# Synthesis of Some New Pyrido[2,3d]pyrimidines and Their Ribofuranosides as Possible Antimicrobial Agents

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ABSTRACT: The ribofuranosides, namely, 4-amino-5,7-disubstituted-1-[2',3',5'-tri-O-benzoyl-α-D-ribofuranosyl]pyrido-[2,3-d] pyrimidine-2(1H)-thiones, have been synthesized by the condensation of trimethylsilyl derivatives of 5,7-disubstituted pyrido[2,3-d]pyr*imidine-2(1H)-thiones with* β-D-ribofuranose-1-acetate-2,3,5-tribenzoate in the presence of SnCl<sub>4</sub>. The heterocyclic bases, namely, 4-amino-5,7-disubstituted pyrido[2,3-d]pyrimidine-2(1H)-thiones, were synthesized by the treatment of 2-amino-3-cyano-4,6-disubstituted pyridines with thiourea. The structures of all the synthesized ribofuranosides and their precursors have been established by elemental analysis, IR, and <sup>1</sup>H NMR spectral data. The <sup>13</sup>C NMR data of ribofuranosides has also been presented. All the synthesized heterocyclic bases and their ribofuranosides have been screened for their antibacterial and antifungal activities. © 2001 John Wiley & Sons, Inc. Heteroatom Chem 12:52-56, 2001

## INTRODUCTION

The importance of the pyrido[2,3-*d*]pyrimidine nucleus has been well proved and is illustrated by the large number of patents of it. Perusal of the literature on pharmacological studies reported for the pyrido[2,3-*d*]pyrimidines class of compounds reveals that these compounds have immense chemotherapeutic importance as antibacterial, [1,2] anti-

fungal [3,4], antitumor [5], antiinflammatory [6], anticancer [7], and phosphodiesterase inhibitor compounds [8].

In recent years, there has been significant interest in the synthesis of nucleosides of various heterocyclic bases as antiherpes [9], antifungal [10], antibacterial [11], and antiviral [12] agents. Motoo et al. [13] reported the anticancer activity of dioxo derivatives of pyrido[2,3-*d*]pyrimidine. The importance of various heterocyclic bases such as purines, pyrimidines, and pteridinones as intermediates for the synthesis of biologically active nucleosides and the utility of pyrido[2,3-*d*]pyrimidines as promising drugs prompted our interest in exploring some new pyrido[2,3-*d*]pyrimidines and their nucleoside analogues, with the attempt to discover some potential compounds of medicinal importance.

### RESULTS AND DISCUSSION

2-Amino-3-cyano-4,6-disubstituted pyridines II were prepared from chalcones I [14] on treatment with ammonium acetate in ethanol via Michael-type condensation. Compounds II, upon condensation with thiourea, gave 4-amino-5,7-disubstituted pyrido[2,3d]pyrimidine-2(1H)-thiones III [15]. The ribofuranosides, namely, 4-amino-5,7-disubstituted-1-[2',3', 5'-tri-O-benzoyl- $\alpha$ -D-ribofuranosyl]pyrido[2,3-d]pyrimidine-2(1H)-thiones VI [16] were prepared by the condensation of trimethylsilyl derivatives of pyrido[2,3-d]pyrimidines IV with a sugar, that is,  $\beta$ -Dribofuranose-1-acetate-2,3,5-tribenzoate V, in presence of tin tetrachloride at 0°C. The proposed

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structures of the synthesized compounds are well supported by spectroscopic and elemental analysis data (Table 1).

#### SPECTRAL STUDIES

#### IR Spectra

The characteristic IR bands of compounds are given in Table 2. In the IR spectra of compounds II, bands due to -CN and  $NH_2$  groups appeared in the region 2210–2200 cm<sup>-1</sup> and 3450–3300 cm<sup>-1</sup>, respectively. The disappearance of a band due to -CN and appearance of a new band due to >C=S in the region 1250–1200 cm<sup>-1</sup> indicated the formation of compounds III from II. In compounds III, bands due to  $-NH_2$  and >NH groups appeared in the region 3460–3300 cm<sup>-1</sup> and 3200–3150 cm<sup>-1</sup>, respectively.

Disappearance of a band due to >NH in compounds VI and appearance of a new band at 1750– 1730 cm<sup>-1</sup> due to >C=O confirms the ribosilation by the substitution of the >NH proton. The symmetric and asymmetric stretching vibrations due to the C–O–C linkage in compounds VI appeared in the region 1070–1030 cm<sup>-1</sup>

#### <sup>1</sup>H NMR Spectra

The <sup>1</sup>H NMR data for the compounds are given in Table 2. In the <sup>1</sup>H NMR spectra of compounds III, a multiplet due to aromatic protons appeared in the region  $\delta$  6.87–8.02. Signals due to  $-NH_2$  protons were found to be merged with aromatic protons. The >NH proton in compounds III appeared as a singlet at  $\delta$  7.62–8.13.

In the <sup>1</sup>H NMR spectra of ribofuranosides VI, a multiplet due to aromatic protons appeared in the region  $\delta$  6.95–8.18. A peak due to >NH was found to be absent, indicating the site of attachment of the sugar V. The  $\alpha$ -configuration of the ribofuranosides was confirmed by a doublet at  $\delta$  6.40–6.53 (J = 8Hz) [17] due to C'<sub>1</sub>-H. C'<sub>4</sub>-H and C'<sub>5</sub>-2H protons of the sugar moiety causing a multiplet in the region  $\delta$  4.37–4.84, and protons at C'<sub>2</sub> and C'<sub>3</sub> appeared in the region  $\delta$  5.71–5.93 as a multiplet. A sharp singlet due to -CH<sub>3</sub> protons in compounds IIIa and VIa was observed at  $\delta$  2.01 and  $\delta$  2.06, respectively, while a singlet due to the -OH proton in compounds IIId and VId was noticed at  $\delta$  9.96 and 10.02, respectively.

The <sup>13</sup>C NMR data for compounds VIa–d are presented in Table 3 and these data are in reasonable agreement for their values.

#### ANTIMICROBIAL ACTIVITY

The compounds III and ribofuranosides VI were screened for their antimicrobial activity following the paper disc method of Varma and Nobles [18]. The concentration applied was 100  $\mu$ g per disk. Streptomycin and mycostatin were used as reference compounds while testing antibacterial and antifungal activity, respectively.

All the compounds were found to be moderately active against various bacteria, such as *Escherichia coli* (gram negative) and *Staphylococcus aureus* (gram positive) and fungi (*Aspergillus niger, Aspergillus flavus*). The comparison of activity indices of ribofuranosides with the activity indices of their parent bases reveal that the ribosylation enhances the activity in almost all the cases, except in the case of ribofuranoside **VId** against *E. coli*. Against *A. niger,* compound **VIb** showed a remarkable increase in activity.

These results have been tabulated in the form of inhibition zone and activity indices (Table 4).

#### EXPERIMENTAL

Melting points were determined in open capillary tubes and are uncorrected. The IR spectra were recorded in KBr disks on a NICOLET-MEGNA FT-IR 550 spectrometer. The <sup>1</sup>H NMR spectra were obtained from an FX 90 Q JEOL type spectrophotom-

							Elemental Analysis % Found (Calcd.)		
Compound	$R^{1}$	R <sup>2</sup>	Molecular Formula	Molecular Weight	Yield (%)	M.P. (°C)	С	Н	Ν
Illa	$C_4H_3O$	4-CH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub>	$C_{18}H_{14}N_4 \text{ OS}$	334	75	105	64.70 (64.67)	4.21 (4 19)	16.75 (16.77)
lllb	$C_4H_3O$	$2-F-C_6H_4$	$C_{17}H_{11}\ N_4OSF$	338	73	178	60.39 (60.35)	3.28	16.54 (16.57)
llic	$C_4H_3O$	$4-Br-C_6H_4$	$C_{17}H_{11} N_4OSBr$	399	69	116	51.15 (51.13)	2.78 (2.76)	14.01 (14.03)
llld	$C_4H_3O$	2-OH–C <sub>6</sub> H <sub>4</sub>	$C_{17}H_{12} \ N_4O_2S$	336	67	138	`60.75 <sup>´</sup> (60.71)	3.60 (3.57)	16.63 (16.67)
Vla	$C_4H_3O$	$4-CH_3-C_6H_4$	$C_{44}H_{34}\:N_4O_8S$	778	71	75	67.90 (67.87)	4.42 (4.37)	7.18 (7.20)
VIb	$C_4H_3O$	$2-F-C_6H_4$	$C_{43}H_{31}N_4O_8SF$	782	68	73	66.03 (65.98)	3.99 (3.96)	7.13 (7.16)
VIc	$C_4H_3O$	$4-Br-C_6H_4$	$C_{43}H_{31}N_4O_8SBr$	843	72	148	61.23 (61.21)	3.72 (3.68)	6.62 (6.64)
Vld	$C_4H_3O$	2-OH–C <sub>6</sub> H <sub>4</sub>	$C_{43}H_{32}N_4O_9S$	780	63	105	66.19 (66.15)	4.12 (4.10)	7.15 (7.18)

**TABLE 1** Characterization Data of Pyrido[2,3-*d*]pyrimidines and Their Ribofuranosides

TABLE 2 The IR and <sup>1</sup>H NMR Spectral Data of Synthesized Compounds

		<sup>1</sup> H NMR (	$\delta$ ppm fi	rom TMS	6)	IR (KBr: $v_{max}$ cm <sup>-1</sup> )				
Compound	>NH	Ar-H (multiplet)	$-CH_3$	-OH	C <sub>1</sub> '-H (doublet)	>NH	-NH <sub>2</sub>	>C $=$ S	>C=O	C-O-C
IIIa IIIb IIIc IIId Via	8.09 7.62 8.13 7.92	7.15–8.00 6.87–7.56 6.93–8.02 6.97–7.69 7.03–8.08	2.01   2.06	  9.96 	  6.42	3180 3150 3200 3170	3460–3340 3455–3320 3450–3335 3445–3320 3450–3335	1250 1230 1225 1230 1235	   1745	  1030
VIb	_	7.06-8.09	_	_	(J = 8Hz) 6.53 (J = 8Hz) 6.43	_	3430–3300 3445–3325	1200 1220	1730 1750	1070 1040
Vld	_	7.11–8.06	_	10.02	(J = 8Hz) 6.40 (J = 8Hz)	_	3440–3310	1215	1730	1035

eter in CDCl<sub>3</sub>/DMSO-d<sub>6</sub>, and <sup>13</sup>C NMR spectra were recorded in CH<sub>3</sub>OH solution, using tetramethylsilane as an internal standard. The purity of each compound was checked by thin-layer chromatography (TLC) using silica gel "G" as adsorbent, and visualization was accomplished by UV light or iodine. Chalcones were synthesized by reported methods [14].

## *Synthesis of 2-Amino-3-cyano-4,6-disubstituted Pyrimidines* **II**

A mixture of the appropriate chalcone (0.045 mol), malononitrile (0.045 mol), and ammonium acetate (0.36 mol) in ethanol (80 mL) was refluxed in a water bath for 8–10 hours. After cooling, the contents of

the flask were poured onto crushed ice with constant stirring to obtain a solid mass, which was washed with water and cold ethanol. The residue was recrystallized from ethanol.

## *Synthesis of 4-Amino-5,7-disubstituted pyrido[2,3-d]pyrimidine-2(1H)-thiones* **III**

A mixture of II (0.01 mol) and thiourea (0.02 mol) was refluxed on an oil bath at 120–130°C for 2 hours with constant stirring. The temperature of the reaction mixture was raised gradually (2 hours) to 180°C, and finally the mixture was heated at 210–220°C for 2 hours. The product so obtained was washed with water, saturated sodium bicarbonate solution, and

Compound	<b>C</b> <sub>2</sub>	$C_4$	$C_5$	$C_6$	<b>C</b> <sub>7</sub>	C <sub>9</sub>	C <sub>10</sub>	Furan–C₅	Ar–C <sub>7</sub>	$CH_3$ –Ar at $C_7$
Vla	159.76	158.83	134.87	130.70	134.17	134.01	133.85	146.41, 109.15	128.05–134.44	21.3
VIb	160.12	158.92	134.97	131.21	134.85	134.50	134.10	148.67, 110.36 116.28 144.31	130.27–153.62	—
VIc	160.04	158.79	134.77	130.55	134.26	134.36	134.05	148.37, 110.21 116.21, 144.17	126.85–135.67	—
Vld	159.68	158.71	134.81	130.85	134.57	134.21	133.81	147.36, 109.26 116.13, 144.36	134.21–150.67	—
	$C'_1$		$C_2'$	$C'_3$		$C'_4$	$C_5'$	_	0    -C-	Ar (OB <sub>z</sub> )
Vla Vlb Vlc Vld	100.80 103.65 102.93 100.67	78 78 79 78	8.45 8.63 9.41 8.96	77.07 77.51 77.83 76.85		73.55 74.85 73.97 73.86	65.0 66.1 66.1 65.4	5 167.52 5 167.21 0 167.64 5 167.83	2, 166.35 , 166.38 , 166.21 , 166.47	126.82–136.02 125.21–136.71 125.38–136.53 127.09–136.82

**TABLE 3** <sup>13</sup>C NMR Spectral Data of Ribofuranosides VIa–VId in CH<sub>3</sub>OH ( $\delta$  ppm from TMS)

TABLE 4 Antimicrobial Activity of Synthesized Pyrido[2,3-d]pyrimidines and Ribofuranosides

		Bacteria	Fungi			
Compound	Escherichia coli	Staphylococcus aureus	Aspergillus niger	Aspergillus flavus. 9.5		
Illa	14.1	19.3	9.9			
IIIb	(0.97) 13.4 (0.92)	(0.92) 18 (0.86)	(1.02) 9.6 (0.99)	(0.96) 9.8 (0.99)		
llic	(0.92) 13.2 (0.91)	(0.86) 22 (1.05)	(0.99) 11.0 (1.13)	(0.99) 10.6 (1.07)		
llld	(0.31) 18 (1.24)	21.5	10.2	(1.07) 10.0 (1.01)		
Vla	14.5	24.8	11.8	(1.07) 11.1 (1.12)		
VIb	16.2 (1.12)	23.7 (1.13)	12.1 (1.25)	(11.7 (1.18)		
VIc	16 (1.10)	24 (1.14)	11.5 (1.18)	11.5 (1.16)		
Vld	15.3 (1.05)	22.5 (1.07)	11.2 (1.15)	10.8 (1.09)		

Note: Values in parenthesis represent activity index; activity index = inhibition area of sample/inhibition area of the standard.

finally, with cold ethanol, and was recrystallized from DMF-ethanol (1:2).

## Synthesis of 4-Amino-5,7-disubstituted-1-[2',3',5'-tri-O-benzoyl-α-D-ribofuranosyl]pyrido-[2,3-d]pyrimidine-2(1H)-thiones **VI**

Compounds III (0.01 mol) were refluxed by stirring under anhydrous conditions for 24 hours with hexamethyldisilazane (HMDS) (60 mL) and  $(NH_4)_2SO_4$  (0.02 g). The clear solution obtained was cooled, and the solvent was removed in vacuo. The resulting tri-

methylsilylated pyrido[2,3-*d*]pyrimidines V were dissolved in anhydrous acetonitrile (40 mL), and a solution of  $\beta$ -D-ribofuranose-1-acetate-2,3,5-triben-zoate (0.011 mol) in dry acetonitrile (20 mL) was then added with stirring. The mixture was cooled to 0°C, a solution of SnCl<sub>4</sub> (1.6 mL) in anhydrous acetonitrile 5 mL was added dropwise, the mixture was stirred until the reaction was determined complete by TLC (2–3 hr) and then poured into saturated NaHCO<sub>3</sub> solution and extracted with CHCl<sub>3</sub>. The organic layer was dried over anhydrous MgSO<sub>4</sub> and concentrated to give the crude nucleosides, which

were recrystallized from ethanol to afford pale yellow needle crystals (VI).

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