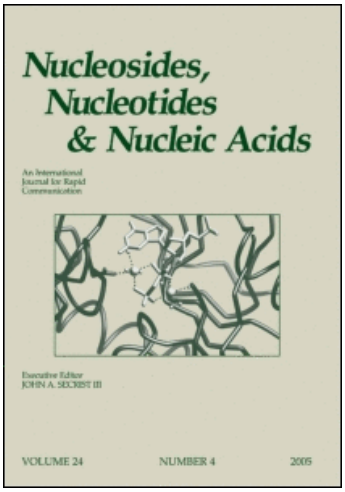

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Aminopyrimidines and Derivatives. 27[1].-Synthesis, Anticancer and Antimicrobiological Activities of 7-Glycopyranosyl-Pyrrolo[2,3-D]Pyrimidines[2]

M. L. Quijano[a]; M. Nogueras[a]; M. Melguizo[a]; G. Alvarez de Cienfuegos[a]; M. Melgarejo[a]; A. Sánchez[a]
[a] Deptos. Química Orgánica y Microbiologia, Colegio Universitario de Jaén Universidad de Granada, Jaén, Spain

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AMINOPYRIMIDINES AND DERIVATIVES. 27[1].-SYNTHESIS, ANTICANCER AND ANTIMICROBIOLOGICAL ACTIVITIES OF 7-GLYCOPYRANOSYL-PYRROLO[2,3-d]PYRIMIDINES[2]

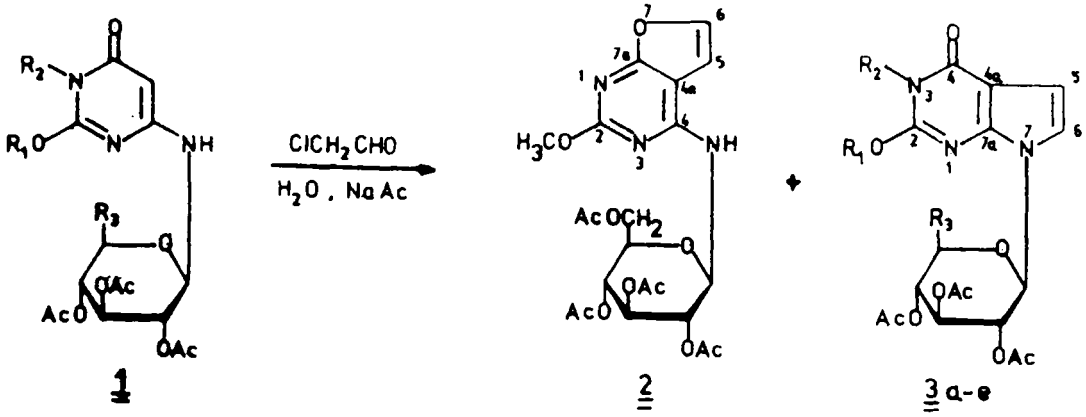
Mª. L. Quijano, M. Nogueras, M. Melguizo, G. Alvarez de Cienfuegos, M. Melgarejo and A. Sánchez

Deptos. Química Orgánica y Microbiologia. Colegio Universitario de Jaén Universidad de Granada. 23071 Jaén. Spain

Abstract : Reaction between 4-(O-acetyl-β-D-glycopyranosylamino)-6-oxopyrimidines **1** and chloroacetaldehyde leads to the corresponding 7-glycopyranosyl-4-oxopyrrolo[2,3-d]pyrimidines **3** in moderate yields. The reaction of **1a** yields also 4-glucopyranosylaminofuro[2,3-d]pyrimidine **2**. The anticancer and antimicrobiological activities of these products are noticed.

Pyrrolo[2,3-d]pyrimidines, which are often described as 7-deazapurines because of their structural analogy to purines, have been reported as potential purine antagonists[3]. Many pyrrolopyrimidine derivatives have been reported to possess antibiotic and antitumor activity[4], central nervous system depressant properties[5], diuretic, cardiac and central nervous system stimulating properties[6] as well as plant growth regulating properties[7].

In an earlier publication in this series, we have recorded the synthesis of some 7-glycopyranosyl-5-oxopyrrolo[2,3-d]pyrimidines by treatment of the corresponding 4-glycopyranosylaminopyrimidines with chloroacetylchloride and subsequent cyclization of the formed 5-α-chloroacetyl derivatives in DMF with anhydrous K_2CO_3 [8]. Similarly, we have also reported the obtained results in the anticancerogenic tests



1 2 3 a-e

	R_1	R_2	R_3
a	CH_3	H	CH_2OAc
b	CH_3	CH_3	H
c	CH_3	CH_3	CH_2OAc
d	H	H	CH_2OAc
e	H	CH_3	CH_2OAc

of the above products againts L 1210 Leukemia previously implanted into mice. As a fellow up, in the present paper we report the obtained results in the reaction of compounds 1 with chloroacetaldehyde as well as the results of the anticancer "in vivo" tests againts L 1210 Leukemia of the obtained compounds and their antimicrobiological activity againts several straims of bacteria and yeasts.

The treatment of **1b,c,d** and **e** with an excess of chloroacetaldehyde in refluxing water/sodium acetate has led to the corresponding 7-glycopyranosylpyrrolo[2,3-d]pyrimidines **3b,c,d** and **e** (Scheme 1) in moderate yields (Table 1). The reaction of **1a** leads, in the same conditions, to the 7-glucopyranosylpyrrolo[2,3-d]pyrimidine **3a** and a fluorescent compound identified as 2-methoxy-4-(tetra-O-acetyl-β-D-glucopyranosylamino)furo[2,3-d]pyrimidine **2** in 43 % and 38% yields respectively. In the reaction of **1d**, the compound **3d** is obtained in 32% yield when the described conditions in the experimental part are used, nevertheless it is possible to obtain up to 60% yield if the reactive

TABLE 1.- Reaction time, yields and analytical data

Compound	Reaction time(m)	Yield (%)	MP (°C) (solvent)	Formula (Mw)	Analysis (%) Calcd (Found) C	H	N
2	45	39	165 EtOH	$C_{21}H_{25}N_3O_{11}$ 495	50.90 (50.67)	5.08 (5.02)	8.48 (8.50)
3a	45	43	118-120 EtOH	$C_{21}H_{25}N_3O_{11}$ 495	50.90 (50.62)	5.08 (4.91)	8.48 (8.32)
3b	90	55	178-180 EtOH	$C_{19}H_{23}N_3O_9$ 437	52.17 (51.89)	5.03 (5.60)	9.61 (9.37)
3c	60	58	138-140 Ethyl ether	$C_{22}H_{27}N_3O_{11}$ 509	51.86 (51.55)	5.34 (5.17)	8.25 (7.98)
3d	20	32	155-160 Ethyl ether	$C_{20}H_{23}N_3O_{11}$ 481	49.89 (49.56)	4.81 (4.87)	8.73 (7.98)
3e	30	62	225 (d) EtOH	$C_{21}H_{25}N_3O_{11}$ 495	50.91 (50.80)	5.09 (5.30)	8.48 (8.47)

amount is decreased. In the first situation, secondary products have been detected by TLC, but at the present we have not succeeded in isolating them with enough purity to their identification. The decrease reactive amount does not produce significant variations in the remaining reactions. On the other hand, when prolonged reaction times are used, a progressive complication, probably due to glycosidic bond hydrolysis, CH_3O hydrolysis (when this group is present) and polymerization, is observed (TLC) in all the reactions.

The structures 2 and 3 are supported by their ^{1}H-NMR and ^{13}C-NMR spectra (Table 2). The ^{1}H-NMR spectrum of 2 shows an exchangeable doublet at 6.35 ppm ($J_{NH,1'}$=9.8 Hz) corresponding to the C_4-NH proton, whereas 3a shows an exchangeable broad singlet at 11.3 ppm attributed to N_3-H; these signals indicate that the cyclization acurrs "via" C_6-O in 2 and "via" C_4-NH in 3a. Moreover, the chemical shift of the hydrogens of the system $-C_6H=C_5H-$ is significative: in 2,$\Delta\delta$ H_5-H_6 is 0.8 ppm whereas the increase in 3a is only 0.2 ppm just as the rest of pyrrolo[2,3-d] pyrimidines. The C_5-C_6 system shows

Table 2.- ^{1}H-NMR and ^{13}C-NMR data

Compound (solvent)	H-6 H-5 $J_{5,6}$	H-1' $J_{1',2'}$	-NH[a] -OH	OAc	C-6 C-5	C-1'
2 ($CDCl_3$)	7.4 d 6.6 d J=2.3 Δδ=0.8	6.35 d[a] J=9.8 5.7 d J=8.9		2.05 s (12H) Δδ =0	140.64 102.65 Δδ=37.99	79.92
3a ($CDCl_3$)	6.9 d 6.7 d J=3.4 Δδ=0.2	5.9 d J=9.5	11.3s broad	2.0 s(9H) 1.75s(3H) Δδ=0.25	118.60 104.44 Δδ =14.16	80.52
3b ($CDCl_3$)	6.8 d 6.6 d J=3.5 Δδ =0.2	5.6[b]		2.05 s(6H) 1.80 s(3H) Δδ =0.25	118.34 104.52 Δδ =13.82	81.32
3c ($CDCl_3$)	6.85d 6.65 d J=3.5 Δδ=0.2	5.7[b]		2.05 s(9H) 1.75 s(3H) Δδ =0.3	118.36 104.50 Δδ=13.86	80.68
3d ($DMSO-d_6$)	6.70d 3.30 d J=3.5 Δδ =0.4	5.6[b]	11.5s broad 10.7s broad	2.00 s(9H) 1.80 s(3H) Δδ=0.2	118.50 103.59 Δδ =14.91	82.68
3e ($DMSO-d_6$)	6.75d 6.35 d J=3.5 Δδ =0.4	5.6[b]	11.8s broad	2.00 s(9H) 1.80 s(3H) Δδ=0.2	117.15 105.78 Δδ =11.37 ($CDCl_3$)	82.56

a)with D_2O dissappears. d=doublet, s=singlet; b)appears with other sugar protons. J(Hz)

remarkable differences too in the ^{13}C-NMR chemical shifts: in compound **2** $\Delta\delta$ C_5-C_6 is 37.99 ppm whereas the increase in **3a** is 14.16 ppm, like the rest of pyrrolo[2,3-d]pyrimidines. Likewise, all the obtained 7-(β-D-O-acetylglycopyranosyl)pyrrolo [2,3-d] pyrimidines show, in their ^{1}H-NMR spectra one of the methylic of the acetate groups upfield shifted about 0.2-0.3 ppm with regard to the remaining acetate groups

which appear as a singlet about 2 ppm; this fact is typical when the cyclizations take place on C_4-NH as we have observed in other cases[9] this shift is not observed in **2** neither in similar products[10]

As we have described, the reaction of **1a** yields a deazapurine and a furopyrimidine, however **1d** and **e** which have also NH which could allow cyclization to C_6-O (N_1-H and N_3-H for **1d** and HO-C=N_3 ⇄ O=C-N_3-H for **1e**) yield deazapurines and no furopyrimidines. In the possible intermediate, C_5-CH_2CHO, formed by electrophilic aromatic substitution at C-5 of the pyrimidine ring, the C_6=O group can give rise to strong interaction with the solvent by intermolecular hydrogen bonds, whereas this is not possible for C_4-NH due to the presence of the C-5 sustituent as well as the glycosidic rest. This fact allows the nucleophilic addition of the C_4-NH group to the carbonyl group of C_5-CH_2CHO to form the corresponding deazapurine instead of the HN_1-C_6=O ⇄ N_1=C_6-OH group, which in no hydroxylic solvents is more reactive[10] . A similar behavior has been observed in other reactions of the same type[9]. The formation of **2**, although in smaller yield than **3a** is a result of is greater stability due to the total aromatization.

Compounds **2**, **3a-e**, 5-α-chloroacetyl-2,6-dioxo-(tetra-O-acetyl-β-D-glucopyranosylamino)-1-methyl-1,2,3,6-tetrahydropyrimidine[8] **4** and 4,5-dioxo-3-methyl-2-methoxy-3,4,5,6-tetrahydro-7-(tetra-O-acetyl-β-D-glucopyranosyl)pyrrolo[2,3-d]pyrimidine[8] **5**, have been tested "in vivo" as inhibitors of the **L 1210** Leukemia at the National Cancer Institute (NCI) acording to standard methods. The T/C percent values have ranged between 93 (**3c**, 100.00 mg/Kg) and 124 (**4**, 240.00 mg/Kg) and none of the products have shown significant anticancer activity.

The antimicrobial activity of these compounds against some bacteria and yeasts straims have been investigated. The compounds **2a** and **3d** have shown some activity towards genus Proteus. The MIC of compound **2** has been 100 μg/ml and 50 μg/ml in **3d**, however these compounds have shown a lack of inhibitory activity in other microorganisms. The rest of tested compounds have presented a weak or lack of the antimicrobial activity againts Gram positive bacteria and yeast especially.

EXPERIMENTAL

Melting points were determined in a Melting Point Apparatus Gallenkamp and are uncorrected. Proton nuclear magnetic resonance

spectra were recorded with Hitachi Perkin-Elmer R-600 and Bruker AM-300 Spectrometers, using tetramethylsilane as an internal standard. Carbon-13 nuclear magnetic resonance spectra were recorded with Bruker AM-300 Spectrometer. Specific rotation values were determined with a Perkin-Elmer 141 polarimeter. Ultraviolet and visible spectra were recorded with a Model 25 Beckman Spectrophotometer. Infrared spectra were recorded with a Beckman 4250 spectrophotometer (KBr pellets). The analysis of C, H, and N have been performed in "Servicios Técnicos de la Universidad de Granada" in Granada. Mass spectra were recorded with Hewlett-Packar HP-5988-A spectrometer. Thin layer chromatography (TLC) was performed on Merck pre-coated TLC aluminum sheets silica gel 60 F_{254}, visualization was accomplished by ultraviolet absorbance followed by charring with a 4% sulfuric acid/methanol solution. Column chromatography was done on Merck silica gel 60 (70-230 mesh) using the solvent systems indicated in each case. Compounds **1** were prepared by previously reported methods[11].

General procedure for the synthesis of **2** and **3**

To 10 ml of distilled water, 2.34 ml (20 mmol) of 2-chloroacetaldehyde dimethylacetal and 1 ml of concentrated chloride acid were added. The mixture was heated till a homogeneous solution was obtained; sodium acetate was then added (pH=6). This mixture was added to a suspension containing **1** (2 mmol) and sodium acetate (0.164 g, 2 mmol) in water (15ml). The reaction was stirred under reflux for appropiate time until no departure product was detected by TLC (Table 1). The reaction mixture was extracted with four portions of 10 ml of CH_2Cl_2 (the extraction was not necessary for **1e** because **3e** precipitate directly). The organic layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. To the crude of the reaction of **1a** and **1d**, 1 g of silica gel and hexane were added; the mixture was then evaporated under reduced pressure, poured into a chromatographic column (4 cmx 50 cm) which contained 50 g of silica gel and next eluted with petroleum ether, petroleum ether:CH_2Cl_2(grow amount of CH_2Cl_2), CH_2Cl_2:EtOH (0-2%) mixtures for **1a** and CH_2Cl_2:EtOH (0-4%) mixtures for **1d**. The CH_2Cl_2 solution (1ml) of the crude reaction of **1b** and **c** was applied on the chromatographic column using CH_2Cl_2: AcOEt (0-30%) mixtures as eluent for **1b** and CH_2Cl_2:EtOH (0-2.5%) mix-

tures for **1c**.The fractions containing the desired products were pooled, evaporated and crystallized from the appropiated solvent (Table 1).

2-methoxy-4-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosylamino) furo[2,3-d]pyrimidine (**2**)

$[\alpha]_D^{20}$=-21.3°(c 1, $CHCl_3$). Rf 0.63, Cl_2CH_2/EtOH (5:0.3). UV (MeOH): λ_{max} nm (ε): 214 (16200), 253 (12400), 272 (13200). IR (KBr) ν_{max} (cm^{-1}): 3370 (N-H), 3140 (C-H), 2960 (C-H), 1750 (C=O), 1625,1600 (C=N, C=C), 1450 (CH_3), 1360 (CH_3), 1225, 1160 (C-N), 1095, 1070, 1035 (C-O). Mass spectrum, m/z (abundance %): 495 (5) M^+, 375 (21), 376 (22), 331 (2), 193 (36), 165 (24), 109 (25), 43 (100).

3,4-dihydro-2-methoxy-4-oxo-7-(2,3,4,6-tetra-O-acetyl- β-D-glucopyranosyl)pyrrolo[2,3-d]pyrimidine (**3a**)

$[\alpha]_D^{20}$=+1.2° (c, 1, Cl_3CH). Rf 0.55, Cl_2CH_2/EtOH (5:0.3). UV (MeOH): λ_{max} nm (ε): 215 (21200), 254 (12500), 268 (shoulder). IR (KBr) ν_{max} (cm^{-1}): 3600-3400 (N-H, O-H), 3120 (C-H), 2950 (C-H), 1750 (C=O), 1690 $(C=O)_{ring}$, 1605, 1550 (C=N, C=C), 1445, 1425 (CH_3), 1375 (CH_3), 1230, 1035 (C-N), 1075, 1065, 1035 (C-O). Mass spectrum, m/z (abundance %): 495 (7) M^+, 331 (4), 168 (77), 164 (45), 108 (75), 43 (100).

3,4-dihydro-3-methyl-2-methoxy-4-oxo-7-(2,3,4-tri-O-acetyl-β -D-xylopyranosyl)pyrrolo[2,3-d]pyrimidine (**3b**)

$[\alpha]_D^{20}$=+9.7° (c 1, Cl_3CH). Rf 0.49, Cl_2CH_2/AcOEt (2:3). UV (MeOH): λ_{max} nm (ε): 219 (7000), 253 (10000), 269 (shoulder). IR (KBr) ν_{max} (cm^{-1}): 3650-3300 (O-H), 3110 (C-H), 2960 (C-H), 1735, 1755 (C=O), 1700 $(C=O)_{ring}$, 1580, 1550, 1515 (C=N, C=C), 1420, 1040 (C-O). Mass spectrum, m/z (abundance %): 437 (15) M^+, 259 (4), 179 (89), 156 (53), 139 (58), 135 (4), 97 (100).

3,4-dihydro-3-methyl-2-methoxy-4-oxo-7-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)pyrrolo[2,3-d]pyrimidine (**3c**)

$[\alpha]_D^{20}$=-2.3°(c 1, Cl_3CH). Rf 0.43, Cl_2CH_2/EtOH (5:0.3). UV (MeOH): λ_{max} nm (ε): 219 (7900), 254 (11100), 268 (shoulder). IR (KBr) ν_{max} (cm^{-1}): 3650-3400 (O-H), 3140 (C-H), 2960 (C-H), 1760 (C=O), 169) $(C=O)_{ring}$, 1575, 1565, 1510 (C=N, C=C), 1410 (CH_3), 1370 (CH_3), 1230 (C-N), 1090, 1065, 1035 (C-O). Mass spectrum, m/z (abundance %): 509 (13) M^+, 331 (3), 178 (83), 169 (100), 164 (7), 127 (30), 148 (6), 109 (88), 43 (81).

1,2,3,4-tetrahydro-2,4-dioxo-7-(2,3,4,6-tetra-O-acetyl-β -D-glucopyranosyl)pyrrolo[2,3-d]pyrimidine (**3d**)

$[\alpha]_D^{20}$=-37.9°(c 1, DMSO). Rf 0.29, Cl_2CH_2/EtOH (5:0.3). UV (MeOH): λ_{max} nm (ε): 215 (16500), 241 (87000), 270 (62000). IR (KBr) ν_{max} (cm^{-1}): 3120 (C-H), 2950 (C-H), 1755 (C=O), 1685 $(C=O)_{ring}$, 1620, 1535 (C=N, C=C), 1440 (CH_3), 1365 (CH_3), 1225 (C-N), 1090, 1060, 1030 (C-O). Mass spectrum, m/z (abundance %): 481 (2) M^+, 331 (2), 169 (39), 151 (4), 127 (17), 109 (47), 43 (100).

1,2,3,4-tetrahydro-3-methyl-2,4-dioxo-7-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)pyrrolo[2,3-d]pyrimidine (**3e**)

$[\alpha]_D^{20}$=-8.7°(c 1, Cl_3CH). Rf 0.37, Cl_2CH_2/EtOH (5:0.3). UV (MeOH): λ_{max} nm (ε): 219 (10300), 242 (8700), 269 (6800). IR (KBr) ν_{max} (cm^{-1}): 2950 (C-H), 1760 (C=O), 1705 $(C=O)_{ring}$, 1650, 1575 (C=N, C=C), 1455, 1425 (CH_3), 1370 (CH_3), 1225, 1210 (C-N), 1065, 1035 (C-O). Mass spectrum, m/z (abundance %): 495 (3) M^+, 169 (52), 165 (8), 127 (19), 109 (57), 43 (100).

"In vivo" antitumor activity against **L 1210** Leukemia was determined by the NCI according to the protocol described in instruction 14. The **L 1210** Leukemia was implanted into CDF_1 mice and each mouse was innoculated once at various dose levels and observed for 20 d. The result was evaluated as %T/C=(median survival time (MST) treated/MST control)x100, and compound is considered active if %T/C exceed 125.

The determination of minimal inhibitory concentration (MIC) of these compounds against Pseudomones, E. Coli, Proteus, Salmonella, Micrococcus, Staphylococcus, Bacillus and Candida was performed with some modifications of the technique described by Jones et al.[12].

Briefly, a working antimicrobial solution is prepared by dilution of the compound in Mueller-Hinton broth (MH) to the highest final concentration (2000 μg/ml). In some compounds dimethylsulfoxide at 10% in MH is required for his dissolution. For each microorganism tubes at 1000, 500, 100, 50, 25, and 5 μg/ml of each compound were prepared by successive dilutions with MH and were inoculated with 10 μl of MH containg 10^4 colony forming units of microorganism tested. The microorganism concentration was determined by turbidimetric method. After 20 h of incubation at 37°C or 20°C by Pseudomonas tests, the

tubes were examined for MIC determination. MIC is defined as the lowest concentration of compound resulting in complete inhibition of visible growth.

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