC 15313), and *Enterobacter aerogenes*. The in vitro antibacterial activity was studied by determining their minimum inhibitory concentrations (MICs) by means of the broth microdilution method. Briefly, exponentially growing bacteria were diluted in a liquid sterile medium to obtain a final inoculum of 1×10^4 CFU/mL and subsequently cultured with varying dilutions of compounds for 16−20 h. The MICs were defined as the lowest concentration at which bacterial growth was no longer evident.

Cell Cytotoxicity Assay. Cell viability was measured using the cell proliferation kit 1 (MTT, Boehringer Mannheim), as per manufacturer's instructions. Briefly, a 96-well plate was seeded with Vero cells or HFF cells at a density of 2.5×10^5 cells per well. Cells were allowed to attach for 6−8 h, and the medium was replaced with medium containing drugs at concentrations of 100, 50, 25, 12.5, 6.3, and 1.5 μg/mL. DMSO was also included as control. Plates were incubated for 3 days at 37 °C. The color reaction involved adding 10 μL of MTT reagent per well, incubating 4 h at 37 °C, and then adding 100 μL of solubilization reagent. Plates were read on an ELISA plate reader (absorption at 560−650 nm) following an overnight incubation at 37 °C.

Acknowledgment. We are grateful to the Canadian Institutes of Health Research (CIHR) for an operating grant (Grant MOP-49415) for the financial support of this research. B.A. is the recipient of Alberta Heritage Foundation for Medical Research (AHFMR) Senior Scholar Award.

Supporting Information Available: Elemental analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Shah, N. S.; Wright, A.; Bai, G. H.; Barrera, L.; Boulahbal, F.; Martín-Casabona, N.; Drobniewski, F.; Gilpin, C.; Havelková, M.; Lepe, R.; Lumb, R.; Metchock, B.; Portaels, F.; Filomena, M.; Rüsch-Gerdes, S.; Rodrigues, S.; et al. Worldwide Emergence of Extensively Drug-Resistant Tuberculosis. *Emerging Infect. Dis.* **2007**, *13*, 380−387. (b) Rouhi, A. M. Tuberculosis: A Tough Adversary. *Chem. Eng. News* **1999**, May 17, 52−69. (c) Kling, J. Enlisting a Genome to Fight MDR-Tuberculosis. *Modern Drug Discovery* **1999**, Jan/Feb, 32−45. (d) Campos-Outcalt, D. Tuberculosis: Old Problem, New Concerns. *J. Family Pract.* **2003**, *52*, 792−798. (e) Tomioka, H. Current Status of Some Antituberculosis Drugs and the Development of New Antituberculous Agents. *Curr. Pharm. Des.* **2006**, *12*, 4047−4070.

(2) (a) Valadas, E.; Antunes, F. Tuberculosis a Re-emergent Disease. *Eur. J. Radiol.* **2005**, *55*, 154−157. (b) *HIV, Tuberculosis and Malaria*; WHO Fact Sheet No. 104; World Health Organization Information Resource Center: Geneva, Switzerland, 2004. (c) Grassi, C. Anti-Infectives: New Drugs for Tuberculosis. *Expert Opin. Invest. Drugs* **1997**, *6*, 1211−1226.

(3) (a) Long, R.; Houston, S.; Hershfield, E. Canadian Tuberculosis Committee of the Centre for Infectious Disease Prevention and Control, Population and Public Health Branch, Health Canada. Recommendations for Screening and Prevention of Tuberculosis in Patients with HIV and for Screening for HIV in Patients with Tuberculosis and their Contacts. *Can. Med. Assoc. J.* **2003**, *169*, 789−791. (b) Nguyen, D.; Proulx, J. F.; Westley, J.; Thibert, L.; Dery, S.; Behr, M. A. Tuberculosis in the Inuit Community of Quebec, Canada. *Am. J. Respir. Crit. Care Med.* **2003**, *168*, 1353−1357.

(4) (a) Maher, D.; Raviglione, M. Global Epidemiology of Tuberculosis. *Clin. Chest Med.* **2005**, *26*, 168−182. (b) Pilheu, J. A. Tuberculosis 2000: Problems and Solutions. *Int. J. Tuberculosis Lung Dis.* **1998**, *2*, 696−703.

(5) (a) *Global Tuberculosis Control: Surveillance, Planning, Financing*; WHO Report 2004: World Health Organization: Geneva, Switzerland, 2004. (b) Raviglione, M. C.; Snider, D. E.; Kochi, A. Global Epidemiology of Tuberculosis. Morbidity and Mortality of a Worldwide Epidemic. *JAMA, J. Am. Med. Assoc.* **1995**, *273*, 220−226.

(6) (a) Schlesinger, N.; Lardizabal, A.; Rao, J.; McDonald, R. Tuberculosis of the Spine: Experience in an Inner City Hospital. *J. Clin. Rheumatol.* **2005**, *11*, 17−20. (b) Willcox, P. A. Drug-Resistant Tuberculosis. *Curr. Opin. Pulm. Med.* **2000**, *6*, 198−202. (c) Méndez, A. P.; Raviglione, M. C.; Laszlo, A.; Binkin, N.; Rieder, H. L.; Bustreo, F.; Cohn, D. L.; Lambregts-van Weezenbeek, C. S. B.; Kim, S. J.; Chaulet, P.; Nunn, P. Global Surveillance for Anti-Tuberculosis-Drug Resistance, 1994−1997. World Health Organization−International Union against Tuberculosis and Lung Disease Working Group on Anti-Tuberculosis Drug Resistance Surveillance. *N. Engl. J. Med.* **1998**, *338*, 1641−1649. (d) Inderlied, C. B.; Kemper, C. A.; Bermudez, L. E. The *Mycobacterium avium* Complex. *Clin. Microbiol. Rev.* **1993**, *6*, 266−310. (e) Falkinham, J. O., III. Epidemiology of Infection by Nontuberculous Mycobacteria. *Clin. Microbiol. Rev.* **1996**, *9*, 177−215.

(7) Peloquin, C. A.; Berning, S. E. Infections Caused by *Mycobacterium tuberculosis*. *Ann. Pharmacother.* **1994**, *28*, 72−84.

(8) (a) Guerrin-Tran, E.; Thiolet, J. M.; Rousseau, C.; Henry, S.; Poirier, C.; Che, D.; Vinas, J. M.; Jarlier, V.; Robert, J. An Evaluation of Data Quality in a Network for Surveillance of *Mycobacterium tuberculosis* Resistance to Antituberculosis Drugs in Ile-de-France Region. *Eur. J. Epidemiol.* **2006**, *21*, 783−785. (b) Murray, J. F. Tuberculosis and HIV Iinfection: A Global Perspective. *Respiration* **1998**, *65*, 335−342. (c) Gordin, F. M.; Nelson, E. T.; Matts, J. P.; Cohn, D. L. J.; Benator, E. D.; Besch, C. L.; Crane, L. R.; Sampson, J. H.; Bragg, P. S.; El-Sadr, W. The Impact of HIV Infection on Drug-Resistant Tuberculosis. *Am. J. Respir. Crit. Care Med.* **1996**, *154*, 1478−1483. (d) Moss, A. R.; Alland, D.; Telzak, E.; Hewlett, D., Jr.; Sharp, V.; Chiliade, P.; LaBombardi, V.; Kabus, D.; Hanna, B.; Palumbo, L.; Brudney, K.; Weltman, A.; Stoeckle, K.; Chirgwin, K.; Simberkoff, M.; Moghazeh, S.; Eisner, W.; Lutfey, M.; Kreiswirth, B. A City-Wide Outbreak of a Multiple-Drug-Resistant Strain of *Mycobacterium tuberculosis* in New York. *Int. J. Tuberculosis Lung. Dis.* **1997**, *1*, 115−121. (e) National Survey of Tuberculosis in England and Wales. *Communicable Dis. Rep. Wkly.* **1998**, *8*, 209−212.

(9) (a) Cohen, J. Extensively Drug-Resistant TB Gets Foothold in South Africa. *Science* **2006**, *313*, 1554. (b) Wright, A. Emergence of *Mycobacterium tuberculosis* with Extensive Resistance to Second-Line Drugs Worldwide 2000−2004. *Morbidity Mortality Wkly. Rep.* **2006**, *55*, 301−305. (c) XDR-TB−A Global Threat. Editorial. *Lancet* **2006**, *368*, 964.

(10) (a) Coker, R. J. Multidrug-Resistant Tuberculosis: Public Health Challenges. *Trop. Med. Int. Health* **2004**, *9*, 25−40. (b) Pozniak, A. Mycobacterial Diseases and HIV. *J. HIV Ther.* **2002**, *7*, 13−16.

(11) Colditz, G. A.; Brewer, T. F.; Berkey, C. S.; Wilson, M. E.; Burdick, E.; Fineberg, H. V.; Mosteller, F. Efficacy of BCG Vaccine in the Prevention of Tuberculosis. Meta-Analysis of the Published Literature. *JAMA, J. Am Med. Assoc.* **1994**, *271*, 698−702.

(12) (a) Koehler, C. S. W. Consumption the Great Killer. *Modern Drug Discovery* **2002**, 47−49. (b) Guerrero, A. Nosocomial Transmission of M. Bovis Resistant to 11 Drugs in People with Advanced HIV-1 Infection. *Lancet* **1997**, *350*, 1738−1742.

(13) Rai, D.; Johar, M.; Manning, T.; Agrawal, B.; Kunimoto, D. Y.; Kumar, R. Design and Studies of Novel 5-Substituted Alkynyl Pyrimidine Nucleosides as Potent Inhibitors of Mycobacteria. *J. Med. Chem.* **2005**, *48*, 7012−7017.

(14) Johar, M.; Manning, T.; Kunimoto, D. Y.; Kumar, R. Synthesis and *in vitro* Anti-Mycobacterial Activity of 5-Substituted Pyrimidine Nucleosides. *Bioorg. Med Chem.* **2005**, *13*, 6663−6671.

(15) Kim, C. H.; Marquez, V. E.; Broder, S.; Mitsuya, H.; Driscoll, J. S. Potential Anti-AIDS Drugs. 2′,3′-Dideoxycytidine Analogues. *J. Med. Chem.* **1987**, *30*, 862−866.

(16) Lin, T. S.; Chen, M. S.; McLaren, C.; Gao, Y. S.; Ghazzouli, I.; Prusoff, W. H. Synthesis and Antiviral Activity of Various 3′-Azido, 3′-Amino, 2′,3′-Unsaturated, and 2′,3′-Dideoxy Analogues of Pyrimidine Deoxyribonucleosides against Retroviruses. *J. Med. Chem.* **1987**, *30*, 440−444.

(17) Van Aerschot, A.; Everaert, D.; Balzarini, J.; Augustyns, K.; Jie, L.; Janssen, G.; Peeters, O.; Blaton, N.; De Ranter, C.; De Clercq, E. Synthesis and anti-HIV Evaluation of 2′,3′-Dideoxyribo-5-chloropyrimidine Analogues: Reduced Toxicity of 5-Chlorinated 2′,3′-Dideoxynucleosides. *J. Med. Chem.* **1990**, *33*, 1833−1839.

(18) Balzarini, J.; Van Aerschot, A.; Pauwels, R.; Baba, M.; Schols, D.; Herdewijn, P.; De Clercq, E. 5-Halogeno-3′-fluoro-2′,3′-dideoxyuridines as Inhibitors of Human Immunodeficiency Virus (HIV): Potent and Selective Anti-HIV Activity of 3′-Fluoro-2′,3′-dideoxy-5-chlorouridine. *Mol. Pharmacol.* **1989**, *35*, 571−577.

(19) Meier, C.; Knispel, T.; De Clercq, E.; Balzarini, J. CycloSal-pronucleotides of 2′,3′-Dideoxyadenosine and 2′,3′-Dideoxy-2′,3′-didehydroadenosine: Synthesis and Antiviral Evaluation of a Highly Efficient Nucleotide Delivery System. *J. Med. Chem.* **1999**, *42*, 1604−1614.

(20) Murakami, K.; Shirasaka, T.; Yoshioka, H.; Kojima, E.; Aoki, S.; Ford, H.; Driscoll, J. S.; Kelley, J. A.; Mitsuya, H. *E. coli* Mediated Biosynthesis and in Vitro Anti-HIV Activity of Lipophilic 6-Halo-2′,3′-dideoxypurine Nucleosides. *J. Med. Chem.* **1991**, *34*, 1606−1612.

(21) Shirasaka, T.; Murakami, K.; Ford, H.; Kelley, J. A.; Yoshioka, H.; Kojima, E.; Aoki, S.; Broder, S.; Mitsuya, H. Lipophilic Halogenated Congeres of 2′,3′-Dideoxypurine Nucleosides Active against Human Immunodeficiency Virus in Vitro. *Proc. Natl. Acad. Sci., U.S.A.* **1990**, *87*, 9426−9430.

(22) Robins, M. J.; Wilson, J. S.; Madej, D.; Low, N. H.; Hansske, F.; Wnuk, S. F. Nucleic Acid Related Compounds. 88. Efficient Conversions of Ribonucleosides into Their 2′,3′-Anhydro,2′(and 3′)-deoxy, 2′,3′-Didehydro-2′,3′-dideoxy, and 2′,3′-Dideoxynucleoside Analogs. *J. Org. Chem.* **1995**, *60*, 7902−7908.

(23) Jacobson, K. A.; Siddiqi, S. M.; Ola, M. E.; Ji, X.; Melman, N.; Bellamkonda, K.; Meshulam, Y.; Stiles, G. L.; Kim, H. O. Structure−Activity Relationships of 9-Alkyladenine and Ribose-Modified Adenosine Derivatives at Rat A_3 Adenosine Receptors. *J. Med. Chem.* **1995**, *38*, 1720−1735.

(24) Krawczyk, S. H.; Renau, T. E.; Reza Nassiri, M.; Westreman, A. C.; Wotring, L. L.; Drach, J. C.; Townsend, L. B. Synthesis and Evaluation of Certain Thiosangivamycin Analogs as Potential Inhibitors of Cell Proliferation and Human Cytomegalovirus. *J. Med. Chem.* **1995**, *38*, 4115−4119.

(25) Kojima, E.; Shirasaka, T.; Machida, M.; Yoshioka, H.; Murakami, K.; Mitsuya, H. Synthesis and Structure−Activity Relationships of 2′,3′-Dideoxypurine Nucleosides as Potential Antiretroviral Agents. *Nucleic Acids Symp. Ser.* **1991**, *25*, 91−92.

(26) Ramasamy, K.; Imamura, M.; Hanna, N. B.; Finch, R. A.; Avery, T. L.; Robins, R. K.; Revankar, G. R. Synthesis and Antitumor Evaluation in Mice of Certain 7-Deazapurine (pyrrolo[2,3-*d*]pyrimidine) and 3-Deazapurine (imidazo[4,5-*c*]pyridine) Nucleosides Structurally Related to Sulfenosine, Sulfinosine, and Sulfonosine. *J. Med. Chem.* **1990**, *33*, 1220−1225.

(27) (a) Franzblau, S. G.; Witzig, R. S.; McLaughlin, J. C.; Torres, P.; Madico, G.; Hernandez, A.; Degnan, M. T.; Cook, M. B.; Quenzer, V. K.; Ferguson, R. M.; Gilman, R. H. Rapid, Low-Technology MIC Determination with Clinical *Mycobacterium tuberculosis* Isolates by Using the Microplate Alamar Blue Assay. *J. Clin. Microbiol.* **1998**, *36*, 362−366. (b) Collins, L.; Franzblau, S. G. Microplate Alamar Blue Assay versus BACTEC 460 System for High-Throughput Screening of Compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. *Antimicrob. Agents. Chemother.* **1997**, *41*, 1004−1009. (c) Bermudez, L. E.; Inderlied, C. B.; Kolonoski, P.; Wu, M.; Aralar, P.; Young, L. S. Telithromycin Is Active against *Mycobacterium avium* in Mice Despite Lacking Significant Activity in Standard in Vitro and Macrophage Assays and Is Associated with Low Frequency of Resistance during Treatment. *Antimicrob. Agents Chemother.* **2001**, *45*, 2210−2214.

(28) Ryan, A. J.; Gray, N. M.; Lowe, P. N.; Chung, C. Effect of Detergent on Promiscuous Inhibitors. *J. Med. Chem.* **2003**, *46*, 3448−3451.

(29) Neugebauer, J. M. Detergents: An Overview. *Methods Enzymol.* **1990**, *182*, 239−153.

(30) Cole, S. T.; Brosch, R.; Parkhill, J.; Garnier, T.; Churcher, C.; Harris, D.; Gordon, S. V.; Eiglmeier, K.; Gas, S.; Barry, C. E., III; Tekala, F.; Badcock, K.; Bashman, D.; Brown, D.; Chillingworth, T. R.; Connor, R.; et al. Deciphering the Biology of *Mycobacterium tuberculosis* from the Complete Genome Sequence. *Nature* **1998**, *393*, 537−544.

(31) Herdewijn, P.; Van Aerschot, A.; Kerremans, L. Syntheis of Nucleosides Fluorinated in the Sugar Moiety: The Application of Diethylaminosulfurtrifluoride to the Synthesis of Fluorinated Nucleosides. *Nucleosides Nucleotides* **1989**, *8*, 65−96.

JM070391T