Growth Inhibition of *Mycobacterium bovis, Mycobacterium tuberculosis* and *Mycobacterium avium* In Vitro: Effect of 1-β-D-2′-Arabinofuranosyl and 1-(2′-Deoxy-2′-fluoro-β-D-2′-ribofuranosyl) Pyrimidine Nucleoside Analogs

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The resurgence of tuberculosis and the emergence of multiple-drug-resistant strains of *Mycobacteria* necessitate the search for new classes of antimycobacterial agents. We synthesized a series of 1-β-D-2′-arabinofuranosyl and 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl) pyrimidine nucleosides possessing diverse sets of alkynyl, alkenyl, alkyl, and halo substituents at the C-5 position of the uracil and investigated their effect on activity against *M. tuberculosis*, *M. bovis*, and *M. avium*. Among these molecules, 5-alkynyl-substituted derivatives emerged as potent inhibitors of *M. bovis*, *M. tuberculosis*, and *M. avium*. Nucleosides 1-β-D-2′-arabinofuranosyl-5-dodecynyluracil (**5**), 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)-5-dodecynyluracil (**24**), and 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)-5-tetradecynyluracil (**25**) showed the highest antimycobacterial potency against *M. bovis* and *M. tuberculosis*. The MIC_{90} exhibited by compounds **5**, **24**, and **25** was similar or close to that of the reference drug rifampicin. The most active compounds **5**, **24**, and **25** were also found to retain sensitivity against a rifampicin-resistant strain of *M. tuberculosis* H37Rv at similar concentrations. Some of these analogs also revealed in vitro antimicrobial effect against several other gram-positive pathogens.

Introduction

Mycobacterium tuberculosis (*M. tuberculosis*[a]), *Mycobacterium avium* (*M. avium*), and *Mycobacterium bovis* (*M. bovis*) are clinically significant species of the genus *Mycobacterium* that are causative agents of tuberculosis (TB), killing over 2−3 million people annually worldwide.[1] TB bacilli are highly contagious, airborne, slow-growing, gram-positive, aerobic, acid-fast mycobacteria. The World Health Organization (WHO) estimates that between 1 and 2 billion people are latently infected with TB bacilli.[2−4] Approximately 8 million people develop active disease each year.[5] TB is the world's second most common cause of death from infectious disease, after acquired immuno deficiency syndrome (AIDS).[6]

TB and human immunodeficiency virus (HIV) have formed a new and deadly combination. There is a resurgence in the incidence of TB in developed and developing countries with high rates of HIV−TB coinfection. The increase in TB incidence is strongly associated with the prevalence of HIV infection.[7] *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.[8] For both TB and HIV, misdiagnosis and noncompliance with treatment regimens further compound the problem and facilitate the development of drug resistance.[7,9]

M. avium complex (MAC) infections, in particular, *M. avium* infections, are one of the most serious complications among patients with AIDS.[10,11] MAC infections are disseminated rather than being 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.[10,11] 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.[12,13]

The problem of tuberculosis is further complicated by the emergence of new TB strains, which are not susceptible to a number of available drugs, that is, multidrug-resistant TB (MDR-TB).[2,14,15] Around 50 million people have been infected with MDR-TB. MDR-TB is defined as resistant to at least isoniazid and rifampicin and requires the use of second-line drugs. An outbreak of recently recognized "extensively drug-resistant tuberculosis" or XDR-TB, threatens the TB control globally. XDR-TB is MDR-TB that is also resistant to three or more of the six classes of second-line drugs.[16,17] A 100% correlation has been observed in XDR-TB with HIV coinfection and mortality.[16]

Bacillus Calmette Guerin (BCG)[18] is an attenuated strain of *M. bovis* that is more than 98% homologous to *M. tuberculosis* and, therefore, is closely related to *M. tuberculosis*. 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.[19] 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

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[a] Abbreviations: TB, tuberculosis; *M. bovis*, *Mycobacterium bovis*; *M. tuberculosis*, *Mycobacterium tuberculosis*; *M. avium*, *Mycobacterium avium*; MAC, *Mycobacterium avium* complex; MDR-TB, multidrug resistant tuberculosis; XDR-TB, extensively drug resistant tuberculosis; BCG, Bacillus Calmette Guerin; MABA, microplate alamar blue assay; HFF, human foreskin fibroblast; CMC, critical micellar concentrations; CFU, colony forming unit; GI, growth index; *S. aureus*, *Staphylococcus aureus*; *S. epidermis*, *Staphylococcus epidermis*; *E. faecalis*, *Enterococcus faecalis*; *B. subtilis*, *Bacillus subtilis*; *S. pneumoniae*, *Streptococcus pneumoniae*; *S. pyogenes*, *Streptococcus pyogenes*; *S. typhimurium*, *Salmonella typhimurium*; *E. coli*, *Escherichia coli*; *P. vulgaris*, *Proteus vulgaris*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *L. monocytogenes*, *Lysteria monocytogenes*; *E. aerogenes*, *Enterobacter aerogenes.*

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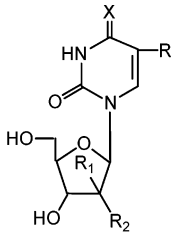
in 19 patients.[19] There is an ever-increasing threat of drug-resistant TB appearing as an epidemic in many developing countries as well as developed countries, particularly because no new drugs have been introduced to treat TB for over 40 years.[4]

Because of the global health problems associated with TB, the increasing rate of multiple drug-resistant TB and the high rate of a co-infection with HIV, the discovery and development of potent new anti-TB agents without cross-resistance with current antimycobacterial agents are urgently needed.

The *M. tuberculosis* genome has been fully sequenced, which has greatly facilitated the identification of potential new and unique targets for the design of new classes of selective anti-TB drugs.[20] Pyrimidine biosynthesis is an essential step in the progression of TB. Significant differences exist in the details and the regulatory mechanisms of the pyrimidine biosynthesis pathway in bacteria and mammals, suggesting that novel nucleoside derivatives could target new biochemical mechanisms, potentially allowing the design and development of novel antituberculosis drugs.[20] It is postulated that unnatural pyrimidine nucleosides can specifically target the mycobacterial enzymes involved in their nucleic acid synthesis by acting as their substrates or inhibitors and inhibit the mycobacterial DNA and RNA synthesis.

A number of pyrimidine nucleoside derivatives in which the 2′-hydroxyl group has the opposite configuration (1-β-D-2′-arabinofuranosyl) to that of a ribonucleoside, exhibit potent antiviral, and anticancer properties.[21] Among them, 1-β-D-2′-arabinofuranosyl-5-(1-propynyl)uracil (**1a**, netivudine) is one of the most effective and selective analogs for the treatment of varicella zoster virus infection, possessing very good pharmacokinetic properties with long plasma half-life.[22] In addition, incorporation of a fluorine atom at the 2′-position of 2′-deoxyuridine derivatives with a variety of 5-substituents has provided compounds that in many instances are excellent substrates for phosphorylation by kinases. In earlier studies, it has been observed that the presence of an arabino hydroxyl group and the ribofluoro group at the C-2′ position greatly enhances resistance to phosphorolysis of the glycosyl bond in the deoxyribose derivatives while retaining biological activities of the parent nucleosides.[23]

In ongoing efforts to develop new and effective therapies for mycobacterial infections, our previous investigations of 5-substituted pyrimidine nucleosides led to the identification of 5-(alkyn-1-yl)uracil 2′-deoxy nucleosides with good inhibitory activity against *M. bovis* (MIC_{90} = 10−50 μg/mL) and *M. avium* (MIC_{75} = 100 μg/mL) in cell-based assays.[24,25] In these studies, we observed an important role of the carbohydrate portion because the corresponding uracil derivatives were inactive.[24] We reasoned that replacement of the 2′-deoxyribose by a sugar moiety resistant to glycosidic bond cleavage might produce compounds with analogous structural features and enhanced biological profile. In the present studies, we now report the synthesis and antituberculosis activity of 1-β-D-2′-arabinofuranosyl (**3**−**8**, **9**−**16**, **19**, **20**) and 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl) (**22**−**26**, **27**−**33**, **36**, **37**) pyrimidine nucleoside analogs with diverse 5-alkynyl, 5-alkenyl, 5-alkyl, and 5-haloalkyl substituents against *M. bovis*, *M. tuberculosis*, and *M. avium* in vitro. Nucleosides possessing alkenyl, alkyl, and halogen groups at the 5-position were moderately active or devoid of antimycobacterial activity. However, our investigations revealed that 1-β-D-2′-arabinofuranosyl-5-dodecynyluracil (**5**), 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)-5-dodecynyluracil (**24**), and 1-(2′-deoxy-2′fluoro-β-D-ribofuranosyl)-5-tridecynyluracil (**25**) display very promising activity against *M. bovis* and *M. tuberculosis* in vitro. Encouragingly, these compounds were also found to retain sensitivity against drug-resistant *M. tuberculosis*. These studies extend our previous observations and suggest that this class of compounds has potential to serve as new antituberculosis agents for drug-sensitive and drug-resistant mycobacteria.



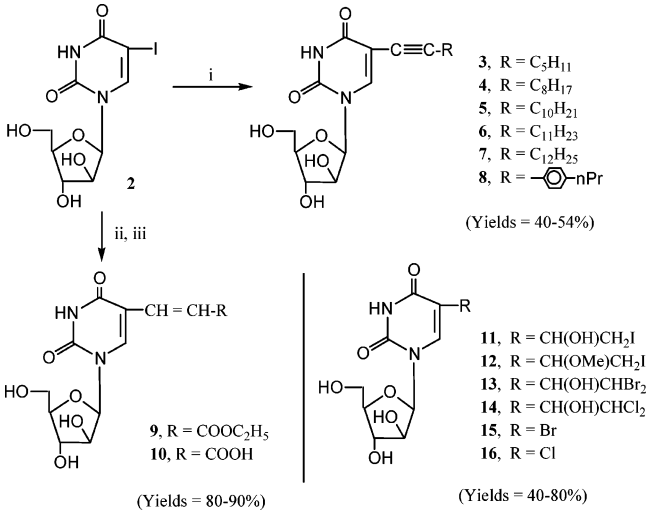
1a, R = C≡C-CH_3, X = O, R_1 = OH, R_2 = H
1b, R = CH=CH_2, X = O, R_1 = OH, R_2 = H
1c, R = CH=CHBr, X = O, R_1 = OH, R_2 = H
1d, R = CH=CHCl, X = O, R_1 = OH, R_2 = H
3, R = C≡C-C_5H_{11}, X = O, R_1 = OH, R_2 = H
4, R = C≡C-C_8H_{17}, X = O, R_1 = OH, R_2 = H
5, R = C≡C-$C_{10}H_{21}$, X = O, R_1 = OH, R_2 = H
6, R = C≡C-$C_{11}H_{23}$, X = O, R_1 = OH, R_2 = H
7, R = C≡C-$C_{12}H_{25}$, X = O, R_1 = OH, R_2 = H
8, R = C≡C-C$_6$H$_4$-nPr, X = O, R_1 = OH, R_2 = H
9, R = CH=$CHCOOC_2H_5$, X = O, R_1 = OH, R_2 = H
10, R = CH=CHCOOH, X = O, R_1 = OH, R_2 = H
11, R = CH(OH)CH_2I, X = O, R_1 = OH, R_2 = H
12, R = CH(OMe)CH_2I, X = O, R_1 = OH, R_2 = H
13, R = CH(OH)$CHBr_2$, X = O, R_1 = OH, R_2 = H
14, R = CH(OH)$CHCl_2$, X = O, R_1 = OH, R_2 = H
15, R = Br, X = O, R_1 = OH, R_2 = H
16, R = Cl, X = O, R_1 = OH, R_2 = H
19, R = H, X = S, R_1 = OH, R_2 = H
20, R = CH_3, X = S, R_1 = OH, R_2 = H
22, R = C≡C-C_5H_{11}, X = O, R_1 = H, R_2 = F
23, R = C≡C-C_8H_{17}, X = O, R_1 = H, R_2 = F
24, R = C≡C-$C_{10}H_{21}$, X = O, R_1 = H, R_2 = F
25, R = C≡C-$C_{11}H_{23}$, X = O, R_1 = H, R_2 = F
26, R = C≡C-$C_{12}H_{25}$, X = O, R_1 = H, R_2 = F
27, R = CH=$CHCOOC_2H_5$, X = O, R_1 = H, R_2 = F
28, R = CH=CHCOOH, X = O, R_1 = H, R_2 = F
29, R = CH(OH)CH(Cl)$COOC_2H_5$, X = O, R_1 = H, R_2 = F
30, R = CH(OH)CH(Br)$COOC_2H_5$, X = O, R_1 = H, R_2 = F
31, R = CH(OH)CH(I)COOH, X = O, R_1 = H, R_2 = F
32, R = Br, X = O, R_1 = H, R_2 = F
33, R = Cl, X = O, R_1 = H, R_2 = F
36, R = H, X = S, R_1 = H, R_2 = F
37, R = CH_3, X = S, R_1 = H, R_2 = F

Chemistry

The target 1-β-D-arabinofuranosyl-5-alkynyluracils (**3**−**8**) were synthesized in one step by the Pd-catalyzed coupling of a series of terminal alkynes with 1-β-D-arabinofuranosyl-5-iodouracil (**2**)[26] in DMF in 40−54% yields (Scheme 1). Structures of **3**−**8** were characterized by ^{1}H NMR, ^{13}C NMR, and microanalysis. Reaction of **2** with ethylacrylate, bis-(triphenyl)palladium(II) chloride, and triethylamine, using a procedure reported by us,[26] yielded the (*E*)-5-(2-ethoxycarbonylvinyl) analog (**9**). Alkaline hydrolysis of **9** afforded the (*E*)-5-(2-carboxyvinyl) derivative (**10**).[27]

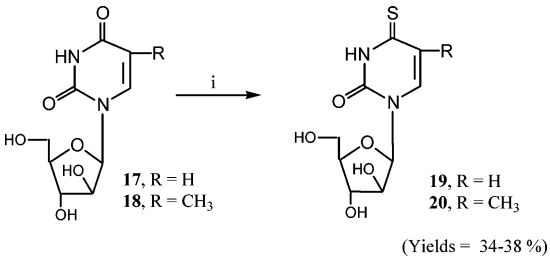
The nucleoside 1-β-D-arabinofuranosyl-5-(1-hydroxy-2-iodoethyl)uracil (**11**)[28] was synthesized by the reaction of 1-β-D-arabinofuranosyl-5-vinyluracil (**1b**) with iodine in the presence of the oxidizing agent, iodic acid. Treatment of (**11**) with methanolic sulfuric acid yielded **12.**[28] The compounds 5-(2,2-dibromo-1-hydroxyethyl)-(**13**) and 5-(2,2-dichloro-1-hydroxyethyl)-(**14**) were prepared by the reaction of (*E*)-5-(2-bromovinyl)-

Scheme 1[a]



[a] Reagents and conditions: (i) H−C ≡ C−R, $Pd(PPh_3)_4$, CuI, $(i\text{-Pr})_2EtN$, DMF, RT; (ii) CH_2═CHCOOEt, $(Ph_3P)_2PdCl_2$, Et_3N (**9**); (iii) 0.5 N KOH (**10**).

Scheme 2[a]

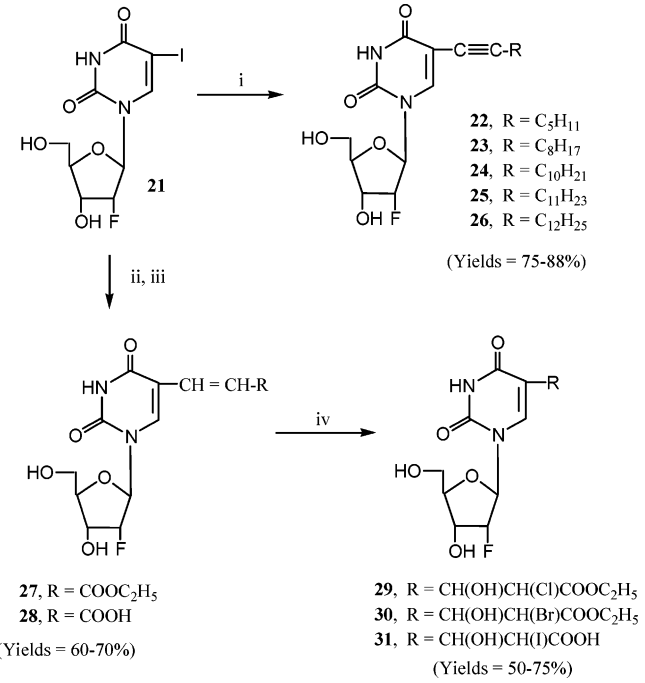


[a] Reagents and conditions: (i) acetic anhydride, dimethylaminopyridine, 25 °C; Lawesson's reagent, 1,4-dioxane, reflux; NH_3, MeOH, 0−25 °C.

(**1c**) and (*E*)-5-(2-chlorovinyl)-(**1d**) 1-β-D-arabinofuranosyluracils, with *N*-bromosuccinimide or *N*-chloro succinimide in 1,4-dioxane, respectively.[26] A mild and efficient reaction of 1-β-D-arabinofuranosyluracil (**17**) with *N*-bromosuccinimide and sodium azide in 1,2-dimethoxyethane (DME) at 25 °C yielded the 5-bromo compound (**15**) in quantitative yield.[29] A similar chlorination reaction of **17** using *N*-chlorosuccinimide and sodium azide in DME at 45 °C gave the 5-chloro product (**16**).[29] The 4-thio arabinosyl nucleosides (**19**, **20**) were prepared starting from commercially available compounds **17** and **18** in a single step without isolating protected nucleoside intermediates. Thus, acetylation of **17** or **18** using acetic anhydride and dimethylaminopyridine at 25 °C, followed by thiolation with Lawesson's reagent in 1,4-dioxane and subsequent deacetylation of the crude products, gave 4-thione products (**19**, **20**) in 38 and 34% yields, respectively (Scheme 2).

For the synthesis of 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)-5-alkynyluracils (**22**−**26**), similar methodologies were used that we had adopted for the preparation of compounds **3**−**8**, involving the Pd-catalyzed coupling reaction of terminal acetylenes with 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)-5-iodouracil (**21**; Scheme 3). A coupling reaction of **21** with ethylacrylate followed by alkaline hydrolysis of the obtained ester (**27**)[30] afforded compound **28**,[30] as described above for arabino analogs (**9**, **10**). The halohydrin derivatives, **29**−**31**, were readily prepared by the regiospecific addition of HO−X (X = Cl, Br, I) to the vinyl substituent of (*E*)-5-(2-ethoxycarbonylvinyl)-**27** and (*E*)-5-(2-carboxyvinyl)-**28**, 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)uracils according to the procedure reported earlier[30] (Scheme 3). Compounds **32** and **33** were

Scheme 3[a]



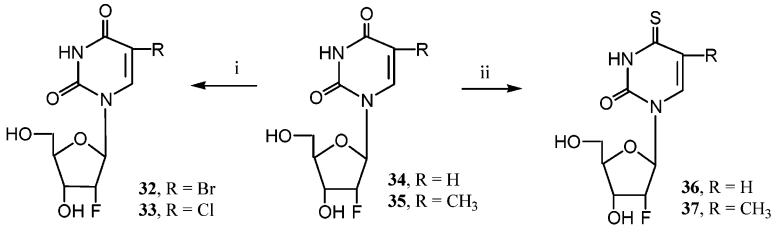
[a] Reagents and conditions: (i) H−C≡C−R, $Pd(PPh_3)_4$, CuI, $(i\text{-Pr})_2EtN$, DMF, RT; (ii) CH_2═CHCOOEt, $(Ph_3P)_2PdCl_2$, Et_3N (**27**); (iii) 0.5 N KOH (**28**); (iv) *N*-chlorosuccinimide, H_2O, 25 °C (**29**); *N*-bromosuccinimide, H_2O, 25 °C, (**30**); I_2, KIO_3, H_2O, H_2SO_4 (**31**).

synthesized by the electrophilic addition reactions of 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)uracil (**34**) with dilute solutions of the appropriate halogen in acetic acid followed by treatment with aqueous ammonia.[23] The preparation of hitherto unknown compounds **36** and **37** proceeded from 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)uracil (**34**) and 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)-5-methyluracil (**35**) and utilized essentially the same methodology developed for the synthesis of corresponding arabinosyl analogs (**19**, **20**; Scheme 4).

Results and Discussion

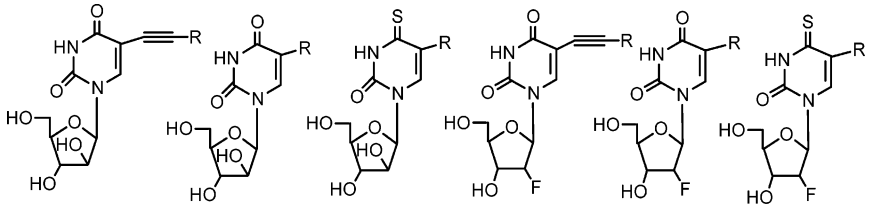
All of the test compounds were evaluated in vitro against *M. bovis*, *M. tuberculosis*, and *M. avium* by the microplate Alamar blue assay (MABA)[31] at 1−100 μg/mL concentrations. Rifampicin and clarithromycin were used as reference standards. Antimycobacterial activity for the compounds **3**−**16**, **19**, **20**, **22**−**33**, **36**, and **37** is summarized in Table 1. The 5-substituted pyrimidine nucleoside derivatives, modified in the carbohydrate and base moiety, investigated here for their antimycobacterial effect, can mainly be divided into four different structural classes: (i) 5-alkynyl analogs (**3**−**8**, **22**−**26**), (ii) 5-alkenyl analogs (**9**, **10**, **27**, **28**), (iii) 5-alkyl analogs (**11**−**14**, **20**, **29**−**31**, **37**), and (iv) 5-halo analogs (**15**, **16**, **32**, **33**). Among the nucleosides possessing 5-alkynyl substituents (**3**−**8**, **22**−**26**), a clear structure−activity relationship (SAR) can be delineated. The compounds containing a longer carbon chain at the C-2 carbon of the 5-substituent, namely, 1-β-D-arabinofuranosyl-5-decynyluracil (**4**), 1-β-D-arabinofuranosyl-5-dodecynyluracil (**5**), 1-β-D-arabinofuranosyl-5-tridecynyluracil (**6**), 1-β-D-arabinofuranosyl-5-tetradecynyluracil (**7**), 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)-5-decynyluracil (**23**), 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)-5-dodecynyluracil (**24**), 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)-5-tridecynyluracil (**25**), and 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)-5-tetradecynyluracil (**26**), are potent inhibitors of *M. bovis* and *M. tuberculosis* (MIC_{90} =1−100 μg/mL range), with the activity depending on the length of the

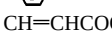
Scheme 4[a]



[a] Reagents and conditions: (i) bromine (**32**) or chlorine (**33**), CH_3COOH, 25 °C, NH_4OH; (ii) acetic anhydride, dry pyridine, 0−25 °C; Lawesson's reagent, 1,4-dioxane, reflux; NH_3, MeOH, 0−25 °C.

Table 1. In Vitro Antimycobacterial Activity of 1-β-D-2′-Arabinofuranosyl and 1-(2′-Deoxy-2′-fluoro-β-D-ribofuranosyl) Pyrimidine Nucleoside Analogs



		antimycobacterial activity					
		M. bovis (BCG)		***M. tuberculosis*** (H37Ra)		***M. avium*** (ATCC 25291)	
cmpd	R	% inhibition[a] (concn μg/mL)	MIC_{90}[d] (μg/mL)	% inhibition[a] (concn μg/mL)	MIC_{90}[d] (μg/mL)	% inhibition[a] (concn μg/mL)	MIC_{90}[d] (μg/mL)
3	C_5H_{11}	25 (100)		25 (100)		0 (100)	
4	C_8H_{17}	100 (100), 50 (50), 30 (10)	100	100 (100), 50 (50), 10 (10)	100	50 (100)	
5	$C_{10}H_{21}$	100 (100, 50, 10), 75 (1)	1−5	100 (100, 50, 10), 75 (1)	1−5	100 (100), 55 (50)	50−100
6	$C_{11}H_{23}$	100 (100, 50), 50 (10)	10−50	100 (100, 50), 50 (10)	10−50	25 (100)	
7	$C_{12}H_{25}$	100 (100, 50), 50 (10)	10−50	100 (100, 50), 50 (10)	10−50	0 (100)	
8		100 (100), 50 (50)	100	100 (100), 50 (50)	100	25 (100)	
9	CH═CHCOOEt	0 (100)		0 (100)		0 (100)	
10	CH═CHCOOH	0 (100)		0 (100)		0 (100)	
11	$CH(OH)CH_2I$	0 (100)		0 (100)		0 (100)	
12	$CH(OMe)CH_2I$	0 (100)		0 (100)		0 (100)	
13	$CH(OH)CHBr_2$	0 (100)		0 (100)		0 (100)	
14	$CH(OH)CHCl_2$	0 (100)		0 (100)		0 (100)	
15	Br	0 (100)		0 (100)		0 (100)	
16	Cl	0 (100)		0 (100)		0 (100)	
19	H	0 (100)		0 (100)		0 (100)	
20	CH_3	0 (100)		0 (100)		0 (100)	
22	C_5H_{11}	50 (100)		50 (100)		0 (100)	
23	C_8H_{17}	100 (100), 75 (50), 50 (10), 30 (1)	50−100	100 (100), 100 (50), 50 (10), 25 (1)	50−100	50 (100)	
24	$C_{10}H_{21}$	100 (100, 50, 10), 70 (1)	1−5	100 (100, 50, 10), 70 (1)	1−5	100 (100), 50 (50)	50−100
25	$C_{11}H_{23}$	100 (100, 50, 10), 90 (1)	1	100 (100, 50, 10), 90 (1)	1	100 (100), 50 (50)	50−100
26	$C_{12}H_{25}$	100 (100, 50), 50 (10)	10−50	100 (100, 50), 50 (10)	10−50	20 (100)	
27	CH═CHCOOEt	0 (100)		0 (100)		0 (100)	
28	CH═CHCOOH	0 (100)		0 (100)		0 (100)	
29	CH(OH)CH(Cl)COOEt	0 (100)		0 (100)		0 (100)	
30	CH(OH)CH(Br)COOEt	0 (100)		0 (100)		0 (100)	
31	CH(OH)CH(I)COOH	0 (100)		0 (100)		0 (100)	
32	Br	0 (100)		0 (100)		0 (100)	
33	Cl	50 (100, 50)		20 (100)		0 (100)	
36	H	25 (100)		25 (100)		0 (100)	
37	CH_3	25 (100)		25 (100)		0 (100)	
std1[b]		100 (0.5−1)	0.5−1	100 (0.5−1)	0.5−1	90 (2)	2
std2[b]		ND[c]	ND	ND	ND	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.

alkynyl side chain. The short chain containing 1-β-D-arabinofuranosyl-5-heptynyluracil (**3**) and 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)-5-heptynyluracil (**22**) derivatives were moderately active for *M. bovis* and *M. tuberculosis* (25−50% inhibition at 100 μg/mL; Table 1). 5-Dodecynyl, 5-tridecynyl, and 5-tetradecynyl side chains appear to provide optimal activity against

both mycobacterial species. The maximum inhibition of bacterial growth in the case of 2′-arabinofuranosyl analogs (**3**−**7**) was obtained by the dodecynyl moiety, whereas in 2′-fluororibofuranosyl derivatives (**22**−**26**), maximum activity was provided by dodecynyl and tetradecynyl chain-bearing derivatives. To delineate the structural requirements for optimal potency, beyond the necessity of a longer alkyl chain at the C-2 of the 5-position, we sought to replace the flexible alkyl chain by a rigid ring system in compounds **3**−**7**, allowing us to examine the effect of a *para*-alkyl-substituted phenyl group at the terminal carbon of the alkynyl chain. We note that inclusion of the N-propylphenyl ring did not improve antimycobacterial activity, as 1-β-D-arabinofuranosyl-5-(4-*n*-propylphenylethynyl)uracil (**8**) was only moderately active (MIC_{90} =100 μg/mL). The most potent compounds of the 5-alkynyl series, **5**, **24** (MIC_{90} =1−5 μg/mL), and **25** (MIC_{90} =1.0 μg/mL), inhibited the growth of *M. bovis* and *M. tuberculosis* at concentrations similar or approaching that of the reference drug, rifampicin (MIC_{90} =0.5−1.0 μg/mL). The 5-tridecynyl- (**25**) analog of 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)uracil displayed superior activity compared to that of the corresponding 1-β-D-arabinofuranosyluracil derivative (**6**) against both mycobacteria. Further, inhibition of *M. bovis* and *M. tuberculosis* by 5-heptynyl- (**22**, 50% inhibition at 100 μg/mL) and 5-decynyl- (**23**, 50% inhibition at 10 μg/mL) analogs of 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)-uracil was improved over 1-β-D-arabinofuranosyl-5-heptynyluracil (**3**, 25% inhibition at 100 μg/mL) and 1-β-D-arabinofuranosyl-5-decynyluracil (**4**, 50% inhibition at 100 μg/mL). These studies suggest that a fluoro substituent at the 2′-position of the sugar moiety is preferred for improved activity.

Interestingly, the activity of 5-dodecynyl analogs of 1-β-D-arabinofuranosyluracil (**5**) and 2′-deoxy-2′-fluoro-β-D-ribofuranosyluracil (**24**) was significantly increased, but it was reduced for the 5-tetradecynyl derivatives (**7**, **26**), as compared to their corresponding 2′-deoxyuridines [90% inhibition at 50−100 μg/mL (5-dodecynyl) and 10 μg/mL (5-tetradecynyl), respectively] against *M. bovis*.[24] The activity of compounds **5** and **24** against *M. tuberculosis* (H37Ra) was also significantly improved as compared to their corresponding 2′-deoxyuridine congeners reported previously[24] (MIC_{90} = 50−100 μg/mL, unpublished data). These studies suggest that potent antimycobacterial activities are not solely dependent on the 5-alkynyl side chain, but are also modulated by the carbohydrate portion of the nucleosides. In our studies, we also synthesized 5-alkynyl (decynyl, dodecynyl, and tetradecynyl) uracils that were found to be inactive against *M. bovis* and *M. tuberculosis* (0% inhibition at 100 μg/mL, unpublished results). These results clearly demonstrate that the glycosyl part is crucial and plays an important role in the antimycobacterial activities of these molecules.

With regard to antimycobacterial activity against *M. avium*, observations similar to those described for activities against *M. bovis* and *M. tuberculosis* were made where 1-β-D-arabinofuranosyl-5-dodecynyluracil (**5**), 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)-5-dodecynyl uracil (**24**), and 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)-5-tridecynyluracil (**25**) exhibited the most potent activity (MIC_{90} = 50−100 μg/mL; Table 1). The influence of the 2′-ribofluoro substituent was again noted on the antituberculosis activity as compound **25** provided significantly enhanced inhibition of *M. avium* (MIC_{90} = 50−100 μg/mL) in comparison to the respective 2′-arabinohydroxyl analog **6** (25% inhibition at 100 μg/mL). We note that the activity of nucleosides **5** and **24** was improved (50% inhibition at 50 μg/mL) but diminished for the compounds **7** and **26** (0−20% inhibition at 100 μg/mL) against *M. avium* when compared with their respective 2′-deoxyribose analogs (75% inhibition at 100 μg/mL), suggesting that variations in the glycosyl part of the pyrimidine nucleosides investigated not only modulate the antimycobacterial activity, but also influence the activity spectrum. Comparison of the activity against *M. avium* with *M. bovis* or *M. tuberculosis* revealed that 5-heptynyl (**3**, **22**), 5-decynyl (**4**, **23**), 5-dodecynyl (**5**, **24**), 5-tridecynyl (**6**, **25**), or 5-tetradecynyl (**7**, **26**) nucleosides possessed much higher inhibitory activity against *M. bovis* and *M. tuberculosis* than *M. avium*.

To extend our understanding of the structural requirements for potent activity, we also examined the effect of alkenyl, alkyl and haloalkyl substituents at the C-5 position of the pyrimidine ring in the 1-β-D-arabinofuranosyl and 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl) nucleosides. Introduction of alkenyl groups led to a dramatic reduction in antimycobacterial efficacy in both 1-β-D-arabinofuranosyluracil (**9**, **10**) as well as 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)uracil (**27**, **28**) derivatives. Similarly, nucleosides with shorter chain alkyl groups at the 5-position of the uracil ring, namely, 1-β-D-arabinofuranosyl-5-(1-hydroxy-(or methoxy)-2-iodoethyl)uracil (**11**, **12**), 1-β-D-arabinofuranosyl-5-(1-hydroxy-2,2-dihaloethyl)uracil (**13**, **14**), 1-β-D-arabinofuranosyl-4-thiothymine (**20**), and 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)-4-thiothymine (**37**) or longer chain alkyl groups, namely, 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)-5-[1-hydroxy-2-chloro-2-(ethoxy carbonyl)ethyl]uracil (**29**), 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)-5-[1-hydroxy-2-bromo-2-(ethoxycarbonyl)ethyl]uracil (**30**), and 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)-5-[1-hydroxy-2-iodo-carboxyethyl]uracil (**31**) were inactive up to a concentration of 100 μg/mL against *M. bovis*, *M. tuberculosis*, and *M. avium*. These results suggest that alkynyl functionalities contribute to antituberculosis activity in compounds **3**−**8** and **22**−**26**.

To further establish the structural requirements at the 5-position for antimycobacterial activity, we sought to determine the potential of 5-halo analogs of 1-β-D-arabinofuranosyluracil (**15**, **16**) and 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)uracil (**32**, **33**). Within these, none of the compounds **15**, **16**, or **32** showed inhibitory effects against *M. bovis*, *M. tuberculosis*, and *M. avium*, except moderate activity of 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)-5-chlorouracil (**33**) against *M. bovis* (50% inhibition at 50 μg/mL). The thio-compounds, 1-β-D-arabinofuranosyl-4-thiouracil (**19**) and 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)-4-thiouracil (**36**) did not exert any antimycobacterial activity similar to other thio derivatives **20** and **37**.

To examine whether the most promising compounds are acting as promiscuous inhibitors whose activity is dependent upon their aggregation in biological medium or specific inhibitors of mycobacteria, we determined the activity of selected nucleosides 1-β-D-arabinofuranosyl-5-dodecynyluracil (**5**) and 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)-5-tridecynyluracil (**25**) in the presence of detergents (Figure 1). Initially, the effect of two detergents (Tween-20 and Triton X-100) on *M. bovis* viability and growth was determined (data not shown) in the presence of concentrations of detergents up to their critical micellar concentrations (CMC).[32,33] The concentrations of detergents that had no effect on *M. bovis* viability and growth were then used in the assay to determine the activity of **5** and **25** (Figure 1). It was interesting to note that the inhibition profiles of the two selected compounds did not change in the presence of both detergents, suggesting that they are not acting as promiscuous inhibitors, but rather have specific antimycobacterial activity.

Finally, the most active inhibitors **5**, **24**, and **25** were also evaluated for their activity toward the rifampicin-resistant strain

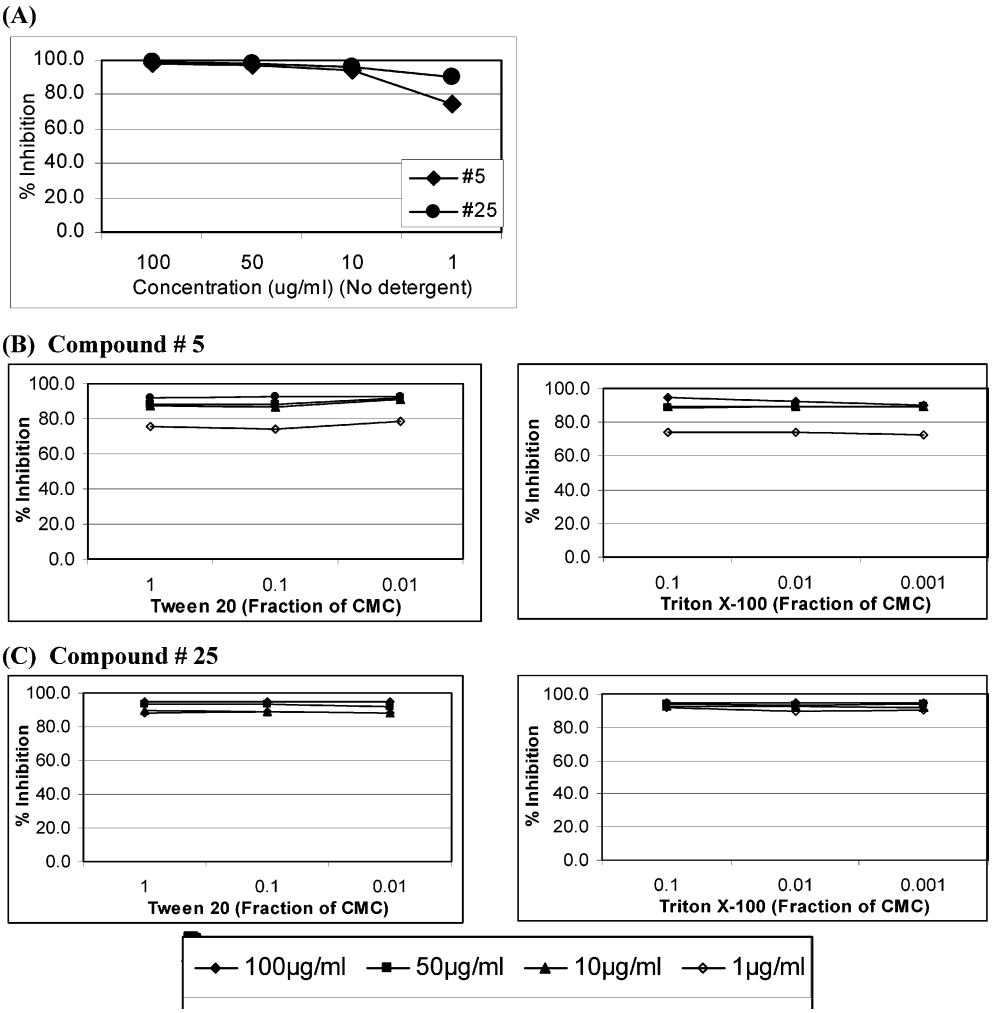


Figure 1. The effect of detergents on the antimycobacterial activity of compounds **5** and **25**. Panel A shows the dose response of compounds **5** and **25** in the absence of detergent. The control bacterial well gave an average reading of 89 897. Panel B shows the activity of compound **5**, and panel C shows the activity of compound **25** in the presence of two different detergents. Both compounds were tested at four different concentrations (depicted by individual lines), as shown in each graph.

Table 2. In Vitro Antibacterial Activities of Pyrimidine Nucleosides against Gram-Positive and Gram-Negative Bacteria

	mean inhibitory concentration[a] (MIC_{100}) μg/mL (% inhibition)											
cmpd	Ec[c]	Ea[c]	Pv[c]	Pa[c]	St[c]	Se[c]	Sa[c]	Bs[c]	Lm[c]	Spy[c]	Spn[c]	Ef[c]
23	>100	>100	>100	>100	>100	100 (100)	100 (100)	>100	100 (100)	100 (100)	100 (100)	100 (100)
24	>100	>100	>100	>100	>100	100 (100)	100 (100)	>100	100 (100)	100 (100)	100 (100)	100 (100)
25	>100	>100	>100	>100	>100	100 (100)	100 (100)	>100	100 (100)	100 (100)	100 (100)	100 (100)
26	>100	>100	>100	>100	>100	100 (100)	100 (100)	>100	>100	>100	>100	>100
ref1[b]	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
ref2[b]	100 (100)	100 (100)	>100	100 (100)	100 (100)	100, 10, 1 (100)	100, 10, 1 (100)	100 (100%)	100, 10, 1 (100)	100, 10, 1 (100)	100, 10, 1	
ref3[b]	>100	>100	>100	>100	(50)	(50)	(100)	(50)	(100)	(100)	(100)	(100)

[a] Antibacterial activity against Gram-positive and Gram-negative bacteria was determined at concentrations 100, 50, 10, and 1 μg/mL using microtiter broth dilution technique. Concentration of compounds exhibiting 100% inhibition in bacterial growth. [b] Positive control drugs; ref 1 = isoniazid, ref 2 = rifampicin, ref 3 = vancomycin at 1 μg/mL. [c] Ec = *E. coli*; Ea = *E. aerogenes*; Pv = *P. vulgaris*; Pa = *P. aeruginosa*; St = *S. typhimurium*; Se = *S. epidermidis*; Sa = *S. aureus*; Bs = *B. subtilis*; Lm = *L. monocytogenes*; Spy = *S. pyogenes*; Spn = *S. pneumoniae*; Ef = *E. faecalis.*

of *M. tuberculosis*, H37Rv by BACTEC assay.[34] Intriguingly, all of the compounds exerted MIC_{90}s against drug-resistant mycobacteria at concentrations ranging from 1 to 10 μg/mL, where rifampicin showed no activity at 2 μg/mL and isoniazid provided 100% inhibition at 1 μg/mL.

An in vitro phosphorolysis study of selected pyrimidine nucleosides **5** and **25**, in the presence of *E. coli* thymidine phosphorylase at 37 °C for 30 min was carried out to determine their glycosidic bond stability.[35] Up to the maximum time investigated, cleavage products of **5** and **25** were not obtained (<10%) in contrast to control thymidine (>70% cleavage in 30 min), suggesting that they are resistant to glycosidic bond cleavage.

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 pyrimidine and purine nucleoside biosynthesis. It is possible that our active nucleoside analogs, after their metabolic conversion to phosphorylated forms by mycobacterial kinases, may be selectively inhibiting its DNA and RNA synthesis, by acting as substrates and inhibitors of metabolic enzymes of DNA/RNA synthesis.

The compounds **3**−**16**, **19**, **20**, **22**−**33**, **36**, and **37** were also tested for MICs for a variety of gram-positive (*S. aureus*, *S. epidermis*, *E. faecalis*, *B. subtilis*, *S. pneumonaie*, *S. pyogenes*, *L. monocytogenes*) and gram-negative (*S. typhimurium*, *E. coli*, *P. vulgaris*, *P. aeruginosa*) bacteria. In these species, compounds **23**−**26** showed a specific spectrum of action, being active mainly toward gram-positive pathogens. Compounds 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)-5-decynyluracil (**23**), 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)-5-dodecynyluracil (**24**), and 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)-5-tridecynyluracil (**25**) exhibited activity against *S. aureus*, *S. epidermis*, *E. faecalis*, *S. pneumonaie*, *S. pyogenes*, and *L. monocytogenes* (MIC_{100} =100 μg/mL; Table 2). Compound 1-(2′ -deoxy-2′-fluoro-β-D-ribofuranosyl)-5-tetradecynyluracil (**26**) possessed activity only against *S. aureus* and *S. epidermis* (MIC_{100} =100 μg/mL). MICs obtained at higher concentrations against these bacteria suggested that they possess higher specificity for mycobacterial species.

The MTT test was performed to evaluate the toxicity of promising compounds (**4**−**8**, **22**−**26**) in vitro against Vero cells and human foreskin fibroblast (HFF cells). No significant toxicity was observed up to a concentration of 100 μg/mL (CC_{50} >100 μg/mL).

Summary

Identification of new lead antimycobacterial compounds that work by different mechanisms of action is necessary for the treatment of tuberculosis due to re-emergence of infection globally and resistance to existing drugs. In this work, among a series of 5-substituted 1-β-D-2′-arabinofuranosyl and 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl) pyrimidine nucleosides, 1-β-D-2′-arabinofuranosyl-5-dodecynyluracil (**5**), 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)-5-dodecynyluracil (**24**), and 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)-5-tridecynyluracil (**25**) emerged with potent antimycobacterial activity at concentrations similar or close to the reference drug. Importantly, the most efficacious analogs are also active against drug-resistant *M. tuberculosis*. The salient feature of the identified molecules, that is, potent broad spectrum activity against drug-sensitive and drug-resistant strains of mycobacteria as well as several other gram-positive bacterial organisms, is an important step for the identification of new antituberculosis agents with different resistance patterns or mechanisms of action. It is envisioned that compounds **5**, **24**, and **25** would have better ADME/T properties as compared to their 2′-deoxyuridine derivatives.[22,23] Further biochemical studies to elucidate the mechanism of action of these compounds and in vivo tests are warranted. The new series of inhibitors identified in this report hold promise for the development of a new class of agents for TB infections.

Experimental Section

Melting points were determined with a Buchi capillary apparatus and are uncorrected. ^{1}H NMR, ^{19}F NMR, and ^{13}C NMR spectra were determined for solutions in Me_2SO-d_6 or CD_3OD on a Bruker AM 300 spectrometer. ^{13}C NMR (J modulated spin echo) spectra were determined for selected compounds, where methyl and methyne carbon resonances appear as positive peaks and 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. 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 Machery−Nagel Alugram SiL G/uv silica gel slides (20 μM thickness). 1-β-D-arabinofuranosyl-5-iodouracil (**2**), 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)-5-iodouracil (**21**), 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)-uracil (**34**), and 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)thymine (**35**) were synthesized using procedures as described earlier.[23,29]

Preparation of 5-Alkynyl Pyrimidine Nucleosides. A full procedure is provided for 1-β-D-arabinofuranosyl-5-heptynyluracil (**3**). For other 5-alkynyl analogs, only brief spectroscopic data are presented.

1-β-D-Arabinofuranosyl-5-heptynyluracil (3). Tetrakis(triphenylphosphine) palladium(0) (94 mg, 0.081 mmol), copper(I) iodide (31 mg, 0.162 mmol), diisopropylethylamine (0.28 mL, 1.62 mmol), and 1-heptyne (0.32 mL, 2.43 mmol) were added to a solution of 1-β-D-arabinofuranosyl-5-iodouracil (**2**; 300 mg, 0.81 mmol) in anhydrous dimethylformamide (30 mL). The orange-colored reaction mixture was stirred at room temperature for 19 h in a nitrogen atmosphere (the progress of the reaction was monitored by TLC in MeOH/$CHCl_3$ (12:88; v/v). After 19 h of stirring, 15 drops of 5% 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 (95:5, v/v) as eluent to yield **3** (120 mg, 44%) as a solid; mp 175−180 °C (dec); ^{1}H NMR (DMSO-d_6) δ 0.87 (t, J = 7.0 Hz, 3H, CH_3), 1.23−1.39 (m, 4H, 2 × CH_2), 1.48 (m, 2H, β-CH_2), 2.34 (t, J = 7.0 Hz, 2H, α-CH_2), 3.50−3.65 (m, 2H, H-5′), 3.72 (m, 1H, H-4′), 3.89 (m, 1H, H-3′), 3.98 (m,1H, H-2′), 5.11 (t, J = 5.2 Hz, 1H, 5′-OH), 5.48 and 5.60 (m, 1H each, 2′-OH, 3′-OH), 5.95 (d, J = 4.3 Hz, 1H, H-1′), 7.79 (s, 1H, H-6), 11.57 (s, 1H, NH); ^{13}C NMR (CD_3-OD) δ 14.33 (CH_3), 20.17, 23.28, 29.49 (3 × CH_2), 32.24 (α-CH_2), 62.38 (C-5′), 72.60 (C-β), 76.96, 77.46 (C-2′ and C-3′), 86.44 (C-4′), 87.58 (C-1′), 94.84 (C-α), 99.84 (C-5), 145.77 (C-6), 151.19 (C-2), 164.77 (C-4). Anal. ($C_{16}H_{22}N_2O_6$) C, H, N.

1-β-D-Arabinofuranosyl-5-decynyluracil (4). Yield 49%; mp 185−189 °C (dec); ^{1}H NMR (DMSO-d_6) δ 0.85 (t, J = 7.0 Hz, 3H, CH_3), 1.22−1.38 (m, 10H, 5 × CH_2), 1.46 (m, 2H, β-CH_2), 2.34 (t, J = 7.0 Hz, 2H, α-CH_2), 3.54−3.63 (m, 2H, H-5′), 3.73 (m, 1H, H-4′), 3.89 (m, 1H, H-3′), 3.98 (m, 1H, H-2′), 5.10 (t, J = 5.3 Hz, 1H, 5′-OH), 5.47 and 5.59 (m, 1H each, 2′-OH, 3′-OH), 5.95 (d, J = 4.3 Hz, 1H, H-1′), 7.78 (s, 1H, H-6), 11.56 (s, 1H, NH); ^{13}C NMR (CD_3OD) δ 14.46 (CH_3), 20.21, 23.72, 29.79, 30.04, 30.26, 30.34 (6 × CH_2), 33.02 (α-CH_2), 62.39 (C-5′), 72.60 (C-β), 76.96, 77.47 (C-2′ and C-3′), 86.46 (C-4′), 87.59 (C-1′), 94.84 (C-α), 99.84 (C-5), 145.61 (C-6), 151.17 (C-2), 164.77 (C-4). Anal. ($C_{19}H_{28}N_2O_6$) C, H, N.

1-β-D-Arabinofuranosyl-5-dodecynyluracil (5). Yield 48%; mp 182−187 °C (dec); ^{1}H NMR (DMSO-d_6) δ 0.85 (t, J = 7.0 Hz, 3H, CH_3), 1.23−1.37 (m, 14H, 7 × CH_2), 1.47 (m, 2H, β-CH_2), 2.34 (t, J = 7.0 Hz, 2H, α-CH_2), 3.53−3.62 (m, 2H, H-5′), 3.73 (m, 1H, H-4′), 3.89 (m, 1H, H-3′), 3.98 (m, 1H, H-2′), 5.11 (t, J = 5.2 Hz, 1H, 5′-OH), 5.48 and 5.60 (m, 1H each, 2′-OH, 3′-OH), 5.96 (d, J = 4.3 Hz, 1H, H-1′), 7.78 (s, 1H, H-6), 11.57 (s, 1H, NH); ^{13}C NMR (CD_3OD) δ 14.45 (CH_3), 20.21, 23.72, 29.79, 30.04, 30.29, 30.46, 30.67, 30.72 (8 × CH_2), 33.07 (α-CH_2), 62.39 (C-5′), 72.60 (C-β), 76.96, 77.47 (C-2′ and C-3′), 86.48 (C-4′), 87.59 (C-1′), 94.85 (C-α), 99.85 (C-5), 145.62 (C-6), 151.18 (C-2), 164.78 (C-4). Anal. ($C_{21}H_{32}N_2O_6$) C, H, N.

1-β-D-Arabinofuranosyl-5-tridecynyluracil (6). Yield 41%; mp 180−186 °C (dec); ^{1}H NMR (DMSO-d_6) δ 0.85 (t, J = 7.0 Hz, 3H, CH_3), 1.18−1.38 (m, 16H, 8 × CH_2), 1.46 (m, 2H, β-CH_2), 2.34 (t, J = 7.0 Hz, 2H, α-CH_2), 3.55−3.63 (m, 2H, H-5′), 3.73 (m, 1H, H-4′), 3.89 (m, 1H, H-3′), 3.97 (m, 1H, H-2′), 5.10, 5.47 and 5.59 (m, 1H each, 2′-OH, 3′-OH, 5′-OH), 5.95 (d, J = 4.27 Hz, 1H, H-1′), 7.79 (s, 1H, H-6), 11.56 (s, 1H, NH); ^{13}C NMR (CD_3OD) δ 14.45 (CH_3), 20.21, 23.75, 29.79, 30.04, 30.29, 30.48,

30.67, 30.74, 30.76 (9 × CH_2), 33.09 (α-CH_2), 62.39 (C-5′), 72.60 (C-*β*), 76.97, 77.48 (C-2′ and C-3′), 86.47 (C-4′), 87.59 (C-1′), 94.84 (C-α), 99.84 (C-5), 145.63 (C-6), 151.18 (C-2), 164.77 (C-4). Anal. ($C_{22}H_{34}N_2O_6$) C, H, N.

1-*β*-D-Arabinofuranosyl-5-tetradecynyluracil (7). Yield 40%; mp 180−185 °C (dec); ^{1}H NMR (DMSO-d_6) *δ* 0.85 (t, *J* = 7.0 Hz, 3H, CH_3), 1.24−1.37 (m, 18H, 9 × CH_2), 1.47 (m, 2H, *β*-CH_2), 2.34 (t, *J* = 7.0 Hz, 2H, α-CH_2), 3.54−3.65 (m, 2H, H-5′), 3.73 (m, 1H, H-4′), 3.89 (m, 1H, H-3′), 3.98 (m,1H, H-2′), 5.09 (t, *J* = 5.2 Hz, 1H, 5′-OH), 5.46 and 5.59 (m, 1H each, 2′-OH, 3′-OH), 5.95 (d, *J* = 4.3 Hz, 1H, H-1′), 7.78 (s, 1H, H-6), 11.56 (s, 1H, NH); ^{13}C NMR (CD_3OD) *δ* 14.47 (CH_3), 20.22, 23.75, 29.79, 30.04, 30.29, 30.49, 30.67, 30.74, 30.77, 30.79 (10 × CH_2), 33.09 (α-CH_2), 62.39 (C-5′), 72.60 (C-*β*), 76.97, 77.48 (C-2′ and C-3′), 86.48 (C-4′), 87.59 (C-1′), 94.85 (C-α), 99.84 (C-5), 145.63 (C-6), 151.17 (C-2), 164.78 (C-4). Anal. ($C_{23}H_{36}N_2O_6$) C, H, N.

1-*β*-D-Arabinofuranosyl-5-(4-*n*-propylphenylethynyl)uracil (8). Yield 54%; mp 217−221 °C (dec); ^{1}H NMR (DMSO-d_6) *δ* 0.88 (t, *J* = 7.0 Hz, 3H, CH_3), 1.60 (m, 2H, CH_2), 2.57 (m, 2H, CH_2), 3.57−3.68 (m, 2H, H-5′), 3.76 (m, 1H, H-4′), 3.92 (m, 1H, H-3′), 4.03 (m, 1H, H-2′), 5.17, 5.49 and 5.65 (m, 1H each, 2′-OH, 3′-OH, 5′-OH), 5.99 (d, *J* = 4.6 Hz, 1H, H-1′), 7.20−7.45 (m, 4H, aromatic), 8.05 (s, 1H, H-6), 11.72 (bs, 1H, NH); ^{13}C NMR (CD_3-OD) *δ* 14.04 (CH_3), 25.53, 38.91 (2 × CH_2), 62.32 (C-5′), 77.03, 77.35 (C-2′ and C-3′), 81.12 (C-*β*), 86.50 (C-4′), 87.74 (C-1′), 93.82 (C-α), 99.39 (C-5), 121.56, 129.53, 132.35, 144.51 (C-phenyl), 146.18 (C-6), 151.10 (C-2), 164.38 (C-4). Anal. ($C_{20}H_{22}N_2O_6$) C, H, N.

1-*β*-D-Arabinofuranosyl-4-thiouracil (19). To a stirred suspension of 1-*β*-D-arabinofuranosyluracil (**17**; 1 g, 4.09 mmol), in acetic anhydride (25 mL), 4-dimethylamino pyridine (50 mg, 0.41 mmol) was added. The reaction mixture was stirred at room temperature for 5 h. Acetic anhydride was removed in vacuo, and the syrup was coevaporated with ethanol (2 × 25 mL). The residue obtained was dried on high vacuum. To this residue, Lawesson's reagent (2.0 g, 4.94 mmol) and dry 1,4-dioxane (50 mL) were added. The mixture was refluxed for 3 h. After the reaction mixture had cooled, the solvent was removed in vacuo. The crude product thus obtained was treated with a saturated solution of ammonia in methanol (20 mL) and stirred at room temp for 3 h. The reaction mixture was concentrated in vacuo, and the residue was purified on a silica gel column using chloroform/methanol (9:1, v/v) as the eluent to afford **19** (400 mg, 38%) as a syrup. ^{1}H NMR (DMSO-d_6) *δ* 3.59 (m, 2H, H-5′), 3.76 (m, 1H, H-4′), 3.89 (m, 1H, H-3′), 4.02 (m, 1H, H-2′), 5.05 (t, *J* = 5.2 Hz, 1H, 5′-OH), 5.49 and 5.63 (m, 1H each, 2′-OH, 3′-OH), 5.95 (d, *J* = 4.3 Hz, 1H, H-1′), 6.28 (d, *J* = 7.3 Hz, 1H, H-5), 7.56 (d, *J* = 7.6 Hz, 1H, H-6), 12.69 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) *δ* 60.64 (C-5′), 74.94, 75.38 (C-2′ and C-3′), 85.17 (C-4′), 85.79 (C-1′), 111.19 (C-5), 137.38 (C-6), 147.55 (C-2), 189.78 (C-4). Anal. ($C_9H_{12}N_2O_5S$) C, H, N.

1-*β*-D-Arabinofuranosyl-4-thio-5-methyluracil (20). This product was synthesized by the procedure described for the synthesis of **19** starting from 1-*β*-D-arabinofuranosyl-5-methyluracil (**18**) as a syrup in 34% yield. ^{1}H NMR (DMSO-d_6) *δ* 1.96 (s, 3H, CH_3), 3.62 (m, 2H, H-5′), 3.75 (m, 1H, H-4′), 3.91 (m, 1H, H-3′), 4.04 (m, 1H, H-2′), 5.13 (t, *J* = 5.2 Hz, 1H, 5′-OH), 5.48 and 5.61 (m, 1H each, 2′-OH, 3′-OH), 5.95 (d, *J* = 4.6 Hz, 1H, H-1′), 7.69 (s, 1H, H-6), 12.70 (s, 1H, NH). Anal. ($C_{10}H_{14}N_2O_5S$) C, H, N.

1-(2′-Deoxy-2′-fluoro-*β*-D-ribofuranosyl)-5-heptynyluracil (22). Compound **22** was prepared from **21** by using the procedure described for the synthesis of **3** in 77% yield as a syrup. ^{1}H NMR (DMSO-d_6) *δ* 0.87 (t, 3H, CH_3), 1.23−1.36 (m, 4H, 2 × CH_2), 1.48 (m, 2H, *β*-CH_2), 2.34 (t, 2H, α-CH_2), 3.57−3.82 (m, 2H, H-5′), 3.87 (m, 1H, H-4′), 4.05−4.22 (m, 1H, H-3′), 5.03 (dm, $J_{2',F}$ = 50.6 Hz, 1H, H-2′), 5.30 (t, $J_{5',OH}$ = 5.2 Hz, 1H, 5′-OH), 5.59 (d, $J_{3',OH}$ = 6.7 Hz, 1H, 3′-OH), 5.86 (d, $J_{1',F}$ = 16.8 Hz, 1H, H-1′), 8.22 (s, 1H, H-6), 11.62 (s, 1H, NH); ^{13}C NMR (CD_3OD) *δ* 14.33 (CH_3), 20.17, 23.28, 29.45 (3 × CH_2), 32.21 (α-CH_2), 60.69 (C-5′), 69.09 and 69.29 (d, C-3′, *J* = 17.6 Hz), 72.39 (C-*β*), 84.79 (C-4′), 90.05 and 89.59 (C-1′, *J* = 35.2 Hz), 96.24 and 93.78 (d, C-2′, *J* = 185.7 Hz), 95.28 (C-α), 101.31 (C-5), 144.05 (C-6), 151.00 (C-2), 164.57 (C-4). Anal. ($C_{16}H_{21}FN_2O_5$) C, H, N.

1-(2′-Deoxy-2′-fluoro-*β*-D-ribofuranosyl)-5-decynyluracil (23). Compound **23** was prepared from **21** by using the procedure described for the synthesis of **3** in 86% yield as a foam. ^{1}H NMR (DMSO-d_6) *δ* 0.85 (t, *J* = 6.7 Hz, 3H, CH_3), 1.21−1.39 (m, 10H, 5 × CH_2), 1.47 (m, 2H, *β*-CH_2), 2.33 (t, *J* = 6.7 Hz, 2H, α-CH_2), 3.55−3.81 (m, 2H, H-5′), 3.86 (m, 1H, H-4′), 4.08−4.22 (m, 1H, H-3′), 5.02 (dm, $J_{2',F}$ = 52.8 Hz, 1H, H-2′), 5.31 (t, $J_{5',OH}$ = 4.9 Hz, 1H, 5′-OH), 5.60 (d, $J_{3',OH}$ = 6.7 Hz, 1H, 3′-OH), 5.85 (d, $J_{1',F}$ = 16.2 Hz, 1H, H-1′), 8.23 (s, 1H, H-6), 11.63 (s, 1H, NH); ^{13}C NMR (CD_3OD) *δ* 14.45 (CH_3), 20.20, 23.72, 29.75, 30.02, 30.26, 30.33 (6 × CH_2), 33.03 (α-CH_2), 60.69 (C-5′), 69.29 and 69.07 (d, C-3′, *J* = 16.5 Hz),72.40 (C-*β*), 84.78 (C-4′), 90.05 and 89.60 (d, C-1′, *J* = 34.1 Hz), 96.24 and 93.77 (C-2′, *J* = 186.8 Hz), 95.28 (C-α), 101.31 (C-5), 144.07 (C-6), 151.02 (C-2), 164.58 (C-4); ^{19}F NMR (DMSO-d_6) (C_6F_6 external standard): *δ* −34.9 (ddd, $J_{2',F}$ = 53.7, $J_{1',F}$ = 17.1, $J_{3',F}$ = 21.9 Hz, F-2′). Anal. ($C_{19}H_{27}FN_2O_5$) C, H, N.

1-(2′-Deoxy-2′-fluoro-*β*-D-ribofuranosyl)-5-dodecynyluracil (24). Compound **24** was prepared from **21** by using the procedure described for the synthesis of **3** in 75% yield as a syrup. ^{1}H NMR (DMSO-d_6) *δ* 0.85 (t, *J* = 6.7 Hz, 3H, CH_3), 1.21−1.39 (m, 14H, 7 × CH_2), 1.47 (m, 2H, *β*-CH_2), 2.33 (t, *J* = 6.7 Hz, 2H, α-CH_2), 3.54−3.80 (m, 2H, H-5′), 3.86 (m, 1H, H-4′), 4.10−4.20 (m, 1H, H-3′), 5.02 (dm, $J_{2',F}$ = 52.8 Hz, 1H, H-2′), 5.32 (t, $J_{5',OH}$ = 4.9 Hz, 1H, 5′-OH), 5.58 (d, $J_{3',OH}$ = 6.7 Hz, 1H, 3′-OH), 5.86 (d, $J_{1',F}$ = 16.2 Hz, 1H, H-1′), 8.23 (s, 1H, H-6), 11.64 (s, 1H, NH). Anal. ($C_{21}H_{31}FN_2O_5$) C, H, N.

1-(2′-Deoxy-2′-fluoro-*β*-D-ribofuranosyl)-5-tridecynyluracil (25). Compound **25** was prepared from **21** by using the procedure described for the synthesis of **3** in 88% yield as a syrup. ^{1}H NMR (DMSO-d_6) *δ* 0.85 (m, 3H, CH_3), 1.22−1.37 (m, 16H, 8 × CH_2), 1.46 (m, 2H, *β*-CH_2), 2.33 (t, *J* = 6.7 Hz, 2H, α-CH_2), 3.55−3.82 (m, 2H, H-5′), 3.87 (m, 1H, H-4′), 4.23−4.09 (m, 1H, H-3′), 5.02 (dm, $J_{2',F}$ = 52.5 Hz, 1H, H-2′), 5.30 (t, $J_{5',OH}$ = 4.9 Hz, 1H, 5′-OH), 5.59 (d, $J_{3',OH}$ = 6.4 Hz, 1H, 3′-OH), 5.86 (d, $J_{1',F}$ = 17.4 Hz, 1H, H-1′), 8.22 (s, 1H, H-6), 11.63 (s, 1H, NH); ^{13}C NMR (CD_3OD) *δ* 14.46 (CH_3), 20.20, 23.75, 29.75, 30.01, 30.29, 30.49, 30.65, 30.75, 30.77 (9 × CH_2), 33.08 (α-CH_2), 60.69 (C-5′), 69.29 and 69.07 (d, C-3′, *J* = 16.5 Hz),72.41 (C-*β*), 84.80 (C-4′), 90.05 and 89.60 (d, C-1′, *J* = 34.1 Hz), 96.24 and 93.77 (d, C-2′, *J* = 186.8 Hz), 95.29 (C-α), 101.32 (C-5), 144.05 (C-6), 151.01 (C-2), 164.58 (C-4); ^{19}F NMR (DMSO-d_6) (C_6F_6 external standard): *δ* −35.0 (ddd, $J_{2',F}$ = 53.7, $J_{1',F}$ = 17.1, $J_{3',F}$ = 21.9 Hz, F-2′). Anal. ($C_{22}H_{33}FN_2O_5$) C, H, N.

1-(2′-Deoxy-2′-fluoro-*β*-D-ribofuranosyl)-5-tetradecynyluracil (26). Compound **26** was prepared from **21** by using the procedure described for the synthesis of **3** in 79% yield as a syrup. ^{1}H NMR (DMSO-d_6) *δ* 0.84 (t, *J* = 6.7 Hz, 3H, CH_3), 1.21−1.37 (m, 18H, 9 × CH_2), 1.45 (m, 2H, *β*-CH_2), 2.33 (t, *J* = 6.7 Hz, 2H, α-CH_2), 3.55−3.79 (m, 2H, H-5′), 3.86 (m, 1H, H-4′), 4.06−4.23 (m, 1H, H-3′), 5.02 (dm, $J_{2',F}$ = 53.4 Hz, 1H, H-2′), 5.29 (t, $J_{5',OH}$ = 5.2 Hz, 1H, 5′-OH), 5.59 (d, $J_{3',OH}$ = 6.4 Hz, 1H, 3′-OH), 5.85 (d, $J_{1',F}$ = 17.1 Hz, 1H, H-1′), 8.22 (s, 1H, H-6), 11.62 (s, 1H, NH); ^{13}C NMR (CD_3OD) *δ* 14.47 (CH_3), 20.22, 23.75, 29.75, 30.00, 30.29, 30.49, 30.65, 30.77, 30.79, 30.93 (10 × CH_2), 33.08 (α-CH_2), 60.69 (C-5′), 69.29 and 69.07 (d, C-3′, *J* = 16.5 Hz), 72.41 (C-*β*), 84.80 (C-4′), 90.04 and 89.59 (d, C-1′, *J* = 34.1 Hz), 96.24 and 93.77 (d, C-2′, *J* = 186.8 Hz), 95.28 (C-α), 101.31 (C-5), 144.04 (C-6), 151.0 (C-2), 164.57 (C-4). Anal. ($C_{23}H_{35}FN_2O_5$) C, H, N.

1-(2′-Deoxy-2′-fluoro-*β*-D-ribofuranosyl)-4-thiouracil (36). To an ice-cold solution of 1-(2′-deoxy-2′-fluoro-*β*-D-ribofuranosyl)-uracil (**34**; 600 mg, 2.44 mmol) in dry pyridine (40 mL) was added acetic anhydride (0.58 mL, 6.1 mmol) dropwise with stirring. The reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 24 h. Pyridine was removed in vacuo. The residues thus obtained were taken in dry 1,4-dioxane (50 mL) and Lawesson's reagent (1.18 g, 2.92 mmol) was added. The reaction mixture was refluxed for 2 h. After the reaction mixture had cooled, solvent

was removed in vacuo. The obtained crude product was treated with a saturated solution of ammonia in methanol (20 mL) and stirred at room temp for 2 h. The reaction mixture was concentrated in vacuo and purified on silica gel column using chloroform/methanol (95:5, v/v) as solvent to yield **36** (280 mg, 44%) as a syrup. ^{1}H NMR (DMSO-d_6) δ 3.56−3.82 (m, 2H, H-5′), 3.89 (m, 1H, H-4′), 4.05−4.19 (m, 1H, H-3′), 5.06 (dm, $J_{2',F}$ = 53.0 Hz, 1H, H-2′), 5.24 (t, $J_{5',OH}$ = 5.2 Hz, 1H, 5′-OH), 5.62 (d, $J_{3',OH}$ = 6.4 Hz, 1H, 3′-OH), 5.84 (d, $J_{1',F}$ = 17.1 Hz, 1H, H-1′), 6.28 (d, J = 7.3 Hz, 1H, H-5), 7.87 (d, J = 7.6 Hz, 1H, H-6), 12.77 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 58.91 (C-5′), 67.10 and 66.88 (d, C-3′, J = 16.5 Hz), 83.14 (C-4′), 87.89 and 87.44 (d, C-1′, J = 34.1 Hz), 94.66 and 92.20 (d, C-2′, J = 185.7 Hz), 112.31 (C-5), 135.38 (C-6), 147.38 (C-2), 190.10 (C-4). Anal. ($C_9H_{11}FN_2O_4S$) C, H, N.

1-(2′-Deoxy-2′-fluoro-β-D-ribofuranosyl)-4-thio-5-methyluracil (37). This product was synthesized according to the procedure described for **36** starting from 1-(2′-deoxy-2′-fluoro-β-D-ribofuranosyl)-5-methyluracil (**35**) as a syrup in 55% yield. ^{1}H NMR (DMSO-d_6) δ 1.94 (s, 3H, CH_3), 3.57−3.85 (m, 2H, H-5′), 3.89 (m, 1H, H-4′), 4.07−4.24 (m, 1H, H-3′), 5.06 (dm, $J_{2',F}$ = 53.0 Hz, 1H, H-2′), 5.33 (t, $J_{5',OH}$ = 5.2 Hz, 1H, 5′-OH), 5.62 (d, $J_{3',OH}$ = 6.7 Hz, 1H, 3′-OH), 5.86 (d, $J_{1',F}$ = 16.5 Hz, 1H, H-1′), 8.02 (s, 1H, H-6), 12.78 (s, 1H, NH). Anal. ($C_{10}H_{13}FN_2O_4S$) C, H, N.

In Vitro Antimycobacterial Activity Assay (*M. tuberculosis*, *M. bovis*, *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 MABA assay.[31] Test compounds were dissolved in DMSO at 10 mg/mL, 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 micrograms/mL in triplicates. 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%. Frozen mycobacterial inocula were diluted in medium 7H9GC and added to each well at final concentration of 2.5×10^5 CFU/mL. Sixteen control wells consisted of **8** with bacteria alone (B) and **8** 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 to 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 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 all *M. bovis*, *M. tuberculosis*, and *M. avium*. Rifampicin and clarithromycin were used as positive controls. As negative controls, DMSO (2 μL, 1 μL, 0.2 μL, 0.02 μL) was added to the B well at concentrations 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 ranging between 60 000 and 100 000.

The antimycobacterial activity of compounds **5** and **25** was also determined against *M. bovis* in the presence of various detergents using MABA assay. For these experiments, titrating amounts of detergents, that is, Tween-20, and Triton X-100 (Fisher Scientific, CMC: 0.05 mM and 0.3 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 microgram/mL (final concentration) for 15 min before adding to the *M. bovis* wells in triplicates. The data is shown as the average of the triplicates, and the standard deviation was within 5%. 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 the 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 CMC. Therefore, the compounds were tested in the presence of nontoxic doses of detergents.

Antimycobacterial Activity against a Drug-Resistant Strain of *M. tuberculosis*: The activity of compounds **5**, **24**, and **25** was determined against rifampicin-resistant *M. tuberculosis* H37Rv (ATCC 35838, resistant to rifampicin at 2 μg/mL) using a radiometric-BACTEC assay.[34] 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) media and incubated at 37 °C. Two-fold dilutions of test compounds were delivered to the inoculum-containing BACTEC vials. Negative control vials consisted of media with bacteria inoculum, media with bacteria inoculum at 1:100, and media alone. As reference drugs, rifampicin and isoniazid were used at their MIC_{90} concentration. 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 (△GI) was recorded for each compound. Percent inhibition was defined as (GI of test sample/GI of control) × 100. For the no drug control, the △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 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* were used for the determination of the in vitro antibacterial activity of the studied compounds. 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 a control. Plates were incubated for 3 days at 37 °C. The color reaction involved adding 10 μL 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 (Abs 560−650 nm) following an overnight incubation at 37 °C.

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

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