Design and Studies of Novel 5-Substituted Alkynylpyrimidine Nucleosides as Potent Inhibitors of Mycobacteria

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We herein report a new category of 5-substituted pyrimidine nucleosides as potent inhibitors of mycobacteria. A series of 5-alkynyl derivatives of 2′-deoxyuridine (**1**−**8**), 2′-deoxycytidine (**9**−**14**), uridine (**15**−**17**), and 2′-*O*-methyluridine (**18**, **19**) were synthesized and evaluated for their antimycobacterial activity in vitro. 5-Decynyl, 5-dodecynyl, and 5-tetradecynyl derivatives showed the highest antimycobacterial potency against *M. bovis* and *M. avium*, with the 2′-deoxyribose derivatives being more effective than the ribose analogues. Nucleosides bearing short alkynyl side chains 5-ethynyl, 5-propynyl, 5-pentynyl, and 5-heptynyl were mostly not inhibitory. Incorporation of a phenylethynyl function at the 5-position diminished the antimicrobial effect. Furthermore, related bicyclic analogues (**20**−**24**) were devoid of antimycobacterial activity, indicating that an acyclic side chain at the C-5 position of the pyrimidine ring is essential for potent activity. Compounds **1**−**17** were synthesized by the Pd-catalyzed coupling reactions of respective alkynes with 5-iodo derivatives of 2′-deoxyuridine, 2′-deoxycytidine, and uridine. Intramolecular cyclization of **1** and **3**−**6** in the presence of Cu afforded the corresponding bicyclic compounds **20**−**24**. The investigated nucleosides are recognized here for the first time to be potent inhibitors of mycobacteria. This class of compounds could be of interest for lead optimization as antimycobacterial agents.

Introduction

Tuberculosis (TB) is an ancient enemy of humans. The World Health Organization (WHO) estimates that one-third of the world's population is infected by *Mycobacterium tuberculosis*. TB infects eight million people and causes 2−3 million deaths annually around the world.[1−3] TB was declared a global health emergency by the WHO in 1993. A number of factors have contributed to the sharp increase in TB infections. The biggest factor is that TB and human immunodeficiency virus (HIV) have formed a new and deadly combination. In immunocompromised people, tuberculosis is much more likely to cause infection, reactivate latent TB, and create a greater number of active TB cases, leading to more people who spread the disease. The increased mobility of people from the developing world to developed countries makes a ready pool for TB dissemination. For both TB and HIV, misdiagnosis and noncompliance with treatment regimens further compound the problem. New TB strains are emerging and spreading that are not susceptible to a number of available drugs, i.e., multidrug-resistant TB (MDR-TB).[1,4,5] 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 death.[6] Bacillus Calmette-Guerin (BCG)[7] 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 B.C. Interestingly, *M. bovis* infections are back and causing TB in humans, particularly those who are HIV-positive. In addition, MDR strains of *M. bovis* have been isolated.[8] 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.[8] 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.[3]

Mycobacterium avium complex (MAC) infections, in particular *M. avium* infections, are one of the most serious complications among patients with AIDS.[9,10] MAC infections are disseminated rather restricted to the lungs. Clinical management of MAC infections is very difficult because many of the first-line anti-TB drugs are ineffective against it.[9,10] New macrolides such as clarithromycin and azithromycin are used for the treatment of MAC; however, resistance occurs at such a rate that single drugs are inadequate for therapy.[11,12]

TB is spread through the air when someone with infectious TB disease coughs or sneezes. The control and management of mycobacterial infections are highly important not only for the survival of AIDS patients but also for the people who are or will be infected with multi-drug-resistant bacteria, people who develop resistance after previous drug treatment, immunocompetent or immunocompromised people in proximity to HIV patients, and patients with MAC infection.

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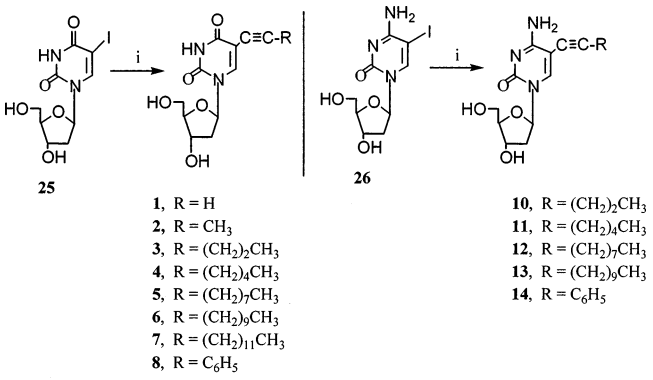
The development of drug-resistant clinical isolates of mycobacteria makes the investigation of new classes of anti-TB agents a high priority, since new agents that work by mechanisms different from those of current drugs and are not cross-resistant with them are likely the best long-term prospect to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

Recently, we reported the in vitro antimycobacterial activity of various 5-substituted pyrimidine nucleosides. 5-(1-Substitiuted alkyl)-2′-deoxyuridines exhibited potent and selective in vitro antimycobacterial activity against *M. avium*. Our studies have shown that novel 5-substituted alkyl side chains at the C-5 position of pyrimidine nucleosides play a crucial role in their antimycobacterial properties.[13] Pyrimidine nucleosides containing a C-5 alkynyl group have been shown to possess significant antiviral and/or anticancer properties.[14] The 5-alkynyl-2′-deoxyuridines with longer chain alkynyl group at the C-5 position expressed appreciable antiviral activity in contrast to the corresponding alkyl derivatives that showed decreasing antiviral activity with increasing C-5 side chain length.[14] In an effort to further explore the structure−activity relationships, in the present investigation we have designed, synthesized, and evaluated a new class of 5-substituted alkynyl derived 2′-deoxyribose and ribose pyrimidine nucleosides (**1**−**19**) for their antimycobacterial activity against *M. bovis* and *M. avium*. It was postulated that unnatural pyrimidine nucleosides can specifically target the mycobacterial enzymes involved in their nucleic acid synthesis by acting as their substrates and/or inhibitors and inhibit the mycobacterial DNA and/or RNA synthesis. We now report that 5-decynyl, 5-dodecynyl, and 5-tridecynyl analogues of 2′-deoxyuridine (**5**−**7**) and 2′-deoxycytidine (**12**,**13**) exhibit marked inhibitory activity against *M. bovis* and *M. avium* in vitro. In contrast, nucleosides possessing shorter alkynyl side chains at the 5-position [5-ethynyl, 5-propynyl, 5-pentynyl, 5-heptynyl, and 5-(2-phenylethynyl)] and related analogues in which the 5-substituent had undergone intramolecular cyclization to provide bicyclic analogues (**20**−**24**) were devoid of antimycobacterial activity.

Chemistry

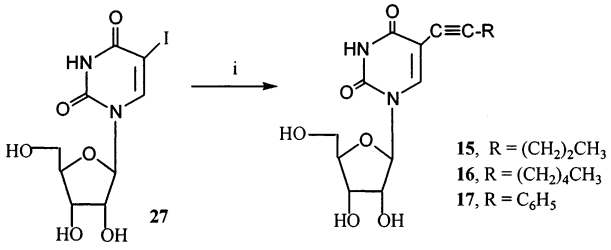
5-Ethynyl-2′-deoxyuridine (**1**) and 5-propynyl-2′-deoxyuridine (**2**) were synthesized by the coupling reaction of 3′,5′-di-*O*-*p*-toluyl-5-iodo-2′-deoxyuridine with trimethylsilylacetylene and propyne, respectively, in triethylamine followed by deprotection with sodium methoxide in methanol at room temperature using the method of Robins et al.[15] 5-Pentynyl- (**3**), 5-heptynyl- (**4**), 5-decynyl- (**5**), 5-dodecynyl- (**6**), 5-tetradecynyl- (**7**), and 5-(2-phenylethynyl)- (**8**) 2′-deoxyuridines were prepared by the treatment of 5-iodo-2′-deoxyuridine (**25**) with respective terminal alkynes as described earlier.[16] The synthetic route for the preparation of 5-alkynyl-2′-deoxycytidines (**10**−**14**) and 5-alkynyluridines (**15**−**17**) was adopted after slight modification over the method used for 2′-deoxyuridine analogues.[15,16] The Pd-catalyzed coupling reaction of terminal alkynes and arylacetylene with 5-iodo-2′-deoxycytidine (**26**) and 5-iododouridine (**27**) proceeded readily to yield hitherto unknown target compounds **10**−**17** in moderate to excellent yields (Scheme 1, 2). Bicyclic compounds **20**−**24** were prepared by the treatment of corresponding 5-alkynyl-2′-deoxyuridines (**1**, **3**−**6**) with copper iodide in methanol and triethylamine using reported procedures[16] (Scheme 3). The ^{1}HNMR spectra of **20**−**24** provided conclusive evidence for the cyclization products because the NH proton disappeared and a new olefinic proton was introduced.

Scheme 1[a]



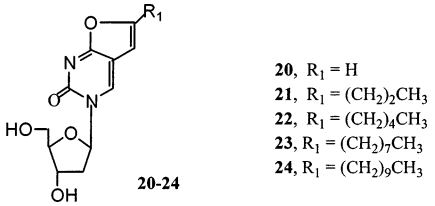
[a] Reagents: (i) H−C≡C−R, $Pd(PPh_3)_4$, CuI, $(i\text{-}Pr)_2EtN$, DMF, room temp.

Scheme 2[a]



[a] Reagents: (i) H−C≡C−R, $Pd(PPh_3)_4$, CuI, $(i\text{-}Pr)_2EtN$, DMF, room temp.

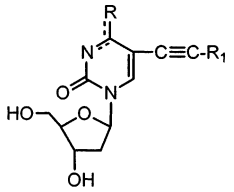
Scheme 3



Results and Discussion

5-Alkynylpyrimidine nucleosides (**1**−**19**) and the bicyclic compounds **20**−**24** were evaluated in culture to determine their antimicrobial effect against the multiplication of two mycobacteria (*M. bovis*, *M. avium*) using the microplate alamar blue assay (MABA)[17] at 1−100 μg/mL concentrations. Rifampicin and clarithromycin were used as reference standards. Antimycobacterial activity for this new class of compounds **1**−**24** is summarized in Table 1. Among the different series of 5-alkynyl substituted 2′-deoxyuridines (**1**−**8**), 2′-deoxycytidines (**9**−**14**), uridines (**15**−**17**), and 2′-*O*-methyuridines (**18**, **19**), a clear structure−activity relationship (SAR) can be delineated. The nucleosides containing a larger carbon chain at the C-2 carbon of the 5-substituent are potent inhibitors of *M. bovis* and *M. avium* in these assays, demonstrating activity depending on

Table 1. In Vitro Antimycobacterial Activity of Test Compounds against *M. bovis* and *M. avium*



			antimycobacterial activity			
			M. bovis		*M. avium*	
compd	R	R_1	% inhibition (concentration, μg/mL)[a]	MIC_{90}[d] (μg/mL)	% inhibition (concentration, μg/mL)	MIC_{90} (μg/mL)
4	O	$(CH_2)_4CH_3$	60 (100, 50)		25 (100)	
5	O	$(CH_2)_7CH_3$	90 (100), 50 (50), 0 (10)	100	30 (100)	
6	O	$(CH_2)_9CH_3$	90 (100, 50), 70 (10), 25 (1)	50	75 (100), 0 (50)	
7	O	$(CH_2)_{11}CH_3$	90 (100, 50,10), 33 (1)	10	75 (100), 30 (50)	
12	NH_2	$(CH_2)_7CH_3$	90 (100, 50), 10 (10)	50	75 (100), 50 (50), 20 (10),	
13	NH_2	$(CH_2)_9CH_3$	90 (100, 50, 10), 25 (1)	10	100 (100), 60 (50), 10 (10)	100
Std1[b]			100 (0.5−1)	0.5−1	90 (2)	2
Std2[b]			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. Std1 = rifampicin, Std2 = clarithromycin. [c] ND = not determined. [d] Concentration of compounds exhibiting 90% inhibition in mycobacterial growth.

the length of the alkynyl side chain. The short chain containing 5-ethynyl (**1**), 5-propynyl (**2**, **9**, **18**, **19**), 5-pentynyl (**3**, **10**, **15**), and heptynyl (**11**,**16**) derivatives were devoid of any antimycobacterial activity, with the exception of 5-heptynyl-2′-deoxyuridine (**4**), which was moderately active for *M. bovis* (60% at 100 and 50 μg/mL) and *M. avium* (25% at 100 μg/mL) (Table 1). 5-Decynyl, 5-dodecynyl, and 5-tetradecynyl side chains appear to provide optimal activity against both mycobacteria. It was observed that increasing the C_{10} (decyne) side chain to C_{12} (dodecyne) and further to C_{14} (tetradecyne) provided significantly enhanced biological activities (Table 1). The inhibition in multiplication of *M. bovis* by the two most potent 2′-deoxyuridine analogues 5-dodecynyl-2′-deoxyuridine (**6**) and 5-tetradecynyl-2′-deoxyuridine (**7**) was 90% (100, 50 μg/mL) and 70% (10 μg/mL) for **6** and 90% (100, 50, 10 μg/mL) for **7**. The 5-decynyl (**5**) and 5-dodecynyl (**6**) analogues of 2′-deoxyuridine displayed moderate activity, while the corresponding 2′-deoxycytidine derivatives (**12**, **13**) exhibited higher activity against both mycobacteria. Interestingly, inhibition of *M. bovis* by 5-dodecynyl-2′-deoxycytidine (**13**) was similar to 5-tetradecynyl-2′-deoxyuridine (**7**), and activity against *M. avium* was superior. These results suggest that a longer carbon chain at the C-2 of the 5-position is determinant of the antimycobacterial activity. In addition, an amino group at C-4 appears to be preferred for improved activity.

Interestingly, inclusion of the phenyl ring at the C-2 carbon atom of the 5-substituent in the present compounds was detrimental to antimycobacterial activity, as exemplified by 5-(2-phenylethynyl) analogues (**8**, **14**, **17**) that were inactive. Modification of the 5-alkynyl substituent in the novel pyrimidine nucleoside (**1**, **3**−**6**) to the corresponding cyclic analogues 3-(2′-deoxy-β-D-ribofuranosyl)-6-octynyl-2,3-dihydrofuro[2,3-*d*]pyimidin-2-one (**20**−**24**) provided compounds that were devoid of antimycobacterial activity, suggesting that an acyclic substituent at the C-5 position of the pyrimidine ring or intact pyrimidine motif [NHC(O)] is essential for potent antimycobacterial activity. It thus appears that in order to confer a substantial antimycobacterial effect, the 5-alkynyl side chain should be linear and exceed seven carbons. Further, the respective 5-alkynyluracils were devoid of antimycobacterial activity (data not shown), suggesting that antimycobacterial activity also depends on the glycosyl part of these molecules.

Comparison of the anti *M. bovis* activity with anti *M. avium* activity revealed that 5-decynyl- (**5**, **12**), 5-dodecynyl- (**6**, **13**), or 5-tetradecynyl- (**7**) 2′-deoxy nucleosides possessed higher inhibitory activity against *M. bovis* than *M. avium*.

To examine whether the most promising compounds **6** and **7** are acting as promiscuous inhibitors[18] or specific inhibitors of mycobacteria, their activity was determined in the presence of various detergents (Figure 1). Initially the effect of three detergents (Tween-20, Triton X-100, and SDS) on BCG viability and growth was determined (data not shown) in the presence of concentrations of detergents up to their critical micellar concentrations (cmc).[19] The concentrations of detergents that had no effect on BCG viability and growth were then used in the assay to determine the activity of **6** and **7** (Figure 1). It was interesting to note that the inhibition profiles of the two selected compounds did not change in the presence of various detergents, suggesting that they are not acting as promiscuous inhibitors but rather have specific antimycobacterial activity. Compound **6** showed increased inhibition (90%) at 10 μg/mL in the presence of all detergents used, compared to that in the absence of detergents (70%). In fact, at the lowest concentration of compounds **6** and **7** (i.e., 1.0 μg/mL) in the presence of detergent Triton X-100, the antimycobacterial activity was also slightly increased compared to that of inhibition in the absence of detergents (Figure 1B,C vs Figure 1A) probably because of the detergent causing a reduction in nonspecific binding of compounds to plates. These observations are consistent with previous studies[18] where inhibition of β-lactamase activity by inhibitors was increased in the presence of detergents. These results clearly indicated that compounds **6** and **7** are not promiscuous inhibitors whose activity is dependent on their aggregation in biological medium but are specific inhibitors of mycobacterial growth and could be

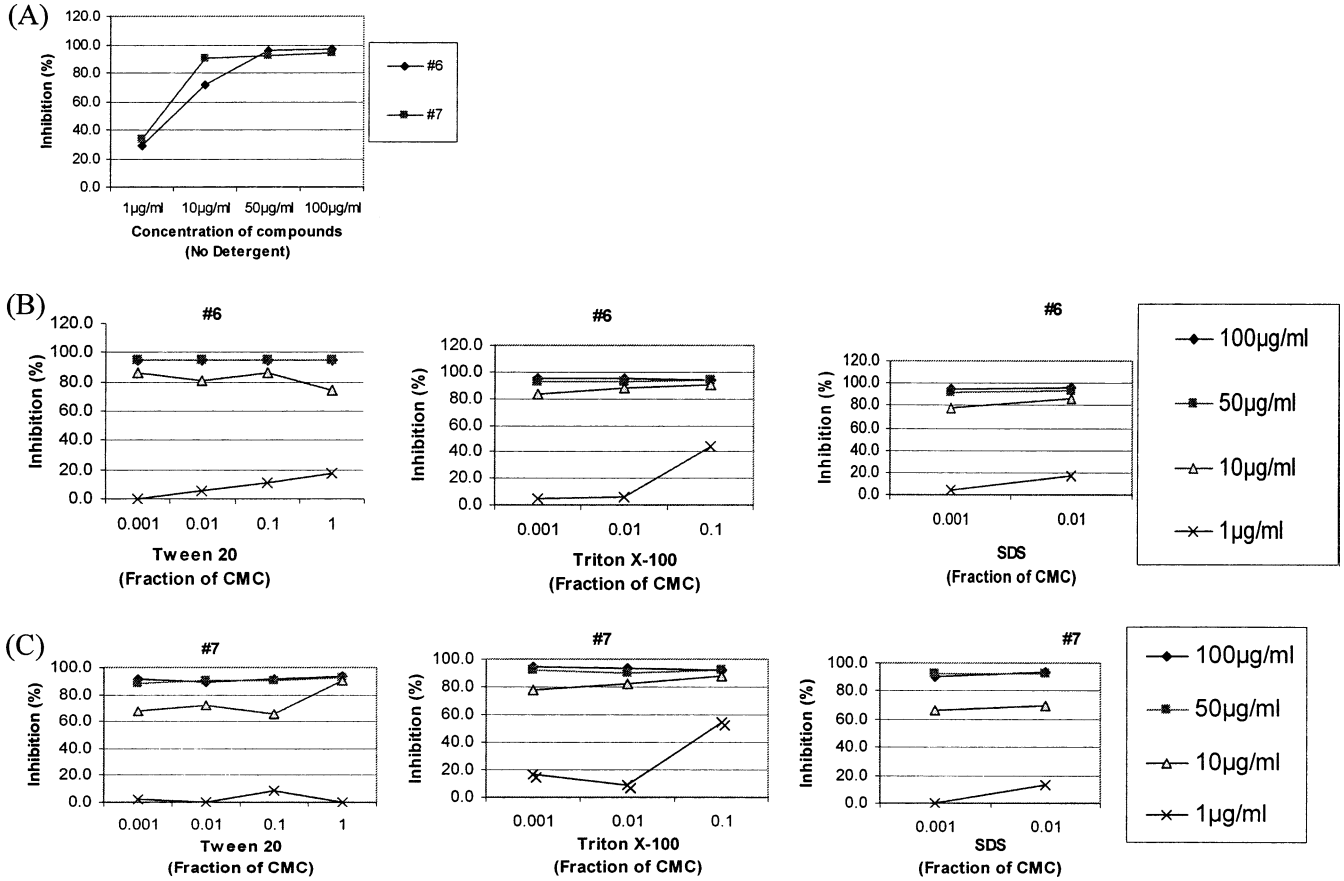


Figure 1. Effect of detergents on antimycobacterial activity of compounds **6** and **7**. Panel A shows the dose response of compounds **6** and **7** in the absence of detergent. The control bacterial well gave an average reading of 90 991. Panel B shows the activity of compound **6**, and panel C shows the activity of compound **7** in the presence of three different detergents. Both compounds were tested at four different concentrations (depicted by individual lines), as shown in each graph.

considered as a starting point 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.[20] 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 its DNA and/or RNA synthesis by acting as substrates and/or inhibitors of metabolic enzymes of DNA/RNA synthesis.

The promising compounds (**4**−**13**) were tested in vitro for their toxicity against monkey kidney (Vero cells), HepG2 cells, and human foreskin fibroblast (HFF cells) cell lines up to 100 μg/mL concentration, where they did not display toxicity up to the highest concentrations tested ($CC_{50} \geq 100$ μg/mL).

Summary

In conclusion, we present design, synthesis, and in vitro biological evaluation of 5-alkynylpyrimidine nucleosides that emerge as potent antimycobacterial agents. 2′-Deoxyuridine and 2′-deoxycytidine analogues possessing 5-decynyl, 5-dodecynyl, and 5-tetradecynyl substituents were found to be strong inhibitors of *M. bovis* multiplication in vitro. It is noteworthy that this series of agents is also active against *M. avium*. This new class of agents merits further studies to explore them as potential chemotherapeutic agents for tuberculosis. Additional in vitro and in vivo tests as well as studies of structure−activity relationships and mechanism of action on this class of compounds are ongoing in our laboratories. Identification of new lead compounds working by different mechanisms of action is required for the treatment of tuberculosis because of the emerging global health threat.

Experimental Section

Melting points were determined with a Buchi capillary apparatus and are uncorrected. ^{1}H NMR spectra were determined for solutions in Me_2SO-d_6 on a Bruker AM 300 spectrometer using Me_4Si as an internal standard. The assignment of all exchangeable protons (OH, NH) was confirmed by the addition of the D_2O. Microanalyses 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 Whatman MK6F silica gel microslides (25 μM thickness). 5-Iodo-2′-deoxyuridine (**25**), 5-iodo-2′-deoxycytidine (**26**), and 5-iodouridine (**27**) were purchased from Sigma-Aldrich Chemical Co. 5-Propynyl-2′-deoxycytidine (**9**), 5-propynyl-2′-*O*-methyluridine (**18**), and 5-propynyl-2′-*O*-methylcytidine (**19**) were purchased from Berry and Associates.

Preparation of 5-Alkynylpyrimidine Nucleosides. A full procedure is provided for 5-pentynyl-2′-deoxycytidine (**10**) and 5-pentynyluridine (**15**). For other analogues, only brief spectroscopic data are presented.

5-Pentynyl-2′-deoxycytidine (10). Tetrakis(triphenylphosphine)palladium(0) (98 mg, 0.084 mmol), copper(I) iodide (32

mg, 0.169 mmol), diisopropylethylamine (0.30 mL, 1.69 mmol), and 1-pentyne (0.35 mL, 3.54 mmol) were added to a solution of 5-iodo-2′-deoxycytidine (**26**) (300 mg, 0.85 mmol) in anhydrous dimethylformamide (25 mL). The orange reaction mixture was stirred at room temperature for 4 h in 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 for 4 h, 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 silica gel column using $CHCl_3$/MeOH (9:1, v/v) as eluent to yield **10** (185 mg, 74%) as a syrup. ^{1}H NMR (DMSO-d_6) δ 8.07 (s, 1H, H-6), 7.67 (s, 1H, NH), 6.72 (s, 1H, NH), 6.12 (t, J = 6.5 Hz, 1H, H-1′), 5.22 (d, J = 4.3 Hz, 1H, 3′-OH), 5.08 (t, J = 5.0 Hz, 1H, 5′-OH), 4.20 (m, 1H, H-3′), 3.78 (m, 1H, H-4′), 3.50−3.68 (m, 2H, H-5′), 2.37 (t, J = 7.0 Hz, 2H, α-CH_2), 2.13 and 1.98 (2m, 2H, H-2′), 1.54 (m, 2H, β-CH_2), 0.95 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 13.41 (CH_3), 20.96 (CH_2), 21.47 (α-CH_2), 40.69 (C-2′), 60.97 (C-5′), 70.06 (C-3′), 72.08 (C-β), 85.17 (C-4′), 87.31 (C-1′), 90.32 (C-α), 95.40 (C-5), 143.35 (C-6), 153.36 (C-2), 164.23 (C-4). Anal. ($C_{14}H_{19}N_3O_4$) C, H, N.

5-Heptynyl-2′-deoxycytidine (11). Yield 96%; ^{1}H NMR (DMSO-d_6) δ 8.05 (s, 1H, H-6), 7.65 (s, 1H, NH), 6.68 (s, 1H, NH), 6.11 (t, J = 6.6 Hz, 1H, H-1′), 5.20 (d, J = 4.0 Hz, 1H, 3′-OH), 5.04 (t, J = 5.0 Hz, 1H, 5′-OH), 4.20 (m, 1H, H-3′), 3.72 (m, 1H, H-4′), 3.68−3.50 (m, 2H, H-5′), 2.40 (t, J = 7.1 Hz, 2H, α-CH_2), 2.15 and 1.97 (2m, 2H, H-2′), 1.54 (m, 2H, β-CH_2), 1.34 (m, 4H, 2 × CH_2), 0.87 (t, J = 7.0 Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 13.82 (CH_3), 19.0, 21.62, 27.73 (3 × CH_2), 30.60 (α-CH_2), 40.69 (C-2′), 61.01 (C-5′), 70.11 (C-3′), 71.90 (C-β), 85.20 (C-4′), 87.34 (C-1′), 90.37 (C-α), 95.62 (C-5), 143.32 (C-6), 153.38 (C-2), 164.25 (C-4). Anal. ($C_{16}H_{23}N_3O_4$) C, H, N.

5-Decynyl-2′-deoxycytidine (12). Yield 82%; ^{1}H NMR (DMSO-d_6) δ 8.05 (s, 1H, H-6), 7.68 (s, 1H, NH), 6.68 (s, 1H, NH), 6.10 (t, J = 6.6 Hz, 1H, H-1′), 5.20 (d, J = 4.4 Hz, 1H, 3′-OH), 5.04 (t, J = 4.9 Hz, 1H, 5′-OH), 4.20 (m, 1H, H-3′), 3.78 (m, 1H, H-4′), 3.65−3.50 (m, 2H, H-5′), 2.38 (t, J = 7.2 Hz, 2H, α-CH_2), 2.12 and 1.96 (2m, 2H, H-2′), 1.54 (m, 2H, β-CH_2), 1.30 (m, 10H, 5 × CH_2), 0.85 (m, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 13.89 (CH_3), 19.01, 22.02, 28.04, 28.40, 28.47, 28.53 (6 × CH_2), 31.20 (α-CH_2), 40.69 (C-2′), 61.01 (C-5′), 70.11 (C-3′), 71.90 (C-β), 85.18 (C-4′), 87.34 (C-1′), 90.35 (C-α), 95.61 (C-5), 143.26 (C-6), 153.35 (C-2), 164.23 (C-4). Anal. ($C_{19}H_{29}N_3O_4$) C, H, N.

5-Dodecynyl-2′-deoxycytidine (13). Yield 80.5%; ^{1}H NMR (DMSO-d_6) δ 8.05 (s, 1H, H-6), 7.67 (s, 1H, NH), 6.70 (s, 1H, NH), 6.12 (t, J = 6.6 Hz, 1H, H-1′), 5.22 (d, J = 4.0 Hz, 1H, 3′-OH), 5.05 (t, J = 5.0 Hz, 1H, 5′-OH), 4.20 (m, 1H, H-3′), 3.79 (m, 1H, H-4′), 3.68−3.52 (m, 2H, H-5′), 2.38 (t, J = 7.1 Hz, 2H, α-CH_2), 2.12 and 1.98 (2m, 2H, H-2′), 1.54 (m, 2H, β-CH_2), 1.28 (m, 14H, 7 × CH_2), 0.86 (m, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 13.89 (CH_3), 19.0, 22.02, 28.01, 28.36, 28.47, 28.61, 28.85, 28.90 (8 × CH_2), 31.21 (α-CH_2), 40.66 (C-2′), 61.00 (C-5′), 70.10 (C-3′), 71.89 (C-β), 85.17 (C-4′), 87.31 (C-1′), 90.30 (C-α), 95.61 (C-5), 143.26 (C-6), 153.29 (C-2), 164.21 (C-4). Anal. ($C_{21}H_{33}N_3O_4$) C, H, N.

5-(2-Phenylethynyl)-2′-deoxycytidine (14). Yield 86%; ^{1}H NMR (DMSO-d_6) δ 8.32 (s, 1H, H-6), 7.80 (s, 1H, NH), 7.38−7.62 (m, 5H, aromatic), 7.05 (s, 1H, NH), 6.12 (t, J = 6.4 Hz, 1H, H-1′), 5.24 (d, J = 4.3 Hz, 1H, 3′-OH), 5.14 (t, J = 4.9 Hz, 1H, 5′-OH), 4.22 (m, 1H, H-3′), 3.80 (m, 1H, H-4′), 3.72−3.53 (m, 2H, H-5′), 2.20 and 2.05 (2m, 2H, H-2′); ^{13}C NMR (DMSO-d_6) δ 40.81 (C-2′), 60.85 (C-5′), 69.87 (C-3′), 81.56 (C-β), 85.37 (C-4′), 87.37 (C-1′), 89.43 (C-α), 93.64 (C-5), 122.38, 128.31, 131.13 (C-phenyl), 144.76 (C-6), 153.26 (C-2), 164.64 (C-4). Anal. ($C_{17}H_{17}N_3O_4$) C, H, N.

5-Pentynyluridine (15). To a solution of 5-iodoridine (**27**) (300 mg, 0.810 mmol) in anhydrous dimethylformamide (25 mL) were added tetrakis(triphenylphosphine)palladium(0) (94 mg, 0.081 mmol), copper(I) iodide (31 mg, 0.162 mmol), diisopropylethylamine (0.3 mL, 1.69 mmol), and 1-pentyne (0.24 mL, 2.43 mmol). The reaction mixture was stirred at room temperature for overnight under 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, 15 drops of 5% of disodium salt of EDTA/H_2O was added to the reaction mixture, and then the mixture was concentrated in vacuo. The residue obtained was purified on silica gel column using $CHCl_3$/MeOH (9:1, v/v) as eluent to yield **15** (120 mg, 48%) as a syrup. ^{1}H NMR (DMSO-d_6) δ 11.60 (s, 1H, NH), 8.20 (s, 1H, H-6), 5.78 (d, J = 5.2 Hz, 1H, H-1′), 5.40, 5.20 and 5.10 (m, 1H each, 5′-OH, 3′-OH, 2′-OH), 4.10−3.84 (m, 3H, H-2′, H-3′, H-4′), 3.70−3.50 (m, 2H, H-5′), 2.35 (t, J = 7.0 Hz, 2H, α-CH_2), 1.52 (m, 2H, β-CH_2), 0.98 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 13.27 (CH_3), 20.71 (CH_2), 21.59 (α-CH_2), 60.39 (C-5′), 69.49 (C-2′), 72.82 (C-β), 73.68 (C-3′), 84.78 (C-4′), 87.99 (C-1′), 93.03 (C-α), 99.03 (C-5), 142.66 (C-6), 149.61 (C-2), 161.55 (C-4). Anal. ($C_{14}H_{18}N_2O_6$) C, H, N.

5-Heptynyluridine (16). Yield 47%; ^{1}H NMR (DMSO-d_6) δ 11.58 (s, 1H, NH), 8.18 (s, 1H, H-6), 5.76 (d, J = 4.9 Hz, 1H, H-1′), 5.40, 5.17, 5.05 (m, 1H each, 5′-OH, 3′-OH, 2′-OH), 4.20−3.80 (m, 3H, H-2′, H-3′, H-4′), 3.70−3.50 (m, 2H, H-5′), 2.35 (t, J = 6.9 Hz, 2H, α-CH_2), 1.52 (m, 2H, β-CH_2), 1.48 (m, 4H, 2 × CH_2), 0.9 (t, J = 6.9 Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 13.77 (CH_3), 18.72, 21.56, 27.80 (3 × CH_2), 30.40 (α-CH_2), 60.43 (C-5′), 69.50 (C-2′), 72.62 (C-β), 73.65 (C-3′), 84.79 (C-4′), 87.99 (C-1′), 93.19 (C-α), 99.04 (C-5), 142.60 (C-6), 149.59 (C-2), 161.52 (C-4). Anal. ($C_{16}H_{22}N_2O_6$) C, H, N.

5-(2-Phenylethynyl)uridine (17). Yield 36.5%; ^{1}H NMR (DMSO-d_6) δ 11.72 (s, 1H, NH), 8.50 (s, 1H, H-6), 7.60−7.38 (m, 5H, aromatic), 5.76 (d, J = 4.6 Hz, 1H, H-1′), 5.48, 5.30, 5.12 (m, 1H each, 5′-OH, 3′-OH, 2′-OH), 4.12−3.98 (m, 3H, H-2′, H-3′, H-4′), 3.80−3.58 (m, 2H, H-5′); ^{13}C NMR (DMSO-d_6) δ 60.11 (C-5′), 69.17 (C-2′), 73.86 (C-3′), 82.29 (C-β), 84.63 (C-4′), 88.40 (C-1′), 91.65 (C-α), 98.08 (C-5), 122.27, 128.38, 128.48, 128.57, 131.00 (C-phenyl), 143.90 (C-6), 149.51 (C-2), 161.18 (C-4) Anal. ($C_{17}H_{16}N_2O_6$) C, H, N.

In Vitro Antimycobacterial Activity Assay (*M. bovis, M. avium*). *M. bovis* (BCG) 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).[17] Test compounds were dissolved in DMSO at 100× of the highest final concentration used, and subsequent dilutions were performed in 7H9GC (Difco Laboratories, Detroit, Michigan) media 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 three times, and the mean percent inhibition is reported in the table. The standard deviations were within 10%. Frozen mycobacterial inocula were diluted in medium 7H9GC and added to each well at 2.5 × 10^5 CFU/mL final concentration. Sixteen control wells consisted of eight with bacteria alone (B) and eight with media alone (M). Plates were incubated for an initial 6 days, and starting from 6 days of incubation, 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 24−48 h for visual color change from blue to pink and read by spectrophotometer (at excitation 530/525 and emission 590/535) 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 (test well-M bkg/B well-M bkg) × 100. The lowest drug concentration effecting an inhibition of ∼90% was considered as the MIC_{90}. Similar methodology was used for both *M. bovis* BCG and *M. avium*. 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 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 of 60000−100000.

The antimycobacterial activity of compounds **6** and **7** was also determined against BCG in the presence of various detergents using MABA assay. For these experiments titrating amounts of detergents, i.e., Tween-20, Triton X-100, and SDS (Fisher Scientific, critical micellar concentrations (cmc): 0.05, 0.3, and 8.2 mM, respectively) at 1.0, 0.1, 0.01, and 0.001 cmc (final concentration) were preincubated with test compounds at 100, 50, 10, and 1 μg/mL (final concentration) for 15 min before adding to the BCG wells in triplicate. The data are shown as the average of the triplicates, and the standard deviation was within 5%. Control wells included BCG cultured with various amounts of all three detergents without compounds. In the initial experiment, we determined the effect of various concentrations of the three detergents on BCG growth. Tween-20 had no effect on BCG growth at all four concentrations, whereas Triton X-100 was toxic at 1 cmc, and SDS was toxic to BCG at 1 and 0.1 cmc. Therefore, the compounds were tested in the presence of nontoxic doses of detergents.

Cell Cytotoxicity Assay. Cell viability was measured using the cell proliferation kit 1 (MTT; Boehringer Mannheim), 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 media was replaced with media 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.

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

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