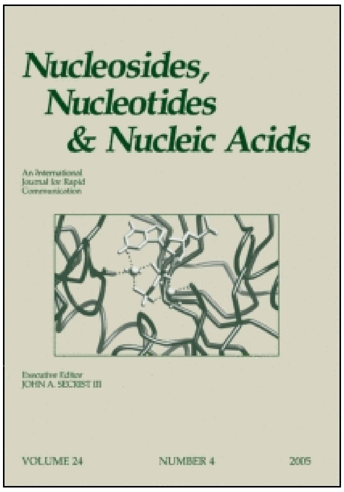

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 4-SUBSTITUTED 1-[1-(2-HYDROXYETHOXY)- METHYL-1,2,3-TRIAZOL-(4 & 5)-YLMETHYL]-1*H*-PYRAZOLO[3,4-d]PYRIMIDINES

O. Moukha-Chafiq[a]; M. L. Taha[a]; H. B. Lazrek[a]; C. Pannecouque[b]; M. Witvrouw[b]; E. De Clercq[b]; J. L. Barascut[c]; J. L. Imbach[c]
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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 4-SUBSTITUTED 1-[1-(2-HYDROXYETHOXY)-METHYL-1,2,3-TRIAZOL-(4 & 5)-YLMETHYL]-1*H*-PYRAZOLO[3,4-d]PYRIMIDINES

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ABSTRACT

The chemical synthesis of some 4-substituted 1-[1-(2-hydroxyethoxy)-methyl-1,2,3-triazol-(4 and 5)-ylmethyl]-1*H*-pyrazolo[3,4-d]pyrimidines **12a,b**, **13a,b** and **14–23** as acyclic nucleosides is described. Treatment of (2-acetoxyethoxy)methylbromide with sodium azide afforded (2-acetoxyethoxy)methylazide **9**. The heterocycles **6a,b** were alkylated, separately, with propargyl bromide to obtain, regioselectively, 4-(methyl and benzyl)thio-1-(prop-2-ynyl)-1*H*-pyrazolo[3,4-d]pyrimidines **7a,b**. These N_1-alkylated products were condensed with compound **9** *via* a 1,3-dipolar cycloaddition reaction to obtain, after separation and deprotection, 1,4- and 1,5-regioisomers **12a,b** and **13a,b**. The deprotected acyclic nucleosides **12a** and **13a** served as precursors for the preparation of 4-amino (**14** and **15**), 4-methylamino (**16** and **17**), 4-benzylamino (**18** and **19**),

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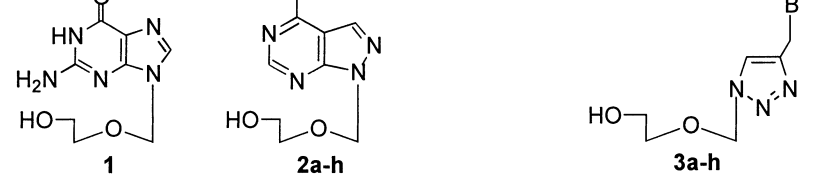
4-methoxy (**20** and **21**) and 4-hydroxy (**22** and **23**) analogues. Compounds **7a,b** and all deprotected acyclic nucleosides were evaluated for their inhibitory effects against the replication of HIV-1(III_B) and HIV-2(ROD) in MT-4 cells and for their anti-tumor activity. No marked activity was found. However, initial evaluation of **6a,b**, **7a,b**, **12a,b**, **13a,b** and **14–23** showed that compound **7b** has marked activity against M. tuberculosis.

INTRODUCTION

In the past few years, intensive efforts of chemists and molecular pharmacologists have been directed towards the synthesis of various acyclonucleosides with different side chains and aglycons in the hope to discover new and more effective antiviral agents[1–3]. Active compounds are generally considered to exhibit antiviral activities after being converted to their triphosphates. For example, the anti-HSV agent acyclovir[4] **1** (Fig. 1), is monophosphorylated by HSV thymidine kinase and then converted to the corresponding triphosphate by cellular enzymes. So far, structure-activity relationship studies have shown that the side chains of acyclonucleosides play a crucial role in the interaction of acyclonucleoside with their antiviral target enzyme (phosphorylation). The orientation of the heterocyclic base about the glycosidic bond in nucleosides is an important conformational parameter, and has been clearly delineated in a variety of enzymatic reactions[5]. In this respect, we recently reported the synthesis of some acyclonucleosides with the alkyl chain of acyclovir, in which the guanine part was replaced by pyrazolo[3,4-d]pyrimidine[6] **2a–h** and N_1/N_9-1,2,3-triazol-4-ylmethylpyrimidine/purine[7] **3a–h** derivatives (Fig. 1).

In this paper, we would like to report the synthesis of some 4-substituted 1-[1-(2-hydroxyethoxy)methyl-1,2,3-triazol-(4 and 5)-ylmethyl]-1*H*-pyrazolo-



a: R = SCH_3, **b**: R = SC_2H_5
c: R = $SCH_2C_6H_5$, **d**: R = NH_2
e: R = $HNCH_3$, **f**: R = $N(CH_3)_2$
g: R = $NHCH_2C_6H_5$, **h**: R = OCH_3

a: B = Thymine, **b**: B = N-Ac guanine
c: B = 5-Chlorouracil, **d**: B = Uracil
e: B = 5-Bromouracil, **f**: B = Adenine
g: B = 5-Iodouracil, **h**: B = 5-Fluorouracil

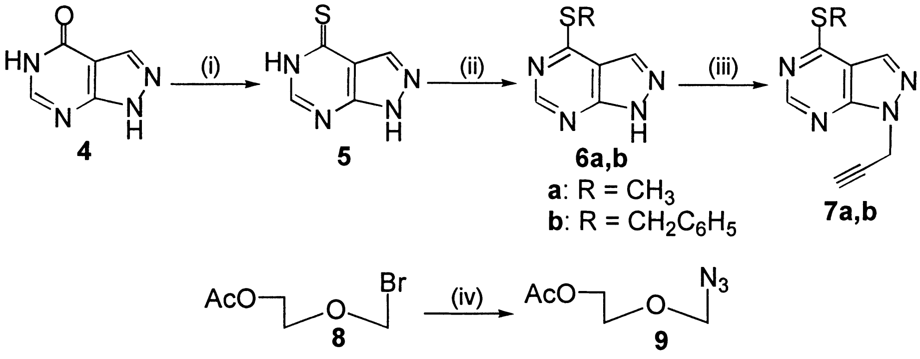
Figure 1.

[3,4-d]pyrimidines **12a,b**, **13a,b** and **14–23** (Scheme 2), in order to determine the influence on biological evaluation of the 1,2,3-triazol-(4 or 5)-ylmethyl moiety as a spacer between the pyrazolo[3,4-d]pyrimidine derivatives and the (2-hydroxyethoxy)methyl chain. The synthesis and biological activity are described herein.

RESULTS AND DISCUSSION

Treatment of pyrazolo[3,4-d]pyrimidin-4-one **4** with phosphorus pentasulfide in pyridine gave pyrazolo[3,4-d]pyrimidine-4-thione **5** in 98% yield (Scheme 1). The methylation[8] and benzylation of this compound with methyl iodide and benzyl bromide in sodium hydroxide solution gave **6a** and **6b** in 78% and 72% yield, respectively (Scheme 1). The preparation of **7a,b** was achieved using the same conditions previously described for the synthesis of the 4-substituted pyrazolo[3,4-d]pyrimidine N_1-acyclonucleosides[9,10]. Thus, the alkylation of heterocycles **6a,b** with propargyl bromide, using potassium carbonate as base and *N,N*-dimethylformamide (DMF) as solvent, afforded regioselectively the N_1-regioisomers **7a,b** in 73% and 78% yield, respectively (Scheme 1). The site of alkylation for compounds **7a,b** was established to be at N_1 by a direct comparison of the UV spectra of the compounds **7a,b** with the UV spectra of the corresponding N_1-acyclonucleosides[9,10]. The N_2-nucleoside (acyclonucleoside) isomer was reported[10–16] to occur during glycosylation (alkylation) of the pyrazolo[3,4-d]pyrimidines. The presumed N_2-regioisomers of **7a,b** were detected but not isolated.

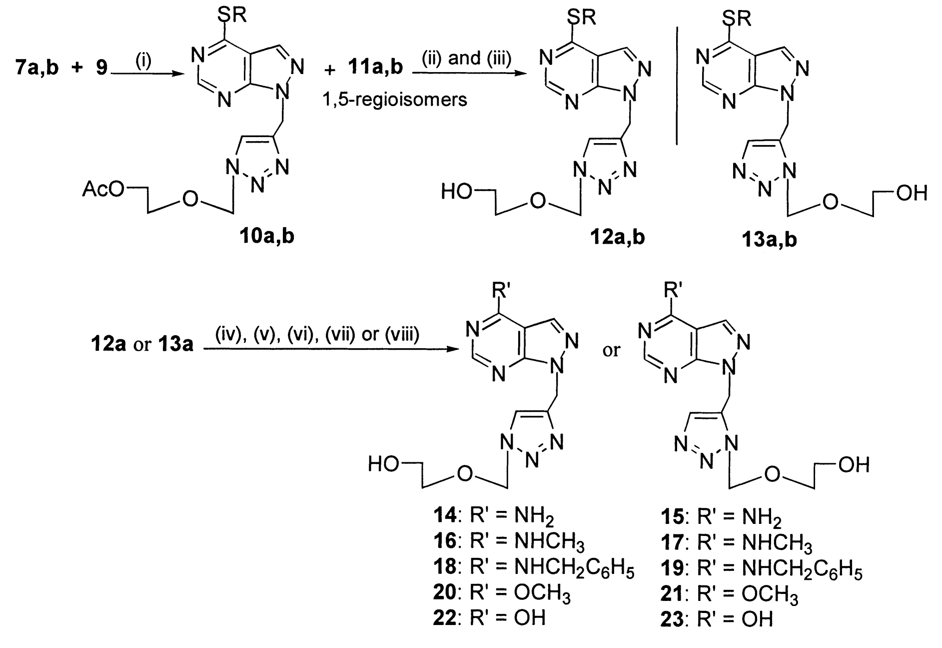
The treatment of (2-acetoxyethoxy)methylbromide[17] **8** with sodium azide in DMF gave the desired compound **9** in 96% yield (Scheme 1).



Ac: acetyl group
(i): P_2S_5, pyridine, reflux; (ii): NaOH (0.8N), CH_3I or $C_6H_5CH_2Br$, r. t.;
(iii): propargyl bromide, K_2CO_3, DMF, r. t.; (iv): NaN_3, DMF, 90°C.

Scheme 1.

The 4-substituted 1-[1-(2-acetoxyethoxy)methyl-1,2,3-triazol-(4 and 5)-ylmethyl]-1*H*-pyrazolo[3,4-d]pyrimidines **10a,b** and **11a,b** (Scheme 2) were prepared by 1,3-dipolar cycloaddition of the azide **9** to the N_1-propargyl derivatives **7a,b**. It is well known from the literature[18] that the addition of azides to unsymmetrical acetylenes is determined by steric and electronic factors. In general, such addition tends to give mainly the isomers with electron-withdrawing groups at the 4-position and electron-donating groups at the 5-position. On the other hand, the sterically less hindered isomer tends to be the major isomer. Thus, cycloaddition of compound **9** with **7a,b**, in anhydrous toluene under reflux, afforded a mixture of two possible protected acyclonucleosides (**10a**+**11a**) and (**10b**+**11b**) in 87% and 89% overall yield, respectively. After their separation on silica gel column chromatography, the products **10a** and **10b** were obtained as the major regioisomers in 68% and 73% yields respectively. The structures of the regioisomers **10a,b** and **11a,b** were established by comparison of the chemical shift values for the triazole ring protons with those available from a known pair of alkyl-1,2,3-triazole derivatives[7,18]. The 1,4 regioisomers **10a,b** showed an H-5 resonance at lower field (8.22 and 8.21 ppm, respectively). For the 1,5-regioisomers **11a,b**, the H-4 resonance was at higher field (7.70 and 7.72 ppm, respectively). The differences in the



(i): toluene, reflux; (ii): separation by chromatography on silica gel column; (iii): NH_3/CH_3OH, r.t; (iv): NH_3/CH_3OH, 100°C; (v): methylamine 40%, reflux; (vi): benzylamine, ethanol, reflux; (vii): CH_3ONa/CH_3OH, r.t; (viii): NaOH (2N), r.t.

Scheme 2.

chemical shifts between the regioisomers are in agreement with literature data[7,18].

The acetyl groups were subsequently removed from the protected acyclic nucleosides **10a,b** and **11a,b** by treatment with methanol saturated with ammonia at 25 °C, affording the required deacetylated acyclonucleosides **12a,b** and **13a,b** in good yield (Scheme 2).

When compounds **12a** and **13a** were treated with methanol saturated with ammonia in a sealed reacting vessel at 100 °C, the 4-amino-1-[1-(2-hydroxyethoxy)-methyl-1,2,3-triazol-(4 and 5)-ylmethyl]-1*H*-pyrazolo[3,4-d]pyrimidines **14** and **15** were obtained in 88% and 81% yield, respectively (Scheme 2). Condensation of the compounds **12a** and **13a** with primary amines in aqueous or alcohol solution afforded, in good yield, the acyclonucleosides **16–19** (Scheme 2).

The acyclonucleosides **20** and **21** were synthesized in 70% yield *via* treatment of **12a** and **13a** with CH_3ONa/CH_3OH solution at room temperature (Scheme 2). Treatment of the compounds **12a** and **13a** with sodium hydroxide solution at room temperature gave the products **22** and **23** in 82% and 77% yield, respectively (Scheme 2). Structure identification of the synthetic products was done by ^{1}H-NMR, mass spectra and elemental analysis.

BIOLOGICAL STUDIES

Compounds **7a,b**, **12a,b**, **13a,b**, and **14–23** were evaluated for their cytotoxycity and their inhibitory effect on HIV-1(III_B) and HIV-2(ROD) replication in MT-4 cells[19,20]. All compounds tested were inactive against the replication of HIV-1(III_B) and HIV-2(ROD) at subtoxic concentrations.

The compounds were also evaluated for their anti-tumor activity using a series of tumor-cell lines (leukemia, colon cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, breast cancer, brain cancer, non-small cell lung cancer). However, none of the compounds showed appreciable anti-tumor activity at compound concentrations lower than 10^{-4} M.

Primary screening of compounds **6a,b**, **7a,b**, **12a,b**, **13a,b** and **14–23** against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) in BACTEC 12B medium revealed that only compound **7b** had potent activity (90% of inhibition at a concentration of 12.5 μg/ml), while **6a,b**, **12b**, **19** and **20** had affected (2%, 50%, 52%, 11% and 57% inhibition at concentration of 12.5 μg/ml for **6a,b** and 6.25 μg/ml for **12b**, **19** and **20**, respectively).

In conclusion, a series of acyclic 1,2,3-triazol(4 & 5)ylmethyl-1*H*-pyrazolo[3,4-d]pyrimidine nucleosides with the same side chain as in acyclovir were synthesized, in good yield, from accessible starting materials, using the 1,3-dipolar cycloaddition reaction.

EXPERIMENTAL

Melting points (mp) were determined on a Electrothermal digital melting point apparatus and were uncorrected. Ultraviolet (UV) spectra were recorded on a Beckman DU-64 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. Key: s (singlet), d (doublet), t (triplet), m (multiplet) and br (broad). Mass spectra were obtained with a JOEL JMS DX 300 instrument using fast atomic bombardment (FAB positive). Infrared (IR) spectra were recorded with a Perkin-Elmer 580 spectrometer. 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. R_f^2 is R_f after two migrations in hexane/ethyl acetate (6/4, v/v). Column chromatography separation were obtained on silica gel 60 (70–230 mesh, Merck). Elemental analysis were determined by the French microanalytical central service.

Preparation of Pyrazolo[3,4-d]pyrimidin-4-thione 5

A freshly pulverized mixture of the allopurinol **4** (10 g, 73.52 mmol) and phosphorus pentasulfide (50 g, 225.28 mmol), in 300 ml of pyridine, was heated overnight under reflux conditions. The pyridine was removed under reduced pressure and the residue was added carefully to one liter of water at 90 °C. The solution was then boiled for ten minutes and enough potassium hydroxide was added to effect complete solution. The solution was stirred with charcoal and filtered. Acidification of the hot filtrate with acetic acid yielded a light tan precipitate which was recrystallized from a 30% ethanol solution to give compound **5** as white crystals in 98% yield. $R_f = 0.22$ ($CHCl_3/CH_3OH$, 9/1, v/v). mp > 360 °C (ethanol 30%) (Lit (6): mp >360 °C). ^{1}H-NMR, $Me_2SO\text{-}d_6$, δ: 8.13 and 8.25 (2s, 2H, H_3 and H_6), 13.60 (s, 1H, S$\underline{H}$), 14.00 (br s,1H, N$\underline{H}$). MS, m/z: $[M+H]^+ = 153$.

Preparation of Compounds 6a,b

Compound **5** (5 g, 32.89 mmol) was dissolved in 42 ml of sodium hydroxide solution (0.8N). To this solution were added 3.92 g (32.94 mmol) of methyl iodide or 4.67 g (32.91 mmol) of benzyl bromide at 0 °C. The mixture was stirred at room temperature for 25 minutes. The solution was filtered and the crude product was recrystallized in water or methanol to give **6a,b** in 78% and 72% yield, respectively.

4-Methylthiopyrazolo[3,4-d]pyrimidine (6a). $R_f = 0.50$ ($CHCl_3/CH_3OH$, 9/1, v/v). mp: 193–194 °C (water) (Lit (8): mp: 193 °C). ^{1}H-NMR, Me_2SO-d_6, δ: 2.73 (s, 3H, SC$\underline{H}_3$), 8.30 and 8.75 (2s, 2H, H_3 and H_6), 14.00 (br s, 1H, N$\underline{H}$). MS, m/z: $[M+H]^+ = 167$.

4-Benzylthiopyrazolo[3,4-d]pyrimidine (6b). $R_f = 0.60$ ($CHCl_3/CH_3OH$, 9/1, v/v). mp: 173–174 °C (methanol). UV (ethanol): λ_{max} 287 nm ($\varepsilon = 18\,100$). ^{1}H-NMR, Me_2SO-d_6, δ: 4.70 (s, 2H, SC$\underline{H}_2$), 7.33 (m, 5H, aromatic protons of phenyl group), 8.26 and 8.76 (2s, 2H, H_3 and H_6), 14.13 (br s, 1H, N$\underline{H}$). MS, m/z: $[M+H]^+ = 243$, $[C_6H_5\text{-}CH_2]^+ = 91$.

General Alkylation Procedure

A mixture of the heterocycle **6a** or **6b** (1 mmol), potassium carbonate (2 mmol) and dry DMF (14 ml) was stirred for 15 minutes at room temperature. The propargyl bromide (1.2 mmol) was added, at 0 °C, and the mixture was stirred overnight at room temperature. After filtration, the solvent was removed *in vacuo*. The residue was then chromatographed on a silica gel column, using chloroform as eluent, to furnish the propargylated product.

4-Methylthio-1-(prop-2-ynyl)-1*H*-pyrazolo[3,4-d]pyrimidine (7a). Yield: 0.149 g (73%). $R_f = 0.53$ ($CHCl_3$). mp: 107–108 °C (ethanol). UV (pH_7): λ_{max} 292 nm ($\varepsilon = 21\,000$), λ_{min} 248 nm ($\varepsilon = 3\,600$). ^{1}H-NMR ($CDCl_3$), δ: 2.72 (s, 3H, SC$\underline{H}_3$), 3.42 (t, 1H, $\underline{H}$CC-CH_2-N, J = 2.48 Hz), 5.28 (d, 2H, N-C$\underline{H}_2$-CC, J = 2.48 Hz), 8.40 (s, 1H, H_3), 8.82 (s, 1H, H_6). MS, m/z: $[M+H]^+ = 205$.

4-Benzylthio-1-(prop-2-ynyl)-1*H*-pyrazolo[3,4-d]pyrimidine (7b). Yield: 0.218 g (78%). $R_f = 0.41$ ($CHCl_3$). mp: 100–101 °C (ethanol). UV (pH_7): λ_{max} 292 nm ($\varepsilon = 20\,300$), λ_{min} 248 nm ($\varepsilon = 6\,000$). ^{1}H-NMR ($CDCl_3$), δ: 3.75 (t, 1H, $\underline{H}$CC-CH_2, J = 2.48 Hz), 4.73 (s, 2H, SC$\underline{H}_2$-C_6H_5), 5.32 (d, 2H, N-C$\underline{H}_2$-CC, J = 2.48 Hz), 7.41 (m, 5H, phenyl group aromatic protons), 8.41 (s, 1H, H_3), 8.89 (s, 1H, H_6). MS, m/z: $[M+H]^+ = 281$, $[C_6H_5\text{-}CH_2]^+ = 91$.

Preparation of (4-Acetoxyethoxy)methylazide 9

To a solution of finely ground NaN_3 (5.44 g, 83.75 mmol) in 240 ml of anhydrous DMF were added 11 g (55.83 mmol) of freshly distilled compound **8** and the resulting mixture was heated at 90 °C under stirring for 4 h. The solid was removed and washed with DMF (3 × 30 ml). To a combined filtrate and washings were added 150 ml of water. The mixture was then extracted with ether (4 × 60 ml). The extract was combined, dried ($MgSO_4$) and then evaporated *in vacuo* to leave a pale yellow oil. The oil was chromatographed on silica gel with hexane as eluent to give compound **9** (8.07 g, 91%) as a clear oil. IR (CCl_4): 2100 cm^{-1} (N_3), 1730 cm^{-1} ($\underline{CO}CH_3$). ^{1}H-NMR, $CDCl_3$, δ: 2.10 (s, 3H, $\underline{CH_3}CO$), 3.80 (m, 2H, O-$\underline{CH_2}$), 4.25 (m, 2H, $\underline{CH_2}$-O), 4.70 (s, 2H, O-$\underline{CH_2}$-N_3).

Preparation of Compounds 10a,b and 11a,b

A mixture of propargylated heterocycle **7a** or **7b** (1 mmol) and azide derivative **9** (1.5 mmol) in anhydrous toluene (60 ml) was refluxed for two days. The reaction was monitored by thin-layer chromatography and was shown to be completed at this time with formation of two isomers. The solution was evaporated to dryness. The mixture of the regioisomers was chromatographed onto a silica gel column, using hexane/ethyl acetate (60/40, v/v) as eluent, to give: the fastest moving band 69 mg (19%) of **11a** (as an amorphous solid) or 70 mg (16%) of **11b** (as a white foam) and the slowest moving band 246 mg (68%) of **10a** (as an amorphous solid) or 320 mg (73%) of **10b** (as an amorphous solid).

1-[1-(2-Acetoxyethoxy)methyl-1,2,3-triazol-4-ylmethyl]-4-methylthio-1*H*-pyrazolo-[3,4-d]pyrimidine (10a). $R_f^2 = 0.41$. mp: 93–94 °C (ethanol). ^{1}H-NMR, $Me_2SO\text{-}d_6$, δ: 1.95 (s, 3H, $C\underline{H}_3CO$), 2.71 (s, 3H, $SC\underline{H}_3$), 3.62 and 4.10 (2m, 4H, O-$C\underline{H}_2$-$C\underline{H}_2$-O), 5.69 (s, 2H, C-$C\underline{H}_2$-N), 5.74 (s, 2H, O-$C\underline{H}_2$-N), 8.22 (s, 1H, aromatic proton of triazol group), 8.35 and 8.83 (2s, 2H, H_3 and H_6). MS, m/z: $[M+H]^+ = 364$, $[CH_3CO]^+ = 43$.

1-[1-(2-Acetoxyethoxy)methyl-1,2,3-triazol-5-ylmethyl]-4-methylthio-1*H*-pyrazolo-[3,4-d]pyrimidine (11a). $R_f^2 = 0.48$. mp: 71–72 °C ($CHCl_3$). ^{1}H-NMR, $Me_2SO\text{-}d_6$, δ: 1.95 (s, 3H, $C\underline{H}_3CO$), 2.72 (s, 3H, $SC\underline{H}_3$), 3.56 and 3.98 (2m, 4H, O-$C\underline{H}_2$-$C\underline{H}_2$-O), 5.88 (s, 2H, C-$C\underline{H}_2$-N), 5.92 (s, 2H, O-$C\underline{H}_2$-N), 7.70 (s, 1H, aromatic proton of triazole group), 8.41 and 8.86 (2s, 2H, H_3 and H_6). MS, m/z: $[M+H]^+ = 364$, $[CH_3CO]^+ = 43$.

1-[1-(2-Acetoxyethoxy)methyl-1,2,3-triazol-4-ylmethyl]-4-benzylthio-1*H*-pyrazolo-[3,4-d]pyrimidine (10b). $R_f^2 = 0.47$. mp: 100–101 °C (ethanol). ^{1}H-NMR, $Me_2SO\text{-}d_6$, δ: 1.92 (s, 3H, $C\underline{H}_3CO$), 3.63 and 4.03 (2m, 4H, O-$C\underline{H}_2$-$C\underline{H}_2$-O), 4.68 (s, 2H, $SC\underline{H}_2$-C_6H_5), 5.68 (s, 2H, C-$C\underline{H}_2$-N), 5.71 (s, 2H, O-$C\underline{H}_2$-N), 7.29 and 7.46 (2m, 5H, aromatic protons of phenyl group), 8.21 (s, 1H, aromatic proton of triazole group), 8.33 and 8.85 (2s, 2H, H_3 and H_6). MS, m/z: $[M+H]^+ = 440$, $[CH_3CO]^+ = 43$.

1-[1-(2-Acetoxyethoxy)methyl-1,2,3-triazol-5-ylmethyl]-4-benzylthio-1*H*-pyrazolo-[3,4-d]pyrimidine (11b). $R_f^2 = 0.56$. ^{1}H-NMR, $Me_2SO\text{-}d_6$, δ: 1.97 (s, 3H, $C\underline{H}_3CO$), 3.56 and 3.93 (2m, 4H, O-$C\underline{H}_2$-$C\underline{H}_2$-O), 4.72 (s, 2H,

$SC\underline{H}_2$-C_6H_5), 5.87 (s, 2H, C-$C\underline{H}_2$-N), 5.90 (s, 2H, O-$C\underline{H}_2$-N), 7.33 and 7.50 (2m, 5H, aromatic proton of phenyl group), 7.72 (s, 1H, aromatic protons of triazole group), 8.39 and 8.89 (2s, 2H, H_3 and H_6). MS, m/z: $[M+H]^+ = 440$, $[CH_3CO]^+ = 43$.

General Deprotection Method

To 45 ml of anhydrous methanol saturated with NH_3 at $-5\,°C$ was added 1 mmol of the acetylated product **10a,b** or **11a,b**. The flask was stopped tightly and the solution was stirred for 16 hours at room temperature. Thin-layer chromatography (tlc) evaluation indicated that complete deprotection of acetylated product had occurred. Volatile materials were evaporated *in vacuo* and the resulting solid was recrystallized or, if necessary, purified on a column of silica gel, using chloroform/methanol (98/2, v/v) as eluent, to obtain the expected deacetylated acyclonucleoside.

1-[1-(2-Hydroxyethoxy)methyl-1,2,3-triazol-4-ylmethyl]-4-methylthio-1*H*-pyrazolo-[3,4-d]pyrimidine (12a). Yield: 0.31 g (97%). $R_f = 0.33$ ($CHCl_3$/MeOH, 9/1, v/v). mp: 120–121 °C (ethanol). ^{1}H-NMR, Me_2SO-d_6, δ: 2.68 (s, 3H, $SC\underline{H}_3$), 3.40 (m, 4H, O-$C\underline{H}_2$-$C\underline{H}_2$-O), 4.68 (t, 1H, O$\underline{H}$, D_2O exchangeable, J = 5.00 Hz), 5.66 (s, 2H, C-$C\underline{H}_2$-N), 5.69 (s, 2H, O-$C\underline{H}_2$-N), 8.19 (s, 1H, aromatic proton of triazole group), 8.33 and 8.79 (2s, 2H, H_3 and H_6). MS, m/z: $[M+H]^+ = 322$. Anal. calcd for $C_{12}H_{15}N_7O_2S$ (321.35): C 44.85, H 4.70, N 30.51. Found: C 44.73, H 4.80, N 30.79.

1-[1-(2-Hydroxyethoxy)methyl-1,2,3-triazol-5-ylmethyl]-4-methylthio-1*H*-pyrazolo-[3,4-d]pyrimidine (13a). Yield: 0.31 g (97%), $R_f = 0.41$ ($CHCl_3$/MeOH, 9/1, v/v). mp: 109–110 °C (ethanol). ^{1}H-NMR, Me_2SO-d_6, δ: 2.74 (s, 3H, $SC\underline{H}_3$), 3.37 (m, 4H, O-$C\underline{H}_2$-$C\underline{H}_2$-O), 4.71 (t, 1H, O$\underline{H}$, D_2O exchangeable, J = 5.00 Hz), 5.87 (s, 2H, C-$C\underline{H}_2$-N), 5.91 (s, 2H, O-$C\underline{H}_2$-N), 7.70 (s, 1H, aromatic proton of triazole group), 8.42 and 8.85 (2s, 2H, H_3 and H_6). MS, m/z: $[M+H]^+ = 322$. Anal. calcd for $C_{12}H_{15}N_7O_2S$ (321.35): C 44.85, H 4.70, N 30.51. Found: C 44.70, H 4.82, N 30.83.

1-[1-(2-Hydroxyethoxy)methyl-1,2,3-triazol-4-ylmethyl]-4-benzylthio-1*H*-pyrazolo-[3,4-d]pyrimidine (12b). Yield: 0.37 g (93%). $R_f = 0.39$ ($CHCl_3$/MeOH, 9/1, v/v). mp: 135–136 °C (ethanol). ^{1}H-NMR, Me_2SO-d_6, δ: 3.48 (m, 4H, O-$C\underline{H}_2$-$C\underline{H}_2$-O), 4.70 (br s, 1H, O$\underline{H}$, D_2O exchangeable), 4.72 (s, 2H, $SC\underline{H}_2$-C_6H_5), 5.70 (s, 2H, C-$C\underline{H}_2$-N), 5.75 (s, 2H, O-$C\underline{H}_2$-N), 7.33 and 7.50 (2m, 5H, aromatic protons of phenyl group), 8.24 (s, 1H, aromatic proton of triazole group), 8.36 and 8.89 (2s, 2H, H_3 and H_6). MS, m/z: $[M+H]^+ = 398$, $[C_6H_5$-$CH_2]^+ = 91$. Anal. calcd for $C_{18}H_{19}N_7O_2S$ (397.45): C 54.39, H 4.81, N 24.66. Found: C 54.16, H 4.93, N 24.96.

1-[1-(2-Hydroxyethoxy)methyl-1,2,3-triazol-5-ylmethyl]-4-benzylthio-1*H*-pyrazolo-[3,4-d]pyrimidine (13b). Yield: 0.37 g (93%). $R_f = 0.47$ ($CHCl_3$/MeOH, 9/1, v/v). mp: 230–131 °C (ethanol). ^{1}H-NMR, Me_2SO-d_6, δ: 3.58 and 3.93 (2m, 4H, O-C$\underline{H}_2$-C$\underline{H}_2$-O), 4.70 (t, 1H, OH, D_2O exchangeable, J = 5.00 Hz), 4.72 (s, 2H, SC$\underline{H}_2$-C_6H_5), 5.86 (s, 2H, C-C$\underline{H}_2$-N), 5.94 (s, 2H, O-C$\underline{H}_2$-N), 7.33 and 7.50 (2m, 5H, aromatic protons of phenyl group), 7.74 (s, 1H, aromatic proton of triazole group), 8.35 and 8.88 (2s, 2H, H_3 and H_6). MS, m/z: $[M+H]^+ = 398$, $[C_6H_5\text{-}CH_2]^+ = 91$. Anal. calcd for $C_{18}H_{19}N_7O_2S$ (397.45): C 54.39, H 4.81, N 24.66. Found: C 54.11, H 4.97, N 24.99.

Preparation of Acyclonucleosides 14 and 15 from 12a and 13a

A solution of **12a** or **13a** (0.15 g, 0.46 mmol) in 30 ml of methanol saturated with ammonia (previously saturated at − 5 °C) was heated at 100 °C for 20 hours in a sealed reacting vessel. After removal of the solvent, the resulting solid was recrystallized from 95% ethanol to provide pure compound **14** (0.12 g, 88%) or compound **15** (0.11 g, 81%).

4-Amino-1-[1-(2-hydroxyethoxy)methyl-1,2,3-triazol-4-ylmethyl]-1*H*-pyrazolo[3,4-d]pyrimidine (14). $R_f = 0.16$ ($CHCl_3$/MeOH, 8/2, v/v). mp: 190–191 °C (ethanol). ^{1}H-NMR, Me_2SO-d_6, δ: 3.48 (m, 4H, O-C$\underline{H}_2$-C$\underline{H}_2$-O), 4.71 (br s, 1H, O$\underline{H}$, D_2O exchangeable), 5.58 (s, 2H, C-C$\underline{H}_2$-N), 5.69 (s, 2H, O-C$\underline{H}_2$-N), 7.78 (s, 2H, N$\underline{H}_2$, D_2O exchangeable), 8.12 (s, 1H, triazole group of aromatic proton), 8.15 and 8.22 (2s, 2H, H_3 and H_6). MS, m/z: $[M+H]^+ = 291$. Anal. calcd for $C_{11}H_{14}N_8O_2$ (290.28): C 45.51, H 4.86, N 38.60. Found: C 45.28, H 4.96, N 38.71.

4-Amino-1-[1-(2-hydroxyethoxy)methyl-1,2,3-triazol-5-ylmethyl]-1*H*-pyrazolo[3,4-d]pyrimidine (15). $R_f = 0.13$ ($CHCl_3$/MeOH, 9/1, v/v). mp: 171–172 °C (ethanol). ^{1}H-NMR, Me_2SO-d_6, δ: 3.40 (m, 4H, O-C$\underline{H}_2$-C$\underline{H}_2$-O), 4.70 (t, 1H, OH, D_2O exchangeable, J = 5.00 Hz), 5.71 (s, 2H, C-C$\underline{H}_2$-N), 5.85 (s, 2H, O-C$\underline{H}_2$-N), 7.60 (s, 1H, aromatic proton of triazole group), 7.78 (s, 2H, N$\underline{H}_2$, D_2O exchangeable), 8.12 and 8.21 (2s, 2H, H_3 and H_6). MS, m/z: $[M+H]^+ = 291$. Anal. calcd for $C_{11}H_{14}N_8O_2$ (290.28): C 45.51, H 4.86, N 38.60. Found: C 45.24, H 4.97, N 38.74.

Preparation of Acylonucleosides 16 and 17 from 12a and 13a

A solution of 0.25 g (0.77 mmol) of **12a** or **13a** in 40% aqueous methylamine (7.8 ml) was refluxed for 10 minutes. The reaction was monitored by tlc, and was completed at this time. After cooling, the residue was coevaporated with benzene (5 × 4 ml) and the resulting solid was recrystallized from ethanol to provide crystalline **16** (0.19 g, 78%) or **17** (0.18 g, 76%).

1-[1-(2-Hydroxyethoxy)methyl-1,2,3-triazol-4-ylmethyl]-4-methylamino-1*H*-pyrazolo[3,4-d]pyrimidine (16). $R_f = 0.13$ ($CHCl_3$/MeOH, 9/1, v/v). mp: 183–184 °C (ethanol). ^{1}H-NMR, Me_2SO-d_6, δ: 2.96 (d, 3H, HN-C$\underline{H}_3$, J = 4.58 Hz), 3.48 (m, 4H, O-C$\underline{H}_2$-C$\underline{H}_2$-O), 4.70 (t, 1H, O$\underline{H}$, D_2O exchangeable, J = 5.00 Hz), 5.56 (s, 2H, C-C$\underline{H}_2$-N), 5.66 (s, 2H, O-C$\underline{H}_2$-N), 8.06 (s, 1H, aromatic proton of triazole proton), 8.10 and 8.28 (2s, 2H, H_3 and H_6), 8.26 (br s,1H, $\underline{H}$N-CH_3, D_2O exchangeable). MS, m/z: $[M+H]^+ = 305$, $[HO\text{-}CH_2\text{-}CH_2\text{-}O\text{-}CH_2]^+ = 75$. Anal. calcd for $C_{12}H_{16}N_8O_2$ (304.30): C 47.36, H 5.30, N 36.82. Found: C 47.07, H 5.41, N 36.92.

1-[1-(2-Hydroxyethoxy)methyl-1,2,3-triazol-5-ylmethyl]-4-methylamino-1*H*-pyrazolo[3,4-d]pyrimidine (17). $R_f = 0.15$ ($CHCl_3$/MeOH, 9/1, v/v). mp: 84–85 °C (ethanol). ^{1}H-NMR, Me_2SO-d_6, δ: 2.96 (d, 3H, HN-C$\underline{H}_3$, J = 4.58 Hz), 3.44 (m, 4H, O-C$\underline{H}_2$-C$\underline{H}_2$-O), 4.70 (t, 1H, O$\underline{H}$, D_2O exchangeable, J = 5.00 Hz), 5.70 (s, 2H, C-C$\underline{H}_2$-N), 5.88 (s, 2H, O-C$\underline{H}_2$-N), 7.58 (s, 1H, aromatic proton of triazole group), 8.10 and 8.30 (2s, 2H, H_3 and H_6), 8.32 (br s,1H, $\underline{H}$N-CH_3, D_2O exchangeable). MS, m/z: $[M+H]^+ = 305$, $[HO\text{-}CH_2\text{-}CH_2\text{-}O\text{-}CH_2]^+ = 75$. Anal. calcd for $C_{12}H_{16}N_8O_2$ (304.30): C 47.36, H 5.30, N 36.82. Found: C 47.01, H 5.45, N 36.95.

Preparation of Acyclonucleosides 18 and 19 from 12a and 13a

To a solution of **12a** or **13a** (0.25 g, 0.65 mmol) in 7.4 ml of absolute ethanol were added 2.43 ml of freshly distilled benzylamine and the mixture was heated at reflux temperature with stirring overnight. The solvent was removed *in vacuo*, the residue was purified on a column of silica gel and then recrystallized from ethanol to give compound **18** (0.24 g, 81%) or compound **19** (0.23 g, 77%).

4-Benzylamino-1-[1-(2-hydroxyethoxy)methyl-1,2,3-triazol-4-ylmethyl]-1*H*-pyrazolo[3,4-d]pyrimidine (18). Eluent: ($CHCl_3$/MeOH, 98/2, v/v). $R_f = 0.26$ ($CHCl_3$/MeOH, 9/1, v/v). mp: 58–59 °C (ethanol). ^{1}H-NMR, Me_2SO-d_6, δ: 3.50 (m, 4H, O-C$\underline{H}_2$-C$\underline{H}_2$-O), 4.72 (t, 1H, O$\underline{H}$, D_2O exchangeable, J = 5.00 Hz), 4.78 (d, 2H, HN-C$\underline{H}_2$-C_6H_5, J = 5.89 Hz), 5.61 (s, 2H, C-C$\underline{H}_2$-N), 5.70 (s, 2H, O-C$\underline{H}_2$-N), 7.33 (m, 5H, aromatic protons of phenyl group), 8.17 (s, 1H, aromatic proton of triazole group), 8.20 and 8.33 (2s, 2H, H_3 and H_6), 8.84 (t, 1H, $\underline{H}$N-CH_2, D_2O exchangeable, J = 5.89 Hz). MS, m/z: $[M+H]^+ = 381$, $[C_6H_5\text{-}CH_2]^+ = 91$. Anal. calcd for $C_{18}H_{20}N_8O_2$ (380.40): C 56.83, H 5.29, N 29.45. Found: C 56.60, H 5.35, N 29.61.

4-Benzylamino-1-[1-(2-hydroxyethoxy)methyl-1,2,3-triazol-5-ylmethyl]-1*H*-pyrazolo[3,4-d]pyrimidine (19). Eluent: ($CHCl_3$/MeOH, 98/2, v/v). $R_f = 0.34$ ($CHCl_3$/MeOH, 9/1, v/v). ^{1}H-NMR, Me_2SO-d_6, δ: 3.50 (m, 4H, O-C$\underline{H}_2$-C$\underline{H}_2$-O), 4.70 (t, 1H, O$\underline{H}$, D_2O exchangeable, J = 5.00 Hz), 4.80 (d, 2H, HN-C$\underline{H}_2$-C_6H_5, J = 5.89 Hz), 5.75 (s, 2H, C-C$\underline{H}_2$-N), 5.90 (s, 2H, O-C$\underline{H}_2$-N), 7.33 (m, 5H, aromatic protons of phenyl group), 7.62 (s, 1H, aromatic proton of triazole group), 8.30 and 8.40 (2s, 2H, H_3 and H_6), 8.86 (t, 1H, $\underline{H}$N-CH_2, D_2O exchangeable, J = 5.89 Hz). MS, m/z: $[M+H]^+ = 381$, $[C_6H_5\text{-}CH_2]^+ = 91$. Anal. calcd for $C_{18}H_{20}N_8O_2$ (380.40): C 56.83, H 5.29, N 29.45. Found: C 56.58, H 5.39, N 29.67.

Preparation of Acyclonucleosides 20 and 21 from 12a and 13a

A solution of 0.2 g (8.6 mmol) of sodium in 50 ml of anhydrous methanol was prepared. To this solution were added 1.73 g (5.38 mmol) of compound **12a** or **13a**. The mixture was stirred at room temperature for 5 hours. The resulting clear solution was neutralized with Amberlite IRN 77. The resin was removed by filtration and washed with hot methanol (3 × 50 ml). The filtrate and washings were combined and evaporated under diminished pressure to provide a colorless solid. This solid was recrystallized from ethanol to furnish deacetylated acyclonucleoside **20** (1.15 g, 70%) or **21** (1.15 g, 70%).

1-[1-(2-Hydroxyethoxy)methyl-1,2,3-triazol-4-ylmethyl]-4-methoxy-1*H*-pyrazolo-[3,4-d]pyrimidine (20). $R_f = 0.36$ ($CHCl_3$/MeOH, 9/1, v/v). mp: 116–117 °C (ethanol). ^{1}H-NMR, Me_2SO-d_6, δ: 3.50 (m, 4H, O-C$\underline{H}_2$-C$\underline{H}_2$-O), 4.12 (s, 3H, OC$\underline{H}_3$), 4.72 (br s, 1H, O$\underline{H}$, D_2O exchangeable), 5.70 (s, 2H, C-C$\underline{H}_2$-N), 5.73 (s, 2H, O-C$\underline{H}_2$-N), 8.23 (s, 1H, aromatic proton of triazole group), 8.28 and 8.66 (2s, 2H, H_3 and H_6). MS, m/z: $[M+H]^+ = 306$. Anal. calcd for $C_{12}H_{15}N_7O_3$ (305.29): C 47.21, H 4.95, N 32.11. Found: C 47.09, H 4.96, N 32.23.

1-[1-(2-Hydroxyethoxy)methyl-1,2,3-triazol-5-ylmethyl]-4-methoxy-1*H*-pyrazolo-[3,4-d]pyrimidine (21). $R_f = 0.45$ ($CHCl_3$/MeOH, 9/1, v/v). mp: 117–118 °C (ethanol). ^{1}H-NMR, Me_2SO-d_6, δ: 3.38 (m, 4H, O-C$\underline{H}_2$-C$\underline{H}_2$-O), 4.11 (s, 3H, OC$\underline{H}_3$), 4.70 (t, 1H, O$\underline{H}$, D_2O exchangeable, J = 5.00 Hz), 5.85 (s, 2H, C-C$\underline{H}_2$-N), 5.87 (s, 2H, O-C$\underline{H}_2$-N), 7.64 (s, 1H, aromatic proton of triazole group), 8.30 and 8.65 (2s, 2H, H_3 and H_6). MS, m/z: $[M+H]^+ = 306$. Anal. calcd for $C_{12}H_{15}N_7O_3$ (305.29): C 47.21, H 4.95, N 32.11. Found: C 47.00, H 4.96, N 32.24.

Preparation of Acyclonucleosides 22 and 23 from 12a and 13a

Compound **12a** or **13a** (0.2 g, 0.62 mmol) was stirred in 2N NaOH (50 ml) at room temperature for 3 hours. The reaction was monitored by tlc and was shown to be complete at this time. After neutralization using a 2N HCl solution and filtration, the solvent was removed *in vacuo*. The residue was coevaporated with benzene (6×4 ml) and the resulting solid was chromatographed on silica gel to give crystalline **22** (0.15 g, 82%) or **23** (0.14 g, 77%).

1-[1-(2-Hydroxyethoxy)methyl-1,2,3-triazol-4-ylmethyl]-4-hydroxy-1*H*-pyrazolo-[3,4-d]pyrimidine (22). Eluent: ($CHCl_3$/MeOH, 98/2, v/v). $R_f = 0.17$ ($CHCl_3$/MeOH, 9/1, v/v). mp: 162–163 °C (ethanol). ^{1}H-NMR, Me_2SO-d_6, δ: 3.48 (m, 4H, O-C$\underline{H}_2$-C$\underline{H}_2$-O), 4.70 (br s, 1H, O$\underline{H}$, D_2O exchangeable), 5.58 (s, 2H, C-C$\underline{H}_2$-N), 5.68 (s, 2H, O-C$\underline{H}_2$-N), 8.08 (s, 1H, aromatic proton of triazole group), 8.12 and 8.23 (2s, 2H, H_3 and H_6), 12.52 (br s, 1H, OH, D_2O exchangeable). MS, m/z: $[M+H]^+ = 292$. Anal. calcd for $C_{11}H_{13}N_7O_3$ (291.26): C 45.36, H 4.50, N 33.66. Found: C 45.10, H 4.61, N 33.76.

1-[1-(2-Hydroxyethoxy)methyl-1,2,3-triazol-5-ylmethyl]-4-hydroxy-1*H*-pyrazolo-[3,4-d]pyrimidine (23). Eluent: ($CHCl_3$/MeOH, 98/2, v/v), $R_f =$ 0.19 ($CHCl_3$/MeOH, 9/1, v/v), mp: 156–157 °C (ethanol). ^{1}H-NMR, Me_2SO-d_6, δ: 3.40 (m, 4H, O-C$\underline{H}_2$-C$\underline{H}_2$-O), 4.70 (br s, 1H, O$\underline{H}$, D_2O exchangeable), 5.70 (s, 2H, C-C$\underline{H}_2$-N), 5.80 (s, 2H, O-C$\underline{H}_2$-N), 7.60 (s, 1H, aromatic proton of triazole group), 8.07 and 8.10 (2s, 2H, H_3 and H_6), 12.52 (br s, 1H, O$\underline{H}$, D_2O exchangeable). MS, m/z: $[M+H]^+ = 292$. Anal. calcd for $C_{11}H_{13}N_7O_3$ (291.26): C 45.36, H 4.50, N 33.66. Found: C 45.05, H 4.63, N 33.79.

Biological Assays

The antiviral experiments using MT-4 cell cultures and HIV(III_B) and HIV-2(ROD) were performed following procedures that have been previously described[19,20].

The anti-tumor experiments were realized in the National Cancer Institute using the procedure published in Seminars in Oncology[21].

The anti-tuberculosis assay is as described previously[22].

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