Synthesis and Antiviral Activity Evaluation of some Novel Acyclic *C*-Nucleosides

Nikolaos Lougiakis,[a] Panagiotis Marakos,[a] Nicole Poul,*[,a] and Jan Balzarini[b]

[a] *Department of Pharmacy, Division of Pharmaceutical Chemistry, University of Athens; Panepistimiopolis Zografou 15771, Athens, Greece: and* [b] *Rega Institute for Medical Research; K.U.Leuven, B-3000 Leuven, Belgium.*

Received November 2, 2007; accepted March 10, 2008; published online March 19, 2008

The preparation of novel 5-amino or 7-hydroxy substituted pyrazolo[4,3-*b*]pyridine and pyrazolo[3,4-*c*]pyridine acyclic *C*-nucleosides is described. Their synthesis was carried out by condensation of suitably substituted lithiated picolines with 2-benzyloxyethoxymethylchloride followed by pyrazole ring annulation. The compounds were evaluated for their antiviral activity against a wide panel of viruses, but were found inactive at subtoxic concentrations.

Key words acyclonucleoside; pyrazolopyridine; antiviral activity

The discovery of 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir, Fig. 1)[1] as the first potent and selective inhibitor of a human herpes virus[2] has stimulated continuing research into the preparation and evaluation of novel acyclic analogues of nucleosides as potential substrates of virus-specific thymidine kinase (TK) and/or inhibition of DNA polymerase after activation by the viral TK and subsequent phosphorylation by cellular enzymes to the 5′-triphosphates.[3,4] Structure–activity relationship studies have been explored extensively and revealed that both the nature of the heterocyclic base and the chemical structure of the aliphatic substituent have a pronounced effect on the antiviral activity of these molecules. Purine nucleosides structurally related to acyclovir that are currently used in the clinic are ganciclovir,[5,6] which is useful against cytomegalovirus (HCMV) infections and the penciclovir prodrug famciclovir,[7] which is marketed for shingles. The antiviral spectrum of these agents is limited to the herpes virus genus. On the other hand, 3-deazaadenosine as well as a number of acyclic and carbocyclic adenosine analogues, such as the *S* enantiomer of 9-(2,3-dihydroxypropyl)-adenine (S-DHPA), aristeromycin, neplanocin A and 3-deazaneplanocin A (Fig. 1) were found to act as reversible inhibitors of *S*-adenosyl-L-homocysteine (SAH) hydrolase.[8] These SAH hydrolase inhibitors exhibit potent antiviral activity against a number of DNA and RNA viruses, which heavily depend on methylation reactions for viral replication. More interestingly, acyclic nucleoside phosphonates have been developed, which retain marked activity against thymidine kinase deficient viral strains and some of them are useful for the treatment of HCMV, retrovirus (HIV) and human hepatitis B virus (HBV) infections.[9]

In the course of our studies, concerning the preparation of a number of *C*-nucleosides structurally related to inosine and adenosine[10,11] and in conjunction with the stimulating results reported for acyclic nucleosides, we were interested in determining whether antiviral activity is retained when the (2-hydroxyethoxy)methyl group of acyclovir is employed in combination with a base structurally related to 3-deazaadenine. Within this context we report here the synthesis and the *in vitro* antiviral evaluation of some new acyclic *C*-nucleoside analogues, bearing the 8-aza-1,9-dideazapurine or the 8-aza-3,9-dideazapurine skeleton.

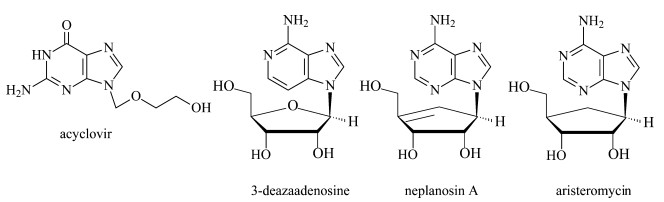
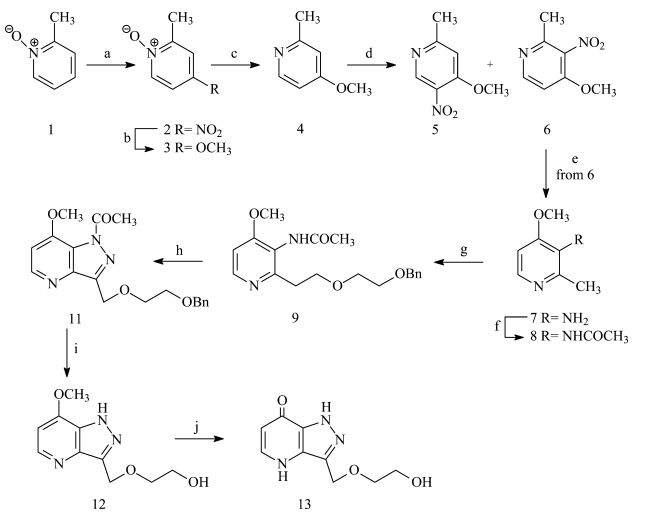


Fig. 1. Structures of Acyclovir and Adenosine Analogues

Results and Discussion

Chemistry For the preparation of the pyrazolopyridinone **13** (Chart 1) we used commercial 2-picoline *N*-oxide (**1**) which was converted to the picoline **4** through known procedures.[12] Subsequent nitration of **4** provided a mixture of the isomeric nitroderivatives **5** and **6** and from this mixture only the desired compound **6** could be isolated in pure form by column chromatography, whereas **5** was contaminated with traces of **6**. The isomer **6** was reduced to the aminoderivative **7**, which has been previously reported as a side-product of the ammonolysis of 3-methoxy-2-acylfuran.[13]



Reagents and conditions: a) H_2SO_4, HNO_3, 160 °C; b) CH_3ONa, CH_3OH, 80 °C; c) Fe, CH_3CO_2H, 130 °C; d) H_2SO_4, HNO_3, 65 °C; e) H_2, Pd–C, EtOH; f) Ac_2O, CH_2Cl_2, rt; g) (1) *n*-BuLi (2.5 eq), THF, −78 °C, (2) 2-benzyloxyethoxymethylchloride, THF; h) AcOK, Ac_2O, isoamyle nitrite, C_6H_6, reflux; i) BCl_3, CH_2Cl_2, −70 °C; j) HCl (36%), EtOH, 80 °C.

Chart 1

* To whom correspondence should be addressed. e-mail: pouli@pharm.uoa.gr

© 2008 Pharmaceutical Society of Japan

Compound **7** was then acetylated to give the acetamide **8**, which was lithiated using *n*-butyllithium in THF solution. The resulting anion was reasonably stable to be trapped by 2-benzyloxyethoxymethylchloride,[14] thus allowing the preparation of the substituted pyridine **9**. From this reaction we have also isolated a significant amount (32%) of a by-product the structure of which was unambiguously assigned as **10** (Fig. 2), on the basis of 1-D and 2-D NMR data. The formation of **10** is due to a competitive anion formation on the acetamide's methyl group.

Compound **9** was then heated at reflux in benzene with isoamyl nitrite, in the presence of acetic anhydride.[15] This furnished the substituted 1-acetylpyrazolo[4,3-*b*]pyridine **11** through a rearrangement of the intermediate *N*-nitroso compound. Both the *N*-acetyl and benzyl groups were easily cleaved upon treatment of **11** with a 1 M solution of BCl_3 in hexane at low temperature. This provided the 7-methoxy-derivative **12** which was then converted to the target pyrazolopyridinone by heating into an ethanolic HCl solution.

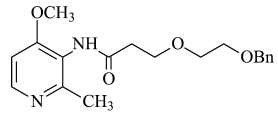
Following an analogous procedure, using the readily prepared acetamide **19** (Chart 2),[16] we prepared the isomeric pyrazolo[3,4-*c*]pyridinone **23** in reasonable overall yield.

In order to prepare the corresponding 5-aminosubstituted derivatives, we used the aminonitropicoline **24**[17] which was first converted to the Boc-protected analogue **25** and then, upon reduction, to the acetamide **27** (Chart 3). The Boc-protecting group is suitable in terms of its stability to anionic reaction conditions. Thus, lithiation of **27** with 3.3 eq of *n*-butyllithium followed by treatment with 2-benzyloxyethoxymethylchloride provided the intermediate pyridine **28**, which was subjected to ring-closure according to the afore-mentioned conditions to result in the pyrazolopyridine **29**. The Boc-group was then easily cleaved by the use of trifluoracetic acid and the resulting pyrazolopyridine **30** was converted to the target compound **31** upon treatment with boron trichloride in dichloromethane solution.

The application of this strategy using the isomeric picoline **33** (Chart 4) which was prepared from the previously reported amino-derivative **32**,[18] resulted in six steps in the 3-substituted 5-aminopyrazolopyridine **39**.

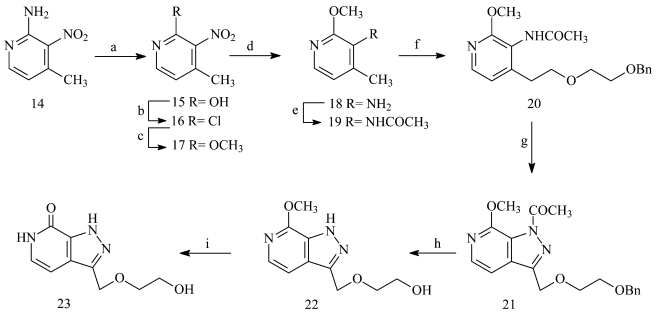
Antiviral and Cytostatic Evaluations The cytotoxicity and antiviral activity of compounds against the replication of Herpes simplex virus-1 (KOS), Herpes simplex virus-2 (G), Vaccinia virus, Vesicular stomatitis virus and Herpes simplex virus-1 ACVr in HEL cell cultures, against the replication of Vesicular stomatitis virus, Coxsackie virus B4, Respiratory syncytial virus in HeLa cell cultures, against the replication of Para-influenza-3 virus, Reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus in Vero cell cultures, against the replication of Feline Corona virus and Human Corona (SARS) virus in feline kidney Crandel cell cultures and against HIV-1- and HIV-2-induced cytopathicity in CEM cell cultures were evaluated. The compounds were antivirally inactive at subtoxic concentrations.

The inhibitory effects of the compounds on the proliferation of murine leukemia cells (L1210) and human T-lympho-



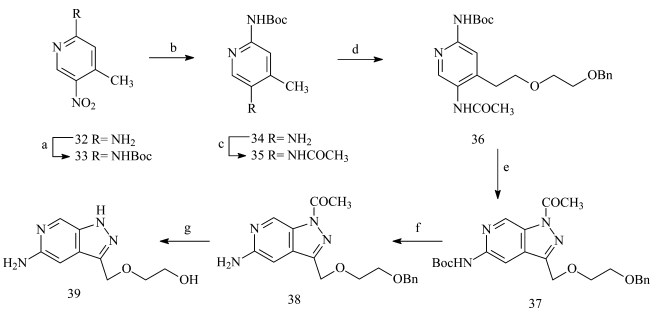
10

Fig. 2. Structure of the By-product **10**



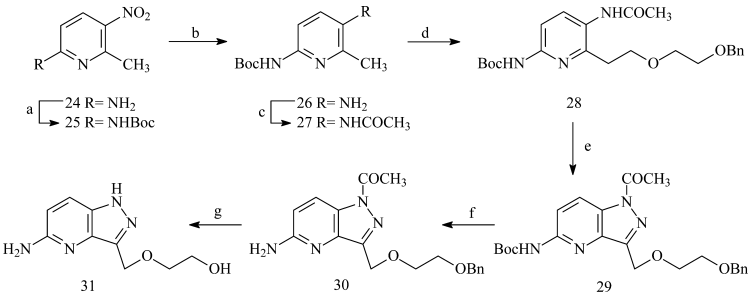
Reagents and conditions: a) $NaNO_2$, H_2SO_4, H_2O; b) PCl_5, $POCl_3$, 130 °C; c) CH_3ONa, CH_3OH, 80 °C; d) H_2, Pd–C, EtOH; e) Ac_2O, CH_2Cl_2, rt; f) (1) *n*-BuLi (2.5 eq), THF, −78 °C, (2) 2-benzyloxyethoxymethylchloride, THF; g) AcOK, Ac_2O, isoamyle nitrite, C_6H_6, reflux; h) BCl_3, CH_2Cl_2, −70 °C; i) HCl/CH_3OH, rt.

Chart 2



Reagents and conditions: a) (1) NaH, THF, 0 °C, (2) Boc_2O, THF; b) H_2, Pd–C, EtOH; c) Ac_2O, CH_2Cl_2, r.t.; d) (1) *n*-BuLi (3.3 eq), THF, −78 °C, (2) 2-benzyloxyethoxymethylchloride, THF; e) AcOK, Ac_2O, isoamyle nitrite, benzene, reflux; f) CF_3CO_2H, CH_2Cl_2, rt; g) BCl_3, CH_2Cl_2, −70 °C.

Chart 4



Reagents and conditions: a) (1) NaH, THF, 0 °C, (2) Boc_2O, THF; b) H_2, Pd–C, EtOH; c) Ac_2O, CH_2Cl_2, rt; d) (1) *n*-BuLi (3.3 eq), THF, −78 °C, (2) 2-benzyloxyethoxymethylchloride, THF; e) AcOK, Ac_2O, isoamyle nitrite, benzene, reflux; f) CF_3CO_2H, CH_2Cl_2, rt; g) BCl_3, CH_2Cl_2, −70 °C.

Chart 3

cyte cells (Molt4/C8, CEM) were also determined. Only compound **13** exhibited a slight antiproliferative activity with IC_{50} values within the range of 126—220 μM against the tested cell lines.

The inactivity of the new compounds is likely due to their inability to be phosphorylated to a potentially active metabolite and to the lack of being efficient substrates for viral enzymes. It seems that a closer similarity of the purine-like nucleus to guanine should be taken under consideration, since the replacement of the imidazole ring of the purine skeleton by the isosteric pyrazole nucleus and the lack of the suitable second substituent on the pyridine ring, are not in favour of the antiviral activity of this class of compounds.

Experimental

All chemicals were purchased from Aldrich Chemical Co. Melting points were determined on a Büchi apparatus and are uncorrected. Flash chromatography was performed on Merck silica gel 60 (0.040—0.063 mm). Analytical thin layer chromatography (TLC) was carried out on precoated (0.25 mm) Merck silica gel F-254 plates. ^{1}H-NMR spectra and 2D spectra were recorded on a Bruker Avanche 400 instrument, whereas ^{13}C-NMR spectra were recorded on a Bruker AC 200 spectrometer in deuterated solvents and were referenced to TMS (δ scale). The signals of ^{1}H and ^{13}C spectra were unambiguously assigned by using 2D NMR techniques: ^{1}H–^{1}H COSY, NOESY HMQC and HMBC. Elemental analyses were performed on a Perkin-Elmer PE 240C Elemental Analyzer (Norwalk, CT, U.S.A.) and were within ±0.4% of the theoretical values. The oily analytical samples, upon the appropriate chromatographic purification were dried *in vacuo* (vacuum pump) at 90 °C in the presence of phosphorous pentoxide for 12 h.

4-Methoxy-2-methyl-3-nitropyridine (6) A mixture of sulfuric acid (98%, 1.6 ml) and nitric acid (65%, 1.6 ml) was added at 0 °C to a solution of **4**[12] (2.4 g, 19.51 mmol) in sulfuric acid (98%, 11.1 ml) and the resulting solution was heated at 65 °C for 12 h. The mixture was then poured into ice-water, neutralized with a 40% NaOH solution and the precipitate was filtered and air-dried. Flash chromatography of the residue, on silica gel, using a mixture of CH_2Cl_2/EtOAc (85/15, v/v) as the eluent, provided pure only the title nitrocompound **6** in 48% yield, whereas the isomeric nitroderivative **5** was eluted in mixture with **6**. mp: 55—56°C (EtOAc–*n*-hexane). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.52 (3H, s, CH_3), 3.95 (3H, s, OCH_3), 6.85 (1H, d, J=5.8 Hz, H-5), 8.45 (1H, d, J=5.8 Hz, H-6). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 20.37 (CH_3), 56.64 (CH_3O), 105.93 (C-5), 138.80 (C-3), 151.42 (C-2), 151.70 (C-6), 157.30 (C-4).

***N*-(4-Methoxy-2-methylpyridin-3-yl)acetamide (8)** To a solution of the amine **7** (1.1 g, 7.97 mmol) in dry CH_2Cl_2 (25 ml) was added acetic anhydride (0.95 ml, 10 mmol) and the mixture was stirred at room temperature for 10 h. The solvent was then vacuum-evaporated and the residue was purified by column chromatography (silica gel) using a mixture of CH_2Cl_2/CH_3OH (98/2, v/v) as the eluent, to give pure **8** (1.32 g, 92%). mp: 136 °C (EtOH). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.15 (3H, s, $COCH_3$), 2.37 (3H, s, CH_3), 3.79 (3H, s, OCH_3), 6.65 (1H, d, J=5.7 Hz, H-5), 7.47 (1H, br s, D_2O exchang., NH), 8.23 (1H, d, J=5.7 Hz, H-6). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 21.10 (CH_3), 23.34 ($\underline{C}H_3CO$), 55.78 (CH_3O), 104.67 (C-5), 120.83 (C-3), 148.52 (C-6), 156.88 (C-2), 160.54 (C-4), 169.12 (CH_3CO). *Anal.* Calcd for $C_9H_{12}N_2O_2$: C, 59.99; H, 6.71; N, 15.55. Found: C, 60.12; H, 6.55; N, 15.43.

***N*-[2-(2-Benzyloxyethoxy)ethyl-4-methoxypyridin-3-yl]acetamide (9)** To a solution of the picoline **8** (0.6 g, 3.33 mmol) in dry THF (30 ml) at −78 °C was added under argon *n*-BuLi (5.2 ml, 8.32 mmol, 1.6 M solution in hexanes). The resulting light yellow solution was stirred at −78 °C for 15 min and the temperature then raised to −50 °C for 50 min. The orange-colored solution was cooled to −78 °C and a solution of 2-benzyloxyethoxymethylchloride[14] (0.86 g, 4.29 mmol) in dry THF (5 ml) was added dropwise. The resulting mixture was stirred at −78 °C for 15 min and the temperature was then allowed to rise to the ambient one. A saturated ammonium chloride solution was then added to the reaction mixture to quench the excess *n*-BuLi. The solvent was vacuum-evaporated, water was added to the residue and this was extracted with CH_2Cl_2. The organic extracts were dried (Na_2SO_4) and concentrated to dryness to give an oil which was purified by flash chromatography (silica gel) using EtOAc as the eluent, to give compounds **9** and **10**.

Data for **9**: Yield: 40%. Oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.01 (3H, s, $COCH_3$), 3.01 (2H, t, J=5.5 Hz, Pyr$\underline{CH}_2CH_2O$), 3.58—3.60 (4H, m, OCH_2CH_2O), 3.81 (2H, t, J=5.5 Hz, Pyr$CH_2\underline{CH}_2O$), 3.84 (3H, s, OCH_3), 4.51 (2H, s, CH_2Ph), 6.74 (1H, d, J=5.7 Hz, H-5), 7.27—7.33 (5H, m, Ph-H), 8.16 (1H, br s, D_2O exchang., NH), 8.31 (1H, d, J=5.7 Hz, H-6). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 23.10 ($\underline{C}H_3CO$), 35.06 (Pyr$\underline{C}H_2CH_2O$), 55.86 (CH_3O), 69.48 ($O\underline{C}H_2CH_2O$), 69.94 ($OCH_2\underline{C}H_2O$), 71.59 (Pyr$CH_2\underline{C}H_2O$), 73.41 (CH_2Ph), 105.72 (C-5), 122.23 (C-3), 127.62 (C-4′), 127.97 (2× Phenyl C), 128.52 (2×Phenyl C), 137.69 (C-1′), 149.05 (C-6), 157.34 (C-2), 161.41 (C-4), 169.18 ($CH_3\underline{C}O$). *Anal.* Calcd for $C_{19}H_{24}N_2O_4$: C, 66.22; H, 7.02; N, 8.13. Found: C, 66.03; H, 6.91; N, 8.07.

Data for 3-[3-(2-Benzyloxyethoxy)-1-oxopropanamine]-4-methoxy-2-methylpyridine (**10**): Yield: 32%. Oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.38 (3H, s, CH_3), 2.67 (2H, t, J=5.7 Hz, NH(O=)C$\underline{C}H_2CH_2O$), 3.64—3.66 (2H, m, O$\underline{C}H_2CH_2O$), 3.71—3.73 (2H, m, $OCH_2\underline{C}H_2O$), 3.76 (3H, s, OCH_3), 3.85 (2H, t, J=5.7 Hz, NH(O=)CC$H_2\underline{C}H_2O$), 4.47 (2H, s, CH_2Ph), 6.63 (1H, d, J=5.7 Hz, H-5), 7.17—7.24 (5H, m, Ph-H), 8.05 (1H, br s, D_2O exchang., NH), 8.26 (1H, d, J=5.7 Hz, H-6). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 21.19 (NHCO$\underline{C}H_2$), 37.13 (PyrCH_3), 55.72 (CH_3O), 67.31 ($COCH_2\underline{C}H_2O$), 69.15 ($O\underline{C}H_2CH_2O$), 70.40 ($OCH_2\underline{C}H_2O$), 73.35 (CH_2Ph), 104.69 (C-5), 120.79 (C-3), 127.77 (C-4′), 127.93 (2×Phenyl C), 128.41 (2×Phenyl C), 137.76 (C-1′), 148.57 (C-6), 156.78 (C-2), 160.54 (C-4), 170.59 ($CH_3\underline{C}O$). *Anal.* Calcd for $C_{19}H_{24}N_2O_4$: C, 66.22; H, 7.02; N, 8.13. Found: C, 65.94; H, 7.17; N, 8.32.

1-Acetyl-3-(2-benzyloxyethoxy)methyl-7-methoxy-1*H*-pyrazolo[4,3-*b*]pyridine (11) Potassium acetate (23 mg, 0.233 mmol) and acetic anhydride (0.07 ml, 0.74 mmol) were added to a solution of **9** (70 mg, 0.20 mmol) in dry benzene (20 ml) under Ar. The reaction mixture was heated at reflux, isoamyl nitrite (0.06 ml, 0.44 mmol) was added and the resulting mixture was refluxed for 7 h. The insoluble material was then filtered off, the solvent was vacuum-evaporated and the residue was purified by column chromatography (silica gel) using a mixture of cyclohexane/EtOAc (7/3, v/v) as the eluent, to give **11** (65 mg, 87%) as an oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.81 (3H, s, $COCH_3$), 3.70 (2H, t, J=4.7 Hz, $OCH_2\underline{CH}_2OBn$), 3.87 (2H, t, J=4.7 Hz, $O\underline{CH}_2CH_2OBn$), 4.05 (3H, s, OCH_3), 4.57 (2H, s, CH_2Ph), 5.02 (2H, s, pyrazol-CH_2O), 6.88 (1H, d, J=5.3 Hz, H-6), 7.29—7.35 (5H, m, Ph-H), 8.56 (1H, d, J=5.3 Hz, H-5). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 23.72 ($CO\underline{C}H_3$), 56.36 (OCH_3), 64.33 (pyrazol-CH_2O), 69.41 ($OCH_2\underline{C}H_2OBn$), 70.73 ($O\underline{C}H_2CH_2OBn$), 73.34 (CH_2Ph), 104.98 (C-6), 124.83 (C-7α), 127.66 (C-4′), 127.84 (2×Phenyl C), 128.43 (2×Phenyl C), 138.28 (C-1′), 145.26 (C-3α), 148.06 (C-3), 150.04 (C-5), 154.31 (C-7), 169.23 ($\underline{C}OCH_3$). *Anal.* Calcd for $C_{19}H_{21}N_3O_4$: C, 64.21; H, 5.96; N, 11.82. Found: C, 63.89; H, 5.90; N, 11.73.

3-(2-Hydroxyethoxy)methyl-7-methoxy-1*H*-pyrazolo[4,3-*b*]pyridine (12) A solution of BCl_3 (1 M in hexane, 0.84 ml, 0.84 mmol) was added dropwise under argon to a solution of **11** (150 mg, 0.42 mmol) in dry CH_2Cl_2 (25 ml) at −70 °C. The reaction mixture was stirred at −70 °C for 1 h, the temperature was then raised to −25 °C and a cold solution of CH_2Cl_2/MeOH (4 ml, 1/1 v/v) was added. The solvents were evaporated and the residue was purified by flash chromatography (silica gel) using a mixture of CH_2Cl_2/MeOH (95/5, v/v) as the eluent to give pure **12** (79.6 mg, 85%) as a white solid. mp: 139—140 °C (EtOH). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.80—3.85 (4H, m, OCH_2CH_2O), 4.00 (3H, s, OCH_3), 5.09 (2H, s, pyrazol-CH_2O), 6.66 (1H, d, J=5.4 Hz, H-6), 8.42 (1H, d, J=5.4 Hz, H-5), 11.60 (1H, br s, D_2O exchang., NH). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 56.03 (OCH_3), 61.54 ($OCH_2\underline{C}H_2OH$), 65.10 (pyrazol-CH_2O), 72.82 ($O\underline{C}H_2CH_2OH$), 101.31 (C-6), 127.18 (C-7α), 140.30 (C-3α), 143.01 (C-3), 147.18 (C-5), 152.80 (C-7). *Anal.* Calcd for $C_{10}H_{13}N_3O_3$: C, 53.81; H, 5.87; N, 18.82. Found: C, 53.57; H, 5.93; N, 18.61.

1,4-Dihydro-3-(2-hydroxyethoxy)methyl-7*H*-pyrazolo[4,3-*b*]pyridin-7-one (13) A mixture of HCl (36%, 1 ml) in ethanol (2 ml) was added to a solution of **12** (50 mg, 0.22 mmol) in ethanol (15 ml) and the resulting solution was heated at reflux for 36 h. The mixture was then neutralized with a saturated $NaHCO_3$ solution, the inorganic material was filtered off, the solvents were evaporated and the residue was purified by column chromatography (silica gel) using a mixture of CH_2Cl_2/CH_3OH (9/1, v/v) as the eluent, to give **13** (25 mg, 54%) as a white solid. mp: 200—201 °C (EtOH). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.45—3.55 (4H, m, OCH_2CH_2O), 4.69 (2H, s, pyrazol-CH_2O), 5.94 (1H, d, J=7.43 Hz, H-6), 7.69 (1H, m, H-5), 11.82 (1H, br s, D_2O exchang., NH-4), 13.86 (1H, br s, D_2O exchang., NH-1). ^{13}C-NMR (50 MHz, DMSO-d_6) δ: 60.14 ($OCH_2\underline{C}H_2OH$), 64.21 (pyrazol-CH_2O), 71.62 ($O\underline{C}H_2CH_2OH$), 109.12 (C-6), 127.54 (C-3α), 133.45 (C-7α), 136.09 (C-3), 137.23 (C-5), 168.55 (C-7). *Anal.* Calcd for $C_9H_{11}N_3O_3$: C, 51.67; H, 5.30; N, 20.09. Found: C, 51.36; H, 5.12; N, 19.84.

***N*-[4-(2-Benzyloxyethoxy)ethyl-2-methoxypyridin-3-yl]acetamide (20)** This compound was prepared by a procedure analogous to that of **9**, starting

from **19**[16] (700 mg, 3.89 mmol), using 2.5 eq of *n*-BuLi. The product was purified by column chromatography (silica gel) using a mixture of cyclohexane/EtOAc (6/4, v/v) as the eluent to provide **20** (575 mg, 43%) as an oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.04 (3H, s, $COCH_3$), 2.82 (2H, t, J=5.9 Hz, Pyr$\underline{CH_2}CH_2O$), 3.57—3.60 (4H, m, OCH_2CH_2O), 3.71 (2H, t, J=5.9 Hz, Pyr$CH_2\underline{CH_2}O$), 3.96 (3H, s, OCH_3), 4.53 (2H, s, CH_2Ph), 6.78 (1H, d, J=5.1 Hz, H-5), 7.28—7.35 (5H, m, Ph-H), 7.78 (1H, br s, D_2O exchang., NH), 7.97 (1H, d, J=5.1 Hz, H-6). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 23.24 ($\underline{C}H_3CO$), 32.05 (Pyr$\underline{C}H_2CH_2O$), 54.05 (CH_3O), 69.47 ($O\underline{C}H_2CH_2O$), 70.09 ($OCH_2\underline{C}H_2O$), 71.41 (Pyr$CH_2\underline{C}H_2O$), 73.44 (CH_2Ph), 118.15 (C-5), 120.36 (C-3), 127.96 (3×Phenyl C), 128.54 (2×Phenyl C), 137.80 (C-1′), 144.33 (C-6), 147.34 (C-4), 159.67 (C-2), 169.20 (CH_3CO). *Anal.* Calcd for $C_{19}H_{24}N_2O_4$: C, 66.22; H, 7.02; N, 8.13. Found: C, 65.97; H, 7.19; N, 8.28.

1-Acetyl-3-(2-benzyloxyethoxy)methyl-7-methoxy-1*H*-pyrazolo[3,4-*c*]pyridine (21) This compound was prepared by a procedure analogous to that of **11**, starting from **20** (500 mg, 1.45 mmol). The product was purified by column chromatography (silica gel) using a mixture of cyclohexane/EtOAc (8/2, v/v) as the eluent to provide **21** (475 mg, 92%) as an oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.78 (3H, s, $COCH_3$), 3.65—3.69 (2H, m, $OCH_2\underline{CH_2}O$), 3.71—3.75 (2H, m, $O\underline{CH_2}CH_2O$), 4.13 (3H, s, OCH_3), 4.56 (2H, s, CH_2Ph), 4.89 (2H, s, pyrazol-CH_2O), 7.30—7.34 (5H, m, Ph-H), 7.36 (1H, d, J=5.5 Hz, H-4), 7.99 (1H, d, J=5.5 Hz, H-5). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 24.12 ($CO\underline{C}H_3$), 54.34 ($O\underline{C}H_3$), 66.32 (pyrazol-CH_2O), 69.48 ($OCH_2\underline{C}H_2O$), 70.32 ($O\underline{C}H_2CH_2O$), 73.45 (CH_2Ph), 108.51 (C-4), 127.84 (2×Phenyl C), 128.50 (2×Phenyl C), 129.79 (C-4′), 133.28 (C-3α), 136.22 (C-7α), 138.17 (C-1′), 140.45 (C-5), 147.58 (C-3), 151.77 (C-7), 168.75 ($\underline{C}OCH_3$). *Anal.* Calcd for $C_{19}H_{21}N_3O_4$: C, 64.21; H, 5.96; N, 11.82. Found: C, 64.33; H, 5.86; N, 11.67.

3-(2-Hydroxyethoxy)methyl-7-methoxy-1*H*-pyrazolo[3,4-*c*]pyridine (22) This compound was prepared by a procedure analogous to that of **12**, starting from **21** (420 mg, 1.18 mmol). The product was purified by column chromatography (silica gel) using a mixture of EtOAc/CH_3OH (95/5, v/v) as the eluent to provide **22** (220 mg, 85%) as an amorphous solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.75 (1H, br s, D_2O exchang., OH), 3.69 (2H, t, J=4.8 Hz, $OCH_2\underline{CH_2}OH$), 3.78 (2H, t, J=4.8 Hz, $O\underline{CH_2}CH_2OH$), 4.15 (3H, s, OCH_3), 4.94 (2H, s, pyrazol-CH_2O), 7.27 (1H, d, J=5.9 Hz, H-4), 7.81 (1H, d, J=5.9 Hz, H-5), 10.85 (1H, br s, D_2O exchang., NH). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 53.68 (OCH_3), 61.95 ($OCH_2\underline{C}H_2OH$), 66.01 (pyrazol-CH_2O), 72.12 ($O\underline{C}H_2CH_2OH$), 108.38 (C-4), 127.35 (C-3α), 128.27 (C-7α), 136.54 (C-5), 143.84 (C-3), 150.76 (C-7). *Anal.* Calcd for $C_{10}H_{13}N_3O_3$: C, 53.81; H, 5.87; N, 18.82. Found: C, 53.62; H, 5.79; N, 18.97.

1,6-Dihydro-3-(2-hydroxyethoxy)methyl-7*H*-pyrazolo[3,4-*c*]pyridin-7-one (23) Compound **22** (50 mg, 0.22 mmol) was added at 0 °C to a saturated solution of HCl in dry methanol (10 ml) and the resulting solution was stirred at room temperature for 18 h. The solvent was vacuum-evaporated and the residue was purified by column chromatography (silica gel) using a mixture of CH_2Cl_2/CH_3OH (95/5, v/v) as the eluent to give **23** (40 mg, 85%). mp: 235 °C (EtOH). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.45 (2H, t, J=4.9 Hz, $OCH_2\underline{CH_2}OH$), 3.50 (2H, t, J=4.9 Hz, $O\underline{CH_2}CH_2OH$), 4.68 (2H, s, pyrazol-CH_2O), 6.58 (1H, d, J=6.6 Hz, H-4), 6.92 (1H, m, H-5), 11.25 (1H, br s, D_2O exchang., NH-6), 13.88 (1H, br s, D_2O exchang., NH-1). ^{13}C-NMR (50 MHz, DMSO-d_6) δ: 60.20 ($OCH_2\underline{C}H_2OH$), 64.99 (pyrazol-CH_2O), 71.62 ($O\underline{C}H_2CH_2OH$), 98.44 (C-4), 124.03 (C-3α), 125.59 (C-5), 132.79 (C-7α), 143.32 (C-3), 154.18 (C-7). *Anal.* Calcd for $C_9H_{11}N_3O_3$: C, 51.67; H, 5.30; N, 20.09. Found: C, 51.41; H, 5.24; N, 19.94.

***tert*-Butyl-*N*-(6-methyl-5-nitropyridin-2-yl) Carbamate (25)** To a solution of the aminopicoline **24**[17] (2 g, 13.07 mmol) in dry THF (40 ml) sodium hydride (0.78 g, 19.4 mmol, 60% suspension in paraffin oil) was added under argon at 0 °C and the resulting mixture was stirred at rt for 2 h. It was then cooled at 0 °C, a solution of di-*tert*-butyl dicarbonate (3.3 ml, 14.38 mmol) in dry THF (10 ml) was added dropwise and the mixture was stirred at rt for an additional 4 h. The solvent was vacuum-evaporated, a solution of HCl (0.5 N, 20 ml) was added to the residue and the product was extracted with ethyl acetate. The organic extracts were dried (Na_2SO_4) and concentrated to dryness and the residue was purified by column chromatography (silica gel) using a a mixture of cyclohexane/EtOAc (98/2, v/v) as the eluent, to give **25** (3.15 g, 95%). mp: 127—128 °C (EtOAc). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.47 [9H, s, $(CH_3)_3$], 2.73 (3H, s, CH_3), 7.93 (1H, d, J=9.12 Hz, H-3), 8.32 (1H, d, J=9.12 Hz, H-4), 8.37 (1H, br s, D_2O exchang., NH). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 24.29 (CH_3), 28.11 [$(CH_3)_3$], 82.27 [$(CH_3)_3\underline{C}$], 109.63 (C-3), 135.85 (C-4), 140.43 (C-5), 151.76 (OCONH), 154.28 (C-6), 154.34 (C-2). *Anal.* Calcd for $C_{11}H_{15}N_3O_4$: C, 52.17; H, 5.97; N, 16.59. Found: C, 52.34; H, 5.81; N, 16.38.

***tert*-Butyl-*N*-(5-amino-6-methylpyridin-2-yl) Carbamate (26)** A solution of **25** (2.5 g, 9.88 mmol) in dry ethanol (40 ml) was hydrogenated in the presence of 10% Pd/C (130 mg) under a pressure of 50 psi at rt for 5 h. The solution was filtered through a celite pad to remove the catalyst and the filtrate was evaporated to dryness to give pure **9** (2.18 g, 99%). mp: 122—123 °C (Et_2O) ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.47 [9H, s, $(CH_3)_3$], 2.29 (3H, s, CH_3), 3.40 (2H, br s, D_2O exchang., NH_2), 6.95 (1H, d, J=8.61 Hz, H-4), 7.30 (1H, br s, D_2O exchang., NH), 7.54 (1H, d, J=8.61 Hz, H-3). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 19.94 (CH_3), 28.42 [$(CH_3)_3$], 80.30 [$(CH_3)_3\underline{C}$], 111.07 (C-3), 124.74 (C-4), 136.18 (C-5), 141.57 (C-6), 143.45 (C-2), 152.88 (OCONH). *Anal.* Calcd for $C_{11}H_{17}N_3O_2$: C, 59.17; H, 7.67; N, 18.82. Found: C, 58.84; H, 7.79; N, 18.97.

***tert*-Butyl-*N*-(5-acetamido-6-methylpyridin-2-yl) Carbamate (27)** To a solution of **26** (2 g, 8.97 mmol) in dry dichloromethane (20 ml) acetic anhydride (0.95 ml, 10 mmol) was added and the resulting solution was stirred at rt for 10 h. The solvent was vacuum-evaporated and the residue was purified by column chromatography (silica gel) using a a mixture of cyclohexane/EtOAc (6/4, v/v) as the eluent, to give **27** (2.15 g, 91%). mp: 131 °C (Et_2O). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.50 [9H, s, $(CH_3)_3$], 2.18 (3H, s, $COCH_3$), 2.35 (3H, s, CH_3), 7.00 (1H, br s, D_2O exchang., NHAc), 7.42 (1H, br s, D_2O exchang., NHBoc), 7.73 (1H, d, J=8.97 Hz, H-3), 7.88 (1H, d, J=8.97 Hz, H-4). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 20.25 (CH_3), 23.56 ($\underline{C}H_3CO$), 28.25 [$(CH_3)_3$], 80.90 [$(CH_3)_3\underline{C}$], 109.97 (C-3), 126.93 (C-5), 135.06 (C-4), 148.68 (C-2), 150.51 (C-6), 152.54 (OCONH), 169.40 ($\underline{C}OCH_3$). *Anal.* Calcd for $C_{13}H_{19}N_3O_3$: C, 58.85; H, 7.22; N, 15.84. Found: C, 58.61; H, 7.09; N, 15.68.

***tert*-Butyl-*N*-[5-acetamido-6-(2-benzyloxyethoxy)ethylpyridin-2-yl] Carbamate (28)** This compound was prepared by a procedure analogous to that of **9**, starting from **27** (650 mg, 2.45 mmol), using 3.3 eq of *n*-BuLi. The anion formation was complete at −20 °C. The product was purified by column chromatography (silica gel) using a mixture of cyclohexane/EtOAc (7/3, v/v) as the eluent to provide **28** (420 mg, 40%) as an oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.51 [9H, s, $(CH_3)_3$], 2.01 (3H, s, $COCH_3$), 2.93 (2H, t, J=5.3 Hz, Pyr$\underline{CH_2}CH_2O$), 3.60—3.63 (4H, m, OCH_2CH_2O), 3.80 (2H, t, J=5.3 Hz, Pyr$CH_2\underline{CH_2}O$), 4.50 (2H, s, CH_2Ph), 7.09 (1H, br s, D_2O exchang., NHBoc), 7.27—7.36 (5H, m, Ph-H), 7.77 (1H, d, J=8.6 Hz, H-3), 8.03 (1H, d, J=8.6 Hz, H-4), 8.86 (1H, br s, D_2O exchang., NHAc). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 23.94 ($\underline{C}H_3CO$), 28.42 [$(CH_3)_3$], 35.76 (Pyr$\underline{C}H_2CH_2O$), 69.29 ($O\underline{C}H_2CH_2O$), 70.40 ($OCH_2\underline{C}H_2O$), 72.09 (Pyr$CH_2\underline{C}H_2O$), 73.30 (CH_2Ph), 80.98 [$(CH_3)_3\underline{C}$], 110.72 (C-3), 127.84 (2×Phenyl C), 128.06 (C-4′), 128.62 (2×Phenyl C), 128.88 (C-5), 134.61 (C-4), 137.73 (C-1′), 147.95 (C-2), 150.37 (C-6), 152.36 (OCONH), 169.04 ($CH_3\underline{C}O$). *Anal.* Calcd for $C_{23}H_{31}N_3O_5$: C, 64.32; H, 7.27; N, 9.78. Found: C, 64.21; H, 7.23; N, 9.60.

***tert*-Butyl-*N*-[1-acetyl-3-(2-benzyloxyethoxy)methyl-1*H*-pyrazolo[4,3-*b*]pyridin-5-yl] Carbamate (29)** This compound was prepared by a procedure analogous to that of **11**, starting from **28** (400 mg, 0.93 mmol). The product was purified by column chromatography (silica gel) using a mixture of cyclohexane/EtOAc (7/3, v/v) as the eluent to provide **29** (370 mg, 90%) as an oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.53 [9H, s, $(CH_3)_3$], 2.76 (3H, s, $COCH_3$), 3.68 (2H, t, J=4.7 Hz, $OCH_2\underline{CH_2}O$), 3.83 (2H, t, J=4.7 Hz, $O\underline{CH_2}CH_2O$), 4.56 (2H, s, CH_2Ph), 4.93 (2H, s, pyrazol-CH_2O), 7.27—7.34 (5H, m, Ph-H), 7.49 (1H, br s, D_2O exchang., NH), 8.23 (1H, d, J=9.2 Hz, H-6), 8.61 (1H, d, J=9.2 Hz, H-7). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 22.43 ($CO\underline{C}H_3$), 28.35 [($\underline{C}H_3)_3C$], 64.30 (pyrazol-CH_2O), 69.48 ($OCH_2\underline{C}H_2O$), 70.62 ($O\underline{C}H_2CH_2O$), 73.37 (CH_2Ph), 81.50 [$(CH_3)_3\underline{C}$], 114.02 (C-6), 125.60 (C-7), 127.81 (3×Phenyl C), 128.47 (2×Phenyl C), 130.82 (C-7α), 138.28 (C-1′), 140.67 (C-3α), 147.69 (C-3), 150.74 (C-5), 152.43 (OCONH), 170.95 ($\underline{C}OCH_3$). *Anal.* Calcd for $C_{23}H_{28}N_4O_5$: C, 62.71; H, 6.41; N, 12.72. Found: C, 62.58; H, 6.36; N, 12.57.

1-Acetyl-3-(2-benzyloxyethoxy)methyl-1*H*-pyrazolo[4,3-*b*]pyridin-5-yl-amine (30) Trifluoroacetic acid (0.1 ml, 1.28 mmol) was added at 0 °C to a solution of **29** (60 mg, 0.14 mmol) in dry dichloromethane (10 ml) and the mixture was stirred at rt for 10 h. The solvent was vacuum-evaporated and the residue was treated with a saturated $NaHCO_3$ solution and dichloromethane. The organic layer was dried (Na_2SO_4) and concentrated to dryness. The residue was purified by flash chromatography (silica gel) using a mixture of cyclohexane/EtOAc (1/1, v/v) as the eluent to give **30** (45 mg, 97%). mp: 103 °C (EtOAc). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.72 (3H, s, $COCH_3$), 3.71 (2H, t, J=4.7 Hz, $OCH_2\underline{CH_2}O$), 3.83 (2H, t, J=4.7 Hz, $O\underline{CH_2}CH_2O$), 4.57 (2H, s, CH_2Ph), 4.87 (2H, s, pyrazol-CH_2O), 5.55 (2H, br s, D_2O exchang., NH_2), 6.45 (1H, d, J=9 Hz, H-6), 7.28—7.36 (5H, m, Ph-H), 8.28 (1H, d, J=9 Hz, H-7). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 22.36 ($CO\underline{C}H_3$), 64.41 (pyrazol-CH_2O), 69.63 ($OCH_2\underline{C}H_2O$), 70.51 ($O\underline{C}H_2CH_2O$), 73.48 (CH_2Ph), 112.11 (C-6), 125.78 (C-7), 127.81 (C-4′), 127.99 (2×

Phenyl C), 128.54 (2×Phenyl C), 128.91 (C-7α), 138.17 (C-1′), 141.04 (C-3α), 147.03 (C-3), 157.80 (C-5), 170.92 (COCH$_3$). *Anal.* Calcd for $C_{18}H_{20}N_4O_3$: C, 63.52; H, 5.92; N, 16.46. Found: C, 63.34; H, 6.15; N, 16.63.

3-(2-Hydroxyethoxy)methyl-1*H*-pyrazolo[4,3-*b*]pyridin-5-yl-amine (31) This compound was prepared by a procedure analogous to that of **12**, starting from **30** (40 mg, 0.12 mmol) using 5 eq of BCl_3. The product was purified by column chromatography (silica gel) using a mixture of CH_2Cl_2/CH_3OH (95/5, v/v) as the eluent to provide **31** (20 mg, 82%). mp: 146—148 °C (EtOH). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.48 (4H, m, OCH_2CH_2O), 4.63 (2H, s, pyrazol-CH_2O), 4.72 (1H, br s, D_2O exchang., OH), 5.95 (2H, br s, D_2O exchang., NH_2), 6.60 (1H, d, J=8.9 Hz, H-6), 7.62 (1H, d, J=8.9 Hz, H-7), 12.78 (1H, br s, D_2O exchang., NH-1). ^{13}C-NMR (DMSO-d_6) δ: 60.09 (OCH_2CH_2-OH), 63.24 (pyrazol-CH_2O), 71.40 (OCH_2CH_2OH), 110.99 (C-6), 120.77 (C-7), 129.07 (C-7α), 136.91 (C-3α), 138.71 (C-3), 156.31 (C-5). *Anal.* Calcd for $C_9H_{12}N_4O_2$: C, 51.92; H, 5.81; N, 26.91. Found: C, 52.17; H, 5.76; N, 26.82.

***tert*-Butyl-*N*-(4-methyl-5-nitropyridin-2-yl) Carbamate (33)** This compound was prepared by a procedure analogous to that of **25**, starting from **32**[18] (3 g, 19.6 mmol). The product was purified by column chromatography (silica gel) using a mixture of cyclohexane/EtOAc (4 : 6, v/v) as the eluent, to give pure **33** (4.71 g, 95%). mp: 186 °C (Et_2O/*n*-pentane); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.59 [9H, s, $(CH_3)_3$], 2.69 (3H, s, CH_3), 8.10 (1H, s, H-3), 9.12 (1H, s, H-6), 10.43 (1H, br s, D_2O exchang., NH). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 21.59 (CH_3), 28.42 [(CH_3)$_3$CO], 82.74 [(CH_3)$_3$C], 114.39 (C-3), 140.71 (C-5), 146.00 (C-6), 147.02 (C-4), 152.35 (OCONH), 155.87 (C-2). *Anal.* Calcd for $C_{11}H_{15}N_3O_4$: C, 52.17; H, 5.97; N, 16.59. Found: C, 52.34; H, 5.81; N, 16.38.

***tert*-Butyl-*N*-(5-amino-4-methylpyridin-2-yl) Carbamate (34)** A solution of **33** (1 g, 3.95 mmol) in dry ethanol (40 ml) was hydrogenated in the presence of 10% Pd/C (130 mg) under a pressure of 50 psi at rt for 5 h. The solution was filtered through a celite pad to remove the catalyst and the filtrate was evaporated to dryness to give pure **34** (880 mg, 98%). mp: 167 °C (EtOAc); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.50 [9H, s, $(CH_3)_3$], 2.14 (3H, s, CH_3), 3.34 (2H, br s, D_2O exchang., NH_2), 7.69 (1H, s, H-3), 7.78 (1H, s, H-6), 9.28 (1H, br s, D_2O exchang., NH). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 17.38 (CH_3), 28.47 [(CH_3)$_3$C], 80.08 [(CH_3)$_3$C], 114.01 (C-3), 133.86 (C-6), 134.49 (C-5), 136.96 (C-4), 145.25 (C-2), 153.26 (OCONH). *Anal.* Calcd for $C_{11}H_{17}N_3O_2$: C, 59.17; H, 7.67; N, 18.82. Found: C, 58.84; H, 7.79; N, 18.97.

***tert*-Butyl-*N*-(5-acetamido-4-methylpyridin-2-yl) Carbamate (35)** To a solution of **34** (880 mg, 3.95 mmol) in dry dichloromethane (20 ml) acetic anhydride (0.56 ml, 5.93 mmol) was added and the resulting solution was stirred at rt for 12 h. The solvent was vacuum-evaporated and the residue was triturated with diethyl ether to give pure **35** (0.98 g, 93%). mp: 208—210 °C (EtOAc); ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.46 [9H, s, $(CH_3)_3$], 2.05 (3H, s, $COCH_3$), 2.19 (3H, s, CH_3), 7.69 (1H, s, H-3), 8.15 (1H, s, H-6), 9.43 (1H, br s, D_2O exchang., NHAc), 9.83 (1H, br s, D_2O exchang., NHBoc). ^{13}C-NMR (50 MHz, DMSO-d_6) δ: 17.95 (CH_3), 23.02 (CH_3CO), 28.09 [(CH_3)$_3$C], 79.65 [(CH_3)$_3$C], 113.22 (C-3), 128.70 (C-5), 143.78 (C-4), 144.55 (C-6), 149.62 (C-2), 152.80 (OCONH), 168.85 (COCH$_3$). *Anal.* Calcd for $C_{13}H_{19}N_3O_3$: C, 58.85; H, 7.22; N, 15.84. Found: C, 58.61; H, 7.09; N, 15.68.

3-*tert*-Butyl-*N*-[5-acetamido-4-(2-benzyloxyethoxy)ethylpyridin-2-yl] Carbamate (36) This compound was prepared by a procedure analogous to that of **9**, starting from **35** (600 mg, 2.26 mmol), using 3.3 eq of *n*-BuLi. The anion formation was complete at −20 °C. The product was purified by column chromatography (silica gel) using a mixture of cyclohexane/EtOAc (1/1, v/v) as the eluent to provide **36** (390 mg, 40%) as an oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.51 [9H, s, $(CH_3)_3$], 1.99 (3H, s, $COCH_3$), 2.82 (2H, t, J=5.2 Hz, PyrCH_2CH_2O), 3.56—3.59 (4H, m, OCH_2CH_2O), 3.73 (2H, t, J=5.2 Hz, PyrCH_2CH_2O), 4.49 (2H, s, CH_2Ph), 7.23—7.36 (5H, m, Ph-H), 7.81 (1H, s, H-3), 8.41 (1H, br s, D_2O exchang., NHBoc), 8.58 (1H, s, H-6), 8.75 (1H, br s, D_2O exchang., NHAc). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 23.78 (CH_3CO), 28.43 [(CH_3)$_3$C], 33.37 (PyrCH_2CH_2O), 69.21 (OCH_2CH_2O), 70.39 (OCH_2CH_2O), 72.60 (PyrCH_2CH_2O), 73.32 (CH_2Ph), 81.04 [(CH_3)$_3$C], 112.97 (C-3), 127.87 (2×Phenyl C), 128.08 (C-4′), 128.63 (2×Phenyl C), 129.39 (C-5), 137.68 (C-1′), 144.07 (C-4), 144.54 (C-6), 148.95 (C-2), 152.78 (OCONH), 169.08 (CH_3CO). *Anal.* Calcd for $C_{23}H_{31}N_3O_5$: C, 64.32; H, 7.27; N, 9.78. Found: C, 64.41; H, 7.19; N, 9.93.

***tert*-Butyl-*N*-[1-acetyl-3-(2-benzyloxyethoxy)methyl-1*H*-pyrazolo[3,4-*c*]pyridin-5-yl] Carbamate (37)** This compound was prepared by a procedure analogous to that of **11**, starting from **36** (350 mg, 0.82 mmol). The product was purified by column chromatography (silica gel) using a mixture of cyclohexane/EtOAc (95/5, v/v) as the eluent to provide **37** (315 mg, 88%) as an amorphous solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.55 [9H, s, $(CH_3)_3$], 2.75 (3H, s, $COCH_3$), 3.71 (2H, t, J=4.7 Hz, OCH_2CH_2OBn), 3.79 (2H, t, J=4.7 Hz, OCH_2CH_2OBn), 4.59 (2H, s, CH_2Ph), 4.92 (2H, s, pyrazol-CH_2O), 7.31—7.35 (5H, m, Ph-H), 8.25 (1H, br s, D_2O exchang., NH), 8.37 (1H, s, H-4), 9.41 (1H, s, H-7). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 22.61 ($COCH_3$), 28.48 [(CH_3)$_3$C], 65.95 (pyrazol-CH_2O), 69.52 (OCH_2CH_2OBn), 70.65 (OCH_2CH_2OBn), 73.47 (CH_2Ph), 81.19 [(CH_3)$_3$C], 101.87 (C-4), 127.73 (C-4′), 127.87 (2×Phenyl C), 128.50 (2×Phenyl C), 133.05 (C-3α), 133.33 (C-7α), 136.42 (C-7), 138.31 (C-1′), 147.65 (C-5), 148.59 (C-3), 152.73 (OCONH), 170.23 (COCH$_3$). *Anal.* Calcd for $C_{23}H_{28}N_4O_5$: C, 62.71; H, 6.41; N, 12.72. Found: C, 62.80; H, 6.44; N, 12.77.

1-Acetyl-3-(2-benzyloxyethoxy)methyl-1*H*-pyrazolo[3,4-*c*]pyridin-5-yl-amine (38) This compound was prepared by a procedure analogous to that of **30**, starting from **37** (260 mg, 0.59 mmol). The product was purified by column chromatography (silica gel) using a mixture of cyclohexane/EtOAc (4/6, v/v) as the eluent to provide **38** (175 mg, 87%). mp: 98—100 °C (dec.) (EtOAc). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.69 (3H, s, $COCH_3$), 3.67—3.70 (2H, m, OCH_2CH_2OBn), 3.72—3.75 (2H, m, OCH_2CH_2OBn), 4.36 (2H, br s, D_2O exchang., NH_2), 4.57 (2H, s, CH_2Ph), 4.86 (2H, s, pyrazol-CH_2O), 6.85 (1H, s, H-4), 7.30—7.36 (5H, m, Ph-H), 9.22 (1H, s, H-7). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 22.51 ($COCH_3$), 66.58 (pyrazol-CH_2O), 69.59 (OCH_2CH_2OBn), 70.32 (OCH_2CH_2OBn), 73.41 (CH_2Ph), 96.60 (C-4), 127.84 (3×Phenyl C), 128.61 (2×Phenyl C), 131.56 (C-7α), 133.72 (C-3α), 136.63 (C-7), 138.24 (C-1′), 147.95 (C-3), 154.71 (C-5), 169.93 (COCH$_3$). *Anal.* Calcd for $C_{18}H_{20}N_4O_3$: C, 63.52; H, 5.92; N, 16.46. Found: C, 63.66; H, 5.71; N,16.65.

3-(2-Hydroxyethoxy)methyl-1*H*-pyrazolo[3,4-*c*]pyridin-5-yl-amine (39) This compound was prepared by a procedure analogous to that of **12**, starting from **38** (120 mg, 0.35 mmol) using 5 eq of BCl_3. The product was purified by column chromatography (silica gel) using a mixture of CH_2Cl_2/CH_3OH (9/1, v/v) as the eluent to provide **39** (60 mg, 83%) as an amorphous solid. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.44 (2H, t, J=4.9 Hz, OCH_2CH_2OH), 3.50 (2H, t, J=4.9 Hz, OCH_2CH_2OH), 4.69 (2H, s, pyrazol-CH_2O), 5.40 (2H, br s, D_2O exchang., NH_2), 6.65 (1H, s, H-4), 8.47 (1H, s, H-7), 12.98 (1H, br s, D_2O exchang., NH-1). ^{13}C-NMR (50 MHz, DMSO-d_6) δ: 60.16 (OCH_2CH_2OH), 65.15 (pyrazol-CH_2O), 71.49 (OCH_2CH_2OH), 92.56 (C-4), 128.99 (C-3α), 131.97 (C-7), 133.59 (C-7α), 140.01 (C-3), 152.90 (C-5). *Anal.* Calcd. for $C_9H_{12}N_4O_2$: C, 51.92; H, 5.81; N, 26.91. Found: C, 51.77; H, 5.60; N, 27.18.

Antiviral and Cytostatic Assays Antiviral activity against vesicular stomatitis virus, HSV-1, HSV-2, Vaccinia virus, VZV and CMV in HEL cell cultures, Coxsackie B4 virus, respiratory syncytial virus, reovirus-1, Sindbis virus and Punta Toro virus in Vero and HeLa cell cultures was determined by adding virus (100 $CCID_{50}$) to confluent human cell cultures in 96-well microtiter plates. After a 1—2 h incubation period, residual virus was removed and the infected cells were further incubated with the medium containing different concentrations of the compounds. After incubation at 37 °C, virus-induced cytopathogenicity was monitored microscopically when the infected control cell cultures reached full cytopathicity. For the anti-HIV assays, the viruses were administered to CEM cell cultures in the presence of different dilutions of the test compounds, and virus-induced cytopathicity (syncytia formation) was microscopically recorded. Antiviral activity was expressed as the EC_{50} or compound concentration required to reduce virus-induced cytopathogenicity by 50%. EC_{50} values were calculated from graphic plots of the percentage of cytopathogenicity as a function of concentration of the compounds.

Cytostatic activity against L1210 (murine leukemia), Molt4/C8 and CEM (human T-lymphocytes) cells were measured by adding *ca.* 50000 to 75000 cells per 200-μl well in 100-μl growth medium. Then, medium (100 μl) containing different concentrations of the test compounds were added. After 2—3 d of incubation at 37 °C, the cell number was determined with a Coulter counter. The cytostatic concentration was calculated as the CC_{50} or the compound concentration required to reduce cell growth by 50% relative to the number of cells in the untreated controls. CC_{50} values were estimated from graphic plots of the number of cells (percentage of control) as a function of the concentration of the test compounds.

References

1) Elion G. B., Furman P. A., Fyfe J. A., de Miranda P., Beauchamp L., Schaeffer H. J., *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 5716—5720 (1977).
2) Elion G. B., *J. Med. Virol. Suppl. I*, **1993**, 2—6 (1993).
3) Chu C. K., Cutler S. J., *J. Heterocycl. Chem.*, **23**, 289—319 (1986).
4) Shugar D., *Pharmacol. Ther.*, **82**, 315—335 (1999).

5) Martin J. C., Dvorak C. A., Smee D. F., Matthews T. R., Julien P. H., Verheyden J. P. H., *J. Med. Chem.*, **26**, 759—761 (1983).
6) McGavin J. K., Goa K. L., *Drugs*, **61**, 1153—1183 (2001).
7) Hodge R., Sutton D., Boyd M. R., Harnden M. R., Jarvest R. L., *Antimicrob. Agents Chemother.*, **33**, 1765—1773 (1989).
8) Cools M., De Clercq E., *Biochem. Pharmacol.*, **38**, 1061—1067 (1989).
9) De Clercq E., *Antiviral Res.*, **75**, 1—13 (2007).
10) Kourafalos V. N., Marakos P., Pouli N., Townsend L. B., *J. Org. Chem.*, **68**, 6466—6469 (2003).
11) Kourafalos V. N., Marakos P., Pouli N., Townsend L. B., *Synlett*, **2002**, 1479—1482 (2002).
12) Olah G. A., Arvanaghi M., Vankar Y. D., *Synthesis*, **8**, 660—661 (1980).
13) Fischer B. E., Hodge J. E., *J. Org. Chem.*, **29**, 776—781 (1964).
14) Emerson T. R., Rees C. W., *J. Chem. Soc.*, **1964**, 2319—2325 (1964).
15) Marakos P., Pouli N., Wise D. S., Townsend L. B., *Synlett*, **1997**, 561—562 (1997).
16) Chapman D., Hurst J., *J. Chem. Soc. Perkin Trans. I*, **1980**, 2398—2404 (1980).
17) Besly D. M., Goldberg A. A., *J. Chem. Soc.*, **1954**, 2448—2455 (1954).
18) Pino L. N., Zehrung W. S. III, *J. Am. Chem. Soc.*, **77**, 3154—3155 (1955).