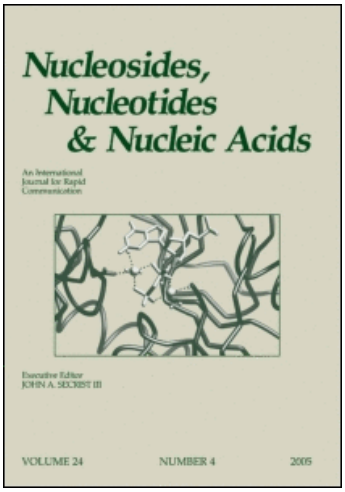


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Pyrazole Related Nucleosides 5.[1] Synthesis and Biological Activity of 2'-Deoxy- 2', 3'-dideoxy- and Acyclo-analogues of 4-Iodo-1-β-D-ribofuranosyl-3-carboxymethyl Pyrazole (IPCAR)

Stefano Manfredini[a]; Pier Giovanni Baraldi[a]; Rita Bazzanini[b]; Elisa Durini[a]; Silvia Vertuani[a]; Alessandra Pani[c]; Tiziana Marceddu[c]; Francesca Demontis[c]; Laura Vargiu[c]; Paolo La Colla[c]
[a] Dipartimento di Scienze Farmaceutiche, Università di Ferrara, Ferrara [b] Consiglio Nazionale delle Ricerche, Bologna [c] Dipartimento di Biologia Sperimentale, Sez. di Microbiologia, Cittadella Universitaria di Monferrato, Cagliari, ITALY

To cite this Article Manfredini, Stefano , Baraldi, Pier Giovanni , Bazzanini, Rita , Durini, Elisa , Vertuani, Silvia , Pani, Alessandra , Marceddu, Tiziana , Demontis, Francesca , Vargiu, Laura and La Colla, Paolo(2000) 'Pyrazole Related Nucleosides 5.[1] Synthesis and Biological Activity of 2'-Deoxy- 2', 3'-dideoxy- and Acyclo-analogues of 4-Iodo-1-β-D-ribofuranosyl-3-carboxymethyl Pyrazole (IPCAR)', Nucleosides, Nucleotides and Nucleic Acids, 19: 4, 705 — 722
To link to this Article: DOI: 10.1080/15257770008035019
URL: http://dx.doi.org/10.1080/15257770008035019

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 19(4), 705–722 (2000)

PYRAZOLE RELATED NUCLEOSIDES 5.[1] SYNTHESIS AND BIOLOGICAL ACTIVITY OF 2'-DEOXY- 2', 3'-DIDEOXY- AND ACYCLO-ANALOGUES OF 4-IODO-1-β-D-RIBOFURANOSYL-3-CARBOXYMETHYL PYRAZOLE (IPCAR).

Stefano Manfredini,* Pier Giovanni Baraldi, Rita Bazzanini,§
Elisa Durini, Silvia Vertuani, Alessandra Pani,# Tiziana Marceddu,#
Francesca Demontis,# Laura Vargiu# and Paolo La Colla.#

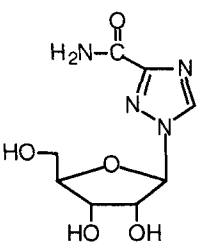
Dipartimento di Scienze Farmaceutiche, Università di Ferrara, Via Fossato di Mortara 17-19, I-44100 Ferrara. §*Consiglio Nazionale delle Ricerche, I.Co.C.E.A, Via P. Gobetti 101, I-40129 Bologna.* #*Dipartimento di Biologia Sperimentale, Sez. di Microbiologia, Cittadella Universitaria di Monferrato SS 554 Km 4.500, I-09042, Cagliari. ITALY*

Abstract. Continuing our studies on the structure-activity relationships (SAR) of 4-iodo-1-β-D-ribofuranosyl-3-carboxymethyl pyrazole (IPCAR), the ribofuranosyl moiety has been substituted with acyclic chains, namely 1-[(2-hydroxyethoxy)methyl]- and 1-[(1,3-dihydroxy-2-propoxy)methyl]-pyrazole derivatives (**4**, **5** and **8**, **9** respectively), with the 2'-deoxy-β- D-ribofuranosyl group (**12** and **13**) and finally with the 2',3'-dideoxy-D-glycero-pentofuranosyl-moiety (**16** and **17**). None of the new compounds display any interesting biological activity.

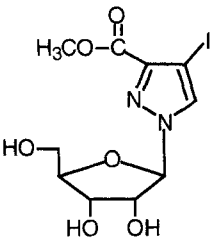
Introduction

Azole nucleosides constitute a class of antimetabolites structurally related to 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (AICAR), the 5'-monophosphate of which is a key intermediate in purine biosynthesis. Important members of this class are bredinin, pyrazofurin and ribavirin,[2] which are endowed with immunosuppressive, antitumor and antiviral activity, respectively.

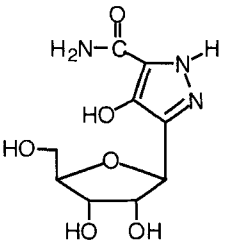
Figure 1



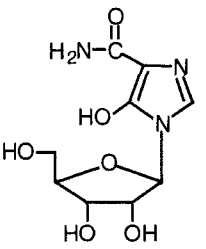
Ribavirin



IPCAR



Pyrazofurin



Bredinin

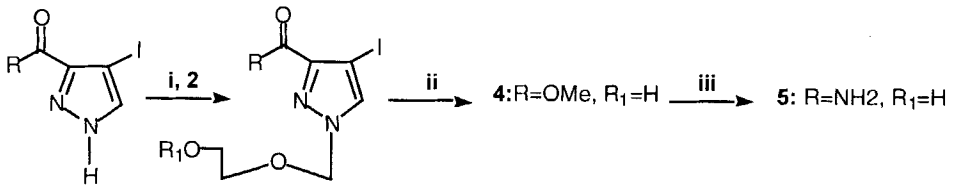
In view of the interest of this class of compounds, we have started a project that has led to the discovery of IPCAR and the related amide, pyrazole nucleosides endowed with an interesting antiproliferative activity associated with an interesting potentiation of the antiviral activity of important antiviral drugs such are ddI and ACG.[1,3-6] Although the structural similarities with ribavirin and tiazofurin might suggest that IPCAR is targeted to inosine monophosphate dehydrogenase (IMPDH), several lines of evidence indicate that it inhibits one of the early steps of the *de novo* purine biosynthesis.[6] This latter idea implies that IPCAR may act, *in toto*, as an antagonist of one of the imidazole nucleoside intermediates of the purine ring biosynthesis. To inhibit RNA synthesis a nucleoside antimetabolite should be recognized as a substrate and/or an inhibitor by enzymes that metabolize ribonucleosides and ribonucleotides or that are devoted to their *de novo* biosynthesis. Therefore, such an inhibitor should have two hydroxy groups at the 2'- and 3'-positions. However, a well recognized strategy to investigate structure-activity relationships (SAR) in the field of nucleoside analogs involves the modification of the glycosylic portion. In recent years, this strategy has lead to the discovery of novel

antimetabolites of particular interest in the field of antiviral and antitumor nucleosides, such as acyclic derivatives (i.e. acyclovir, gancyclovir, PMEA), arabinofuranosides (i.e. araA, araC), 2',3'-dideoxyribofuranosides (i.e. ddC, ddI), 2',3'-dideoxydidehydro-ribofuranosides(i.e. d4T), 3'-azido-2',3'-dideoxyribofuranosides (i.e. AZT) and so forth.[7] Taking these considerations into account we started the present study with the aim to apply these modifications to our lead compounds.

Chemistry

The synthesis of the acyclic derivatives **4**, **5** and **8**, **9** was achieved by alkylation of 3-methoxycarbonyl-4-iodo-pyrazole (**1**).[1] Thus, the preparation of 4-iodo-3-methoxycarbonyl-1-[(2-hydroxyethoxy)methyl]-pyrazole and related 3-carboxamide (**4** and **5**) involved the base catalyzed condensation of (2-acetoxyethoxy)methyl bromide (**2**)[8] with 4-iodo-3-methoxycarbonyl-pyrazole (**1**). The condensation proceeded in low yield to give a mixture of the two N1 and N2 regioisomers which proved difficult to separate. However, the amount of the N1 isomer obtained, was sufficient for the continuation of the study and thus yields were not optimized. 4-Iodo-3-methoxycarbonyl-1-[(2-acetoxyethoxy)methyl]pyrazole (**3**) was next deprotected, with sodium methoxide, to give the 4-iodo-3-methoxycarbonyl-1-[(2-hydroxyethoxy)methyl]pyrazole (**4**) in 87%. Next conversion into the corresponding amide (**5**) was obtained under mild conditions with methanolic ammonia (Scheme 1).

Scheme 1



1: R=OMe **3:** R=OMe, R_1=CH_3CO

i: CH_3CN, NaH; **ii:** MeONa, MeOH; **iii:** NH_3/MeOH

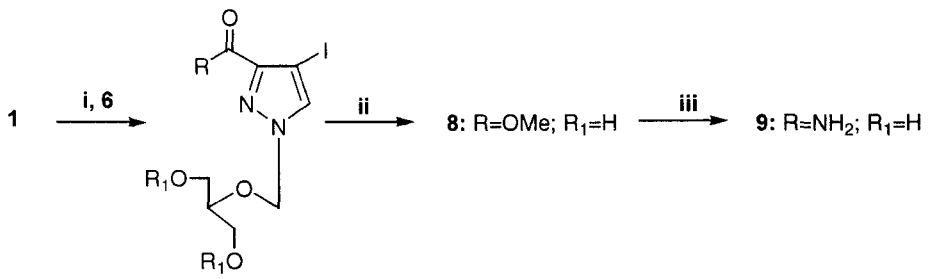
The 4-iodo-3-methoxycarbonyl-1-[(1,3-dihydroxy-2-propoxy)methyl]pyrazole (**8**) was obtained through condensation of 2-*O*-(acetoxymethyl)-1,3-di-*O*-benzylglycerol (**6**),[9] prepared as described in literature, and 4-iodo-3-methoxycarbonyl-pyrazole (**1**). The condensation was first attempted through base catalysis as described above, however, under these conditions, very low yields and mixtures of the two regioisomers, namely N1 and N2 derivatives, were obtained.

To overcome this difficulty we studied an alternative approach based on the condensation of the above cited glycerol derivative **6** on the silylated heterocyclic base in the presence of trimethylsilyl trifluoromethanesulfonate as the catalyst. Under these conditions, 4-iodo-3-methoxycarbonyl-1-{[(1,3-bis(benzyloxy)-2-propoxy]methyl}pyrazole (**7**) was obtained in 96% yield without any detectable amount of the corresponding N2 derivative. Next deprotection, by removal of the benzyl groups, under reductive conditions (Pd/C or Pd-hydroxide) failed due to the concomitant reduction of the 4-position to give the corresponding deiodinated product.

The inability to apply the standard hydrogenation prompted us to investigate an alternative procedure, based on the use of BCl_3 at -73 °C, which allowed us to obtain **8** in satisfactory yields (58%) with short reaction times. The corresponding amide derivative **9** was obtained, in 94% yield, by treatment with methanolic ammonia at room temperature (Scheme 2).

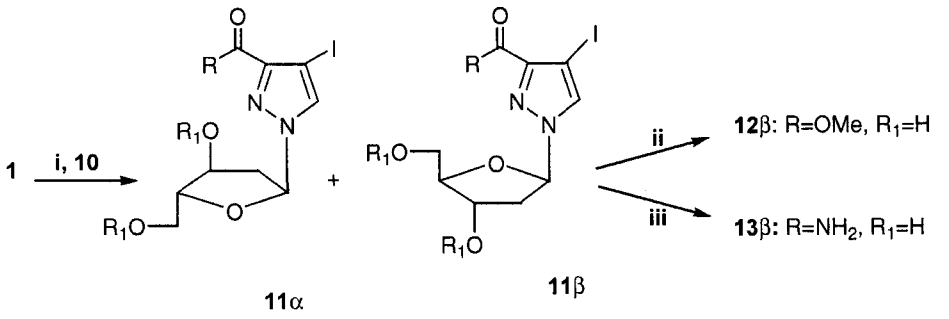
The derivatives **12** and **13**, 1-(2'-deoxy-β-D-ribofuranosyl)-pyrazoles, were obtained in two steps by condensation of 2-deoxy-3,5-di-*O*-p-toluoyl-α-D-ribofuranosyl chloride

Scheme 2



i: CH_3CN, HMDS, TMSOTf, $(NH_4)_2SO_4$; **ii:** BCl_3, CH_2Cl_2; **iii:** NH_3/MeOH

Scheme 3



i: CH_3CN, HMDS, TMSOTf, $(NH_4)_2SO_4$; **ii:** MeONa/MeOH; **iii:** NH_3/MeOH

(**10**)[10] with 3-methoxycarbonyl-4-iodo-pyrazole (**1**) in the presence of trimethylsilyl trifluoromethanesulfonate as the catalyst (Scheme 3) to give 4-iodo-3-methoxycarbonyl-1-(3,5-di-*O*-p-toluoyl-2'-deoxy-D-ribofuranosyl) pyrazole (**11**) in 62% yield as a α:β mixture (1:1.7 ratio, by HPLC).

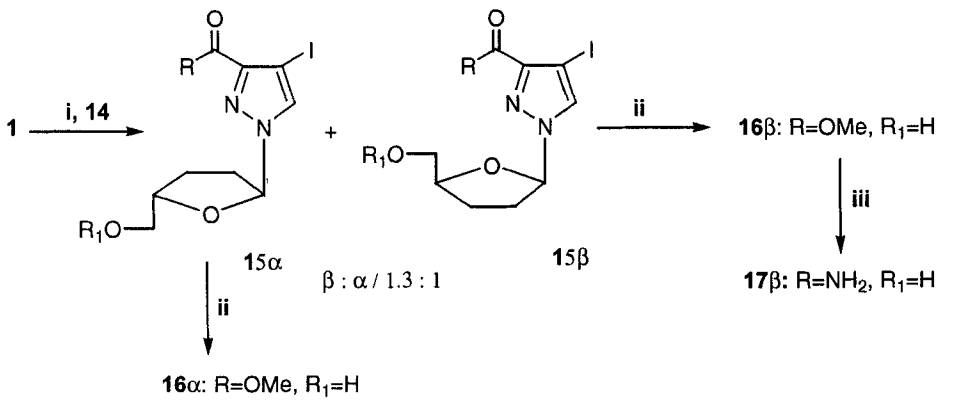
These results are comparable to those obtained with other reported methods[11,12] for coupling of **10** with heterocyclic bases. Also in this case no detectable amounts of the corresponding N2 regioisomers were recovered.

Deprotection of **11** (β-isomer) with sodium methoxide in methanol gave 4-iodo-3-methoxycarbonyl-1-(2'-deoxy-β-D-ribofuranosyl)pyrazole (**12**) in 48% yield; whereas deprotection with methanolic ammonia gave the corresponding 4-iodo-3-carboxamido-1-(2'-deoxy-β-D-ribofuranosyl)pyrazole (**13**) in 68% yield.

The α- and β-2',3'-dideoxy analogues **16** were simply prepared through the direct condensation of (S)-(+)-5-(tert-butyldimethylsilyloxy)methyl-4,5-dihydrofuran (**14**)[13] with the starting compound **1**, in the presence of pyridinium p-toluenesulfonate (PPTS) as the catalyst,[14] (Scheme 4).

This approach is an alternative for other non-stereoselective methods,[15] involving the activation step of the anomeric position (i.e., through preparation of 1-halo- or 1-acetoxy-sugars). However, although simple and efficient, this procedure gave rise to 1 : 1.3 α/β mixtures (^{1}H-NMR analysis, based on the two anomeric-protons centered at

Scheme 4



i: TsOH, MeCN; ii: TBAF, THF; iii: NH_3/MeOH

δ 6.08 and 6.04 respectively) and thus it is not an alternative for other stereoselective methods based on the use of 2-substituted furanoid glycals.[16]

Attempts to use other catalysts, such as Lewis acids [i.e. trimethylsilyl triflate (TMSOTf)] on the silylated **1** did not improve the stereoselectivity of the reaction.[17] Also in this case no traces of the corresponding N2 regioisomers were recovered. The resulting crude coupling product was deprotected with tetrabutylammonium fluoride (TBAF) in THF to give after purification the α– and β-anomers in 53 % overall yield. The corresponding carboxamido derivative **17**(β) was simply obtained, in 86% yield, by treatment of compound **16**(β) with methanolic ammonia.

All structural assignments were based upon NMR analysis in comparison with our previous data.[1,3,4] The regiochemistry and stereochemistry of the reactions were determined by NMR analysis in comparison with our previous data.[1,3,4] In particular, the ^{13}C NMR C-3 signal in N2 derivatives is generally shifted to fields lower than the C-3 on the corresponding N1 derivatives.[1] Moreover, the assignment of anomeric configuration for compounds **11** rested on ^{1}H NMR data. In particular, the H1' proton in the α-anomer was observed as a dd centered at about δ 6.37 whereas for the β anomer a pseudo triplet was observed centered at about δ 6.29.[18] In the case of compounds **15** the assignment of anomeric configuration was based on the downfield

shift of the H4' signal and of the Me signals of the (t-Bu)Me_2Si groups, and on the upfield shift of H5' signals, as usually observed in the case of the α-anomer.[19,20]

Biology

The study compounds were evaluated for their capability to inhibit the multiplication of DNA and RNA viruses, HIV-1 included, and for their cytotoxicity and antiproliferative activity on leukemia/lymphoma (WIL2-NS, Raji, CCRF-CEM, MT-4) and solid tumors (HT-29, HeLa, ACHN, 5637) cell lines.

Title compounds were also evaluated for their capability to inhibit the multiplication of various human pathogenic fungi (*Candida albicans*, *Candida parapsilosis*, *Candida paratropicalis*, *Trycophyton mentagrophytes*, *Microsporum canis*, *Aspergillus fumigatus* and *Criptococcus neoformans*) and bacteria (*Staphylococcus aureus*, group D *Streptococcus*, *Pseudomonas aeruginosa* and *Escherichia coli*). Miconazole and Streptomycin were used as reference compounds in antimicotic and antibacterial assays, respectively.

None of the compounds displayed any biological activity up to 300 μM.

Results and Discussion

We started the present study in order to investigate the role of the glycosyl portion in our model compound IPCAR and related amide; this was achieved by preparing the corresponding acyclic, 2'-deoxy- and 2',3'-dideoxyribofuranosyl derivatives as depicted in the Schemes 1-4.

As described above the compounds proved inactive. However, inactive compounds might be expected if the D-ribofuranosyl moiety is a prerequisite for the recognition of IPCAR by enzymes involved in ribonucleotide and/or ribonucleoside biosynthesis. On the other hand, the conversion of IPCAR to 2'-deoxyribofuranosyl derivatives **12** and **13**, did not shift the antimetabolic activity on DNA synthesis. Moreover, the inactivity of the 1-(2',3'-dideoxy-β-D-glycero-pentofuranosyl)-derivatives **16** and **17**, of the 1-[(2-hydroxyethoxy)methyl]- and 1-[(1,3-dihydroxy-2-propoxy)methyl]-acyclo derivatives **4**, **5** and **8**, **9**, is in contrast with other known nucleoside analogues having similar structural-modifications.[7] In our opinion, these data may be considered as an indirect confirmation that the possible target of IPCAR activity is one or more of the steps of the

ribonucleosides and/or ribonucleotides *de novo* biosynthesis. Preliminary studies on the isolated enzyme (data not shown) indicate that IPCAR is not targeted at IMPDH, thus a possible targeting at one of the early enzymatic steps of the purine *de novo* biosynthesis, appears reasonable. This hypothesis is currently being investigated and results will be reported in due course.

Experimental Section

Chemistry: Reaction courses and product mixtures were routinely monitored by thin-layer chromatography (TLC) on silica gel precoated Durasil-25 UV_{254} Macherey-Nagel plates with detection under 254 nm UV lamp and/or by spraying the plates with 10% H_2SO_4/MeOH and/or with 5% $KMnO_4/H_2O$ solutions and heating. Melting points were obtained in open capillary tubes and are uncorrected. Column chromatographies were performed with Macherey-Nagel 70-230 mesh silica gel. Nuclear magnetic resonance (^1H NMR) spectra were determined in $CDCl_3$ or DMSO-*d6* solution on a Bruker AC-200 spectrometer and peak positions are given in parts per million (δ) downfield from tetramethylsilane as internal standard. Ultraviolet spectra were recorded on a Jasco 510 spectrometer. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectra were taken on a Hewlett-Packard HPG2025A mass spectrometer operating in a positive linear mode. Analytical HPLC were performed on a Waters 600E instrument on a Beckman C_{18} column. Ambient temperature was 22-25°C. Unless otherwise stated all drying operations were performed over anhydrous magnesium sulphate.

Starting Materials: Compounds **1**,[1] **2**,[8] **6**,[9] **10**[10] and **14**[13] were synthesized following known procedures.

4-Iodo-3-methoxycarbonyl-1-[(2-acetoxyethoxy)methyl]pyrazole (3)

Sodium hydride (90 mg, 60% in mineral oil, 2.25 mmol) was added to a solution of 4-iodo-3-methoxycarbonyl-pyrazole (**1**, 370 mg, 1.46 mmol) in dry DMF (40 mL) and the mixture was stirred under argon atmosphere at 80°C. After all hydrogen evolution had ceased (1 hr), (2-acetoxyethoxy)methylbromide (**2**, 270 mg, 1.37 mmol) in dry DMF (10 mL) was added dropwise and the mixture was stirred at 80°C for 15 hrs. The solvent was removed under reduced pressure and the residue, dissolved in CH_2Cl_2, was washed

with water. The organic phase was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The resulting crude material was purified by silica gel column chromatography (EtOAc/Hexane, 2:8) to give 170 mg of the title compound (N1-isomer), yield 30%, and 200 mg of an inseparable mixture of both N1- and N2-isomers.

White solid; mp 95-97°C (Et_2O/Hexane). ^1H NMR ($CDCl_3$): δ 2.04 (s, 3H, Me), 3.70 (t, 2H, J = 4.9 Hz, CH_2O), 3.96 (s, 3H, MeO), 4.14 (t, 2H, J = 5.0 Hz, CH_2OCO), 5.89 (s, 2H, CH_2N), 7.64 (s, 1H, H5).

4-Iodo-3-methoxycarbonyl-1-[(2-hydroxyethoxy)methyl]pyrazole (4)

4-Iodo-3-methoxycarbonyl-1-[(2-acetoxyethoxy)methyl]pyrazole (**3**, 90 mg, 0.24 mmol) was added to a solution of sodium methoxide (40 mg, 0.72 mmol) in dry MeOH (15 mL). The reaction mixture was stirred at 0°C for 1 hr, then neutralized by addition of small portions of Dowex-50 H^+ form. The solution was filtered and the resin washed with MeOH. The solvent was removed under reduced pressure and the crude material purified by silica gel column chromatography (CH_2Cl_2/MeOH, 9.5:0.5) to give 68 mg of colorless syrup, yield 87%.

UV (MeOH): λ_{max} 258 (ε 3000), λ_{min} 242 (ε 2200). ^1H NMR ($CDCl_3$): δ 3.60-3.70 (m, 5H, 2 x CH_2O, OH), 3.96 (s, 3H, MeO), 5.91 (s, 2H, CH_2N), 7.65 (s, 1H, H5).

MALDI-TOF MS: *m/z* 349.3 $(M+Na)^+$, 365.2 $(M+K)^+$, $C_8H_{11}IN_2O_4$ requires 326.09.

3-Carboxamido-4-iodo-1-[(2-hydroxyethoxy)methyl]pyrazole (5)

4-Iodo-3-methoxycarbonyl-1-[(2-acetoxyethoxy)methyl]pyrazole (**3**, 70 mg, 0.18 mmol) was dissolved in methanolic ammonia (10 mL) and was stirred in a firmly capped flask at room temperature overnight. The solvent was evaporated to dryness *in vacuo* and the crude material purified by silica gel column chromatography (CH_2Cl_2/MeOH, 9.5:0.5) to give 40 mg of gray solid, yield 71 %.

White solid; mp 115-117°C (MeOH/Et_2O). UV (MeOH): λ_{max} 254 (ε 2800), λ_{min} 240 (ε 2200). ^1H NMR (DMSO-*d6*): δ 3.20-3.50 (m, 3H, CH_2O, OH), 4.60-4.70 (s, 2H, CH_2O), 5.57 (s, 2H, CH_2N), 7.64 (s, 1H, H5), 7.88 and 7.99 (brs, 2H, NH_2). MALDI-TOF MS: *m/z* 334.1 $(M+Na)^+$, 350.2 $(M+K)^+$, $C_7H_{10}IN_3O_3$ requires 311.08.

4-Iodo-3-methoxycarbonyl-1-{[1,3-bis(benzyloxy)-2-propoxy]methyl}-pyrazole (7)

To a stirred suspension of 4-iodo-3-methoxycarbonyl-pyrazole (**1**, 100 mg, 0.40 mmol) and 2-*O*-(acetoxymethyl)-1,3-di-*O*-benzylglycerol (**6**, 202 mg, 0.59 mmol) in freshly distilled CH_3CN (25 mL), hexamethyldisilazane (0.14 mL, 0.67 mmol) and a catalytic amount of ammonium sulfate were added under argon atmosphere. The mixture was heated at reflux until complete dissolution (15 min). To the resulting solution, cooled at room temperature, was added of trimethylsilyl trifluoromethanesulfonate (0.12 mL, 0.62 mmol) and then the mixture was refluxed for 1.5 hrs under argon atmosphere. The cooled solution was diluted with CH_2Cl_2 (15 mL) and washed with saturated $NaHCO_3$ aqueous solution (15 mL). After a further extraction of the aqueous phase with CH_2Cl_2 (2 x 15 mL), the combined organic phases were washed with brine (15 mL), dried over anhydrous $MgSO_4$ and evaporated to dryness. The residue was purified by silica gel column chromatography (Hexane/EtOAc, 8:2 → 7:3) to give 0.2 g of pale orange syrup, yield 96%.

^1H NMR ($CDCl_3$): δ 3.50 (d, 4H, J = 5.0 Hz, $\underline{CH_2}$-CH-$\underline{CH_2}$), 3.93 (m, 4H, MeO and CHO), 4.47 (s, 4H, 2 x CH_2Ph), 5.65 (s, 2H, CH_2N), 7.25-7.40 (m, 10 H, aromatic), 7.79 (s, 1H, H5).

4-Iodo-3-methoxycarbonyl-1-[(1,3-dihydroxy-2-propoxy)methyl]pyrazole (8)

A solution of 4-iodo-3-methoxycarbonyl-1-{[(1,3-bis(benzyloxy)-2-propoxy]methyl} pyrazole (**7**, 420 mg, 0.78 mmol) in anhydrous CH_2Cl_2 (40 mL) was cooled at –78°C and BCl_3 (6.24 mL of 1M solution in CH_2Cl_2, 6.24 mmol) was added dropwise. After 10 min the reaction, monitored by TLC (CH_2Cl_2/MeOH, 9.5:0.5), was completed. The solution was then diluted with CH_2Cl_2/MeOH, 1:1 (50 mL) and after 10 min, Et_3N (4 mL) was added and the mixture was allowed to warm to room temperature. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 9.5:0.5) to give the title compound as an off-white waxy solid, yield 58%.

White solid; mp 100-104°C (MeOH/Et_2O); UV (MeOH): λ_{max} 255 (ε 2800), λ_{min} 240 (ε 2000). ^1H NMR (DMSO-d_6), δ 3.25-3.55 (m, 5H, CH_2CHCH_2), 3.80 (s, 3H, MeO), 4.65 (t, 2H, J = 5.5 Hz, 2 x OH), 5.58 (s, 2H, CH_2N), 8.25 (s, 1H, H5). MALDI-TOF MS: *m/z* 357.2 $(M+H)^+$, $C_9H_{13}IN_2O_5$ requires 356.11.

4-Iodo-3-carboxamido-1-[(1,3-dihydroxy-2-propoxy)methyl]pyrazole (9)

4-Iodo-3-methoxycarbonyl-1-[(1,3-dihydroxy-2-propoxy)methyl]pyrazole (**8**, 90 mg, 0.25 mmol) was deprotected as described above for **5**, to give after purification by silica gel column chromatography (CH_2Cl_2/MeOH, 9:1) 80 mg of a green syrup, yield 94%. UV (MeOH): λ_{max} 252 (ε 2700), λ_{min} 242 (ε 1900). ^1H NMR (DMSO-*d6*): δ 3.25-3.45 (m, 5H, CH_2CHCH_2), 4.64 (t, 2H, J = 5.5 Hz, 2 x OH), 5.54 (s, 2H, CH_2N), 7.32 and 7.52 (sbr, 2H, NH_2), 8.16 (s, 1H, H5). MALDI-TOF MS: *m/z* 364.2 $(M+Na)^+$, 380.2 $(M+K)^+$, $C_8H_{12}IN_3O_4$ requires 341.10.

4-Iodo-3-methoxycarbonyl-1-(3,5-di-*O*-p-toluoyl-2'-deoxy-D-ribofuranosyl) pyrazole (11α, 11β)

A suspension of 4-iodo-3-methoxycarbonyl-pyrazole (**1**, 125 mg, 0.5 mmol) in hexamethyldisilazane (1.5 mL) was heated under reflux until complete dissolution. The solvent was removed under reduced pressure and the residue was co-evaporated (three times) with CH_3CN. The crude silylated derivative was dissolved in freshly distilled CH_3CN (5 mL) and 2-deoxy-3,5-di-*O*-p-toluoyl-α-D-ribofuranosyl chloride (**10**, 195 mg, 0.5 mmol) in dry CH_2Cl_2 (2 mL) was added. This solution was cooled at 0°C then trimethylsilyl trifluoromethanesulfonate (0.1 mmol freshly prepared from trifluoromethane sulfonate and trimethylsilyl chloride in 5 mL of anhydrous CH_3CN) was added. The reaction mixture was stirred at room temperature under argon atmosphere overnight, then was diluted with CH_2Cl_2 (15 mL) and washed with saturated $NaHCO_3$ aqueous solution (2 x 15 mL). The organic phase was dried over anhydrous $MgSO_4$ and evaporated to dryness. The residue was purified by silica gel column chromatography (Hexane/EtOAc, 8.5:1.5 → 8:2) to give 125 mg of β-anomer and 65 mg of α-anomer.

α-anomer: colorless syrup, yield 21%. ^1H NMR ($CDCl_3$): δ 2.40 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 2.90-2.95 (m, 2H, H2', H2''), 3.92 (s, 3H, MeO), 4.55-4.60 (m, 2H, H5' and H5''), 4.80-4.90 (m, 1H, H4'), 5.60-5.65 (m, 1H, H3'), 6.37 (dd, 1H, J = 7.4, 3.0 Hz, H1'), 7.20-7.30 (m, 4H, toluoyl), 7.61 (d, 2H, J = 8.2 Hz, toluoyl), 7.93 (d, 2H, J = 8.2 Hz, toluoyl), 7.96 (s, 1H, H5).

β-anomer: colorless syrup, yield 41%. ^{1}H NMR ($CDCl_3$): δ 2.39 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 2.70-3.00 (m, 2H, H2', H2''), 3.91 (s, 3H, MeO), 4.55-4.70 (m, 3H, H4', H5' and H5''), 5.60-5.75 (m, 1H, H3'), 6.29 (t, 1H, J = 6.8 Hz, H1'), 7.20-7.30 (m, 4H, toluoyl), 7.80-95 (m, 2H, J = 8.4 Hz, toluoyl), 7.85-7.95 (s, 2H, toluoyl and H5).

4-Iodo-3-methoxycarbonyl-1-(2'-deoxy-β-D-ribofuranosyl)pyrazole (12)

4-Iodo-3-methoxycarbonyl-1-(3,5-di-*O*-p-toluoyl-2'-deoxy-β-D-ribofuranosyl) pyrazole (**11**β, 0.12 g, 0.2 mmol) was dissolved in dry MeOH (5 mL) then was added to a solution of sodium methoxide (20 mg, 0.36 mmol) in dry MeOH (5 mL). The reaction mixture, stirred at room temperature for 1 hr under argon atmosphere, was neutralized by addition of small portions of Dowex-50 H^+ form. The solution was filtered and the resin washed with MeOH. After evaporation of the solvent *in vacuo* the crude material was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 9.5:0.5). The appropriate fractions were evaporated and dissolved in water to give, upon freeze drying, 36 mg of **12,** yield 48 %.

Pale yellow solid; mp 111-114 °C (MeOH/Et_2O); UV (MeOH): λ_{max} 258 nm (ε 2700), λ_{min} 245 nm (ε 1800). ^{1}H NMR (DMSO-*d6*): δ 2.30 (dt, 1H, J = 14.0 and 3.5 Hz, H2'), 2.60-2.75 (m, 1H, H2''), 3.35-3.45 (m, 2H, H5' and H5''), 3.79 (s, 3H, MeO), 4.00-4.10 (m, 1H, H4'), 4.15-4.25 (m, 1H, H3'), 4.84 (t, 1H, J = 5.8 Hz, OH5'), 5.39 (d, 1H, J = 4.5 Hz, OH3'), 6.37 (t, 1H, J = 7.0 Hz, H1'), 8.28 (s, 1H, H5). MALDI-TOF MS: *m/z* 391.3 $(M+Na)^+$, $C_{10}H_{13}IN_2O_5$ requires 368.12.

4-Iodo-3-carboxamido-1-(2'-deoxy-β-D-ribofuranosyl)pyrazole (13)

4-Iodo-3-methoxycarbonyl-1-(2'-deoxy-β-D-ribofuranosyl) pyrazole (**12**, 0.12 g, 0.2 mmol)) was deprotected as described above for **5**, and purified by silica gel column chromatography (CH_2Cl_2/MeOH, gradient 9.5:0.5 →9:1). The appropriate fractions were evaporated and dissolved in water to give, upon freeze drying, 48 mg of **13,** yield 68%.

Off-white glassy solid; mp 123-125°C (MeOH/Et_2O). UV (MeOH): λ_{max} 255 nm (ε 2800), λ_{min} 247 nm (ε 2000). ^{1}H NMR (DMSO-*d6*): δ 2.30 (dt, 1H, J=14.0 and 3.5 Hz, H2'), 2.60-2.75 (m, 1H, H2''), 3.40-3.60 (m, 2H, H5' and H5''), 4.00.4.05 (m, 1H,

H4'), 4.15-4.25 (m, 1H, H3'), 4.84 (t, 1H, J = 5.7 Hz, OH5'), 5.36 (d, 1H, J = 4.4 Hz, OH3'), 6.33 (t, 1H, J = 7.2 Hz, H1'), 7.32 (sbr, 1H, NH), 7.53 (sbr, 1H, NH), 8.20 (s, 1H, H5). MALDI-TOF MS: *m/z* 376.4 $(M+Na)^+$, 392.3 $(M+K)^+$, $C_9H_{12}IN_3O_4$ requires 353.11.

4-Iodo-methoxycarbonyl-1-(5'-*O*-tert-butyldimethylsilyl-2',3'-dideoxy-D-glycero-pentofuranosyl)pyrazole (15α , 15β)

To a solution of 4-iodo-3-methoxycarbonyl-pyrazole (**1**, 0.5 g, 2 mmol) in anhydrous CH_3CN (50 mL) a catalytic amount of pyridinium p-toluenesulfonate was added under argon atmosphere. Then a solution of 5-*O*-TBDMS-2,3-dideoxy-D-glycero-pentofuranose (**14**, 0.5 g, 2.3 mmol) in anhydrous CH_3CN (25 mL) was slowly added at 0°C to the mixture. The mixture was refluxed for 6 hrs. After evaporation of the solvent, the residue was dissolved in $CHCl_3$ (100 mL) and washed with saturated $NaHCO_3$ solution (50 mL), brine (50 mL), then dried over anhydrous $MgSO_4$ and evaporated to dryness. The crude oily residue, containing the mixture of the two **15α** and **15β** anomers, was deprotected as described below, without any further purification. A small amount of the crude residue was purified by silica gel column chromatography (EtOAc/Hexane gradient 1:1 → 6:4) for analytical purposes.

α-anomer: syrup; ^1H NMR ($CDCl_3$): δ 0.02 and 0.03 (2 x s, 2 x 3H, $SiMe_2$), 0.83 (s, 9H, Si-tBut), 1.90-2.10 (m, 2H, H3' and H3''), 2.35-2.45 (m, 2H, H2' and H2''), 3.50-3.70 (m, 2H, H5' and H5''), 3.86 (s, 3H, MeO), 4.30-4.40 (m, 1H, H4'), 6.10 (dd, 1H, J = 6.1 and 3.3 Hz, H1'), 7.66 (s, 1H, H5).

β-anomer: syrup; ^1H NMR ($CDCl_3$): δ 0.04 and 0.05 (2 x s, 2 x 3H, $SiMe_2$), 0.87 (s, 9H, Si-tBut), 1.80-2.10 (m, 2H, H3' and H3''), 2.25-2.35 (m, 2H, H2' and H2''), 3.64 (dd, 1H, J = 11.5 and 3.0 Hz, H5'), 3.85 (s, 3H, MeO), 3.93 (dd, 1H, J = 11.4 and 3.0 Hz, H5''), 4.10-4.20 (m, 1H, H4'), 6.00 (t, 1H, J = 3.5 Hz, H1'), 7.08 (s, 1H, H5).

4-Iodo-3-methoxycarbonyl-1-(2',3'-dideoxy-D-glycero-pentofuranosyl) pyrazole (16α and 16β)

The crude 4-iodo-3-methoxycarbonyl-1-(5'-*O*-tert-butyldimethylsilyl-2',3'-dideoxy-D-glycero-pentofuranosyl)pyrazole (**15α** and β, 250 mg, corresponding to a theoretic 0.53

mmol) was dissolved in freshly distilled THF (50 mL) and treated under stirring with TBAF x 3 H_2O (0.95 g, 0.53 mmol) at room temperature. After 30 min the reaction was complete (TLC, EtOAc/Hexane, 8:2). The solvent was evaporated to dryness and the residue, dissolved in EtOAc (100 mL), was washed with water (50 mL) then dried over anhydrous $MgSO_4$, filtered and then evaporated to dryness. The crude material was purified by column chromatography (EtOAc/Hexane, gradient 6:4 → 8:2) to give 56 mg of the β-anomer, and 43 mg of the α-anomer.

α-anomer: colorless syrup, yield 23%. UV (MeOH): λ_{max} 205 (ε 13400), 257 (ε 2900); $\lambda_{shoulder}$ 250 (ε 2600), λ_{min} 245 (ε 2000); ^{1}H-NMR ($CDCl_3$): δ 1.80-2.10 (m, 3H, H3', H3'' and OH), 2.40-2.60 (m, 2H, H2' and H2''), 3.59 (dd, 1H, J = 12.0 and 5.5 Hz, H5'), 3.79 (dd, 1H, J = 12.0 and 3.2 Hz, H5''); 3.94 (s, 3H, MeO); 4.40-4.50 (m, 1H, H4'), 6.09 (dd, 1H, J = 6.2 and 3.0 Hz, H1'), 7.75 (s, 1H, H5). MALDI-TOF MS: *m/z* 375.3 $(M+Na)^+$, 391.2 $(M+K)^+$, $C_{10}H_{13}IN_2O_4$ requires 352.12.

β-anomer: white solid, yield 30%; mp 92-95°C (Et_2O/Hexane). UV (MeOH): λ_{max} 205 (ε 14700), 257 (ε 3400); $\lambda_{shoulder}$ 250 (ε 2400), λ_{min} 245 (ε 2100). ^{1}H NMR ($CDCl_3$): δ 1.90-2.00 (m, 1H, H3'), 2.10-2.30 (m, 1H, H3''), 2.35-2.45 (m, 2H, H2' and H2''), 3.35 (brs, 1H, OH), 3.60 (dd, 1H, J = 12.0 and 5.4 Hz, H5'), 3.84 (s, 3H, MeO), 3.92 (dd, 1H, J = 12.2 and 3.6 Hz, H5''), 4.20-4.30 (m, 1H, H4'), 5.94 (t, 1H, J = 4.0 Hz, H1'), 7.73 (s, 1H, H5). MALDI-TOF MS: *m/z* 375.3 $(M+Na)^+$, 391.2 $(M+K)^+$, $C_{10}H_{13}IN_2O_4$ requires 352.12.

4-Iodo-3-carboxamido-1-(2',3'-dideoxy-β-D-glycero-pentofuranosyl)-pyrazole (17)

4-Iodo-3-methoxycarbonyl-1-(2',3'-dideoxy-β-D-glycero-pentofuranosyl)pyrazole (**16β**, 50 mg, 0.14 mmol)) was deprotected as described above for **5**, to give after purification by silica gel column chromatography (EtOAc/Hexane, 8:2) 20 mg of a brownish syrup, yield 41%.

UV (MeOH): λ_{max} 205 (ε 14700), 259 (ε 3500); λ_{min} 245 (ε 3000). ^{1}H NMR ($CDCl_3$): δ 2.00-2.15 (m, 1H, H3'), 2.20-2.35 (m, 1H, H3''), 2.45-2.60 (m, 2H, H2' and H2''), 3.05 (brs, 1H, OH), 3.64 (dd, 1H, J = 12.3 and 5.3 Hz, H5'), 3.94 (dd, 1H, J = 12.2 and 2.6 Hz, H5''), 4.30-4.40 (m, 1H, H4'), 5.54 (brs, 1H, NH_2), 5.96 (t, 1H, J =

4.8 Hz, H1'), 6.66 (brs, 1H, NH_2), 7.74 (s, 1H, H5). MALDI-TOF MS: *m/z* 360.3 $(M+Na)^+$, 376.3 $(M+K)^+$, $C_9H_{12}IN_3O_3$ requires 337.11.

Biology: Test compounds were dissolved in DMSO at an initial concentration of 200 mM and then were serially diluted in culture medium.

Cells. Cell lines were from American Type Culture Collection (ATCC); bacterial and fungal strains were either clinical isolates (obtained from Clinica Dermosifilopatica, University of Cagliari) or collection strains from ATCC. H9/IIIB and MT-4 cells (grown in RPMI 1640 containing 10% fetal calf serum (FCS), 100 UI/mL penicillin G and mg/mL streptomycin) were used for anti-HIV assays.

The following cell lines were used for antiproliferative assay: Wil2-NS, human splenic B-lymphoblastod cells; Raji, human Burkitt lymphoma; CCRF-CEM, human acute T-lymphoblastic leukemia; HT-29, human colon adenocarcinoma; HeLa, human cervix carcinoma; ACHN, human renal adenocarcinoma; 5637, human bladder carcinoma. Leukemia- and lymphoma-derived cells were grown in RPMI-1640 medium supplemented with 10% FCS, 100 units/ml penicillin and 100 μg/mL streptomycin. Slid tumor-derived cells were grown in their specific media supplemented with 10 % FCS and antibiotics. Cell cultures were incubated at 37°C in a humidified, 5% CO_2 atmosphere. Cell cultures were checked penodically for the absence of mycoplasma contamination with a MycoTect Kit (Gibco).

Viruses. Human immunodeficiency virus type-l (HIV-l, III_B strain) was obtained from supernatants of persistently infected H9/III_B cells. HIV-l stock solutions had a titre of 6 x 10^6 cell culture infectious dose fifty ($CCID_{50}$)/mL.

Antiviral Assays. Activity of compounds against the HIV-1 multiplication in acutely infected cells was based on inhibition of virus-induced cytopathogenicity in MT-4 cells. Briefly, 50 mL of RPMI 10% FCS containing 1 x 10^4 cells were added to each well of flat-bottomed microtiter trays containing 50 mL of medium with or without various concentrations of the test compounds. A suspension of HIV-1 (20 mL) containing 100 $CCID_{50}$, was then added. After 4 days incubation at 37°C, the number of viable cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method, as described by Dezinot and Lang[21] and by Pauwels.[22]

The cytotoxicity of compounds was evaluated in parallel with their antiviral activity. It was based on the viability of mock-infected cells, as monitored by the MTT method.

Antiproliferative assay. Exponentially growing leukemia and lymphoma cells were resuspended at a density of $1x10^5$ cells/mL in growth medium containing serial dilutions of the drugs. Cell viability was determined after 96 hrs at 37°C by the MTT method.[21, 22] Activity against solid tumor-derived cells was evaluated in exponentially growing cultures seeded at $5x10^4$ cells/mL and allowed to adhere for 16 hrs to culture plates before addition of the drugs. Cell viability was determined by the MTT method[21, 22] four days later. Cell growth at each drug concentration was expressed as percentage of untreated controls and the concentration resulting in 50% growth inhibition (IC_{50}) was determined by linear regression analysis.

Antibacterial Assays. *Staphylococcus aureus*, group D *Streptococcus*, *Salmonella* sp. and *Shigella* sp. were recent clinical isolates. Tests were carried out in nutrient broth, pH 7.2, with an inoculum of 10^3 cells/tube. MICs were determined after 18 hrs incubation at 37°C in the presence of serial dilutions of the test compounds.

Antimycotic Assays. Yeast blastospores were obtained from a 30 hrs old shaken culture incubated at 30 °C in Sabouraud dextrose broth. The dermatophyte inoculum was scraped aseptically with a spatula from a 7 day-old culture on agar and the macerate was finely suspended in Sabouraud dextrose broth using a glass homogenizer. Glicerol, final concentration 10%, was added as a cryoprotective agent to both suspensions, yeast and dermatophyte, aliquots of which were then stored in liquid nitrogen. Test tubes were inoculated with 10^3 blastospores or colony forming units (CFU)/tube. The minimal inhibitory concentration (MIC) was determined by serial dilutions using Sabouraud dextrose broth pH 5.7) and incubating at 37°C. The growth control for yeasts was read after 1 day and for dermatophytes after 3 days (5 days for Cryptococcus neoformans). The MIC was defined as the compound concentration at which no macroscopic signs of fungal growth were detected. The minimal germicidal concentration (MGC) was determined by subcultivating negative test tubes in Sabouraud dextrose agar.

ACKNOWLEDGMENTS

This work was supported by the University of Ferrara (research grants 1997-1998) and by ISS progetto AIDS 1998 (grant n° 40A055).

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