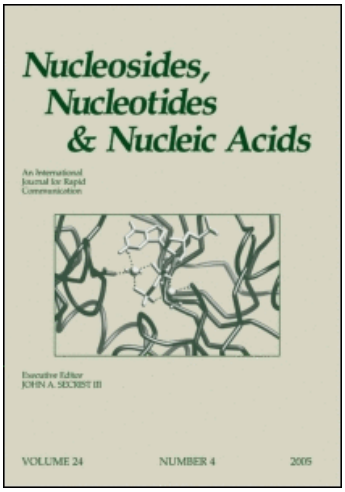

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Synthesis and Antiviral Activity of Some C_2-, C_4-, and C_6-Substituted Pyrazolo[3,4-D]Pyrimidine Acyclonucleosides with the Alkylating Chains of ACV, HBG, and ISO-DHPG

Omar Moukha-chafiq[a]; Mohamed Labd Taha[a]; Hassan Bihi Lazrek[b]; Jean-Jacques Vasseur[c]; Erik De Clercq[d]
[a] Laboratoire de Chimie Bio-Organique, Faculte des Sciences, Agadir, Maroc [b] Laboratoire de Chimie Bio-Organique, Faculte des Sciences I, Marrakech, Maroc [c] Lab. de Chimie Organique Biomoleculaire de synthèse, U.S.T. Montpellier II, Moukhao, Montpellier, France [d] Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium

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SYNTHESIS AND ANTIVIRAL ACTIVITY OF SOME C_2-, C_4-, AND C_6-SUBSTITUTED PYRAZOLO[3,4-D]PYRIMIDINE ACYCLONUCLEOSIDES WITH THE ALKYLATING CHAINS OF ACV, HBG, AND ISO-DHPG

Omar Moukha-chafiq and Mohamed Labd Taha □ *Laboratoire de Chimie Bio-Organique, Faculte des Sciences, Agadir, Maroc*

Hassan Bihi Lazrek □ *Laboratoire de Chimie Bio-Organique, Faculte des Sciences I, Marrakech, Maroc*

Jean-Jacques Vasseur □ *Lab. de Chimie Organique Biomoleculaire de synthèse, U.S.T. Montpellier II, Moukhao, Montpellier, France*

Erik De Clercq □ *Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium*

□ *A useful route to obtain trisubstituted pyrazolo[3,4-d]pyrimidines* ***14–17*** *is described. Those later were coupled with the alkylating agents* ***18–20*** *as in ACV, HBG, and iso-DHPG to give, after deprotection, the desired acylonucleosides* ***33–44****. Almost all of the new compounds were evaluated for their inhibitory effects against the replication of various DNA viruses in culture.*

Keywords C_2-, C_4-, and C_6-substituted pyrazolo[3,4-d]pyrimidines; Acyclonucleoside; Analogues of ACV, HBG, and iso-DHPG

INTRODUCTION

The structural diversity and biological importance of acyclonucleosides have made them attractive targets for synthesis over many years. Recent development of physiologically highly potent acyclonucleoside analogues with interesting antiviral and/or anticancer activities have promoted a great current interest in facile and general routes to these molecules in synthetically useful yields.

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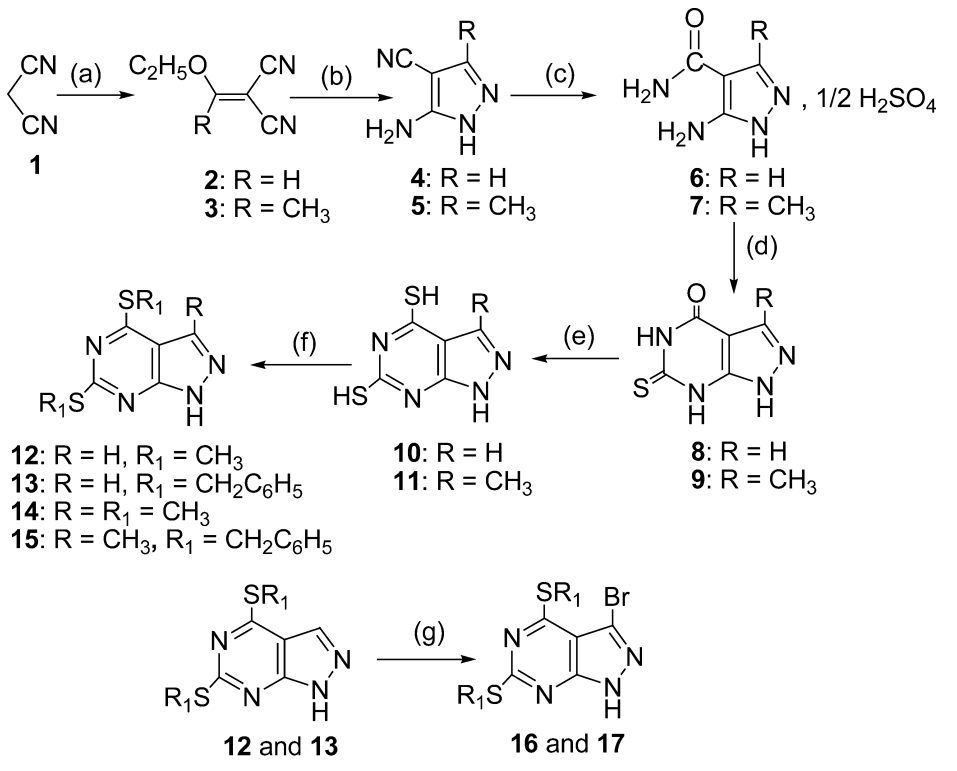
Address correspondence to Omar Moukha-chafiq, Drug Discovery Division, Southern Research Institute, 2000 Ninth Ave. S., Birminghanm, AL 35205. E-mail: moukhachafiq@sri.org

As purine analogues, the pyrazolo[3,4-d]pyrimidines are of considerable chemical and pharmacological important due to their anti-tumor activities[1–3] and their strong therapeutic activity against various diseases.[4] Only a few acyclic pyrazolo[3,4-d]pyrimidine nucleosides have been reported. As an extension of our studies on mono- and disubstituted pyrazolo[3,4-d]pyrimidine acyclonycleosides,[5–7] in which some of them showed an interesting antiviral, anti-tumor, and/or anti-tuberculosis activity, we decided to use C_3-, C_4-, and C_6-substituted pyrazolo[3,4-d]pyrimidines as new aglycons to study any variation in biological activity.

CHEMISTRY

We first prepared the heterocycles **10** and **11** from commercially available malononitrile **1** and triethyl orthoformate or triethyl orthacetate following a synthetic pathway previously described by Robins *et al.*[8] The C_4 and

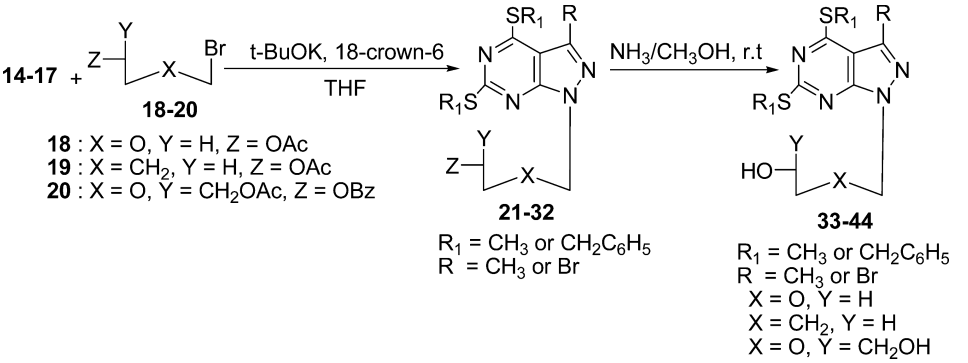


(a) : triethylorthoformate or triethylorthoacetate/acetic anhydride/ reflux;
(b) : H_2NNH_2, r.t.; (c) : H_2SO_4; (d) : thiourea / reflux; (e) : P_2S_5 / pyridine;
(f) CH_3I or $C_6H_5CH_2Br$ in NaOH (1N), r.t. (g) : NBS/$ClCH_2CH_2Cl$, reflux.

SCHEME 1

C_6 sulfur atoms of compounds **10** and **11** were alkylated with methyl iodide and benyl bromide in a sodium hydroxide solution to give compounds **12–15**, respectively, in 83–86% yields. Treatment of **12** and **13** with *N*-bromosuccenimide in 1,2-dichloroethan led to bromated heterocycles **16** and **17** in 85 and 83% yields, respectively (Scheme 1).

The condensation, separately, between the nucleobases **14–17** with the alkylating agents **18**,[9] **19**[10] and **20**[11] was carried out using solid-liquid phase transfer catalysis method in which potassium tert-butoxide was used as alkali, tetrahydrofuran as solvent and 18-crown-6 as catalyst, to afford regioselectively the N_1-regioisomers **21–32**, respectively, in good yields (Scheme 2).



Compound	R	R_1	X	Y
33	CH_3	CH_3	O	H
34	CH_3	$CH_2C_6H_5$	O	H
35	CH_3	CH_3	CH_2	H
36	CH_3	$CH_2C_6H_5$	CH_2	H
37	CH_3	CH_3	O	CH_2OH
38	CH_3	$CH_2C_6H_5$	O	CH_2OH
39	Br	CH_3	O	H
40	Br	$CH_2C_6H_5$	O	H
41	Br	CH_3	CH_2	H
42	Br	$CH_2C_6H_5$	CH_2	H
43	Br	CH_3	O	CH_2OH
44	Br	$CH_2C_6H_5$	O	CH_2OH

SCHEME 2

Finally, the treatment of N_1-protected acyclic nucleosides **21–32** with a solution of methanolic ammonia at room temperature gave deprotected the acyclic nucleosides **33–44** in quantitative yields (Scheme 2).

The site of alkylation in some of these compounds was established to be at N_1 by a direct comparison of the UV spectra of the compounds **33–44**

with the UV spectra of the corresponding N_1-pyrazolo[3,4-d]pyrimidine nucleosides.[12]

All structures of the synthetic products were identified by ^{1}H NMR, mass spectra, UV, and/or elemental analysis.

ANTIVIRAL ACTIVITY

The target acyclonucleosides **33**, **35–39**, **41**, and **43** were evaluated for their antiviral activity in a wide variety of assay systems: herpes simplex virus type 1 (HSV-1) (KOS) and (HSV-2) (G), vaccinia virus, vesicular stomatitis virus (VSV), thymidine kinase-deficient (TK^-) strain of HSV-1 (B2006 and VWM1837) in human embryonic skin muscle fibroblasts (E_6MS), Coxackie virus B4 virus in Hela cell cultures, parainfluenza virus type 3, reovirus type 1, Sindbis virus, Coxsackie B4 virus, and Punta Toro virus in Vero cell cultures (Tables 1 and 2).

Data for ribavirine, DHPG, and BVDU are shown for comparison. None of the tested compounds showed any significant activity except for compound **35**, which was slightly active toward vaccinia virus (MIC = 240 μg/mL) (Table 1).

The in vitro antiviral activity of acyclonucleosides **33**, **35–39**, **41**, and **43** against cytomegalovirus (CMV) and varicella-zoster (VZV) in human embryonic lung (HEL) cells is summarized in Tables 3 and 4. Data for DHPG, HPMPC, ACV, and BVDU are also shown for comparison. Compounds **33**, **35**, **36**, **38**, **39**, **41**, and **43** showed very interesting anti-cytomegalovirus activities (IC_{50} = 0.5–15 μg/mL); however, these compounds were found cytotoxic (CC_{50} = 5–50 μg/mL) (Table 3).

TABLE 1 Cytotoxicity and Antiviral Activity in Human Embryonic Lung (HEL) Cell Cultures

		Minimum inhibitory concentration[b] (μg/mL)				
Compound	MCC[a] (μg/mL)	HSV-1 (KOS)	HSV-1 (G)	Vaccinia virus	Vesicular stomatitis virus	HSV-1 (TK^- KOS)
33	400	>80	>80	>80	>80	>80
35	400	>80	>80	240	>80	>80
36	≥80	>16	>16	>16	>16	>16
37	400	>80	>80	>80	>80	>80
38	80	>16	>16	>16	>16	>16
39	400	>80	>80	>80	>80	>80
41	≥80	>16	>16	>16	>16	>16
43	80	>16	>16	>16	>16	>16
Ribavirine	>400	0.0768	240	16	>400	>400
DHPG	>100	0.032	0.096	>100	>100	12

[a]MCC: minimum cytotoxic concentration: required to cause a microscopically detectable alteration of normal cell morphology.

[b]Required to reduce virus-induced cytopathogenecity by 50%.

TABLE 2 Cytotoxicity and Antiviral Activity in HEL Cell Cultures

		Minimum inhibitory concentration[b] (μg/mL)					
Compound	MCC[a] (μg/mL)	Parainfluenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus	Respiratory syncytial virus
33	400	>80	>80	>80	>80	>80	>80
35	≥80	>16	>16	>16	>16	>16	>16
36	≥80	>80	>80	>80	>80	>80	>80
37	400	>80	>80	>80	>80	>80	>80
38	≥400	>80	>80	>80	>80	>80	>80
39	400	>80	>80	>80	>80	>80	>80
41	400	>80	>80	>80	>80	>80	>80
43	400	>80	>80	>80	>80	>80	>80
Ribavirine	>400	>400	>400	>400	48	>400	9.6
BVDU	>400	>400	16	>400	>400	>400	>400

[a]MCC: minimum cytotoxic concentration: required to cause a microscopically detectable alteration of normal cell morphology.

[b]Required to reduce virus-induced cytopathogenecity by 50%.

Compounds **33**, **35**, **36**, **38**, and **41** showed some activities against VZV (TK$^-$ VZV: YS/R strain; IC_{50} = 1–12 μg/mL) comparable or better than ACV and BVDU. Also, these compounds were found to be cytotoxic (CC_{50} = 5–50 μg/mL) (Table 4).

In conclusion, we have regioselectively synthesized some new trisubstituted pyrazolo[3,4-d]pyrimidines acyclonucleosides with the alkylating

TABLE 3 Activity Against Cytomegalovirus in HEL Cell Culture

	Antiviral activity IC_{50} (μg/mL)[a]		Cytotoxicity (μg/mL)	
Compound	AD-169 strain	Davis strain	Cell morphology (MCC)[b]	Cell growth (CC_{50})[c]
33	15	ND	50	>50
35	1.5	1	20	24
36	0.5	1	5	>50
37	>50	ND	>50	>50
38	0.9	0.7	5	18
39	8.6	>5	50	>50
41	2	2	20	37
43	>5	ND	20	>50
DHPG	0.9	0.8	>50	>50
HPMPC	0.16	0.5	>50	ND

[a]Inhibitory concentration required to reduce virus plaque formation by 50%. Virus input was 100 plaque-forming units (PFU).

[b]MCC: minimum cytotoxic concentration: required to cause a microscopically detectable alteration of normal cell morphology.

[c]Required to reduce virus-induced cytopathogenecity by 50%.

TABLE 4 Activity Against Varicella-Zoster Virus in HEL Cell Cultures

	Antiviral activity IC_{50} (μg/mL)[a]					
	TK^+ VZV		TK^- VZV		Cytotoxicity (μg/mL)	
Compound	YS strain	OKA strain	07/1 strain	YS/R strain	Cell morphology (MCC)[b]	Cell growth (CC_{50})[c]
33	>20	>20	>20	12	50	>50
35	>2	>2	>2	2	5	24
36	>2	>2	>2	2	5	>50
37	>20	>20	>20	>20	50	>50
38	>2	>2	>2	1	5	18
39	>5	>5	>5	>5	20	>50
41	>5	>5	>5	3.2	20	37
43	>5	>5	>5	>5	20	>50
ACV	0.56	0.41	7.9	3.2	>50	>200
BVDU	0.003	0.003	38	>28	>50	>200

[a]Inhibitory concentration required to reduce virus plaque formation by 50%. Virus input was 100 plaque-forming units (PFU).

[b]Minimum cytotoxic concentration required to cause a microscopically detectable alteration of normal cell morphology.

[c]Required to reduce virus-induced cytopathogenecity by 50%.

chains of acyclovir, HBG, and iso-DHPG. Their anti-SARS, anti-tumor, and anti-tuberculosis evaluations are in progress.

EXPERIMENTAL

Melting points (mp) were determined on an electrothermal digital melting point apparatus and are uncorrected. Ultraviolet (UV) spectra were recorded on a HP 845x spectrophotometer. The ^{1}H-NMR spectra were recorded using a Bruker AC 250 spectrometer. The chemical shifts were reported as parts per million (δ ppm) from $(CH_3)_4Si$ (TMS) as an internal standard.* Mass spectra were obtained with a JOEL JMS DX 300 instrument using fast atomic bombardment (FAB positive). Thin-layer chromatography (tlc) was performed on plates of Merck Kieselgel 60 F_{254} and short wavelength UV light (254 nm) was used to detect the UV-absorbing spots. Column chromatography separation were obtained on silica gel 60 (70–230 mesh, Merck, Montpellier, France). Elemental analysis was determined by the French microanalytical central service.

General Preparation Procedure of 12–15

The 1*H*-pyrazolo[3,4-d]pyrimidin-4,6-dithione **10** and 3-methyl-1*H*-pyrazolo[3,4-d]pyrimidin-4,6-dithione **11** (20 mmol) were dissolved,

*Key: s (singlet), d (doublet), t (triplet), m (multiplet), and br (broad).

separately, in 1N sodium hydroxide solution (40 mL). To this solution were added 40 mmol of methyl iodide or benzyl bromide at 0°C and the mixture was stirred at room temperature for 3 h. The reaction was monitored by thin-layer chromatography and was shown to be complete at this time. The excess of the solvent was removed *in vacuo*. The residue was coevapored with benzene (3 × 20 mL) and chromatographed on a silica gel column using chloroform:methanol (98:2) as eluent to furnish the expected heterocyclic bases **12–15**.

4,6-Dimethylthio-1H-pyrazolo[3,4-d]pyrimidine ***12***. Yield: 88%. R_f: 0.25 ($CHCl_3$:CH_3OH, 98:2, v/v). Mp: 193–194°C (methanol). UV (ethanol) λ_{max}: 250 nm (ε: 16,700). ^{1}H-NMR (Me_2SO-d_6) δ: 2.61(s, 3H, 6-SCH_3), 2.72 (s, 3H, 4-SCH_3), 8.30 (s, 1H, H_3), 14.00 (sl, 1H, NH). MS (FAB^+, NBA) *m/z*: 213 $[M+H]^+$.

4,6-Dibenzylthio-1H-pyrazolo[3,4-d]pyrimidine ***13***. Yield: 86%. R_f: 0.30 ($CHCl_3$:CH_3OH, 98:2, v:v) Mp: 163–164°C (ethanol). UV (ethanol) λ_{max}: 253 nm (ε: 21,700). ^{1}H-NMR (Me_2SO-d_6) δ: 4.51 (s, 2H, 6-SCH_2), 4,61 (s, 2H, 4-SCH_2), 7.25–7.51 (m, 10H, 2 C_6H_5), 8.21 (s, 1H, H_3), 13.95 (sl, 1H, NH). MS (FAB^+, NBA) *m/z*: 365 $[M+H]^+$.

3-Methyl-4.6-dimethylthio-1H-pyrazolo[3,4-d]pyrimidine ***14***. Yield: 85%. R_f: 0.40 ($CHCl_3$:CH_3OH, 98:2, v:v). Mp: 237–238°C (methanol). UV (ethanol) λ_{max}: 241 nm (ε: 18,700). ^{1}H-NMR (Me_2SO-d_6) δ: 2.50 (s, 3H, CH_3), 2.52 (s, 3H, 6-SCH_3), 2.60 (s, 3H, 4-SCH_3), 13.37 (sl, 1H, NH). MS (FAB^+, NBA) *m/z*: 227 $[M+H]^+$.

3-Methyl-4.6-dibenzylthio-1H-pyrazolo[3,4-d]pyrimidine ***15***. Yield: 83%. R_f: 0.46 ($CHCl_3$:CH_3OH, 98:2, v/v). Mp: 173–174°C (ethanol). UV (ethanol) λ_{max}: 246 nm (ε: 20,300). ^{1}H-NMR (Me_2SO-d_6) δ: 2.48 (s, 3H, CH_3), 4.43 (s, 2H, 6-SCH_2), 4,54 (s, 2H, 4-SCH_2), 7.19–7.43 (m, 10H, 2 C_6H_5), 13.43 (sl, 1H, NH). MS (FAB^+, NBA) *m/z*: 415 $[M+H]^+$.

Preparation Procedure of 16 and 17

A solution of 10 mmol of compound **12** or **13** and 15.5 mmol of *N*-bromosuccenimide in 30 mL of anhydrous 1,2-dichloromethan was refluxed during 30 min. The reaction mixture was evaporated to dryness *in vacuo* and the obtained residue was chromatographed on a silica gel column, using dichloromethane as eluent, to give **16** or **17,** respectively.

3-Bromo-4,6-diméthylthio-1H-pyrazolo[3,4-d]pyrimidine ***16***. Yield: 85%. R_f: 0.40 ($CHCl_3$:CH_3OH, 98:2, v/v). Mp: 231–232°C (methanol). UV (ethanol) λ_{max}: 247 nm (ε: 20,000), ^{1}H-NMR (Me_2SO-d_6) δ: 2.50 (s, 3H, CH_3), 2.52 (s, 3H, 6-SCH_3), 2.60 (s, 3H, 4-SCH_3), 13.37 (sl, 1H, NH). MS (FAB^+, NBA) *m/z*: 227 $[M+H]^+$.

4,6-Dibenzylthio-3-bromo-1H-pyrazolo[3,4-d]pyrimidine ***17***. Yield: 83%. R_f: 0.46 ($CHCl_3$:CH_3OH, 98:2, v/v). Mp: 201–202°C (ethanol). UV (methanol) λ_{max} 246 nm (ε = 18,500). ^{1}H-NMR (Me_2SO-d_6) δ: 2.48 (s, 3H, CH_3), 4.43

(s, 2H, 6-SCH_2), 4.54 (s, 2H, 4-SCH_2), 7.19–7.43 (m, 10H, 2 C_6H_5), 13.43 (sl, 1H, NH). MS (FAB^+, NBA) *m/z*: 415 $[M+H]^+$.

General Alkylation Procedure

To a solution of 0.66 g (2.5 mmol) of 18-crown-6 in 140 mL of anhydrous tetrahydrofuran was added 1.13 g (10 mmol) of potassium *tert*-butoxide. Then 10 mmol of heterocycle **14**, **15**, **16,** or **17** was added and the reaction mixture was stirred at room temperature for 15 mins. At this time the reaction mixture was cooled to 0°C and 10 mmol of compound **18**, **19,** or **19** in 20 mL of anhydrous THF was added dropwise with stirring. When the addition was finished, the reaction mixture was stirred for 1 h at 40°C. The reaction mixture was then filtrated and the filtrate was evaporated to dryness *in vacuo*. The residue was chromatographed on a silica gel column, using chloroform as eluent, to give the N_1-protected acyclic nucleoside.

1-(2-Acetoxyethoxy) methyl-3-methyl-4, 6-dimethylthio-1H-pyrazolo[3, 4-d]pyrimidine ***21***. Yield: 80%. R_f: 0.54 (CH_2Cl_2:CH_3OH, 99:1, v:v). Mp: 86–87°C (ethanol). ^{1}H-NMR (Me_2SO-d_6) δ: 1.99 (s, 3H, CH_3CO), 2.57 (s, 3H, CH_3), 2.63 (s, 3H, 6-SCH_3), 2.69 (s, 3H, 4-SCH_3), 3.71 and 4.17 (2m, 4H, OCH_2CH_2O), 5.65 (s, 2H, OCH_2N). MS (FAB^+, GT) *m/z:* 343 $[M+H]^+$.

1-(2-Acetoxyethoxy) methyl-3-methyl-4, 6-dibenzylthio-1H-pyrazolo[3, 4-d]pyrimidine ***22***. Yield: 78%. R_f*:* 0.56 ($CHCl_3$:CH_3OH, 98:2, v:v). Mp: 74–75°C (ethanol). ^{1}H-NMR (Me_2SO-d_6) δ: 1.96 (s, 3H, CH_3CO), 2.50 (s, 3H, CH_3), 3.69 and 4.08 (2m, 4H, OCH_2CH_2O), 4.54 (s, 2H, 6-$SCH_2C_6H_5$), 4.63 (s, 3H, 4-$SCH_2C_6H_5$), 5.68 (s, 2H, OCH_2N), 7.28–7.53 (m, 10H, 2 C_6H_5). MS (FAB^+, GT) *m/z:* 495 $[M+H]^+$.

1-(4-Acetoxybutyl)-3-methyl-4, 6-dimethylthio-1H-pyrazolo[3, 4-d]pyrimidine ***23***. Yield: 79%. R_f*:* 0.60 ($CHCl_3$:CH_3OH, 98:2, v:v). ^{1}H-NMR (Me_2SO-d_6) δ: 1.44 (m, 2H, $AcOCH_2CH_2$), 1.79 (m, 2H, CH_2CH_2N), 1.93 (s, 3H, CH_3CO), 2.49 (s, 3H, CH_3), 2.54 (s, 3H, 6-SCH_3), 2.60 (s, 3H, 4-SCH_3), 3.95 (t, *J*: 6.51 Hz, 2H, $AcOCH_2CH_2$), 4.21 (t, *J*: 6.78 Hz, 2H, CH_2N). MS (FAB^+, GT) *m/z:* 341 $[M+H]^+$.

1-(4-Acetoxybutyl)-3-methyl-4, 6-dibenzylthio-1H-pyrazolo[3, 4-d]pyrimidine ***24***. Yield: 77%. R_f*:* 0.68 ($CHCl_3$:CH_3OH, 98:2, v:v). Mp: 52–53°C (ethanol). ^{1}H-NMR (Me_2SO-d_6) δ: 1.51 (m, 2H, $AcOCH_2CH_2$), 1.85 (m, 2H, CH_2CH_2N), 1.99 (s, 3H, CH_3CO), 2.55 (s, 3H, CH_3), 4.01 (t, *J*: 6.50 Hz, 2H, $AcOCH_2CH_2$), 4.31 (t, *J*: 6.73 Hz, 2H, CH_2N), 4.50 (s, 3H, 6-$SCH_2C_6H_5$), 4.59 (s, 3H, 4- $SCH_2C_6H_5$), 7.22–7.52 (m, 10H, 2 C_6H_5). MS (FAB^+, GT) *m/z*: 497 $[M+H]^+$.

1-(3-Acetoxy-2-O-benzoyl-1-propoxy)methyl-3-methyl-4, 6-dimethylthio-1H-pyrazolo[3,4-d]pyrimidine ***25***. Yield: 76%. R_f*:* 0.56 ($CHCl_3$:CH_3OH, 98:2, v:v). ^{1}H-NMR (Me_2SO-d_6) δ: 1.94 (s, 3H, CH_3CO), 3.89 (d, *J*: 4.69 Hz, 2H, OCH_2CH), 2.45 (s, 3H, CH_3), 2.54 (s, 3H, 6-SCH_3), 2.60 (s, 3H,

4-SCH_3), 4.18–4.40 (m, 2H, CH_2OAc), 5.28 (m, 1H, CH_2CHOBz), 5.70 (s, 2H, OCH_2N), 7.45–7.92 (m, 5H, C_6H_5). MS (FAB^+, GT) *m/z:* 477 $[M+H]^+$.

1-(3-Acetoxy-2-O-benzoyl-1-propoxy)methyl-3-methyl-4, 6-dibenzylthio-1H-pyrazolo[3,4-d]pyrimidine ***26***. Yield: 74%. R_f: 0.60 ($CHCl_3$:CH_3OH, 98:2, v:v). ^{1}H-NMR (Me_2SO-d_6) δ: 1.94 (s, 3H, CH_3CO), 2.45 (s, 3H, CH_3), 3.89 (d, J: 4.69 Hz, 2H, OCH_2CH), 4.18–4.40 (m, 2H, CH_2OAc), 4.51 (s, 3H, 6-SCH_3), 4.59 (s, 3H, 4-SCH_3), 5.28 (m, 1H, CH_2CHOBz), 5.70 (s, 2H, OCH_2N), 7.25–7.82 (m, 15H, 3 C_6H_5). MS (FAB^+, GT) *m/z:* 629 $[M+H]^+$.

1-(2-Acetoxyethoxy)methyl-3-bromo-4, 6-dimethylthio-1H-pyrazolo[3, 4-d]pyrimidine ***27***. Yield: 85%. R_f: 0.54 (CH_2Cl_2:CH_3OH, 99:1, v:v). Mp: 89–90°C (ethanol). ^{1}H-NMR (Me_2SO-d_6) δ: 1.96 (s, 3H, CH_3CO), 2.61 (s, 3H, 6-SCH_3), 2.71 (s, 3H, 4-SCH_3), 3.73 and 4.10 (2m, 4H, OCH_2CH_2O), 5.72 (s, 2H, OCH_2N). MS (FAB^+, GT) *m/z:* 408 $[M+H]^+$.

1-(2-Acetoxyethoxy)methyl-3-bromo-4,6-benzylthio-1H-pyrazolo[3,4-d]pyrimidine ***28***. Yield: 81%. R_f: 0.58 (CH_2Cl_2:CH_3OH, 99:1, v:v). Mp: 65–66°C (ethanol). ^{1}H-NMR (Me_2SO-d_6) δ: 1.88 (s, 3H, CH_3CO), 3.64 and 4.01 (2m, 4H, OCH_2CH_2O), 4.49 (s, 2H, 6-$SCH_2C_6H_5$), 4.57 (s, 3H, 4-$SCH_2C_6H_5$), 5.72 (s, 2H, OCH_2N), 7.20–7.49 (m, 10H, 2 C_6H_5). MS (FAB^+, GT) *m/z:* 460 $[M+H]^+$.

1-(4-Acetoxybutyl)-3-bromo-4,6-dimethylthio-1H-pyrazolo[3, 4-d]pyrimidine ***29***. Yield: 82%. R_f: 0.68 (CH_2Cl_2:CH_3OH, 99:1, v:v). Mp: 70–71°C (ethanol). ^{1}H-NMR (Me_2SO-d_6) δ: 1.48 (m, 2H, $AcOCH_2CH_2$), 1.86 (m, 2H, CH_2CH_2N), 1.98 (s, 3H, CH_3CO), 2.58 (s, 3H, 6-SCH_3), 2.65 (s, 3H, 4-SCH_3), 3.99 (t, J: 6.51 Hz, 2H, $AcOCH_2CH_2$), 4.34 (t, J: 6.75 Hz, 2H, CH_2N). MS (FAB^+, GT) *m/z:* 405 $[M+H]^+$.

1-(4-Acetoxybutyl)-3-bromo-4,6-dibenzylthio-1H-pyrazolo[3, 4-d]pyrimidine ***30***. Yield: 80%. R_f: 0.73 (CH_2Cl_2:CH_3OH, 99:1, v:v). Mp: 72–73°C (ethanol). ^{1}H-NMR (Me_2SO-d_6) δ: 1.48 (m, 2H, $AcOCH_2CH_2$), 1.86 (m, 2H, CH_2CH_2N), 1.96 (s, 3H, CH_3CO), 3.99 (t, J: 6.49 Hz, 2H, $AcOCH_2CH_2$), 4.38 (t, J: 6.74 Hz, 2H, CH_2N), 4.49 (s, 3H, 6-$SCH_2C_6H_5$), 4.58 (s, 3H, 4-$SCH_2C_6H_5$), 7.26–7.50 (m, 10H, 2 C_6H_5). MS (FAB^+, GT) *m/z:* 562 $[M+H]^+$.

1-(3-Acetoxy-2-O-benzoyl-1-propoxy)methyl-3-bromo-4,6-dimethylthio-1H-pyrazolo[3,4-d]pyrimidine ***31***. Yield: 78%. R_f: 0.56 (CH_2Cl_2:CH_3OH, 99:1, v:v). ^{1}H-NMR (Me_2SO-d_6) δ: 1.95 (s, 3H, CH_3CO), 2.60 (s, 3H, 6-SCH_3), 2.68 (s, 3H, 4-SCH_3), 3.92 (d, J: 4.80 Hz, 2H, OCH_2CH), 4.17–4.41 (m, 2H, CH_2OAc), 5.32 (m, 1H, CH_2CHOBz), 5.73 (s, 2H, OCH_2N), 7.45–8.00 (m, 5H, C_6H_5). MS (FAB^+, GT) *m/z:* 542 $[M+H]^+$.

1-(3-Acetoxy-2-O-benzoyl-1-propoxy)methyl-3-bromo-4, 6-dibenzylthio-1H-pyrazolo[3,4-d]pyrimidine ***32***. Yield: 76%. R_f: 0.60 (CH_2Cl_2:CH_3OH, 99:1, v:v). ^{1}H-NMR (Me_2SO-d_6) δ: 1.90 (s, 3H, CH_3CO), 3.88 (m, 2H, OCH_2CH), 4.27 (m, 2H, CH_2OAc), 4.48 (s, 2H, 6-$SCH_2C_6H_5$), 4.58 (s, 2H, 4-$SCH_2C_6H_5$), 5.24 (m, 1H, CH_2CHOBz), 5.76 (s, 2H, OCH_2N), 7.21–7.98 (m, 15H, 3 C_6H_5). MS (FAB^+, GT) *m/z:* 694 $[M + H]^+$.

General Deprotection Procedure

To 80 mL of dry methanol saturated with ammonia at −5°C was added 1 mmol of the protected acyclic nucleoside **21–32**. The flask was stopped tightly and the solution was stirred for 16–20 h at room temperature. Thin-layer chromatography indicated that complete deprotection of protected product had occurred. Volatile materials were evaporated *in vacuo*. The residue was purified by column chromatography on silica gel, using chloroform:methanol (98:2) as eluent, to obtain the expected acyclic nucleoside.

*1-(2-Hydroxyethxy)meythl-3-methyl-4, 6-dimethylthio-1H-pyrazolo[3, 4-d]pyrimidine **33**.* Yield: 89%. R_f: 0.54 ($CHCl_3$:CH_3OH, 90:10, v:v). Mp: 97–98°C (ethanol). UV (methanol) λ_{max}: 253, 293, 308 nm (ε: 19,300, 8,700, 4 300). ^{1}H-NMR (Me_2SO-d_6) δ: 2.57 (s, 3H, CH_3), 2.63 (s, 3H, 6-SCH_3), 2.69 (s, 3H, 4-SCH_3), 3.34–3.51 (m, 4H, OCH_2CH_2O), 4.61 (t, J: 5.39 Hz, 1H, HO, D_2O exchangeable), 5.65 (s, 2H, OCH_2N). MS (FAB^+, GT) *m/z:* 301 $[M+H]^+$.

*4,6-Dibenzylthio-1-(2-hydroxyethoxy)methyl-3-methyl-1H-pyrazolo[3,4-d]pyrimidine **34**.* Yield: 87%. R_f: 0.59 ($CHCl_3$:CH_3OH, 90:10, v:v). Mp: 84–85°C (ethanol). UV (methanol) λ_{max}: 256, 293, 309 nm (ε: 18,000, 7,900, 4,800). ^{1}H-NMR (Me_2SO-d_6) δ: 2.50 (s, 3H, CH_3), 3.34–3.51 (m, 4H, OCH_2CH_2O), 4.54 (s, 2H, 6-$SCH_2C_6H_5$), 4.60 (s, 3H, 4-$SCH_2C_6H_5$), 4.61 (t, J: 5.39 Hz, 1H, HO, D_2O exchangeable), 5.68 (s, 2H, OCH_2N), 7.28–7.53 (m, 10H, 2 C_6H_5). MS (FAB^+, GT) *m/z:* 453 $[M+H]^+$.

*1-(4-Hydroxybutyl)-3-methyl-4,6-dimethylthio-1H-pyrazolo[3,4-d]pyrimidine **35**.* Yield: 89%. R_f: 0.65 ($CHCl_3$:CH_3OH, 90:10, v:v). Mp: 78–79°C (ethanol). UV (methanol) λ_{max}: 254, 296, 312 nm (ε: 20,200, 9,600, 5,100). ^{1}H-NMR (Me_2SO-d_6) δ: 1.34 (m, 2H, $HOCH_2CH_2$), 1.79 (m, 2H, CH_2CH_2N), 2.46 (s, 3H, CH_3), 2.54 (s, 3H, 6-SCH_3), 2.60 (s, 3H, 4-SCH_3), 3.37 (m, 2H, $HOCH_2$), 4.24 (t, J: 6.86 Hz, 2H, CH_2N), 4.38 (m, 1H, HO, D_2O exchangeable). MS (FAB^+, GT) *m/z:* 299 $[M+H]^+$.

*4,6-Dibenzylthio-1-(4-hydroxybutyl)-3-methyl-1H-pyrazolo[3, 4-d]pyrimidine **36**.* Yield: 88%. R_f: 0.68 ($CHCl_3$:CH_3OH, 90:10, v:v). Mp: 60–61°C (ethanol). UV (methanol) λ_{max}: 253, 296, 310 nm (ε: 19,900, 9,300, 4,900). ^{1}H-NMR (Me_2SO-d_6) δ: 1.34 (m, 2H, $HOCH_2CH_2$), 1.79 (m, 2H, CH_2CH_2N), 2.41 (s, 3H, CH_3), 3.37 (m, 2H, $HOCH_2$), 4.24 (t, J: 6.86 Hz, 2H, CH_2N), 4.38 (m, 1H, HO, D_2O exchangeable), 4.44 (s, 3H, 6-$SCH_2C_6H_5$), 4.53 (s, 3H, 4-$SCH_2C_6H_5$), 7.21–7.46 (m, 10H, 2 C_6H_5). MS (FAB^+, GT) *m/z:* 451 $[M+H]^+$.

*1-(2, 3-Dihydroxy-1-propoxy)methyl-3-methyl-4, 6-dimethylthio-1H-pyrazolo[3,4-d]pyrimidine **37**.* Yield: 85%. R_f: 0.60 ($CHCl_3$:CH_3OH, 90:10, v:v). UV (methanol) λ_{max}: 251, 296, 311 nm (ε: 21,400, 10,200, 4,600). ^{1}H-NMR (Me_2SO-d_6) δ: 2.45 (s, 3H, CH_3), 2.54 (s, 3H, 6-SCH_3), 2.60 (s, 3H, 4-SCH_3), 3.25–3.55 (m, 5H, OCH_2CHCH_2), 4.50 (t, J: 5.61 Hz, 1H, $HOCH_2$, D_2O

exchangeable), 4.73 (d, *J*: 4.64 Hz, 1H, HOCH, D_2O exchangeable), 5.70 (s, 2H, OCH_2N). MS (FAB^+, GT) *m/z:* 331 $[M+H]^+$.

4,6-Dibenzylthio-1-(2, 3-dihydroxy-1-propoxy)methyl-3-methyl-1H-pyrazolo[3, 4-d]pyrimidine. ***38***. Yield: 83%. R_f: 0.63 (CH_2Cl_2:CH_3OH, 90:10, v:v). UV (methanol) λ_{max}: 255, 298, 309 nm (ε: 19,500, 8,800, 4,700). ^{1}H-NMR (Me_2SO-d_6) δ: 2.45 (s, 3H, CH_3), 3.25–3.55 (m, 5H, OCH_2CHCH_2), 4.50 (t, *J*: 5.61 Hz, 1H, $HOCH_2$, D_2O exchangeable), 4.51 (s, 3H, 6-SCH_3), 4.59 (s, 3H, 4-SCH_3), 4.73 (d, *J*: 4.64 Hz, 1H, HOCH, D_2O exchangeable), 5.70 (s, 2H, OCH_2N), 7.25–7.82 (m, 10H, 2 C_6H_5). MS (FAB^+, GT) *m/z:* 483 $[M+H]^+$.

3-Bromo-1-(2-hydroxyethoxy)methyl-4, 6-dimethylthio-1H-pyrazolo[3, 4-d]pyrimidine ***39***. Yield: 88%. R_f: 0.58 (CH_2Cl_2:CH_3OH, 90:10, v:v). Mp: 109–110°C (ethanol). UV (methanol) λ_{max}: 246 nm (ε: 11,300). ^{1}H-NMR (Me_2SO-d_6) δ: 2.61 (s, 3H, 6-SCH_3), 2.71 (s, 3H, 4-SCH_3), 3.34–3.51 (m, 4H, OCH_2CH_2O), 4.61 (t, *J*: 5.39 Hz, 1H, HO, D_2O exchangeable), 5.72 (s, 2H, OCH_2N). MS (FAB^+, GT) *m/z:* 366 $[M+H]^+$.

4, 6-Dibenzylthio-3-bromo-1-(2-hydroxyethoxy)methyl-1H-pyrazolo[3, 4-d]pyrimidine ***40***. Yield: 88%. R_f: 0.62 (CH_2Cl_2:CH_3OH, 90:10, v:v). Mp: 75–76°C (ethanol). UV (methanol) λ_{max}: 249 nm (ε: 13,500). ^{1}H-NMR (Me_2SO-d_6) δ: 3.34–3.51 (m, 4H, OCH_2CH_2O), 4.49 (s, 2H, 6-$SCH_2C_6H_5$), 4.57 (s, 3H, 4-$SCH_2C_6H_5$), 4.61 (t, *J*: 5.39 Hz, 1H, HO, D_2O exchangeable), 5.72 (s, 2H, OCH_2N), 7.20–7.49 (m, 10H, 2 C_6H_5). MS (FAB^+, GT) *m/z:* 518 $[M+H]^+$.

3-Bromo-1-(4-hydroxybutyl)-4, 6-dimethylthio-1H-pyrazolo[3, 4-d]pyrimidine ***41***. Yield: 88%. R_f: 0.69 (CH_2Cl_2:CH_3OH, 90:10, v:v). Mp: 89–89°C (ethanol). UV (methanol) λ_{max}: 245 nm (ε: 10,000). ^{1}H-NMR (Me_2SO-d_6) δ: 1.34 (m, 2H, $HOCH_2CH_2$), 1.79 (m, 2H, CH_2CH_2N), 2.58 (s, 3H, 6-SCH_3), 2.65 (s, 3H, 4-SCH_3), 3.37 (m, 2H, $HOCH_2$), 4.24 (t, *J*: 6.86 Hz, 2H, CH_2N), 4.38 (m, 1H, HO, D_2O exchangeable). MS (FAB^+, GT) *m/z:* 364 $[M+H]^+$.

4, 6-Dibenzylthio-3-bromo-1-(4-hydroxybutyl)-1H-pyrazolo[3, 4-d]pyrimidine ***42***. Yield: 86%. R_f: 0.75 (CH_2Cl_2:CH_3OH, 90:10, v:v). Mp: 82–83°C (ethanol). UV (methanol) λ_{max}: 250 nm (ε: 14,200). ^{1}H-NMR (Me_2SO-d_6) δ: 1.34 (m, 2H, $HOCH_2CH_2$), 1.79 (m, 2H, CH_2CH_2N), 3.37 (m, 2H, $HOCH_2$), 4.24 (t, *J*: 6.86 Hz, 2H, CH_2N), 4.38 (m, 1H, HO, D_2O exchangeable), 4.49 (s, 3H, 6-$SCH_2C_6H_5$), 4.58 (s, 3H, 4-$SCH_2C_6H_5$), 7.26–7.50 (m, 10H, 2 C_6H_5). MS (FAB^+, GT) *m/z:* 516 $[M+H]^+$.

3-Bromo-1-(2,3-dihydroxy-1-propoxy)methyl-4, 6-dimethylthio-1H-pyrazolo[3,4-d]pyrimidine ***43***. Yield: 84%. R_f: 0.60 (CH_2Cl_2:CH_3OH, 90:10, v:v). UV (methanol) λ_{max}: 252 nm (ε: 15,100). ^{1}H-NMR (Me_2SO-d_6) δ: 2.60 (s, 3H, 6-SCH_3), 2.68 (s, 3H, 4-SCH_3), 3.25–3.55 (m, 5H, OCH_2CHCH_2), 4.50 (t, *J*: 5.61 Hz, 1H, $HOCH_2$, D_2O exchangeable), 5.73 (s, 2H, OCH_2N). MS (FAB^+, GT) *m/z:* 396 $[M+H]^+$.

*4, 6-Dibenzylthio-3-bromo-1-(2, 3-dihydroxy-1-propoxy)methyl-1H-pyrazolo[3, 4-d]pyrimidine **44**.* Yield: 81%. R_f: 0.66 (CH_2Cl_2:CH_3OH, 90:10, v:v). UV (methanol) λ_{max}: 250 nm (ε: 13,200). ^{1}H-NMR (Me_2SO-d_6) δ: 3.25–3.55 (m, 5H, OCH_2CHCH_2), 4.47 (s, 2H, 6-S$CH_2C_6H_5$), 4.51 (t, J: 5.61 Hz, 1H, $HOCH_2$, D2O exchangeable), 4.58 (s, 2H, 4-S$CH_2C_6H_5$), 4.73 (d, J: 4.64 Hz, 1H, $HOCH$, D_2O exchangeable), 5.76 (s, 2H, OCH_2N), 7.21–7.98 (m, 10H, 2 C_6H_5). MS (FAB$^+$, GT) *m/z:* 548 $[M+H]^+$.

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