Heteroatom Chemistry
Volume 12, Number 1, 2001

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

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Received 27 March 2000; revised 28 September 2000

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]pyrimidine-2(1H)-thiones with β-D-ribofuranose-1-acetate-2,3,5-tribenzoate in the presence of $SnCl_4$. 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 ^1H NMR spectral data. The ^{13}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] antifungal [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[2,3-*d*]pyrimidine-2(1H)-thiones **III** [15]. 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 **VI** [16] were prepared by the condensation of trimethylsilyl derivatives of pyrido[2,3-*d*]pyrimidines **IV** with a sugar, that is, β-D-ribofuranose-1-acetate-2,3,5-tribenzoate **V**, in presence of tin tetrachloride at 0°C. The proposed

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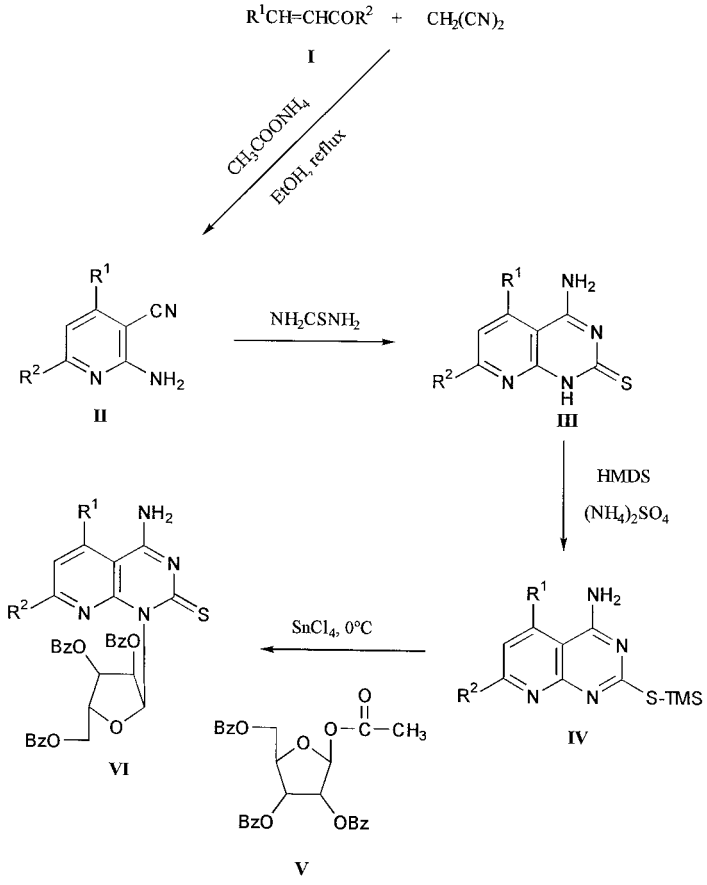

SCHEME 1

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^{-1} and 3450–3300 cm^{-1}, 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^{-1} 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^{-1} and 3200–3150 cm^{-1}, respectively.

Disappearance of a band due to $>NH$ in compounds **VI** and appearance of a new band at 1750–1730 cm^{-1} 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^{-1}

^{1}H NMR Spectra

The ^{1}H NMR data for the compounds are given in Table 2. In the ^{1}H NMR spectra of compounds **III**, a multiplet due to aromatic protons appeared in the region δ 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 δ 7.62–8.13.

In the ^{1}H NMR spectra of ribofuranosides **VI**, a multiplet due to aromatic protons appeared in the region δ 6.95–8.18. A peak due to $>NH$ was found to be absent, indicating the site of attachment of the sugar **V**. The α-configuration of the ribofuranosides was confirmed by a doublet at δ 6.40–6.53 (J = 8Hz) [17] due to C_1'-H. C_4'-H and C_5'-2H protons of the sugar moiety causing a multiplet in the region δ 4.37–4.84, and protons at C_2' and C_3' appeared in the region δ 5.71–5.93 as a multiplet. A sharp singlet due to $-CH_3$ protons in compounds **IIIa** and **VIa** was observed at δ 2.01 and δ 2.06, respectively, while a singlet due to the $-OH$ proton in compounds **IIId** and **VId** was noticed at δ 9.96 and 10.02, respectively.

The ^{13}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 μg 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 ^{1}H NMR spectra were obtained from an FX 90 Q JEOL type spectrophotom-

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

Compound	R^1	R^2	Molecular Formula	Molecular Weight	Yield (%)	M.P. (°C)	*Elemental Analysis % Found (Calcd.)* C	H	N
IIIa	C_4H_3O	4-CH_3–C_6H_4	$C_{18}H_{14}N_4$ OS	334	75	105	64.70 (64.67)	4.21 (4.19)	16.75 (16.77)
IIIb	C_4H_3O	2-F–C_6H_4	$C_{17}H_{11}$ N_4OSF	338	73	178	60.39 (60.35)	3.28 (3.25)	16.54 (16.57)
IIIc	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)
IIId	C_4H_3O	2-OH–C_6H_4	$C_{17}H_{12}$ N_4O_2S	336	67	138	60.75 (60.71)	3.60 (3.57)	16.63 (16.67)
VIa	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)
VId	C_4H_3O	2-OH–C_6H_4	$C_{43}H_{32}$ N_4O_9S	780	63	105	66.19 (66.15)	4.12 (4.10)	7.15 (7.18)

TABLE 2 The IR and ^{1}H NMR Spectral Data of Synthesized Compounds

Compound	^{1}H NMR (δ ppm from TMS) >NH	Ar-H (multiplet)	-CH_3	-OH	C_1'-H (doublet)	IR (KBr: ν_{max} cm^{-1}) >NH	-NH_2	>C=S	>C=O	C–O–C
IIIa	8.09	7.15–8.00	2.01	—	—	3180	3460–3340	1250	—	—
IIIb	7.62	6.87–7.56	—	—	—	3150	3455–3320	1230	—	—
IIIc	8.13	6.93–8.02	—	—	—	3200	3450–3335	1225	—	—
IIId	7.92	6.97–7.69	—	9.96	—	3170	3445–3320	1230	—	—
VIa	—	7.03–8.08	2.06	—	6.42 (J = 8Hz)	—	3450–3335	1235	1745	1030
VIb	—	7.06–8.09	—	—	6.53 (J = 8Hz)	—	3430–3300	1200	1730	1070
VIc	—	6.95–8.18	—	—	6.43 (J = 8Hz)	—	3445–3325	1220	1750	1040
VId	—	7.11–8.06	—	10.02	6.40 (J = 8Hz)	—	3440–3310	1215	1730	1035

eter in $CDCl_3$/DMSO-d_6, and ^{13}C NMR spectra were recorded in CH_3OH 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

TABLE 3 ^{13}C NMR Spectral Data of Ribofuranosides **VIa–VId** in CH_3OH (δ ppm from TMS)

Compound	C_2	C_4	C_5	C_6	C_7	C_9	C_{10}	Furan–C_5	Ar–C_7	CH_3–Ar at C_7
VIa	159.76	158.83	134.87	130.70	134.17	134.01	133.85	146.41, 109.15 115.36, 143.30	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	—
VId	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'	$\overset{O}{\overset{\|\|}{-C-}}$	*Ar* (OB_z)
VIa	100.80	78.45	77.07	73.55	65.05	167.52, 166.35	126.82–136.02
VIb	103.65	78.63	77.51	74.85	66.15	167.21, 166.38	125.21–136.71
VIc	102.93	79.41	77.83	73.97	66.10	167.64, 166.21	125.38–136.53
VId	100.67	78.96	76.85	73.86	65.45	167.83, 166.47	127.09–136.82

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

	Bacteria		*Fungi*	
Compound	*Escherichia coli*	*Staphylococcus aureus*	*Aspergillus niger*	*Aspergillus flavus.*
IIIa	14.1 (0.97)	19.3 (0.92)	9.9 (1.02)	9.5 (0.96)
IIIb	13.4 (0.92)	18 (0.86)	9.6 (0.99)	9.8 (0.99)
IIIc	13.2 (0.91)	22 (1.05)	11.0 (1.13)	10.6 (1.07)
IIId	18 (1.24)	21.5 (1.02)	10.2 (1.05)	10.0 (1.01)
VIa	14.5 (1.0)	24.8 (1.18)	11.8 (1.22)	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)
VId	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 trimethylsilylated pyrido[2,3-*d*]pyrimidines **V** were dissolved in anhydrous acetonitrile (40 mL), and a solution of β-D-ribofuranose-1-acetate-2,3,5-tribenzoate (0.011 mol) in dry acetonitrile (20 mL) was then added with stirring. The mixture was cooled to 0°C, a solution of $SnCl_4$ (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 $NaHCO_3$ solution and extracted with $CHCl_3$. The organic layer was dried over anhydrous $MgSO_4$ and concentrated to give the crude nucleosides, which

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

ACKNOWLEDGMENT

Authors are thankful to the Head, Department of Chemistry, University of Rajasthan Jaipur, for providing laboratory facilities.

REFERENCES

[1] Kuyper, L. F.; Garvey, J. M.; Baccanari, D. P.; Champness, J. N.; Stammers, D. K.; Beddell, C. R. Bio Org Med Chem 1996, 4 (4), 593; Chem Abstr 1996, 125, 86587.
[2] Blankley, C. J.; Doherty, A. M.; Hamby, J. M.; Panek, R. L.; Schroeder, M. C.; Showalker, H. D. H.; Connolly, C. PCT Int Appl WO 9615, 128(Cl. CO 7D, 471/04) May 23, 1996, U.S. Patent Appl. 339 051, Nov 14, 1994; Chem Abstr 1996, 125, 114688.
[3] Sharma, R.; Goyal, R. D.; Prakash, L. Indian J Chem 1992, 31B, 719.
[4] Corell, F.; Massa, S.; Stefancich, M. A.; Panico, S.; Simonelti, N. Farmaco Ed 1984, 39, 95.
[5] Tomita, K.; Chiba, K.; Kashimoto, S.; Shibamori, K.; Tsuzuki, Y. PCT Int Appl WO 95,35,559 (Cl CO 7D 417/04) Dec 21, 1995, Jpn. Patent Appl. 94/156, 578 Jun 14, 1994; Chem Abstr 1996, 124 289559.
[6] Degraw, J. I.; Colwell, W. T.; Sirtonok, F. M.; Smith, R. L.; Piper, J. R. U.S. Patent US 5,536, 724 (Cl 514-258; A 61 K 31/505) July 16, 1996, Chem Abstr 1996, 125, 196375.
[7] Bar, T.; Zimmermann, P.; Boer, R.; Gekeler, V.; Isc, W.; Boss, H.; Ulrich, W. R. PCT Int Appl WO 97, 19, 946 (Cl. CO 7 D 471/04) Jun 5, 1997, CH Appl. 95/3, 322, Nov 24, 1995; Chem Abstr 1997, 127, 81463.
[8] Takayama, K.; Hisamichi, H.; Iwata, M.; Kubota, H.; Aoki, M. PCT Int Appl WO 97, 19, 078 (Cl. CO 7D 471/04), May 29, 1997, Jpn Patent Appl. 96/141, 468, Jun 4, 1996; Chem Abstr 1997, 127, 65784.
[9] Verheggen, I.; Aerschot, A. V.; Snoeck, R.; Janssen, G.; Balzarini, J.; Declereq, E.; Herdewijn, P. J Med Chem 1993, 36, 2033.
[10] Agrofoglio, L.; Suhas, E.; Farese, A.; Condom, R.; Challand, R. S.; Earl, R. A.; Guedi, R. Tetrahedron 1994, 50, 10611.
[11] Mansour, T. S.; Storer, R. Curr Pharm Des 1997, 3, 227.
[12] Huryn, D. M.; Okabe, M. Chem Rev 1992, 92, 1745.
[13] Motoo, H.; Yashio, P.; Isuneo, I.; Iii Meritsuku Ohanj, R.; Ichiro, I.; Yoshihisa, M.; Tokuo, K.; Haruo, O. (Meiji Seika Kaisha Ltd.). Jpn Kokoi Tokkyo Koho 01, 143, 895 [89, 143, 895] (Cl CO 7H 19/23), Jun 6, 1989 6pp; Chem Abstr 1990, 112, 7859.
[14] Furniss, B. S.; Hannaford, A. J.; Rogers, V.; Smith, P. W. G.; Tatchell, A. R.; Vogel's Textbook of Practical Organic Chemistry; Longman Group Ltd., London, 1980; p 796.
[15] Singh, G.; Sharma, A. K.; Mishra, A. K.; Prakash, L. Phosphorus Sulfur Silicon 1998, 133, 83.
[16] Attia, A. M.; Elgemeie, G. H.; Shahada, L. A. Tetrahedron 1997, 53 (51) 17441.
[17] Fei, C. P.; Chan, T. H. Tetrahedron Let 1987, 28, 849.
[18] Varma, R. S.; Nobles, W. L. J Pharm Sci 1972, 61, 112.